Electronic supplementary information (ESI) for:

Controlled aggregation of peptide substituted perylene-bisimides

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General methods

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used as received, unless otherwise stated. Amino acid derivatives were purchased from Merck KGaA (Darmstadt, Germany). Derivatized resin was purchased from AAPPTec (Louisville, KY, USA) and O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) was purchased from Nova Biochem. ^{1}H and ^{13}C NMR data were collected on a Varian Unity Inova operating at 500 MHz, and full 1D- and 2D NMR data were obtained on a Varian Direct-Drive spectrometer operating at 600 MHz for ^{1}H and equipped with a HCN coldprobe. All NMR spectra were referenced internally to residual solvent peaks. Fourier-transform infrared (FT-IR) spectroscopy was performed with a Perkin Elmer Spectrum One spectrometer using freshly prepared potassium bromide pellets and recorded in the range of 450-4000 cm^{-1} and averaged over eight scans. Mass spectra were collected on a Waters Q-TOF Premier Tandem Mass Spectrometer for **1b, 2b,** and **3b,** an Agilent 6530 Series Q-TOF mass spectrometer for **4**, and MALDI-MS was collected on an AB SCIEX TOF/TOF 5800 for **1**, **2**, and **3** using 2,5-dihydroxybenzoic acid (DHB) as matrix.

Absorption spectroscopy measurements

Absorption spectroscopy (UV-Vis) was performed with an Agilent 8453 UV-Visible spectrophotometer over the range 220-1100 nm using a quartz curvette with 1 cm path length. All sample solutions analysed had a total volume of 5 mL. Aqueous samples of each **1**, **2** and **3** were

prepared by having the desired solution (e.g. altered pH or aqueous salt solution) then 20 µL of a stock solution (1.3 mg/mL in H_2O) of the relevant peptide-PBI conjugate was added. Solvent composition samples of peptide-PBI conjugates were prepared by mixing water and the organic solvent to the desired percentage (by volume) and then adding 20 µL of a stock solution (1.3mg/mL in H_2O) of the relevant peptide-PBI conjugate to give a series of individual solution from 0-100% solvent percentage. Samples of **4** were prepared by adding 100 µL of a stock solution (0.07 mg/mL in $CHCl_3$) into 5 mL of solvent.

Fluorescence spectroscopy measurements

Fluorescence spectroscopy was performed with a Shimadzu RF-5301PC spectrofluorophotometer using a quartz cuvette with 1 cm path length and four polished sides, the slit widths on the spectrofluorophotometer were set to 1.5 nm resolution (for both excitation and emission). All excitation wavelengths were at the [0-0] vibronic peak position, unless otherwise stated. Samples were prepared as described above.

Circular Dichroism (CD) spectroscopy measurements

CD spectra were recorded on a Chirascan CD Spectrometer. Experiments were performed in a quartz cell with a 1 cm path length over the range of 205-500 nm at 25°C. Samples were prepared as described above.

Dynamic Light Scattering (DLS) measurements

DLS measurements were recorded on a Malvern Zetasizer Nano-ZS. Experiments were performed in a quartz cell with a 1 cm path length at 25°C. The size for each sample was averaged over eight measurements. Samples were prepared as described above.

Data Analysis

Absorption data*:* All spectra were baseline corrected ($Abs_{700\ nm}$ = 0) before subtracting an aggregate signal that may be convoluting the spectrum. The absorption spectrum in acetonitrile

(MeCN) was used as the aggregate reference spectrum in all cases. This aggregated spectrum was scaled to match the 'raw' absorption at 575 nm and subsequently subtracted from the absorption spectrum of interest with the resulting spectrum being considered the 'vibronically structured' component of the absorption spectrum. From this 'vibronically structured' component the vibronic ratio ($A_{0\text{-}1}/A_{0\text{-}0}$) and the value of the 'vibronically structured' fraction was calculated by dividing its integrated area (on an x-axis of energy) by that area of the raw absorption spectrum.

Emission data*:* All emission spectra were corrected for absorption by dividing by the optical density at the fluorescence excitation wavelength.

Preparation of Organogel

The gelation sample was prepared by dissolving 3.8 mg of peptide **1b** in 1 mL of acetonitrile (MeCN) and sonicating until all the solid was dissolved (approximately 1.5 hours), the self supporting organogel was formed overnight (Figure S1).

Fig S1 – Inversion of vial demonstrates self supporting organogel formation of **1b** in MeCN**.**

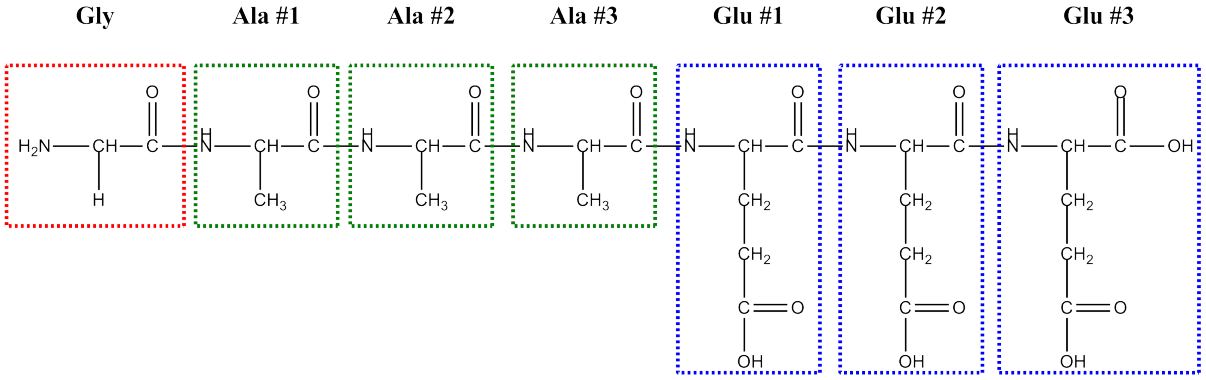
Peptide synthesis procedures:

General procedure:

The peptide sequences were synthesised via standard Fmoc-based solid phase peptide synthesis (SPPS) protocols.[1] Briefly, approximately 0.5 g of Fmoc-Glu(OtBu) Wang resin (loading of 0.57 $mmolg^{-1}$) was swelled in dichloromethane (DCM) for 30 minutes and subsequently washed with dimethylformamide (DMF). Fmoc-deprotection steps were carried out using 30% piperidine (in DMF) with gentle shaking for 30 minutes and then washed five times with fresh DMF. Each amino acid coupling step used 4 mole equivalents (to the loading of resin) of the desired amino acid, 3.95 mole equivalents of HBTU, and 6 mole equivalents of DIPEA and shaken for 20 minutes. The Kaiser test was used to confirm the coupling had gone to completion. Amino acid derivatives used: Fmoc-Glu(OtBU)-OH, Fmoc-Ala-OH, Fmoc-Leu-OH and Fmoc-Gly-OH.

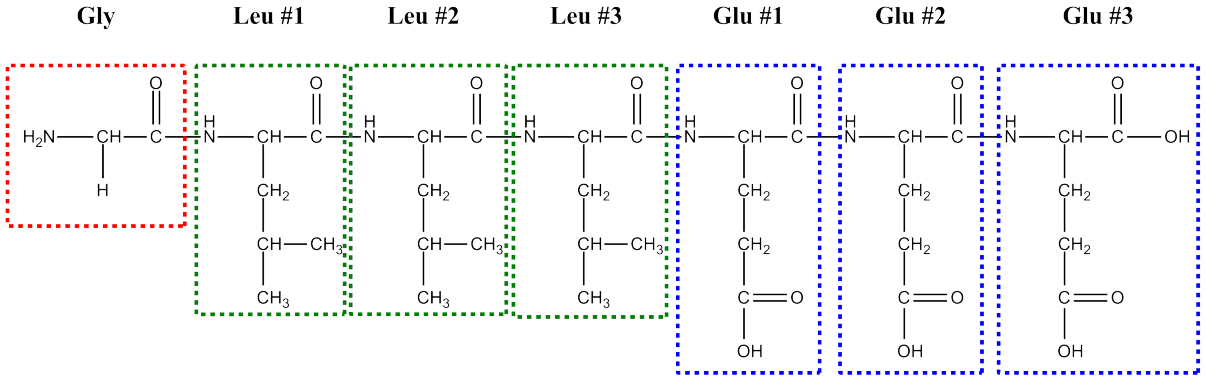
Cleavage of the complete peptide sequence involved removing the Fmoc protection on the terminal amino acid residue, rinsing the resin in DMF (5x) and then DCM (5x) and allowing the resin to dry by passing a stream of nitrogen gas through the reaction column. The cleavage solution was a freshly prepared cocktail of 3:4 trifluoroacetic acid:DCM. This was shaken with the resin for 3 hours and then collected. Fresh cleavage solution was passed through the column and the resin was rinsed with DCM. The collected solution was reduced in volume by half and then added dropwise to 20 mL of cold diethyl ether and allowed to crystallise overnight to afford the fully deprotected peptide sequence which was collected via vacuum filtration.

H_2N-Gly-(Ala)$_3$-(Glu)$_3$-OH (1b):



Peptide **1b** was obtained using the general procedure described above. (Yield: 0.21 g, 55%). ^{1}H NMR (600 MHz, DMSO-d_6) δ= 8.54 (d, J=7.5 Hz, N-H Ala #1), 8.24 (d, J=7.5 Hz, N-H Ala #2), 8.21 (d, J=7.5 Hz, N-H Glu #3), 7.95 (m, 5H, N-H_2 Gly; N-H Glu #1, #2; N-H Ala #3), 4.40 (quin, J= 7.2 Hz, C-H Ala #1), 4.26 (m, 4H, C-H Ala #2, #3; C-H Glu #1, #2), 4.16 (m, 1H, C-H Glu #3), 3.56 (q, J= 5.3 Hz, C-H_2 Gly), 2.23 (m, 6H, γ-CH_2 Glu #1, #2, #3), 1.84 (m, 6H, β-CH_2 Glu #1, #2, #3), 1.19 (m, 9H, CH_3 Ala #1, #2, #3); ^{13}C NMR (150 MHz, DMSO-d_6) 174.0, 173.8, 173.1, 171.8, 171.5, 171.1, 170.5, 170.0, 165.5, 51.7, 51.6, 51.3, 48.1, 48.1, 48.0, 40.1, 30.1, 30.1, 29.9, 27.5, 27.4, 26.2, 18.9, 18.1, 18.1; HR-ESI-MS: calcd for $C_{26}H_{42}N_7O_{14}$ $[M+H]^+$ 676.2784 *m/z*, found 676.2794; IR (KBr) 3290, 3085, 2983, 2940, 2637, 1716, 1634, 1533, 1443, 1412, 1338, 1201, 1142, 965, 929, 827, 799.

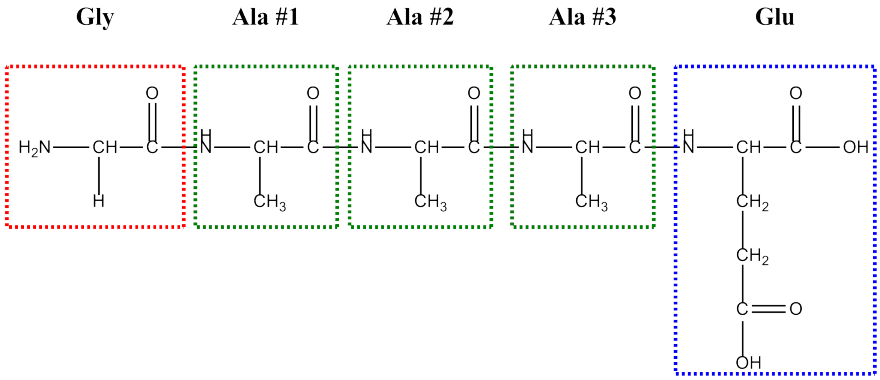
H_2N-Gly-(Leu)$_3$-(Glu)$_3$-OH (2b):



Peptide **2b** was obtained using the general procedure described above. (Yield: 0.15 g, 52%). ^{1}H NMR (600 MHz, DMSO-d_6) δ= 8.47 (d, J=8.5 Hz, N-H Leu #1), 8.24 (d, J=8.2 Hz, N-H Leu #2), 8.22 (d, 7.6 Hz,

Glu #3), 7.95 (m, 5H, N-H_2 Gly; N-H Glu #1, #2; N-H Ala #3), 4.44 (m, 1H, C-H Leu #1), 4.28 (m, 3H, C-H Leu #2; Leu #3; Glu #2), 4.17 (m, 2H, C-H Glu #1; Glu #3), 3.56 (q, 5.6 Hz, C-H_2 Gly), 2.24 (m, 6H, γ-CH_2 Glu #1, #2, #3), 1.81 (m, 6H, β-CH_2 Glu #1, #2, #3), 1.57 (m, 3H, γ-C-H Leu #1, #2, #3), 1.42 (m, 6H β-C-H_2 Leu #1, #2, #3), 0.85 (m, 18H, CH_3 Leu #1, #2, #3); ^{13}C NMR (150 MHz, DMSO-d_6) 174.5, 174.4, 174.1, 173.5, 172.0, 171.8, 171.5, 171.1, 165.9, 52.0, 51.7, 51.2, 51.2, 41.9, 40.8, 40.5, 30.5, 30.4, 28.0, 27.8, 26.6, 24.6, 24.5, 24.5, 23.6, 23.4, 22.2, 22.1, 21.9; HR-ESI-MS: calcd for $C_{35}H_{60}N_7O_{14}$ $[M+H]^+$ 802.4193 *m/z*, found 802.4177.

H_2N-Gly-(Ala)$_3$-Glu-OH (3b):



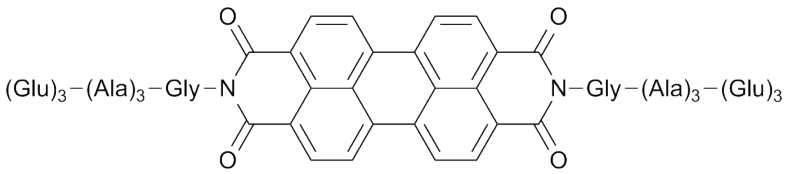
Peptide **3b** was obtained using the general procedure described above. (Yield: 0.12 g, 93%). (600 MHz, DMSO-d_6) δ= 8.56 (d, J=7.5 Hz, N-H Ala #1), 8.24 (d, J=7.6 Hz, N-H Ala #2), 8.11 (d, J=7.9 Hz, N-H Glu), 7.99 (m, 2H, N-H_2 Gly), 7.91 (d, 7.3 Hz, N-H Ala #3), 4.40 (quin, J= 7.2 Hz, C-H Ala #1), 4.26 (m, 2H, C-H Ala #2, #3), 4.19 (m, 1H, C-H Glu), 3.56 (q, J= 4.9 Hz, C-H_2 Gly), 2.27 (m, 2H, γ-CH_2 Glu), 1.86 (m, 2H, β-CH_2 Glu), 1.21 (m, 9H, CH_3 Ala #1, #2, #3); ^{13}C NMR (150 MHz, DMSO-d_6) 174.1, 173.5, 172.6, 171.9, 165.9, 51.5, 48.5, 48.3, 48.2, 40.4, 30.4, 26.8, 19.0, 18.6, 18.5; HR-ESI-MS: calcd for $C_{16}H_{27}N_5O_8$ $[M+H]^+$ 418.1932 *m/z*, found 418.1936

PBI synthesis procedures:

General procedure for Peptide-PBI conjugates

Peptide-PBI conjugates were synthesised using the standard method of amine condensation reported in the literature for the synthesis of symmetric perylene bisimide derivatives, developed by Langhals and co-workers.[2] A mixture of the relevant peptide sequence, perylene tetracarboxylic dianhydride (PTCDA) and imidazole were added in a round bottom flask and stirred at 120°C for 24 hours under a nitrogen atmosphere, after which the mixture was allowed to cool to 90°C and 3 mL of water was added under nitrogen and allowed to cool to room temperature. The dark red solution was acidified with acetic acid to afford a very fine red precipitate. This solution was then passed through an HP20ss column with addition of increasing amounts of water. Once the crude mixture was loaded onto the column, 3% (400 mL total) and 5% (300 mL) acetic acid washings were passed through the column. The column was then washed with acetone and the desired product was extracted from the column by rinsing the column with pyridine, subsequently removing the solvent via reduced pressure.

$(Glu)_3$-$(Ala)_3$-Gly-PTCDI- Gly-$(Ala)_3$-$(Glu)_3$ (1):



Compound **1** was synthesised using the general procedure outlined above, using 0.191 g (0.28 mmol) peptide **1b**, 0.0597 g (0.15 mmol) PTCDA, and 2.049 g imidazole (Yield: 0.20 g, 84%). MALDI-TOF-MS: calcd for $C_{76}H_{86}N_{14}O_{32}Na$ $[M+Na]^+$ 1730 *m/z*, found 1730; IR (KBr) υ 3432, 3295, 3070, 2930, 1693, 1661, 1634, 1595, 1578, 1545, 1440, 1402, 1384, 1367, 1341, 1304, 1249, 1175, 858 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$:TFA-*d* (2:1 *v/v*)) δ= 8.90-8.77 (m, Ar-H, N-H), 5.27-4.97 (m, 4H, C-H (Gly)), 4.80-4.50 (m, C-H), 2.72-1.98 (m, α- and β- CH_2 Glu), 1.63-0.96 (m, CH_3). In spite of the challenges associated

with the poor solubilty of **1**, its structure identity was confirmed through the use of ^{1}H NMR, MALDI-TOF-MS and FT-IR spectroscopy. Spectra are included below, along with a discussion of the key data used for characterisation.

Fig S2 shows the IR spectrum of **1** compared with the anhydride precurse PTCDA between 2000-400 cm^{-1}, with important stretching frequencies labelled. This IR spectrum shows the disappearance of the characteristic bands of the anhydride carbonyl stretching bands (1774 cm^{-1}), and the appearance of the *N*-imide carbonyl stretching bands at 1661 cm^{-1}. Similarly, the C--O--C stretching band (1025 cm^{-1}) has disappeared and been replaced by the C--N stretching band at 1366 cm^{-1}. These spectral features indicate the conversion of the anhydride on PTCDA, to an imide on PBI.

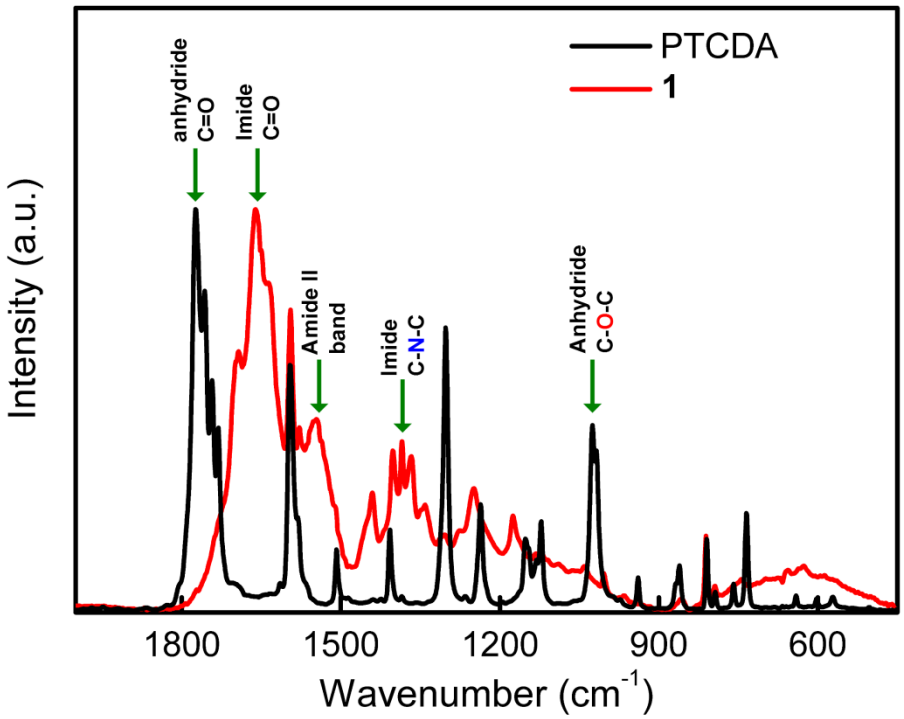


Fig S2 – FT-IR of **1** showing the fingerprint region indicating the replacement of anhydride stretches by imide stretches and evidence of amide stretching frequencies.

The results from MALDI-MS yielded the expectant mass-to-charge ratio for **1**. Further supporting evidence was obtained upon performing an MS-MS spectrum and analysing the fragmentation of the peptide-PBI conjugate, which exhibited the expected fragmentation of the peptide sequence. Finally,

the structure was confirmed through the use of 2D NMR experiments, namely HSQC, HMBC, and COSY. Fig S3 shows a small section of **1** highlighting the correlations that confirm the link between peptide sequence and the PBI core. The connection of these two building blocks is confirmed upon the observation of an HMBC correlation from the H_5 protons (*i.e.* the protons attached to C_5) to C_3 (*i.e.* the PBI carbonyl carbon). These chemical shifts were deduced from the NMR spectra through using the other correlations depicted.

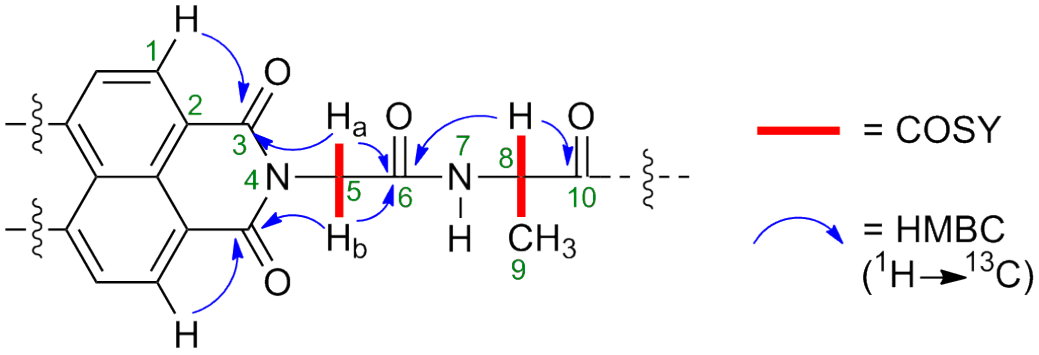


Fig S3 – Portion of **1** showing the HMBC and COSY correlations which confirm the coupling of the peptide sequence to the PBI core

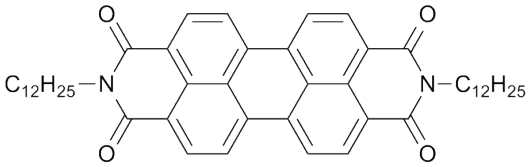
$(Glu)_3$-$(Leu)_3$-Gly-PTCDI- Gly-$(Leu)_3$-$(Glu)_3$ (2):

Compound **2** was synthesised using the general procedure outlined above, using 0.1441 g (0.18 mmol) peptide **2b**, 0.0353 g (0.09 mmol) PTCDA, and 2.2023 g imidazole (Yield: 0.12 g, 71%). MALDI-TOF-MS: calcd for $C_{94}H_{122}N_{14}O_{32}Na$ $[M+Na]^+$ 1982 *m/z*, found 1982; IR (KBr) υ 3426, 3291, 3074, 2959, 2931, 2872, 1703, 1663, 1642, 1595, 1542, 1438, 1402, 1369, 1341, 1248, 1174, 1130, 1038, 1002, 811 cm^{-1}.

Glu-(Ala)$_3$-Gly-PTCDI- Gly-(Ala)$_3$-Glu (3):

Compound **3** was synthesised using the general procedure outlined above, using 0.1002 g (0.24 mmol) peptide **3b**, 0.0471 g (0.12 mmol) PTCDA, and 4.66 g imidazole (Yield: 0.11 g, 74%). MALDI-TOF-MS: calcd for $C_{56}H_{58}N_{10}O_{20}Na$ $[M+Na]^+$ 1213 *m/z*, found 1214; IR (KBr) υ 3445, 3280, 3060, 2965, 2928, 1698, 1658, 1630, 1594, 1543, 1439, 1401, 1364, 1340, 1302, 1245, 1172, 1054, 853, 810 cm^{-1}.

N,N'-di(dodecyl)-perylene-3,4,9,10-tetracarboxylic diimide (4):



Compound **4** was synthesised following a similar condensation method reported by Balakrishnan *et al*.[3] Briefly, a mixture of PTCDA (3.92 g, 10 mmol), dodecylamine (5.53g, 30 mmol), $Zn(OAc)_2$ (0.988 g) and imidazole (40.6 g) were placed in a flask and stirred at 130°C for 24 hours under argon atmosphere. The reaction mixture was allowed to cool to room temperature and dispersed in 500 mL ethanol with subsequent addition of 2 M HCl until pH 3-4 was reached. A red/brown waxy solid was collected via vacuum filtration and dried in an oven overnight at 120°C. (Yield: 6.8 g, 93.6%). ^{1}H NMR (600 MHz, $CDCl_3$) δ= 8.77 (d, 7.9Hz, Ar-H), 8.66 (d, 8.1 Hz, Ar-H), 4.21 (t, 7.7 Hz, α-CH_2), 1.47 (m, 40H, CH_2), 0.83 (m, 6H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) 163.4, 134.7, 131.5, 123.3, 123.1, 40.7, 31.9, 29.7, 29.7, 29.6, 29.6, 29.4, 27.2, 22.7, 14.1; IR (KBr) υ 3426, 3070, 2957, 2923, 2850, 1697, 1657, 1594, 1439, 1405, 1345, 1255, 1090, 853, 809, 747; HR-ESI-MS: calcd for $C_{48}H_{58}N_2O_4Na$ [M+Na] 749.4294 *m/z*, found 749.4315.

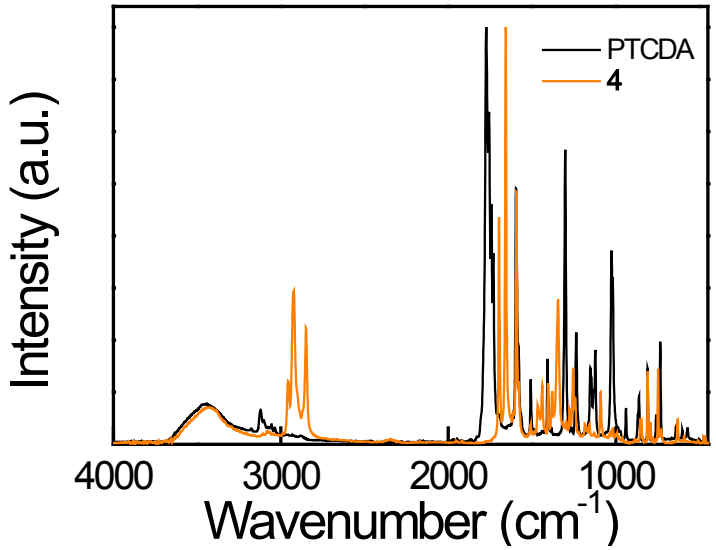


Fig S4 – *Left:* Full FT-IR spectra of PTCDA (starting material) and compound **4** indicating the replacement of anhydride stretches by imide stretches.

Additional figures

Fig S5. PL spectra of **1** (3.0 x 10^{-6} M) in MeCN, and H_2O and **3** (2.0x10^{-6} M) in chloroform. Also included the excitation spectra of **1** in MeCN (emission wavelength 655 nm).

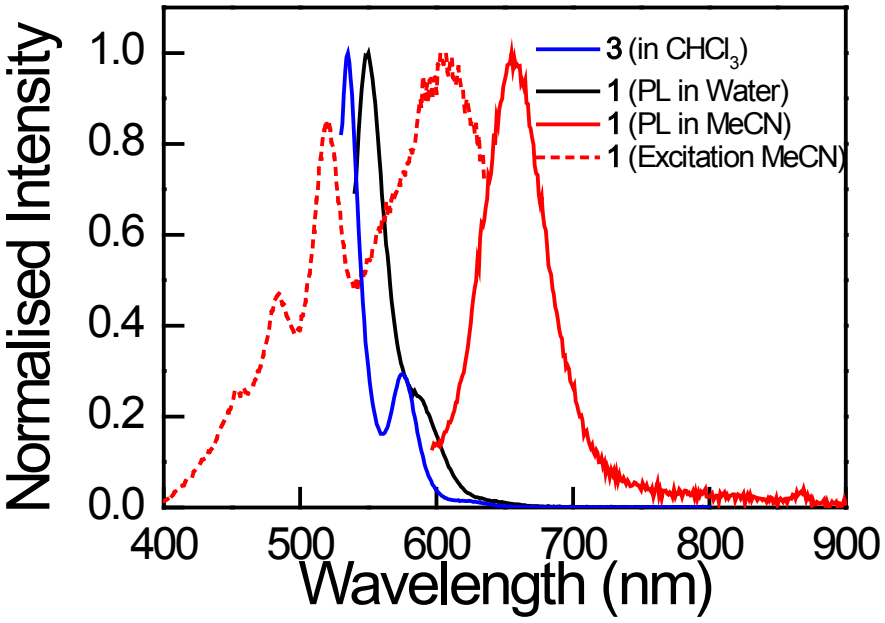


Fig S6. CD spectra of **1** (3.0x10-6 M) in DMSO, MeCN and H_2O

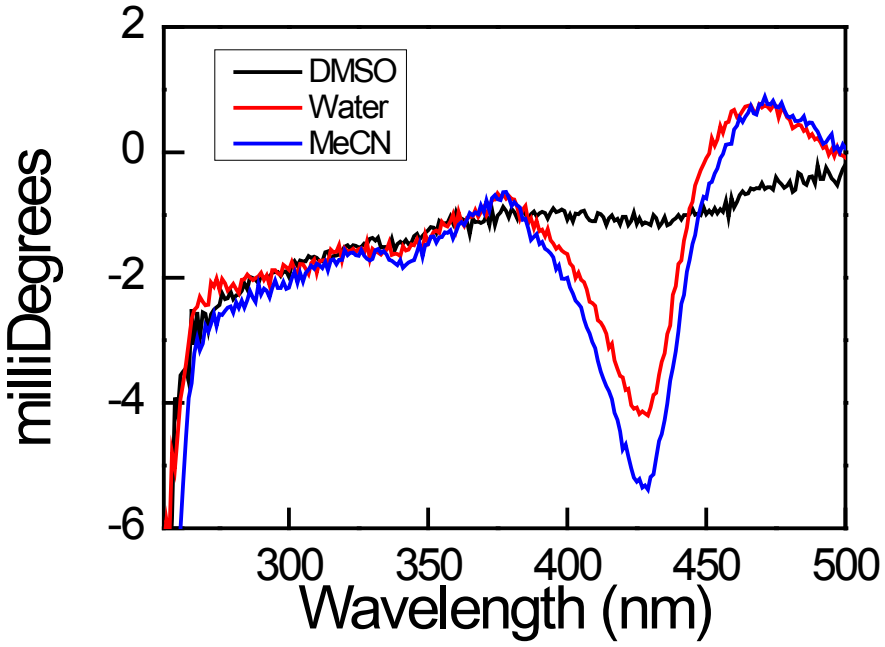
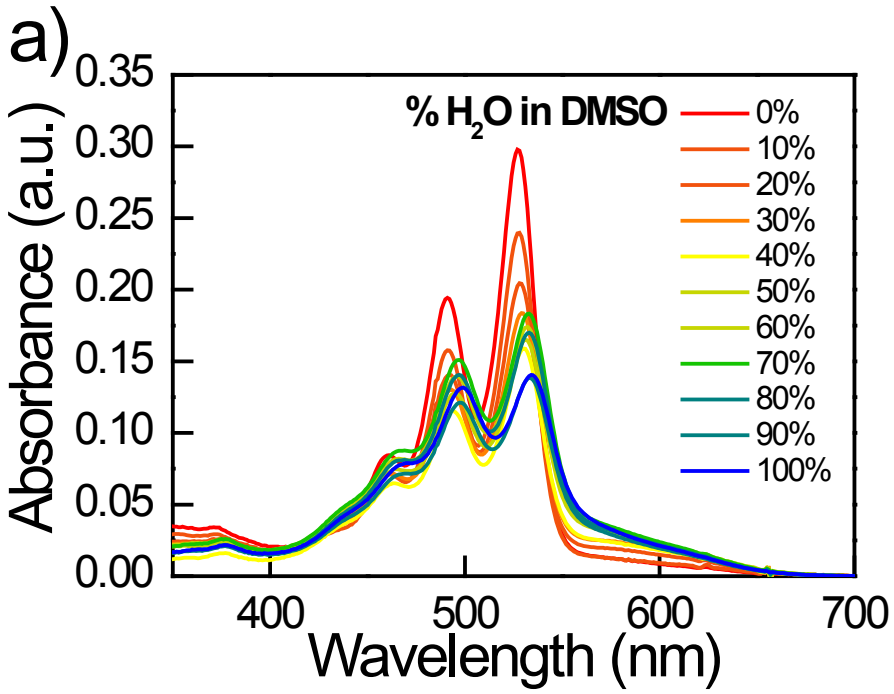


Fig S7. a) UV-vis absorption spectra of **1** in mixtures of DMSO and H_2O. b) Fraction of vibronically structured chomophores extracted from the spectral series in a). Also shown are the vibronic peak ratio of the vibronically structured chromophores



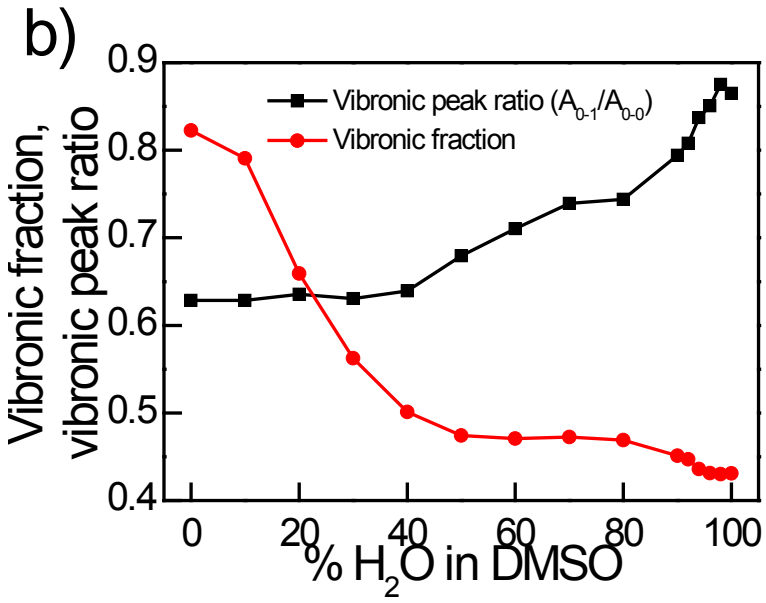


Fig. S8 Change in vibronic fraction of **3** (3.0 x 10^{-6} M) as a function of solvent composition, with and without $NaOCl_4$. The comparison of products **1** and **3** confirms the dominant reorganising role of electrostatic repulsions because with fewer ionisable glutamic acid residues, product **3** is slower to respond to water addition and its response is virtually independent of ionic strength

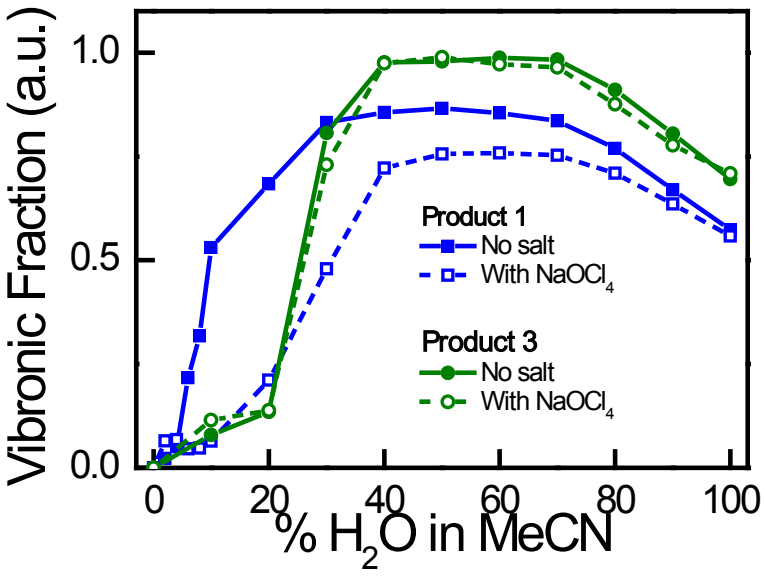
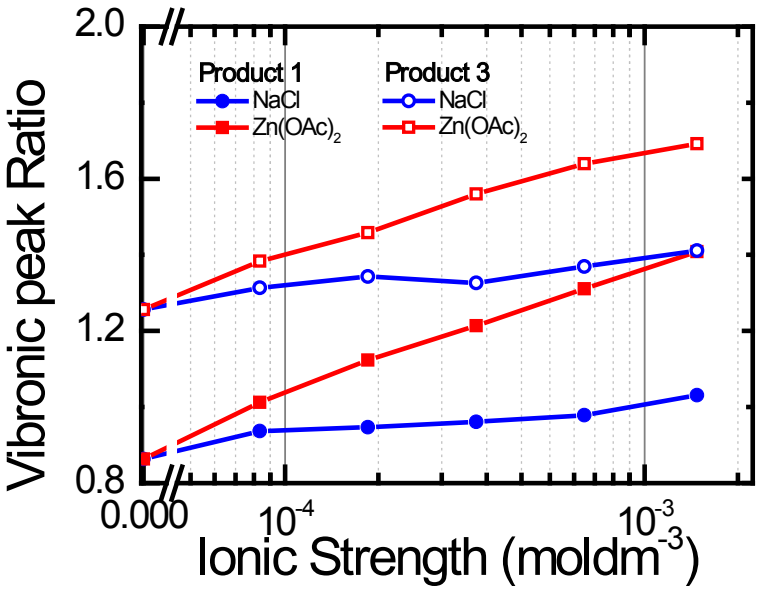


Fig. **S9** Changes in vibronic peak ratios of **1** and **3** (3.0 x 10-6 M) with $Zn(OAc)_2$ and NaCl as a function of ionic strength. The fact that both products exhibit enhanced sensitivity to Zn^{2+} cations in spite of product **3** only having one glutamic acid residue on each peptide arm is consistent with the proposed intermolecular binding mode where Zn^{2+} cations cross-link glutamate residues of neighbouring molecules in the aggregate to tune their interaction. The two products have different starting vibronic peak ratios in the absence of salts due to their different initial aggregate configurations.



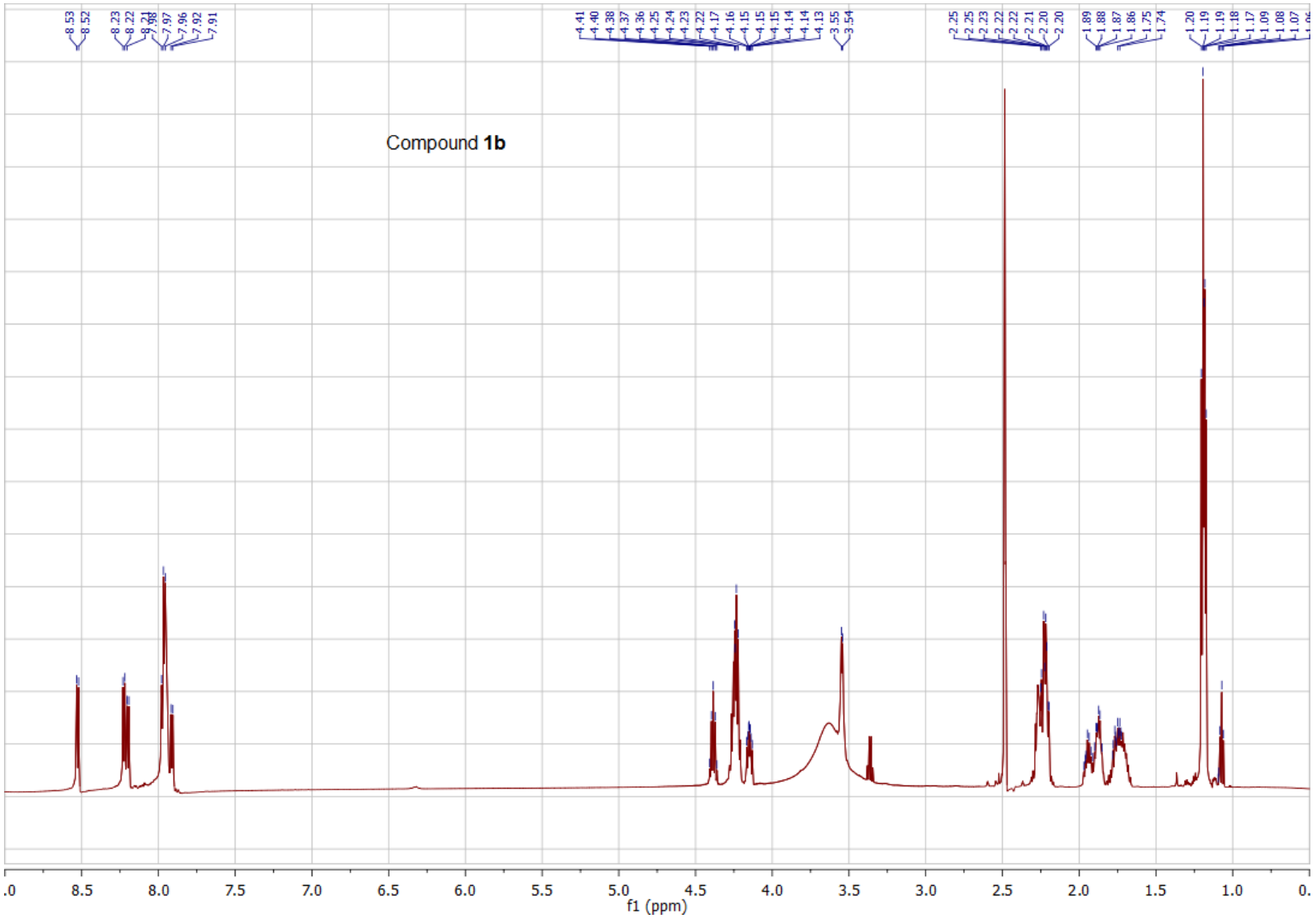


Fig **S10** – ^{1}H NMR spectrum of compound **1b**

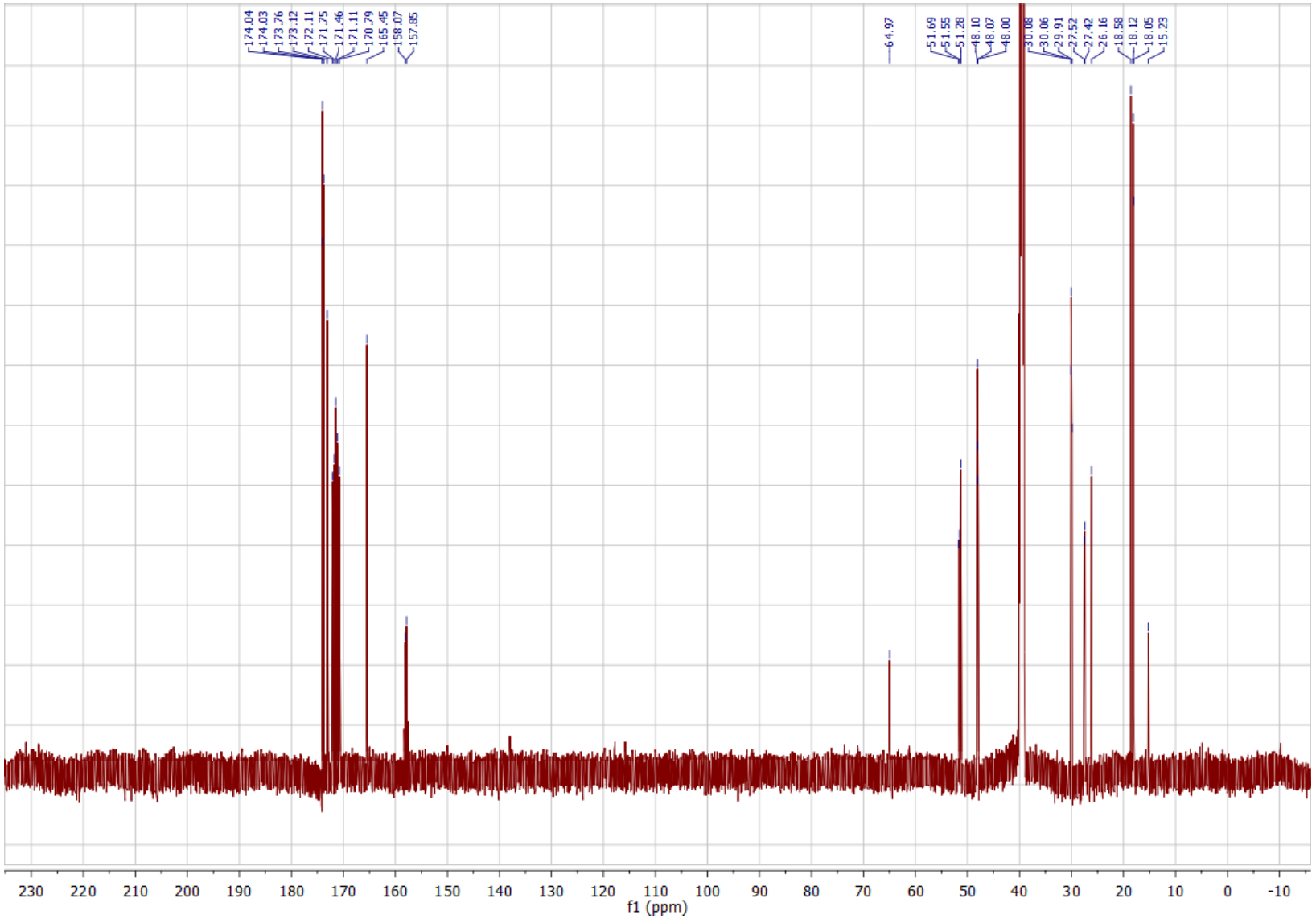


Fig **S11** – ^{13}C NMR spectrum of compound **1b**

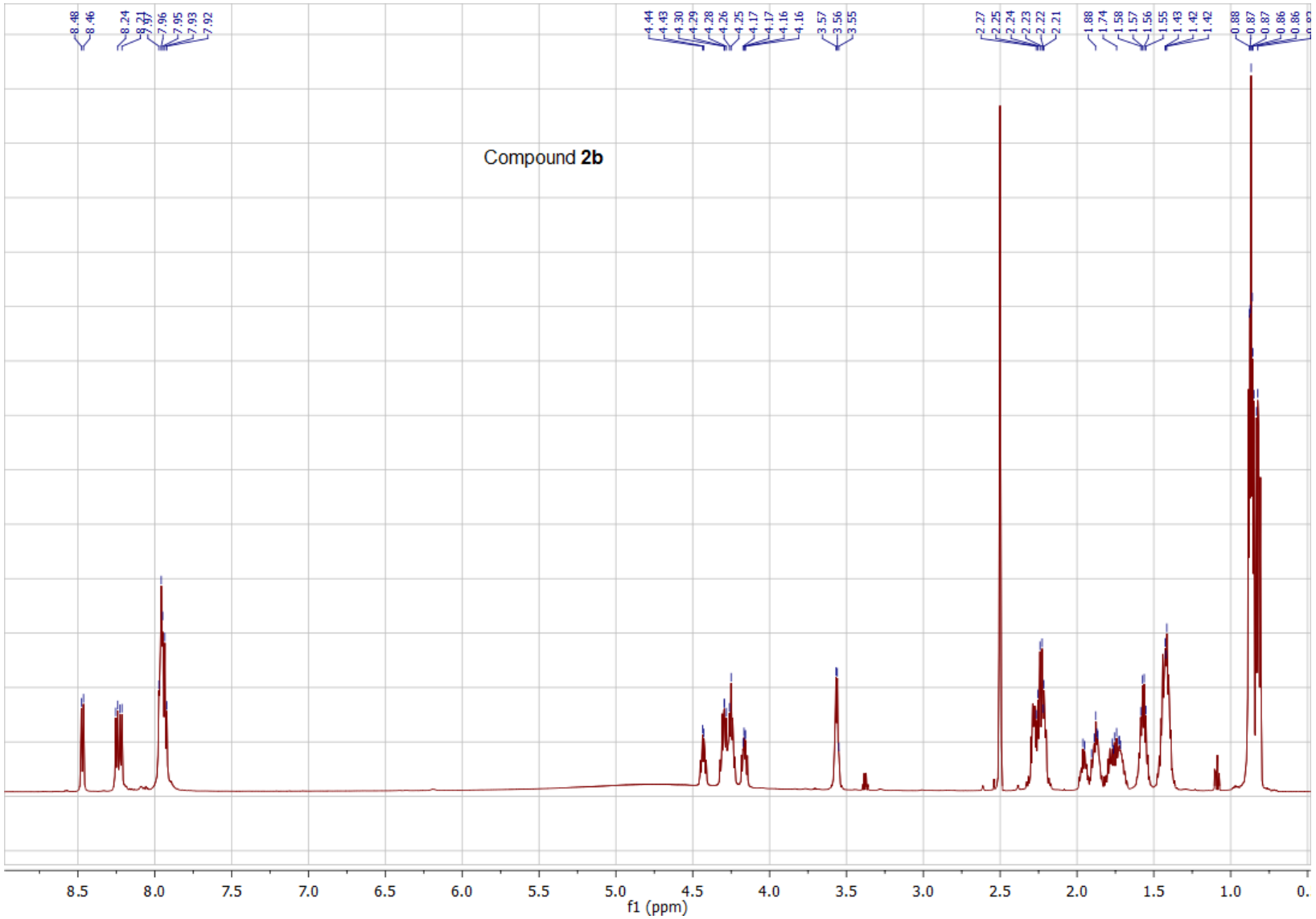


Fig **S12** – ^{1}H NMR spectrum of compound **2b**

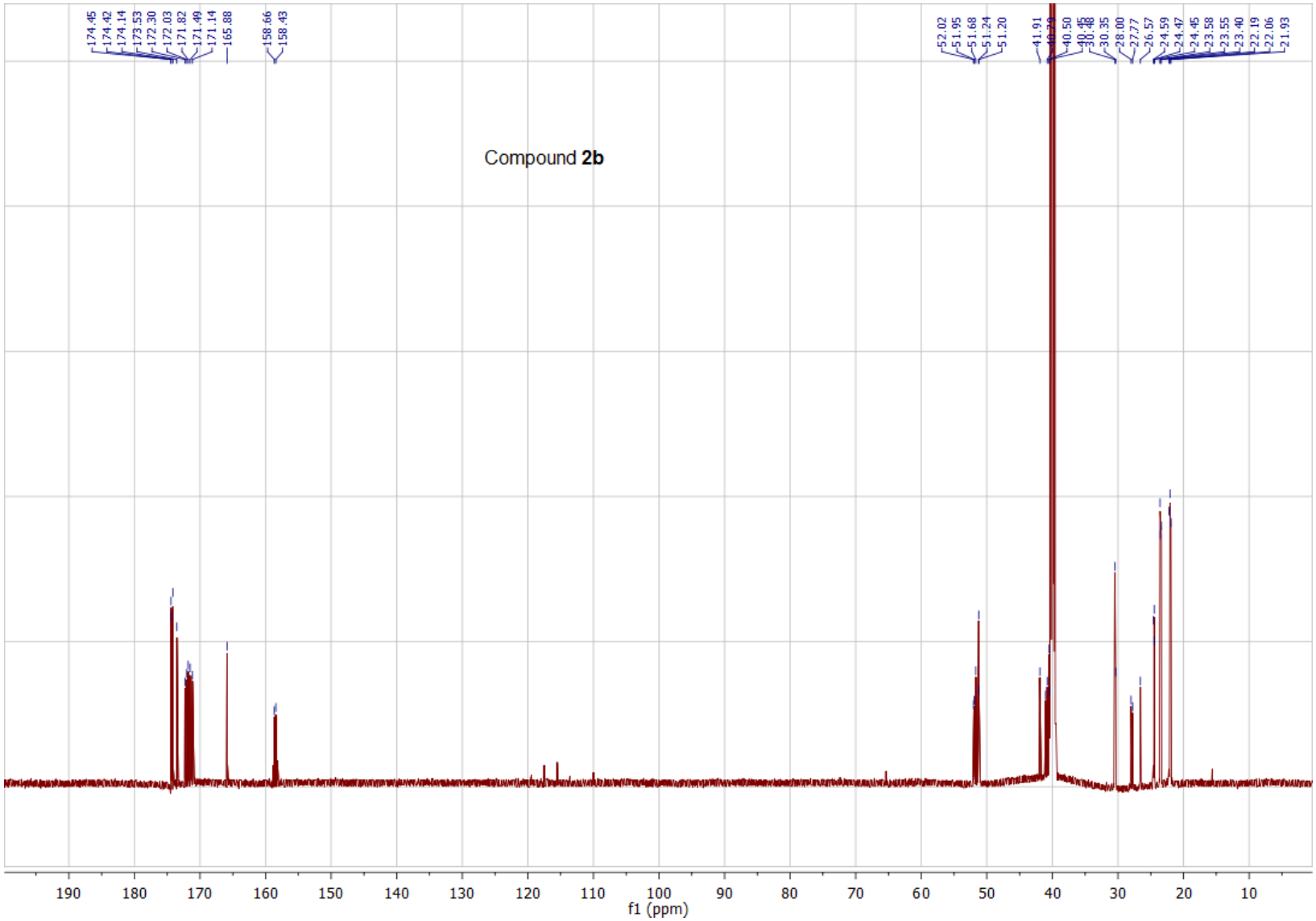


Fig **S13** – ^{13}C NMR spectrum of compound **2b**

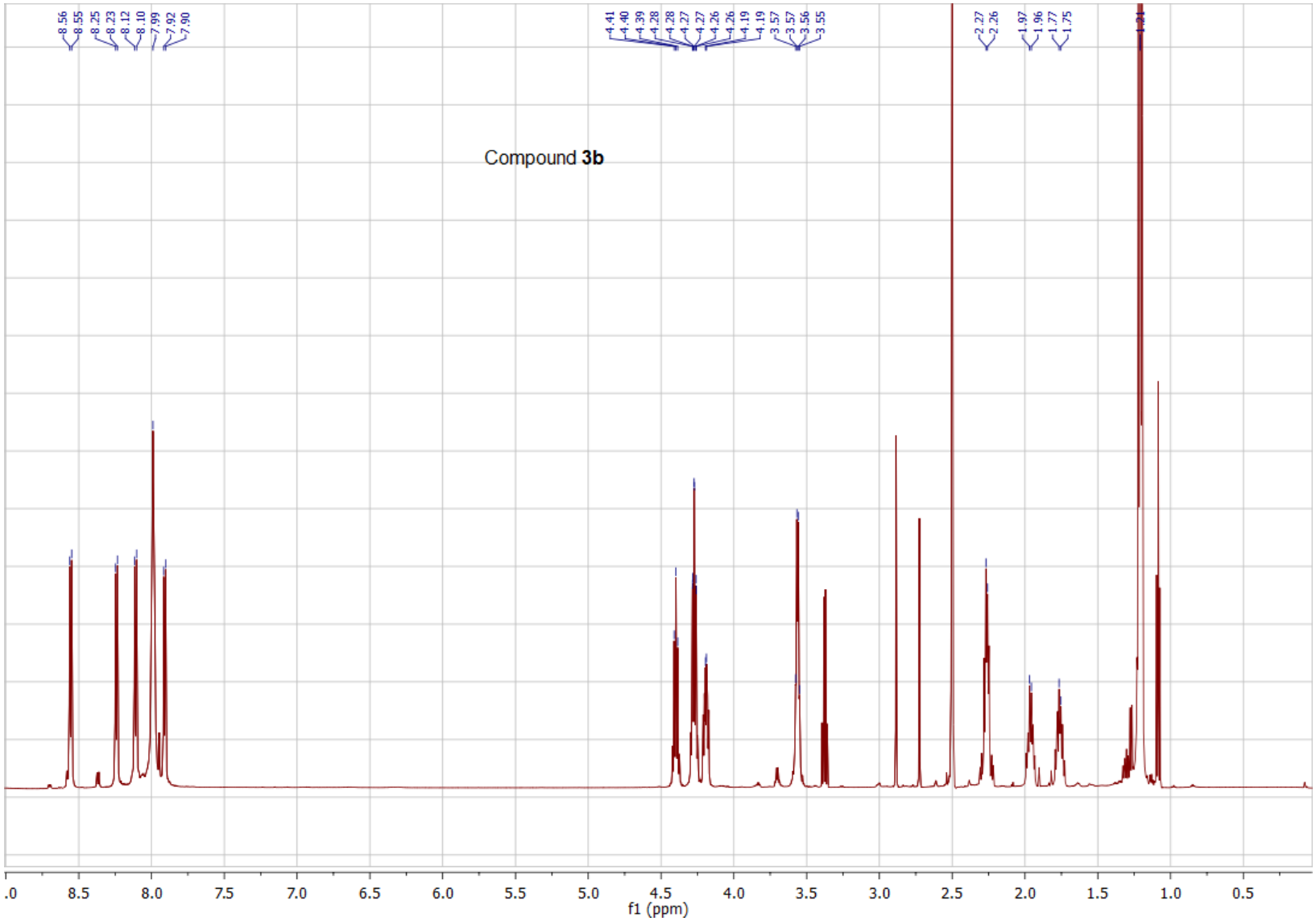


Fig **S14** – ^{1}H NMR spectrum of compound **3b**

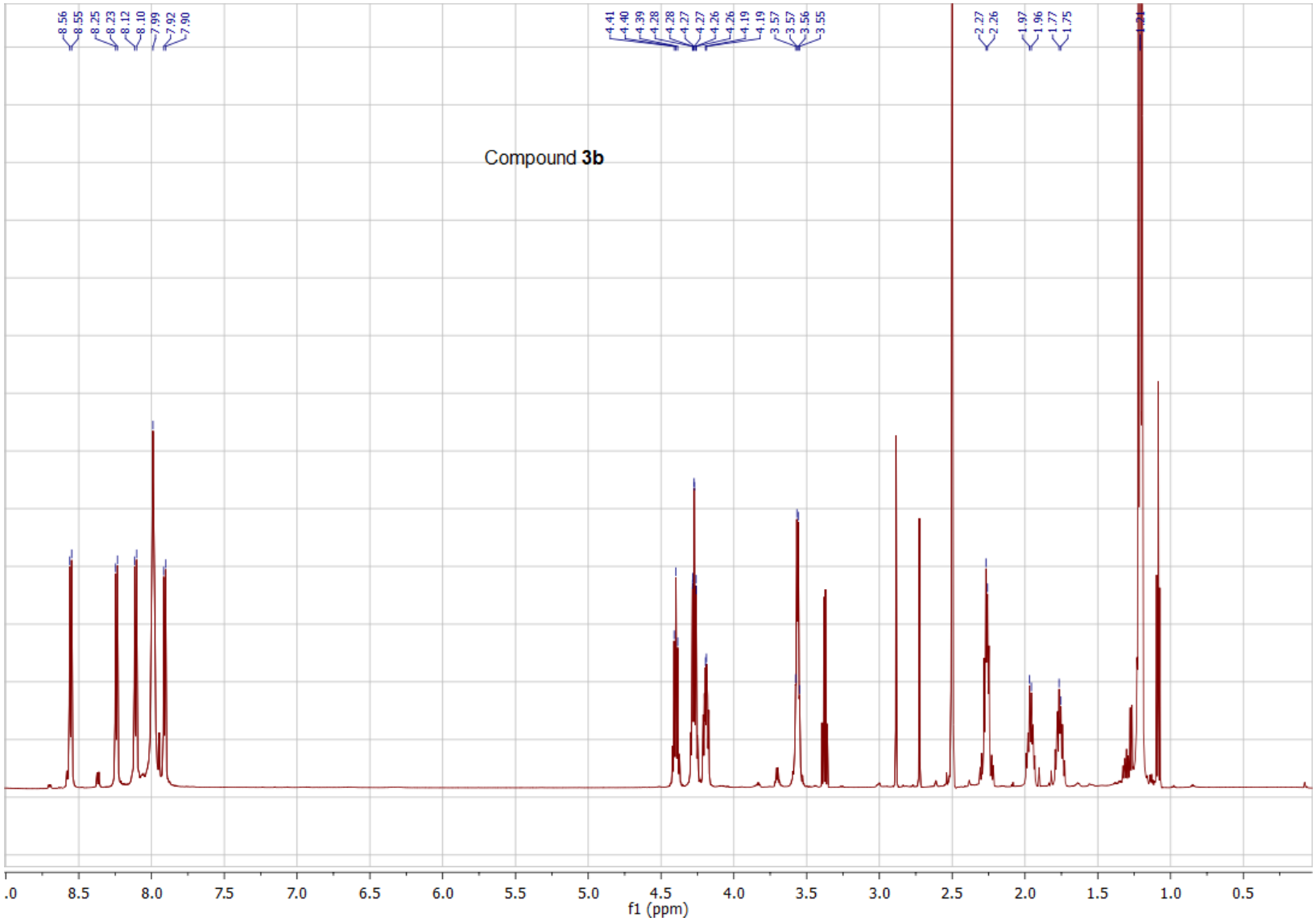


Fig **S15** – ^{13}C NMR spectrum of compound **3b**

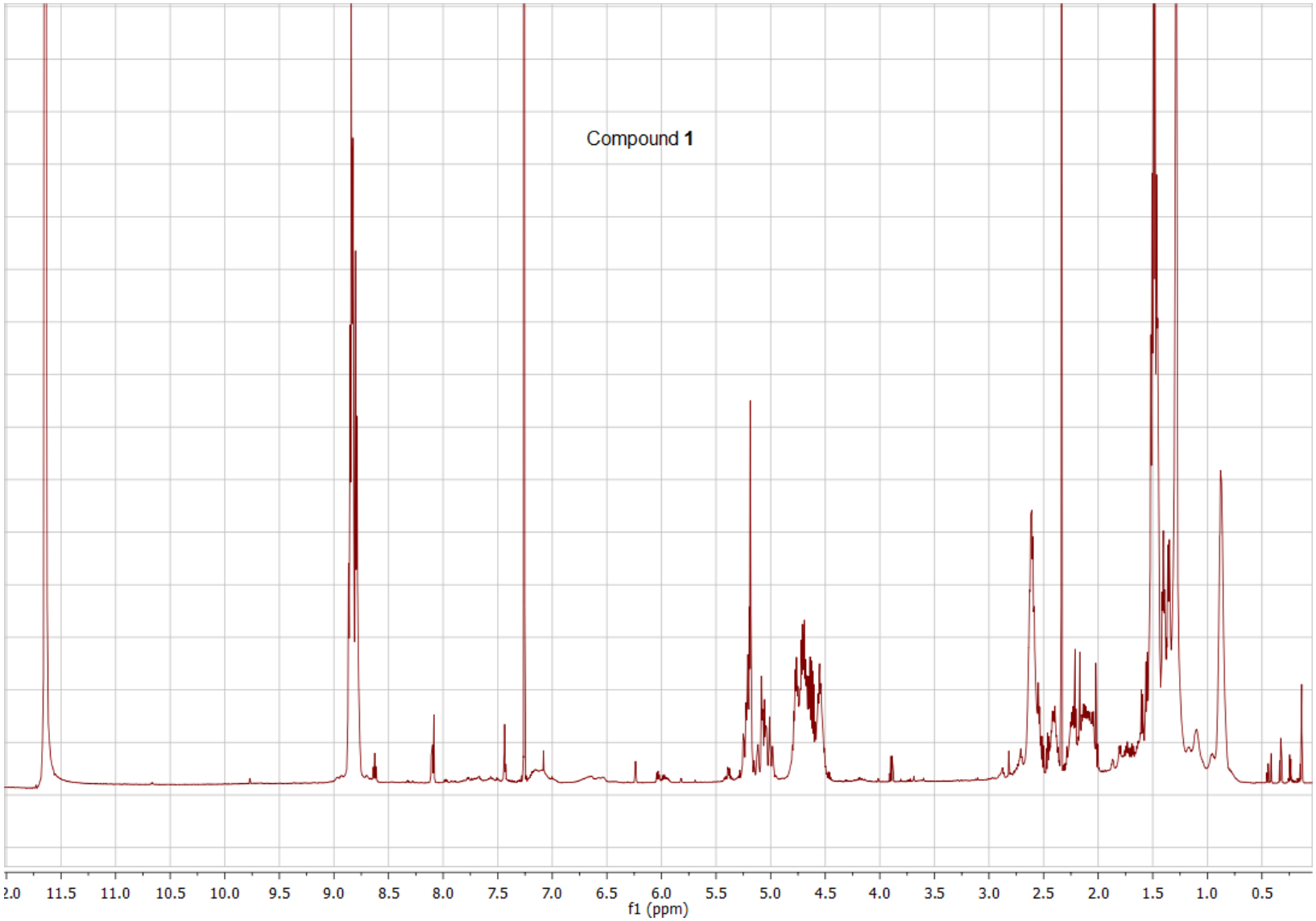


Fig **S16** – ^{1}H NMR spectrum of compound **1**

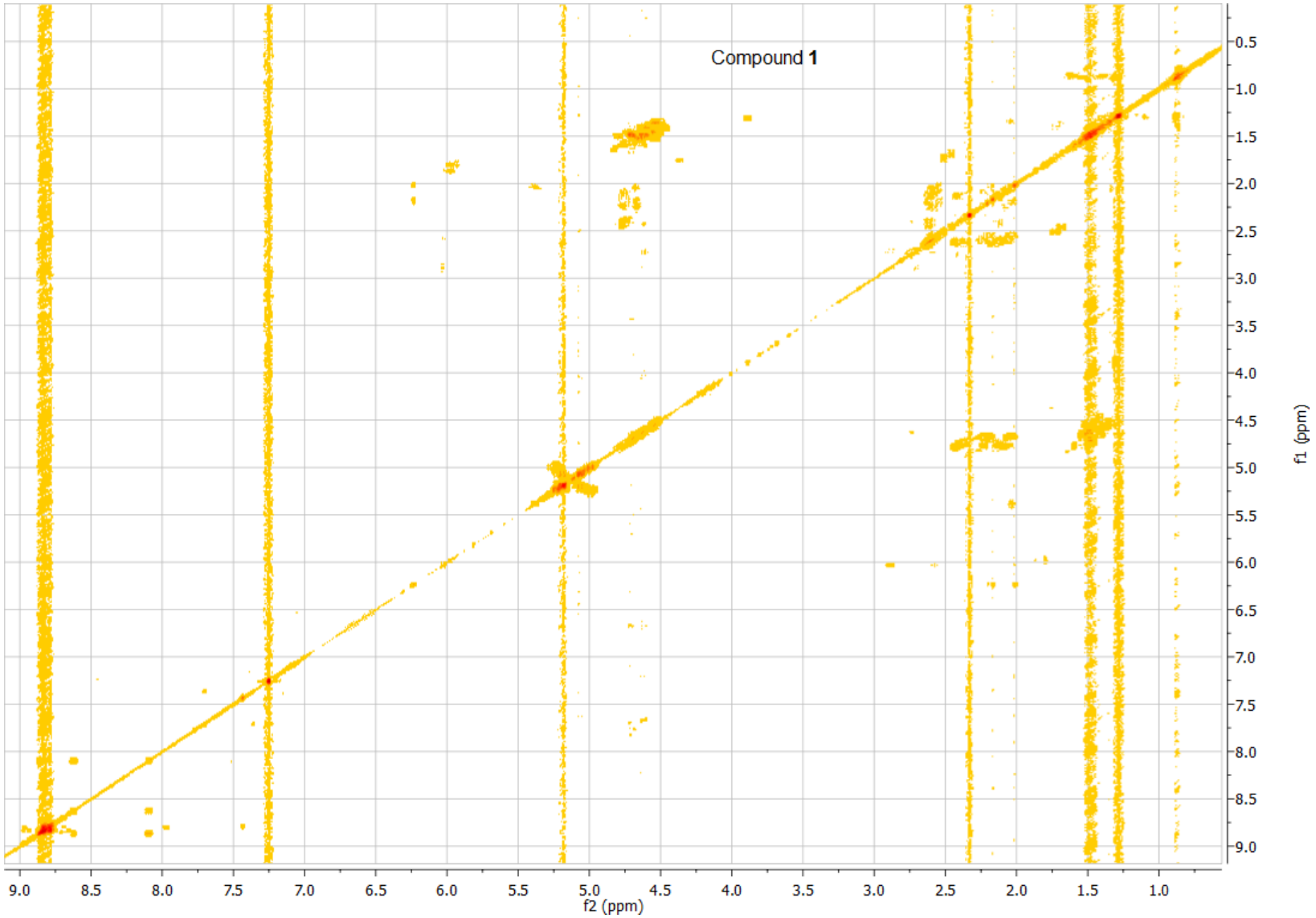


Fig **S17** – COSY NMR spectrum of compound **1**

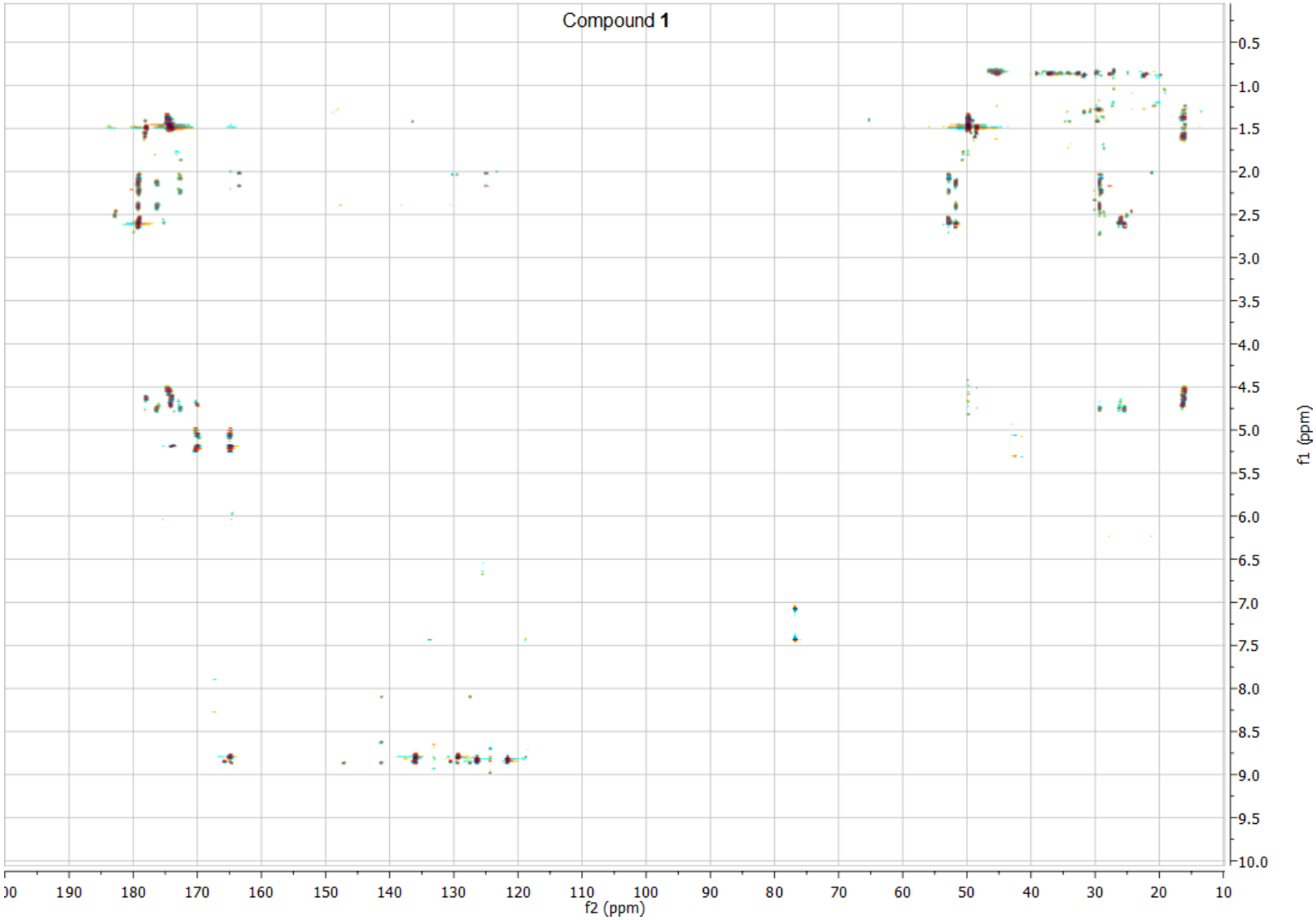


Fig **S18** – HMBC NMR spectrum of compound **1**

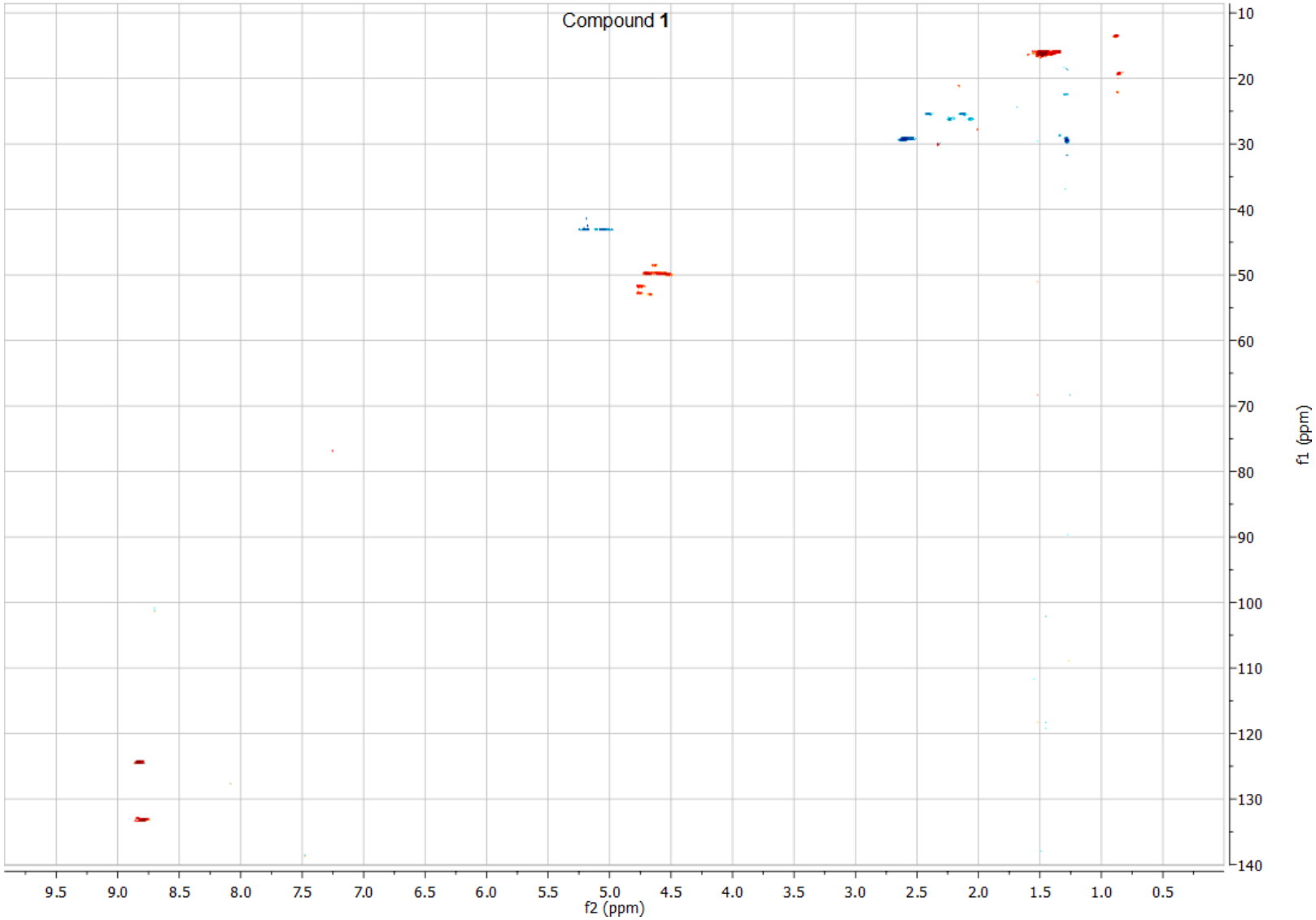


Fig **S19** – HSQC NMR spectrum of compound **1**

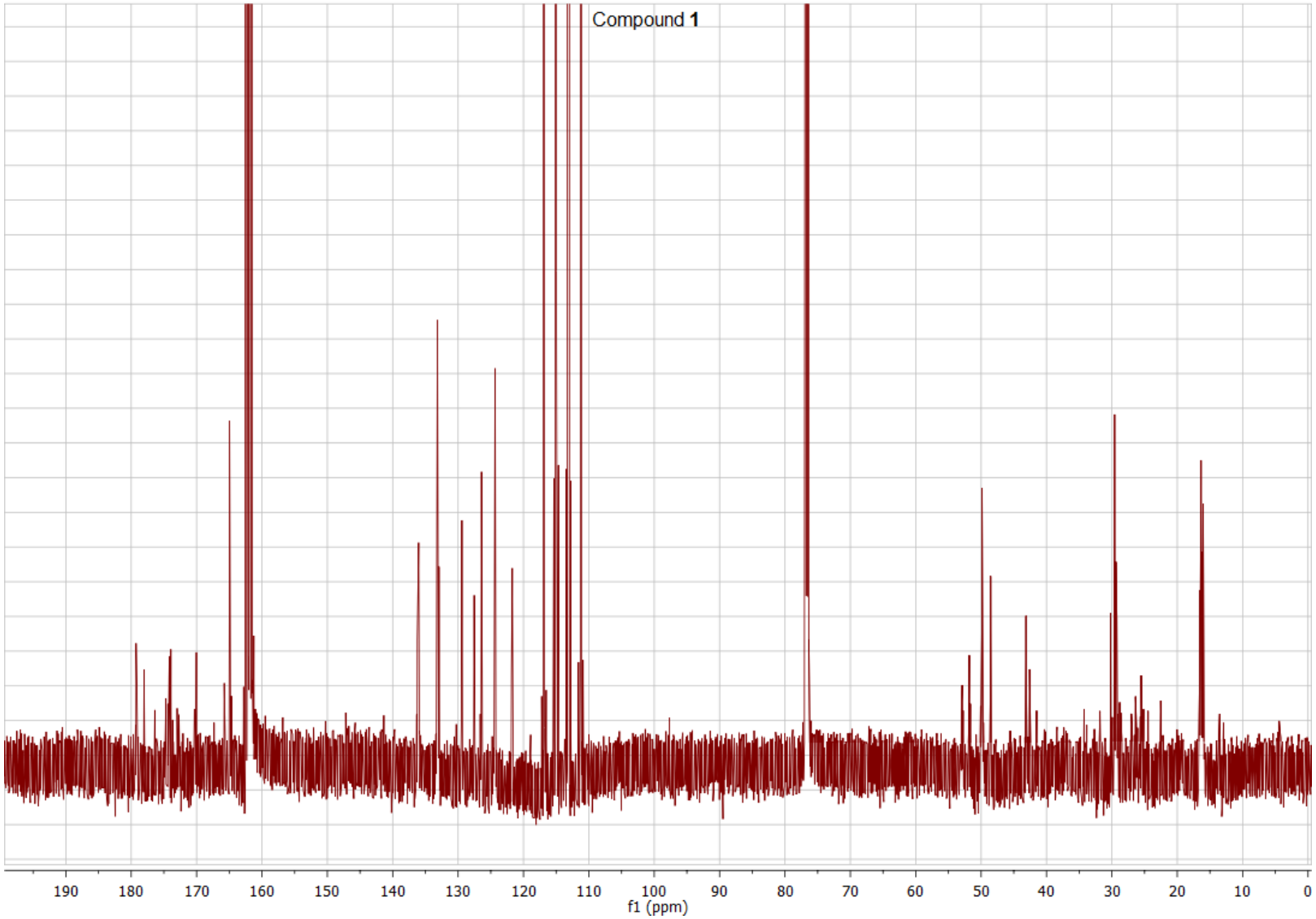


Fig **S20** – ^{13}C NMR spectrum of compound **1**

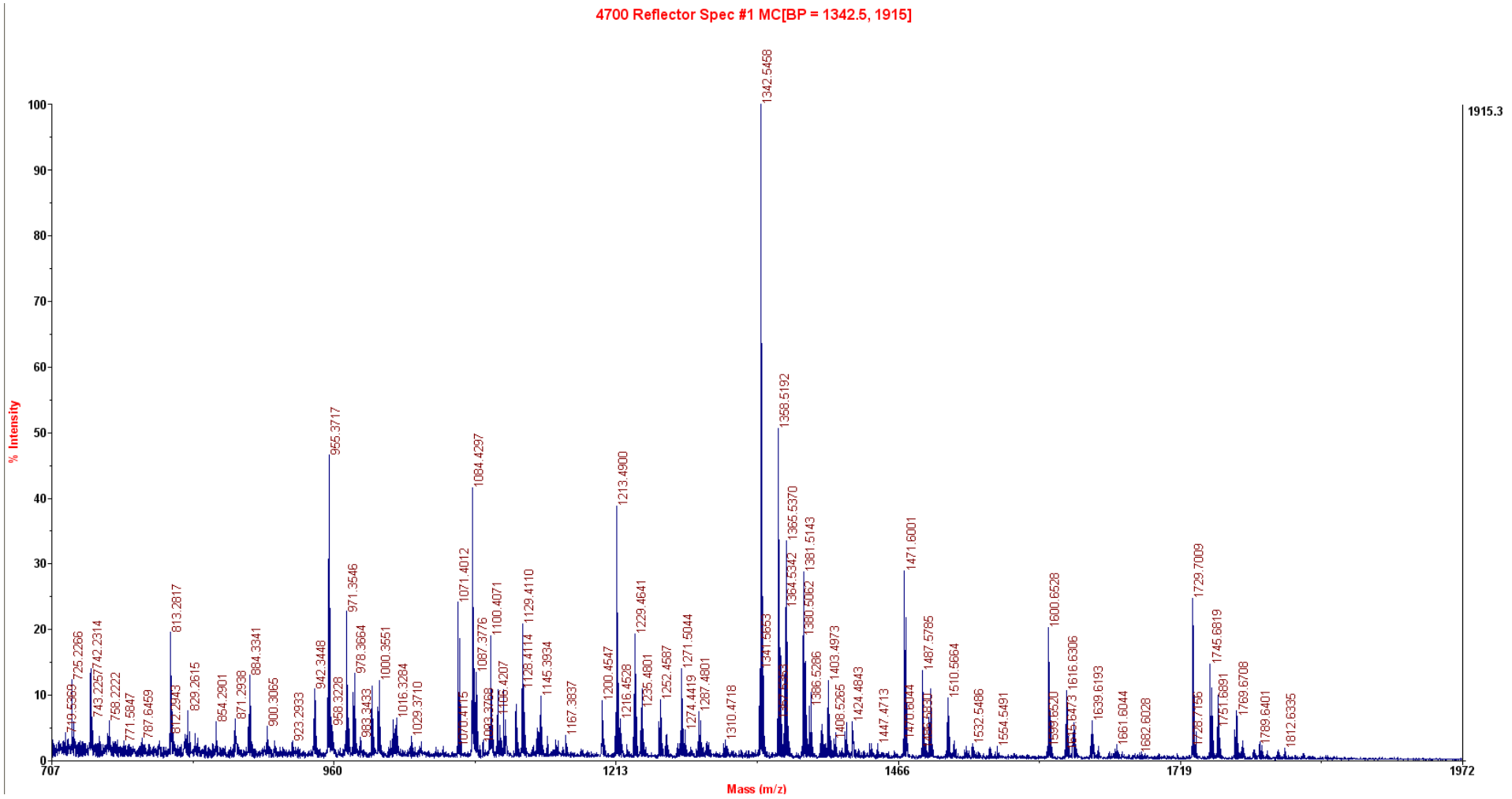


Fig **S21** – MALDI-MS spectrum of compound **1**

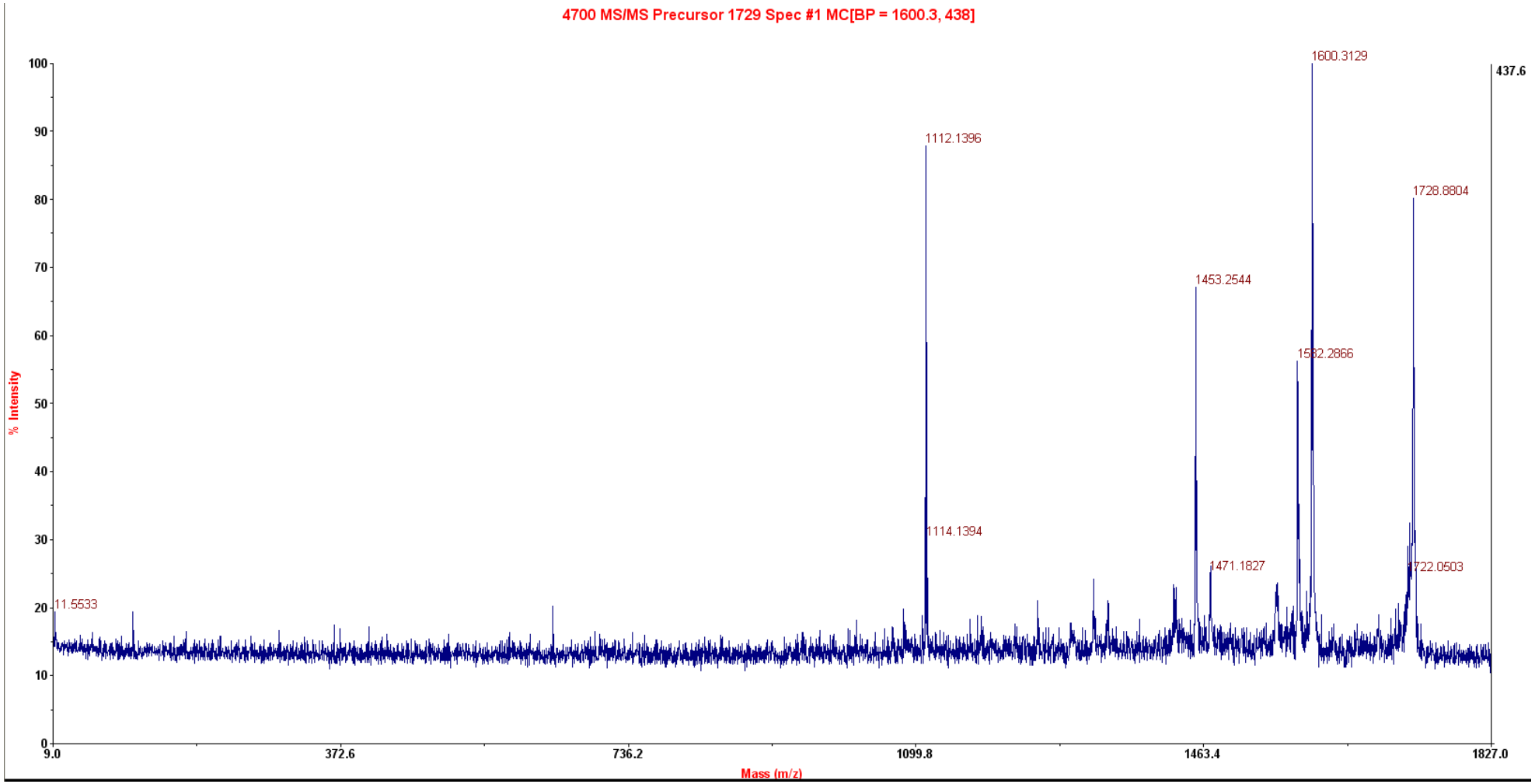


Fig **S22** – MS-MS spectrum of compound **1**

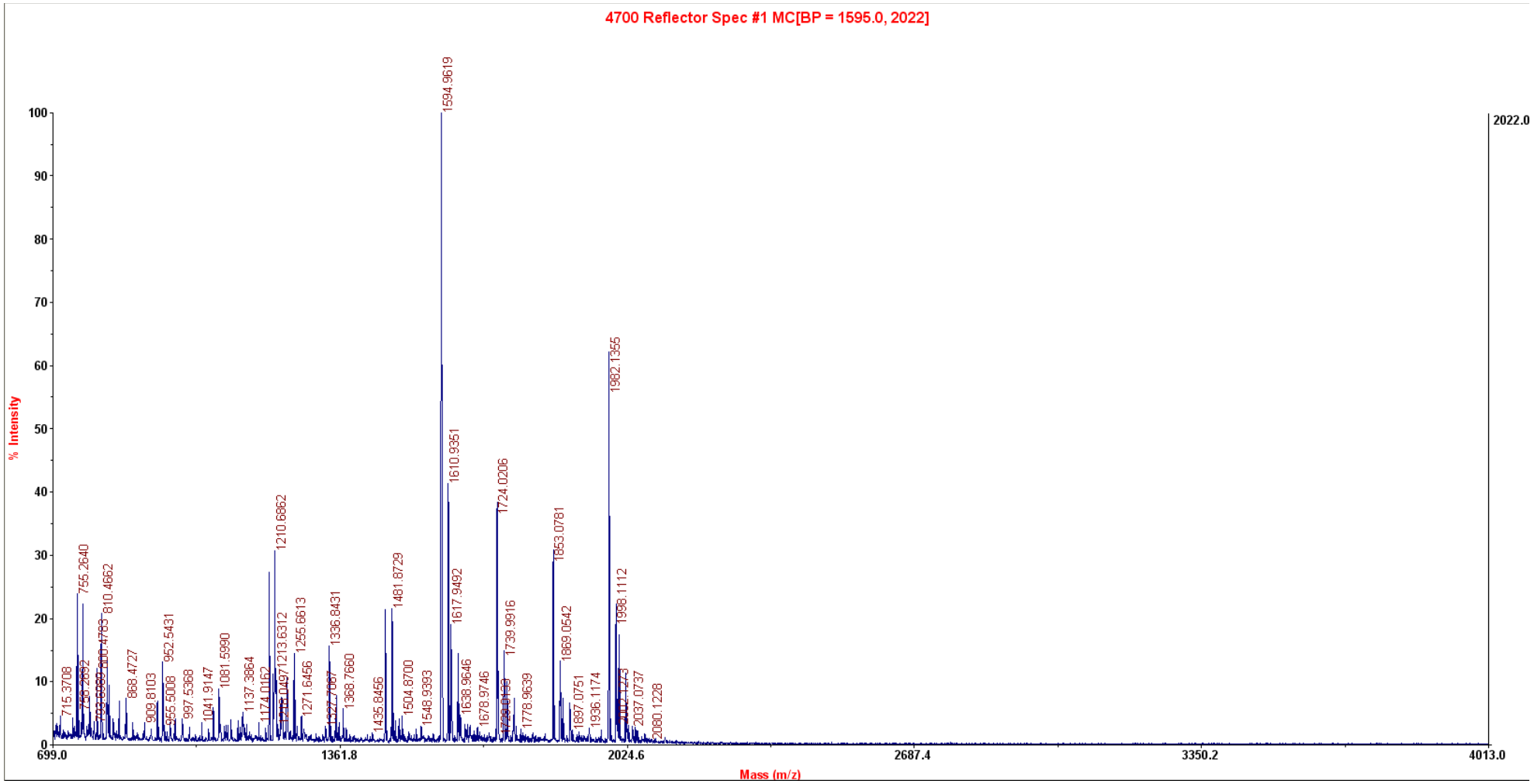


Fig **S23** – MALDI-MS spectrum of compound **2**

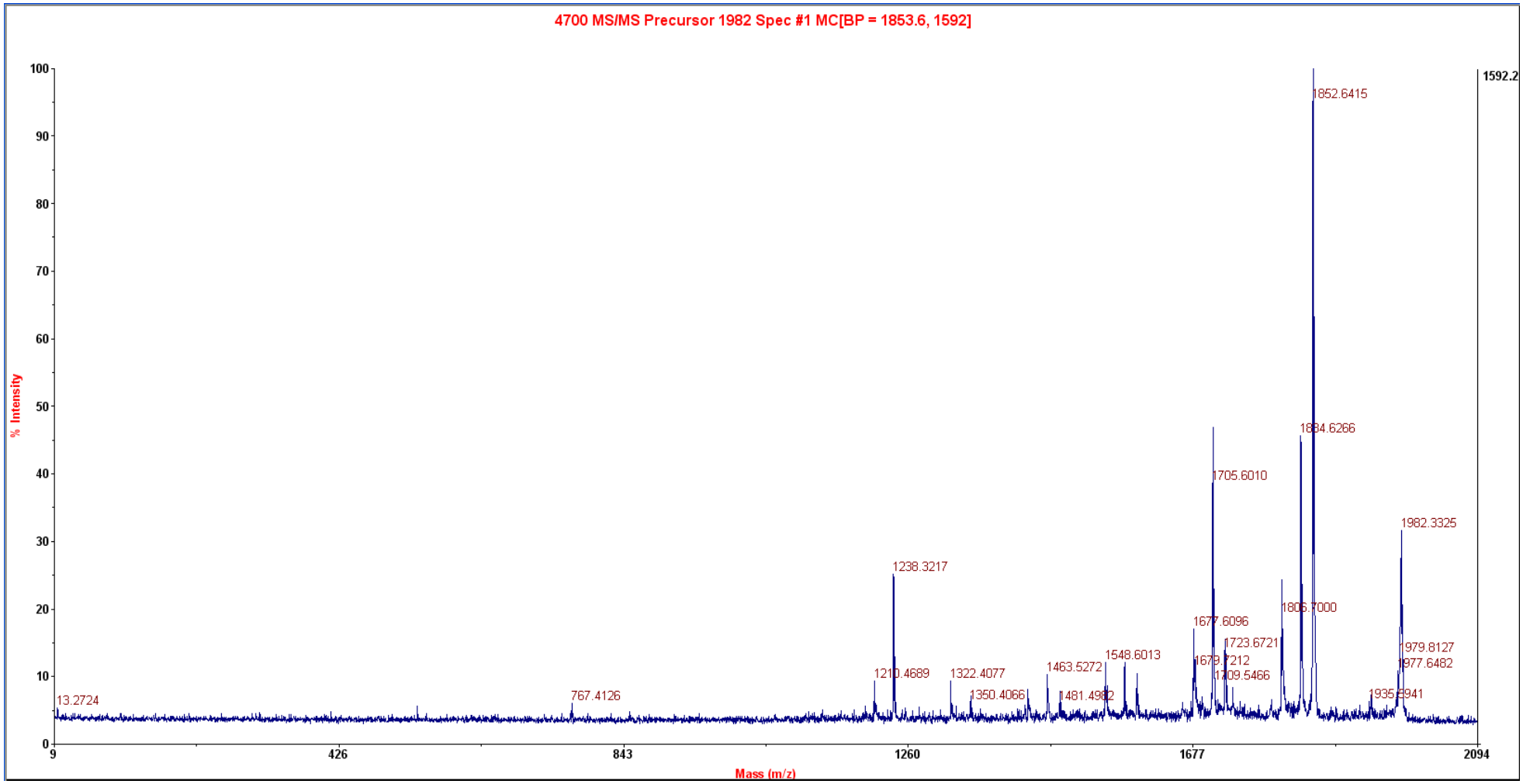


Fig **S24** – MS-MS spectrum of compound **2**

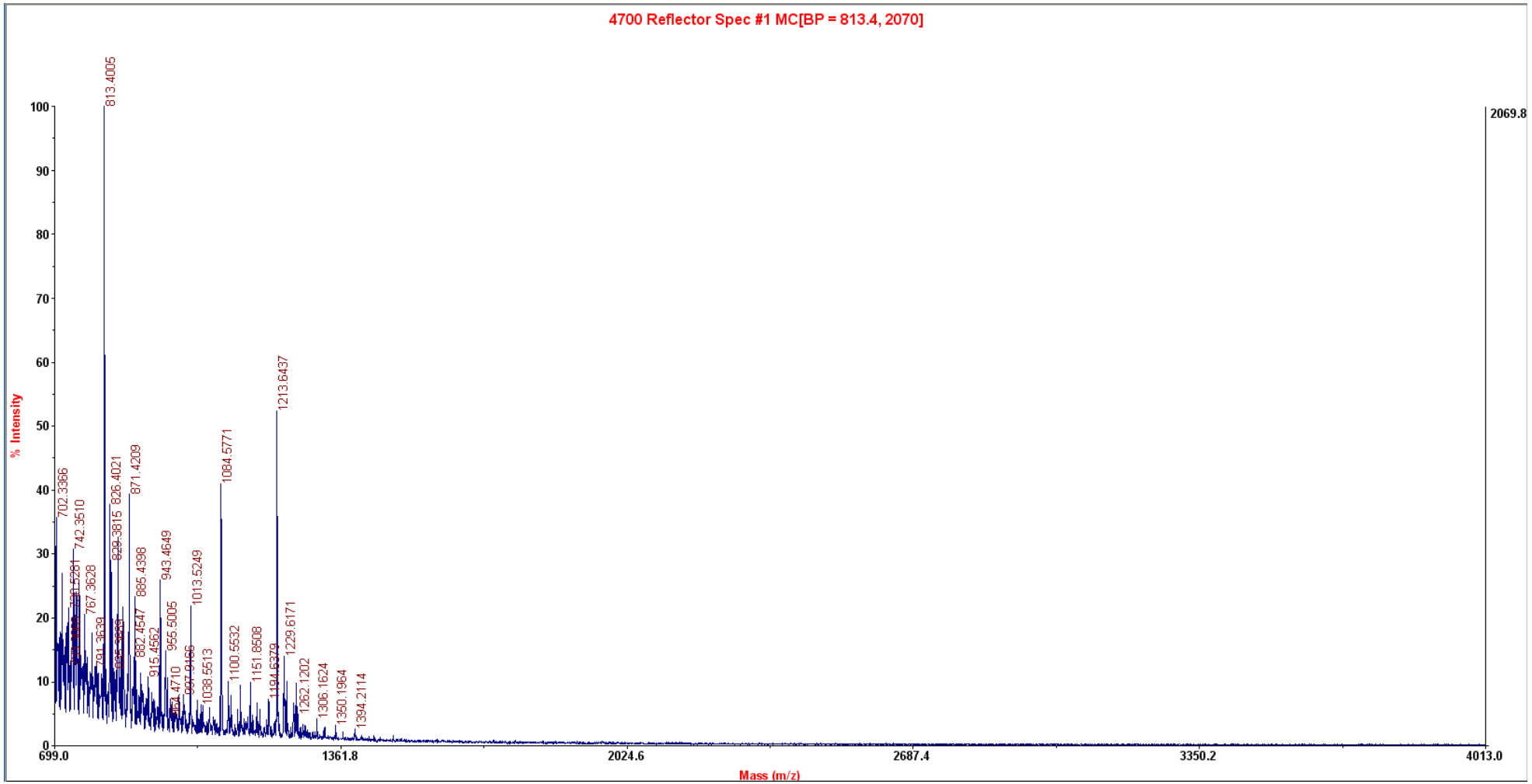


Fig **S25** – MALDI-MS spectrum of compound **3**

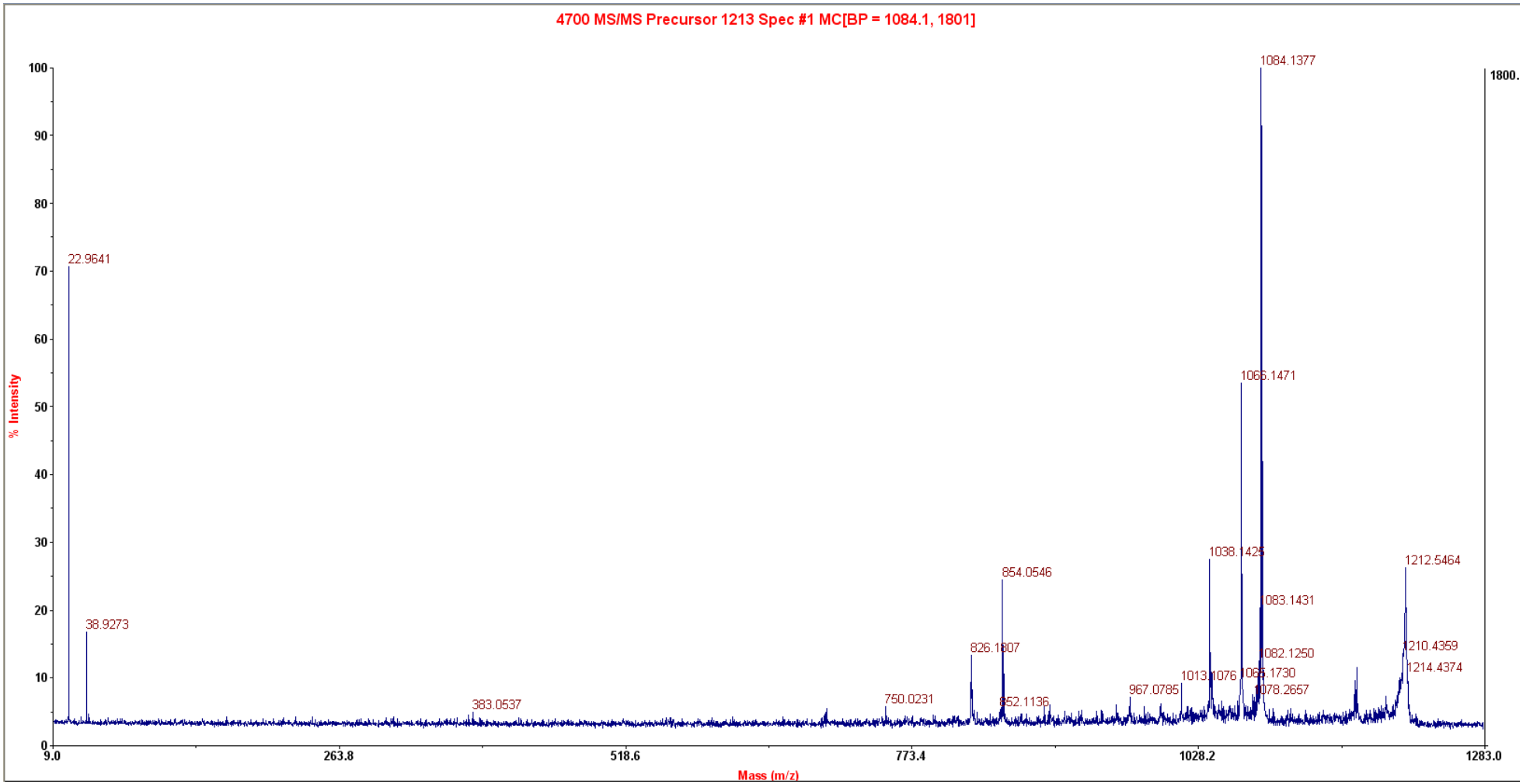


Fig **S26** – MS-MS spectrum of compound **3**

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