# Supporting Information

## Tulongicin, an Antibacterial Tri-Indole Alkaloid from a Deep Water *Topsentia* sp. Sponge

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Contents:



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**Sponge taxonomy.**

The sponge was collected with the manned submersible *Deepworker*, by Patrick L. Colin (Coral Reef Research Foundation), from the outer exposed face of Ulong Rock, just north of Ulong Channel, on the west barrier reef of Palau, from a depth of 140 m on 25 August 2008. The morphology of the sponge was a thick, flattened, V-shaped mass, 20 cm long and 5 cm thick. The surface was slightly fuzzy to the touch, overall undulating and sculpted. The surface was encrusted with a very thin, relatively smooth sponge, closely comparable to *Poecillastra incrustans* Sollas, 1888 (Demospongiae, Tetractinellida, Vulcanellidae), encrusting to about 1–2 mm deep and integrated into the supporting sponge. A single, 1 cm diameter oscule, with a thin collagenous rim and septa, was visible on the upper surface. The internal texture was dense and siliceous, the overall texture compressible and relatively easily broken. External color in life was oak-brown and the interior was tan, but darker where the encrusting sponge lies; preservative turning a very pale pinkish orange. There was no obvious odour. *Poecillastra incrustans* is characterised by a thin ectosome of roughened centrotylote microxeas, about 90 µm long, orientated tangentially and interspersed with streptasters and metasters about 20–22 µm long and spirasters, about 12–17 µm long. The choanosome consists of oxeas about 400 to more than 2000 µm long, in disarray, interspersed with calthrops, the clads of which are about 400 µm long. The great bulk of the supporting sponge has a granular choanosome and oxeas in what appear to be two size categories, the first up to about 300 µm long, and the second up to about 1000µm long. The arrangement of the oxeas is highly disorganised. The specimen is most likely an undescribed species of *Topsentia* (Demospongiae, Suberitida, Halichondriidae), most closely comparable to *Topsentia halichondrioides* (Dendy, 1905), but differing in the larger size of the oxeas (*T. halichondriodes*: 120–645 µm long; Hooper *et al*., 1997). The calthrops of this species are intermingled with the oxeas of the supporting sponge, especially in the outer choanosome. A voucher specimen has been deposited at NIWA Invertebrate Collection (NIC) at the National Institute of Water & Atmospheric Research (NIWA), Auckland, New Zealand (NIWA 112371), and the California Academy of Sciences (CAS302370).

**ECD calculation for compounds 1, 2, and 2a-2d.**

**Generation of the conformers.** Maestro 10.5[i] was employed to build the 3D chemical structures of **1**, **2**, and **2a**-**2d**, that were optimized with MacroModel 11.1,[ii] using the OPLS force field[iii] and the Polak-Ribier conjugate gradient algorithm (PRCG, maximum derivative less than 0.001 kcal/mol). Once the starting 3D structures of **1**, **2** and **2a**-**2d** were obtained, exhaustive conformational searches were performed at the empirical molecular mechanics (MM) level with the Monte Carlo Multiple Minimum (MCMM) method (50,000 steps) to allow a full exploration of the conformational space. The Low Mode Conformational Search (LMCS) method (50,000 steps) was also employed to integrate the conformational sampling. Also, molecular dynamics simulations were performed at 450, 600, 700, 750 K, with a time step of 2.0 fs, an equilibration time of 0.1 ns, and a simulation time of 10 ns. For each compound, all the conformers obtained from the conformational searches were minimized (PRCG,

maximum derivative less than 0.001 kcal/mol) and then compared, using the “Redundant Conformer Elimination” module of Macromodel 11.1i to select non-redundant conformers. In detail, the conformers differing more than 21.0 kJ/mol (5.02 kcal/mol) from the most energetically favoured conformation were discarded, and a RMSD (root-mean-square deviation) minimum cut-off of 0.5 Å was set for saving structures.

**DFT calculations and prediction of the ECD spectra** The conformers obtained from MM calculations were optimized at the quantum mechanical (QM) level using the MPW1PW91 functional and the 6-31G(d) basis set and the integral equation formalism version of the polarizable continuum model (methanol IEFPCM). The new obtained geometries were visually inspected in order to remove further possible redundant conformers, and then those selected were used for the prediction of the ECD spectra at TDDFT (NStates=60) MPW1PW91/6-31g(d,p) level and methanol IEFPCM. Final ECD spectra for **1**, **2**, and **2a**-**2d** were built considering the influence of each on the total Boltzmann distribution taking into account the relative energies. SpecDisc software[iv] was used to simulate the ECD curve, applying a Gaussian band-shape function with the exponential half-width of 0.25 eV. All QM calculations were performed using Gaussian 09 software package.[v]

**Table S1** $^{1}H$ (500 MHz) and $^{13}C$ (125 MHz) NMR data of compounds **1**-**2** in $CD_3OD$

| | **1** | | **2** | |
|---|---|---|---|---|
| No. | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ |
| 2 | | 172.5 | | 173.6 |
| 4 | 5.71 dd (12.0, 8.3) | 56.0 | 5.67 dd (11.9, 8.4) | 56.1 |
| 5 | a 3.96 dd (11.8, 8.3) | 52.2 | a 3.94 dd (11.7, 8.4) | 52.2 |
| | b 4.37 dd (12.0, 11.8) | | b 4.37 dd (11.9, 11.7) | |
| 6 | 5.91 s | 34.9 | 5.97 s | 64.2 |
| 2' | 7.18 s | 126.8 | 7.47 s | 126.8 |
| 3' | | 111.1 | | 113.0 |
| 3a' | | 126.3 | | 125.3 |
| 4' | 7.46 d (8.5) | 120.8 | 7.60 d (8.5) | 121.7 |
| 5' | 7.18 dd (8.5, 1.7) | 123.9 | 7.13 dd (8.5, 1.8) | 124.0 |
| 6' | | 116.7 | | 116.8 |
| 7' | 7.62 d (1.7) | 115.8 | 7.62 d (1.8) | 115.7 |
| 7a' | | 139.2 | | 139.6 |
| 2'' | 7.25 s | 126.1 | 7.35 s | 126.1 |
| 3'' | | 113.9 | | 114.0 |
| 3a'' | | 124.6 | | 124.7 |
| 4'' | 6.93 d (8.5) | 120.6 | 7.09 d (8.5) | 120.7 |
| 5'' | 6.82 dd (8.5, 1.8) | 123.6 | 6.94 dd (8.5, 1.8) | 123.7 |
| 6'' | | 116.6 | | 116.6 |
| 7'' | 7.53 d (1.8) | 115.8 | 7.57 d (1.8) | 115.8 |
| 7a'' | | 139.5 | | 139.3 |
| 2''' | 7.26 s | 126.8 | | |
| 3''' | | 111.1 | | |
| 3a''' | | 126.2 | | |
| 4''' | 7.46 d (8.5) | 120.9 | | |
| 5''' | 7.21 dd (8.5, 1.8) | 123.9 | | |
| 6''' | | 116.7 | | |
| 7''' | 7.65 d (1.8) | 115.9 | | |
| 7a''' | | 139.3 | | |

**Table S2**.$^{1}$H (600 MHz) and $^{13}$C (600 MHz) NMR data of **2a**-**2d** in $CD_3OD$.

| | **2a** | | **2b** | | **2c** | | **2d** | |
|---|---|---|---|---|---|---|---|---|
| No. | $\delta_H$ (J in Hz) | $\delta_C$ | $\delta_H$ (J in Hz) | $\delta_C$ | $\delta_H$ (J in Hz) | $\delta_C$ | $\delta_H$ (J in Hz) | $\delta_C$ |
| 1-$CH_3$ | | | 3.48 s, 3H | 35.1 | 3.39 s, 3H | 34.4 | 3.28 s, 3H | 34.7 |
| 2 | | 171.4 | | 167.5 | | 167.8 | | 162.3 |
| 3-$CH_3$ | 2.66 s, 3H | 30.6 | 3.01 s, 3H | 32.3 | 2.95 s, 3H | 32.1 | 2.86 s, 3H | 32.1 |
| 4 | 5.50 dd, (12.2, 9.4) | 63.3 | 5.49 dd, (12.4, 9.9) | 60.9 | 5.48 dd, (12.3, 9.6) | 60.3 | 5.77 dd, (12.3, 10.5) | 61.7 |
| 5 | 4.38 dd, (12.2, 11.6) | 50.1 | 4.38 dd, (12.4, 11.9) | 57.9 | 4.34 dd, (12.3, 11.8) | 57.3 | 4.60 dd, (12.3, 10.9) | 58.1 |
| | 4.06 (dd, 11.6, 9.4) | | 4.11 dd, (11.9, 9.9) | | 4.05 dd, (11.8, 9.6) | | 4.19 dd, (10.9, 10.5) | |
| 6 | 6.12 s | 63.4 | 6.42 s | 63.0 | 4.30 d, (16.2) | 21.7 | | 173.8 |
| | | | | | 4.27 d, (16.2) | | | |
| 1'-$CH_3$ | | | | | | | 4.01 s, 3H | 34.8 |
| 2' | 7.55 s | 127.3 | 7.45 s | 125.9 | 7.33 s | 126.0 | 8.57 s | 144.7 |
| 3' | | 111.4 | | 111.2 | | 105.1 | | 114.7 |
| 3a' | | 125.4 | | 125.6 | | 126.6 | | 125.4 |
| 4' | 7.70 d, (8.5) | 121.1 | 8.20 d, (8.6) | 120.9 | 7.52 d, (8.5) | 120.4 | 8.20 brd, (8.2) | 124.4 |
| 5' | 7.27 dd, (8.5, 1.9) | 124.4 | 7.27 dd, (8.6, 1.9) | 124.4 | 7.24 dd, (8.5, 1.8) | 124.1 | 7.58 brd, (8.2) | 128.9 |
| 6' | | 116.9 | | 117.0 | | 116.9 | | 119.9 |
| 7' | 7.63 d, (1.9) | 115.9 | 7.64 d, (1.9) | 115.9 | 7.62 d, (1.8) | 115.8 | 7.93 brs | 115.9 |
| 7a' | | 139.3 | | 139.2 | | 138.9 | | 141.1 |
| 2'' | 7.46 s | 128.3 | 7.51 s | 128.6 | 7.48 s | 128.3 | 7.60 s | 128.5 |
| 3'' | | 110.9 | | 110.5 | | 110.6 | | 110.2 |
| 3a'' | | 124.8 | | 124.7 | | 124.7 | | 124.8 |
| 4'' | 7.37 d, (8.5) | 120.4 | 7.18 d, (8.5) | 120.4 | 7.13 d, (8.5) | 120.5 | 7.50 d, (8.5) | 120.4 |
| 5'' | 7.18 dd, (8.5, 1.8) | 124.3 | 7.06 dd, (8.5, 1.9) | 124.2 | 7.01 dd, (8.5, 1.7) | 124.1 | 7.31 brd, (8.5) | 124.7 |
| 6'' | | 117.0 | | 117.0 | | 116.9 | | 117.2 |
| 7'' | 7.63 d, (1.8) | 116.2 | 7.62 d, (1.9) | 116.2 | 7.60 d, (1.7) | 116.2 | 7.68 brs | 116.4 |
| 7a'' | | 139.8 | | 139.8 | | 139.7 | | 139.8 |

**Figure S1.** $^{1}$H NMR spectrum of **1** in DMSO-$d_6$ at 298 K

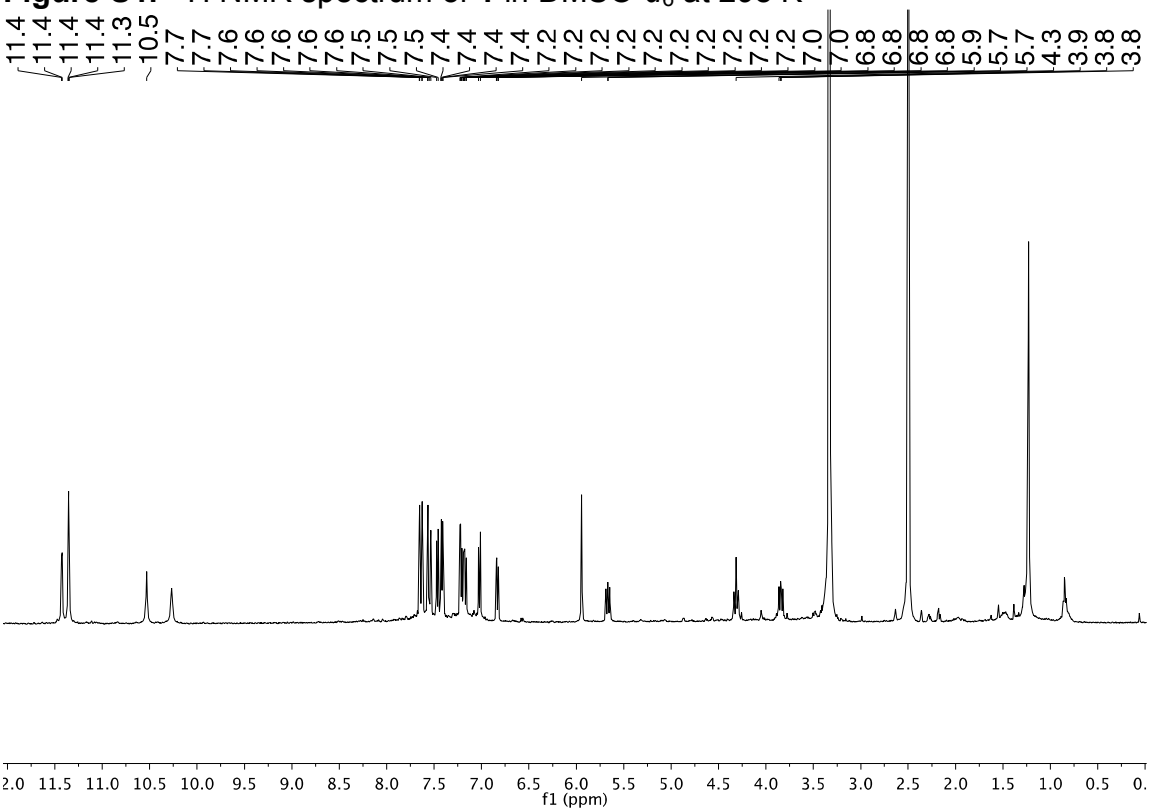



**Figure S2.** $^{1}$H NMR spectrum of **1** in DMSO-$d_6$ at 328 K

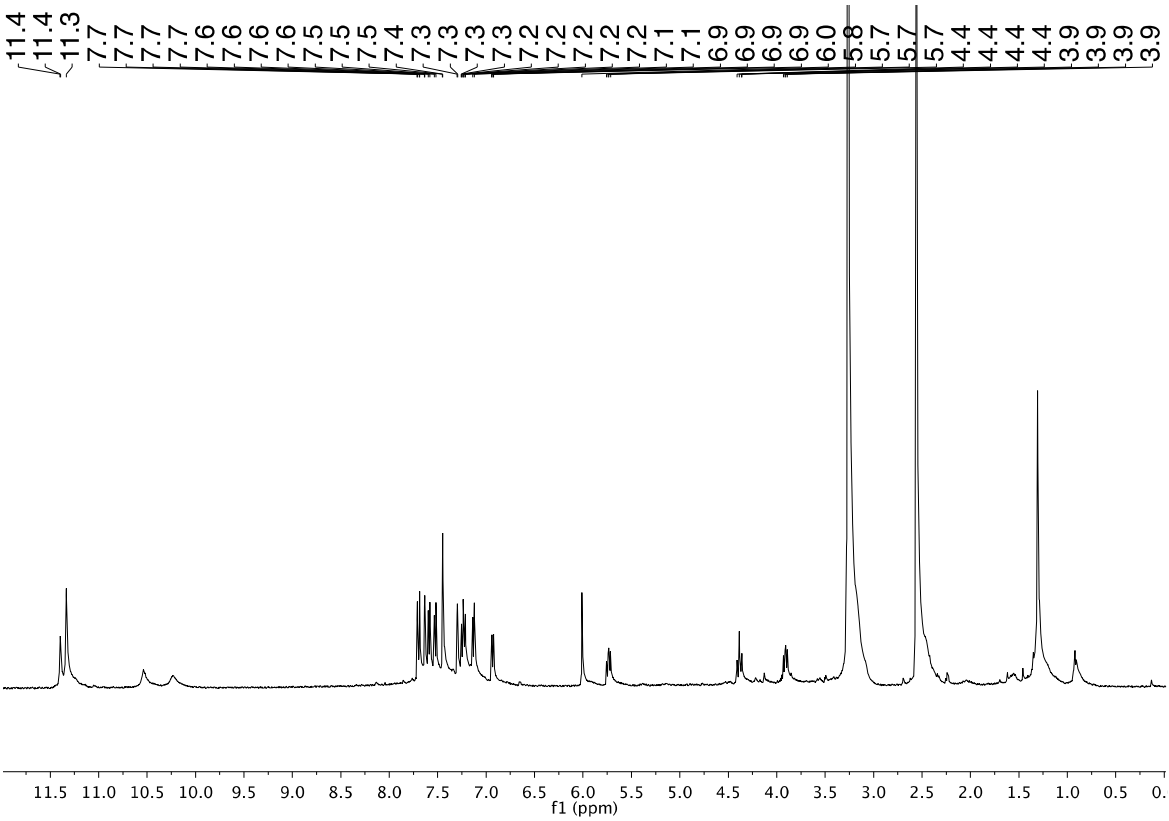


**Figure S3.** $^{13}C$ NMR spectrum of **1** in DMSO-$d_6$ at 298 K

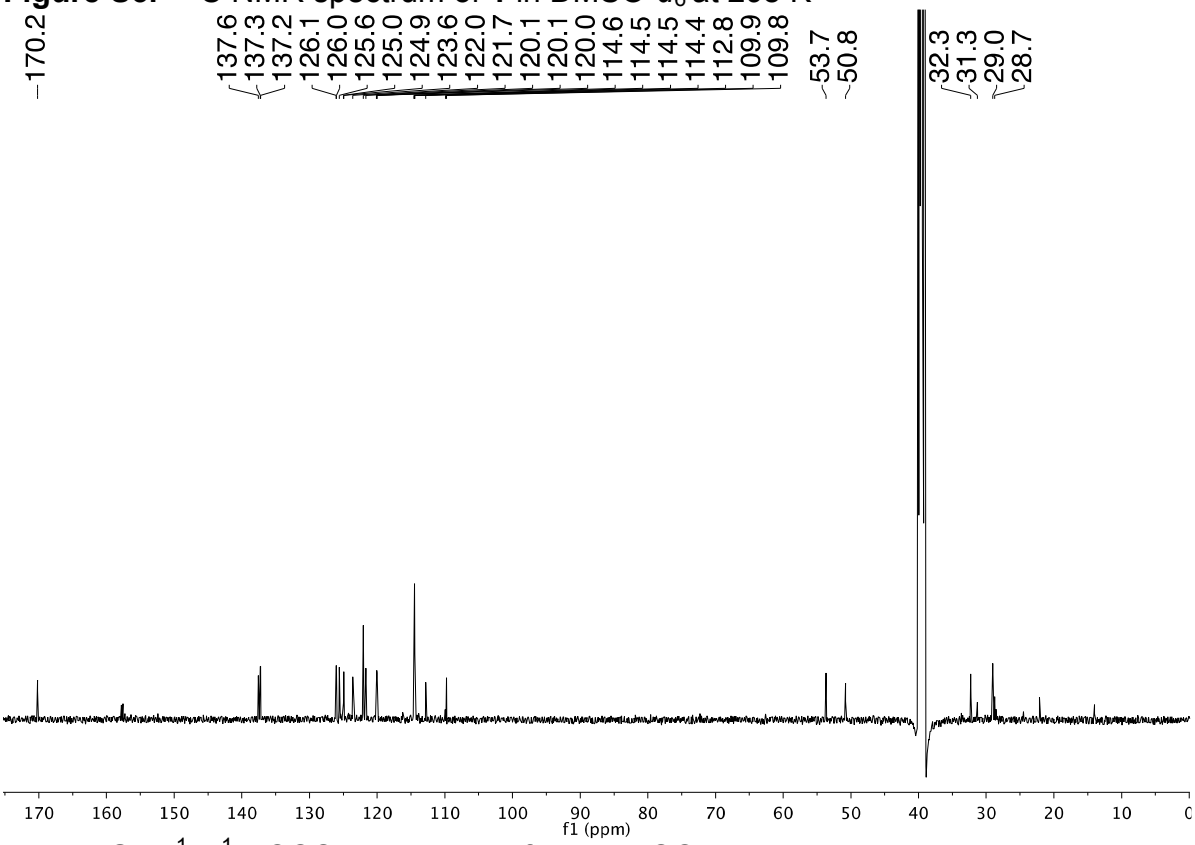



**Figure S4.** $^{1}H$-$^{1}H$ COSY spectrum of **1** in DMSO-$d_6$ at 298 K

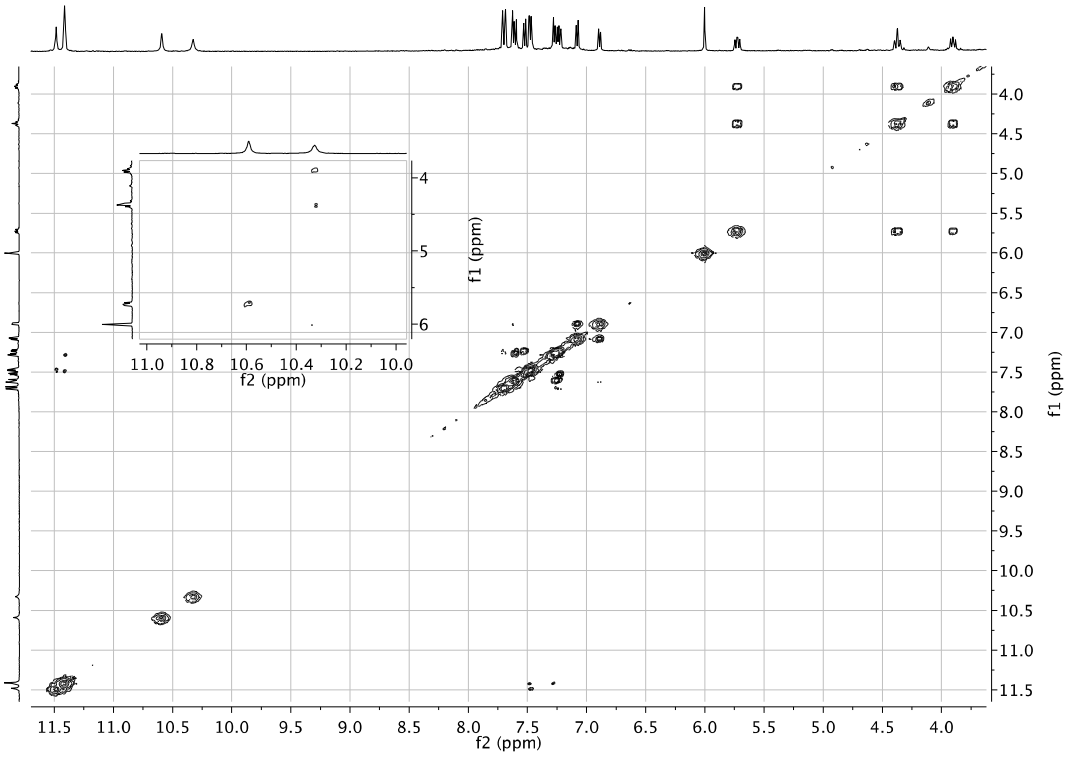


**Figure S5.** HSQC spectrum of **1** in DMSO-$d_6$ at 298 K

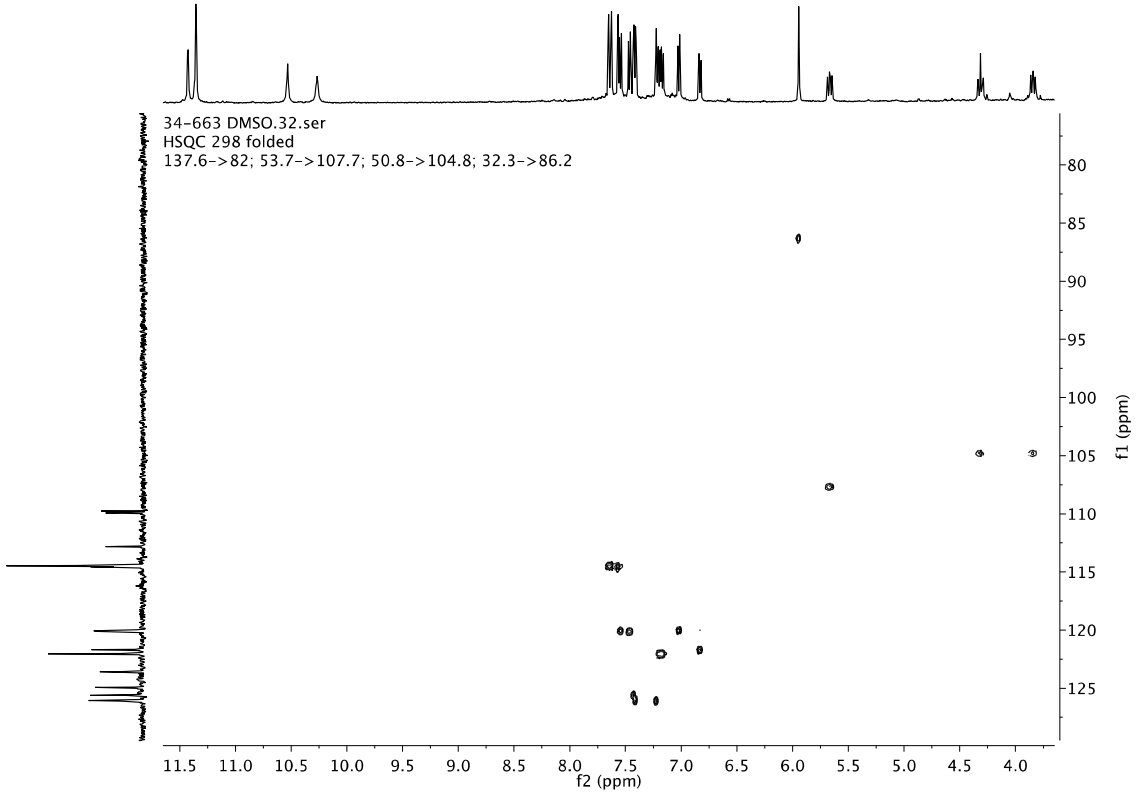



**Figure S6.** HMBC spectrum of **1** in DMSO-$d_6$ at 298 K

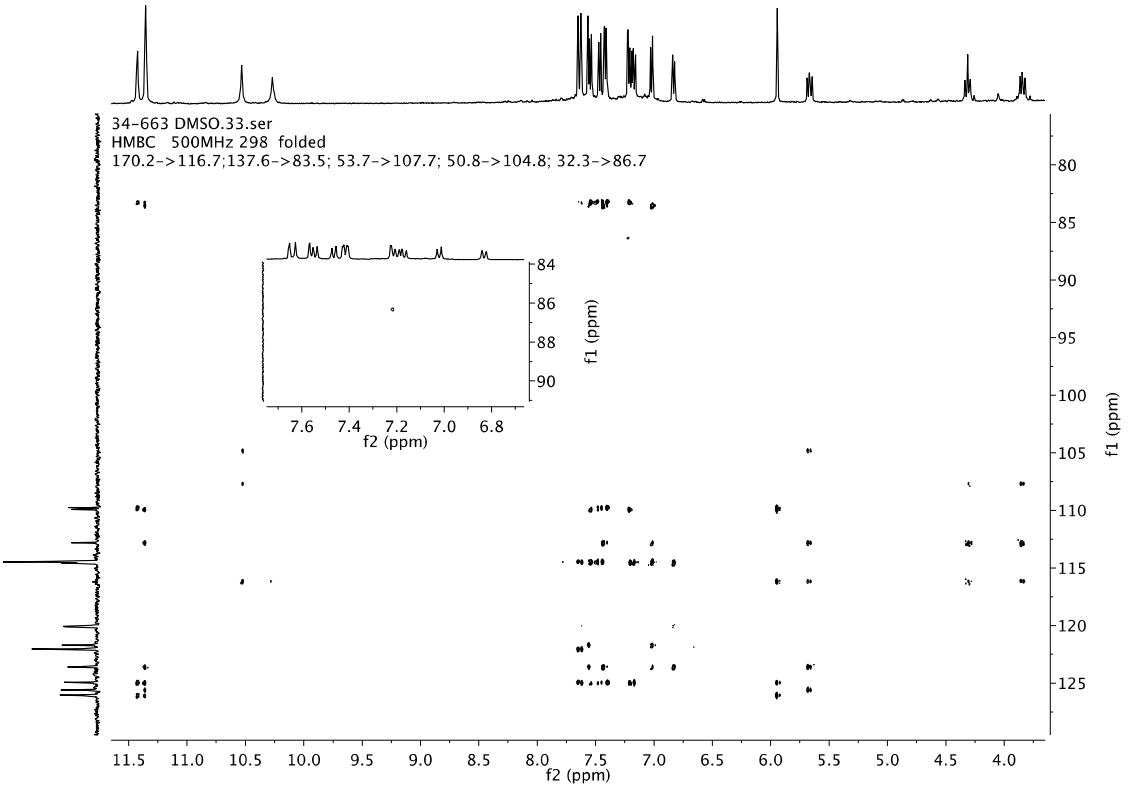


**Figure S7.** 1D NOE spectrum of **1** in DMSO-$d_6$

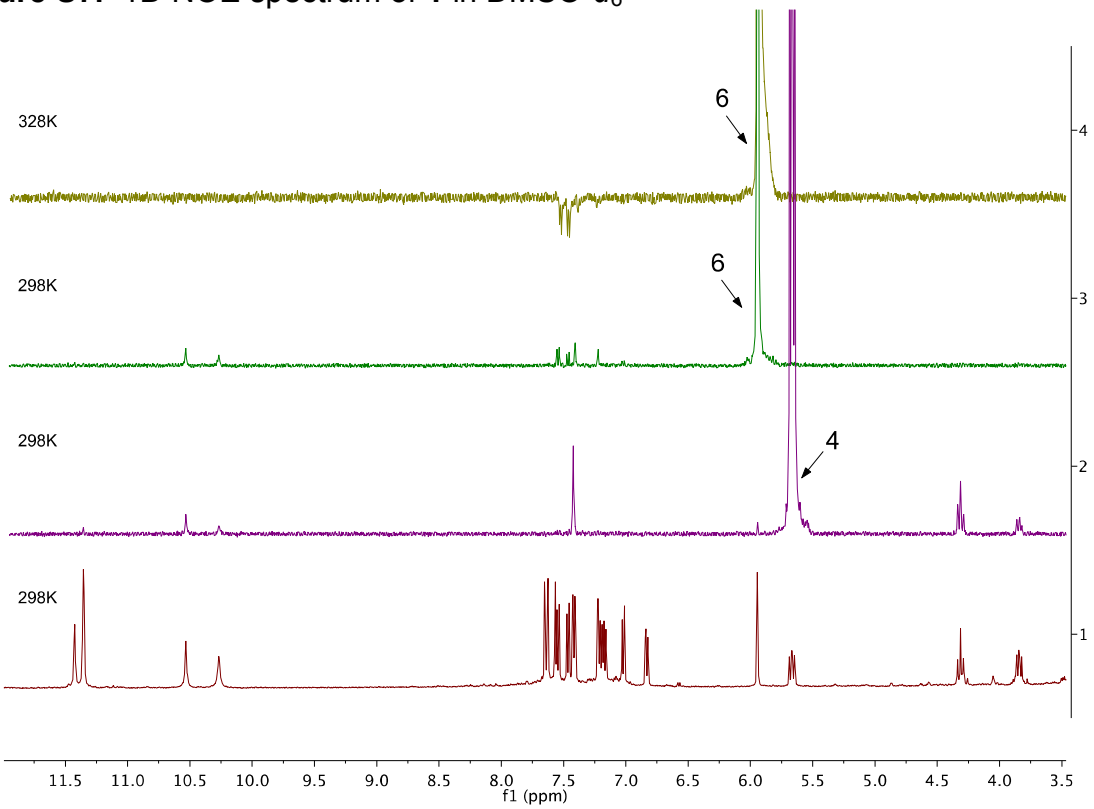



**Figure S8.** $^1$H NMR spectrum of **1** in $CD_3OD$ at 298 K

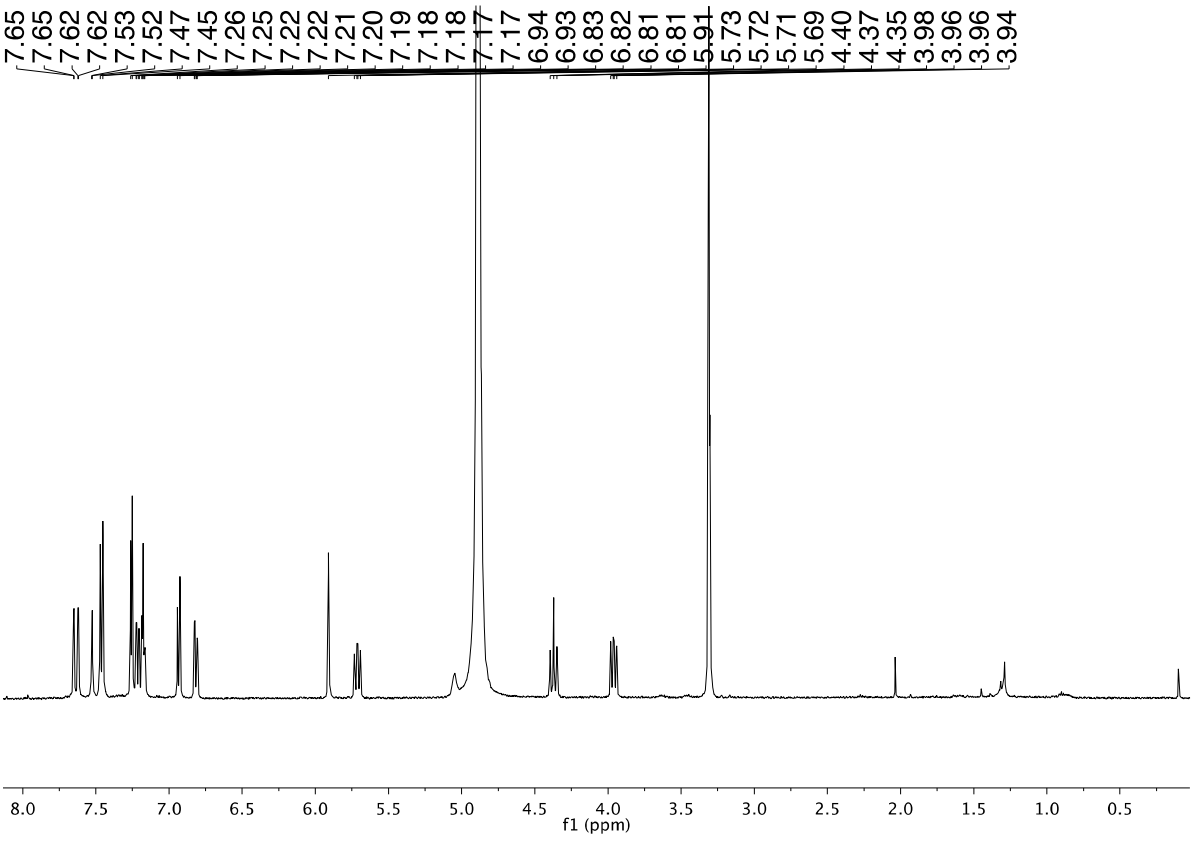


**Figure S10.** $^{13}C$ NMR spectrum of **1** in $CD_3OD$ at 298 K

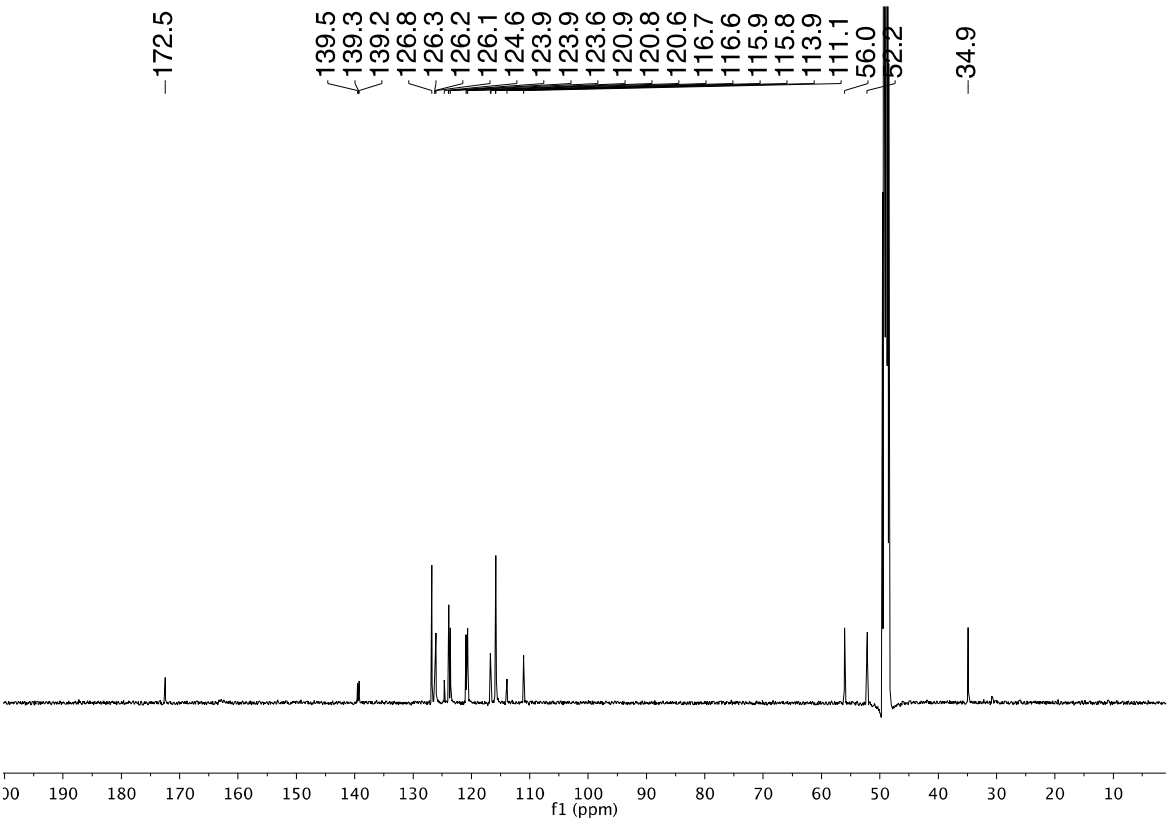



**Figure S11.** $^{1}H$-$^{1}H$ COSY spectrum of **1** in $CD_3OD$ at 298 K

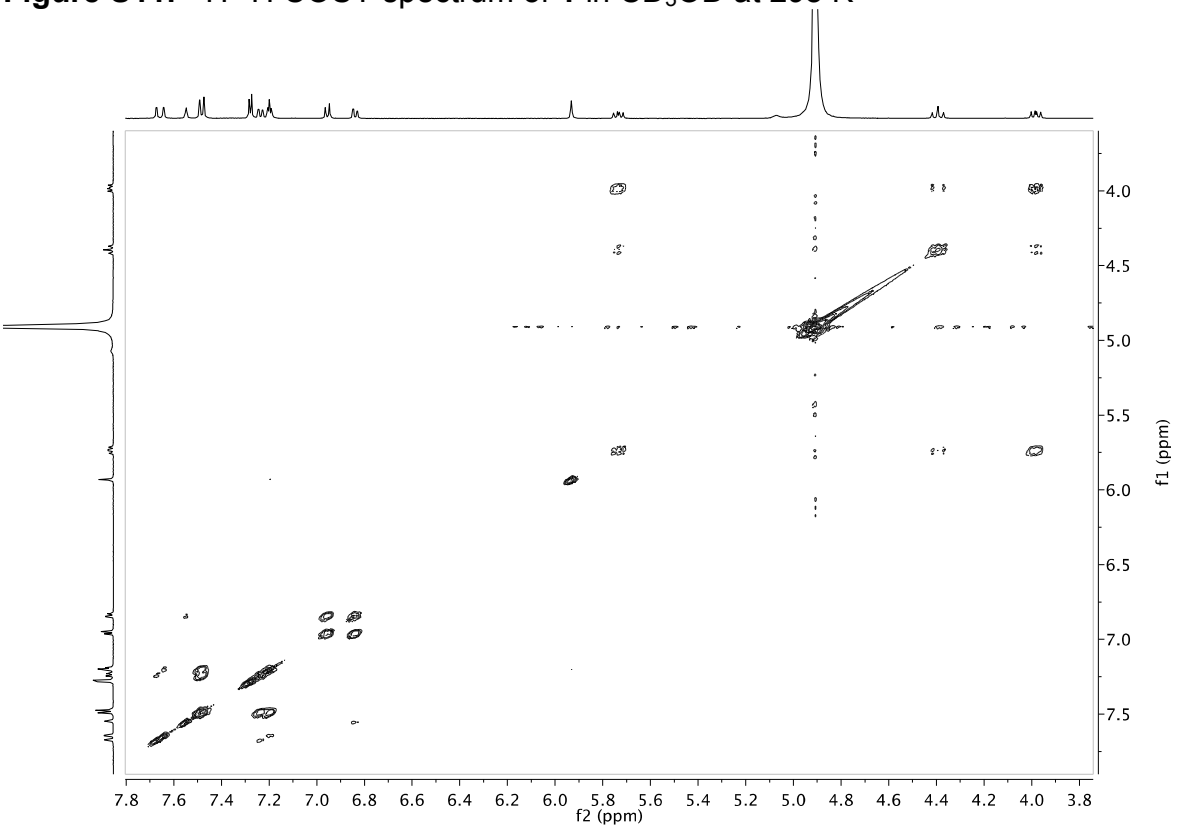


**Figure S12.** HSQC spectrum of **1** in $CD_3OD$ at 298 K

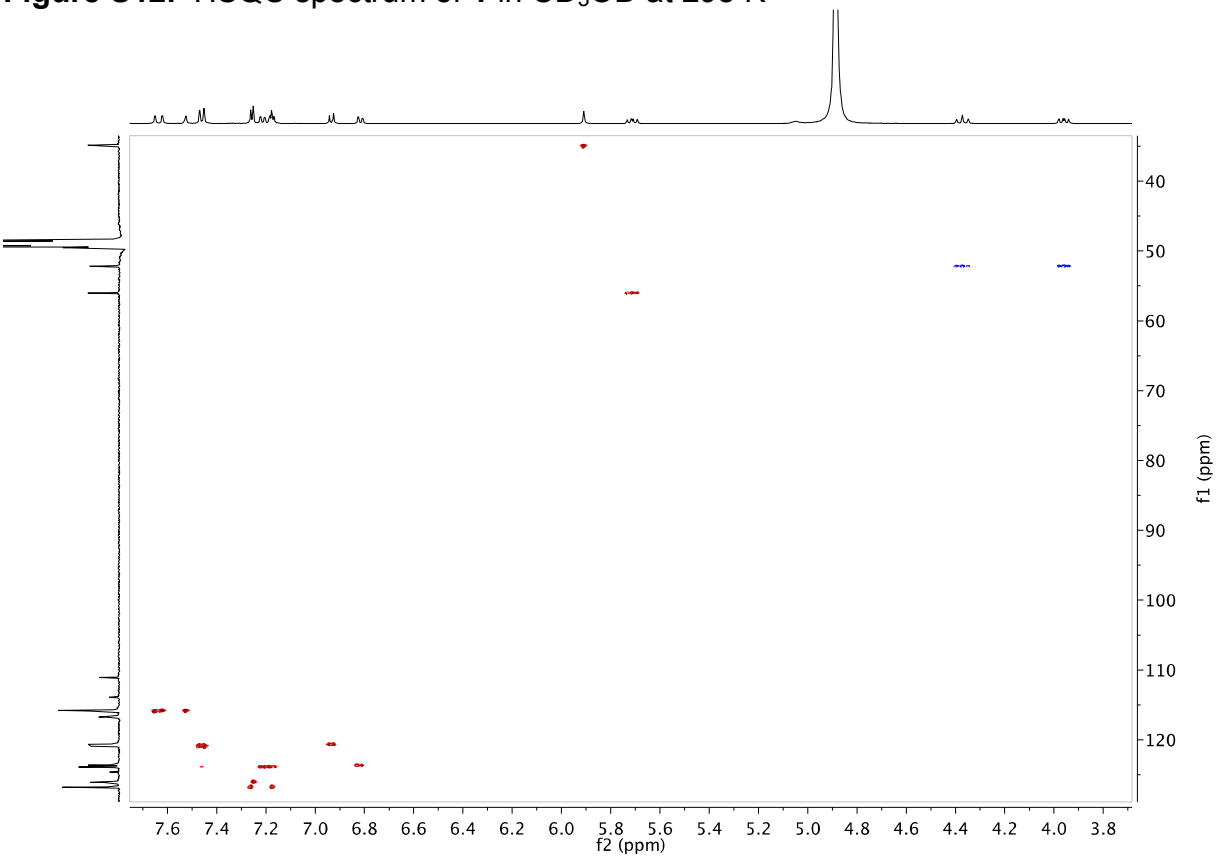



**Figure S13.** HMBC spectrum of **1** in $CD_3OD$ at 298 K

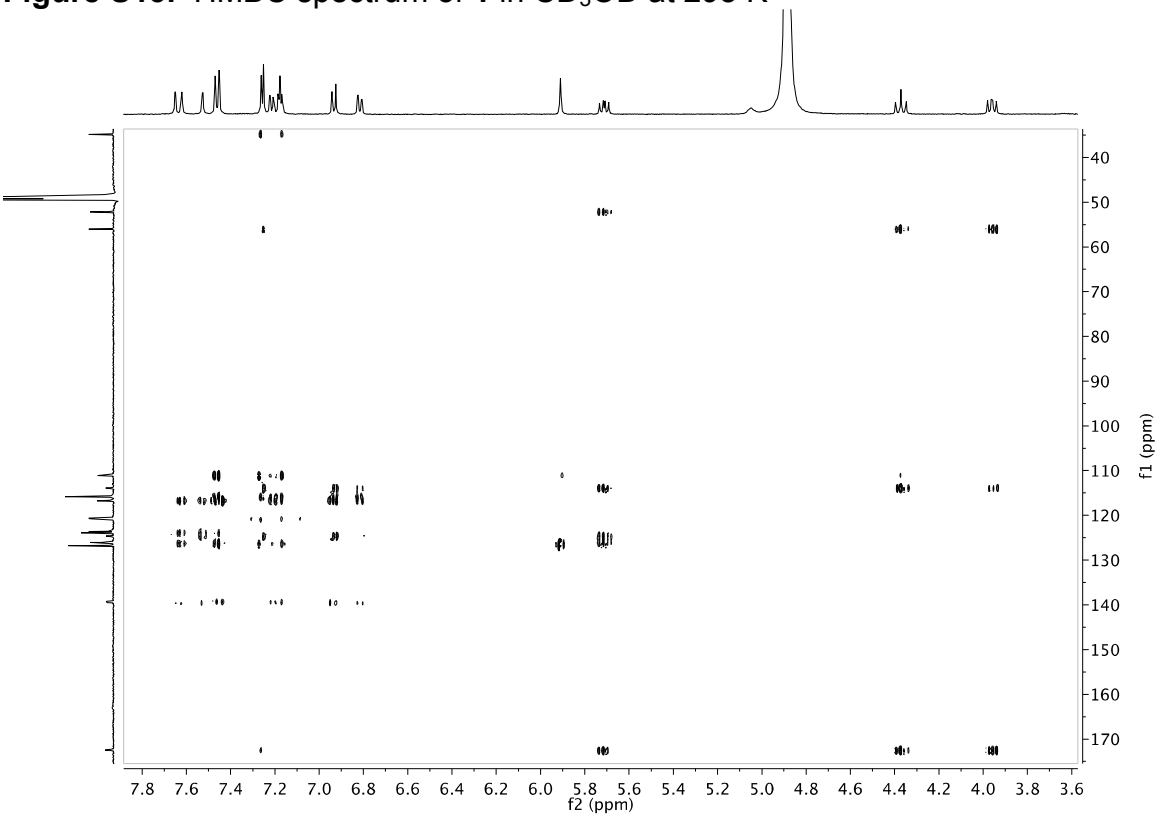


**Figure S14.** 1D NOE spectra of **1** in $CD_3OD$

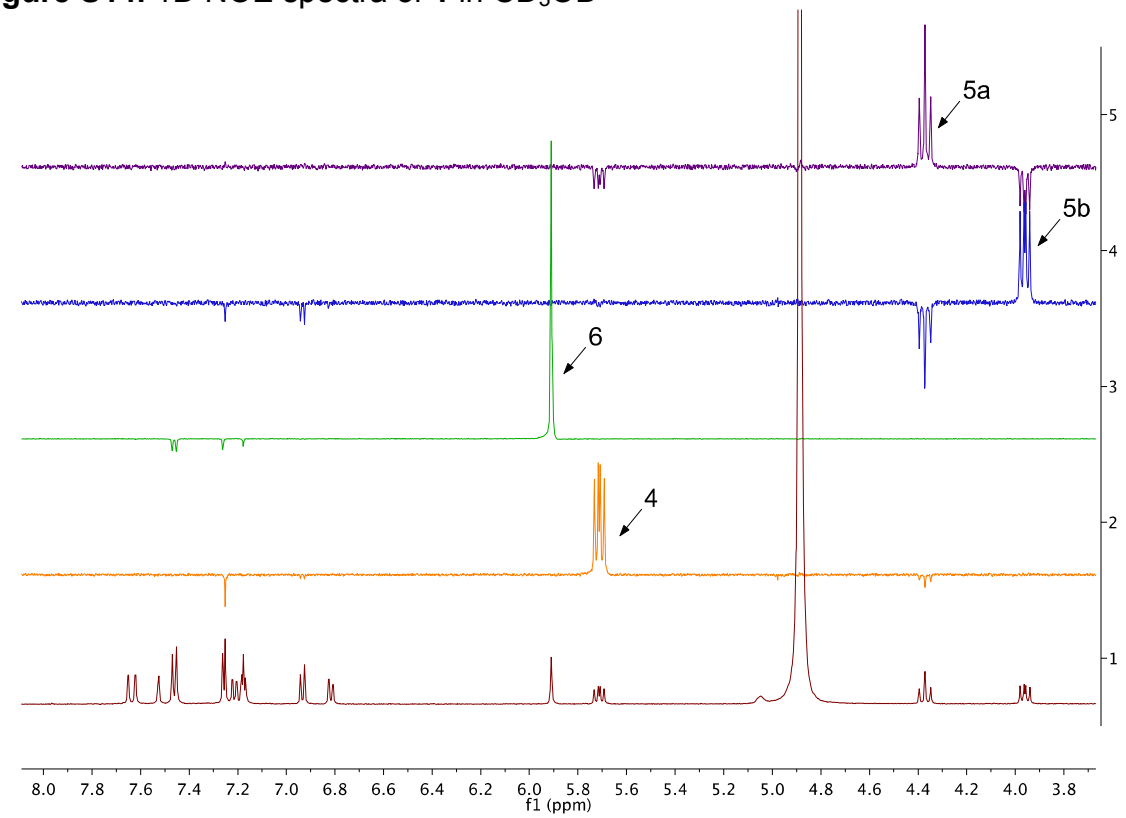



**Figure S15.** HRESIMS spectrum of **1**

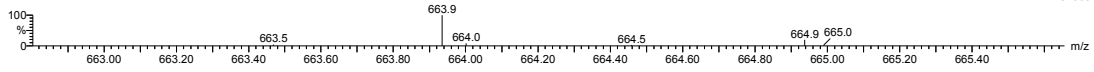
**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 10.0 mDa / DBE: min = -2.0, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
76 formula(e) evaluated with 3 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-120 H: 0-200 N: 5-5 O: 0-40 79Br: 3-3

28-Sep-2015
hbl-28sep15----34-663-pos 82 (1.516) Cn (Cen,5, 50.00, Ar); Sm (SG, 1x2.00); Sb (12,5.00 )

TOF MS ES+
1.11e+003




| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -2.0 | | |
| Maximum: | | 10.0 | 10.0 | 500.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 663.9347 | 663.9347 | 0.0 | 0.0 | 19.5 | n/a | C28 H21 N5 79Br3 |
| | 663.9406 | -5.9 | -8.9 | 10.5 | n/a | C21 H25 N5 O5 79Br3 |
| | 663.9253 | 9.4 | 14.2 | 6.5 | n/a | C17 H25 N5 O8 79Br3 |

**Figure S16.** $^{1}$H NMR spectrum of **2** in DMSO-$d_6$ at 298 K

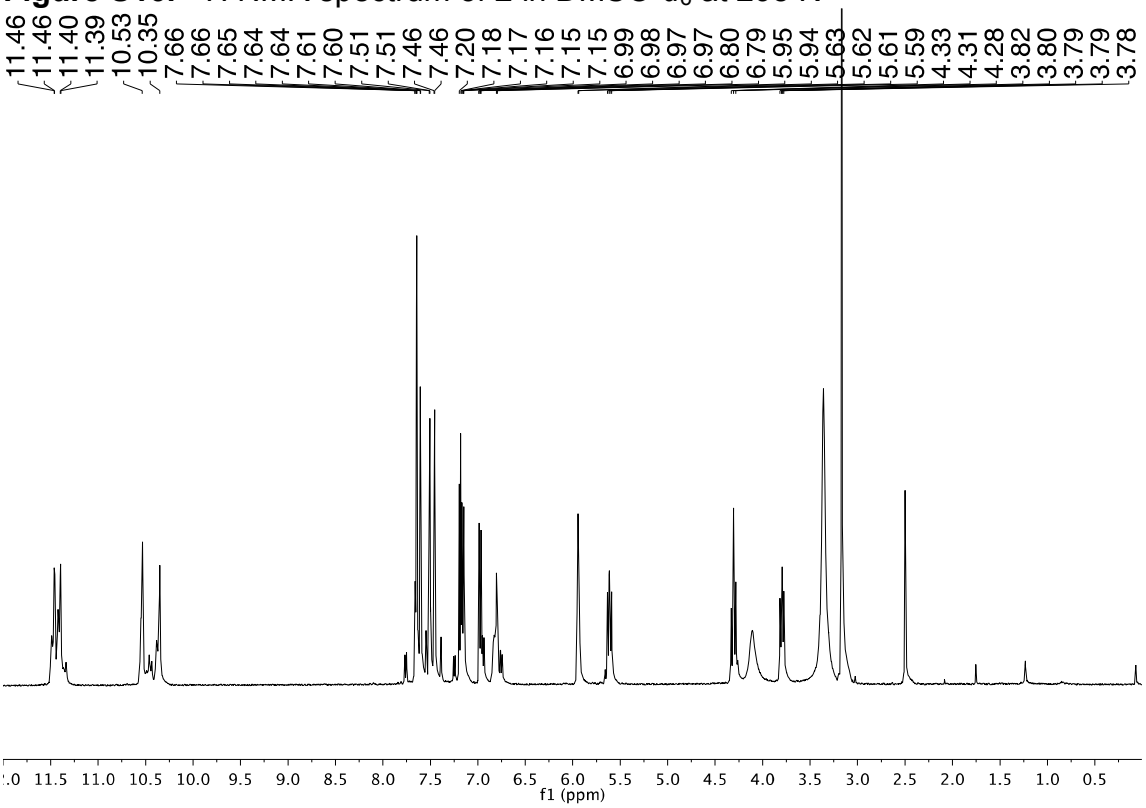



**Figure S17.** $^{13}$C NMR spectrum of **2** in DMSO-$d_6$ at 298 K

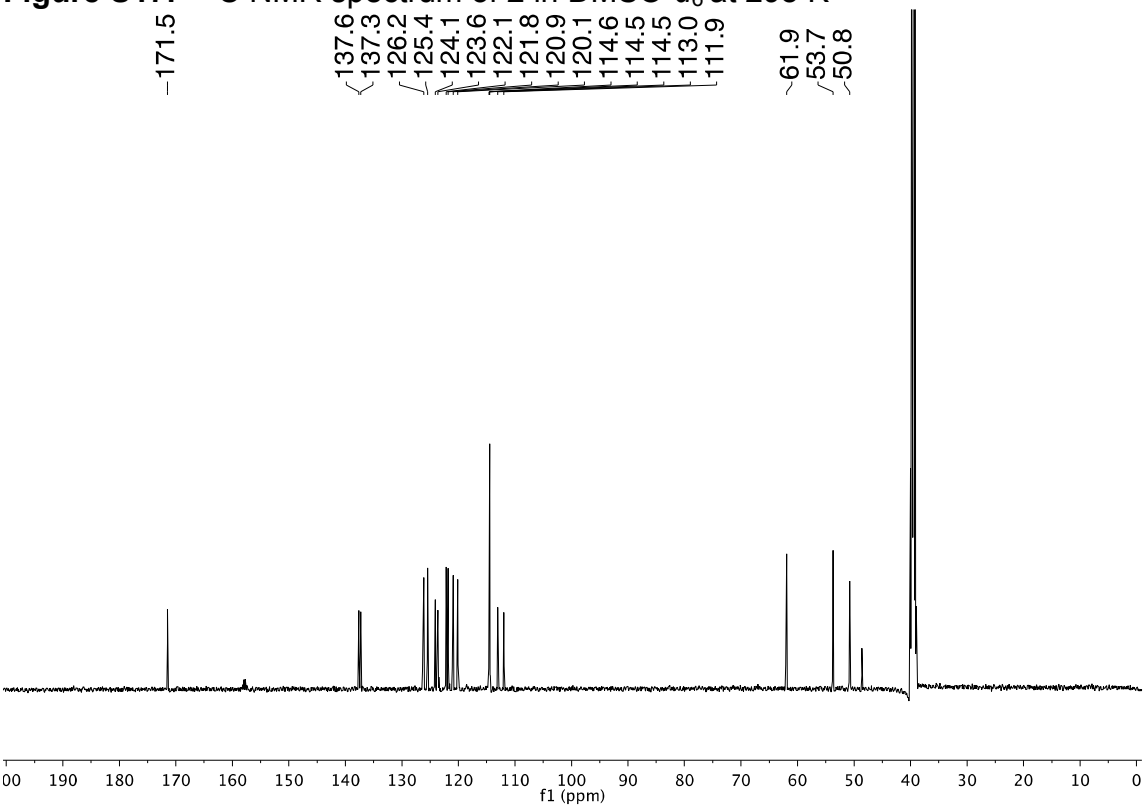


**Figure S18.** $^{1}$H-$^{1}$H COSY spectrum of **2** in DMSO-$d_6$ at 298 K

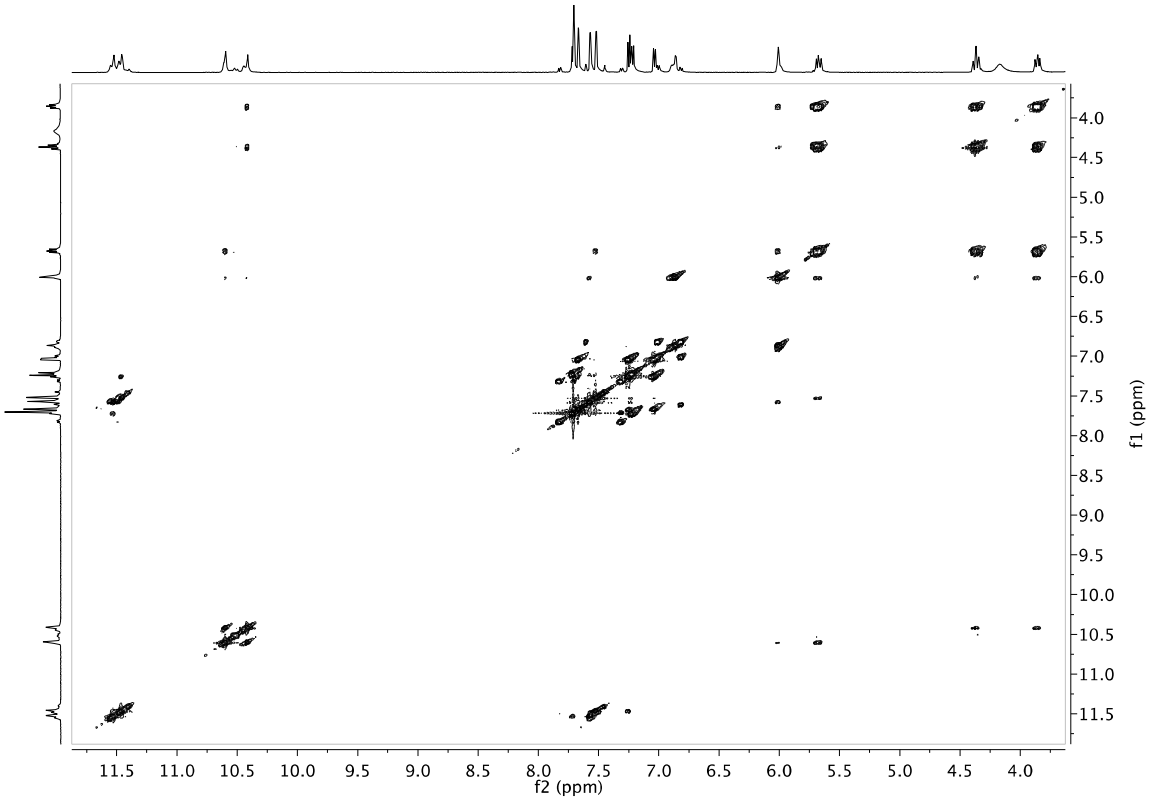



**Figure S19.** HSQC spectrum of **2** in DMSO-$d_6$ at 298 K

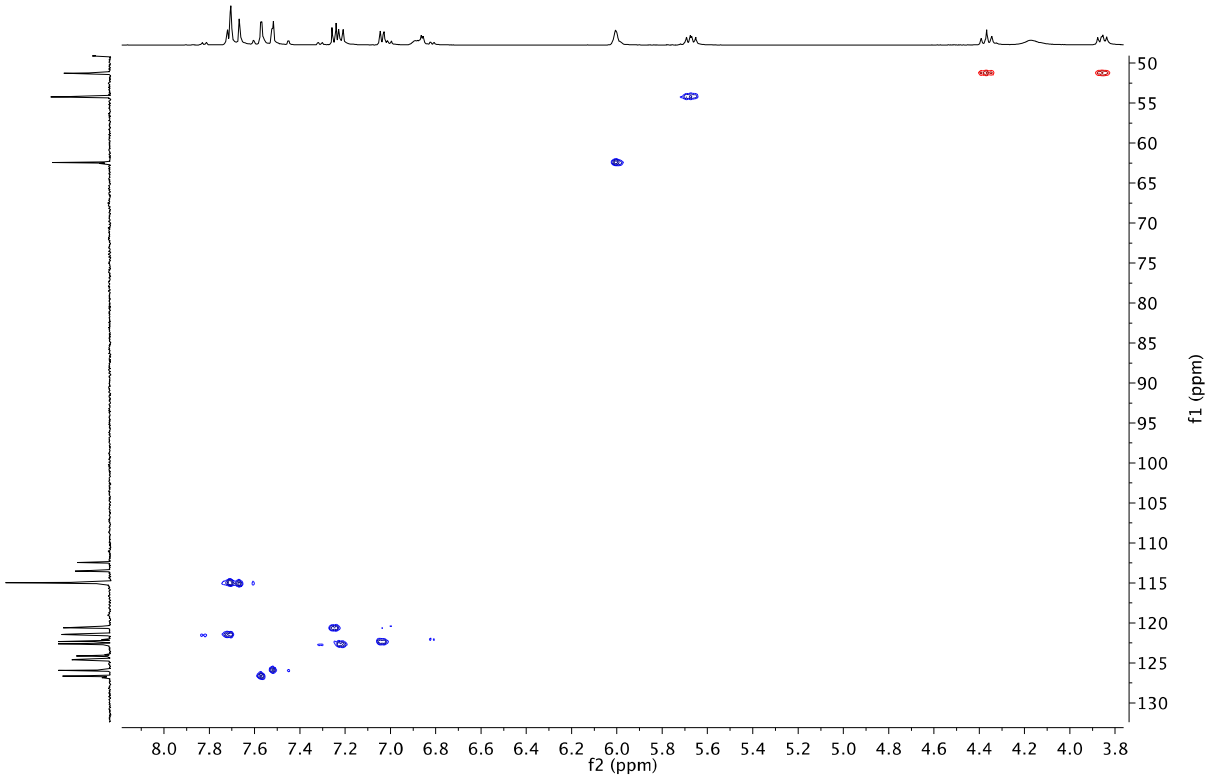


**Figure S20.** HMBC spectrum of **2** in DMSO-$d_6$ at 298 K

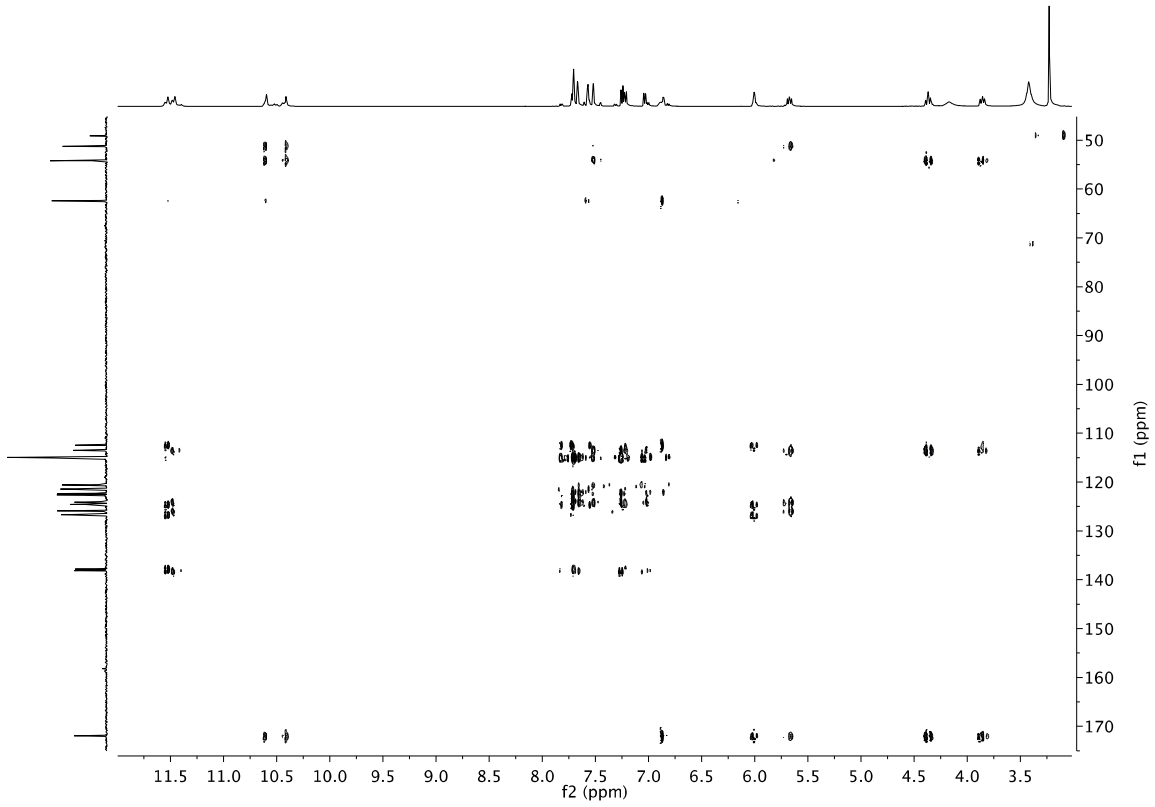



**Figure S21.** $^1$H NMR spectrum of **2** in $CD_3OD$ at 298 K

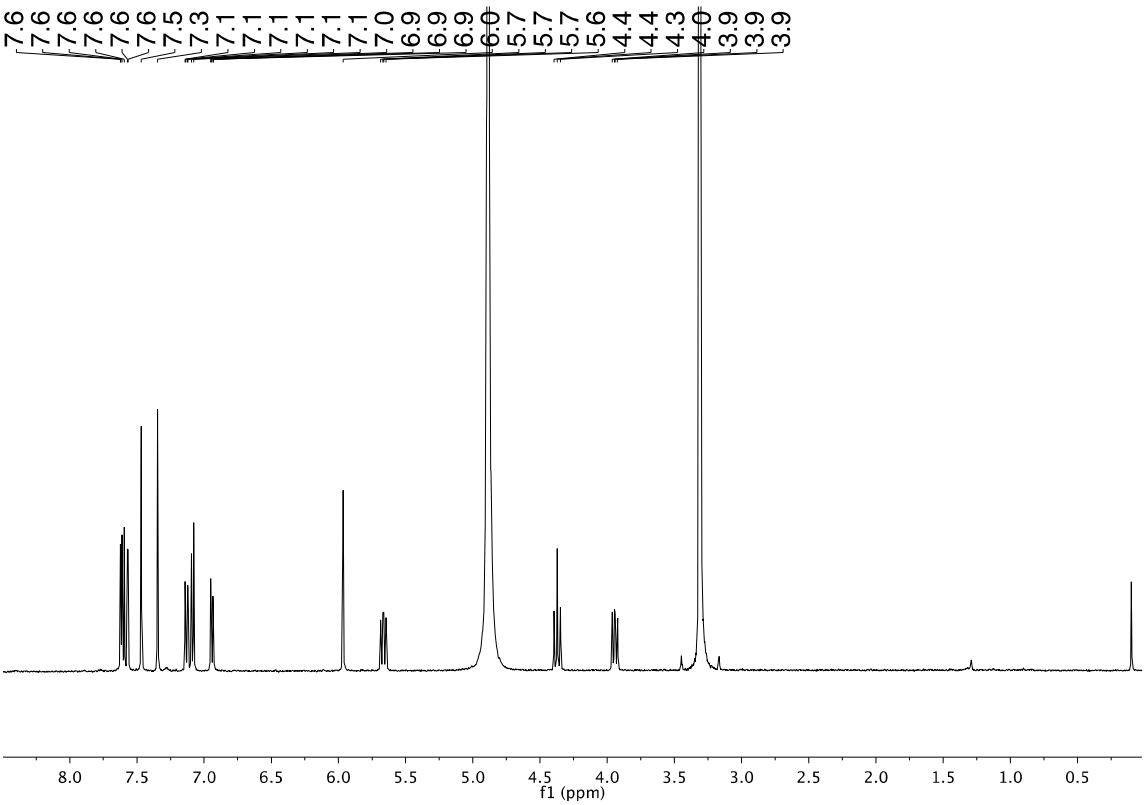


**Figure S22.** $^{13}C$ NMR spectrum of **2** in $CD_3OD$ at 298 K

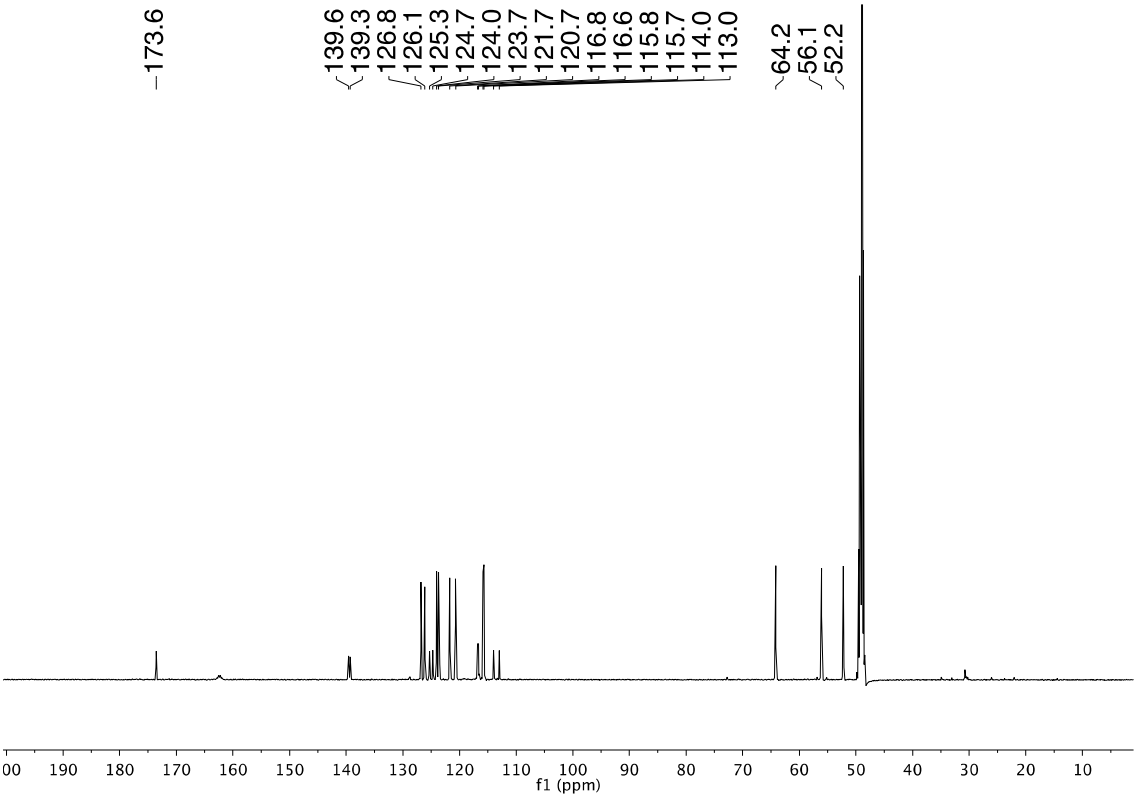



**Figure S23.** $^{1}H$-$^{1}H$ COSY spectrum of **2** in $CD_3OD$ at 298 K

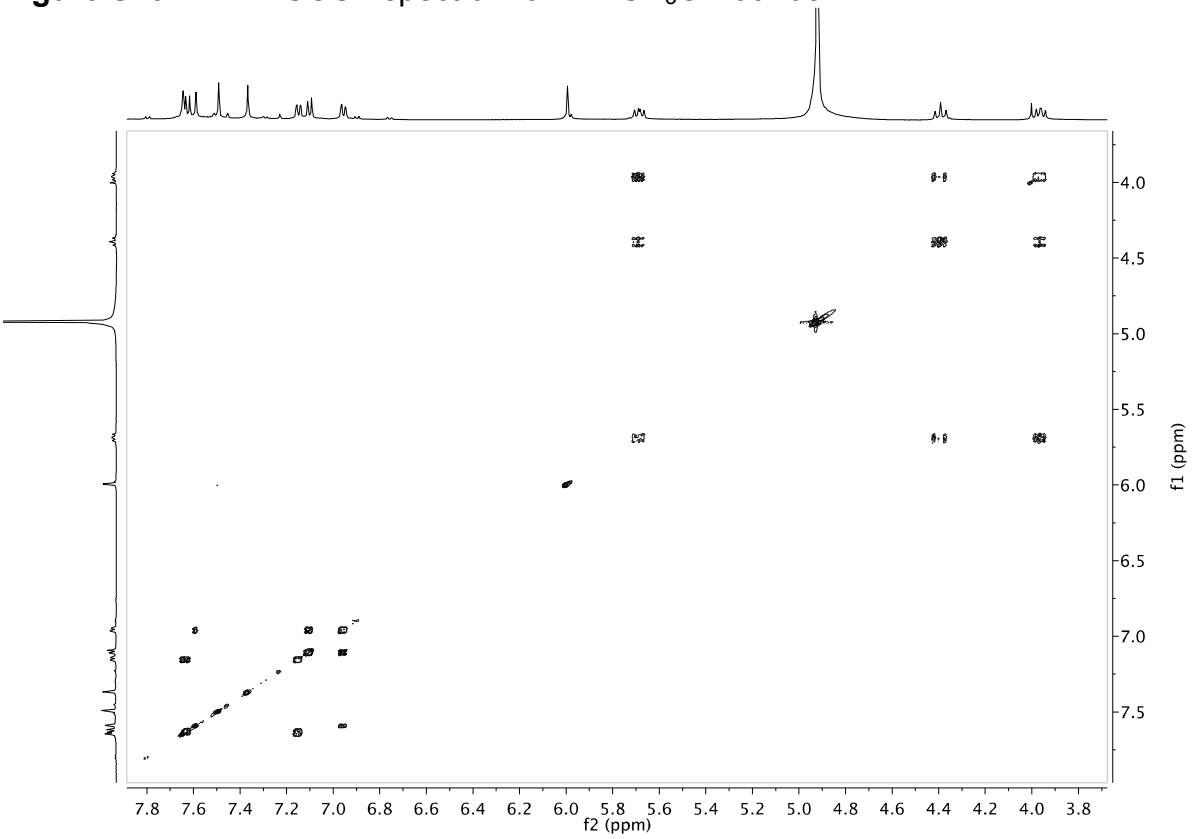


**Figure S24.** HSQC spectrum of **2** in $CD_3OD$ at 298 K

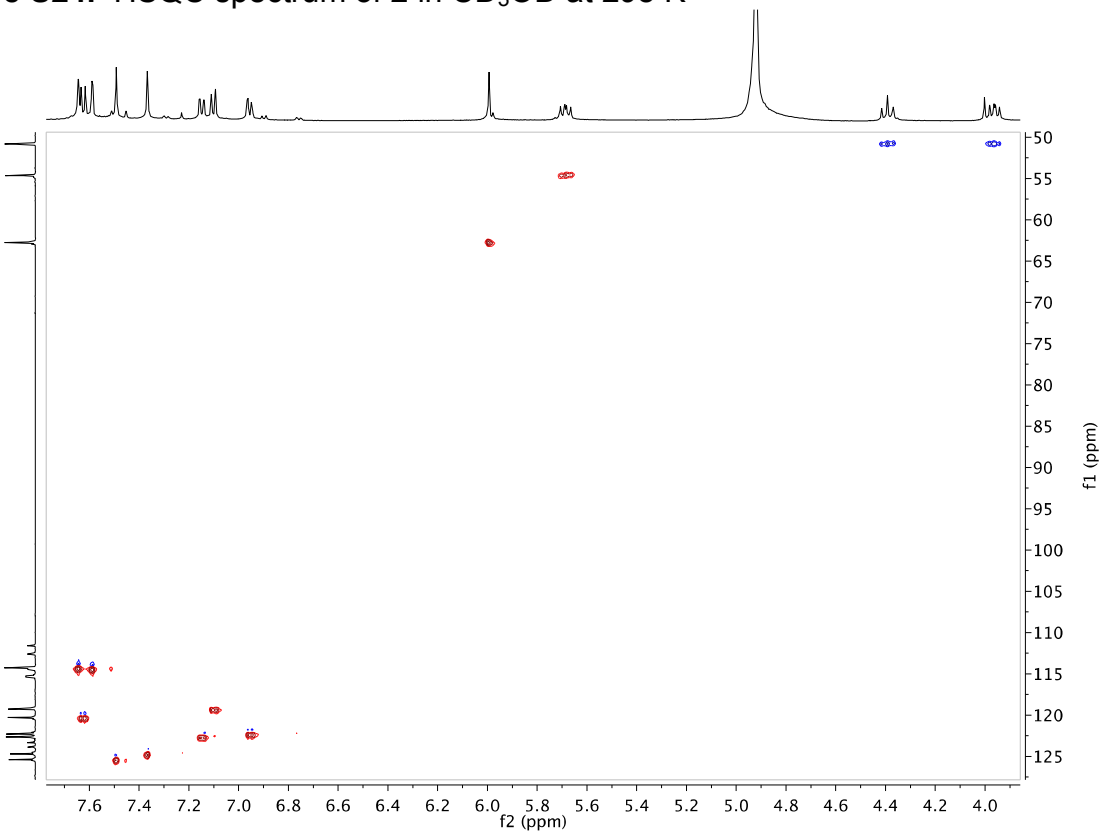



**Figure S25.** HMBC spectrum of **2** in $CD_3OD$ at 298 K

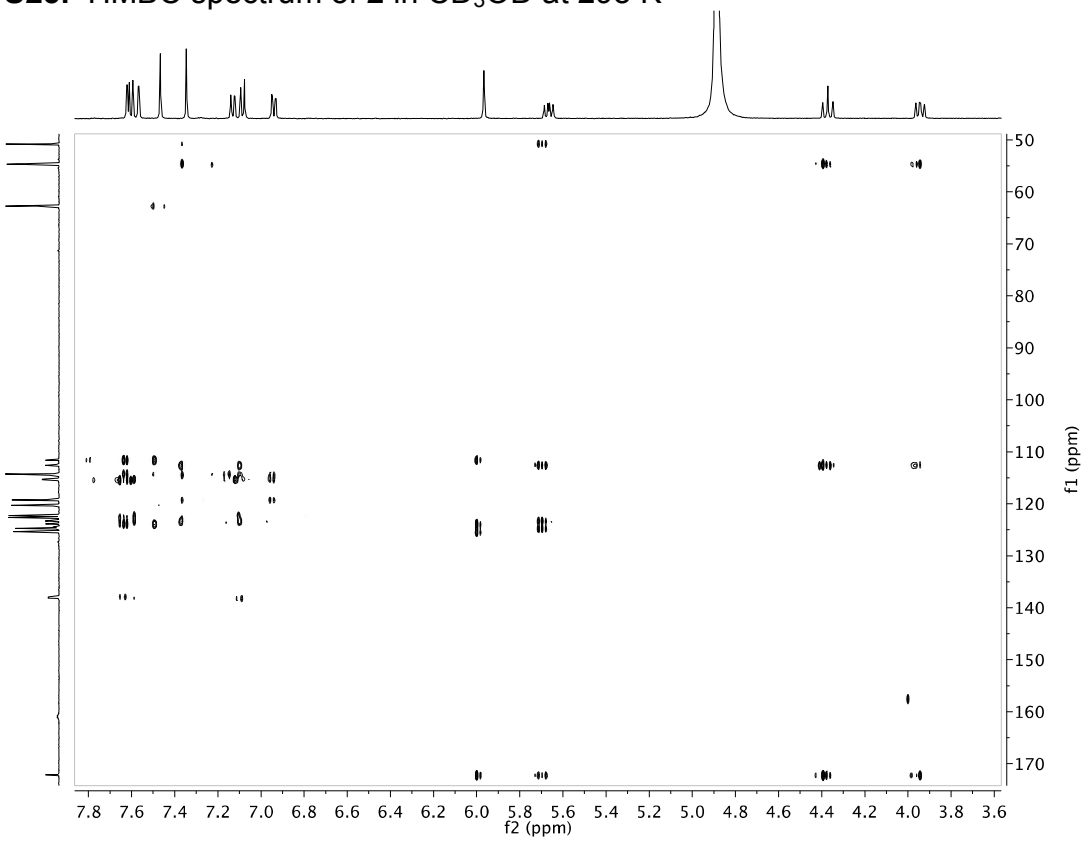


**Figure S26.** 1D NOE spectra of **2** in $CD_3OD$

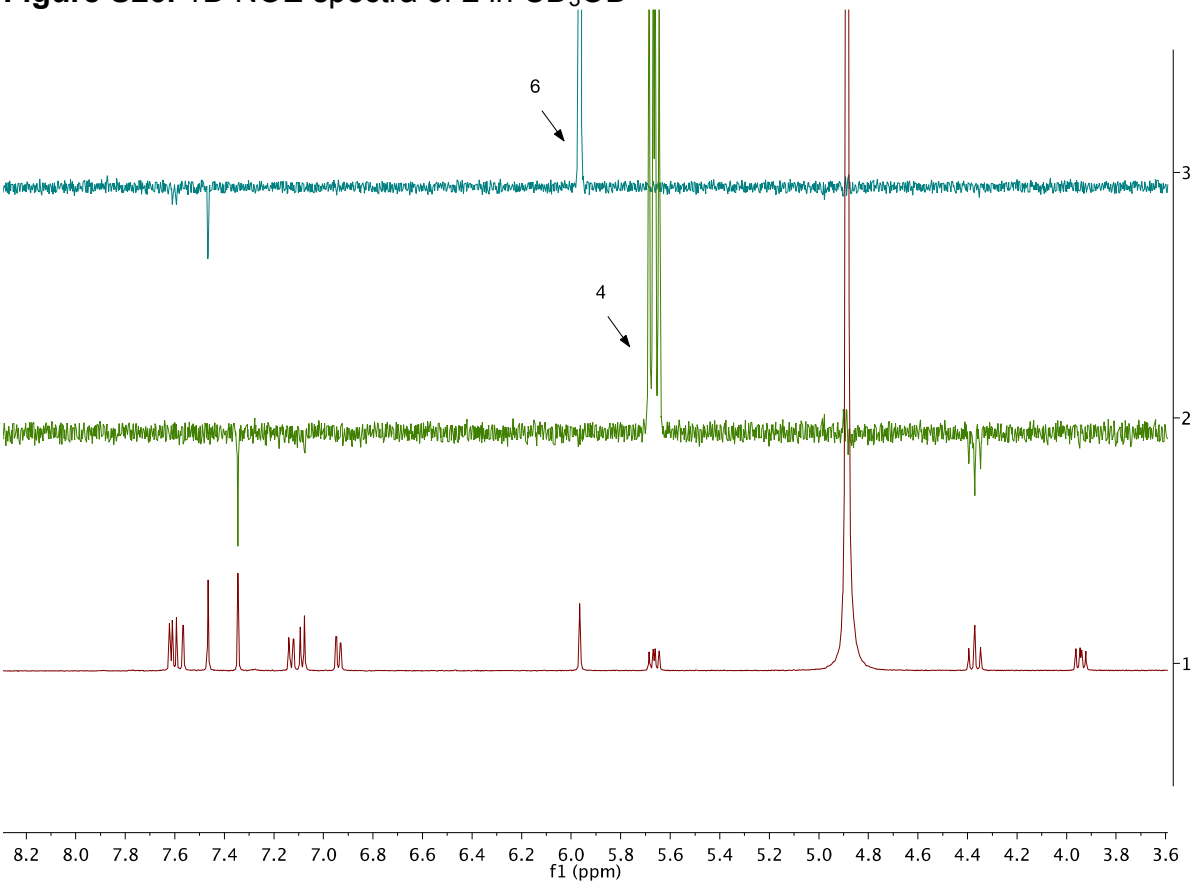


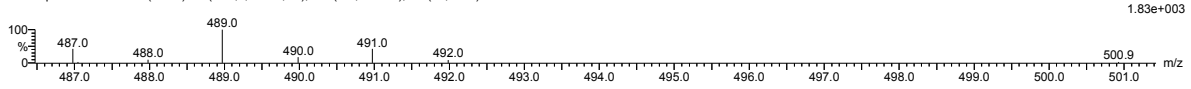

**Figure S27.** HRESIMS spectrum of **2**

**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 10.0 mDa / DBE: min = -2.0, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
50 formula(e) evaluated with 3 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-120 H: 0-200 N: 4-4 O: 0-40 79Br: 2-2
29-Sep-2015
hbl-29ep15---34-485 155 (2.866) Cn (Cen,5, 50.00, Ar); Sm (SG, 1x2.00); Sb (12,5.00 )

TOF MS ES+
1.83e+003




| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -2.0 | | |
| Maximum: | | 10.0 | 10.0 | 500.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 486.9767 | 486.9769 | -0.2 | -0.4 | 13.5 | 896.4 | C20 H17 N4 O 79Br2 |
| | 486.9828 | -6.1 | -12.5 | 4.5 | 905.8 | C13 H21 N4 O6 79Br2 |
| | 486.9675 | 9.2 | 18.9 | 0.5 | 911.8 | C9 H21 N4 O9 79Br2 |

**Figure S28.** $^{1}H$ NMR spectrum of **2a** in $CD_3OD$ at 298 K

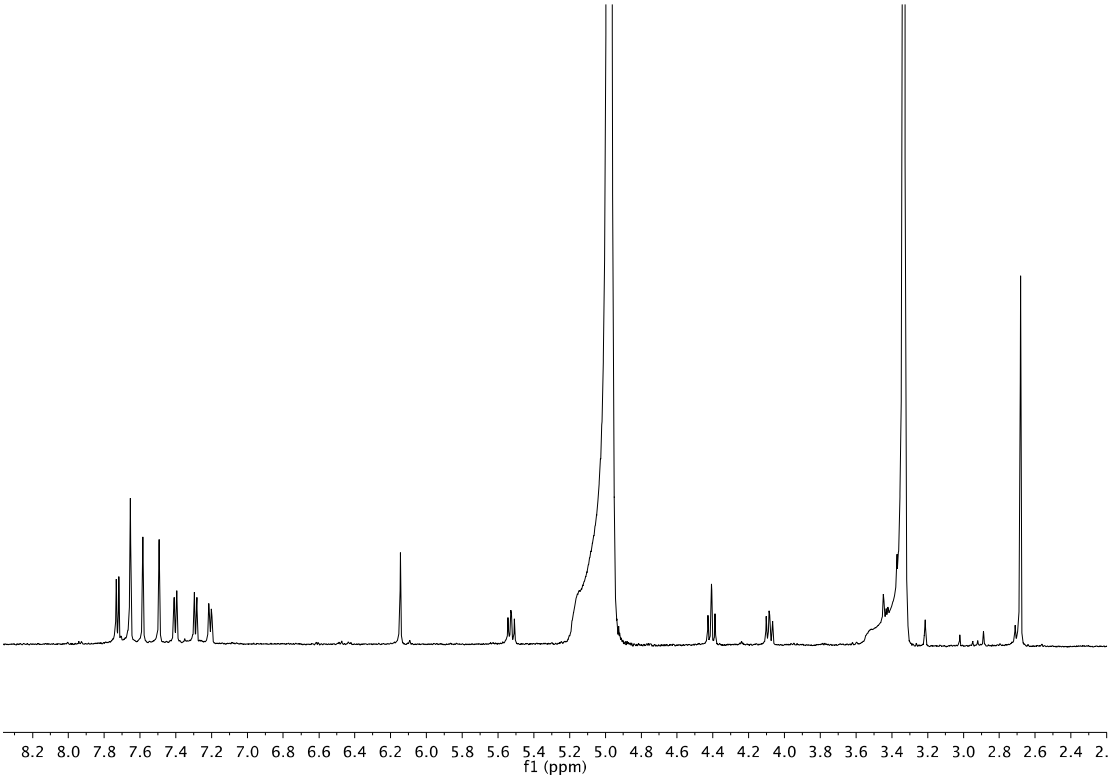



**Figure S29.** $^{13}C$ NMR spectrum of **2a** in $CD_3OD$ at 298 K

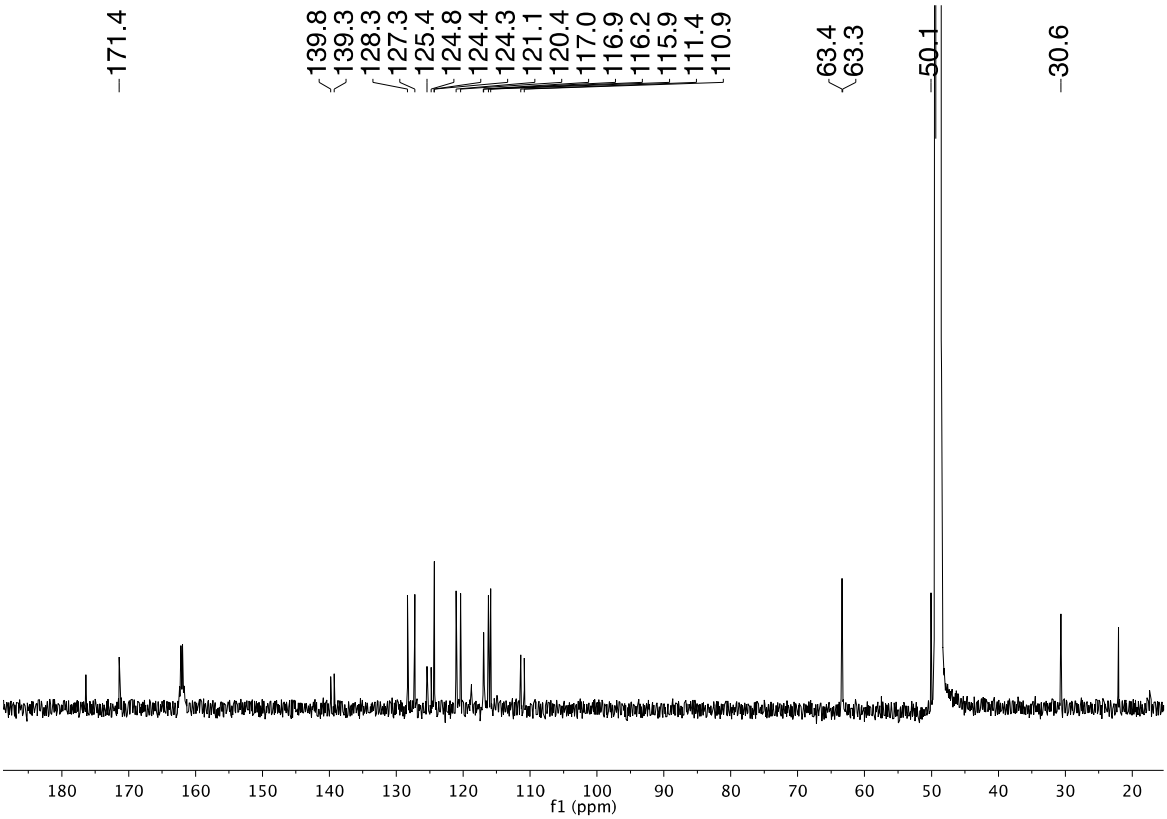


**Figure S30.** $^{1}H$-$^{1}H$ COSY spectrum of **2a** in $CD_3OD$ at 298 K

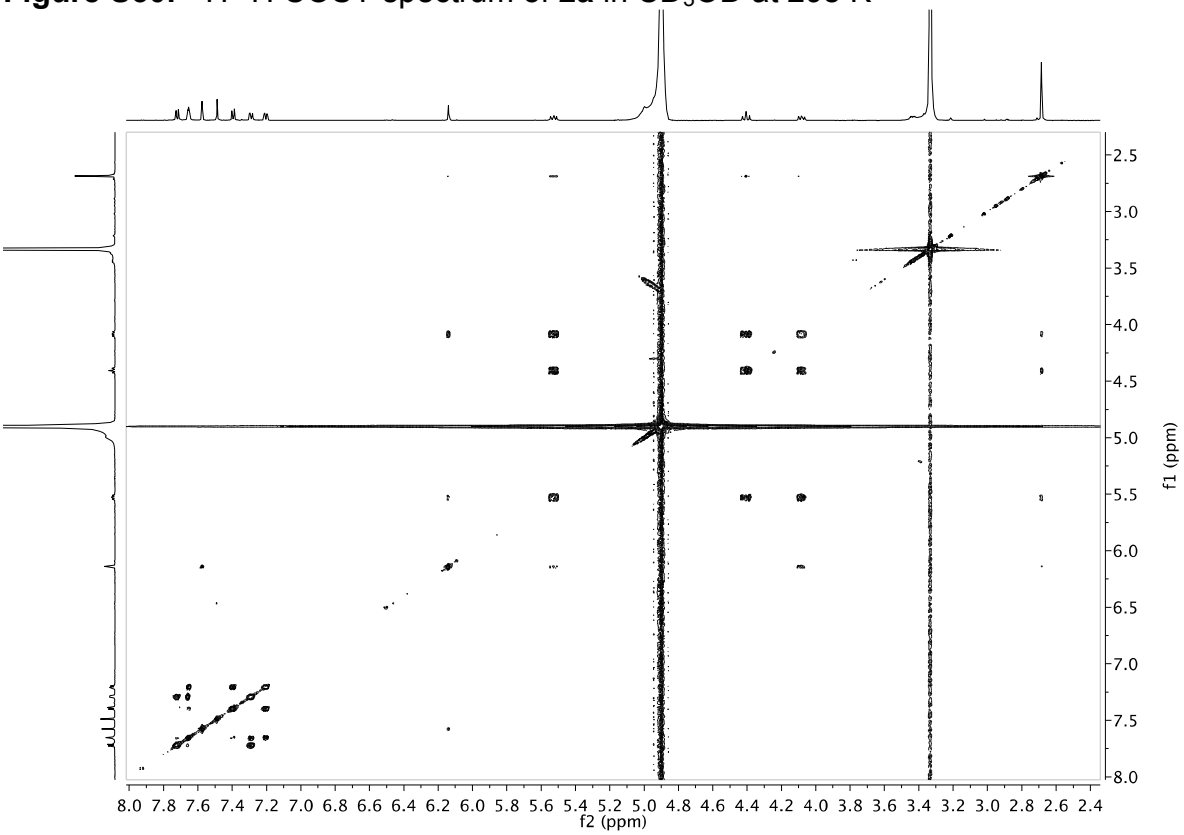



**Figure S31.** HSQC spectrum of **2a** in $CD_3OD$ at 298 K

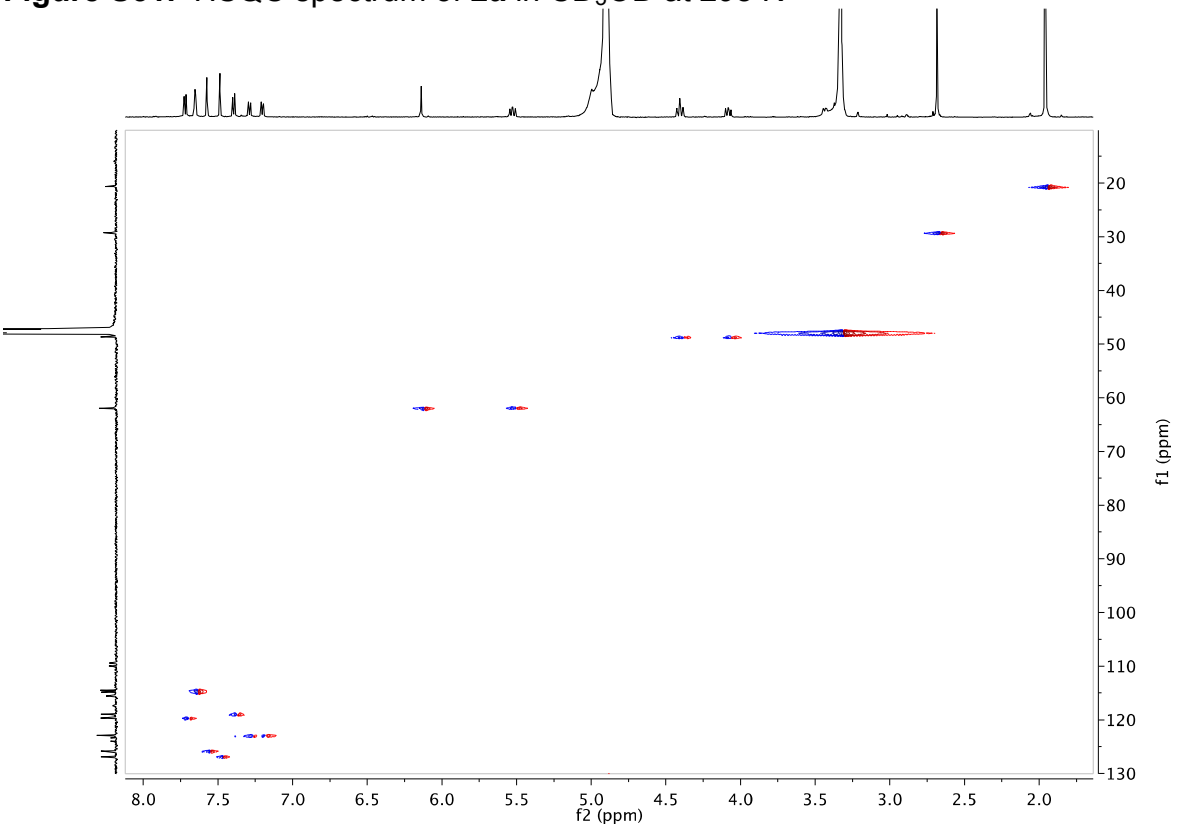


**Figure S32.** HMBC spectrum of **2a** in $CD_3OD$ at 298 K

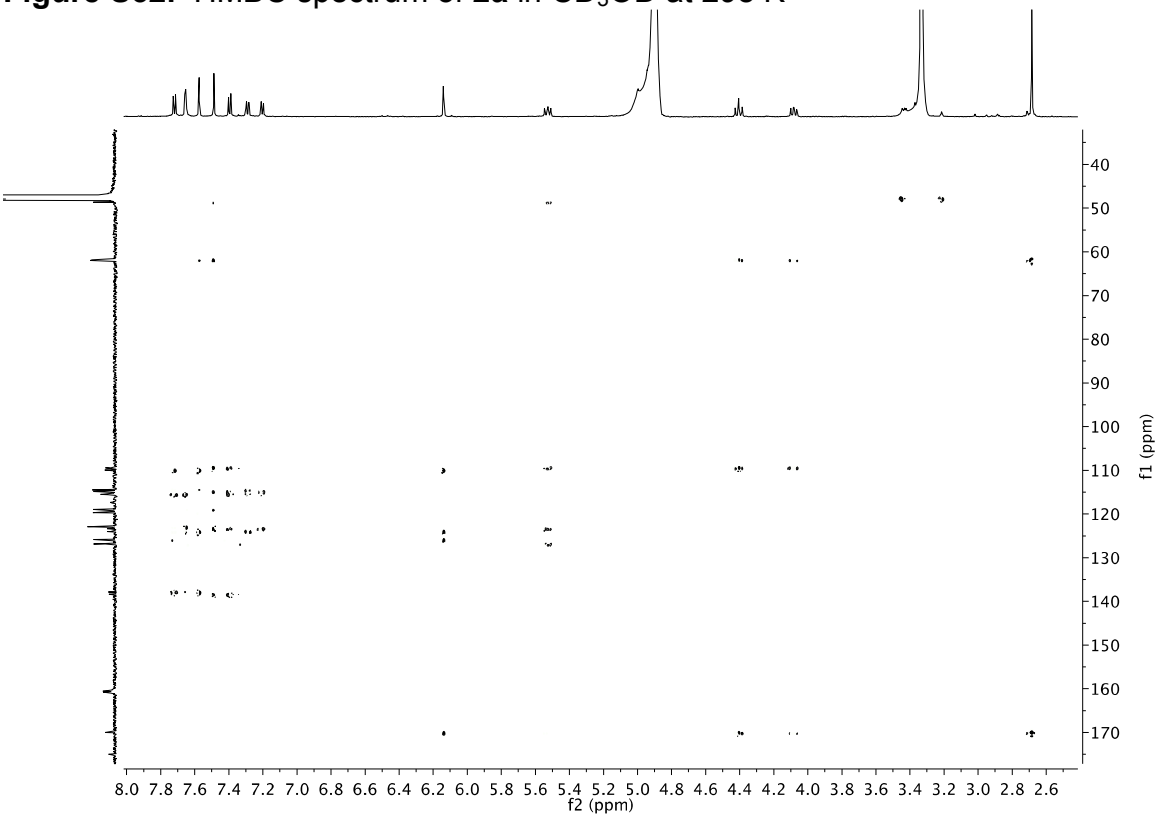


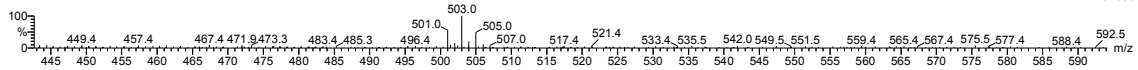

**Figure S33.** HRESIMS spectrum of **2a**

**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 30.0 mDa / DBE: min = -2.0, max = 1000.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
56 formula(e) evaluated with 6 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 N: 4-4 O: 0-25 79Br: 2-2

09-Jan-2017
hbl-09jan17-34-485-methylate-1 140 (2.590) Cn (Cen,5, 50.00, Ar); Sm (SG, 1x3.00); Sb (12,5.00 )
TOF MS ES+
4.17e+003




| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
|---|---|---|---|---|---|---|
| Minimum: | | | | -2.0 | | |
| Maximum: | | 30.0 | 10.0 | 1000.0 | | |
| 500.9931 | 500.9926 | 0.5 | 1.0 | 13.5 | 2016.0 | C21 H19 N4 O 79Br2 |
| | 500.9984 | -5.3 | -10.6 | 4.5 | 2068.5 | C14 H23 N4 O6 79Br2 |
| | 500.9832 | 9.9 | 19.8 | 0.5 | 2100.5 | C10 H23 N4 O9 79Br2 |
| | 500.9773 | 15.8 | 31.5 | 9.5 | 2038.4 | C17 H19 N4 O4 79Br2 |
| | 501.0137 | -20.6 | -41.1 | 8.5 | 2088.1 | C18 H23 N4 O3 79Br2 |
| | 501.0196 | -26.5 | -52.9 | -0.5 | 2174.8 | C11 H27 N4 O8 79Br2 |

**Figure S34.** $^{1}H$ NMR spectrum of **2b** in $CD_3OD$ at 298 K

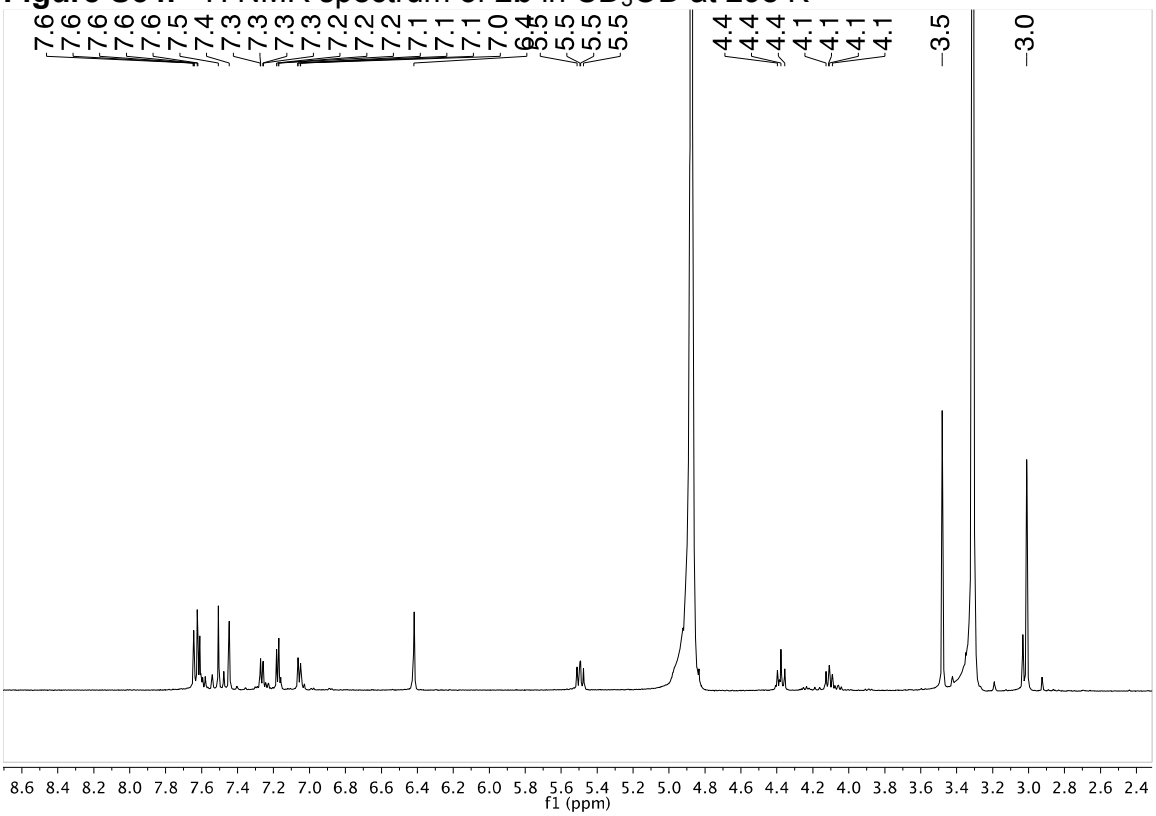



**Figure S35.** $^{13}C$ NMR spectrum of **2b** in $CD_3OD$ at 298 K

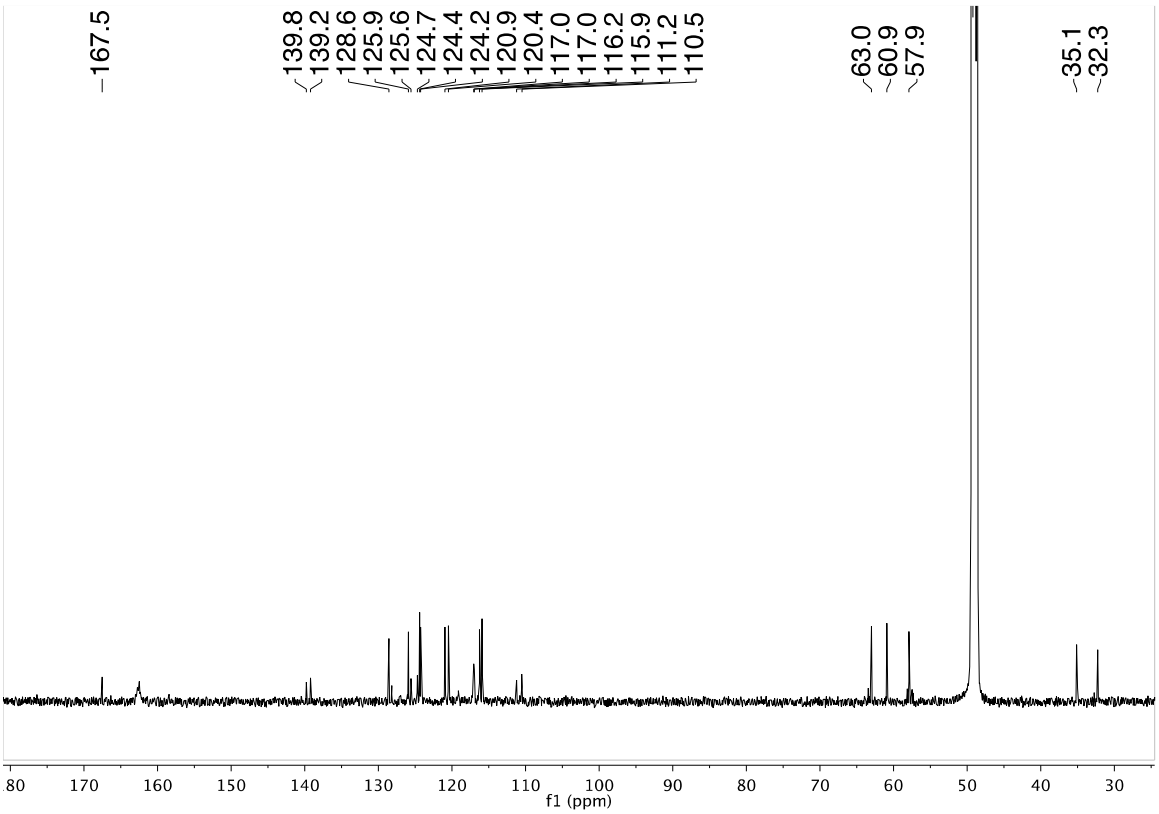


**Figure S36.** $^{1}H$-$^{1}H$ COSY spectrum of **2b** in $CD_3OD$ at 298 K

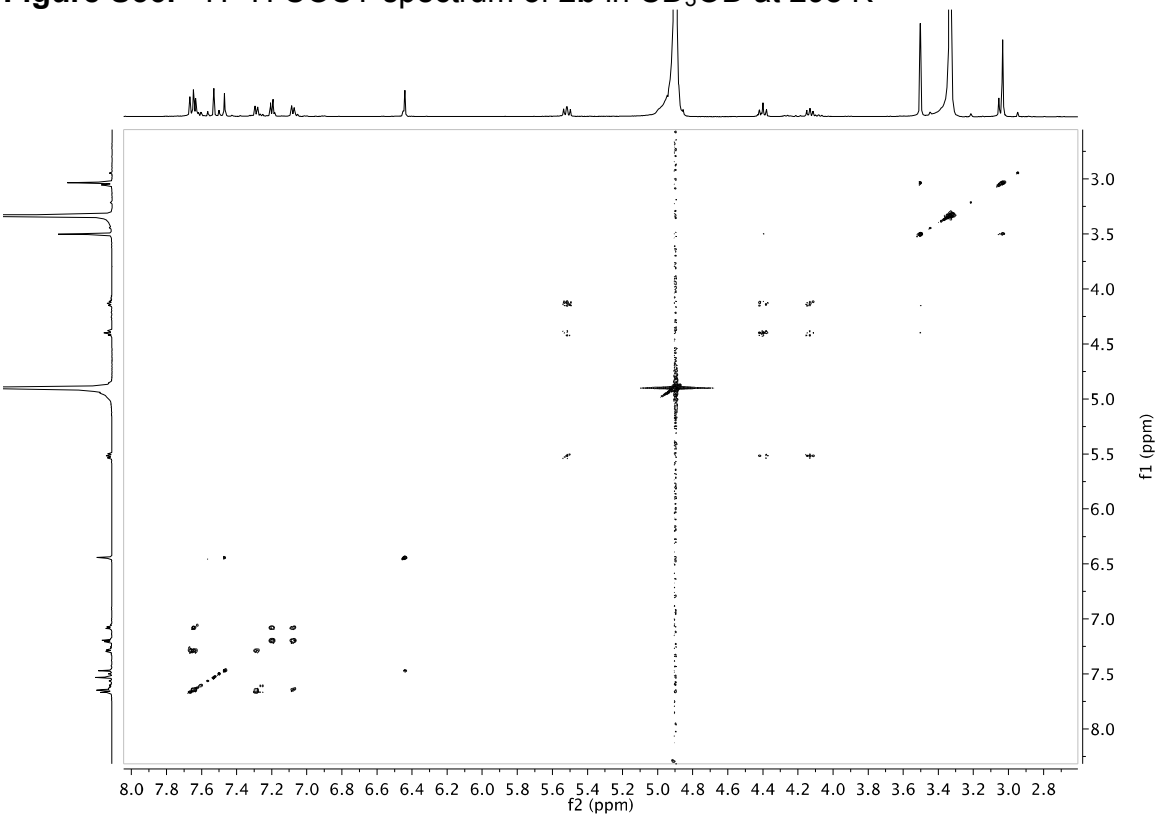



**Figure S37.** HSQC spectrum of **2b** in $CD_3OD$ at 298 K

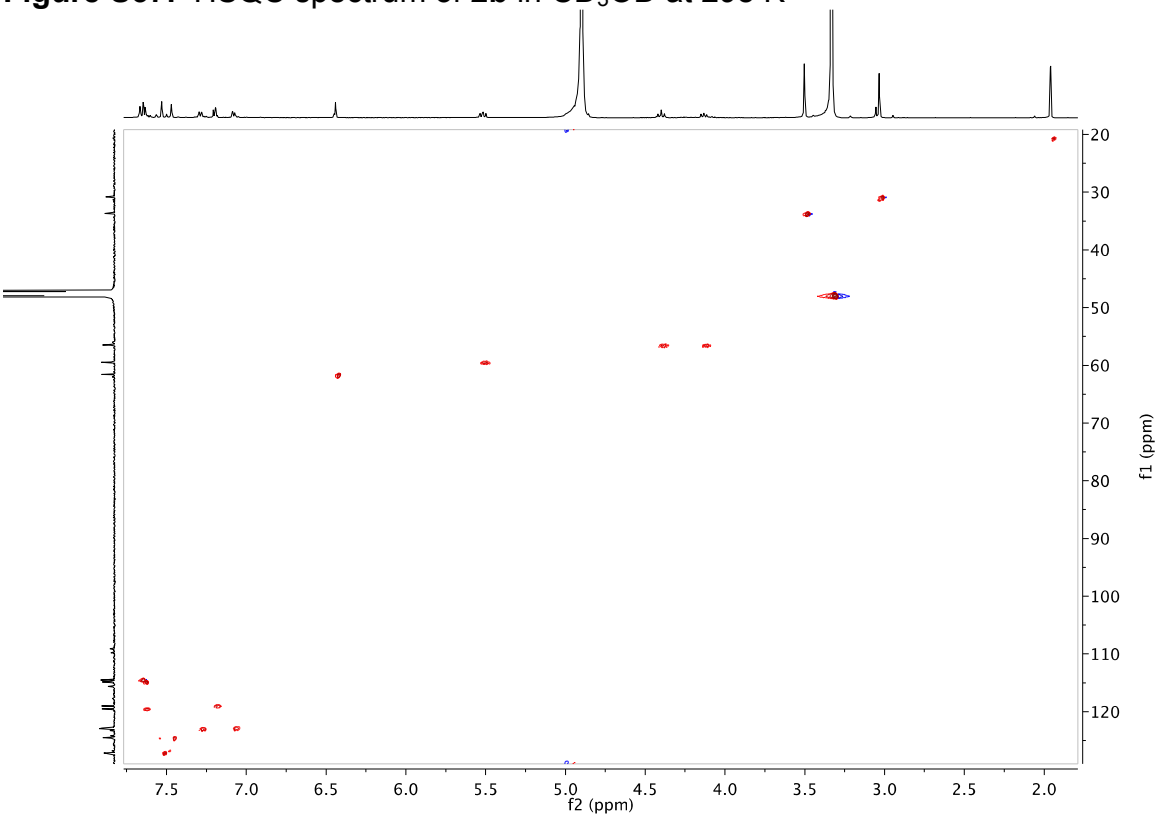


**Figure S38.** HMBC spectrum of **2b** in $CD_3OD$ at 298 K

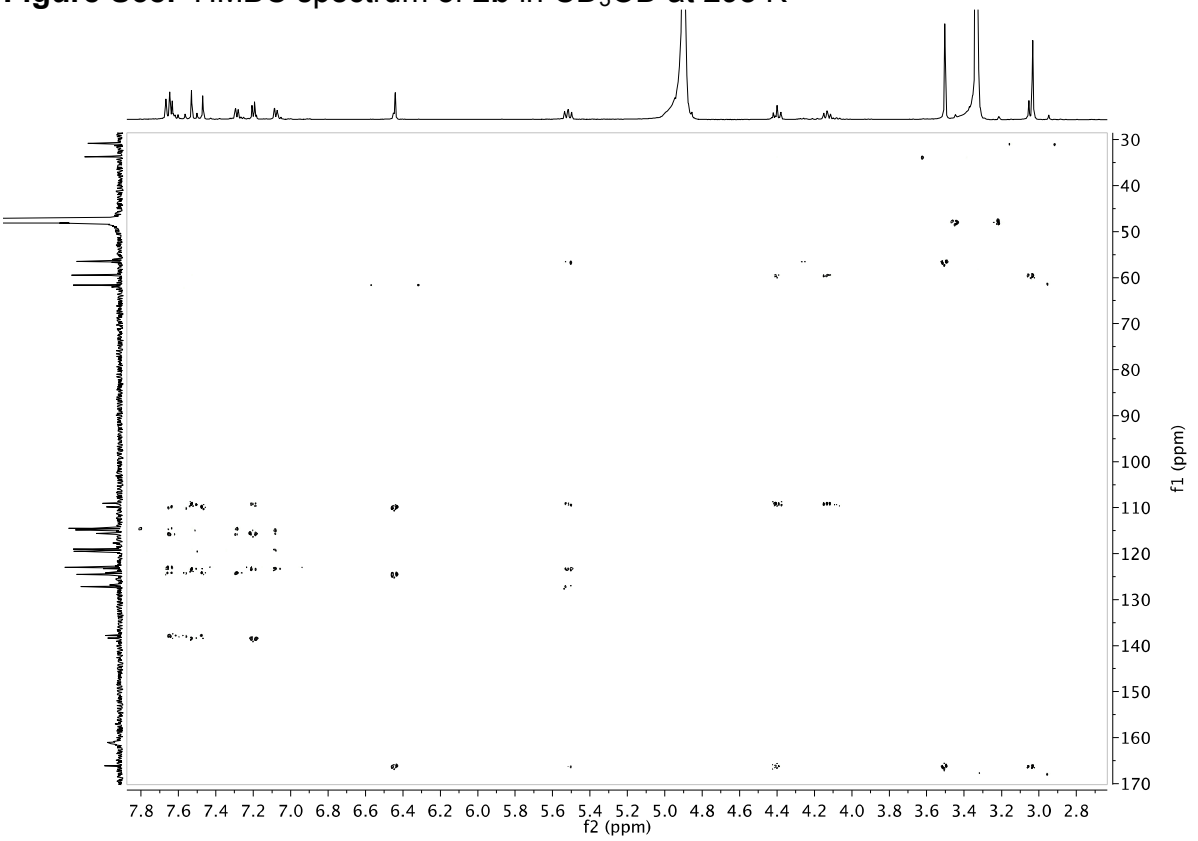



**Figure S39.** HRESIMS spectrum of **2b**

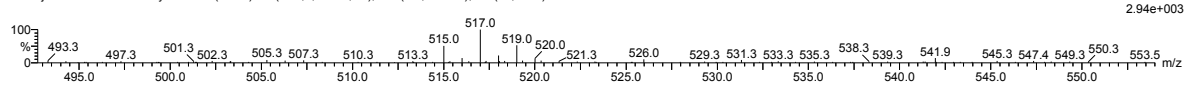
**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 30.0 mDa / DBE: min = -2.0, max = 1000.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
58 formula(e) evaluated with 6 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 N: 4-4 O: 0-25 79Br: 2-2

09-Jan-2017
hbl-09jan17--34-485-bimethylate 188 (3.477) Cn (Cen,5, 50.00, Ar); Sm (SG, 1x3.00); Sb (12,5.00 )

TOF MS ES+
2.94e+003




| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -2.0 | | |
| Maximum: | | 30.0 | 10.0 | 1000.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 515.0079 | 515.0082 | -0.3 | -0.6 | 13.5 | 1422.4 | C22 H21 N4 O 79Br2 |
| | 515.0141 | -6.2 | -12.0 | 4.5 | 1457.3 | C15 H25 N4 O6 79Br2 |
| | 514.9988 | 9.1 | 17.7 | 0.5 | 1477.3 | C11 H25 N4 O9 79Br2 |
| | 514.9930 | 14.9 | 28.9 | 9.5 | 1437.2 | C18 H21 N4 O4 79Br2 |
| | 515.0293 | -21.4 | -41.6 | 8.5 | 1470.0 | C19 H25 N4 O3 79Br2 |
| | 515.0352 | -27.3 | -53.0 | -0.5 | 1526.2 | C12 H29 N4 O8 79Br2 |

**Figure S40.** $^{1}H$ NMR spectrum of **2c** in $CD_3OD$ at 298 K

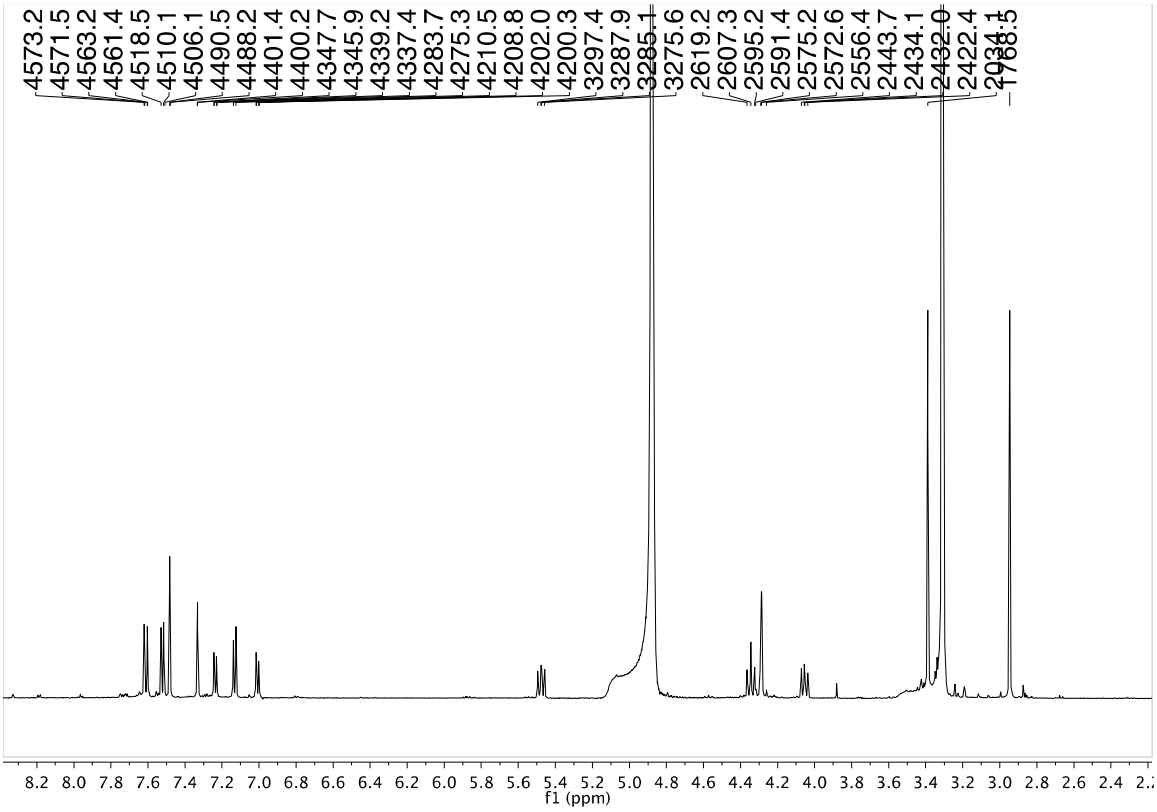



**Figure S41.** $^{13}C$ NMR spectrum of **2c** in $CD_3OD$ at 298 K

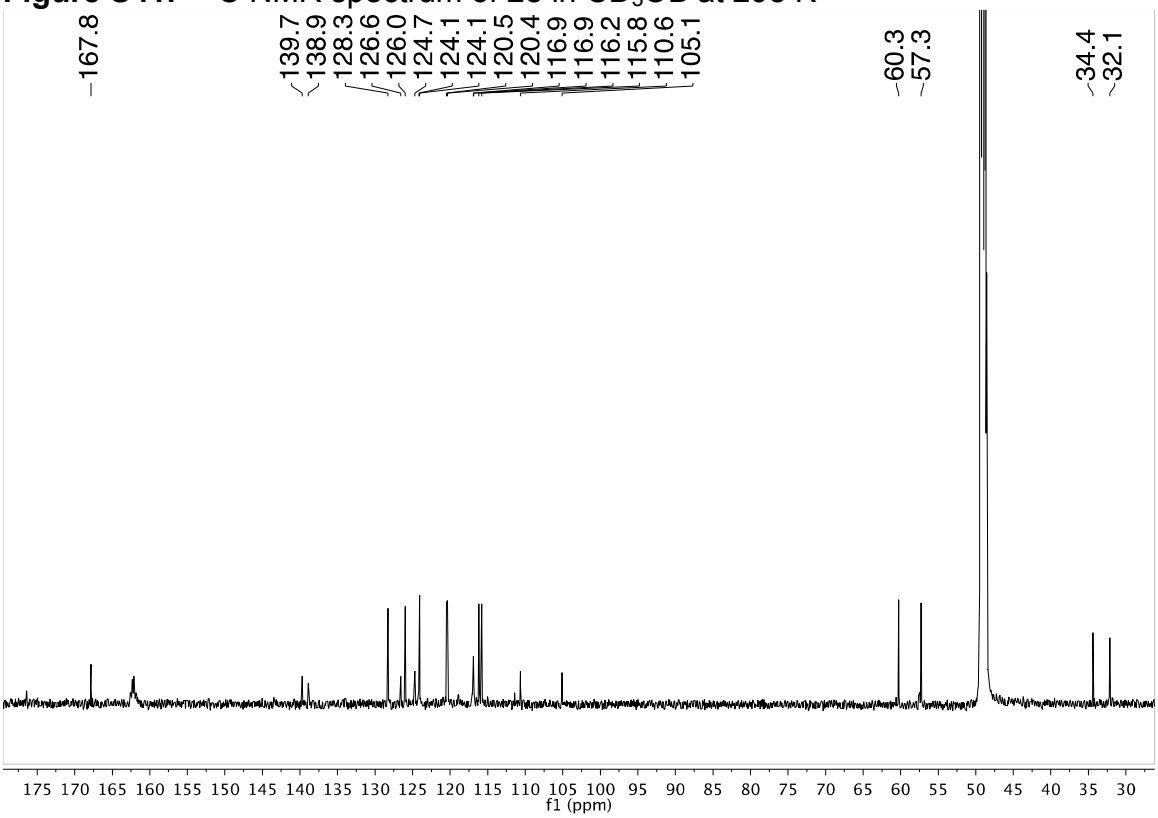


**Figure S42.** $^{1}H$-$^{1}H$ COSY spectrum of **2c** in $CD_3OD$ at 298 K

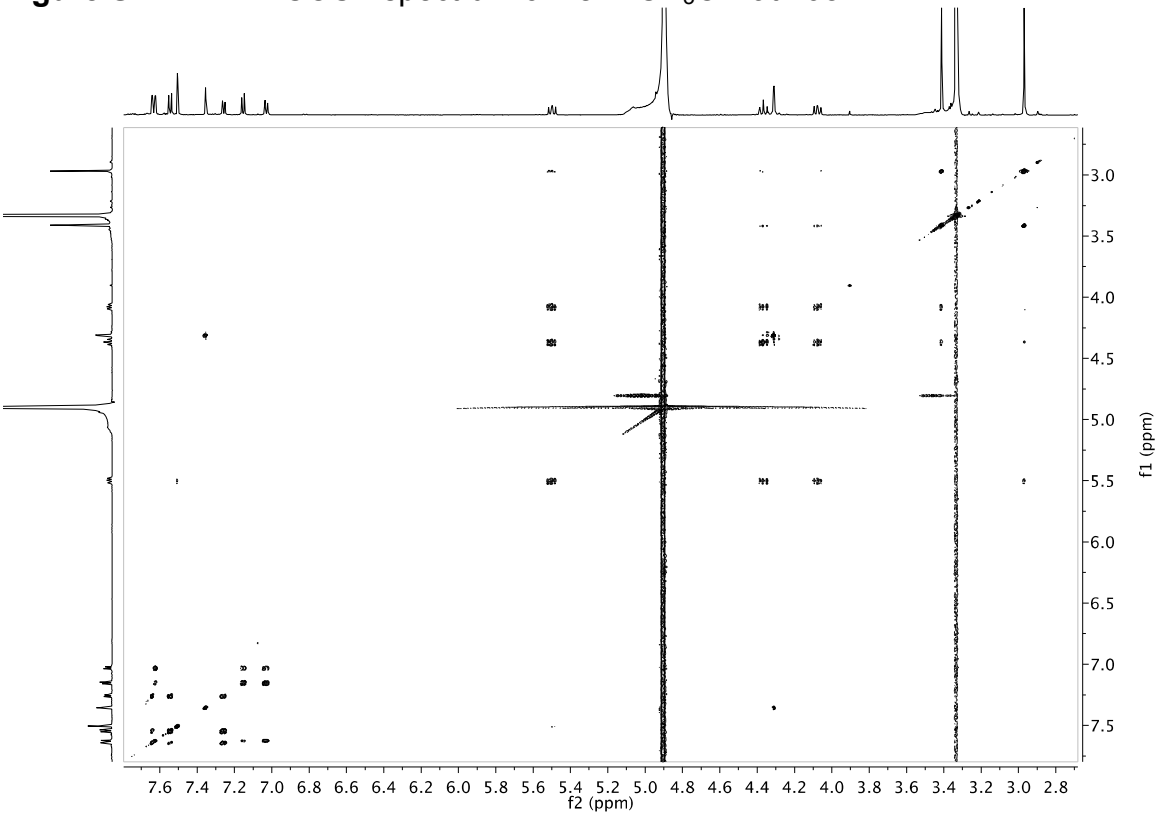



**Figure S43.** HSQC spectrum of **2c** in $CD_3OD$ at 298 K

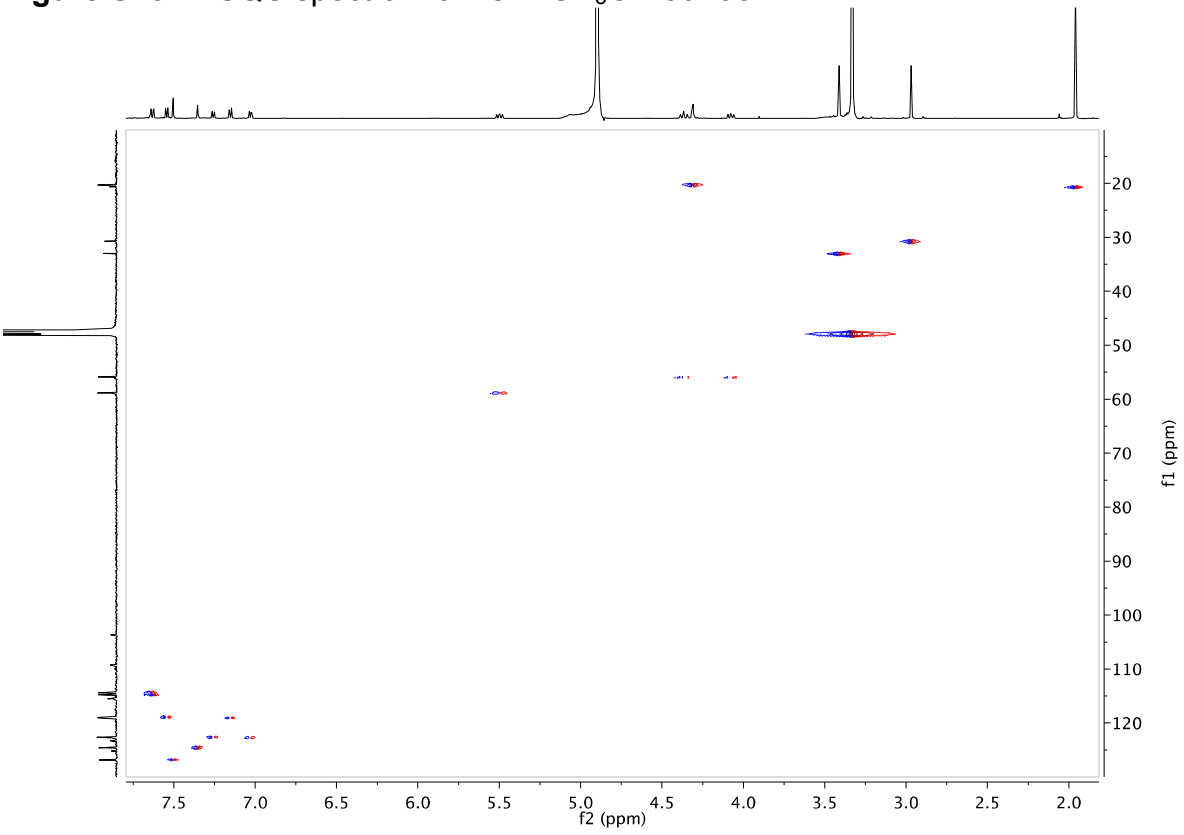


**Figure S44.** HMBC spectrum of **2c** in $CD_3OD$ at 298 K

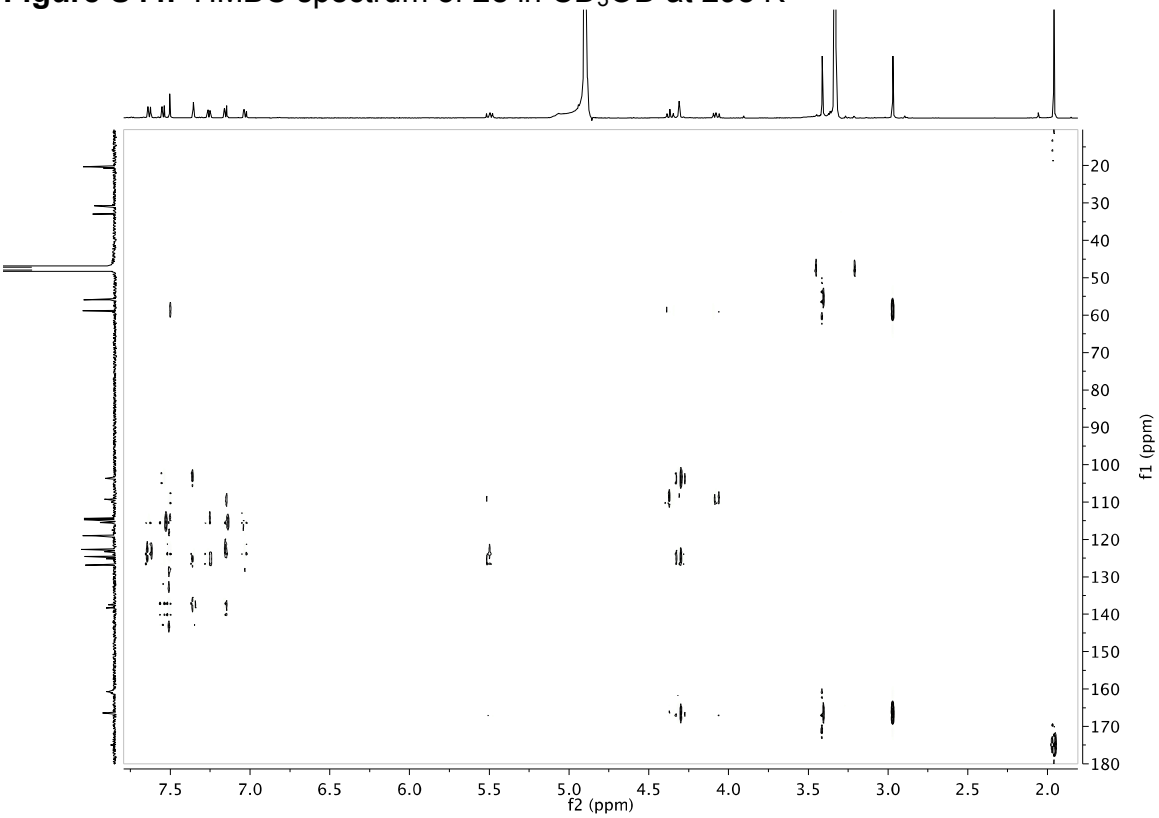



**Figure S45.** HRESIMS spectrum of **2c**

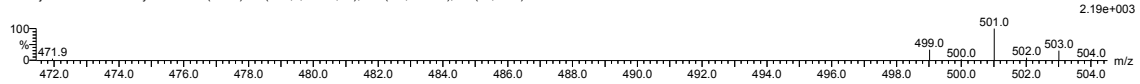
**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 30.0 mDa / DBE: min = -2.0, max = 1000.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
53 formula(e) evaluated with 7 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 N: 4-4 O: 0-25 79Br: 2-2

09-Jan-2017
hbl-09jan17--34-485-methylate-2 123 (2.275) Cn (Cen,5, 50.00, Ar); Sm (SG, 1x3.00); Sb (12,5.00 )

TOF MS ES+
2.19e+003




| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -2.0 | | |
| Maximum: | | 30.0 | 10.0 | 1000.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 499.0124 | 499.0133 | -0.9 | -1.8 | 13.5 | 1153.3 | C22 H21 N4 79Br2 |
| | 499.0192 | -6.8 | -13.6 | 4.5 | 1115.0 | C15 H25 N4 O5 79Br2 |
| | 499.0039 | 8.5 | 17.0 | 0.5 | 1088.3 | C11 H25 N4 O8 79Br2 |
| | 498.9980 | 14.4 | 28.9 | 9.5 | 1127.7 | C18 H21 N4 O3 79Br2 |
| | 499.0344 | -22.0 | -44.1 | 8.5 | 1161.6 | C19 H25 N4 O2 79Br2 |
| | 499.0403 | -27.9 | -55.9 | -0.5 | 1133.2 | C12 H29 N4 O7 79Br2 |
| | 498.9828 | 29.6 | 59.3 | 5.5 | 1119.4 | C14 H21 N4 O6 79Br2 |

**Figure S46.** $^{1}H$ NMR spectrum of **2d** in $CD_3OD$ at 298 K

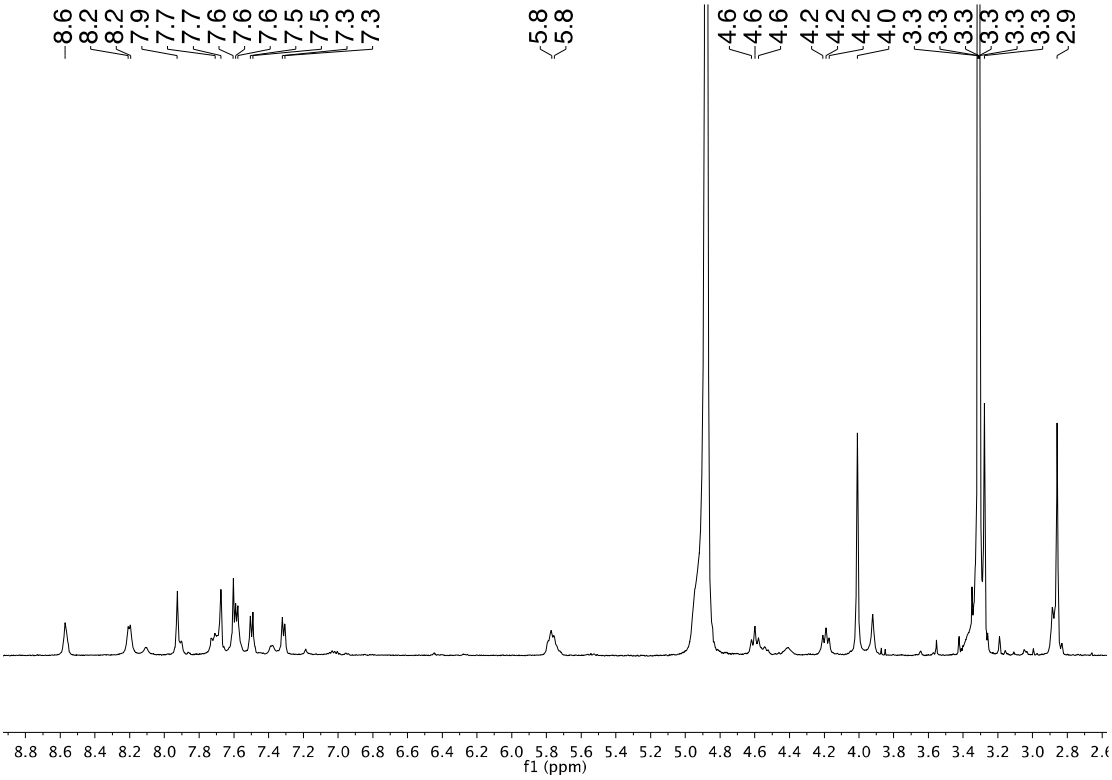



**Figure S47.** $^{13}C$ NMR spectrum of **2d** in $CD_3OD$ at 298 K

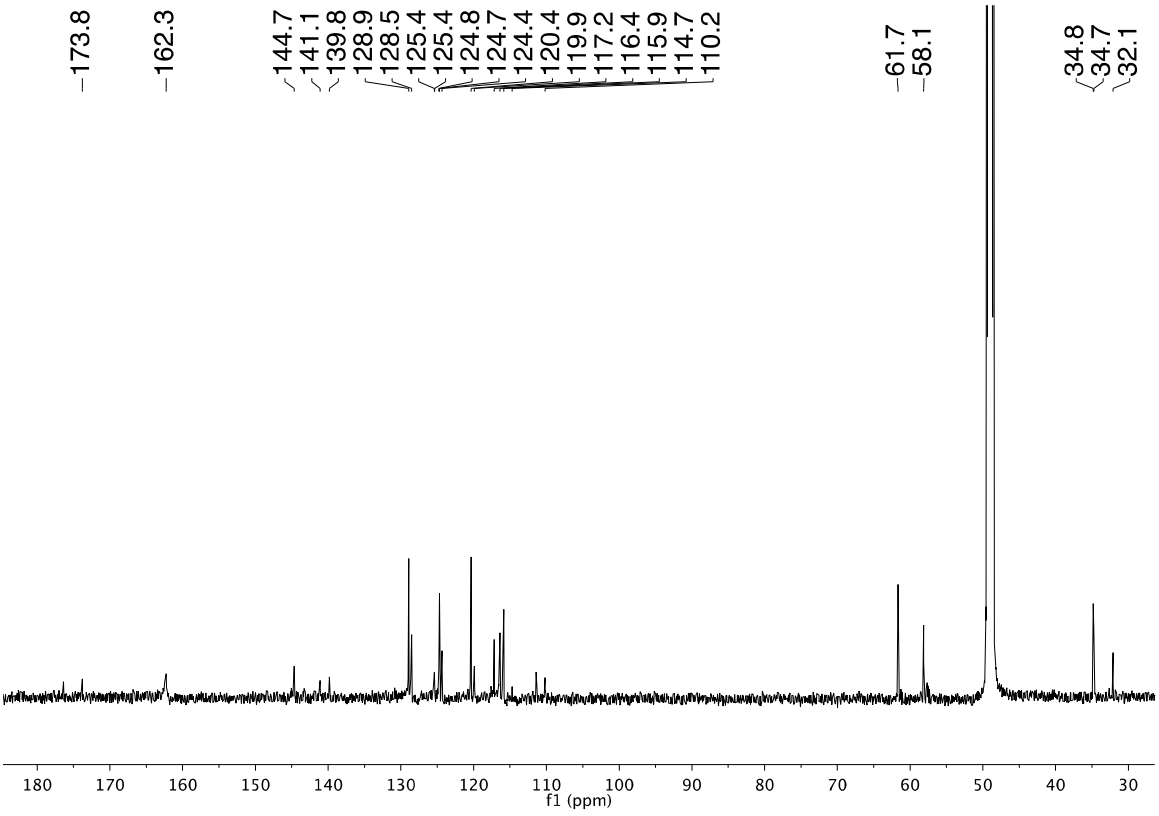


**Figure S48.** $^{1}H$-$^{1}H$ COSY spectrum of **2d** in $CD_3OD$ at 298 K

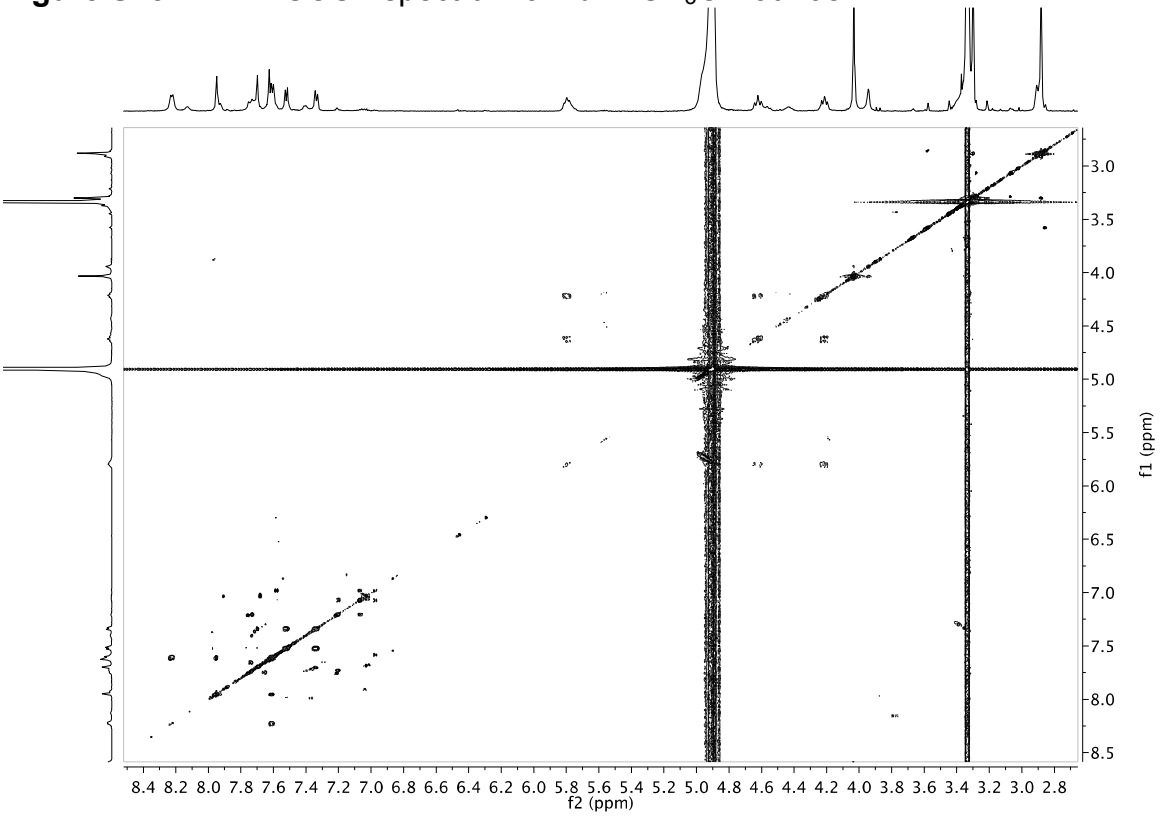



**Figure S49.** HSQC spectrum of **2d** in $CD_3OD$ at 298 K

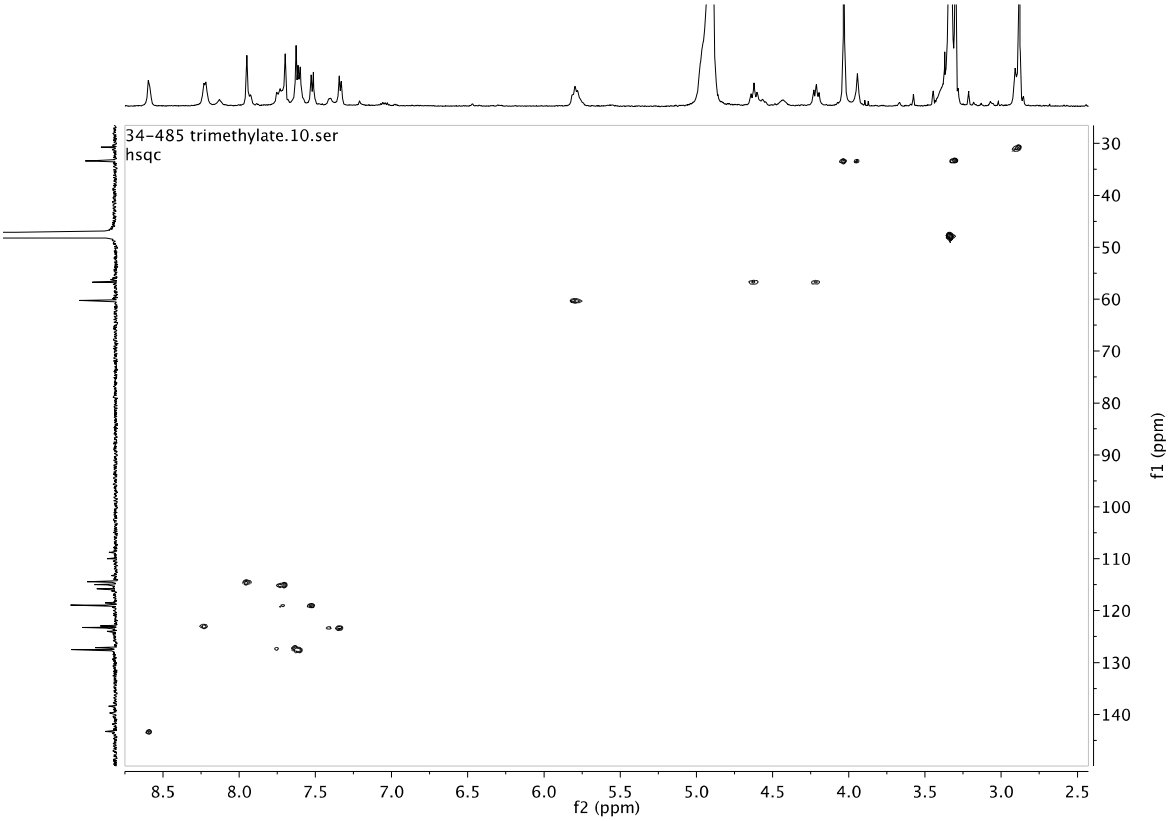


**Figure S50.** HMBC spectrum of **2d** in $CD_3OD$ at 298 K

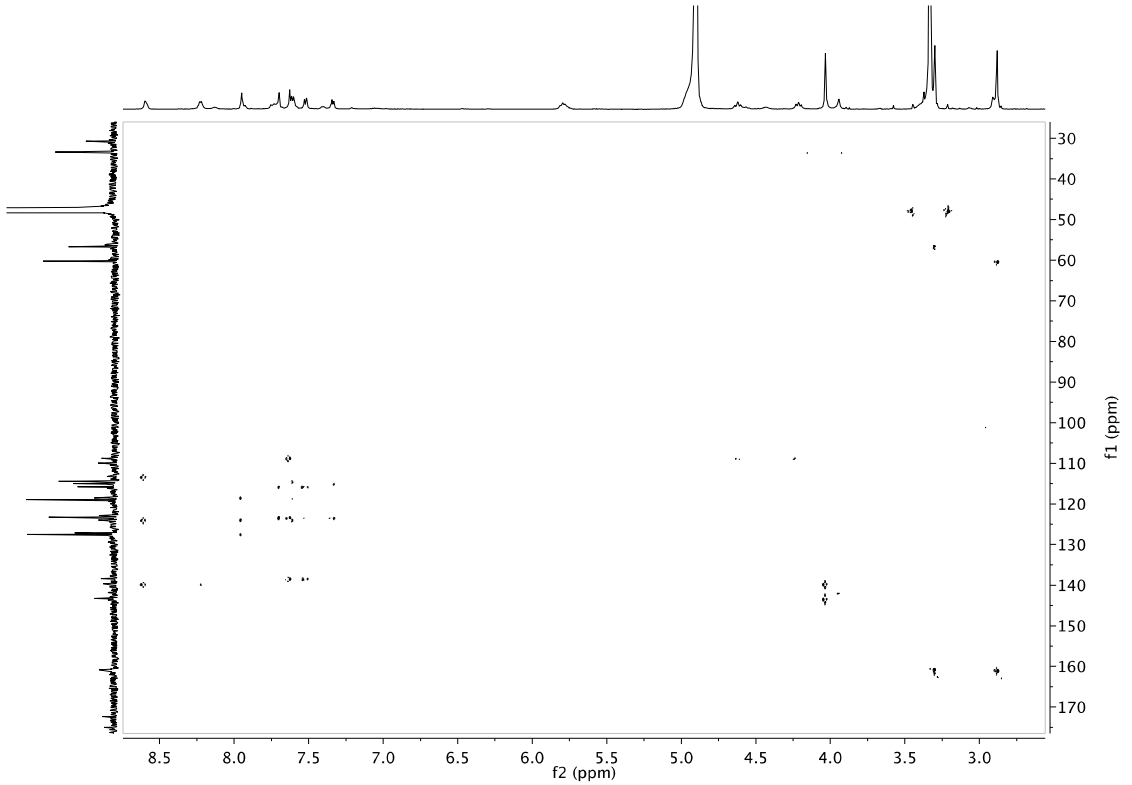


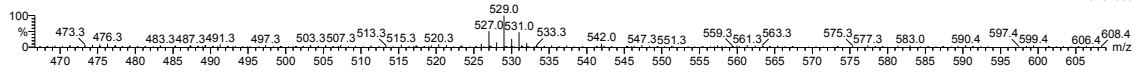

**Figure S51.** HRESIMS spectrum of **2d**

**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 30.0 mDa / DBE: min = -2.0, max = 1000.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
62 formula(e) evaluated with 6 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 N: 4-4 O: 0-25 79Br: 2-2

09-Jan-2017
hbl-09jan17--34-485-trimethylate 148 (2.737) Cn (Cen,5, 50.00, Ar); Sm (SG, 1x3.00); Sb (12,5.00 ) TOF MS ES+ 2.91e+003




| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Minimum: | | | | -2.0 | | | | | | | |
| Maximum: | | 30.0 | 10.0 | 1000.0 | | | | | | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula | | | | | |
| 527.0088 | 527.0082 | 0.6 | 1.1 | 14.5 | 1401.3 | C23 | H21 | N4 | O | 79Br2 | |
| | 527.0141 | -5.3 | -10.1 | 5.5 | 1434.2 | C16 | H25 | N4 | O6 | 79Br2 | |
| | 526.9988 | 10.0 | 19.0 | 1.5 | 1455.3 | C12 | H25 | N4 | O9 | 79Br2 | |
| | 526.9930 | 15.8 | 30.0 | 10.5 | 1417.6 | C19 | H21 | N4 | O4 | 79Br2 | |
| | 527.0293 | -20.5 | -38.9 | 9.5 | 1443.8 | C20 | H25 | N4 | O3 | 79Br2 | |
| | 527.0352 | -26.4 | -50.1 | 0.5 | 1496.9 | C13 | H29 | N4 | O8 | 79Br2 | |

**Figure S52.** Comparison of experimental (black) and calculated (blue) CD spectra of 4*S*, 6*S*-**2**.

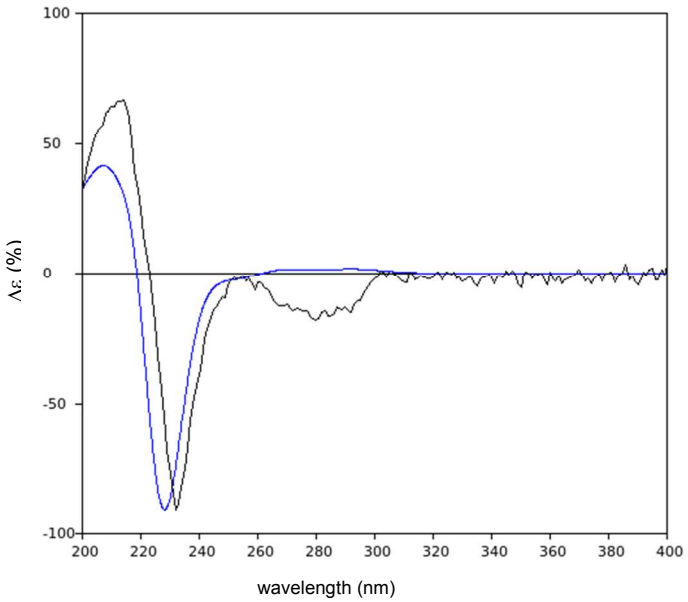



**Figure S53.** Comparison of experimental (red) and calculated (black) CD spectra of 4*S*, 6*R*-**2**.

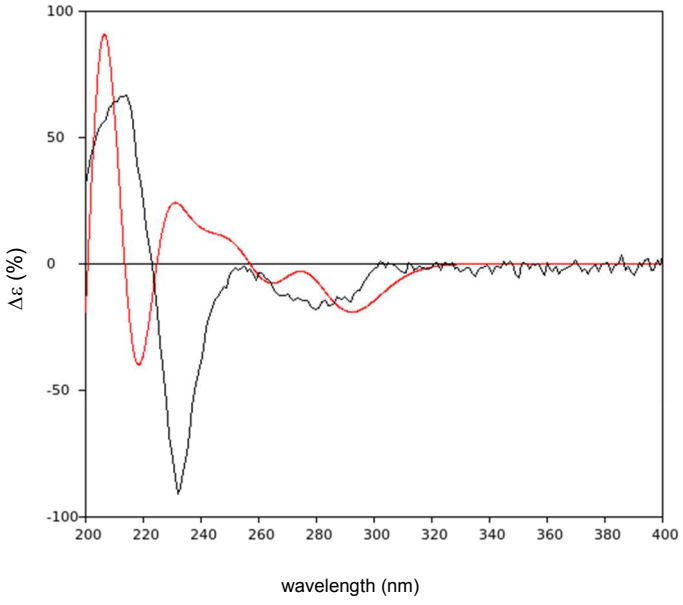


**Figure S54.** Comparison of experimental (black) and calculated (blue) CD spectra of 4*S,* 6*S*-**2a**.

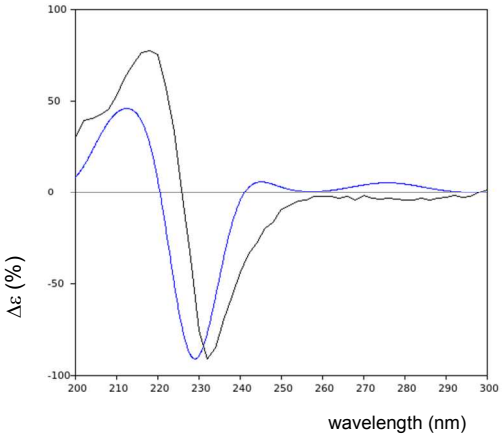



**Figure S55.** Comparison of experimental (black) and calculated (red) CD spectra of 4*S,* 6*R*-**2a**.

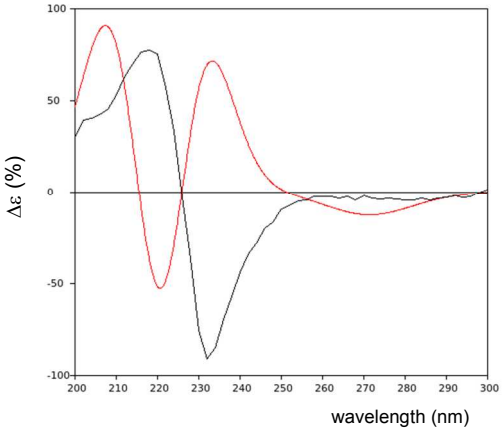



**Figure S56.** Comparison of experimental (black) and calculated (blue) CD spectra of 4*S,* 6*S*-**2b**.

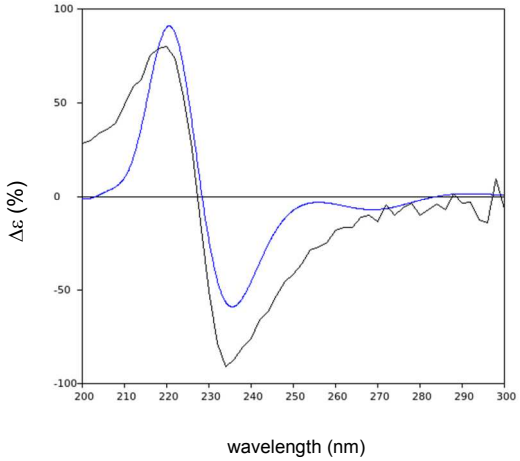


**Figure S57.** Comparison of experimental (black) and calculated (red) CD spectra of 4*S,* 6*R*-**2b**.

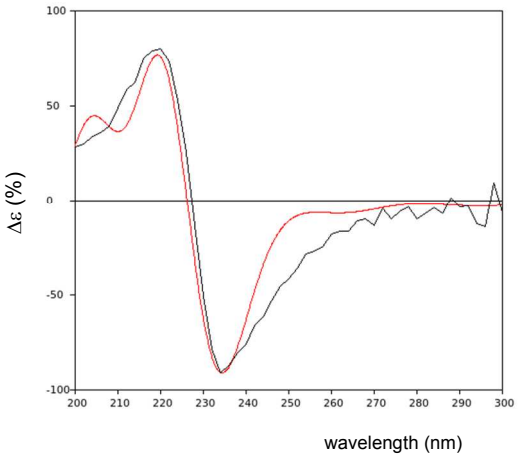



**Figure S58.** Comparison of experimental (black) and calculated CD spectra of 4*R*-**2c** (red) and 4*S*-**2c** (blue).

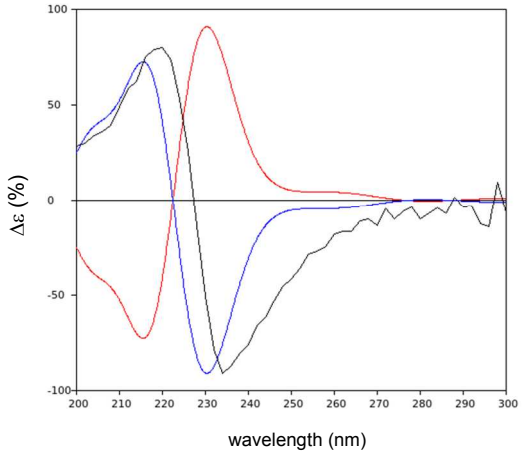



**Figure S59.** Comparison of experimental (black) and calculated CD spectra of 4*R*-**2d** (red) and 4*S*-**2d** (blue).

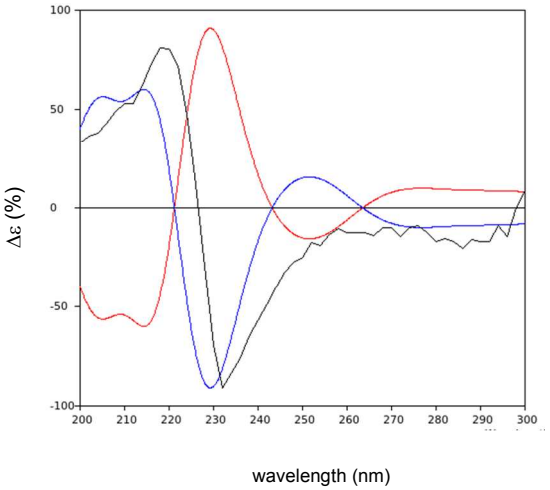


**Figure S59.** IR spectrum of **1**.

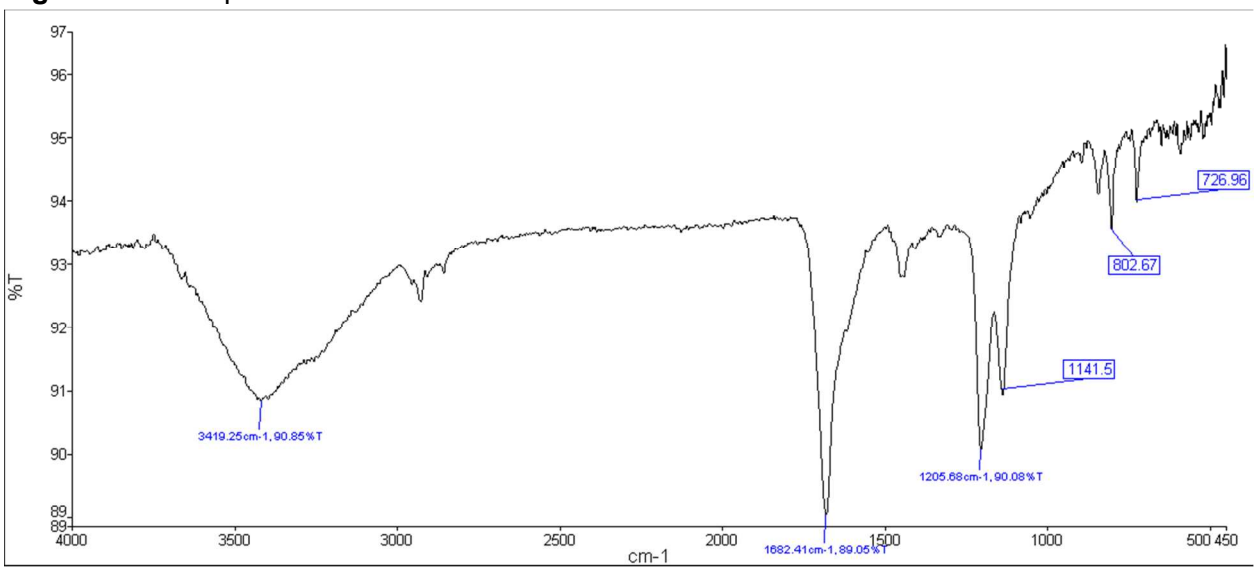
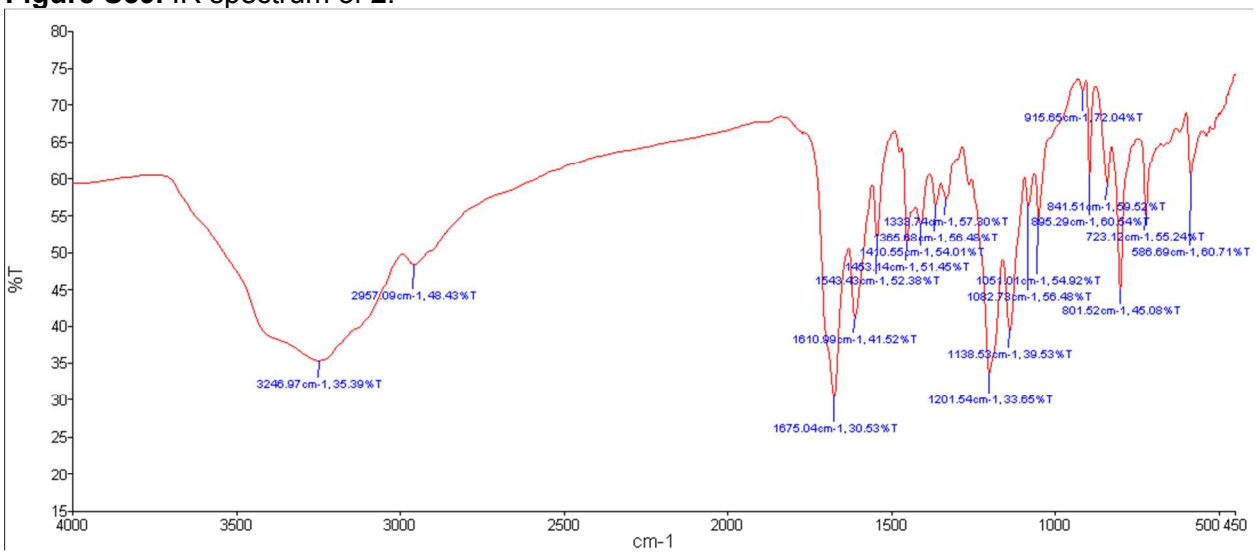



**Figure S59.** IR spectrum of **2**.




[i] Maestro, version 10.5, Schrödinger *LLC New York NY* **2016**.

[ii] MacroModel, version 11.1, Schrödinger *LLC New York NY*, **2016**.

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