Synthesis of mycobacterial phenolic glycolipids

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Chapter 3

Synthesis of Phthiocerol alkyne

Introduction

Phthiocerol is a methoxyglycol that was first isolated from chloroform extracts of *Mycobacterium tuberculosis*,[1,2] and its structure was later established to be the methoxydiol shown in Figure 1, using infrared spectroscopy, GC-MS and degradative experiments.[3–6] Minor variations such as phthiotriol and phthiodiolone in which the methyl ether is replaced by either an alcohol or a ketone, respectively, and shorter versions (B) have also been found (Figure 1).[7–11] Phthiocerol is the backbone of Phthiocerol Dimycocerosate[12] (PDIM) and Phenolic Glycolipids (PGLs) which are phenolphthiocerol dimycocerosate based glycolipids.

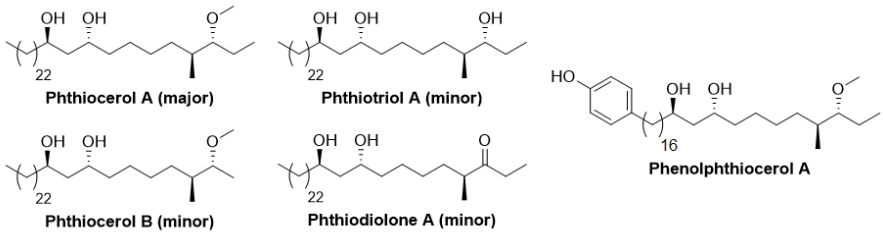


Figure 1. Phthiocerol and variations thereof.

Phenolic glycolipids have a well preserved lipid structure among mycobacteria with an *anti*-diol on the phthiocerol backbone and multiple *R*-configured *C*-methyls on

mycocerosic acid, with 4 methyls being the most prevalent.[13–16] *M. marinum* and *M. ulcerans* on the other hand contain phenolic glycolipids with a *syn*-diol on the phthiocerol backbone and *S*-configured methyls on mycocerosic acid (Figure 2).[17–20]

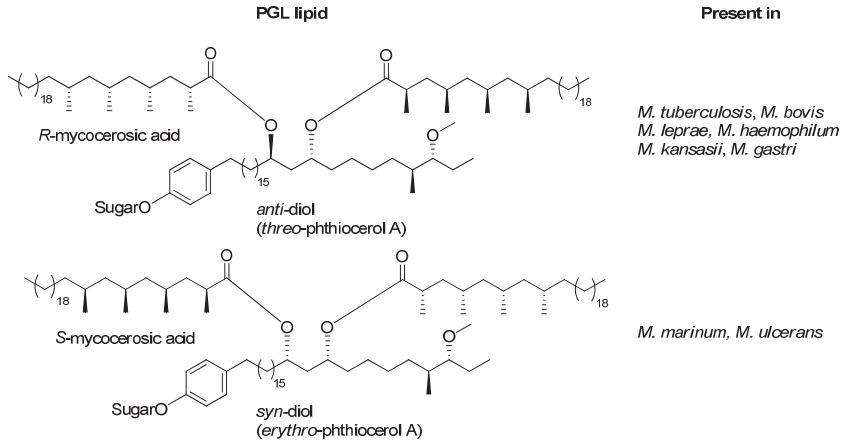
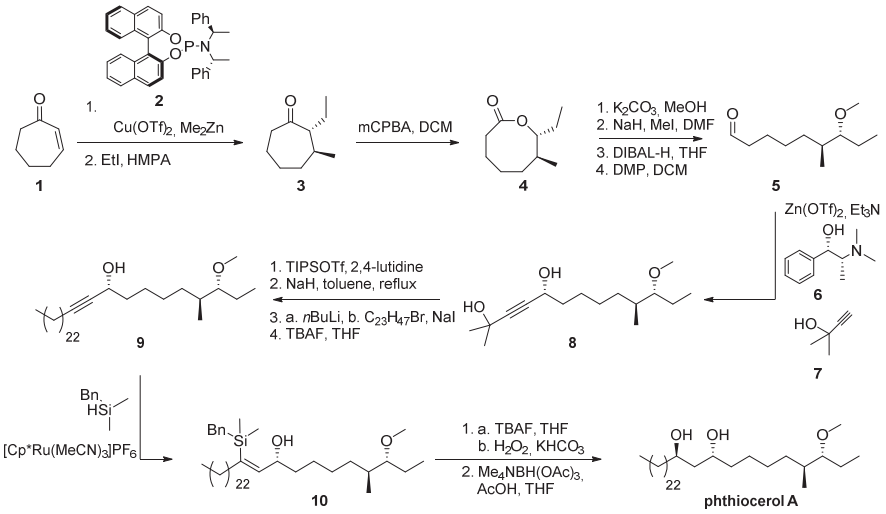


Figure 2. Lipid backbones of PGLs and the corresponding mycobacteria.

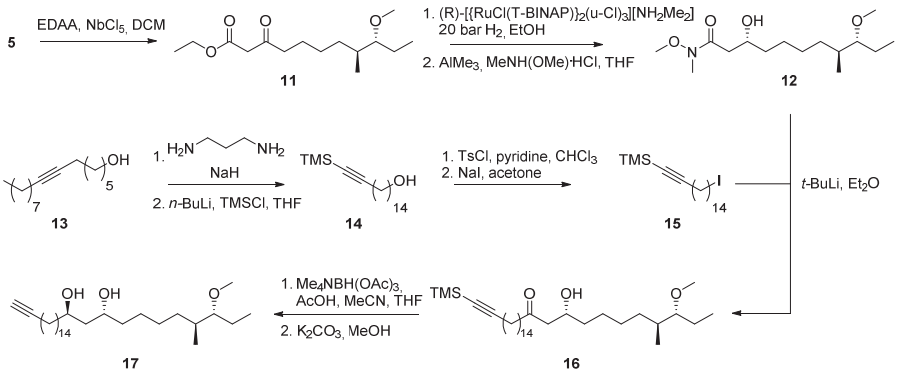
In order to confirm its structure, the chemical synthesis of PDIM A, and thereby also phthiocerol A, has been performed by Minnaard and coworkers in 2008 (Scheme 1).[21,22] Their synthetic strategy was based on a tandem copper/phosphoramidite-catalyzed asymmetric conjugation addition to cycloheptenone providing the *anti*-methoxy methyl unit in phthiocerol.[21,23–25] After a Baeyer-Villiger oxidation, opening of the resulting 8-membered lactone **4**, methylation of the freed alcohol and reduction and oxidation of the methyl ester gave aldehyde **5**, alkyne **7** could then stereoselectively be added to the aldehyde using a procedure developed by Carreira and co-workers.[26] The secondary alcohol that was formed was protected with a silyl ether, whereafter the alkyne was deprotected under basic conditions and elongated by alkylating the corresponding alkynyllithium compound with $CH_3(CH_2)_{22}Br$ in the presence of NaI. After removal of the silyl ether, compound **9** was hydrosilylated with benzyldimethylsilane catalyzed by $[Cp^*Ru(MeCN)_3]PF_6$.[27,28] The mixture of silanes was treated with TBAF and subjected to a Fleming-Tamao oxidation with H_2O_2 and $KHCO_3$ to give the corresponding hydroxy ketones that were separated by chromatography.[29] Thereafter an Evans-Saksena reduction[30] of the β–hydroxy ketone selectively produced the *anti*-diol which was then

coupled to mycocerosic acid[22] using Steglich esterification conditions[31] to complete the first total synthesis of PDIM A.



Scheme 1. Synthesis of phthiocerol A as performed by Casas-Arce et al.[32]

While this was an efficient synthesis of PDIM A, the phthiocerol that was synthesized was not suited for conjugation to glycans, which is required to synthesize complete PGLs. Therefore when Minnaard and coworkers set out to synthesize PGL-tb1, a new route had to be designed which would yield phthiocerol derivative **17** with a terminal alkyne as a conjugation handle (Scheme 2).[33] This route also made use of key aldehyde **5** which was elongated with ethyl diazoacetate and $NbCl_5$[34] to give β-keto ester **11**. This ketone was then stereoselectively reduced using a chiral ruthenium catalyst[35,36] and the resulting β-hydroxy ester was transformed to a Weinreb amide.



Scheme 16. Synthesis of phthiocerol containing a terminal alkyne as performed by Barroso et al.[33]

Iodide **15** was made in 4 steps from hexadec-7-yn-1-ol (**13**) by means of a Zipper reaction,[37] protection of the terminal alkyne with a TMS group[38] and the substitution of the primary alcohol with an iodide. Coupling of lithiated iodide **15** to Weinreb amide **12** produced β-hydroxy ketone **16** which was stereoselectively transformed to the *anti*-diol by means of an Evans-Saksena reduction similarly as described above in the synthesis of phthiocerol by Casas-Arce et al.[30] After deprotection of the terminal alkyne this phthiocerol derivative **17** could be connected through a Sonogashira cross-coupling to a glycan bearing an iodophenol on the reducing end. Esterification of the resulting diol with mycocerosic acid[22] and subsequent global deprotection produced the first total synthesis of PGL-tb1.

In order to synthesize all PGLs outlined in this thesis, a large amount of the phthiocerol alkyne derivative is needed. Unfortunately, hexadec-7-yn-1-ol was difficult to obtain commercially, especially in large quantities. Therefore, a new synthesis had to be devised. A route was envisaged which introduced the terminal alkyne of iodide **15** by means of a homologation of an aldehyde which in turn could be produced in large amounts from pentadecanolide (Figure 3). A potential method for this reaction could be the Seyferth-Gilbert homologation, which makes use of dimethyl (diazomethyl)phosphonate.[39] While this method produces the alkyne in a single step from the aldehyde, the terminal alkyne would then have to be protected in a separate step. Another disadvantage of this method is that the required reagent is not shelf stable. The

more stable Bestmann-Ohira reagent (dimethyl diazo-2-oxopropylphosphonate)[40,41] could be used, but this reagent needs to be synthesized beforehand with relatively expensive reagents, compromising scaling up of the synthesis. Therefore, a route was chosen which uses the Corey-Fuchs reaction[42] to transform the aldehyde to a 1,1-dibromoalkene with PPh_3 and CBr_4. This alkene could then be transformed to an alkyne via an *n*-Buli mediated 1,2-hydride shift (Fritsch-Buttenberg-Wiechell rearrangement)[43–46] and the resulting alkynyllithium intermediate could react with TMSCl in the same pot. For the homologation reaction the primary alcohol would have to be protected with a moiety that could withstand the extremely basic conditions required for the hydride shift and possible nucleophilic attack by PPh_3. Furthermore, because of the TMS-protected terminal alkyne the protecting group should not require hydrogenation, protic bases or fluoride-based conditions to be removed. It was therefore opted to use a *para*-methoxybenzyl (PMB) or benzyl ether as these groups can be removed with oxidative conditions or alternatively in the case of the PMB ether with mild acidic conditions.[47,48]

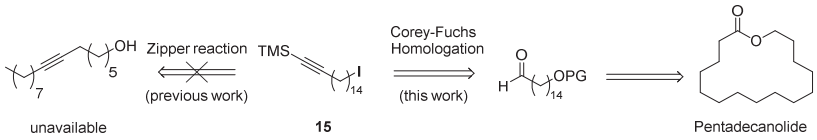
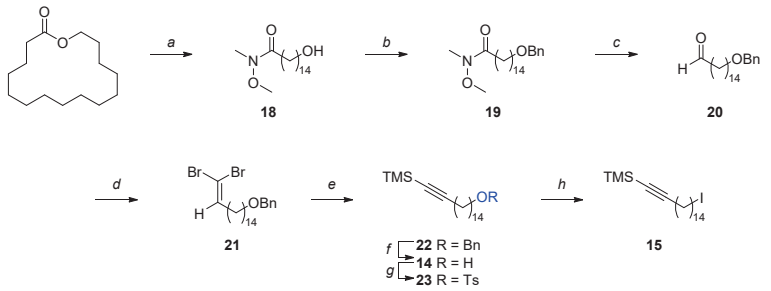


Figure 3. Retrosynthetic analysis of iodide **15**. (PG = protecting group)

Results and discussion

The successful synthesis of iodide **15** is depicted in Scheme 3. The opening of pentadecanolide with *N,O*-dimethyl hydroxylamine hydrochloride and *iso*-propylmagnesium chloride in THF[49] gave Weinreb amide **18** in near quantitative yield. At first, the resulting free alcohol was protected with a PMB-ether. Later on in the synthesis, when acidic conditions were used to remove this group, only moderate yields were obtained. A DDQ mediated oxidation proceeded smoothly but the *p*-anisaldehyde which was liberated as a side-product had the same retention factor as the product during column chromatography, which greatly hindered purification. Therefore, a benzyl ether was then tried as an alternative protecting group. Thus, Weinreb amide **18** was protected with a benzyl ether using BnBr and NaH in DMF to give **19** in 77% yield. The protected Weinreb amide was then reduced with $LiAlH_4$ to give aldehyde **20** in quantitative yield. It

was found that this reaction is best performed in Et_2O instead of THF, as this significantly facilitates the work-up, leading to an improved yield. Using a 1 M HCl washing step instead of Rochelle's salt further improved the work-up as the latter resulted in a thick gel that was very difficult to separate into two layers.

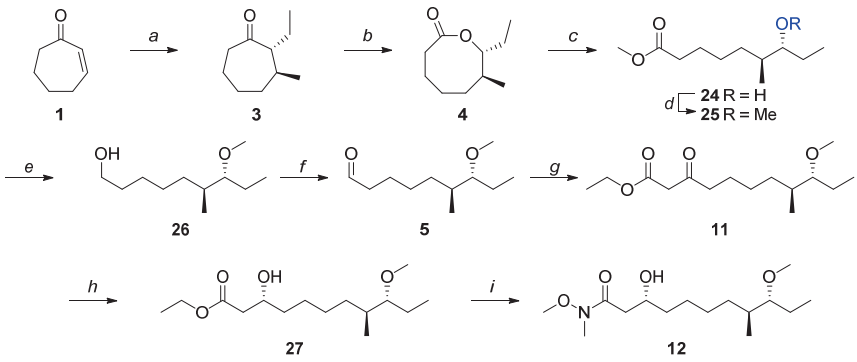


Scheme 3 Synthesis of iodide **16**. Reagents and conditions: (a) *N,O*-dimethylhydroxylamine hydrochloride, *iso*-propylMgCl, THF, 99%, (b) NaH, BnBr, DMF, 0 °C → RT, 77%, (c) $LiAlH_4$, Et_2O, 0 °C → RT, 100%, (d) CBr_4, PPh_3, DCM, 0 °C → RT, 91%, (e) *n*-BuLi, TMSCl, Et_2O, 0 °C, 94%, (f) DDQ, DCM/H_2O, 86%, (g) *p*-TsCl, pyridine, $CHCl_3$, 95%, (h) NaI, acetone, 96%.

Next, aldehyde **20** could be transformed to dibromoalkene **21** with PPh_3 and CBr_4, a reaction which could easily be scaled up to >25 grams. The Fritsch-Buttenberg-Wiechell rearrangement and subsequent TMS protection were first attempted at -78 °C, but at this temperature, the starting material partially precipitates which hampered the reaction. When the reaction was performed at 0 °C, the starting material was well soluble and the reaction produced protected alkyne **22** in 89% yield. As noted before in the reduction of the Weinreb amide to the aldehyde, the use of Et_2O instead of THF improved the yield. The benzyl ether was then removed using DDQ to produce primary alcohol **15** in 86% yield. The tosylation and iodide substitution that followed produced iodide **16** in 90% yield over two steps.

The synthesis of Weinreb amide **12** (Scheme 4) was performed as reported by Barroso *et al.*[33] with only minor modifications. It was attempted to improve the Baeyer-Villiger oxidation of **3** by using other oxidative reagents (TFPAA, 2-iodobenzenesulfonic acid together with Oxone) but these attempts were to no avail. Direct reduction of ester **25** to the aldehyde **5** with DIBAL-H was attempted but overreduction occurred. Therefore, reduction of the ester with $LiAlH_4$ to the alcohol was followed by an oxidation with Dess-

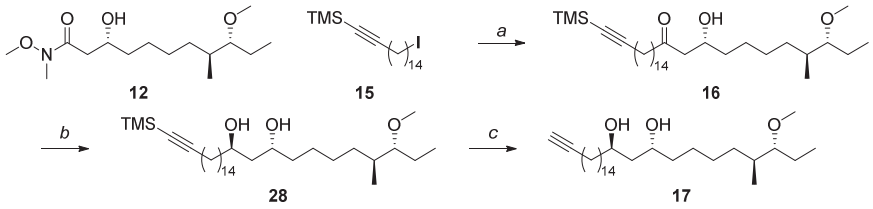
Martin Periodinane to give aldehyde **5** in 82% yield over 2 steps. The coupling of this aldehyde with ethyl diazoacetate under the agency of $NbCl_5$ produced ß-keto ester **11** in 86% yield. Because $NbCl_5$ is highly hygroscopic, the best results were obtained if the reagent was not weighted. Next the asymmetric hydrogenation was performed with (*R*)-[(RuCl(tol-BINAP))$_2$(μ-Cl)$_3$][NH_2Me_2] as catalyst, which gave ß-hydroxy ester **27** in 87% yield. This ester was transformed to the corresponding Weinreb amide with *N,O*-dimethylhydroxylamine hydrochloride and $AlMe_3$ in 89% yield.



Scheme 4. Synthesis of Weinreb amide **13**. Reagents and conditions: (a) 1. Phosphoramidite **2**, $Cu(OTf)_2$, Me_2Zn, -25 °C, toluene 2. EtI, HMPA, 0 °C. (b) *m*CPBA, DCM reflux, (c) K_2CO_3, MeOH, 43% over 3 steps, (d) NaH, MeI, DMF, 0 °C → RT, 84%, (e) $LiAlH_4$, Et_2O, 0 °C, 98%, (f), DMP, DCM, 84%, (g) ethyl diazoacetate, $NbCl_5$, DCM, 86%, (h) (R)-[(RuCl(tol-BINAP))$_2$(μ-Cl)$_3$[NH_2Me_2], H_2, EtOH, 87%, (i) *N,O*-dimethylhydroxylamine hydrochloride, $AlMe_3$, THF, 0 °C → RT, 89%.

The final steps of the synthesis of the phthiocerol alkyne are depicted in Scheme 5. Coupling of lithiated **15** to Weinreb amide **12** turned out to be a challenge. Iodide **15** partially precipitates at the low temperatures required for the reaction, hampering the reaction. Two equivalents of *t*-BuLi per iodide are required to activate it and more than two equivalents of the activated species are required for the coupling because the first equivalent will deprotonate the alcohol in **12**. However, if too much *t*-BuLi is added a *tert*-butyl ketone can be formed. It was observed that this *tert*-butyl ketone has the same retention factor as product **16**, complicating purification of **16** but also of **28** and **17**, generated in the next two steps. Despite these challenges ß-hydroxy ketone **16** was obtained in 60% yield and it could be reduced with $NMe_4BH(OAc)_3$ to stereoselectively

produce 1,3-*anti* diol **28** in 80% yield. Thereafter, removal of the TMS-group using basic conditions gave the desired phthiocerol alkyne derivative in 97% yield.



Scheme 5. Synthesis of phthiocerol alkyne **18**. Reagents and conditions: (a) *t*-BuLi, Et_2O -78 °C, 60%, (b) $NMe_4BH(OAc)_3$, THF/MeCN/AcOH, 0 °C, 80%, (c) K_2CO_3, MeOH, 97%.

Conclusion

In conclusion, in order to synthesize phthiocerol alkyne derivative **17**, a new route had to be devised for iodide **15** which did not rely on hexadec-7-yn-1-ol. A route was chosen which started from pentadecanolide to give the desired molecule in 51% yield over 8 steps, with a Corey-Fuchs reaction as the key step, which could be easily scaled up. Weinreb amide **12** was synthesized from cycloheptenone in 19% yield over 9 steps according to previously reported procedures. After the coupling of these two building blocks and the final reduction and deprotection, phthiocerol alkyne derivative **17** was successfully synthesized in 4.3 % over 20 steps and could be used for the total synthesis of phenolic glycolipids as will be reported in the coming chapters of this thesis.

EXPERIMENTAL:

General procedures

All reactions were carried out in oven-dried glassware (80 °C). Prior to reactions, traces of water and solvent were removed by co-evaporation with toluene where appropriate. Reactions sensitive to air or moisture were carried out under N_2 atmosphere (balloon). Commercially available reagents and solvents (Aldrich Chemistry, Honeywell, Merck, Fisher Scientific, Biosolve, Fluka, VWR Chemicals, Acros Organics, Fluorochem, Brunschwig, Carbosynth) were used as received unless stated otherwise.

Solvents for reactions were reagent grade and dried by storage over flame dried 4 Å molecular sieves when needed. Et_2O used for column chromatography was distilled before use and stored over iron filings.

Reaction progress was monitored using aluminium-supported silica gel TLC plates (Merck, Kieselgel 60, F254); visualization was carried out by irradiation with UV light (254 nm), and spraying with 20% H_2SO_4 in EtOH (w/v) or $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/L) in 10% H_2SO_4, followed by charring. Additional analysis with TLC-MS was used when needed.

Column chromatography was carried out using silica gel (Fluka, 40-63 μm mesh). The column was prepared using the apolar component mentioned in the corresponding experimental. If the apolar component was pentane the product was brought up in toluene. If the apolar component was DCM the product was brought up in DCM, possibly with a few drops of methanol if needed. Colum chromatography was performed using a gradient ranging from 0% polar component up to the ratio mentioned in the corresponding experimental in 2 to 5 steps depending on the ease of separation.

NMR spectra were recorded at ambient temperature on a Bruker AV-400LIQ spectrometer. Samples were prepared in $CDCl_3$ unless stated otherwise. Chemical shifts (δ) in $CDCl_3$ are reported in ppm relative to Me_4Si (δ: 0.00 ppm) for ^{1}H-NMR and $CDCl_3$ (δ: 77.16 ppm) for ^{13}C-APT NMR. Chemical shifts in CD_3OD are reported in ppm relative to H_2O (δ: 4.87 ppm) for ^{1}H-NMR and CD_3OD (δ: 49.00 ppm) for ^{13}C-APT NMR. ^{13}C-APT spectra are ^{1}H decoupled and structural assignments were achieved using HH-COSY and HSQC 2D experiments. Coupling constants (*J*) are given in Hz. Coupling constants of anomeric carbon atoms ($J_{H1,C1}$) were determined using HMBC-GATED experiments. Optical rotations were measured on an Anton Paar Modular Circular Polarimeter MCP 100/150. High resolution mass spectra were recorded on a Synapt G2-Si or a Q Exactive HF Orbitrap equipped with an electron spray ion source positive mode. Infrared spectra were recorded on a Perkin Elmer Spectrum 2 FT-IR.

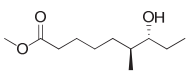
(2*R*)-ethyl-(3*S*)-methylcycloheptanone (3)

(S,R,R)-Leggy phosphoramidite (**2**, 200 mg, 0.37 mmol, 0.6 mol%) and $Cu(OTf)_2$ (65 mg, 0.18 mmol, 0.3 mol%) were dissolved in dry toluene (60 ml) and stirred for 15 min under nitrogen at room temperature. The mixture was cooled to -25 °C and Me_2Zn (2 M in toluene, 50 ml, 100 mmol, 1.5 eq) was added dropwise under nitrogen flow. After stirring for 10 min, a solution of cycloheptenone (7.5 ml, 66 mmol, 1.0 eq) in dry toluene (60 ml) was added over 8 h by syringe pump and the resulting mixture was stirred for 24 hours at -25 °C. Ethyl iodide (54 ml, 660 mmol, 10.0 eq) and HMPA (115 ml, 660 mmol, 10.0 eq) were added, the mixture was warmed up to 0 °C and stirred for 60 h. The reaction was quenched with sat. aq. NH_4Cl, extracted with Et_2O (3x), washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 49:1) to give **3** as a clear oil. Due to the volatile nature of the product it was used in the next reaction without further analysis.

(8*R*)-ethyl-(7*S*)-methyloxocan-2-one (4)

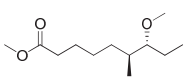
Compound **3** (10.2 g, 66 mmol, 1.0 eq) was dissolved in DCM (250 mL, 0.26 M) and mCPBA (81.4 g, 330 mmol, 5.0 eq) was added to the solution. The mixture was refluxed for 3 days after which it was cooled to rt. The mixture was washed with sat. aq. $NaHCO_3$, sat. aq. NaS_2O_3 and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 49:1) to give the **4** as a clear oil. Due to the volatile nature of the product it was used in the next reaction without further analysis.

Methyl (7*R*)-hydroxy-(6*S*)-methylnonanoate (24)



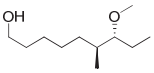
Compound **4** (11.2 g, 18.5 mmol, 1.0 eq) was dissolved in MeOH (250 mL, 0.26 M), this solution was cooled to 0 °C and K_2CO_3 (3.48 g, 27.8 mmol, 1.5 eq) was added. The reaction was allowed to stir overnight while slowly warming to rt. The reaction was quenched with sat. aq. NH_4Cl, extracted with Et_2O (3x). The combined organic layers were washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 4:1) to give **25** (5.72 g, 28.3 mmol, 43% over 3 steps) as a clear oil. Spectroscopic data were in accordance with those previously reported in the literature.[33]

Methyl (7*R*)-methoxy-(6*S*)-methylnonanoate (25)



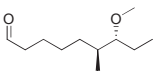
Compound **24** (1.59 g, 7.84 mmol, 1.0 eq) was dissolved in dry DMF (78 mL, 0.1 M) and MeI (1.46 mL, 23.5 mmol, 3.0 eq) was added to the solution. The mixture was cooled to 0 °C, and NaH (60%, 0.63 g, 15.7 mmol, 2.0 eq) was added. The reaction mixture was warmed to rt while stirring for 6 hours. The reaction was quenched by addition of MeOH, partitioned between water and Et_2O and extracted with Et_2O (2x). The organic layers were combined, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 6:4) gave the title compound (1.42 g, 6.57 mmol, 84%) as a clear oil. Spectroscopic data were in accordance with those previously reported in the literature.[33]

(7*R*)-methoxy-(6*S*)-methylnonan-1-ol (26)



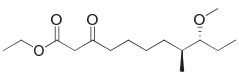
Compound **25** (1.42 g, 6.57 mmol, 1.0 eq) was dissolved in Et_2O (50 mL, 0.13 M), and the solution was cooled to 0 °C. $LiAlH_4$ (4.0 M in Et_2O, 1.7 mL, 6.9 mmol, 1.05 eq) was added and the reaction was allowed to stir for 2 hours. The reaction was quenched by addition of MeOH and washed with 1 M HCl, sat. aq. $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*, giving the product as a clear oil (1.22 g, 6.46 mmol, 98%). The product was used in the next reaction without further purification. Spectroscopic data were in accordance with those previously reported in the literature.[21]

(7*R*)-methoxy-(6*S*)-methylnonanal (5)



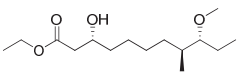
Compound **26** (1.22 g, 6.46 mmol, 1.0 eq) was dissolved in DCM (32 mL, 0.2 M) and DMP (3.02 g, 7.11 mmol, 1.1 eq) was added to the solution. The reaction was allowed to stir for 4 hours after which it was quenched by addition of a 1:1 mixture of sat. aq. $NaHCO_3$ and sat. aq. NaS_2O_3. The layers were separated and the organic layer was washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 9:1) to give **5** (1.01 g, 5.41 mmol, 84%) as a clear oil. Spectroscopic data were in accordance with those previously reported in the literature.[33]

(8*S*,9*R*)-ethyl-9-methoxy-8-methyl-3-oxoundecanoate (11)



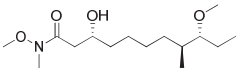
Compound **5** (0.613 g, 3.29 mmol, 1.0 eq) was dissolved in DCM (35 mL, 0.1 M) and a catalytic amount [the amount was not weighed due to tendency for hydrolysis] of $NbCl_5$ was added to the solution and it was cooled to 0 °C. EDAA (87%, 0.6 mL, 4.94 mmol, 1.5 eq) was slowly added and the reaction was allowed to stir for 4 hours after which it was diluted with H_2O and extracted with DCM (3x). The combined organic layers were washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 9:1) to give **11** (0.735 g, 2.7 mmol, 86%) as a clear oil. Spectroscopic data were in accordance with those previously reported in the literature.[33]

(3*R*,8*S*,9*R*)-ethyl-3-hydroxy-9-methoxy-8-methyl-oxoundecanoate (27)



Compound **11** (1.13 g, 4.15 mmol, 1.0 eq) was dissolved in EtOH (20 mL, 0.2 M) and (*R*)-[(RuCl(tol-BINAP))$_2$(μ-Cl)]$_3$[NH_2Me_2] (74 mg, 42 μmol, 0.01 eq) was added to the solution. The mixture was purged with N_2 after which it was stirred under 22 bar of H_2 atmosphere for 24 hours. The mixture was then diluted with toluene, concentrated *in vacuo* and purification by means of column chromatography (*n*-pentane-Et_2O 6:4) to give **28** (0.99 g, 3.61 mmol, 87%) as a slightly green oil. Spectroscopic data were in accordance with those previously reported in the literature.[33]

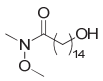
(3*R*,8*S*,9*R*)-3-hydroxy-*N*,9-dimethoxy-*N*,8-dimethylundecanamide (12)



N,O-dimethylhydroxylamine hydrochloride (1.06 g, 10.8 mmol, 3.0 eq) was dissolved in dry THF (36 mL) and the solution was cooled to 0 °C. $AlMe_3$ (2 M in toluene, 5.4 mL, 10.8 mmol, 3.0 eq) was added. This mixture was allowed to stir for 1 hour after which compound **27** (0.99 g, 3.61 mmol, 1.0 eq) was dissolved in THF (3.6

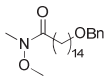
mL, 1 M) and added to the solution. The reaction was allowed to stir overnight while slowly warming to rt. The reaction was then quenched by addition of MeOH and the resulting mixture was diluted with Et_2O. The organic layer was washed with 1 M HCl and the resulting aqueous layer was extracted with Et_2O (2x). The organic layers were combined, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (Et_2O) to give **12** (935 mg, 3.23 mmol, 89%) as a clear oil. Spectroscopic data were in accordance with those previously reported in the literature.[33]

15-hydroxy-*N*-methoxy-*N*-methylpentadecanamide (18)



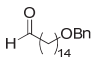
Pentadecanolide (62.74 g, 261 mmol, 1.0 eq) was dissolved in dry THF (1.0 L, 0.26 M) after which *N,O*-dimethylhydroxylamine hydrochloride (38.24 g, 392 mmol, 1.5 eq) was added to the solution. The mixture was then cooled to 0 °C after which isopropylmagnesium chloride (2.0 M in THF, 392 mL, 783 mmol, 3.0 eq) was added. After stirring for three hours the reaction was quenched by addition of sat. aq. NH_4Cl, and extracted with Et_2O (3x). The combined organic layers were then washed with brine, dried with $MgSO_4$ and concentrated *in vacuo* to give the title compound (77.3 g, 256 mmol, 98%) as a white waxy solid. The compound was used in the next step without further purification.

15-((benzyl(oxy)-*N*-methoxy-*N*-methylpentadecanamide (19)



Weinreb amide **18** (30.24 g, 100 mmol, 1.0 eq) was dissolved in dry DMF (0.5 L, 0.2 M) after which BnBr (23.8 mL, 200 mmol, 2.0 eq) was added to the solution. The mixture was cooled to 0 °C and NaH (60% dispersion in mineral oil, 8.00 g, 200 mmol, 2.0 eq) was added. The reaction was stirred for 40 hours while warming to RT after which the reaction was quenched by addition of H_2O and extracted with Et_2O (3x). The combined organic layers were then washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 6:4) gave the title compound (29.98 g, 76.6 mmol, 77%) as a white waxy solid. ^{1}H-NMR (500 MHz) δ: 7.33-7.28 (m, 4H, C*H*$_{arom}$); 7.26-7.21 (m, 1H, C*H*$_{arom}$); 4.48 (s, 2H, PhC*H*$_2$); 3.65 (s, 3H, OC*H*$_3$); 3.46 (t, 2H, *J* = 5.2 Hz, OC*H*$_2$); 3.15 (s, 3H, NC*H*$_3$); 2.39 (t, 2H, *J* = 6.0 Hz, C*H*$_2$); 1.66-1.58 (m, 4H, C*H*$_2$); 1.37-1.26 (m, 20H, C*H*$_2$). ^{13}C-BBD NMR (125 MHz) δ: 174.9 (RN*C*O); 138.8 (C$_{q,arom}$); 128.4, 127.6, 127.5 (*C*H$_{arom}$); 72.9 (Ph*C*H$_2$); 70.6 (O*C*H$_2$); 61.2 (O*C*H$_3$); 32.2 (N*C*H$_3$); 32.0, 29.8, 29.7, 29.7, 29.6, 29.5, 26.3, 24.7 (*C*H$_2$). IR (thin film, cm^{-1}): 1102, 1384, 1454, 1668, 2853, 2925. HRMS calculated for $C_{24}H_{42}NO_3$ 392.3165 $[M+H]^+$; found 392.3156.

15-((benzyl(oxy)pentadecanal (20)



Compound **19** (21.4 g, 50.7 mmol, 1.0 eq) was dissolved in Et_2O (500 mL, 0.1 M) and the reaction was cooled to 0 °C. After stirring for a few minutes $LiAlH_4$ (4.0 M in Et_2O, 6.58 mL, 26.3 mmol, 0.5 eq) was slowly added to the solution. After TLC-analysis (*n*-pentane-Et_2O 7:3) indicated complete conversion of the starting material the reaction was quenched with MeOH. The organic layer was washed with 1 M HCl, sat. aq. $NaHCO_3$ and brine, after which it was dried with $MgSO_4$ and concentrated *in vacuo* to give the title compound (25.3 g, 76.2 mmol, 100%) as a white waxy solid. ^{1}H-NMR (400 MHz) δ: 9.76 (t, 1H, *J* = 1.8 Hz, C*H*O); 7.37-7.30 (m, 5H, C*H*$_{arom}$); 4.50 (s, 2H, PhC*H*$_2$); 3.46 (t, 2H, *J* = 6.6 Hz); 2.42 (dt, 2H, *J* = 1.8, 7.4 Hz); 1.66-1.58 (m, 4H); 1.37-1.25 (m, 20H). ^{13}C-APT NMR (101 MHz) δ: 203.2 (*C*HO); 138.9 (C$_{q,arom}$); 128.5, 127.8, 127.6 (*C*H$_{arom}$); 73.0, 70.7, 44.1, 29.9, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5,

29.3, 26.3, 22.2 (CH_2). IR (thin film, cm^{-1}): 1029, 1043, 1100, 1235, 1262, 1362, 1455, 1497, 1507, 1747, 2853, 2925. HRMS calculated for $C_{22}H_{37}O_2$ 333.2794 $[M+H]^+$; found 333.2785.

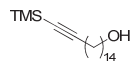
1,1-dibromo-16-((benzyl(oxy)hexadec-1-ene (21)

CBr_4 (1.62 g, 4.88 mmol, 2.0 eq) was dissolved in DCM (6.1 mL, 0.81 M) and the solution was cooled to 0 °C after which PPh_3 (2.63 g, 9.76 mmol, 4.0 eq) was added. The mixture was stirred for 40 minutes after which a solution of aldehyde **20** (0.812 g, 2.44 mmol, 1.0 eq) in DCM (5.0 mL, 0.5 M) was added. After stirring for 45 minutes the reaction mixture was diluted with hexane and Et_2O after which it was filtered over celite. The filtrate was concentrated *in vacuo*, diluted with Et_2O, and filtered again. The filtrate was then concentrated *in vacuo* and purification by means of column chromatography (*n*-pentane-Et_2O 49:1) gave the title compound (1.08 g, 2.22 mmol, 91%) as a clear oil. ^{1}H-NMR (400 MHz) δ: 7.37-7.30 (m, 5H, CH_{arom}); 6.38 (t, 1H, *J* = 7.2 Hz, CBr_2CH); 4.50 (s, 2H, PhCH_2); 3.46 (t, 2H, *J* = 6.6 Hz); 2.08 (dt, 2H, 7.2, 7.6 Hz); 1.65-1.59 (m, 2H); 1.43-1.26 (m, 22H). ^{13}C-APT NMR (101 MHz) δ: 139.1 (CBr_2CH); 138.9 ($C_{q,arom}$); 128.5, 127.8, 127.6 (CH_{arom}); 88.5 (CBr_2); 73.0, 70.7, 33.2, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 29.2, 27.9, 26.3 (CH_2). IR (thin film, cm^{-1}): 1029, 1102, 1362, 1454, 2853, 2925. HRMS calculated for $C_{23}H_{37}Br_2O$ 489.1180 $[M+H]^+$; found 489.1185.

1-trimethylsilyl-16-((benzyl(oxy)hexadec-1-yne (22)

Compound **21** (23.6 g, 48.3 mmol, 1.0 eq) was dissolved in Et_2O (500 mL, 0.1 M) and the solution was cooled to 0 °C. After stirring for a few minutes *n*-BuLi (2.5 M in hexanes, 48.3 mL, 121 mmol, 2.5 eq) was slowly added to the solution. After stirring for 1.5 hours TMSCl (24.5 mL, 193 mmol, 4.0 eq) was added. The reaction was allowed to stir for 2 hours while warming to RT. The reaction was then quenched by addition of sat. aq. NH_4Cl and extracted with Et_2O (3x). The combined organic layers were then washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 49:1) gave the title compound (18.2 g, 45.3 mmol, 94%) as a clear liquid. ^{1}H-NMR (400 MHz) δ: 7.31-7.27 (m, 5H, CH_{arom}); 4.51 (s, 2H, PhCH_2); 3.47 (t, 2H, *J* = 6.6 Hz); 2.21 (t, 2H, *J* = 7.2 Hz); 1.65-1.58 (m, 2H); 1.55-1.48 (m, 2H); 1.36-1.26 (m, 22H). ^{13}C-APT NMR (101 MHz) δ: 138.9 ($C_{q,arom}$); 128.5, 127.8, 127.6 (CH_{arom}); 108.0, 84.4 ($C_{q,alkyne}$); 73.0, 70.7, 29.9, 29.8, 29.8, 29.6, 29.2, 29.0, 28.8, 26.3, 20.0 (CH_2); 0.3 ($CH_{3,TMS}$). IR (thin film, cm^{-1}): 1029, 1102, 1202, 1249, 1362, 1455, 1497, 2175, 2853, 2924. HRMS calculated for $C_{26}H_{48}NOSi$ 418.3505 $[M+NH_4]^+$; found 418.3499.

1-trimethylsilylhexadec-1-yn-16-ol (14)



Compound **22** (18.16 g, 45.3 mmol, 1.0 eq) was dissolved in DCM/H_2O (20:1, 440 mL, 0.1 M) and the solution was cooled to 0 °C. After stirring for a few minutes DDQ (15.42 g, 68.0 mmol, 1.5 eq) was added to the solution. The reaction was stirred vigorously overnight after which it was quenched by addition of sat. aq. $NaHCO_3$. The organic layer was then washed sat. aq. $NaHCO_3$, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 4:1) gave the title compound (12.1 g, 39.0 mmol, 86%) as a white waxy solid. The spectroscopic data were in accordance with those previously reported in the literature.[33]

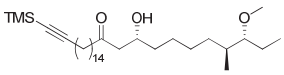
16-(trimethylsilyl)hexadec-15-yn-1-yl 4-toluenesulfonate (23)

Compound **14** (1.77 g, 5.7 mmol, 1.0 eq) was dissolved in $CHCl_3$ (30 mL, 0.2 M) together with *p*-TsCl (2.17 g, 11.4 mmol, 2.0 eq) and pyridine (1.38 mL, 17.1 mmol, 3.0 eq) was added to the solution. The reaction was left to stir overnight and was then diluted with Et_2O. The organic layer was then washed with H_2O, 1 M HCl, sat. aq. $NaHCO_3$ and brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 19:1) gave the title compound (2.52 g, 5.42 mmol, 95%) as a white waxy solid. The spectroscopic data were in accordance with those previously reported in the literature.[33]

(16-iodohexadec-1-yn-1-yl)trimethylsilane (15)

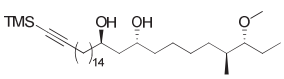
Compound **23** (11.1 g, 23.9 mmol, 1.0 eq) was dissolved in acetone (250 mL, 0.1 M) and NaI (14.3 g, 95.6 mmol, 4.0 eq) was added to the solution. The reaction was left to stir overnight after which it was diluted with EtOAc and concentrated *in vacuo*. The residue was then diluted with EtOAc and washed with sat. aq. $Na_2S_2O_3$, H_2O and brine. The organic layer was then dried with $MgSO_4$ and concentrated *in vacuo* after which the product was purified by means of column chromatography (*n*-pentane-Et_2O 49:1) gave the title compound (9.70 g, 23.1 mmol, 96%) as a clear oil. The spectroscopic data were in accordance with those previously reported in the literature.[33]

(3*R*,4*S*,9*R*)-9-hydroxy-3-methoxy-4-methyl-27-(trimethylsilyl)heptacos-26-yn-11-one (16)



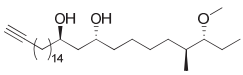
Compound **15** (3.19 g, 7.58 mmol, 3.0 eq) was dissolved in Et_2O (76 mL, 0.1 M) and the solution was cooled to -78 °C. *t*-BuLi (1.6 M in hexane, 7.9 mL, 12.6 mmol, 5.0 eq) was added to the solution and the mixture was allowed to stir for 30 minutes. After this time a solution of compound **12** (0.731 g, 2.53 mmol, 1.0 eq) in Et_2O (5.1 mL, 0.5 M) was slowly added and the reaction was allowed to stir for 1 hour. The reaction was then quenched by the addition of a 4:1 mixture of MeOH/sat. aq. NH_4Cl and allowed to warm to rt. The layers were then separated and the aqueous layer was extracted with Et_2O (2×). The combined organic layers were washed with H_2O and brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification of the product by means of column chromatography (*n*-pentane-Et_2O 3:2) gave the title compound (0.798 g, 1.53 mmol, 60%) as a white waxy solid. The spectroscopic data were in accordance with those previously reported in the literature. [33]

(3*R*,4*S*,9*R*,11*R*)-3-methoxy-4-methyl-27-(trimethylsilyl)heptacos-26-yne-9,11-diol (28)



Compound **16** (304 mg, 0.581 mmol, 1.0 eq) was dissolved in a 12:12:1 mixture of MeCN, AcOH and THF (194 mL, 0.003 M) and this solution was cooled to 0 °C. $Me_4NBH(OAc)_3$ (0.92 g, 3.49 mmol, 6.0 eq) was added in 5 portions over 60 minutes and the reaction was allowed to stir for 90 more minutes. The reaction was quenched by the addition of H_2O and the layers were separated. The aqueous layer was extracted with Et_2O (3×) and the combined organic layers were washed with sat. aq. $NaHCO_3$ (3×) and brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification of the product by means of column chromatography (*n*-pentane-Et_2O 1:1) gave the title compound (245 mg, 0.467 mmol, 80%) as a white waxy solid. The spectroscopic data were in accordance with those previously reported in the literature.[33]

(3*R*,4*S*,9*R*,11*R*)-3-methoxy-4-methylheptacos-26-yne-9,11-diol (17)



Compound **28** (0.491 g, 0.935 mmol, 1.0 eq) was dissolved in MeOH (20 mL, 0.05 M) and K_2CO_3 (0.65 g, 4.68 mmol, 5.0 eq) was added solution and the reaction was allowed to stir overnight. The mixture was then diluted with Et_2O and H_2O, the layers were separated, and the aqueous layer was extracted with Et_2O (2×). The combined organic layers were washed with H_2O and brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 1:1) gave the title compound (0.41 g, 0.91 mmol, 97%) as a white waxy solid. The spectroscopic data were in accordance with those previously reported in the literature.[33]

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