Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Modified *N*-Acyl-homoserine lactones as chemical probes for the elucidation of plant-microbe interactions

Heike Thomanek,[a] Sebastian T. Schenk,[b] Elke Stein,[b] Karl-Heinz Kogel,[b] Adam Schikora*[b] and Wolfgang Maison*[a]

[a] Pharmaceutical and Medicinal Chemistry, University of Hamburg, Bundesstr. 45, 20146 Hamburg, Germany. Fax: (+) 4940 42838 3477; Tel: (+) 4940 42838 3497; E-mail: maison@chemie.uni-hamburg.de

[b] Institute for Phytopathology and Applied Zoology, University of Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany. Fax: (+) 49641 9937499; Tel. (+) 49641 9937497; E-mail: adam.schikora@agrar.uni-giessen.de

Table of Contents

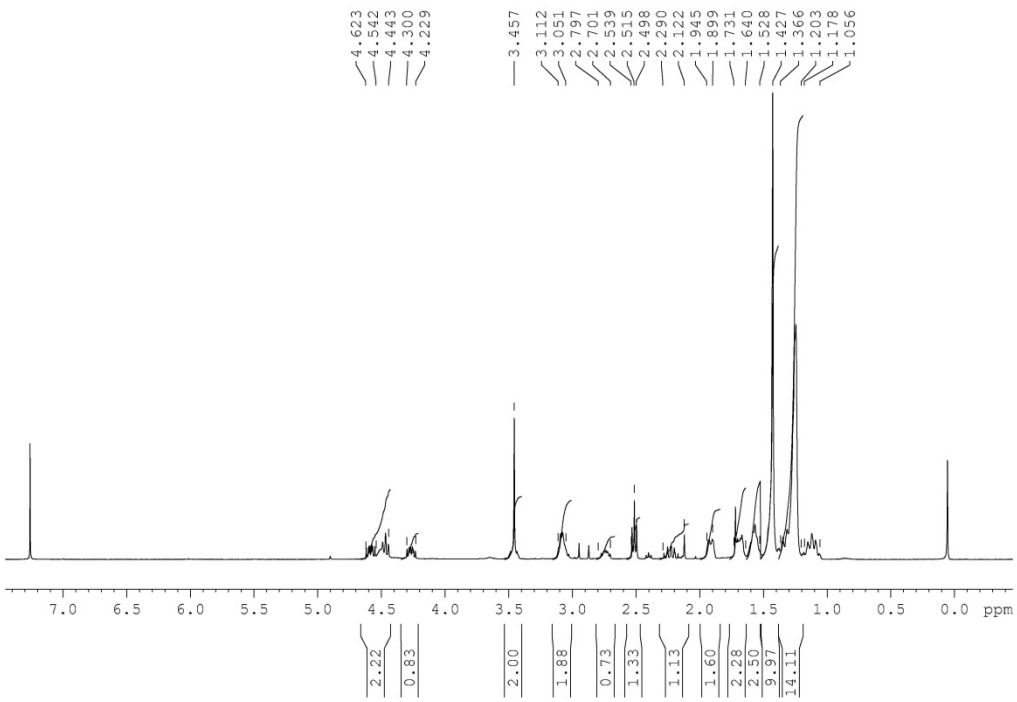
Material and Methods

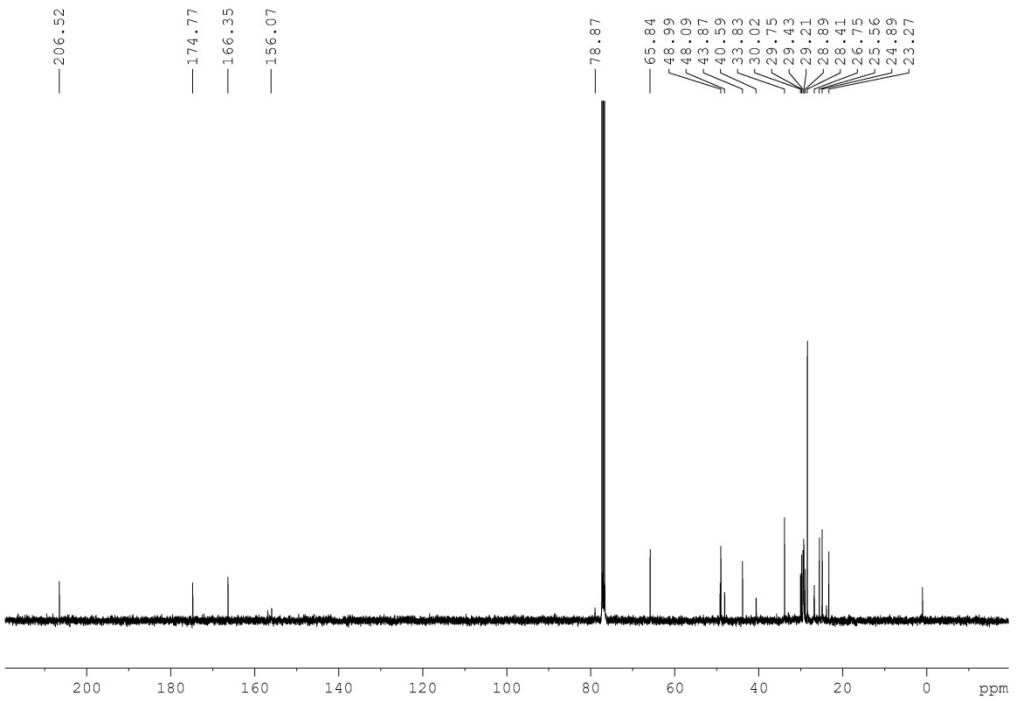
TLC was performed on silica gel aluminum sheets. Reagents used for developing plates include cer-reagent (5 g molybdatophosphoric acid, 2.5 g ceric sulfate tetrahydrate, 25 mL sulfuric acid and 225 mL water), potassium permanganate (0.5% in 1 N NaOH w/v) and detection by UV light was used when applicable. Flash column chromatography was performed on silica gel (60 – 200 µm). ^{1}H chemical shifts are referenced to residual non-deuterated solvent ($CDCl_3$, δ_H = 7.26 ppm; DMSO-d_6, δ_H = 2.50 ppm; CD_3OD, δ_H = 3.31 ppm). ^{13}C chemical shifts are referenced to the solvent signal ($CDCl_3$, δ_C = 77.16 ppm; DMSO-d_6, δ_C = 128.06 ppm; CD_3OD, δ_H = 49.00 ppm). NMR spectra were recorded on 400 (100) MHz instruments. ESI mass spectra were recorded on a TOF instrument operated in positive mode (MicrOTOF Q Bruker; ESI COMPASS 1.3). Samples were dissolved MeOH or H_2O/MeCN-mixtures and directly injected *via* syringe. Analytical HPLC analysis was recorded on a VWR HITACHI ELITE LaChrom L-2130 HPLC (RI Detector: L2490; Programme EZ ChromElite). The following chiral column was used: CHIRALPAK IA (DAICEL Chemical Industries; Particle size: 5µm; Dimensions: 4.6 mm ϕ x 150 mm). Solvents were dried by distillation from sodium under nitrogen atmosphere prior to application.

The following compounds were prepared according to literature procedures: Boc-protected acid **1**,[1] biotin-NHS ester,[2] azido acid **6**,[3] TFA-glycine allylester **11a**,[4] TFA-allyl glycine **12a**,[4] TFA-iodolactone **13a**,[4] Boc-protected iodolactone **13c**,[5] Boc-protected lactone **16c**[5]

NMR-Spectra for new compounds

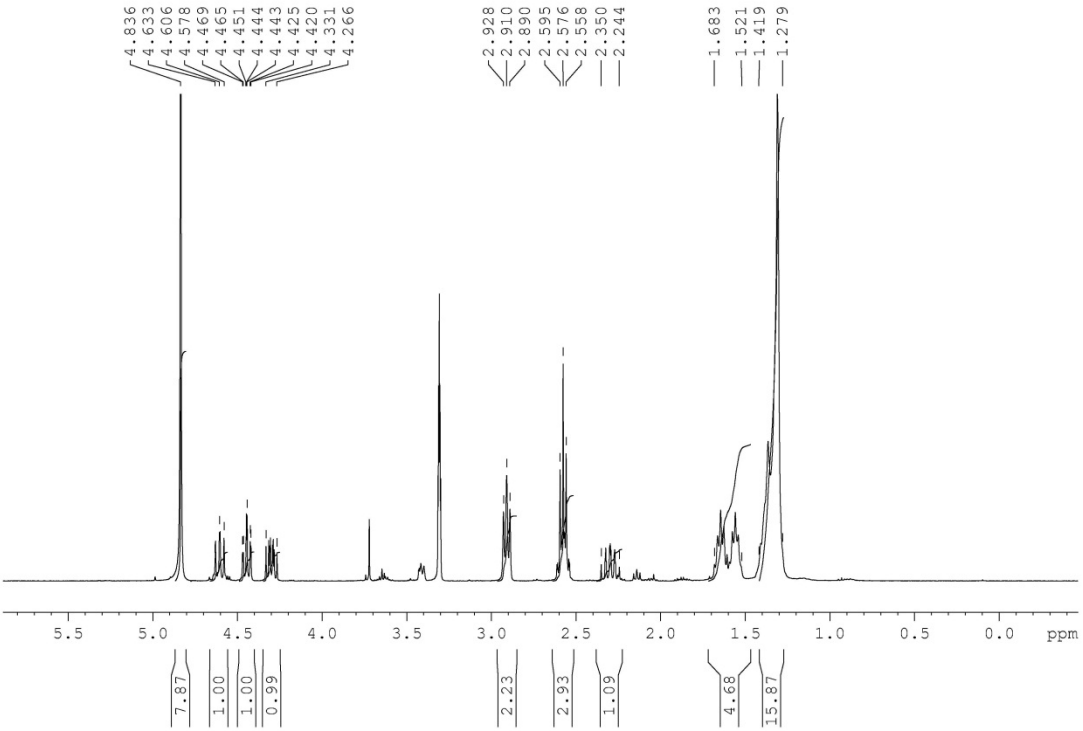
AHL-derivative 2:

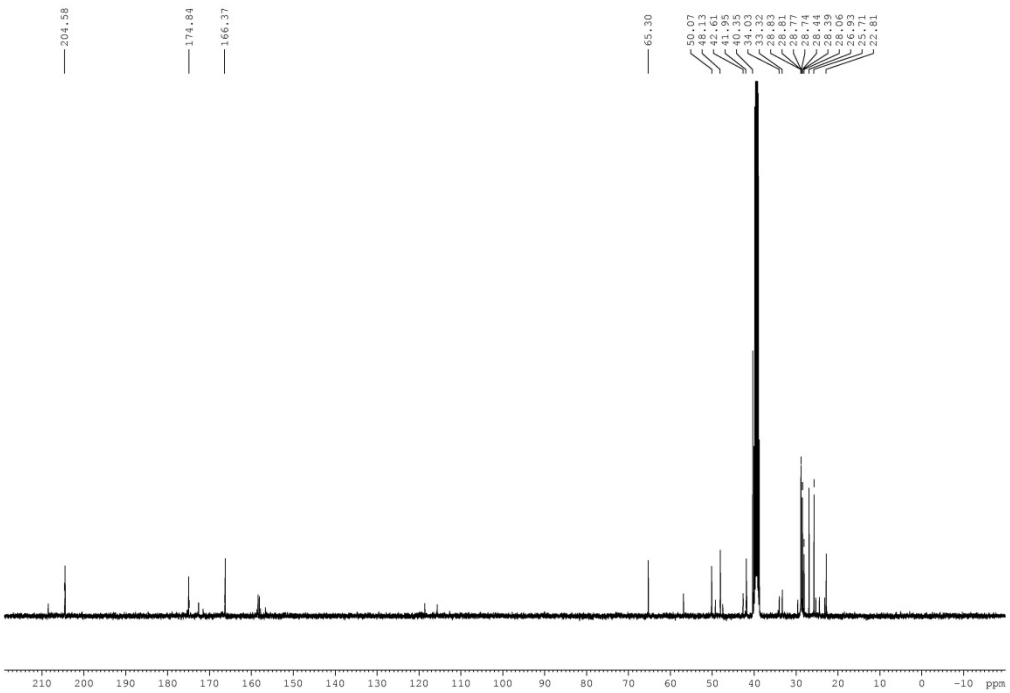




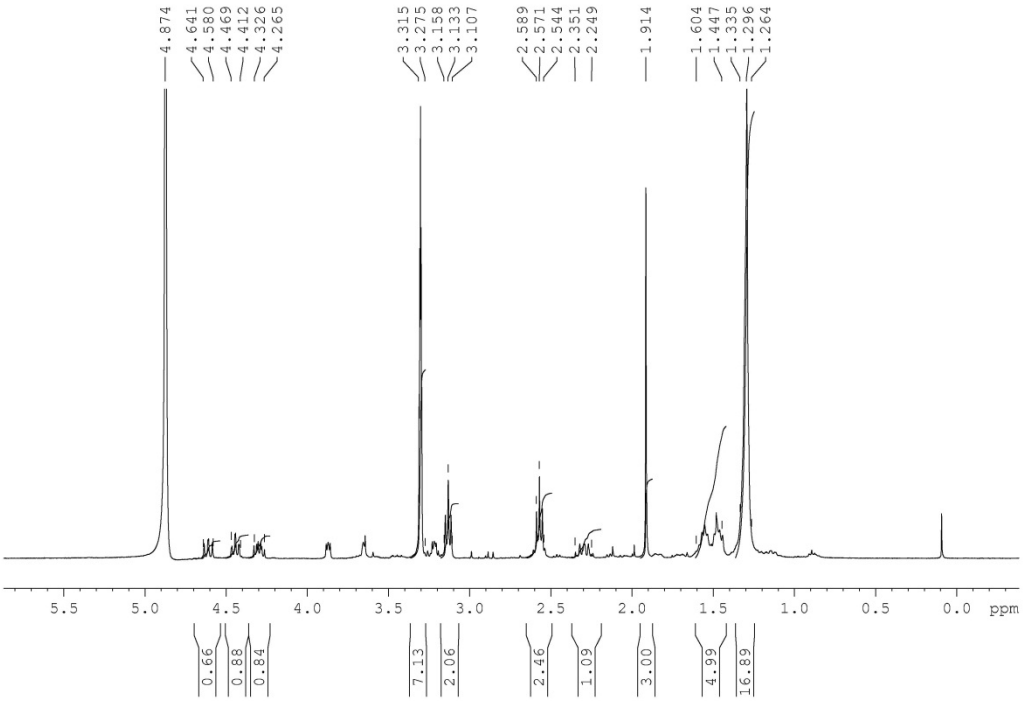
5

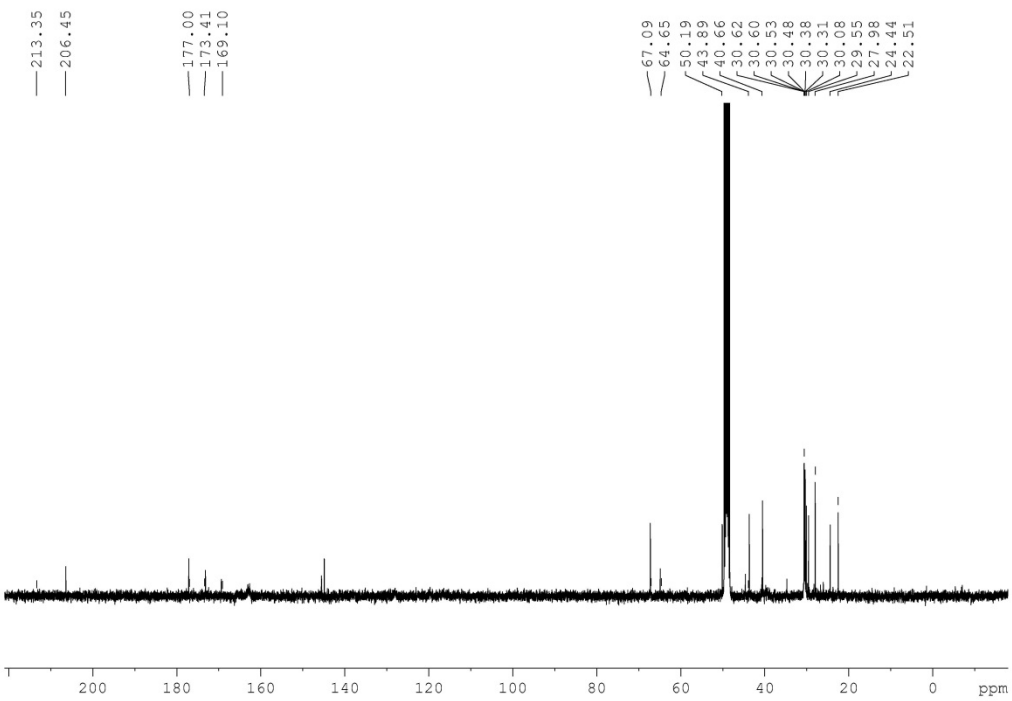
AHL-derivative 3:





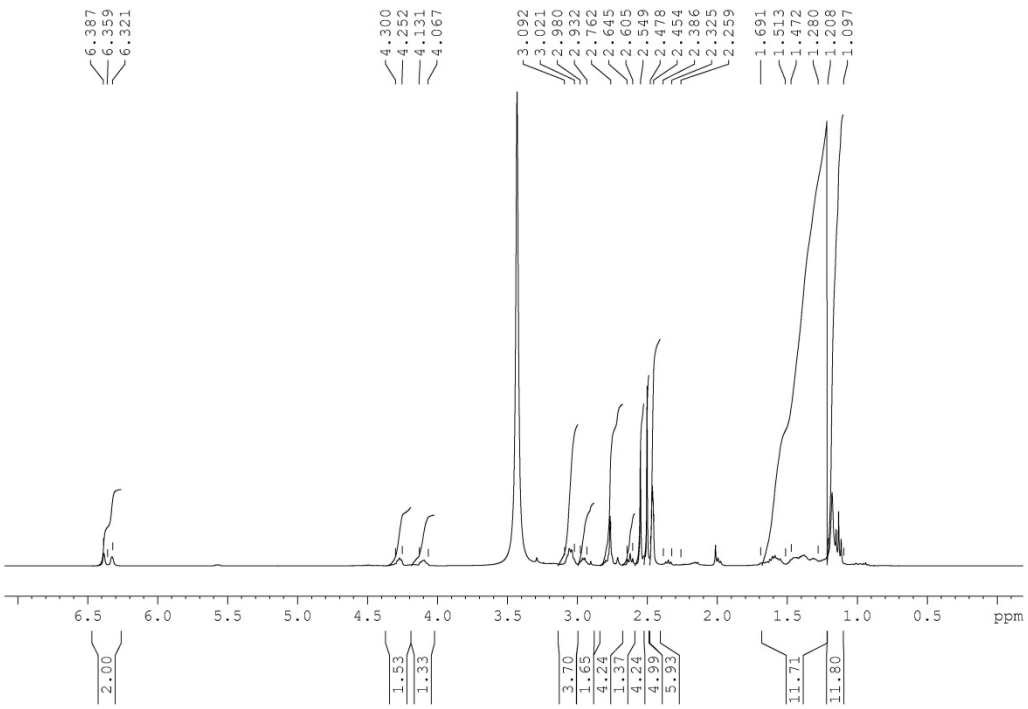
AHL-derivative 4:

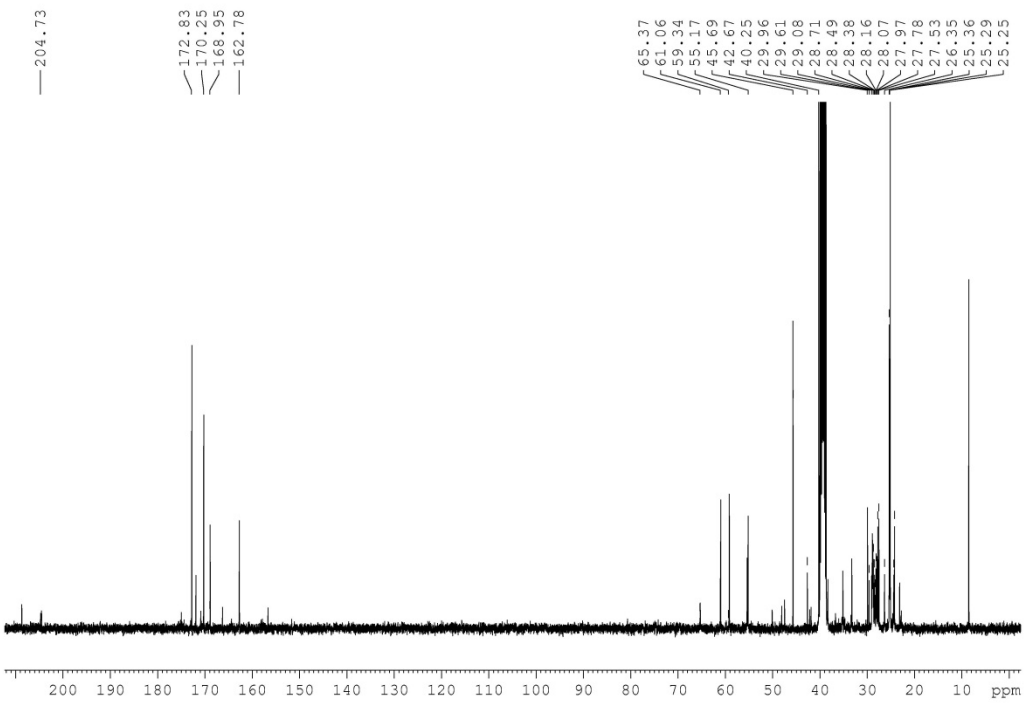




5

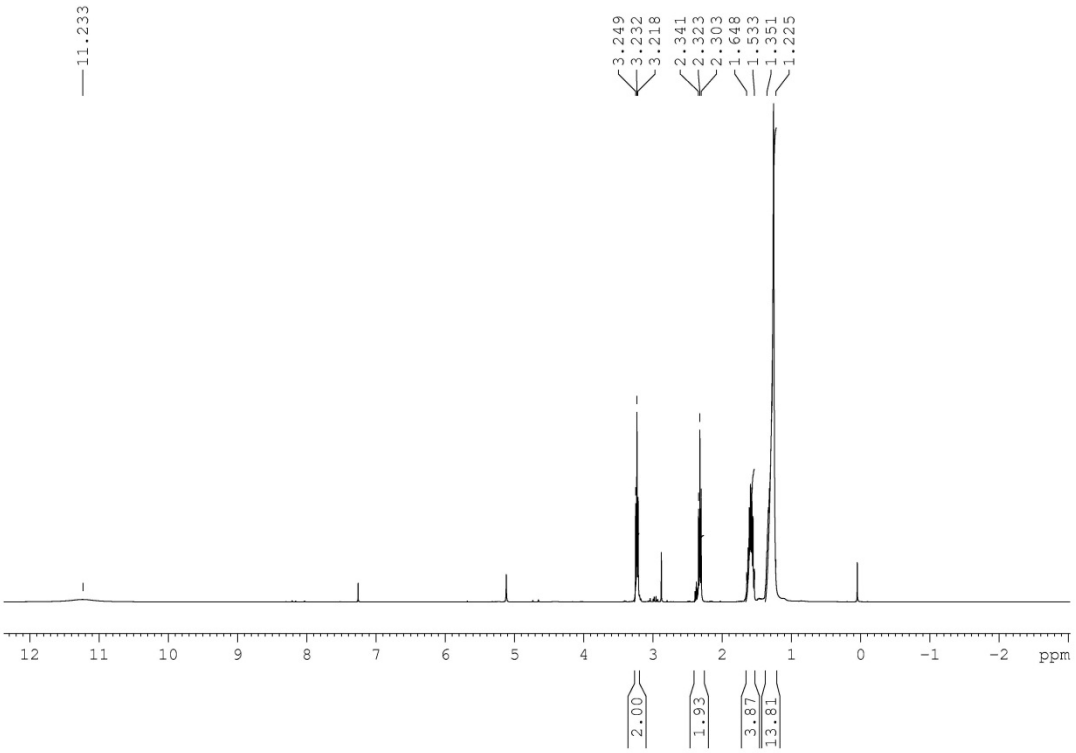
Biotin-labeled-AHL 5:



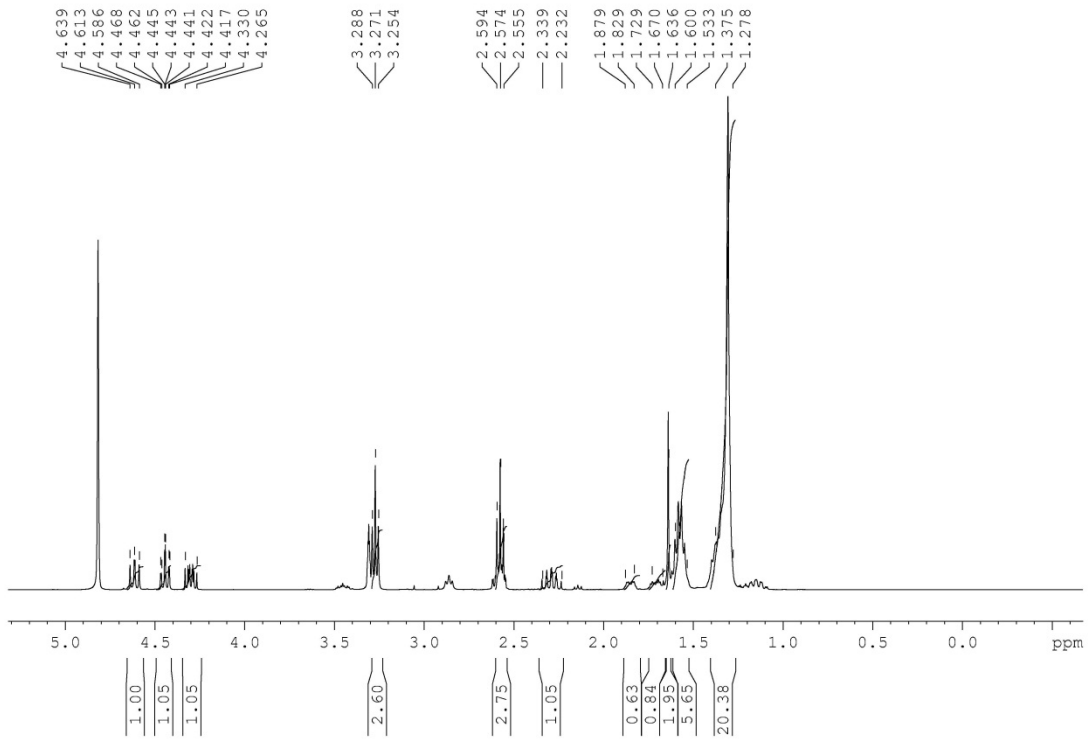


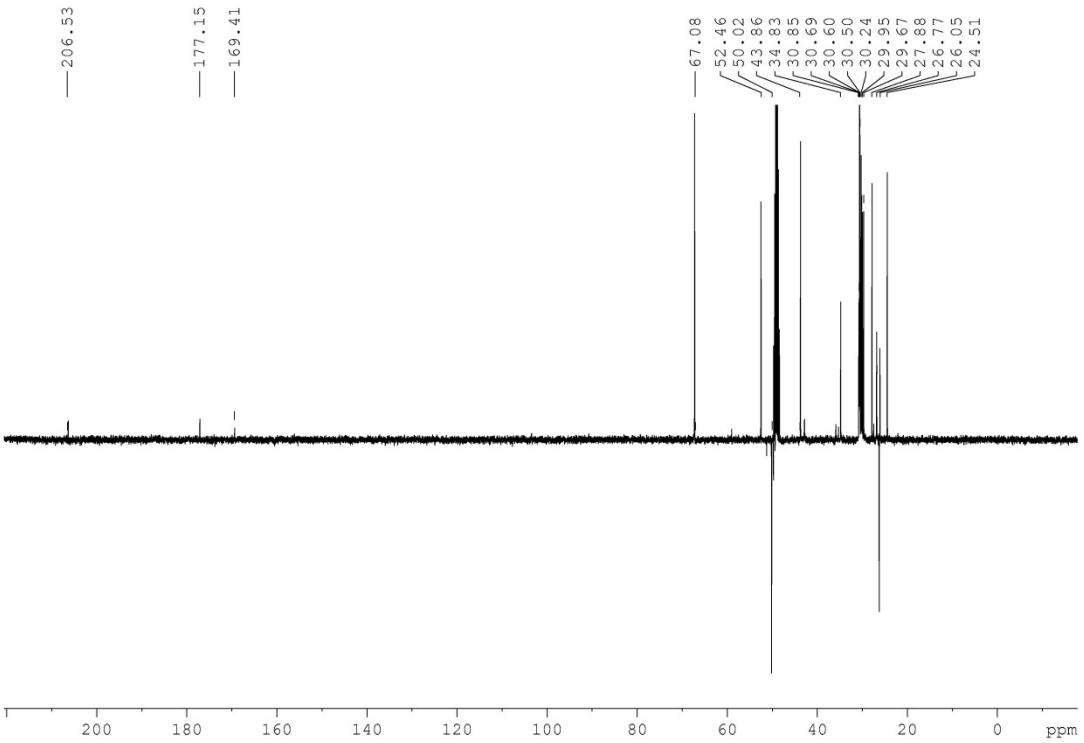
5

Azid Carboxylic acid 6

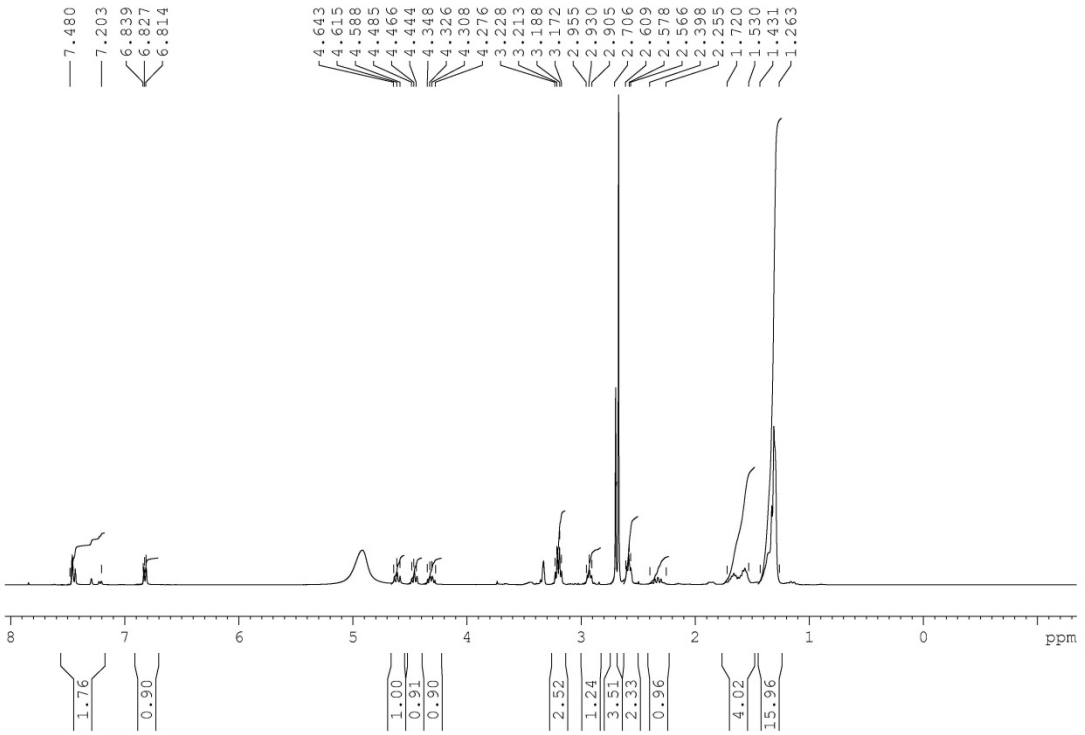


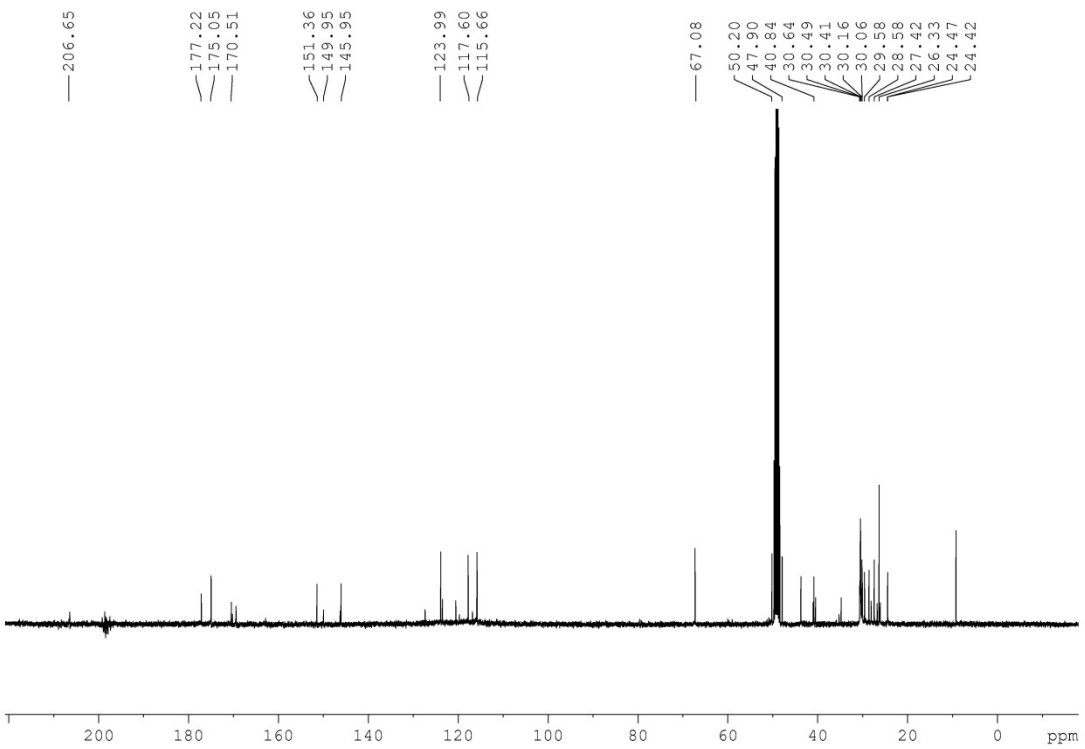
AHL derivative 7



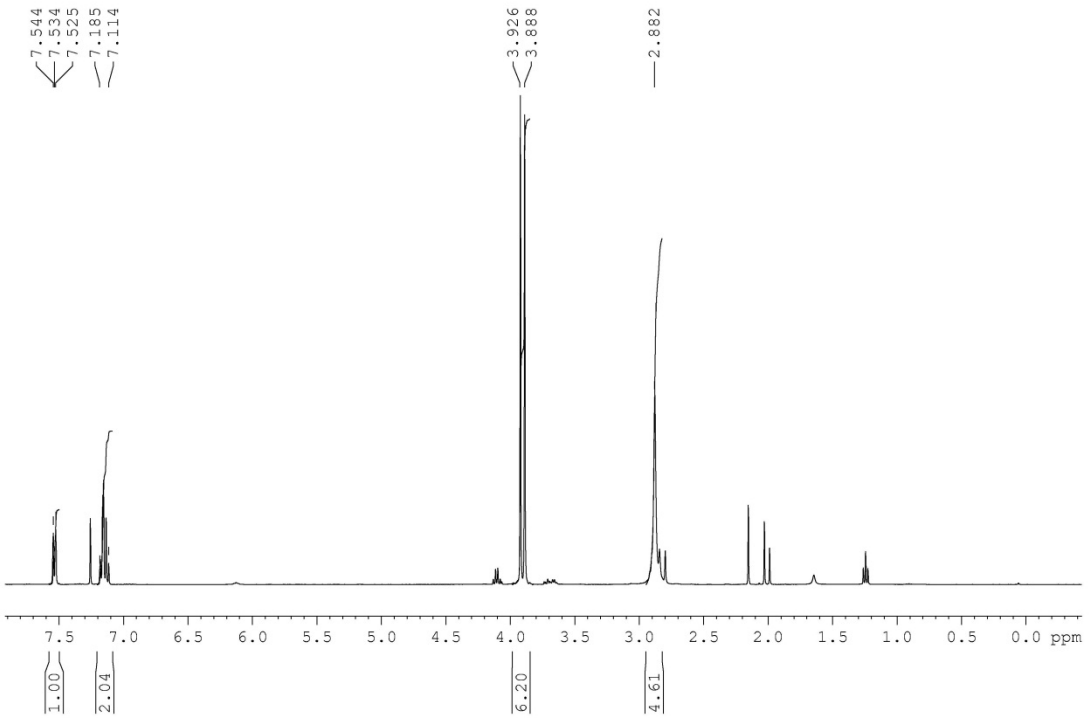


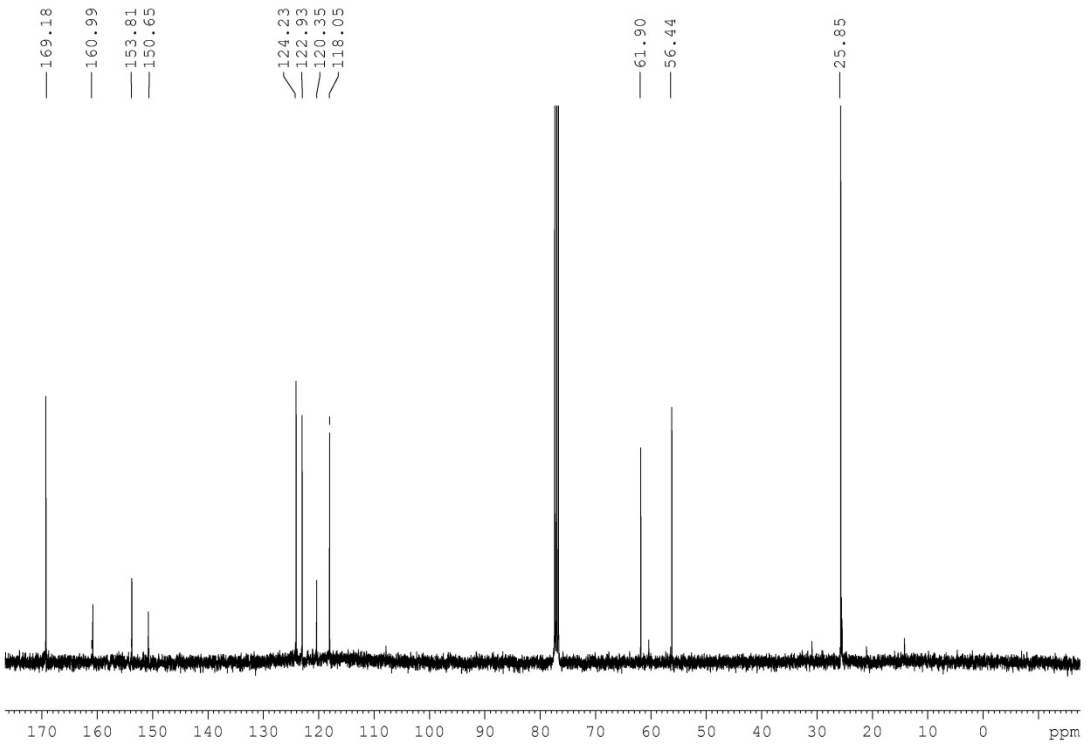
AHL derivative 8



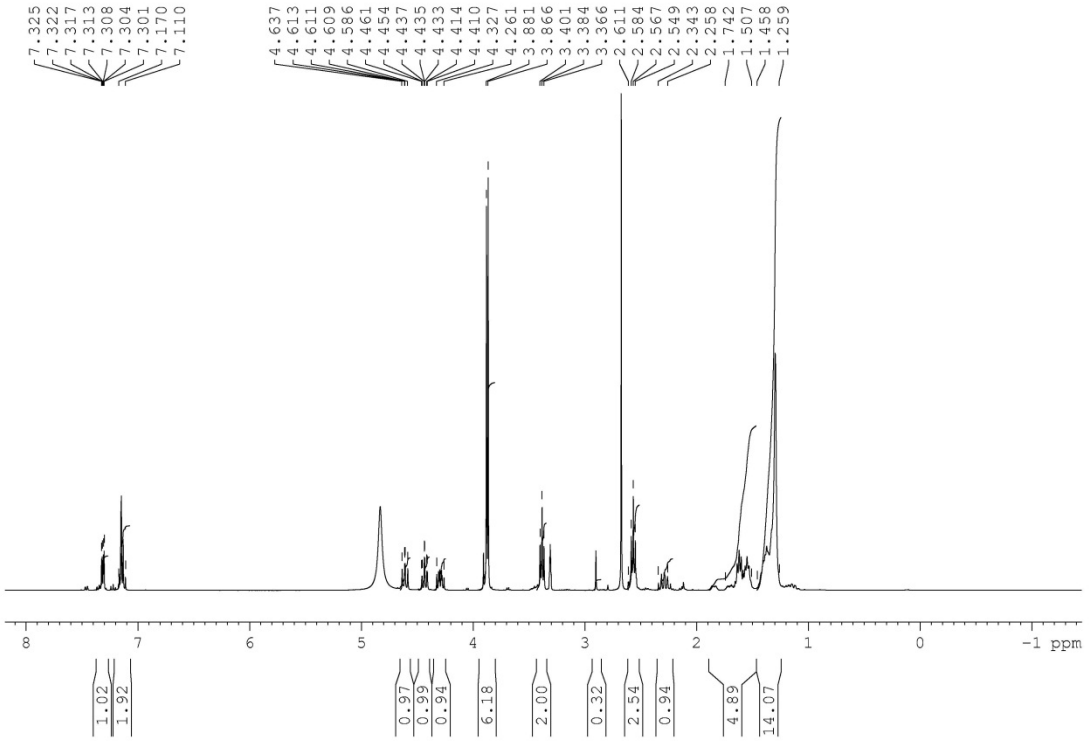


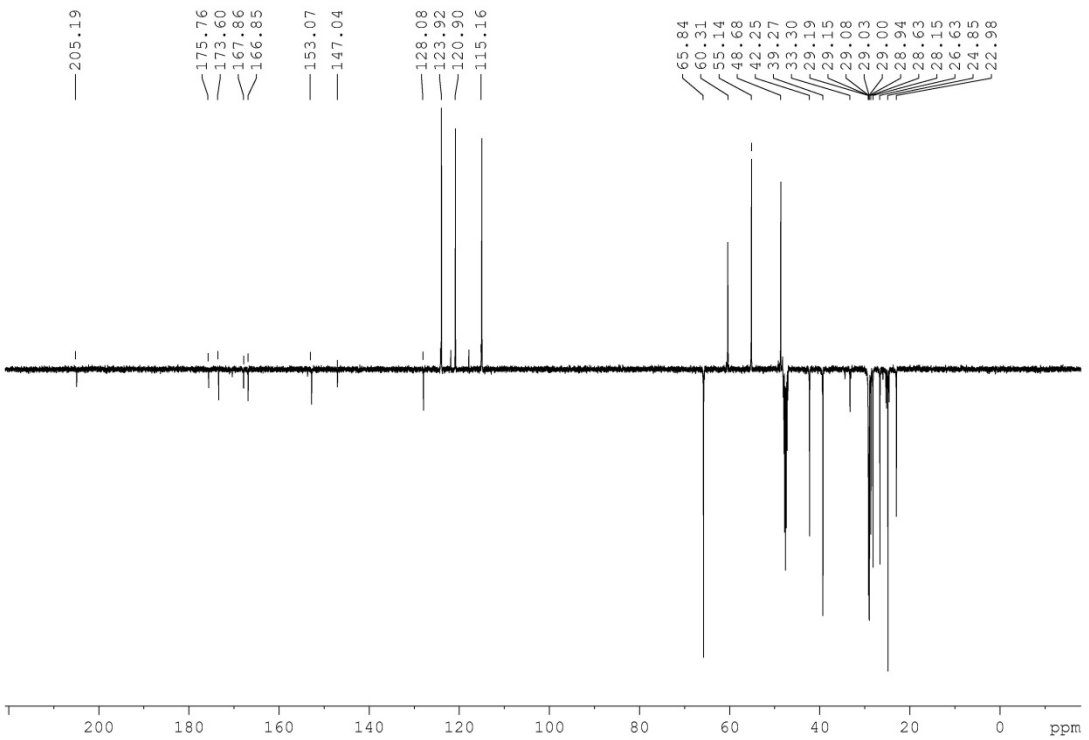
Protected NHS-Ester



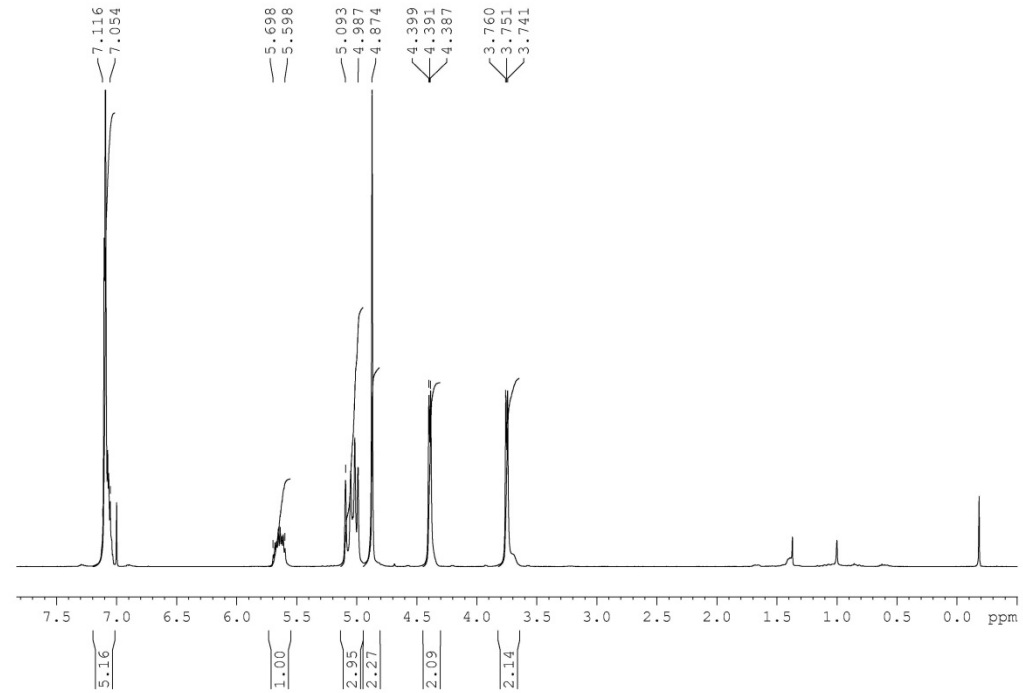


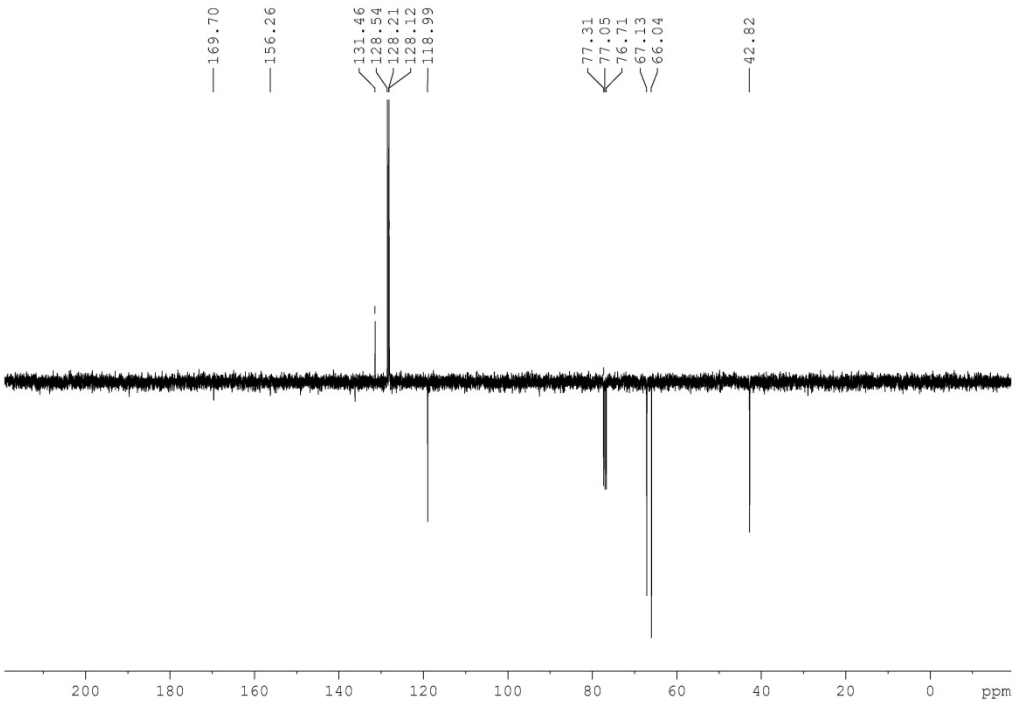
AHL-derivative 9



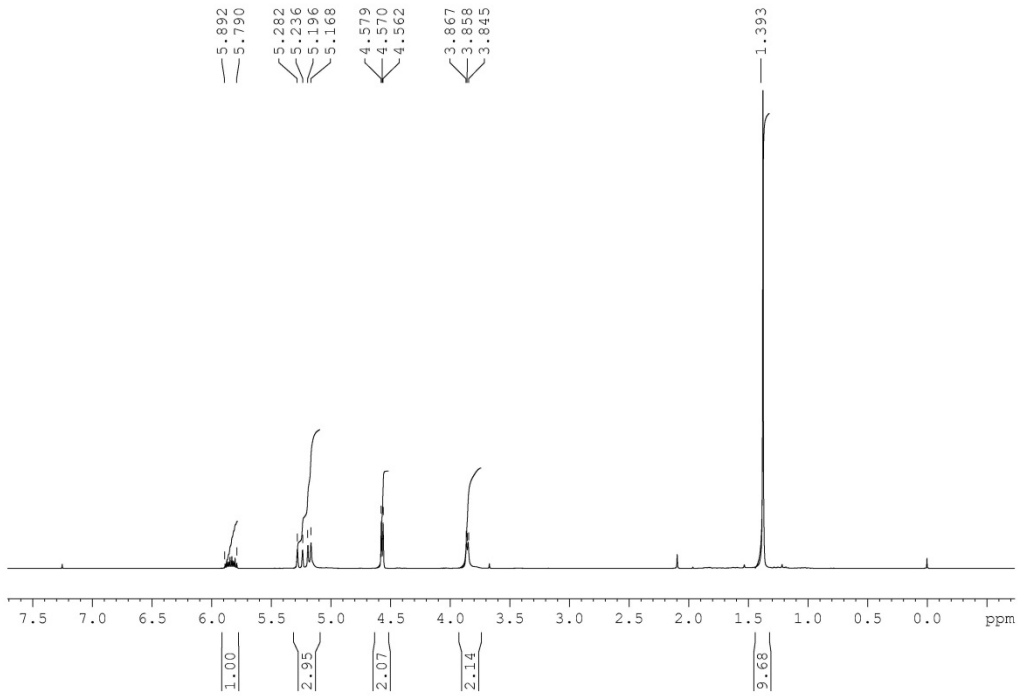


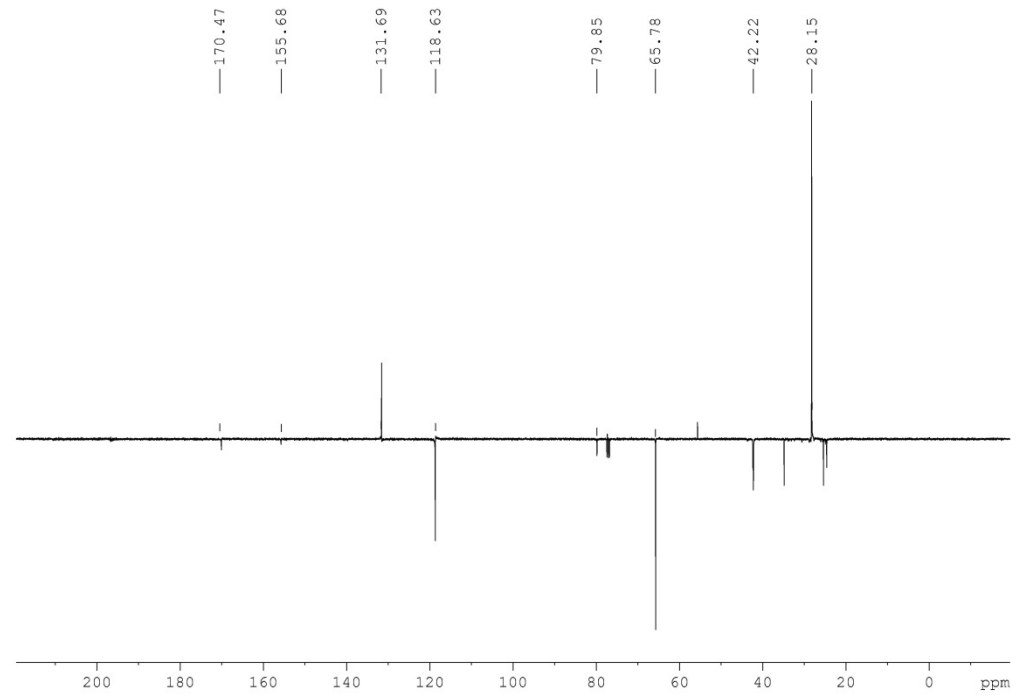
Cbz-glycine allylester 11b





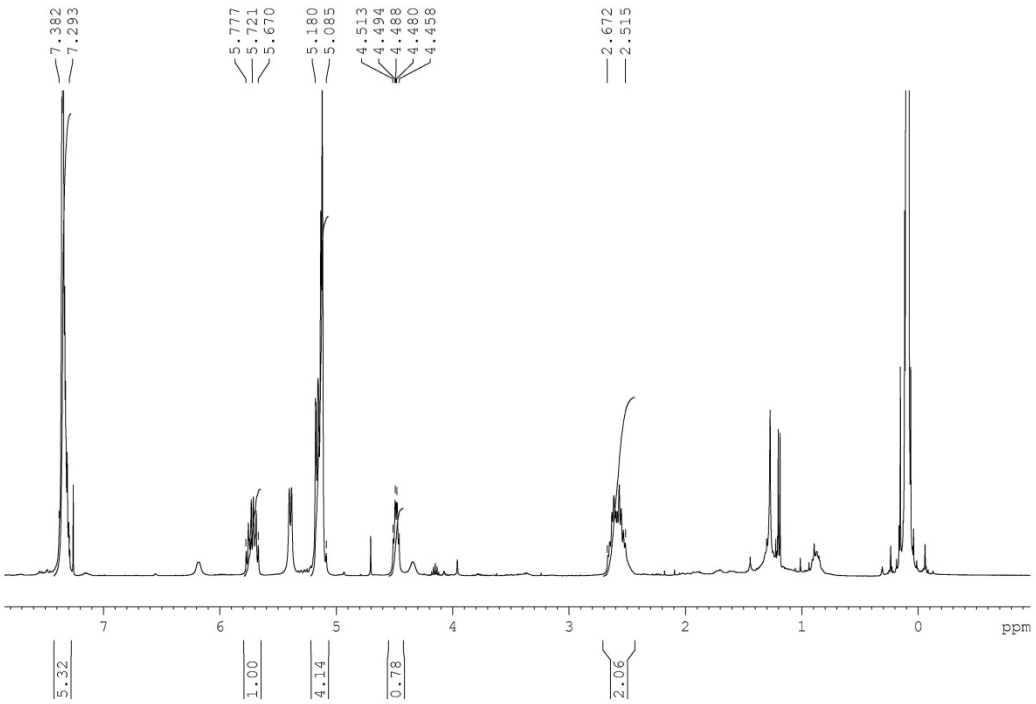
Boc-glycine allylester 11c

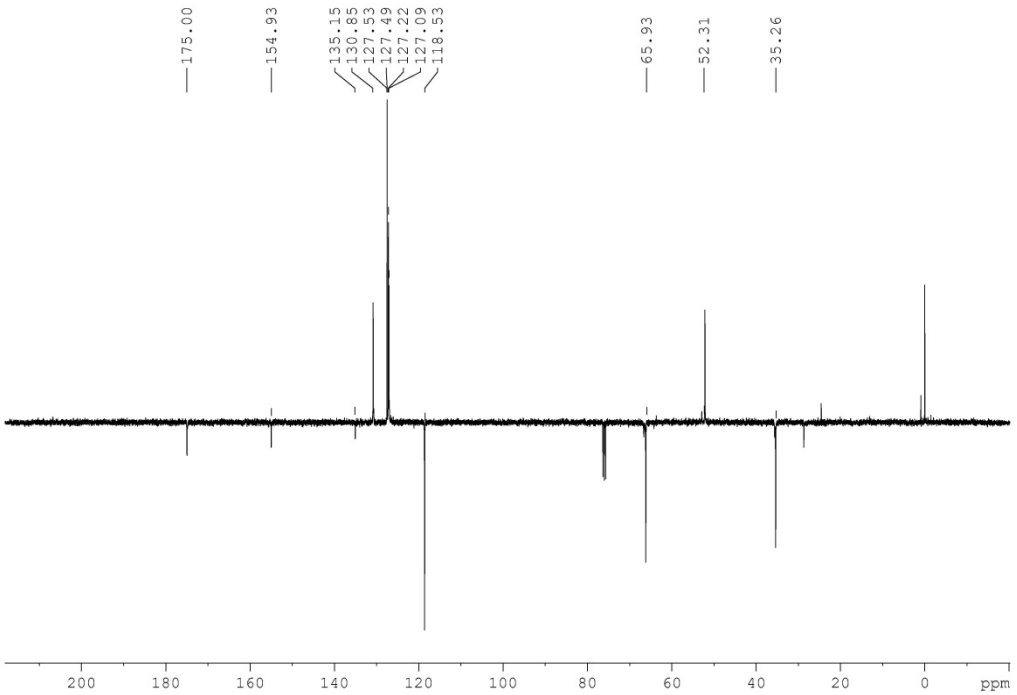




5

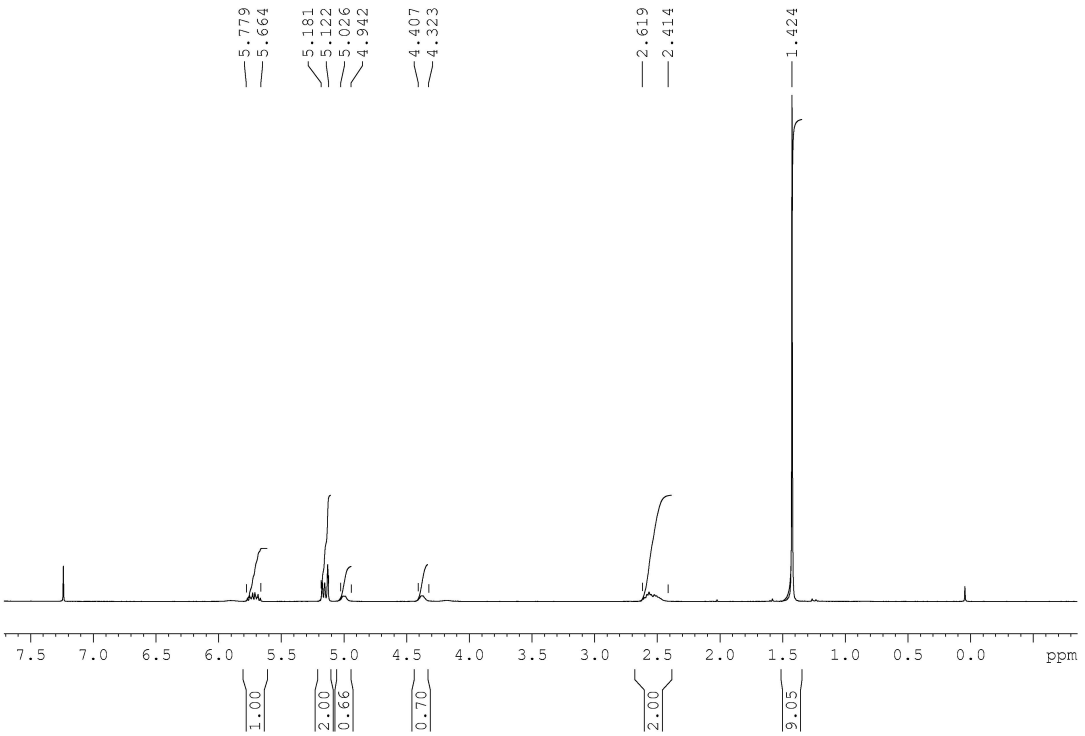
Cbz-protected allyl glycine 12b

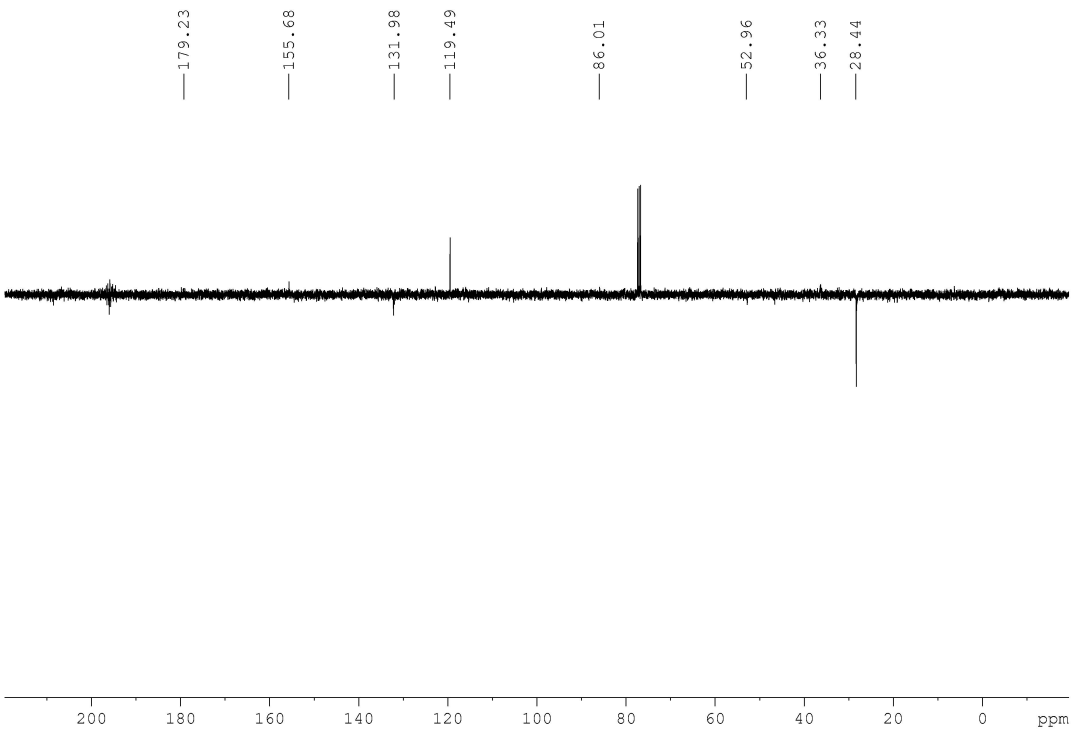




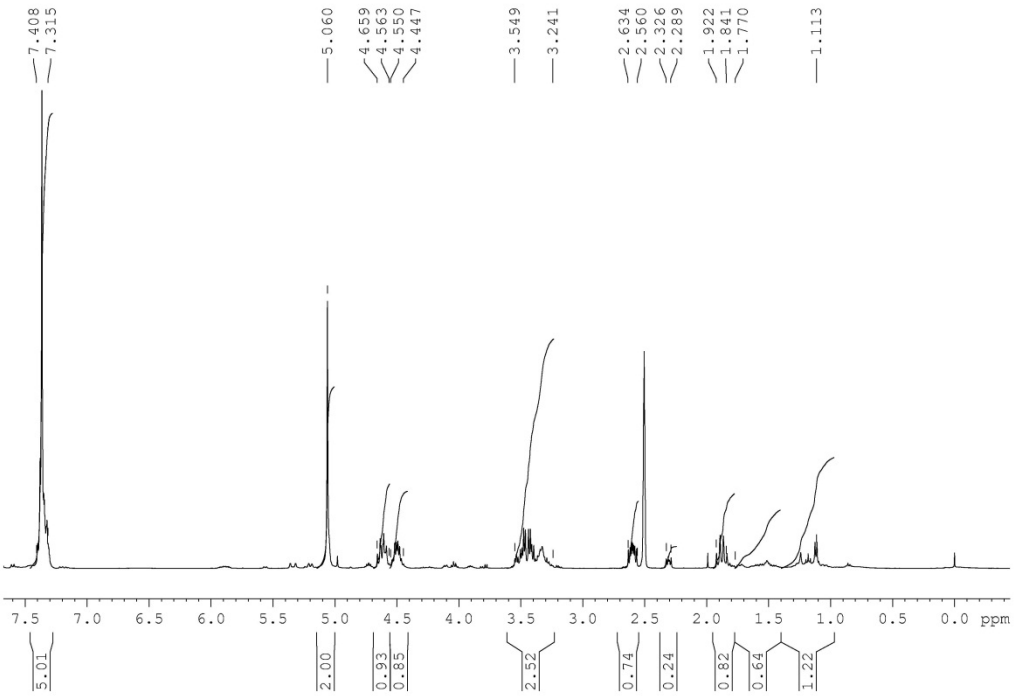
5

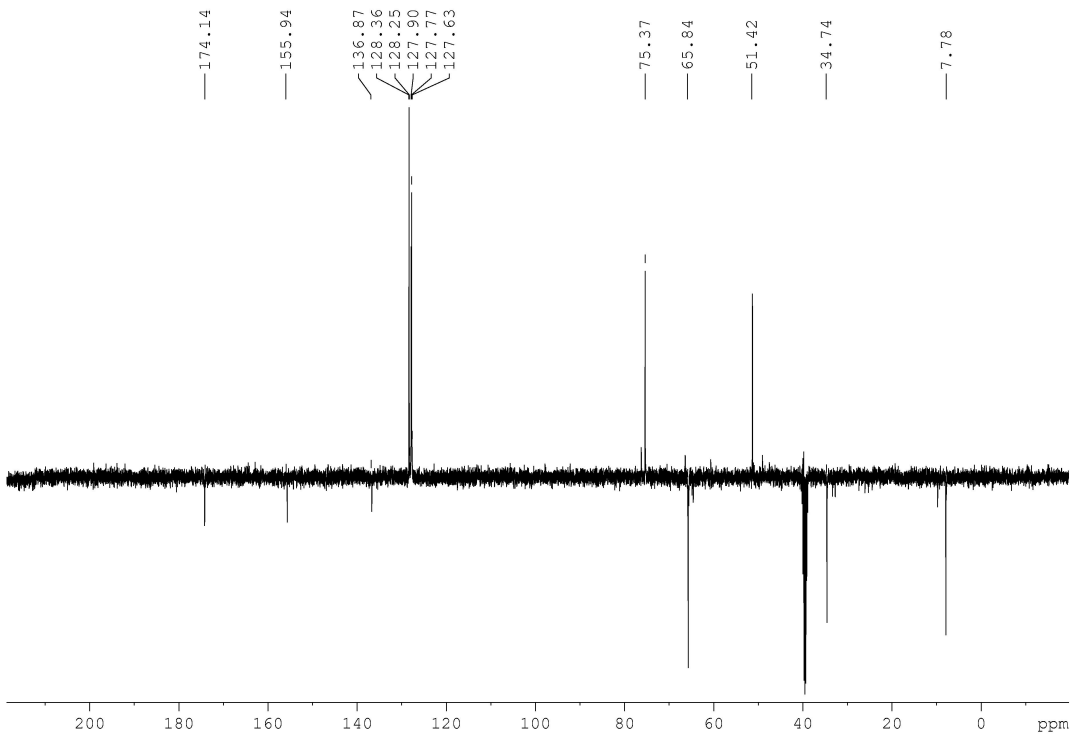
Boc-protected allyl glycine 12c





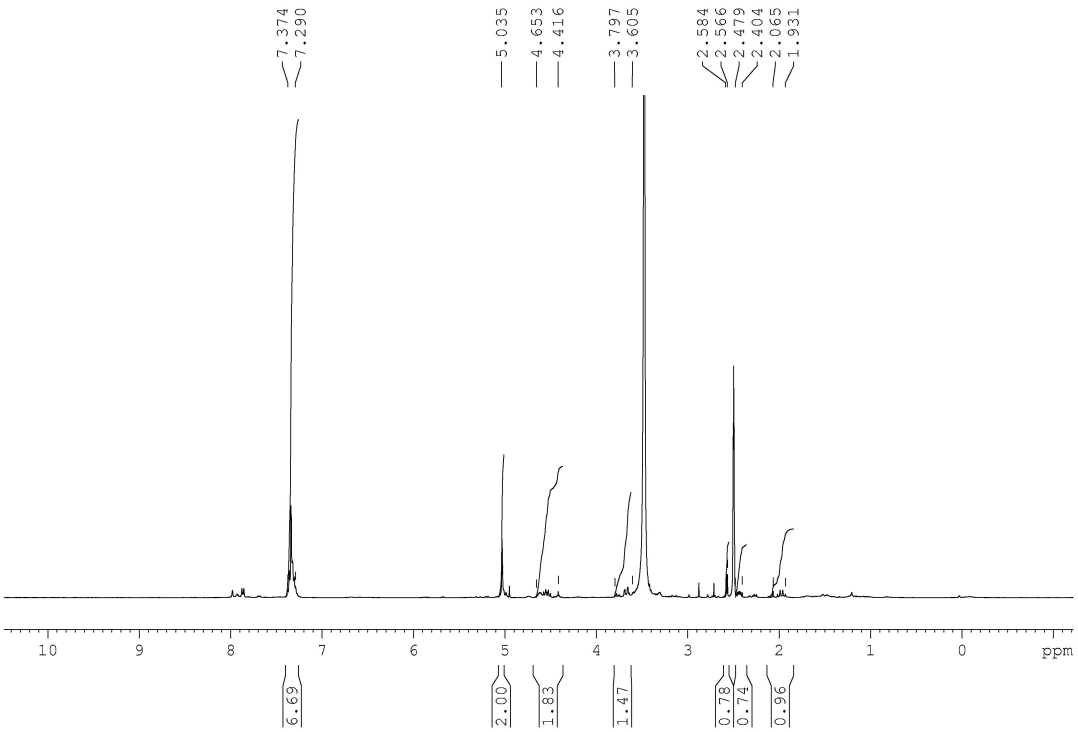
Cbz-protected iodolactone 13b

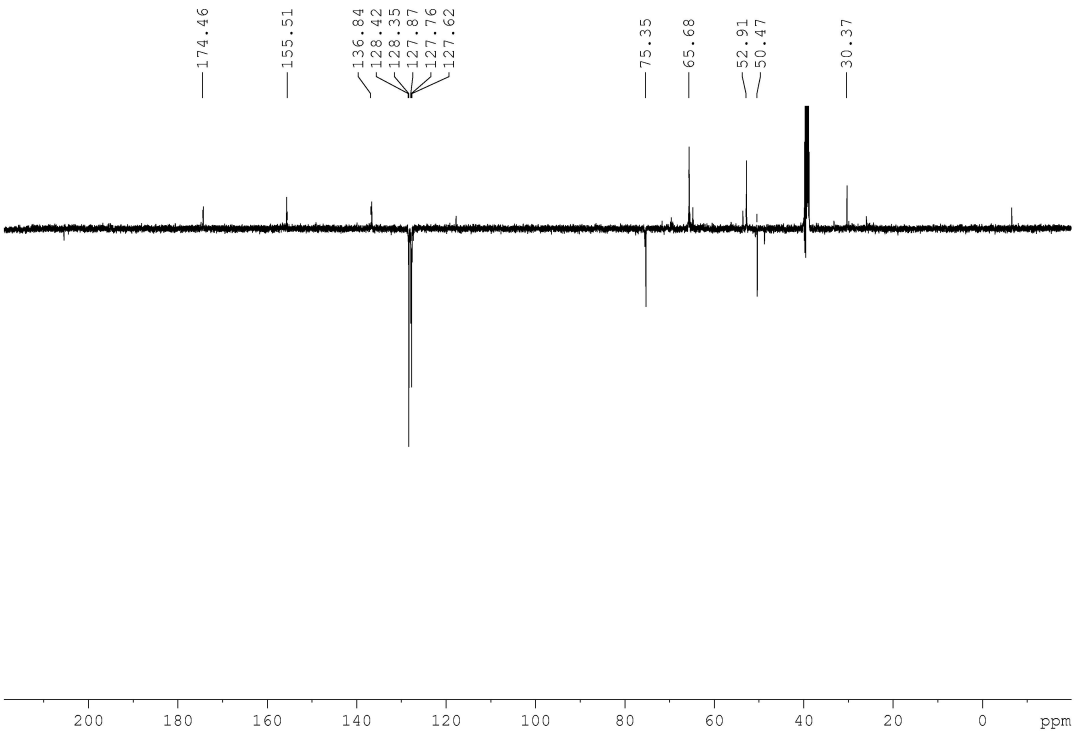




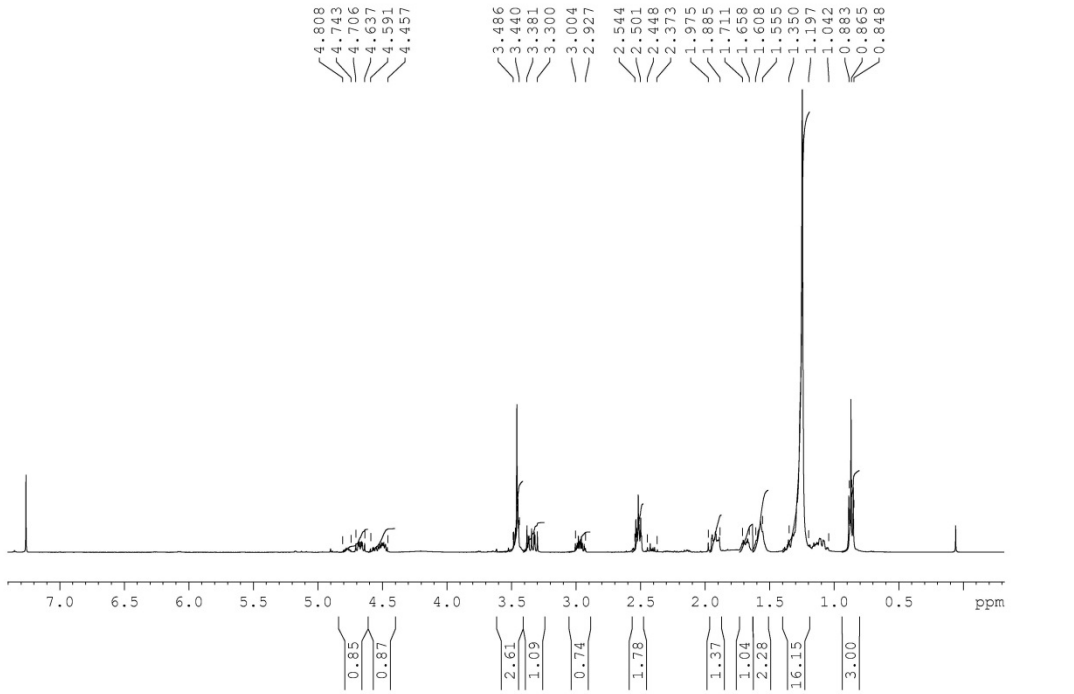
5

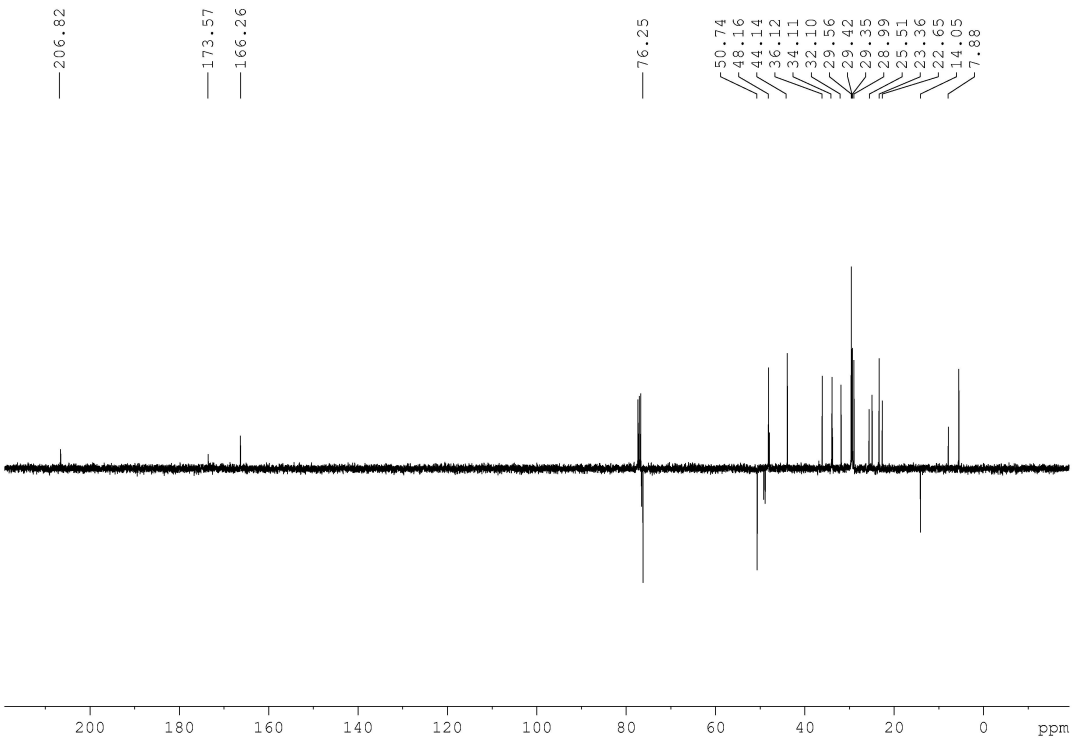
Cbz-protected azide 16b





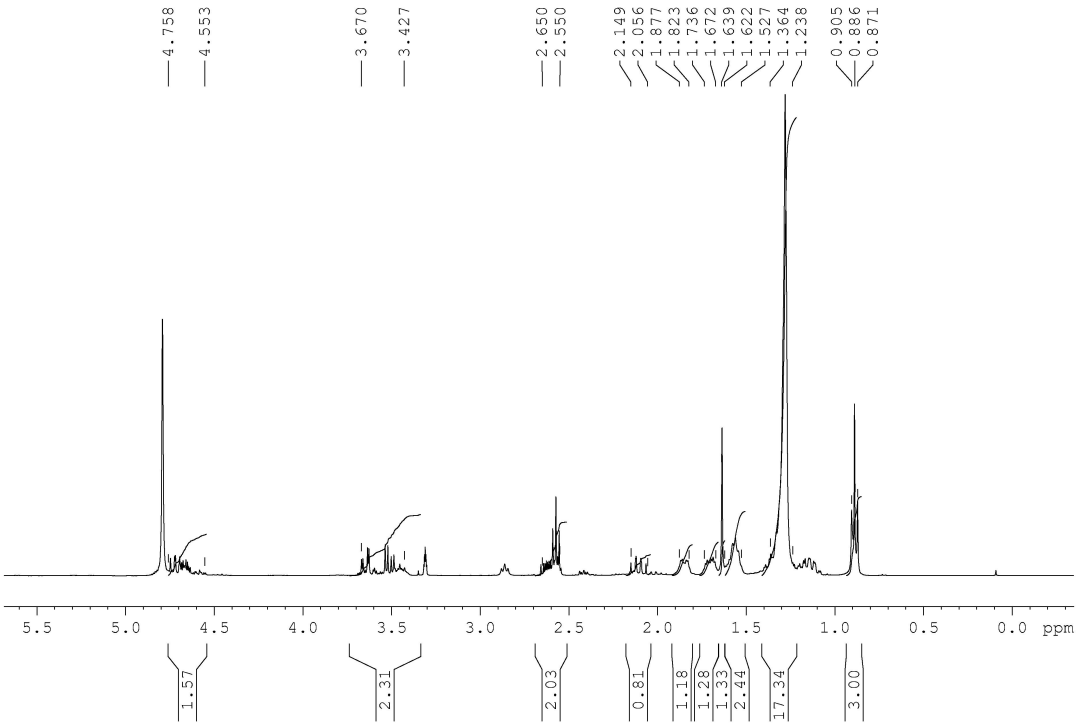
AHL-derivative 15

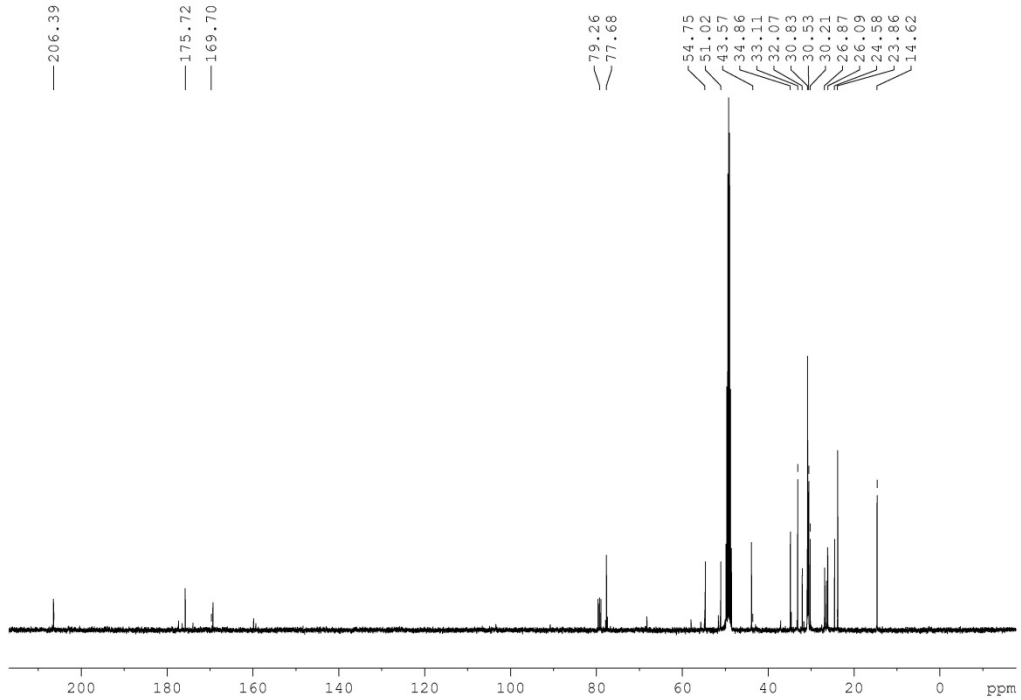




5

AHL-derivative 18





HPLC analysis

Sample: Boc-protected allyl glycine **12c**: 4.7 mg/1mL

Column: CHIRALPAK IA

Detector: RI Detector

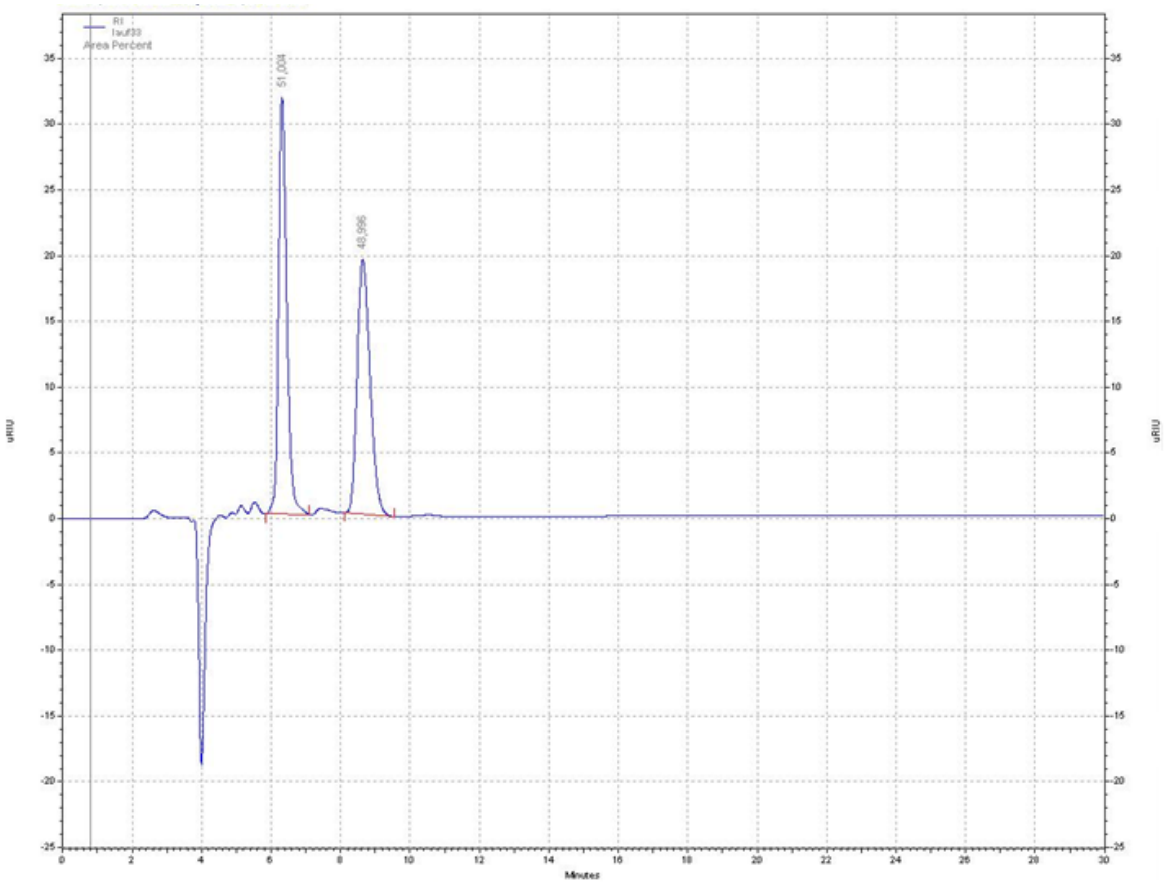
Run time: 30 min

Solvent: *n*-hexane: *i*-PrOH: TFA (80:20:0.1)

Flow rate: 0.5 mL/min

Peak 1: Retention time = 6.50 min; area%=51.004

Peak 2: Retention time = 7.0 min; area%= 48.996



Biological Evaluation

HSL Detection

Detection of *N*-acyl-homoserine lactones and their derivatives was done using two bacterial strains: *Pseudomonas putida* strain F117, an AHL-negative derivative of the IsoF strain mutated in the *ppuI* AHL synthase gene, carrying the pKR-C12 plasmid, which is a modified version of pBBR1MCS-5 carrying P_{lasB}-*gfp*(ASV)-P_{lac}-*lasR*; Gm^r, and the *Escherichia coli* strain MT102 carrying the pJBA89 plasmid (Ap^r; pUC18Not-*luxR*-P_{luxI}-RBSII-*gfp*(ASV)-T_0-T_1). Bacteria are detecting a range of homoserines lactones from C6-HSL to oxo-C14-HSL. Reporter bacteria were grown on LB medium with specific antibiotics. Five µl of different homoserine lactones or derivatives (**2** to **15**) were dropped on the bacteria lawn. Fluorescence was observed 2 hours after incubation using an ex: 480/40 nm, em: 510 nm filter.

Purification of recombinant Sinme_0536

Sinorhizobium meliloti genomic DNA was used as matrix for the cloning and production of recombinant 6xHis-tagged Sinme_0536 protein. Full open reading frame of *Sinme_0536* was cloned into pDEST17 vectors (Invitrogen) and resulting expression vectors were transfected into *E. coli* BL21 cells. Protein expression was induced with 1 mM IPTG over night at 30 °C. Cells were lysed and protein purified accordingly to the manufacturers' protocols (Qiagen for Ni-beads purifications). Beads were washed 3 times and Ni-binding complexes separated on SDS-PAGE gel for size control.

Pull-down assay

In the pull-down assay streptavidin beads (sepharose beads conjugate) from Cell Signaling Technology® Inc. were first washed in 200 µl protein extraction buffer (25mM Tris-HCl pH 7.8, 10 mM $MgCl_2$, 15mM EGTA, 75mM NaCl, 1mM DTT, Protease Inhibitors Roche®) with 10 µg BSA, and subsequently coated with **5** using a 600 µM solution of **5** for 40 min. As a control, streptavidin beads were coated with 600 µM free biotin. Coated beads were incubated over night with 2 µg of purified recombinant protein (6xHis-LuxR) together with (or without) 200 µg total *Arabidopsis thaliana* protein extract in the presence of 15 µg BSA in a final volume of 300 µl at 4 °C. After 5x wash, beads were denatured with protein loading dye at 95 °C and deposed on SDS gel. Ni-binding complexes were probed in a Western blot analysis for the presence of 6xHis-LuxR using a specific anti-His antibody. Each experiment was repeated 3 times.

Quantitative RT-PCR

Arabidopsis thaliana Col-0 seedlings were germinated on sterile MS/2 medium[6] with 0.3 % Gelrite for 2 weeks at 22°C with 150 µmol/m^2/s light in 8/16h day/night photoperiod. Seedlings were then transferred into 6-well plates with 5 ml liquid MS/2 medium per well and were pretreated with *N*-3-oxo-tetradecanoyl-*L*-homoserine lactone (oxo-C14-AHL) (Sigma-Aldrich, Deisenhofen, Germany) or **5** in different concentrations for 3 days. Oxo-C14-AHL was dissolved in acetone or AHL-derivative **5** in DMSO as 60 mM stock solution and added to the growth medium to 6 µM final concentration. HSL-pretreated plants were treated with 100 nM flg22 and harvested at time point as indicated. 50-100 mg of plant material were homogenized and total RNA extraction performed using the Trizol system. Two µg of total RNA was used for DNAse digestion. cDNA synthesis was done according to the qScript cDNA Synthesis Kit from Quanta BioScience Inc. Quantitative RT-PCR was done using specific primers *WRKY22* (*At4g01250* fwd.: ATCTCCGACGACCACTATTG rev.: TCATCGCTAACCACCGTA TC) and *WRKY29* (*At4g23550* fwd.: TCCGGTACGTTTTCACCTTC rev.: AGAGAC CGAGCTTGTGAGGA). Annealing temperature was set to 60°C. All expression values were normalized to expression of *UBQ4* (*At5g25760* fwd.: GCTTGGAGTCCTGCTTGGACG rev.: CGCAGTTAAGAGGACTGTCCGGC) and to the 0 hours post infection (hpi) values. The experiments were performed 3 times and error bars indicate standard deviation between the biological replicates.

Experimental Procedures

Detection of AHLs and AHL-derivatives with biosensor bacteria

Two different bacterial strains were used to detect the *N*-acyl-homoserine lactones. The bacteria carry plasmids with gene coding for an AHL receptor and the *Green Fluorescent Protein* (*GFP*) gene under control of AHL-inducible promoters, allowing the visualization and partial quantification of specific molecules. The range from 6 to 14 carbons in lipid chain was tested using C6-AHL to oxo-C14-AHL in different concentrations on all bacterial strains. Molecules were dissolved in acetone, except **5**, which was dissolved in DMSO, and 5 µL were dropped on bacterial lawns for 2 h. Green Fluorescent Protein (GFP) signals were observed with fluorescent binocular usinf GFP filter Em: 505-550 nm. While *Pseudomonas putida* strain F117 carrying the pKR-C12 plasmid (P_{lasB}-*gfp*(ASV)-P_{lac}-*lasR*)[7] recognizes C10 to C14, *Escherichia coli* strain MT102 carrying the pJBA89 plasmid (Ap^r; pUC18Not-*luxR*-P_{luxI}-RBSII-*gfp*(ASV)-T_0-T_1),[8] recognize C6 to C14.

References

(1) H. Kikuchi, K. Sasaki, J. i. Sekiya, Y. Maeda, A. Amagai, Y. Kubohara and Y. Oshima, *Bioorg. Med. Chem.*, 2004, **12**, 3203.

(2) T. Karlsson, M. V. Turkina, O. Yakymenko, K.-E. Magnusson and E. Vikström, *PLoS Pathog.*, 2012, **8**, e1002953.

(3) N. Amara, R. Mashiach, D. Amar, P. Krief, S. p. A. H. Spieser, M. J. Bottomley, A. Aharoni and M. M. Meijler, *J. Am. Chem. Soc.*, 2009, **131**, 10610.

(4) H. Mues and U. Kazmaier, *Synthesis*, 2001, 487.

(5) G. Yoon, T. M. Zabriskie and S. H. Cheon, *J. Labelled Compd. Rad.*, 2009, **52**, 53.

(6) T. Murashige and F. Skoog, *Physiol. Plant.*, 1962, **15**, 473.

(7) A. Steidle, K. Sigl, R. Schuhegger, A. Ihring, M. Schmid, S. Gantner, M. Stoffels, K. Riedel, M. Givskov, A. Hartmann, C. Langebartels and L. Eberl, *Appl. Environ. Microbiol.*, 2001, **67**, 5761-5770.

(8) J. B. Andersen, A. Heydorn, M. Hentzer, L. Eberl, O. Geisenberger, B. B. Christensen, S. Molin and M. Givskov, *Appl. Environ. Microbiol.*, 2001, **67**, 575-585.