

Non-toxic antimicrobials that evade drug resistance

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Supplementary Information

Supplementary Results

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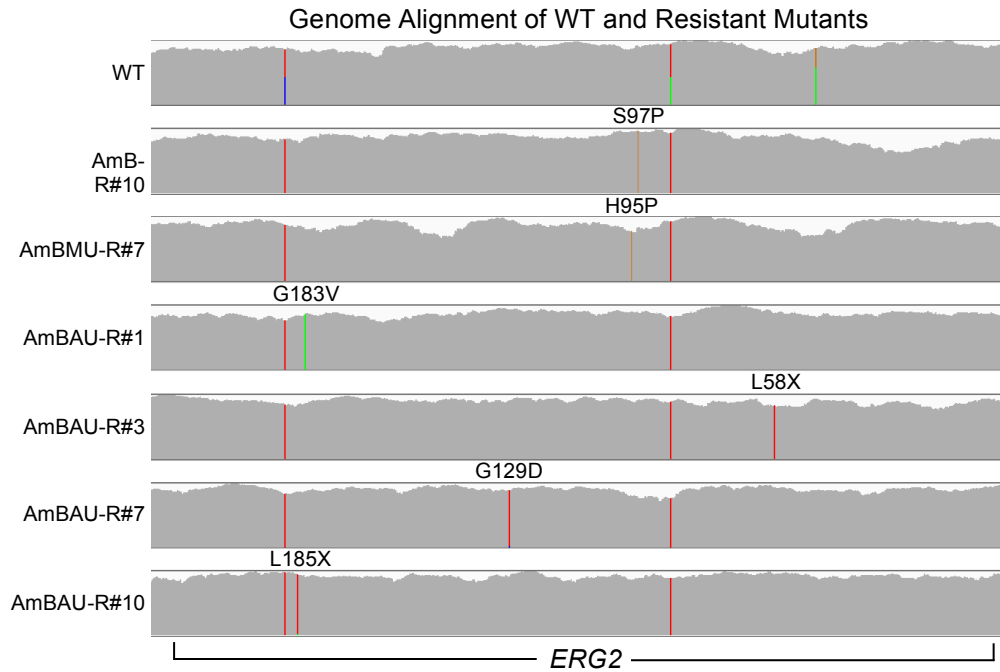
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Supplementary Results

Supplementary Table 1. Amphotericin Resistant Yeast Summary

Strain	Identified Mutations	AmB MIC (μM)	AmBAU MIC (μM)	AmBMU MIC (μM)	tBOOH MIC (μM)	Geldanamycin MIC (μM)	Radicalol MIC (μM)
SC5314-WT	n/a	0.25	0.25	0.5	4	32	32
ABR-1	<i>orf19.7235 D216Y</i>	0.5	0.5	1	4	16	32
ABR-2	<i>erg2 W79X</i>	2	8	8	1	2	4
ABR-3	<i>erg2 G129D</i>	1	8	16	1	2	4
ABR-4	<i>orf19.7235 D216Y</i>	0.5	0.5	1	2	32	32
ABR-10	<i>erg2 S97P</i>	2	8	8	1	1	4
ABR-o1	<i>erg6 D60N</i>	2	8	4	1	1	4
ABR-o13	<i>erg2 I16N</i>	2	8	8	1	1	4
MUR-6	<i>orf19.7235 D216Y</i>	0.5	0.5	0.5	2	16	32
MUR-7	<i>erg2 H95P</i>	1	8	8	1	1	2
MUR-9	<i>RPP1A D106E, D97E</i>	1	2	2	2	8	16
MUR-N2	<i>erg6 I222V, Y366X</i>	2	4	4	0.5	1	1
MUR-o3	<i>erg6 Y194F</i>	1	8	4	1	0.5	4
MUR-o16	not found (WT <i>ERG2/6</i>)	4	8	16	0.25	0.125	0.5
AUR-1	<i>erg2 G183V</i>	1	8	16	1	2	4
AUR-3	<i>erg2 L58X</i>	1	8	16	1	2	4
AUR-5	<i>erg2 G129D</i>	1	8	16	1	2	4
AUR-7	<i>erg2 G129D</i>	2	8	16	1	1	4
AUR-10	<i>erg2 L185X</i>	2	8	16	1	2	4



Supplementary Figure 1: Complete genome sequencing of parental wild-type (SC5314) and mutants selected for resistance to AmB, AmBMU or AmBAU. *ERG2* ORF is shown; color coded mutations represent blue=C, red=T, brown=G, green=A in the mutant strain.

Supplementary Note

I. General Methods

Materials

Commercially available materials were purchased from Aldrich Chemical Co. (Milwaukee, WI), AKSci (Union City, CA), Fisher Scientific (Hampton, NH), Lipoid (Luwigshafen, Germany), and Silicycle (Quebec, Canada) and used without further purification unless noted otherwise. All solvents were dispensed from a solvent purification as described by Pangborn and coworkers¹ (THF, Et₂O: dry neutral alumina; DMSO, DMF, CH₃OH : activated molecular sieves). Triethylamine and pyridine were freshly distilled under nitrogen from CaH₂. Camphorsulfonic acid was recrystallized from ethanol. Water was doubly distilled or obtained from a Millipore (Billerica, MA) MilliQ water purification system.

Reactions

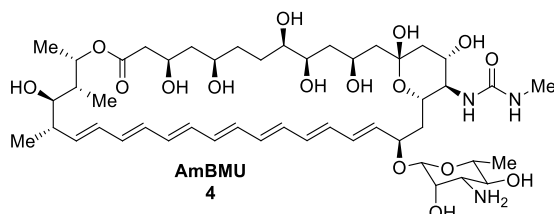
Due to the light and air sensitivity of amphotericin B (AmB), all manipulations were carried out under low light conditions and compounds were stored under an anaerobic atmosphere. All reactions were performed in oven- or flame-dried glassware under an atmosphere of argon unless otherwise indicated. Reactions were monitored by RP-HPLC using an Agilent 1200 Series HPLC system equipped with an Agilent Zorbax Eclipse C₁₈ 3.5- μ m, 4.6 x 75 mm column with UV detection at 383 nm at 1.2 mL/min, or an Agilent 6230 ESI TOF LC/MS system equipped with an Agilent Zorbax Eclipse C₁₈ 1.8- μ m, 2.1 x 50 mm column with UV detection at 383 nm at 0.4 mL/min.

Purification and Analysis

¹H NMR spectra were recorded at room temperature on a Varian Unity Inova Narrow Bore spectrometer operating at a ¹H frequency of 500 MHz with a Varian 5 mm ¹H{¹³C/¹⁵N} pulsed-field gradient Z probe or an Agilent VNMRS 750 MHz spectrometer with a Varian 5mm indirect-detection probe ¹H{¹³C/¹⁵N} probe equipped with X,Y,Z-field gradient capability. Chemical shifts (δ) are reported in parts per million (ppm) downfield added tetramethylsilane ($\delta = 0.0$). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet, b = broad, app = apparent), coupling constant (*J*) in Hertz (Hz) and integration. ¹³C spectra were recorded at room temperature with a Varian Unity Inova spectrometer operating at a ¹³C frequency of 125 MHz with a 5 mm Nalorac gradient {¹³C/¹⁵N}¹H quad probe or a Varian Unity Inova spectrometer operating at a ¹³C frequency of 150 MHz and equipped with a Varian 5 mm 600 DB Auto X probe. AmBMU and AmBAU ¹³C spectra were acquired in a 1:1 mixture of pyridine:methanol and chemical shifts (δ) are reported downfield of added tetramethylsilane ($\delta = 0.00$). Due to solubility reasons the AmBCU ¹³C spectrum was recorded in DMSO and is referenced to the carbon resonances in the NMR solvent ((CD₃)₂SO, $\delta = 39.52$, center line). All two dimensional NMR spectra were acquired in a 1:1 mixture of pyridine:methanol on an Agilent VNMRS 750 MHz spectrometer with a Varian 5mm indirect-detection probe ¹H{¹³C/¹⁵N} probe equipped with X,Y,Z-field gradient capability. Proton assignments are indicated directly on each spectrum. Diastereotopic methylene protons are designated a and b, but stereochemical assignments are not made. MS analysis was performed with an Applied Biosystems Micromass Ultima system with ESI ionization. IR spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR with the Perkin-Elmer Universal ATR Sampling Accessory. Instrument operation and data analysis were performed using Perkin-Elmer Spectrum version 6.3.5 software. Sample was placed on the ATR crystal and a force of 80-90 Newtons were applied to the sample. Spectra are a composite of four scans. High resolution mass spectra (HRMS) were obtained at the University of Illinois mass spectrometry facility. All synthesized compounds gave HRMS within 5 ppm of the calculated values. Large scale prep purification of amphotericin B and its derivatives was carried out by preparative HPLC purification using either an agilent 1100 or 1200 series instrument equipped with either a Waters SunFire Prep C₁₈ OBD 5- μ m, 30 x 150 mm at 25 mL/min, or a Waters Sunfire Prep C₁₈ OBD 5- μ m, 50 x 250 mm column at 75 mL/min with detection at 383 nm and an eluent of acetonitrile and 0.3% formic acid. The purity of amphotericin B and its derivatives was determined by HPLC analysis using an Agilent Zorbax Eclipse C₁₈ 1.8- μ m, 2.1 x 50 mm column with detection at 383 nm and an eluent of acetonitrile and 0.1% formic acid in water unless otherwise indicated.

II. Synthesis of AmB Derivatives

AmBMU (4)



A round bottom flask was charged with amphotericin B (1 g, ca. 1.082 mmol, 1 eq) and Fmoc-succinimide (0.55 g, 1.62 mmol, 1.5 eq) which were dissolved in a 2:1 mixture of DMF:MeOH (33.8 mL) at room temperature. Pyridine (0.5 mL, 6.21 mmol, 5.74 eq) was subsequently added and the reaction was stirred for 12 hours at room temperature. The reaction mixture was then poured into diethyl ether (1.0 L). After stirring for 30 minutes, the resulting yellow precipitate was isolated via Büchner filtration using Whatman #50 filter paper to afford a yellow solid. The filter cake was dried on the filter for 10 minutes and then stored under vacuum for one hour.

The resulting powder was dissolved in 1:1 THF:MeOH (35 mL) and cooled to 0°C. To this solution was added camphorsulfonic acid (138 mg, 0.59 mmol, 0.55 eq) and the resulting mixture was stirred for 1 hour at 0°C. The reaction was then quenched at 0°C with triethylamine (0.14 mL, 0.59 mmol, 0.55 eq). The reaction was concentrated *in vacuo* removing approximately half of the solvent. The resulting saturated solution was poured into 1:1 hexanes:diethyl ether (1.0 L) and the yellow precipitate was collected via Büchner filtration using Whatman #50 filter paper and washed with diethyl ether (200 mL) to yield a yellow solid.

The resulting solid was dissolved in THF (54 mL, 0.02M). To this solution was added triethylamine (0.15 mL, 1.08 mmol, 1 eq) and then diphenyl phosphoryl azide (0.70 mL, 3.25 mmol, 3 eq). The reaction was heated to 50°C and stirred for 12 hours. After 12 hours the reaction was cooled to room temperature and methylamine (2.0M in THF, 4.33 mL, 8.8 mmol, 8 eq) was added. The reaction then stirred at room temperature for 8 hours, slowly evolving a yellow precipitate. The reaction mixture was then poured into diethyl ether (1.0 L), and the resulting yellow precipitate was isolated via Büchner filtration using Whatman #50 filter paper to afford a yellow solid. The solid was dissolved in DMSO (~100 mg/mL) and purified by a single prep-HPLC purification (C₁₈, 5- μ m, 50 x 250 mm, 75 mL/min, 80:20 to 59:41 0.3% HCO₂H (aq):MeCN over 9 minutes), Following HPLC purification, the solvent was removed *in vacuo* at 40°C. Upon complete solvent removal, residual formic acid was removed via azeotroping with milliQ water (10 mL) and toluene (50 mL). This process was repeated three times to ensure formic acid removal. During the course of this HPLC purification the methyl ketal was quantitatively converted to a hemiketal, yielding **AmBMU** as a yellow solid (264.3 mg, 0.278 mmol, 64% average yield per step).

¹H NMR (750 MHz, 1:1 Pyridine *d*-5: Methanol *d*-4) δ 6.64 (dd, *J* = 14.7, 11.2 Hz, 1H), 6.60 (dd, *J* = 14.8, 10.0 Hz, 1H), 6.52 (t, *J* = 12.1 Hz, 1H), 6.48 – 6.37 (m, 6H), 6.36 – 6.25 (m, 4H), 5.69 – 5.63 (m, 1H), 5.50 (dd, *J* = 14.3, 9.8 Hz, 1H), 4.97 (s, 1H), 4.82-4.76 (m, 1H), 4.65 (app t, *J* = 10.3 Hz, 1H), 4.53 (bs, 1H), 4.49 (app tt, *J* = 9.8, 2.9 Hz, 1H), 4.42 (app t, *J* = 9.1 Hz, 1H), 4.29 – 4.23 (m, 1H), 3.98 (app t, *J* = 10.0 Hz, 1H), 3.90 – 3.84 (m, 2H), 3.80 – 3.72 (m, 1H), 3.62 – 3.56 (m, 1H), 3.56 – 3.51 (m, 1H), 3.47 – 3.44 (m, 1H), 3.38 (app d, *J* = 9.5 Hz, 1H), 2.79 (s, 3H), 2.71 – 2.65 (m, 1H), 2.61 – 2.55 (m, 1H), 2.51 (dd, *J* = 16.7, 9.8 Hz, 1H), 2.39 – 2.34 (m, 2H), 2.21 – 2.14 (m, 1H), 2.06 – 1.99 (m, 2H), 1.96 (dd, *J* = 14.8, 7.3 Hz, 1H), 1.85 (dd, *J* = 13.9, 10.9 Hz, 1H), 1.84-1.79 (m, 1H), 1.73 – 1.65 (m, 3H), 1.66 – 1.61 (m, 1H), 1.61 – 1.56 (m, 1H), 1.53 (app dt, *J* = 14.0, 3.0 Hz, 1H), 1.47-1.45 (m, 1H), 1.46 (d, *J* = 6.2 Hz, 3H), 1.37 (d, *J* = 6.5 Hz, 3H), 1.25 (d, *J* = 6.4 Hz, 3H), 1.18 (d, *J* = 7.1 Hz, 3H).

¹³C NMR (125 MHz, 1:1 Pyridine *d*-5: Methanol *d*-4) δ 172.31, 161.63, 137.57, 137.33, 134.82, 134.20, 134.17, 133.99, 133.91, 133.67, 133.59, 133.28, 132.98, 131.04, 129.73, 128.99, 98.04, 98.26, 79.10, 77.44, 76.32, 75.24, 74.64, 72.32, 70.92, 70.45, 69.98, 69.26, 68.97, 68.69, 68.46, 58.35, 57.35, 47.22, 45.74, 44.90, 44.06, 42.89, 41.28, 40.80, 36.72, 36.38, 31.53, 27.02, 19.11, 18.10, 17.35, 12.64.

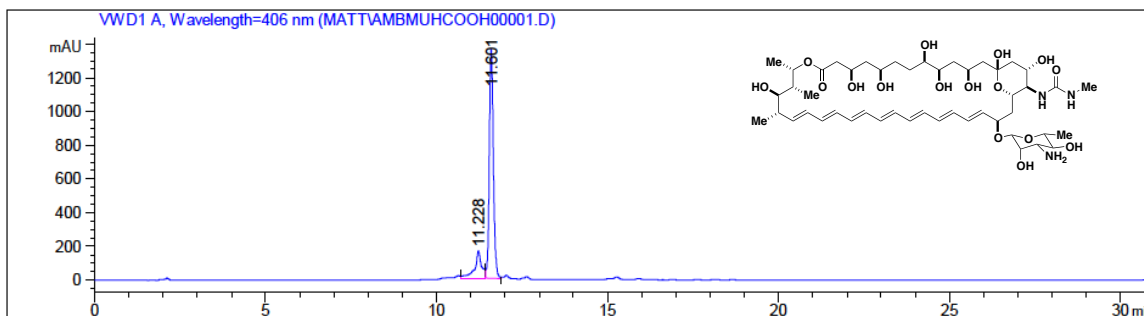
IR (cm⁻¹) 3332.5, 2930.4, 1710.4, 1635.8, 1571.4, 1490.4, 1447.9, 1420.9, 1377.1, 1344.9, 1314.1, 1263.9, 1172.5, 1128.7, 1068.3, 1041.2, 1009.1, 908.6, 888.1, 848.2, 769.7, 729.8, 692.5.

HRMS (ESI) Calculated ($C_{48}H_{77}N_3O_{16} + H$)⁺. 952.5382 Observed. 952.5385

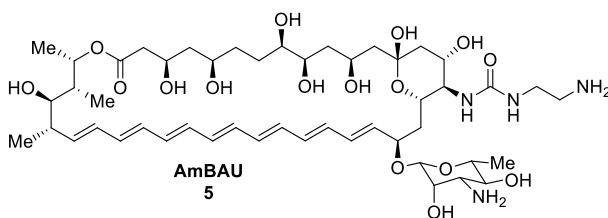
Analytical HPLC (Sunfire C₁₈, 5.0- μ m, 10 x 250 mm, 1.2 mL/min, 95:5 to 5:95 H₂O:MeCN (H₂O containing 0.1% HCO₂H) over 30 minutes)

AmB Retention Time = 12.7 min.

AmBMU Retention Time = 11.6 min.



AmBAU (5)



A round bottom flask was charged with amphotericin B (1 g, ca. 1.082 mmol, 1 eq) and Fmoc-succinimide (0.55 g, 1.62 mmol, 1.5 eq) which were dissolved in a 2:1 mixture of DMF:MeOH (33.8 mL) at room temperature. Pyridine (0.5 mL, 6.21 mmol, 5.74 eq) was subsequently added and the reaction was stirred for 12 hours at room temperature. The reaction mixture was then poured into diethyl ether (1.0 L). After stirring for 30 minutes, the resulting yellow precipitate was isolated via Büchner filtration using Whatman #50 filter paper to afford a yellow solid. The filter cake was dried on the filter for 10 minutes and then stored under vacuum for one hour.

The resulting powder was dissolved in 1:1 THF:MeOH (35 mL) and cooled to 0°C. To this solution was added camphorsulfonic acid (138 mg, 0.59 mmol, 0.55 eq) and the resulting mixture was stirred for 1 hour at 0°C. The reaction was then quenched at 0°C with triethylamine (0.14 mL, 0.59 mmol, 0.55 eq). The reaction was concentrated *in vacuo* removing approximately half of the solvent. The resulting saturated solution was poured into 1:1 hexanes:diethyl ether (1.0 L) and the yellow precipitate was collected via Büchner filtration using Whatman #50 filter paper and washed with diethyl ether (200 mL) to yield a yellow solid.

The resulting solid was dissolved in THF (54 mL, 0.02M). To this solution was added triethylamine (0.15 mL, 1.08 mmol, 1 eq) and then diphenyl phosphoryl azide (0.70 mL, 3.25 mmol, 3 eq). The reaction was heated to 50°C and stirred for 12 hours. After 12 hours, ethylene diamine (0.29 mL, 4.33 mmol, 4 eq) was added, and the reaction continued stirring at 50°C for 3 hours, slowly evolving a yellow precipitate. The reaction mixture was then poured into diethyl ether (1.0 L), and the resulting yellow precipitate was isolated via Büchner filtration using Whatman #50 filter paper to afford a yellow solid which was dissolved in DMSO (~66 mg/mL) and purified by prep-HPLC (C₁₈, 5- μ m, 50 x 250 mm, 75 mL/min, 80:20 to 50:50 0.3% HCO₂H (aq):MeCN over 9 minutes). After HPLC purification the solvent was removed *in vacuo* at 40°C. Upon complete solvent removal, residual formic acid was removed via azeotroping with milliQ water (10 mL) and toluene (50 mL). This process was repeated three times to ensure formic acid removal. During the course of this HPLC purification the methyl ketal was quantitatively converted to a hemiketal, yielding **AmBAU** as a yellow solid (236.2 mg, 0.241 mmol, 61% average yield per step).

^1H NMR (750 MHz, 1:1 Pyridine *d*-5: Methanol *d*-4) δ 6.64 (dd, J = 14.7, 11.2 Hz, 1H), 6.60 (dd, J = 15.2, 8.8 Hz, 1H), 6.55 – 6.47 (m, 1H), 6.47 – 6.35 (m, 7H), 6.35 – 6.25 (m, 3H), 5.68 – 5.61 (m, 1H), 5.49 (dd, J = 15.0, 10.2 Hz, 1H), 4.91 (s, 1H), 4.79 – 4.73 (m, 1H), 4.64 (app t, J = 10.7 Hz, 1H), 4.49 – 4.42 (m, 3H), 4.28 – 4.23 (m, 1H), 3.96 (app t, J = 10.4 Hz, 1H), 3.88 – 3.82 (m, 1H), 3.82 – 3.73 (m, 3H), 3.55 – 3.50 (m, 1H), 3.51 – 3.46 (m, 1H), 3.47 – 3.43 (m, 1H), 3.39 (app d, J = 8.8 Hz, 1H), 3.36 (app d, J = 9.2 Hz, 1H), 3.32 – 3.26 (m, 1H), 3.23 – 3.16 (m, 1H), 2.66 (dd, J = 15.4, 4.9 Hz, 1H), 2.59 – 2.53 (m, 1H), 2.49 (dd, J = 16.8, 9.8 Hz, 1H), 2.38 – 2.33 (m, 2H), 2.18 – 2.12 (m, 1H), 2.04 – 1.97 (m, 2H), 1.90 (dd, J = 14.9, 7.8 Hz, 1H), 1.84 (dd, J = 14.0, 11.0 Hz, 1H), 1.82 – 1.75 (m, 1H), 1.71–1.65 (m, 3H), 1.65 – 1.60 (m, 1H), 1.60 – 1.55 (m, 1H), 1.52 (app dt, J = 13.8, 2.9 Hz, 1H), 1.48 – 1.44 (m, 1H), 1.44 (d, J = 6.2 Hz, 3H), 1.36 (d, J = 6.5 Hz, 3H), 1.24 (d, J = 6.4 Hz, 3H), 1.17 (d, J = 7.1 Hz, 3H).

^{13}C NMR (150 HMz, 1:1 Pyridine *d*-5: Methanol *d*-4) δ 172.34, 161.35, 137.58, 137.08, 134.78, 134.16, 134.11, 134.02, 133.93, 133.71, 133.62, 133.26, 133.00, 130.85, 130.62, 98.26, 98.07, 79.12, 77.60, 76.37, 75.24, 74.63, 72.28, 71.43, 70.47, 70.00, 69.26, 68.70(2), 68.62, 58.16, 57.32, 47.18, 45.77, 44.93, 44.05, 42.86, 41.67, 40.80, 40.49, 39.31, 36.73, 36.35, 31.56, 19.08, 18.08, 17.32, 12.63.

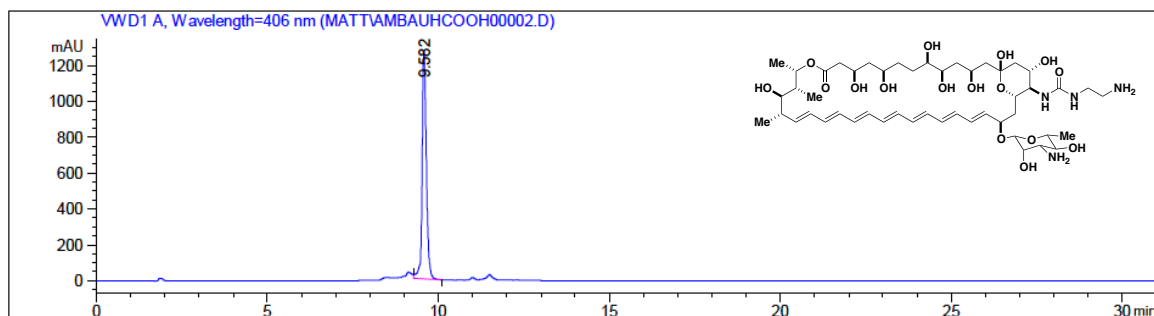
IR (cm^{-1}) 3293.8, 2925.3, 1709.2, 1650.0, 1576.6, 1490.4, 1450.5, 1374.5, 1342.4, 1317.9, 1249.7, 1200.8, 1173.8, 1068.3, 1043.8, 1009.1, 906.1, 889.4, 850.8, 765.8, 731.1, 692.5.

HRMS (ESI) Calculated ($\text{C}_{49}\text{H}_{80}\text{N}_4\text{O}_{16} + \text{H}$)⁺. 981.5648 Observed. 981.5641

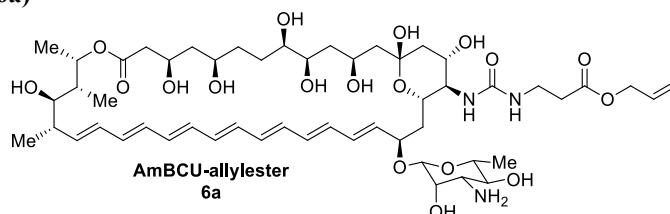
Analytical HPLC (Sunfire C_{18} , 5.0- μm , 10 x 250 mm, 1.2 mL/min, 95:5 to 5:95 $\text{H}_2\text{O}:\text{MeCN}$ (H_2O containing 0.1% HCO_2H) over 30 minutes)

AmB Retention Time = 12.7 min.

AmBAU Retention Time = 9.6 min.



AmBCU-allylester (6a)



A round bottom flask was charged with amphotericin B (1 g, ca. 1.08 mmol, 1 eq) and Fmoc-succinimide (0.55 g, 1.62 mmol, 1.5 eq) which were dissolved in a mixture of 2:1 DMF:MeOH (33.8 mL) at room temperature. Pyridine (0.5 mL, 6.21 mmol, 5.74 eq) was subsequently added and the reaction was stirred for 12 hours at room temperature. The reaction mixture was then poured into diethyl ether (1.0 L). After stirring for 30 minutes, the resulting yellow precipitate was isolated via Büchner filtration using Whatman #50 filter paper to afford a yellow solid. The filter cake was dried on the filter for 10 minutes and then stored under vacuum for one hour.

The resulting powder was dissolved in 1:1 THF:MeOH (35 mL) and cooled to 0°C. To this solution was added camphorsulfonic acid (138 mg, 0.60 mmol, 0.55 eq) and the resulting mixture was stirred for 1 hour at 0°C. The reaction was then quenched at 0°C with triethylamine (0.14 mL). The

reaction was concentrated *in vacuo* removing approximately half of the solvent. The resulting saturated solution was poured into 1:1 hexanes:diethyl ether (1.0 L) and the yellow precipitate was collected via Büchner filtration using Whatman #50 filter paper and washed with diethyl ether (20 mL) to yield a yellow solid.

The resulting solid (1.06 g, ca. 1.08 mmol, 1 eq) was added to a 40 mL vial followed by THF (54 mL, 0.02M), triethylamine (0.16 mL, 1.14 mmol, 1.05 eq), and lastly diphenyl phosphoryl azide (0.70 mL, 3.25 mmol, 3 eq). The reaction was then heated to 50°C and stirred for 15 hours.

To a separate 40 mL vial was added β -alanine allylester hydrochloride (7.16 g, 43.3 mmol, 40 eq), sodium carbonate (13.75 g, 129.8 mmol, 120 eq), and THF (14 mL). The resulting suspension stirred then at room temperature for 20 minutes. The suspension was then filtered through celite followed by filtration through a syringe tip 0.2- μ m filter. The resulting β -alanine allylester free base was then added to the 50°C reaction mixture and allowed to stir for 8 hours. After 8 hours, the volatiles were removed *in vacuo* yielding a red oil. This was dissolved in DMSO and purified directly by prep HPLC (C₁₈, 5- μ m, 50 x 250 mm, 80 mL/min, 80:20 to 40:60 0.3% HCO₂H (aq):MeCN over 9 minutes). Upon removal of the acetonitrile and aqueous formic acid solution *in vacuo* at 35°C, the C-13 methyl ketal is quantitatively converted to a hemiketal yielding **AmBCU-allylester** as a yellow solid (370 mg, 0.352 mmol, 68% average yield per step).

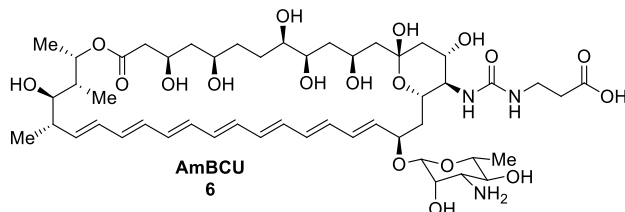
¹H NMR (500 MHz, 10:1 Pyridine-*d*₅: Methanol-*d*₄) δ 6.71 – 6.25 (m, 13H), 6.01 – 5.89 (m, 1H), 5.70 – 5.64 (m, 1H), 5.54 – 5.48 (m, 1H), 5.33 (m, 1H), 5.20 (m, 1H), 4.97 (s, 1H), 4.79 (bs, 1H), 4.70 – 4.59 (m, 4H), 4.50 (app t, *J* = 10.0 Hz, 1H), 4.43 (app t, *J* = 8.8 Hz, 1H), 4.26 – 4.20 (m, 1H), 3.99 (app t, *J* = 10.0 Hz, 1H), 3.88 (app d, *J* = 10.8 Hz, 1H), 3.82-3.77 (m, 2H), 3.65-3.60 (m, 3H), 3.47 (m, 1H), 3.42 – 3.35 (m, 1H), 3.35 – 3.31 (m, 1H), 2.71 – 2.62 (m, 3H), 2.58 (m, 1H), 2.52 (dd, *J* = 16.8, 9.7 Hz, 1H), 2.41 – 2.33 (m, 2H), 2.23-2.13 (m, 1H), 2.07 – 1.91 (m, 3H), 1.91 – 1.77 (m, 2H), 1.75 – 1.57 (m, 5H), 1.57 – 1.51 (m, 1H), 1.48 – 1.44 (m, 4H), 1.37 (d, *J* = 6.3 Hz, 3H), 1.26 (d, *J* = 6.3 Hz, 3H), 1.18 (d, *J* = 7.1 Hz, 3H).

¹³C NMR (125 MHz, 10:1 Pyridine-*d*₅: MeOH-*d*₄) δ 172.31, 171.92, 160.41, 136.99, 136.83, 136.75, 134.46, 133.84, 133.57, 133.47, 133.45, 133.20, 133.03, 132.91, 132.61, 130.57, 129.55, 117.73, 98.06, 97.90, 77.08, 75.96, 74.89, 74.31, 71.92, 71.61, 70.08, 69.59, 68.65, 68.29, 68.19, 65.31(2), 57.96, 57.09, 46.84, 45.53, 44.62, 43.67, 42.59, 41.29, 40.89, 40.48, 36.52, 36.41, 36.07, 35.42, 32.27, 31.22, 18.74, 17.89, 17.02, 12.32.

HRMS (ESI) Calculated (C₅₃H₈₃N₃O₁₈ + H)⁺. 1050.570 Observed. 1050.5756.

Analytical HPLC (Zorbax Eclipse C₁₈, 1.8- μ m, 2.1 x 50 mm, 0.4 mL/min, 95:5 to 5:95 H₂O:MeCN (both containing 0.1% HCO₂H) over 8 minutes) Retention Time = 6.4 min

AmBCU (6)



To a 40 mL vial was added **AmBCU-allylester** (370 mg, 352.3 μ mol, 1 eq), and thiosalicylic acid (203.4 mg, 1.76 mmol, 5 eq). The vial was then brought into a glove box and Pd(PPh₃)₄ was added (205 mg, 0.18 mmol, 0.5 eq). The vial was sealed with a septa cap, removed from the glovebox, and DMF was added (17.6 mL, 0.2 M) via syringe. The reaction then stirred at room temperature for 1 hour. The reaction was then poured into Et₂O (370 mL) in multiple 50 mL centrifuge tubes. The resulting suspension was then centrifuged at 3700 G for 5 minutes. The pale red supernatant was decanted and the resulting yellow/orange solid was dissolved in DMSO and purified by prep HPLC (C₁₈, 5- μ m, 50 x 250 mm, 80 mL/min, 80:20 to 40:60 0.3% HCO₂H (aq):MeCN over 9 minutes) yielding **AmBCU** as a yellow solid

(124.4 mg, 0.123 mmol, 35% yield, 58% average yield per step from 1 g AmB).

^1H NMR (750 MHz, 1 Pyridine *d*-5: Methanol *d*-4) δ 6.65 (dd, J = 14.6, 11.1 Hz, 1H), 6.59 (dd, J = 14.8, 9.8 Hz, 1H), 6.55 – 6.47 (m, 1H), 6.47 – 6.35 (m, 7H), 6.36 – 6.25 (m, 3H), 5.69 – 5.62 (m, 1H), 5.49 (dd, J = 15.2, 10.3 Hz, 1H), 4.94 (s, 1H), 4.74 – 4.68 (m, 1H), 4.62 (app t, J = 10.9 Hz, 1H), 4.49 – 4.42 (m, 2H), 4.39 (app t, J = 9.4 Hz, 1H), 4.25 – 4.17 (m, 1H), 3.95 (app t, J = 10.0 Hz, 1H), 3.87 – 3.78 (m, 2H), 3.75 – 3.61 (m, 4H), 3.48 – 3.44 (m, 2H), 3.36 (app d, J = 9.8 Hz, 1H), 2.63 – 2.59 (m, 2H), 2.60 – 2.53 (m, 1H), 2.51 – 2.46 (m, 2H), 2.39 – 2.33 (m, 1H), 2.35 – 2.30 (m, 1H), 2.18 – 2.09 (m, 1H), 2.02 – 1.96 (m, 2H), 1.92 – 1.85 (m, 1H), 1.85 – 1.81 (m, 1H), 1.81 – 1.75 (m, 1H), 1.72 – 1.63 (m, 3H), 1.64 – 1.55 (m, 2H), 1.55 – 1.49 (m, 1H), 1.46 – 1.44 (m, 1H), 1.44 (d, J = 5.9 Hz, 3H), 1.36 (d, J = 6.6 Hz, 3H), 1.25 (d, J = 6.5 Hz, 3H), 1.17 (d, J = 7.2 Hz, 3H).

^{13}C NMR (150 MHz, DMSO-*d*₆) δ 175.38, 170.58, 158.41, 136.81, 136.33, 133.92, 133.76, 133.64, 133.25, 133.16, 132.57, 132.40, 132.25, 132.17, 131.87, 131.23, 130.28, 129.00, 97.04, 96.87, 77.20, 76.06, 73.86, 73.48, 73.05, 69.50, 69.16, 68.80, 67.98, 67.79, 67.44, 66.84, 66.20, 59.75, 56.23, 55.25, 45.84, 44.88, 44.80, 42.52, 42.01, 40.43, 39.52, 35.11, 30.96, 29.05, 18.52, 17.80, 16.96, 12.10.

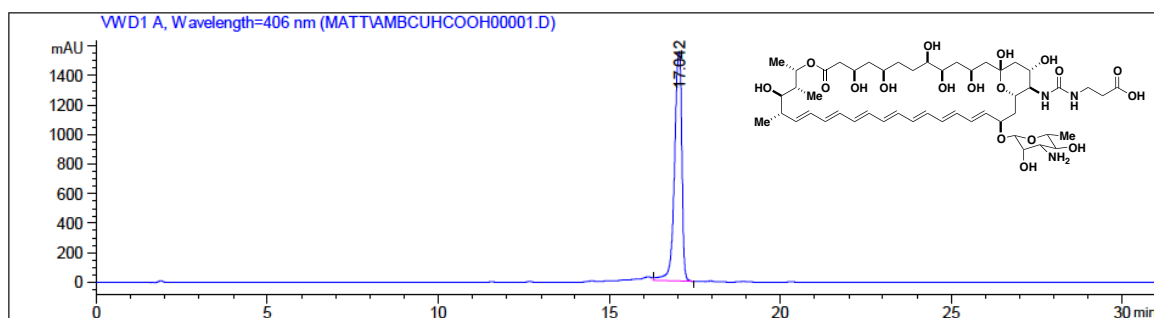
IR (cm⁻¹) 3311.9, 2927.8, 1720.7, 1642.2, 1559.9, 1438.9, 1400.3, 1377.1, 1312.8, 1261.3, 1172.5, 1109.4, 1067.0, 1046.4, 1005.2, 888.1, 852.0, 822.4, 760.7, 719.5, 691.2.

HRMS (ESI) Calculated (C₅₀H₇₉N₃O₁₈ + H)⁺ 1010.5437 Found. 1010.5449.

Analytical HPLC (Sunfire C₁₈, 5.0- μm , 10 x 250 mm, 1.2 mL/min, 95:5 to 5:95 H₂O:MeCN (H₂O containing 0.1% HCO₂H) over 30 minutes)

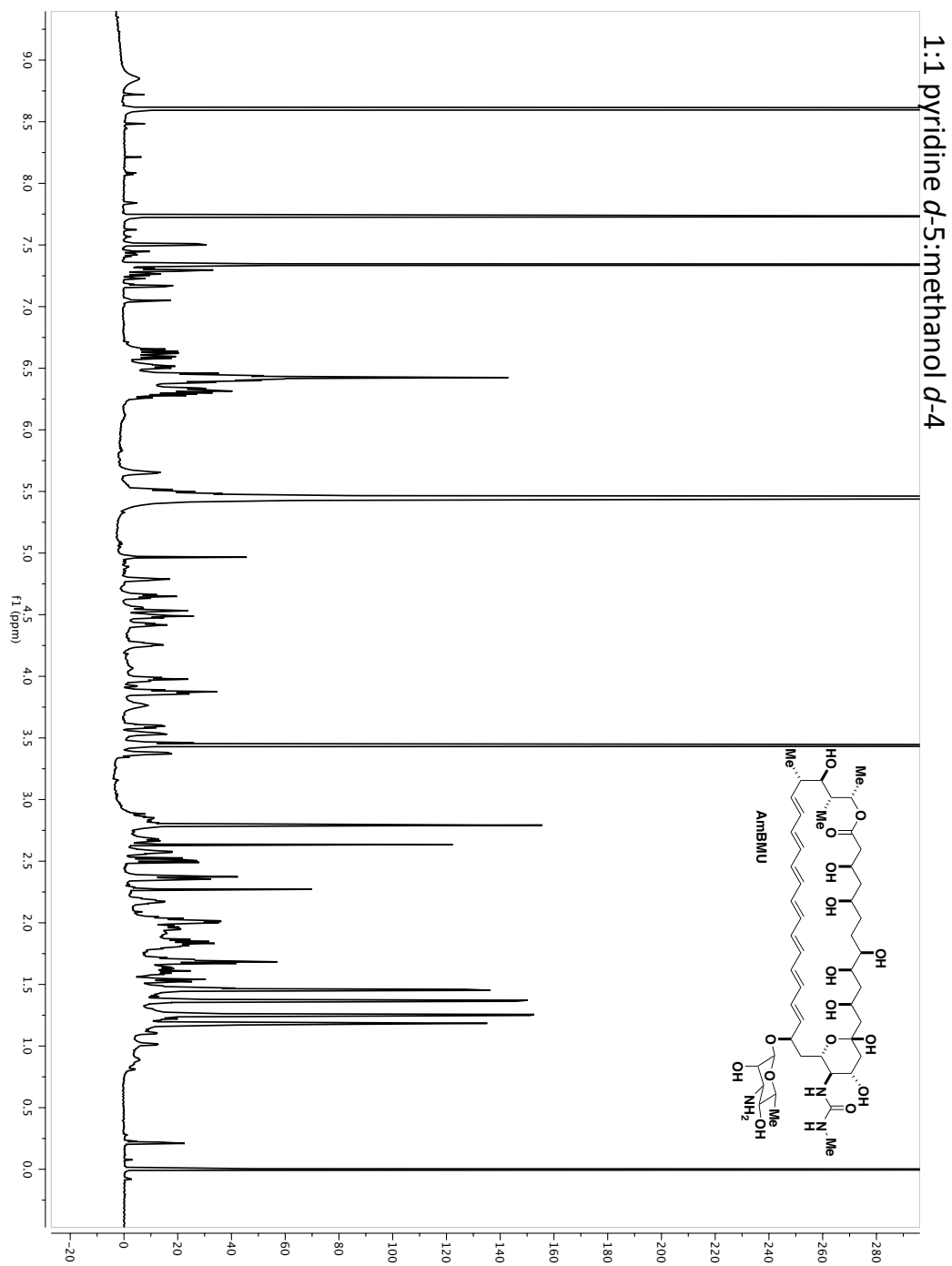
AmB Retention Time = 12.7 min.

AmBCU Retention Time = 17.0 min.

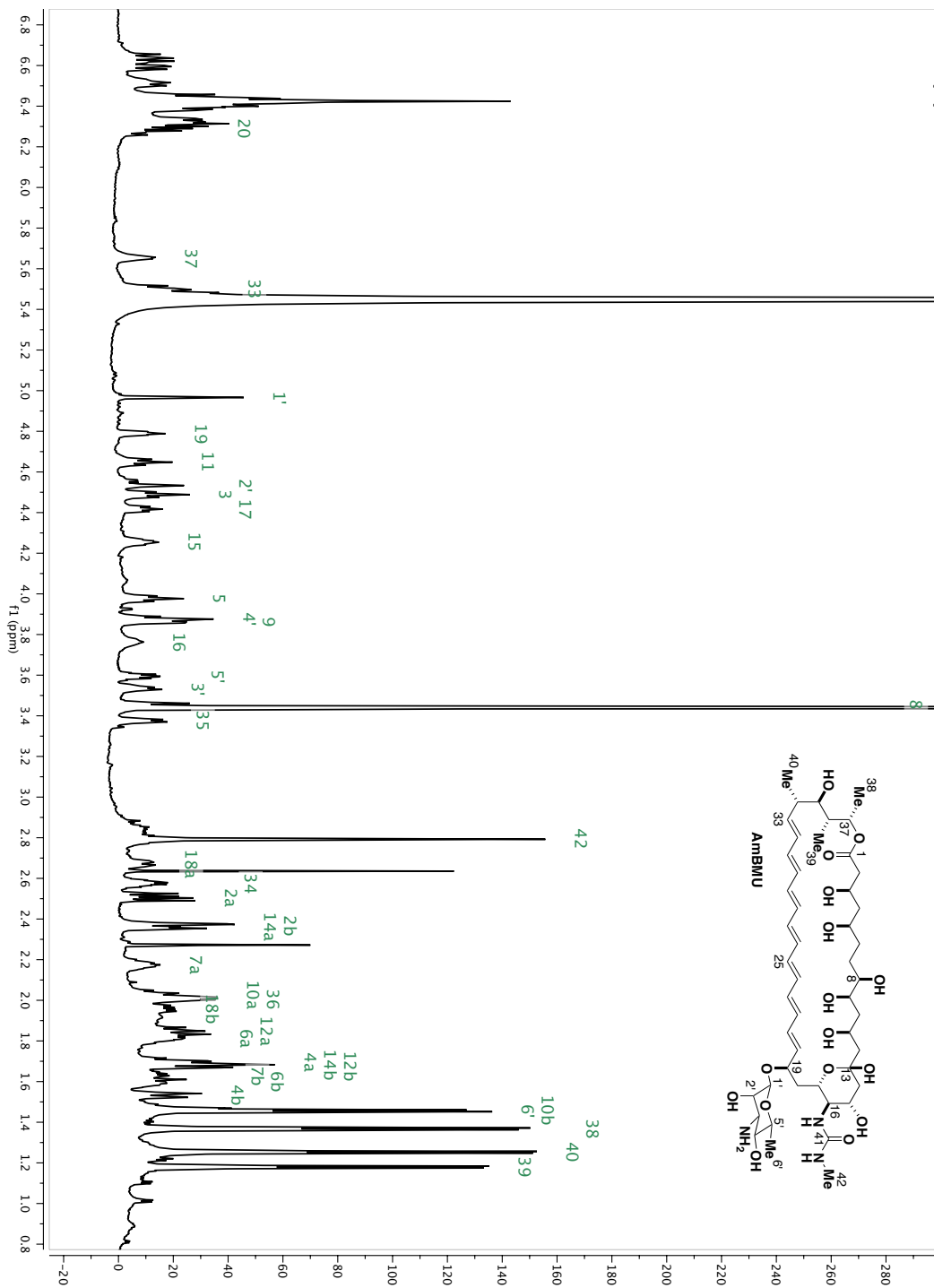


Synthesis of AmdeB (**7**)³, AmBME (**8**)⁴, C41MeAmB (**9**)³, AmBMA (**10**)⁵, and AmBTABA (**11**)⁶ was accomplished using previously reported procedures; characterization following HPLC purification matched literature values.

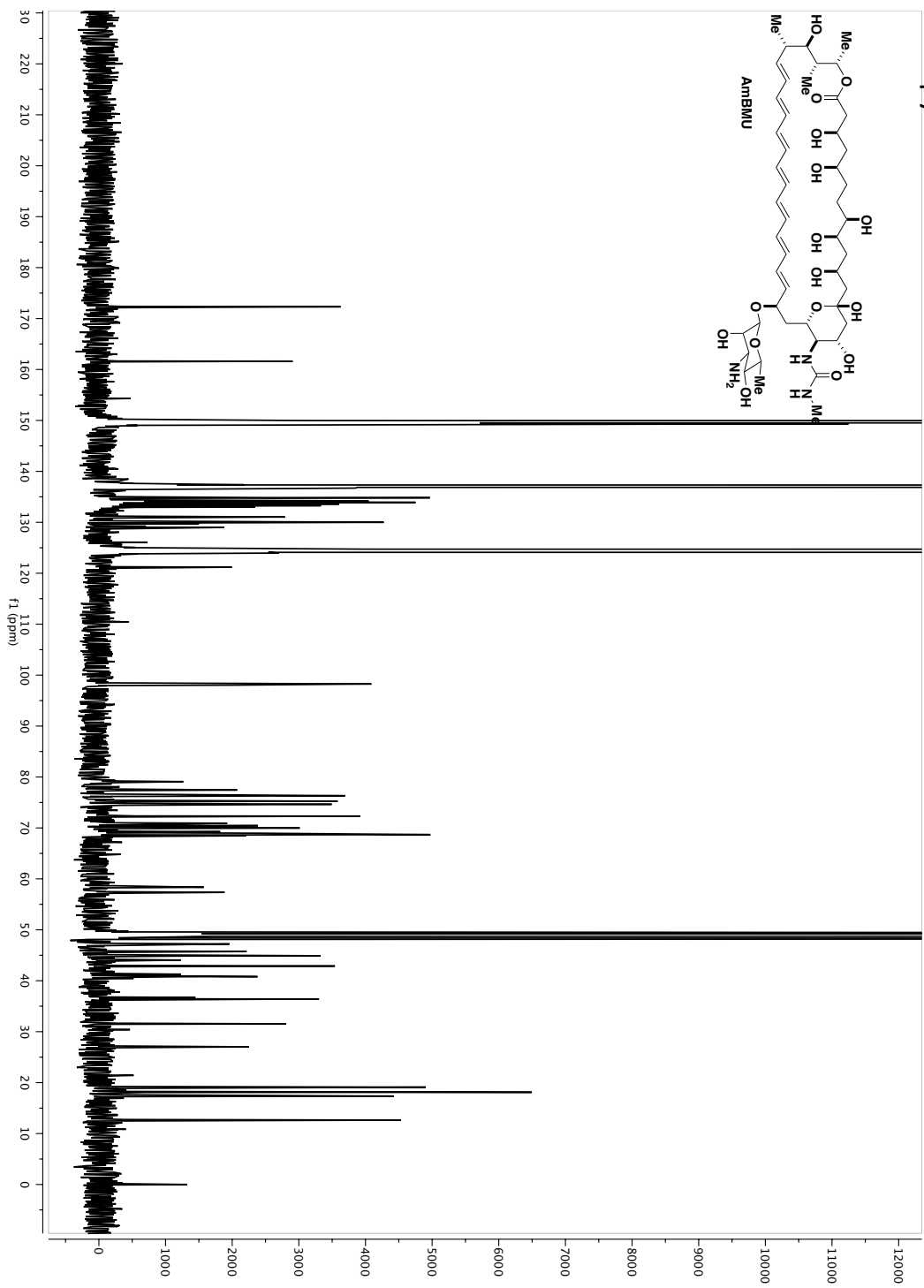
III. NMR spectra



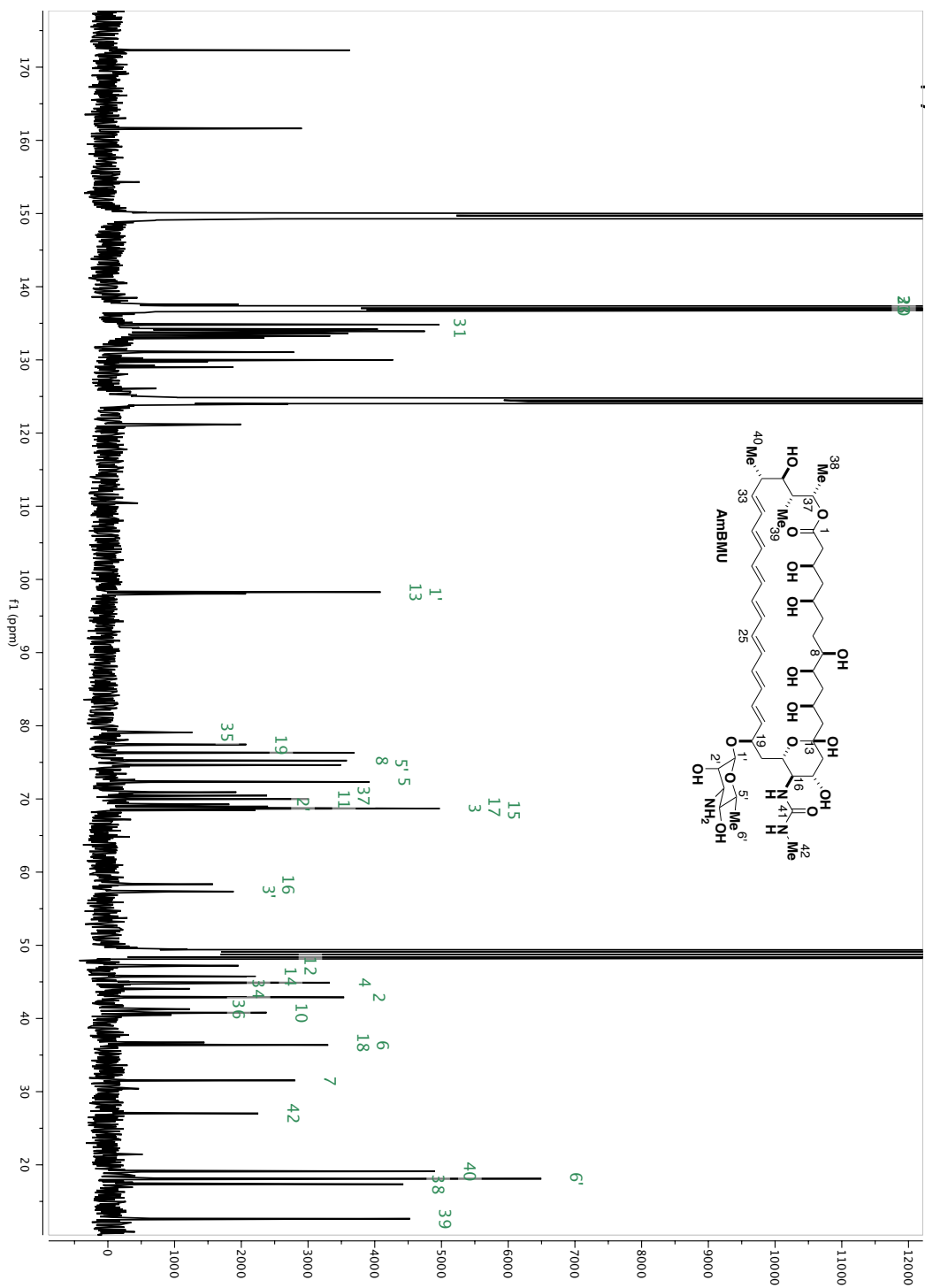
1:1 pyridine *d*-5:methanol *d*-4



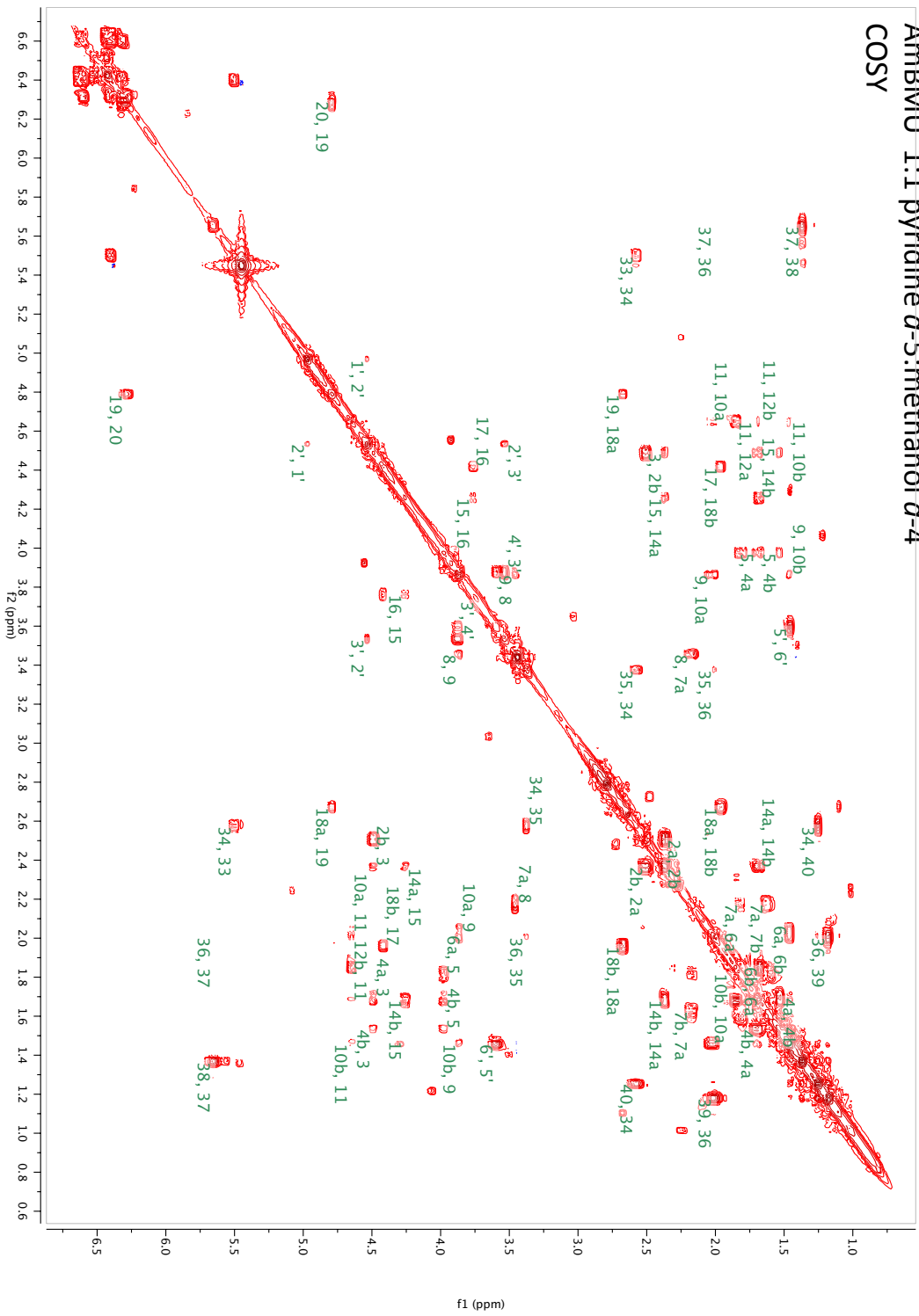
1:1 pyridine *d*-5:methanol *d*-4



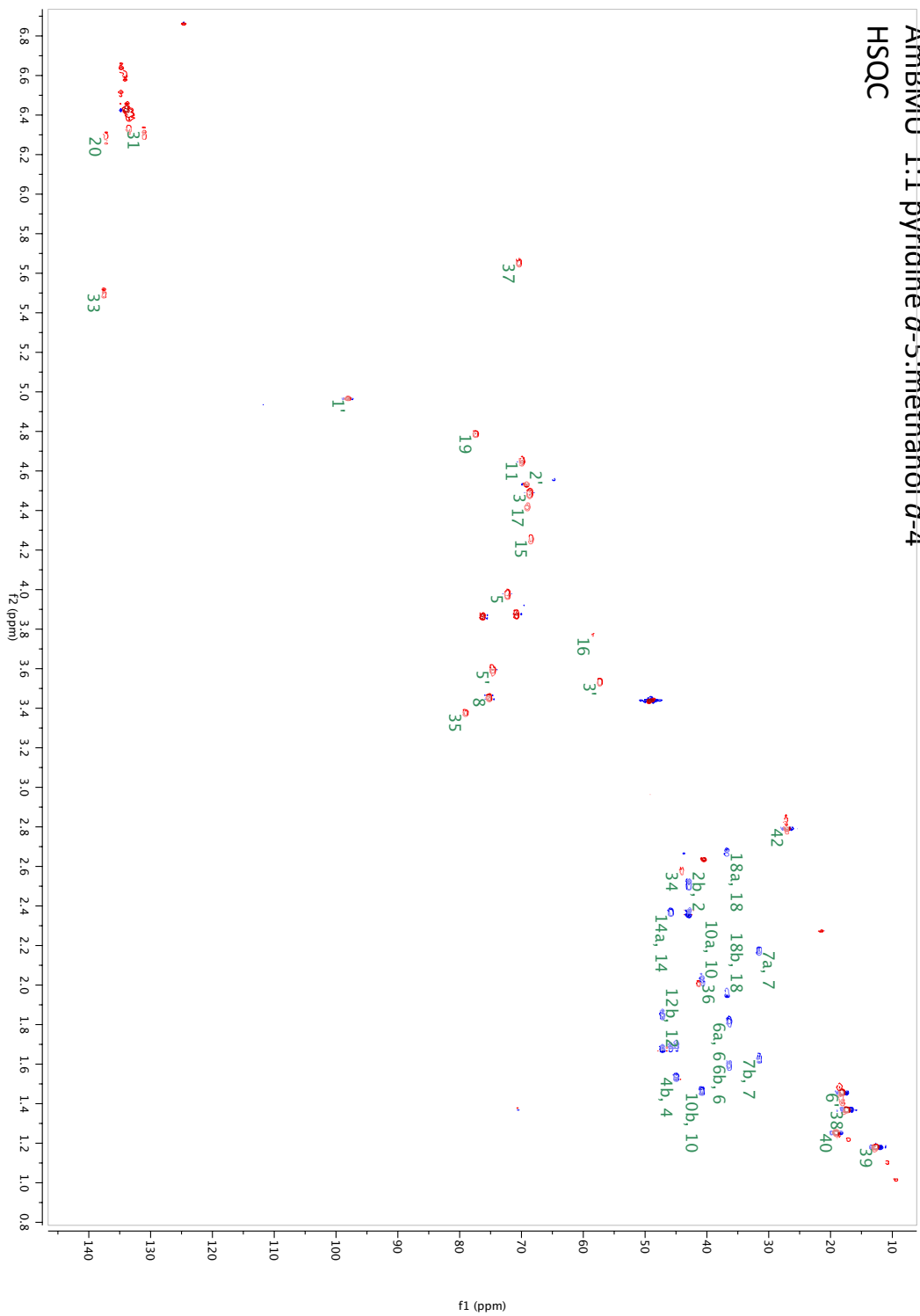
1:1 pyridine *d*-5:methanol *d*-4



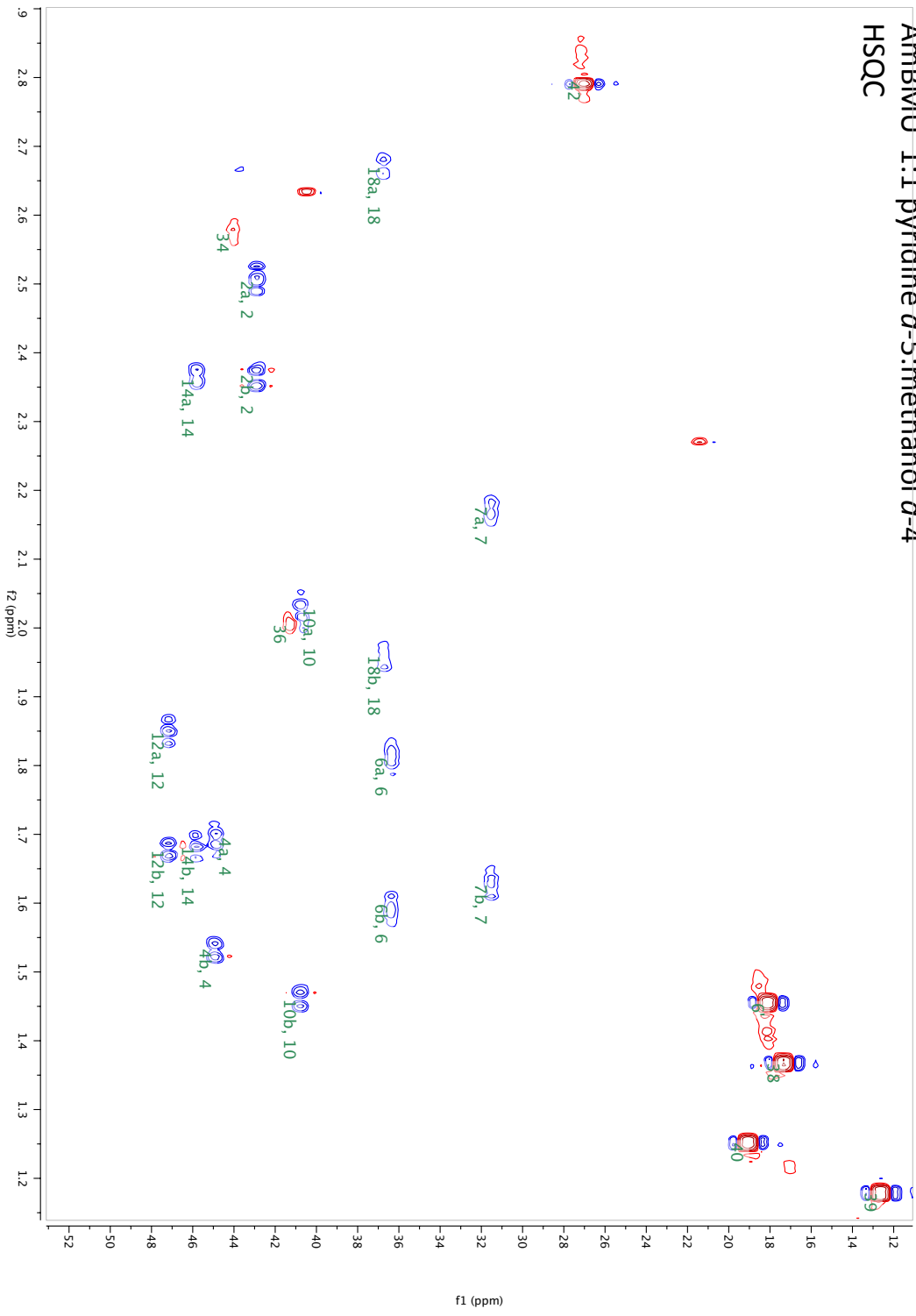
AmbMU 1:1 pyridine *d*-5:methanol *d*-4
COSY



AmbMU 1:1 pyridine *d*-5:methanol *d*-4
HSQC

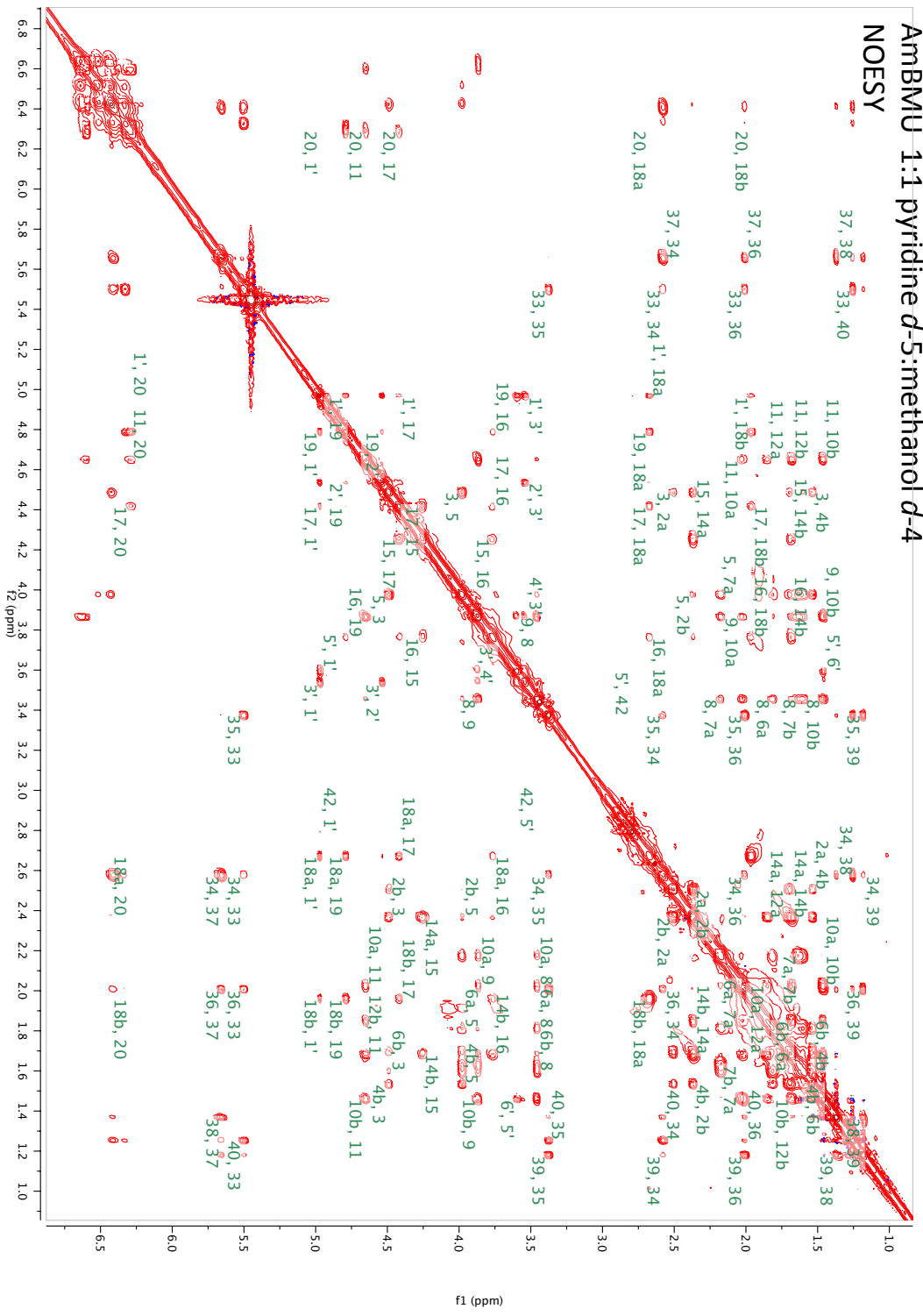


AmBMU 1:1:1 pyridine *d*-5:methanol *d*-4
HSQC

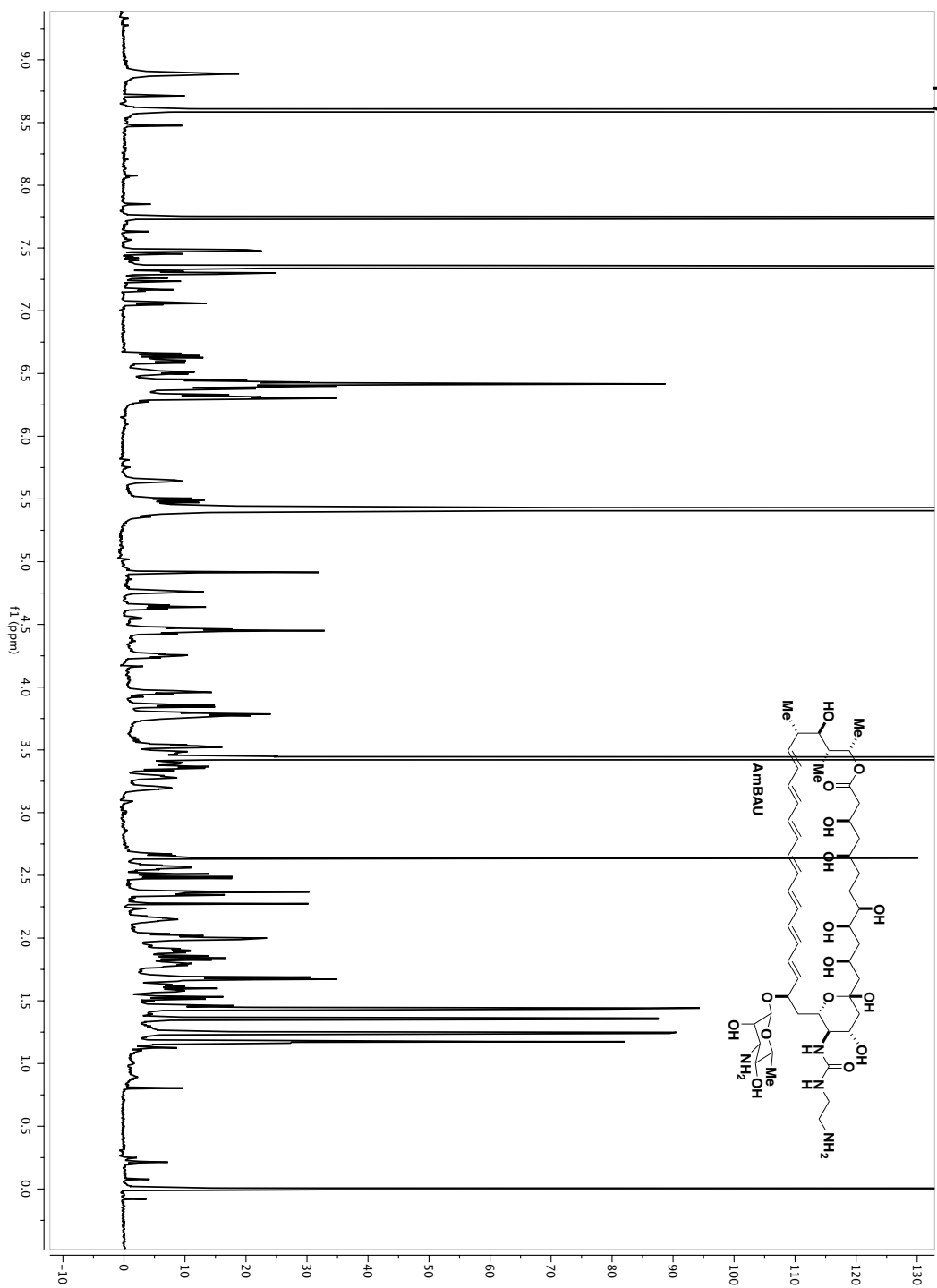


AmbMU 1:1 pyridine d-5:methanol d-4

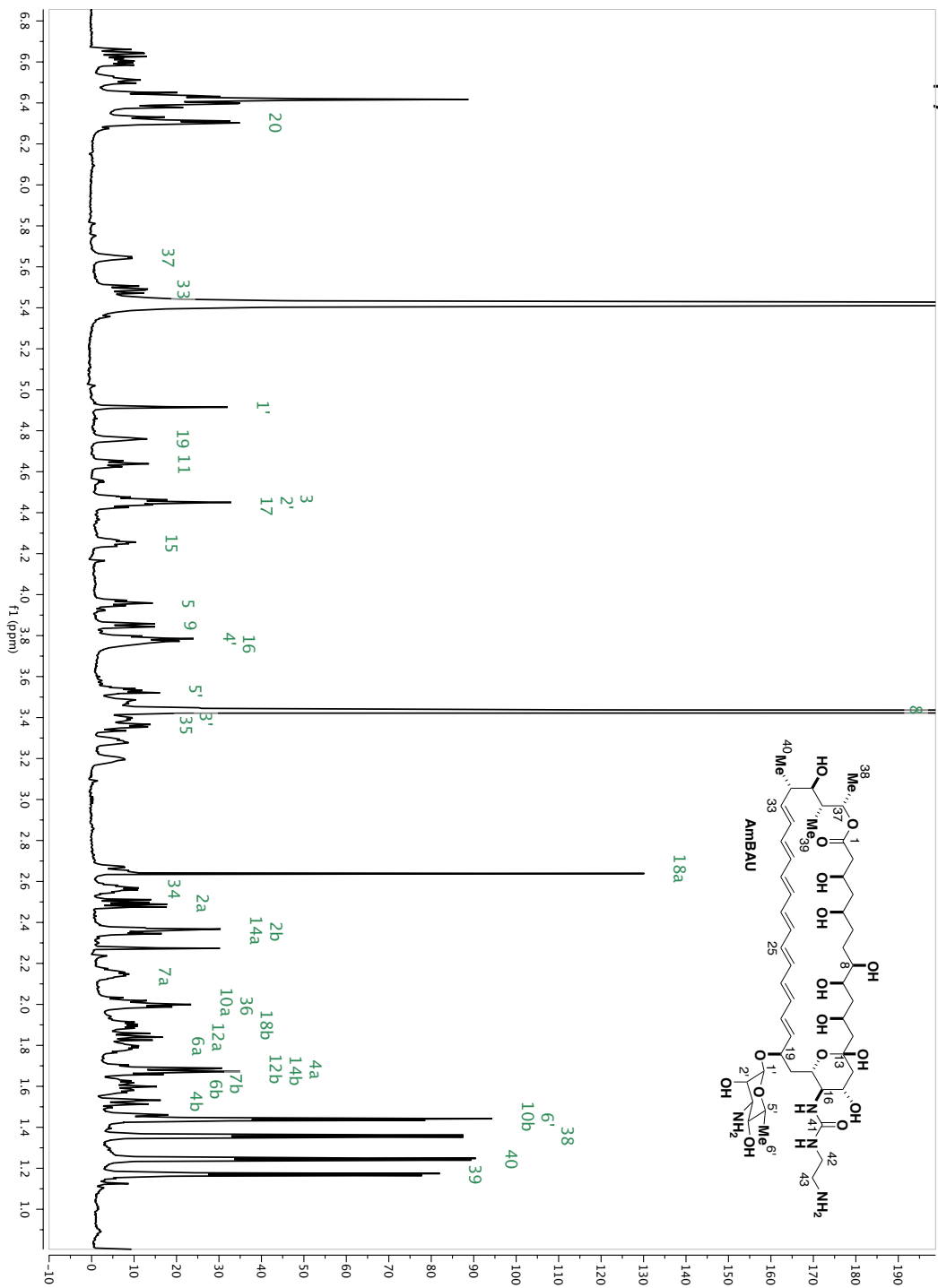
NOESY



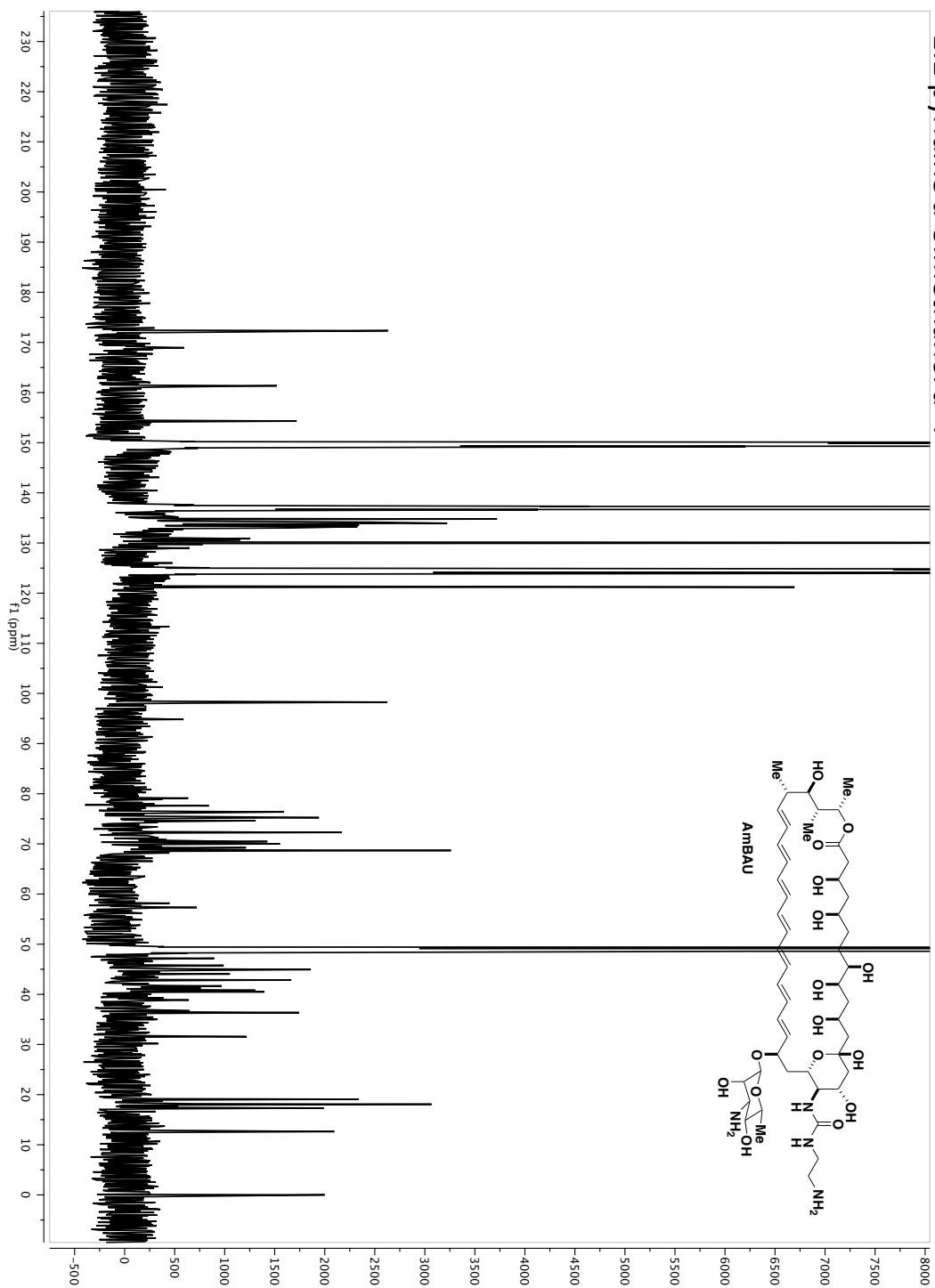
1:1 pyridine *d*-5:methanol *d*-4



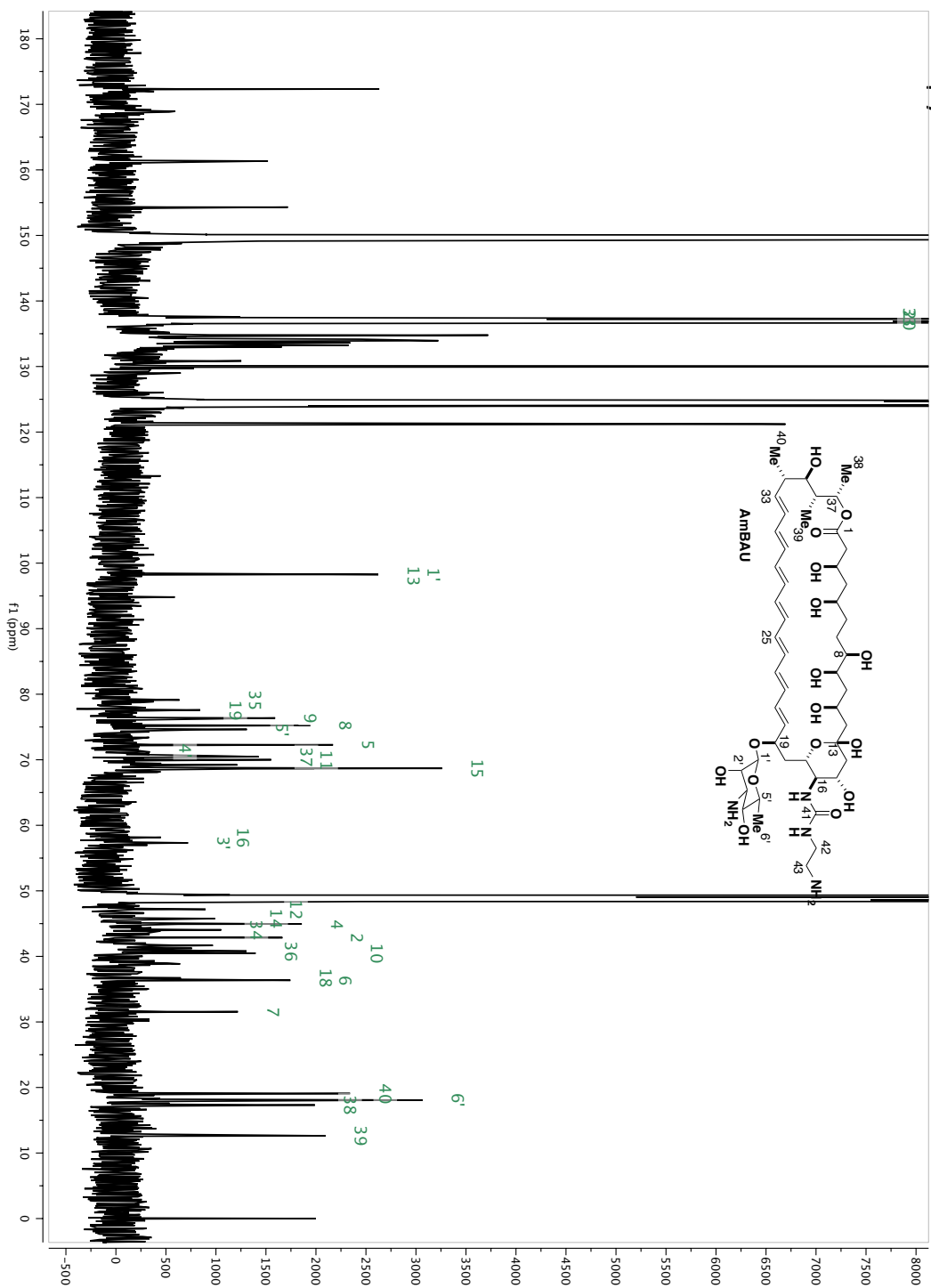
1:1 pyridine *d*-5:methanol *d*-4



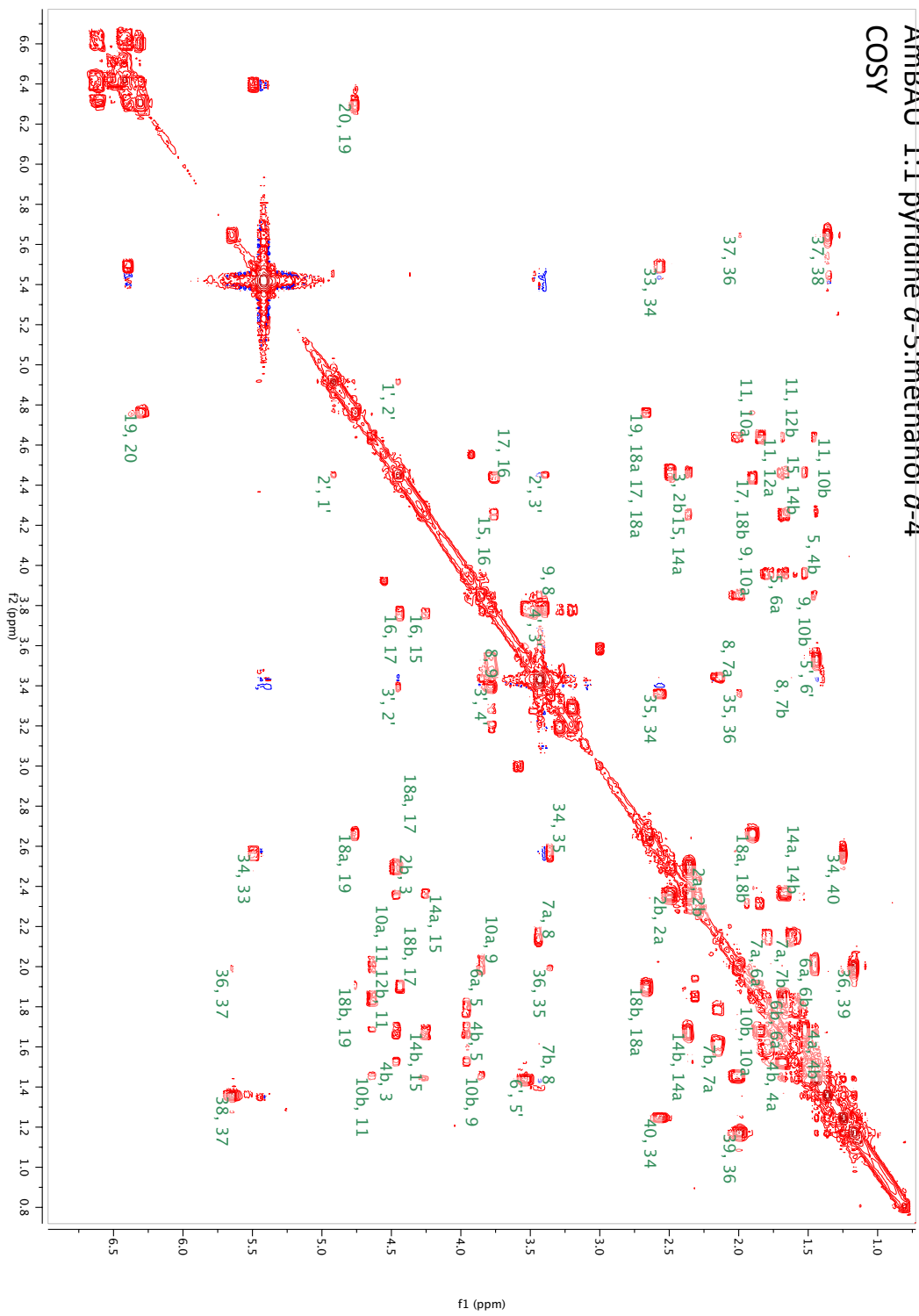
1:1 pyridine *d*-5:methanol *d*-4



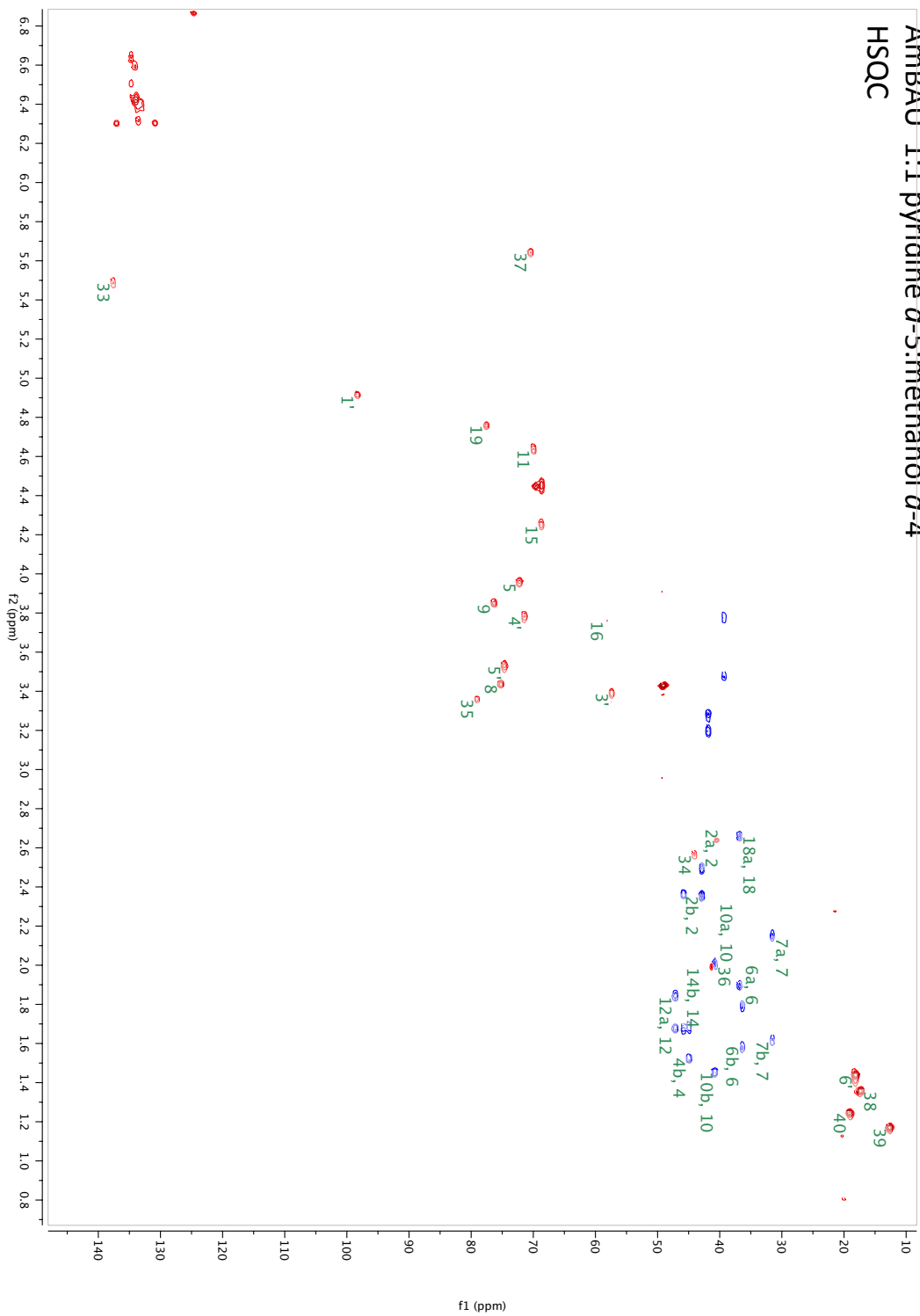
1:1 pyridine *d*-5:methanol *d*-4



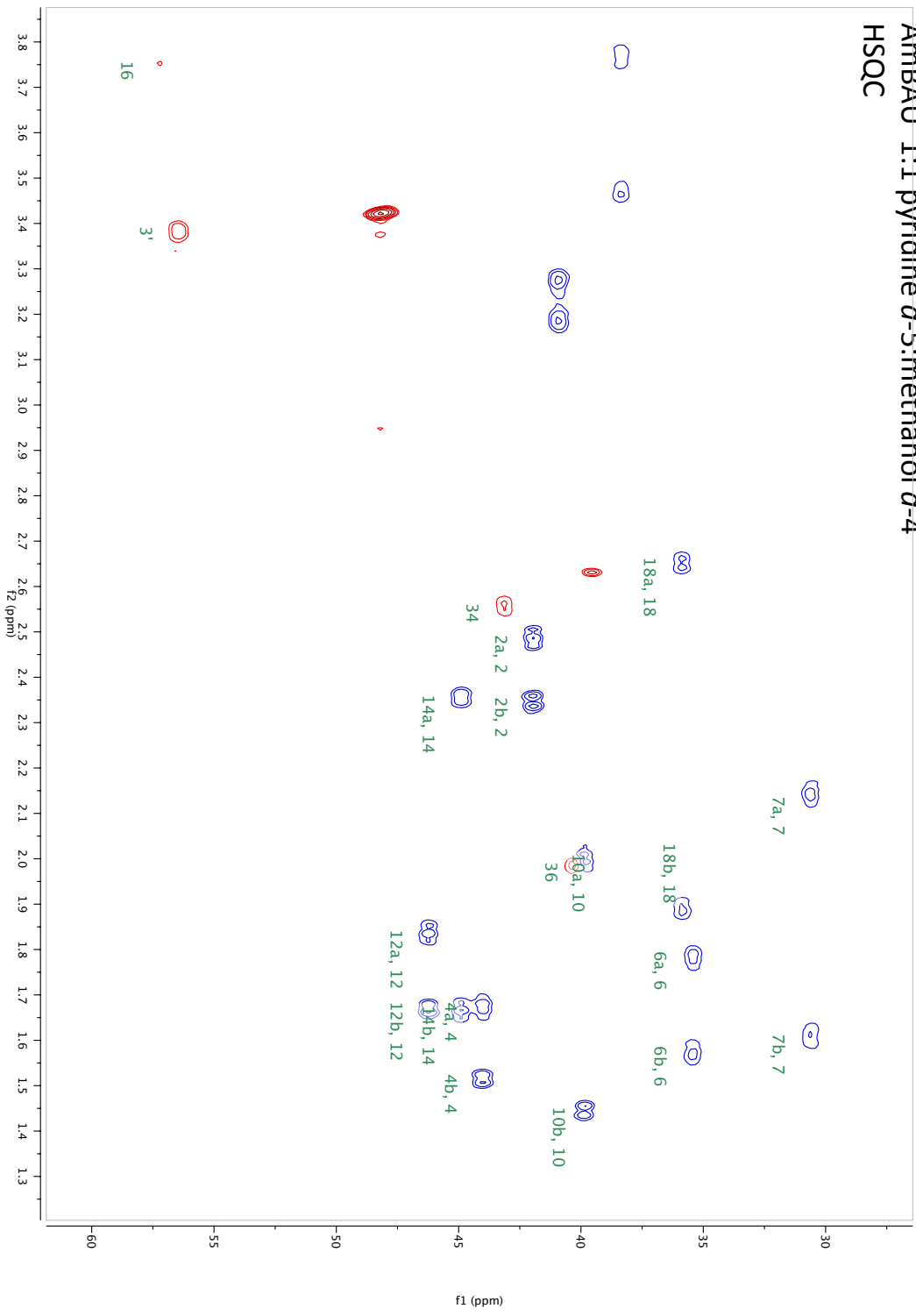
AmbAU 1:1 pyridine *d*-5:methanol *d*-4
COSY

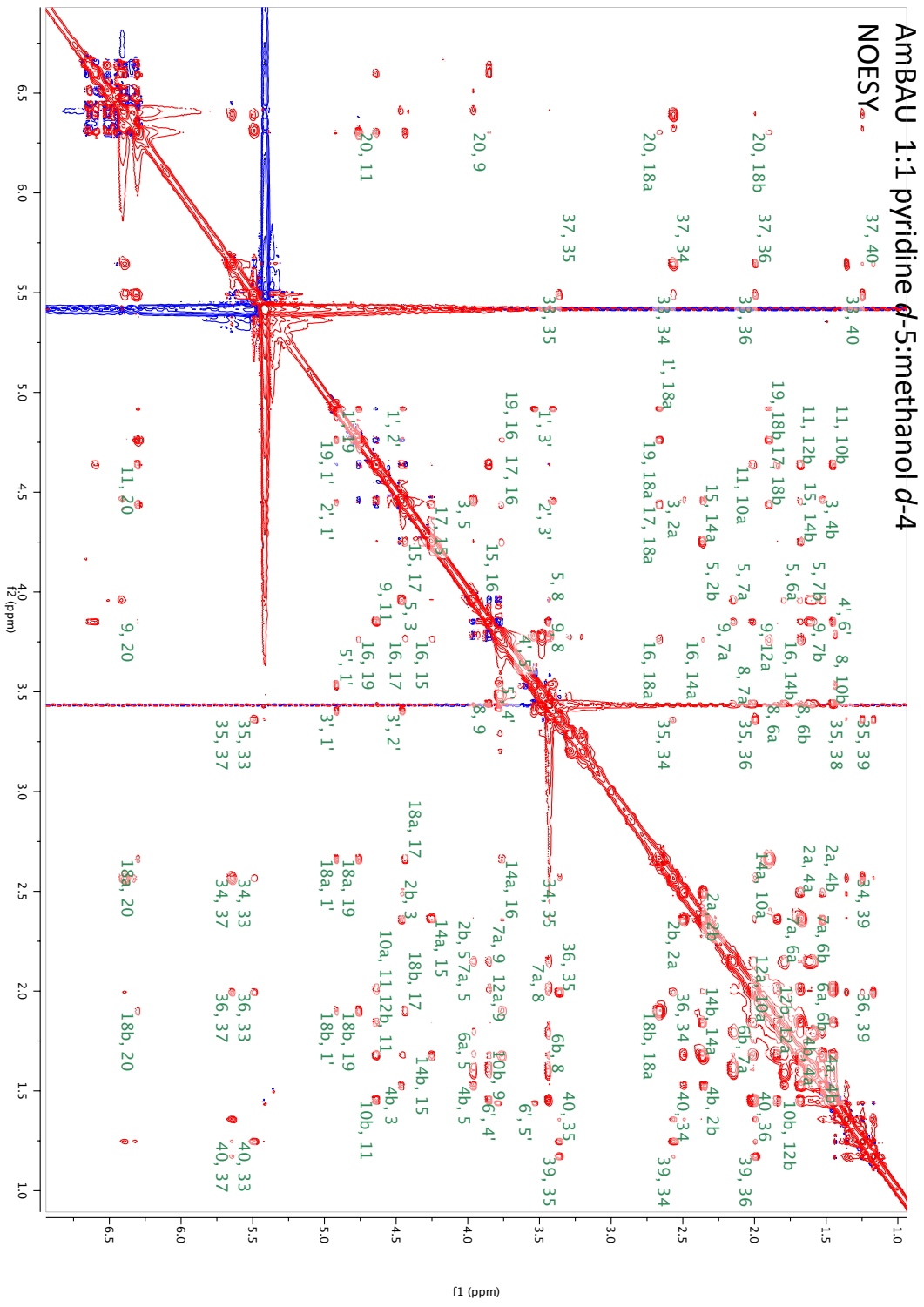


AmbAU 1:1 pyridine *d*-5:methanol *d*-4
HSQC

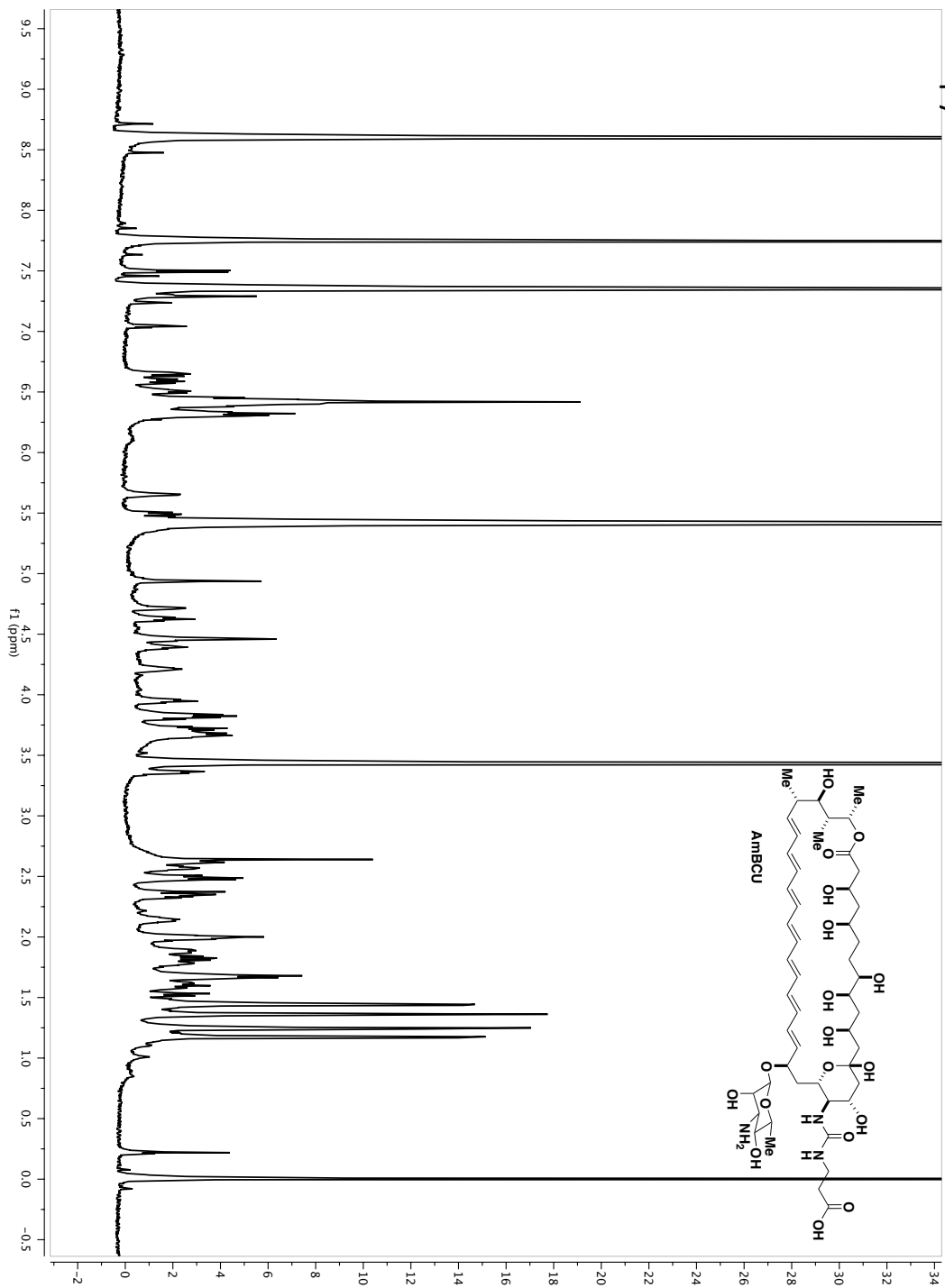


AmbAU 1:1 pyridine *d*-5:methanol *d*-4
HSQC

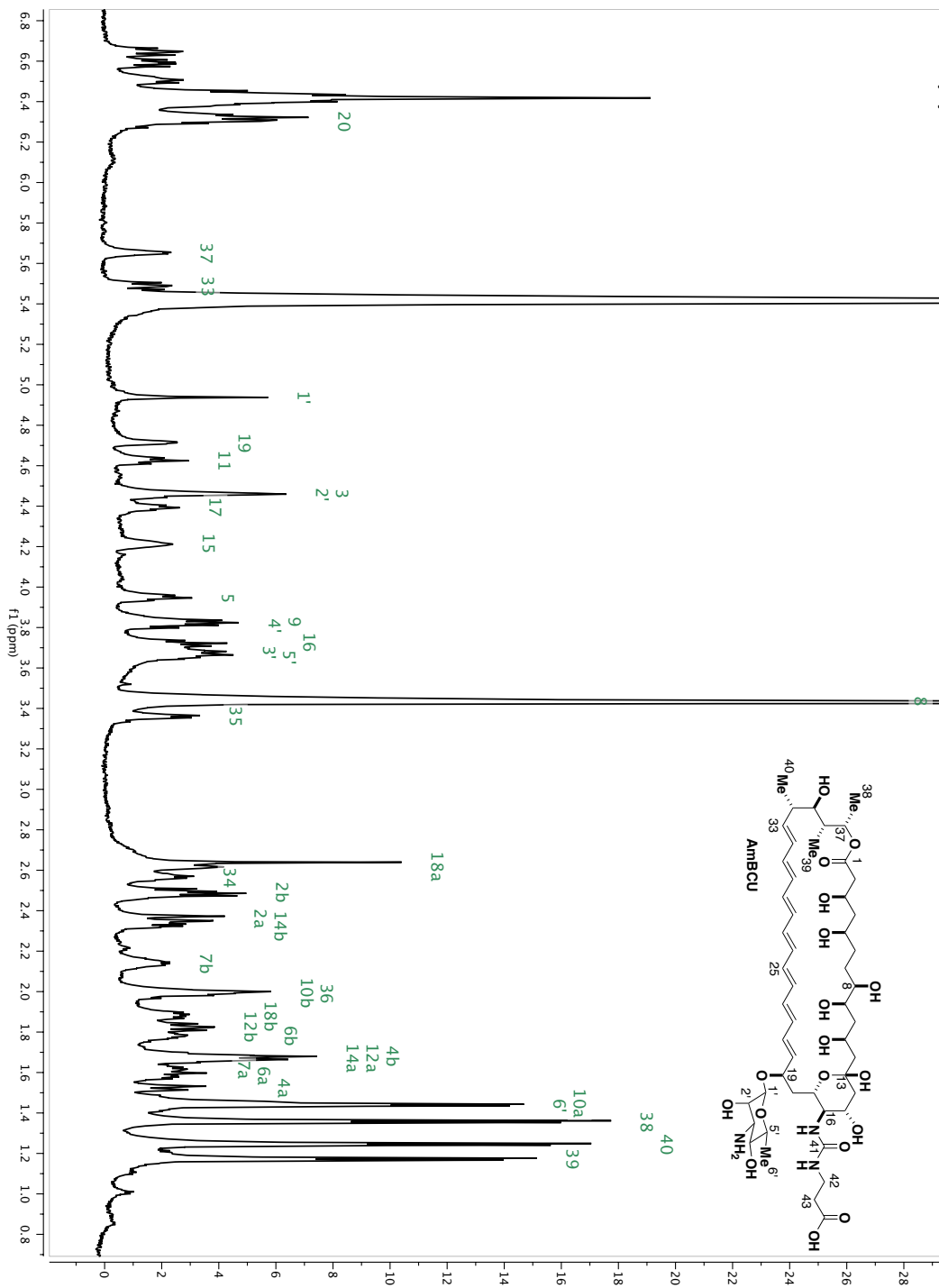




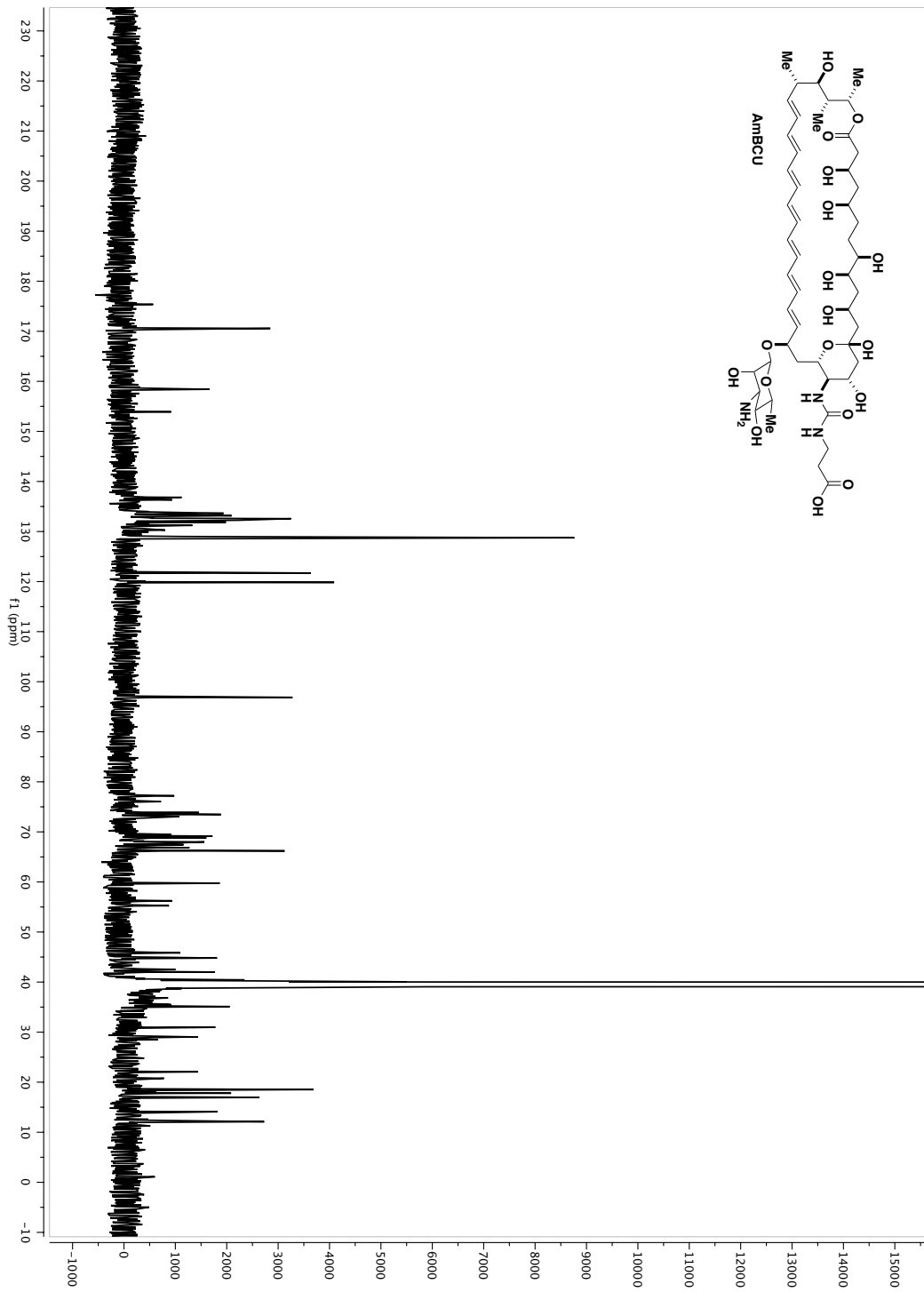
1:1 pyridine *d*-5:methanol *d*-4



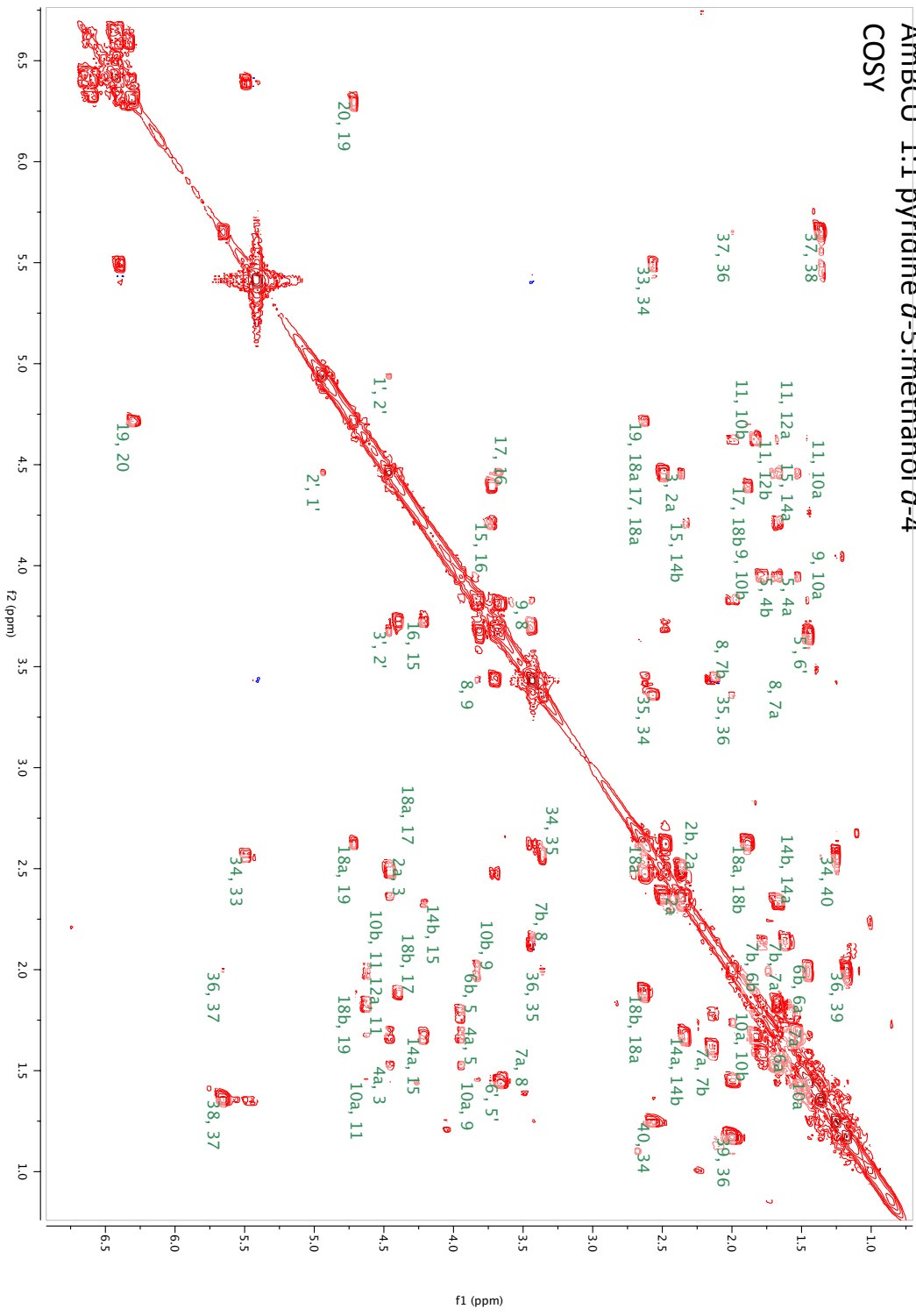
1:1 pyridine *d*-5:methanol *d*-4



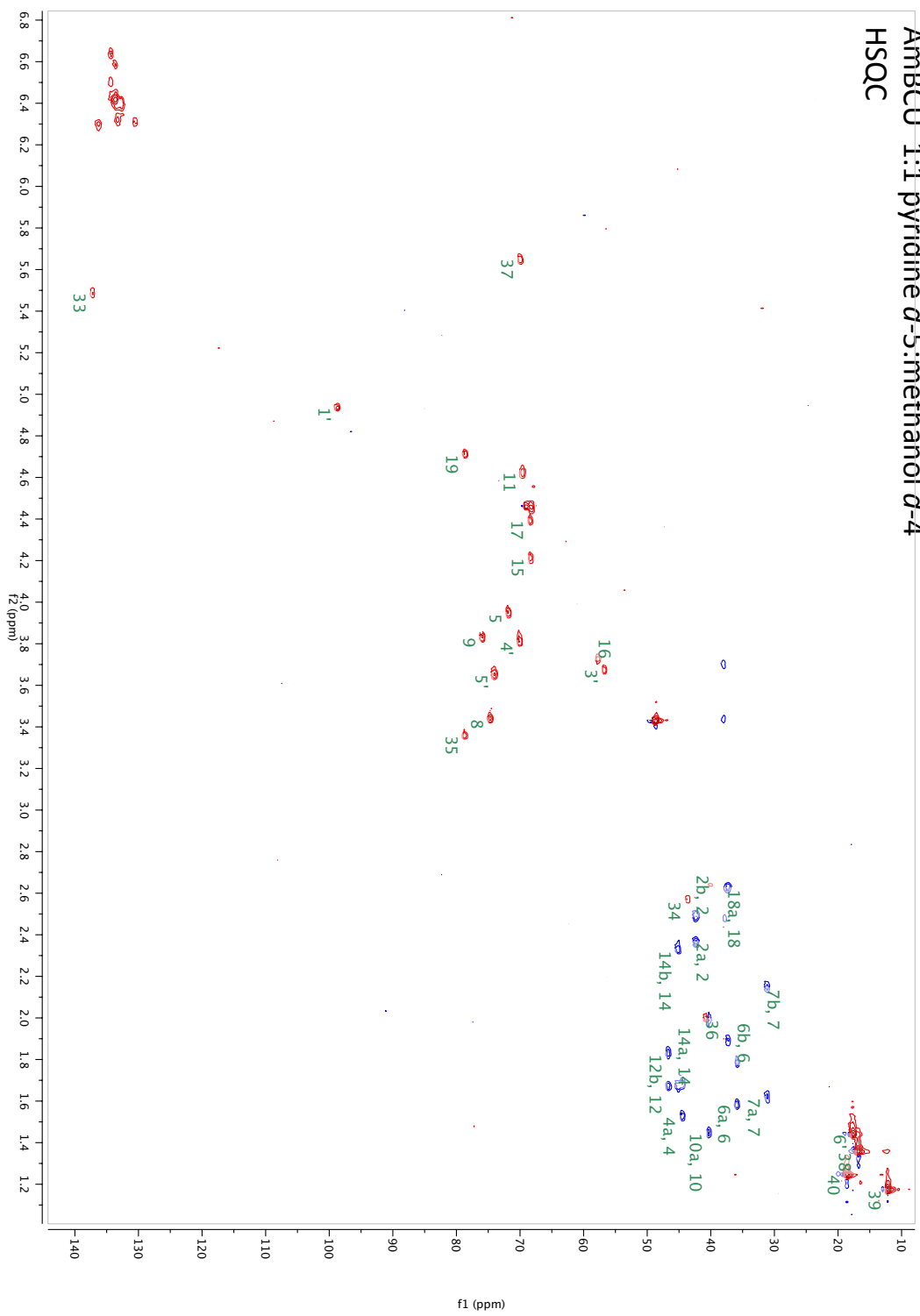
DMSO *d*-6



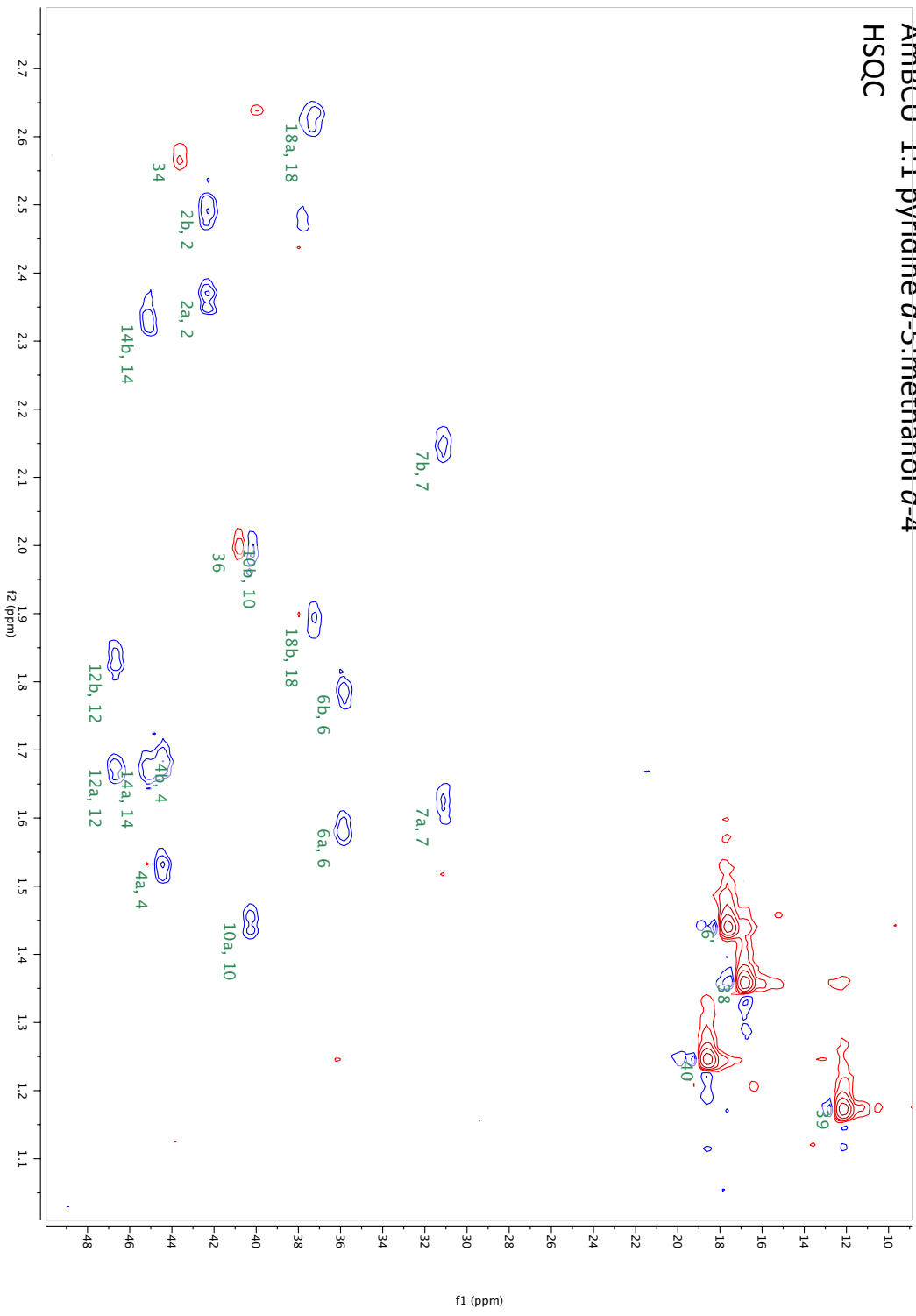
AmbCU 1:1 pyridine *d*-5:methanol *d*-4
 COSY



AmbCU 1:1 pyridine *d*-5:methanol *d*-4
HSQC

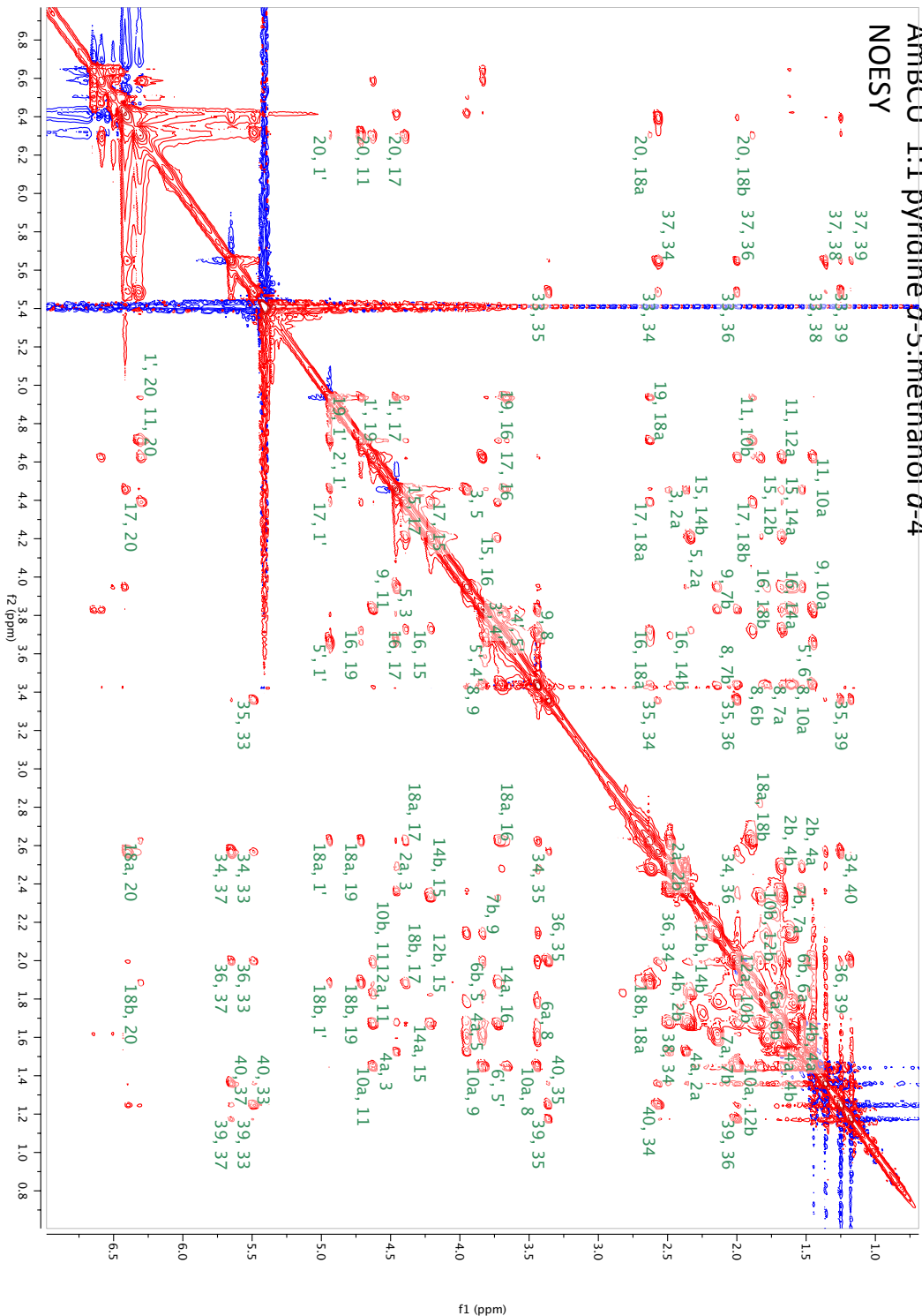


AmbCU 1:1 pyridine *d*-5: methanol *d*-4
HSQC



AmbCU 1:1 pyridine *d*-5:methanol *d*-4

NOESY



- 1 Pangborn, A. B., Giardello, M. A., Grubbs, R. H., Rosen, R. K. & Timmers, F. J. Safe and Convenient Procedure for Solvent Purification. *Organometallics* **15**, 1518-1520, (1996).
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- 4 Bonner, D. P., Mechlinski, W. & Schaffner, C. P. Polyene Macrolide Derivatives .3. Biological Properties of Polyene Macrolide Ester Salts. *J. Antibiot.* **25**, 261-262, (1972).
- 5 Tevyashova, A. N. *et al.* Structure-Antifungal Activity Relationships of Polyene Antibiotics of the Amphotericin B Group. *Antimicrob. Agents Chemother.* **57**, 3815-3822, (2013).
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