KIT, Campus North, Institute of Functional Interfaces (IFG)

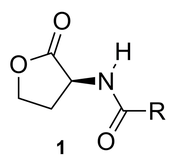
Synthesis of isotope labeled *N*-acyl-L-homoserine lactones (AHLs)

Dorota Jakubczyk[a,b], Stefan Bräse[a], Gerald Brenner-Weiß[b]

[a] *Karlsruhe Institute of Technology, Institute of Organic Chemistry, Fritz-Haber-Weg 6, 76131 Karlsruhe, Germany, Fax: +49 721 608 8581;E-mail:braese@kit.edu;*
[b] *Karlsruhe Institute of Technology, Institute of Functional Interfaces, Hermann von Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany*

Introduction

***N*-acyl-L-homoserine lactones 1 (AHLs)** are natural products which



R – acyl or alkyl chain

belong to **semiochemicals** (signal molecules or infochemical compounds). They act as messengers within (**pheromones**) or between (**allomones**) species.

AHL is so-called quorum sensing molecule, which enables inter-bacterial and inter-kingdom communication (Inter-kingdom Signalling).

Inter-bacterial comunication - biofilm formation

Biofilm - an aggregate of microorganisms (Fig. 1)

- Common cause of persistent infections
- Chronic, destructive inflammatory processes
- Antibiotic resistant

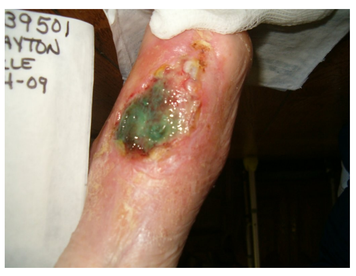
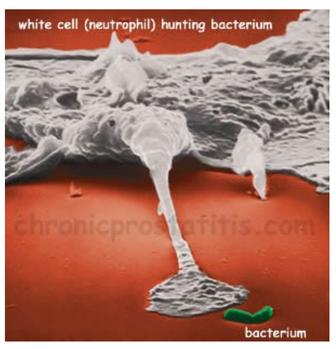


Fig. 1. Biofilm



Inter-kingdom Signalling

Interacts with a variety of mammalian cells:

- Induction of apoptosis
- Induces the chemotaxis of neutrophils (Fig. 2)

Fig. 2. Chemotaxis of neutrophils

Aim

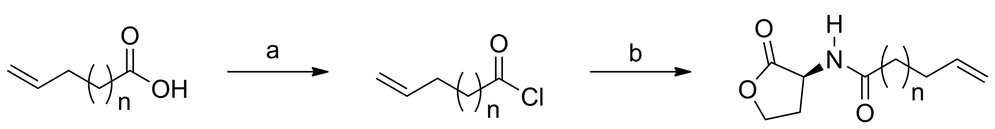
Synthesis and isotopic labeling of *N*-acyl-L-homoserine lactones - detection of AHLs crossing eukaryotic cell membranes

⇩

Elucidating the mechanism of Inter-kingdom Signalling

Results and discussion

1.1. Synthesis of terminally unsaturated *N*-acyl-L-homoserine lactones – substrates for the isotopic labeling (scheme 1).



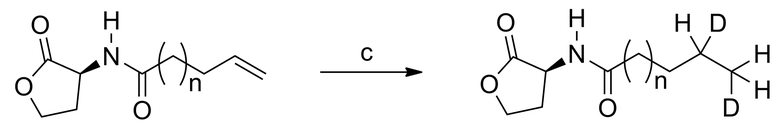
Scheme 1. a) Oxalyl chloride, hexan, RT-45°C; b) L-homoserine lactone hydrobromide, Et_3N, CH_2Cl_2, 0°C-RT; n = 1, 3, 4, 6.

Entry	n	Yield (%)		Time (h)	
		Step a	Step b	Step a	Step b
1	1	80	63	20	4,5
2	3	56	52	22	5
3	4	45	51	21	4,5
4	6	71	88	22	5,5

Table 1. Results for the first two steps – the synthesis of the substrates for the isotopic labeling.

Results and discussion

1.2. Deuterium labeling via catalytic reduction of the double bond (scheme 2).



Scheme 2. c) $Pd(OAc)_2$, THF, CH_3COOH, MeOH, $NaBD_4$, $NaOH_{aq}$, -196 °C – RT.

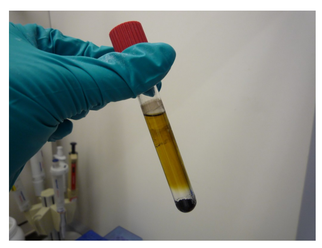
Entry	n	Time (h)	Yield (%)	D content (%)[a] pre-terminal C	D content (%)[a] terminal C
1	1	18	90	90	>99
2	3	19	86	72	>99
3	4	16	83	65	>99
4	6	20	92	85	>99

Table 2. Results for the deuterium labeling of the terminally unsaturated AHLs; [a] Determined by ^{1}HNMR and mass spectrometry.

Unconventional conditions of the reaction (Fot. 1, 2, 3).

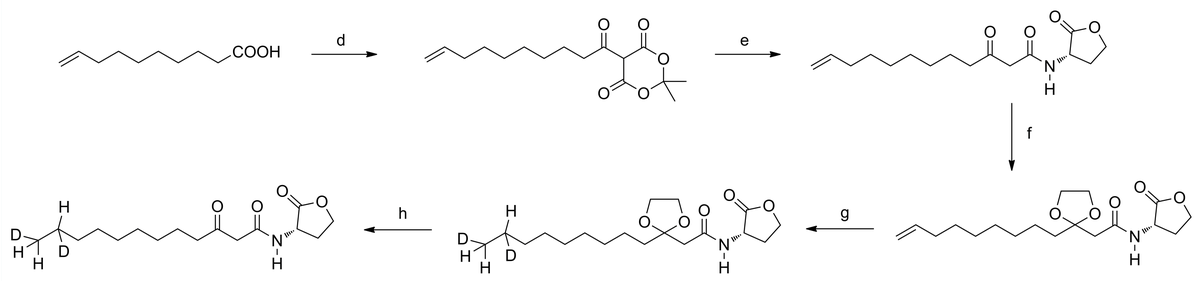
Fot. 1. Reaction starts in the liquid nitrogen.

Fot. 2. Agitation in a room temperature.



Fot. 3. Reduced palladium residue.

2.1. Synthesis of a highly biologically active, deuterium labeled AHL: *N*-(3-oxododecanoyl)-L-[D_2]-homoserine lactone (scheme 3).



Scheme 3. d) 4-Dimethylaminopyridine (DMAP); 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC); CH_2Cl_2;RT; e) L-homoserine lactone hydrobromide, Et_3N; CH_3CN; RT-80°C; f) Ethylene glycol, *p*-TsOH, $CH(OMe)_3$, PhMe, 110°C-RT; g) $Pd(OAc)_2$, THF, CH_3COOH, MeOH, $NaBD_4$, $NaOH_{aq}$, -196 °C – RT; h) $HClO_4$, CH_2Cl_2, 0°C-RT.

	Step				
	d	e	f	g	h
Yield (%)	76	63	64	79	91
Time (h)	5	6	22	17	2,5
D content (%)[a] pre-terminal C	-	-	-	66	-
D content (%)[a] terminal C	-	-	-	>99	-

Table 3. Results for the synthesis of the *N*-(3-oxododecanoyl)-L-[D_2]-homoserine lactone ; [a] Determined by ^{1}HNMR and mass spectrometry.

Conclusions

- The new methods of isotopic labeling of AHL was developed. The methods are efficient and enable further biological investigations;
- Structures of the products were confirmed by TLC, ^{1}H NMR, ^{13}C NMR, ESI-TOF MS, HRMS, IR and Raman spectroscopy, elemental analysis and optical rotation.

References

[1] Schwarzmann, G. *Biochim. Biophys. Acta* **1978**, 529, 106;
[2] Chhabra, S. R.; Stead, P.; Bainton, N. J.; Salmond, G. P. C.; Stewart, G. S. A. B.; Williams, P.; Bycroft, B. W. *J. Antibiot.* **1993**, 46, 441;
[3] Costerton J. W., Stewart Philip S., Greenberg E. P., *Science*, **1999**, 284(5418), 1318 – 1322;
[4] Chhabra, S. R., C. Harty, et al., *Journal of Medicinal Chemistry*, **2003**, 46(1) 97-104.