Electronic Supplementary Material for Organic & Biomolecular Chemistry

The Stereocontrolled Total Synthesis of Altohyrtin A/Spongistatin 1. Part 4: Fragment Couplings, Completion of the Synthesis, Analogue Generation and Biological Evaluation

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General Experimental Details

^{1}H nuclear magnetic resonance (NMR) spectra were recorded at either 250, 400 500 or 800 MHz on Bruker DPX 250, DPX 400, DRX 500 or DRX 800 spectrometers at ambient temperature using an internal deuterium lock. The following internal references were used for the residual protons in the following solvents: $CDCl_3$ (δ_H 7.26), C_6D_6 (δ_H 7.16) and CD_3CN (δ_H 1.94). Data are presented as follows: chemical shift (in ppm on the δ scale relative to tetramethylsilane $\delta_{TMS} = 0$), integration, multiplicity, coupling constant and interpretation XX-C<u>H</u> where XX refers to the carbon no. to which the proton in question is attached. Where reasonable, this numbering is based on the spongistatin skeleton. The following abbreviations for splitting patterns are used: s, singlet; d, doublet; t, triplet; q, quartet; quin., quintet; m, multiplet; br, broad. When the multiplet is derived from couplings to non-equivalent protons with coincidentally the same coupling constants then the multiplet is referred to as app, apparent. Assignments were determined either on the basis of unambiguous chemical shift or coupling pattern, COSY experiments or by analogy to fully interpreted spectra for related compounds. ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 100.6 MHz or 62.5 MHz on Bruker AM 400 or DPX 250 spectrometers respectively at ambient temperature using an internal deuterium lock, and all chemical shift values are reported in parts per million (δ) downfield relative to tetramethylsilane (TMS, $\delta_{TMS} = 0$). An internal reference was used for $CDCl_3$ (δ_C 77.16) and C_6D_6 (δ_C 128.06).

Infra-red spectra were recorded on Perkin-Elmer 1620 (FT-IR) spectrometers using 0.5 cm sodium chloride plates. Absorbance bands are reported in wavenumbers (cm^{-1}) relative to polystyrene as the calibrant, and the following abbreviations are used to describe their appearance: w, weak; s, strong; br, broad. Only the most significant bands are reported.

High and low resolution mass spectra were acquired using positive chemical ionisation using NH_4^+ (+CI, NH_3) by the EPSRC National Mass Spectrometry Service Centre, Swansea, UK and the Departmental Mass Spectrometry Service, University Chemical Laboratory, Cambridge, using

electron impact (EI), electrospray (+ESI), chemical ionisation (+CI) or fast atom bombardment (+FAB) ionisation techniques. The parent ion $[M]^+$ or $[MH]^+$ or $[M + NH_4]^+$ is quoted, followed by significant fragments with their relative intensities.
Optical rotations were recorded on a Perkin Elmer 241 polarimeter at the sodium D line (589 nm) and are reported as follows: $[\alpha]_D^{20}$, concentration (*c* in g/100 mL) and solvent (all the rotations were measured at a temperature of 20 °C). Melting points were recorded on a Kofler hot-stage and are uncorrected.
Analytical thin layer chromatography (TLC) was carried out using Merck Kieselgel 60 F_{254} plates with visualisation either by ultra violet light (254 nm), anisaldehyde or Goofy's dips. Flash column chromatography was carried out using Merck Kieselgel 60 (230-400 mesh) under a positive pressure using distilled solvents and in this thesis the term implies subsequent removal of the solvents *in vacuo* unless otherwise stated. High Performance Liquid Chromatography (HPLC) was carried out using a Rainin Instrument Co. Inc. DYNAMAX Macro-HPLC column (internal diameter: 21.4 mm), prepacked with 8 micron irregular silica particles, and equipped with a Gilson refractive index detector (Model 131) or a Gilson UV detector (Model 111B) at a wavelength of 254 nm. A flow rate of 10 mL min^{-1} was used and all solvents were vacuum-filtered and degassed prior to use.
Reagents and solvents were prepared using standard means.[1] Anhydrous CH_2Cl_2, MeOH and hexane were distilled from CaH_2 and stored under argon; ether was distilled from sodium metal/benzophenone ketyl and stored under an argon atmosphere; THF was distilled from either $LiAlH_4$ or potassium metal/benzophenone ketyl and stored under an argon atmosphere. Triethylamine (Et_3N), *i*-Pr_2NEt, pyridine and 2,6-lutidine were distilled from and stored over CaH_2. Acetic acid (AcOH) was distilled from CrO_3 and Ac_2O and stored under an argon atmosphere. Simple aldehydes were distilled from calcium chloride immediately prior to use. All other reagents were used as received except where noted in the experimental procedure.
All experiments were performed under anhydrous conditions, utilising anhydrous solvents, under an atmosphere of argon, except where stated, using oven-dried glassware and employing standard techniques in handling air-sensitive materials. All reactants added *via* cannula were added using a positive pressure of argon. Where a reaction temperature is not specified the reaction was performed at room temperature. Where a compound has been published in the literature, all spectroscopic and physical properties matched those reported.

Experimental Procedures and Product Characterisation Data

(2*R*,4*S*,5*S*,6*R*)-4-(*t*-Butyldimethylsiloxy)-6-(4-(triphenylphosphonium)-butyl)-2-methoxy-2-[[4,5-(*R*,*R*)-bis-(*p*-methoxybenzyloxy)-3-(*R*)-methyl-6-(*R*)-(2-methylallyl)-tetrahydropyran-2-(*R*)-yl]-((*S*)-*p*-methoxybenzyloxy)-methyl]-5-methyl-tetrahydropyran iodide (14)

To a solution of chloride **13**[2] (29.8 mg, 0.032 mmol) in MeCN/MeOH (9:1, 0.5 mL) was added $^iPr_2NEt$ (11 μL, 0.064 mmol, 2 equiv.), NaI (121 mg, 0.8 mmol, 25 equiv.) and PPh_3 (422 mg, 1.6 mmol, 50 equiv.). The resultant mixture was heated at reflux for 20 h then cooled to RT and concentrated *in vacuo*. The resultant residue was taken up in CH_2Cl_2 (10 mL + 3 x 2 mL washings) and filtered through sinter to partially remove the excess NaI. The filtrate was concentrated *in vacuo* and flash chromatography (20:80 → 70:30 MeCN/EtOAc) afforded phosphonium salt **14** which contained trace amounts of NaI. The residue was taken up in CH_2Cl_2 (10 mL + 3 x 2 mL washings) and filtered to remove any remaining NaI. The filtrate was concentrated *in vacuo* to give a glassy solid. Lyophilisation with C_6H_6 (3x) afforded phosphonium salt **14** (38.9 mg, 94%) as a pale yellow powder: $\mathbf{R_f}$ 0.67 (30:70 EtOAc/MeCN); $[\alpha]_D^{20}$ +9.3 (*c* 0.94, $CHCl_3$); **IR** (liquid film) 2932, 1612, 1585, 1513 cm^{-1}; **^{1}H NMR** δ (400 MHz, C_6D_6) 7.73–7.84 (9H, m, ArH), 7.57 (2H, d, *J* = 8.5 Hz, ArH), 7.35 (2H, d, *J* = 8.5 Hz, ArH), 7.25 (2H, d, *J* = 8.6 Hz, ArH), 6.98–7.08 (6H, m, ArH), 6.94

[1] D. A. Perrin and W. L. F. Armarego, *Purification of Laboratory Chemicals*, Pergamon Press, Oxford, 1988.
[2] I. Paterson, M. J. Coster, D. Y.-K. Chen, J. L. Aceña, J. Bach, L. E. Keown, and T. Trieselmann, *Org. Biomol. Chem.*, 2005, **X**, XX-XX.

(2H, J = 8.5 Hz, ArH), 6.85 (2H, d, J = 8.5 Hz, ArH), 6.79 (2H, d, J = 8.6 Hz, ArH), 5.13 (1H, d, J = 11.2 Hz, OCH_aH_bAr), 5.01 (2H, br s, 45-C=CH_2), 4.90–4.94 (2H, m, OCH_aH_bAr + OCH_aH_bAr), 4.88 (1H, d, J = 11.2 Hz, OCH_aH_bAr), 4.61–4.65 (1H, m, 29-CH_aH_b), 4.61 (1H, d, J = 10.8 Hz, OCH_aH_bAr), 4.53 (1H, d, J = 11.2 Hz, OCH_aH_bAr), 4.32 (1H, m, 33-CH), 4.16–4.23 (1H, m, 29-CH_aH_b), 4.03 (1H, s, 38-CH), 3.95 (1H, br d, J = 1.8 Hz, 35-CH), 3.54 (1H, br t, J = 8.8 Hz, 43-CH), 3.44 (3H, s, OCH_3), 3.43 (1H, m, 39-CH), 3.38 (3H, s, OCH_3), 3.34 (3H, s, OCH_3), 3.32 (1H, m, 42-CH), 3.30 (3H, s, OCH_3), 3.16 (1H, t, J = 9.6, 41-CH), 2.69 (1H, d, J = 14.9 Hz, 44-CH_aH_b), 2.61 (1H, dd, J = 15.4, 3.6 Hz, 36-CH_aH_b), 2.39–2.45 (2H, m, 30-CH_aH_b + 44-CH_aH_b), 2.23 (1H, m, 40-CH), 2.06 (1H, br d, J = 15.4 Hz, 36-CH_aH_b), 1.94 (3H, s, 46-CH_3), 1.90 (1H, m, 30-CH_aH_b), 1.74 (1H, m, 32-CH_aH_b), 1.56–1.65 (3H, m, 31-CH_2 + 34-CH), 1.21 (1H, m, 32-CH_aH_b), 1.08 (9H, s, SiC(CH_3)$_3$), 1.05 (3H, d, J = 6.2 Hz, 40-CHCH_3), 1.01 (3H, d, J = 7.1 Hz, 34-CHCH_3), 0.25 (3H, s, SiCH_3), 0.15 (3H, s, SiCH_3); **^{13}C NMR** δ (62.5 MHz, $CDCl_3$) 159.3, 159.2, 142.8, 135.2 (C *para*- to P^+), 133.7 (d, $^3J_{P–C}$ = 9.9 Hz, C *meta*- to P^+), 130.8, 130.7, 130.6 (d, $^2J_{P–C}$ = 12.4 Hz, C *ortho*- to P^+), 129.5, 129.3, 118.1 (d, $^1J_{P–C}$ = 85.4 Hz, C *ipso*- to P^+), 113.8, 113.8, 113.8, 112.5, 102.5, 86.9, 82.8, 79.6, 74.8, 74.6, 74.4, 73.4, 70.3, 66.3, 55.4, 55.3, 55.3, 47.5, 40.0, 38.5, 38.2, 32.9, 30.6, 27.3, 27.0, 25.8, 23.9, 223.0, 22.7, 17.9, 11.6 (d, $^1J_{P–C}$ = 152.4 Hz, 29-C), –4.7, –4.7; **m/z** (+ESI) 1301 ($[M + Na]^+$, 42), 1151 (67), 587 (100).

Side-chain truncated Wittig product (15)

CaH_2 (spatula tip) was added to phosphonium salt **14** (18.3 mg, 0.014 mmol) in a glove box and the system was evacuated and recharged with Ar. O_2-free THF (200 μL) was added, followed by HMPA (20 μL) and the mixture was stirred at RT for 2 h. The phosphonium salt/CaH_2 suspension was cooled to –78 °C and a freshly prepared solution of LiHMDS (0.253 M in THF, 90 μL, 0.023 mmol, 1.6 equiv.) was added, upon which the solution developed an intense yellow-orange colour. The reaction mixture was stirred at –78 °C for further 10 min. A 500 μL gas-tight syringe was flushed with a solution of aldehyde **2** (20.1 mg, 0.018 mmol, 1.3 equiv.) in THF (250 mL) and then the aldehyde solution was added to the ylide solution *via* this syringe. The resultant mixture was removed from the cooling bath and allowed to warm to RT. The mixture was stirred at RT for 40 min by which time a deep red colouration had developed. The reaction was quenched by transferring it, by pipette, to a cold (0 °C), vigorously stirred mixture of sat. aq. NH_4Cl/20% aq. $Na_2S_2O_3$ (2:1, 10 mL), and washing the flask with Et_2O (3 x 5 mL). The layers were separated and the aqueous phase was extracted with Et_2O (3 x 20 mL), the combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo*. Flash chromatography (10:90 → 60:40 EtOAc/hexanes) afforded the desired Wittig product **15** (20 mg, 71%) as a white amorphous solid: **R_f** 0.54 (50:50 EtOAc/hexanes); $[\alpha]_D^{20}$ –13.0 (*c* 2.69, $CDCl_3$); **IR** (liquid film) 3416, 2953, 1734, 1612, 1513, 1461, 1371, 1249 cm^{-1}, **^{1}H NMR** δ (500 MHz, C_6D_6) 7.44 (2H, d, J = 8.0 Hz, ArH), 7.35 (2H, d, J = 8.3 Hz, ArH), 7.25 (2H, d, J = 8.2 Hz, ArH), 6.81–6.85 (4H, m, ArH), 6.79 (2H, d, J = 8.3 Hz, ArH), 5.75 (1H, dd, J = 10.9, 7.1 Hz, 28-CH), 5.61–5.65 (2H, m, 15-CH + 27-CH), 5.55 (1H, m, 29-CH), 5.22 (1H, s, 13-C=CH_aH_b), 4.94–5.06 (6H, m, 5-CH + 13-C=CH_aH_b + 45-C=CH_2 + 2 x OCH_aH_bAr), 4.87 (1H, d, J = 10.6 Hz, OCH_aH_bAr), 4.77 (1H, d, J = 12.0 Hz, OCH_aH_bCCl$_3$), 4.75 (1H, m, OCH_aH_bAr), 4.62 (1H, d, J = 10.5 Hz, OCH_aH_bAr), 4.55 (1H, d, J = 10.6 Hz, OCH_aH_bAr), 4.49 (1H, m, 3-CH), 4.47 (1H, d, J = 12.0 Hz, OCH_aH_bCCl$_3$), 4.33–4.36 (2H, m, 11-CH + 33-CH), 4.19 (1H, m, 19-CH), 3.91–3.94 (2H, m, 25-CH + 35-CH), 3.71 (1H, s, 38-CH), 3.54 (1H, m, 43-CH), 3.32 (6H, s, 2 x ArOCH_3), 3.30–3.34 (2H, m, 39-CH + 42-CH), 3.29 (3H, s, ArOCH_3), 3.25 (1H, m, 21-CH), 3.23 (3H, s, 37-COCH_3), 3.12–3.20 (1H, m, 41-CH), 3.06 (3H, s, 21-COCH_3), 3.05 (1H, m, 18-CH_aH_b), 2.92 (1H, m, 16-CH), 2.74–2.81 (2H, m), 2.58–2.73 (2H, m), 2.48 (2H, app d, J = 5.5 Hz, 12-CH_2), 2.34–2.45 (4H, m), 2.28–2.34 (2H, m), 2.21 (1H, m, 40-CH), 2.00–2.15 (3H, m), 1.95 (3H, s, COCH_3), 1.94 (3H, s, 46-CCH_3), 1.92 (1H, m, 6-CH_aH_b), 1.79 (3H, s, COCH_3), 1.71–1.80 (4H, m), 1.49–1.68 (6H, m), 1.42 (1H, d, J = 14.2 Hz, 10-CH_aH_b), 1.23 (3H, d, J = 6.8 Hz, 14-CHCH_3), 1.22–1.24 (1H, m, 6-CH_aH_b), 1.18 (3H, d, J = 6.9 Hz, 16-CHCH_3), 1.17 (1H, m, 4-CH_aH_b), 1.12 (9H, s, SiC(CH_3)$_3$), 1.11–1.14 (1H, m, 24-CH_aH_b), 1.03–1.06 (1H, m, 20-

CH_aH_b), 1.03 (9H, t, J = 7.0 Hz, $Si(CH_2CH_3)_3$), 1.02 (9H, s, $SiC(CH_3)_3$), 0.99 (3H, s, 9-CCH_3), 0.97–1.03 (5H, m, 8-CH_aH_b + 10-CH_aH_b + 34-$CHCH_3$), 0.82 (3H, d, J = 6.0 Hz, 40-$CHCH_3$), 0.57–0.60 (6H, m, $Si(CH_2CH_3)_3$), 0.24 (3H, s, $SiCH_3$), 0.14 (3H, s, $SiCH_3$), 0.08 (6H, s, 2 x $SiCH_3$); **^{13}C NMR** δ (62.5 MHz, C_6D_6) 209.3, 169.9, 168.9, 159.6, 159.3, 147.7, 143.1, 132.0, 131.5, 131.3, 130.7, 130.4, 129.2, 129.0, 113.8, 113.8, 113.8, 113.5, 112.8, 102.8, 98.1, 96.9, 95.3, 86.9, 83.1, 79.7, 77.9, 76.2, 74.6, 74.4, 74.2, 73.8, 73.6, 70.9, 70.4, 67.3, 66.6, 66.4, 65.0, 64.3, 62.0, 60.6, 54.9, 54.5, 54.5, 54.5, 50.9, 47.7, 47.6, 47.0, 45.2, 44.3, 42.7, 40.6, 40.1, 39.2, 38.9, 38.4, 37.7, 34.7, 33.9, 33.0, 31.9, 31.3, 30.2, 29.9, 28.5, 26.6, 25.9, 23.0, 21.1, 20.3, 18.1, 13.4, 13.2, 12.0, 10.3, 7.3, 6.9, –4.6, –4.7, –5.0, –5.0; **HRMS** (+ESI) Calc. for $C_{103}H_{161}O_{24}Cl_3Si_3Na$ $[M + Na]^+$: 1993.9643, found: 1993.9435.

Side-chain truncated analogue – *tris*-PMB deprotection (16)

To a cooled (0 °C) solution of *tris*-PMB ether **15** (9.4 mg, 4.76 μmol) in CH_2Cl_2/pH7 buffer (10:1, 1.1 mL) was added DDQ (97 mg, 0.43 mmol, 90 equiv.) over a period of 1.5 h (10 equiv. every 10 min). The reaction was quenched by pouring into sat. aq. $NaHCO_3$ (10 mL) and the layers were separated. The aqueous phase was extracted with Et_2O (3 x 15 mL), combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. Flash chromatography (20:80 → 100:0 EtOAc/hexanes) afforded the desired *tris*-PMB deprotected material **16** as an inseparable mixture (5.3 mg, 69%) as a white amorphous solid consisting of an inseparable *ca*. 1.1:1 mixture of methyl and hemiacetal at C37: **R_f** 0.50 (80:20 EtOAc/hexanes); **IR** (liquid film) 3449, 2954, 1735 (C=O), 1654 cm^{-1}; **^{1}H NMR** δ (500 MHz, C_6D_6) 5.77 (1H, m, 28-CH), 5.71 (1H, br d, J = 9.7 Hz, 15-CH), 5.67 (1H, m, 27-CH), 5.59 (1H, m, 29-CH), 5.31 (1H, s, 13-C=CH_aH_b), 5.22 (0.5H, s, 37-OH), 5.08 (1H, s, 13-C=CH_aH_b), 5.06 (1H, br s, 5-CH), 5.01 (1H, s, 45-C=CH_aH_b), 4.98 (1H, s, 45-C=CH_aH_b), 4.87 (1H, d, J = 11.9 Hz, $CH_aH_bCCl_3$), 4.58 (1H, d, J = 12.0 Hz, $CH_aH_bCCl_3$), 4.58 (1H, m, 3-CH), 4.43 (2H, m, 33-CH + 11-CH), 4.27 (1H, m, 19-CH), 3.98 (2H, m, 25-CH + 35-CH), 3.89 (0.5H, d, J = 6.2 Hz), 3.86 (0.5H, d, J = 10.3 Hz), 3.72 (0.5H, d, J = 8.6 Hz), 3.54 (0.5H, m, 43-CH), 3.31–3.46 (2H, m), 3.26 (1.5 H, s, 37-OCH_3), 3.01–3.22 (4H, m), 3.16 (3H, 2 x s, 21-OCH_3), 2.84–2.93 (2H, m), 2.65–2.76 (3H, m), 2.56 (2H, d, J = 6.2 Hz), 2.29–2.46 (6H, m), 2.20 (4H, m), 2.05 (3H, 2 x s, $COCH_3$), 2.04 (1H, m), 1.92 (3H, 2 x s, $COCH_3$), 1.92 (3H, s, 46-CCH_3), 1.80–1.92 (4H, m), 1.61–1.74 (6H, m), 1.47–1.53 (4H, m), 1.33 (3H, m, 14-$CHCH_3$), 1.29 (3H, m, 16-$CHCH_3$), 1.22–1.34 (2H, m), 1.06–1.18 (33H, m), 0.95 (3H, m, 40-$CHCH_3$), 0.68 (6H, q, J = 7.6 Hz, $Si(CH_2CH_3)_3$), 0.13–0.29 (12H, m, 3 x $Si(CH_3)_2$); **m/z** (+ESI) 1635 (for **16Me**, $[M + Na]^+$, 43), 1621 (for **16H**, $[M + Na]^+$, 83), 1151 (18), 822 (100).

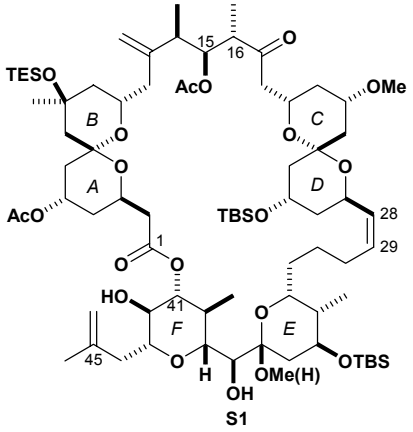
Side-chain truncated analogue – TCE deprotection (17)

To a solution of **16** (36.8 mg, max. 0.023 mmol) in THF (1.0 mL) was added Zn dust (1.0 g, excess). The resultant suspension was stirred at RT for 2 min and aq. NH_4OAc (1M, 100 μL) was added. The reaction mixture was stirred at RT for 30 min and then diluted with EtOAc (10 mL). The supernatant was separated with a pipette and the resultant Zn residue was washed with EtOAc (3 x 10 mL). The combined organics were dried (Na_2SO_4), filtered through sinter, and concentrated *in vacuo*. Flash column chromatography (40:60 → 100:0 EtOAc/hexanes, then 1:99 AcOH/EtOAc) afforded *seco*-acid **17** (20.9 mg, 62%), as a mixture of methyl acetal and hemiacetal at C37, and partially dechlorinated esters (5.7 mg, 16%) as an amorphous solid and a colourless oil, respectively.

Seco-acid **17**: **R_f** 0.36 (80:20 EtOAc/hexanes); **IR** (liquid film) 3458, 2932, 1732 (C=O), 1372 cm^{-1}; **^{1}H NMR** δ (500 MHz, C_6D_6) 5.79 (2H, m, 15-CH +28-CH), 5.66 (1H, m, 27-CH), 5.57 (1H, m, 29-CH), 5.04–5.30 (5.5H, m, 37-OH + 5-CH + 13-C=CH_2, 45-C=CH_2), 4.62, 4.53, 4.29–4.40 (4H, 3 x m, 3-CH + 11-CH + 19-CH + 33-CH), 3.81–4.00 (3H, m, 25-CH + 35-CH + 38-CH + 39-CH), 3.23–3.57 (3H, m), 3.31 (1.5H, s, 37-OCH_3), 2.97–3.19 (3H, m), 3.14, 3.15 (3H, 2 x s, 21-OCH_3), 2.75–2.94 (3H, m), 2.67 (2H, m), 2.43–2.56 (4H, m), 2.25–2.42 (3H, m), 2.19 (4H, m), 2.04 (3H, s, $COCH_3$), 2.02 (1H, m), 1.98, 1.93 (3H, 2 x s, $COCH_3$), 1.95 (3H, s, 46-CH_3), 1.75–1.85 (4H, m),

1.68 (6H, m), 1.02–1.52 (46H, m), 0.73 (6H, m, Si(C<u>H</u>$_2$CH$_3$)$_3$), 0.10–0.29 (12H, m, 3 x Si(C<u>H</u>$_3$)$_2$). The absence of doublet peaks at 4.58 and 4.87 indicated the loss of the 2,2,2-trichloroethyl ester moiety; **m/z** (+ESI) 1503 (for **17Me**, [M + Na]$^+$, 34), 1489 (for **17H**, [M + Na]$^+$, 67), 756 (56), 681 (94), 469 (100).

Side-chain truncated analogue – macrolactonisation (S1)



S1

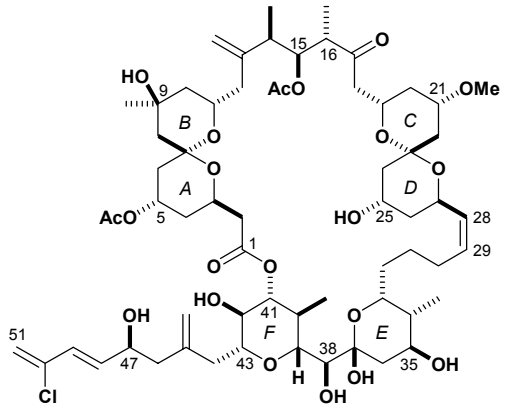
To a solution of *seco*-acid **17** (20.9 mg, max. 0.014 mmol) in THF (200 μL) was added freshly prepared solution of Et_3N (0.5M in THF, 141 μL, 0.07 mol, 5 equiv.) and a solution of 2,4,6-trichlorobenzoyl chloride (0.5M in THF, 85 μL, 0.042 mmol, 3 equiv.). The resultant solution was left to stir at RT for 1 h before a further aliquot of reagents (Et_3N and 2,4,6-trichlorobenzoyl chloride, same stoichiometry as above) were added. This process was repeated for a third time such that a total of Et_3N (15 equiv.) and 2,4,6-trichlorobenzoyl chloride (9 equiv.) were added in three portions over a period of 3 h. The reaction mixture was diluted with PhMe (1 mL) and this solution was then added to a refluxing solution of DMAP (34 mg, 0.28 mmol, 20 equiv.) in PhMe (10 mL) over a period of 3 minutes. The anhydride flask was rinsed with PhMe (2 x 1 mL) and added to the refluxing reaction. The resultant cloudy white suspension was heated at reflux for 16 h and then cooled to RT before being quenched with sat. $NaHCO_3$ (20 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3 x 20 mL). The combined organic extracts were washed with brine (20 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Flash chromatography (20:80 → 80:20 EtOAc/hexanes) afforded macrolactone **S1** (9.7 mg, 47%) as a colourless oil, consisting of an inseparable mixture of methyl acetal and hemiacetal at C37: $\mathbf{R_f}$ 0.32 (50:50 EtOAc/hexanes); **IR** (liquid film) 3455, 2953, 1736 (C=O), 1459 cm^{-1}; **^{1}H NMR** δ (500MHz, CD_3CN) 5.42 (1H, m, 28-C<u>H</u>), 5.36 (1H, m, 29-C<u>H</u>), 5.18 (1H, m, 27-C<u>H</u>), 5.11 (1H, dd, *J* = 10.5, 1.5 Hz, 15-C<u>H</u>), 4.91 (1H, br s, 5-C<u>H</u>), 4.85 (2H, br s, 13-C=C<u>H</u>$_2$), 4.78–4.83 (2H, m, 45-C=C<u>H</u>$_2$), 4.73, 4.68 (1H, m, dd, *J* = 10.6, 9.1 Hz, 41-C<u>H</u>), 4.29 (2H, m, 3-C<u>H</u> + 11-C<u>H</u>), 4.13 (2H, m), 4.04 (1H, m), 3.89 (1.5H, m), 3.80 (0.5H, m), 3.60 (1H, d, *J* = 10.3 Hz, 39-C<u>H</u>), 3.49 (1H, m, 21-C<u>H</u>), 3.39 (2H, m, 38-C<u>H</u> + 43-C<u>H</u>), 3.31 (1H, d, *J* = 6.1 Hz), 3.25, 3.24 (3H, 2 x s, 21-OC<u>H</u>$_3$), 3.03–3.21 (3H, m), 2.92 (1H, m), 2.84 (1H, d, *J* = 6.3 Hz), 2.67–2.80 (3H, m), 2.36–2.55 (3H, m), 2.31 (2H, m), 2.04–2.18 (7H, m), 1.95 (3H, s, COC<u>H</u>$_3$), 1.87 (3H, s, COC<u>H</u>$_3$), 1.87 (2H, m), 1.75 (2H, m), 1.55–1.66 (6H, m), 1.32–1.48 (8H, m), 1.21 (3H, s, 9-CC<u>H</u>$_3$), 1.19 (3H, d, *J* = 7.0 Hz, 16-CHC<u>H</u>$_3$), 1.14 (1H, m), 1.08 (2H, m), 1.08 (3H, d, *J* = 6.9 Hz, 14-CHC<u>H</u>$_3$), 0.84–0.97 (33H, m), 0.59 (6H, m, Si(C<u>H</u>$_2$CH$_3$)$_3$), 0.02–0.12 (12H, m, 3 x Si(C<u>H</u>$_3$)$_2$). The appearance of double-doublet signal at δ 4.68 (*J* = 10.6, 9.1 Hz) indicated the formation of the macrolactones; **m/z** (+ESI) 1485 (for **S1Me**, [M + Na]$^+$, 19), 1471 (for **S1H**, [M + Na]$^+$, 93), 747 (100).

Side-chain truncated analogue (18)

To a cold (0 °C) solution of macrolactone **S1** (2.4 mg, max. 1.64 μmol) in MeCN (0.5 mL) was added a freshly prepared HF solution (40% $HF_{(aq)}$/H_2O/MeCN, 0.5/0.9/8.6, 50 μL) and the reaction mixture was stirred at 0 °C for 4 h before being quenched with sat. aq. $NaHCO_3$ (5 mL). The

resultant mixture was diluted with CH_2Cl_2 (5 mL) and the layers were separated. The aqueous phase was extracted with CH_2Cl_2 (3 x 10 mL), combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. Flash chromatography (5:95 → 10:90 MeOH/CH_2Cl_2) afforded **18** as a white solid: $\mathbf{R_f}$ 0.24 (5:95 MeOH/CH_2Cl_2); **^{1}H NMR** δ (500 MHz, CD_3CN) 5.47 (1H, m, 29-C<u>H</u>), 5.36 (1H, br t, *J* = 9.7 Hz, 28-C<u>H</u>), 5.18 (1H, dd, *J* = 10.6, 1.8 Hz, 15-C<u>H</u>), 5.00 (1H, m, 27-C<u>H</u>), 4.94 (1H, br s, 5-C<u>H</u>), 4.85 (2H, br s, 13-C=C<u>H$_2$</u>), 4.84 (1H, br s, 45-C=C<u>H$_a$</u>H$_b$), 4.77 (1H, dd, *J* = 11.0, 9.2 Hz, 41-C<u>H</u>), 4.74 (2H, br s, 45-C=CH$_a$<u>H$_b$</u> + 37-O<u>H</u>), 4.35 (1H, br t, *J* = 11.0 Hz, 11-C<u>H</u>), 4.29 (1H, d, *J* = 10.7 Hz, 25-O<u>H</u>), 4.26 (1H, br s, 9-O<u>H</u>), 4.26 (1H, obsc., 3-C<u>H</u>), 4.19 (1H, br d, *J* = 5.2 Hz, 42-O<u>H</u>), 4.16 (1H, m, 33-C<u>H</u>), 4.02 (1H, br t, *J* = 11.1 Hz, 19-C<u>H</u>), 3.92 (1H, m, 25-C<u>H</u>), 3.82 (1H, d, *J* = 9.3 Hz, 35-O<u>H</u>), 3.74 (1H, br d, *J* = 10.4 Hz, 39-C<u>H</u>), 3.66 (1H, m, 35-C<u>H</u>), 3.49 (1H, m, 21-C<u>H</u>), 3.38 (1H, obsc., 43-C<u>H</u>), 3.37 (1H, d, *J* = 10.7 Hz, 38-C<u>H</u>), 3.27 (3H, s, 21-OC<u>H$_3$</u>), 3.14 (1H, td, *J* = 9.1, 5.2 Hz, 42-C<u>H</u>), 3.04 (1H, dq, *J* = 10.7, 6.9 Hz, 16-C<u>H</u>), 2.82–2.90 (3H, m, 14-C<u>H</u> + 18-C<u>H$_a$</u>H$_b$ + 38-O<u>H</u>), 2.61–2.65 (2H, m, 18-C<u>H$_a$</u>H$_b$ + 44-C<u>H$_a$</u>H$_b$), 2.56 (1H, dd, *J* = 16.7, 1.8 Hz, 2-C<u>H$_a$</u>H$_b$), 2.49 (1H, dd, *J* = 16.7, 10.5 Hz, 2-CH$_a$<u>H$_b$</u>), 2.29 (2H, m, 24-C<u>H$_a$</u>H$_b$ + 12-C<u>H$_a$</u>H$_b$), 2.14 (3H, obsc., 45-CC<u>H$_3$</u>), 2.04–2.10 (4H, m, 12-CH$_a$<u>H$_b$</u> + 30-CH$_2$ + 44-CH$_a$<u>H$_b$</u>), 1.90–1.99 (3H, m, 20-C<u>H$_a$</u>H$_b$ + 22-C<u>H$_a$</u>H$_b$ + 36-C<u>H$_a$</u>H$_b$), 1.85 (3H, s, COC<u>H$_3$</u>), 1.73 (3H, s, COC<u>H$_3$</u>), 1.68 (1H, m, 4-C<u>H$_a$</u>H$_b$), 1.54–1.62 (9H, m, 4-CH$_a$<u>H$_b$</u> + 8-C<u>H$_a$</u>H$_b$ + 10-C<u>H$_a$</u>H$_b$ + 24-CH$_a$<u>H$_b$</u> + 26-C<u>H$_2$</u> + 31-C<u>H$_a$</u>H$_b$ + 34-C<u>H</u> + 36-CH$_a$<u>H$_b$</u>), 1.48 (1H, d, *J* = 14.0 Hz, 8-CH$_a$<u>H$_b$</u>), 1.41 (1H, m, 32-C<u>H$_a$</u>H$_b$), 1.27–1.32 (3H, m, 10-CH$_a$<u>H$_b$</u> + 31-CH$_a$<u>H$_b$</u> + 32-CH$_a$<u>H$_b$</u>), 1.17 (3H, d, *J* = 7.0 Hz, 16-CHC<u>H$_3$</u>), 1.11 (1H, t, *J* = 11.9 Hz, 22-CH$_a$<u>H$_b$</u>), 1.07 (3H, s, 9-CC<u>H$_3$</u>), 1.04 (3H, d, *J* = 6.9 Hz, 14-CHC<u>H$_3$</u>), 0.96 (1H, ddd, *J* = 12.0, 12.0, 12.0 Hz, 20-CH$_a$<u>H$_b$</u>), 0.85 (3H, d, *J* = 7.2 Hz, 34-CHC<u>H$_3$</u>), 0.77 (3H, d, *J* = 6.7 Hz, 40-CHC<u>H$_3$</u>).

Table S1: NMR assignments for natural and synthetic altohyrtin A/spongistatin 1 (**1**) recorded in CD_3CN. Coupling constants are in Hz (in parentheses)

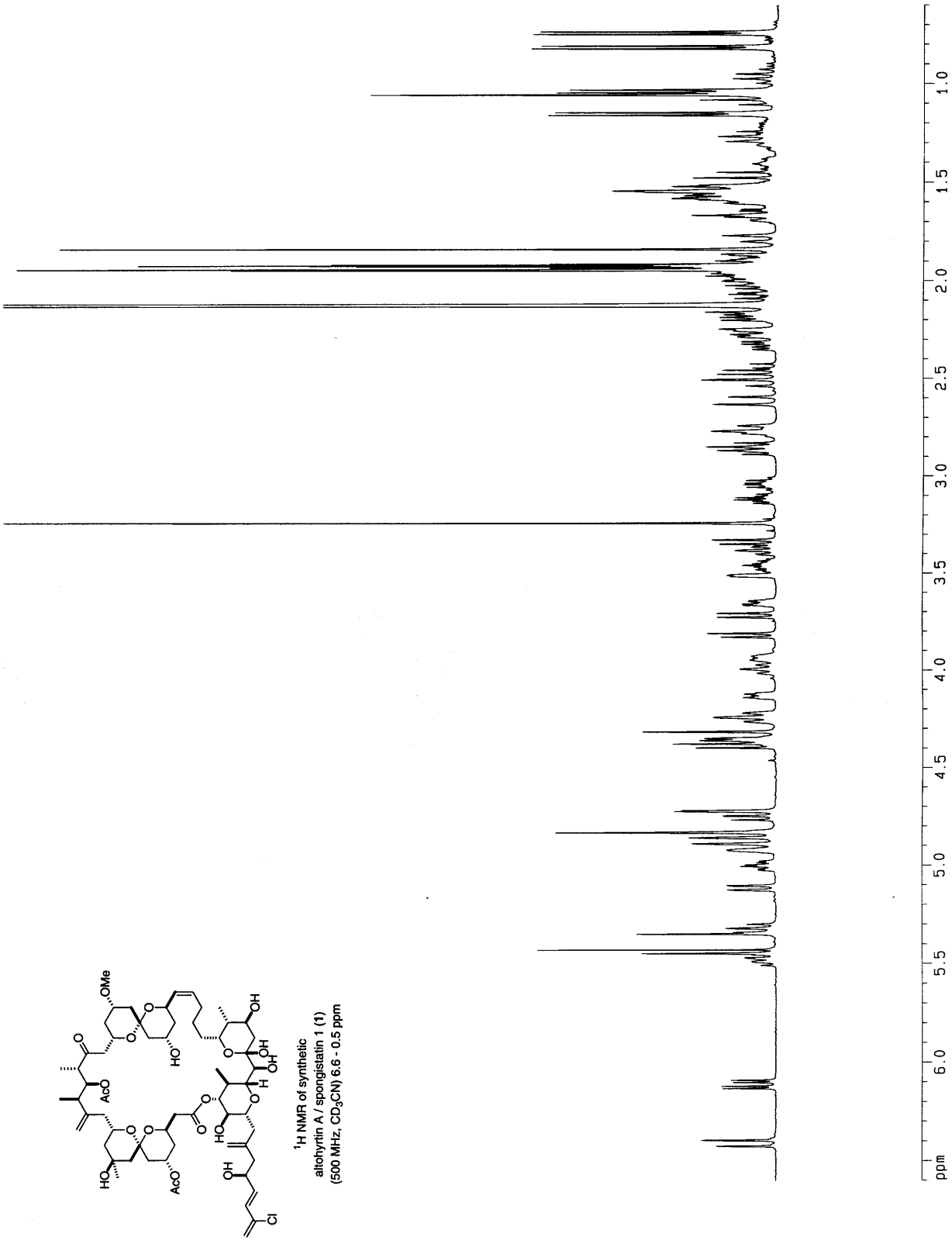


1: altohyrtin A/spongistatin 1

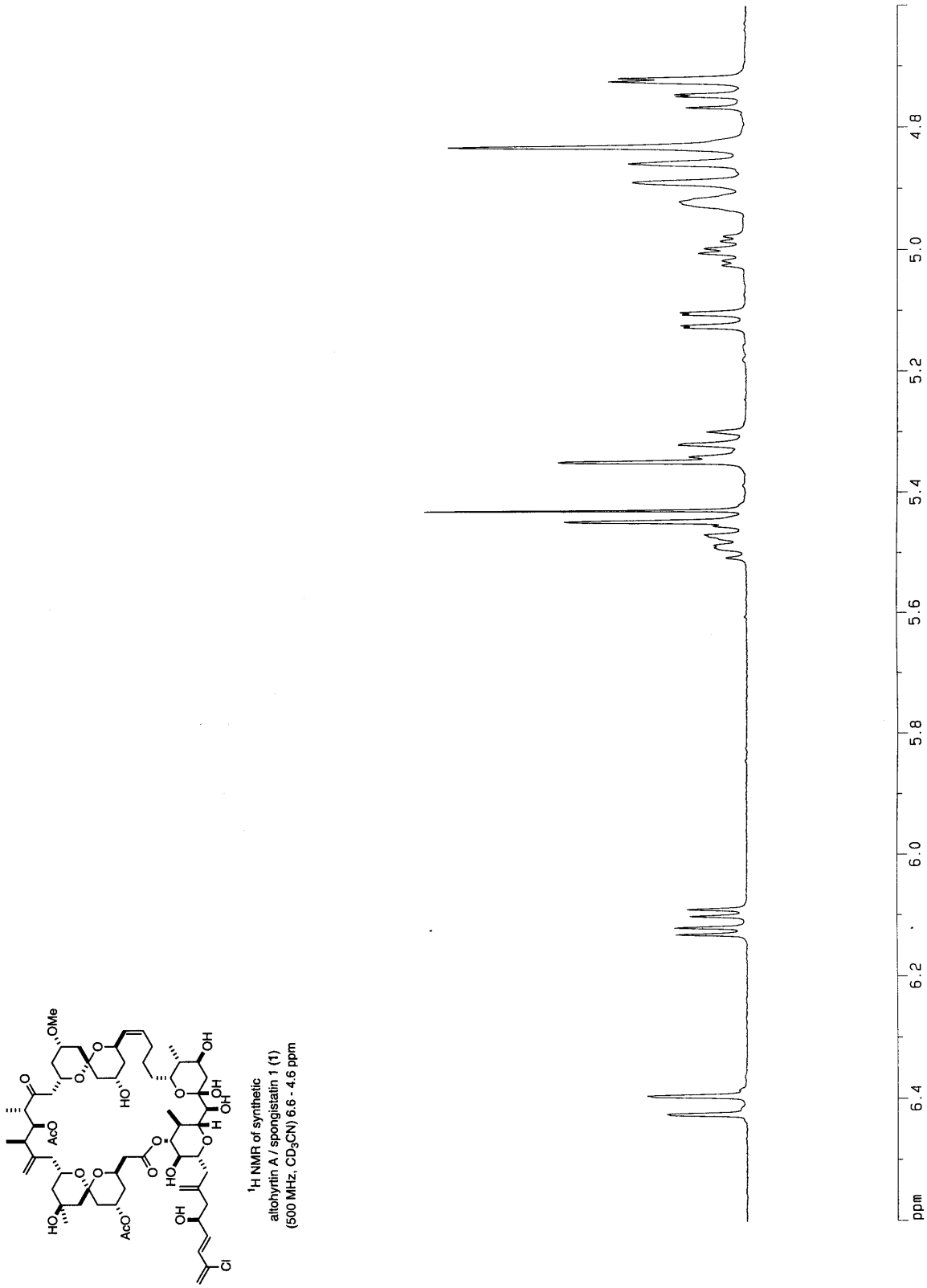
$^1H/^{13}C$ No.	Natural	Synthetic	Natural	Synthetic
	^{13}C (100 MHz)	^{13}C (125 MHz)	^1H (400 MHz)	^1H (500 MHz)
1	173.07	173.07		
2	40.86	40.86	2.44 dd (10, 18)	2.45 dd (10.5, 16.2)
			2.53 dd (2, 18)	2.52 dd (1.8, 16.2)
3	63.59	63.59	4.25 brt (10)	4.24 brt (10.7)
4	34.65	34.65	1.55*, 1.68*	1.55*, 1.68*
5	67.06	67.06	4.92 brs	4.92 brs
6	38.17	38.16	1.67 dd (5, 14); 1.78 brd (14)	1.66 dd (4.4, 15.2); 1.78 d (15.1)
7	99.26	99.25		
8	46.76	46.75	1.47 d (14), 1.60*	1.46 d (14.0), 1.60*
9	69.64	69.63		
9a	30.21	30.21	1.06 s	1.06 s
10	44.96	44.95	1.28*, 1.55*	1.28*, 1.55*
11	64.70‡	64.70	4.25 brt (10)	4.24 brt (10.7)
12	44.24	44.24	1.99*; 2.27 brd (14)	1.99*; 2.26 brd (14.0)
13	148.03	148.03		
13a	114.86	114.86	4.83 brs; 4.83 brs	4.83 brs; 4.83 brs
14	36.60	36.59	2.78*	2.78*
14a	12.09	12.09	1.04 d (6.9)	1.04 d (6.9)
15	75.34	75.34	5.12 dd (1.7, 11)	5.11 dd (1.8, 10.7)
16	47.62	47.61	3.04 dq (7, 11)	3.04 dq (6.9, 10.7)
16a	13.73	13.73	1.15 d (7)	1.15 d (6.9)
17	213.52	213.52		
18	51.94	51.95	2.62 brd (18); 2.86 dd (11, 18)	2.61 brd (18.2); 2.86 dd (10.1, 19.4)
19	66.16	66.15	4.00 brt (11)	3.99 brt (11.1)
20	37.70	37.70	0.97 ddd (12, 12, 12); 1.98*	0.96 ddd (12, 12, 12); 1.98*
21	73.98	73.98	3.46 tt (4, 4, 12, 12)	3.46 tt (4.2, 4.2, 11.6, 11.6)
22	44.18	44.18	1.08 t (12); 1.99*	1.08 t (12.1), 1.99*
23	99.91	99.91		
24	34.91	34.90	1.55*; 2.28*	1.55*; 2.27*
25	64.41	64.41	3.93 brm	3.94 brm
26	39.11	39.11	1.57*; 1.57*	1.57*; 1.57*
27	61.22	61.21	5.00 ddd (4.3, 10, 10)	5.00 ddd (3.8, 9.9, 9.9)
28	131.22	131.21	5.32 brt (10)	5.32 brt (10.8)
29	133.42	133.41	5.48 ddd (10, 10, 10)	5.48 ddd (9.9, 9.9, 10.6)
30	28.07	28.08	2.00*; 2.19*	2.00*; 2.18*
31	27.04	27.04	1.23*; 1.60*	1.23*; 1.60*

32	32.82	32.83	1.30 m; 1.42 m	1.30 m; 1.42 m
33	67.15	67.15	4.13 dt (3.4, 3.4, 8)	4.13 dt (3.0, 3.0, 9.0)
34	39.32	39.32	1.57 m	1.57 m
34a	11.55	11.55	0.81 d (7)	0.81 d (7.2)
35	71.47	71.47	3.65 brs	3.65 m
36	33.79	33.78	1.61*; 1.89*	1.61*; 1.89*
37	99.41	99.41		
38	73.11	73.09	3.34 brs	3.34 d (10.7)
39	81.30	81.30	3.72 brd (10)	3.72 brd (10.2)
40	37.26	37.26	1.91*	1.91*
40a	12.69	12.69	0.74 d (7)	0.74 d (6.6)
41	80.60	80.60	4.75 dd (9, 11)	4.75 dd (9.2, 11.0)
42	73.11	73.09	3.12 t (9)	3.11 dt (5.4, 9.1)
43	78.72	78.70	3.39 brt (9)	3.38 dt (2.2, 8.6)
44	40.24	40.23	2.08*; 2.76 brd (13)	2.08*; 2.75 brd (13.9)
45	144.00	143.98		
45a	116.61	116.63	4.86 brs; 4.89 brs	4.86 brs; 4.89 brs
46	43.93	43.92	2.33 brdd (7, 14); 2.19*	2.33 brdd (6.3, 13.6); 2.18*
47	70.13	70.12	4.36 ddd (6, 7, 11)	4.36*
48	139.21	139.20	6.11 dd (6, 15)	6.11 dd (5.7, 15.0)
49	126.99	126.98	6.41 brd (15)	6.41 dd (1.1, 15.0)
50	139.21	139.20		
51	116.48	116.49	5.35 brs; 5.45 brs	5.35 brs; 5.45 brs
OMe	55.72	55.71	3.24 s	3.24 s
OAc	21.78	27.78	1.94 s	1.94 s
	171.61	171.61		
OAc	21.00	21.00	1.84 s	1.84 s
	170.21	170.19		
OH (C9)			4.32 brs	4.31 brs
OH (C25)			4.39 d (9.9)	4.39 d (10.0)
OH (C35)			3.83 brm	3.82 d (9.4)
OH (C37)			4.73 d (2)	4.72 d (2.5)
OH (C38)				2.86 brd (10.1)
OH (C42)				4.35 brd (5.3)
OH (C47)				3.51 brd (2.5)

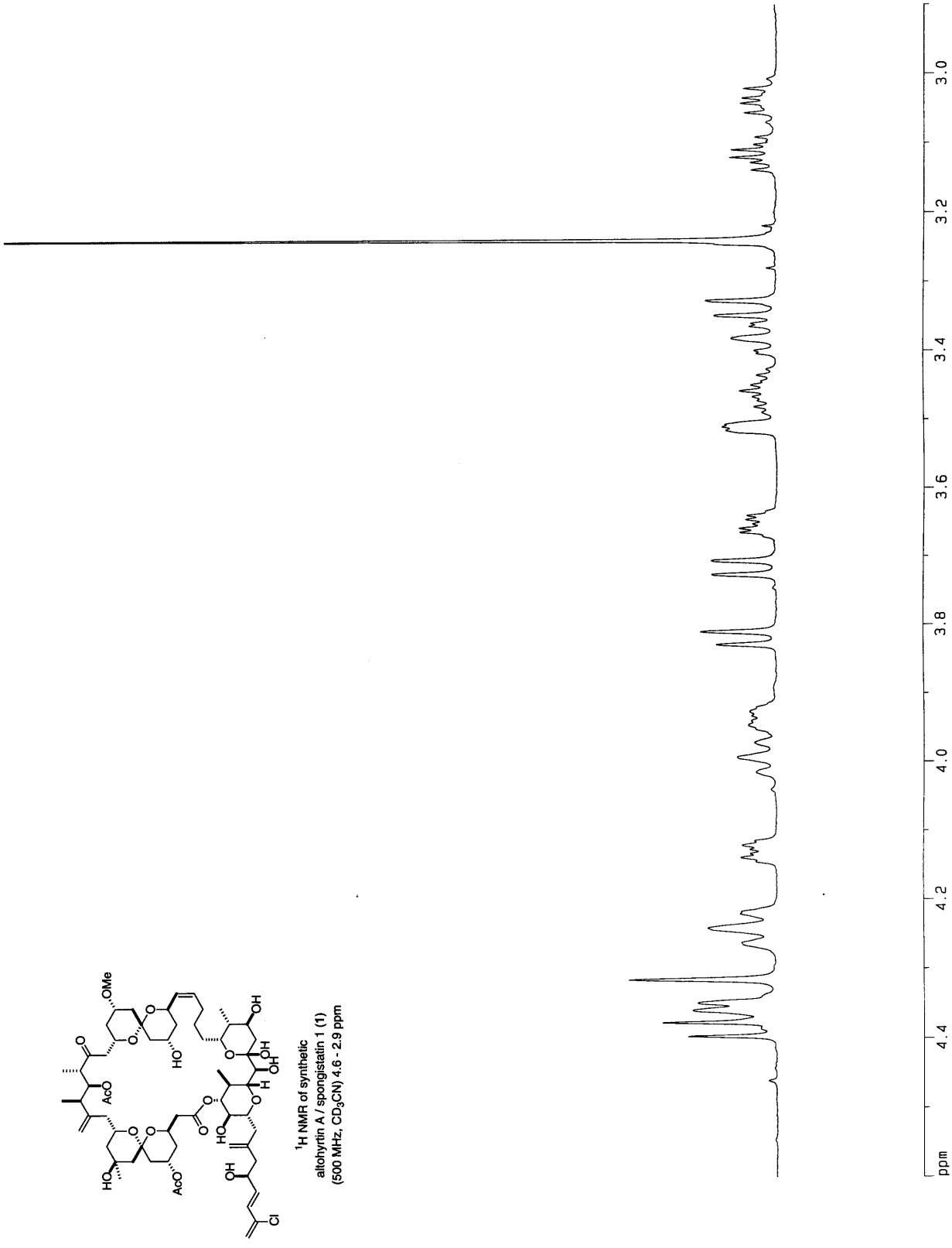
* coupling constants for these signals were not measured due to overlapping.
‡ correction to Pettit's published value.



^{1}H NMR of synthetic altohyrtin A / spongistatin 1 (1) (500 MHz, CD_3CN) 6.6 - 0.5 ppm



^{1}H NMR of synthetic altohyrtin A / spongistatin 1 (1) (500 MHz, CD_3CN) 6.6 - 4.6 ppm



^{1}H NMR of synthetic
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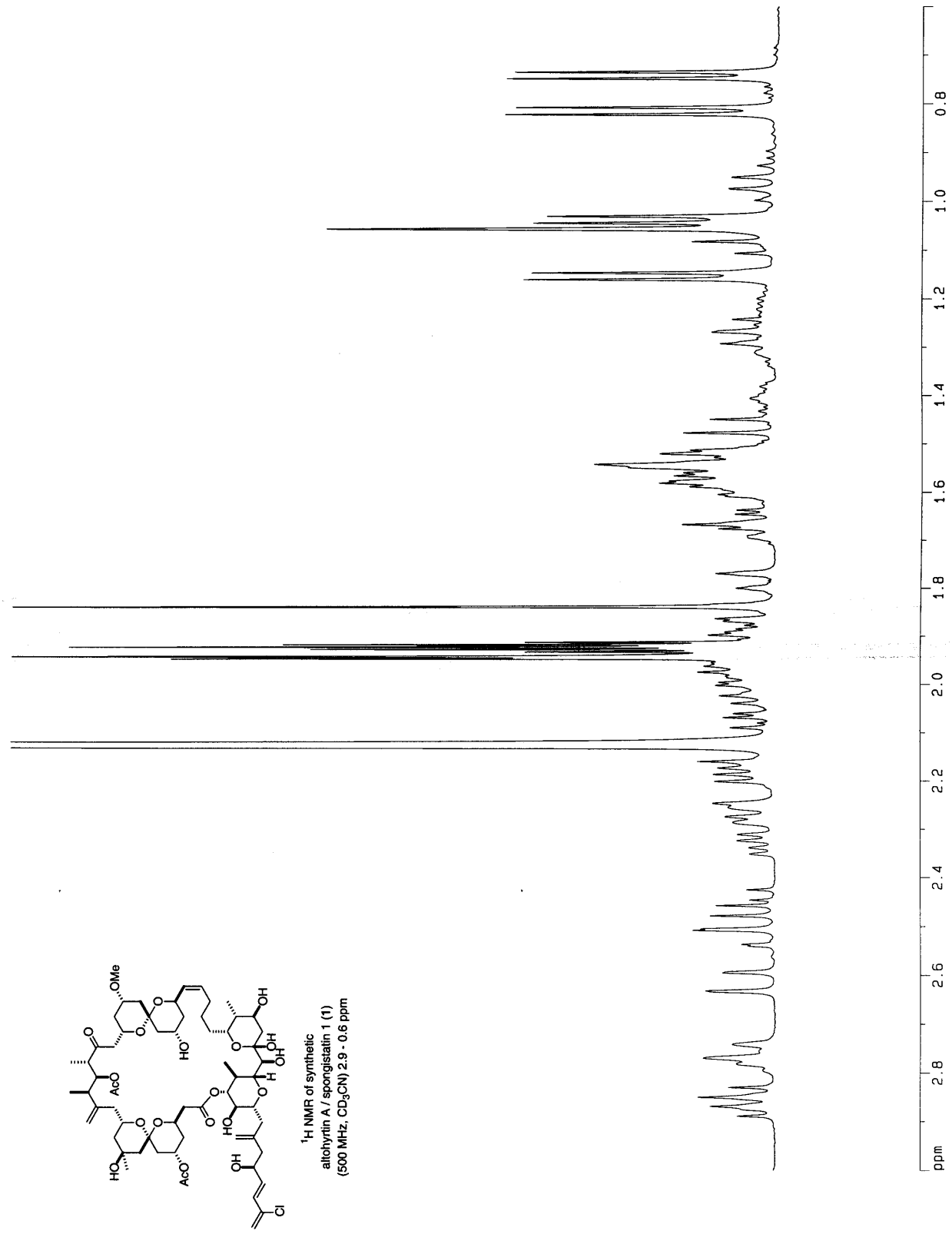
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(500 MHz, CD_3CN) 2.9 - 0.6 ppm
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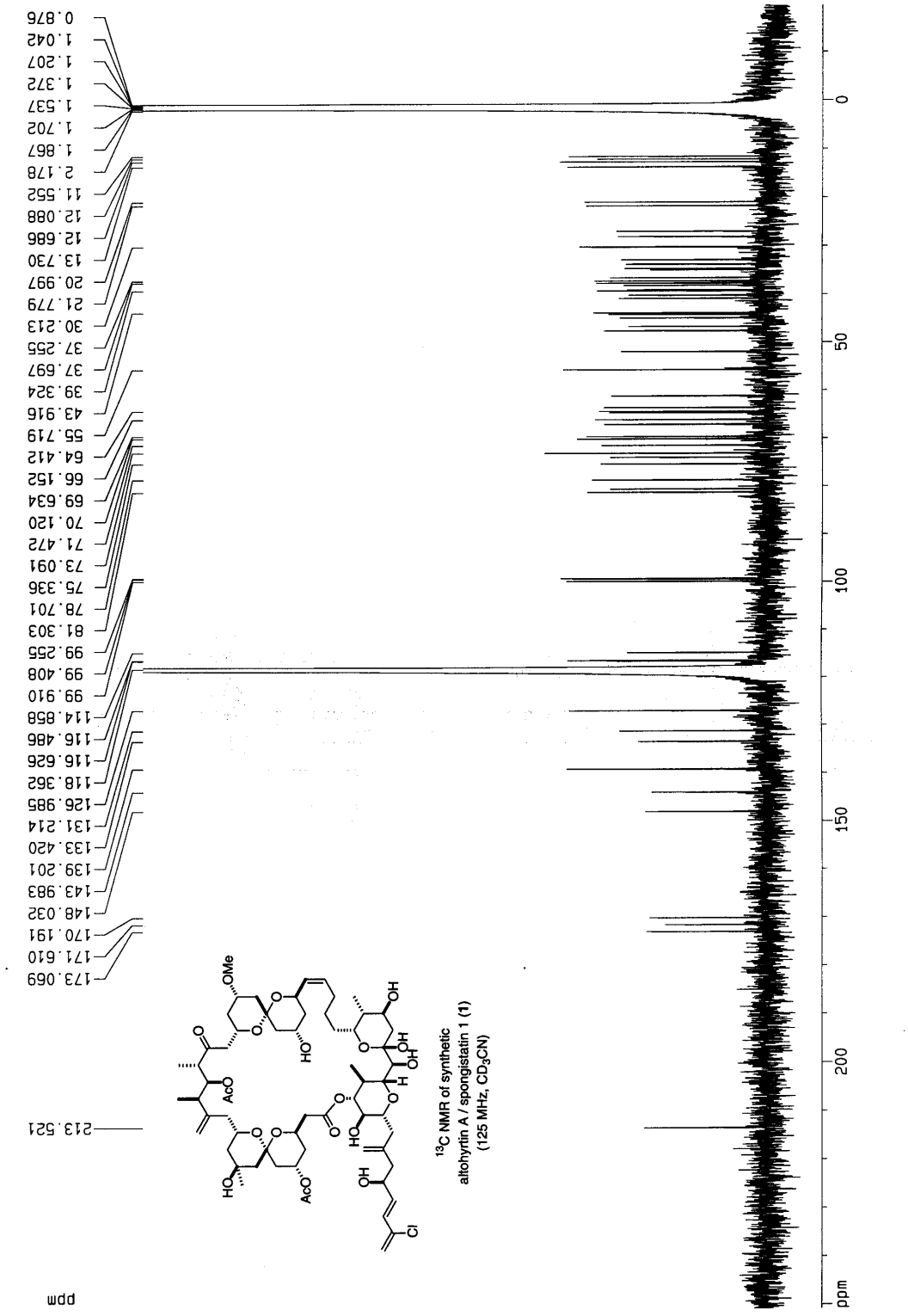
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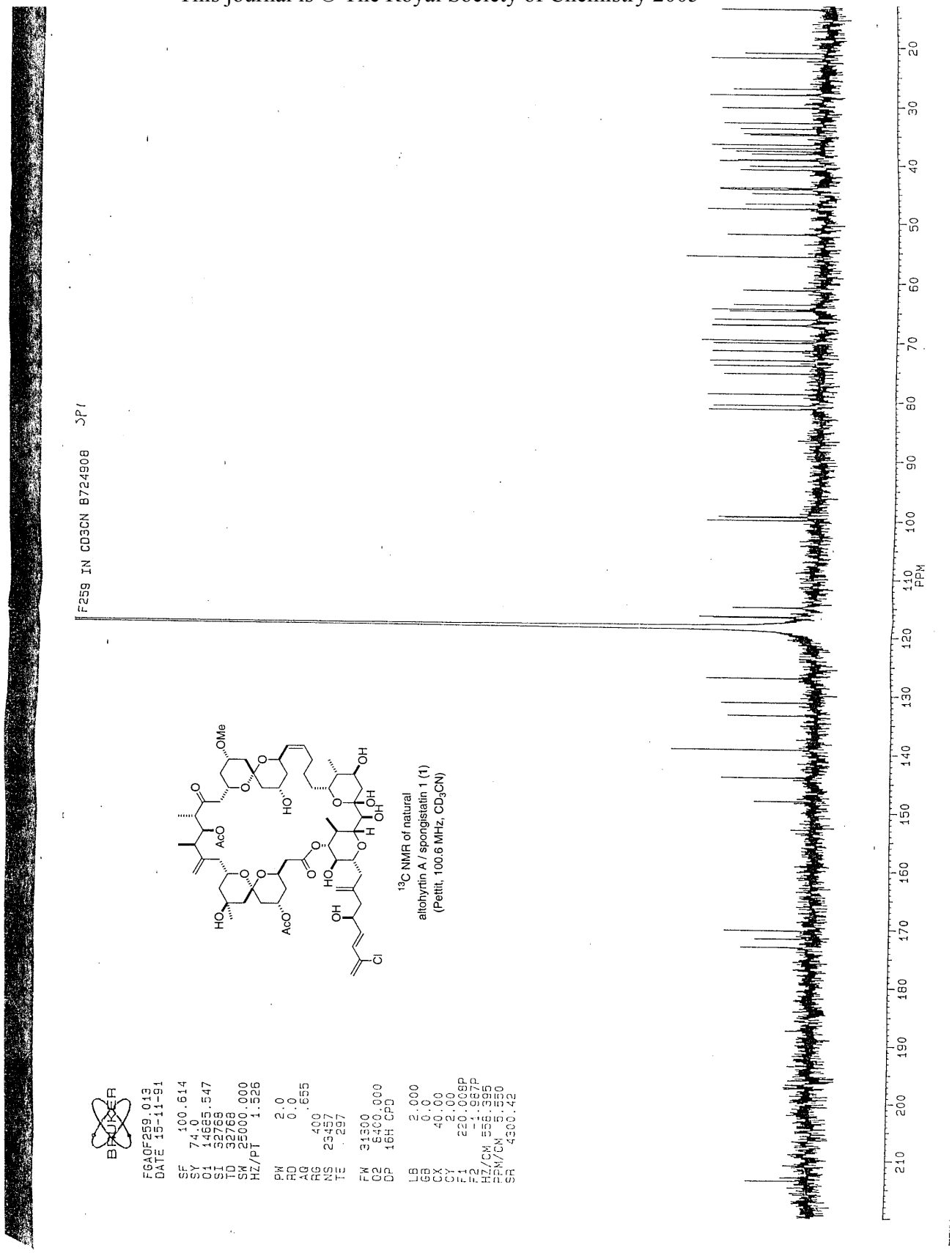
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13C NMR of natural
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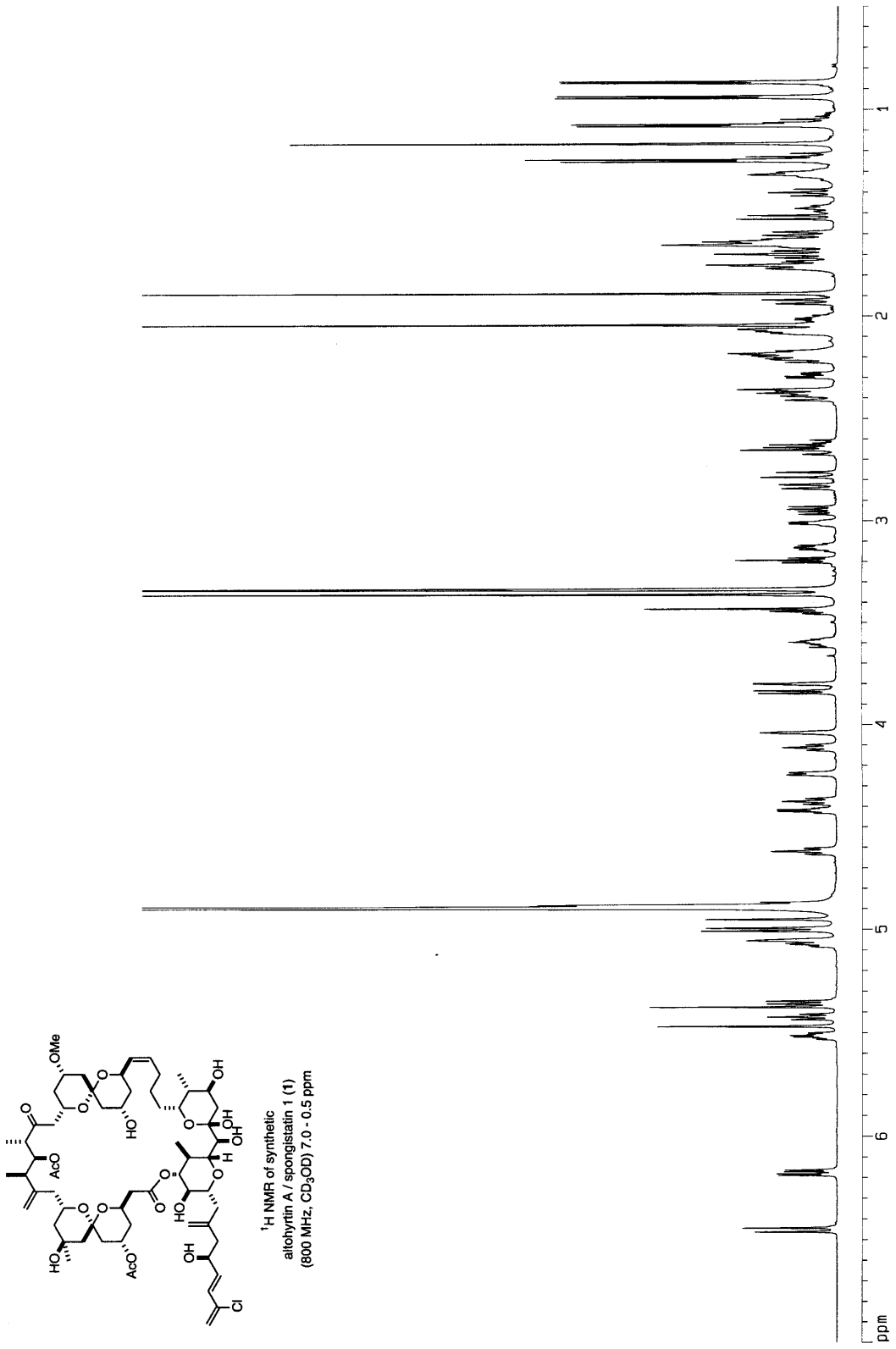
(Pettit, 100.6 MHz, CD3CN)
PPM



^{1}H NMR of synthetic
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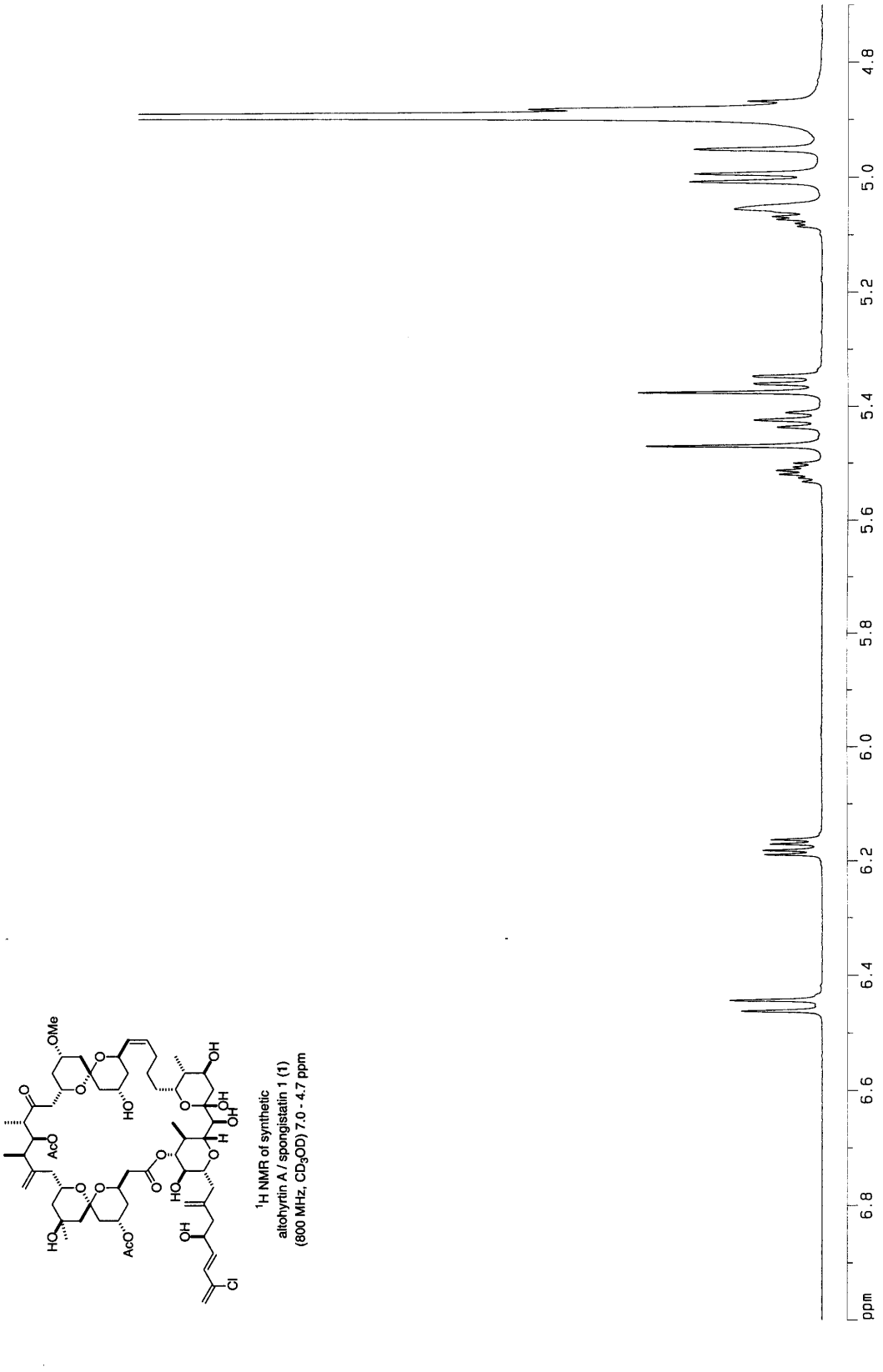
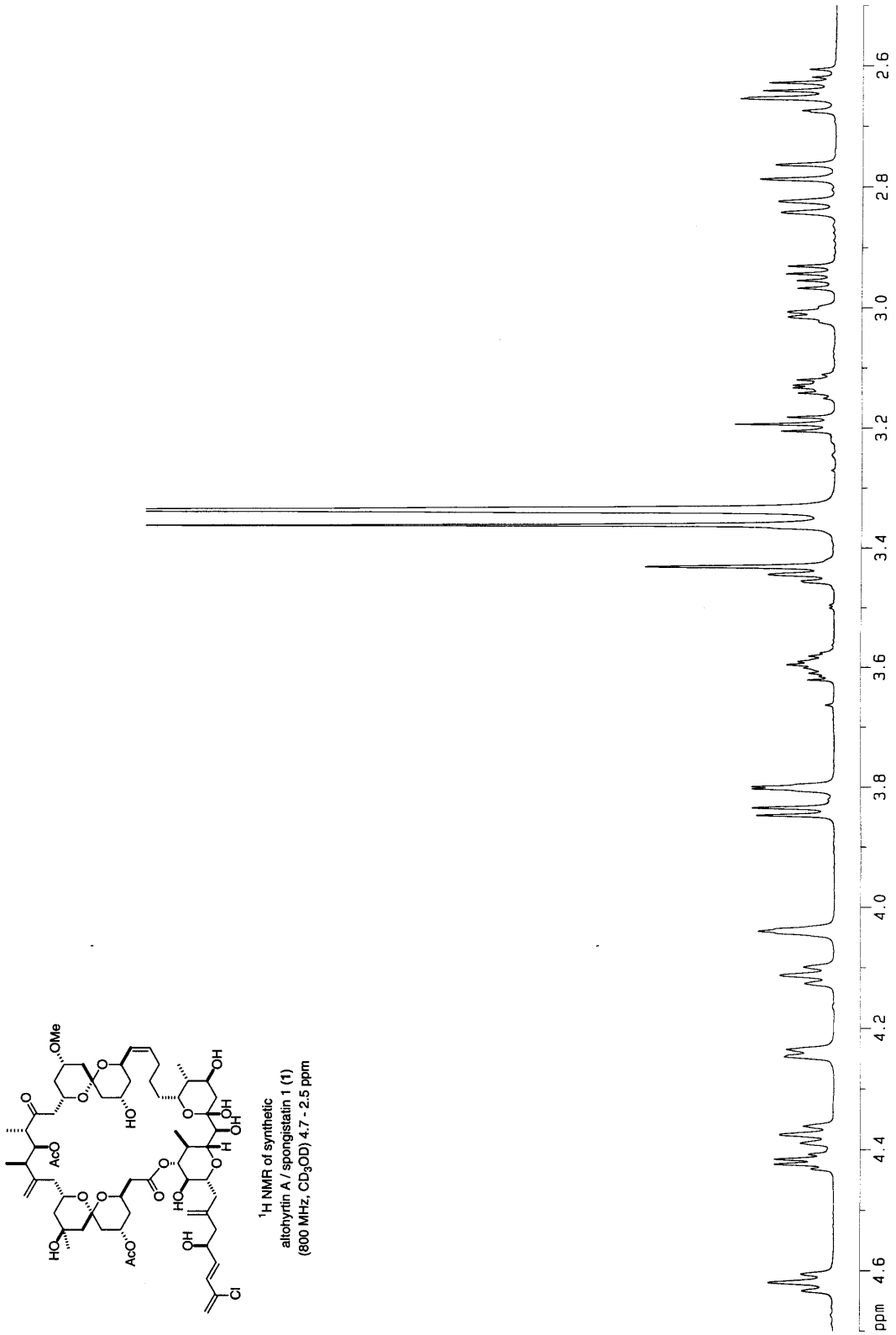


^{1}H NMR of synthetic altohyrtin A / spongistatin 1 (1) (800 MHz, CD_3OD) 7.0 - 4.7 ppm



^{1}H NMR of synthetic
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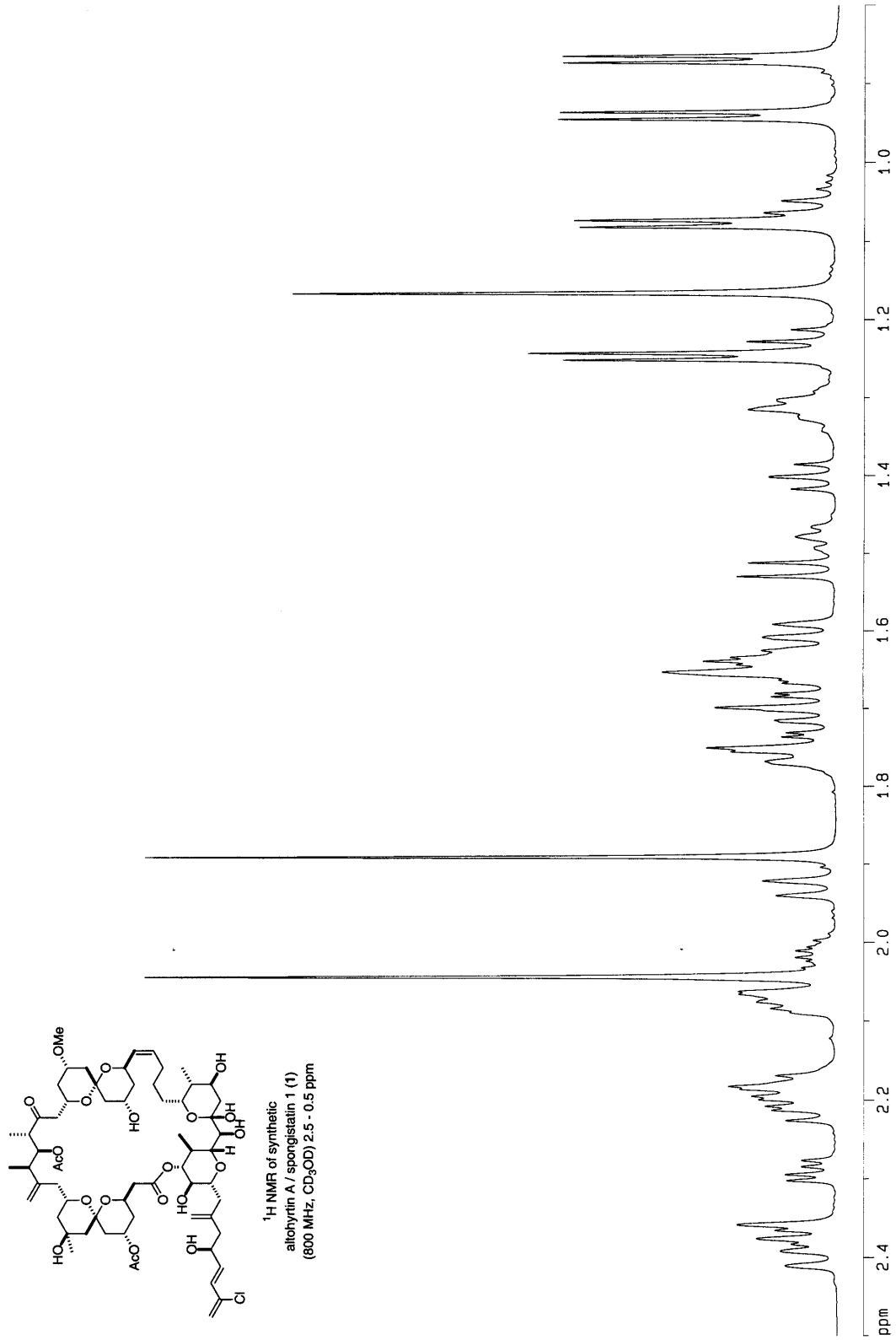
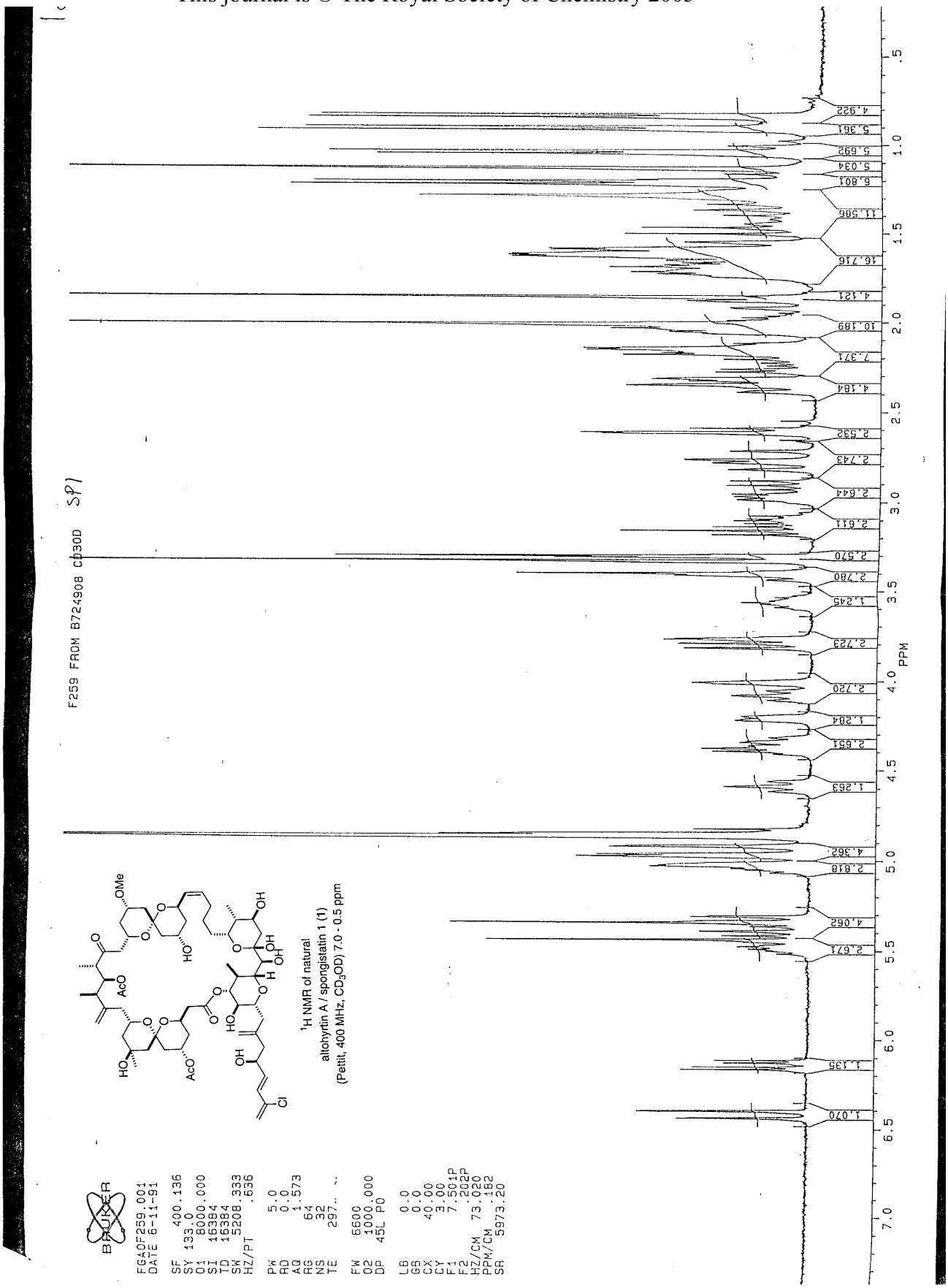
(800 MHz, CD_3OD) 4.7 - 2.5 ppm



^{1}H NMR of synthetic altohyrtin A / spongistatin 1 (1) (800 MHz, CD_3OD) 2.5 - 0.5 ppm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4.184
2.532
2.743
2.644
2.611
2.570
2.780
1.245
2.723
2.720
1.284
2.651
1.263
4.362
2.818
4.062
2.671
1.135
1.070
OMe
AcO
HO
OH
Cl
^{1}H NMR of natural
altohyrtin A / spongistatin 1 (1)
(Pettit, 400 MHz, CD_3OD) 7.0 - 0.5 ppm
BRUKER
FG40F259.001
DATE 6-11-91
SF 400.136
SY 133.0
O1 8000.000
SI 16384
TD 16384
SW 5208.333
HZ/PT .636
PW 5.0
RD 0.0
AQ 1.573
RG 64
NS 32
TE 297.. K
FW 6600
O2 1000.000
DP 45L PO
LB 0.0
GB 0.0
CX 40.00
CY 3.00
F1 7.5001P
F2 .202P
HZ/CM 73.020
PPM/CM .182
SR 5973.20