An Organometallic Isostere of an Amino Acid.

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Supplementary Information

General Information

Experimental

All reagents and starting materials were purchased from commercial vendors and used without further purification. The 5′-Amino-2,2′-bipyridine-5-carboxylic acid ligand was prepared as previously described. * Deuterated solvents were purchased from Cambridge Isotope Laboratories and used as received. NMR spectra were recorded on 500 MHz, and 300 MHz spectrometers and chemical shifts were given in ppm relative to residual solvent resonances (^{1}H NMR and ^{13}C{^{1}H} NMR spectra). High-resolution mass spectrometry experiments were performed on a Bruker MicroTOF-III instrument. Infrared spectra were collected on Thermo Scientific Nicolet iS5 which was equipped with an iD5 ATR. UV-visible spectra were recorded on a Hitachi 3010 spectrometer. CD spectra were recorded in AVIV Circular Dichroism Spectrophotometer, FV 1000.

X-ray intensity data were measured on a Bruker CCD-based diffractometer with dual Cu/Mo ImuS microfocus optics (Cu Kα radiation, λ = 1.54178 Å, Mo Kα radiation, λ =0.71073 Å). Crystals were mounted on a cryoloop using Paratone oil and placed under a steam of nitrogen at 100 K (Oxford Cryosystems). The detector was placed at 5.00 cm from the crystal. The data were corrected for absorption with the SADABS program. The structures were refined using the Bruker SHELXTL Software Package (Version 6.1) and were solved using direct methods until the final anisotropic full-matrix, least squares refinement of F^2 converged. Table 1 lists the X-ray data collection and structure parameters for all structures in this report.

Separation of Enantiomers

Enantiomeric separation of isostere molecule was done by HPLC purification process performed on a GE Äkta Purifier equipped with a UV900 detector and a 5 μm CHIRALPAK IE (Daicel) column. The solvent system used was 60%/40%/0.1%/0.1% n-Hexane/Ethanol/Trifluoroacetic acid/Diethylamine v/v/v. The detector was set to detect absorption at 254 nm.

Peptide synthesis

Tripeptide was produced using standard Fmoc solid phase peptide synthesis methods (Scheme 2). The completeness of each coupling reaction was monitored by the Kaiser test. Fmoc removal between each cycle was performed by a 30-minute treatment with 20% piperidine in dimethyl formamide (DMF). Each synthesis was initiated by using 100 milligrams of Fmoc clear amide resin. The resin was soaked in DMF for 1–2 h to swell the resin beads. Prior to coupling, Fmoc groups were removed using 20% piperidine in DMF for 30 minutes followed by washing with DMF and dichloromethane several times. Two equivalents of Fmoc-amino acid along with two equivalents of HBTU and HOBt and four equivalents of 4-methylmorpholine were used in this synthesis. The coupling process was performed overnight followed by washing and the Kaiser test was carried out prior to the next cycle. All reactions were performed on a mechanical shaker. In cases where free NH_2 termini remained present on the resin, the coupling process was repeated. In each case the resin was moved to a clean round bottom flask and air-dried. Methylene chloride was added into the flask and the resin was left until swollen with the solvent (1 hour). Methylene chloride was removed under vacuum and the TFA (trifluoroacetic acid) cleavage method was started immediately. The TFA/water/triisoproplysilane (95 : 2.5 : 2.5% v/v/v) cocktail solution (10 mL: 1 mL per 10 mg resin) was added to dry resins. The suspension was gently stirred for three hours. The mixture was filtered, and resins were washed twice with a small amount of fresh TFA

cocktail. The filtrate was kept, and the volume was reduced to be around 1/4 of the original volume. An excess amount of cold ether (>10 times the initial TFA cocktail volume) was added causing the crude yellow peptide to precipitate. The suspension was cooled and centrifuged for 5 minutes at 5000 rpm and the supernatant was discarded. The peptide was lyophilized to give yellow-orange powder.

HPLC Purification

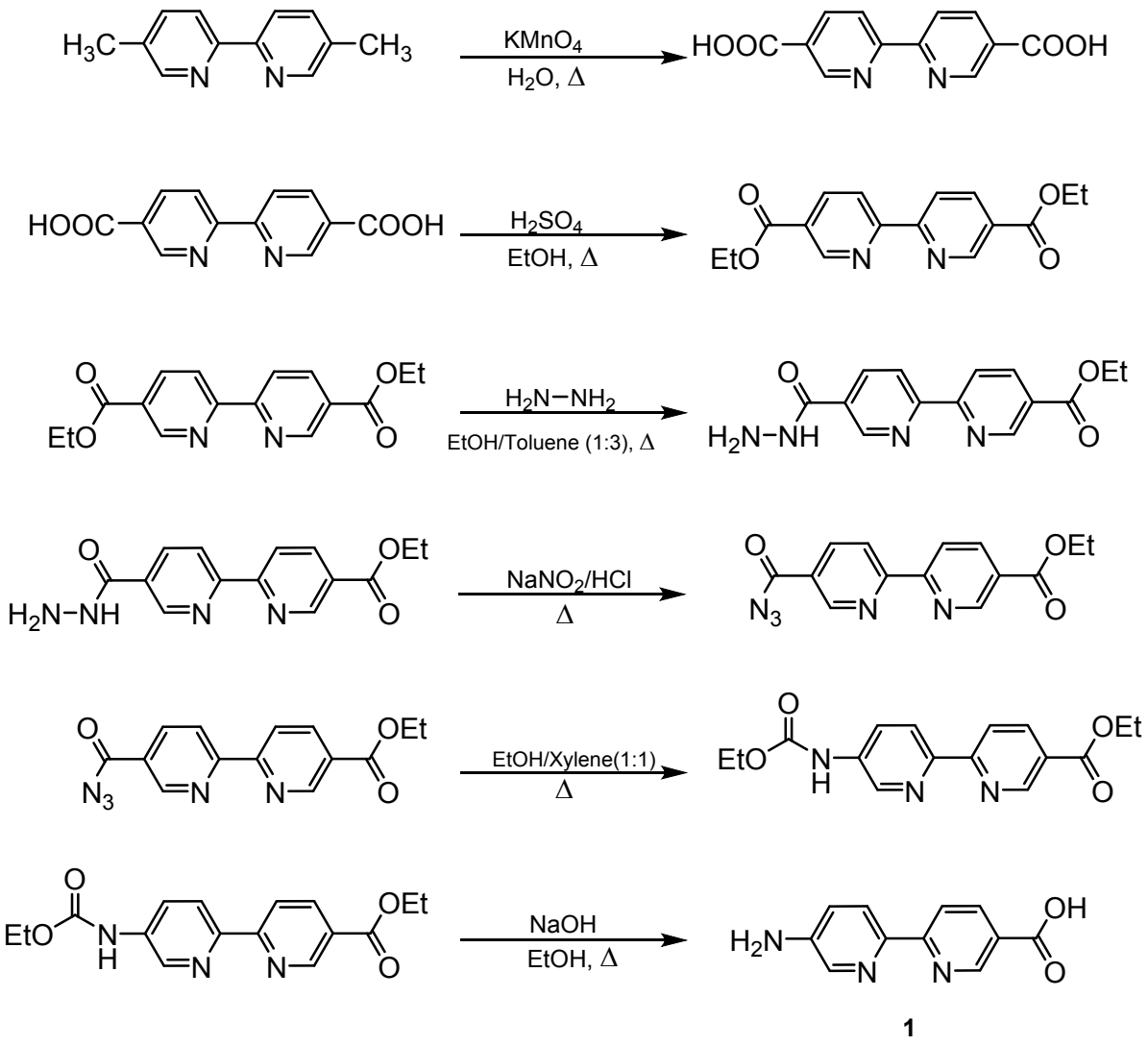
Peptide conjugates were purified by HPLC. The purification process was performed on a GE Äkta Purifier equipped with a UV900 detector using a semipreparative Vydac 218TP C18 reversed-phase column. The solvent system used was 60%/40%/0.1%/0.1% n-Hexane/Ethanol/Trifluoroacetic acid/Triethylamine v/v/v. The detector was set to detect absorption at 254 nm.

Characterization of peptide conjugate

Tripeptide was characterized by m/z values obtained from High-resolution mass spectrometry performed on a Bruker MicroTOF-III instrument. Mass spectra of the purified tripeptide showed the expected m/z value.

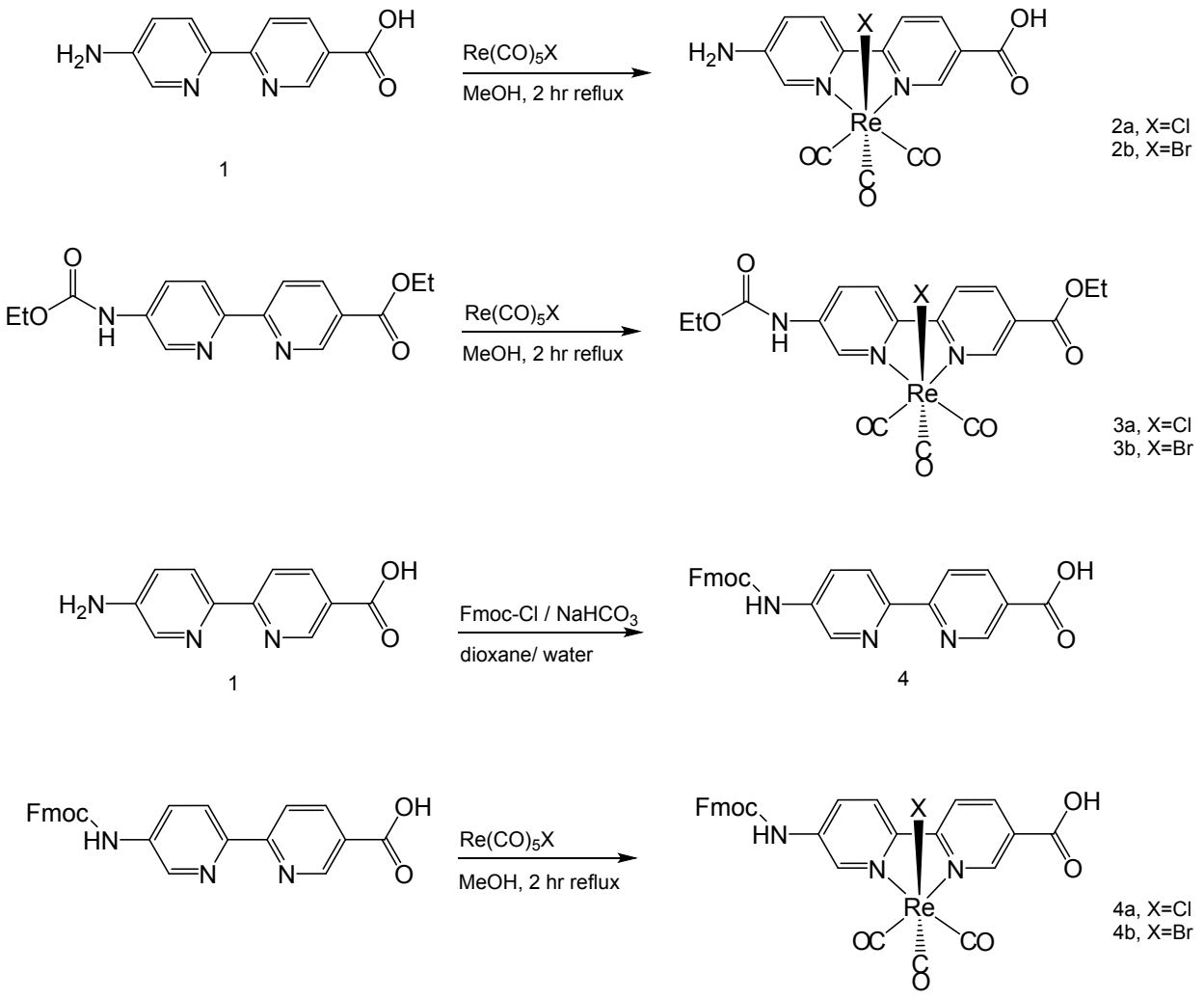
Syntheses:

Ligand synthesis scheme:



1

Synthesis of Re complexes and Fmoc protection:



1: Ligand was synthesized according to a previous method.[1] Yield 1.22 g (62%). ESI MS: calculated $C_{11}H_9N_3O_2$, 216.0773 m/z, found 216.0788 m/z. ^{1}H NMR: δ = 8.95 (d, 1H), 8.31 (dd, 1H), 8.03 (d, 1H), 7.89 (m, 2H), 7.54 (dd, 1H). ^{13}C NMR (500 MHz, DMSO-d_6): δ = 165.66, 149.76, 148.27, 139.22, 126.49, 125.22, 120.14.

2a: 50mg of $Re(CO)_5Cl$ (0.138 mmol) dissolved in 10mL of hot methanol and 29 mg of compound 1 (0.138mmol), were mixed together and allowed to reflux for 2 hrs. After cooling to room temperature, it was filtered, washed with cold methanol, and dried in the vacuum oven. Yield: 47 mg (66%). IR (CO stretch, 1891.23, 1928.39, 2019.60 cm^{-1}): ESI MS calculated $[C_{14}H_8ClN_3Na_2O_5Re]^+$ 565.9500 m/z, found 565.9469 m/z. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.16 (d, 1H), 8.40 (m, 4H), 7.28 (d, 1H), 6.92 (s, 2H). ^{13}C NMR (500 MHz, DMSO-d_6): δ =

198.12, 190.57, 164.76, 159.60, 153.05, 149.63, 140.85, 140.10, 139.09, 127.25, 121.77. Crystals suitable for X-ray diffraction were grown in DMF.

2b. 50mg of $Re(CO)_5Br$ (0.123 mmol) dissolved in 10mL of hot methanol and 26 mg of compound 1 (0.138mmol), were mixed together and allowed to reflux for 2 hrs. After cooling to room temperature, it was filtered, washed with cold methanol, and dried in the vacuum oven. Yield: 45 mg (65%). IR (CO stretch, 1891.84, 1928.72, 2019.38 cm^{-1}): ESI MS calculated $[C_{14}H_8N_3NaO_5Re]^+$ 507.9914 m/z, found 507.9939 m/z. ^1H NMR (300 MHz, DMSO-d_6): δ = 9.17 (s 1H), 8.39 (m 4H), 7.28 (d, 2H), 6.88 (s, 2H). ^{13}C NMR (500 MHz, DMSO-d_6): δ = 198.30, 190.57, 164.76, 159.60, 152.88,149.63, 140.91,140.10, 139.09, 127.25, 121.77.

3a. 50mg of $Re(CO)_5Cl$ (0.123 mmol) dissolved in 10mL of hot toluene and 43 mg of compound 3 (0.137 mmol), were mixed together and allowed to reflux for 2 hrs. After cooling to room temperature, it was filtered, washed with cold toluene, and dried in the vacuum oven. Yield: 67 mg (76%). IR (CO stretch, 1888.99, 1956.33, 2019.75 cm^{-1}): ESI MS calculated $[C_{19}H_{17}ClN_3O_7ReNa]^+$ 644.020 m/z, found 644.0232 m/z. ^1H NMR (300 MHz, DMSO-d_6): δ = 10.68(s, 1H), 9.32 (d, 1H), 8.61 (m, 3H), 8.26 (m, 1H), 4.41 (q, 2H), 4.19 (q, 2H), 1.37 (t, 3H), 1.25 (t, 3H). ^{13}C NMR (500 MHz, DMSO-d_6): δ = 196.72, 193.04, 188.78, 162.41, 158.05, 153.05, 147.07, 141.99, 139.99, 139.83, 127.51, 126.69, 125.91, 123.12, 61.90, 61.27, 14.07, 13.76. Crystals suitable for X-ray diffraction were grown in DMF

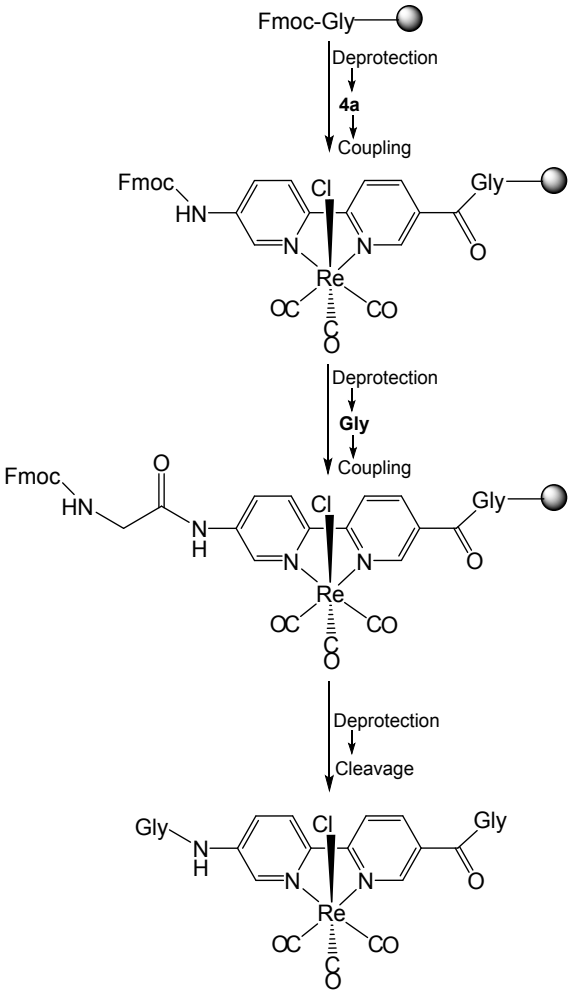
3b. 50mg of $Re(CO)_5Br$ (0.123 mmol) dissolved in 10mL of hot toluene and 26 mg of compound 1 (0.138mmol), were mixed together and allowed to reflux for 2 hrs. After cooling to room temperature, it was filtered, washed with cold toluene, and dried in the vacuum oven. Yield: 71 mg (86%). IR (CO stretch, 1885.94, 1924.32, 2025.19 cm^{-1}): ESI MS calculated $[C_{19}H_{17}BrN_3O_7ReNa]^+$ 687.9680 m/z, found 687.9616 m/z. ^1H NMR (300 MHz, DMSO-d_6): δ = 10.68 (s 1H), 9.22 (d, 1H), 8.68 (m, 3H), 8.27 (m 1H), 4.40 (q, 2H), 4.18 (q, 2H), 1.36 (t, 3H), 1.25 (t, 3H). ^{13}C NMR (500 MHz, DMSO-d_6): δ = 197.64, 197.41, 198.46, 163.09, 158.74, 153.73, 153.04, 147.75, 142.67, 140.67, 128.19, 127.37, 126.59, 123.80, 62.58, 61.95, 14.75, 14.44.

4. Fmoc protected ligand was synthesized according to a previous method.[2] Yield 201 mg (56 %). ^1H NMR (300 MHz, DMSO-d_6): δ = 7.54 (dd, 1H), 7.89 (m, 4H), 8.31 (dd, 1H), 8.95 (s 1H, NH). ^{13}C NMR (300 MHz, DMSO-d_6) δ = 165.84, 157.58, 151.38, 150.01, 148.91, 145.66, 141.10, 139.79, 138.95, 134.08, 127.55, 125.64, 121.61, 120.75, 120.28, 64.21, 50.54.

4a. 50mg of $Re(CO)_5Cl$ (0.138 mmol) dissolved in 10mL of hot methanol and 60 mg of compound 3 (0.138mmol), were mixed together and allowed to reflux for 2 hrs. After cooling to room temperature, it was filtered, washed with cold methanol, and dried in the vacuum oven. Yield: 78 mg (79%). IR (CO stretch, 1888.33, 2017.75 cm^{-1}): ESI MS calculated $[C_{29}H_{18}ClN_3Na_2O_7Re]^+$ 788.0181 m/z, found 788.0157. ^1H NMR (300 MHz, DMSO-d_6): δ = 10.67(s 1H), 9.24 (d, 2H), 8.40 (m 5H), 7.74 (dd 3H), 7.33(m 3H), 4.62 (d 2H), 4.34 (t 1H). ^{13}C NMR (500 MHz, DMSO-d_6): δ = 198.58, 198.30, 190.58, 164.72, 159.50, 157.49, 152.88, 149.68, 145.65, 141.12, 140.07, 138.98, 130.75, 127.56, 125.64, 121.45, 120.27, 64.21, 50.54. Crystals suitable for X-ray diffraction were grown in DMF.

4b. 50mg of $Re(CO)_5Br$ (0.123 mmol) dissolved in 10mL of hot methanol and 29 mg of compound 3 (0.123 mmol), were mixed together and allowed to reflux for 2 hrs. After cooling to room temperature, it was filtered, washed with cold methanol, and dried in the vacuum oven. Yield: 68 mg (70 %). IR (CO stretch, 1888.81, 2019.73 cm^{-1}): ESI MS calculated $[C_{29}H_{19}BrN_3O_7Re]^-$ 786.9964 m/z, found 785.9980. ^1H NMR (300 MHz, DMSO-d_6): δ = 10.68 (s 1H), 9.24 (d 2H), 8.55 (m 3H), 7.90 (d 2H), 7.77 (d 2H), 7.36 (m 4H), 4.62 (d 2H), 4.34 (t 1H). ^{13}C NMR (500 MHz, DMSO-d_6): δ =198.30, 198.02, 190.30, 164.44, 159.22, 157.21, 152.60, 149.40, 145.37, 140.84, 139.79, 138.70, 127.28, 125.36, 121.17, 119.99, 63.93, 50.26.

5. Peptide Gly-Iso-Gly: Tripeptide was synthesized using solid phase peptide synthesis method.[3,4] After lyophilization and HPLC purification 4 mg of peptide was obtained which was run through a Chiral HPLC for enantiomeric separation. IR (CO stretch, 1888.76, 2016.17 cm^{-1}). ESI MS calculated $[C_{18}H_{15}N_5O_7Re]^+$ 600.0520 m/z, found 600.0512 m/z. Peptide synthesis scheme is provided below:



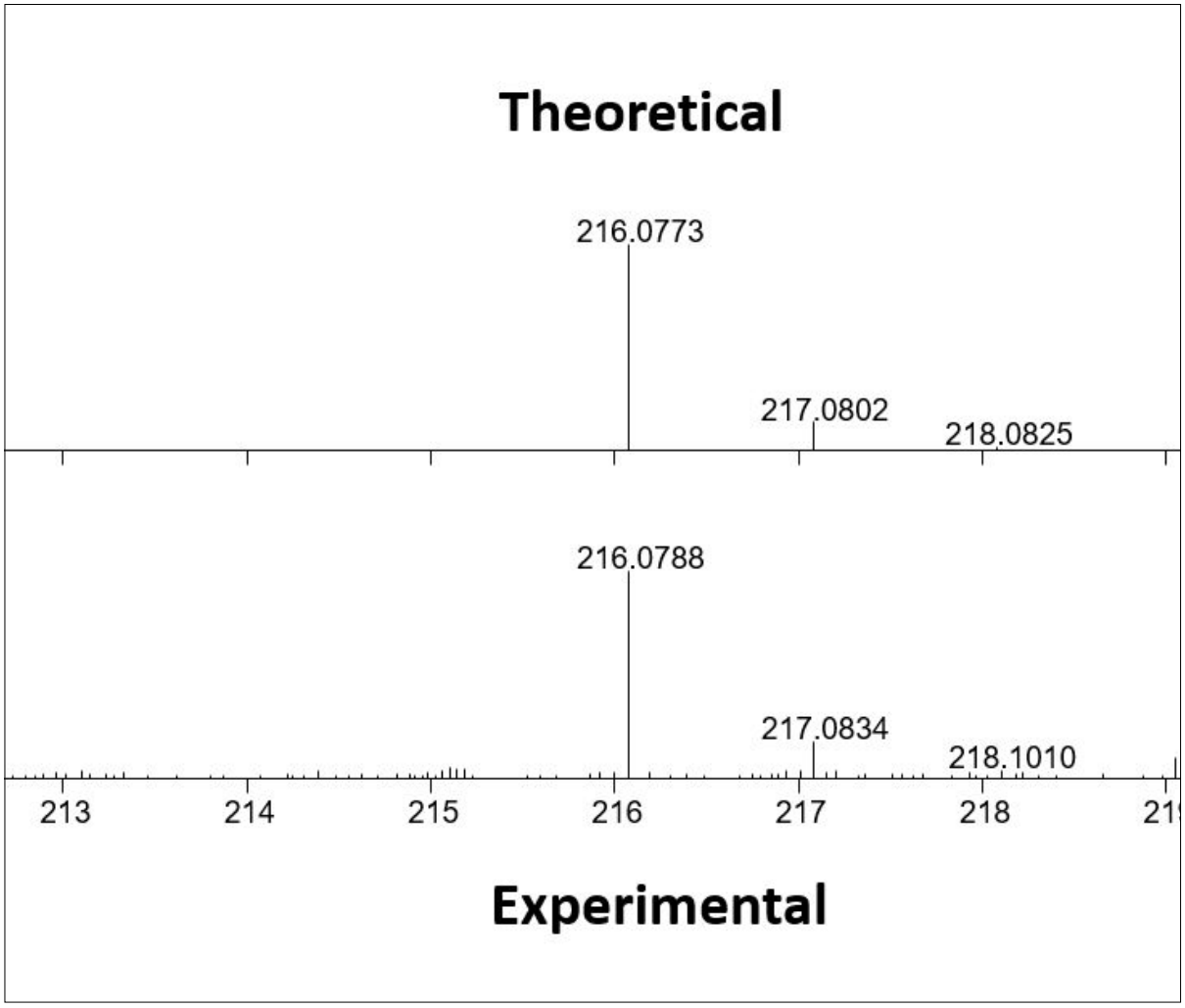


Figure S1: ESI mass spectra of **1.** Top: calculated spectrum. Bottom: experimental spectrum.

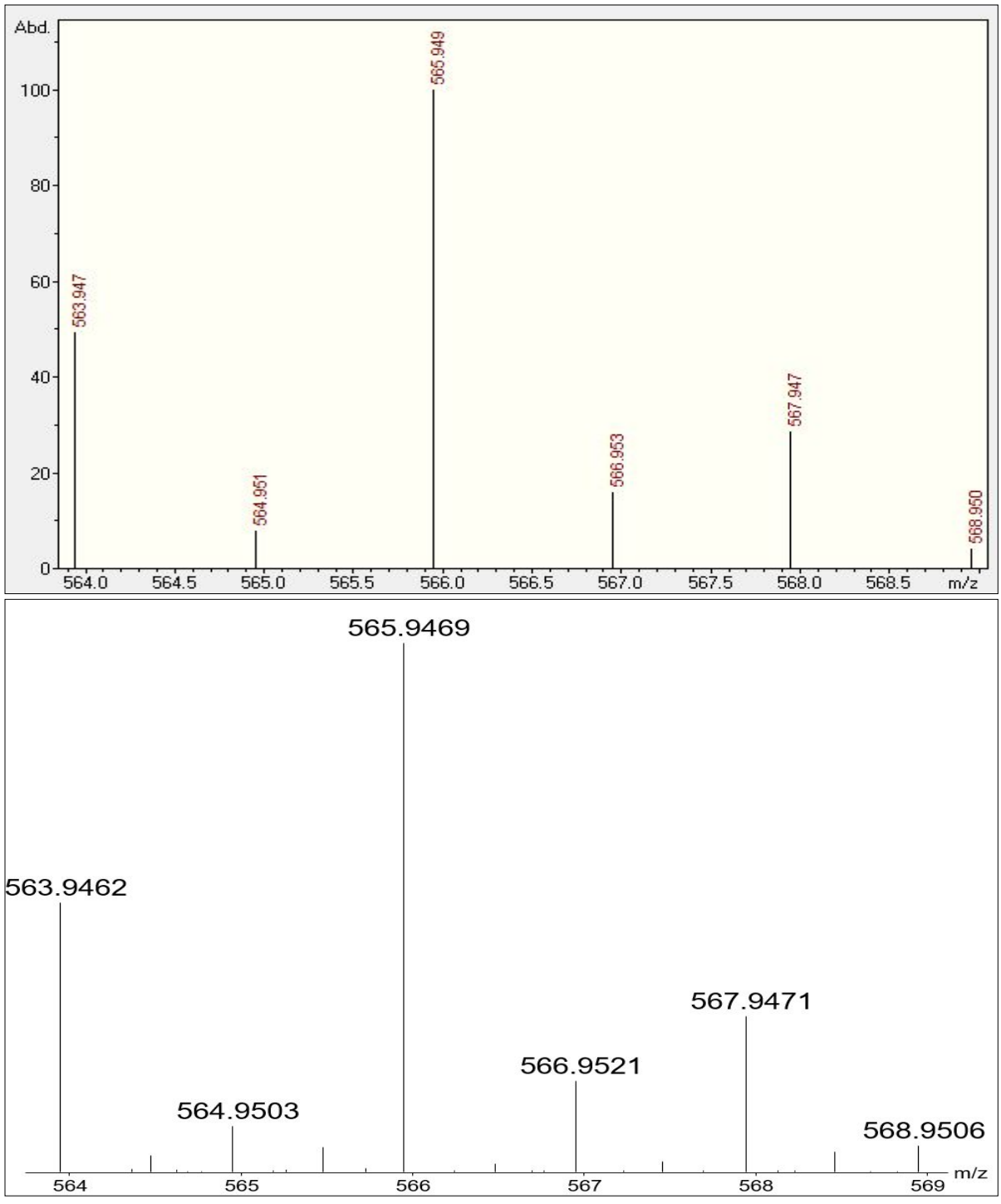


Figure S2: High-resolution ESI mass spectra of **2a.** Top: calculated spectrum. Bottom: experimental spectrum

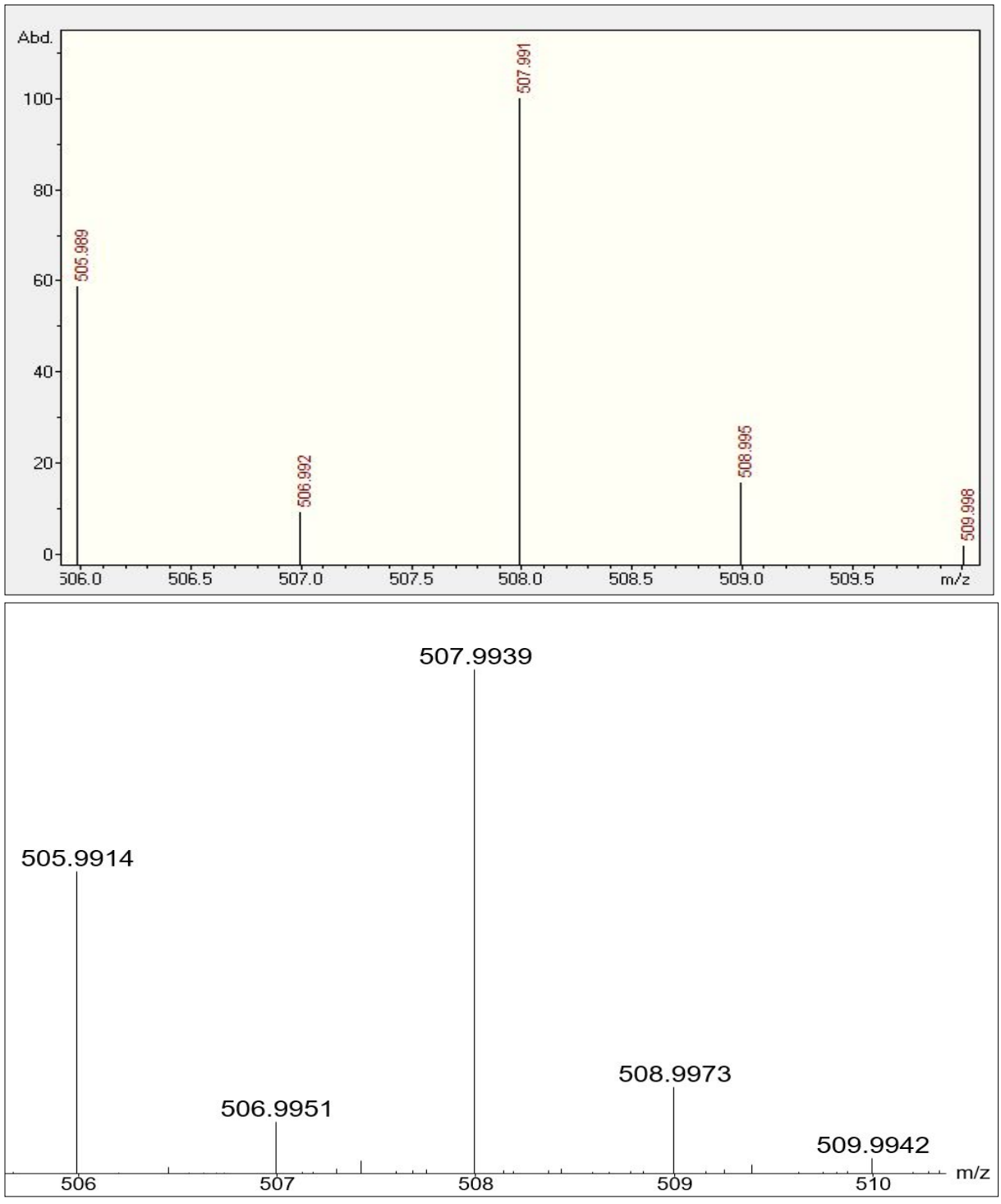


Figure S3: High-resolution ESI mass spectra of **2b.** Top: calculated spectrum. Bottom: experimental spectrum

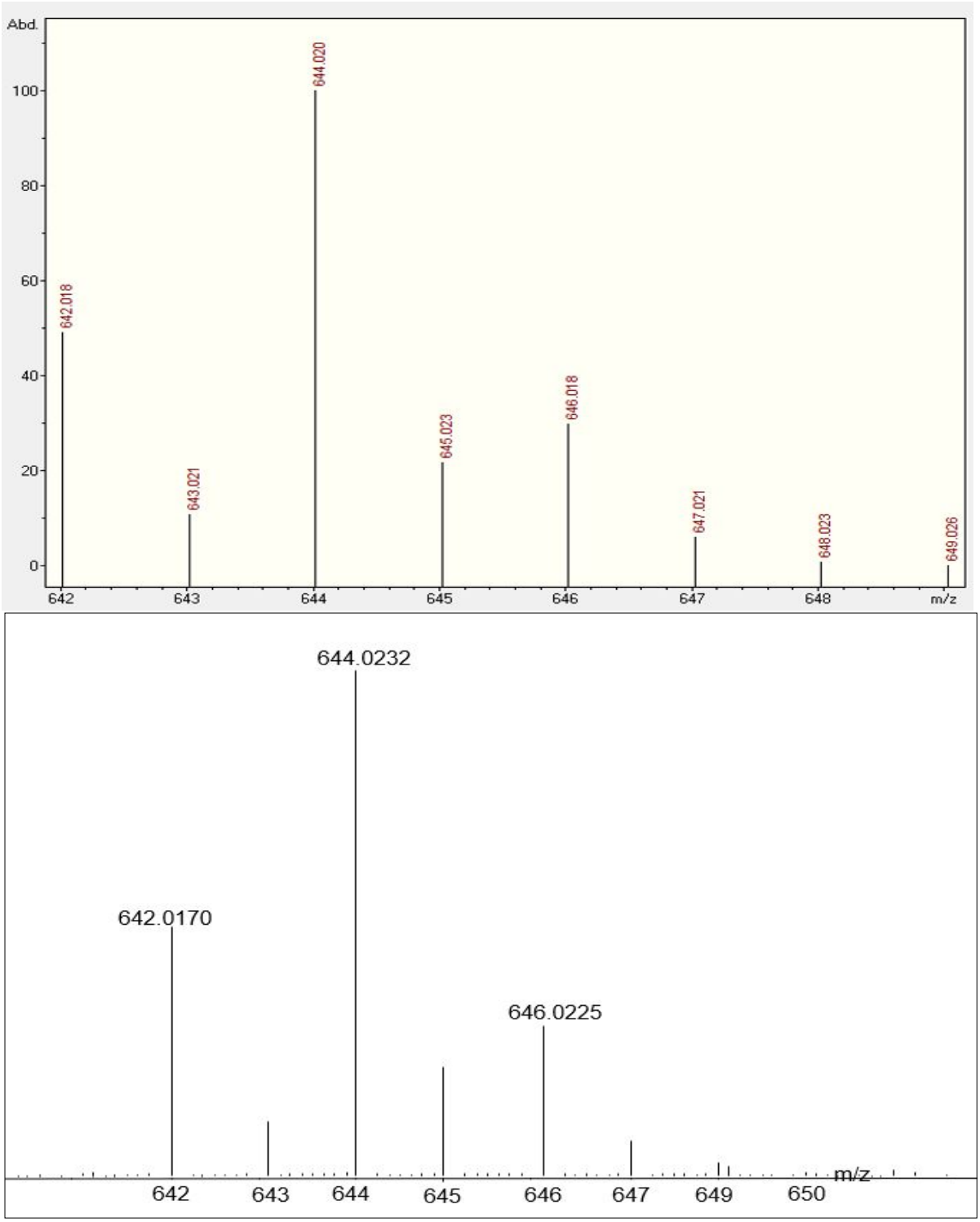


Figure S4: High-resolution ESI mass spectra of **3a.** Top: calculated spectrum. Bottom: experimental spectrum

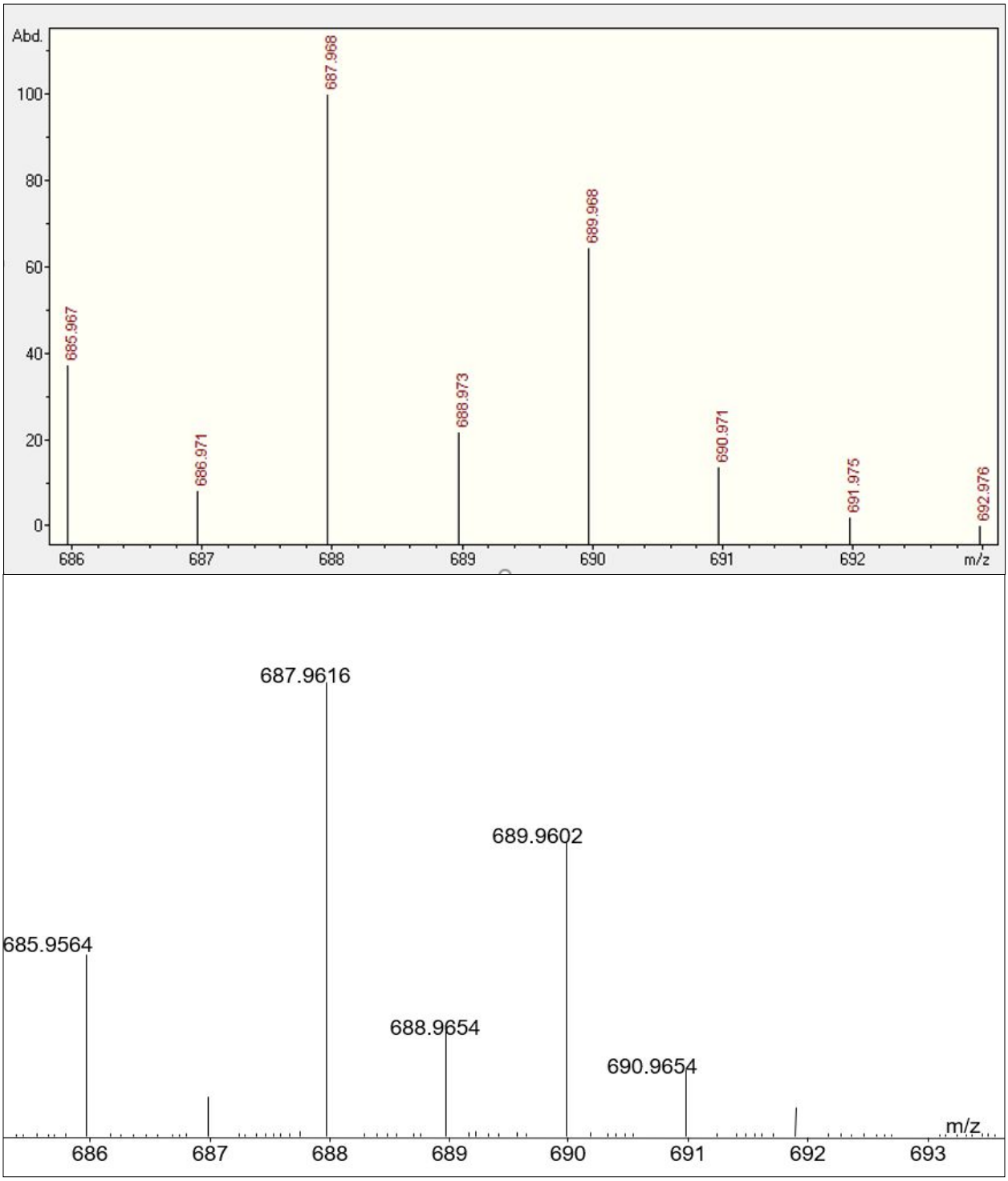


Figure S5: High-resolution ESI mass spectra of **3b.** Top: calculated spectrum. Bottom: experimental spectrum.

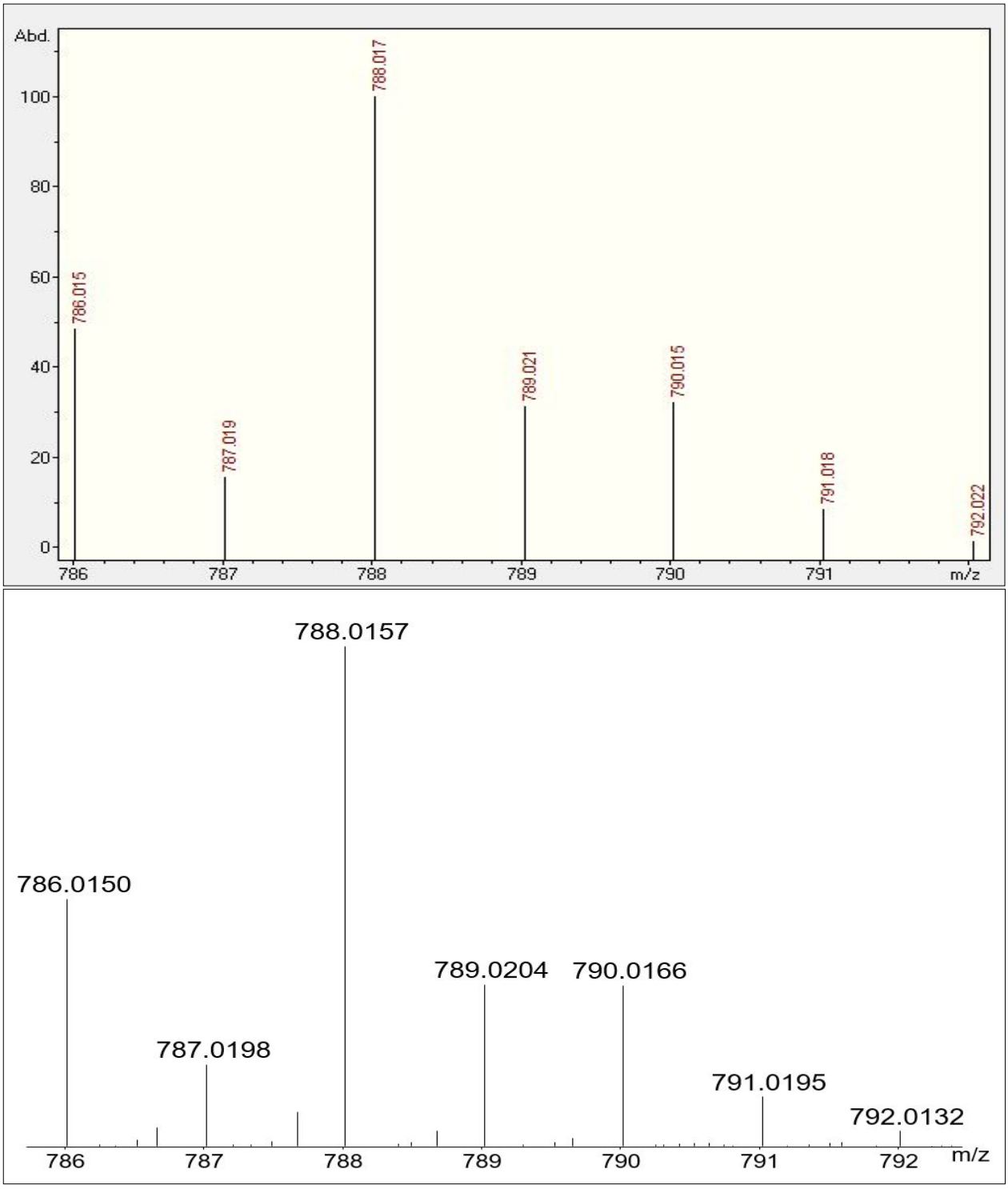


Figure S6: High-resolution ESI mass spectra of **4a.** Top: calculated spectrum. Bottom: experimental spectrum

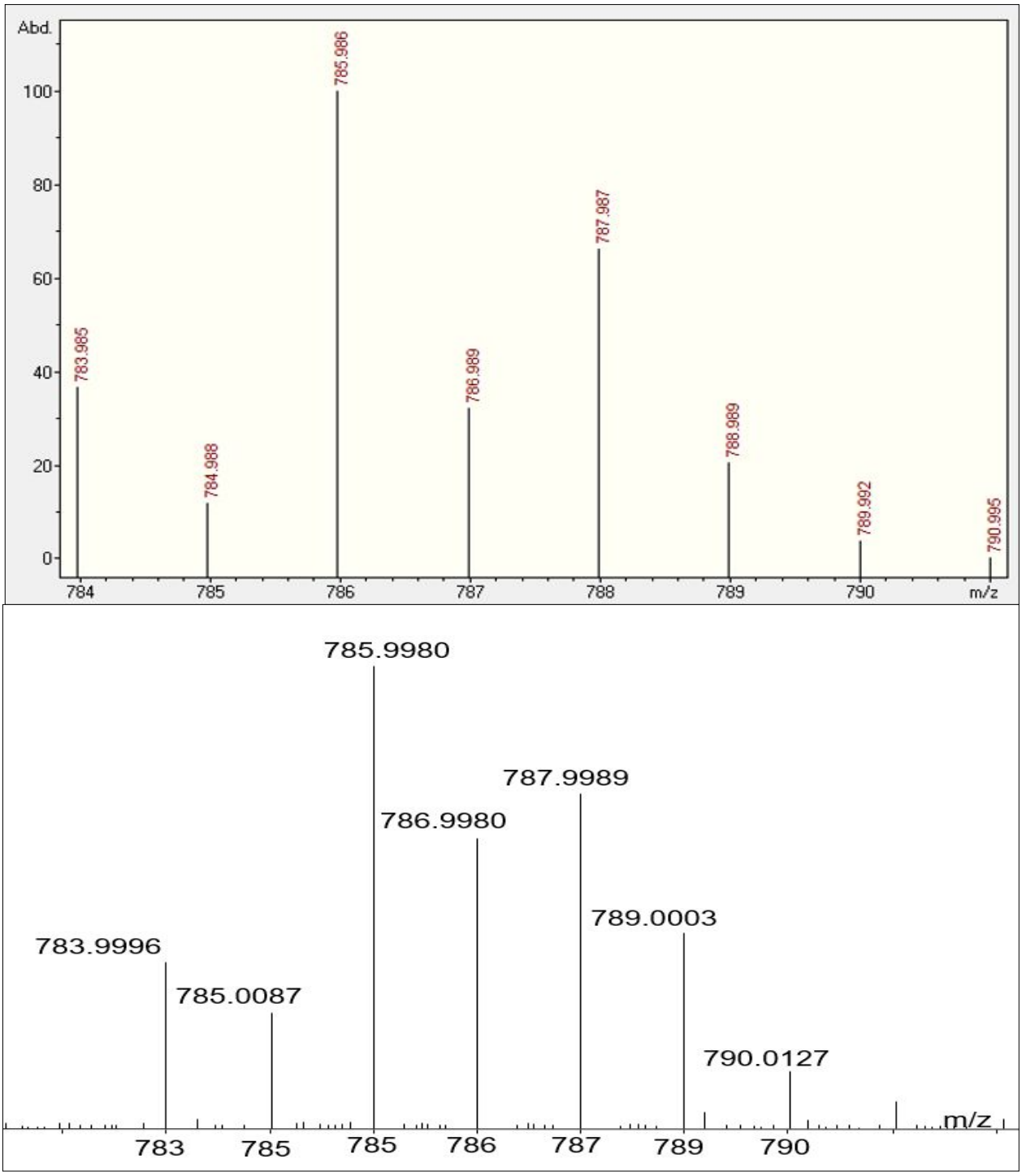


Figure S7: High-resolution ESI mass spectra of **4b.** Top: calculated spectrum. Bottom: experimental spectrum

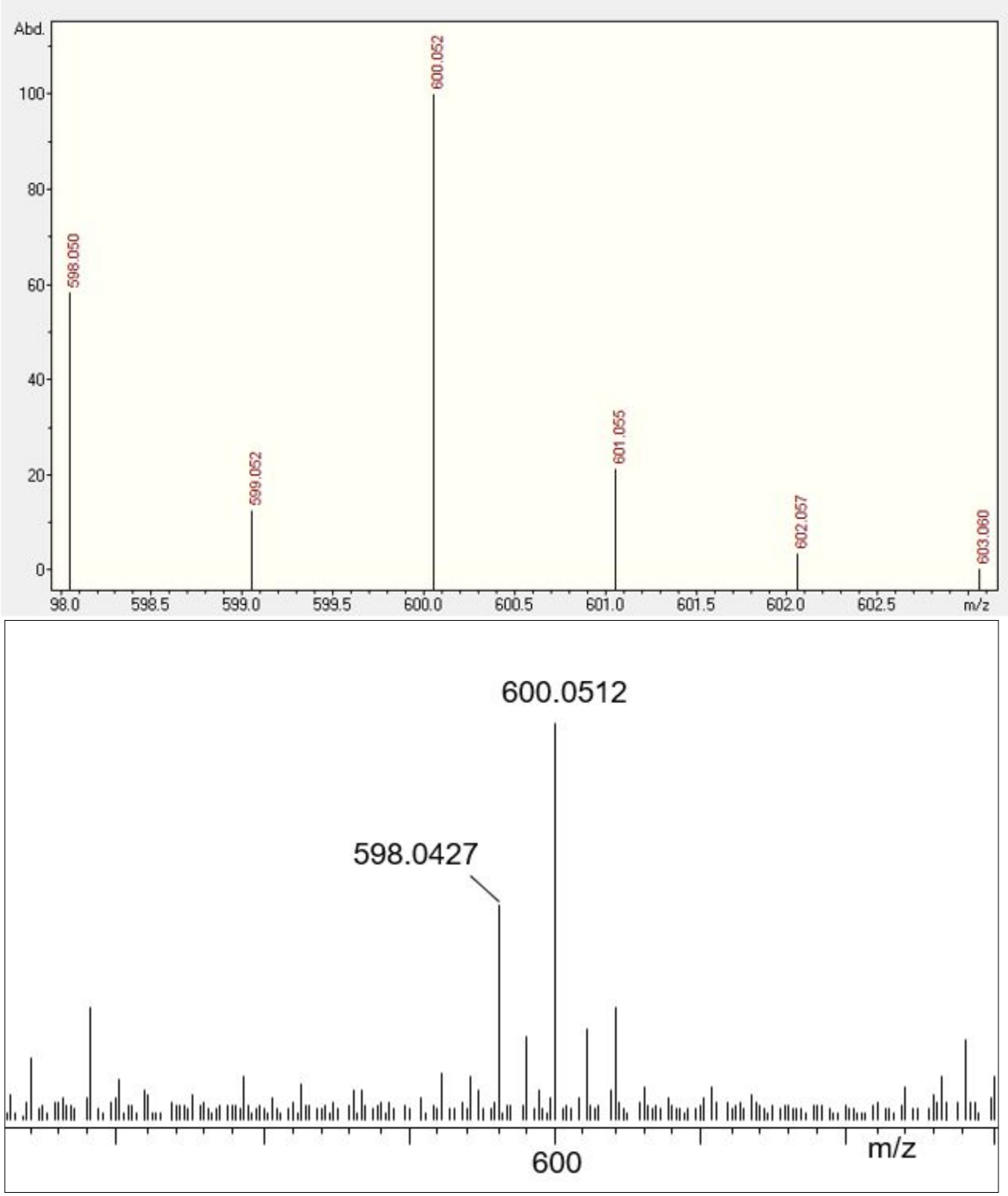


Figure S8: High-resolution ESI mass spectra of **tripeptide.** Top: calculated spectrum. Bottom: experimental spectrum.

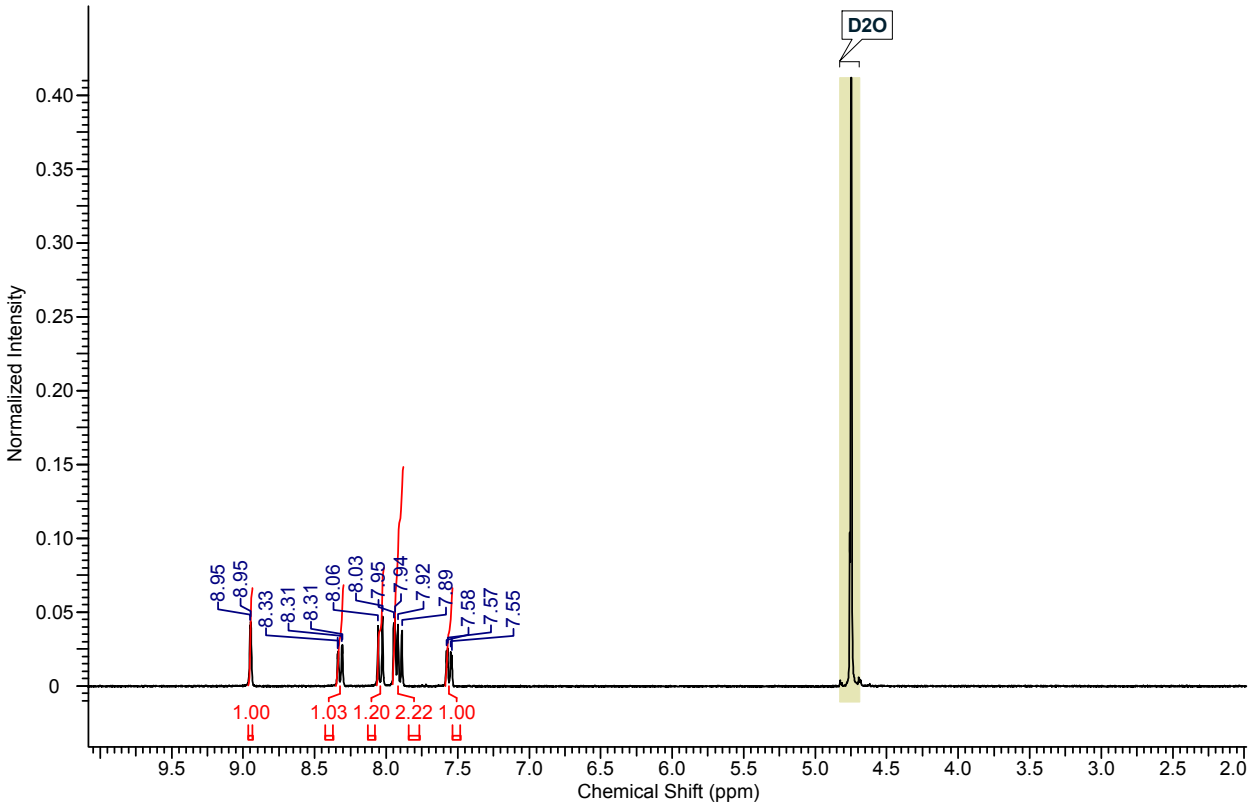


Figure S9: ^{1}H NMR (300 MHz) of **1** in DMSO-d_6.

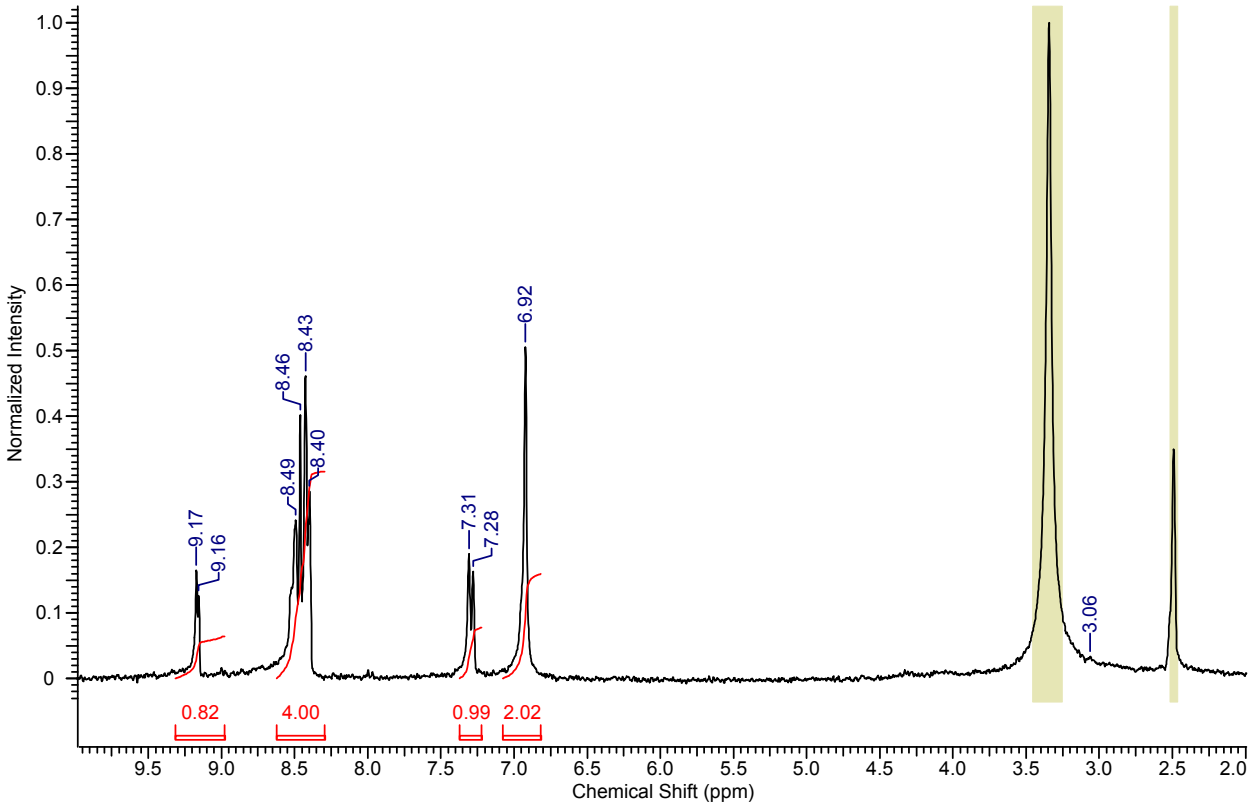


Figure S10: ^{1}H NMR (300 MHz) of **2a** in DMSO-d_6.

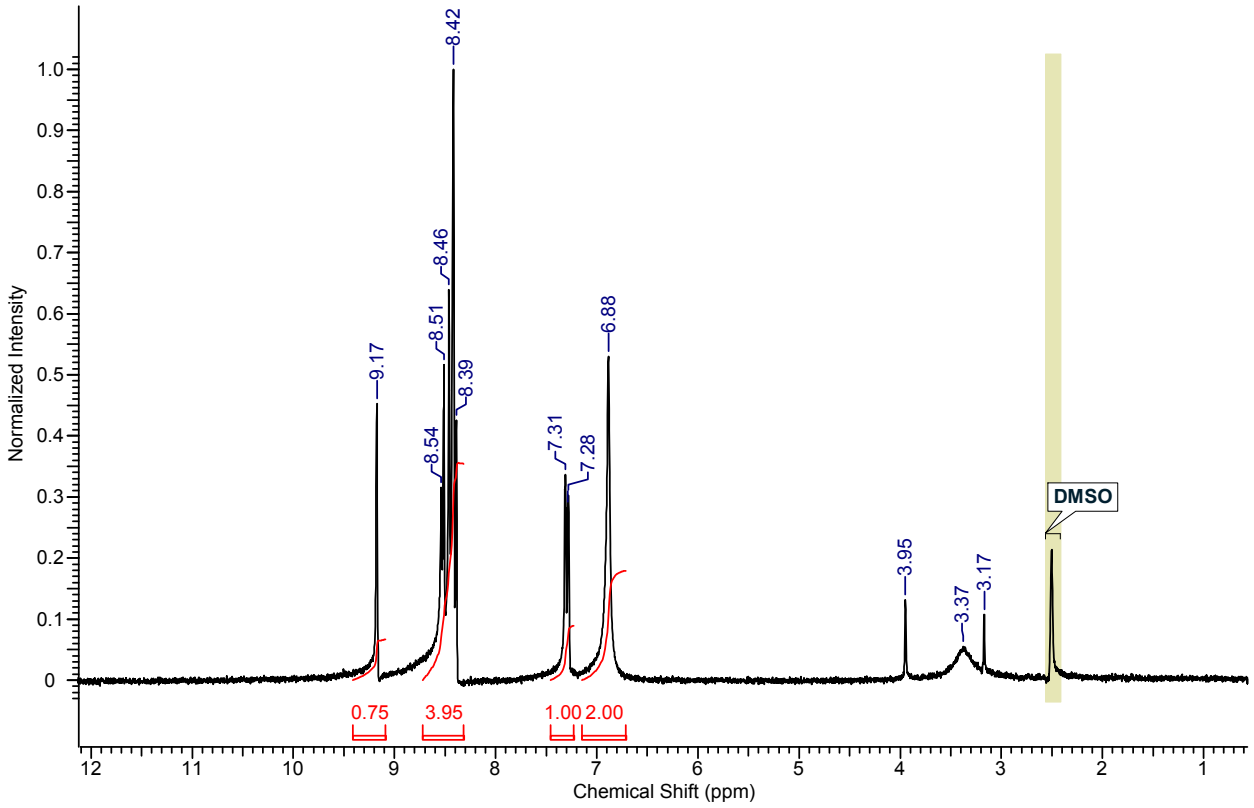


Figure S11: ^{1}H NMR (300 MHz) of **2b** in DMSO-d_6.

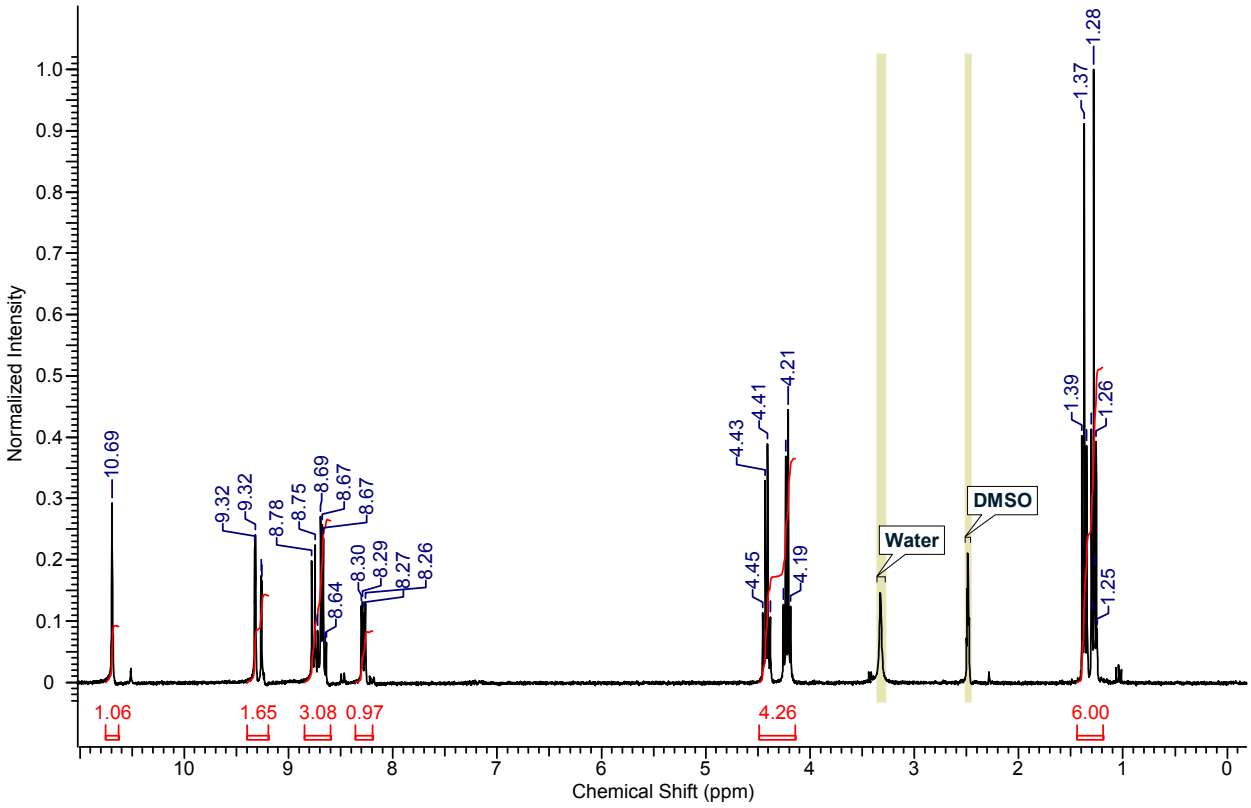


Figure S12: ^{1}H NMR (300 MHz) of **3a** in DMSO-d_6

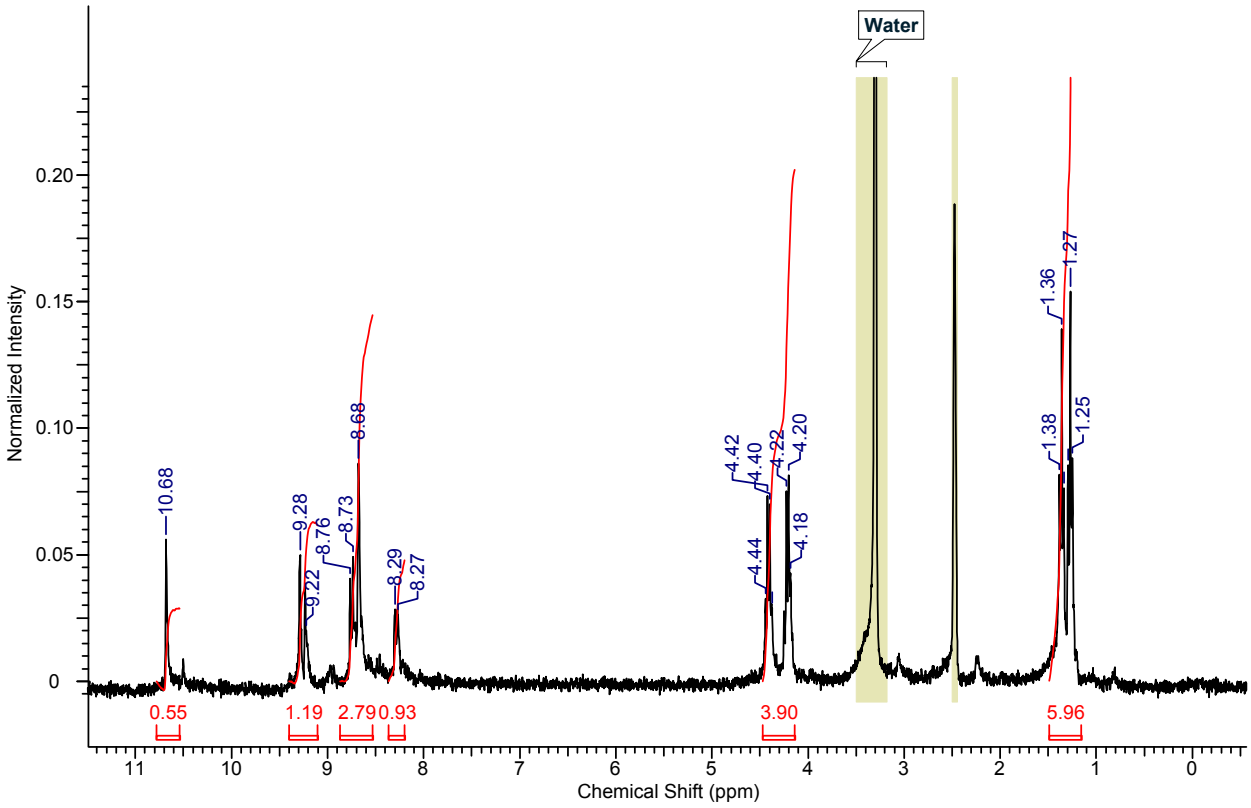


Figure S13: ^{1}H NMR (300 MHz) of **3b** in DMSO-d_6

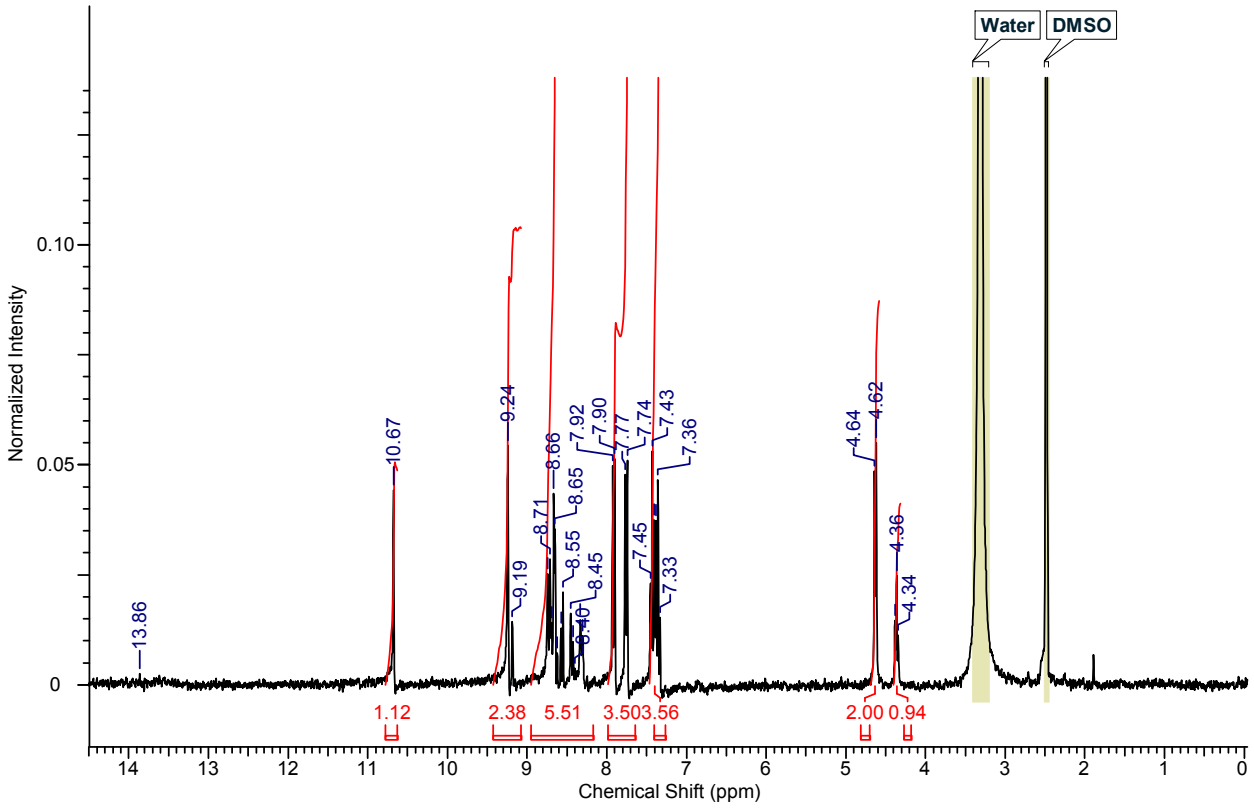


Figure S14: ^{1}H NMR (300 MHz) of **4a** in DMSO-d_6.

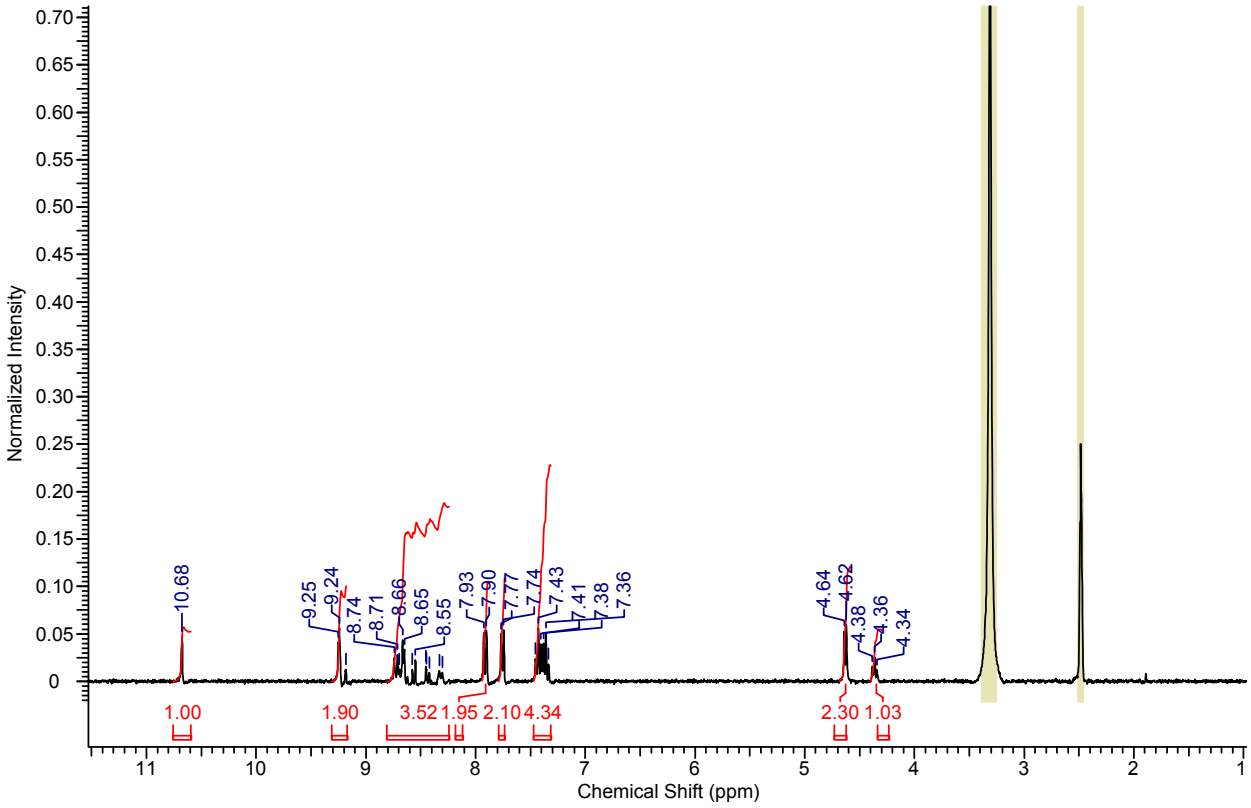


Figure S15: ^{1}H NMR (300 MHz) of **4b** in DMSO-d_6

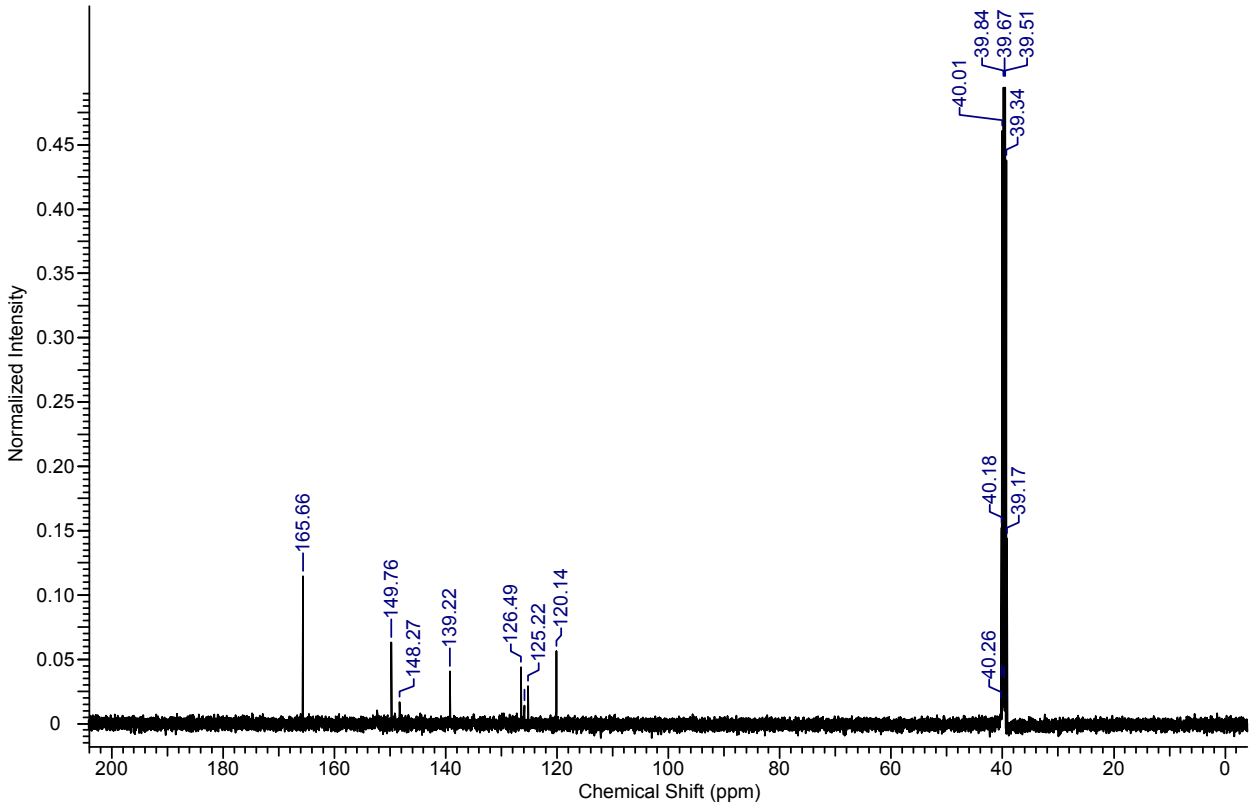


Figure S16: $^{13}C\{^{1}H\}$ NMR (500 MHz) of **1** in DMSO-d_6.

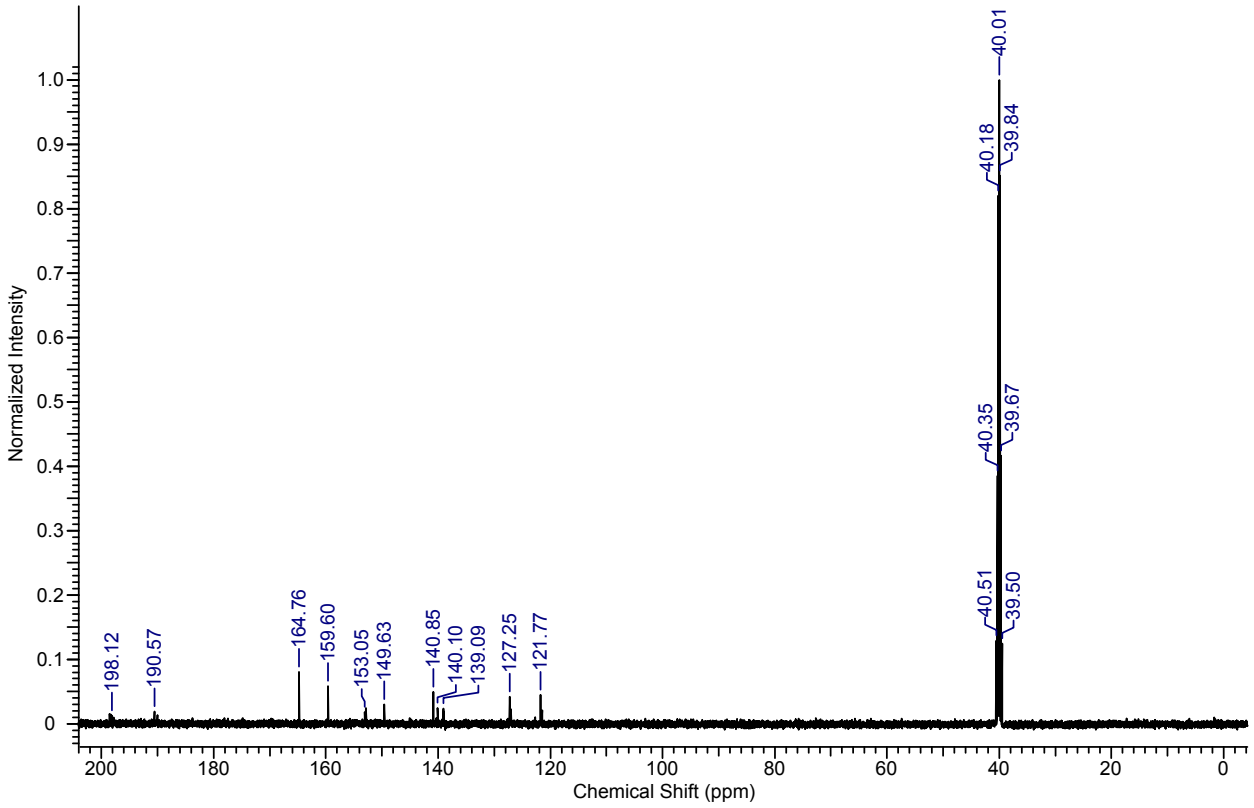


Figure S17: $^{13}C\{^{1}H\}$ NMR (500 MHz) of **2a** in DMSO-d_6.

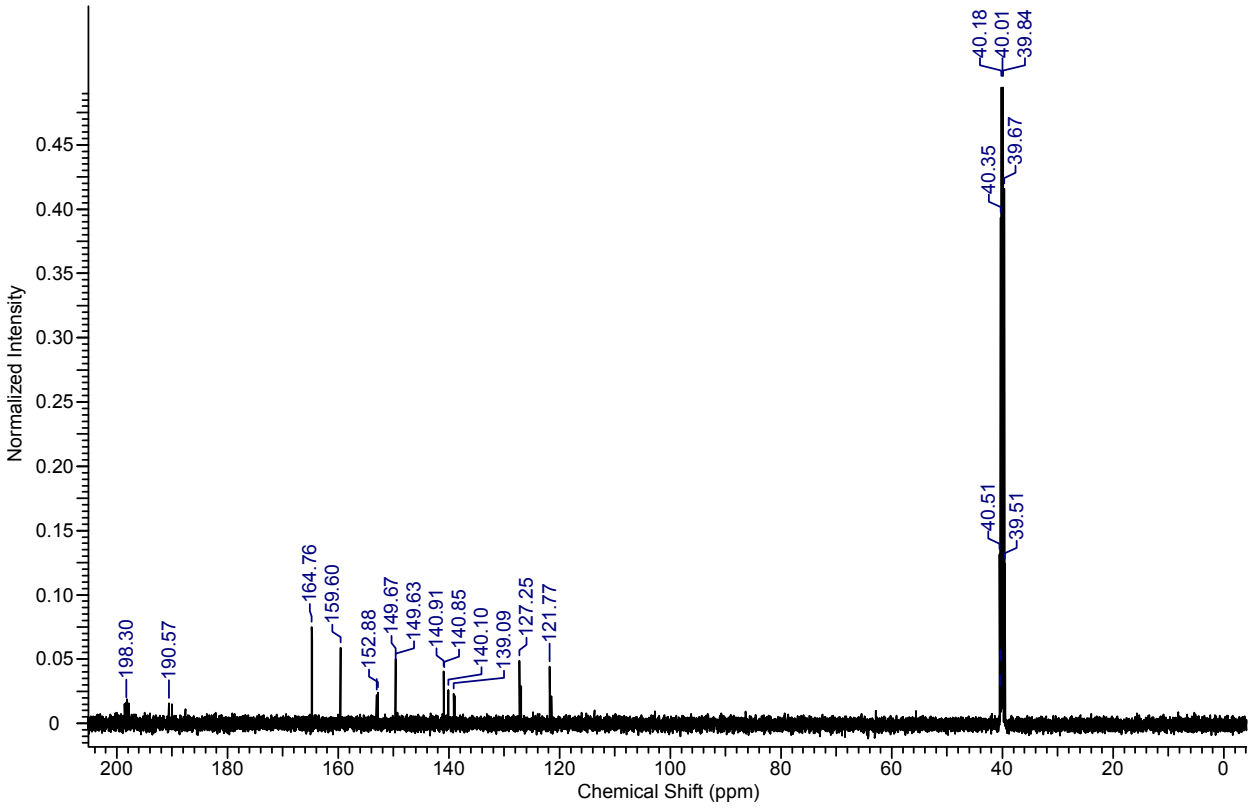


Figure S18: $^{13}C\{^{1}H\}$ NMR (500 MHz) of **2b** in DMSO-d_6.

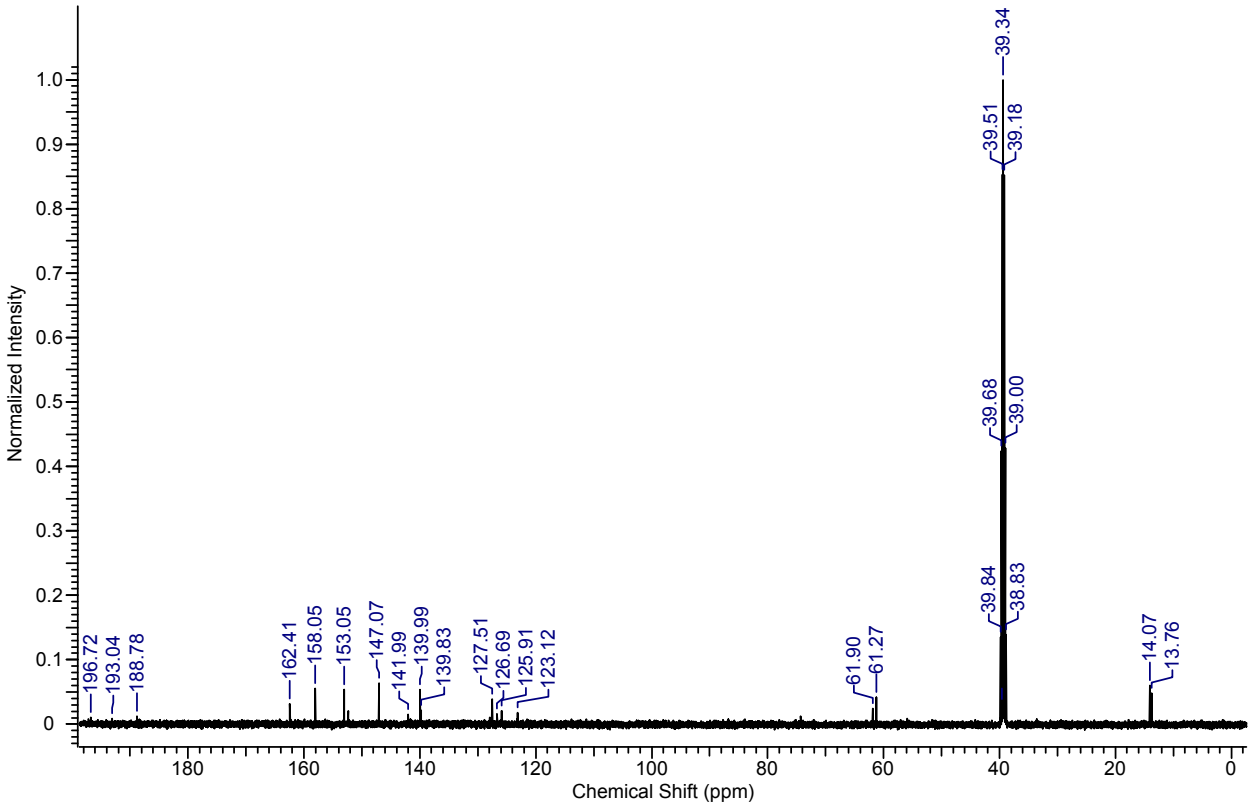


Figure S19: $^{13}C\{^{1}H\}$ NMR (500 MHz) of **3a** in DMSO-d_6.

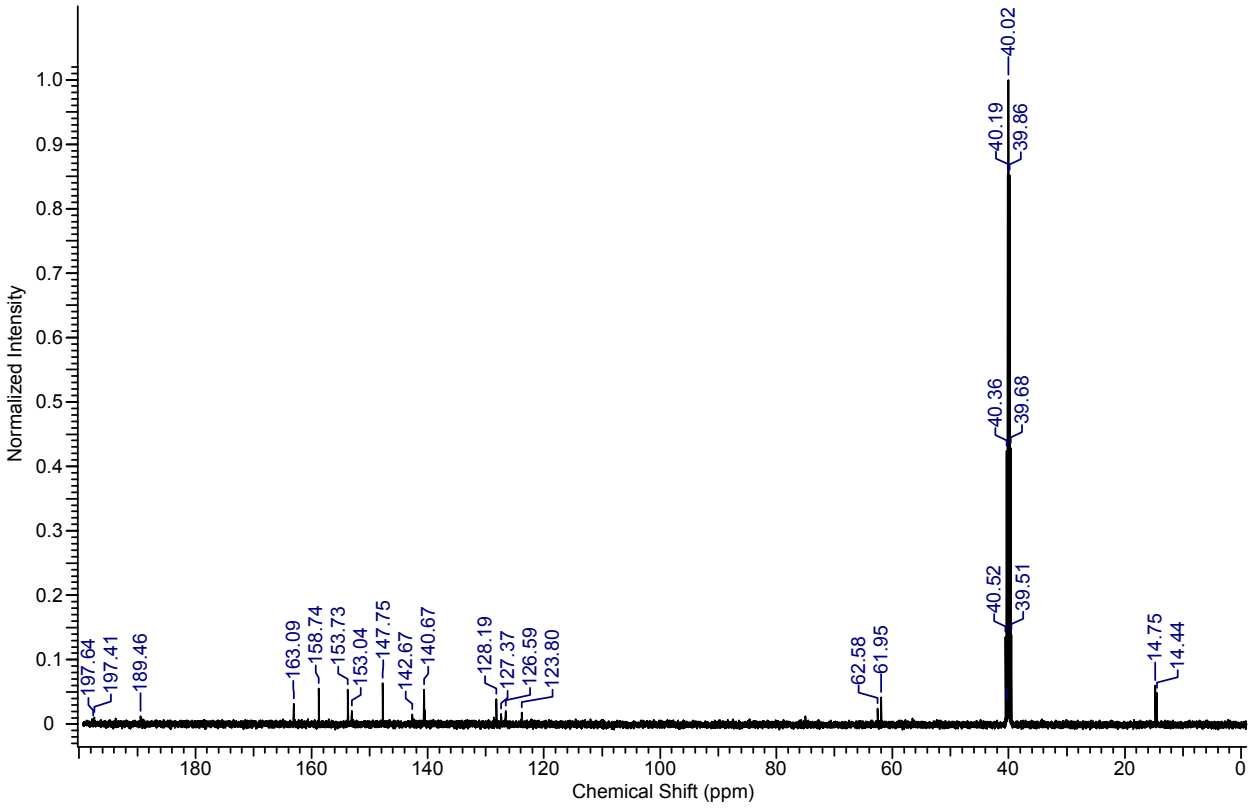


Figure S20: $^{13}C\{^{1}H\}$ NMR (500 MHz) of **3b** in DMSO-d_6.

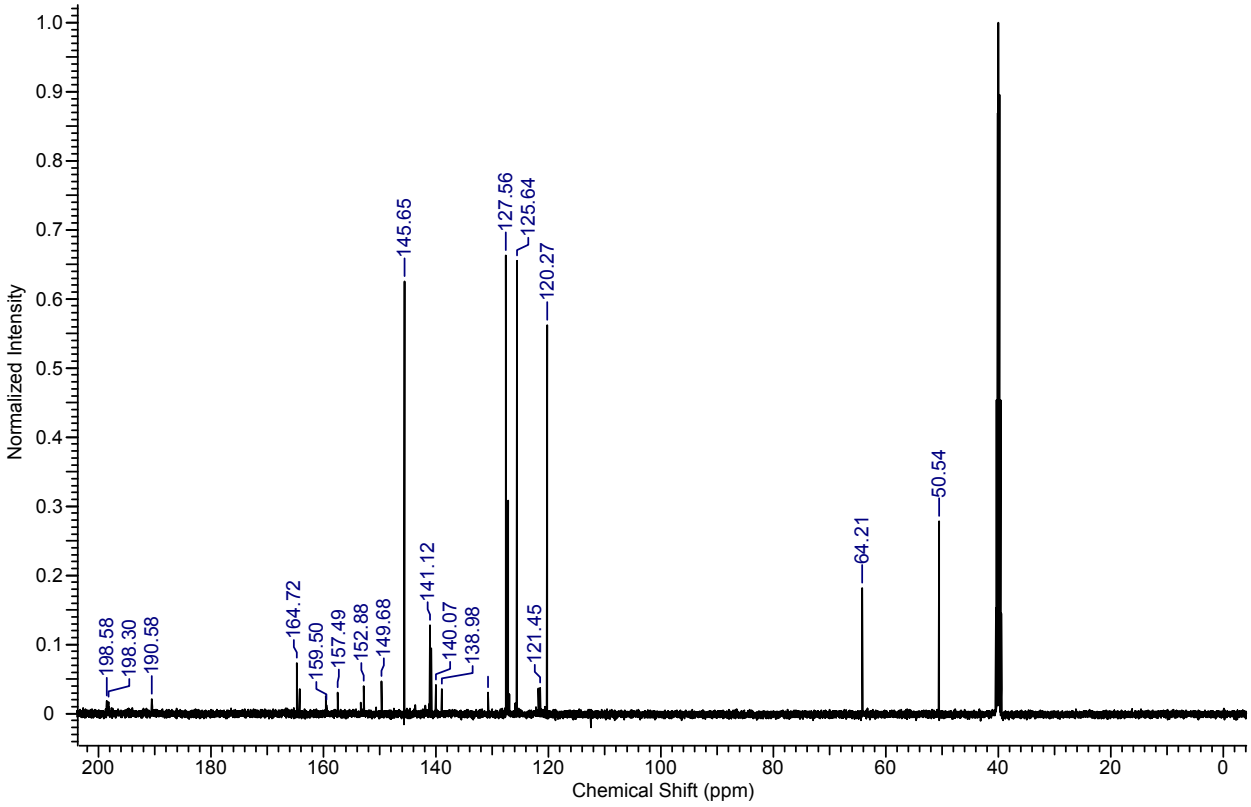


Figure S22: $^{13}C\{^{1}H\}$ NMR (500 MHz) of **4a** in DMSO-d_6.

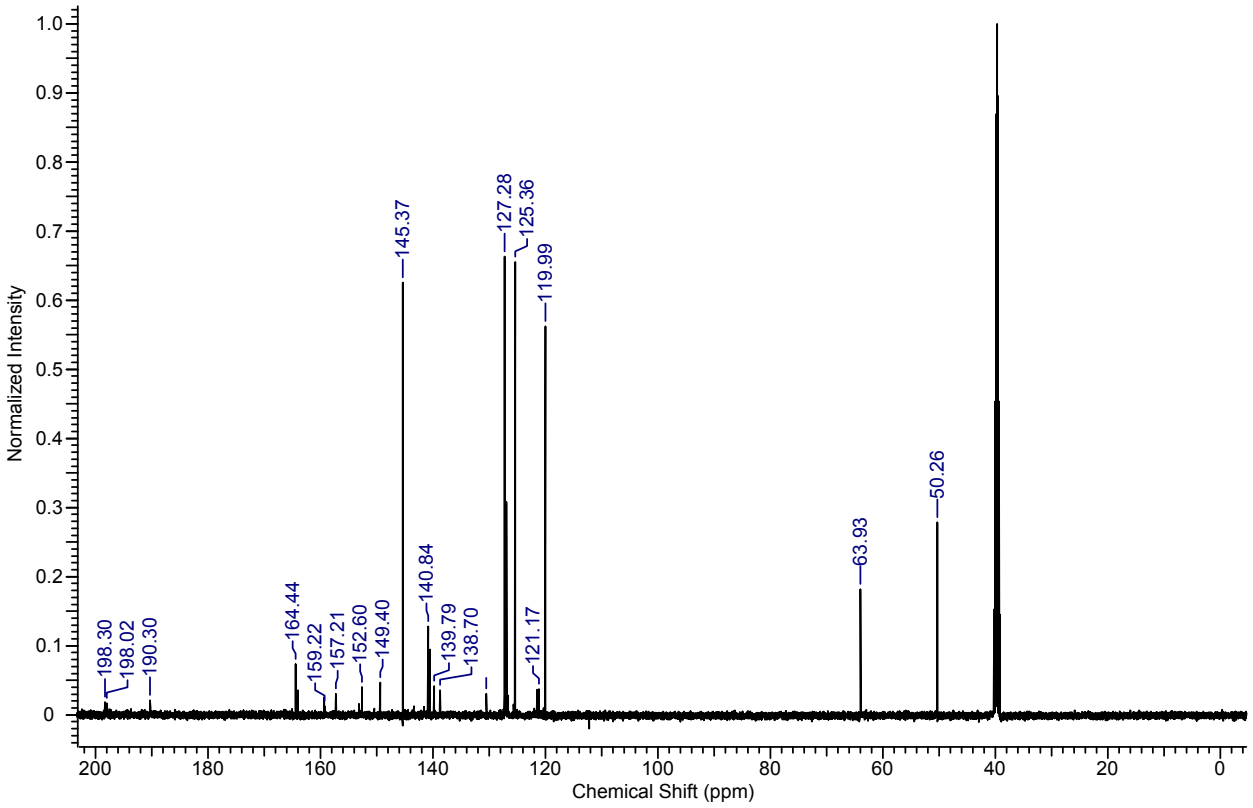
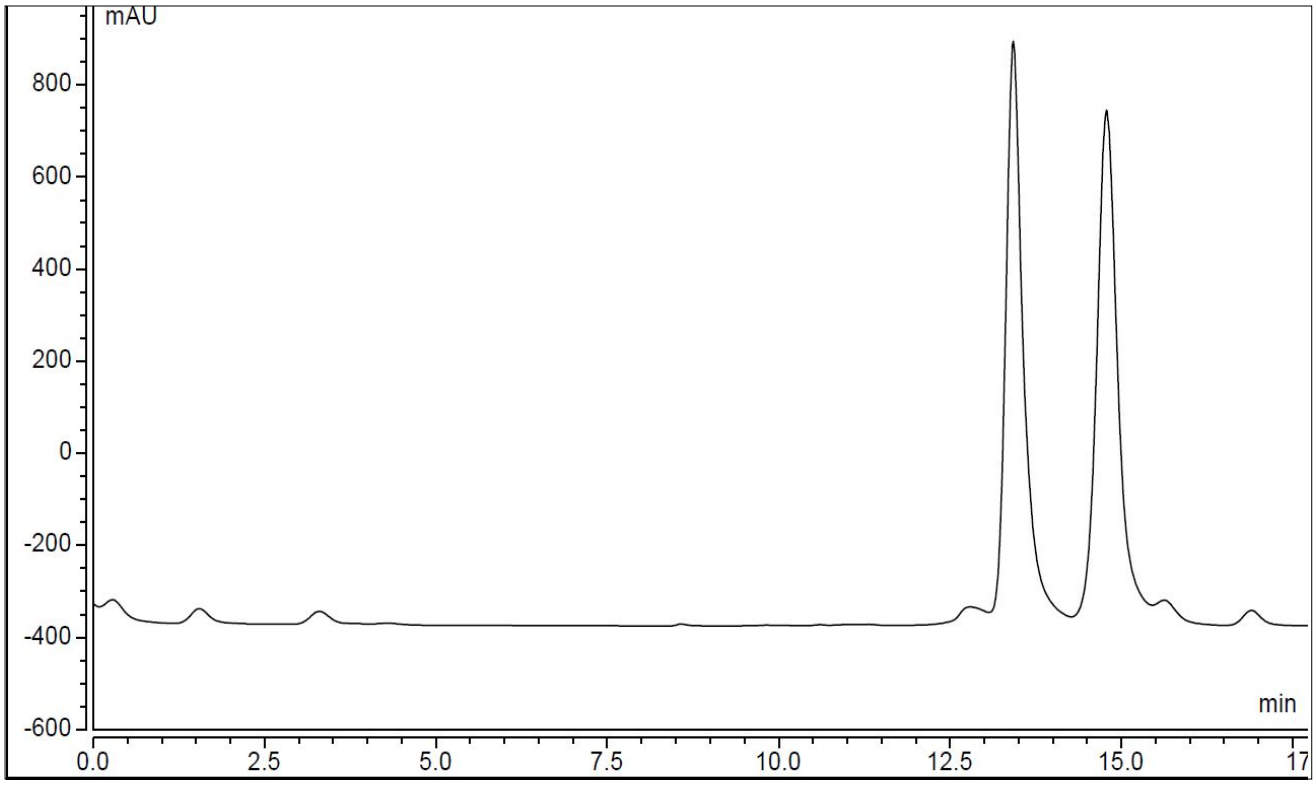


Figure S23: $^{13}C\{^{1}H\}$ NMR (500 MHz) of **4b** in DMSO-d_6.



F**igure S24:** Enantiomeric separation of 2a**.**

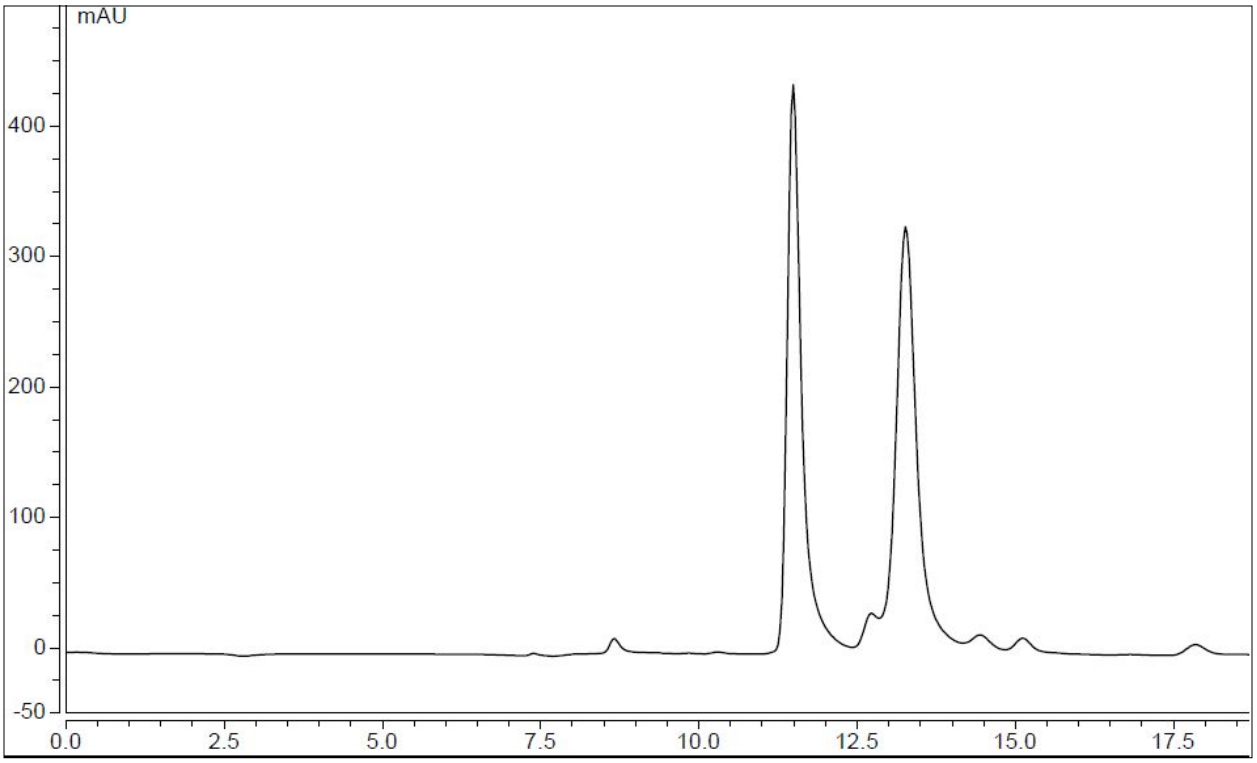


Figure S25: Enantiomeric separation of 2b.

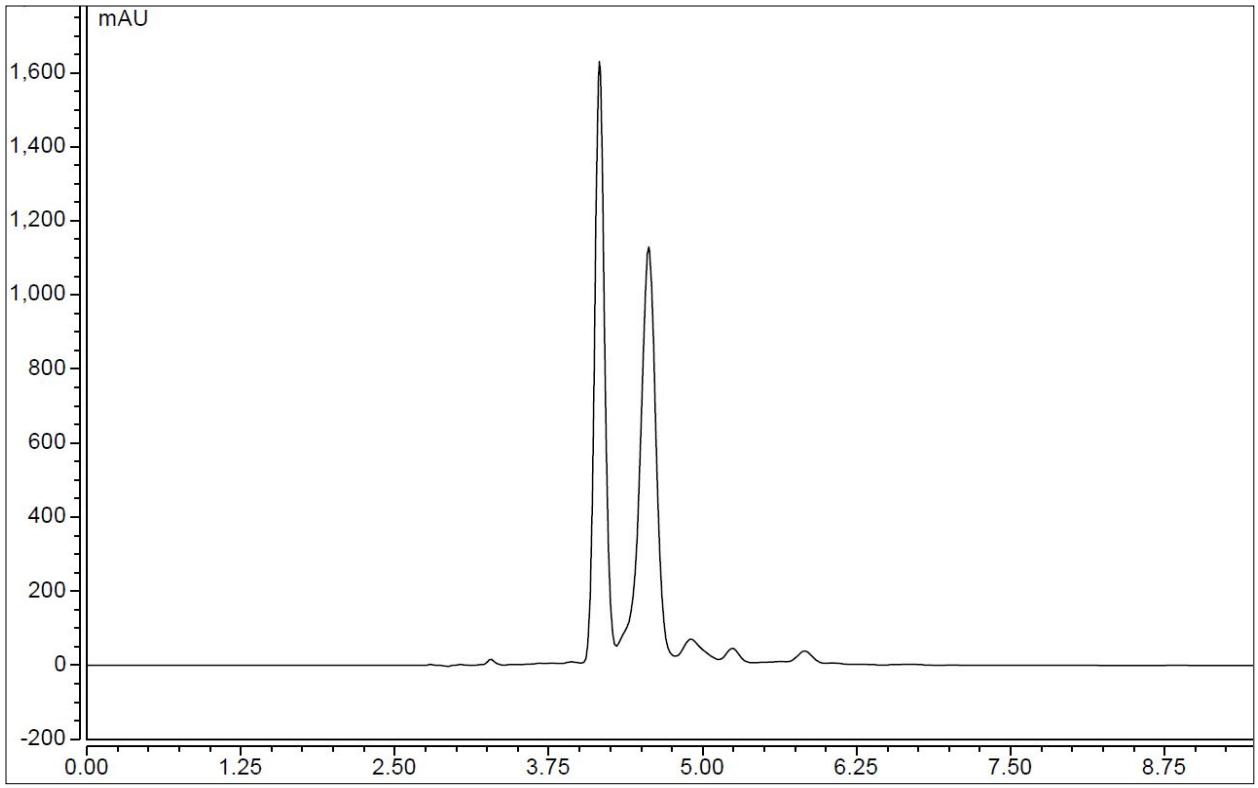


Figure S26: Enantiomeric separation of Gly-Iso-Gly.

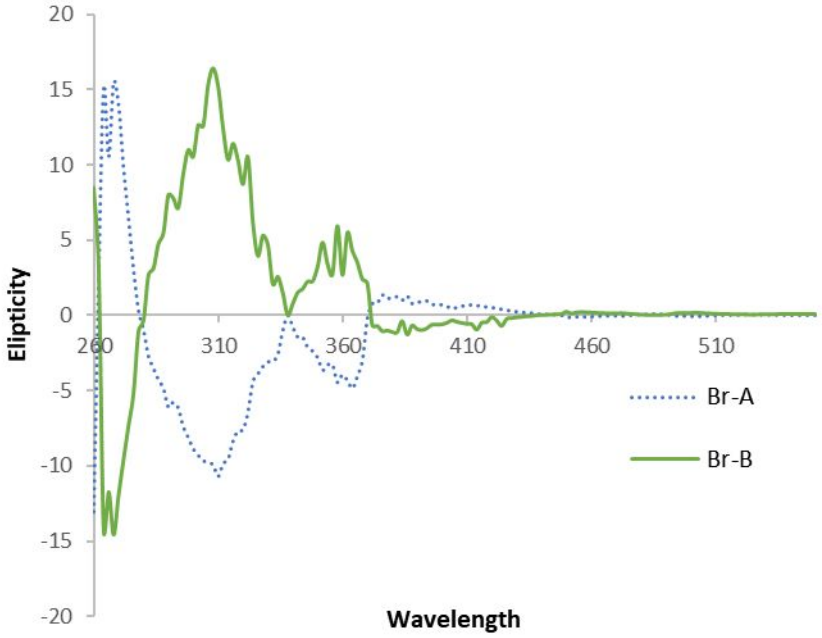


Figure S27: CD Spectra of two enantiomers of **2b**.

Table S1: X-ray crystal data and structure parameters for compounds **2a, 3a and 4a.**

Compound	**2a**	**3a**	**4a**
Empirical formula	$C_{17}H_{16}ClN_4O_6Re$	$C_{20}H_{21}ClN_3O_8Re$	$C_{32}H_{26}ClN_4O_8Re$
CCDC	2074599	2074600	2074601
Formula weight	593.99	653.05	816.22
Crystal system	Monoclinic	Triclinic	Triclinic
Space group	$P2_1/c$	P-1	P-1
a/ Å	17.97(5)	9.4576(7)	10.6721(6)
b/ Å	9.43(2)	10.1283(8)	12.4699(7)
c/ Å	12.13(3)	12.4646(9)	13.8576(7)
α(°)	90	92.936(5)	73.492(3)
β(°)	101.21(4)	96.876(5)	84.789(3)
γ(°)	90	105.926(5)	78.667(3)
Volume ($Å^3$)	2015(9)	1135.46(15)	1732.48(17)
Z	4	2	2
Dc (Mg/m^3)	1.958	1.910	1.565
μ (mm^{-1})	6.204	5.520	3.636
F(000)	1144	636	804
reflns collected	18065	16891	32153
indep. reflns	4954	16891	8553
GOF on F^2	0.968	1.081	1.005
R1 (on F_o^2, $I > 2\sigma(I)$)	0.0475	0.0577	0.0718
wR2 (on F_o^2, $I > 2\sigma(I)$)	0.0793	0.1182	0.1856
R1 (all data)	0.0894	0.0747	0.1075
wR2 (all data)	0.0940	0.1279	0.2063

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