Increasing the data storage capacity of sequence-defined macromolecules

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„Die Kunst ist,

einmal mehr aufzustehen,

als man umgeworfen wird.“

Sir Winston Churchill

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Declaration of Authorship

Die vorliegende Arbeit wurde von Februar 2018 bis März 2021 unter Anleitung von Prof. Dr. Michael A. R. Meier am Institut für Organische Chemie (IOC) des Karlsruher Instituts für Technologie (KIT) angefertigt.

Erklärung

Hiermit versichere ich, dass ich die Arbeit selbstständig angefertigt, nur die angegebenen Quellen und Hilfsmittel benutzt und mich keiner unzulässigen Hilfe Dritter bedient habe. Insbesondere habe ich wörtlich oder sinngemäß aus anderen Werken übernommene Inhalte als solche kenntlich gemacht. Die Satzung des Karlsruher Instituts für Technologie (KIT) zur Sicherung wissenschaftlicher Praxis habe ich beachtet. Des Weiteren erkläre ich, dass ich mich derzeit in keinem laufenden Promotionsverfahren befinde, und auch keine vorausgegangenen Promotionsversuche unternommen habe. Die elektronische Version der Arbeit stimmt mit der schriftlichen Version überein und die Primärdaten sind gemäß Abs. A (6) der Regeln zur Sicherung guter wissenschaftlicher Praxis des KIT beim Institut abgegeben und archiviert.

Karlsruhe, den 2. März 2021

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Maximiliane Frölich

Abstract

The field of sequence-defined macromolecules is inspired by the high molecular definition of biomacromolecules that occur in nature, such as DNA and peptides. Their complex and perfectly defined structure enable their biochemical functions, which play an important and relevant role in living organisms. The sequencing and the complete read-out of the DNA are considered one of the most significant scientific discoveries over the last years, and DNA can be described as one of the most prominent examples for storing information on a molecular scale. The resulting idea of storing data in sequence-defined molecules and subsequent increase of data storage density in different systems of sequence-defined macromolecules emerged in recent years to become a major research topic. This thesis investigates sequence-defined Passerini molecules in the field of data storage by exploiting an established iterative synthesis cycle to ultimately enhance data storage capacity. In the first step of the iterative cycle, a Passerini three component reaction (P-3CR) with a carboxylic acid, an aldehyde and an isocyanide bearing a benzyl ester is performed and afterwards in the second step a hydrogenolytic deprotection yields the intended oligomers. Using the iterative step cycle, different systems like side chain defined, backbone defined, and dual sequence-defined oligomers were synthesized and analyzed. Analytical methods were investigated regarding there detection threshold to track and identify impurities. Afterwards, the molecular data storage application was in focus, investigating and developing a successful read-out strategy for the application of the oligomers in the field of data storage. Therefore, tandem electron spray ionization (ESI-MS/MS) measurements of different Passerini systems, such as side chain defined, backbone defined and dual sequence-defined oligomers, had to be analyzed and fragmentation patterns assigned accordingly. Thereby, two common patterns were observed and used in the subsequent development of the read-out process. Hence, the read-out of sequence-defined Passerini oligomers was demonstrated and the data storage capacity of the different systems were compared with each other. Moreover, the investigated system was capable of a simultaneous and automated read-out of oligomer mixtures. Therefore, different sequence-defined oligomers (six trimer, twelve tetramers and three hexamers) with varying side chains and mass markers were synthesized. Due to the application of different mass markers, the analysis of the ESI-MS/MS measurements were simplified allowing the unambiguous read-out of mixtures of sequence-defined macromolecules. Through the successful read-out of three hexamer in a mixture, by hand and automated, an increase of

the storage capacity up to 64.5 bits was achieved. Additionally, the read-out of a oligomer mixture with up to six oligomers was achieved.

Zusammenfassung

Das Feld der sequenzdefinierten Makromoleküle wurde durch die Natur inspiriert, in der hochdefinierte Biomakromoleküle, wie zum Beispiel DNA oder Peptide, vorkommen. Die durch ihre komplexe und perfekt definierte Struktur entstehenden biochemischen Eigenschaften spielen eine bedeutende Rolle in unserem Leben. Als eine der bedeutendsten wissenschaftlichen Entdeckungen der letzten Jahre gilt die Sequenzierung und das anschließend vollständige Auslesen der DNA. Sie stellt damit eines der prominentesten Beispiele für die Datenspeicherung in Molekülen dar. Der daraus entwickelte Ansatz der Speicherung von Daten in sequenzdefinierten Molekülen und die anschließende Erhöhung der Datendichte erweckte in den vergangenen Jahren immer größeres Interesse. In dieser Arbeit werden neuartige Ansätze zur Etablierung sequenzdefinierte Passerini Makromoleküle im Bereich der Datenspeicherung untersucht, indem ein bereits etablierter iterativer Zyklus zur Synthese der Makromoleküle verwendet wird um die Datenspeicherdichte zu erhöhen. Der erste Schritt des iterativen Zyklus beinhaltet die Passerini-Dreikomponentenreaktion, in der eine Carbonsäure, ein Aldehyd und ein Benzylester geschütztes Isocyanide miteinander reagieren. Im zweiten Schritt findet eine Entschützung des Benzylesters statt, um erneut die freie Säure zu erhalten. Durch die Verwendung des iterativen Reaktionszyklus konnten verschiedene Seitenketten-definierte sequenzdefinierte Makromoleküle synthetisiert, sowie weitere Rückgrat-definierte, und Dual-sequenzdefinierte Makromoleküle analysiert werden. Analytische Methoden wurden hinsichtlich ihrer Nachweisgrenze untersucht, um Verunreinigungen nachzuweisen und zu identifizieren. Anschließend stand die Anwendung der sequenzdefinierten Makromoleküle im Fokus, insbesondere die Entwicklung einer erfolgreichen Strategie zum Auslesen der Information von Oligomeren und der damit folgende Einsatz im Bereich der Datenspeicherung. Dafür wurden Tandem Elektronen Spray Ionisation (ESI-MS/MS) Messungen vom verschiedene Passerini Systemen, wie Seitenketten-definiert, Rückgrat-definiert oder Dual-sequenzdefiniert durchgeführt und analysiert, sowie die Fragmentierungsmuster entsprechend zugeordnet. Dabei konnten zwei signifikante Fragmentierungsmuster beobachtet und zur Entwicklung eines Ausleseverfahrens verwendet werden. Dies ermöglichte das Auslesen von sequenzdefinierten Passerini-Oligomeren und den Vergleich der Datenspeicherkapazität verschiedener Systeme miteinander. Anschließend konnte ein simultanes und automatisiertes Auslesen von Oligomer Mischungen demonstriert werden, wofür sequenzdefinierte Oligomere (sechs

Trimere, zwölf Tetramere und drei Hexamere) mit unterschiedlichen Seitenketten und Massemarkern verwendet wurden. Durch die Verwendung verschiedener Massenmarker wurde die Auswertungen der ESI-MS/MS-Daten vereinfacht und dies ermöglichte das eindeutige Auslesen von Oligomer Mischungen. Mittels manueller und automatisierter Auslesung von Oligomer Mischungen wurde eine Datenspeicherdichte von bis zu 64.5 Bits erzielt. Zusätzlich konnte das Auslesen von Oligomer Mischungen mit bis zu sechs verschieden Oligomeren gezeigt werden.

Table of content

1 Introduction

How to store data safely and for a long time? This is one of humanity's main questions in the last decades. Through the fast growth of digitalization, data storage capacity and data storage in general has become more and more important.[1] Compact discs can get broken, electronic files can be overwritten, so to ensure a safe and robust way of storing of our data for a long time, new systems must be developed. Nature achieves such functions with biomacromolecules, like DNA and peptides, and is an interesting inspiration. Due to its complexity and its perfectly defined sequences, DNA enables a robust and efficient storage architecture[2,3] and the storage of our genetic code is the best example of data storage in molecules.[4,5] The sequencing and full read-out of the DNA was a key topic in the scientific world in the 20st century.[6] In the field of sequence-defined macromolecules, which is inspired by the precision found in DNA, data storage is an interesting potential application and is already discussed in literature.[7–11] Sequence-defined macromolecules with a distinct order of repeating units and a defined length are called "uniform" molecules.[12] In the beginning, the synthesis of these defined complex structures was the focus of the scientific community and different synthesis pathways were developed increasing the degree of precision and control achieved.[13–19] Three different approaches are mostly used: iterative exponential growth, bidirectional growth and iterative stepwise approach.[16,20–22] The use of the iterative stepwise approach enables the highest possible control over the repeating units. Multicomponent reactions are a suitable tool for the synthesis of sequence-defined macromolecules due to their high yield and minimal side reactions. Furthermore, due to their highly modular character, different functionalities can be easily introduced in the side chains or in the backbone to increase the structural variety of the oligomers.[14,23] A well-established multicomponent reaction for the synthesis of sequence-defined oligomers is the Passerini three-component reaction and it can be performed in solution or on solid phase.[10,24–26] Furthermore, its combination with other reactions offers a versatile tool for the synthesis of different architectures and sequences.[15,26–29] After the report of many different synthesis approaches for sequence-defined oligomers, it was possible to demonstrate the application in the field of data storage.[11,30–33] For the read-out process, tandem mass spectrometry is generally used,[34–37] however, recently, the read-out of small sequences by single mass spectrometry was also reported.[10] In this thesis, novel approaches for data storage in sequence-defined macromolecules are investigated and the already established iterative synthesis approach using a Passerini three-component reaction is used for the oligomer

synthesis. Furthermore, the increase of the data storage capacity by the variation of the oligomers, but also by the readout of more complex systems, is herein investigated.

2 Theoretical Background

2.1 Multicomponent reactions

Multicomponent reactions (MCRs) can be defined as a class of reactions, where three or more starting compounds react to form one complex product.[38] MCRs became increasingly interesting over time because of their significant advantages compared to conventional multistep syntheses. First of all, they have a highly modular character. The individual components commonly have easily accessible functional groups and are available on large scales and a great variety.[39,40] Additionally, MCRs show high atom efficiency and are often time saving since no intermediate has to be isolated and later purified. In recent years, MCRs gained more interest, for example in the field of green chemistry, due to the minimal waste generated during the reaction and the possible use of sustainable compounds in these reactions.[41–44] Furthermore, MCRs are used in pharmaceutical chemistry for the generation of component libraries,[45–47] in the field of sequence-definition, due to their high yields and simple reaction procedures, as well as in polymerizations.[26,48–52] In general, MCRs can be divided into three different reaction types depending on the reaction mechanism, as shown in Table 1.

Table 1: The three types of MCRs[38]

Type of MCR	Reaction scheme
I[a]	A + B ⇌ C ⇌ … ⇌ P
II[b]	A + B ⇌ C ⇌ D …… ⟶ P
III[c]	A ⟶ C + D ⟶ E ⟶ … ⟶ P

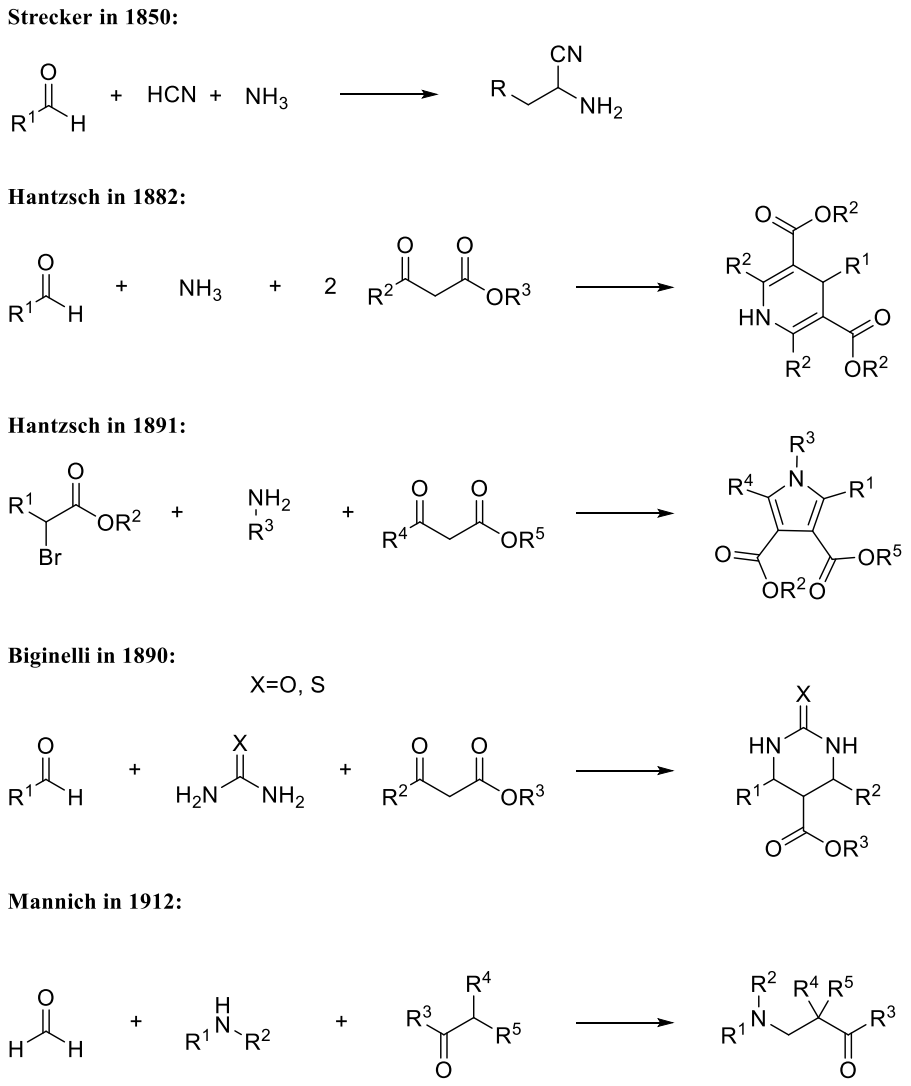
[a] All steps are reversible, [b] only the last step is irreversible, [c] all steps are irreversible. A, B: starting material; C,D,E: intermediates; P: product

In type I of MCRs, all reaction steps are reversible, the starting material, intermediates and product are in equilibrium. Low yields are common for type I reactions because of the difficult isolation of the product, since mixtures of product, intermediates, and starting material are obtained. In type II reactions, the last step is irreversible. The advantage of this type is that the equilibrium is shifted to the side of the product. Examples for the irreversible steps can be a ring-closing, aromatization, or a highly exothermic reaction. In the following chapters, examples of type II reactions are described, like the Passerini-three component reaction

(P-3CR) or the Ugi-four component reaction (U-4CR). In reactions of type III, all steps are irreversible. Biochemical reactions in the living world offer some examples for this type.[38]

2.1.1 History of MCRs

In 1850, the synthesis of α-amino acids *via* amino cyanides was published by Strecker as the first MCR.[53] In this report, hydrogen cyanide, an aldehyde and ammonia were reacted to form the corresponding α-amino acids. Around 40 years later, Hantzsch described a dihydropyridine synthesis in 1882.[54] Another eight years later, Hantzsch published a pyrrole synthesis that described the reaction of a β-ketoester with an amine and α-ketoester.[55] In 1891, the reaction of an urea, aldehydes and a β-ketoester, leading to the formation of heterocyclic dihydropyrimidones, was published by Biginelli.[56] In 1912, Mannich described one of the most known and interesting MCRs.[57] In this report, an amine, formaldehyde and an oxo-component, like an aldehyde or a ketone, react to form a β-aminocarbonyl compound. An overview of the history of these reactions is shown in Scheme 1. Furthermore, in 1921, Mario Passerini published the first isocyanide-based multicomponent reaction (IMCR).[24] This and another IMCRs are further discussed in chapter 2.1.2.2.



Scheme 1: Historical important MCRs.[38]

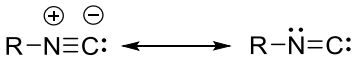
2.1.2 Isocyanide-based multicomponent reactions (IMCRs)

IMCRs are an important subclass of MCRs, if not the most important. The reactivity and characteristics of the isocyanide itself, as well as its synthesis and use in IMCRs are discussed in this chapter.

2.1.2.1 Isocyanides

In 1859, the synthesis of the functional group of isocyanides, also known as isonitriles, was first described by Lieke.[58] They are one among the few groups with a formally divalent carbon atom. Other than isocyanides, only carbon monoxide and carbenes exhibit this structural feature.[38] The α-acidity, the ability to perform α-addition and the easy formation of radicals is a token to the diversity of isocyanides. This extraordinary reactivity can be explained by their

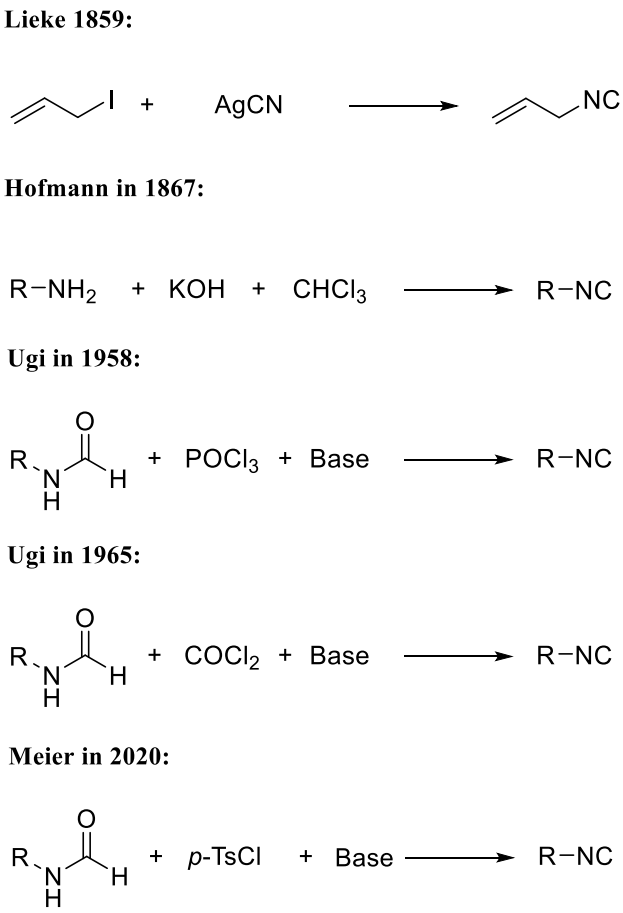
resonance structures: on one hand, the zwitterionic structure and the carbenoid structure on the other, which are shown in Scheme 2.



Scheme 2: Resonance structure of the isocyanide: zwitterionic and carbenoid structure.[59]

Interestingly, naturally occurring marine isocyanides show antibiotic, antineoplastic, fungicidal, or antifouling effects,[38] *e.g.* the antibiotic Xanthocillin from Penicillium Chrysogenum or the marine diterpenoid isocyanide Kalihinene.[60,61] Due to the unique reactivity of isocyanides, they are also used in different kinds of syntheses, *e.g.* in the synthesis of heterocycles, oxazoles and imidazoles.[38,62,63] Most often, they are used in the field IMCRs, as described in the following.

As previously mentioned, Lieke synthesized the first isocyanide in 1859 by reacting an allyl halogen with silver cyanide.[58] Interestingly, the silver cation acted as a protecting group for the cyanide carbon and the nitrogen attacked the halide as a nucleophile to yield the isocyanide. In 1867, Hofmann described the synthesis of an isocyanide without silver cyanide by the reaction of a primary amine with chloroform and potassium hydroxide. [64] Around 100 years later, Ugi discovered a synthesis involving the reaction of *N*-formamides with a base and phosphorus oxychloride ($POCl_3$) to yield isocyanides.[65,66] In 1965, Ugi also described a synthesis with phosgene, instead of $POCl_3$, and a base for a successful and variable synthesis of isocyanides.[59] Nowadays, *N*-formamides are often used as starting materials for the synthesis of isocyanides and, to avoid the use of highly toxic phosgene, especially in laboratory work, $POCl_3$ is used. In 2015, Dömling *et al.* described an interesting route for the *N*-formamide synthesis. Instead of an amine as starting material, an oxo-component was used. In a Leuckart-Wallach reaction, the oxo-component reacted with formamide and formic acid to yield the *N*-formamide. Afterwards, the corresponding isocyanides were synthesized, again with $POCl_3$ and triethylamine, even *in situ*.[67] With this approach, variable structural motifs can be synthesized and it has thus been employed also in this thesis. Furthermore, a more sustainable isocyanide synthesis for non-sterically demanding aliphatic *N*-formamides as starting materials was established in 2020 by Meier *et al*.[68] In this report, *p*-toluenesulfonyl chloride (*p*-TsCl) was used instead of $POCl_3$ and pyridine as a base. An overview of the history and development of the isocyanide synthesis is shown in Scheme 3.

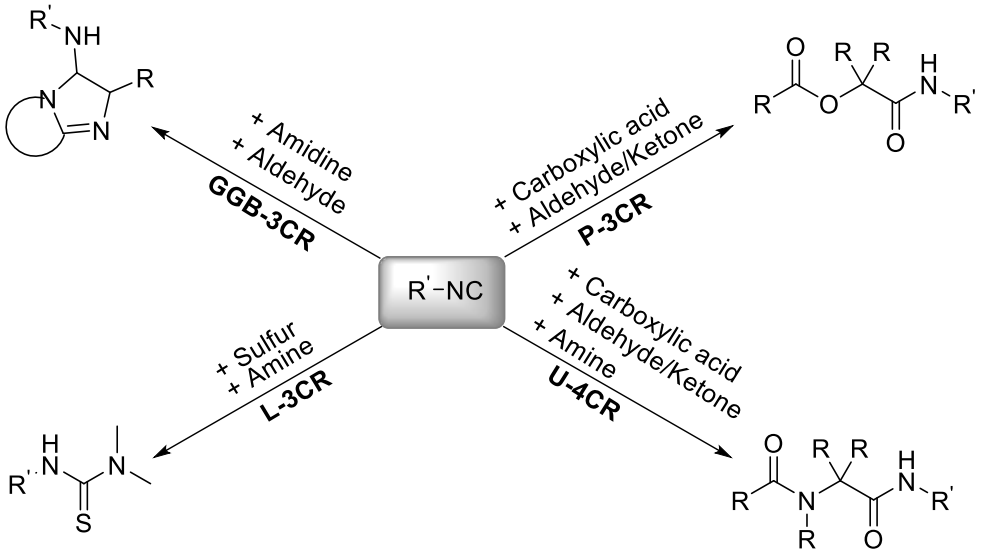


Scheme 3: Historical overview of the isocyanide synthesis.[58,59,64–66,68]

Another interesting synthesis was described by Dömling *et al.* in 2015.[69] To avoid the noxious smell, especially for low molecular weight isocyanides, the isocyanide was synthesized *in-situ* from *N*-formamide with triphosgene, and then used directly in an IMCR.

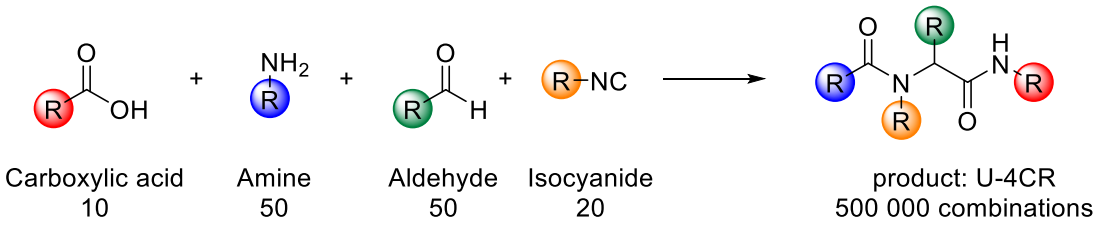
2.1.2.2 Isocyanides multicomponent reactions

The most popular examples of IMCRs are the P-3CR and the U-4CR.[24,70] Nevertheless, also other interesting IMCRs like the Groebke-Blackburn-Bienaymé (GGB-3CR) and the Lipp three component reaction (L-3CR) are frequently used for different applications.[71–74] In Scheme 4, an overview of different IMCRs is depicted, while the reactions and their applications are discussed in the following section.



Scheme 4: Four important ICMRs: P-3CR, U-4CR, GBB-3CR and L-3CR.[69,74]

In 1959, the U-4CR was discovered by Ugi.[70] In this reaction, the aldehyde initially reacts with the amine to form an imine and afterwards with the isocyanide and the carboxylic acid to yield an α-amino acylamide. The last step of the reaction is irreversible and is named Mumm rearrangement. Due to its molecular diversity and synthetic potential, the U-4CR ranks among the most important MCRs with a lot of different applications.[38,69] Some applications are, *e.g.*, in the field of molecular data storage, medical chemistry, sequence-definition, green chemistry and polymer chemistry.[23,33,75–79] An example that showcases the modular character of U-4CR was reported in 2018, when Meier *et al*. used the U-4CR for data storage.[33] They described an example database of 130 commercially available components, which can be potentially be combined to 10 × 50 × 50 × 20 = 500,000 different molecules. This displays the large combinatorial scope of the U-4CR that is practically only limited by the number of commercially available components.

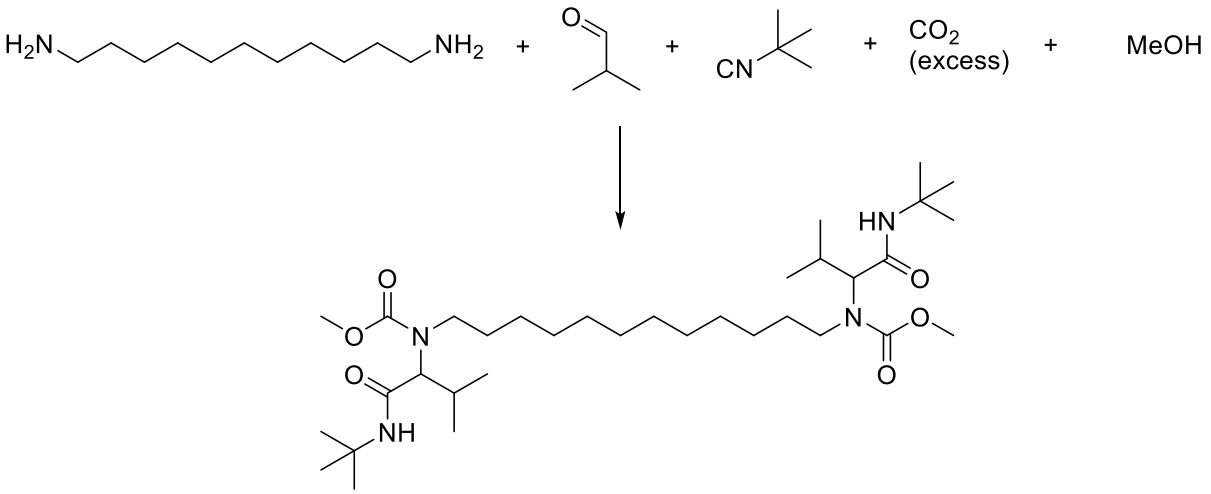


Scheme 5: Schematic of the U-4CR showcasing its large combination potential with the possibility to yield 500,000 different molecules.[33]

Especially in the field of medical chemistry, different variations of the reaction have been employed. One example is the Ugi tetrazole variation (UT-4CR) in the field of bioactive compounds, such as inhibitor of γ-aminobutyric acid transporters.[80,81] Furthermore, the Ugi

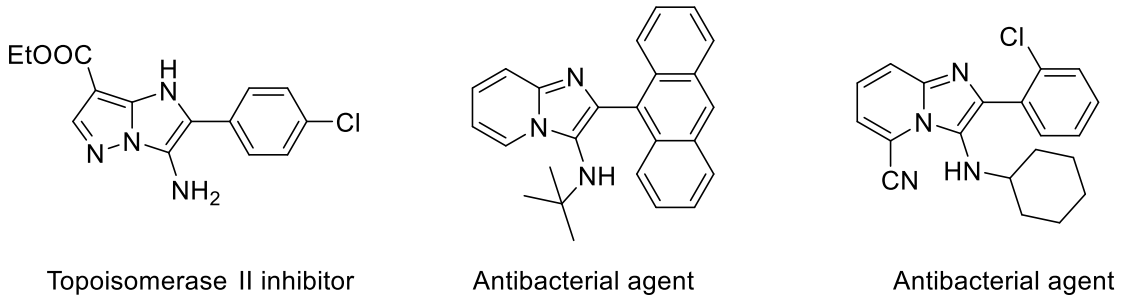
five-center-four-component reaction (U-5C-4CR) involving a free α-amino acid, an aldehyde, an alcohol and isocyanide yields iminodicarboxylic acid monoamide monoesters, which are used in the industrial synthesis of clinical oxytocin receptor antagonists Epelsiban and Atosiban.[82,83]

Furthermore, variations of the U-4CR are also reported in the field of polymer chemistry. One example is the Ugi five-component condensation (U-5CC) between a diamine, isobutyraldehyde, *tert*-butyl isocyanide, methanol and carbon dioxide, leading to substituted dicarbamates (see Scheme 6).[84] This compound was subsequently polymerized to yield non-isocyanate polyurethanes (NIPUs). The direct polymerization *via* the U-5CC to yield polyhydantoins has also been reported.[84]



Scheme 6: U-5CC for the synthesis of dimethyl carbamates as monomer for the synthesis of polyurethanes.[84]

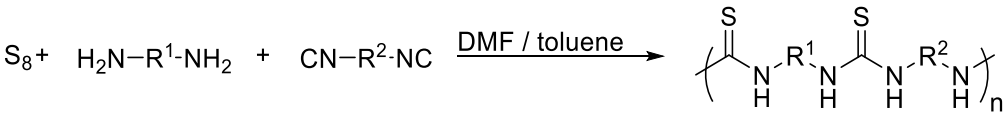
The GGB-3CR, with an imidazole[1,2-*a*]-heterocycle as core structure, has also been described as a variation of the U-4CR and was discovered in 1998 by three independently groups (Groebke−Blackburn−Bienaymé).[71–73] In this reaction, heteroaromatic amidines react with an aldehyde and an isocyanide to yield *N*-bridged imidazoles. Nowadays, the GGB-3CR is often used for the synthesis of bioactive molecules, like kinase inhibitors, antibacterial agents, or HIV-1 reverse transcriptase inhibitors.[85–88] In Figure 1, three examples of the bioactive molecules synthesized *via* the GBB-3CR are displayed.



Topoisomerase II inhibitor | Antibacterial agent | Antibacterial agent

Figure 1: Bioactive molecules, synthesized *via* the GBB-3CR.[86–88]

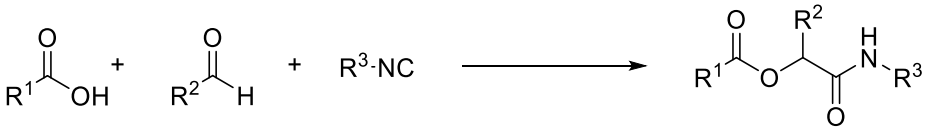
In 1958, another relevant ICMR was described by Lipp *et al.*[74] Elemental sulfur was reacted with an amine and an isocyanide to yield a thiourea,[89] which are often used as catalysts or in polymer chemistry.[90,91] Furthermore, an alcohol or a thiol instead of the amine were also used to yield *O*-thiocarbamates or dithiocarbamates, respectively.[92] A multicomponent polymerization was demonstrated by Hu and Tang in 2018.[90] They reported the reaction of sulfur, an aliphatic diamine and a diisocyanide at room temperature to yield polythioureas, as depicted in Scheme 7. Furthermore, the reaction was a good example for how to use the excess elemental sulfur produced by the crude oil industry.[93,94] There sulfur is produced in a large scale as a byproduct in the oil and gas production and available as an interesting and inexpensive compound.



Scheme 7: Multicomponent polymerization with sulfur, a diamine and a diisocyanide to yield a polythiourea.[90]

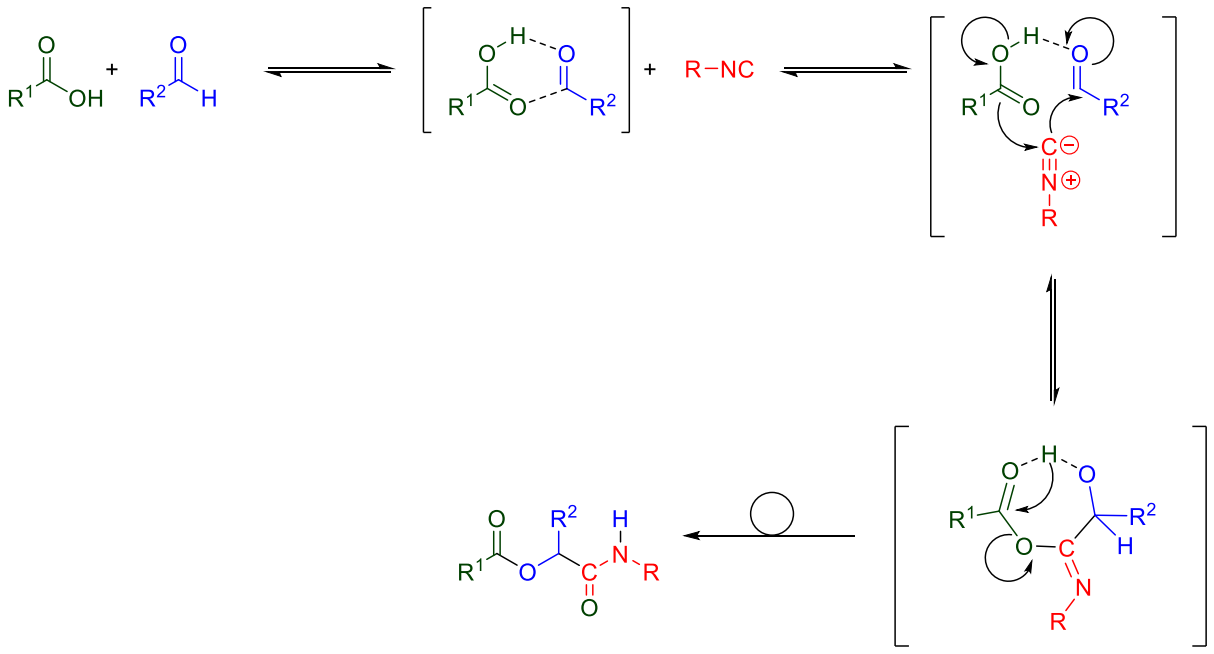
2.1.3 Passerini three component reaction

In 1921, the P-3CR was discovered as the first IMCR by Mario Passerini.[24] A carboxylic acid and an oxo-compound were reacted with an isocyanide to yield an α-acyloxy amine (see Scheme 8). With simple reaction conditions, such as performing the reaction at room temperature, high concentration, and the use of aprotic solvents, *e.g.* dichloromethane (DCM), excellent yields were obtained.[38]



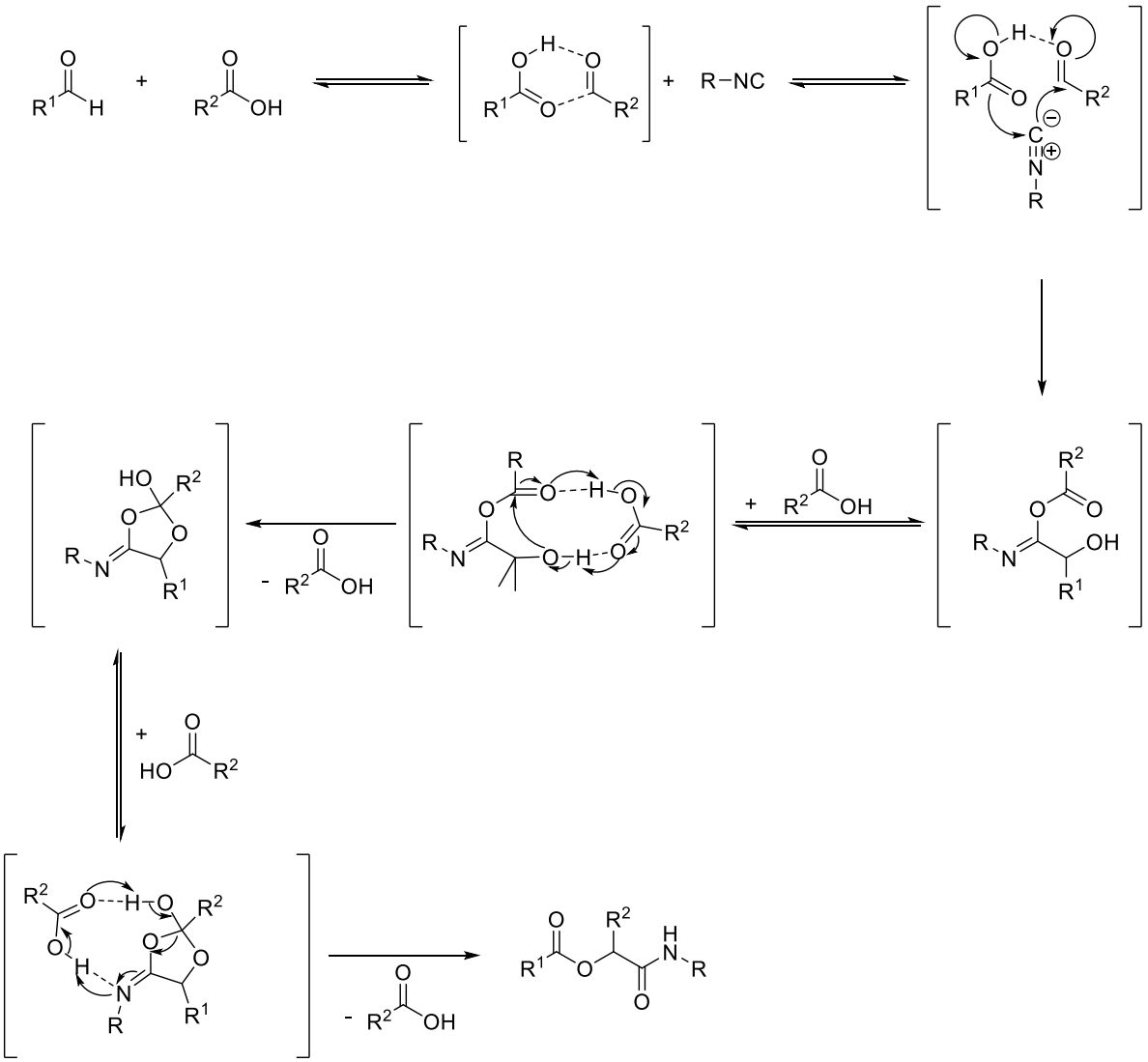
Scheme 8: P-3CR with a carboxylic acid, an oxo-compound, and an isocyanide to yield an α-acyloxy amine.[24]

Although the P-3CR has been known for 100 years, the mechanism is not fully understood yet. Passerini himself proposed a plausible mechanism and further investigations by Baker and Ugi supported it.[24,95,96] Accordingly, the oxo-compound initially reacts with the carboxylic acid to form a hydrogen bonded adduct. Subsequently, the isocyanide reacts in an α-addition and a cyclic transition state is formed, which was not isolated. Afterwards, an irreversible rearrangement takes place and the final Passerini α-acyloxy amine is formed. The described mechanism is displayed in Scheme 9.



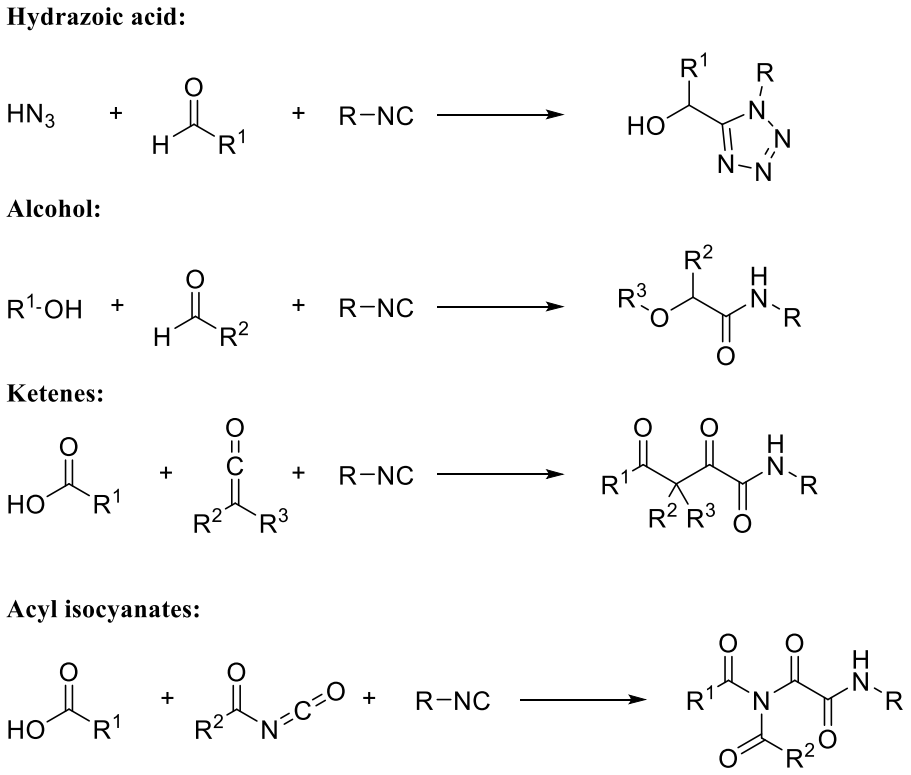
Scheme 9: Proposed mechanism of the P-3CR. First, a hydrogen bonded adduct is formed. Afterwards, the isocyanide reacts in an α-addition to form a cyclic transition state. Subsequently, the irreversible rearrangement takes place to form the Passerini product.[24,95]

In 1965, Eholzer proposed another mechanism, where the isocyanide is first protonated by the carboxylic acid.[97] This assumption was based on the fact that the Passerini reaction is faster with a mineral acid as catalyst. Furthermore, it agrees with the report that P-3CRs are accelerated in water.[98] However, this contradicts Ugi's statement that the Passerini reaction proceeds best in aprotic, non-polar solvents. In 2011, Maeda *et al.* postulated another mechanism based on calculations in the gas phase, where a fourth component is involved (see Scheme 10).[99] In this mechanism, two carboxylic acids react, one of which acts as the a catalyst. During the rearrangement, the second acid is introduced to decrease the individual barrier. The activation and α-addition steps are similar to the mechanism proposed by Passerini. Therefore, the reaction has been described as an organo-catalyzed three-component reaction. Density functional theory (DFT) calculations further support this postulated mechanism.[100]



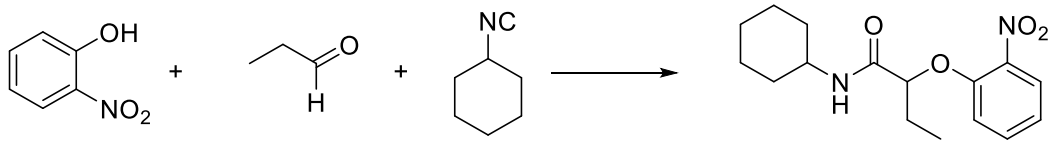
Scheme 10: Postulated mechanism of the P-3CR as a organo-catalyzed three-component reaction.[99]

Due to the structural diversity generated in a single-step synthesis and its easy implementation, the P-3CR gained a lot of interest over time. The wide range of applications of the P-3CR are found in medical chemistry, combinatorial chemistry, sequence-definition, polymerization, and green chemistry, some of which will be described in the following section. Furthermore, many variations of the P-3CR have been reported, for example replacing the carboxylic acid with hydrazoic acid to yield a 5-(1-hydroxyalkly)tetrazole (see Scheme 11)[101,102] or using alcohols instead of carboxylic acids for the synthesis of α-alkoxy amide derivates.[103–105] Moreover, the use of ketenes or acyl isocyanates instead of aldehydes has been reported, leading to α,γ-diketo-carboxamides or *N*,*N*-diacyloxamides (see Scheme 11).[96,106]



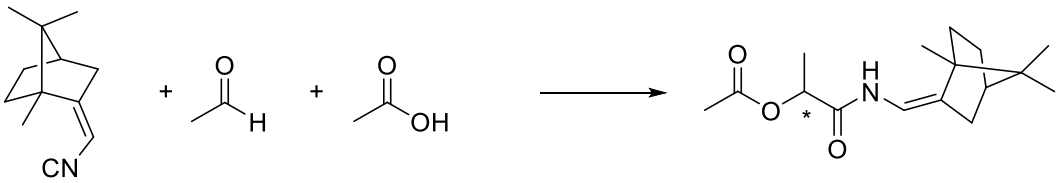
Scheme 11: Four variations of the P-3CR using hydrazoic acid or alcohols instead of carboxylic acids and ketenes or acyl isocyanates instead of aldehydes. [96,101–106]

Furthermore, the general P-3CR has also been performed with alcohols, which were oxidized *in-situ* to the corresponding aldehyde with 2-iodoxybenzoic acid (IBX) as oxidation agent.[107] This reaction is used as an alternative for unstable or difficult to synthesize aldehydes. Soeta *et al.* also reported an oxidative Passerini reaction with meta-chloroperoxybenzoic acid (mCPBA) as oxidation agent.[108] The aldehyde, isocyanide and sulfinic acid, in place of carboxylic acid, reacted with the mCPBA to form α-(sulfonyloxy)amides. Furthermore, Soeta *et al.* reported the successful synthesis of P-3CR with phosphinic acid or silanols instead of carboxylic acids to form α-(phosphinyloxy)amides or α-siloxyamides.[108,109] Another interesting variation of the P-3CR is the Passerini-Smiles reaction.[110,111] There, electron poor phenol derivatives are used instead of the carboxylic acid to react with an aldehyde and an isocyanide to form an O-arylated compounds. In Scheme 12, the reaction of *o*-nitrophenol, with cyclohexyl isocyanide and propionadehyde to yield the α-aryloxyamide is shown. The key step of the reaction is the irreversible Smiles rearrangement of the intermediate. The Smiles rearrangement is also observed in the analogous Ugi-Smiles reaction.[110]



Scheme 12: Passerini-Smiles reaction of o-nitrophenol, propionaldehyde and cyclohexyl isocyanide to yield an α-aryloxyamide.[110,111]

For the introduction of stereoselectivity in IMCRs, it was observed that a chiral amine must be used for the U-4CR, while chirality in the other components only resulted in low yields or no product was isolated. However, in the P-3CR, no amines are used, therefore a chiral isocyanide was instead employed and the Passerini product was obtained in high diastereoselectivity. With the same chiral isocyanide, no product was obtained in the U-4CR. This result further confirmed the different reaction mechanisms of the U-4CR and the P-3CR.[38,40,112] In Scheme 13, the highly diastereoselective reaction of the chiral isocyanide, which can be synthesized in a two-step reaction from camphor, with acetic acid and acetaldehyde is shown.



Scheme 13: Diastereoselective P-3CR with a chiral isocyanide.[112]

Furthermore, in the field of medical chemistry, different important drugs can be synthesized by the P-3CR. Casodex® (see Figure 2) as the leading drug in the treatment of prostate cancer is one of the most prominent examples.[45] The active pharmaceutical ingredient bicalutamide is synthesized in a $TiCl_4$-mediated type of P-3CR.[113]

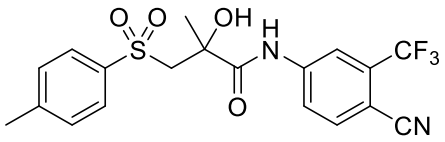


Figure 2: Chemical structure of Casodex®.[45]

The P-3CR is also applied in the field of green chemistry,[44,114] *e.g.* in the synthesis of glycomimetics[114] or in the modification of cellulose.[41–43] A lot of sustainable synthesis examples using P-3CR are also known in the field of polymer chemistry, which is described in the following.

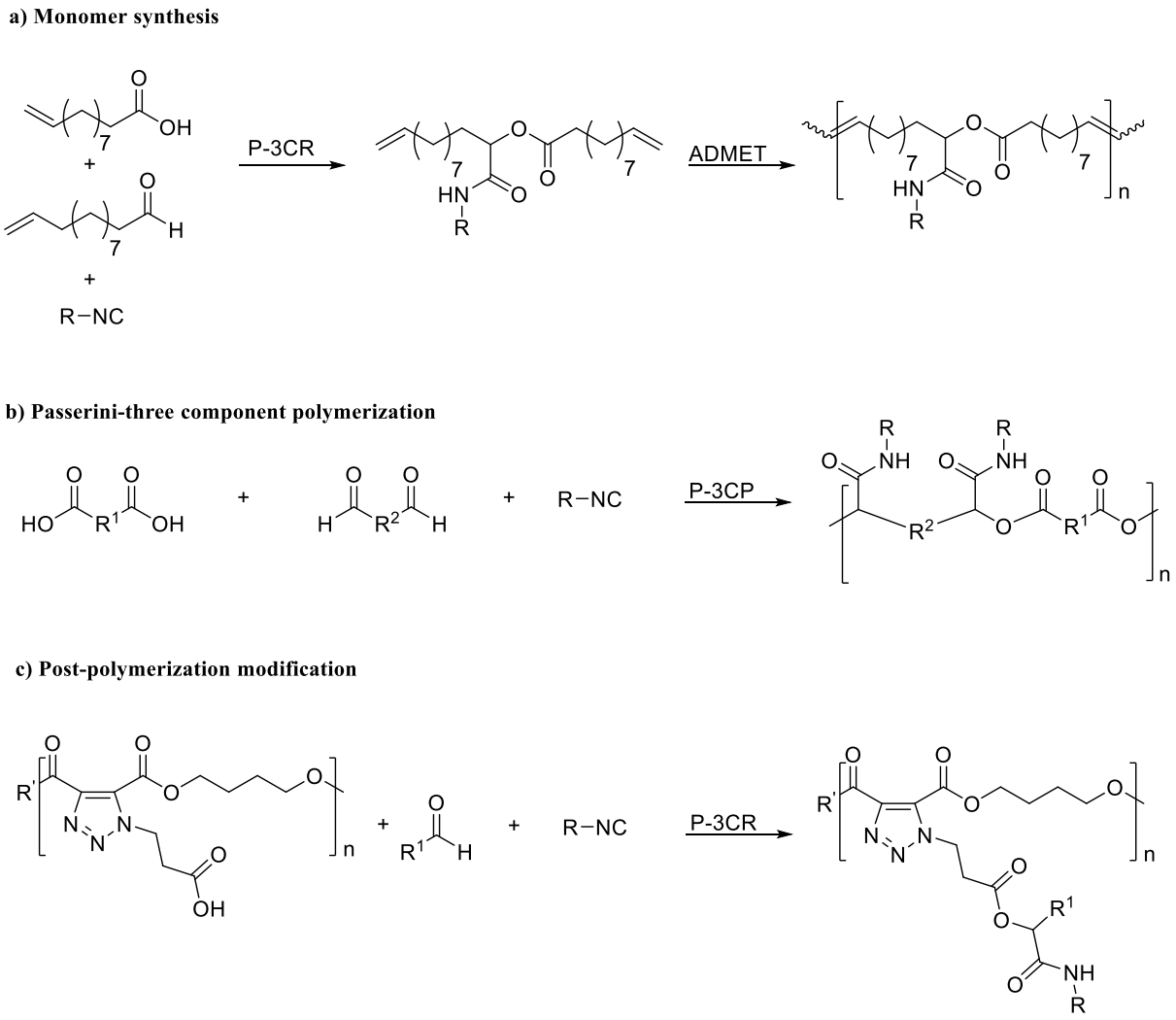
2.1.3.1 P-3CR in polymer synthesis

In the literature, three general ways are mentioned, how the P-3CR has been applied in the field of polymer synthesis.[115–117] These entailed the monomer synthesized by the P-3CR and afterwards used for the polymerization, the polymerization itself involving multiple P-3CR steps, or a post-polymerization modification performed *via* the P-3CR.

In 2010, the first use of a MCR for the synthesis of a monomer capable of undergoing polymerization was reported involving a Passerini-type condensation.[118] In 2011, Meier *et al*. described the successful synthesis of acyclic diene metathesis (ADMET) monomers *via* the P-3CR (see Scheme 14a).[119] Furthermore, it was possible to synthesize a broad variety of monomers with the P-3CR. Vinyl monomers, α,ω-dienes, functionalized hemilactides, acrylate monomers and photo-cleavable cross linkers, were polymerized by ring-opening polymerization (ROP), ADMET and radical polymerization.[120–124] Additionally, a library of (meth)acrylated with the variation of isocyanide, and aldehydes or ketones were synthesized.[123,125,126] Also, styrenic monomers were synthesized with 3-vinylbenzaldehyde, different isocyanides and carboxylic acids.[126]

P-3CR as a direct polymerization method was first mentioned by Meier *et al*. in 2011[119] and further examples were published over the last ten years.[116,127–136] Meier *et al.* reported an addition polymerization *via* a step-growth polymerization mechanism with bifunctional monomers: diacids as AA-type monomers and dialdehydes as BB-type monomers were used with different isocyanides to yield α-amide polyesters (see Scheme 14b).[119] Li and co-workers reported on the one hand side a polymerization with carboxylic diacids and diisocyanides with different aldehydes, as well as a polymerization with dialdehydes and diisocyanides and a various carboxylic acids.[128,136] Different polymer structures were synthesized by the variation of the components, like ester or amide moieties in the main and/or side chains.[119,128,136] Moreover, other similar approaches have been reported in the literature[127,129]

Post-polymerization modification is also a possible application of the P-3CR in the field of polymer chemistry. Several publications have emerged in the last ten years concerning this topic.[119,137] One post-polymerization possibility is to perform the P-3CR with an isocyanide and an aldehyde on a polyester with an acid functionality (see Scheme 14c). The functional group was introduced to the polyester *via* a Huisgen 1,3-dipolar cycloaddition.[138]



Scheme 14: Three different examples from the literature showing how P-3CR has been applied in the field of polymer chemistry:[119,138] **a.** In the monomer synthesis. **b.** As Passerini multicomponent polymerization. **c.** P-3CR as post-polymerization modification.

As shown in the above examples, the P-3CR can be used as interesting tool for the field of polymerization. The versatility of the P-3CR is demonstrated with the different applications like the monomer synthesis, polymerization itself or the post modification. Another important application of the P-3CR is in the field of the sequence-defined macromolecules. The sequence-definition and use of the P-3CR in this field will be discussed in detail in the following chapter.

2.2 Sequence-definition in polymer chemistry

In 2013, Lutz, Ouchi and Sawamoto reported for the first time the definition of sequence-controlled polymers,[17] describing it as follows:

"…macromolecules in which monomer units of different chemical nature are arranged in an ordered fashion"[17]

The definition of sequence-controlled polymers shows that the term can be used as a generic term for each level of control in polymers, ranging from perfectly defined macromolecules, like "biopolymers", to less defined polymers, like block copolymers, alternating copolymers, and periodic copolymers, with a varying $Đ$ (dispersity index). Thus, more strict definitions were necessary to distinguish the different degrees of control in copolymers and as a result the terms "disperse" and "uniform" were introduced (see Figure 3).[139,140]

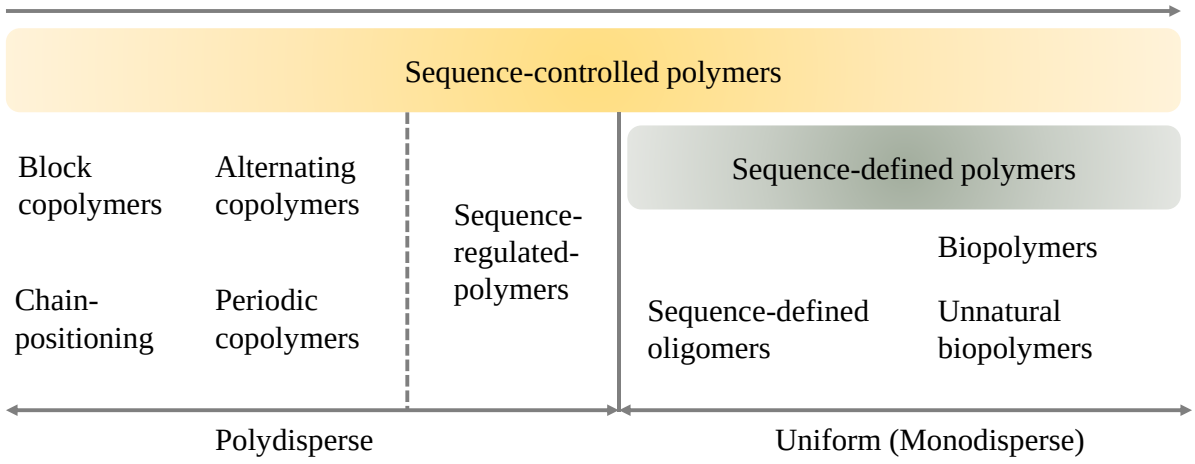
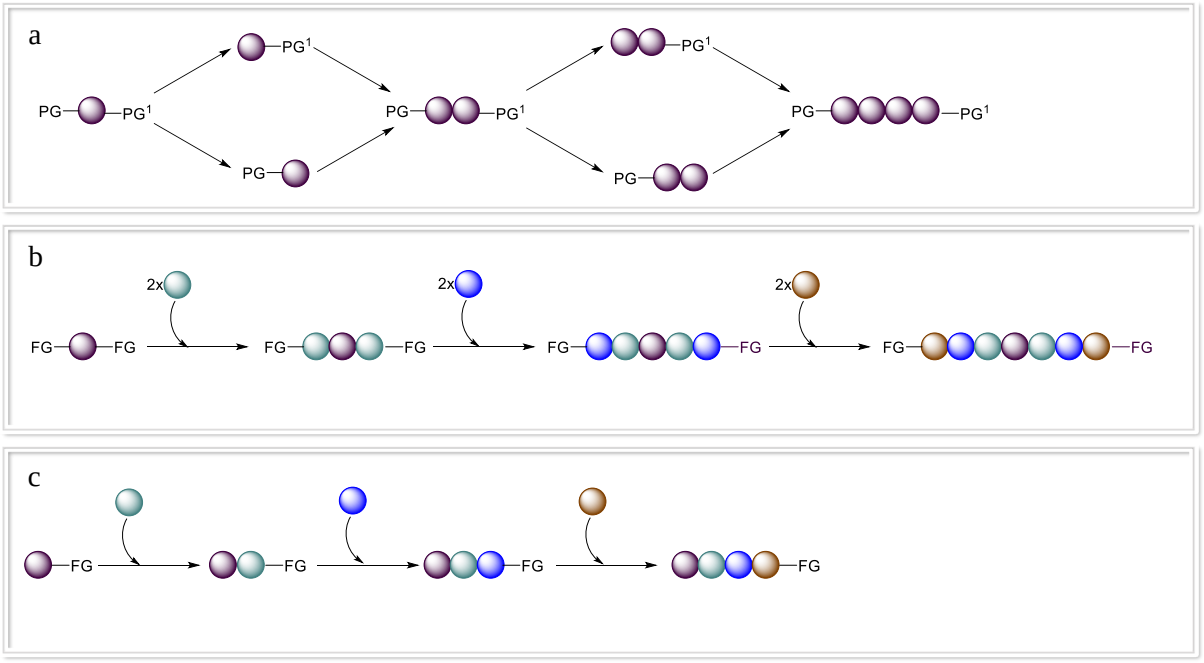


Figure 3: Classification of polymers with different levels of control.[139]

Most of the sequence-controlled polymers, like block copolymers, alternating copolymers or periodic copolymers, have a disperse chain-length distribution. Also, sequence-regulated polymers with a higher degree of control still have a dispersity in their chain-length.[128] However, this does not apply to a particular group of sequence-controlled polymers, the sequence-defined macromolecules. These "polymers" are strictly uniform in size and composition meaning they have fully controlled sequence, in which all chains are the same length, and each monomer is placed at an exact position in the chain.[140] The term polymer is still frequently used also for such highly defined systems, yet by definition polymers consist of different macromolecules. The terms sequence-ordered or uniform polymers are used as well to describe uniformity due to the fact that the nomenclature for this young field is not strictly defined yet.[141] However, the international union of pure and applied chemistry (IUPAC) recently defined monodisperse macromolecules as macromolecules with a uniform size and

therefore are not to be confused with the terms uniform or sequence-ordered since the degree of control is lower.[12] In this work, the synthesis of uniform macromolecules is described and thus this term is applied throughout.

The field of sequence-definition is inspired by nature; biomolecules, like DNA and RNA, are important for life. For this reason, in the following chapter, 2.2.1, sequence-defined non-natural biomacromolecules inspired by nature are described. The synthesis and the application of non-natural sequence-defined macromolecules will be discussed in more detail in the following chapters.[35,142] Especially the application in the field of data storage is described in detail (see 2.2.6). The approaches for the preparation of sequence-defined macromolecules can be divided in solid-phase synthesis, liquid-phase synthesis, fluorous-phase synthesis and polymer-tethered approaches.[143] Furthermore, three main synthetic approaches that are used in the synthesis of uniform sequence-defined macromolecules can be described. The iterative exponential growth (IEG) (Scheme 15a), bidirectional growth (Scheme 15b) and the linear approach (Scheme 15c).[142] The three different approaches are discussed in detail in chapter 2.2.2, 2.2.3, 2.2.4. In the linear approach, one monomer is installed per iterative step cycle, which is discussed in detail in chapter 2.2.4, or by a single unit monomer insertion (SUMI). However, SUMI will not be discussed further in this work, as it is not relevant for the synthesis, but also the subsequent application of sequence-defined molecules in the field of data storage.[137,144–150]



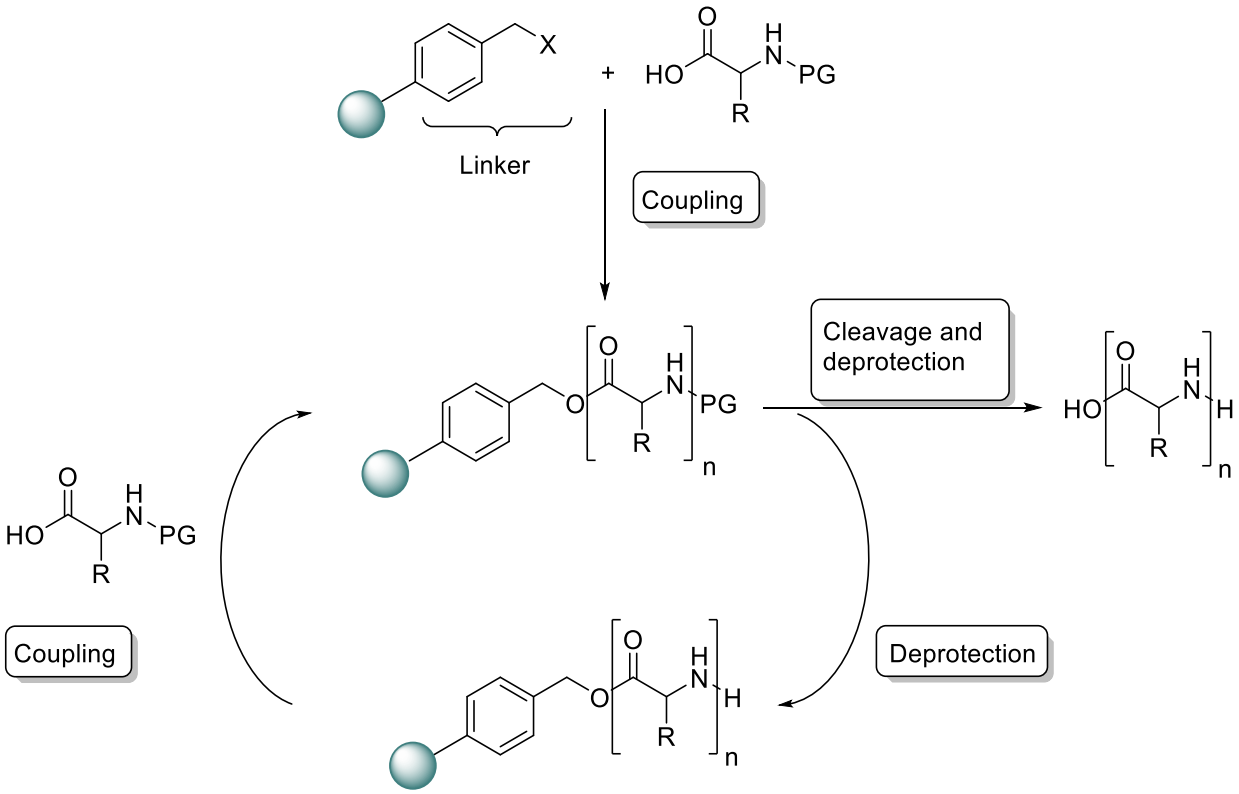
Scheme 15: Schematic overview of the main synthesis approaches used for sequence-defined macromolecules.[142,143] **a**. Iterative exponential growth. **b**. Bidirectional growth. **c**. Linear growth. PG = protecting group, FG = functional group

Furthermore, it is important to mention that there are other strategies for the synthesis of sequence-defined molecules besides the main approaches.[11,151] However, these will not be considered further herein.

2.2.1 Sequence-defined biomacromolecules, inspired by nature

In 1963, Merrifield described the first approach of sequence-defined synthesis, the solid phase peptide synthesis (SPPS).[152] In 1984 he was awarded with the Nobel prize for this achievement.[153] SPPS allowed the synthesis of oligopeptides and later also oligopeptoids and oligonucleotides.[152,154,155] Furthermore, this concept was adapted in combinatorial chemistry, whereby in 1966, Merrifield reported the automation of the process, which allowed the fast synthesis of even longer sequences.[156] This was another major milestone leading to sequence-definition, since SPPS allows the facile work up of the product by simple separation, *i.e.* filtration and washing, which was easily automated. Due to the possibility of using a large excess of reagents, quantitative conversion was ensured, thus decreasing side-products.[157] SPPS, in general, follows an iterative cycle with a coupling and a deprotection step. For this concept, protecting groups, like the commonly used base-labile 9-fluorenyl methoxy carbonyl (Fmoc), are applied for the protection of the *N*-terminus. In the first step of the synthesis, the Fmoc-protected amino acid reacts in a S_N2 with a linker molecule, which is coupled to the resin. Afterwards, the amine group is deprotected and reacted with the activated carboxylic acid group of another Fmoc-protected amino acid, followed by a second deprotection step, and so on. In Scheme 16, the synthesis of a tetramer oligopeptide is shown as an example, whereby the product is obtained in a final step by cleaving it from the resin. Since a carboxylic acid and a basic amine would react immediately to the corresponding salt in an acid-base reaction resulting in the inhibition of amide formation under mild conditions, the prior activation of the carboxylic acid is crucial.[158,159] Usually, activation is achieved by forming an active ester, which allows the peptide bond formation due to an increased electrophilicity in the carboxy group and shift of the equilibrium. Several activating agents have been established, the most common being *N*,*N*'-dicyclohexylcarbodiimide (DCC) or uronium- and phosphonium-based coupling agents, like 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate and benzotriazol-1-yl-oxytripyrrolidino phosphonium hexafluorophosphate. By exploiting automation combined with the optimized synthesis concepts, longer sequences were reported. For instance, Merrifield and co-workers reported the synthesis of bovine insulin with a sequence of 52 amino acids as well as a ribonuclease A with a sequence of 124 amino acids.[160,161] Further

improvements and developments demonstrated that the SPPS approach is an important tool in the synthesis of peptides, but also other uniform macromolecules.[162,163]



Scheme 16: Schematic overview of the SPPS by Merrifield.[152,157] PG = protection group, X = leaving group, green dot = resin.

In general, highly crosslinked copolymers are used for the solid support resin. The resins exhibit swelling in organic solvents and thus the growing chains on the surface are solvated, enabling them to react with the employed reagents. Examples of commonly used resin linkers, like the chloromethyl, Wang and the Rink resin are depicted in Figure 4.[152,164,165]

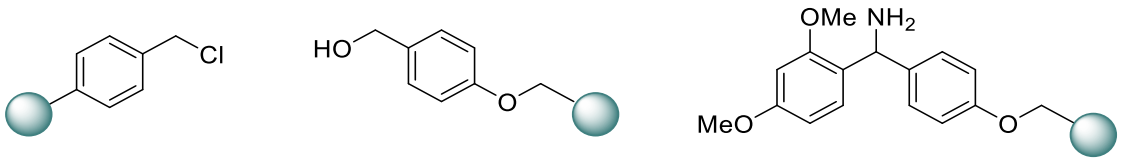


Figure 4: Examples of the commonly used resin linkers. left: chloromethyl resin, center: Wang resin, right: Rink resin.[152,164,165]

Besides peptides, another important class of biomacromolecules are peptoids. These non-naturally occurring macromolecules exhibit a structure, which is analogous to the peptide structure, however differing in the backbone substitution. While peptides have *C*-substituted backbones, peptoids bear *N*-substituted ones.[166] The different structures of a peptide and a peptoid are displayed in Figure 5.

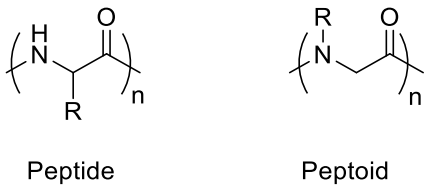


Figure 5: Chemical structures of a peptide and a peptoid.[116]

This small change in the positioning of the side chains in the backbone has a large impact on the properties. Some peptoids are biologically active and, compared to a peptide, they can be transported faster into cells, while they are more resistant against enzymatic degradation.[154,166,167] This example demonstrates that, in addition to the diverse functionality in the sidechain of a macromolecule, the backbone diversity is also important for its properties and functions. The synthesis of peptoids has been performed, like the synthesis of peptides, on a solid phase. However, in the synthesis of peptoids, the coupling steps are much slower[154] and due to this fact, Zuckermann *et al.* reported the synthesis of peptoids with a sub monomer approach in 1992.[166] There, the glycine sequences were formed in a reaction of two sub monomers: an amine and a haloacetic acid. In the first step of the reaction, a secondary amine linked on the resin reacted in an acylation step with a haloacetic acid through activation with diisopropylcarbodiimide (DIC). In the next step, the halogen was replaced by nucleophilic substitution of another amine, forming the first monomer unit. Subsequently, further acylation steps take place to complete the sequence. This approach has also been performed in an automated setup and polypeptoids with up to 50 glycine units were synthesized.[166,168–170]

Another important class of biomacromolecules are oligo(nucleotide)s, which were first reported by Letsinger and Mahadevan in 1966.[171] The synthesis was then further improved and finally the phosphoramidite chemistry was developed, which is still the most used.[172,173] There, a solid support is used, similarly to the SPPS for the synthesis, however with different resins. These are in general non-swelling glass beads with controlled pores. Furthermore, unlike in biological systems, in chemistry, the synthesis of oligonucleotides is performed by utilizing an orthogonal protecting group strategy. In the first step of the synthesis, a nucleotide on solid phase is deprotected on the 5’ position and afterwards the deprotected primary OH-group reacts with an excess of tetrazole-activated phosphoramidite to yield a phosphite triester. As a result of incomplete conversion of the coupling step, a capping and an oxidation step are necessary to yield the product, the desired phosphite triester, and to ensure the continuing of the cycle for the synthesis of the sequence. The first automated synthesis of DNA was reported in 1985.[174,175] Nowadays, the synthesis of oligo(nucleotide)s is fully automated and can be controlled by a computer.[176–179]

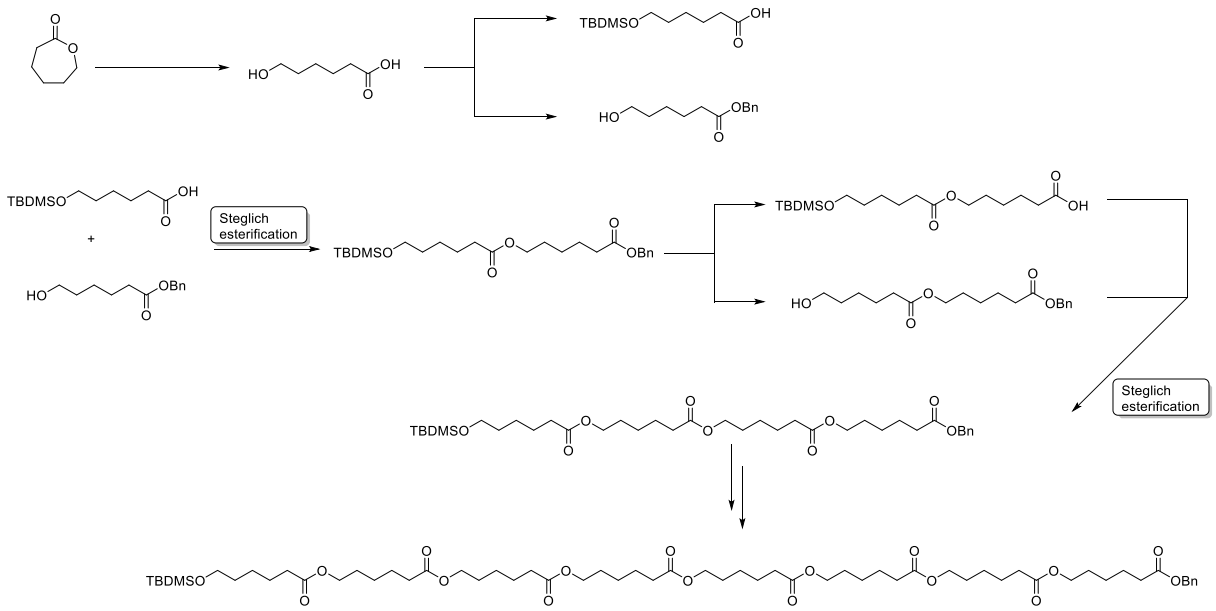
Many important achievements in the synthesis of biomacromolecules were developed and this allows the usage of synthetic biomacromolecules for different applications. Further, some important synthesis routes can be transferred to the synthesis of non-natural macromolecules, like sequence-defined macromolecules.

2.2.2 Iterative exponential growth

For the rapid synthesis of large macromolecules, synthesis *via* an iterative exponential growth (IEG) is often used. It is noted that IEG approach is named divergent/convergent in the field of conjugated, uniform macromolecules.[32,143] The synthesis of uniform macromolecules with IEG is often used as a niche method, however there is a considerable advantage.[21,180–191] Monomers with orthogonal protecting or activated functional groups (at least two) are synthesized and afterwards split into two parts (see Scheme 15). A separate orthogonal deprotection or activation of the two parts is performed and subsequently combined in a coupling reaction. Therefore, addition of monomers is performed in the steps (deprotection/activation of PG1 and PG2 and coupling). Due to the exponential character of this approach, reaction of two monomers yields a dimer, of two dimers a tetramer, of two tetramers an octamer, and so on (see Scheme 15). Macromolecules with a degree of polymerization, which is not present in the exponential growth of the number two, are also accessible by adding the respective monomer or dimer.[181] Using the IEG approach, introduction of defined side chains is challenging and limited to repetitive sequences. Nevertheless, uniform macromolecules are synthesized in a few steps in contrast to the other approaches.

The synthesis of a long aliphatic chain compound with the IEG approach was first reported by Whiting *et al.* in 1982.[192] They used C_{12}-bromacetal as precursor-monomer. Subsequently, the batch was split and in one half the bromine was transformed into a phosphine and in the other half the deprotection of the acetal group was performed. Afterwards, the phosphine group was reacted with an aldehyde in a Wittig-olefination step to yield a dimer, which included again a bromine and acetal functionality. Further repetition of these steps led to the synthesis of an octamer. In the last step, the bromine and the acetal groups were removed, and the double bonds were hydrogenated to yield pure aliphatic chains. This early example of the IEG approach led to highly defined aliphatic oligomers. With this approach, further syntheses have been described, like the synthesis of *n*-paraffins with different chain lengths.[193]

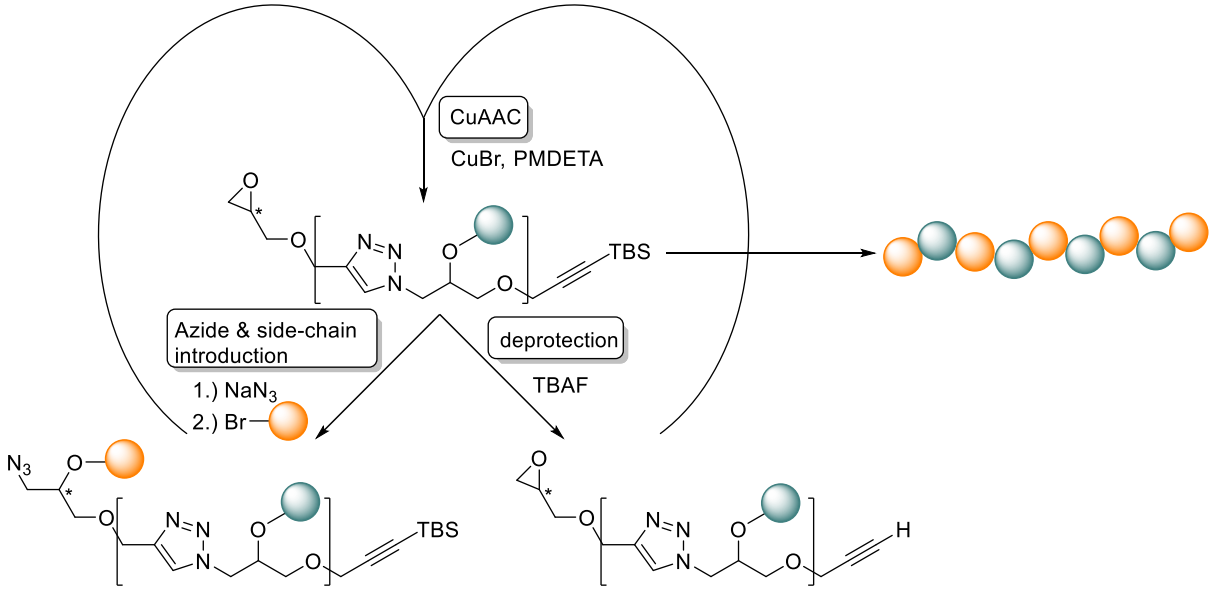
Another interesting approach involving the IEG concerned the synthesis of a uniform 64-mer oligo(ε-caprolactone) was reported by Hawker *et al.* in 2008.[184] From the ε-caprolactone, two orthogonally protected monomers were synthesized, one with *tert*-butyl dimethyl silyl (TBDMS) protected hydroxyl groups and a terminal carboxylic functionality, and one with a benzyl ester–protected group and an alcohol functionality. After the reaction of the monomers in a Steglich esterification, the orthogonally protected dimer was obtained. Subsequently, 50% of the dimer was used for the deprotection of the benzyl ester protection group and the other 50% in the removal of the TBDMS group. Afterwards, the dimers were coupled again in a Steglich esterification to yield the tetramer and so on. Following this procedure, a 64-mer oligo(ε-caprolactone) in high purity 96% (detected by size exclusion chromatography (SEC)) was synthesized (see Scheme 17). Applying the same approach, the group of Hawker and coworkers reported the synthesis of a uniform 64-mer poly(lacticde) in high purity (SEC traces).[194]



Scheme 17: Overview of the synthesis of sequence-defined ε-caprolactone oligomers. Monomer synthesis of ε-caprolactone, its protected derivatives and the following chain growth *via* IEG.[184] Reagents are not displayed to retain clarity.

Furthermore, the IEG strategies were applied in the synthesis of uniform poly(ethylene glycol)s (PEG)s.[183,186,195] Burns and co-workers reported the synthesis of uniform PEGs by the reaction of protected tri- or tetra(ethylene glycol) with another glycol building block, bearing a protecting and a leaving group. Afterwards, the obtained PEGs were fully deprotected to yield the uniform PEG or selectively deprotected for a further chain growth. In 2004, Hill *et al.* developed the synthesis of uniform PEGs in an approach similar to that of Bruns and coworkers

to obtain a 24-mer PEG after the coupling of two orthogonally protected dodecamers.[185] In 2015, Johnson and co-workers reported the synthesis of a semi-automated and scalable IEG for the synthesis of sequence-defined macromolecules.[186] First a monomer was synthesized in an esterification step to yield a monomer with a bromine and a triisopropylsilyl protected alkyne. The bromine was transformed into an azide in a nucleophilic substitution reaction and the protected alkyne orthogonally deprotected with a fluorine agent. Due to the semi-automated synthesis approach, it was possible to split the monomer in two parts for an orthogonal transformation to an alkyne and an azide, in-line purification and afterwards the coupling steps of the deprotected monomers. In the coupling step, a copper-assisted azide-alkyne cycloaddition (CuAAC) took place and the coupling product needed to be purified in a conventional way, *via* column chromatography. Furthermore, the group of Johnson *et al.* reported the IEG+ strategy in 2015.[21] This strategy demonstrated an exponential growth of molecular weight along with side chain variation. For this method, a chiral monomer with an epoxide group and a TBDMS-protected alkyne was used, which was obtained in a two-step synthesis. For the elimination of the TBDMS group, fluorine reagents were used and the epoxide was ring-opened by sodium azide to yield a secondary alcohol that was subsequently converted into an acetyl-, or benzyl ether group. Due to the functionalization of the secondary alcohol, side chain variation was possible and the CuAAC allowed the exponential growth. The overview of the IEG+ approach is displayed in Scheme 18.[21]

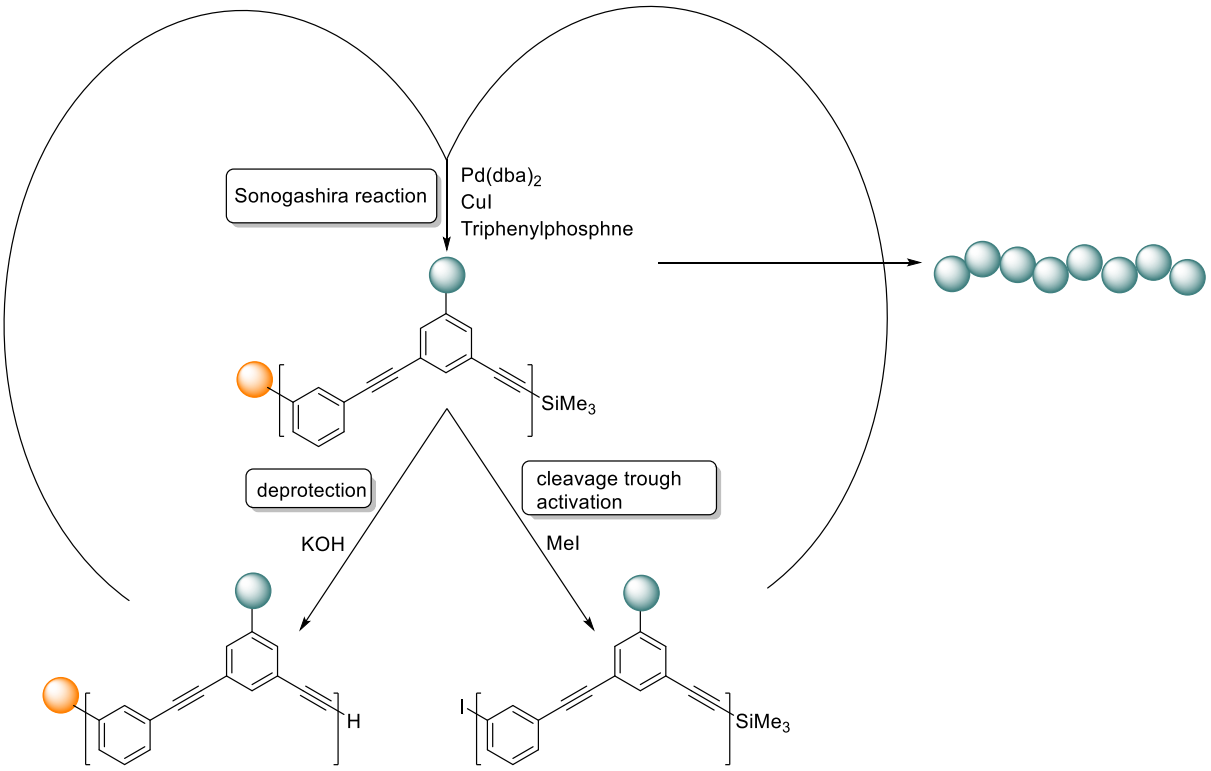


Scheme 18: Overview of the IEG+ approach, which demonstrates an exponential growth of molecular weight along with a side chain variation.[21,143] The used monomer bearing a TBDMS protected alkyne group and a epoxy group.

In 2016, further improvement of the IEG+ approach was reported, allowing the synthesis of a uniform block copolymer in large scale (up to 1 g).[196] By the allylation of the secondary alcohol and consecutive thiol-ene reaction, the side chain variety was amplified. The IEG approach introduced by Johnson *et al*. was subsequently used in multiple reports varying the functional groups employed.[180,182,187]

As already mentioned in the beginning of this chapter, in the field of conjugated molecules, like oligoacetylenes, oligo(*para*-phenylene)s, oligofluorenes and oligophenylene ethylenes (OPEs)s, the IEG approach is often employed and referred to as divergent/convergent approach.[143,197,198]

In 1996, Schlüter *et al.* reported the synthesis of oligo(*para*-phenylene)s *via* Suzuki cross-coupling in a divergent/convergent approach.[199] For the synthesis, a bifunctional biphenyl with a trimethylsilyl (TMS) group and a bromine group was used as starting molecule. Furthermore, in one part of the starting molecule, the TMS protecting group was converted to an iodine and, in the other part, the bromine was transformed to a boronic acid. Subsequently, the two products were coupled in a Suzuki coupling. By following this approach, it was possible to synthesize an octamer with 16 aromatic rings. Other examples for the synthesis of conjugated molecules (in this case OPEs) *via* a convergent/divergent followed.[200–203] The group of Moore reported several syntheses of OPEs in solution but also in solid phase[190,191]. For instance, they reported the solid-phase synthesis of sequence-defined phenylacetylene oligomers.[190] For the linkage to the solid phase, a triazene linkage system bearing a bromine-substituted benzene, which was further reacted to form a phenylacetylene. Afterwards, the phenylacetylene on the solid phase was reacted with TMS-protected 1-(*tert*-butyl)-3-ethynyl-5-iodobenzene to lead to a dimer. Subsequently, the dimer was divided into two pots: one was deprotected with potassium hydroxide and the other was cleaved from the solid-support by methyl iodine resulting in phenyl iodine moiety *via ipso* substitution. Then, the two pots were reacted again in a Sonogashira coupling to yield the solid phase–supported tetramer. Following this cycle, up to a 32-mer was obtained. In Scheme 19, the convergent/divergent approach for the OPEs synthesis with the Sonogashira reaction in solid phase is depicted.



Scheme 19: Convergent/divergent approach of Moore *et al.* for the OPEs synthesis with the Sonogashira reaction in solid phase.[143,190] Blue dots symbolize the *tert*-butyl groups introduced.

To date, new publications using this method constantly emerge. For example, in 2015, Lutz *et al.* reported the synthesis of poly(alkoxyamine amide)s in solid phase for the digitally-encoding polymers.[204] In 2020, the group of Szostak reported the synthesis of sequence-defined oligomers in solid phase with the Sonogashira reaction as coupling step.[205] Furthermore, Kim *et al.* demonstrated the synthesis of large cyclic polymers and block copolymers *via* the IEG approach,[151] using *rac*-lactide with a benzyl ester and TBDMS ether groups.

The IEG is a broadly used approach for the synthesis of sequence-defined oligomers, through their robustness and versatility. However, it is still limited by the fact that multiple steps and purifications are required for each unit added to the growing macromolecules. An aspect that *e.g.* bidirectional growth overcomes.

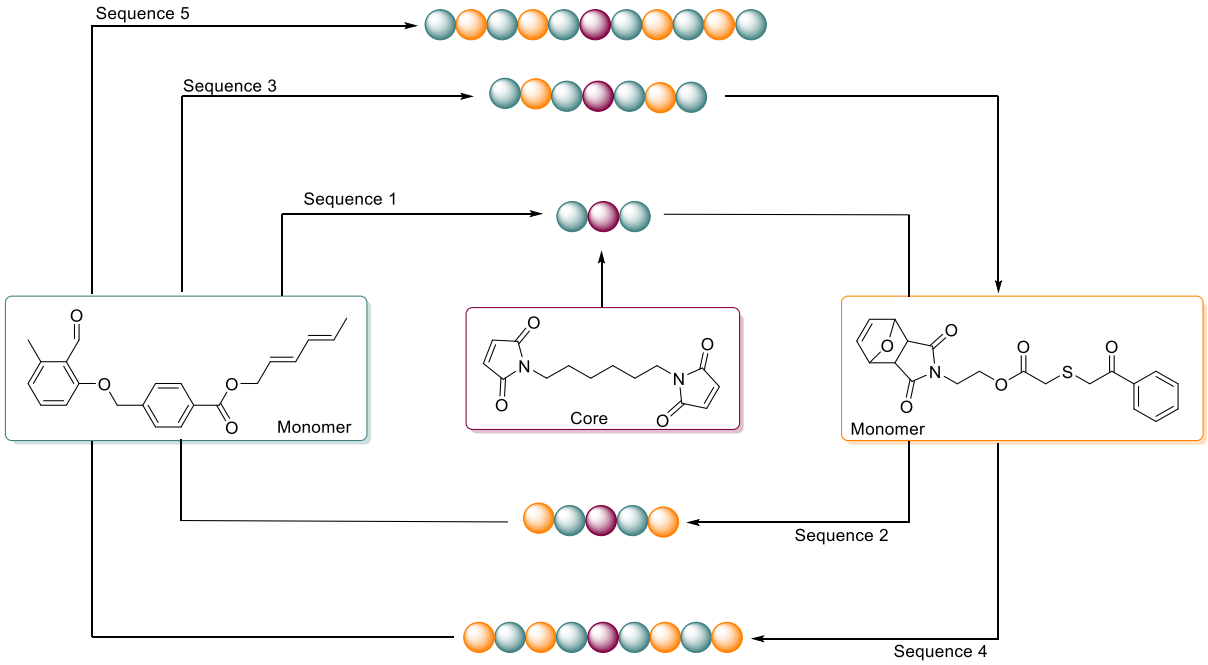
2.2.3 Bidirectional growth

As already mentioned in chapter 2.2, the bidirectional growth represents the second important approach for the synthesis of sequence-defined oligomers.[142,143] In the bidirectional growth, a bifunctional starting molecule reacts with two monomer units per step to reach high molecular weights in fewer steps then in other approaches, like the iterative step synthesis. Nonetheless, the restriction to symmetric molecules limits the level of control in this approach.

The synthesis of PEGs has been performed with a bidirectional growth and different approaches have been reported.[206–210] For example, in 1992, Jenneskens *et al.* published the synthesis of a dodeca(ethylene glycol) with a monoprotected and a ditosylated tetra(ethylene glycol) *via* bidirectional growth.[208] Baker *et al.* reported a similar approach like Jenneskens, however, in a more sustainable way.[211,212] Furthermore, in 2006, Tanaka *et al.* published the synthesis of a 44-mer PEG.[209] There, two equivalents (eq.) of monoprotected tosylated tetra(ethylene glycol) reacted with another glycol and afterwards a deprotected step. Repetition of this cycle yielded a uniform 44-mer PEG. In 2015, Jiang *et al.* reported the synthesis of uniform PEGs *via* a macrocyclic sulfate (MCS)-based approach.[207] For the synthesis of the MCS, tetra(ethylene glycol) was reacted with thionyl chloride ($SOCl_2$) and triethylamine (Et_3N), leading to a 14-membered macrocyclic sulfite. Subsequently, nucleophilic ring-opening of the MCS took place with a nucleophile and a base to obtain the uniform PEG with a nucleophilic end group. Furthermore, the bidirectional growth approach has also been used for the synthesis of oligo(1,4-phenylene ethynylene)s, as reported by Tour and Huang in 1999.[146] In the first step, a symmetric core molecule with TMS protecting groups on both sides was synthesized. After the deprotection of the TMS and a subsequent Sonogashira coupling with 1-bromo-4-iodobenzene, a symmetric trimer was obtained. Afterwards, the trimer was reacted with a palladium catalyst and trimethylsilyl acetylene to yield the trimer with TMS protected alkynes on both ends. By continuing the cycle, it was possible to synthesize an OPE hexamer. The bidirectional growth has since been reported for the synthesis of various uniform OPEs and oligo(thiophene ethylene-*alt*-bipyridine ethylene)s.[213–216]

In 2015, Barner-Kowollik *et al*. reported a photochemical approach *via* bidirectional growth.[22] There, a symmetric core unit with two maleimides was used as starting material, while two bifunctional monomers were synthesized: one bearing a sorbyl ester group and an *ortho* methyl benzaldehyde moiety, as acting as a diene in a photo enolization, and one bearing a phenacylsulfide and a masked maleimide group. Sequence-defined oligomers were obtained using consecutive Diels-Alder reactions. First, the symmetric core molecule was reacted with the photo enol group of the sorbyl ester monomer (sequence 1 in Scheme 20). Secondly, the other monomer generated a thioketone *via* Norrish-type II cleavage which formed a Diels-Alder adduct with the sorbyl ester group of the growing chain (sequence 2 in Scheme 20). Subsequently, a thermal retro-Diels-Alder was performed, liberating a maleimide functionality while eliminating furan, which then reacted with the first monomer (sequence 3 in Scheme 20), and so on. Following this cycle, it was possible to synthesize a sequence-defined decamer. In

Scheme 20, the photo-induced reaction cycle for the synthesis of sequence-defined oligomers is depicted.[22]



Scheme 20: Overview of the photochemical reaction approach for the synthesis of sequence-defined macromolecules. Symmetric core unit with two maleimides (in the red box), one monomer with a phenacylsulfide and a protected maleimide group (in the orange box) and the other monomer with a sorbyl ester group and a photo enol (in the blue box).[22]

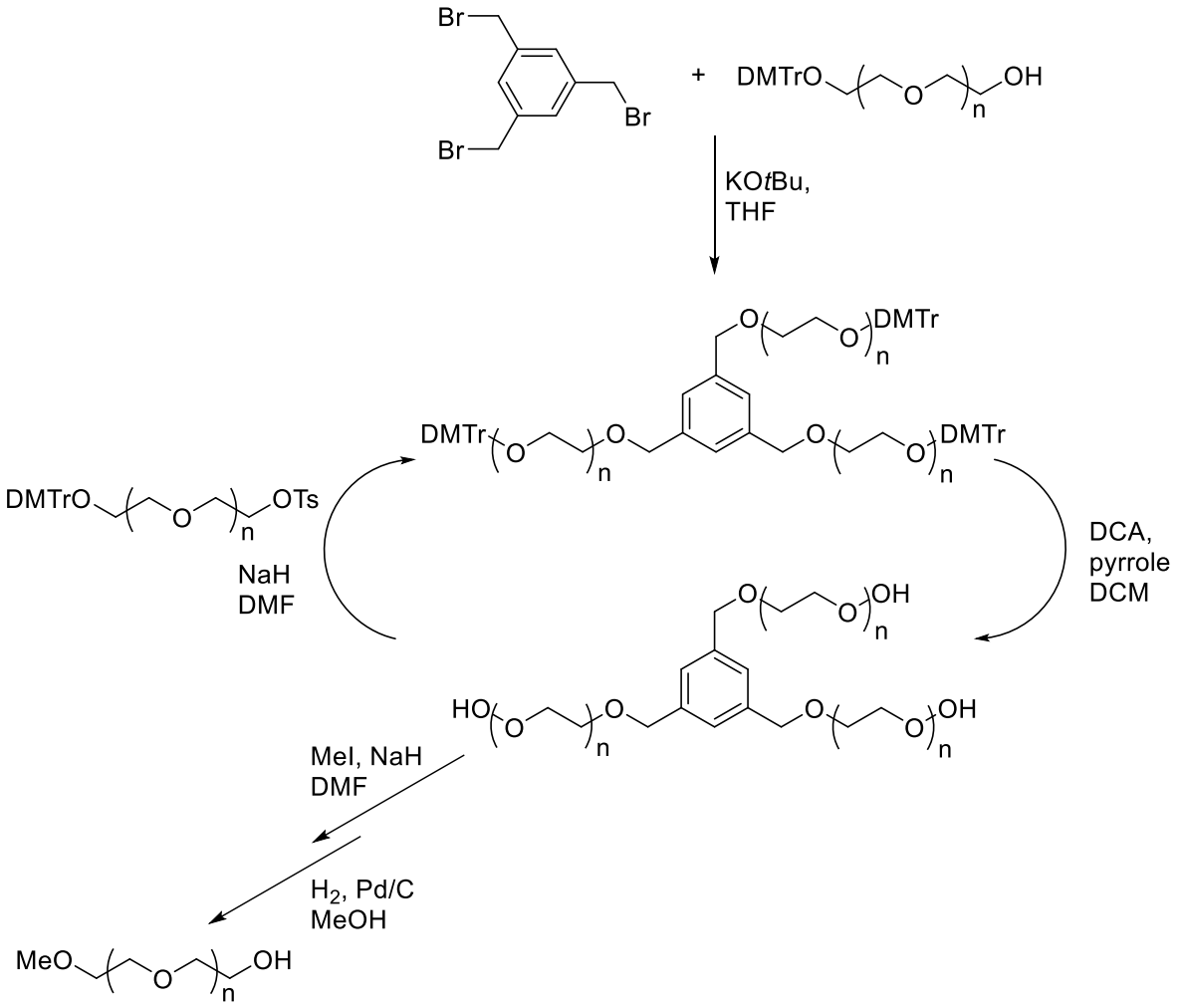
Furthermore, in 2019, Barner-Kowollik *et al.* published a protection group–free synthesis for sequence-defined macromolecules by precision orthogonal photochemistry.[217] Here, a symmetric core molecule with two carboxylic acids and two monomers was used. One monomer was synthesized with a pyrene-functionalized visible light–responsive tetrazole and a diene, while the other monomer with a carboxylic acid and fumarate. In the first step, the core molecule reacted with the tetrazole of the monomer at 410 nm in a nitrile imine-carboxylic acid litigation. Afterwards, the remaining diene group reacted with the fumarate-based sequence at 365 nm in a Diels-Alder cycloaddition. Following this cycle, a sequence-defined decamer was synthesized without protecting groups using selective photochemistry. Further different approach for the synthesis *via* bidirectional growth were published in the literature.[218,219]

The bidirectional growth can be used for the fast synthesis of sequence-defined macromolecules with a higher molecular weight in less steps. However, the restriction of the symmetric of the macromolecules is a downside of these approach.

2.2.4 Iterative stepwise approach

In an iterative stepwise approach, the monofunctional staring material is elongated step-by-step and the monomer units are coupled one after the other. Compared to the IEG or bidirectional growth, the formation of higher molecular weights takes more steps and time, however, the stepwise iterative approach is not restricted to symmetric sequences. Different parameters, like side chain substitution and monomers, are independently controlled by each repeating unit.[14,35,50,142,143,220–222] In chapter 2.2.2 and 2.2.3, the synthesis of PEGs *via* IEG and bidirectional growth were described, however the synthesis *via* iterative stepwise approach is also prominent in literature.[222,223]

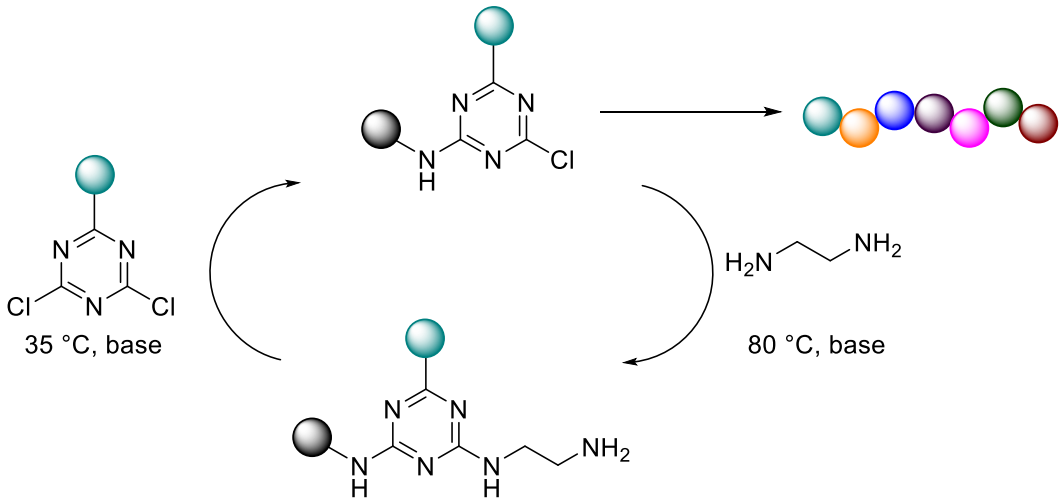
In 2014, Livingston *et al.* published their synthesis of uniform PEGs *via* iterative stepwise synthesis.[222] A three-arm benzylic star was used as core and a monoprotected octa(ethylene glycol), which reacted with the core in a nucleophilic substitution, was used as monomer for the synthesis of the linear uniform PEGs. Afterwards, the protected chain ends were deprotected with dichloroacetic acid, pyrrole and DCM to obtain the hydroxy groups. Subsequently, the hydroxy groups were reacted in a coupling step with a tosylated and monoprotected octa(ethylene glycol). By continuing this cycle, a three-arm star-shaped 24-mer was synthesized. The decoupling of the linear PEG from the benzylic core was only demonstrated in a milligram scale, however, a 24-mer oligo(ethylene glycol) was obtained. The synthesis of linear PEGs with a star core is shown in Scheme 21. In 2017, Fang and Khanal reported the synthesis of 12mer-PEGs on solid phase, with a stepwise addition of tetraethylene glycol monomers on the solid support.[224] There, a tetraethylene glycol monomer with a tosyl group on the one side and a dimethoxy trityl (DMTr) group on the other side was synthesized and a Wang resin with a 4-benzoloxy alcohol function was used. The reaction cycle comprised three steps. First, the alcohol group of the resin was deprotonated with potassium *tert*-butoxide (*t*BuOK) and was reacted with the tosyl group of the monomer *via* a Williamson ether synthesis. In the next step, the DMTr was cleaved with an acid to yield the hydroxy group, which was then reacted again in a Williamson ether synthesis. By continuing this cycle, an unsymmetric PEG was obtained. In the last step, the PEG was functionalized with an acid-stable protecting group and subsequently cleaved from the resin to obtain an asymmetric PEG.



Scheme 21: Synthesis of uniform PEG with a benzylic core.[222]

In 2019, again Livingston *et al.* reported an important new synthesis approach for PEGs and sequence-defined molecules, in general.[225] They published a unique liquid-phase synthesis, combined with molecular sieving and monitoring to yield uniform sequence-defined PEGs. For the iterative two-step approach, they used a benzylic ether linkage between the polyether chain and the core molecule to ensure cleavage at the end of the reaction. Different monomers with a hydrophilic tetrahydropyran-1-yl (THP) acetal on the one side of the monomer and a toluene sulfonate group on the other side were synthesized. In the first step of the reaction cycle, the hydroxy group of the core unit reacted with the toluene sulfonate group of the monomer in a Williamson ether synthesis, followed by deprotection of the THP to yield again the hydroxy group. To avoid purification *via* chromatography, they used the idea of molecular sieving in organic solvents to separated impurities. However, it is important to note that the separation capacity of the membrane depended on the size difference of the molecules. For this reason, the use of a bulky three-armed star core for chain growth was an important step for the success of this system.

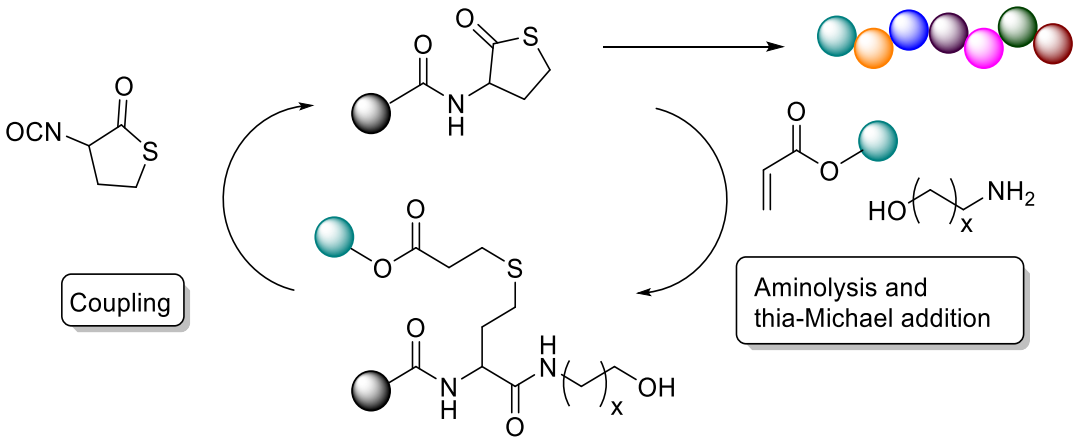
In 2016, Grate and coworkers published the synthesis of triazine-based sequence-defined polymers with the introduction of specific side chains to the backbone.[226] There, a cyanuric chloride was used in a reaction with a diamine. Since each reaction deactivates the cyanuric chloride, higher temperatures were needed for the next coupling step. In the first step, a cyanuric chloride on a resin reacted at 80 °C with a diamine. Afterwards, the unit reacted with another cyanuric chloride (sub monomer) at 35 °C and deactivated the cyanuric chloride. Consequently, 80 °C were necessary for the next step (see Scheme 22). Because of the deactivation of the cyanuric chloride, no protecting groups were necessary. Sequence-definition was enabled by variation of the side chains in the diamine sub monomer. After the cleavage from the resin, a sequence-defined hexamer based on triazine was obtained with this approach.



Scheme 22: Synthesis of a triazine-based sequence-defined hexamer.[20,226]

Recently, Porel *et al.* reported the synthesis of sequence-defined dithiocarbamate oligomers.[227] There, a chloroacetyl chloride was used as a co-monomer and different monomers were synthesized in a two-step synthesis. For the monomer synthesis, the co-monomer reacted with an amine to form a chloroterminal amide, which further reacted with an ethanolamine to obtain an amine-hydroxyl monomer. For the first step of the reaction cycle, the co-monomer reacted with a secondary amine to form a chloroacetyl amide which reacted in the second step with a monomer in presence of carbon disulfide (CS_2) to yield the dimer. In the third step, the hydroxyl group of the monomer reacted with the co-monomer and Et_3N. Subsequent iteration of the second and third step yielded a sequence-defined dithiocarbamate pentamer in a short time (~ 6 hours).

In 2013, Du Prez, Madder and coworkers reported the synthesis of multifunctionalized sequence-defined oligomers.[16] They used an iterative two-step approach based on thiolactone chemistry on solid phase. In the first step of the reaction cycle, the amine function reacted in an aminolysis, in which the thiolactone was ring-opened to form a thiol. Subsequently, the thiol reacted in a thia-Michael addition with a thiolactone-acrylamide building block. Continuing these steps, different sequence-defined trimers and tetramers with different side chains were synthesized. The side chain variation was achieved by introducing different primary amines in the aminolysis reaction. Three years later, in 2016, Du Prez *et al.* published again the synthesis of sequence-defined macromolecules with thiolactone chemistry, however with an expanded and improved system.[228] Again, a two-step iterative system without protecting groups was used. First, two thiolactone building blocks were synthesized, one with an alcohol function, the other with an isocyanate group. For the synthesis on solid phase, the resin was functionalized *via* reaction of the thiolactone building block with the alcohol function with the 2-chlortritylchloride resin. Afterwards, the reaction cycle started with the ring-opening of the thiolactone with an amino alcohol to form a thiol, which reacted with an acrylamide or an acrylate in a thia-Michael addition. Since many acrylates and acrylamides are commercially available, different side chains were introduced in this step. The use of different amino alcohols led to variation in the backbone. In the second step of the synthesis cycle, the hydroxy group reacted with the isocyanate group of the thiolactone building block. Using this iterative two-step reaction approach, the synthesis of different sequence-defined decamers was demonstrated. The two-step iterative cycle is depicted in Scheme 23. Furthermore, a decamer was synthesized in an automated process with a synthesizer and afterwards compared with the non-automated synthesis–obtained decamers. Some impurities were observed in the decamer prepared by the synthesizer; however, it was possible to reduce the reaction time from 3-5 days to 33 hours. With this interesting approach, functionalized sequence-defined oligomers were synthesized using a simple and straightforward synthesis method. In 2017, the same group reported the synthesis of thioacrylates, however, in a four-step iterative synthesis protocol.[229] There, the modification of the side chain was included in the iterative stepwise synthesis and the aminolysis and the thia-Michael addition were described as two-steps.



Scheme 23: Synthetic protocol of a two-step iterative solid phase reaction based on thiolactone chemistry.[228]

In 2019, also Du Prez *et al.* reported the synthesis of sequence-defined oligourethane amides.[230] Different resins were used for the synthesis, *N*-hydroxyethyl acrylamide was used instead of acrylates or acrylamides, and the variation of the side chains was achieved with different amines. This approach enabled automation that not only improved purity, but also offered the possibility to increase throughput and scale up. Up to 72 reactions were simultaneously processed. Recently, the same group published the automated synthesis of stereo controlled, multifunctional sequence-defined molecules based on thiolactone chemistry.[231] The application of the thiolactone chemistry iterative approach in the field of data storage was subsequently published in 2018 and will be discussed more in detail in chapter 2.2.6.[9]

A large part of the literature concerning sequence-defined polymers, especially in the field of chemical data storage, derives from the work of the group of Jean-Francois Lutz. In 2015, they published the synthesis of non-natural polyphosphates on an insoluble cross-linked polystyrene resin.[143] The idea of non-natural phosphoramidite was inspired by biological polymers and was reported before in the literature.[172,232–236] For the synthesis of the sequence-defined oligomers, three different phosphoramidite monomers were used and defined as 0, 1 and 1' to enable the read-out of the oligomers after the synthesis. In the iterative cycle, a deprotection step followed by a coupling step and an oxidation step were performed. Sequence-defined oligomers, up to a 24-mer, were obtained and read-out. The read-out of molecules in the field of data storage will be discussed in detail in chapter 2.2.6. Further improvements and developments of this approach are reported in the literature.[8,237–239] Moreover, Lutz *et al.* described the synthesis of oligoalkoxyamine amides and polyurethanes in an iterative stepwise cycle, also for the application in the field of data storage.[30,240–242] Different approaches with

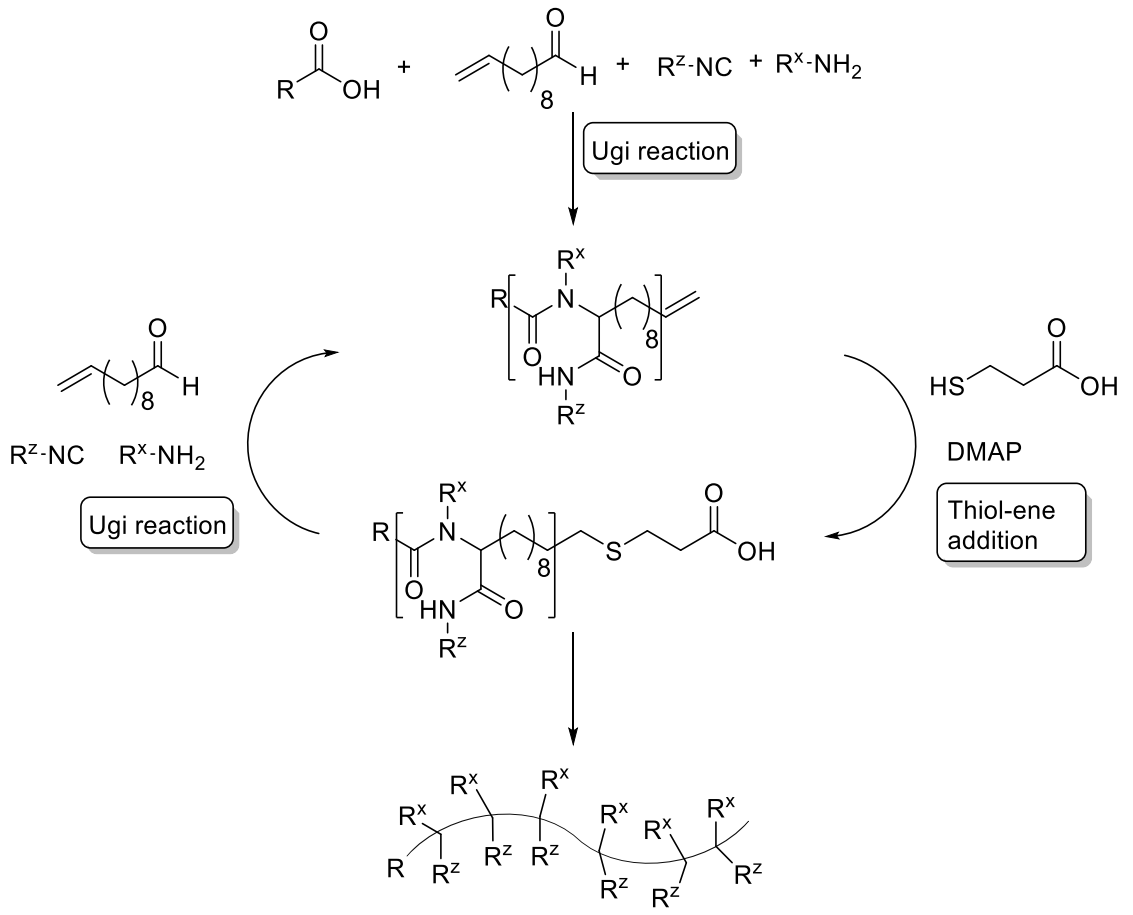
an iterative stepwise synthesis were reported in our group mainly based on MCR. They will be discussed more in detail in the following chapter about MCRs in sequence-definition.

The iterative stepwise synthesis is a commonly used approach for the synthesis of sequence-defined macromolecules. The building of long sequences is more time consuming, instead of the IEG approach and the bidirectional growth, because more repeating steps are necessary. However, the sequences are not limited to the symmetric macromolecules and with the iterative stepwise approach different parameters, like side chain substitution and monomers, are independently controlled for each repeating unit.

2.2.5 MCRs in sequence-definition

Multicomponent reactions offer a wide range of applications, as already discussed in chapter 2.1. In the field of sequence-definition, they play a major role due to their ease of implementation and the wide range of components that can be used. Different groups demonstrated the applications of MCRs in the field of sequence-definition.[11,13,26,50,52,243,244] In general, the reactions are carried out either on the solid phase or in solution. Different approaches in solid phase, solution, or the comparison of both are described in the following.

In 2014, our group reported the synthesis of a sequence-defined macromolecule *via* the P-3CR.[13] There, stearic acid was used as starting material for the first P-3CR, 10-undecenal as aldehyde and an isocyanide, which can be varied production each synthesis cycle. Subsequently, the double bond of the Passerini product side chain reacted with 3-mercaptopropionic acid in a thiol-ene addition to yield again a free acid, which reacted in the next P-3CR. By continuing the iterative cycle, a sequence-defined tetramer was obtained in an overall yield of 26%. One year later, the improvement of these iterative syntheses was published by our group.[48] To introduce greater variance, the P-3CR was changed with the U-4CR, which offers variation not only of the isocyanide, but also the amine group in the reaction, leading to a dual side chain control. The iterative two-step reaction cycle of the U-4CR and the thiol-ene addition is displayed in Scheme 24. A tetramer was obtained in an overall yield of 15%, while only the amine compound was varied. Furthermore, a pentamer with the variation of the isocyanide and the amine was obtained also in an overall yield of 15%. It was mentioned that it is important, which amine/isocyanide is used to ensure high yields in the U-4CR. Especially in the iterative stepwise approaches, high yields are important to ensure the possibility to build up long sequences.

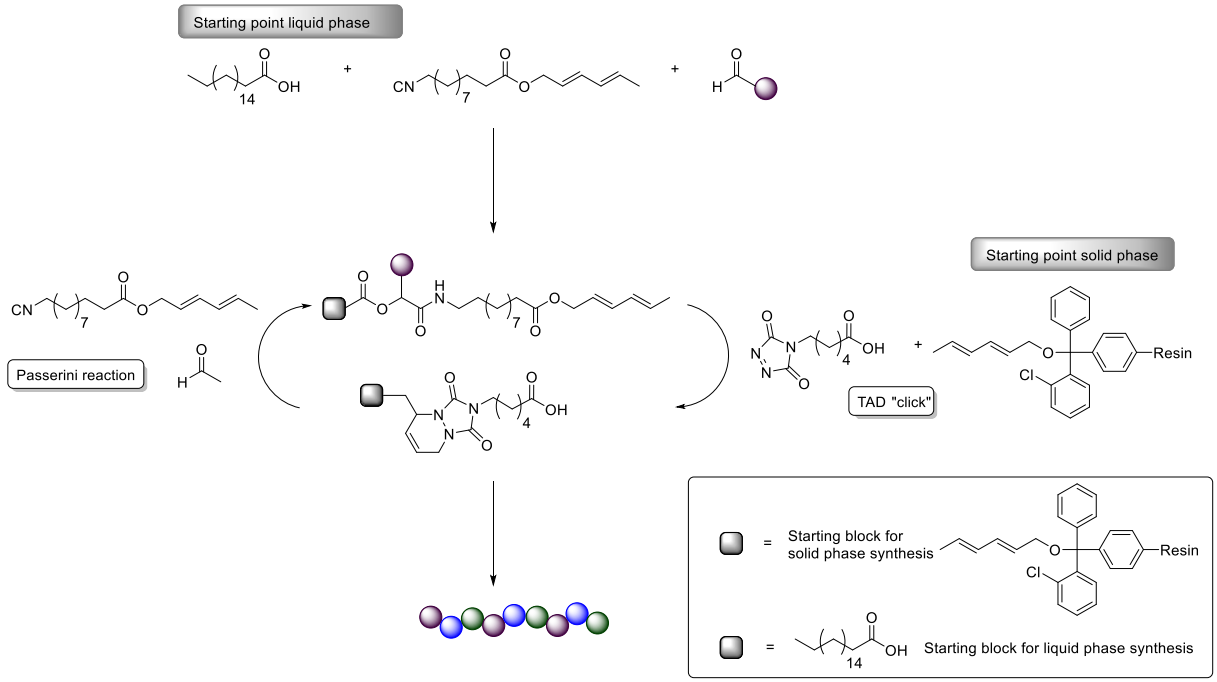


Scheme 24: Synthesis strategy for a two-step iterative U-4CR approach for dual side chain control in sequence-defined oligomers.[48]

In 2017, Meier and Du Prez reported another interesting approach.[15] There, the two important approaches of the groups for sequence-defined molecules were combined:[13,16] on the one hand, the P-3CR with a thiol-ene addition, on the other hand aminolysis and a thia-Michael addition. First, α,ω-functionalized isocyanide building blocks were synthesized. A thiolactone acid was used as starter acid to react in the iterative cycle of P-3CR and thiol-ene addition. After the third Passerini reaction, the product thiolactone of the trimer reacted with an amine in an aminolysis to yield the thiol. This reacted directly in a thia-Michael addition with an isocyanide-functional acrylate. Furthermore, the carboxylic acid trimer reacted with the α,ω-functionalized isocyanide building block and an aldehyde in a P-3CR, followed by a thiol-ene addition and again a P-3CR with another α,ω-functionalized isocyanide building block. With this approach it was possible to combine the two different approaches and obtain a sequence-defined 15mer.

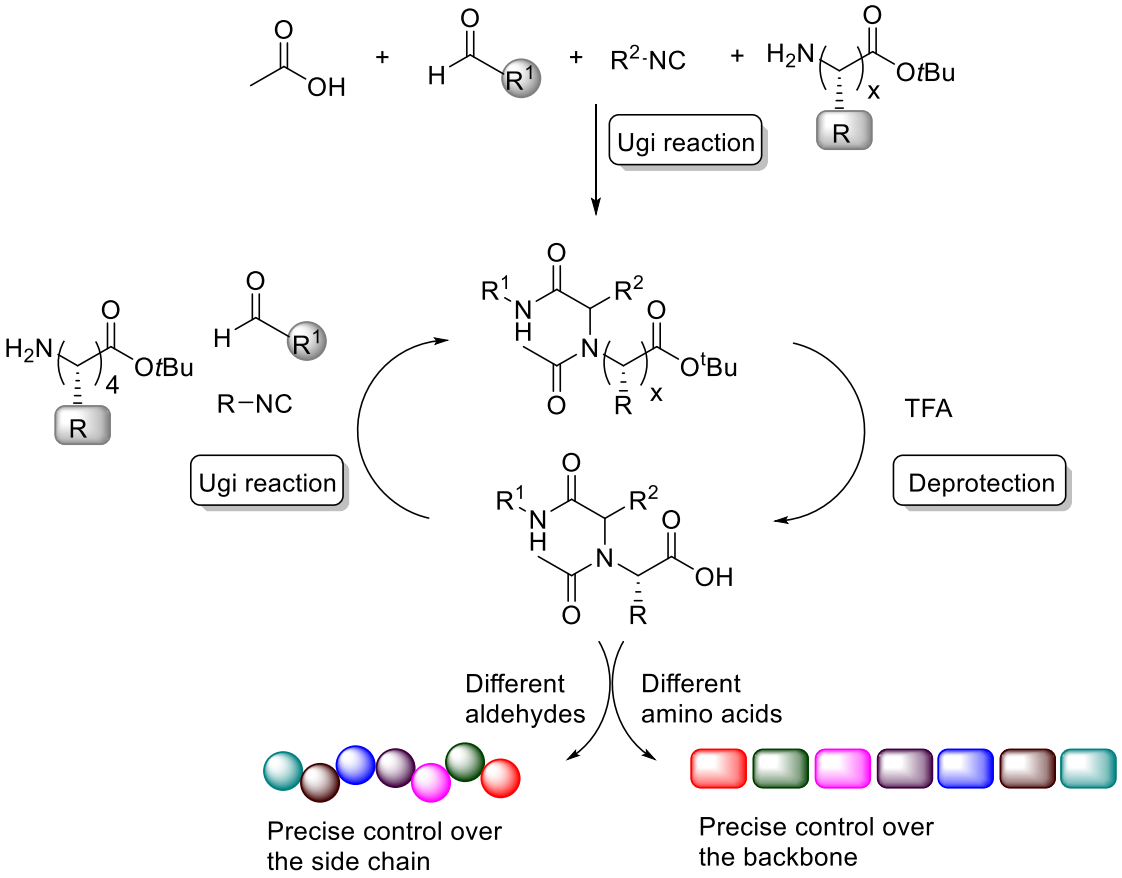
In 2018, another interesting combination of two different synthesis approaches was reported. This time Meier, Barner-Kowollik and co-workers combined the strategies of the P-3CR and a Diels-Alder reaction based on photocaged dienes.[22,117] First, a Passerini linker was synthesized with a diisocyanide, a maleimide equipped with a carboxylic acid group and a aliphatic aldehydes. Afterwards, the Passerini product reacted *via* the free isocyanide group with an aromatic aldehyde and a dicarboxylic acid to form a symmetric trimer. Subsequently, the maleimide functions of the trimer reacted in a bidirectional step in a photochemically induced Diels-Alder reaction with a photo monomer/dimer, leading to different functional oligomers.

In 2019, Du Prez, Meier and coworkers published the comparison of the synthesis of sequence-defined oligomers on solid phase and in solution.[26] For the iterative cycle, a 1,2,4-triazoline-3,5-dione (TAD) "click" reaction and a P-3CR were used. For the synthesis in solution, stearic acid was used as starting material and reacted in a first P-3CR with an aldehyde and a linker molecule equipped with an isocyanide and a conjugated diene. The obtained Passerini product reacted with a second linker molecule (TAD and carboxylic acid moiety). The introduced free acid group of the sequence-unit enabled again a P-3CR with another aldehyde and the first linker molecule. Repeating this cycle, it was possible to synthesize a sequence-defined nonamer in solution and each reaction step was thoroughly analyzed. After each P-3CR step, the product was purified *via* column chromatography. For the synthesis on solid phase, in the first step of the reaction, a functionalized resin reacted with the second linker molecule in a TAD-click reaction introducing a free acid functionality. All further steps were similar to the synthesis in solution. On solid phase, a dodecamer was synthesized, but only the trimer, nonamer and dodecamer were analyzed by SEC, NMR and mass spectrometry. In Scheme 25, the iterative stepwise cycle for both systems, solid phase and solution, are displayed. The advantages of the synthesis in solution compared to the synthesis on solid phase included high purity of the product (≥99% compared to 84%), larger scale (200 mg compared to 50 mg), and higher overall yield (18% compared to 5%). The advantages of the synthesis on solid phase included the easy purification after the P-3CR by a washing step, compared to column chromatography, shorter reaction times for the P-3CR (30-120 min compared to 8-48 h) and shorter overall time required (2 days compared to 3 weeks). This comprehensive study demonstrated the successful combination of the TAD-"click" reaction and the P-3CR. Since both systems carry several advantages and disadvantages, the choice of system must therefore be made depending on the characteristics that are important for each procedure.



Scheme 25: Iterative two-step reaction cycle with the TAD-“click” reaction and the P-3CR. Synthesis was carried out both in solution or on solid phase and compared.[26]

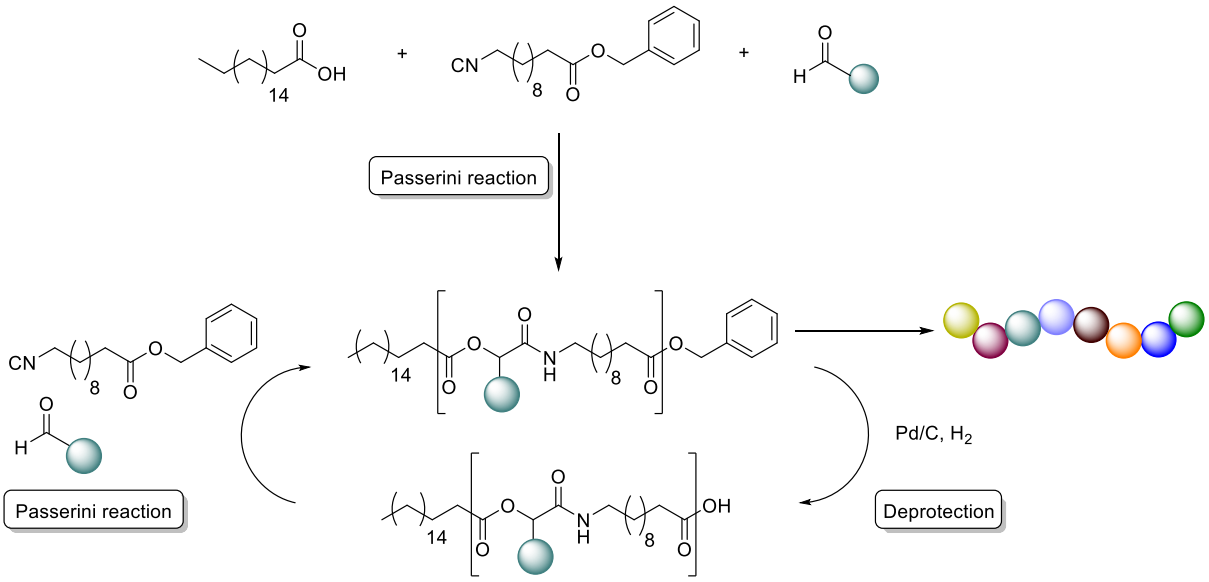
In 2019, Tao *et al.* published the synthesis of sequence-defined peptoids *via* the U-4CR. A side chain and backbone variation with amino acid building blocks was described. First, acetic acid was used as starter acid and reacted with an aldehyde, isocyanide and an acid-protected amino acid in the Ugi reaction. Deprotection with trifluoroacetic acid yielded the free acid, which reacted in the next step again in a U-4CR with an isocyanide, aldehyde, and acid-protected amino acid as amine. Depending on which group was controlled, either the aldehyde or the protected amino acid was varied in the following reaction cycles. Thus, by aldehyde variation, a side chain varied sequence-defined decamer was obtained as well, while by varying the amino acid, a backbone defined pentamer was prepared. The schematic reaction cycle for the synthesis of side chain and backbone defined peptoids is displayed in Scheme 26.



Scheme 26: Synthesis strategy for sequence-defined peptoids for side chain and backbone control.[23]

In 2016, our group reported the synthesis of sequence-defined macromolecules with an improved P-3CR approach, which leads to high yields and allows the synthesis in a multigram scale.[51] Therefore first, a monoprotected AB monomer with an isocyanide functionality and a benzyl ester was synthesized in a three-step synthesis. As such, 11-aminoundecanoic acid was reacted with benzyl alcohol and thionyl chloride to yield the benzyl ester. In the next step, the formamide was formed and reacted subsequently with $POCl_3$ and diisopropylamine to yield the monoprotected monomer with the isocyanide function. For the iterative stepwise growth, stearic acid was used as starter acid to synthesize the first Passerini product with the AB monomer and an aldehyde. In the deprotection step, the benzyl ester was cleaved by palladium on carbon with hydrogen gas. The resulting free carboxylic acid was used again in a Passerini reaction with the monomer and a different aldehyde. By continuing this iterative two-step reaction cycle, it was possible to synthesize a sequence-defined decamer with 9 different side chains in a high purity, confirmed by SEC, NMR, and mass spectrometry. The reaction scheme is depicted in Scheme 27. With this approach, it was possible to synthesize a decamer with an overall yield of 44% which was significantly higher when compared with the P-3CR/thiol-ene approach that had an overall yield for a pentamer of 15%. Moreover, by introduction of a *cis* double bond in the side

chain of the tenth Passerini reaction, it was possible to perform self-metathesis to yield a 20-mer.

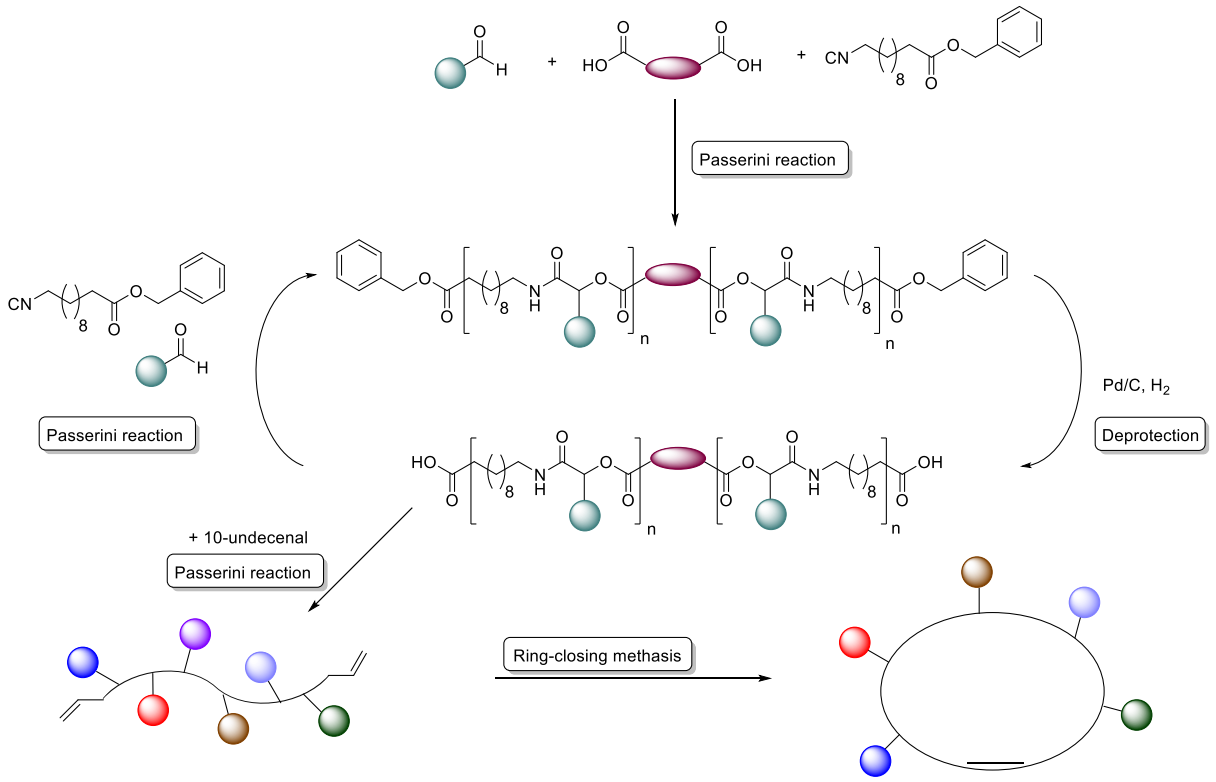


Scheme 27: Two-step iterative reaction cycle with the P-3CR and a deprotection.[14] For the usage in a iterative cycle a monoprotected isocyanide was used as AB monomer. The variety of aldehydes is marked in gray.

Since this system delivered very promising results, further approaches adapted it according to the respective targeted applications. The synthesis of sequence-defined macromolecules in this thesis is based on this approach and further discussed in the results and discussion.

In 2018, our group reported the use of the iterative stepwise cycle with the P-3CR and the deprotection combined with another MCR, the Biginelli reaction.[28,245] A Biginelli acid was first synthesized with an aldehyde, ureido carboxylic acid and acetoacetate benzyl ester. Afterwards, the acid was reacted with different aldehydes and a diisocyanide in a P-3CR to obtain a monomer with a benzyl ester protecting group and an isocyanide function. Subsequently, a P-3CR was performed with an aldehyde and a carboxylic acid followed by the deprotection step. The obtained free carboxylic acid was used again in a P-3CR with the monomer, and an aldehyde. The monomer carried five functionalities, while after the P-3CR a sixth functionality was introduced, therefore, this approach is important especially for the field of data storage and the applications there will be discussed in the following chapter 2.2.6. Another approach with the iterative stepwise cycle with the P-3CR and the deprotection was reported in 2019,[219] in which the synthesis of sequence-defined macromolecules in a bidirectional growth and a subsequent ring closure metathesis were discussed. Linear oligomers of different lengths (namely comprising 2, 4, 6, 8, or 10 monomer units) were synthesized in the bidirectional growth with three different side chains. As starting material, sebacic acid was

used for the first P-3CR with an aldehyde and the well-established AB monomer. Again, deprotection with palladium on activated carbon and hydrogen gas was performed to yield the free acid for the next P-3CR. At different stages of the reaction cycle, a terminal double bond was introduced with 10-undecenal in the P-3CR. Ring-closing metathesis was performed with the two terminal double bonds of the symmetric oligomer with a Grubbs catalyst to obtain sequence-defined macrocycles with different ring sizes. With this approach, it was possible to synthesize a cyclic tetramer, hexamer, octamer and decamer. The reaction scheme for the synthesis of sequence-defined macromolecules is displayed in Scheme 28 and depicts the successful synthesis of sequence-defined macrocycles.



Scheme 28: Synthesis of sequence-defined macrocycles with a different ring size.[219]

Recently, further syntheses using the P-3CR approach with a monoprotected monomer have been published.[49,50,246]

Through their huge variability and their easy implementation MCR can be used as a strong tool for the synthesis of sequence-defined macromolecules. High yields and a wide range of components allow the synthesis of long and variable sequences. Further the synthesis can be carried out in milligram scales, but also in multigram scales.

2.2.6 Sequence-definition in the field of data storage

Since we are producing more and more data due to digitalization, the field of data storage has become more and more popular in the last years. Especially the application of sequence-defined macromolecules for data storage is steadily growing. The idea of storing information in molecules is inspired by DNA, storing our genetic code, *i.e.* information.[4,247,248] In 2001, the DNA-sequencing of the human genome was one of the most important and impressive achievements of the twentieth century.[249] The "code" of the genetic information in living organisms uses a quaternary system consisting of four different nucleobases, namely adenine, guanine, cytosine and thymine, connected in a precise sequence. Investigations were made in the pursuit to create artificial molecules capable of storing a similar amount of data. To compare the capacity of systems, it is important to use the number of possible permutations as a benchmark, from which the number of bits can then be calculated.[33,34] The number of bits is calculated by the logarithm of the number of permutations divided to the log of two (see Equation 3). In Figure 6, the different systems for the chemical data storage are displayed. So far; the binary system is mostly used for artificial data storage.[204,237,250–252] There, "0" and "1" are used as "on" and "off" and thus it is possible to store 1 bit in each repeating unit. To yield high data storage capacity in the binary, but also in the quaternary system, long sequences of the respective repeating units are necessary in a specific order. Due to these facts, also other approaches were published in the last years, for instance the sequence-defined molecules with a higher amount of varying number per units.[34] For instance, Figure 6a shows a DNA sequence with a data storage capacity of 2 bit per repeating unit, thus obtaining data storage of 8 bits by synthesizing a tetramer.

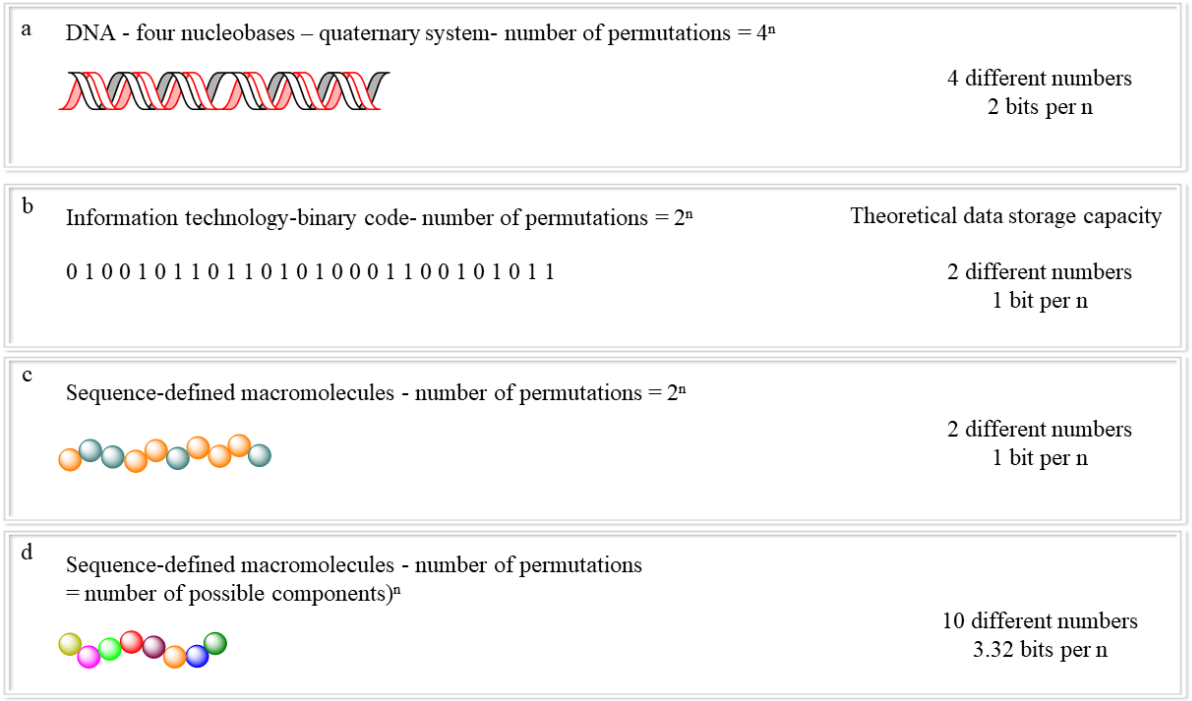


Figure 6: Comparison of the data storage capacity per repeating unit of different systems for data storage.[34] **a.** DNA with the quaternary system. **b.** Binary system used in computer technology. **c.** Binary system in a non-natural macromolecule with two varying compounds per unit. **d.** Sequence-defined macromolecules varying ten components.

The read-out of the encoded molecules that are used in data storage is often one of the most difficult steps, while the synthesis steps are often well established. Data storage is a rather young field, thus in the following section, the important developments and publications are presented in a chronological order. The field is presented into two parts: one concerning small molecules and the other sequence-defined macromolecules.

In 2016, Margulies *et al.* reported the idea of a message in a molecule.[253] In 2018, our group published the usage of MCR-obtained molecules as keys for secret communication.[33,254] There, using the U-4CR (see 2.1.2.2), a virtual library of 500,000 key molecules with the use of 130 commercially available different components (50 aldehydes, 20 isocyanides, 10 carboxylic acids and 50 amines) was presented. Through a synthesized library of 27 molecules, the proof of concept was demonstrated. To simplify the work-up for the synthesis and the extraction out of other media, perfluorinated acids were used. Thus, it was demonstrated that the molecules could be hidden by absorption onto coffee, paper, sugar, or dissolved in perfume or blood. These were subsequently extracted with organic solvents and analyzed *via* ESI-MS/MS, showing the practicality of the concept. The read-out was successfully performed and was assisted by a computer software. With this approach, it was possible to show the application of small molecules for secret communication with a data storage capacity of up to 18 bits in

one molecule. The number of 18 bit was calculated based on the 500,000 possible permutations within these molecules.

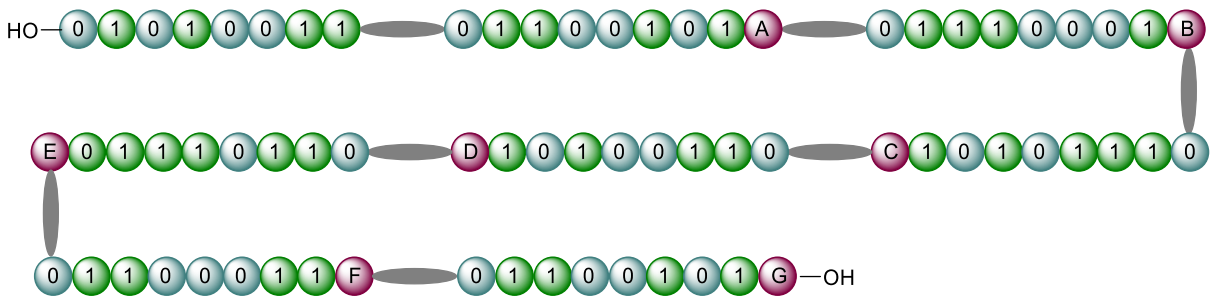
In 2020, Rosenstein *et al.* also published a MCR-based approach for molecular memory.[76,255] There, the U-4CR was also used for the synthesis of a library of up to 1,500 compounds in an automated process. By using a mixture of the molecules printed on a MALDI plate, they were able to encode a picture of Picasso and demonstrated an impressively high data storage capacity of up to 0.8 million bits. However, it must be noted that the decoding of these molecules was only possible with an accuracy of 97.6%. The accuracy of the readout process is enormously important for application in cryptography, as well as in the field of data storage in general, making a 100% accuracy crucial for the read-out. For optical read-outs, as in this example, less accuracy can be tolerated.

Small molecules are mostly used in the fields of secret communication, cryptography, or as molecular memory. To ensure high accuracy of the read-out and achieving high data density, however, sequence-defined macromolecules are another interesting approach. Inspired by the sequencing of DNA, suitable approaches for synthesis of long sequences have been established, as well as their respective read-out.

In 2015, Lutz reported the coding of macromolecules with the usage of polyphosphoramidite chemistry in a binary system.[252] His group was one of the first introducing sequence-defined macromolecules for data storage by read-out *via* tandem electrospray ionization (ESI-MS/MS). For the synthesis of the sequence-defined macromolecules, an iterative step approach was used, as already described in 2.2.4. The successful read-out of the molecules was achieved with ESI-MS/MS, while the associated fragments of the molecules were clearly assigned to their respective moieties. In 2016, Barner-Kowollik *et al.* published the synthesis of sequence-defined copolymers and the successful coding and read-out.[32] For their synthesis, bidirectional growth was used, as described in chapter 2.2.3. The read-out was performed *via* tandem-MS, this time by MALDI-TOF-TOF. The fragmentation pattern of the molecule was observed in the MS/MS spectra allowing the read-out of the molecule with the associated monomer units. The symmetry of the used tetramers facilitated the interpretation and complete read-out of the molecules. In 2016, Lutz *et al.* reported the synthesis of a sequence-defined poly(alkoxyamine phosphodiester) *via* an orthogonal iterative synthesis based on a successive phosphoramidite and radical-radical coupling step.[237] Two years later, this concept was used to simplify the read-out through the favored formation of fragments that contain two bits instead of one.[242]

With these approaches, it was possible to generate a storage capacity of 16 bits per macromolecule.

In 2017, the same group published another important step in the field of data-storage in synthetic sequence-defined macromolecules.[7] Through the use of mass markers (TAG), which were introduced at precise positions, it was possible to simplify the read-out process, thus allowing a data storage capacity up to 64 bits. With this approach, the read-out *via* ESI-MS/MS of long sequences was optimized and demonstrated for the first time. After a sequence of eight units, a TAG was introduced to mark this sequence, followed by eight more units and a subsequent TAG. A schematic overview of the concept of sequences bearing TAGs in a binary system is displayed in Scheme 29. Furthermore, in 2017, the same group reported the "millisecond sequencing" of a binary-coded synthetic polymer using a computer program.[256] The program used an easy algorithm, allowing fast and accurate read-out of the sequence-defined molecules. The superiority of an automated read-out was demonstrated on 84 different sequence-defined polymers, which took only milliseconds per sequence for processing. Furthermore, at the same time, Lutz and co-workers reported several different approaches for the read-out of synthetic polymers, which however are not discussed in detail.[31,240,257,258]



Scheme 29: Schematic overview of the 64 bits macromolecule with TAGs using the binary system.[7] Green dots symbolize each unit marked with a "0", cyan dots symbolize "1" and red dots symbolize the different TAGs.

In 2018, the group of Du Prez published the synthesis of multifunctional sequence-defined macromolecules for application in chemical data storage.[9] There, the automated iterative step approach was used, as discussed in detail in chapter 2.2.4, relying on thiolactone chemistry to synthesize 70 oligomers (11 pentamers, 59 hexamers, in addition one monomer was synthesized) using a binary system. Afterwards, the oligomers were fragmented in MALDI-MS/MS experiments and an automated read-out was established with a computer program. The program was able to combine the different sequences of the oligomers to yield one long sequence, which then generated a QR code. The automation of the read-out was done in a few seconds and can be also applied to other synthesis approaches. In 2018, our group published another approach to increase the data storage capacity of sequence-defined molecules, through

the combination of two MCRs, namely the Biginelli and the P-3CR.[33] The explanation of the synthesis was already discussed in detail in chapter 2.2.5. Through the combination of the two MCRs, it was possible to introduce up to six different functional groups per unit, resulting in a significant increase of the data storage capacity. If a large library with 100 possible compounds is assumed, up to 24 bits can be stored in one repeating unit. With this approach, it was possible to synthesize and fully read-out a tetramer *via* ESI-MS/MS with a theoretical data storage capacity of 97 bits. In 2020, the group of Du Prez reported another interesting approach for storing information in sequence-defined molecules.[10] There, the focus was not set on the storage density, but rather on simplifying the reading of the molecules and concentrating on the encryption. For the sequence synthesis, the established approach of P-3CR and TAD-"click" reaction was used.[26] When the molecule was heated up, the TAD-indole covalent bonds randomly reshuffled and the information was encrypted. To decrypt the code, another heating step was necessary and afterwards the code was deciphered by ESI-MS. This approach of encryption/decryption presented a new approach in the field of data storage. However, only a rather small number of 4 bits can be stored in the molecule and thus application in the field of cryptography is more sensible.

In 2020, Lutz *et al.* published the storage of images in single molecules and the, up to now, highest data storage capacity of 144 bits.[238] There, polyphosphodiesters were synthesized using stepwise automated phosphoramidite chemistry resulting in a library of four or eight phosphoramidite monomers that were used in the synthesis of sequence-defined macromolecules to yield a data density of two or three bits per monomer unit. Furthermore, TAGs were implemented in the sequences to allow a successful read-out *via* ESI-MS/MS. Black and white pictures were encoded in the polymer chains as a proof of concept. By the use of the library of eight monomers, it was possible to store 144 bits in a 57-mer polyphosphodiester. Besides the aforementioned approaches, other approaches and synthesis strategies for sequence-defined molecules in the field of data-storage were recently published;[205,259–265] for example, the storage of information in polyesters and *N*-substituted polyurethanes.[11,241] It becomes clear that the still young research field of data storage in sequence-defined macromolecules arouses great interest and offers a growing number of exciting new approaches. This thesis is focused on increasing the data storage capacity with new approaches like read-out of mixtures (see 4.3) or the synthesis of dual sequence-defined molecules (see 4.2). In general, different approaches have been used for the sequencing of macromolecules. These include NMR spectroscopy,[266–270] cleavage/depolymerization,[271–273] nanopore

analysis[274–277] or mass spectrometry.[278–281] Recently, the sequencing *via* Raman scattering was also reported.[282]

It has to be mentioned that in the field of sequence-defined macromolecules and small molecules with application in data storage, mainly the use of mass spectrometry has been reported up till now.[9,28,30,33,238,242,243,263] Especially for the read-out of sequence-defined molecules, soft ionization tandem mass spectrometry, like MADLI-TOF-TOF or ESI-MS/MS, were used to ensure a successful read-out.[7,9,11,28] As already mentioned, the group of Du Prez and Rosenstein reported the successful readout of a sequence-defined molecule and small molecules by using ESI-MS, without the use of tandem MS.[76,243] Due to the frequent application of mass spectrometry in the field of sequencing, more and more methods and approaches have been investigated to simplify and improve its use,[37,283–287] for example by optimizing the conditions of tandem MS or by changing the positive/negative mode as well as other settings.

In summary, it is clear how interesting the application of sequence-defined macromolecules is in the field of data storage. They offer a novel and versatile possibility to store data in the future.

In conclusion, versatile approaches for the synthesis of high defined macromolecules have been developed in different systems. Each system carries its certain advantages and disadvantages, which must be considered in the design of the synthesis to achieve the perfect yield subsequently. However, for the synthesis of sequence-defined macromolecules highly efficient reactions are necessary to yield high conversions and long sequences without by-products.

3 Aim

The aim of this thesis was to investigate the potential application of sequence-defined Passerini oligomers in the field of data storage, followed by the establishment of novel approaches to increase their data storage capacity. For the oligomer synthesis, a well-established iterative cycle was used, consisting of a P-3CR and a deprotection step, as a powerful toolbox for sequence-defined molecules. Due to experiences in preliminary investigations, a thorough study of analytical methods regarding their information and detection thresholds was planned to track and identify products and potentially interfering impurities, respectively. Furthermore, the application was in focus, investigating and developing a successful read-out strategy for the application of the oligomers in the field of data storage. Therefore, ESI-MS/MS measurements of different Passerini systems, such as side chain defined, backbone defined and dual sequence-defined oligomers, had to be analyzed and fragmentation patterns assigned accordingly. Only by detection of a common fragmentation pattern of all investigated system, a read-out protocol could be established and used in the field of data storage. To increase data storage capacity, a method for the read-out of oligomer mixtures was supposed to be developed. In contrast to individual and isolated oligomers, the challenge here is to be able to clearly separate the oligomers from each other in the readout process and to ensure the unambiguous read-out of each oligomer. In addition, to reduce the time of the readout process and further to eases the process, a less time-consuming automation was supposed to be developed. Different mass markers are required to ensure the exact assignment of the oligomers. After the proof of concept of the automated read-out of mixtures, the number of oligomers should be increased to show the limit of oligomer mixing. Further, it was supposed to be demonstrated that reading mixtures is a powerful way for the data storage in the future and thus data storage density can be increased.

4 Results and Discussion

4.1 Impurity studies of sequence-defined macromolecules

Abstract:

An impurity study using a sequence-defined pentamer, which is contaminated by different wt% amounts of a known impurity, i.e. the corresponding sequence-defined tetramer, is described in this chapter. For the synthesis of the sequence-defined pentamer and the corresponding tetramer, the established iterative stepwise synthesis approach was used, which combines a P-3CR and a subsequent deprotection step. After the synthesis of the tetramer and pentamer, they were fully characterized and afterwards used for the impurity studies. Thus, the pentamer was contaminated with different amounts of the tetramer and the mixtures were analyzed by SEC and ^{1}H NMR. By measuring different amounts of contamination, it was possible to detect an impurity of ≥1% by SEC and ≥5% in the ^{1}H-NMR. Furthermore, the importance of a complete characterization of sequence-defined macromolecules and the need for clear definition for purity statements for sequence-defined macromolecules are demonstrated.

4.1.1.1 Concept and synthesis

The purification and especially the purity itself is an important key element in the synthesis of sequence-defined macromolecules. To confirm a uniform macromolecule, it is important to use different characterization methods to ensure the uniformness and the successful synthesis of the molecule. In a review of our group published in 2017, different systems for the synthesis of sequence-defined macromolecules were discussed and compared with each other.[20] Since many groups still publish incomplete characterizations, for instance only mass spectrometry or only NMR data, the review pointed out the importance of a complete characterization of the sequence-defined molecules. For a complete characterization, the use of liquid chromatography (like SEC or high-pressure liquid chromatography), mass spectrometry and NMR are necessary. Especially for the determination of the purity of the products, the full analysis is important, because without a full analysis the purity of the product cannot be unambiguously proven. Thus, in this chapter, the importance of the analytic methods is shown and furthermore the limit of the detection of an impurity in SEC and the ^{1}H NMR is demonstrated.

For the comparison studies of the purity, a sequence-defined pentamer **IP** was synthesized. Therefore, a iterative stepwise cycle, which was already established in our group, was used.[51] The iterative approach is based on the P-3CR and followed by a deprotection step (see Figure 7).

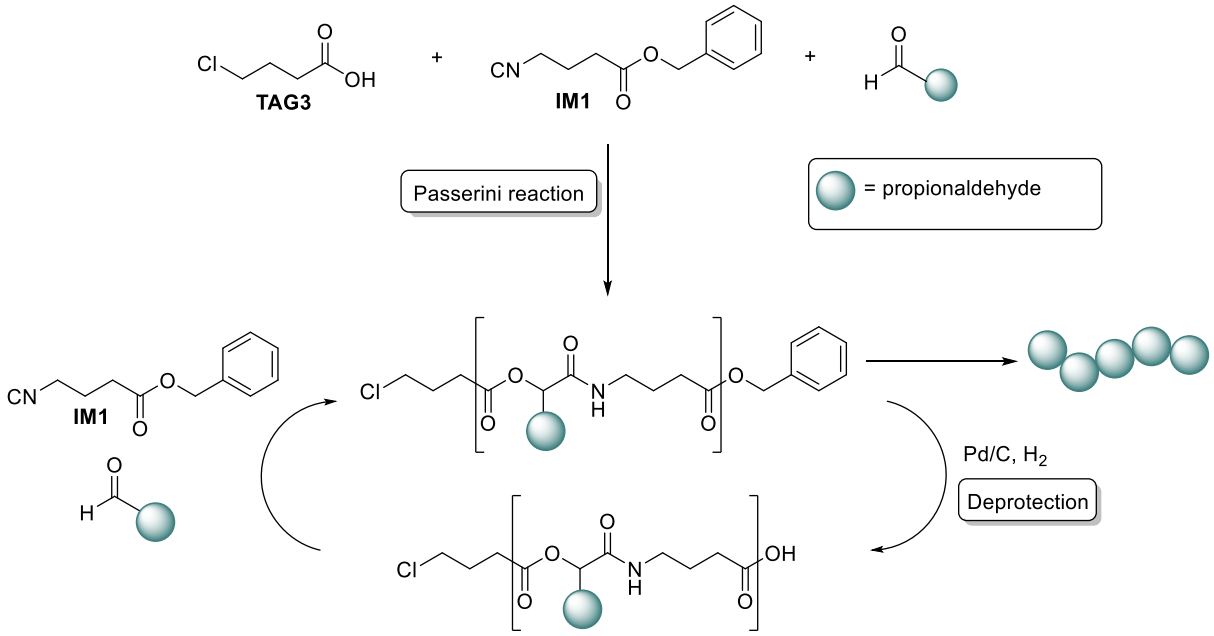


Figure 7: Iterative step approach with a P-3CR and deprotection. For the P-3CR 4-chlorobutyric acid **TAG3** was used as starter acid, a monoprotected isocyanide **IM1**.

For the iterative cycle, a monoprotected monomer was necessary. Therefore, an isocyanide **IM1** with a benzyl ester protected acid was synthesized in a three-step synthesis with an overall yield of 49% in a 13 g scale (see 6.3.1).[50]

For the synthesis of the pentamer **IS5**, first the Passerini product was synthesized and afterwards deprotected to the carboxylic acid. The respective synthesis and characterization of the mono-, di-, trimer etc. are then iterative, using propionaldehyde **A2** and **IM1** in each Passerini step (see 6.3.1.2). For the synthesis of the pentamer **IS5**, 4-chlorobutyric acid **TAG3** was used as starting acid in the first Passerini reaction and reacted with propionaldehyde **A2** and isocyanide **IM1**, which were used in small excess relative to the acid. The reaction was stirred at room temperature for one day and after purification *via* column chromatography, the Passerini product **IS1** was obtained in a yield of 92% and high purity (≥99%). The successful synthesis of **IS1** was confirmed by ^{1}H-, ^{13}C-NMR, SEC, IR and mass spectrometry (see 6.3.1.2). Afterwards, the Passerini product **IS1** was deprotected with palladium on activated carbon **1** and hydrogen gas overnight. Then, the deprotected Passerini monomer **ISD1** was purified *via* filtration over celite® and obtained in a yield of 98%. Subsequently the deprotected Passerini product **ISD1** was used in the further iterative cycle. After nine reaction steps in total, the pentamer **IS5** was synthesized in a yield of 44%. All products of the steps were fully characterized, for further information see 6.3.1.2. The chemical structure of **IS5**, SEC-traces after each P-3CR step and the high-resolution isotopic pattern obtained by ESI-MS compared with the calculated isotopic pattern are depicted in Figure 8.

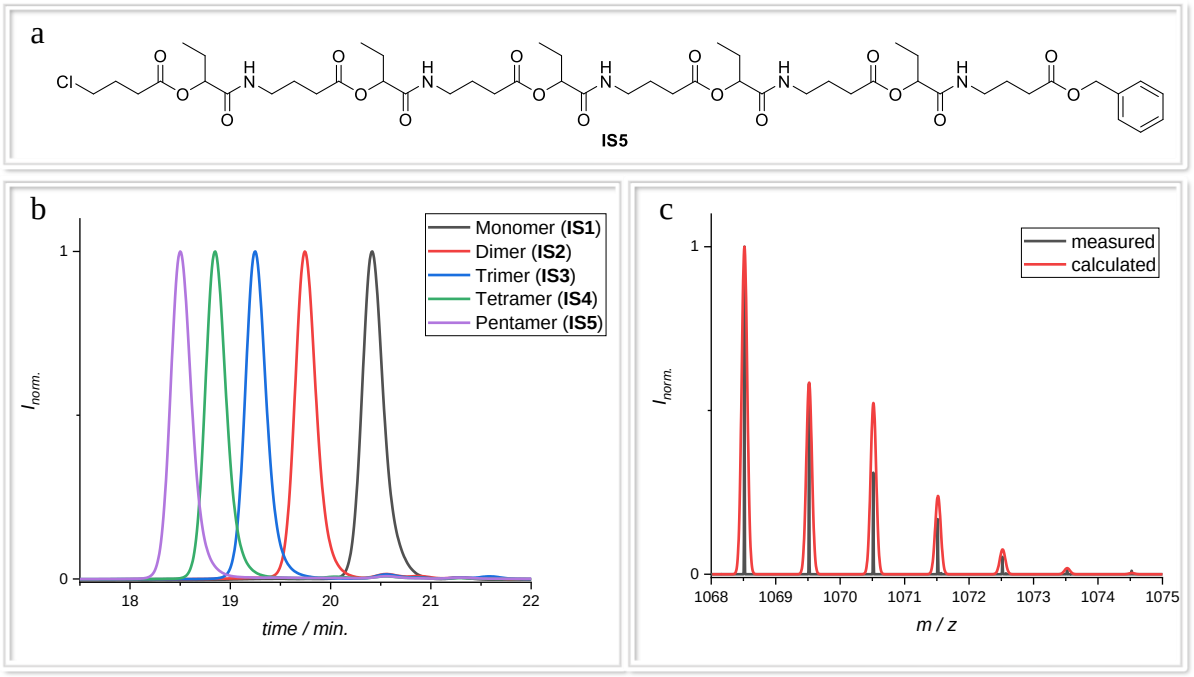


Figure 8: Characterization of the sequence-defined pentamer **IS5**. **a.** Chemical Structure of the sequence-defined pentamer **IS5**. **b.** SEC traces of each P-3CR product. Each unit with its specific aldehyde moiety is colored with the same color of the respective SEC-traces. **c.** An overlay of the calculated isotopic pattern of -resolution ESI-MS measurement of **IS5**; calculated isotopic pattern (red) and measured isotopic pattern (black).

In Figure 9, the ^{1}H-NMR spectrum of pentamer **IS5** is depicted and all significant signals could be assigned. The resonances of the benzyl ester can be observed at 7.41-7.30 ppm for the phenyl group (signal 1) and at 5.16 – 5.05 ppm for the CH_2-group of the benzyl ester (signal 4). The CH protons (signals 3) visible at 5.16 – 5.05 ppm overlap with the CH_2-group and indicate the successful formation of the Passerini product. The formation of the amide protons (signal 2) at a ppm range of 7.90 – 7.44 ppm and 6.40 – 6.30 ppm further confirm the formation of the Passerini product **IS5**. In Figure 9, the comparison between ^{1}H-NMR spectra of the Passerini monomer **IS1** (top, marked in green) and the Passerini pentamer **IS5** (bottom, marked in red) is shown. It can be observed that the amide signals of the longer sequences are shifting between 7.90 – 7.44 ppm. Moreover, the formation of isomers can be observed at the amide proton (signal 2), and also for the CH_2-group next to amide (signal 6). This is also visible in the ^{13}C-NMR spectrum (see Supplementary Figure 121).

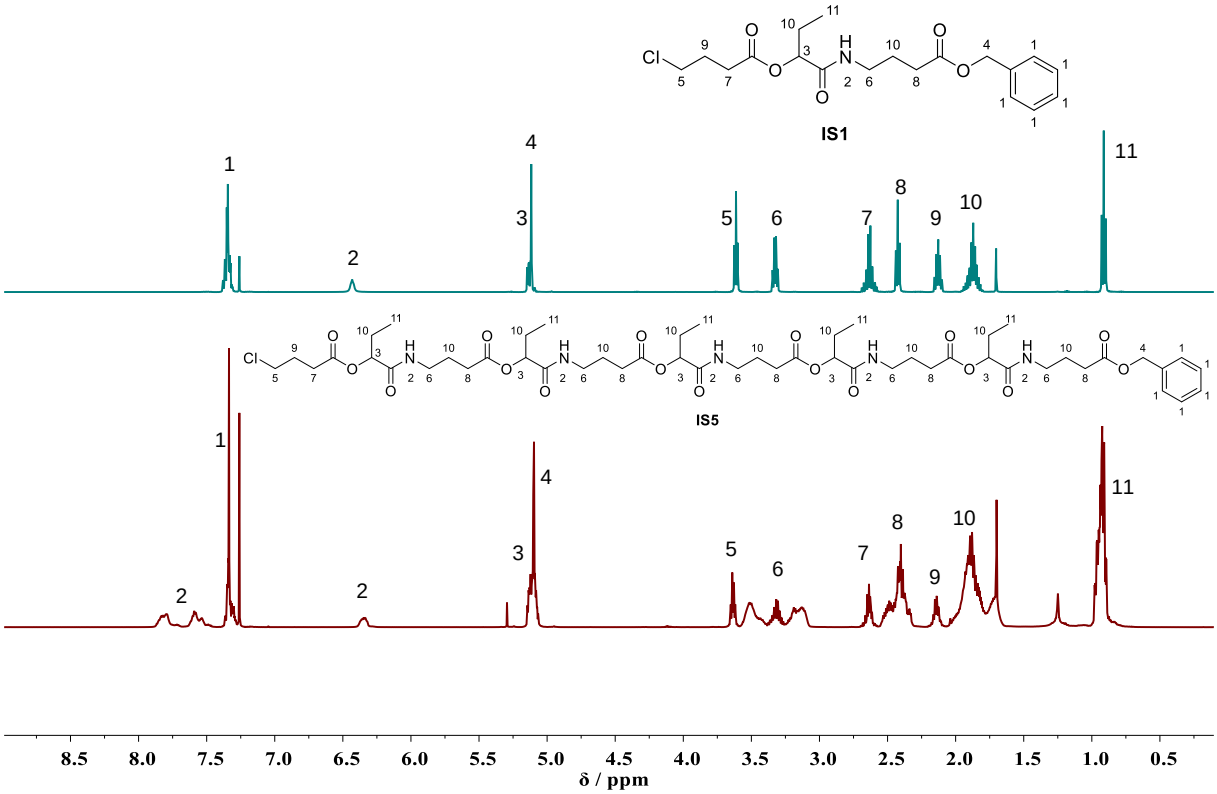


Figure 9: Comparison of the ^{1}H-spectra of Passerini monomer **IS1** (top, marked in green) and Passerini pentamer **IS5** (top, marked in red)

Furthermore, in the comparison, it can be observed that in the spectrum of the pentamer impurities of solvent and water are visible. Due to a high viscosity of the pentamer, it was not possible to completely remove the solvent.

4.1.1.2 Impurity studies

To demonstrate the need of a full characterization for sequence-defined macromolecules, especially for the indication of the purity, an impurity study was performed. In particular, the importance of using liquid chromatography to determine the purity of macromolecules is highlighted.

For the impurity studies, a test series with ten different percent additions of impurity were used. In the beginning, the two pure oligomers, the tetramer **IS4** and the pentamer **IS5**, were analyzed by ^{1}H, ^{13}C- NMR, SEC, IR and mass spectrometry. The pentamer **IS5** was then contaminated with different amounts of wt% of impurity of the corresponding sequence-defined tetramer **IS4** (0 wt%-100 wt%). The different amounts of impurity, which are used for the measurements, are displayed in Table 2.

Table 2: Summary of the pentamer **IS5** contaminated with different amounts of impurity of the tetramer **IS4**.

Impurity	Pentamer IS5	Tetramer IS4	Detectable in SEC	Detectable in NMR
0 wt%	100%	0%		
1 wt%	99%	1%	✓	✗
2 wt%	98%	2%	✓	✗
3 wt%	97%	3%	✓	✗
4 wt%	96%	4%	✓	✗
5 wt%	95%	5%	✓	✓
7 wt%	93%	7%	✓	✓
9 wt%	91%	9%	✓	✓
11 wt%	89%	11%	✓	✓
13 wt%	87%	13%	✓	✓
15 wt%	85%	15%	✓	✓
100 wt%	0%	100%	✓	✓

The impurity studies were performed *via* SEC and ^{1}H-NMR. For the SEC measurements, the samples were prepared in a concentration of 2 mg*mL^{-1} and measured on a THF-SEC with Oligo columns at 30 °C. In Figure 10, the SEC traces of the pentamer **IS5** plus the impurity measurements up to 15% are shown.

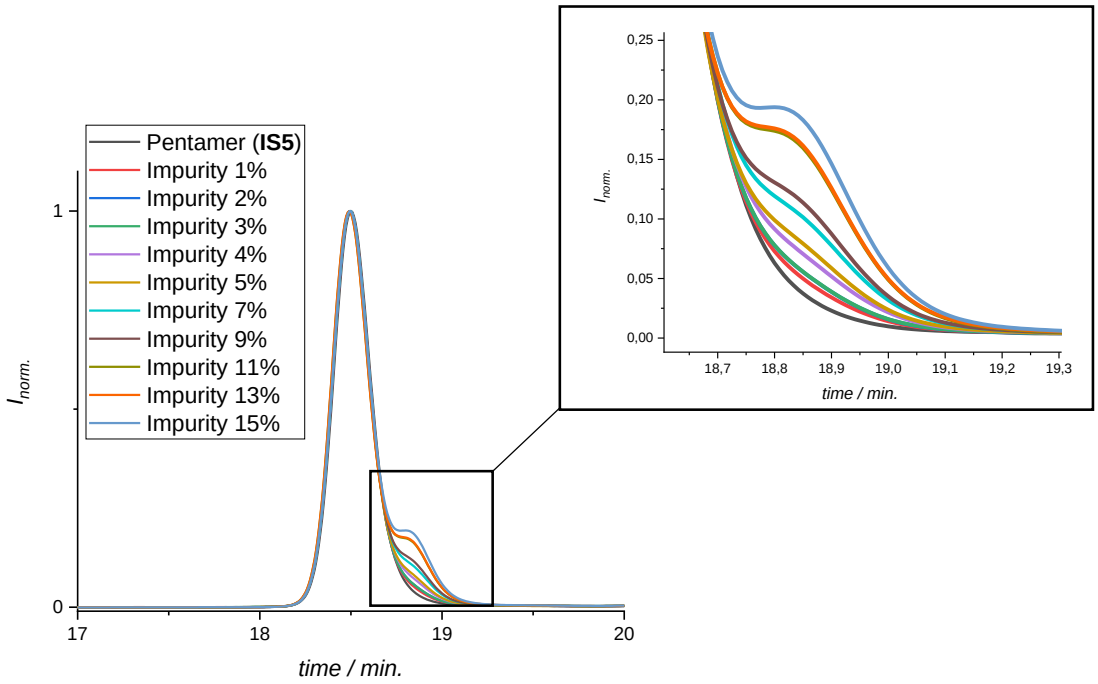


Figure 10: Comparison of the SEC traces of a sequence-defined Passerini pentamer **IS5** containing different amounts of wt% of impurity of the corresponding sequence-defined tetramer **IS4**.

In Figure 10, the SEC traces of the measurement series are depicted. Comparing the SEC traces of pentamer **IS5** (black) containing different amounts of wt% of the corresponding sequence-defined tetramer **IS4**, it can be observed that it is possible to detect an impurity with an amount of as low as 1 wt% (Figure 10 red curve). From a contamination of 7 wt% (turquoise) and onwards, a clear shoulder can already be observed in the SEC measurements. With this measurement series, it can be demonstrated that it is possible to identify an impurity of ≥1% in the SEC. Until now, our group demonstrated only the possibility of the identification of 2% impurity in uniform PEGs.[210]

In the ^{1}H-NMR measurements, it was more challenging to distinguish oligomers with only one additional repeating unit due to the high number of protons for a Passerini pentamer **IS5** and also the Passerini tetramer **IS4** and only a small shift, which can be observed in the spectra. The measurements were performed with a concentration of 20 mg*mL. By comparing the integrals of the end group, it was possible to identify the impurity with 5 wt% and more. However, this only provides initial indications of how suited the NMR is for purity determination. In order to be able to make more substantiated statements, the cooperation with an NMR expert would be necessary to perform further measurements, such das DOSY experiments.

As already discussed in the ^{1}H-NMR measurements, it was more challenging to distinguish oligomers with only one additional repeating unit. However, it is important to note that the

NMR is a strong analytic tool for impurities, like solvents or side products. This is demonstrated in Figure 9. For **IS5** in the SEC, the solvent cannot be detected, whereas in the NMR it is clearly visible. What became clear is that we have analysis tools, which can detect even the smallest amount of contamination ≥1%. However, each analysis tool has its strengths and weaknesses. For example, the NMR can detect only 5% of impurity of the smaller sequence, however different kind of impurities like solvents or small side product can be detected more easily. Also, it is necessary to proof the successful synthesis of a molecule. The SEC can detect even the smallest contamination, but characterization only *via* SEC is difficult. Furthermore, impurities with very different retention times cannot always be detected. Moreover, it would be interesting to measure the impurity series also *via* ESI-MS to check if there the impurities can also be detected. In general, however, the impurity measurements show how important it is to combine the various analytical methods in order to demonstrate a meaningful result about the synthesis process and the purity of the molecules.

Since the impurities studies were only performed for sequence-defined Passerini molecule, it would be interesting to perform the same studies with different systems in the future, like conjugated molecules or PEGs. For example, our group demonstrated the detection of 2% impurity in the SEC for a uniform PEG, which would be characterized as pure when only using MS and NMR data. It would be interesting to observe how well the different systems can be studied by the different analytical methods and how much percent of the impurity can be detected with them. This could provide a general approach for describing the purity of sequence-defined molecules. Furthermore, it offers the possibility that a uniform specification of the purity makes the results more comparable with each other.

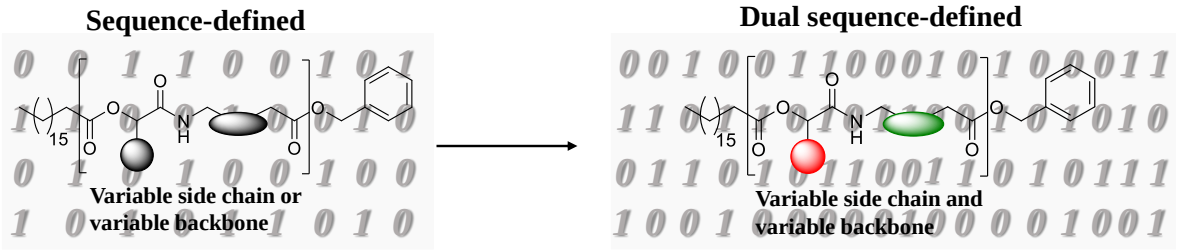
4.1.1.3 Conclusion

In summary, the synthesis of a sequence-defined pentamer **IS5** without side chain variation was shown, followed by the full characterization. Impurity studies were then performed to analyze the detection limit of an impurity amount. The pentamer **IS5** was thus contaminated with different amounts of wt% of impurity of the corresponding sequence-defined tetramer **IS4**. *Via* SEC analysis, it was possible to detect an impurity with an amount as low as 1 wt%. Furthermore, with ^{1}H-NMR measurements, it was possible to detected impurities with 5% and more by comparing the ^{1}H-spectras of the different measurements. The investigations confirmed the assumptions made by our group in 2017 that only a complete characterization of sequence-defined molecules can provide information about successful synthesis and purity. Therefore, a mass spectrometry, NMR and a liquid chromatography is necessary.

4.2 Identifying the most common fragmentation patterns of sequence-defined Passerini macromolecules, to increase the data storage capacity of dual sequence-defined macromolecules

Parts of this chapter and the associated parts in the experimental section were published before: Dual sequence-definition – increasing the data storage capacity of sequence-defined macromolecules, Katharina S. Wetzel, Maximiliane Frölich, Susanne C. Solleder, Roman Nickisch, Philipp Treu & Michael A. R. Meier, *Communication Chemistry*, 2020, 3, 63.[50] (The first two authors contributed equally).

The project was a collaboration within the group of Prof. Dr. Meier, the synthetic part was performed by Dr. Katharina S. Wetzel, Dr. Susanne C. Solleder, Roman Nickisch and Philipp Treu. The tandem MS measurements and the read-out were performed by the author (Maximiliane Frölich).

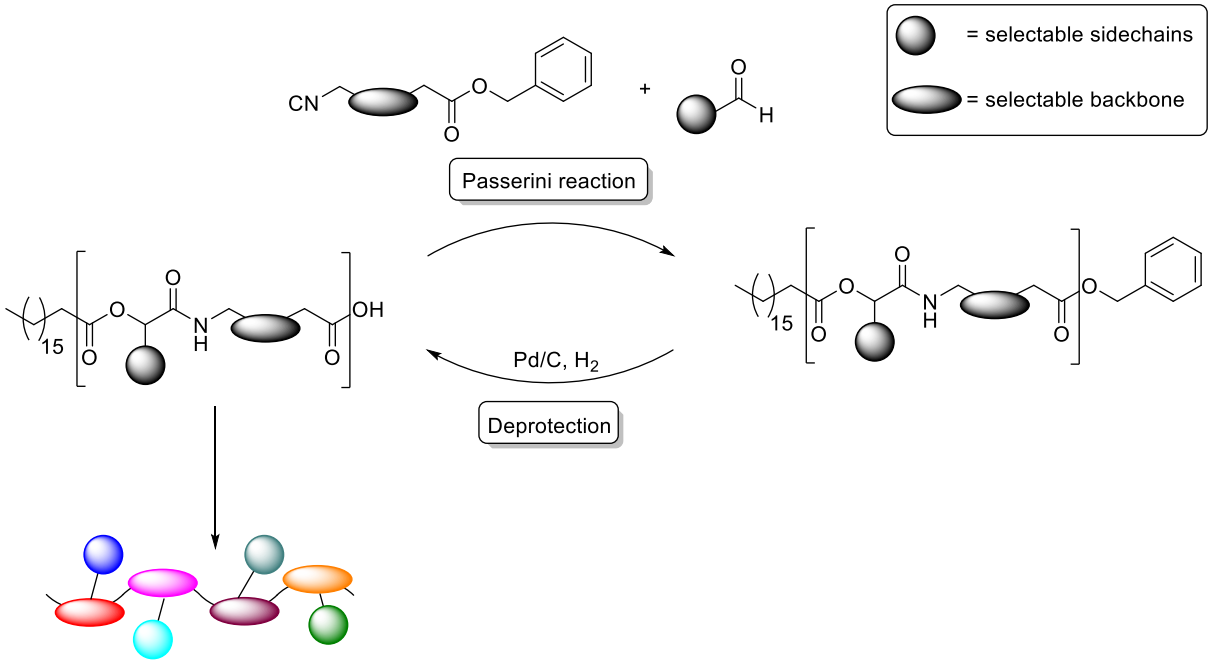


Abstract:

The analysis of common fragmentation patterns of sequence-defined macromolecules and their potential applications in data storage is demonstrated in this chapter. Using an established iterative stepwise synthesis approach that combines P-3CR and deprotection step, it was possible to increase the degree of definition of sequence-defined macromolecules. Therefore, backbone defined, side chain defined, and dual sequence-defined molecules were synthesised by the above mentioned cooperation partners. Afterwards, the most common fragmentation patterns of these molecules observed in ESI-MS/MS were carefully analysed. With this information, it was possible to establish full read-out of the molecules. For the dual sequence-defined pentamer, it was possible to demonstrate 33 bits of storage capacity in one molecule. Furthermore, comparing different systems and molecules, it was demonstrated that with increasing molecular diversity the data storage capacity is also increased.

4.2.1.1 Synthesis concept

Multicomponent reactions are suitable for applications in the field of data storage due to their modular character. Different components can easily be introduced to incorporate a large variety of different moieties into a growing oligomer. This leads to an increase of the structural variety and thus of the data storage capacity. For a successful application of multicomponent reactions in the field of data storage, two criteria must be fulfilled. First, they need to be orthogonal and must achieve nearly quantitative yields in the oligomer synthesis. Secondly, in the ESI-MS/MS spectra, they must show a distinct fragmentation pattern and the fragments must be distinguishable for a clear assignment and for allowing reconstruction of the molecule structure. To analyze the common fragmentation patterns of sequence-defined Passerini products, it was necessary to synthesize various molecules. Therefore, an established iterative step-wise approach was used, which combines the P-3CR and a subsequent deprotection step *via* hydrolysis (see 2.2.5).[51] First, a high variety of different highly defined molecules was synthesized varying the aldehyde component in each iterative synthesis cycle to achieve a different the side chain in each repeating unit.[51] Also, backbone-defined macromolecules were synthesized by utilizing different monomers. Moreover, it was possible to obtain dual sequence-defined macromolecules by varying both the side chain and the backbone independently at the same time. The synthesis concept is shown in Scheme 30. Stearic acid was used as starter moiety, then different aldehydes and/or monomers were used to generate sequence-defined macromolecules.



Scheme 30: Two-step iterative reaction cycle for the synthesis of sequence-defined macromolecules, with the P-3CR and a subsequent deprotection step. The reaction cycle allows for introducing different side chains and backbones.

A summary of all molecules analyzed *via* ESI-MS/MS is displayed in Figure 11. A side chain defined pentamer **SC5** and decamer **SC10** were analyzed, as well as a backbone-defined pentamer **BB5** and heptamer **BB7.** For the dual sequence-definition, a dual sequence-defined pentamer **DS5** was synthesized and analyzed.[50,51]

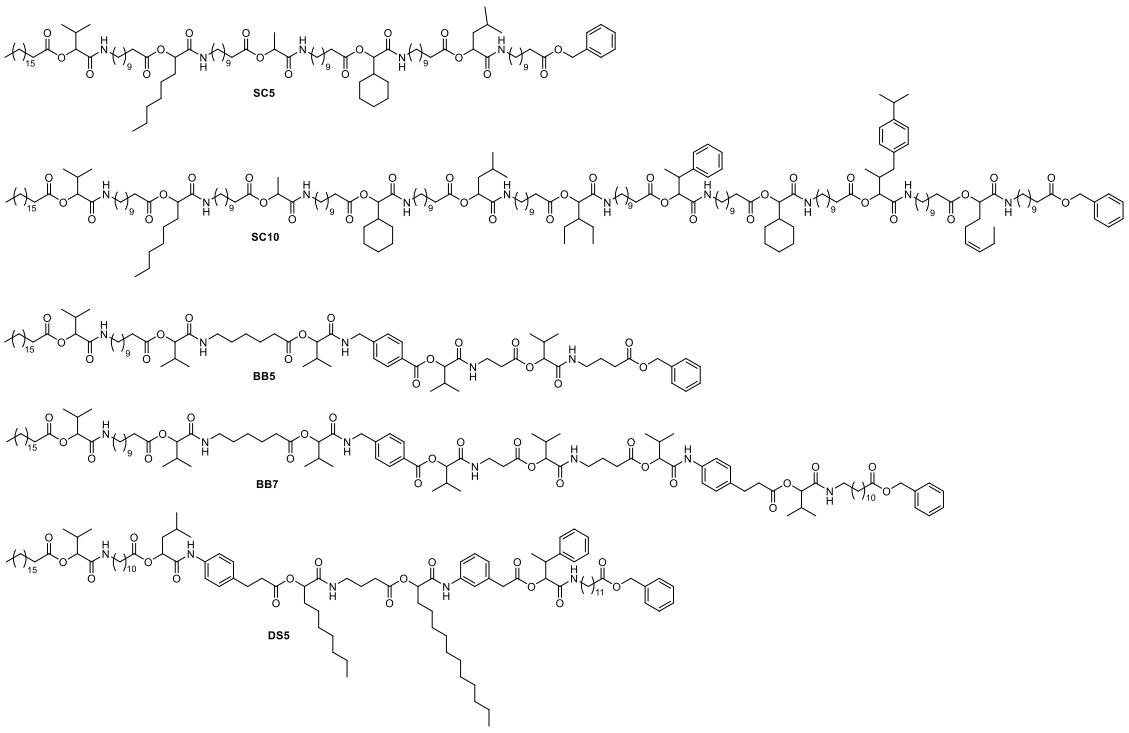


Figure 11: Library of the sequence-defined macromolecules that were analyzed by ESI-MS/MS.

4.2.1.2 Sequential read-out *via* ESI-MS/MS

The application of sequence-defined molecules in data storage evokes increasing interest during the last years (see 2.2.6). In this work, sequential read-out of sequence-defined Passerini products *via* ESI-MS/MS was investigated.

The analysis and identification of the most common fragmentation patterns of Passerini molecules in ESI-MS/MS experiments was the first important step. First results about the fragmentation of Passerini molecules were achieved by the author (Maximiliane Frölich) during her Master's thesis,[288] where small side chain defined molecules – four dimers and one trimer – were analyzed *via* ESI-MS/MS. The most common fragmentation observed in that work was the cleavage at the ester group in the Passerini product, next to the amide. This specific fragmentation was forced by the addition of sodium trifluoroacetate as additive during the measurements. With this preliminary information, further measurements were performed. Again, side chain defined molecules were analyzed, therefore the pentamer **SC5** and the decamer **SC10** were first used. This time, for technical reasons, the measurements were performed without sodium trifluoroacetate as an additive. In Figure 12, the fragmentation *via* ESI-MS/MS of decamer **SC10** is displayed and the mass peak of the molecule $[M+H]^+$ at 3563.7336 *m/z* was fragmented with a normalized collision energy (NCE) of 20. The read-out from both ends of the oligomer is demonstrated, focusing on the fragmentation next to the carbonyl. Starting from the mass peak of the molecule $[M+H]^+$ at 3563.7336 *m/z*, when performing the read-out from the right side of the molecule the cleavage at the carbonyl of the last monomer unit (marked as green spheres) was detected at 3132.4291 *m/z*. Further the cleavage at the carbonyl of the next monomer unit (marked as yellow spheres) at 2731.1321 *m/z* is shown. This common fragmentation is visible at each monomer unit, which are marked as colored spheres. The splitting from the left side also follows these rules of fragmentation. For the successful read-out, the masses of the single mass fragments were recombined to re-establish the initial structure. Through this strategy the stored information can be read.

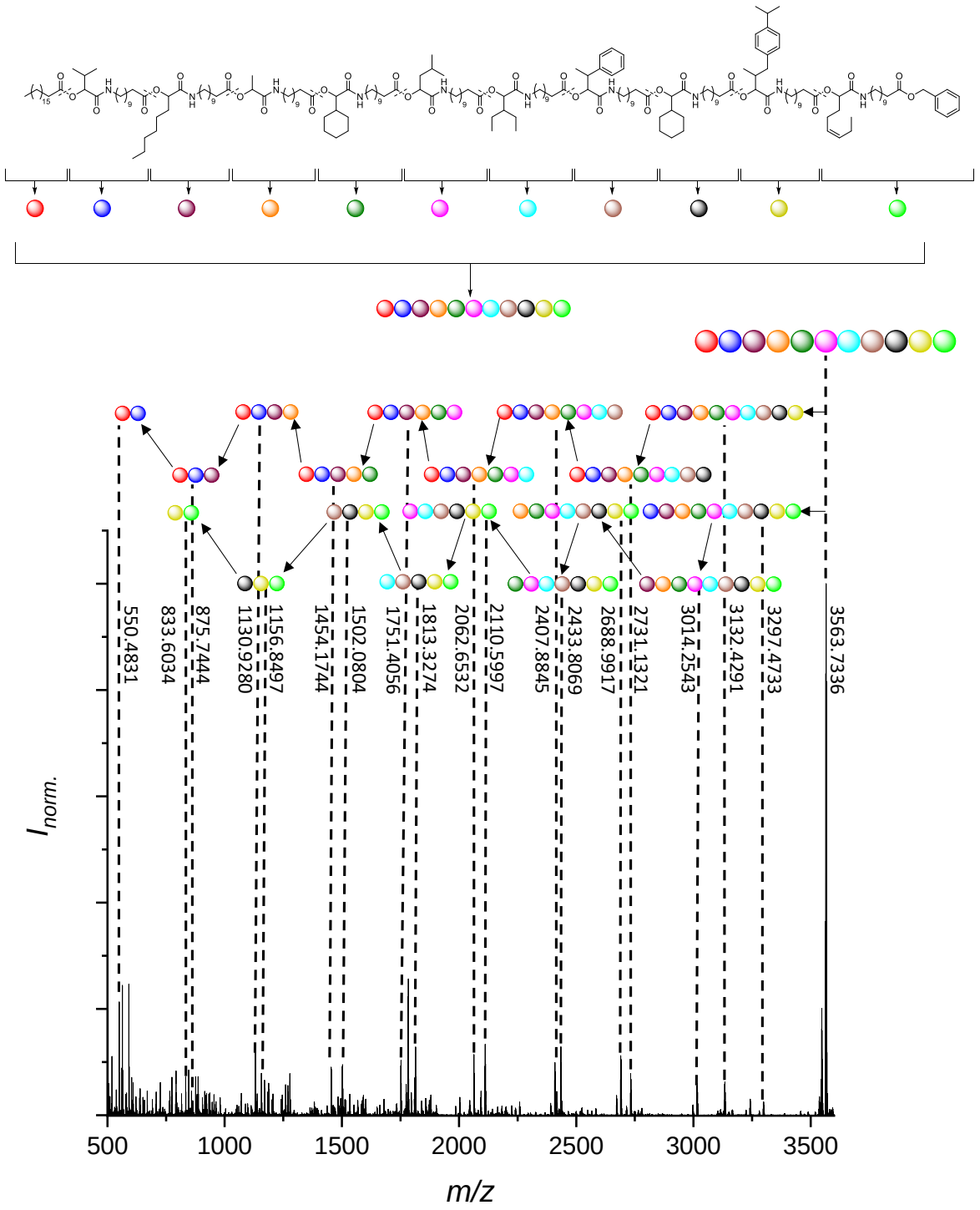


Figure 12: Fragmentation of the side chain defined decamer **SC10** *via* ESI-MS/MS with NCE of 20. In the spectrum, the read-out from both ends of the oligomer is shown, focusing on the fragmentation next to the carbonyl. With the recombination of the fragments, the initial structure can be re-established and thus the stored information can be read.

The measurements of decamer **SC10** and pentamer **SC5** without additive revealed that another fragmentation pathway of the Passerini molecule was predominant. During the measurements without sodium additive, cleavage next to the carbonyl was the most common fragmentation pattern. On the other hand, when measurements were performed with the additive, fragmentation next to the ester was observed. Furthermore, during the measurements without additive, fragmentation next to the ester took place as well, but to a much lesser extent. Moreover, measuring with and without an additive confirmed that the type of cleavage depends on the presence or absence of additive, respectively. As a next step, ESI-MS/MS measurements of backbone defined molecules were performed. Therefore, a backbone defined pentamer **BB5** and heptamer **BB7** were analyzed. In Figure 13, the read-out of the pentamer **BB5** is depicted as an example (measurement performed without additive, $[M+H]^+$ at 1460.9759 *m/z*, NCE 18). Furthermore, the read-out of the heptamer **BB7** was successfully demonstrated and is displayed in chapter 6.3.2.2.2. The analysis of the backbone defined molecules revealed the same fragmentation patterns, independent of the used monomer. In measurements without additive, the fragmentation next to the carbonyl was identified to be the predominant one also in this case.

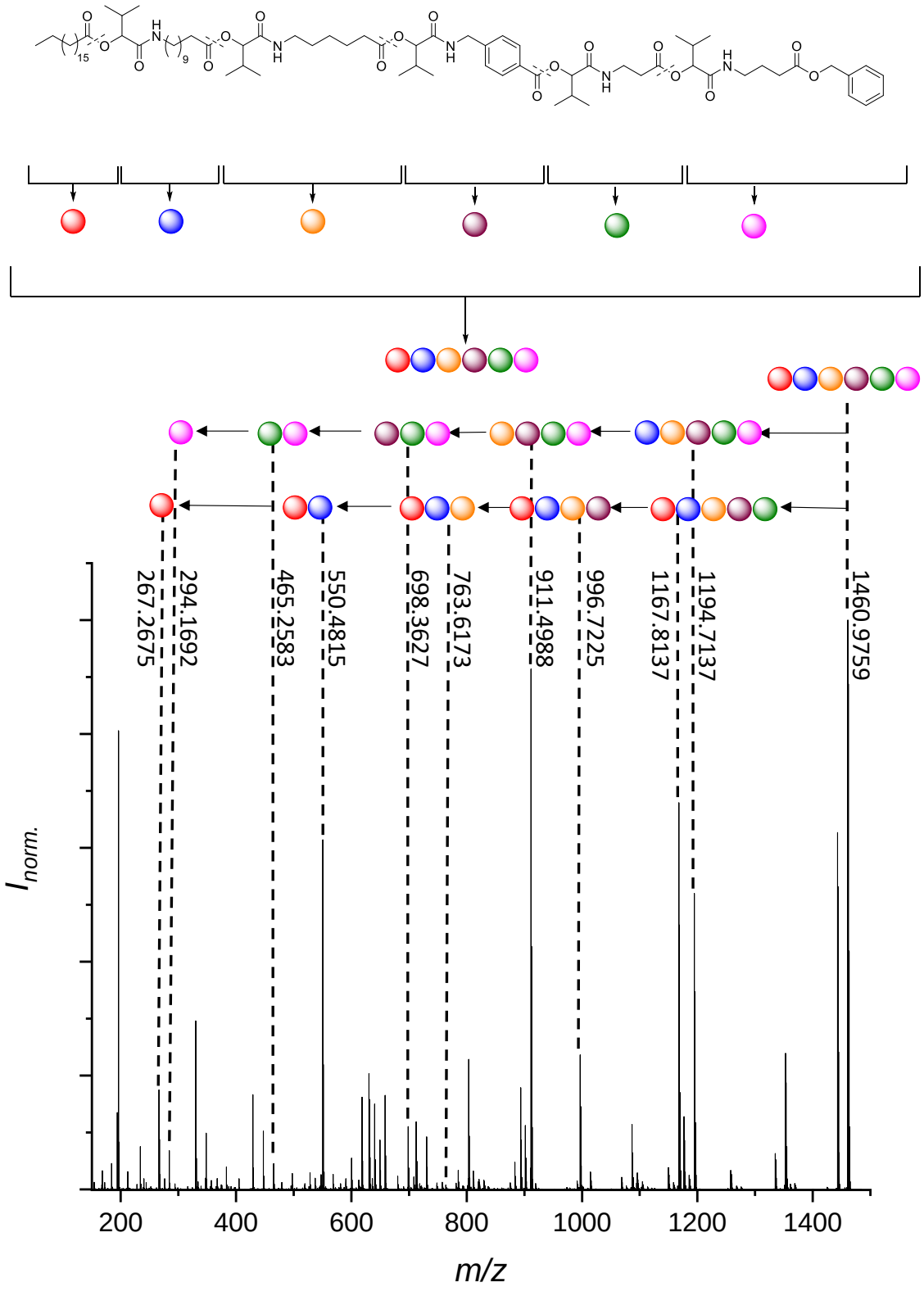


Figure 13: Fragmentation of the backbone defined pentamer **BB5** *via* ESI-MS/MS with an NCE of 18. In the spectrum, the read-out from both ends of the oligomer is shown, focusing on the fragmentation next to the carbonyl. By recombining the fragments, the initial structure can be re-established and thus the stored information is read.

Having gathered the information about fragmentation pathways of side chain and backbone defined macromolecules, the ESI-MS/MS measurement of the dual sequence-defined pentamer **DS5** was performed. Again, it was observed, that the fragmentation follows the same rules. With this important information, it was confirmed that the fragments of the dual sequence-defined molecules are not too complex for manual analysis.The read-out remains possible even though the molecular structure is significantly more complex. The most common fragmentation next to the carbonyl is displayed in Figure 14 and the fragmentation next to the ester is shown in Figure 15.

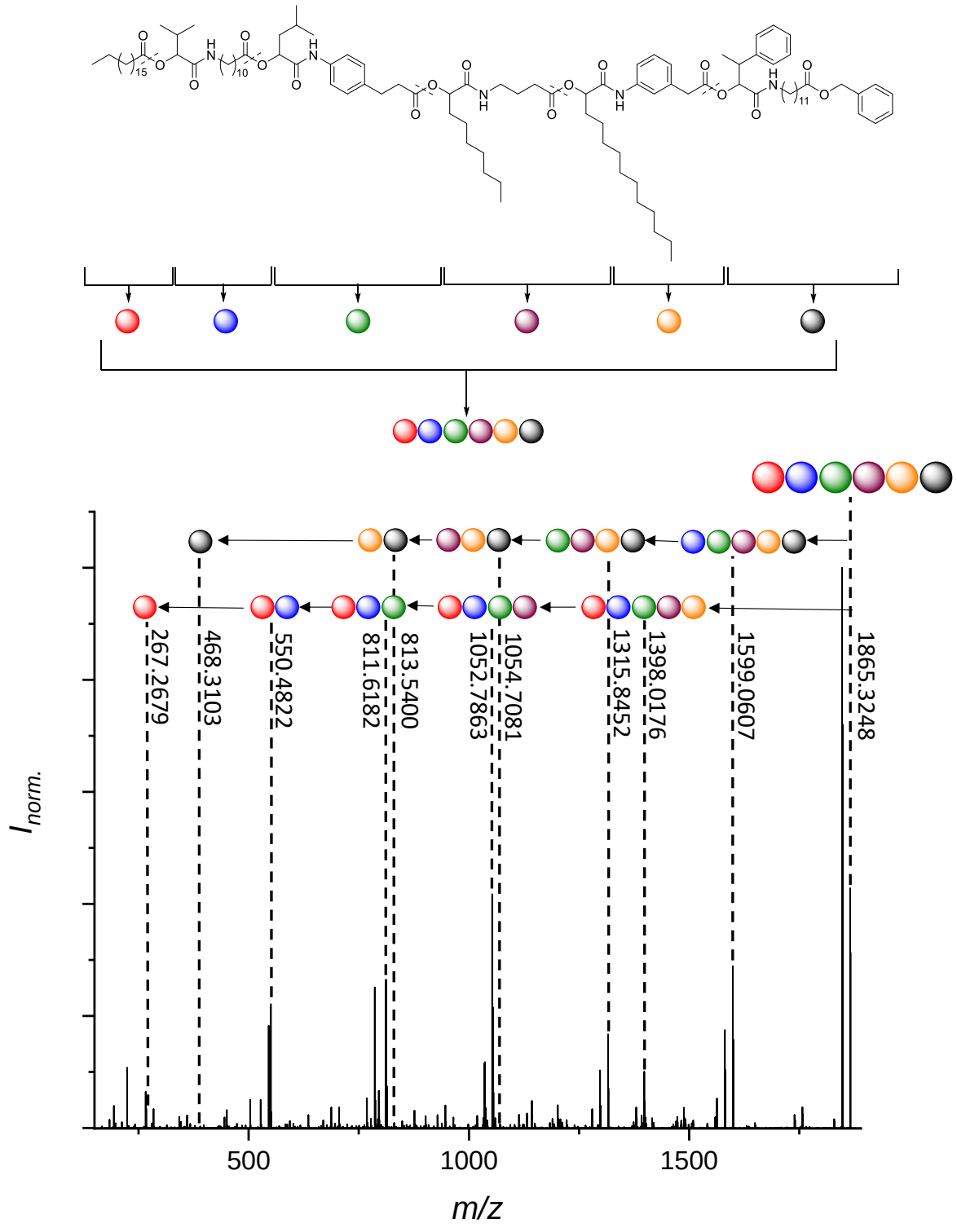


Figure 14: Fragmentation of the dual sequence-defined pentamer **DS5** *via* ESI-MS/MS with NCE of 18. In the spectrum, the read-out from both ends of the oligomer is shown, focusing on the fragmentation next to the carbonyl. By recombining the fragments, the initial structure can be re-established and thus the stored information can be read.

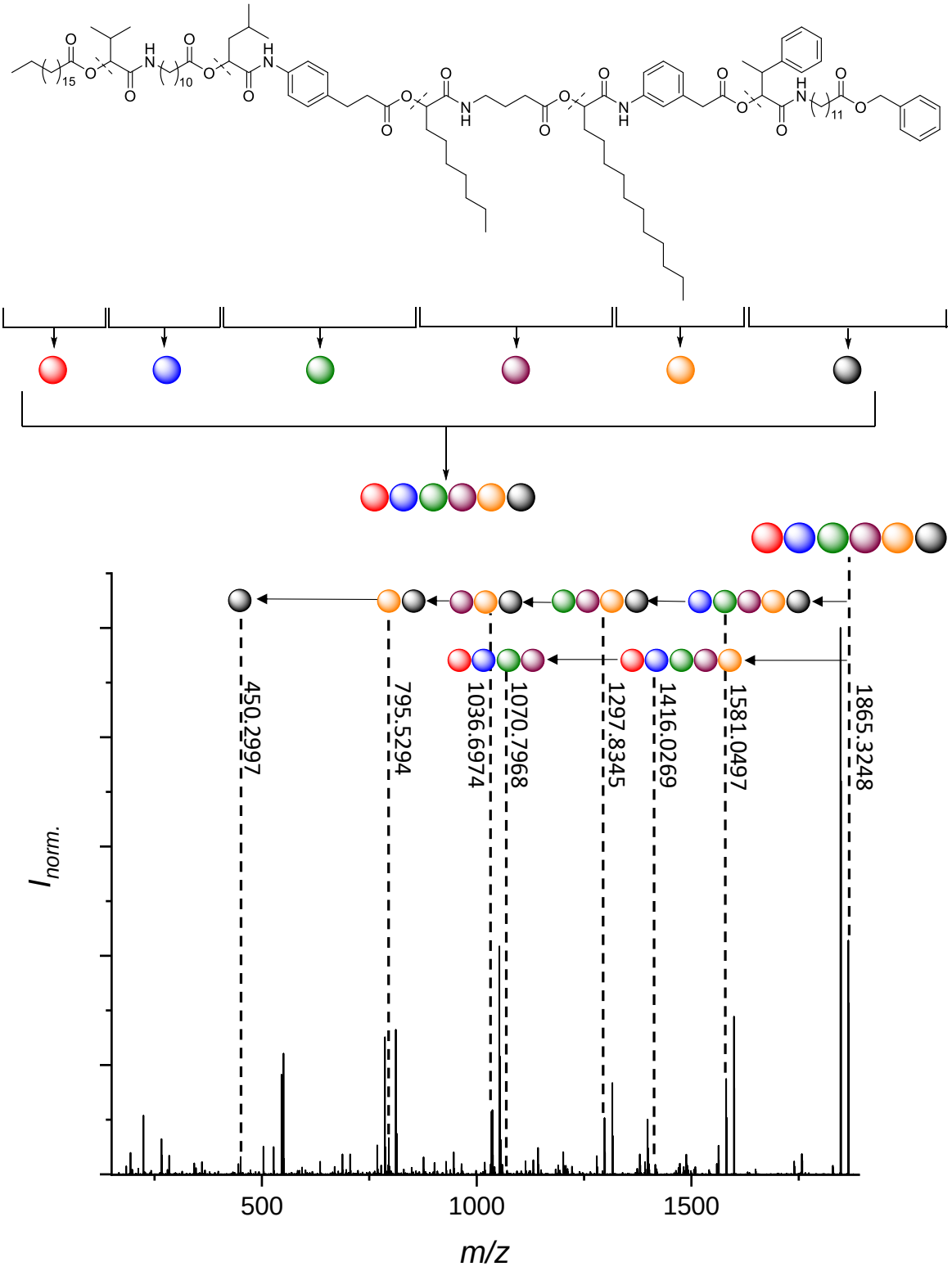


Figure 15: Fragmentation of the dual sequence-defined pentamer **DS5** *via* ESI-MS/MS with NCE of 18. In the spectrum, the read-out from both ends of the oligomer is shown, focusing on the fragmentation next to the ester. By recombining the fragments, the initial structure can be re-established and thus the stored information can be read.

As shown in Figure 14 and Figure 15, the fragmentation of the pentamer **DS5** follows the discussed distinct rules: By recombining the masses of the fragments, it was possible to re-establish the initial structure. Both types of fragmentation can be read similarly, thus allowing for error correction. First, the start and end fragments were identified. Then, the masses of possible middle fragments were added which includes the respective backbone and side chains of the Passerini moiety. Thus, it was possible to calculate the mass of the initial molecule. The masses of the fragments were calculated with Equation 1 (for further information see chapter 6.3.3.5.6).

$$[M_{Molecule} + H]^+ = \left[\left(M_{Start} + \sum_{i=1}^{i=n} M_{Backbone}^{i} + \sum_{i=1}^{i=n} M_{Sidechain}^{i} + M_{End} + y \times M(H)\right) + \mathrm{H}\right]^+$$

Equation 1

With Equation 1, the mass of the molecule and the masses of the expected fragments were calculated and then tracked in the ESI-MS/MS spectrum, thus allowing for the successful read-out of the molecule. With the identification of two significant fragmentation patterns, one next to the carbonyl (see Figure 16a) and one next to the ester group (see Figure 16b), error correction was possible by applying both fragmentation patterns for one molecule independently. It was also possible to assign the middle fragments of the molecules in the ESI-MS/MS spectra; however, for the sake of clarity this is displayed in a separate spectrum (see 6.3.2.3.2). The middle fragment is created when the fragmentation takes place on both ends of the Passerini product. For example, at the starter moiety (first Passerini unit) and the end (last Passerini unit).

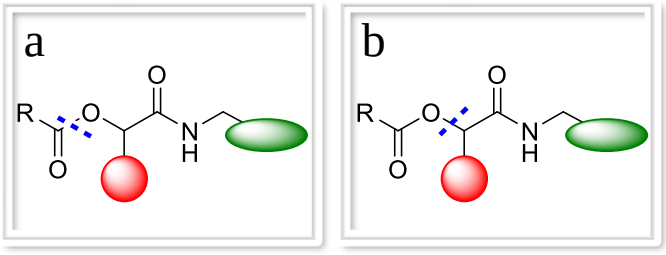


Figure 16: Most common fragmentation patterns of the oligomer during fragmentation *via* ESI-MS/MS. **a.** Fragmentation next to the carbonyl, which is favorable in measurements without additives. **b.** Fragmentation next to the ester group is preferred when sodium trifluoroacetate is used as additive.

4.2.1.3 Comparison of the data storage capacity of different systems

Once the writing and read-out process was established, it was important to compare the maximal data storage capacity of the investigated oligomers with other common systems. Common information technology today is based on the binary code with "0"s and "1"s. For storing information in the binary code system, very long sequences are needed, because only 1 bit is stored in one repeating unit. A sequence of eight binary digits represents 1 byte, which is 8 bit or 2^8 and that represent 256 permutations. The number of permutations is an important benchmark in this context and can be calculated as shown in Equation 2. From Equation 2, it is obvious that the degree of oligomerization has a bigger influence on the number of permutations than the molecular diversity.

$$n_{permutationts} = \left(n_{variations\ per\ repeating\ unit}\right)^{Degree\ of\ Oligomerization}$$

Equation 2

The number of permutations can be translated into bit and byte by the following equations:

$$bit = \frac{\log\left(n_{permutations}\right)}{\log(2)}$$

Equation 3

and

$$8\ bits = 1\ byte$$

Equation 4

The naturally occurring DNA is an example for a quaternary system. It uses the four nucleobases adenine, guanine, cytosine, and thymine. With such a system, a tetramer achieves the same number of permutations as achievable with eight binary digits, since according to Equation 2 the number of permutations is calculated with variation per repeating unit = 4 and number of degrees = 4 to be 4^4 = 256 permutations. In Figure 17, the number of permutations of five different tetramers (binary, quaternary and side chain defined, backbone defined, dual sequence-defined) are compared. For the side chain defined tetramer, 11 possible and synthetically established side chains were used for the calculation, thus 11^4 = 14.641 permutations were obtained. In case of the backbone defined molecule, 9^4 = 6561 permutations were calculated with 9 possible and synthetically demonstrated backbones. And finally, for the dual sequence-defined tetramer, 9 backbone and 11 side chain possibilities were used and thus $(11*9)^4$ = 96.059.601 permutations were obtained. With the dual sequence-defined system, the

number of permutations can be significantly increased, because of the increasing molecular diversity.

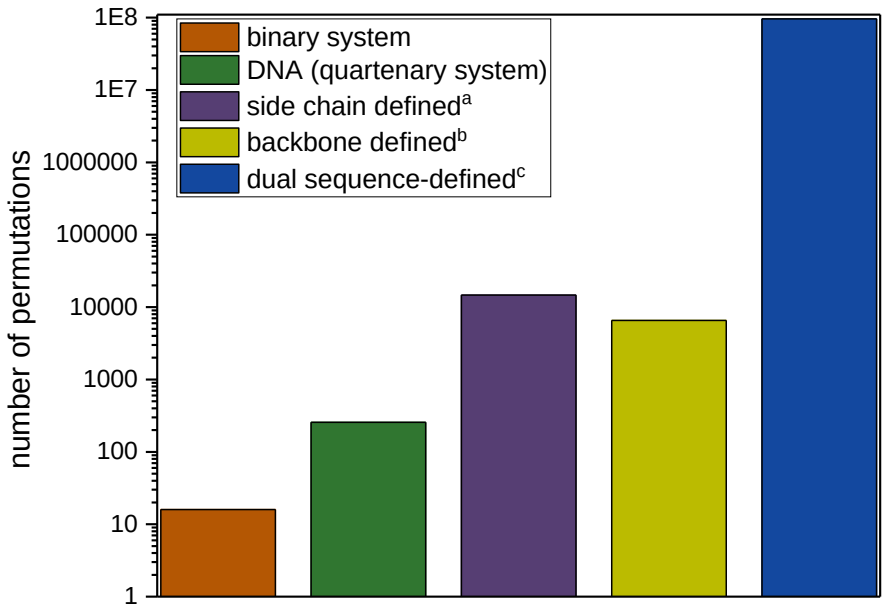


Figure 17: Comparison of the different data storage systems. Comparison of the artificial (*i.e.* binary system) as well as naturally applied data storage system (*i.e.* DNA) with the data storage system established and applied in this work. For all different systems, the number of permutations is calculated for a tetramer. [a] 11 possible side chains were used for the calculation, [b] 9 possible backbones were used for the calculation, [c] 11 possible side chains and 9 possible backbones were used for the calculation. Please note the logarithmic scale.

In order to compare the influence of the sequence length and of the number of selectable functionalities per repeating unit on the number of permutations and thus on the data storage capacity, the different systems were compared. As can be seen in Equation 2, increasing the length of sequences has a more pronounced influence on the data storage capacity than increasing the chemical variety in each monomer. As shown by the collaboration partners, the synthesis of longer sequences can be challenging. In the synthesis of the backbone defined molecules for example, until now, the heptamer **BB7** was the limit and in case of the dual sequence-defined molecules a pentamer **DS5** was achieved.[50] While elongating the molecules, it becomes more and more complicated to maintain a high purity. Increasing the chemical variety can help circumvent this synthesis problem, while maintaining excellent data storage capacity. With the help of dual sequence-definition, the synthetic effort could be reduced by half. The influence of the chemical variety can be demonstrated by an easy calculation example. For a pentamer with 5 possible side chains (5^5), 11.6 bit can be stored but by increasing the possible side chains to 10, (10^5) = 16.6 bit can be obtained. In case of the dual sequence-defined

molecule, 5 possibilities for the side chain and 5 possibilities for the backbone were used for the calculation and this results in $(5*5)^5$ = 23.2 bit, thus a significant increase in storage capacity.

In Table 3, the comparison of the herein discussed sequence-defined molecules (**SC5**, **SC10**, **BB5**, **BB7**, **DS5**) and their data storage capacity is summarized. With the dual sequence-defined pentamer **DS5**, it was possible to achieve 4.14 byte. With the decamer **SC10** on the other hand, 4.32 byte were achieved, but it needs to be noticed that the synthetic effort for **SC10** was twice as high as for **DS5**.

Table 3: Comparison of the demonstrated data storage capacity of side chain defined, backbone defined and dual sequence-defined molecules. The number of different degrees of definition must be considered.

	pentamer (SC5)[a]	**pentamer (BB5)[b]**	**pentamer (DS5)[c]**	**decamer (SC10)[a]**	**heptamer (BB7)[b]**
permutations	161.051	59.049	9.509.900.499	25.937.424.601	4.782.969
bit	17.30	15.85	33.15	34.59	22.19
byte	2.16	1.98	4.14	4.32	2.77

[a] for calculation 11 possible side chains, [b] for calculation 9 possible backbones, [c] for calculation 11 possible side chains and 9 possible backbones

The comparison of the different systems visualized the potential of dual sequence-defined macromolecules in the field of data storage. The monomers and side chains had to be chosen carefully. On one hand, they have to offer orthogonality and quantitative yields in the synthesis. On the other hand, they must show a distinct fragmentation pattern in the ESI-MS/MS spectra and the fragments must be distinguishable. Furthermore, the implementation of an automated program for the sequential read-out might become necessary for more complex structures. For long sequences, manual read-out is very time-consuming and will at some point become too complicated.

4.3 Reading mixtures of uniform sequence-defined macromolecules

In this section, the read-out of oligomer mixtures is described. In chapter 4.3, the synthesis of twelve tetramers and three hexamers with three different TAGs is described, followed by the development of the concept for the read-out of mixtures and the establishment of a computer program-based evaluation is discussed. In chapter 4.3.2, the question of how many different oligomers can be mixed together, so that the readout still remains possible, is discussed. The same synthetic concept is used and further oligomers with additional three different TAGs were synthesized. Furthermore, an automated read-out of these oligomers was established. In a systematic test series of up to six oligomers, each of them carrying a different TAG, were mixed together and a full read-out was demonstrated.

4.3.1 Reading mixtures of uniform sequence-defined macromolecules to increase data storage capacity

Parts of this chapter and the associated parts in the experimental part were published before: Reading mixtures of uniform sequence-defined macromolecules to increase data storage capacity, Maximiliane Frölich, Dennis Hofheinz & Michael A. R. Meier, *Communication Chemistry*, **2020**, *3*, 184.

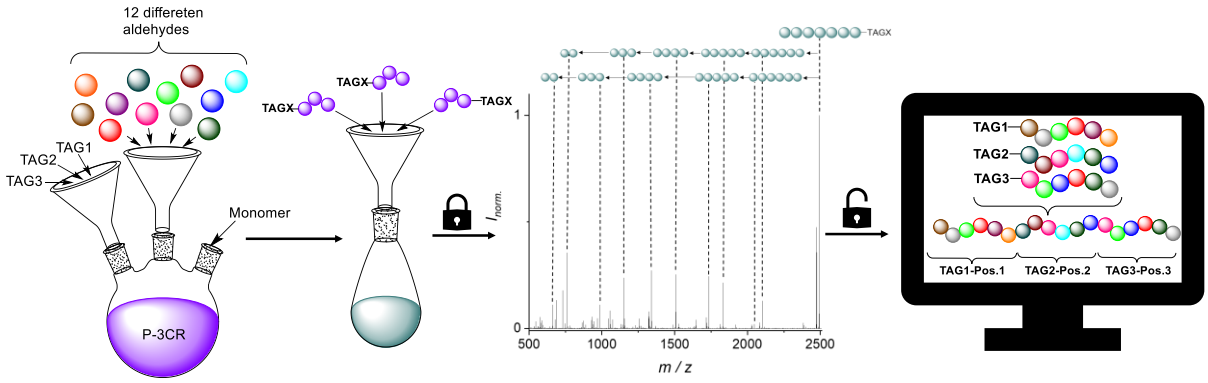
A part of the project has been started in the Master Thesis of the author (Maximiliane Frölich) and was continued during the PhD thesis. Some of the molecules were synthesized by Vertiefer- or Bachelor student under the co-supervision of Maximiliane Frölich, which are marked with footnotes in the experimental part. The computer program (python script) was written by Prof. Dr. Hofheinz, which is marked with footnotes in the experimental part.

Abstract

In recent years, the field of molecular data storage has emerged from a niche to a vibrant research topic. Herein, a simultaneous and automated read-out of data stored in mixtures of sequence-defined oligomers is described. Therefore, different sequence-defined oligomers (twelve tetramers and three hexamers) with varying mass markers and side chains were successfully synthesized via iterative Passerini three-component reactions and subsequent deprotection steps. By programming a straightforward python script for ESI-MS/MS analysis, it is possible to automatically sequence and thus, read-out the information stored in these oligomers within one second. Most importantly, the use of mass-markers as starting compounds eases MS/MS data interpretation and furthermore allows the unambiguous reading of sequences of mixtures of sequence-defined oligomers. Hence, high data storage capacity (up to 64.5 bit in our examples), considering the field of synthetic macromolecules, can be obtained without the need of synthesizing long sequences, yet by analyzing a mixture of shorter, sequence-defined oligomers.

4.3.1.1 Concept and synthesis

The data storage capacity of sequence-defined macromolecules directly correlates with the variation possibilities per repeat unit, *i.e.* the X in the X^Y notation of possible permutations, where X is the base describing the available different repeat units (commonly different side chains) and Y is the degree of oligomerization (for further information see 4.2). To achieve higher data storage capacity within sequence-defined macromolecules, either longer sequences need to be synthesized and subsequently read-out, as recently demonstrated for the longest sequences analyzed so far,[238] or the amount of data stored per repeat unit needs to be increased, as recently shown by our group for dual sequence-defined macromolecules.[50] Both approaches are challenging and laborious while concomitantly synthetically limited. For instance, the practically accessible degree of polymerization (DP) will suffer from lower yields exponentially as it increases even considering high yields in each iterative cycle, which were demonstrated for the approaches discussed in chapter 2.2.5, (assuming the yield of each iterative cycle (P-3CR plus deprotection) to be 90%, the overall yield of a 20-mer would be only 12%). However, this is not necessarily an issue, at least for data storage applications, if the sequence is established by other means (Scheme 31).



Scheme 31: Concept of the automated read-out of a mixture of sequence-defined molecules by varying twelve different aldehydes and specifically designed mass markers (TAGs). Iterative step synthesis with the P-3CR, using twelve different aldehydes and three different TAGs. The aldehydes can be introduced at any desired position of the oligomer and provide the sidechains of the macromolecule and thus differentiate each repeating unit. Subsequently, the individual sequences of an oligomer mixture can be analyzed *via* ESI-MS and ESI-MS/MS, followed by fully automated read-out in silico with a clearly defined position of the TAGs.

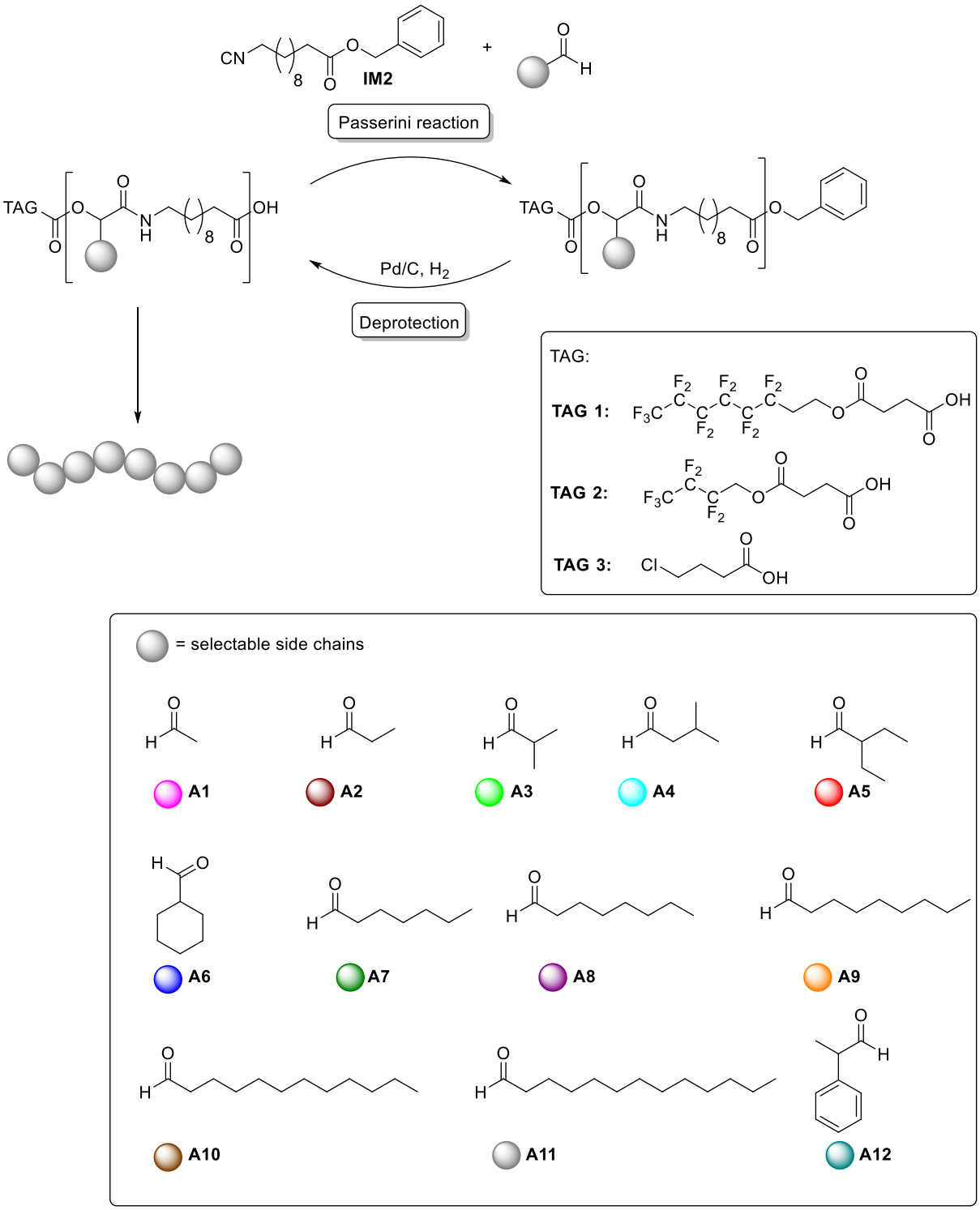
Here, molecular tags suitable for an unambiguous identification and distinction between different oligomers by high resolution MS were used. These molecular tags are used to construct a nominally longer sequence (*i.e.* **TAG1** defines position 1 in a virtually higher DP oligomer, and so on), based on the notion that the data storage capacity of three different

hexamers is the same as that of an 18-mer, however without the associated strenuous synthesis. Thus, to achieve our goal of a simultaneous analysis of mixtures of sequence-defined oligomers, the position of these specifically designed mass markers (TAGs) must be clearly defined for the read-out of the mixture. Therefore, **TAG1** is utilized to define position 1, **TAG 2** defines position 2, and in a similar fashion for **TAG3**. The herein employed TAGs were halide bearing carboxylic acids for use in an initial P-3CR. **TAG3**, a monochlorinated carboxylic acid, was commercially available, while the two perfluorinated carboxylic acids (**TAG1** and **TAG2**) were synthesized in one-step syntheses as described in chapter 6.3.3.2, 0. The three different **TAGs** were selected to increase the molar mass difference of the respective molecules to allow their distinction in mixtures during MS-experiments. Furthermore, the individual halogenated **TAGs** impart the molecule a characteristic isotopic pattern, unique for each **TAG**, allowing an unambiguous MS assignment. The three different **TAGs** were selected to increase the molar mass difference of the respective molecules to allow distinguishing molecules in mixtures during MS-experiments. Furthermore, the selection of halogenated **TAGs** provides the molecule a characteristic isotopic pattern, unique for each **TAG**, that allows to unambiguously assign the mass of an investigated oligomer to a certain **TAG**. Interestingly, the group of Du Prez recently reported the use of halogenated **TAGs** to write a pin code.[10] There, a mono-chlorinated, a mono-brominated, and a di-brominated indole were used, in addition to a nonhalogenated indole. By using ESI-MS and the specific isotopic pattern, it was possible to carry out the read-out without tandem mass analysis. Herein, for example the perfluorinated **TAG2** and chlorinated **TAG3** can be easily distinguished due to the characteristic isotopic pattern of the chlorinated **TAG** in the high resolution ESI-MS spectrum. In fact, by increasing the molecular weight of the investigated molecules, the specific isotopic pattern of the Cl-marker cannot be resolved anymore. However, the fragmentation *via* ESI-MS/MS results in smaller molecular fragments, where the characteristic pattern of the Cl can be found again, which allows to distinguish the TAGs. Additionally, the high mass difference between the **TAGs** makes them distinguishable. For the proof of concept, the mass differences between the perfluorinated **TAG1** and **TAG2** were sufficient (164 g*mol^{-1}) to distinguish the two TAGs. This would also be possible with other commercially available acids, like stearic acid, but the use of perfluorinated acids was preferred because of the simplified workup as demonstrated previously. For a mixture of three oligomers with **TAG1-3**, the mass differences, and the isotopic pattern of the chlorinated **TAG** allowed a read-out of the oligomers. For a mixture of more than three molecules, it would be highly beneficial to introduce **TAGs** with another

characteristic and different isotopic pattern, for instance a brominated **TAG**. This will be discussed in chapter 4.3.2.

4.3.1.2 Oligomer synthesis

For the oligomer synthesis, a stepwise iterative approach based on the P-3CR and a subsequent hydrogenolytic deprotection were carried out (see Scheme 32).[51] With this synthesis protocol, a variety of aldehydes **A1**-**A12** can be used to introduce different sidechains.[50,51,219] Furthermore, different repeating units can be introduced to the defined oligomers at predefined positions. This synthesis procedure and the isocyanide synthesis are well established in the group and been demonstrated also in 4.1, 4.2. Therefore, isocyanide **IM2** with a benzyl ester protected acid group was synthesized in a three step synthesis (see 6.3.3.1), with an overall yield of 41% in a 15 g scale.[51] Using this approach, twelve aldehyde derivatives were carefully selected (see Scheme 32) to be reacted with **IM2** to provide side chain variation and to allow the simplified read-out of the sequence by tandem mass spectrometry. In addition, aldehyde derivatives were omitted that potentially yield identical mass fragments. The aldehydes can be introduced at any desired position of the sequence, which we demonstrated in the synthesis of the oligomers **T1/1**-**T3/4**. Consequently, the number of the aldehydes represents the freely selectable repeating units at each position of the sequence-defined oligomers.

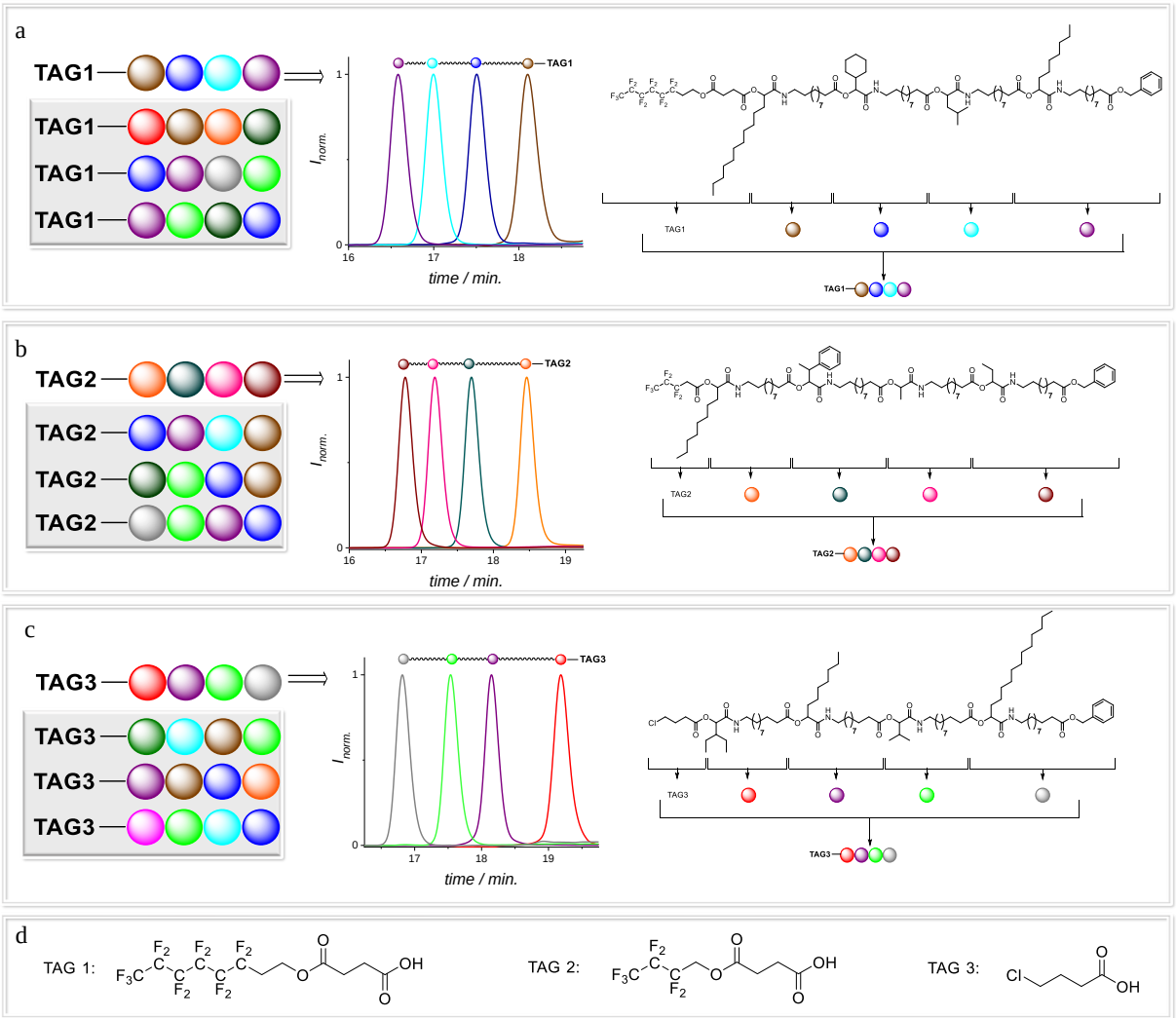


Scheme 32: Iterative step approach with a P-3CR and afterwards a deprotection *via* hydrogenolysis to obtain sequence-defined oligomers with tailorable side chains and.[51] A carboxylic acid with **TAG 1-3** as moiety is used as the starting component for the chain**.**

4.3.1.3 Tetramer synthesis

A library of twelve tetramers **T1/1-T3/4** (see Figure 18, Figure 19, Figure 20) (in total four tetramers per **TAG**) were prepared in high purity (97-99% determined by SEC). After each

reaction step of the tetramer synthesis, the products were thoroughly characterized using proton and carbon NMR, SEC, mass spectrometry and infrared spectroscopy (IR) (see 6.3.3). A detailed discussion on the applied Passerini synthesis protocol, including ^{1}H-NMR and the deprotection, is provided in the following chapter 4.3.1.4. One selected example of a tetramer for each **TAG1-3** is shown in Scheme 33, illustrating SEC data after each iterative cycle of the oligomer synthesis. Furthermore, the side chain variation of the twelve aldehydes **A1-A12** within the library of the twelve tetramers **T1/1-T3/4** are visualized by the different colors of the spheres in Scheme 33.



Scheme 33: Schematic representation of the variation of the twelve different aldehydes (colored spheres) and SEC traces of three different tetramers, one for each tag exemplarily. **a.** Chemical structure and SEC traces of **T1/2** with **TAG1** and the sidechain variation of the aldehydes for **T1/1**, **T1/3**, **T1/4**. **b.** Chemical structure and SEC traces of **T2/1** with **TAG2** and the sidechain variation of the aldehydes for **T2/2**, **T2/3**, **T2/4**. **c.** Chemical structure and SEC traces of **T3/1** with **TAG3** and the sidechain variation of the aldehydes for **T3/2**, **T3/3**, **T3/4**. **d.** Chemical structures of **TAG1-3**.

The SEC traces clearly demonstrate the successful synthesis and high purity (see 4.1) of the prepared oligomers in a scale of 45 mg up to 2 g, respectively. A comprehensive overview of all TAG labelled oligomers **T1/1-T3/4** and the respective SEC traces of each P-3CR stage after chromatographic purification is shown in Figure 18, Figure 19, Figure 20.

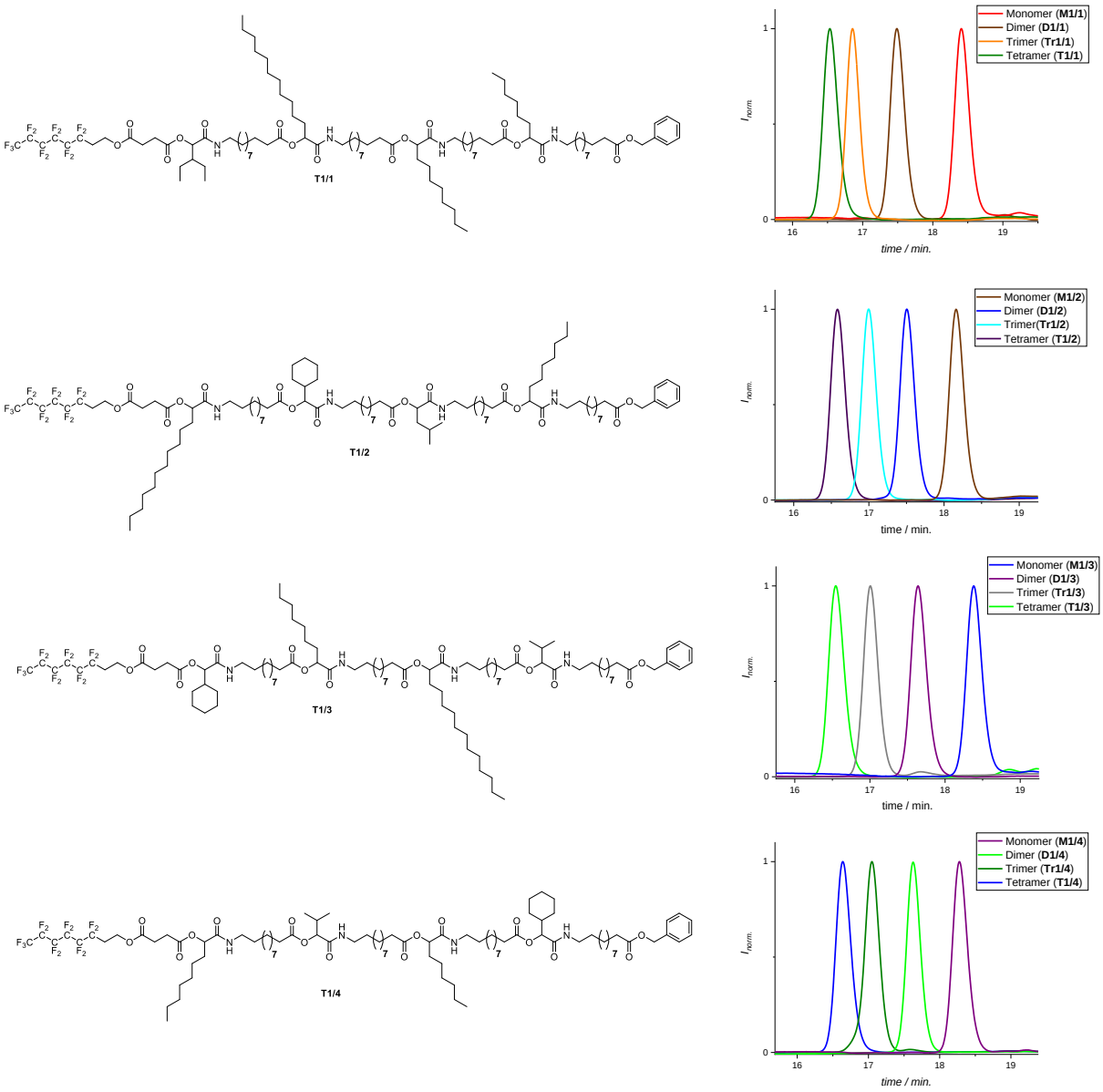


Figure 18: Illustration of the Passerini tetramers **T1/1-T1/4** with **TAG1**. The associated SEC traces of each Passerini reaction according to color code of the aldehyde employed in the reaction.

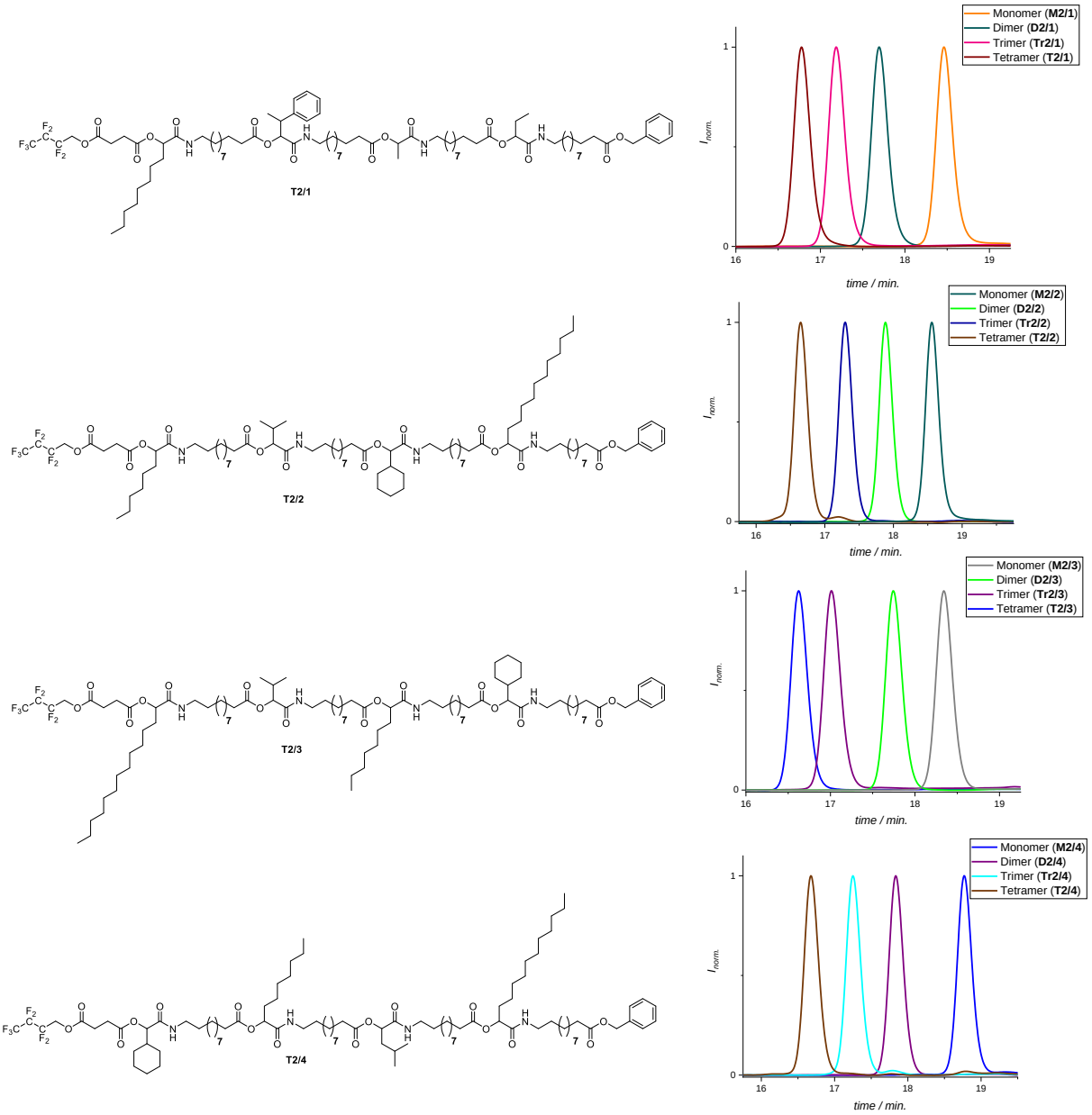


Figure 19: Illustration of the four Passerini tetramers **T2/1-T2/4** with **TAG2**. The associated SEC traces of each Passerini reaction are shown with the colored indication of the aldehyde which is used in this reaction step.

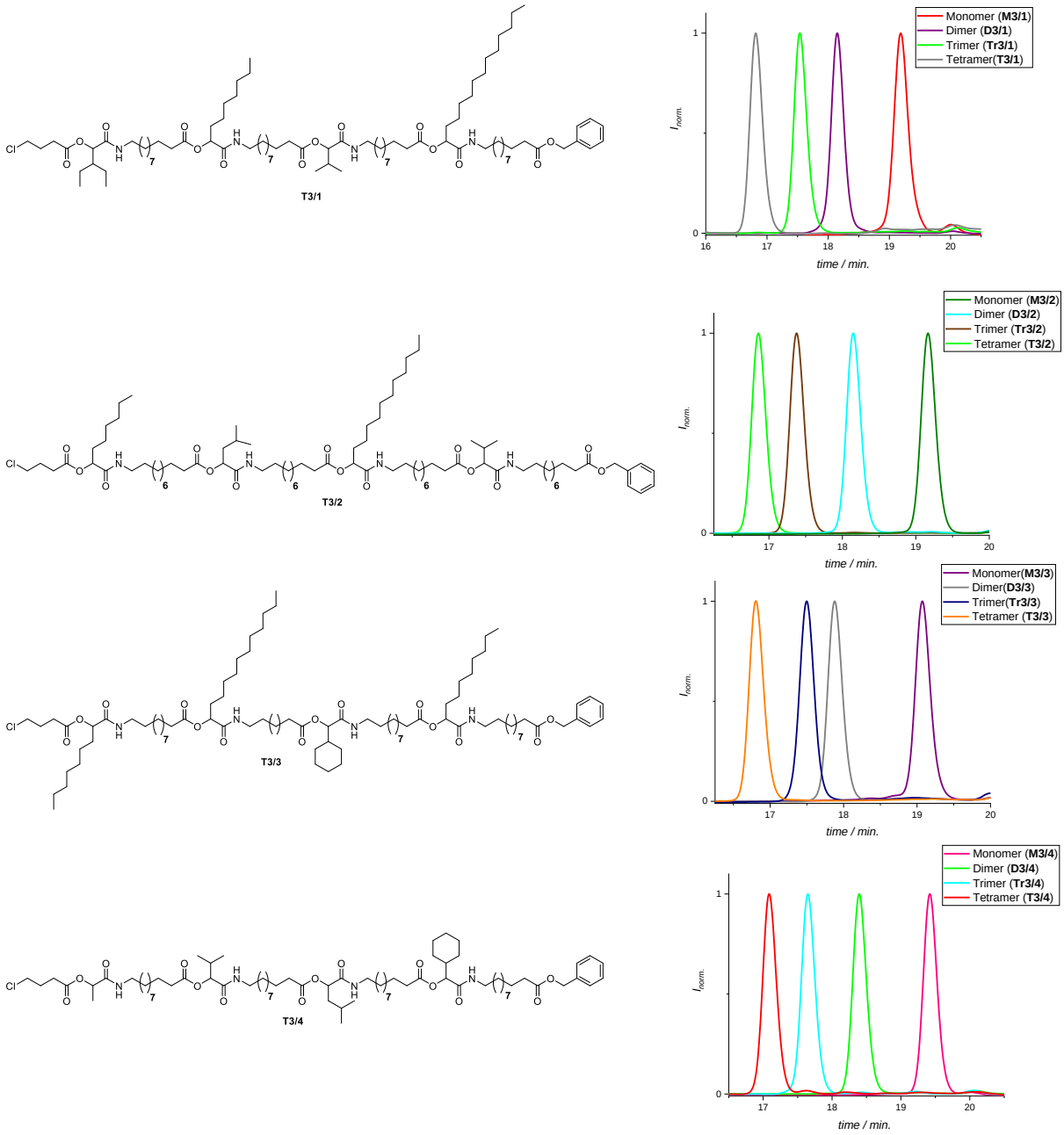
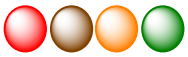
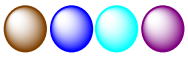
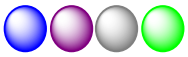
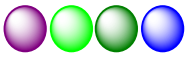
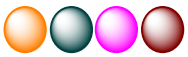
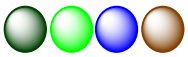
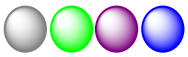
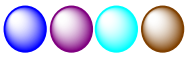
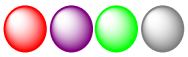
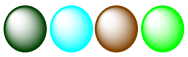
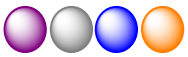
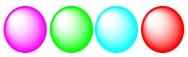


Figure 20: Illustration of the four Passerini tetramers **T3/1-T3/4** with **TAG3**. The associated SEC traces of each Passerini reaction is shown with the colored indication of the aldehyde which is used in this reaction step.

All tetramers **T1/1-T3/4** were obtained in high purity (99-97%, determined by SEC, refer to Table 4). Please note that the SEC instrumentation involved in this study has an impurity detection threshold of 2%.[210] The high purity and molecular integrity of the oligomers were also confirmed by mass spectrometry and ^{1}H and ^{13}C-NMR.

Table 4: Summary of the purity and overall yield of the tetramers **T1/1-T3/4**, the order of the incorporated aldehydes (visualized by color code) and used **TAGs**.

TAG	Tetramer	Overall yield [%][a]	Purity [%][b]	Aldehydes[c]
TAG1	**T1/1**	78	99	
TAG1	**T1/2**	62	99	
TAG1	**T1/3**	11	99	
TAG1	**T1/4**	56	99	
TAG2	**T2/1**	58	99	
TAG2	**T2/2**	40	97	
TAG2	**T2/3**	47	99	
TAG2	**T2/4**	46	99	
TAG3	**T3/1**	64	99	
TAG3	**T3/2**	28	99	
TAG3	**T3/3**	64	99	
TAG3	**T3/4**	35	97	

[a] after seven reaction steps, [b] confirmed by SEC, [c] marked with the color code

In order to gather information on the fragmentation patterns, each of the twelve oligomers with different sidechains and TAGs was first analyzed by tandem ESI-MS/MS. The manual analysis of the MS/MS results was important as we needed to ensure that the herein used set of aldehydes did not produce overlapping mass fragments, that would hinder the unambiguous assignment of all peaks. Furthermore, we sought to ensure that all oligomers showed the same fragmentation patterns, independent of side groups or TAGs. The gained information was later used to write a program for a significantly accelerated, automated, and simplified read-out of

the sequence-defined molecules, as described below. Thus, the program was written using the gained information about the fragmentation pattern. The storage capacity of one of the described tetramers can be calculated as follow taking twelve possible side chains into account: resulting in $(12)^4$ = 20.736 permutations, relating to 14.3 bit according to Equation 6.

4.3.1.4 Increasing data storage capacity by synthesizing hexamers

To further increase data storage capacity, three sequence-defined hexamers **H1/1-H3/1**, each of which carried one of the different **TAGs**, were then synthesized. These were later also used to demonstrate the readability of mixtures of different oligomers. The reaction steps and a short cut of the characterization of the hexamers **H1/1-H3/1** are described in the following parts.

The syntheses of hexamers **H1/1** P-3CR is discussed more in detailed. First, Passerini product **M1/1** was prepared and subsequently deprotected to the carboxylic acid **MD1/1**. The respective synthesis and the characterization of the mono, di, trimers, etc. are then iterative, using a different aldehyde in each Passerini step (refer to section 6.3.3 for comprehensive synthesis and characterization). For the synthesis of the first hexamer **H1/1**, **TAG1** was used as starting acid. The isocyanide **IM2** and 2-ethylbutyraldehyde **A5** were used in a small excess relative to the carboxylic acid **TAG1** (see Figure 21).

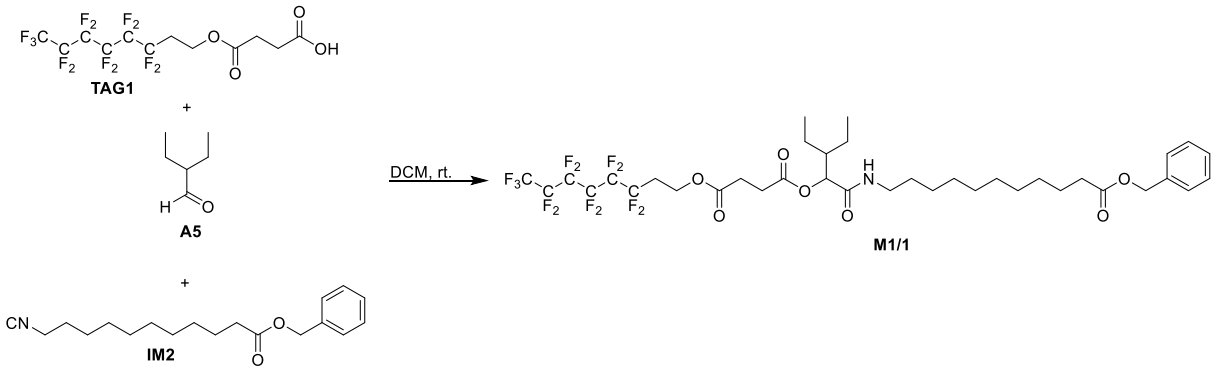


Figure 21: P-3CR with **TAG1** as carboxylic acid, isocyanide **IM2** and 2-ethylbutyralehyde **A5** stirred at room temperature and DCM as solvent.

The reaction was stirred at room temperature for one day and after purification *via* column chromatography, the Passerini product **M1/1** was obtained in a yield of 96% and high purity (≥99%). The successful synthesis of **M1/1** were confirmed by ^{1}H-, ^{13}C-NMR, IR and mass spectrometry (see Figure 23). The first deprotection of the Passerini product **M1/1** was performed by hydrogenation using EA/ THF (1:1) as solvent and 20 wt.% of the heterogeneous catalyst palladium on activated carbon **1** (see Figure 22). The reaction was purged with

hydrogen and stirred under hydrogen atmosphere overnight. The deprotected oligomer **MD1/1** was purified *via* filtration over celite® to remove the heterogeneous catalyst and obtained in quantitative yield.

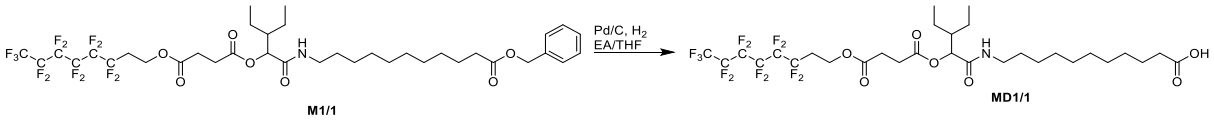


Figure 22: Hydrogenolytic deprotection of the Passerini product **M1/1** with palladium on activated carbon **1** and hydrogen to yield the deprotected Passerini product **MD1/1**.

In Figure 23, the comparison between the ^{1}H-NMR spectra of the protected Passerini monomer **M1/1** (top, marked in purple) and deprotected Passerini monomer **MD1/1** (bottom, marked in green) is shown. The relevant signals of the Passerini product of the amide (signal 2), at around 6.46 ppm, **TAG1** (signal 5, 7, 8), and the doublet of the CH proton of aldehyde sidechain (signal 3) at 5.32 ppm remain unchanged. The full deprotection of the Passerini product **M1/1** can be confirmed by the resonances at 5.32 and 7.41-7.30 ppm. In the aromatic range of 7.41-7.30 ppm the resonances of the aromatic protons (marked in blue, signal 1) disappeared as well as the CH_2-group of the benzyl ester (marked in red, signal 4) at 5.32 ppm.

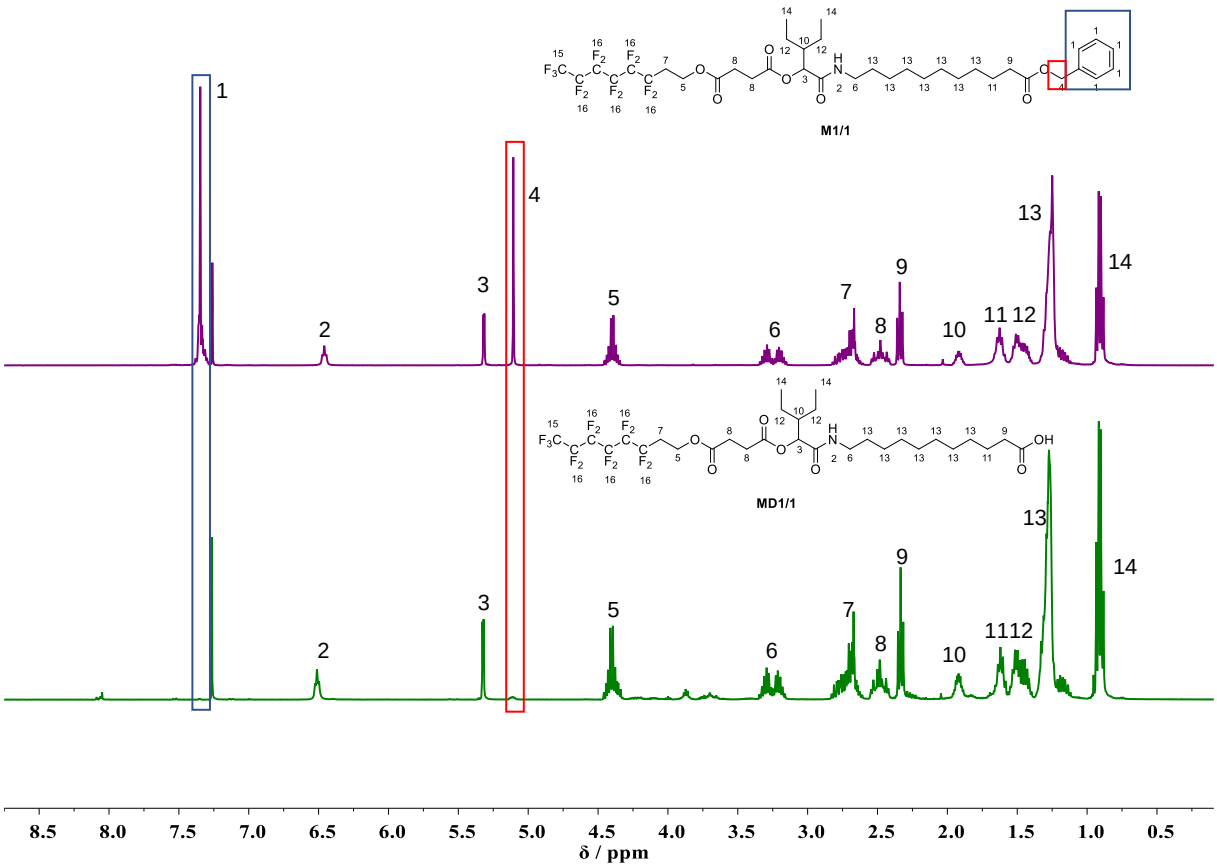


Figure 23: Comparison of the ^{1}H-NMR spectra of the protected **M1/1** (purple) and unprotected **MD1/1** (green) Passerini product: The disappearance of the resonances of the phenyl group (blue box) and the CH_2 signal of the benzyl ester (red box) is highlighted, measured in $CDCl_3$.

The deprotected Passerini product **MD1/1** was then used in further iterative reaction cycles. For the further cycles, different aldehydes were inserted in the following order: dodecanal **A10**, nonanal **A9**, heptanal **A7**, propionaldehyde **A2** and isobutyraldehyde **A3**. The reaction times were increased up to six days, since the reaction rate decreased with higher DPs, presumably due to declining solubility in the reaction medium.[26] After eleven steps, the hexamer **H1/1** was obtained in an overall yield of 58%. All intermediates and final products were fully characterized, see 6.3.3.3.2 for further information. In Figure 24, the chemical structure of the hexamer with **TAG1**, the SEC traces after each P-3CR and the high-resolution isotopic pattern obtained by ESI-MS compared with the calculated isotopic pattern are shown. The SEC traces verify the high purity of the product (≥99%). Furthermore, the comparison of the isotopic pattern confirms the successful synthesis of the **H1/1**.

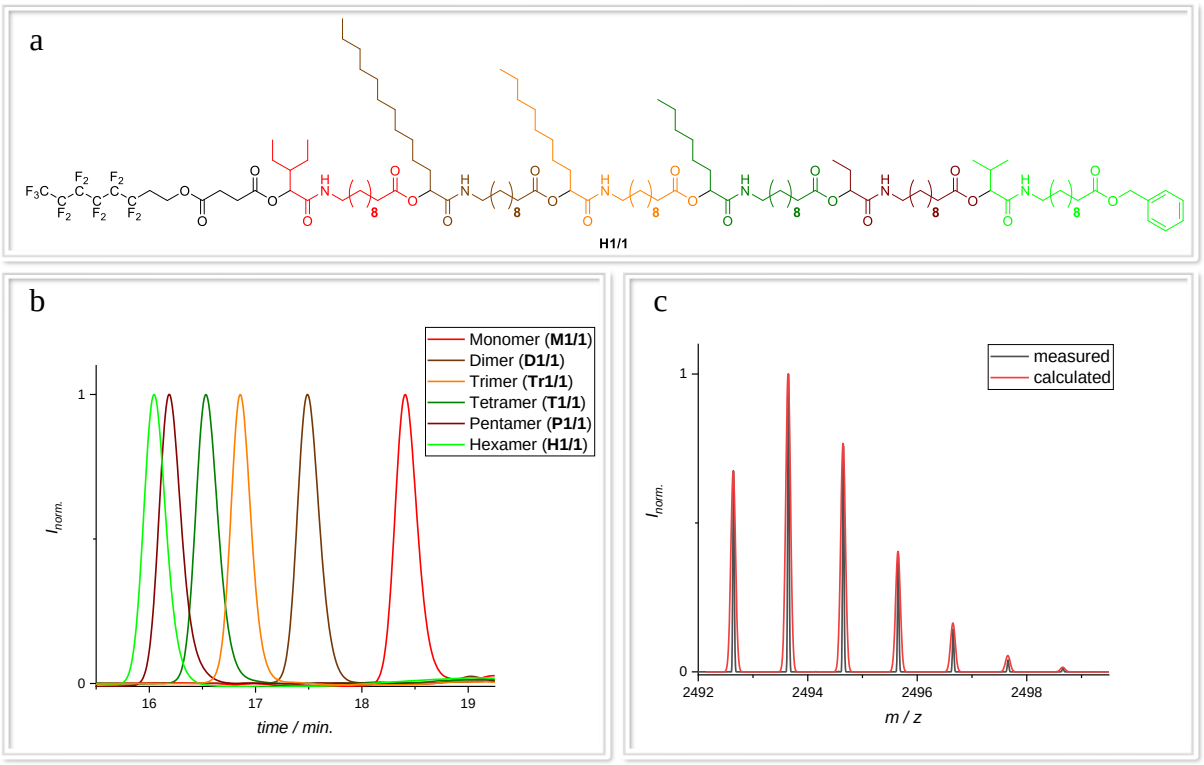


Figure 24: Characterization of the sequence-defined hexamer **H1/1** with **TAG1**. **a.** Chemical Structure of the sequence-defined hexamer **H1/1**. **b.** SEC traces of each P-3CR product. Each unit with its specific aldehyde moiety is colored with the same color of the respective SEC-traces. **c.** An overlay of the calculated isotopic pattern of - resolution ESI-MS measurement of **H1/1**; calculated isotopic pattern (red) and measured isotopic pattern (black).

In Figure 25, the ^{1}H-NMR spectrum of hexamer **H1/1** is depicted, and all significant signals were assigned. Again, the resonances of the benzyl ester can be observed at 7.38-7.28 ppm for the phenyl group (signal 1) and at 5.10 ppm for the CH_2-group of the benzyl ester (signal 4). The formation of the amide protons (signal 2) at a ppm range of 6.48 and 6.10– 5.93 indicate the successful formation of the Passerini product. The CH protons (signals 3) further confirm the formation of the Passerini product **H1/1**.

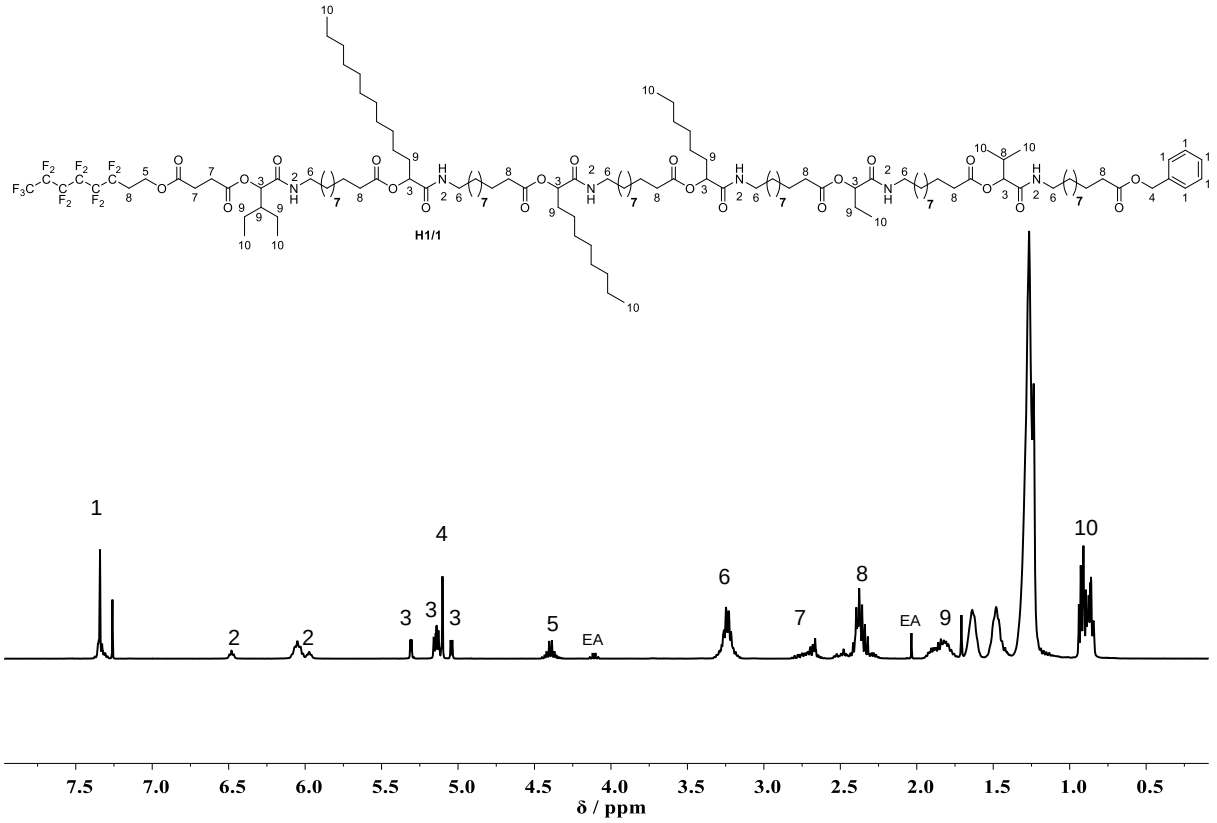


Figure 25: ^{1}H-NMR spectrum of hexamer **H1/1**: Measured in $CDCl_3$, all characteristic signals were assigned and marked in the spectrum and the molecule. Neglectable residuals of ethyl acetate (EA) were highlighted.

For the synthesis of hexamer **H2/1**, **TAG2** was used as the starter acid. Aldehydes were incorporated in the following order: Nonanal **A9**, 2-phenyl-propionaldehyde **A12**, acetaldehyde **A1**, propionaldehyde **A2**, tridecanal **A11**, and dodecanal **A10**. The yields of each individual step for hexamer **H2/1** are listed in Table 5. After eleven steps, the hexamer **H2/1** was obtained in an overall yield of 11%. The yield was significantly lowered due to less efficient separation of the crude mixture in column chromatography in comparison to **H1/1**. Especially in the 4th, 5th, and 6th P-3CR, the column chromatography was challenging to yield the products in a purity of ≥99%. With the SEC traces in Figure 26, the high purity of **H2/1** with ≥99% and all steps of the P-3CR were confirmed. Also, the comparison of the isotopic pattern confirms the successful synthesis of the **H2/1**.

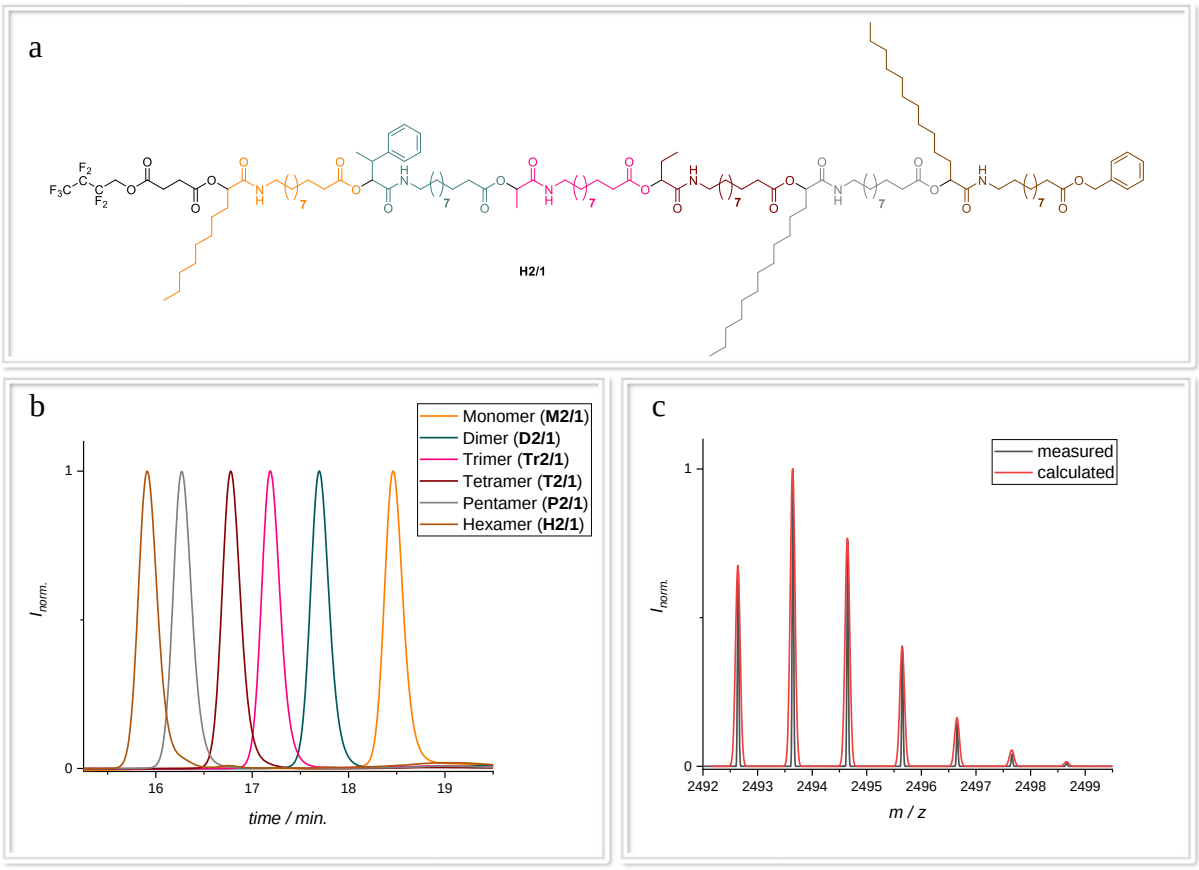


Figure 26: Characterization of the sequence-defined hexamer **H2/1** with **TAG2**. **a.** Chemical Structure of the sequence-defined hexamer **H2/1**. **b.** SEC traces of each P-3CR product. Each unit with its specific aldehyde moiety is colored with the same color of the respective SEC-traces. **c.** An overlay of the calculated isotopic pattern of -resolution ESI-MS measurement of **H2/1**; calculated isotopic pattern (red) and measured isotopic pattern (black).

Ultimately, hexamer **H3/1** was synthesized with **TAG3** as starter carboxylic acid using an identical synthesis protocol. Aldehydes were incorporated as sidechains in the following order: 2-ethylbutyraldehyde **A5**, heptanal **A7**, isobutyraldehyde **A3**, tridecanal **A11**, 2-phenyl-propionaldehyde **A12**, acetaldehyde **A1**. After eleven steps, hexamer **H3/1** was synthesized in an overall yield of 33%. In Table 5, the yields of each reaction step for the synthesis of **H3/1** are listed. Except for the ^{6th} P-3CR reaction step, all yields were high (≥87%), as expected. The moderate yield of the ^{6th} P-3CR can be explained by the difficult purification *via* column chromatography. Careful isolation of the product yielded a pure product, as observed by SEC. The successful synthesis of **H3/1** were confirmed by ^{1}H-, ^{13}C-NMR, IR and mass spectrometry (see 6.3.3.5.2). Furthermore, the purity of **H3/1** and all Passerini products (**M3/1-H3/1**) were determined by the SEC in a high purity of ≥99%. Furthermore, the comparison of the calculated and measured isotopic pattern confirms the successful synthesis (see Figure 27).

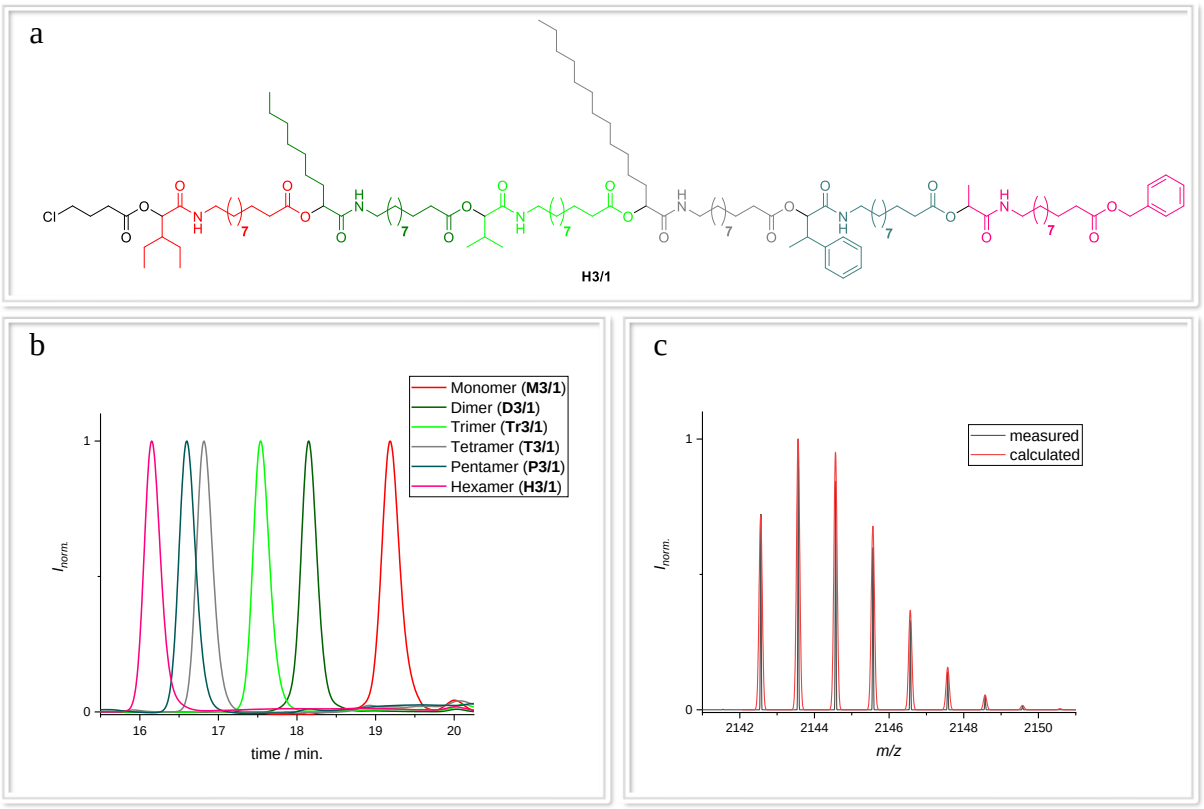


Figure 27: Characterization of the sequence-defined hexamer **H3/1** with **TAG3**. **a.** Chemical Structure of the sequence-defined hexamer **H3/1**. **b.** SEC traces of each P-3CR product. Each unit with its specific aldehyde moiety is colored with the same color of the respective SEC-traces. **c.** An overlay of the calculated isotopic pattern of **H3/1** with the obtained pattern *via* high-resolution ESI-MS measurement of **H3/1**; calculated isotopic pattern (red) and measured isotopic pattern (black).

In Table 5, a comparison of the hexamer **H1/1-H3/1** synthesis is displayed as an overview of the used aldehydes and yield of the reaction's steps. Up to now, it was not possible to indicate any trend, that the yield might on the used aldehyde. For example, dodecanal **A10** is used in a ^{2nd} P-3CR with a yield of 96%, compared to a ^{6th} P-3CR with a yield of 41%. However, propionaldehyde **A2** in a ^{5th} P-3CR with a yield of 91% and in a ^{4th} P-3CR with 68%. It seems that the yield decreases with higher DP, as can be explained by the difficult purification by column chromatography. With the 2-phenyl-propionaldehyde **A12,** excellent yields (^{2nd} P-3CR, quantitative) and ^{5th} P-3CR (93%)), but due to the building of isomers the purification in the next steps gets more difficult. Also, sterically hindrance and solubility problems can affect the yield of the P-3CR. For a detailed analysis of the impact of the aldehydes in the P-3CR, a focused library with systematic variation of the aldehydes would be necessary. Since the focus of this work was not on the optimization of the reaction synthesis, as the system was already established, further investigations were not carried out.

Table 5: Summary of the hexamer **H1/1-H3/1** synthesis.

Product	**Hexamer H1/1**		**Hexamer H2/1**		**Hexamer H3/1**	
	Aldehyde	Yield [%]	Aldehyde	Yield [%]	Aldehyde	Yield [%]
1st P-3CR	**A5**	96	**A9**	97	**A5**	91
1st Deprotection		Quant.		98		99
2nd P-3CR	**A10**	96	**A12**	Quant.	**A7**	87
2nd Deprotection		99		99		96
3rd P-3CR	**A9**	92	**A1**	92	**A3**	89
3rd Deprotection		98		Quant.		99
4th P-3CR	**A7**	95	**A2**	68	**A11**	96
4th Deprotection		99		97		98
5th P-3CR	**A2**	91	**A11**	53	**A12**	93
5th Deprotection		99		91		99
6th P-3CR	**A3**	84	**A10**	41	**A1**	57
Over all steps		58		11		33

4.3.1.5 Establishing a read-out protocol

As already mentioned, for a successful read-out of the oligomers *via* tandem ESI-MS/MS, it was essential to identify the characteristic patterns occurring during fragmentation. As discussed in 4.2, two dominant fragmentation patterns were identified: type I fragmentation next to the carbonyl group and type II fragmentation next to the ester (Figure 28).[50] Further investigations showed that the type II fragmentation was dominant when sodium trifluoroacetate **2** (0.013 mg/mL) was added during the measurement. In experiments without addition of any additive, the type I fragmentation was dominant. Therefore, depending on the

MS sample preparation, one of the two pathways can be selected for computer-assisted read-out providing error proofing by comparison of the independent read-out routes.

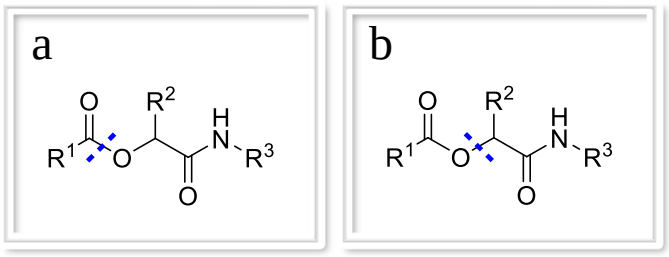


Figure 28: Most common fragmentation patterns of the oligomer by tandem ESI-MS/MS.[50] **a.** Fragmentation next to the carbonyl, which is preferred in measurements without any additives. **b.** Fragmentation next to the ester group is preferred when sodium trifluoroacetate **2** is used as additive.

As an example, hexamer **H1/1** measurements were conducted without additive, *i.e.* type I fragmentation was present (refer to Figure 28). The tandem ESI-MS/MS fragmentation spectrum of **H1/1** is displayed in Figure 29. The mass peak of the molecule **H1/1** at 2492.6395 m/z was fragmentated using a NCE of 18. The sequence of the hexamer can be read from the left (starting at the TAG) or from the right (starting at the benzyl protection group) and the information is complementary, thus providing a further error-proof mechanism, as already discussed in this thesis (see 4.2). Furthermore, the middle fragments without start and end blocks were observed, further confirming the structure of the molecule.

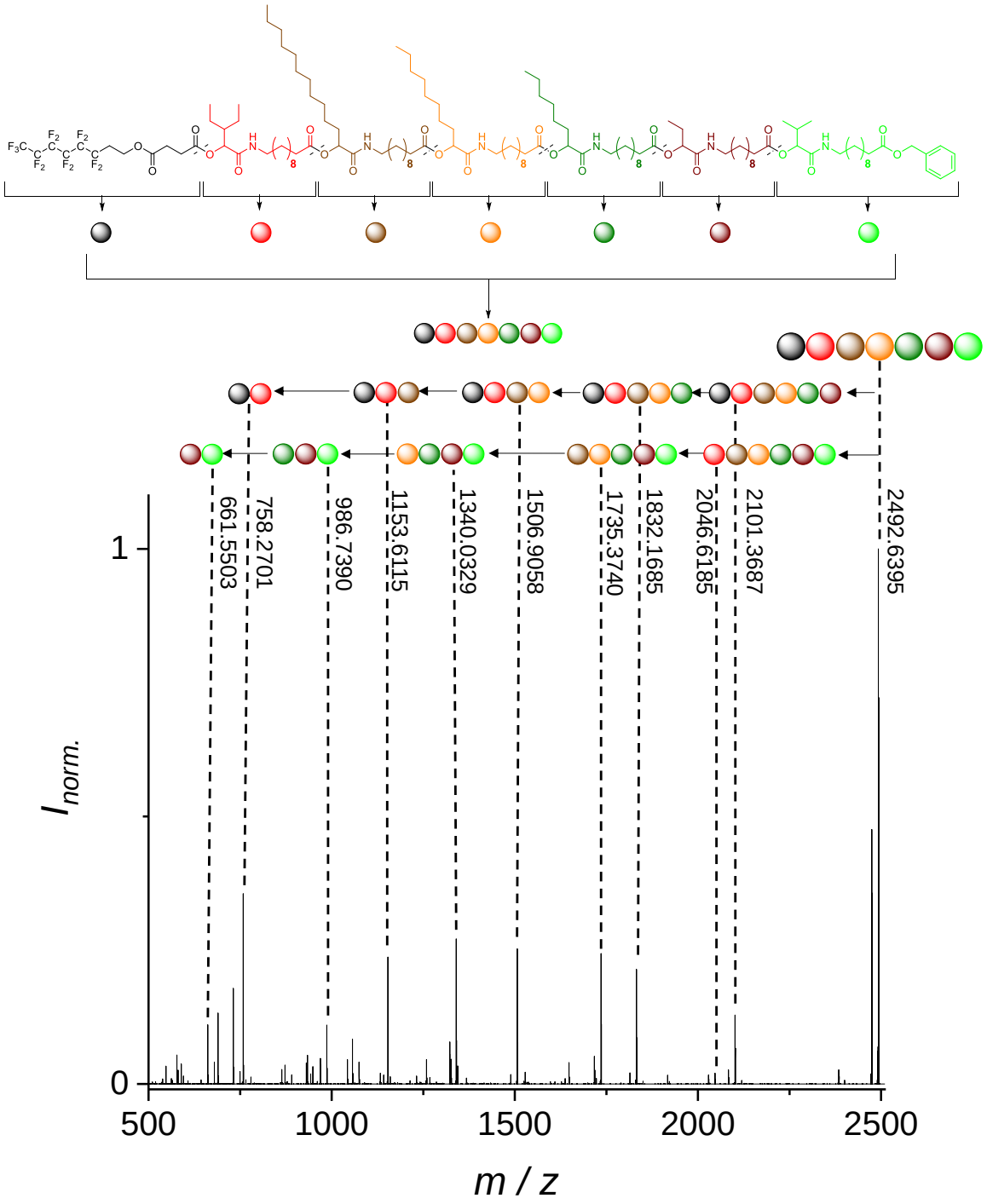


Figure 29: Read-out of the sequence-defined hexamer **H1/1**. Read-out of the hexamer **H1/1** *via* tandem ESI-MS/MS with an NCE of 18. In the spectrum the read-out from both ends of the oligomer is shown, using the type I fragmentation.

As shown in Figure 29, the fragmentation of the oligomers occurs in a distinct pattern. The reconstruction of the oligomers can subsequently perform *via* the combination of all observed mass fragments. Consequently, the fragmentation rules can be converted into Equation 5**,** providing a means for calculating the molecular mass of each fragment. More details about the equation and the exact description are shown in 6.3.3.5.6.

$$[M_{Molecule} + H]^+ = \left[\left(M_{Start} + n \times (M_{Backbone}) + \sum_{i=1}^{i=n} M_{Sidechain}^{i} + M_{End} + y \times M(H)\right) + H\right]^+$$

Equation 5

n = number of repeating units, y =(n-1)

With Equation 5, the fundamental sequencing rules are established, allowing to transfer this empiric information to a computer program for automated read-out. In order to compare different data storage systems, the data storage capacity needs to be calculated. The storage capacity of one of the described hexamers is calculated as follows: twelve possible side chains were taken into account, as well as six repeating units, resulting in $(12)^6$ = 2.985.984 permutations. The number of permutations can be translated into bit or byte nomenclature by using the following Equation 6.

$$bit = \frac{\log(n_{permutations})}{\log(2)}$$

Equation 6

Thus, the storage capacity of the described hexamers (**H1/1-H3/1**) was calculated to be 21.5 bits (2.7 bytes), compared with a tetramer (**T1/1-T3/4**), with a capacity of 14.3 bits.

4.3.1.6 Program for automated read-out of sequence-defined oligomers[i]

To simplify the read-out process of oligomers and the analysis of larger molecules as well as mixtures of molecules, it was crucial to establish a computational software for MS analysis in close collaboration with D. Hofheinz. Only a few specific fragments are necessary to re-establish the initial sequence of the oligomer.

It was possible to implement a reconstruction algorithm, which takes an ESI-MS/MS spectrum as input, in combination with the absolute molecular weights of possible markers **TAG1-3**, sidechains **A1-A12**, backbone of **IM2** and the ending (benzyl group). The algorithm attempts a reconstruction of the molecule (see 6.3.3.5.6) taking into account the aforementioned components and fragments extracted from the mass spectrum. Our algorithm proceeds with a directed brute-force search: All possible combinations of side chains are enumerated and then compared to the associated masses with peaks from the corresponding ESI-MS/MS spectrum. Molecule peak and fragments were detected, the possible oligomer can be released. The

[i] Program was written by Prof. Dr. Dennis Hofheinz

algorithm was implemented in Python® allowing its utilization on a common office computer. Du Prez *et al.* also described a reconstruction algorithm.[36] Unlike our read-out procedure, their algorithm only matches and compares the total mass candidates and ignores the information of the full spectrum. Moreover, Lutz *et al.* described a "millisecond sequencing" of binary coded polymers using a program with implemented algorithm.[256,258] This algorithm searches for the mass of the starting molecule. Afterwards, the mass of the starter plus the mass of the first backbone plus one of the possible side chains must be found. Subsequently, the next repeating unit is checked, etc.[256] For the binary system, such an easy algorithm is feasible. In contrast to Lutz´s approach, the implementation of side chain variation in our case requires a more complex algorithm. A "filter system" and some criteria, such as fragments bearing no TAGs, have to be considered in our algorithm to allow for the analysis of more advanced sequence-defined structures. The developed code is flexible and can be easily adjusted to other molecular architectures in the future.

The output of our program reveals the employed **TAGs1-3** and each individual sequence, including name and the mass of the incorporated aldehyde in the side chain at a defined position. Inherently, the automatic *in silico* read-out can be carried out faster with three hexamers than one eighteen-mer. With increasing DP, the calculation time rises exponentially, and a hexamer represents the more feasible material.

4.3.1.7 Read-out of mixtures of hexamers

As discussed above, the reading of mixtures of sequence-defined macromolecules could significantly increase the data storage capacity while minimizing synthetic efforts. This challenge was explicitly communicated by Du Prez *et al.* in 2018 in a publication about multifunctional sequence-defined macromolecules for chemical data storage:[36]

"As every oligomer had to be analyzed separately, a future challenge would be to combine techniques for the analysis of much more complex samples, in order to guarantee a high data density."

As already mentioned, (see 4.3.1.3), the individually read-out of the twelve tetramers (**T1/1-T3/4**) was performed successfully. Furthermore, the analysis of more complex samples was sought, like the read-out of a mixture. We decided to combine two different tetramers, for a test, each bearing a different TAG, to increase the data storage capacity. For the sample preparation, two stock solutions of two tetramers **T1/1, T3/1** with a concentration of

0.05 mg*mL^{-1} were combined. Afterwards, high-resolution ESI-MS of the tetramer mixture was performed and verified by comparing with the calculated masses. Afterwards each tetramer in the mixture was fragmentated *via* tandem ESI-MS/MS. With the combination of the two tetramers **T1/1, T3/1** (see 6.3.3.5.7), it was possible to increase the data storage capacity from 14.3 bit for one tetramer to 28.6 bit for the tetramer mixture, while keeping appealing yields and the synthetic effort to an absolute minimum. Due to the fact of purification problems in a longer chain and the needed of a huge amount of product to ensure to have enough product for the synthesis steps.[50] It is important to note that a mixture of oligomers with the same TAGs would of course be indistinguishable with the herein presented read-out algorithm. To demonstrate the full potential of the read-out of mixtures and to ultimately increase the data storage capacity of the system, the three synthesized hexamers **H1/1-H3/1** were mixed and analyzed in a similar fashion as the two tetramers. The samples were prepared as a mixture of stock solutions with a concentration of 0.05 mg*mL^{-1} of each hexamer and the mixture was analyzed *via* ESI-MS (Figure 30). Afterwards, the high-resolution mass of each hexamer was analyzed and compared with the calculated masses. Subsequently, each hexamer in the mixture was fragmentated *via* tandem ESI-MS/MS and the read-out is shown in Figure 30.

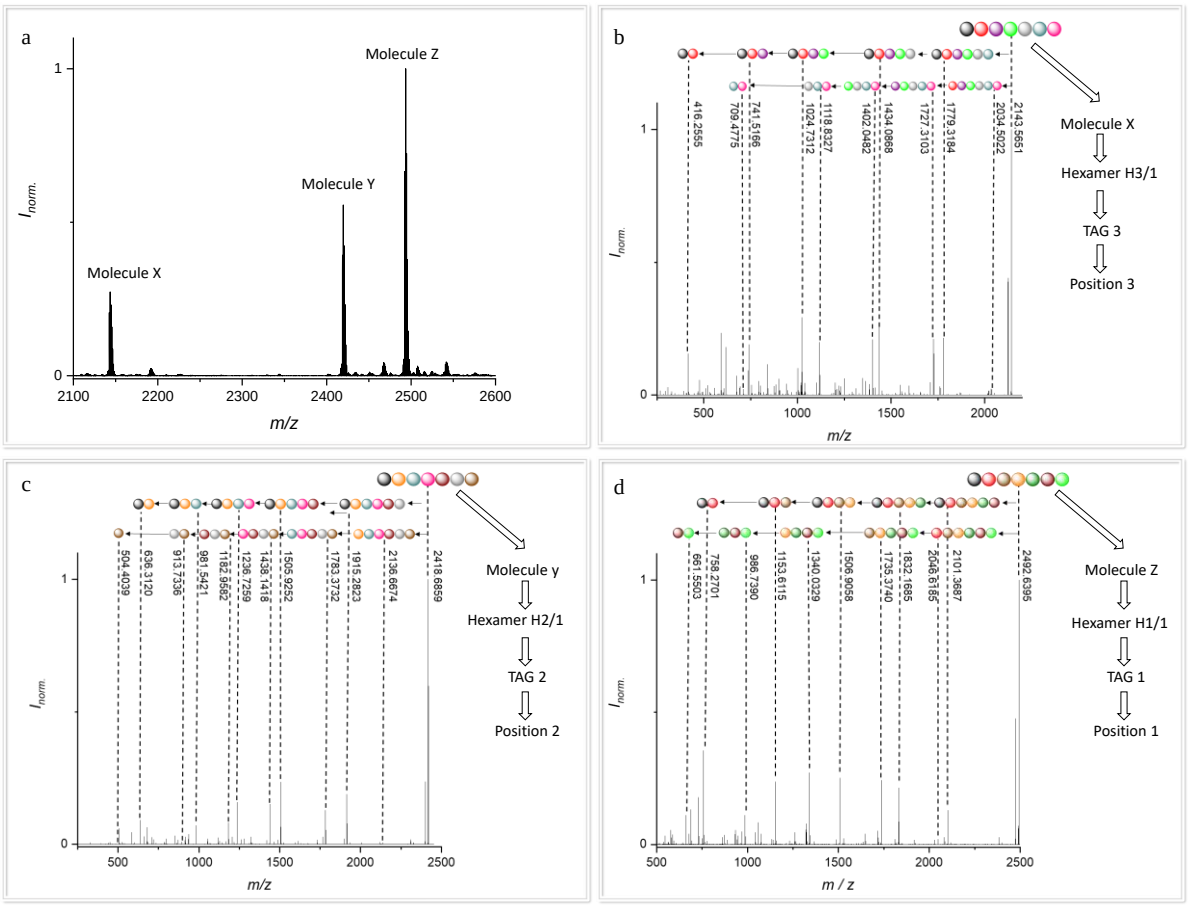


Figure 30: Read-out of a mixture of three hexamers **H1/1-H3/1**, with clearly defined positions of the **TAGs** to increase the data storage capacity. **a.** ESI-MS spectrum of a mixture of three different hexamers **H1/1-H3/1** that was used for subsequent tandem ESI-MS/MS fragmentation. For the fragmentation, each molecule peak was selected individually. **b.** fragmentation of hexamer **H3/1**. **c.** fragmentation of hexamer **H2/1**. **d.** fragmentation of hexamer **H1/1**.

Afterwards, it was possible to perform the read-out of each hexamer in the mixture manually as shown in Figure 30. To increase the data storage capacity, it was necessary to clearly define a position of each TAGs in the mixture to generate a successful read-out (*i.e.* **TAG1** was position one, etc.). Ultimately, the full *in silico* analysis was successfully conducted and the output of the program acquired the chosen TAG and each defined sequence, such as the name and the mass of the employed aldehyde in the side chain at the defined position in a few seconds. Screenshots of the output of the program for each hexamer **H1/1-H3/1** are depicted in Figure 31. In the top of the output of the program, the matched molecular mass is displayed, afterwards the peaks were analyzed, and the program stops when 1 solution is found. Then, the molecular mass of the starter, plus the positions and names of the sidechains are shown.

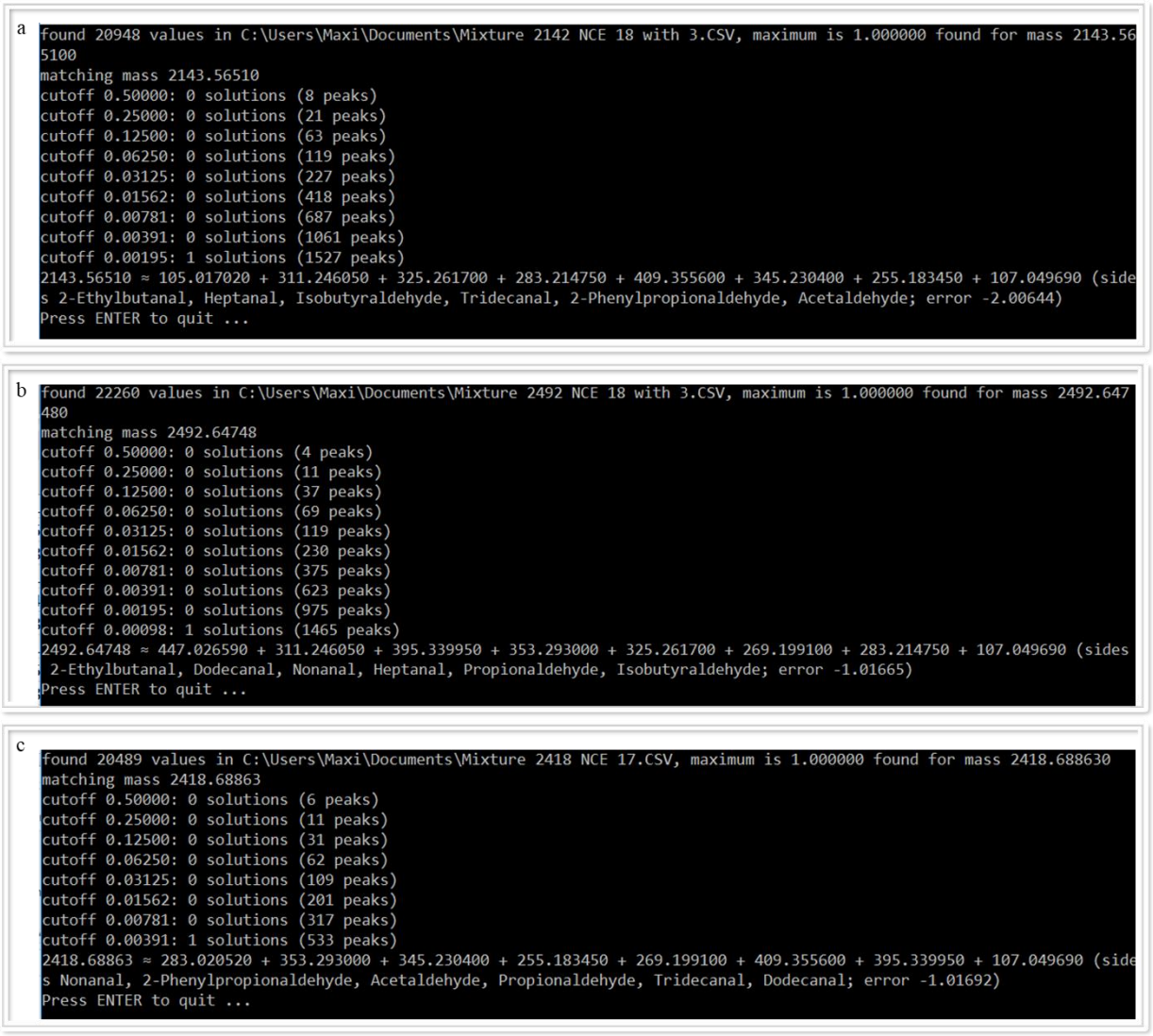


Figure 31: Screenshot of the read-out for all hexamers. **a.** Read-out of hexamer **H3/1**. **b.** Read-out of hexamer **H2/1**. **c.** Read-out of hexamer **H3/1**.

This full read-out of the mixture of three different hexamers allowed to increase the data storage capacity significantly from 21.5 bit for a single hexamer to 64.5 bit for the mixture of the three hexamers with different TAGs which could be calculated as follows: For the mixture, the permutations can be calculated as $(12)^6 \times (12)^6 \times (12)^6 = (12)^{18} = 26.623.333.280.885.243.904$ permutations. With Equation 6**,** the number of permutations can be easily translated into 64.5 bit or 8.06 byte. As shown by the calculation, the data storage capacity of the hexamer mixture corresponds to the data storage capacity of one 18-mer. However, the synthesis of an 18-mer would be significantly more complex and very time consuming in comparison to the synthesis of three hexamers, considering longer reaction times and purification drawbacks. Furthermore, *in silico* analysis of the 18-mer would not be feasible in this simple fashion, as it would require in the order of 2^{64} operations.

4.3.1.8 Conclusion

In summary, we have shown the synthesis of twelve different sequence-defined tetramers and three different hexamers with three different TAGs and twelve individual side chains in high purity (97-99%) and yields. The oligomers were subsequently utilized for sequential read-out by tandem mass spectrometry, further demonstrating the versatility of our well-established approach. The acquired information by manual read-out of the sequences was successfully implemented in a program for automatic read-out. Using this program, the stored information of all tetramers was read automatically in brief computing time. Afterwards, the three sequence-defined hexamers were mixed and utilized for read-out. Our method, as well as the computer program, were shown to be successful and powerful tool for the automated read-out of highly complex structures and even mixtures of sequence-defined molecules. We therefore developed a general method to increase the data storage capacity of sequence-defined macromolecules by using mixtures of such compounds. The example of three hexamers provided an increase from 21.5 bit for a single hexamer up to 64.5 bit for a mixture of three hexamer mixture, which corresponds to the data storage capacity of an eighteen-mer. Furthermore, the mixing of different sequence-defined oligomers (*i.e.* different degree of oligomerization) as well as different numbers of oligomers (*i.e.* one, two or three oligomers, as presented) for data storage allows for a straightforward adjustment of the data storage capacity without the need of further synthesis.

4.3.2 Reading mixtures, small molecules conquer the field of data storage

Some of the molecules which were used in this chapter and the used computer program (python script), were published before: The Reading mixtures of uniform sequence-defined macromolecules to increase data storage capacity macromolecules, Maximiliane Frölich, Dennis Hofheinz & Michael A. R. Meier, *Communication Chemistry*, **2020**, *3*, 184.

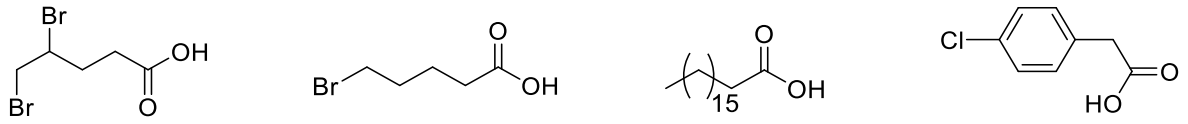
Some of the molecules were synthesized by a Bachelor student (Felix Bauer) under the co-supervision of Maximiliane Frölich. The respective molecules are marked with footnotes in the experimental part. The computer program (python script) was written by Prof. Dr. Hofheinz, which is also marked with footnotes in the experimental part.

Abstract

Further development of the read-out of mixtures from sequence-defined oligomers for data storage is demonstrated in this chapter. Using an established iterative stepwise synthesis approach, three sequence-defined trimers with varying new mass markers were successfully synthesized. Afterwards, the trimers were analyzed by ESI-MS/MS and an automated read-out with the already established computer program was demonstrated. The use of six different mass markers allows the unambiguous read-out of a mixture of up to six different oligomers. A high data storage capacity of up to 64.5 bit was thus demonstrated by mixing short sequences.

4.3.2.1 Oligomer Synthesis

In chapter 4.3.1, the proof of concept of the read-out of a mixture to increase the data storage capacity was demonstrated. Three different TAGs were used to establish an automated read-out process by a computer program. In this chapter, the concept will be further improved to demonstrate the mixing of more than three different oligomers. Therefore, already synthesized oligomers with **TAG1-3** were used and further oligomers with different TAGs (**TAG4-7**) were synthesized using the same two-step iterative cycle as described in 4.3.1.2. The selection of TAGs with different characteristic isotopic patterns was necessary, *e.g.* a brominated TAG, but also a long aliphatic and an aromatic one, as already discussed in 4.3.1.1. Therefore, four new TAGs (**TAG4-7**) were selected (see Figure 32) and tested in a first iterative cycle with the P-3CR and a subsequent deprotection. **TAG5-7** were commercially available, while the dibrominated **TAG4** was synthesized in a one-step synthesis. Using 4-pentenoic acid as starting agent and elemental bromine, **TAG4** was synthesized on a 5 g scale with a yield of 55%.[289]



TAG4 **TAG5** **TAG6** **TAG7**

Figure 32: Overview of four different TAGs as starter acid in the oligomer synthesis.

In order to evaluate whether the respective TAG is suitable to be used in the iterative synthesis and in the characterization and read-out *via* ESI-MS and ESI-MS-MS, one test experiment per TAG was carried out first. Therefore, **TAG4-7** were used in a P-3CR, with isocyanide **IM2** and aldehydes **A1**-**A12**. The Passerini monomers **T4/1-T6/1** with **TAG4-6** as starting acids were purified by column chromatography, except for the Passerini monomer **T7/1** with **TAG7**. In this case, first an aqueous work-up was necessary, followed by column chromatography to yield the pure product. The four monomers **T4/1-T7/1** were analyzed by NMR, IR, ESI-MS and SEC. A subsequent deprotection using palladium on activated carbon **1** and hydrogen gas yielded the deprotected Passerini monomers **TD4/1-TD7/1**. These were further analyzed by NMR, IR, ESI-MS and SEC. During the ESI-MS analysis of the deprotected Passerini monomer **MD7/1** with **TAG7**, some issues were observed. In the ESI-MS, the product peak plus sodium could be assigned at 490.2334 *m/z*, however another intense peak at 456.2714 *m/z* was observed. This peak can be assigned to the mass of the molecule without Cl plus sodium (calculated $[M+H+Na-Cl]^+$ = 456.2720), see Figure 33. This indicates a cleavage of the Cl bond in the ESI and thus, the molecule is not stable enough for ESI-MS analysis. More difficult purification after the

P-3CR in combination with this instability during ESI-MS led to the decision, that **TAG7** is not suitable for the synthesis of a trimer and the application in mixtures for molecular data storage.

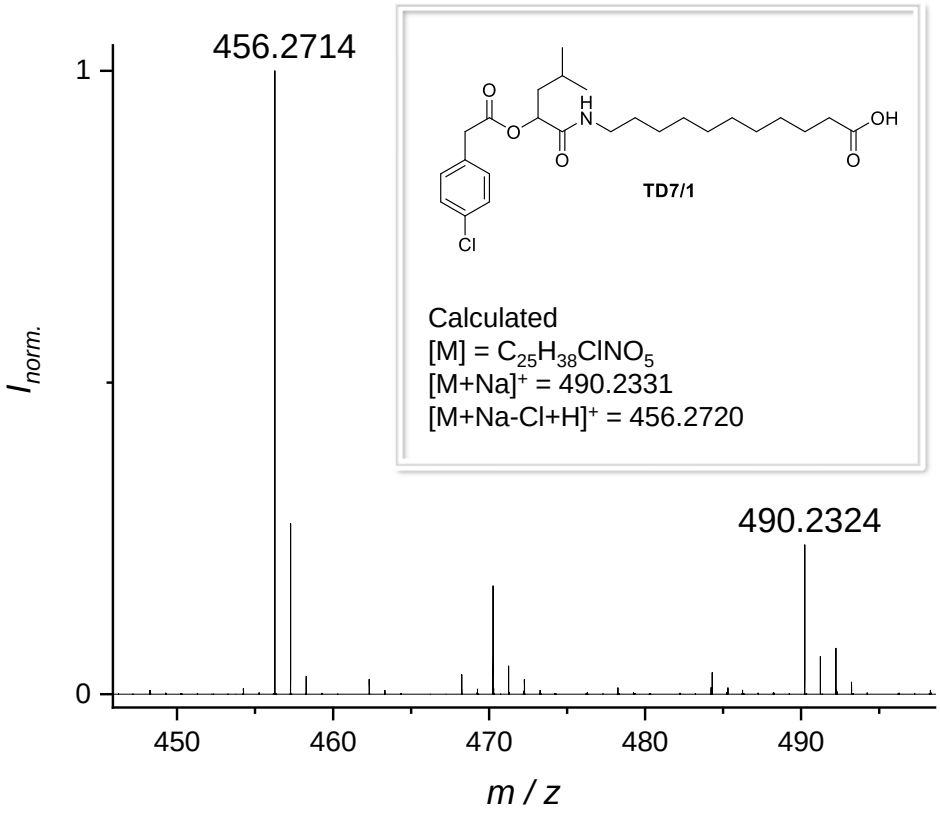
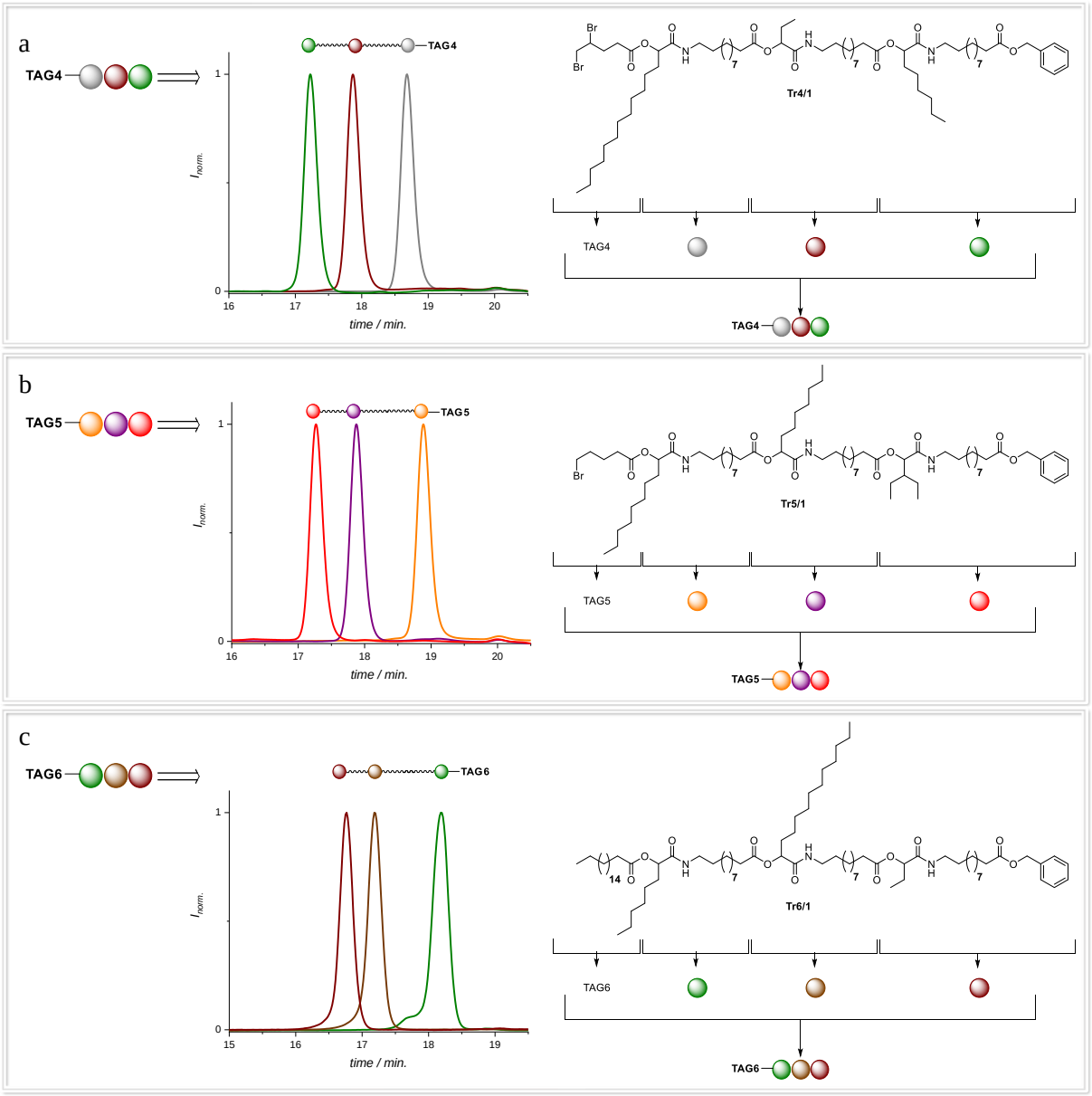


Figure 33: ESI-MS spectrum of the deprotected Passerini monomer **M7/1**. Cleavage of the Cl bond can be observed.

The difficulties with **TAG7** demonstrate the importance of selecting TAGs carefully, especially for their specific application in ESI-MS and ESI-MS/MS. This also shows how important preliminary tests of selected TAGs are in order to identify difficulties at an early stage.

After the suitability of the other TAGs (**TAG4-6**) was confirmed by ESI-MS and ESI-MS/MS analysis, they were used as starting acids in the iterative step approach (4.3.1.1). First, the P-3CR with the monoprotected isocyanide **IM2**, one of the **TAGs4-6** as starting acid and an aldehyde was performed. Afterwards, the Passerini monomer **Tr4/1-T6/1** was deprotected with palladium on activated carbon **1** and hydrogen gas to yield the free acid, which can react further in the next iterative step (see Scheme 32 and chapter 4.3.1.1 for the detailed explanation of the synthetic cycle and the used compounds). To further increase the number of oligomers that can be combined in a mixture of three sequence-defined trimers, **Tr4/1-Tr6/1** were synthesized with different **TAGs** as starting acid, respectively. The reaction steps and the SEC traces of the trimers **Tr4/1-Tr6/1** are described in the following parts. Due to the analogy to the synthesis of the hexamers **H1/1-H3/1**, which were described in detail in chapter 4.3.1.4, the synthesis of

Tr4/1-Tr6/1 is not explained in detail in this chapter. However, specific findings related to the synthesis of the herein presented trimers **Tr4/1-Tr6/1** will of course be discussed in detail.



Scheme 34: Overview of the introduced aldehydes (colored spheres represent the differently functionalized repeating units), the chemical structure and SEC traces of the three different trimers, each with a different TAG. **a.** Chemical structure of **Tr4/1** with **TAG4** and the associated SEC traces of each Passerini reaction according to color code of the aldehyde employed in the reaction. **b.** Chemical structure of **Tr5/1** with **TAG5** and the associated SEC traces of each Passerini reaction according to color code of the aldehyde employed in the reaction **c.** Chemical structure of **Tr6/1** with **TAG6** and the associated SEC traces of each Passerini reaction according to color code of the aldehyde employed in the reaction.

For the synthesis of trimer **Tr4/1**, **TAG4** was used as starter acid and the aldehydes were incorporated in the following order: Tridecanal **A11**, propionaldehyde **A2** and heptanal **A7**. The yields for each individual step of trimer **Tr4/1** are listed in Table 6. After five steps, the trimer

Tr4/1 was obtained in an overall yield of 9%. The yield in the second and third P-3CR were significantly lower compared to the synthesis of the tetramers **T1/1-T3/4** described in chapter 4.3.1.3. In the SEC measurements of the crude mixture of the dimer **D4/1** and trimer **Tr4/1** of the Passerini product, different side-products were observed. After intense purification *via* column chromatography, the pure products were obtained. Furthermore, the purity of **Tr4/1** and all Passerini products (**M4/1-Tr4/1**) were determined by SEC to be of high purity above 99% (see Scheme 34). The successful synthesis of **Tr4/1** was confirmed by ^{1}H-, ^{13}C-NMR, IR spectroscopy and mass spectrometry (see 6.3.4.2).

For the synthesis of trimer **Tr5/1**, the commercially available 5-bromovaleric acid (**TAG5**) was used as starting material in the iterative synthesis and the aldehydes were incorporated in the following order: Nonanal **A9**, octanal **A8** and 2-ethylbutyraldehyde **A5**. **Tr5/1** was synthesized in five steps with an overall yield of 6% (yield of all steps in Table 6). The significantly lower yield in the 3rd P-3CR can be explained by a side reaction in the second deprotection step. A solvent mixture of methanol MeOH/EA instead of THF/EA was used during the deprotection with palladium on activated carbon **1** and purging with hydrogen gas. Afterwards, impurities of the side reaction were observed in the NMR spectrum. It was found that the deprotected acid reacted with methanol to form the methyl ester during the deprotection. Purification of **Tr5/1** was challenging due to the methyl ester impurity. However, through a time consuming and more challenging purification *via* column chromatography, it was possible to separate the methyl ester protected dimer and yield the product in high purity. The purity of **Tr5/1** as well as of the respective intermediates (**M5/1-Tr5/1**) was determined by SEC (≥99%, see Scheme 34) and the structure of **Tr5/1** was confirmed by ^{1}H-, ^{13}C-NMR, IR and mass spectrometry (see 6.3.4.3).

Trimer **Tr6/1** was synthesized with stearic acid (**TAG6**) as starting material and the aldehydes were incorporated in the following order: Heptanal **A7**, dodecanal **A10** and propionaldehyde **A2**. The trimer was synthesized in five steps with an overall yield of 47% (see Table 6). The purity of **Tr6/1** and all intermediates (**M6/1-Tr61**) was determined by SEC (see Scheme 34). In the SEC chromatogram of the first Passerini product **M6/1** a small impurity was determined after purification *via* column chromatography. The dimer and trimer **Tr6/1** P-3CR product was obtained in a high purity of ≥99% as determined by SEC after purification *via* column chromatography. The successful synthesis of **Tr6/1** was confirmed by ^{1}H-, ^{13}C-NMR, IR spectroscopy and mass spectrometry (see 6.3.4.4). A summary of the used aldehydes, the obtained yields and synthetic procedure for the trimers **Tr4/1-Tr6/1** is shown in Table 6.

Table 6: Summary of the synthesis of trimer **Tr4/1-Tr6/1**.

Product	**Trimer Tr4/1**		**Trimer Tr5/1**		**Trimer Tr6/1**	
	Aldehyde	Yield [%]	Aldehyde	Yield [%]	Aldehyde	Yield [%]
1st P-3CR	**A11**	99	**A9**	96	**A7**	86
1st Deprotection		95		97		93
2nd P-3CR	**A2**	66	**A8**	96	**A10**	67
2nd Deprotection		99		75		97
3rd P-3CR	**A7**	22	**A5**	9	**A2**	91
Over all steps		9		6		47

4.3.2.2 Automated read-out of trimers

After the successful synthesis of **Tr4/1-Tr6/1**, each trimer was first analyzed separately by tandem ESI-MS/MS as a pure compound, in order to ensure a successful read-out. The already established "read-out protocol" (see 4.3.1.5) was used for the analysis of the ESI-MS/MS spectra. Therefore, the read-out was first performed manually to proof the common fragmentation pattern of the oligomers (see Figure 28). The sequences of the trimers can be read from both sides: from the left (starting at the TAG) or from the right (starting at the benzyl protection group). The information is complementary, thus providing a further error-proof mechanism. The middle fragments without start and end blocks were also observed, further confirming the structure of the molecule. The computer program was adjusted for the read-out of **Tr4/1-Tr6/1** by simply implementing the masses of the starter acid **TAG4-6** into the program and subsequently, the automatic read-out was performed within a few seconds. Screenshots of the output of the program for each trimer **Tr4/1-Tr6/1** are displayed in Figure 34 and the manual read-out is displayed in the experimental part (see 6.3.4.2, 6.3.4.3, 6.3.4.4).

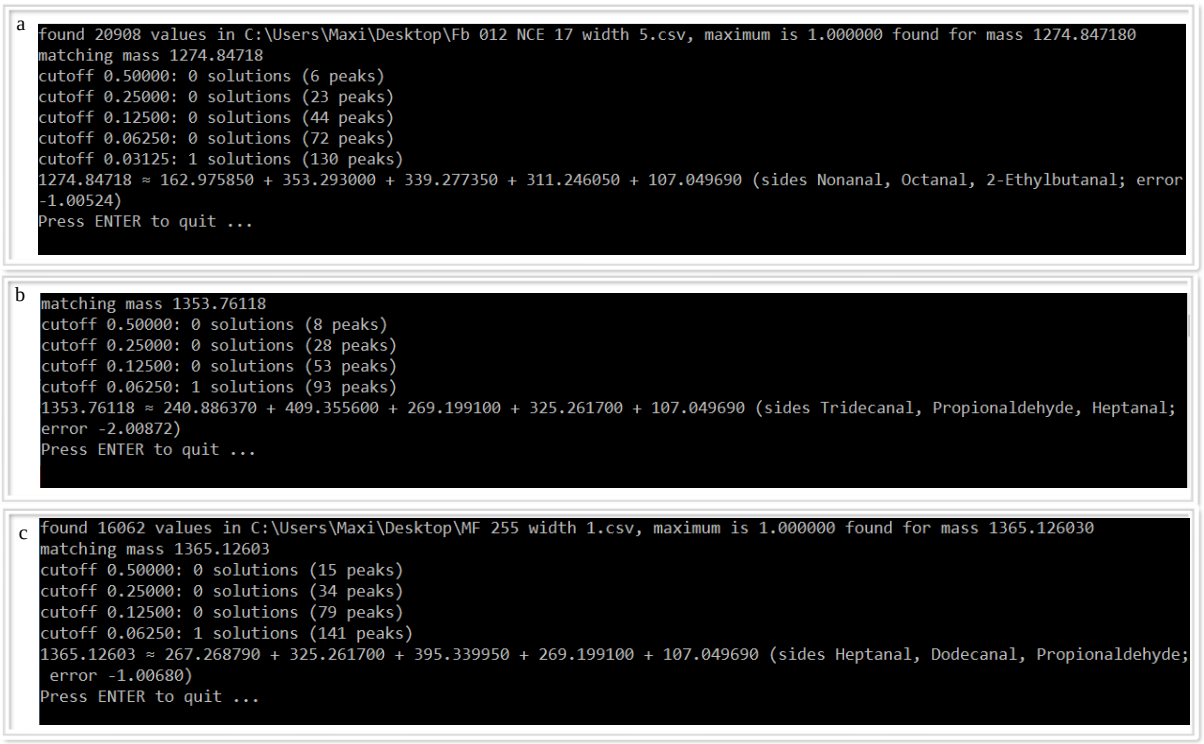


Figure 34: Screenshot of the program based read-out of the trimers **Tr4/1-Tr6/1**. **a.** Read-out of trimer **Tr4/1**. **b.** Read-out of trimer **Tr5/1**. **c.** Read-out of trimer **Tr6/1**.

4.3.2.3 Reading the mixture of up to six trimers

In order to find out how many different molecules, with one specifically assigned TAG each, can be read in a mixture, a test series was carried out. In chapter 4.3, the read-out of three different molecules was demonstrated. Here, for a further proof of concept, mixing of up to six different molecules, *i.e.* trimers **Tr1/1-Tr3/1** with **TAG1-3** and the new molecules **Tr4/1-Tr6/1** were used. An overview of the chemical structures of all six trimers **Tr1/1-Tr6/1,** which were used in different mixtures, are displayed in Figure 35.

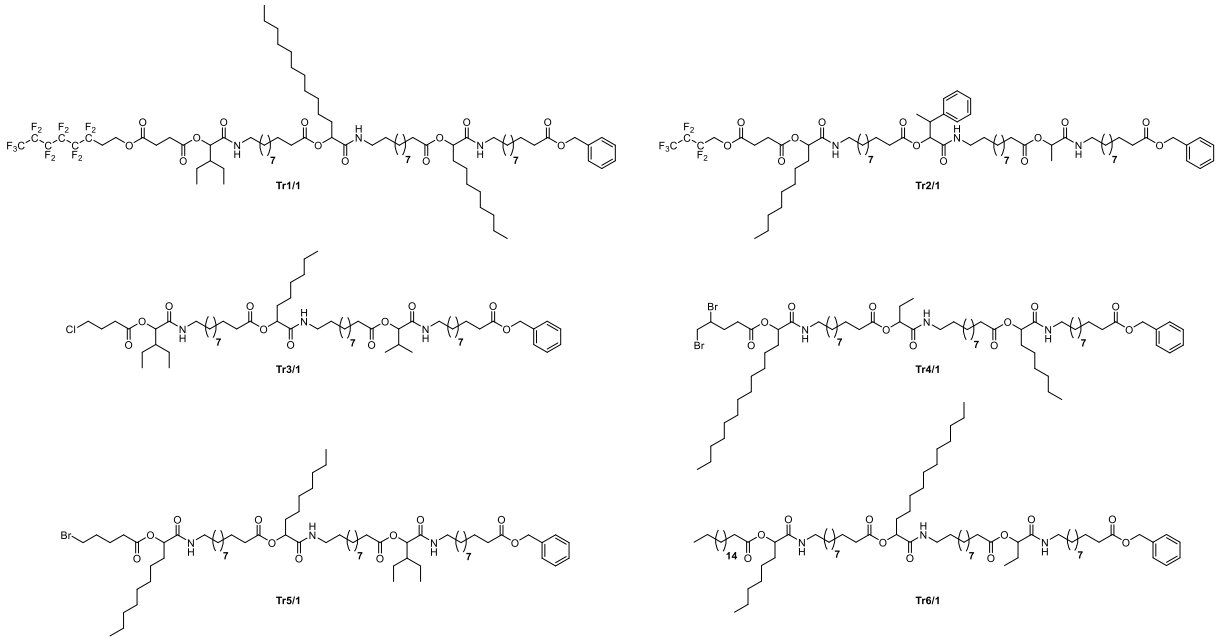


Figure 35: Set of six trimers **Tr1/1-Tr6/1**, each with a different TAG (**TAG1-6**) which were used for the read-out of mixtures.

First, it was necessary to check whether the new trimers **Tr4-6** with their TAGs **TAG4-6** can also be read in a mixture of three different oligomers. Therefore, the two trimers **Tr2/1-Tr3/1** of the already established **TAG2-3** were used together with one of the trimers **Tr4-6**, respectively. In Table 7, the combinations (mixtures 1-3) of the new trimers **Tr4/1-Tr6/1** with **Tr2/1** and **Tr3/1** are depicted. The samples were prepared as a mixture of stock solutions with a concentration of 0.05 mg*mL^{-1} of each trimer and the mixture was analyzed *via* ESI-MS. The high-resolution mass of each trimer was analyzed and compared with the calculated masses. Subsequently, each trimer in the mixture was fragmentated *via* tandem ESI-MS/MS and the read-out was demonstrated. After the successful use of the new trimers **Tr4/1-Tr6/1** in the read-out of a mixture, mixing more than three oligomers was investigated. Therefore, different combinations (see Table 7) were used to show the read-out of five oligomers in one mixture. Mixtures 4-6 were mixed and analyzed in a similar fashion as mixtures 1-3. The samples were prepared as a mixture of stock solutions with a concentration of 0.05 mg*mL^{-1} of each trimer and the mixture was then analyzed as described above. Therefore, each trimer in the mixture was analyzed, and the read-out was proofed in the similar way like in Figure 36 displayed for the mixture of six oligomers. With this proof of different combinations of trimer in one mixture, the successful read-out of mixtures 4-6, the mixing of five oligomers was demonstrated and the versatility of the system could be clarified.

Table 7: Overview of different mixtures of trimers **Tr1/1-Tr6/1** used for the read-out.

	Compounds of the molecular mixture						Read-out
Mixture 1	Tr2/1	Tr3/1	Tr6/1				✓
Mixture 2	Tr2/1	Tr3/1	Tr4/1				✓
Mixture 3	Tr2/1	Tr3/1	Tr5/1				✓
Mixture 4	Tr1/1	Tr2/1	Tr3/1	Tr4/1	Tr6/1		✓
Mixture 5	Tr1/1	Tr2/1	Tr3/1	Tr4/1	Tr5/1		✓
Mixture 6	Tr1/1	Tr2/1	Tr3/1	Tr5/1	Tr6/1		✓
Mixture 7	Tr1/1	Tr2/1	Tr3/1	Tr4/1	Tr5/1	Tr6/1	✓

To demonstrate the full potential of the read-out of mixtures and to ultimately increase the data storage capacity of the system, all six synthesized trimers **Tr1/1-Tr6/** were mixed and analyzed (see Table 7, mixture 7). Therefore, samples were prepared again as a mixture of stock solutions with a concentration of 0.05 mg*mL^{-1} of each trimer **Tr1-Tr6/1** and the mixture was analyzed *via* ESI-MS (see Figure 36). Afterwards, the high-resolution mass of each trimer was analyzed and compared with the calculated masses. Each trimer in the mixture was fragmentated *via* tandem ESI-MS/MS. The manual read-out of each trimer in the mixture was performed as shown in Figure 36. It was thus demonstrated that the read-out of the molecules is also possible, even if they display only small mass differences of around 10 m/z between the different trimers. To increase the data storage capacity, it was necessary to clearly define a position of each TAG in the mixture to generate a successful read-out (*i.e.* **TAG1** was position one, etc.). With the read-out of the mixture of six different trimers, it was possible to obtain a data storage capacity of 64.5 bit, which can be calculated as follows: number of permutations $((12)^3 \times (12)^3 \times (12)^3 \times (12)^3 \times (12)^3 \times (12)^3 = (12)^{18}$ = 26.623.333.280.885.243.904 permutations. With Equation 6, the number of permutations can be translated into 64.5 bit or 8.06 byte storage capacity for mixture 7. The data storage capacity of the trimer mixture corresponds to the data storage capacity of the hexamer mixture (see 4.3.1.7) and consequently of an 18-mer. It was

demonstrated that up to six different molecules each with another TAG can be read in one mixture. Thereby, the application of short sequences in the field of data storage capacity is demonstrated. It would be interesting to build up further hexamers out of the trimers **Tr4/1-Tr6/1** and use them in the read-out of a mixture of six hexamers to yield a storage capacity of up to 129 bits. Furthermore, investigations on the limitation of mixing the molecules would be the next step to find out how many different molecules can be stored and analyzed in one molecular mixture. Therefore, different TAGs with a characteristic isotopic pattern, like iodine for example, should be used.

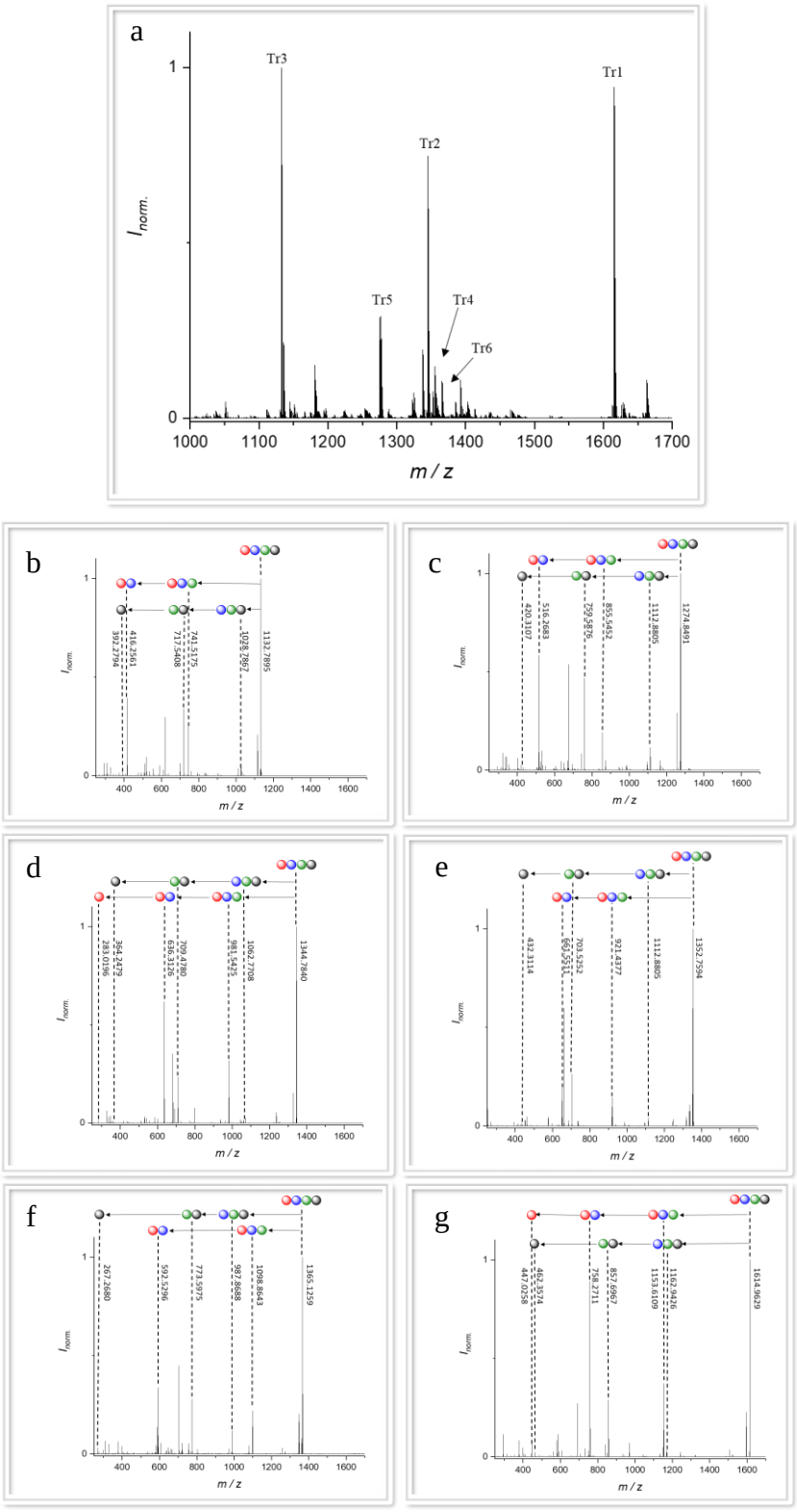


Figure 36: Read-out of a mixture of six trimers **Tr1/1-Tr6/1**, with clearly defined positions of the **TAGs** to increase the data storage capacity. **a.** ESI-MS spectrum of a mixture of six different trimers **Tr1/1-Tr6/1** that was used for subsequent tandem ESI-MS/MS fragmentation. For the fragmentation, each molecule peak was selected individually. **b.** Fragmentation of trimer **Tr3/1**. **c.** Fragmentation of trimer **Tr5/1**. **d.** Fragmentation of trimer **Tr2/1. e.** Fragmentation of trimer **Tr4/1**. **f.** Fragmentation of trimer **Tr6/1**. **g.** Fragmentation of trimer **Tr1/1**.

In summary, three additional sequence-defined trimers with three different TAGs were synthesized in high purity as determined by SEC. Furthermore, it was shown how important the careful selection and the proving of the TAGs is, to ensure an error-free application in the field of data storage. The synthesized trimers were used for a sequential read-out by tandem ESI-MS/MS, which was performed manually as well as automatically. For the automatic read-out, the already established computer program was used after implementation of the masses of the new TAGs. Afterwards, different mixtures of trimers were prepared and utilized for a subsequent read-out. A read-out of up to six sequence-defined trimers in a mixture was demonstrated. With this method a powerful tool for the read-out of complex mixtures was shown. Moreover, a method to store 64.5 bit in a mixture of six trimers instead of in a mixture of three hexamers was developed. Furthermore, the mixing of up to six different oligomers allows the usage of short sequences instead of long oligomers, thus facilitating the synthesis significantly.

5 Conclusion and Outlook

Sequence-defined oligomers are a promising and *via*ble tool for the storage of information in molecules and the full read-out is mostly performed *via* tandem mass spectrometry. To increase the data storage capacity in sequence-defined molecules, alternatives to the synthesis of ever-longer sequences are desirable. In this thesis, the application of sequence-defined Passerini oligomers in the field of data storage, followed by the establishment of novel approaches to increase their data storage capacity was investigated and successfully demonstrated. For the synthesis of the sequence-defined oligomers, a well-established iterative cycle was used, consisting of a P-3CR and a deprotection step. By using six different starter acids, twelve aldehydes and two monoprotected isocyanides, a large variety of oligomers of different length and side chain arrangement could be synthesized. In order to track and identify products or potentially interfering impurities, a thorough investigation of the analytical methods with respect to their information and detection thresholds was planned and carried out. To determine the impurity detection limits, a sequence-defined pentamer without side chain variation was synthesized and fully characterized. In the impurities studies, the pentamer was intentionally contaminated with different amounts of a impurity of the corresponding sequence-defined tetramer and analyzed by SEC and NMR. *Via* SEC, it was possible to detect an impurity of ≥1%, by NMR impurities of 5% and more. Moreover, it was shown that each analytic method has it's strengths and weaknesses, and it is necessary to apply at least mass spectrometry, NMR and liquid chromatography for sequence-defined molecules to ensure the successful synthesis and high purity of the molecules. Furthermore, the application was in focus and a read-out strategy for the application of the oligomers in the field of data storage was established. Therefore, different Passerini systems, like side chain defined, backbone defined, and dual sequence-defined oligomers were analyzed *via* ESI-MS/MS. There, it became apparent that fragmentation of the Passerini oligomers consistently follows a fixed scheme and a distinct fragmentation pattern was observed. By detection of the two common fragmentation patterns, a read-out protocol was established, which enabled the unambiguous read-out of sequence-defined oligomers. Thereby, a data storage capacity of 33 bits for a dual sequence-defined pentamer was determined. To increase data storage capacity, the read-out of oligomer mixtures was established and further developed. In order to enable the read-out of large complex systems and to reduce processing time, the read-out was automated. To develop an automated read-out, a library of twelve different sequence-defined tetramers and three different hexamers with individual side chains and three different starting acids as mass markers (TAGs) were

synthesized at high purity (97-99%). The oligomers were analyzed *via* ESI-MS/MS and following the protocol, the successful read-out by hand was translated and compiled into a computer program for the automatic read-out. By utilizing this computer program, the stored information of the oligomers was read automatically in less than three seconds. To increase the data storage capacity the three different hexamers, each with another TAG were mixed and the unambiguous recovery of information was achieved. In comparison to individual oligomers, the mixing of three hexamers shows an increase from 21.5 bit for a single hexamer up to 64.5 bit for the hexamer mixture. After the successful demonstration of the concept of reading mixtures, the possible number of oligomers was further investigated. Therefore, three sequence-defined trimers with further three different TAGs were synthesized and implemented in the program for the automatically read-out. Furthermore, it was demonstrated, that the selection of the TAGs is important to ensure an error-free application in the read-out process. By mixing up to six different trimers, each with a different TAG, a data storage capacity of 64.5 bit was established. This pointed out that the read-out of mixtures is a powerful tool to increase the data storage capacity, especially through the possible usage of oligomers with a different degree of oligomerization (up to a hexamer), but also different numbers of oligomers (up to six). The read-out of mixtures will be the future in the field of data storage of sequence-defined macromolecules, due easier and faster synthesis.

6 Experimental Section

6.1 Materials

The following chemicals were used as received from the following suppliers unless otherwise noted: propionaldehyde (97% Merck), isobutyraldehyde (98%, Sigma-Aldrich), 3-methylbutyraldehyde (≥ 98%, VWR), cyclohexancarboxaldehyde (98%), heptanal (≥ 95%,Sigma-Aldrich), octanal (99%, Sigma-Aldrich), dodecanal (≥ 95%, VWR), 2-phenylpropanal (98%, Fisher Scientific), 4-chlorobutyric acid (99% Sigma-Aldrich), succinic anhydride (>99.0%, Sigma-Aldrich), 4-(dimethylamino)-pyridin (DMAP) (99% Alfa Aesar), sodium sulfate (Merck), 2,2,3,3,4,4-heptafluro-1-butanol (98%, Alfa Aesar), 1*H*,1*H*,2*H*,2*H*-perfluoro-1-octanol (97%, Alfa Aesar), acetaldehyde (99.5%, Fluka), 2-ethylbutanal (98%, TCI), nonanal (97%, Alfa Aesar), tridecanal (96%, Alfa Aesar), 11-aminoundecanoic acid (97%, Sigma-Aldrich), benzyl alcohol (99%, Sigma-Aldrich), thionyl chloride (99%, Sigma-Aldrich), trimethyl orthoformate (99%, Sigma-Aldrich), phosphoryl trichloride **8** (99%, Sigma-Aldrich), diisopropylamine (> 99.5%, Sigma-Aldrich), palladium on activated carbon (10% palladium basis, Sigma-Aldrich), bromine (reagent grade, Fisher Scientific), 4-pentenoic acid (97%, Sigma Aldrich), 5-bromvaleric acid (97%, Sigma-Aldrich), Aminobutyric acid (+99%, Acros Organics), hydrogen (99,999%, Air Liquide), TLC silica gel F_{254} (Sigma-Aldrich), silica gel 60 (0.040 - 0.063, Sigma-Aldrich and Rocc), cerium(IV)-sulfate (99%, Sigma-Aldrich), phosphomolybdic acid hydrate (99%, Sigma-Aldrich), sodium carbonate (98%, Sigma-Aldrich), sodium hydrogen carbonate (> 95%, Sigma-Aldrich), sodium sulfate (> 99%, anhydrous, Sigma-Aldrich), magnesium sulfate (≥ 99%, Carl Roth), DMSO-d6 (≥ 99.8%, Euriso-top), MeOH-d4 (≥ 99.8%, Euriso-top), CDCl3 (≥ 99.8%, Euriso-top), dichloromethane (DCM, HPLC grade ≥ 99.9%, Sigma-Aldrich), methanol (HPLC grade 99.8%, Acros Organics), tetrahydrofuran (THF, 99.5%, extra dry over molecular sieves, Acros Organics), ethanol (analytical reagent grade, Fisher Scientific), diethyl ether (analytical reagent grade, Fisher Scientific), cyclohexane (technical grade), ethyl acetate (technical grade). All solvents were used without further purification, unless otherwise noted. Water, when used in the synthesis, was de-ionised.

6.2 Instrumentation

Nuclear magnetic resonance (NMR)

^{1}H, ^{19}F and ^{13}C spectra were recorded at the Karlsruhe Institute of Technology (KIT, Germany) on a Bruker Avance 400 NMR instrument at 400 MHz for ^{1}H-NMR, at 376 MHz for ^{19}F and 101 MHz for ^{13}C-NMR. ^{1}H spectra were recorded on a Bruker Avance 300 NMR instrument at 300 MHz for ^{1}H-NMR or on a Bruker AVANCE DRX at 500 MHz for ^{1}H-NMR and 126 MHz for ^{13}C-NMR. $CDCl_3$or CD_3OD were used as solvents. Chemical shifts are presented in parts per million (ppm, δ) relative to the resonance signal at 7.26 ppm (^{1}H, $CDCl_3$) and 77.16 ppm (^{13}C, $CDCl_3$) or 3.31 ppm (^{1}H, CD_3OD) and 49.00 ppm (^{13}C, CD_3OD), respectively. The spin multiplicity and corresponding signal patterns were abbre*via*ted as follows: s = singlet, d = doublet, t = triplet, q = quartet, quint. = quintet, sext. = sextet, m = multiplet and br = broad signal. Coupling constants (*J*) are reported in Hertz (Hz). All measurements were recorded in a standard fashion at 25 °C unless otherwise stated. Full assignment of structures was aided by 2D-NMR analysis (COSY, HSQC and HMBC). If isomers of a substance were observed, all species which could be assigned clearly were labelled with additional appendices (a, b, c. etc.). Hereby, the main isomer was labelled with the appendix “a”, the second isomer with appendix “b” and so on.

Size exclusion chromatography (SEC)

Measurements were performed on a Shimadzu Size Exclusion Chromatography (SEC) system equipped with a Shimadzu isocratic pump (LCYCLO20AD), a Shimadzu refractive index detector (24°C) (RID-20A), a Shimadzu autosampler (SIL-20A) and a Varian column oven (510, 50°C or 30°C). For separation, a three-column setup was used with one SDV 3 µm, 8×50 mm precolumn and two SDV 3 µm, 1000 Å, 3×300 mm columns supplied by PSS, Germany. Tetrahydrofuran (THF) stabilized with 250 ppm butylated hydroxytoluene (BHT, ≥99.9%) supplied by Sigma-Aldrich was used at a flow rate of 1.0 mL min^{-1} injection volume 20µL. Calibration was carried out by injection of eight narrow polymethylmethacrylate (PMMA) standards ranging from 102 to 58300 kDa.

Orbitrap electrospray-ionisation mass spectrometry (ESI-MS)

mass spectra were recorded on a Q Exactive (Orbitrap) mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with an atmospheric pressure ionisation source operating in the nebuliser assisted electrospray mode. The instrument was calibrated in the m/z-range 150-2000 using a standard containing caffeine, Met-Arg-Phe-Ala acetate (MRFA)

and a mixture of fluorinated phosphazenes (Ultramark 1621, all from Sigma-Aldrich). A constant spray voltage of 3.5 kV, a dimensionless sheath gas of 6, and a sweep gas flow rate of 2 were applied. The capillary voltage and the S-lens RF level were set to 68.0 V and 320 °C, respectively. For the interpretation of the spectra, molecular peaks $[M]^+$, peaks of pseudo molecules $[M+H]^+$ and $[M+Na]^+$ characteristic fragment peaks are indicated with their mass to charge ratio (*m/z*) and their intensity in percent, relative to the most intense peak (100%).

Electron ionisation (EI)

Mass spectra were recorded on a Finnigan instrument, model MAT 90 (70 eV). 3-nitrobenzyl alcohol (3-NBA) was used as matrix. For the interpretation of the spectra, molecular peaks $[M]^+$, peaks of pseudo molecules $[M+H]^+$ and characteristic fragment peaks are indicated with their mass to charge ratio (*m/z*) and their intensity in percent, relative to the most intense peak (100%).

Fast atom bombardment (FAB)

Mass spectra were recorded on a Finnigan MAT 95 instrument. The protonated molecule ion is expressed by the term: $[M+H]^+$ and $[M+Na]^+$

Infrared spectra (IR)

IR were recorded on a Bruker Alpha-p instrument in a frequency range from 3998 to 374 cm^{-1} applying KBr and Attenuated Total Reflection (ATR) technology. IR (Type of measurement) ν / cm^{-1} = wave number (signal intensity, molecular oscillation assignment). The signal shape and intensity is reported relative to the signal of highest intensity and was abbre*via*ted in the following pattern: br = brought, vs = very strong, s = strong, m = medium, w = weak, vw = very weak.

Thin layer chromatography (TLC)

All TLC experiments were performed on silica gel coated aluminium foil (silica gel 60 F_{254}, Sigma-Aldrich). Compounds were visualized first by fluorescence quenching (λ =254 nm and 365 nm). Also by staining with Seebach-solution (mixture of phosphomolybdic acid hydrate, cerium(IV)-sulfate, sulfuric acid and water) or Vanillin staining solution (mixture of Vanillin, ethanol, sulfuric acid).

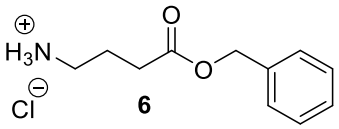
6.3 Experimental procedures

6.3.1 Impurity studies of sequence-defined macromolecules

6.3.1.1 Synthesis of monomer **IM1**

Esterification

Monomer **IM1** was synthesised according to the reported procedure from Meier *et al*.[50]

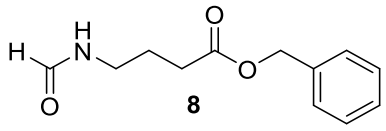


6

In a 500 mL three necked flask 13.0 g aminobutyric acid **3** (126 mmol, 1.00 eq.) were suspended in 126 mL THF and 136 g benzyl alcohol **4** (1.26 mol, 10.0 eq.) were added. The suspension was cooled in an ice bath and subsequently 27.4 mL thionyl chloride **5** (45.0 g, 378 mmol, 3.00 eq.) were added dropwise at 0 °C. After addition of the thionyl chloride **5**, the solution was warmed to room temperature and stirred overnight. The yellow solution was then poured into 500 mL diethyl ether and stored in the freezer for one hour. The precipitate was filtered off and dried under high vacuum. The product **6** was obtained as a white solid in a yield of 93.5% (27.1 g, 118 mmol).

^{1}H-NMR (300 MHz, CDO_3D): δ / ppm = 7.41 – 7.29 (m, 5 H), 5.16 (s, 2 H), 3.00 (t, J = 7.7 Hz, 2 H), 2.55 (t, J = 7.2 Hz, 2 H), 1.98 (q, J = 7.3 Hz, 2 H). ^{1}H-NMR was in accordance to the literature.[50]

***N*-Formylation**

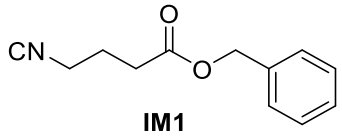


8

In a 250 mL round bottom flask 27.1 g of **6** (118 mmol, 1.00 eq.) were dissolved in 129 mL trimethyl orthoformate **7** (125 g, 1.18 mol, 10.0 eq.) and heated to 100 °C for 12 hours. Trimethyl orthoformate **7** was removed under reduced pressure and the product was used without further purification. The product **8** was obtained in quantitative yield (26.2 g, 118 mmol).

^{1}H-NMR (400 MHz, CDO_3D): δ / ppm = 8.04 (s, 1 H, CH), 7.45 – 7.25 (m, 5 H, CH_{Ar}), 5.14 (s, 2 H, CH_2), 3.27 (t, J = 6.9 Hz, 2 H, CH_2), 2.48 – 2.36 (m, 2 H, CH_2), 1.85 (q, J = 7.2 Hz, 2 H, CH_2). ^{1}H-NMR was in accordance to the literature.[50]

Dehydration



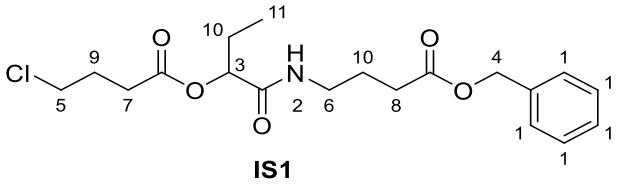
IM1

In a 500 mL three necked flask, 26.2 g of **8** (118 mmol, 1.00 eq.) were dissolved in 393 mL DCM, 66.3 mL diisopropylamine **9** (47.7 g, 572 mmol, 4.00 eq.) were added and the reaction mixture was cooled to 0 °C. Subsequently, 16.1 mL phosphorus oxychloride **10** (27.1 g, 177 mmol, 1.50 eq.) were added dropwise and the reaction mixture was then stirred at room temperature for two hours. The reaction was quenched by addition of sodium carbonate solution **11** (5 wt%, 100 mL) at 0 °C. After stirring this mixture for 30 min, 100 mL water and 100 mL DCM were added. The aqueous phase was separated, and the organic layer was washed with water (3 × 100 mL). The combined organic layers were dried over sodium sulfate **12** and the solvent was evaporated under reduced pressure. The crude product was then purified by column chromatography (hexane / ethyl acetate 6:1 → 3:1). The product monomer **IM2** was obtained as slightly yellow oil in a yield of 52.8% (12.7 g, 62.6 mmol).

^{1}H-NMR (400 MHz, $CDCl_3$): δ / ppm = 7.47 – 7.28 (m, 5 H, CH_{Ar}), 5.14 (s, 2 H, CH_2), 3.53 – 3.43 (m, 2 H, CH_2), 2.56 (t, J = 7.1 Hz, 2 H, CH_2), 2.08 – 1.96 (m, 2 H, CH_2). ^{1}H-NMR was in accordance to the literature.[50]

6.3.1.2 Synthesis of pentamer **IS5**

Passerini reaction



IS1

In a 50 mL round bottom flask 605 µL of 4-chlorobutyric acid **TAG3** (750 mg, 6.12 mmol, 1.00 eq.) were stirred in 3.00 mL DCM. Subsequently 1.32 mL propionaldehyde **A2** (1.07 g, 18.4 mmol, 3.00 eq.) and 1.87 g of monomer **IM1** (9.18 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 1 day. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (5:1 → 3:1) to yield the Passerini product **IS1** as a pale highly viscous oil. (2.15 g, 5.67 mmol, 92.0%).

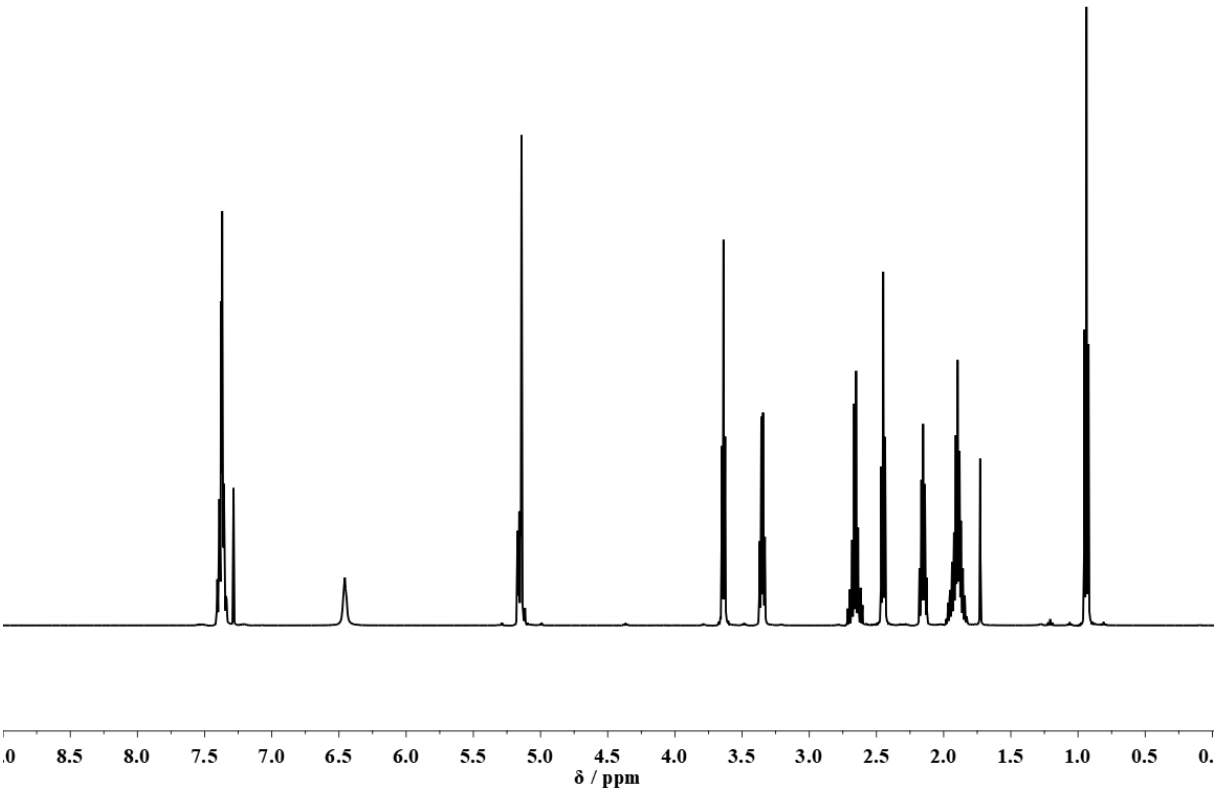
R_f = 0.37 in cyclohexane/ethyl acetate (3:2).

IR (ATR): ν / cm^{-1} = 3305.6 (vw), 2938.4 (w), 1732.3 (vs), 1658.4 (s), 1532.4 (m), 1454.4 (w), 1382.4 (w), 1296.8 (w), 1163.5 (vs), 1100.3 (m), 1048.5 (m), 979.5 (m), 907.4 (vw), 787.1 (vw), 736.8 (m), 697.2 (m), 646.8 (w), 421.0 (vw), 405.8 (vw).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.45 – 7.28 (m, 5 H, CH_{Ar}^1), 6.43 (t, J = 5.9 Hz, 1 H, NH^2), 5.19 – 5.08 (m, 3 H, CH^3, CH_2^4), 3.61 (t, J = 6.2 Hz, 2 H, CH_2^5), 3.32 (td, J = 6.8, 5.7 Hz, 2 H, CH_2^6), 2.71 – 2.56 (m, 2 H, CH_2^7), 2.43 (t, J = 7.0 Hz, 2 H, CH_2^8), 2.17 – 2.07 (m, 2 H, CH_2^9), 1.97 – 1.79 (m, 4 H, CH_2)^{10}, 0.91 (t, J = 7.4 Hz, 3 H, CH_3^{11}).

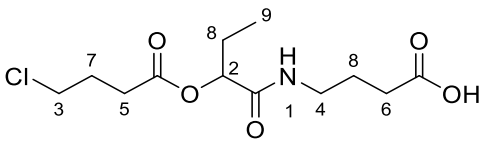
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 173.52, 171.66, 169.84, 135.83, 128.74, 128.47, 128.32, 75.16, 66.64, 44.11, 38.92, 31.91, 31.21, 27.48, 25.23, 24.38, 9.19.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{19}{}^1H_{26}{}^{16}O_5{}^{14}N^{35}Cl$, 384.1572; found, 348.1566, Δ = 0.6 mmu.



Supplementary Figure 1: ^{1}H-NMR of compound **IS1** measured in $CDCl_3$.

Deprotection



ISD1

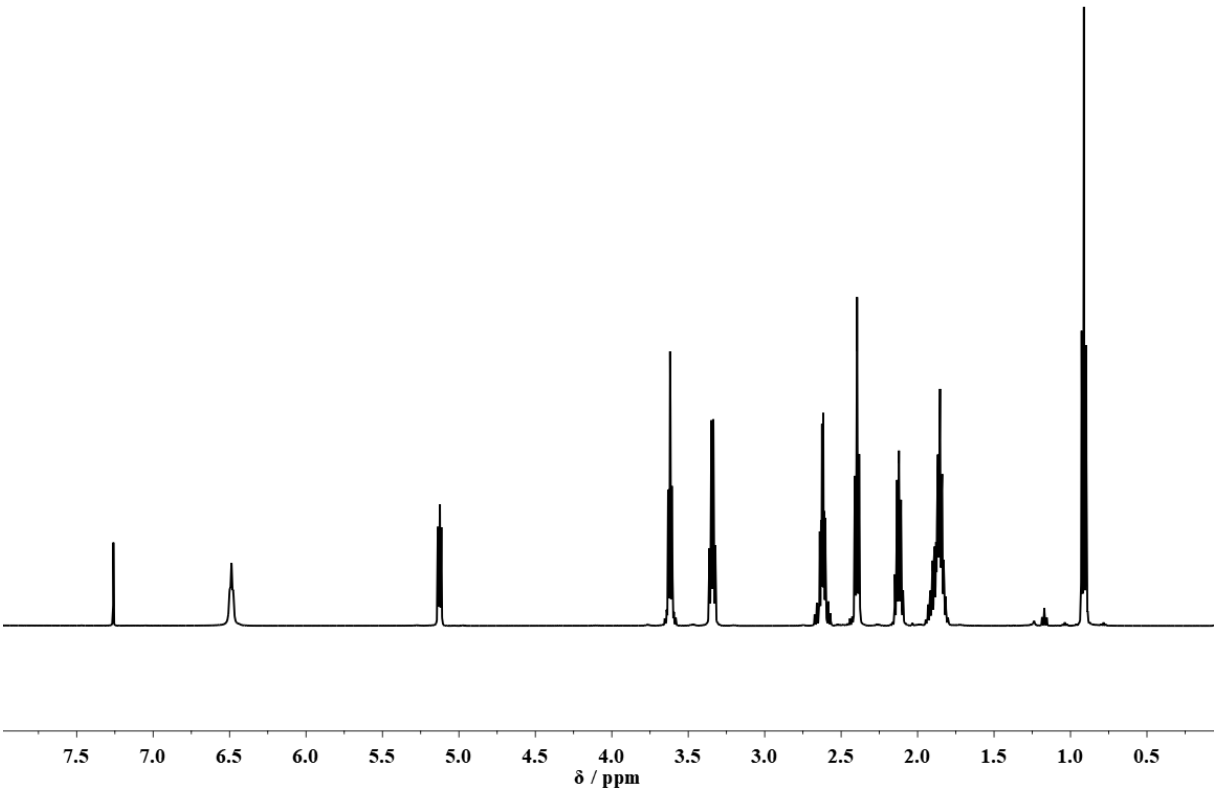
In a 25 mL round bottom flask 4.44 g of **IS1** (11.7 mmol, 1.00 eq.) was dissolved in 8.00 mL ethyl acetate and 8.00 mL THF. Subsequently, 888 mg (20 wt%) palladium on activated carbon **1** were added to the solution. The resulting mixture was purged with hydrogen gas (3 balloons) and stirred for one day at room temperature under hydrogen atmosphere. The crude reaction mixture was filtered over celite® and flushed with 50 mL dichloromethane. After evaporation of the solvents and drying under reduced pressure the product **ISD1** was obtained as a colorless solid (3.32 g, 11.3 mmol, 97.9%).

IR (ATR): ν / cm^{-1} = 3306.2 (w), 2970.4 (m), 2938.1 (m), 2880.5 (w), 2175.0 (vw), 2074.2 (vw), 1727.6 (vs), 1642.1 (vs), 1540.7 (s), 1440.5 (m), 1413.4 (m), 1382.2 (m), 1297.5 (m), 1173.0 (vs), 1141.4 (vs), 1101.9 (s), 1048.8 (m), 980.2 (m), 909.1 (w), 873.9 (w), 786.7 (w), 729.3 (w), 647.2 (m), 431.5 (vw).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 6.49 (t, J = 5.8 Hz, 1 H, NH^2), 5.17 – 5.09 (m, 1 H, CH^2), 3.62 (td, J = 6.3, 1.3 Hz, 2 H, $CH_2{}^3$), 3.34 (q, J = 6.6 Hz, 2 H, $CH_2{}^4$), 2.70 – 2.56 (m, 2 H, $CH_2{}^5$), 2.40 (t, J = 7.0 Hz, 2 H, $CH_2{}^6$), 2.18 – 2.05 (m, 2 H, $CH_2{}^7$), 1.96 – 1.78 (m, 4 H, $CH_2{}^8$), 0.91 (t, J = 7.4 Hz, 3 H, $CH_3{}^9$).

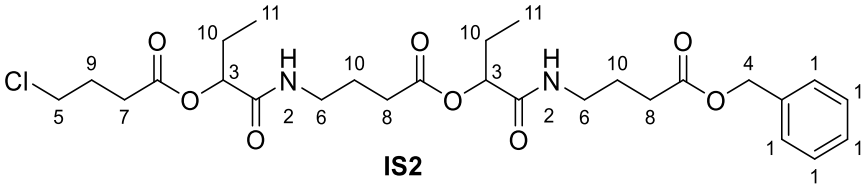
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 177.93, 171.80, 170.40, 75.14, 44.12, 38.79, 31.46, 31.18, 27.45, 25.21, 24.40, 9.17.

ESI-MS [m/z]: $[M + H]^+$ calculated for $^{12}C_{12}{}^{1}H_{20}{}^{16}O_5{}^{14}N{}^{35}Cl$, 294.1103; found, 294.1091, Δ = 1.2 mmu.



Supplementary Figure 2: ^{1}H-NMR of compound **ISD1** measured in $CDCl_3$.

Passerini reaction



IS2

In a 50 mL round bottom flask 3.19 g of **ISD1** (10.9 mmol, 1.00 eq.) were stirred in 10.9 mL DCM. Subsequently 1.17 mL propionaldehyde **A2** (947 mg, 16.3 mmol, 1.50 eq.) and 3.31 g of monomer **IM1** (16.3 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 1 day. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (1:1 → 1:2) to yield the Passerini product **IS2** as a pale highly viscous oil. (5.61 g, 10.1 mmol, 92.9%).

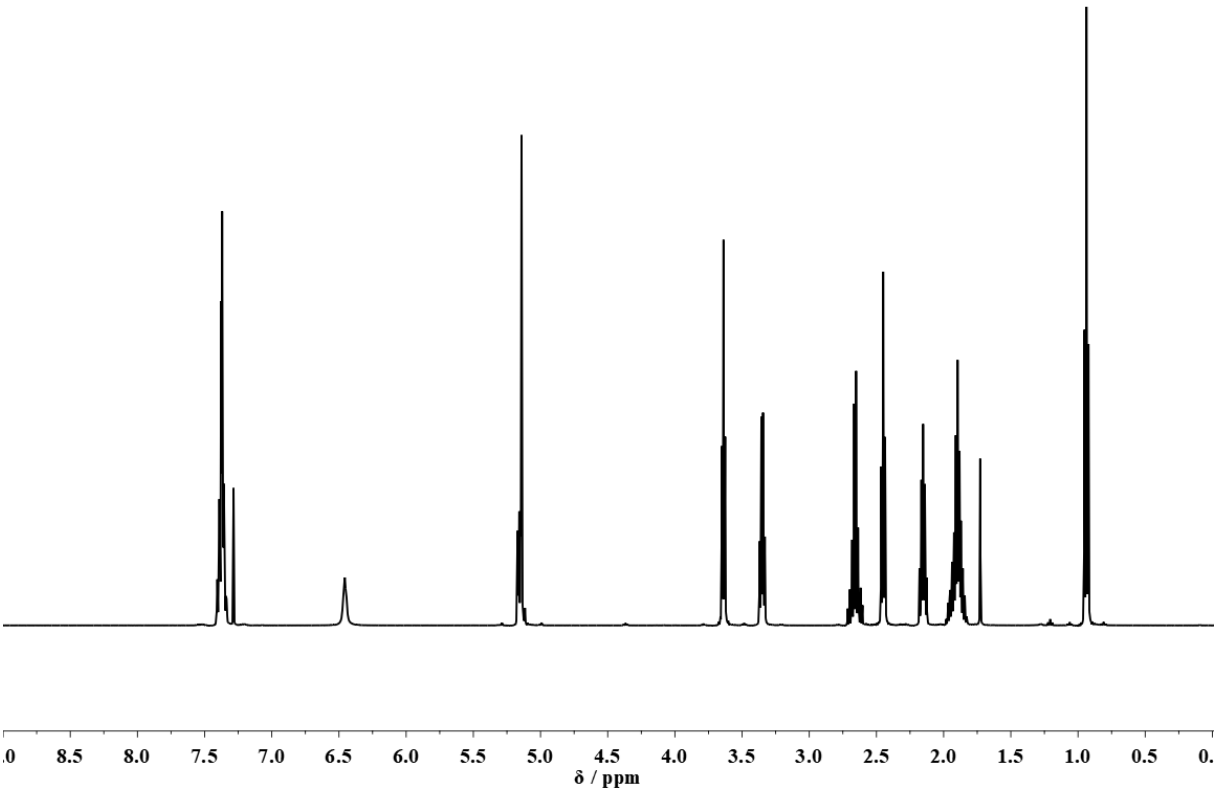
R_f = 0.34 in cyclohexane/ethyl acetate (1:2).

IR (ATR): ν / cm^{-1} = 3305.7 (w), 3088.5 (vw), 2969.7 (w), 2937.0 (w), 2878.6 (vw), 2111.6 (vw), 1733.2 (vs), 1653.9 (vs), 1534.7 (s), 1439.3 (m), 1381.5 (m), 1296.6 (m), 1232.3 (s), 1161.9 (vs), 1101.8 (s), 1048.4 (m), 977.5 (m), 908.1 (w), 787.0 (w), 737.6 (m), 697.9 (w), 650.3 (w).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.42 – 7.29 (m, 5 H, CH_{Ar}^1), 7.08 (dt, J = 23.6, 5.9 Hz, 1 H, NH^2), 6.39 (dt, J = 20.4, 6.4 Hz, 1 H, NH^2), 5.17 – 5.03 (m, 4 H, CH^3, CH_2^4), 3.68 – 3.55 (m, 2 H, CH_2^5), 3.51 – 3.15 (m, 4 H, CH_2^6), 2.69 – 2.54 (m, 2 H, CH_2^7), 2.51 – 2.35 (m, 4 H, CH_2^8), 2.18 – 2.07 (m, 2 H, CH^9), 1.98 – 1.70 (m, 8 H, CH_2^{10}), 1.01 – 0.83 (m, 6 H, CH_3^{11}).

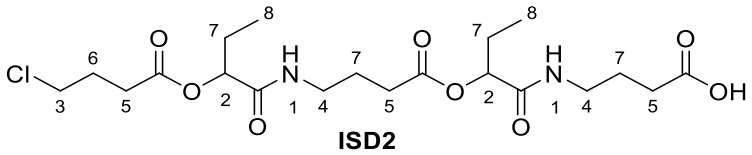
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 173.44, 172.40, 171.77 ($C_{quart.}^a$), 171.71 ($C_{quart.}^b$), 170.42 ($C_{quart.}^a$), 170.41 ($C_{quart.}^b$), 170.15 ($C_{quart.}^a$), 170.09 ($C_{quart.}^b$), 136.01 (C_{Ar}^a), 135.98 (C_{Ar}^b), 128.68 (CH_{Ar}), 128.69 (CH_{Ar}), 128.37 (CH_{Ar}), 128.35 (CH_{Ar}), 128.26 (CH_{Ar}), 75.31 ($CH^{3\text{ or }4,b}$), 75.30 ($CH^{3\text{ or }4,b}$), 75.25 ($CH^{3\text{ or }4,a}$), 75.19 ($CH^{3\text{ or }4,b}$), 66.46 ($CH_2^{4,a}$), 66.44 ($CH_2^{4,b}$), 44.11 ($CH_2^{5,a}$), 44.09 ($CH_2^{5,b}$), 38.80, 38.07, 31.84 (CH_2^8), 31.18 ($CH_2^{7,a}$), 31.17 ($CH_2^{7,b}$), 31.00 (CH_2^8), 27.45 ($CH_2^{9,a}$), 27.41 ($CH_2^{9,b}$), 25.27, 25.24, 25.19, 25.09, 25.07, 24.59, 9.39 ($CH_3^{11,a}$), 9.35 ($CH_3^{11,b}$), 9.23 ($CH_3^{11,a}$), 9.22 ($CH_3^{11,b}$).

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{27}{}^1H_{39}{}^{16}O_8{}^{14}N_2{}^{35}Cl$, 554.2468; found, 554.2463, Δ = 0.5 mmu.



Supplementary Figure 3: ^{1}H-NMR of compound **IS2** measured in $CDCl_3$.

Deprotection



In a 25 mL round bottom flask 5.41 g of **IS2** (9.76 mmol, 1.00 eq.) was dissolved in 8.00 mL ethyl acetate and 8.00 mL THF. Subsequently, 1.08 g (20 wt%) palladium on activated carbon **1** were added to the solution. The resulting mixture was purged with hydrogen gas (3 balloons) and stirred for one day at room temperature under hydrogen atmosphere. The crude reaction mixture was filtered over celite® and flushed with 50 mL dichloromethane. After evaporation of the solvents and drying under reduced pressure the product **ISD2** was obtained as a colorless solid (4.42 g, 9.52 mmol, 97.5%).

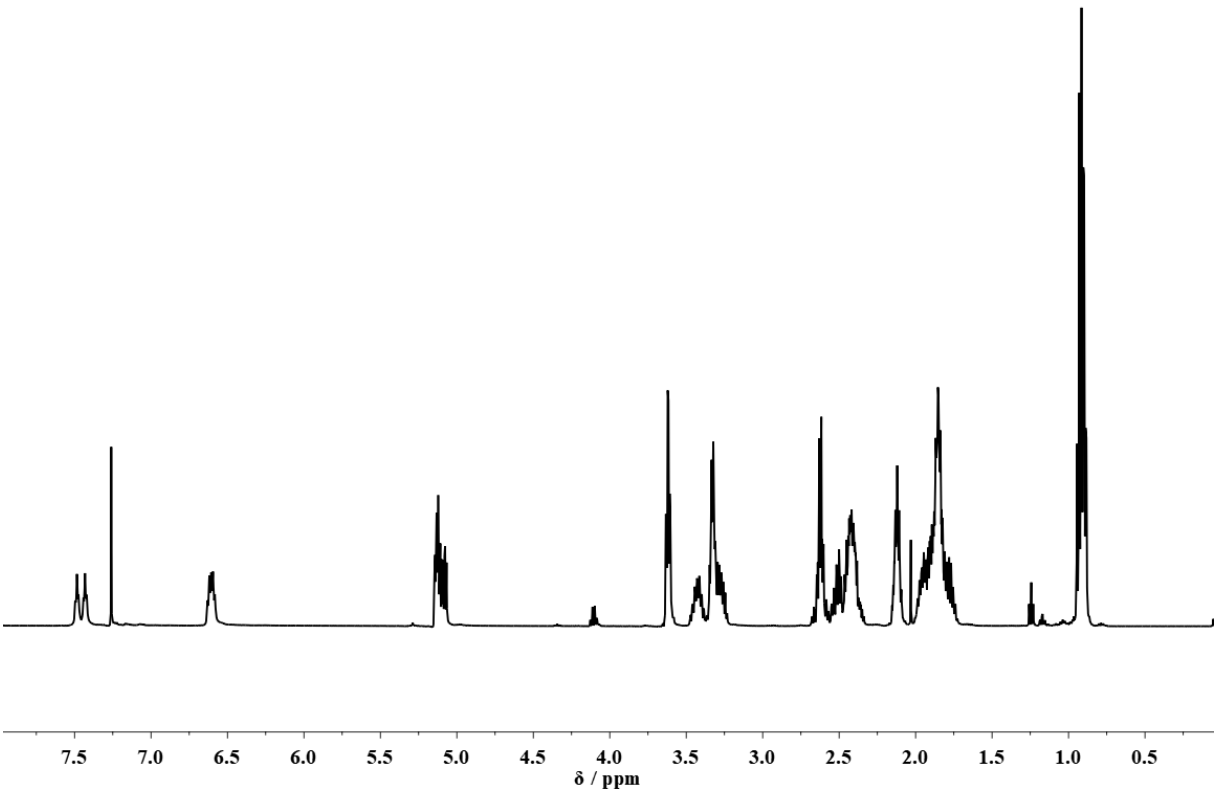
Please note, that the complex mixtures of several isomers cannot be distinguished in the NMR.

IR (ATR): ν / cm^{-1} = 3305.4 (w), 3084.0 (w), 2971.0 (m), 2937.7 (m), 2880.3 (w), 2096.7 (vw), 1732.0 (vs), 1649.3 (vs), 1539.2 (vs), 1439.7 (m), 1416.1 (m), 1380.6 (m), 1297.8 (m), 1164.9 (vs), 1102.1 (s), 1048.1 (m), 978.3 (m), 908.3 (w), 786.7 (w), 646.6 (w), 430.1 (vw), 404.8 (vw).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.48 (t, J = 5.5 Hz, 1 H, $NH^{1,a}$), 7.43 (t, J = 5.5 Hz, 1 H, $NH^{1,b}$), 6.61 (dt, J = 12.1, 6.2 Hz, 1 H, NH^{1}), 5.17 – 5.04 (m, 2 H, CH^{2}), 3.67 – 3.58 (m, 2 H, CH_2^{3}), 3.48 – 3.21 (m, 4 H, CH_2^{4}), 2.69 – 2.32 (m, 6 H, CH_2^{5}), 2.17 – 2.07 (m, 2 H, CH_2^{6}), 2.01 – 1.71 (m, 8 H, CH_2^{7}), 0.96 – 0.86 (m, 6 H, CH_3^{8}).

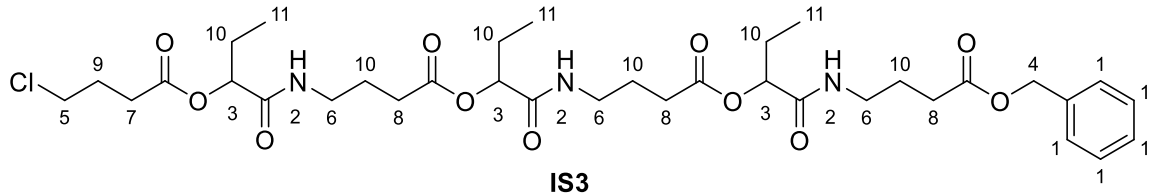
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 176.99, 172.45, 172.35, 171.89, 171.88, 171.10, 171.05, 170.68, 170.62, 75.16, 75.08, 75.03, 44.10, 39.42 (CH_2^{4}), 38.51 ($CH_2^{4,a}$), 38.46 ($CH_2^{4,b}$), 32.04 ($CH_2^{5,b}$), 32.03 ($CH_2^{5,a}$), 31.18, 31.07 ($CH_2^{5,a}$), 31.01 ($CH_2^{5,b}$), 27.42 ($CH_2^{6,a}$), 27.39 ($CH_2^{6,b}$), 25.25 ($CH_2^{7,a}$), 25.24 ($CH_2^{7,b}$), 25.21 ($CH_2^{7,a}$), 25.15 ($CH_2^{7,b}$), 24.89 ($CH_2^{7,a}$), 24.81 ($CH_2^{7,b}$), 23.76 ($CH_2^{7,a}$), 23.72 ($CH_2^{7,b}$), 9.28 ($CH_3^{8,a}$), 9.24 ($CH_3^{8,b}$), 9.22 ($CH_3^{8,a}$), 9.20 ($CH_3^{8,b}$).

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{20}{}^{1}H_{33}{}^{16}O_8{}^{14}N_2{}^{35}Cl$, 465.1998; found, 465.1995, Δ = 0.3 mmu.



Supplementary Figure 4: ^{1}H-NMR of compound **ISD2** measured in $CDCl_3$.

Passerini reaction



IS3

In a 50 mL round bottom flask 3.96 g of **ISD2** (8.52 mmol, 1.00 eq.) were stirred in 8.00 mL DCM. Subsequently 811 µL propionaldehyde **A2** (495 mg, 8.52 mmol, 1.00 eq.) and 2.60 g of monomer **IM1** (12.8 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 1 day. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (1:1 → 1:4) to yield the Passerini product **IS3** as a pale highly viscous oil. (4.96 g, 6.82 mmol, 80.1%).

R_f = 0.50 in cyclohexane/ethyl acetate (0:1).

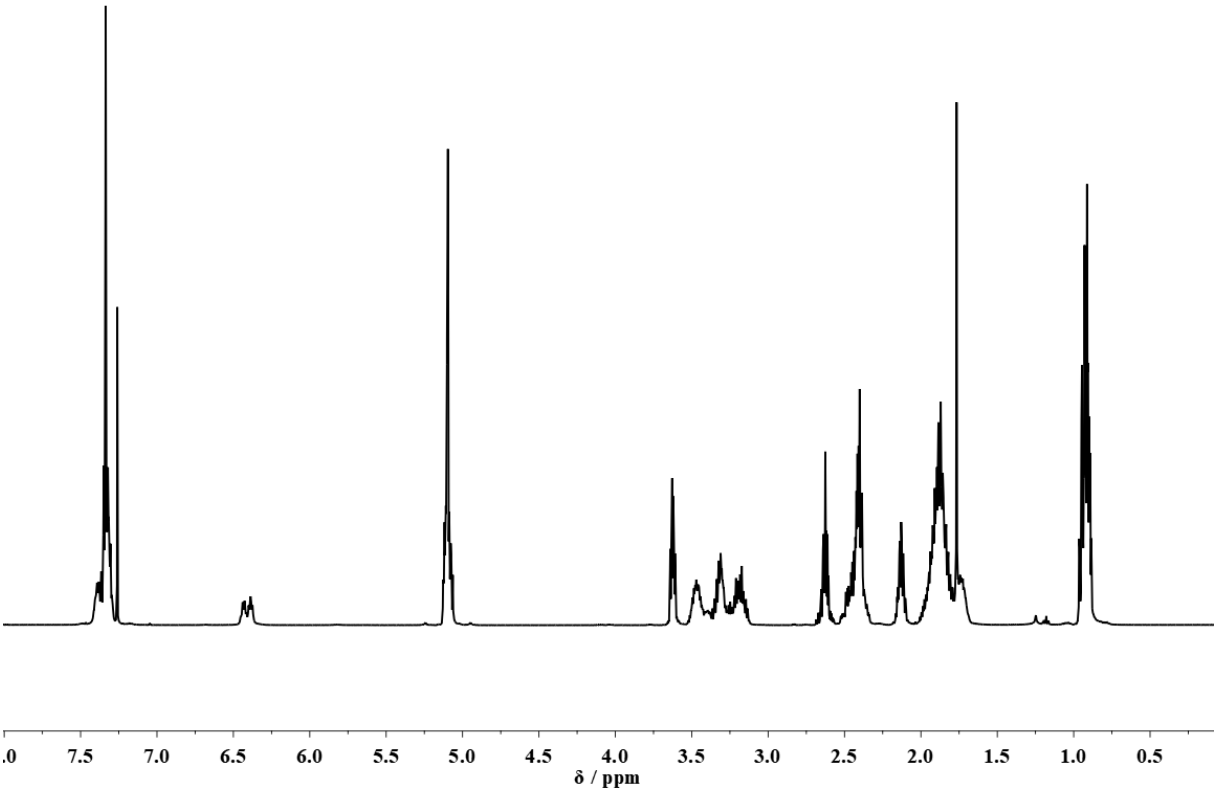
Please note, that the complex mixtures of several isomers cannot be distinguished in the NMR.

IR (ATR): ν / cm^{-1} = 3307.4 (w), 3088.0 (vw), 2970.6 (w), 2937.1 (w), 2878.7 (vw), 1733.7 (vs), 1652.8 (vs), 1535.0 (vs), 1438.9 (m), 1381.1 (m), 1228.3 (s), 1160.3 (vs), 1101.8 (s), 1047.8 (m), 977.1 (m), 907.7 (w), 786.8 (w), 737.7 (w), 698.0 (m), 647.4 (w), 400.6 (vw).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.45 – 7.28 (m, 7 H, CH_{Ar}^1, NH^2), 6.48 – 6.34 (m, 1 H, NH^2), 5.17 – 5.03 (m, 5 H, CH^3, CH_2^4), 3.68 – 3.58 (m, 2 H, CH_2^5), 3.54 – 3.11 (m, 6 H, CH_2^6), 2.70 – 2.55 (m, 2 H, CH_2^7), 2.54 – 2.33 (m, 6 H, CH_2^8), 2.19 – 2.08 (m, 2 H, CH_2^9), 2.02 – 1.68 (m, 12 H, CH_2^{10}), 0.98 – 0.87 (m, 9 H, CH_3^{11}).

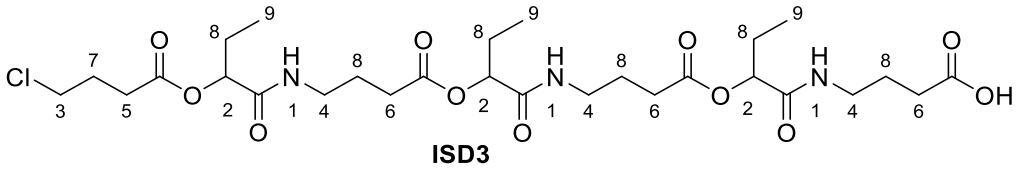
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 173.47 – 173.27 (m, $C_{quart.}$), 172.72 – 172.23 (m, $C_{quart.}$), 171.92 – 171.68 (m, $C_{quart.}$), 170.80 – 170.57 (m, $C_{quart.}$), 170.37 – 170.13 (m, $C_{quart.}$), 136.18 – 135.95 (m, $C_{quart.}$), 128.66 (CH_{Ar}^1), 128.39 – 128.14 (m, CH_{Ar}^1), 75.87 – 74.85 (m, CH^3), 66.61 – 66.00 (m, CH_2^4), 44.28 – 43.61 (m, CH_2^5), 38.75 (CH_2^6), 38.33 – 37.52 (m, CH_2^6), 32.11 – 31.63 (m, $CH_2^{7 \text{ or } 8}$), 31.63 – 30.47 (m, $CH_2^{7 \text{ or } 8}$), 27.86 – 27.10 (m, CH_2^9), 25.59 – 24.91 (m, CH_2^{10}), 24.78 – 24.49 (m, CH_2^{10}), 9.98 – 8.62 (m, CH_2^{11}).

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{35}{}^1H_{52}{}^{16}O_{11}{}^{14}N_3{}^{35}Cl$, 726.3363; found, 726.3351, Δ = 1.2 mmu.



Supplementary Figure 5: ^{1}H-NMR of compound **IS3** measured in $CDCl_3$.

Deprotection



ISD3

In a 25 mL round bottom flask 3.57 g of **IS3** (5.61 mmol, 1.00 eq.) was dissolved in 8.00 mL ethyl acetate and 8.00 mL THF. Subsequently, 814 mg (20 wt%) palladium on activated carbon **1** were added to the solution. The resulting mixture was purged with hydrogen gas and stirred for one day at room temperature under hydrogen atmosphere (3 balloons). The crude reaction mixture was filtered over celite® and flushed with 50 mL dichloromethane. After evaporation of the solvents and drying under reduced pressure the product **ISD3** was obtained as a colorless solid (3.46 g, 5.43 mmol, 96.9%).

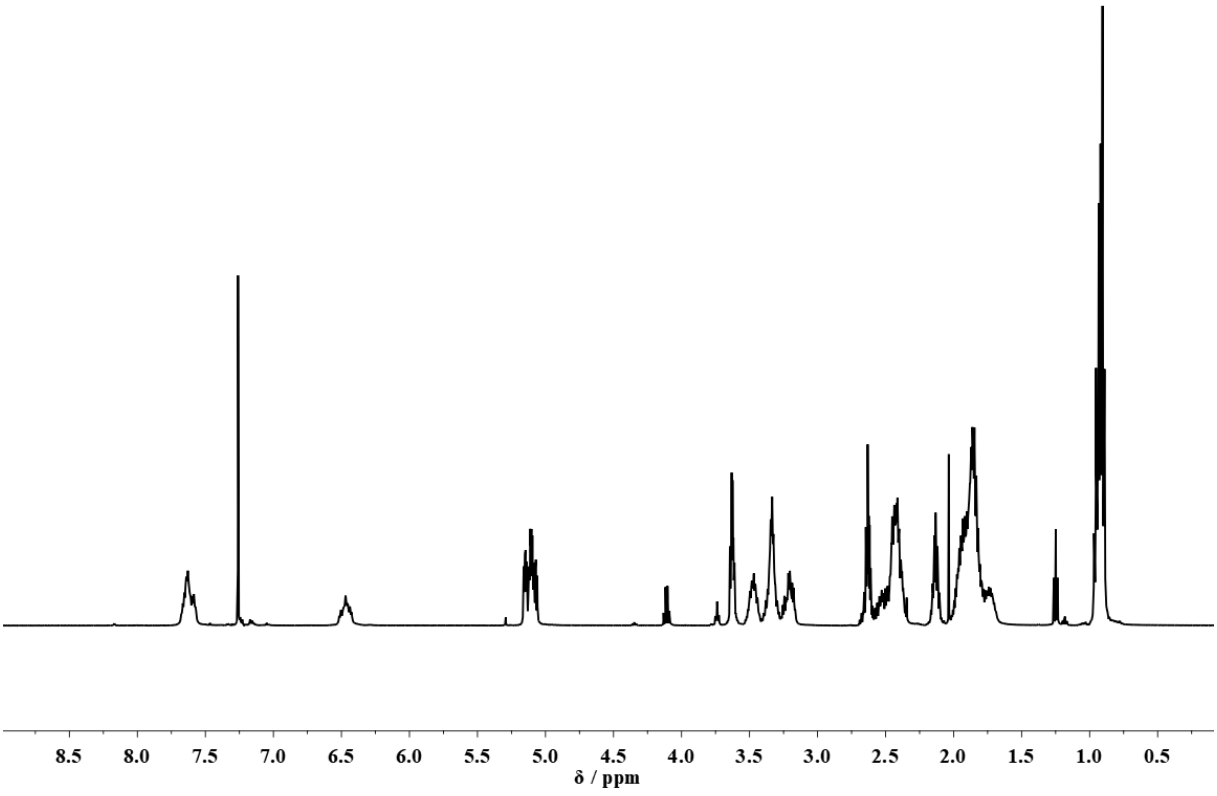
Please note, that the complex mixtures of several isomers cannot be distinguished in the NMR.

IR (ATR): ν / cm^{-1} = 3306.8 (w), 3085.2 (vw), 2970.4 (w), 2936.9 (w), 2879.4 (vw), 1734.4 (s), 1651.8 (s), 1537.9 (s), 1439.3 (w), 1380.6 (w), 1162.2 (s), 1101.8 (m), 1047.6 (w), 977.5 (w), 908.1 (vw), 787.9 (vw), 644.3 (w).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.72 – 7.55 (m, 2 H, NH^1), 6.56 – 6.38 (m, 1 H, NH^1), 5.20 – 5.03 (m, 3 H, CH^2), 3.68 – 3.57 (m, 2 H, $CH_2{}^3$), 3.53 – 3.16 (m, 6 H, $CH_2{}^4$), 2.70 – 2.59 (m, 2 H, $CH_2{}^5$), 2.58 – 2.32 (m, 6 H, $CH_2{}^6$), 2.19 – 2.08 (m, 2 H, $CH_2{}^7$), 2.02 – 1.65 (m, 12 H, $CH_2{}^8$), 1.02 – 0.78 (m, 9 H, $CH_3{}^9$).

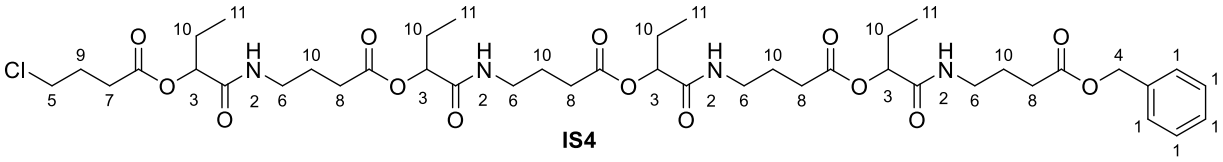
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 176.66 – 176.46 (m, C_{quart}), 172.66 – 172.39 (m, C_{quart}), 171.97 – 171.79 (m, C_{quart}), 171.54 – 171.33 (m, C_{quart}), 170.96 – 170.68 (m, C_{quart}), 75.34 – 75.11 (m, CH^2), 75.07 – 74.81 (m, CH^2), 44.19 – 44.02 (m, $CH_2{}^3$), 39.64 – 39.47 (m, $CH_2{}^4$), 38.67 – 38.46 (m, $CH_2{}^4$), 38.02 – 37.78 (m, $CH_2{}^4$), 32.32 – 32.13 (m, $CH_2{}^{5\text{ or }6}$), 31.45 – 30.98 (m, $CH_2{}^{5\text{ or }6}$), 30.84 – 30.39 (m, $CH_2{}^{5\text{ or }6}$), 27.57 – 27.29 (m, $CH_2{}^7$), 25.41 – 25.03 (m, $CH_2{}^8$), 24.82 (s, $CH_2{}^8$), 23.76 (s, $CH_2{}^8$), 23.58 (s, $CH_2{}^8$), 9.47 – 9.19 (m, $CH_3{}^9$).

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{28}{}^{1}H_{46}{}^{16}O_{11}{}^{14}N_3{}^{35}Cl$, 636.2894; found, 636.2893, Δ = 0.1 mmu.



Supplementary Figure 6: ^{1}H-NMR of compound **ISD3** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask 3.27 g of **ISD3** (5.14 mmol, 1.00 eq.) were stirred in 5.00 mL DCM. Subsequently 553 µL propionaldehyde **A2** (448 mg, 7.71 mmol, 1.00 eq.) and 1.56 g of monomer **IM1** (7.71 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 1 day. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (1:2 → 0:1) to yield the Passerini product **IS4** as a pale highly viscous oil. (4.43 g, 4.94 mmol, 96.1%).

R_f = 0.81 in cyclohexane/ethyl acetate (1:1).

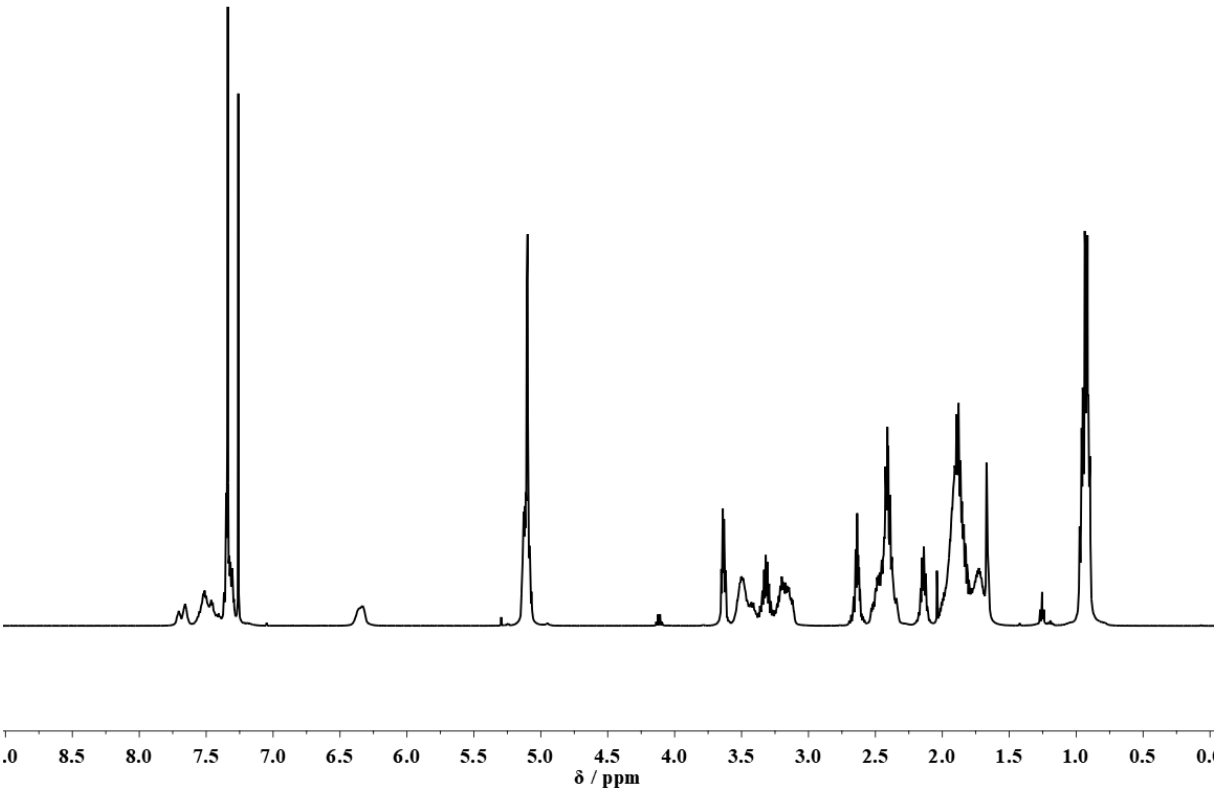
Please note, that the complex mixtures of several isomers cannot be distinguished in the NMR.

IR (ATR): ν / cm^{-1} = 3306.7 (w), 3084.5 (vw), 2970.8 (w), 2936.6 (w), 2879.4 (vw), 1734.4 (vs), 1652.0 (vs), 1535.3 (vs), 1438.7 (m), 1380.8 (m), 1225.1 (s), 1159.2 (vs), 1101.7 (s), 1047.5 (m), 976.9 (m), 907.6 (w), 786.6 (vw), 734.8 (vw), 697.6 (m).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.77 – 7.28 (m, 8 H, CH_{Ar}^1, NH^2), 6.43 – 6.27 (m, 1 H, NH^2), 5.20 – 5.02 (m, 6 H, CH^3, CH_2^4), 3.68 – 3.59 (m, 2 H, CH_2^5), 3.57 – 3.08 (m, 8 H, CH_2^6), 2.71 – 2.57 (m, 2 H, CH_2^7), 2.55 – 2.31 (m, 8 H, CH_2^8), 2.20 – 2.08 (m, 2 H, CH_2^9), 2.03 – 1.65 (m, 16 H, CH_2^{10}), 1.02 – 0.87 (m, 12 H, CH_2^{11}). *Small impurities of EA, DCM and THF are visible in the NMR.*

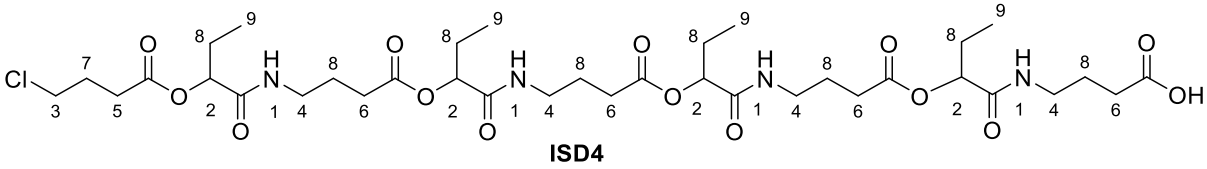
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 173.47 – 173.27 (m, $C_{quart.}$), 172.81 – 172.19 (m, C_{quart}), 171.89 – 171.66 (m, C_{quart}), 171.07 – 170.71 (m, C_{quart}), 170.50 – 170.37 (m, C_{quart}), 170.36 – 170.25 (m, C_{quart}), 136.14 (C_{quart}), 128.67 (CH_{Ar}^1), 128.34 – 128.22 (m, CH_{Ar}^1), 75.51 – 75.07 (m, CH^3), 66.43 – 66.27 (m, CH_2^4), 44.21 – 43.84 (m, CH_2^5), 38.75 (CH_2^6), 37.94 – 37.54 (m, CH_2^6), 31.90 – 31.77 (m, $CH_2^{7\text{ or }8}$), 31.23 – 31.12 (m, $CH_2^{7\text{ or }8}$), 31.06 – 30.49 (m, $CH_2^{7\text{ or }8}$), 27.49 – 27.30 (m, CH_2^9), 25.48 – 25.10 (m, CH_2^{10}), 24.85 – 24.67 (m, CH_2^{10}), 9.60 – 9.14 (m, CH_3^{11}).

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{43}{}^1H_{65}{}^{16}O_{14}{}^{14}N_4{}^{35}Cl$, 897.4259; found, 897.4258, Δ = 0.1 mmu.



Supplementary Figure 7: ^{1}H-NMR of compound **IS4** measured in $CDCl_3$.

Deprotection



In a 25 mL round bottom flask 1.51 g of **IS4** (1.69 mmol, 1.00 eq.) was dissolved in 5.00 mL ethyl acetate and 5.00 mL THF. Subsequently, 302 mg (20 wt%) palladium on activated carbon **1** were added to the solution. The resulting mixture was purged with hydrogen gas (3 balloons) and stirred for one day at room temperature under hydrogen atmosphere. The crude reaction mixture was filtered over celite® and flushed with 50 mL dichloromethane. After evaporation of the solvents and drying under reduced pressure the product **ISD4** was obtained as a colorless solid (1.01 g, 1.25 mmol, 74.1%).

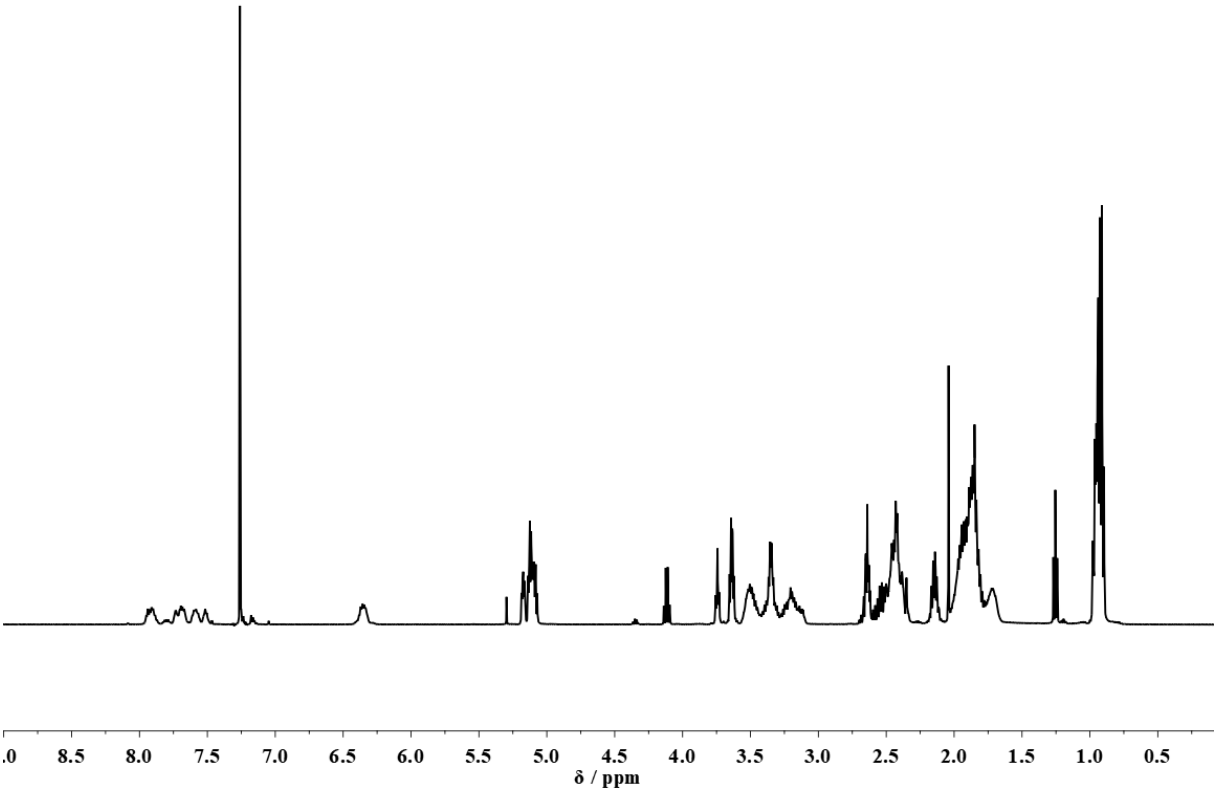
Please note, that the complex mixtures of several isomers cannot be distinguished in the NMR.

IR (ATR): ν / cm^{-1} = 3305.2 (vw), 2970.5 (vw), 2938.0 (vw), 2879.2 (vw), 1735.9 (m), 1653.8 (w), 1539.2 (w), 1439.0 (vw), 1380.8 (vw), 1235.0 (vw), 1162.2 (w), 1102.1 (vw), 1047.5 (vw), 977.2 (vw), 649.4 (vw).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.98 – 7.43 (m, 3 H, NH^1), 6.43 – 6.28 (m, 1 H, NH^1), 5.21 – 5.04 (m, 4 H, CH^2), 3.67 – 3.60 (m, 2 H, $CH_2{}^3$), 3.59 – 3.06 (m, 8 H, $CH_2{}^4$), 2.72 – 2.60 (m, 2 H, $CH_2{}^5$), 2.60 – 2.30 (m, 8 H, $CH_2{}^6$), 2.19 – 2.10 (m, 2 H, $CH_2{}^7$), 2.02 – 1.59 (m, 16 H, $CH_2{}^8$), 1.02 – 0.86 (m, 12 H, $CH_3{}^9$). *Impurities of EA, DCM and THF are visible in the NMR.*

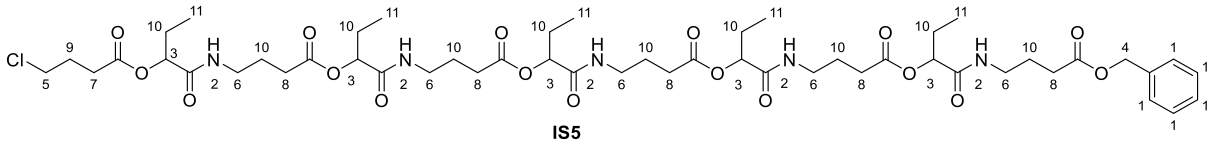
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 176.33 – 175.98 (m, $C_{quart.}$), 172.84 – 172.21 (m, $C_{quart.}$), 171.89 – 171.51 (m, $C_{quart.}$), 171.31 – 171.05 (m, $C_{quart.}$), 171.05 – 170.62 (m, $C_{quart.}$), 75.48 – 74.79 (m, CH^2), 44.84 – 43.03 (m, $CH_2{}^3$), 39.90 – 39.45 (m, $CH_2{}^4$), 38.83 – 38.32 (m, $CH_2{}^4$), 38.11 – 37.42 (m, $CH_2{}^4$), 32.51 – 32.17 (m, $CH_2{}^{5\text{ or }6}$), 31.51 – 31.00 (m, $CH_2{}^{5\text{ or }6}$), 30.96 – 30.41 (m, $CH_2{}^{5\text{ or }6}$), 27.64 – 27.19 (m, $CH_2{}^7$), 25.53 – 24.78 (m, $CH_2{}^8$), 23.76 – 23.42 (m, $CH_2{}^8$), 9.61 – 9.10 (m, $CH_3{}^9$).

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{36}{}^{1}H_{59}{}^{16}O_{14}{}^{14}N_4{}^{35}Cl$, 807.3789; found, 807.3781, Δ = 0.8 mmu.



Supplementary Figure 8: ^{1}H-NMR of compound **ISD4** measured in $CDCl_3$. *Impurities of EA, DCM and THF are visible in the NMR.*

Passerini reaction



IS5

In a 50 mL round bottom flask 886 mg of **ISD4** (1.07 mmol, 1.00 eq.) was stirred in 4.00 mL DCM, subsequently 231 µL propionaldehyde **A2** (187 mg, 3.32 mmol, 1.00 eq.) and 328 mg of the monomer **IM1** (1.61 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 1 day. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (1:2 → 0:1), ethyl acetate and acetone (1:1) yield the passerini product **IS5** as a pale highly viscous oil. (1.13 g, 1.05 mmol, 97.8%).

R_f = 0.59 in ethyl acetate/acetone (1:1).

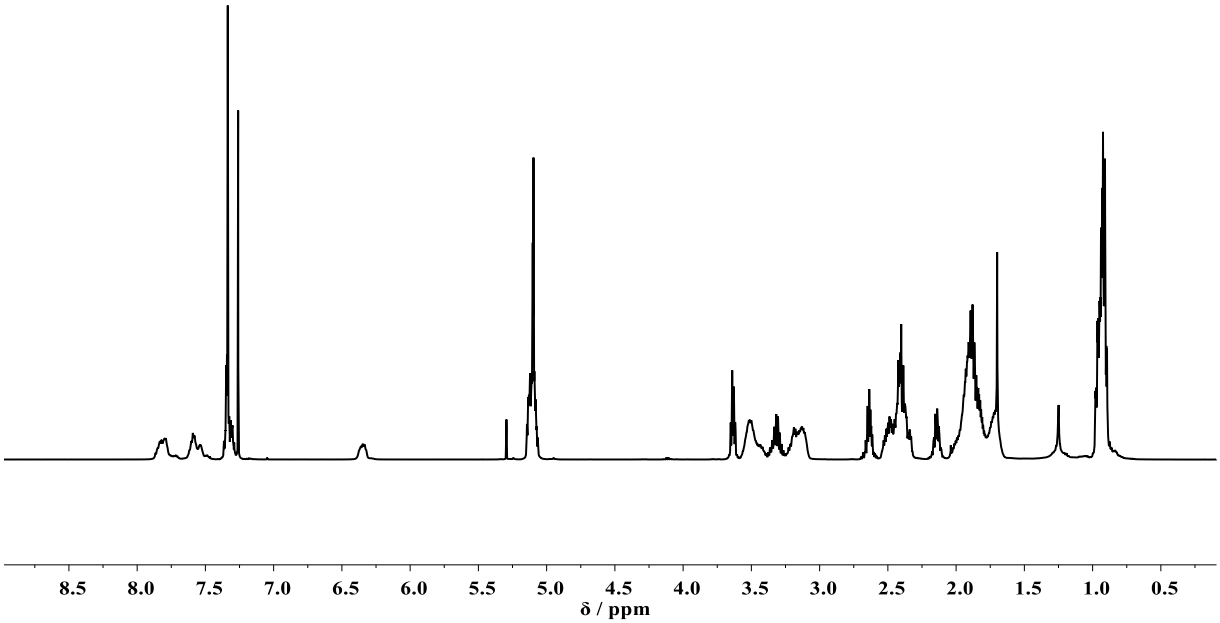
Please note, that the complex mixtures of several isomers cannot be distinguished in the NMR.

IR (ATR): ν / cm^{-1} = 3306.9 (w), 3088.6 (vw), 2971.2 (w), 2937.3 (w), 2879.1 (w), 1734.1 (vs), 1651.3 (vs), 1534.9 (s), 1438.5 (m), 1380.6 (m), 1225.5 (s), 1158.8 (vs), 1101.5 (s), 1047.5 (m), 976.9 (m), 907.8 (w), 786.2 (vw), 736.0 (w), 697.9 (m), 642.3 (w).

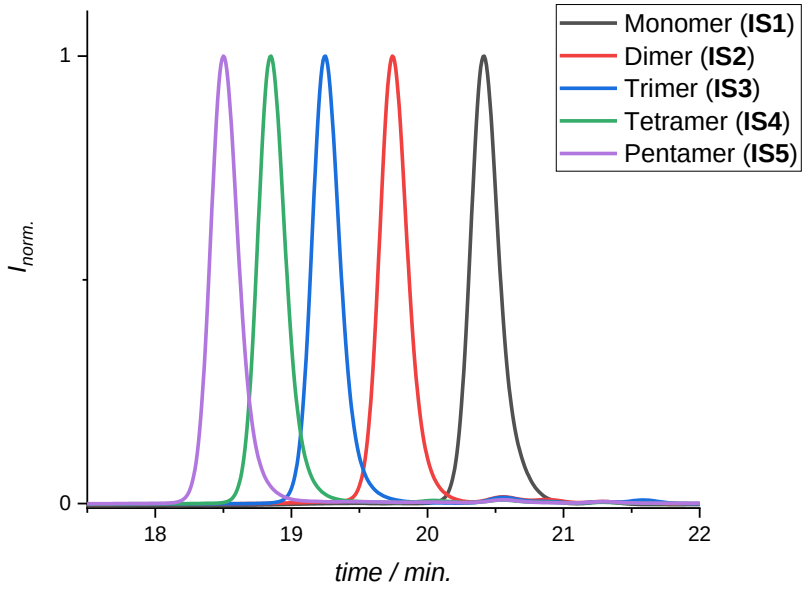
^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.90 – 7.69 (m, 2 H, NH^2), 7.64 – 7.46 (m, 2 H, NH^2), 7.39 – 7.27 (m, 5 H, $CH_{Ar}{}^1$), 6.41 – 6.28 (m, 1 H, NH^2), 5.18 – 5.02 (m, 7 H, CH^3, $CH_2{}^4$), 3.68 – 3.59 (m, 2 H, $CH_2{}^5$), 3.58 – 3.03 (m, 10 H, $CH_2{}^6$), 2.68 – 2.57 (m, 2 H, $CH_2{}^7$), 2.56 – 2.30 (m, 10 H, $CH_2{}^8$), 2.20 – 2.09 (m, 2 H, $CH_2{}^9$), 2.06 – 1.63 (m, 20 H, $CH_2{}^{10}$), 1.01 – 0.78 (m, 15 H, $CH_2{}^{11}$). *Impurities of EA, DCM and THF are visible in the NMR.*

^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 173.35, 172.92 – 172.17 (m, $C_{quart.}$), 171.87 – 171.67 (m, C_{quart}), 171.24 – 170.77 (m, C_{quart}), 170.52 – 170.27 (m, C_{quart}), 136.16 (C_{quart}), 128.65 ($CH_{Ar}{}^1$), 128.27 (d, J = 2.7 Hz, $CH_{Ar}{}^1$), 75.69 – 74.50 (m, CH^3), 66.32 (d, J = 3.0 Hz, $CH_2{}^4$), 44.09 (d, J = 3.7 Hz, $CH_2{}^5$), 38.73 (s, $CH_2{}^6$), 37.98 – 37.23 (m, $CH_2{}^6$), 31.83 (d, J = 4.6 Hz, $CH_2{}^{7\text{ or }8}$), 31.27 – 30.31 (m, $CH_2{}^{7\text{ or }8}$), 27.37 (d, J = 10.2 Hz, $CH_2{}^9$), 25.53 – 24.61 (m, $CH_2{}^{10}$), 9.65 – 8.86 (m, $CH_3{}^{11}$).

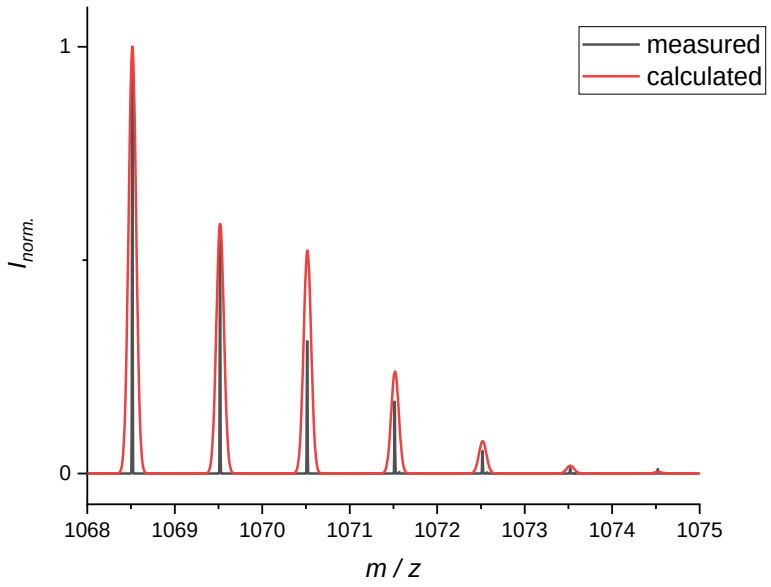
ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{51}{}^1H_{78}{}^{16}O_{17}{}^{14}N_5{}^{35}Cl$, 1068.5154; found, 1068.51445, Δ = 0.9 mmu.



Supplementary Figure 9: ^{1}H-NMR of compound **IS5** measured in $CDCl_3$. *Impurities of EA, DCM and THF are visible in the NMR.*



Supplementary Figure 10: SEC traces of the intermediates after each P-3CR in the synthesis of product **IS5**.

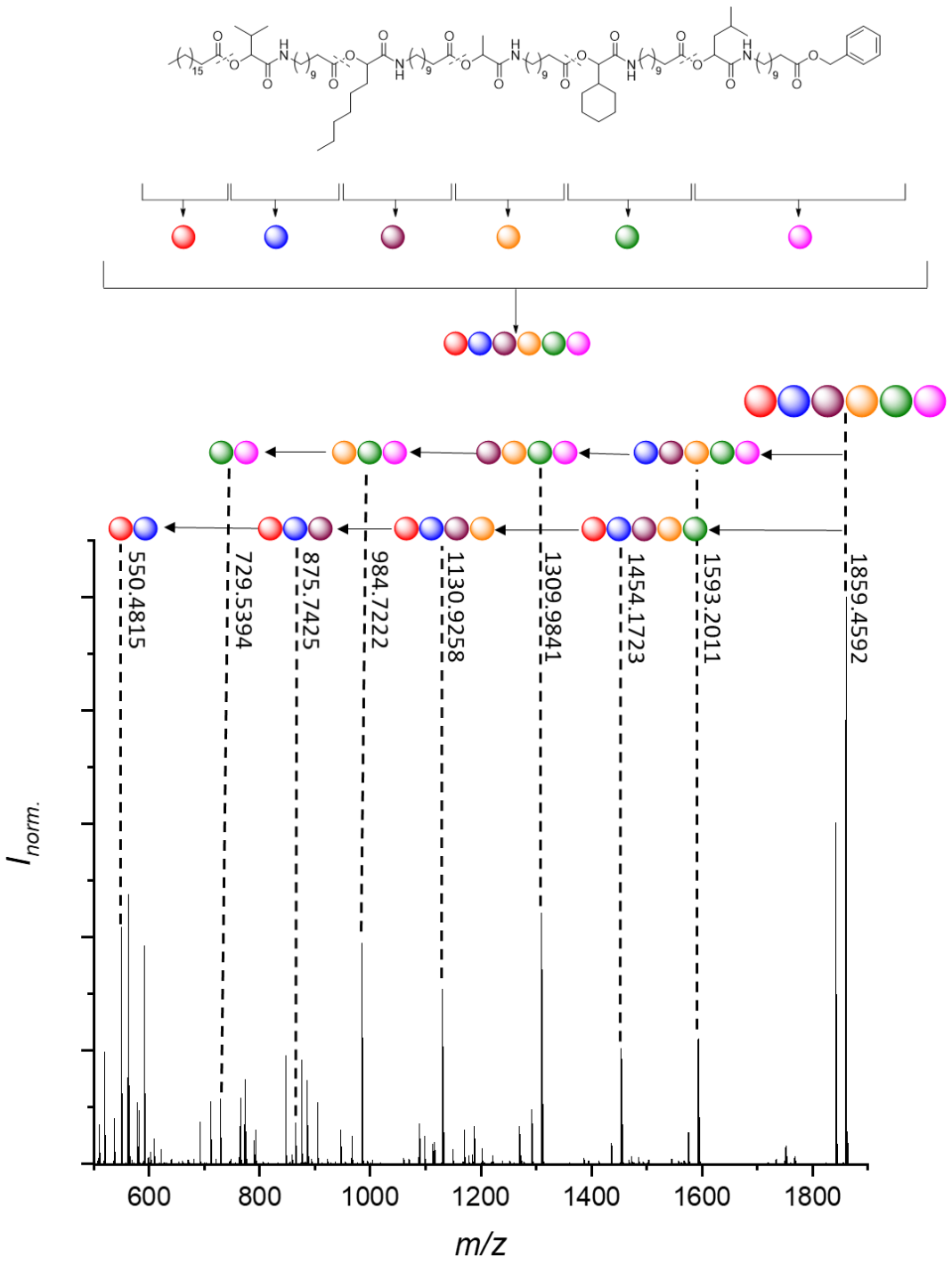


Supplementary Figure 11: High resolution ESI-MS measurement of **IS5**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (red).

6.3.2 Identifying the most common fragmentation patterns of sequence-defined Passerini macromolecules

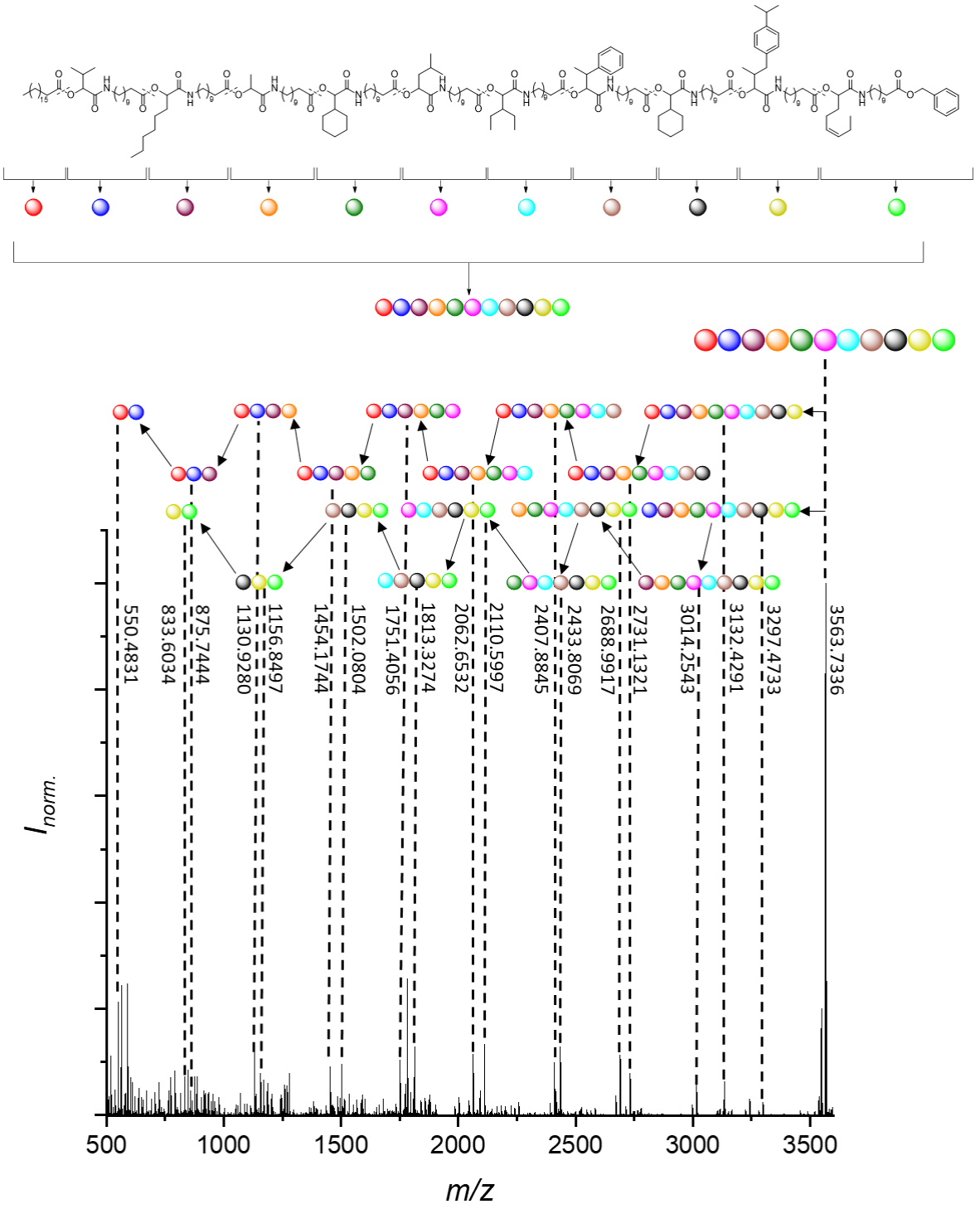
6.3.2.1 Side chain defined

6.3.2.1.1 Side chain defined pentamer **SC5**



Supplementary Figure 12: Structure and ESI-MS/MS fragmentation of the side chain-defined pentamer **SC5**. The assigned peaks belong to the most prominent fragmentation pattern (in this case: fragmentation next to the carbonyl) from both ends of the molecule. Other intense peaks belong to the other prominent fragmentation pattern and to the middle fragments and can be assigned analogously (for the sake of clarity not shown in this graph).

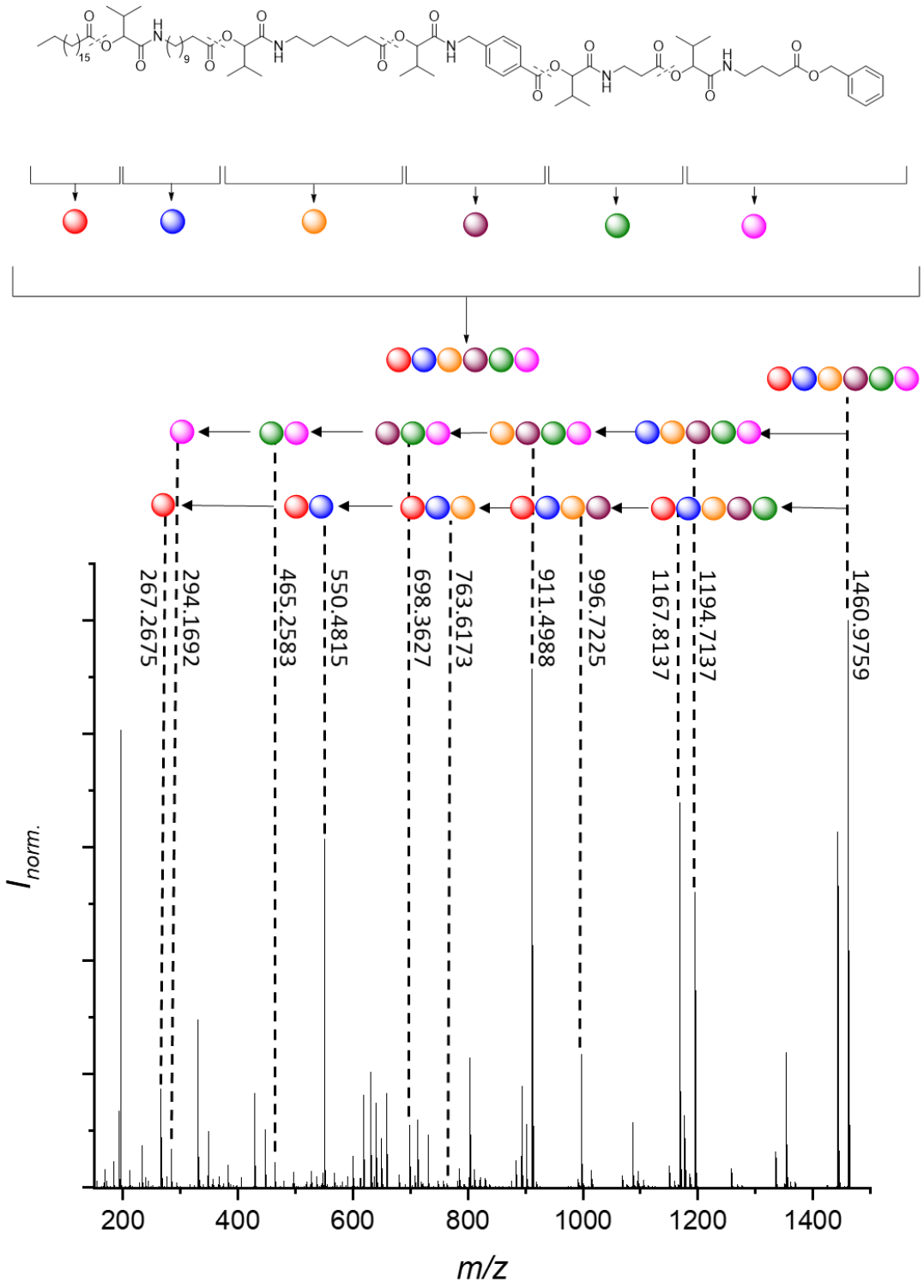
6.3.2.1.2 Side chain defined decamer **SC10**



Supplementary Figure 13: Structure and ESI-MS/MS fragmentation of the side chain-defined decamer **SC10**. The assigned peaks belong to the most prominent fragmentation pattern (in this case: fragmentation next to the carbonyl) from both ends of the molecule. Other intense peaks belong to the other prominent fragmentation pattern and to the middle fragments and can be assigned analogously (for the sake of clarity not shown in this graph).

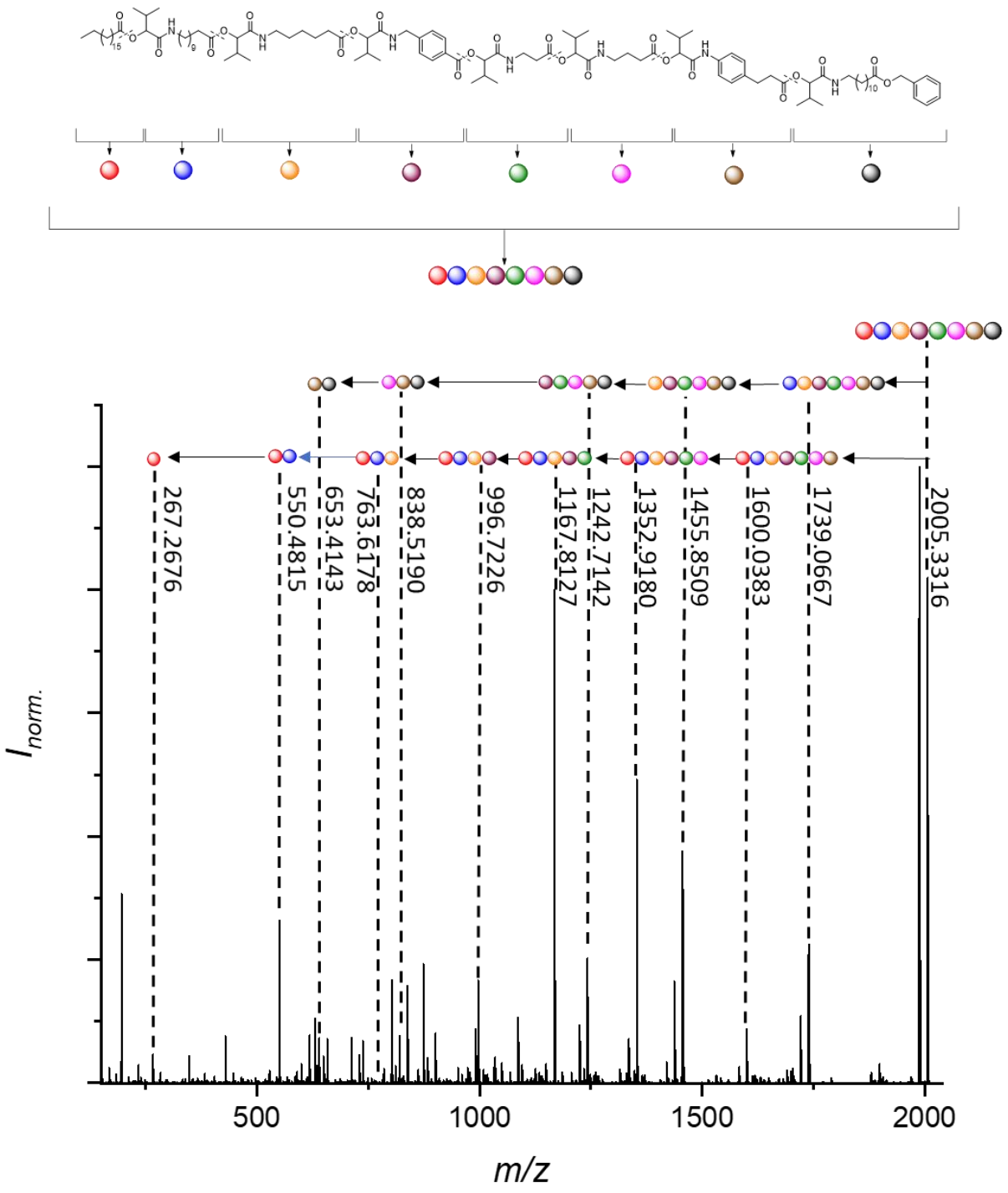
6.3.2.2 Backbone defined

6.3.2.2.1 Backbone defined pentamer **BB5**



Supplementary Figure 14: Structure and ESI-MS/MS fragmentation of the backbone-defined pentamer **BB5**. The assigned peaks belong to the most prominent fragmentation pattern (in this case: fragmentation next to the carbonyl) from both ends of the molecule. Other intense peaks belong to the other prominent fragmentation pattern and to the middle fragments and can be assigned analogously (for the sake of clarity not shown in this graph).

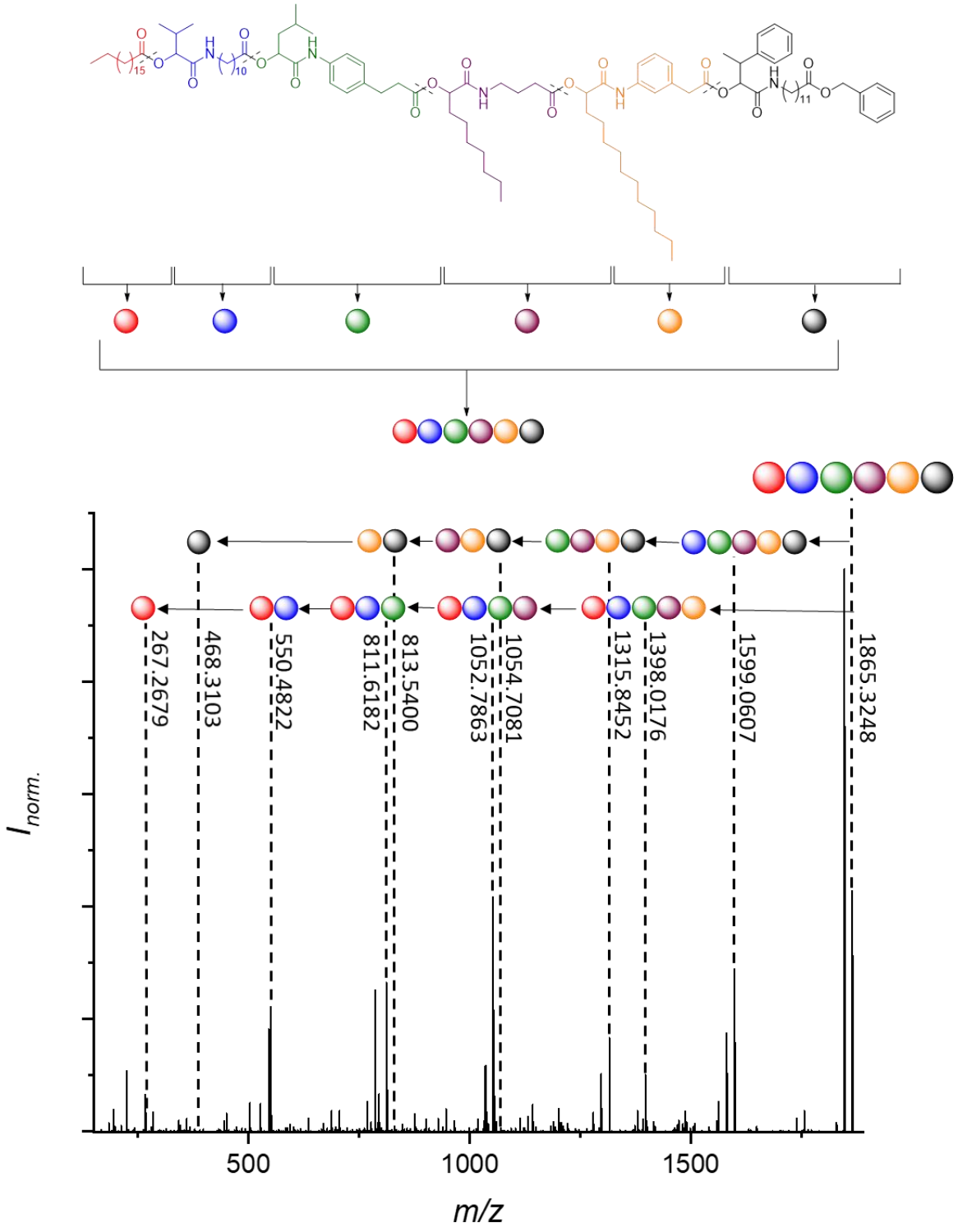
6.3.2.2.2 Backbone defined heptamer **BB7**



Supplementary Figure 15: Structure and ESI-MS/MS fragmentation of the backbone-defined heptamer **BB7**. The assigned peaks belong to the most prominent fragmentation pattern (in this case: fragmentation next to the carbonyl) from both ends of the molecule. Other intense peaks belong to the other prominent fragmentation pattern and to the middle fragments and can be assigned analogously (for the sake of clarity not shown in this graph).

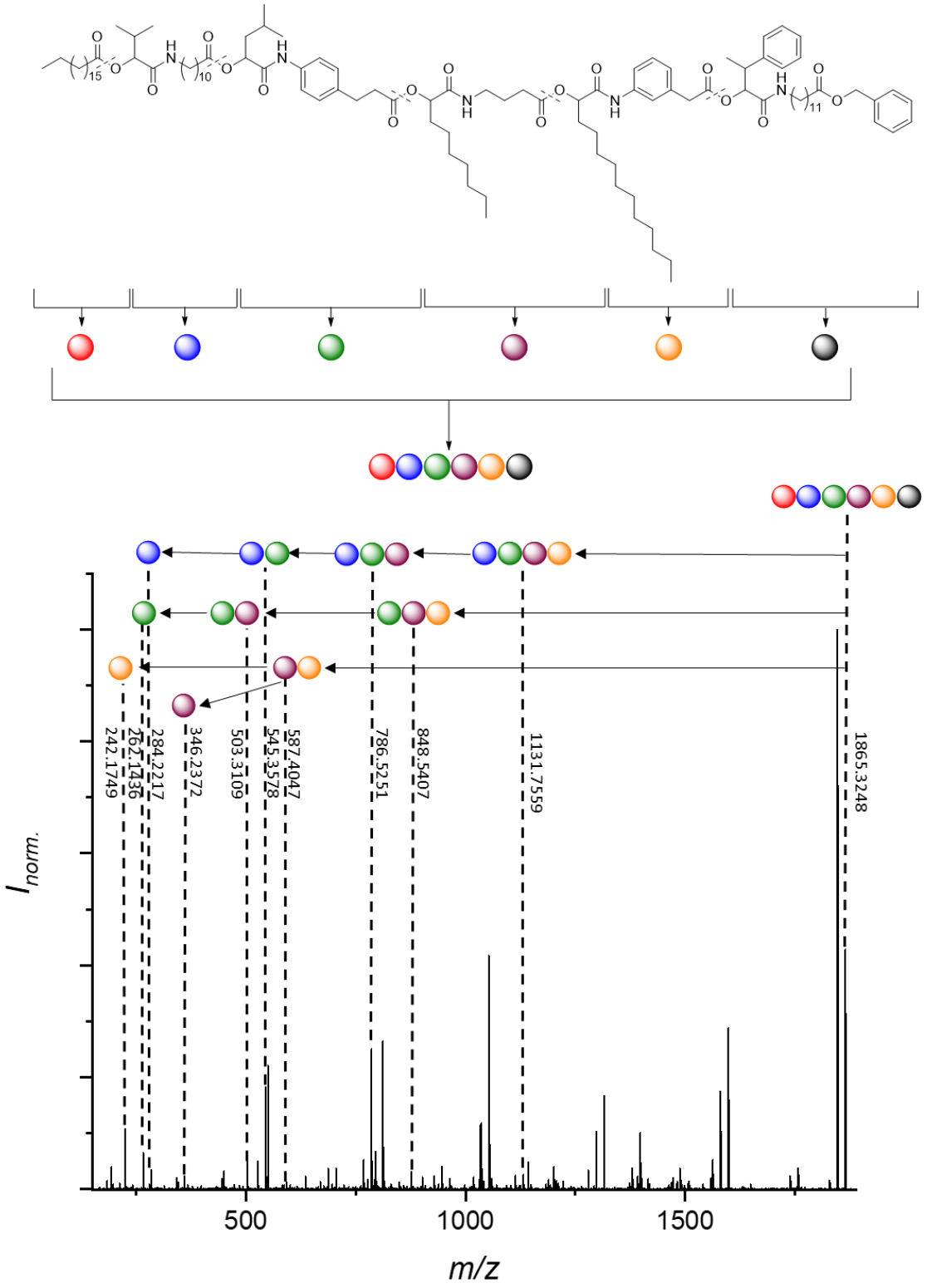
6.3.2.3 Dual sequence-defined pentamer **DS5**

6.3.2.3.1 Fragments with start and end block



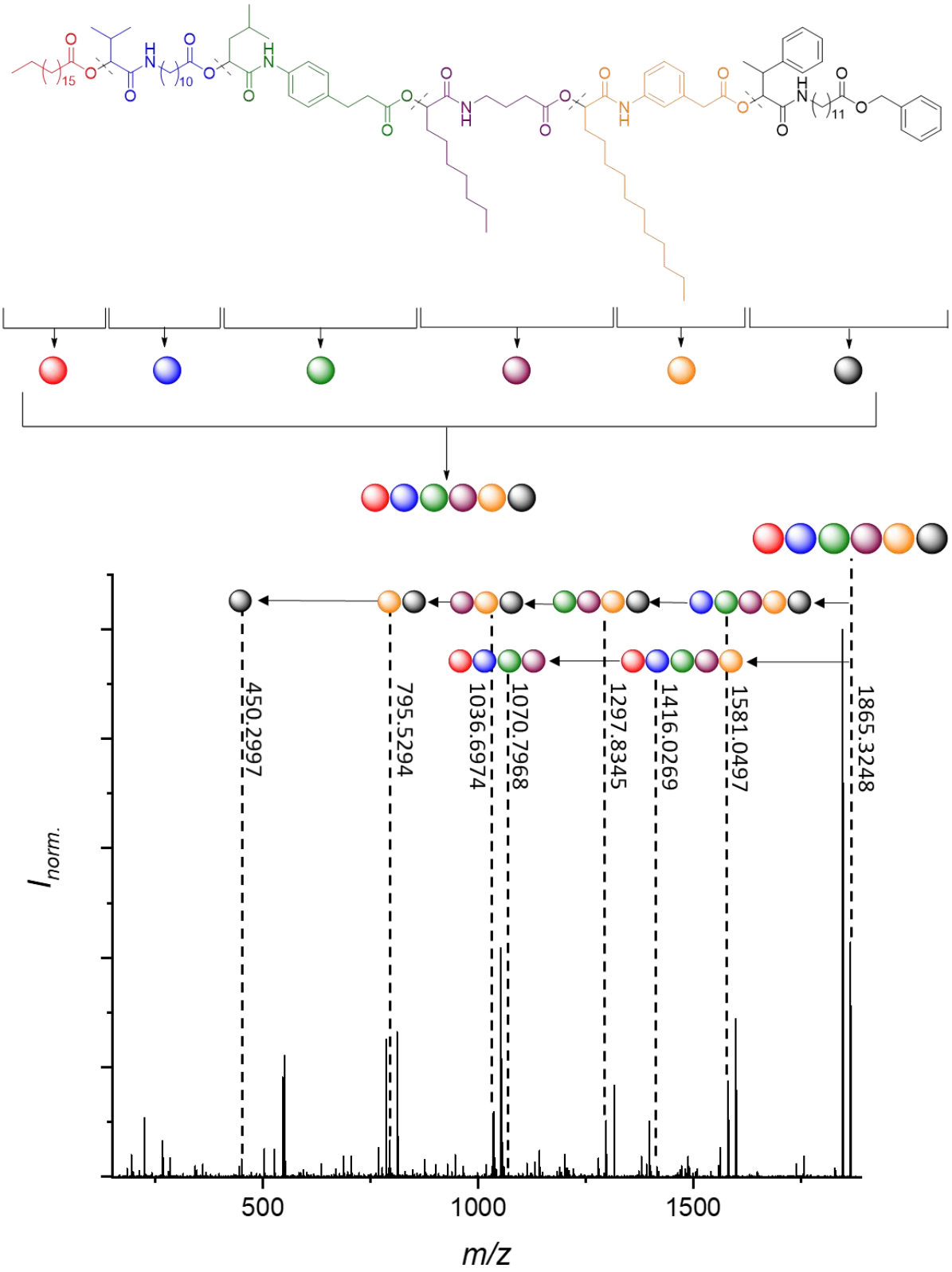
Supplementary Figure 16: Structure and ESI-/MS/MS fragmentation of the dual sequence-defined pentamer **DS5**. The assigned peaks belong to the most prominent fragmentation pattern (in this case: fragmentation next to the carbonyl) from both ends of the molecule. Other intense peaks belong to the other prominent fragmentation pattern and to the middle fragments and can be assigned analogously (for the sake of clarity not shown in this graph).

6.3.2.3.2 Middle fragments (without start and end block)



Supplementary Figure 17: Structure and ESI-MS/MS fragmentation of the dual sequence-defined pentamer **DS5**. The assigned peaks belong to the middle parts of the most prominent fragmentation pattern (in this case: fragmentation next to the carbonyl). Other intense peaks belong to the other prominent fragmentation pattern and to the fragments with start and end block and can be assigned analogously (for the sake of clarity not shown in this graph).

6.3.2.3.3 Fragmentation next to the ester



Supplementary Figure 18: Structure and ESI-MS/MS fragmentation of the dual sequence-defined pentamer **DS5**. The assigned peaks belong to the fragmentation next to the ester. Other more intense peaks belong to the fragmentation next to the carbonyl and are assigned analogously (for the sake of clarity not shown in this graph).

6.3.2.4 Equations

6.3.2.4.1 Equations dual sequence-defined molecules

Mass calculation of the molecule

$$[M_{Molecule} + H]^+ = \left[\left(M_{Start} + \sum_{i=1}^{i=n} M^i_{Backbone} + \sum_{i=1}^{i=n} M^i_{Sidechain} + M_{End} + y \times M(H) \right) + \mathrm{H} \right]^+$$

x =(n), (n-1), (n-2), (...), 0

y =(n-1)

n = number of repeating units

M_{Start} = M (**1**)

M_{End} = M (C_7H_7)

$M_{Backbone}$ = (M (**IM2**) or M (**IM2**) or M (**M3**) or M (**M4**) or M (**M5**) or M (**M6**) or M (**M7**) or M (**M8**) or M (**M9**)) – M(C_7H_7)

$M_{Sidechain}$ = M (**2a**) or M (**2b**) or M (**2c**) or M (**2d**) or M (**2e**) or M (**2f**) or M (**2g**) or M (**2h**) or M (**2i**) or M (**2j**) or M (**2k**)

$M_{Backbone}$ is calculated with the mass of the monomer which incorporates the protected acid (benzyl ester); however, in the iterative cycle, the benzyl ester is deprotected and further converted as the free acid compound. In order to take that into consideration in the formula, y is introduced as additional summand.

Fragmentation

Fragmentation next to the carbonyl:

From left to the right:

$$[M + H]^+ = \left[\left((M_{start} - M\ (OH)) + \sum_{i=1}^{i=x} M^i_{Backbone} + \sum_{i=1}^{i=x} M^i_{Sidechain} + (x-1) \times M(H) \right) + H \right]^+$$

From right to the left:

$$[M + H]^+ = \left[\left(M_{End} + \sum_{i=1}^{i=x} M^i_{Backbone} + \sum_{i=1}^{i=x} M^i_{Sidechain} + M(OH) + x \times M(H) \right) + H \right]^+$$

Fragmentation next to the ester:

From left to the right:

$$[M+H]^+ = \left[\left((M_{start} - M\ (H)) + \sum_{i=1}^{i=x} M^i_{Backbone} + \sum_{i=0}^{i=x} M^i_{Sidechain} + (x+1) \times M(H)\right) + H\right]^+$$

From right to the left:

$$[M+H]^+ = \left[\left(M_{End} + \sum_{i=1}^{i=x} M^i_{Backbone} + \sum_{i=0}^{i=x} M^i_{Sidechain} + (x-1) \times M(H)\right) + H\right]^+$$

Calculation example for the dual sequence-defined pentamer DS5

Calculation of the molecule mass and finding the respective mass peak in the mass spectrum.

$$M_{Molecule} = M_{Start} + \sum_{i=1}^{i=x} M^i_{Backbone} + \sum_{i=1}^{i=x} M^i_{Sidechain} + M_{End} + y * M(H)$$

For **DS5**:

M_{DS5}= (284.27153 + (301.20418 - 91.05478) + (265.11028 - 91.05478) + (203.09463 - 91.05478) + (251.09463 - 91.05478) + (315.21983 – 91.05478) + 72.05751 + 86.07316 + 128.12012 + 184.18272 + 134.07316 + 91.05478 + 4*1.00783) Da

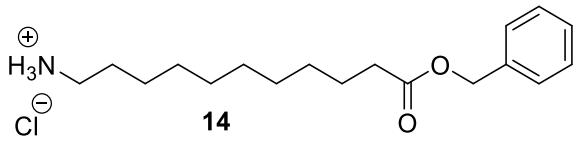
M_{DS5}= 1864.31395 Da

6.3.3 Reading the mixture

6.3.3.1 Synthesis of monomer **IM2**

Esterification

Monomer **IM2** was synthesised according to the reported procedure from Meier *et al*.[51]

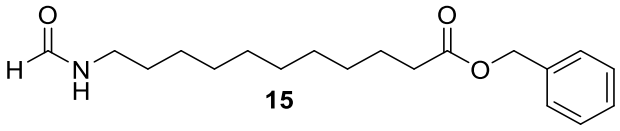


14

In a 500 mL three necked flask 15.0 g 11-aminoundecanoic acid **13** (74.5 mmol, 1.00 eq.) were suspended in 75 mL THF and 96.7 g (895 mmol, 12.0 eq.) benzyl alcohol **4** were added. The suspension was cooled in an ice bath and subsequently 16.5 mL thionyl chloride **5** (27.1 g, 231 mmol, 3.10 eq.) were added dropwise at 0 °C. After addition of the thionyl chloride **5** the solution was warmed to room temperature and stirred overnight. The yellow solution was then poured into 500 mL diethylether and stored in the freezer for one hour. The product was the filtered off and dried under high vacuum. The 11-(benzyloxy)-11-oxoundecan-1-aminium chloride **14** was obtained as a white solid in a yield of 72.4% (17.6 g, 53.9 mmol).

^{1}H-NMR (300 MHz, CDO_3D): δ / ppm = 7.53 – 6.77 (m, 5 H, CH_{Ar}), 5.08 (s, 2 H, CH_2), 3.03 – 2.71 (m, 2 H, CH_2), 2.39 – 2.08 (m, 2 H, CH_2), 1.76 – 1.45 (m, 4 H, CH_2), 1.42 – 1.02 (m, 12 H, CH_2). ^{1}H-NMR was in accordance to the literature.[51]

***N*-Formylation**

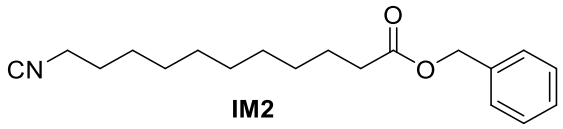


15

In a 250 mL round bottom flask 17.6 g 11-(benzyloxy)-11-oxoundecan-1-aminium chloride 1**4** (53.9 mmol, 1.00 eq.) were dissolved in 58.9 mL trimethyl orthoformate **6** (57.2 g, 539 mmol, 10.0 eq.) and heated to 100 °C for 12 hours. Trimethyl orthoformate **6** was removed under reduced pressure and the product was used without further purification. The product **15** was obtained in quantitative yield (17.2 g, 53.9 mmol).

^{1}H-NMR (300 MHz, $CDCl_3$): δ / ppm = 8.01 (s, 1 H, CH), 7.38 – 7.08 (m, 5 H, CH_{Ar}), 5.03 (s, 2 H, CH_2), 4.52 (s, 1 H, NH), 3.31 – 3.18 (m, 2 H, CH_2), 2.26 (t, J = 7.6 Hz, 2 H, CH_2), 1.67 – 1.30 (m, 4 H, CH_2), 1.18 (s, 12 H, CH_2). ^{1}H-NMR was in accordance to the literature.[51]

Dehydration



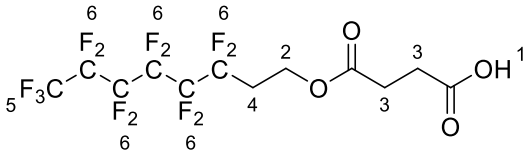
IM2

In a 500 mL three necked flask, 17.2 g of benzyl 11-formamidoundecanoate **15** (53.8 mmol, 1.00 eq.) were dissolved in 200 mL DCM, 24.7 mL diisopropylamine **8** (17.7 g, 167 mmol, 3.10 eq.) were added and the reaction mixture was cooled to 0 °C. Subsequently, 6.54 mL phosphorus oxychloride **9** (10.7 g, 69.9 mmol, 1.30 eq.) were added dropwise and the reaction mixture was then stirred at room temperature for two hours. The reaction was quenched by addition of sodium carbonate solution **10** (20 %, 75 mL) at 0 °C. After stirring this mixture for 30 min, water (50 mL) and DCM (50 mL) were added. The aqueous phase was separated, and the organic layer was washed with water (3 × 80 mL) and brine (80 mL). The combined organic layers were dried over sodium sulfate **11** and the solvent was evaporated under reduced pressure. The crude product was then purified by column chromatography (hexane / ethyl acetate 19:1 → 8:1). The product monomer **IM2** was obtained as slightly yellow oil in a yield of 57.1% (9.30 g, 30.8 mmol).

^{1}H-NMR (300 MHz, $CDCl_3$): δ / ppm = 7.47 – 7.03 (m, 5 H, CH_{Ar}), 5.00 (s, 2 H, CH_2), 3.24 (s, 2 H, CH_2), 2.33 – 2.13 (m, 2 H, CH_2), 1.62 – 1.47 (m, 4 H, CH_2), 1.36 – 1.31 (m, 12 H, CH_2). ^{1}H-NMR was in accordance to the literature.[51]

6.3.3.2 TAG synthesis

TAG1 synthesis



TAG1

In a 50.0 mL round bottom flask, 606 µL 1*H*,1*H*,2*H*,2*H*-perfluoro-1-octanol **16** (1.00 g, 2.75 mmol), 316 mg succinic anhydride **17** (3.16 mmol, 1.15 eq.) and 26.8 mg DMAP **18** (219 µmol, 0.08 eq.) were dissolved in 6.00 mL DCM. After 2 days stirring at room temperature, the solution was washed with $NaHSO_4$ **19** (10 %, 15 mL) and DCM (10 mL). The aqueous phase was separated and washed with DCM (2 × 60 mL). The organic layer was washed with water (3 × 80 mL). The combined organic layers were dried over sodium sulfate **11** and the solvent was evaporated under reduced pressure. The product **TAG1** was obtained as a colourless solid in a yield of 83.7% (1.06 g, 2.30 mmol).

R_f = 0.48 in cyclohexane / ethyl acetate (2:1).

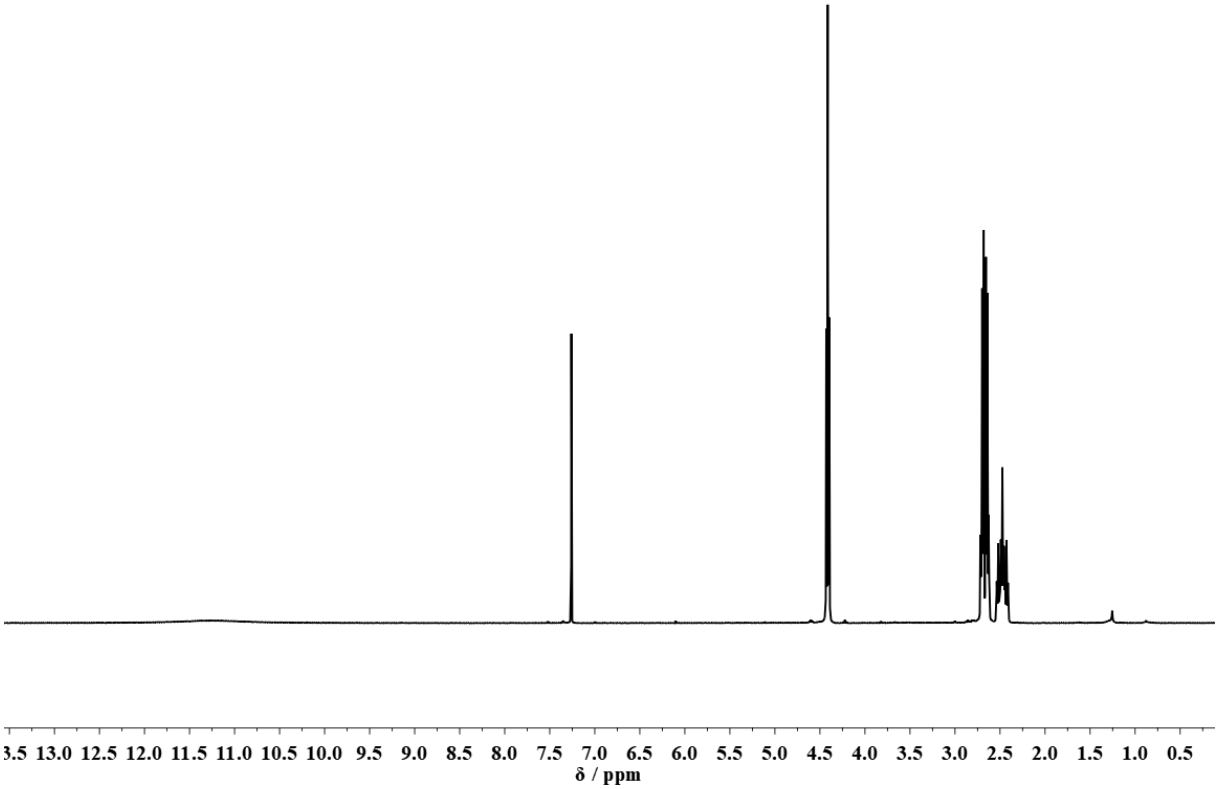
IR (ATR): ν / cm^{-1} = 2971.4 (w), 1731.5 (vs), 1697.1 (vs), 1435.2 (w), 1405.6 (m), 1364.0 (s), 1173.0 (vs), 1137.5 (vs), 1080.8 (vs), 1022.1 (s), 947.0 (s), 871.5 (w), 834.0 (s), 781.1 (s), 734.2 (vs), 706.2 (vs), 646.5 (vs), 563.7 (s), 532.2 (s), 506.3 (m), 463.1 (m).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 11.25 (s, 1 H, OH^1), 4.41 (t, J = 6.5 Hz, 2 H, CH_2^2), 2.76 – 2.59 (m, 4 H, CH_2^3), 2.56 – 2.38 (m, 2 H, CH_2^4).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 178.06, 171.82, 56.81, 30.60, 28.83, 28.79.

^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm =-84.82 – -85.49 (m, 3 F, CF_3^5), -117.80 – -118.24 (m, 2 F, CF_2^6), 125.96 – -126.62 (m, 2 F, CF_2^6), -126.68 – -127.49 (m, 2 F, CF_2^6), -127.68 – -128.41 (m, 2 F, CF_2^6), -130.13 – -130.91 (m, 2 F, CF_2^6). Total integral of CF_2 region normalized with respect to the CF_3^5 group = 10.

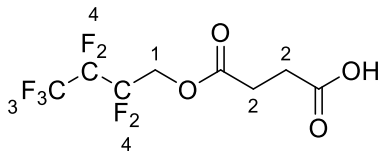
ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{12}{}^{1}H_9{}^{16}O_4{}^{19}F_{13}$, 465.0366; found, 465.0354, Δ = 1.2 mmu.



Supplementary Figure 19: ^{1}H-NMR of compound TAG1 measured in $CDCl_3$.

TAG2 synthesis

TAG2 was synthesised according to the reported procedure from the Master thesis of the author.[288]



TAG2

In a round bottom flask, 625 µL 2,2,3,3,4,4-heptafluro-1-butanol **19** (1.00 g, 4.99 mmol, 1.00 eq.), 575 mg succinic anhydride **17** (5.75 mmol, 1.15 eq.) and 48.9 mg DMAP **18** (400 µmol, 0.08 eq.) were dissolved in 6.00 mL DCM. After 2 days of stirring at room temperature, the solution was washed with $NaHSO_4$ **19** (10 %, 15 mL) and DCM (10 mL). The aqueous phase was separated and washed with DCM (2 × 60 mL). The organic layer was washed with water (3 × 80 mL). The combined organic layers were dried over sodium sulfate **11** and the solvent was evaporated under reduced pressure. The product **TAG2** was obtained as a colourless solid in a yield of 85.0% (1.28 g, 424 mmol).

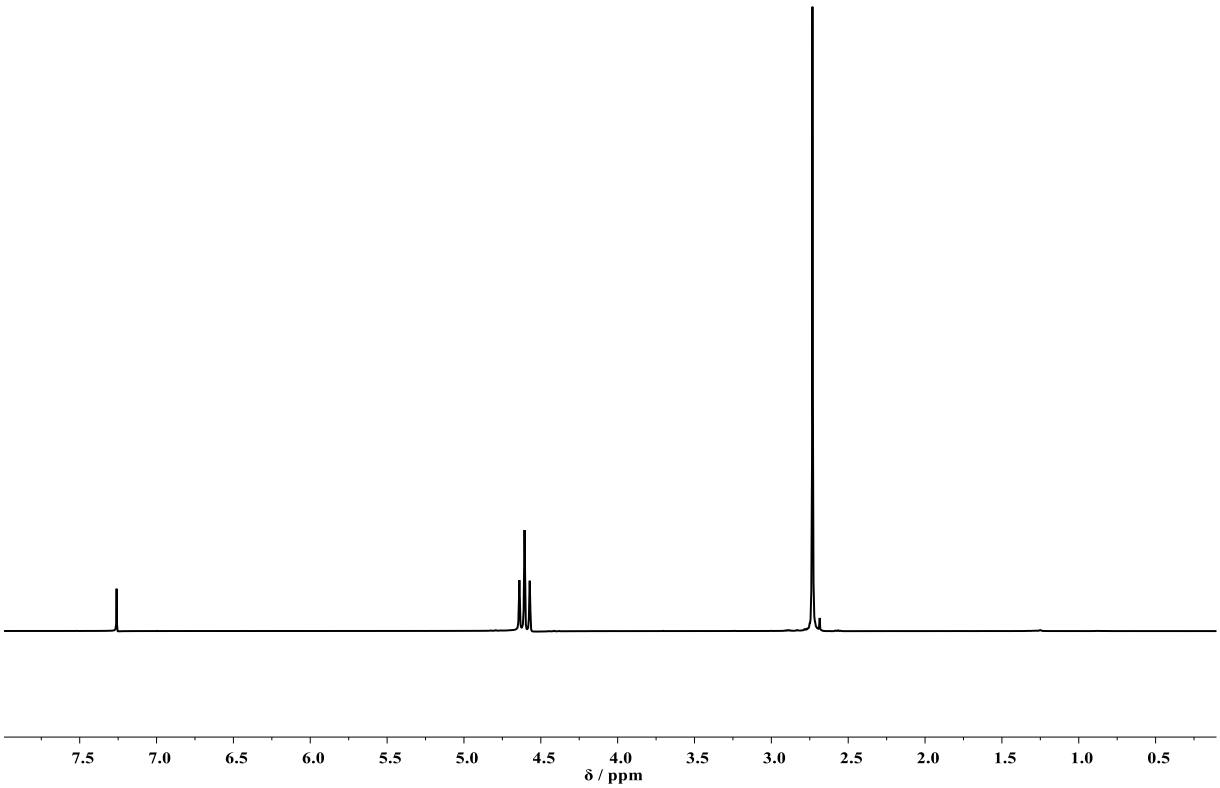
IR (ATR): ν / cm^{-1} = 2934.2 (w), 1749.3 (s), 1690.9 (s), 1454.2 (w), 1420.8 (m), 1400.3 (m), 1367.9 (m), 1350.8 (m), 1302.6 (m), 1280.5 (m), 1255.6 (s), 1223.6 (vs), 1175.1 (vs), 1143.5 (vs), 1120.4 (vs), 1030.1 (m), 1018.2 (m), 991.7 (s), 974.6 (m), 950.2 (s), 913.0 (vs), 846.3 (m), 780.6 (w), 733.2 (vs), 689.4 (m), 654.5 (w), 631.6 (w), 588.6 (w), 539.9 (s), 437.3 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 4.76 – 4.39 (m, 2 H, CH_2^1), 2.73 (s, 4 H, CH_2^2).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 177.97, 170.71, 59.67, 28.73, 28.47.

^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.27 (t, J = 9.7 Hz, 3 F, CF_3^3), -123.85 – -126.58 (m, 2 F, CF_2^4), -131.47 – -134.72 (m, 2 F, CF_2^4). Total integral of CF_2 region normalized with respect to the CF_3^3 group = 4.

ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_8{}^{1}H_7{}^{16}O_4{}^{19}F_7$, 323.0125; found, 323.0117, Δ = 0.8 mmu.

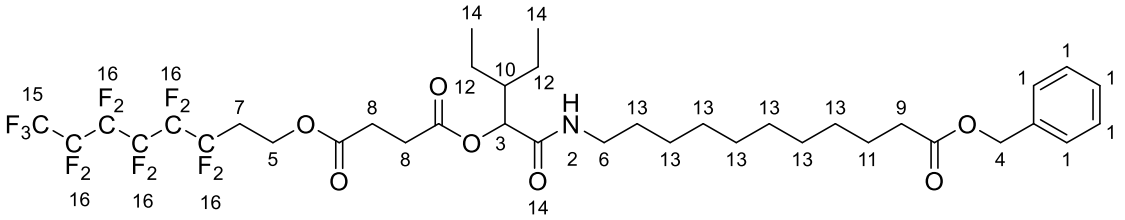


Supplementary Figure 20: ^{1}H-NMR of compound **TAG2** measured in $CDCl_3$.

6.3.3.3 Oligomer synthesis with TAG1

6.3.3.3.1 Synthesis of tetramer T1/1

Passerini reaction



M1/1

In a 10 mL round bottom flask, 1.20 g **TAG1** (2.59 mmol, 1.00 eq.) were stirred in 3.00 mL DCM. Subsequently, 477 µL 2-ethylbutyraldehyde **A5** (388 mg, 3.88 mmol, 1.50 eq.) and 1.17 g of monomer **IM2** (3.88 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 6 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (7:1 → 5:1) to yield the Passerini product **M1/1** as a pale highly viscous oil. (2.16 g, 2.49 mmol, 96.3%).

R_f = 0.46 in cyclohexane / ethyl acetate (3:1).

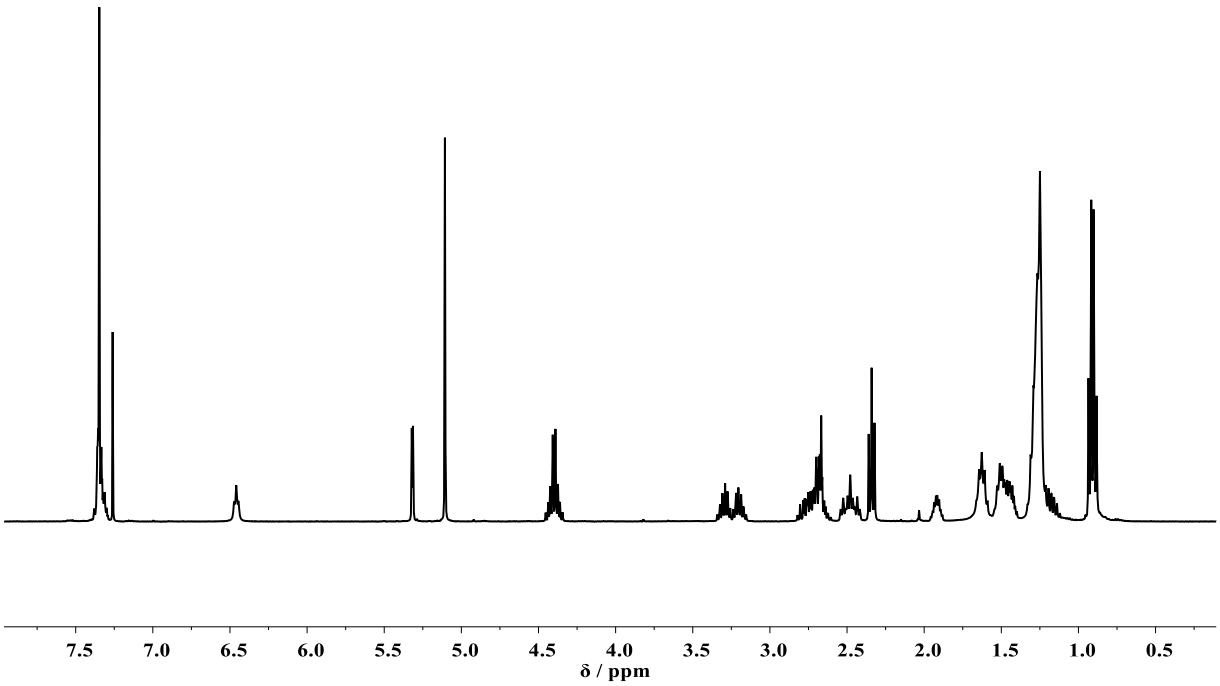
IR (ATR): ν / cm^{-1} = 2929.0 (w), 2856.2 (w), 1737.2 (s), 1656.7 (m), 1533.7 (w), 1458.1 (w), 1358.2 (w), 1234.5 (vs), 1191.7 (vs), 1144.2 (vs), 1005.0 (m), 841.9 (w), 808.7 (w), 732.9 (m), 697.4 (s), 651.0 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.41 – 7.20 (m, 5 H, CH_{Ar}^1), 6.39 (t, J = 5.8 Hz, 1 H, NH^2), 5.25 (d, J = 3.1 Hz, 1 H, CH^3), 5.04 (s, 2 H, CH_2^4), 4.47 – 4.18 (m, 2 H, CH_2^5), 3.34 – 3.05 (m, 2 H, CH_2^6), 2.81 – 2.54 (m, 2 H, CH_2^7), 2.50 – 2.33 (m, 4 H, CH_2^8), 2.27 (t, J = 7.5 Hz, 2 H, CH_2^9), 1.85 (td, J = 5.0, 3.0 Hz, 1 H, CH^{10}), 1.61 – 1.52 (m, 2 H, CH_2^{11}), 1.50 – 1.35 (m, 4 H, CH_2^{12}), 1.29 – 1.04 (m, 14 H, CH_2^{13}), 0.85 (t, J = 7.3, 3 H, CH_3^{14}), 0.83 (t, J = 7.3, 3 H, CH_3^{14}).

^{13}C NMR (101MHz, $CDCl_3$): δ / ppm = 174.57, 173.59, 171.98, 170.36, 137.03, 129.42, 129.04, 76.56, 66.95, 57.75, 44.12, 40.25, 35.21, 31.56, 31.34, 31.12, 30.60, 30.36, 30.26, 30.25, 30.12, 30.11, 30.00, 29.96, 29.91, 27.75, 25.83, 23.32, 22.93, 12.62, 12.50.

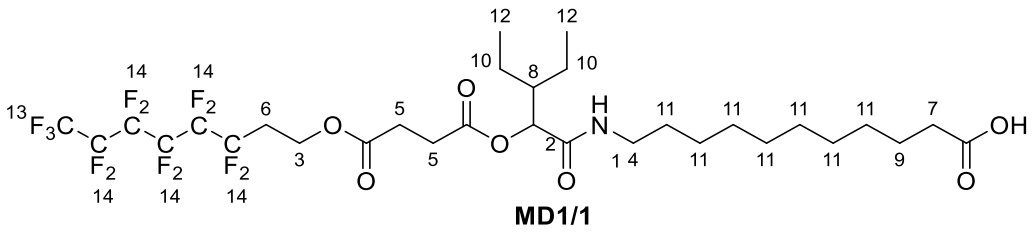
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -84.73 – -85.64 (m, 3 F, CF_3^{15}), -117.60 – -119.25 (m, 2 F, CF_2^{16}), -126.03 – -126.50 (m, 2 F, CF_2^{16}), -126.75 – -127.62 (m, 2 F, CF_2^{16}), -127.46 – -128.50 (m, 2 F, CF_2^{16}), -130.04 – -131.52 (m, 2 F, CF_2^{16}). Total integral of CF_2 region normalized with respect to the CF_3^{15} group = 10.

ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{37}{}^{1}H_{48}{}^{16}O_{7}{}^{14}N^{19}F_{13}$, 888.3115; found, 888.3144, Δ = 2.9 mmu.



Supplementary Figure 21: ^{1}H-NMR of compound **M1/1** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 2.02 g of **M1/1** (2.33 mmol, 1.00 eq.) were dissolved in 3.00 mL ethyl acetate and 3.00 mL THF. Afterwards, 403 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloon) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **MD1/1** was obtained as a pale highly viscous oil in a quantitative yield.

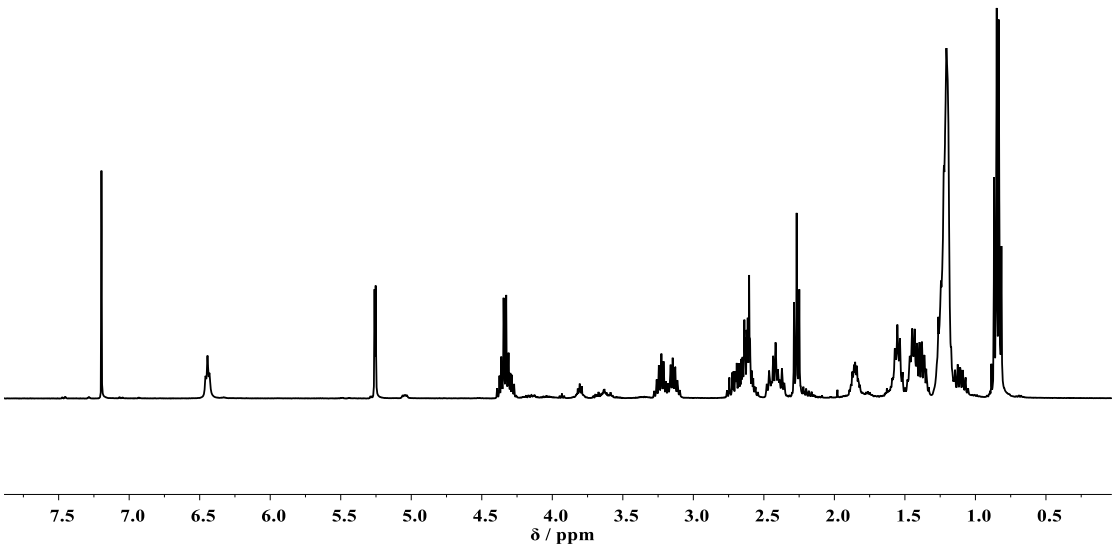
IR (ATR): ν / cm^{-1} = 2928.2 (m), 2856.4 (w), 1738.7 (s), 1649.6 (m), 1540.7 (w), 1461.4 (w), 1360.9 (w), 1234.0 (vs), 1192.5 (vs), 1144.2 (vs), 1082.8 (m), 1006.9 (m), 841.9 (w), 808.8 (w), 732.4 (w), 697.7 (w), 651.4 (w), 530.7 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.51 (t, J = 5.8 Hz, 1 H, NH^{1}), 5.32 (d, J = 3.1 Hz, 1 H, CH^{2}), 4.48 – 4.28 (m, 2 H, $CH_2{}^{3}$), 3.39 – 3.11 (m, 2 H, $CH_2{}^{4}$), 2.87 – 2.60 (m, 4 H, $CH_2{}^{5}$), 2.59 – 2.40 (m, 2 H, $CH_2{}^{6}$), 2.33 (t, J = 7.5 Hz, 2 H, $CH_2{}^{7}$), 1.97 – 1.86 (m, 1 H, CH^{8}), 1.72 – 1.56 (m, 2 H, $CH_2{}^{9}$), 1.58 – 1.38 (m, 4 H, $CH_2{}^{10}$), 1.39 – 1.14 (m, 14 H, $CH_2{}^{11}$), 0.99 – 0.79 (m, 6 H, $CH_3{}^{12}$).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 178.81, 172.91, 171.29, 169.78, 75.76, 57.01, 43.32, 39.51, 34.01, 30.56 (t, J = 21.8 Hz), 29.46, 29.40, 29.34, 29.23, 29.20, 29.15, 29.06, 26.92, 24.93, 24.78, 22.15, 11.85, 11.73.

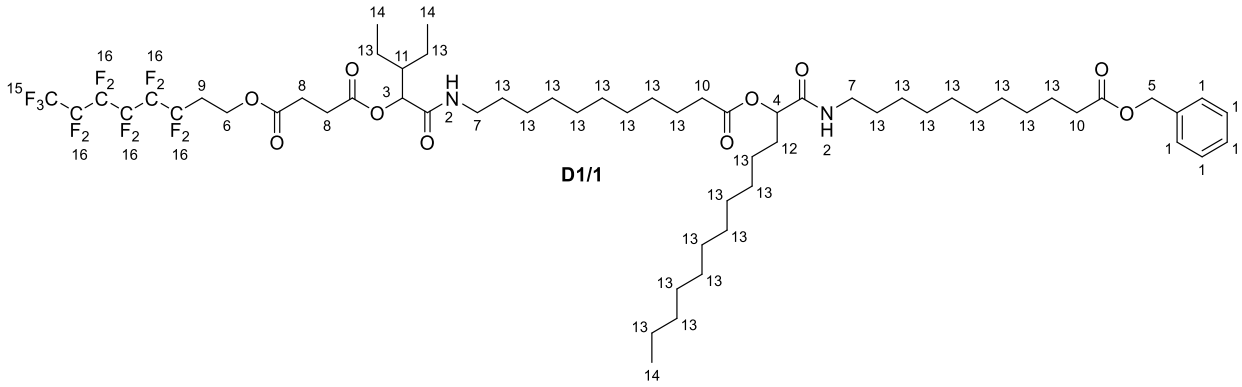
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.11 (t, J = 10.2 Hz, 3 F, $CF_3{}^{13}$), -117.83 – -118.35 (m, 3 F, $CF_3{}^{14}$), -126.02 – -126.56 (m, 2 F, $CF_2{}^{14}$), -126.73 – -127.51 (m, 2 F, $CF_2{}^{14}$), -127.64 – -128.11 (m, 2 F, $CF_2{}^{14}$), -130.37 – -130.95 (m, 2 F, $CF_2{}^{14}$). Total integral of CF_2 region normalized with respect to the $CF_3{}^{13}$ group = 10.

ESI-MS [m/z]: $[M + H]^+$ calculated for $^{12}C_{30}{}^{1}H_{42}{}^{16}O_7{}^{14}N{}^{19}F_{13}$, 776.2826; found, 776.2811, Δ = 1.5 mmu.



Supplementary Figure 22: ^{1}H-NMR of compound **MD1/1** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 1.20 g of **MD1/1** (1.55 mmol, 1.00 eq.) was dissolved in 3.00 mL DCM and 395 mg octanal **A8** (2.32 mmol, 1.50 eq.) and 699 mg of monomer **IM2** (2.32 mmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 3 days. Subsequently, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane / ethyl acetate 6:1 → 4:1) to afford product **D1/1** as a pale highly viscous oil in a yield of 96.4% (1.88 g, 1.49 mmol).

R_f = 0.29 in cyclohexane / ethyl acetate (3:1).

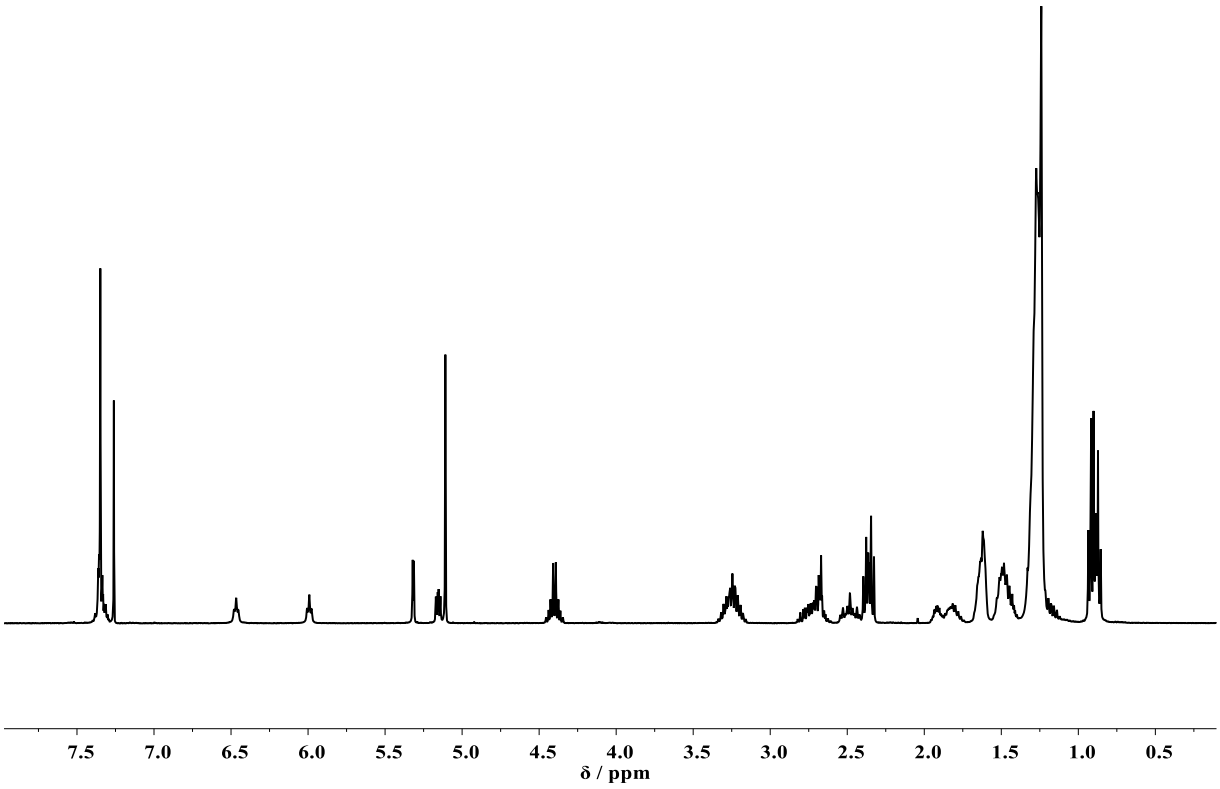
IR (ATR): ν / cm^{-1} = 3296.1 (w), 2916.7 (s), 2849.1 (m), 1741.0 (vs), 1655.0 (vs), 1560.8 (w), 1468.4 (w), 1359.5 (w), 1235.4 (vs), 1204.7 (vs), 1143.0 (vs), 1083.7 (s), 1010.3 (m), 949.2 (w), 841.9 (vw), 803.4 (vw), 746.5 (w), 696.8 (vs), 652.0 (m), 566.1 (w), 528.3 (w), 439.6 (vw), 389.8 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.48 – 7.27 (m, 5 H, CH_{Ar}^1), 6.47 (t, J = 5.8 Hz, 1 H, NH^2), 6.00 (t, J = 5.8 Hz, 1 H, NH^2), 5.31 (d, J = 3.1 Hz, 1 H, CH^3), 5.17 – 5.13 (m, 1 H, CH^4), 5.11 (s, 2 H, CH_2^5), 4.47 – 4.33 (m, 2 H, CH_2^6), 3.34 – 3.15 (m, 4 H, CH_2^7), 2.85 – 2.61 (m, 4 H, CH_2^8), 2.58 – 2.40 (m, 2 H, CH_2^9), 2.41 – 2.30 (m, 4 H, CH_2^{10}), 1.97 – 1.73 (m, 3 H, CH^{11}, CH_2^{12}), 1.70 – 1.58 (m, 6 H, CH_2^{13}), 1.57 – 1.38 (m, 6 H, CH_2^{13}), 1.36 – 1.10 (m, 42 H, CH_2^{13}), 0.96 – 0.80 (m, 9 H, CH_3^{14}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.80, 172.83, 172.57, 171.22, 169.98, 169.61, 136.27, 128.67, 128.28, 75.79, 74.07, 66.20, 56.99, 43.37, 39.47, 39.33, 34.45, 32.05, 30.59 (t, J = 21.9 Hz), 29.75, 29.69, 29.67, 29.62, 29.58, 29.57, 29.51, 29.49, 29.48, 29.39, 29.37, 29.35, 29.25, 29.20, 29.15, 26.99, 26.97, 25.08, 24.89, 22.82, 22.56, 22.18.

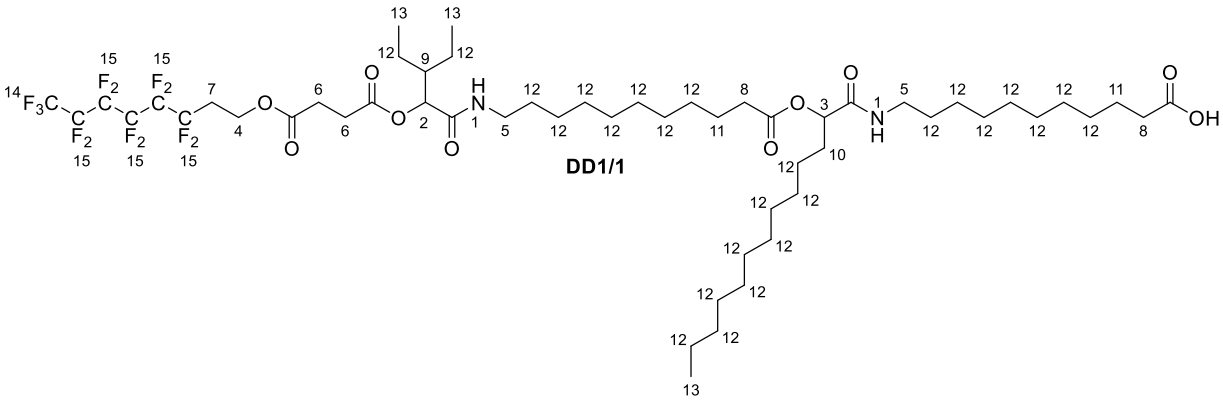
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -84.73 – -85.52 (m, 3 F, CF_3[15]), -117.70 – -118.32 (m 2 F, CF_2[16]), -125.97 – -126.42 (m 2 F, CF_2[16]), -126.99 – -127.39 (m 2 F, CF_2[16]), -127.77 – -128.04 (m 2 F, CF_2[16]), -130.30 – -130.60 (m 2 F, CF_2[16]). Total integral of CF_2 region normalized with respect to the CF_3[15] group = 10.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{61}{}^{1}H_{93}{}^{16}O_{10}{}^{14}N_2{}^{19}F_{13}$, 1261.6695 found, 1261.6692, Δ = 0.3 mmu.



Supplementary Figure 23: ^{1}H-NMR of compound **D1/1** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 1.80 g of **D1/1** (1.43 mmol, 1.00 eq.) were dissolved in 3.00 mL ethyl acetate and 3.00 mL THF. Afterwards, 360 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **DD1/1** was obtained as a highly viscous oil in a yield of 98.6% (1.65 g, 1.41 mmol).

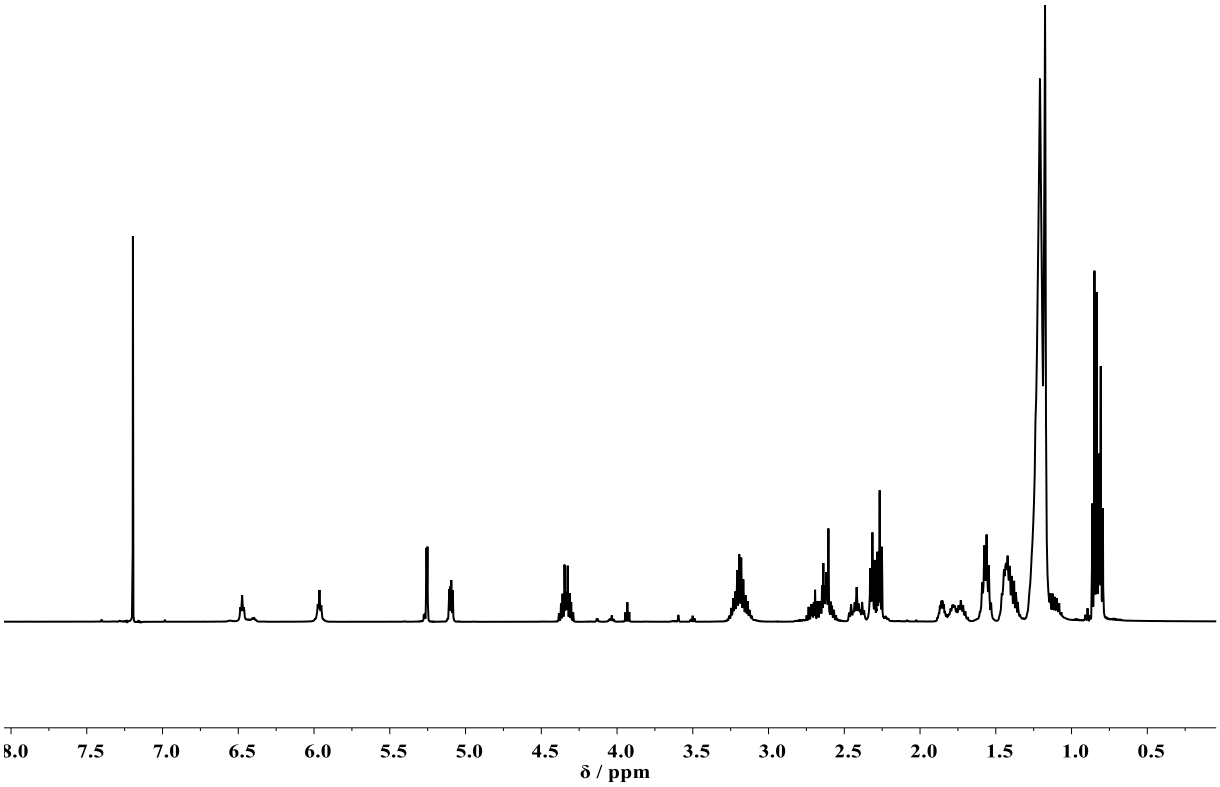
IR (ATR): ν / cm^{-1} = 2924.6 (s), 2854.3 (m), 1740.3 (s), 1654.0 (m), 1539.1 (w), 1462.3 (w), 1361.4 (w), 1235.6 (vs), 1197.6 (vs), 1144.7 (vs), 1007.1 (w), 841.9 (vw), 808.5 (w), 697.6 (w), 651.5 (w).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 6.54 (t, J = 5.8 Hz, 1 H, NH^{1}), 6.03 (t, J = 5.8 Hz, 1 H, NH^{1}), 5.32 (d, J = 3.2 Hz, 1 H, CH^{2}), 5.22 – 5.12 (m, 1 H, CH^{3}), 4.48 – 4.32 (m, 2 H, CH_2^{4}), 3.36 – 3.14 (m, 4 H, CH_2^{5}), 2.81 – 2.62 (m, 4 H, CH_2^{6}), 2.55 – 2.44 (m, 2 H, CH_2^{7}), 2.43 – 2.19 (m, 4 H, CH_2^{8}), 1.97 – 1.75 (m, 3 H, CH^{9}, CH_2^{10}), 1.71 – 1.58 (m, 4 H, CH_2^{11}), 1.56 – 1.40 (m, 6 H, CH_2^{12}), 1.37 – 1.15 (m, 44 H, CH_2^{12}), 0.96 – 0.82 (m, 9 H, CH_3^{13}).

^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 177.43, 172.92, 172.61, 171.29, 170.10, 169.87, 75.76, 74.09, 57.02, 43.31, 39.56, 39.30, 34.47, 33.94, 32.05, 32.01, 30.57 (t, J = 21.8 Hz), 29.76, 29.68, 29.65, 29.57, 29.56, 29.54, 29.49, 29.44, 29.40, 29.36, 29.34, 29.28, 29.21, 29.21, 29.15, 29.08, 27.00, 26.88, 25.13, 24.90, 24.87, 22.83, 22.54, 22.16, 14.25, 11.85, 11.73.

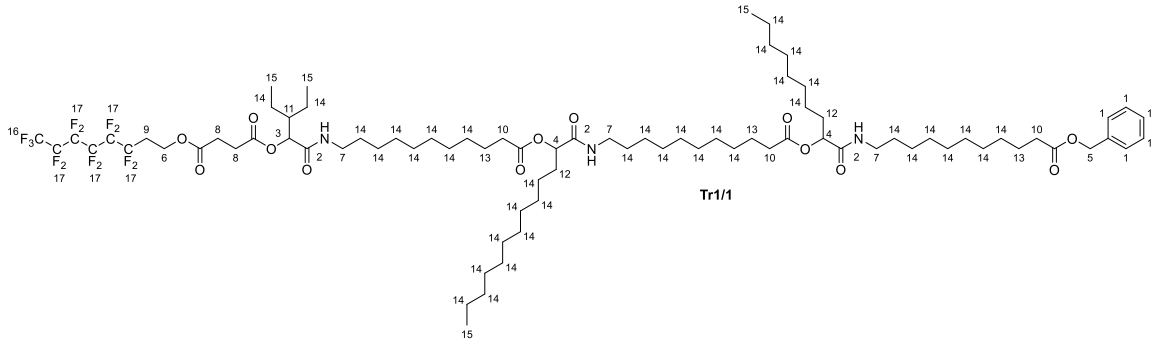
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm =-85.09 (t, J = 9.7 Hz, 3 F, CF_3^{14}), -117.79 – -118.40 (m, 2 F, CF_2^{15}), -125.89 – -126.53 (m, 2 F, CF_2^{15}), -126.69 – -127.45 (m, 2 F, CF_2^{15}), -127.74 – -128.14 (m, 2 F, CF_2^{15}), -130.00 – -130.81 (m, 2 F, CF_2^{15}). Total integral of CF_2 region normalized with respect to the CF_3^{14} group = 10.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{54}{}^{1}H_{87}{}^{16}O_{10}{}^{14}N_2{}^{19}F_{13}$, 1171.6226; found, 1171.6216, Δ = 1.0mmu.



Supplementary Figure 24: ^{1}H-NMR of compound **DD1/1** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 1.58 g of Passerini **DD1/1** (1.35 mmol, 1.00 eq.) was dissolved in 5.00 mL DCM and 347 µL nonanal **A9** (287 mg, 2.02 mmol, 1.50 eq.) and 608 mg of monomer **IM2** (2.02 mmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 2 d and subsequently the solvent was removed under reduced pressure. The crude product

was purified by column chromatography (cyclohexane / ethyl acetate 5:1→ 2:1) to afford product **Tr1/1** as a white solid in a yield of 91.9% (1.99 g, 1.24 mmol).

R_f = 0.26 in cyclohexane / ethyl acetate (3:1).

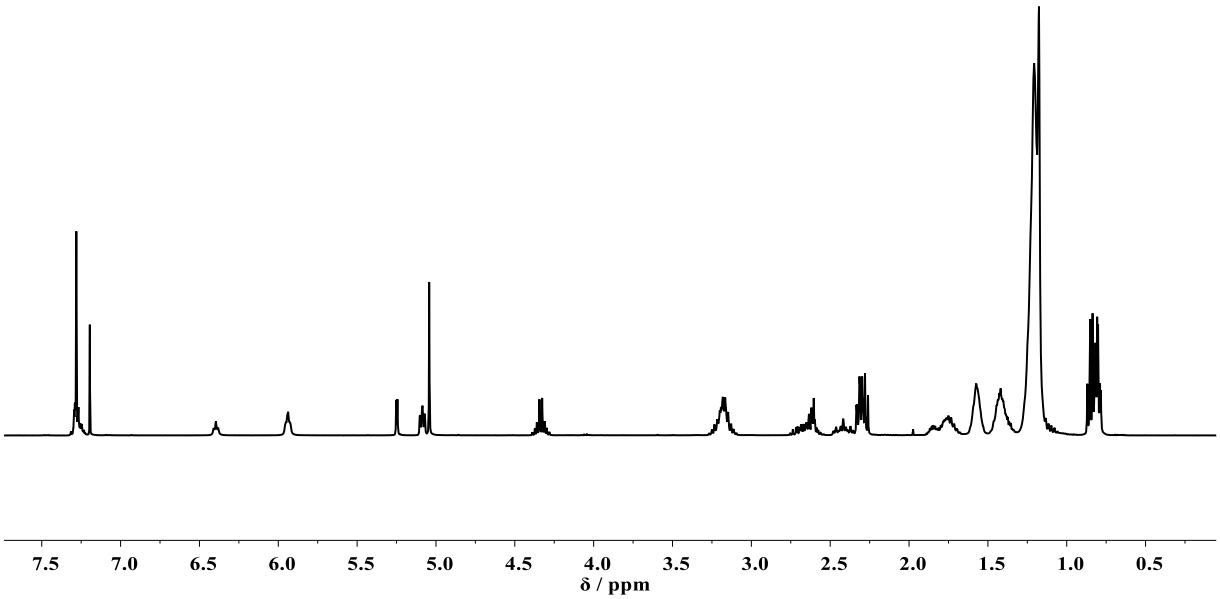
IR (ATR): ν / cm^{-1} = 3300.8 (vw), 2919.0 (s), 2851.1 (m), 1737.1 (vs), 1655.0 (vs), 1541.3 (w), 1465.7 (w), 1362.8 (w), 1236.9 (s), 1204.7 (s), 1145.0 (vs), 1005.9 (w), 842.1 (vw), 809.1 (vw), 697.4 (m), 652.7 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.39 – 7.29 (m, 5 H, CH_{Ar}[1]), 6.47 (t, J = 5.7 Hz, 1 H, NH[2]), 6.07 – 5.98 (m, 2 H, NH[2]), 5.31 (d, J = 3.1 Hz, 1 H, CH[3]), 5.17 – 5.13 (m, 2 H, CH[4]), 5.11 (s, 2 H, CH_2[5]), 4.46 – 4.34 (m, 2 H, CH_2[6]), 3.35 – 3.14 (m, 6 H, CH_2[7]), 2.85 – 2.62 (m, 4 H, CH_2[8]), 2.57 – 2.42 (m, 2 H, CH_2[9]), 2.42 – 2.30 (m, 6 H, CH_2[10]), 1.95 – 1.75 (m, 5 H, CH_2[11], CH[12]), 1.70 – 1.58 (m, 6 H, CH_2[13]), 1.54 – 1.40 (m, 8 H, CH_2[14]), 1.38 – 1.15 (m, 68 H, CH_2[14]), 0.98 – 0.79 (m, 12 H, CH_3[15]).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.80, 172.83, 172.59, 171.22, 170.00, 169.98, 169.62, 136.27, 128.67, 128.28, 75.79, 74.08, 66.20, 56.99, 43.38, 39.47, 39.33, 34.46, 32.05, 31.96, 30.82, 30.60, 30.38, 29.75, 29.72, 29.70, 29.67, 29.63, 29.62, 29.59, 29.57, 29.52, 29.50, 29.48, 29.39, 29.38, 29.35, 29.32, 29.26, 29.25, 29.20, 29.15, 26.99, 26.97, 25.10, 25.09, 25.08, 24.91, 24.90, 22.82, 22.78, 22.57, 22.10, 14.23, 11.87, 11.75.

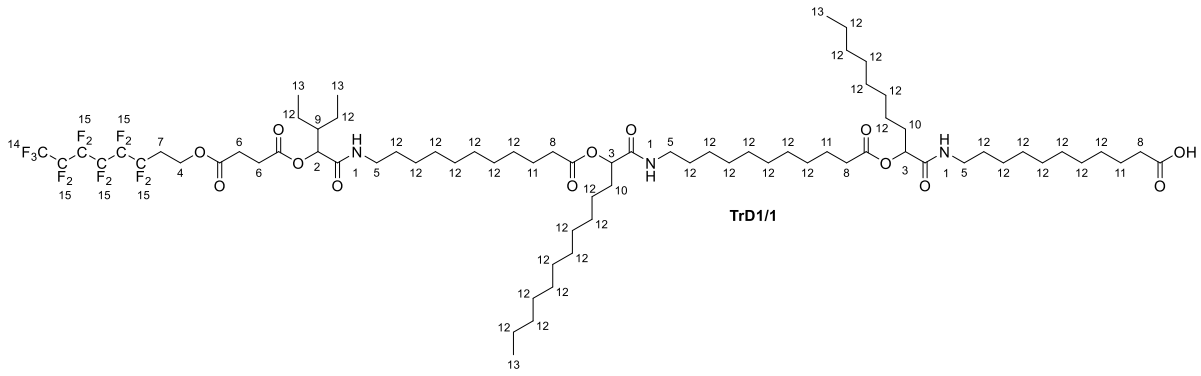
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -84.62 – -85.83 (m, 3 F, CF_3[16]), -117.63 – -118.90 (m, 2 F, CF_2[17]), -126.02 – -126.52 (m, 2 F, CF_2[17]), -126.81 – -127.48 (m, 2 F, CF_2[17]), -127.63 – -128.33 (m, 2 F, CF_2[17]), -130.01 – -131.02 (m, 2 F, CF_2[17]). Total integral of CF_2 region normalized with respect to the CF_3[17]group = 10.

ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{82}{}^{1}H_{132}{}^{16}O_{13}{}^{14}N_3{}^{19}F_{13}$, 1636.9445; found, 1636.9430, Δ = 1.5 mmu.



Supplementary Figure 25: ^{1}H-NMR of compound **Tr1/1** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 1.94 g of **Tr1/1** (1.20 mmol, 1.00 eq.) were dissolved in 4.00 mL ethyl acetate and 4.00 mL THF. Afterwards, 388 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **TrD1/1** was obtained as a highly viscous oil in a yield of 97.5% (1.79 g, 1.17 mmol).

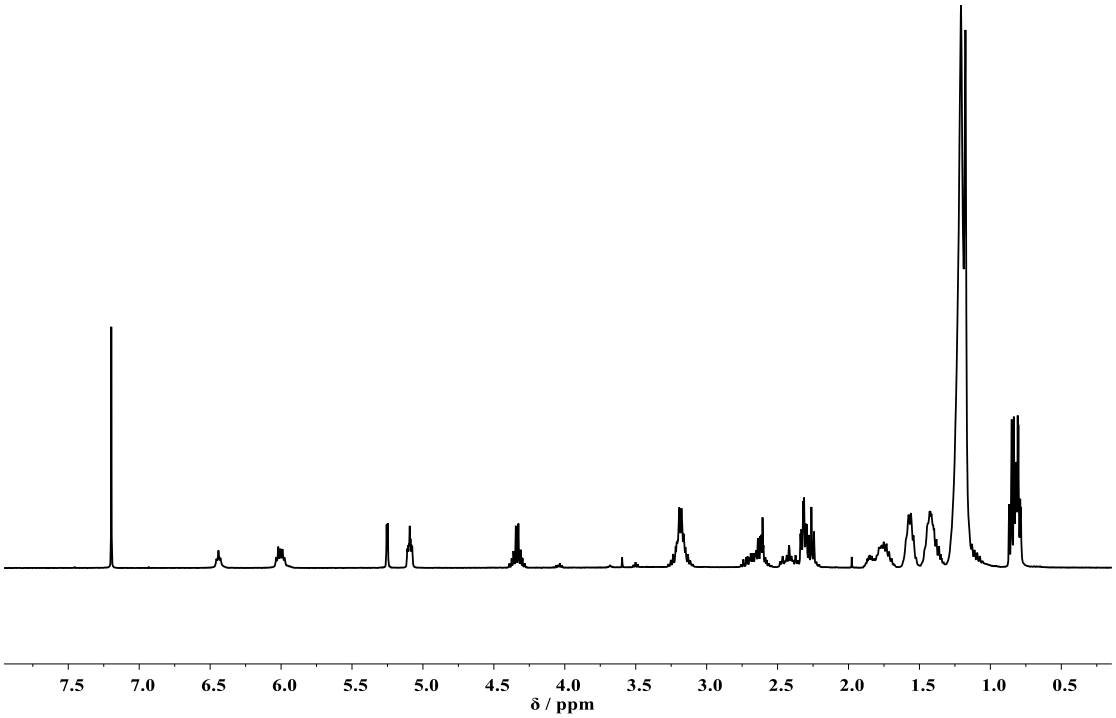
IR (ATR): ν / cm^{-1} = 3297.8 (vw), 2919.6 (s), 2851.4 (m), 1738.1 (s), 1655.5 (vs), 1555.1 (w), 1465.1 (w), 1364.1 (w), 1236.6 (vs), 1144.6 (vs), 1007.4 (w), 842.2 (vw), 809.3 (vw), 697.4 (w), 651.8 (w), 566.0 (vw), 530.6 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.49 – 6.39 (m, 1 H, NH^{1}), 6.06 – 5.94 (m, 2 H, NH^{1}), 5.25 (d, J = 3.2 Hz, 1 H, CH^{2}), 5.12 – 5.04 (m, 2 H, CH^{3}), 4.43 – 4.26 (m, 2 H, CH_2^{4}), 3.28 – 3.09 (m, 6 H, CH_2^{5}), 2.79 – 2.55 (m, 4 H, CH_2^{6}), 2.51 – 2.37 (m, 2 H, CH_2^{7}), 2.36 – 2.20 (m, 6 H, CH_2^{8}), 1.92 – 1.67 (m, 5 H, CH^{9}, CH_2^{10}), 1.64 – 1.50 (m, 6 H, CH_2^{11}), 1.51 – 1.30 (m, 8 H, CH_2^{12}), 1.31 – 1.02 (m, 68 H, CH_2^{12}), 0.89 – 0.76 (m, 12 H, CH_3^{13}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 176.95, 172.88, 172.70, 172.62, 171.25, 170.19, 170.08, 169.75, 75.77, 74.10, 74.07, 57.01, 43.35, 39.51, 39.40, 39.30, 34.48, 34.45, 33.96, 32.05, 32.02, 31.97, 29.76, 29.68, 29.63, 29.57, 29.56, 29.53, 29.51, 29.49, 29.43, 29.39, 29.37, 29.34, 29.28, 29.25, 29.22, 29.16, 29.09, 26.98, 26.89, 25.14, 25.08, 24.91, 22.83, 22.79, 22.56, 22.17, 14.25, 14.24, 11.87, 11.74.

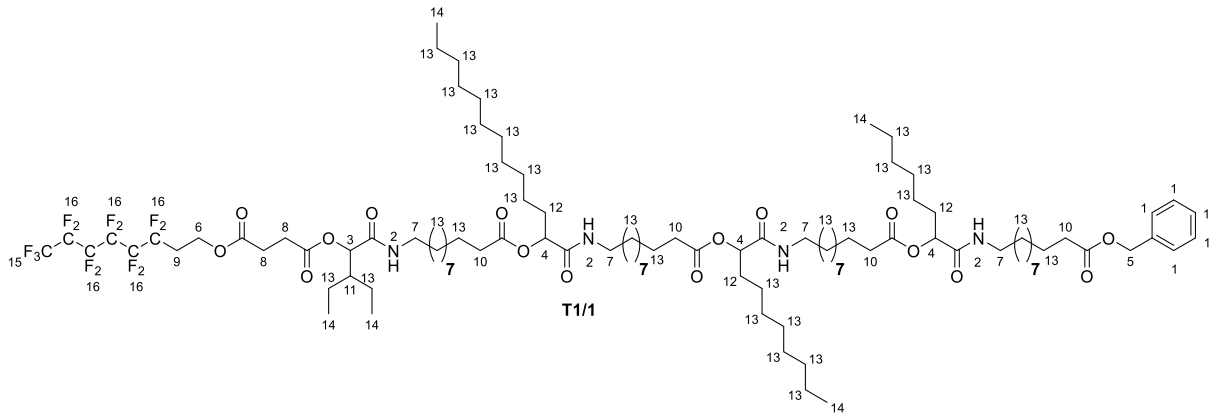
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -84.04 – -86.23 (m, 3 F, CF_3^{14}), -117.79 – -118.50 (m, 2 F, CF_2^{15}), -125.95 – -126.50 (m, 2 F, CF_2^{15}), -127.05 – -127.38 (m, 2 F, CF_2^{15}), -127.70 – -128.23 (m, 2 F, CF_2^{15}), -130.34 – -130.71 (m, 2 F, CF_2^{15}). Total integral of CF_2 region normalized with respect to the CF_3^{14} group = 10.

ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{75}{}^{1}H_{126}{}^{16}O_{13}{}^{19}F_{13}{}^{14}N_3$, 1546.8975; found, 1546.8952, Δ = 2.3 mmu.



Supplementary Figure 26: ^{1}H-NMR of compound **TrD1/1** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 1.71 g of **TrD1/1** (1.12 mmol, 1.00 eq.) was dissolved in 5.00 mL DCM and 235 µL heptanal **A7** (192 mg, 1.69 mmol, 1.50 eq.) and 508 mg of monomer **IM2** (1.69 mmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 2 d and subsequently the solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane / ethyl acetate 5:1 → 1:1) to afford product **T1/1** as a white solid in a yield of 94.6% (2.06 g, 1.06 mmol).

R_f = 0.21 in cyclohexane / ethyl acetate (2:1).

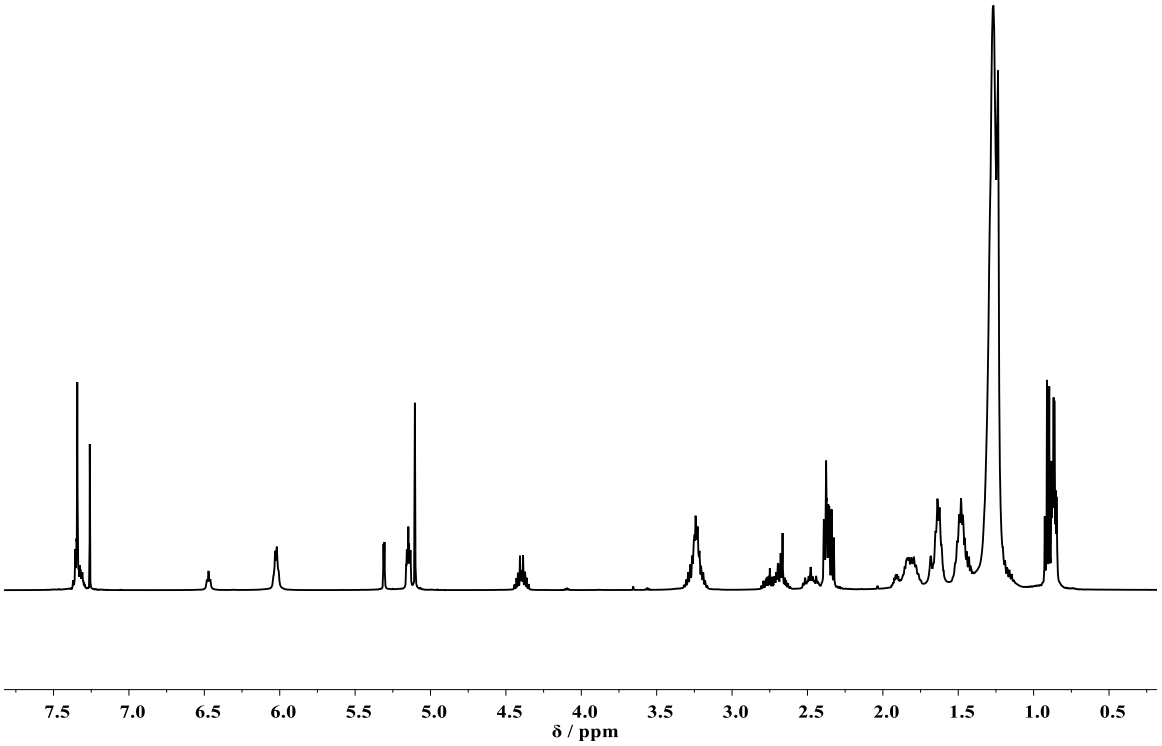
IR (ATR): ν / cm^{-1} = 3304.4 (vw), 2923.3 (s), 2853.1 (m), 1738.7 (s), 1655.1 (s), 1535.6 (m), 1464.1 (w), 1362.8 (w), 1236.8 (s), 1145.3 (vs), 697.1 (w), 651.1 (w), 396.3 (vw).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.45 – 7.28 (m, 5 H, CH_{Ar}[1]), 6.47 (t, J = 5.8 Hz, 1 H, NH[2]), 6.06 – 5.96 (m, 3 H, NH[2]), 5.31 (d, J = 3.2 Hz, 1 H, CH[3]), 5.17 – 5.13 (m, 3 H, CH[4]), 5.10 (s, 2 H, CH_2[5]), 4.47 – 4.33 (m, 2 H, CH_2[6]), 3.33 – 3.14 (m, 8 H, CH_2[7]), 2.82 – 2.60 (m, 4 H, CH_2[8]), 2.56 – 2.41 (m, 2 H, CH_2[9]), 2.42 – 2.30 (m, 8 H, CH_2[10]), 1.96 – 1.74 (m, 7 H, CH[11], CH_2[12]), 1.71 – 1.59 (m, 12 H, CH_2[13]), 1.55 – 1.40 (m, 14 H, CH_2[13]), 1.38 – 1.11 (m, 80 H, CH_2[13]), 0.96 – 0.82 (m, 15 H, CH_3[14]).

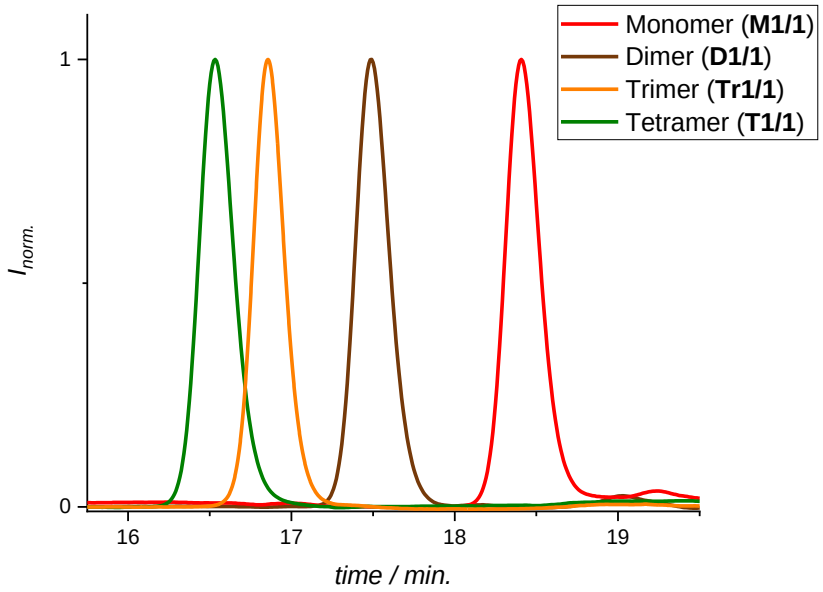
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 173.70, 172.74, 172.48, 171.11, 169.88, 169.85, 169.51, 136.13, 128.55, 128.17, 75.64, 73.93, 66.08, 56.86, 43.23, 39.33, 39.19, 34.32, 31.92, 31.83, 31.63, 30.44 (t, J = 21.8 Hz), 29.62, 29.58, 29.56, 29.54, 29.49, 29.46, 29.44, 29.39, 29.39, 29.35, 29.26, 29.22, 29.20, 29.13, 29.06, 29.01, 28.92, 26.84, 24.97, 24.96, 24.77, 24.71, 22.69, 22.65, 22.54, 22.42, 22.04, 14.12, 14.11, 14.05, 11.74, 11.62.

^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -80.74 (t, J = 10.0 Hz, 3 F, CF_3[15]), -113.42 – -113.95 (m, 2 F, CF_2[16]), -121.56 – -122.14 (m, 2 F, CF_2[16]), -122.38 – -123.21 (m, 2 F, CF_2[16]), -123.25 – -123.72 (m, 2 F, CF_2[16]), -125.70 – -126.66 (m, 2 F, CF_2[16]). Total integral of CF_2 region normalized with respect to the CF_3[16]group = 10.

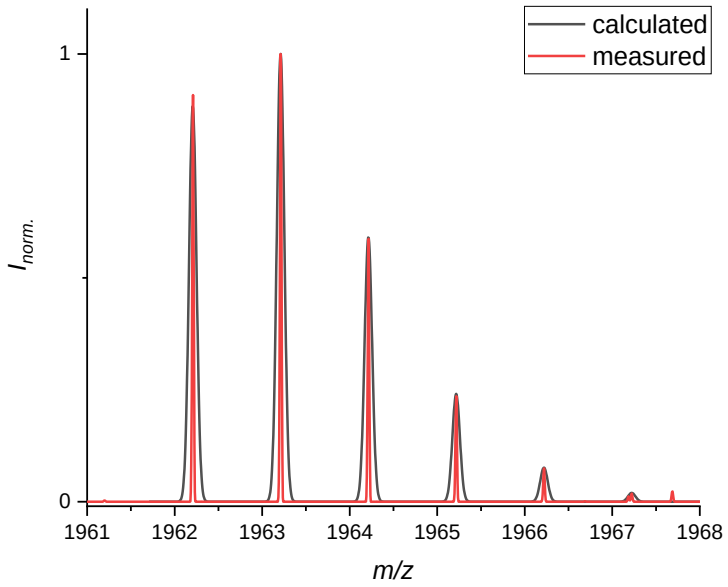
ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{101}{}^{1}H_{167}{}^{19}F_{13}{}^{14}N_4{}^{16}O_{16}$, 1962.2062; found, 1962.2090, Δ = 2.8 mmu.



Supplementary Figure 27: ^{1}H-NMR of compound **T1/1** measured in $CDCl_3$.



Supplementary Figure 28: SEC traces of the intermediates after each P-3CR in the synthesis of product **T1/1**.

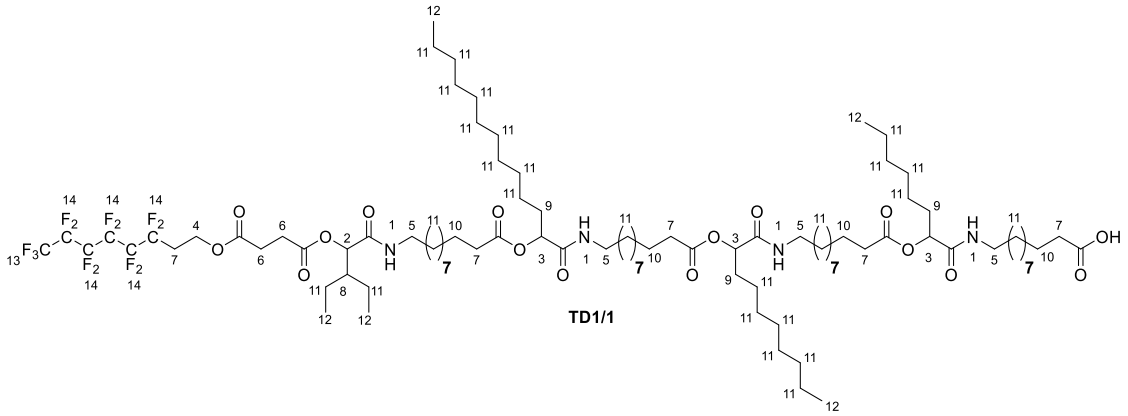


Supplementary Figure 29: High resolution ESI-MS measurement of **T1/1**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (black).

```
aximum is 1.000000 found for mass 1963.205820
matching mass 1963.20582
cutoff 0.50000: 0 solutions (12 peaks)
cutoff 0.25000: 0 solutions (51 peaks)
cutoff 0.12500: 0 solutions (109 peaks)
cutoff 0.06250: 0 solutions (179 peaks)
cutoff 0.03125: 0 solutions (262 peaks)
cutoff 0.01562: 1 solutions (354 peaks)
1963.20582 ≈ 463.021500 + 311.246050 + 395.339950 + 353.293000 + 325.261700 + 91.054780 (sides 2-Ethylbutanal, Dodecanal,
 Nonanal, Heptanal; error -23.98884)
Press ENTER to quit ...
```

Supplementary Figure 30: Screenshot of the automated read-out of **T1/1**, sodium trifluoroacetate **2** was used as additive during the measurement.

6.3.3.3.2 Synthesis of hexamer H1/1



In a 50 mL round bottom flask, 1.45 g of **T1/1** (748 µmol, 1.00 eq.) were dissolved in 5.00 mL ethyl acetate and 5.00 mL THF. Afterwards, 290 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **TD1/1** was obtained as a pale highly viscous oil in a yield of 99.5% (1.38 g, 745 µmol).

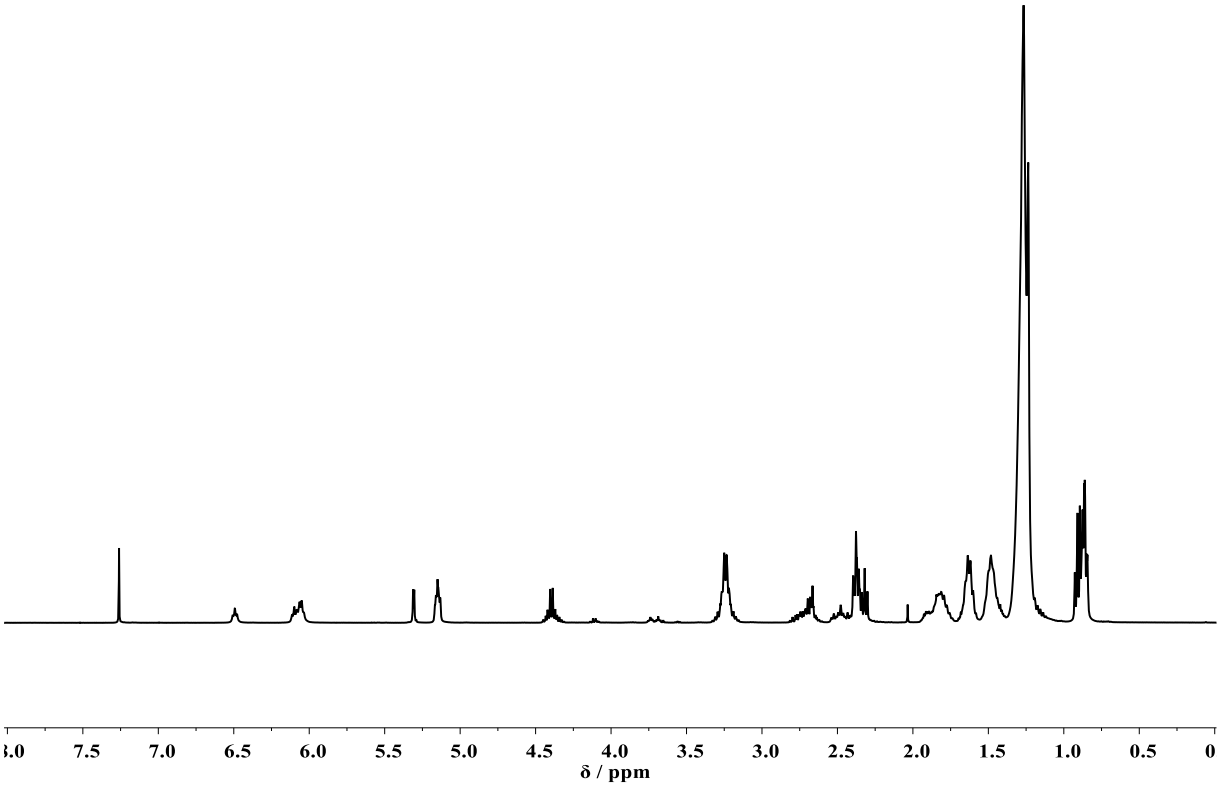
IR (ATR): ν / cm^{-1} = 3293.4 (vw), 2922.3 (vs), 2852.7 (s), 1738.6 (s), 1655.5 (vs), 1540.7 (m), 1465.3 (w), 1364.0 (w), 1236.5 (vs), 1145.1 (vs), 842.2 (vw), 697.5 (w), 651.7 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.43 (t, J = 5.8 Hz, 1 H, NH^1), 6.09 – 5.94 (m, 3 H, NH^1), 5.25 (d, J = 3.2 Hz, 1 H, CH^2), 5.14 – 5.05 (m, 3 H, CH^3), 4.43 – 4.24 (m, 2 H, CH_2^4), 3.30 – 3.05 (m, 8 H, CH_2^5), 2.78 – 2.54 (m, 4 H, CH_2^6), 2.51 – 2.16 (m, 10 H, CH_2^7), 1.91 – 1.66 (m, 7 H, CH^8, CH_2^9), 1.65 – 1.51 (m, 8 H, CH_2^{10}), 1.49 – 0.98 (m, 101 H, CH_2^{11}), 0.92 – 0.74 (m, 15 H, CH_3^{12}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 177.11, 172.86, 172.68, 172.62, 171.24, 170.17, 170.11, 170.07, 169.72, 75.75, 74.07, 74.05, 56.98, 43.34, 34.43, 33.97, 32.03, 31.95, 31.75, 30.57, 29.74, 29.67, 29.61, 29.56, 29.51, 29.48, 29.46, 29.36, 29.35, 29.24, 29.18, 29.13, 29.10, 29.03, 26.96, 26.89, 25.11, 25.08, 24.89, 24.84, 22.80, 22.77, 22.65, 22.54, 22.15, 14.23, 14.16, 11.85, 11.72.

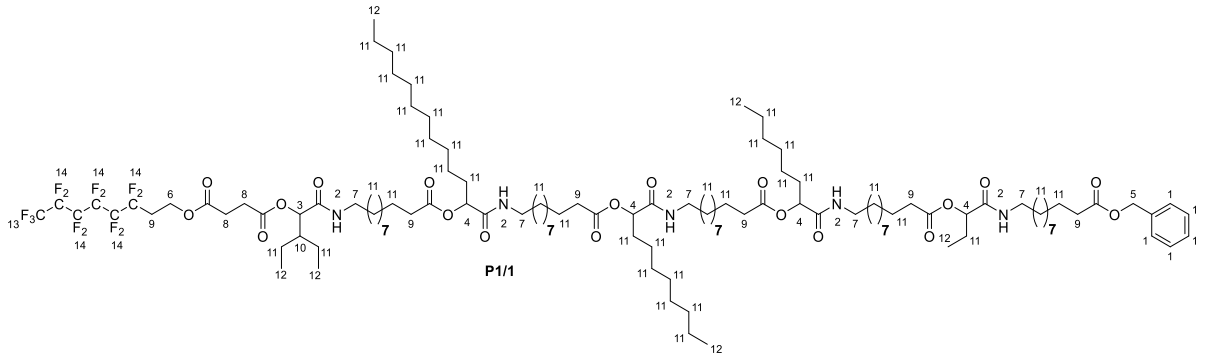
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -80.74 (t, J = 9.9 Hz, 3 F, CF_3^{13}), -113.52 – -113.84 (m, 2 F, CF_2^{14}), -121.68 – -121.98 (m, 2 F, CF_2^{14}), -122.48 – -122.99 (m, 2 F, CF_2^{14}), -123.39 – -123.65 (m, 2 F, CF_2^{14}), -125.48 – -126.44 (m, 2 F, CF_2^{14}). Total integral of CF_2 region normalized with respect to the CF_3^{14} group = 10.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{94}{}^{1}H_{161}{}^{16}O_{16}{}^{14}N_4{}^{19}F_{13}$, 1850.1773; found, 1850.1772, Δ = 0.1 mmu.



Supplementary Figure 31: ^{1}H-NMR of compound **TD1/1** measured in $CDCl_3$.

Passerini reaction



In a 10 mL round bottom flask, 1.28 g of **TD1/1** (690 µmol, 1.00 eq.) were stirred in 2.10 mL DCM. Subsequently, 148 µL propionaldehyde **A2** (120 mg, 2.07, 3.00 eq.) and 312 mg of monomer **IM2** (1.04 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 3 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel

eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (4:1 → 1:1) to yield the Passerini product **P1/1** as a pale highly viscous oil. (1.39 g, 626 µmol, 90.7%).

R_f = 0.61 in cyclohexane / ethyl acetate (3:2).

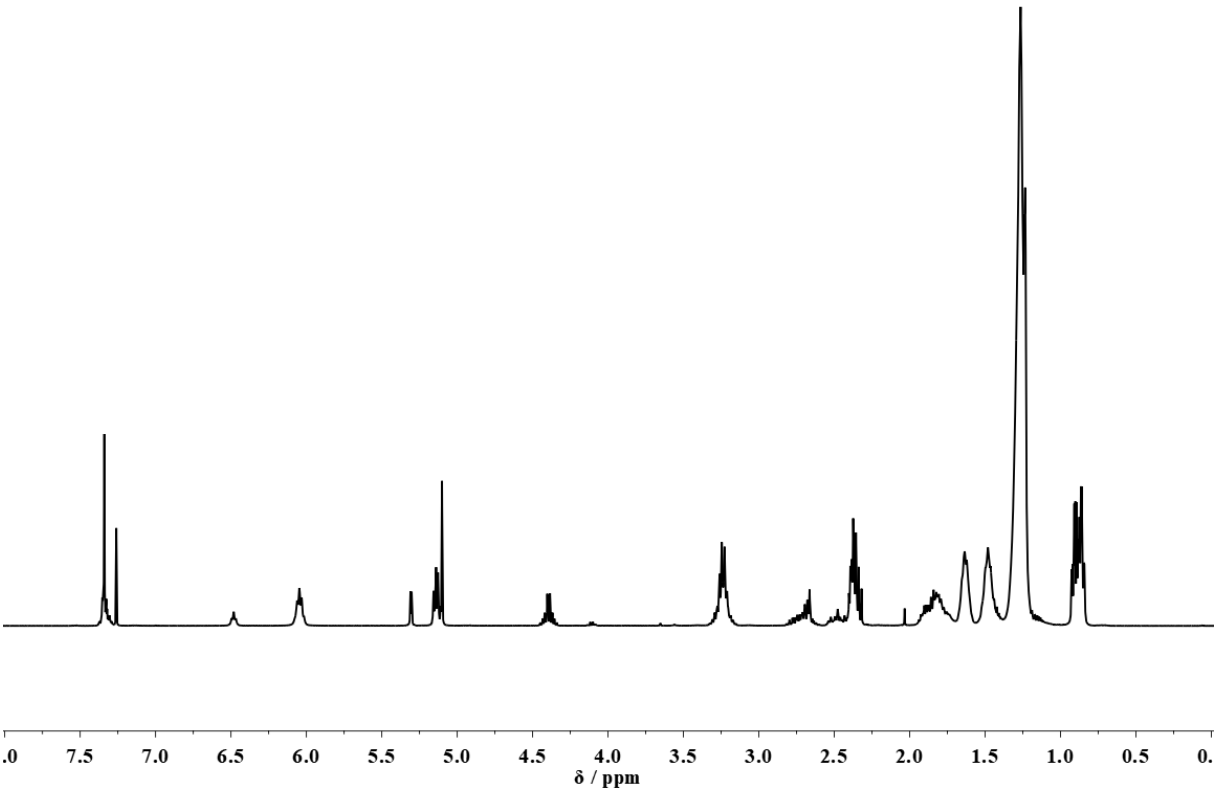
IR (ATR): ν / cm^{-1} = 3304.8 (vw), 2923.9 (s), 2853.8 (m), 1739.6 (s), 1654.1 (s), 1535.4 (m), 1459.0 (w), 1374.5 (w), 1236.5 (s), 1145.2 (vs), 697.1 (w), 651.2 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.39 – 7.23 (m, 5 H, $CH_{Ar}{}^{1}$), 6.42 (t, J = 5.8 Hz, 1 H, NH^{2}), 6.12 – 5.89 (m, 4 H, NH^{2}), 5.25 (d, J = 3.1 Hz, 1 H, CH^{3}), 5.16 – 4.98 (m, 6 H, CH^{4}, $CH_2{}^{5}$), 4.45 – 4.26 (m, 2 H, $CH_2{}^{6}$), 3.33 – 3.08 (m, 10 H, $CH_2{}^{7}$), 2.79 – 2.53 (m, 4 H, $CH_2{}^{8}$), 2.50 – 2.24 (m, 12 H, $CH_2{}^{9}$), 1.90 – 1.51 (m, 21 H, CH^{10}, $CH_2{}^{11}$), 1.50 – 1.02 (m, 112 H, $CH_2{}^{11}$), 0.90 – 0.70 (m, 18 H, $CH_3{}^{12}$).

^{13}C NMR (101MHz, $CDCl_3$): δ / ppm = 173.80, 172.84, 172.60, 172.55, 171.22, 170.00, 169.74, 169.62, 136.24, 128.66, 128.27, 75.75, 74.92, 74.04, 66.18, 56.97, 43.34, 39.45, 39.30, 34.43, 32.03, 31.94, 31.74, 29.73, 29.69, 29.65, 29.60, 29.57, 29.55, 29.50, 29.46, 29.37, 29.33, 29.24, 29.22, 29.18, 29.12, 29.03, 26.97, 26.95, 25.21, 25.08, 24.88, 24.83, 22.80, 22.76, 22.65, 22.53, 22.15, 14.22, 14.22, 14.16, 11.85, 11.73, 9.13.

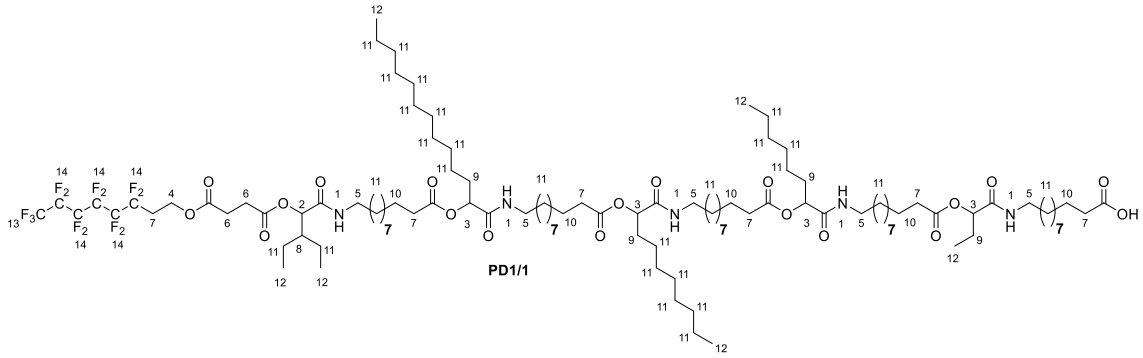
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -80.64 – -81.03 (m, 3 F, $CF_3{}^{13}$), -113.54 – -113.82 (m, 2 F, $CF_2{}^{14}$), -121.66 – -122.01 (m 2 F, $CF_2{}^{14}$), -122.72 – -122.99 (m 2 F, $CF_2{}^{14}$), -123.41 – -123.70 (m 2 F, $CF_2{}^{14}$), -125.95 – -126.19 (m 2 F, $CF_2{}^{14}$). Total integral of CF_2 region normalized with respect to the $CF_3{}^{15}$ group = 10.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{116}{}^{1}H_{194}{}^{16}O_{19}{}^{14}N^{19}F_{13}$, 2209.4233; found, 2209.4261, Δ = 2.8 mmu.



Supplementary Figure 32: ^{1}H-NMR of compound **P1/1** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 1.04 g of **P1/1** (469 µmol, 1.00 eq.) were dissolved in 5.00 mL ethyl acetate and 5.00 mL THF. Afterwards, 207 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **PD1/1** was obtained as a pale highly viscous oil in a yield of 98.7% (981 mg, 462 µmol).

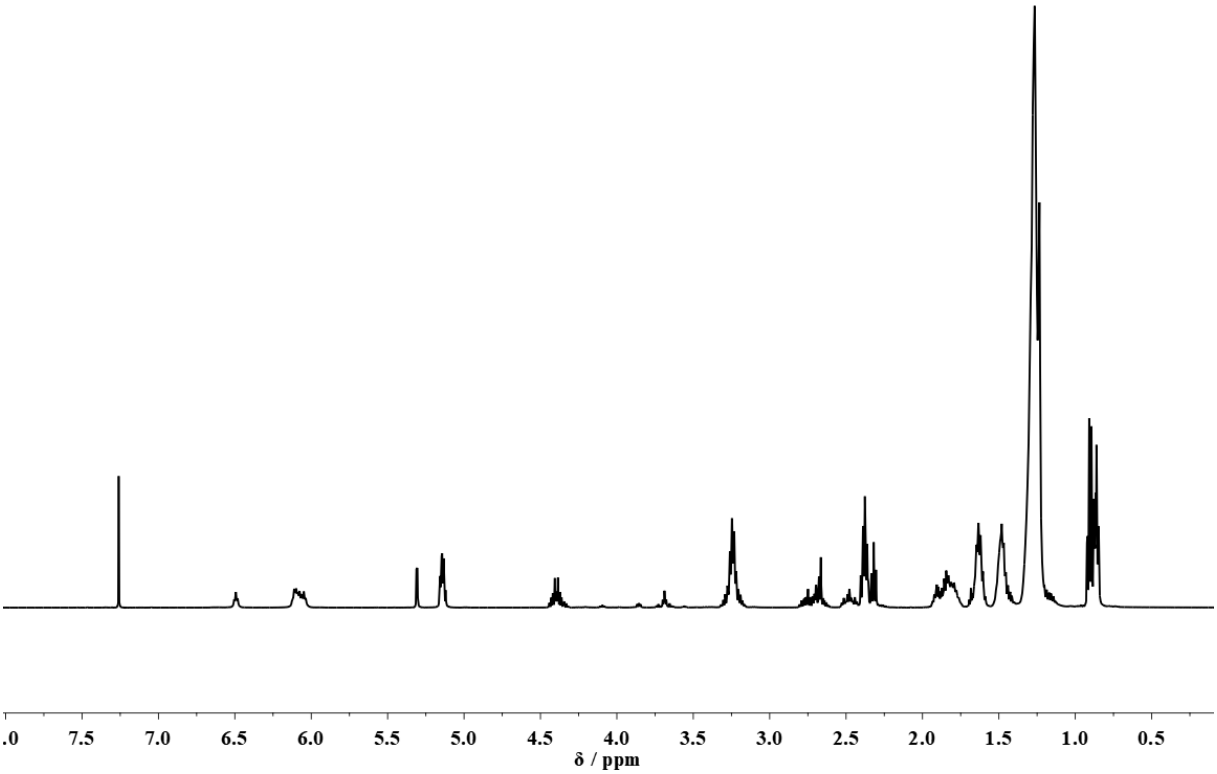
IR (ATR): ν / cm^{-1} = 3305.4 (vw), 2924.0 (s), 2854.0 (m), 1740.7 (s), 1654.4 (s), 1539.7 (m), 1463.5 (w), 1374.8 (w), 1236.9 (vs), 1145.3 (vs), 1008.3 (w), 841.8 (vw), 808.9 (vw), 720.6 (w), 651.0 (w), 400.3 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.49 (t, J = 5.8 Hz, 1 H, NH^{1}), 6.17 – 5.99 (m, 4 H, NH^{1}), 5.31 (d, J = 3.2 Hz, 1 H, CH^{2}), 5.16 – 5.08 (m, 4 H, CH^{3}), 4.49 – 4.30 (m, 2 H, CH_2^{4}), 3.35 – 3.14 (m, 10 H, CH_2^{5}), 2.84 – 2.58 (m, 4 H, CH_2^{6}), 2.55 – 2.25 (m, 12 H, CH_2^{7}), 1.98 – 1.72 (m, 9 H, CH^{8}, CH_2^{9}), 1.70 – 1.56 (m, 10 H, CH_2^{10}), 1.53 – 1.12 (m, 127 H, CH_2^{11}), 0.96 – 0.82 (m, 18 H, CH_3^{12}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 176.86, 172.87, 172.70, 172.64, 172.63, 172.59, 171.25, 170.18, 170.11, 170.09, 169.86, 169.70, 75.74, 74.94, 74.04, 62.92, 56.98, 43.34, 39.48, 39.37, 39.34, 39.29, 34.43, 33.96, 32.02, 32.01, 31.95, 31.75, 30.73, 30.56, 30.39, 30.00, 29.74, 29.69, 29.66, 29.61, 29.59, 29.56, 29.52, 29.50, 29.47, 29.42, 29.37, 29.37, 29.33, 29.24, 29.21, 29.18, 29.13, 29.09, 29.03, 26.98, 26.95, 26.87, 25.19, 25.11, 25.08, 24.91, 24.90, 24.84, 22.66, 22.53, 22.15, 14.24, 14.23, 14.17, 11.85, 11.73, 9.15.

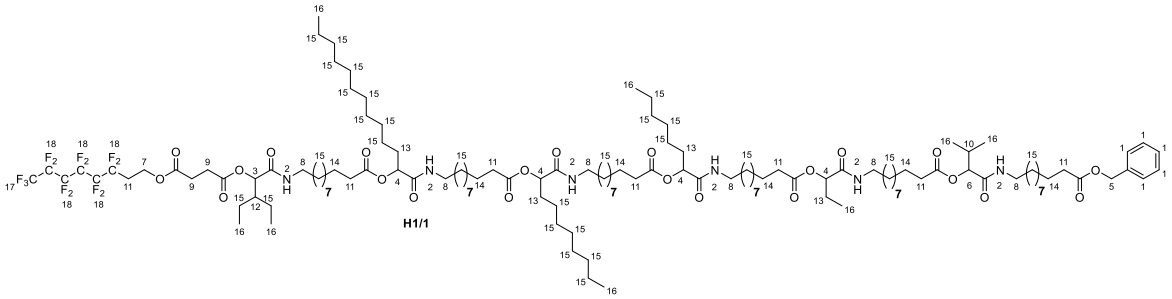
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -80.56 – -80.94 (m, 3 F, CF_3), -113.44 – -113.80 (m, 2 F, CF_2), -121.63 – -122.01 (m), -122.60 – -123.02 (m), -123.25 – -123.84 (m), -125.82 – -126.33 (m). Total integral of CF_2 region normalized with respect to the CF_3^{14} group = 10.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{109}{}^{1}H_{188}{}^{16}O_{19}{}^{14}N_5{}^{19}F_{13}$, 2119.3764; found, 2119.3829, Δ = 6.5 mmu.



Supplementary Figure 33: ^{1}H-NMR of compound **PD1/1** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 877 mg of **PD1/1** (414 µmol, 1.00 eq.) was dissolved in 2.50 mL DCM and 57.0 µL isobutyraldehyde **A3** (44.8 mg, 621 µmol, 1.50 eq.) and 187 mg of monomer **IM2** (621 µmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 2 days and subsequently the solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane / ethyl acetate 4:1→ 1:2) to afford product **H1/1** as a white solid in a yield of 83.8% (863 mg, 347 µmol).

R_f = 0.71 in cyclohexane / ethyl acetate (1:1).

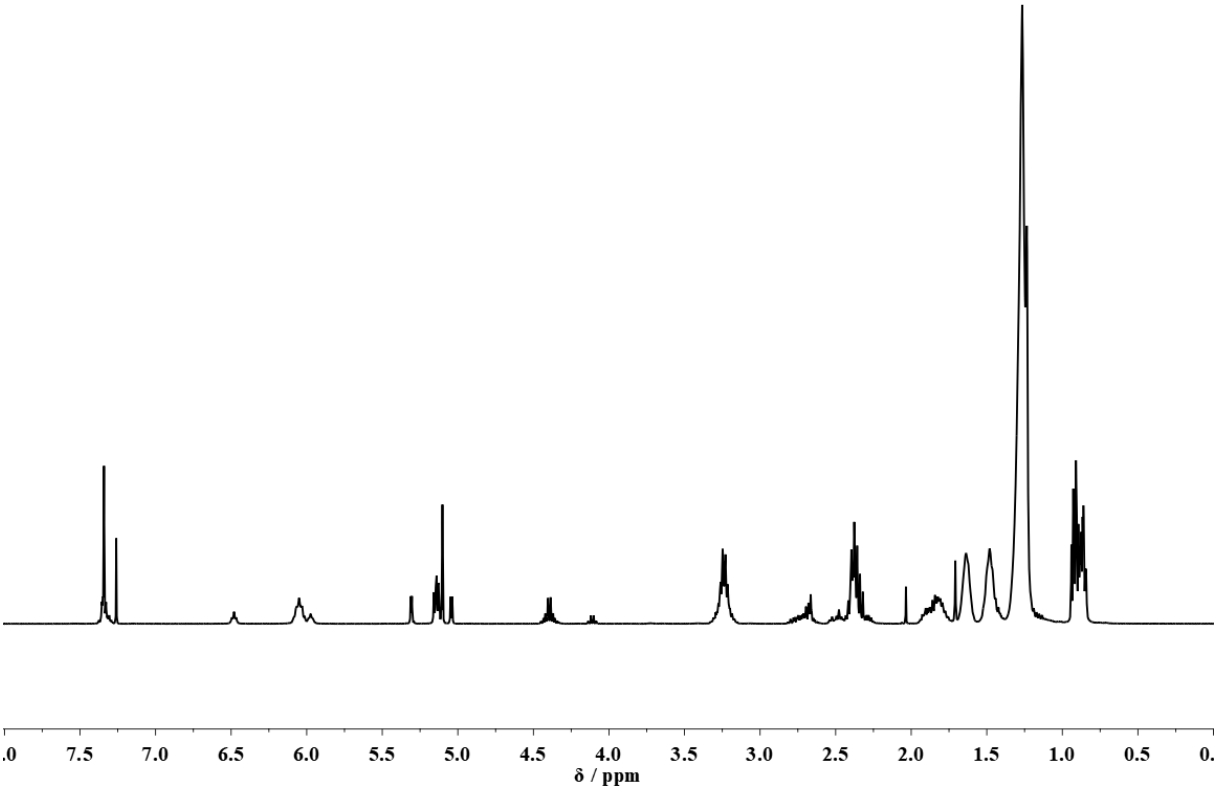
IR (ATR): ν / cm^{-1} = 3306.1 (vw), 2924.1 (s), 2853.9 (m), 1740.5 (s), 1654.5 (s), 1535.9 (m), 1462.7 (w), 1370.2 (w), 1237.3 (s), 1145.6 (s), 697.4 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.43 – 7.31 (m, 5 H, CH_{Ar}[1]), 6.50 (t, J = 5.8 Hz, 1 H, NH[2]), 6.13 – 5.96 (m, 5 H, NH[2]), 5.33 (d, J = 3.1 Hz, 1 H, CH[3]), 5.20 – 5.14 (m, 4 H, CH[4]), 5.12 (s, 2 H, CH_2[5]), 5.07 (d, J = 4.5 Hz, 1 H, CH[6]), 4.50 – 4.33 (m, 2 H, CH_2[7]), 3.38 – 3.16 (m, 12 H, CH_2[8]), 2.84 – 2.61 (m, 4 H, CH_2[9]), 2.58 – 2.25 (m, 15 H, CH[10], CH_2[11]), 1.99 – 1.75 (m, 9 H, CH[12], CH_2[13]), 1.71 – 1.60 (m, 12 H, CH_2[14]), 1.57 – 1.11 (m, 126 H, CH_2[15]), 0.98 – 0.83 (m, 24 H, CH_3[16]).

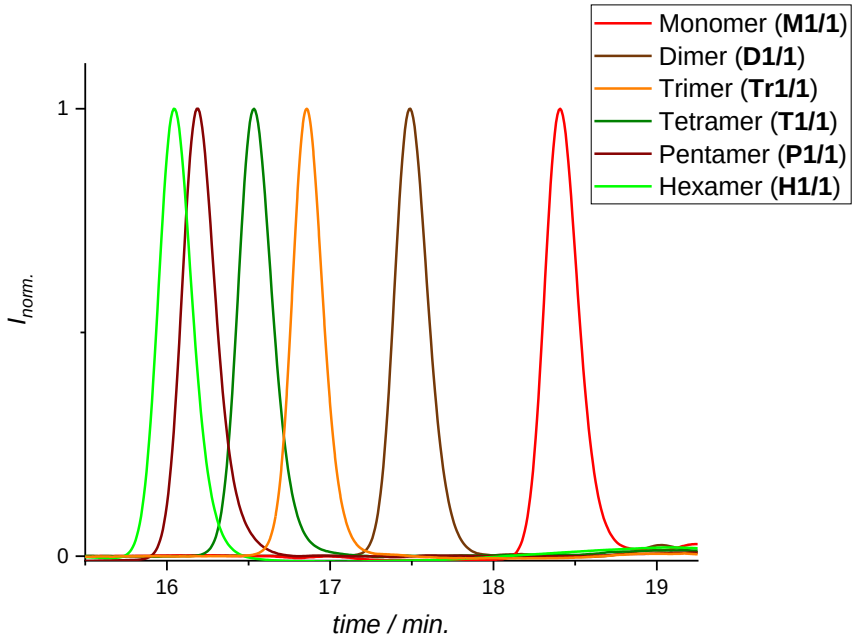
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.81, 172.85, 172.68, 172.60, 172.57, 171.23, 169.99, 169.75, 169.61, 169.38, 136.24, 128.66, 128.28, 78.03, 75.74, 74.92, 74.04, 66.19, 56.97, 43.34, 39.45, 39.30, 39.28, 34.44, 34.41, 32.04, 31.95, 31.75, 30.64, 29.74, 29.70, 29.66, 29.61, 29.58, 29.56, 29.51, 29.47, 29.37, 29.33, 29.31, 29.24, 29.22, 29.18, 29.13, 29.03, 26.96, 25.21, 25.12, 25.08, 25.06, 24.89, 24.84, 22.80, 22.76, 22.65, 22.53, 22.15, 18.91, 17.08, 14.23, 14.21, 14.17, 11.86, 11.73, 9.15.

^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.08 (t, J = 9.9 Hz, 3 F, CF_3[17]), -117.53 – -118.42 (m, 2 F, CF_2[18]), -125.96 – -126.57 (m, 2 F, CF_2[18]), -126.82 – -127.46 (m, 2 F, CF_2[18]), -127.67 – -128.17 (m, 2 F, CF_2[18]), -129.91 – -130.82 (m, 2 F, CF_2[18]). Total integral of CF_2 region normalized with respect to the CF_3[18]group = 10.

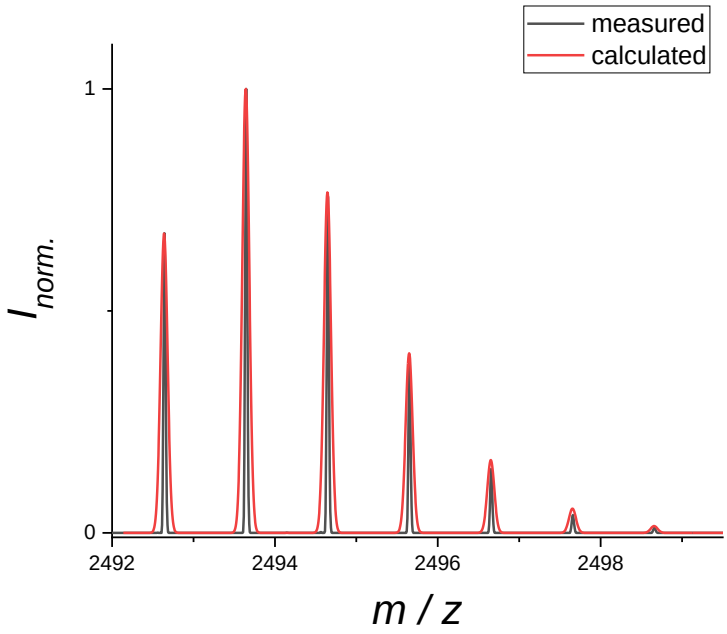
ESI-MS [m/z]: $[M + H]^+$ calculated for $^{12}C_{132}{}^{1}H_{223}{}^{19}F_{13}{}^{14}N_6{}^{16}O_{22}$, 2492.6381; found, 2492.6446, Δ = 6.5 mmu.



Supplementary Figure 34: ^{1}H-NMR of compound H1/1 measured in $CDCl_3$.



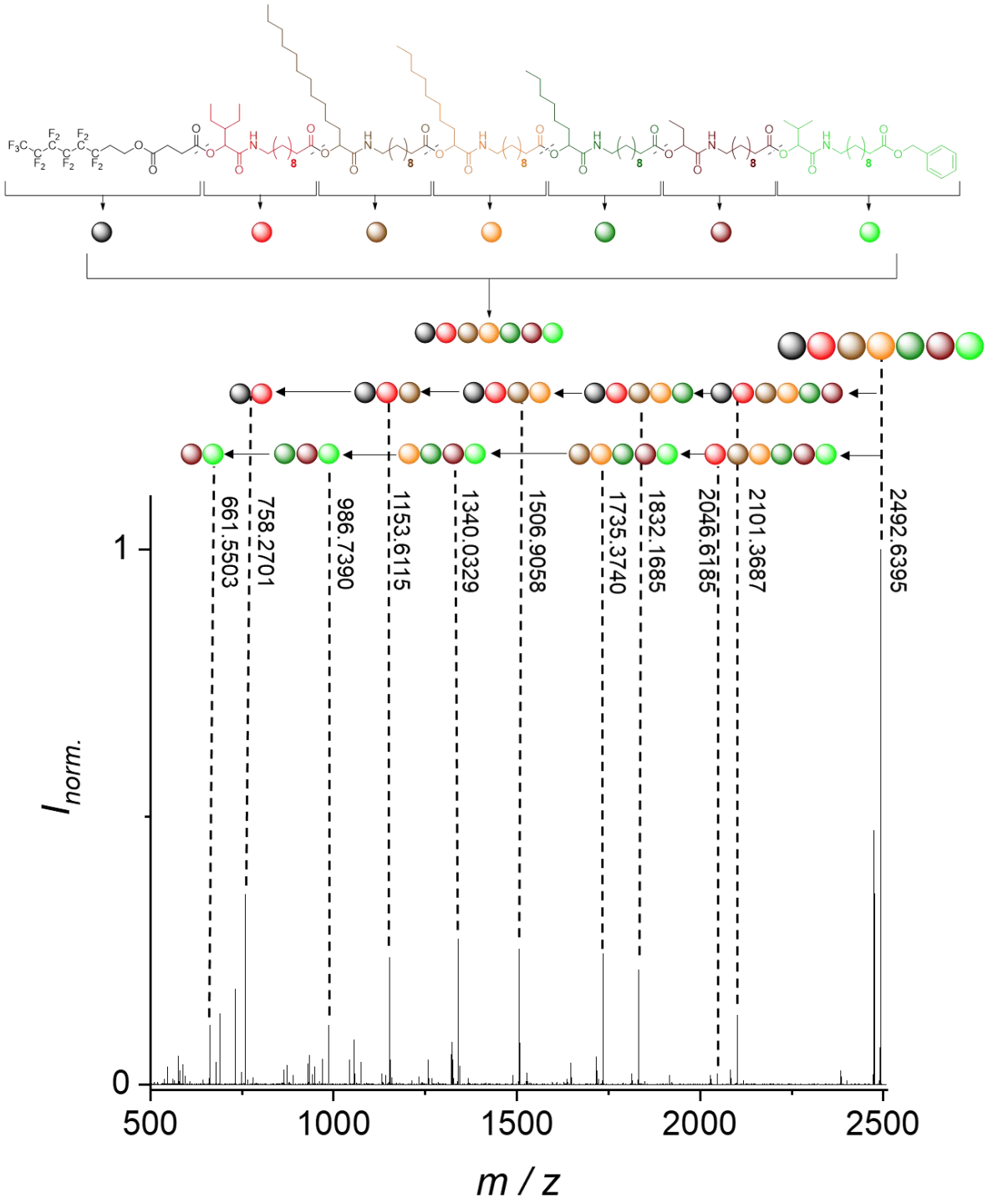
Supplementary Figure 35: SEC traces of the intermediates after each P-3CR in the synthesis of product **H1/1**.



Supplementary Figure 36: High resolution ESI-MS measurement of **H1/1**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (black).

```
found 22260 values in C:\Users\Maxi\Documents\Mixture 2492 NCE 18 with 3.CSV, maximum is 1.000000 found for mass 2492.647
480
matching mass 2492.64748
cutoff 0.50000: 0 solutions (4 peaks)
cutoff 0.25000: 0 solutions (11 peaks)
cutoff 0.12500: 0 solutions (37 peaks)
cutoff 0.06250: 0 solutions (69 peaks)
cutoff 0.03125: 0 solutions (119 peaks)
cutoff 0.01562: 0 solutions (230 peaks)
cutoff 0.00781: 0 solutions (375 peaks)
cutoff 0.00391: 0 solutions (623 peaks)
cutoff 0.00195: 0 solutions (975 peaks)
cutoff 0.00098: 1 solutions (1465 peaks)
2492.64748 ≈ 447.026590 + 311.246050 + 395.339950 + 353.293000 + 325.261700 + 269.199100 + 283.214750 + 107.049690 (sides
 2-Ethylbutanal, Dodecanal, Nonanal, Heptanal, Propionaldehyde, Isobutyraldehyde; error -1.01665)
Press ENTER to quit ...
```

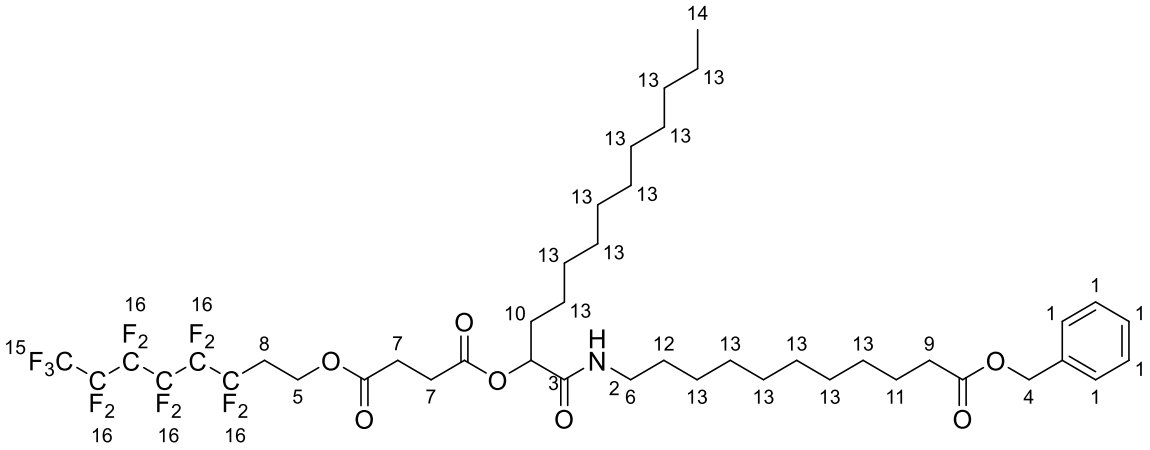
Supplementary Figure 37: Screenshot of the automated read-out of **H1/1**.



Supplementary Figure 38: Read-out of the sequence-defined hexamer **H1/1**. Read-out of the hexamer **H1/1** *via* tandem ESI-MS/MS with an NCE of 18. In the spectrum, the read-out from both ends of the oligomer using the fragmentation next to the carbonyl are shown.

6.3.3.3.3 Synthesis of tetramer T1/2

Passerini reaction



In 50.0 mL round bottom flak, 1.00 g **TAG1** (2.15 mmol, 1.00 eq.) were dissolved in 6.00 mL DCM and 550 mg dodecanal **A11** (3.23 mmol, 1.50 eq.) and 974 mg of monomer **IM2** (3.23 mol, 1.50 eq.) were added. The mixture was stirred at room temperature for 2 days. Subsequently, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (hexane / ethyl acetate 6:1 → 4:1) to afford product **M1/2** as a yellow oil in a yield of 94.0% (1.93 g, 2.02 mmol).

R_f = 0.60 in cyclohexane / ethyl acetate (3:1).

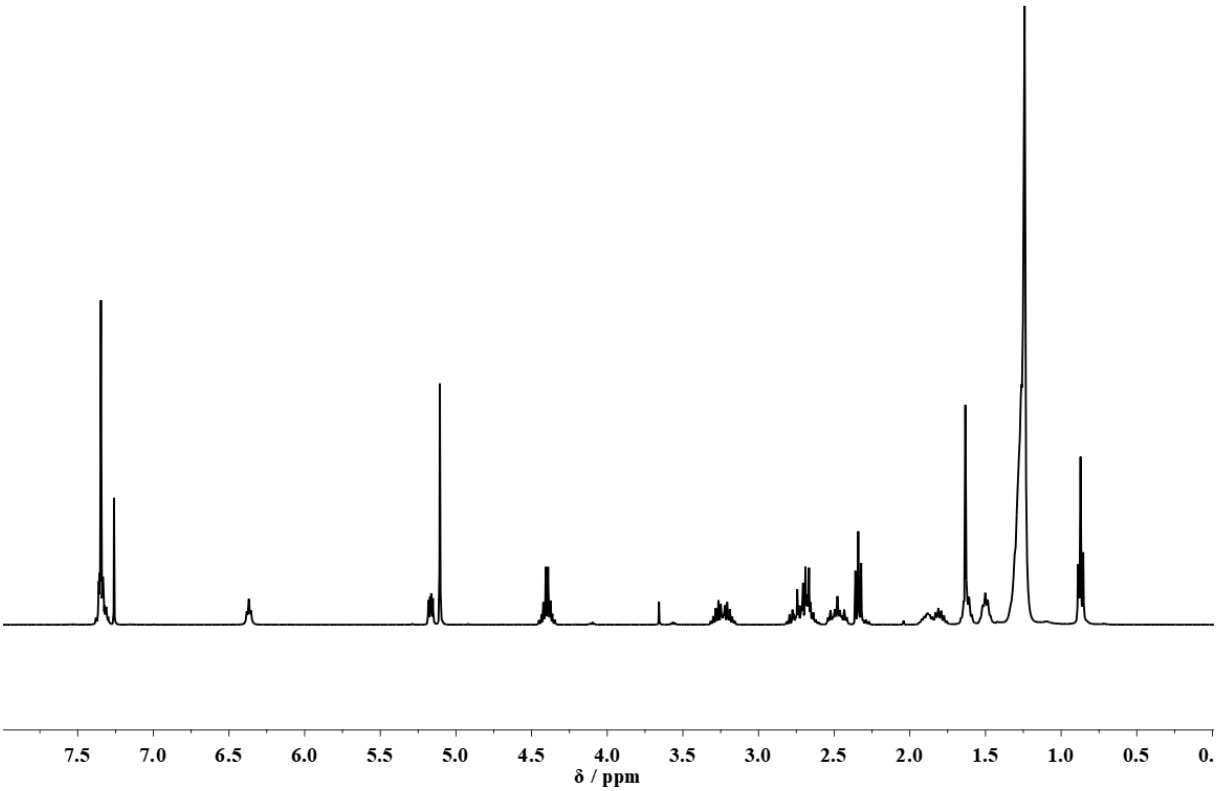
IR (ATR): ν / cm^{-1} = 2925.0 (m), 2854.4 (w), 1738.1 (s), 1658.1 (w), 1535.5 (w), 1457.7 (w), 1359.5 (w), 1235.3 (vs), 1202.8 (vs), 1144.6 (vs), 1081.9 (m), 1005.2 (w), 842.0 (vw), 808.9 (vw), 732.4 (w), 697.2 (m), 651.2 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.41 – 7.28 (m, 5 H, $CH_{Ar}{}^1$), 6.37 (t, J = 5.8 Hz, 1 H, NH^2), 5.19 – 5.15 (m, 1 H, CH^3), 5.11 (s, 2 H, $CH_2{}^4$), 4.48 – 4.32 (m, 2 H, $CH_2{}^5$), 3.34 – 3.15 (m, 2 H, $CH_2{}^6$), 2.82 – 2.60 (m, 4 H, $CH_2{}^7$), 2.48 (s, 2 H, $CH_2{}^8$), 2.34 (t, J = 7.5 Hz, 2 H, $CH_2{}^9$), 1.96 – 1.74 (m, 2 H, $CH_2{}^{10}$), 1.68 – 1.58 (m, 2 H, $CH_2{}^{11}$), 1.56 – 1.44 (m, 2 H, $CH_2{}^{12}$), 1.36 – 1.19 (m, 30 H, $CH_2{}^{13}$), 0.87 (t, J = 6.8 Hz, 3 H, $CH_3{}^{14}$).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.81, 172.63, 171.24, 169.71, 136.26, 128.66, 128.28, 74.75, 66.19, 56.95, 39.46, 34.44, 32.04, 31.94, 30.57, 29.75, 29.69, 29.60, 29.58, 29.53, 29.49, 29.48, 29.38, 29.35, 29.24, 29.22, 29.13, 26.97, 25.06, 25.03, 22.82, 14.24.

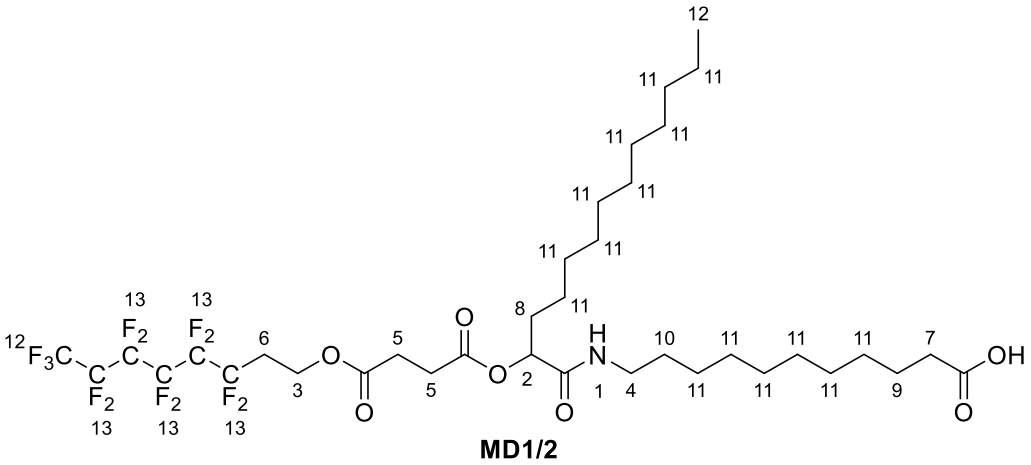
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -83.76 – -86.21 (m, 3 F, CF_3^{15}), -117.01 – -118.71 (m, 2 F, CF_2^{16}), -126.05 – -126.35 (m, 2 F, CF_2^{16}), -127.05 – -127.34 (m, 2 F, CF_2^{16}), -127.79 – -128.06 (m, 2 F, CF_2^{16}), -130.36 – -130.58 (m, 2 F, CF_2^{16}). Total integral of CF_2 region normalized with respect to the CF_3^{15} group = 10.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{43}{}^{1}H_{60}{}^{16}O_7{}^{14}N{}^{19}F_{13}$, 950.4235; found, 950.4210, Δ = 2.5 mmu.



Supplementary Figure 39: ^{1}H-NMR of compound **M1/2** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 1.82 g of **M1/2** (1.91 mmol, 1.00 eq.) were dissolved in 3.00 mL ethyl acetate and 3.00 mL THF. Afterwards, 363 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **MD1/2** was obtained as a highly viscous oil in a yield of 96.3% (1.58 g, 1.84 mmol).

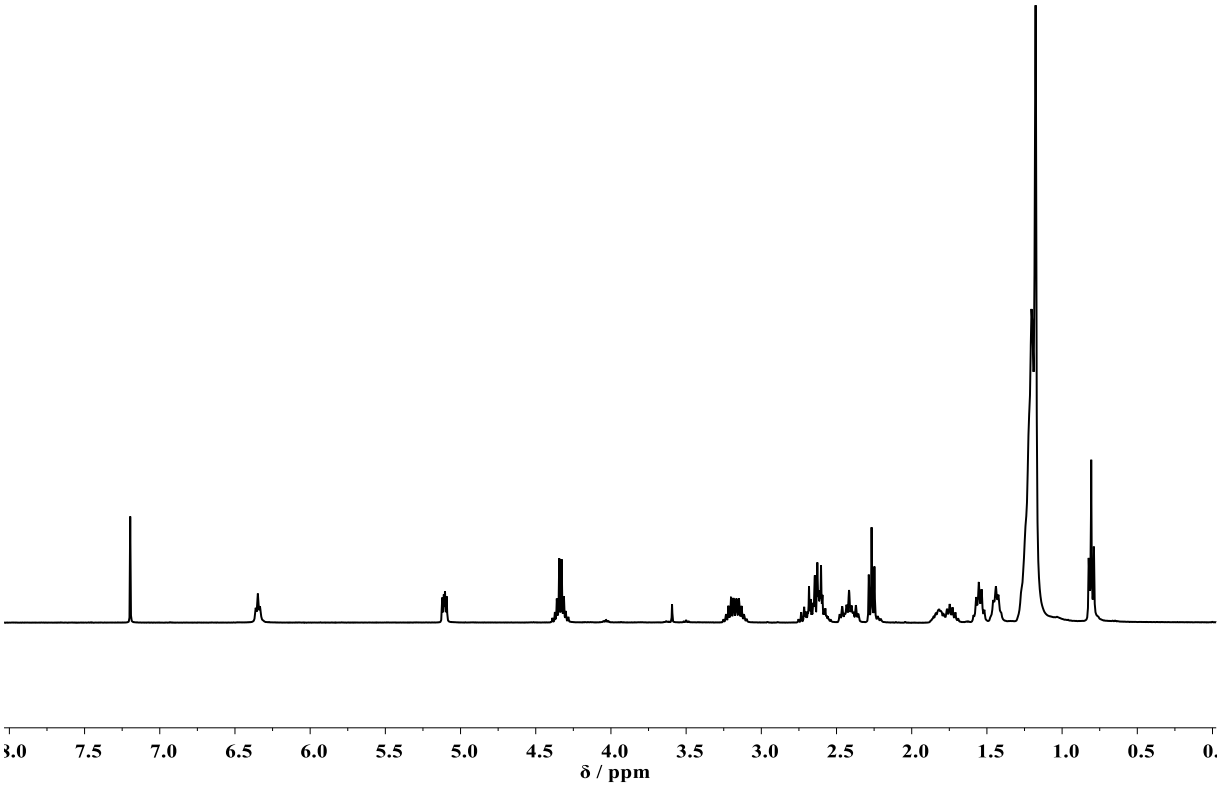
IR (ATR): ν / cm^{-1} = 3291.4 (w), 2913.2 (vs), 2847.8 (s), 1740.8 (vs), 1695.6 (s), 1659.5 (vs), 1556.4 (m), 1469.3 (m), 1414.0 (w), 1360.7 (m), 1188.8 (vs), 1162.5 (vs), 1141.8 (vs), 1080.1 (s), 905.0 (w), 839.7 (w), 808.4 (w), 732.6 (m), 698.7 (s), 651.0 (m), 565.9 (w), 529.5 (w), 460.5 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.41 (t, J = 5.8 Hz, 1 H, NH^{1}), 5.21 – 5.13 (m, 1 H, CH^{2}), 4.50 – 4.31 (m, 2 H, CH_2^{3}), 3.33 – 3.14 (m, 2 H, CH_2^{4}), 2.84 – 2.60 (m, 4 H, CH_2^{5}), 2.56 – 2.40 (m, 2 H, CH_2^{6}), 2.33 (t, J = 7.5 Hz, 2 H, CH_2^{7}), 1.96 – 1.74 (m, 2 H, CH_2^{8}), 1.67 – 1.56 (m, 2 H, CH_2^{9}), 1.54 – 1.45 (m, 2 H, CH_2^{10}), 1.39 – 1.17 (m, 30 H, CH_2^{11}), 0.87 (t, J = 6.7 Hz, 3 H, CH_3^{12}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 178.65, 172.69, 171.28, 74.76, 56.98, 39.49, 33.99, 32.05, 31.93, 30.60, 29.76, 29.70, 29.59, 29.49, 29.46, 29.38, 29.35, 29.25, 29.23, 29.21, 29.15, 29.08, 26.91, 25.04, 24.79, 22.82, 14.24.

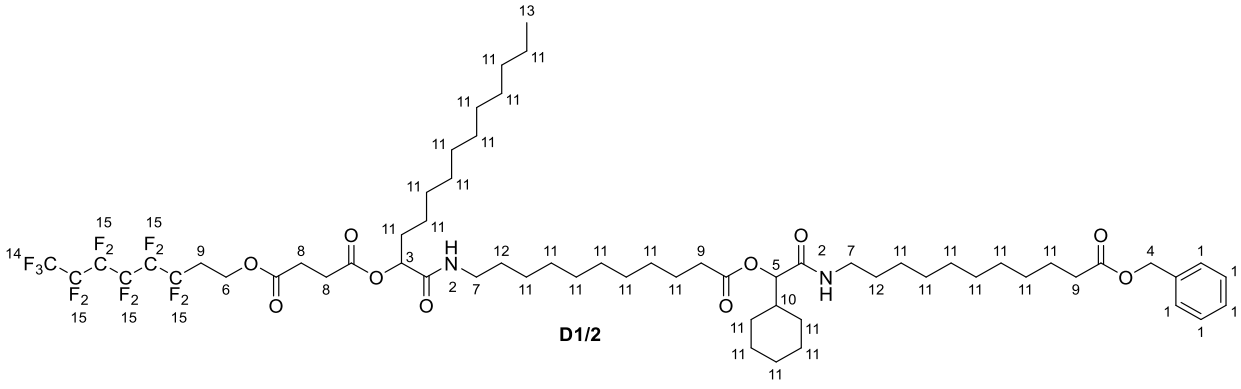
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -84.77 – -85.47 (m, 3 F, CF_3^{12}), -117.91 – -118.15 (m, 2 F, CF_2^{13}), -126.04 – -126.37 (m, 2 F, CF_2^{13}), -127.07 – -127.35 (m, 2 F, CF_2^{13}), -127.78 – -128.08 (m, 2 F, CF_2^{13}), -130.31 – -130.62 (m, 2 F, CF_2^{13}). Total integral of CF_2 region normalized with respect to the CF_3^{13} group = 10.

ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{36}{}^{1}H_{54}{}^{16}O_{7}{}^{14}N^{19}F_{13}$, 882.3585; found, 882.3559, Δ = 2.6 mmu.



Supplementary Figure 40: ^{1}H-NMR of compound **MD1/2** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 1.53 g of **MD1/2** (1.78 mmol, 1.00 eq.) was dissolved in 4.00 mL DCM and 323 µL cyclohexanecarboxaldeyhde **A6** (319 mg, 2.67 mmol, 1.50 eq.) and 857 mg of monomer **IM2** (2.67 mmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 3 days. Subsequently, the solvent was removed under reduced pressure. The

crude product was purified by column chromatography (cyclohexane / ethyl acetate 5:1 → 2:1) to afford product **D1/2** as a pale highly viscous oil in a yield of 91.1% (2.06 g, 1.62 mmol).

R_f = 0.50 in cyclohexane / ethyl acetate (3:1).

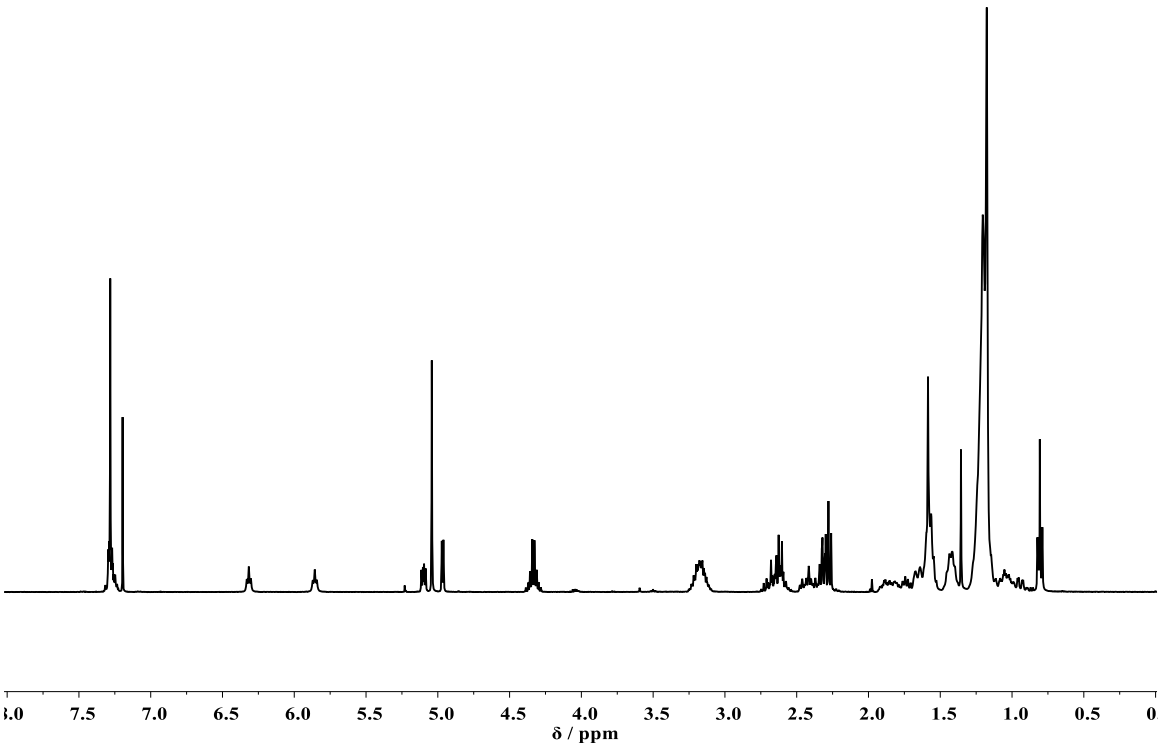
IR (ATR): ν / cm^{-1} = 3285.3 (vw), 2919.4 (s), 2850.8 (m), 1737.6 (vs), 1652.4 (s), 1552.7 (w), 1466.8 (w), 1362.9 (w), 1235.9 (vs), 1143.8 (vs), 1082.8 (m), 1005.2 (w), 842.4 (vw), 809.6 (vw), 697.3 (s), 652.3 (w), 567.2 (vw), 455.5 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.36 – 7.22 (m, 5 H, CH_{Ar}[1]), 6.32 (t, J = 5.8 Hz, 1 H, NH[2]), 5.86 (t, J = 5.9 Hz, 1 H, NH[2]), 5.12 – 5.07 (m, 1 H, CH[3]), 5.04 (s, 2 H, CH_2[4]), 4.97 (d, J = 4.6 Hz, 1 H, CH[5]), 4.43 – 4.25 (m, 2 H, CH_2[6]), 3.25 – 3.06 (m, 4 H, CH_2[7]), 2.78 – 2.55 (m, 4 H, CH_2[8]), 2.51 – 2.21 (m, 6 H, CH_2[9]), 1.95 – 1.51 (m, 13 H, CH[10], CH_2[11]), 1.47 – 1.37 (m, 4 H, CH_2[12]), 1.30 – 0.93 (m, 46 H, CH_2[11]), 0.84 – 0.76 (m, 3 H, CH_3[13]).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.83, 172.65, 171.25, 169.74, 169.34, 136.26, 128.67, 128.29, 77.74, 74.75, 66.20, 56.94, 40.12, 39.44, 39.27, 34.45, 34.42, 32.04, 31.94, 30.57 (t, J = 21.6 Hz), 29.75, 29.70, 29.68, 29.61, 29.57, 29.54, 29.52, 29.47, 29.36, 29.32, 29.27, 29.24, 29.22, 29.13, 27.39, 27.05, 26.97, 26.19, 26.12, 26.01, 25.12, 25.07, 25.04, 22.81, 14.24.

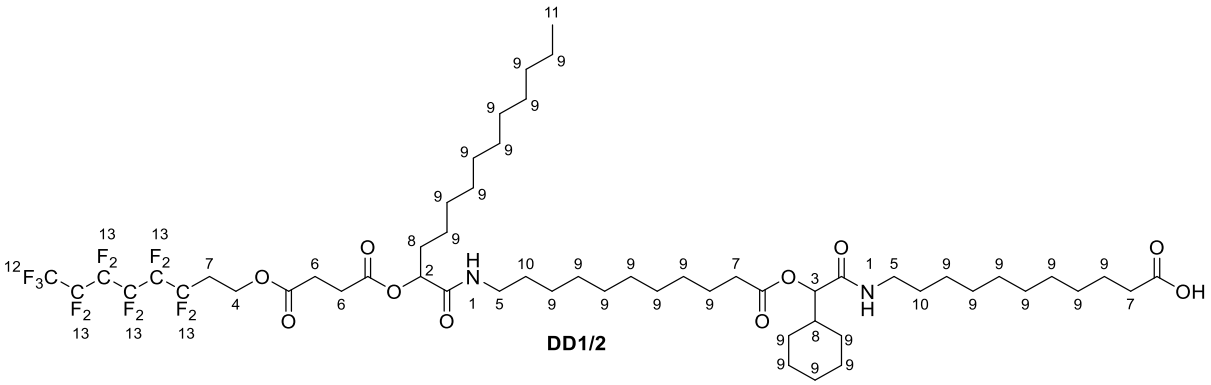
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -84.31 – -85.80 (m, 3 F, CF_3[14]), -117.77 – -118.47 (m, 2 F, CF_2[15]), -125.71 – -126.57 (m, 2 F, CF_2[15]), -126.86 – -127.43 (m, 2 F, CF_2[15]), -127.48 – -128.34 (m, 2 F, CF_2[15]), -129.95 – -130.75 (m, 2 F, CF_2[15]). Total integral of CF_2 region normalized with respect to the CF_3[14]group = 10.

ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{62}{}^{1}H_{93}{}^{16}O_{10}{}^{14}N_2{}^{19}F_{13}$, 1295.6515; found, 1261.6500, Δ = 1.5 mmu.



Supplementary Figure 41: ^{1}H-NMR of compound **D1/2** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 1.98 g of **D1/2** (1.56 mmol, 1.00 eq.) were dissolved in 4.00 mL ethyl acetate and 4.00 mL THF. Afterwards, 396 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **DD1/2** was obtained as a highly viscous oil in a yield of 98.7% (1.82 g, 1.54 mmol).

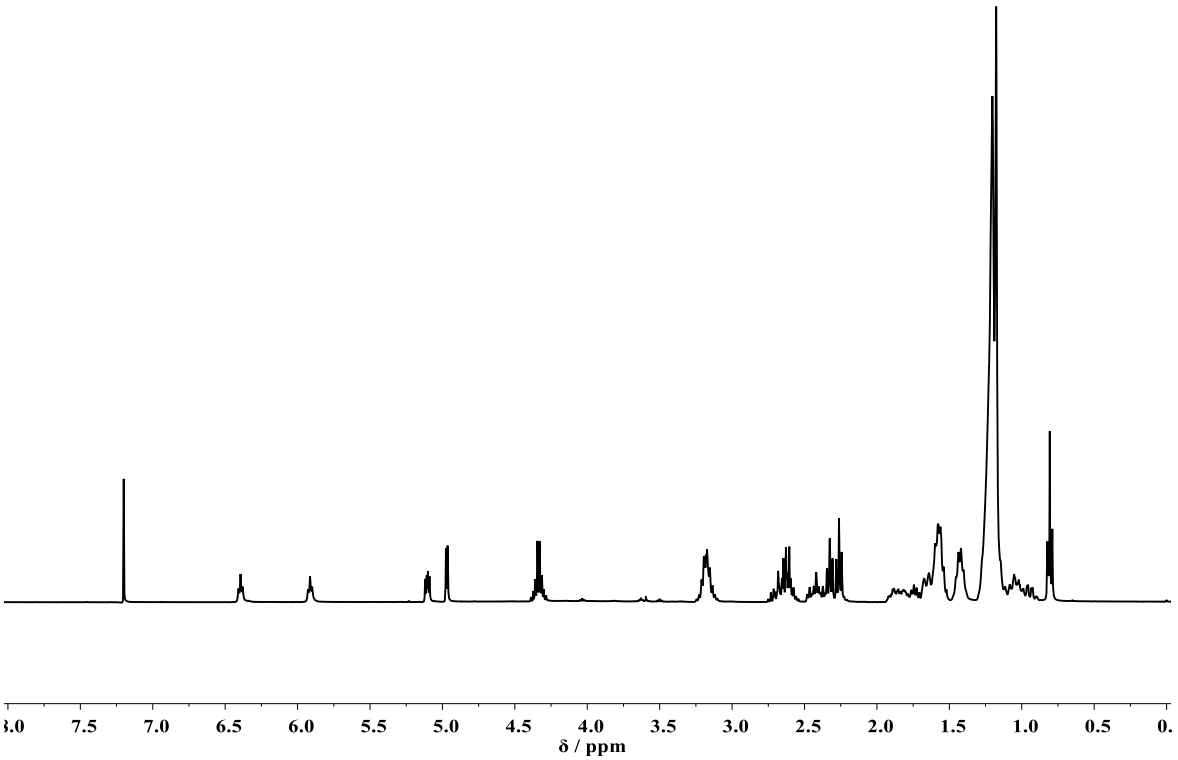
IR (ATR): ν / cm^{-1} = 3297.6 (w), 2919.4 (s), 2851.0 (m), 1736.5 (vs), 1655.2 (vs), 1555.0 (m), 1466.3 (w), 1364.5 (m), 1235.5 (vs), 1164.6 (vs), 1143.4 (vs), 1082.3 (m), 1006.9 (w), 842.1 (vw), 810.2 (vw), 697.9 (m), 652.2 (w), 567.0 (vw), 530.7 (vw), 453.2 (vw), 394.1 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.45 (t, J = 5.8 Hz, 1 H, NH^{1}), 5.97 (t, J = 5.8 Hz, 1 H, NH^{1}), 5.19 – 5.13 (m, 1 H, CH^{2}), 5.03 (d, J = 4.6 Hz, 1 H, CH^{3}), 4.46 – 4.33 (m, 2 H, CH_2^{4}), 3.31 – 3.15 (m, 4 H, CH_2^{5}), 2.81 – 2.58 (m, 4 H, CH_2^{6}), 2.56 – 2.28 (m, 6 H, CH_2^{7}), 2.00 – 1.56 (m, 13 H, CH^{8}, CH_2^{9}), 1.55 – 1.42 (m, 4 H, CH_2^{10}), 1.37 – 0.97 (m, 46 H, CH_2^{9}), 0.93 – 0.82 (m, 3 H, CH_3^{11}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 178.65, 173.46, 172.07, 170.72, 170.23, 78.52, 75.49, 57.73, 40.84, 40.28, 40.02, 35.19, 34.81, 32.80, 32.67, 31.34 (t, J = 21.8 Hz), 30.51, 30.44, 30.38, 30.35, 30.33, 30.28, 30.27, 30.23, 30.21, 30.12, 30.04, 29.99, 29.97, 29.89, 29.86, 28.17, 27.73, 27.66, 26.95, 26.87, 26.76, 25.90, 25.79, 25.64, 23.57, 14.98.

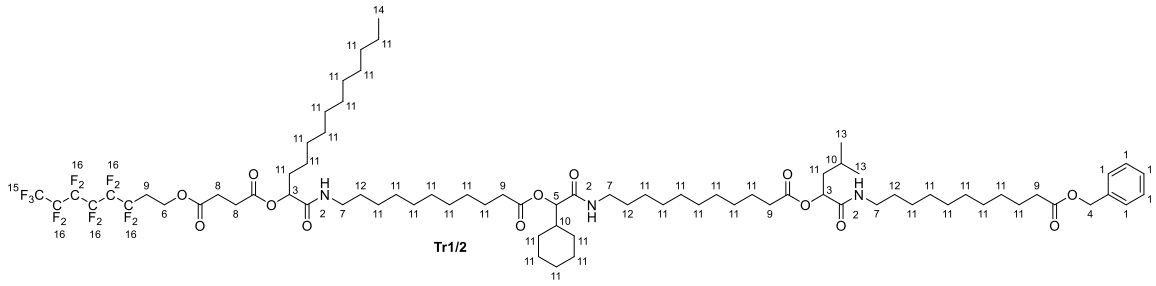
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.10 (t, J = 9.8 Hz, 3 F, CF_3^{12}), -117.60 – -118.27 (m, 2 F, CF_2^{13}), -125.80 – -126.54 (m, 2 F, CF_2^{13}), -126.84 – -127.47 (m, 2 F, CF_2^{13}), -127.62 – -128.11 (m, 2 F, CF_2^{13}), -130.25 – -130.87 (m, 2 F, CF_2^{13}). Total integral of CF_2 region normalized with respect to the CF_3^{12}group = 10.

ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{55}{}^{1}H_{87}{}^{16}O_{10}{}^{14}N_2{}^{19}F_{13}$, 1205.6045; found, 1205.6026, Δ = 1.9 mmu.



Supplementary Figure 42: ^{1}H-NMR of compound **DD1/2** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 1.05 g **54** (886 µmol, 1.00 eq.) was dissolved in 2.00 mL DCM and 143 µL 3-methylbutyraldehyde **A4** (114 mg, 1.33 mmol, 1.50 eq.) and 401 mg of monomer **IM2** (1.33 mmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 3 days. Subsequently, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane / ethyl acetate 5:1 → 2:1) to afford product **Tr1/2** as a pale highly viscous oil in a yield of 86.2% (1.20 g, 764 µmol).

R_f = 0.34 in cyclohexane / ethyl acetate (3:1).

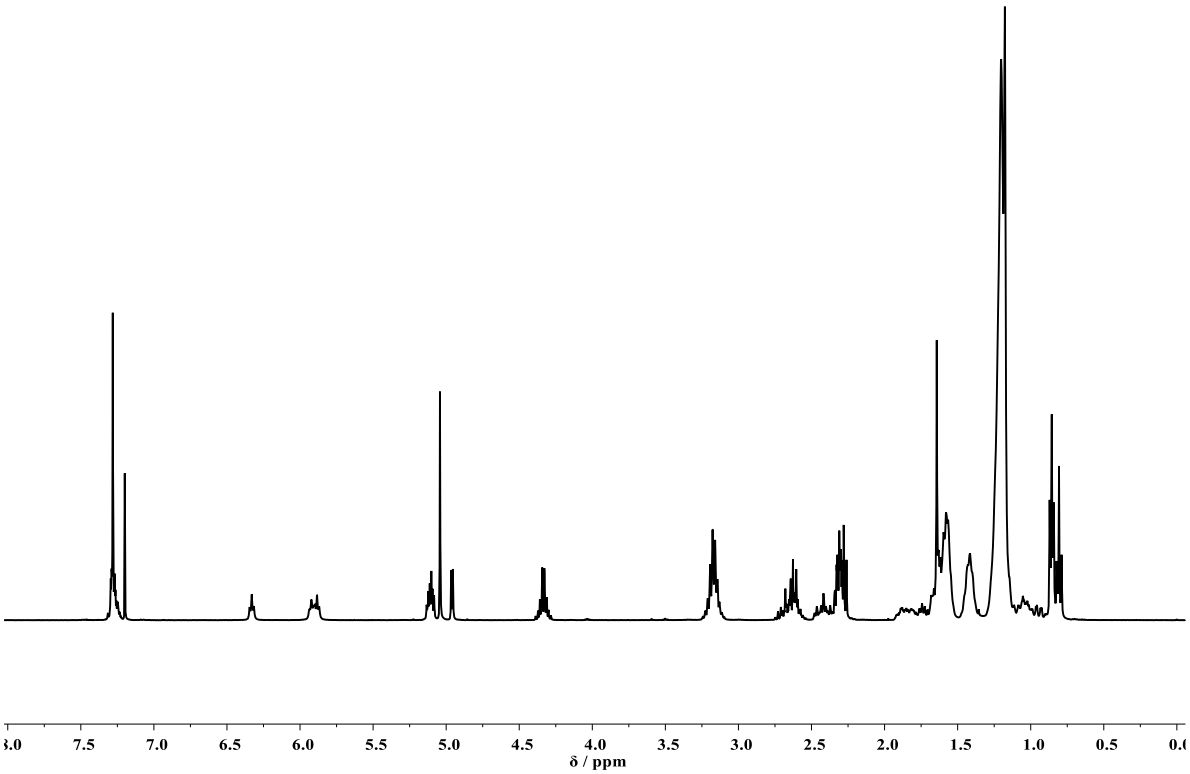
IR (ATR): ν / cm^{-1} = 3299.8 (vw), 2920.7 (s), 2851.3 (m), 1736.8 (s), 1655.3 (vs), 1553.2 (w), 1465.8 (w), 1365.0 (w), 1236.2 (s), 1206.9 (s), 1143.7 (vs), 1005.2 (w), 697.1 (m), 652.1 (w), 567.5 (vw), 450.7 (vw), 394.2 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.35 – 7.22 (m, 5 H, CH_{Ar}^{1}), 6.33 (t, J = 5.8 Hz, 1 H, NH^{2}), 5.97 – 5.82 (m, 2 H, NH^{2}), 5.15 – 5.06 (m, 2 H, CH^{3}), 5.04 (s, 2 H, CH_2^{4}), 4.96 (d, J = 4.6 Hz, 1 H, CH^{5}), 4.42 – 4.25 (m, 2 H, CH_2^{6}), 3.25 – 3.07 (m, 6 H, CH^{7}), 2.76 – 2.53 (m, 4 H, CH_2^{8}), 2.50 – 2.23 (m, 8 H, CH_2^{9}), 1.95 – 1.51 (m, 16 H, CH^{10}, CH_2^{11}), 1.48 – 1.35 (m, 6 H, CH_2^{12}), 1.32 – 0.96 (m, 60 H, CH_2^{11}), 0.86 (t, J = 5.7 Hz, 6 H, CH_3^{13}), 0.81 (t, J = 6.8 Hz, 3 H, CH_3^{14}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.82, 172.77, 172.67, 172.65, 171.25, 170.34, 169.74, 169.36, 136.25, 128.66, 128.28, 77.74, 74.73, 72.76, 66.19, 56.94, 40.98, 40.10, 39.44, 39.35, 39.26, 34.44, 34.41, 32.03, 31.93, 30.57 (t, J = 21.7 Hz), 29.74, 29.70, 29.67, 29.64, 29.60, 29.56, 29.53, 29.51, 29.47, 29.35, 29.33, 29.29, 29.26, 29.22, 29.12, 27.40, 26.96, 26.92, 26.19, 26.11, 26.00, 25.11, 25.06, 25.05, 25.03, 24.67, 23.25, 22.80, 21.91, 14.23.

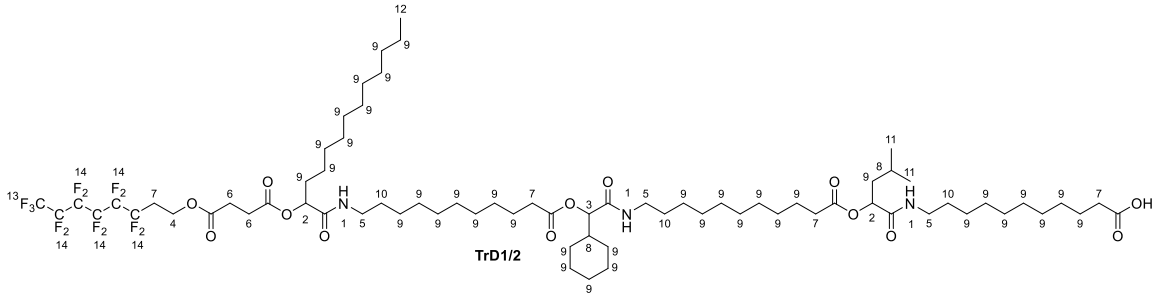
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.09 (t, J = 9.9 Hz, 3 F, CF_3^{15}), -117.70 – -118.56 (m, 2 F, CF_2^{16}), -126.00 – -126.49 (m, 2 F, CF_2^{16}), -126.86 – -127.42 (m, 2 F, CF_2^{16}), -127.72 – -127.97 (m, 2 F, CF_2^{16}), -130.25 – -130.99 (m, 2 F, CF_2^{16}). Total integral of CF_2 region normalized with respect to the CF_3^{16} group = 10.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{79}{}^{1}H_{124}{}^{16}O_{13}{}^{14}N_3{}^{19}F_{13}$, 1570.8999; found, 1570.8988, Δ = 1.1 mmu.



Supplementary Figure 43: ^{1}H-NMR of compound **Tr1/2** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 1.51 g of **Tr1/2** (964 µmol, 1.00 eq.) were dissolved in 7.00 mL ethyl acetate and 7.00 mL THF. Afterwards, 303 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **TrD1/2** was obtained as a highly viscous oil in a yield of 99.5% (1.42 g, 959 µmol).

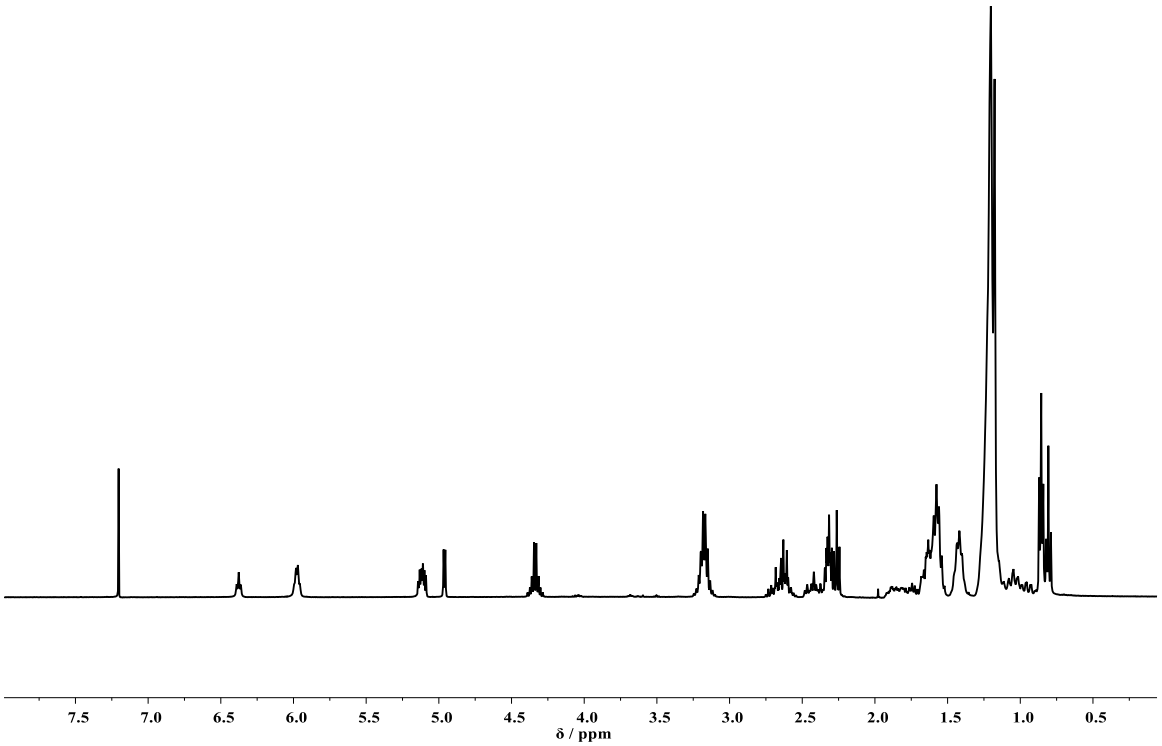
IR (ATR): ν / cm^{-1} = 3307.3 (vw), 2924.4 (s), 2853.4 (m), 1739.3 (s), 1655.0 (s), 1540.6 (m), 1465.2 (w), 1366.2 (w), 1235.8 (vs), 1144.5 (vs), 842.9 (vw), 808.8 (vw), 697.6 (w), 651.3 (w), 396.6 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.43 (t, J = 5.8 Hz, 1 H, NH^{1}), 6.09 – 5.99 (m, 2 H, NH^{1}), 5.23 – 5.13 (m, 2 H, CH^{2}), 5.02 (d, J = 4.7 Hz, 1 H, CH^{3}), 4.46 – 4.33 (m, 2 H, CH_2^{4}), 3.35 – 3.16 (m, 6 H, CH_2^{5}), 2.82 – 2.59 (m, 4 H, CH_2^{6}), 2.55 – 2.28 (m, 8 H, CH_2^{7}), 2.00 – 1.56 (m, 16 H, CH^{8}, CH_2^{9}), 1.54 – 1.41 (m, 6 H, CH_2^{10}), 1.38 – 1.01 (m, 60 H, CH_2^{9}), 0.91 (t, J = 5.8 Hz, 6 H, CH_3^{11}), 0.89 – 0.83 (m, 3 H, CH_3^{12}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 177.49, 172.81, 172.78, 172.68, 171.27, 170.45, 169.88, 169.52, 77.75, 74.71, 72.78, 56.95, 40.93, 40.06, 39.47, 39.34, 39.32, 34.42, 34.40, 34.01, 32.03, 31.92, 30.57 (t, J = 21.7 Hz), 29.74, 29.67, 29.59, 29.56, 29.53, 29.50, 29.46, 29.43, 29.34, 29.29, 29.24, 29.23, 29.12, 29.10, 27.42, 26.95, 26.86, 26.18, 26.09, 25.98, 25.10, 25.07, 25.02, 24.88, 24.67, 23.24, 22.80, 21.90, 14.22.

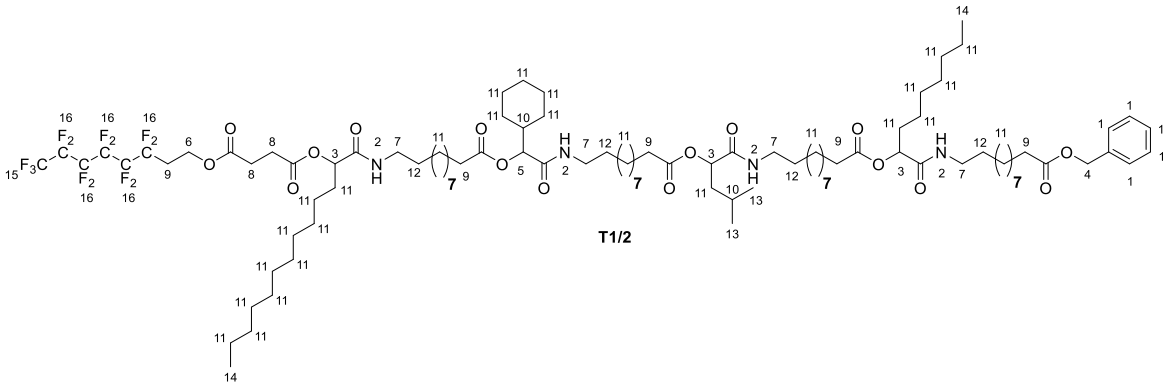
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.10 (t, J = 9.8 Hz, 3 F, CF_3^{13}), -117.52 – -118.47 (m, 2 F, CF_2^{14}), -125.86 – -126.49 (m, 2 F, CF_2^{14}), -126.92 – -127.34 (m, 2 F, CF_2^{14}), -127.44 – -128.06 (m, 2 F, CF_2^{14}), -130.27 – -130.61 (m, 2 F, CF_2^{14}). Total integral of CF_2 region normalized with respect to the CF_3^{13} group = 10.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{72}{}^{1}H_{118}{}^{16}O_{13}{}^{14}N_2{}^{19}F_{13}$, 1480.8530; found, 1480.8527, Δ = 0.3 mmu.



Supplementary Figure 44: ^{1}H-NMR of compound **TrD1/2** measured in $CDCl_3$.

Passerini reaction



In a 25 mL round bottom flask, 1.34 g of **Tr1/2** (853 µmol, 1.00 eq.) was dissolved in 5.00 mL DCM. Afterwards, 200 µL octanal **A8** (164 mg, 1.28 mol, 1.50 eq.) and 386 mg of monomer **IM2** (1.28 mmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 3 days and subsequently the solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane / ethyl acetate 4:1 → 1:1) to afford product **T1/2** as a white solid in a yield of 89.4% (1.46 g, 763 µmol).

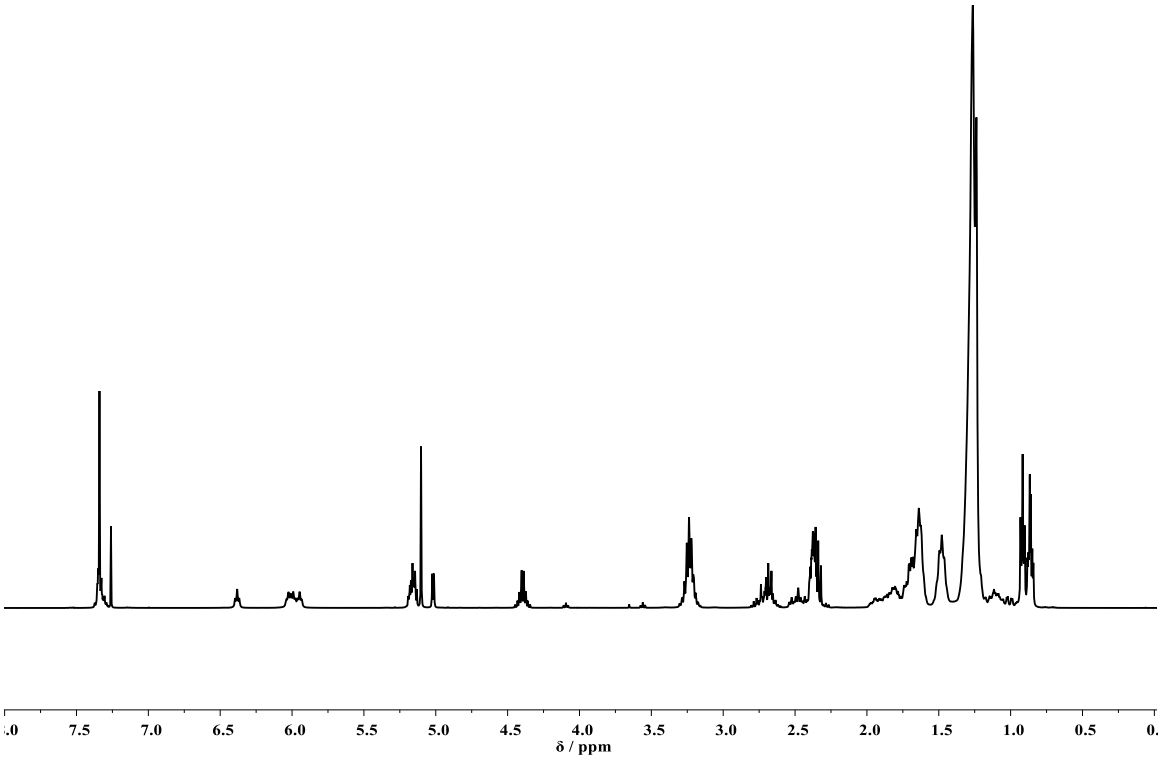
IR (ATR): ν / cm^{-1} = 3302.5 v(w), 2922.1 (s), 2851.9 (m), 2364.7 (vw), 2354.7 (vw), 2324.5 (vw), 1738.1 (s), 1655.8 (vs), 1555.9 (w), 1465.9 (w), 1365.9 (w), 1237.3 (s), 1207.8 (s), 1164.7 (vs), 1144.9 (vs), 1006.8 (vw), 697.6 (w), 653.2 (w), 568.5 (vw), 457.0 (vw), 389.9 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.41 – 7.28 (m, 5 H, CH_{Ar}^{1}), 6.38 (t, J = 5.7 Hz, 1 H, NH^{2}), 6.09 – 5.88 (m, 3 H, NH^{2}), 5.22 – 5.12 (m, 3 H, CH^{3}), 5.10 (s, 2 H, CH_2^{4}), 5.02 (d, J = 4.6 Hz, 1 H, CH^{5}), 4.47 – 4.32 (m, 2 H, CH_2^{6}), 3.32 – 3.14 (m, 8 H, CH^{7}), 2.85 – 2.59 (m, 4 H, CH_2^{8}), 2.58 – 2.27 (m, 10 H, CH_2^{9}), 1.99 – 1.57 (m, 22 H, CH^{10}, CH_2^{11}), 1.55 – 1.40 (m, 8 H, CH_2^{12}), 1.36 – 1.03 (m, 80 H, CH_2^{11}), 0.92 (t, J = 5.8 Hz, 6 H, CH_3^{13}), 0.89 – 0.80 (m, 6 H, CH_3^{14}).

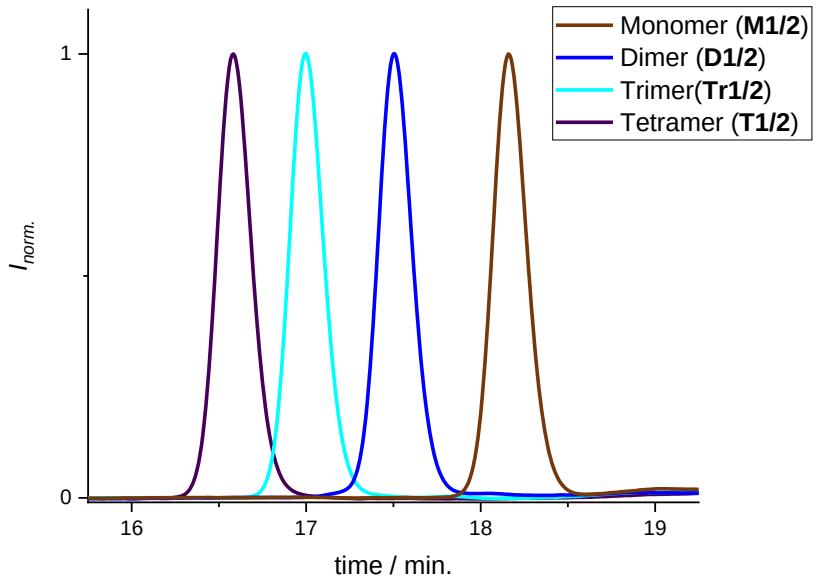
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.81, 172.77, 172.65, 172.64, 172.59, 171.24, 170.35, 169.98, 169.74, 169.35, 136.24, 128.66, 128.28, 77.73, 74.73, 74.06, 72.76, 66.19, 56.94, 40.98, 40.10, 39.44, 39.34, 39.32, 39.26, 34.44, 34.41, 32.03, 31.94, 31.86, 29.74, 29.71, 29.68, 29.61, 29.59, 29.52, 29.49, 29.48, 29.35, 29.23, 29.13, 27.41, 26.96, 26.19, 26.12, 26.00, 25.11, 25.09, 25.08, 25.06, 24.89, 24.67, 23.26, 22.81, 22.74, 21.91, 14.23, 14.21.

^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -84.86 – -85.51 (m, 3 F, CF_3^{15}), -117.86 – -118.51 (m, 2 F, CF_2^{16}), -125.81 – -126.56 (m, 2 F, CF_2^{16}), -126.89 – -127.43 (m, 2 F, CF_2^{16}), -127.81 – -128.18 (m, 2 F, CF_2^{16}), -130.22 – -130.82 (m, 2 F, CF_2^{16}). Total integral of CF_2 region normalized with respect to the CF_3^{16} group = 10.

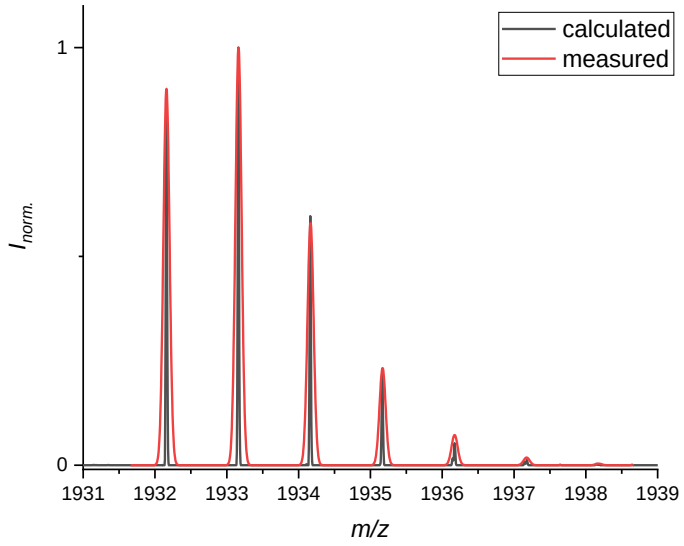
ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{99}{}^{1}H_{161}{}^{16}O_{16}{}^{14}N_4{}^{19}F_{13}$, 1932.1592; found, 1932.1591 Δ = 0.1 mmu.



Supplementary Figure 45: ^{1}H-NMR of compound **T1/2** measured in $CDCl_3$.



Supplementary Figure 46: SEC traces of the intermediated in the synthesis after each P-3CR of product **T1/2**.



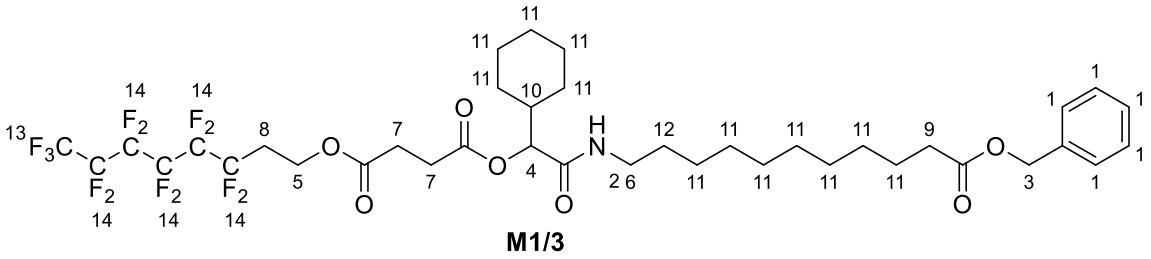
Supplementary Figure 47: High resolution ESI-MS measurement of **T1/2**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (black).

```
matching mass 1933.15486
cutoff 0.50000: 0 solutions (14 peaks)
cutoff 0.25000: 0 solutions (53 peaks)
cutoff 0.12500: 0 solutions (114 peaks)
cutoff 0.06250: 0 solutions (191 peaks)
cutoff 0.03125: 1 solutions (298 peaks)
1933.15486 ≈ 463.021500 + 395.339950 + 323.246050 + 297.230400 + 339.277350 + 91.054780 (sides Dodecanal,
Cyclohexancarboxaldehyde, 3-Methylbutanal, Octanal; error -23.98483)
Press ENTER to quit ...
```

Supplementary Figure 48: Screenshot of the automated read-out of **T1/2**, sodium trifluoroacetate **2** was used as additive during the measurement.

6.3.3.3.4 Synthesis of tetramer T1/3

Passerini reaction



M1/3

In a 50 mL round bottom flask, 300 mg **TAG1** (646 µmol, 1.00 eq.) were stirred in 2.00 mL DCM. Subsequently, 117 µL cyclohexanecarboxaldehyde **A6** (109 mg, 969 µmol, 1.50 eq.) and 292 mg of monomer **IM2** (969 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 6 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (6:1 → 5:1) to yield the Passerini product **M1/3** as a pale highly viscous oil. (489 mg, 557 µmol, 86.2%).

R_f = 0.77 in cyclohexane / ethyl acetate (2:1).

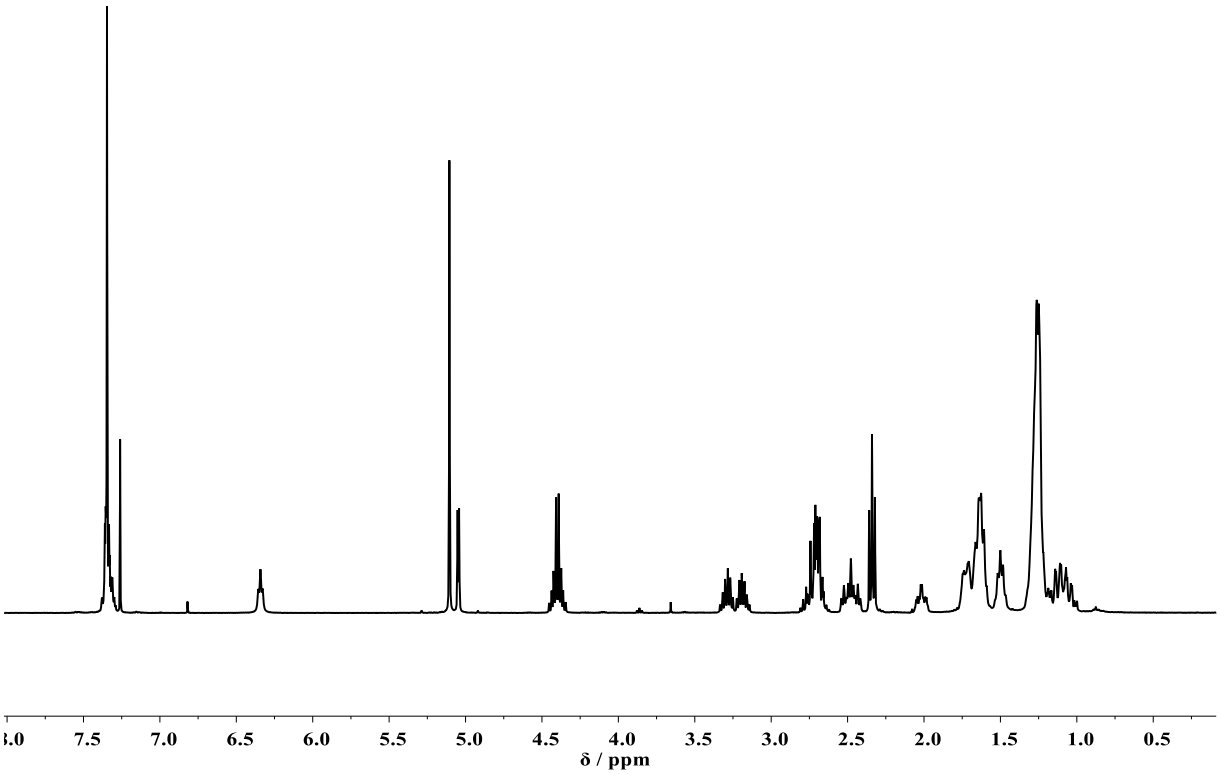
IR (ATR): ν / cm^{-1} = 2927.3 (m), 2854.9 (w), 1737.0 (vs), 1656.6 (m), 1534.7 (w), 1453.1 (w), 1359.5 (w), 1234.2 (vs), 1144.0 (vs), 1082.6 (s), 1002.9 (m), 842.2 (w), 808.9 (w), 733.0 (m), 697.3 (s), 651.4 (m), 566.0 (m).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.43 – 7.30 (m, 5 H, $CH_{Ar}{}^1$), 6.34 (t, J = 5.7 Hz, 1 H, NH^2), 5.11 (s, 2 H, $CH_2{}^3$), 5.05 (d, J = 3.9 Hz, 1 H, CH^4), 4.47 – 4.33 (m, 2 H, $CH_2{}^5$), 3.35 – 3.13 (m, 2 H, $CH_2{}^6$), 2.83 – 2.62 (m, 4 H, $CH_2{}^7$), 2.56 – 2.40 (m, 2 H, $CH_2{}^8$), 2.34 (t, J = 7.6 Hz, 2 H, $CH_2{}^9$), 2.08 – 1.96 (m, 1 H, CH^{10}), 1.79 – 1.58 (m, 8 H, $CH_2{}^{11}$), 1.55 – 1.47 (m, 2 H, $CH_2{}^{12}$), 1.35 – 0.96 (m, 16 H, $CH_2{}^{11}$).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.82, 172.69, 171.24, 169.03, 136.26, 128.66, 128.28, 78.44, 66.19, 56.96, 39.88, 39.41, 34.45, 30.57, 29.59, 29.55, 29.48, 29.35, 29.24, 29.17, 29.14, 27.04, 26.98, 26.18, 26.16, 26.04, 25.07.

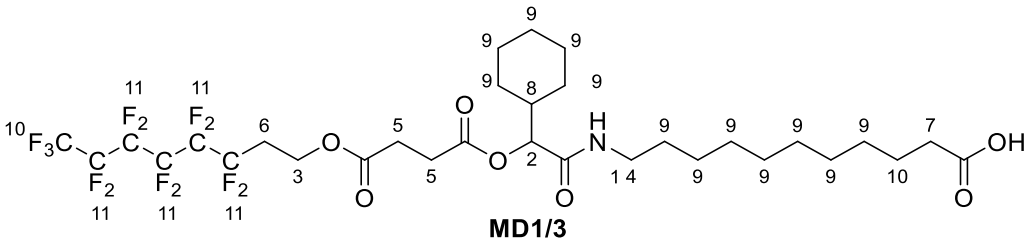
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -84.01 – -86.07 (m, 3 F, $CF_3{}^{13}$), -117.1 – -118.64 (m, 2 F, $CF_2{}^{14}$), -126.05 – -126.37 (m, 2 F, $CF_2{}^{14}$), -127.08 – -127.35 (m, 2 F, $CF_2{}^{14}$), -127.75 – -128.08 (m, 2 F, $CF_2{}^{14}$), -130.35 – -130.63 (m, 2 F, $CF_2{}^{14}$). Total integral of CF_2 region normalized with respect to the $CF_3{}^{13}$ group = 10.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{38}{}^{1}H_{48}{}^{16}O_7{}^{14}N{}^{19}F_{13}$, 878.3296; found, 878.3271, Δ = 2.5 mmu.



Supplementary Figure 49: ^{1}H-NMR of compound **M1/3** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 419 mg of **M1/3** (478 µmol, 1.00 eq.) were dissolved in 3.00 mL ethyl acetate and 3.00 mL THF. Afterwards, 83.8 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **MD1/3** was obtained as a pale highly viscous oil in a yield of 98.7% (372 mg, 472 µmol).

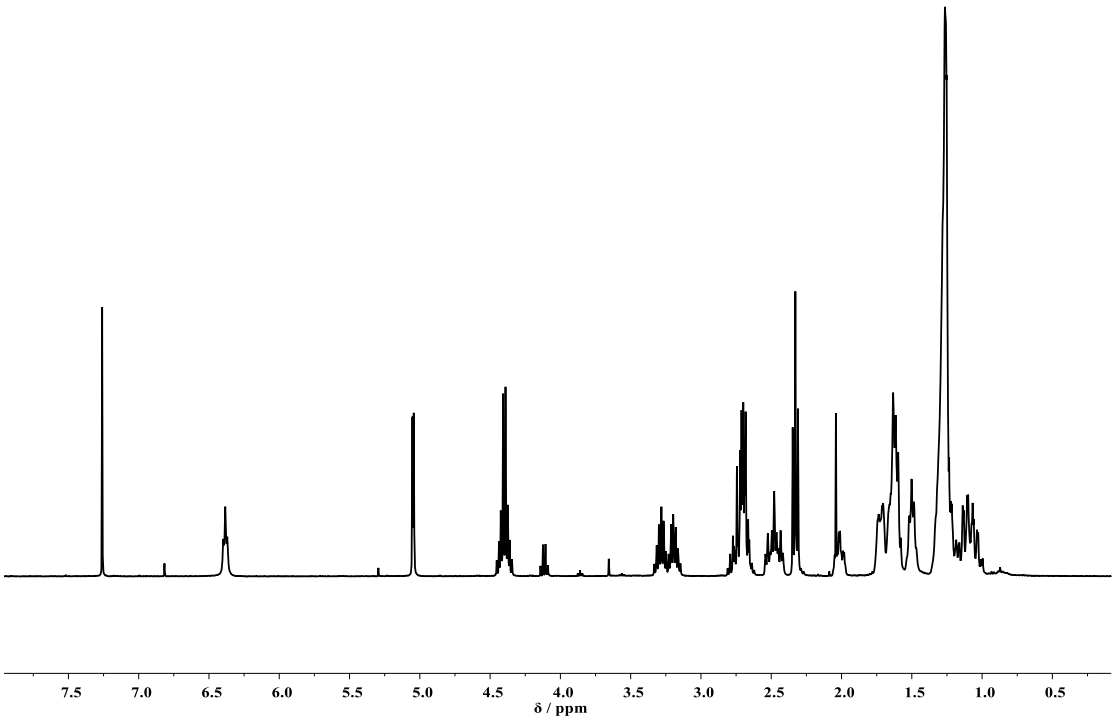
IR (ATR): ν / cm^{-1} = 2927.4 (m), 2855.3 (w), 1737.9 (s), 1650.1 (m), 1541.7 (w), 1451.4 (w), 1362.1 (w), 1233.6 (vs), 1192.2 (vs), 1143.8 (vs), 1082.9 (m), 1003.7 (w), 842.5 (w), 808.9 (w), 732.4 (w), 697.6 (m), 651.3 (w), 566.3 (w), 531.4 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.38 (t, J = 5.8 Hz, 1 H, NH^{1}), 5.05 (d, J = 3.9 Hz, 1 H, CH^{2}), 4.49 – 4.32 (m, 2 H, CH_2^{3}), 3.38 – 3.12 (m, 2 H, CH_2^{4}), 2.84 – 2.62 (m, 4 H, CH_2^{5}), 2.57 – 2.40 (m, 2 H, CH_2^{6}), 2.33 (t, J = 7.5 Hz, 2 H, CH_2^{7}), 2.11 – 1.94 (m, 1 H, CH^{8}), 1.78 – 1.68 (m, 2 H, CH_2^{9}), 1.68 – 1.54 (m, 4 H, $CH_2^{9,10}$), 1.56 – 1.43 (m, 2 H, CH_2^{9}), 1.37 – 0.96 (m, 18 H, CH_2^{9}).

^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 179.59, 173.49, 172.03, 169.93, 79.19, 57.73, 40.61, 40.18, 34.78, 31.34, 30.22, 30.09, 29.98, 29.96, 29.94, 29.90, 29.83, 27.80, 27.67, 26.93, 26.91, 26.78, 25.54.

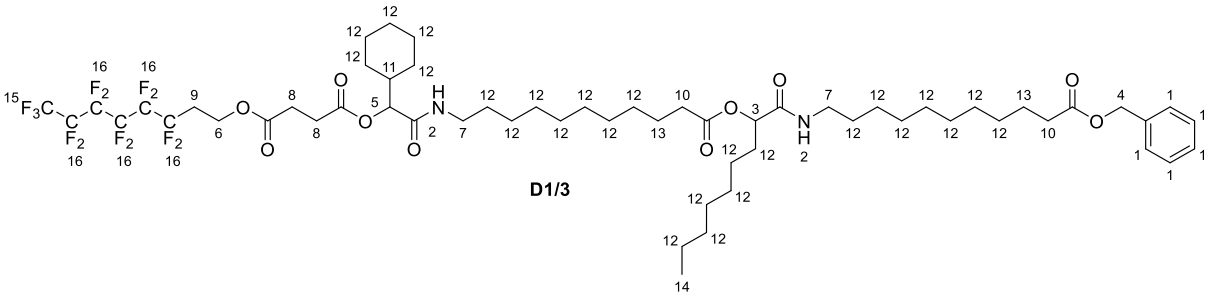
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.12 (t, J = 9.9 Hz, 3 F, CF_3^{10}), -117.58 – -118.68 (m, 2 F, CF_2^{11}), -125.95 – -126.43 (m, 2 F, CF_2^{11}), -127.08 – -127.31 (m, 2 F, CF_2^{11}), -127.70 – -128.07 (m, 2 F, CF_2^{11}), -130.32 – -131.42 (m, 2 F, CF_2^{11}). Total integral of CF_2 region normalized with respect to the CF_3^{10} group = 10.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{31}{}^{1}H_{42}{}^{16}O_7{}^{14}N{}^{19}F_{13}$, 788.2826; found, 788.2803, Δ = 2.3 mmu.



Supplementary Figure 50: ^{1}H-NMR of compound **MD1/3** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 338 mg of **MD1/3** (429 µmol, 1.00 eq.) were stirred in 2.00 mL DCM. Subsequently, 101 µL octanal **A8** (109 mg, 644 µmol, 1.50 eq.) and 194 mg of monomer **IM2** (644 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 6 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (6:1 → 5:1) to yield the Passerini product **D1/3** as a pale highly viscous oil. (420 mg, 344 µmol, 80.2%).

R_f = 0.50 in cyclohexane / ethyl acetate (2:1).

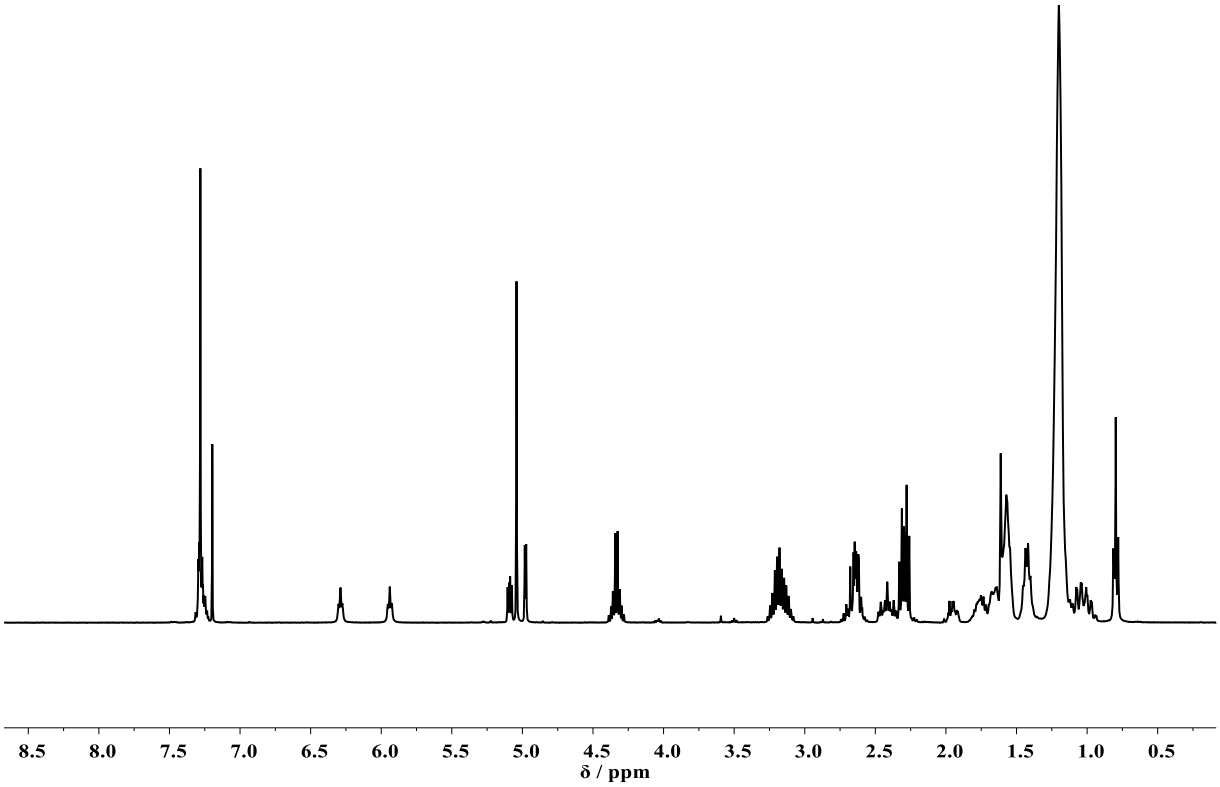
IR (ATR): ν / cm^{-1} = 3293.3 (vw), 2918.1 (s), 2851.0 (m), 1736.5 (vs), 1678.6 (m), 1651.2 (s), 1532.6 (m), 1466.9 (w), 1362.3 (w), 1235.6 (vs), 1143.9 (vs), 1082.2 (s), 1005.7 (m), 843.0 (w), 808.4 (w), 733.5 (m), 697.4 (s), 651.3 (w), 567.1 (vw), 531.6 (vw), 445.8 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.41 – 7.28 (m, 5 H, $CH_{Ar}{}^1$), 6.35 (t, J = 5.8 Hz, 1 H, NH^2), 6.00 (t, J = 5.9 Hz, 1 H, NH^2), 5.18 – 5.13 (m, 1 H, CH^3), 5.10 (s, 2 H, $CH_2{}^4$), 5.04 (d, J = 3.9 Hz, 1 H, CH^5), 4.48 – 4.33 (m, 2 H, $CH_2{}^6$), 3.33 – 3.15 (m, 4 H, $CH_2{}^7$), 2.79 – 2.64 (m, 4 H, $CH_2{}^8$), 2.54 – 2.41 (m, 2 H, $CH_2{}^9$), 2.41 – 2.31 (m, 4 H, $CH_2{}^{10}$), 2.08 – 1.96 (m, 1 H, CH^{11}), 1.90 – 1.57 (m, 12 H, $CH_2{}^{12}$), 1.55 – 1.43 (m, 4 H, $CH_2{}^{13}$), 1.36 – 0.99 (m, 38 H, $CH_2{}^{12}$), 0.91 – 0.82 (m, 3 H, $CH_3{}^{14}$).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.81, 172.69, 172.57, 171.23, 169.97, 169.04, 136.25, 128.66, 128.28, 78.43, 74.05, 66.20, 56.95, 39.87, 39.38, 39.32, 34.45, 32.04, 31.86, 30.57, 29.69, 29.61, 29.58, 29.55, 29.49, 29.48, 29.35, 29.34, 29.24, 29.23, 29.17, 29.13, 27.04, 26.97, 26.96, 26.17, 26.15, 26.03, 25.09, 25.07, 24.88, 22.74, 14.19.

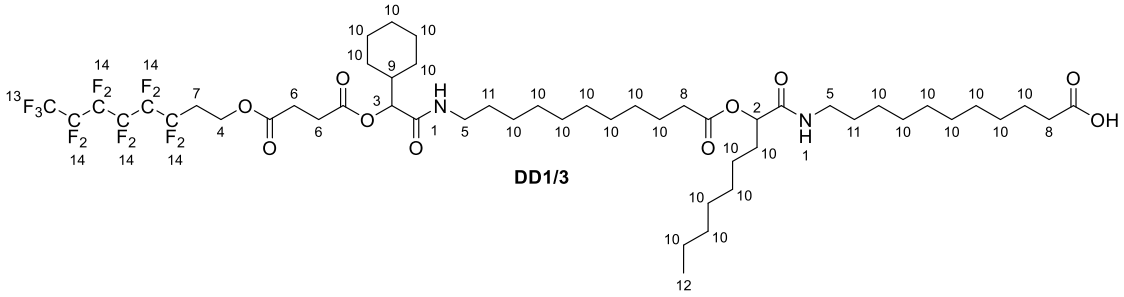
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.01 – -85.18 (m, 3 F, $CF_3{}^{15}$), -117.85 – -118.19 (m, 2 F, $CF_2{}^{16}$), -126.01 – -126.38 (m, 2 F, $CF_2{}^{16}$), -127.05 – -127.36 (m, 2 F, $CF_2{}^{16}$), -127.74 – -128.06 (m, 2 F, $CF_2{}^{16}$), -130.32 – -130.63 (m, 2 F, $CF_2{}^{16}$). Total integral of CF_2 region normalized with respect to the $CF_3{}^{15}$ group = 10.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{58}{}^{1}H_{85}{}^{16}O_{10}{}^{14}N_2{}^{19}F_{13}$, 1217.6069; found, 1217.6050, Δ = 1.9 mmu.



Supplementary Figure 51: ^{1}H-NMR of compound **D1/3** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 333 mg of **D1/3** (273 µmol, 1.00 eq.) were dissolved in 3.00 mL ethyl acetate and 3.00 mL THF. Afterwards, 66.6 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **DD1/3** was obtained as a pale highly viscous oil in a yield of 74.4% (230 mg, 203 µmol).

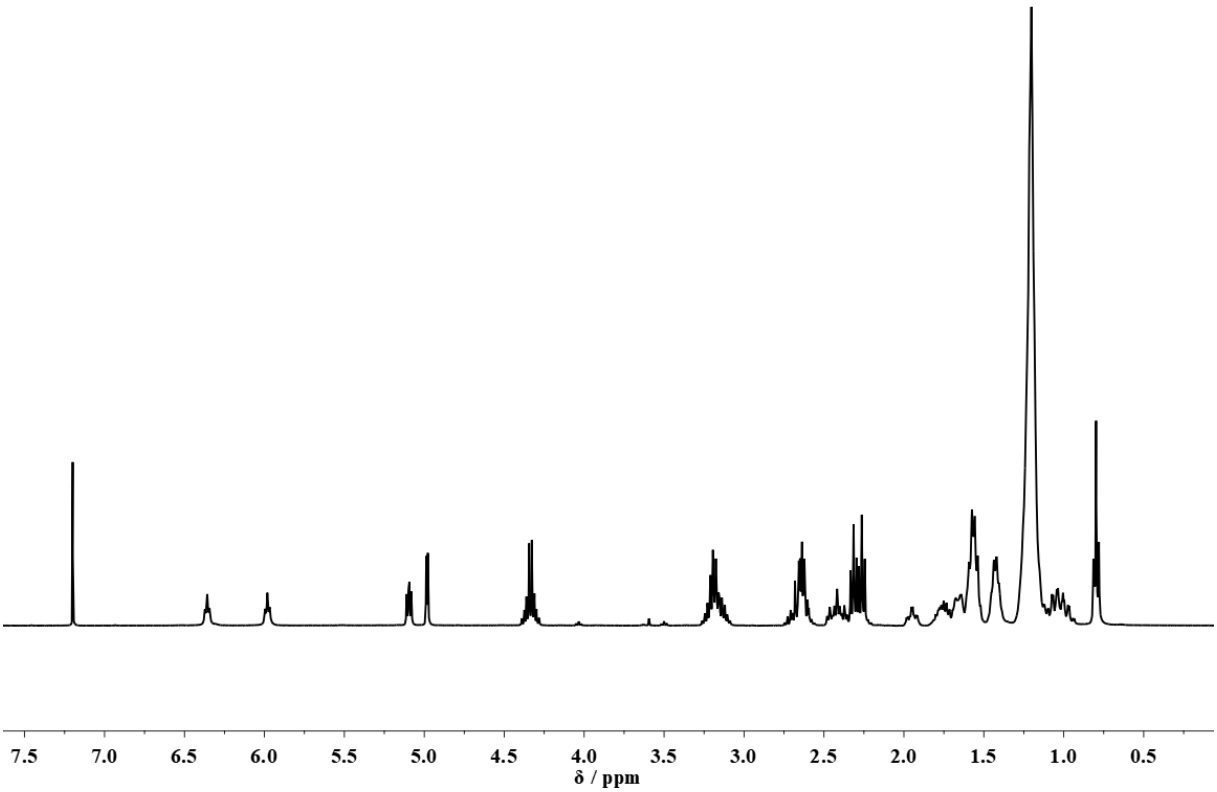
IR (ATR): ν / cm^{-1} = 2917.1 (s), 2849.7 (m), 1738.4 (vs), 1655.0 (m), 1552.6 (m), 1467.3 (w), 1364.4 (w), 1234.4 (vs), 1190.7 (vs), 1143.9 (vs), 1091.8 (s), 1007.4 (w), 843.1 (vw), 809.9 (vw), 732.7 (w), 697.9 (w), 651.6 (w), 619.5 (w), 531.3 (vw), 445.6 (vw), 388.9 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.42 (t, J = 5.8 Hz, 1 H, NH^{1}), 6.04 (t, J = 5.8 Hz, 1 H, NH^{1}), 5.19 – 5.12 (m, 1 H, CH^{2}), 5.04 (d, J = 3.9 Hz, 1 H, CH^{3}), 4.49 – 4.33 (m, 2 H, CH_2^{4}), 3.34 – 3.13 (m, 4 H, CH_2^{5}), 2.83 – 2.62 (m, 4 H, CH_2^{6}), 2.55 – 2.27 (m, 6 H, $CH_2^{8,7}$), 2.06 – 1.96 (m, 1 H, CH^{9}), 1.91 – 1.55 (m, 12 H, CH_2^{10}), 1.54 – 1.42 (m, 4 H, CH_2^{11}), 1.38 – 0.98 (m, 38 H, CH_2^{10}), 0.89 – 0.83 (m, 3 H, CH_3^{12}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 178.71, 173.51, 173.37, 172.06, 170.86, 170.01, 79.18, 74.84, 57.73, 40.61, 40.22, 40.07, 35.22, 34.79, 32.77, 32.62, 31.57, 31.35, 31.13, 30.38, 30.34, 30.29, 30.27, 30.23, 30.13, 30.09, 30.01, 29.98, 29.93, 29.90, 29.87, 27.82, 27.74, 27.66, 26.93, 26.91, 26.78, 25.87, 25.64, 23.49.

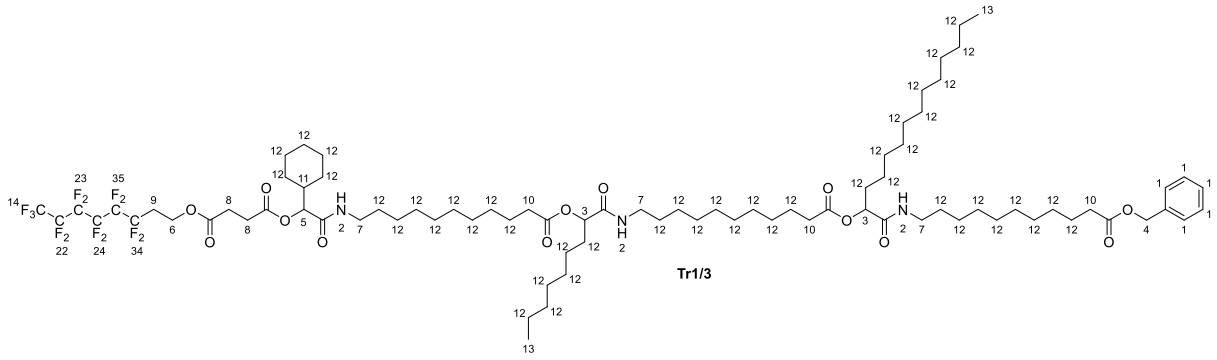
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.11 (t, J = 10.0 Hz, 3 F, CF_3^{13}), -117.41 – -118.41 (m, 2 F, CF_2^{14}), -125.94 – -126.53 (m, 2 F, CF_2^{14}), -126.87 – -127.33 (m, 2 F, CF_2^{14}), -127.67 – -128.39 (m, 2 F, CF_2^{14}), -130.26 – -130.98 (m, 2 F, CF_2^{14}). Total integral of CF_2 region normalized with respect to the CF_3^{13} group = 10.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{51}{}^{1}H_{79}{}^{16}O_{10}{}^{14}N_2{}^{19}F_{13}$, 1127.5600; found, 1127.5581, Δ = 1.9 mmu.



Supplementary Figure 52: ^{1}H-NMR of compound **DD1/3** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 252 mg of **DD1/3** (224 µmol, 1.00 eq.) were stirred in 2.00 mL DCM. Subsequently, 79.7 µL tridecanal **A11** (66.5 mg, 335 µmol, 1.50 eq.) and 101 mg of monomer **IM2** (335 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 3 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (4:1 → 2:1) to yield the Passerini product **Tr1/3** as a pale highly viscous oil. (255 mg, 157 µmol, 70.1%).

R_f = 0.15 in cyclohexane / ethyl acetate (2:1).

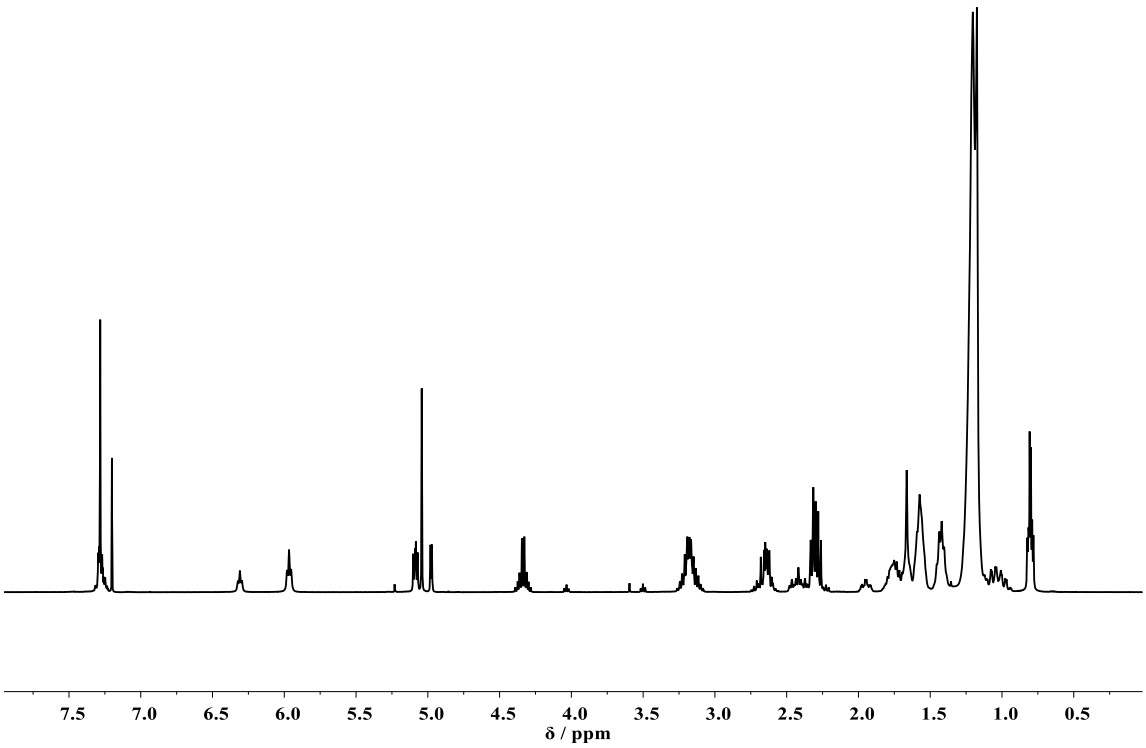
IR (ATR): ν / cm^{-1} = 3292.0 (w), 2917.4 (vs), 2850.3 (s), 1737.0 (vs), 1654.5 (vs), 1556.9 (m), 1466.7 (w), 1362.8 (w), 1236.8 (vs), 1205.0 (vs), 1144.7 (vs), 1082.6 (m), 1007.7 (w), 809.0 (vw), 696.6 (m), 652.3 (w), 566.2 (vw), 529.3 (vw), 440.8 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.36 – 7.23 (m, 5 H, CH_{Ar}[1]), 6.31 (t, J = 5.8 Hz, 1 H, NH[2]), 5.97 (t, J = 5.9 Hz, 2 H, NH[2]), 5.17 – 5.12 (m, 2 H, CH[3]), 5.04 (s, 2 H, CH_2[4]), 4.98 (d, J = 3.9 Hz, 1 H, CH[5]), 4.42 – 4.25 (m, 2 H, CH_2[6]), 3.28 – 3.07 (m, 6 H, CH_2[7]), 2.77 – 2.55 (m, 4 H, CH_2[8]), 2.49 – 2.21 (m, 8 H, CH_2[9,10]), 2.08 – 1.91 (m, 1 H, CH[11]), 1.90 – 0.94 (m, 92 H, CH_2[12]), 0.84 – 0.74 (m, 6 H, CH_3[13]).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 174.59, 173.47, 173.37, 172.02, 170.78, 170.76, 169.83, 137.02, 129.43, 129.05, 79.20, 74.84, 74.82, 66.96, 57.72, 40.64, 40.15, 40.09, 35.21, 32.81, 32.62, 31.55, 31.34, 31.12, 30.56, 30.54, 30.52, 30.47, 30.45, 30.37, 30.34, 30.32, 30.28, 30.25, 30.16, 30.11, 30.01, 29.99, 29.93, 29.90, 27.82, 27.74, 27.73, 26.94, 26.91, 26.80, 25.85, 25.85, 25.66, 23.58, 23.50, 15.01, 14.95.

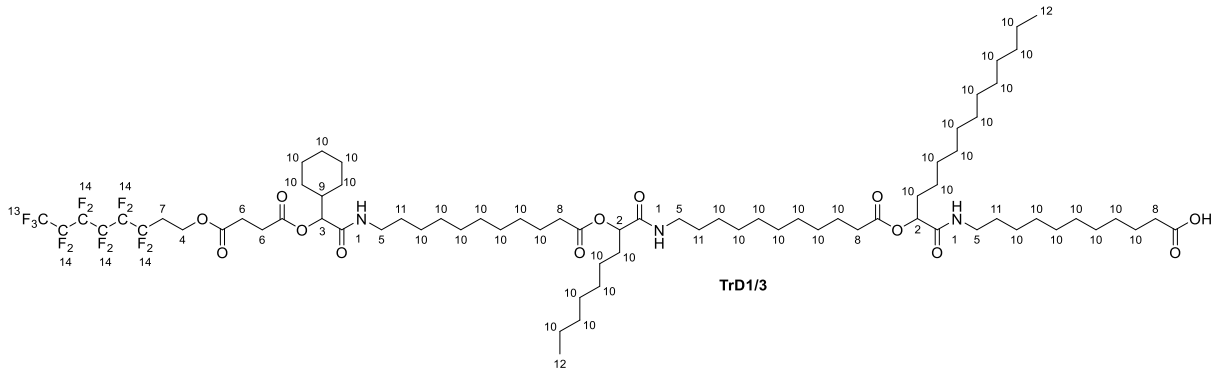
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.10 (t, J = 10.0 Hz, 3 F, CF_3[14]), -117.98 – -118.64 (m, 2 F, CF_2[15]), -126.01 – -126.41 (m, 2 F, CF_2[15]), -126.96 – -127.26 (m, 2 F, CF_2[15]), -127.76 – -128.52 (m, 2 F, CF_2[15]), -130.27 – -130.87 (m, 2 F, CF_2[15]) Total integral of CF_2 region normalized with respect to the CF_3[14] group = 10.

ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{83}{}^{1}H_{132}{}^{16}O_{13}{}^{14}N_3{}^{9}F_{13}$, 1648.9445; found, 1648.9487, Δ = 4.2 mmu.



Supplementary Figure 53: ^{1}H-NMR of compound **Tr1/3** measured in $CDCl_3$.

Deprotection



In a 25 mL round bottom flask equipped with a magnetic stir bar, 175 mg of **Tr1/3** (108 µmol, 1.00 eq.) was dissolved in 2.00 mL ethyl acetate and 2.00 mL THF. Subsequently, 35.2 mg (20 wt%) palladium on activated carbon **1** was added to the solution. The resulting mixture was purged with hydrogen gas and stirred for 1 day at room temperature under hydrogen atmosphere (3 balloons). The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **TrD1/3** was obtained as a yellow highly viscous oil in a yield of 97.2% (160 mg, 105 µmol).

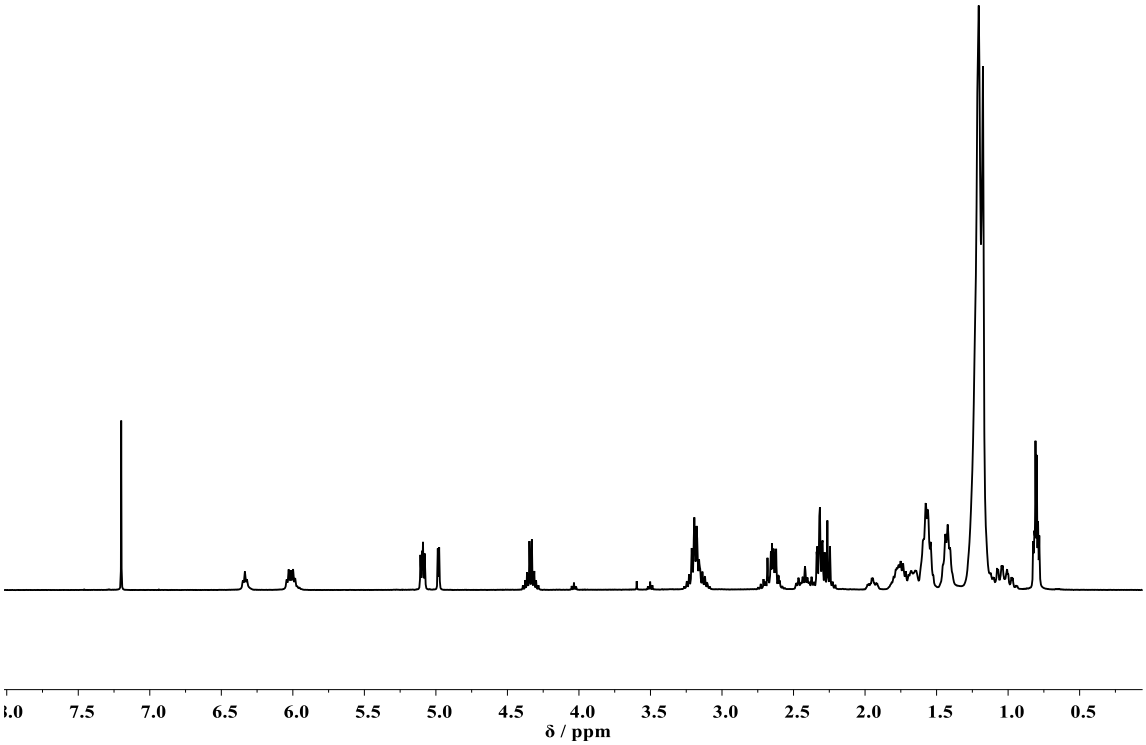
IR (ATR): ν / cm^{-1} = 3270.4 (vw), 2917.7 (m), 2850.0 (w), 2355.9 (vw), 2329.9 (vw), 1741.8 (m), 1651.7 (w), 1547.9 (w), 1466.9 (vw), 1365.0 (vw), 1238.2 (m), 1204.7 (m), 1146.3 (m), 1120.5 (w), 809.9 (vw), 721.8 (vw), 653.6 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.39 (t, J = 5.8 Hz, 1 H, NH^{1}), 6.15 – 6.00 (m, 2 H, NH^{1}), 5.21 – 5.10 (m, 2 H, CH^{2}), 5.04 (d, J = 3.8 Hz, 1 H, CH^{3}), 4.49 – 4.31 (m, 2 H, CH_2^{4}), 3.33 – 3.12 (m, 6 H, CH_2^{5}), 2.82 – 2.65 (m, 4 H, CH_2^{6}), 2.57 – 2.26 (m, 8 H, $CH_2^{7,8}$), 2.06 – 1.96 (m, 1 H, CH^{9}), 1.90 – 1.56 (m, 14 H, CH_2^{10}), 1.54 – 1.43 (m, 6 H, CH_2^{11}), 1.43 – 1.03 (m, 72 H, CH_2^{10}), 0.95 – 0.80 (m, 6 H, CH_3^{12}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 177.15, 172.73, 172.70, 172.63, 171.27, 170.19, 170.10, 169.18, 78.42, 74.10, 74.06, 56.97, 39.87, 39.42, 39.39, 39.30, 34.46, 34.45, 33.95, 32.05, 32.01, 31.86, 30.80, 30.58, 30.37, 29.79, 29.78, 29.68, 29.62, 29.57, 29.54, 29.49, 29.44, 29.39, 29.34, 29.27, 29.22, 29.17, 29.14, 29.10, 27.05, 26.97, 26.89, 26.17, 26.15, 26.03, 25.12, 25.08, 24.89, 22.82, 22.74, 14.24, 14.19.

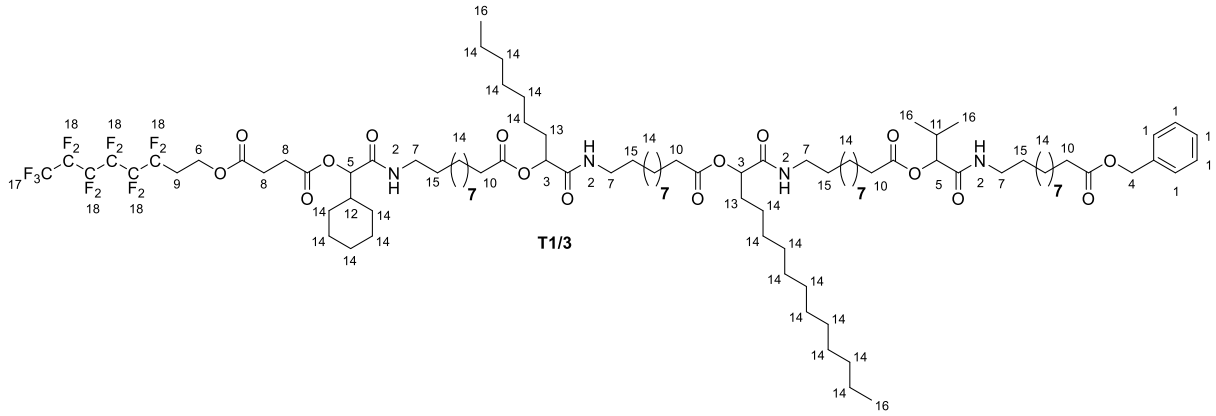
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -83.45 – -86.27 (m, 3 F, CF_3^{13}), -117.14 – -118.77 (m, 2 F, CF_2^{14}), -125.77 – -126.36 (m, 2 F, CF_2^{14}), -126.88 – -127.40 (m, 2 F, CF_2^{14}), -127.40 – -128.01 (m, 2 F, CF_2^{14}), -130.23 – -131.00 (m, 2 F, CF_2^{14}). Total integral of CF_2 region normalized with respect to the CF_3^{13}group = 10.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{76}{}^{1}H_{126}{}^{16}O_{13}{}^{14}N_3{}^{19}F_{13}$, 1536.9156; found, 1536.9133, Δ = 2.3 mmu.



Supplementary Figure 54: ^{1}H-NMR of compound **TrD1/3** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 118 mg of **TrD1/3** (77.0 µmol, 1.00 eq.) were stirred in 2.00 mL DCM. Subsequently, 10.5 µL isobutyraldehyde **A3** (8.30 mg, 115 µmol, 1.50 eq.) and 34.7 mg of monomer **IM2** (115 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 3 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (3:1 → 1:1) to yield the Passerini product **T1/3** as a pale highly viscous oil. (45.5 mg, 23.9 µmol, 31.0%).

R_f = 0.55 in cyclohexane / ethyl acetate (3:2).

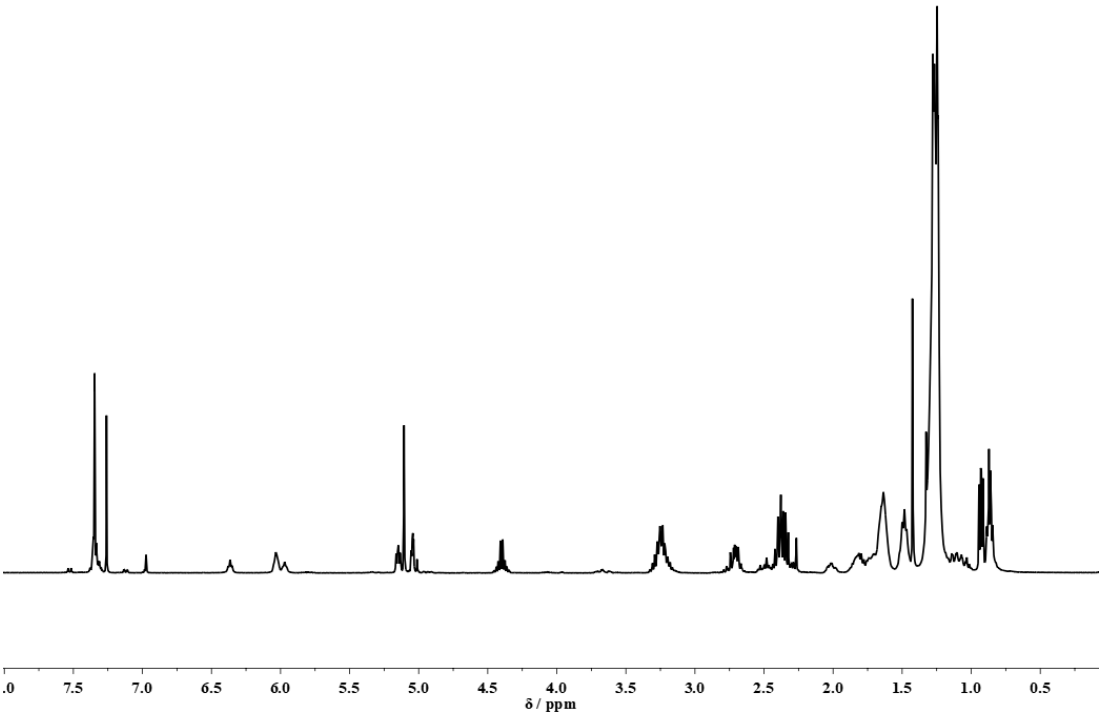
IR (ATR): ν / cm^{-1} = 3292.9 (vw), 2920.8 (m), 2851.5 (m), 1737.1 (m), 1655.5 (m), 1555.5 (w), 1465.7 (w), 1364.5 (vw), 1237.2 (m), 1206.0 (m), 1145.5 (m), 1008.4 (vw), 808.7 (vw), 697.2 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.42 – 7.29 (m, 5 H, CH_{Ar}^1), 6.44 – 6.30 (m, 1 H, NH^2), 6.11 – 5.92 (m, 3 H, NH^2), 5.19 – 5.09 (m, 2 H, CH^3), 5.11 (s, 2 H, CH_2^4), 5.08 – 5.02 (m, 2 H, CH^5), 4.49 – 4.34 (m, 2 H, CH_2^6), 3.37 – 3.15 (m, 8 H, CH_2^7), 2.82 – 2.63 (m, 4 H, CH_2^8), 2.57 – 2.44 (m, 2 H, CH_2^9), 2.44 – 2.24 (m, 9 H, CH^{10}, CH_2^{11}), 2.10 – 1.96 (m, 1 H, CH^{12}), 1.90 – 1.57 (m, 20 H, CH_2^{13}, CH_2^{14}), 1.55 – 1.44 (m, 8 H, CH_2^{15}), 1.37 – 1.02 (m, 80, CH_2^{14}), 0.97 – 0.82 (m, 12 H, CH_3^{16}).

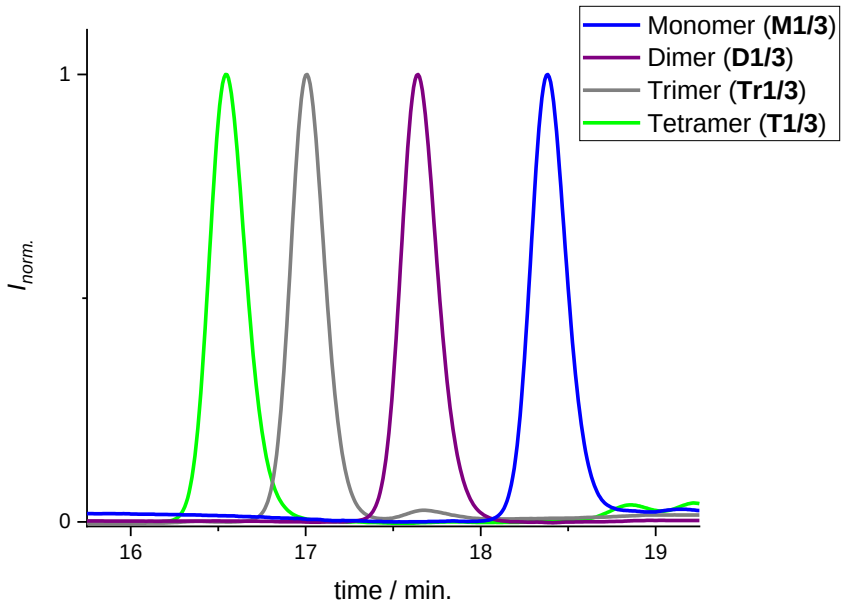
^{13}C NMR (101 MHz, $CDCl_3$): δ/ ppm = 173.83, 172.71, 172.71, 172.61, 171.25, 170.01, 169.40, 169.06, 136.24, 128.67, 128.29, 125.65, 78.42, 78.04, 74.05, 74.03, 66.20, 56.95, 39.87, 39.38, 39.31, 39.31, 34.44, 34.42, 32.05, 31.86, 31.57, 30.65, 30.44, 30.31, 29.83, 29.80, 29.78, 29.76, 29.71, 29.68, 29.61, 29.59, 29.57, 29.52, 29.50, 29.49, 29.38, 29.35, 29.33, 29.24, 29.17, 29.13, 27.03, 26.97, 26.17, 26.14, 26.03, 25.14, 25.09, 25.07, 24.91, 24.90, 22.82, 22.74, 18.92, 17.08, 14.26, 14.20.

^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -83.70 – -85.90 (m, 3 F, CF_3^{17}), -117.79 – -118.30 (m, 2 F, CF_2^{18}), -125.87 – -126.56 (m, 2 F, CF_2^{18}), -127.01 – -127.46 (m, 2 F, CF_2^{18}), -127.81 – -128.10 (m, 2 F, CF_2^{18}), -130.33 – -130.97 (m, 2 F, CF_2^{18}). Total integral of CF_2 region normalized with respect to the CF_3^{17} group = 10.

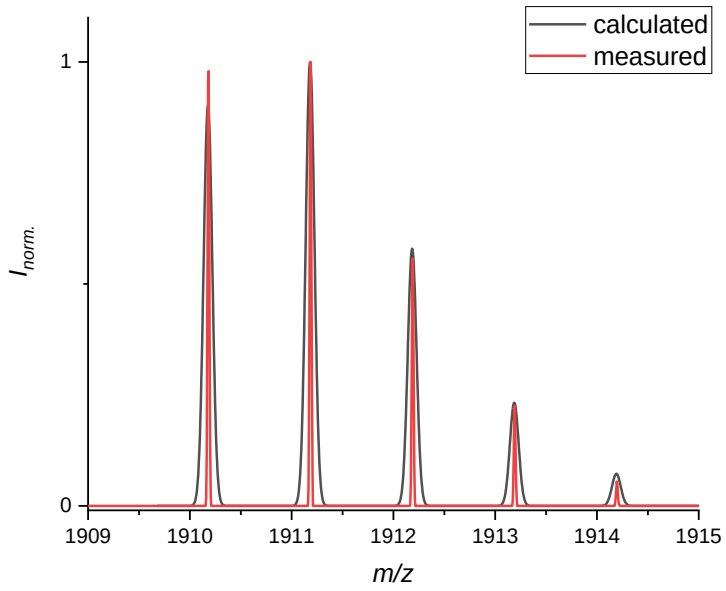
ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{99}{}^1H_{161}{}^{16}O_{16}{}^{14}N_4{}^9F_{13}$, 1910.1773; found, 1910.1826, Δ = 5.3 mmu.



Supplementary Figure 55: ^{1}H-NMR of compound **T1/3** measured in $CDCl_3$.



Supplementary Figure 56: SEC traces of the intermediates after each P-3CR in the synthesis of product **T1/3**.



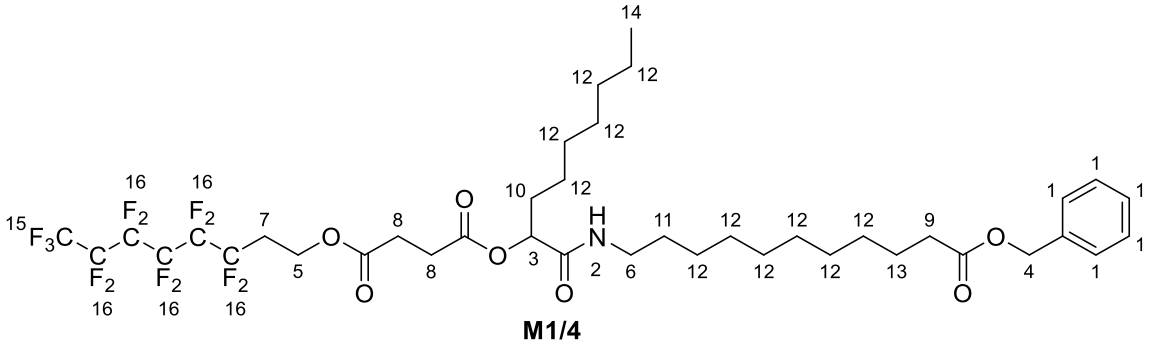
Supplementary Figure 57: High resolution ESI-MS measurement of **T1/3**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (black).

```
matching mass 1910.18541
cutoff 0.50000: 0 solutions (8 peaks)
cutoff 0.25000: 0 solutions (19 peaks)
cutoff 0.12500: 0 solutions (41 peaks)
cutoff 0.06250: 0 solutions (76 peaks)
cutoff 0.03125: 0 solutions (138 peaks)
cutoff 0.01562: 0 solutions (238 peaks)
cutoff 0.00781: 1 solutions (368 peaks)
1910.18541 ≈ 447.026590 + 323.246050 + 339.277350 + 409.355600 + 283.214750 + 107.049690 (sides Cyclohexancarboxaldehyde,
 Octanal, Tridecanal, Isobutyraldehyde; error -1.01538)
Press ENTER to quit ...
```

Supplementary Figure 58: Screenshot of the automated read-out of **T1/3**, sodium trifluoroacetate **2** was used as additive during the measurement.

6.3.3.3.5 Synthesis of tetramer T1/4

Passerini reaction



In a 50.0 mL round bottom flaks, 300 mg **TAG1** (646 µmol, 1.00 eq.) were dissolved in 2.00 mL DCM and 151 µL octanal **A8** (109 mg, 969 µmol, 1.50 eq.) and 292 mg of monomer **IM2** (969 µmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 3 days. Subsequently, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (hexane / ethyl acetate 6:1 → 5:1) to afford product **M1/4** as a yellow oil in a yield of 97.1% (560 mg, 627 µmol).

R_f = 0.40 in cyclohexane / ethyl acetate (3:1).

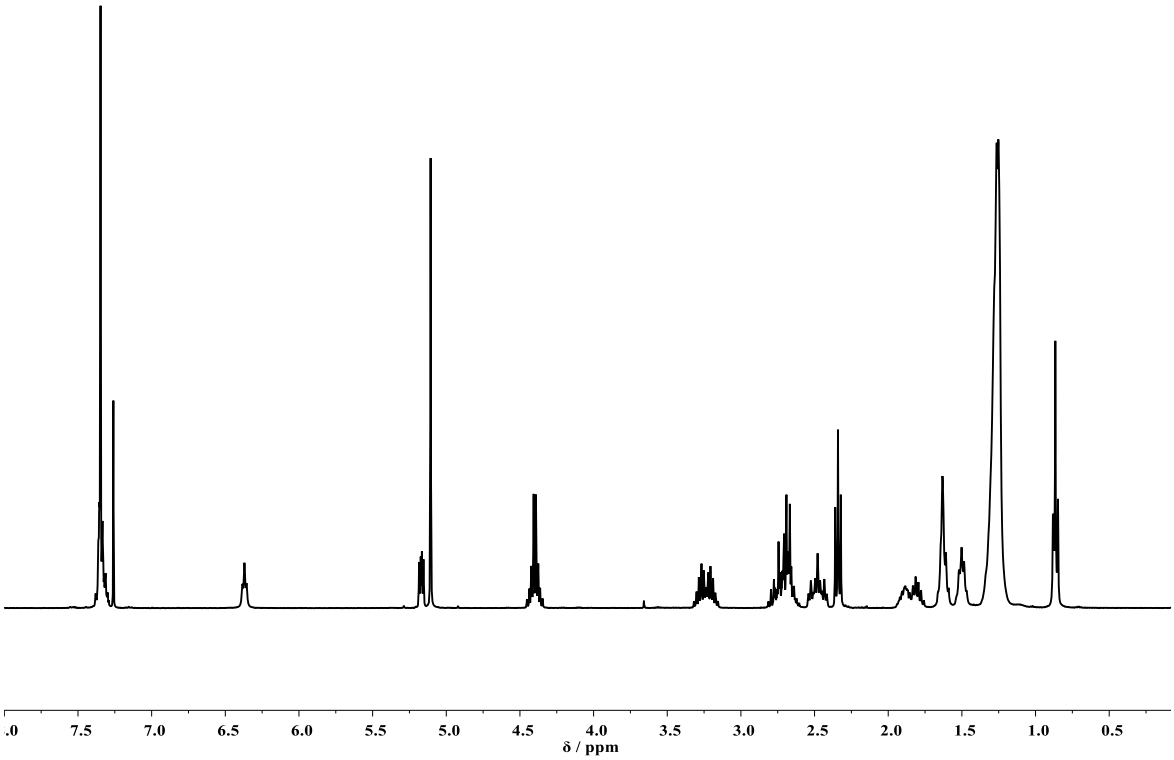
IR (ATR): ν / cm^{-1} = 2927.0 (m), 2855.8 (w), 1737.6 (s), 1659.3 (w), 1536.1 (w), 1456.7 (vw), 1359.7 (w), 1234.7 (vs), 1190.6 (vs), 1144.1 (vs), 1081.2 (m), 1003.3 (w), 841.5 (vw), 808.5 (vw), 732.4 (w), 696.9 (m), 650.7 (w), 565.4 (vw), 530.2 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.43 – 7.28 (m, 5 H, $CH_{Ar}{}^1$), 6.37 (t, J = 5.8 Hz, 1 H, NH^2), 5.17 (q, J = 7.6, 4.3 Hz, 1 H, CH^3), 5.11 (s, 2 H, $CH_2{}^4$), 4.47 – 4.33 (m, 2 H, $CH_2{}^5$), 3.34 – 3.14 (m, 2 H, $CH_2{}^6$), 2.85 – 2.60 (m, 2 H, $CH_2{}^7$), 2.55 – 2.40 (m, 4 H, $CH_2{}^8$), 2.34 (t, J = 7.6 Hz, 2 H, $CH_2{}^9$), 1.95 – 1.74 (m, 2 H, $CH_2{}^{10}$), 1.69 – 1.58 (m, 4 H, $CH_2{}^{11,12}$), 1.55 – 1.43 (m, 2 H, $CH_2{}^{13}$), 1.38 – 1.21 (m, 20 H, $CH_2{}^{12}$), 0.86 (t, J = 7.0 Hz, 3 H, $CH_3{}^{14}$)

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.82, 172.64, 171.24, 169.71, 136.27, 128.67, 128.29, 76.84, 66.20, 56.96, 39.47, 34.45, 31.94, 31.88, 30.80, 30.59, 30.37, 29.60, 29.54, 29.50, 29.39, 29.35, 29.33, 29.25, 29.23, 29.15, 26.98, 25.07, 25.02, 22.74.

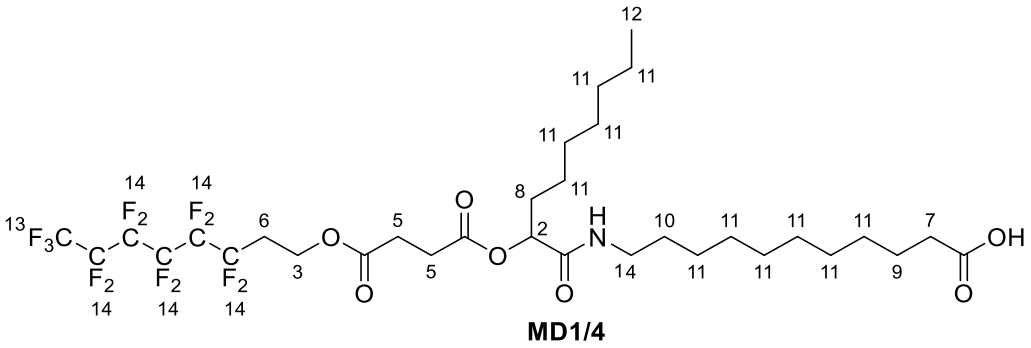
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -83.86 – -86.34 (m, 3 F, $CF_3{}^{15}$), -117.65 – -118.28 (m, 2 F, $CF_2{}^{16}$), -125.99 – -126.38 (m, 2 F, $CF_2{}^{16}$), -127.05 – -127.38 (m, 2 F, $CF_2{}^{16}$), -127.71 – -128.09 (m, 2 F, $CF_2{}^{16}$), -130.31 – -130.71 (m, 2 F, $CF_2{}^{16}$). Total integral of CF_2 region normalized with respect to the $CF_3{}^{15}$ group = 10.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{39}{}^{1}H_{52}{}^{16}O_7{}^{14}N{}^{19}F_{13}$, 894.3609; found, 894.3597, Δ = 1.2 mmu.



Supplementary Figure 59: ^{1}H-NMR of compound **M1/4** measured in $CDCl_3$.

Deprotection



In a 25 mL round bottom flask equipped with a magnetic stir bar, 457 mg of the **M1/4** (511 µmol, 1.00 eq.) was dissolved in 2.00 mL ethyl acetate and 2.00 mL THF. Subsequently, 91.4 mg (20 wt%) palladium on activated carbon **1** was added to the solution. The resulting mixture was purged with hydrogen gas (3 balloons) and stirred for 5 days at room temperature under hydrogen atmosphere. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **MD1/4** was obtained as a yellow highly viscous oil in a yield of 94.3%. (388 mg, 482 µmol).

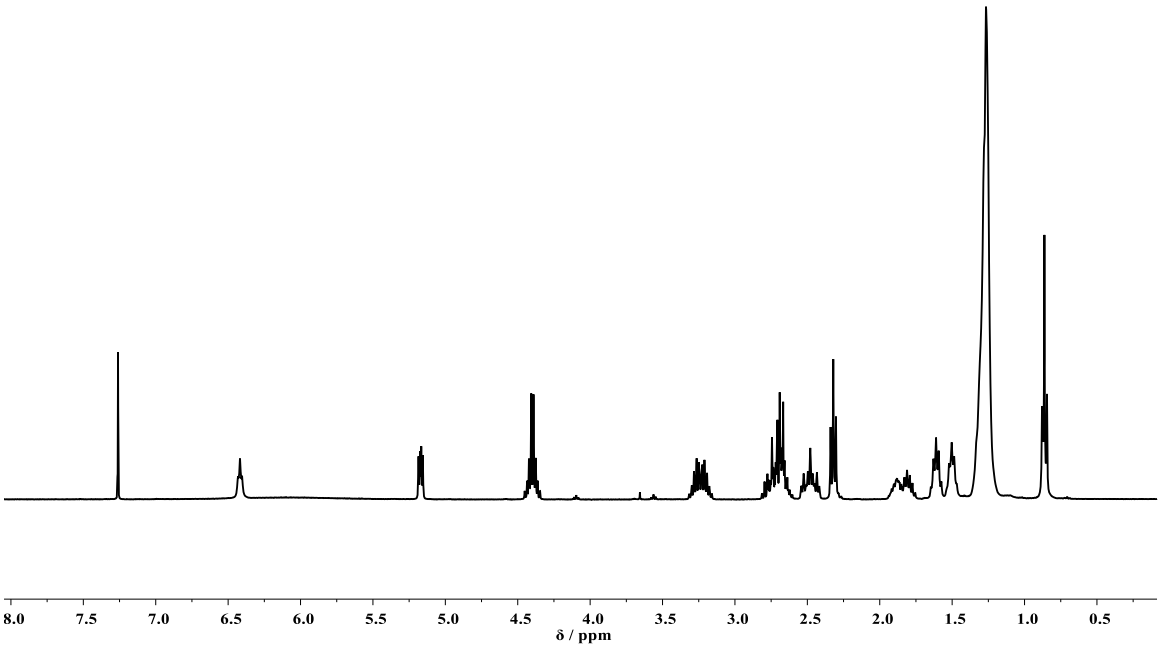
IR (ATR): ν / cm^{-1} = 3296.2 (w), 2923.7 (s), 2851.9 (m), 1742.9 (vs), 1696.1 (s), 1653.5 (s), 1560.3 (w), 1468.4 (w), 1411.5 (w), 1358.8 (m), 1233.0 (vs), 1189.4 (vs), 1141.7 (vs), 1082.3 (vs), 1009.6 (w), 938.0 (w), 841.3 (w), 698.3 (vs), 651.3 (s), 567.5 (w), 528.7 (w), 437.6 (w), 389.0 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.35 (t, J = 5.9 Hz, 1 H, NH^1), 5.19 – 5.01 (m, 1 H, CH^2), 4.41 – 4.23 (m, 2 H, $CH_2{}^3$), 3.34 – 3.02 (m, 2 H, $CH_2{}^4$), 2.77 – 2.54 (m, 4 H, $CH_2{}^5$), 2.49 – 2.35 (m, 2 H, $CH_2{}^6$), 2.30 – 2.23 (m, 2 H, $CH_2{}^7$), 1.90 – 1.67 (m, 2 H, $CH_2{}^8$), 1.61 – 1.49 (m, 2 H, $CH_2{}^9$), 1.49 – 1.39 (m, 2 H, $CH_2{}^{10}$) 1.34 – 1.14 (m, 22 H, $CH_2{}^{11}$), 0.80 (t, J = 6.7 Hz, 3 H, $CH_3{}^{12}$).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.43, 172.02, 170.60, 116.31, 75.50, 57.72, 40.23, 34.77, 32.67, 32.63, 31.56, 31.34, 31.13, 30.20, 30.10, 30.08, 29.98, 29.90, 29.83, 27.66, 25.77, 25.55, 23.49, 14.93.

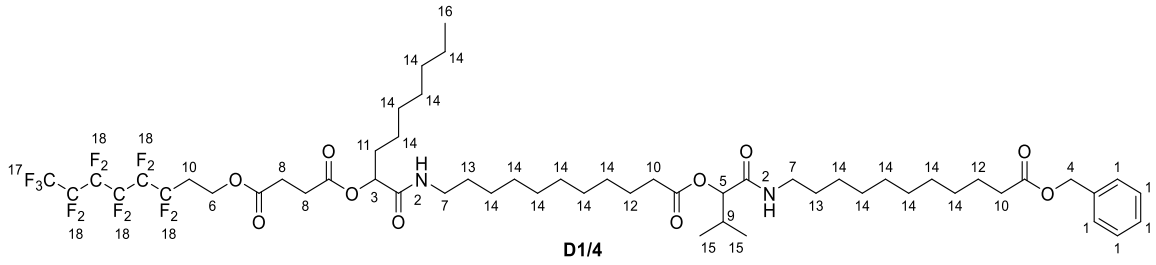
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.01 – -85.29 (m, 3 F, $CF_3{}^{13}$), -117.90 – -118.20 (m, 2 F, $CF_2{}^{14}$), -125.96 – -126.43 (m, 2 F, $CF_2{}^{14}$), -126.99 – -127.42 (m, 2 F, $CF_2{}^{14}$), -127.82 – -128.11 (m, 2 F, $CF_2{}^{14}$), -130.29 – -130.76 (m, 2 F, $CF_2{}^{14}$). Total integral of CF_2 region normalized with respect to the $CF_3{}^{13}$ group = 10.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{32}{}^{1}H_{46}{}^{16}O_7{}^{14}N^{19}F_{13}$, 804.3139; found, 804.3115, Δ = 2.4 mmu.



Supplementary Figure 60: ^{1}H-NMR of compound **MD1/4** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 413 mg of **MD1/4** (514 µmol, 1.00 eq.) was dissolved in 2.00 mL DCM and 70.4 µL isobutyraldehyde **A3** (55.6 mg, 772 µmol, 1.50 eq.) and 233 mg of monomer **IM2** (772 mmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 3 days. Subsequently, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane / ethyl acetate 5:1 → 1:1) to afford product **D1/4** as a pale highly viscous oil in a yield of 96.5% (555 mg, 496 µmol).

R_f = 0.20 in cyclohexane / ethyl acetate (3:1).

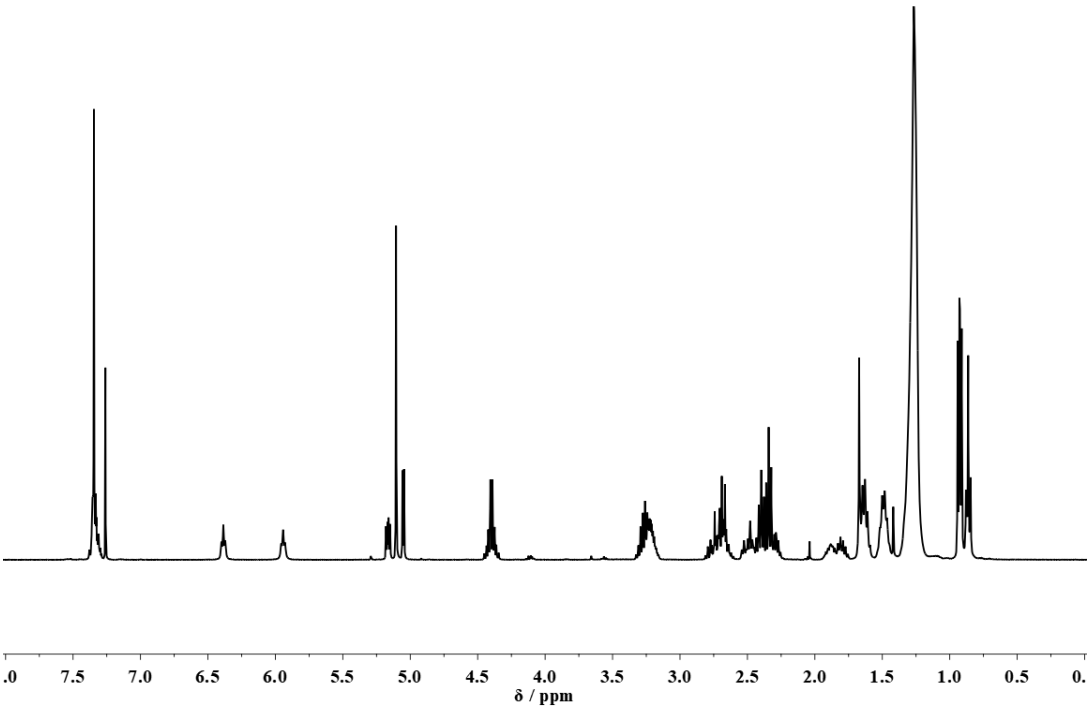
IR (ATR): ν / cm^{-1} = 3315.8 (vw), 2923.9 (m), 2852.2 (w), 1736.6 (vs), 1656.1 (vs), 1548.7 (w), 1466.1 (w), 1363.6 (w), 1236.4 (vs), 1144.2 (vs), 1009.0 (m), 841.8 (w), 809.6 (vw), 732.3 (w), 697.3 (m), 652.0 (w), 567.3 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.41 – 7.28 (m, 5 H, CH_{Ar}^{1}), 6.39 (t, J = 5.8 Hz, 1 H, NH^{2}), 5.94 (t, J = 5.9 Hz, 1 H, NH^{2}), 5.20 – 5.14 (m, 1 H, CH^{3}), 5.11 (s, 2 H, CH^{4}), 5.05 (d, J = 4.4 Hz, 1 H, CH^{5}), 4.46 – 4.34 (m, 2 H, CH^{6}), 3.36 – 3.15 (m, 4 H, CH^{7}), 2.83 – 2.60 (m, 4 H, CH_2^{8}), 2.56 – 2.24 (m, 7 H, CH^{9}, CH_2^{10}), 1.97 – 1.74 (m, 2 H, CH_2^{11}), 1.72 – 1.61 (m, 4 H, CH_2^{12}), 1.55 – 1.45 (m, 4 H, CH_2^{13}), 1.38 – 1.20 (m, 34 H, CH_2^{14}), 0.95 – 0.90 (m, 6 H, CH_3^{15}), 0.89 – 0.84 (m, 3 H, CH_3^{16}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.82, 172.66, 172.64, 171.24, 169.72, 169.39, 136.25, 128.66, 128.28, 78.03, 74.74, 66.20, 56.94, 39.44, 39.29, 34.45, 34.42, 31.93, 31.87, 30.65, 29.72, 29.60, 29.56, 29.54, 29.50, 29.47, 29.35, 29.33, 29.26, 29.23, 29.14, 26.97, 25.14, 25.07, 25.02, 22.74, 18.90, 17.05, 14.19.

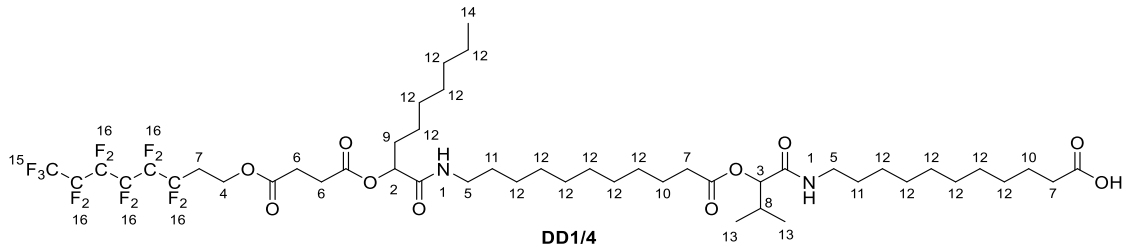
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -84.77 – -85.55 (m, 3 F, CF_3^{17}), -117.72 – -118.51 (m, 2 F, CF_2^{18}), -126.00 – -126.49 (m, 2 F, CF_2^{18}), -126.94 – -127.43 (m, 2 F, CF_2^{18}), -127.58 – -128.18 (m, 2 F, CF_2^{18}), -129.96 – -130.99 (m, 2 F, CF_2^{18}). Total integral of CF_2 region normalized with respect to the CF_3^{17} group = 10.

ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{55}{}^{1}H_{81}{}^{16}O_{10}{}^{14}N_2{}^{19}F_{13}$, 1199.5576; found, 1199.5555, Δ = 2.1 mmu.



Supplementary Figure 61: ^{1}H-NMR of compound **D1/4** measured in $CDCl_3$.

Deprotection



In a 25 mL round bottom flask, 505 mg of **D1/4** (429 µmol, 1.00 eq.) was dissolved in 2.00 mL ethyl acetate and 2.00 mL THF. Subsequently, 101 mg (20 wt%) palladium on activated carbon **1** was added to the solution. The resulting mixture was purged with hydrogen gas and stirred for 1 day at room temperature under hydrogen atmosphere (3 balloons). The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **DD1/4** was obtained as a yellow highly viscous oil in a yield of 97.4%. (445 mg, 418 µmol).

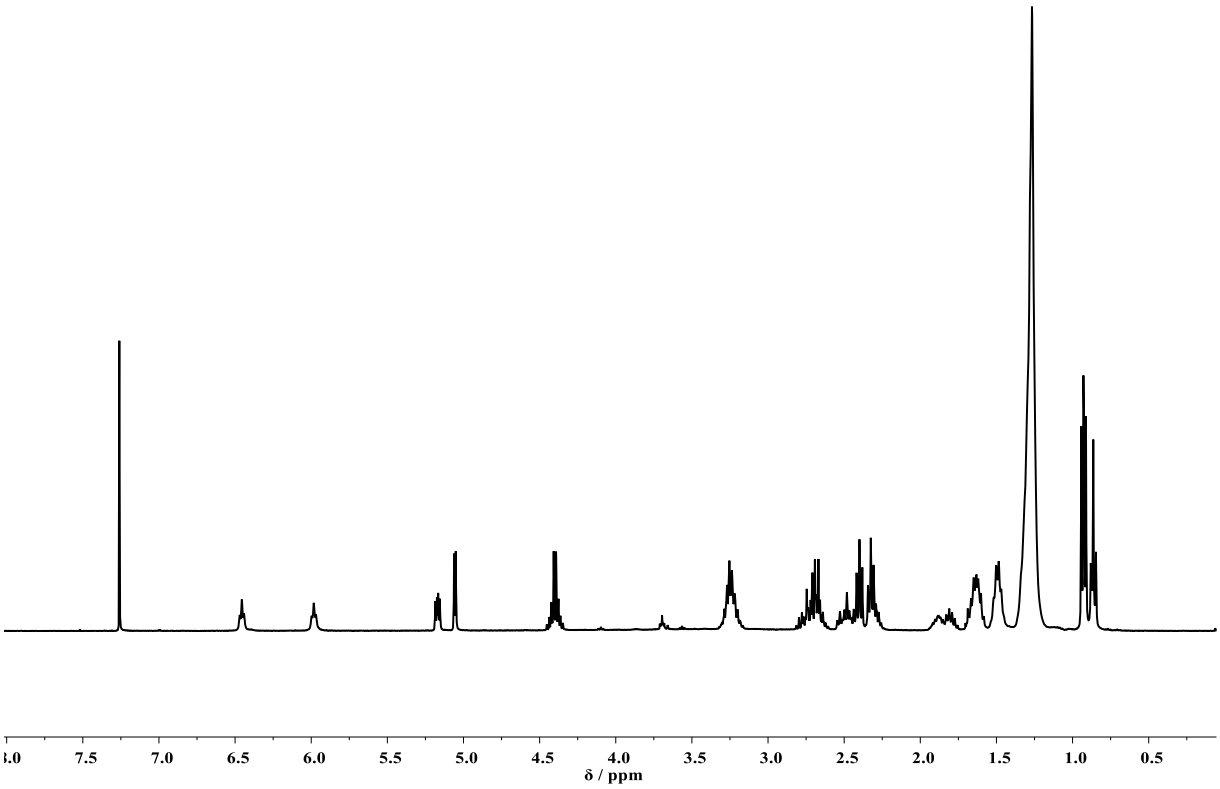
IR (ATR): ν / cm^{-1} = 3304.8 (vw), 2925.6 (m), 2854.6 (m), 1738.4 (s), 1654.9 (s), 1540.6 (w), 1464.3 (w), 1365.1 (w), 1235.0 (vs), 1144.6 (vs), 1007.2 (w), 841.4 (vw), 808.8 (w), 697.7 (w), 651.2 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.46 (t, J = 5.8 Hz, 1 H, NH^{1}), 5.98 (t, J = 5.9 Hz, 1 H, NH^{1}), 5.21 – 5.12 (m, 1 H, CH^{2}), 5.06 (d, J = 4.4 Hz, 1 H, CH^{3}), 4.48 – 4.32 (m, 2 H, CH_2^{4}), 3.34 – 3.15 (m, 4 H, CH_2^{5}), 2.83 – 2.58 (m, 4 H, CH_2^{6}), 2.56 – 2.20 (m, 7 H, CH^{7}, CH_2^{8}), 1.95 – 1.72 (m, 2 H, CH_2^{9}), 1.70 – 1.55 (m, 4 H, CH_2^{10}), 1.54 – 1.40 (m, 4 H, CH_2^{11}), 1.39 – 1.14 (m, 34 H, CH_2^{12}), 0.97 – 0.90 (m, 6 H, CH_3^{13}), 0.89 – 0.78 (m, 3 H, CH_3^{14}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 172.72, 171.32, 169.97, 169.52, 78.07, 74.73, 56.98, 39.52, 39.26, 34.43, 34.01, 31.90, 31.87, 30.62, 29.62, 29.58, 29.51, 29.41, 29.37, 29.35, 29.32, 29.29, 29.21, 29.14, 29.08, 26.97, 26.89, 25.16, 25.02, 24.88, 22.74, 18.90, 17.05, 14.18.

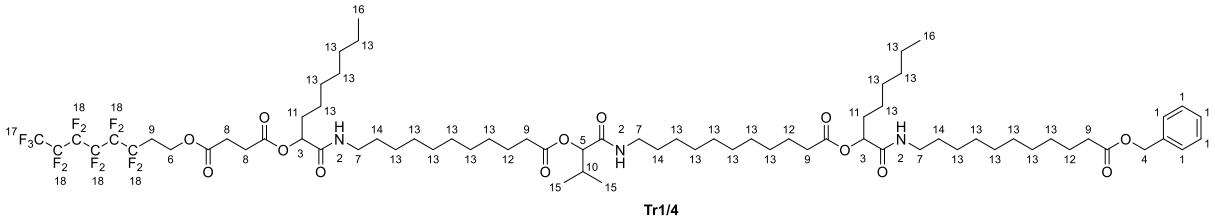
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.09 (t, J = 9.9 Hz, 3 F, CF_3^{13}), -117.74 – -118.31 (m, 2 F, CF_2^{14}), -125.91 – -126.37 (m, 2 F, CF_2^{14}), -126.61 – -127.43 (m, 2 F, CF_2^{14}), -127.48 – -128.13 (m, 2 F, CF_2^{14}), -130.00 – -130.75 (m, 2 F, CF_2^{14}). Total integral of CF_2 region normalized with respect to the CF_3^{13} group = 10.

ESI-MS [m/z]: $[M + Na]^+$ calculated for $^{12}C_{48}{}^{1}H_{75}{}^{16}O_{10}{}^{14}N_2{}^{19}F_{13}$, 1109.5106; found, 1109.5092, Δ = 1.4 mmu.



Supplementary Figure 62: ^{1}H-NMR of compound **DD1/4** measured in $CDCl_3$.

Passerini reaction



Tr1/4

In a 50 mL round bottom flask, 274 mg of **DD1/4** (232 µmol, 1.00 eq.) was dissolved in 3.00 mL DCM and 41.9 µL heptanal **A7** (39.7 mg, 348 µmol, 1.50 eq.) and 105 mg of monomer **IM2** (348 µmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 3 days. Subsequently, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane / ethyl acetate 4:1 → 2:1) to afford product **Tr1/4** as a pale highly viscous oil in a yield of 90.1% (314 mg, 209 µmol).

R_f = 0.13 in cyclohexane / ethyl acetate (2:1).

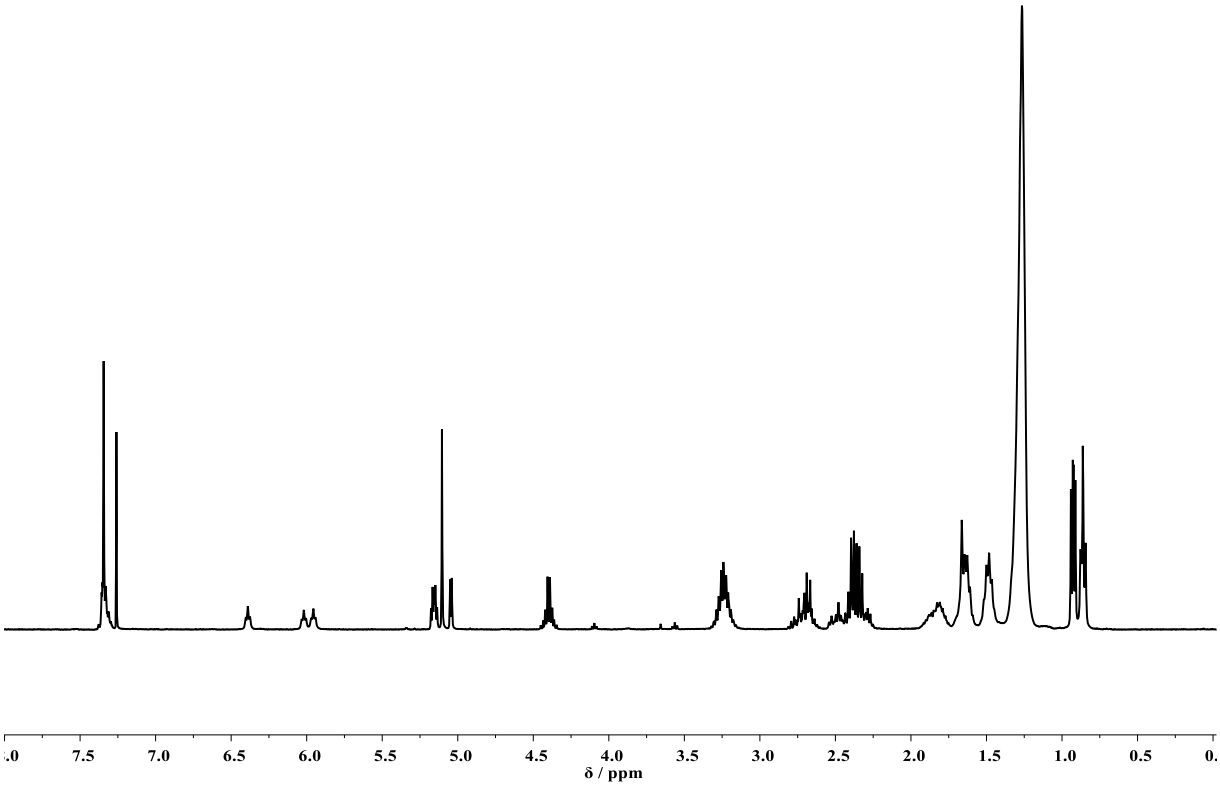
IR (ATR): ν / cm^{-1} = 3291.1 (vw), 2923.5 (s), 2852.7 (m), 1736.5 (vs), 1655.0 (vs), 1540.3 (m), 1465.1 (w), 1365.4 (w), 1235.0 (vs), 1144.9 (vs), 1005.5 (w), 842.2 (vw), 697.7 (m), 652.8 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.44 – 7.29 (m, 5 H, $CH_{Ar}{}^1$), 6.39 (t, J = 5.8 Hz, 1 H, NH^2), 6.02 (t, J = 5.9 Hz, 1 H, NH^2), 5.96 (t, J = 5.9 Hz, 1H, NH^2), 5.22 – 5.15 (m, 2 H, CH^3), 5.10 (s, 2 H, $CH_2{}^4$), 5.05 (d, J = 4.4 Hz, 1 H, CH^5), 4.49 – 4.34 (m, 2 H, $CH_2{}^6$), 3.32 – 3.14 (m, 6 H, $CH_2{}^7$), 2.83 – 2.58 (m, 4 H, $CH_2{}^8$), 2.55 – 2.23 (m, 9 H, CH^9, $CH_2{}^{10}$), 1.96 – 1.75 (m, 4 H, $CH_2{}^{11}$), 1.75 – 1.56 (m, 8 H, $CH_2{}^{12}$, $CH_2{}^{13}$), 1.56 – 1.39 (m, 6 H, $CH_2{}^{14}$), 1.37 – 1.16 (m, 52 H, $CH_2{}^{13}$), 0.96 – 0.90 (m, 6 H, $CH_3{}^{15}$), 0.89 – 0.83 (m, 6 H, $CH_2{}^{16}$).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.82, 172.69, 172.66, 172.60, 171.25, 169.99, 169.74, 169.42, 136.26, 128.67, 128.29, 78.04, 74.74, 74.06, 66.20, 56.94, 39.44, 39.32, 39.28, 34.45, 34.41, 32.04, 31.93, 31.86, 31.75, 30.64, 30.57, 29.72, 29.68, 29.60,29.58, 29.53, 29.49, 29.35, 29.32, 29.26, 29.23, 29.22, 29.13, 29.04, 26.96, 25.13, 25.09, 25.07, 25.02, 24.83, 22.73, 22.66, 18.89, 17.06, 14.17.

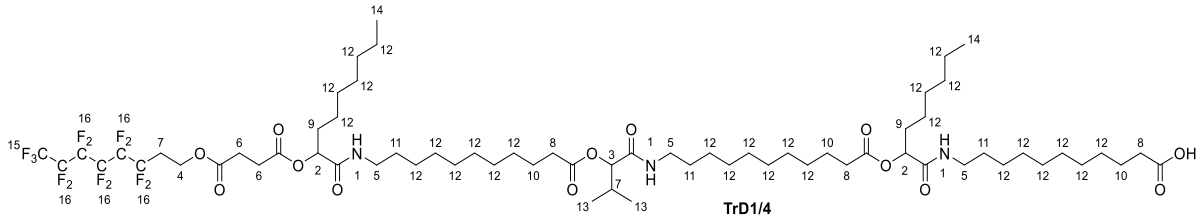
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.09 (t, J = 9.9 Hz, 3 F, $CF_3{}^{17}$), -117.75 – -118.21 (m, 2 F, $CF_2{}^{18}$), -125.91 – -126.45 (m, 2 F, $CF_2{}^{18}$), -126.96 – -127.40 (m, 2 F, $CF_2{}^{18}$), -127.70 – -128.26 (m, 2 F, $CF_2{}^{18}$), -130.02 – -130.86 (m, 2 F, $CF_2{}^{18}$). Total integral of CF_2 region normalized with respect to the $CF_3{}^{17}$ group = 10.

ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{74}{}^{1}H_{116}{}^{16}O_{13}{}^{14}N_3{}^{19}F_{13}$, 1524.8193; found, 1524.8176, Δ = 1.7 mmu.



Supplementary Figure 63: ^{1}H-NMR of compound **Tr1/4** measured in $CDCl_3$.

Deprotection



In a 25 mL round bottom flask, 225 mg of **Tr1/4** (150 µmol, 1.00 eq.) was dissolved in 3.00 mL ethyl acetate and 3.00 mL THF. Subsequently, 91.4 mg (20 wt%) palladium on activated carbon **1** was added to the solution. The resulting mixture was purged with hydrogen gas and stirred for 1 day at room temperature under hydrogen atmosphere (3 balloons). The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **TrD1/4** was obtained as a pale highly viscous oil in a yield of 95.3%. (201 mg, 143 µmol).

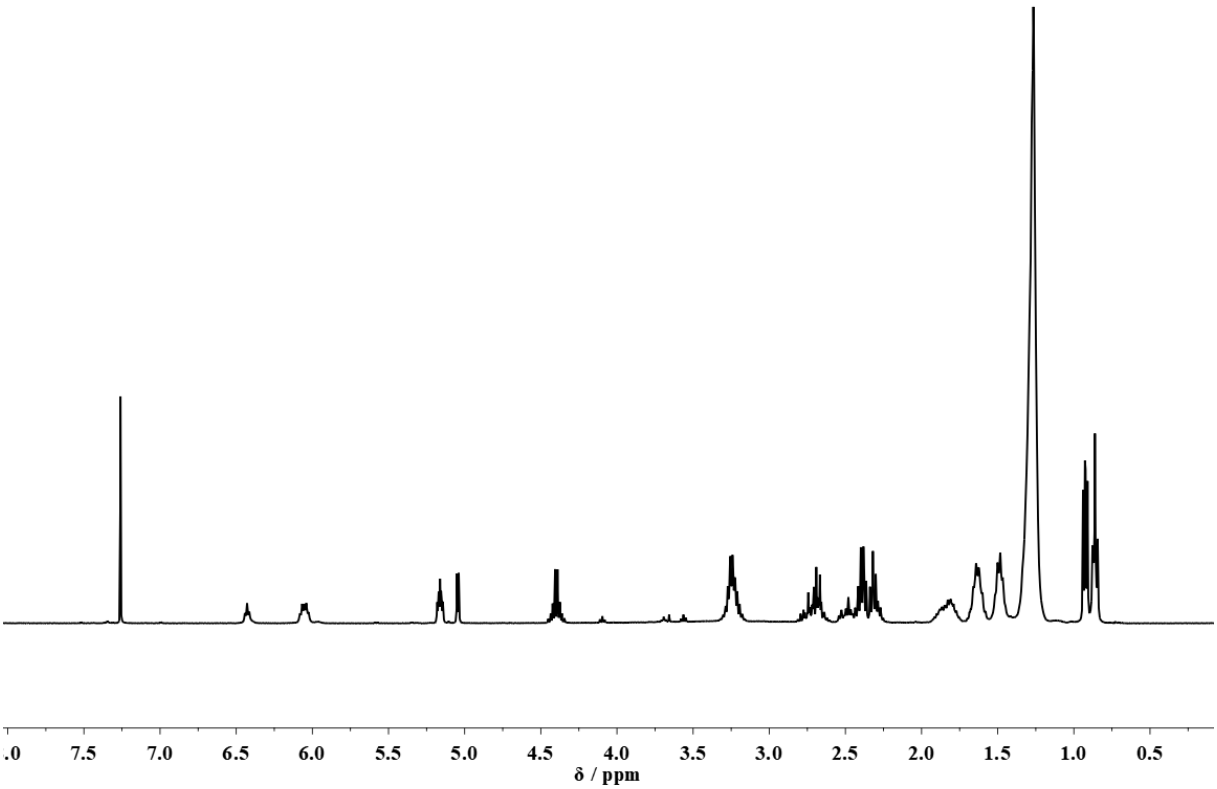
IR (ATR): ν / cm^{-1} = 3290.0 (vw), 2922.6 (m), 2852.3 (m), 2335.9 (vw), 1737.7 (s), 1655.3 (s), 1554.2 (w), 1466.3 (w), 1366.0 (w), 1235.6 (s), 1144.9 (vs), 1008.2 (w), 842.6 (vw), 809.8 (vw), 698.5 (w), 652.2 (w), 567.4 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.43 (t, J = 5.8 Hz, 1 H, NH^{1}), 6.13 – 5.93 (m, 2 H, NH^{1}), 5.21– 5.12 (m, 2 H, CH_2^{2}), 5.04 (d, J = 4.5 Hz, 1 H, CH^{3}), 4.48 – 4.32 (m, 2 H, CH_2^{4}), 3.35 – 3.15 (m, 6 H, CH_2^{5}), 2.84 – 2.59 (m, 4 H, CH_2^{6}), 2.56 – 2.22 (m, 9 H, CH^{7}, CH_2^{8}), 1.98 – 1.70 (m, 4 H, CH_2^{9}), 1.70 – 1.56 (m, 6 H, CH_2^{10}), 1.54 – 1.41 (m, 6 H, CH_2^{11}), 1.40 – 1.16 (m, 54 H, CH_2^{12}), 0.95 – 0.90 (m, 6 H, CH_3^{13}), 0.89 – 0.83 (m, 6 H, CH_3^{14}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 177.26, 172.80, 172.68, 172.64, 171.27, 170.10, 169.88, 169.61, 78.07, 74.73, 74.09, 56.96, 39.48, 39.35, 39.30, 34.47, 34.41, 34.07, 32.01, 31.92, 31.87, 31.75, 30.62, 29.69, 29.59, 29.55, 29.51, 29.43, 29.37, 29.34, 29.32, 29.25, 29.22, 29.14, 29.10, 29.04, 26.97, 26.96, 26.88, 25.12, 25.01, 24.94, 24.85, 22.73, 22.66, 18.88, 17.08, 14.17.

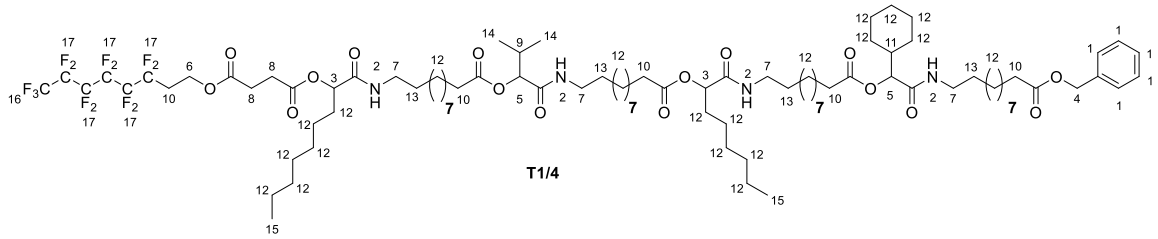
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.09 (t, J = 9.8 Hz, 3 F, CF_3^{15}), -117.60 – -118.73 (m, 2 F, CF_2^{16}), -125.73 – -126.37 (m, 2 F, CF_2^{16}), -126.86 – -127.47 (m, 2 F, CF_2^{16}), -127.73 – -128.10 (m, 2 F, CF_2^{16}), -130.03 – -130.85 (m, 2 F, CF_2^{16}). Total integral of CF_2 region normalized with respect to the CF_3^{15} group = 10.

ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{67}{}^{1}H_{110}{}^{16}O_{13}{}^{14}N_3{}^{19}F_{13}{}^{23}Na$, 1434.7723; found, 1434.7705, Δ = 1.8 mmu.



Supplementary Figure 64: ^{1}H-NMR of compound **TrD1/4** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 164 mg **TrD1/4** (116 µmol, 1.00 eq.) was dissolved in 2.00 mL DCM and 21.1 µL cyclohexancarboxaldehyde **A6** (19.5 mg, 174 µmol, 1.50 eq.) and 52.5 mg of monomer **IM2** (174 µmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 3 days. Subsequently, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane / ethyl acetate 4:1 → 1:1) to afford product **T1/4** as a pale highly viscous oil in a yield of 75.5% (160 mg, 87.6 µmol).

R_f = 0.53 in cyclohexane / ethyl acetate (3:2).

IR (ATR): ν / cm^{-1} = 3293.4 8 (w), 2922.3 (s), 2851.6 (m), 1735.9 (vs), 1654.5 8 (vs), 1537.5 (m), 1466.3 (w), 1362.4 (w), 1236.3 (vs), 1145.2 (vs), 1009.2 (w), 809.0 (vw), 697.2 (m), 652.2 (w), 566.9 (vw), 451.0 (vw).

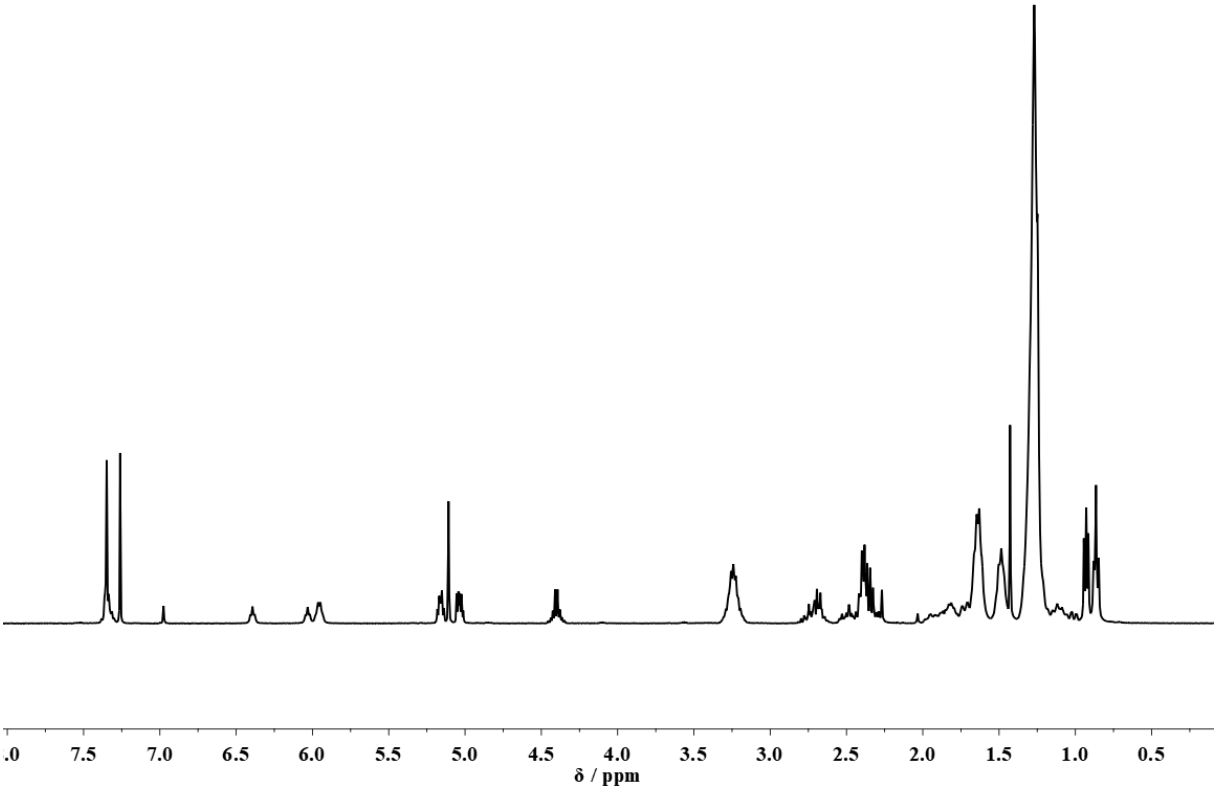
^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.41 – 7.30 (m, 5 H, CH_{Ar}^{1}), 6.39 (t, J = 5.8 Hz, 1 H, NH^{2}), 6.09 – 5.91 (m, 3 H, NH^{2}), 5.19 – 5.13 (m, 2 H, CH_2^{3}), 5.11 (s, 2 H, CH_2^{4}), 5.07 – 4.98 (m, 2 H, CH_2^{5}), 4.47 – 4.34 (m, 2 H, CH_2^{6}), 3.33 – 3.15 (m, 8 H, CH_2^{7}), 2.83 – 2.62 (m, 4 H, CH_2^{8}), 2.56 – 2.24 (m, 11 H, CH^{9}, CH_2^{10}), 2.00 – 1.59 (m, 21 H, CH^{11}, CH_2^{12}), 1.54 – 1.44 (m, 8 H, CH_2^{13}), 1.38 – 1.06 (m, 68 H, CH_2^{12}), 0.97 – 0.90 (m, 6 H, CH_2^{14}), 0.90 – 0.82 (m, 6 H, CH_2^{15}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.84, 172.68, 172.62, 171.54, 171.53, 171.26, 170.00, 169.74, 169.42, 169.34, 136.26, 128.68, 128.30, 78.04, 77.75, 74.74, 74.06, 66.21, 40.11, 39.44, 39.32, 39.28, 34.46, 34.43, 32.05, 31.94, 31.87, 31.76, 30.65, 30.45, 29.85, 29.74, 29.71, 29.59, 29.54, 29.51, 29.48, 29.35, 29.33, 29.27, 29.24, 29.23, 29.14, 29.05, 27.41, 26.97, 26.21, 26.13, 26.01, 25.13, 25.10, 25.08, 25.03, 24.85, 22.74, 22.67, 18.91, 17.06, 14.19.

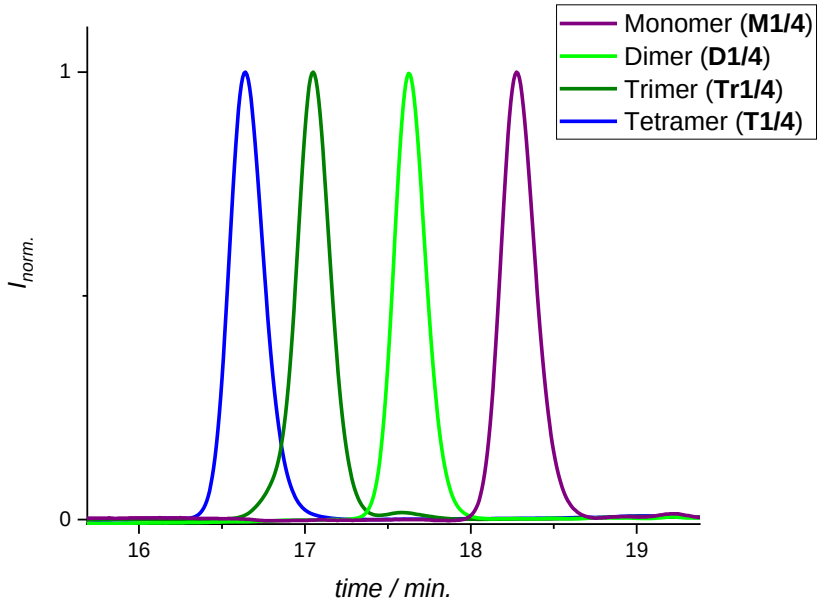
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -84.73 – -85.47 (m, 3 F, CF_3^{16}), -117.67 – -118.35 (m, 2 F, CF_2^{17}), -125.60 – -126.44 (m, 2 F, CF_2^{17}), -126.83 – -127.22 (m, 2 F, CF_2^{17}), -127.54 – -128.48 (m, 2 F, CF_2^{17}), -130.28 – -131.34 (m, 2 F, CF_2^{17}).

Total integral of CF_2 region normalized with respect to the CF_3^{16} group = 10.

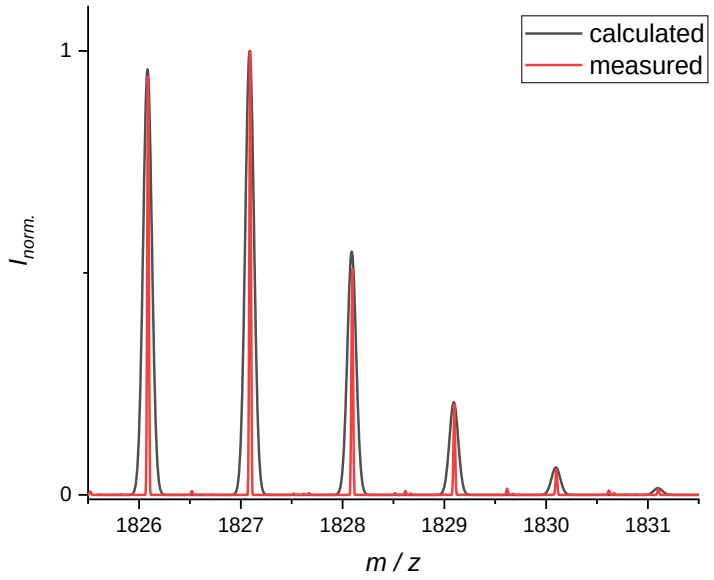
ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{93}{}^{1}H_{149}{}^{16}O_{16}{}^{14}N_4{}^{19}F_{13}$, 1826.0834; found, 1826.0885 Δ = 5.1 mmu.



Supplementary Figure 65: ^{1}H-NMR of compound **T1/4** measured in $CDCl_3$.



Supplementary Figure 66: SEC traces of the intermediates after each P-3CR in the synthesis of product **T1/4**.



Supplementary Figure 67: High resolution ESI-MS measurement of **T1/4**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (black).

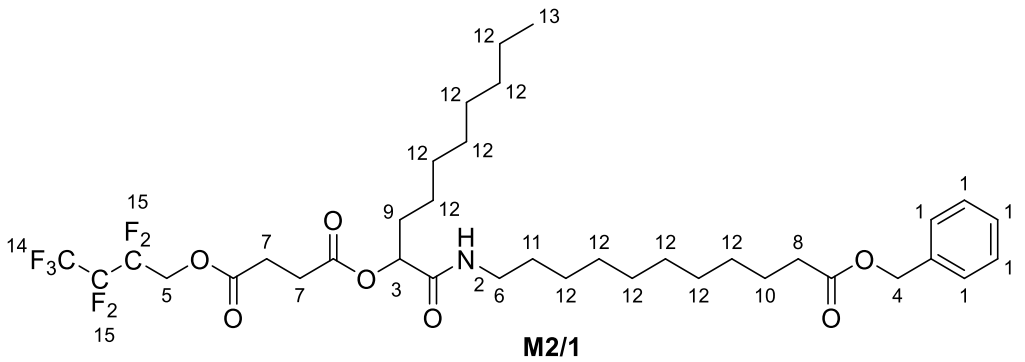
```
imum is 1.000000 found for mass 1827.090720
matching mass 1827.09072
cutoff 0.50000: 0 solutions (9 peaks)
cutoff 0.25000: 0 solutions (20 peaks)
cutoff 0.12500: 0 solutions (54 peaks)
cutoff 0.06250: 0 solutions (103 peaks)
cutoff 0.03125: 0 solutions (155 peaks)
cutoff 0.01562: 0 solutions (287 peaks)
cutoff 0.00781: 0 solutions (423 peaks)
cutoff 0.00391: 0 solutions (568 peaks)
cutoff 0.00195: 1 solutions (1147 peaks)
1827.09072 ≈ 447.026590 + 339.277350 + 283.214750 + 325.261700 + 323.246050 + 107.049690 (sides Octanal,
 Isobutyraldehyde, Heptanal, Cyclohexancarboxaldehyde; error -2.01459)
Press ENTER to quit ...
```

Supplementary Figure 68: Screenshot of the automated read-out of **T1/4**.

6.3.3.4 Oligomer synthesis with TAG2

6.3.3.4.1 Synthesis of tetramer T2/1

Passerini reaction



M2/1

In a 50 mL round bottom flask, 1.10 g of **TAG2** (3.68 mmol, 1.00 eq.) were stirred in 4.00 mL dichloromethane. Subsequently, 2.01 mL nonanal **A9** (758 mg, 5.52 mmol, 1.50 eq.) and 1.66 g of monomer **IM2** (5.52 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (9:1 → 4:1) to yield the Passerini product **M2/1** as a pale highly viscous oil (2.65 g, 3.56 mmol, 96.7%).

R_f = 0.32 in cyclohexane / ethyl acetate (3:1).

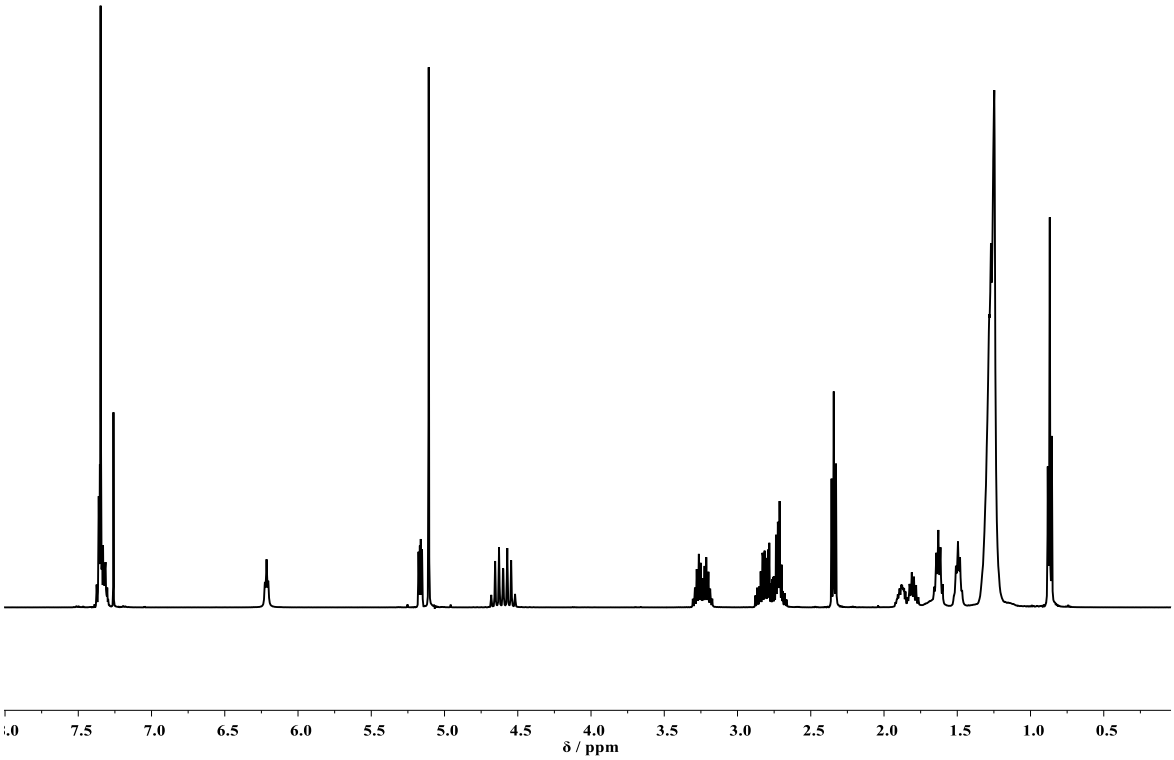
IR (ATR): ν / cm^{-1} = 3303.5 (vw), 2925.5 (m), 2854.8 (w), 1739.1 (s), 1657.5 (m), 1536.4 (w), 1456.0 (w), 1352.3 (w), 1226.4 (vs), 1144.5 (vs), 1020.1 (m),), 909.6 (w), 736.0 (m), 697.2 (m).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.42 – 7.32 (m, 5 H, $CH_{Ar}{}^1$), 6.24 (t, J = 5.8 Hz, 1 H, NH^2), 5.23 – 5.16 (m, 1 H, CH^3), 5.13 (s, 2 H, $CH_2{}^4$), 4.74 – 4.52 (m, 2 H, $CH_2{}^5$), 3.36 – 3.17 (m, 2 H, $CH_2{}^6$), 2.92 – 2.66 (m, 4 H, $CH_2{}^7$), 2.37 (t, J = 7.6 Hz, 2 H, $CH_2{}^8$), 1.96 – 1.78 (m, 2 H, $CH_2{}^9$), 1.70 – 1.61 (m, 2 H, $CH_2{}^{10}$), 1.57 – 1.47 (m, 2 H, $CH_2{}^{11}$), 1.40 – 1.21 (m, 24 H, $CH_2{}^{12}$), 0.89 (t, J = 6.9 Hz, 3 H, $CH_3{}^{13}$).

^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 173.70, 171.37, 170.77, 169.48, 136.14, 128.55, 128.17, 74.76, 66.07, 59.56 (t, J = 26.9 Hz), 39.32, 34.32, 31.82, 29.46, 29.42, 29.38, 29.37, 29.24, 29.21, 29.20, 29.11, 28.92, 28.66, 26.83, 24.94, 24.85, 22.64, 14.09.

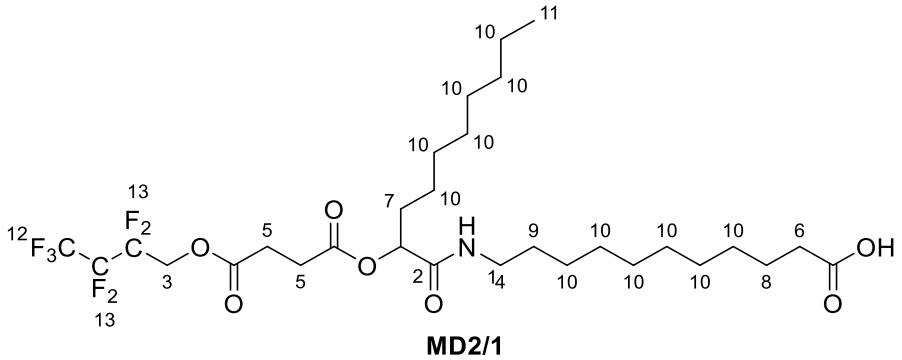
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm =-80.89 (t, J = 9.3 Hz, 3F, $CF_3{}^{14}$), -119.83 – -121.39 (m, 2 F, $CF_2{}^{15}$), -127.31 – -128.95 (m, 2 F, $CF_2{}^{15}$). Total integral of CF_2 region normalized with respect to the $CF_3{}^{14}$ group = 4.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{36}{}^{1}H_{52}{}^{16}O_7{}^{14}N^{19}F_7$, 744.3705; found, 744.3693, Δ = 1.2 mmu.



Supplementary Figure 69: ^{1}H-NMR of compound **M2/1** measured in $CDCl_3$.

Deprotection



In a 25 mL round bottom flask, 2.08 g of **M2/1** (3.29 mmol, 1.00 eq.) was dissolved in 4.00 mL ethyl acetate and 4.00 mL THF. Subsequently, 488 mg (20 wt%) palladium on activated carbon **1** were added to the solution. The resulting mixture was purged with hydrogen gas and stirred for one day at room temperature under hydrogen atmosphere (balloon). The crude reaction mixture was filtered over celite® and flushed with 50 mL dichloromethane. After evaporation

of the solvents and drying under reduced pressure the corresponding acid **MD2/1** was obtained as a colorless solid (2.11 g, 3.22 mol, 98.0%).

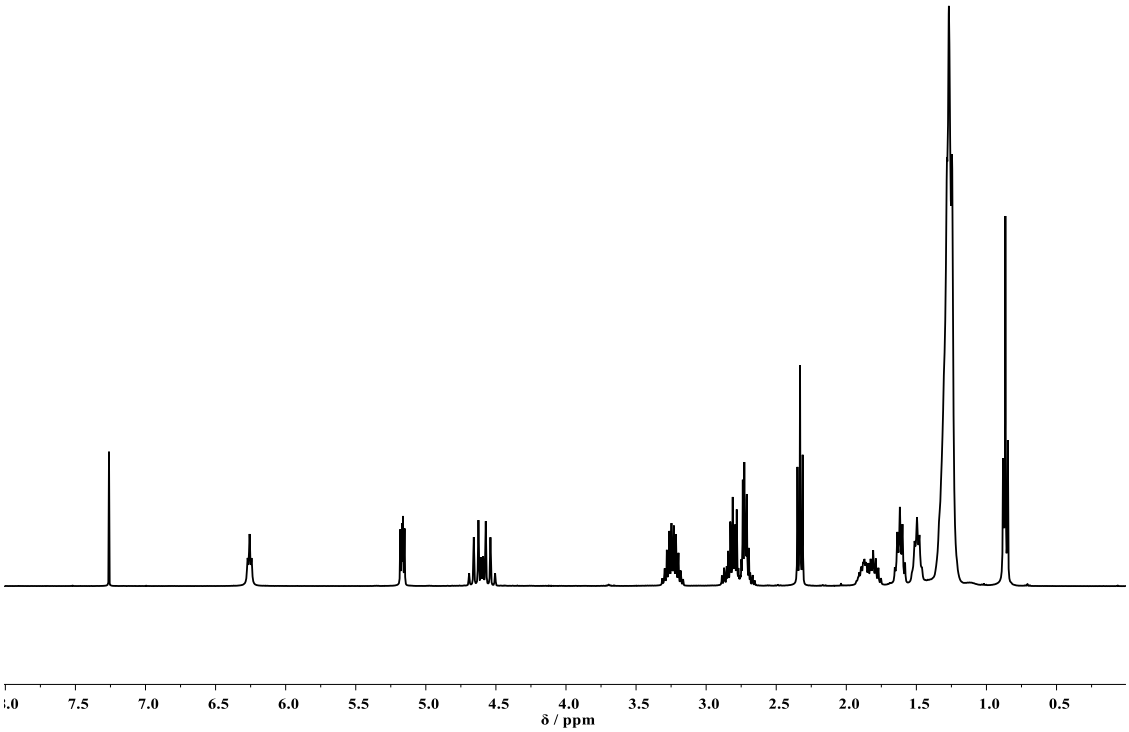
IR (ATR): ν / cm^{-1} = 3298.2 (w), 2920.6 (s), 2850.8 (s), 1744.3 (vs), 1692.7 (vs), 1651.4 (vs), 1557.3 (m), 1468.7 (w), 1412.0 (m), 1218.3 (vs), 1159.3 (vs), 1022.3 (s), 911.8 (m), 723.0 (w), 671.8 (w), 535.7 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.26 (t, J = 5.8 Hz, 1H, NH^{1}), 5.22 – 5.09 (m, 1 H, CH^{2}), 4.70 – 4.49 (m, 2 H, CH_2^{3}), 3.34 – 3.15 (m, 2 H, CH_2^{4}), 2.92 – 2.64 (m, 4 H, CH_2^{5}), 2.33 (t, J = 7.5 Hz, 2 H, CH_2^{6}), 1.95 – 1.74 (m, 2 H, CH_2^{7}), 1.68 – 1.57 (m, 2 H, CH_2^{8}), 1.56 – 1.42 (m, 2 H, CH_2^{9}), 1.41 – 1.17 (m, 24 H, CH_2^{10}), 0.91 – 0.79 (m, 3 H, CH_3^{11}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 179.20, 171.52, 170.93, 169.77, 74.87, 59.69 (t, J = 26.8 Hz), 39.47, 34.08, 31.94, 31.92, 29.49, 29.47, 29.38, 29.35, 29.32, 29.27, 29.24, 29.10, 29.04, 28.79, 26.90, 24.97, 24.79, 22.76, 14.20.

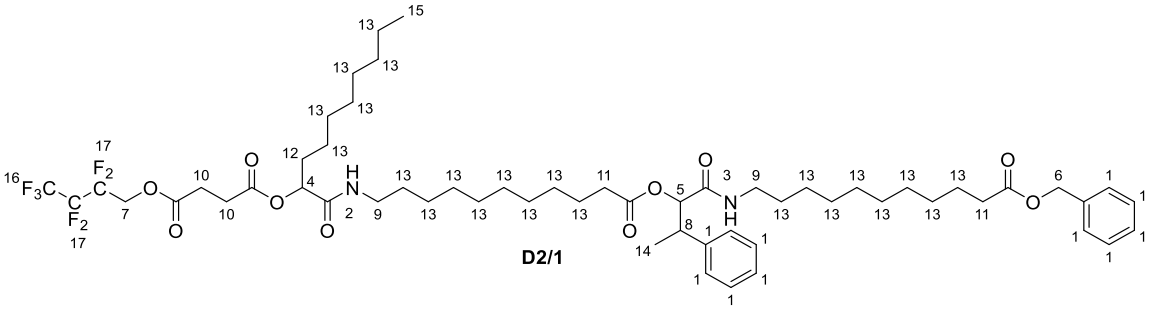
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -80.84 (t, J = 9.3 Hz, 3 F, CF_3^{12}), -120.14 – -121.45 (m, 2 F, CF_2^{13}), -127.34 – -128.12 (m, 2 F, CF_2^{13}). Total integral of CF_2 region normalized with respect to the CF_3^{12} group = 4.

ESI-MS [m/z]: $[M + H]^+$ calculated for $^{12}C_{29}{}^{1}H_{46}{}^{16}O_7{}^{14}N{}^{19}F_7$, 654.3235; found, 654.3219, Δ = 1.4 mmu.



Supplementary Figure 70: ^{1}H-NMR of compound **MD2/1** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 2.05 g of **MD2/1** (3.13 mmol, 1.00 eq.) were stirred in 3.00 mL dichloromethane. Subsequently, 629 µL 2-phenylpropionaldehyde **A12** (630 mg, 4.70 mmol, 1.50 eq.) and 1.42 g of monomer **IM2** (4.70 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (7:1 → 1:1) to yield the Passerini product **D2/1** as a pale highly viscous oil. (3.41 g, 3.13 mmol, 99.9%).

R_f = 0.30 in cyclohexane / ethyl acetate (1:1).

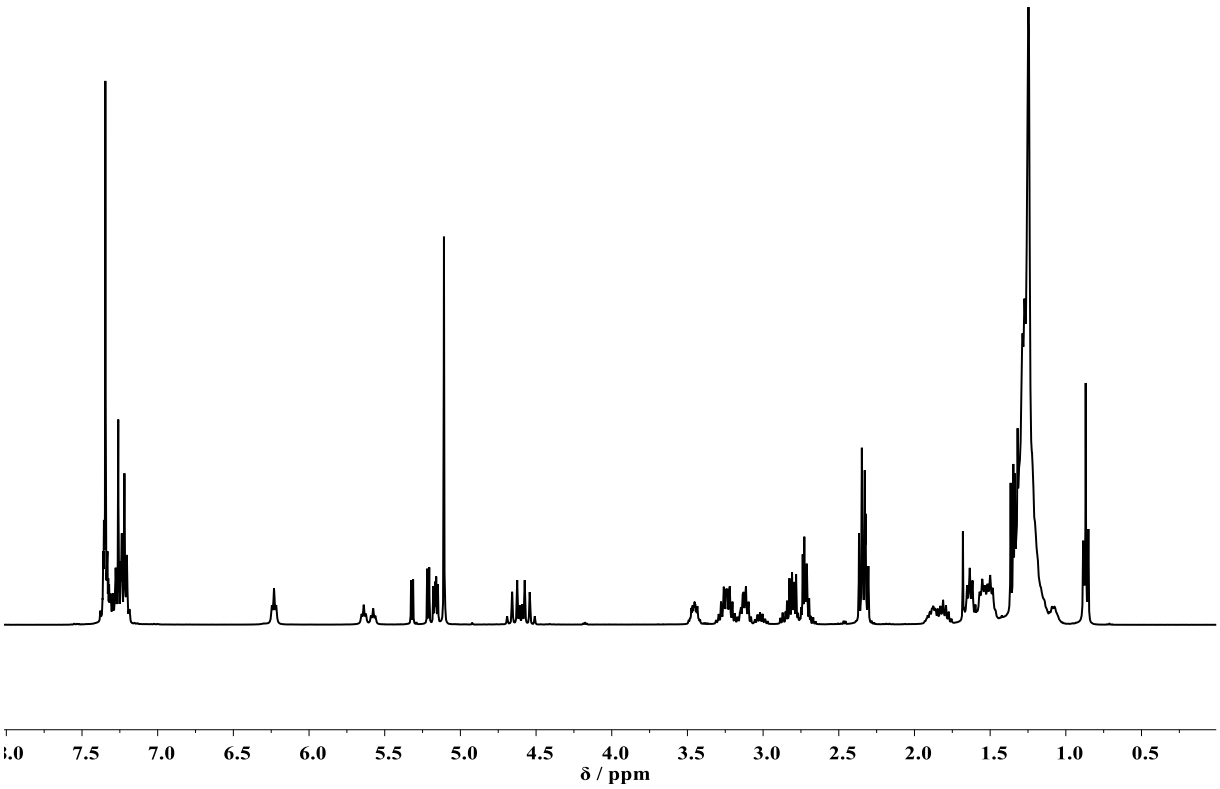
IR (ATR): ν / cm^{-1} = 3307.3 (vw), 2925.3 (s), 2854.5 (m), 1738.9 (s), 1655.6 (s), 1535.1 (w), 1496.9 (vw), 1454.8 (w), 1352.6 (w), 1226.4 (vw), 1144.0 (vs), 1020.0 (m), 909.5 (w), 735.3 (w), 698.2 (s), 534.5 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.43 – 7.15 (m, 10 H, CH_{Ar}^1), 6.23 (t, J = 5.8 Hz, 1 H, NH^2), 5.64 (t, J = 5.8 Hz, 0.5 H, NH^{3a}), 5.58 (t, J = 5.9 Hz, 0.5 H, NH^{3b}), 5.32 (d, J = 5.4 Hz, 0.5 H, CH^{4a}), 5.21 (d, J = 5.4 Hz, 0.5 H, CH^{4b}), 5.19 – 5.14 (m, 1 H, CH^5), 5.11 (s, 2 H, CH_2^6), 4.73 – 4.46 (m, 2 H, CH_2^7), 3.52 – 3.39 (m, 1 H, CH^8), 3.35 – 2.96 (m, 4 H, CH_2^9), 2.90 – 2.64 (m, 4 H, CH_2^{10}), 2.41 – 2.28 (m, 4 H, CH_2^{11}), 1.96 – 1.74 (m, 2 H, CH_2^{12}), 1.72 – 1.00 (m, 47 H, CH_2^{13}, CH_3^{14}), 0.91 – 0.83 (m, 3 H, CH_3^{15}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.80, 172.57, 172.42, 171.47, 170.88, 169.61, 168.82, 168.61, 141.73, 141.18, 136.26, 128.66, 128.52, 128.32, 128.28, 127.97, 127.13, 127.05, 77.85, 74.88, 66.19, 59.67 (t, J = 27.0 Hz), 41.58, 41.29, 39.43, 39.29, 39.20, 34.44, 34.35, 34.30, 31.94, 29.59, 29.55, 29.51, 29.52, 29.48, 29.47, 29.39, 29.35, 29.30, 29.23, 29.18, 29.16, 29.04, 28.78, 26.96, 26.86, 26.80, 25.06, 24.98, 24.91, 22.75, 17.62, 15.30, 14.20.

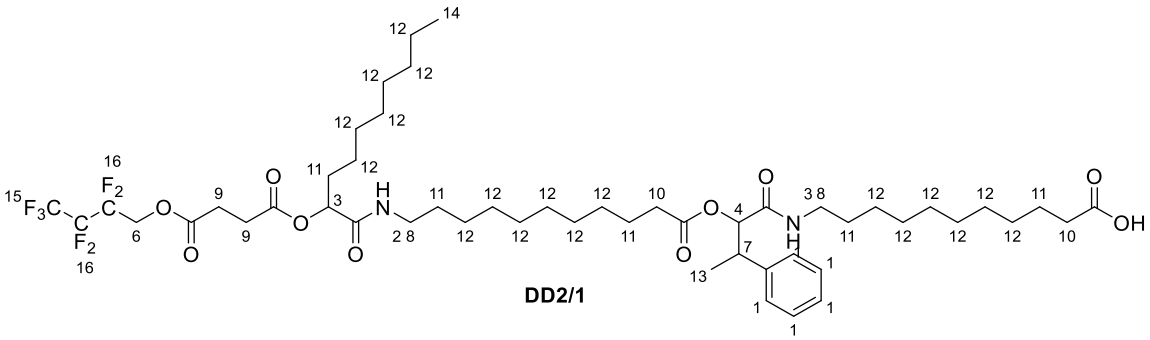
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -80.81 (t, J = 9.1 Hz, 3 F, CF_3), -120.24 – -120.74 (m, 2 F, CF_2), -127.46 – -127.96 (m, 2 F, CF_2). Total integral of CF_2 region normalized with respect to the CF_3[14] group = 4.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{57}{}^{1}H_{83}{}^{16}O_{10}{}^{14}N_2{}^{19}F_7$, 1089.6009; found, 1089.5993, Δ = 1.6 mmu.



Supplementary Figure 71: ^{1}H-NMR of compound **D2/1** measured in $CDCl_3$.

Deprotection



In a 25 mL round bottom flask, 3.26 g of **D2/1** (2.99 mmol, 1.00 eq.) was dissolved in 5.00 mL ethyl acetate and 5.00 mL THF. Subsequently, 652 mg (20 wt%) palladium on activated carbon **1** were added to the solution. The resulting mixture was purged with hydrogen gas and stirred for one day at room temperature under hydrogen atmosphere (balloon). The crude reaction mixture was filtered over celite® and flushed with 50 mL dichloromethane. After evaporation of the solvents and drying under reduced pressure the product **DD2/1** was obtained as a colorless solid (2.97 g, 2.97 mol, 99.3%).

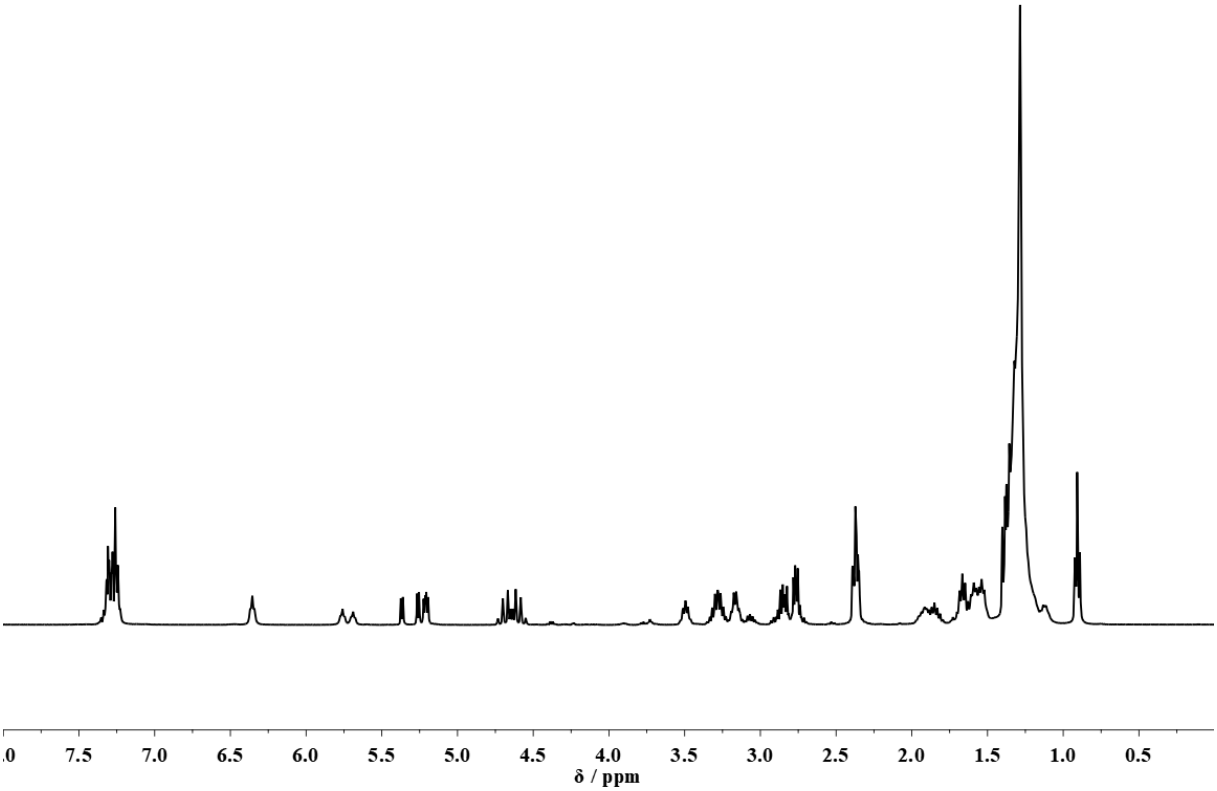
IR (ATR): ν / cm^{-1} = 3306.6 (vw), 2924.9 (d), 2854.3 (m), 2164.0 (vw), 2111.2 (vw), 2016.9 (vw), 1741.2 (s), 1651.4 (s), 1540.4 (m), 1495.6 (vw), 1454.8 (w), 1410.5 (w),1352.8 (w), 1226.4 (vs), 1179.0 (vs), 1144.2 (vs), 1020.6 (m), 979.1 (w), 909.8 (w), 759.9 (w), 735.8 (w), 700.2 (m), 630.7 (w), 536.7 (w), 411.1 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.40 – 7.20 (m, 5 H, $CH_{Ar}{}^{1}$), 6.35 (t, J = 5.8 Hz, 1 H, NH^{2}), 5.76 (t, J = 5.8 Hz, 0.5 H, NH^{3a}), 5.69 (t, J = 5.9 Hz, 0.5 H, NH^{3b}), 5.37 (d, J = 5.3 Hz, 0.5 H, CH^{4a}), 5.26 (d, J = 5.3 Hz, 0.5 H, CH^{4b}), 5.23 – 5.14 (m, 1 H, CH^{5}), 4.78 – 4.53 (m, 2 H, $CH_2{}^{6}$), 3.56 – 3.43 (m, 1 H, CH^{7}), 3.38 – 3.00 (m, 4 H, $CH_2{}^{8}$), 2.95 – 2.68 (m, 4 H, $CH_2{}^{9}$), 2.43 – 2.27 (m, 4 H, $CH_2{}^{10}$), 2.03 – 1.45 (m, 10 H, $CH_2{}^{11}$), 1.44 – 1.06 (m, 39 H, $CH_2{}^{12}$, $CH_3{}^{13}$), 0.91 (t, J = 6.7 Hz, 3 H, $CH_3{}^{14}$).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 178.45, 172.60, 172.45, 171.51, 170.92, 169.81, 168.97, 168.74, 141.66, 141.12, 128.50, 128.29, 127.95, 127.11, 127.05, 77.82, 74.82, 59.65 (t, J = 26.8 Hz), 41.54, 41.23, 39.47, 39.28, 39.19, 34.33, 34.27, 34.08, 31.91, 31.89, 29.57, 29.49, 29.46, 29.43, 29.37, 29.32, 29.25, 29.19, 29.16, 29.14, 29.11, 29.01, 28.76, 26.93, 26.79, 26.72, 24.95, 24.89, 24.84, 22.73, 17.59, 15.26, 14.18.

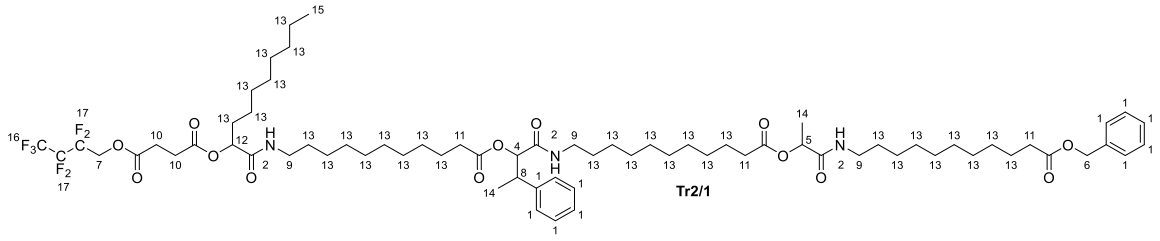
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.17 (t, J = 9.2 Hz, 3 F, $CF_3{}^{15}$), -124.68 – -125.12 (m, 2 F, $CF_2{}^{16}$), -131.78 – -132.20 (m, 2 F, $CF_2{}^{16}$). Total integral of CF_2 region normalized with respect to the $CF_3{}^{15}$ group = 4.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{50}{}^{1}H_{77}{}^{16}O_{10}{}^{14}N_2{}^{19}F_7$, 999.5539; found, 999.5526, Δ = 1.3 mmu.



Supplementary Figure 72: ^{1}H-NMR of compound **DD2/1** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 2.84 g of **DD2/1** (2.84 mmol, 1.00 eq.) were stirred in 3.00 mL dichloromethane. Subsequently, 482 µL acetaldehyde **A1** (376 mg, 8.53 mmol, 3.00 eq.) and 1.29 g of monomer **IM2** (4.27 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 1 day. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (4:1 → 1:1) to yield the Passerini product **Tr2/1** as a pale highly viscous oil. (3.50 g, 2.60 mmol, 91.6%).

R_f = 0.60 in cyclohexane / ethyl acetate (1:1).

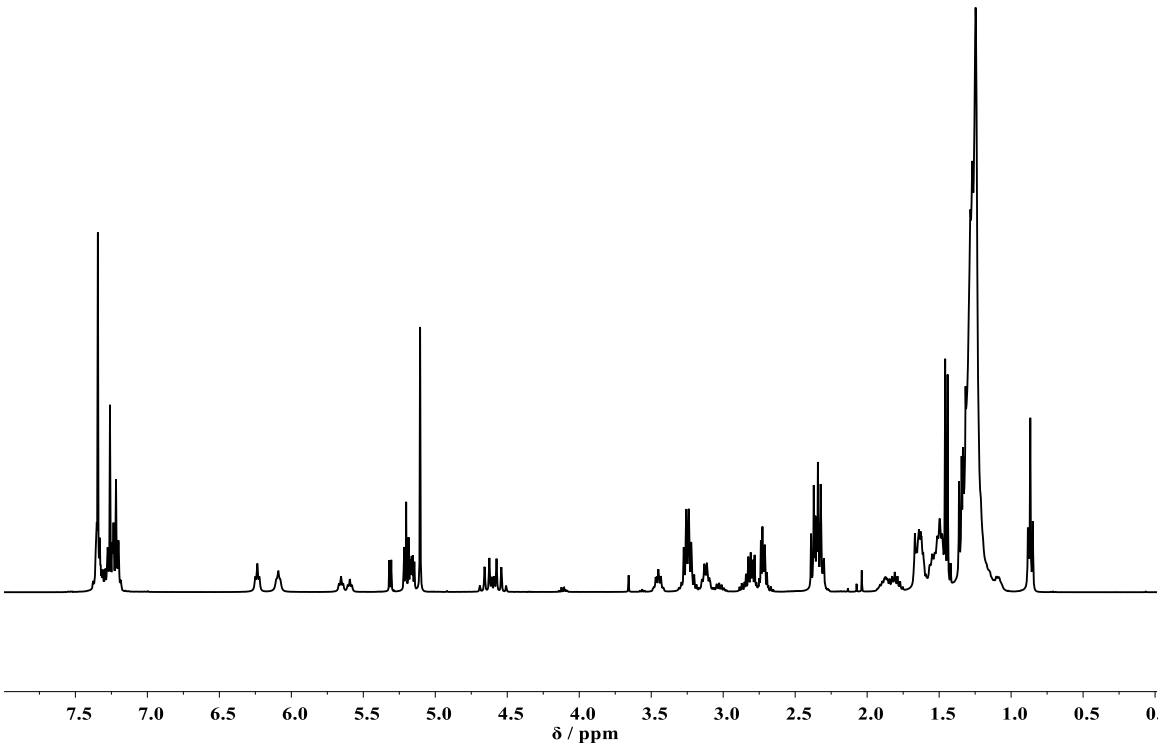
IR (ATR): ν / cm^{-1} = 3305.7 (vw), 3086.0 (vw), 2925.0 (s), 2853.9 (m), 2097.9 (vw), 1739.2 (s), 1655.6 (s), 1535.4 (m), 1497.2 (vw), 1454.8 (w), 1353.3 (w), 1226.9 (vs), 1145.6 (vs), 1020.8 (m), 909.8 (w), 736.0 (w), 699.1 (m), 538.7 (vw), 405.4 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.44 – 7.15 (m, 10 H, CH_{Ar}[1]), 6.24 (t, J = 5.8 Hz, 1 H, NH[2]), 6.09 (t, J = 5.8 Hz, 1 H, NH[2]), 5.65 (t, J = 5.9 Hz, 0.5 H, NH[3a]), 5.59 (t, J = 5.9 Hz, 0.5 H, NH[3b]), 5.31 (d, J = 5.4 Hz, 0.5 H, CH[4a]), 5.25 – 5.14 (m, 0.5 H, 2 H, CH[4b], CH[5]), 5.11 (s, 2 H, CH_2[6]), 4.72 – 4.49 (m, 2 H, CH_2[7]), 3.51 – 3.39 (m, 1 H, CH[8]), 3.34 – 2.97 (m, 6 H, CH_2[9]), 2.90 – 2.64 (m, 4 H, CH[10]), 2.43 – 2.26 (m, 6 H, CH_2[11]), 1.95 – 1.73 (m, 2 H, CH_2[12]), 1.71 – 1.05 (m, 66 H, CH_2[13], CH_3[14]), 0.90 – 0.82 (m, 3 H, CH_3[15]).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.80, 172.58, 172.44, 172.38, 171.48, 170.89, 170.42, 169.62, 168.85, 168.64, 141.74, 141.21, 136.27, 128.67, 128.53, 128.32, 128.29, 127.97, 127.13, 127.05, 77.86, 74.88, 70.59, 66.19, 59.68 (t, J = 26.9 Hz), 41.58, 41.29, 39.43, 39.36, 39.29, 39.20, 34.46, 34.45, 34.36, 34.30, 31.94, 29.67, 29.59, 29.56, 29.53, 29.49, 29.47, 29.41, 29.33, 29.31, 29.28, 29.23, 29.20, 29.19, 29.16, 29.04, 28.79, 26.95, 26.86, 26.80, 25.06, 25.01, 24.98, 24.92, 22.76, 18.09, 17.63, 15.31, 14.21.

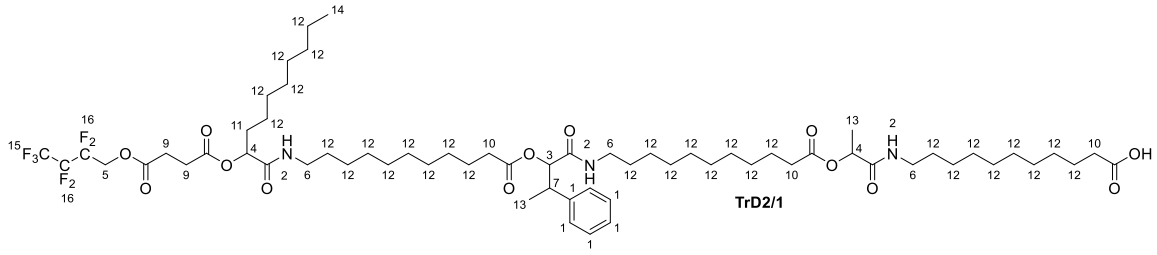
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -80.81 (t, J = 9.3 Hz, 3 F, CF_3[16]), -120.19 – -120.65 (m, 2 F, CF_2[17]), -127.59 – -127.72 (m, 2 F, CF_2[17]). Total integral of CF_2 region normalized with respect to the CF_3[17] group = 4.

ESI-MS [m/z]: $[M + H]^+$ calculated for $^{12}C_{71}{}^{1}H_{108}{}^{16}O_{13}{}^{14}N_3{}^{19}F_7$, 1344.7843; found, 1344.7813, Δ = 3.0 mmu.



Supplementary Figure 73: ^{1}H-NMR of compound **Tr2/1** measured in $CDCl_3$.

Deprotection



In a 25 mL round bottom flask, 3.32 g of **Tr2/1** (2.47 mmol, 1.00 eq.) was dissolved in 6.00 mL ethyl acetate and 6.00 mL THF. Subsequently, 764 mg (20 wt%) palladium on activated carbon **1** were added to the solution. The resulting mixture was purged with hydrogen gas and stirred for one day at room temperature under hydrogen atmosphere (3 balloons). The crude reaction mixture was filtered over celite® and flushed with 50 mL dichloromethane. After evaporation of the solvents and drying under reduced pressure the product **TrD2/1** was obtained as a colorless solid (3.08 g, 2.46 mol, 99.5%).

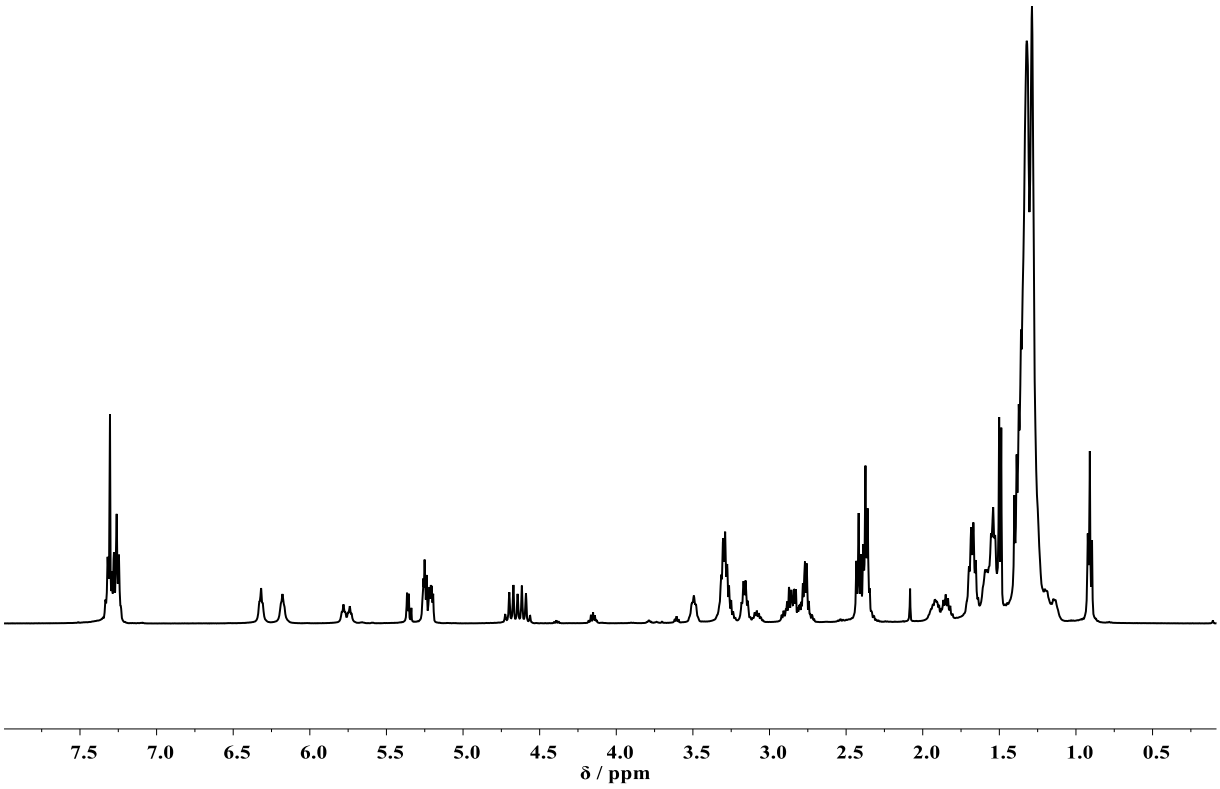
IR (ATR): ν / cm^{-1} = 3305.5 (vw), 2924.5 (s), 2854.0 (m), 1740.6 (s), 1654.0 (s), 1539.0 (m), 1454.6 (w), 1371.5 (w), 1226.7 (vs), 1145.2 (vs), 1020.8 (m), 909.9 (w), 759.8 (w), 735.7 (w), 700.3 (m), 536.7 (vw).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.25 – 7.10 (m, 5 H, CH_{Ar}^{1}), 6.21 (t, J = 5.9 Hz, 1 H, NH^{2}), 6.07 (t, J = 6.0 Hz, 1 H, NH^{2}), 5.76 – 5.60 (m, 0.5 H, CH^{3a}), 5.30 – 5.21 (m, 2.5 H, CH^{3b}, CH^{4}), 5.18 – 5.06 (m, 2 H, CH_2^{5}), 4.68 – 4.43 (m, 6 H, CH_2^{6}), 3.42 – 3.35 (m, 1 H, CH^{7}), 3.29 – 2.93 (m, 6 H, CH_2^{8}), 2.81 – 2.60 (m, 4 H, CH_2^{9}), 2.39 – 2.22 (m, 6 H, CH_2^{10}), 1.90 – 1.68 (m, 2 H, CH_2^{11}), 1.62 – 0.98 (m, 12H, CH_2^{12}, CH_3^{13}), 0.80 (t, J = 6.8 Hz, 3 H, CH_3^{14}).

^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 177.35, 172.71, 172.58, 172.42, 171.52, 170.93, 170.56, 169.76, 169.04, 168.82, 141.66, 141.17, 128.54, 128.31, 127.97, 127.14, 127.06, 77.88, 74.85, 70.59, 59.68 (t, J = 26.9 Hz), 41.55, 41.27, 39.47, 39.34, 39.27, 34.48, 34.35, 34.29, 33.96, 31.94, 31.92, 29.58, 29.53, 29.49, 29.43, 29.35, 29.31, 29.28, 29.22, 29.17, 29.14, 29.09, 29.03, 28.79, 26.95, 26.86, 26.85, 26.80, 25.04, 24.98, 24.96, 24.90, 24.87, 22.76, 18.06, 17.61, 15.33, 14.21.

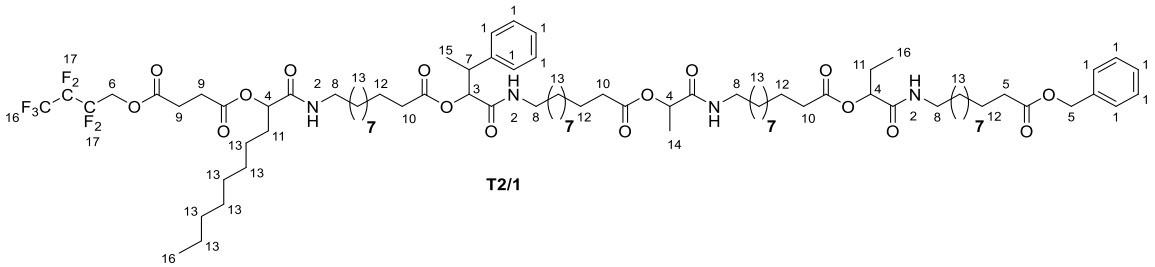
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -80.81 (t, J = 9.3 Hz, 3 F, CF_3^{15}), -119.20 – -121.40 (m, 2 F, CF_2^{16}), -126.52 – -130.07 (m, 2 F, CF_2^{16}). Total integral of CF_2 region normalized with respect to the CF_3^{15} group = 4.

ESI-MS [m/z]: $[M + H]^+$ calculated for $^{12}C_{64}{}^{1}H_{102}{}^{16}O_{13}{}^{14}N_3{}^{19}F_7$, 1254.7374; found, 1254.7351, Δ = 2.3 mmu.



Supplementary Figure 74: ^{1}H-NMR of compound **TrD2/1** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 428 g of **TrD2/1** (341 µmol, 1.00 eq.) were stirred in 2.00 mL DCM. Subsequently, 36.7 µL propionaldehyde **A2** (29.7 mg, 512 µmol, 3.00 eq.) and 154 mg of monomer **IM2** (512 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 1 day. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetatecyclohexane and ethyl acetate (3:1 → 1:1) to yield the Passerini product **T2/1** as a pale highly viscous oil. (372 mg, 230 µmol, 67.6%).

R_f = 0.52 in cyclohexane / ethyl acetate (1:1).

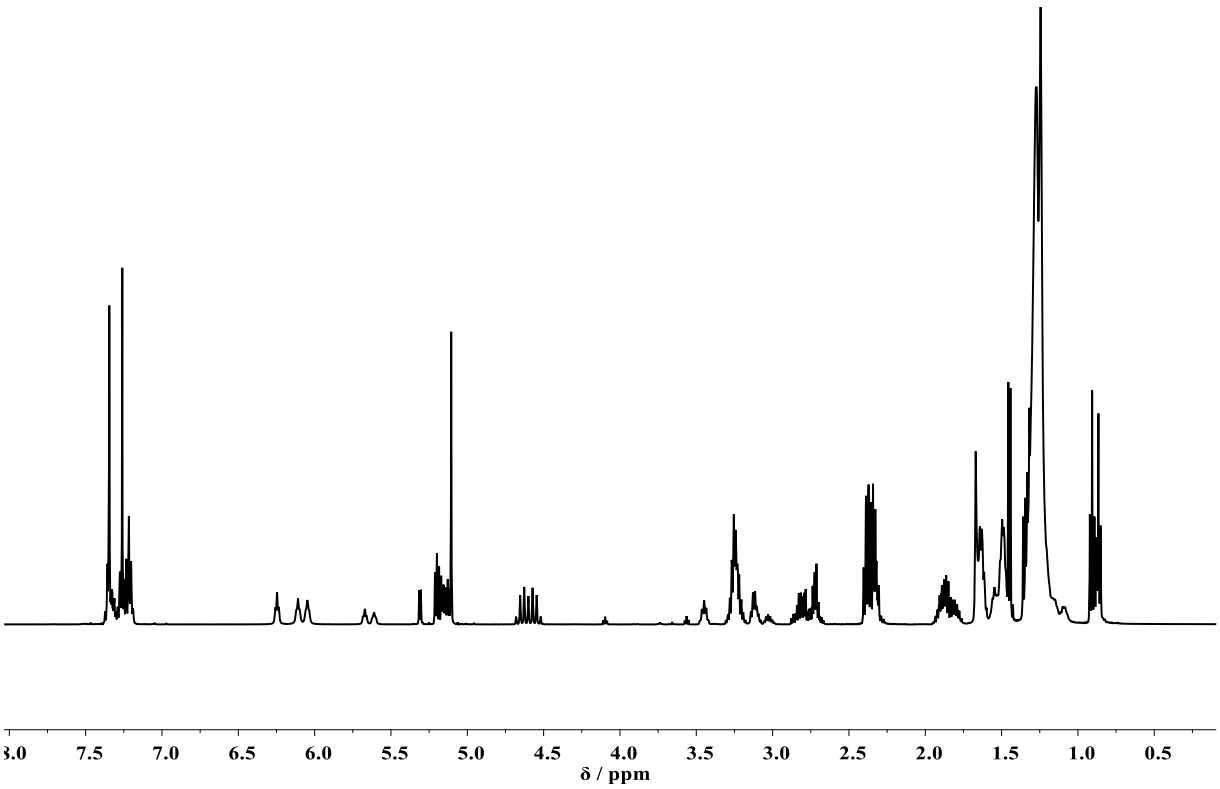
IR (ATR): ν / cm^{-1} = 3304.8 (w), 2924.6 (s), 2853.8 (m), 1738.8 (s), 1655.0 (s), 1535.6 (m), 1455.2 (w), 1373.2 (w), 1227.0 (s), 1146.1 (vs), 1020.8 (m), 735.6 (w), 699.1 (m).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.41 – 7.16 (m, 10 H, CH_{Ar}^1), 6.25 (t, J = 5.8 Hz, 1 H, NH^2), 6.11 (t, J = 5.9 Hz, 1 H, NH^2), 6.05 (t, J = 6.0 Hz, 1 H, NH^2), 5.73 – 5.56 (m, 1 H, NH^2), 5.31 (d, J = 5.4 Hz, 0.5 H, CH^{3a}), 5.24 – 5.07 (m, 5.5 H, CH^{3a}, CH^4, CH_2^5), 4.69 – 4.51 (m, 2 H, CH_2^6), 3.49 – 3.38 (m, 1 H, CH^7), 3.32 – 2.97 (m, 8 H, CH_2^8), 2.89 – 2.66 (m, 4 H, CH_2^9), 2.43 – 2.25 (m, 8 H, CH_2^{10}), 1.95 – 1.75 (m, 4 H, CH_2^{11}), 1.71 – 1.40 (m, 19 H, $CH_2^{12,13}$, CH_3^{14}), 1.38 – 1.06 (m, 28 H, CH_2^{13}, CH_3^{15}), 0.95 – 0.84 (m, 6 H, CH_3^{16}).

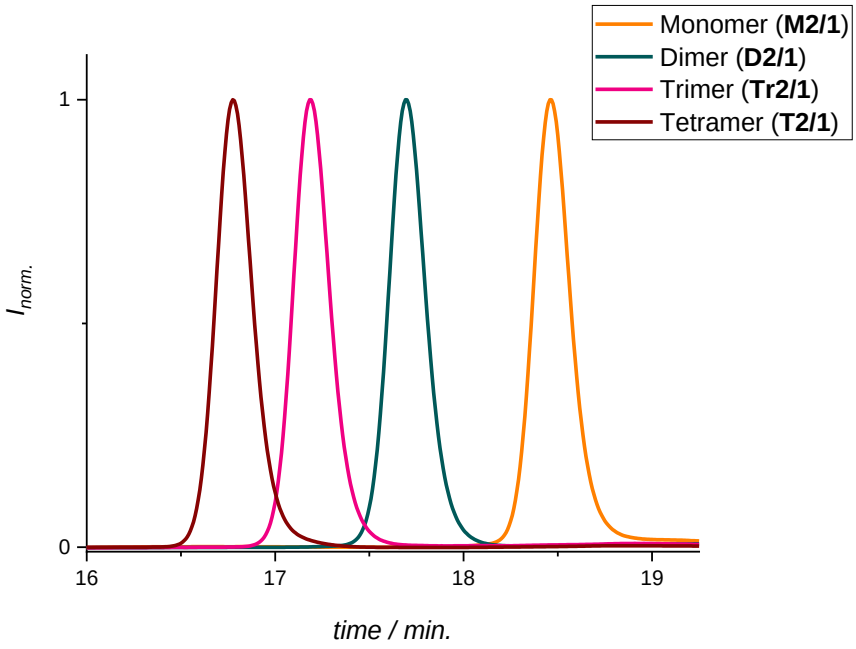
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 173.83, 172.61, 172.57, 172.46, 172.41, 171.50, 170.91, 170.46, 169.75, 169.64, 168.87, 168.66, 141.72, 141.19, 136.25, 128.67, 128.53, 128.32, 128.30, 128.29, 127.97, 127.13, 127.05, 77.85, 74.93, 74.87, 70.58, 66.20, 59.67 (t, J = 26.5 Hz), 41.57, 41.28, 39.43, 39.35, 39.32, 39.29, 39.20, 34.45, 34.43, 34.35, 34.30, 31.94, 29.70, 29.68, 29.59, 29.56, 29.49, 29.47, 29.41, 29.35, 29.33, 29.28, 29.23, 29.18, 29.16, 29.04, 28.78, 26.95, 26.86, 26.80, 25.22, 25.08, 25.06, 25.00, 24.98, 24.91, 22.76, 17.62, 15.30, 14.22, 9.15.

^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -80.81 (t, J = 9.1 Hz, 3 F, CF_3[16]), -119.77 – -123.82 (m, 2 F, CF_2[17]), -124.53 – -130.56 (m, 2 F, CF_2[17]). Total integral of CF_2 region normalized with respect to the CF_3[17] group = 4.

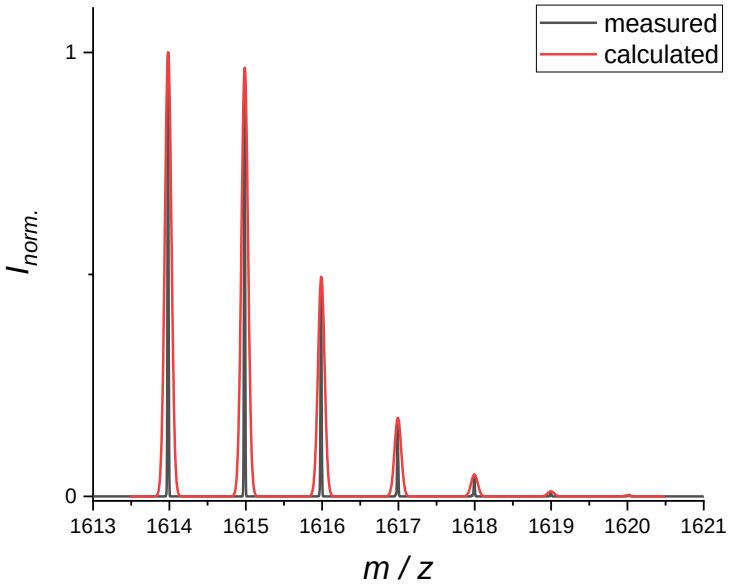
ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{86}{}^{1}H_{135}{}^{16}O_{16}{}^{14}N_4{}^{19}F_7$, 1613.9834; found, 1613.9821, Δ = 1.3 mmu.



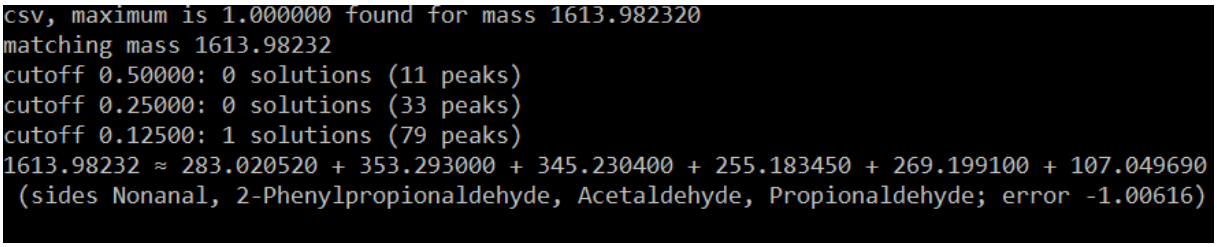
Supplementary Figure 75: ^{1}H-NMR of compound **T2/1** measured in $CDCl_3$.



Supplementary Figure 76: SEC traces of the intermediates after each P-3CR in the synthesis of product **T2/1**.



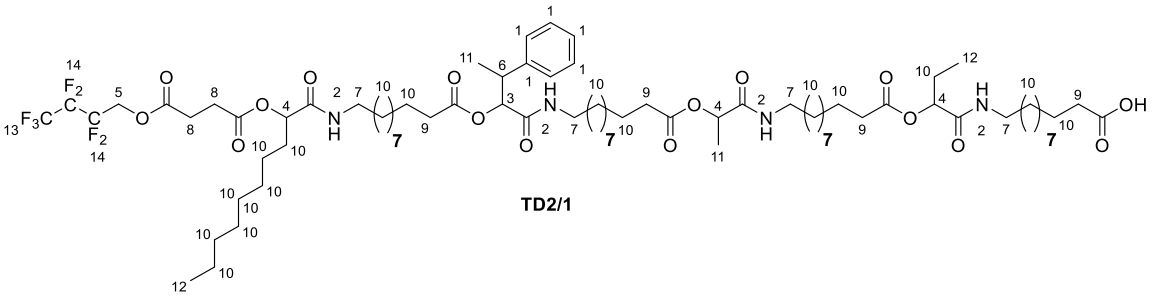
Supplementary Figure 77: High resolution ESI-MS measurement of **T2/1**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (red).



Supplementary Figure 78: Screenshot of the automated read-out of **T2/1**.

6.3.3.4.2 Synthesis of hexamer H2/1

Deprotection



In a 25 mL round bottom flask, 323 mg of **T2/1**(200 µmol, 1.00 eq.) was dissolved in 3.00 mL ethyl acetate and 3.00 mL THF. Subsequently, 64.5 mg (20 wt%) palladium on activated carbon **1** were added to the solution. The resulting mixture was purged with hydrogen gas and stirred for one day at room temperature under hydrogen atmosphere (3 balloons). The crude reaction mixture was filtered over celite® and flushed with 50 mL dichloromethane. After evaporation of the solvents and drying under reduced pressure the product **TD2/1** was obtained as a colorless solid (296 mg, 1940 µmol, 97.1%).

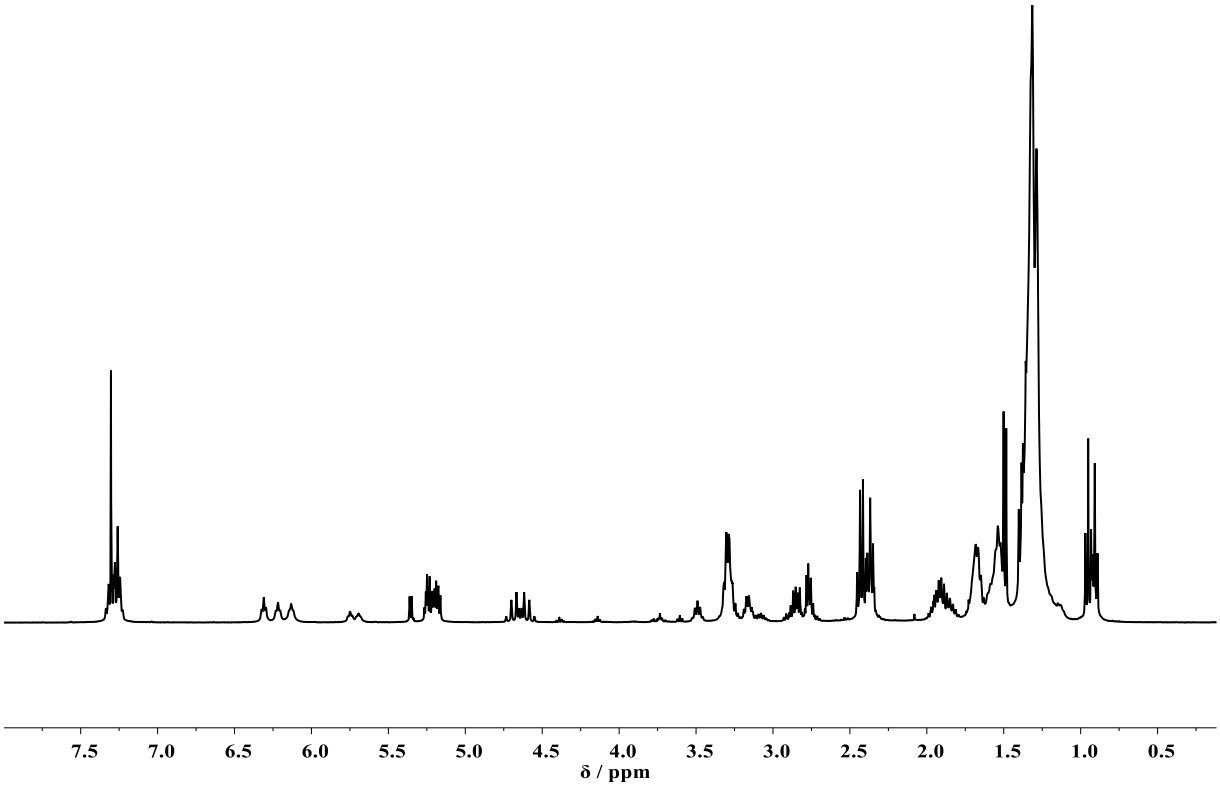
IR (ATR): ν / cm^{-1} = 3300.1 (vw), 2926.1 (w), 2854.6 (vw), 1741.9 (w), 1655.1 (w), 1541.6 (vw), 1457.2 (vw), 1228.3 (w), 1147.3 (vw), 700.5 (vw), 426.6 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.38 – 7.18 (m, 5 H, CH_{Ar}^{1}), 6.31 (t, J = 5.9 Hz, 1 H, NH^{2}), 6.22 (t, J = 5.9 Hz, 1 H, NH^{2}), 6.13 (t, J = 6.0 Hz, 1 H, NH^{2}), 5.81 – 5.61 (m, 1 H, NH^{2}), 5.36 (d, J = 5.5 Hz, 0.5 H, CH^{3a}), 5.29 – 5.12 (m, 3.5 H, CH^{3b}, CH^{4}), 4.79 – 4.51 (m, 2 H, CH_2^{5}), 3.61 – 3.43 (m, 1 H, CH^{6}), 3.38 – 2.99 (m, 8 H, CH_2^{7}), 2.95 – 2.67 (m, 4 H, CH_2^{8}), 2.48 – 2.26 (m, 8 H, CH_2^{9}), 2.02 – 1.07 (m, 86 H, CH_2^{10}, CH_3^{11}), 1.04 – 0.82 (m, 6 H, CH_3^{12}).

^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 176.95, 172.65, 172.61, 172.51, 172.48, 171.52, 170.93, 170.66, 169.86, 169.74, 168.99, 168.77, 141.69, 141.18, 128.54, 128.31, 127.97, 127.14, 127.07, 77.85, 74.95, 74.86, 70.56, 59.67 (t, J = 26.9 Hz), 41.57, 41.27, 39.46, 39.41, 39.30, 39.23, 34.44, 34.35, 34.29, 33.94, 31.93, 29.64, 29.57, 29.53, 29.49, 29.41, 29.35, 29.33, 29.26, 29.23, 29.20, 29.08, 29.03, 28.78, 26.95, 26.94, 26.86, 26.79, 25.18, 25.11, 24.98, 24.90, 22.76, 18.07, 17.62, 15.30, 14.21, 9.15.

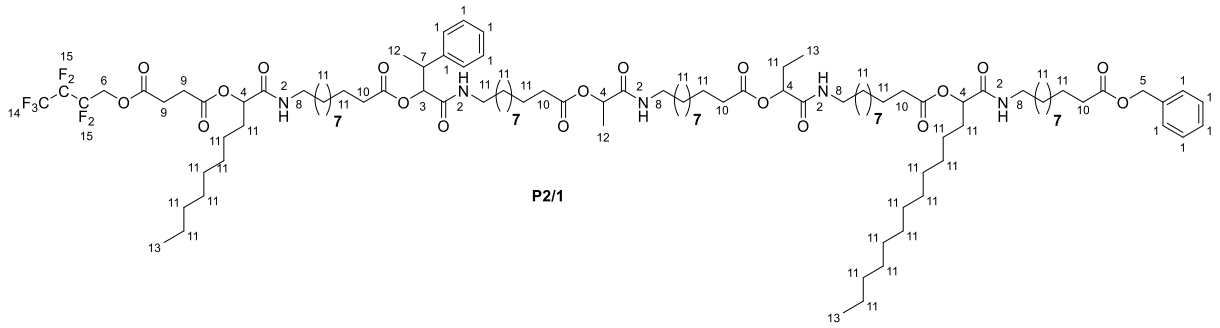
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -80.81 (t, J = 9.2 Hz, 3 F, CF_3^{13}), -117.22 – -122.54 (m, 2 F, CF_2^{14}), -125.88 – -129.43 (m, 2 F, CF_2^{14}). Total integral of CF_2 region normalized with respect to the CF_3^{13} group = 4.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{79}{}^{1}H_{129}{}^{16}O_{16}{}^{14}N_4{}^{19}F_7$, 1523.9365; found, 1523.9361, Δ = 0.4 mmu.



Supplementary Figure 79: ^{1}H-NMR of compound **TD2/1** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 260 mg of **TD2/1** (171 µmol, 1.00 eq.) were stirred in 2.00 mL DCM. Subsequently, 40.5 µL tridecanal **A11** (33.8 mg, 171 µmol, 1.00 eq.) and 51.4 mg of monomer **IM2** (171 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 1 day. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel

eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (3:1 → 1:1) to yield the Passerini product **P2/1** as a pale highly viscous oil. (183 mg, 90.6 µmol, 53.1%).

R_f = 0.33 in cyclohexane / ethyl acetate (1:1).

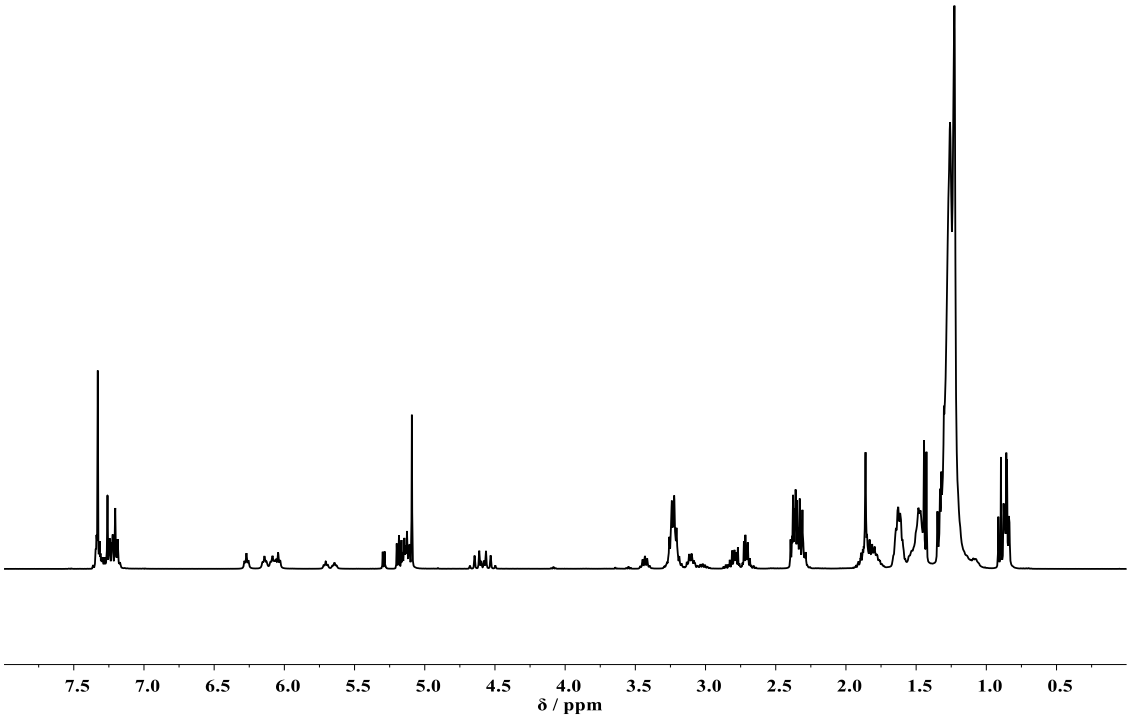
IR (ATR): ν / cm^{-1} = 2925.3 (m), 2854.3 (m), 1741.7 (w), 1649.3 (m), 1587.1 (m), 1534.1 (s), 1454.9 (m), 1337.8 (w), 1225.8 (s), 1145.7 (m), 981.3 (w), 782.1 (w), 725.8 (w), 699.6 m), 635.1 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.39 – 7.14 (m, 10 H, CH_{Ar}^1), 6.27 (t, *J* = 5.8 Hz, 1 H, NH^2), 6.19 – 5.99 (m, 3 H, NH^2), 5.76 – 5.60 (m, 1 H, NH^2), 5.29 (d, *J* = 5.5 Hz, 0.5 H, CH^{3a}), 5.24 – 5.04 (m, 6.5 H, CH^{3a}, CH^4, CH_2^5), 4.71 – 4.47 (m, 2 H, CH_2^6), 3.52 – 3.37 (m, 1 H, CH^7), 3.33 – 2.98 (m, 10 H, CH_2^8), 2.90 – 2.63 (m, 4 H, CH_2^9), 2.42 – 2.26 (m, 10 H, CH_2^{10}), 1.99 – 1.72 (m, 8 H, CH_2^{11}), 1.68 – 1.01 (m, 116 H, CH_2^{11}, CH_3^{12}), 0.96 – 0.80 (m, 9 H, CH_3^{13}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 172.38, 171.45, 170.87, 170.45, 169.97, 169.75, 169.62, 168.85, 168.64, 141.69, 141.18, 136.21, 128.62, 128.48, 128.26, 128.25, 128.23, 127.92, 127.08, 127.01, 77.81, 74.89, 74.82, 74.03, 70.52, 66.15, 59.62 (t, *J* = 26.7 Hz), 41.55, 41.26, 39.40, 39.31, 39.28, 39.17, 34.31, 34.25, 32.00, 31.90, 29.75, 29.72, 29.67, 29.63, 29.52, 29.43, 29.35, 29.31, 29.23, 29.19, 29.19, 29.11, 29.00, 28.74, 26.91, 26.90, 26.82, 26.76, 25.18, 25.04, 24.95, 24.86, 22.77, 22.71, 18.05, 17.59, 15.27, 14.20, 14.16, 9.12.

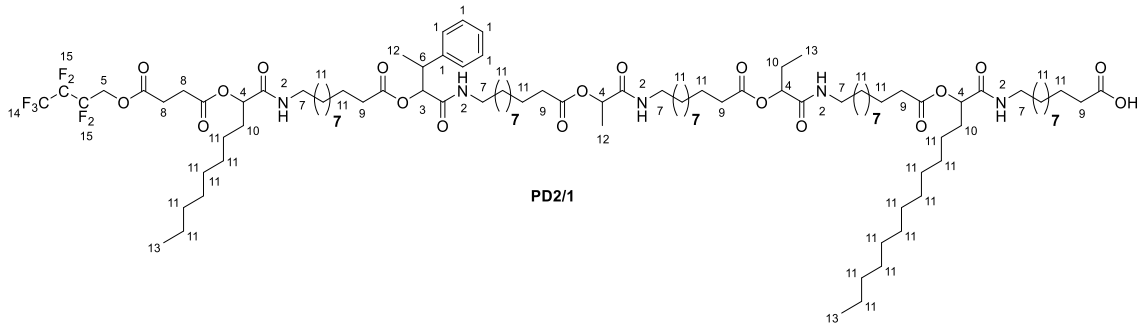
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -80.82 (t, *J* = 9.1 Hz, 3 F, CF_3^{14}), -119.33 – -121.63 (m, 2 F, CF_2^{15}), -127.21 – -128.37 (m, 2 F, CF_2^{15}). Total integral of CF_2 region normalized with respect to the CF_3^{16} group = 4.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{111}{}^1H_{182}{}^{16}O_{19}{}^{14}N_5{}^{19}F_7$, 2023.3390; found, 2023.3392, Δ = 0.2 mmu.



Supplementary Figure 80: ^{1}H-NMR of compound **P2/1** measured in $CDCl_3$.

Deprotection



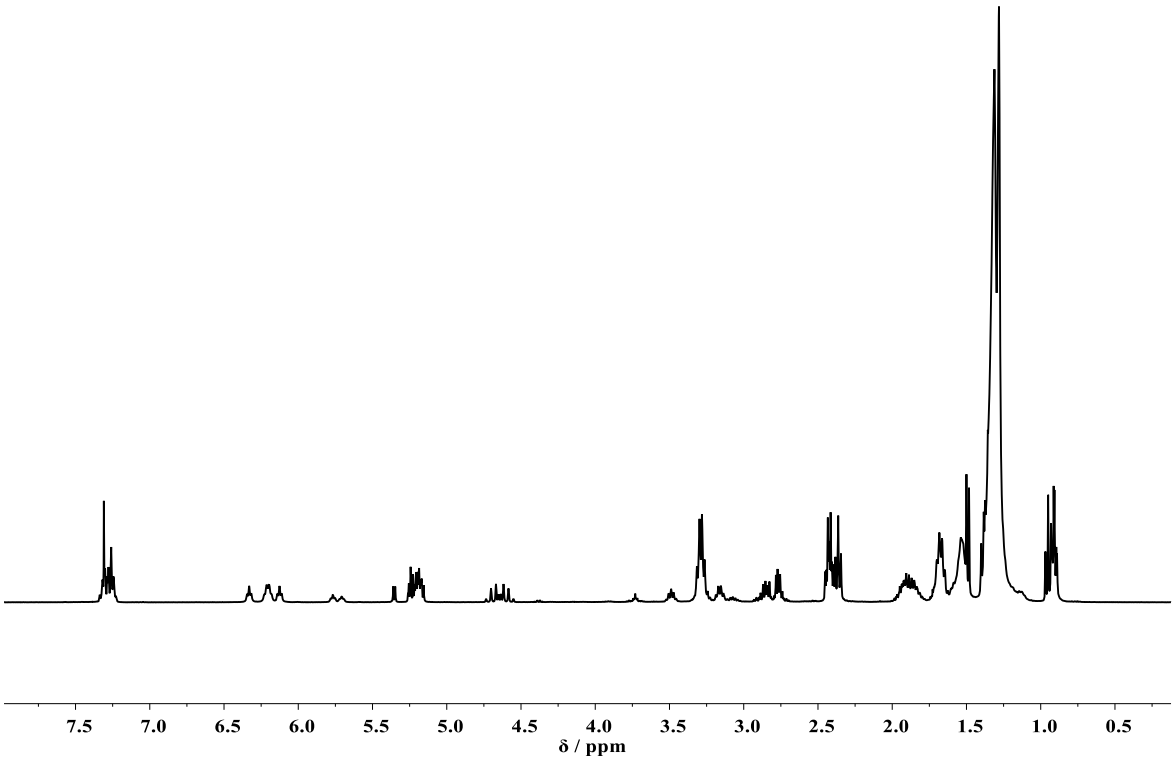
In a 25 mL round bottom flask, 77.1 mg of **P2/1** (38.0 µmol, 1.00 eq.) was dissolved in 2.00 mL ethyl acetate and 2.00 mL THF. Subsequently, 15.4 mg (20 wt%) palladium on activated carbon **1** were added to the solution. The resulting mixture was purged with hydrogen gas and stirred for one day at room temperature under hydrogen atmosphere (3 balloons). The crude reaction mixture was filtered over celite® and flushed with 50 mL dichloromethane. After evaporation of the solvents and drying under reduced pressure the product **PD2/1** was obtained as a colorless solid (67.2 mg, 34.7 µmol, 91.2%).

IR (ATR): ν / cm^{-1} = 3291.0 (vw), 2923.9 (s), 2853.7 (m), 1741.6 (m), 1650.2 (s), 1535.7 (s), 1456.1 (m), 1373.8 (w), 1226.7 (s), 1145.4 (s), 1021.5 (w), 980.5 (w), 781.8 (vw), 723.6 (w), 699.8 (m), 634.8 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.41 – 7.15 (m, 5 H, CH_{Ar}^{1}), 6.33 (t, J = 5.8 Hz, 1 H, NH^{2}), 6.26 – 6.16 (m, 2 H, NH^{2}), 6.13 (t, J = 5.9 Hz, 1 H, NH^{2}), 5.74 (dt, J = 24.0, 6.1 Hz, 1 H, NH^{2}), 5.35 (d, J = 5.5 Hz, 0.5 H, CH^{3a}), 5.28 – 5.14 (m, 4.5 H, CH^{3b}, CH^{4}), 4.75 – 4.52 (m, 2 H, CH_2^{5}), 3.56 – 3.43 (m, 1 H, CH^{6}), 3.38 – 3.01 (m, 10 H, CH_2^{7}), 2.95 – 2.68 (m, 4 H, CH_2^{8}), 2.49 – 2.30 (m, 10 H, CH_2^{9}), 2.00 – 1.77 (m, 6 H, CH_2^{10}), 1.75 – 1.05 (m, 118 H, CH_2^{11}, CH_3^{12}), 1.00 – 0.81 (m, 9 H, CH_3^{13}).

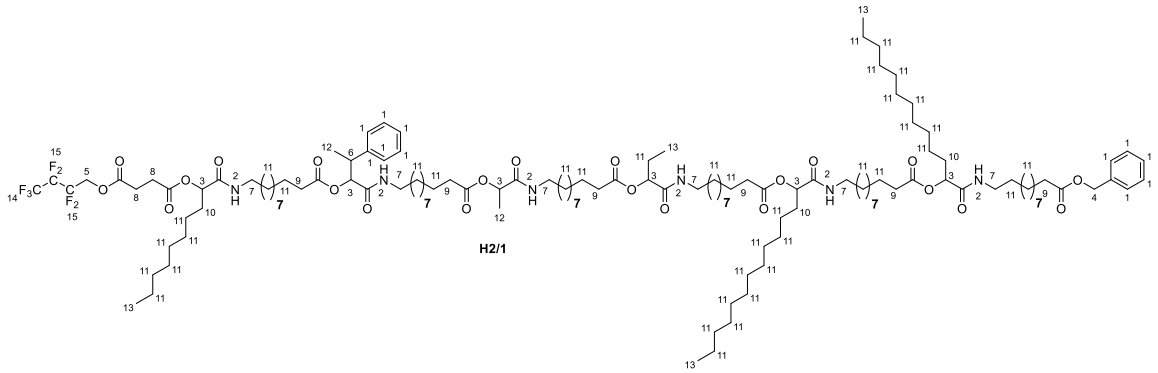
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 176.94, 172.66, 172.64, 172.49, 172.43, 171.50, 170.91, 170.59, 170.09, 169.95, 169.72, 168.96, 168.74, 141.68, 141.17, 128.52, 128.29, 127.95, 127.12, 127.04, 77.84, 77.36, 74.91, 74.84, 74.07, 70.54, 59.65 (t, J = 26.6 Hz), 41.56, 39.44, 39.37, 39.29, 39.21, 34.43, 34.40, 34.33, 34.28, 33.97, 32.03, 32.00, 31.92, 29.78, 29.76, 29.74, 29.66, 29.63, 29.58, 29.55, 29.51, 29.46, 29.45, 29.34, 29.31, 29.30, 29.26, 29.23, 29.19, 29.16, 29.14, 29.10, 29.02, 28.77, 26.94, 26.91, 26.88, 26.84, 26.78, 25.19, 25.09, 25.05, 24.98, 24.97, 24.89, 22.80, 22.74, 18.07, 17.60, 15.29, 14.24, 14.20, 9.14.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{104}{}^{1}H_{176}{}^{16}O_{19}{}^{14}N_5{}^{19}F_7$, 193.2921; found, 193.2935, Δ = 1.4 mmu.



Supplementary Figure 81: ^{1}H-NMR of compound **PD2/1** measured in $CDCl_3$.

Passerini reaction



In a 10 mL round bottom flask, 56.2 mg of **PD2/1** (29.1 µmol, 1.00 eq.) were stirred in 1.00 mL DCM. Subsequently, 8.04 mg dodecanal **A10** (43.6 µmol, 1.00 eq.) and 13.1 mg of monomer **IM2** (43.6 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (3:1 → 1:1) to yield the Passerini product **H2/1** as a pale highly viscous oil. (28.7 mg, 11.8 µmol, 40.5%).

R_f = 0.46 in cyclohexane / ethyl acetate (1:1)

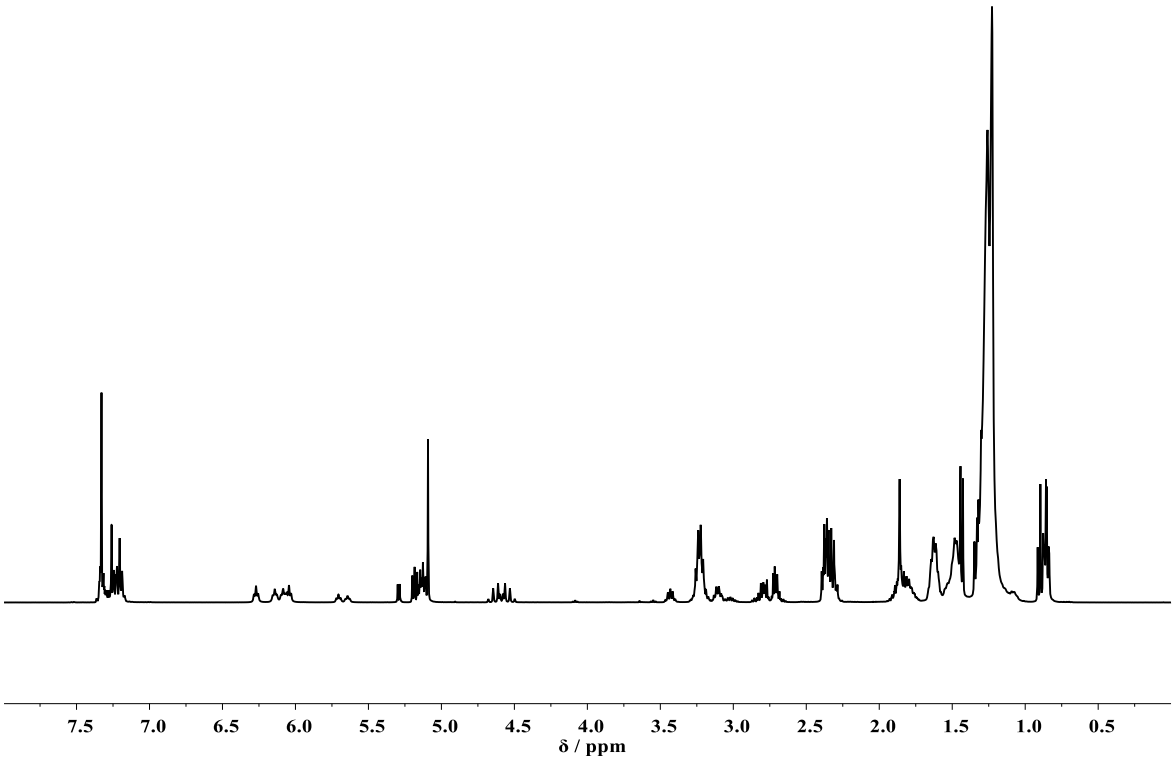
IR (ATR): ν / cm^{-1} = 3138.8 (w), 2962.1 (w), 1648.2 (w), 1587.1 (m), 1535.6 (m), 1456.1 (w), 1338.2 (w), 1278.7 (w), 1224.0 (w), 1146.3 (w), 1063.5 (w), 981.8 (vw), 791.7 (vw), 726.0 (vw), 700.9 (w), 635.7 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.33 – 7.09 (m, 10 H, CH_{Ar}[1]), 6.18 (t, J = 5.7 Hz, 1 H, NH[2]), 6.09 – 5.90 (m, 4 H, NH[2]), 5.66 – 5.50 (m, 1 H, NH[2]), 5.31 – 5.00 (m, 8 H, CH[3], CH_2[4]), 4.66 – 4.43 (m, 2 H, CH_2[5]), 3.47 – 3.32 (m, 1 H, CH[6]), 3.27 – 2.91 (m, 12 H, CH_2[7]), 2.88 – 2.59 (m, 4 H, CH_2[8]), 2.38 – 2.23 (m, 12 H, CH_2[9]), 1.91 – 1.67 (m, 8 H, CH_2[10]), 1.66 – 0.93 (m, 152 H, CH_2[11], CH_3[12]), 0.89 – 0.71 (m, 12 H, CH_3[13]).

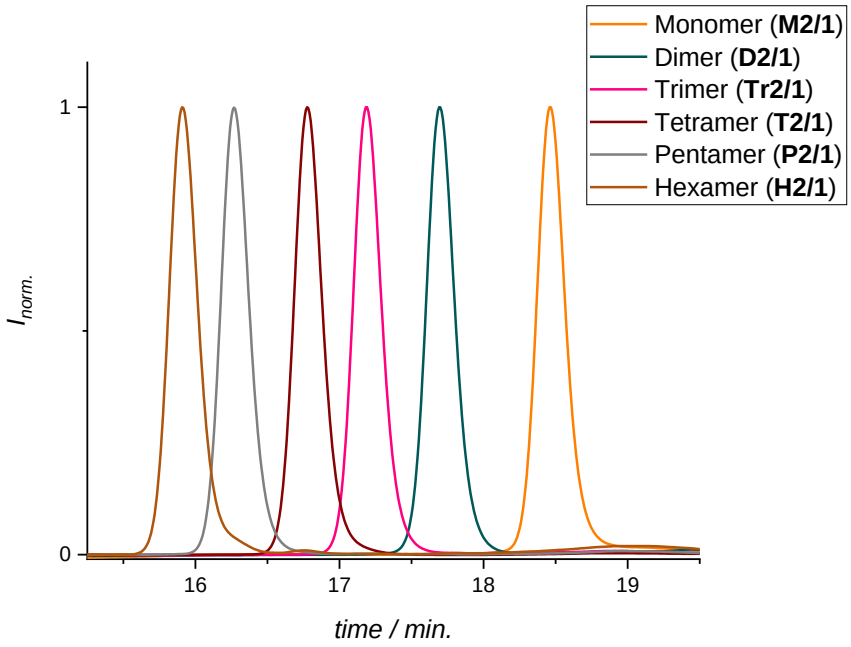
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.83, 172.62, 172.43, 171.51, 170.91, 170.49, 170.02, 169.79, 169.65, 168.88, 136.27, 128.69, 128.55, 128.33, 128.31, 127.98, 77.88, 77.36, 74.95, 74.90, 74.09, 70.60, 66.22, 41.60, 39.46, 39.37, 39.34, 39.22, 34.47, 32.06, 31.96, 29.81, 29.79, 29.77, 29.72, 29.70, 29.61, 29.59, 29.50, 29.41, 29.37, 29.26, 29.06, 28.81, 27.06, 26.98, 26.88, 26.82, 25.24, 25.10, 25.02, 25.01, 24.93, 22.83, 22.78, 18.11, 15.33, 14.27, 14.23, 9.17.

^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -83.96 – -86.17 (m, 3 F, CF_3[14]), -124.42 – -125.22 (m, 2 F, CF_2[15]), -131.64 – -132.55 (m, 2 F, CF_2[15]). Total integral of CF_2 region normalized with respect to the CF_3[16] group = 4.

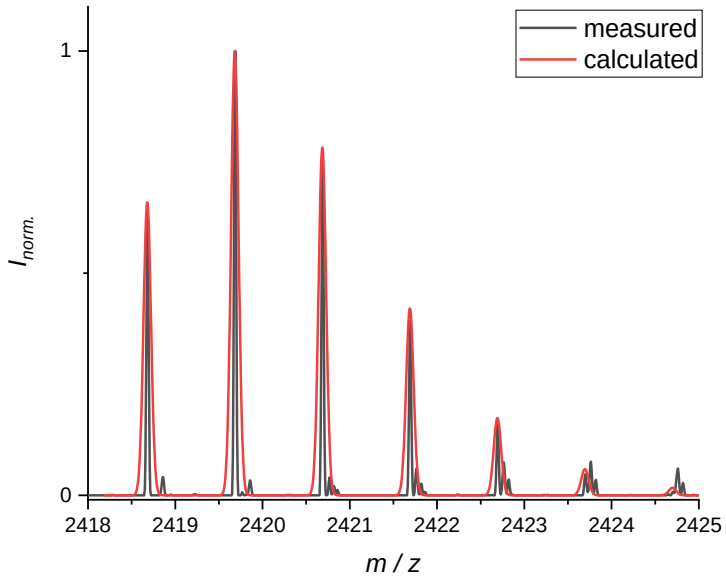
ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{135}{}^{1}H_{227}{}^{16}O_{22}{}^{14}N_6{}^{19}F_7$, 2418.6789; found, 2418.6818, Δ = 2.9 mmu.



Supplementary Figure 82: ^{1}H-NMR of compound **H2/1** measured in $CDCl_3$.



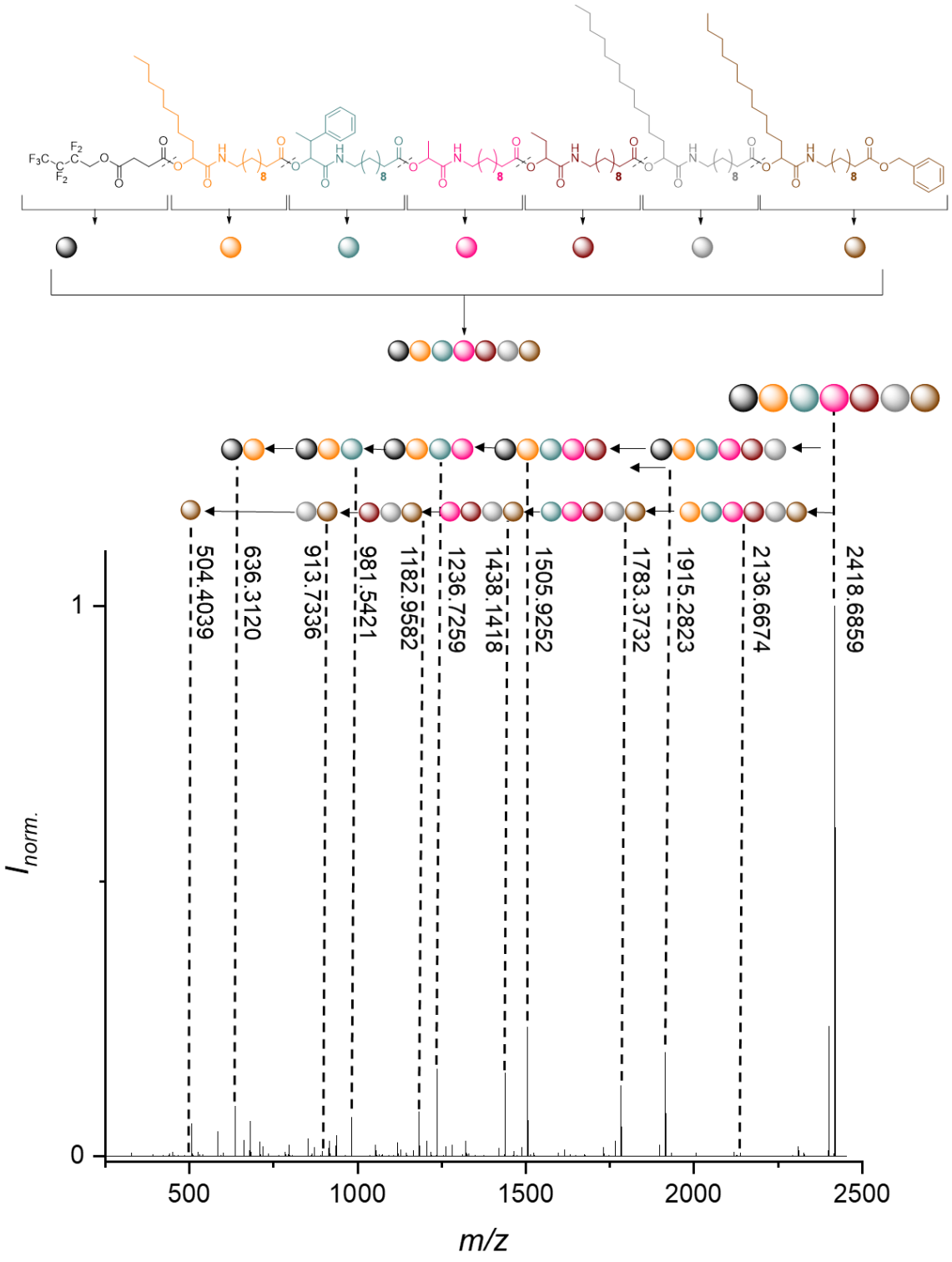
Supplementary Figure 83: SEC traces of the intermediates after each P-3CR in the synthesis of product **H2/1**.



Supplementary Figure 84: High resolution ESI-MS measurement of **H2/1**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (red).

```
matching mass 2418.68173
cutoff 0.50000: 0 solutions (7 peaks)
cutoff 0.25000: 0 solutions (12 peaks)
cutoff 0.12500: 0 solutions (34 peaks)
cutoff 0.06250: 0 solutions (68 peaks)
cutoff 0.03125: 0 solutions (117 peaks)
cutoff 0.01562: 0 solutions (217 peaks)
cutoff 0.00781: 0 solutions (328 peaks)
cutoff 0.00391: 1 solutions (513 peaks)
2418.68173 ≈ 283.020520 + 353.293000 + 345.230400 + 255.183450 + 269.199100 + 409.355600 + 395.339950 + 107.049690 (sides
 Nonanal, 2-Phenylpropionaldehyde, Acetaldehyde, Propionaldehyde, Tridecanal, Dodecanal; error -1.01002)
Press ENTER to quit ...
```

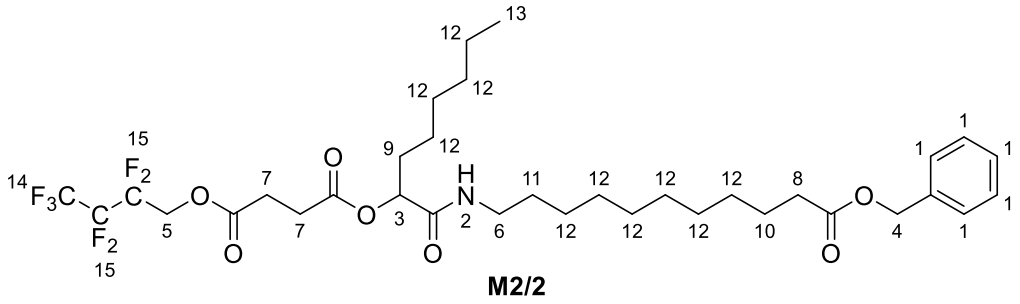
Supplementary Figure 85: Screenshot of the automated read-out of **H2/1**.



Supplementary Figure 86: Read-out of the sequence-defined hexamer **H2/1**. Read-out of the hexamer **H2/1** *via* tandem ESI-MS/MS with an NCE of 17. In the spectrum, the read-out from both ends of the oligomer using the fragmentation next to the carbonyl are shown.

6.3.3.4.3 Synthesis of tetramer T2/2

Passerini reaction



In a 50 mL round bottom flask 500 mg, **TAG2** (1.67 mmol, 1.00 eq.) were stirred in 5.00 mL DCM. Subsequently, 372 µL heptanal **A7** (321 mg, 2.50 mmol, 1.50 eq.) and 754 mg of monomer **IM2** (2.50 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (6:1 → 4:1) to yield the Passerini product **M2/2** as a pale highly viscous oil. (1.04 g, 1.46 mmol, 87.4%).

R_f = 0.38 in cyclohexane / ethyl acetate (3:1).

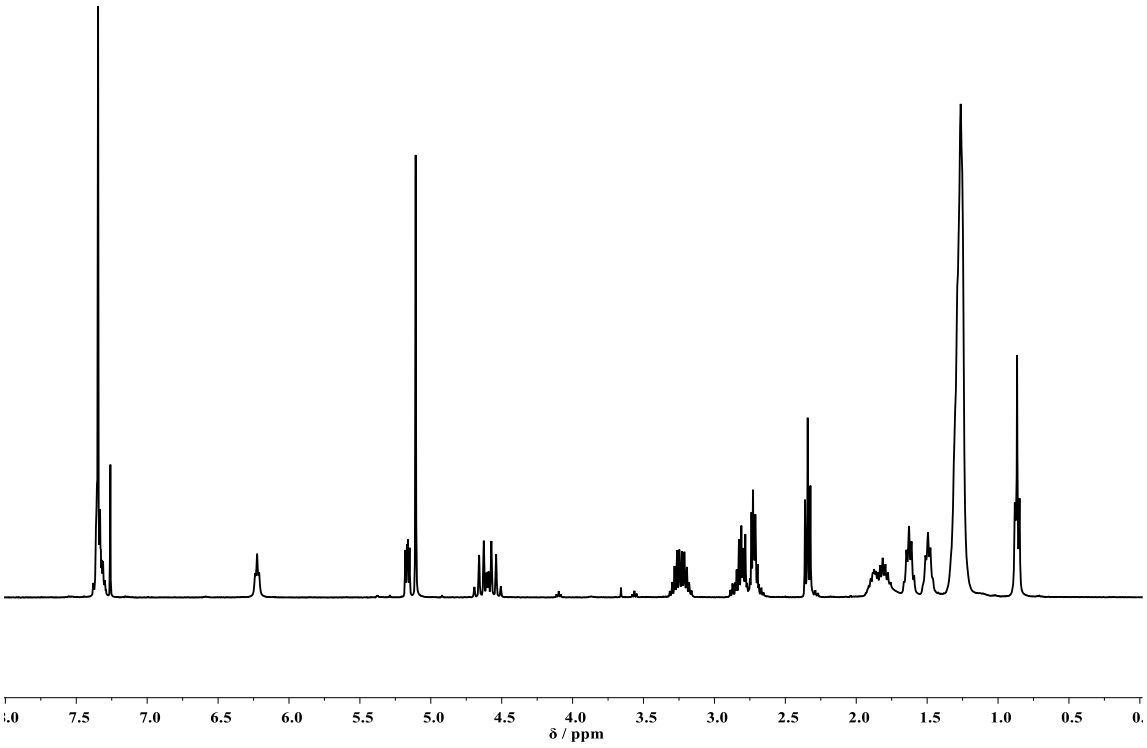
IR (ATR): ν / cm^{-1} = 3306.1 (vw), 2927.1 (m), 2856.0 (w), 1738.6 (s), 1657.5 (m), 1536.5 (w), 1456.0 (w), 1352.2 (w), 1226.3 (vs), 1144.2 (vs), 1019.9 (m), 909.7 (w), 735.9 (m), 697.2 (m).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.41 – 7.29 (m, 5 H, $CH_{Ar}{}^1$), 6.22 (t, J = 5.8 Hz, 1 H, NH^2), 5.19 – 5.14 (m, 1 H, CH^3), 5.11 (s, 2 H, $CH_2{}^4$), 4.71 – 4.48 (m, 2 H, $CH_2{}^5$), 3.32 – 3.11 (m, 2 H, $CH_2{}^6$), 2.94 – 2.64 (m, 4 H, $CH_2{}^7$), 2.34 (t, J = 7.6 Hz, 2 H, $CH_2{}^8$), 1.95 – 1.76 (m, 2 H, $CH_2{}^9$), 1.67 – 1.57 (m, 2 H, $CH_2{}^{10}$), 1.54 – 1.43 (m, 2 H, $CH_2{}^{11}$), 1.39 – 1.13 (m, 20 H, $CH_2{}^{12}$), 0.87 (t, J = 6.6 Hz, 3 H, $CH_3{}^{13}$).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 174.58, 172.23, 171.64, 170.37, 137.03, 129.42, 129.04, 75.64, 66.95, 60.45, 40.21, 35.21, 32.70, 32.49, 30.34, 30.30, 30.24, 30.13, 30.09, 29.99, 29.81, 29.77, 29.55, 27.72, 25.83, 25.69, 23.42, 14.90.

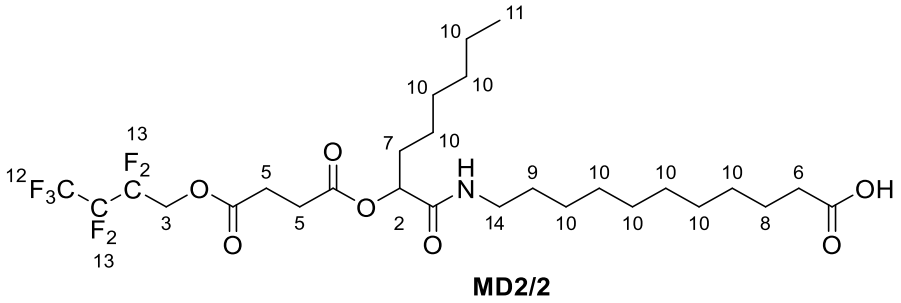
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -84.54 – -86.48 (m, 3 F, $CF_3{}^{14}$), -124.50 – -126.45 (m, 2 F, $CF_2{}^{15}$), -131.22 – -133.88 (m, 2 F, $CF_2{}^{15}$). Total integral of CF_2 region normalized with respect to the $CF_3{}^{14}$ group = 4.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{34}{}^{1}H_{48}{}^{16}O_7{}^{14}N{}^{19}F_7$, 716.3392; found, 716.3371, Δ = 2.1 mmu.



Supplementary Figure 87: ^{1}H-NMR of compound **M2/2** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 995 mg of **M2/2** (1.39 mmol, 1.00 eq.) were dissolved in 3.00 mL ethyl acetate and 3.00 mL THF. Afterwards, 199 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **MD2/2** was obtained as a pale highly viscous oil in a yield of 96.4% (835 mg, 1.34 mmol).

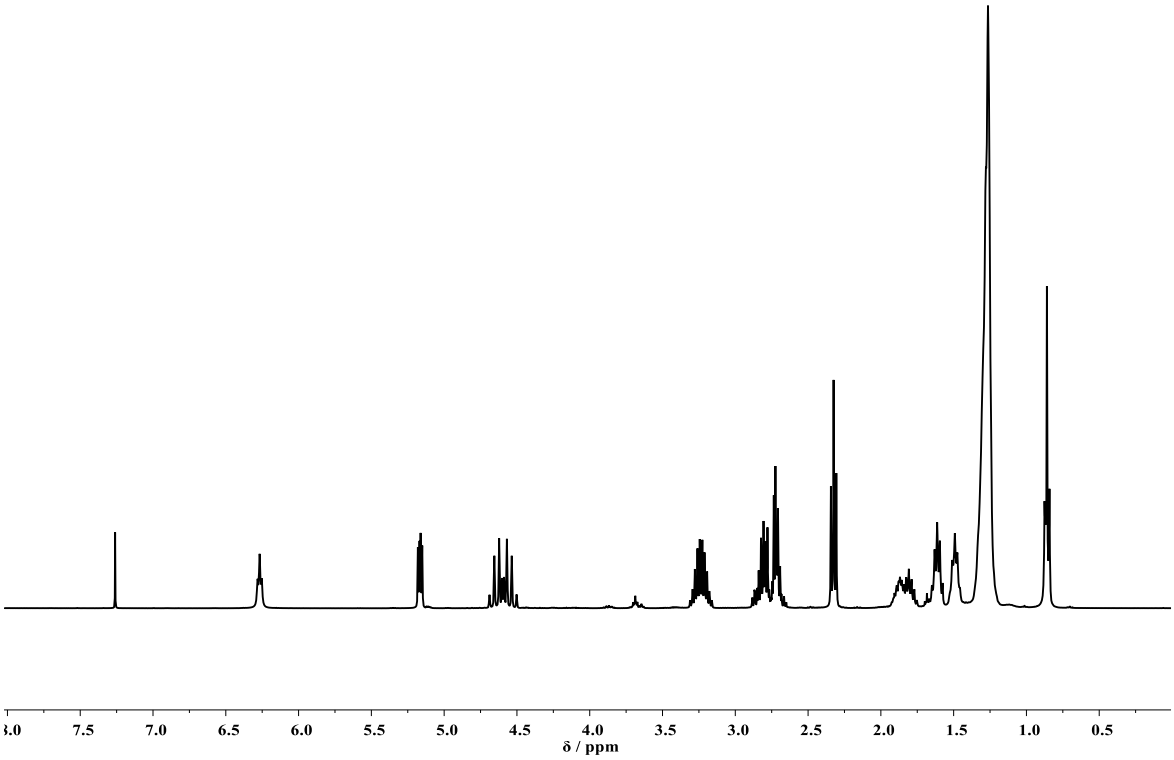
IR (ATR): ν / cm^{-1} = 2926.9 (m), 2856.3 (m), 1743.1 (s), 1652.0 (m), 1542.6 (w), 1352.4 (w), 1226.0 (vs), 1179.7 (vs), 1144.2 (vs), 1020.0 (m), 909.4 (w), 735.9 (w), 628.5 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.27 (t, J = 5.8 Hz, 1 H, NH^{1}), 5.22 – 5.10 (m, 1 H, CH^{2}), 4.71 – 4.49 (m, 2 H, CH^{3}), 3.31 – 3.13 (m, 2 H, CH_2^{4}), 2.93 – 2.63 (m, 4 H, CH_2^{5}), 2.32 (t, J = 7.5 Hz, 2 H, CH_2^{6}), 2.00 – 1.74 (m, 2 H, CH_2^{7}), 1.69 – 1.56 (m, 2 H, CH_2^{8}), 1.54 – 1.43 (m, 2 H, CH_2^{9}), 1.39 – 1.16 (m, 20 H, CH_2^{10}), 0.98 – 0.80 (m, 3 H, CH_3^{11}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 178.91, 171.52, 170.93, 169.78, 74.88, 59.70 (t, J = 26.8 Hz), 39.48, 34.12, 31.92, 31.73, 29.47, 29.37, 29.26, 29.22, 29.10, 29.05, 29.01, 28.80, 26.90, 24.92, 24.84, 22.66, 14.14.179.16, 171.69, 169.77, 75.53, 44.09, 43.62, 39.41, 34.18, 31.22, 29.57, 29.46, 29.36, 29.23, 29.10, 27.46, 26.92, 24.83, 22.35, 22.00, 11.71, 11.68.

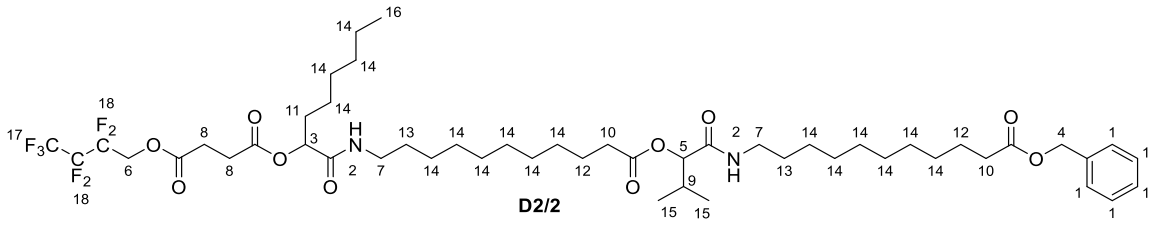
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -80.85 (t, J = 9.2 Hz, 3 F, CF_3^{12}), -120.39 – -120.91 (m, 2 F, CF_2^{13}), -127.07 – -129.04 (m, 2 F, CF_2^{13}). Total integral of CF_2 region normalized with respect to the CF_3^{12} group = 4.

ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{27}{}^{1}H_{42}{}^{16}O_7{}^{14}N{}^{19}F_7$, 648.2742; found, 648.2729, Δ = 1.3 mmu.



Supplementary Figure 88: ^{1}H-NMR of compound **MD2/2** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 794 mg of **MD2/2** (1.27 mmol, 1.00 eq.) were stirred in 4.00 mL DCM. Subsequently, 174 µL isobutyraldehyde **A3** (137 mg, 1.90 mmol, 1.50 eq.) and 577 mg of monomer **IM2** (1.90 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (5:1 → 2:1) to yield the Passerini product **D2/2** as a pale highly viscous oil. (1.12 g, 1.12 mol, 88.2%).

R_f = 0.21 in cyclohexane / ethyl acetate (2:1).

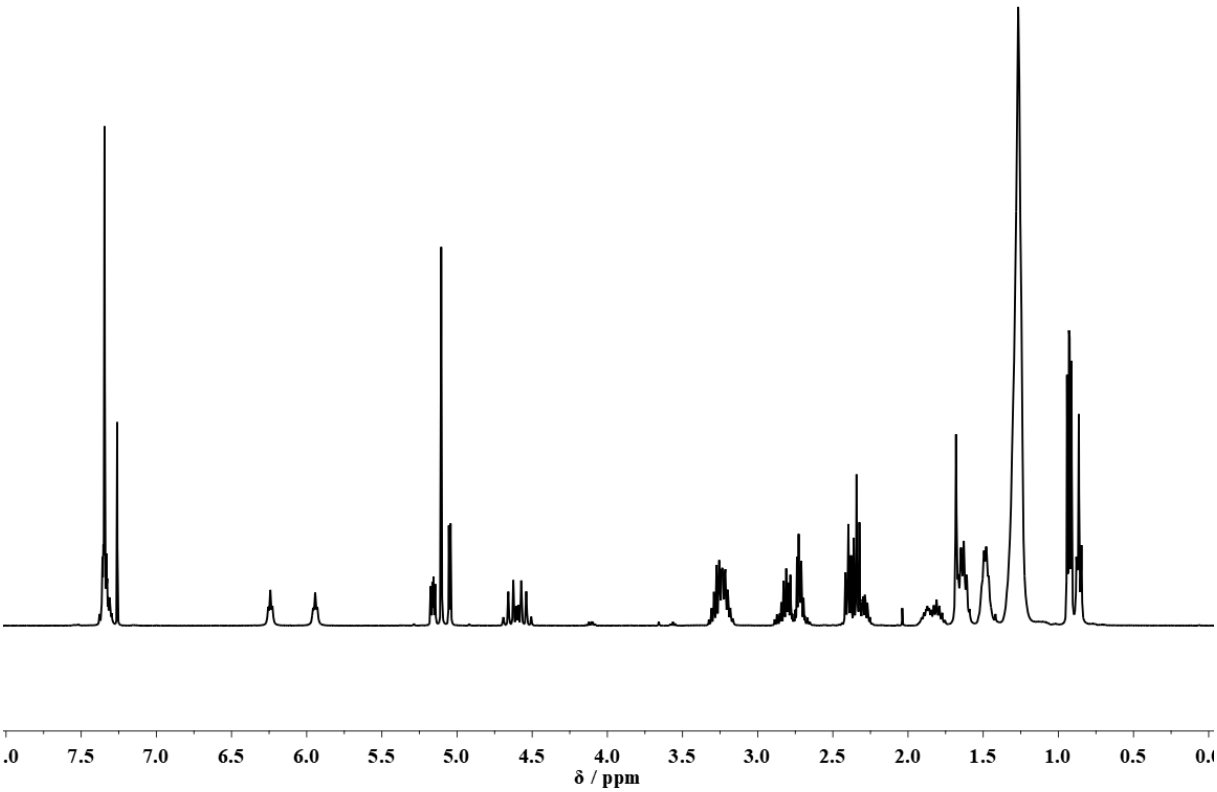
IR (ATR): ν / cm^{-1} = 3308.0 (vw), 2926.1 (m), 2854.9 (w), 1738.8 (s), 1655.3 (s), 1534.8 (m), 1457.1 (w), 1352.5 (w), 1226.6 (vs), 1144.7 (vs), 1019.3 (m), 909.9 (w), 735.7 (w), 697.3 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.36 – 7.21 (m, 5 H, $CH_{Ar}{}^1$), 6.18 (t, J = 5.8 Hz, 1 H, NH^2), 5.88 (t, J = 5.9 Hz, 1 H, NH^2), 5.13 – 5.07 (m, 1 H, CH^3), 5.04 (s, 2 H, $CH_2{}^4$), 4.99 (d, J = 4.4 Hz, 1 H, CH^5), 4.66 – 4.43 (m, 2 H, $CH_2{}^6$), 3.30 – 3.09 (m, 4 H, CH^7), 2.83 – 2.59 (m, 4 H, $CH_2{}^8$), 2.39 – 2.19 (m, 5 H, CH^9, $CH_2{}^{10}$), 1.90 – 1.69 (m, 2 H, $CH_2{}^{11}$), 1.67 – 1.58 (m, 4 H, $CH_2{}^{12}$), 1.49 – 1.35 (m, 4 H, $CH_2{}^{13}$), 1.29 – 1.10 (m, 32 H, $CH_2{}^{14}$), 0.92 – 0.84 (m, 6 H, $CH_3{}^{15}$), 0.83 – 0.77 (m, 3 H, $CH_3{}^{16}$).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.82, 172.68, 171.49, 170.89, 169.63, 169.40, 136.26, 128.67, 128.28, 78.05, 74.88, 66.20, 59.68 (t, J = 26.8 Hz), 39.43, 39.29, 34.45, 34.42, 31.94, 31.72, 30.65, 29.71, 29.58, 29.55, 29.54, 29.49, 29.46, 29.33, 29.25, 29.23, 29.04, 29.01, 28.79, 26.96, 26.95, 25.13, 25.07, 24.93, 22.65, 18.90, 17.07, 14.14.

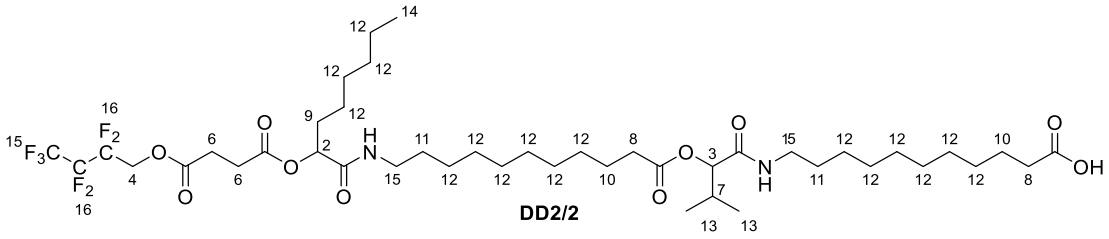
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.16 (t, J = 9.3 Hz, 3 F, $CF_3{}^{17}$), -124.27 – -125.33 (m, 2 F, $CF_2{}^{18}$), -131.44 – -132.73 (m, 2 F, $CF_2{}^{18}$). Total integral of CF_2 region normalized with respect to the $CF_3{}^{17}$ group = 4.

ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{50}{}^1H_{77}{}^{16}O_{10}{}^{14}N_2{}^{19}F_7$, 1021.5359; found, 1021.5337, Δ = 2.2 mmu.



Supplementary Figure 89: ^{1}H-NMR of compound **D2/2** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 1.03 g of **D2/2** (1.03 mmol, 1.00 eq.) were dissolved in 5.00 mL ethyl acetate and 5.00mL THF. Afterwards, 206 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **DD2/2** was obtained as a pale highly viscous oil in a yield of 98.1% (918 mg, 1.01 mmol).

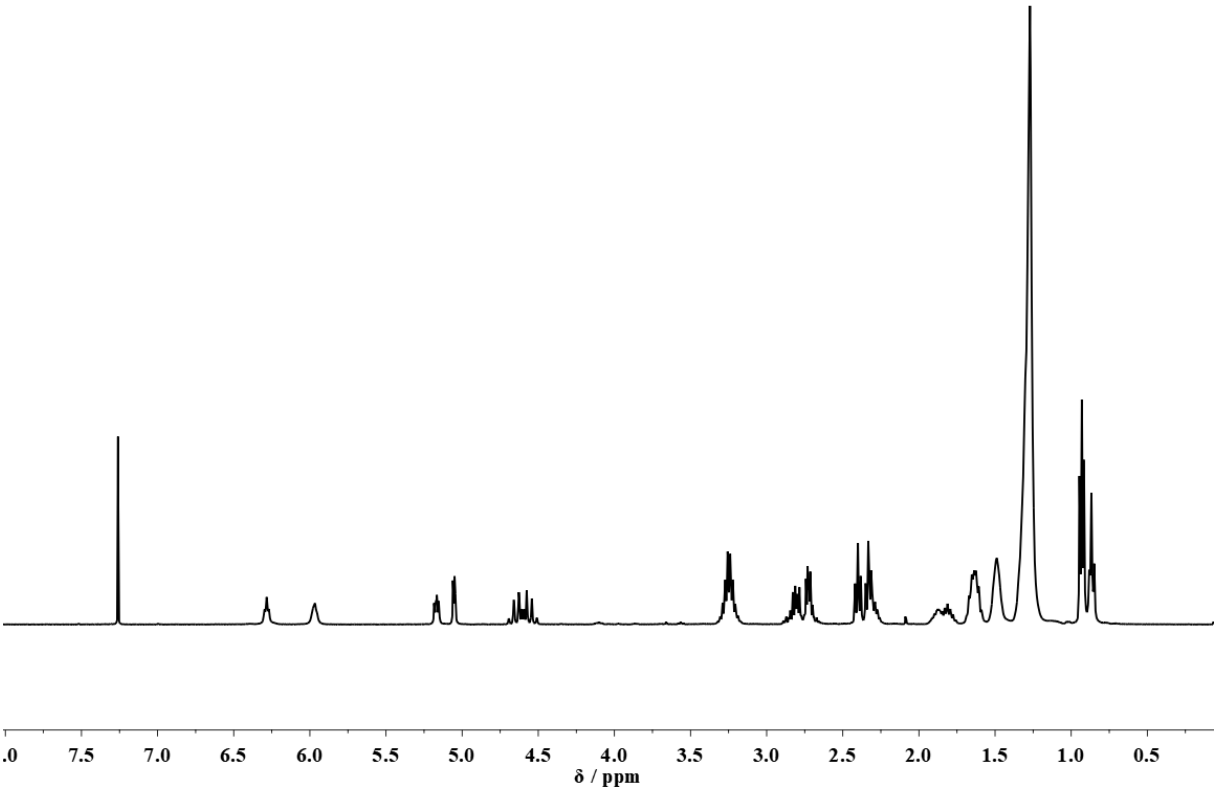
IR (ATR): ν / cm^{-1} = 3305.8 (vw), 2926.4 (s), 2855.3 (m), 1741.5 (s), 1652.3 (s), 1540.2 (m), 1463.6 (w), 1353.0 (w), 1226.3 (vs), 1144.1 (vs), 1019.3 (m), 909.7 (w), 735.6 (w), 627.5 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.22 (t, J = 5.8 Hz, 1 H, NH^{1}), 5.97 – 5.86 (m, 1 H, NH^{2}), 5.13 – 5.06 (m, 1 H, CH^{2}), 5.01 – 4.96 (m, 1 H, CH^{3}), 4.64 – 4.43 (m, 2 H, CH_2^{4}), 3.30 – 3.09 (m, 4 H, CH_2^{5}), 2.85 – 2.59 (m, 4 H, CH_2^{6}), 2.37 – 2.17 (m, 5 H, CH^{7}, CH_2^{8}), 1.90 – 1.68 (m, 2 H, CH_2^{9}), 1.64 – 1.50 (m, 4 H, CH_2^{10}), 1.48 – 1.35 (m, 4 H, CH_2^{11}), 1.33 – 1.07 (m, 32 H, CH_2^{12}), 0.89 – 0.84 (m, 6 H, CH_3^{13}), 0.83 – 0.75 (m, 3 H, CH_3^{14}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 172.71, 171.53, 170.94, 169.83, 169.51, 78.08, 74.87, 59.70 (t, J = 26.9 Hz), 39.51, 39.28, 34.43, 34.00, 33.96, 31.91, 31.72, 30.62, 29.60, 29.52, 29.51, 29.43, 29.42, 29.36, 29.35, 29.28, 29.21, 29.20, 29.09, 29.05, 29.00, 28.80, 26.95, 26.90, 25.16, 24.93, 24.85, 22.66, 18.90, 17.07, 14.14.

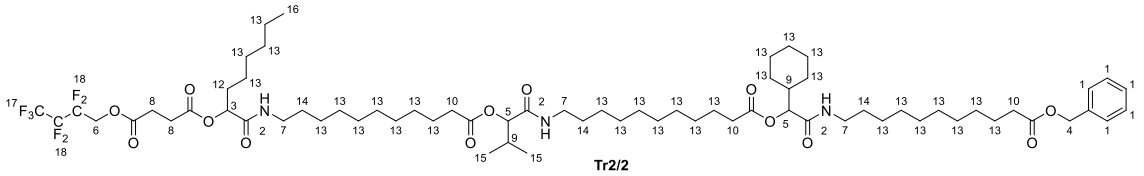
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -84.58 – -86.46 (m, 3 F, CF_3^{15}), -124.16 – -125.27 (m, 2 F, CF_2^{16}), -131.44 – -132.90 (m, 2 F, CF_2^{16}). Total integral of CF_2 region normalized with respect to the CF_3^{15} group = 4.

ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{43}{}^{1}H_{71}{}^{16}O_{10}{}^{14}N_2{}^{19}F_7$, 931.4889; found, 931.4871, Δ = 1.8 mmu.



Supplementary Figure 90: ^{1}H-NMR of compound **DD2/2** measured in $CDCl_3$.

Passerini reaction



Tr2/2

In a 50 mL round bottom flask, 831 mg **DD2/2** (895 µmol, 1.00 eq.) were stirred in 4.00 mL DCM. Subsequently, 163 µL cyclohexanecarboxaldehyde **A6** (151 mg, 1.34 mmol, 1.50 eq.) and 407 mg of monomer **IM2** (1.34 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (4:1 → 2:1) to yield the Passerini product **Tr2/2** as a pale highly viscous oil. (880 mg, 665 µmol, 74.3%).

R_f = 0.13 in cyclohexane / ethyl acetate (2:1).

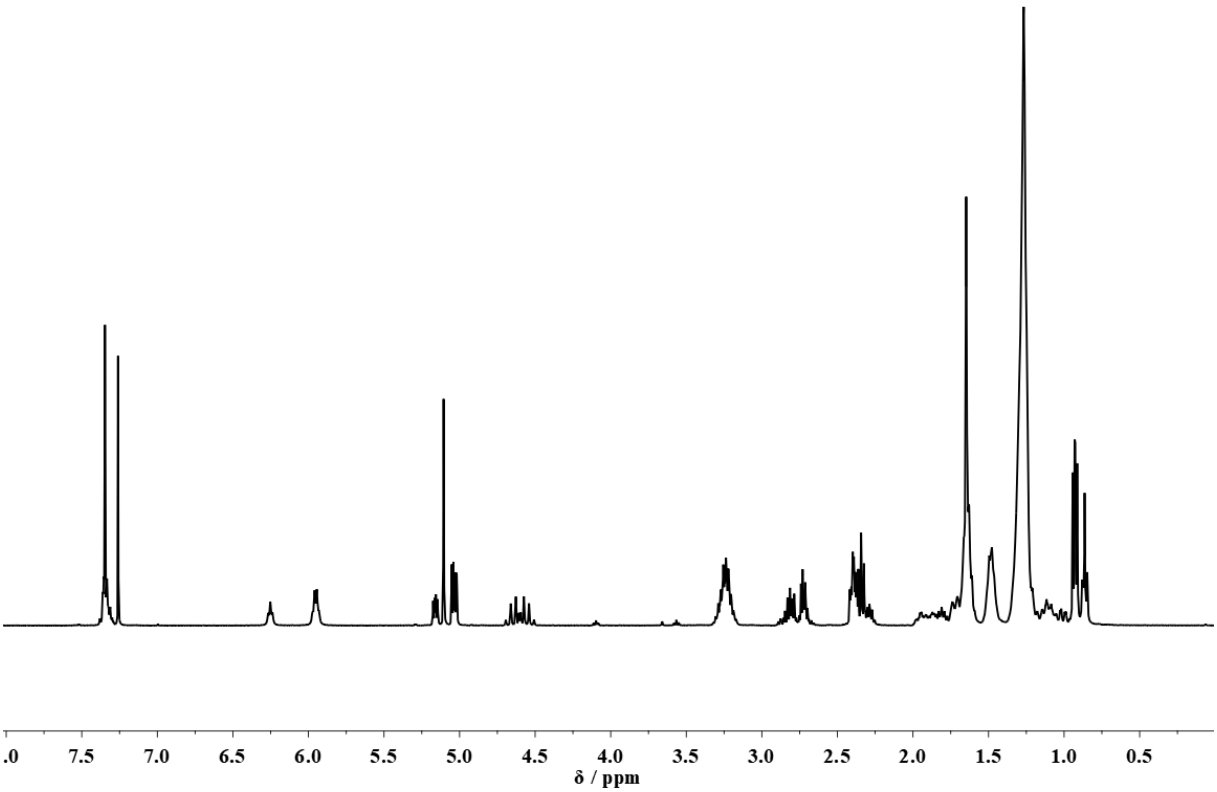
IR (ATR): ν / cm^{-1} = 3306.2 (vw), 2925.2 (s), 2853.8 (m), 1738.9 (s), 1654.4 (s), 1534.8 (m), 1454.6 (w), 1352.8 (w), 1226.9 (s), 1145.7 (vs), 1019.3 (m), 735.6 (w), 697.4 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.43 – 7.28 (m, 5 H, $CH_{Ar}{}^1$), 6.25 (t, J = 5.8 Hz, 1 H, NH^2), 6.02 – 5.90 (m, 2 H, NH^2), 5.19 – 5.14 (m, 1 H, CH^3), 5.11 (s, 2 H, $CH_2{}^4$), 5.08 – 5.00 (m, 2 H, $CH_2{}^5$), 4.72 – 4.50 (m, 2 H, $CH_2{}^6$), 3.34 – 3.16 (m, 6 H, $CH_2{}^7$), 2.90 – 2.65 (m, 4 H, $CH_2{}^8$), 2.43 – 2.24 (m, 7 H, CH^9, $CH_2{}^{10}$), 2.01 – 1.77 (m, 3 H, CH^{11}, $CH_2{}^{12}$), 1.76 – 1.58 (m, 12 H, $CH_2{}^{13}$), 1.56 – 1.41 (m, 6 H, CH^{14}), 1.37 – 1.04 (m, 48 H, $CH_2{}^{13}$), 0.96 – 0.90 (m, 6 H, $CH_3{}^{15}$), 0.90 – 0.82 (m, 3 H, $CH_3{}^{16}$).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.83, 172.70, 172.69, 171.51, 170.91, 169.65, 169.43, 169.35, 136.26, 128.68, 128.29, 78.06, 77.76, 74.88, 66.21, 59.69, 40.12, 39.44, 39.29, 34.46, 34.43, 31.94, 31.73, 30.65, 29.72, 29.72, 29.58, 29.55, 29.50, 29.48, 29.35, 29.31, 29.26, 29.24, 29.05, 29.02, 28.80, 27.43, 26.98, 26.97, 26.21, 26.13, 26.02, 25.13, 25.08, 24.94, 22.66, 18.91, 17.08, 14.15.

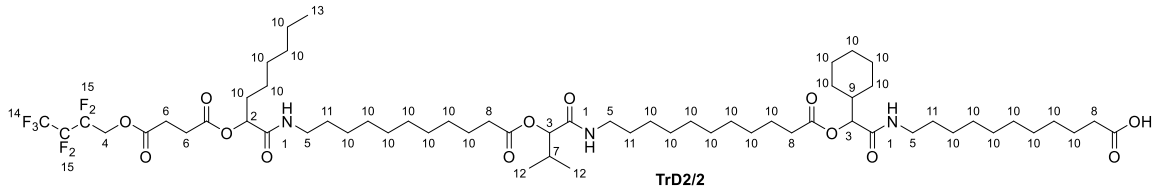
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.15 (t, J = 9.2 Hz, 3 F, $CF_3{}^{17}$), -124.51 – -125.62 (m, 2 F, $CF_2{}^{18}$), -131.79 – -133.84 (m, 2 F, $CF_2{}^{18}$). Total integral of CF_2 region normalized with respect to the $CF_3{}^{17}$ group = 4.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{69}{}^{1}H_{110}{}^{16}O_{13}{}^{14}N_3{}^{19}F_7$, 1322.8000; found, 1322.7981, Δ = 1.9 mmu.



Supplementary Figure 91: ^{1}H-NMR of compound **Tr2/2** measured in $CDCl_3$.

Deprotection



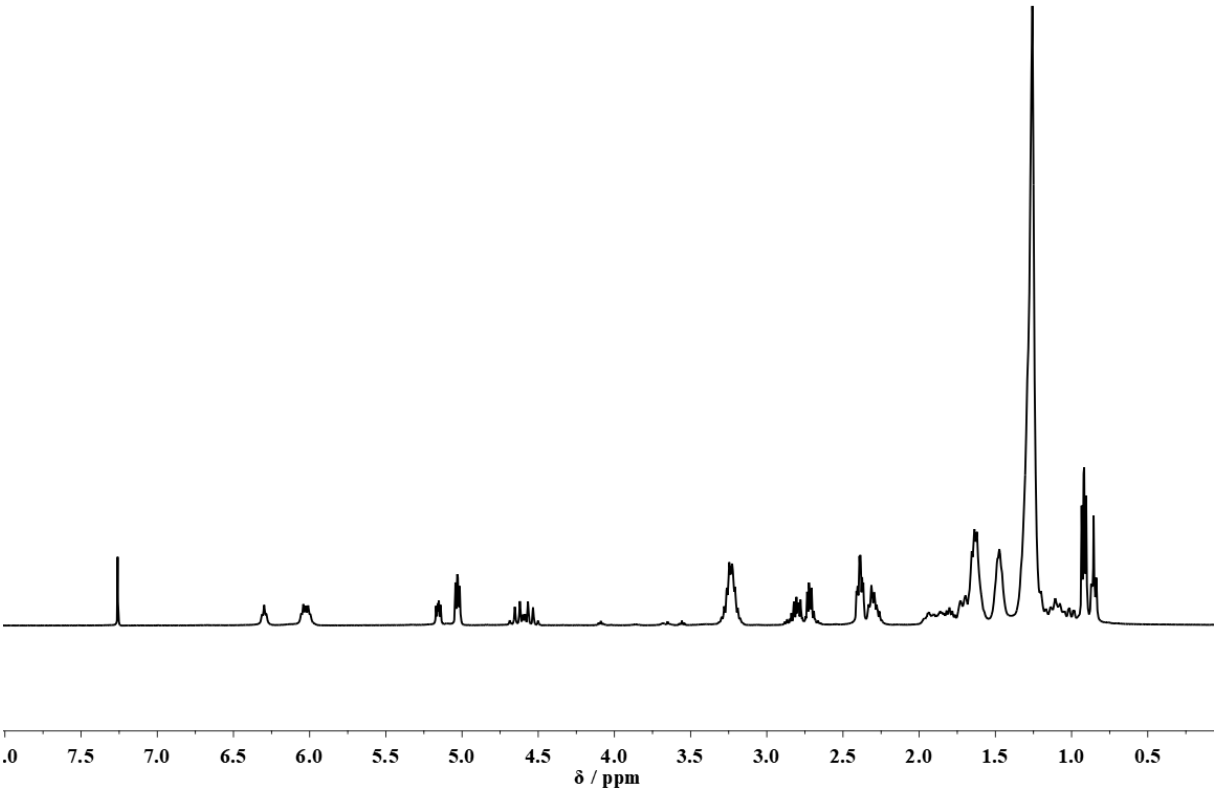
In a 50 mL round bottom flask, 829 mg of **Tr2/2** (627 µmol, 1.00 eq.) were dissolved in 5.00 mL ethyl acetate and 5.00 mL THF. Afterwards, 166 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **TrD2/2** was obtained as a pale highly viscous oil in a quant. yield. (772 mg, 627 mmol).

IR (ATR): ν / cm^{-1} = 3294.0 (vw), 2925.3 (s), 2854.1 (m), 1740.3 (s), 1651.1 (s), 1538.6 (m), 1452.6 (w), 1370.2 (w), 1226.3 (vs), 1145.1 (vs), 1019.1 (w), 909.9 (w), 735.4 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.24 (t, J = 5.8 Hz, 1 H, NH^{1}), 6.06 – 5.88 (m, 2 H, NH^{1}), 5.14 – 5.07 (m, 1 H, CH^{2}), 5.03 – 4.92 (m, 2 H, CH^{3}), 4.70 – 4.39 (m, 2 H, CH_2^{4}), 3.26 – 3.08 (m, 6 H, CH_2^{5}), 2.84 – 2.58 (m, 4 H, CH_2^{6}), 2.39 – 2.16 (m, 7 H, CH^{7}, CH_2^{8}), 1.94 – 1.50 (m, 15 H, CH^{9}, CH_2^{10}), 1.48 – 1.33 (m, 6 H, CH_2^{11}), 1.33 – 0.94 (m, 48 H, CH_2^{10}), 0.91 – 0.83 (m, 6 H, CH_3^{12}), 0.83 – 0.77 (m, 3 H, CH_3^{13}).

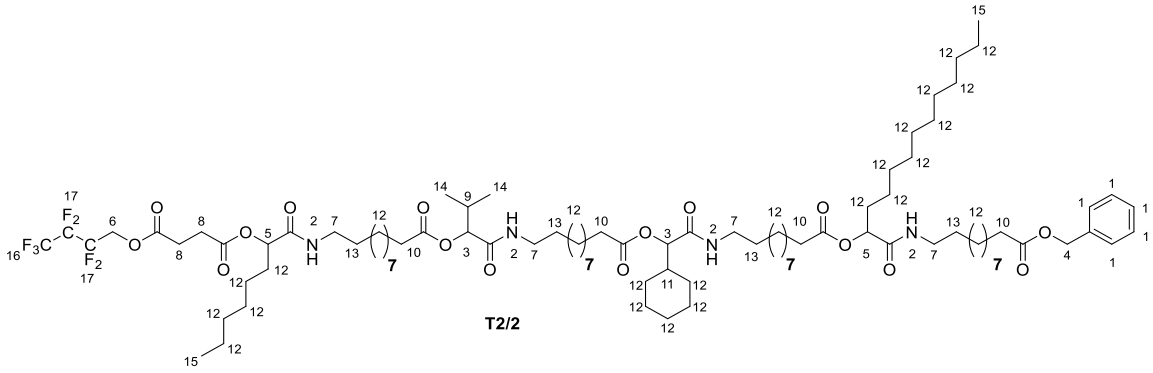
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 172.77, 172.71, 171.52, 170.92, 169.77, 169.59, 169.45, 78.03, 77.74, 74.82, 59.65 (t, J = 27.0 Hz), 40.05, 39.45, 39.32, 39.25, 34.40, 34.08, 31.91, 31.70, 30.61, 29.67, 29.56, 29.49, 29.46, 29.34, 29.31, 29.29, 29.24, 29.21, 29.11, 29.02, 28.99, 28.77, 27.39, 26.94, 26.92, 26.90, 26.17, 26.10, 25.98, 25.12, 25.11, 24.90, 22.64, 18.87, 17.06, 14.13.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{62}{}^{1}H_{104}{}^{16}O_{13}{}^{14}N_3{}^{19}F_7$, 1232.7530; found, 1232.7502, Δ = 2.8 mmu.



Supplementary Figure 92: ^{1}H-NMR of compound **TrD2/2** measured in $CDCl_3$.

Passerini reaction[ii]



In a 50 mL round bottom flask, 686 mg of **TrD2/2** (557 µmol, 1.00 eq.) were stirred in 4.00 mL DCM. Subsequently, 154 mg dodecanal **A10** (835 µmol, 1.50 eq.) and 252 mg of monomer **IM2** (835 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 1 day. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (4:1 → 2:1) to yield the Passerini product **T2/2** as a pale highly viscous oil. (707 mg, 412 µmol, 74.0%).

R_f = 0.66 in cyclohexane / ethyl acetate (1:1).

IR (ATR): ν / cm^{-1} = 3290.4 (vw), 2920.8 (m), 2851.4 (w), 1736.9 (m), 1655.8 (m), 1557.1 (w), 1466.8 (vw), 1377.1 (vw), 1228.5 (w), 1205.6 (w), 1174.5 (m), 1019.8 (vw), 722.6 (vw), 696.6 (vw).

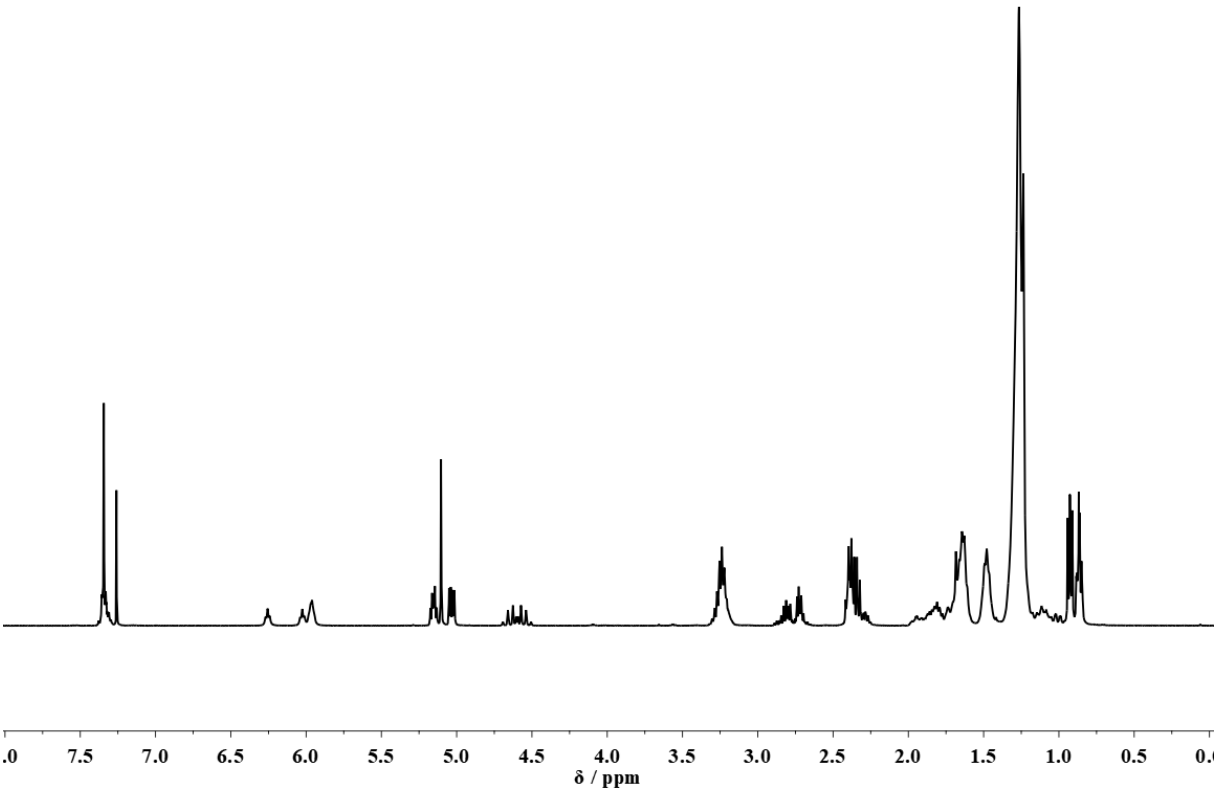
^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.41 – 7.28 (m, 5 H, $CH_{Ar}{}^1$), 6.26 (t, J = 5.8 Hz, 1 H, NH^2), 6.06 – 5.93 (m, 3 H, NH^2), 5.19 – 5.13 (m, 2 H, CH^3), 5.10 (s, 2 H, $CH_2{}^4$), 5.07 – 4.99 (m, 2 H, CH^5), 4.72 – 4.48 (m, 2 H, $CH_2{}^6$), 3.34 – 3.14 (m, 8 H, $CH_2{}^7$), 2.90 – 2.65 (m, 4 H, $CH_2{}^8$), 2.43 – 2.24 (m, 9 H, CH^9, $CH_2{}^{10}$), 2.00 – 1.58 (m, 19 H, CH^{11}, $CH_2{}^{12}$), 1.54 – 1.43 (m, 8 H, $CH_2{}^{13}$), 1.37 – 1.04 (m, 78 H, $CH_2{}^{12}$), 0.95 – 0.90 (m, 6 H, $CH_3{}^{14}$), 0.89 – 0.83 (m, 6 H, $CH_3{}^{15}$).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.82, 172.69, 172.60, 171.50, 170.90, 169.98, 169.63, 169.41, 169.35, 136.24, 128.67, 128.28, 78.03, 77.74, 74.86, 74.06, 66.20, 59.66, 40.10, 39.42, 39.32, 39.27, 34.44, 32.04, 31.93, 31.72, 30.64, 29.75, 29.72, 29.68, 29.67, 29.54, 29.49, 29.49, 29.34, 29.31, 29.26, 29.24, 29.04, 29.01, 28.79, 27.41, 26.96, 26.95, 26.20, 26.12, 26.01, 25.12, 25.07, 24.93, 24.89, 22.81, 22.65, 18.91, 17.07, 14.25, 14.15.

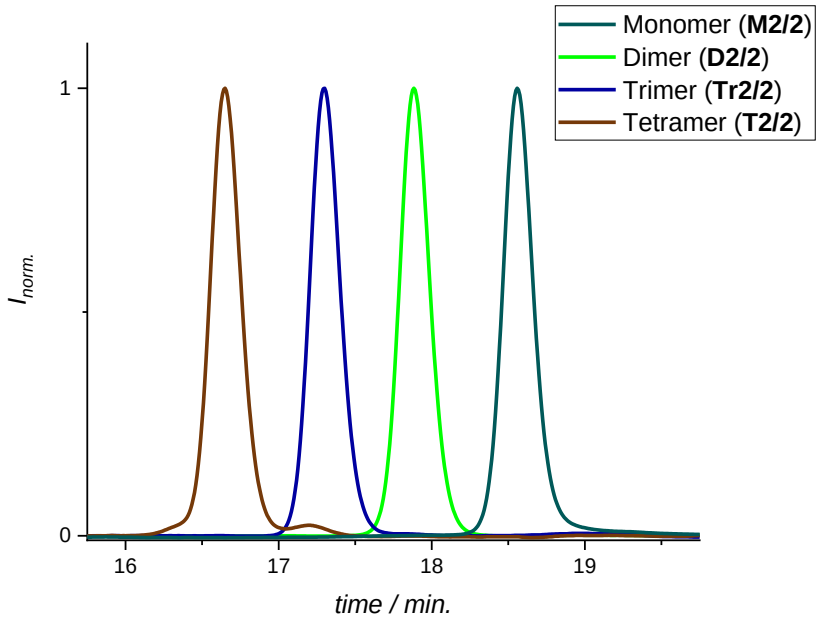
[ii] Synthesis was carried out by Lara Faden in the Vertieferarbeit "Synthesis and characterization of monodisperse sequence-defined oligomers" under the laboratory supervision of Maximiliane Frölich.[290]

^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -83.46 – -86.58 (m, 3 F, CF_3[16]), -123.51 – -126.45 (m, 2 F, CF_2[16]), -131.44 – -133.84 (m, 2 F, CF_2[17]). Total integral of CF_2 region normalized with respect to the CF_3[16] group = 4.

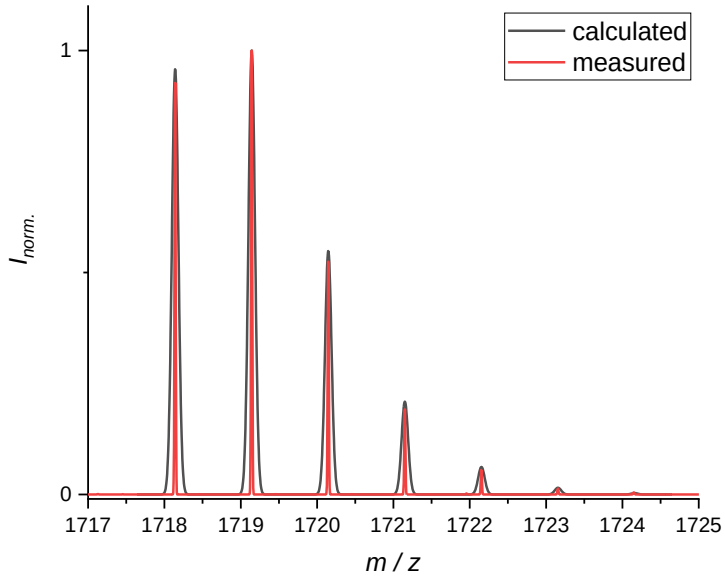
ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{93}{}^{1}H_{155}{}^{16}O_{16}{}^{14}N_4{}^{19}F_7$, 1718.1399; found, 1718.1400, Δ = 0.1 mmu.



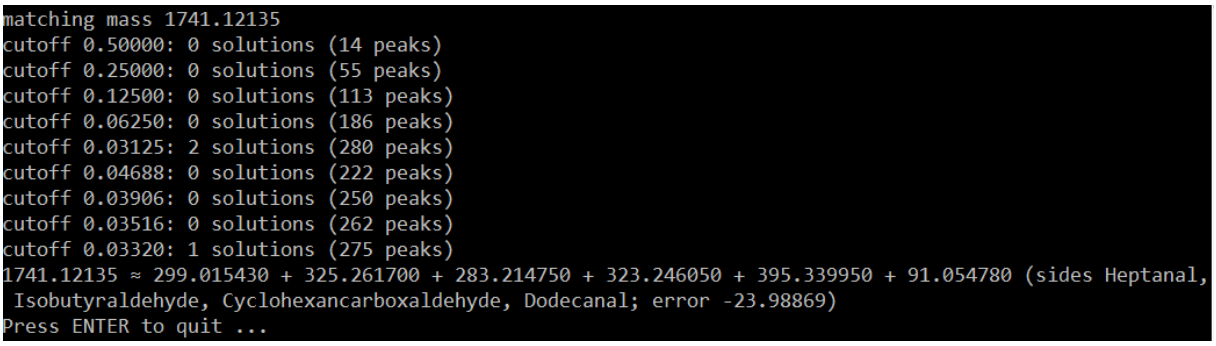
Supplementary Figure 93: ^{1}H-NMR of compound **T2/2** measured in $CDCl_3$.



Supplementary Figure 94: SEC traces of the intermediates after each P-3CR in the synthesis of product **T2/2**.



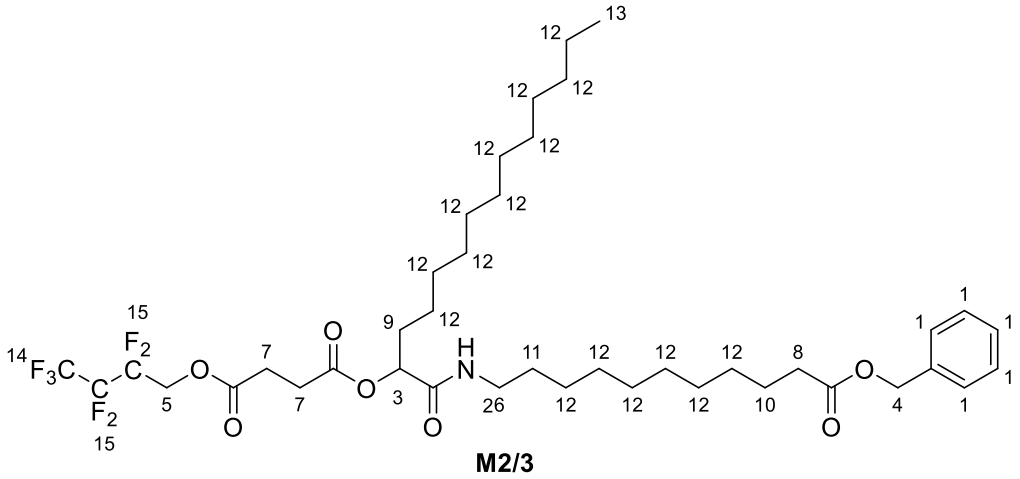
Supplementary Figure 95: High resolution ESI-MS measurement of **T2/2**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (black).



Supplementary Figure 96: Screenshot of the automated read-out of **T2/2**, sodium trifluoroacetate **2** was used as additive during the measurement.

6.3.3.4.4 Synthesis of tetramer T2/3

Passerini reaction



In a 50 mL round bottom flask, 500 mg **TAG2** (1.67 mmol, 1.00 eq.) were stirred in 2.00 mL DCM. Subsequently, 593 µL tridecanal **A13** (496 mg, 2.50 mmol, 1.50 eq.) and 754 mg of monomer **IM2** (2.50 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (7:1 → 5:1) to yield the Passerini product **M2/3** as a pale highly viscous oil. (1.03 g, 1.30 mmol, 78.0%).

R_f = 0.45 in cyclohexane / ethyl acetate (3:1).

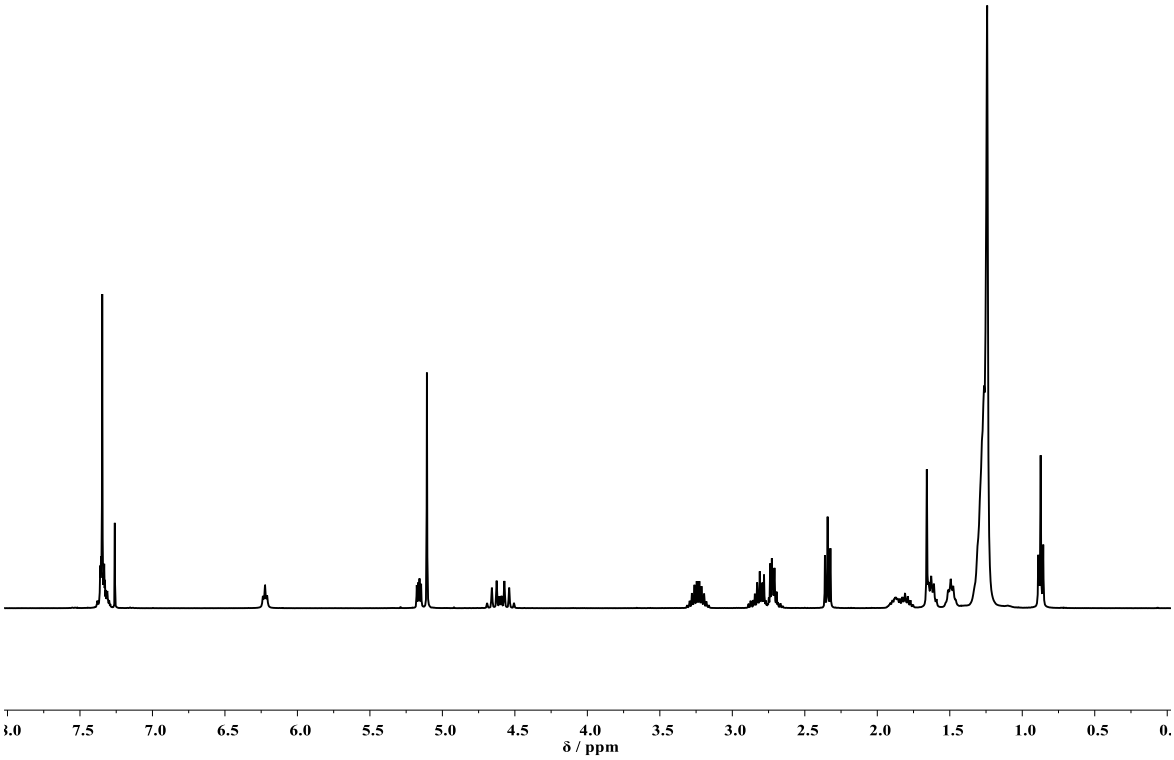
IR (ATR): ν / cm^{-1} = 3305.5 (vw), 2924.2 (s), 2853.9 (m), 1739.8 (s), 1656.6 (m), 1535.6 (w), 1456.5 (w), 1352.2 (w), 1226.6 (vs), 1144.2 (vs), 1019.9 (m), 909.7 (w), 735.7 (m), 696.9 (m).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.44 – 7.30 (m, 5 H, CH_{Ar}[1]), 6.22 (t, J = 5.8 Hz, 1 H, NH[2]), 5.19 – 5.13 (m, 1 H, CH[3]), 5.11 (s, 2 H, CH_2[4]), 4.73 – 4.49 (m, 2 H, CH_2[5]), 3.34 – 3.15 (m, 2 H, CH_2[6]), 2.91 – 2.66 (m, 4 H, CH_2[7]), 2.34 (t, J = 7.6 Hz, 2 H, CH_2[8]), 1.95 – 1.74 (m, 2 H, CH_2[9]), 1.68 – 1.57 (m, 2 H, CH_2[10]), 1.54 – 1.44 (m, 2 H, CH_2[11]), 1.36 – 1.15 (m, 32 H, CH_2[12]), 0.92 – 0.83 (m, 3 H, CH_3[13]).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.82, 171.48, 170.89, 169.62, 136.27, 128.67, 128.29, 74.90, 66.19, 59.69 (t, J = 27.1 Hz), 39.45, 34.45, 32.05, 31.95, 29.78, 29.77, 29.75, 29.68, 29.58, 29.56, 29.54, 29.48, 29.37, 29.33, 29.24, 29.04, 28.79, 26.96, 25.06, 25.00, 22.82, 14.24.

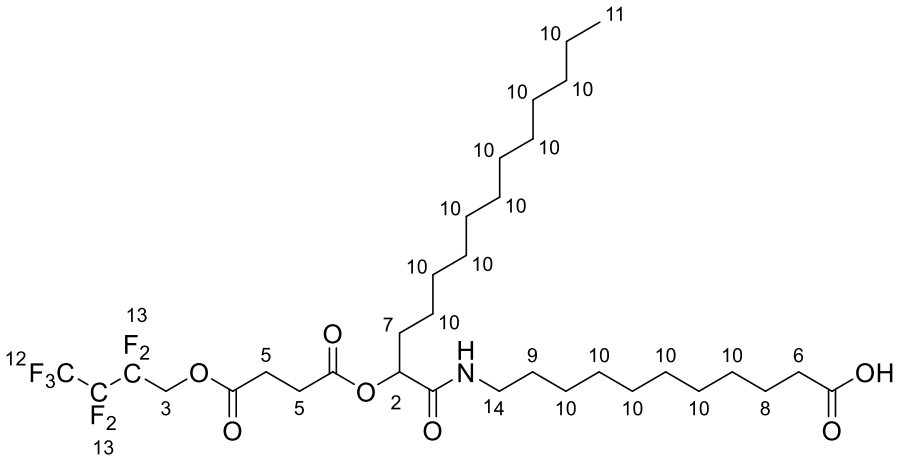
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.16 (t, J = 9.2, 3 F, CF_3^{14}),), -123.69 – -125.74 (m, 2 F, CF_2^{15}), -131.26 – -133.08 (m, 2 F, CF_2^{15}). Total integral of CF_2 region normalized with respect to the CF_3^{14} group = 4.

ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{40}{}^{1}H_{60}{}^{16}O_7{}^{14}N{}^{19}F_7$, 822.4150; found, 822.4133, Δ = 1.7 mmu.



Supplementary Figure 97: ^{1}H-NMR of compound **M2/3** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 1.03 g of **M2/3** (1.28 mmol, 1.00 eq.) were dissolved in 4.00 mL ethyl acetate and 4.00 mL THF. Afterwards, 103 mg (10 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **MD2/3** was obtained as a pale highly viscous oil in a yield of 98.4% (894 mg, 1.26 mmol).

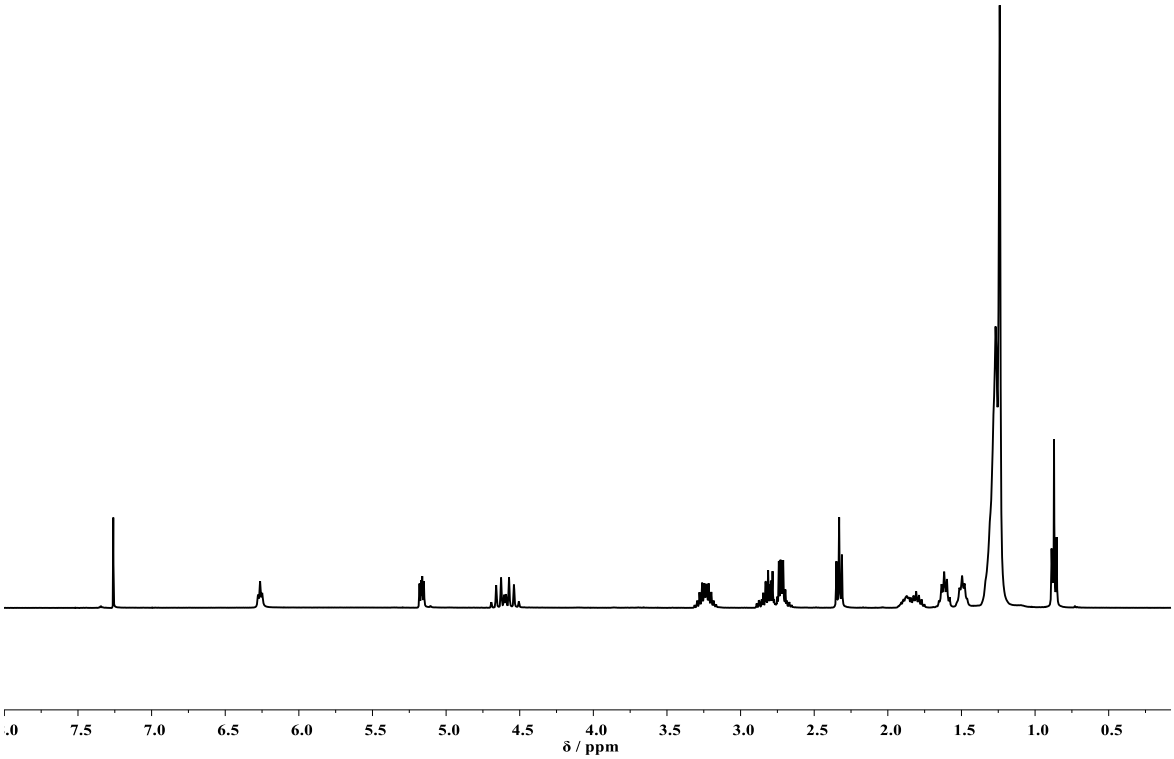
IR (ATR): ν / cm^{-1} = 3293.4 (vw), 2918.0 (s), 2851.6 (s), 1736.1 (vs), 1694.9 (s), 1657.2 (vs), 1560.9 (w), 1470.0 (w), 1419.7 (w), 1343.8 (m), 1278.1 (m), 1220.9 (vs), 1176.8 (vs), 1131.7 (vs), 1021.9 (m), 929.3 (m), 800.9 (w), 722.1 (w), 678.8 (w), 624.1 (w), 528.8 (w), 462.8 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.26 (t, J = 5.8 Hz, 1 H, NH^1), 5.21 – 5.13 (m, 1 H, CH^2), 4.75 – 4.48 (m, 2 H, $CH_2{}^3$), 3.35 – 3.15 (m, 2 H, $CH_2{}^4$), 2.91 – 2.64 (m, 4 H, $CH_2{}^5$), 2.33 (t, J = 7.5 Hz, 2 H, $CH_2{}^6$), 1.94 – 1.73 (m, 2 H, $CH_2{}^7$), 1.71 – 1.57 (m, 2 H, $CH_2{}^8$), 1.54 – 1.42 (m, 2 H, $CH_2{}^9$), 1.38 – 1.14 (m, 32 H, $CH_2{}^{10}$), 0.91 – 0.83 (m, 3 H, $CH_3{}^{11}$).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 179.82, 172.29, 171.70, 170.54, 75.64, 60.46 (t, J = 26.9 Hz), 40.24, 34.86, 32.81, 32.69, 30.54, 30.54, 30.52, 30.45, 30.32, 30.24, 30.13, 30.03, 29.99, 29.86, 29.81, 29.55, 27.66, 25.76, 25.58, 23.58, 15.00.

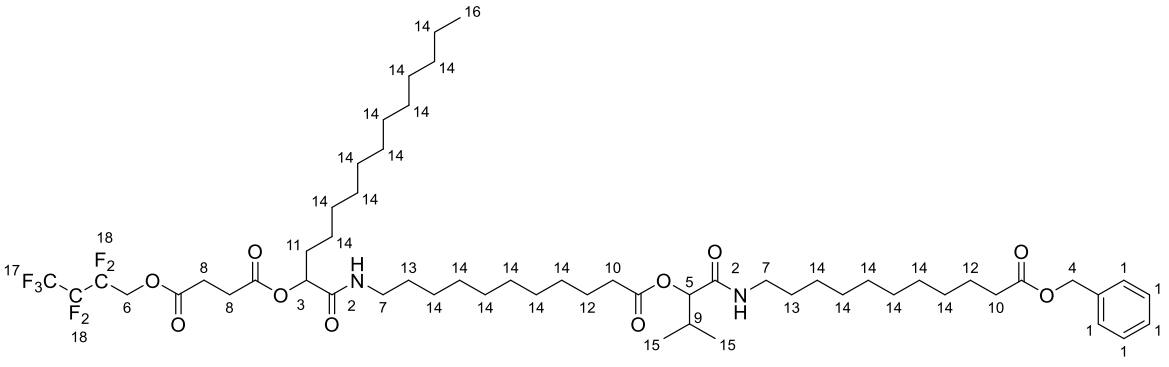
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.17 (t, J = 9.2 Hz, 3 F, $CF_3{}^{12}$), -124.45 – -128.85 (m, 2 F, $CF_2{}^{13}$), -131.44 – -136.02 (m, 2 F, $CF_2{}^{13}$). Total integral of CF_2 region normalized with respect to the $CF_3{}^{12}$ group = 4.

ESI-MS [m/z]: $[M + H]^+$ calculated for $^{12}C_{33}{}^{1}H_{54}{}^{16}O_7{}^{14}N{}^{19}F_7$, 710.3861; found, 710.3848, Δ = 1.3 mmu.



Supplementary Figure 98: ^{1}H-NMR of compound **MD2/3** measured in $CDCl_3$.

Passerini reaction



D2/3

In a 50 mL round bottom flask, 420 mg **MD2/3** (592 µmol, 1.00 eq.) were stirred in 3.00 mL DCM. Subsequently, 81.1 µL isobutyraldehyde **A3** (64.0 mg, 888 µmol, 1.50 eq.) and 268 mg of monomer **IM2** (888 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (5:1 → 1:1) to yield the Passerini product **D2/3** as a pale highly viscous oil. (618 g, 571 µmol, 96.4%).

R_f = 0.36 in cyclohexane / ethyl acetate (2:1).

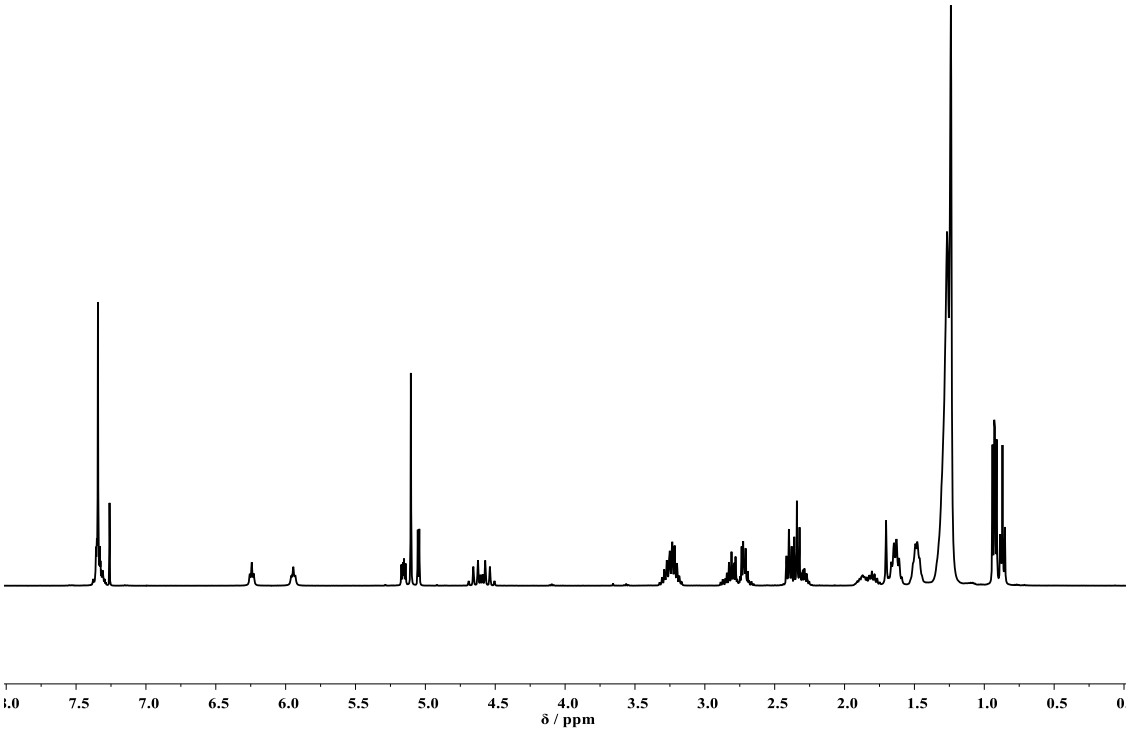
IR (ATR): ν / cm^{-1} = 3306.9 (w), 2917.5 (s), 2850.1 (s), 1732.5 (vs), 1656.2 (vs), 1544.3 (m), 1467.2 (w), 1312.7 (w), 1228.4 (vs), 1148.4 (vs), 1020.4 (m), 979.6 (m), 913.5 (m), 800.8 (vw), 735.0 (m), 696.5 (m), 536.6 (vw), 474.9 (vw), 422.4 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.43 – 7.28 (m, 5 H, CH_{Ar}[1]), 6.24 (t, J = 5.8 Hz, 1 H, NH[2]), 5.95 (t, J = 5.9 Hz, 1 H, NH[2]), 5.19 – 5.13 (m, 1 H, CH[3]), 5.10 (s, 2 H, CH[4]), 5.05 (d, J = 4.4 Hz, 1 H, CH[5]), 4.71 – 4.49 (m, 2 H, CH_2[6]), 3.36 – 3.14 (m, 4 H, CH_2[7]), 2.89 – 2.65 (m, 4 H, CH_2[8]), 2.44 – 2.22 (m, 5 H, CH[9], CH_2[10]), 1.94 – 1.73 (m, 2 H, CH_2[11]), 1.69 – 1.57 (m, 4 H, CH_2[12]), 1.54 – 1.43 (m, 4 H, CH_2[13]), 1.36 – 1.20 (m, 44 H, CH[14]), 0.96 – 0.90 (m, 6 H, CH_3[15]), 0.89 – 0.83 (m, 3 H, CH_2[16]).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.81, 172.66, 171.48, 170.89, 169.63, 169.39, 136.27, 128.66, 128.28, 78.05, 74.88, 66.19, 59.67 (t, J = 27.0 Hz), 39.43, 39.28, 34.44, 34.42, 32.04, 31.94, 30.65, 29.76, 29.74, 29.71, 29.67, 29.58, 29.55, 29.49, 29.47, 29.36, 29.33, 29.26, 29.23, 29.04, 28.79, 26.96, 26.95, 25.13, 25.06, 25.00, 22.81, 18.90, 17.06, 14.23.

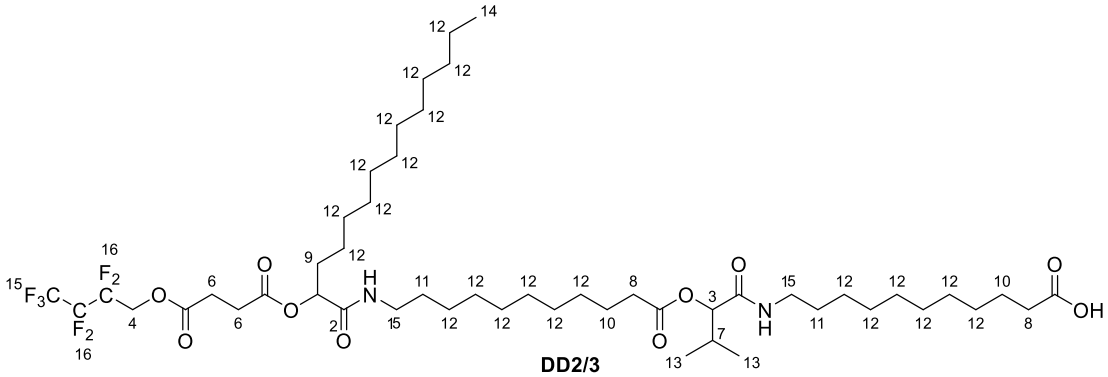
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.16 (t, J = 9.2 Hz, 3 F, CF_3[17]), -124.27 – -125.74 (m, 2 F, CF_2[18]), -131.26 – -133.08 (m, 2 F, CF_2[18]). Total integral of CF_2 region normalized with respect to the CF_3[17] group = 4.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{56}{}^{1}H_{89}{}^{16}O_{10}{}^{14}N_2{}^{19}F_7$, 1083.6478; found, 1083.6459, Δ = 1.9 mmu.



Supplementary Figure 99: ^{1}H-NMR of compound **D2/3** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 537 mg of **D2/3** (496 µmol, 1.00 eq.) were dissolved in 4.00 mL ethyl acetate and 4.00 mL THF. Afterwards, 107 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **DD2/3** was obtained as a pale highly viscous oil in a yield of 98.2% (484 mg, 487 µmol).

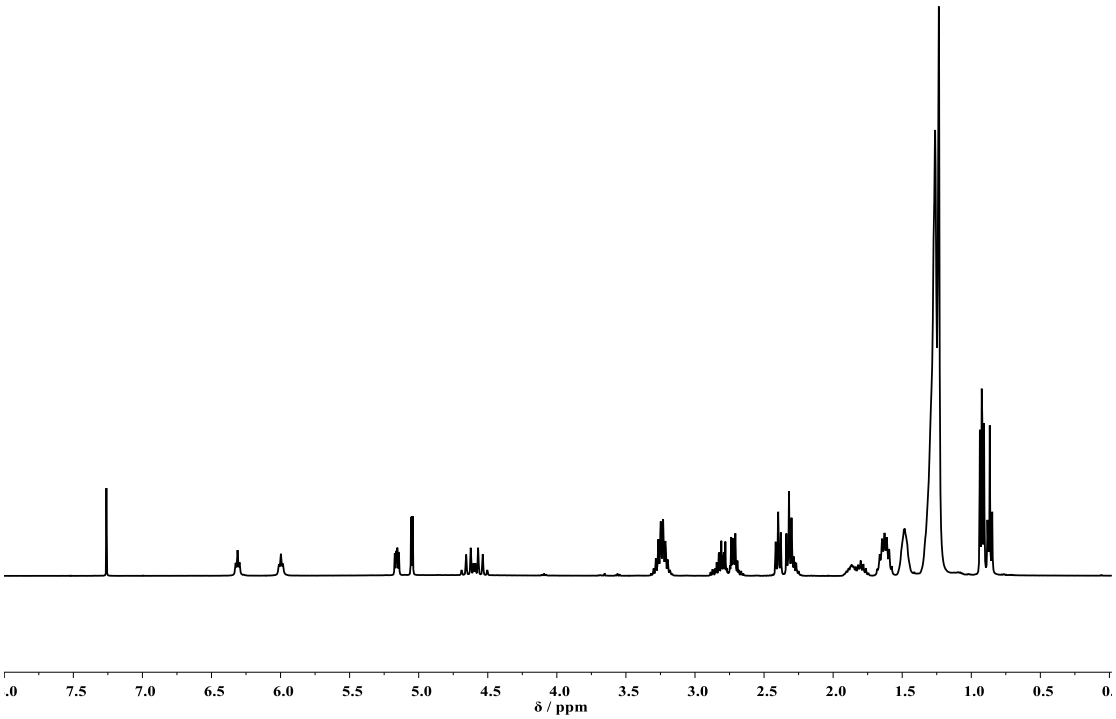
IR (ATR): ν / cm^{-1} = 3324.9 (vw), 2920.4 (s), 2850.9 (s), 1741.3 (vs), 1702.3 (m), 1650.7 (vs), 1540.1 (m), 1466.9 (w), 1433.6 (w), 1354.3 (w), 1291.7 (m), 1228.4 (vs), 1145.5 (vs), 1020.8 (m), 955.5 (w), 721.0 (m), 658.5 (w), 534.0 (vw), 473.2 (vw), 403.9 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.31 (t, J = 5.8 Hz, 1 H, NH^{1}), 6.00 (t, J = 5.8 Hz, 1 H, NH^{1}), 5.20 – 5.13 (m, 1 H, CH^{2}), 5.05 (d, J = 4.4 Hz, 1 H, CH^{3}), 4.72 – 4.49 (m, 2 H, CH_2^{4}), 3.36 – 3.14 (m, 4 H, CH_2^{5}), 2.92 – 2.64 (m, 4 H, CH_2^{6}), 2.45 – 2.24 (m, 5 H, CH^{7}, CH_2^{8}), 1.95 – 1.71 (m, 2 H, CH_2^{9}), 1.68 – 1.56 (m, 4 H, CH_2^{10}), 1.54 – 1.40 (m, 4 H, CH_2^{11}), 1.40 – 1.14 (m, 44 H, CH_2^{12}), 0.95 – 0.89 (m, 6 H, CH_3^{13}), 0.89 – 0.84 (m, 3 H, CH_3^{14}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 178.15, 172.71, 171.54, 170.96, 169.84, 169.53, 78.05, 74.85, 59.68 (t, J = 26.7 Hz), 39.49, 39.28, 34.41, 34.08, 32.04, 31.91, 30.61, 29.78, 29.77, 29.74, 29.67, 29.59, 29.55, 29.50, 29.47, 29.44, 29.34, 29.33, 29.27, 29.22, 29.10, 29.03, 28.78, 26.94, 26.90, 25.14, 24.99, 24.87, 22.81, 18.89, 17.05, 14.23.

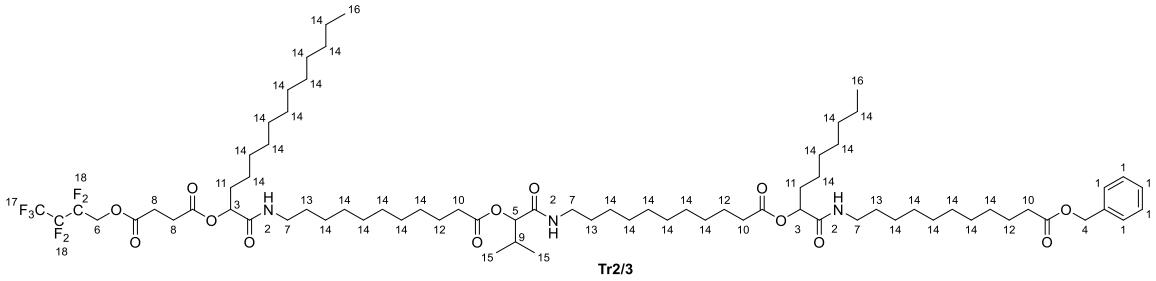
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.16 (t, J = 9.2 Hz, 3 F, CF_3^{15}), -123.16 – -126.45 (m, 2 F, CF_2^{16}), -130.67 – -138.42 (m, 2 F, CF_2^{16}). Total integral of CF_2 region normalized with respect to the CF_3^{15} group = 4.

ESI-MS [m/z]: $[M + H]^+$ calculated for $^{12}C_{49}{}^{1}H_{83}{}^{16}O_{10}{}^{14}N_2{}^{19}F_7{}^{23}Na$, 1015.5828; found, 1015.5812, Δ = 1.6 mmu.



Supplementary Figure 100: ^{1}H-NMR of compound **DD2/3** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 403 mg of **DD2/3** (406 µmol, 1.00 eq.) were stirred in 4.00 mL dichloromethane. Subsequently, 95.2 µL octanal **A8** (78.1 mg, 609 µmol, 1.50 eq.) and 184 mg of monomer **IM2** (609 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (6:1 → 4:1) to yield the Passerini product **Tr2/3** as a pale highly viscous oil. (430 mg, 302 µmol, 74.4%).

R_f = 0.53 in cyclohexane / ethyl acetate (2:1).

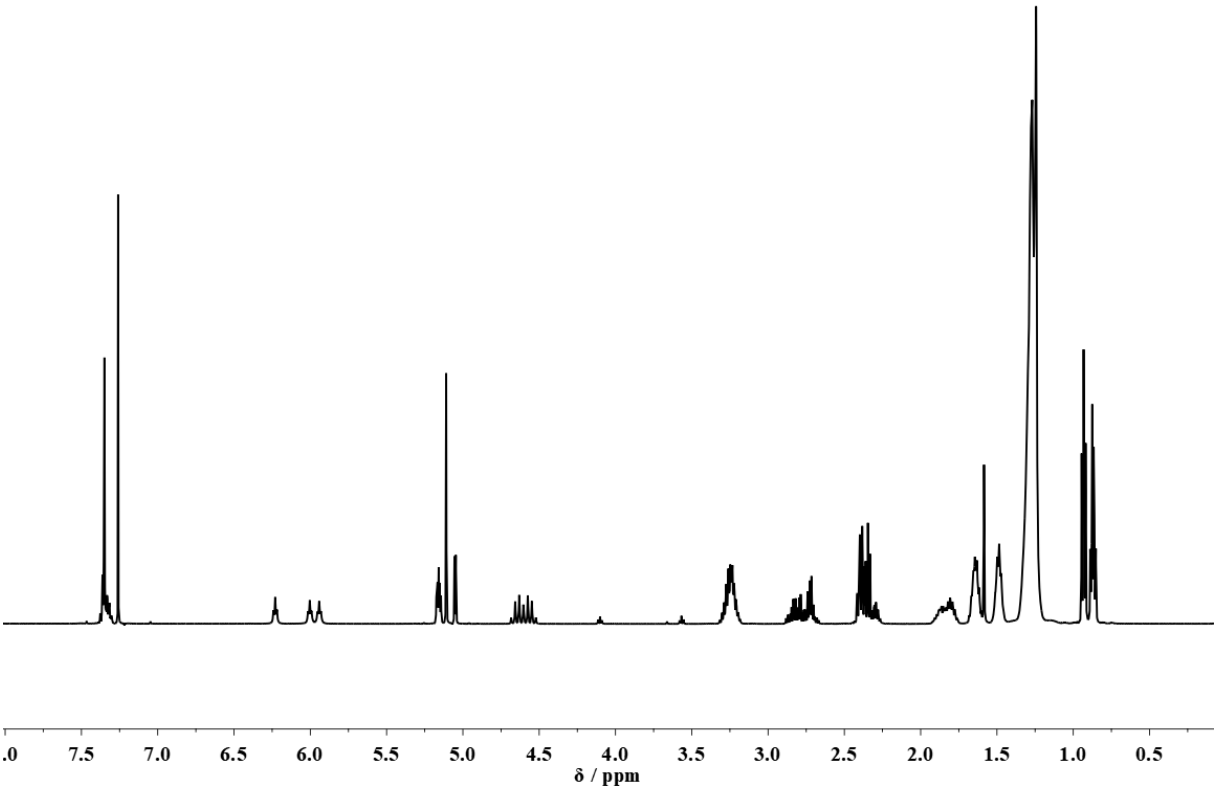
IR (ATR): ν / cm^{-1} = 3300.5 (vw), 2919.6 (w), 2850.9 (w), 2367.7 (vw), 2358.7 (vw), 2339.6 (vw), 2123.3 (vw), 1739.2 (w), 1655.9 (w), 1556.2 (vw), 1466.6 (vw), 1366.9 (vw), 1301.3 (vw), 1225.7 (w), 1164.1 (w), 1118.0 (w), 1019.5 (vw), 912.3 (vw), 803.3 (vw), 738.2 (vw), 696.7 (vw), 417.8 (vw).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.35 – 7.21 (m, 5 H, CH_{Ar}^1), 6.16 (t, J = 5.8 Hz, 1 H, NH^2), 5.94 (t, J = 5.9 Hz, 1 H, NH^2), 5.88 (t, J = 5.9 Hz, 1 H, NH^2), 5.12 – 5.07 (m, 2 H, CH^3), 5.04 (s, 2 H, CH_2^4), 4.98 (d, J = 4.4 Hz, 1 H, CH^5), 4.68 – 4.43 (m, 2 H, CH_2^6), 3.32 – 3.10 (m, 6 H, CH_2^7), 2.85 – 2.60 (m, 4 H, CH_2^8), 2.37 – 2.18 (m, 7 H, CH^9, CH_2^{10}), 1.91 – 1.67 (m, 4 H, CH_2^{11}), 1.66 – 1.54 (m, 6 H, CH_2^{12}), 1.48 – 1.37 (m, 6 H, CH_2^{13}), 1.32 – 1.11 (m, 66 H, CH^{14}), 0.87 (t, J = 6.5 Hz, 6 H, CH_3^{15}), 0.83 – 0.74 (m, 6 H, CH_3^{16}).

^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 173.83, 172.69, 172.60, 171.52, 170.91, 169.98, 169.63, 169.41, 136.27, 128.69, 128.30, 78.05, 74.89, 74.08, 66.21, 59.69 (t, J = 26.9 Hz), 39.44, 39.33, 39.28, 34.46, 34.43, 32.06, 31.95, 31.87, 30.66, 29.80, 29.78, 29.76, 29.75, 29.70, 29.60, 29.57, 29.50, 29.36, 29.33, 29.28, 29.25, 29.05, 28.80, 26.98, 25.14, 25.11, 25.08, 25.02, 24.90, 22.83, 22.76, 18.92, 17.08, 14.26, 14.22.

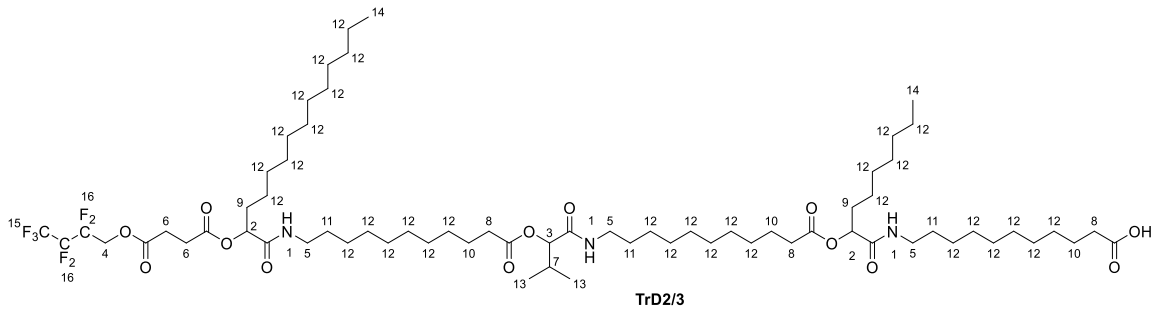
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -84.05 – -85.87 (m, 3 F, CF_3^{14}), -123.33 – -127.03 (m, 2 F, CF_2^{15}), -131.26 – -133.96 (m, 2 F, CF_2^{15}). Total integral of CF_2 region normalized with respect to the CF_3^{14} group = 4.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{76}{}^{1}H_{126}{}^{16}O_{13}{}^{14}N_3{}^{19}F_7$, 1422.9252; found, 1422.9239, Δ = 1.3 mmu.



Supplementary Figure 101: ^{1}H-NMR of compound **Tr2/3** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 363 mg of **Tr2/3** (255 µmol, 1.00 eq.) were dissolved in 5.00 mL ethyl acetate and 5.00 mL THF. Afterwards, 166 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and

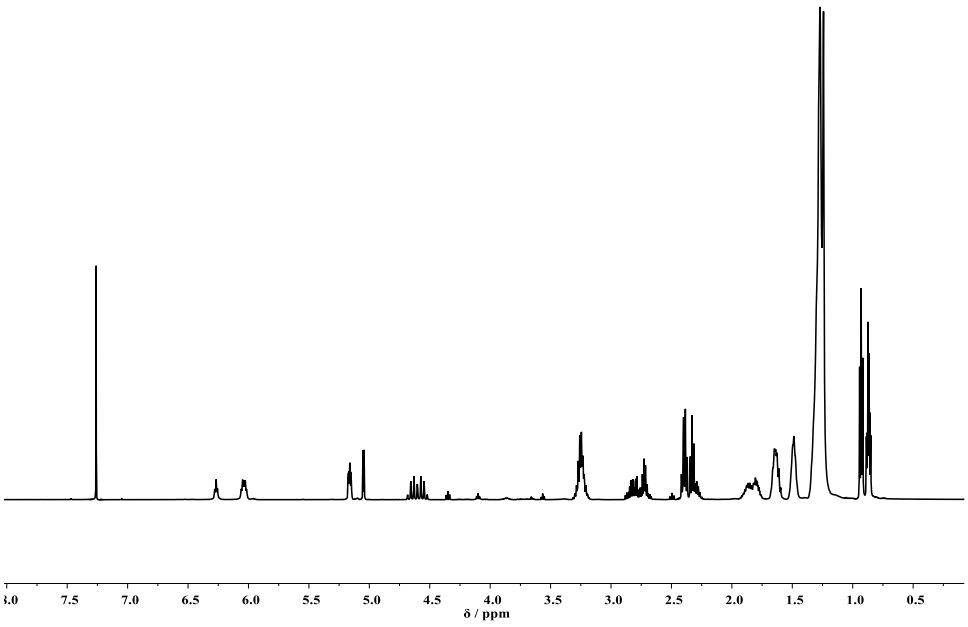
stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **TrD2/3** was obtained as a pale highly viscous oil in a yield of 97.3% (331 mg, 249 µmol).

IR (ATR): ν / cm^{-1} = 3274.6 (vw), 2919.2 (vs), 2850.9 (s), 1740.1 (vs), 1654.7 (vs), 1545.2 (m), 1466.5 (w), 1365.9 (w), 1227.7 (vs), 1146.3 (vs), 1020.3 (m), 911.5 (w), 721.6 (w), 535.5 (vw), 474.0 (vw), 407.2 (vw).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 6.20 (t, J = 5.8 Hz, 1 H, NH^{1}), 6.03 – 5.93 (m, 2 H, NH^{1}), 5.14 – 5.07 (m, 2 H, CH^{2}), 4.98 (d, J = 4.5 Hz, 1 H, CH^{3}), 4.67 – 4.43 (m, 2 H, CH_2^{4}), 3.28 – 3.12 (m, 6 H, CH_2^{5}), 2.85 – 2.60 (m, 4 H, CH_2^{6}), 2.37 – 2.18 (m, 7 H, CH^{7}, CH_2^{8}), 1.91 – 1.68 (m, 4 H, CH_2^{9}), 1.64 – 1.50 (m, 6 H, CH_2^{10}), 1.48 – 1.37 (m, 6 H, CH_2^{11}), 1.33 – 1.06 (m, 66 H, CH_2^{12}), 0.87 (t, J = 6.4 Hz, 6 H, CH_3^{13}), 0.83 – 0.76 (m, 6 H, CH_3^{14}).

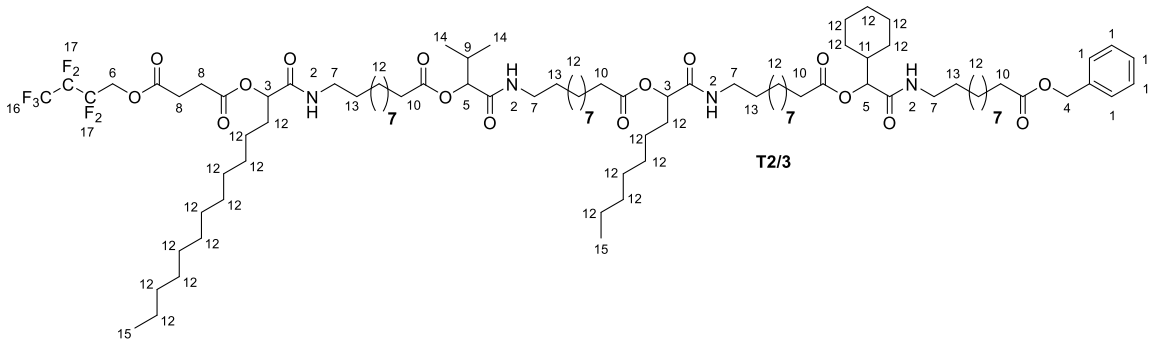
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 177.05, 172.80, 172.63, 171.54, 170.93, 170.08, 169.76, 169.61, 78.07, 74.87, 74.09, 59.69 (t, J = 26.9 Hz), 39.47, 39.35, 39.29, 34.47, 34.42, 33.91, 32.05, 32.01, 31.93, 31.87, 30.62, 29.79, 29.78, 29.75, 29.70, 29.69, 29.60, 29.58, 29.56, 29.52, 29.51, 29.49, 29.43, 29.38, 29.37, 29.33, 29.26, 29.24, 29.21, 29.08, 29.04, 28.79, 26.97, 26.94, 26.88, 25.13, 25.12, 25.01, 24.90, 24.88, 22.82, 22.75, 18.89, 17.09, 14.25, 14.21.

ESI-MS [m/z]: $[M + H]^+$ calculated for $^{12}C_{70}{}^{1}H_{120}{}^{16}O_{11}{}^{14}N_3{}^{35}Cl$, 1214.8684; found, 1214.8655, Δ = 2.9 mmu.



Supplementary Figure 102: ^{1}H-NMR of compound **TrD2/3** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 281 mg of **TrD2/3** (211 µmol, 1.00 eq.) were stirred in 3.00 mL dichloromethane. Subsequently, 38.3 µL cyclohexancarboxaldehyde **A6** (35.5 mg, 316 µmol, 1.50 eq.) and 95.3 mg of monomer **IM2** (316 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (4:1 → 2:1) to yield the Passerini product **T2/3** as a pale highly viscous oil. (331 mg, 190 µmol, 90.0%).

R_f = 0.46 in cyclohexane / ethyl acetate (2:1).

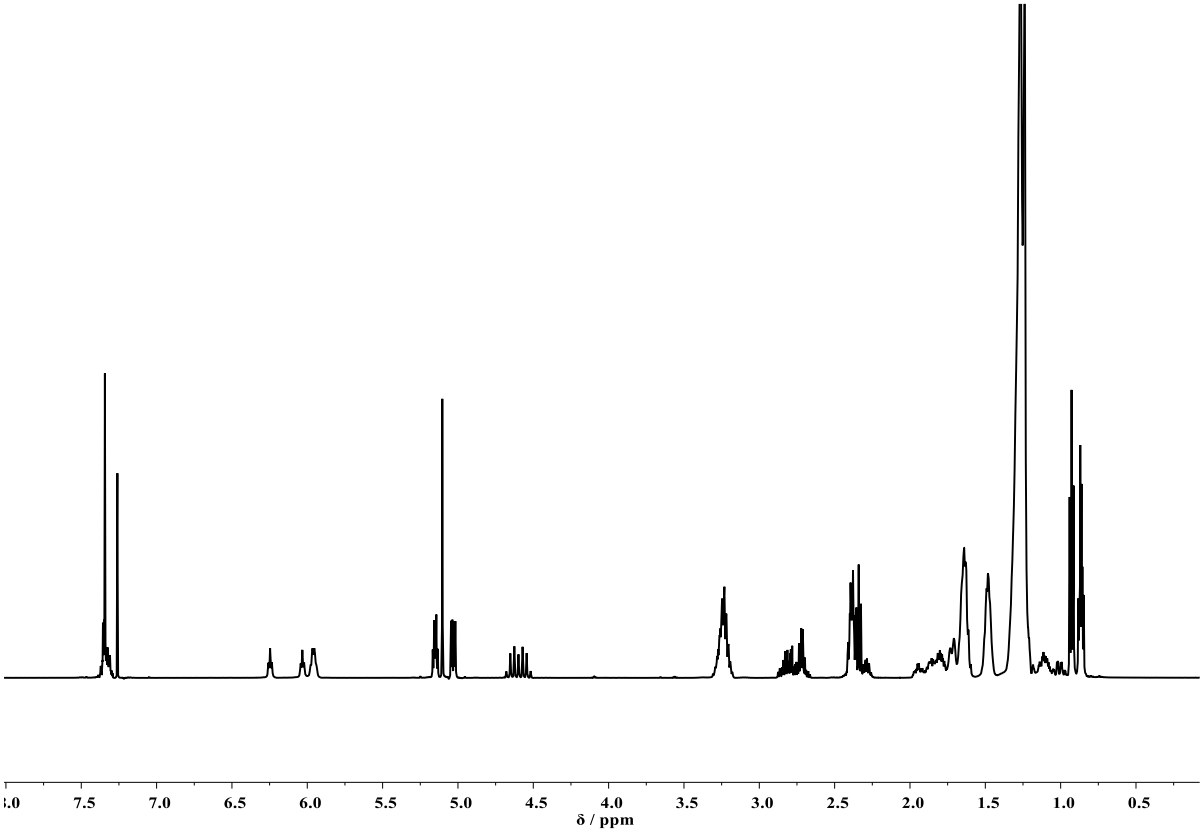
IR (ATR): ν / cm^{-1} = 3302.4 (w), 2920.6 (vs), 2851.2 (s), 1735.2 (vs), 1655.3 (vs), 1555.1 (m), 1466.2 (m), 1377.7 (w), 1228.1 (vs), 1207.0 (vs), 1172.6 (vs), 1020.5 (w), 722.7 (w), 696.5 (m).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.35 – 7.22 (m, 5 H, CH_{Ar}^1), 6.19 (t, J = 5.8 Hz, 1 H, NH^2), 5.97 (t, J = 5.9 Hz, 1 H, NH^2), 5.93 – 5.81 (m, 2 H, NH^2), 5.12 – 5.07 (m, 2 H, CH^3), 5.04 (s, 2 H, CH_2^4), 4.98 (d, J = 4.4 Hz, 1 H, CH^5), 4.96 (d, J = 4.6 Hz, 1 H, CH^5), 4.65 – 4.43 (m, 2 H, CH_2^6), 3.29 – 3.09 (m, 8 H, CH_2^7), 2.84 – 2.60 (m, 4 H, CH_2^8), 2.41 – 2.18 (m, 9 H, CH^9,CH_2^{10}), 1.95 – 1.52 (m, 21 H, CH^{11}, CH_2^{12}), 1.48 – 1.36 (m, 8 H, CH_2^{13}), 1.30 – 0.94 (m, 18 H, CH_2^{12}), 0.87 (t, J = 6.5 Hz, 6 H, CH_3^{14}), 0.85 – 0.76 (m, 6 H, CH_3^{15}).

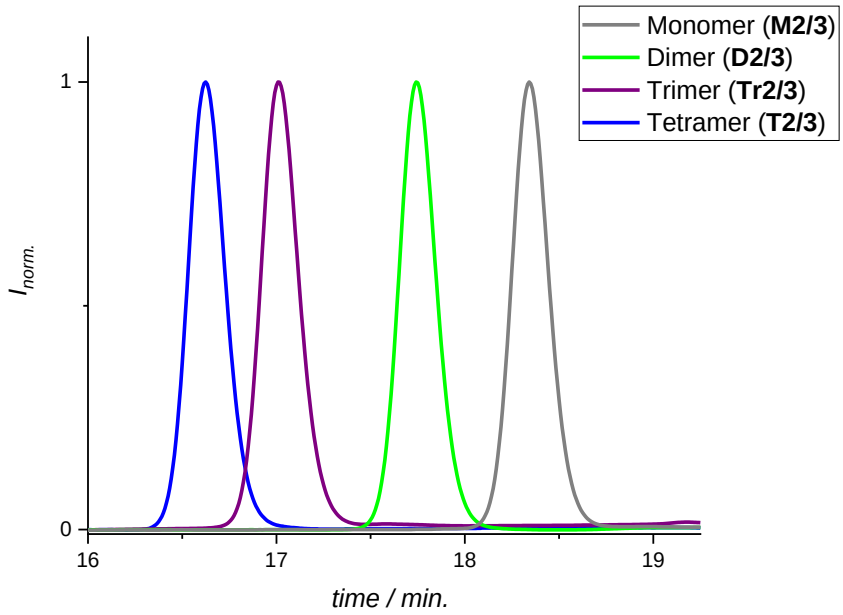
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 173.80, 172.67, 172.66, 172.60, 171.49, 170.89, 169.98, 169.62, 169.40, 169.32, 136.24, 128.66, 128.28, 128.27, 78.03, 77.74, 74.86, 74.05, 66.19, 59.66 (t, J = 27.0 Hz), 40.10, 39.43, 39.31, 39.27, 34.44, 34.41, 32.04, 31.94, 31.86, 30.64, 29.78, 29.77, 29.74, 29.72, 29.70, 29.67, 29.58, 29.57, 29.55, 29.54, 29.51, 29.49, 29.47, 29.36, 29.34, 29.32, 29.26, 29.23, 29.03, 28.78, 27.41, 26.97, 26.95, 26.20, 26.12, 26.01, 25.12, 25.09, 25.07, 25.01, 24.89, 22.81, 22.74, 18.90, 17.07, 14.25, 14.21.

^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.14 (t, J = 9.2 Hz, 3 F, CF_3[16]), -124.10 – -125.56 (m, 2 F, CF_2[17]), -131.03 – -133.61 (m, 2 F, CF_2[17]). Total integral of CF_2 region normalized with respect to the CF_3[16] group = 4.

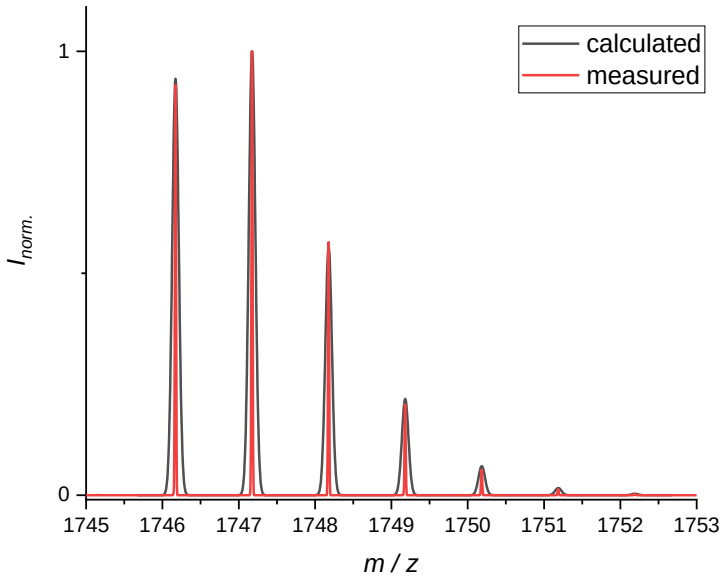
ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{95}{}^{1}H_{159}{}^{16}O_{16}{}^{14}N_4{}^{19}F_7$, 1746.1712; found, 1746.1708, Δ = 0.4 mmu.



Supplementary Figure 103: ^{1}H-NMR of compound **T2/3** measured in $CDCl_3$.



Supplementary Figure 104: SEC traces of the intermediates after each P-3CR during the synthesis of product **T2/3**.



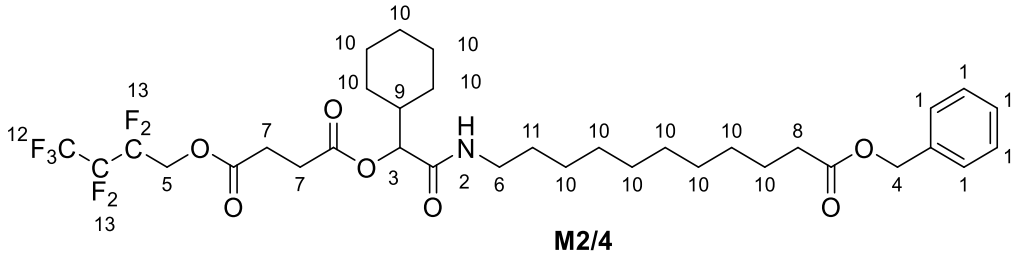
Supplementary Figure 105: High resolution ESI-MS measurement of **T2/3**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (black).

```
matching mass 1747.17426
cutoff 0.50000: 0 solutions (16 peaks)
cutoff 0.25000: 0 solutions (42 peaks)
cutoff 0.12500: 0 solutions (106 peaks)
cutoff 0.06250: 0 solutions (216 peaks)
cutoff 0.03125: 0 solutions (394 peaks)
cutoff 0.01562: 0 solutions (654 peaks)
cutoff 0.00781: 1 solutions (1071 peaks)
1747.17426 ≈ 283.020520 + 409.355600 + 283.214750 + 339.277350 + 323.246050 + 107.049690 (sides Tridecanal,
 Isobutyraldehyde, Octanal, Cyclohexancarboxaldehyde; error -2.01030)
Press ENTER to quit ...
```

Supplementary Figure 106: Screenshot of the automated read-out of **T2/3**.

6.3.3.4.5 Synthesis of tetramer T2/4

Passerini reaction



M2/4

In a 50 mL round bottom flask, 304 mg **TAG2** (1.01 mmol, 1.00 eq.) were stirred in 3.00 mL dichloromethane. Subsequently, 204 µL cyclohexancarboxaldehyde **A6** (170 mg, 1.52 mmol, 1.50 eq.) and 458 mg of monomer **IM2** (1.52 mol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (7:1 → 5:1) to yield the Passerini product **M2/4** as a pale highly viscous oil. (663 mg, 927 µmol, 91.8%).

R_f = 0.47 in cyclohexane / ethyl acetate (3:1).

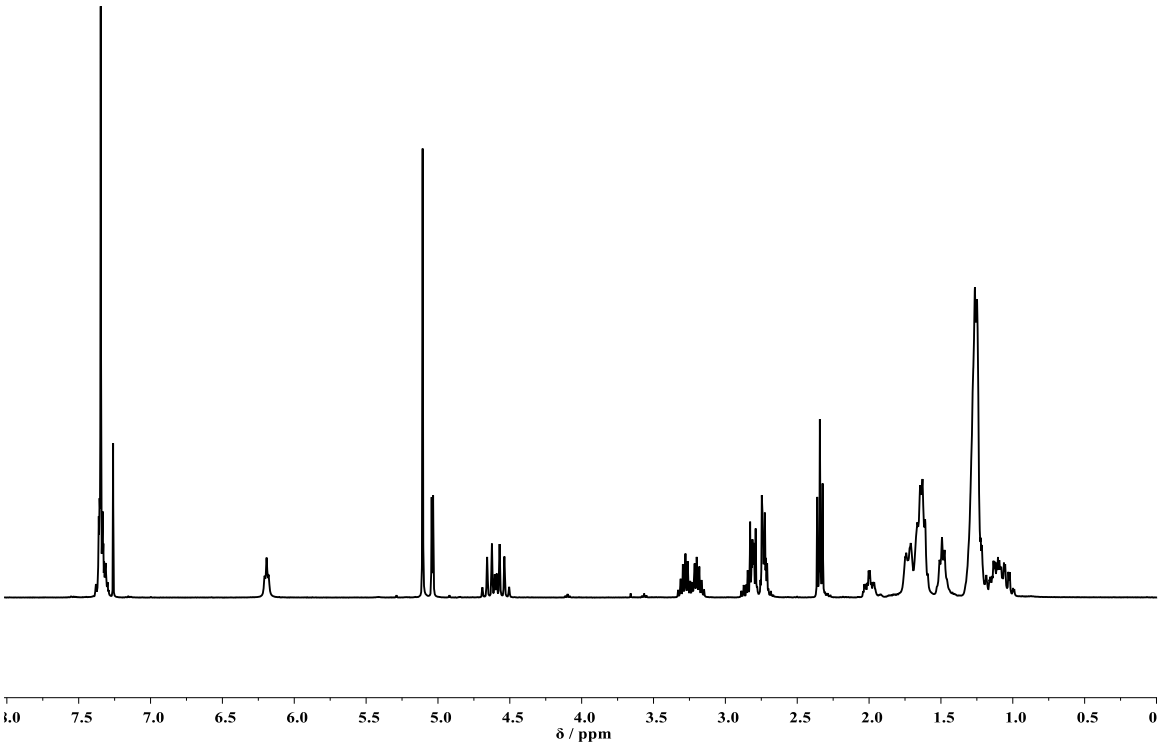
IR (ATR): ν / cm^{-1} = 3306.8 (vw), 2927.0 (m), 2854.6 (w), 1737.7 (s), 1655.8 (m), 1534.5 (w), 1452.0 (w), 1351.8 (w), 1226.0 (vs), 1142.5 (vs), 1019.7 (m), 910.1 (m), 735.9 (m), 697.2 (m).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.46 – 7.29 (m, 5 H, CH_{Ar}^1), 6.19 (t, J = 5.8 Hz, 1 H, NH^2), 5.11 (s, 2 H, CH_2^3), 5.04 (d, J = 4.0 Hz, 1 H, CH^4), 4.74 – 4.46 (m, 2 H, CH_2^5), 3.35 – 3.12 (m, 2 H, CH_2^6), 2.91 – 2.66 (m, 4 H, CH_2^7), 2.34 (t, J = 7.6 Hz, 2 H, CH_2^8), 2.07 – 1.92 (m, 1 H, CH_2^9), 1.80 – 1.57 (m, 8 H, CH_2^{10}), 1.55 – 1.42 (m, 2 H, CH_2^{11}), 1.37 – 0.96 (m, 16 H, CH_2^{10}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.83, 171.54, 170.93, 168.95, 136.27, 128.67, 128.29, 78.58, 66.19, 59.71 (t, J = 27.1 Hz), 39.91, 39.40, 34.45, 29.57, 29.56, 29.47, 29.42, 29.33, 29.23, 29.00, 28.80, 27.14, 26.97, 26.15, 26.01, 25.06.

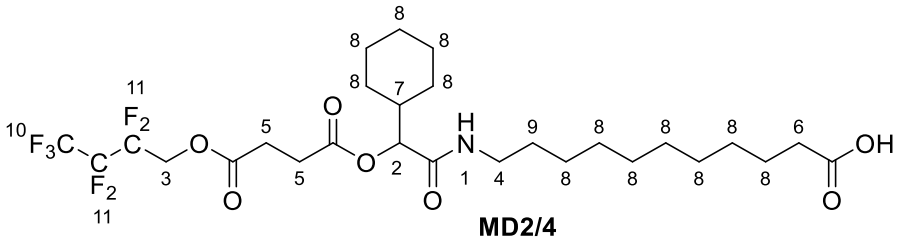
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.16 (t, J = 9.2 Hz, 3 F, CF_3^{12}), -124.49 – -125.85 (m, 2 F, CF_2^{13}), -131.00 – -132.82 (m, 2 F, CF_2^{13}).Total integral of CF_2 region normalized with respect to the CF_3^{13} group = 4.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{34}{}^1H_{46}{}^{16}O_7{}^{14}N^{19}F_7$, 714.3235; found, 714.3226, Δ = 0.9 mmu.



Supplementary Figure 107: ^{1}H-NMR of compound **M2/4** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 625 mg of **M2/4** (876 µmol, 1.00 eq.) were dissolved in 3.00 mL ethyl acetate and 3.00 mL THF. Afterwards, 125 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **MD2/4** was obtained as a pale highly viscous oil in a yield of 99.6% (544 mg, 872 µmol).

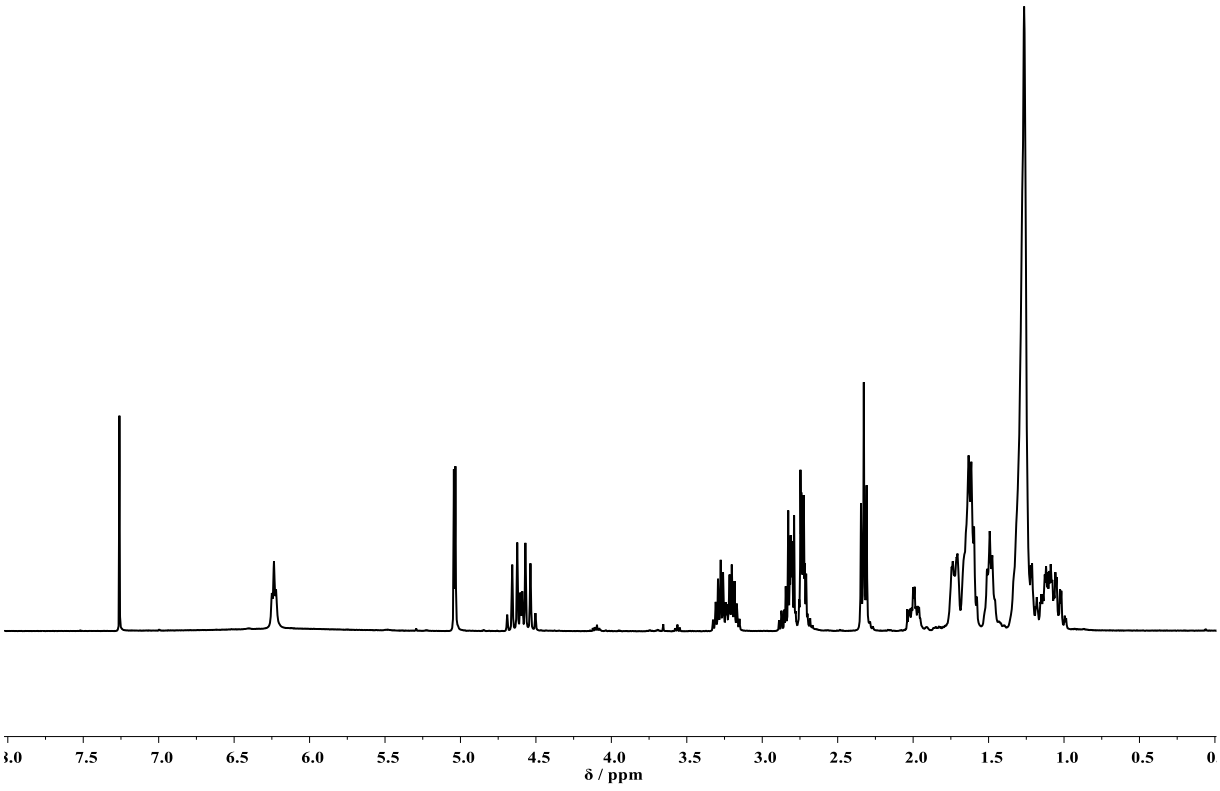
IR (ATR): ν / cm^{-1} = 3313.2 (w), 2926.8 (s), 2853.3 (m), 1765.1 (s), 1736.3 (vs), 1686.1 (vs), 1655.2 (vs), 1552.0 (s), 1447.0 (w), 1381.9 (w), 1352.7 (m), 1298.0 (m), 1217.5 (vs), 1144.9 (vs), 1115.9 (vs), 1084.8 (m), 1023.4 (w), 984.8 (s), 955.5 (m), 912.4 (m), 872.9 (w), 843.8 (vw), 784.1 (w), 737.0 (vs), 671.5 (w), 573.5 (w), 540.4 (w), 451.3 (w), 381.1 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.17 (t, J = 5.8 Hz, 1 H, NH^{1}), 4.98 (d, J = 4.1 Hz, 1 H, CH^{2}), 4.68 – 4.41 (m, 2 H, CH_2^{3}), 3.31 – 3.07 (m, 2 H, CH_2^{4}), 2.84 – 2.59 (m, 4 H, CH_2^{5}), 2.27 (t, J = 7.5 Hz, 2 H, CH_2^{6}), 2.00 – 1.87 (m, 1 H, CH^{7}), 1.73 – 1.51 (m, 6 H, CH_2^{8}), 1.47 – 1.37 (m, 2 H, CH_2^{9}), 1.30 – 0.90 (m, 18 H, CH_2^{8}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 179.13, 171.58, 170.98, 169.10, 78.56, 59.71 (t, J = 27.0 Hz), 39.88, 39.42, 34.12, 29.47, 29.40, 29.35, 29.23, 29.22, 29.09, 28.99, 28.79, 27.12, 26.91, 26.13, 26.00, 24.81.

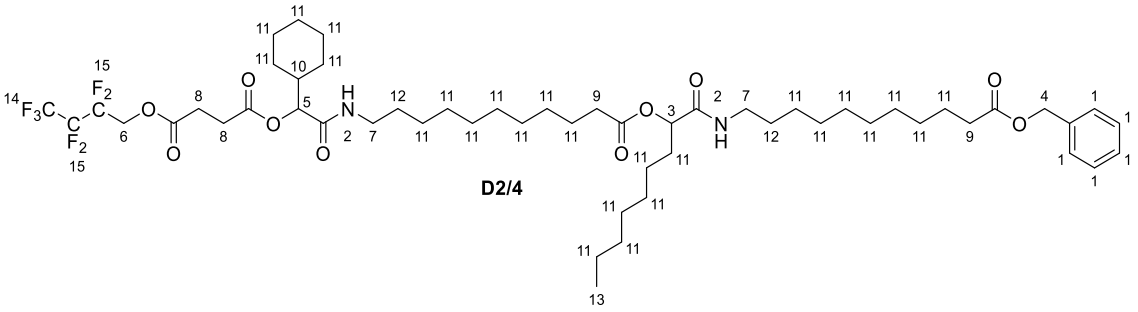
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.18 (t, J = 9.2 Hz, 3 F, CF_3^{10}), -124.03 –125.46 (m, 2 F, CF_2^{11}), -131.58 – -132.63 (m, 2 F, CF_2^{11}). Total integral of CF_2 region normalized with respect to the CF_3^{10} group = 4.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{27}{}^{1}H_{40}{}^{16}O_7{}^{14}N{}^{19}F_7$, 624.2766; found, 624.2752, Δ = 1.4 mmu.



Supplementary Figure 108: ^{1}H-NMR of compound **MD2/4** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 419 mg of **MD2/4** (671 µmol, 1.00 eq.) were stirred in 3.00 mL dichloromethane. Subsequently, 194 µL octanal **A8** (160 mg, 1.25 mmol, 1.85 eq.) and 374 mg of monomer **IM2** (1.25 mol, 1.85 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (4:1 → 2:1) to yield the Passerini product **D2/4** as a pale highly viscous oil. (663 mg, 927 µmol, 91.8%).

R_f = 0.32 in cyclohexane / ethyl acetate (2:1).

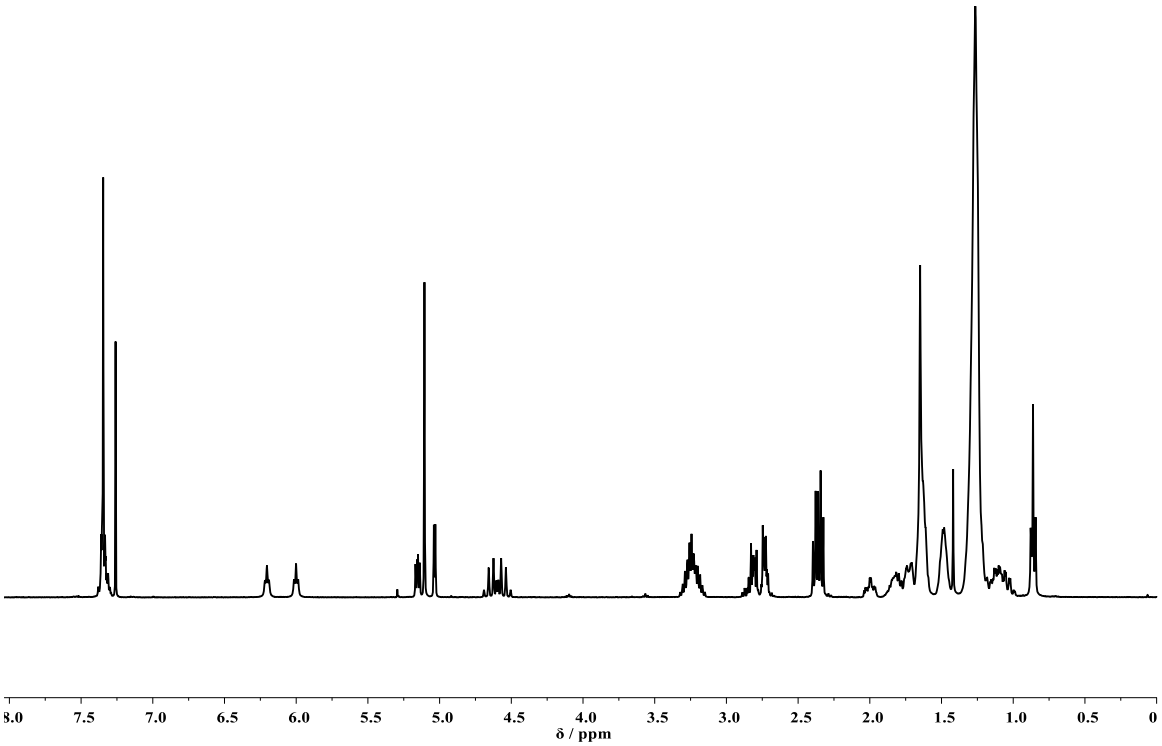
IR (ATR): ν / cm^{-1} = 3293.7 (w), 2918.5 (s), 2850.5 (s), 1728.6 (vs), 1679.6 (m), 1651.3 (vs), 1533.4 (m), 1466.9 (w), 1320.5 (m), 1227.7 (vs), 1158.9 (vs), 1021.9 (m), 957.0 (w), 909.9 (w), 803.6 (vw), 735.6 (w), 720.1 (w), 697.8 (m), 628.5 (w), 539.8 (w), 449.2 (vw), 383.7 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.45 – 7.28 (m, 5 H, $CH_{Ar}{}^1$), 6.20 (t, J = 5.8 Hz, 1 H, NH^2), 6.00 (t, J = 5.8 Hz, 1 H, NH^2), 5.19 – 5.13 (m, 1 H, CH^3), 5.11 (s, 2 H, $CH_2{}^4$), 5.03 (d, J = 4.1 Hz, 1 H, CH^5), 4.72 – 4.48 (m, 2 H, $CH_2{}^6$), 3.36 – 3.13 (m, 4 H, $CH_2{}^7$), 2.90 – 2.66 (m, 4 H, $CH_2{}^8$), 2.44 – 2.30 (m, 4 H, $CH_2{}^9$), 2.08 – 1.93 (m, 1 H, CH^{10}), 1.90 – 1.56 (m, 14 H, $CH_2{}^{11}$), 1.56 – 1.44 (m, 4 H, $CH_2{}^{12}$), 1.38 – 0.98 (m, 36 H, $CH_2{}^{11}$), 0.90 – 0.83 (m, 3 H, $CH_3{}^{13}$).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.83, 172.59, 171.55, 170.93, 169.99, 168.96, 136.26, 128.68, 128.29, 78.58, 74.07, 66.21, 59.84 (t, J = 27.0 Hz), 39.91, 39.39, 39.33, 34.46, 32.04, 31.86, 29.69, 29.60, 29.58, 29.57, 29.49, 29.42, 29.35, 29.33, 29.24, 29.23, 29.00, 28.80, 27.14, 27.05, 26.97, 26.15, 26.02, 25.09, 25.08, 24.88, 22.74, 14.20.

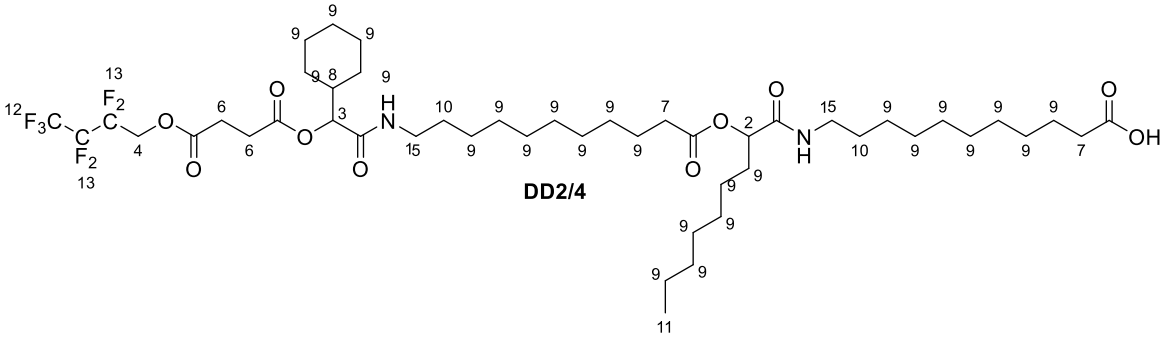
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.15 (t, J = 9.7 Hz, 3 F, $CF_3{}^{14}$), -124.10 – -125.74 (m, 2 F, $CF_2{}^{15}$), -130.32 – -133.43 (m, 2 F, $CF_2{}^{15}$). Total integral of CF_2 region normalized with respect to the $CF_3{}^{14}$ group = 4.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{54}{}^{1}H_{83}{}^{16}O_{10}{}^{14}N_2{}^{19}F_7$, 1053.6009; found, 1053.6000, Δ = 0.9 mmu.



Supplementary Figure 109: ^{1}H-NMR of compound **D2/4** measured in $CDCl_3$.

Deprotection



In a 25 mL round bottom flask, 497 mg of **D2/4** (472 µmol, 1.00 eq.) was dissolved in 4.00 mL ethyl acetate and 4.00 mL THF. Subsequently, 99.4 mg (20 wt%) palladium on activated carbon **1** was added to the solution. The resulting mixture was purged with hydrogen gas and stirred for 1 day at room temperature under hydrogen atmosphere (3 balloons). The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **DD2/4** was obtained as a yellow high viscos oil. (429 mg, 445 µmol, 94.4%).

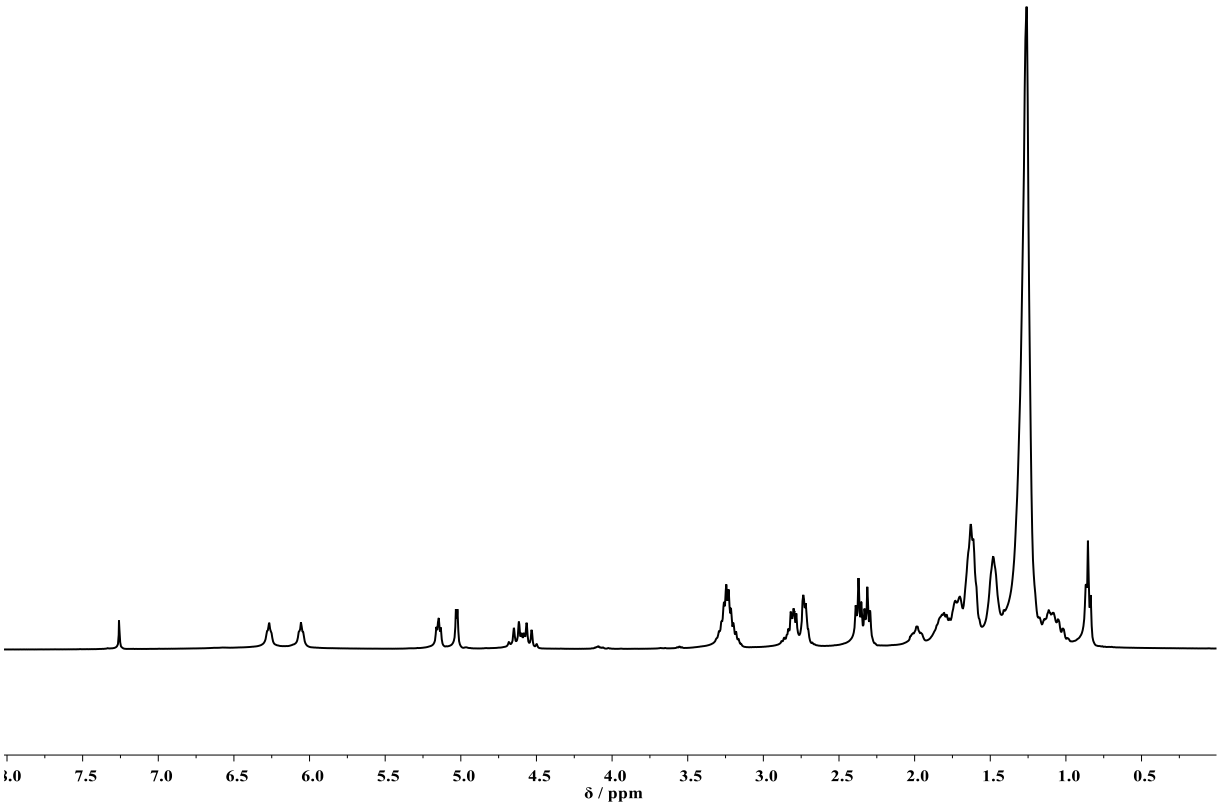
IR (ATR): ν / cm^{-1} = 3316.2 (vw), 2927.8 (w), 2855.4 (vw), 2360.7 (w), 2343.5 (vw), 2328.7 (vw), 2154.8 (vw), 1743.0 (w), 1656.5 (vw), 1544.0 (vw), 1453.7 (vw), 1354.3 (vw), 1229.6 (w), 1181.7 (vw), 1146.8 (w), 1020.7 (vw), 736.0 (vw), 443.1 (vw), 418.4 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.29 (t, J = 6.0 Hz, 1 H, NH^{1}), 6.07 (t, J = 6.0 Hz, 1 H, NH^{1}), 5.16 (t, J = 6.1 Hz, 1 H, CH^{2}), 5.05 (d, J = 4.2 Hz, 1 H, CH^{3}), 4.76 – 4.47 (m, 2 H, CH_2^{4}), 3.41 – 3.12 (m, 4 H, CH_2^{5}), 2.93 – 2.69 (m, 4 H, CH_2^{6}), 2.48 – 2.28 (m, 4 H, CH_2^{7}), 2.10 – 1.95 (m, 1 H, CH^{8}), 1.92 – 0.99 (m, 54 H, CH_2^{9}), 0.87 (t, J = 6.6 Hz, 3 H, CH_3^{10}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 178.20, 172.61, 171.54, 170.96, 170.11, 169.12, 78.57, 74.08, 59.71 (t, J = 27.1 Hz), 39.89, 39.44, 39.33, 34.45, 34.13, 32.01, 31.84, 29.59, 29.53, 29.48, 29.38, 29.35, 29.31, 29.25, 29.20, 29.13, 28.99, 28.79, 27.17, 26.96, 26.90, 26.13, 26.00, 25.10, 24.89, 22.72, 14.16.

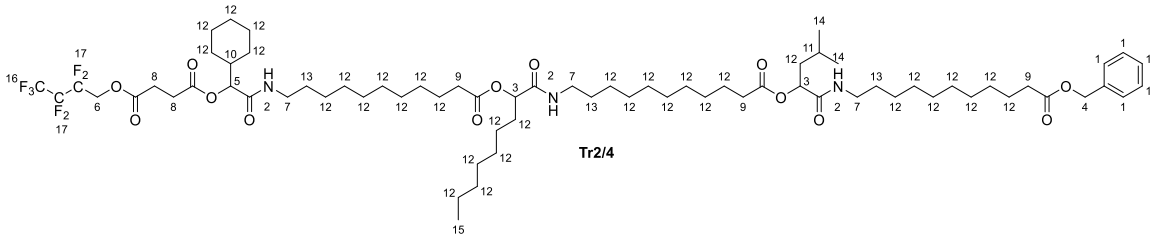
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -84.58 – -85.52 (m, 3 F, CF_3^{11}), -124.10 – -125.92 (m, 2 F, CF_2^{12}), -130.67 – -133.43 (m, 2 F, CF_2^{12}). Total integral of CF_2 region normalized with respect to the CF_3^{11} group = 4.

ESI-MS [m/z]: $[M + Na]^+$ calculated for $^{12}C_{47}{}^{1}H_{77}{}^{16}O_{10}{}^{14}N_2{}^{19}F_7{}^{23}Na$, 985.5359; found, 985.5341, Δ = 1.8 mmu.



Supplementary Figure 110: ^{1}H-NMR of compound **DD2/4** measured in $CDCl_3$.

Passerini reaction[iii]



In a 50 mL round bottom flask, 361 mg of **DD2/4** (375 µmol, 1.00 eq.) were stirred in 4.00 mL dichloromethane. Subsequently, 60.5 µL 3-methylbutyraldehyde **A4** (48.4 mg, 562 µmol, 1.50 eq.) and 169 mg of monomer **IM2** (562 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (5:1 → 2:1) to yield the Passerini product **Tr2/4** as a pale highly viscous oil. (377 mg, 279 µmol, 74.5%).

R_f = 0.30 in cyclohexane / ethyl acetate (2:1).

IR (ATR): ν / cm^{-1} = 3307.8 (vw), 2926.3 (w), 2854.6 (w), 1740.3 (w), 1654.7 (w), 1537.7 (w), 1454.8 (vw), 1353.3 (vw), 1227.3 (m), 1144.9 (m), 1020.0 (vw), 910.7 (vw), 735.7 (vw), 697.4 (vw), 453.0 (vw), 431.1 (vw).

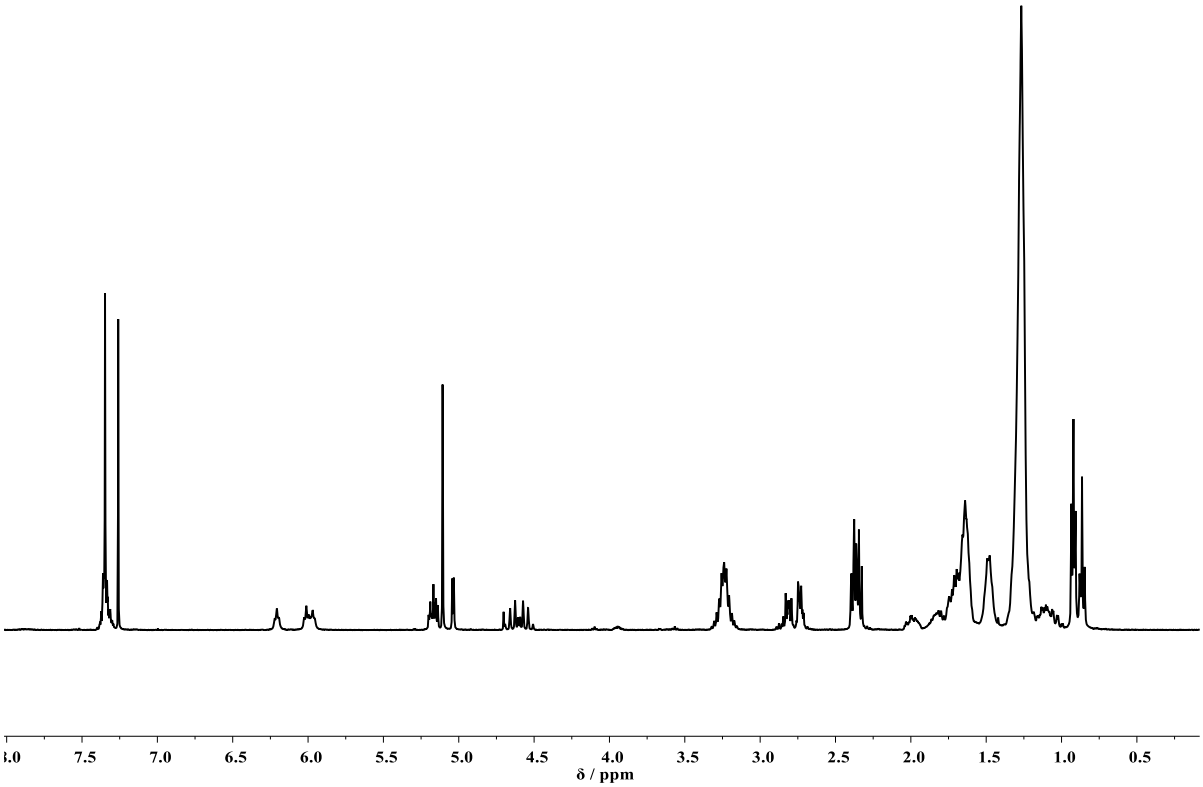
^{1}H-NMR (400 MHz, $CDCl_3$): δ / ppm = 7.44 – 7.28 (m, 5 H, CH_{Ar}^1), 6.21 (t, J = 5.8 Hz, 1 H, NH^2), 6.07 – 5.91 (m, 2 H, NH^2), 5.26 – 5.14 (m, 2 H, CH^3), 5.11 (s, 2 H, CH_2^4), 5.04 (d, J = 4.0 Hz, 1 H, CH^5), 4.74 – 4.45 (m, 2 H, CH_2^6), 3.35 – 3.14 (m, 6 H, CH_2^7), 2.91 – 2.68 (m, 4 H, CH_2^8), 2.44 – 2.30 (m, 6 H, CH_2^9), 2.08 – 1.91 (m, 1 H, CH^{10}), 1.90 – 1.56 (m, 17 H, CH^{11}, CH_2^{12}), 1.56 – 1.38 (m, 6 H, CH_2^{13}), 1.38 – 0.99 (m, 50 H, CH_2^{12}), 0.92 (t, J = 5.7 Hz, 6 H, CH_3^{14}), 0.89 – 0.79 (m, 3 H, CH_3^{15}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.82, 172.77, 172.60, 171.55, 170.93, 170.34, 170.00, 168.95, 136.27, 128.68, 128.30, 78.58, 74.07, 72.78, 66.21, 59.71, 40.99, 39.91, 39.39, 39.37, 39.32, 34.46, 32.05, 31.86, 30.23, 29.70, 29.66, 29.59, 29.57, 29.50, 29.48, 29.43, 29.34, 29.24, 29.00, 28.81, 27.14, 26.97, 26.94, 26.15, 26.02,25.09, 25.08, 25.06, 24.90, 24.69, 23.82, 23.27, 22.74, 21.94, 14.20.

[iii] Synthesis was carried out by Lara Faden in the Vertieferarbeit "Synthesis and characterization of monodisperse sequence-defined oligomers" under the laboratory supervision of Maximiliane Frölich.[290]

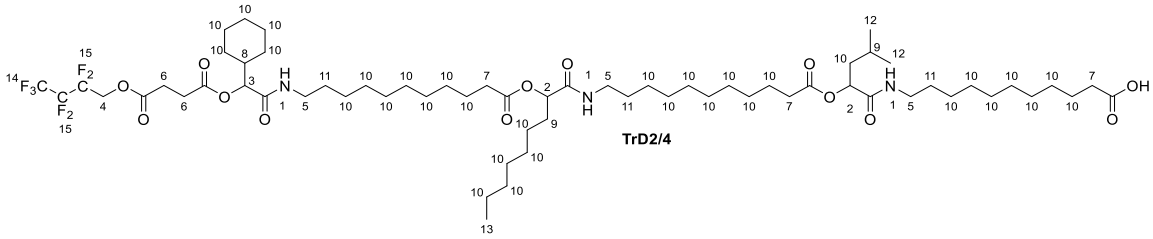
^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.14 (t, J = 9.5 Hz, 3 F, CF_3[16]), -124.79 – -124.91 (m, 2 F, CF_2[17]), -131.94 – -132.04 (m, 2 F, CF_2[17]). Total integral of CF_2 region normalized with respect to the CF_3[16] group = 4.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{71}{}^{1}H_{114}{}^{16}O_{13}{}^{14}N_3{}^{19}F_7$, 1350.8313; found, 1350.8312, Δ = 0.1 mmu.



Supplementary Figure 111: ^{1}H-NMR of compound **Tr2/4** measured in $CDCl_3$.

Deprotection[iv]



In a 25 mL round bottom flask, 335 mg of **Tr2/4** (248 µmol, 1.00 eq.) was dissolved in 4.00 mL ethyl acetate and 4.00 mL THF. Subsequently, 84.6 mg (20 wt%) palladium on activated carbon **1** was added to the solution. The resulting mixture was purged with hydrogen gas and stirred for 1 day at room temperature under hydrogen atmosphere (3 balloons). The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **TrD2/4** was obtained as a yellow high viscos oil (308 mg, 244 µmol, 98.5%).

IR (ATR): ν / cm^{-1} = 3309.2, 2925.5, 2854.6, 2036.6, 1987.3, 1742.0, 1654.5, 1540.1, 1465.2, 1369.3, 1227.8, 1145.9, 1021.0, 910.8, 735.9, 472.7, 422.0.

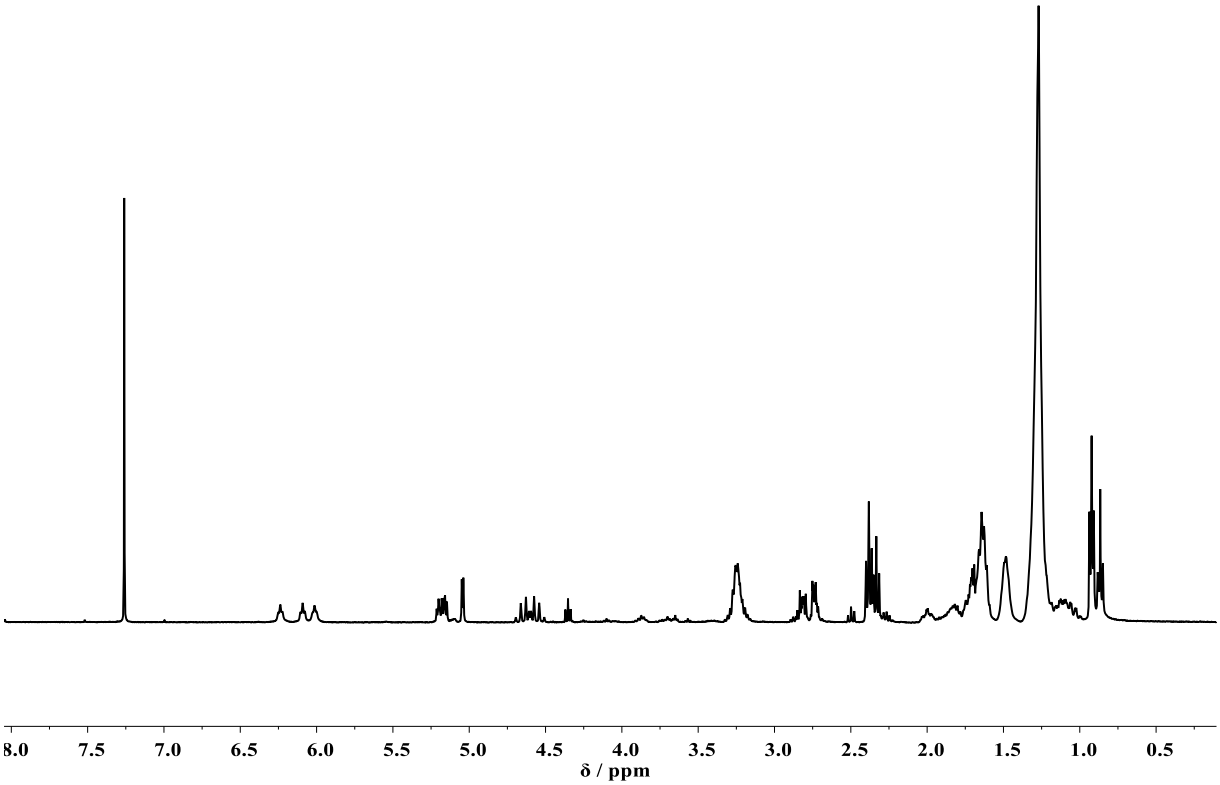
^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.28 – 6.17 (m, 1 H, NH^1), 6.11 – 6.07 (m, 1 H, NH^1), 6.03 – 5.97 (m, 1 H, NH^1), 5.24 – 5.11 (m, 2 H, CH^2), 5.04 (d, J = 4.0 Hz, 1 H, CH^3), 4.71 – 4.48 (m, 2 H, CH_2^4), 3.33 – 3.15 (m, 6 H, CH_2^5), 2.91 – 2.67 (m, 4 H, CH_2^6), 2.43 – 2.29 (m, 6 H, CH_2^7), 2.04 – 1.94 (m, 1 H, CH^8), 1.93 – 1.59 (m, 17 H, CH^9, CH_2^{10}), 1.56 – 1.42 (m, 6 H, CH_2^{11}), 1.34 – 0.90 (m, 50 H, CH_2^{10}), 0.92 (t, J = 5.7 Hz, 6 H, CH_3^{12}), 0.87 (t, J = 6.8 Hz, 3 H, CH_3^{13}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 176.62, 172.82, 172.72, 171.59, 170.96, 170.43, 170.21, 169.08, 78.56, 74.07, 72.80, 40.93, 39.89, 39.42, 39.32, 34.45, 33.85, 32.01, 31.87, 29.67, 29.61, 29.60, 29.55, 29.52, 29.51, 29.42, 29.38, 29.34, 29.32, 29.29, 29.27, 29.23, 29.18, 29.05, 29.00, 28.81, 27.13, 26.97, 26.84, 26.15, 26.02, 25.10, 25.09, 24.89, 24.69, 23.27, 22.75, 21.92, 14.21.

^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.14 (t, J = 9.6 Hz, 3 F, CF_3^{14}), -124.79 – -124.90 (m, 2 F, CF_2^{15}), -131.90 – -132.10 (m, 2 F, CF_2^{15}). Total integral of CF_2 region normalized with respect to the CF_3^{14} group = 4.

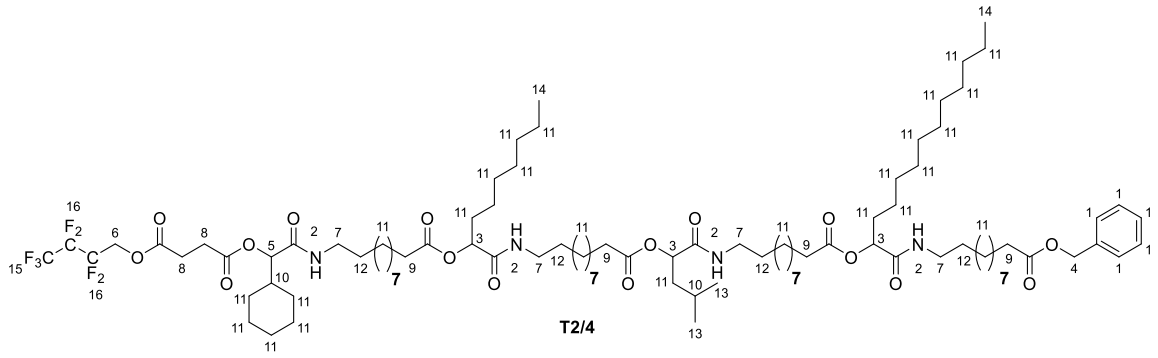
ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{64}{}^{1}H_{108}{}^{16}O_{13}{}^{14}N_3{}^{19}F_7$, 1260.7843; found, 1260.7826, Δ = 1.7 mmu.

[iv] Synthesis was carried out by Lara Faden in the Vertieferarbeit "Synthesis and characterization of monodisperse sequence-defined oligomers" under the laboratory supervision of Maximiliane Frölich.[290]



Supplementary Figure 112: ^{1}H-NMR of compound **TrD2/4** measured in $CDCl_3$.

Passerini reaction[v]



In a 50 mL round bottom flask, 264 mg of **TrD2/4** (210 µmol, 1.00 eq.) were stirred in 3.00 mL DCM. Subsequently, 57.9 mg dodecanal **A10** (314 µmol, 1.50 eq.) and 94.7 mg of monomer **IM2** (314 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting

[v] Synthesis was carried out by Lara Faden in the Vertieferarbeit "Synthesis and characterization of monodisperse sequence-defined oligomers" under the laboratory supervision of Maximiliane Frölich.[290]

with a gradual solvent mixture of cyclohexane and ethyl acetate (3:1 → 2:1) to yield the Passerini product **T2/4** as a pale highly viscous oil. (292 mg, 167 µmol, 79.7%).

R_f = 0.68 in cyclohexane / ethyl acetate (1:1).

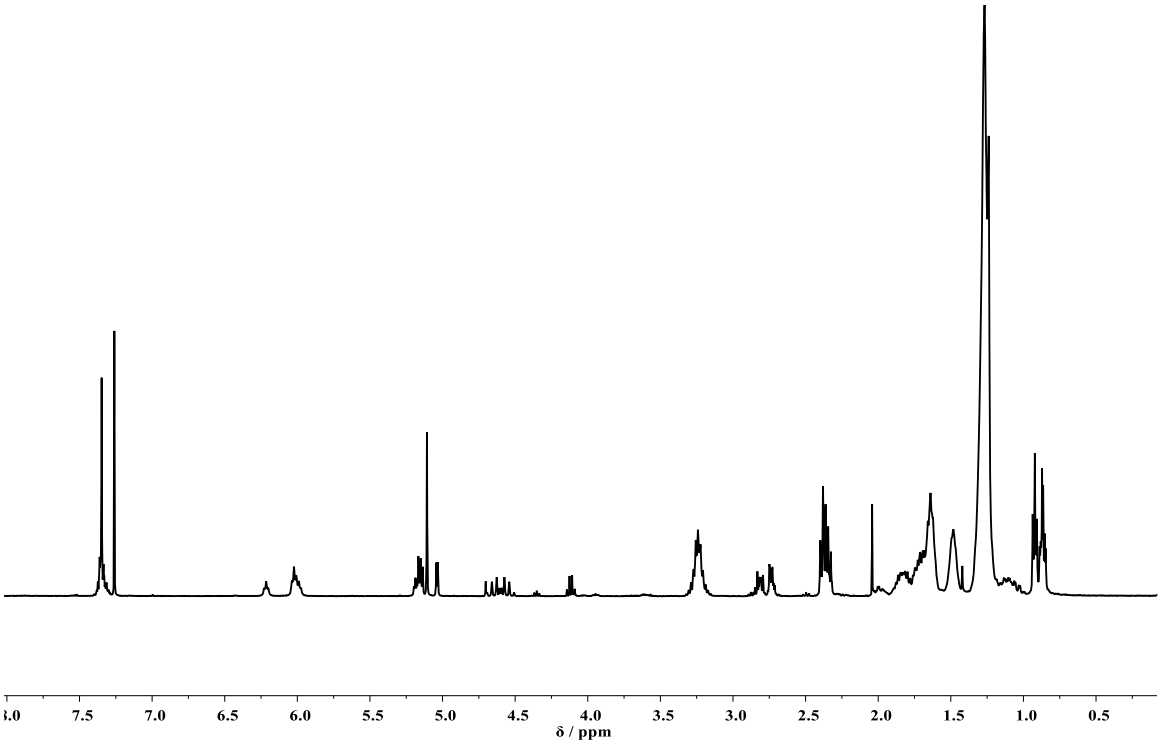
IR (ATR): ν / cm^{-1} = 3302.0, 2922.6, 2852.3, 2165.9, 1739.0, 1656.3, 1540.6, 1466.3, 1369.9, 1228.7, 1171.8, 1022.2, 722.6, 697.9 (vw).

^{1}H-NMR (400 MHz, $CDCl_3$): δ / ppm = 7.41 – 7.28 (m, 5 H, $CH_{Ar}{}^1$), 6.21 (t, J = 5.9 Hz, 1 H, NH^2), 6.08 – 5.93 (m, 3 H, NH^2), 5.22 – 5.13 (m, 3 H, CH^3), 5.11 (s, 2 H, $CH_2{}^4$), 5.04 (d, J = 4.1 Hz, 1 H, CH^5), 4.72 – 4.46 (m, 2 H, $CH_2{}^6$), 3.34 – 3.14 (m, 8 H, $CH_2{}^7$), 2.91 – 2.68 (m, 4 H, $CH_2{}^8$), 2.43 – 2.29 (m, 8 H, $CH_2{}^9$), 2.03 – 1.57 (m, 26 H, CH^{10}, $CH_2{}^{11}$), 1.55 – 1.43 (m, 8 H, $CH_2{}^{12}$), 1.38 – 1.00 (m, 76 H, $CH_2{}^{11}$), 0.92 (t, J = 5.8 Hz, 6 H, $CH_3{}^{13}$), 0.90 – 0.84 (m, 6 H, $CH_3{}^{14}$).

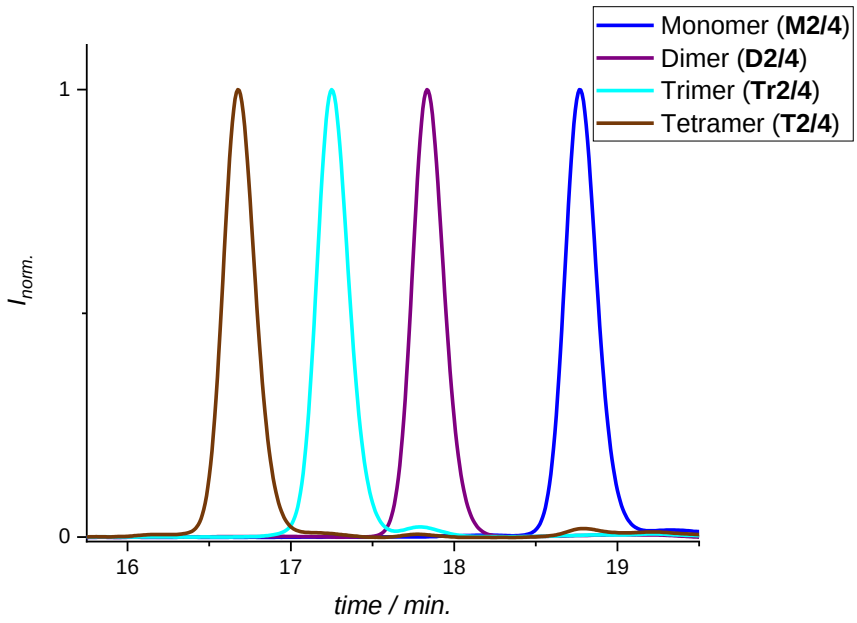
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.82, 172.79, 172.61, 171.56, 170.93, 170.36, 170.01, 169.99, 168.96, 136.26, 128.68, 128.30, 78.58, 74.07, 72.77, 66.21, 42.13, 40.99, 39.91, 39.39, 39.36, 39.33, 34.46, 32.05, 31.86, 29.76, 29.71, 29.68, 29.60, 29.58, 29.51, 29.48, 29.43, 29.40, 29.35, 29.24 (d, J = 1.9 Hz), 29.00, 28.81, 27.15, 26.97, 26.94, 26.15, 26.02, 25.10, 25.08, 25.06, 24.90, 24.69, 23.27, 22.82, 22.75, 21.93, 14.26, 14.21.

^{19}F NMR (376 MHz, $CDCl_3$): δ / ppm = -85.14 (t, J = 9.6 Hz, 3 F, $CF_3{}^{15}$), -123.51 – -127.56 (m, 2 F, $CF_2{}^{16}$), -130.32 – -134.20 (m, 2 F, $CF_2{}^{16}$). Total integral of CF_2 region normalized with respect to the $CF_3{}^{15}$ group = 4.

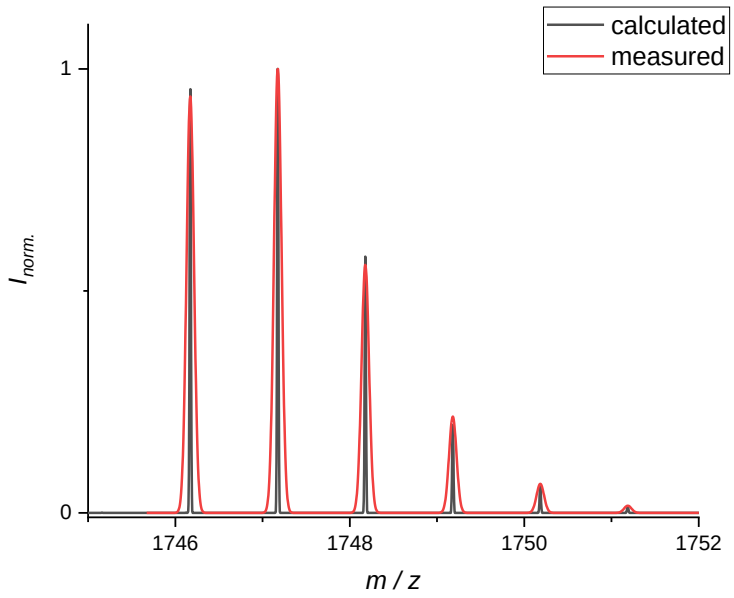
ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{95}{}^1H_{159}{}^{16}O_{16}{}^{14}N_4{}^{19}F_7$, 1746.1712; found, 1746.1712, Δ = 0.0 mmu.



Supplementary Figure 113: ^{1}H-NMR of compound **T2/4** measured in $CDCl_3$.



Supplementary Figure 114: SEC traces of the intermediates after each P-3CR in the synthesis of product **T2/4**.



Supplementary Figure 115: High resolution ESI-MS measurement of **T2/4**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (black).

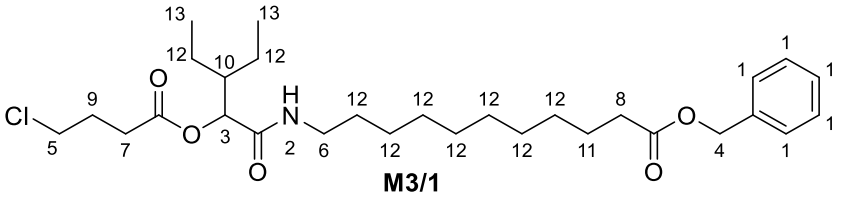
```
found 22108 values in C:\Users\Maxi\bwSyncShare\Dennis\Daten für Programm\LFMF 015 nce 18.csv,
maximum is 1.000000 found for mass 1746.171390
matching mass 1746.17139
cutoff 0.50000: 0 solutions (10 peaks)
cutoff 0.25000: 0 solutions (30 peaks)
cutoff 0.12500: 0 solutions (66 peaks)
cutoff 0.06250: 0 solutions (128 peaks)
cutoff 0.03125: 0 solutions (206 peaks)
cutoff 0.01562: 1 solutions (367 peaks)
1746.17139 ≈ 283.020520 + 323.246050 + 339.277350 + 297.230400 + 395.339950 + 107.049690 (sides
 Cyclohexancarboxaldehyde, Octanal, 3-Methylbutanal, Dodecanal; error -1.00743)
Press ENTER to quit ...
```

Supplementary Figure 116: Screenshot of the automated read-out of **T2/4**.

6.3.3.5 Oligomer synthesis with TAG3

6.3.3.5.1 Synthesis of tetramer T3/1[vi]

Passerini reaction



In a 50 mL round bottom flask, 205 µL 4-chlorobutyric acid **TAG3** (254 mg, 2.07 mmol, 1.00 eq.) were stirred in 2.00 mL DCM, subsequently 328 µL 2-ethybutanal **A5** (311 mg, 3.11 mmol, 1.50 eq.) and 936 mg of monomer **IM2** (3.11 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 3 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradient solvent mixture of cyclohexane and ethyl acetate (8:1 → 6:1) to yield product **M3/1** as a yellow, highly viscous oil. (992 mg, 1.88 mmol, 90.8%).

R_f = 0.52 in cyclohexane / ethyl acetate (2:1).

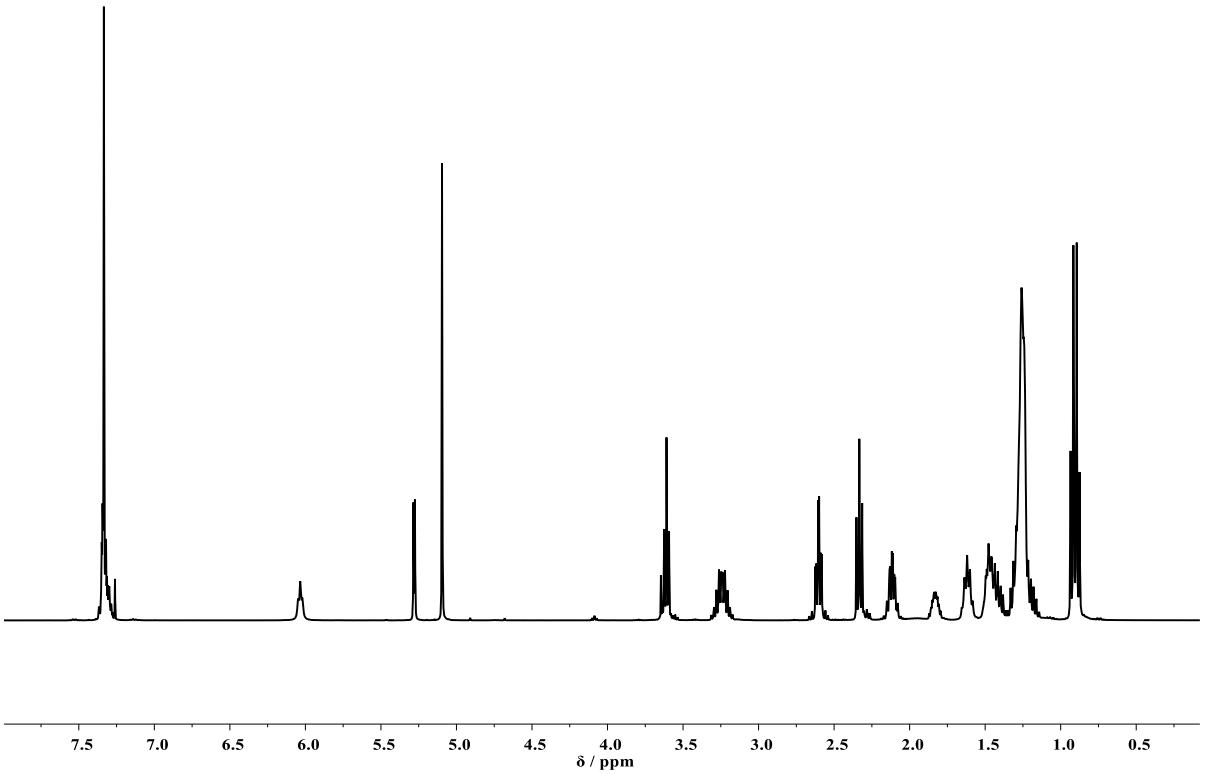
IR (ATR): ν / cm^{-1} = 3326.0 (vw), 2926.4 (m), 2854.3 (w), 1735.5 (vs), 1654.2 (s), 1531.1 (m), 1456.1 (m), 1379.6 (w), 1142.9 (s), 1004.2 (m), 785.6 (vw), 735.0 (m), 697.1 (m), 650.8 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.40 – 7.28 (m, 5 H, CH_{Ar}^1), 6.03 (t, J = 5.9 Hz, 1 H, NH^2), 5.28 (d, J = 3.8 Hz, 1 H, CH^3), 5.10 (s, 2 H, CH_2^4), 3.68 – 3.50 (m, 2 H, CH_2^5), 3.36 – 3.15 (m, 2 H, CH_2^6), 2.60 (td, J = 7.1, 2.5 Hz, 2 H, CH_2^7), 2.33 (t, J = 7.5 Hz, 2 H, CH_2^8), 2.18 – 2.07 (m, 2 H, CH_2^9), 1.88 – 1.77 (m, 1 H, CH^{10}), 1.69 – 1.33 (m, 2 H, CH_2^{11}), 1.53 – 1.13 (m, 18 H, CH_2^{12}), 0.95 – 0.86 (m, 6 H, CH_3^{13}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.74, 171.58, 169.57, 136.21, 128.60, 128.22, 75.47, 66.12, 63.43, 44.02, 43.60, 39.32, 34.38, 31.17, 29.57, 29.49, 29.39, 29.26, 29.15, 26.91, 25.00, 22.33, 21.97, 11.67, 11.65.

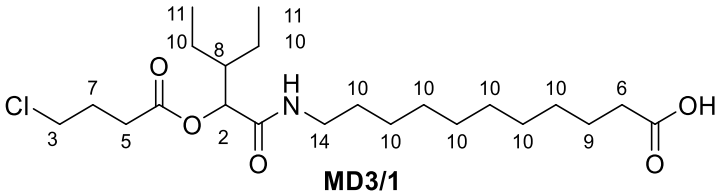
ESI-MS [*m*/z]: $[M+H]^+$ calculated for $^{12}C_{29}{}^{1}H_{46}{}^{16}O_5{}^{14}N^{35}Cl$: 524.3137; found: 524.3126; Δ = 1.1 mmu.

[vi] Synthesis up to the trimer was carried out by Nico Zuber in the Bachelor thesis "Synthesis of sequence-defined oligomers with 4-chlorobutyric acid as starter acid" under the laboratory supervision of Maximiliane Frölich.[291]



Supplementary Figure 117: ^{1}H-NMR of compound **M3/1** measured in $CDCl_3$.

Deprotection



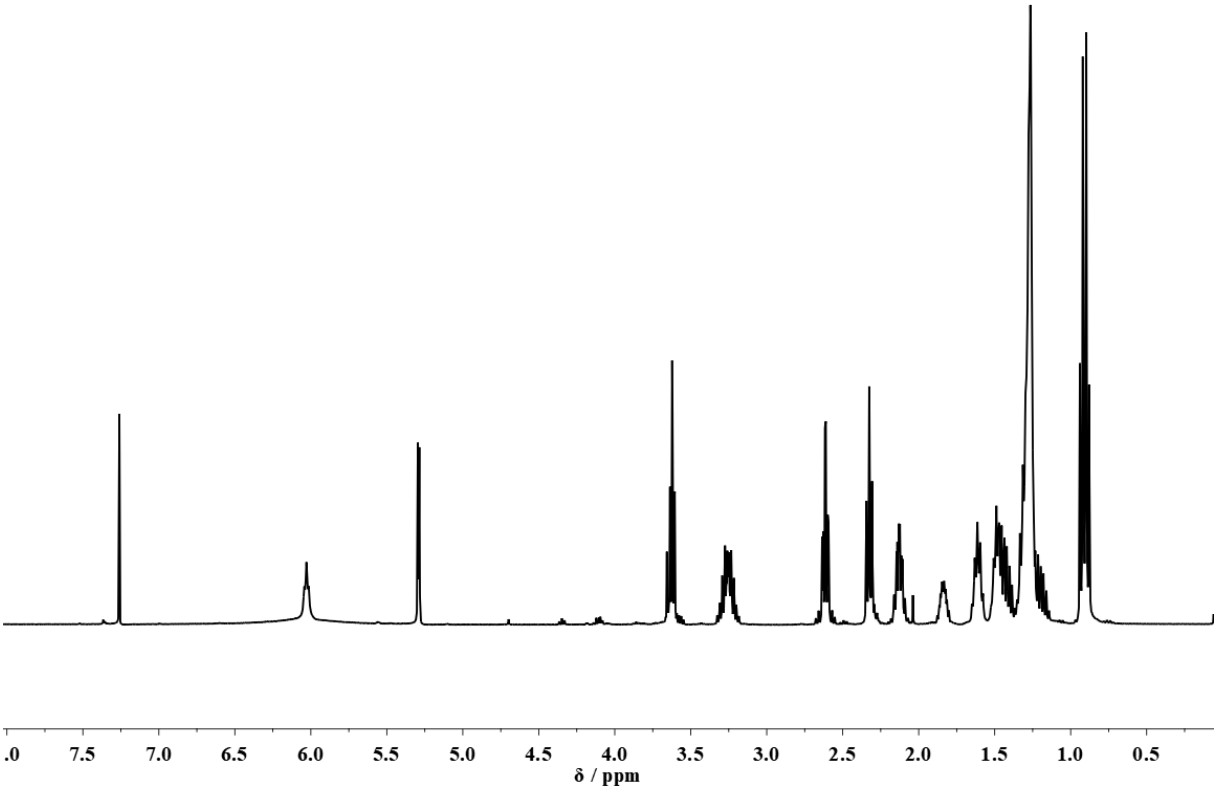
In a 50 mL round bottom flask, 444 mg of the Passerini product **M3/1** (850 µmol, 1.00 eq.) were dissolved in 2.00 mL ethyl acetate and 2.00 mL THF. Afterwards, 99.0 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **MD3/1** was obtained as a pale highly viscous oil in a yield of 99.2% (365 mg, 843 µmol).

IR (ATR): ν / cm^{-1} = 3307.3 (w), 2926.2 (m), 2854.6 (w), 1736.9 (m), 1648.5 (m), 1535.7 (w), 1459.4 (w), 1379.7 (vw), 1175.9 (w), 1140.4 (w), 1047.1 (vw), 1006.5 (vw), 784.9 (vw), 722.2 (vw), 649.7 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 5.97 (t, J = 5.9 Hz, 1 H, NH^{1}), 5.23 (d, J = 3.8 Hz, 1 H, CH^{2}), 3.62 – 3.52 (m, 2 H, CH_2^{3}), 3.30 – 3.11 (m, 2 H, CH_2^{4}), 2.56 (td, J = 7.1, 2.3 Hz, 2 H, CH_2^{5}), 2.27 (t, J = 7.5 Hz, 2 H, CH_2^{6}), 2.13 – 2.01 (m, 2 H, CH_2^{7}), 1.83 – 1.72 (m, 1 H, CH^{8}), 1.60 – 1.51 (m, 2 H, CH_2^{9}), 1.47 – 1.09 (m, 18 H, CH_2^{10}), 0.91 – 0.79 (m, 6 H, CH_2^{11}).

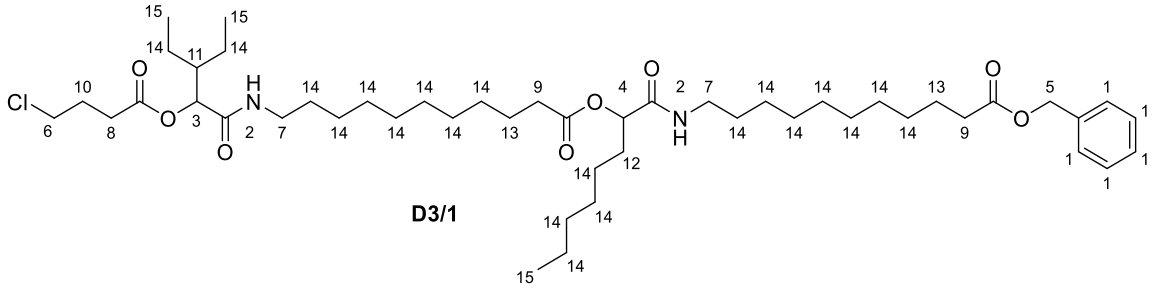
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 179.16, 171.69, 169.77, 75.53, 44.09, 43.62, 39.41, 34.18, 31.22, 29.57, 29.46, 29.36, 29.23, 29.10, 27.46, 26.92, 24.83, 22.35, 22.00, 11.71, 11.68.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{22}{}^{1}H_{40}{}^{16}O_5{}^{14}N{}^{35}Cl$, 434.2668; found, 434.2659, Δ = 0.9 mmu.



Supplementary Figure 118: ^{1}H-NMR of compound **MD3/1** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 313 mg **MD3/1** (722 µmol, 1.00 eq.) were stirred in 2.00 mL DCM, subsequently 151 µL heptanal **A7** (124 mg, 1.08 mmol, 1.50 eq.) and 326 mg of monomer **IM2** (1.08 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 3 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (6:1 → 2:1) to yield the Passerini product **D3/1** as a yellow highly viscous oil. (533 mg, 630 µmol, 87.3%).

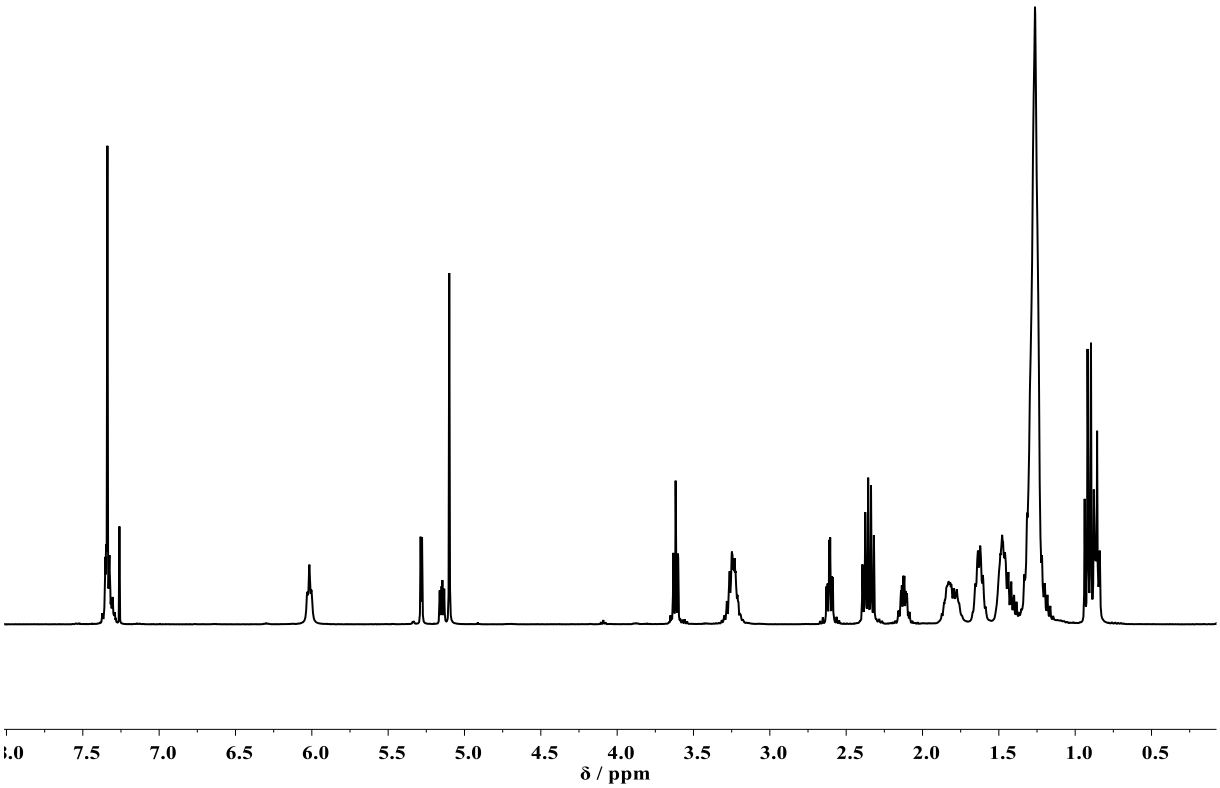
R_f: 0.20 in cyclohexane / ethyl acetate (3:1).

IR (ATR): ν / cm^{-1} = 3305.8 (vw), 2924.9 (s), 2854.1 (m), 1737.2 (vs), 1653.5 (s), 1533.8 (m), 1456.8 (m), 1377.3 (w), 1163.3 (s), 1005.5 (w), 732.4 (w), 696.8 (m), 651.4 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.40 – 7.28 (m, 5 H, CH_{Ar}^1), 6.04 – 5.95 (m, 2 H, NH^2), 5.28 (d, J = 3.8 Hz, 1 H, CH^3), 5.18 – 5.12 (m, 1 H, CH^4), 5.10 (s, 2 H, CH_2^5), 3.62 (t, J = 6.2 Hz, 2 H, CH_2^6), 3.33 – 3.17 (m, 4 H, CH_2^7), 2.61 (td, J = 7.1, 2.7 Hz, 2 H, CH_2^8), 2.41 – 2.30 (m, 4 H, CH_2^9), 2.18 – 2.07 (m, 2 H, CH_2^{10}), 1.90 – 1.74 (m, 3 H, CH^{11}, CH_2^{12}), 1.68 – 1.57 (m, 4 H, CH_2^{13}), 1.54 – 1.15 (m, 40H, CH_2^{14}), 0.96 – 0.82 (m, 9 H, CH_3^{15}).

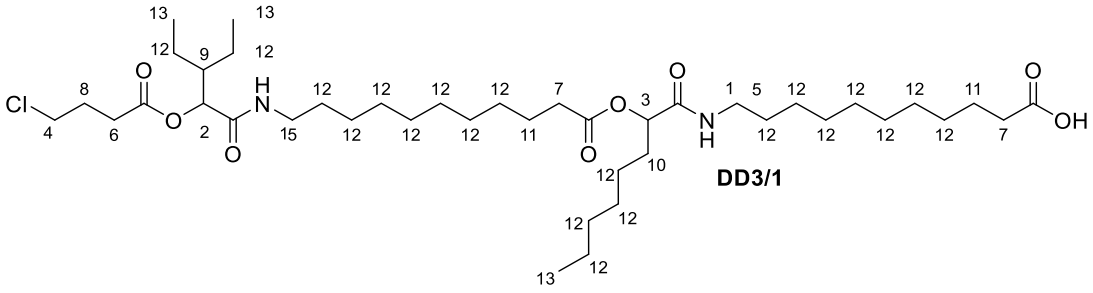
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.79, 172.57, 171.62, 169.96, 169.62, 136.23, 128.64, 128.27, 128.26, 75.52, 74.04, 66.17, 44.06, 43.64, 39.35, 39.30, 34.43, 32.02, 31.73, 31.20, 29.65, 29.62, 29.55, 29.45, 29.32, 29.28, 29.20, 27.46, 26.95, 26.94, 25.07, 25.04, 24.81, 22.63, 22.37, 22.01, 14.15, 11.70, 11.69.

ESI-MS [m/z]: $[M+H]^+$ calculated for $^{12}C_{48}{}^{1}H_{81}{}^{16}O_8{}^{14}N_2{}^{35}Cl$: 849.5754; found: 849.5734; Δ = 2.0 mmu.



Supplementary Figure 119: ^{1}H-NMR of compound **D3/1** measured in $CDCl_3$.

Deprotection



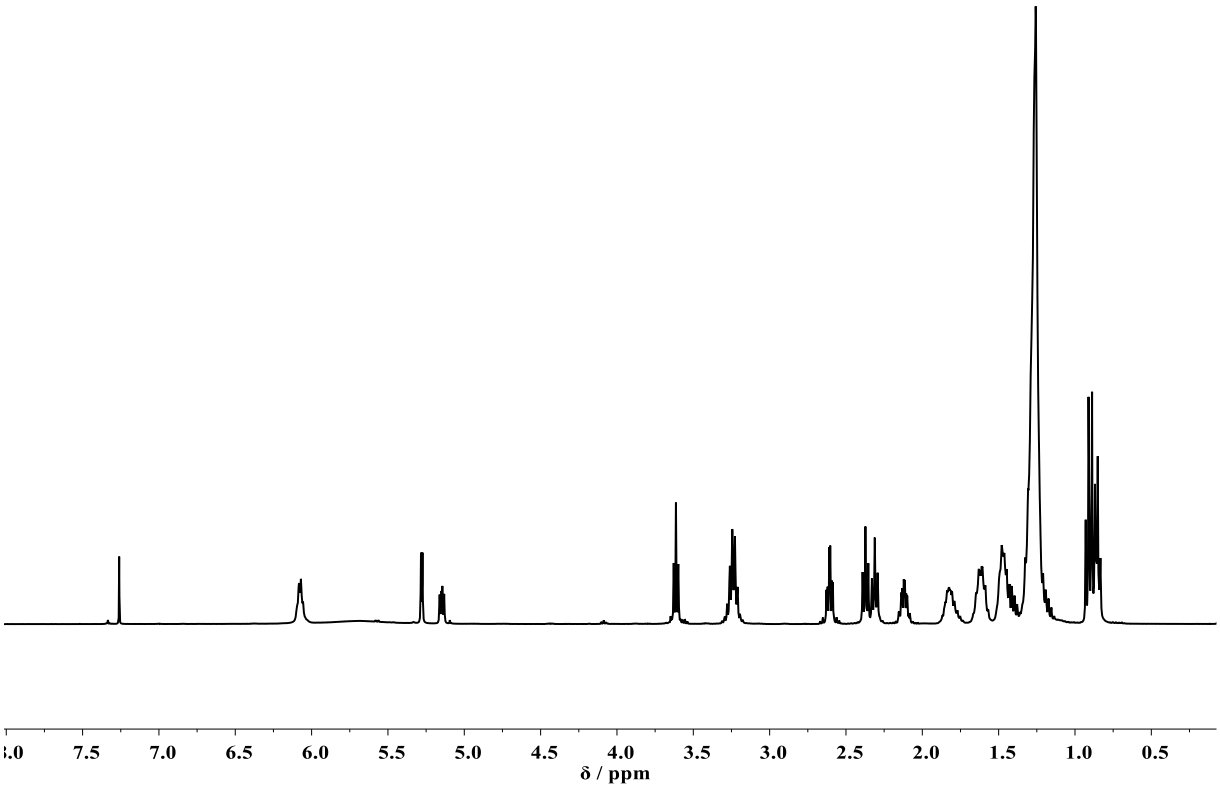
In a 50 mL round bottom flask, 474 mg of Passerini product **D3/1** (560 µmol, 1.00 eq.) were dissolved in 3.00 mL ethyl acetate and 3.00 mL THF. Afterwards, 95.0 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **DD3/1** was obtained as a pale highly viscous oil in a yield of 96.2% (407 mg, 537 µmol).

IR (ATR): ν / cm^{-1} = 3307.0 (vw), 2924.9 (s), 2854.2 (m), 1738.8 (m), 1650.5 (m), 1536.4 (w), 1459.5 (w), 1376.3 (w), 1142.4 (m), 722.2 (vw), 643.9 (vw), 384.8 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.15 – 6.02 (m, 2 H, NH^{1}), 5.28 (d, J = 3.9 Hz, 1 H, CH^{2}), 5.21 – 5.10 (m, 1 H, CH^{3}), 3.61 (t, J = 6.1 Hz, 2 H, CH_2^{4}), 3.33 – 3.15 (m, 4 H, CH_2^{5}), 2.61 (td, J = 7.1, 2.8 Hz, 2 H, CH_2^{6}), 2.37 (t, J = 7.5 Hz, 2 H, CH_2^{7}), 2.31 (t, J = 7.5 Hz, 2 H, CH_2^{7}), 2.18 – 2.05 (m, 2 H, CH_2^{8}), 1.88 – 1.74 (m, 3 H, CH^{9}, CH_2^{10}), 1.69 – 1.54 (m, 4 H, CH_2^{11}), 1.52 – 1.14 (m, 40 H, CH_2^{12}), 0.95 – 0.81 (m, 9 H, CH_3^{13}).

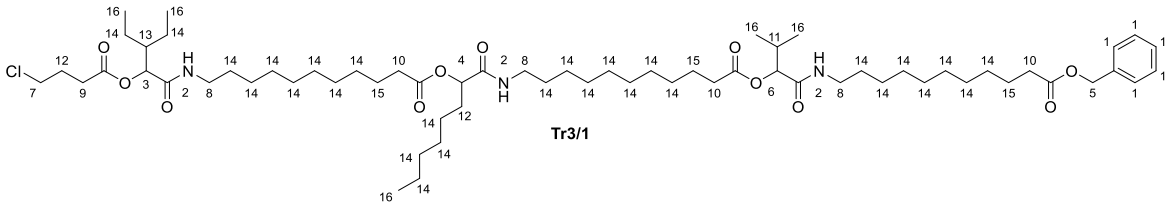
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 178.33, 172.62, 171.70, 170.11, 169.81, 75.50, 74.03, 44.07, 43.59, 39.41, 39.31, 34.43, 34.16, 31.99, 31.72, 31.20, 29.58, 29.56, 29.47, 29.37, 29.33, 29.28, 29.25, 29.21, 29.12, 29.01, 27.45, 26.95, 26.89, 25.08, 24.88, 24.81, 22.63, 22.33, 21.97, 14.15, 11.67, 11.65.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{41}{}^{1}H_{75}{}^{16}O_8{}^{14}N_2{}^{35}Cl$, 759.5285; found, 759.5267, Δ = 1.8 mmu.



Supplementary Figure 120: ^{1}H-NMR of compound **DD3/1** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 325 mg **DD3/1** (460 µmol, 1.00 eq.) were stirred in 4.00 mL DCM. Subsequently, 63.0 µL isobutyraldehyde **A3** (50.0 mg, 690 µmol, 1.50 eq.) and 210 mg of monomer **IM2** (690 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 3 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (5:1 → 2:1) to yield the Passerini product **Tr3/1** as a yellow highly viscous oil. (460 mg, 410 µmol, 89.1%).

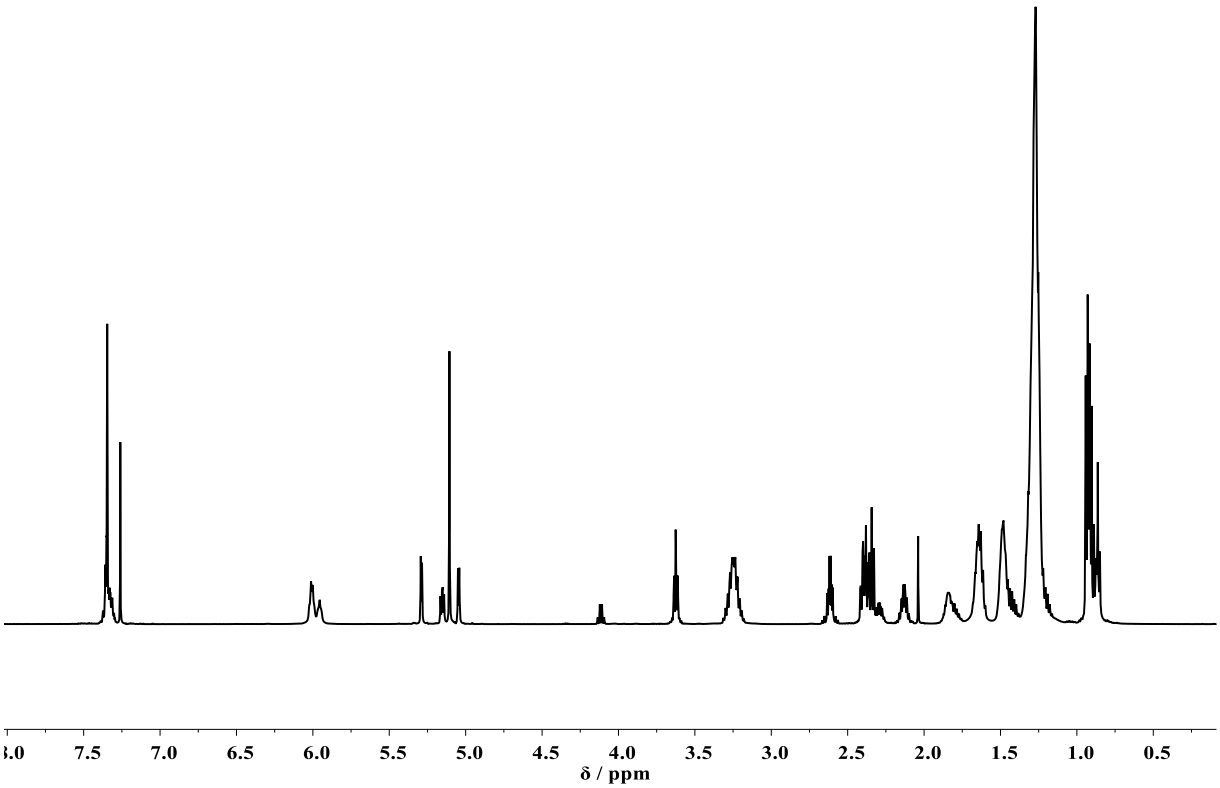
R_f = 0.13 in cyclohexane / ethyl acetate (2:1).

IR (ATR): ν / cm^{-1} = 3306.7 (vw), 2924.8 (s), 2853.8 (m), 1737.6 (vs), 1652.7 (vs), 1533.9 (m), 1458.0 (w), 1373.7 (w), 1162.4 (s), 1905.3 (w), 724.8 (w), 697.0 (w), 650.1 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.35 – 7.23 (m, 5 H, CH_{Ar}^1), 6.05 – 5.82 (m, 3 H, NH^2), 5.23 (d, J = 3.8 Hz, 1 H, CH^3), 5.12 – 5.05 (m, 1 H, CH^4), 5.04 (s, 2 H, CH_2^5), 4.98 (d, J = 4.4 Hz, 1 H, CH_2^6), 3.56 (t, J = 6.2 Hz, 2 H, CH_2^7), 3.27 – 3.09 (m, 6 H, CH_2^8), 2.55 (td, J = 7.1, 3.9 Hz, 2 H, CH_2^9), 2.39 – 2.18 (m, 7 H, CH^{10}, CH_2^{11}), 2.12 – 2.02 (m, 2 H, CH_2^{12}), 1.87 – 1.67 (m, 5 H, CH^{13}, CH_2^{14}), 1.66 – 1.51 (m, 6 H, CH_2^{15}), 1.48 – 1.08 (m, 50 H, CH_2^{14}), 0.91 – 0.77 (m, 15 H, CH_3^{16}).

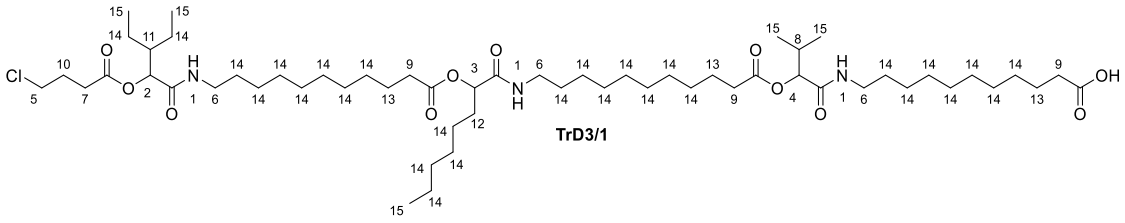
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.82, 172.69, 172.60, 171.64, 170.00, 169.64, 169.39, 136.22, 128.66, 128.27, 78.03, 75.50, 74.03, 66.19, 60.52, 44.09, 43.63, 39.36, 39.29, 39.28, 34.43, 34.40, 32.02, 31.74, 31.20, 30.63, 29.69, 29.68, 29.63, 29.57, 29.55, 29.47, 29.47, 29.33, 29.32, 29.24, 29.21, 29.03, 27.46, 26.95, 26.93, 25.12, 25.08, 25.05, 24.82, 22.65, 22.35, 22.00, 21.18, 18.90, 17.07, 14.32, 14.17, 11.71, 11.70.

ESI-MS [*m*/z]: $[M+Na]^+$ calculated for $^{12}C_{64}{}^1H_{110}{}^{16}O_{11}{}^{14}N_3{}^{35}Cl$: 1154.7721 found: 1154.7698; Δ = 2.3 mmu.



Supplementary Figure 121: ^{1}H-NMR of compound **Tr3/1** measured in $CDCl_3$.

Deprotection



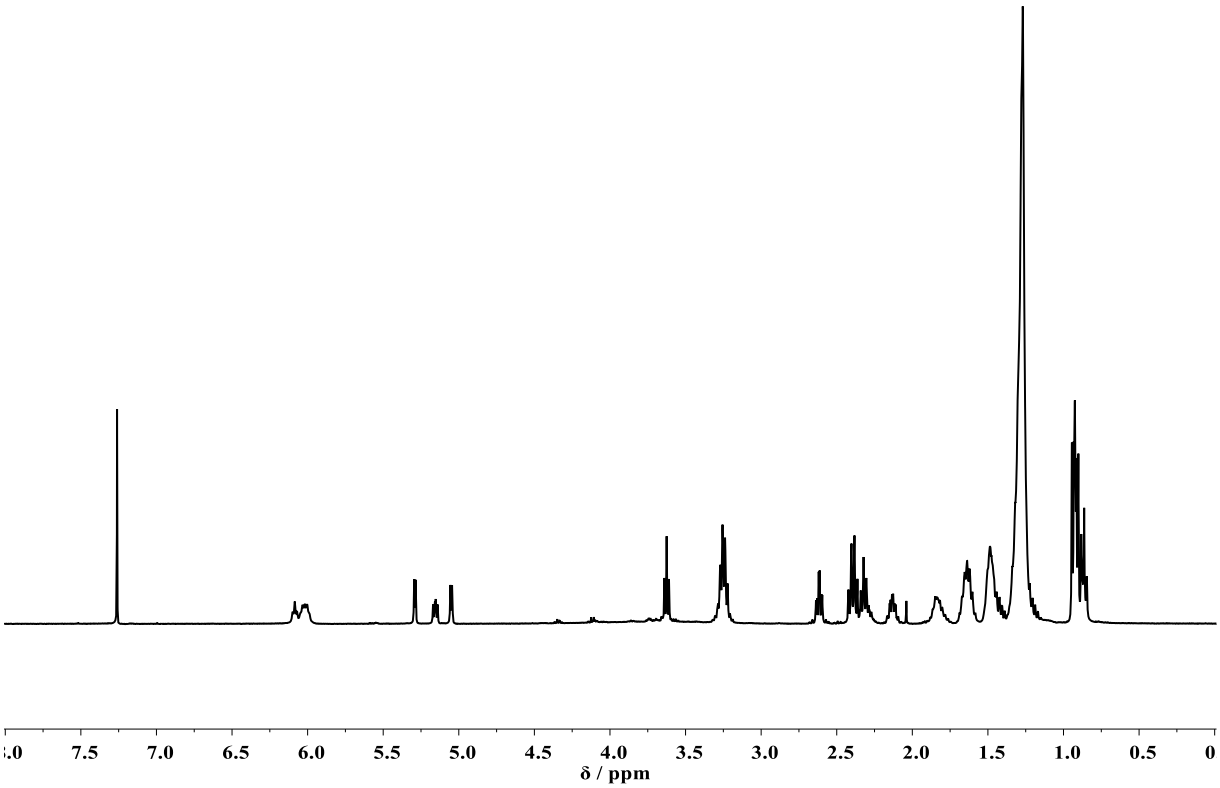
In a 50 mL round bottom flask, 400 mg of the Passerini product **Tr3/1** (353 µmol, 1.00 eq.) were dissolved in 2.00 mL ethyl acetate and 2.00 mL THF. Afterwards, 80.0 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **TrD3/1** was obtained as a pale highly viscous oil in a yield of 98.6% (363 mg, 348 µmol).

IR (ATR): ν / cm^{-1} = 3305.7 (vw), 2924.5 (s), 2853.6 (m), 1738.3 (s), 1650.5 (s), 1536.1 (s), 1460.6 (m), 1371.4 (w), 1165.6 (s), 1008.1 (w), 722.1 (w), 650.1 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.08 (t, J = 5.9 Hz, 1 H, NH^{1}), 6.06 – 5.98 (m, 2 H, NH^{1}), 5.29 (d, J = 3.8 Hz, 1 H, CH^{2}), 5.18 – 5.13 (m, 1 H, CH^{3}), 5.05 (d, J = 4.5 Hz, 1 H, CH^{4}), 3.62 (t, J = 6.2 Hz, 2 H, CH_2^{5}), 3.34 – 3.18 (m, 6 H, CH_2^{6}), 2.62 (td, J = 7.1, 2.6 Hz, 2 H, CH_2^{7}), 2.44 – 2.25 (m, 7 H, CH^{8},CH_2^{9}), 2.18 – 2.08 (m, 2, CH_2^{10}), 1.94 – 1.74 (m, 3 H, CH^{11}, CH_2^{12}), 1.71 – 1.57 (m, 6 H, CH_2^{13}), 1.37 – 1.14 (m, 54 H, CH_2^{14}), 0.98 – 0.82 (m, 15 H, CH_3^{15}).

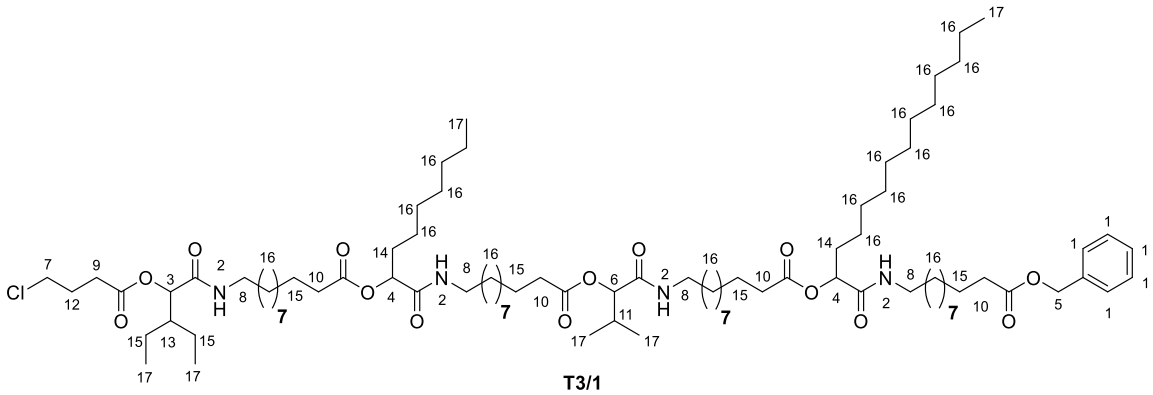
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 172.71, 172.70, 171.67, 170.17, 169.74, 169.49, 78.09, 75.55, 74.07, 44.10, 43.65, 39.41, 39.39, 39.27, 34.46, 34.44, 32.02, 31.76, 31.23, 30.62, 29.67, 29.62, 29.51, 29.48, 29.42, 29.37, 29.28, 29.23, 29.21, 29.10, 29.04, 27.49, 26.97, 26.90, 25.17, 25.10, 24.92, 24.85 22.67, 22.39, 22.03, 18.92, 17.10, 14.18, 11.73.

ESI-MS [m/z]: $[M + H]^+$ calculated for $^{12}C_{57}{}^{1}H_{104}{}^{16}O_{11}{}^{14}N_3{}^{35}Cl$, 1042.7432; found, 1042.7412, Δ = 2.0 mmu.



Supplementary Figure 122: ^{1}H-NMR of compound **TrD3/1** measured in $CDCl_3$.

Passerini reaction



T3/1

In a 50 mL round bottom flask, 688 mg **TrD3/1** (659 µmol, 1.00 eq.) were stirred in 2.00 mL DCM. Subsequently, 235 µL tridecanal **A11** (196 mg, 989 µmol, 1.50 eq.) and 298 mg of monomer **IM2** (989 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (5:1 → 1:1) to yield the Passerini product **T9** as a yellow highly viscous oil. (981 mg, 635 µmol, 96.4%).

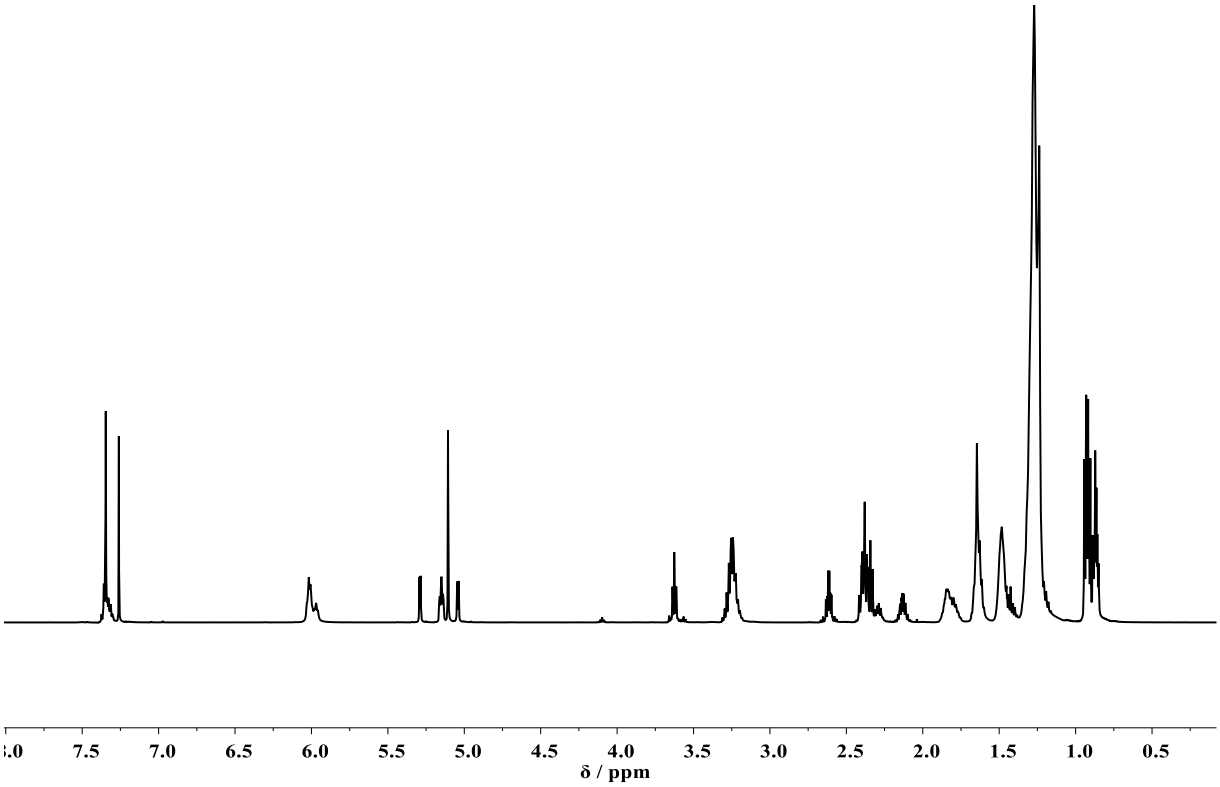
R_f = 0.75 in cyclohexane / ethyl acetate (1:1).

IR (ATR): ν / cm^{-1} = 3304.4 (vw), 2923.0 (s), 2852.8 (m), 1738.5 (s), 1653.1 (s), 1534.7 (m), 1458.5 (m), 1372.7 (w), 1163.1 (s), 1006.7 (w), 723.1 (w), 696.9 (w).

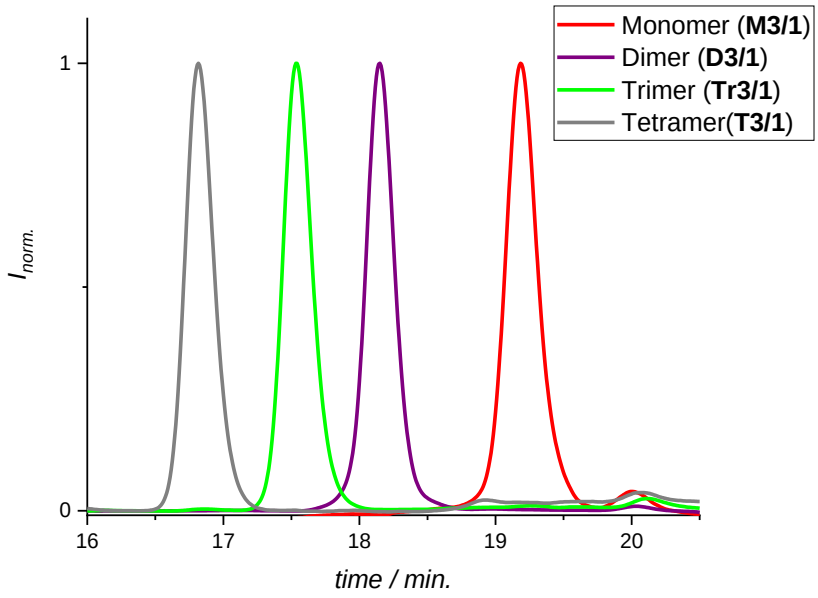
^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.43 – 7.29 (m, 5 H, CH_{Ar}^1), 6.08 – 5.93 (m, 4 H, NH^2), 5.29 (d, J = 3.8 Hz, 1 H, CH^3), 5.17 – 5.12 (m, 2 H, CH^4), 5.11 (s, 2 H, CH_2^5), 5.04 (d, J = 4.5 Hz, 1 H, CH^6), 3.63 (t, J = 6.2 Hz, 2 H, CH_2^7), 3.33 – 3.17 (m, 8 H, CH_2^8), 2.68 – 2.56 (m, 2 H, CH_2^9), 2.43 – 2.26 (m, 9 H, CH^{10}, CH_2^{11}), 2.18 – 2.08 (m, 2 H, CH_2^{12}), 1.91 – 1.74 (m, 5 H, CH^{13}, CH_2^{14}), 1.69 – 1.58 (m, 12 H, CH_2^{15}), 1.54 – 1.16 (m, 84 H, CH_2^{16}), 0.96 – 0.82 (m, 18 H, CH_3^{17}).

^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 173.82, 172.70, 172.61, 171.65, 169.99, 169.98, 169.64, 169.40, 136.25, 128.67, 128.30, 128.29, 78.05, 75.53, 74.07, 74.05, 66.20, 44.10, 43.65, 39.37, 39.31, 39.28, 34.45, 34.42, 32.05, 31.76, 31.22, 30.65, 29.80, 29.78, 29.76, 29.73, 29.69, 29.68, 29.65, 29.58, 29.50, 29.49, 29.39, 29.35, 29.33, 29.32, 29.31, 29.26, 29.24, 29.04, 27.47, 26.97, 26.96, 25.13, 25.09, 25.07, 24.89, 24.84, 22.82, 22.66, 22.38, 22.02, 18.92, 17.09, 14.26, 14.18, 11.73, 11.71.

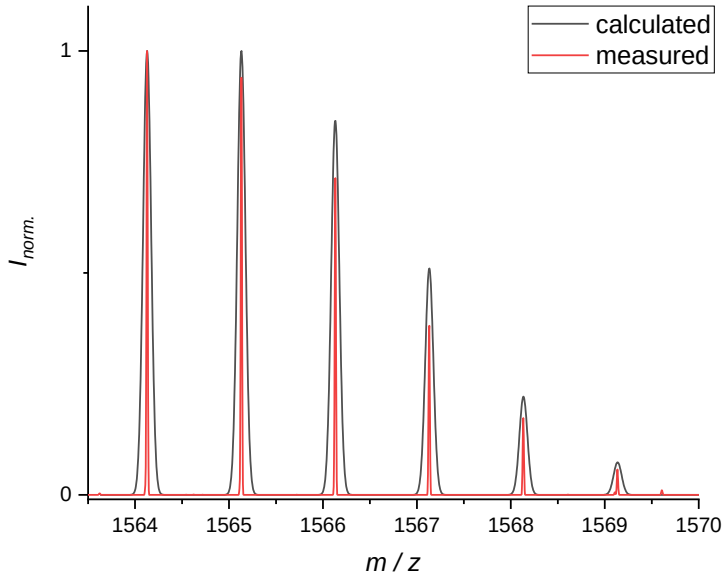
ESI-MS [*m*/z]: $[M+Na]^+$ calculated for $^{12}C_{89}{}^{1}H_{157}{}^{16}O_{14}{}^{14}N_4{}^{35}Cl$: 1564.1277; found: 1564.1282; Δ = 0.5 mmu.



Supplementary Figure 123: ^{1}H-NMR of compound **T3/1** measured in $CDCl_3$.



Supplementary Figure 124: SEC traces of the intermediates after each P-3CR in the synthesis of product **T3/1**.



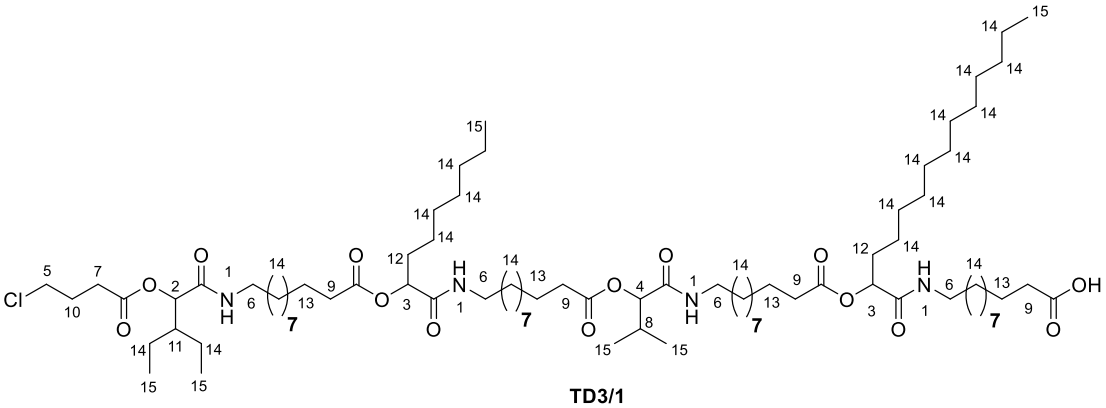
Supplementary Figure 125: High resolution ESI-MS measurement of **T3/1**. The observed isotopic pattern is compared with the calculated isotopic pattern from mMass (black).

```
.csv, maximum is 1.000000 found for mass 1564.124010
matching mass 1564.12401
cutoff 0.50000: 0 solutions (12 peaks)
cutoff 0.25000: 0 solutions (35 peaks)
cutoff 0.12500: 0 solutions (74 peaks)
cutoff 0.06250: 0 solutions (144 peaks)
cutoff 0.03125: 0 solutions (229 peaks)
cutoff 0.01562: 0 solutions (326 peaks)
cutoff 0.00781: 1 solutions (467 peaks)
1564.12401 ≈ 121.005630 + 311.246050 + 325.261700 + 283.214750 + 409.355600 + 91.054780 (sides 2-Ethylbutanal, Heptanal,
 Isobutyraldehyde, Tridecanal; error -22.98550)
Press ENTER to quit ...
```

Supplementary Figure 126: Screenshot of the automated read-out of **T3/1**, sodium trifluoroacetate **2** was used as additive during the measurement.

6.3.3.5.2 Synthesis of hexamer H3/1

Deprotection



TD3/1

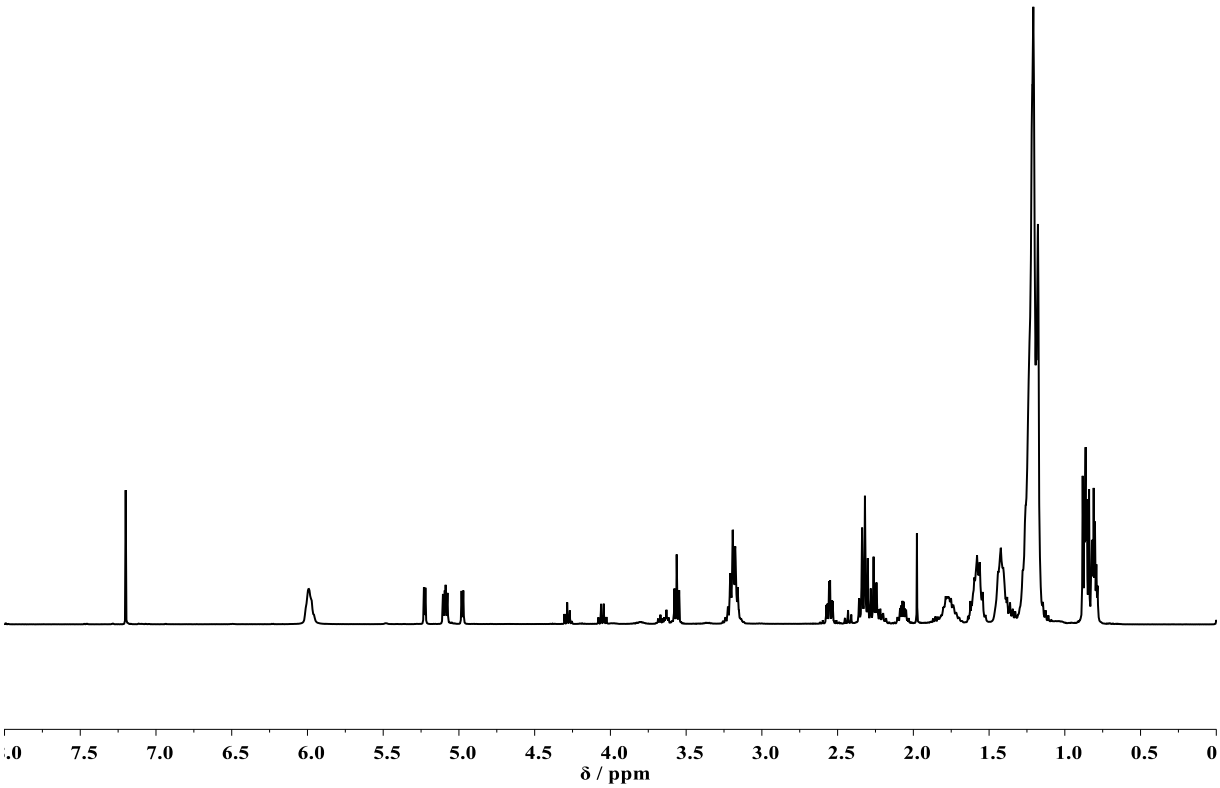
In a 25 mL round bottom flask, 874 mg of the Passerini product **T3/1** (566 µmol, 1.00 eq.) was dissolved in 5.00 mL ethyl acetate and 5.00 mL THF. Subsequently, 174 mg (20 wt%) palladium on activated carbon **1** were added to the solution. The resulting mixture was purged with hydrogen gas (3 balloons) and stirred for one day at room temperature under hydrogen atmosphere. The crude reaction mixture was filtered over celite® and flushed with 50 mL dichloromethane. After evaporation of the solvents and drying under reduced pressure the corresponding acid **TD3/1** was obtained as a colorless solid. (806 mg, 555 µmol, 98.1%).

IR (ATR): ν / cm^{-1} = 3305.8 (vw), 2922.9 (vs), 2852.8 (s), 1739.6 (s), 1651.6 (s), 1536.8 (m), 1461.1 (m), 1371.7 (w), 1163.7 (s), 722.0 (w), 652.9 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.14 – 5.98 (m, 4 H, NH^1), 5.29 (d, J = 3.8 Hz, 1 H, CH^2), 5.19 – 5.11 (m, 2 H, CH^3), 5.04 (d, J = 4.5 Hz, 1 H, CH^4), 3.62 (t, J = 6.2 Hz, 2 H, CH_2^5), 3.33 – 3.16 (m, 8 H, CH_2^6), 2.68 – 2.57(m, 2 H, CH_2^7), 2.50 – 2.23 (m, 9 H, CH^8, CH_2^9), 2.21 – 2.06 (m, 2 H, CH_2^{10}), 1.94 – 1.73 (m, 5 H, CH^{11}, CH_2^{12}), 1.73 – 1.56 (m, 8 H, CH_2^{13}), 1.57 – 1.17 (m, 88 H, CH_2^{14}), 0.98 – 0.82 (m, 18 H, CH_3^{15}).

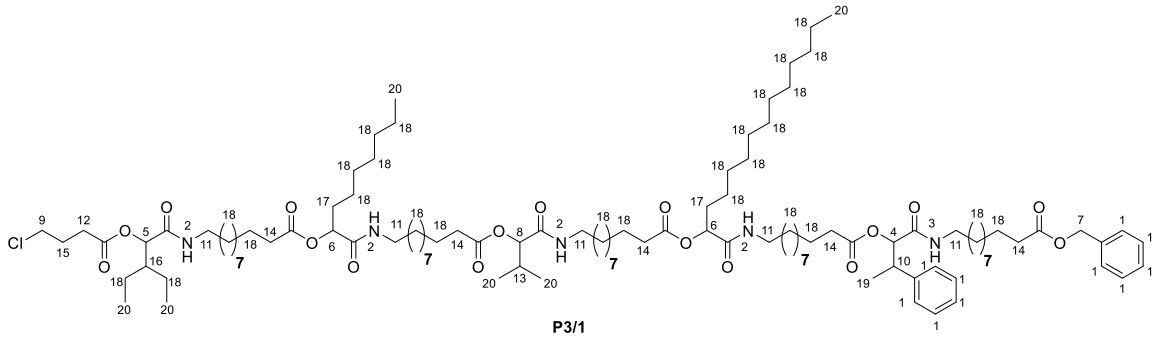
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 177.00, 172.80, 172.63, 171.67, 170.11, 170.08, 169.72, 169.58, 78.08, 75.53, 74.09, 74.04, 44.09, 43.64, 39.39, 39.34, 39.30, 34.45, 34.41, 33.95, 32.04, 32.03, 32.01, 31.75, 31.22, 30.62, 29.79, 29.77, 29.75, 29.69, 29.67, 29.63, 29.60, 29.57, 29.50, 29.48, 29.44, 29.38, 29.35, 29.32, 29.25, 29.23, 29.10, 29.03, 27.48, 26.97, 26.94, 26.89, 25.12, 25.09, 24.91, 24.90, 24.84, 22.82, 22.66, 22.37, 22.01, 18.89, 17.11, 14.25, 14.17, 11.71, 11.70.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{82}{}^{1}H_{151}{}^{16}O_{14}{}^{14}N_4{}^{35}Cl$, 1452.0988; found, 1452.0990, Δ = 0.2 mmu.



Supplementary Figure 127: ^{1}H-NMR of compound **TD3/1** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask 752 mg **TD3/1** (518 µmol, 1.00 eq.) were stirred in 2.00 mL DCM. Subsequently, 104 µL 2-phenylpropionaldehyde **A12** (104 mg, 776 µmol, 1.50 eq.) and 234 mg of monomer **IM2** (776 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (4:1 → 1:1) to yield the Passerini product **P3/1** as a yellow highly viscous oil (911 mg, 482 µmol, 93.2%).

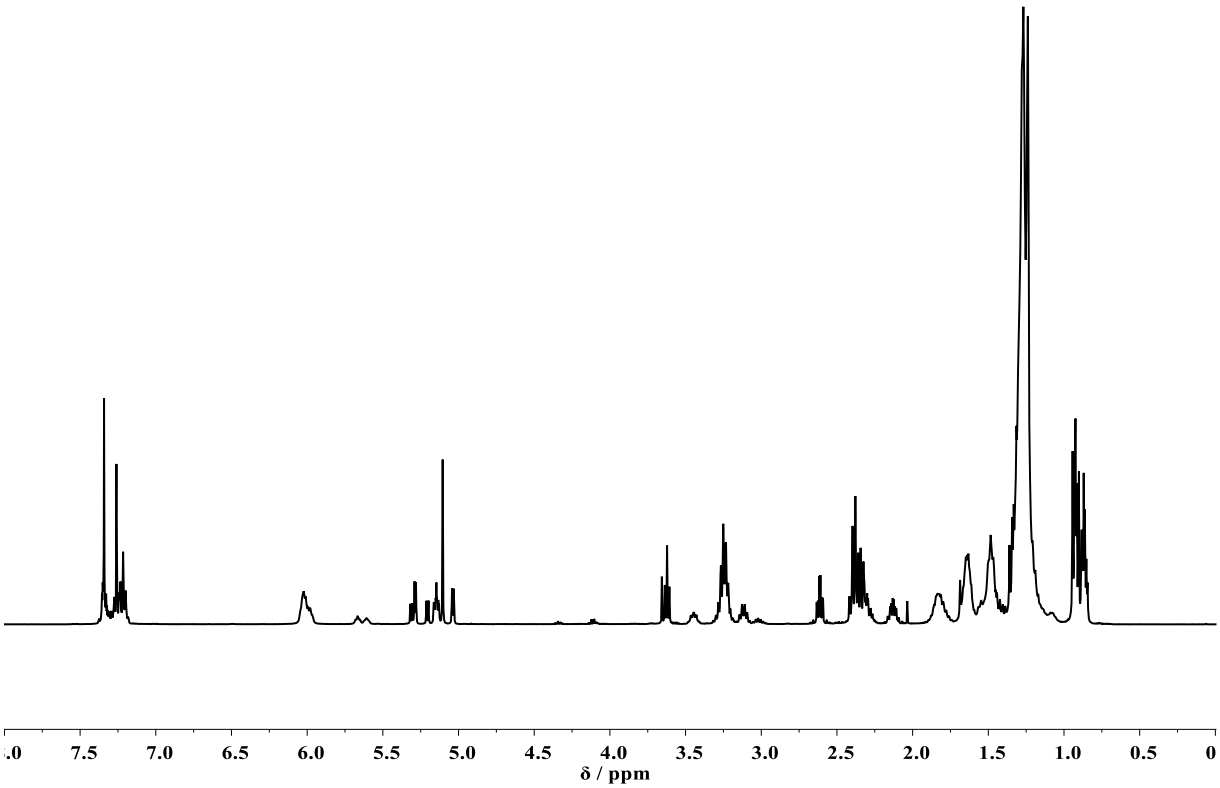
R_f: 0.41 in cyclohexane / ethyl acetate (3:2).

IR (ATR): ν / cm^{-1} = 3306.0 (vw), 3087.6 (vw), 2923.2 (vs), 2853.0 (s), 2314.0 (vw), 2078.9 (vw), 1948.6 (vw), 1738.7 (vw), 1652.5 (vs), 1534.6 (s), 1456.2 (m), 1373.7 (w), 1231.6 (m), 1161.3 (s), 1107.7 (m), 1005.2 (w), 722.3 (w), 698.6 (m), 653.3 (w), 537.7 (vw), 429.7 (vw), 400.4 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.44 – 7.16 (m, 10 H, CH_{Ar}^{1}), 6.12 – 5.93 (m, 4 H, NH^{2}), 5.67 (t, *J* = 5.9 Hz, 0.5 H, NH^{3a}), 5.64 – 5.58 (m, 0.5 H, NH^{3b}), 5.31 (d, *J* = 5.4 Hz, 0.5 H, CH^{4a}), 5.29 (d, *J* = 3.8 Hz, 1 H, CH^{5}), 5.21 (d, *J* = 5.4 Hz, 0.5 H, CH^{4b}), 5.17 – 5.12 (m, 2 H, CH^{6}), 5.10 (s, 2 H, CH_2^{7}), 5.04 (d, *J* = 4.4 Hz, 1 H, CH^{8}), 3.75 – 3.53 (m, 2 H, CH_2^{9}), 3.50 – 3.39 (m, 1 H, CH_2^{10}), 3.34 – 2.95 (m, 10 H, CH_2^{11}), 2.69 – 2.55 (m, 2 H, CH_2^{12}), 2.52 – 2.22 (m, 11 H, CH^{13}, CH_2^{14}), 2.20 – 2.06 (m, 2 H, CH_2^{15}), 1.96 – 1.72 (m, 5 H, CH^{16}, CH_2^{17}), 1.72 – 1.04 (m, 115 H, CH_2^{18}, CH_3^{19}), 0.97 – 0.82 (m, 18 H, CH_3^{20}).

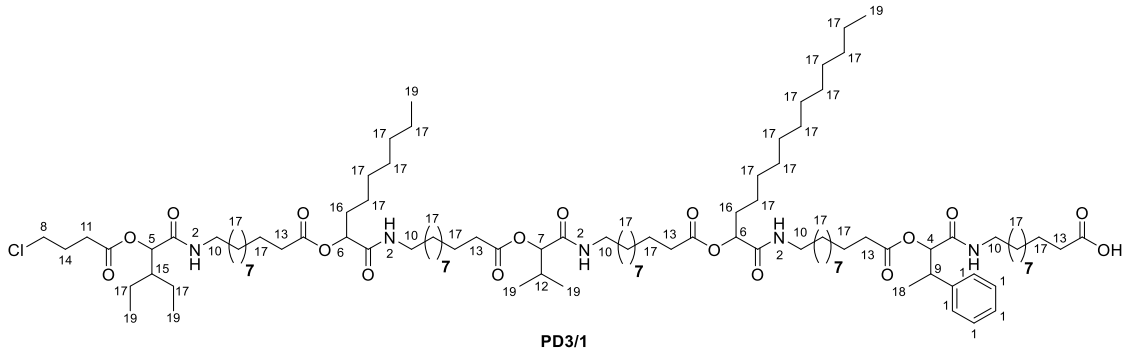
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.79, 172.69, 172.60, 172.43, 171.63, 169.98, 169.61, 169.38, 168.82, 168.61, 141.73, 141.20, 136.26, 128.66, 128.52, 128.31, 128.30, 128.29, 127.97, 127.12, 127.05, 78.06, 77.86, 75.54, 74.06, 66.19, 44.08, 43.66, 41.58, 41.30, 39.36, 39.30, 39.28, 39.21, 34.45, 34.41, 34.35, 34.30, 32.04, 31.75, 31.22, 30.64, 29.79, 29.77, 29.74, 29.70, 29.67, 29.65, 29.58, 29.52, 29.51, 29.47, 29.38, 29.34, 29.33, 29.32, 29.24, 29.18, 29.16, 29.03, 27.48, 26.96, 29.65, 26.87, 26.80, 25.13, 25.09, 25.07, 24.98, 24.91, 24.84, 22.81, 22.65, 22.39, 22.03, 18.91, 17.63, 17.10, 15.34, 14.25, 14.17, 11.72, 11.71.

ESI-MS [*m*/z]: $[M+H]^+$ calculated for $^{12}C_{110}{}^{1}H_{188}{}^{16}O_{17}{}^{14}N_5{}^{35}Cl$: 1887.3762; found 1887.3793; Δ = 3.1 mmu.



Supplementary Figure 128: ^{1}H-NMR of compound **P3/1** measured in $CDCl_3$.

Deprotection



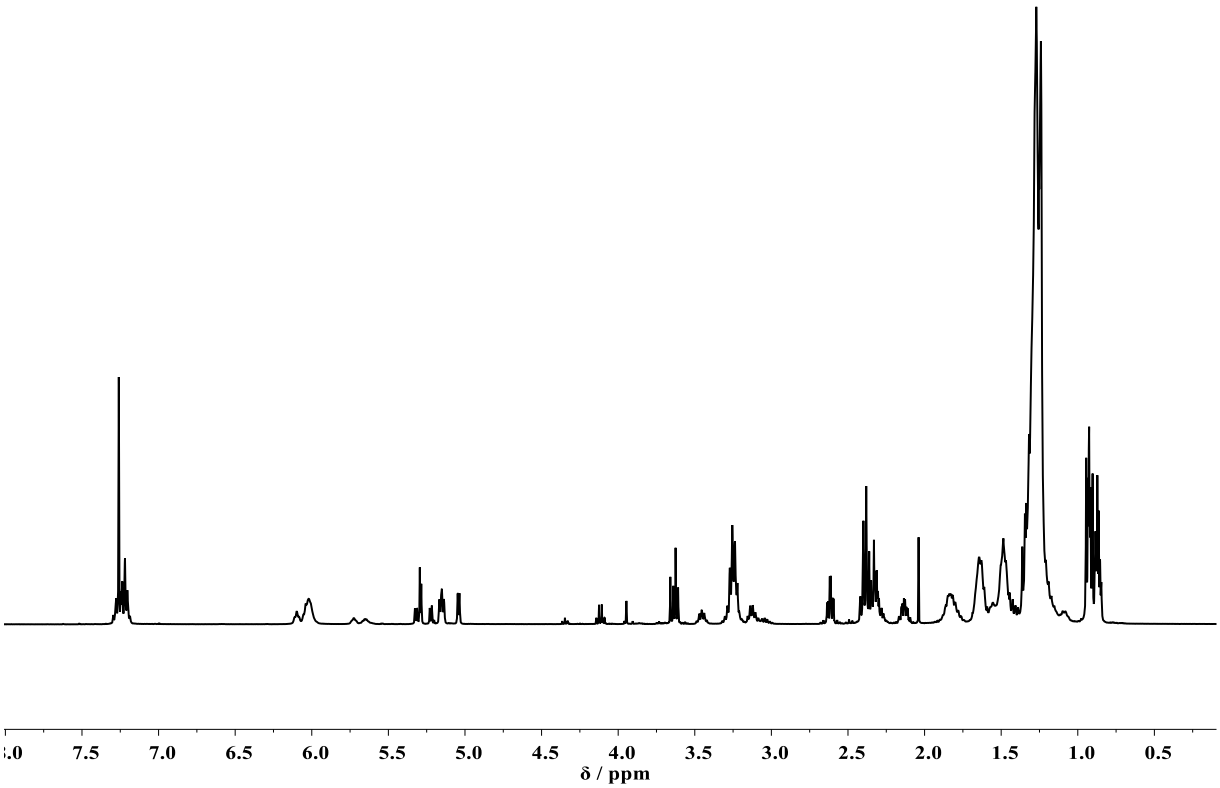
In a 50 mL round bottom flask, 718 mg of **P3/1** (414 µmol, 1.00 eq.) were dissolved in 3.00 mL ethyl acetate and 3.00 mL THF. Afterwards, 156 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen gas (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **PD3/1** was obtained as a high viscous oil in a yield of 99.3% (739 mg, 411 µmol).

IR (ATR): ν / cm^{-1} = 3305.9 (vw), 3085.2 (vw), 2923.4 (vs), 2853.1 (s), 2075.0 (vw), 1739.2 (s), 1460.2 (m), 1373.3 (w), 1237.3 (m), 1162.2 (s), 1106.6 (m), 1021.1 (w), 923.9 (vw), 761.0 (vw), 721.2 (w), 699.8 (m), 653.9 (w), 538.2 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.34 – 7.15 (m, 5 H, CH_{Ar}^{1}), 6.15 – 5.94 (m, 4 H, NH^{2}), 5.76 – 5.59 (m, 1 H, NH^{3}), 5.34 – 5.27 (m, 0.5 H, 1 H, CH^{4a}, CH^{5}), 5.22 (d, J = 5.5 Hz, 0.5 H, CH^{4b}), 5.18 – 5.12 (m, 2 H, CH^{6}), 5.04 (d, J = 4.5 Hz, 1 H, CH^{7}), 3.67 – 3.59 (m, 2 H, CH_2^{8}), 3.52 – 3.40 (m, 1 H, CH^{9}), 3.35 – 2.97 (m, 10 H, CH_2^{10}), 2.68 – 2.56 (m, 2 H, CH_2^{11}), 2.44 – 2.23 (m, 11 H, CH^{12}, CH_2^{13}), 2.18 – 2.08 (m, 2 H, CH_2^{14}), 1.93 – 1.73 (m, 5 H, CH^{15}, CH_2^{16}), 1.71 – 1.04 (m, 115 H, CH_2^{17}, CH_3^{18}), 0.99 – 0.82 (m, 18 H, CH_3^{19}).

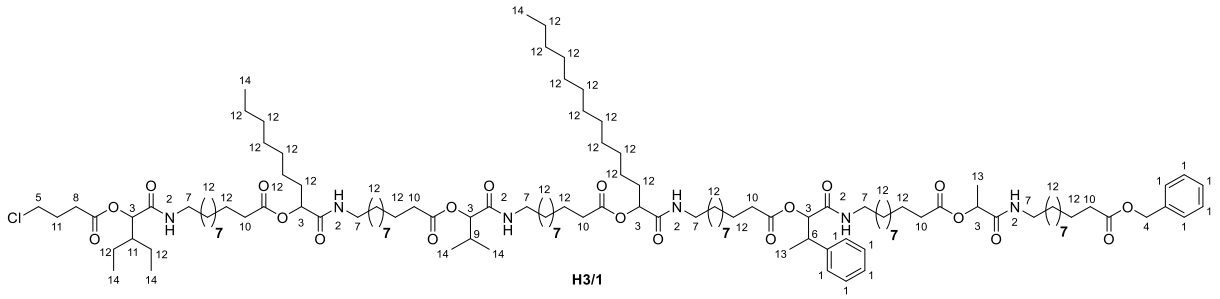
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 176.41, 172.74, 172.71, 172.63, 171.66, 170.07, 169.69, 169.51, 168.71, 141.19, 128.53, 128.33, 127.98, 127.14, 127.07, 78.07, 77.87, 75.55, 74.08, 74.06, 44.09, 43.66, 41.56, 41.26, 39.39, 39.34, 39.33, 39.26, 39.17, 34.46, 34.42, 34.41, 34.36, 34.24, 33.87, 32.05, 31.76, 31.23, 30.65, 29.80, 29.78, 29.76, 29.72, 29.68, 29.65, 29.59, 29.49, 29.39, 29.36, 29.33, 29.27, 29.24, 29.20, 29.14, 29.08, 29.04, 27.49, 26.97, 26.78, 26.71, 25.14, 25.10, 24.92, 24.91, 24.85, 22.82, 22.67, 22.40, 22.03, 18.92, 17.65, 17.12, 15.31, 14.26, 14.18, 11.73, 11.71.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{103}{}^{1}H_{182}{}^{16}O_{17}{}^{14}N_5{}^{35}Cl$, 1797.3292; found, 1797.3300, Δ = 1.8 mmu.



Supplementary Figure 129: ^{1}H-NMR of compound **PD3/1** measured in $CDCl_3$.

Passerini reaction



In a 25 mL round bottom flask, 641 mg **PD3/1** (356 µmol, 1.00 eq.) were stirred in 3.00 mL DCM. Subsequently, 59.8 µL acetaldehyde **A1** (47.1 mg, 1.07 mmol, 3.00 eq.) and 161 mg of monomer **IM2** (535 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 3 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (4:1 → 1:2) to yield the Passerini product **H3/1** as a pale highly viscous oil. (433.1 mg, 626 µmol, 56.7%).

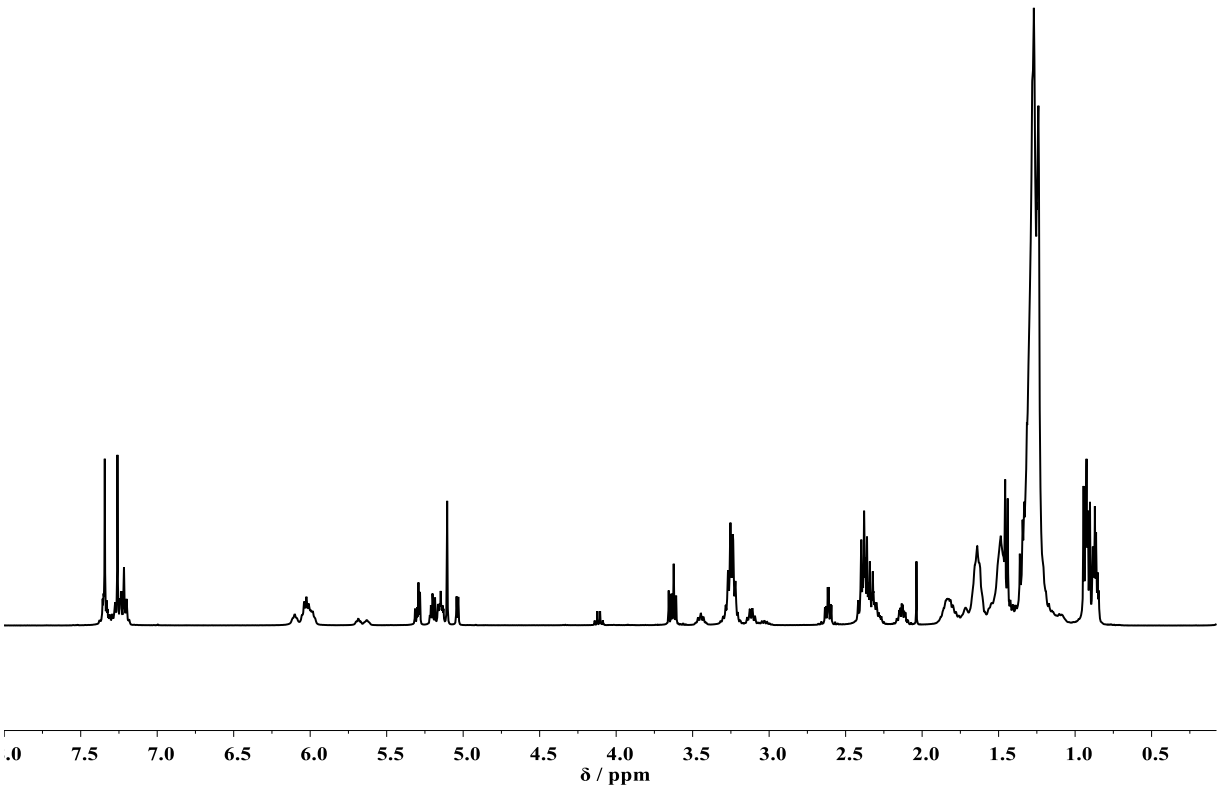
R_f = 0.39 in cyclohexane / ethyl acetate (1:1).

IR (ATR): ν / cm^{-1} = 3304.9 (vw), 2323.4 (vs), 2852.9 (s), 1738.7 (vs), 1652.6 (vs), 1535.3 (s), 1456.1 (m), 1372.2 (w), 1232.5 (m), 1161.6 (s), 1105.5 (m), 722.1 (w), 698.9 (m).

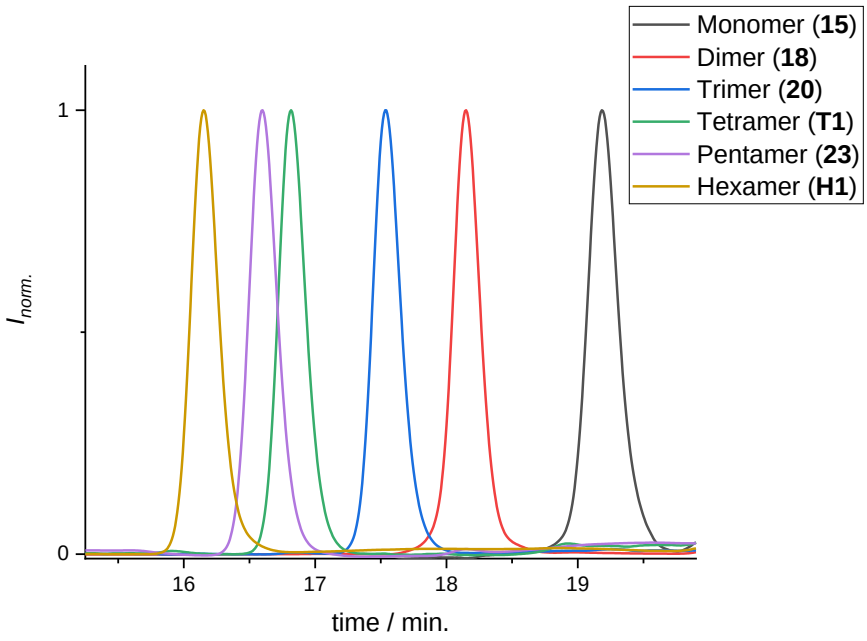
^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.46 – 7.13 (m, 10 H, CH_{Ar}^{1}), 6.20 – 5.92 (m, 5 H, NH^{2}), 5.75 – 5.59 (m, 1 H, NH^{2}), 5.34 – 5.08 (m, 7 H, CH^{3}, CH_2^{4}), 5.04 (d, J = 4.5 Hz, 1 H, CH^{3}), 3.69 – 3.58 (m, 2 H, CH_2^{5}), 3.49 – 3.39 (m, 1 H, CH^{6}), 3.34 – 2.97 (m, 12 H, CH_2^{7}), 2.66 – 2.57 (m, 2 H, CH_2^{8}), 2.45 – 2.23 (m, 13 H, CH^{9}, CH_2^{10}), 2.19 – 2.07 (m, 2 H, CH_2^{11}), 1.92 – 1.05 (m, 138 H, CH^{12}, CH_2^{12}, CH_3^{13}), 0.99 – 0.82 (m, 18 H, CH_3^{14}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.80, 172.71, 172.61, 172.46, 172.38, 171.64, 170.43, 170.00, 169.63, 169.40, 168.85, 168.65, 141.74, 141.23, 136.26, 128.67, 128.53, 128.31, 128.28, 127.97, 127.13, 127.05, 78.07, 77.87, 75.55, 74.07, 70.59, 66.19, 44.09, 43.66, 41.59, 41.30, 39.37, 39.32, 39.31, 39.22, 34.45, 34.42, 34.36, 34.30, 32.05, 31.75, 31.23, 30.65, 29.80, 29.75, 29.70, 29.69, 29.58, 29.53, 29.48, 29.35, 29.28, 29.26, 29.23, 29.21, 29.16, 29.04, 27.49, 26.97, 26.87, 26.81, 25.14, 25.10, 25.07, 25.01, 24.92, 24.85, 22.82, 22.66, 22.39, 22.03, 18.91, 18.10, 17.64, 17.11, 15.35, 14.25, 14.17, 11.73, 11.71.

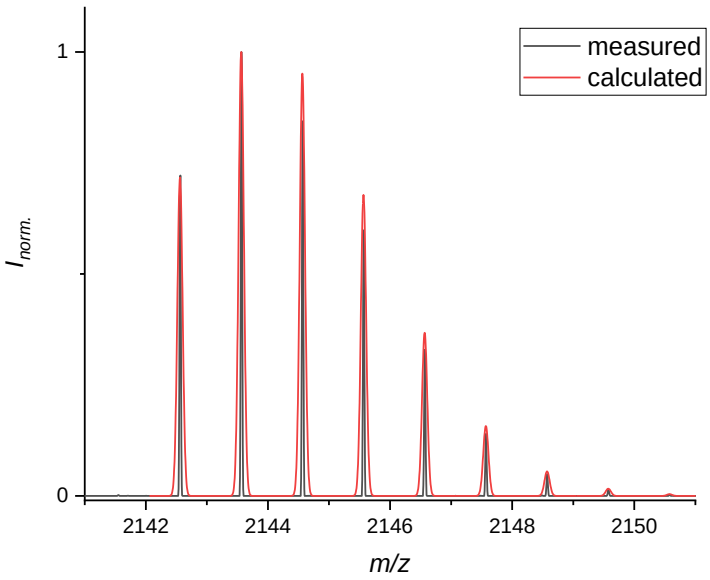
ESI-MS [m/z]: $[M + H]^+$ calculated for $^{12}C_{124}{}^{1}H_{213}{}^{16}O_{20}{}^{14}N_6{}^{35}Cl$, 2142.5596; found, 2142.5607, Δ = 1.1 mmu.



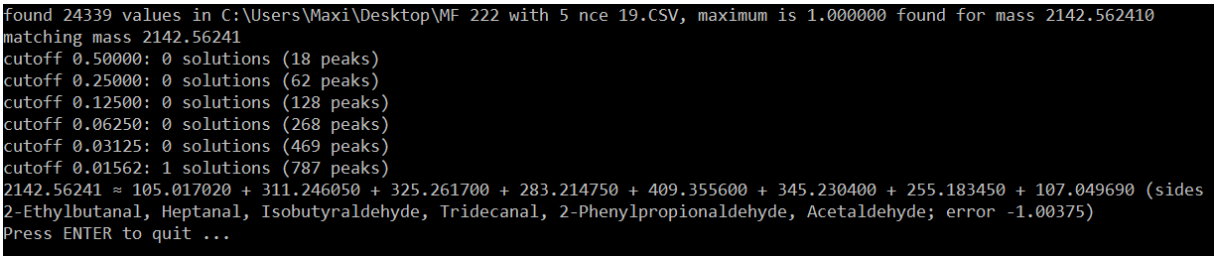
Supplementary Figure 130: ^{1}H-NMR of compound **H3/1** measured in $CDCl_3$.



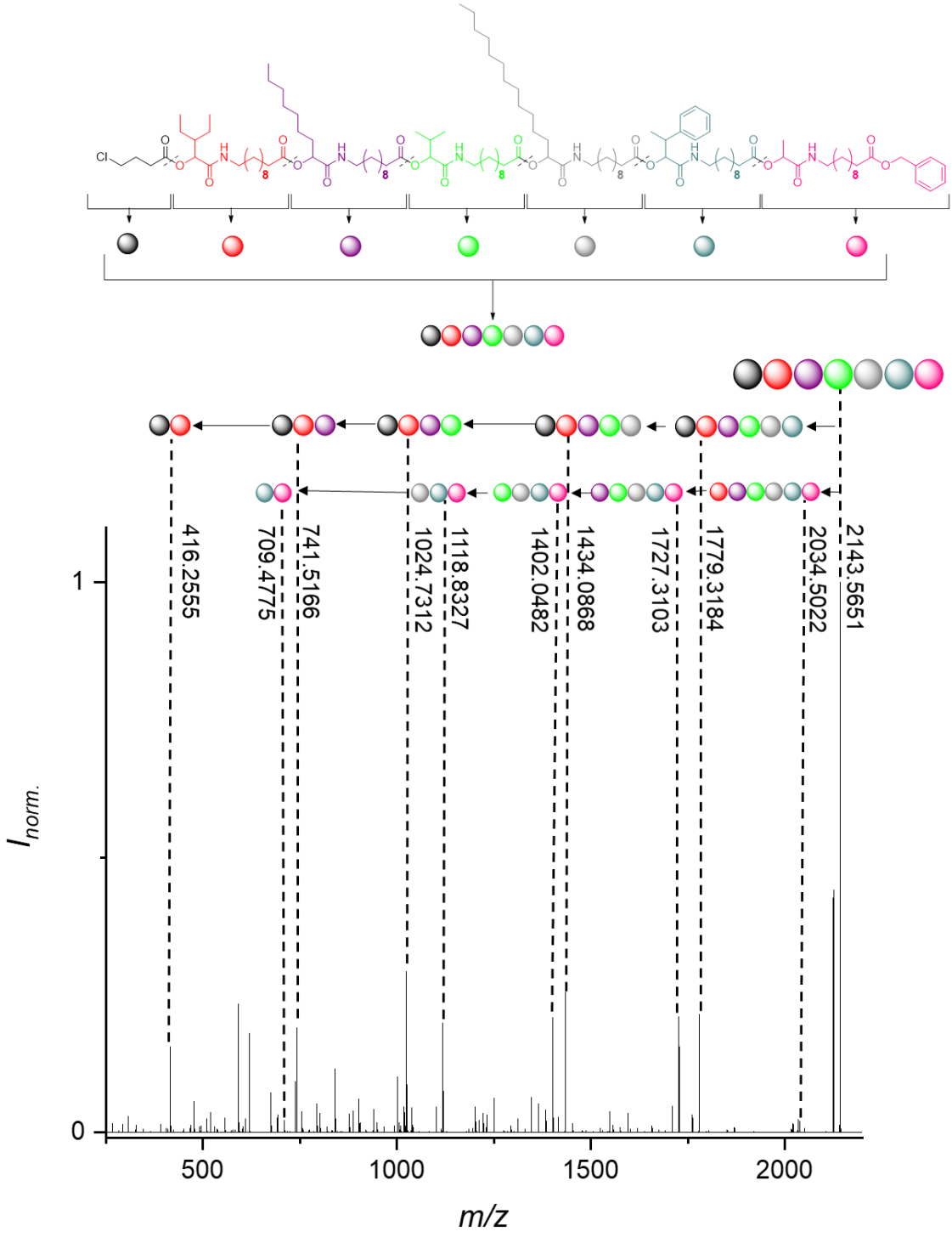
Supplementary Figure 131: SEC traces of the intermediates after each P-3CR in the synthesis of product **H3/1**.



Supplementary Figure 132: High resolution ESI-MS measurement of **H3/1**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (red).



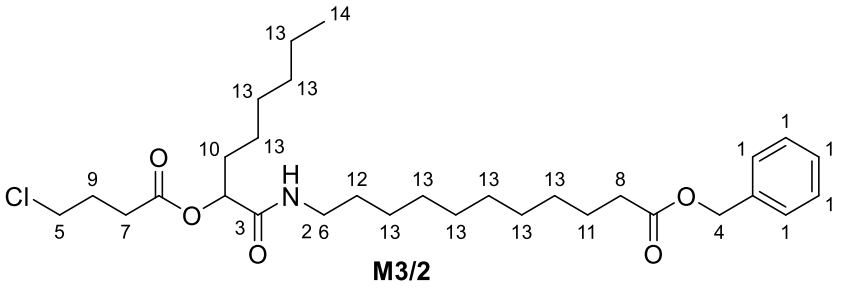
Supplementary Figure 133: Screenshot of the automated read-out of **H3/1**.



Supplementary Figure 134: Read-out of the sequence-defined hexamer **H3/1**. Read-out of the hexamer **H3/1** *via* tandem ESI-MS/MS with an NCE of 18. In the spectrum, the read-out from both ends of the oligomer using the fragmentation next to the carbonyl are shown.

6.3.3.5.3 Synthesis of tetramer T3/2[vii]

Passerini reaction



In a 50 mL round bottom flask, 74.5 mg 4-chlorobutyric acid **TAG3** (608 µmol, 1.00 eq.) was dissolved in 2.00 mL DCM and 130 µL heptanal **A7** (104 mg, 912 µmol, 1.50 eq.) and 275 mg of monomer **IM2** (912 µmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 3 days. Subsequently, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane / ethyl acetate 8:1 → 6:1) to afford product **M3/2** as a high viscous oil in a yield of 78.9% (258 mg, 480 µmol).

R_f = 0.32 in cyclohexane / ethyl acetate (3:1).

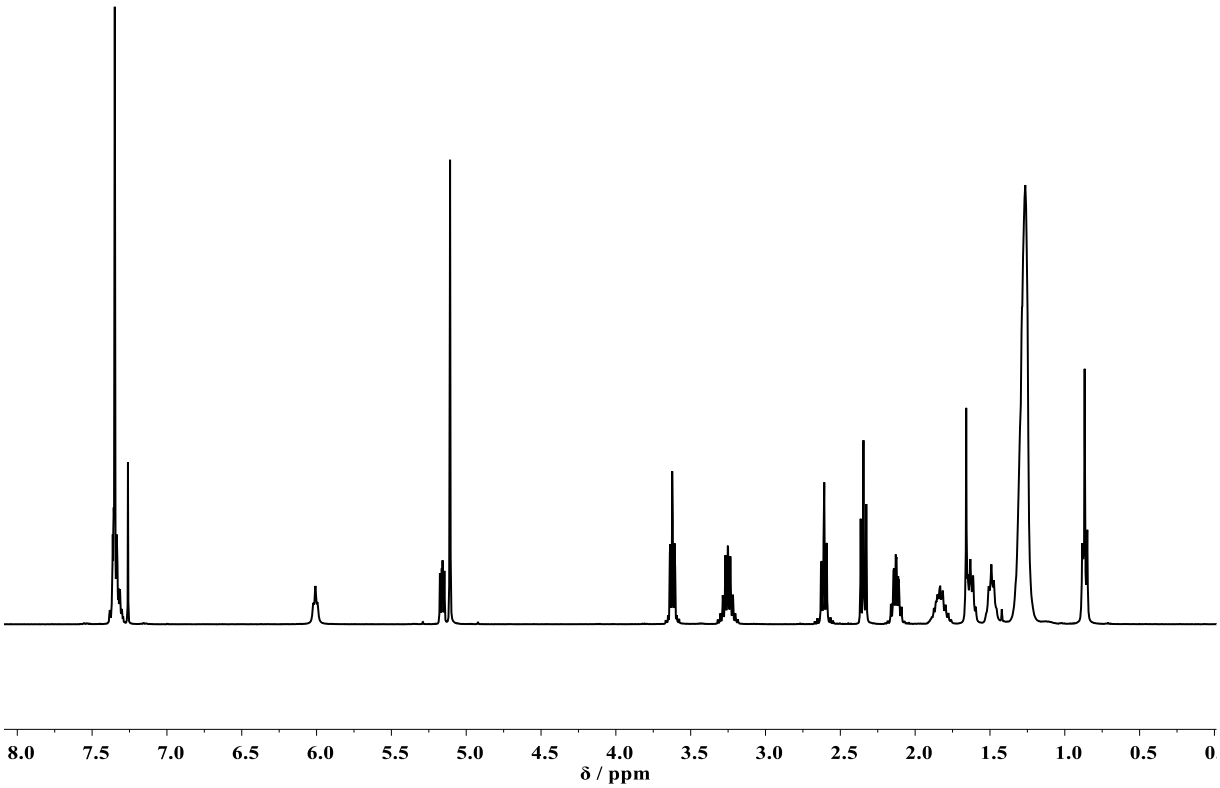
IR (ATR): ν / cm^{-1} = 3306.6 (vw), 2924.7 (s), 2854.1 (m), 1735.9 (vs), 1655.6 (s), 535.1 (m), 1455.4 (w), 1377.2 (w), 1143.9 (s), 734.2 (w), 696.9 (m), 648.9 (w), 401.0 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.42 – 7.29 (m, 5 H, CH_{Ar}^1), 6.01 (t, *J* = 5.9 Hz, 1 H, NH^2), 5.18 – 5.14 (m, 1 H, CH^3), 5.11 (s, 2 H, CH_2^4), 3.62 (td, *J* = 6.3, 1.4 Hz, 2 H, CH_2^5), 3.36 – 3.17 (m, 2 H, CH_2^6), 2.61 (td, *J* = 7.1, 1.5 Hz, 2 H, CH_2^7), 2.35 (t, *J* = 7.5 Hz, 2 H, CH_2^8), 2.18 – 2.06 (m, 2 H, CH_2^9), 1.93 – 1.74 (m, 2 H, CH_2^{10}), 1.69 – 1.59 (m, 2 H, CH_2^{11}), 1.54 – 1.44 (m, 2 H, CH_2^{12}), 1.40 – 1.13 (m, 20 H, CH_2^{13}), 0.91 – 0.83 (m, 3 H, CH_3^{14}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.81, 171.58, 169.74, 136.25, 128.66, 128.28, 74.47; 66.20, 44.07, 39.37, 34.45, 32.05, 31.73, 31.25, 29.65, 29.56, 29.47, 29.33, 29.23, 29.03, 27.50, 26.95, 25.07, 24.86, 22.66, 14.17.

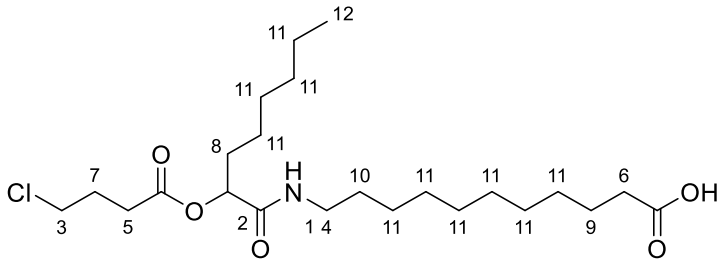
ESI-MS [m/z]: $[M + H]^+$ calculated for $^{12}C_{30}{}^{1}H_{48}{}^{16}O_5{}^{14}N^{35}Cl$, 538.3294; found, 538.3282, Δ = 1.2 mmu.

[vii] Synthesis up to the trimer was carried out by Nico Zuber in the Bachelorthesis "Synthesis of sequence-defined oligomers with 4-chlorobutyric acid as starter acid" under the laboratory supervision of Maximiliane Frölich.[291]



Supplementary Figure 135: 1H-NMR of compound **M3/2** measured in CDCl3.

Deprotection



In a 50 mL round bottom flask, 209 mg of **M3/2** (389 µmol, 1.00 eq.) were dissolved in 2.00 mL ethyl acetate and 2.00 mL THF. Afterwards, 41.8 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **MD3/2** was obtained as a pale highly viscous oil in a yield of 99.0% (172 mg, 385 µmol).

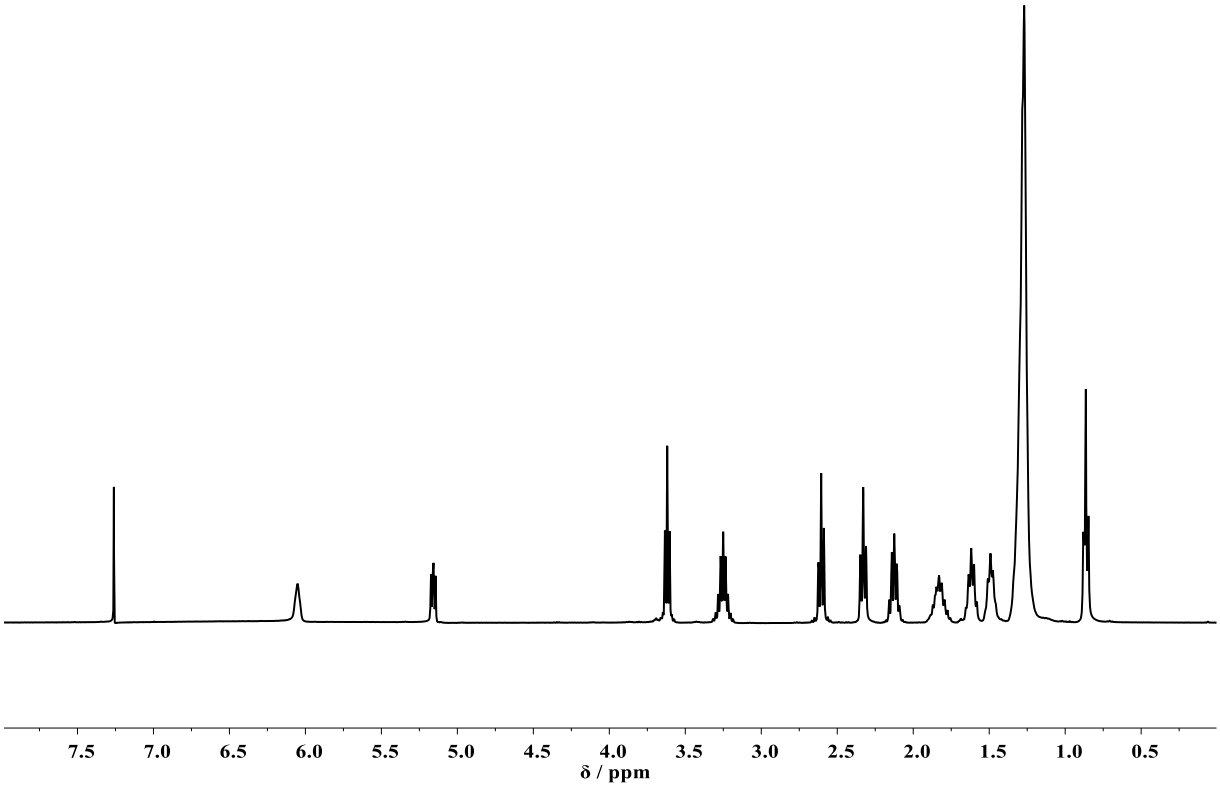
IR (ATR): ν / cm^{-1} = 3296.9 (w), 2918.7 (vs), 2850.5 (s), 1731.7 (vs), 1694.2 (vs), 1651.8 (vs), 1557.9 (s), 1468.6 (m), 1430.0 (m), 1378.9 (m), 1330.2 (m), 1277.1 (s), 1219.4 (vs), 1173.2

(vs), 1082.2 (m), 925.5 (m), 794.1 (w), 722.8 (m), 683.4 (m), 650.5 (m), 473.8 (vw), 437.5 (w), 388.7 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.11 – 6.01 (m, 1 H, NH^1), 5.20 – 5.11 (m, 1 H, CH^2), 3.62 (t, J = 6.1 Hz, 2 H, CH_2^3), 3.35 – 3.17 (m, 2 H, CH_2^4), 2.60 (t, J = 6.6 Hz, 2 H, CH_2^5), 2.36 – 2.28 (m, 2 H, CH_2^6), 2.19 – 2.06 (m, 2 H, CH_2^7), 1.92 – 1.75 (m, 2 H, CH_2^8), 1.67 – 1.56 (m, 2 H, CH_2^9), 1.56 – 1.43 (m, 2 H, CH_2^{10}), 1.38 – 1.12 (m, 20 H, CH_2^{11}), 0.92 – 0.81 (m, 3 H, CH_3^{12}).

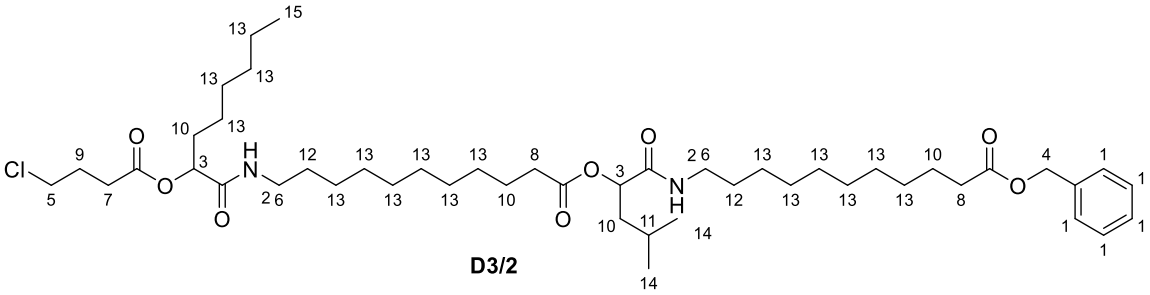
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 179.09, 171.64, 169.91, 74.47, 44.05, 39.41, 34.13, 32.03, 31.72, 31.26, 29.59, 29.48, 29.37, 29.25, 29.11, 29.02, 27.51, 26.91, 24.85, 24.83, 22.65, 14.15.

ESI-MS [m/z]: $[M + Na]^+$ calculated for $^{12}C_{23}{}^{1}H_{42}{}^{16}O_5{}^{14}N^{35}Cl$, 470.2644; found, 470.2639, Δ = 0.5 mmu.



Supplementary Figure 136: ^{1}H-NMR of compound **MD3/2** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 172 mg **MD3/2** (384 µmol, 1.00 eq.) was dissolved in 2.00 mL DCM and 62.1 µL 3-methylbutanal **A4** (49.7 mg, 577 µmol, 1.50 eq.) and 174 mg of monomer **IM2** (577 µmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 3 days. Subsequently, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane / ethyl acetate 5:1 → 2:1) to afford product **D3/2** as a high viscous oil in a yield of 70.6% (227 mg, 271 µmol).

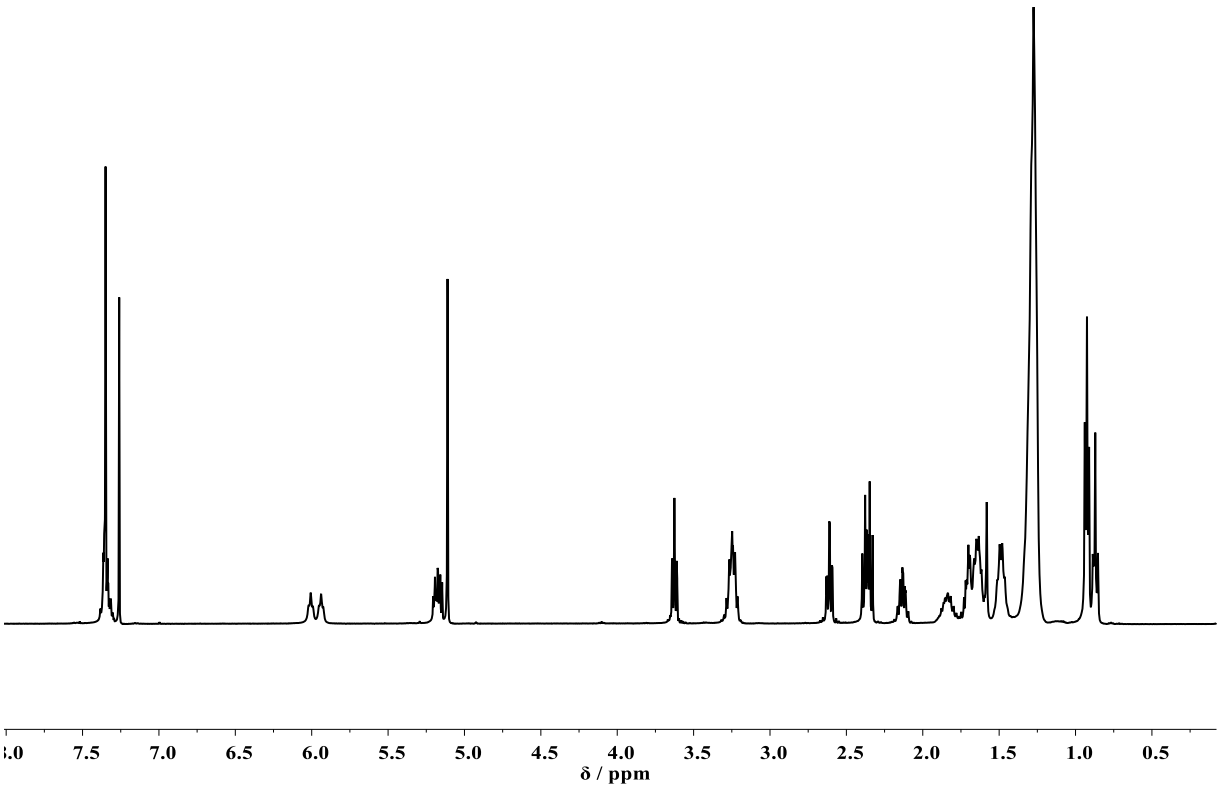
R_f = 0.39 in cyclohexane / ethyl acetate (2:1).

IR (ATR): ν / cm^{-1} = 304.3 (vw), 2924.8 (s), 2854.1 (m), 1737.4 (vs), 1654.9 (vs), 1536.0 (m), 1456.3 (m), 1371.1 (w), 1164.8 (s), 1061.8 (w), 732.8 (w), 697.0 (m).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.37 – 7.21 (m, 5 H, CH_{Ar}^1), 5.98 – 5.91 (m, 1 H, NH^2), 5.90 – 5.81 (m, 1 H, NH^2), 5.16 – 5.06 (m, 2 H, CH^3), 5.04 (s, 2 H, CH_2^4), 3.56 (t, J = 5.7 Hz, 2 H, CH_2^5), 3.26 – 3.11 (m, 4 H, CH_2^6), 2.58 – 2.49 (m, 2 H, CH_2^7), 2.35 – 2.24 (m, 4 H, CH_2^8), 2.11 – 2.00 (m, 2 H, CH_2^9), 1.85 – 1.52 (m, 9 H, CH^{10}, CH_2^{11}), 1.47 – 1.36 (m, 4 H, CH_2^{12}), 1.31 – 1.12 (m, 32 H, CH_2^{13}), 0.86 (t, J = 5.6 Hz, 6 H, CH_3^{14}), 0.83 – 0.78 (m, 3 H, CH_3^{15}).

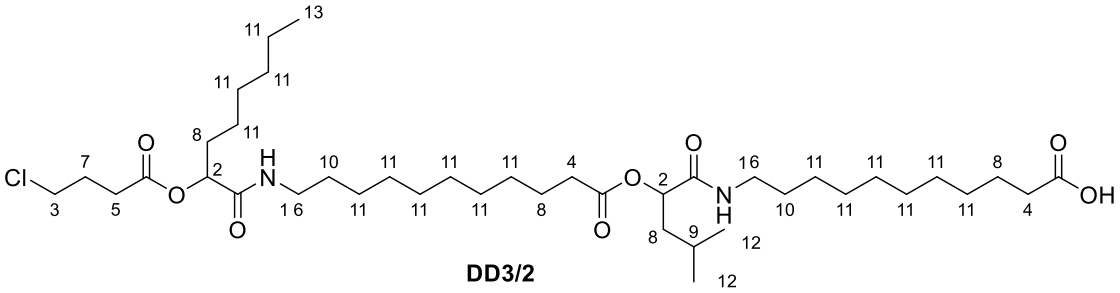
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.84, 172.78, 171.61, 170.34, 169.78, 128.69, 128.31, 74.49, 72.80, 66.22, 44.09, 40.99, 39.38, 34.47, 34.45, 32.07, 31.74, 31.26, 29.67, 29.58, 29.49, 29.35, 29.25, 29.24, 29.04, 27.51, 26.96, 26.95, 25.08, 24.88, 24.70, 23.28, 22.67, 21.95, 14.18.

ESI-MS [m/z]: $[M + H]^+$ calculated for $^{12}C_{47}{}^{1}H_{79}{}^{6}O_8{}^{14}N_2{}^{35}Cl$, 835.5598; found, 835.5588, Δ = 1.0 mmu.



Supplementary Figure 137: ^{1}H-NMR of compound **D3/2** measured in $CDCl_3$.

Deprotection



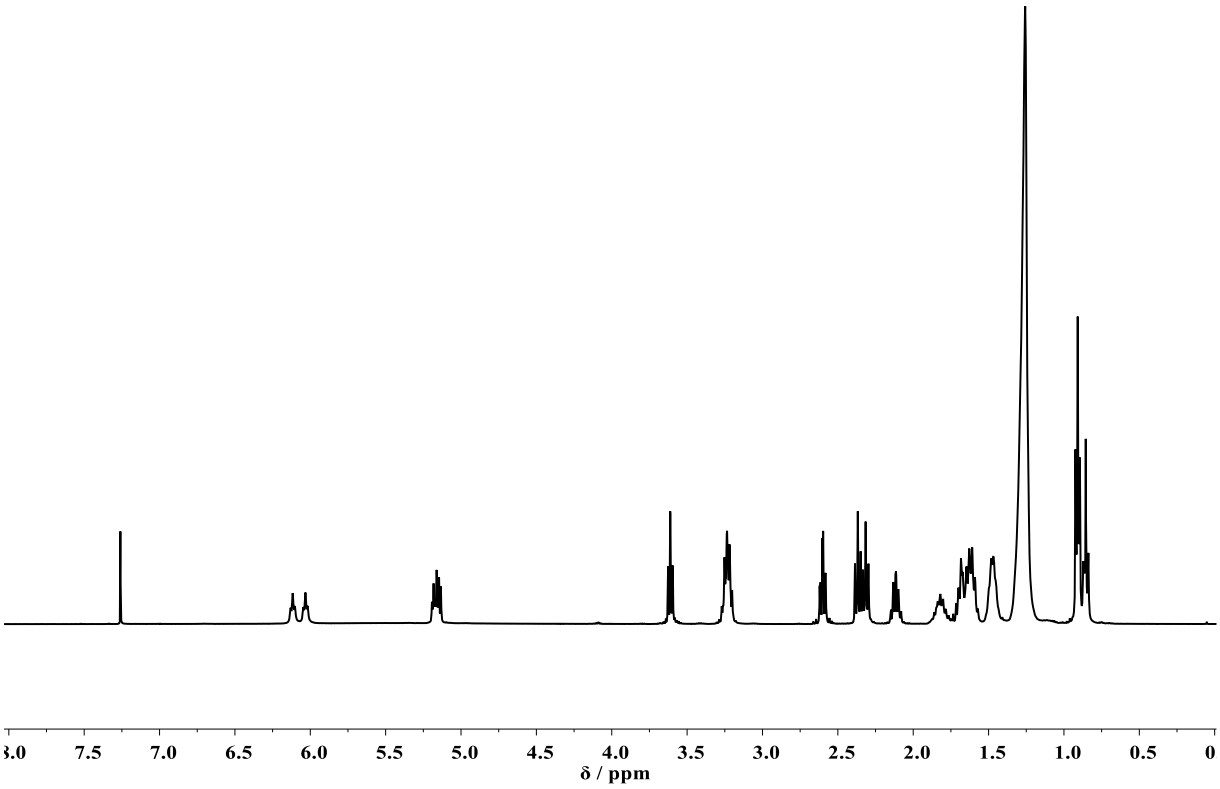
In a 50 mL round bottom flask, 176 mg of **D3/2** (211 µmol, 1.00 eq.) were dissolved in 2.00 mL of ethyl acetate and 2.00 mL THF. Afterwards, 35.2 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **DD3/2** was obtained as a pale highly viscous oil in a yield of 97.6% (153 mg, 206 mmol).

IR (ATR): ν / cm^{-1} = 3306.8 (vw), 2924.2 (s), 2853.7 (m), 1738.2 (s), 1651.7 (s), 1540.2 (m), 1463.1 (w), 1370.1 (w), 1143.5 (s), 1061.2 (w), 722.6 (w), 650.3 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.12 – 6.03 (m, 1 H, NH^{1}), 5.98 (t, J = 5.8 Hz, 1 H, NH^{1}), 5.16 – 5.07 (m, 2 H, CH^{2}), 3.62 – 3.52 (m, 2 H, CH_2^{3}), 3.24 – 3.13 (m, 4 H, CH_2^{4}), 2.59 – 2.50 (m, 2 H, CH_2^{5}), 2.36 – 2.22 (m, 4 H, CH_2^{6}), 2.11 – 2.01 (m, 2 H, CH_2^{7}), 1.85 – 1.69 (m, 2 H, CH_2^{8}), 1.68 – 1.50 (m, 7 H, CH^{9}, CH_2^{8}), 1.50 – 1.34 (m, 4 H, CH_2^{10}), 1.31 – 1.13 (m, 32 H, CH_2^{11}), 0.86 (t, J = 5.8 Hz, 6 H, CH_3^{12}), 0.83 – 0.77 (m, 3 H, CH_3^{13}).

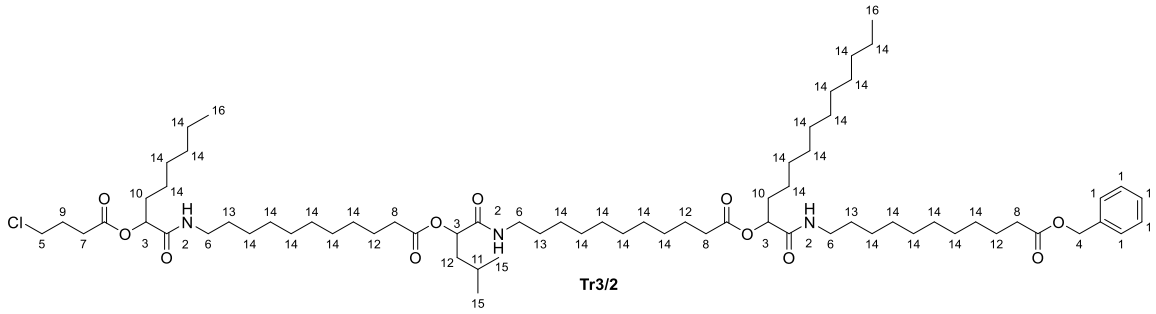
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 178.33, 172.81, 171.67, 170.47, 169.97, 74.42, 72.75, 44.05, 40.92, 39.40, 39.35, 34.40, 34.11, 32.01, 31.70, 31.22, 29.60, 29.56, 29.53, 29.46, 29.45, 29.36, 29.31, 29.30, 29.23, 29.21, 29.10, 29.00, 27.47, 26.93, 26.86, 25.04, 24.86, 24.84, 24.65, 23.23, 22.63, 21.89, 14.15.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{40}{}^{1}H_{73}{}^{6}O_8{}^{14}N_2{}^{35}Cl^{23}$, 745.5128; found, 745.5113, Δ = 1.5 mmu.



Supplementary Figure 138: ^{1}H-NMR of compound **DD3/2** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 91.2 mg **DD3/2** (122 µmol, 1.00 eq.) was dissolved in 2.00 mL DCM and 40.8 µL dodecanal **A10** (33.8 mg, 184 µmol, 1.50 eq.) and 55.3 mg of monomer **IM2** (184 µmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 3 days. Subsequently, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane / ethyl acetate 8:1 → 6:1) to afford product **Tr3/2** as a high viscous oil in a yield of 82.8% (124 mg, 101 µmol).

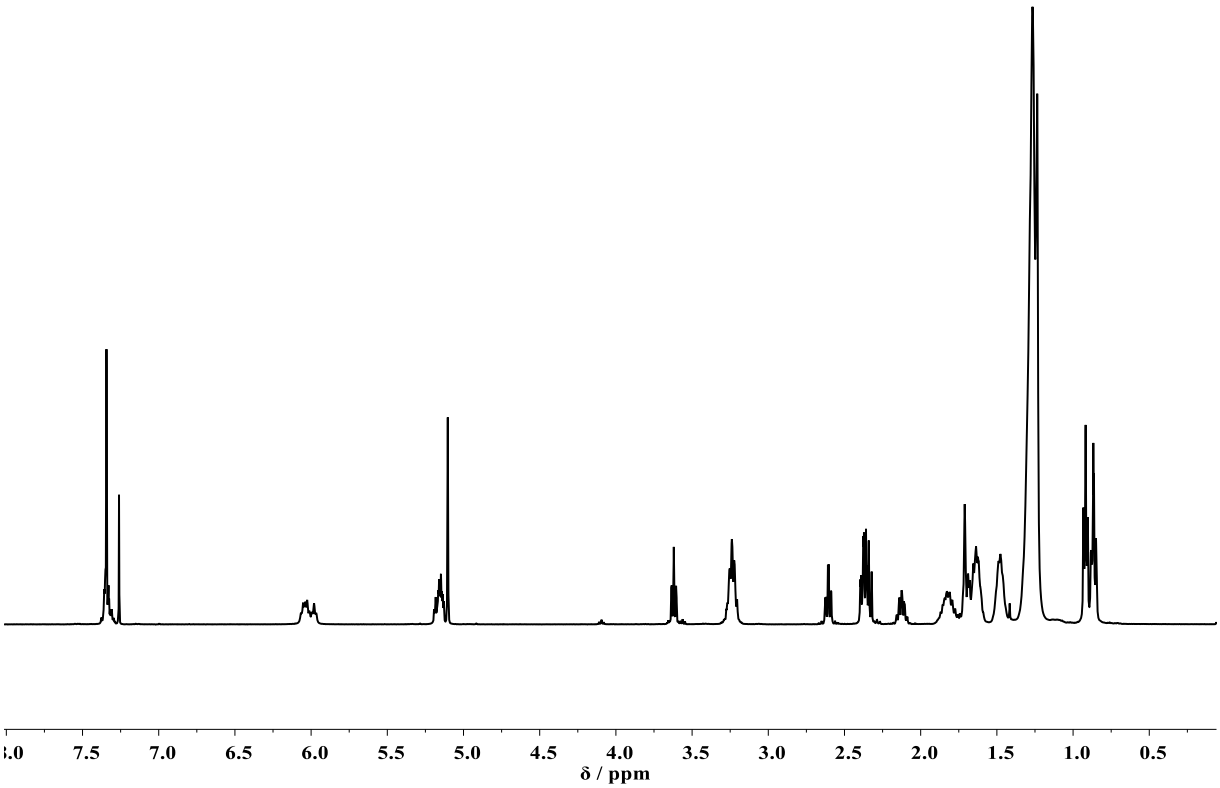
R_f = 0.77 in cyclohexane / ethyl acetate (1:1).

IR (ATR): ν / cm^{-1} = 3294.1 (w), 2923.0 (s), 2852.9 (m), 1738.2 (s), 1654.5 (s), 1535.6 (m), 1457.0 (w), 1371.2 (w), 1163.3 (s), 723.5 (w), 697.0 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.36 – 7.23 (m, 5 H, CH_{Ar}^1), 6.04 – 5.88 (m, 3 H, NH^2), 5.16 – 5.05 (m, 3 H, CH^3), 5.04 (s, 2 H, CH_2^4), 3.62 – 3.53 (m, 2 H, CH_2^5), 3.27 – 3.12 (m, 6 H, CH_2^6), 2.59 – 2.50 (m, 2 H, CH_2^7), 2.34 – 2.24 (m, 6 H, CH_2^8), 2.12 – 2.01 (m, 2 H, CH_2^9), 1.84 – 1.67 (m, 4 H, CH_2^{10}), 1.64 – 1.51 (m, 9 H, CH^{11}, CH_2^{12}), 1.47 – 1.37 (m, 6 H, CH_2^{13}), 1.30 – 1.14 (m, 62 H, CH_2^{14}), 0.86 (t, J = 5.8 Hz, 6 H, CH_3^{15}), 0.83 – 0.77 (m, 6 H, CH_3^{16}).

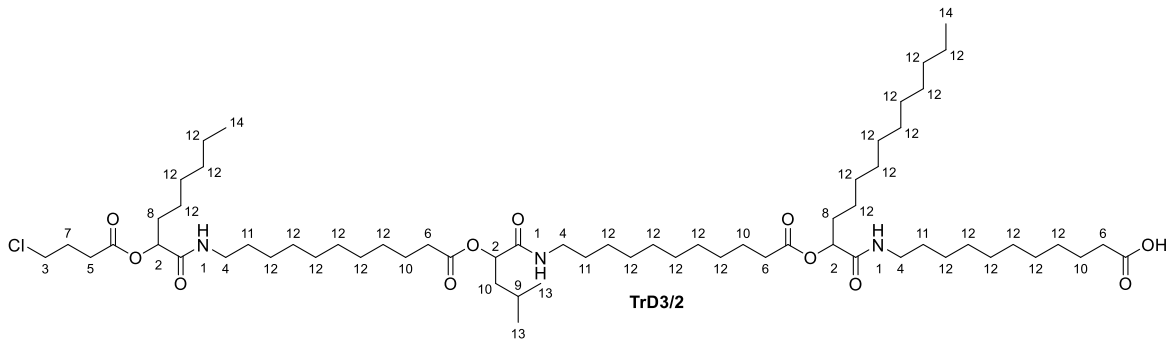
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.82, 172.78, 172.61, 171.61, 170.36, 169.99, 169.78, 136.23, 128.66, 128.29, 128.28, 74.44, 74.06, 72.75, 66.19, 44.07, 40.97, 39.33, 39.32, 34.44, 32.03, 31.72, 31.22, 29.74, 29.66, 29.65, 29.57, 29.55, 29.47, 29.38, 29.34, 29.32, 29.23, 29.21, 29.02, 27.48, 26.95, 26.92, 25.08, 25.05, 25.04, 24.88, 24.86, 24.66, 23.26, 22.81, 22.65, 21.91, 14.25, 14.16.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{71}{}^1H_{124}{}^{16}O_{11}{}^{14}N_3{}^{35}Cl$, 1230.8997; found, 1230.8976, Δ = 2.1 mmu.



Supplementary Figure 139: ^{1}H-NMR of compound **Tr3/2** measured in $CDCl_3$.

Deprotection



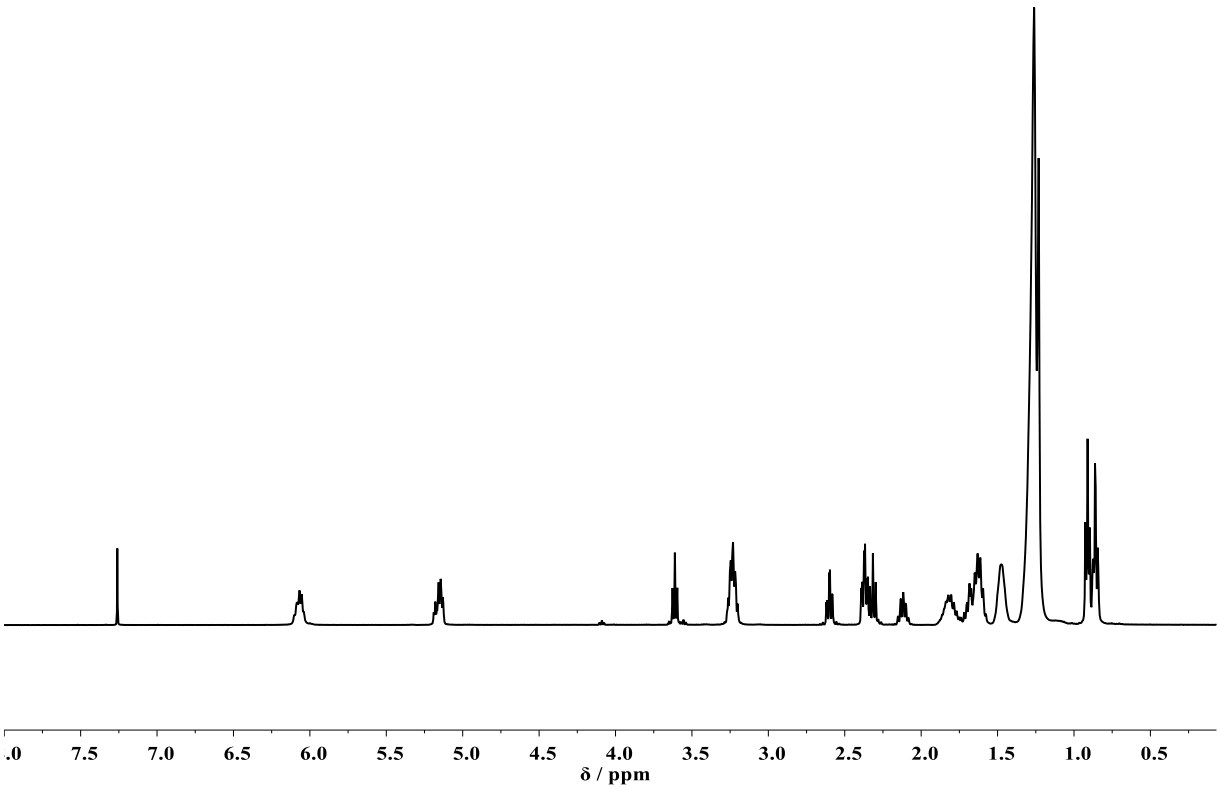
In a 50 mL round bottom flask, 75.3 mg of **Tr3/2** (61.0 µmol, 1.00 eq.) were dissolved in 2.00 mL ethyl acetate and 2.00 mL THF. Afterwards, 15.6 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **TrD3/2** was obtained as a pale highly viscous oil in a yield of 89.2% (62.3 mg, 54.4 mmol).

IR (ATR): ν / cm^{-1} = 3292.6 (vw), 2822.8 (vs), 2852.9 (s), 1739.4 (s), 1652.5 (s), 1539.6 (m), 1463.5 (w), 1370.3 (w), 1165.3 (m), 722.0 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.10 – 5.95 (m, 3 H, NH^{1}), 5.18 – 5.01 (m, 3 H, CH^{2}), 3.61 – 3.49 (m, 2 H, CH_2^{3}), 3.25 – 3.12 (m, 6 H, CH_2^{4}), 2.60 – 2.50 (m, 2 H, CH^{5}), 2.36 – 2.20 (m, 6 H, CH_2^{6}), 2.13 – 2.00 (m, 2 H, CH_2^{7}), 1.85 – 1.67 (m, 4 H, CH_2^{8}), 1.67 – 1.50 (m, 9 H, CH^{9}, CH_2^{10}), 1.48 – 1.37 (m, 6 H, CH_2^{11}), 1.30 – 1.13 (m, 62 H, CH_2^{12}), 0.86 (t, J = 5.9 Hz, 6 H, CH_3^{13}), 0.84 – 0.77 (m, 6 H, CH_3^{14}).

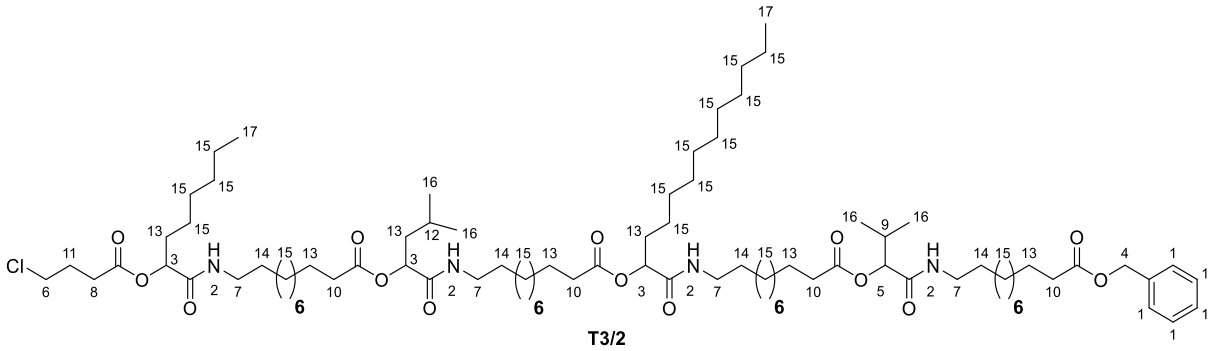
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 177.57, 172.86, 172.62, 171.63, 170.50, 170.09, 169.90, 74.44, 74.08, 72.77, 44.04, 40.93, 39.40, 39.39, 39.31, 34.44, 34.40, 34.01, 32.02, 31.99, 31.71, 31.24, 29.73, 29.65, 29.61, 29.57, 29.55, 29.49, 29.45, 29.36, 29.30, 29.24, 29.20, 29.11, 29.00, 27.50, 26.92, 26.91, 26.89, 25.10, 25.02, 24.89, 24.87, 24.85, 24.66, 23.23, 22.79, 22.63, 21.91, 14.22, 14.14.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{64}{}^{1}H_{118}{}^{16}O_{11}{}^{14}N_3{}^{35}Cl^{23}$, 1140.8528; found, 1140.8505, Δ = 2.3 mmu.



Supplementary Figure 140: ^{1}H-NMR of compound **Tr3/2** measured in $CDCl_3$.

Passerini reaction[viii]



In a 50 mL round bottom flask, 62.0 mg **TrD3/2** (54.0 µmol, 1.00 eq.) was dissolved in 2.00 mL DCM and 8.00 µL isobutyraldehyde **A3** (5.90 mg, 81.0 µmol, 1.50 eq.) and 29.2 mg of monomer **IM2** (81.0 µmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 24 hours. Subsequently, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (hexane / ethyl acetate 4:1 → 2:1) to afford product **T3/2** as a highly viscous oil in a yield of 70.7% (58.2 mg, 38.2 µmol).

R_f = 0.53 in cyclohexane / ethyl acetate (2:1).

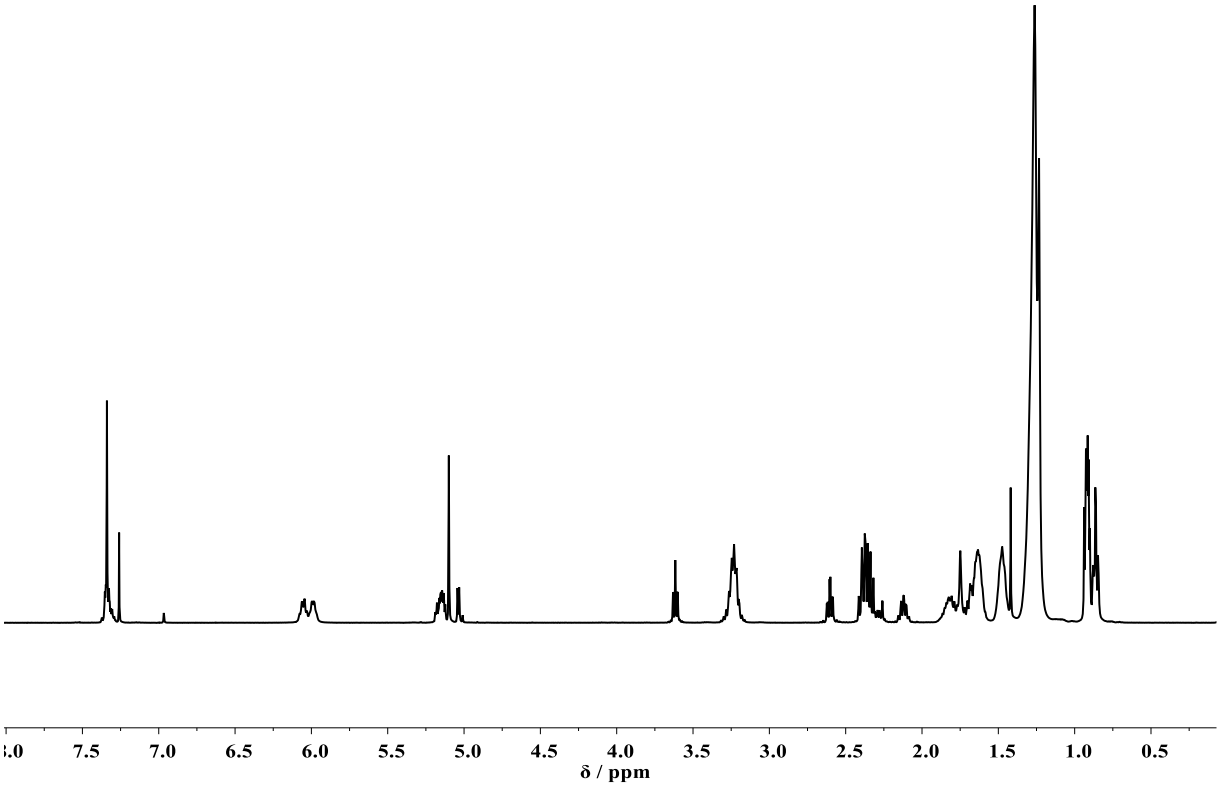
IR (ATR): ν / cm^{-1} = 3293.3 (vw), 2919.9 (m), 2851.4 (w), 1736.3 (m), 1654.9 (s), 1556.3 (w), 1466.3 (w), 1374.3 (w), 1243.1 (w), 1207.6(w), 1176.0 (m), 1059.7 (w), 1000.8 (w), 879.3 (vw), 841.2 (vw), 721.1 (w), 689.3 (w), 430.7 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.35 – 7.23 (m, 5 H, CH_{Ar}^1), 6.07 – 5.89 (m, 4 H, NH^2), 5.16 – 5.07 (m, 3 H, CH^3), 5.04 (s, 2 H, CH_2^4), 4.98 (d, J = 4.4 Hz, 1 H, CH^5), 3.56 (t, J = 6.1 Hz, 2 H, CH_2^6), 3.27 – 3.09 (m, 8 H, CH_2^7), 2.60 – 2.50 (m, 2 H, CH_2^8), 2.38 – 2.18 (m, 9 H, CH^9, CH_2^{10}), 2.11 – 2.02 (m, 2 H, CH_2^{11}), 1.85 – 1.51 (m, 15 H, CH^{12}, CH_2^{13}), 1.48 – 1.37 (m, 8 H, CH_2^{14}), 1.32 – 1.10 (m, 74 H, CH_2^{15}), 0.90 – 0.83 (m, 12 H, CH_3^{16}), 0.83 – 0.78 (m, 6 H, CH_3^{17}).

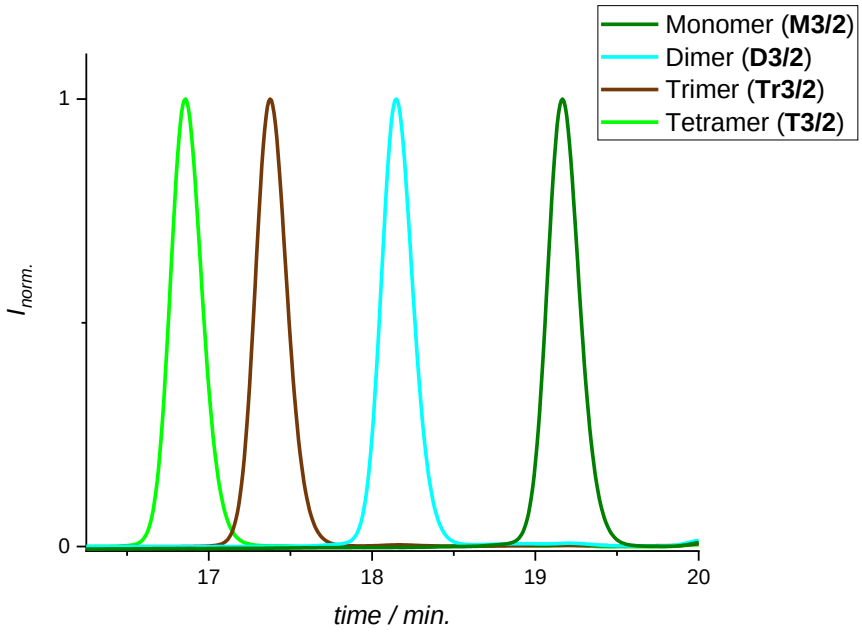
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.81, 172.78, 172.69, 172.62, 171.61, 170.36, 170.00, 169.78, 169.39, 136.24, 128.65, 128.28, 128.27, 125.63, 78.05, 74.44, 74.06, 72.75, 66.18, 44.06, 40.97, 39.34, 39.28, 34.43, 34.40, 32.03, 31.72, 31.23, 30.64, 30.44, 29.74, 29.69, 29.68, 29.65, 29.57, 29.55, 29.48, 29.46, 29.38, 29.37, 29.35, 29.34, 29.33, 29.31, 29.25, 29.23, 29.22, 29.01, 27.49, 26.96, 26.92, 25.12, 25.07, 25.05, 25.04, 24.90, 24.86, 24.66, 23.25, 22.80, 22.64, 21.91, 18.90, 17.09, 14.24, 14.15.

[viii] Synthesis was carried out by Lara Faden in the Vertieferarbeit "Synthesis and characterization of monodisperse sequence-defined oligomers" under the laboratory supervision of Maximiliane Frölich.[290]

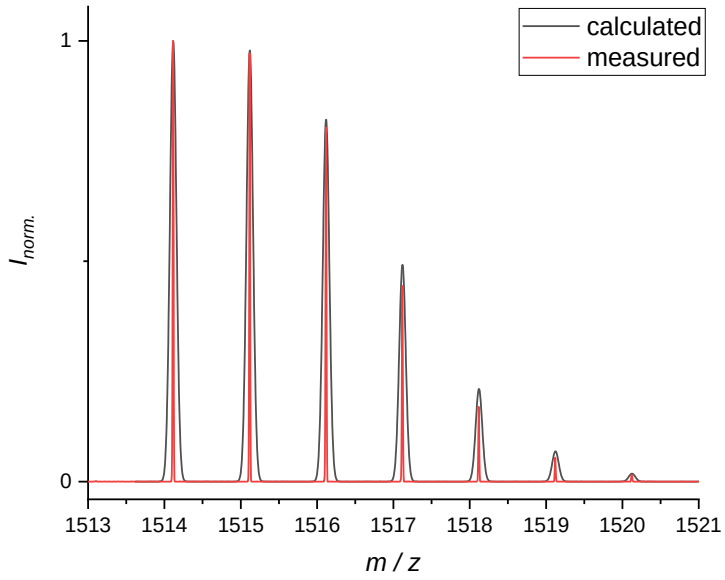
ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{87}{}^{1}H_{153}{}^{16}O_{14}{}^{14}N_4{}^{35}Cl$, 1514.1145; found, 1514.1137, Δ = 0.8 mmu.



Supplementary Figure 141: ^{1}H-NMR of compound **T3/2** measured in $CDCl_3$.



Supplementary Figure 142: SEC traces of the intermediates after each P-3CR in the synthesis of product **T3/2**.



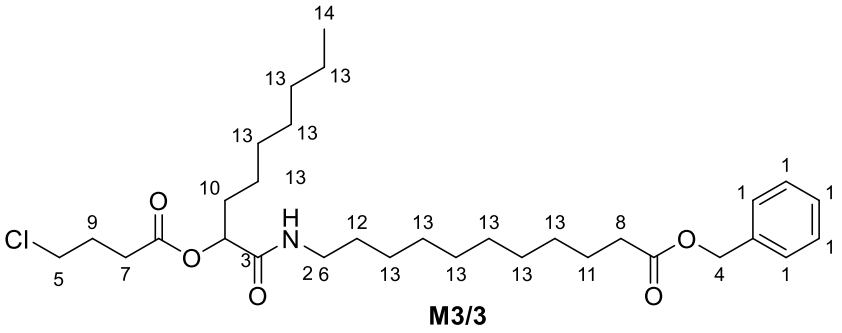
Supplementary Figure 143: High resolution ESI-MS measurement of **T3/2**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (black).

```
er.CSV, maximum is 100.000000 found for mass 1536.089620
matching mass 1536.08962
cutoff 0.50000: 0 solutions (22 peaks)
cutoff 0.25000: 0 solutions (75 peaks)
cutoff 0.12500: 0 solutions (140 peaks)
cutoff 0.06250: 1 solutions (239 peaks)
1536.08962 ≈ 121.005630 + 325.261700 + 297.230400 + 395.339950 + 283.214750 + 91.054780 (sides Heptanal, 3-Methylbutanal
, Dodecanal, Isobutyraldehyde; error -22.98241)
Press ENTER to quit ...
```

Supplementary Figure 144: Screenshot of the automated read-out of **T3/2**, sodium trifluoroacetate **2** was used as additive during the measurement.

6.3.3.5.4 Synthesis of tetramer T3/3

Passerini reaction



In a 50 mL round bottom flask, 161 mg 4-chlorobutyric acid **TAG3** (1.31 mmol, 1.00 eq.) were stirred in 2.00 mL DCM. Subsequently, 308 µL octanal **A8** (252 mg, 1.97 mmol, 1.50 eq.) and 594 mg of monomer **IM2** (1.97 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 3 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (8:1 → 6:1) to yield the Passerini product **M3/3** as a yellow highly viscous oil. (675 mg, 1.22 mmol, 86.2%).

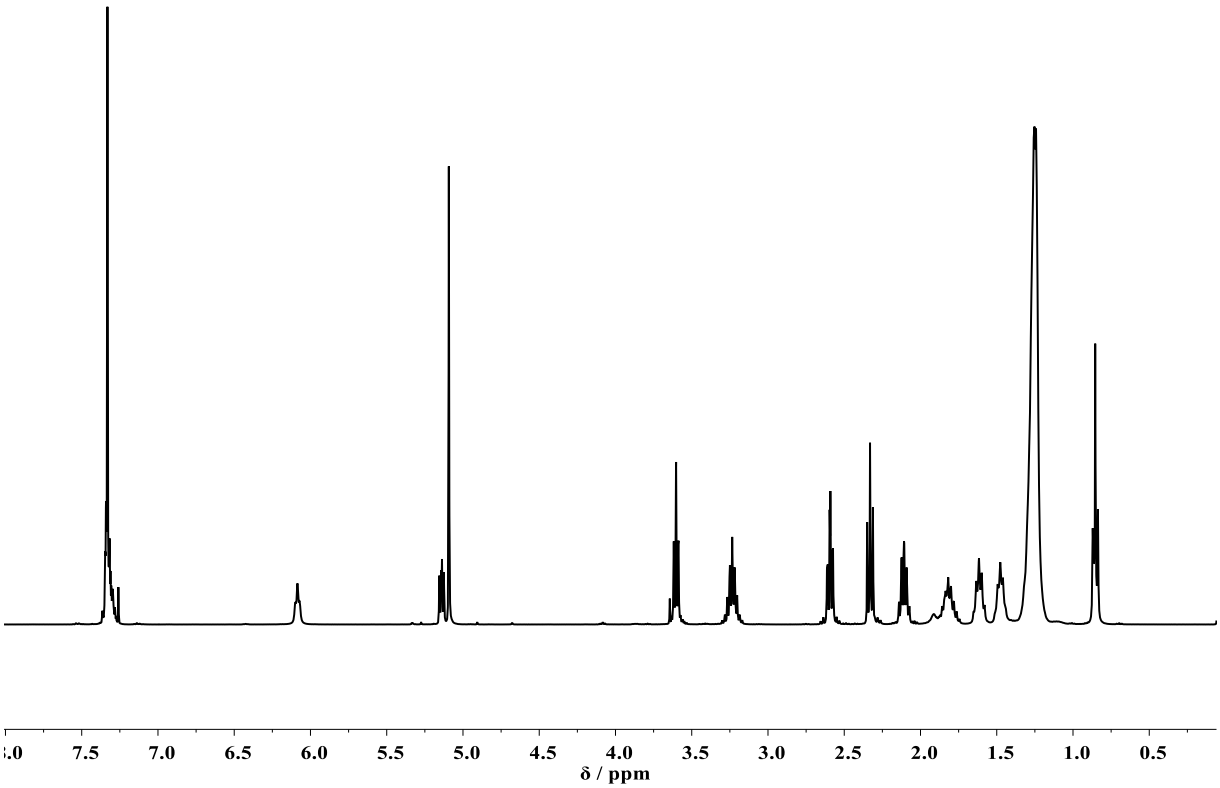
R_f = 0.34 in cyclohexane / ethyl acetate (3:1).

IR (ATR): ν / cm^{-1} = 3306.6 (vw), 2924.1 (s), 2853.7 (m), 1736.1 (vs), 1655.7 (s), 1535.9 (m), 1455.5 (w), 1377.0 (w), 1144.0 (s), 733.8 (m), 697.0 (m), 650.8 (w). 1455.5 (w), 1377.0 (w), 1144.0 (s), 733.8 (m), 697.0 (m), 650.8 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.41 – 7.27 (m, 5 H, CH_{Ar}^1), 6.09 (t, J = 5.9 Hz, 1 H, NH^2), 5.18 – 5.12 (m, 1 H, CH^3), 5.09 (s, 2 H, CH_2^4), 3.60 (td, J = 6.3, 1.2 Hz, 2 H, CH_2^5), 3.32 – 3.16 (m, 2 H, CH_2^6), 2.59 (td, J = 7.1, 1.7 Hz, 2 H, CH_2^7), 2.33 (t, J = 7.5 Hz, 2 H, CH^8), 2.16 – 2.06 (m, 2 H, CH_2^9), 1.95 – 1.73 (m, 2 H, CH_2^{10}), 1.66 – 1.56 (m, 2 H, CH_2^{11}), 1.52 – 1.45 (m, 2 H, CH_2^{12}), 1.37 – 1.14 (m, 22 H, CH_2^{13}), 0.92 – 0.81 (m, 3 H, CH_3^{14}).

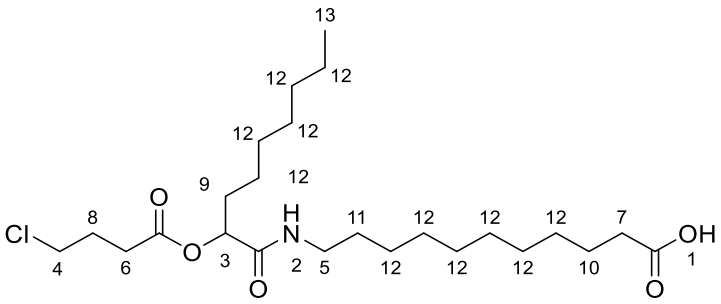
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.74, 171.54, 169.72, 136.19, 128.59, 128.20, 74.37, 66.11, 44.00, 39.30, 34.37, 31.99, 31.78, 31.16, 29.57, 29.49, 29.39, 29.26, 29.15, 29.14, 27.44, 26.88, 24.99, 24.85, 22.66, 14.13.

ESI-MS [*m*/z]: $[M+H]^+$ calculated for $^{12}C_{31}{}^{1}H_{50}{}^{16}O_5{}^{14}N^{35}Cl$, 552.3450; found: 552.3438; Δ = 1.2 mmu.



Supplementary Figure 145: ^{1}H-NMR of compound **M3/3** measured in $CDCl_3$.

Deprotection



MD3/3

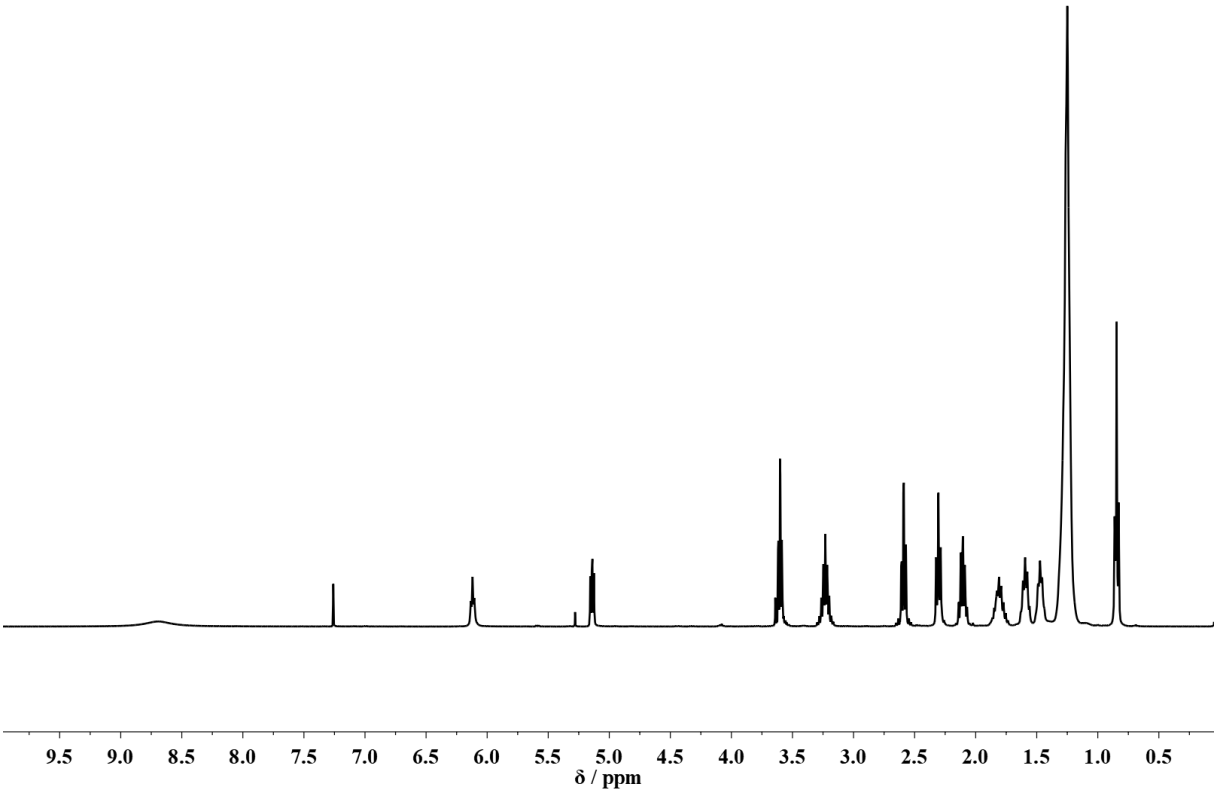
In a 50 mL round bottom flask, 606 mg of **M3/3** (1.10 mmol, 1.00 eq.) were dissolved in 2.00 mL ethyl acetate and 2.00 mL THF. Afterwards, 121 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **MD3/3** was obtained as a pale highly viscous oil in a yield of 98.2% (502 mg, 1.08 mmol).

IR (ATR): ν / cm^{-1} = 3294.8 (vw), 2923.7 (s), 2853.7 (m), 1737.3 (s), 1649.0 (s), 1541.6 (m), 1457.9 (w), 1376.1 (w), 1296.9 (w), 1173.7 (s), 1141.8 (s), 787.3 (w), 722.9 (w), 652.1 (w), 427.9 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 8.65 (br, 1 H, OH^{1}), 6.08 (t, J = 5.9 Hz, 1 H, NH^{2}), 5.13 – 5.02 (m, 1 H, CH^{3}), 3.61 – 3.51 (m, 2 H, CH_2^{4}), 3.28 – 3.11 (m, 2 H, CH_2^{5}), 2.62 – 2.49 (m, 2 H, CH_2^{6}), 2.26 (t, J = 7.5 Hz, 2 H, CH_2^{7}), 2.13 – 2.01 (m, 2 H, CH_2^{8}), 1.86 – 1.69 (m, 2 H, CH_2^{9}), 1.61 – 1.50 (m, 2 H, CH_2^{10}), 1.47 – 1.38 (m, 2 H, CH_2^{11}), 1.32 – 1.13 (m, 22 H, CH_2^{12}), 0.84 – 0.76 (m, 3 H, CH_3^{13}).

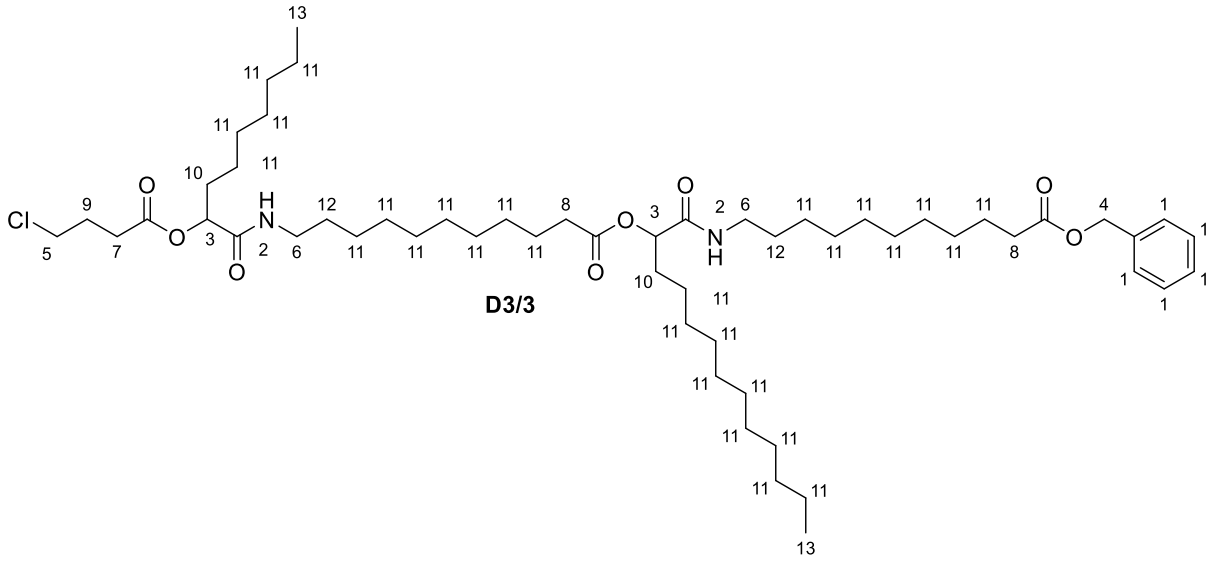
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 179.33, 171.62, 169.96, 74.37, 44.02, 39.37, 34.23, 31.98, 31.80, 31.18, 29.53, 29.47, 29.37, 29.27, 29.24, 29.16, 29.10, 27.44, 26.87, 24.85, 24.82, 22.68, 14.15.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{24}{}^{1}H_{44}{}^{16}O_5{}^{14}N{}^{35}Cl$, 462.2981; found, 462.2971, Δ = 1.0 mmu.



Supplementary Figure 146: ^{1}H-NMR of compound **MD3/3** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 882 mg **MD3/3** (815 µmol, 1.00 eq.) were stirred in 3.00 mL DCM. Subsequently, 564 mg dodecanal **A10** (1.22 mmol, 1.50 eq.) and 922 mg of monomer **IM2** (1.22 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 3 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (7:1 → 4:1) to yield the Passerini product **D3/3** as a yellow highly viscous oil. (1.65 g, 755 µmol, 92.6%).

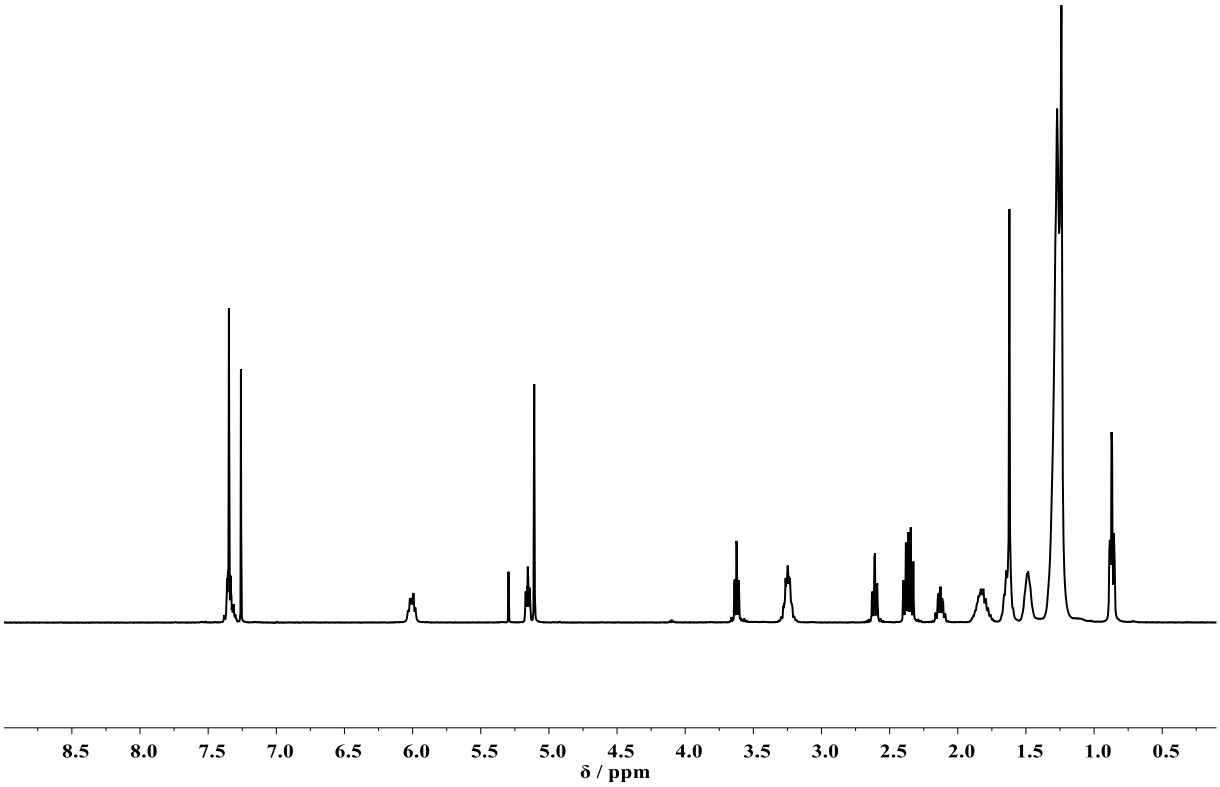
R_f: 0.21 in cyclohexane / ethyl acetate (3:1).

IR (ATR): ν / cm^{-1} = 3304.4 (w), 2922.2 (s), 2852.3 (m), 1737.3 (s), 1654.0 (s), 1536.8 (m), 1456.1 (w), 1376.6 (w), 1166.4 (m), 723.0 (w), 696.9 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.41 – 7.29 (m, 5 H, CH_{Ar}^1), 6.08 – 5.92 (m, 2 H, NH^2), 5.21 – 5.14 (m, 2 H, CH^3), 5.11 (s, 2 H, CH^4), 3.66 – 3.59 (m, 2 H, CH_2^5), 3.32 – 3.19 (m, 4 H, CH_2^6), 2.61 (td, J = 7.1, 1.7 Hz, 2 H, CH_2^7), 2.41 – 2.31 (m, 4 H, CH_2^8), 2.19 – 2.07 (m, 2 H, CH_2^9), 1.92 – 1.73 (m, 4 H, CH_2^{10}), 1.69 – 1.57 (m, 8 H, CH_2^{11}), 1.54 – 1.42 (m, 4 H, CH_2^{12}), 1.36 – 1.19 (m, 48 H, CH_2^{11}), 0.91 – 0.83 (m, 6 H, CH_3^{13}).

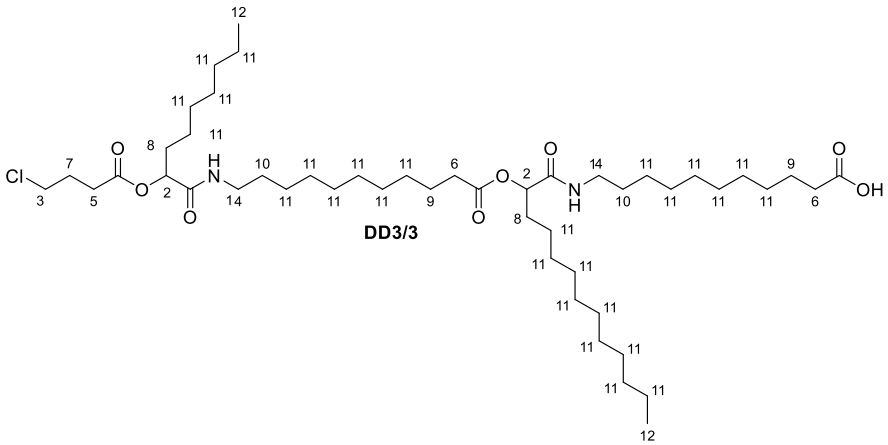
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 172.68, 171.45, 170.46, 168.84, 168.62, 135.11, 127.52, 127.15, 73.32, 72.93, 65.05, 42.92, 38.21, 38.18, 33.30, 30.89, 30.70, 30.09, 28.60, 28.53, 28.52, 28.43, 28.42, 28.34, 28.33, 28.24, 28.19, 28.18, 28.09, 28.06, 26.35, 25.81, 23.94, 23.92, 23.77, 23.74, 21.67, 21.59, 13.10, 13.05.

ESI-MS [*m*/z]: $[M+H]^+$ calculated for $^{12}C_{55}{}^{1}H_{95}{}^{16}O_8{}^{14}N_2{}^{35}Cl$, 947.6850; found: 947.6831; Δ = 1.9 mmu.



Supplementary Figure 147: ^{1}H-NMR of compound **D3/3** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 525 mg of **D3/3** (610 µmol, 1.00 eq.) were dissolved in 3.00 mL ethyl acetate and 3.00 mL THF. Afterwards, 117 mg (20 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred

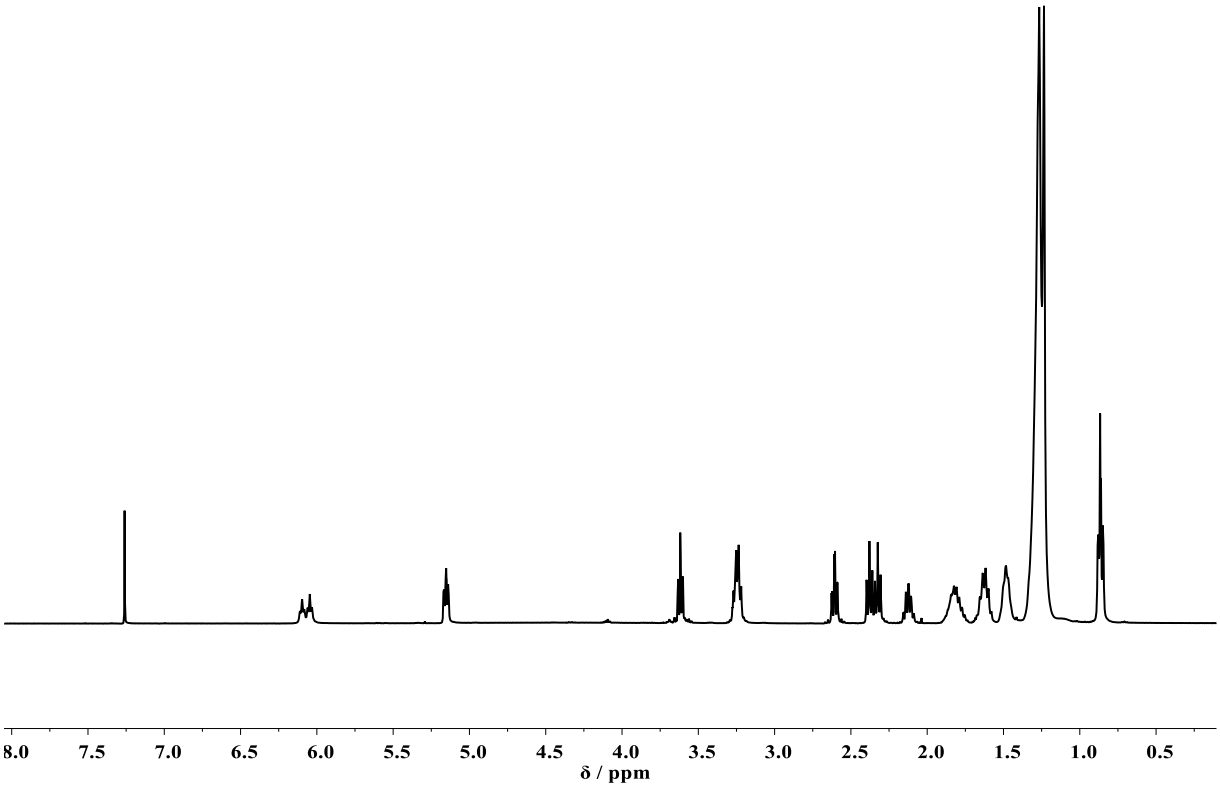
under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was evaporated under reduced pressure. The product **DD3/3** was obtained as a pale highly viscous oil in a yield of 96.7% (502 mg, 590 µmol).

IR (ATR): ν / cm^{-1} = 3291.2 (w), 2919.5 (vs), 2850.7 (s), 1736.6 (s), 1698.2 (m), 1655.5 (vs), 1560.4 (m), 1466.9 (m), 1377.2 (w), 1302.3 (w), 1170.6 (s), 938.1 (vw), 721.1 (w), 445.9 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.04 (t, J = 5.9 Hz, 1 H, NH^{1}), 5.99 (t, J = 5.8 Hz, 1 H, NH^{1}), 5.14 – 5.06 (m, 2 H, CH^{2}), 3.67 – 3.48 (m, 2 H, CH_2^{3}), 3.25 – 3.10 (m, 4 H, CH_2^{4}), 2.55 (td, J = 7.1, 1.9 Hz, 2 H, CH_2^{5}), 2.32 (t, J = 7.5 Hz, 2 H, CH_2^{6}), 2.27 (t, J = 7.5 Hz, 2 H, CH_2^{6}), 2.11 – 2.00 (m, 2 H, CH_2^{7}), 1.87 – 1.65 (m, 4 H, CH_2^{8}), 1.65 – 1.49 (m, 4 H, CH_2^{9}), 1.50 – 1.31 (m, 4 H, CH_2^{10}), 1.32 – 0.98 (m, 52 H, CH_2^{11}), 0.84 – 0.74 (m, 6 H, CH_3^{12}).

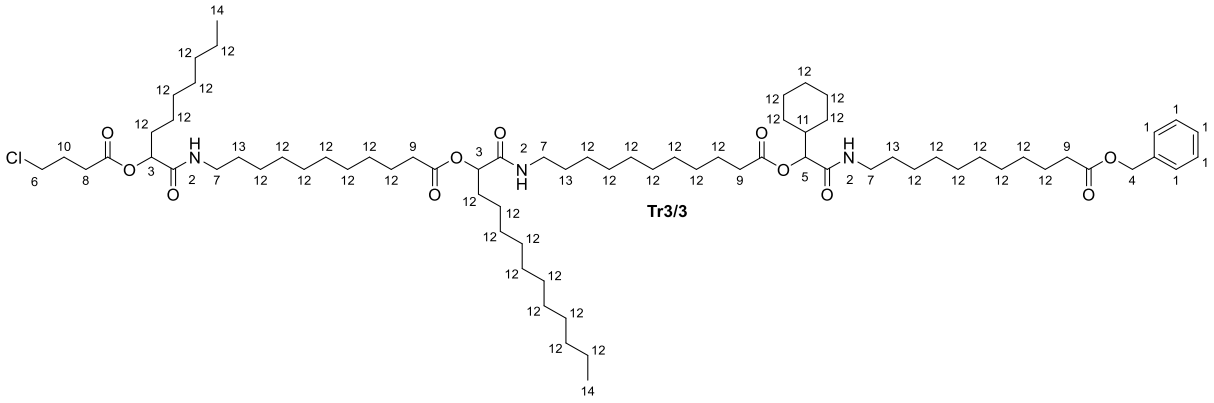
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 178.05, 172.63, 171.68, 170.11, 169.97, 74.45, 74.08, 44.06, 39.42, 39.32, 34.45, 34.05, 32.04, 31.84, 31.24, 29.75, 29.66, 29.63, 29.58, 29.57, 29.50, 29.47, 29.37, 29.32, 29.24, 29.20, 29.11, 27.49, 26.95, 26.89, 25.10, 24.90, 24.89, 24.87, 22.81, 22.73, 14.25, 14.20.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{48}{}^{1}H_{89}{}^{16}O_8{}^{14}N_2{}^{35}Cl$, 857.6380 found, 857.6366, Δ = 1.4 mmu.



Supplementary Figure 148: ^{1}H-NMR of compound **DD3/3** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 1.35 g **DD3/3** (1.58 mmol, 1.00 eq.) were stirred in 2.00 mL DCM. Subsequently, 286 µL cyclohexanecarboxaldehyde **A6** (265 mg, 2.37 mmol, 1.50 eq.) and 714 mg of monomer **IM2** (2.37 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 3 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (4:1 → 2:1) to yield the Passerini product **Tr3/3** as a yellow highly viscous oil. (1.88 g, 1.48 mmol, 93.7%).

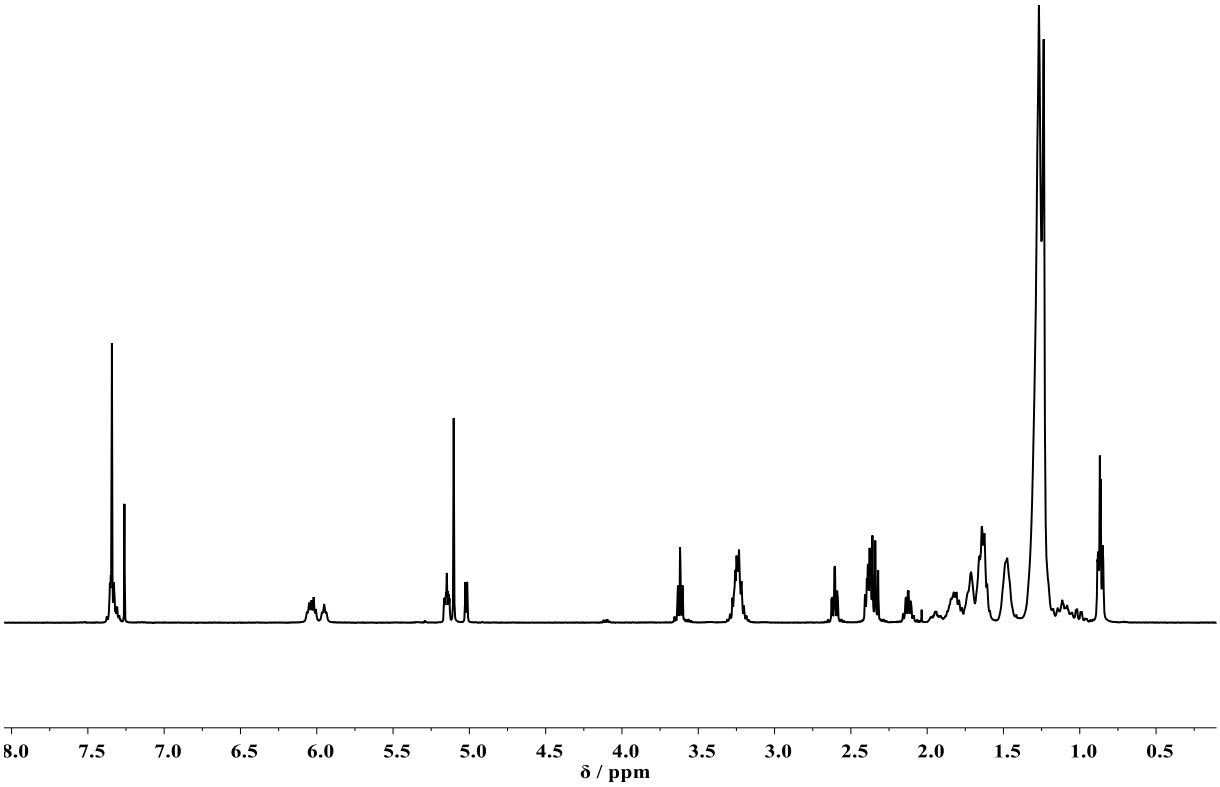
R_f: 0.20 in cyclohexane / ethyl acetate (3:1).

IR (ATR): ν / cm^{-1} = 3293.1 (w), 2918.0 (vs), 2850.4 (s), 1733.1 (vs), 1655.0 (vs), 1558.1 (m), 1466.2 (m), 1378.1 (m), 1238.1 (m), 1207.0 (s), 1172.8 (vs), 980.7 (w), 721.9 (m), 695.9 (m), 453.1 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.41 – 7.29 (m, 5 H, $CH_{Ar}{}^1$), 6.09 – 6.00 (m, 2 H, NH^2), 5.95 (t, J = 6.0 Hz, 1 H, NH^2), 5.18 – 5.12 (m, 2 H, CH^3), 5.10 (s, 2 H, $CH_2{}^4$), 5.02 (d, J = 4.6 Hz, 1 H, CH^5), 3.67 – 3.58 (m, 2 H, $CH_2{}^6$), 3.32 – 3.16 (m, 6 H, $CH_2{}^7$), 2.61 (td, J = 7.1, 1.9 Hz, 2 H, $CH_2{}^8$), 2.44 – 2.29 (m, 6 H, $CH_2{}^9$), 2.19 – 2.07 (m, 2 H, $CH_2{}^{10}$), 2.00 – 1.58 (m, 17 H, CH^{11}, $CH_2{}^{12}$), 1.54 – 1.42 (m, 6 H, $CH_2{}^{13}$), 1.39 – 1.05 (m, 68 H, $CH_2{}^{12}$), 0.91 – 0.82 (m, 6 H, $CH_3{}^{14}$).

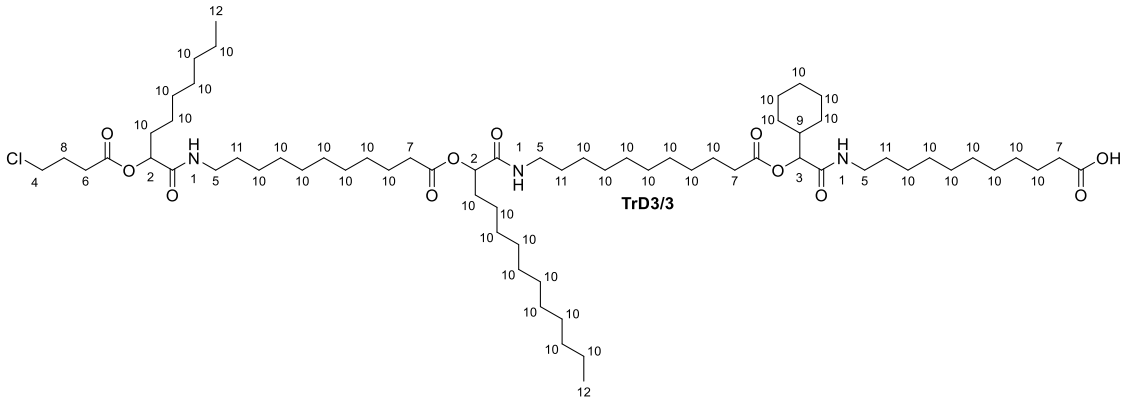
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.81, 172.67, 172.60, 171.61, 170.00, 169.77, 169.33, 136.23, 128.66, 128.29, 128.28, 77.74, 74.44, 74.05, 66.19, 44.07, 40.09, 39.35, 39.30, 39.27, 34.44, 34.41, 32.04, 32.03, 31.84, 31.22, 29.74, 29.68, 29.66, 29.58, 29.57, 29.49, 29.46, 29.38, 29.35, 29.32, 29.25, 29.22, 29.20, 27.49, 27.41, 26.96, 26.95, 26.19, 26.11, 26.00, 25.11, 25.08, 25.06, 24.90, 24.89, 22.81, 22.73, 14.25, 14.20.

ESI-MS [*m*/z]: $[M+Na]^+$ calculated for $^{12}C_{74}{}^{1}H_{128}{}^{16}O_{11}{}^{14}N_3{}^{35}Cl$, 1292.9130; found: 1292.9134; Δ = 0.4 mmu.



Supplementary Figure 149: ^{1}H-NMR of compound **Tr3/3** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 1.49 g of **Tr3/3** (1.17 mmol, 1.00 eq.) were dissolved in 4.00 mL ethyl acetate and 4.00 mL THF. Afterwards, 149 mg (10 wt%) palladium on activated carbon **1** were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and

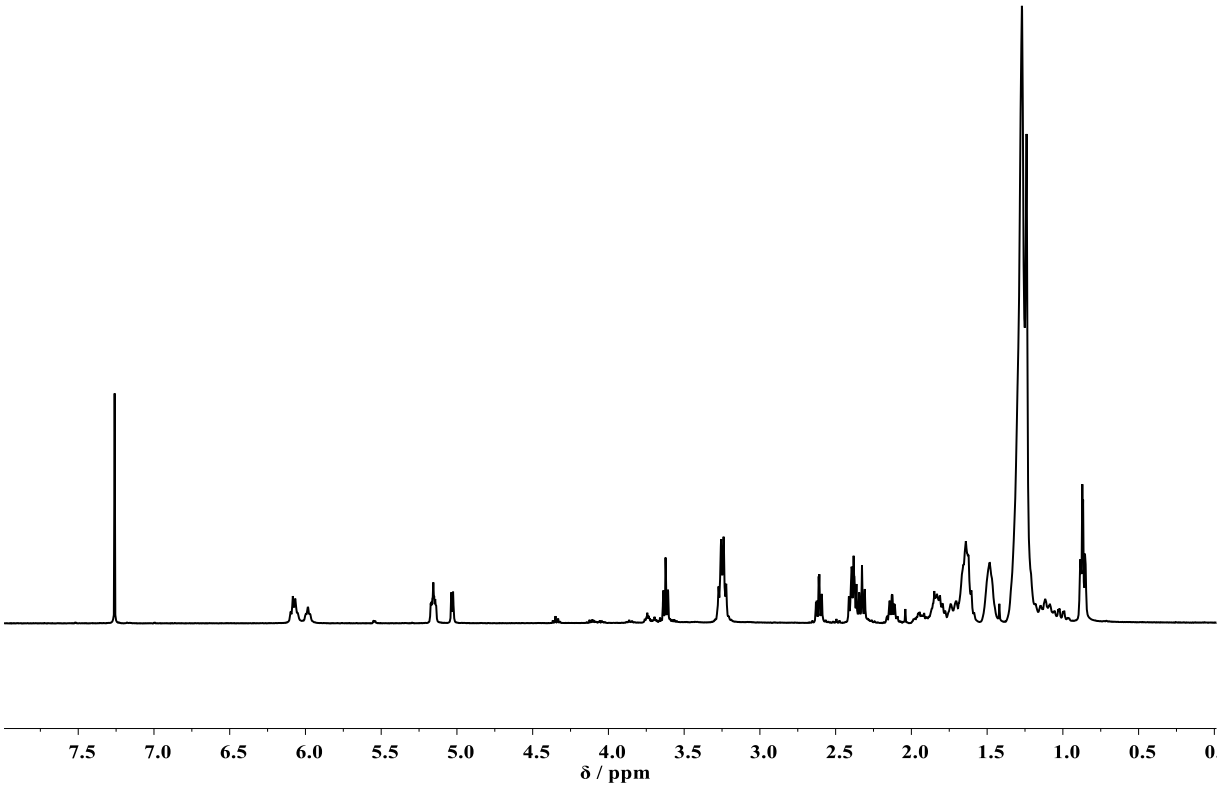
the solvent was evaporated under reduced pressure. The product **TrD3/3** was obtained as a pale highly viscous oil in a yield of 99.1% (1.37 g, 1.16 mmol).

IR (ATR): ν / cm^{-1} = 3292.4 (w), 2918.6 (vs), 2850.6 (vs), 1735.6 (s), 1654.9 (vs), 1556.3 (m), 1466.0 (m), 1377.5 (m), 1244.8 (m), 1207.9 (s), 1177.3 (vs), 941.3 (vw), 721.6 (w), 454.0 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 6.06 – 5.97 (m, 2 H, NH^{1}), 5.92 (t, J = 6.0 Hz, 1 H, NH^{1}), 5.13 – 5.06 (m, 2 H, CH^{2}), 4.97 (d, J = 4.6 Hz, 1 H, CH^{3}), 3.62 – 3.51 (m, 2 H, CH_2^{4}), 3.24 – 3.09 (m, 6 H, CH_2^{5}), 2.55 (td, J = 7.1, 1.8 Hz, 2 H, CH_2^{6}), 2.36 – 2.22 (m, 6 H, CH_2^{7}), 2.12 – 2.01 (m, 2 H, CH_2^{8}), 1.93 – 1.51 (m, 15 H, CH^{9}, CH_2^{10}), 1.50 – 1.38 (m, 6 H, CH_2^{11}), 1.32 – 0.93 (m, 70 H, CH_2^{10}), 0.85 – 0.74 (m, 6 H, CH_3^{12}).

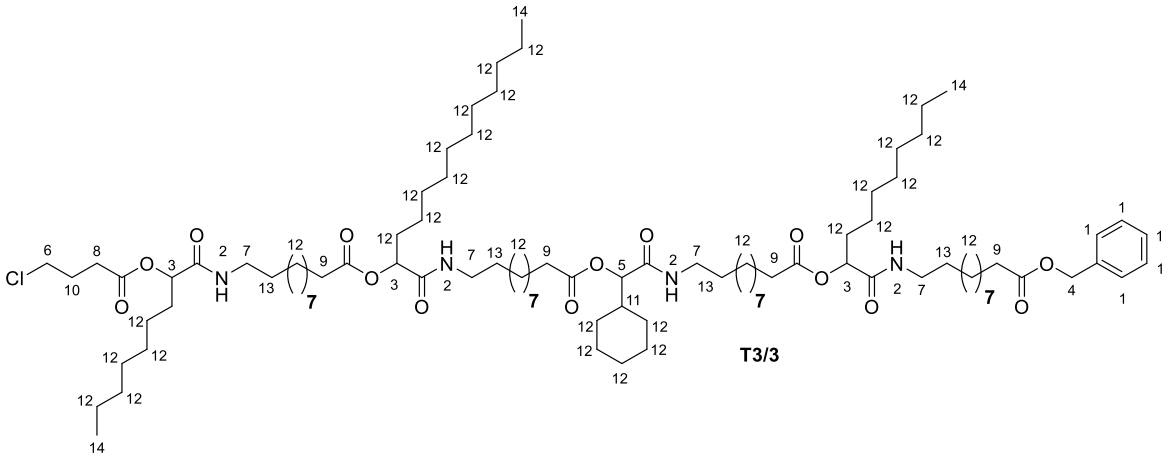
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 172.71, 171.65, 170.19, 169.90, 169.44, 77.78, 74.46, 74.08, 44.08, 40.06, 39.40, 39.24, 34.45, 33.96, 32.05, 32.01, 31.86, 31.25, 29.76, 29.68, 29.65, 29.61, 29.58, 29.53, 29.50, 29.49, 29.41, 29.38, 29.35, 29.34, 29.29, 29.24, 29.21, 29.20, 29.08, 27.50, 27.42, 26.96, 26.89, 26.20, 26.12, 26.01, 25.16, 25.09, 24.92, 24.91, 22.82, 22.74, 14.26, 14.21.

ESI-MS [m/z]: $[M + H]^+$ calculated for $^{12}C_{67}{}^{1}H_{122}{}^{16}O_{11}{}^{14}N_3{}^{35}Cl$, 1180.8841 found, 1180.8837, Δ = 0.4 mmu.



Supplementary Figure 150: ^{1}H-NMR of compound **TrD3/3** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask, 415 mg **TrD3/3** (351 mol, 1.00 eq.) were stirred in 2.00 mL DCM. Subsequently, 91.6 µL nonanal **A9** (74.9 mg, 527 µmol, 1.50 eq.) and 159 mg of monomer **IM2** (517 µmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 3 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (5:1 → 2:1) to yield the Passerini product **T3/3** as a yellow highly viscous oil. (527 mg, 323 µmol, 92.0%).

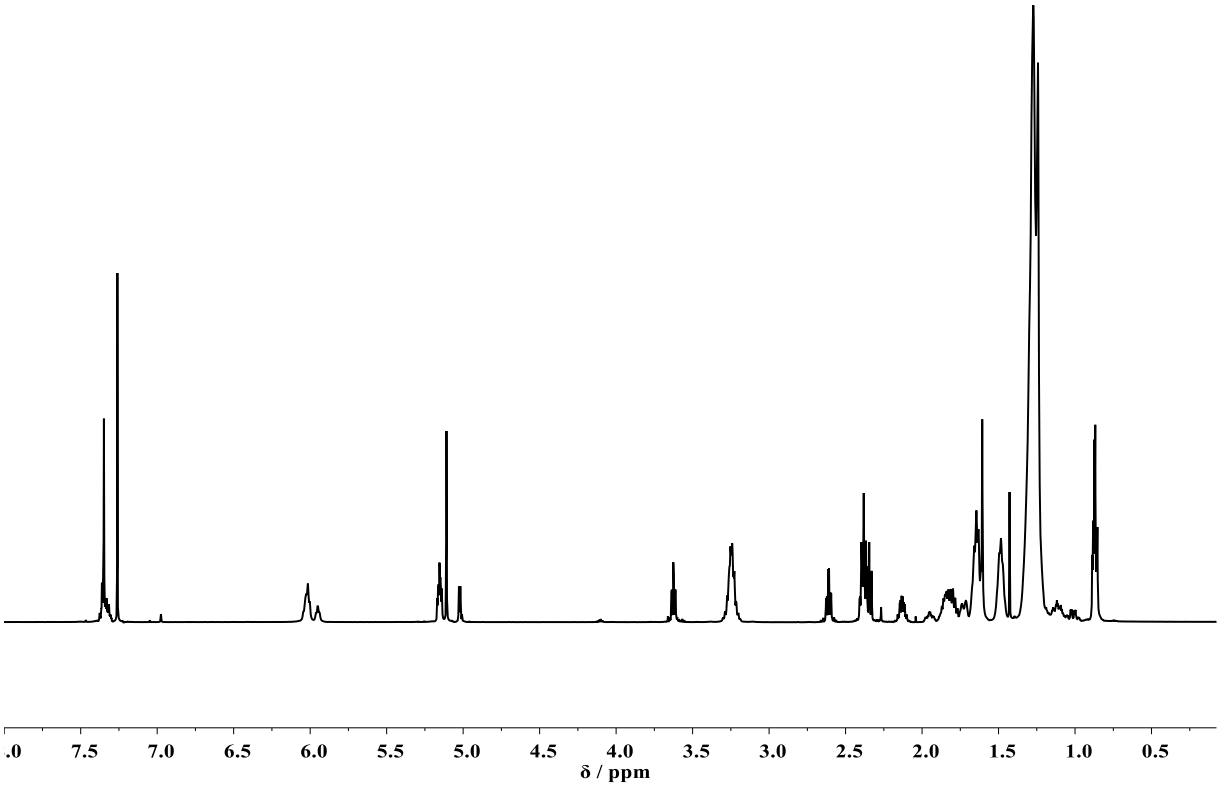
R_f: 0.17 in cyclohexane / ethyl acetate (2:1).

IR (ATR): ν / cm^{-1} =3291.6 (w), 2920.0 (vs), 2851.1 (s), 1736.4 (vs), 1654.9 (vs), 1557.0 (m), 1465.8 (m), 1377.3 (w), 1243.8 (m), 1207.8 (m), 1173.6 (vs), 1112.7 (m), 721.9 (w), 696.4 (m), 453.3 (vw), 384.2 (vw).

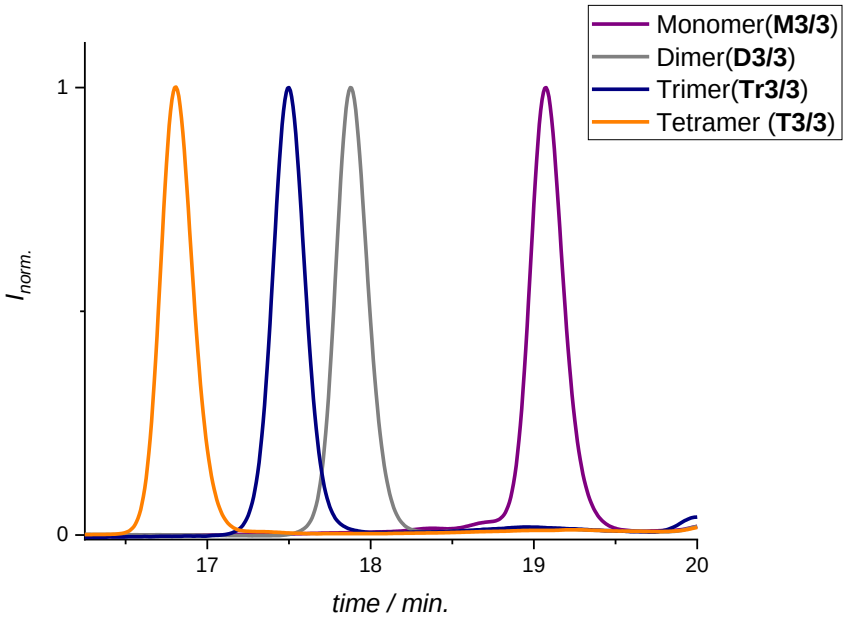
^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.44 – 7.31 (m, 5 H, CH_{Ar}^1), 6.10 – 5.99 (m, 3 H, NH^2), 5.97 – 5.87 (m, 1 H, NH^2), 5.18 – 5.13 (m, 3 H, CH^3), 5.11 (s, 2 H, CH_2^4), 5.02 (d, J = 4.6 Hz, 1 H, CH^5), 3.67 – 3.56 (m, 2 H, CH_2^6), 3.33 – 3.16 (m, 8 H, CH_2^7), 2.67 – 2.56 (m, 2 H, CH_2^8), 2.44 – 2.29 (m, 8 H, CH_2^9), 2.19 – 2.10 (m, 2 H, CH_2^{10}), 2.01 – 1.58 (m, 23 H, CH^{11}, CH_2^{12}), 1.54 – 1.44 (m, 8 H, CH_2^{13}), 1.38 – 0.97 (m, 88 H, CH_2^{12}), 0.94 – 0.80 (m, 9 H, CH_3^{14}).

^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 173.83, 172.70, 172.61, 171.62, 170.01, 169.98, 169.78, 169.35, 136.26, 128.68, 128.30, 77.76, 74.47, 74.07, 66.21, 44.09, 40.11, 34.46, 34.43, 32.07, 32.06, 31.96, 31.86, 31.24, 30.46, 29.76, 29.73, 29.71, 29.70, 29.68, 29.60, 29.58, 29.53, 29.51, 29.49, 29.40, 29.36, 29.35, 29.33, 29.28, 29.25, 29.22, 27.50, 27.43, 26.99, 26.97, 26.21, 26.13, 26.02, 25.13, 25.10, 25.08, 24.92, 24.89, 22.83, 22.78, 22.75, 14.27, 14.25, 14.22.

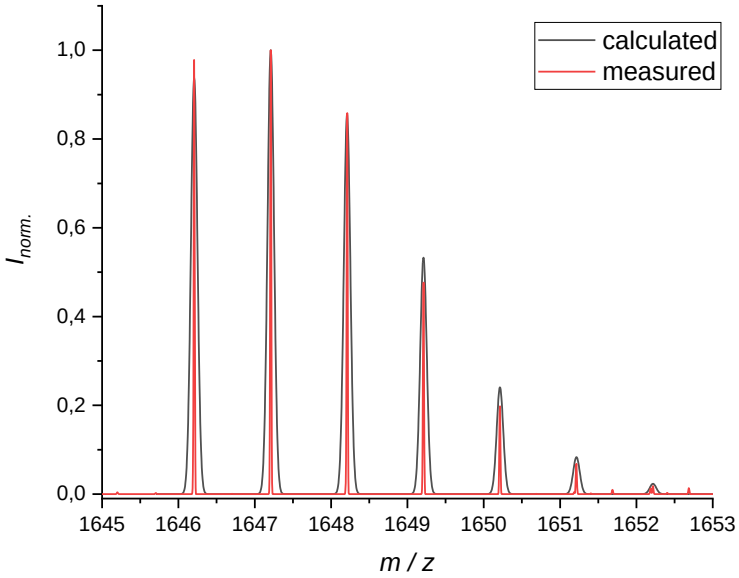
ESI-MS [*m*/z]: $[M+Na]^+$ calculated for $^{12}C_{95}{}^{1}H_{167}{}^{16}O_{14}{}^{14}N_4{}^{35}Cl$, 1646.2060; found: 1646.2066; Δ = 0.6 mmu.



Supplementary Figure 151: ^{1}H-NMR of compound **T3/3** measured in $CDCl_3$.



Supplementary Figure 152: SEC traces of the intermediates after each P-3CR in the synthesis of product **T3/3**.



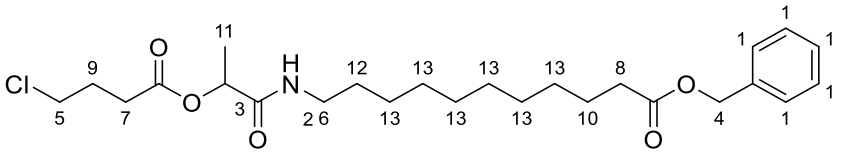
Supplementary Figure 153: High resolution ESI-MS measurement of **T3/3**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (black).

```
tramer.CSV, maximum is 1.000000 found for mass 1647.207720
matching mass 1647.20772
cutoff 0.50000: 0 solutions (10 peaks)
cutoff 0.25000: 0 solutions (24 peaks)
cutoff 0.12500: 0 solutions (62 peaks)
cutoff 0.06250: 0 solutions (121 peaks)
cutoff 0.03125: 0 solutions (193 peaks)
cutoff 0.01562: 0 solutions (305 peaks)
cutoff 0.00781: 1 solutions (436 peaks)
1647.20772 ≈ 121.005630 + 339.277350 + 395.339950 + 323.246050 + 353.293000 + 91.054780 (sides Octanal, Dodecanal,
 Cyclohexancarboxaldehyde, Nonanal; error -23.99096)
Press ENTER to quit ...
```

Supplementary Figure 154: Screenshot of the automated read-out of **T3/3**, sodium trifluoroacetate **2** was used as additive during the measurement.

6.3.3.5.5 Synthesis of tetramer T3/4[ix]

Passerini reaction



M3/4

In a 50 mL round bottom flask, 125 mg 4-chlorobutyric acid **TAG3** (1.02 mmol, 1.00 eq.) was dissolved in 2.00 mL dichloromethane and 171 µL acetaldehyde **A1** (461 mg, 3.06 mmol, 3.00 eq.) and 461 mg of monomer **IM2** (1.53 mol, 3.00 eq.) were added. The mixture was stirred at room temperature for 3 days. Subsequently, the solvent was removed under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (6:1 → 2:1) to yield the Passerini product **M3/4** as a pale highly viscous oil with 92.3% (441 mg, 941 µmol).

R_f = 0.16 in cyclohexane / ethyl acetate (3:1).

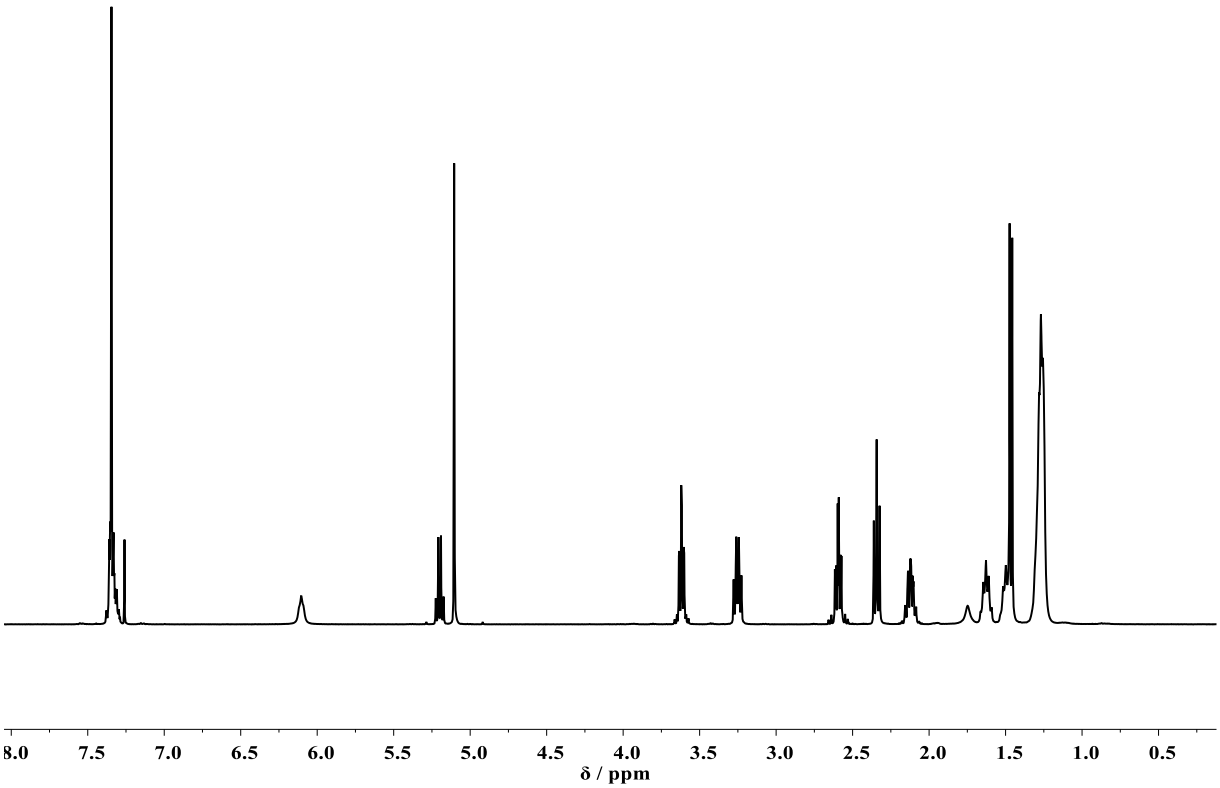
IR (ATR): ν / cm^{-1} = 3317.5 (vw), 2926.7 (w), 2854.4 (w), 1734.8 (s), 1660.0 (m), 1538.4 (w), 1498.1 (vw), 1454.9 (w), 1373.5 (w), 1298.4 (w), 1166.8 (m), 1144.5 (s), 1097.3 (w), 1031.5 (vw), 878.2 (vw), 787.6 (vw), 735.8 (w), 697.2 (m), 648.5 (vw), 432.2 (vw).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.40 – 7.28 (m, 5 H, CH^1), 6.10 (t, J = 5.9 Hz, 1H, NH^2), 5.20 (q, J = 6.8 Hz, 1 H, CH^3), 5.10 (s, 2 H, CH_2^4), 3.71 – 3.55 (m, 2 H, CH_2^5), 3.32 – 3.19 (m, 2 H, CH_2^6), 2.59 (td, J = 7.1, 2.7 Hz, 2 H, CH_2^7), 2.34 (t, J = 7.5 Hz, 2 H, CH_2^8), 2.21 – 2.04 (m, 2 H, CH_2^9), 1.69 – 1.56 (m, 2 H, CH_2^{10}), 1.55 – 1.43 (m, 5 H, CH^{11}, CH_2^{12}), 1.34 – 1.21 (m, 12 H, CH_2^{13}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.81, 171.36, 170.21, 136.23, 128.65, 128.27, 70.93, 66.18, 44.06, 39.39, 34.43, 31.29, 29.60, 29.52, 29.43, 29.31, 29.30, 29.20, 27.46, 26.92, 25.04, 18.09.

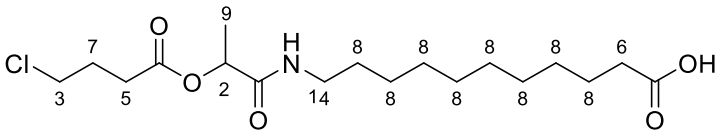
ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{25}{}^{1}H_{38}{}^{16}O_5{}^{14}N^{35}Cl$, 468.2511; found, 468.2511, Δ = 0.0 mmu.

[ix] Synthesis was carried out by Lara Faden in the Vertieferarbeit "Synthesis and characterization of monodisperse sequence-defined oligomers" under the laboratory supervision of Maximiliane Frölich



Supplementary Figure 155: ^{1}H-NMR of compound **M3/4** measured in $CDCl_3$.

Deprotection



MD3/4

In a 25 mL round bottom flask, 394 mg of Passerini product **M3/4** (841 µmol, 1.00 eq.) was dissolved in 4.00 mL ethyl acetate and 4.00 mL THF. Subsequently, 99.4 mg (20 wt%) palladium on activated carbon **1** was added to the solution. The resulting mixture was purged with hydrogen gas and stirred for 2 d at room temperature under hydrogen atmosphere (3 balloons). The heterogeneous catalyst was filtered over celite® and the solvent was evaporated under reduced pressure. The product **MD3/4** was obtained as a pale highly viscous oil in a yield of 97.5% (310 mg, 820 µmol).

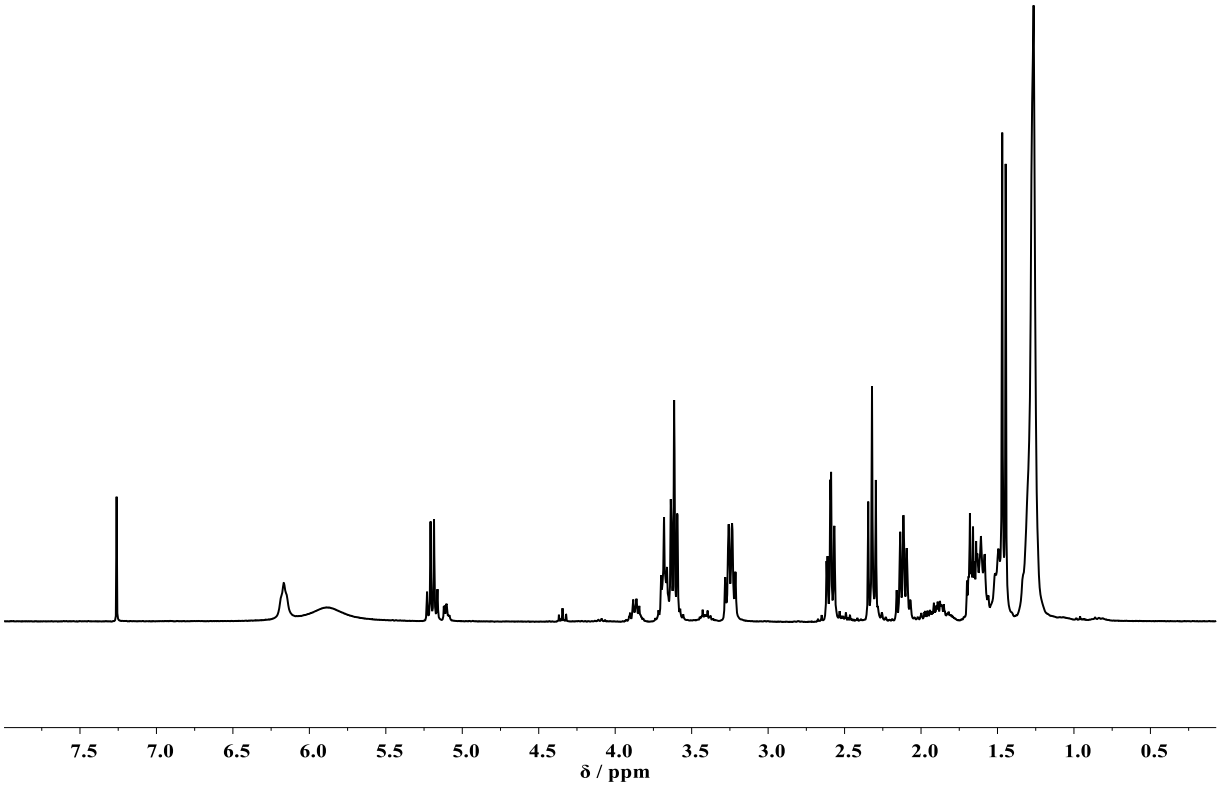
IR (ATR): ν / cm^{-1} = 3295.3 (w), 2919.4 (m), 2850.1 (w), 1729.4 (w), 1690.9 (w), 1653.4 (m), 1550.8 (m), 1453.5 (w), 1435.5 (w), 1407.6 (w), 1372.9 (w), 1330.3 (w), 1278.1 (w), 1256.2

(w), 1235.8 (w), 1187.4 (w), 1145.0(w), 1092.2 (w), 1057.9 (w), 1037.6 (w), 999.7 (w), 880.21 (vw), 840.8 (vw), 721.4 (w), 686.6 (w), 542.3(vw), 431.0 (vw).

^{1}H NMR (300 MHz, $CDCl_3$): δ / ppm = 6.26 – 5.72 (m, 1 H, NH^1), 5.27 – 5.06 (m, 1 H, CH^2), 3.92 – 3.53 (m, 2 H, CH_2^3), 3.49 – 3.17 (m, 2 H, CH_2^4), 2.69 – 2.45 (m, 2 H, CH_2^5), 2.32 (t, J = 7.5 Hz, 2 H, CH_2^6), 2.19 – 1.86 (m, 2 H, CH_2^7), 2.03 – 0.77 (m, 19 H, CH_2^8, CH_3^9).

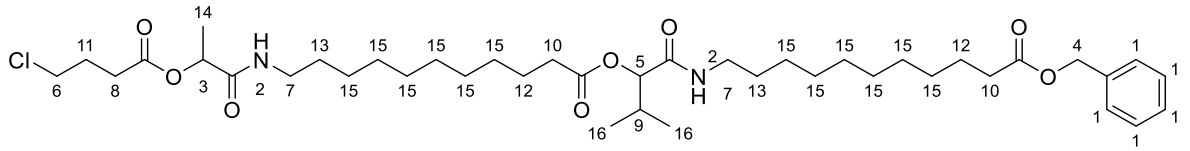
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 178.74, 171.41, 170.44, 70.89, 67.31, 67.12, 64.18, 62.76, 62.38, 44.05, 39.43, 34.08, 31.27, 29.92, 29.51, 29.43, 29.34, 29.22, 29.08, 27.44, 26.86, 18.06.

ESI-MS [m/z]: $[M + H]^+$ calculated for $^{12}C_{18}{}^{1}H_{33}{}^{16}O_5{}^{14}N{}^{35}Cl$, 378.2042; found, 378.2030, Δ = 1.2 mmu.



Supplementary Figure 156: ^{1}H-NMR of compound **MD3/4** measured in $CDCl_3$.

Passerini reaction



D3/4

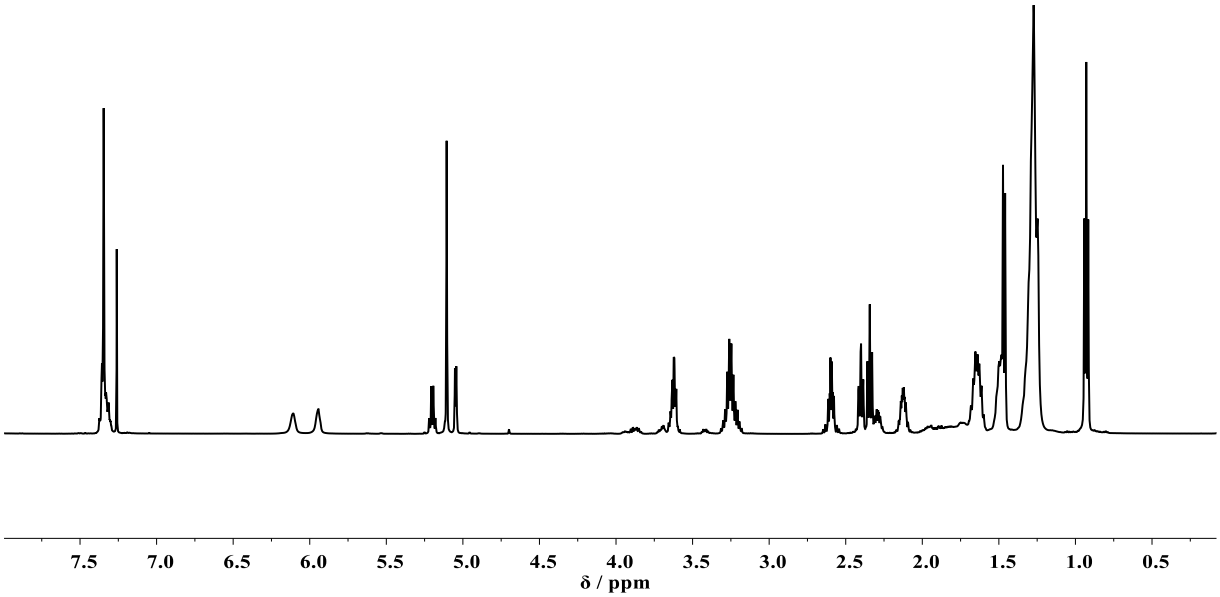
In a 25 mL round bottom flask, 263 mg of **MD3/4** (695 µmol, 1.00 eq.) was dissolved in 3.00 mL dichloromethane (DCM) and 37.6 µL isobutyraldehyde **A3** (30.1 mg, 417 µmol, 0.60 eq.) and 126 mg of monomer **IM2** (417 µmol, 0.60 eq.) were added. The mixture was stirred at room temperature for 24 hours and subsequently the solvent was removed under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (4:1 → 2:1) to yield the Passerini product **D3/4** with 73.9% (232 mg, 514 µmol).

R_f = 0.64 in cyclohexane / ethyl acetate (1:1).

IR (ATR): ν / cm^{-1} = 3304.8 (vw), 2926.0 (m), 2854.2 (m), 1737.0 (vs), 1656.0 (s), 1537.2 (m), 1455.2 (w), 1371.1 (w), 1166.4 (s), 1098.9 (m), 1034.3 (w), 734.6 (w), 697.3 (m), 646.2 (vw).^{1}H-NMR (500 MHz, $CDCl_3$): δ / ppm = 7.35 – 7.22 (m, 5 H, $CH_{Ar}{}^1$), 6.13 – 6.00 (m, 1 H, NH^2), 5.96 – 5.79 (m, 1 H, NH^2), 5.14 (q, J = 6.8 Hz, 1 H, CH^3), 5.04 (s, 2 H, $CH_2{}^4$), 4.98 (d, J = 4.4 Hz, 1 H, CH^5), 3.69 – 3.46 (m, 2 H, $CH_2{}^6$), 3.28 – 3.07 (m, 4 H, $CH_2{}^7$), 2.58 – 2.43 (m, 2 H, $CH_2{}^8$), 2.41 – 2.16 (m, 5 H, CH^9, $CH_2{}^{10}$), 2.11 – 1.97 (m, 2 H, $CH_2{}^{11}$), 1.65 – 1.46 (m, 4 H, $CH_2{}^{12}$), 1.49 – 1.37 (m, 7 H, $CH_2{}^{13}$, $CH_3{}^{14}$), 1.32 – 1.08 (m, 24 H, $CH_2{}^{15}$), 0.87 (t, J = 6.5 Hz, 6 H, $CH_3{}^{16}$).

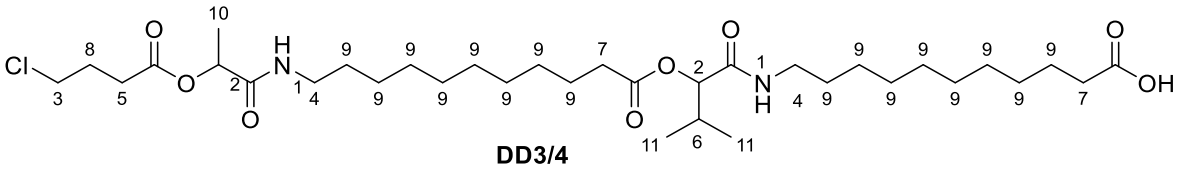
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.83, 172.68, 171.39, 170.24, 169.40, 136.27, 128.68, 128.29, 78.06, 70.97, 66.20, 44.08, 39.40, 39.30, 34.46, 34.43, 31.32, 30.81, 30.65, 29.98, 29.71, 29.64, 29.56, 29.47, 29.33, 29.24, 27.49, 26.97, 26.94, 25.89, 25.14, 25.07, 24.09, 23.98, 18.92, 18.10, 17.09.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{41}{}^1H_{67}{}^{16}O_8{}^{14}N_2{}^{35}Cl$, 751.4659; found, 751.4636, Δ = 2.3 mmu.



Supplementary Figure 157: ^{1}H-NMR of compound **D3/4** measured in $CDCl_3$.

Deprotection



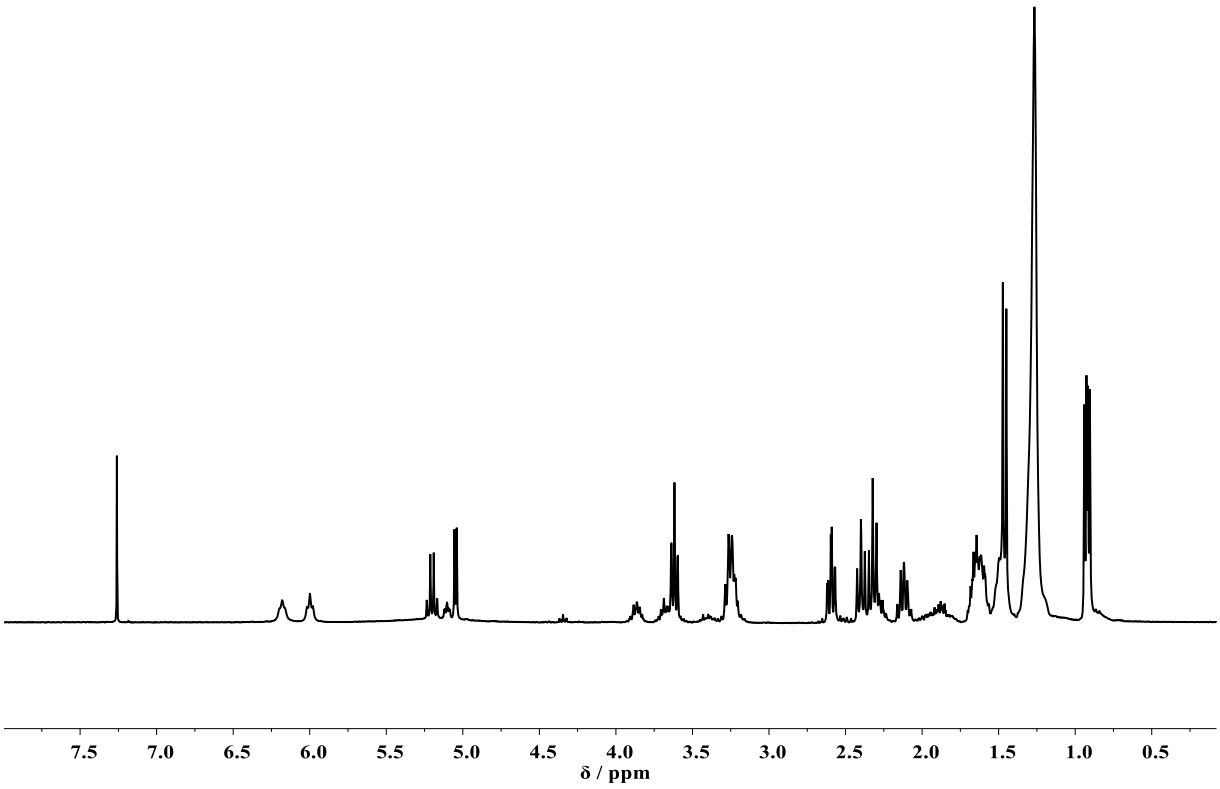
In a 25 mL round bottom flask, 186 mg of **D3/4** (247 µmol, 1.00 eq.) was dissolved in 2.00 mL ethyl acetate and 2.00 mL THF. Subsequently, 37.2 mg (20 wt%) palladium on activated carbon **1** was added to the solution. The resulting mixture was purged with hydrogen gas and stirred for 2 days at room temperature under hydrogen atmosphere (balloon). The crude reaction mixture was filtered over celite® and flushed with 50 mL DCM. After evaporation of the solvents and drying under reduced pressure, the corresponding acid **DD3/4** was obtained in a yield of 94.8% (155 mg, 234 µmol).

IR (ATR): ν / cm^{-1} = 3311.0 (vw), 2925.9 (w), 2854.2 (vw), 2123.5 (vw), 2050.5 (vw), 2031.8 (vw), 2012.5 (vw), 1984.6 (vw), 1738.9 (w), 1652.2 (w), 1541.2 (vw), 1462.4 (vw), 1371.6 (vw), 1171.43 (vw), 1145.1 (vw), 1099.1 (vw), 921.6 (vw), 723.7 (vw), 649.7 (vw), 451.9 (vw), 405.9 (vw).

^{1}H-NMR (300 MHz, $CDCl_3$): δ / ppm = 6.19 – 6.08 (m, 1 H, NH^1), 6.01 – 5.85 (m, 1 H, NH^1), 5.21 – 4.96 (m, 2 H, CH^2), 3.70 – 3.51 (m, 2 H, CH_2^3), 3.42 – 3.08 (m, 4 H, CH_2^4), 2.65 – 2.43 (m, 2 H, CH_2^5), 2.40 – 2.16 (m, 5 H, CH^6, CH_2^7), 2.14 – 2.00 (m, 2 H, CH_2^8), 1.93 – 1.31 (m, 13 H, CH_2^9, CH_3^{10}), 1.32 – 1.08 (m, 22 H, CH_2^9), 0.87 (dd, J = 6.8, 4.1 Hz, 6 H, CH_3^{11}).

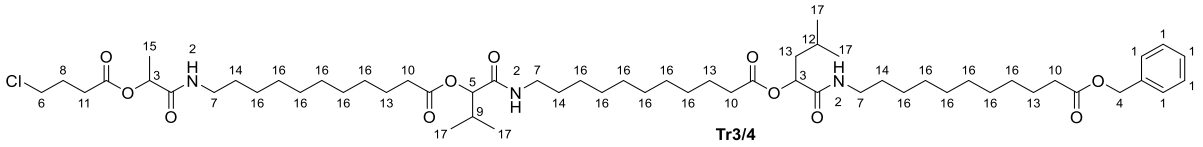
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm =177.46, 172.72, 171.45, 170.44, 169.51, 78.06, 70.94, 44.09, 39.46, 39.27, 34.43, 31.30, 30.61, 29.61, 29.59, 29.57, 29.40, 29.35, 29.31, 29.26, 29.19, 27.46, 26.93, 26.88, 25.15, 24.85, 18.92, 18.09, 17.08.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{34}{}^{1}H_{61}{}^{16}O_8{}^{14}N_2{}^{35}Cl$; 661.4189 found, 661.4197, Δ = 0.8 mmu.



Supplementary Figure 158: ^{1}H-NMR of compound **DD3/4** measured in $CDCl_3$.

Passerini reaction



In a 25 mL round bottom flask, 117 mg of **DD3/4** (177 µmol, 1.00 eq.) was dissolved in 2.00 mL DCM and 28.5 µL 3-methylbutanal **A4** (22.8 mg, 265 µmol, 1.50 eq.) and 79.9 mg of monomer **IM2** (265 µmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 1 day and subsequently the solvent was removed under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (3:1 → 1:1) to yield the Passerini product **Tr3/4** with 64.1% (119 mg, 113 µmol).

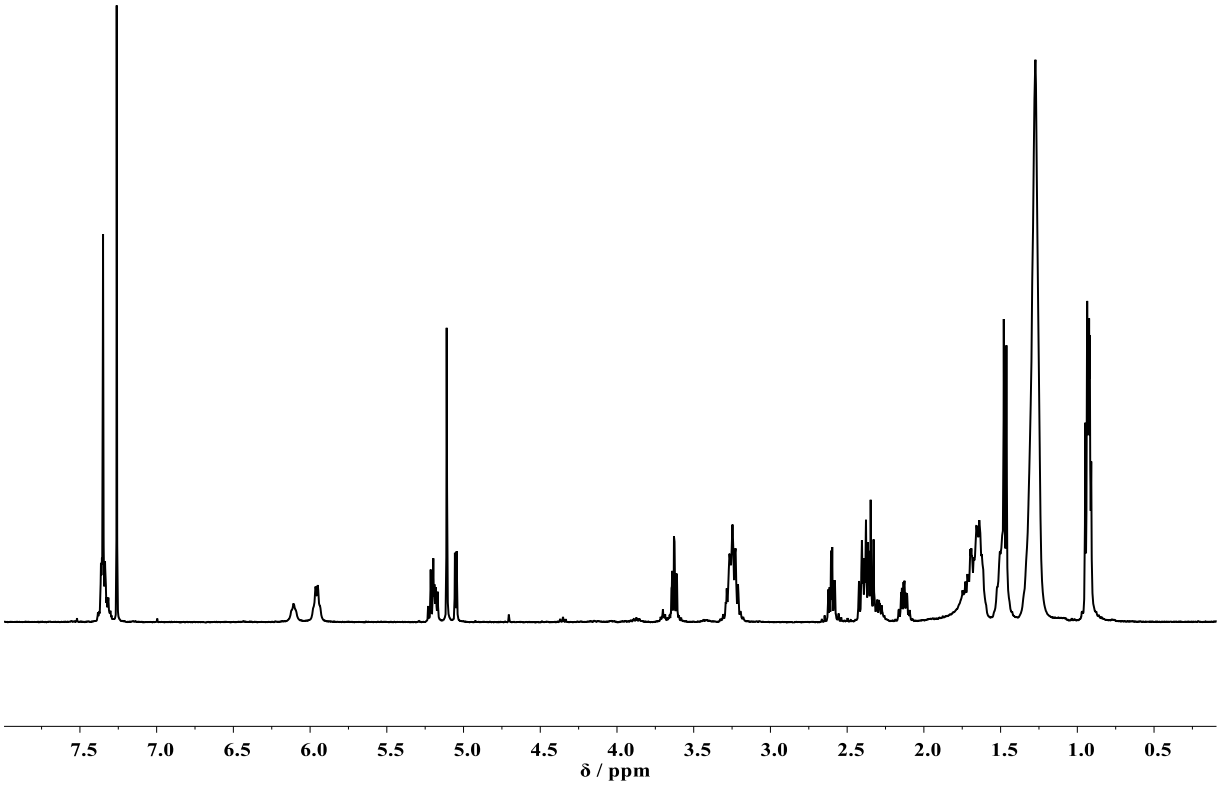
R_f = 0.64 in cyclohexane / ethyl acetate (1:1).

IR (ATR): ν / cm^{-1} = 3292.6 (vw), 2925.4 (w), 2853.9 (vw), 1737.8 (w), 1655.1 (w), 1536.4 (w), 1456.2 (vw), 1370.4 (vw), 1164.8 (w), 1100.7 (vw), 733.0 (vw), 697.6 (vw), 650.2 (vw), 463.2 (vw).

^{1}H-NMR (400 MHz, $CDCl_3$): δ / ppm = 7.42 – 7.29 (m, 5 H, CH_{Ar}^1), 6.11 (s, 1 H, NH^2), 5.96 (d, J = 6.3 Hz, 2 H, NH^2), 5.26 – 5.16 (m, 2 H, CH^3), 5.11 (s, 2 H, CH_2^4), 5.05 (d, J = 4.4 Hz, 1 H, CH^5), 3.63 (td, J = 6.2, 1.6 Hz, 2 H, CH_2^6), 3.32 – 3.15 (m, 6 H, CH_2^7), 2.60 (td, J = 7.1, 3.0 Hz, 2 H, CH_2^8), 2.47 – 2.23 (m, 7 H, CH^9, CH_2^{10}), 2.21 – 2.08 (m, 2 H, CH_2^{11}), 1.86 – 1.59 (m, 9 H, CH^{12}, CH_2^{13}), 1.55 – 1.42 (m, 9 H, CH_2^{14}, CH_3^{15}), 1.36 – 1.15 (m, 36 H, CH_2^{16}), 1.07 – 0.57 (m, 12 H, CH_3^{17}).

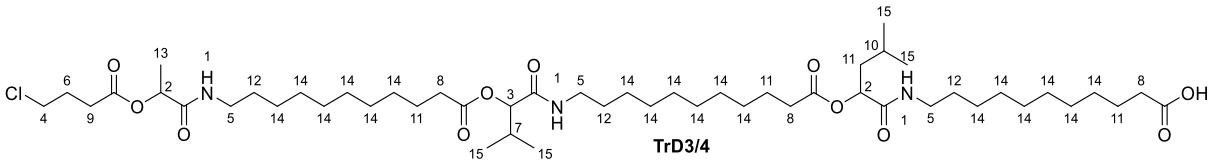
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.83, 172.78, 172.70, 171.40, 170.34, 170.24, 169.42, 136.26, 128.69, 128.30, 78.06, 72.78, 70.97, 66.21, 44.09, 41.00, 39.41, 39.37, 39.29, 34.46, 34.44, 31.32, 30.66, 29.73, 29.66, 29.58, 29.49, 29.35, 29.34, 29.25, 27.49, 26.97, 26.95, 25.15, 25.08, 24.69, 23.28, 21.94, 18.93, 18.12, 17.10.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{58}{}^1H_{98}{}^{16}O_{11}{}^{14}N_3{}^{35}Cl$, 1048.6963; found, 1048.6944, Δ = 1.9 mmu.



Supplementary Figure 159: ^{1}H-NMR of compound **Tr3/4** measured in $CDCl_3$.

Deprotection



In a 25 mL round bottom flask, 88.9 mg of **Tr3/4** (85.0 µmol, 1.00 eq.) was dissolved in 2.00 mL ethyl acetate and 2.00 mL THF. Subsequently, 17.8 mg (20 wt%) palladium on activated carbon **1** was added to the solution. The resulting mixture was purged with hydrogen gas and stirred for 2 days at room temperature under hydrogen atmosphere (balloon). The crude reaction mixture was filtered over celite®. After evaporation of the solvents and drying under reduced pressure the product **TrD3/4** was obtained in a yield of 98.2% (79.8 mg, 83.0 µmol).

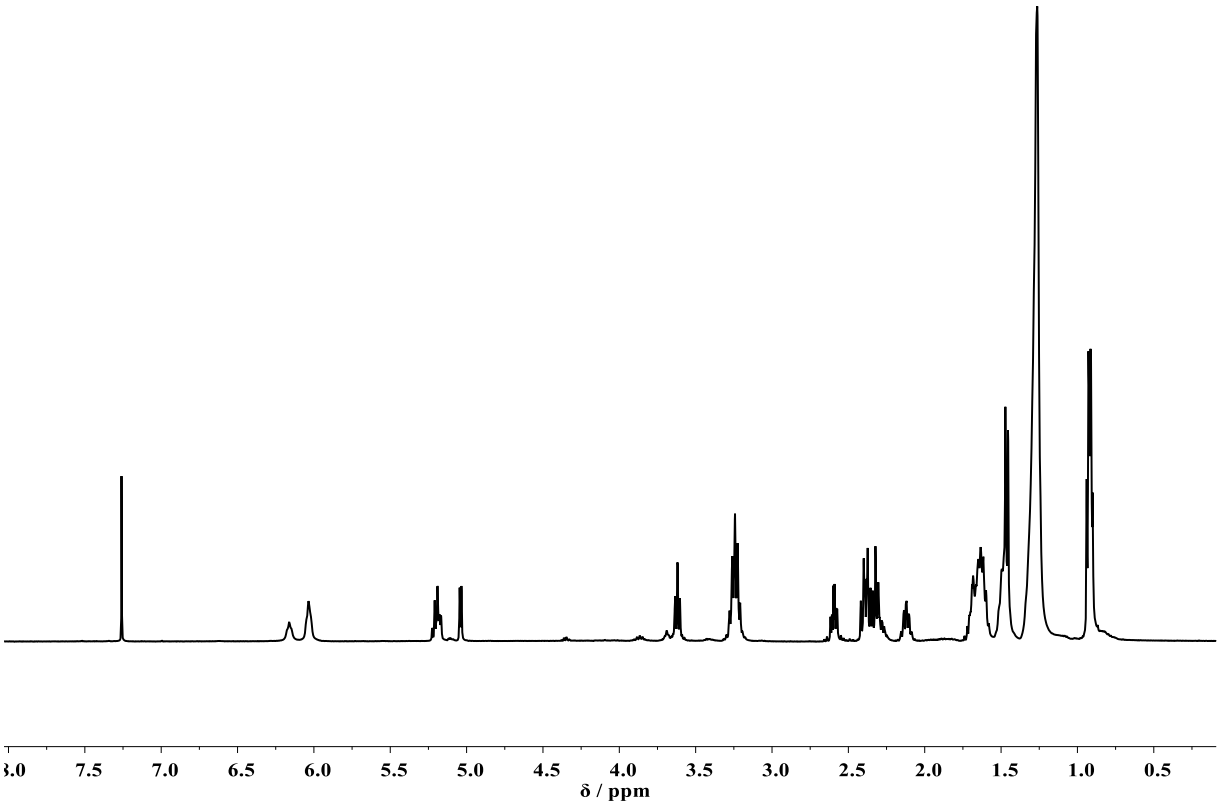
IR (ATR): ν / cm^{-1} = 3306.7 (vw), 2925.3 (m), 2854.0 (w), 1739.2 (m), 1653.2 (m), 1539.6 (w), 1463.9 (w), 1370.3 (w), 1168.9 (m), 722.3 (vw), 650.7 (vw), 426.7 (vw).

^{1}H-NMR (400 MHz, $CDCl_3$): δ / ppm = 6.25 – 6.11 (m, 1 H, NH^{1}), 6.09 – 5.98 (m, 2 H, NH^{1}), 5.26 – 5.14 (m, 2 H, CH^{2}), 5.04 (d, J = 4.5 Hz, 1 H, CH^{3}), 3.62 (td, J = 6.4, 1.6 Hz, 2 H, CH_2^{4}),

3.32 – 3.17 (m, J = 6.1 Hz, 6 H, CH_2^5), 2.59 (td, J = 7.1, 3.2 Hz, 2 H, CH_2^6), 2.44 – 2.23 (m, 7 H, CH^7, CH_2^8), 2.19 – 2.06 (m, 2 H, CH_2^9), 1.74 – 1.56 (m, 9 H, CH^{10}, CH_2^{11}), 1.54 – 1.40 (m, 9 H, CH_2^{12}, CH_3^{13}), 1.39 – 1.12 (m, 36 H, CH_2^{14}), 1.01 – 0.85 (m, 12 H, CH_3^{15}).

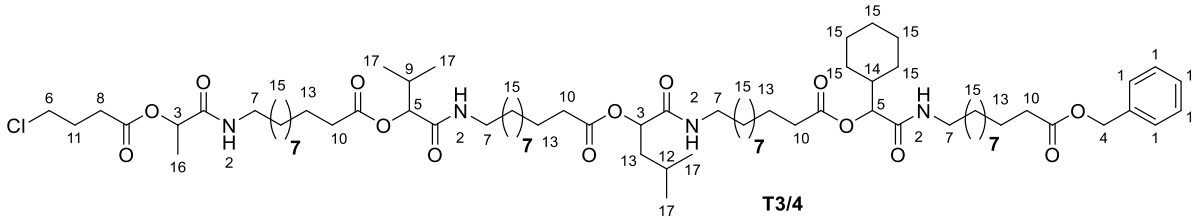
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 177.43, 172.82, 171.42, 170.45, 170.38, 169.59, 78.07, 72.79, 70.93, 44.08, 40.94, 39.43, 39.34, 34.42, 33.98, 31.30, 30.62, 29.68, 29.61, 29.57, 29.53, 29.48, 29.45, 29.34, 29.31, 29.22, 29.09, 27.48, 26.96, 26.92, 26.86, 25.11, 25.07, 24.87, 24.68, 23.25, 21.92, 18.89, 18.09, 17.11.

ESI-MS [m/z]: $[M + H]^+$ calculated for $^{12}C_{51}{}^{1}H_{92}{}^{16}O_{11}{}^{14}N_3{}^{35}Cl$, 958.6493; found, 958.6476, Δ = 1.7 mmu.



Supplementary Figure 160: ^{1}H-NMR of compound **TrD3/4** measured in $CDCl_3$.

Passerini reaction



In a 25 mL round bottom flask, 51.6 mg of **TrD3/4** (54.0 µmol, 1.00 eq.) was dissolved in 2.00 mL DCM and 9.10 mg cyclohexancarboxaldehyde **A6** (81.0 µmol, 1.50 eq.) and 24.3 mg of monomer **IM2** (81.0 µmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 1 day and subsequently the solvent was removed under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (2:1 → 0:1) to yield the Passerini product **T3/4** with 88.5% (65.4 mg, 48.0 µmol).

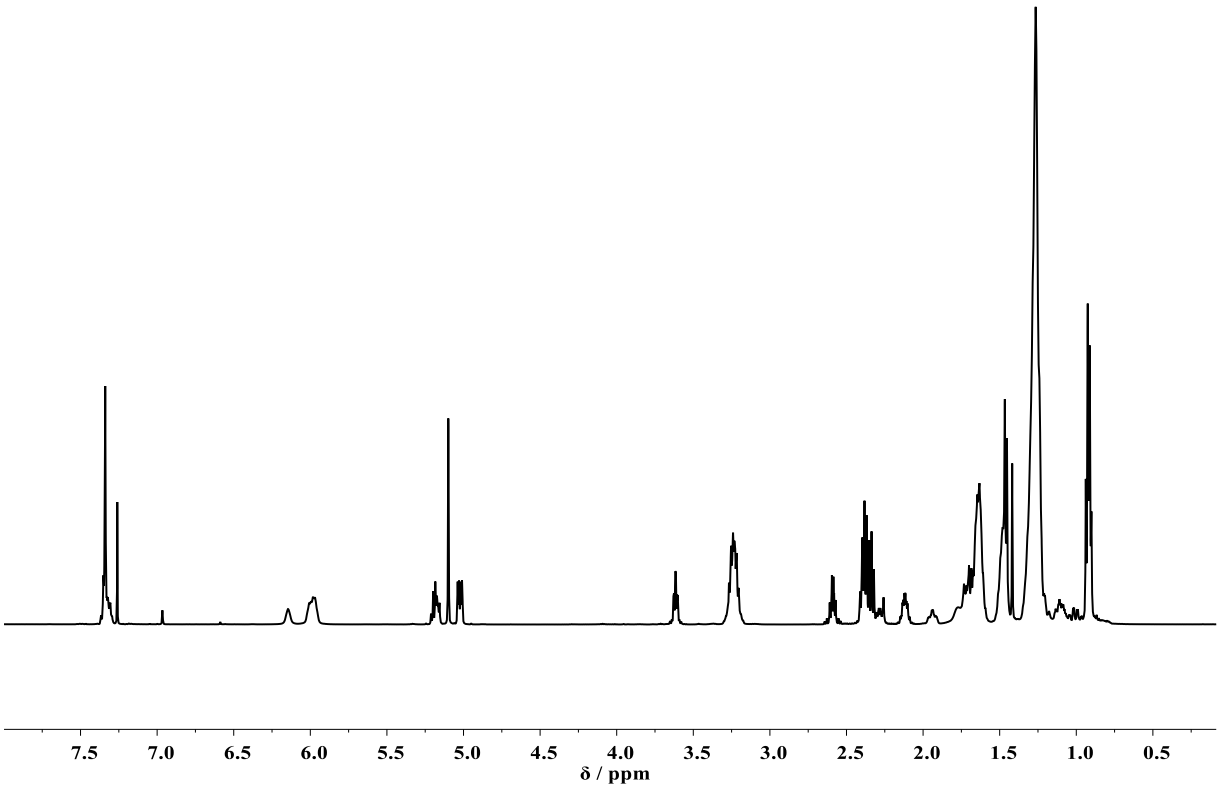
R_f = 0.76 in cyclohexane / ethyl acetate (1:2).

IR (ATR): ν / cm^{-1} = 3299.7 (vw), 2924.9 (w), 2853.4 (w), 1738.5 (w), 1654.9 (w), 1536.8 (w), 1453.9 (vw), 1370.5 (vw), 1165.7 (w), 1101.3 (vw), 722.8 (vw), 697.9 (vw), 488.6 (vw), 461.0 (vw), 417.6 (vw).

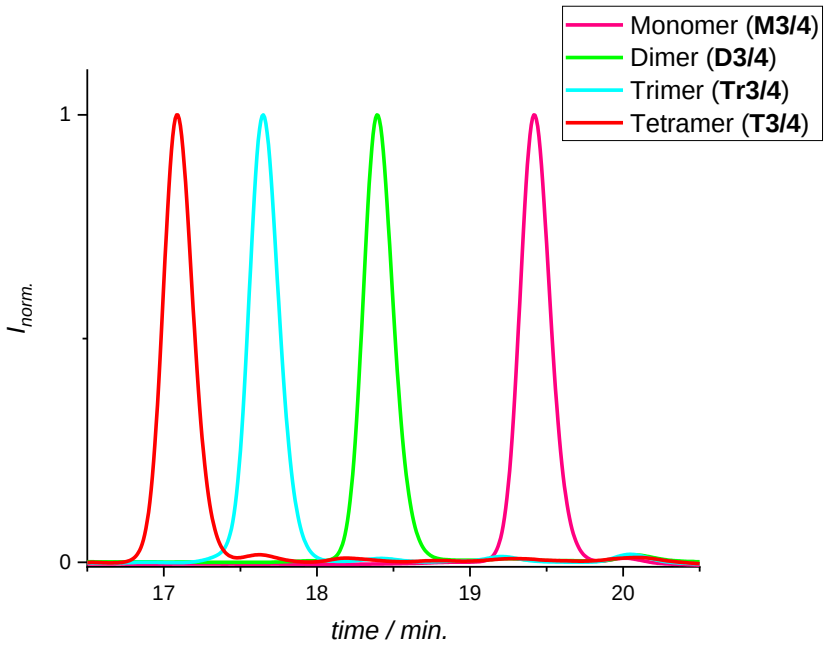
^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.40 – 7.28 (m, 5 H, CH_{Ar}^1), 6.15 (t, J = 6.0 Hz, 1 H, NH^2), 6.06 – 5.90 (m, 3 H, NH^2), 5.25 – 5.13 (m, 2 H, CH^3), 5.10 (s, 2 H, CH_2^4), 5.05 – 4.98 (m, 2 H, CH^5), 3.67 – 3.57 (m, 2 H, CH_2^6), 3.35 – 3.14 (m, 8 H, CH_2^7), 2.66 – 2.53 (m, 2 H, CH_2^8), 2.44 – 2.25 (m, 9 H, CH^9, CH_2^{10}), 2.16 – 2.06 (m, 2 H, CH_2^{11}), 2.01 – 1.90 (m, 1 H, CH^{12}), 1.84 – 1.58 (m, 10 H, CH_2^{13}), 1.56 – 1.38 (m, 12 H, CH^{14}, CH_2^{15}, CH_3^{16}), 1.35 – 1.05 (m, 50 H, CH_2^{15}), 0.98 – 0.88 (m, 12 H, CH_3^{17}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 173.73, 172.72, 172.64, 171.35, 170.31, 170.21, 169.35, 169.28, 136.16, 135.86, 128.58, 128.20, 77.96, 77.66, 72.66, 70.82, 66.11, 44.01, 40.91, 40.03, 39.31, 39.27, 39.20, 34.36, 34.32, 31.22, 30.56, 30.37, 29.61, 29.49, 29.39, 29.24, 29.15, 27.42, 27.35, 26.87, 26.12, 26.05, 25.93, 25.66, 25.04, 24.98, 24.59, 23.19, 21.84, 18.84, 18.02, 17.03.

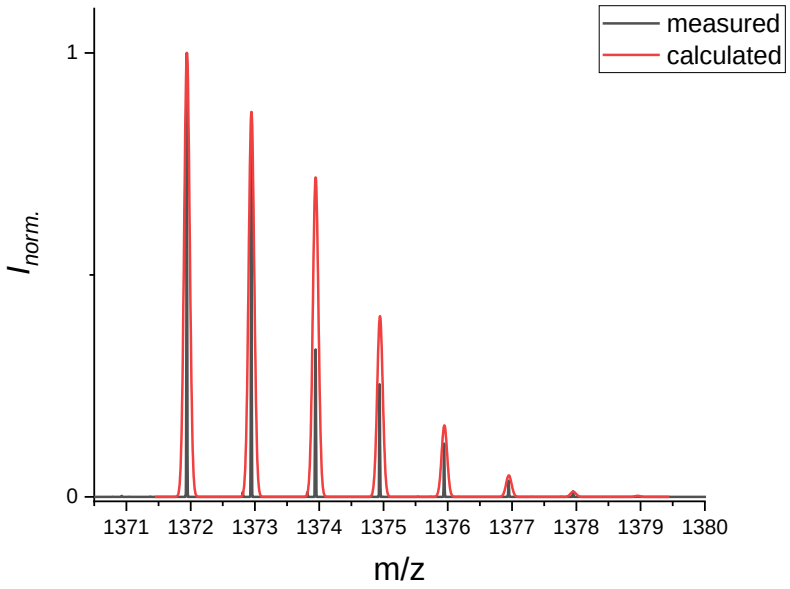
ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{77}{}^1H_{131}{}^{16}O_{14}{}^{14}N_4{}^{35}Cl$, 1371.9423; found, 1371.9403, Δ = 2.0 mmu.



Supplementary Figure 161: ^{1}H-NMR of compound **T3/4** measured in $CDCl_3$.



Supplementary Figure 162: SEC traces of the intermediates after each P-3CR in the synthesis of product **T3/4**.



Supplementary Figure 163: High resolution ESI-MS measurement of **T3/4**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (red).

```
ximum is 1.000000 found for mass 1371.940480
matching mass 1371.94048
cutoff 0.50000: 0 solutions (7 peaks)
cutoff 0.25000: 0 solutions (27 peaks)
cutoff 0.12500: 0 solutions (60 peaks)
cutoff 0.06250: 0 solutions (105 peaks)
cutoff 0.03125: 0 solutions (175 peaks)
cutoff 0.01562: 0 solutions (281 peaks)
cutoff 0.00781: 1 solutions (454 peaks)
1371.94048 ≈ 105.017020 + 255.183450 + 283.214750 + 297.230400 + 323.246050 + 107.049690 (sides
Acetaldehyde, Isobutyraldehyde, 3-Methylbutanal, Cyclohexancarboxaldehyde; error -0.99912)
Press ENTER to quit ...
```

Supplementary Figure 164: Screenshot of the automated read-out of **T3/4**.

6.3.3.5.6 Equations

$$[M_{Molecule} + H]^+ = \left[\left(M_{Start} + n \times (M_{Backbone}) + \sum_{i=1}^{i=n} M_{Sidechain}^{i} + M_{End} + y \times M(H)\right) + \mathrm{H}\right]^+$$

n = number of repeating units,

y = (n-1)

M_{Start} = M (**Tag X**)

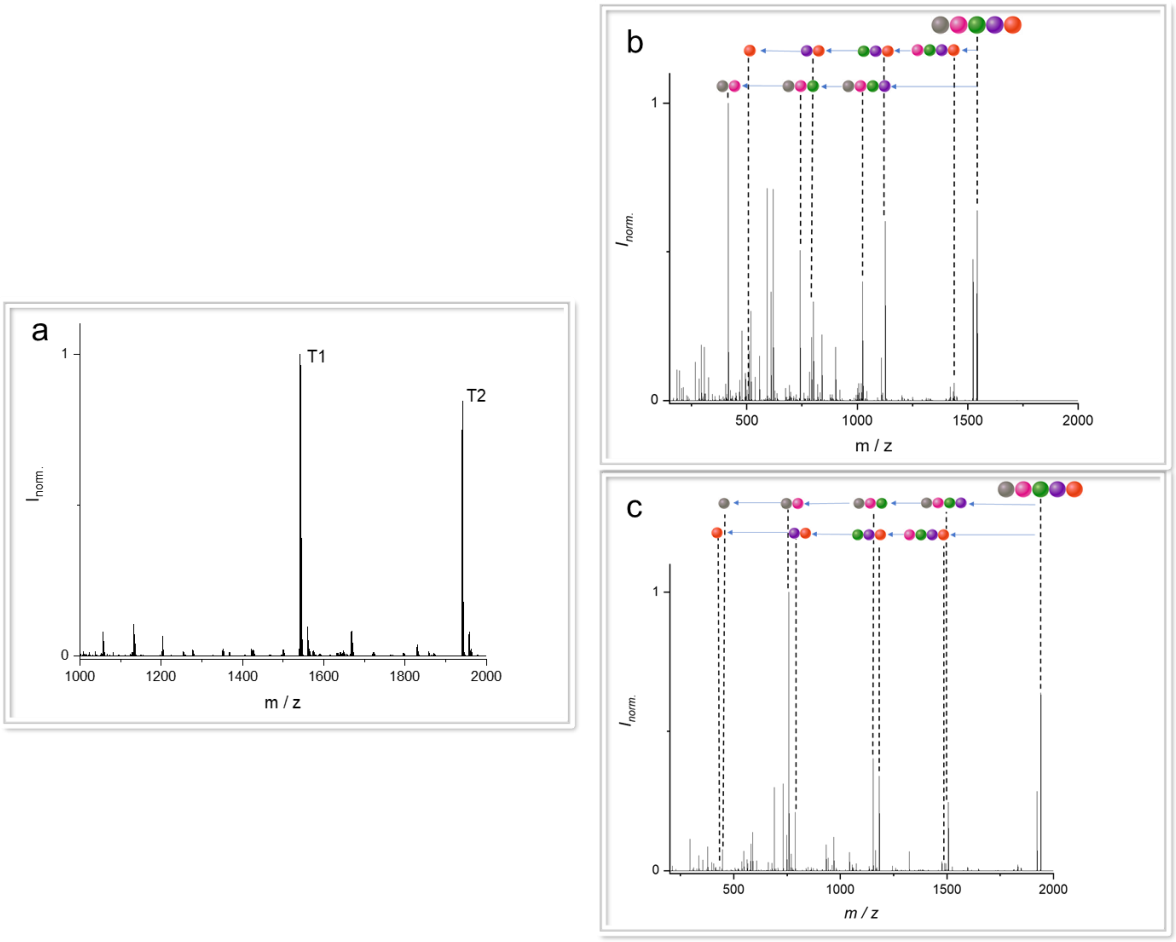
M_{End} = M (C_7H_7)

$M_{Backbone}$ = (M (monomer **IM2**)) – M(C_7H_7)

$M_{Sidechain}$ = M (**2a**) or M (**2b**) or M (**2c**) or M (**2d**) or M (**2e**) or M (**2f**) or M (**2g**) or M (**2h**) or M (**2i**) or M (**2j**) or M (**2k**) or M (2**l**)

$M_{Backbone}$ is calculated with the mass of the monomer, which incorporates the protected acid (benzyl ester); however, in the iterative cycle, the benzyl ester is deprotected and further converted as the free acid compound. In order to take this into consideration in the formula, y is introduced as additional summand.

6.3.3.5.7 Tetramer mixture

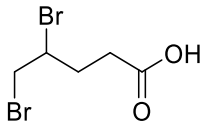


Supplementary Figure 165: Read-out of a mixture of two tetramers. **a.** ESI-MS spectrum of a mixture of two different tetramers **T1/1** and **T3/1** that was used for subsequent tandem ESI-MS/MS fragmentation. For the fragmentation, one of the respective molecule peaks was chosen at a time. **b.** fragmentation and read-out of tetramer **T3/1**. **c.** fragmentation and read-out of tetramer **T1/1**.

6.3.4 Reading mixtures, small molecules conquer the field of data storage

6.3.4.1 TAG synthesis[x]

TAG4 was synthesised according to the reported procedure from Nikishin *et al*.[289]



22

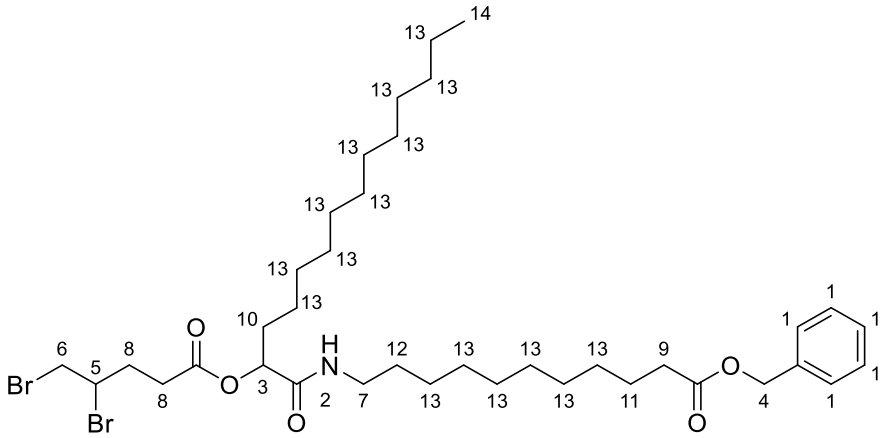
In a 50.0 mL round bottom flask 4.88 g 4-pentenoic acid **20** (48.8 mmol, 1.00 eq.) were dissolved in 19.5 mL DCM and cooled to –40°C. Afterwards, 2.50 mL bromine **21** (7.80 g, 48.8 mmol, 1.00 eq.), dissolved in 9.75 mL DCM, were added dropwise over 1 hour with intense stirring. After full addition of the bromine, the reaction mixture was stirred for another 0.5 hour and the bromine residues were quenched using sodium thiosulfate solution. The aqueous phase was separated and washed with DCM (3 × 50 mL). The organic layer was washed with water (1 × 50 mL). The combined organic layers were dried over sodium sulfate 11 and the solvent was evaporated under reduced pressure. The product **22** was purified *via* column chromatography (cyclohexane / ethyl acetate 8:1 → 4:1) and was obtained as a white solid in a yield of 55.4% (7.19 g, 27.7 mmol).

^{1}H-NMR (400 MHz, $CDCl_3$): δ / ppm = 4.34 – 4.19 (m, 1 H), 3.88 (dd, *J* = 10.4, 4.3 Hz, 1 H), 3.63 (t, *J* = 10.1 Hz, 1 H), 2.80 – 2.48 (m, 3 H), 2.12 – 1.96 (m, 1 H). ^{1}H-NMR was in accordance to the literature.[289]

[x] Synthesis was carried out by Felix Bauer in the Bachelorthesis "Synthesis of sequence-defined macromolecules using multicomponent reactions" under the laboratory supervision of Maximiliane Frölich.[292]

6.3.4.2 Synthesis of Trimer Tr4/1 with TAG4[xi]

Passerini reaction



In a 50 mL round bottom flask 1.50 g 4,5-dibromopentoic acid **23** (5.77 mmol, 1.00 eq.) were dissolved in 5.77 mL DCM (1 M) and 2.04 mL tridecanal **A11** (1.72 g, 8.66 mmol, 1.50 eq.) and 2.61 g of monomer **IM2** (8.66 mmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 1 day. Subsequently the solvent was removed under reduced pressure and the crude product was purified by column chromatography (cyclohexane / ethyl acetate 5:1 → 2:1) to afford the Passerini product **M4/1** as a colourless oil in a yield of 99.4% (4.36 g, 5.73 mmol).

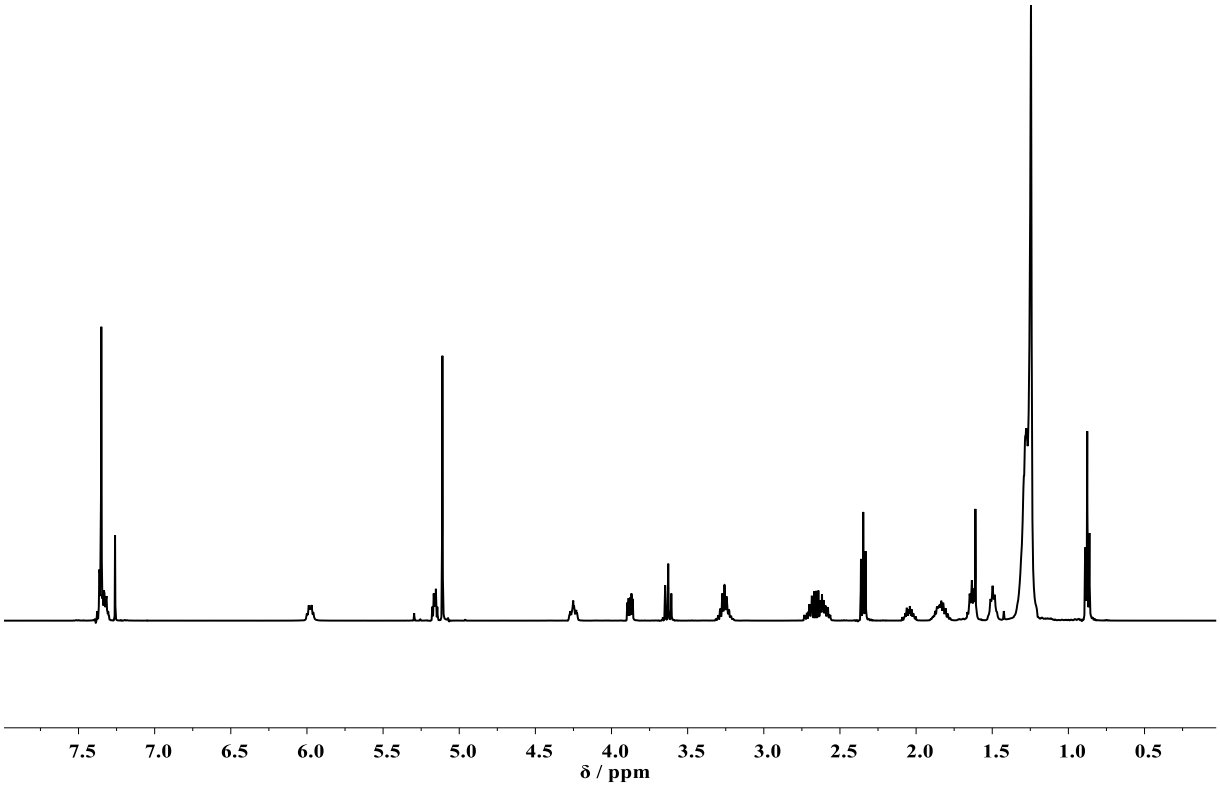
IR (ATR): ν / cm^{-1} = 3306.2 (vw), 2922.0 (s), 2852.0 (m), 1736.4 (s), 1655.4 (m), 1535.4 (w), 1455.8 (w), 1377.7 (w), 1164.3 (s), 732.9 (w), 696.7 (m), 568.2 (w).

^{1}H-NMR (500 MHz, $CDCl_3$): δ / ppm = 7.38 – 7.30 (m, 5 H, CH_{Ar}^{1}), 6.06 – 5.90 (m, 1 H, NH^{2}), 5.19 – 5.13 (m, 2 H, CH^{3}), 5.11 (s, 2 H, CH_2^{4}), 4.31 – 4.20 (m, 1 H, CH^{5}), 3.91 – 3.85 (m, 1 H, CH_2^{6}), 3.63 (t, *J* = 10.1 Hz, 1 H, CH_2^{6}), 3.32 – 3.19 (m, 2 H, CH_2^{7}), 2.74 – 2.55 (m, 3 H, CH_2^{8}), 2.35 (t, *J* = 7.6 Hz, 2 H, CH_2^{9}), 2.10 – 1.99 (m, 1 H, CH_2^{8}), 1.91 – 1.75 (m, 2 H, CH_2^{10}), 1.69 – 1.56 (m, 2 H, CH_2^{11}), 1.54 – 1.44 (m, 2 H, CH_2^{12}), 1.37 – 1.19 (m, 32 H, CH_2^{13}), 0.88 (t, *J* = 6.9 Hz, 3 H, CH_3^{14}).

^{13}C-NMR (126 MHz, $CDCl_3$): δ / ppm = 173.70, 171.10, 169.51, 136.14, 128.56, 128.18, 74.53, 66.08, 51.41, 51.28, 39.27, 35.81, 35.78, 34.33, 31.93, 31.88, 31.86, 31.32, 31.29, 29.68, 29.66, 29.64, 29.56, 29.46, 29.44, 29.37, 29.27, 29.23, 29.12, 26.91, 26.85, 24.95, 24.82, 24.79, 22.71, 14.15.

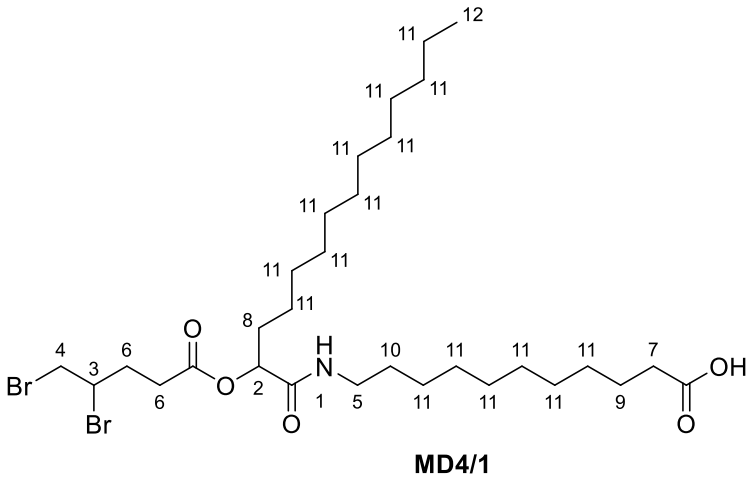
[xi] Synthesis was carried out by Felix Bauer in the Bachelorthesis "Synthesis of sequence-defined macromolecules using multicomponent reactions" under the laboratory supervision of Maximiliane Frölich

ESI-MS [*m/z*]: $[M+H]^+$ calculated for $^{12}C_{37}{}^{1}H_{61}{}^{16}O_5{}^{14}N{}^{79}Br_2$, 758.2989; found 758.2979, Δ = 1.0 mmu.



Supplementary Figure 166: ^{1}H-NMR of compound **M4/1** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask 4.18 g of the Passerini product **M4/1** (5.94 mmol, 1.00 eq.) was dissolved in 5.95 mL ethyl acetate and 5.95 mL THF. Afterwards 418 mg (10 wt%) palladium on activated carbon **1** were added. The mixture was purged with hydrogen (balloon) and stirred

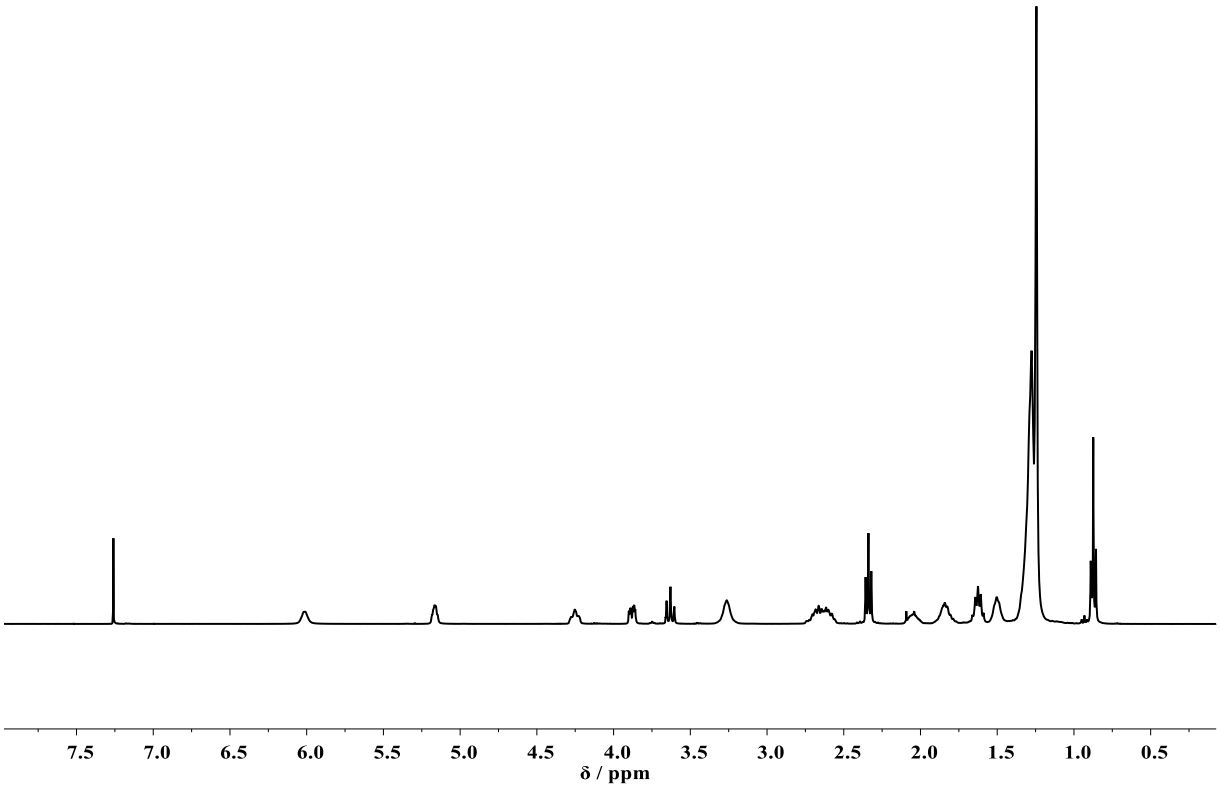
under hydrogen atmosphere overnight. The catalyst was filtered off and the solvent was removed under reduced pressure. The product **MD4/1** was obtained as a red oil in a yield of 95.3% (3.51 g, 5.24 mmol).

IR (ATR): ν / cm^{-1} = 3305.9 (w), 2915.7 (vs), 2849.1 (vs), 1741.7 (s), 1703.6 (vs), 1649.3 (vs), 1539.4 (s), 1467.2 (m), 1432.3 (s), 1373.8 (w), 1273.1 (s), 1248.0 (s), 1220.9 (s), 1160.4 (s), 929.5 (w), 720.0 (m), 566.9 (m).

^{1}H-NMR (400 MHz, $CDCl_3$) δ / ppm = 6.08 – 5.94 (m, 1 H, NH^1), 5.20 – 5.12 (m, 1 H, CH^2), 4.31 – 4.21 (m, 1 H, CH^3), 3.92 – 3.83 (m, 1 H, CH_2^4), 3.63 (t, *J* = 10.1 Hz, 1 H, CH_2^4), 3.34 – 3.19 (m, 2 H, CH_2^5), 2.77 – 2.53 (m, 3 H, CH_2^6), 2.34 (t, *J* = 7.5 Hz, 2 H, CH_2^7), 2.12 – 1.98 (m, 1 H, CH_2^6), 1.91 - 1.75 (m, 2 H, CH_2^8), 1.63 (p, *J* = 7.4 Hz, 2 H, CH_2^9), 1.56 – 1.43 (m, 2 H, CH_2^{10}), 1.43 – 1.07 (m, 32 H, CH_2^{11}), 0.95 – 0.82 (m, 3 H, CH_3^{12}).

^{13}C-NMR (101 MHz, $CDCl_3$): δ / ppm = 178.94, 171.16, 171.12, 169.67, 74.55, 51.41, 51.28, 39.30,35.81, 35.78, 33.93, 31.93, 31.91, 31.89, 31.87, 31.33, 31.30, 29.67, 29.65, 29.63, 29.55, 29.50, 29.44, 29.36, 29.26, 29.14, 29.12, 28.98, 26.80, 24.81, 24.79, 24.67, 22.70, 14.13.

ESI-MS [*m/z*]: $[M+H]^+$ calculated for $^{12}C_{30}{}^{1}H_{55}{}^{16}O_5{}^{14}N^{79}Br_2$, 668.2520; found 668.2505, Δ = 1.5 mmu.



Supplementary Figure 167: ^{1}H-NMR of compound **MD4/1** measured in $CDCl_3$.

Passerini reaction

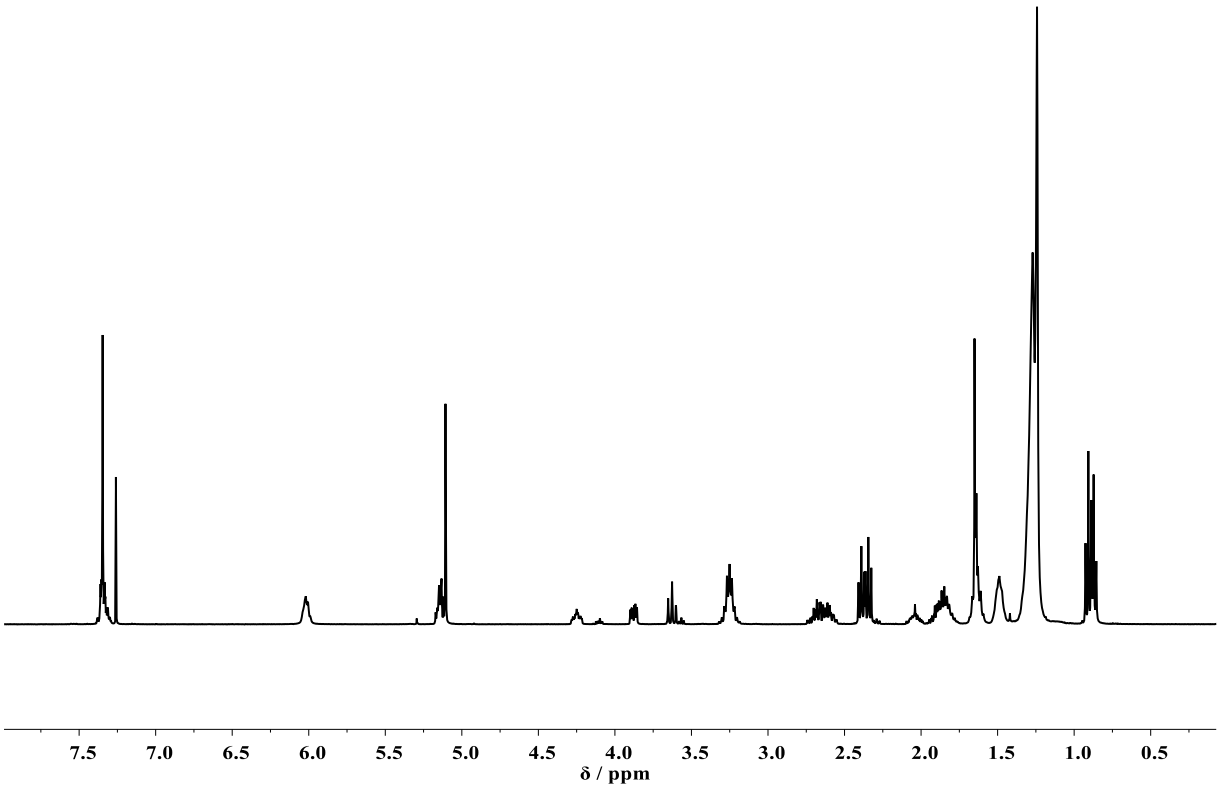
In a 50 mL round bottom flask 3.51 g of the Passerini product **MD4/1** (5.24 mmol, 1.00 eq.) was dissolved in 5.20 mL DCM (1 M) and 564 µL propionaldehyde **A2** (457 mg, 7.86 mmol, 1.50 eq.) and 2.37 g of monomer **IM2** (8.66 mmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 1 day. Subsequently the solvent was removed under reduced pressure and the crude product was purified by column chromatography (cyclohexane / ethyl acetate 5:1 → 1:1) to afford the Passerini product **D4/1** as a colourless solid in a yield of 66.4% (3.58 g, 3.74 mmol).

IR (ATR): ν / cm^{-1} = 3261.3 (vw), 3094.2 (vw), 2918.3 (vs), 2849.8 (vs), 1733.7 (vs), 1653.2 (vs), 1542.9 (m), 1467.0 (m), 1381.6 (w), 1266.8 (m), 1239.3 (s), 1205.8 (m), 1161.9 (vs), 1114.2 (s), 972.7 (w), 949.4 (w), 740.5 (m), 720.9 (m), 696.9 (s), 565.7 (w), 474.2 (vw).

^{1}H-NMR (400 MHz, $CDCl_3$) δ / ppm = 7.39 – 7.28 (m, 5 H, $CH_{Ar}{}^{1}$), 6.06 – 5.97 (m, 2 H, NH^{2}), 5.18 – 5.12 (m, 2 H, CH^{3}), 5.11 (s, 2 H, $CH_2{}^{4}$), 4.29 – 4.21 (m, 1 H, CH^{5}), 3.88 (ddd, J = 10.4, 4.3, 2.7 Hz, 1 H, $CH_2{}^{6}$), 3.63 (t, J = 10.1 Hz, 1 H, $CH_2{}^{6}$), 3.33 – 3.17 (m, 4 H, $CH_2{}^{7}$), 2.75 – 2.54 (m, 3 H, $CH_2{}^{8}$), 2.43 – 2.31 (m, 4 H, $CH_2{}^{9}$), 2.10 – 1.98 (m, 1 H, $CH_2{}^{8}$), 1.97 – 1.74 (m, 2 H, $CH_2{}^{10}$), 1.72 – 1.57 (m, 4 H, $CH_2{}^{11}$), 1.54 – 1.44 (m, 4 H, $CH_2{}^{12}$), 1.36 – 1.18 (m, 44 H, $CH_2{}^{13}$), 0.94 – 0.84 (m, 6 H, CH_3, ^{14}).

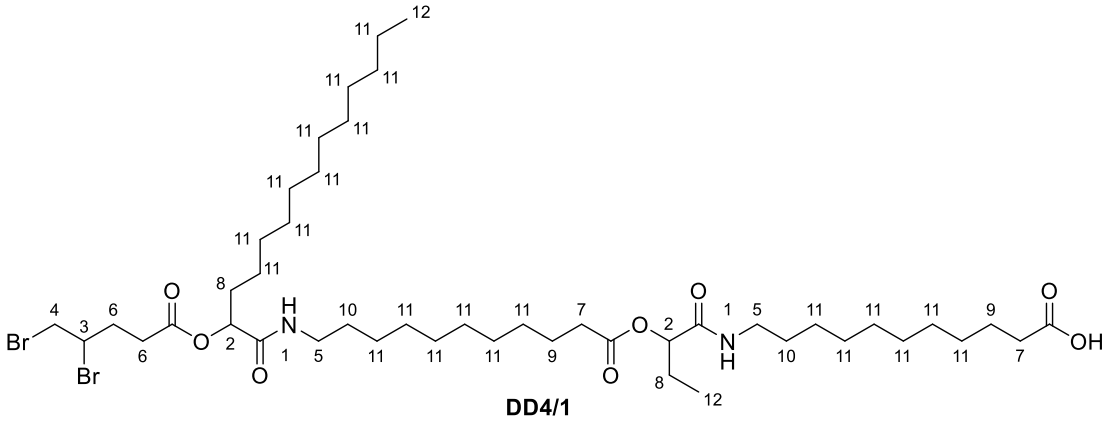
^{13}C-NMR (101 MHz, $CDCl_3$) δ / ppm = 173.71, 172.43, 171.13, 171.10, 169.63, 169.57, 169.54, 136., 128.55, 128.16, 74.81, 74.52, 66.08, 51.42, 51.29, 39.26, 39.20, 35.81, 35.78, 34.32, 31.92, 31.86, 31.31, 31.28, 29.67, 29.65, 29.63, 29.65, 29.58, 29.55, 29.45, 29.44, 29.36, 29.26, 29.21, 29.10, 26.84, 26.83, 26.10, 25.09 24.94, 24.82, 24.80, 22.70, 14.14.

ESI-MS [*m/z*]: $[M+H]^+$ calculated for $^{12}C_{52}{}^{1}H_{88}{}^{16}O_8{}^{14}N_2{}^{79}Br_2$, 1027.4980; found 1027.4964, Δ = 1.6 mmu.



Supplementary Figure 168: ^{1}H-NMR of compound **D4/1** measured in $CDCl_3$.

Deprotection



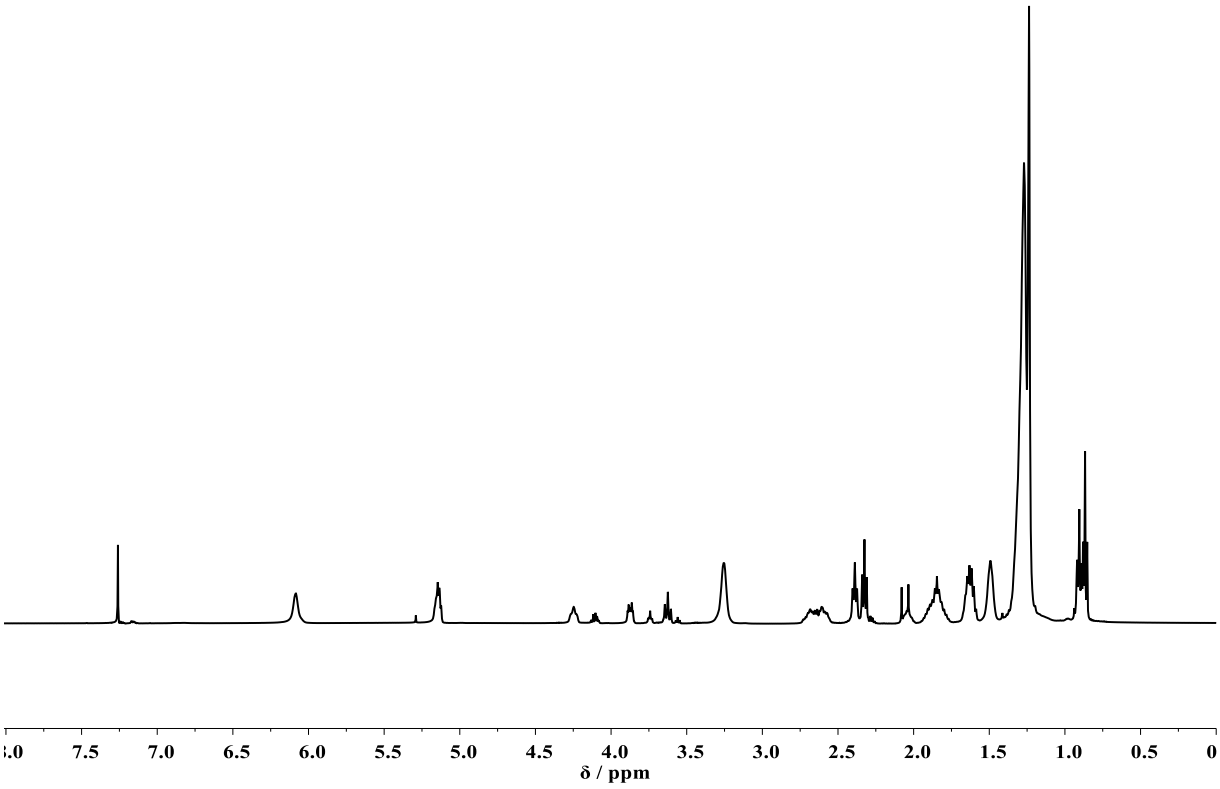
In a 50 mL round bottom flask 3.58 g of the Passerini product **D4/1** (3.43 mmol, 1.00 eq.) were dissolved in 3.40 mL ethyl acetate and 3.40 mL THF. Afterwards 358 mg (10 wt%) palladium on activated carbon **1** were added. The mixture was purged with hydrogen (balloon) and stirred under hydrogen atmosphere overnight. The catalyst was filtered off and the solvent was removed under reduced pressure. The product **DD4/1** was obtained as a red oil in a yield of 98.5% (3.22 g, 3.42 mmol).

IR (ATR): ν / cm^{-1} = 3259.5 (w), 3094.6 (w), 2918.5 (vs), 2850.3 (vs), 1741.5 (vs), 1700.5 (s), 1653.0 (vs), 1542.5 (m), 1466.9 (m), 1435.3 (m), 1378.5 (w), 1265.3 (m), 1238.9 (s), 1161.2 (vs), 1114.5 (s), 971.5 (w), 721.1 (m), 693.5 (m), 566.8 (w).

^{1}H-NMR (500 MHz, $CDCl_3$) δ / ppm = 6.17 – 6.01 (m, 2 H, NH^{1}), 5.21 – 5.10 (m, 2 H, CH^{2}), 4.29 – 4.19 (m, 1 H, CH^{3}), 3.91 – 3.84 (m, 1 H, CH_2^{4}), 3.62 (t, *J* = 10.0 Hz, 1 H, CH^{4}), 3.33 – 3.19 (m, 4 H, CH_2^{5}), 2.75 – 2.52 (m, 3 H, CH_2^{6}), 2.45 – 2.29 (m, 4 H, CH_2^{7}), 2.09 – 2.00 (m, 1 H, CH_2^{6}), 1.97 – 1.74 (m, 4 H, CH_2^{8}), 1.71 – 1.57 (m, 4 H, CH_2^{9}), 1.57 – 1.44 (m, 4 H, CH_2^{10}), 1.39 – 1.09 (m, 44 H, CH_2^{11}), 0.96 – 0.81 (m, 6 H, CH_3^{12}).

^{13}C-NMR (126 MHz, $CDCl_3$) δ / ppm = 172.48, 171.23, 171.19, 169.82, 169.77, 74.82, 74.51, 51.45, 51.31, 39.37, 39.26, 35.86, 35.83, 34.37, 33.94, 31.93, 31.90, 29.68, 29.66, 29.64, 29.55, 29.50, 29.48, 29.45, 29.38, 29.37, 29.35, 29.26, 29.25, 29.22, 29.13, 28.99, 26.86, 26.84, 26.79, 25.11, 25.00, 24.97, 24.84, 24.81, 22.25, 14.14, 9.06.

ESI-MS [*m/z*]: $[M+H]^+$ calculated for $^{12}C_{45}{}^{1}H_{82}{}^{16}O_8{}^{14}N_2{}^{79}Br_2$, 937.4511; found 937.4503, Δ = 0.8 mmu.



Supplementary Figure 169: ^{1}H-NMR of compound **DD4/1** measured in $CDCl_3$.

Passerini reaction

Tr4/1

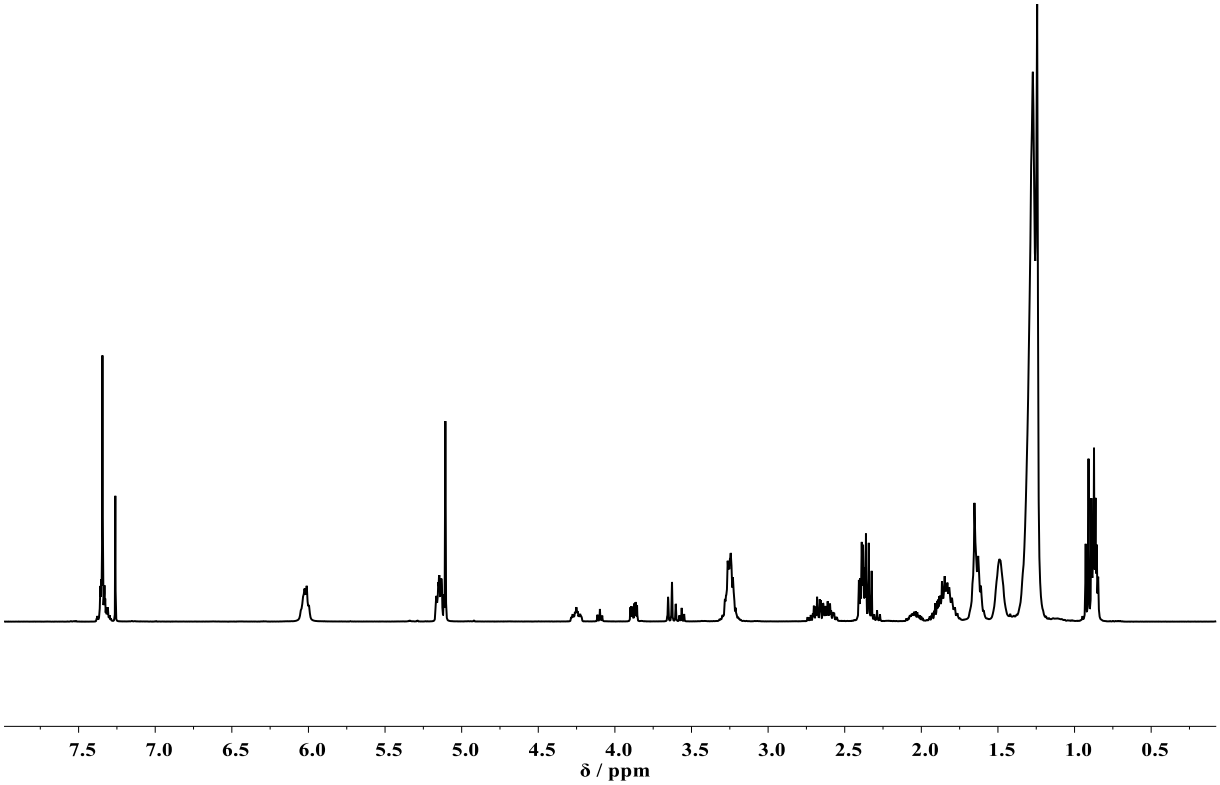
In a 50 mL round bottom flask 3.05 g (3.25 mmol, 1.00 eq.) of the Passerini product **DD4/1** was dissolved in 3.25 mL DCM (1M) and 688 µL heptanal **A7** (556 mg, 4.87 mmol, 1.50 eq.) and 1.47 g of monomer **IM2** (4.87 mmol, 1.50 eq.) were added. The mixture was then stirred at room temperature for 1 day. Subsequently the solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane / ethyl acetate 6:1 → 1:1) to afford the Passerini product **Tr4/1** in a yield of 953 mg (747 µmol, 21.7%).

IR (ATR): ν / cm^{-1} = 3259.5 (vw), 2922.5 (vs), 2852.5 (s), 1737.7 (s), 1654.1 (s), 1536.2 (m), 1456.8 (m), 1376.2 (w), 1162.9 (s), 1101.6 (m), 722.2 (w), 696.9 (w), 569.2 (vw).

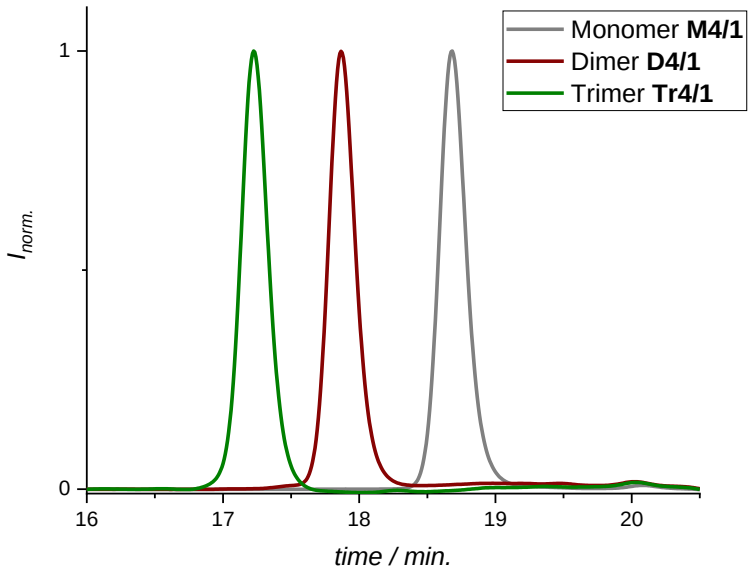
^{1}H-NMR (400 MHz, $CDCl_3$) δ / ppm = 7.39 – 7.28 (m, 5 H, CH_{Ar}^{1}), 6.10 – 5.96 (m, 3 H, NH^{2}), 5.18 – 5.12 (m, 3 H, CH^{3}), 5.11 (s, 2 H, CH_2^{4}), 4.29 – 4.21 (m, 1 H, CH^{5}), 3.91 – 3.85 (m, 1H, CH_2^{6}), 3.63 (t, *J* = 10.1 Hz, 1 H, CH_2^{6}), 3.32 – 3.17 (m, 6 H, CH_2^{7}), 2.75 – 2.54 (m, 3 H, CH_2^{8}), 2.42 – 2.26 (m, 6 H, CH_2^{9}), 2.10 – 1.98 (m, 1 H, CH_2^{8}), 1.96 – 1.73 (m, 6 H, CH_2^{10}), 1.73 – 1.57 (m, 6 H, CH_2^{11}), 1.56 – 1.43 (m, 6 H, CH_2^{12}), 1.39 – 1.17 (m, 64 H, CH_2^{13}), 0.96 – 0.82 (m, 9 H, CH_3^{14}).

^{13}C NMR (101 MHz, $CDCl_3$) δ / ppm = 173.68, 172.47, 172.43, 171.13, 171.10, 169.84, 169.63, 169.56, 169.53, 136.14, 128.54, 128.16, 74.81, 74.52, 73.95, 66.07, 51.42, 51.29, 39.26, 39.20, 35.81, 35.79, 34.32, 31.92, 31.87, 31.63, 31.33, 29.67, 29.65, 29.63, 29.60, 29.56, 29.55, 29.46, 29.43, 29.36, 29.26, 29.22, 29.20, 29.14, 29.11, 28.92, 26.84, 26.83, 25.09, 24.97, 24.95, 24.83, 24.81, 24.72, 22.69, 22.53, 14.13, 14.05, 9.03.

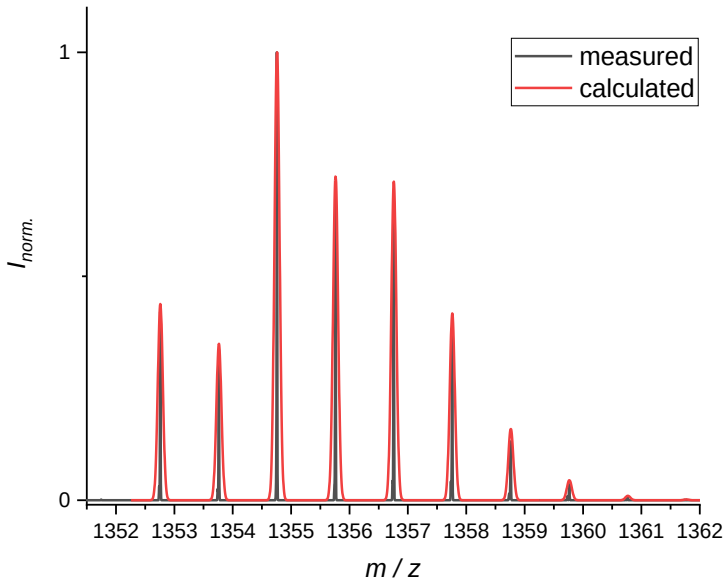
ESI-MS [m/z]: $[M+H]^+$ calculated for $^{12}C_{71}{}^{1}H_{123}{}^{16}O_{11}{}^{14}N_3{}^{79}Br_2$, 1352.7597; found 1352.7589, Δ = 0.8 mmu.



Supplementary Figure 170: ^{1}H-NMR of compound **Tr4/1** measured in $CDCl_3$.



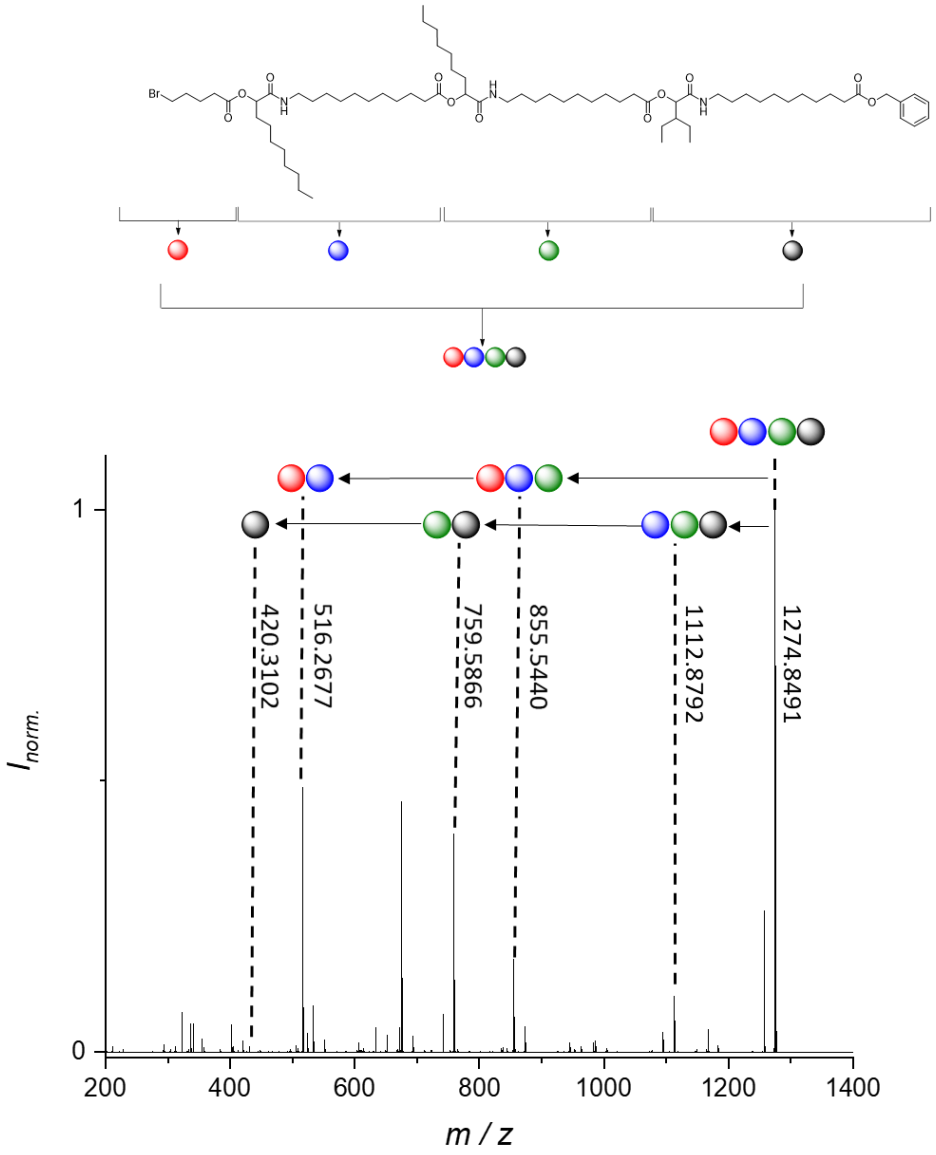
Supplementary Figure 171: SEC traces of the intermediates after each P-3CR in the synthesis of product **Tr4/1**.



Supplementary Figure 172: High resolution ESI-MS measurement of **Tr4/1**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (red).

```
found 20908 values in C:\Users\Maxi\Desktop\Fb 012 NCE 17 width 5.csv, maximum is 1.000000 found for mass 1274.847180
matching mass 1274.84718
cutoff 0.50000: 0 solutions (6 peaks)
cutoff 0.25000: 0 solutions (23 peaks)
cutoff 0.12500: 0 solutions (44 peaks)
cutoff 0.06250: 0 solutions (72 peaks)
cutoff 0.03125: 1 solutions (130 peaks)
1274.84718 ≈ 162.975850 + 353.293000 + 339.277350 + 311.246050 + 107.049690 (sides Nonanal, Octanal, 2-Ethylbutanal; error
-1.00524)
Press ENTER to quit ...
```

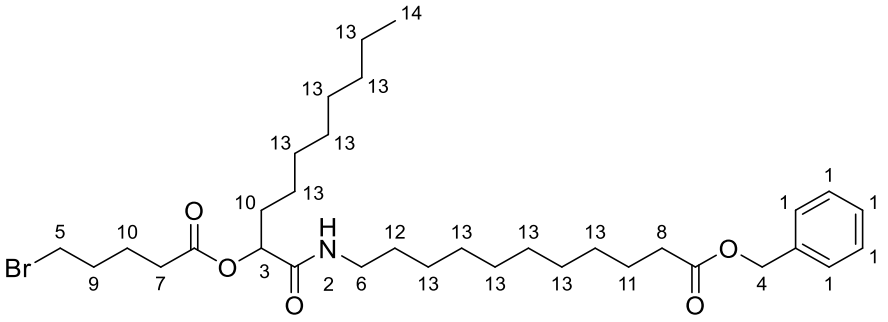
Supplementary Figure 173: Screenshot of the automated read-out of **Tr4/1**.



Supplementary Figure 174: Read-out of the sequence-defined hexamer **Tr4/1**. Read-out of the hexamer **Tr4/1** *via* tandem ESI-MS/MS with an NCE of 17. In the spectrum, the read-out from both ends of the oligomer using the fragmentation next to the carbonyl are shown.

6.3.4.3 Synthesis of Trimer Tr5/1 with TAG5[xii]

Passerini reaction



M5/1

In a 50.0 mL round bottom flask 2.00 g 5-bromovaleric acid **TAG7** (11.1 mmol, 1.00 eq.) was dissolved in 11.1 mL DCM (1 M) and 2.85 mL nonanal **A9** (2.36 g, 16.6 mmol, 1.50 eq.) and 5.00 g of monomer **IM2** (16.6 mmol, 1.50 eq.) were added. The mixture was stirred at room temperature for 1 day. Subsequently the solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane / ethyl acetate 8:1 → 4:1) to afford the Passerini product **M5/1** as a white solid in a yield of 95.9% (6.62 g, 10.6 mmol).

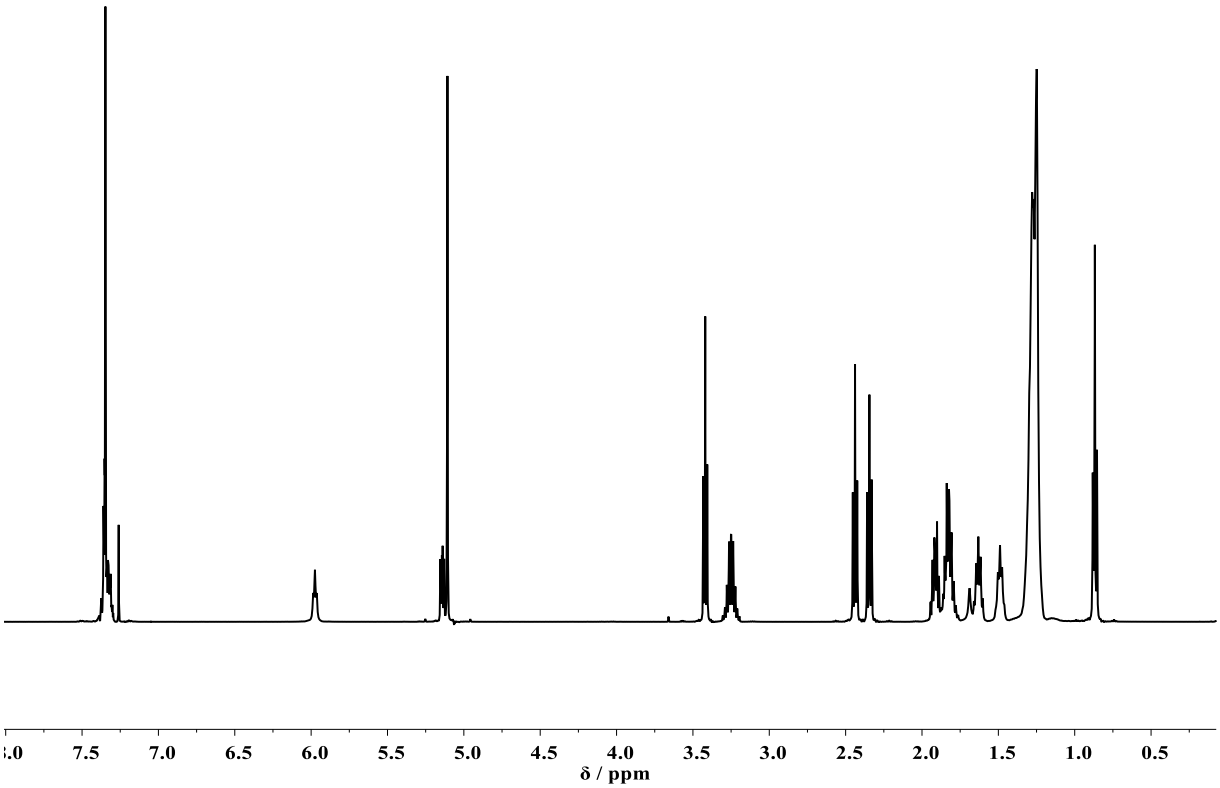
IR (ATR): ν / cm^{-1} = 3276.4 (w), 2198.4 (vs), 2851.5 (s), 1729.5 (vs), 1647.3 (vs), 1551.5 (m), 1466.6 (m), 1362.6 (m), 1295.6 (m), 1262.5 (m), 1240.3 (m), 1204.1 (s), 1167.2 (vs), 1074.8 (m), 1002.9 (m), 742.5 (vs), 697.9 (vs), 559.1 (w), 473.9 (w).

^{1}H-NMR (500 MHz, $CDCl_3$): δ / ppm = 7.56 – 7.28 (m, 5 H, $CH_{Ar}{}^{1}$), 5.97 (t, *J* = 5.9 Hz, 1 H, NH^{2}), 5.17 – 5.12 (m, 1 H, CH^{3}), 5.11 (s, 2 H, $CH_2{}^{4}$), 3.42 (t, *J* = 6.5 Hz, 2 H, $CH_2{}^{5}$), 3.33 – 3.15 (m, 2 H, $CH_2{}^{6}$), 2.44 (t, *J* = 7.2 Hz, 2 H, $CH_2{}^{7}$), 2.34 (t, *J* = 7.5 Hz, 2 H, $CH_2{}^{8}$), 2.02 – 1.87 (m, 2 H, $CH_2{}^{9}$), 1.87 – 1.75 (m, 4 H, $CH_2{}^{10}$), 1.67 – 1.58 (m, 2 H, $CH_2{}^{11}$), 1.53 – 1.43 (m, 2 H, $CH_2{}^{12}$), 1.37 – 1.13 (m, 24 H, $CH_2{}^{13}$), 0.87 (t, *J* = 6.9 Hz, 3H, $CH_3{}^{14}$).

^{13}C-NMR (126 MHz, $CDCl_3$): δ / ppm = 173.70, 171.83, 169.68, 136.14, 128.47, 74.22, 66.08, 39.24, 34.33, 33.30, 32.93, 31.93, 31.83, 29.57, 29.46, 29.40, 29.36, 29.26, 29.22, 29.11, 24.95, 23.47, 22.66, 14.12.

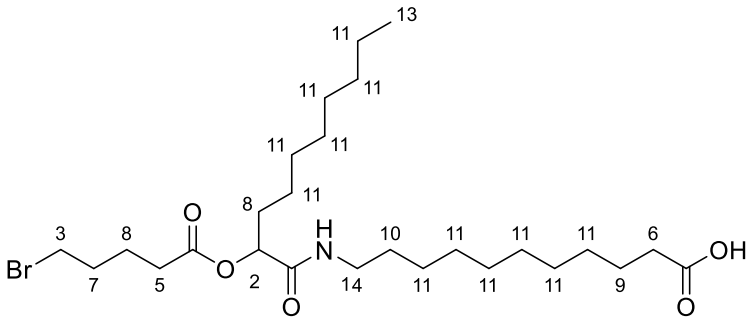
ESI-MS [*m/z*]: $[M+H]^+$ calculated for $^{12}C_{33}{}^{1}H_{54}{}^{16}O_5{}^{14}N^{79}Br$, 624.3258; found 624.3247, Δ = 1.1mmu.

[xii] Synthesis was carried out by Felix Bauer in the Bachelorthesis "Synthesis of sequence-defined macromolecules using multicomponent reactions" under the laboratory supervision of Maximiliane Frölich.[292]



Supplementary Figure 175: ^{1}H-NMR of compound **M5/1** measured in $CDCl_3$.

Deprotection



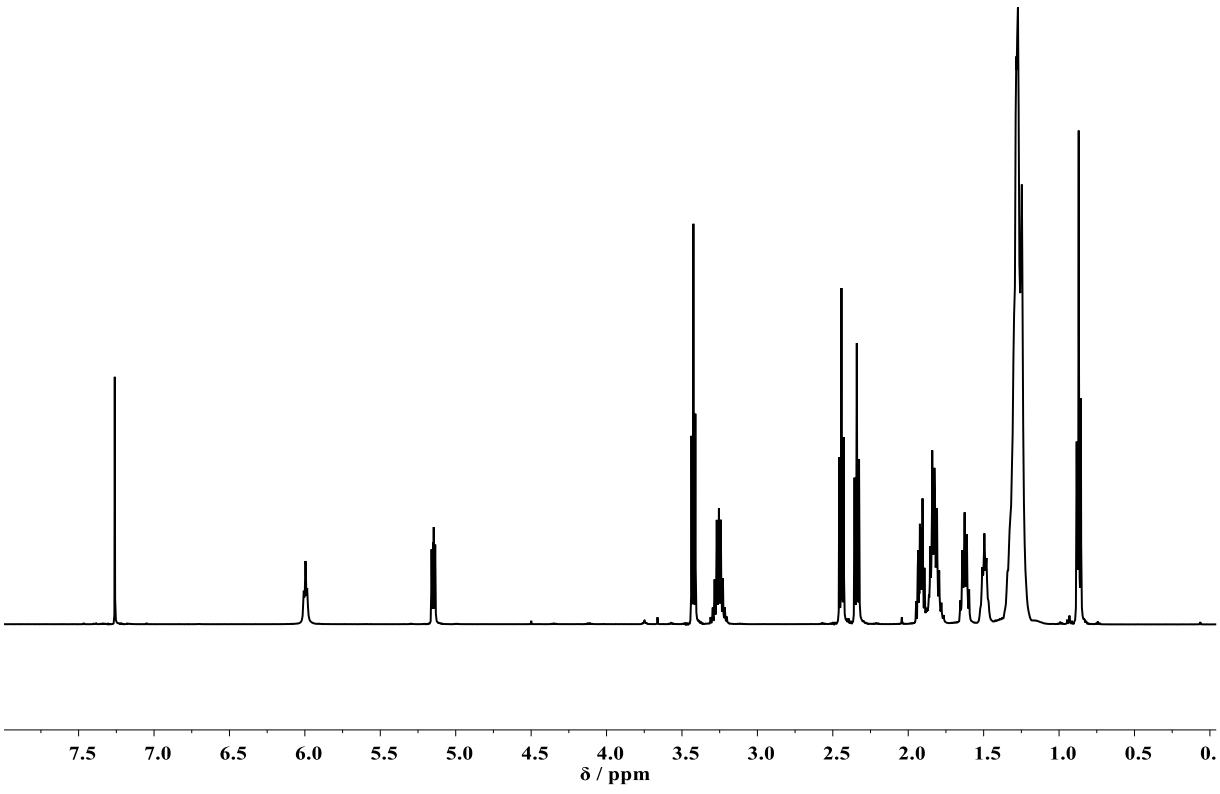
In a 50.0 mL round bottom flask 4.44 g of the Passerini product **M5/1** (7.02 mmol, 1.00 eq.) were dissolved in 7.00 mL ethyl acetate and 7.00 mL THF. Afterwards 444 mg (10wt%) palladium on activated carbon **1** were added. The mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent was removed under reduced pressure. The product **MD5/1** was obtained as a colorless solid in a yield of 96.9% (3.68 g, 6.88 mmol).

IR (ATR): ν / cm^{-1} = 3296.1 (w), 2919.6 (vs), 2849.8 (vs), 1737.2 (vs), 1692.5 (vs), 1651.3 (vs), 1555.8 (s), 1466.8 (m), 1431.3 (m), 1412.8 (m), 1378.0 (w), 1273.6 (s), 1244.0 (s), 1217.8 (s), 1163.9 (vs), 1077.5 (m), 925.0 (m), 799.8 (vw), 722.5 (m), 680.4 (m), 561.6 (w), 473.4 (vw).

^{1}H-NMR (500 MHz, $CDCl_3$): δ / ppm = 6.00 (t, *J* = 5.9 Hz, 1 H, NH^{1}), 5.28 – 5.04 (m, 1 H, CH^{2}), 3.42 (t, *J* = 6.4 Hz, 2 H, CH_2 ^{3}), 3.31 – 3.20 (m, 2 H, CH_2^{4}), 2.44 (t, *J* = 7.2 Hz, 2 H, CH_2^{5}), 2.34 (t, *J* = 7.5 Hz, 2 H, CH_2^{6}), 2.02 – 1.87 (m, 2 H, CH_2^{7}), 1.87 – 1.75 (m, 4 H, CH_2^{8}), 1.63 (p, *J* = 7.4 Hz, 2 H, CH_2^{9}), 1.54 – 1.45 (m, 2 H, CH_2^{10}), 1.39 – 1.16 (m, 24 H, CH_2^{11}), 0.87 (t, *J* = 6.9 Hz, 3 H, CH_3^{12}).

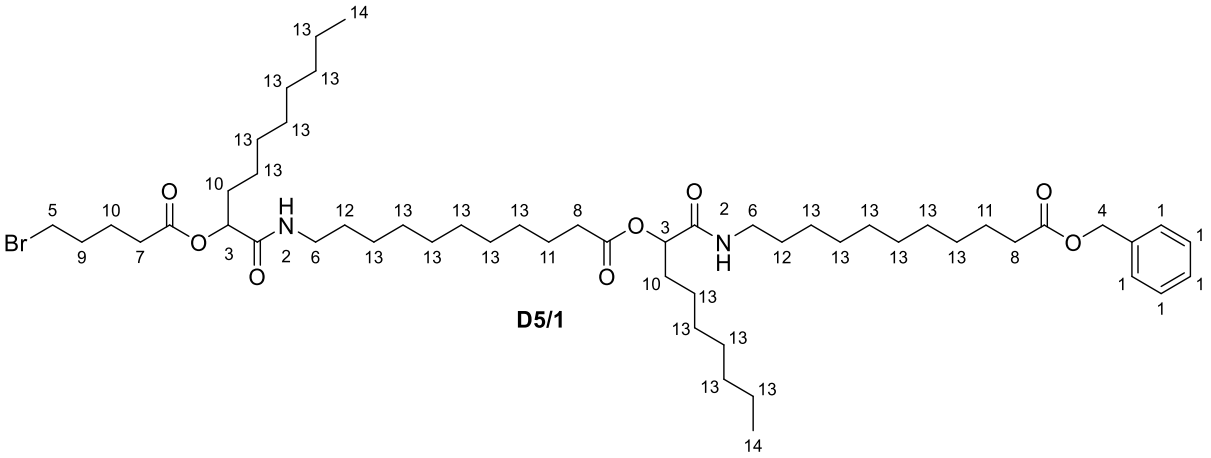
^{13}C-NMR (126 MHz, $CDCl_3$): δ / ppm = 178.87, 171.88, 169.79, 74.22, 39.26, 33.91, 33.30, 32.93, 31.90, 31.83, 29.50, 29.39, 29.35, 29.20, 29.12, 28.96, 26.78, 24.78, 23.47, 22.66, 14.12.

ESI-MS [*m/z*]: $[M+H]^+$ calculated for $^{12}C_{26}{}^{1}H_{48}{}^{16}O_5{}^{14}N{}^{79}Br$, 534.2789; found 534.2782, Δ = 0.7 mmu.



Supplementary Figure 176: ^{1}H-NMR of compound **MD5/1** measured in $CDCl_3$.

Passerini reaction



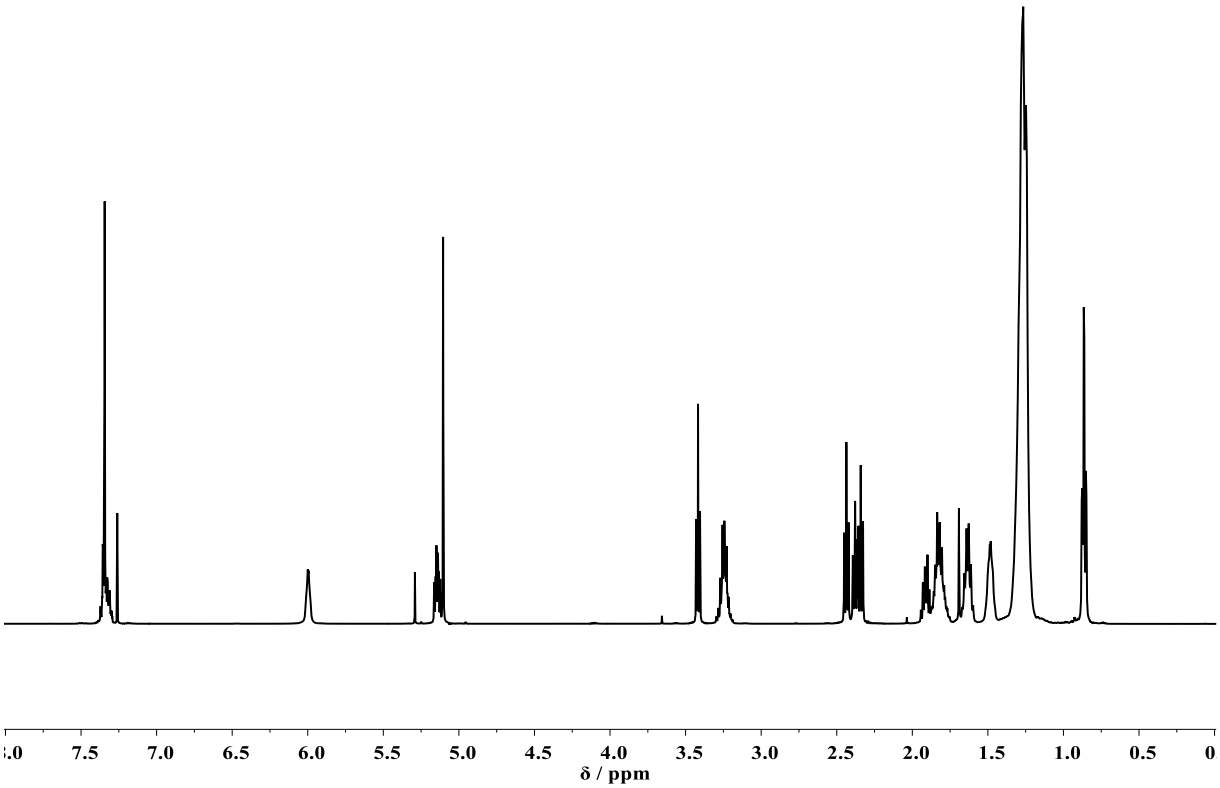
In a 50 mL round bottom flask 3.68 g of **MD5/1** (6.88 mmol, 1.00 eq.) were dissolved in 6.88 mL DCM (1 M) and 1.61 mL octanal **A8** (1.32 g, 10.3 mmol, 1.50 eq.) and 3.10 g of monomer **IM2** (10.3 mmol, 1.50 0eq.) were added. The mixture was stirred at room temperature for 1 day. Subsequently the solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane / ethyl acetate 6:1 → 4:1) to afford the Passerini product **D5/1** as a slight yellowish oil in a yield of 96.1% (6.39 g, 6.63 mmol).

IR (ATR): ν / cm^{-1} = 3304.9 (vw), 2923.0 (s), 2853.1 (m), 1737.7 (s), 1654.9 (s), 1535.9 (m), 1456.5 (w), 1376.4 (w), 1163.7 (s), 734.5 (w), 697.3 (w).

^{1}H-NMR (500 MHz, $CDCl_3$): δ / ppm = 7.38 – 7.29 (m, 5 H, CH_{Ar}^{1}), 6.05 – 5.95 (m, 2 H, NH^{2}), 5.17 – 5.12 (m, 2 H, CH^{3}), 5.10 (s, 2 H, CH_2^{4}), 3.42 (t, *J* = 6.5 Hz, 2 H, CH_2^{5}), 3.31 – 3.18 (m, 4 H, CH_2^{6}), 2.44 (t, *J* = 7.2 Hz, 2 H, CH_2^{7}), 2.41 – 2.28 (m, 4 H, CH_2^{8}), 1.98 – 1.87 (m, 2 H, CH_2^{9}), 1.87 – 1.74 (m, 6 H, CH_2^{10}), 1.68 – 1.57 (m, 4 H, CH_2^{11}), 1.54 – 1.42 (m, 4 H, CH_2^{12}), 1.40 – 1.14 (m, 46 H, CH_2^{13}), 0.90 – 0.82 (m, 6 H, CH_3^{14}).

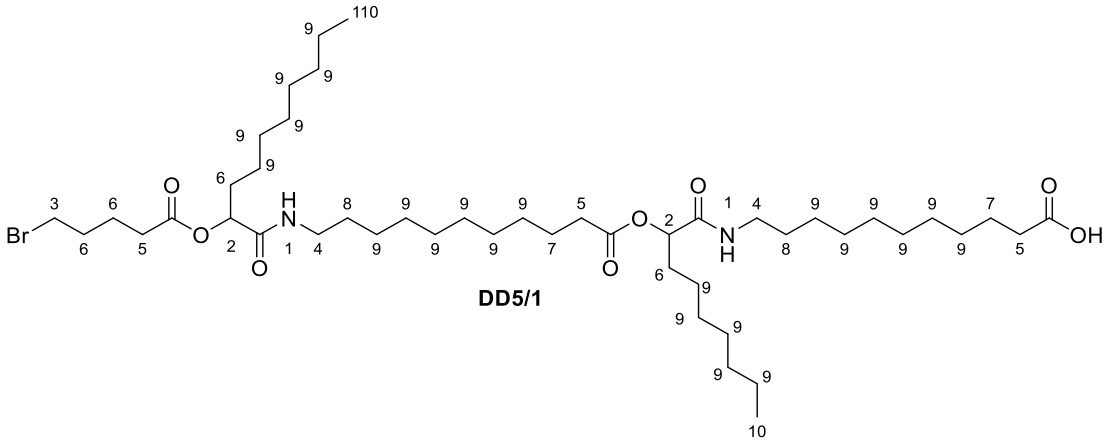
^{13}C-NMR (126 MHz, $CDCl_3$): δ / ppm = 173.70, 172.46, 171.84, 169.85, 169.69, 136.13, 128.55, 128.18, 128.16, 74.22, 73.94, 66.08, 39.23, 39.20, 34.33, 33.29, 32.93, 31.92, 31.83, 31.74, 29.58, 29.57, 29.48, 29.46, 29.39, 29.37, 29.26, 29.24, 29.22, 29.19, 29.12, 26.84, 24.97, 24.95, 24.80, 24.76, 23.47, 22.65, 22.63, 14.12, 14.09.

ESI-MS [*m/z*]: $[M+H]^+$ calculated for $^{12}C_{53}{}^{1}H_{91}{}^{16}O_8{}^{14}N_2{}^{79}Br$, 963.6032; found 963.6019, Δ = 1.3 mmu.



Supplementary Figure 177: ^{1}H-NMR of compound **D5/1** measured in $CDCl_3$.

Deprotection



In a 50 mL round bottom flask, 4.00 g (4.15 mmol, 1.00 eq.) of the passerine product **D5/1** were dissolved in 4.15 mL ethyl acetate and 4.15 mL MeOH. Afterwards 400 mg (10 wt%) palladium on activated carbon **1** were added. The mixture was purged with hydrogen (balloon) and stirred under hydrogen atmosphere overnight. The catalyst was filtered off and the solvent was removed under reduced pressure. The process was repeated with ethyl acetate and THF as solvent to afford the product in a 3:1 ratio of hydrogenated compound and product **DD5/1** as a

colorless oil in a yield of 3.64 g. Excluding the side product by calculations leads to 2.72 g of hydrogenated product (107) (3.11 mmol, 75.0%).

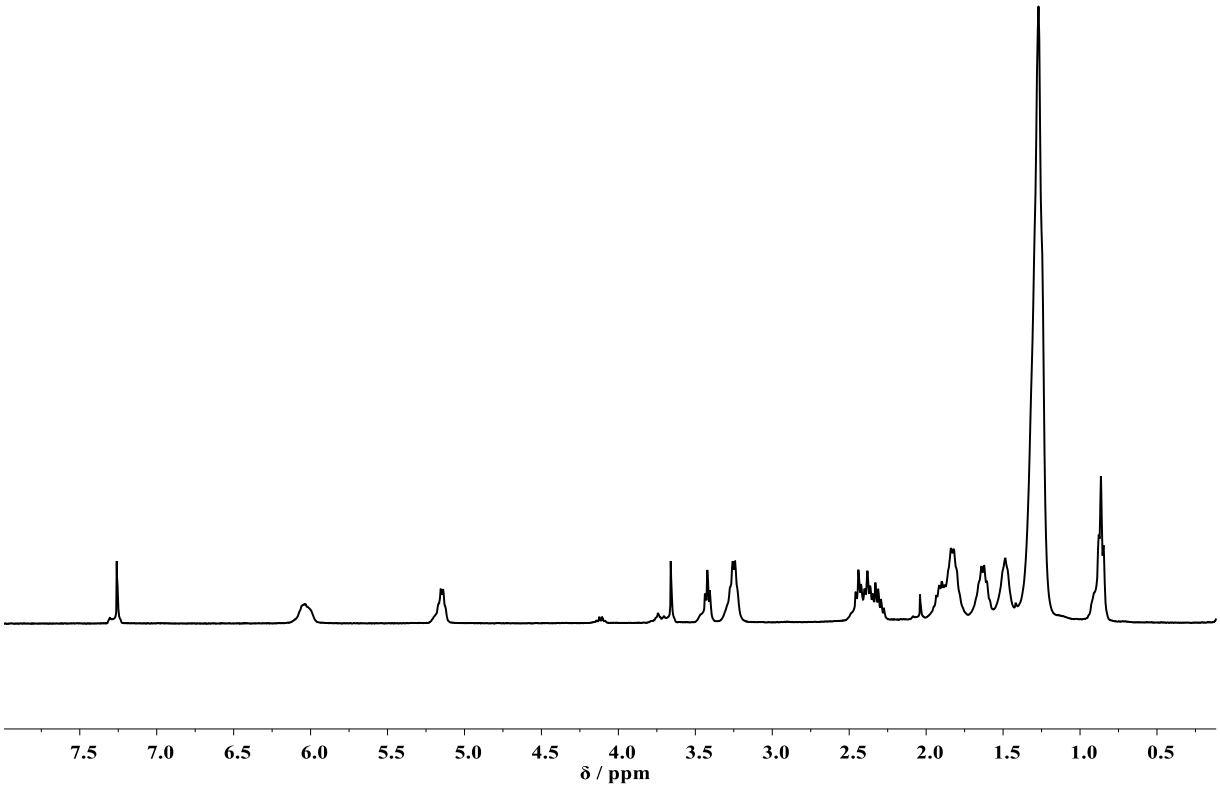
Impurity of the methyl ester is visible in the NMR. In the ^{1}H-NMR 3.66ppm in the ^{13}C-NMR 51.46 ppm.

IR (ATR): ν / cm^{-1} = 3293.8 (vw), 2922.8 (vs), 2853.1 (s), 1738.6 (s), 1652.8 (s), 1538.7 (m), 1457.8 (w), 1374.9 (w), 1163.4 (s), 721.8 (w).

^{1}H-NMR (400 MHz, $CDCl_3$): δ / ppm = 6.09 – 5.97 (m, 2 H, NH^1), 5.19 – 5.11 (m, 2 H, CH^2), 3.42 (t, *J* = 6.4 Hz, 2 H, CH_2^3), 3.29 – 3.20 (m, 4 H, CH_2^4), 2.47 – 2.25 (m, 6 H, CH_2^5), 1.97 – 1.73 (m, 8 H, CH_2^6), 1.69 – 1.57 (m, 4 H, CH_2^7), 1.53 – 1.44 (m, 4 H, CH_2^8), 1.38 – 1.07 (m, 46 H, CH_2^9), 0.91 – 0.82 (m, 6 H, CH_3^{10}).

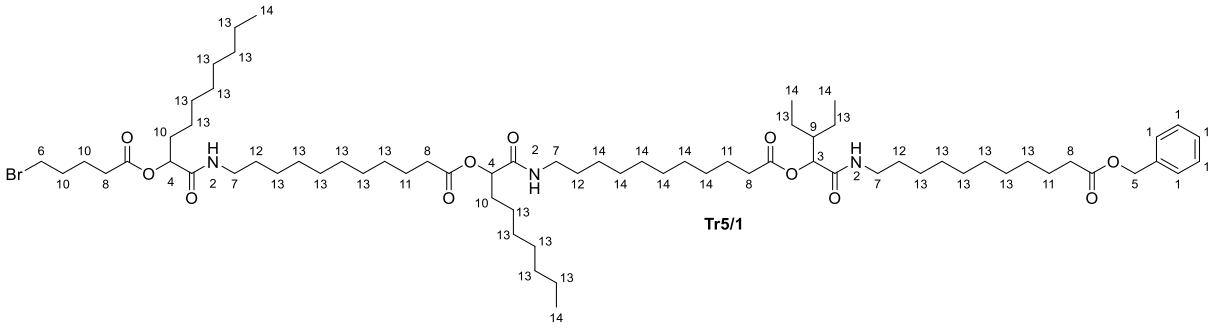
^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 176.70, 172.50, 171.96, 169.95, 169.89, 74.23, 73.98, 39.32, 39.21, 39.17, 34.36, 34.11, 33.69, 33.30, 32.91, 31.87, 31.83, 31.74, 29.55, 29.49, 29.38, 29.36, 29.26, 29.22, 29.19, 29.17, 29.14, 29.10, 29.05, 28.92, 26.84, 26.74, 25.01, 24.95, 24.79, 24.73, 23.46, 22.65, 22.62, 14.10, 14.08.

ESI-MS [*m/z*]: $[M+H]^+$ calculated for $^{12}C_{46}{}^{1}H_{85}{}^{16}O_8{}^{14}N_2{}^{79}Br$ 873.5562, found 873.5550; Δ = 1.2 mmu.



Supplementary Figure 178: ^{1}H-NMR of compound **DD5/1** measured in $CDCl_3$.

Passerini reaction



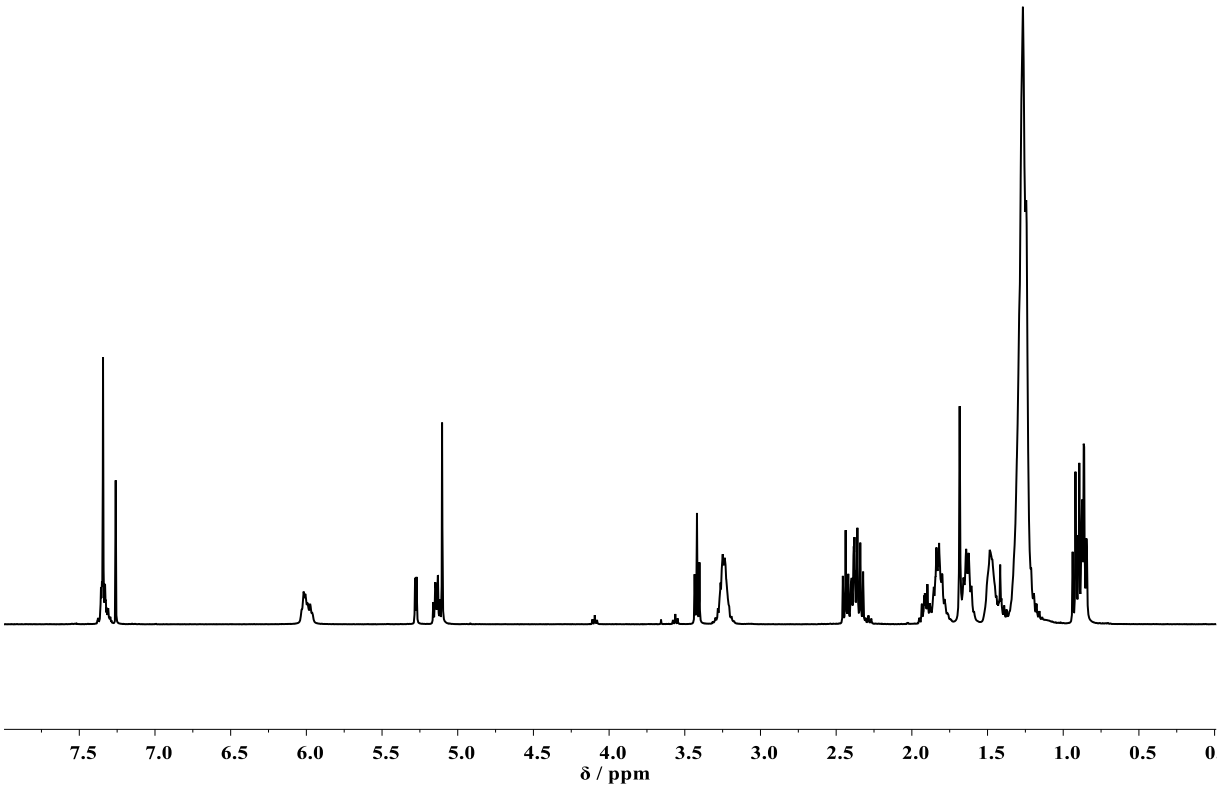
In a 50 mL round bottom flask 3.47 g of **DD5/1** (3.97 mmol, 1.00 eq.) was dissolved in 3.97 mL DCM and 732 µL 2–ethylbutyraldehyde **A5** (596 mg, 5.95 mmol, 1.50 eq.) and 1.79 g of monomer **IM2** (5.95 mmol, 1.50 eq.) were added. The mixture was then stirred at room temperature for 1 day. Subsequently the solvent was removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane / ethyl acetate, first column 6:1 → 1:1) to afford the Passerini product **Tr5/1** in a yield of 9.01% (358 mg, 280 µmol).

IR (ATR): ν / cm^{-1} = 3305.6 (vw), 2923.3 (vs), 2853.2 (m), 1738.0 (s), 1652.7 (s), 1534.4 (m), 1457.3 (m), 1376.2 (w), 1160.7 (s), 722.8 (w), 697.1 (w).

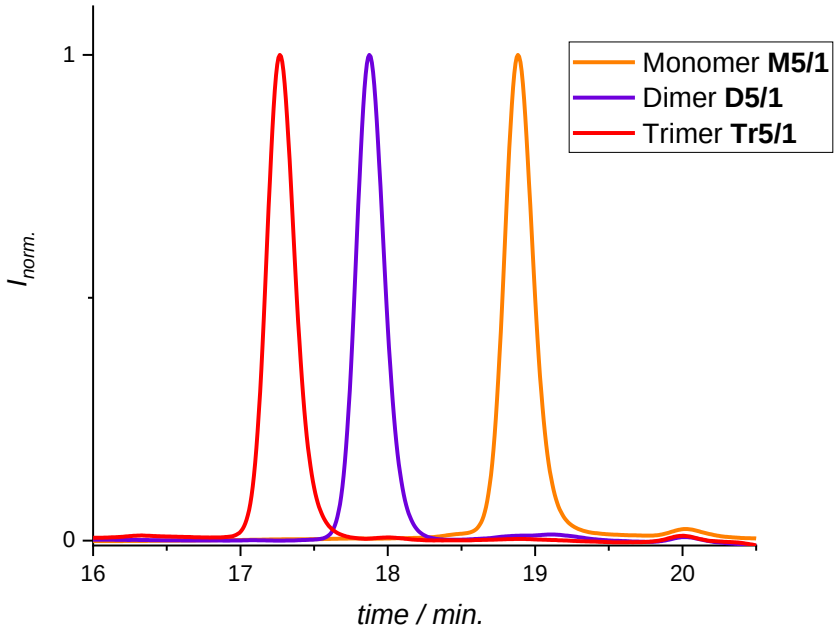
^{1}H-NMR (400 MHz, $CDCl_3$): δ / ppm = 7.40 – 7.27 (m, 5 H, CH_{Ar}^{1}), 6.05 – 5.94 (m, 3 H, NH^{2}), 5.28 (d, *J* = 3.9 Hz, 1 H, CH^{3}), 5.20 – 5.12 (m, 2 H, CH^{4}), 5.10 (s, 2 H, CH_2^{5}), 3.42 (t, *J* = 6.3 Hz, 2 H, CH_2^{6}), 3.32 – 3.15 (m, 6 H, CH_2^{7}), 2.48 – 2.31 (m, 8 H, CH_2^{8}), 1.96 – 1.73 (m, 9 H, CH^{9}, CH_2^{10}), 1.72 – 1.57 (m, 6 H, CH_2^{11}), 1.54 – 1.38 (m, 6 H, CH_2^{12}), 1.38 – 1.07 (m, 62 H, CH_2^{13}), 0.97 – 0.82 (m, 12 H, CH_3^{14}).

^{13}C-NMR (101 MHz, $CDCl_3$): δ / ppm = 173.71, 172.49, 171.86, 169.88, 169.74, 169.71, 136.11, 128.54, 128.16, 75.00, 74.21, 73.93, 66.07, 43.50, 39.23, 39.21, 39.19, 34.34, 34.32, 33.33, 31.91, 31.82, 31.73, 29.57, 29.54, 29.47, 29.45, 29.38, 29.35, 29.25, 29.21, 29.19, 29.13, 29.11, 26.91, 26.85, 26.83, 25.01, 24.96, 24.94, 24.79, 24.77, 23.46, 22.65, 22.62, 21.90, 14.11, 14.09, 11.62, 11.57.

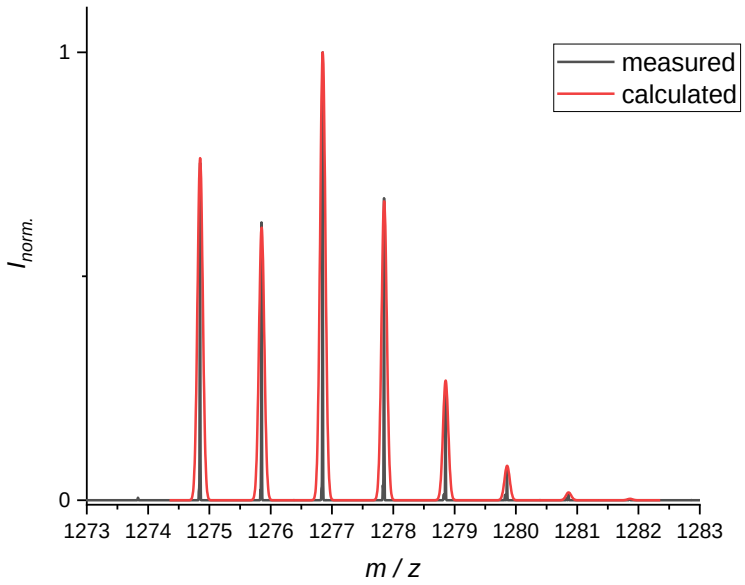
ESI-MS [*m/z*]: $[M+H]^+$ calculated for $^{12}C_{71}{}^{1}H_{124}{}^{16}O_{11}{}^{14}N_3{}^{79}Br$, 1274.8492; found 1274.8477, Δ = 1.5 mmu.



Supplementary Figure 179: ^{1}H-NMR of compound **Tr5/1** measured in $CDCl_3$.



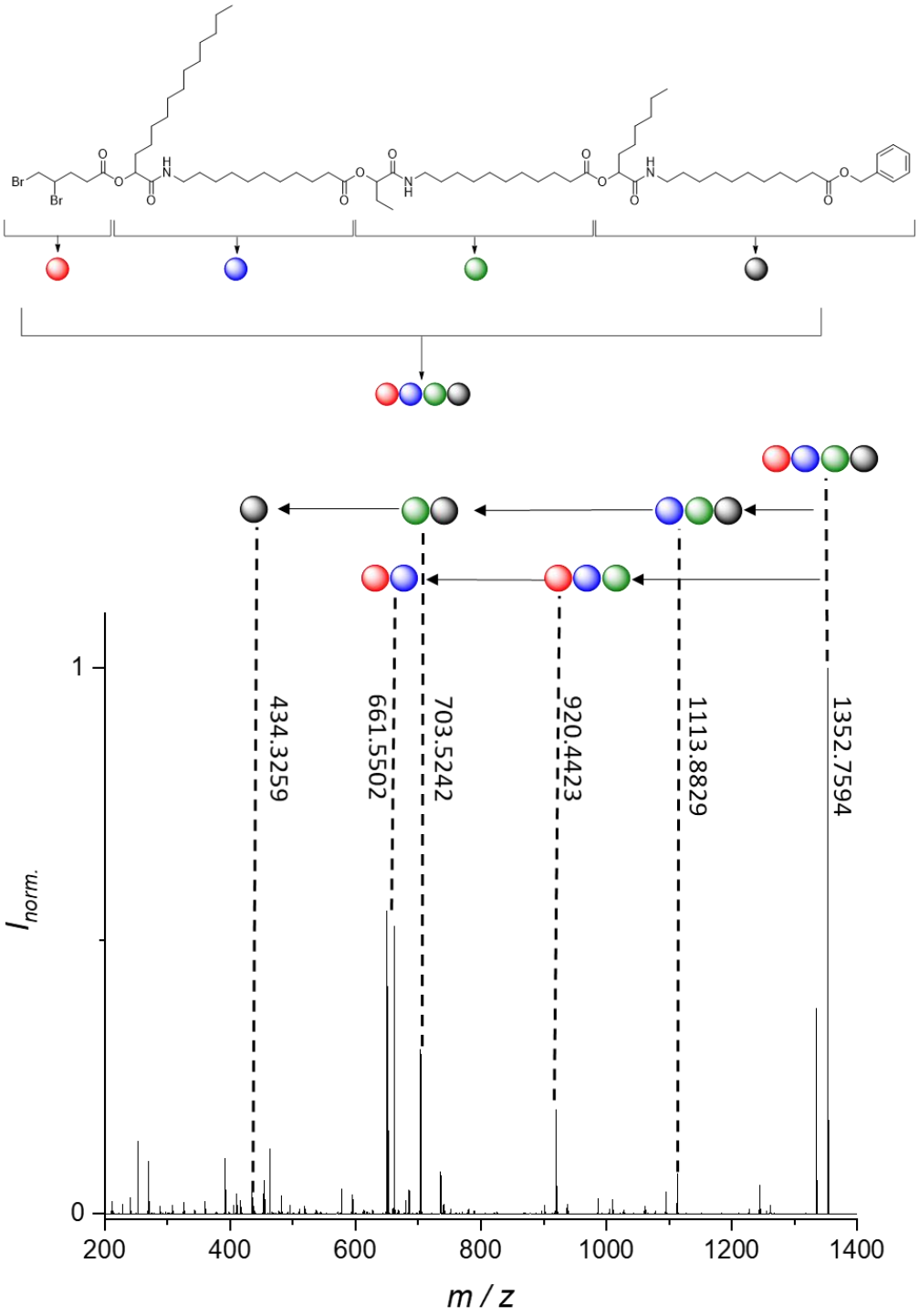
Supplementary Figure 180: SEC traces of the intermediates after each P-3CR in the synthesis of product **Tr5/1**.



Supplementary Figure 181: High resolution ESI-MS measurement of **Tr5/1**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (red).

```
matching mass 1353.76118
cutoff 0.50000: 0 solutions (8 peaks)
cutoff 0.25000: 0 solutions (28 peaks)
cutoff 0.12500: 0 solutions (53 peaks)
cutoff 0.06250: 1 solutions (93 peaks)
1353.76118 ≈ 240.886370 + 409.355600 + 269.199100 + 325.261700 + 107.049690 (sides Tridecanal, Propionaldehyde, Heptanal;
error -2.00872)
Press ENTER to quit ...
```

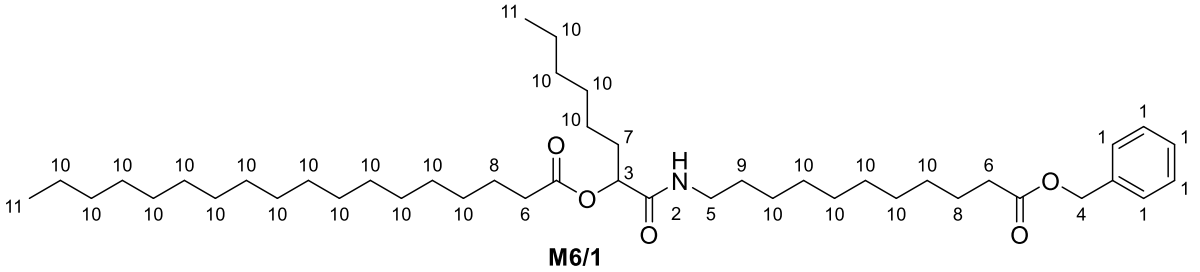
Supplementary Figure 182: Screenshot of the automated read-out of **Tr5/1**.



Supplementary Figure 183: Read-out of the sequence-defined hexamer **Tr5/1**. Read-out of the hexamer **Tr5/1** *via* tandem ESI-MS/MS with an NCE of 17. In the spectrum, the read-out from both ends of the oligomer using the fragmentation next to the carbonyl are shown.

6.3.4.4 Synthesis of Trimer Tr6/1 with TAG 6

Passerini reaction



M6/1

In a 50 mL round bottom flask 1.50 g stearic acid **TAG6** (5.27 mmol, 1.00 eq.) were stirred in 5.27 mL DCM, subsequently 841 µL heptanal **A7** (809 mg, 7.08 mmol, 1.50 eq.) and 2.38 g of monomer **IM2** (7.08 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 1 day. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (11:1 → 9:1) to yield the Passerini product **M6/1** as a pale highly viscous oil. (3.18 g, 4.54 mmol, 86.2%).

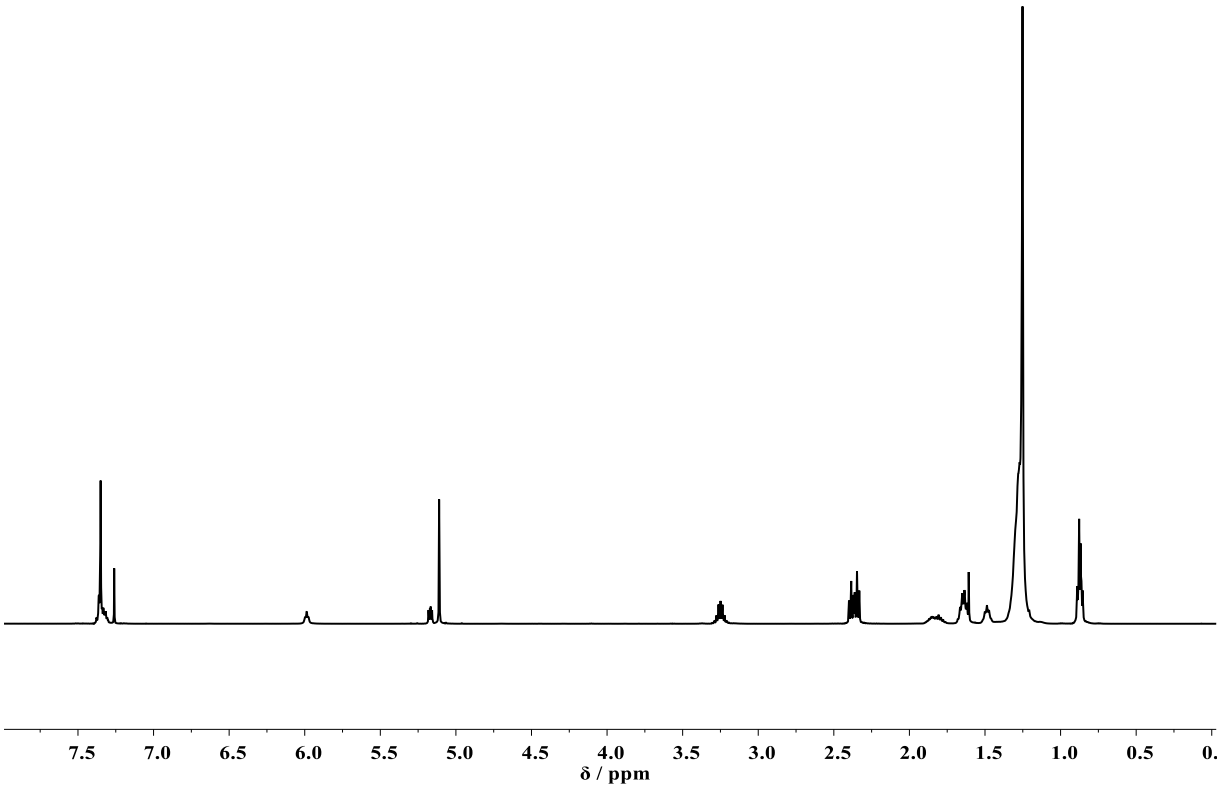
R_f = 0.30 in cyclohexane/ethyl acetate (5:1).

IR (ATR): ν / cm^{-1} = 3247.8 (vw), 2916.7 (vs), 2849. (vs), 1731.9 (vs), 1652.2 (s), 1571.2 (w), 1466.6 (m), 1416.9 (w), 1362.2 (m), 1287.6 (w), 1263.6 (m), 1241.1 (m), 1207.4 (m), 1160.1 (vs), 1113.4 (m), 966.5 (w), 813.4 (vw), 740.9 (m), 722.0 (s), 696.6 (s), 581.1 (vw), 475.0 (vw).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.42 – 7.29 (m, 5 H, $CH_{Ar}{}^1$), 5.99 (t, J = 5.9 Hz, 1 H, NH^2), 5.22 – 5.15 (m, 1 H, CH^3), 5.11 (s, 2 H, $CH_2{}^4$), 3.33 – 3.16 (m, 2 H, $CH_2{}^5$), 2.43 – 2.30 (m, 4 H, $CH_2{}^6$), 1.93 – 1.72 (m, 2 H, $CH_2{}^7$), 1.71 – 1.57 (m, 4 H, $CH_2{}^8$), 1.53 – 1.44 (m, 2 H, $CH_2{}^9$), 1.38 – 1.13 (m, 48 H, $CH_2{}^{10}$), 0.94 – 0.83 (m, 6 H, $CH_3{}^{11}$).

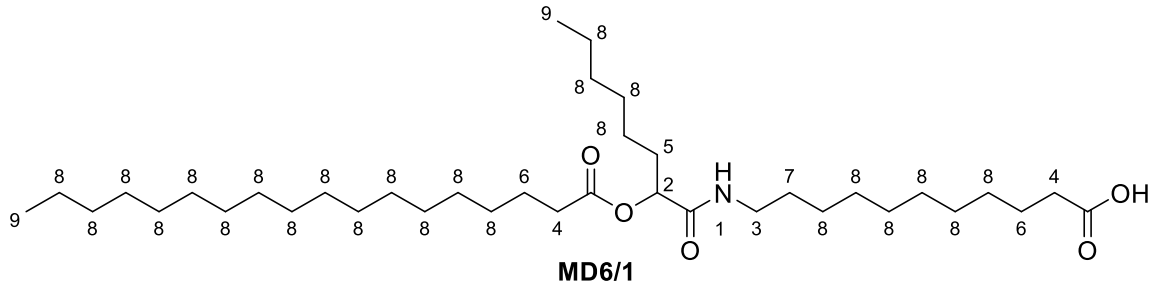
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 173.81, 172.59, 169.97, 136.27, 128.68, 128.30, 74.05, 66.20, 39.32, 34.50, 34.45, 32.06, 32.04, 31.76, 29.84, 29.82, 29.80, 29.76, 29.69, 29.63, 29.59, 29.50, 29.43, 29.36, 29.28, 29.25, 29.05, 26.97, 25.15, 25.07, 24.83, 22.83, 22.67, 14.27, 14.18.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{44}{}^1H_{77}{}^{16}O_5{}^{14}N$, 700.5875; found, 700.5872, Δ = 0.3 mmu.



Supplementary Figure 184: ^{1}H-NMR of compound **M6/1** measured in $CDCl_3$.

Deprotection



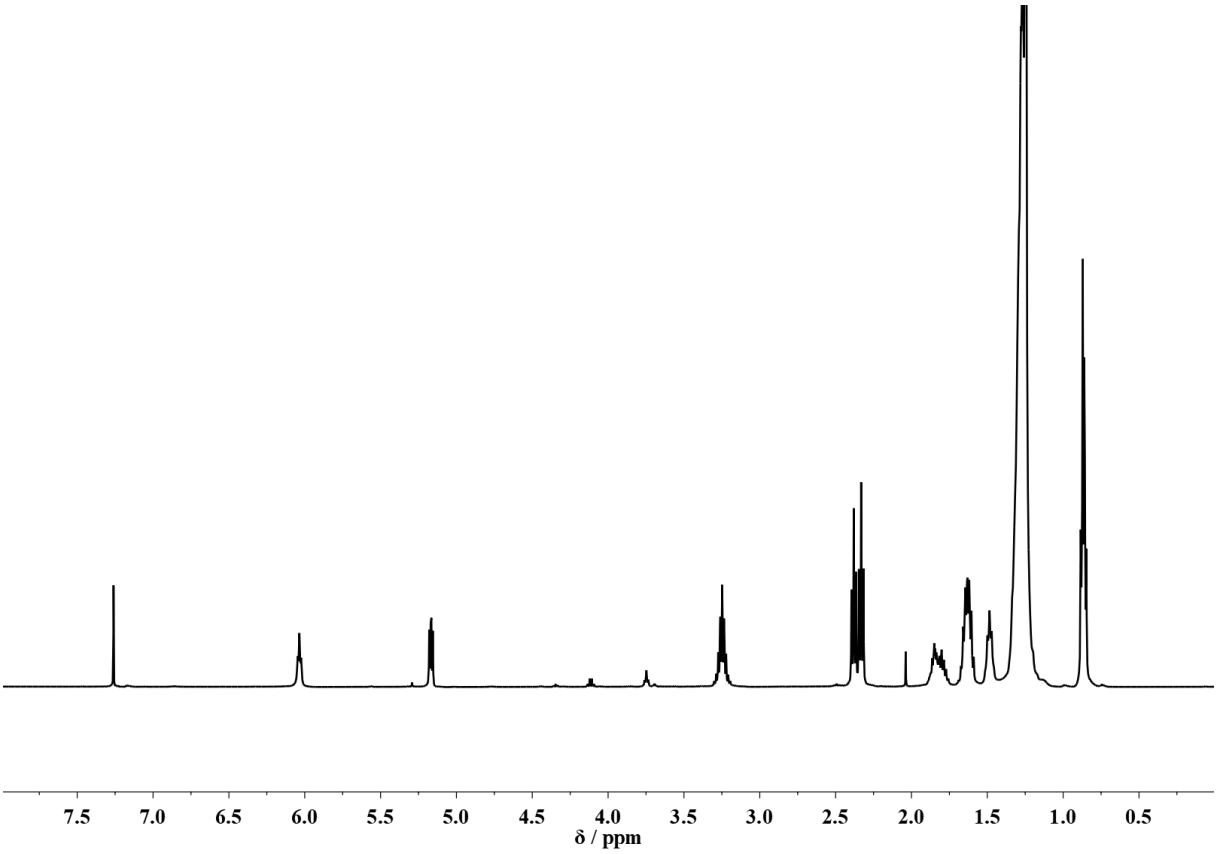
In a 25 mL round bottom flask 1.49 g of **M6/1** (2.13 mmol, 1.00 eq.) was dissolved in 5.00 mL ethyl acetate and 5.00 mL THF. Subsequently, 298 mg (20 wt%) palladium on activated carbon **1** were added to the solution. The resulting mixture was purged with hydrogen gas (3 balloons) and stirred for one day at room temperature under hydrogen atmosphere. The crude reaction mixture was filtered over celite® and flushed with 50 mL dichloromethane. After evaporation of the solvents and drying under reduced pressure the product **MD6/1** was obtained as a colorless solid (1.21 g, 1.97 mmol, 92.8%).

IR (ATR): ν / cm^{-1} = 3297.9 (w), 2915.6 (vs), 2848.8 (vs), 1737.0 (s), 1701.4 (vs), 1645.5 (vs), 1560.2 (m), 1466.4 (s), 1415.0 (w), 1376.2 (w), 1306.4 (w), 1244.3 (m), 1220.8 (m), 1194.6 (m), 1173.7 (vs), 1114.2 (w), 929.4 (w), 722.6 (m), 682.8 (w), 451.9 (vw).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 6.06 (t, J = 5.9 Hz, 1 H, NH^{1}), 5.22 – 5.15 (m, 1 H, CH^{2}), 3.36 – 3.19 (m, 2 H, CH_2^{3}), 2.41 (t, J = 7.5 Hz, 2 H, CH_2^{4}), 2.36 (t, J = 7.5 Hz, 2 H, CH_2^{4}), 1.94 – 1.77 (m, 2 H, CH_2^{5}), 1.72 – 1.60 (m, 4 H, CH_2^{6}), 1.54 – 1.47 (m, 2 H, CH^{7}), 1.39 – 1.20 (m, 48 H, CH_2^{8}), 0.98 – 0.83 (m, 6 H, CH_3^{9}).

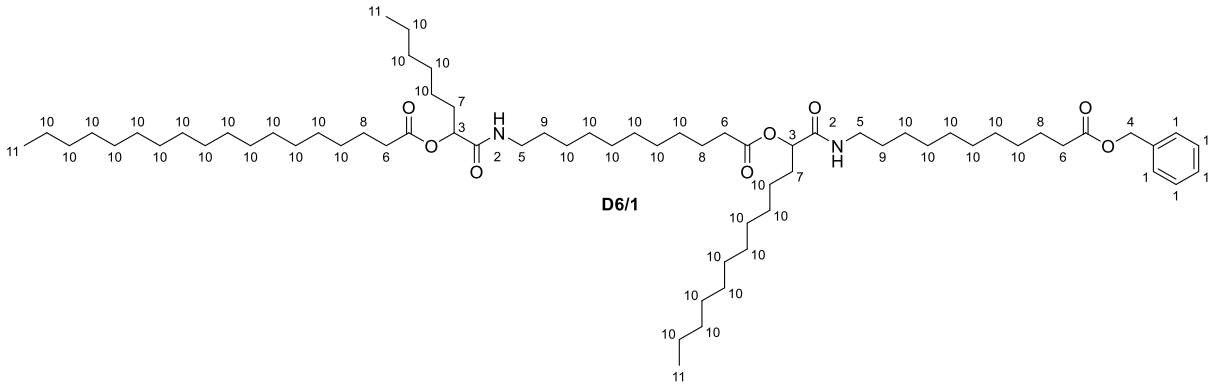
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 179.28, 172.63, 170.11, 74.03, 39.34, 34.48, 34.11, 32.05, 32.01, 31.75, 29.83, 29.81, 29.79, 29.75, 29.62, 29.50, 29.42, 29.40, 29.27, 29.12, 29.04, 26.92, 25.14, 24.81, 24.81, 22.82, 22.66, 14.25, 14.17.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{37}{}^{1}H_{71}{}^{16}O_5{}^{14}N$, 610.5405; found, 610.5401, Δ = 0.4 mmu.



Supplementary Figure 185: ^{1}H-NMR of compound **MD6/1** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask 2.26 g **MD6/1** (3.70 mmol, 1.00 eq.) were stirred in 3.70 mL DCM, subsequently 1.04 g dodecanal **A10** (5.55 mmol, 1.50 eq.) and 1.68 g of monomer **IM2** (5.55 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 1 day. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (11:1 → 9:1) to yield the Passerini product **D6/1** as a pale highly viscous oil. (2.73 g, 2.49 mmol, 67.4%).

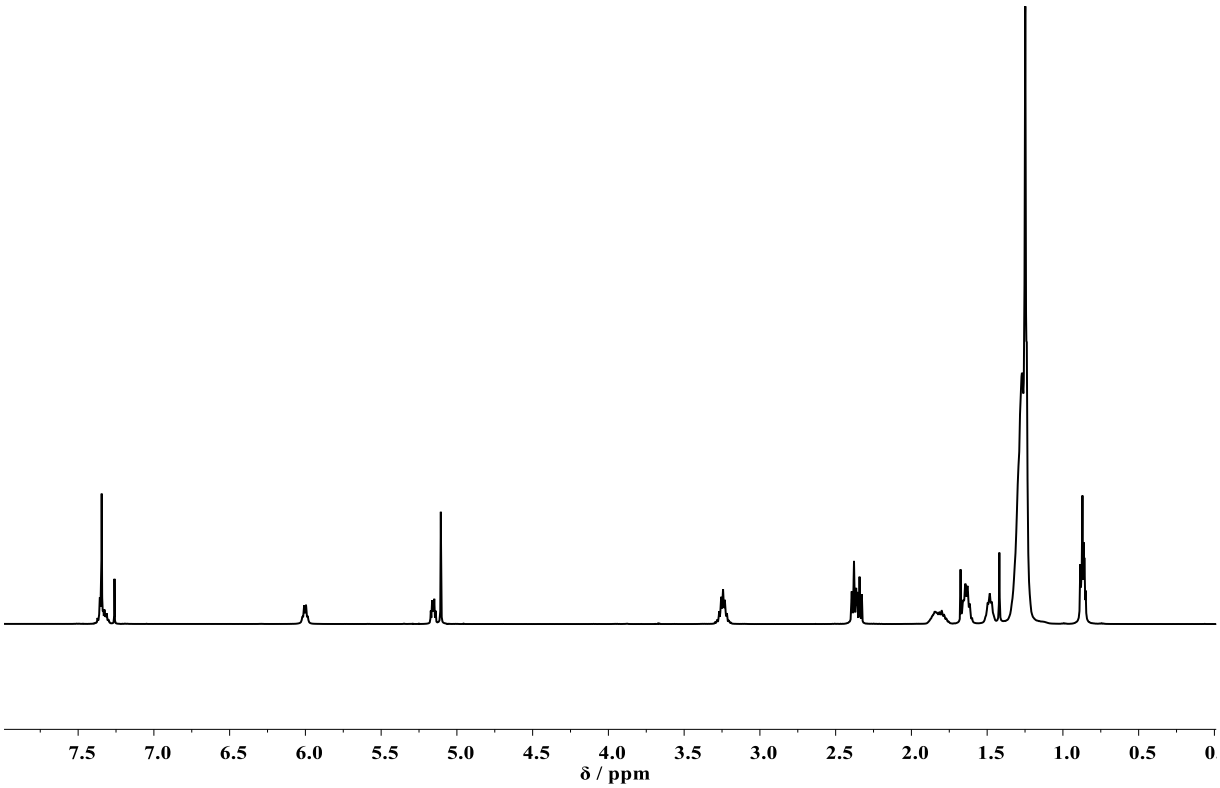
R_f = 0.43 in cyclohexane/ethyl acetate (4:1).

IR (ATR): ν / cm^{-1} = 3295.4 (vw), 3092.3 (vw), 2917.3 (vs), 2849.7 (vs), 1736.7 (vs), 1653.9 (vs), 1559.4 (m), 1466.7 (m), 1379.0 (w), 1240.5 (m), 1207.5 (m), 1165.7 (vs), 1113.1 (m), 721.7 (m), 694.7 (m), 474.7 (vw).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.39 – 7.28 (m, 5 H, $CH_{Ar}{}^1$), 6.05 – 5.94 (m, 2 H, NH^2), 5.20 – 5.12 (m, 2 H, CH^3), 5.12 (s, 2 H, $CH_2{}^4$), 3.32 – 3.19 (m, 4 H, $CH_2{}^5$), 2.43 – 2.30 (m, 6 H, $CH_2{}^6$), 1.91 – 1.74 (m, 4 H, $CH_2{}^7$), 1.67 – 1.58 (m, 6 H, $CH_2{}^8$), 1.54 – 1.44 (m, 4 H, $CH_2{}^9$), 1.36 – 1.19 (m, 78 H, $CH_2{}^{10}$), 0.91 – 0.83 (m, 9 H, $CH_3{}^{11}$).

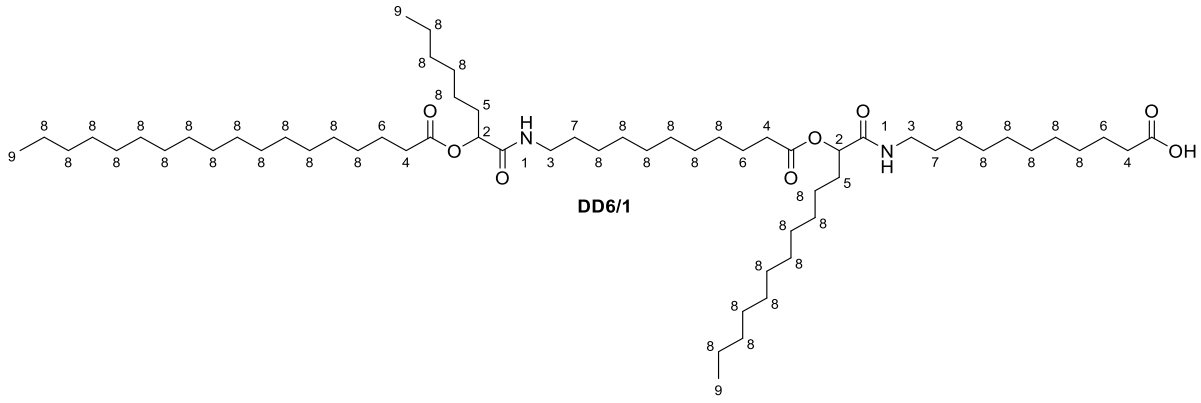
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 173.80, 172.59, 172.56, 169.98, 169.95, 136.25, 128.66, 128.28, 74.06, 74.03, 66.19, 39.31, 39.30, 34.48, 34.44, 32.05, 32.04, 31.75, 29.82, 29.78, 29.75, 29.70, 29.68, 29.67, 29.61, 29.60, 29.57, 29.51, 29.48, 29.42, 29.38, 29.37, 29.34, 29.27, 29.24, 29.04, 27.04, 26.96, 25.13, 25.09, 25.06, 24.88, 24.83, 22.82, 22.66, 14.25, 14.17.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{68}{}^1H_{122}{}^{16}O_8{}^{14}N_2$, 1095.9274; found, 1095.9263, Δ = 1.1 mmu.



Supplementary Figure 186: ^{1}H-NMR of compound **D6/1** measured in $CDCl_3$.

Deprotection



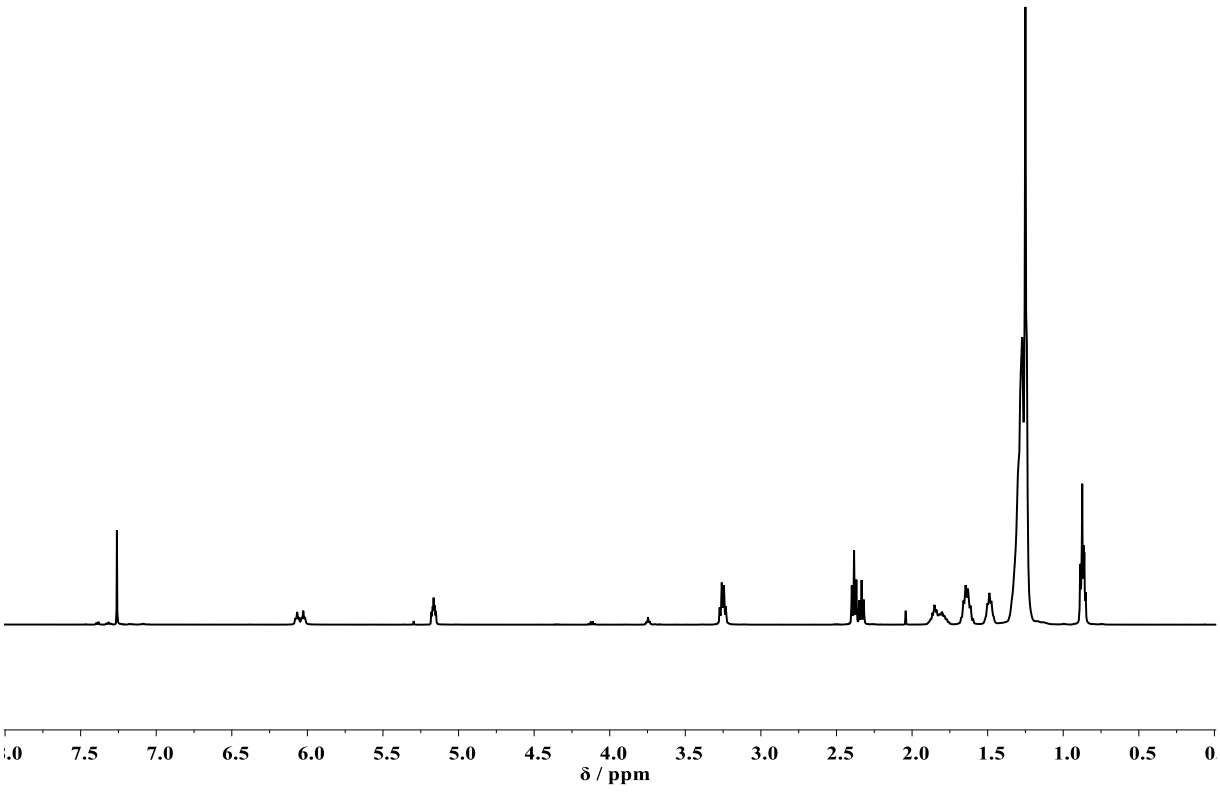
In a 25 mL round bottom flask 2.59 g of **D6/1** (2.36 mmol, 1.00 eq.) was dissolved in 7.00 mL ethyl acetate and 7.00 mL THF. Subsequently, 518 mg (20 wt%) palladium on activated carbon **1** were added to the solution. The resulting mixture was purged with hydrogen gas (3 balloons) and stirred for one day at room temperature under hydrogen atmosphere. The crude reaction mixture was filtered over celite® and flushed with 50 mL dichloromethane. After evaporation of the solvents and drying under reduced pressure the product **DD6/1** was obtained as a colorless solid (2.30 g, 2.29 mmol, 96.7%).

IR (ATR): ν / cm^{-1} = 3294.0 (vw), 2918. (vs), 2850.2 (vs), 1737.7 (s), 1655.3 (vs), 1547.3 (m), 1466.1 (m), 1376.9 (w), 1242.0 (m), 1170.3 (s), 1113.5 (w), 929.5 (vw), 721.1 (m).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 6.09 (t, J = 5.9 Hz, 1 H, NH^1), 6.05 (t, J = 5.9 Hz, 1 H, NH^1), 5.24 – 5.15 (m, 2 H, CH_2^2), 3.33 – 3.20 (m, 4 H, CH_2^3), 2.41 (t, J = 7.5 Hz, 4 H, CH_2^4), 2.36 (t, J = 7.5 Hz, 2 H, CH_2^4), 1.94 – 1.77 (m, 4 H, CH_2^5), 1.71 – 1.61 (m, 6 H, CH_2^6), 1.55 – 1.46 (m, 4 H, CH_2^7), 1.40 – 1.21 (m, 78 H, CH_2^8), 0.94 – 0.86 (m, 9 H, CH_2^9).

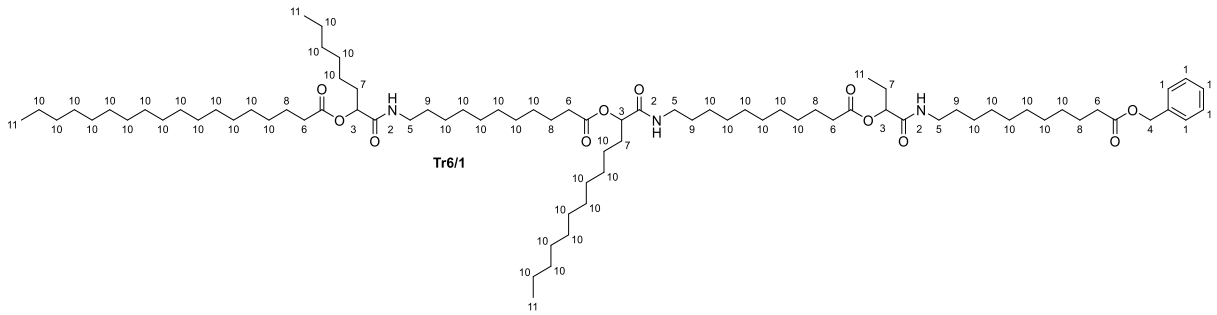
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 172.71, 172.62, 170.20, 170.08, 74.09, 74.04, 39.39, 39.31, 34.49, 34.48, 33.96, 32.06, 32.01, 31.76, 29.84, 29.83, 29.80, 29.76, 29.68, 29.63, 29.58, 29.54, 29.50, 29.49, 29.44, 29.43, 29.40, 29.35, 29.28, 29.22, 29.09, 29.04, 26.97, 26.89, 25.13, 24.90, 24.85, 24.84, 22.83, 22.67, 14.27, 14.18.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{61}{}^{1}H_{116}{}^{16}O_8{}^{14}N_2$, 1005.8804; found, 1005.8795, Δ = 0.9 mmu.



Supplementary Figure 187: ^{1}H-NMR of compound **DD6/1** measured in $CDCl_3$.

Passerini reaction



In a 50 mL round bottom flask 857 mg of **DD6/1** (852 µmol, 1.00 eq.) were stirred in 2.00 mL DCM, subsequently 1.83 µL propionaldehyde **A2** (2.56 mmol, 1.50 eq.) and 385 mg of monomer **IM2** (2.56 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 1 day. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (7:1 → 2:1) to yield the Passerini product **Tr6/1** as a pale highly viscous oil (1.06 g, 775 µmol, 91.0%).

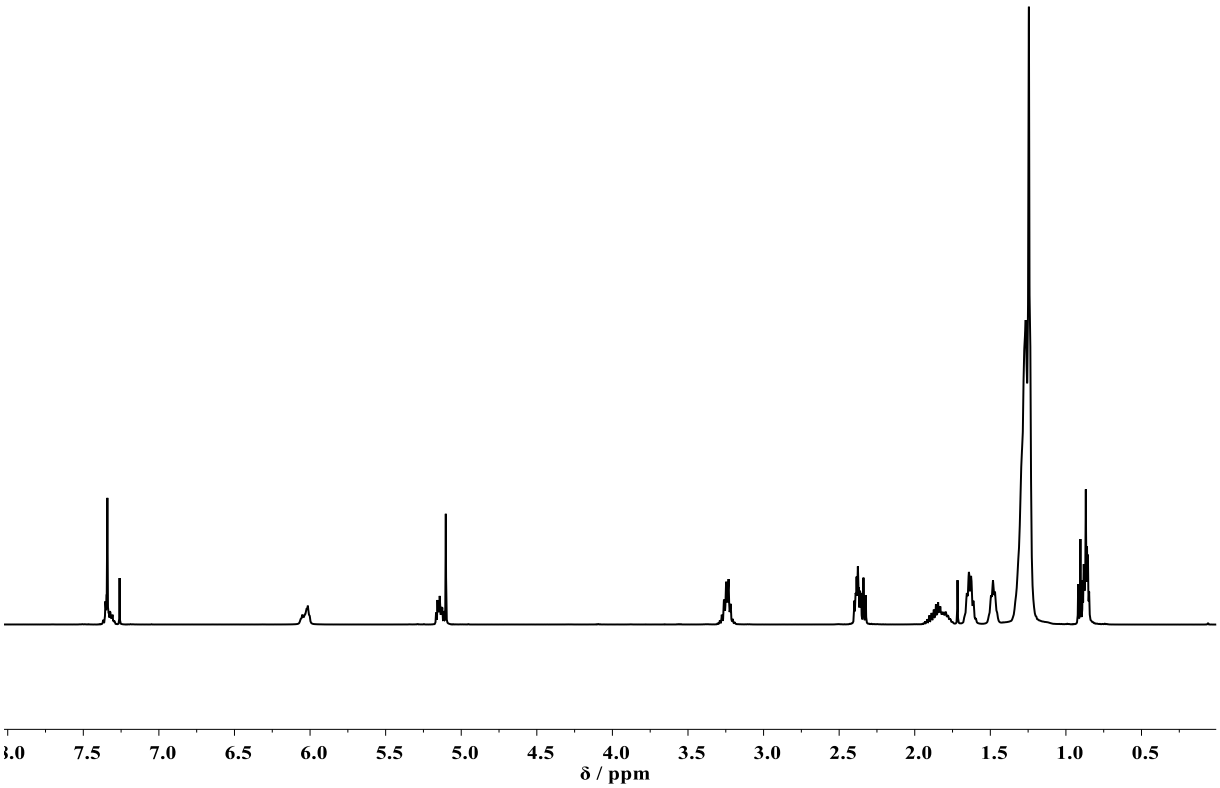
R_f = 0.43 in cyclohexane/ethyl acetate (4:1).

IR (ATR): ν / cm^{-1} = 3290.5 (w), 3093.2 (vw), 2918.6 (vs), 2850.2 (vs), 1738.1 (vs), 1654.5 (s), 1558.4 (m), 1466.2 (m), 1377.9 (w), 1239.4 (m), 1207.5 (m), 1164.7 (s), 1111.8 (m), 721.5 (m), 695.9 (m), 474.4 (vw).

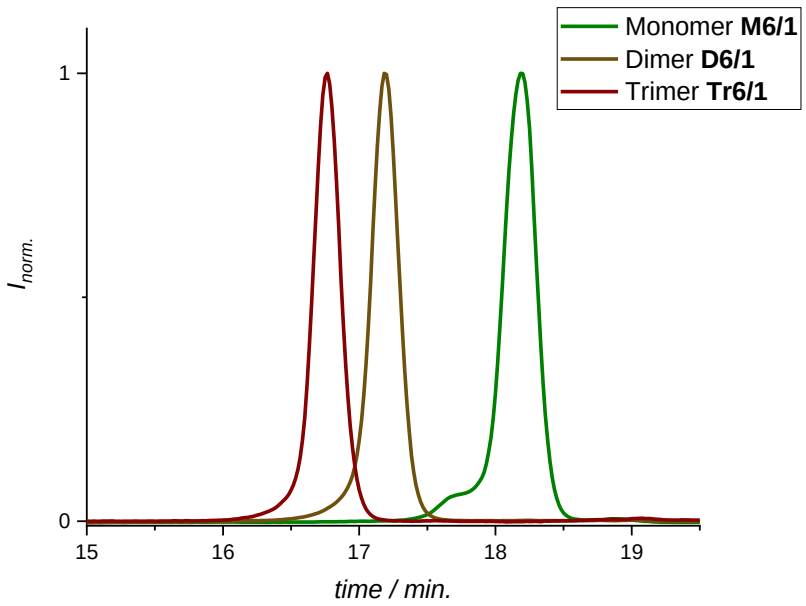
^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.44 – 7.31 (m, 5 H, CH_{Ar}^1), 6.11 – 5.99 (m, 3 H, NH^2), 5.20 – 5.12 (m, 3 H, CH^3), 5.13 (s, 2 H, CH_2^4), 3.36 – 3.20 (m, 6 H, CH^5), 2.45 – 2.32 (m, 8 H, CH_2^6), 1.99 – 1.76 (m, 6 H, CH_2^7), 1.71 – 1.59 (m, 8 H, CH_2^8), 1.58 – 1.45 (m, 6 H, CH_2^9), 1.42 – 1.15 (m, 90 H, CH_2^{10}), 0.97 – 0.84 (m, 12 H, CH_3^{11}).

^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 173.80, 172.60, 172.58, 172.54, 169.98, 169.72, 136.23, 128.65, 128.27, 74.92, 74.05, 74.02, 66.18, 39.31, 39.29, 34.47, 34.43, 32.03, 31.74, 29.82, 29.80, 29.78, 29.74, 29.69, 29.66, 29.60, 29.55, 29.50, 29.48, 29.46, 29.41, 29.33, 29.26, 29.23, 29.22, 29.03, 26.95, 26.94, 25.20, 25.13, 25.08, 25.05, 24.89, 24.83, 22.81, 22.65, 14.24, 14.16, 9.14.

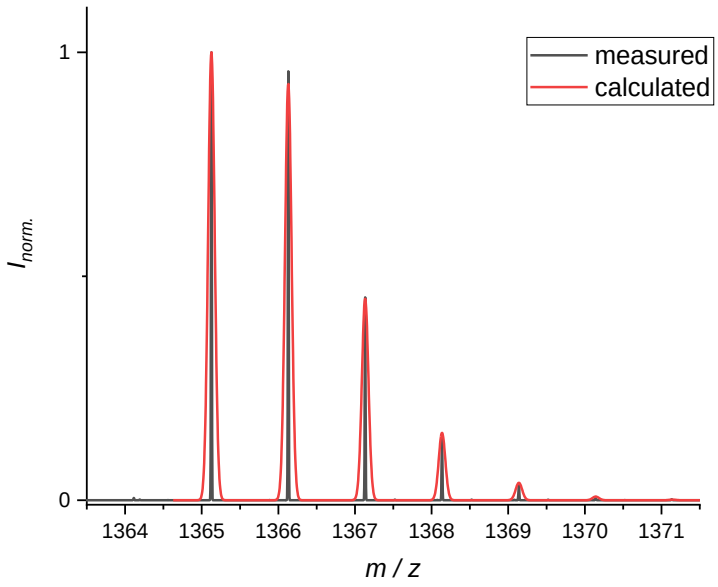
ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{83}{}^1H_{149}{}^{16}O_{11}{}^{14}N_3$, 1365.1265; found, 1365.1264, Δ = 0.1 mmu.



Supplementary Figure 188: ^{1}H-NMR of compound **Tr6/1** measured in $CDCl_3$.



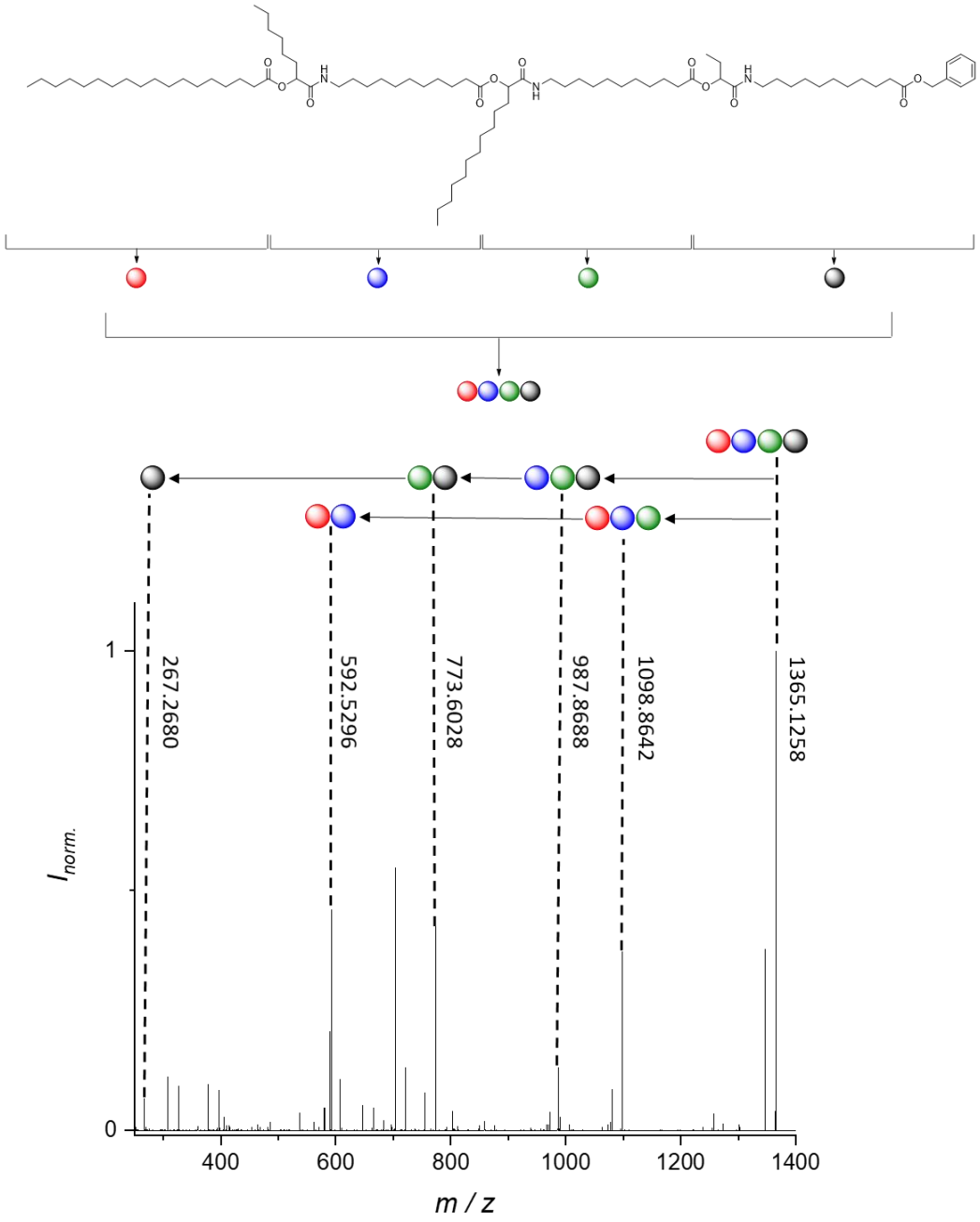
Supplementary Figure 189: SEC traces of the intermediates after each P-3CR in the synthesis of product **Tr6/1**.



Supplementary Figure 190: High resolution ESI-MS measurement of **Tr6/1**. The observed isotopic pattern is compared with the calculated isotopic pattern obtained from mMass (red).

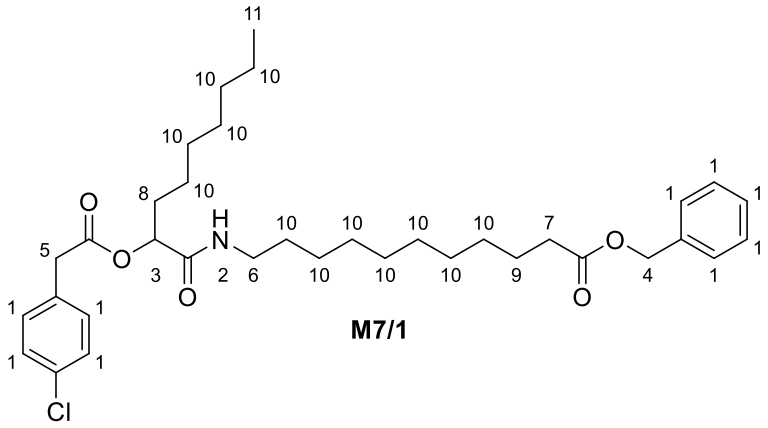
```
found 16062 values in C:\Users\Maxi\Desktop\MF 255 width 1.csv, maximum is 1.000000 found for mass 1365.126030
matching mass 1365.12603
cutoff 0.50000: 0 solutions (15 peaks)
cutoff 0.25000: 0 solutions (34 peaks)
cutoff 0.12500: 0 solutions (79 peaks)
cutoff 0.06250: 1 solutions (141 peaks)
1365.12603 ≈ 267.268790 + 325.261700 + 395.339950 + 269.199100 + 107.049690 (sides Heptanal, Dodecanal, Propionaldehyde;
 error -1.00680)
Press ENTER to quit ...
```

Supplementary Figure 191: Screenshot of the automated read-out of **Tr6/1**



Supplementary Figure 192: Read-out of the sequence-defined hexamer **Tr6/1**. Read-out of the hexamer **Tr6/1** *via* tandem ESI-MS/MS with an NCE of 18. In the spectrum, the read-out from both ends of the oligomer using the fragmentation next to the carbonyl are shown.

6.3.4.5 Synthesis of Monomer M7/1 with TAG7



In a 25 mL round bottom flask 150 mg 2-(4-chlorophenyl)acetic acid **TAG7** (882 µmol, 1.00 eq.) were stirred in 3.00 mL DCM, subsequently 207 µL octanal **A8** (170 mg, 1.33 mmol, 1.50 eq.) and 401 mg of monomer **IM2** (1.33 mmol, 1.50 eq.) were added. The resulting reaction mixture was stirred at room temperature for 2 days. Afterwards, the crude mixture was dried under reduced pressure. The residue was adsorbed onto celite® and purified *via* column chromatography on silica gel eluting with a gradual solvent mixture of cyclohexane and ethyl acetate (7:1 → 3:1). Afterwards 50 ml DCM and 50 ml of 2% potassium carbonate solution were added to the crude product. The aqueous phase was separated, and the organic layer was washed with water (3 × 50 mL). The combined organic layers were dried over sodium sulfate **11** and the solvent was evaporated under reduced pressure. The Passerini product **M7/1** was obtained as a pale highly viscous oil. (479 mg, 798 µmol, 90.5%).

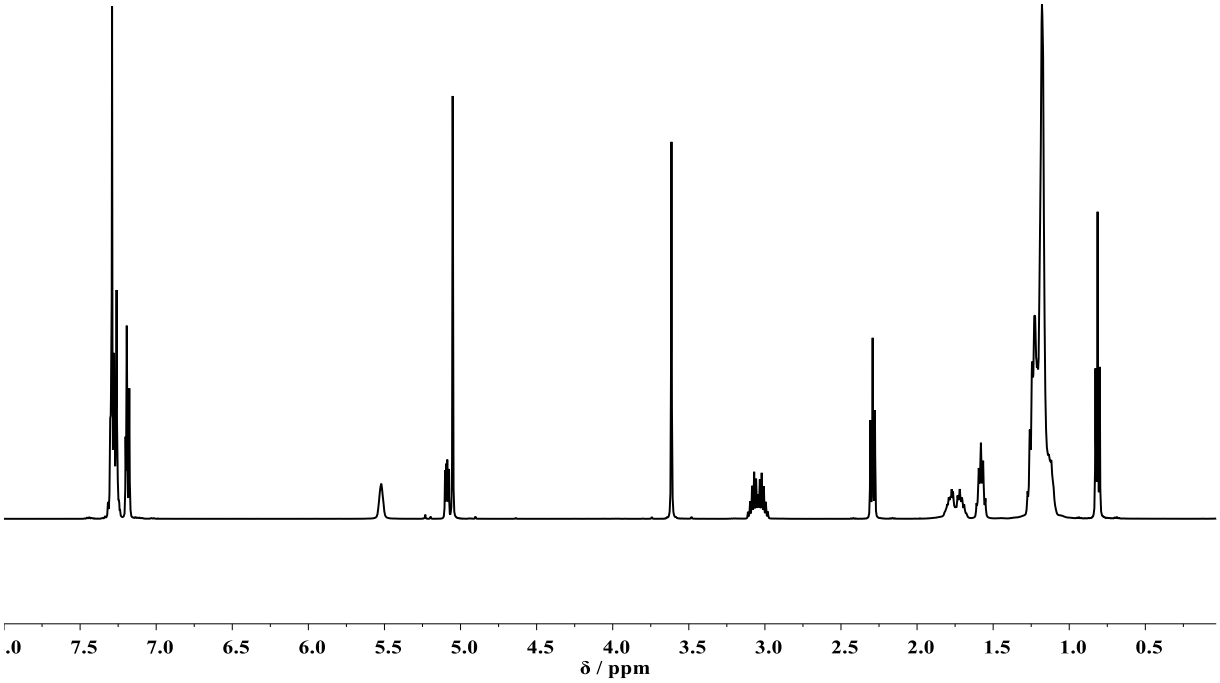
R_f = 0.56 in cyclohexane/ethyl acetate (2:1).

IR (ATR): ν / cm^{-1} = 3293.7 (vw), 2924.1 (s), 2853.1 (m), 1736.2 (s), 1655.5 (m), 1534.8 (w), 1492.3 (m), 1455.7 (w), 1240.9 (m), 1149.8 (s), 1091.2 (m), 1016.0 (m), 806.7 (w), 735.4 (w), 696.7 (m), 579.1 (w), 497.4 (w), 409.9 (vw).

^{1}H NMR (500 MHz, $CDCl_3$): δ / ppm = 7.36 – 7.15 (m, 9 H, CH_{Ar}^1), 5.54 – 5.50 (m,1 H, NH^2), 5.11 – 5.07 (m, 1 H, CH^3), 5.05 (s, 2 H, CH_2^4), 3.61 (s, 2 H, CH_2^5), 3.16 – 2.94 (m, 2 H, CH_2^6), 2.29 (t, J = 7.5 Hz, 2 H, CH_2^7), 1.84 – 1.67 (m, 2 H, CH_2^8), 1.63 – 1.53 (m, 2 H, CH_2^9), 1.29 – 1.09 (m, 24 H, CH_2^{10}), 0.81 (t, J = 7.0 Hz, 3 H, CH_3^{11}).

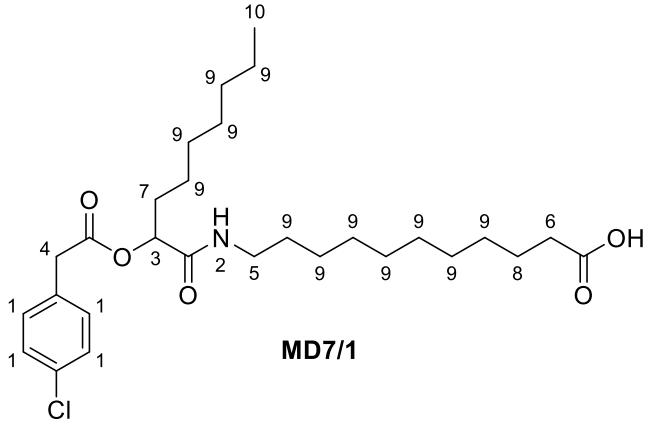
^{13}C NMR (126 MHz, $CDCl_3$): δ / ppm = 173.80, 169.58, 169.48, 136.22, 133.66, 132.17, 130.66, 129.14, 128.65, 128.27, 74.59, 66.18, 41.05, 39.25, 34.43, 31.91, 31.82, 29.55, 29.47, 29.45, 29.33, 29.30, 29.27, 29.23, 29.19, 26.86, 25.06, 24.78, 22.73, 14.21.

ESI-MS [*m/z*]: $[M + H]^+$ calculated for $^{12}C_{35}{}^{1}H_{50}{}^{16}O_5{}^{14}N^{35}Cl^{23}$, 600.3450; found, 600.3450, Δ = 0.0 mmu.



Supplementary Figure 193: ^{1}H-NMR of compound **M7/1** measured in $CDCl_3$.

Deprotection



In a 25 mL round bottom flask 383 mg of **M7/1** (638 µmol, 1.00 eq.) were dissolved in 3.00 mL ethyl acetate and 3.00 mL THF. Afterwards 77.0 mg palladium on activated carbon **1** (20 wt%) were added. Subsequently, the mixture was purged with hydrogen (3 balloons) and stirred under hydrogen atmosphere overnight. The heterogeneous catalyst was filtered off and the solvent

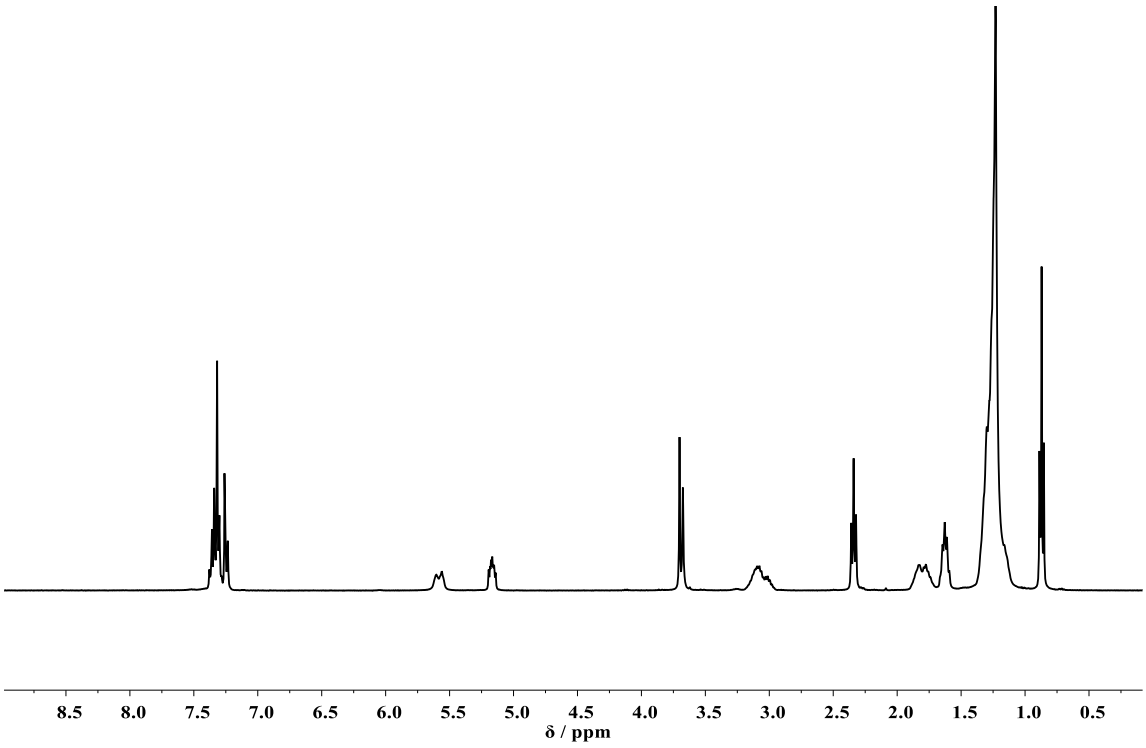
was evaporated under reduced pressure. The product **MD7/1** was obtained as brownish solid in a yield of 289 mg (88.4%, 564 µmol).

IR (ATR): ν / cm^{-1} = 3290.1 (w), 2917.3 (s), 2849.0 (m), 1740.7 (s), 1695.6 (vs), 1652.8 (vs), 1563.6 (m), 1491.4 (w), 1468.4 (w), 1431.0 (w), 1407.9 (w), 1278.2 (m), 1244.9 (s), 1192.6 (w), 1126.3 (s), 1090.6 (w), 1016.6 (w), 929.1 (w), 806.8 (w), 772.2 (vw), 719.5 (m), 691.5 (m), 555.9 (vw), 499.8 (w), 481.5 (vw), 429.4 (vw), 393.2 (w).

^{1}H NMR (400 MHz, $CDCl_3$): δ / ppm = 7.43 – 7.18 (m, 4 H, CH_{Ar}^{1}), 5.68 – 5.50 (m, 1 H, NH^{2}), 5.23 – 5.11 (m, 1 H, CH^{3}), 3.69 (d, J = 10.5 Hz, 2 H, CH_2^{4}), 3.18 – 2.93 (m, 2 H, CH_2^{5}), 2.34 (t, J = 7.5 Hz, 2 H, CH_2^{6}), 1.94 – 1.71 (m, 2 H, CH_2^{7}), 1.69 – 1.55 (m, 2 H, CH_2^{8}), 1.42 – 1.08 (m, 24 H, CH_2^{9}), 0.87 (t, J = 6.8 Hz, 3 H, CH_3^{10}).

^{13}C NMR (101 MHz, $CDCl_3$): δ / ppm = 179.36, 169.89 (s, C_{quart}^{a}), 169.81 (s, C_{quart}^{b}), 169.64(s, C_{quart}^{a}), 169.61(s, C_{quart}^{b}), 133.79 (s, C_{quart}^{a}), 133.67 (s, C_{quart}^{b}), 132.16 (s, C_{quart}), 130.67 (s, CH_{Ar}), 129.38 – 128.94 (m, CH_{Ar}), 127.64 (s, CH_{Ar}), 74.58 (CH^{3a}), 74.31 (CH^{3b}), 41.88 (CH_2^{4a}), 41.07 (CH_2^{4b}), 39.29 (CH_2^{5a}), 39.19 (CH_2^{5b}), 34.10, 31.89, 31.82, 29.47, 29.40, 29.33, 29.26, 29.23, 29.18, 29.11, 26.82, 26.78, 24.78, 24.77, 24.74, 22.73, 14.19.

ESI-MS [*m/z*]: $[M + Na]^+$ calculated for $^{12}C_{28}{}^{1}H_{44}{}^{16}O_5{}^{14}N{}^{35}Cl$, 532.2789; found, 532.2800, Δ = 0.1 mmu.



Supplementary Figure 194: ^{1}H-NMR of compound **MD7/1** measured in $CDCl_3$.

7 Abbreviations

7.1 List of abbreviation

Ar	Aromatic
ADMET	Acyclic diene metathesis
Bit	Binary digit
$CDCl_3$	Deuterated chloroform
CDO_3D	Deuterated methanol
COSY	Correlation spectroscopy
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIC	Diisopropylcarbodiimide
DMAP	4-Dimethylaminopyridine
DMTr	Dimethoxy trityl
DP	Degree of polymerization
CuAAC	Copper-assisted azide-alkyne cycloaddition
DFT	Density functional theory
DMF	Dimethyl formamide
DNA	Deoxyribonucleic acid
EA	Ethyl acetate
EI	Electron ionization
ESI-MS	Electrospray ionization mass spectrometry
ESI-MS/MS	Tandem electrospray ionization mass spectrometry
e.g.	exempli gratia, Lat: for example
et al.	et *alii/aliae/alia*. lat.: and others
FG	Functional group
GGB-3CR	Groebke-Blackburn-Bienaymé three component reaction

Abbreviations

HIV	Human immunodeficiency virus
HMBC	Heteronuclear multiple bond correlation
HSQC	Heteronuclear multiple quantum coherence
HRMS	High resolution mass spectrometry
IEG	Iterative exponential growth
IBX	2-iodoxybenzoic acid
i.e.	*id est* Lat.: that is
IMCR	Isocyanide based multicomponent reaction
in situ	Lat: on site, locally without isolation
in silico	Lat: in silicon
IOC	Institute of organic chemistry
IUPAC	International union of pure and applied chemistry
IR	Infrared spectroscopy
KIT	Karlsruher Institut für Technologie
L-3CR	Lipp-three component reaction
MALDI	Matrix-assistend laser desorption/ionization
MALDI-MS/MS	Tandem matrix-assistend laser desorption/ionization
MeOH	Methanol
MCR	Multicomponent reaction
mCPBA	meta-chloroperoxybenzoic acid
MS	Mass spectroscopy
NCE	Normalized collision energy
NIPU	Non-isocyanate polyurethanes
NMR	Nuclear magnetic resonance spectroscopy
OPE	Oligo(phenylene ethynylene
P-3CP	Passerini-three component polymerization
P-3CR	Passerini-three component reaction

PEG	Poly(ethylene glycol)s
PG	Protecting group
$POCl_3$	Phosphoroxycloride
RNA	Ribonucleic acid
ROP	Ring opening polymerization
RT	Room temperature
SEC	Size exclusion chromatography
SPPS	Solid phase peptide synthesis
SUMI	Single unit monomer insertion
TAD	1,2,4-triazoline-3,5-dione
TAG	Mass marker
TBDMS	*tert*-butyl dimethyl silyl
THF	Tetrahydrofuran
THP	Tetrahydropyran-1-yl acetal
TLC	Thin layer chromatography
TMS	Trimethyl silyl
p-TsCl	para-toluenesulfonylchloride
U-4CR	Ugi-four component reaction
U-5C-4CR	Ugi-five-center-four component reaction
U-5CC	Ugi-five-component condensation
UT-4CR	Ugi tetrazole four component reaction
via	Lat: By way of, using
QR-code	Quick Response-code

7.2 List of symbols

d	Doublet
g	Gram
h	Hours
Hz	Hertz
h	sextet
L	Liter
m	multiplet
m/z	Mass-to-charge ratio
mg	Milligram
MHz	Megahertz
mL	Milliliter
mol	Mol
mmol	Millimol
mmu	Milli mass unit
ppm	Parts per million
s	singlet
R_f	Retarding-front
t	triplet
ν	Wavenumber
µg	Microgram
µmol	Micromol
µL	Mikroliter
δ	Chemical shift in NMR spectroscopy
°C	Degrees Celsius

8 List of Figures, Schemes and Tables

8.1 List of figures

8.2 List of schemes

8.3 List of tables

9 Publication List

- M. Frölich, D. Hofheinz, M. A. R. Meier*, *Commun. Chem.* **2020**, *3*, 184.
- K. S. Wetzel, M. Frölich, S. C. Solleder, R. Nickisch, P. Treu, M. A. R. Meier*, *Commun. Chem.* **2020**, *3*, 63.
- M. Frölich and M.A.R Meier*, *Nachr. Chem.* **2019**, *67*, 45-46.
- C. Boukis, K. Reiter, M. Frölich, D. Hofheinz, M. A. R. Meier*, *Nature Communications* **2018**, *9*, 1439.

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