

# On the mechanism of inter-kingdom signalling - synthesis of isotope labelled *N*-acyl-L-homoserine lactones (AHLs)

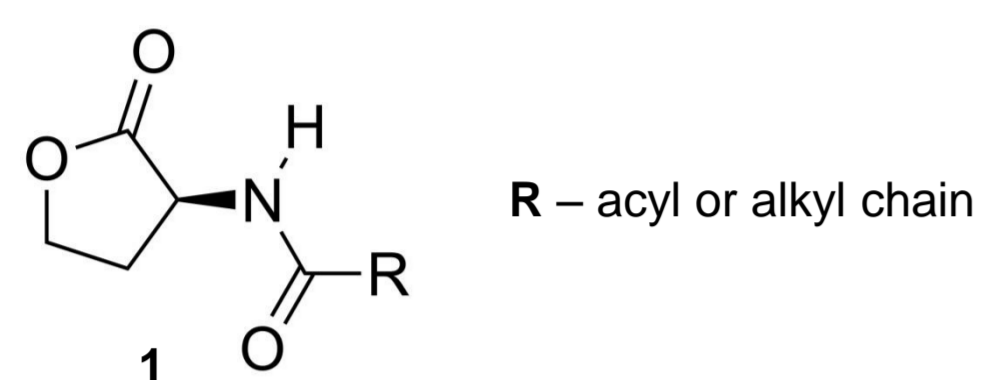
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## Introduction

*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.

AHLs are so-called autoinducer molecules, enabling inter-bacterial communication (**Quorum Sensing**) and inter-kingdom communication (**Inter-kingdom Signalling**).

**Inter-bacterial communication - biofilm formation**

**Biofilm** - an aggregate of microorganisms (Fig. 1-3)

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



Fig. 1. Biofilm



Fig. 2. Biofilm

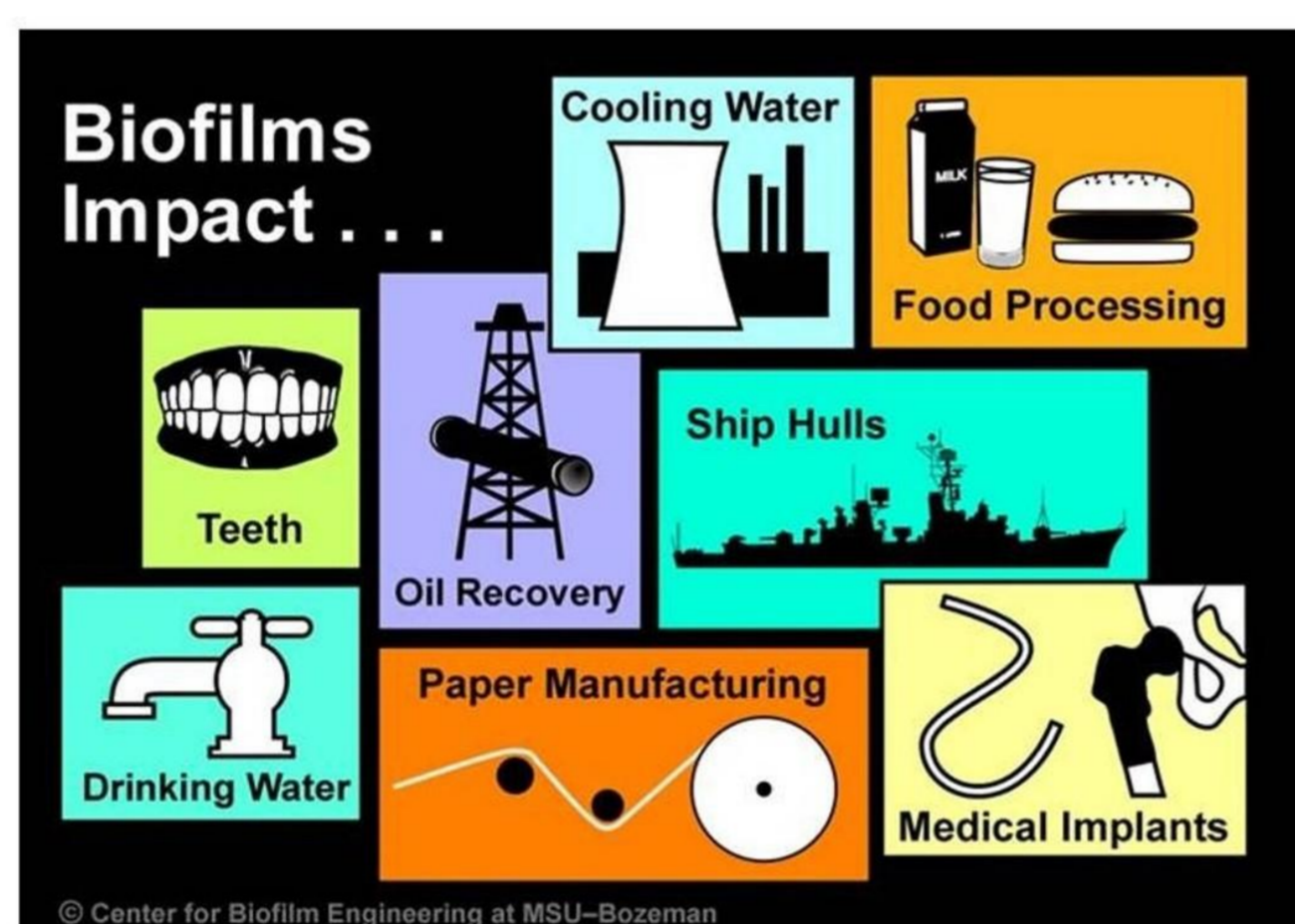
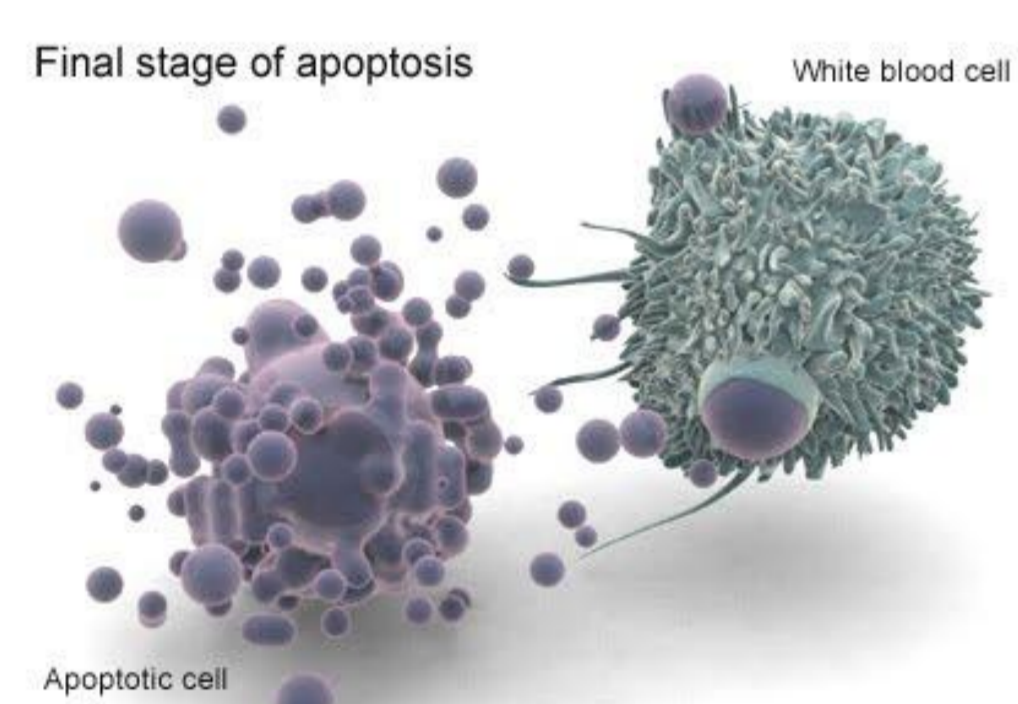


Fig. 3. Occurrence of biofilm

**Inter-kingdom Signalling** – communication between Prokaryotes and Eukaryotes



Interactions of bacteria with a variety of mammalian cells:

- Induction of apoptosis (Fig. 4)
- Induction of the chemotaxis of neutrophils (Fig. 5,6)

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Fig. 4. Apoptosis of the cell

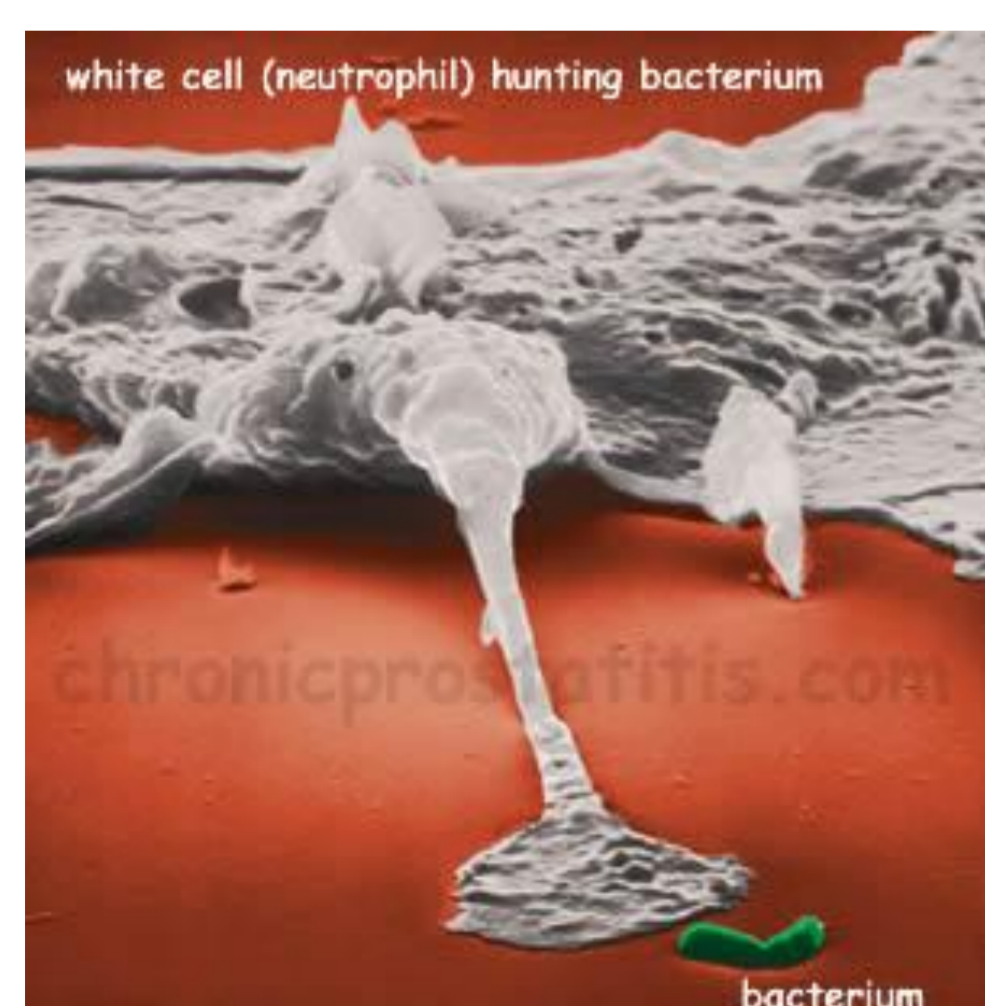


Fig. 5. Chemotaxis of neutrophils

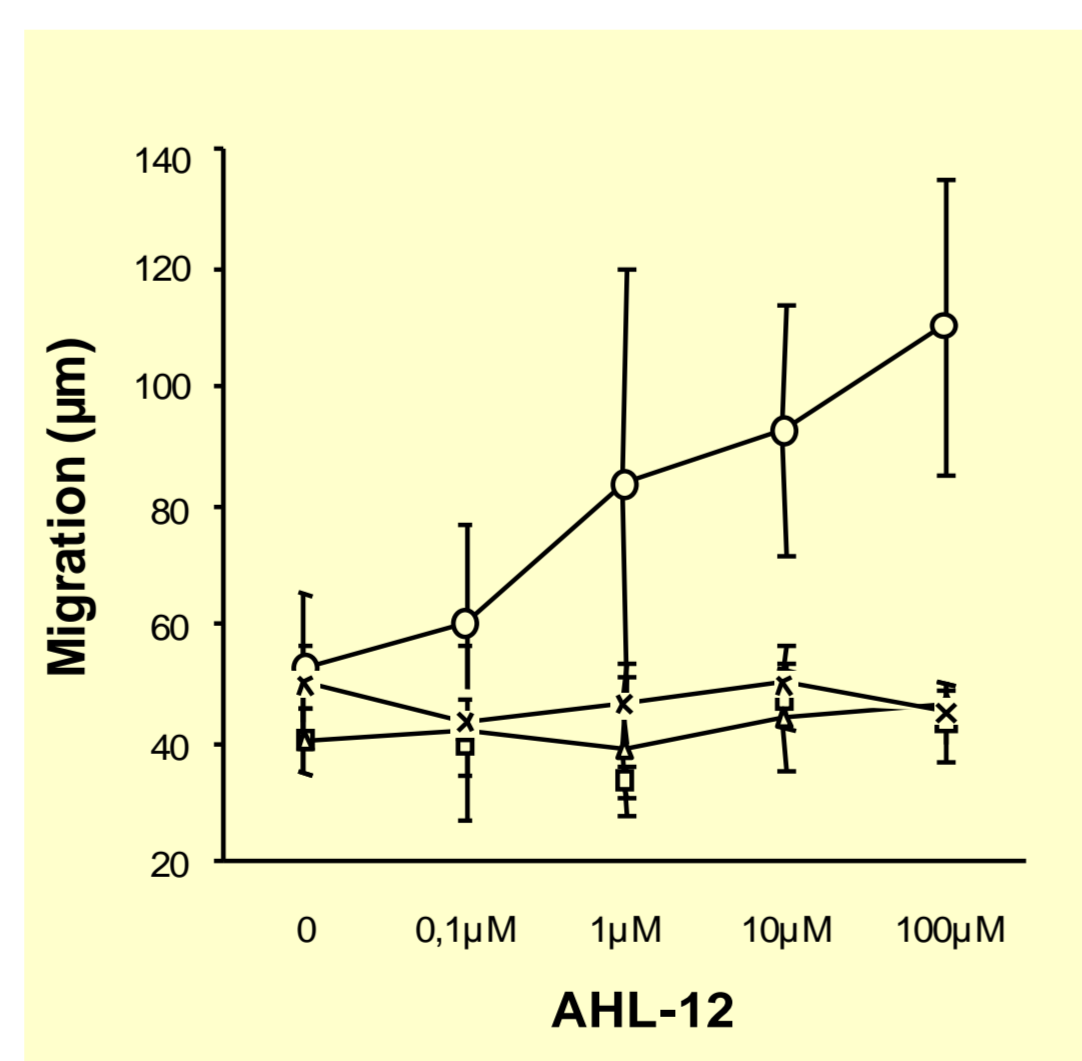


Fig. 6. Migration of human polymorphonuclear neutrophils (PMN) toward increasing concentrations of AHLs with vary chain length.

## Aim

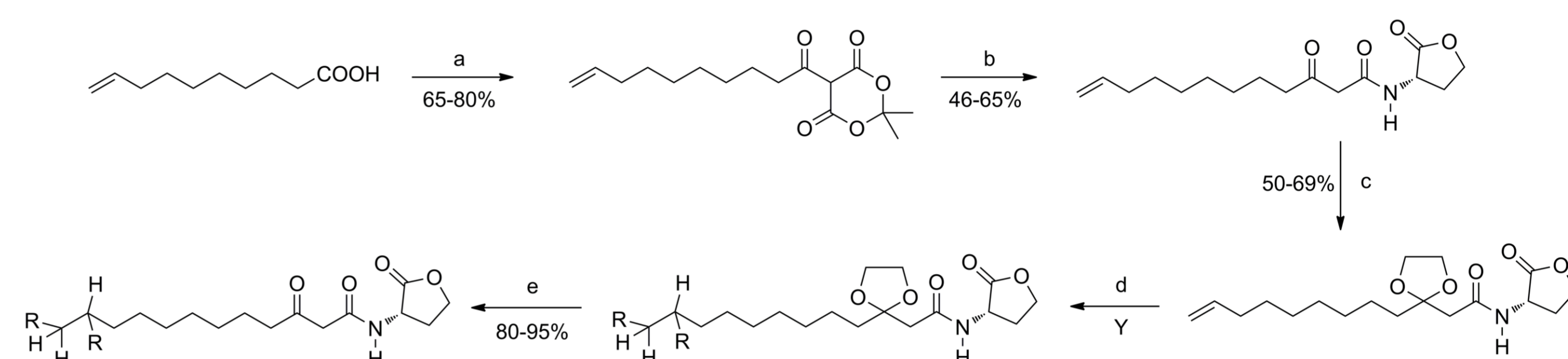
**Synthesis and isotopic labelling of *N*-acyl-L-homoserine lactones - detection of AHLs crossing eukaryotic cell membranes**



Elucidating the mechanism of Inter-kingdom Signalling

## Results and discussion

**Synthesis of a highly biologically active, deuterium and tritium labelled *N*-(3-oxododecanoyl)-L-homoserine lactone (scheme 1).**

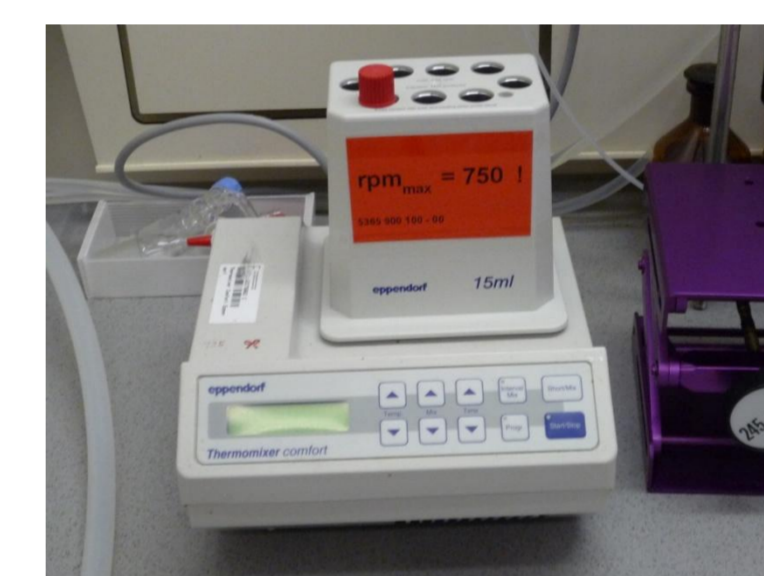


Scheme 1. a) 4-Dimethylaminopyridine (DMAP); 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC);  $\text{CH}_2\text{Cl}_2$ ; RT; b) L-homoserine lactone hydrobromide,  $\text{Et}_3\text{N}$ ;  $\text{CH}_3\text{CN}$ ; RT;  $80^\circ\text{C}$ ; c) Ethylene glycol, *p*-TsOH,  $\text{CH}(\text{OMe})_3$ , PhMe,  $110^\circ\text{C}$ ; d)  $\text{Pd}(\text{OAc})_2$ , THF,  $\text{CH}_3\text{COOH}$ , MeOH,  $\text{NaBR}_4$ ,  $\text{NaOH}_{\text{aq}}$ ,  $-196^\circ\text{C}$ ; e)  $\text{HClO}_4$ ,  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$ ; R = D or T; Y = yield (see Table 1).

**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.

Step d									
Entry	R	Time [h]	Additives notes	Other deuterated reagents	Yield [%]	D content <sup>a</sup> at preterminal C atom [%]	D content <sup>a</sup> at terminal C atom [%]	Isomers <sup>b</sup>	Specific radioactivity <sup>c</sup> [mCi/mmol]
1	D	16	$\text{NaBD}_4$	-	65	45-60	99	$[\text{M}+(1-6)+\text{H}^+]$ $[\text{M}-(1,2)+\text{H}^+]$	-
2	D	16,5	Excess of $\text{NaBD}_4$	1M NaOD (30% NaOD in $\text{D}_2\text{O}$ diluted in MeOH)	79	90	99	$[\text{M}+(1,2)+\text{H}^+]$ $[\text{M}-(1,2)+\text{H}^+]$	-
3	T	16	$\text{NaBT}_4$	-	78	-	-	$[\text{M}+1+\text{H}^+]$ $[\text{M}-1+\text{H}^+]$	588,5

Table 1. Results for the synthesis of the deuterium and tritium labelled *N*-(3-oxododecanoyl)-L-homoserine lactones; <sup>a</sup> Determined by <sup>1</sup>H NMR and mass spectrometry. <sup>b</sup> Determined by ESI-TOF MS. <sup>c</sup> Determined by Liquid Scintillation counter.

**Work with tritium - the radioactive isotope (Fot. 4, 5, 6).**



Fot. 4. Sodium borotritide ( $\text{NaBT}_4$ ).



Fot. 5. Preparative thin layer chromatography.



Fot. 6. Scintillation counter - measurement of radioactivity.

## Conclusions

- The new methods of isotopic labelling of AHL was developed. The methods are efficient and enable further biological investigations;
- Structures of the products were confirmed by TLC, <sup>1</sup>H NMR, <sup>13</sup>C NMR, ESI-TOF MS, HRMS, IR and Raman spectroscopy, TLC / autoradiography and Liquid Scintillation Counter.

## References

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