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Synthesis and hydrogen isotope labelling of *N*-acyl-L-homoserine lactones (AHLs)

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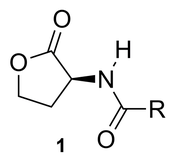
Introduction

***N*-acyl-L-homoserine lactones 1 (AHLs)** are natural products which belong to **semiochemicals** (signal molecules or infochemical compounds). They act as messengers within (**pheromones**) or between (**allomones**) species.



R – acyl or alkyl chain

1

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

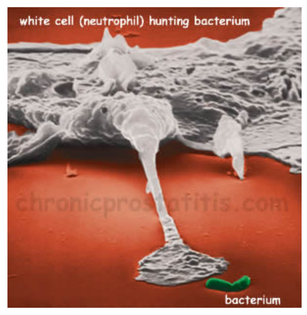


Fig. 1. Chemotaxis of neutrophils

Inter-kingdom Signalling

Interactions with a variety of mammalian cells:

- Induction of apoptosis
- Induces the chemotaxis of neutrophils (Fig. 1)

Deuterium and tritium labelled compounds have several important applications in many branches of science e.g. in the investigation of drug metabolism, biological activity, reaction mechanisms or kinetic studies.

Contrary to most of the isotopic labelling methods based especially on the use of tritium gas, this procedure does not require sophisticated apparatuses, complicated work-up and the tritium gas is generated *in situ* from a solid reagent.
Moreover the uncommon catalytic reduction of the double bond is presented herein.

Aim

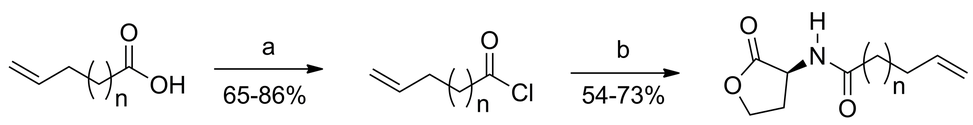
Synthesis and hydrogen isotope labelling of *N*-acyl-L-homoserine lactones

⇨ Detection of AHLs crossing eukaryotic cell membranes

⇨ Elucidating the mechanism of Inter-kingdom Signalling

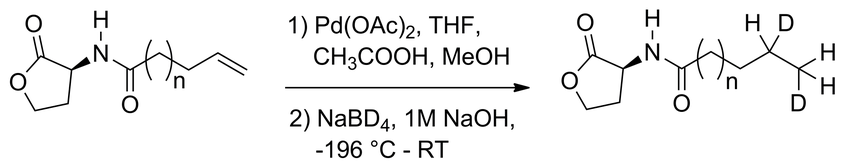
Results and discussion

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



Scheme 1. a) Oxalyl chloride, hexane, RT-45°C, 20-22 h; b) L-homoserine lactone hydrobromide, Et_3N, CH_2Cl_2, 0°C-RT, 4,5-5,5 h; n = 1, 3, 4, 6.

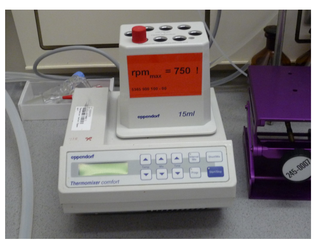
1.2. Deuterium labelling via catalytic reduction of the double bond (scheme 2)



Scheme 2. Deuterium labelling of AHLs via catalytic reduction of the double bond (See Table 1).

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

Fot. 1. Reaction starts in the liquid nitrogen



Fot. 2. Agitation in the thermomixer

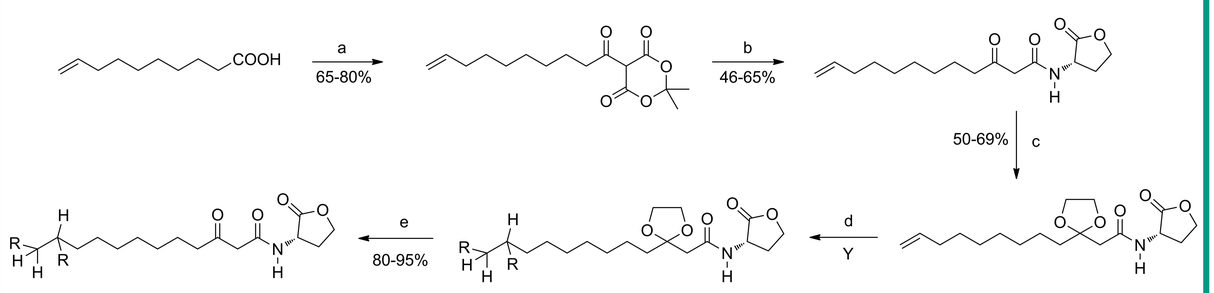
Fot. 3. Special control area for radiation

Results and discussion

Table 1. Results for the deuterium labelling of the terminally unsaturated AHLs; [a] Determined by ^{1}HNMR and mass spectrometry; [b] Determined by ESI-TOF MS.

Entry	n	Time [h]	Additives, notes	Other deuterated reagents	Yield [%]	D content[a] at preterminal C atom [%]	D content[a] at terminal C atom [%]	Isomers[b]
1	1	25	H_2O	-	56	34-56	99	$[M+(1,2,3,4)+H^+]$ $[M-(1,2)+H^+]$
2	1	22,5	excess of $NaBD_4$	D_2O , MeOD, 1M NaOD in D_2O	94	96	99	$[M+(1,2,3,4,5,6)+H^+]$ $[M-(1,2)+H^+]$
3	1	17	excess of $NaBD_4$	MeOD, 30% NaOD in D_2O diluted in MeOD (1 M NaOD)	89	70	99	$[M+(1,2)+H^+]$ $[M-(1,2)+H^+]$
4	3	22	H_2O	-	83	54	99	$[M+(1,2,3,4)+H^+]$ $[M-(1,2)+H^+]$
5	4	18	H_2O	-	74	41	99	$[M+(1,2,3,4)+H^+]$ $[M-(1,2)+H^+]$
6	6	20	H_2O	-	94	51	99	$[M+(1,2,3,4)+H^+]$ $[M-(1,2)+H^+]$
7	6	23	excess of $NaBD_4$	D_2O, CD_3COOD, MeOD,1M NaOD in D_2O	90	69	99	$[M+(1,2,3,4,5,6)+H^+]$ $[M-(1,2)+H^+]$

2.1. Synthesis of a highly biologically active, deuterium and tritium labelled *N*-(3-oxododecanoyl)-L-homoserine lactones (scheme 3)



Scheme 3. a) 4-Dimethylaminopyridine (DMAP); 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC); CH_2Cl_2;RT; b) L-homoserine lactone hydrobromide, Et_3N; CH_3CN; RT-80°C; c) Ethylene glycol, *p*-TsOH, $CH(OMe)_3$, PhMe, 110°C-RT; d) $Pd(OAc)_2$, THF, CH_3COOH, MeOH, $NaBR_4$, $NaOH_{aq}$, -196 °C – RT; e) $HClO_4$, CH_2Cl_2, 0°C-RT; R = H, D or T; Y = yield (see Table 2).

Table 2. Results for the synthesis of the deuteriumand tritum labelled *N*-(3-oxododecanoyl)-L-homoserine lactone ; [a] Determined by ^{1}HNMR and mass spectrometry; [b] Determined by ESI-TOF MS; [c] Determined by Liquid Scintillation counter.

			Step d						
Entry	R	Time [h]	Additives,notes	Other deuterated reagents	Yield [%]	D content[a] at preterminal C atom [%]	D content[a] at terminal C atom [%]	Isomers[b]	Specific radioactivity[c] [mCi/mmol]
1	H	13	$NaBH_4$	-	81	-	-	-	-
2	D	16	excess of $NaBD_4$	1M NaOD (30% NaOD in D_2O diluted in MeOH)	79	90	99	$[M+(1,2)+H^+]$ $[M-(1,2)+H^+]$	-
3	D	20	excess of $NaBD_4$	MeOD, 30% NaOD in D_2O diluted in MeOD (1 M NaOD)	89	68	99	$[M+(1,2)+H^+]$ $[M-(1,2)+H^+]$	-
4	D	20	$NaBD_4$ $Pd(PPh_3)_4$	-	Failed	-	-	-	-
5	T	16	$NaBT_4$	-	78	95	99	$[M+1+H^+]$ $[M-1+H^+]$	588,5

Conclusion

- The new methods of isotopic labelling of AHL was developed. The method is 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, TLC / autoradiography and Liquid Scintillation Counter.

References

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