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

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Gram-negative bacteria often use *N*-acyl-homoserine lactones (AHLs) as signal molecules to monitor their local population densities and to regulate gene-expression in a process called "Quorum Sensing" (QS). This cell-to-cell communication allows bacteria to adapt to environmental changes and to behave as multicellular communities. QS plays a key role in both bacterial virulence towards the host and symbiotic interactions with other organisms. Plants also perceive AHLs and respond to them with changes in gene expression or modifications in development. Herein, we report the synthesis of new AHL-derivatives for the investigation and identification of AHL-interacting proteins. We show that our new compounds are still recognised by different bacteria and that a novel biotin-tagged-AHL derivative interacts with a bacterial AHL receptor.

Introduction

Bacteria produce signal molecules of low molecular weight to report information on their local population densities in order to control and coordinate their behaviour. These signal molecules, also known as autoinducers, play a key role in a complex cell-to-cell communication process.[1] Fuqua *et al.* were the first to describe and investigate bacterial communication and defined it as the cell density dependent alteration of gene expression.[2] The signalling process also termed "Quorum Sensing" allows the bacteria to sense and respond to their local population density. The small, pheromone-like autoinducers pass the cell membrane and, once a certain threshold concentration is reached, the signal molecules bind to a cognate receptor to form a complex which induces alteration of gene expression.[3,4] Examples of QS-regulated behaviours are biofilm production,[5–9] induction of bioluminescence,[10,11] antibiotic production and virulence factor expression. QS plays a central role in the interaction of bacteria with their hosts with often significant effects on human health and crop plant production.[12–15] Gram-negative bacteria use *N*-acyl-L-homoserine lactones (AHLs) as their primary QS signal molecules.[11,16–19] Bacteria produce different AHLs and one species may produce one or more autoinducers varying in constitution and effects on the host. Here, we focus on AHLs of plant-associated bacteria and their biological relevance for the plant hosts.[20]

Recent studies showed that bacteria not only use AHLs for their communication but that plants are also able to detect bacterial signal molecules and respond to the autoinducers with altered gene expression or modifications in development, which can be beneficial for the plant.[21,22] von Rad *et al.* have shown that *Arabidopsis*, for example, takes up short-chained AHLs and allows their systemic distribution in the plant. On the one hand, short-chained AHLs promote root growth and alter root hair development.[23,24] On the other hand, some AHL-producing bacteria have a beneficial effect on resistance against microbial pathogens.[25] Recently, oxo-C14-AHL has been found to induce resistance in *Arabidopsis* and barley plants towards biotrophic and hemibiotrophic pathogens.[26] Despite its importance for plant resistance and development, the underlying molecular processes of AHL-perception by plants are widely unexplored and plant receptors for AHLs have not been isolated so far. The identification and investigation of these putative plant receptors will be crucial to understand the plant response to bacterial pathogens. As a critical step to elucidate the plants' perception system for bacterial autoinducers, we present syntheses of modified oxo-C14-

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†Electronic supplementary information (ESI) available: Copies of NMR spectra for new compounds, HPLC data for the separation of racemic **12c**, experimental details of biological evaluation. See DOI: 10.1039/c3ob41215f

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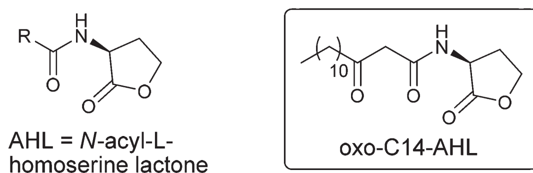


Fig. 1 Generalized AHL-structure and oxo-C14-AHL as a functionalized derivative.

AHLs and evaluate their biological activity in bacteria and plants (Fig. 1).

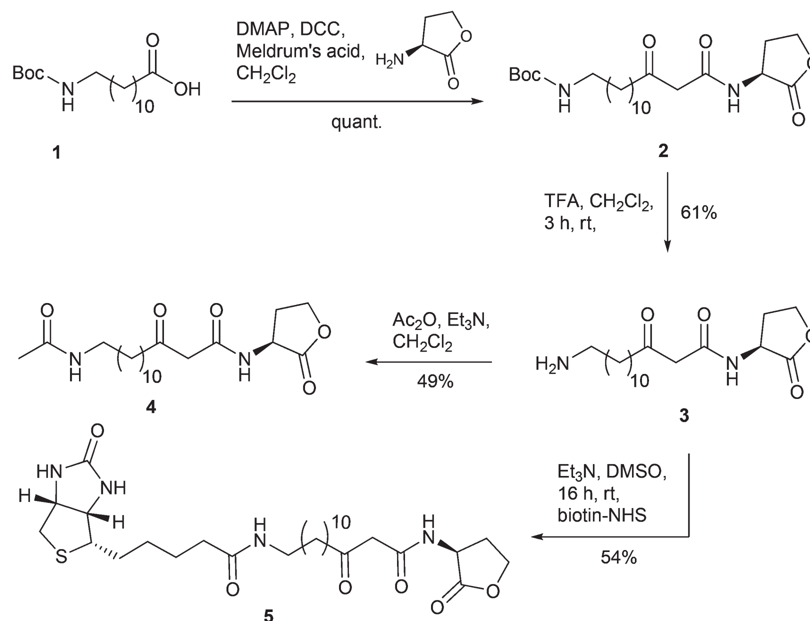
Results and discussion

Different acyl chain modified AHL-derivatives have been synthesized.[27–33] For the synthesis of acyl chain modified oxo-C14-AHLs we chose the Meldrum's acid approach.[34–37]

Following a route by Amara *et al.*[38] we started with the Boc-protected acid **1** which was converted to the novel oxo-C14-AHL analogue **2** by treatment with Meldrum's acid and (*S*)-homoserine lactone. Boc-deprotection of **2** with TFA gave the free amine **3** (Scheme 1). From this intermediate, different functionalized AHL-mimetics were prepared. Acylation with acetic anhydride gave the *N*-acetyl derivative **4**, which was synthesized as a simple and sterically non-demanding analogue of oxo-C14-AHL. Most notably, the biotin-labeled analogue **5** was prepared, which is an interesting molecular probe for a pull-down assay for putative plant AHL receptors. Only a few biotin-tagged-AHLs can be found in literature and derivatives of oxo-C14-AHL are unknown.[39,40] We found NHS-ester coupling of biotin to be the best method for conjugation.[41] Standard peptide coupling conditions (*e.g.* HOBt, EDC) did not give the desired target compound **5** and lead to complex product mixtures.

Three additional functionalized AHL-derivatives **7–9** have been prepared as depicted in Scheme 2. Azide **7** is a versatile intermediate for copper catalyzed click-functionalization of oxo-C14-AHL and was prepared from azidoacid **6**. The two catechol derivatives **8** and **9** were synthesized by NHS-ester coupling of amine **3**. The first catechol-AHL-derivatives have recently been introduced by Gademann.[42] The catechol group is a surface anchor[43,44] and may be used for the immobilization of **8** and **9** on metal oxide surfaces (nanoparticles or bulk materials). The resulting materials are anticipated to be interesting for the modulation of plant root growth or as stationary phases for the affinity purification of human, bacterial and putative plant AHL-receptors.

Substituents at the lactone ring are tolerated in a number of known AHL-mimetics and the crystal structure of LasR from *P. aeruginosa* with oxo-C12-AHL suggested to us that a *cis*-C5-substitution pattern might be suitable for the introduction of functional groups to our AHL-analogues.[45] A number of lactone ring modifications have been described in the literature. Most of them concentrate on the variation of the lactone ring size or replacing the lactone scaffold with other moieties.[27,28,36,46] In 2002 Olsen *et al.* described the synthesis of 3- and 4-hydroxy substituted C6-AHL and some carbamate derivatives starting from serine.[47] Our approach is depicted in Scheme 3. We planned to construct the modified lactone moiety according to the Kazmaier method from *N*-protected allylglycines **10** *via* a Claisen rearrangement of glycine allyl-esters **11**.[48] Following the Kazmaier synthesis we started with TFA-protected glycine **10a** to synthesize glycine allyl ester **11a** (Scheme 3). The following Claisen rearrangement to **12a** proceeded in varying yields up to 74%. In some cases we observed



Scheme 1 Synthesis of amine **3** and its conversion to the *N*-acyl derivative **4** and biotin-labeled molecular probe **5**.

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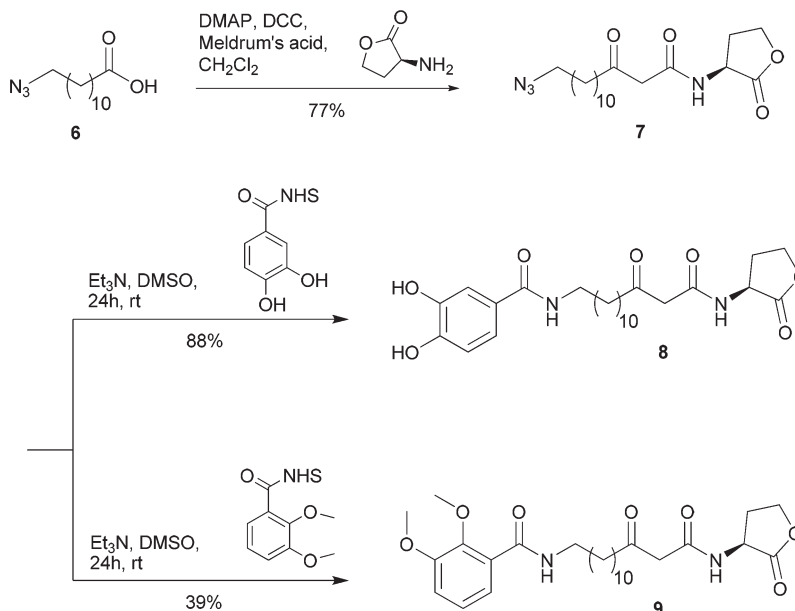
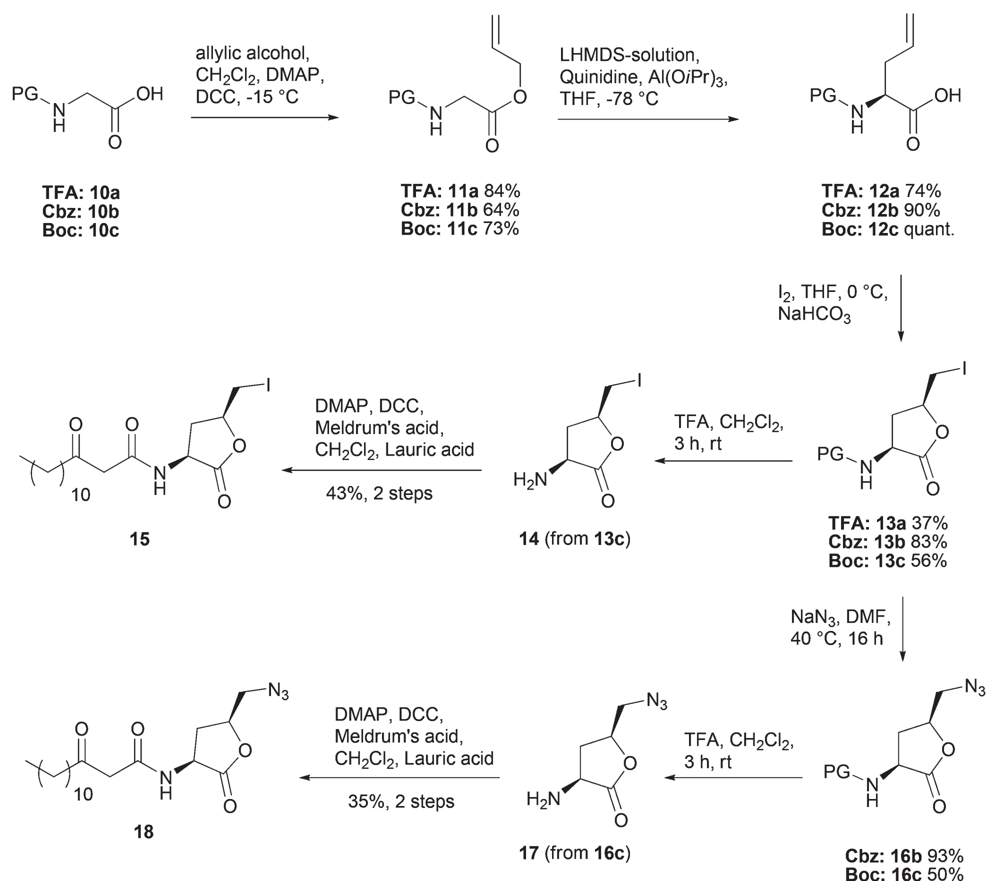


Scheme 2 Synthesis of azide **7** for click-functionalization and two catechol derivatives **8** and **9** for surface immobilisation.



Scheme 3 Synthesis of lactone ring modified AHL-derivatives (only one enantiomer of racemic mixtures is depicted).

deprotection of the TFA group after work up. In addition, the following iodolactonization to **13a** was accomplished with only 37% yield. In consequence, we decided to switch protecting groups and prepared Cbz-protected glycine allyl ester **11b** and the Boc-protected analogue **11c**. With both derivatives, the rearrangement worked fine and gave the expected allylglycines

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12b and **12c**. Particularly Boc-allyl ester **11c** gave consistently high yields of Boc-allylglycine **12c**. Unfortunately, as already reported by Kazmaier for similar carbamates,[49] the enantioselectivity of these conversions is low. For Boc- and Cbz-allylglycine we obtained almost racemic mixtures. However, both enantiomers of Boc-allylglycine **12c** are easy to separate by HPLC on a chiral phase (see ESI†) and are thus available in a pure form if needed. For a first evaluation of our 4-substituted AHL analogues, we used the racemic compounds **12b** and **12c**. Iodolactonization worked well for the Cbz- and the Boc-protected compounds and gave iodolactones **13b** and **13c** in good yields and with good diastereoselectivities for the *cis*-derivatives ($dr_{(cis:trans)} = 10:1$).[50] Deprotection with TFA gave the free amine **14** which was coupled to lauric acid in the presence of Meldrum's acid. The final AHL-analogue **15** was obtained in 43% yield over two steps. Alternatively, an azide moiety was introduced to iodolactone **13** *via* nucleophilic displacement of iodide with NaN_3 to give the desired products **16b** and **16c**. Deprotection of **16c** to the free amine **17** followed by coupling to lauric acid in the presence of Meldrum's acid gave AHL-analogue **18**. Both derivatives **15** and **18** are versatile AHL analogues because the iodide in **15** may be easily substituted with various nucleophiles and the azide in **18** is an excellent precursor for click functionalization or reduction to the amine and subsequent functionalization *via* amide formation.

Modifications of the molecular structure often lead to altered activity of biologically active molecules. To assess whether bacteria still recognize the modified AHL derivatives, we used two reporter strains. These bacteria carry plasmids with gene coding for an AHL receptor and the *Green Fluorescent Protein* (*GFP*) gene under control of AHL-inducible promoters: *Pseudomonas putida* strain F117 carrying the pKR-C12 plasmid (P_{lasB}-*gfp*(ASV)-P_{lac}-*lasR*; Gm^r),[51] and *Escherichia coli* strain MT102 carrying the pJBA89 plasmid (Ap^r; pUC18Not-*luxR*-P_{luxI}-RBSII-*gfp*(ASV)-T_0-T_1).[52] These bacteria are detecting nanomolar–micromolar concentrations of AHLs from C6-AHL to C14-AHL.[26] Both strains were treated with five native AHL molecules (see Fig. S1 of the ESI†). AHLs were dissolved in acetone and applied to lawns of reporter bacteria in different concentrations. GFP signals were recorded 2 h after application. Analysis of the obtained results revealed that reporter bacteria slightly differ in their AHL perception. While *P. putida* recognizes oxo-C10-AHL to oxo-C14-AHL, *E. coli* perceives oxo-C8-AHL but also all other tested native AHLs. *E. coli* and *P. putida* also recognize oxo-C14-AHL. Next, we addressed the question, whether the bacterial strains recognize the modified oxo-C14-AHL derivatives. *P. putida* recognizes **2** in a similar concentration range to oxo-C14-AHL as well as **3** and **4** at high concentration. *E. coli*, on the other hand, recognizes **3** and **5** comparably to oxo-C14-AHL. *E. coli* also recognized **4** and **15**, though only at high concentration (Fig. 2).

In order to verify the potential of biotinylated **5**, we performed an exploratory pull-down experiment with a member of the LuxR AHL-receptor family (Sinme_0536) from *Sinorhizobium meliloti*, a soil borne bacterium known to produce and perceive oxo-C14-AHL (Fig. 3).[29] *Sinme_0536* was cloned and

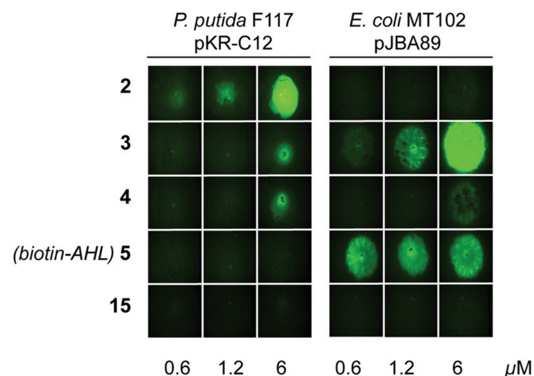

Fig. 2 Detection of AHL-derivatives with biosensor bacteria. Three different bacterial strains were used to detect the derivatives of *N*-acyl-homoserine lactones. Molecules were dissolved in acetone, or DMSO, and 5 μL were dropped on bacterial lawns for 2 h. Green fluorescent protein (GFP) signals were observed with fluorescent binocular using GFP filter Em: 505–550 nm.

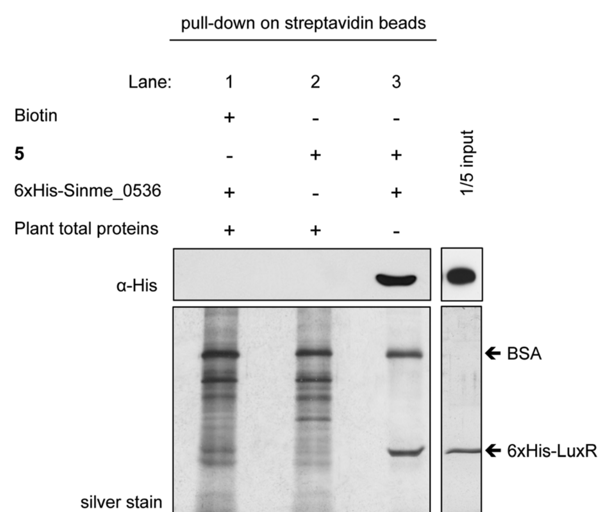

Fig. 3 Pull-down of the His-tagged oxo-C14-AHL receptor 6xHis-Sinme_0536 from *S. meliloti*. The biotinylated AHL-derivative **5** was immobilized on streptavidin beads, which were pretreated with BSA to minimize unspecific protein binding. Protein bands were visualized with silver stain and 6xHis-Sinme_0536 was detected by a His-specific antibody (α-His). BSA: bovine serum albumin.

purified as a 6xHis-tagged recombinant protein. Using streptavidin-coated beads, we attempted to precipitate the protein in the presence of solely biotin (negative control, lane 1) or in the presence of **5** (lane 3). As an additional control we used a pull-down setup without 6xHis-Sinme_0536 (lane 2). Proteins pulled down in the presence of **5** were probed for the occurrence of 6xHis-Sinme_0536 with a specific anti-His antibody. As shown in Fig. 2, in the presence of **5** (lane 3) but not free biotin (lane 1) 6xHis-Sinme_0536 was found in the precipitate (αHis, lane 3).

Recent reports suggest that AHLs produced by soil bacteria can actively affect plant health and development.[22,25,26,53–56] An early plant response to pathogen attack is transcriptional activation of defense related genes. Among them are pathogen-inducible WRKY transcription factors that are activated by

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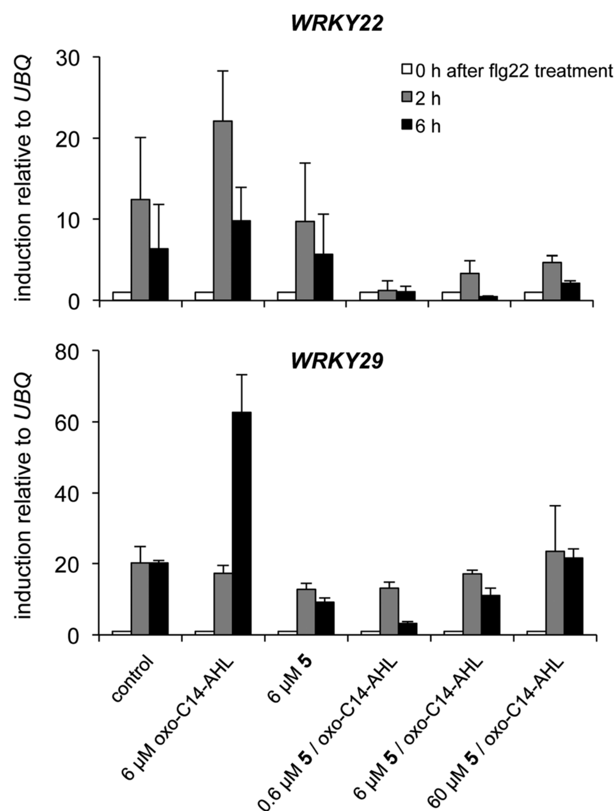

Fig. 4 The biotinylated AHL derivative **5** acts as an antagonist for plant AHL-induced resistance responses, measured by the transcriptional activation of plant transcription factors *WRKY22* and *WRKY29* in the presence of the natural autoinducer oxo-C14-AHL and the modified analogue **5**. Error bars represent SD from three independent experiments.

mitogen-activated protein kinases (MAPK) which are key elements in early defense signalling. Consistent with this, activation (phosphorylation) of MPK6 by a combined treatment of *Arabidopsis thaliana* with oxo-C14-AHL and the bacterial signal flg22 (flagellin 22) resulted in transcriptional upregulation of both *WRKY22* and *WRKY29*.[57–60] This scenario is thought to be the molecular base for AHL-induced resistance.[26] In order to compare the activity of native oxo-C14-AHL and its derivatives, we examined the impact of **5** on the relative expression levels of *WRKY22* and *WRKY29* (Fig. 4). Two-week-old *Arabidopsis* seedlings were pretreated with 6 μM oxo-C14-AHL (positive control), AHL derivatives or combinations of both and subsequently treated with 100 nM flg22. Total RNA was extracted and transcript levels of *WRKYs* normalized to the expression of *UBQ4* (*At5g25760*). Pretreatment with the AHL followed by treatment with 100 nM flg22 resulted in upregulation of *WRKY22* and *WRKY29* (Fig. 4). In contrast, pretreatment with modified AHL derivative **5** had no impact on *WRKYs* expression pattern. Notably, however, when **5** was added in addition to oxo-C14-AHL the observed AHL effect was abolished. We suspect that **5** is an antagonist to oxo-C14-AHL in plants. Given the fact that **5** induced GFP expression in bacterial reporter strains (agonistic action) this antagonistic effect in plants is remarkable and might be the consequence of the two different signaling pathways involved. It is likely that **5** binds to the cognate AHL receptor(s) preventing oxo-C14-AHL binding and initiation of defense priming in the plant. Whether **5** is a direct antagonist will be the subject of future studies.

Conclusions

In summary, we have synthesized new AHL derivatives with modified acyl chains and lactone moieties. The former are attractive molecular probes for the elucidation of molecular communication between plants and bacteria. The biotinylated AHL-derivative **5** turned out to be particularly valuable. It is still recognized by different bacteria as a signal molecule and can be used for pull-down systems on streptavidin beads. This was demonstrated with the specific pull-down of the LuxR-type receptor Sinme_0536 from *S. meliloti*. In addition, we have shown that **5** acts as an antagonist for native oxo-C14-AHL in *Arabidopsis* seedlings.

In addition to the biotinylated derivative **5**, the catechol derivatives **8** and **9** are particularly attractive molecular probes because they can easily be immobilized on metal surfaces and may thus be used for affinity catching of putative AHL-interacting proteins in plants.

Experimental

Material and methods

TLC was performed on silica gel aluminum sheets. Reagents used for developing plates include cerium stain (5 g molybdatophosphoric acid, 2.5 g cerium 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 using 400 (100) MHz instruments. ESI mass spectra were recorded using a TOF instrument operated in positive mode (Bruker MicrOTOF Q). Samples were dissolved in MeOH or H_2O–MeCN-mixtures and directly injected *via* a syringe. Analytical HPLC analysis was recorded using a VWR HITACHI ELITE LaChrom L-2130 HPLC (RI Detector: L2490). The following chiral column was used: CHIRALPAK IA (DAICEL Chemical Industries; particle size: 5 μm; dimensions: 4.6 mm ϕ × 150 mm). Solvents were dried by distillation from sodium under a nitrogen atmosphere prior to application.

The following compounds were prepared according to literature procedures: Boc-protected acid **1**,[49] biotin-NHS ester,[39] azido acid **6**,[38] TFA-glycine allylester **11a**,[48] TFA-allyl glycine

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12a,[48] TFA-iodolactone **13a**,[48] Boc-protected iodolactone **13c**,[50] and Boc-protected lactone **16c**.[50]

General procedure 1: coupling with Meldrum's acid

1 eq. of the appropriate fatty acid was dissolved in CH_2Cl_2 and 1 eq. DMAP, 1 eq. DCC and 1 eq. of Meldrum's acid were added to the mixture. The solution was stirred overnight at room temperature and then filtered to remove the *N*,*N*-dicyclohexyl urea formed in the reaction. The filtrate was concentrated *in vacuo* and the resulting residue was dissolved in DMF. α-Amino-γ-butyrolactone hydrobromide was added and the mixture was stirred at room temperature for 1 h and additional 4 h at 60 °C. The solvent was distilled off *in vacuo* and the residue was dissolved in EtOAc. The organic phase was washed three times with saturated sodium bicarbonate solution, 1 M sodium hydrogen sulfate solution and brine. Afterwards, the organic phase was dried over Na_2SO_4, filtered and then the solvent was distilled off. The crude product was purified by flash chromatography if necessary.

General procedure 2: Boc deprotection

The Boc protected amine was dissolved in TFA–CH_2Cl_2 (1 : 1; 5 mL per 0.1 mmol educt) and stirred at room temperature for 3 h. The solvent was distilled off *in vacuo*. The crude product was purified by flash chromatography if necessary.

General procedure 3: preparation of glycine allyl esters

1 eq. *N*-protected glycine was dissolved in CH_2Cl_2 and cooled to 0 °C. 1 eq. of allylic alcohol was added and the solution was cooled to −15 °C. A solution of 1 eq. DCC and 0.1 eq. DMAP in CH_2Cl_2 was added and the reaction was stirred at room temperature for 12 h. The precipitated *N*,*N*-dicyclohexyl urea was filtered off and washed with CH_2Cl_2. The organic layer was washed with 1 M HCl and saturated $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated *in vacuo*.

General procedure 4: Claisen rearrangement

LHMDS solution was prepared by adding 1 eq. 1.6 M *n*-BuLi in hexane at room temperature under an argon atmosphere to 1.2 eq. hexamethyldisilazane in abs. THF. The solution was stirred for 20 min. In a second flask 0.2 eq. of the *N*-protected glycine ester, 0.2 eq. $Al(OPr\text{-}i)_3$ and 0.5 eq. quinidine were dissolved in abs. THF under an argon atmosphere and cooled to −78 °C. The LHMDS solution was added slowly and the reaction was stirred at room temperature for 12 h. The mixture was treated with 1 M $KHSO_4$ and the organic layer was dried over Na_2SO_4. After filtration the solvent was distilled off *in vacuo* and the crude product was purified by flash chromatography if necessary.

General procedure 5: iodolactonization

The Claisen product was dissolved in THF at 0 °C and 1 eq. I_2 was added. The reaction was stirred for 12 h at room temperature and then diluted with EtOAc. Afterwards the mixture was quenched with saturated $Na_2S_2O_4$ solution. The organic layer was washed with brine, dried over Na_2SO_4 and filtered. The solvent was evaporated *in vacuo* and the crude product was purified by flash chromatography.

General procedure 6: preparation of azido lactones

To a solution of the iodide in DMF, NaN_3 was added and the mixture was stirred at 40 °C for 24 h. The solvent was distilled off *in vacuo* and the residue was dissolved in EtOAc. The organic layer was washed with water and brine. The organic phase was then dried over Na_2SO_4, filtered and the solvent was evaporated *in vacuo*. The crude product was purified by flash chromatography.

General procedure 7: NHS-ester coupling

1 eq. AHL **3**, 1 eq. NHS-ester and 1.3 eq. Et_3N were dissolved in DMSO. After stirring at room temperature for 24 h the solvent was distilled off *in vacuo* and the crude product was purified by flash chromatography if necessary.

AHL-derivative (2)

According to general procedure 1, the title compound **2** (278 mg, quant.) was obtained from carboxylic acid **1** (200 mg, 0.6 mmol), DMAP (77.5 mg, 0.6 mmol), DCC (131 mg, 0.6 mmol), Meldrum's acid (91 mg, 0.6 mmol) and α-amino-γ-butyrolactone hydrobromide (115 mg, 0.6 mmol) as a colourless solid. δ_H ($CDCl_3$, 400 MHz) 4.44–4.62 (m, 2H), 4.23–4.30 (m, 1H), 3.46 (s, 2H), 3.05–3.11 (m, 2H), 2.70–2.79 (m, 1H), 2.51 (t, 1H, J = 9.4 Hz), 2.12–2.29 (m, 1H), 1.90–1.94 (m, 1H), 1.64–1.73 (m, 2H), 1.53–1.64 (m, 2H), 1.43 (s, 9H), 1.18–1.20 (m, 14H); δ_C ($CDCl_3$, 100 MHz) 206.5, 174.8, 166.4, 156.1, 78.9, 65.8, 48.9, 48.1, 43.9, 40.6, 33.8, 30.0, 29.8, 29.4, 29.2, 28.9, 28.4, 26.8, 25.6, 24.9, 23.3; HRMS (ESI) *m*/*z* calcd for $C_{23}H_{40}N_2NaO_6$ $[M + Na]^+$ 463.2779, found 463.2784.

AHL-derivative (3)

According to general procedure 2, the title compound **3** (51 mg, 61%) was obtained as a brown oil from AHL **2** (108 mg, 0.2 mmol). δ_H (CD_3OD, 400 MHz, $COCH_2CO$ signal is hidden under solvent peak) 4.61 (t, 1H, J = 10.0 Hz), 4.44 (t, 1H, J = 2.3 Hz, J = 8.8 Hz), 4.27–4.33 (m, 1H), 2.91 (t, 2H, J = 8.0 Hz), 2.58 (t, 2H, J = 5.3 Hz), 2.24–2.35 (m, 1H), 1.52–1.68 (m, 5H), 1.28–1.42 (m, 14H); δ_C (DMSO-d_6, 100 MHz) 204.6, 174.8, 166.4, 65.3, 50.0, 48.1, 42.6, 41.9, 34.0, 33.3, 28.8, 28.7, 28.4, 28.3, 28.1, 26.9, 25.7, 22.8; HRMS (ESI) *m*/*z* calcd for $C_{18}H_{33}N_2O_4$ $[M + H]^+$ 341.2435, found 341.2440.

AHL-derivative (4)

AHL-derivative **3** (95 mg, 0.3 mmol), acetic anhydride (0.1 mL, 5 eq., 1.4 mmol) and Et_3N (0.4 mL, 10 eq., 2.8 mmol) were dissolved in CH_2Cl_2 (30 mL) and stirred for 12 h at room temperature. The solvent was evaporated *in vacuo* and the crude residue was purified by flash chromatography (EtOAc–MeOH, 9 : 1, R_f = 0.3) to give the title compound **4** (52 mg; 49%) as a brown oil. δ_H (CD_3OD, 400 MHz) 4.58–4.64 (m, 1H), 4.41–4.47 (m, 1H), 4.27–4.33 (m, 1H), 3.28 (s, 2H), 3.13 (t, 2H, J = 8.2 Hz), 2.57 (t, 3H, J = 8.2 Hz), 2.25–2.35 (m, 1H), 1.91 (s, 3H),

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1.44–1.60 (m, 4H), 1.26–1.29 (m, 14H); δ_C (CD_3OD, 100 MHz) 213.4, 206.5, 177.0, 173.4, 169.1, 67.1, 64.7, 50.2, 43.9, 40.7, 30.6, 30.5, 30.4, 30.3, 30.2, 30.0, 29.6, 28.0, 24.4, 22.5; HRMS (ESI) *m*/*z* calcd for $C_{20}H_{34}N_2NaO_5$ $[M + Na]^+$ 405.2360, found 405.2382.

Biotin-labeled-AHL (5)

Biotin-NHS ester (109 mg, 0.3 mmol) was dissolved in DMSO (50 mL). **3** (103 mg, 0.3 mmol) and Et_3N (0.06 mL, 1.3 eq., 0.4 mmol) were added to the solution and the mixture was stirred at room temperature for 24 h. The solvent was distilled off *in vacuo* to give the title compound **5** (97 mg, 54%) as a brown oil. δ_H (DMSO-d_6, 400 MHz) 6.36 (d, 2H, J = 12.3 Hz), 4.25–4.30 (m, 1H), 4.07–4.13 (m, 1H), 3.02–3.09 (m, 3H), 2.93–2.96 (m, 1H), 2.65–2.76 (m, 4H), 2.54–2.60 (m, 2H), 2.45–2.47 (m, 6H), 1.24–1.64 (m, 12H), 1.10–1.21 (m, 12H); δ_C (DMSO-d_6, 100 MHz) 204.7, 172.8, 170.2, 170.0, 162.8, 65.4, 61.1, 59.3, 55.2, 45.7, 42.7, 40.3, 30.0, 29.6, 29.1, 28.7, 28.5, 28.4, 28.2, 28.0, 27.97, 27.9, 27.5, 26.4, 25.4, 25.3, 25.25, 25.2; HRMS (ESI) *m*/*z* calcd for $C_{28}H_{46}N_4NaO_6S$ $[M + Na]^+$ 589.3030, found 589.3038.

Azido acid (6)[38]

12-Bromododecanoic acid (0.50 g, 1.8 mmol) was dissolved in CH_2Cl_2–DMF (100 mL, 6 : 4) and NaN_3 (1.17 g, 18 mmol) was added to the mixture. After stirring for 24 h at 60 °C the solvent was evaporated *in vacuo* and CH_2Cl_2 (60 mL) was added. The organic phase was washed three times with 1 M HCl (30 mL) and saturated NaCl solution (30 mL). The organic layer was then dried over Na_2SO_4, filtered and the solvent was distilled off *in vacuo* to give the title compound **6** (0.41 g, 94%) as a colourless oil. δ_H ($CDCl_3$, 400 MHz) 11.23 (b, 1H), 3.23 (t, 2H, J = 6.6 Hz), 2.32 (t, 2H, J = 7.4 Hz), 1.53–1.64 (m, 4H), 1.23–1.35 (m, 14H); MS (ESI) *m*/*z* 264.2 ($M + Na^+$, 100%).

AHL-derivative (7)

According to general procedure 1, the title compound **7** (480 mg, 77%) was obtained from carboxylic acid **6** (408 mg, 1.7 mmol), DMAP (207 mg, 1.7 mmol), DCC (349 mg, 1.7 mmol), Meldrum's acid (244 mg, 1.7 mmol) and α-amino-γ-butyrolactone hydrobromide (308 mg, 1.7 mmol) as a colourless solid after flash chromatography (EtOAc–MeOH 9 : 1, R_f = 0.3). δ_H (CD_3OD, 400 MHz) 4.61 (t, 1H, J = 10.3 Hz), 4.44 (dt, 1H, J = 3.0 Hz, J = 8.1 Hz), 4.27–4.33 (m, 1H), 3.27 (t, 2H, J = 5.9 Hz), 2.57 (t, 2H, J = 8.0 Hz), 2.23–2.34 (m, 1H), 1.67–1.73 (m, 1H), 1.53–1.63 (m, 6H), 1.28–1.38 (m, 14H); δ_C (CD_3OD, 100 MHz) 206.5, 171.2, 169.4, 67.1, 52.5, 50.0, 43.9, 34.8, 30.9, 30.7, 30.6, 30.5, 30.2, 30.0, 29.7, 27.9, 26.8, 24.5; HRMS (ESI) *m*/*z* calcd for $C_{18}H_{30}N_4NaO_4$ $[M + Na]^+$ 389.2160, found 389.2160.

Dihydroxybenzoic acid-NHS ester

3,4-Dihydroxybenzoic acid (350 mg, 2.3 mmol), *N*-hydroxysuccinimide (261 mg, 2.3 mmol) and DCC (468 mg, 2.3 mmol) were dissolved in DMF (40 mL). After stirring for 48 h at room temperature the solvent was distilled off *in vacuo* and H_2O (20 mL) was added. DCU was filtered off and the solvent was evaporated *in vacuo* to give the crude product as a colourless oil, which was used without further purification. MS (ESI) *m*/*z* 274.1 ($M + Na^+$, 100%).

AHL-derivative (8)

According to general procedure 7, the title compound **8** (180 mg, 88% over 2 steps) was obtained as a colourless oil from dihydroxybenzoic acid NHS-ester (147 mg, 0.6 mmol), **3** (200 mg, 0.6 mmol) and Et_3N (0.1 mL, 0.8 mmol). δ_H (CD_3OD, 400 MHz) 7.20–7.48 (m, 2H), 6.83 (d, 1H, J = 7.4 Hz), 4.62 (t, 1H, J = 10.0 Hz), 4.47 (t, 1H, J = 11.0 Hz), 4.28–4.35 (m, 1H) 3.17–3.22 (m, 2H), 2.93 (t, 1H, J = 11.0 Hz), 2.71 (s, 2H), 2.57–2.61 (m, 2H), 2.26–2.40 (m, 1H), 1.53–1.72 (m, 4H), 1.26–1.43 (m, 14H). δ_C (CD_3OD, 100 MHz) 206.7, 177.2, 175.1, 170.5, 151.4, 150.0, 146.0, 124.0, 117.6, 115.7, 67.1, 50.2, 47.9, 40.8, 30.6, 30.5, 30.4, 30.2, 30.1, 29.6, 28.6, 27.4, 26.3, 24.5, 24.4; HRMS (ESI) *m*/*z* calcd for $C_{25}H_{36}N_2NaO_7$ $[M + Na]^+$ 499.2415, found 499.2396.

Dimethoxybenzoic acid NHS-ester

2.3-Dimethoxybenzoic acid (300 mg, 2.6 mmol), *N*-hydroxysuccinimide (303 mg, 2.6 mmol) and EDC*HCl (503 mg, 2.6 mmol) were dissolved in DMF (50 mL). After stirring for 24 h at room temperature the solvent was distilled off *in vacuo* and EtOAc (20 mL) was added. The organic phase was washed three times with 20 mL 1 M $KHSO_4$ solution, saturated $NaHCO_3$ solution and saturated NaCl solution. The organic layer was then dried over Na_2SO_4, filtered and the solvent was evaporated *in vacuo* to give the title compound (373 mg, 81%) as a colourless solid. δ_H ($CDCl_3$, 400 MHz) 7.53 (d, 1H, J = 4.7 Hz), 7.11–7.19 (m, 2H), 3.93 (s, 3H), 3.89 (s, 3H), 2.88 (s, 4H). δ_C ($CDCl_3$, 100 MHz) 169.2, 161.0, 153.8, 150.7, 124.2, 122.9, 120.4, 118.1, 61.9, 56.4, 25.9; HRMS (ESI) *m*/*z* calcd for $C_{13}H_{13}NNaO_6$ $[M + Na]^+$ 302.0635, found 302.0639.

AHL-derivative (9)

According to general procedure 7, the title compound **9** (127 mg, 39%) was obtained as a colourless oil from dimethoxybenzoic acid NHS-ester (178 mg, 0.6 mmol), **3** (217 mg, 0.6 mmol) and Et_3N (0.1 mL, 0.8 mmol). The crude product was purified by flash chromatography (EtOAc 100%, R_f = 0.2). δ_H (CD_3OD, 400 MHz, $COCH_2CO$ signal is hidden under solvent peak) 7.32 (dd, 1H, J = 2.5 Hz, J = 4.0 Hz), 7.11–7.17 (m, 2H), 4.61 (t, 1H, J = 10.0 Hz), 4.44 (dt, 1H, J = 2.0 Hz, J = 9.0 Hz), 4.26–4.43 (m, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.38 (t, 2H, J = 6.8 Hz), 2.57 (t, 2H, J = 7.8 Hz), 2.27–2.34 (m, 1H), 1.51–1.74 (m, 5H), 1.60–1.45 (m, 14H). δ_C (CD_3OD, 100 MHz) 205.2, 175.8, 173.6, 166.9, 153.1, 147.0, 128.1, 123.9, 120.9, 115.2, 65.8, 60.3, 55.1, 48.7, 42.3, 42.3, 33.3, 29.2, 29.1, 29.0, 28.9, 28.6, 28.2, 26.6, 23.0; HRMS (ESI) *m*/*z* calcd for $C_{27}H_{40}N_2NaO_7$ $[M + Na]^+$ 527.2728, found 527.2735.

Cbz-glycine allylester (11b)

According to general procedure 3, the title compound **11b** (458 mg, 64%) was obtained from Cbz-glycine (600 mg,

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2.9 mmol), allylic alcohol (0.2 mL, 2.9 mmol), DMAP (35 mg, 2.9 mmol) and DCC (603 mg, 2.9 mmol). The crude product was purified by flash chromatography CH_2Cl_2–MeOH (10 : 0.2, R_f = 0.6) to give the product as a colourless oil. δ_H ($CDCl_3$, 400 MHz) 7.05–7.12 (m, 5H), 5.60–5.70 (m, 1H), 4.99–5.09 (m, 2H), 4.87 (s, 2H), 4.39 (d, 2H, J = 6.0 Hz), 3.75 (d, 2H, J = 6.0 Hz); δ_C ($CDCl_3$, 100 MHz) 169.7, 156.3, 136.2, 131.54, 128.54, 128.51, 128.21, 128.15, 128.1, 119.0, 67.1, 66.0, 42.8.

Boc-glycine allylester (11c)

According to general procedure 3, the title compound **11c** (893 mg, 73%) was obtained as a colourless oil from Boc-glycine (1.0 g, 5.7 mmol), allylic alcohol (0.4 mL, 5.7 mmol), DMAP (70 mg, 5.7 mmol) and DCC (1.2 g, 5.7 mmol). δ_H ($CDCl_3$, 400 MHz) 5.70–5.89 (m, 1H), 5.17–5.28 (m, 3H, NH, $-CH_2$), 4.57 (d, 2H, J = 6.7 Hz), 3.86 (d, 2H, J = 9.0 Hz), 1.39 (s, 9H); δ_C ($CDCl_3$, 100 MHz) 170.5, 155.7, 131.7, 118.6, 79.9, 65.8, 42.2, 28.2.

Cbz-protected allyl glycine (12b)

According to general procedure 4, the title compound **12b** (263 mg, 90%) was obtained as a brown oil. LHMDS solution was prepared freshly from hexamethyldisilazane (1.5 mL, 7 mmol) in abs. THF (5 mL) with *n*-BuLi (5 mL, 6 mmol). Cbz-glycine allylester **11b** (300 mg, 1.2 mmol), Al(OPr-i)$_3$ (269 mg, 1.3 mmol) and quinidine (972 mg, 3 mmol) were dissolved in abs. THF (50 mL). The LHMDS solution was added slowly to the reaction mixture. δ_H ($CDCl_3$, 400 MHz) 7.29–7.38 (m, 5H), 5.67–5.78 (m, 1H), 5.09–5.18 (m, 4H, 7-H), 4.46–4.51 (m, 1H), 2.52–2.67 (m, 2H); δ_C ($CDCl_3$, 100 MHz) 175.0, 154.9, 135.2, 130.9, 127.5, 127.4, 127.3, 127.2, 127.1, 118.5, 65.9, 52.3, 35.3.

Boc-protected allyl glycine (12c)

According to general procedure 4, the title compound **12c** (300 mg, quant.) was obtained as a brown oil. LHMDS solution was prepared freshly from hexamethyldisilazane (1.7 mL, 8.1 mmol) in abs. THF (5 mL) with *n*-BuLi (7 mL, 7 mmol). Boc-glycine allylester **12b** (300 mg, 1.4 mmol), Al(OPr-i)$_3$ (314 mg, 1.5 mmol) and quinidine (1.13 g, 3.5 mmol) were dissolved in abs. THF (50 mL). The LHMDS solution was added slowly to the reaction mixture. δ_H ($CDCl_3$, 400 MHz) 5.67–5.78 (m, 1H), 5.02–5.18 (m, 2H), 4.94 (1H, NH), 4.32–4.41 (m, 1H), 2.41–2.62 (m, 2H), 1.42 (s, 9H); δ_C ($CDCl_3$, 100 MHz) 179.2, 155.7, 132.0, 119.5, 86.0, 53.0, 36.3, 28.4.

Cbz-protected iodolactone (13b)

According to general procedure 5, the title compound **13b** (330 mg, 83%) was obtained from Cbz-protected allyl glycine **12b** (264 mg, 1.0 mmol) and I_2 (536 mg, 2.0 mmol). The crude product was purified by flash chromatography PE–EtOAc (1 : 1, R_f = 0.4) to give **13b** as a brown oil. δ_H (DMSO-d_6, 400 MHz) 7.32–7.41 (m, 5H), 5.06 (s, 2H), 4.56–4.67 (m, 1H), 4.44–4.45 (m, 1H), 3.24–3.55 (m, 2H), 2.56–2.63 (m, 1H), 1.77–1.92 (1H); δ_C (DMSO-d_6, 100 MHz) 174.2, 155.9, 136.9, 128.4, 128.3, 127.9, 127.8, 127.6, 75.4, 65.8, 51.4, 34.7, 7.8; HRMS (ESI) m/z calcd for $C_{13}H_{14}INNaO_4$ $[M + Na]^+$ 397.9860, found 397.9861.

Cbz-protected azide (16b)

According to general procedure 6, the title compound **16b** (296 mg, 93%) was obtained from lactone **13b** (400 mg, 1.1 mmol) and NaN_3 (347 mg, 5.3 mmol). The crude product was purified by flash chromatography PE–EtOAc (1 : 1, R_f = 0.3) to give **16b** as a brown oil. δ_H (DMSO-d_6, 400 MHz) 7.29–7.37 (m, 5H), 5.03 (s, 2H), 4.41–4.65 (m, 2H), 3.61–3.80 (m, 2H), 2.40–2.48 (m, 1H), 1.93–2.07 (m, 1H); δ_C (DMSO-d_6, 100 MHz) 174.5, 155.5, 136.8, 128.4, 128.35, 127.9, 127.8, 127.7, 75.4, 65.7, 52.9, 50.5, 30.4; HRMS (ESI) m/z calcd for $C_{13}H_{14}N_4O_4$ $[M + Na]^+$ 313.0907, found 313.0907.

Iodolactone (14)

Deprotection of the Boc-group was performed according to general procedure 2. Boc-protected iodolactone **13c** (209 mg, 0.6 mmol) was dissolved in TFA–CH_2Cl_2 (30 mL, 1 : 1) to give the crude product which was used in the next step without further purification. HRMS (ESI) m/z calcd for $C_5H_8INO_2$ $[M + H]^+$ 241.9672, found 241.9668.

Azide lactone (17)

Deprotection of the Boc-group was performed according to general procedure 2. Boc-protected azide **16c** (129 mg, 0.5 mmol) was dissolved in TFA–CH_2Cl_2 (30 mL, 1 : 1). The product was not isolated and used in the next step without further purification.

AHL-derivative (15)

According to general procedure 1, the title compound **15** (120 mg, 43% over 2 steps) was obtained from lauric acid (119 mg, 0.6 mmol), DMAP (72 mg, 0.6 mmol), DCC (122 mg, 0.6 mmol), Meldrum's acid (85 mg, 0.6 mmol) and iodolactone **14** (143 mg, 0.6 mmol). The crude product was purified by flash chromatography (PE–EtOAc 9 : 1, R_f = 0.2). δ_H ($CDCl_3$, 400 MHz) 4.46–4.80 (m, 2H), 3.44–3.49 (m, 2H), 3.30–3.38 (m, 1H), 2.93–3.00 (m, 1H), 2.44–2.54 (m, 2H), 1.89–1.98 (m, 1H), 1.55–1.71 (m, 3H), 1.19–1.35 (m, 16H), 0.87 (t, 3H, J = 8.1 Hz). δ_C ($CDCl_3$, 100 MHz) 206.8, 173.6, 166.3, 76.3, 50.7, 48.2, 44.1, 36.1, 34.1, 32.1, 29.6, 29.4, 29.3, 29.0, 25.5, 23.36, 22.65, 14.1, 7.9; HRMS (ESI) m/z calcd $C_{19}H_{32}INO_4$ $[M + Na]^+$ 488.1268, found 488.1256.

AHL-derivative (18)

According to general procedure 1, the title compound **18** (106 mg, 35% over 2 steps) was obtained from lauric acid (163 mg, 0.8 mmol), DMAP (99 mg, 0.8 mmol), DCC (167 mg, 0.8 mmol), Meldrum's acid (117 mg, 0.8 mmol) and azide **17** (127 mg, 0.8 mmol). The crude product was purified by flash chromatography (PE–EtOAc 1 : 1, R_f = 0.2). δ_H ($CDCl_3$, 400 MHz) 4.55–4.76 (m, 2H), 3.43–3.67 (m, 2H), 2.55–2.65 (m, 2H), 2.06–2.15 (m, 1H), 1.82–1.88 (m, 1H), 1.67–1.74 (m, 1H), 1.53–1.64 (m, 3H), 1.24–1.36 (m, 16H), 0.89 (t, 3H, J = 8.1 Hz); δ_C ($CDCl_3$, 100 MHz) 206.4, 175.7, 169.7, 79.3, 77.7, 54.8, 51.0, 43.6, 34.9, 33.1, 32.1, 30.8, 30.5, 30.2, 26.9, 26.1, 24.6, 23.9,

14.6; HRMS (ESI) m/z $C_{19}H_{32}N_4O_4$ $[M + Na]^+$ 403.2316, found: 403.2329.

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