Supplementary Material (ESI) for Chemical Communications

Total Syntheses of Amythiamicins A, B and C

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Supporting Information Available

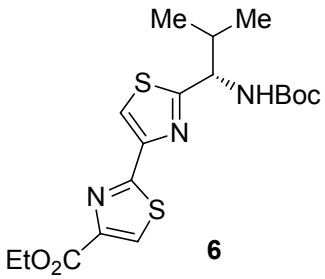
I) Experimental Section

Experimental Data for Compounds

General Procedures. All reactions were carried out under a nitrogen or argon atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Dry ethylene glycol dimethyl ether (DME), methylene chloride (CH_2Cl_2) and toluene were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. *N*,*N*′-dimethylformamide (DMF) and pyridine were purchased in anhydrous form and used without further purification. Water (H_2O), methanol (MeOH) and ethyl acetate (EtOAc) were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Reactions were monitored by thin-layer

chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as visualizing agent and an ethanolic solution of phosphomolybdic acid and cerium sulfate and heat as developing agents. E. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography. Preparative thin-layer chromatography separations were carried out on 0.25 or 2.0 mm E. Merck silica gel plates (60F-254). NMR spectra were recorded on Bruker AV-600 instrument and calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, pent = pentet, hex = hexet, br = broad. IR spectra were recorded on a Perkin-Elmer Spectrum One FTIR spectrometer with diamond ATR accessory. Optical rotation ($[\alpha]_D$) were recorded on a Perkin-Elmer Model 341 polarimeter at 25 °C using thermostable optical glass cell (100mm path length). LC/MS data were recorded on an Agilent 1100 series LC system coupled to a multimode ESI/APCI Agilent MSD. High-resolution mass spectra (HRMS) were recorded on an Agilent ESI TOF (time of flight) mass spectrometer at 3500 V emitter voltage.

Bis-thiazole 6: To a solution of thioamide **5** (300 mg, 1.29 mmol) in DMF (5 mL) were added pre-dried 4Å MS (1.0 g) and thiazole **4** (540 mg, 1.94 mmol) at 0 °C. The reaction mixture was warmed to room temperature over a period of 18 h before it was concentrated in *vacuo*. The residue was dissolved in EtOAc/H_2O (3:1, 40 mL) and the resulting solution was filtered through a mixture of silica gel and Celite®. The organic layer was separated, the aqueous was layer extracted with EtOAc (2 × 30 mL), and the combined organic layers were dried (Na_2SO_4) and then concentrated in *vacuo*. To a solution of the resulting residue in CH_2Cl_2



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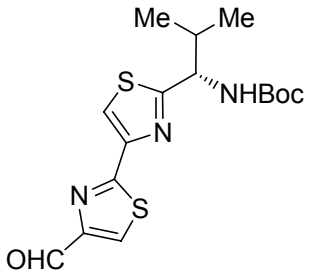
(5 mL) were added pyridine (0.31 mL, 3.87 mmol) and TFAA (0.27 mL, 1.93 mmol) at 0 °C. The resulting mixture was stirred for 3 h, and then Et_3N was added until pH 7–8 was reached. The mixture was concentrated in *vacuo* and the resulting residue was dissolved in EtOAc (40 mL) and brine (10 mL). The aqueous layer was extracted with EtOAc (2 × 20 mL), and the combined organic layers were dried (Na_2SO_4) and then concentrated in *vacuo*. Flash column chromatography (silica, hexanes:EtOAc 5:1 → 4:1) afforded bis-thiazole **6** (414 mg, 78 %) as a yellow oil. **6**: R_f = 0.30 (silica, hexanes:EtOAc 4:1); $[\alpha]_D^{25}$ = −16.7(CH_2Cl_2, *c* = 1.2); IR (film) ν_{max} 3443, 3119, 2957, 2930, 2857, 1714, 1486, 1367, 1295, 1236, 1207, 1167, 1096, 1072, 1020, 837, 777 cm^{-1}; ^1H NMR (600 MHz, CD_3CN): δ = 8.26 (s, 1 H), 8.00 (s, 1 H), 6.06–6.02 (m, 1 H), 4.85–4.77 (m, 1 H), 4.37 (q, *J* = 7.2 Hz, 2 H), 2.38–2.30 (m, 1 H), 1.47 (s, 9 H), 1.39 (t, *J* = 7.2 Hz, 3 H), 0.97 (d, *J* = 6.6 Hz, 3 H), 0.95 ppm (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR (150 MHz, CD_3CN): δ = 175.2, 163.0, 161.1, 155.8, 148.2, 147.8, 128.3, 116.9, 79.2, 73.9, 61.3, 58.5, 32.8, 27.6, 18.7, 17.1, 13.6 ppm; HRMS(ESI): calcd for $C_{18}H_{25}N_3O_4S_2\,Na^+$ [M + Na^+] 434.1178, found 434.1188.

Aldehyde 7: To a solution of ethyl ester **6** (350 mg, 0.85 mmol) in toluene (10 mL) was added DIBAL-H (1.0 M in toluene, 0.85 mL, 0.85 mmol) at −78 °C. The reaction mixture was stirred for 3 h, further DIBAL-H (1.0 M in toluene, 0.85 mL, 0.85 mmol) was added and after further stirring at −78 °C for 1 h, the reaction was quenched with MeOH (2 mL) at −78 °C. The resulting mixture was diluted with sodium potassium tartrate (5 mL, sat. aq) and warmed to room temperature over a period of 12 h. The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 40 mL), and the combined



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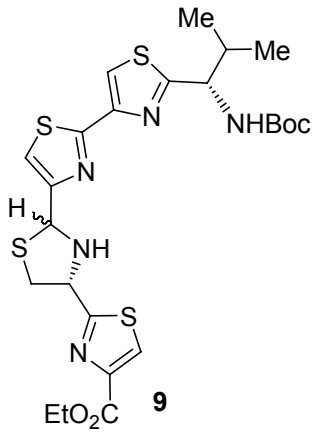
organic layers were dried (Na_2SO_4) and then concentrated in *vacuo*. Flash column chromatography (silica, hexanes:EtOAc 5:1 → 4:1) afforded aldehyde **7** (265 mg, 85 %) as a yellow foam. **7**: R_f = 0.32 (silica, hexanes:EtOAc 4:1); $[\alpha]_D^{25}$ = –25.0 (CH_2Cl_2, *c* = 1.0); IR (film) ν_{max} 2931, 1702, 1483, 1368, 1250, 1167, 1100, 1072, 839, 780, 692 cm^{-1}; ^1H NMR (600 MHz, CD_3CN): δ = 9.97 (s, 1 H), 8.37 (s, 1 H), 8.05 (s, 1 H), 6.06–6.00 (m, 1 H), 4.85–4.77 (m, 1 H), 2.38–2.28 (m, 1 H), 1.44 (s, 9 H), 0.98 (d, *J* = 6.6 Hz, 3 H), 0.96 ppm (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR (150 MHz, CD_3CN): δ = 184.6, 175.3, 163.9, 155.8, 148.0, 130.5, 79.2, 58.5, 32.8, 27.6, 18.7, 17.1 ppm; HRMS(ESI): calcd for $C_{16}H_{21}N_3O_3S_2Na^+$ [M +Na^+] 390.0916, found 390.0902.

Thiazolidine 9: To a solution of thiazole (290 mg, 0.78 mmol) in CH_2Cl_2 (2 mL) was added TFA (2 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 4 h before it was concentrated in *vacuo*. The residue was dissolved in MeOH (5 mL) and H_2O (5 mL) and then concentrated in *vacuo*. Addition and evaporation of MeOH (5 mL) and H_2O (5 mL) was repeated two more times. To a solution of the resulting crude thioamine (**8**) in H_2O/MeOH (1:3.75, 12.5 mL) were added aldehyde **7** (240 mg, 0.65 mmol), and $KHCO_3$ (650 mg, 6.5 mmol) at 0 °C. The reaction mixture was warmed to room temperature over a period of 16 h before it was concentrated in *vacuo*. The residue was extracted with CH_2Cl_2 (2 × 25 mL), and the combined organic layers were dried (Na_2SO_4) and then concentrated in *vacuo*. Flash column chromatography (silica, hexanes:EtOAc 3:1 → 1:1) afforded thiazolidine **9** (304 mg, 80 %; ca. 7:3 mixture of diastereoisomers) as a yellow foam. **9**: R_f = 0.39 and 0.29 (silica, hexanes:EtOAc 1:1); IR (film) ν_{max} 3442, 3112, 2954, 2929, 2857, 1719, 1493, 1366, 1249, 1216, 1167, 1097,



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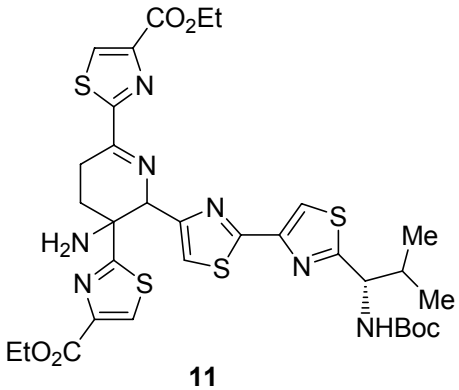
1071, 838, 777, 701 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6, 50 °C): δ = 8.49 (s, 0.7 H), 8.46 (s, 0.3 H), 8.13 (s, 0.7 H), 8.05 (s, 0.3 H), 7.74 (d, *J* = 8.4 Hz, 1 H), 7.73 (s, 0.7 H), 7.64 (s, 0.3 H), 5.88 (d, *J* = 9.6 Hz, 0.3 H), 5.71 (d, *J* = 9.6 Hz, 0.7 H), 5.32–5.27 (m, 1 H), 4.85 (dd, *J* = 10.8, 7.2 Hz, 0.3 H), 4.66–4.60 (m, 1 H), 4.57 (dd, *J* = 10.8, 7.2 Hz, 0.7 H), 4.35–4.25 (m, 2 H), 3.63 (dd, *J* = 10.8, 3.6 Hz, 0.7 H), 3.59 (dd, *J* = 10.8, 3.6 Hz, 0.3 H), 3.51 (dd, *J* = 10.2, 6.0 Hz, 1 H), 2.28–2.19 (m, 1 H), 1.41 (s, 9 H), 1.32 (t, *J* = 7.2 Hz, 3 H), 0.96 (d, *J* = 7.2 Hz, 3 H), 0.91 ppm (d, *J* = 7.2 Hz, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6, 25 °C): δ = 176.0, 174.3, 174.2, 170.8, 162.8, 161.2, 156.2, 156.1, 148.2, 146.6, 130.3, 118.5, 117.3, 116.8, 79.0, 66.8, 65.7, 61.2, 60.2, 59.0, 55.4, 32.7, 28.7, 21.2, 19.7, 18.6, 14.7, 14.6 ppm; HRMS(ESI): calcd for $C_{24}H_{31}N_5O_4S_4Na^+$ [M + Na^+] 604.1151, found 604.1142.

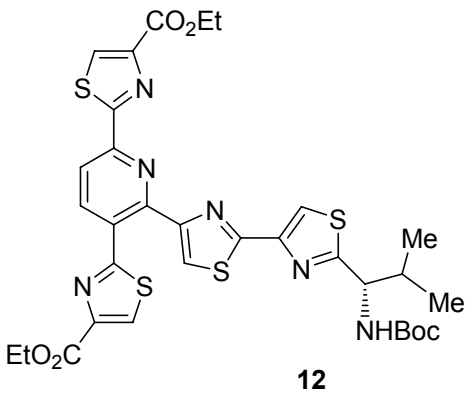
Dehydropiperidine 11: To a solution of thiazolidine **9** (150 mg, 0.257 mmol) in pyridine (15 mL) were added $BnNH_2$ (56 μL, 0.51 mmol), Ag_2CO_3 (71 mg, 0.257 mmol) and DBU (10 μL, 0.065 mmol) at −12 °C. The resulting mixture was stirred for 1 h. Cold brine (10 mL) was added to the black reaction mixture while maintaining the internal solution temperature at −12 °C, followed by addition of EtOAc (30 mL). The reaction mixture was allowed to warm to room temperature over a period of 1 h before it was filtered through a mixture of Celite® and silica gel. The organic layer was separated, washed with H_2O (2 × 10 mL), brine (10 mL), dried (Na_2SO_4) and then concentrated in *vacuo* while maintaining the water bath temperature at 28 °C. Flash column chromatography (silica, hexanes:EtOAc 1:1 → 3:7, 0.5 % Et_3N) afforded dehydropiperidine **11** (49 mg, 51 %; ca. 1:1 mixture of diastereoisomers) as a



11

yellow foam. **11**: R_f = 0.26 (silica, hexanes:EtOAc 3:7); IR (film) ν_{max} 2853, 2052, 1695, 1672, 1620, 1496, 1251, 1166, 1096, 840, 778 cm^{-1}; ^1H NMR (600 MHz, CD_3CN): δ = 8.43–8.38 (m, 1 H), 8.19–8.11 (m, 1 H), 7.75 (s, 1 H), 7.38–7.18 (m, 3 H), 6.15–6.05 (br s, 1 H), 4.80–4.70 (m, 1 H), 4.40–4.25 (m, 2 H), 3.15–2.95 (m, 2 H), 2.88–2.80 (m, 1 H), 2.55–2.40 (m, 7 H), 1.43 (s, 9 H), 1.36 (t, *J* = 6.6 Hz, 3 H), 0.94 ppm (d, *J* = 6.6 Hz, 6 H), ^{13}C NMR (150 MHz, CD_3CN): δ = 170.2, 163.5, 162.4, 161.6, 161.4, 161.1, 160.8, 155.7, 147.7, 147.2, 139.5, 131.6, 130.9, 128.4, 128.2, 127.3, 126.9, 118.2, 115.7, 115.5, 79.2, 69.7, 67.2, 61.3, 61.0, 60.6, 58.4, 56.7, 46.2, 42.6, 36.3, 32.8, 32.2, 27.6, 23.9, 22.0, 18.7, 17.1, 13.6, 8.1 ppm; HRMS(ESI) calcd for $C_{32}H_{39}N_7O_6S_4Na^+$ [M + Na^+] 768.1736, found 768.1736.

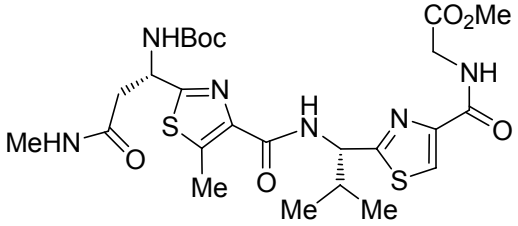
Pyridine 12: To a solution of dehydropiperidine **11** (20 mg, 0.027 mmol) in EtOAc (2 mL) was added DBU (20 μL, 0.135 mmol) at room temperature. The resulting mixture was heated at reflux for 5 h. After cooling to room temperature, the reaction mixture was concentrated in *vacuo*. Flash column chromatography (silica, hexanes:EtOAc 2:1 → 1:2) afforded pyridine **12** (7.0 mg, 36 %) as a yellow foam. **12**: R_f = 0.40 (silica, EtOAc/hexane, 1:1); $[\alpha]_D^{25}$ = –14.6 (*c* = 2.0, $CHCl_3$); IR (film): ν_{max} = 2926, 2855, 1719, 1492, 1244 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ = 8.43 (d, *J* = 7.8 Hz, 1 H), 8.35 (d, *J* = 7.8 Hz, 1 H), 8.34 (s, 1 H), 8.29 (s, 1 H), 8.02 (s, 1 H), 7.53 (s, 1 H), 5.24 (d, *J* = 9.0 Hz, 1 H), 4.93–4.88 (m, 1 H), 4.48 (q, *J* = 7.2 Hz, 2 H), 4.44 (q, *J* = 7.2 Hz, 2 H), 2.45–2.38 (m, 1 H), 1.51 (s, 9 H), 1.46 (t, *J* = 7.2 Hz, 3 H), 1.41 (t, *J* = 7.2 Hz, 3 H), 1.02 (d, *J* = 6.6 Hz, 3 H), 0.95 ppm (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 173.2, 168.8, 165.7, 162.4, 161.4, 155.5, 152.9, 150.8,



12

150.1, 148.6, 148.2, 147.1, 140.2, 130.2, 129.2, 129.2, 127.2, 121.8, 119.3, 116.7, 80.2, 61.6, 57.9, 33.3, 29.7, 28.4, 19.3, 17.4, 14.4 ppm; HRMS (ESI): calcd for $C_{32}H_{34}N_6O_6S_4Na^+$ [M + Na^+]: 749.1314 found: 749.1313.

Tripeptide 14: To a solution of bis-thiazole carboxylic acid **13** (200 mg, 0.38 mmol) in CH_2Cl_2 (5 mL) was added glycine methyl ester (40 mg, 0.46 mmol), HATU (175 mg, 0.46 mmol) and *i*Pr_2NEt (0.33 mL, 1.9 mmol) at room temperature. The resulting mixture was stirred for 12 h before it was concentrated in *vacuo*. Flash column chromatography (silica, hexanes:EtOAc 1:2 → 0:1) afforded tripeptide **14** (136 mg, 60 %) as an oil. **14**: R_f = 0.40 (silica, EtOAc); $[\alpha]_D^{25}$ = –35.0 ($CHCl_3$, *c* = 0.8); IR (film) ν_{max} 3333, 2962, 2925, 1746, 1712, 1660, 1547, 1500 cm^{-1}; ^{1}H NMR (600 MHz, CD_3CN): δ = 8.12 (d, *J* = 8.4 Hz, 1 H), 8.07–8.02 (m, 1 H), 8.06 (s, 1 H), 6.60 (d, *J* = 6.6 Hz, 1 H), 6.55 (br s, 1 H), 5.27 (dd, *J* = 9.0, 6.0 Hz, 1 H), 5.20–5.13 (m, 1 H), 4.13 (t, *J* = 5.4 Hz, 2 H), 3.87 (d, *J* = 5.4 Hz, 1 H), 3.73 (s, 3 H), 2.84 (d, *J* = 6.0 Hz, 1 H), 2.75 (s, 3 H), 2.63 (d, *J* = 4.8 Hz, 3 H), 2.53–2.42 (m, 1 H), 1.45 (s, 9 H), 1.03 (d, *J* = 6.6 Hz, 3 H), 1.02 ppm (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR (150 MHz, CD_3CN): δ = 172.4, 170.6, 170.5, 168.7, 162.0, 161.0, 155.2, 149.2, 141.8, 141.7, 123.6, 79.5, 56.1, 51.8, 49.9, 48.9, 40.7, 38.9, 36.3, 33.1, 27.6, 25.3, 18.7, 17.4, 11.8 ppm; HRMS (ESI): calcd for $C_{25}H_{36}N_6O_7S_2Na^+$ [M +Na^+] 619.1979, found 619.1974.



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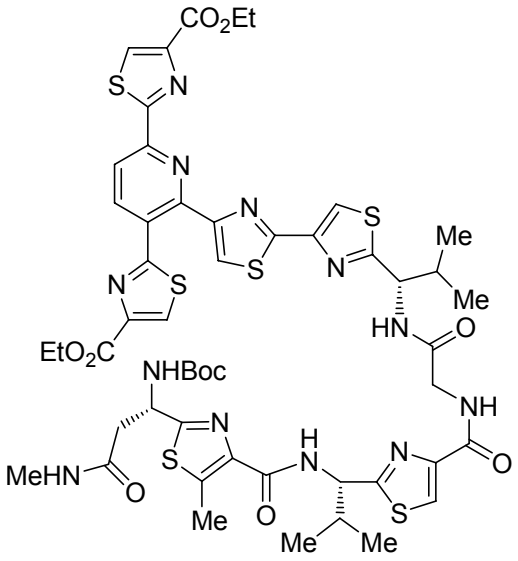
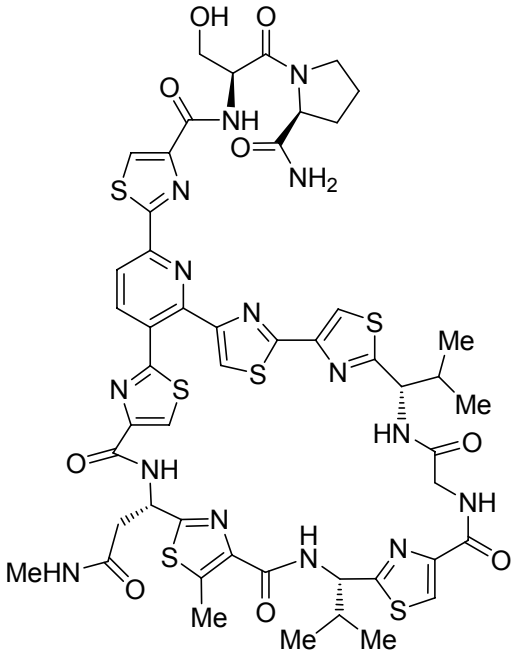
Tetrapeptide 17: To a solution of bis-thiazole **14** (143 mg, 0.24 mmol) in DME/H_2O (4:1, 5 mL) was added LiOH (50 mg, 1.2 mmol) at room temperature. The reaction mixture was stirred for 2 h before it was concentrated in *vacuo*. HCl (1.0 N aq) was added until pH 3 was reached. The resulting mixture was extracted with EtOAc (3 × 5 mL), the combined organic layers were dried (Na_2SO_4) and the solution was concentrated in *vacuo*. The resulting crude carboxylic acid **15** was used directly without further purification. To a solution of Boc-carbamate **12** (145 mg, 0.2 mmol) in CH_2Cl_2 (4 mL) was added TFA (1 mL) at room temperature. The reaction mixture was stirred for 2 h before it was concentrated in *vacuo*. The resulting crude amine **16** was used directly without further purification. To a solution of **16** in CH_2Cl_2 (5 mL) were added crude carboxylic acid **15**, HATU (114 mg, 0.3 mmol) and *i*Pr_2NEt (0.17 mL, 1.0 mmol) at room temperature. The resulting mixture was stirred for 16 h before it was concentrated in *vacuo*. Flash column chromatography (silica, hexanes:EtOAc 1:2 → 0:1) afforded tetrapeptide **17** (142 mg, 60 % for the two steps) as a white foam. **17**: R_f = 0.30 (silica, EtOAc); $[\alpha]_D^{25}$ = –45.3 ($CHCl_3$, *c* = 1.1); IR (film) ν_{max} 3351, 2968, 2925, 1715, 1667, 1547, 1494 cm^{-1}; ^1H NMR (600 MHz, CD_3CN): δ = 8.43 (s, 1 H), 8.37 (s, 1 H), 8.32 (d, *J* = 8.4 Hz, 1 H), 8.26 (d, *J* = 8.4 Hz, 1 H), 8.18–8.13 (m, 2 H), 8.12 (s, 1 H), 8.07 (s, 1 H), 7.51 (s, 1 H), 7.45 (d, *J* = 8.4 Hz, 1 H), 6.67–6.60 (m, 2 H), 5.28 (dd, *J* = 9.0, 6.0 Hz, 1 H), 5.19-5.09 (m, 1 H), 4.41 (q, *J* = 7.2 Hz, 2 H), 4.33 (q, *J* = 7.2 Hz, 2 H), 4.19 (dd, *J* = 17.4, 4.8 Hz, 1 H), 4.10 (dd, *J* = 17.4, 4.8 Hz, 1 H), 2.85–2.75 (m, 2 H), 2.71 (s, 3 H), 2.57 (d, *J* = 4.8 Hz, 3 H), 2.45–2.30 (m, 2 H), 1.42 (s, 9 H),



17

1.41 (t, J = 7.2 Hz, 3 H), 1.33 (t, J = 7.2 Hz, 3 H), 0.98 (d, J = 6.6 Hz, 6 H), 0.96 ppm (d, J = 6.6 Hz, 6 H); ^{13}C NMR (150 MHz, CD_3CN): δ = 173.1, 171.9, 170.5, 169.5, 168.7, 168.4, 165.3, 162.0, 162.0, 161.9, 161.7, 161.1, 161.1, 161.0, 153.5, 150.8, 149.3, 148.5, 148.3, 146.8, 141.7, 140.3, 130.8, 130.1, 129.2, 123.6, 122.4, 118.7, 116.3, 79.4, 61.2, 61.1, 56.9, 55.9, 49.8, 42.5, 38.8, 33.4, 32.9, 27.6, 25.3, 18.7, 18.6, 17.2, 13.6, 11.8 ppm; HRMS (ESI): calcd for $C_{51}H_{58}N_{12}O_{10}S_6$ [M + H$^+$] 1191.2795, found 1191.2820.

Amythiamicin B (2): To a solution of **17** (100 mg, 0.084 mmol) in DME/H_2O (4:1, 5 mL) was added LiOH (35 mg, 0.84 mmol) at room temperature. The resulting mixture was stirred for 5 h, at room temperature before it was acidified with HCl (1.0 N, aq). The crude bis-carboxylic acid **18** was used directly without further purification. To a solution of bis-acid **18** in CH_2Cl_2 (4.0 mL) was added TFA (1.0 mL) at room temperature. The reaction mixture was stirred for 2 h before it was concentrated in *vacuo*. To a solution of the resulting crude amino bis-acid in CH_2Cl_2 (67 mL) and DMF (17 mL) were added HATU (160 mg, 0.42 mmol) and *i*Pr_2NEt (0.14 mL, 0.84 mmol) at 0 °C. The reaction mixture was stirred for 3 h at 0 °C before HCl salt of prolinamide-serine conjugate (**21**) (100 mg, 0.42 mmol) was added. The reaction mixture was stirred for 24 h before it was concentrated in *vacuo*. Flash column chromatography (silica, EtOAc:MeOH 3:1) afforded amythiamicin B (**2**) (25 mg, 25 % for the three steps) as a white powder. **2**: R_f = 0.32 (silica,



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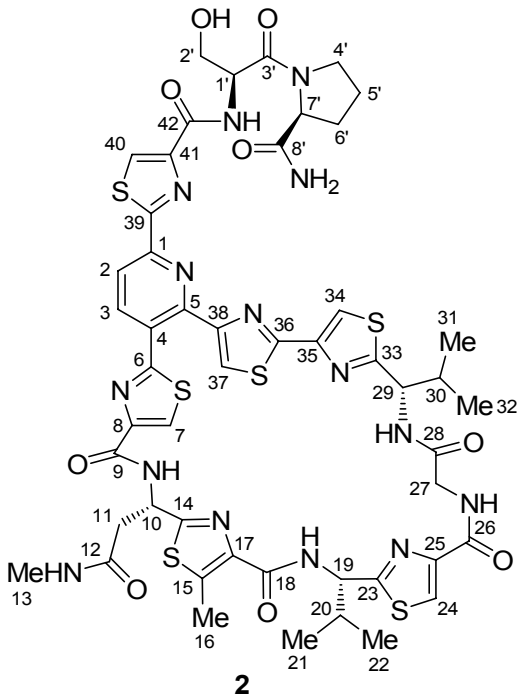
EtOAc:MeOH 3:1); $[\alpha]_D^{25}$ = +154.0 (MeOH, *c* = 0.1); IR (film) ν_{max} 3397, 2964, 1658, 1539, 1070 cm^{-1}; ^1H NMR (600 MHz, pyridine-d^5): δ = 10.19 (d, *J* = 6.6 Hz, 1 H), 9.57 (d, *J* = 9.0 Hz, 1 H), 9.19 (d, *J* = 7.2 Hz, 1 H), 9.14 (d, *J* = 7.2 Hz, 1 H), 9.13–9.11 (m, 1 H), 8.84 (s, 1 H), 8.67 (s, 1 H), 8.43 (s, 1 H), 8.37 (s, 1 H), 8.23 (br s, 1 H), 8.20 (br s, 1 H), 8.15 (d, *J* = 7.8 Hz, 1 H), 8.07 (m, 1 H), 8.03 (d, *J* = 7.8 Hz, 1 H), 7.70 (s, 1 H), 7.36 (m, 1 H), 5.73 (m, 1 H), 5.64 (m, 1 H), 5.47 (m, 1 H), 5.40 (m, 1 H), 5.33 (m, 1 H), 4.53 (m, 1 H), 4.47 (m, 1 H), 4.20 (m, 1 H), 4.02 (m, 1 H), 3.84 (m, 1 H), 3.45 (d, *J* = 15.6 Hz, 1 H), 2.66 (d, *J* = 3.6 Hz, 3 H), 2.61 (s, 3 H), 2.30 (m, 1 H), 2.26 (m, 1 H), 2.13 (m, 1 H), 2.11 (m, 1 H), 1.97 (m, 1 H), 1.75 (m, 1 H), 1.71 (m, 1 H), 1.05 (d, *J* = 6.6 Hz, 3 H), 0.94 (d, *J* = 6.6 Hz, 3 H), 0.93 (d, *J* = 6.6 Hz, 3 H), 0.80 ppm (d, *J* = 7.2 Hz, 3 H); ^{13}C NMR (150 MHz, pyridine-d^5): δ = 175.5, 175.0, 171.3, 170.5, 170.4, 169.5, 169.0, 168.5, 165.7, 162.0, 161.7, 161.6, 161.0, 160.8, 154.8, 152.1, 151.1, 149.1, 143.2, 141.0, 140.3, 128.0, 127.4, 126.4, 124.6, 118.6, 115.6, 63.8, 61.0, 60.0, 56.2, 54.0, 49.2, 47.8, 42.0, 39.0, 35.3, 33.9, 30.0, 26.3, 25.1, 19.7, 19.3, 18.5, 18.2, 12.2 ppm; HRMS (ESI): calcd for $C_{50}H_{53}N_{15}O_9S_6Na^+$ [M + Na^+] 1222.2367, found 1222.2388.



2

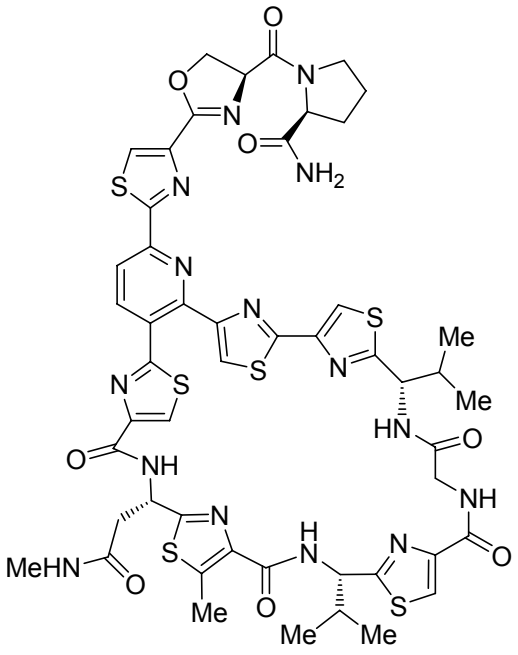
Table 1. NMR comparison for natural versus synthetic amythiamicin B (**2**)

Proton	Natural[1] (Pyridine-d^5, 600 MHz, 25 °C) δ ^{1}H [ppm, mult, J (Hz)]	Synthetic (Pyridine-d^5, 600 MHz, 25 °C) δ ^{1}H [ppm, mult, J (Hz)]	Position	Natural[1] (Pyridine-d^5, 150 MHz, 25 °C) δ ^{13}C (ppm)	Synthetic (Pyridine-d^5, 150 MHz, 25 °C) δ ^{13}C (ppm)
2-H	8.01 (d, J = 8.1 Hz)	8.03 (d, J = 7.8 Hz)	1	150.9	obscured[a]
3-H	8.15 (d, J = 8.1 Hz)	8.15 (d, J = 7.8 Hz)	2	118.6	118.6
7-H	8.84 (s)	8.84 (s)	3	141.0	141.0
10-H	5.73 (dt, J = 8.9, 3.1 Hz)	5.73 (m)	4	128.0	128.0
11-H_a	1.70 (br dd)	1.71 (m)	5	150.6	obscured[a]
11-H_b	3.45 (dd, J = 16.5, 3.1 Hz)	3.45 (d, J = 15.6 Hz)	6	165.6	165.7
13-H_3	2.62 (d, J = 4.6 Hz)	2.66 (d, J = 3.6 Hz)	7	126.3	126.4
16-H_3	2.61 (s)	2.61 (s)	8	151.0	151.1
19-H	5.47 (dd, J = 7.7, 4.7 Hz)	5.47 (m)	9	161.5	161.6
20-H	2.30 (m)	2.30 (m)	10	49.1	49.2
21-H_3	0.94 (d, J = 6.7 Hz)	0.93 (d, J = 6.6 Hz)	11	38.9	39.0
22-H_3	0.95 (d, J = 6.7 Hz)	0.94 (d, J = 6.6 Hz)	12	170.4	170.5
24-H	8.43 (s)	8.43 (s)	13	26.2	26.3
27-H_a	4.18 (dd, J = 17.1, 3.7 Hz)	4.20 (m)	14	169.5	169.5
27-H_b	5.40 (dd, J = 17.1, 9.3 Hz)	5.40 (m)	15	140.2	140.3
29-H	5.33 (dd, J = 7.8, 6.7 Hz)	5.33 (m)	16	12.1	12.2
30-H	2.11 (m)	2.11 (m)	17	143.1	143.2
31-H_3	0.80 (d, J = 6.6 Hz)	0.80 (d, J = 7.2 Hz)	18	162.0	162.0
32-H_3	1.05 (d, J = 6.6 Hz)	1.05 (d, J = 6.6 Hz)	19	56.2	56.2
34-H	7.71 (s)	7.70 (s)	20	35.3	35.3
37-H	8.38 (s)	8.37 (s)	21	18.2	18.2
40-H	8.67 (s)	8.67 (s)	22	18.5	18.5
1′-H	5.64 (ddd, J = 7.7, 7.1, 6.7 Hz)	5.64 (m)	23	169.0	169.0
2′-H_a	4.47 (m)	4.47 (m)	24	124.6	124.6

2′-H_b	4.55 (m)	4.53 (m)	25	150.0	obscured[a]
2′-OH	7.36 (t, *J* = 5.2 Hz)	7.36 (m)	26	161.7	161.7
4′-H_a	3.85 (m)	3.84 (m)	27	41.9	42.0
4′-H_b	4.03 (m)	4.02 (m)	28	171.3	171.3
5′-H_a	1.76 (m)	1.75 (m)	29	60.0	60.0
5′-H_b	1.96 (m)	1.97 (m)	30	33.8	33.9
6′-H_a	2.14 (m)	2.13 (m)	31	19.3	19.3
6′-H_b	2.26 (m)	2.26 (m)	32	19.7	19.7
7′-H	5.10 (dd, *J* = 7.5, 4.0 Hz)	Obscured[a]	33	175.5	175.5
10-NH	9.58 (d, *J* = 8.9 Hz)	9.57 (d, *J* = 9.0 Hz)	34	115.6	115.6
12-NH	8.07 (br q, *J* = 4.5 Hz)	8.07 (m)	35	149.0	149.1
19-NH	9.14 (d, *J* = 7.7 Hz)	9.14 (d, *J* = 7.2 Hz)	36	160.8	160.8
27-NH	9.08 (dd, *J* = 9.3, 3.7 Hz)	9.13–9.11 (m)	37	124.2	obscured[a]
29-NH	10.18 (d, *J* = 6.7 Hz)	10.19 (d, *J* = 6.6 Hz)	38	154.8	154.8
1′-NH	9.19 (d, *J* = 7.7 Hz)	9.19 (d, *J* = 7.2 Hz)	39	168.4	168.5
8′-NH_a	8.21	8.20 (br s)	40	127.4	127.4
8′-NH_b	8.22	8.23 (br s)	41	152.1	152.1
			42	160.9	161.0
			1′	53.9	54.0
			2′	63.8	63.8
			3′	170.4	170.4
			4′	47.8	47.8
			5′	25.0	25.1
			6′	29.8	30.0
			7′	61.1	61.0
			8′	174.9	175.0

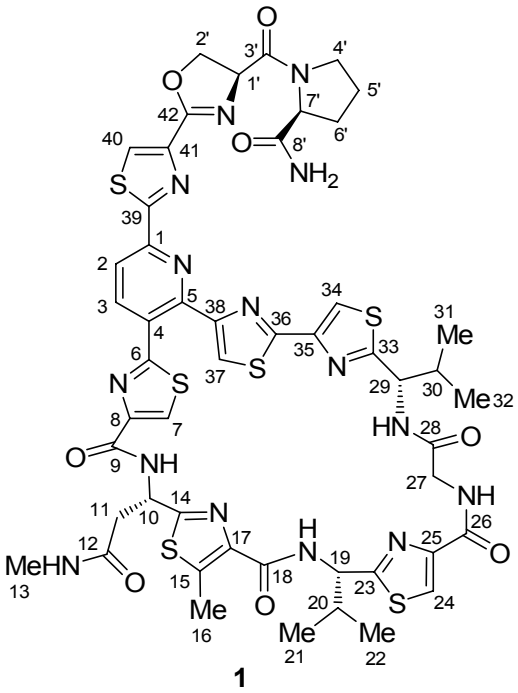
a. signal obscured by NMR solvent

Amythiamicin A (1): To a solution of amythiamicin B **(2)** (10 mg, 0.0083 mmol) in CH_2Cl_2 (2 mL) was added DAST (2 μL, 0.013 mmol) at 25 °C. The reaction mixture was stirred for 1 h before it was concentrated in *vacuo*. Flash column chromatography (silica, EtOAc:MeOH 3:1) afforded amythiamicin A (**1**) (6.9 mg, 70 %) as a white powder.



1

1: R_f = 0.34 (silica, EtOAc:MeOH 3:1); $[\alpha]_D^{25}$ = +124 (DMSO, *c* = 0.1); IR (film) ν_{max} 3300, 2980, 1657, 1539, 1495, 990 cm^{-1}; ^1H NMR (600 MHz, pyridine-d^5): δ = 10.15 (br s, 1 H), 9.56 (d, *J* = 9.0 Hz, 1 H), 9.12 (d, *J* = 7.8 Hz, 1 H), 9.08 (br s, 1 H), 8.81 (s, 1 H), 8.40 (s, 1 H), 8.40 (s, 1 H), 8.40 (br s, 1 H), 8.38 (s, 1 H), 8.37 (m, 1 H), 8.34 (br s, 1 H), 8.12 (d, *J* = 7.8 Hz, 1 H), 8.03 (m, 1 H), 7.68 (s, 1 H), 5.72 (m, 1 H), 5.46 (m, 1 H), 5.46 (m, 1 H), 5.36 (m, 1 H), 5.32 (m, 1 H), 5.30 (m, 1 H), 4.40 (m, 1 H), 4.38 (m, 1 H), 4.18 (dd, *J* = 16.8, 3.0 Hz, 1 H), 3.98 (m, 1 H), 3.42 (dd, *J* = 14.4 Hz, 1 H), 2.64 (d, *J* = 4.2 Hz, 3 H), 2.58 (s, 3 H), 2.29 (m, 1 H), 2.23 (m, 1 H), 2.12 (m, 1 H), 2.12 (m, 1 H), 1.97 (m, 1 H), 1.78 (m, 1 H), 1.69 (br dd, 1 H), 1.04 (d, *J* = 6.6 Hz, 3 H), 0.93 (d, *J* = 6.7 Hz, 3 H), 0.92 (d, *J* = 6.7 Hz, 3 H), 0.78 ppm (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR (150 MHz, pyridine-d^5): δ = 175.4, 174.7, 171.3, 170.4, 167.5, 169.1, 169.0, 168.5, 165.6, 161.9, 161.7, 161.6, 160.8, 160.4, 154.7, 151.2, 151.0, 149.0, 146.1, 143.1, 141.0, 140.1, 128.2, 127.9, 126.2, 124.5, 124.2, 118.7, 115.5, 69.5, 69.4, 61.1, 60.0, 56.3, 49.1, 47.7, 41.9, 38.9, 35.2, 33.8, 29.8, 26.2, 25.0, 19.7, 19.7, 18.5, 18.2, 12.1 ppm; HRMS (ESI): calcd for $C_{50}H_{51}N_{15}O_8S_6Na^+$ [M + Na^+] 1204.2261, found 1204.2292.



1

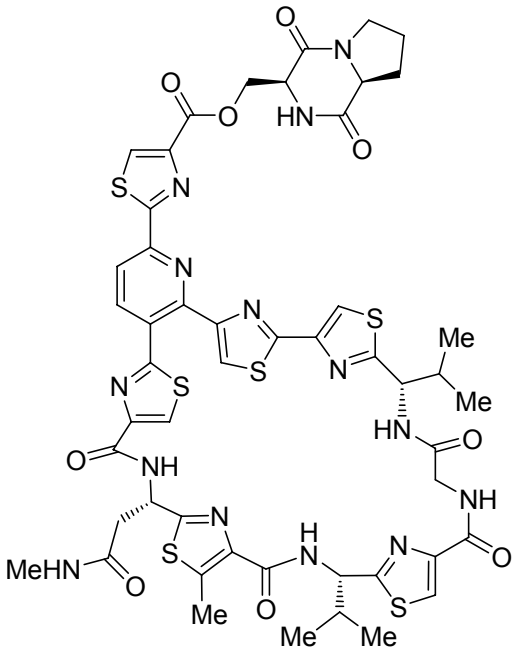
Table 2. NMR comparison for natural versus synthetic amythiamicin A (**1**)

Proton	Natural[1] (Pyridine-d^5, 600 MHz, 25 °C) δ ^{1}H [ppm, mult, J (Hz)]	Synthetic (Pyridine-d^5, 600 MHz, 25 °C) δ ^{1}H [ppm, mult, J (Hz)]	Position	Natural[1] (Pyridine-d^5, 150 MHz, 25 °C) δ ^{13}C (ppm)	Synthetic (Pyridine-d^5, 150 MHz, 25 °C) δ ^{13}C (ppm)
2-H	8.37 (d, J = 7.9 Hz)	8.37 (m)	1	151.1	151.2
3-H	8.15 (d, J = 7.9 Hz)	8.12 (d, J = 7.8 Hz)	2	118.6	118.7
7-H	8.83 (s)	8.81 (s)	3	141.1	141.0
10-H	5.73 (dt, J = 9.1, 3.0 Hz)	5.72 (m)	4	127.9	127.9
11-H_a	1.70 (br dd)	1.69 (br dd)	5	150.6	obscured[a]
11-H_b	3.44 (dd, J = 16.5, 3.0 Hz)	3.42 (dd, J = 14.4 Hz)	6	165.6	165.6
13-H_3	2.66 (d, J = 4.3 Hz)	2.64 (d, J = 4.2 Hz)	7	126.3	126.2
16-H_3	2.61 (s)	2.58 (s)	8	151.0	151.0
19-H	5.46 (dd, J = 7.6, 4.6 Hz)	5.46 (m)	9	161.5	161.6
20-H	2.30 (m)	2.29 (m)	10	49.0	49.1
21-H_3	0.94 (d, J = 6.7 Hz)	0.92 (d, J = 6.7 Hz)	11	38.9	38.9
22-H_3	0.94 (d, J = 6.7 Hz)	0.93 (d, J = 6.7 Hz)	12	170.4	170.4
24-H	8.43 (s)	8.40 (s)	13	26.2	26.2
27-H_a	4.18 (dd, J = 17.1, 3.7 Hz)	4.18 (dd, J = 16.8, 3.0 Hz)	14	169.5	169.5
27-H_b	5.46 (dd, J = 17.1, 8.8 Hz)	5.46 (m)	15	140.2	140.1
29-H	5.30 (dd, J = 7.8, 7.0 Hz)	5.30 (m)	16	12.1	12.1
30-H	2.12 (m)	2.12 (m)	17	143.1	143.1
31-H_3	0.81 (d, J = 6.6 Hz)	0.78 (d, J = 6.6 Hz)	18	162.0	161.9
32-H_3	1.05 (d, J = 6.6 Hz)	1.04 (d, J = 6.6 Hz)	19	56.3	56.3
34-H	7.70 (s)	7.68 (s)	20	35.3	35.2
37-H	8.38 (s)	8.38 (s)	21	18.2	18.2
40-H	8.42 (s)	8.40 (s)	22	18.5	18.5
1′-H	5.36 (dd, J = 9.5, 7.4 Hz)	5.36 (m)	23	169.0	169.0
2′-H_a	4.40 (dd, J = 17.1, 9.5 Hz)	4.40 (m)	24	124.6	124.5

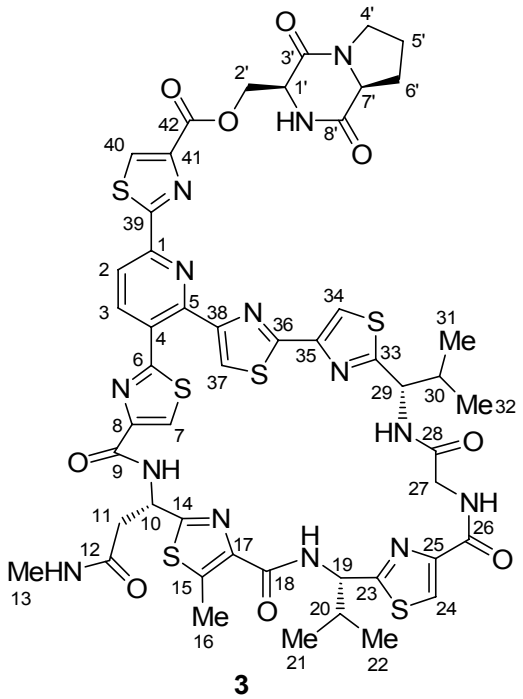
2′-H_b	5.32 (dd, J = 17.1, 7.4 Hz)	5.32 (m)	25	150.0	obscured[a]
4′-H_a	3.98 (m)	3.98 (m)	26	161.7	161.7
4′-H_b	4.38 (m)	4.38 (m)	27	41.9	41.9
5′-H_a	1.79 (m)	1.78 (m)	28	171.3	171.3
5′-H_b	2.12 (m)	2.12 (m)	29	60.0	60.0
6′-H_a	1.97 (m)	1.97 (m)	30	33.8	33.8
6′-H_b	2.25 (m)	2.23 (m)	31	19.3	19.3
7′-H	4.93 (dd, J = 8.4, 3.6 Hz)	Obscured[a]	32	19.7	19.7
10-NH	9.57 (d, J = 9.1 Hz)	9.56 (d, J = 9.0 Hz)	33	175.4	175.4
12-NH	8.05 (br q, J = 4.3 Hz)	8.03 (m)	34	115.5	115.5
19-NH	9.14 (d, J = 7.6 Hz)	9.12 (d, J = 7.8 Hz)	35	149.0	149.0
27-NH	9.08 (dd, J = 8.8, 3.7 Hz)	9.08 (br s)	36	160.8	160.8
29-NH	10.16 (d, J = 7.0 Hz)	10.15 (br s)	37	124.2	124.2
8′-NH_a	8.21	8.34 (br s)	38	154.8	154.7
8′-NH_b	8.40	8.40 (br s)	39	169.1	169.1
			40	128.2	128.2
			41	146.0	146.1
			42	160.4	160.4
			1′	69.3	69.4
			2′	69.5	69.5
			3′	168.5	168.5
			4′	47.7	47.7
			5′	25.0	25.0
			6′	29.9	29.8
			7′	61.1	61.1
			8′	174.7	174.7

a. signal obscured by NMR solvent

Amythiamicin C (3): Amythiamicin A (**1**) (3.0 mg, 0.0025 mmol) was treated with HC1 (1.0 N, aq, 3 ml) at 110 °C for 1 h in a sealed tube before it was concentrated under reduced pressure. Preparative TLC purification (silica, EtOAc:MeOH 3:1) afforded amythiamicin C (**3**) (1.8 mg, 60 %) as a white powder. **3**: R_f = 0.2 (silica, EtOAc:MeOH 3:1); ^{1}H NMR (600 MHz, pyridine-d^5): δ = 10.18 (d, J = 6.6 Hz, 1 H), 9.82 (br s, 1 H), 9.57 (d, J = 8.4 Hz, 1 H), 9.14 (d, J = 7.8 Hz, 1 H), 9.08 (m, 1 H), 8.84 (s, 1 H), 8.53 (s, 1 H), 8.42 (s, 1 H), 8.35 (s, 1 H), 8.30 (d, J = 8.4 Hz, 1 H), 8.17 (d, J = 8.4 Hz, 1 H), 8.06 (m, 1 H), 7.70 (s, 1 H), 5.73 (m, 1 H), 5.46 (dd, J = 7.8, 4.8 Hz, 1 H), 5.40 (m, 1 H), 5.40 (m, 1 H), 5.32 (dd, J = 7.8, 7.2 Hz, 1 H), 5.15 (dd, J = 10.8, 5.4 Hz, 1 H), 4.85 (m, 1 H), 4.29 (t, J = 7.8 Hz, 1 H), 4.18 (d, J = 15.6 Hz, 1 H), 3.63 (m, 1 H), 3.48 (m, 1 H), 3.45 (m, 1 H), 2.65 (br s, 3 H), 2.59 (s, 3 H), 2.30 (m, 1 H), 2.30 (m, 1 H), 2.23 (m, 1 H), 2.10 (m, 1 H), 1.69 (m, 1 H), 1.67 (m, 1 H), 1.65 (m, 1 H), 1.04 (d, J = 6.0 Hz, 3 H), 0.93 (d, J = 6.6 Hz, 3 H), 0.93 (d, J = 6.6 Hz, 3 H), 0.80 ppm (d, J = 6.6 Hz, 3 H).



3



3

Table 3. NMR comparison for natural versus synthetic amythiamicin C (**3**)

Proton	Natural[1] (Pyridine-d^5, 600 MHz, 25 °C) δ ^{1}H [ppm, mult, J (Hz)]	Synthetic (Pyridine-d^5, 600 MHz, 25 °C) δ ^{1}H [ppm, mult, J (Hz)]
2-H	8.30 (d, J = 8.2 Hz)	8.30 (d, J = 8.4 Hz)
3-H	8.17 (d, J = 8.2 Hz)	8.17 (d, J = 8.4 Hz)
7-H	8.85 (s)	8.84 (s)
10-H	5.73 (dt, J = 9.1, 3.2 Hz)	5.73 (m)
11-H_a	1.70 (m)	1.69 (m)
11-H_b	3.45 (dd, J = 16.1, 3.2 Hz)	3.45 (m)
13-H_3	2.66 (d, J = 4.6 Hz)	2.65 (br d)
16-H_3	2.61 (s)	2.59 (s)
19-H	5.46 (dd, J = 7.9, 4.6 Hz)	5.46 (dd, J = 7.8, 4.8 Hz)
20-H	2.30 (m)	2.30 (m)
21-H_3	0.94 (d, J = 6.7 Hz)	0.93 (d, J = 6.6 Hz)
22-H_3	0.94 (d, J = 6.7 Hz)	0.93 (d, J = 6.6 Hz)
24-H	8.43 (s)	8.42 (s)
27-H_a	4.18 (dd, J = 17.1, 3.7 Hz)	4.18 (d, J = 15.6 Hz)
27-H_b	5.40 (dd, J = 17.1, 9.0 Hz)	5.40 (m)
29-H	5.30 (dd, J = 7.8, 7.1 Hz)	5.32 (dd, J = 7.8, 7.2 Hz)
30-H	2.10 (m)	2.10 (m)
31-H_3	0.81 (d, J = 6.7 Hz)	0.80 (d, J = 6.6 Hz)
32-H_3	1.05 (d, J = 6.7 Hz)	1.04 (d, J = 6.0 Hz)
34-H	7.71 (s)	7.70 (s)
37-H	8.36 (s)	8.35 (s)
40-H	8.54 (s)	8.53 (s)
1′-H	4.86 (br dd, J = 6.4, 3.3 Hz)	4.85 (m)
2′-H_a	5.15 (dd, J = 11.6, 6.4 Hz)	5.15 (dd, J = 10.8, 5.4 Hz)
2′-H_b	5.40 (dd, J = 11.6, 3.3 Hz)	5.40 (m)
4′-H_a	3.50 (m)	3.48 (m)

4′-H_b	3.63 (m)	3.63 (m)
5′-H_a	1.67 (m)	1.67 (m)
5′-H_b	2.30 (m)	2.30 (m)
6′-H_a	1.65 (m)	1.65 (m)
6′-H_b	2.23 (m)	2.23 (m)
7′-H	4.29 (t, J = 8.2 Hz)	4.29 (t, J = 7.8 Hz)
10-NH	9.57 (d, J = 9.1 Hz)	9.57 (d, J = 8.4 Hz)
12-NH	8.06 (br q, J = 4.6 Hz)	8.06 (m)
19-NH	9.14 (d, J = 7.7 Hz)	9.14 (d, J = 7.8 Hz)
27-NH	9.08 (dd, J = 9.0, 3.7 Hz)	9.08 (m)
29-NH	10.18 (d, J = 7.1 Hz)	10.18 (d, J = 6.6 Hz)
1′-NH	9.82 (br s)	9.82 (br s)

II) Abbreviations

TFA = trifluoroacetic acid

TFAA = trifluoroacetic anhydride

DIBAL-H = diisobutylaluminium hydride

Bn = benzyl

DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene

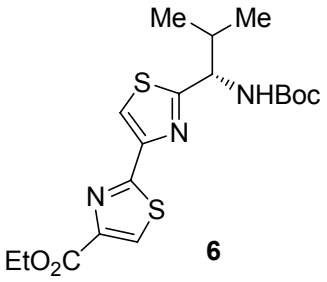
HATU = *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

DAST = *N,N′*-diethylaminosulfur trifluoride

III) References

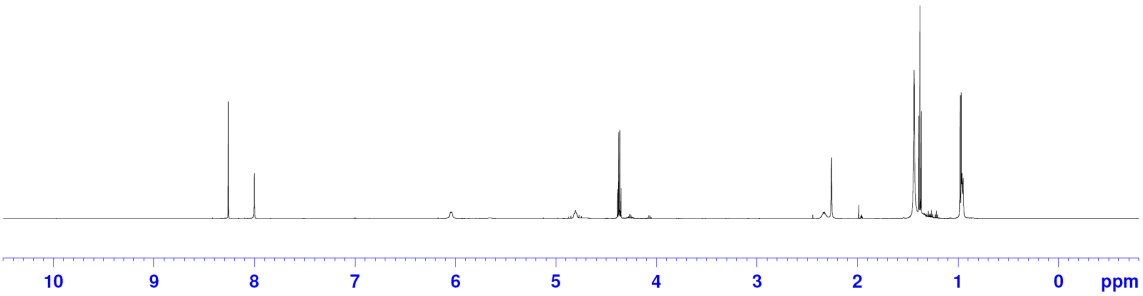
[1] K. Shimanaka, Y. Takahashi, H. Iinuma, H. Naganawa and T. Takeuchi, *J. Antibiot*. **1994**, *47*, 1153–1159.

IV) ^{1}H and ^{13}C NMR of all compounds

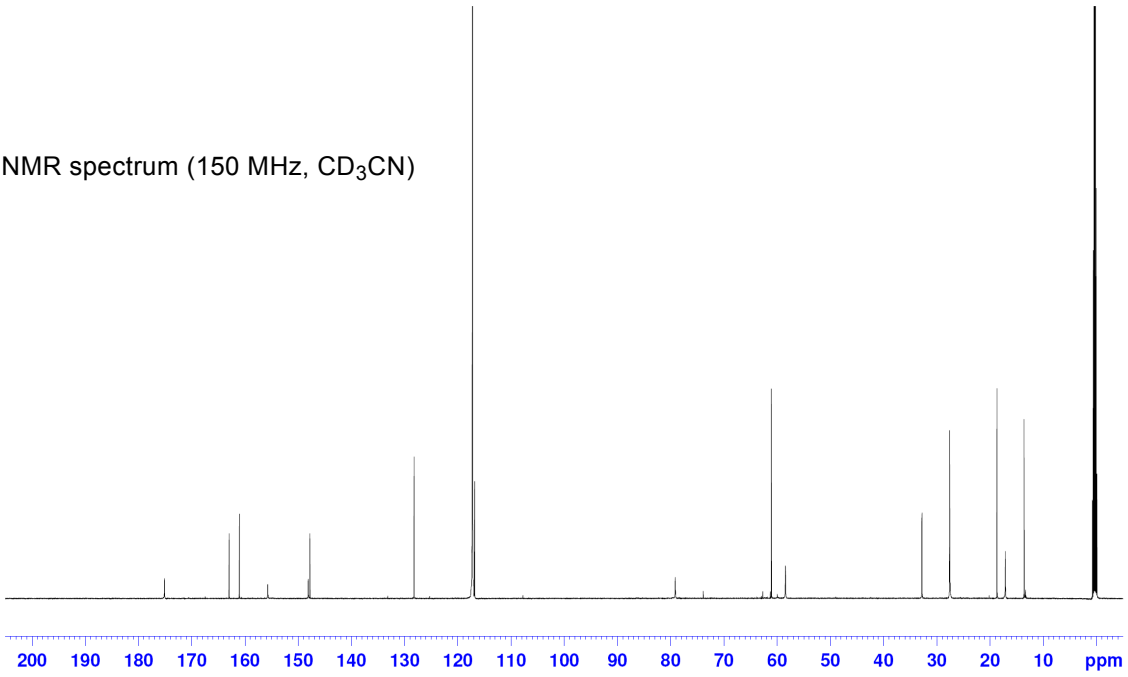


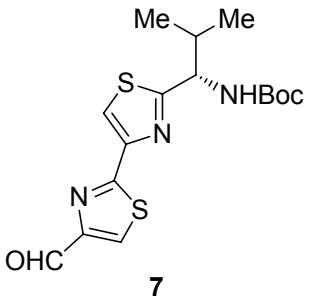
6

^{1}H NMR spectrum (600 MHz, CD_3CN)



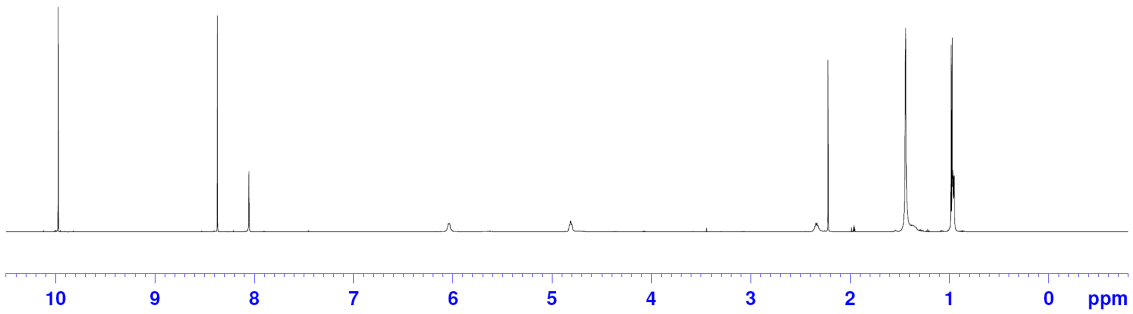
^{13}C NMR spectrum (150 MHz, CD_3CN)



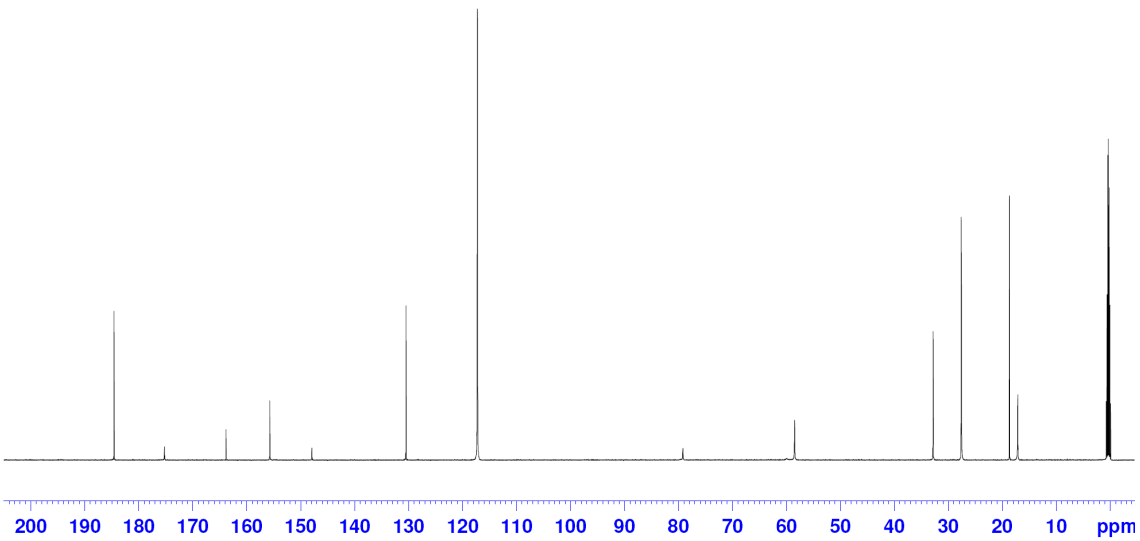


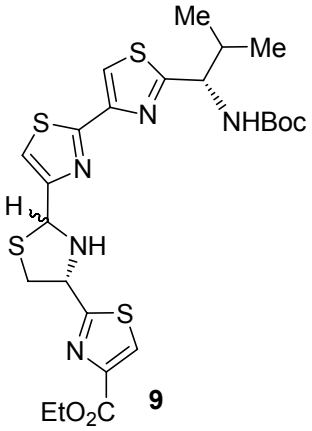
7

^{1}H NMR spectrum (600 MHz, CD_3CN)



^{13}C NMR spectrum (150 MHz, CD_3CN)

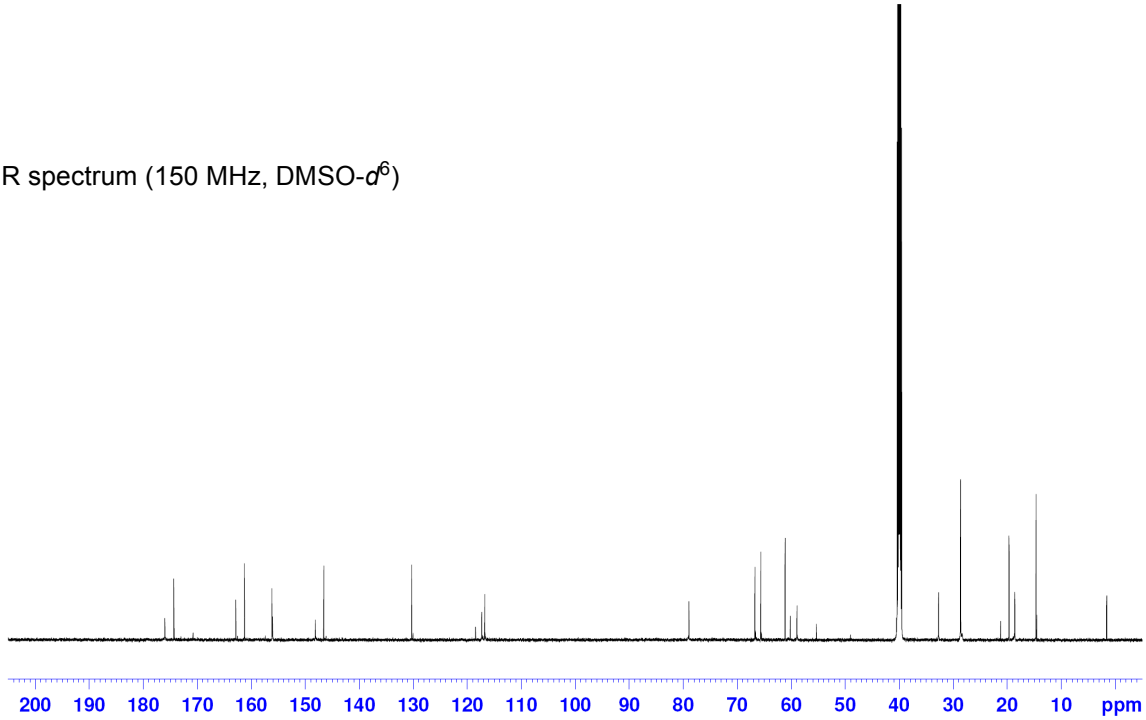


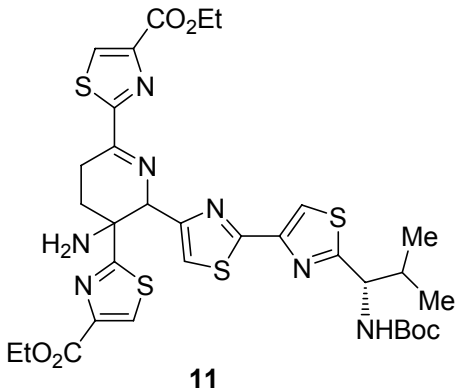


9

^{1}H NMR spectrum (600 MHz, DMSO-*d*^{6}, 50 ^{o}C)

^{13}C NMR spectrum (150 MHz, DMSO-*d*^{6})

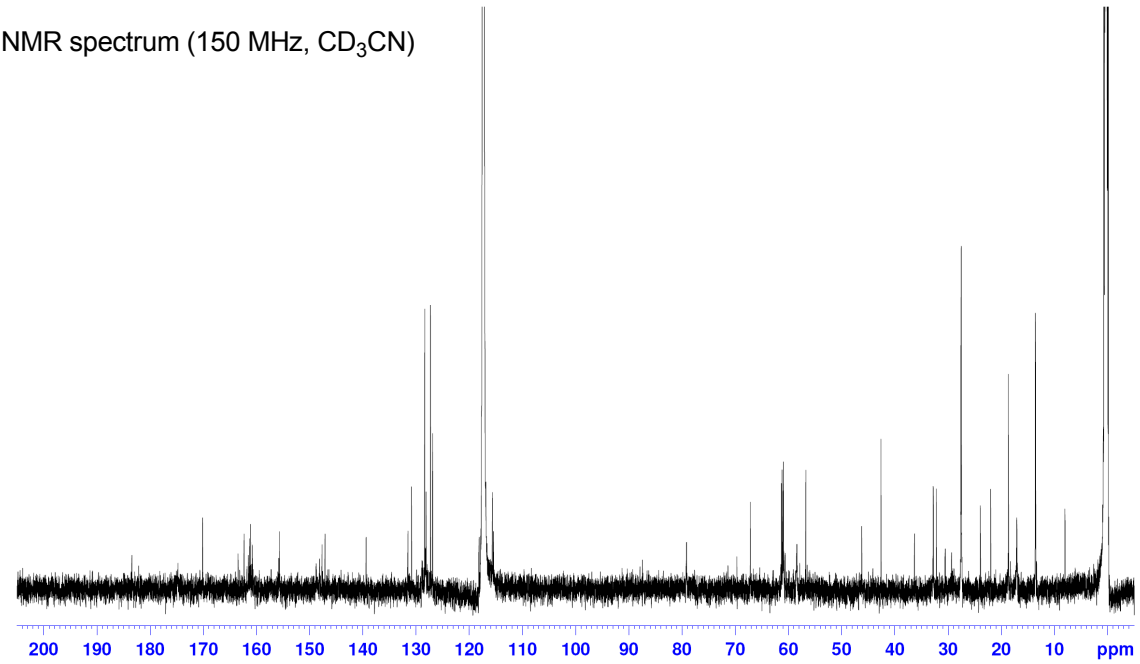


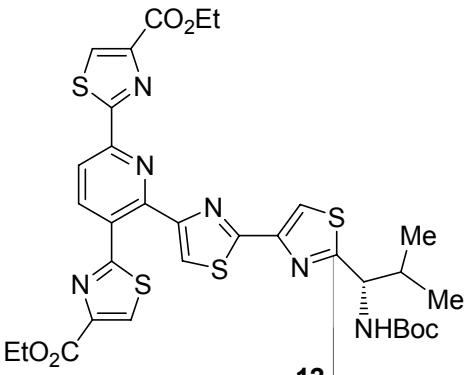


11

^{1}H NMR spectrum (600 MHz, CD_3CN)

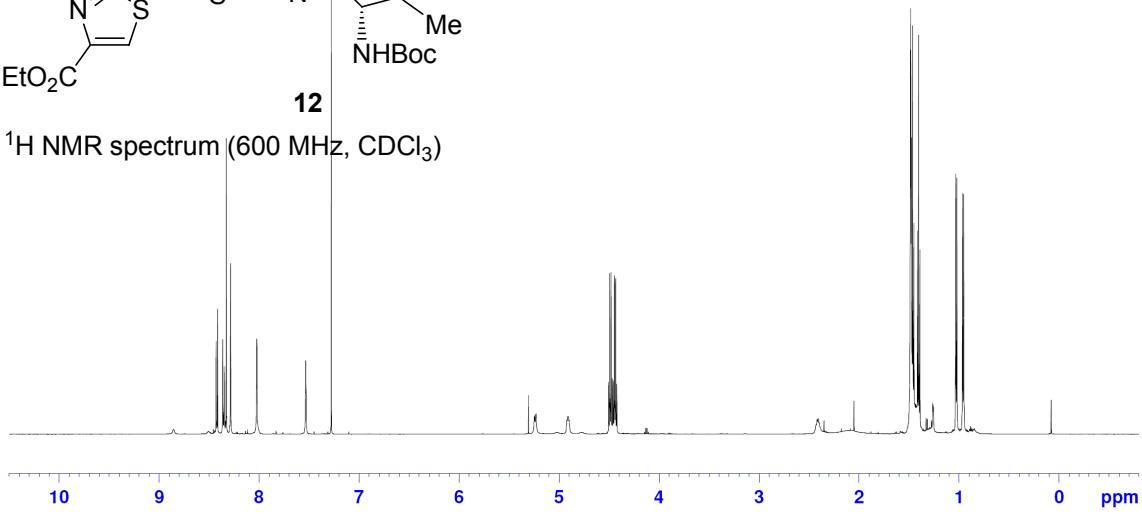
^{13}C NMR spectrum (150 MHz, CD_3CN)



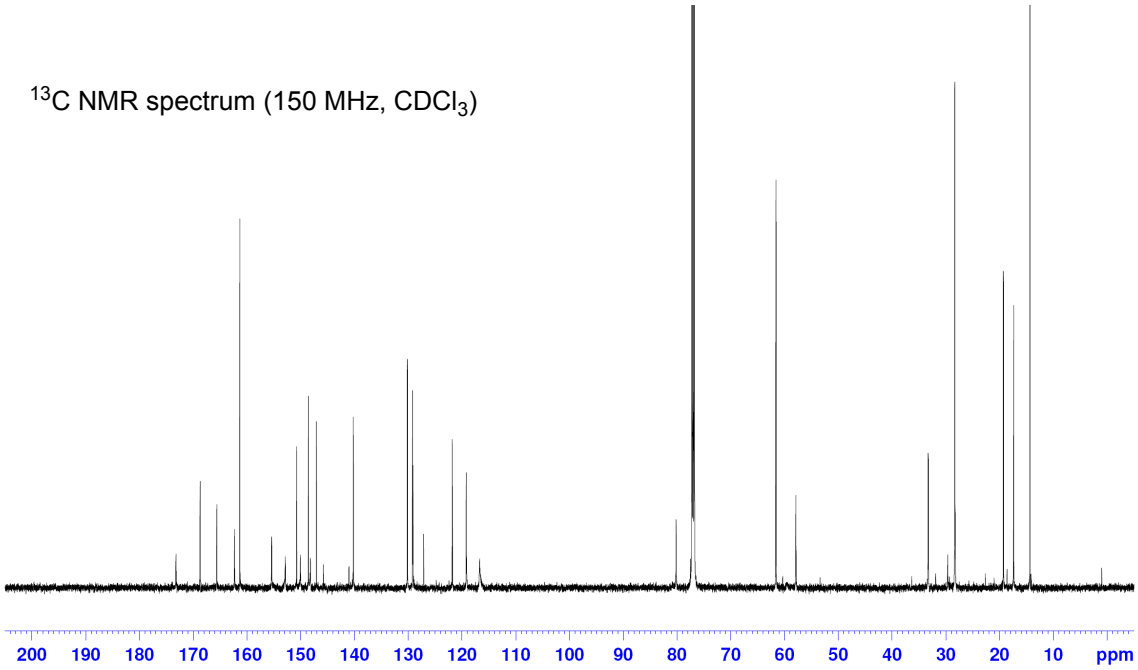


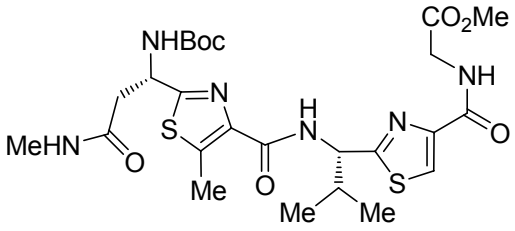
12

^{1}H NMR spectrum (600 MHz, $CDCl_3$)



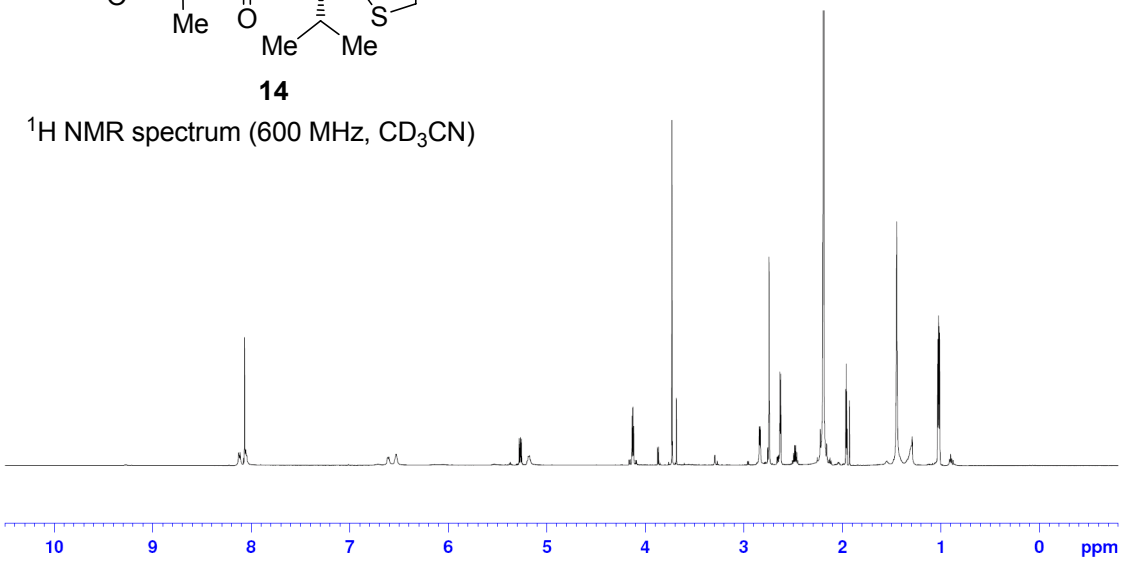
^{13}C NMR spectrum (150 MHz, $CDCl_3$)



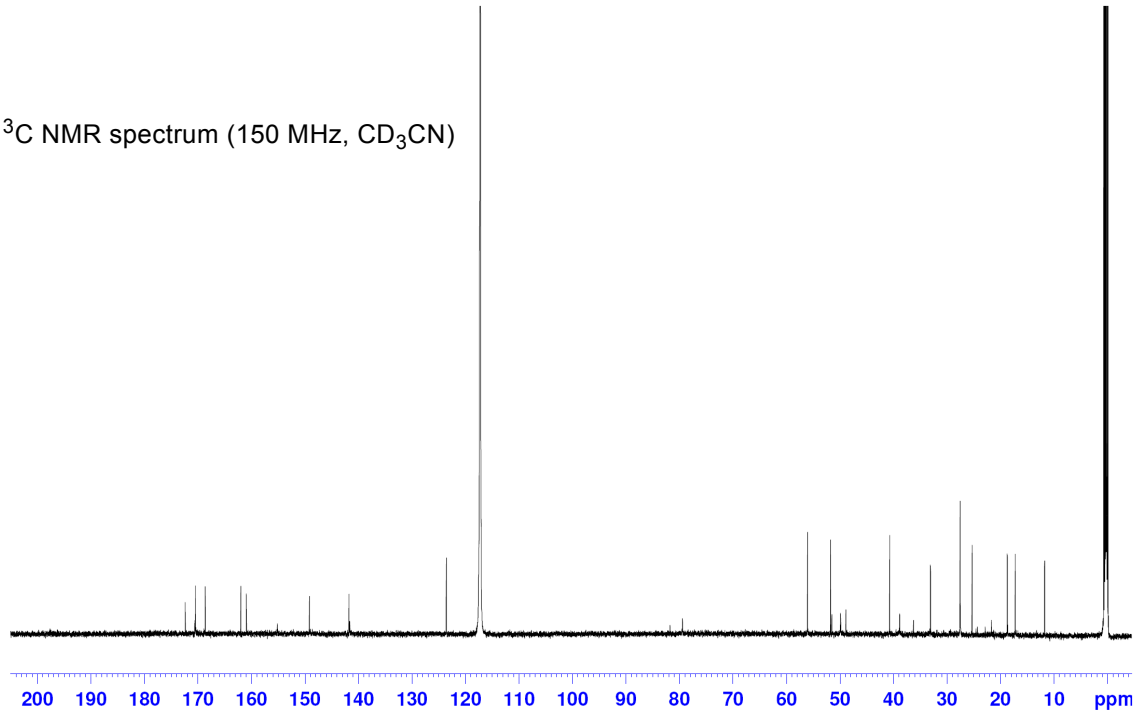


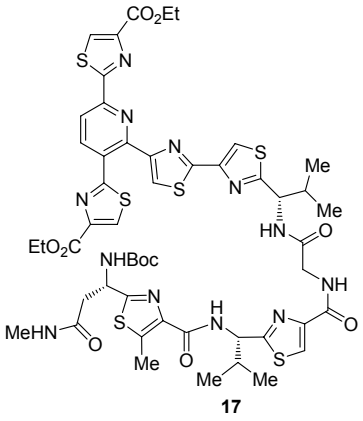
14

^{1}H NMR spectrum (600 MHz, CD_3CN)



^{13}C NMR spectrum (150 MHz, CD_3CN)

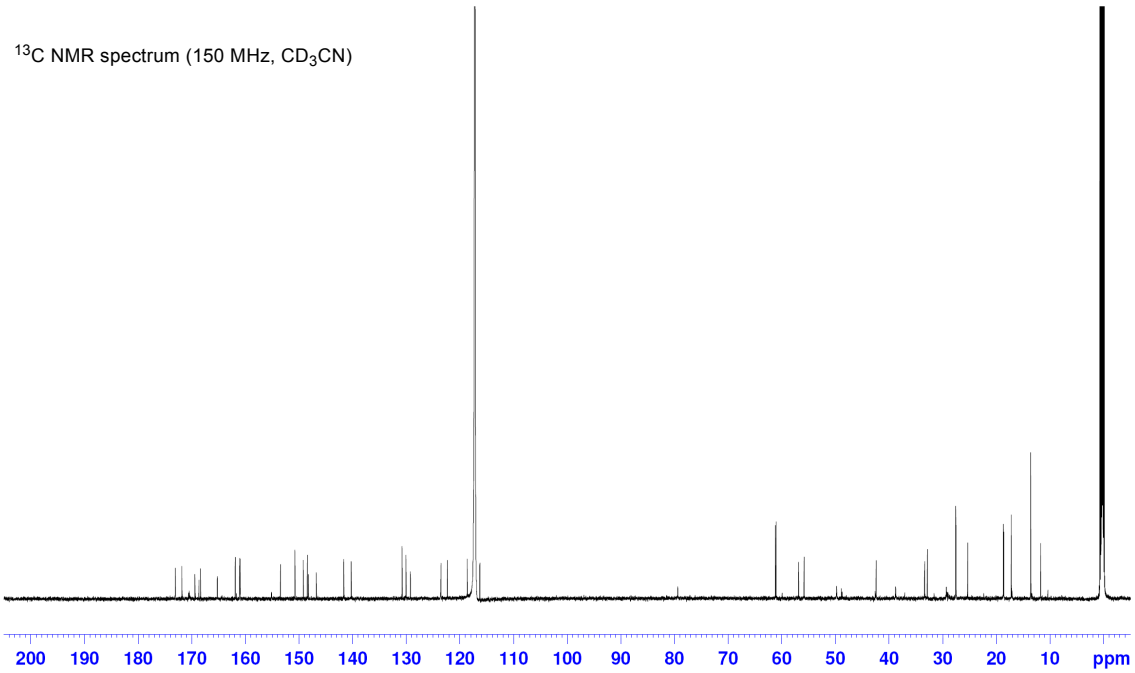


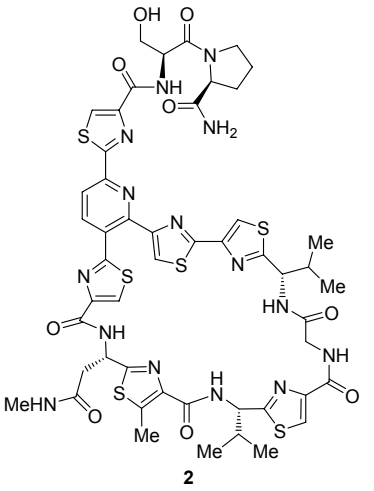


17

^{1}H NMR spectrum (600 MHz, CD_3CN)

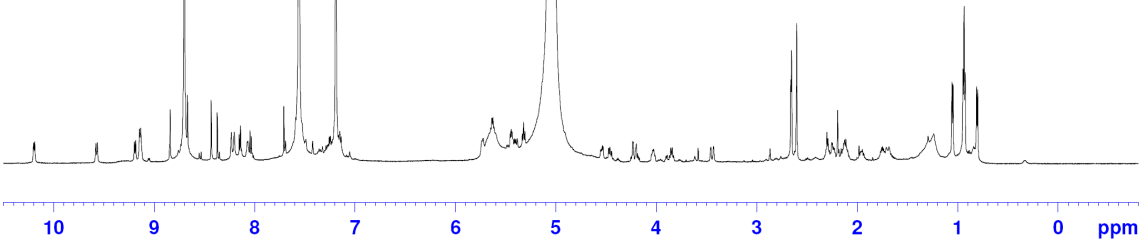
^{13}C NMR spectrum (150 MHz, CD_3CN)



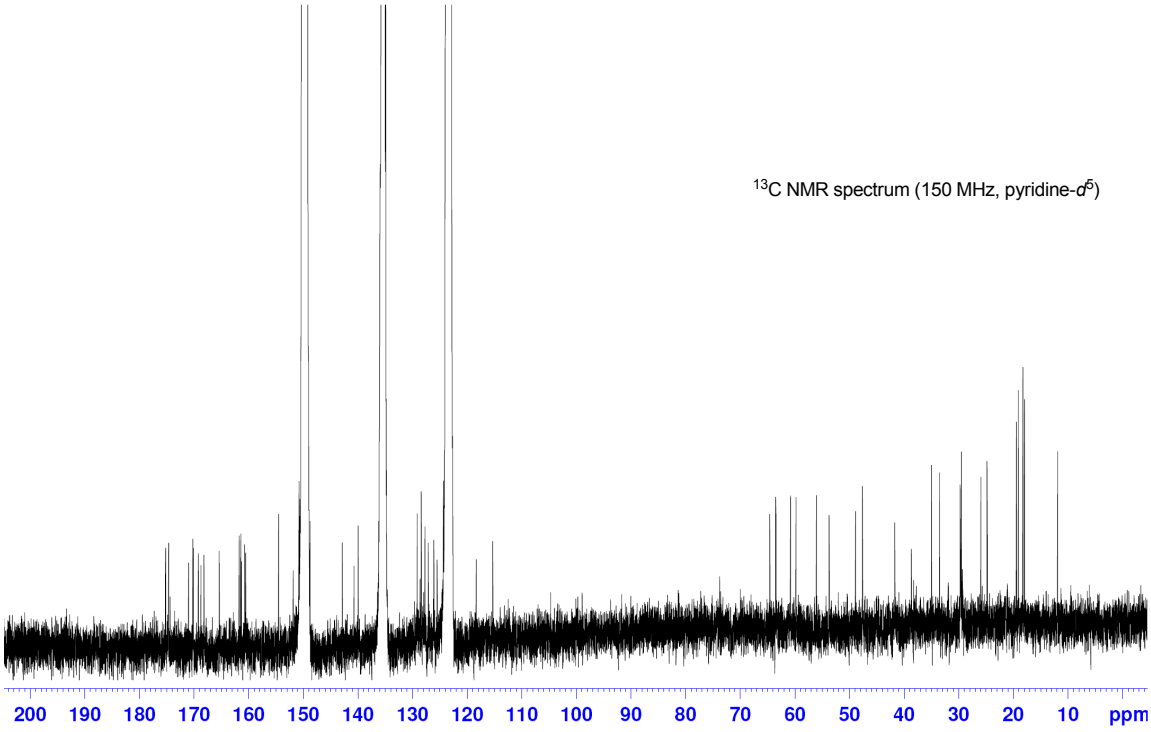


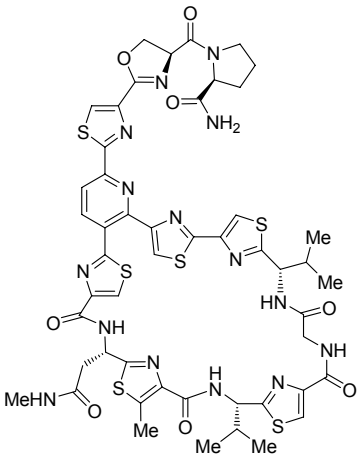
2

^{1}H NMR spectrum (600 MHz, pyridine-d^5)



^{13}C NMR spectrum (150 MHz, pyridine-d^5)

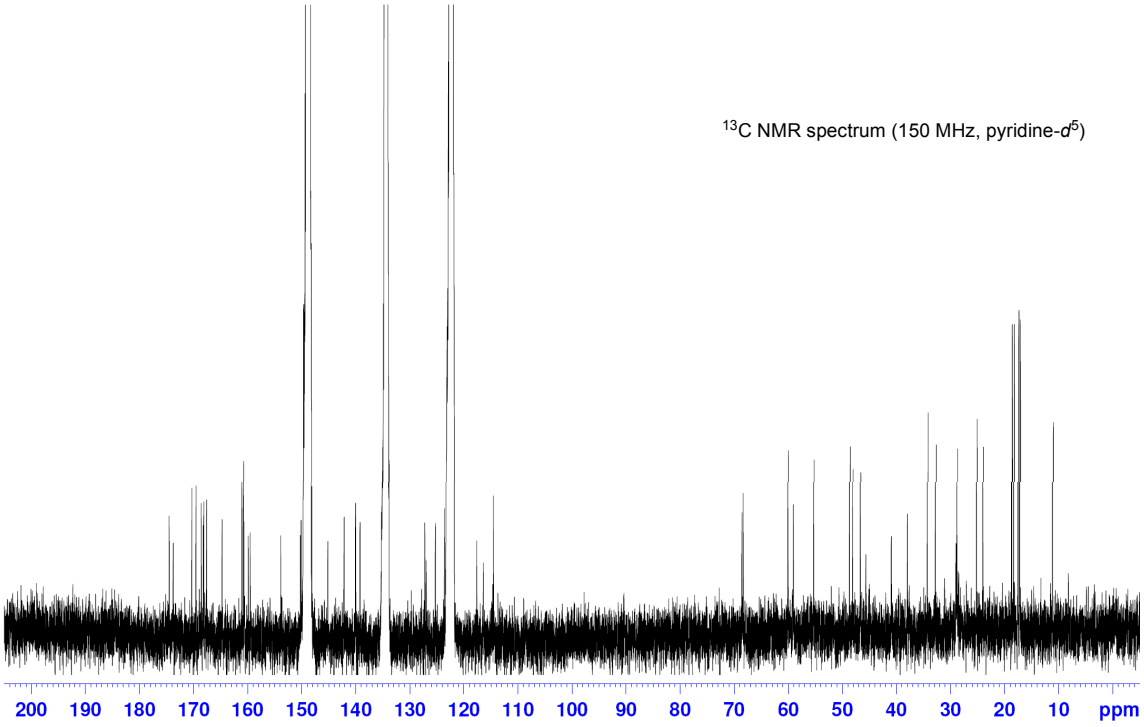


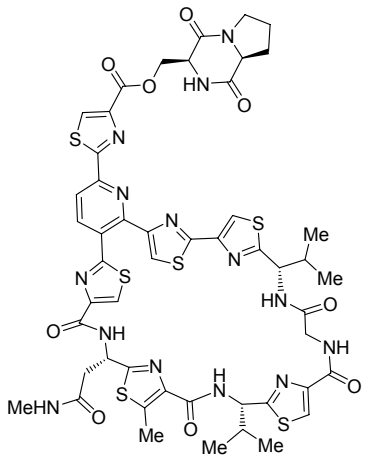


1

^{1}H NMR spectrum (600 MHz, pyridine-d^5)

^{13}C NMR spectrum (150 MHz, pyridine-d^5)





3

^{1}H NMR spectrum (600 MHz, pyridine-d^5)