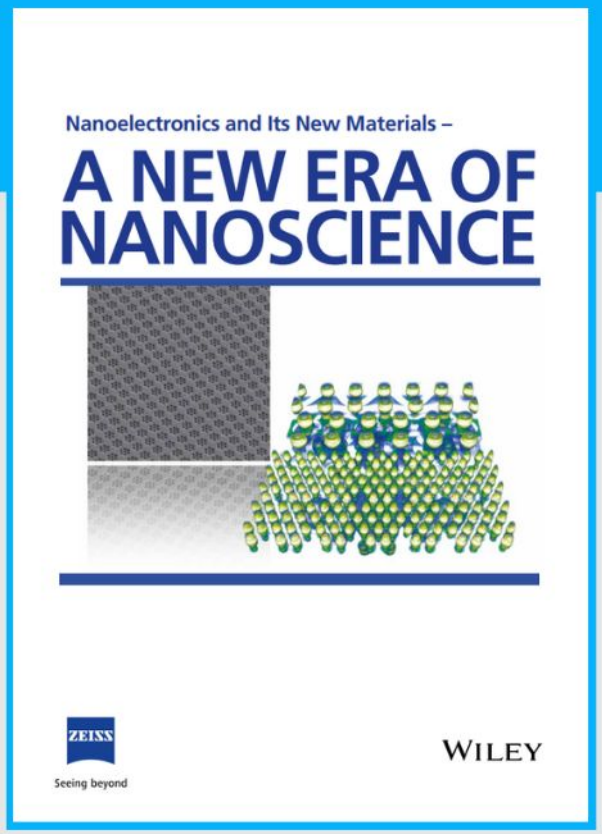
Nanoelectronics and Its New Materials – A NEW ERA OF NANOSCIENCE



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Palladium-Catalyzed Combinatorial Synthesis of Biphenyls on Droplet Microarrays at Nanoliter Scale

*Julius Höpfner, Marius Brehm, and Pavel A. Levkin**

The rising costs of pharmaceutical research are currently limiting the productivity of drug discovery and development, but can potentially be diminished via miniaturization of the synthesis and screening of new compounds. As droplet microarrays already present themselves as a versatile tool for highly miniaturized biological screening of various targets, their use for chemical synthesis is still limited. In this study, the influential palladium-catalyzed Suzuki–Miyaura reaction is successfully implemented at the nanoliter scale on droplet microarrays for the synthesis of an 800-compound library of biphenyls. Each reaction is carried out in individual 150 nL droplets. Remarkably, the synthesis of these 800 compounds requires a minimal amount of reagents, totaling 80 µmol, and a solvent volume of 400 µL. Furthermore, the cleavage kinetics and purity of the obtained biphenylic compounds are investigated. Via the solid-phase synthesis approach, the compounds could be purified from excess reactants and catalyst prior to the analysis and a UV-cleavable linker allows for fast and additive-free cleavage of each compound into the individual 100 nL droplet. This novel approach expands the toolbox of the droplet microarray for miniaturized high-throughput chemical synthesis and paves the way for future synthesis and screening of chemical compounds in a single platform.

1. Introduction

Miniaturization of compound library synthesis and its subsequent screening against a biological target is a promising strategy to withstand the constantly rising costs of pharmaceutical research, which is already titled as the "productivity crisis in pharmaceutical R&D."[1] While the industry standard in high-throughput screening is the microwell plate with either 1536 or 384 wells and working volumes of around 2 or 50 µL per well, respectively, further downsizing is still essential. One approach is the droplet microarray (DMA) platform, which was developed by Levkin et al.[2–4] and is based on superhydrophobic-hydrophilic patterning, therefore enabling up to thousands of nanoliter-sized droplets to stand freely without physical barriers on a glass slide. While it was established in recent years for a wide range of biological systems such as adherend and suspension cells,[2] spheroids,[5] embryoid bodies,[6] bacteria,[7,8] and even zebrafish embryos,[9] its utilization for parallel and miniaturized chemical synthesis just recently began.[10–12] In order to expand the range of reliable reactions available on this platform, we propose the inclusion of the Suzuki–Miyaura reaction (SMR) as a valuable addition to its toolbox. The SMR is a well-established cross-coupling reaction in organic synthesis that utilizes a palladium catalyst to form a new carbon–carbon bond between an organoboron compound and an aryl or vinyl halide or triflate.[13,14] After being introduced in 1979, it rapidly took over medicinal chemistry and is now the most used reaction for C–C coupling[15] and occurs in 20% of all synthetic steps in drug production.[16] Only one type of reaction is used even more in the production steps: the amide bond formation with an occurrence of 32%. Although of significant importance there have been minimal efforts to scale down the SMR to the sub-microliter range in open formats like the DMA. It has been employed in high-throughput experimentation in 1536 well-plates,[17] as well as in enclosed microfluidic setups.[18,19] The SMR is particularly useful in synthesizing biaryl compounds, which are crucial building blocks for a wide range of natural products and pharmaceuticals. In particular, two characteristics that have contributed to the success of the SMR are its great selectivity and tolerance against functional groups as well as the mild reaction conditions. The resulting biphenyl structure of the SMR is a common motif in biological active compounds and can be seen in FDA-approved drugs such as losartan, flurbiprofen or telmisartan. A special case is the class of the hydroxylated biphenyls, which are acting as nonsteroidal ligands for estrogen receptors.[20,21] Since those molecules can influence the growth

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and behavior of estrogen- or androgen-sensitive cancer cells, the ongoing discovery of such compounds is important. Although biphenylic compounds are not currently used as antibiotics in clinical settings, they have exhibited antibacterial properties in multiple studies,[22–24] which showcase their great potential for pharmaceutical applications. The combination of the advantages of the DMA with the synthetic capabilities of the SMR provides a potent platform for the high-throughput synthesis and screening of a broad range of pharmaceutically relevant biphenyl derivatives while minimizing starting materials and generating only minimal waste products.

Therefore, in this manuscript, we utilized the DMA platform to optimize the reaction conditions of the SMR in this nanoliter scale format, investigated the cleavage kinetics and the removal of the participated palladium from the surface and finally to synthesize in parallel an array of 800 carboxylic acid or amide containing biphenyls via the palladium-catalyzed SMR. The chosen set of starting materials represents a broad variety of aryl halides and boronic acids to emphasize the robustness of the SMR using our approach. The palladium precatalyst was also prepared on-chip prior to the synthesis, thus minimizing the usage of precious palladium or laborious pre-synthesis. Our chip design offers 672 (14 × 48) hydrophilic square spots with an edge length of 1 mm and superhydrophobic borders of 500 µm, which allowed us to synthesize almost all combinations of the 41 starting materials on one single chip. Furthermore, employing a solid-phase synthesis approach and the usage of a photolabile linker allowed for the determination of the UV induced release rate from the hydrophilic spots. This synthetic strategy leads to a facile way to control the amount of compound that is released into the droplets, which is a critical factor in many experiments. This study exemplifies the complementary nature of utilizing both the droplet microarray (DMA) and the SMR for the high-throughput production of potent biologically active compounds. This approach offers a more efficient, cost-effective, and streamlined method for generating compounds with therapeutic potential for a range of clinical applications. By integrating both techniques into a single platform for synthesis and screening, it is possible to produce a library of compounds that can be quickly and easily screened for biological activity in the future. This innovative approach holds significant promise for the discovery of new and effective drugs in a more rapid and economical manner.

2. Results and Discussion

2.1. Synthetic Solid-Phase Approach Using DMA

The DMA we developed as a miniaturized high throughput synthesis screening platform is a common microscopic glass slide (75 × 25 × 1 mm) coated with a thin layer of nanoporous poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) (HEMA-*co*-EDMA) functionalized with alkyne groups. The topmost nonporous portion of the polymer is removed using adhesive tape to enhance hydrophobicity and permeability. The coating thickness, controlled by the volume of polymerization mixture, is ≈10 µm. The surface then undergoes UV-induced thiol-yne reactions using different thiols to create hydrophilic (HL) spots on a superhydrophobic (SH) background (**Figure 1**e, Figure S2, Supporting Information). Thus, to create a superhydrophobic (SH) pattern, a 10% v/v solution of 1H,1H,2H,2H-perfluorodecanethiol in isopropanol was applied onto the polymer surface. The slide was then exposed to 254 nm UV light for 3 min through a quartz photomask. Superhydrophilic (SL) spots were created by applying a 15 wt% solution of cysteaminium chloride in a 1:1 v/v mixture of water and ethanol onto the patterned surface. The slide was irradiated with 254 nm UV light for 3 min. Slides with 80 spots (5 × 16, round, d = 3 mm) were used for manual pipetting and slides with 672 spots (14 × 48, square, side length = 1 mm) for automated liquid dispensing via a non-contact liquid dispenser. These spots effectively confine high-surface tension liquids with volumes ranging from single-digit microliters to 10 nL (Figure 1d).

To synthesize biphenylic compounds on DMA, the generalized workflow shown in Figure 1f was adapted. First, the hydrophilic spots were treated with 150 nL linker coupling solution each for 18 h (**Figure 2**a). The linker coupling solution contained 0.1 M photolinker (HEPL or FAPL) and 0.1 M 1-hydroxybenzotriazole (HOBt) in *N*-methyl-2-pyrrolidone (NMP) and was freshly mixed with 5% (v/v) diisopropylcarbodiimide (DIC) before dispensing. By the end of the coupling, the DMA was rinsed with ethanol and acetone and dried in air flow. The FAPL had to be deprotected with a solution of 20% (v/v) piperidine in NMP. Subsequently, the aromatic halides (containing a carboxylic acid) were coupled to the linker by adding in each spot a solution containing 0.1 M of the carboxylic acid, 0.01 M 4-(dimethylamino)pyridine (4-DMAP) and 5% (v/v) DIC in NMP and letting it react for 18 h (Figure 2b).

The attached aryl halides were then employed in the SMR. In every spot, 40 nL of each 0.5 M dibenzyl diisopropylphosphoramidite and 0.2 M solution of disodium tetrachloropalladate in water were dispensed and incubated for 15 min at room temperature to synthesize the catalyst on chip. Subsequently, 80 nL of a solution containing 0.5 M boronic acid derivative in NMP and 40 nL of a saturated sodium carbonate solution were added and the DMA was incubated in the dark at room temperature for 18 h (Figure 2c). The reactions were ended by washing off the mixtures prior to immersion of the whole slide in a 0.1 M solution of potassium cyanide (KCN) in dimethyl sulfoxide (DMSO)/water (1:1) for 3 h to remove the precipitated palladium.

The photoinduced cleavage from the surface using UV light at 365 nm released the biphenylic compounds of interest into the distinct droplets, leaving the cleaved linker on the surface of the HL spots (Figure 2d).

2.2. Performance of the Suzuki–Miyaura Reaction on the DMA

Table 1 and **Scheme 1** shows the structures of all the educts used in this work. We used 16 different carboxylic acid derivatives as surface-bound aryl halides (**A1–A16**) and 25 boronic acid derivatives (**B1–B25**). In combination with the use of HEPL and FAPL, this approach allows for a total of 800 unique biphenylic products of the SMR. For each compound, a corresponding number of spots were functionalized with either HEPL or FAPL and were used to synthesize a particular product. To monitor the synthetic success and purity of the products, we performed 25 reactions on a larger scale using round spots with a diameter of 3 mm. These spots can hold up to 10 µL of solvent and are big enough to be filled and emptied by manual pipetting. Collecting

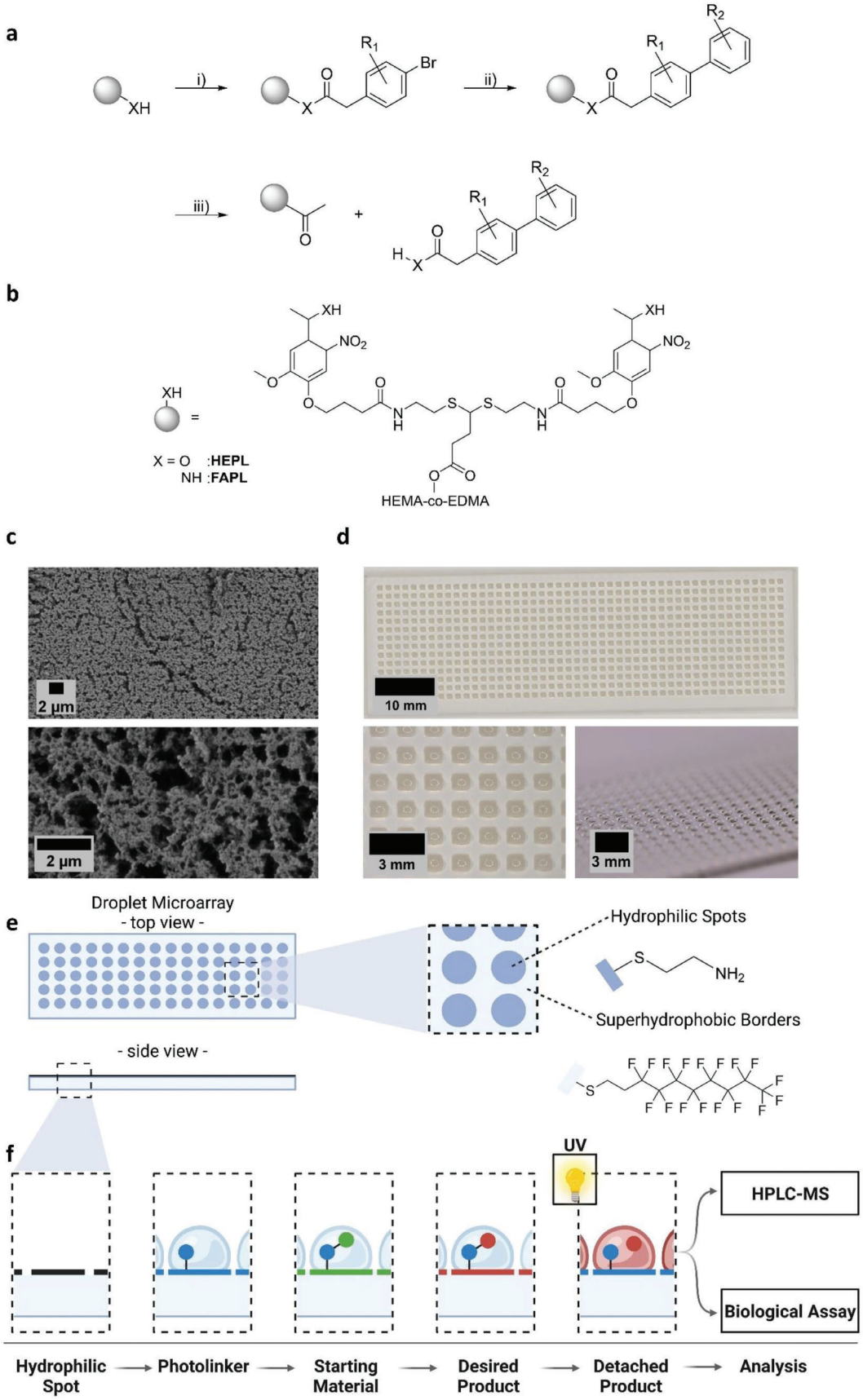
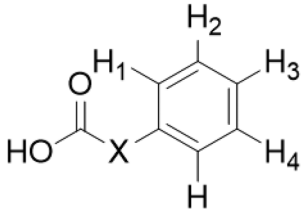
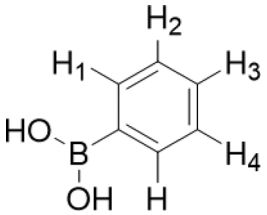


Table 1. Structures of the aryl halide (A_X) and the boronic acid (B_X) entries.





Aryl halide	H_1	H_2	H_3	H_4	X =
A_1	H	H	Br	H	$-(CH_2)_2-(CO)-$
A_2	OMe	H	H	Br	$-(CH_2)-$
A_3	H	H	Br	H	$-(CH_2)_3-$
A_4	H	F	Br	H	$-(CH_2)-$
A_5	F	H	H	Br	$-(CH_2)-$
A_6	F	H	H	Br	–
A_7	H	H	Br	H	$-(CH_2)-O-$
A_8	H	H	Br	H	$-(CH_2)_2-$
A_9	H	Br	H	H	$-(CH_2)_2-$
A_{10}	H	Br	H	H	$-(CH_2)-$
A_{11}	CF_3	H	Br	H	–
A_{12}	CH_3	H	H	Br	–
A_{13}	Cl	H	Br	H	$-(CH_2)-$
A_{14}	OMe	H	Br	H	$-(CH_2)-$
A_{15}	H	H	Br	H	$-(CH_2)-$
A_{16}	H	H	I	H	–

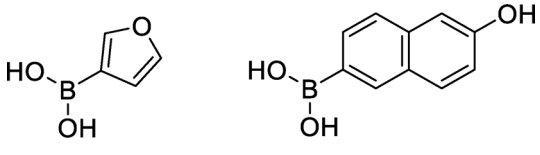
Boronic acid	H_1	H_2	H_3	H_4
B_1	F	H	CH_3	H
B_2	CH_3	H	OMe	H
B_3	H	H	$-(CO)-NH_2$	H
B_4	H	$-(CH)-(CH_3)_2$	H	H
B_5	H	OMe	H	OMe
B_6	H	F	F	H
B_7	H	H	$-(CH_2)_2-CH_3$	H
B_8	H	F	OMe	H
B_9	H	Cl	H	F
B_{10}	H	OH	H	H
B_{11}	H	OEt	H	H
B_{12}	H	H	O^iPr	H
B_{13}	H	H	Cl	H
B_{14}	H	H	CH_3	H
B_{15}	H	CF_3	H	H
B_{16}	H	F	F	F
B_{17}	H	H	$-O-(CH_2)_2-OMe$	H
B_{18}	H	H	H	H
B_{19}	$-O-(CH_2)_3-CH_3$	H	H	CH_3
B_{20}	OMe	H	OMe	H
B_{21}	H	$-O-(CH_2)-O-$		H
B_{22}	H	H	CF_3	H
B_{23}	H	H	OH	H

the solution of 5 spots per product yielded 50 µL of sample after cleavage, which was enough to be handled by our LC-MS autosampler. Purity was calculated as the fraction of the product's peak integral with respect to the sum of integrals of all peaks at 280 nm absorbance. The chromatograms are shown in Supporting Information 1 (compound **1–25**), the respective mass spectra are shown in Supporting Information 2 (products **1–25**). High-resolution mass spectra of ten different compounds synthesized as described in the Experimental Section are shown in Supporting Information 3. The results for the purities of the different

Figure 1. a) Scheme of the synthetic sequence for the on-chip high-throughput synthesis of a chemical library of biphenyls: i) immobilization of different aryl halides to the surface anchored photolinker. ii) SMR with variable boronic acids. iii) UV induced cleavage of the desired biphenylic compound into individual 100 nL droplets. b) Representation of the solid-phase chemistry: UV-cleavable linker (hydroxyethyl photolinker, HEPL; FMOC protected amino photolinker, FAPL) is covalently immobilized to the porous polymer surface within the hydrophilic spots, forming the anchor of the solid-phase synthesis. c) Top-down scanning electron microscopy (SEM) images of the porous polymer of DMA. Top image was taken at 5000× magnification. Bottom image was taken at 20 000× magnification. d) Photographs of a slide with 14 × 48 HL square spots with an area of 1 mm^2. Each spot is filled with 150 nL water. e) Schematic representation of the DMA: A porous net-poly(HEMA-*co*-EDMA) thin film (12 µm thin) is functionalized with fluoroalkyl and cysteamine moieties to generate omniphobic and reactive hydrophilic regions, respectively. f) Scheme showing a generalized workflow using the DMA platform. Covalent immobilization of a photolabile linker in the hydrophilic (HL) spots forms the anchor for the solid phase combinatorial synthesis. Synthesized compounds can be released by UV irradiation at 365 nm into distinct nanodroplets formed in the hydrophilic spots to be analyzed, e.g., via liquid-chromatography mass spectrometry (LC-MS).

Table 2. Purity of exemplary compounds synthesized in 3 mm spots to monitor synthetic success via LC-MS analysis. Product was identified by MS, while purity was calculated as the fraction of the product's peak integral with respect to the sum of integrals of all peaks at 280 nm absorbance.

Product	Entry A	Entry B	Linker	Purity	Product	Entry A	Entry B	Linker	Purity
1	A_{15}	B_{19}	**HEPL**	90%	**14**	A_{16}	B_{23}	**FAPL**	79%
2	A_{15}	B_{20}	**HEPL**	87%	**15**	A_{15}	B_{23}	**FAPL**	87%
3	A_{15}	B_{21}	**HEPL**	91%	**16**	A_{6}	B_{23}	**FAPL**	55%
4	A_{6}	B_{23}	**HEPL**	87%	**17**	A_{7}	B_{23}	**FAPL**	65%
5	A_{15}	B_{23}	**HEPL**	97%	**18**	A_{16}	B_{21}	**FAPL**	76%
6	A_{16}	B_{18}	**HEPL**	95%	**19**	A_{15}	B_{21}	**FAPL**	94%
7	A_{9}	B_{16}	**HEPL**	89%	**20**	A_{6}	B_{21}	**FAPL**	79%
8	A_{9}	B_{7}	**HEPL**	84%	**21**	A_{7}	B_{21}	**FAPL**	86%
9	A_{15}	B_{16}	**HEPL**	88%	**22**	A_{16}	B_{22}	**FAPL**	78%
10	A_{15}	B_{7}	**HEPL**	85%	**23**	A_{15}	B_{22}	**FAPL**	89%
11	A_{9}	B_{14}	**HEPL**	90%	**24**	A_{6}	B_{22}	**FAPL**	66%
12	A_{3}	B_{14}	**HEPL**	88%	**25**	A_{7}	B_{22}	**FAPL**	84%
13	A_{16}	B_{23}	**HEPL**	88%					



B_{24} **B_{25}**

Scheme 1. Structure of boronic acids B24 and B25.

products are presented in **Table 2** (products **1–25**). Despite the fact that the starting materials absorb UV light and can also be detected in the chromatograms, the resulting purities of the products were between 55% and 95%, reflecting the robustness of the SMR. Moreover, there was no combination of starting materials tested that did not yield the desired product.

2.3. Removal of Precipitated Palladium after the Suzuki–Miyaura Reaction

The formation of metallic palladium particles during the SMR on the surface (**Figure 3**c) was blocking the UV light from reaching the polymer and therefore inhibiting the photoinduced cleavage from the surface. To remove the precipitated palladium, the surface was treated with a 0.1 M KCN solution in DMSO/water (1:1, v/v). The removal of palladium by treatment with this solution was monitored via energy-dispersive X-ray spectroscopy (EDX). Figure 3 shows the results of the EDX analysis of a hydrophilic spot a) before and b) after the removal of the precipitated palladium.

Typical peaks of carbon, oxygen, and sulfur are visible in both samples from the polymer backbone. While a strong peak from the palladium could be detected in the spectrum of the sample without the KCN wash, only traces of palladium were visible after the treatment, indicating its sufficient removal.

To quantify the amount of palladium remaining in the cleaved samples after washing with the KCN solution, inductively coupled plasma optical emission spectrometry (ICP-OES) was used. The results showed that the palladium content in the cleaved samples was 35 ± 4 ppm. These findings demonstrate the effectiveness of using a KCN solution to remove the palladium particles from the polymer surface, enabling successful photoinduced cleavage and subsequent release of the synthesized compounds.

2.4. Photorelease from the Surface of the DMA

In addition to investigating the success and purity of the products obtained using the miniaturized on-chip palladium-catalyzed SMR, we also assessed the kinetics of cleavage of the photocleavable linker by analyzing the amount of exemplary compound **26** (**Figure 4**a) released into the droplets after different UV irradiation times. **26** was synthesized according to the procedure described in the Experimental Section and each four spots containing 5 µL of water were exposed to UV light at 365 nm for different times. The combined cleavage solution of the four spots was analyzed via LC-MS and measured for its absorbance at 280 nm, which refers to the wavelength of the previously determined modified extinction coefficient (ϵ = 70 000 L µmol^{-1}, Figure S1, Supporting Information). To generate the modified extinction coefficient, compound **26** was also synthesized in flask in a milligram scale, purified and measured as standard for quantitative analysis of photoinduced cleavage. Related structures and NMR spectra can be found in Supporting Information 4 and 6. By using the Beer–Lambert law we determined the loading as nmol per round 3 mm spot (with an area of 7.01 mm^2) and plotted against irradiation time in Figure 4b. The results showed that the photolinker's half-life under UV exposure at 365 nm with 2.5 mW cm^{-2} was 5 min. The maximum loading of 0.18 nmol per spot of compound **26** was achieved, which was released into the droplets after 15 min of UV irradiation time (365 nm, 2.5 mW cm^{-2}). Filling up a 3 mm round spot with 5 µL of solvent results in a maximum concentration of 36 × 10^{-6} M. These findings suggest that the photocleavable linker was efficient and reliable for releasing the synthesized compounds into the droplets.

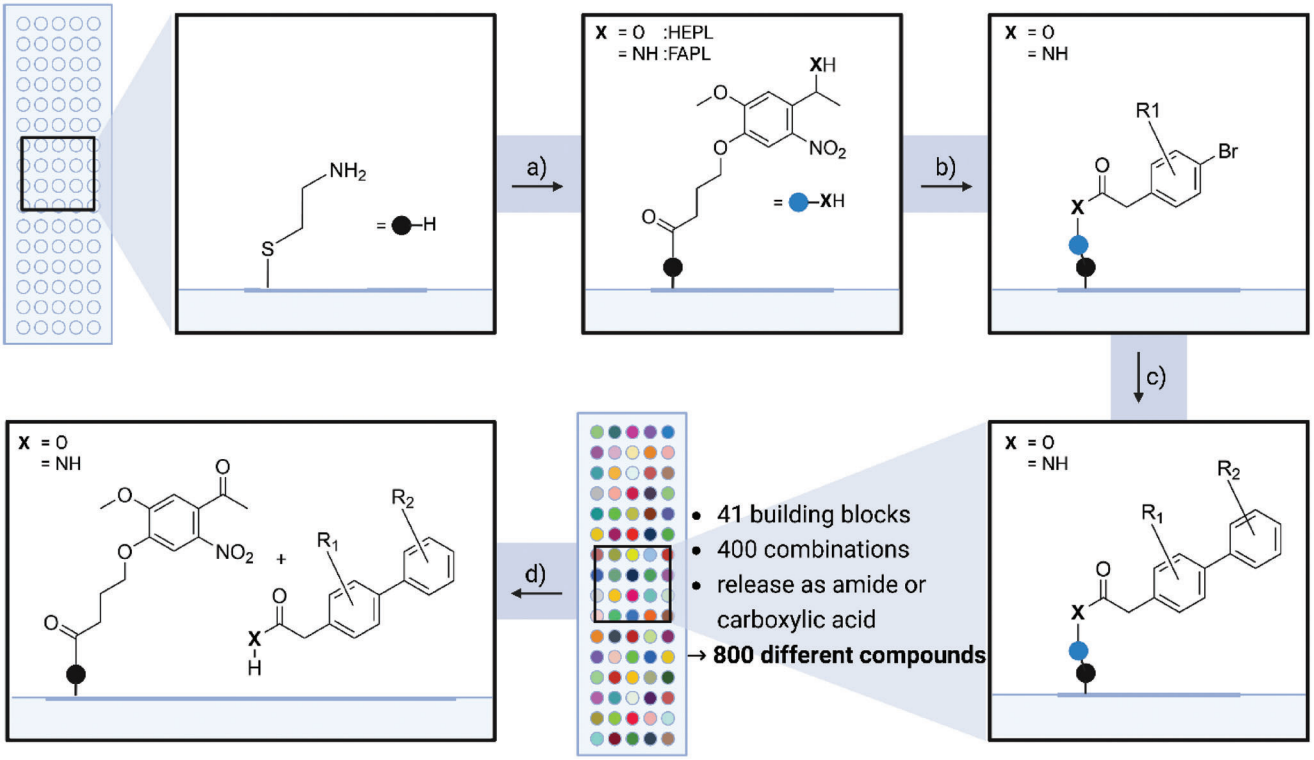


Figure 2. Overview of the SMR performed on the DMA. Aryl bromides containing a carboxylic acid moiety are esterified to the photolinker which is present only in the hydrophilic spots. Subsequent SMR with boronic acids gives a variety of biphenyls that can be purified by washing and immersion of the DMA slide prior to the UV-triggered release. a) HEPL or FAPL, HOBt, DIC in NMP, b) carboxylic acid, DIC, 4-DMAP in NMP, c) aryl boronic acid, Na_2CO_3, Na_2PdCl_4, dibenzyl diisopropylphosphoramidite, tetrabutyl ammoniumbromide in NMP/H_2O (9:1), d) 365 nm, 2.5 mW cm^{-2} for 20 min in H_2O.

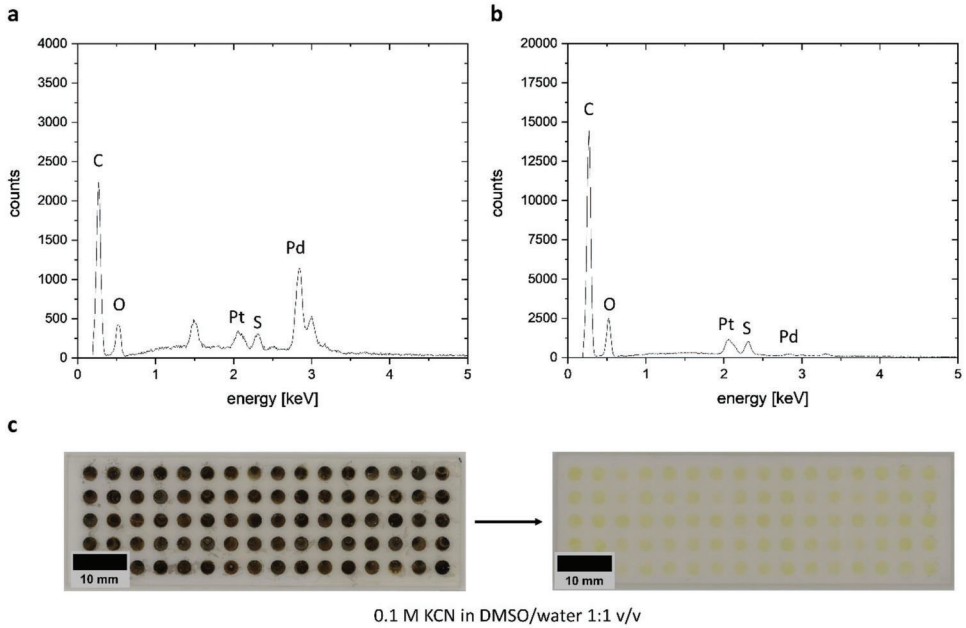


Figure 3. EDX spectra of a 3 mm round hydrophilic spot after the SMR a) before and b) after removal of the precipitated palladium (Pd) via washing with a 0.1 M solution of KCN in DMSO/water (1:1, v/v). Signals representing carbon (C), oxygen (O), and sulfur (S) come from the polymer backbone, while platinum (Pt) comes from the pretreatment of the sample. c) Photograph of hydrophilic spots before (left) and after (right) washing with KCN solution.

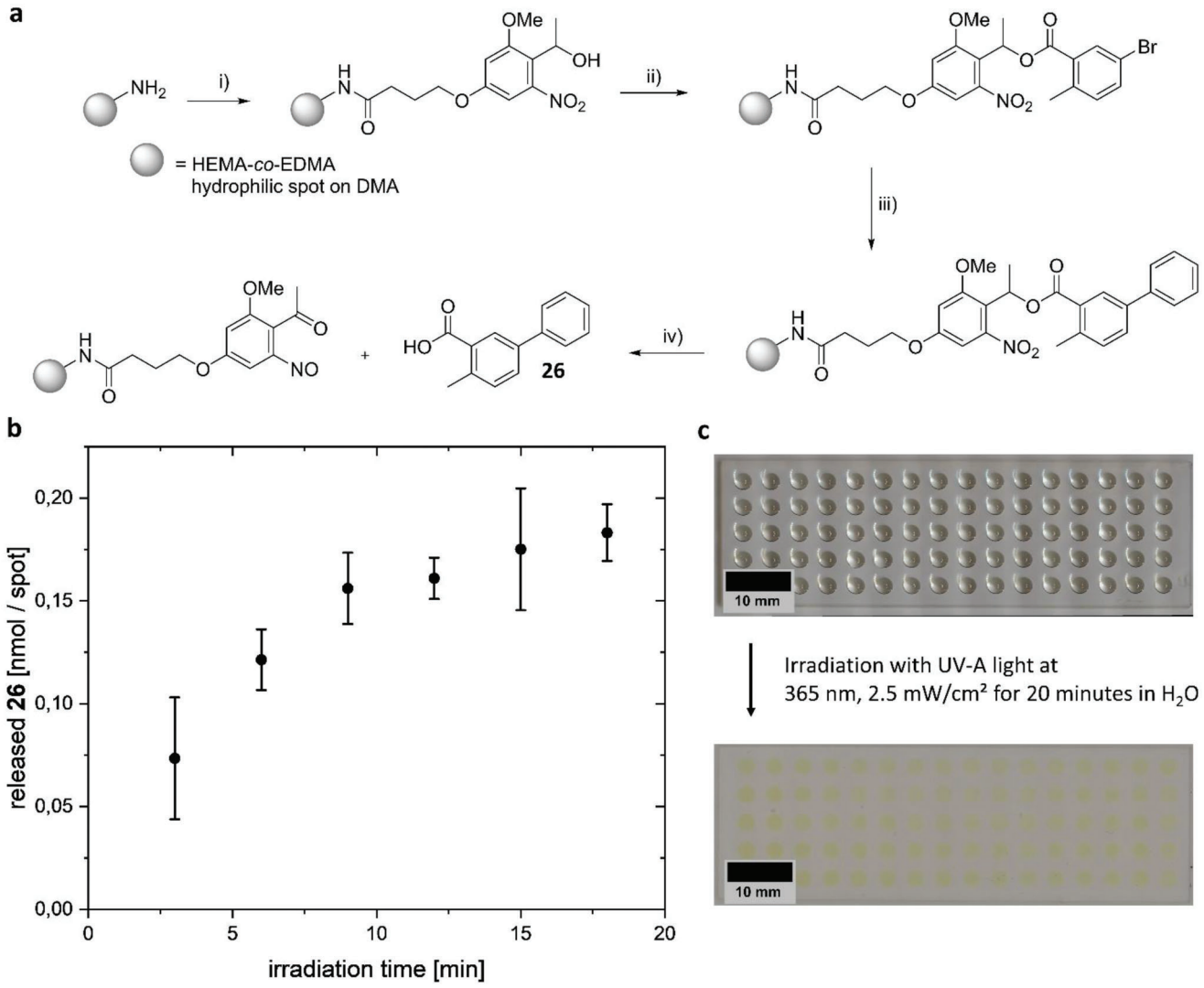


Figure 4. a) Scheme of the synthetic sequence for the synthesis of biphenyl 26 on-chip to determine the UV induced photorelease kinetics: i) immobilization of HEPL to the hydrophilic spots on DMA. ii) Covalent attachment of 5-bromo-2-methyl benzoic acid to the photolinker. iii) Solid-phase SMR using phenylboronic acid. iv) UV induced cleavage from the surface to yield the desired biphenylic compound **26**. b) Kinetics of the UV-triggered release of **26** into the 5 µL droplets after different irradiation times, determined by spectroscopic analysis of the solutions at 280 nm using a LC/MS system. Each data point is the average of three measurements. Error bars represent standard deviation. c) HEMAcoEDMA DMA with 3 mm round spots before (top, filled with 5 µL H_2O) and after (bottom) UV induced cleavage. The yellow color is developed during the cleavage.

2.5. Miniaturization to Nanoliter Compartments Using Droplet Microarrays

To demonstrate the feasibility of miniaturization on our platform, we made a switch from using round HL spots with a diameter of 3 mm, which could only accommodate 5 µL of reactants, to using square spots with an edge length of 1 mm. These square spots allowed for a remarkably lower reaction volume of around 100 nL, thus enabling us to perform chemical reactions on a much smaller scale (**Figure 5**d). This switch was made possible by the exceptional precision that is achieved during the photopatterning of the surface and by the use of a high-precision liquid dispenser. By using these square spots, we were able to achieve a higher density of reaction vessels and reduced needs of starting materials and produced waste. To be precise, a quantity of 20–40 nmol of starting materials was utilized, along with a mere 8 nmol of precious palladium precatalyst per spot. Moreover, by implementing this variation, it is possible to significantly increase the number of individual reactions on a single DMA slide. Specifically, the number of spots increased from 80 to 672, and the spot density increased from 4 to 34 spots cm^{-2}. This increase in spot density allows us to perform more reactions in parallel, thus increasing the throughput of our platform and enabling us to explore a wider range of chemical reactions in a shorter amount of time.

Based on our initial tests, we found that the product purities using 3 mm round spots were around 90% for most of the products, which we aimed to maintain upon further downsizing of

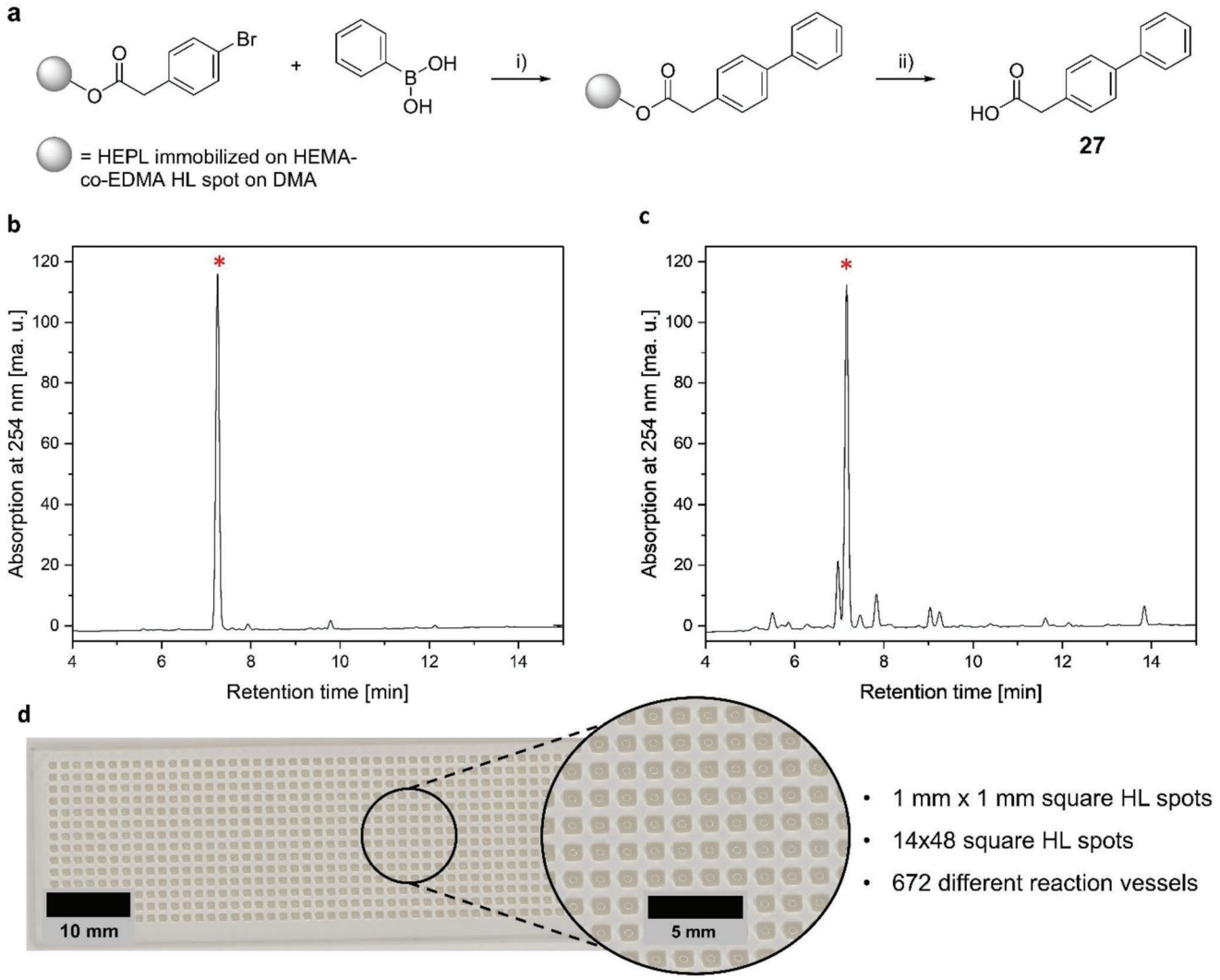


Figure 5. a) Reaction scheme of the synthetic pathway used to create biphenylic compound **27**: i) Solid-phase SMR using immobilized 4-bromophenylacetic acid and phenylboronic acid. ii) UV induced cleavage from the surface to yield the desired biphenylic compound **27**. b) UV chromatogram at 254 nm. After irradiation, the volume of five spots with a diameter of 3 mm (50 µL in total) was pipetted off the surface and analyzed via LC-MS. The red asterisk indicates the corresponding peak. c) UV chromatogram at 254 nm. After irradiation, a total of 50 µL has been collected from all HL spots and was analyzed via LC-MS. The red asterisk indicates the corresponding peak. d) Photograph of a slide with 14 × 48 HL square spots with an area of 1 mm^2. Each spot is filled with 150 nL water.

the reaction volume (Table 2). To further evaluate the benefits of miniaturization, we conducted an exemplary reaction (Figure 5a) using 3 mm round spots and then repeated the same reaction using 1 mm square spots to compare the purities of the obtained exemplary biphenylic compound **27** between them. We used a non-contact liquid dispenser to print 40 nL of sodium tetrachloropalladate solution (0.2 M in H_2O) and 40 nL of dibenzyl diisopropylphosphoramidite solution (0.5 M in NMP) into each spot on the DMA. We incubated the whole slide in a closed Petri dish for 15 min to form the precatalyst. Next, we printed 80 nL of phenylboronic acid solution (0.5 M in NMP) and 40 nL of saturated sodium carbonate solution (in H_2O) into each spot to start the reaction. The DMA was then incubated for 18 h in the dark at room temperature in a closed Petri dish, which contained an NMP-soaked paper tissue to prevent evaporation. After 18 h, the reaction mixtures were washed off with acetone and ethanol before immersing the entire slide in a 0.1 M solution of KCN in DMSO/water (1:1) for 3 h to remove the precipitated palladium. For the final washing step, we used only water and ethanol, and then printed 100 nL of deionized water into each spot. To release the product, we irradiated the whole DMA with UV light of 365 nm (2.5 mW cm^{-2}) for 20 min, causing the products to be released into individual separate droplets. To prepare our slide for sample collection, we needed to evaporate the water using reduced pressure. Once the slide was completely dry, we spread 250 µL of water and 250 µL of DMF evenly across the entire slide. After this, we carefully pipetted off each 25 µL of the water and DMF into a LC-MS vial, leading to a total volume of 50 µL, to mimic the procedure used for larger spot sizes.

The results showed that the purities using the 1 mm square spots (Figure 5d) were comparable to those obtained using the larger 3 mm round spots (Figure 5c). This suggests that miniaturization on our platform does not compromise the quality of the reaction products.

To highlight the possibilities and advantages of miniaturization on the DMA platform, we synthesized a library comprising 800 unique biphenylic compounds. This synthesis involved employing 16 different surface-bound aryl halides as carboxylic acid derivatives (**A1–A16**, Table 2) and 25 boronic acid derivatives (**B1–B25**, Table 2). By incorporating two different photolinkers (HEPL, FAPL), we achieved a total of $16 \times 25 \times 2 = 800$ distinct compounds. Remarkably, using just two DMAs with 1 mm spot size enabled the synthesis of all compounds, while still allowing room for positive and negative controls, as well as blanks on each slide for potential subsequent biological assays.

By dispensing nanoliter volumes during each reaction step, the total volume required for the entire reaction cascade of each synthesized compound was only 500 nL. This encompassed immobilization of the photolinker, covalent attachment of the aromatic halide to the photolinker, and subsequent SMR. A mere 400 µL of reaction solutions were utilized to synthesize all 800 unique biphenylic compounds.

Overall, the synthesis process involved a modest consumption of ≈80 µmol of reagents, with only 6.4 µmol of precious palladium precatalyst being used for the entire synthesis of the 800 compounds. By employing a 0.2 M Na_2PdCl_4 solution, the amount of palladium reagent utilized throughout the library synthesis amounted to less than 2 mg.

3. Conclusion

In summary, we developed a novel method for conducting miniaturized on-chip palladium-catalyzed Suzuki–Miyaura reaction using the droplet microarray platform. The application of the Suzuki–Miyaura reaction is a very important milestone for the synthesis of more complex and potentially biological active reagents using the droplet micorarray platform. Our approach enables the high-throughput synthesis of an 800 membered library of biphenylic compounds using only 80 µmol of starting materials including 6.4 µL of precious palladium catalyst, while generating only sub milliliter amounts of waste. Utilizing this approach, we were able to synthesize 25 exemplary biphenylic compounds in higher amount using 3 mm round spots. The synthesized compounds exhibited an average purity of 84%. To emphasize the possibility of miniaturization on our DMA platform, we conducted the same reaction using 1 mm square spots and compared the results. We found that the miniaturization of the Suzuki–Miyaura reaction applying our developed strategy produced similar purities compared to the bigger spots, demonstrating the efficacy of our approach. This downscaling heavily increased the spot density per slide. Overall, this switch represents an important step forward in the miniaturization of chemical synthesis on our platform. Sufficient removal of the precipitated palladium during the synthesis from the chip by immersion in KCN solution allows for further employment of the synthesized compounds in biological assays. Additionally, by investigating the UV induced release of the compounds from the surface we gained control over the maximum achievable concentration after irradiation of the DMA for different time frames. Our results demonstrate the effectiveness of our methodology in achieving precise control over reaction kinetics and in optimizing reaction conditions to achieve maximum yields. The solid-phase approach we established presents a convenient way for conducting miniaturized, high-throughput synthesis and subsequent photorelease of biphenylic compounds into individual 100 nL droplets. By utilizing the developed approach, upscaling of the number of synthesized compounds can be achieved by increasing the number of unique starting materials. It is feasible to expand the compound library to 8000 by combining 250 aromatic halides and 160 boronic acids, while employing only 12 DMA slides with a spot size of 1 mm. Remarkably, this upscaling would require less than 10 mL of solvents, utilizing less than 1 mmol of reagents for the total synthesis of these 8000 compounds. Furthermore, the integration of the DMA platform with the Suzuki–Miyaura reaction presents a highly efficient approach for the high-throughput production of biphenyl derivatives, which are of great interest in the pharmaceutical industry for the synthesis of anti-cancer, antipsychotic, and nonsteroidal anti-inflammatory drugs. This novel strategy allows for the simultaneous synthesis and screening of a library of compounds, facilitating the identification of potential therapeutic agents in a more streamlined and rapid manner. The innovative approach presented in this work holds great promise for the discovery of new and effective drugs for various clinical applications, while reducing costs and generating less waste.

4. Experimental Section

Glass slides were purchased from Schott Nexterion (Jena, Germany).

4-Pentynoic acid was purchased from Apollo Scientific (Bredbury, UK).

Ethanol, acetone, 4-(dimethylamino)pyridine, 4-[4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy]butanoic acid (hydroxyethyl photolinker), piperidine, *N*,*N*-dimethylformamide, *N*-methyl-2-pyrrolidone, and diisopropyl carbodiimide were purchased from Merck (Darmstadt, Germany).

1-Hydroxybenzotriazole was purchased from Molekula (Newcastle upon Tyne, UK).

Sodium hydroxide, 3-(trimethoxysilyl)propyl methacrylate, 2-hydroxyethyl methacrylate, ethylene dimethacrylate, 1-decanol, cyclohexanol, 2,2-dimethoxy-2-phenylacetophenone, 1*H*,1*H*,2*H*,2*H*-perfluorodecanethiole, cysteaminium chloride, sodium tetrachloropalladate, dibenzyl diisopropylphosphoramidite, and potassium cyanide were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

Deuterated solvents (chloroform, dimethyl sulfoxide) were purchased from VWR International, Radnor, PA.

Aryl bromides and boronic acids building blocks were purchased from Enamine Ltd. (Kiev, Ukraine).

If not stated otherwise, all chemicals have been used without further purification.

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra of the synthesized molecules were recorded on a Bruker Avance III HD 500 MHz (500 and 126 MHz, respectively) at room temperature. The peak shifts were declared in parts per million (ppm). The solvent peak served as a reference. For multiplets, the signal area was declared, for centrosymmetric signals, the center of the signal was declared. The description of the proton splitting occurred by using the abbreviations s for singlet, d for duplet, t for triplet, q for quartet, and m for multiplet.

The morphology of the HEMA-*co*-EDMA film on a silanized glass substrate was characterized by a scanning electron microscope (Zeiss LEO 1530) at an operating voltage of 2 kV. Prior to the SEM measurements, the samples were coated with a 10 nm thick platinum layer.

Automated liquid dispensing was performed by Certus Flex by Fritz Gyger AG (Gwatt, Switzerland). The precision and the CV of the dispensed volumes in the range between 50 and 500 nL is < ±3.0%, that is ± 6 nL for 200 nL droplets according to the manufacturer.

Figures were partially created with BioRender.com.

Manufacturing of Droplet Microarrays (DMAs)[12]: The droplet microarrays were prepared according to the already published protocol, which shall be shortly explained here.[4]

A microscopic glass slide (75 × 26 × 1 mm) was activated by immersing in 1 M sodium hydroxide (NaOH) solution for 1 h, followed by immersing in 1 M hydrochloric acid (HCl), followed by washing with water.

To modify the activated glass slide, a method involving the application of a 20% v/v ethanol solution of 3-(trimethoxysilyl)propyl methacrylate was employed. The solution was spread over the activated glass slides and allowed to incubate for a duration of 30 min. Subsequently, the slides were washed with ethanol and dried under a stream of nitrogen.

In order to create a porous polymer layer according to a previously published work,[4] the following polymerization mixture was utilized: 24 wt% 2-hydroxyethyl methacrylate (HEMA), 16 wt% ethylene dimethacrylate (EDMA), 12 wt% 1-decanol, 48 wt% cyclohexanol, and 0.4 wt% 2,2-dimethoxy-2-phenylacetophenone. Subsequently, 30 µL of the polymerization mixture was applied to a fluorinated glass slide and covered with a modified slide. Polymerization took place by exposing the glass mold to UV irradiation at a wavelength of 254 nm for 15 min using a Biolink BLX UV chamber (Witec AG, Sursee, Switzerland) with 2.5 mW cm^{-2}). The fluorinated glass slide was then removed, and the polymer surface was cleansed with ethanol and dried using an air gun. To enhance the roughness of the polymer surface, thereby increasing its hydrophobicity and hydrophilicity, the smooth top layer of the polymer was eliminated by applying adhesive tape (EAN 4042448036223, Tesa, Offenburg, Germany) to the surface and swiftly removing it.

Prior to the patterning step, the surface underwent modification by immersing the slides in a solution composed of 50 mL of acetone, 56 mg of 4-(dimethylamino)pyridine, 111.6 mg of 4-pentynoic acid, and 180 µL of *N*,*N*′-diisopropylcarbodiimide. The incubation took place for 4 h at room temperature with stirring.

To create a superhydrophobic (SH) pattern, a 10% v/v solution of 1H,1H,2H,2H-perfluorodecanethiol in isopropanol was applied onto the polymer surface. The slide was then exposed to 254 nm UV light using a Biolink BLX UV chamber (Witec AG, Sursee, Switzerland) with 2.5 mW cm^{-2}) through a quartz photomask (Figure S2a, Supporting Information). Subsequently, the porous polymer surface was thoroughly rinsed with acetone and dried using an air gun.

Superhydrophilic (SL) spots were created by applying a 15 wt% solution of cysteaminium chloride in a 1:1 v/v mixture of water and ethanol onto the patterned surface (Figure S2b, Supporting Information). The slide was irradiated with 254 nm UV light using a Biolink BLX UV chamber (Witec AG, Sursee, Switzerland) with 2.5 mW cm^{-2}) through a quartz slide.

Patterns with 5 × 16 spots (round, d = 3 mm) were used for manual pipetting and patterns with 14 × 48 spots (square, side length = 1 mm) for automated liquid dispensing via a Certus Flex (Fritz Gyger AG, Gwatt, Switzerland).

Preparation of the DMA for Synthesis[12]: The hydrophilic spots were treated with 5 µL linker coupling solution per spot for 18 h. The linker coupling solution contained 0.1 M photolinker (HEPL or FAPL) and 0.1 M 1-hydroxybenzotriazole (HOBt) in *N*-methyl-2-pyrrolidone (NMP) and was freshly mixed with 5 v% diisopropylcarbodiimide (DIC) before dispensing. By the end of the coupling, the DMA was rinsed with ethanol and acetone and dried in air flow. The Fmoc amine photolinker was then deprotected with 5 µL of 20 v% piperidine in NMP for 4 h and washed again. Subsequently, the aromatic halides (containing a carboxylic acid) were coupled to the linker by adding in each spot 5 µL of a solution containing 0.1 M of the carboxylic acid, 0.01 M 4-(dimethylamino)pyridine (4-DMAP) and 5 v% DIC in NMP and let react for 18 h. Washing and drying was carried out in the way described above. Volumes for the 1 mm spot sizes were 150 nL in all steps. To avoid the evaporation of the solvents during the incubation step the printed slide was immediately placed into a Petri dish (Corning, USA) with controlled humidity. The humidified Petri dish was prepared by placing a wetted humidifying pad, saturated with a mixture of deionized water and NMP, on the lid of the Petri dish. The lid was then securely sealed with Parafilm (Bemis, USA). Subsequently, the Petri dish containing the slide was allowed to incubate at room temperature for the given reaction time.

Suzuki–Miyaura Reaction[25]: In every 3 mm round spot, 1.5 µL of a 0.2 M solution of Na_2PdCl_4 in H_2O and 1.5 µL of a 0.5 M solution of dibenzyl diisopropylphosphoramidite in NMP were pipetted and incubated for 15 min at room temperature to form the precatalyst (40 nL each for 900 µm pattern).[26] Subsequently, 3 µL of a solution containing 0.5 M boronic acid derivate in NMP and 2 µL of saturated Na_2CO_3 solution were added and the DMA was incubated in the dark at room temperature for 18 h (80 and 40 nL for 1 mm pattern). To avoid the evaporation of the solvents during the incubation step the printed slide was immediately placed into a Petri dish (Corning, USA) with controlled humidity. The humidified Petri dish was prepared by placing a wetted humidifying pad, saturated with a mixture of deionized water and NMP, on the lid of the Petri dish. The lid was then securely sealed with Parafilm (Bemis, USA). Subsequently, the Petri dish containing the slide was allowed to incubate at room temperature for the given reaction time. The reactions were ended by washing off the mixtures with acetone and ethanol prior to immersion of the whole slide in a 0.1 M solution of KCN in DMSO/water (1:1) for 3 h to remove the precipitated palladium. Only water and ethanol were used for the final washing step. The waste was collected separately and treated with hydrogen peroxide and sodium hydroxide.

Cleavage from the Surface[12]: To release the compounds from the polymer layer for analysis, the hydrophilic spots (round, d = 3 mm) were filled with 5 µL deionized water and irradiated with UV light at 365 nm in a Biolink BLX UV chamber (Witec AG, Sursee, Switzerland) with 2.5 mW cm^{-2} at 365 nm for 20 min. The solution was then pipetted off the surface and each spot was filled with additional 5 µL of DMF to dissolve all cleavage products from the polymer layer. The water and DMF were combined and analyzed via LC-MS. For the smaller 1 mm spot sized 100 nL of water was dispensed in every spot.

LC-MS Analysis[12]: The following LC-MS setup was used: HP Series 1100 (Hewlett-Packard, Palo Alto, USA) with a 100 × 4.60 mm Kinetex 2.6 µm XB-C18 100 Å column and DAD and API-MS detector. Set flow rate was 1 mL min^{-1} and the mobile phase gradient was starting at 10% acetonitrile (containing 0.1 v% formic acid) in water (containing 0.1 v% formic acid) and increased to 99% over 10 min, remained constant for 5 min and decreased back to 10% over 5 min. Spectra were analyzed via "Spectrus Processor" software (Advanced Chemistry Development Inc., Toronto, Canada). The UV detector wavelength for purity analysis was 280 nm. Injected sample volumes were 10 µL.

Palladium Removal from Polymer Film: Sufficient removal of the precipitated palladium from the polymer film via washing with a 0.1 M solution of KCN in DMSO/water (1:1) for 1 h was investigated using EDX spectroscopy. Measurement was carried out on a system containing of a "LEO 1530" REM by Zeiss (Jena, Germany) and a "NORAN System SIX" EDX by Thermo Electron GmbH (Karlsruhe, Germany). Samples were coated with 5 nm platinum on an EM ACE600 by Leica (Wetzlar, Germany) prior to the analysis.

After the immersion in the KCN solution, spots of 3 different products were filled with 5 µL water each and irradiated with UV light to cleave off the compounds. The solution was then analysed via inductively coupled plasma optical emission spectrometry (ICP-OES) to determine the amount of residual palladium. The 3 samples showed a Pd content of 35 ± 4 ppm, indicating a sufficient removal of the catalyst.

Synthesis of Standard Compound for Kinetic Analysis: Compound **26** was synthesized in flask, purified and measured as standard for quantitative analysis of photoinduced cleavage. Related structures and plotted NMR spectra can be found in Supporting Information 4 and 6.

*Synthesis of Intermediate **26a***: To 5-bromo-2-methyl benzoic acid (1.00 g, 4.65 mmol, 1.00 equiv) in methanol (30 mL) was added p-toluenesulfonic acid (44 mg, 0.23 mmol, 0.05 equiv) and the reaction mixture was refluxed under stirring for 18 h. Then stirring was stopped and the solvent was removed under reduced pressure to get the crude product as a colorless liquid. It was purified by column chromatography using

silica gel (mesh 60–120) as a stationary phase and cyclohexane/ethyl acetate (10:1, v/v) as eluent. After removing the solvent under reduced pressure, 756 mg (3.30 mmol, 71%) of a colorless liquid was obtained. NMR spectroscopy: ^{1}H NMR (500 MHz, CHLOROFORM-d) δ in ppm: 2.57 (s, 3 H), 3.92 (s, 3 H), 7.15 (d, J = 8.09 Hz, 1 H), 7.54 (dd, J = 8.16, 2.21 Hz, 1 H), 8.07 (d, J = 2.14 Hz, 1 H). ^{13}C NMR (126 MHz, CHLOROFORM-d) δ in ppm: 21.23, 52.12, 119.12, 131.18, 133.31, 133.36, 134.82, 139.20, 166.71.

Synthesis of Intermediate ***26b***: A solution of palladium(II) acetate (2 mg, 0.01 mmol, 0.01 equiv) and triphenylphosphine (16 mg, 0.06 mmol, 0.05 equiv) in 6 mL tetrahydrofuran/water (9:1, v/v) was stirred under an argon atmosphere for 10 min at room temperature. Then phenylboronic acid (160 mg, 1.31 mmol, 1.20 equiv), **26a** (250 mg, 1.09 mmol, 1.00 equiv) and potassium carbonate (452 mg, 3.27 mmol, 3.00 equiv) were added to the solution and the reaction mixture was refluxed under argon atmosphere for 18 h. Then stirring was stopped and the solvent was removed under reduced pressure to obtain the crude product. It was purified by column chromatography using silica gel (mesh 60–120) as a stationary phase and cyclohexane/ethyl acetate (10:1, v/v) as eluent. After removing the solvent under reduced pressure, 185 mg (0.82 mmol, 82%) of a colorless liquid was obtained. NMR spectroscopy: ^{1}H NMR (500 MHz, CHLOROFORM-d) δ in ppm: 2.67 (s, 3 H), 3.95 (s, 3 H), 7.35 (d, J = 7.93 Hz, 1 H), 7.36–7.41 (m, 1 H), 7.48 (t, J = 7.20 Hz, 2 H), 7.61–7.68 (m, 3 H), 8.19 (d, J = 1.98 Hz, 1 H). ^{13}C NMR (126 MHz, CHLOROFORM-d) δ in ppm: 21.43, 51.94, 123.14, 126.97, 127.48, 128.86, 129.22, 130.44, 132.25, 144.93, 169.13, 170.13, 180.15, 191.62.

Synthesis of Standard Compound ***26***: To a solution of **26b** (185 mg, 0.82 mmol, 1.00 equiv) in 5 mL dichloromethane/methanol (9:1, v/v) was added a solution of sodium hydroxide (131 mg, 3.28 mmol, 4.00 equiv) in 5 mL methanol. The reaction mixture was stirred for 2 h at room temperature. The solvents were then removed under reduced pressure, the residue was diluted with water. The aqueous solution was then cooled, acidified to pH 2 with dilute hydrochloric acid. A white solid precipitated after the addition of the hydrochloric acid. The precipitate was isolated by filtration and redissolved in ethyl acetate. The solvent was removed under reduced pressure to obtain 133 mg (0.63 mmol, 77%) of a white powder. NMR spectroscopy: ^{1}H NMR (500 MHz, DMSO-d6) δ in ppm: 2.57 (s, 3 H), 7.37–7.43 (m, 2 H), 7.49 (t, J = 7.71 Hz, 2 H), 7.68 (d, J = 7.37 Hz, 2 H), 7.76 (dd, J = 7.93, 2.14 Hz, 1 H), 8.08 (d, J = 2.14 Hz, 1 H), 12.98 (br s, 1 H). ^{13}C NMR (126 MHz, DMSO-d6) δ in ppm: 14.54, 21.36, 40.24, 40.41, 40.57, 51.50, 80.78, 97.35, 113.91, 126.98, 129.52, 130.23, 138.75, 196.72.

Photorelease Kinetics Analysis: A DMA with on chip synthesized compound **26** was irradiated for different times. After irradiation, the volume of four spots (40 µL in total) was pipetted off the surface and analyzed via LC-MS. To calculate the concentration of **26** in a droplet, a calibration curve was constructed. 4.5 mg of **26** was dissolved in 10 mL DMF/water (1:1). This stock solution was then diluted with different ratios to obtain twelve solutions with different concentrations (50–2000 µM). The UV detector wavelength was 280 nm. Injected sample volumes were 10 µL. Peak areas in the chromatograms at 280 nm corresponding to compound **26** were calculated to obtain the modified extinction coefficient.

Nano-ESI MS Analysis: Compounds were released from the from the polymer layer for high-resolution electrospray ionization mass spectrometry (ESI-MS) analysis. The combined volume of 5 spots (50 µL in total, DMF/water; 1:1) was pipetted off the surface and analyzed via ESI-MS. Measurement was carried out using a ThermoFisher MSD (QExactive Plus). The following parameters were used: drying gas temperature: 80 K, capillary temperature: 350 K, probe heater temperature: 120 K, capillary voltage: 8000 V. Spectra were analyzed via "Spectrus Processor" software (Advanced Chemistry Development Inc., Toronto, Canada).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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nanomolar, on-chip, palladium, Suzuki–Miyaura

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[1] F. Pammolli, L. Magazzini, M. Riccaboni, *Nat. Rev. Drug Discovery* **2011**, *10*, 428.
[2] A. A. Popova, C. Depew, K. M. Permana, A. Trubitsyn, R. Peravali, J. Á. G. Ordiano, M. Reischl, P. A. Levkin, *SLAS Technol.* **2017**, *22*, 163.
[3] A. A. Popova, K. Demir, T. G. Hartanto, E. Schmitt, P. A. Levkin, *RSC Adv.* **2016**, *6*, 38263.
[4] A. A. Popova, S. M. Schillo, K. Demir, E. Ueda, A. Nesterov-Mueller, P. A. Levkin, *Adv. Mater.* **2015**, *27*, 5217.
[5] A. A. Popova, T. Tronser, K. Demir, P. Haitz, K. Kuodyte, V. Starkuviene, P. Wajda, P. A. Levkin, *Small* **2019**, *15*, 1901299.
[6] T. Tronser, K. Demir, M. Reischl, M. Bastmeyer, P. A. Levkin, *Lab Chip* **2018**, *18*, 2257.
[7] W. Lei, K. Demir, J. Overhage, M. Grunze, T. Schwartz, P. A. Levkin, *Adv. Biosyst.* **2020**, *4*, 2000073.
[8] W. Lei, J. Bruchmann, J. L. Rüping, P. A. Levkin, T. Schwartz, *Adv. Sci.* **2019**, *6*, 1900519.
[9] A. A. Popova, D. Marcato, R. Peravali, I. Wehl, U. Schepers, P. A. Levkin, *Adv. Funct. Mater.* **2018**, *28*, 1703486.
[10] A. Rosenfeld, M. Brehm, A. Welle, V. Trouillet, S. Heissler, M. Benz, P. A. Levkin, *Mater. Today Bio* **2019**, *3*, 100022.
[11] M. Benz, M. R. Molla, A. Böser, A. Rosenfeld, P. A. Levkin, *Nat. Commun.* **2019**, *10*, 2879.
[12] M. Brehm, S. Heissler, S. Afonin, P. A. Levkin, *Small* **2020**, *16*, 1905971.
[13] N. Miyaura, A. Suzuki, *J. Chem. Soc., Chem. Commun.* **1979**, *19*, 866.
[14] N. Miyaura, K. Yamada, A. Suzuki, *Tetrahedron Lett.* **1979**, *20*, 3437.
[15] N. Schneider, D. M. Lowe, R. A. Sayle, M. A. Tarselli, G. A. Landrum, *J. Med. Chem.* **2016**, *59*, 4385.

[16] D. G. Brown, J. Boström, *J. Med. Chem.* **2016**, *59*, 4443.
[17] N. Gesmundo, K. Dykstra, J. L. Douthwaite, Y.-T. Kao, R. Zhao, B. Mahjour, R. Ferguson, S. Dreher, B. Sauvagnat, J. Saurí, T. Cernak, *Nat Synth* **2023**, https://doi.org/10.1038/s44160-023-00351-1.
[18] A. B. Theberge, G. Whyte, M. Frenzel, L. M. Fidalgo, R. C. R. Wootton, W. T. S. Huck, *Chem. Commun.* **2009**, *41*, 6225.
[19] E. S. Isbrandt, R. J. Sullivan, S. G. Newman, *Angew. Chem., Int. Ed.* **2019**, *58*, 7180.
[20] R. J. Edsall, H. A. Harris, E. S. Manas, R. E. Mewshaw, *Bioorg. Med. Chem.* **2003**, *11*, 3457.
[21] K. Connor, K. Ramamoorthy, M. Moore, M. Mustain, I. Chen, S. Safe, T. Zacharewski, B. Gillesby, A. Joyeux, P. Balaguer, *Toxicol. Appl. Pharmacol.* **1997**, *145*, 111.
[22] A. Khalid, A. S. Khan, *Phytochemistry* **1984**, *23*, 765.
[23] D. A. G. Cortez, B. A. A. Filho, C. V. Nakamura, B. P. D. Filho, A. Marston, K. Hostettmann, *Pharm. Biol.* **2002**, *40*, 485.
[24] C. Chizzali, L. Beerhues, *Beilstein J. Org. Chem.* **2012**, *8*, 613.
[25] R. Frei, A. S. Breitbach, H. E. Blackwell, *Chem. Sci.* **2012**, *3*, 1555.
[26] M. Guo, Q. Zhang, *Tetrahedron Lett.* **2009**, *50*, 1965.