Total synthesis and biological evaluation of simplified aplyronine analogues as synthetically tractable anticancer agents

Talia R. Pettigrew[†], Rachel J. Porter[†], Stephen J. Walsh, Michael P. Housden, Nelson Y. S. Lam, Jason S. Carroll, Jeremy S. Parker, David R. Spring and Ian Paterson[*]

Table of Contents

Detailed rationale for the simplification of the aplyronines

1.1. Summary of SAR studies conducted to date

A summary of structural features important for the bioactivity of the aplyronines is given below.[1–5]

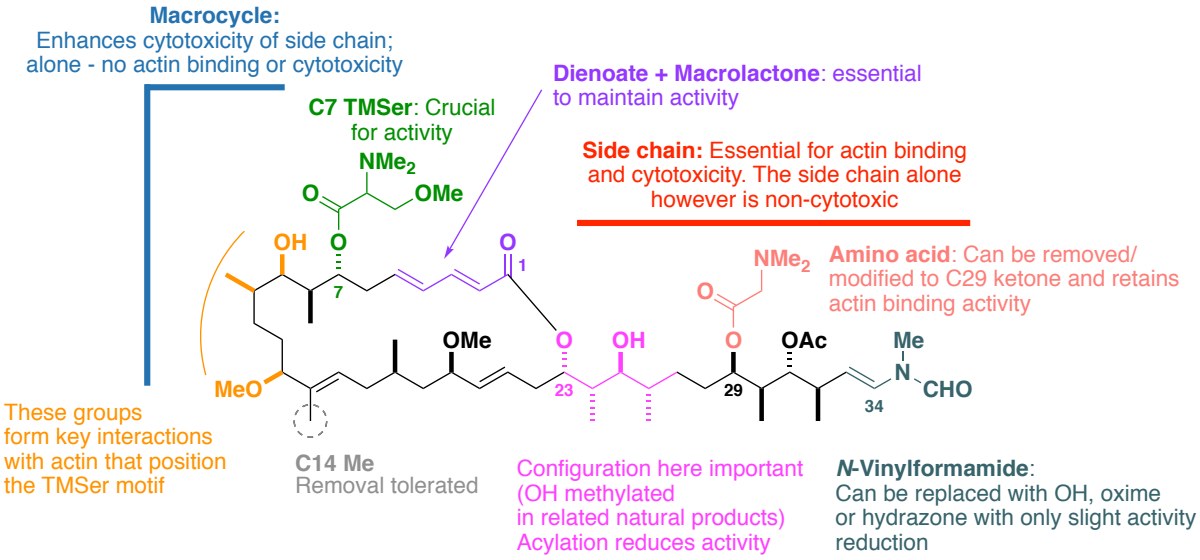


Figure 1. *Summary of SAR studies conducted for the aplyronines as overlayed onto aplyronine D*

1.2. Analysis of the bound Aplyronine A-actin crystal structure

The bound structure of aplyronine A in actin was visualised using Pymol using the Protein Databank (PDB) code: 1WUA.[3] The ATP binding site of actin is identified with the ATP ligand in **red**. Aplyronine A is denoted in **green**.

The aplyronine A bound crystal structure of actin verifies the importance of side chain in binding to a key binding pocket in actin. The C7 TMSer vital for the activity of aplyronines A/D points directly out into bulk solvent, presumably as a binding site to recruit tubulin. It is clear that the macrocycle is required to confer overall geometric control, with the northern hemisphere appearing to contribute to key contacts with actin. The southern hemisphere however did not appear to form relevant contacts as it projects *away* from both actin and where tubulin binds (TMSer) into bulk solvent. Alongside with the SAR study presented above, this observation forms the major rationale for our function-oriented simplification of the aplyronines (see below for annotated diagram).

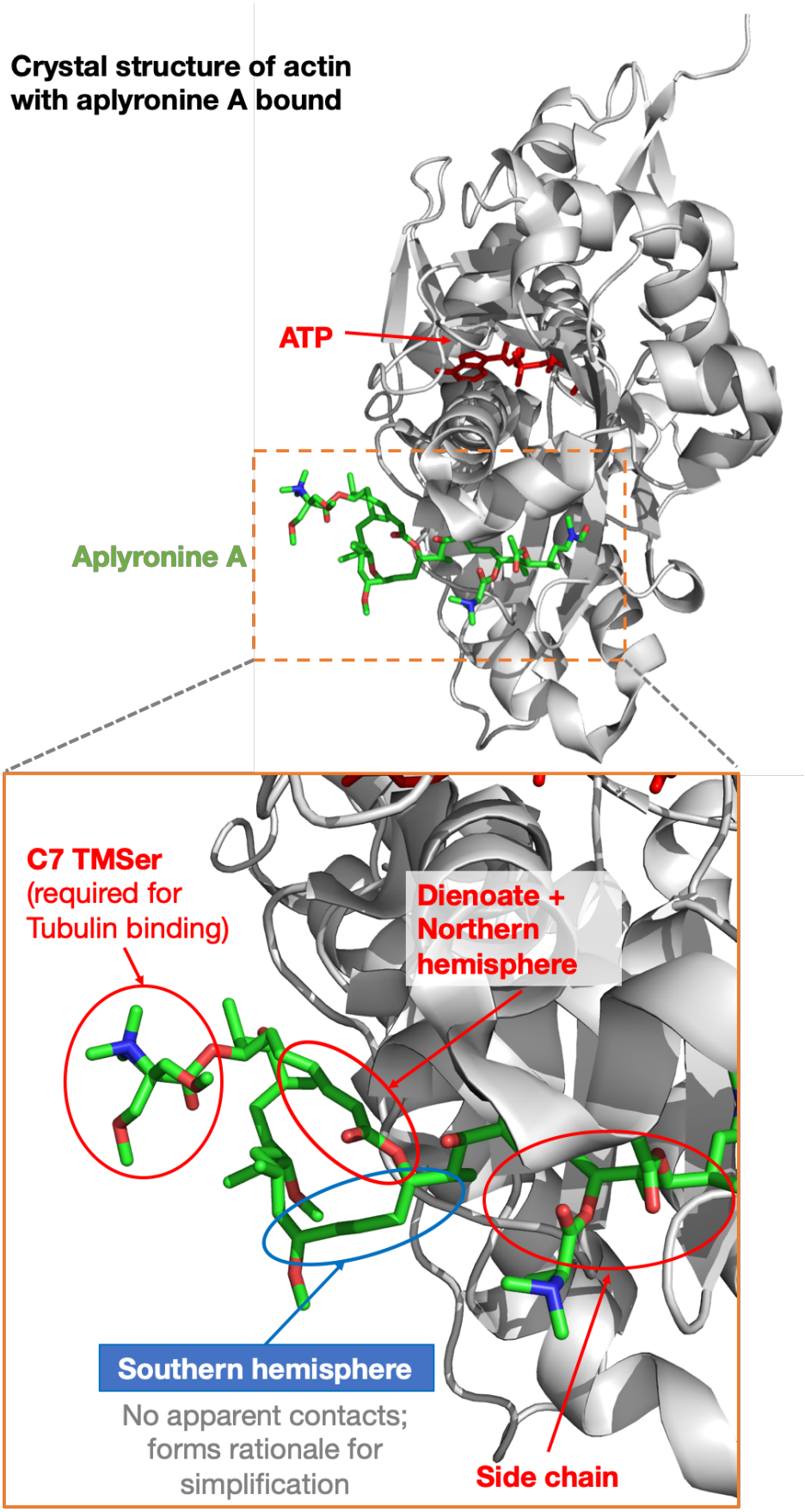


Figure 2. *Annotated figure of the aplyronine-bound actin crystal structure, highlighting the key binding areas of aplyronine to the relevant biomolecules, as well as the lack of apparent biological contacts of the southern hemisphere*

Experimental

2. General comments

2.1. General experimental procedures

All experiments were performed under anhydrous conditions and under an inert atmosphere of argon, except where stated or when water or aqueous solutions were used, using oven-dried apparatus and employing standard techniques for handling air-sensitive materials.

Purification of reagents and solvents was carried out according to standard procedures. Acetonitrile (MeCN), benzene, dichloromethane (CH_2Cl_2), and dimethyl sulfoxide (DMSO) were distilled from calcium hydride (CaH_2) and stored under an argon atmosphere. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from benzophenone ketyl radical and sodium or potassium wire, respectively, under an argon atmosphere. Solvents used in workup, extraction, recrystallisation and column chromatography were distilled prior to use.

Triethylamine (Et_3N), pyridine (py), diethyl ethylphosphonate and hexamethyldisilazane (HMDS) were distilled from and stored over CaH_2. 2,6-lutidine was distilled from CaH_2 and stored neat under argon. Diisopropylethylamine (iPr$_2$NEt) was first distilled from ninhydrin, then distilled from and stored over CaH_2. Titanium tetrachloride ($TiCl_4$), titanium tetraisopropoxide (Ti(OiPr)$_4$) and oxalyl chloride ($(COCl)_2$) were distilled and stored at −20 °C under an argon atmosphere. Acetaldehyde and propionaldehyde were distilled from calcium chloride ($CaCl_2$) immediately prior to use. Proton Sponge was recrystallised from methanol. DDQ was recrystallised from chloroform. Barium hydroxide ($Ba(OH)_2$) was dried under high vacuum at 130 °C and stored under argon. All other chemicals were used as received, except where noted other- wise.

Sodium bicarbonate ($NaHCO_3$), ammonium chloride (NH_4Cl), sodium/potassium (Na^+/K^+) tartrate, brine (NaCl) and sodium thiosulfate ($Na_2S_2O_3$) were used as saturated aqueous solutions, unless otherwise stated in the text. Buffer solutions were prepared as directed from stock tablets with deionised water.

Flash column chromatography was carried out on Merck Kieselgel 60 (230−400 mesh) silica gel under a positive pressure of regulated compressed air. Merck Kieselgel F254 plates were used for preparative thin layer chromatography. All solvent mixtures are reported as volume ratios. Solvents were subsequently evaporated *in vacuo*.

2.2. General analytical procedures

Analytical thin layer chromatography (TLC) was carried out using Merck Kieselgel 60 F_{254} plates which were visualised using UV light (254 nm) and stained with potassium permanganate, vanillin, anisaldehyde or phosphomolybdic acid / cerium (III) sulfate $Ce_2(SO_4)_3$ dips.

Proton nuclear magnetic resonance (NMR) spectra were recorded using an internal deuterium lock at ambient probe temperature (298 K) on the following instruments: Bruker Avance BB, or Bruker Avance TCI (500 MHz and Bruker DPX400 (400 MHz). An internal reference of δ_H = 7.26 ppm was used for residual solvent protons in $CDCl_3$ and δ_H = 7.26 2.50 ppm for DMSO. All ^1H NMR data are represented as: chemical shift (in ppm on the δ scale relative to δ_{TMS} = 0 ppm), integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, obs = obscured, app = apparent), coupling constant (*J* in Hz), and assignment. Assignments were determined on the basis of unambiguous chemical shift or coupling pattern, ^1H-^1H COSY, HSQC and HMBC experiments, or by analogy to fully interpreted data for related compounds.

Proton-decoupled ^{13}C NMR spectra were recorded using an internal deuterium lock at ambient probe temperature (298K) on the following instruments: Bruker Avance BB and Bruker Avance TCI (125 MHz). An internal reference of δ_C = 77.0 ppm was used for carbons in $CDCl_3$ and δ_C = 79.5 ppm for DMSO. All chemical shift values are reported in ppm on the δ scale relative to δ_{TMS} = 0 ppm.

Infrared spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer. Absorbance frequencies (ν_{max}) are reported in cm^{-1}.

Optical rotations were measured on an Anton Parr MCP100 polarimeter at 589 nm and are reported as follows: $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ at 20 °C (unless otherwise noted), concentration (*c* in g dL^{-1}) and solvent.

High resolution mass spectra (HRMS) were recorded at the EPSRC Mass Spectrometry Service (Swansea, UK) or at the departmental mass spectrometry service (University Chemical Laboratories, Cambridge) using electron impact (EI) and electrospray ionization (ESI) techniques. The parent ion is quoted with the indicated cation: $[M+H]^+$, $[M+Na]^+$ or $[M+NH_4]^+$

2.3. Compound numbering nomenclature

The numbering system used for aplyronine intermediates and analogues follows that proposed by Yamada and co-workers in their original publication covering the isolation and characterisation of the aplyronines as shown below:

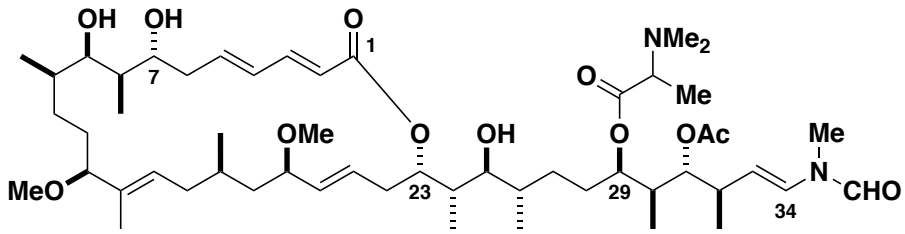
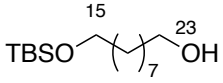


Figure 3. *Structure of aplyronine C, highlighting the compound numbering as proposed by Yamada et al.*

3. Experimental Procedures for the Synthesis of Aplyrologues 7, *R*-/*S*-8 and 9

3.1. Synthesis of the C15-C27 aldehyde 12

TBS ether 14a

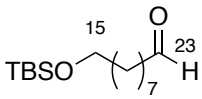


To a solution of 1,9-nonanediol (12.0 g, 74.9 mmol) and imidazole (2.04 g, 68.1 mmol) in THF (80 mL) at 0 °C was added a solution of TBSCl (3.77 g, 25.0 mmol) in THF (70 mL) dropwise over 40 min. The mixture was slowly warmed to rt and allowed to stir for 65 h, then quenched by addition of H_2O. The phases were separated and the aqueous layer extracted with EtOAc (3 × 100 mL). The combined organics were washed with H_2O (50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography (1:3 → 1:2 EtOAc/PE) gave alcohol **14a** as a colourless oil (5.69 g, 83%).

R_f 0.27 (1:6 EtOAc/PE); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 3.64 (2H, t, J = 6.6 Hz, H_{23}), 3.59 (2H, t, J = 6.6 Hz, H_{15}), 1.61–1.45 (4H, m, H_{16},H_{22}), 1.39–1.24 (10H, m, $H_{17–21}$), 0.89 (9H, s, $SiC(CH_3)_3$), 0.04 (6H, s, $Si(CH_3)_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C 63.3, 63.0, 32.8, 32.8, 29.6, 29.3, 29.3, 26.0, 25.8, 25.7, 18.4, −5.3; **HRMS** calc. for $C_{15}H_{35}O_2Si$ $[M+H]^+$ 275.2401, found 275.2402.

These data are in agreement with those reported by Diab *et al*.[6]

Aldehyde 14



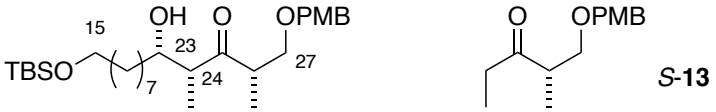
To a solution of oxalyl chloride (2.39 mL, 28.2 mmol) in CH_2Cl_2 (50 mL) at −78 °C was added DMSO (2.67 mL, 37.6 mmol). The mixture was stirred for 30 min, then a solution of alcohol **14a** (5.16 g, 18.8 mmol) in CH_2Cl_2 (25 mL) was added *via* cannula and stirred for a further 1 h. Addition of Et_3N (7.86 mL, 56.4 mmol) dropwise at −78 °C caused the reaction mixture to seize. After warming to rt with stirring and addition of CH_2Cl_2 (20 mL), the resulting yellow solution was allowed to stir for 16 h. The reaction was quenched carefully with NH_4Cl (40 mL). The phases were separated and the aqueous layer extracted with CH_2Cl_2 (2 × 80 mL).

The combined organics were washed with $NaHCO_3$ (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash chromatography (1:15 EtOAc/PE) to give aldehyde **14** as a colourless oil (4.68 g, 91%).

R_f 0.50 (1:8 EtOAc/PE); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 9.76 (1H, t, J = 1.9 Hz, H_{23}), 3.59 (2H, t, J = 6.6 Hz, H_{15}), 2.41 (2H, td, J = 7.4, 1.9 Hz, H_{22}), 1.68–1.57 (2H, m, H_{16}), 1.55–1.43 (2H, m, H_{21}), 1.37–1.24 (8H, m, $H_{17–20}$), 0.89 (9H, s, $SiC(CH_3)_3$), 0.04 (6H, s, $Si(CH_3)_2$); **HRMS** calc. for $C_{15}H_{33}O_2Si$ $[M+H]^+$ 273.2244, found 273.2248.

These data are in agreement with those reported by Cryle *et al.*[7]

Aldol adduct 15



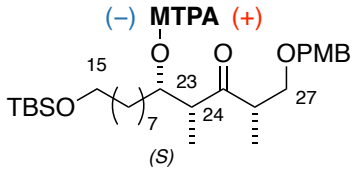
***S*-13**

To a solution of $TiCl_4$ (1.37 mL, 12.5 mmol) in CH_2Cl_2 (50 mL) at 0 °C was added Ti(O*i*-Pr)$_4$ (1.23 mL, 4.17 mmol). The mixture was stirred at 0 °C for 10 min, then at rt for 30 min. The resulting colourless solution was added dropwise to a solution of ketone *S*-**13** (3.52 g, 14.9 mmol, stirred over CaH_2 immediately before use) in CH_2Cl_2 (150 mL) at −78 °C, during which the solution gradually turned through yellow to light orange. Dropwise addition of *i*-Pr_2NEt (2.85 mL, 16.4 mmol) caused a further colour change to dark red. The reaction mixture was stirred at −78 °C for 30 min to allow full enolisation. Aldehyde **14** (4.46 g, 16.4 mmol, stirred over CaH_2 immediately before use) in CH_2Cl_2 (100 mL) was then added *via* cannula down the side of the reaction flask over 10 min. Shortly thereafter the solution was observed to return to a light orange colour. The mixture was stirred at −78 °C for a further 40 min. Upon completion the reaction was quenched with $NaHCO_3$/Na^+/K^+ tartrate (1:1, 90 mL) and warmed to rt. The biphasic mixture was stirred vigorously for 2 h and then left to stand overnight. The layers were separated and the organic phase washed with brine (100 mL). The combined aqueous fractions were then extracted with EtOAc (2 × 100 mL). The organics were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography (1:10 → 1:5 EtOAc/PE) afforded aldol adduct **15** as a colourless oil (6.42 g, 85%, 18:1 *dr*).

R_f 0.26 (1:5 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.18 (2H, d, J = 8.7 Hz, ArH), 6.85 (2H, d, J = 8.7 Hz, ArH), 4.39 (1H, d, J = 11.4 Hz, $ArCH_aH_bO$), 4.36 (1H, d, J = 11.5 Hz, $ArCH_aH_bO$), 4.00–3.95 (1H, m, H_{23}), 3.79 (3H, s, ArOMe), 3.61 (1H, app t, J = 8.7 Hz, H_{27a}), 3.59 (2H, t, J = 6.7 Hz, H_{15} ×2), 3.42 (1H, dd, J = 8.7, 4.7 Hz, H_{27b}), 3.15 (1H, dqd, J = 9.3,

6.9, 4.9 Hz, H_{26}), 2.88 (1H, d, J = 3.9 Hz, $C_{23}OH$), 2.72 (1H, qd, J = 7.1, 2.7 Hz, H_{24}), 1.50 (2H, app quint, J = 6.8 Hz, H_{16} × 2), 1.47–1.34 (2H, m, H_{22} × 2), 1.34–1.18 (10H, m, H_{17-21}), 1.05 (3H, d, J = 7.1 Hz, Me_{24}), 1.00 (3H, d, J = 6.9 Hz, Me_{26}), 0.89 (9H, s, $SiC(CH_3)_3$), 0.04 (6H, s, $Si(CH_3)_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C 218.1, 159.3, 129.6, 129.3, 113.8, 73.1, 72.9, 70.6, 63.3, 55.2, 50.9, 44.8, 33.6, 32.9, 29.6, 29.6, 29.4, 26.2, 25.9, 25.8, 18.3, 13.6, 8.8, −5.3; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ −2.0 (*c* 0.10, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 3500, 2929, 2853, 1709, 1615, 1514, 1461, 1360, 1302, 1249, 1173, 1037, 1005, 991, 834, 777; **HRMS** calc. for $C_{29}H_{56}NO_5Si$ $[M+NH_4]^+$ 526.3922, found 526.3909.

*The configuration at C23 was confirmed by synthesising the diastereomeric Mosher esters (S/R-MTPA-**15**) for analysis:*



To a solution of alcohol **15** (10.1 mg, 19.8 μmol) and (*S*)-α-methoxy-α-trifluoromethylphenylacetic acid (13.9 mg, 59.4 μmol) in CH_2Cl_2 (1 mL) was added DCC (60 μL, 1.0 M in CH_2Cl_2, 60.0 μmol) followed by DMAP (8.4 mg, 68.8 μmol). The reaction was stirred at rt for 19 h, then filtered and concentrated. Flash chromatography (1:30 → 1:20 EtOAc/PE) gave the (*S*)-MTPA ester *S*-MTPA-**15** as a colourless oil (10.6 mg, 74%). The analogous procedure gave the (*R*)-MTPA ester *R*-MTPA-**15** in 47% yield.

S-MTPA-**15**:

R_f 0.65 (1:4 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.58–7.53 (2H, m, PhH), 7.40–7.36 (3H, m, PhH), 7.19 (2H, d, J = 8.6 Hz, PMB ArH), 6.85 (2H, d, J = 8.7 Hz, PMB ArH), 5.44 (1H, app dt, J = 7.6, 5.2 Hz, H_{23}), 4.39 (1H, d, J = 11.6 Hz, $ArCH_aH_bO$), 4.33 (1H, d, J = 11.6 Hz, $ArCH_aH_bO$), 3.79 (3H, s, ArOMe), 3.59 (2H, t, J = 6.7 Hz, H_{15} × 2), 3.56 (1H, dd, J = 8.9, 8.2 Hz, H_{27a}), 3.52 (3H, s, OMe), 3.39 (1H, dd, J = 9.0, 5.4 Hz, H_{27b}), 3.08–2.99 (1H, m, H_{26}), 2.93 (1H, dq, J = 7.1, 6.7 Hz, H_{24}), 1.60–1.53 (1H, m, H_{22a}), 1.53–1.43 (3H, m, H_{16} × 2,H_{22b}), 1.31–1.10 (10H, m, $H_{17–21}$), 1.09 (3H, d, J = 7.2 Hz, Me_{24}), 1.03 (3H, d, J = 7.0 Hz, Me_{26}), 0.89 (9H, s, $SiC(CH_3)_3$), 0.05 (6H, s, $Si(CH_3)_2$).

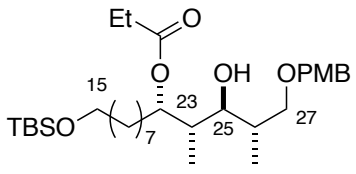
R-MTPA-**15**:

R_f 0.65 (1:4 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.59–7.52 (2H, m, PhH), 7.41–7.35 (3H, m, PhH), 7.19 (2H, d, *J* = 8.7 Hz, PMB ArH), 6.85 (2H, d, *J* = 8.7 Hz, PMB ArH), 5.42 (1H, app q, *J* = 6.1 Hz, H_{23}), 4.38 (1H, d, *J* = 11.5 Hz, $ArCH_aH_bO$), 4.34 (1H, d, *J* = 11.6 Hz, $ArCH_aH_bO$), 3.79 (3H, s, ArOMe), 3.58 (2H, t, J = 6.7 Hz, H_{15} × 2), 3.56–3.51 (4H, m, OMe, H_{27a}), 3.36 (1H, dd, *J* = 9.0, 5.4 Hz, H_{27b}), 3.00–2.92 (1H, m, H_{26}), 2.90 (1H, dq, J = 7.3, 6.9 Hz, H_{24}), 1.62–1.52 (2H, m, H_{22} × 2), 1.48 (2H, app quint, *J* = 7.1 Hz, H_{16} × 2), 1.31–1.12 (10H, m, $H_{17–21}$), 1.02 (3H, d, J = 7.2 Hz, Me_{24}), 0.97 (3H, d, *J* = 7.0 Hz, Me_{26}), 0.89 (9H, s, $SiC(CH_3)_3$), 0.04 (6H, s, $Si(CH_3)_2$).

Table 1. Diagnostic ^{1}H NMR signals for the configurational assignment of 23S

Proton	**δH (*S*)-MTPA** (ppm)	**δH (*R*)-MTPA** (ppm)	$\Delta\delta_{S-R}$ (ppm)
H22a	1.57	1.60	–0.03
H22b	1.51	1.60	–0.09
H23	5.44	5.42	+0.02
H24	2.93	2.90	+0.03
Me24	1.09	1.02	+0.07
H26	3.04	2.96	+0.08
Me26	1.03	0.97	+0.06

Alcohol 16

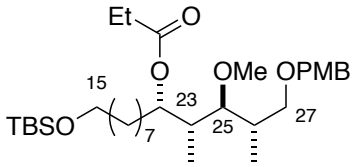


Freshly prepared SmI_2 (18.7 mL, *ca.* 0.1 M in THF, 1.87 mmol) was added dropwise to a solution of propionaldehyde (8.1 mL, 112 mmol, distilled from $CaCl_2$ immediately prior to use) in THF (120 mL) at 0 °C. The blue-green colour of SmI_2 faded upon addition to give a golden yellow solution, which was stirred at 0 °C for 15 min then cooled to −20 °C. Ketone **15** (9.34 g, 18.3 mmol) was dissolved in THF (70 mL) and added to the reaction mixture *via* cannula. The reaction was kept at −20 °C for 1 h, then quenched with $NaHCO_3$ (100 mL) and warmed

to rt. The aqueous fraction was extracted with Et_2O (3 × 100 mL). The combined organics were dried over Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography (1:15 EtOAc/PE) to give alcohol **16** as a pale yellow oil (9.91 g, 95%, >20:1 *dr*).

R_f 0.48 (1:5 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.23 (2H, d, J = 8.6 Hz, ArH), 6.87 (2H, d, J = 8.6 Hz, ArH), 5.27 (1H, ddd, J = 8.7, 5.0, 1.5 Hz, H_{23}), 4.43 (1H, d, J = 11.6 Hz, $ArCH_aH_bO$), 4.39 (1H, d, J = 11.6 Hz, $ArCH_aH_bO$), 3.80 (3H, s, ArOMe), 3.59 (2H, t, J = 6.6 Hz, H_{15} × 2), 3.55 (1H, dd, J = 9.2, 5.1 Hz, H_{27a}), 3.43 (1H, dd, J = 9.2, 5.8 Hz, H_{27b}), 3.35 (1H, d,J=5.7Hz,$C_{25}OH$),3.10(1H,ddd,J=8.9,5.7,3.5Hz,H_{25}),2.33(2H,q,J=7.6Hz,$H_{2'}$×2), 2.05–1.98 (1H, m, H_{26}), 1.76 (1H, dqd, J = 8.5, 6.9, 1.3 Hz, H_{24}), 1.72–1.63 (1H, m, H_{22a}), 1.50 (2H, app quint, J = 6.9 Hz, H_{16} × 2), 1.45–1.36 (1H, m, $H_{22\,b}$), 1.34–1.20 (10H, m, $H_{17\,-\,21}$), 1.14 (3H, t, J = 7.6 Hz, $H_{3'}$ × 3), 1.06 (3H, d, J = 7.0 Hz, Me_{26}), 0.89 (9H, s, $SiC(CH_3)_3$), 0.87 (3H, d, J = 6.9 Hz, Me_{24}), 0.04 (6H, s, $Si(CH_3)_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C 175.3, 159.1, 130.4, 129.1, 113.7, 76.3, 73.7, 72.9, 71.7, 63.3, 55.2, 40.2, 34.7, 32.8, 32.6, 29.5, 29.4, 29.3, 27.8, 26.0, 26.0, 25.7, 18.3, 16.3, 10.1, 9.3, −5.3; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ −3.5 (*c* 1.00, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 3504, 2929, 2849, 1728, 1711, 1617, 1514, 1463, 1364, 1247, 1203, 1096, 1039, 1001, 832, 777; **HRMS** calc. for $C_{32}H_{62}NO_6Si$ $[M+NH_4]^+$ 584.4341, found 584.4325.

Methyl ether 16a

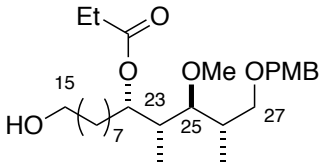


Proton Sponge (9.30 g, 43.4 mmol) and $Me_3O{\cdot}BF_4$ (4.28 g, 28.9 mmol) were charged to a flask in an argon-filled glove box, then a solution of alcohol **16** (8.17 g, 14.4 mmol) in CH_2Cl_2 (300 mL) was added at rt. The reaction was stirred for 3 h, during which time it gradually turned yellow, then bright orange. The mixture was then quenched by addition of NH_4Cl (150 mL) and stirred overnight. The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL), then the combined organics were washed with citric acid (2 × 200 mL, 10% w/v aq.), dried over $MgSO_4$, and concentrated *in vacuo*. Purification by flash chromatography (1:20 EtOAc/PE) provided the methyl ether **16a** as a colourless oil (7.42 g, 89%, 99% brsm).

R_f 0.48 (1:8 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.24 (2H, d, J = 8.7 Hz, ArH), 6.86 (2H, d, J = 8.7 Hz, ArH), 5.18 (1H, app td, J = 7.4, 2.0 Hz, H_{23}), 4.43 (1H, d, J = 11.5 Hz, $ArCH_aH_bO$), 4.39 (1H, d, J = 11.6 Hz, $ArCH_aH_bO$), 3.80 (3H, s, ArOMe), 3.59 (2H, t, J = 6.7 Hz, H_{15} × 2), 3.53 (1H, dd, J = 9.2, 4.9 Hz, H_{27a}), 3.38 (3H, s, $C_{25}OMe$), 3.32 (1H, dd, J = 9.1,

7.3 Hz, H_{27b}), 2.87 (1H, dd, J = 8.5, 3.6 Hz, H_{25}), 2.31 (2H, q, J = 7.6 Hz, $H_{2'}$ × 2), 2.12–2.04 (1H, m, H_{26}), 1.78 (1H, dqd, J = 8.9, 7.0, 2.0 Hz, H_{24}), 1.66–1.58 (1H, m, H_{22a}), 1.49 (2H, app quint, J = 7.0 Hz, H_{16} × 2), 1.46–1.39 (1H, m, $H_{22\,b}$), 1.33–1.19 (10H, m, H_{17-21}), 1.14 (3H, t, J = 7.6 Hz, $H_{3'}$ × 3), 1.06 (3H, d, J = 7.1 Hz, Me_{26}), 0.92–0.88 (12H, m, Me_{24}, $SiC(CH_3)_3$), 0.04 (6H, s, $Si(CH_3)_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C 174.1, 159.0, 130.8, 129.0, 113.7, 85.9, 73.2, 72.7, 71.6, 63.3, 61.3, 55.2, 38.8, 35.9, 32.9, 32.7, 29.5, 29.5, 29.4, 28.0, 26.0, 25.8, 25.7, 18.4, 16.3, 10.6, 9.4, −5.3; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ −4.4 (*c* 0.79, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 2932, 2858, D 1732, 1614, 1513, 1463, 1247, 1193, 1090, 1038, 834, 776; **HRMS** calc. for $C_{33}H_{64}NO_6Si$ $[M+NH_4]^+$ 598.4497, found 598.4482.

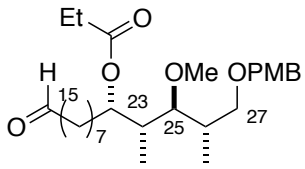
Alcohol 16b



Methyl ether **16a** (2.03 g, 3.49 mmol) was dissolved in THF (50 mL) and cooled to 0 °C. HCl (30 mL, 1.0 M aq.) was added, then the reaction was stirred at rt. After 1 h the reaction was quenched by careful addition of $NaHCO_3$ (80 mL) with cooling in an ice bath. The resulting mixture was then diluted with H_2O (100 mL) and Et_2O (50 mL) and extracted with Et_2O (3 × 100 mL). The combined organics were dried over Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography (1:3 EtOAc/PE) to give alcohol **16b** as a colourless oil (1.62 g, 99%).

R_f 0.15 (1:3 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.24 (2H, d, J = 8.5 Hz, ArH), 6.86 (2H, d, J = 8.5 Hz, ArH), 5.18 (1H, app td, J = 7.0, 1.6 Hz, H_{23}), 4.42 (1H, d, J = 11.6 Hz, $ArCH_aH_bO$), 4.39 (1H, d, J = 11.5 Hz, $ArCH_aH_bO$), 3.80 (3H, s, ArOMe), 3.62 (2H, t, J = 6.6 Hz, H_{15} × 2), 3.53 (1H, dd, J = 9.2, 4.8 Hz, H_{27a}), 3.37 (3H, s, $C_{25}OMe$), 3.32 (1H, dd, J = 9.0, 7.4 Hz, H_{27b}), 2.86 (1H, dd, J = 8.5, 3.5 Hz, H_{25}), 2.31 (2H, q, J = 7.6 Hz, $H_{2'}$ × 2), 2.12–2.04 (1H, m, H_{26}), 1.78 (1H, dqd, J = 8.6, 7.1, 1.6 Hz, H_{24}), 1.69–1.59 (1H, m, H_{22a}), 1.55 (2H, app quint, J = 6.9 Hz, H_{16} ×2), 1.47–1.39 (1H, m, H_{22b}), 1.39–1.19 (10H, m, $H_{17–21}$), 1.14 (3H, t, J = 7.6Hz, $H_{3'}$×3),1.05(3H, d, J = 7.0 Hz, Me_{26}),0.90 (3H, d, J = 7.0 Hz, Me_{24});**^{13}C NMR** (125 MHz, $CDCl_3$) δ_C 174.1, 159.0, 130.8, 129.0, 113.7, 85.9, 73.1, 72.7, 71.6, 63.0, 61.4, 55.2, 38.8, 35.9, 32.7, 32.7, 29.4, 29.3, 29.2, 28.0, 25.6, 25.6, 16.3, 10.6, 9.4; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ −8.0 (*c* 0.10, D $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 3452, 2972, 2933, 2857, 1730, 1613, 1588, 1514, 1463, 1423, 1364, 1300, 1247, 1195, 1086, 1037, 1003, 820; **HRMS** calc. for $C_{27}H_{47}O_6$ $[M+H]^+$ 467.3367, found 467.3359.

Aldehyde 12

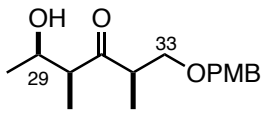


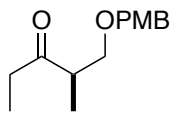
Oxalyl chloride (381 μL, 4.50 mmol) and CH_2Cl_2 (40 mL) were charged to a flask and cooled to −78 °C. DMSO (426 μL, 6.00 mmol) was added dropwise, and the mixture was stirred for 30 min. A solution of alcohol **16b** (1.41 g, 3.03 mmol) in CH_2Cl_2 (20 mL) was added and stirring continued for 20 min. After addition of Et_3N (1.25 mL, 9.00 mmol), the reaction was warmed to rt and stirred for 1.5 h. Addition of NH_4Cl (60 mL) to quench was followed by extraction with Et_2O (3 × 50 mL). The combined organics were washed with HCl (100 mL, 0.5 M aq.), brine (100 mL), and $NaHCO_3$(100 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. The product could be used crude or purified by flash chromatography (1:8 EtOAc/PE) to yield the aldehyde **12** as a colourless oil (1.32 g, 94%).

R_f 0.45 (1:3 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) **δ_H** 9.75 (1H, t, *J* = 1.8 Hz, H_{15}), 7.24 (2H, d, *J* = 8.6 Hz, ArH), 6.86 (2H, d, *J* = 8.7 Hz, ArH), 5.17 (1H, app td, *J* = 7.0, 1.9 Hz, H_{23}), 4.43 (1H, d, *J* = 11.5 Hz, $ArCH_2O$), 4.39 (1H, d, *J* = 11.6 Hz, $ArCH_2O$), 3.80 (3H, s, ArOMe), 3.53 (1H, dd, *J* = 9.2, 4.9 Hz, H_{27a}), 3.38 (3H, s, $C_{25}OMe$), 3.32 (1H, dd, *J* = 9.1, 7.4 Hz, H_{27b}), 2.86 (1H, dd, *J* = 8.5, 3.5 Hz, H_{25}), 2.40 (2H, td, *J* = 7.4, 1.8 Hz, H_{16} × 2), 2.31 (2H, q, *J* = 7.6 Hz, $H_{2'}$ × 2), 2.12–2.04 (1H, m, H_{26}), 1.78 (1H, dqd, *J* = 8.9, 7.0, 1.9 Hz, H_{24}), 1.66–1.58 (3H, m, H_{17} × 2, H_{22a}), 1.47–1.38 (1H, m, H_{22b}), 1.33–1.20 (8H, m, $H_{18\text{-}21}$), 1.14 (3H, t, *J* = 7.6 Hz, $H_{3'}$ × 3), 1.05 (3H, d, *J* = 7.0 Hz, Me_{26}), 0.90 (3H, d, *J* = 7.0 Hz, Me_{24}); **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C 202.9, 174.1, 159.0, 130.8, 129.0, 113.7, 85.9, 73.1, 72.7, 71.6, 61.4, 55.3, 43.9, 38.8, 35.9, 32.7, 29.3, 29.2, 29.0, 28.0, 25.6, 22.0, 16.3, 10.6, 9.4; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ −5.6 (*c* 0.65, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 2936, 2858, 1726, 1614, 1511, 1461, 1364, 1247, 1195, 1088, 1038, 822; **HRMS** calc. for $C_{27}H_{48}O_6N$ $[M+NH_4]^+$ 482.3476, found 482.3471.

3.2. Synthesis of the C28-C34 side chain *E*-11

Aldol adduct 17





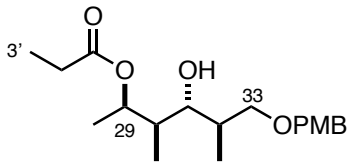
R-**13**

To a solution of $TiCl_4$ (175 μL, 1.78 mmol) in CH_2Cl_2 (8.4 mL) at 0 °C was added Ti(O-*i*Pr)$_4$ (175 μL, 0.59 mmol). The mixture was stirred at 0 °C for 10 min, then at rt for 20 min. The resulting colourless solution was added dropwise to a solution of ketone *R*-**13** (500 mg, 2.12 mmol, dried under vacuum for 2 h and stirred over CaH_2 immediately prior to use) in CH_2Cl_2 (13 mL) at −78 °C. To the yellow solution was added *i*-PrNEt$_2$ (0.41 mL) dropwise and the reaction mixture was allowed to enolise at −78 °C for 30 min. Acetaldehyde (1.12 mL, 21.2 mmol, distilled from $CaCl_2$ immediately prior to use) was dissolved in CH_2Cl_2 (10 mL) and added to the reaction mixture dropwise, causing the deep red solution to gradually turn pale orange. The mixture was stirred at −78 °C for 30 min before MeOH (8 mL) was added. After warming to rt Na^+/K^+ tartrate was added (20 mL) and the biphasic mixture was vigorously stirred overnight. The layers were separated, and the organic phase washed with brine (20 mL) and the combined aqueous layers were extracted with CH_2Cl_2 (3 × 20 mL). The combined organics were dried (Na_2SO_4) and concentrated *in vacuo* and purified by flash column chromatography (1:3 EtOAc/PE) to give aldol adduct **17** as a colourless oil (456 mg, 77%, 17:1 *dr*).

R_f 0.24 (3:1EtOAc/PE); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.18 (2H, d, *J* = 8.6 Hz, ArH), 6.87 (2H, d, *J* = 8.9 Hz, ArH), 4.39 (2H, ABq, *J* = 8.6 Hz, OCH_2Ar), 4.19 (1H, qdd, *J* = 6.6, 4.1, 3.2 Hz, H_{29}), 3.80 (3H, s, OMe), 3.61 (1H, t, *J* = 9.1 Hz, H_{33a}), 3.42 (1H, dd, *J* = 8.7, 4.6 Hz, H_{33b}), 3.15 (1H, dqd, *J* = 9.4, 7.0, 4.7 Hz, H_{32}), 2.94 (1H, d, *J* = 4.2 Hz, OH), 2.73 (1H, qd, *J* = 7.1, 3.0 Hz, H_{30}), 1.12 (3H, d, *J* = 6.7 Hz, Me_{28}), 1.08 (3H, d, *J* = 7.1 Hz, Me_{30}), 1.00 (3H, d, *J* = 6.7 Hz, Me_{32}).

These data are in agreement with those reported by Fink *et al*.[8]

Alcohol 18

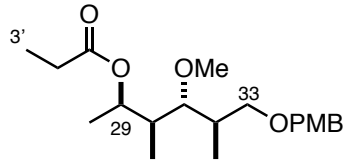


To a solution of propionaldehyde (freshly distilled from $CaCl_2$, 1.54 mL, 21.4 mmol) in THF (20 mL) at 0 °C was added freshly prepared SmI_2 (2.56 mL, 0.1 M in THF, 0.256 mmol). The solution was stirred for 5 min until the deep blue colouration had subsided. A solution of aldol adduct **17** (1.00 g, 3.57 mmol) in THF (10 mL) was then added *via* cannula. The reaction was stirred at 0 °C for 1 h, before being quenched with $NaHCO_3$ (10 mL) and extracted with Et_2O (3 × 10 mL). The combined organic layers were dried ($MgSO_4$), concentrated *in vacuo* and purified by flash column chromatography (1:4 EtOAc/PE) to give alcohol **18** as a colourless oil (1.17 g, 97%, >95:5 *dr*).

R_f 0.31 (1:4 EtOAc/PE); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.23 (2H, d, J = 8.4 Hz, ArH), 6.87 (2H, d, J = 8.7 Hz, ArH), 5.40 (1H, qd, J = 6.6, 2.1 Hz, H_{29}), 4.41 (2H, ABq, J = 11.6 Hz, OCH_2Ar), 3.80 (3H, s, OMe), 3.54-3.48 (2H, m, H_{33} × 2), 3.21-3.15 (2H, m, H_{31}, OH), 2.30 (2H, q, J = 7.7 Hz, $H_{2'}$ × 2), 2.03-1.94 (1H, m, H_{32}), 1.70-1.59 (1H, m, H_{30}), 1.22 (3H, d, J = 6.7 Hz, Me_{28}), 1.12 (3H, t, J = 7.5 Hz, $H_{3'}$ × 3), 1.08 (3H, d, J = 7.0 Hz, Me_{32}), 0.89 (3H, t, J = 6.9 Hz, Me_{30}).

These data are in agreement with those reported by Fink *et al*.[8]

Methyl ether 18a

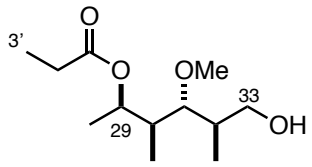


To a solution of Meerwein salt (Me_3OBF_4, 874 mg, 5.92 mmol) and Proton Sponge® (1.90 g, 8.88 mmol) in CH_2Cl_2 (50 mL) was added alcohol **18** (1.00 g, 2.96 mmol). The reaction was stirred for 4 h before being quenched with NH_4Cl (100 mL). The layers were separated, and the aqueous phase extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried ($MgSO_4$), concentrated *in vacuo* and purified by flash column chromatography (1:4 EtOAc/PE) to give methyl ether **18a** as a colourless oil (0.939 g, 90%).

R_f 0.29 (1:3 EtOAc/PE); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.24 (2H, d, J = 8.6 Hz, ArH), 6.87 (2H, d, J = 8.6 Hz, ArH), 5.27-5.20 (1H, m, H_{29}), 4.41 (2H, ABq, J = 11.7 Hz, OCH_2Ar), 3.80 (3H, s, OMe), 3.54 (1H, dd, J = 9.1, 4.9 Hz, H_{33a}), 3.36 (3H, s, OMeAr), 3.31 (1H, dd, J = 8.7,

2.3 Hz, H_{33b}), 2.89 (1H, dd, J = 8.8, 3.2 Hz, H_{31}), 2.30 (2H, q, *J* = 7.8 Hz, $H_{2'}$ × 2), 2.12-2.03 (1H, m, H_{32}), 1.74-1.66 (1H, m, H_{30}), 1.21 (3H, d, *J* = 6.0 Hz, Me_{28}), 1.21 (3H, t, *J* = 7.6 Hz, $H_{3'}$ × 3), 1.10 (3H, d, *J* = 6.7 Hz, Me_{32}), 0.94 (3H, t, *J* = 7.4 Hz, Me_{30}). **^{13}C NMR** (125 MHz, $CDCl_3$) $\boldsymbol{\delta}_C$ 173.8, 158.9, 130.7, 128.9, 113.6, 85.9, 72.6, 71.3, 69.6, 61.3, 55.1, 40.8, 35.8, 27.9, 18.2, 16.2, 10.3, 9.2; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ +2.3 (c 0.98, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 2974, 2937, 1731, 1613, D 1513, 1462, 1367, 1302, 1246, 1195, 1172, 1083, 1036, 1011, 819; **HRMS** calc. for $C_{20}H_{36}O_5N$ $[M+NH_4]^+$ 370.2588, found 370.2589.

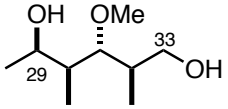
Alcohol 18b



To a solution of PMB ether **18a** (380 mg, 1.08 mmol) in CH_2Cl_2 (12 mL) was added pH 7.0 buffer (6 mL) and DDQ (490 mg, 2.16 mmol) at 0 °C. The reaction was warmed to rt and stirred for 1 h before being quenched with $NaHCO_3$ (15 mL). The layers were separated, and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. The crude material was purified by flash column chromatography (1:3 EtOAc/PE) to yield alcohol **18b** as a colourless oil (245 mg, 93%).

R_f 0.21 (1:3 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) $\boldsymbol{\delta}_H$ 5.24 (1H, dq, *J* = 6.5, 2.5 Hz, H_{29}), 3.84-3.78 (1H, m, H_{33a}), 3.58-3.52 (1H, m, H_{33b}), 3.47 (3H, s, OMe), 2.98 (1H, dd, *J* = 8.8, 3.2 Hz, H_{31}), 2.78 (1H, br s, OH), 2.32 (2H, q, *J* = 7.8 Hz, $H_{2'}$ × 2), 1.92-1.85 (1H, m, H_{32}), 1.82-1.75 (1H, m, H_{30}), 1.24 (3H, d, *J* = 6.5 Hz, Me_{28}), 1.15 (3H, d, *J* = 7.2 Hz, Me_{32}), 1.14 (3H, t, *J* = 7.5 Hz, $H_{3'}$ × 3), 0.92 (3H, d, *J* = 7.0 Hz, Me_{30}). **^{13}C NMR** (125 MHz, $CDCl_3$) $\boldsymbol{\delta}_C$ 173.9, 88.6, 69.6, 64.5, 61.7, 41.3, 36.0, 28.0, 18.3, 16.0, 10.3, 9.2;; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ 8.8 (c 1.00, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 3425, 2977, 2938, 2881, 2829, 1731, 1462, 1376, 1276, 1194, 1158, 1123, 1083, 1032, 966, 936, 870, 807; **HRMS** calc. for $C_{12}H_{25}O_4$ $[M+H]^+$ 233.1747, found 233.1749.

Diol 19

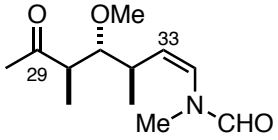


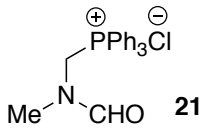
To a solution of ester **18b** (233 mg, 1.00 mmol) in CH_2Cl_2 (10 mL) was added DIBAL (4 mL, 4.00 mmol, 1.0 M in CH_2Cl_2) at – 78 °C. Upon completion, the reaction was quenched with

Na^+/K^+ tartrate (20 mL) and allowed to warm to rt. The layers were separated and the aqueous was extracted with CH_2Cl_2 (4 × 30 mL) and the combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. The crude was purified by flash column chromatography (1:1 EtOAc/PE) to afford diol **19** as a colourless oil (153 mg, 87%).

R_f 0.20 (1:1 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 4.19 (1H, q, J = 6.7 Hz, H_{29}), 3.76-3.64 (2H, m, H_{33} × 2), 3.56 (3H, s, OMe), 3.18 (1H, dd, J = 7.7, 4.5 Hz, H_{31}), 2.95 (1H, br s, OH), 2.27 (1H, br s, OH), 2.02-1.95 (1H, m, H_{32}), 1.71-1.64 (1H, m, H_{30}), 1.56 (3H, d, J = 6.4 Hz, Me_{28}), 1.03 (3H, d, J = 7.2 Hz, Me_{32}), 0.96 (3H, d, J = 6.7 Hz, Me_{30}); **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C 90.6, 66.6, 65.3, 61.8, 39.7, 37.6, 20.7, 14.9, 11.0; $[\alpha]_D^{20}$ +8.5 (*c* 1.00, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 3217, 2972, 2930, 1456, 1370, 1076, 1032, 1010, 998, 937, 900; **HRMS** calc. for $C_9H_{20}O_3Na$ $[M+Na]^+$ 199.1305, found 199.1299.

Enamide *E/Z*-11





21

To a solution of DMSO (0.52 mL, 7.38 mmol) in CH_2Cl_2 (10 mL) at –78 °C was added oxalyl chloride (0.31 mL, 3.69 mmol). After stirring for 30 min, a solution of diol **19** (216 mg, 1.23 mmol) in CH_2Cl_2 (10 mL) was added. The mixture was stirred for 30 min and then triethylamine (2.06 mL, 14.7 mmol) was added. The reaction mixture was stirred at –78 °C for 30 min, then warmed to rt for 1 h before being quenched with NH_4Cl solution (15 mL). The layers were separated, and the aqueous phase was extracted with Et_2O (3 × 15 mL). The combined organics were dried over $MgSO_4$ and concentrated *in vacuo* to afford aldehyde **20** (180 mg, 85%) which was used immediately crude in the subsequent Wittig reaction.

To a suspension of phosphonium salt **21 (**4.48 g, 11.3 mmol) in THF (25 mL) at –78 °C was added freshly prepared LiHMDS (12.5 mL, 1.0 M in THF, 12.5 mmol). The ylide solution was stirred at –78 °C for 30 min, warmed to –40 °C and stirred for 30 min, warmed to –20 °C and stirred for 30 min and finally warmed to 0 °C for 20 min after which the yellow suspension was cooled back down to –78 °C. A solution of ketoaldehyde **20** (976 mg, 5.67 mmol, pre-dried over CaH_2 for 1 h) in THF (25 mL) was added slowly. The reaction mixture was stirred at –78 °C for 2 h before being quenched with pH 7.0 buffer (50 mL) and diluted with Et_2O (10 mL). The layers were separated and the aqueous layer extracted with EtOAc (3 × 50 mL). The combined organics were dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was purified by flash column chromatography (1:1 EtOAc/PE) to afford enamide *E/Z*-**11** as a pale

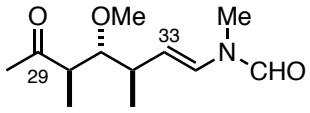
yellow oil as an inseparable mixture of geometric isomers (800 mg, 62% over 2 steps, 3:1 *Z*/*E*).

While a full characterisation has been carried out for enamide *E/Z*-**11**, an analytically pure sample for spectroscopic characterisation was obtained, and a spectroscopically pure sample was only obtained for *E*-**11** after the I_2-mediated isomerisation, described below.

R_f 0.27 (1:1 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 8.10 (0.85H, s, NCHO), [8.01] (0.1H, s, NCHO*), [6.19] (0.12H, d, *J* = 9.1 Hz, H_{34}*), 5.92 (0.81H, d, *J* = 8.7 Hz, H_{34}), 5.27 (0.84H, dd, *J* = 10.8, 8.7 Hz, H_{33}), [5.24-5.19] (0.13H, m, H_{33}*), 3.32 (3H, s, OMe), 3.20 (1H, dd, *J* = 9.4, 3.1 Hz, H_{31}), [2.98] (0.15H, s, NMe*), 2.95 (2.45H, s, NMe), [2.74-2.68] (0.16H, m, H_{32}*), 2.67-2.57 (2H, m, H_{32}, H_{30}), 2.14 (3H, s, Me_{28}), 1.09 (3H, d, *J* = 7.0 Hz, Me_{30}), [0.88] (0.67H, d, *J* = 7.0 Hz, Me_{32}*), 0.84 (2.40H, d, *J* = 7.0 Hz, Me_{32}); **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C [212.4], 211.8, 162.5, [162.4], 127.7, 125.1, [124.5], [124.1], [87.2], 86.9, [61.2], 61.3, [49.7], 49.8, 36.2, [34.9], 33.8, 31.5, [31.1], 30.9, 19.0, [18.3], [13.4], 13.2; $[\alpha]_D^{20}$ +11.0 (*c* 1.00, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 2972, 2929, 1682, 1653, 1457, 1355, 1088, 1054; **HRMS** calc. for $C_{12}H_{21}NO_3Na$ $[M+Na]^+$ 250.1414, found 250.1412.

Distinguishable resonances of the minor rotamer (3:1 ratio) are given in brackets and assignments denoted with an asterisk.

C28-C34 fragment *E*-11



Enamide *E/Z*-**86** (810 mg, 3.56 mmol) was dissolved in CH_2Cl_2 (100 mL) and a solution of iodine (45 mg, 0.18 mmol) in CH_2Cl_2 (20 mL) was added. The reaction mixture was stirred in the dark at room temperature for 16 h before being quenched with sodium thiosulfate (100 mL, sat. aq.). The biphasic mixture was stirred rapidly for 2 h and the organic layer separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL) and the combined organics dried ($MgSO_4$) and concentrated *in vacuo*. The crude was purified by flash column chromatography (1:1 EtOAc/PE) to afford (*E*)-enamide **11** as a pale-yellow oil (700 mg, 86%, >20:1 *E*/*Z*).

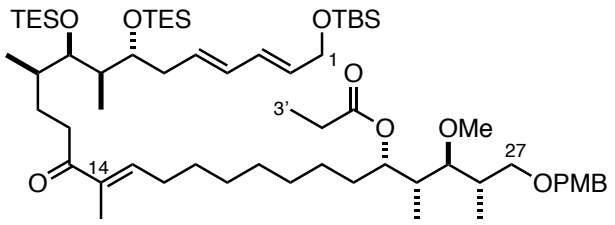
R_f 0.27 (1:1 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 8.25 (0.59H, s, NCHO), [8.04] (0.33H, s, NCHO*), [7.09] (0.32H, d, *J* = 14.5 Hz, H_{34}*), 6.44 (0.59H, d, *J* = 14.5 Hz, H_{34}), 5.09 (1H, dd, *J* = 14.5, 8.5 Hz, H_{33}), 3.34 (3H, s, OMe), 3.26 (1H, dd, *J* = 10.5, 2.7 Hz, H_{31}), [3.04] (1H, s, NMe*), 3.00 (2H, s, NMe), 2.72-2.61 (1H, m, H_{32}), 2.44-2.31 (1H, m, H_{30}), 2.16 (3H, s, Me_{28}), 1.12 (3H, d, *J* = 6.7 Hz, Me_{32}), 0.92 (3H, d, *J* = 7.1 Hz, Me_{30}); **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C [212.4], 212.3, 162.1, 160.8, 128.7, 124.7, [113.1], 111.3, [87.4], 87.3, 61.2, 61.2, [49.7], 49.6, 37.6, 37.5, 33.0, [31.0], 30.9, 29.6, 27.5, 19.3, [13.4], 13.3; $[\alpha]_D^{20}$ –74.4 (*c*

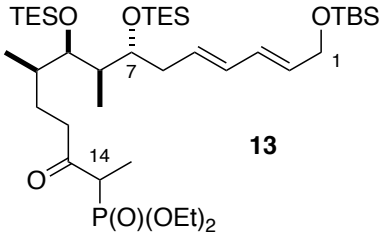
0.84, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 2964, 2928, 1691, 1651, 1457, 1353, 1318, 1275, 1193, 1170, 1092, 1068, 956, 725; **HRMS** calc. for $C_{12}H_{21}NO_3Na$ $[M+Na]^+$ 250.1414, found 250.1412.

Distinguishable resonances of the minor rotamer (2:1 ratio) are given in brackets and assignments denoted with an asterisk.

3.3. Fragment assembly and synthesis of the macrocycle 25

Enone 22





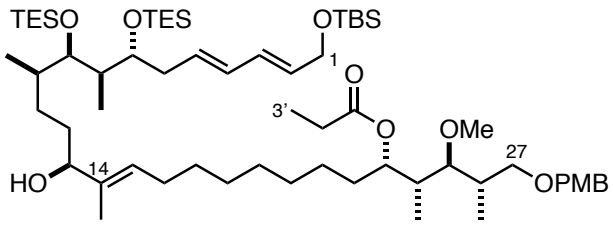
13

To $Ba(OH)_2$ (592 mg, 3.46 mmol) was added a solution of phosphonate **13** (2.24 g, 2.88 mmol, 3:1 mixture with regioisomer **77**) in THF (40 mL) and the suspension was stirred for 1 h at rt. Aldehyde **12** (1.10 g, 2.37 mmol) in THF/H_2O (40:1, 25 mL) was added and the reaction was stirred for 72 h. The reaction was quenched by the addition of NH_4Cl (75 mL) and the layers separated. The aqueous phase was extracted with EtOAc (3 × 100 mL) and the combined organics were dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was purified by flash column chromatography (1:5 → 1:4 EtOAc/PE) to yield enone **22** (1.96 g, 85% >20:1) as a colourless oil.

R_f 0.76 (1:3 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.24 (2H, d, J = 8.5 Hz, ArH), 6.86 (2H, d, J = 8.5 Hz, ArH), 6.59 (1H, t, J = 7.1 Hz, H_{15}), 6.18 (1H, dd, J = 14.9, 10.5 Hz, H_3), 6.04 (1H, dd, J = 15.2, 10.5 Hz, H_4), 5.68 (1H, dt, J = 14.7, 7.5 Hz, H_5), 5.64 (1H, dt, J = 14.7, 5.7 Hz, H_2), 5.18 (1H, td, J = 6.7, 2.0 Hz, H_{23}), 4.41 (2H, ABq, J = 11.0 Hz, OCH_2Ar), 4.20 (2H, d, J = 5.1 Hz, H_1 × 2), 3.80 (3H, s, OMe), 3.66-3.60 (1H, m, H_7), 3.56-3.51 (2H, m, H_9, H_{27a}), 3.38 (3H, s, OMe), 3.32 (1H, dd, J = 8.9, 7.2 Hz, H_{27b}), 2.87 (1H, dd, J = 8.8, 3.8 Hz, H_{25}), 2.73 (1H, ddd, J = 15.7, 9.7, 6.0 Hz, H_{12a}), 2.56 (1H, ddd, J = 15.7, 9.7, 6.0 Hz, H_{12b}), 2.31 (2H, q, J = 7.6 Hz, $H_{2'}$ × 2), 2.21 (2H, q, J = 7.4 Hz, H_{16} × 2), 2.18-2.14 (2H, m, H_6 × 2), 2.11-2.06 (1H, m, H_{26}), 1.81-1.70 (3H, m, H_8, H_{11a}, H_{24}), 1.76 (3H, s, Me_{14}), 1.67-1.58 (1H, m, H_{22a}), 1.48-1.37 (4H, m, H_{11b}, H_{22b}, H_{17} × 2), 1.34-1.20 (8H, m, $H_{18\text{-}21}$), 1.14 (3H, t, J = 7.6 Hz, $H_{3'}$ × 3), 1.06 (3H, d, J = 7.1 Hz, Me_{26}), 0.95 (9H, t, J = 8.2 Hz, $Si(CH_2C\underline{H}_3)_3$), 0.93 (9H, t, J =

8.2 Hz, Si($CH_2C\underline{H}_3$)$_3$), 0.92-0.89 (15H, m, SiC($C\underline{H}_3$)$_3$, Me_{24}, Me_{10}), 0.86 (3H, d, J = 6.9 Hz, Me_8), 0.61 (6H, q, J = 7.8 Hz, Si($C\underline{H}_2CH_3$)$_3$), 0.56 (6H, q, J = 7.5 Hz, Si($C\underline{H}_2CH_3$)$_3$), 0.07 (6H, s, Si($C\underline{H}_3$)$_2$). **^{13}C NMR** (125 MHz, CDCl3) $\boldsymbol{\delta}_C$ 202.1, 174.1, 159.0, 142.1, 137.1, 131.5, 131.4, 130.9, 130.3, 130.3, 129.0, 113.7, 85.9, 77.5, 74.5, 73.1, 72.8, 71.6, 63.7, 61.4, 55.2, 41.8, 38.9, 38.2, 36.0, 35.9, 35.4, 32.8, 29.5, 29.5, 29.4, 29.1, 28.7, 28.0, 26.9, 26.0, 25.8, 18.4, 16.3, 16.3, 11.4, 10.6, 10.5, 9.4, 7.2, 7.0, 5.6, 5.3, −5.2; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ –2.6 (*c* 1.01, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 2952, 2931, 2876, 2857, 1732, 1670, 1614, 1513, 1461, 1378, 1247, 1192, 1083, 1040, 1005, 990, 835, 775, 738, 725; **HRMS** calc. for $C_{62}H_{118}O_9Si_3N$ $[M+NH_4]^+$ 1104.8109, found 1104.8091.

Alcohol 22a

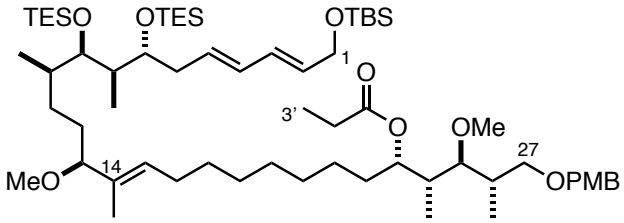


To a solution of enone **22** (1.96 g, 1.80 mmol) in THF (47 mL) was added (*R*)-Me-CBS catalyst (2.16 mL, 1 M in PhMe, 2.16 mmol) at –10 °C and the solution stirred for 5 min before dropwise addition of $BH_3{\cdot}SMe_2$ (188 μL, 1.98 mmol). The solution was stirred at –10 °C for 1.5 h then carefully quenched by the addition of MeOH (50 mL). The solution was warmed to rt and concentrated *in vacuo*. The residue was re-dissolved in MeOH (40 mL) and was again concentrated *in vacuo*. This process was repeated twice further. Purification *via* flash column chromatography (1:6 EtOAc/PE) gave alcohol **22a** (1.79 g, 93%, >20:1 *dr*) as a colourless oil.

R_f 0.72 (1:3 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) $\boldsymbol{\delta}_H$ 7.24 (2H, d, J = 8.5 Hz, ArH), 6.86 (2H, d, J = 8.5 Hz, ArH), 6.18 (1H, dd, J = 15.2, 10.4 Hz, H_3), 6.04 (1H, dd, J = 15.3, 10.2 Hz, H_4), 5.68 (1H, dt, J = 14.7, 7.4 Hz, H_5), 5.64 (1H, dt, J = 15.3, 5.6 Hz, H_2), 5.36 (1H, t, J = 6.4 Hz, H_{15}), 5.18 (1H, td, J = 7.2, 1.8 Hz, H_{23}), 4.41 (2H, ABq, J = 11.1 Hz, OCH_2Ar), 4.20 (2H, d, J = 5.2 Hz, H_1 × 2), 3.94 (1H, td, J = 6.6, 2.5 Hz, H_{13}), 3.80 (3H, s, OMe), 3.62-3.58 (1H, m, H_7), 3.53 (1H, dd, J = 9.2, 4.8 Hz, H_{27a}), 3.50 (1H, dd, J = 5.1, 3.5 Hz, H_9), 3.38 (3H, s, OMe), 3.32 (1H, dd, J = 9.1, 7.4 Hz, H_{27b}), 2.87 (1H, dd, J = 8.6, 3.4 Hz, H_{25}), 2.31 (2H, q, J = 7.7 Hz, $H_{2'}$ × 2), 2.18-2.12 (2H, m, H_6 × 2), 2.12-2.06 (1H, m, H_{26}), 2.03-1.94 (2H, m, H_{16} × 2), 1.82-1.70 (2H, m, H_8, H_{24}), 1.66-1.60 (2H, m, H_{12a}, H_{22a}), 1.58 (3H, br s, Me_{14}), 1.54-1.48 (1H, m, H_{10}), 1.48-1.37 (4H, m, H_{11} × 2, H_{12b}, H_{22b}), 1.35-1.29 (2H, m, H_{17} × 2), 1.29-1.24 (8H, m, H_{18-21}), 1.14 (3H, t, J = 7.6 Hz, $H_{3'}$ × 3), 1.05 (3H, d, J = 7.0 Hz, Me_{26}), 0.96 (9H, t, J = 8.0 Hz, Si($CH_2C\underline{H}_3$)$_3$), 0.93 (9H, t, J = 7.9 Hz, Si($CH_2C\underline{H}_3$)$_3$), 0.91 (9H, s, SiC($C\underline{H}_3$)$_3$), 0.90-0.84 (9H, m, Me_8, Me_{24}, Me_{10}), 0.60 (6H, q, J = 8.0 Hz, Si($C\underline{H}_2CH_3$)$_3$), 0.57 (6H, q, J = 7.8 Hz,

Si(C<u>H</u>$_2$CH$_3$)$_3$), 0.07 (6H, s, Si(C<u>H</u>$_3$)$_2$). **^{13}C NMR** (125 MHz, CDCl3) $\boldsymbol{\delta}_C$ 174.1, 159.0, 136.7, 131.6, 131.4, 130.8, 130.3, 130.3, 129.0, 127.5, 113.7, 85.9, 78.7, 77.6, 74.6, 73.1, 72.7, 71.6, 63.7, 61.3, 55.2, 41.7, 38.8, 38.5, 35.9, 35.9, 33.0, 32.7, 29.5, 29.5, 29.4, 29.3, 28.0, 27.8, 27.5, 25.9, 25.7, 18.4, 16.3, 16.2, 10.8, 10.6, 10.3, 9.4, 7.1, 7.0, 5.6, 5.2, −5.2; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ +1.9 (*c* 1.01, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 2952, 2930, 2876, 2856, 1733, 1614, 1514, 1462, 1378, 1362, 1248, 1193, 1084, 1041, 1005, 990, 835, 776, 737, 725; **HRMS** calc. for $C_{62}H_{120}O_9Si_3N$ $[M+NH_4]^+$ 1106.8265, found 1106.8257.

Methyl ether 23

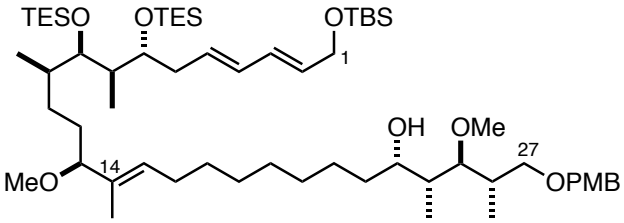


To a solution of Meerwein salt (143 mg, 0.96 mmol) and proton sponge (309 mg, 1.44 mmol) in CH_2Cl_2 (30 mL) was added alcohol **22a** (525 mg, 0.48 mmol). The reaction was stirred for 4 h at rt before being quenched with NH_4Cl (50 mL). The layers were separated, and the aqueous phase extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried ($MgSO_4$), concentrated *in vacuo* and purified by flash column chromatography (1:20 → 1:9 EtOAc/PE) to give methyl ether **23** as a colourless oil (445 mg, 87%).

R_f 0.79 (1:3 EtOAc/PE); **^{1}H NMR** (400 MHz, $CDCl_3$) $\boldsymbol{\delta}_H$ 7.24 (2H, d, *J* = 8.7 Hz, ArH), 6.86 (2H, d, *J* = 8.7 Hz, ArH), 6.18 (1H, dd, *J* = 14.9, 10.6 Hz, H_3), 6.03 (1H, dd, *J* = 15.3, 10.6 Hz, H_4), 5.68 (1H, dt, *J* = 14.8, 7.1 Hz, H_5), 5.64 (1H, dt, *J* = 14.8, 5.9 Hz, H_2), 5.31 (1H, t, *J* = 7.2 Hz, H_{15}), 5.18 (1H, td, *J* = 7.2, 1.7 Hz, H_{23}), 4.41 (2H, ABq, *J* = 11.6 Hz, OCH_2Ar), 4.20 (2H, d, *J* = 5.0 Hz, H_1 × 2), 3.80 (3H, s, OMe), 3.62-3.56 (1H, m, H_7), 3.53 (1H, dd, *J* = 8.9, 4.5 Hz, H_{27a}), 3.48 (1H, dd, *J* = 5.0, 3.2 Hz, H_9), 3.38 (3H, s, OMe), 3.36-3.30 (2H, m, H_{13}, H_{27b}), 3.14 (3H, s, OMe), 2.87 (1H, dd, *J* = 8.2, 3.9 Hz, H_{25}), 2.31 (2H, q, *J* = 7.6 Hz, $H_{2'}$ × 2), 2.19-2.12 (2H, m, H_6 × 2), 2.12-1.93 (3H, m, H_{16} × 2, H_{26}), 1.83-1.68 (2H, m, H_8, H_{24}), 1.68-1.57 (2H, m, H_{12a}, H_{22a}), 1.54-1.40 (4H, m, H_{10}, H_{12} × 2, H_{22b}), 1.49 (3H, s, Me_{14}), 1.39-1.31 (4H, m, H_{11} × 2, H_{17} × 2), 1.31-1.24 (8H, m, $H_{18\text{-}21}$), 1.14 (3H, t, *J* = 7.4 Hz, $H_{3'}$ × 3), 1.06 (3H, d, *J* = 7.1 Hz, Me_{26}), 0.94 (9H, t, *J* = 8.1 Hz, Si($CH_2C\underline{H}_3$)$_3$), 0.93 (9H, t, *J* = 8.1 Hz, Si($CH_2C\underline{H}_3$)$_3$), 0.91 (9H, s, SiC(C<u>H</u>$_3$)$_3$), 0.90-0.83 (9H, m, Me_8, Me_{10}, Me_{24}), 0.60 (6H, q, *J* = 7.6 Hz, Si(C<u>H</u>$_2$CH$_3$)$_3$), 0.57 (6H, q, *J* = 7.6 Hz, Si(C<u>H</u>$_2$CH$_3$)$_3$), 0.07 (6H, s, Si(C<u>H</u>$_3$)$_2$). **^{13}C NMR** (125 MHz, CDCl3) $\boldsymbol{\delta}_C$ 174.1, 159.0, 133.8, 131.7, 131.3, 130.9, 130.3, 130.3, 129.6, 129.0, 113.7, 88.3, 85.9, 77.6, 74.7, 73.1, 72.8, 71.6, 63.7, 61.4, 55.5, 55.2, 41.7, 38.9, 38.6, 35.9, 35.8, 32.8, 31.8, 29.6, 29.6, 29.5, 29.4, 28.0, 27.9, 27.6, 26.0, 25.8, 18.4, 16.3, 16.1, 10.6, 10.3, 10.1, 9.4, 7.2, 7.0, 5.6, 5.2,

5.2; $[\alpha]_D^{20}$ +1.5 (c 0.89, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 2952, 2926, 2876, 2855, 1734, 1615, 1514, 1462, 1378, 1362, 1248, 1192, 1171, 1093, 1043, 1006, 990, 836, 776, 739, 725; **HRMS** calc. for $C_{63}H_{122}O_9Si_3N$ $[M+NH_4]^+$ 1120.8422, found 1120.8420.

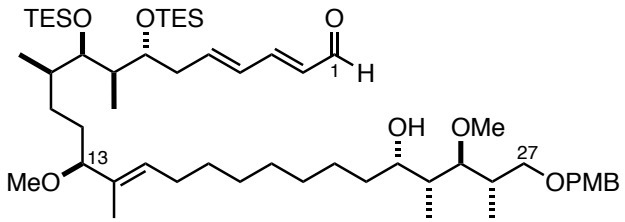
Alcohol 23a



To a solution of ester **23** (1.58 g, 1.43 mmol) in CH_2Cl_2 (28 mL) was added DIBAL (2.86 mL, 2.86 mmol, 1.0 M in hexanes) at –78 °C. After 1.5 h, the reaction was quenched with NH_4Cl (25 mL) and Na^+/K^+ tartrate (25 mL) and allowed to warm to rt. The layers were separated and the aqueous was extracted with CH_2Cl_2 (3 × 25 mL) and the combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. The crude was purified by flash column chromatography (1:4 EtOAc/PE) to afford alcohol **23a** as a colourless oil (1.51 g, 99%).

$\mathbf{R_f}$ 0.62 (1:3 EtOAc/PE); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.26 (2H, d, *J* = 8.9 Hz, ArH), 6.88 (2H, d, *J* = 8.6 Hz, ArH), 6.18 (1H, dd, *J* = 15.2, 10.7 Hz, H_3), 6.04 (1H, dd, *J* = 15.2, 10.7 Hz, H_4), 5.68 (1H, dt, *J* = 14.4, 7.0 Hz, H_5), 5.64 (1H, dt, *J* = 15.2, 5.0 Hz, H_2), 5.31 (1H, t, *J* = 7.0 Hz, H_{15}), 4.44 (2H, s, OCH_2Ar), 4.20 (2H, d, *J* = 5.4 Hz, H_1 × 2), 3.89 (1H, t, *J* = 6.6 Hz, H_{23}), 3.80 (3H, s, OMe), 3.62-3.56 (1H, m, H_7), 3.54-3.50 (1H, m, H_{27a}), 3.50-3.46 (3H, m, H_9, H_{27b}, OH,), 3.44 (3H, s, OMe), 3.34 (1H, t, *J* = 7.1 Hz, H_{13}), 3.19 (1H, dd, *J* = 9.0, 3.0 Hz, H_{25}), 3.14 (3H, s, OMe), 2.19-2.10 (2H, m, H_6 × 2), 2.10-1.97 (3H, m, H_{26}, H_{16} × 2), 1.76-1.70 (2H, m, H_8, H_{24}), 1.55-1.44 (4H, m, H_{10}, H_{12} × 2, H_{22a}), 1.49 (3H, s, Me_{14}), 1.41-1.31 (5H, m, H_{11} × 2, H_{17} × 2, H_{22b}), 1.31-1.26 (8H, m, $H_{18\text{-}21}$), 1.04 (3H, d, *J* = 6.9 Hz, Me_{24}), 0.95 (9H, t, *J* = 8.1 Hz, $Si(CH_2C\underline{H}_3)_3$), 0.94 (9H, t, *J* = 8.1 Hz, $Si(CH_2C\underline{H}_3)_3$), 0.94-0.91 (3H, obs m, Me_{26}), 0.91 (9H, s, $SiC(C\underline{H}_3)_3$), 0.87 (3H, d, *J* = 7.5 Hz, Me_{10}), 0.85 (3H, d, *J* = 7.6 Hz, Me_8), 0.60 (6H, q, *J* = 8.0 Hz, $Si(C\underline{H}_2CH_3)_3$), 0.57 (6H, q, *J* = 8.1 Hz, $Si(C\underline{H}_2CH_3)_3$), 0.07 (6H, s, $Si(C\underline{H}_3)_2$). **^{13}C NMR** (125 MHz, CDCl3) δ_C 159.1, 133.7, 131.7, 131.3, 130.6, 130.3, 130.2, 129.6, 129.1, 113.7, 89.0, 88.2, 77.6, 74.7, 72.7, 72.0, 70.6, 63.7, 61.7, 55.5, 55.2, 41.6, 38.6, 37.1, 36.7, 35.8, 34.7, 31.7, 29.8, 29.6, 29.5, 29.4, 27.9, 28.5, 26.2, 25.9, 18.4, 16.0, 14.9, 11.4, 10.2, 10.0, 7.1, 7.0, 5.6, 5.2, 5.2; $[\alpha]_D^{20}$ 1.8 (c 1.01, $CHCl_3$)); **IR** (thin film) ν_{max} (cm^{-1}) 3507, 2952, 2928, 2876, 2853, 1613, 1514, 1460, 1413, 1377, 1359, 1300, 1246, 1169, 1084, 1042, 1006, 990, 965, 834, 776, 739, 725, 675; **HRMS** calc. for $C_{60}H_{118}O_8Si_3N$ $[M+NH_4]^+$ 1064.8160, found 1064.8128.

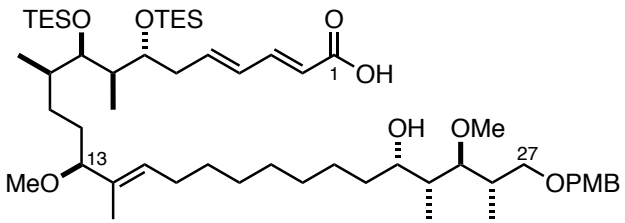
Aldehyde 23b



To a solution of TBS ether **23a** (836 mg, 0.80 mmol) in CH_2Cl_2 (12 mL) and pH 9.2 buffer (3 mL) at 0 °C was added DDQ (200 mg, 0.88 mmol). The solution was stirred at this temperature for 15 min, then quenched with $NaHCO_3$ (60 mL) and warmed to rt. The mixture was extracted with CH_2Cl_2 (3 × 50 mL) and the combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. Purification *via* flash column chromatography (1:6 EtOAc/PE) gave aldehyde **23b** (560 mg, 75%, 91% brsm) as a colourless oil.

R_f 0.62 (1:3 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) $\boldsymbol{\delta}_H$ 9.55 (1H, d, J = 7.9 Hz, H_1), 7.25 (2H, d, J = 7.8 Hz, ArH), 7.08 (1H, dd, J = 15.3, 10.1 Hz, H_3), 6.88 (2H, d, J = 8.5 Hz, ArH), 6.34-6.28 (2H, m, H_4, H_5), 6.08 (1H, dd, J = 15.4, 8.2 Hz, H_2), 5.31 (1H, t, J = 7.0 Hz, H_{15}), 4.43 (2H, s, OCH_2Ar), 3.90 (1H, app t, J = 6.6 Hz, H_{23}), 3.81 (3H, s, OMe), 3.70-3.63 (1H, m, H_7), 3.55-3.45 (4H, m, H_9, H_{27} × 2, OH), 3.44 (3H, s, OMe), 3.34 (1H, t, J = 6.1 Hz, H_{13}), 3.18 (1H, dd, J = 9.3, 3.7 Hz, H_{25}), 3.14 (3H, s, OMe), 2.37-2.24 (2H, m, H_6 × 2), 2.11-1.93 (3H, m, H_{16} × 2, H_{26}), 1.80-1.71 (1H, m, H_8), 1.71-1.64 (1H, m, H_{24}), 1.57-1.51 (4H, m, H_{10}, H_{22a}, H_{12} × 2), 1.49 (3H, s, Me_{14}), 1.45-1.33 (5H, m, H_{17} × 2, H_{11} × 2, H_{22b}), 1.33-1.25 (8H, m, $H_{18\text{-}21}$), 1.04 (3H, d, J = 7.1 Hz, Me_{24}), 0.95 (9H, t, J = 7.9 Hz, $Si(CH_2C\underline{H}_3)_3$), 0.94 (9H, t, J = 7.9 Hz, $Si(CH_2C\underline{H}_3)_3$), 0.93 (3H, obs d, J = 7.8 Hz, Me_{26}), 0.88 (3H, d, J = 6.8 Hz, Me_{10}), 0.87 (3H, d, J = 6.8 Hz, Me_8), 0.60 (6H, q, J = 7.8 Hz, $Si(C\underline{H}_2CH_3)_3$), 0.57 (6H, q, J = 7.8 Hz, $Si(C\underline{H}_2CH_3)_3$)); **^{13}C NMR** (125 MHz, CDCl3) $\boldsymbol{\delta}_C$ 193.9, 159.1, 152.5, 144.8, 133.7, 130.6, 130.3, 130.2, 129.6, 129.2, 113.7, 89.0, 88.2, 77.4, 74.3, 72.7, 72.0, 70.6, 61.7, 55.5, 55.2, 41.5, 38.9, 37.1, 36.7, 36.4, 34.7, 31.8, 29.8, 29.6, 29.5, 29.4, 28.3, 27.5, 26.2, 15.9, 14.9, 11.4, 10.1, 10.0, 7.1, 6.9, 5.6, 5.1; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ 2.4 (c 1.01, CHCl3); **IR** (thin film) ν_{max} (cm^{-1}) 3486, 2952, 2931, 2876, 2853, 1685, 1640, 1613, 1514, 1459, 1416, 1378, 1367, 1302, 1247, 1171, 1159, 1087, 1038, 1009, 988, 820, 739, 725; **HRMS** calc. for $C_{54}H_{102}NO_8Si_2$ $[M+NH_4]^+$ 948.7138, found 948.7133.

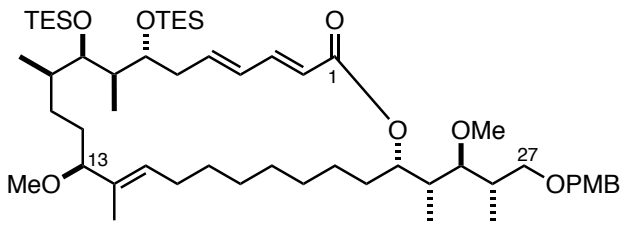
***Seco* acid 24**



To aldehyde **23b** (510 mg, 0.55 mmol) was added 2-methyl-2-butene (8.71 mL, 82.6 mmol) and the solution was cooled to 0 °C. $NaClO_2$ (1.86 g, 80% technical grade, 16.4 mmol) and $NaH_2PO_4{\cdot}H_2O$ (3.43 g, 22 mmol) were dissolved in H_2O/*t*-BuOH (34 mL, 1:1) and added to the reaction mixture. The reaction was warmed to rt and stirred for 16 h before being diluted with EtOAc (50 mL) and brine (50 mL). The mixture was extracted with EtOAc (3 × 10 mL) and the combined organics were dried (Na_2SO_4) and concentrated *in vacuo* to give *seco*-acid **24** (*ca*. 521 mg) as a pale-yellow oil. The crude material was used directly in the next reaction without further purification.

R_f 0.21 (1:4:1 EtOAc/PE/AcOH); **^{1}H NMR** (500 MHz, $CDCl_3$) $\boldsymbol{\delta}_H$ 7.32 (1H, dd, *J* = 15.2, 9.9 Hz, H_3), 7.25 (2H, d, *J* = 8.5 Hz, ArH), 6.88 (2H, d, *J* = 8.5 Hz, ArH), 6.27-6.12 (2H, m, H_4, H_5), 5.79 (1H, d, *J* = 15.5 Hz, H_2), 5.31 (1H, t, *J* = 7.0 Hz, H_{15}), 4.44 (2H, s, OCH_2Ar), 3.98-3.91 (1H, t, *J* = 7.1 Hz, H_{23}), 3.81 (3H, s, OMe), 3.67-3.60 (1H, m, H_7), 3.55-3.40 (2H, m, H_9, H_{27b}), 3.49-3.45 (1H, m, H_{27b}), 3.45 (3H, s, OMe), 3.35 (1H, t, *J* = 6.9 Hz, H_{13}), 3.19 (1H, dd, *J* = 8.8, 3.1 Hz, H_{25}), 3.14 (3H, s, OMe), 2.31-2.22 (2H, m, H_6 × 2), 2.12-2.06 (2H, m, H_{16a}, H_{26}), 2.01-1.93 (1H, m, H_{16b}), 1.76-1.70 (2H, m, H_8, H_{24}), 1.65-1.54 (4H, m, H_{10}, H_{12} × 2, H_{22a},), 1.50 (3H, s, Me_{14}), 1.40-1.20 (13H, m, H_{11} × 2, $H_{17\text{-}21}$, H_{22b}), 1.04 (3H, d, *J* = 7.3 Hz, Me_{24}), 0.95 (9H, t, *J* = 7.9 Hz, $Si(CH_2C\underline{H}_3)_3$), 0.94 (9H, t, *J* = 7.9 Hz, $Si(CH_2C\underline{H}_3)_3$), 0.94 (3H, obs d, *J* = 8.0 Hz, Me_{26}), 0.88 (3H, d, *J* = 6.8 Hz, Me_{10}), 0.84 (3H, d, *J* = 7.1 Hz, Me_8), 0.59 (6H, q, *J* = 7.1 Hz, $Si(C\underline{H}_2CH_3)_3$), 0.57 (6H, q, *J* = 7.8 Hz, $Si(C\underline{H}_2CH_3)_3$); **^{13}C NMR** (125 MHz, CDCl3) $\boldsymbol{\delta}_C$ 170.9, 159.1, 146.5, 142.7, 133.6, 130.6, 130.0, 129.8, 129.2, 118.9, 113.7, 89.1, 88.1, 77.2, 74.2, 72.8, 72.0, 70.8, 61.7, 55.5, 55.3, 41.3, 39.1, 37.1, 36.6, 36.3, 34.6, 31.7, 29.7, 29.7, 29.4, 29.3, 28.3, 27.6, 26.1, 15.6, 14.9, 11.5, 10.2, 10.0, 7.1, 7.0, 5.6, 5.2; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ 6.0 (c 0.99, $CHCl_3$); IR (thin film) n max (cm–1) 2948, 2931, 2876, 2853, 1712, 1690, 1643, 1614, 1513, 1459, 1415, 1377, 1302, 1246, 1173, 1084, 1038, 1009, 820, 739, 727; **HRMS** calc. for $C_{54}H_{102}NO_9Si_2$ $[M+NH_4]^+$ 964.7081, found 964.7088.

Macrocycle 25

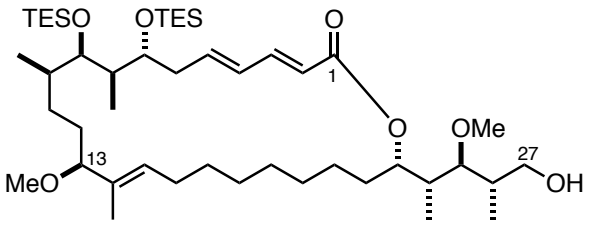


Crude *seco*-acid **24** (*ca*. 568 mg, 0.60 mmol) was dissolved in THF (15 mL) and NEt_3 (836 µL, 6.00 mmol) and TCBC (656 µL, 4.20 mmol) were added. The mixture was stirred at rt for 1 h, then diluted with PhMe (100 mL) and the resulting cloudy orange solution added to a solution of DMAP (733 mg, 6.00 mmol) in PhMe (50 mL) *via* syringe pump at a rate of 4.6 mL/h. Once complete the reaction was stirred for a further 16 h before being quenched with NH_4Cl (100 mL). The layers were separated, and the aqueous phase was extracted with EtOAc (3 × 100 mL) and the combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. Flash column chromatography (1:50 → 1:9 EtOAc/PE) yielded macrocycle **25** (386 mg, 70% over two steps) as a colourless oil.

R_f 0.52 (1:5 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) $\boldsymbol{\delta}_H$ 7.28 (1H, dd, *J* = 15.3, 10.0 Hz, H_3), 7.24 (2H, d, *J* = 8.9 Hz, ArH), 6.86 (2H, d, *J* = 8.5 Hz, ArH), 6.25-6.15 (2H, m, H_4, H_5), 5.83 (1H, d, *J* = 15.7 Hz, H_2), 5.38 (1H, dt, *J* = 10.8, 2.3 Hz, H_{23}), 5.24 (1H, dd, *J* = 8.0, 6.0 Hz, H_{15}), 4.41 (2H, ABq, *J* = 11.4 Hz, OCH_2Ar), 3.80 (3H, s, OMe), 3.67-3.61 (1H, m, H_7), 3.56-3.50 (2H, m, H_9, H_{27a}), 3.45 (3H, s, OMe), 3.32 (1H, dd, *J* = 9.2, 7.2 Hz, H_{27b}), 3.31 (1H, t, *J* = 8.7 Hz, H_{13}), 3.13 (3H, s, OMe), 2.90 (1H, dd, *J* = 8.3, 3.8 Hz, H_{25}), 2.35-2.26 (1H, m, H_{6a}), 2.18-2.03 (3H, m, H_{6b}, H_{16a}, H_{26}), 1.88-1.80 (1H, m, H_{16b}), 1.78-1.73 (1H, m, H_{24}), 1.73-1.64 (2H, m, H_8, H_{22a}), 1.62-1.57 (2H, m, H_{10}, H_{12a}), 1.53-1.48 (1H, m, H_{12b}), 1.48 (3H, s, Me_{14}), 1.43-1.33 (3H, m, H_{11a}, H_{21a}, H_{22b}), 1.33-1.20 (10H, m, H_{11b}, $H_{17\text{-}20}$, H_{21b},), 1.06 (3H, d, *J* = 7.1 Hz, Me_{26}), 0.96 (9H, t, *J* = 8.2 Hz, $Si(CH_2C\underline{H}_3)_3$), 0.95 (9H, t, *J* = 8.2 Hz, $Si(CH_2C\underline{H}_3)_3$), 0.94 (9H, m, Me_8, Me_{10}, Me_{24}), 0.61 (6H, q, *J* = 7.8 Hz, $Si(C\underline{H}_2CH_3)_3$), 0.58 (6H, q, *J* = 7.8 Hz, $Si(C\underline{H}_2CH_3)_3$); **^{13}C NMR** (125 MHz, $CDCl_3$) $\boldsymbol{\delta}_C$ 167.1, 158.9, 144.8, 141.8, 133.2, 130.8, 130.0, 129.9, 119.7, 113.6, 88.0, 85.6, 73.1, 72.6, 71.5, 61.0, 55.5, 55.1, 40.9, 40.7, 36.4, 35.9, 33.8, 31.3, 28.9, 28.5, 28.4, 28.2, 27.8, 27.7, 27.6, 24.5, 23.8, 20.7, 17.4, 17.2, 16.1, 14.6, 11.7, 11.1, 9.7, 7.0, 6.9, 5.3, 5.1; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ +10.9 (*c* 1.00, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 2930, 2876, 1711, 1643, 1614, 1580, 1513, 1458, 1367, 1300, 1245, 1171, 1134, 1091, 1038, 1001; **HRMS** calc. for $C_{54}H_{96}O_8Si_2Na$ $[M+Na]^+$ 951.6536, found 951.6541.

3.4. Completion of synthesis of the aplyologues

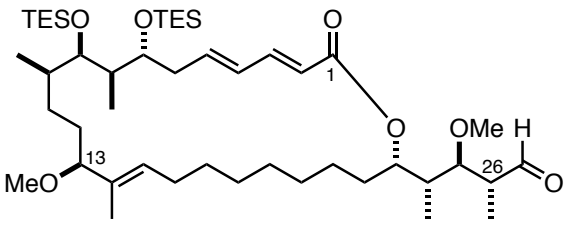
Alcohol 25a



To a solution of PMB ether **25** (335 mg, 0.36 mmol) in CH_2Cl_2 (20 mL) and pH 7.0 buffer (10 mL) was added DDQ (164 mg, 0.72 mmol). The reaction was stirred for 2 h before being quenched with $NaHCO_3$ (50 mL). The solution was diluted with H_2O (100 mL) and the layers were separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL) and the combined organics dried (Na_2SO_4) and concentrated *in vacuo*. The crude residue was purified by flash column chromatography (1:50 EtOAc/PhMe → 1:2 EtOAc/PE) to give alcohol **25a** (238 mg, 82%) as a colourless oil.

R_f 0.21 (1:5 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.28 (1H, dd, J = 15.1, 10.3 Hz, H_3), 6.23-6.18 (2H, m, H_4, H_5), 5.84 (1H, d, J = 14.8 Hz, H_2), 5.38 (1H, dt, J = 10.9, 2.6 Hz, H_{23}), 5.25 (1H, dd, J = 8.4, 5.7 Hz, H_{15}), 3.80 (1H, dd, J = 10.9, 3.5 Hz, H_{27a}), 3.68-3.62 (1H, m, H_7), 3.59-3.55 (2H, m, H_9, H_{27b}), 3.53 (3H, s, OMe), 3.31 (1H, dd, J = 8.4, 5.8 Hz, H_{13}), 3.14 (3H, s, OMe), 3.00 (1H, dd, J = 8.1, 3.5 Hz, H_{25}), 2.87 (1H, br s, OH), 2.35-2.27 (1H, m, H_{6a}), 2.23-2.06 (2H, m, H_{6b}, H_{16a}), 1.94-1.80 (3H, m, H_{16b}, H_{24}, H_{26}), 1.77-1.64 (2H, m, H_8, H_{22a}), 1.64-1.49 (4H, m, H_{10}, H_{12} × 2, H_{22b}), 1.47 (3H, s, Me_{14}), 1.45-1.36 (2H, m, H_{11a}, H_{21a}) 1.35-1.18 (10H, m, H_{11b}, $H_{17\text{-}20}$, H_{21b},), 1.13 (3H, d, J = 7.2 Hz, Me_{26}), 0.96 (9H, t, J = 7.9 Hz, $Si(CH_2C\underline{H}_3)_3$), 0.95 (9H, t, J = 7.9 Hz, $Si(CH_2C\underline{H}_3)_3$), 0.93 (3H, obs d, Me_{24}), 0.91 (3H, d, J = 7.6 Hz, Me_{10}), 0.89 (3H, d, J = 7.0 Hz, Me_8), 0.60 (6H, q, J = 7.8 Hz, $Si(C\underline{H}_2CH_3)_3$), 0.58 (6H, q, J = 7.8 Hz, $Si(C\underline{H}_2CH_3)_3$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C 167.3, 145.1, 141.8, 133.3, 130.1, 129.9, 129.1, 128.7, 119.6, 88.7, 88.1, 77.2, 73.4, 73.1, 65.1, 61.5, 41.7, 41.1, 39.6, 36.2, 36.2, 33.6, 31.4, 29.0, 28.6, 28.3, 27.9, 27.8, 27.7, 24.5, 16.2, 14.5, 11.7, 11.2, 9.8, 7.1, 7.0, 5.4, 5.2; $[\alpha]_D^{20}$ +19.2 (*c* 1.00, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 3452, 2935, 2876, 1712, 1642, 1458, 1415, 1367, 1300, 1238, 1134, 1092, 842, 739; **HRMS** calc. for $C_{46}H_{88}O_7Si_2Na$ $[M+Na]^+$ 831.5961, found 831.5957.

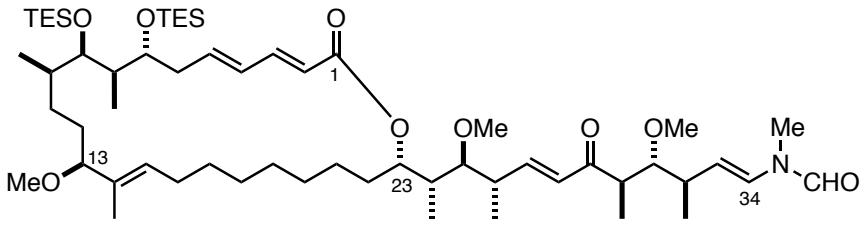
Aldehyde 26



To a solution of alcohol **25a** (144 mg, 0.18 mmol) in CH_2Cl_2 (17 ml) was added DMP (377 mg, 0.89 mmol) and $NaHCO_3$ (74 mg, 0.89 mmol). The mixture was stirred for 2 h before being quenched with $Na_2S_2O_3$ (50 mL). The quenching mixture was stirred for 1 h and the layers were separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL) and the combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. Aldehyde **26** was used immediately and crude subsequent aldol reaction.

R_f 0.68 (1:1 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 9.74 (1H, d, J = 1.4 Hz, CHO), 7.29 (1H, dd, J = 15.0, 10.0 Hz, H_3), 6.27-6.15 (2H, m, H_4, H_5), 5.84 (1H, d, J = 14.4 Hz, H_2), 5.45 (1H, dt, J = 10.8, 2.2 Hz, H_{23}), 5.25 (1H, dd, J = 8.4, 5.7 Hz, H_{15}), 3.68-3.61 (1H, m, H_7), 3.56-3.49 (1H, m, H_9), 3.44 (3H, s, OMe), 3.34-3.26 (2H, m, H_{13}, H_{25}), 3.13 (3H, s, OMe), 2.74-2.64 (1H, m, H_{26}), 2.37-2.25 (1H, m, H_{6a}), 2.25-2.04 (2H, m, H_{6b}, H_{16a}), 1.90-1.78 (2H, m, H_{16b}, H_{24}), 1.78-1.65 (2H, m, H_8, H_{22a}), 1.65-1.49 (3H, m, H_{10}, H_{12} × 2,) 1.47 (3H, s, Me_{14}), 1.45-1.35 (3H, m, H_{11a}, H_{21a}, H_{22b}), 1.35-1.19 (10H, m, H_{11b}, $H_{17\text{-}20}$, H_{21b}), 1.16 (3H, d, J = 7.0 Hz, Me_{26}), 0.96 (18H, t, J = 7.6 Hz, $Si(CH_2C\underline{H}_3)_3$ × 2), 0.93 (3H, d, J = 7.2 Hz, Me_{10}), 0.89 (3H, d, J = 7.0 Hz, Me_8), 0.83 (3H, d, J = 7.0 Hz, Me_{24}), 0.61 (6H, q, J = 7.9 Hz, $Si(C\underline{H}_2CH_3)_3$), 0.57 (6H, q, J = 7.9 Hz, $Si(C\underline{H}_2CH_3)_3$). **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C 203.9, 167.2, 145.2, 141.9, 133.3, 130.1, 129.9, 119.4, 88.0, 83.4, 77.2, 73.4, 72.6, 59.2, 55.4, 48.1, 40.8, 39.5, 36.4, 33.2, 31.4, 29.7, 29.0, 28.6, 28.3, 27.8, 27.8, 27.6, 24.6, 14.5, 11.6, 10.4, 9.9, 9.8, 7.0, 7.0, 5.4, 5.2; $[\alpha]_D^{20}$ 1.9 (c 1.00, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 2948, 2932, 2876, 1714, 1641, 1615, 1458, 1413, 1361, 1300, 1260, 1236, 1171, 1133, 1096, 1058, 1002, 739, 725; **HRMS** calc. for $C_{46}H_{90}O_7Si_2N$ $[M+NH_4]^+$ 824.6250, found 824.6250.

Enone 26a



Ketone *E*-**11** was azeotropically dried with benzene and placed under vacuum for 16 h. To a solution of ketone *E*-**11** (125 mg, 0.55 mmol) in Et_2O (5 mL) at 0 °C was added a freshly prepared solution of $BCy_2Cl{\cdot}NEt_3$ (275 μL, 0.55 mmol, 2.0 M in Et_2O). The resulting cloudy yellow solution of enolate was stirred at 0 °C for 1 h before being cooled to –78 °C. A solution of crude aldehyde **26** (ca. 140 mg) in Et_2O (3 mL) was added and the reaction mixture was stirred at –78 °C for 2 h, then warmed to –10 °C over 1 h before being quenched with SiO_2 (*ca.* 1 g). The slurry was stirred for 1 h then filtered and rinsed with Et_2O (10 mL) before being concentrated *in vacuo.* The crude material was purified by flash column chromatography (3:1→1:1 EtOAc/PE). As the aldol adduct and ketone *E*-**11** were inseparable by chromatography, all fractions containing the intermediary aldol adduct and ketone *E*-**11** were combined and used subsequently in the elimination reaction.

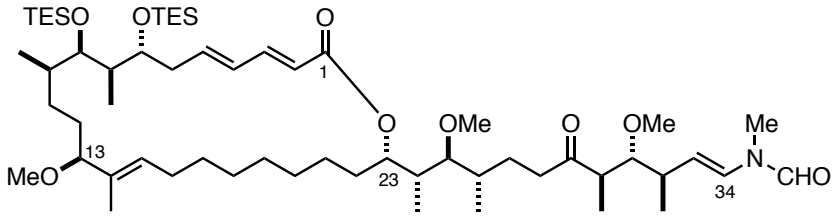
To a solution of the crude aldol adduct and ketone *E*-**11** (*ca.* 184 mg) in THF (6 mL) was added Burgess reagent (83 mg, 0.36 mmol). The reaction mixture was stirred at room temperature for 16 h then quenched with NH_4Cl (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (15 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash column chromatography (2:1→1:1 EtOAc/PE) yielding enone **26a** (117 mg, 65% over three steps) and recovered ketone *E*-**11** (75 mg).

R_f 0.89 (1:1 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 8.30 (0.60H, s, NCHO), [8.08] (0.30H, s, NCHO*), 7.30 (1H, dd, J = 15.4, 9.1 Hz, H_3), [7.15] (0.33H, d, J = 14.6 Hz, H_{34}*), 6.91 (1H, dd, J = 15.1, 8.1 Hz, H_{27}), 6.48 (0.67H, d, J = 14.3 Hz, H_{34}), 6.25-6.10 (3H, m, H_4, H_5, H_{28}), 5.83 (1H, d, J = 15.2 Hz, H_2), 5.40 (1H, br d, J = 10.7 Hz, H_{23}), 5.24 (1H, t, J = 6.0 Hz, H_{15}), 5.15 (1H, dd, J = 13.5, 9.9 Hz, H_{33}), 3.68-3.61 (1H, m, H_7), 3.56-3.51 (1H, m, H_9), 3.49 (3H, s, OMe), 3.33-3.27 (5H, m, H_{13}, H_{31}, OMe), 3.13 (3H, s, OMe), [3.10] (1H, s, NMe*), 3.06 (2H, s, NMe), 3.03-2.95 (1H, m, H_{30}), 2.95-2.89 (1H, m, H_{25}), 2.66-2.57 (1H, m, H_{26}), 2.50-2.37 (1H, m, H_{32}), 2.36-2.24 (1H, m, H_{6a}), 2.22-2.07 (2H, m, H_{6b}, H_{16a}), 1.89-1.79 (1H, m, H_{16b}), 1.74-1.65 (2H, m, H_8, H_{22a}), 1.64-1.57 (3H, m, H_{10}, H_{12a}, H_{24},) 1.47 (3H, s, Me_{14}), 1.44-1.33 (2H, m, H_{11a}, H_{22b}), 1.32-1.19 (11H, m, H_{11b}, $H_{17\text{-}21}$), 1.18-1.12 (6H, m, Me_{26}, Me_{32}), 0.96 (18H, t, J = 7.7 Hz, $Si(CH_2C\underline{H}_3)_3) \times 2$), 0.95 (3H, d, Me_{30}), 0.92 (3H, d, J = 7.1 Hz, Me_{10}),

0.89 (3H, d, J = 7.1 Hz, Me_8), 0.83 (3H, d, J = 7.4 Hz, Me_{24}), 0.60 (6H, q, J = 7.4 Hz, $Si(C\underline{H}_2CH_3)_3$), 0.58 (6H, q, J = 7.4 Hz, $Si(C\underline{H}_2CH_3)_3$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C [203.5], 203.4, [167.2], 162.2, [160.9], 149.3, [149.2], 145.1, 141.9, 133.2, [130.5], 130.1, 129.9, 128.8, [124.7], 119.5, [113.3], 111.4, 88.0, [87.6], 87.5, 86.0, 77.1, 73.4, 72.9, 61.2, 61.1, 55.4, [46.1], 46.0, 41.4, 39.6, 39.5, [37.9], 37.7, 36.5, 33.5, [33.0], 31.4, 29.0, 28.6, 28.3, 27.9, 27.8, 27.7, 27.5, 24.6, 19.5, 17.4, 14.5, 13.8, [13.7], 11.7, 10.4, [10.3], 9.8, 7.1, 7.0, 5.4, 5.2; $[\alpha]_D^{20}$ –39.7 (c 1.00, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 2924, 2875, 1696, 1656, 1458, 1370, 1237, 1134, 1095, 1069, 1003, 913; **HRMS** calc. for $C_{58}H_{105}NO_9Si_2Na$ $[M+Na]^+$ 1038.7220, found 1038.7230.

Distinguishable resonances of the minor rotamer (2:1 ratio) are given in brackets and assignments denoted with an asterisk.

Ketone 10



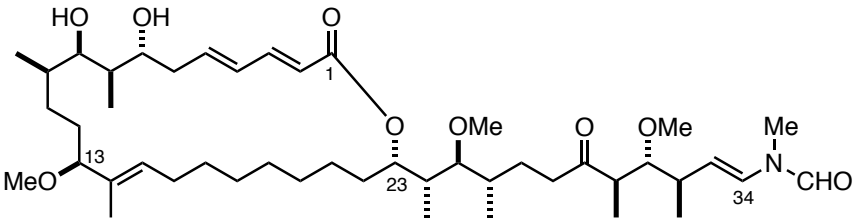
Stryker's reagent solution (104 μL, 0.0026 mmol, 0.025 M in PhMe) was added to enone **26a** (54 mg, 0.052 mmol) and the reaction mixture stirred at room temperature for 1 h. Further aliquots of Stryker's reagent solution (100 μL) were added every hour for a further 4 h. The solution was applied directly to a silica gel column and the product eluted with EtOAc/CH_2Cl_2 (1:4) to afford ketone **10** (48 mg, 89%) as a colourless oil.

R_f 0.88 (1:1 EtOAc/PE); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 8.29 (0.67H, s, NCHO), [8.08] (0.33H, s, NCHO*), 7.28 (1H, dd, J = 15.4, 10.6 Hz, H_3), [7.15] (0.33H, d, J = 14.6 Hz, H_{34}*), 6.46 (0.67H, d, J = 14.6 Hz, H_{34}), 6.24-6.12 (2H, m, H_4, H_5), 5.83 (1H, d, J = 15.3 Hz, H_2), 5.42 (1H, d, J = 11.5 Hz, H_{23}), 5.23 (1H, t, J = 8.4 Hz, H_{15}), 5.15 (1H, dd, J = 14.6, 9.8 Hz, H_{33}), 3.67-3.59 (1H, m, H_7), 3.55-3.49 (1H, m, H_9), 3.47 (3H, s, OMe), 3.33 (3H, s, OMe), 3.23-3.27 (2H, m, H_{13}, H_{31}), 3.14 (3H, s, OMe), [3.07] (1H, s, NMe*), 3.03 (2H, s, NMe), 2.82-2.74 (1H, m, H_{25}), 2.72-2.62 (1H, m, H_{30}), 2.61-2.50 (1H, m, H_{28a}), 2.50-2.35 (2H, m, H_{28b}, H_{32}), 2.35-2.26 (1H, m, H_{6a}), 2.16-1.97 (2H, m, H_{6b}, H_{16a}), 1.88-1.79 (1H, m, H_{16b}), 1.78-1.49 (8H, m, H_8, H_{10}, H_{12} × 2, H_{22a}, H_{24}, H_{26}, H_{27a}), 1.47 (3H, s, Me_{14}), 1.43-1.33 (3H, m, H_{11a}, H_{22b}, H_{27b}), 1.32-1.19 (11H, m, $H_{17\text{-}21}$, H_{11b}), 1.15 (3H, d, J = 7.5 Hz, Me_{32}), 0.96-0.87 (33H, m, Me_8, Me_{10}, Me_{24}, Me_{26}, Me_{30}, $Si(CH_2C\underline{H}_3)_3$), 0.60 (6H, q, J = 7.7 Hz, $Si(C\underline{H}_2CH_3)_3$), 0.58 (6H, q, J

= 7.7 Hz, Si(C<u>H</u>$_2$CH$_3$)$_3$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C [214.3], 214.2, 167.2 162.1, [160.8], 144.9, 134.9, 133.2, 130.2, 130.0, 128.9, [124.5], 119.8, [113.2], 111.3, 88.0, 87.4, 87.3, 74.4, 73.4, 73.2, 61.4, 61.3, 55.5, [49.2], 49.1, 42.0, 41.0, 40.4, [37.7], 37.5, 36.5, 34.4, 33.7, [33.1], 31.4, 29.7, 29.0, 28.6, 28.3, 28.0, 27.8, 27.7, [27.4], 24.6, 23.4, [19.4], 19.3, 17.4, 14.5, 13.6, 13.5, 10.8, 9.8, 7.1, 7.0, 5.4, 5.2; $[\alpha]_D^{20}$ –22.5 (*c* 1.00, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 2934, 2869, 1706, 1657, 1458, 1369, 1238, 1068, 1004, 725; **HRMS** calc. for $C_{58}H_{107}NO_9Si_2Na$ $[M+Na]^+$ 1040.7377, found 1040.7353.

Distinguishable resonances of the minor rotamer (2:1 ratio) are given in brackets and assignments denoted with an asterisk.

Diol (Aplyronine C/scytophycin hybrid) 9



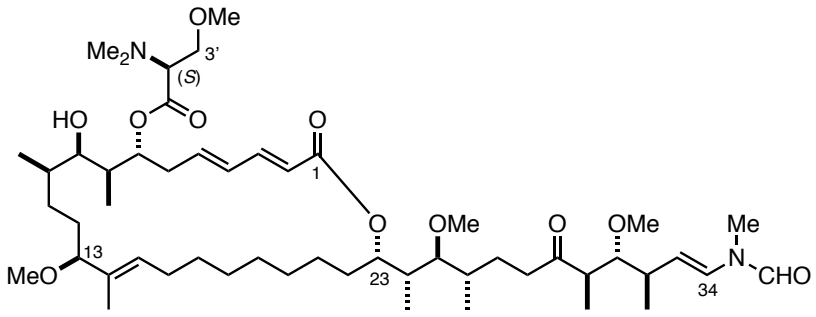
A stock solution of HF·pyridine was prepared by adding HF·pyridine (100 μL) to a solution of pyridine (200 μL) in THF (1 mL) at 0 °C before warming to rt and stirring for 30 min. This solution was added to *bis*-TES ether **10** (8 mg, 7.9 μmol) in a plastic reaction vessel at 0 °C and the reaction stirred at rt overnight. The reaction was quenched at 0 °C with $NaHCO_3$ solution (5 mL), stirred vigorously at rt for 30 min and the aqueous phase extracted with EtOAc (3 × 5 mL). The combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. Purification *via* flash column chromatography (1:1:0 → 10:10:1 EtOAc/CH_2Cl_2/MeOH) yielded diol **9** (5.3 mg, 85%) as a colourless oil.

R_f 0.52 (5:5:1 EtOAc/CH_2Cl_2/MeOH); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 8.29 (0.67H, s, NCHO), [8.07] (0.33H, s, NCHO*), 7.27 (1H, obs dd, H_3), [7.13] (0.33H, d, *J* = 14.8 Hz, H_{34}*), 6.46 (0.67H, d, *J* = 13.6 Hz, H_{34}), 6.28 (1H, dd, *J* = 15.4, 11.1 Hz, H_4), 6.11 (1H, dt, *J* = 15.1, 7.4 Hz, H_5), 5.84 (1H, d, *J* = 15.0 Hz, H_2), 5.35 (1H, td, *J* = 10.2, 2.6 Hz, H_{23}), 5.30 (1H, t, *J* = 7.9 Hz, H_{15}), 5.11 (1H, dd, *J* = 14.3, 9.5 Hz, H_{33}), 3.89-3.82 (1H, m, H_7), 3.70-3.64 (1H, m, H_9), 3.46 (3H, s, OMe), 3.38-3.35 (1H, m, H_{13}), 3.34 (3H, s, OMe), 3.31 (1H, br d, *J* = 9.3 Hz, H_{31}), 3.15 (3H, s, OMe), [3.08] (1H, s, NMe*), 3.04 (2H, s, NMe), 2.82-2.74 (2H, m, H_{25}, OH), 2.71-2.62 (1H, m, H_{30}), 2.59-2.52 (3H, m, H_6 × 2, H_{28a}), 2.49-2.32 (3H, m, H_{28b}, H_{32}, OH), 2.09-1.94 (2H, m, H_{16} × 2), 1.80-1.54 (8H, m, H_8, H_{10}, H_{12} × 2, H_{22a}, H_{24}, H_{26}, H_{27a}), 1.53-1.47 (1H, m, H_{11a}), 1.47 (3H, s, Me_{14}), 1.47-1.33 (2H, m, H_{22b}, H_{27b}), 1.32-1.19 (11H, m, H_{11b}, $H_{17\text{-}21}$,),

1.16 (3H, d, J = 6.8 Hz, Me_{32}), 1.04 (3H, d, J = 7.1 Hz, Me_{24}), 0.97 (3H, d, J = 6.8 Hz, Me_8), 0.90 (3H, d, J = 7.1 Hz, Me_{30}), 0.90 (3H, d, J = 7.1 Hz, Me_{26}), 0.79 (3H, d, J = 7.2 Hz, Me_{10}); **^{13}C NMR** (125 MHz, $CDCl_3$) $\boldsymbol{\delta}_C$ [214.3], 214.2, 167.0 162.2, [160.9], 144.3, 138.9, 133.7, 131.0, 129.9, 128.8, [124.7], 120.6, [113.3], 111.4, 87.9, 87.5, 87.3, 75.3, 75.2, 73.8, 61.4, 61.3, 55.7, 49.1, 42.1, 40.7, 39.0, [37.7], 37.5, 37.0, 34.5, 33.7, [33.1], 30.9, 29.7, 28.8, 28.7, 28.5, 27.8, 27.6, 27.3, 25.1, 23.5, 19.5, 17.4, 15.8, 13.5, 11.6, 10.9, 10.3; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ –46.8 (c 0.21, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 3349, 2939, 2853, 1703, 1655, 1461, 1360, 1277, 1134, 1065, 1002, 970, 800, 725; **HRMS** calc. for $C_{46}H_{79}NO_9Na$ $[M+Na]^+$ 812.5647, found 812.5643.

Distinguishable resonances of the minor rotamer (2:1 ratio) are given in brackets and assignments denoted with an asterisk.

Aplyronine A,D/scytophycin hybrid (bearing *S*-TMSer) *S*-8



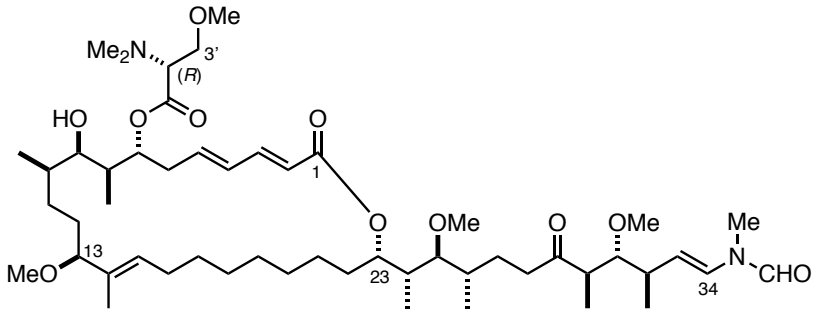
(*S*)-Trimethyl serine (3.9 mg, 26.5 μmol) and DMAP (3.2 mg, 26.3 μmol) were dissolved in CH_2Cl_2 (3 mL), then TCBC (8 μL, 51.1 μmol) and NEt_3 (11 μL, 78.9 μmol) were added to form a stock solution of mixed anhydride. Diol **9 (**2 mg, 2.65 μmol) was dissolved in benzene (0.30 mL) and an aliquot of the stock solution (0.30 mL) was added at 0 °C. Further aliquots of stock solution (0.15 mL) were added hourly for 4 h. The reaction was then quenched with MeOH (0.5 mL) and concentrated *in vacuo*. The crude residue was purified by preparative thin layer chromatography (4:4:1 EtOAc/CH_2Cl_2/MeOH) to give ester ***S*-8** as a colourless oil (1.5 mg, 62%).

R_f 0.57 (4:4:1 EtOAc/CH_2Cl_2/MeOH); **^{1}H NMR** (500 MHz, $CDCl_3$) $\boldsymbol{\delta}_H$ 8.29 (0.67H, s, NCHO), [8.07] (0.33H, s, NCHO*), 7.23 (1H, obs dd, H_3), [7.12] (0.33H, d, J = 14.8 Hz, H_{34}*), 6.45 (0.67H, d, J = 14.2 Hz, H_{34}), 6.26 (1H, dd, J = 14.7, 11.2 Hz, H_4), 6.04 (1H, dt, J = 14.7, 6.7 Hz, H_5), 5.86 (1H, d, J = 15.1 Hz, H_2), 5.38 (1H, br d, J = 10.8 Hz, H_{23}), 5.28 (1H, t, J = 7.5 Hz, H_{15}), 5.11 (1H, dd, J = 14.5, 9.6 Hz, H_{33}), 5.00-4.95 (1H, m, H_7), 3.67 (1H, dd, J = 9.9, 2.8

Hz, $H_{3',a}$), 3.64 (1H, dd, J = 8.3, 4.0 Hz, $H_{3',b}$), 3.61-3.58 (1H, m, H_9), 3.46 (3H, s, OMe), 3.42-3.39 (1H, m, $H_{2'}$), 3.38-3.35 (4H, m, H_{13}, OMe), 3.34 (3H, s, OMe), 3.31 (1H, br d, J = 9.1 Hz, H_{31}), 3.14 (3H, s, OMe), [3.08] (1H, s, NMe*), 3.04 (2H, s, NMe), 2.78 (1H, dt, J = 9.1, 2.5 Hz, H_{25}), 2.72-2.61 (1H, m, H_{30}), 2.61-2.51 (2H, m, H_{6a}, H_{28a}), 2.50-2.40 (3H, m, H_{6b}, H_{28b}, H_{32}), 2.40-2.35 (7H, m, OH, $C_{2'}NMe_2$), 1.95-1.90 (2H, m, H_{16} × 2), 1.80-1.70 (3H, m, H_{24}, H_{26}, H_{27a}) 1.65-1.58 (3H, m, H_{10}, H_{12} × 2), 1.50 (3H, s, Me_{14}), 1.46-1.35 (4H, m, H_{11a}, H_{22} × 2, H_{27b}), 1.35-1.19 (11H, m, H_{11b}, H_{17-21},), 1.16 (3H, d, J = 6.9 Hz, Me_{32}), 0.99-0.95 (6H, m, Me_8, Me_{10}), 0.92 (3H, d, J = 6.5 Hz, Me_{30}), 0.90 (3H, d, J = 6.1 Hz, Me_{24}), 0.88 (3H, d, J = 6.9 Hz, Me_{26}); **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C [214.3], 214.2, 170.2, 167.0, 162.2, [160.9], 144.0, 137.8, 133.8, 131.4, 129.9, 128.7, 128.2, [124.7], 121.2, [113.2], 111.4, 87.8, 87.4, 87.4, 87.3, 74.0, 73.7, 70.5, 67.6, 67.4, 61.4, [61.3], 59.2, 55.6, [49.2], 49.1, 42.5, 42.4, 42.1, [40.8], 40.8, [37.7], 37.5, 36.7, 36.4, 34.6, 34.5, 33.8, [33.1], 31.6, 29.7, 29.4, 28.9, 28.4, 28.1, 27.7, 27.6, 27.4, 22.7, [22.7], [19.4], 19.4, 17.4, 15.5, 13.5, 11.8, 10.9, 10.1; $[\alpha]_D^{20}$ –22.1 (c 0.032, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 3390, 2451, 2896, 2853, 1711, 1678, 1651, 1462, 1360, 1272, 1220, 1079, 1002, 957, 891, 829, 701; **HRMS** calc. for $C_{52}H_{90}N_2O_{11}K$ $[M+K]^+$ 957.6176, found 957.6189.

Distinguishable resonances of the minor rotamer (2:1 ratio) are given in brackets and assignments denoted with an asterisk.

Aplyronine A,D/scytophycin hybrid (bearing *R*-TMSer) *R*-8



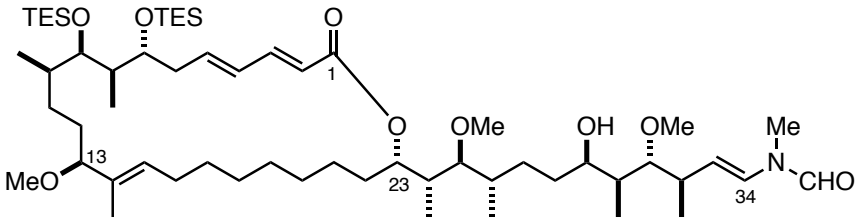
(*R*)-Trimethyl serine (3.9 mg, 26.5 μmol) and DMAP (3.2 mg, 26.3 μmol) were dissolved in CH_2Cl_2 (3 mL), then TCBC (8 μL, 51.1 μmol) and NEt_3 (11 μL, 78.9 μmol) were added to form a stock solution of mixed anhydride. Diol **9** (2.1 mg, 2.67 μmol) was dissolved in benzene (0.30 mL) and an aliquot of the stock solution (0.30 mL) was added at 0 °C. Further aliquots of stock solution (0.30 mL) were added every 30 min for 1.5 h. The reaction was then quenched with MeOH (1.00 mL) and concentrated *in vacuo*. The crude residue was purified by flash

column chromatography (1:1:0 → 4:4:1 EtOAc/CH_2Cl_2 /MeOH) to give ester ***R*-8** as a colourless oil (1.8 mg, 73%).

R_f 0.42 (5:5:1 EtOAc/CH_2Cl_2/MeOH); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 8.29 (0.67H, s, NCHO), [8.07] (0.33H, s, NCHO*), 7.26 (1H, obs dd, H_3), [7.13] (0.33H, d, J = 14.0 Hz, H_{34}*), 6.46 (0.67H, d, J = 14.1 Hz, H_{34}), 6.26 (1H, dd, J = 14.6, 11.9 Hz, H_4), 6.06-6.00 (1H, m, H_5), 5.86 (1H, d, J = 15.5 Hz, H_2), 5.38 (1H, br d, J = 10.7 Hz, H_{23}), 5.32-5.26 (1H, m, H_{15}), 5.09 (1H, dd, J = 14.3, 9.1 Hz, H_{33}), 4.99-4.95 (1H, m, H_7), 3.69 (2H, app d, J = 6.4 Hz, $H_{3'}$ × 2), 3.59-3.56 (1H, m, H_9), 3.46 (3H, s, OMe), 3.46-3.40 (1H, m, $H_{2'}$), 3.37-3.35 (4H, m, H_{13}, OMe), 3.34 (3H, s, OMe), 3.31 (1H, br d, J = 10.9 Hz, H_{31}), 3.14 (2H, s, OMe), [3.12] (1H, s, OMe*), [3.08] (1H, s, NMe*), 3.04 (2H, s, NMe), 2.78 (1H, dt, J = 8.2, 3.1 Hz, H_{25}), 2.72-2.62 (1H, m, H_{30}), 2.61-2.42 (5H, m, H_6 × 2, H_{28} × 2, H_{32}), 2.40-2.35 (7H, m, OH, $C_{2'}NMe_2$), 1.96-1.90 (2H, m, H_{16} × 2), 1.82-1.71 (3H, m, H_{24}, H_{26}, H_{27a}) 1.65-1.59 (3H, m, H_{10}, H_{12} × 2), 1.51 (3H, s, Me_{14}), 1.43-1.35 (4H, m, H_{11a}, H_{22} × 2, H_{27b}), 1.35-1.17 (11H, m, H_{11b}, $H_{17\text{-}21}$,), 1.15 (3H, d, J = 7.0 Hz, Me_{32}), 0.99-0.95 (6H, m, Me_8, Me_{10}), 0.92 (3H, d, J = 6.6 Hz, Me_{30}), 0.90 (3H, d, J = 6.4 Hz, Me_{24}), 0.88 (3H, d, J = 7.0 Hz, Me_{26}); **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C [214.3], 214.2, 169.9, 166.9, 162.2, [160.8], 143.8, 138.8, 137.6, 133.8, 132.1, 131.5, 129.9, 128.7, 128.5, 127.7, 125.0, [124.7], 121.2, [113.2], 111.4, 87.8, 87.4, 87.4, 87.3, 74.0, 73.7, 71.3, 67.2, 61.4, [61.4], 61.3, 59.1, 55.6, [49.2], 49.1, 42.3, 42.1, 40.8, [37.7], 37.5, 36.6, 36.3, 34.6, 34.5, 33.8, [33.1], 30.5, 29.7, 29.4, 29.0, 28.4, 28.2, 27.7, 27.6, 27.5, 22.7, [19.4], 19.4, 17.4, 15.5, [13.6], 13.5, 11.6, 10.9, [10.9], 10.1; $[\alpha]_D^{20}$ −7.1 (*c* 0.028, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 3392, 2896, 2846, 1710, 1675, 1651, 1461, 1343, 1288, 1221, 1082, 1003, 960, 892, 829, 700; **HRMS** calc. for $C_{52}H_{90}N_2O_{11}Na$ $[M+Na]^+$ 941.6442, found 941.6402.

Distinguishable resonances of the minor rotamer (2:1 ratio) are given in brackets and assignments denoted with an asterisk.

Alcohol 27a



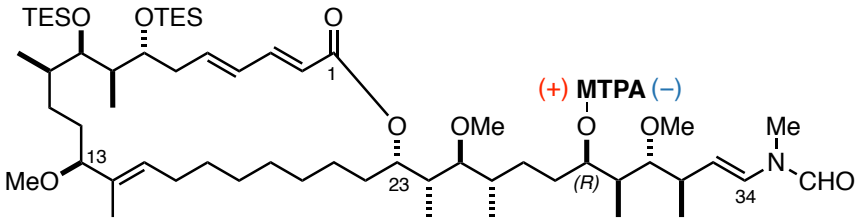
$Zn(BH_4)_2$ solution (1.12 mL, 0.175 M in Et_2O, 0.196 mmol) was added to ketone **10** (20 mg, 0.0196 mmol) at 0 °C and the reaction stirred at this temperature for 5 h before being quenched with NH_4Cl (5 mL) and Na^+/K^+ tartrate solution (10 mL). The quenching mixture was stirred vigorously for 3 h then extracted with CH_2Cl_2 (3 × 10 mL). The combined organics were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash chromatography (1:0 → 4:1 → 1:1 CH_2Cl_2/EtOAc) to yield alcohol **27a** as a colourless oil (11.5 mg, 58%, 2:1 *dr*).

R_f 0.45 (1:2 EtOAc/CH_2Cl_2); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 8.29 (0.66H, s, NCHO), [8.07] (0.33H, s, NCHO*), 7.28 (1H, dd, *J* = 15.2, 9.8 Hz, H_3), [7.15] (0.33H, d, *J* = 14.7 Hz, H_{34}*), 6.49 (0.66H, d, *J* = 14.3 Hz, H_{34}), 6.24-6.14 (2H, m, H_4, H_5), 5.84 (1H, d, *J* = 15.1 Hz, H_2), 5.42 (1H, dd, *J* = 10.9, 2.0 Hz, H_{23}), 5.24 (1H, t, *J* = 7.8 Hz, H_{15}), [5.15] (0.37H, dd, *J* = 15.2, 8.8 Hz, H_{33}*), 5.07 (0.60H, dd, *J* = 14.2, 8.3 Hz, H_{33}), 3.90-3.83 (1H, m, H_{29}), 3.67-3.61 (1H, m, H_7), 3.55-3.40 (1H, m, H_9), 3.48 (3H, s, OMe), 3.46 (3H, s, OMe), 3.30 (1H, dd, *J* = 8.1, 5.9 Hz, H_{13}), [3.06] (1H, s, NMe*), 3.02 (2H, s, NMe), 3.01 (1H, obs dd, H_{31}), 2.79 (1H, dd, *J* = 11.0, 2.1 Hz, H_{25}), 2.60-2.46 (1H, m, H_{32}), 2.38-2.27 (1H, m, H_{6a}), 2.17-2.00 (3H, m, H_{6b}, H_{16} × 2), 1.88-1.77 (1H, m, H_{22a}), 1.75-1.61 (4H, m, H_8, H_{22b}, H_{24}, H_{26}), 1.61-1.55 (4H, m, H_{10}, H_{12} × 2, H_{30}), 1.51-1.48 (2H, m, H_{28} × 2), 1.47 (3H, s, Me_{14}), 1.43-1.33 (4H, m, H_{11} × 2, H_{27} × 2), 1.31-1.20 (10H, m, H_{17-21}), [1.10] (1H, d, *J* = 6.7 Hz, Me_{32}*), 1.07 (2H, d, *J* = 6.7 Hz, Me_{32}), 1.00 (3H, d, *J* = 6.7 Hz, Me_{26}), 0.97 (3H, obs d, Me_8), 0.96 (9H, t, *J* = 8.3 Hz, $Si(CH_2C\underline{H}_3)_3$), 0.95 (9H, t, *J* = 8.3 Hz, $Si(CH_2C\underline{H}_3)_3$), 0.92-0.86 (9H, m, Me_{10}, Me_{24}, Me_{30}), 0.60 (6H, q, *J* = 8.0 Hz, $Si(C\underline{H}_2CH_3)_3$), 0.58 (6H, q, *J* = 8.0 Hz, $Si(C\underline{H}_2CH_3)_3$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C 167.3, 162.2, [160.8], 144.9, 135.2, 134.5, 133.1, 132.1, 131.9, 130.1, 129.9, 128.5, 125.0, [124.5], 122.3, 119.8, 114.5, [113.0], 90.5, 90.1, 88.0, [87.6], 87.5, 74.3, 73.6, 73.3, 71.8, 71.5, 61.9, [61.2], 55.4, [40.9], 40.4, 40.1, 39.3, 38.8, 38.1, 35.5, 33.7, [33.1], 31.9, 31.4, 30.7, 29.7, 29.6, 29.1, 28.9, 28.7, 28.3, 27.9, 27.7, 27.5, 26.6, 25.7, 24.6, 23.4, 22.7, 18.8, 17.7, 14.5, 14.1, [10.9], 10.8, 9.8, 9.7, 7.1, 7.0, 5.4, 5.2; $[\alpha]_D^{20}$ −15.1 (*c* 0.46, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1})

3445, 2933, 2876, 1698, 1657, 1461, 1240, 1225, 1006, 727, 669; **HRMS** calc. for $C_{58}H_{109}NO_9Si_2Na$ $[M+Na]^+$ 1042.7533, found 1042.7539.

Distinguishable resonances of the minor rotamer (2:1 ratio) are given in brackets and assignments denoted with an asterisk.

The configuration at C23 was confirmed by synthesising the diastereomeric Mosher esters for analysis:



To a solution of alcohol **27** (2 mg, 1.96 µmol) in CH_2Cl_2 (0.10 mL) was added DMAP (1 crystal), DMAP hydrochloride (1 crystal), DCC (7 µL, 1M in CH_2Cl_2, 9.82 µmol) and (*R*)-(-)-MTPA OH (2 mg, 9.82 µmol) and the mixture was stirred for 16 h. Once complete, the reaction was purified by flash column chromatography (1:4 EtOAc/CH_2Cl_2) to give (*R*)-Mosher ester *R*-MTPA-**27a** as a colourless oil (1.5 mg, 63%). The corresponding (*S*)-Mosher ester was synthesised from **27a** (1.8 mg, 1.77 µmol) to generate *S*-MTPA-**27a** as a colourless oil (1.6 mg, 72%).

***S*-MTPA-27a**

R_f 0.78 (1:3 EtOAc/CH_2Cl_2); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 8.27 (0.66H, s, NCHO), [8.05] (0.33H, s, NCHO*), 7.62-7.56 (2H, m, ArH), 7.43-7.37 (3H, m, ArH), 7.29 (1H, obs dd, H_3), [7.10] (0.33H, d, J = 14.5 Hz, H_{34*}), 6.41 (0.66H, d, J = 14.5 Hz, H_{34}), 6.24-6.14 (2H, m, H_4, H_5), 5.83 (1H, d, J = 15.5 Hz, H_2), 5.39 (1H, dd, J = 10.7, 2.4 Hz, H_{23}), 5.35 (1H, app q, J = 8.4 Hz, H_{29}), 5.24 (1H, t, J = 8.2 Hz, H_{15}), [5.10] (0.37H, dd, J = 14.3, 8.2 Hz, H_{33*}), 5.04 (0.6H, dd, J = 14.7, 8.2 Hz, H_{33}), 3.67-3.59 (1H, m, H_7), 3.57 (3H, s, OMe), 3.55-3.40 (1H, m, H_9), 3.45 (3H, s, OMe), 3.39 (3H, s, OMe), 3.31 (1H, dd, J = 8.4, 5.7 Hz, H_{13}), 3.15 (3H, s, OMe), [3.03] (1H, s, NMe*), 3.00 (2H, s, NMe), 2.75 (1H, dt, J = 8.9, 2.2 Hz, H_{25}), 2.64 (1H, td, J = 9.6, 1.7 Hz, H_{31}), 2.41-2.24 (3H, m, $H_6 \times 2$, H_{32}), 2.17-2.08 (2H, m, $H_{16} \times 2$), 1.90-1.72 (2H, m, H_8, H_{22a}), 1.65-1.55 (7H, m, H_{10}, $H_{12} \times 2$, H_{22b}, H_{24}, H_{26}, H_{30}), 1.51-1.44 (5H, m, $H_{28} \times 2$, Me_{14}), 1.43-1.35 (4H, m, $H_{11} \times 2$, $H_{27} \times 2$), 1.32-1.22 (10H, m, $H_{17\text{-}21}$,), 1.09 (3H, d, J = 6.8 Hz, Me_{32}), 1.00 (3H, d, J = 7.0 Hz, Me_{26}), 0.97 (3H, obs d, Me_8), 0.96 (9H, t, J = 8.2 Hz, $Si(CH_2C\underline{H}_3)_3$),

0.95 (9H, t, J = 8.2 Hz, Si($CH_2C\underline{H}_3$)$_3$), 0.89 (3H, d, J = 6.6 Hz, Me_{24}), 0.84 (3H, d, J = 6.6 Hz, Me_{10}), 0.76 (3H, d, J = 6.6 Hz, Me_{30}), 0.60 (6H, q, J = 8.2 Hz, Si($C\underline{H}_2CH_3$)$_3$), 0.58 (6H, q, J = 8.2 Hz, Si($C\underline{H}_2CH_3$)$_3$).

***R*-MTPA-27a**

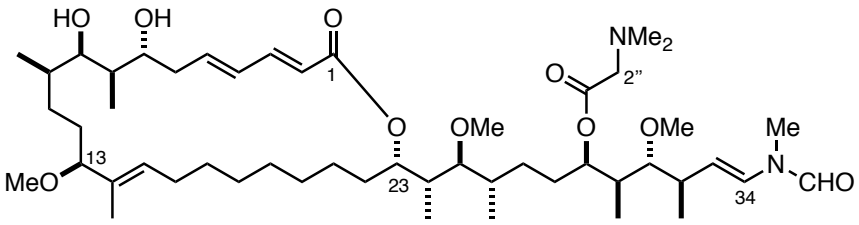
R_f 0.78 (1:3 EtOAc/CH_2Cl_2); **^{1}H NMR** (500 MHz, $CDCl_3$) $\boldsymbol{\delta}_H$ 8.27 (0.66H, s, NCHO), [8.05] (0.33H, s, NCHO*), 7.61-7.56 (2H, m, ArH), 7.44-7.37 (3H, m, ArH), 7.30 (1H, obs dd, H_3), [7.11] (0.33H, d, J = 14.7 Hz, H_{34*}), 6.43 (0.66H, d, J = 14.5 Hz, H_{34}), 6.24-6.14 (2H, m, H_4, H_5), 5.84 (1H, d, J = 15.1 Hz, H_2), 5.38 (1H, dd, J = 10.9, 2.1 Hz, H_{23}), 5.36 (1H, app q, J = 8.4 Hz, H_{29}), 5.24 (1H, t, J = 7.4 Hz, H_{15}), [5.13] (0.47H, dd, J = 15.2, 8.8 Hz, H_{33*}), 5.06 (1H, dd, J = 13.9, 7.9 Hz, H_{33}), 3.68-3.61 (1H, m, H_7), 3.56-3.51 (1H, m, H_9), 3.49 (3H, s, OMe), 3.47 (3H, s, OMe), 3.38 (3H, s, OMe), 3.31 (1H, dd, J = 8.2, 5.9 Hz, H_{13}), 3.15 (3H, s, OMe), [3.04] (1H, s, NMe*), 3.00 (2H, s, NMe), 2.74 (1H, dt, J = 8.8, 2.7 Hz, H_{25}), 2.65 (1H, td, J = 9.6, 1.7 Hz, H_{31}), 2.43-2.22 (3H, m, $H_6 \times 2$, H_{32}), 2.15-2.08 (2H, m, $H_{16} \times 2$), 1.91-1.71 (2H, m, H_8, H_{22a}), 1.65-1.55 (7H, m, H_{10}, $H_{12} \times 2$, H_{22b}, H_{24}, H_{26}, H_{30}), 1.54-1.43 (4H, m, $H_{28} \times 2$, Me_{14}), 1.42-1.36 (4H, m, $H_{11} \times 2$, $H_{27} \times 2$), 1.32-1.22 (10H, m, $H_{17\text{-}21}$,), 1.10 (3H, d, J = 6.8 Hz, Me_{32}), 0.99 (3H, d, J = 7.0 Hz, Me_{26}), 0.97 (3H, obs d, Me_8), 0.96 (9H, t, J = 8.0 Hz, Si($CH_2C\underline{H}_3$)$_3$), 0.95 (9H, t, J = 8.0 Hz, Si($CH_2C\underline{H}_3$)$_3$), 0.87 (3H, d, J = 6.6 Hz, Me_{24}), 0.84 (3H, d, J = 6.6 Hz, Me_{10}), 0.78 (3H, d, J = 6.6 Hz, Me_{30}), 0.60 (6H, q, J = 8.1 Hz, Si($C\underline{H}_2CH_3$)$_3$), 0.58 (6H, q, J = 8.1 Hz, Si($C\underline{H}_2CH_3$)$_3$).

Distinguishable resonances of the minor rotamer (2:1 ratio) are given in brackets and assignments denoted with an asterisk.

Table 2. Diagnostic ^{1}H NMR signals for the configurational assignment of 29R

Proton	**δH (*S*)-MTPA** (ppm)	**δH (*R*)-MTPA** (ppm)	$\Delta\delta_{S\text{-}R}$ (ppm)
H23	5.39	5.38	+0.01
Me24	0.89	0.87	+0.02
H25	2.75	2.74	+0.01
H29	5.35	5.36	–0.01
Me30	0.76	0.78	–0.02
H31	2.64	2.65	–0.01
Me32	1.09	1.10	–0.01

Diol 27



DMAP (11 mg, 8.8 μmol), DMAP hydrochloride (14 mg, 8.8 μmol), dimethylglycine (9 mg, 8.8 μmmol) and alcohol **27a** (9 mg, 8.8 μmmol) were dissolved in CH_2Cl_2 (0.50 mL). DCC (88 μL, 8.8 μmol, 1M in CH_2Cl_2) was added and the reaction stirred for 16 h before being quenched with $NaHCO_3$ (2 mL). The layers were separated, and the aqueous phase extracted with CH_2Cl_2 (3 × 5 mL). The combined organics were dried (Na_2SO_4), concentrated *in vacuo* and the crude material transferred to a plastic reaction vessel.

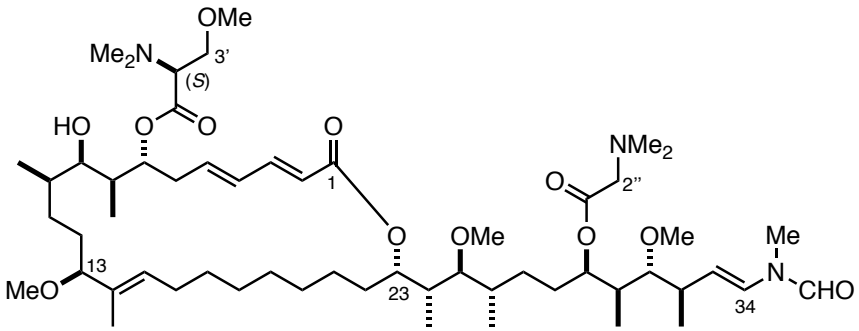
A stock solution of HF·pyridine was prepared by adding HF·pyridine (100 μL) to a solution of pyridine (200 μL) in THF (1 mL) at 0 °C before warming to rt and stirring for 30 min. This solution was added the crude material 0 °C and the reaction stirred at rt overnight. The reaction was quenched at 0 °C with $NaHCO_3$ solution (5 mL), stirred vigorously at rt for 30 min and the aqueous phase extracted with EtOAc (3 × 5 mL). The combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. Purification *via* flash column chromatography (1:1:0 → 4:4:1 EtOAc/CH_2Cl_2/MeOH) yielded diol **27** (5.3 mg, 69%) as a colourless oil.

R_f 0.45 (4:4:1 EtOAc/CH_2Cl_2/MeOH); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 8.28 (0.66H, s, NCHO), [8.06] (0.33H, s, NCHO*), 7.27 (1H, obs dd, H_3), [7.12] (0.33H, d, J = 14.5 Hz, H_{34*}), 6.45 (0.66H, d, J = 14.5 Hz, H_{34}), 6.28 (1H, dd, J = 15.3, 11.0 Hz, H_4), 6.12 (1H, dt, J = 15.2, 7.8 Hz, H_5), 5.84 (1H, d, J = 15.7 Hz, H_2), 5.35-5.27 (2H, m, H_{23}, H_{29}), 5.24 (1H, t, J = 6.9 Hz, H_{15}), 5.11 (1H, dd, J = 14.5, 10.0 Hz, H_{33}), 3.88-3.80 (1H, m, H_7), 3.70-3.59 (1H, m, H_9), 3.53-3.46 (2H, m, $H_{2''}$ × 2), 3.44 (3H, s, OMe), 3.43 (3H, s, OMe), 3.41 (1H, obs dd, H_{13}), 3.15 (3H, s, OMe), [3.05] (1H, s, NMe*), 3.00 (2H, s, NMe), 2.81 (1H, dd, J = 9.8, 2.1 Hz, H_{25}), 2.75 (1H, dd, J = 8.4, 2.4 Hz, H_{31}), 2.59-2.42 (3H, m, H_6 × 2, H_{32}), 2.39 (6H, s, $C_{2''}NMe_2$), 2.10-1.99 (2H, m, H_{16} × 2), 1.73-1.65 (6H, m, H_8, H_{22a}, H_{24}, H_{26}, H_{28} × 2), 1.64-1.51 (5H, m, H_{10}, H_{12} × 2, H_{22b}, H_{30}), 1.38-1.31 (4H, m, H_{11} × 2, H_{27} × 2), 1.48 (3H, s, Me_{14}), 1.30-1.19 (10H, m, H_{17-21},), 1.16 (3H, d, J = 6.6 Hz, Me_{32}), 1.04 (3H, d, J = 6.6 Hz, Me_8), 0.97 (3H, d, J = 7.4 Hz, Me_{26}), 0.88-0.82 (6H, m, Me_{10}, Me_{24}), 0.79 (3H, d, J = 7.4 Hz, Me_{30}); **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C 170.2, 167.0, 162.2, [160.9], 156.7, 144.3, 139.0, 133.7, 130.9, 129.9, 128.8, 128.6, [124.6], 120.5, [113.4], 111.7, 87.9, 87.3, 86.6, 75.3, 74.0, 73.9, 61.3, [61.1], 60.3, 55.6, 49.1, 45.2, 40.6, [39.3], 39.1, 39.0, 38.1, [37.8], 37.0, 36.9, 35.2, 33.9, 33.6, [33.1], 30.9, 29.7, 28.8,

28.7, 28.5, 27.8, 27.6, 27.3, 24.9, 20.3, [17.4], 17.3, 15.7, [11.6], 11.1, 10.3, 9.8, 9.7; $[\alpha]_D^{20}$ −13.9 (*c* 0.16, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 3325, 2952, 2853, 1708, 1690, 1656, 1635, 1559, 1448, 1254, 1159, 1091 975; **HRMS** calc. for $C_{50}H_{88}N_2O_{10}Na$ $[M+Na]^+$ 899.6337, found 899.6324.

Distinguishable resonances of the minor rotamer (2:1 ratio) are given in brackets and assignments denoted with an asterisk.

Aplyrologue D (7)



(*S*)-Trimethyl serine (6.7 mg, 46.1 μmol) and DMAP (5.6 mg, 46.1 μmol) were dissolved in CH_2Cl_2 (3 mL) and TCBC (15 μL, 92.3 μmol) and NEt_3 (13 μL, 92.3 μmol) were added to form a stock solution of mixed anhydride. Diol **27** (2 mg, 46.1 μmol) was dissolved in benzene (0.30 mL) and an aliquot of the stock solution (0.40 mL) was added at 0 °C. Further aliquots of stock solution (0.40 mL) were added every 30 min for 2 h. The reaction was then quenched with MeOH (1.5 mL) and concentrated *in vacuo*. The crude residue was purified by flash column chromatography (1:0:0 → 1:1:0 → 4:4:1 CH_2Cl_2/EtOAc/MeOH) to give aplyrologue D (**7**) as a colourless oil (1.4 mg, 65%).

$\mathbf{R_f}$ 0.31 (4:4:1 EtOAc/CH_2Cl_2/MeOH); **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 8.28 (0.66H, s, NCHO), [8.06] (0.33H, s, NCHO*), 7.24 (1H, obs dd, H_3), [7.12] (0.33H, d, *J* = 14.4 Hz, H_{34*}), 6.46 (0.66H, d, *J* = 14.2 Hz, H_{34}), 6.28 (1H, dd, *J* = 14.8, 11.1 Hz, H_4), 6.03 (1H, dt, *J* = 15.2, 7.8 Hz, H_5), 5.86 (1H, d, *J* = 15.4 Hz, H_2), 5.34 (1H, dt, *J* = 9.5, 2.4 Hz, H_{15}), 5.31-5.25 (2H, m, H_{23}, H_{29}), 5.08 (1H, dd, *J* = 14.6, 9.4 Hz, H_{33}), 5.01-4.95 (1H, m, H_7), 3.82-3.68 (2H, m, $H_{3'}$ × 2, $H_{2''}$ × 2), 3.60-3.55 (1H, m, H_9), 3.44 (3H, s, OMe), 3.43 (3H, s, OMe), 3.37 (3H, s, OMe) 3.42-3.38 (1H, m, $H_{2'}$), 3.35 (1H, br d, *J* = 6.4 Hz, H_{13}), 3.15 (3H, s, OMe), [3.05] (1H, s, NMe*), 3.00 (2H, s, NMe), 2.81 (1H, dd, *J* = 9.1, 2.5 Hz, H_{25}), 2.76 (1H, dd, *J* = 8.4, 2.7 Hz, H_{31}), 2.58 (6H, s, $C_{2'}$ NMe_2), 2.52 (7H, s, $C_{2''}NMe_2$, H_{32}), 2.50-2.41 (2H, m, H_6 × 2), 1.96-1.86 (2H, m, H_{16} × 2), 1.72-1.66 (4H, m, H_8, H_{22a}, H_{24}, H_{26},), 1.64-1.53 (7H, m, H_{10}, H_{12} × 2, H_{22b}, H_{28} × 2, H_{30}), 1.38-1.29 (4H, m, H_{11} × 2, H_{27} × 2), 1.50 (3H, s, Me_{14}), 1.28-1.19 (10H, m, H_{17}-

$_{21}$,), 1.15 (3H, d, *J* = 6.6 Hz, Me_{32}), 0.98 (6H, d, *J* = 7.4 Hz, Me_8, Me_{26}), 0.88 (3H, d, *J* = 6.8 Hz, Me_{10}), 0.86 (3H, d, *J* = 6.8 Hz, Me_{24}), 0.83 (3H, d, *J* = 7.0 Hz, Me_{30}); **^{13}C NMR** (125 MHz, $CDCl_3$) $\boldsymbol{\delta}_C$ 169.1, [168.5], 166.9, 162.2, 160.9, 143.9, 137.7, 135.2, 133.8, 131.4, 129.9, 128.7, 127.6, 125.0, [124.7], 121.1, [113.6], 111.7, 87.7, [87.3], 86.6, 74.1, 73.7, 70.3, 67.0, 61.9, 61.3, [61.3], 59.2, 55.6, 49.2, 45.2, 42.2, 40.8, 39.2, 39.0, 38.1, 37.8, 36.5, 35.2, 34.3, 33.9, 33.7, [33.1], 30.4, 29.7, 29.4, 29.0, 28.4, 28.1, 27.8, 27.6, 27.5, 26.4, 25.9, 23.4, 22.7, 20.1, 17.6, [17.5], 15.5, 14.1, [11.6], 11.1, 10.2, 9.9, 9.8; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ –4.5 (*c* 0.056, $CHCl_3$); **IR** (thin film) ν_{max} (cm^{-1}) 3456, 2940, 2925, 2912, 1714, 1692, 1649, 1613, 1546, 1454, 1368, 1258, 1218, 1129, 1094, 852, 820, 780, 731; **HRMS** calc. for $C_{55}H_{98}N_3O_{11}$ $[M+H]^+$ 992.7151 found 992.7126.

Distinguishable resonances of the minor rotamer (2:1 ratio) are given in brackets and assignments denoted with an asterisk.

4. Experimental Procedures for Cell Viability Studies

4.1. Cell viability studies

HeLa cells were obtained from the American Type Culture Collection (ATCC) and were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated FBS and 2 mM L-glutamine.

Cells were seeded at 2,000 cells/well in 96-well plates for 24 h at 37 °C with 5% CO_2. Serial dilutions of aplyronines (**1-3**) and their analogues (**7-9**) were added to the cells in complete growth medium and incubated at 37 °C with 5% CO_2 for 96 h. Cell viability was measured using CellTiter-Glo viability assay (Promega) according to the manufacturer's instructions. Cell viability was plotted as a percentage of untreated cells. Each measurement was taken in triplicate and three independent repeats were performed from different cell passages. Discodermolide was used as an external control between all tests.

4.2. Cytotoxicity graphs

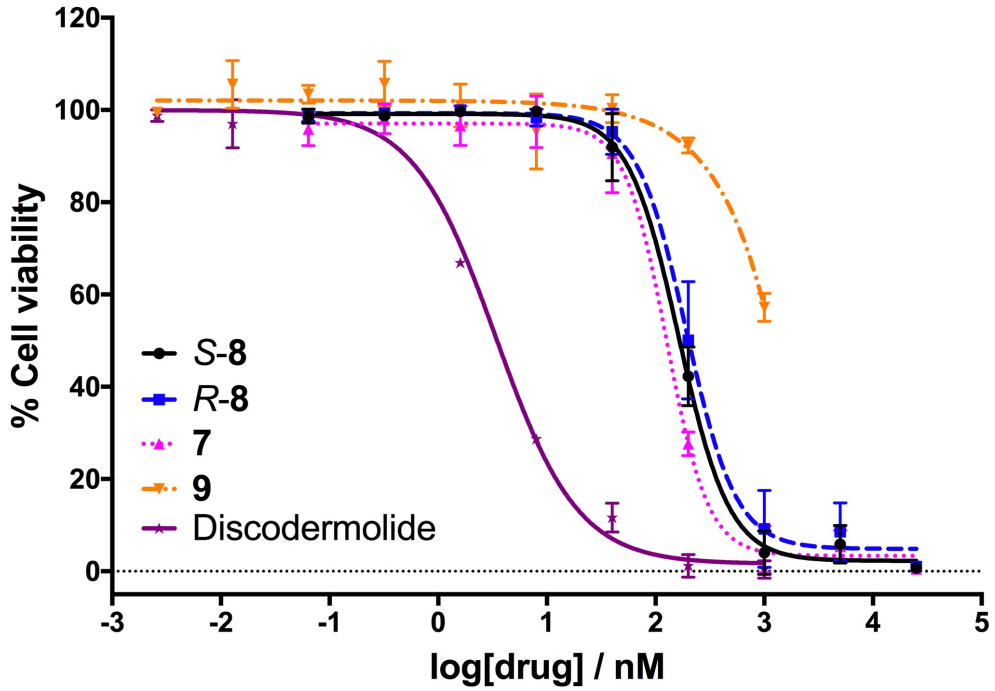


Figure 4. *Graph showing cell viability as a function of log[drug] for* ***7*** *(aplyrologue D), S-/R-****8*** *(aplyronine A,D/scytophycin hybrid) and* ***9*** *(aplyronine C/scytophcin hybrid). Discodermolide was included as an external control*

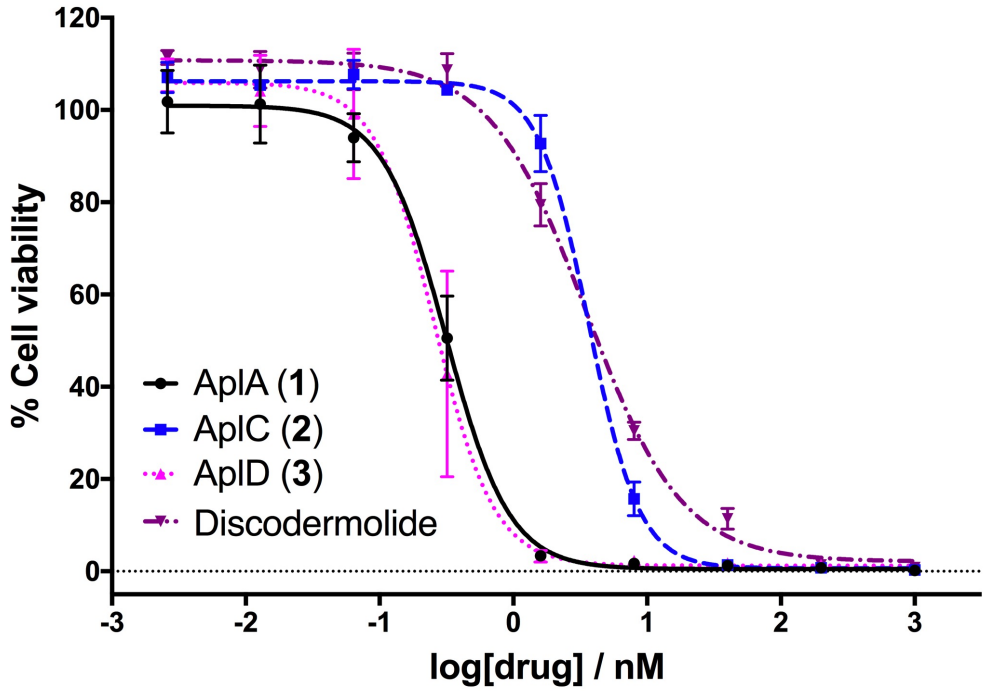


Figure 5. *Graph showing cell viability as a function of log[drug] for synthetic aplyronines A (****1****), C (****2****) and D (****3****)*[8,9]*. Discodermolide was included as an external control*

References

1 H. Kigoshi, K. Suenaga, T. Mutou, T. Ishigaki, T. Atsumi, H. Ishiwata, A. Sakakura, T. Ogawa, M. Ojika and K. Yamada, *J. Org. Chem.*, 1996, **61**, 5326–5351.

2 K. Kobayashi, Y. Fujii, Y. Hirayama, S. Kobayashi, I. Hayakawa and H. Kigoshi, *Org. Lett.*, 2012, **14**, 1290–1293.

3 K. Hirata, S. Muraoka, K. Suenaga, T. Kuroda, K. Kato, H. Tanaka, M. Yamamoto, M. Takata, K. Yamada and H. Kigoshi, *J. Mol. Biol.*, 2006, **356**, 945–954.

4 M. Kita, Y. Hirayama, K. Yoneda, K. Yamagishi, T. Chinen, T. Usui, E. Sumiya, M. Uesugi and H. Kigoshi, *J. Am. Chem. Soc.*, 2013, **135**, 18089–18095.

5 H. Kigoshi, K. Suenaga, M. Takagi, A. Akao, K. Kanematsu, N. Kamei, Y. Okugawa and K. Yamada, *Tetrahedron*, 2002, **58**, 1075–1102.

6 L. Diab, T. Šmejkal, J. Geier and B. Breit, *Angew. Chem. Int. Ed.*, 2009, **48**, 8022–8026.

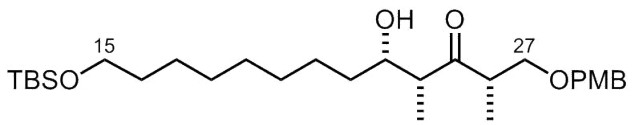
7 M. J. Cryle, P. Y. Hayes and J. J. De Voss, *Chem. Eur. J.*, 2012, **18**, 15994–15999.

8 I. Paterson, S. J. Fink, L. Y. W. Lee, S. J. Atkinson and S. B. Blakey, *Org. Lett.*, 2013, **15**, 3118–3121.

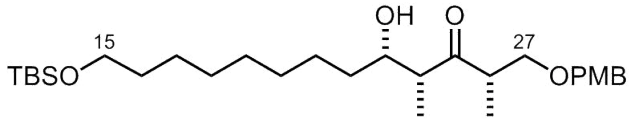
9 N. Anžiček, S. Williams, M. P. Housden and I. Paterson, *Org. Biomol. Chem.*, 2018, **16**, 1343–1350.

NMR Spectra for All New Compounds

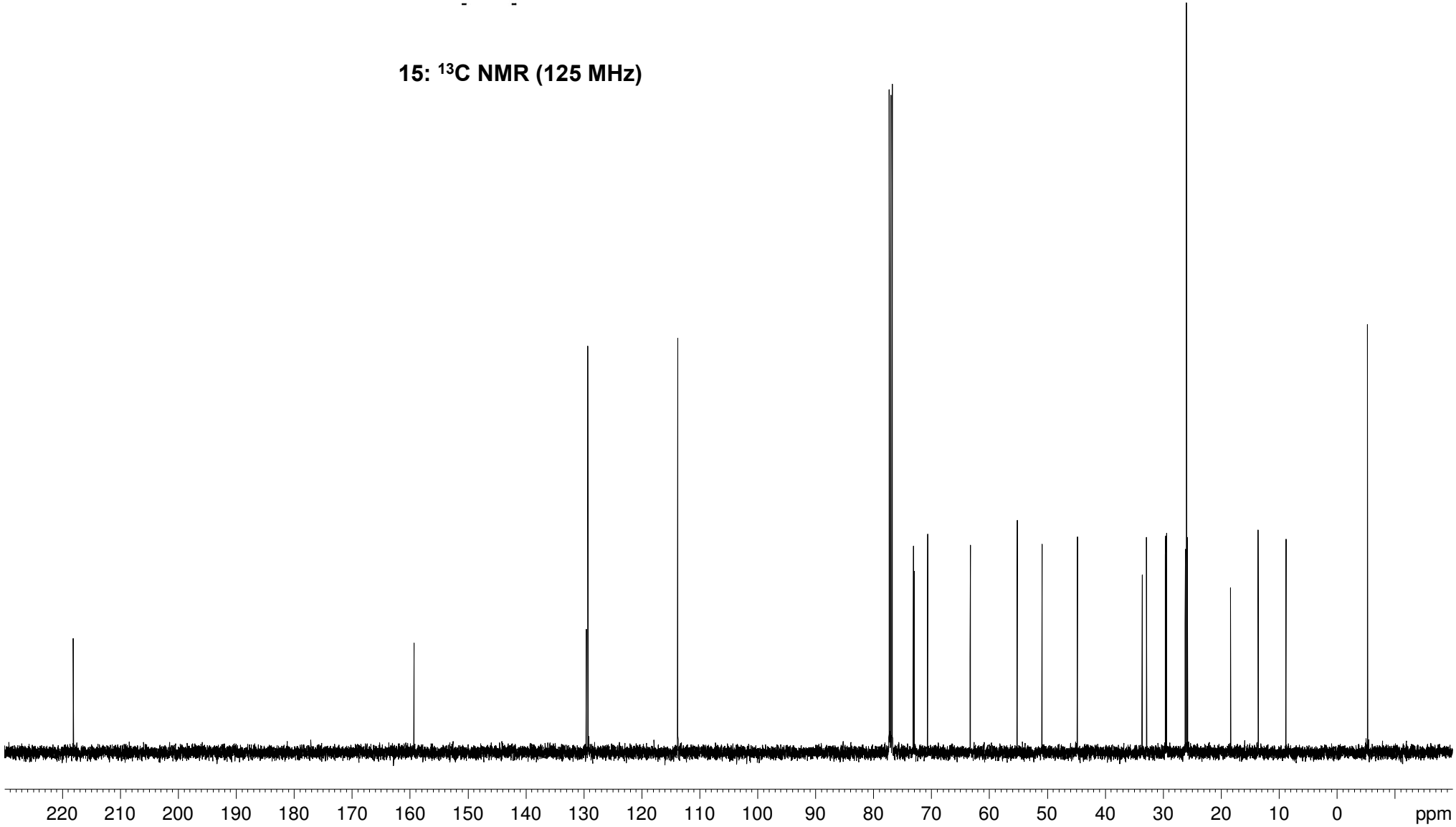
^{1}H and ^{13}C NMR spectra of all new compounds are presented below.

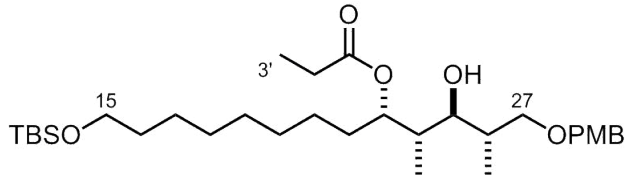


15: ^{1}H NMR (500 MHz)

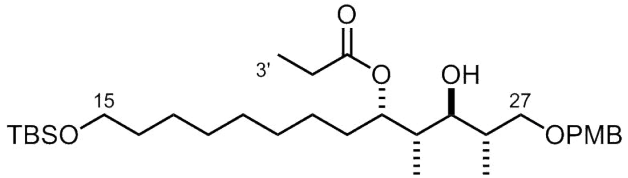


15: ^{13}C NMR (125 MHz)

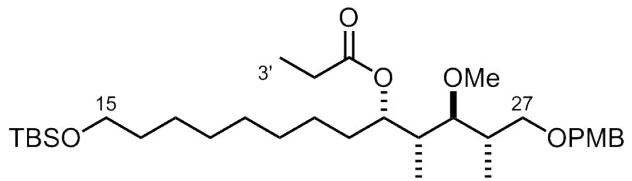




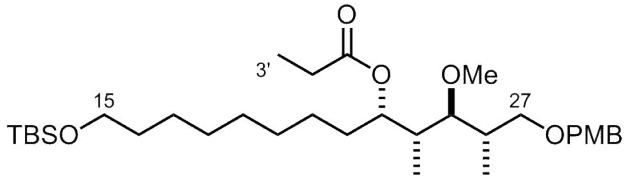
16: ^{1}H NMR (500 MHz)



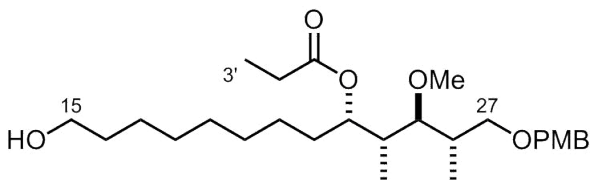
16: ^{13}C NMR (125 MHz)



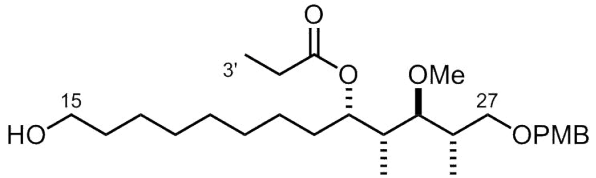
16a: ^{1}H NMR (500 MHz)



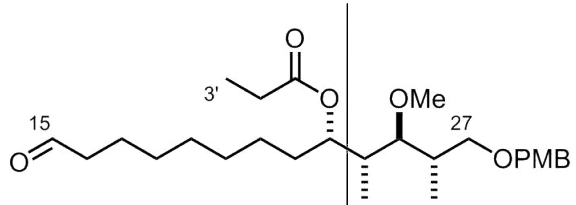
16a: ^{13}C NMR (125 MHz)



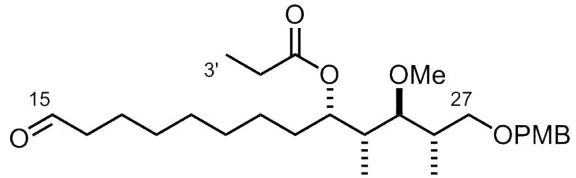
16b: ^{1}H NMR (500 MHz)



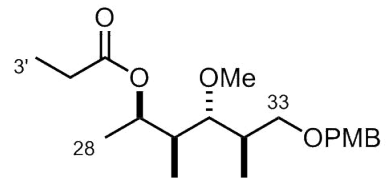
16b: ^{13}C NMR (125 MHz)



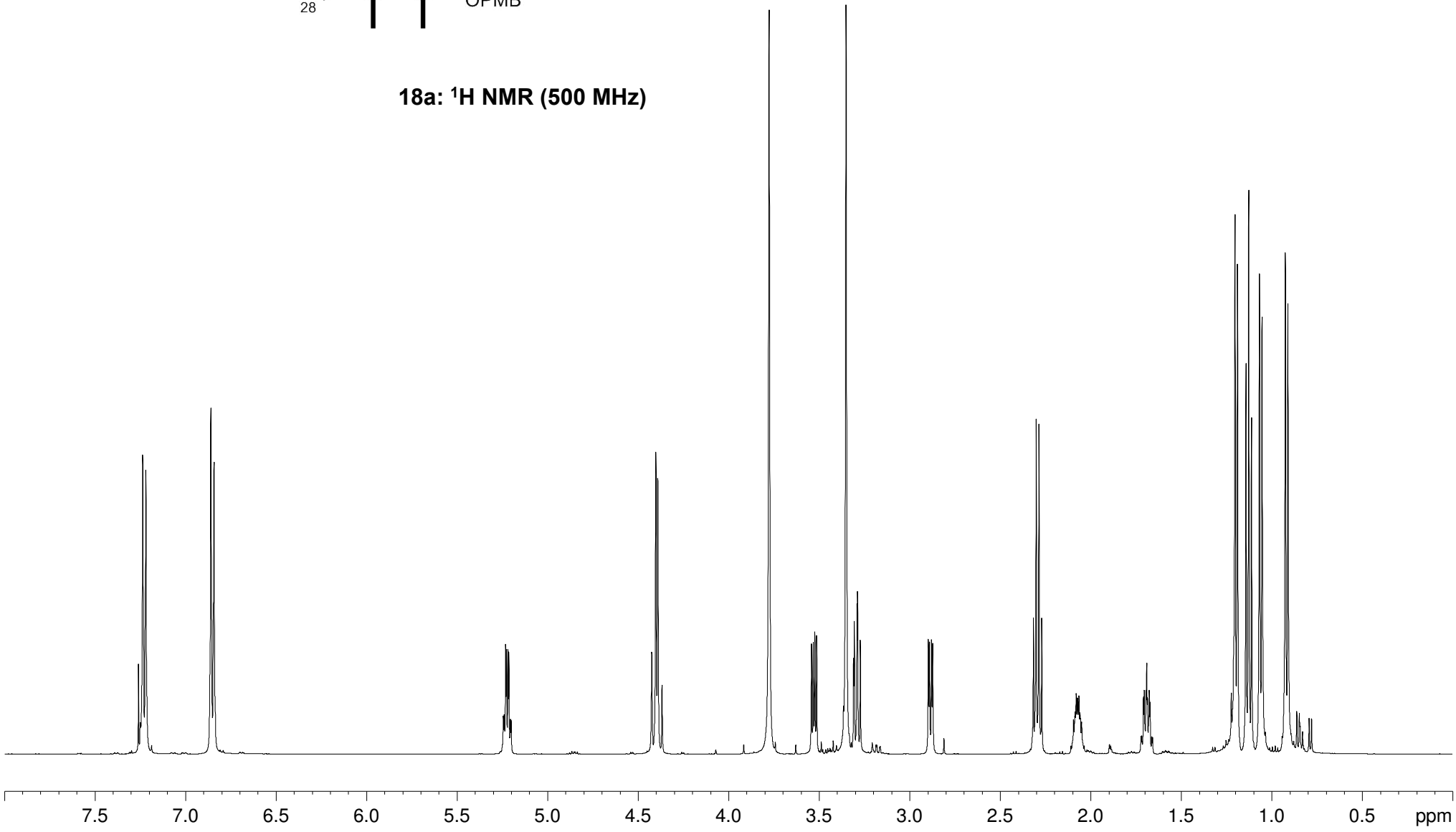
12: ^{1}H NMR (500 MHz)

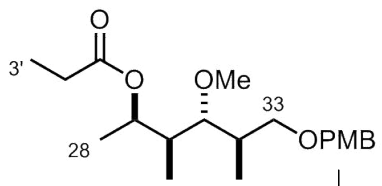


12: ^{13}C NMR (125 MHz)

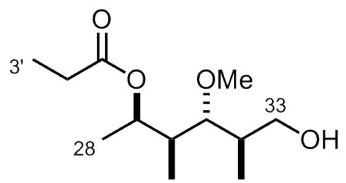


18a: ^{1}H NMR (500 MHz)

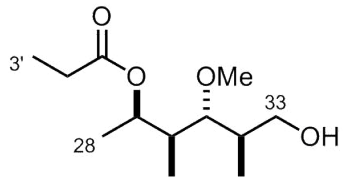




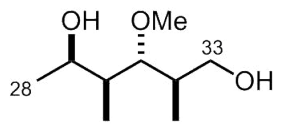
18a: ^{13}C NMR (125 MHz)



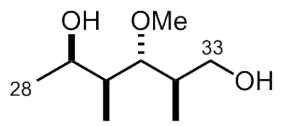
18b: ^{1}H NMR (500 MHz)



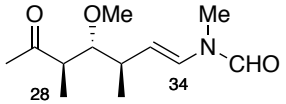
18b: ^{13}C NMR (125 MHz)



19: ^{1}H NMR (500 MHz)

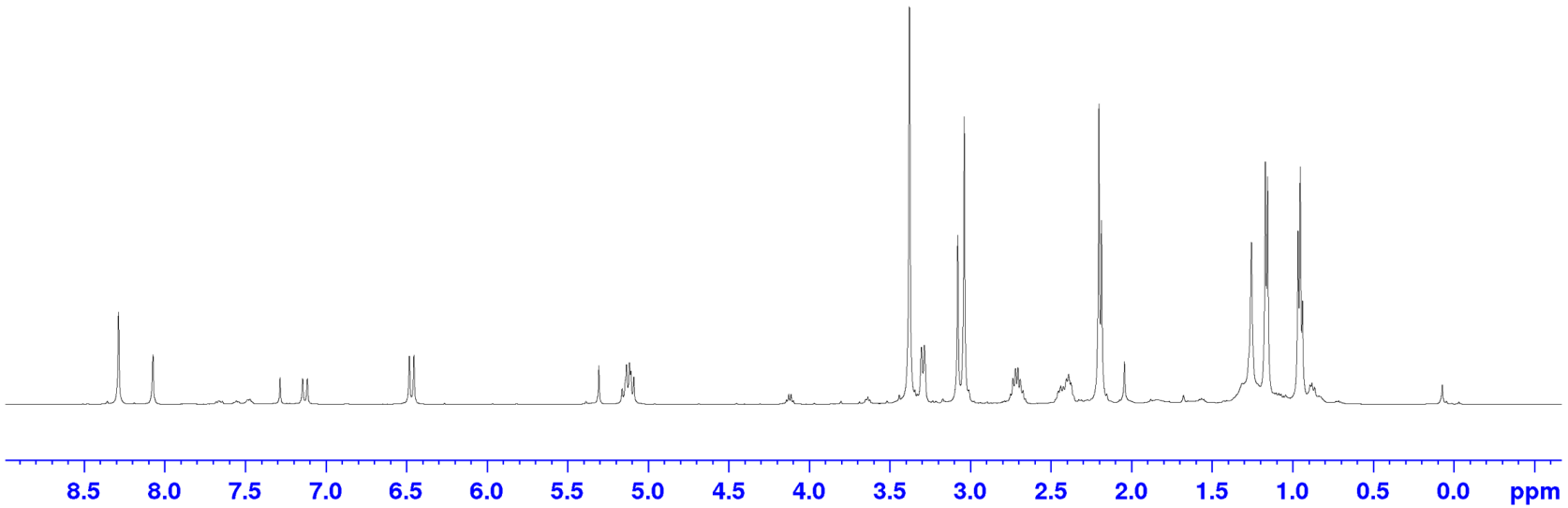


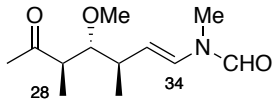
19: ^{13}C NMR (125 MHz)



***E*-11**

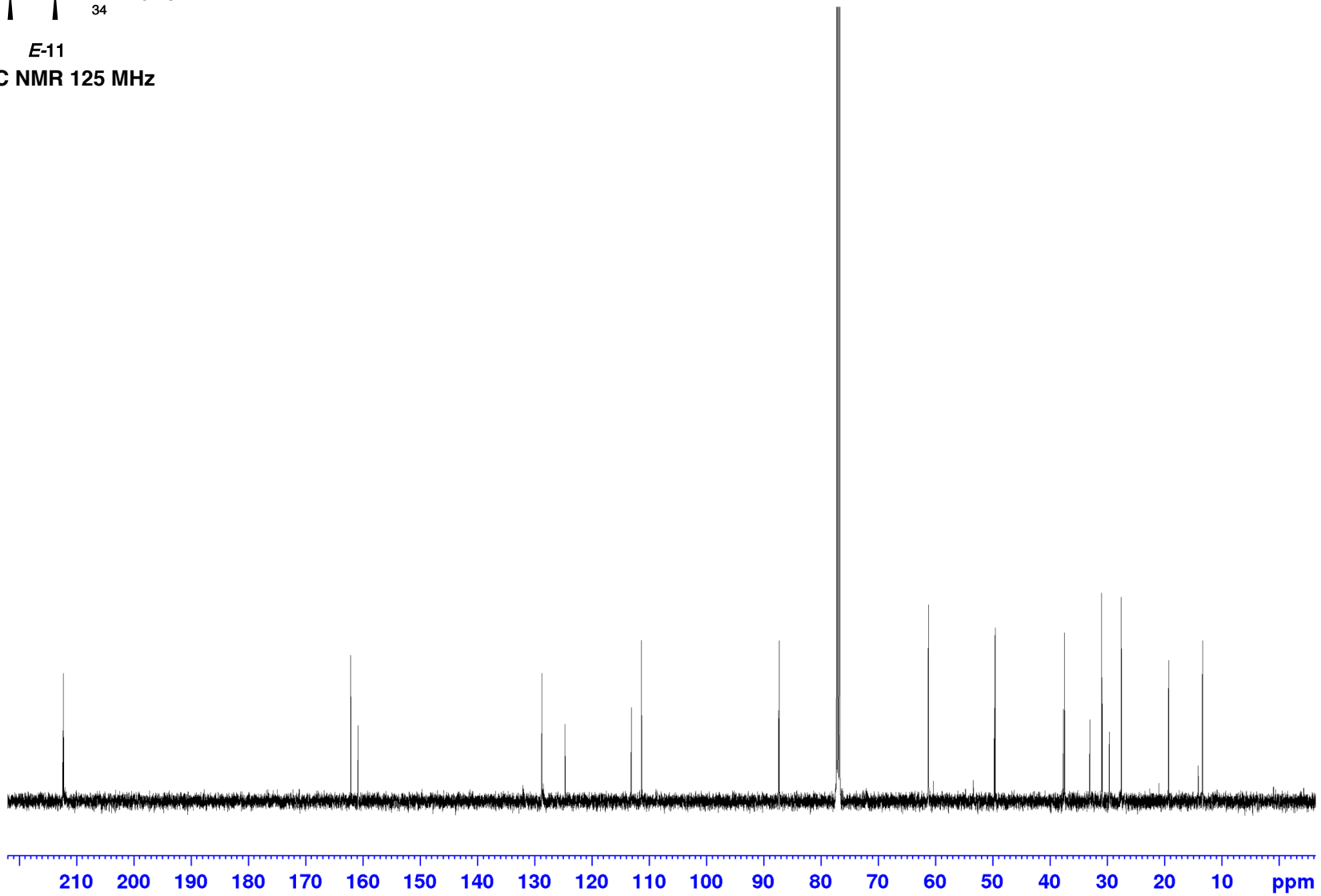
^{1}H NMR 500 MHz

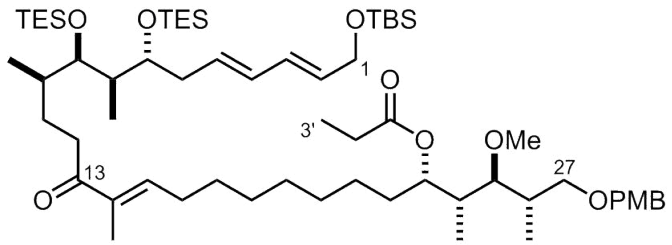




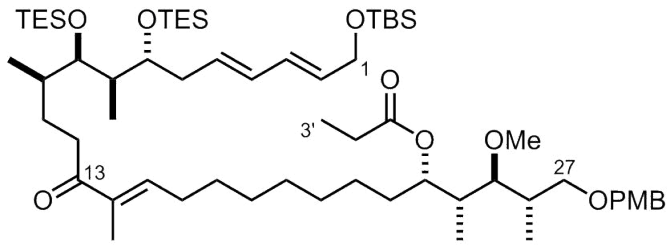
***E*-11**

^{13}C NMR 125 MHz

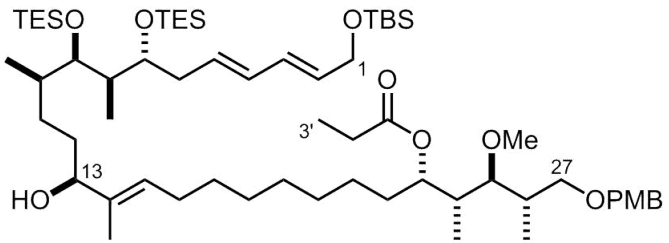




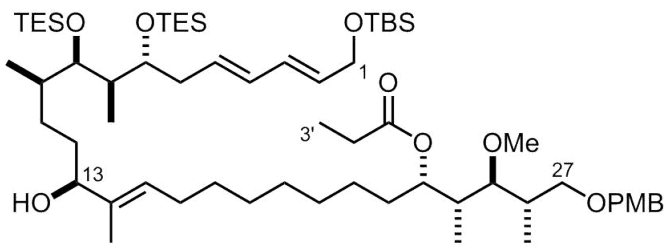
22: ^{1}H NMR (500 MHz)



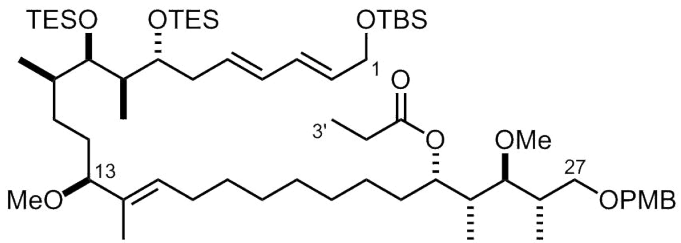
22: ^{13}C NMR (125 MHz)



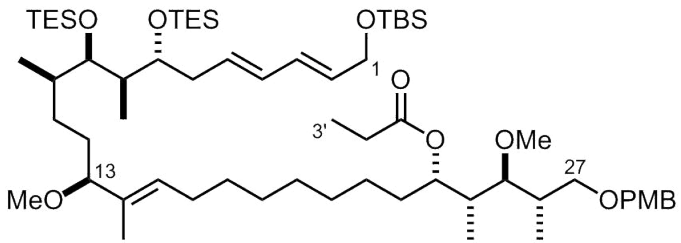
22a: ^{1}H NMR (500 MHz)



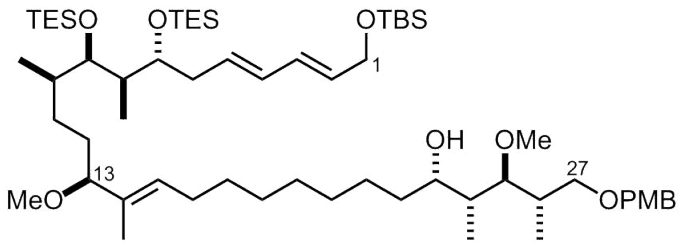
22a: ^{13}C NMR (125 MHz)



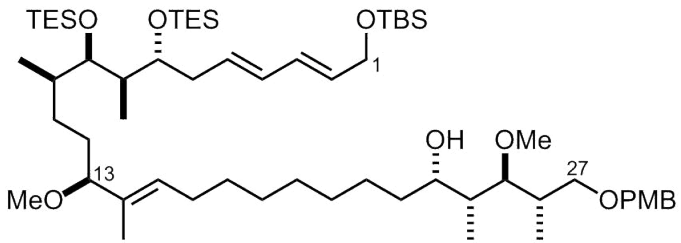
23: ^{1}H NMR (500 MHz)



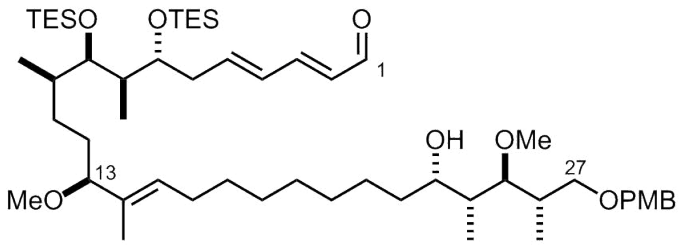
23: ^{13}C NMR (500 MHz)



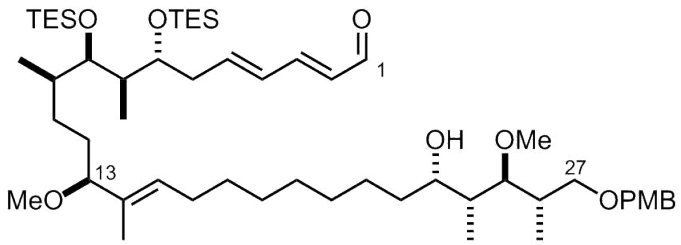
23a: ^{1}H NMR (500 MHz)



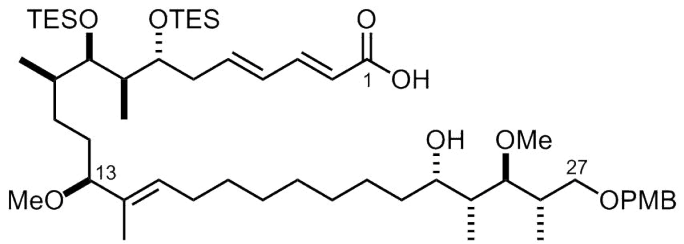
23a: ^{13}C NMR (125 MHz)



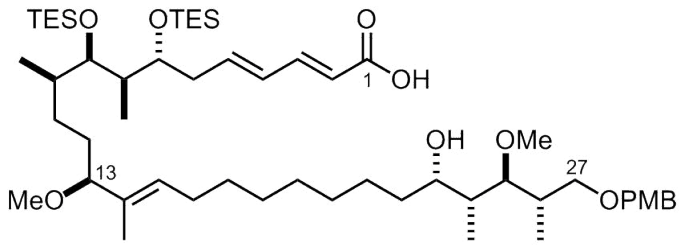
23b: ^{1}H NMR (500 MHz)



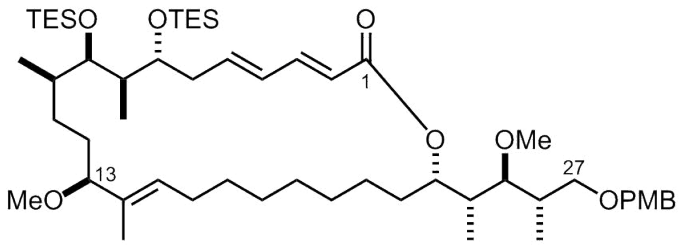
23b: ^{13}C NMR (125 MHz)



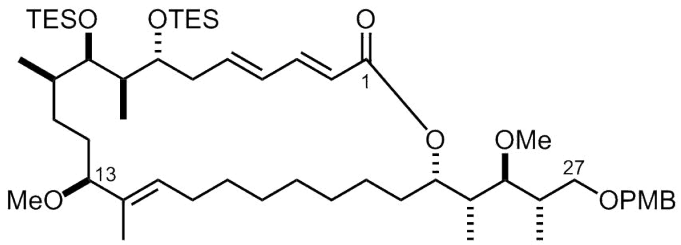
24: ^{1}H NMR (500 MHz)



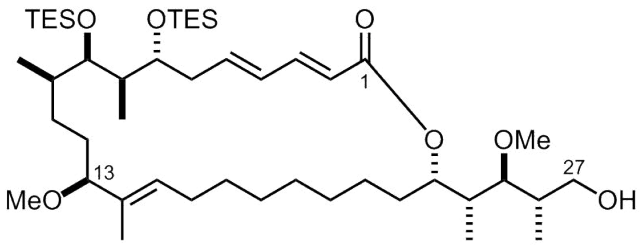
24: ^{13}C NMR (125 MHz)



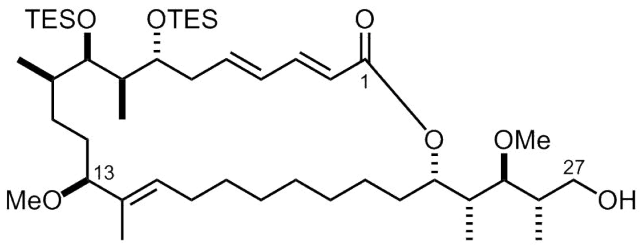
25: ^{1}H NMR (500 MHz)



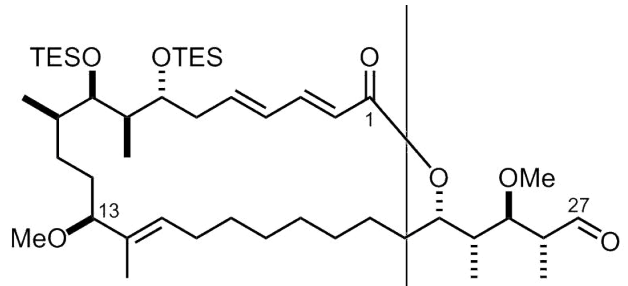
25: ^{13}C NMR (125 MHz)



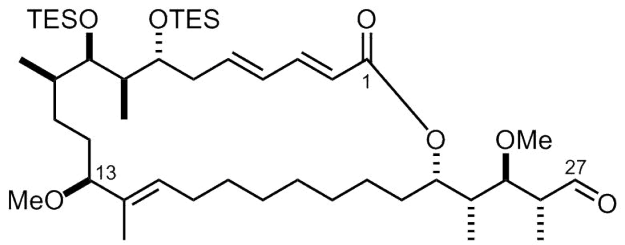
25a: ^{1}H NMR (500 MHz)



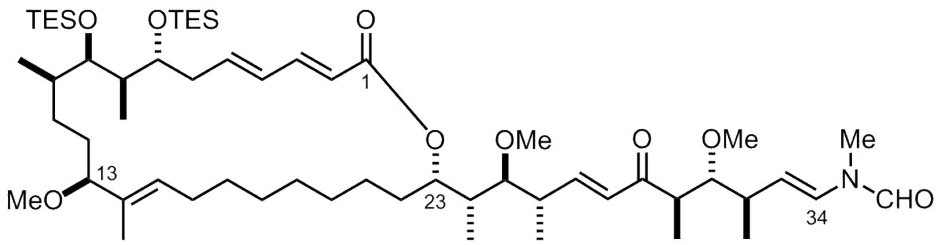
25a: ^{13}C NMR (125 MHz)



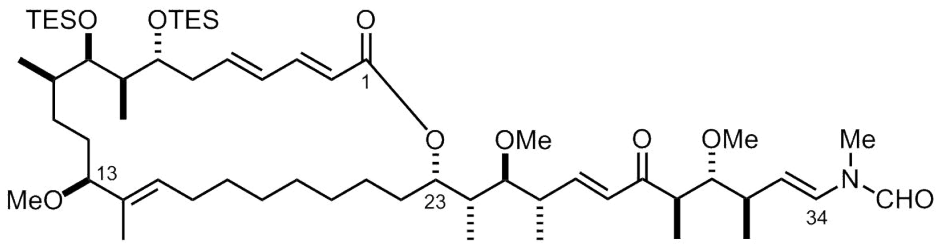
26: ^{1}H NMR (500 MHz)



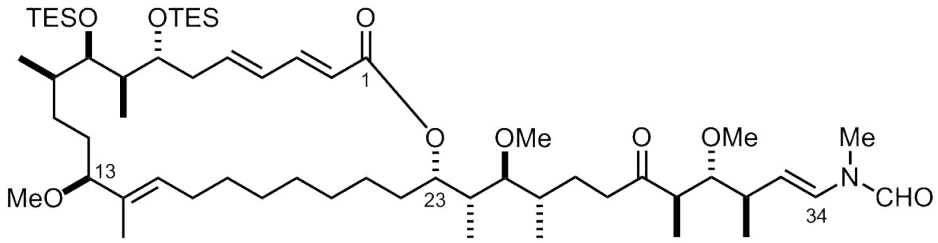
26: ^{13}C NMR (125 MHz)



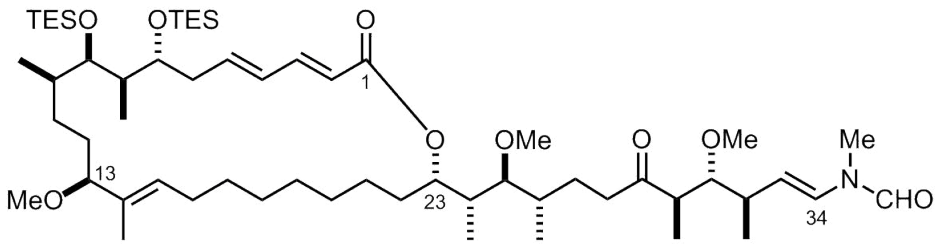
26a: ^{1}H NMR (500 MHz)



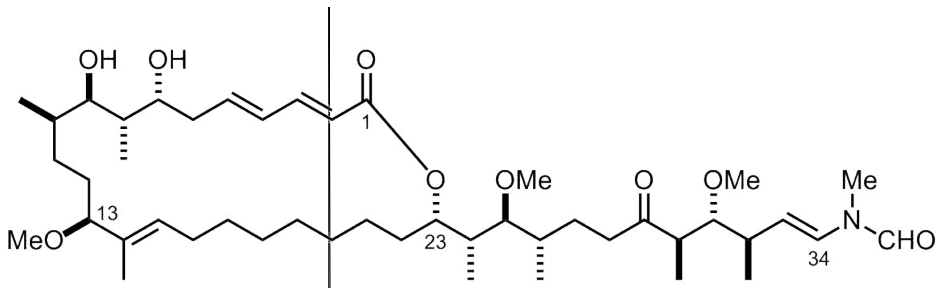
26a: ^{13}C NMR (125 MHz)



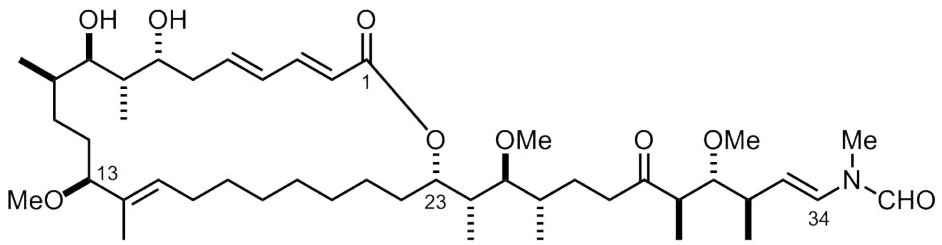
10: ^{1}H NMR (500 MHz)



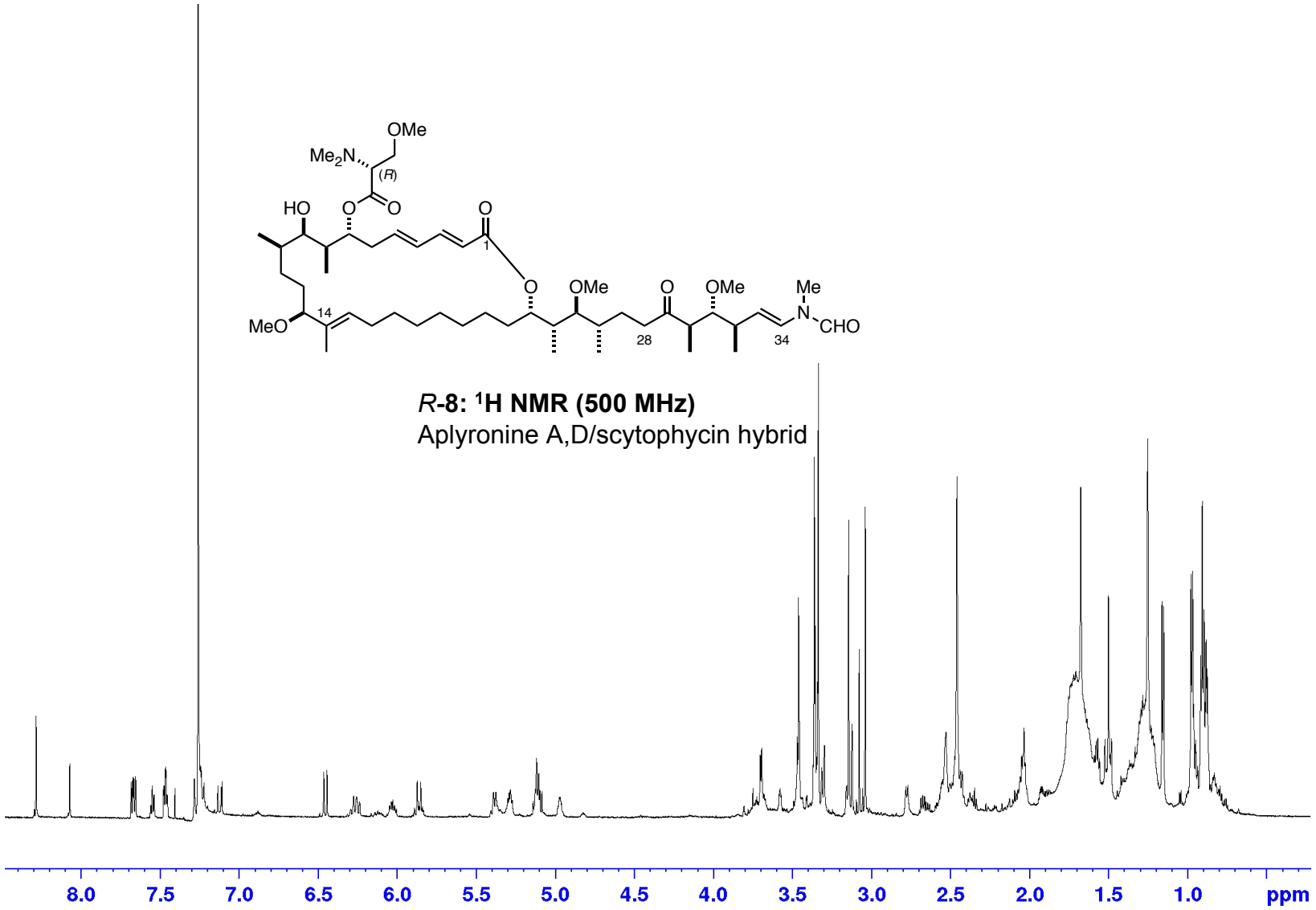
10: ^{13}C NMR (125 MHz)



9: ^{1}H NMR (500 MHz)
Aplyronine C/
scytophycin hybrid

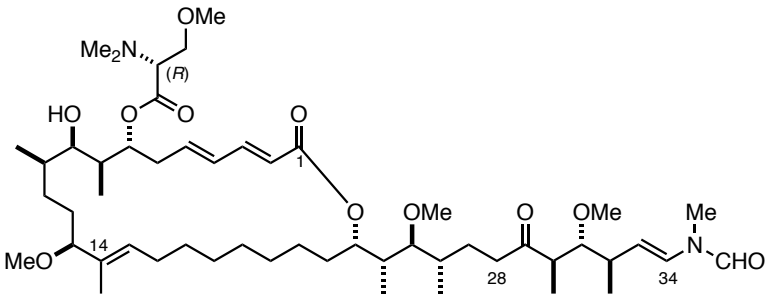


9: ^{13}C NMR (125 MHz)
Aplyronine C/scytophycin hybrid



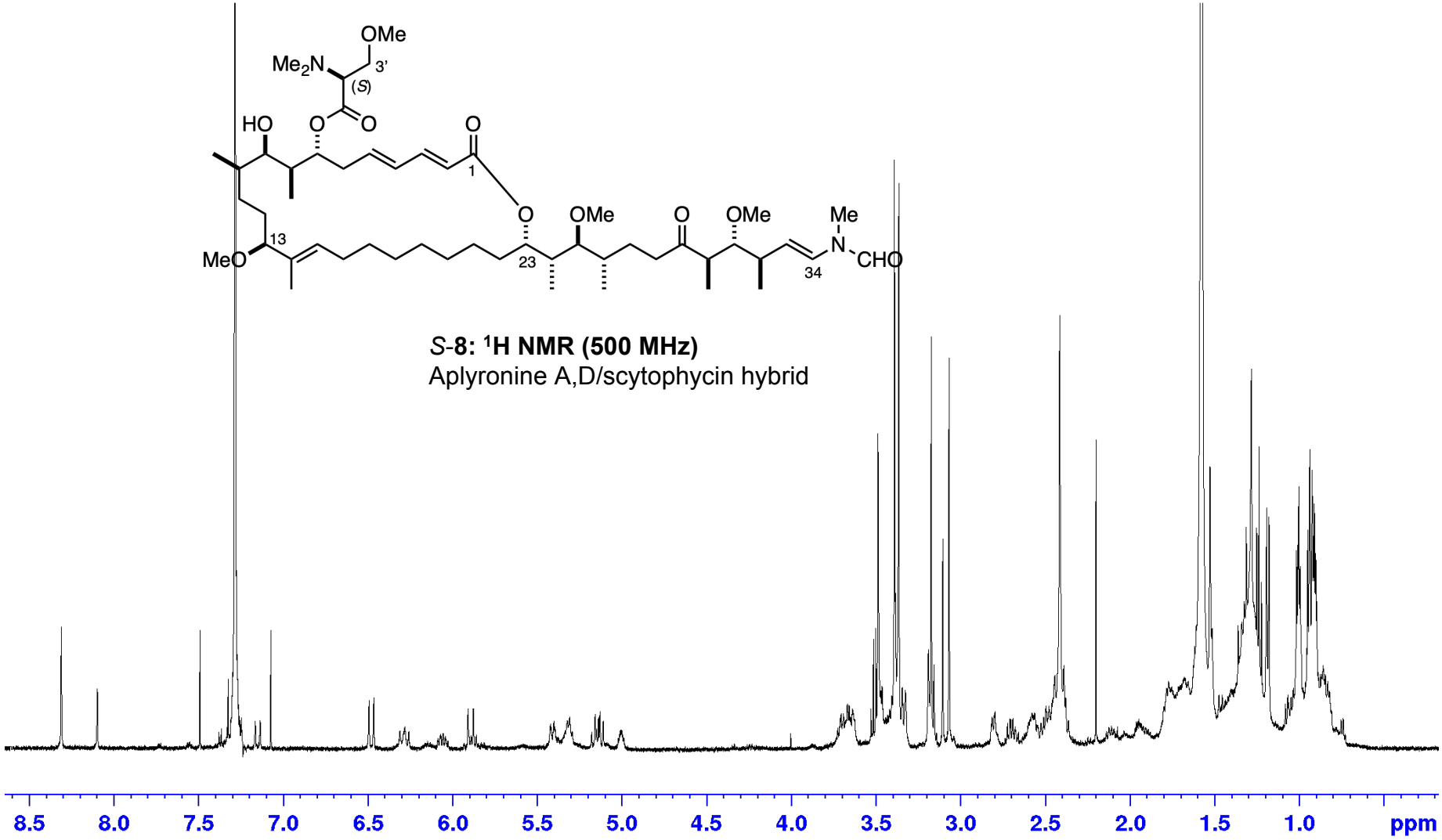
***R*-8: ^{1}H NMR (500 MHz)**

Aplyronine A,D/scytophycin hybrid

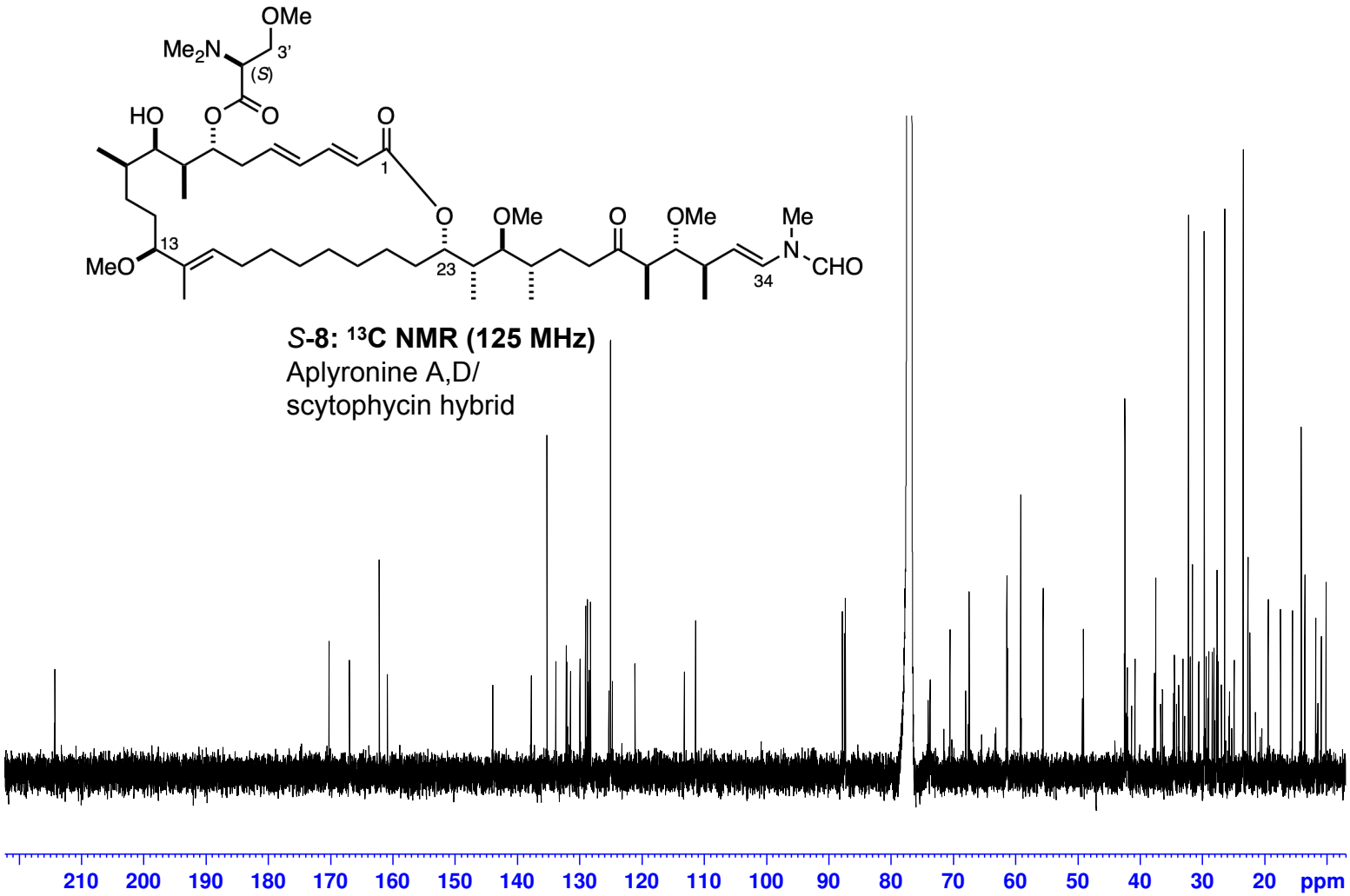


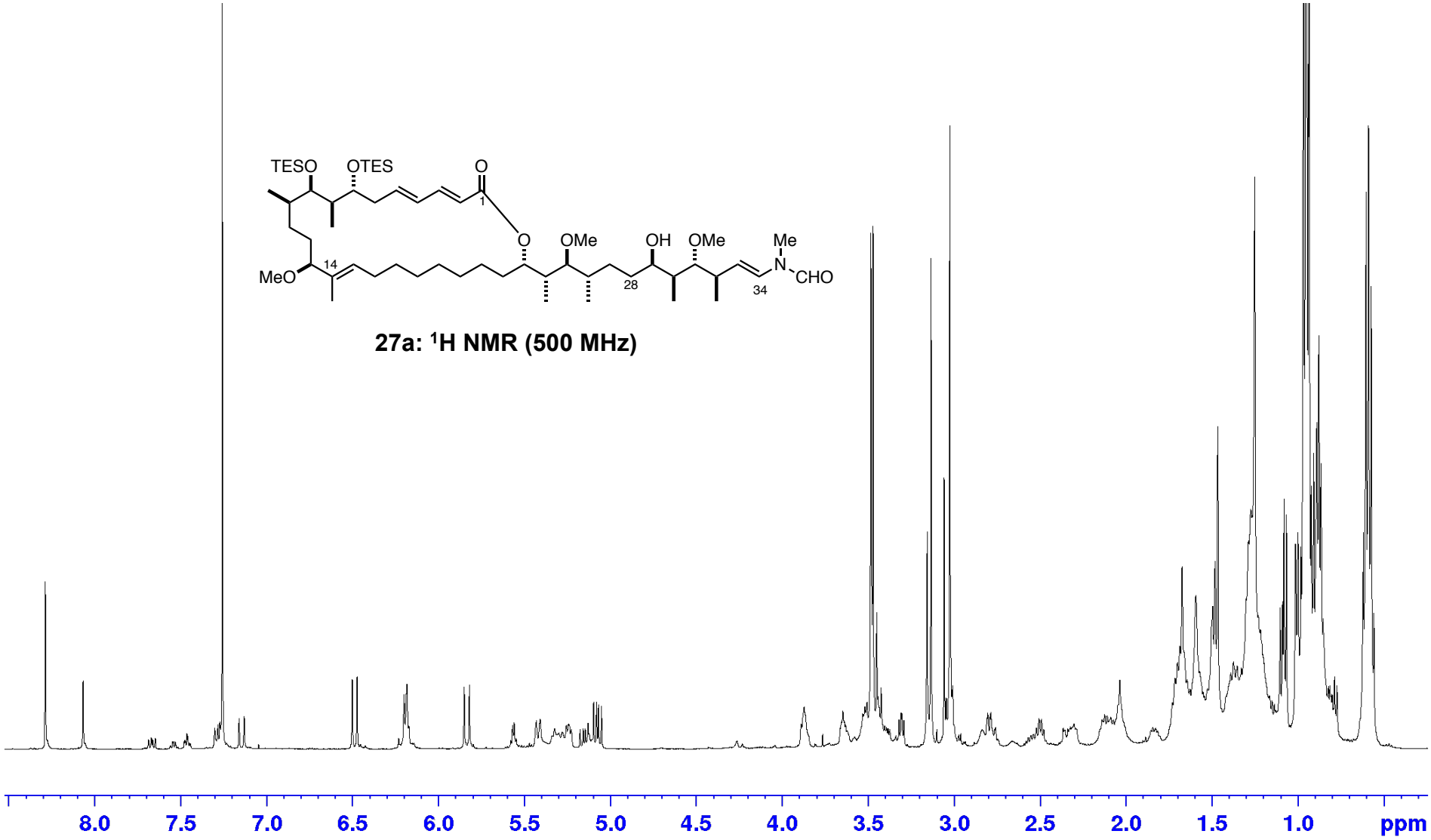
***R*-8: ^{13}C NMR (125 MHz)**

Aplyronine A,D/scytophycin hybrid

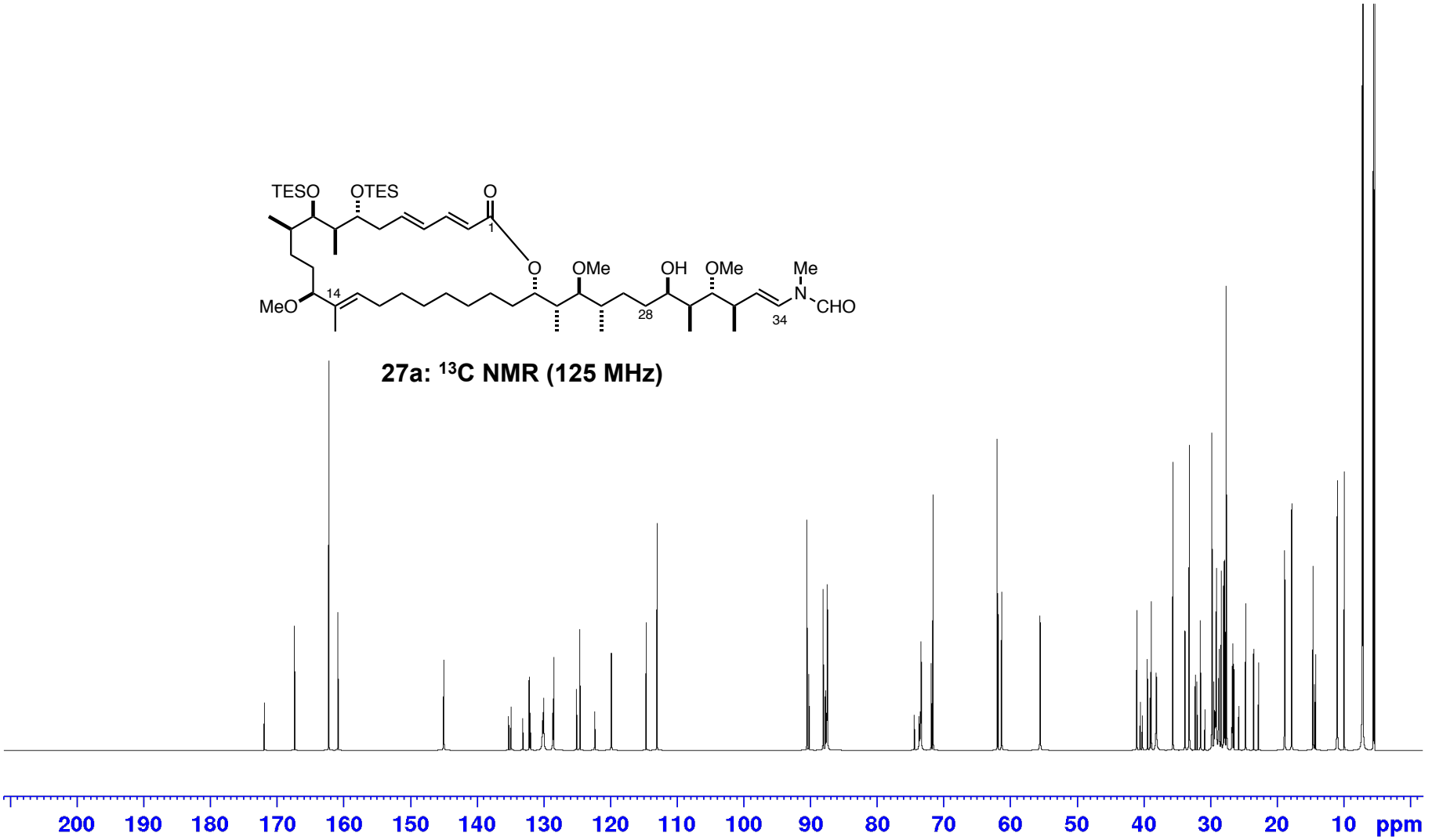


***S*-8: ^{1}H NMR (500 MHz)**
Aplyronine A,D/scytophycin hybrid

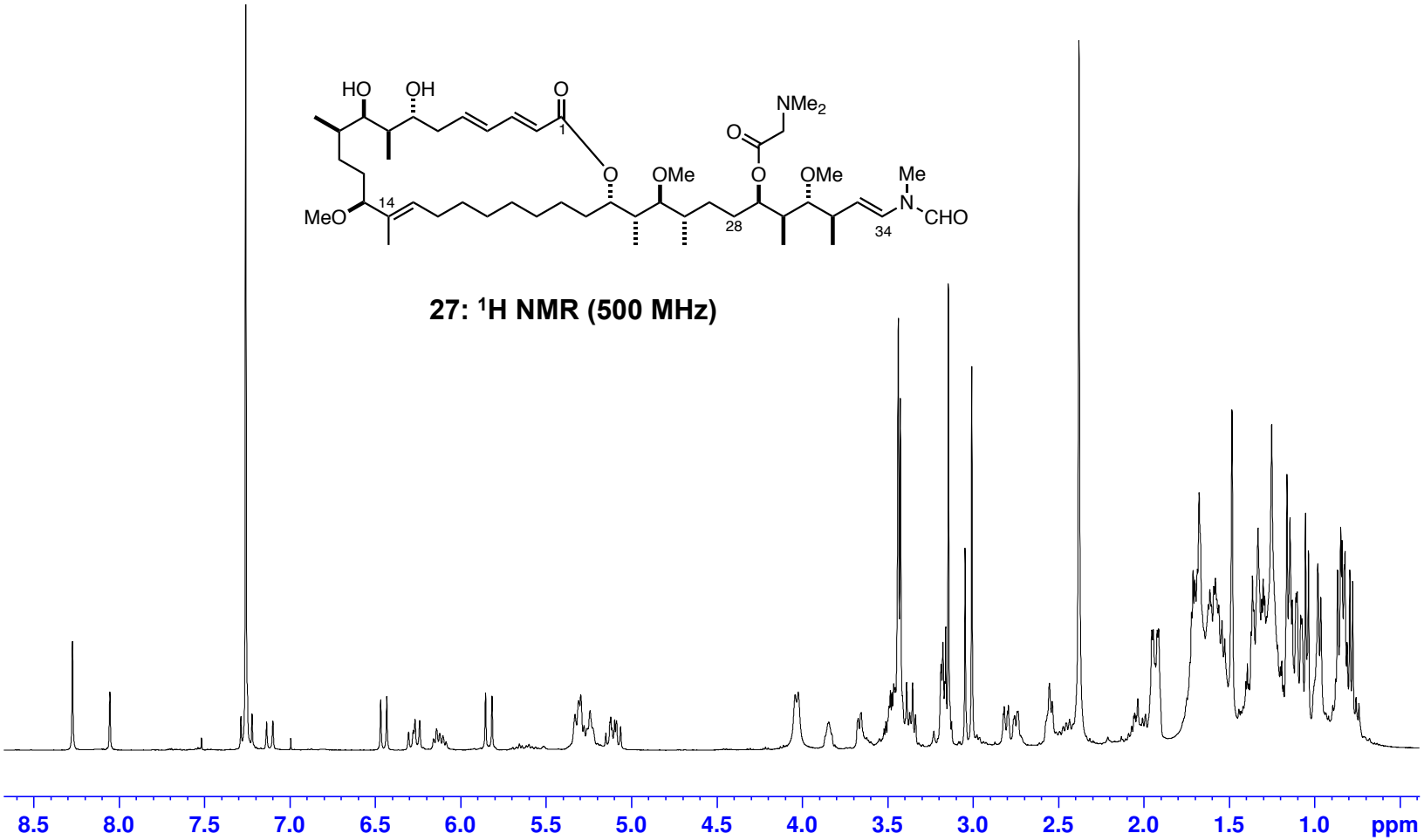

OMe
Me2N
3'
(S)
HO
O
O
O
1
O
OMe
O
OMe
Me
N
CHO
34
23
13
MeO
S-8: 13C NMR (125 MHz)
Aplyronine A,D/
scytophycin hybrid
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

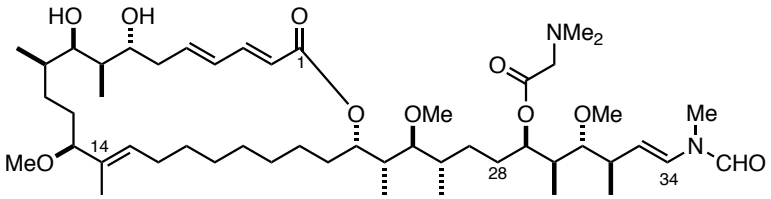


27a: ^{1}H NMR (500 MHz)

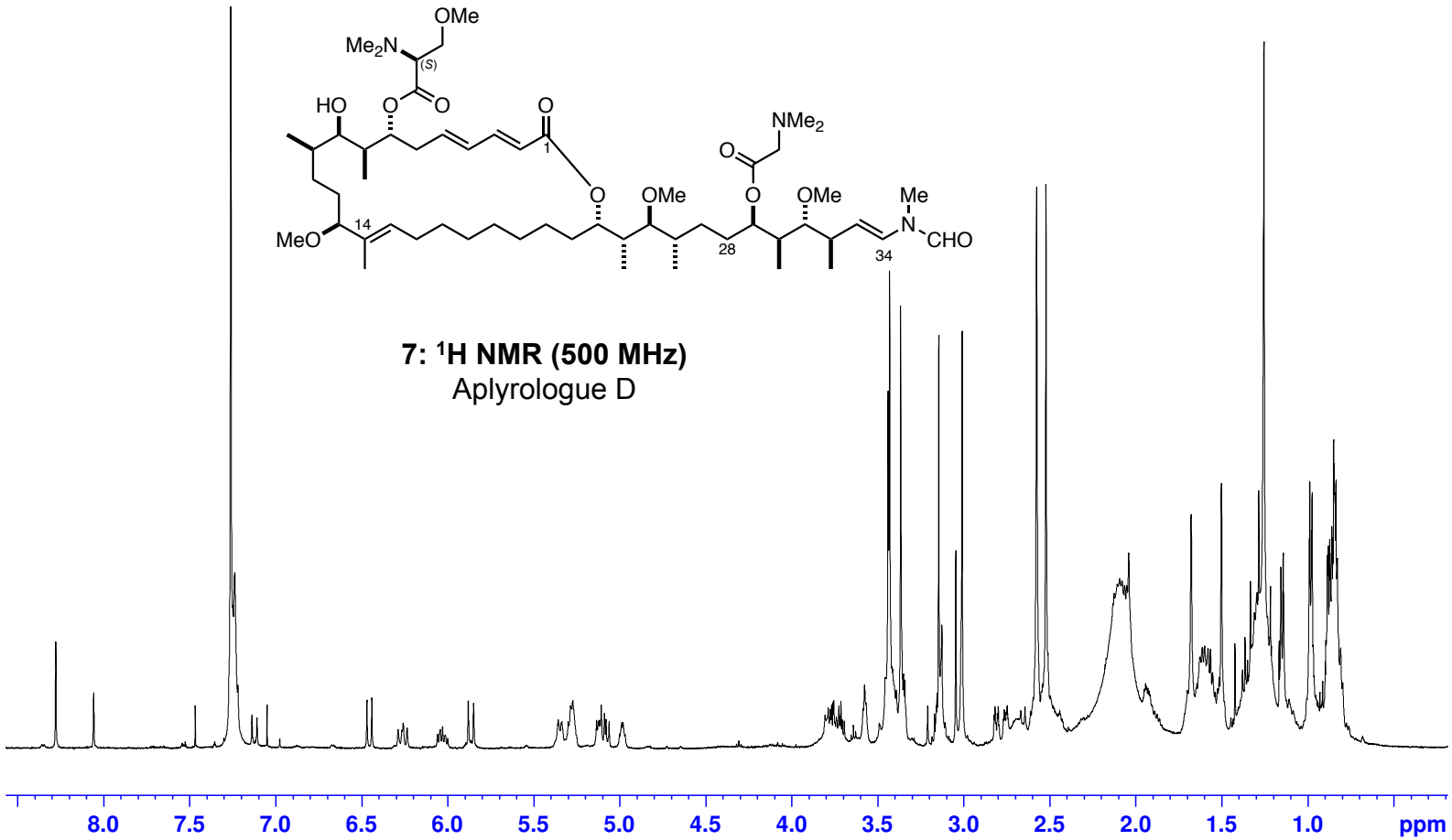


27a: ^{13}C NMR (125 MHz)

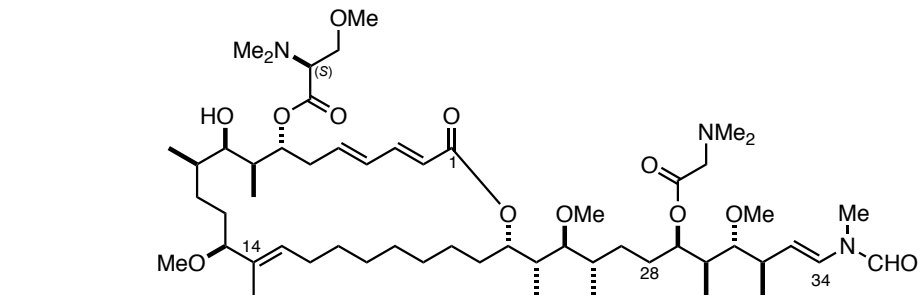

HO
OH
O
1
NMe2
O
O
OMe
O
OMe
Me
N
CHO
MeO
14
28
34
27: 1H NMR (500 MHz)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm



27: ^{13}C NMR (125 MHz)



7: ^{1}H NMR (500 MHz)
Aplyrologue D



7: ^{13}C NMR (125 MHz)

Aplyrologue D