Catch and Release Photosensitizers: Combining Dual Action Ruthenium Complexes with Protease Inactivation for Targeting Invasive Cancers

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Supporting Information

(61 Pages)

Part A. Synthesis

***tert*-butyl ((benzyloxy)carbonyl)-*L*-isoleucyl-*L*-prolinate (10).** A mixture of *tert*-butyl *L*-prolinate (**9**) (5.00 g, 29.2 mmol) and DCM (50 mL) was cooled to 0 °C under a nitrogen atmosphere. DIPEA (17.8 mL, 100.0 mmol) was added dropwise over a period of 5 min. A solution of ((benzyloxy)carbonyl)-*L*-isoleucine (**8**) (7.75 g, 29.2 mmol) in DCM (50 mL) was added dropwise over a period of 10 min followed by addition of HOBt (4.90 g, 32.1 mmol) in one portion. After 15 min a solution of DCC (6.63 g, 32.1 mmol) in DCM (50 mL) was added dropwise over a period of 10 min. The reaction mixture was allowed to warm to rt and stirred overnight for 18 h. After consumption of the starting material, as judged by TLC analysis, the reaction mixture was filtered through celite. The solvent was evaporated *in vacuo*, EtOAc (100mL) was added and stirred for 20 min to precipitate out HOBt and again filtered through celite. The filtrate was washed with 10% aqueous solution of citric acid (2 × 100 mL) and saturated $NaHCO_3$ (2 × 100 mL). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo* to obtain product **10** as clear oil (12.00 g, 28.7 mmol, 98.0 %). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.36–7.26 (m, 5H), 5.08 (part of an AB system, J_{AB} = 12.4 Hz, 1H), 5.04 (part of an AB system, J_{AB} = 12.4 Hz, 1H) 4.32 (dd, J = 8.4, 4.9 Hz, 1H), 4.24 (d, J = 8.8 Hz, 1H), 3.90 (dt, J = 10.1, 6.8 Hz, 1H), 3.66 (dt, J = 9.9, 6.5 Hz, 1H), 2.27–2.16 (m, 1H), 2.10–1.98 (m, 3H), 1.98–1.87 (m, 1H), 1.64–1.60 (m, 1H), 1.45 (s, 9H), 1.27–1.16 (m, 1H), 1.01 (d, J = 6.8 Hz, 3H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 171.7, 171.3, 157.2, 136.8, 128.0, 127.6, 127.4, 81.1, 66.3, 60.1, 56.9, 36.6, 28.8, 26.8, 24.4, 24.3, 14.3, 9.7; $[\alpha]_D^{25}$ –62.0 (c = 1.0, DCM); IR (thin film) 3268, 2967, 1714, 1636, 1528, 1439, 1366, 1253, 1222 cm^{-1}; HRMS (ESMS) calculated for $C_{23}H_{34}N_2O_5$ $[M+H]^+$: 419.2546, found 419.2549.

(2*S*,3*S*)-3-(((2*S*,3*S*)-1-((*S*)-2-(*tert*-butoxycarbonyl)pyrrolidin-1-yl)-3-methyl-1-oxopentan-2-yl)carbamoyl)oxirane-2-carboxylic acid (12). A round bottom flask was purged with nitrogen gas for 2 min. Pd/C (1.20 g, 10% by mass of reactant) was added to the flask. A solution of *tert*-butyl ((benzyloxy)carbonyl)-*L*-isoleucyl-*L*-prolinate (**10**) (12.00 g, 28.7 mmol) in EtOH (200 mL) was added under nitrogen atmosphere. The flask was evacuated just until the solvent began to bubble, then backfilled twice with nitrogen gas. The flask was evacuated again and a the nitrogen balloon was replaced with hydrogen. The flask was evacuated just until the solvent begins to bubble, then backfilled with hydrogen gas. Then suspension of Pd/C was stirred for 5 h. After consumption of the starting material, as judged by TLC analysis, the reaction mixture was filtered through celite (without drying the filter cake). The solvent was evaporated *in vacuo* to obtain a secondary amine (7.90 g, 27.7 mmol). The secondary amine was analyzed by ^{1}H NMR spectroscopy and was used without further purification. ^{1}H NMR (400 MHz, Methanol-d_4) δ 4.33 (dd, J = 8.4, 5.0 Hz, 1H), 3.76–3.65 (m, 1H), 3.67–3.56 (m, 1H), 3.43 (d, J = 6.2 Hz, 1H), 2.29–2.16 (m, 1H), 2.10–1.87 (m, 3H), 1.71–1.61 (m, 2H), 1.45 (s, 9H), 1.25–1.09 (m, 1H), 1.01 (d, J = 6.8 Hz, 3H), 0.93 (t, J = 7.3 Hz, 3H).

A solution of the secondary amine (7.90 g, 27.7 mmol) in DMF (100 mL) was maintained at rt under a nitrogen atmosphere. (2*S*,3*S*)-oxirane-2,3-dicarboxylic acid (**11**, 14.70 g, 111.0 mmol), EDCI (6.40 g, 33.33 mmol) and DIPEA (14.5 mL, 83.3 mmol) were added. The reaction mixture was allowed to stir for 18 h. The reaction mixture was diluted by adding EtOAc (250 mL) and the organic layer was washed with 0.1 M HCl (2 × 100 mL) and 10% LiCl solution in 0.1M HCl (2 × 100 mL). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo* to give a crude mixture which was purified by silica gel chromatography (0.5%: 0% to 6% AcOH:MeOH:DCM) to afford carboxylic acid **12** as a white solid (7.55 g, 18.9 mmol, 66 %)

over two steps. ^{1}H NMR (400 MHz, Methanol-d_4) δ 4.53 (d, J = 8.8 Hz, 1H), 4.32 (dd, J = 8.4, 5.0 Hz, 1H), 3.91 (dt, J = 10.0, 6.8 Hz, 1H), 3.68 (dt, J = 10.0, 6.8 Hz, 1H), 3.66 (d, J = 1.8 Hz, 1H), 3.50 (d, J = 1.8 Hz, 1H), 2.29–2.16 (m, 1H), 2.12–1.84 (m, 4H), 1.69–1.54 (m, 1H), 1.45 (s, 9H), 1.27–1.12 (m, 1H), 1.04 (d, J = 6.8 Hz, 3H), 0.93 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 171.2, 170.6, 169.1, 167.1, 81.2, 60.1, 55.3, 52.6, 51.7, 36.5, 28.8, 26.8, 24.4, 24.3, 14.1, 9.7; $[a]_D^{25}$ - 56.2 (c = 1.0, DCM); IR (thin film) 3267, 2969, 2882, 1735, 1620, 1548, 1450, 1366, 1220, 1216 cm^{-1}; HRMS (ESMS) calculated for $C_{19}H_{30}N_2O_7$ $[M-H]^-$: 397.1975, found 397.1984.

Ethyl (*tert*-butoxycarbonyl)-*L*-isoleucylglycylglycinate (14). A solution of ethyl (*tert*-butoxycarbonyl)-*L*-isoleucylglycinate (**13**, 17.20 g, 54.4 mmol), THF (200 mL) and EtOH (150 mL) was maintained at 0 °C under nitrogen atmosphere. KOH (3.66 g, 65.2 mmol) in EtOH (65 mL) was added dropwise to the reaction mixture. The reaction mixture was stirred for 6 h. After consumption of the starting material, as judged by TLC analysis, the solvent was evaporated *in vacuo* and the residue was dissolved in distilled water (150 mL). The aqueous layer was washed with ethyl acetate (100 mL). The aqueous layer was then acidified to pH ~2 by dropwise addition of 6 M HCl. The aqueous layer was extracted with EtOAc (2 × 150 mL). The combined organic layer was dried over Na_2SO_4 and evaporated *in vacuo* to obtain the carboxylic acid intermediate as a clear oil (12.20 g, 42.3 mmol). The carboxylic acid was analyzed by ^{1}H NMR spectroscopy and was used without further purification. ^{1}H NMR (400 MHz, Methanol-d_4) δ 4.06–3.78 (m, 3H), 1.89–1.71 (m, 1H), 1.62–1.46 (m, 1H), 1.44 (s, 9H), 1.21–1.10 (m, 1H), 0.95 (d, J = 6.8 Hz, 3H), 0.90 (t, J = 7.2 Hz, 3H).

A solution of H-Gly-OEt.HCl (8.00 g, 57.3 mmol) in 20% K_2CO_3 (100 mL) was extracted with EtOAc (2 × 100 mL). The combined layer was dried over Na_2SO_4 and evaporated

in vacuo to obtain free amine H-Gly-OEt (the free amine is a volatile liquid, evaporation should be stopped when the quantitative mass is reached). A solution of H-Gly-OEt (4.36 g, 42.3 mmol) and EDCI (8.11 g, 42.3 mmol) in DCM (250 mL) was maintained at rt under nitrogen atmosphere. The solution of crude carboxylic acid in DCM (100 mL) was added to the reaction mixture. The reaction mixture was stirred for 18 h. The reaction mixture was washed with 1M HCl (2 × 150 mL) and aqueous saturated $NaHCO_3$ (2 × 150 mL). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo* to give product **14** as white solid (13.9 g, 37.2 mmol, 68 %) over two steps. ^{1}H NMR (400 MHz, Methanol-d_4) δ 4.17 (q, J = 7.1 Hz, 2H), 4.03–3.81 (m, 5H), 1.86–1.72 (m, 1H), 1.62–1.48 (m, 1H), 1.43 (s, 9H), 1.26 (t, J = 7.1 Hz, 3H), 1.23–1.11 (m, 1H), 0.93 (d, J = 5.3 Hz, 3H), 0.91 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 173.8, 170.7, 169.6, 157.1, 79.4, 60.9, 59.9, 41.8, 40.6, 36.3, 27.3, 24.7, 14.5, 13.0, 10.1; $[a]_D^{25}$ + 2.6 (c = 1.0, DCM); IR (thin film) 3308, 2969, 1750, 1665, 1529, 1372, 1249, 1171 cm^{-1}; HRMS (ESMS) calculated for $C_{17}H_{31}N_3O_6$ $[M+Na]^+$: 396.2111, found 396.2115.

***tert*-butyl ((14*S*,15*S*)-1-([2,2'-bipyridin]-5-yl)-15-methyl-1,7,10,13-tetraoxo-2,6,9,12-tetraazaheptadecan-14-yl)carbamate (17).** A solution of ethyl ester **14** (13.90 g, 37.2 mmol), THF (150 mL) and EtOH (100 mL) was maintained at 0 ^{o}C under nitrogen atmosphere. KOH (2.50 g, 44.6 mmol) in EtOH (50 mL) was added dropwise to the reaction mixture. The reaction mixture was stirred for 6 h. After consumption of the starting material, as judged by TLC analysis, the solvent was evaporated *in vacuo*. The residue was dissolved in distilled water (100 mL), and the aqueous layer was washed with EtOAc (100 mL). The aqueous layer was then acidified to pH ~2 by dropwise addition of 6 M HCl. The aqueous layer was extracted with EtOAc (2 × 100 mL). The combined organic layer was dried over Na_2SO_4 and evaporated *in vacuo* to obtain the crude carboxylic acid intermediate as clear oil (12.00 g, 34.7 mmol). The

carboxylic acid was analyzed by ^{1}H NMR spectroscopy and was used without further purification. ^{1}H NMR (400 MHz, Methanol-d_4) δ 4.02–3.80 (m, 5H), 1.88–1.71 (m, 1H), 1.62–1.49 (m, 1H), 1.44 (s, 9H), 1.22–1.13 (m, 1H), 0.96–0.85 (m, 6H).

A solution of *N*-(3-aminopropyl)-[2,2'-bipyridine]-5-carboxamide (**15**) (297 mg, 1.16 mmol) and EDCI (222 mg, 1.16 mmol) in DCM (6 mL) was maintained at rt under nitrogen atmosphere. The solution of carboxylic acid (400 mg, 1.16 mmol) in DCM (4 mL) was added to the reaction mixture. The reaction mixture was allowed to stir for 18 h. After consumption of the starting material, as judged by TLC analysis the reaction mixture was washed with 10 % aqueous Na_2CO_3 (2 × 10 mL). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo* to give a crude mixture which was purified by silica gel chromatography (0% to 8% MeOH:DCM) to afford the product **17** as a white solid (352 mg, 0.60 mmol, 48%) over two steps. ^{1}H NMR (400 MHz, Methanol-d_4) δ 9.10 (d, J = 2.3 Hz, 1H), 8.68 (d, J = 4.7 Hz, 1H), 8.43 (t, J = 7.8 Hz, 2H), 8.33 (dd, J = 8.3, 2.3 Hz, 1H), 7.96 (td, J = 7.8, 1.8 Hz, 1H), 7.51–7.43 (m, 1H), 4.01–3.78 (m, 5H), 3.55–3.41 (m, 2H), 1.84 (p, J = 6.8 Hz, 2H), 1.81–1.71 (m, 1H), 1.58–1.49 (m, 1H), 1.41 (s, 9H), 1.26–1.10 (m, 1H), 0.92 (d, J = 6.7 Hz, 3H), 0.88 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 174.7, 170.9, 170.3, 166.6, 157.9, 154.9, 149.1, 148.1, 137.3, 135.9, 129.9, 124.4, 121.6, 120.4, 79.5, 60.0, 48.2, 48.0, 47.8, 47.6, 47.4, 47.2, 47.0, 42.6, 42.3, 36.7, 36.2, 28.5, 27.3, 24.8, 14.4, 10.0; $[a]_D^{25}$ + 1.7 (c = 1.0, MeOH); IR (thin film) 3300, 3071, 2968, 2934, 2878, 1650, 1590, 1536, 1459, 1368, 1294, 1248, 1165 cm^{-1}; HRMS (ESMS) calculated for $C_{29}H_{41}N_7O_6$ $[M+H]^+$: 584.3197, found 584.3219.

***tert*-butyl ((14*S*,15*S*)-15-methyl-1,7,10,13-tetraoxo-1-(pyridin-3-yl)-2,6,9,12-tetraazaheptadecan-14-yl)carbamate (18).** A solution of *N*-(3-aminopropyl)nicotinamide (**16**) (8.35 g, 46.6 mmol), EDCI (6.38 g, 33.3 mmol) and DCM (200 mL) was maintained at rt under nitrogen

atmosphere. The solution of carboxylic acid obtained from compound **14** (11.50 g, 33.3 mmol) in DCM (100 mL) was added to the reaction mixture. The reaction mixture was stirred for 18 h. After consumption of the starting material, as judged by TLC analysis, the reaction mixture was washed with 10 % aqueous Na_2CO_3 (2 × 150 mL). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo* to give a crude mixture which was purified by silica gel chromatography (0% to 6% MeOH:DCM) to afford the product **18** as a white solid (9.70 g, 19.1 mmol, 53 %) over two steps. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.99 (d, J = 2.0 Hz, 1H), 8.68 (dd, J = 5.0, 1.7 Hz, 1H), 8.26 (dt, J = 8.0, 2.0 Hz, 1H), 7.58–7.50 (m, 1H), 4.00–3.77 (m, 5H), 3.55–3.39 (m, 2H), 1.89–1.71 (m, 3H), 1.58–1.49 (m, 1H), 1.41 (s, 9H), 1.26–1.11 (m, 1H), 0.91 (d, J = 6.7 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 174.6, 170.9, 170.3, 166.5, 151.2, 147.7, 135.6, 130.6, 123.7, 79.5, 60.0, 42.6, 42.2, 36.8, 36.2, 28.5, 27.3, 24.8, 14.4, 10.0; $[a]_D^{25}$ + 15.4 (c = 1.0, DCM); IR (thin film) 3293, 3074, 2966, 1648, 1529, 1367, 1294, 1246, 1164 cm^{-1}; HRMS (ESMS) calculated for $C_{24}H_{38}N_6O_6$ $[M+H]^+$: 507.2931, found 507.2911.

***tert*-butyl ((2*S*,3*S*)-3-(((2*S*,3*S*)-1-((2-((2-((3-([2,2'-bipyridine]-5-carboxamido)propyl)amino)-2-oxoethyl)amino)-2-oxoethyl)amino)-3-methyl-1-oxopentan-2-yl)carbamoyl)oxirane-2-carbonyl)-*L*-isoleucyl-*L*-prolinate (19).** A solution of carboxylic acid **12** (225 mg, 0.56 mmol) and *p*-nitrophenol (78 mg, 0.56 mmol) in EtOAc (3 mL) was maintained at 0 °C under nitrogen atmosphere. A solution of DCC (116 mg, 0.56 mmol) in EtOAc (1.5 mL) was added dropwise. The reaction mixture was allowed to warm to rt and stirred overnight for 18 h. After consumption of the starting material, as judged by TLC analysis, the reaction mixture was filtered through celite. The solvent was evaporated *in vacuo* to obtain the *p*-nitrophenol ester as a crude yellow solid (300 mg). The crude product was analyzed by ^{1}H NMR spectroscopy and was used without further purification. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.31 (d, J = 9.2 Hz, 2H),

7.35 (d, *J* = 9.2 Hz, 2H), 6.84 (d, *J* = 9.2 Hz, 1H), 4.66 (dd, *J* = 9.1, 7.2 Hz, 1H), 4.39 (dd, *J* = 8.7, 4.8 Hz, 1H), 3.83 (d, *J* = 1.8 Hz, 1H), 3.81–3.76 (m, 1H), 3.73 (d, *J* = 1.7 Hz, 1H), 3.70–3.65 (m, 1H), 2.25–2.19 (m, 1H), 2.11–1.92 (m, 4H), 1.63–1.50 (m, 1H), 1.46 (s, 9H), 1.15–1.08 (m, 1H), 1.04 (d, *J* = 6.9 Hz, 3H), 0.92 (t, *J* = 7.5 Hz, 3H).

A solution of compound **17** (330 mg, 0.56 mmol) in DCM (1 mL) was maintained at rt under nitrogen atmosphere. TFA (0.44 mL) was added to the reaction mixture. The reaction mixture was allowed to stir for 4 h. The reaction mixture was concentrated *in vacuo* and excess TFA was removed by azeotropic distillation from DCM. The TFA salt was dissolved in DCM (4 mL) and DIPEA (0.30 mL, 1.72 mmol) was added. The reaction mixture was maintained at rt under nitrogen atmosphere. A solution of crude *p*-nitrophenol ester (300 mg) in DCM (2 mL) was added dropwise to the reaction mixture. The reaction was stirred for 8 h. After consumption of the starting material, as judged by TLC analysis, the organic layer was evaporated *in vacuo* to give a crude mixture. The crude reaction mixture was dissolved in EtOAc (20 mL), the organic layer was washed with 10% Na_2CO_3 (2 × 15 mL), dried over Na_2SO_4 and solvent was evaporated *in vacuo* to obtain the crude product. The crude mixture was purified by silica gel chromatography (0% to 8% MeOH:DCM) to afford the product **19** as a white solid (180 mg, 0.21 mmol, 37 %) over three steps. ^{1}H NMR (400 MHz, Methanol-d_4) δ 9.09 (d, *J* = 1.8 Hz, 1H), 8.68 (d, *J* = 4.2 Hz, 1H), 8.43 (d, *J* = 5.8 Hz, 1H), 8.41 (d, *J* = 5.4 Hz, 1H), 8.32 (dd, *J* = 8.3, 2.3 Hz, 1H), 7.96 (td, *J* = 7.8, 1.8 Hz, 1H), 7.50–7.43 (m, 1H), 4.52 (d, *J* = 8.6 Hz, 1H), 4.28 (dd, *J* = 8.3, 4.8 Hz, 1H), 4.23 (d, *J* = 7.5 Hz, 1H), 3.99–3.79 (m, 5H), 3.71 (d, *J* = 1.7 Hz, 1H), 3.68 (d, *J* = 1.7 Hz, 1H), 3.67–3.61 (m, 1H), 3.46 (t, *J* = 6.7 Hz, 2H), 3.34 (t, *J* = 6.7 Hz, 2H), 2.26–2.13 (m, 1H), 2.09–1.78 (m, 7H), 1.67–1.50 (m, 2H), 1.43 (s, 9H), 1.25–1.09 (m, 2H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.89 (t, *J* = 7.4 Hz, 6H); ^{13}C NMR (100 MHz, Methanol-d_4) δ

172.8, 171.1, 170.6, 170.5, 170.3, 167.7, 167.0, 166.5, 157.9, 154.9, 149.1, 148.1, 137.3, 136.0, 130.0, 124.4, 121.6, 120.4, 81.2, 60.1, 58.5, 55.2, 52.9, 52.8, 48.2, 48.0, 47.8, 47.6, 47.4, 47.2, 47.0, 42.5, 42.2, 36.8, 36.6, 36.3, 36.3, 28.8, 28.6, 26.8, 24.7, 24.4, 24.3, 14.4, 14.1, 10.0, 9.8; $[a]_D^{25}$ - 11.5 (c = 1.0, MeOH); IR (thin film) 3289, 3072, 2967, 1654, 1543, 1456, 1368, 1295 cm^{-1}; HRMS (ESMS) calculated for $C_{43}H_{61}N_9O_{10}$ $[M+H]^+$: 864.4620, found 864.4628.

***tert*-butyl ((2*S*,3*S*)-3-(((2*S*,3*S*)-3-methyl-1-((2-((2-((3-(nicotinamido)propyl)amino)-2-oxoethyl)amino)-2-oxoethyl)amino)-1-oxopentan-2-yl)carbamoyl)oxirane-2-carbonyl)-*L*-isoleucyl-*L*-prolinate** (**20**). A solution of carboxylic acid **12** (1.50 g, 3.76 mmol) and *p*-nitrophenol (524 mg, 3.76 mmol) in EtOAc (25 mL) was maintained at 0 °C under nitrogen atmosphere. A solution of DCC (777 mg, 3.76 mmol) in EtOAc (10 mL) was added dropwise. The reaction mixture was allowed to warm to rt and stirred for 18 h. After consumption of the starting material, as judged by TLC analysis, the reaction mixture was filtered through celite. The filtrate was evaporated *in vacuo* to obtain the *p*-nitrophenol ester as a crude yellow solid (2.00 g). The crude product was analyzed by ^{1}H NMR spectroscopy and was used without further purification. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.31 (d, *J* = 9.2 Hz, 2H), 7.35 (d, *J* = 9.2 Hz, 2H), 6.84 (d, *J* = 9.2 Hz, 1H), 4.66 (dd, *J* = 9.1, 7.2 Hz, 1H), 4.39 (dd, *J* = 8.7, 4.8 Hz, 1H), 3.83 (d, *J* = 1.8 Hz, 1H), 3.81–3.76 (m, 1H), 3.73 (d, *J* = 1.7 Hz, 1H), 3.70–3.65 (m, 1H), 2.25–2.19 (m, 1H), 2.11–1.92 (m, 4H), 1.63–1.50 (m, 1H), 1.46 (s, 9H), 1.15–1.08 (m, 1H), 1.04 (d, *J* = 6.9 Hz, 3H), 0.92 (t, *J* = 7.5 Hz, 3H).

A solution of compound **18** (2.10 g, 4.14 mmol) in DCM (6 mL) was maintained at rt under nitrogen atmosphere. TFA (3.17mL) was added to the reaction mixture. The reaction mixture was allowed to stir for 4 h then concentrated *in vacuo* and excess TFA was removed by azeotropic distillation from DCM (3 × 10 mL). The TFA salt was dissolved in DCM (25 mL) and

DIPEA (2 mL, 11.3 mmol) was added. The reaction mixture was maintained at rt under nitrogen atmosphere. The solution of crude *p*-nitrophenol ester (2.00 g) in DCM (10 mL) was added dropwise to the reaction mixture. The reaction was allowed to stir for 8 h. After consumption of the starting material, as judged by TLC analysis, the organic layer was evaporated *in vacuo* to give a crude mixture. The crude reaction mixture was dissolved in EtOAc (60 mL), the organic layer was washed with 10% Na_2CO_3 (2 × 50 mL), dried over Na_2SO_4 and solvent was evaporated *in vacuo* to obtain the crude product. The crude mixture was purified by silica gel chromatography (0% to 8% MeOH:DCM) to afford the product **20** as a white solid (1.10 g, 1.40 mmol, 37 %) over three steps. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.99 (d, J = 2.3 Hz, 1H), 8.68 (dd, J = 4.9, 1.6 Hz, 1H), 8.25 (dt, J = 8.0, 1.9 Hz, 1H), 7.58–7.50 (m, 1H), 4.54 (d, J = 8.6 Hz, 1H), 4.29 (dd, J = 8.3, 5.0 Hz, 1H), 4.23 (d, J = 7.4 Hz, 1H), 3.98–3.77 (m, 5H), 3.72–3.62 (m, 3H), 3.43 (t, J = 6.8 Hz, 2H), 2.28–2.14 (m, 1H), 2.12–1.69 (m, 7H), 1.66–1.50 (m, 2H), 1.44 (s, 9H), 1.26–1.13 (m, 2H), 1.03 (d, J = 6.8 Hz, 3H), 0.94 (d, J = 6.8 Hz, 3H), 0.91 (d, J = 7.4 Hz, 3H), 0.90 (t, J = 7.3 Hz, 6H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 172.7, 171.2, 170.6, 170.5, 170.3, 167.7, 167.0, 166.4, 151.2, 147.8, 135.6, 130.6, 123.7, 81.2, 60.1, 58.4, 55.2, 52.9, 52.8, 42.5, 42.2, 36.8, 36.6, 36.4, 36.3, 28.8, 28.6, 26.8, 24.7, 24.4, 24.3, 14.4, 14.2, 10.0, 9.8; $[a]_D^{25}$ -27.5 (c = 1.0, DCM); IR (thin film) 3282, 3075, 2963, 1735, 1632, 1537, 1447, 1366, 1259 cm^{-1}; HRMS (ESMS) calculated for $C_{38}H_{58}N_8O_{10}$ $[M+H]^+$: 787.4354, found 787.4363.

((2*S*,3*S*)-3-(((2*S*,3*S*)-1-((2-((2-((3-([2,2'-bipyridine]-5-carboxamido)propyl)amino)-2-oxoethyl)amino)-2-oxoethyl)amino)-3-methyl-1-oxopentan-2-yl)carbamoyl)oxirane-2-carbonyl)-*L*-isoleucyl-*L*-proline (**1**). A solution of compound **19** (40.0 mg, 0.05 mmol) in DCM (1.5 mL) was maintained at 0 °C under a nitrogen atmosphere. TFA (71 μL, 0.92 mmol) was added to the reaction mixture. The reaction mixture was allowed to warm to rt and stirred for 8 h.

The reaction mixture was concentrated *in vacuo* and excess TFA was removed by azeotropic distillation from DCM (3 × 5 mL). The TFA salt was dissolved in MeOH (3 mL) and precipitated by adding excess diethyl ether. The product was filtered over celite and washed with diethyl ether (10 mL). The white solid was dissolved in methanol and the solvent was removed *in vacuo* to give product **1** as white solid (42 mg, 88 %). ^{1}H NMR (400 MHz, Methanol-d_4) δ 9.19 (s, 1H), 8.80 (d, J = 5.2 Hz, 1H), 8.62 (d, J = 8.0 Hz, 1H), 8.50 (d, J = 8.4 Hz, 1H), 8.45 (d, J = 7.9 Hz, 1H), 8.35 (t, J = 7.8 Hz, 1H), 7.80 (t, J = 6.4 Hz, 1H), 4.53 (d, J = 8.5 Hz, 1H), 4.38 (dd, J = 8.7, 4.2 Hz, 1H), 4.22 (d, J = 7.4 Hz, 1H), 3.99–3.79 (m, 5H), 3.71–3.65 (m, 1H), 3.69 (d, J = 1.6 Hz, 1H), 3.67 (d, J = 1.6 Hz, 1H), 3.47 (t, J = 6.5 Hz, 2H), 3.34 (t, J = 5.8 Hz, 2H), 2.30–2.17 (m, 1H), 2.08–1.80 (m, 7H), 1.65–1.50 (m, 2H), 1.24–1.12 (m, 2H), 1.01 (d, J = 6.7 Hz, 3H), 0.95 (d, J = 6.7 Hz, 3H), 0.92–0.86 (m, 6H); ^{13}C NMR (101 MHz, Methanol-d_4) δ 173.6, 172.8, 170.7, 170.6, 170.4, 167.8, 167.0, 165.8, 153.1, 151.1, 148.2, 146.0, 142.1, 137.1, 131.4, 126.0, 123.1, 121.4, 59.1, 58.6, 55.3, 52.9, 48.2, 48.0, 47.8, 47.6, 47.4, 47.2, 46.9, 42.5, 42.3, 36.9, 36.6, 36.3, 28.8, 28.5, 24.7, 24.5, 24.3, 14.4, 14.0, 10.0, 9.7; $[a]_D^{25}$ +1.6 (c = 1.0, MeOH); IR (thin film) 3292, 3081, 2967, 2936, 2877, 1738, 1658, 1543, 1455, 1369, 1204, 1134 cm^{-1}; HRMS (ESMS) calculated for $C_{39}H_{54}N_9O_{10}$ $[M+H]^+$: 808.3994, found 808.3984.

((2*S*,3*S*)-3-(((2*S*,3*S*)-3-methyl-1-((2-((2-((3-(nicotinamido)propyl)amino)-2-oxoethyl)amino)-2-oxoethyl)amino)-1-oxopentan-2-yl)carbamoyl)oxirane-2-carbonyl)-*L*-isoleucyl-*L*-proline (2). A solution of compound **20** (200.0 mg, 0.25 mmol) in DCM (4 mL) was maintained at 0 ^{o}C under nitrogen atmosphere. TFA (195 μL, 2.54 mmol) was added to the reaction mixture. The reaction mixture was allowed to warm to rt and stirred for 8 h. The reaction mixture was concentrated *in vacuo* and excess TFA was removed by azeotropic distillation from DCM (3 ×

10 mL). The TFA salt was dissolved in MeOH (5 mL) and precipitated out by adding excess diethyl ether. The product was filtered over celite and washed with diethyl ether (20 mL). The white solid was dissolved in MeOH and the solvent was evaporated *in vacuo* to give product **2** as white solid (166 mg, 89 %).^{1}H NMR (400 MHz, Methanol-d_4) δ 9.09 (s, 1H), 8.78 (d, J = 3.4 Hz, 1H), 8.50 (dt, J = 8.0, 1.9 Hz, 1H), 7.76 (dd, J = 8.1, 5.2 Hz, 1H), 4.54 (d, J = 8.7 Hz, 1H), 4.39 (dd, J = 8.5, 4.1 Hz, 1H), 4.21 (d, J = 7.4 Hz, 1H), 3.98–3.77 (m, 5H), 3.75–3.62 (m, 1H), 3.68 (d, J = 1.6 Hz, 1H), 3.67 (d, J = 1.6 Hz, 1H), 3.45 (t, J = 6.8 Hz, 2H), 2.30–2.20 (m, 1H), 2.12–1.93 (m, 3H), 1.93–1.79 (m, 4H), 1.66–1.51 (m, 2H), 1.28–1.12 (m, 2H), 1.02 (d, J = 6.8 Hz, 3H), 0.95 (d, J = 6.8 Hz, 3H), 0.91 (t, J = 7.4 Hz, 3H), 0.90 (t, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, Methanol-d_4) δ 173.6, 172.8, 170.7, 170.6, 170.4, 167.8, 167.0, 165.2, 148.7, 145.7, 138.5, 131.7, 124.9, 59.1, 58.5, 55.3, 52.9, 48.2, 48.0, 47.8, 47.6, 47.4, 47.2, 46.9, 42.5, 42.2, 36.9, 36.6, 36.3, 28.9, 28.4, 24.7, 24.5, 24.3, 14.4, 14.0, 10.0, 9.7; IR (thin film) 3294, 3076, 2965, 2934, 2882, 1651, 1542, 1450, 1316, 1237, 1197, 1137 cm^{-1}; HRMS (ESMS) calculated for $C_{34}H_{50}N_8O_{10}Na$ $[M+Na]^+$: 753.3572, found 753.3550.

Δ,Λ-[Ru(bpy)$_2$(1)](O$_2$CCF$_3$)$_2$ (3). In a sealable tube, a solution of *cis*-[Ru(bpy)$_2$Cl$_2$] (42 mg, 0.087 mmol), compound **19** (86 mg, 0.099 mmol) and EtOH (6 mL) was maintained at rt and argon was bubbled through the solution for 5 min. The mixture was sealed under an inert atmosphere, wrapped in aluminum foil and heated to 80 °C for 18 h, during which time the color of the reaction mixture changed from dark violet to bright orange. After cooling to rt the solvent was removed *in vacuo* without external heating, resulting in an orange solid. The crude reaction mixture was purified by column chromatography on neutral alumina (0% to 5% MeOH:DCM) to get the intermediate as *t*-butyl ester protected CA-074 analog [Ru(bpy)$_2$(**19**)]Cl$_2$ (53 mg, 0.039 mmol, 45%).

The solution of $[Ru(bpy)_2(\mathbf{19})]Cl_2$ (23 mg, 0.017 mmol) in DCM (1 mL) was maintained at 0 ^oC under argon atmosphere. TFA (26 μL, 0.34 mmol) was added to the reaction mixture. The reaction mixture was allowed to warm to RT and stirred for 18 h. After consumption of the starting material, as judged by TLC analysis, diethyl ether (10 mL) was added to precipitate out an orange solid. The product was filtered over celite and washed with diethyl ether (20 mL). The orange solid was collected by dissolving in MeOH and the solvent was evaporated *in vacuo* to give the product **3** as an orange solid (16 mg, 71 %). mp = 75 °C (decomp); ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.79 (t, J = 8.5 Hz, 2H), 8.71 (d, J = 8.3 Hz, 4H), 8.46 (dd, J = 8.5, 2.0 Hz, 1H), 8.20–8.09 (m, 6H), 7.87 (d, J = 5.6 Hz, 1H), 7.85 (t, J = 4.8 Hz, 2H), 7.80 (t, J = 6.4 Hz, 2H), 7.57–7.45 (m, 5H), 4.53 (d, J = 8.5 Hz, 1H), 4.36 (dd, J = 8.3, 4.4 Hz, 1H), 4.18 (dd, J = 7.3, 2.6 Hz, 1H), 3.95–3.53 (m, 8H), 3.19–3.11 (m, 2H), 2.32–2.12 (m, 1H), 2.12–1.79 (m, 5H), 1.71 (p, J = 6.3 Hz, 2H), 1.66–1.48 (m, 2H), 1.26–1.11 (m, 2H), 1.01 (d, J = 6.7 Hz, 3H), 0.97–0.83 (m, 9H); IR (thin film) 3457, 3013, 2969, 2947, 1740, 1654, 1557, 1443, 1366, 1215, 1094, 900 cm^{-1}; ESMS calcd for $C_{59}H_{69}N_{13}O_{10}Ru$ (M^{+2}) 601.7, found 610.7; UV-vis λ_{max} = 450 nm (ε = 10,000 $M^{-1}cm^{-1}$); Anal. Calcd for $C_{63}H_{81}F_6N_{13}O_{20}Ru$ (**3**·$6H_2O$): C, 48.65; H, 5.25; N, 11.71. Found: C, 48.42; H, 5.04; N, 11.42.

$[Ru(tpy)(bpy)(2)](PF_6)_2$ (4). In a sealable tube, a solution of [Ru(tpy)(bpy)Cl]Cl (550 mg, 0.98 mmol), in EtOH (15 mL) was maintained at RT and argon was bubbled through the solution for 5 min. Silver triflate (755 mg, 2.94 mmol) was added to the reaction mixture and purged with argon for 5 min. The mixture was sealed under an inert atmosphere, wrapped in aluminum foil and heated to 80 °C for 18 h, during which time the color of the reaction mixture changed from dark violet to brown with the appearance of a grey precipitate (AgCl). After cooling to RT, the reaction mixture was filtered over celite to remove AgCl. Diethyl ether was added to the filtrate

resulting in the formation of a brown precipitate. The brown solid was filtered over a Büchner funnel and washed with diethyl ether (50 mL) to obtain [Ru(tpy)(bpy)OTf]OTf (700 mg, 91.0 %). In a sealable tube, a solution of [Ru(tpy)(bpy)OTf]OTf (140 mg, 0.177 mmol), compound **20** (145 mg, 0.185 mmol) in EtOH (8 mL) was maintained at RT and argon was bubbled through the solution for 5 min. The mixture was sealed under an inert atmosphere, wrapped in aluminum foil and heated to 80°C for 6 h, during which time the color of the reaction mixture changed to bright orange from brown. After cooling to rt the solvent was removed *in vacuo* without external heating, resulting in an orange solid. The crude reaction mixture was purified by column chromatography on neutral alumina (0% to 5% MeOH:DCM) to get intermediate as *t*-butyl ester. [Ru(tpy)(bpy)(**20**)](OTf)$_2$. [Ru(tpy)(bpy)(**20**)](OTf)$_2$ was dissolved in distilled water (20 mL) and NH_4PF_6 (100 mg) was added, resulting in the precipitation of an orange solid. The orange solid was filtered over a Büchner funnel and washed with diethyl ether (25 mL) to obtain [Ru(tpy)(bpy)(**20**)](PF$_6$)$_2$ (100 mg, 36%).

A solution of [Ru(tpy)(bpy)(**20**)](PF$_6$)$_2$ (75 mg, 0.047 mmol) in DCM (2 mL) was maintained at 0 ^{o}C under argon atmosphere. TFA (100 μL, 1.44 mmol) was added to the reaction mixture. The reaction mixture was allowed to warm to rt and stirred overnight for 18 h. After consumption of the starting material, as judged by TLC analysis, diethyl ether (15 mL) was added resulting in the formation of an orange precipitate. The orange solid was filtered over celite and washed with diethyl ether (20 mL). The orange solid was collected by dissolving in MeOH and the solvent was evaporated *in vacuo* to give product **4** as an orange solid (54 mg, 75%). mp = 148 °C (decomp); ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.88 (d, J = 8.1 Hz, 1H), 8.77 (d, J = 5.4 Hz, 1H), 8.74 (d, J = 8.2 Hz, 2H), 8.65 (d, J = 8.1 Hz, 2H), 8.62 (d, J = 8.2 Hz, 1H), 8.38 (t, J = 7.8 Hz, 1H), 8.28 (t, J = 8.1 Hz, 1H), 8.23 (d, J = 7.9 Hz, 1H), 8.17 (s, 1H), 8.11

(t, *J* = 7.9 Hz, 2H), 7.98 (d, *J* = 5.6 Hz, 1H), 7.95–7.86 (m, 4H), 7.52 (t, *J* = 6.6 Hz, 2H), 7.43–7.35 (m, 2H), 7.18 (t, *J* = 6.7 Hz, 1H), 4.53 (d, *J* = 8.5 Hz, 1H), 4.36 (dd, *J* = 8.7, 4.4 Hz, 1H), 4.19 (d, *J* = 7.3 Hz, 1H), 3.94–3.59 (m, 8H), 3.28 (t, *J* = 6.7 Hz, 2H), 3.24–3.11 (m, 2H), 2.28–2.18 (m, 1H), 2.11–1.92 (m, 3H), 1.92–1.79 (m, 2H), 1.70 (p, *J* = 6.7 Hz, 2H), 1.66–1.47 (m, 2H), 1.27–1.08 (m, 2H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.92–0.84 (m, 6H); IR (thin film) ν_{max} (cm^{-1}) 3458, 3014, 2969, 2946, 1740, 1659, 1544, 1445, 1367, 1215, 1139, 1092, 900 cm^{-1}; ESMS calcd for $C_{59}H_{69}N_{13}O_{10}Ru$ (M^{+2}) 601.7, found 610.7; UV-vis λ_{max} = 461 nm (ε = 8,800 $M^{-1}cm^{-1}$); Anal. Calcd for $C_{61}H_{74}F_{12}N_{13}O_{10.5}P_2Ru$ (**4**·0.5 Et_2O): C, 47.32; H, 4.82; N, 11.76. Found: C, 47.44; H, 5.06; N, 11.88.

[Ru(tpy)(Me$_2$bpy)(2)](PF$_6$)$_2$ (5) In a sealable tube, a solution of [Ru(tpy)(Me$_2$bpy)Cl]Cl (490 mg, 0.83 mmol), in EtOH (15 mL) was maintained at rt and argon was bubbled through the solution for 5 min. Silver triflate (534 mg, 2.07 mmol) was added to the reaction mixture and the solution purged with argon for 5 min. The mixture was sealed under an inert atmosphere, wrapped in aluminum foil and heated to 80 °C overnight for 18 h, during which time the color of the reaction mixture changed from dark violet to brown with the appearance of a grey precipitate (AgCl). After cooling to rt the reaction mixture was filtered over celite to remove AgCl. Diethyl ether (30 mL) was added to the filtrate resulting in the formation of a brown precipitate. The brown solid was filtered over a Büchner funnel and washed with diethyl ether (50 mL) to obtain [Ru(tpy)(Me$_2$bpy)OTf]OTf (650 mg, 96.0 %). In a sealable tube, solution of [Ru(tpy)(Me$_2$bpy)OTf]OTf (210 mg, 0.26 mmol), compound **20** (212 mg, 0.27 mmol) in EtOH (8 mL) was maintained at rt and argon was bubbled through the solution for 5 min. The mixture was sealed under an inert atmosphere, wrapped in aluminum foil and heated to 80 °C for 6 h, during which time the color of the reaction mixture changed to bright orange from brown. After

cooling to rt the solvent was removed *in vacuo* without external heating, resulting in an orange solid. The crude reaction mixture was purified by column chromatography on neutral alumina (0% to 5% MeOH:DCM) to get intermediate as *t*-butyl ester protected CA-074 analog [Ru(tpy)(Me_2bpy)(**20**)]$(OTf)_2$. [Ru(tpy)(Me_2bpy)(**20**)]$(OTf)_2$ was dissolved in distilled water (20 mL) and NH_4PF_6 (100 mg) was added resulting in the formation of an orange precipitate. The orange solid was filtered over a Büchner funnel and washed with diethyl ether (50 mL) to obtain [Ru(tpy)(Me_2bpy)(**20**)]$(PF_6)_2$ (200 mg, 49.0 %).

A solution of [Ru(tpy)(Me_2bpy)(**20**)]$(PF_6)_2$ (26 mg, 0.016 mmol) in DCM (1 mL) was maintained at 0 ^oC under argon atmosphere. TFA (37 μL, 0.48 mmol) was added to the reaction mixture. The reaction mixture was allowed to warm to RT and stirred overnight for 18 h. After consumption of the starting material, as judged by TLC analysis, diethyl ether (6 mL) was added to precipitate out an orange solid. The product was filtered over celite and washed with diethyl ether (10 mL). The orange solid was dissolved by adding MeOH and was collected in another flask. The solvent was evaporated *in vacuo* to give product **5** as an orange solid (18 mg, 72 %). mp = 151 °C (decomp); ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.78–8.70 (m, 2H), 8.66 (d, J = 7.7 Hz, 2H), 8.60 (d, J = 8.1 Hz, 1H), 8.48 (d, J = 8.1 Hz, 1H), 8.36–8.26 (m, 2H), 8.26–8.19 (m, 2H), 8.19–8.11 (m, 3H), 8.09 (s, 1H), 7.85 (d, J = 5.8 Hz, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.75 (t, J = 7.9 Hz, 1H), 7.69–7.59 (m, 2H), 7.24 (t, J = 6.9 Hz, 1H), 7.02 (d, J = 7.7 Hz, 1H), 4.54 (d, J = 8.6 Hz, 1H), 4.40–4.31 (m, 1H), 4.20 (d, J = 6.8 Hz, 1H), 4.00–3.58 (m, 8H), 3.25 (t, J = 6.0 Hz, 2H) 3.22–3.09 (m, 2H), 2.29–2.17 (m, 1H), 2.10 (s, 3H), 2.07–1.77 (m, 5H), 1.67 (p, J = 6.6 Hz, 2H), 1.63–1.53 (m, 2H), 1.52 (s, 3H), 1.27–1.08 (m, 2H), 1.01 (d, J = 6.4 Hz, 3H), 0.94 (d, J = 6.9 Hz, 3H), 0.92–0.84 (m, 6H); IR (thin film) 3459, 3014, 2969, 2946, 1740, 1655, 1541, 1445, 1366, 1215, 1094, 900 cm^{-1}; ESMS calcd for $C_{61}H_{73}N_{13}O_{10}Ru$ (M^{+2}) 624.7, found 624.7;

UV-vis λ_{max} = 469 nm (ε = 8,700 $M^{-1}cm^{-1}$); Anal. Calcd for $C_{61}H_{75}F_{12}N_{13}O_{11}P_2Ru$ (**5**·H_2O): C, 47.05; H, 4.85; N, 11.69. Found: C, 47.27; H, 5.10; N, 11.50.

[Ru(tpy)(dppn)(2)](PF$_6$)$_2$ (6) In a sealable tube, a solution of [Ru(tpy)(dppn)Cl]Cl (385 mg, 0.52 mmol) and EtOH (12 mL) was maintained at rt and argon was bubbled through the solution for 5 min. Silver triflate (402 mg, 1.56 mmol) was added to the reaction mixture and the solution was purged with argon for 5 min. The mixture was sealed under an inert atmosphere, wrapped in aluminum foil and heated to 80 °C overnight for 18 h, during which time the color of the reaction mixture changed from dark violet to brown with the appearance of a grey precipitate (AgCl). After cooling to rt the reaction mixture was filtered over celite to remove AgCl. Diethyl ether was added to the filtrate to precipitate out a brown solid. The brown solid was filtered over a Büchner funnel and washed with diethyl ether (50 mL) to obtain [Ru(tpy)(dppn)OTf]OTf (490 mg, 97 %). In a sealable tube, a solution of [Ru(tpy)(dppn)OTf]OTf (415 mg, 0.43 mmol), compound **20** (352 mg, 0.45 mmol) and EtOH (15 mL) was maintained at RT and argon was bubbled through the solution for 5 min. The mixture was sealed under an inert atmosphere, wrapped in aluminum foil and heated to 80 °C for 6 h, during which time the color of the reaction mixture changed to from brown to bright orange. After cooling to RT the solvent was removed *in vacuo* without external heating, resulting in an orange solid. The crude reaction mixture was purified by column chromatography on neutral alumina (0% to 6% MeOH:DCM) to give the intermediate *t*-butyl ester [Ru(tpy)(dppn)(**20**)](OTf)$_2$. [Ru(tpy)(dppn)(**20**)](OTf)$_2$ was dissolved in distilled water (25 mL) and NH_4PF_6 (200 mg) was added resulting in the precipitation of an orange solid. The orange solid was filtered over a Büchner funnel and washed with diethyl ether (50 mL) to obtain [Ru(tpy)(dppn)(**20**)](PF$_6$)$_2$ (330 mg, 43%).

A solution of [Ru(tpy)(bpy)(**20**)]$(PF_6)_2$ (73 mg, 0.042 mmol) in DCM (2 mL) was maintained at 0 °C under argon atmosphere. TFA (100 μL, 1.30 mmol) was added to the reaction mixture. The reaction mixture was allowed to warm to rt and stirred overnight for 18 h. After consumption of the starting material, as judged by TLC analysis, diethyl ether (15 mL) was added resulting in the formation of an orange solid. The orange solid was filtered over celite and washed with diethyl ether (20 mL). The orange solid was collected by dissolving in MeOH. The solvent was evaporated *in vacuo* to give the product **6** as an orange solid (52 mg, 73%). mp = 183 °C (decomp); ^{1}H NMR (400 MHz, Methanol-d_4) δ 10.00 (d, J = 8.1 Hz, 1H), 9.47 (d, J = 8.1 Hz, 1H), 9.22 (d, J = 5.2 Hz, 1H), 9.08 (s, 1H), 9.03 (s, 1H), 8.82 (d, J = 8.0 Hz, 2H), 8.70 (d, J = 8.0 Hz, 2H), 8.45–8.25 (m, 6H), 8.16–8.07 (m, 3H), 8.04 (t, J = 6.2 Hz, 2H), 7.83 (d, J = 5.4 Hz, 1H), 7.70–7.58 (m, 3H), 7.53–7.39 (m, 3H), 4.49 (d, J = 8.5 Hz, 1H), 4.32 (dd, J = 8.7, 4.4 Hz, 1H), 4.15 (d, J = 7.2 Hz, 1H), 3.94–3.55 (m, 8H), 3.25–3.13 (m, 2H), 2.24–2.13 (m, 1H), 2.06–1.76 (m, 5H), 1.71 (p, J = 6.6 Hz, 2H), 1.62–1.44 (m, 2H), 1.23–1.07 (m, 2H), 0.99 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 6.7 Hz, 3H), 0.89–0.83 (m, 6H); IR (thin film) 3459, 3013, 2969, 2947, 1740, 1551, 1445, 1367, 1215, 1094, 900 cm^{-1}; ESMS calcd for $C_{71}H_{73}N_{15}O_{10}Ru$ (M^{+2}) 698.7, found 698.7; UV-vis λ_{max} = 471 nm (ε = 13,800 $M^{-1}cm^{-1}$); Anal. Calcd for $C_{72}H_{85}F_{12}N_{15}O_{15}P_2Ru$ (**6**·$4H_2O$·CH_3OH): C, 48.27; H, 4.78; N, 11.73. Found: C, 48.66; H, 4.51; N, 11.37.

[Ru(tpy)(Me$_2$dppn)(2)](PF$_6$)$_2$ (7) In a sealable tube, a solution of [Ru(tpy)(Me$_2$dppn)Cl]Cl (250 mg, 0.33 mmol), in EtOH (8 mL) was maintained at rt and argon was bubbled through the solution for 5 min. Silver triflate (252 mg, 0.98 mmol) was added to the reaction mixture and the solution was purged with argon for 5 min. The mixture was sealed under an inert atmosphere, wrapped in aluminum foil and heated to 80 °C for 18 h, during which time the color of the reaction mixture changed to brown from dark violet with formation of a grey precipitate (AgCl).

After cooling to rt, the reaction mixture was filtered over celite to remove AgCl. Diethyl ether was added to the filtrate resulting in the formation of a brown solid. The brown solid was isolated by filtration over a Büchner funnel and was washed with diethyl ether (30 mL) to obtain [Ru(tpy)(Me_2dppn)OTf]OTf (312 mg, 96%). In a sealable tube, a solution of [Ru(tpy)(Me_2dppn)OTf]OTf (267 mg, 0.269 mmol), compound **20** (220 mg, 0.280 mmol) in EtOH (10 mL) was maintained at rt and argon was bubbled through the solution for 5 min. The mixture was sealed under an inert atmosphere, wrapped in aluminum foil and heated to 80 °C for 6 h, during which time the color of the reaction mixture changed from brown to bright orange. After cooling to rt the solvent was removed *in vacuo* without external heating, resulting in the formation of an orange solid. The crude reaction mixture was purified by column chromatography on neutral alumina (0% to 6% MeOH:DCM) to give intermediate *t*-butyl ester [Ru(tpy)(Me_2dppn)(**20**)]$(OTf)_2$. [Ru(tpy)(Me_2dppn)(**20**)]$(OTf)_2$ was dissolved in distilled water (25 mL) and NH_4PF_6 (200 mg) was added resulting in the formation of an orange precipitate that was filtered over a Büchner funnel and washed with diethyl ether (50 mL) to obtain [Ru(tpy)(Me_2dppn)(**20**)]$(PF_6)_2$ (200 mg, 42%).

A solution of [Ru(tpy)(Me_2dppn)(**20**)]$(PF_6)_2$ (60 mg, 0.033 mmol) in DCM (2 mL) was maintained at 0 °C under argon atmosphere. TFA (78 μL, 1.02 mmol) was added to the reaction mixture. The reaction mixture was allowed to warm to rt and stirred for 18 h. After consumption of the starting material, as judged by TLC analysis, diethyl ether (15 mL) was added resulting in the formation of an orange precipitate. The precipitate was filtered over celite and washed with diethyl ether (20 mL). The orange solid dissolved in methanol and the solvent was evaporated *in vacuo* to give product **7** as an orange solid (36 mg, 62%). mp = 187 °C (decomp); ^{1}H NMR (400 MHz, Methanol-d_4) δ 9.97 (d, J = 8.4 Hz, 1H), 9.43 (d, J = 8.2 Hz, 1H), 9.11 (s, 1H), 9.04 (s,

1H), 8.78 (d, *J* = 8.3 Hz, 1H), 8.74 (d, *J* = 8.0 Hz, 1H), 8.68 (t, *J* = 7.5 Hz, 2H), 8.33–8.25 (m, 5H), 8.25–8.10 (m, 5H), 7.93 (d, *J* = 5.7 Hz, 1H), 7.73–7.64 (m, 2H), 7.50 (t, J = 6.9 Hz, 2H), 7.47 (d, *J* = 8.2 Hz, 1H), 7.34–7.26(m, 1H), 4.54–4.45 (m, 1H), 4.36–4.30 (m, 1H), 4.18–4.11 (m, 1H), 3.93–3.55 (m, 8H), 3.28–3.23 (m, 2H), 3.21–3.10 (m, 2H), 2.38 (s, 3H), 2.26–2.11 (m, 1H), 2.09–1.85 (m, 3H), 1.86–1.81 (m, 3H), 1.82 (s, 3H), 1.67 (p, *J* = 6.6 Hz, 2H), 1.61–1.46 (m, 2H), 1.23–1.07 (m, 2H), 1.05–0.95 (m, 3H) 0.95–0.82 (m, 9H); IR (thin film) 3459, 3014, 2969, 2949, 1740, 1549, 1444, 1366, 1215, 1094, 900 cm^{-1}; ESMS calcd for $C_{73}H_{77}N_{15}O_{10}Ru$ (M^{+2}) 712.7, found 712.7; UV-vis λ_{max} = 482 nm (ε = 13,700 $M^{-1}cm^{-1}$); Anal. Calcd for $C_{77}H_{87}F_{12}N_{15}O_{11}P_2Ru$ (**7**·Et_2O): C, 51.68; H, 4.90; N, 11.74. Found: C, 51.94; H, 5.01; N, 12.13.

Part B. Enzyme Inhibition Studies

Progress curves with Cathepsin B for compound **1** – **7**.

Progress curves for cathepsin B were collected using CSTB (4 nM), Z-Arg-Arg-AMC (100 μM), **1**-**7** (0.00 –10.0 nM) in 0.4 M acetate buffer, pH 5.5, <1 % DMSO, 4 mM EDTA, 0.01% Triton X-100, DTT = 8 mM at 25 °C as described previously.[1]
Data are averages of three independent experiments with errors equal to standard deviations.

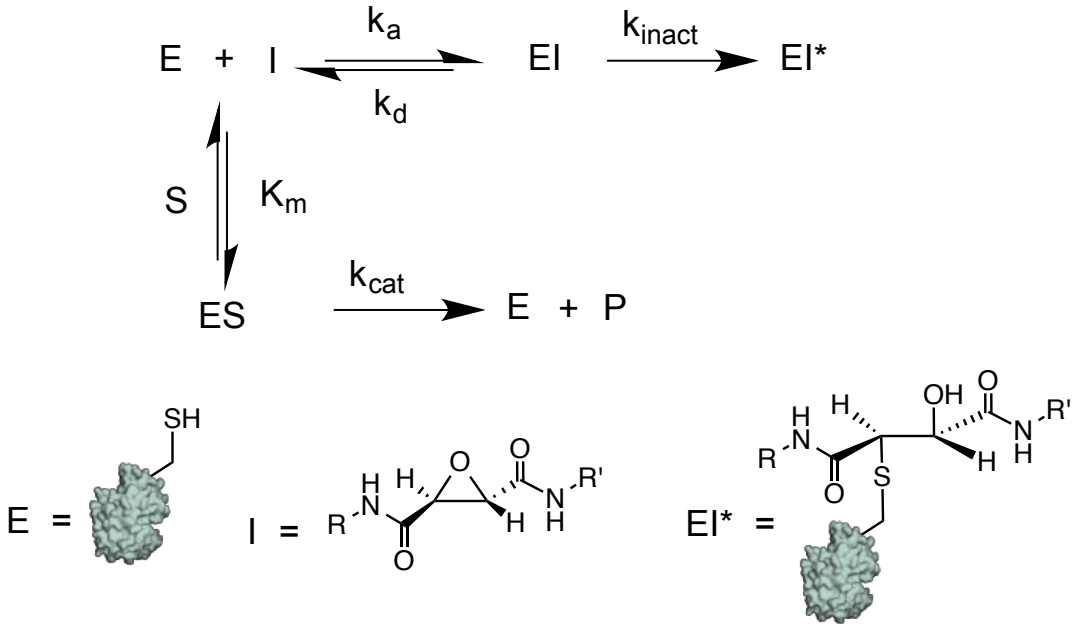

Figure S1. Model for competitive, irreversible inactivation of cathepsin B by epoxysuccinyl inhibitors with reversible formation of the enzyme inhibitor complex (EI) with inhibitor (I) to form irreversible covalent complex (EI*).

```
Cathepsin B:
;____________________________________________________________________________
[task]
   data = progress
   task = fit
   model = two steps ?
[mechanism]
   E + S <==> E.S     :    ka.S  kd.S
   E.S ---> E + P     :    kd.P
   E + I <==> E.I     :    ka.I  kd.I
   E.I --> E-I        :    k.inact
[constants]
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   kd.P = 10 ?
   ka.I = 100 ? , kd.I = 0.1 ?
   k.inact = 0.1 ?
[concentrations] | E = 0.004 ?, S = 100 ?
[responses]
   P = 800 ? (500 .. 2000)
[progress]
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   sheet      033020181.csv
   column 2 | conc I = 0.00 | offset auto ? | label I = 0
   column 3 | conc I = 0.00025 ? (0.0002 .. 0.0003)| offset auto ? | label I = 0.25nm
   column 4 | conc I = 0.0005 ? (0.0004 .. 0.0006)| offset auto ? | label I = 0.5nm
   column 5 | conc I = 0.001 ? (0.0008 .. 0.0012)| offset auto ? | label I = 1nm
   column 6 | conc I = 0.0025 ? (0.002 .. 0.003) | offset auto ? | label I = 2.5nm
   column 7 | conc I = 0.005 ? (0.004 .. 0.006) | offset auto ? | label I = 5nm
   column 8 | conc I = 0.01 ? (0.008 .. 0.012) | offset auto ? | label I = 10nm


[settings] {Constraints} | Concentrations = 0.01
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   task = fit
   model = equilibrium ?
[constants]
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   kd.P = 10 ?
   ka.I = 10, kd.I = 0.1 ?
   k.inact = 0.1 ?
;____________________________________________________________________________
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   task = fit
   model = one step ?
[mechanism]
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   E.S ---> E + P     :    kd.P
   E + I ---> E-I     :    k.inact
[constants]
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   kd.P = 10 ?
   k.inact = 0.1 ?
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;____________________________________________________________________________
;____________________________________________________________________________
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Figure S2. Example of Dynafit script used in fitting progress curve for compounds **1** – **7**.

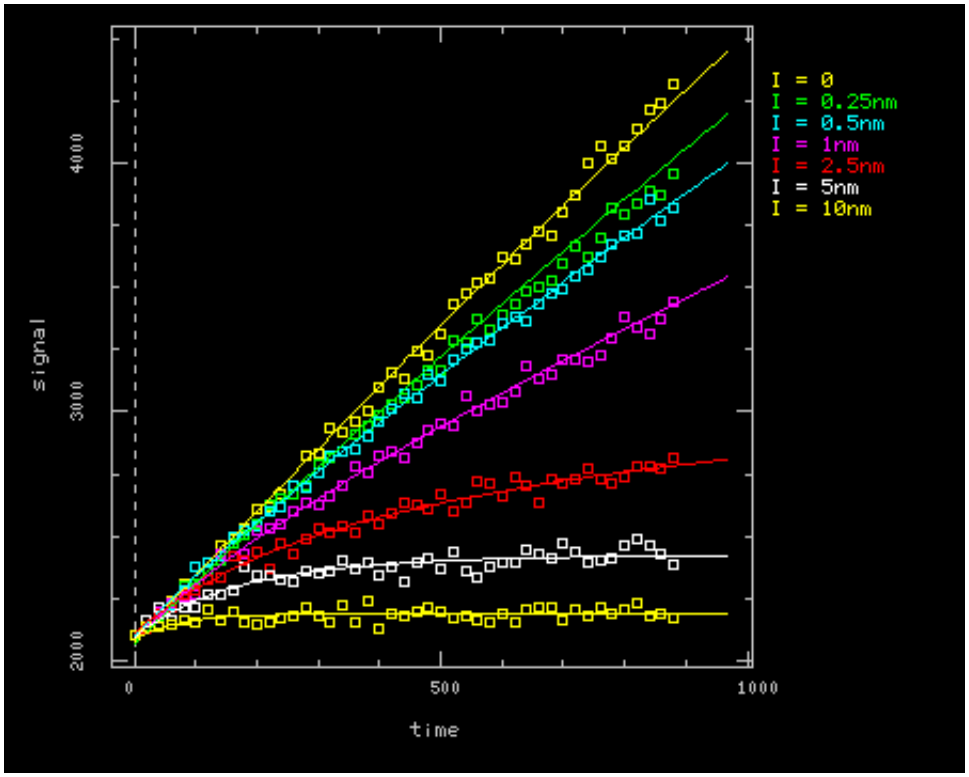

Figure S3. Experimental data (squares) vs. estimated fit lines for **1** (0 – 10 nM) and Cathepsin B using the competitive, twostep irreversible inhibition model shown in Figure S1.

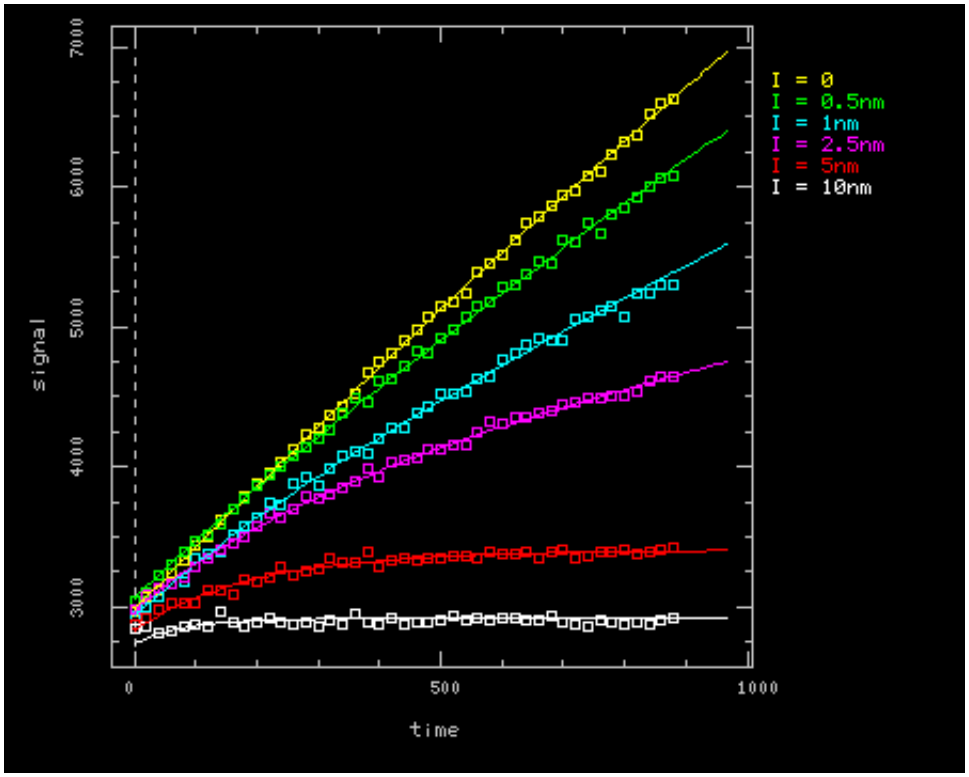

Figure S4. Experimental data (squares) vs. estimated fit lines for **2** (0 – 10 nM) and Cathepsin B using the competitive, twostep irreversible inhibition model shown in Figure S1.

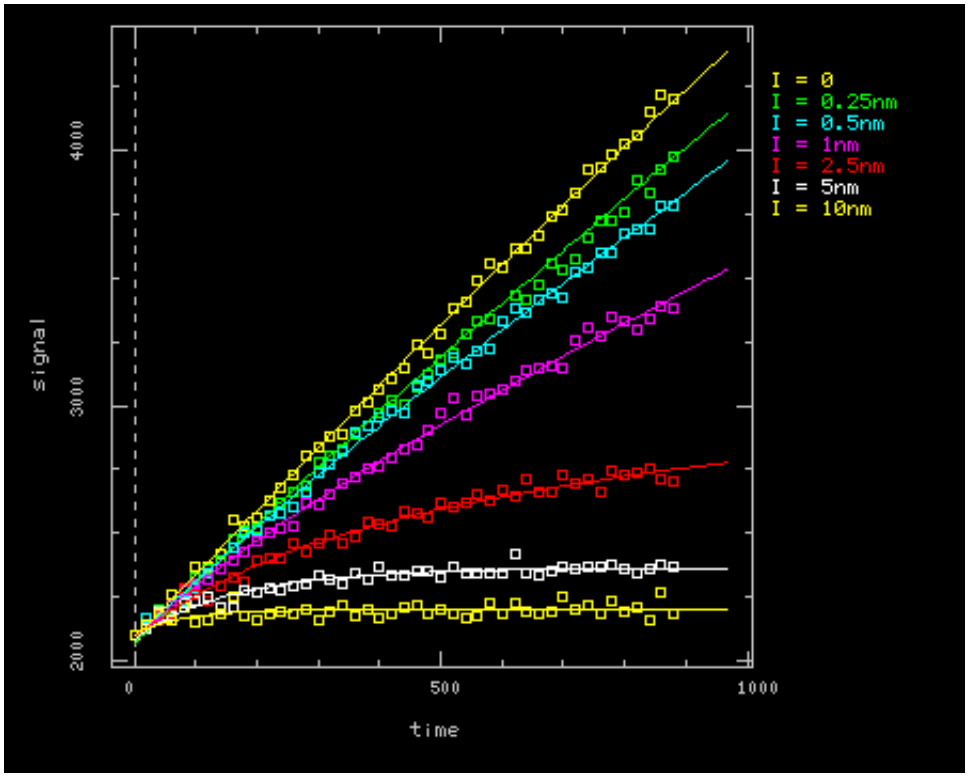

Figure S5. Experimental data (squares) vs. estimated fit lines for **3** (0 – 10 nM) and Cathepsin B using the competitive, twostep irreversible inhibition model shown in Figure S1.

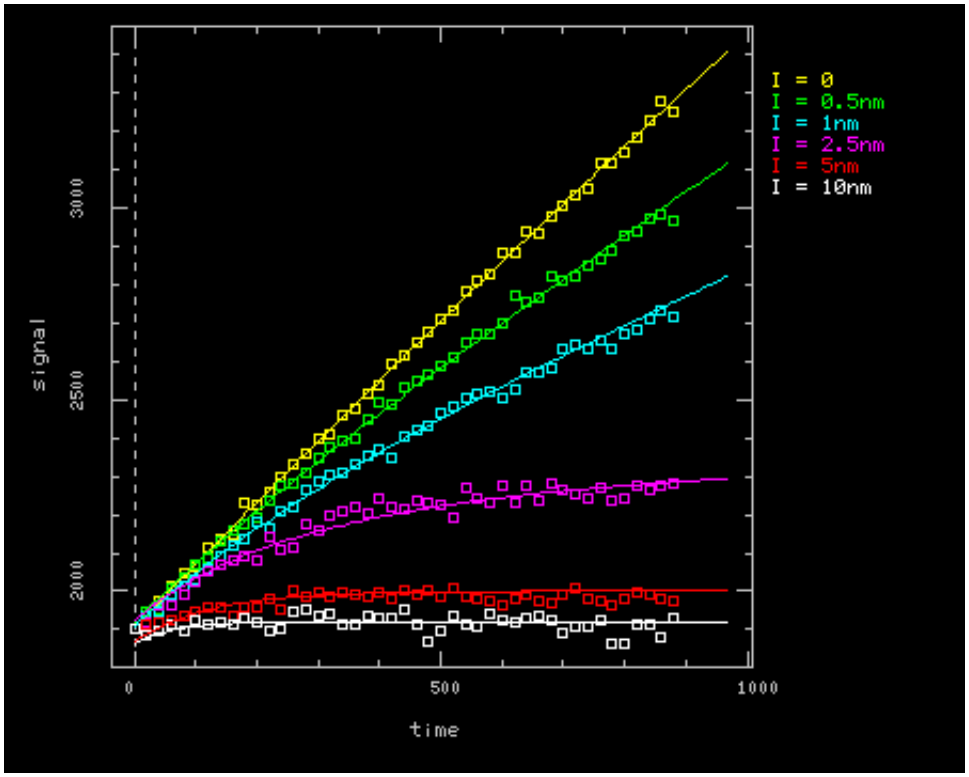

Figure S6. Experimental data (squares) vs. estimated fit lines for **4** (0 – 10 nM) and Cathepsin B using the competitive, twostep irreversible inhibition model shown in Figure S1.

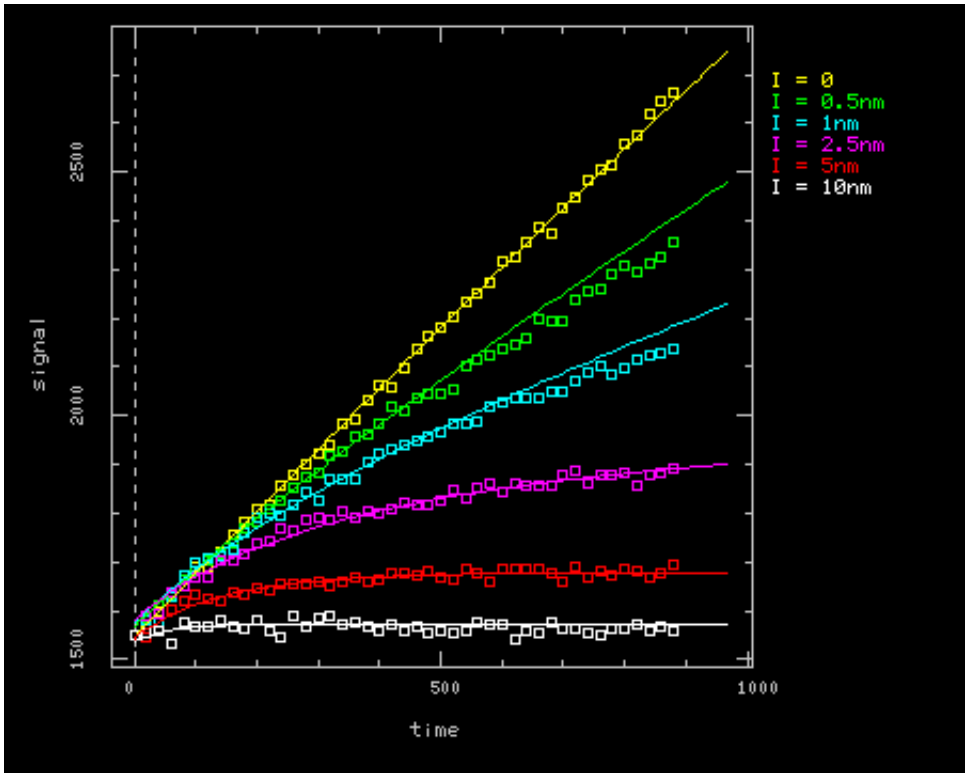

Figure S7. Experimental data (squares) vs. estimated fit lines for **5** (0 – 10 nM) and Cathepsin B using the competitive, twostep irreversible inhibition model shown in Figure S1.

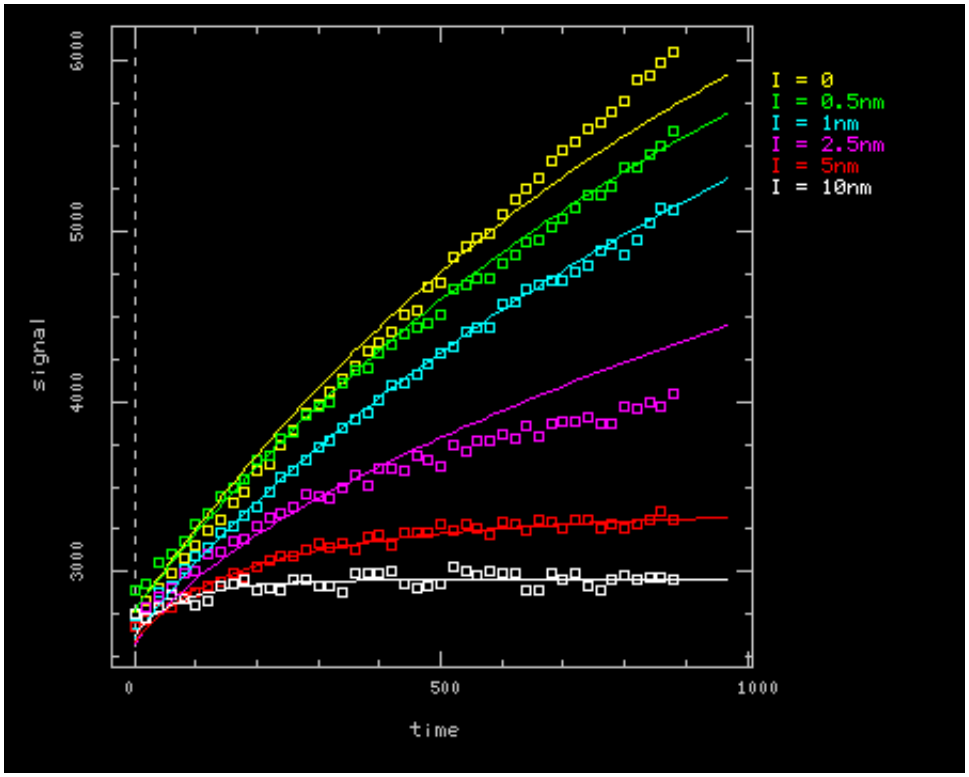

Figure S8. Experimental data (squares) vs. estimated fit lines for **6** (0 – 10 nM) and Cathepsin B using the competitive, twostep irreversible inhibition model shown in Figure S1.

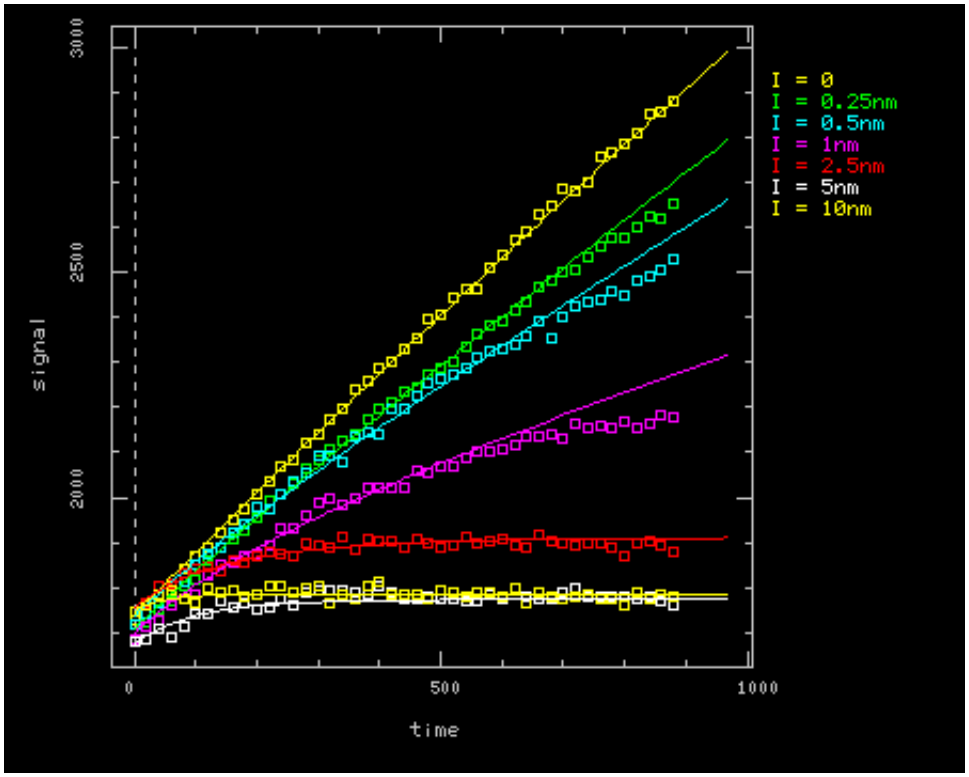

Figure S9. Experimental data (squares) vs. estimated fit lines for **7** (0 – 10 nM) and Cathepsin B using the competitive, twostep irreversible inhibition model shown in Figure S1.

Part C. Cell Studies

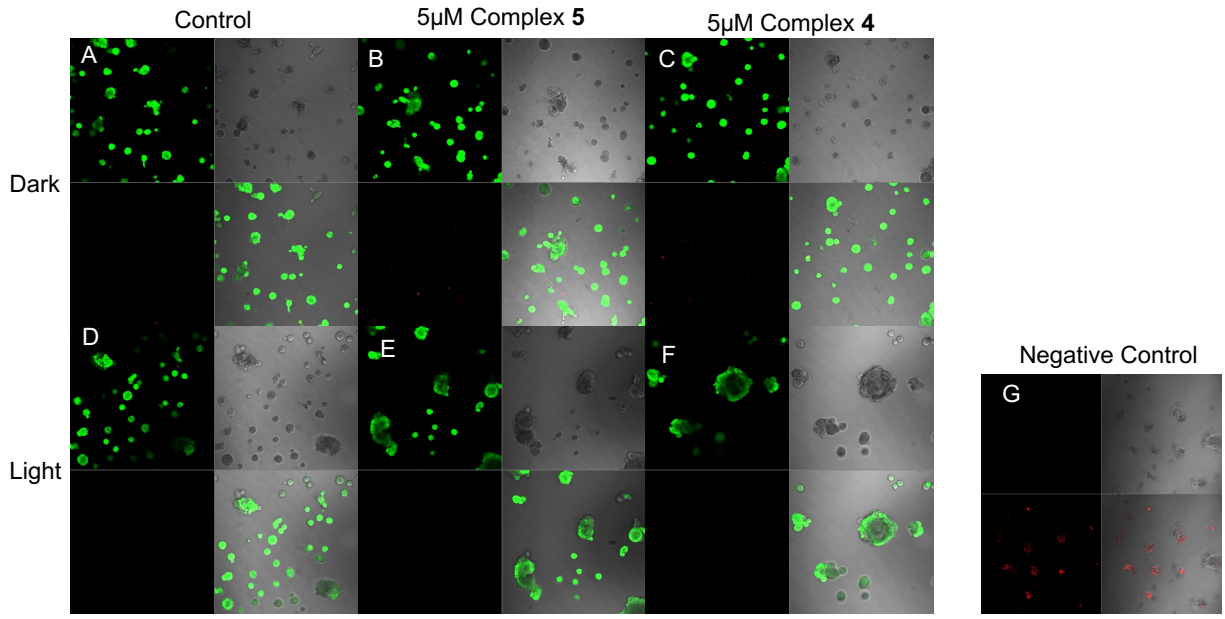

Figure S10: Evaluation of cytotoxic effects in MDA-MB-231 cells. Cells were treated with DMSO (Control, **A,D**), 5μM Complex **5** (**B,E**) or 5μM Complex 4 (**C,F**) and placed in the dark (Dark, **A-C**), or irradiated with light (Light, **D-F**). Cells were stained with 2μM Calcein AM and 5μM Ethidium homodimer-1 for 30 minutes and imaged. Live cells shown as green in top left panels and merged panels. Dead cells shown as red in bottom left panels and merged panels. DIC (differential interference contrast) images show general morphology in top right panels and merged panels. **G)** Cells permeabilized with 0.2% Triton-X were used as negative control.

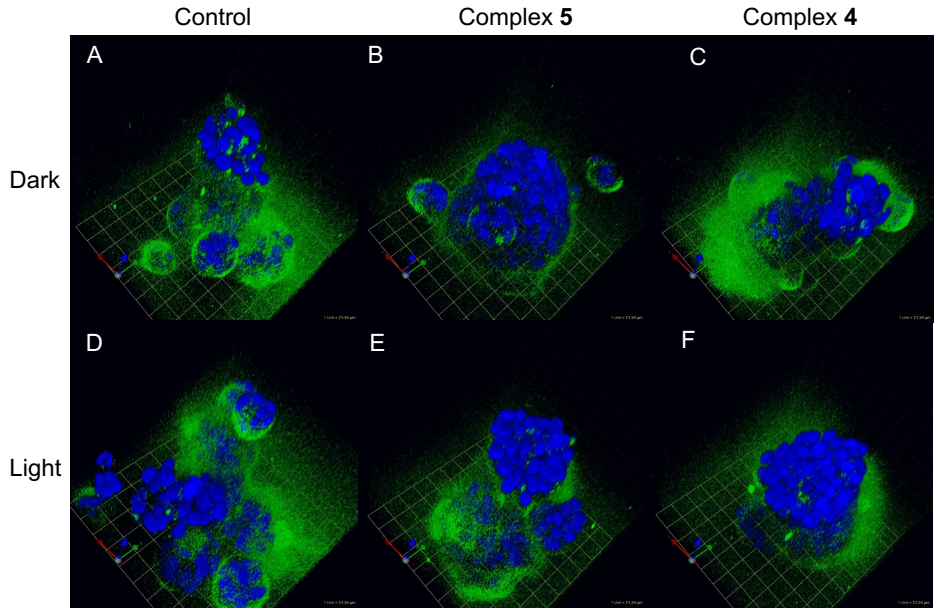

Figure S11: Representative DQIV proteolysis images of MDA-MB-231 cells. Cells were treated with DMSO (**A,D**), 5µM Complex **5** (**B,E**), or 5µM Complex **4 (C,F**) and placed under dark conditions (Dark, **A-C**), or irradiated (Light, **D-F**). Cells were imaged and the z-stacks were reconstructed to show DQIV degradation (green).

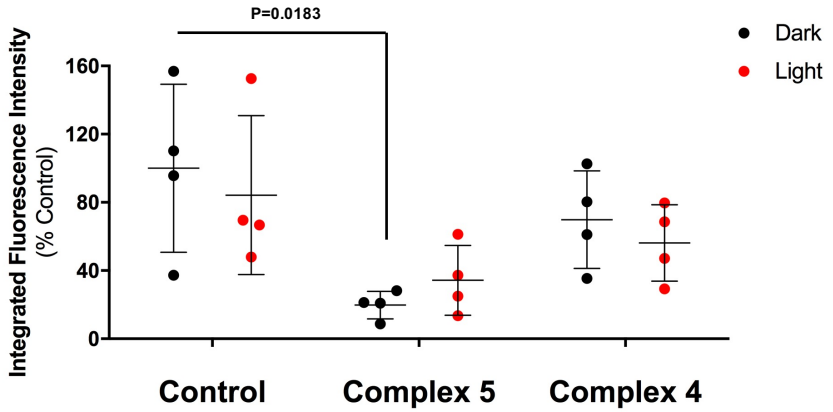

Figure S12: Quantification of DQIV proteolysis. DQ-collagen IV degradation shown as fluorescence intensity/cell shown in comparison to control (DMSO) conditions in dark (black) and light (red) in the presence of 5µM Complex **5** and 5µM Complex **4**. Proteolysis was quantified in each 3D reconstructed spheroid using Volocity Software, and is shown as fluorescence intensity per cell in the entire volume. Data depict representative experiment with four individual spheroid reconstructions.

Part D. ^{1}H, ^{13}C NMR, HRMS spectra and for new compounds

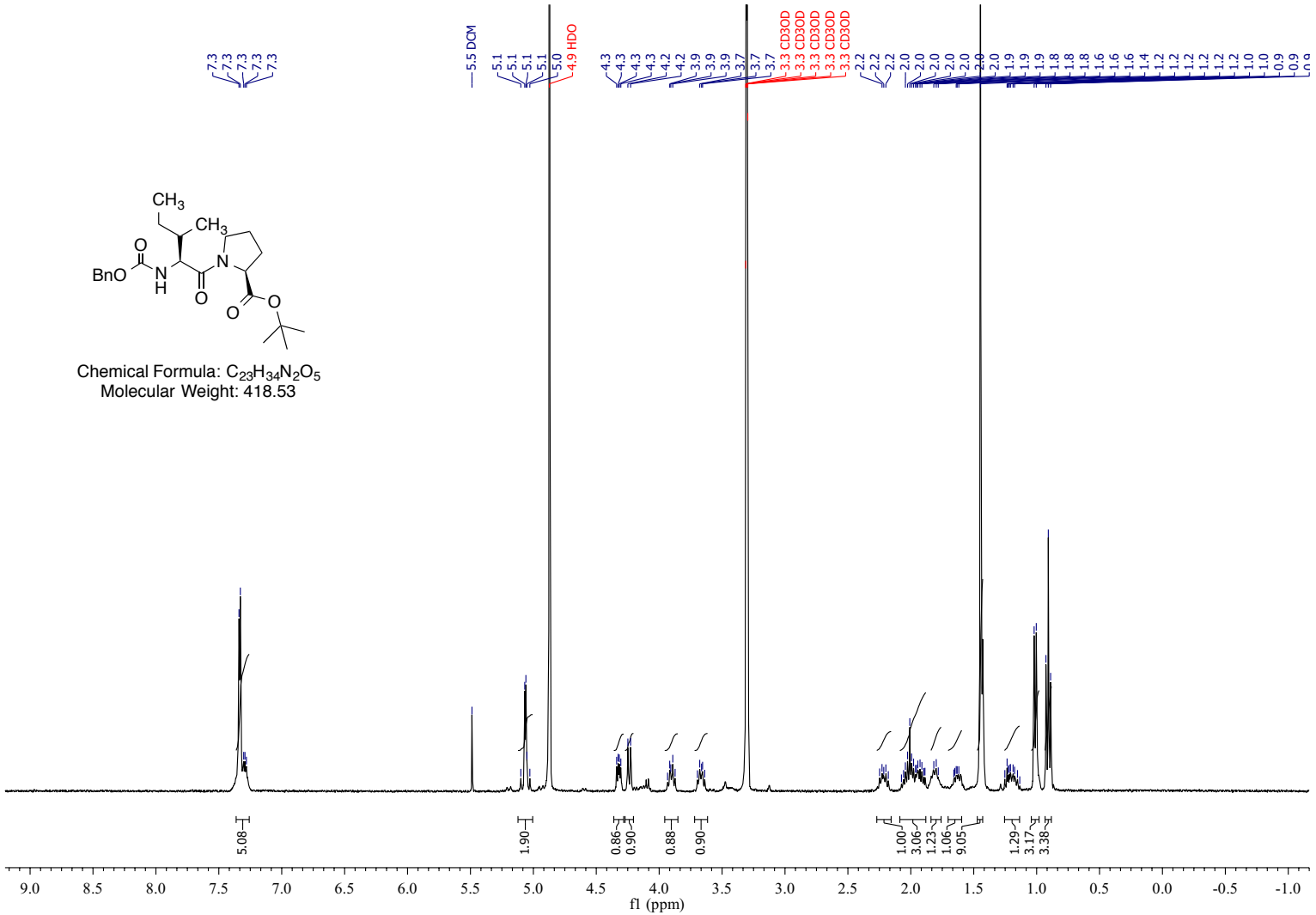

Figure S13: ^{1}H NMR of compound **10** in CD_3OD

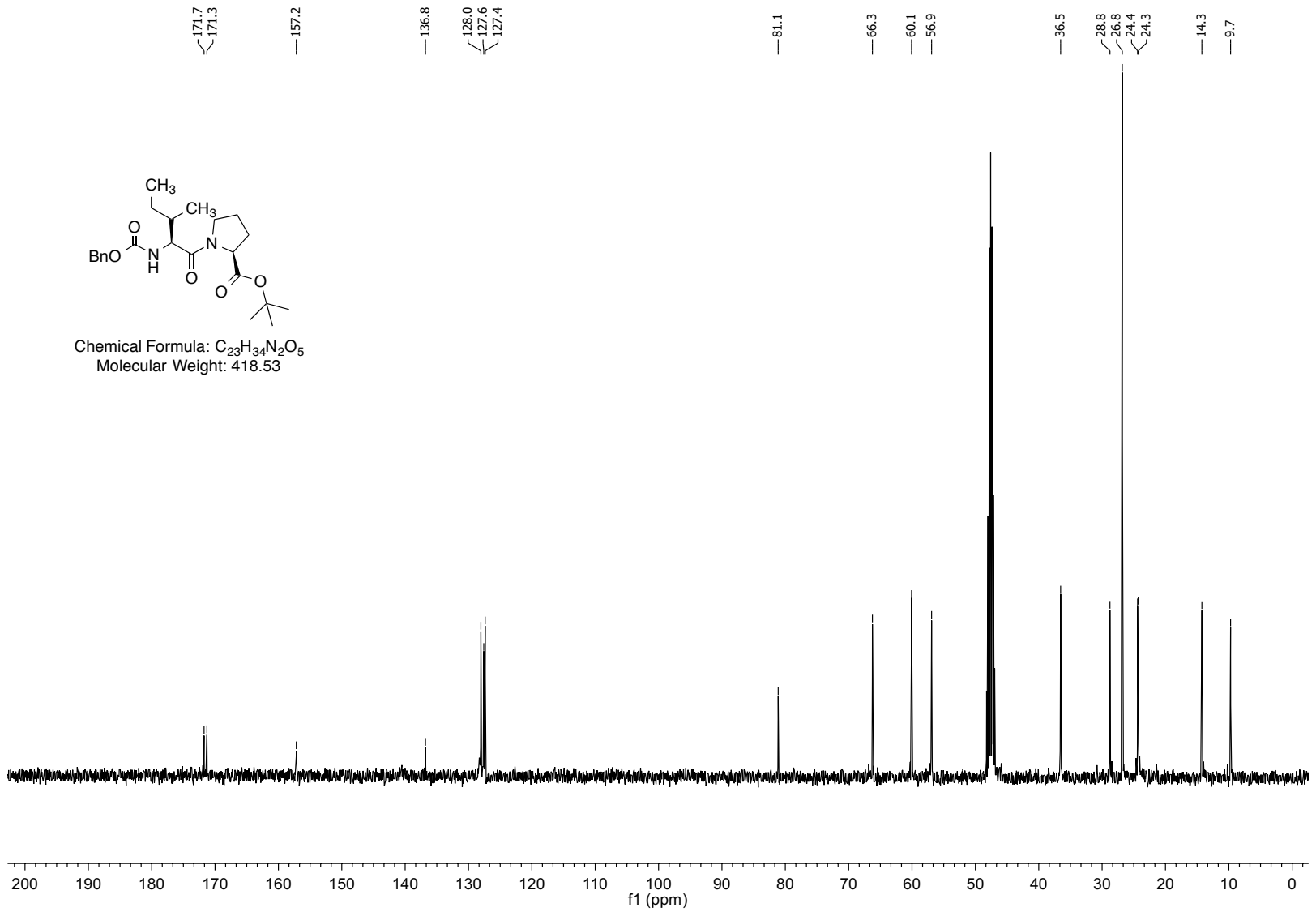

Figure S14: ^{13}C NMR of compound **10** in CD_3OD

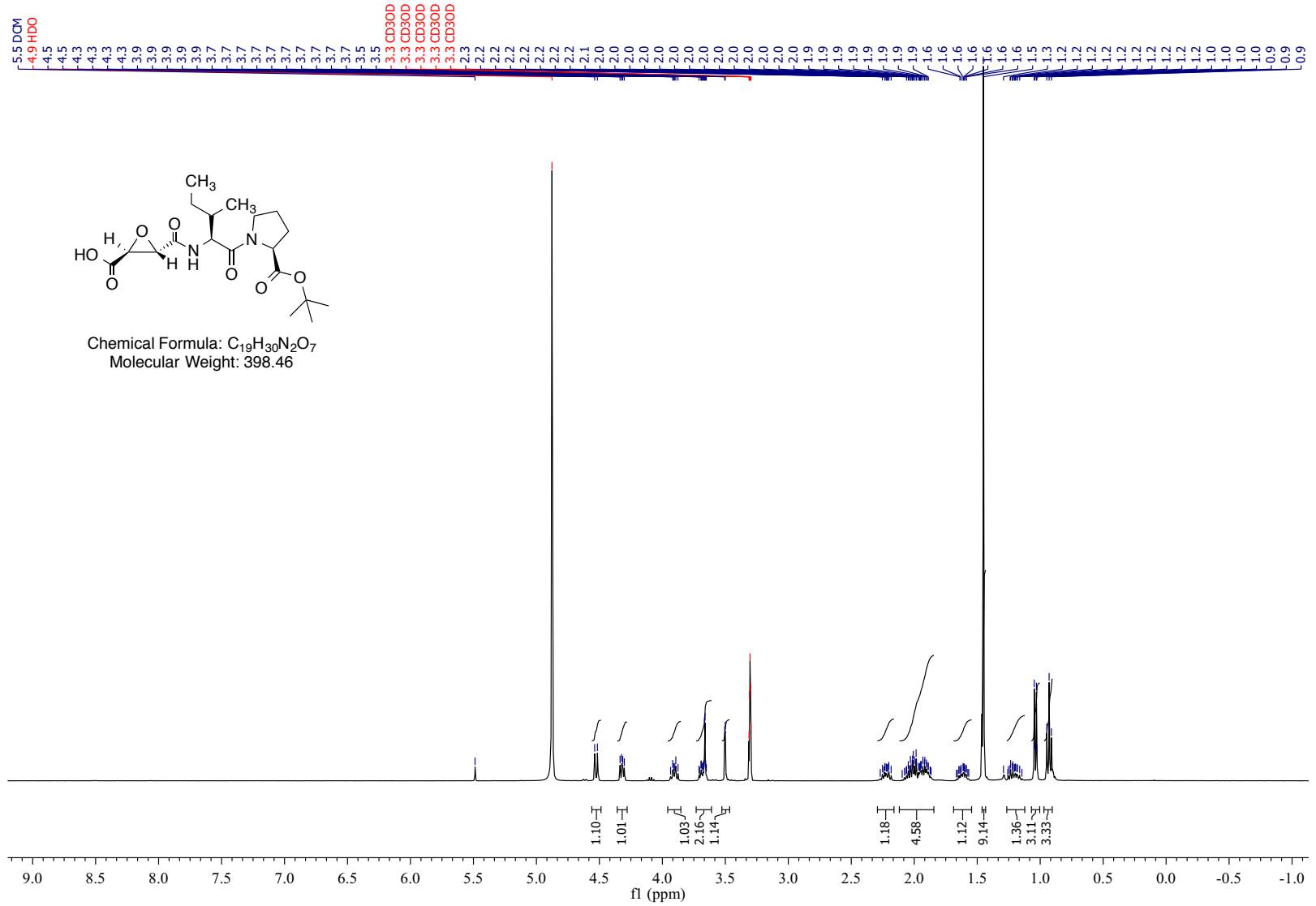

Figure S15: ^{1}H NMR of compound **12** in CD_3OD

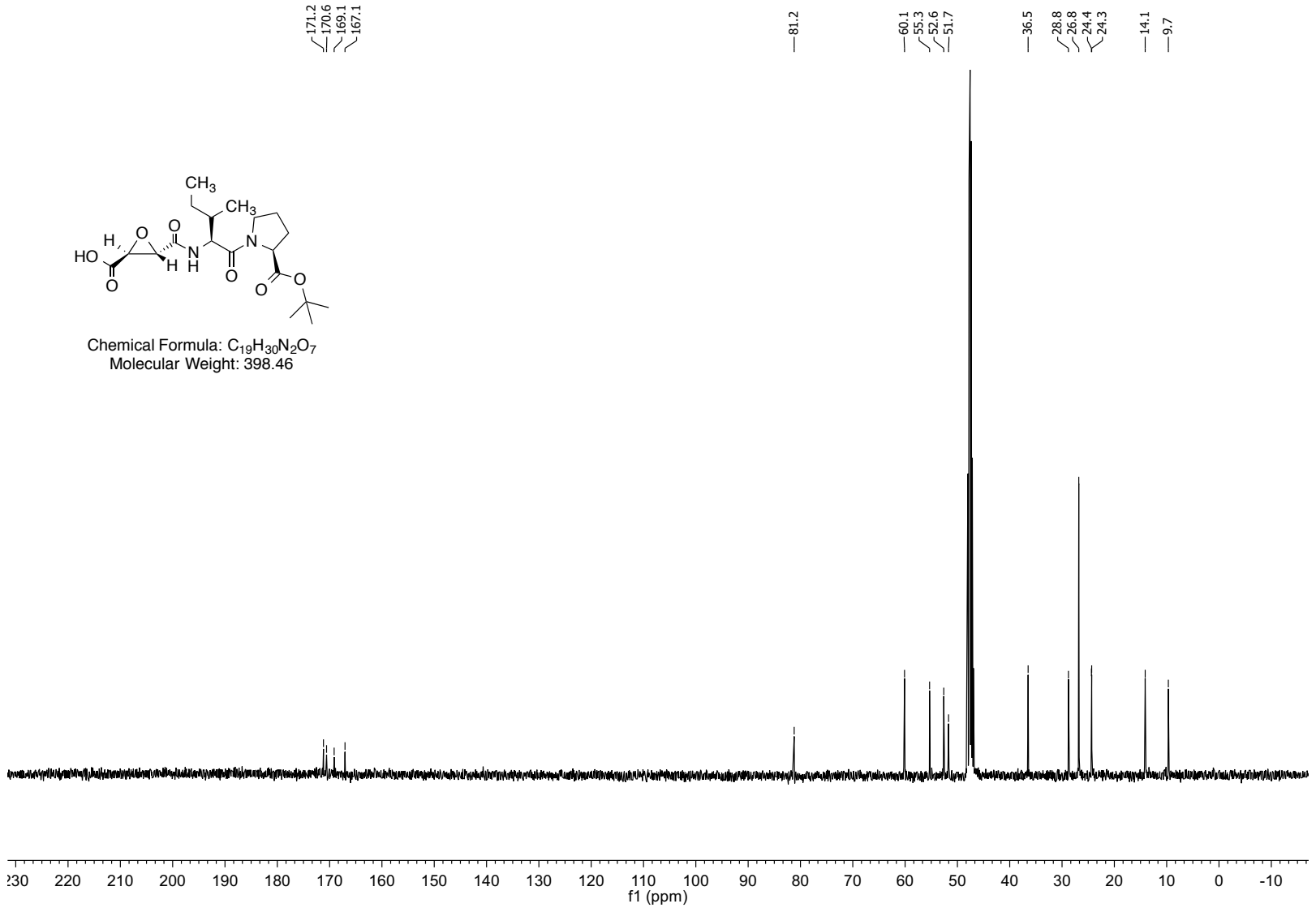

Figure S16: ^{13}C NMR of compound **12** in CD_3OD

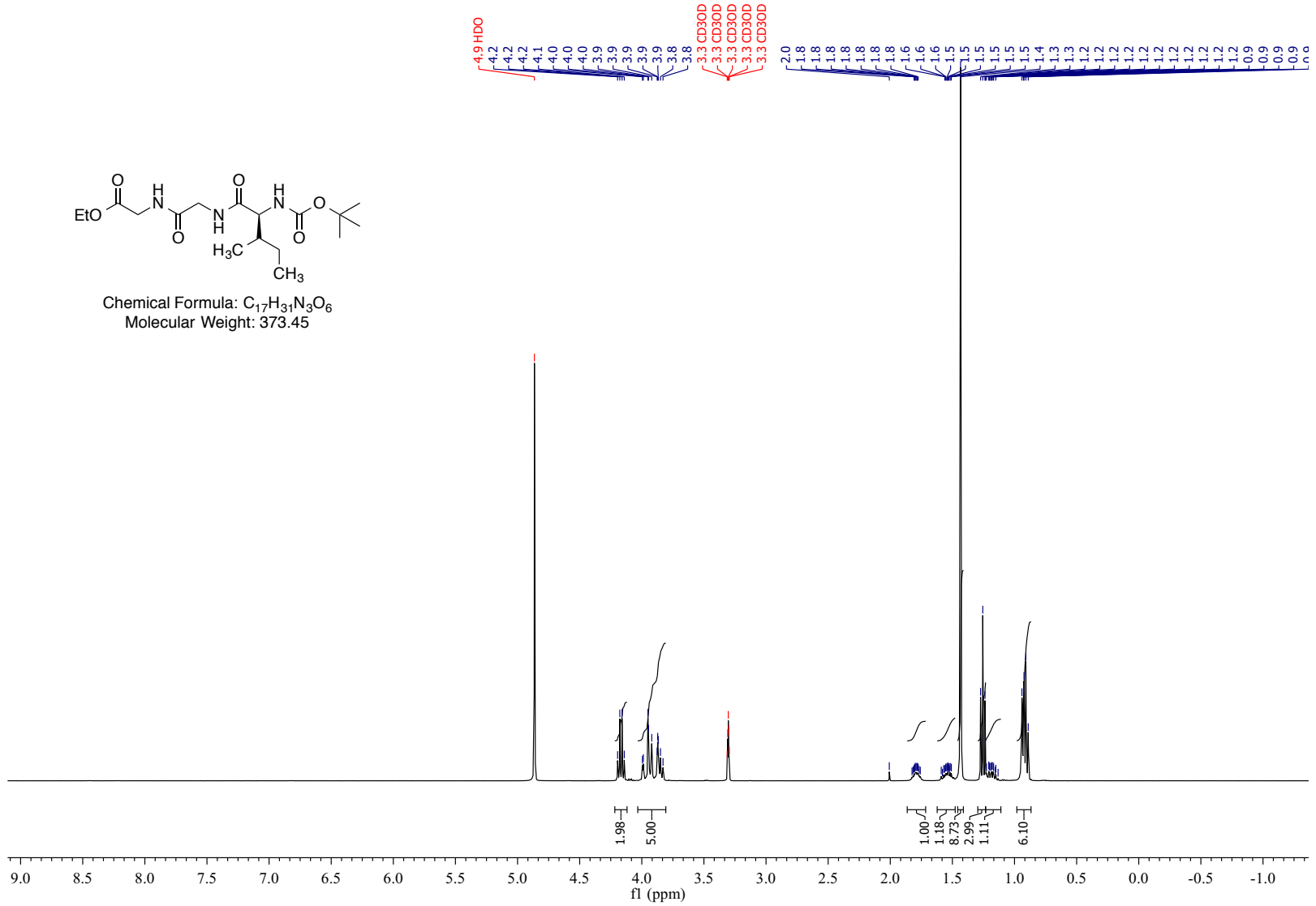

Figure S17: ^{1}H NMR of compound **14** in CD_3OD

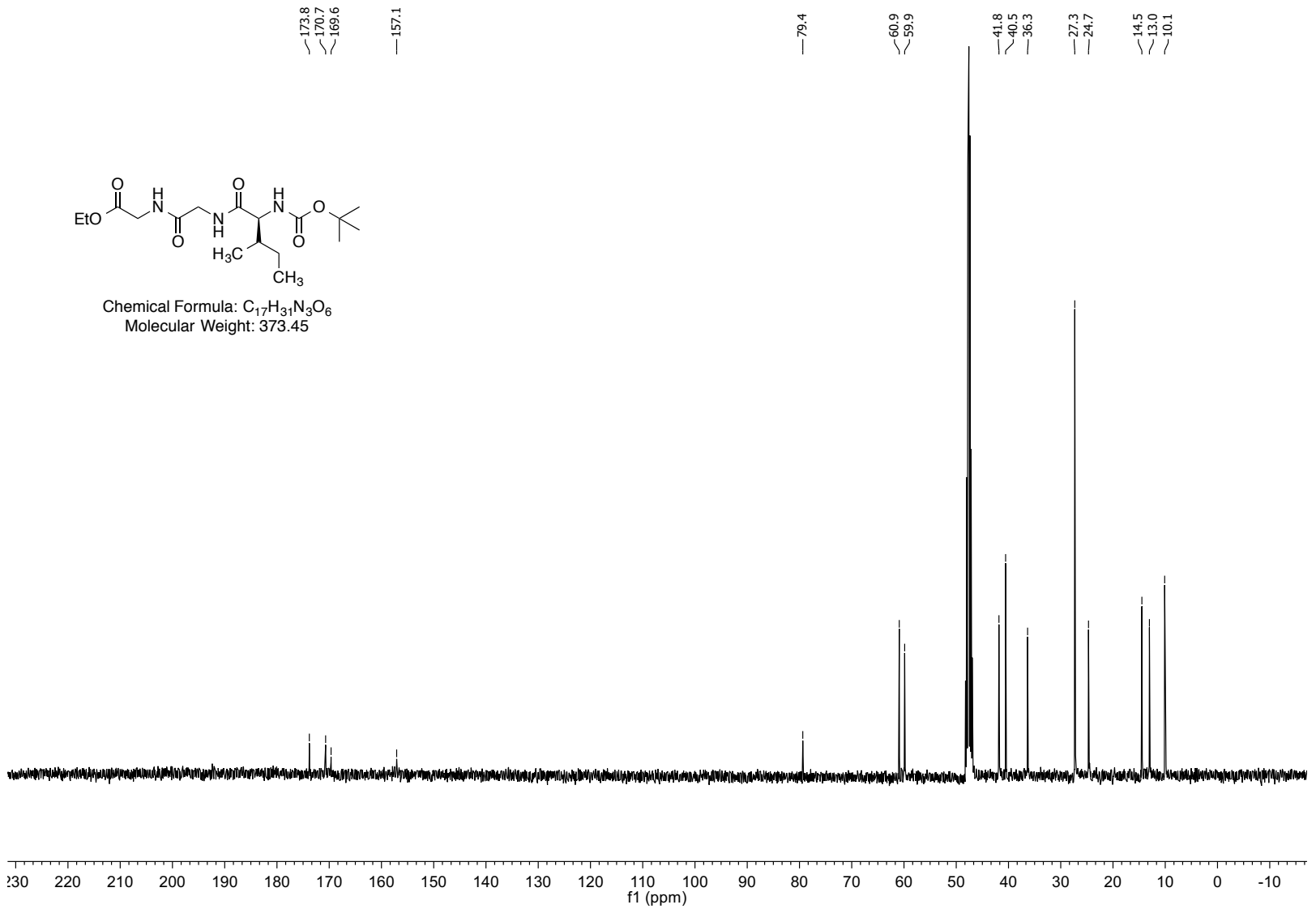

Figure S18: ^{13}C NMR of compound **14** in CD_3OD

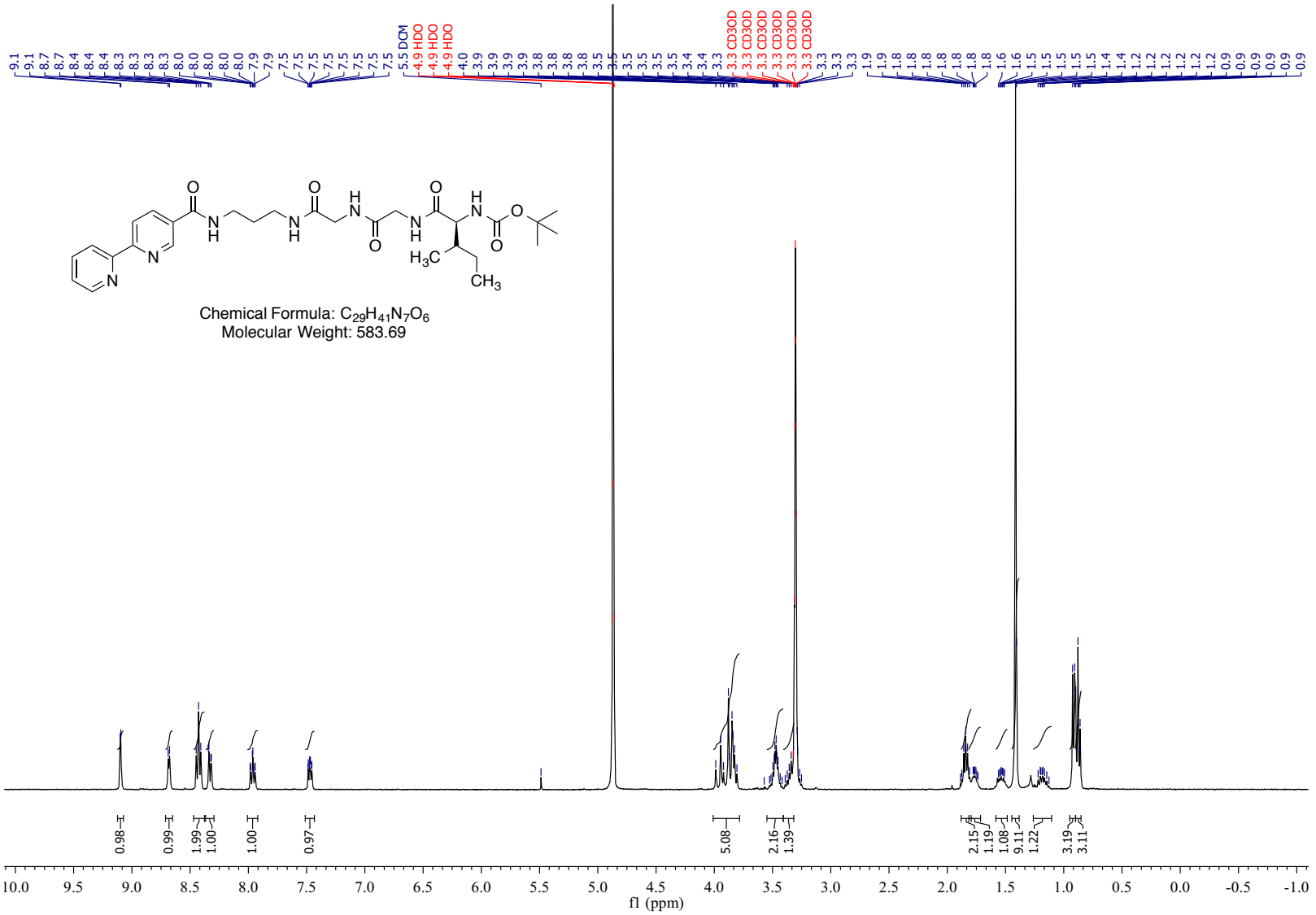

Figure S19: ^{1}H NMR of compound **17** in CD_3OD

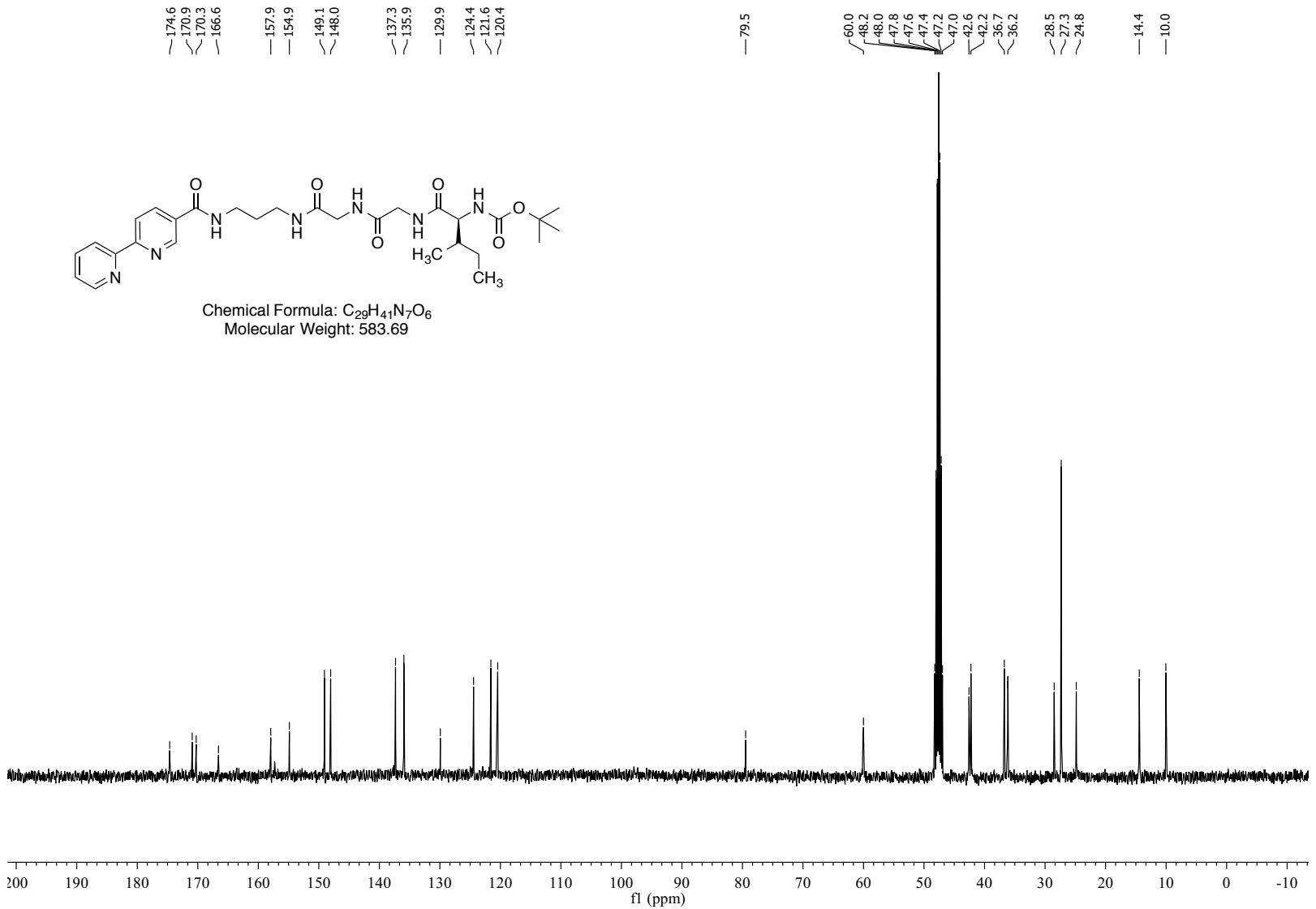

Figure S20: ^{13}C NMR of compound **17** in CD_3OD

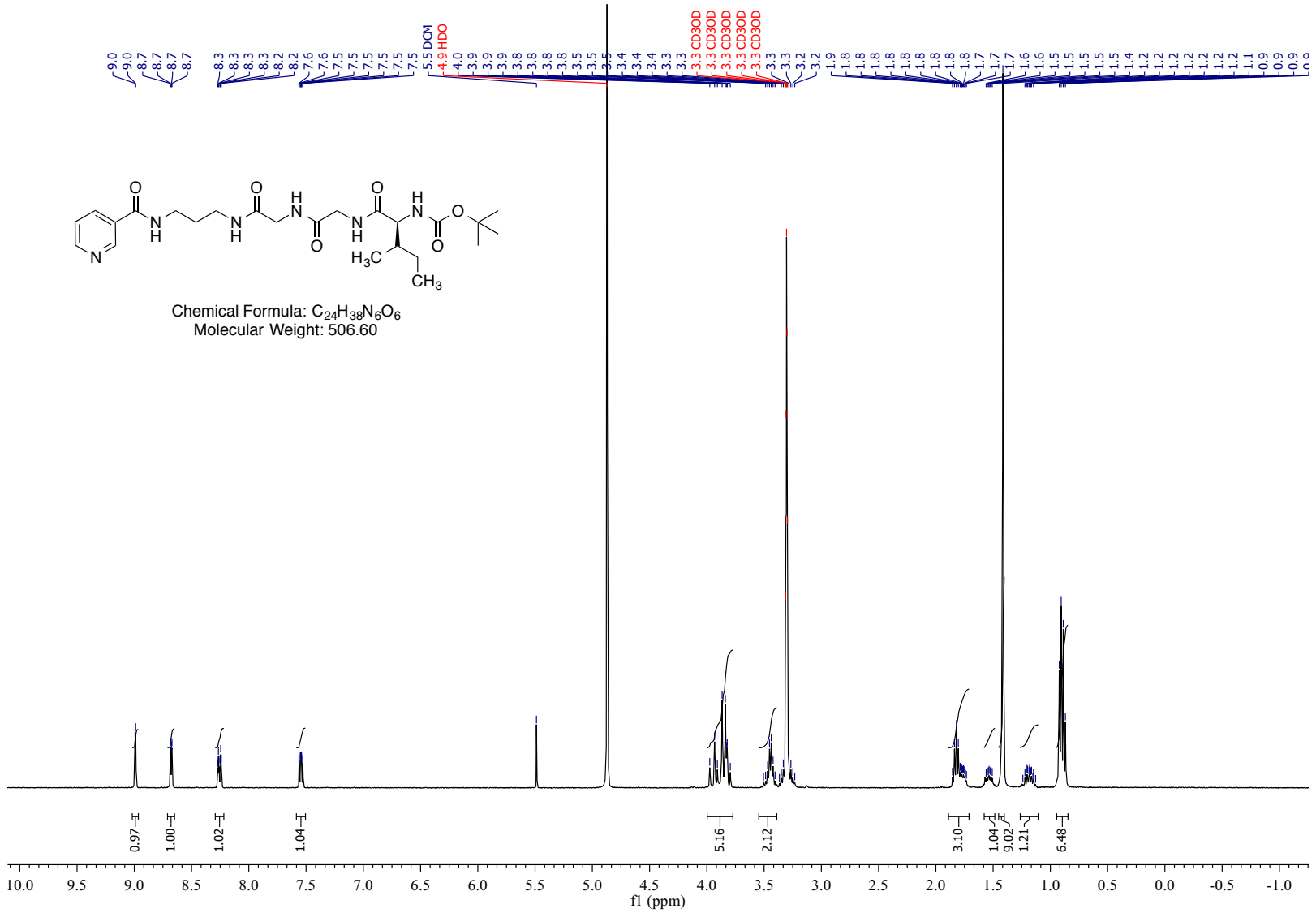

Figure S21: ^{1}H NMR of compound **18** in CD_3OD

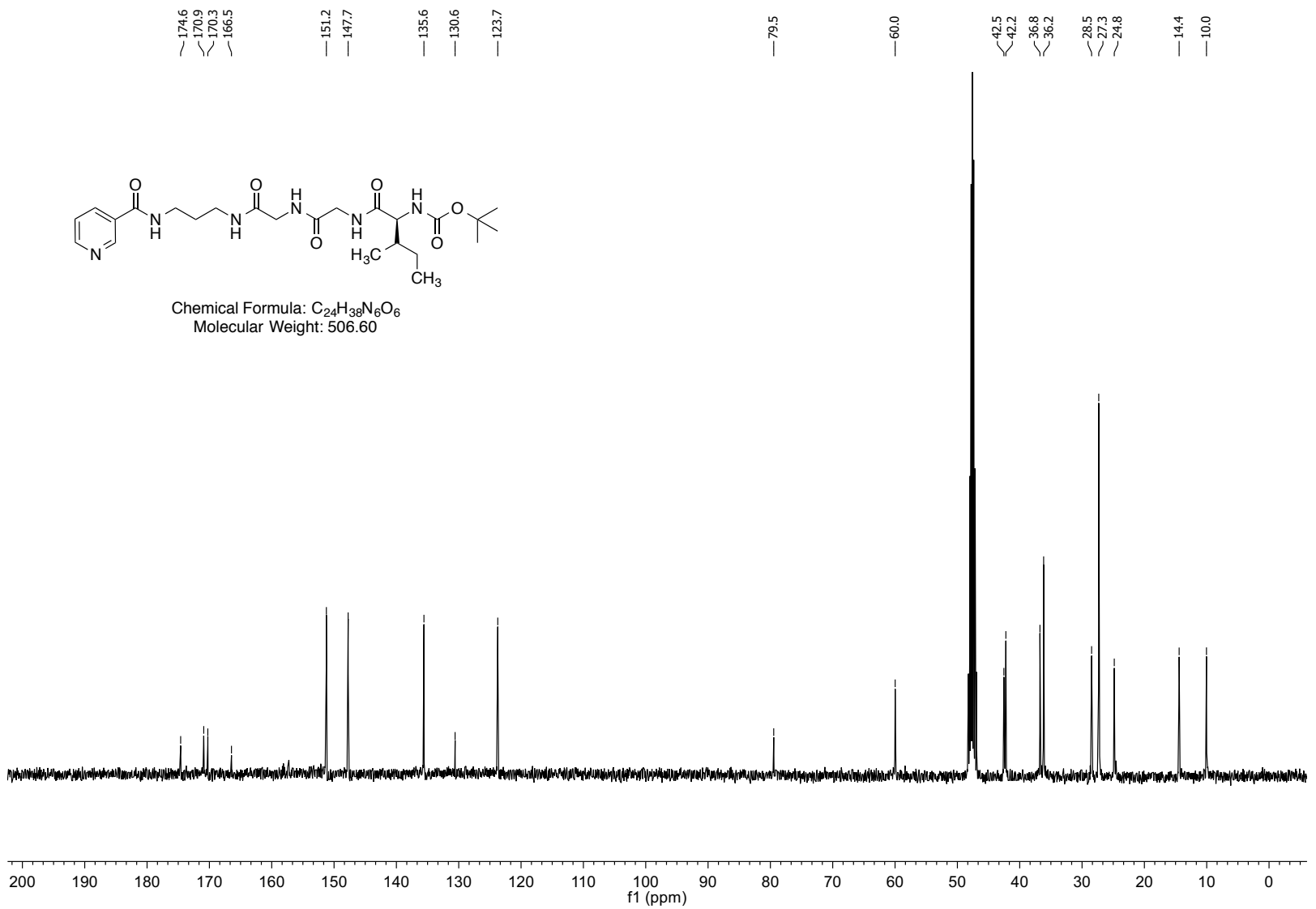

Figure S22: ^{13}C NMR of compound **18** in CD_3OD

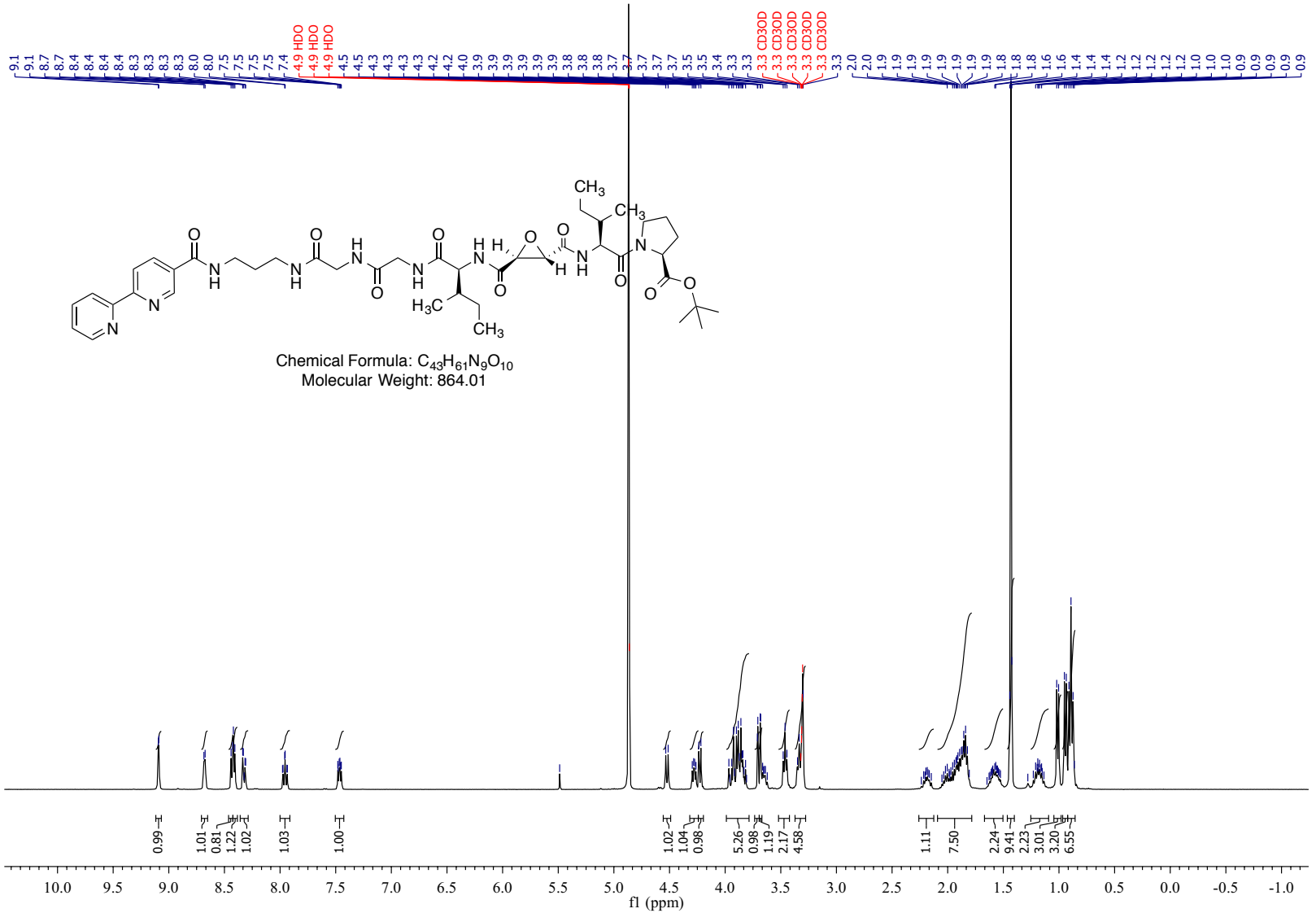

Figure S23: ^{1}H NMR of compound **19** in CD_3OD

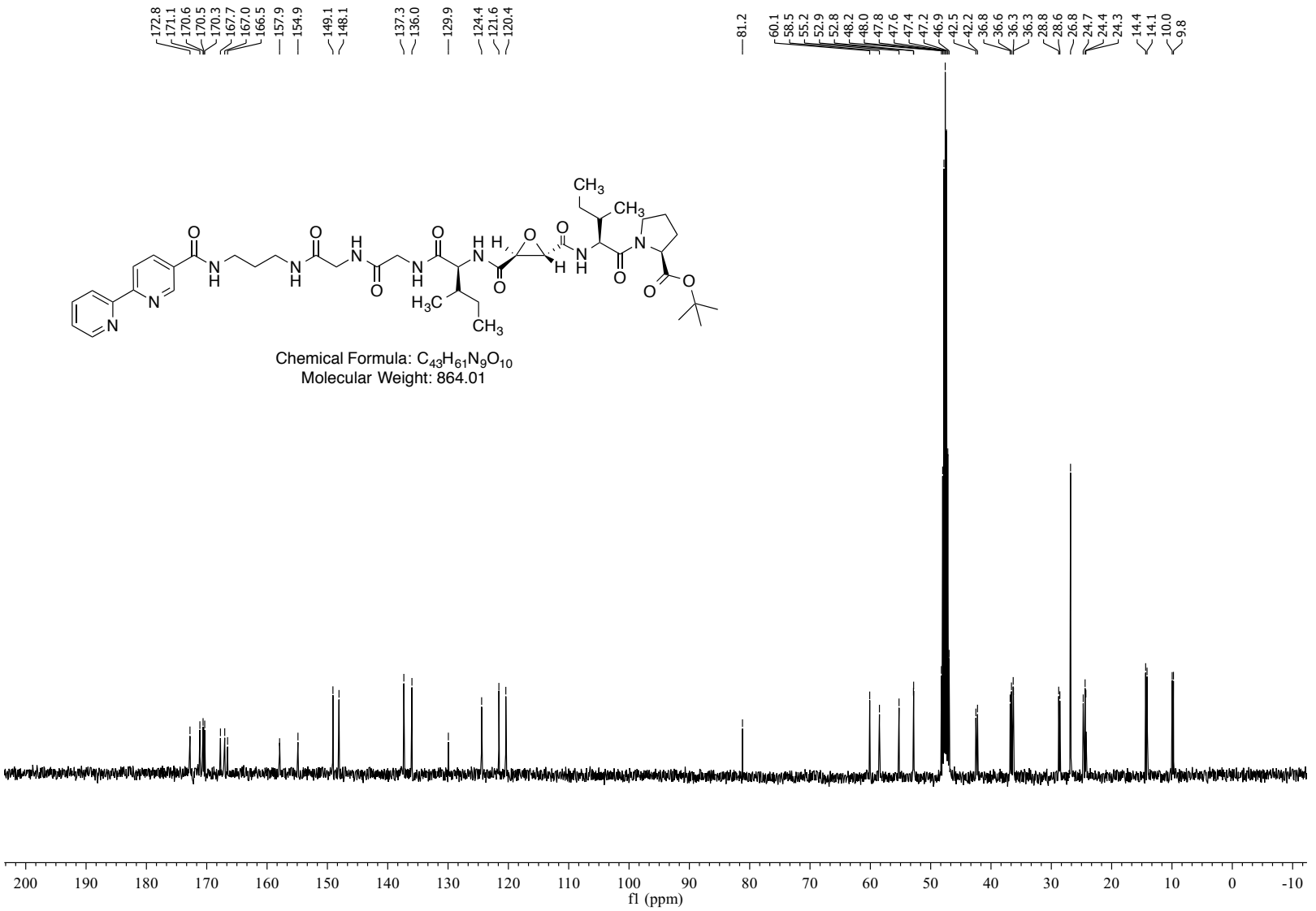

Figure S24: ^{13}C NMR of compound **19** in CD_3OD

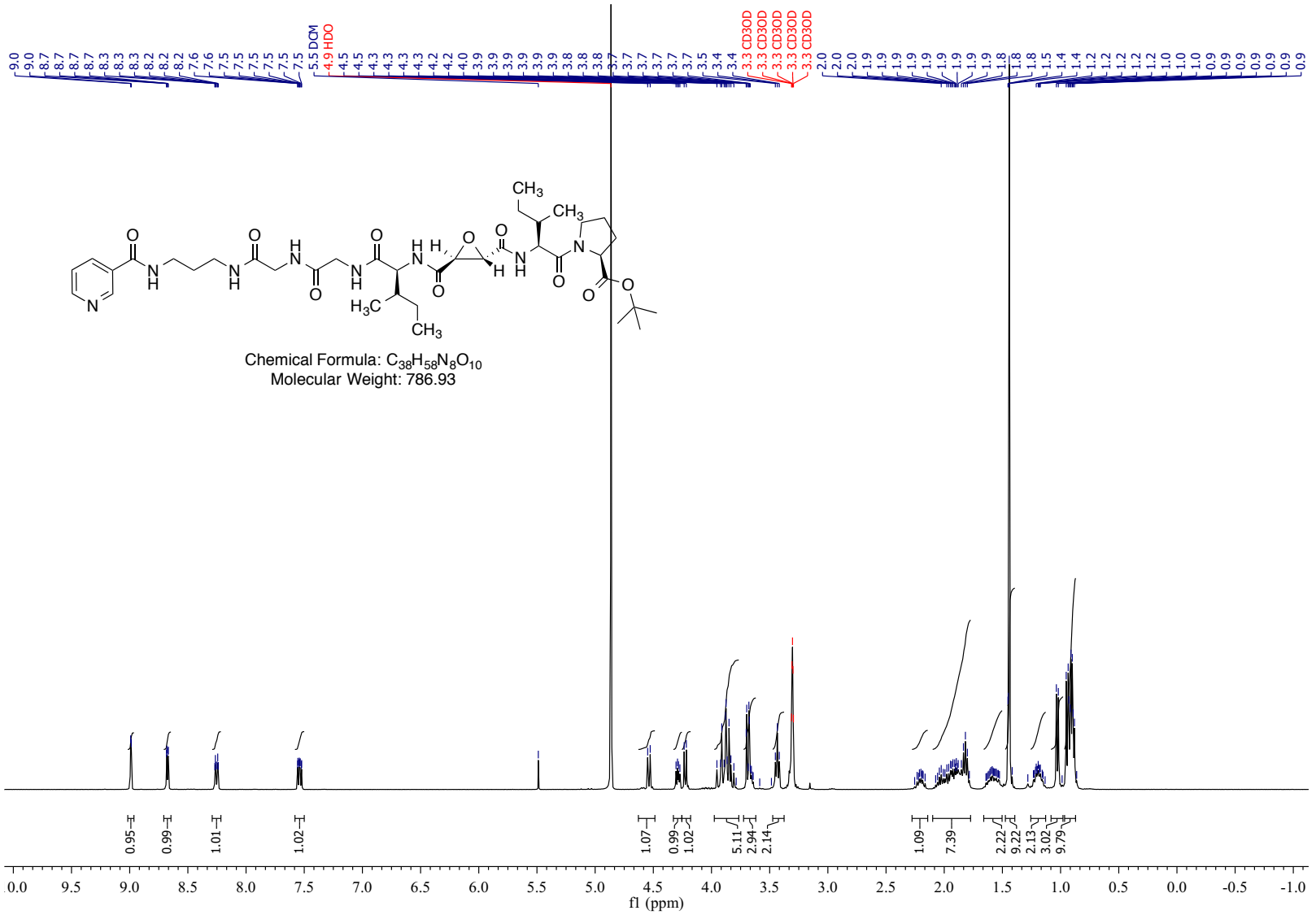

Figure S25: ^{1}H NMR of compound **20** in CD_3OD

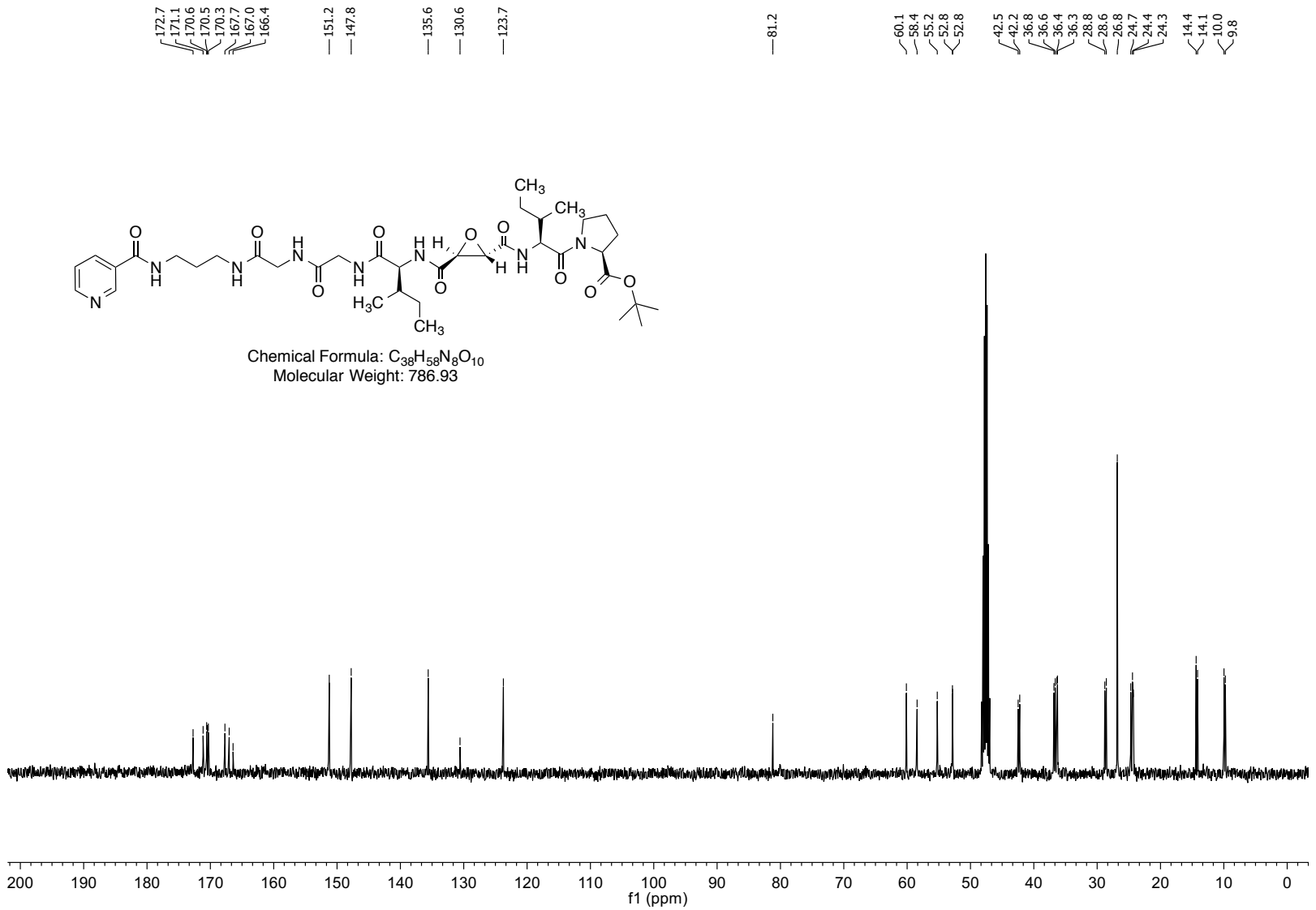

Figure S26: ^{13}C NMR of compound **20** in CD_3OD

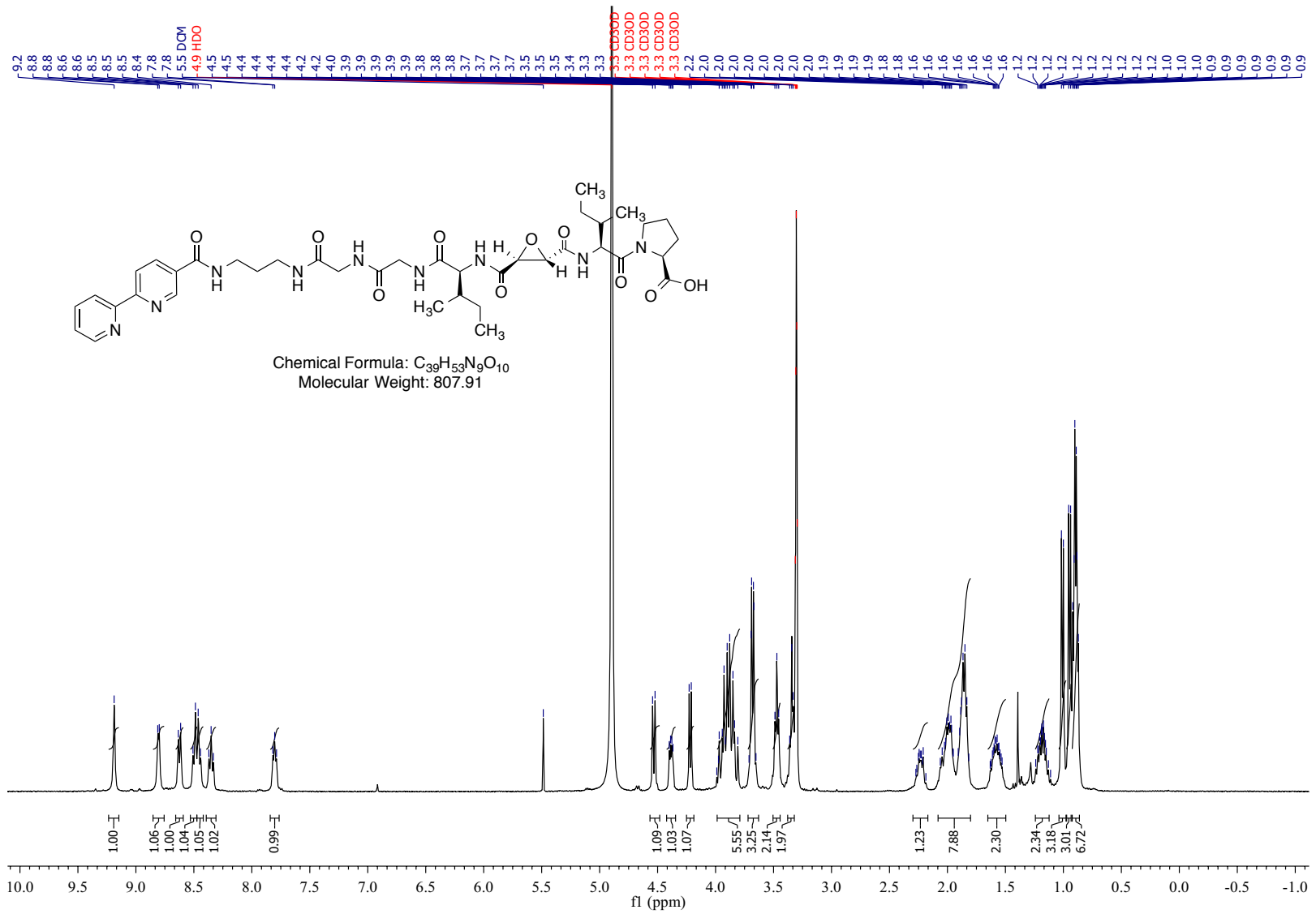

Figure S27: ^{1}H NMR of compound **1** in CD_3OD

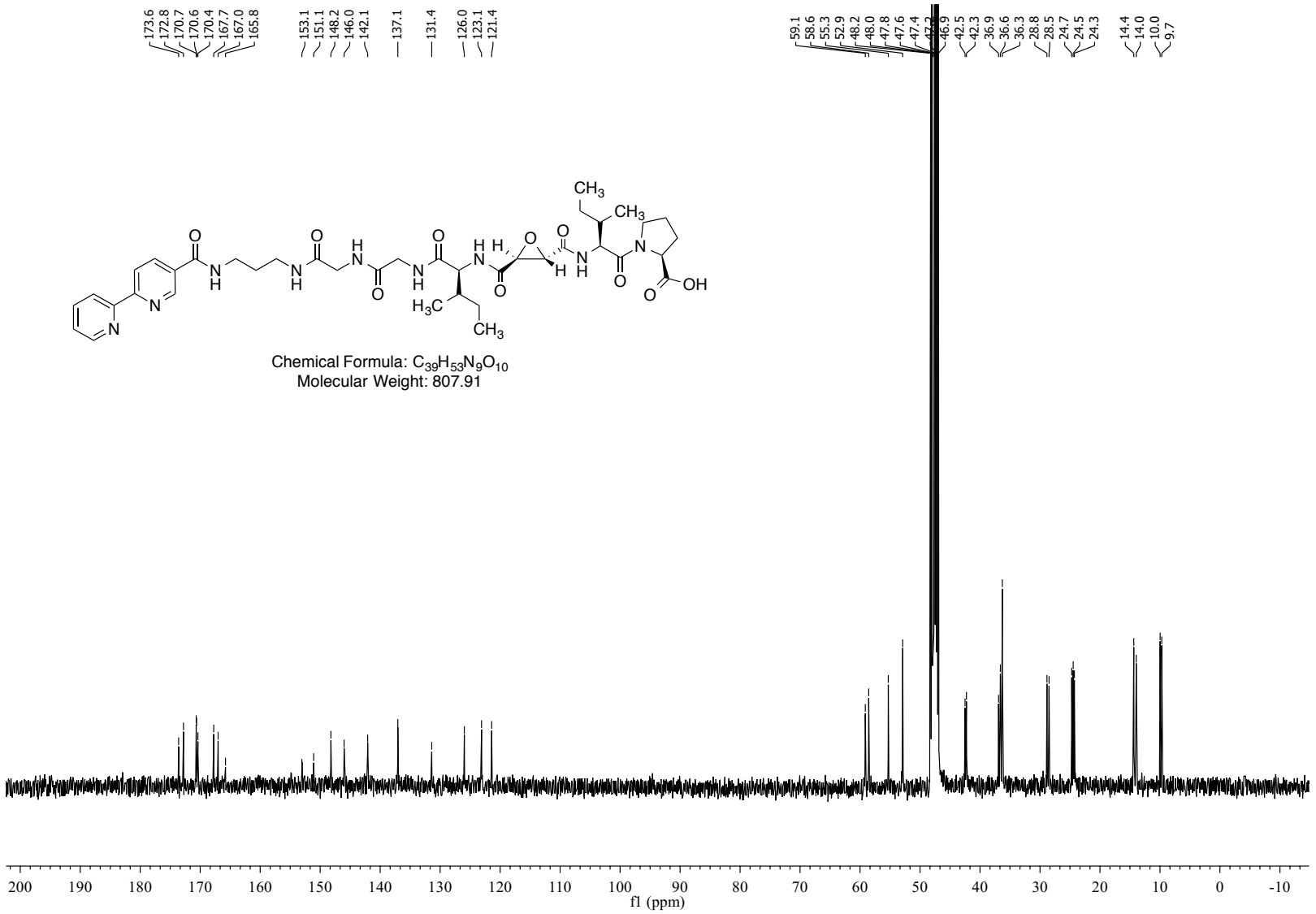

Figure S28: ^{13}C NMR of compound **1** in CD_3OD

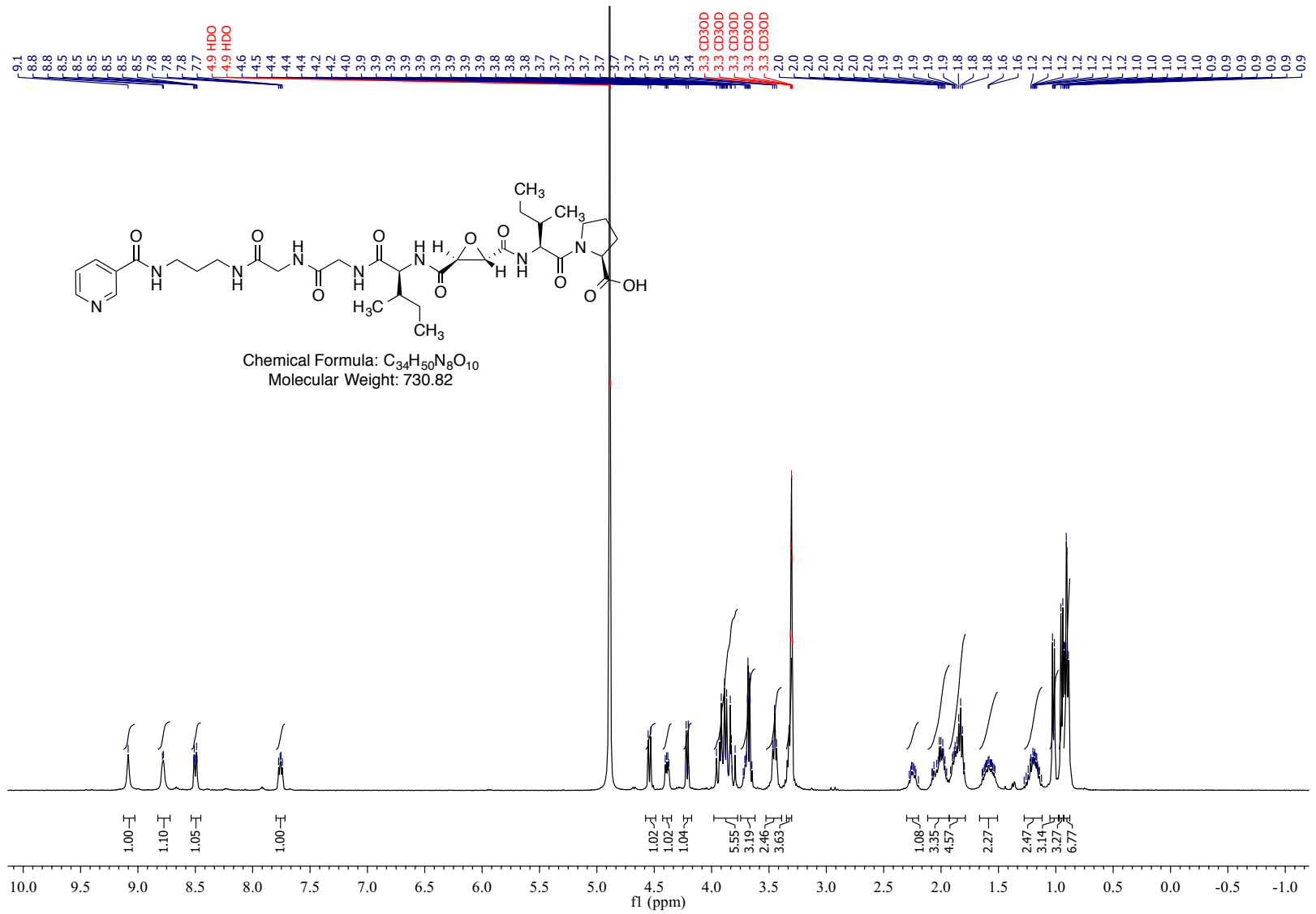

Figure S29: ^{1}H NMR of compound **2** in CD_3OD

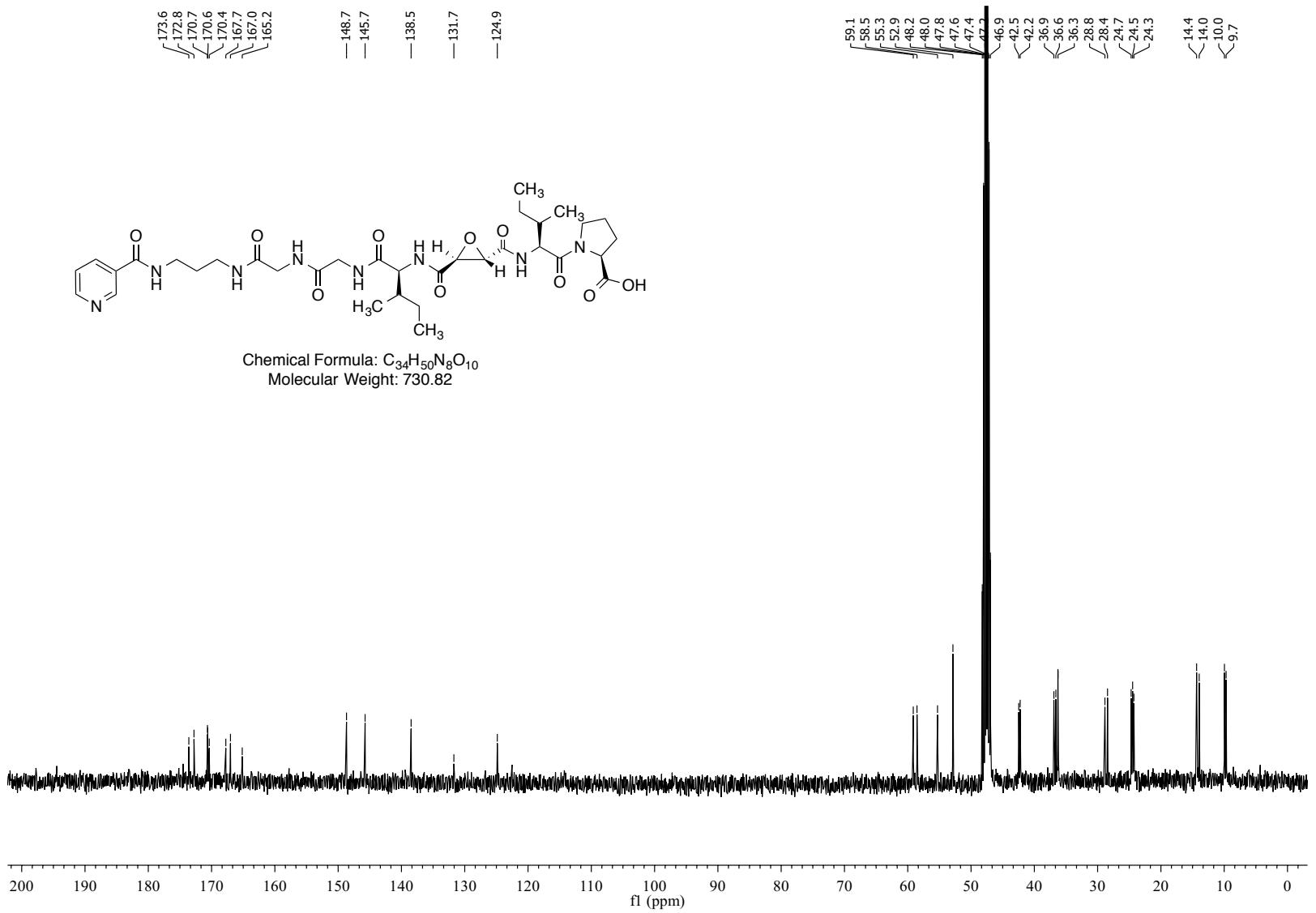

Figure S30: ^{13}C NMR of compound **2** in CD_3OD

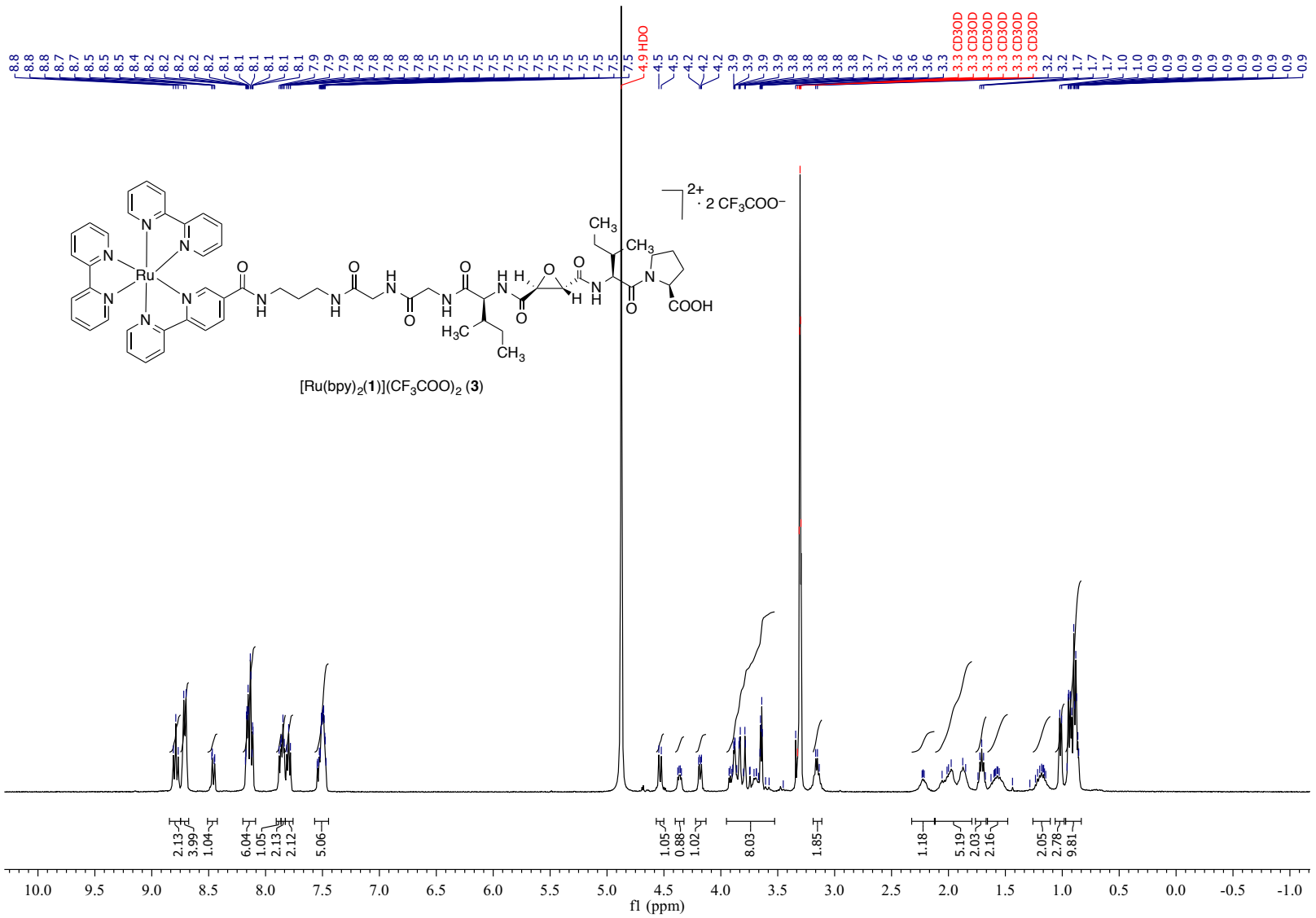

Figure S31: ^{1}H NMR of [Ru(bpy)$_2$(**1**)](CF$_3$COO)$_2$ (**3**) in CD$_3$OD

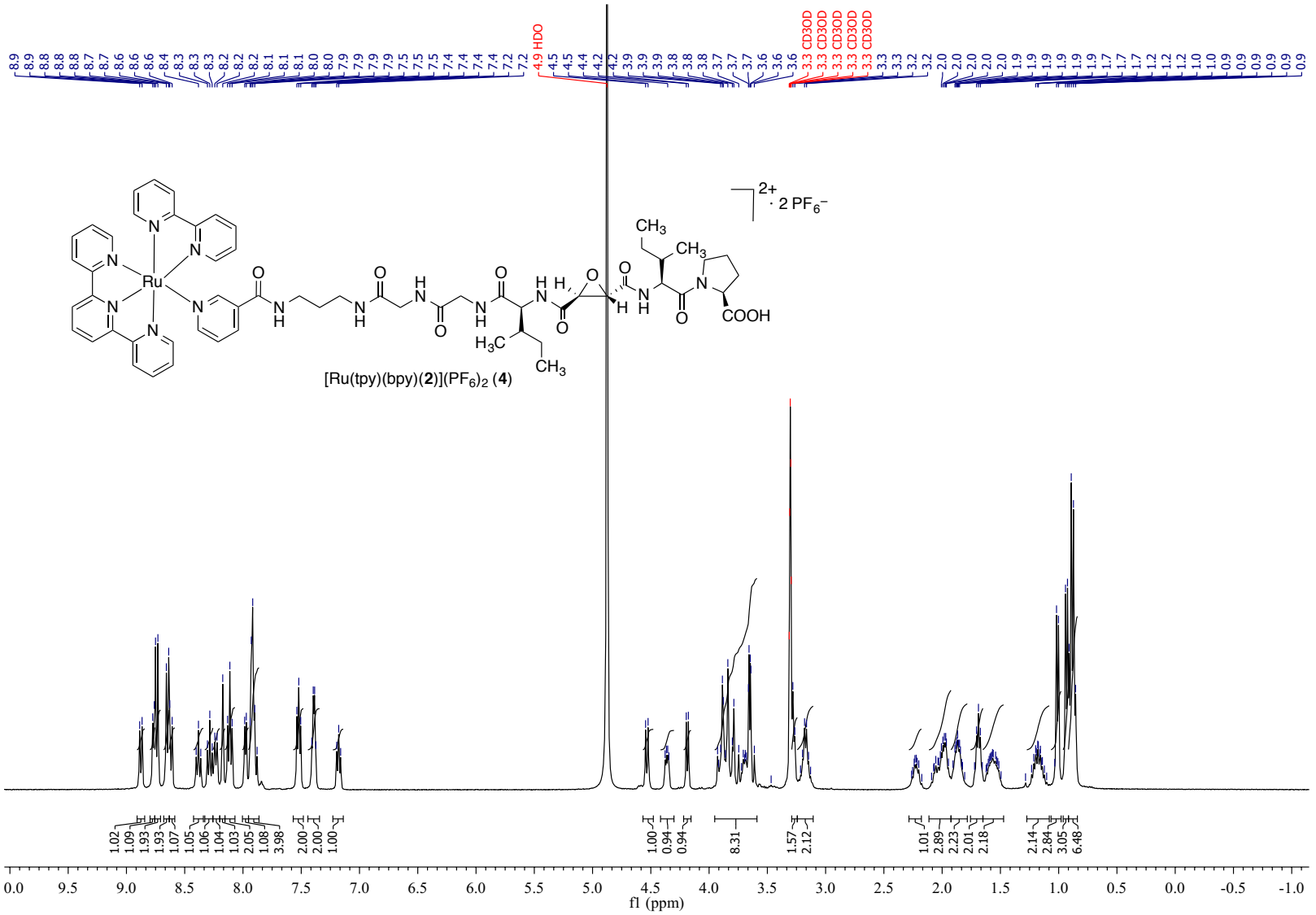

Figure S32: ^{1}H NMR of [Ru(tpy)(bpy)(**2**)](PF$_6$)$_2$ (**4**) in CD$_3$OD

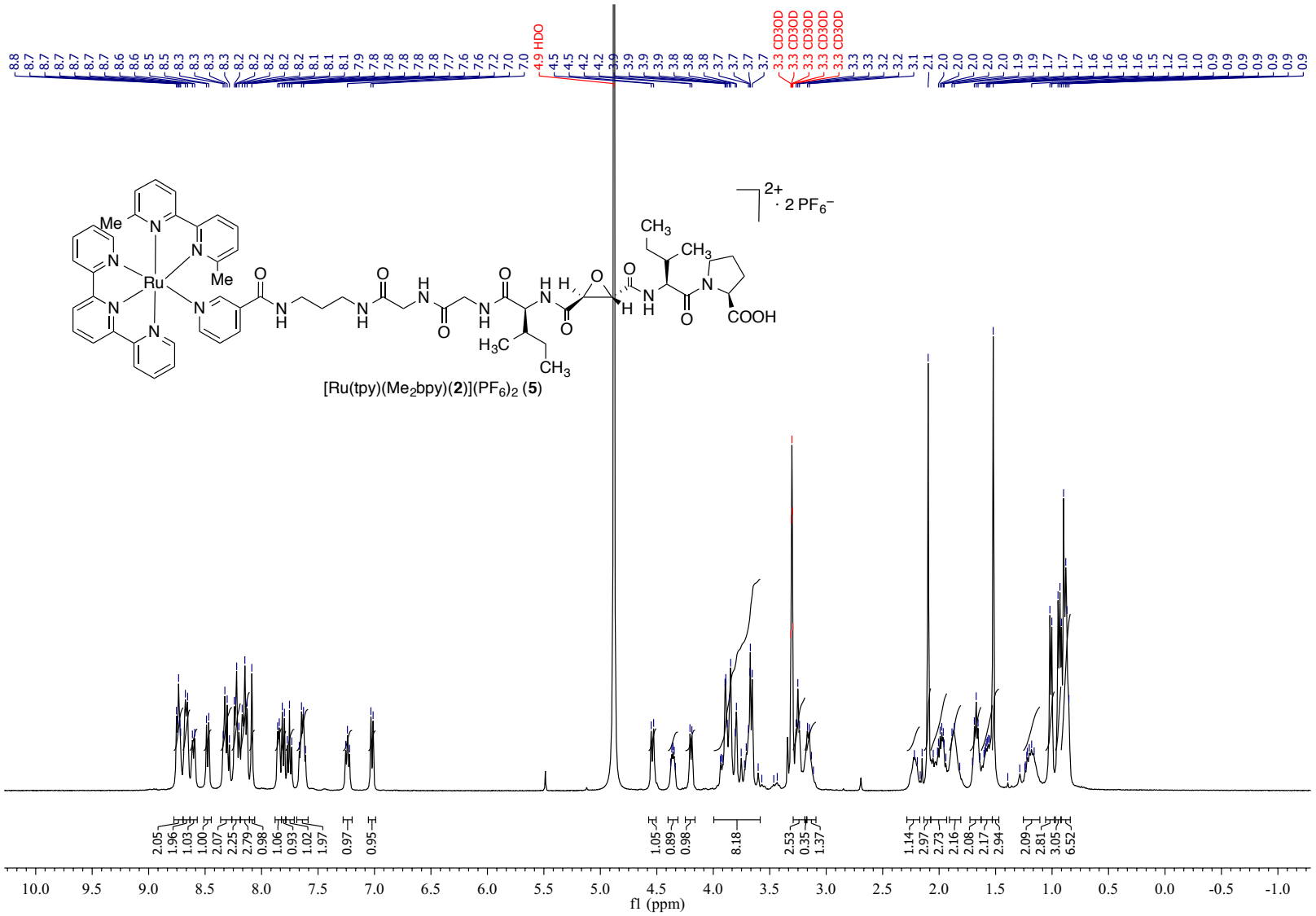

Figure S33: ^{1}H NMR of [Ru(tpy)(Me$_2$bpy)(**2**)](PF$_6$)$_2$ (**5**) in CD$_3$OD

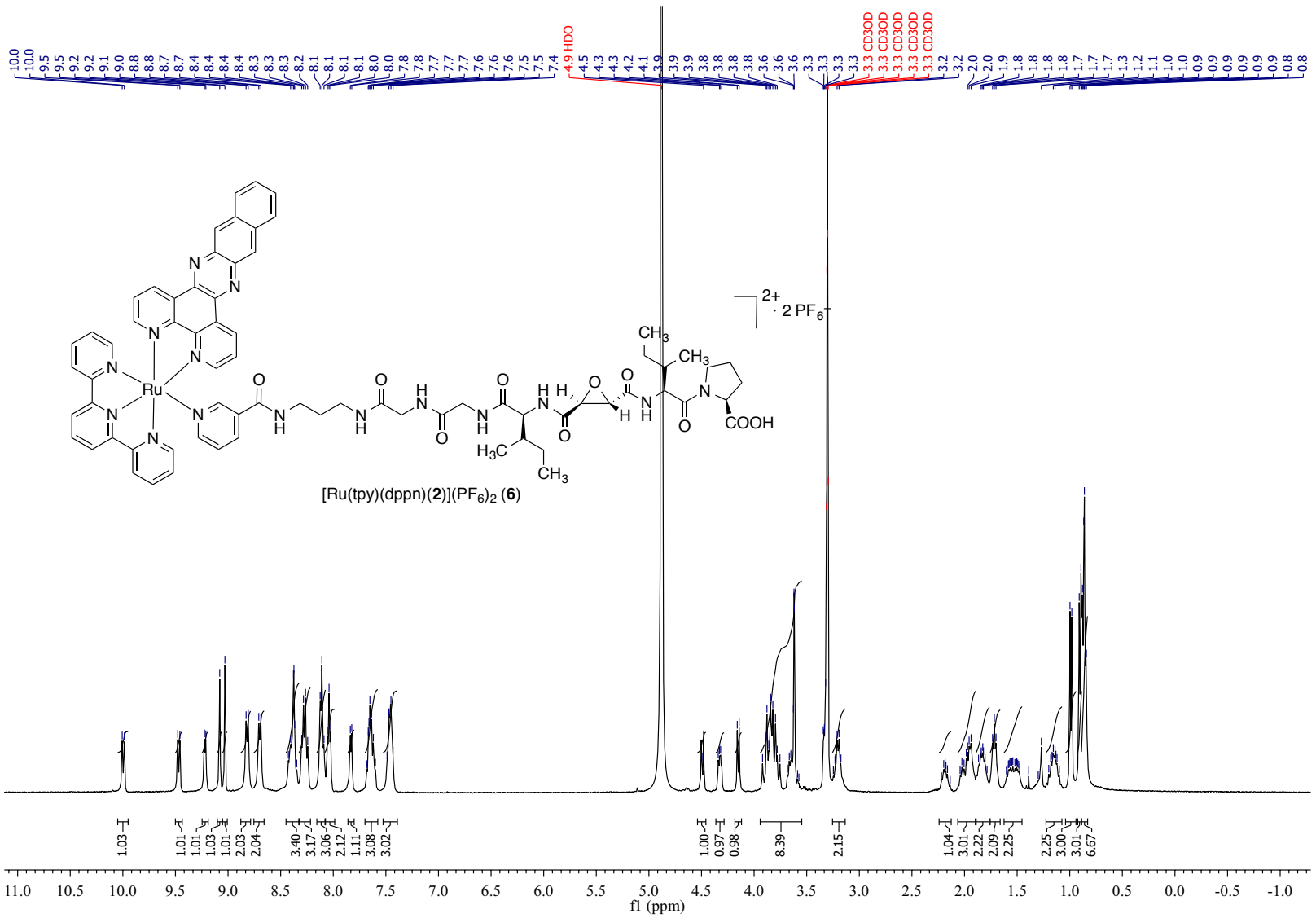

Figure S34: ^{1}H NMR of [Ru(tpy)(dppn)(**2**)](PF$_6$)$_2$ (**6**) in CD$_3$OD

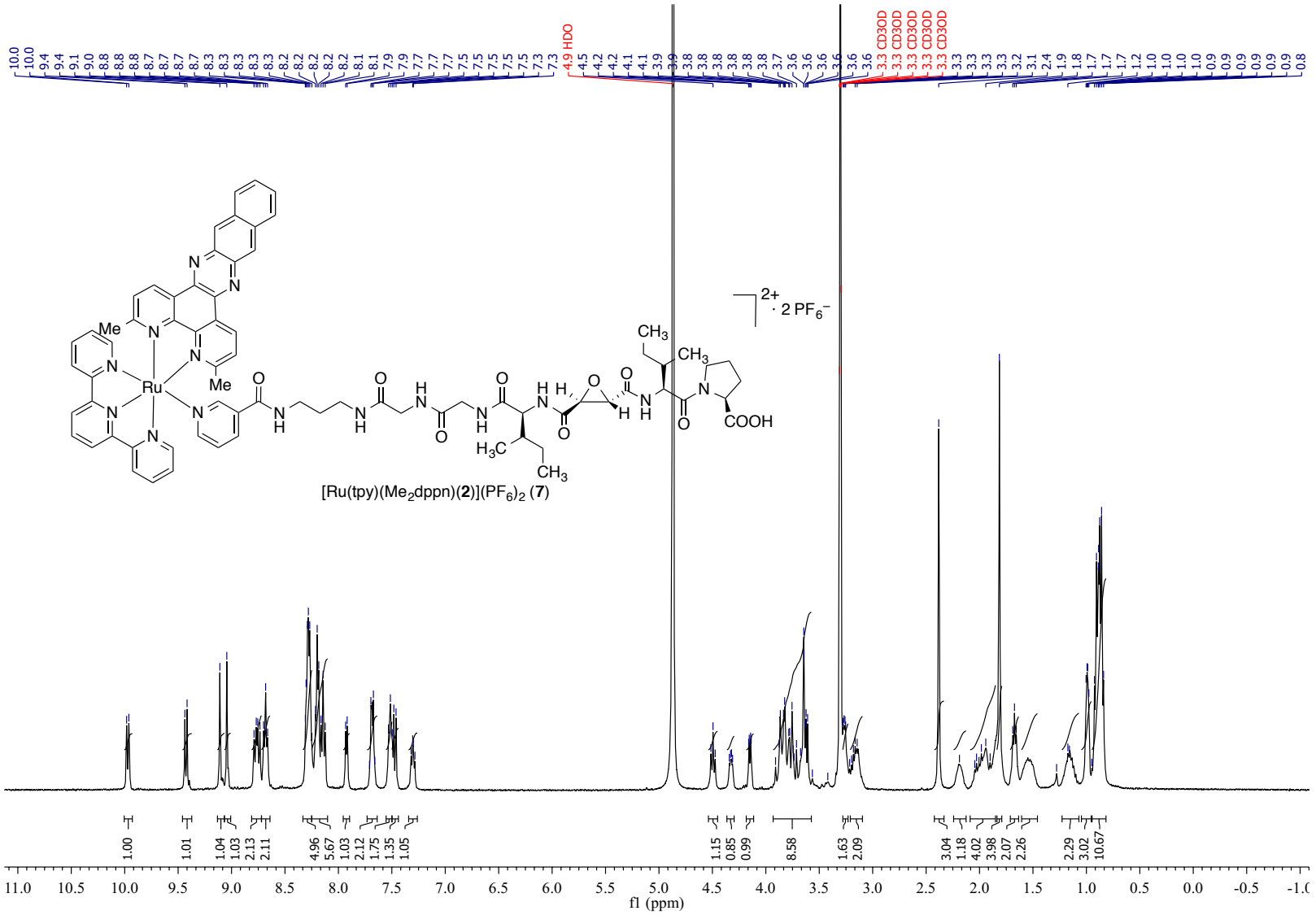

Figure S35: ^{1}H NMR of [Ru(tpy)(Me$_2$dppn)(**2**)](PF$_6$)$_2$ (**7**) in CD$_3$OD

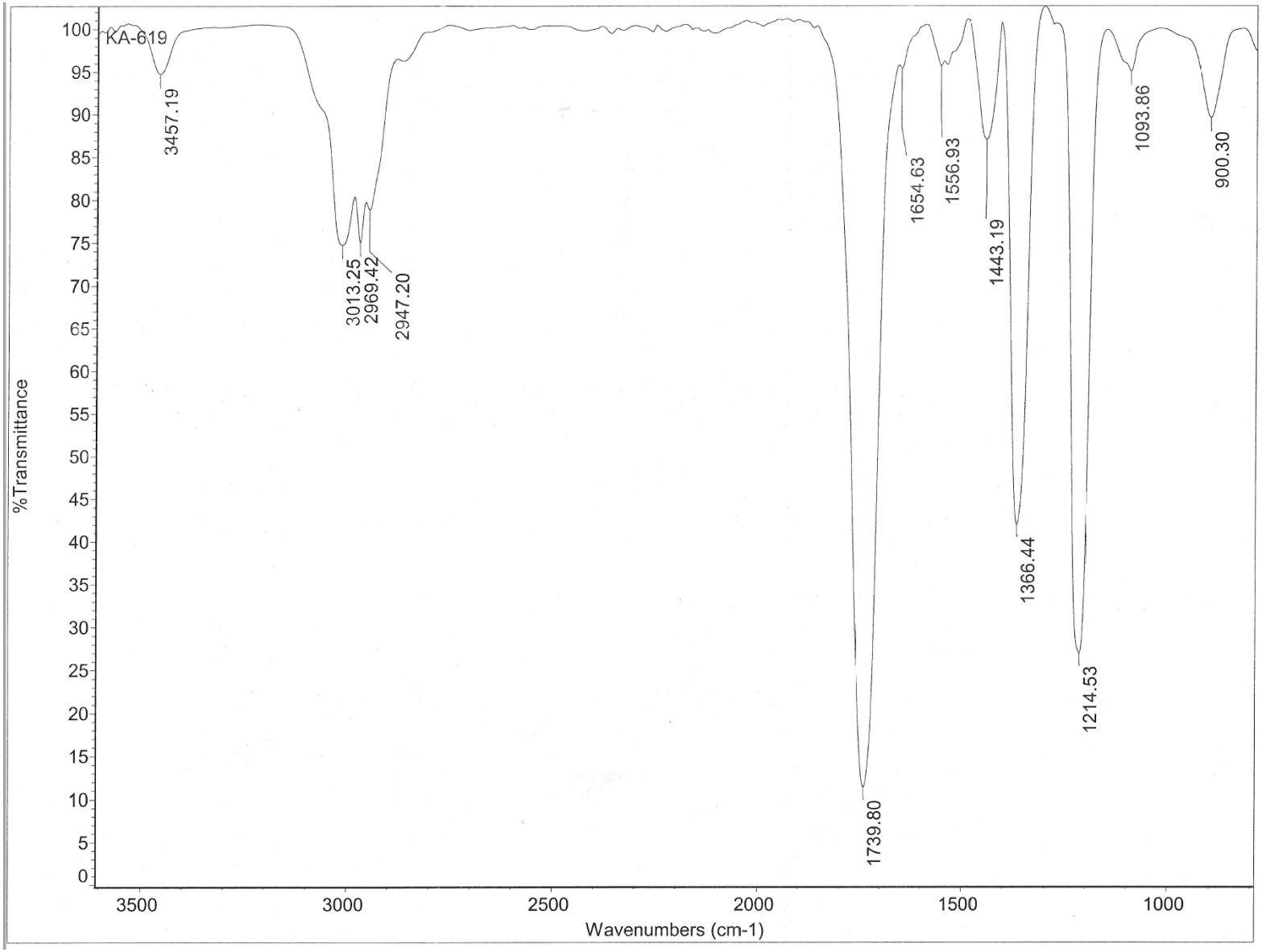

Figure S36: IR Spectrum of the $[Ru(bpy)_2(\mathbf{1})](CF_3COO)_2$ (**3**)

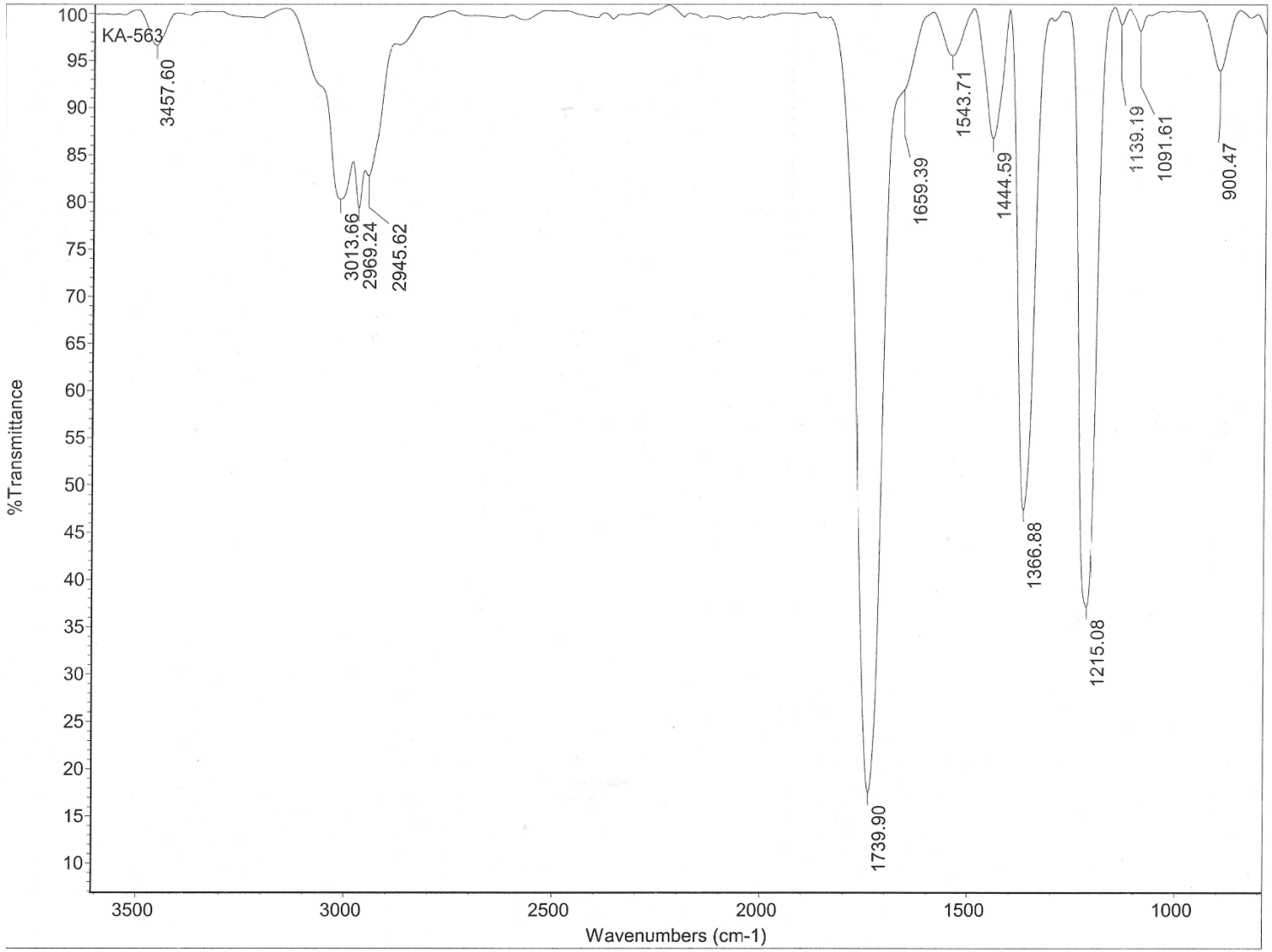

Figure S37: IR Spectrum of the [Ru(tpy)(bpy)(**2**)]$(PF_6)_2$ (**4**)

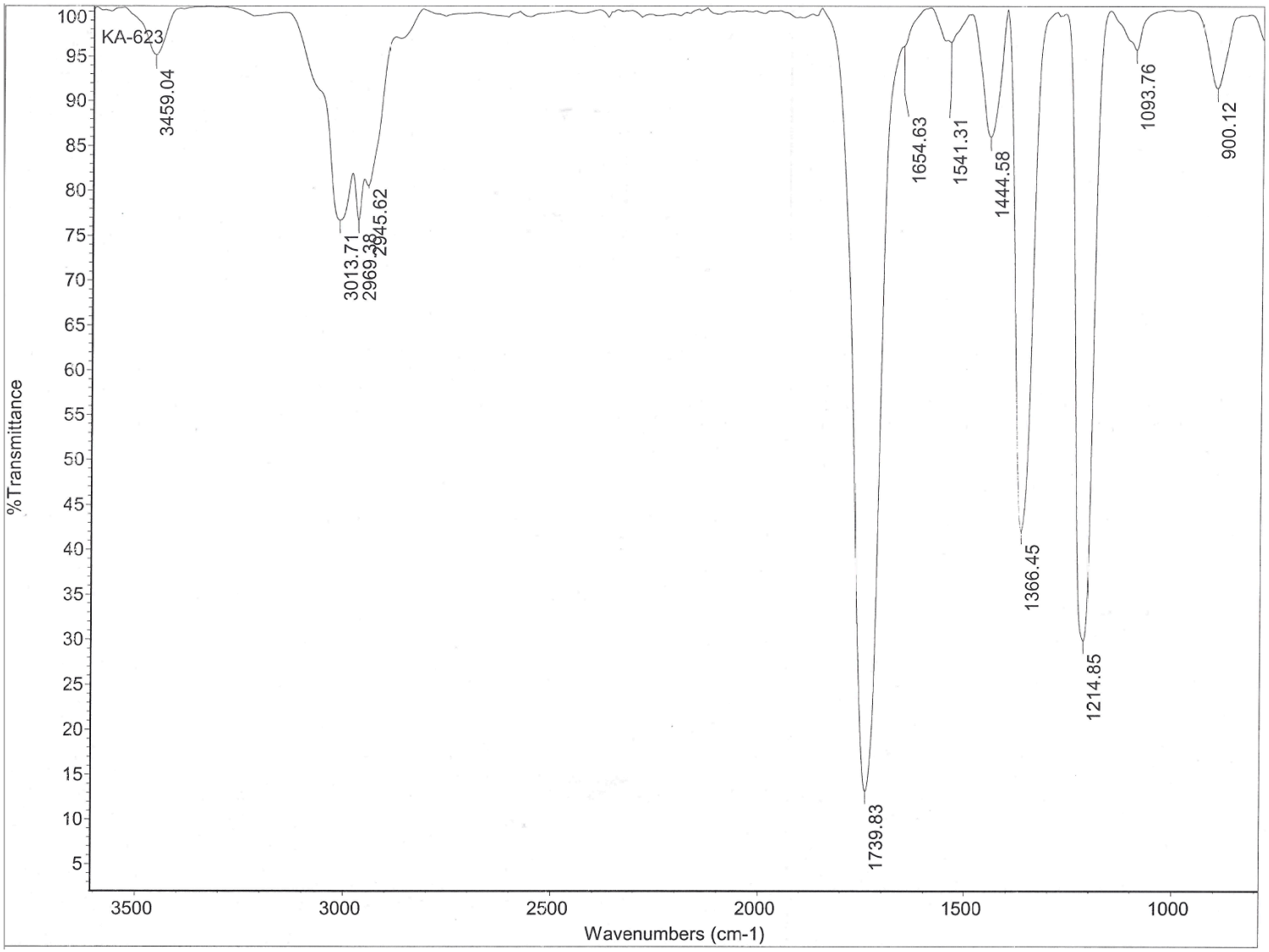

Figure S38: IR Spectrum of the [Ru(tpy)(Me_2bpy)(**2**)](PF_6)$_2$ (**5**)

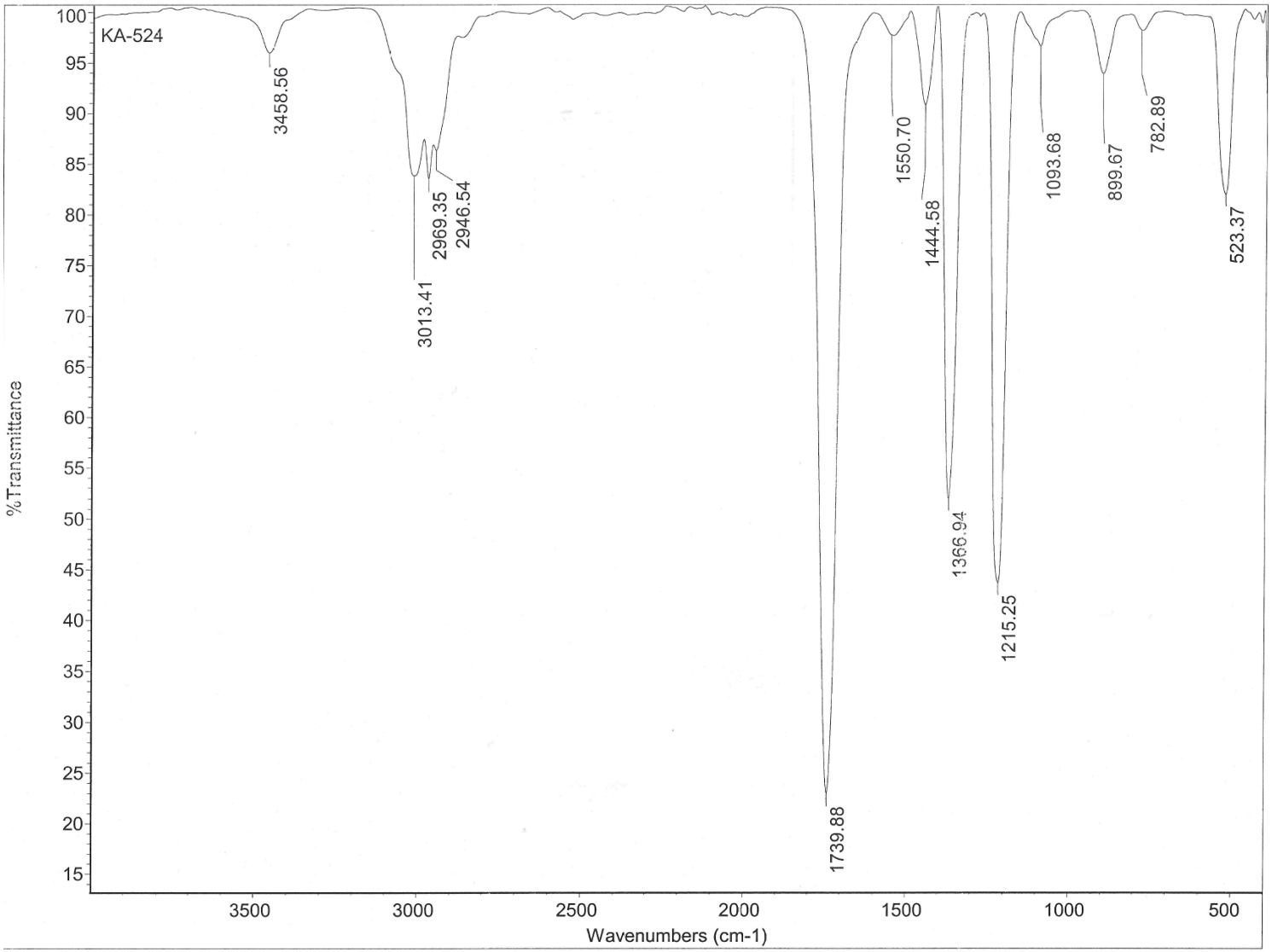

Figure S39: IR Spectrum of the [Ru(tpy)(dppn)(**2**)]$(PF_6)_2$ (**6**)

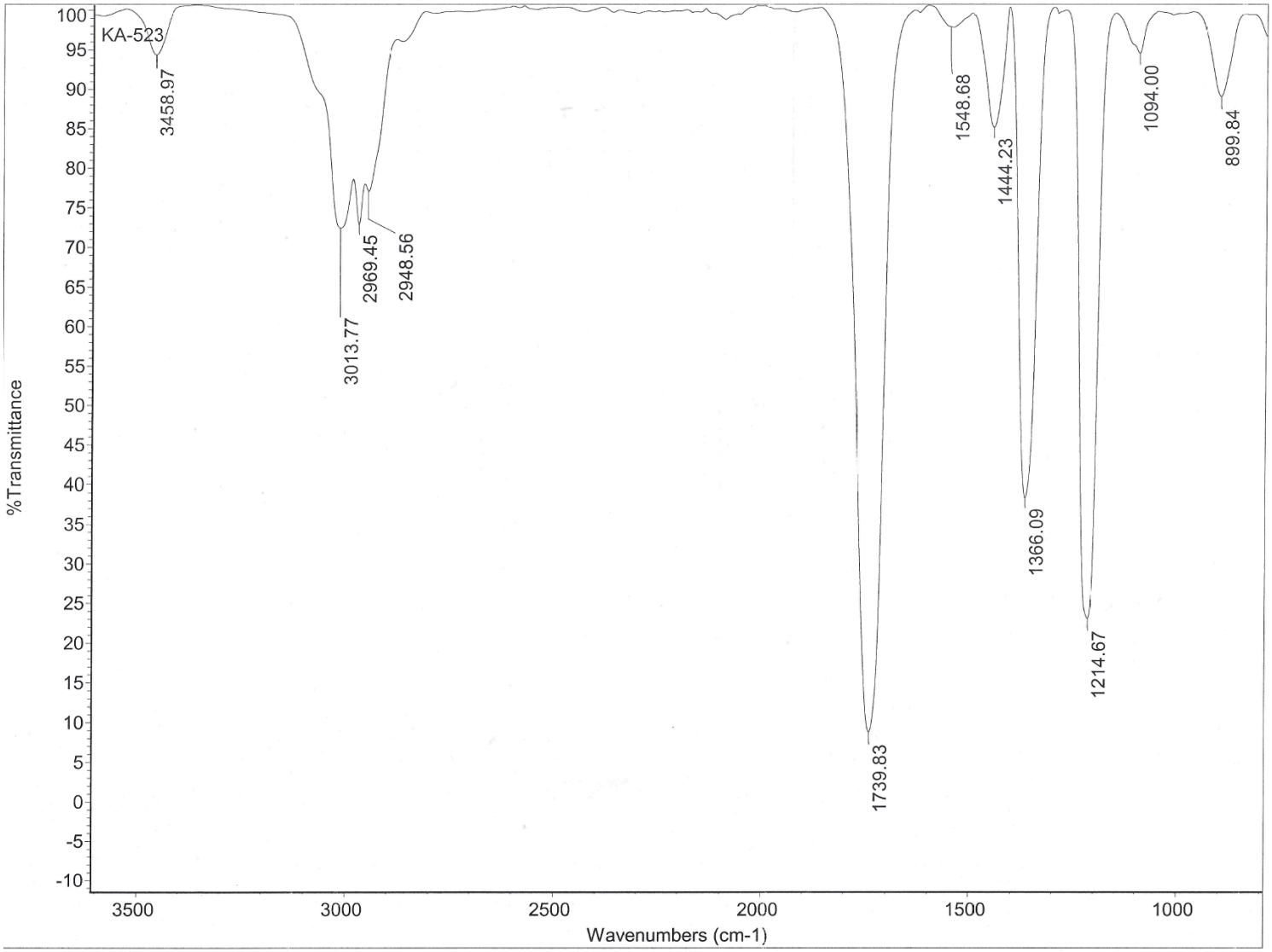

Figure S40: IR Spectrum of the [Ru(tpy)(Me$_2$dppn)(**2**)](PF$_6$)$_2$ (**7**)

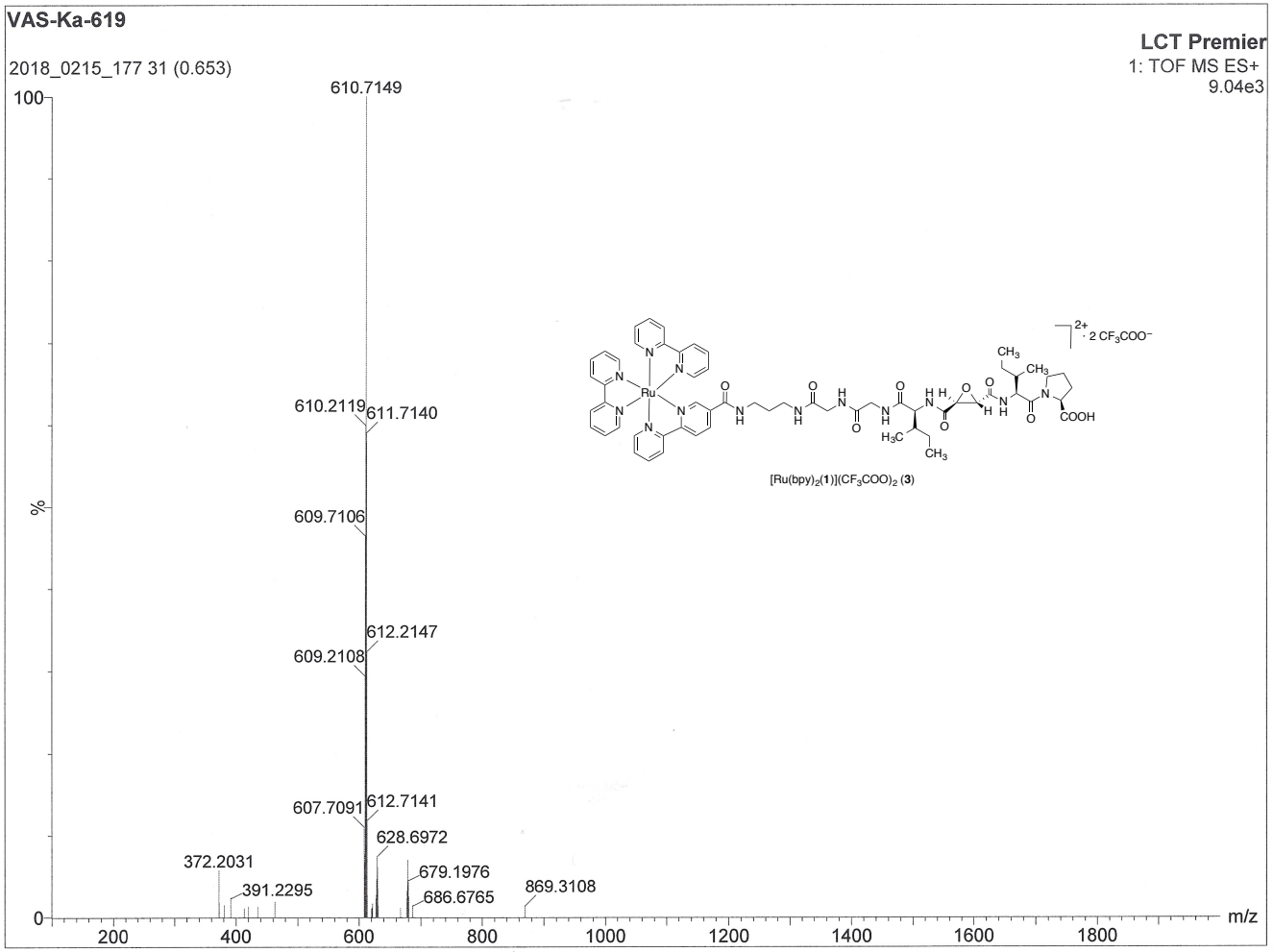

Figure S41: Mass spectrum of the di-cation $[Ru(bpy)_2(\mathbf{1})]^{2+}$ (**3**)

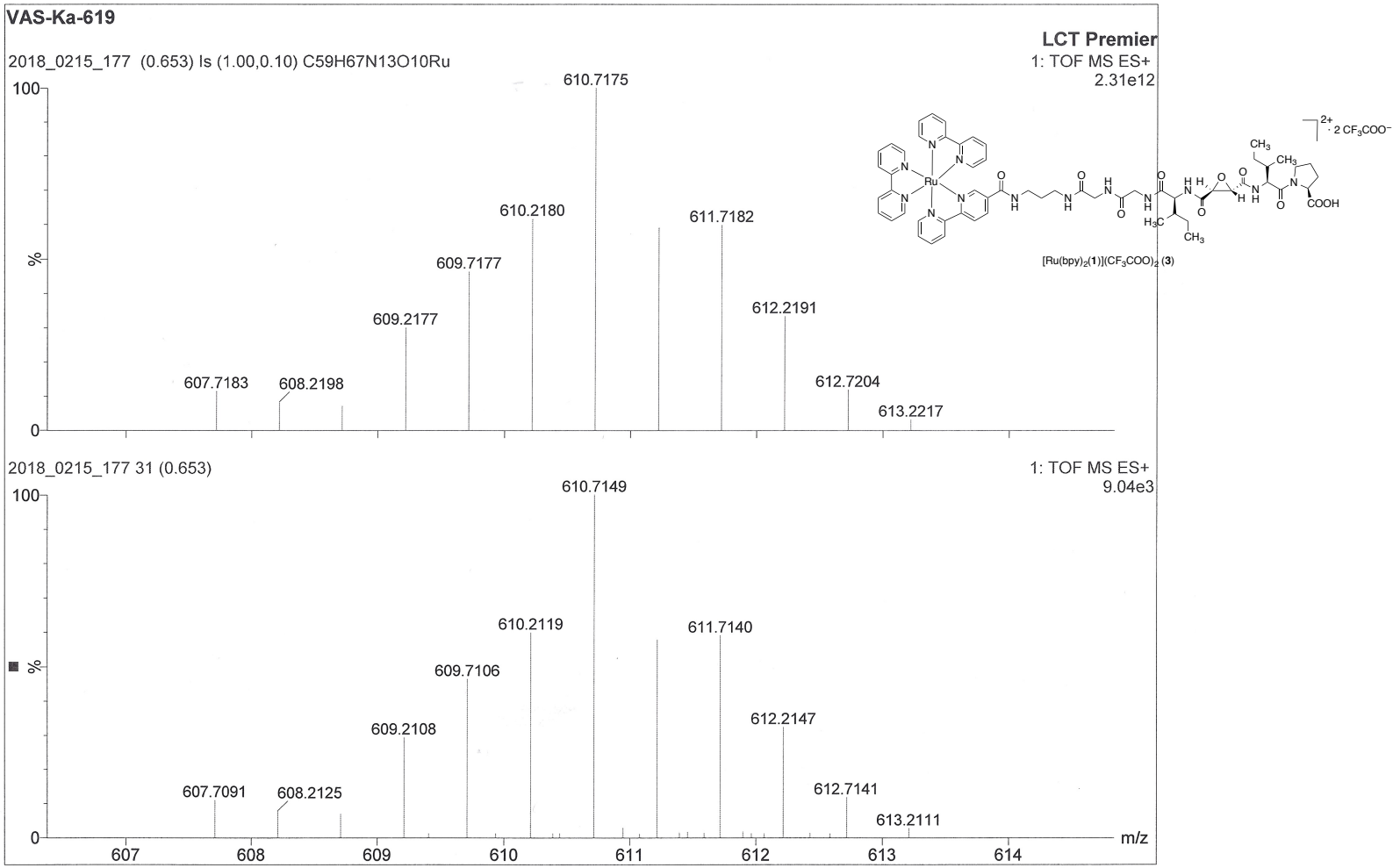

Figure S42: Expansion of mass spectrum of $[Ru(bpy)_2(\mathbf{1})]^{2+}$ (**3**) calculated (above) and observed (below) isotope pattern for major peak with m/z = 611

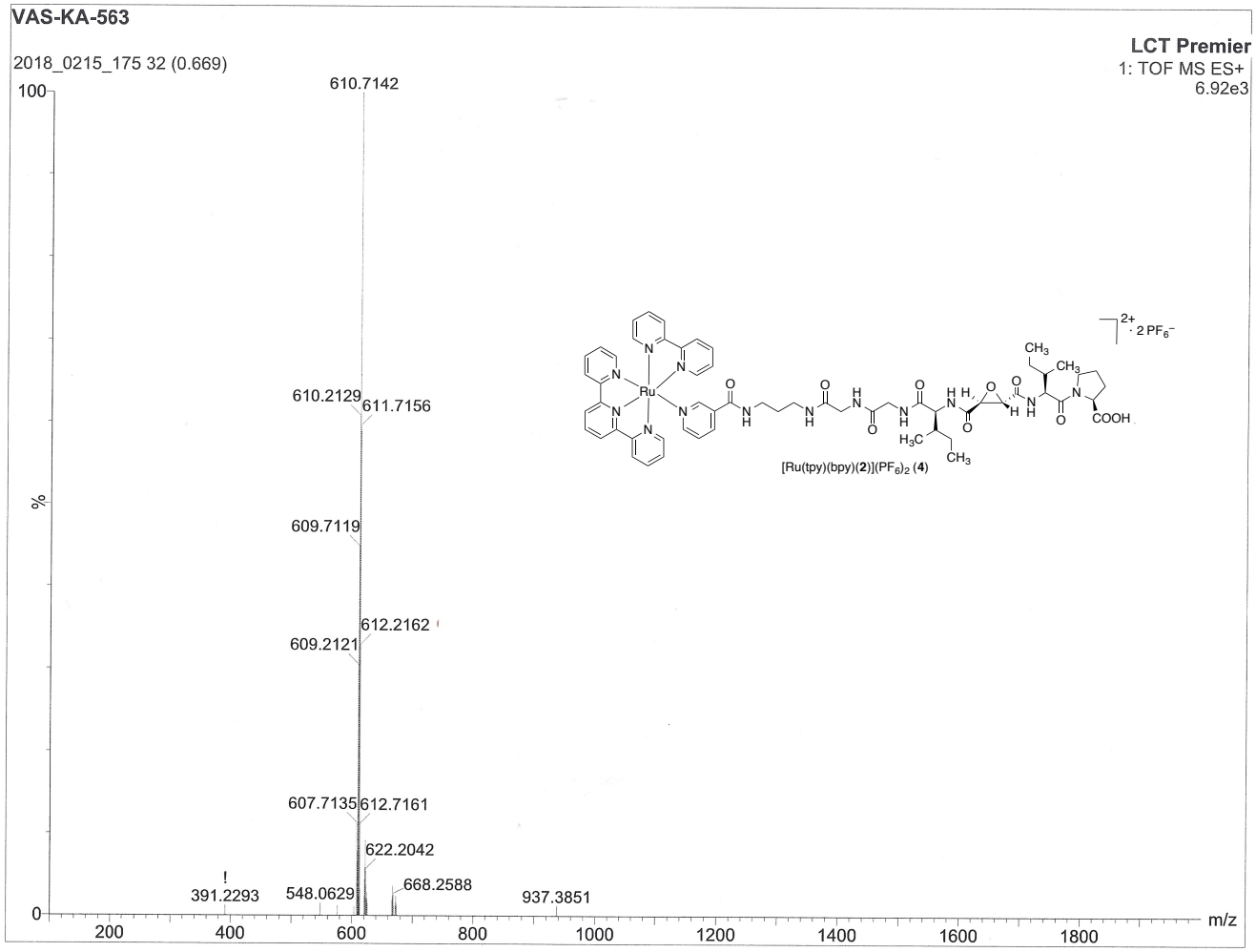

Figure S43: Mass spectrum of the di-cation $[Ru(tpy)(bpy)(\mathbf{2})]^{2+}$ (**4**)

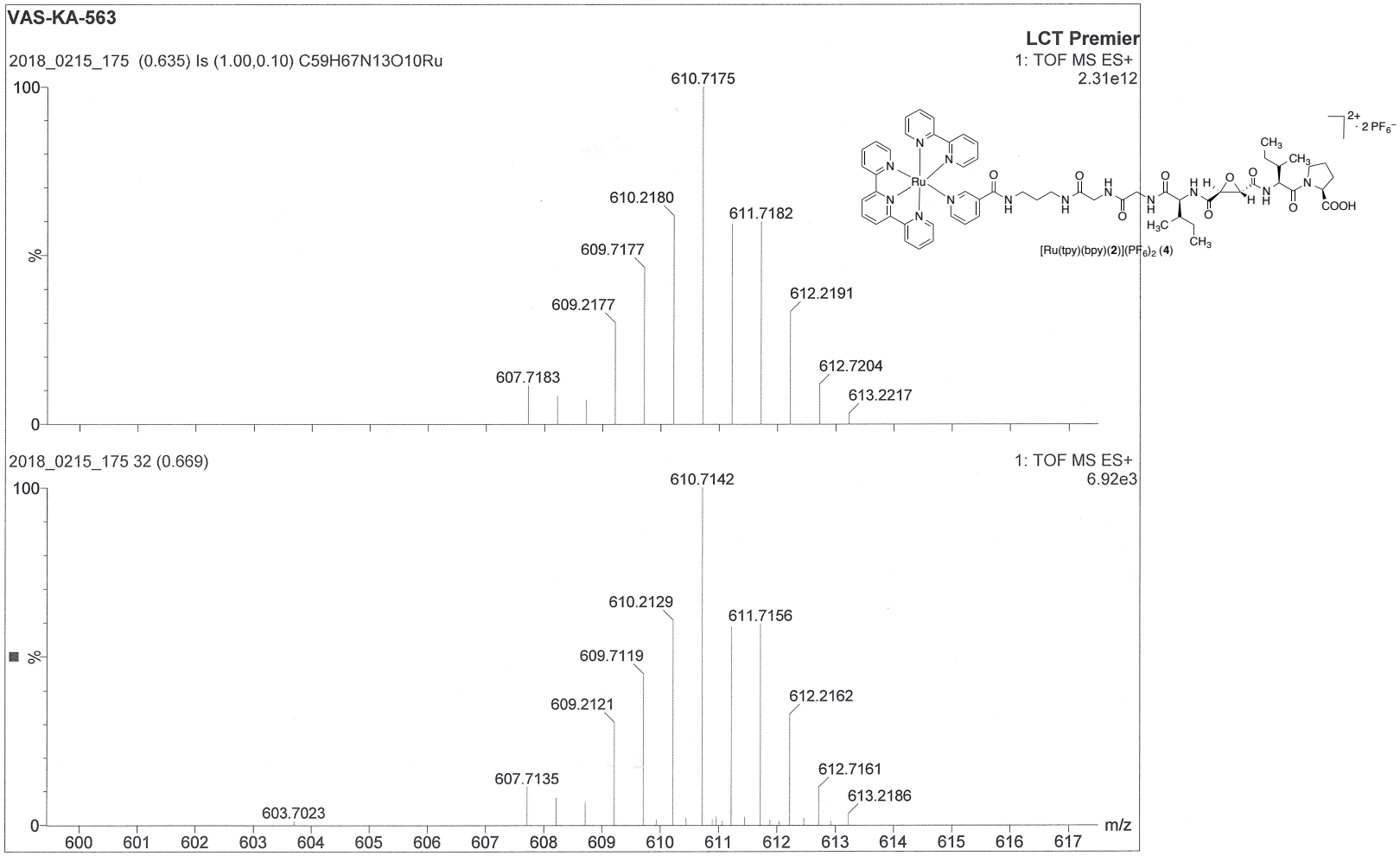

Figure S44: Expansion of mass spectrum of $[Ru(tpy)(bpy)(\mathbf{2})]^{2+}$ (**4**) calculated (above) and observed (below) isotope pattern for major peak with m/z = 611

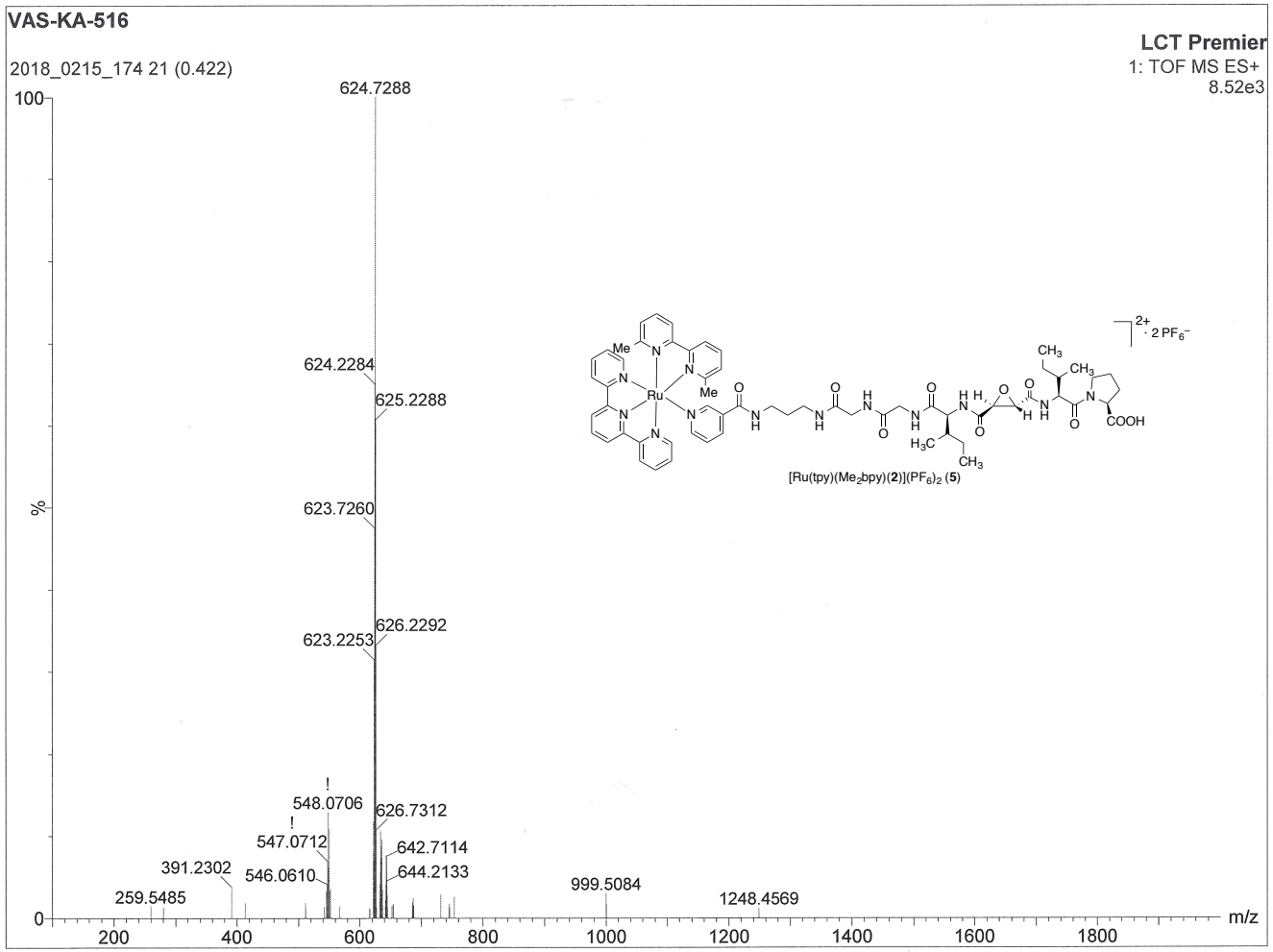

Figure S45: Mass spectrum of the di-cation $[Ru(tpy)(Me_2bpy)(\mathbf{2})]^{2+}$ (**5**)

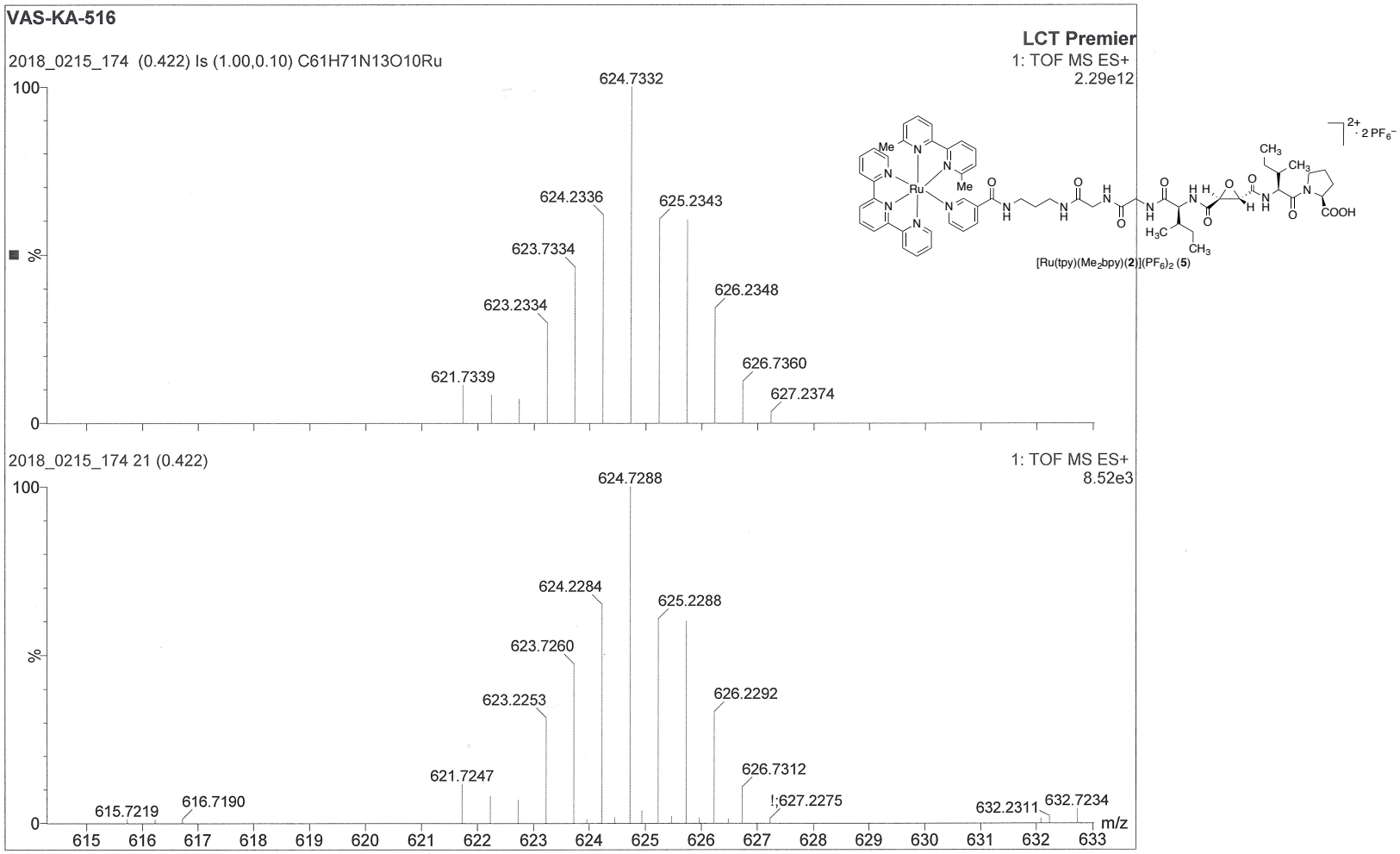

Figure S46: Expansion of mass spectrum of $[Ru(tpy)(Me_2bpy)(\mathbf{2})]^{2+}$ (**5**) calculated (above) and observed (below) isotope pattern for major peak with m/z = 625

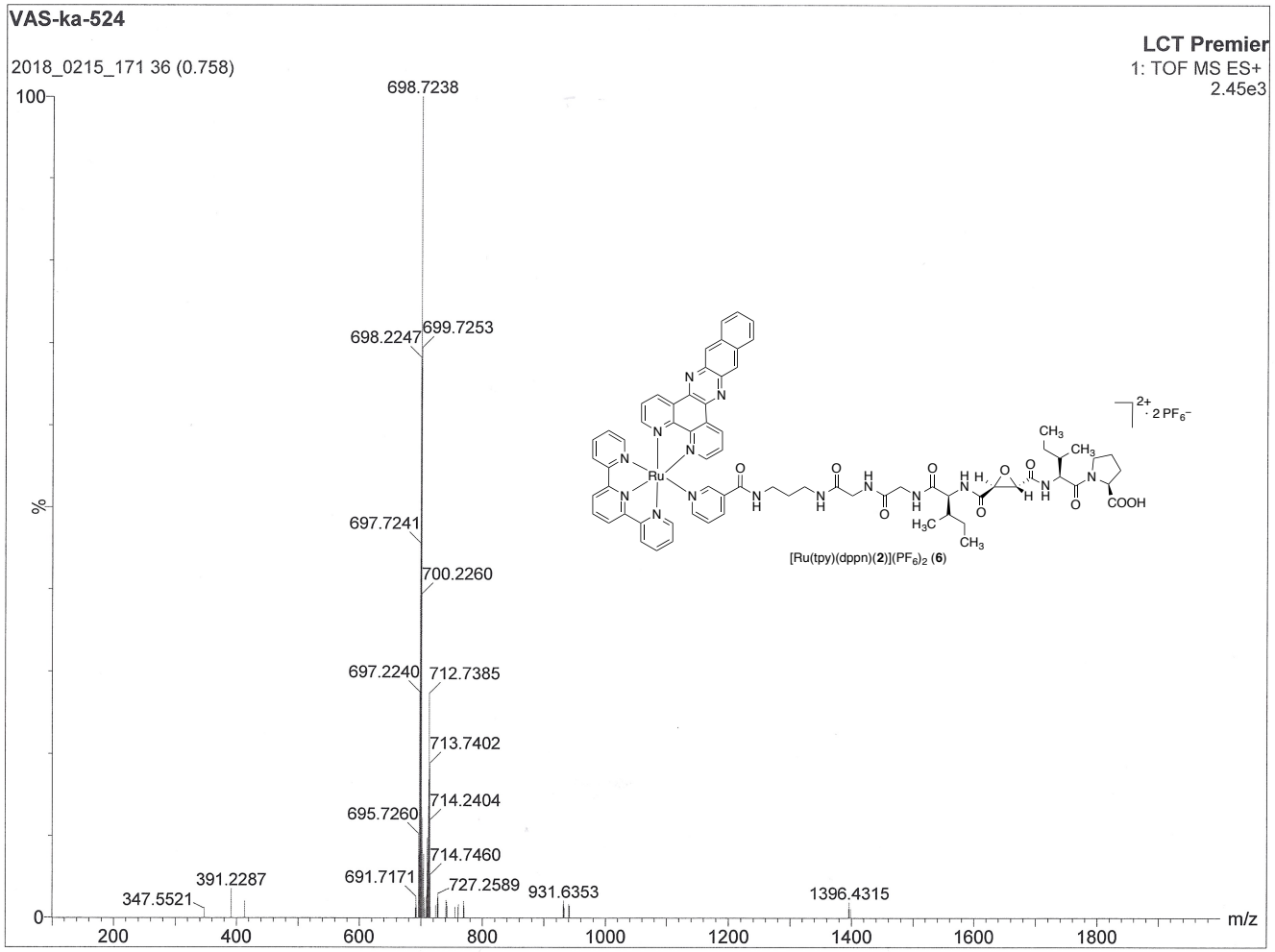

Figure S47: Mass spectrum of the di-cation $[Ru(tpy)(dppn)(\mathbf{2})]^{2+}$ (**6**)

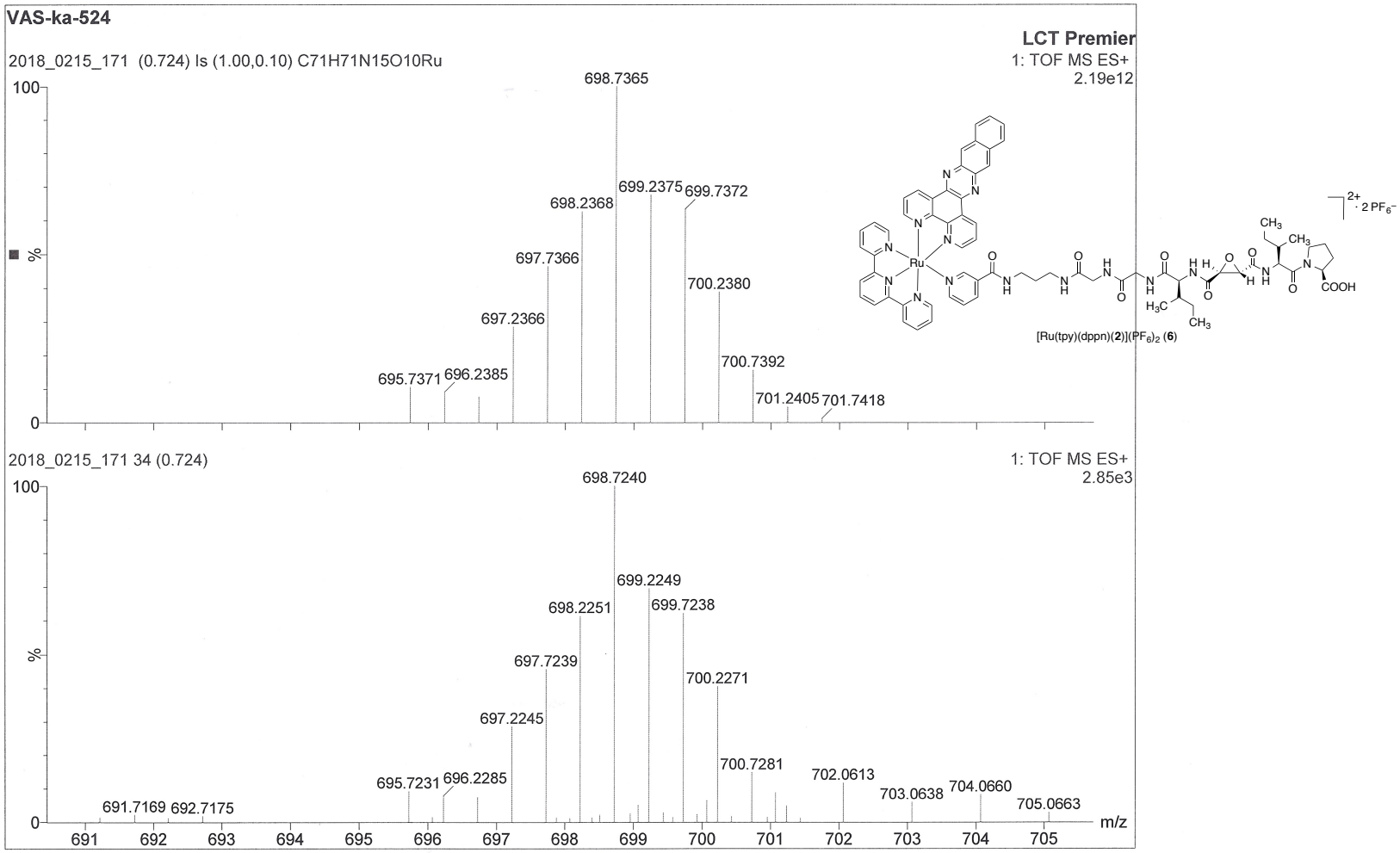

Figure S48: Expansion of mass spectrum of $[Ru(tpy)(dppn)(\mathbf{2})]^{2+}$ (**6**) calculated (above) and observed (below) isotope pattern for major peak with m/z = 699

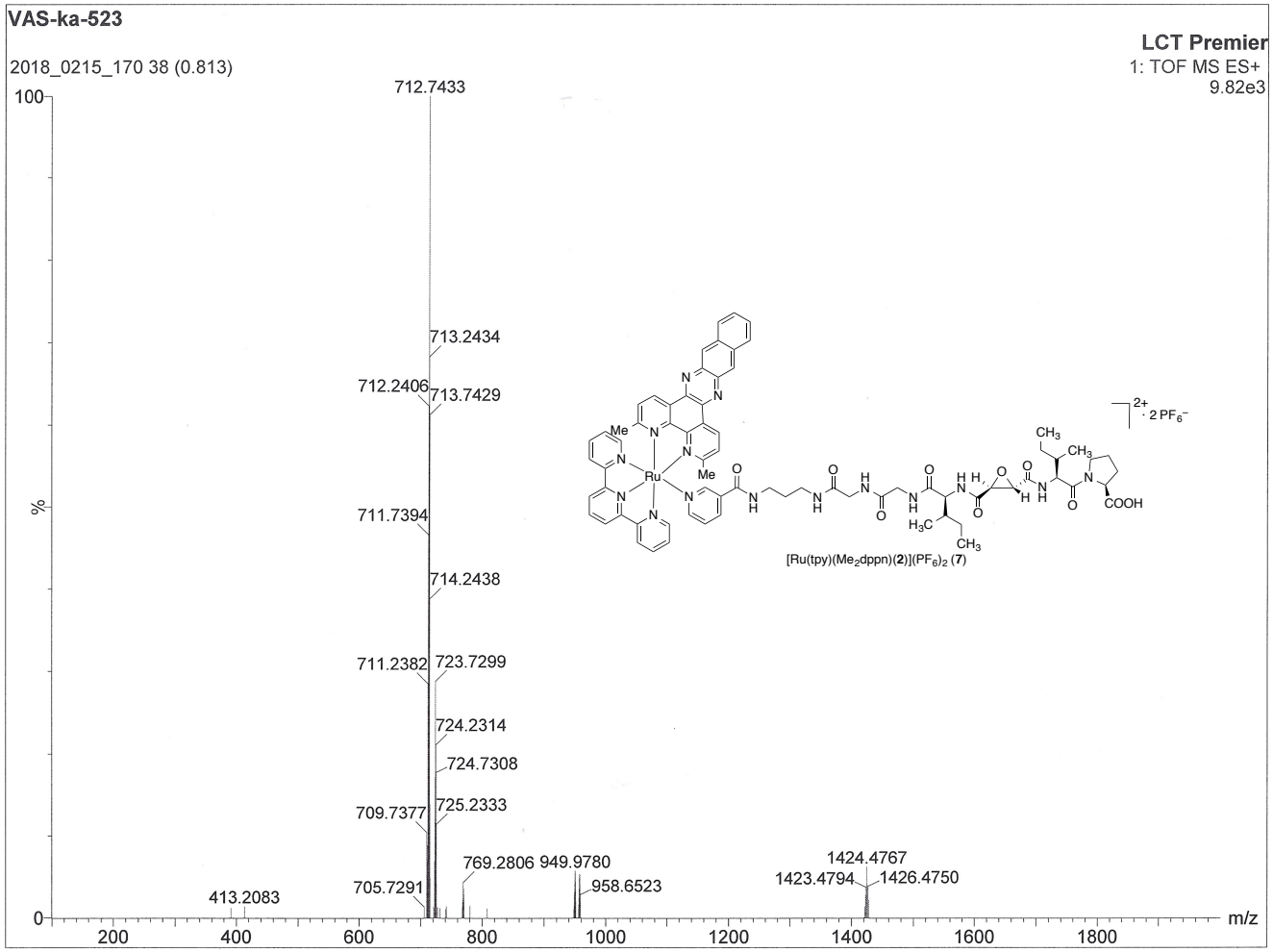

Figure S49: Mass spectrum calculated (above) and observed (below) of the di-cation $[Ru(tpy)(Me_2dppn)(\mathbf{2})]^{2+}$ (**7**)

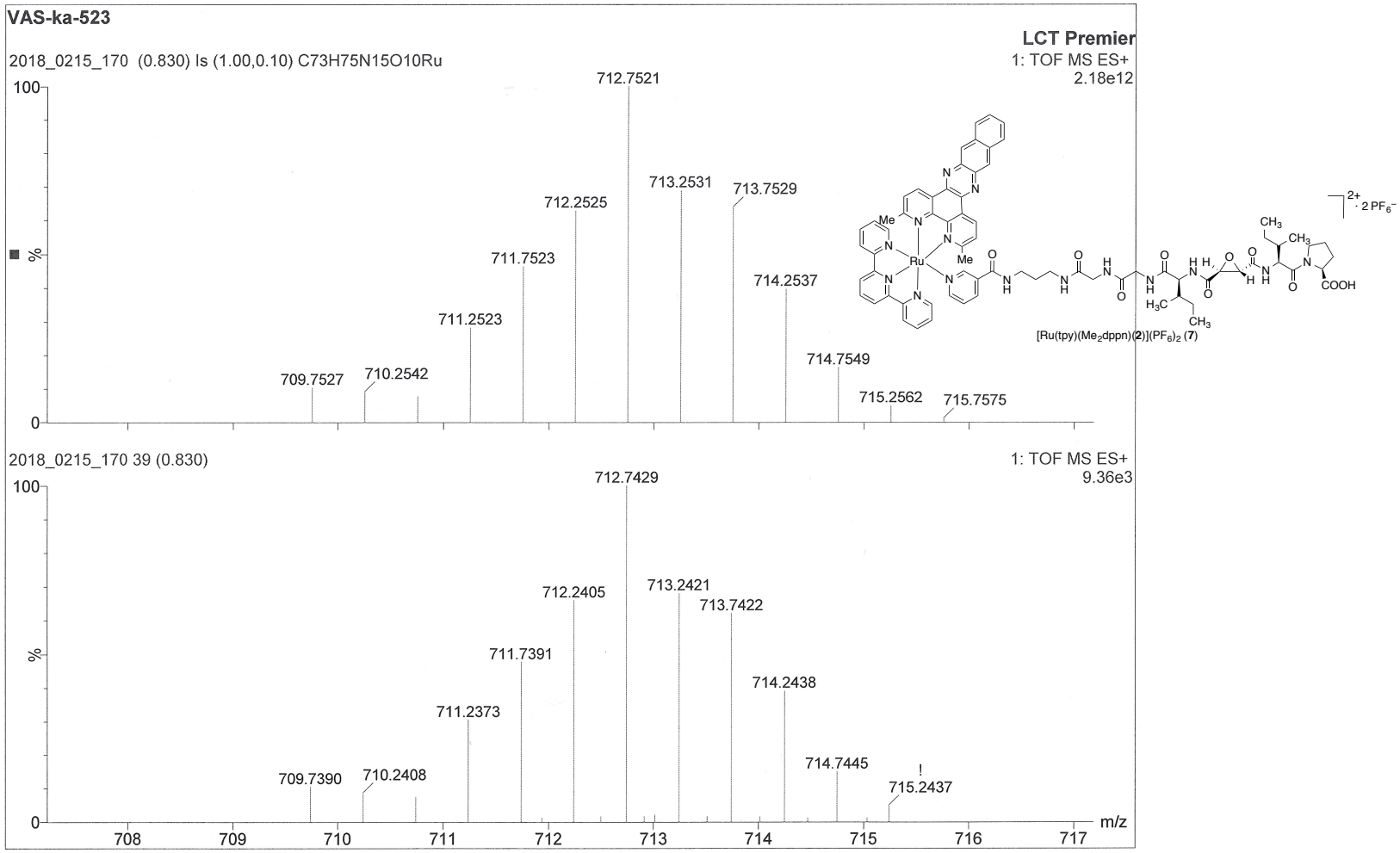

Figure S50: Expansion of mass spectrum of [Ru(tpy)(Me$_2$dppn)(**2**)]$^{2+}$ (**7**) calculated (above) and observed (below) isotope pattern for major peak with m/z = 713

Part E. Photochemistry

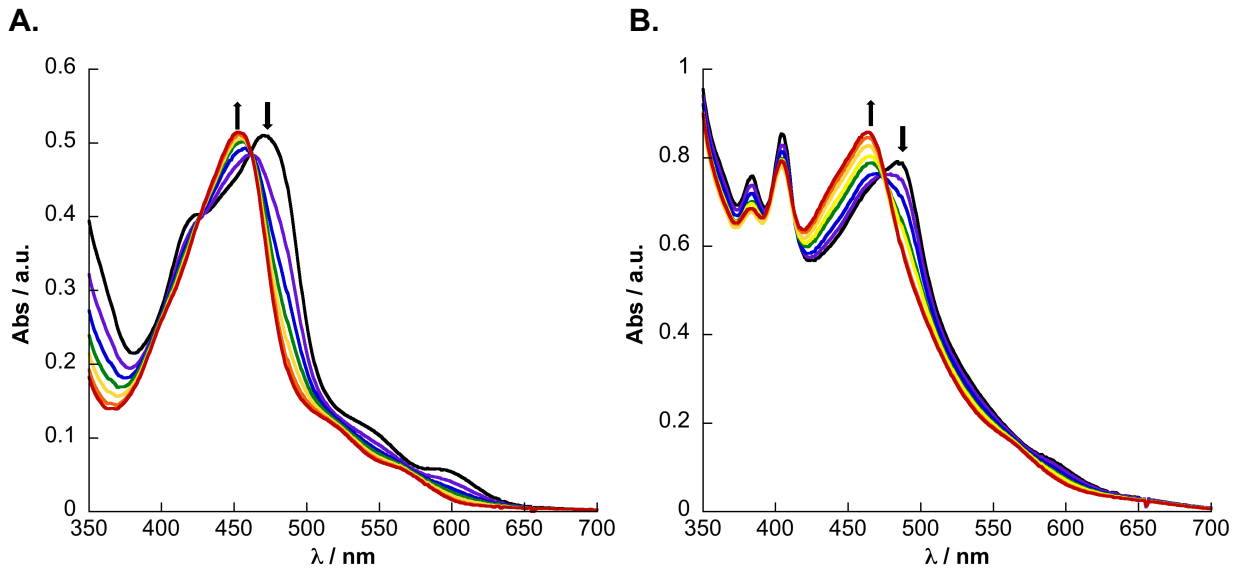

Figure 51. Changes to the electronic absorption spectra of 5 (**A**) and N_2 purged 7 (**B**) in CH_3CN as a function of irradiation time ($\lambda_{irr} \geq 475$ nm) for 0 – 3 min and 0 – 5 min, respectively.

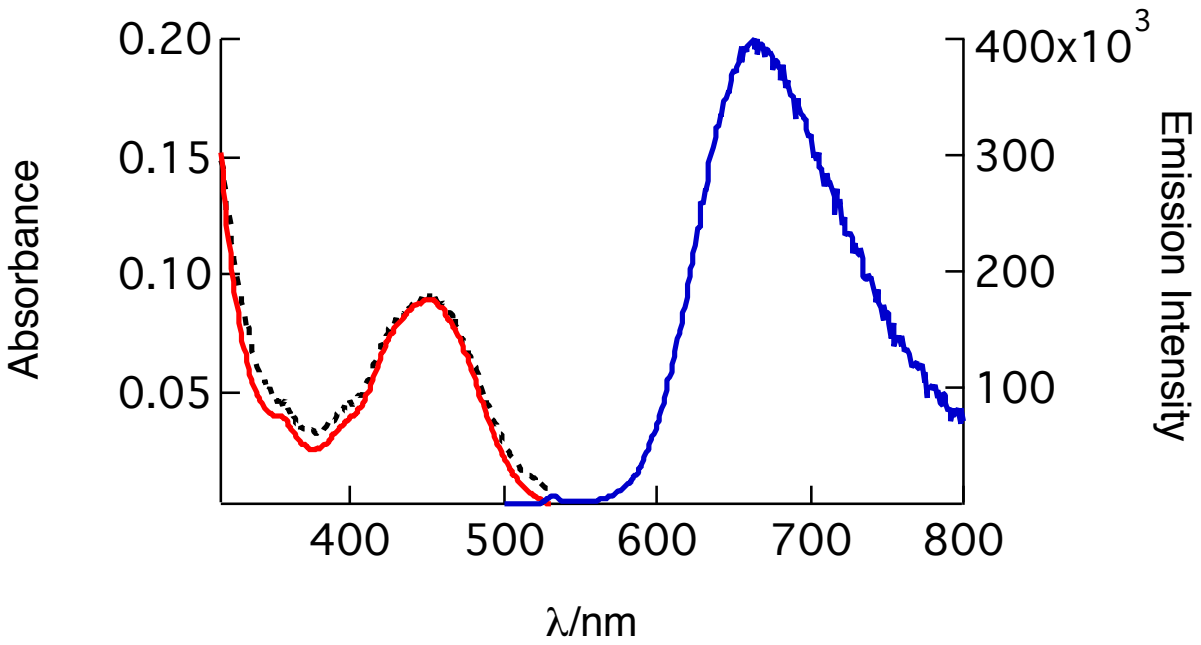

Figure S52. Absorption (—), emission (λ_{ex} = 460 nm, —), and excitation (λ_{em} = 665 nm, - - -) spectra of **3** (8.3 mM) in CH_3OH at room temperature.

▲ = $Ru(tpy)(Me_2bpy)(D_2O)^{2+}$

● = **1**

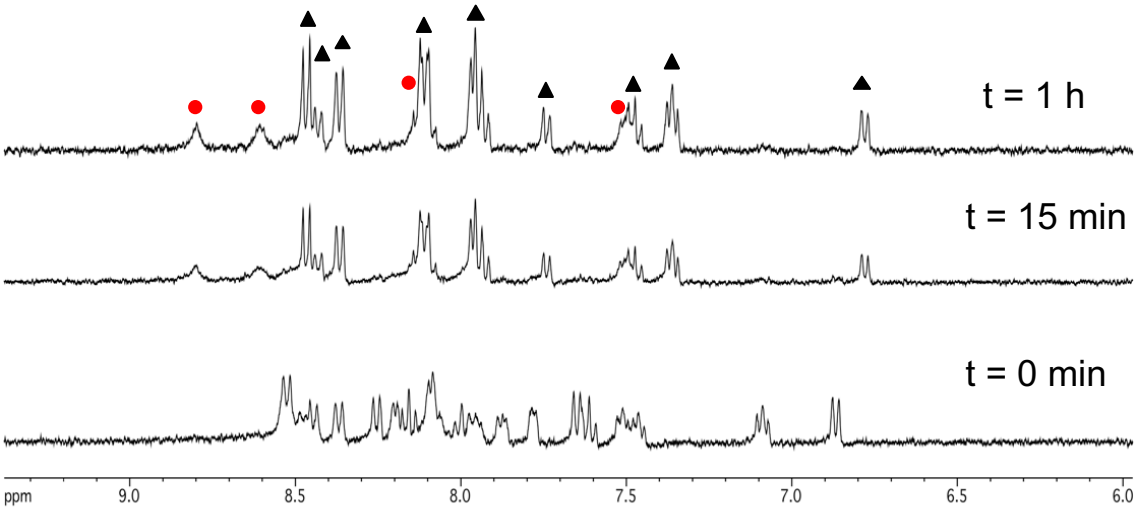

Figure S53. Aromatic region of ^1H NMR spectra of complex **5** in 5% CD_3OD/D_2O upon irradiation ($\lambda_{irr} \geq 475$ nm) at t = 0 (bottom), 15 min (middle), and 1 h (top).

▲ = $Ru(tpy)(Me_2bpy)(D_2O)^{2+}$

● = **1**

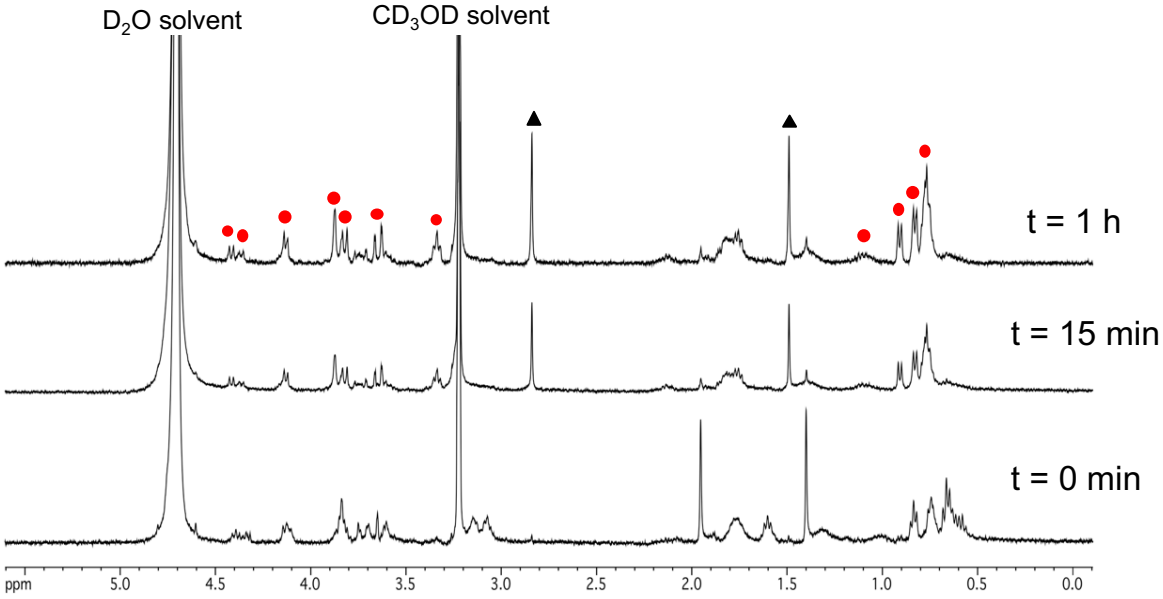

Figure S54. Aliphatic region of ^1H NMR spectra of complex **5** in 5% CD_3OD/D_2O upon irradiation ($\lambda_{irr} \geq 475$ nm) at t = 0 (bottom), 15 min (middle), and 1 h (top).

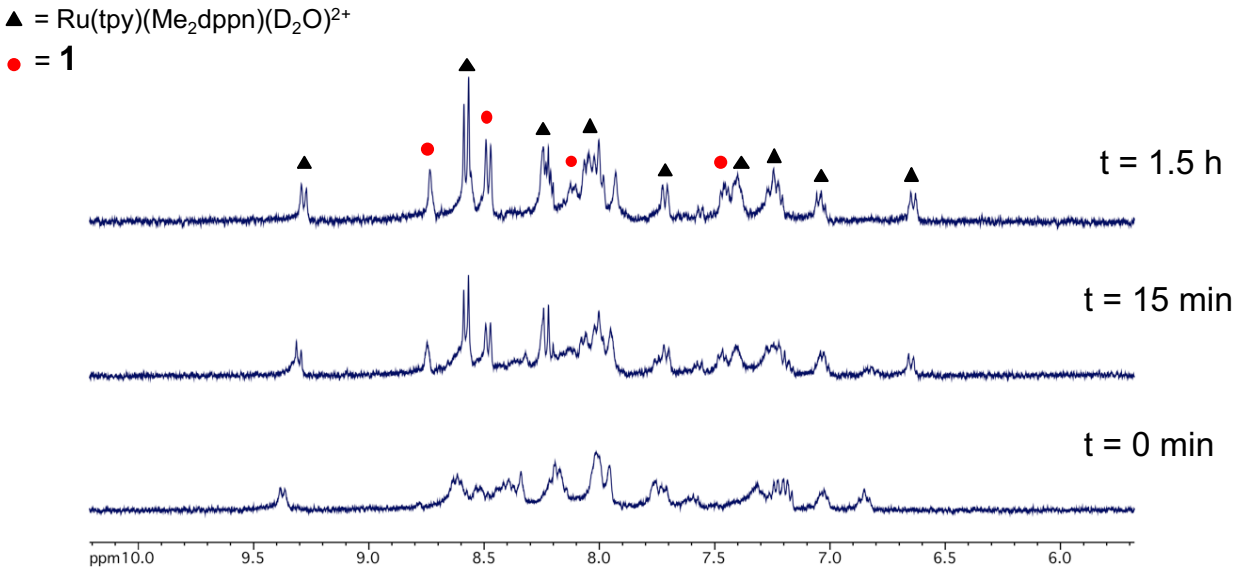

Figure S55. Aromatic region of ^{1}H NMR spectra of complex **7** in 5% CD_3OD/D_2O upon irradiation ($\lambda_{irr} \geq 475$ nm) at t = 0 (bottom), 15 min (middle), and 1.5 h (top).

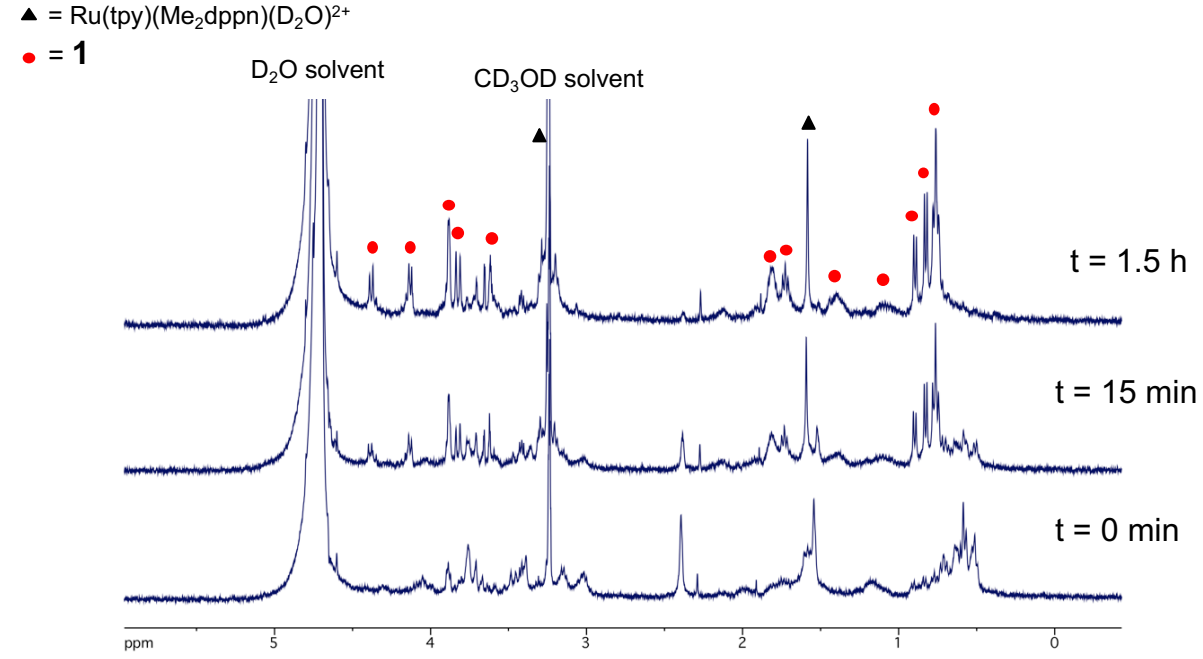

Figure S56. Aliphatic region of ^{1}H NMR spectra of complex **7** in 5% CD_3OD/D_2O upon irradiation ($\lambda_{irr} \geq 475$ nm) at t = 0 (bottom), 15 min (middle), and 1.5 h (top).

▲ = $Ru(tpy)(Me_2dppn)(D_2O)^{2+}$

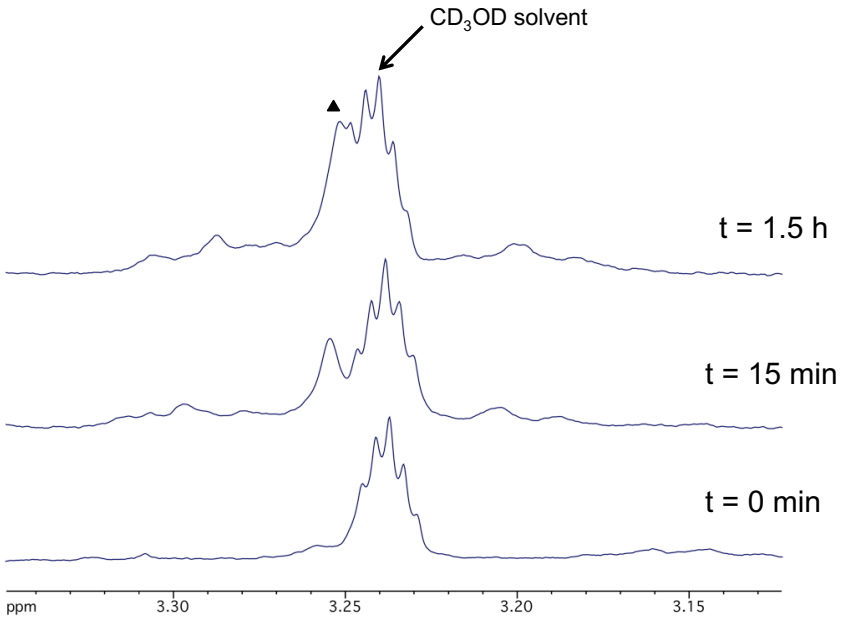

Figure S57. Enhanced aliphatic region of ^{1}H NMR spectra of complex **7** in 5% CD_3OD/D_2O showing the appearance of the methyl group resonance of the Me_2dppn ligand upon irradiation ($\lambda_{irr} \geq 475$ nm).

Part F. Stability Studies

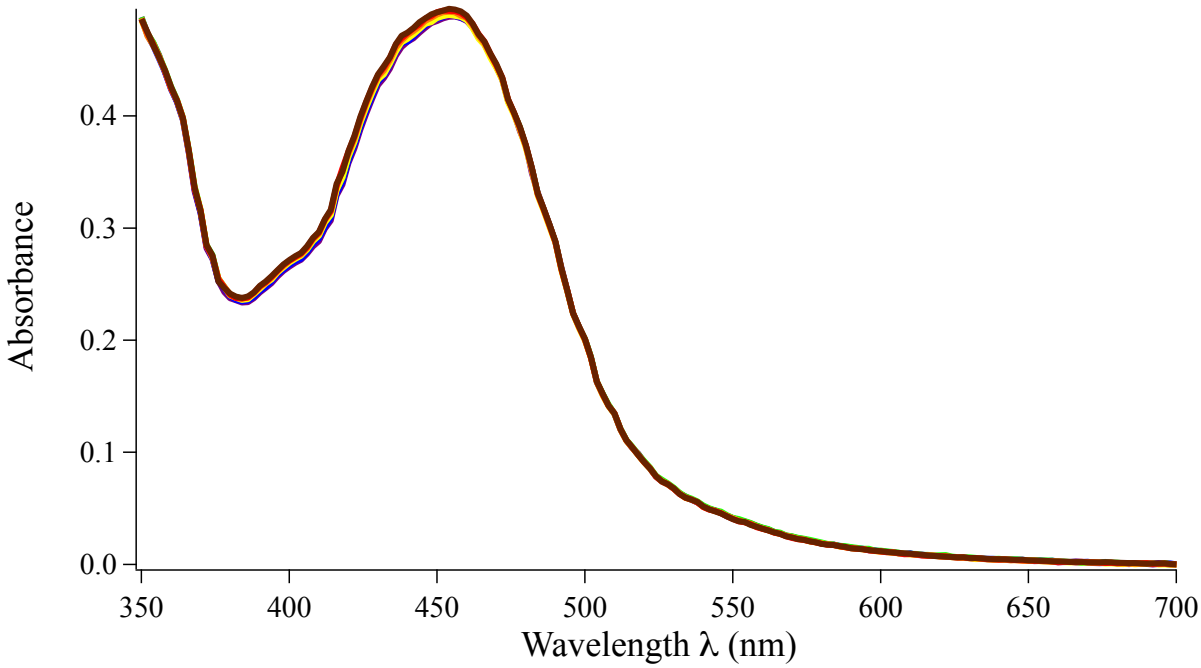

Figure S58. Absorbance scan for complex **3** at 25.0°C in DMSO. The spectra were taken at t = 0 (purple), 4 (blue), 8 (green), 12 (yellow), 16 (orange), 20 (red) and 24 (brown) h.

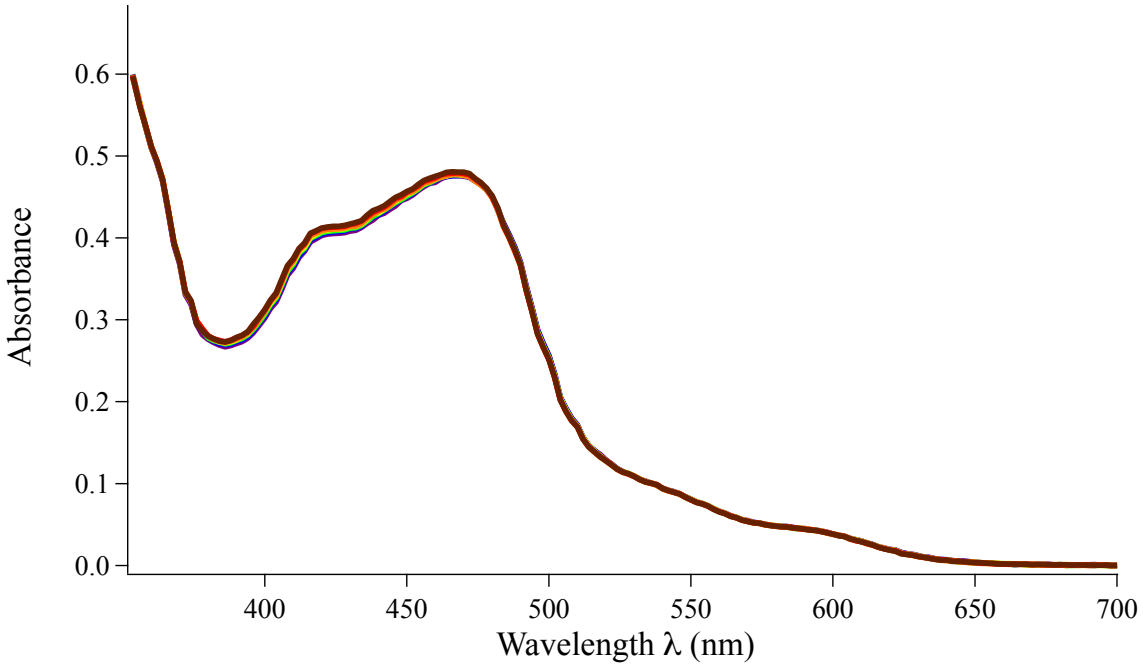

Figure S59. Absorbance scan for complex **4** at 25.0°C in DMSO. The spectra were taken at t = 0 (purple), 4 (blue), 8 (green), 12 (yellow), 16 (orange), 20 (red) and 24 (brown) h.

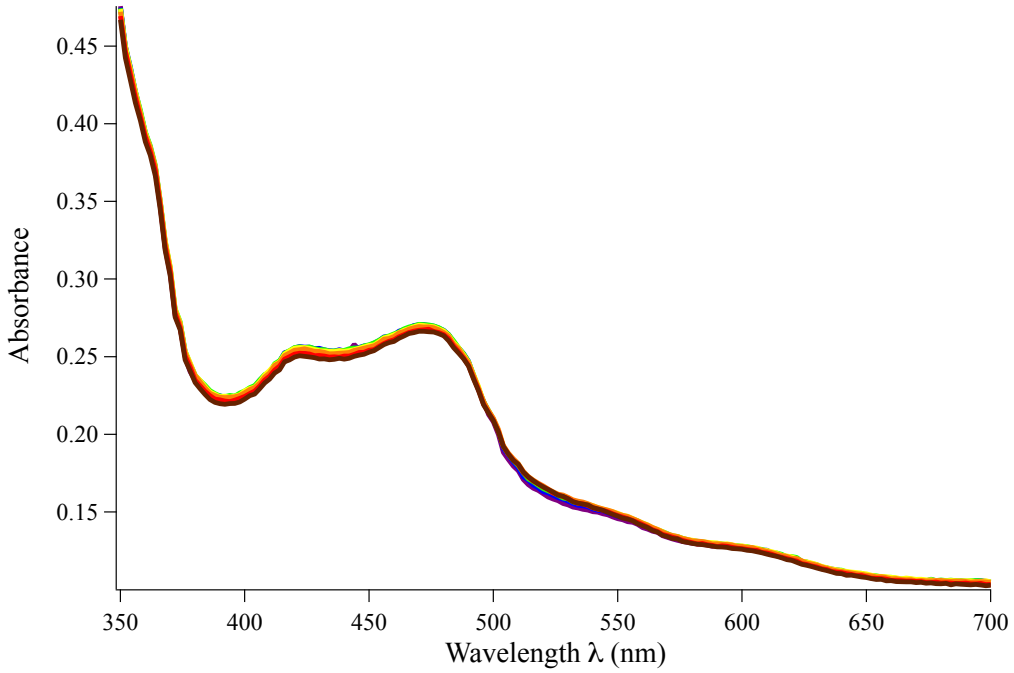

Figure S60. Absorbance scan for complex **5** at 25.0°C in DMSO. The spectra were taken at t = 0 (purple), 4 (blue), 8 (green), 12 (yellow), 16 (orange), 20 (red) and 24 (brown) h.

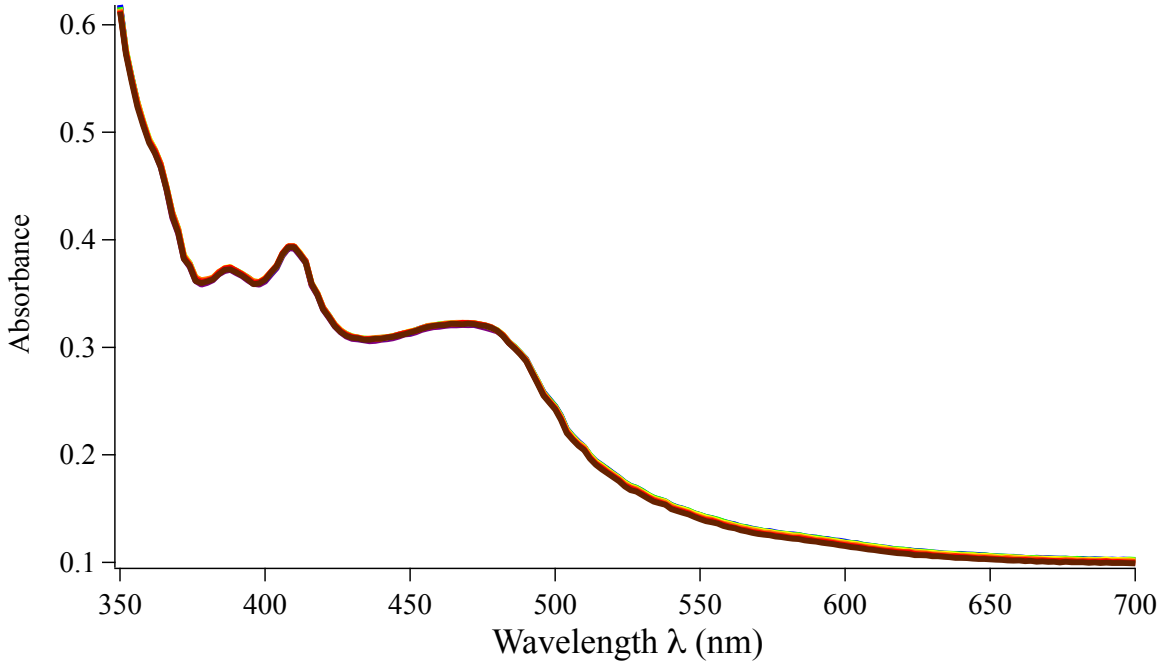

Figure S61. Absorbance scan for complex **6** at 25.0°C in DMSO. The spectra were taken at t = 0 (purple), 4 (blue), 8 (green), 12 (yellow), 16 (orange), 20 (red) and 24 (brown) h.

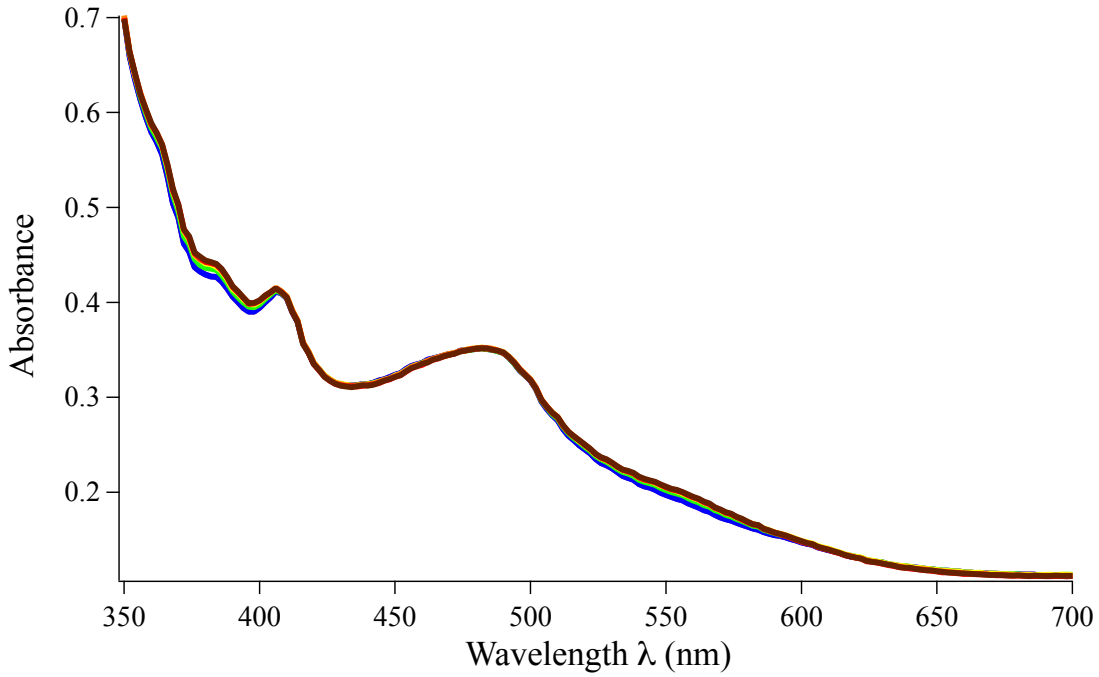

Figure S62. Absorbance scan for complex **7** at 25.0°C in DMSO. The spectra were taken at t = 0 (purple), 4 (blue), 8 (green), 12 (yellow), 16 (orange), 20 (red) and 24 (brown) h.

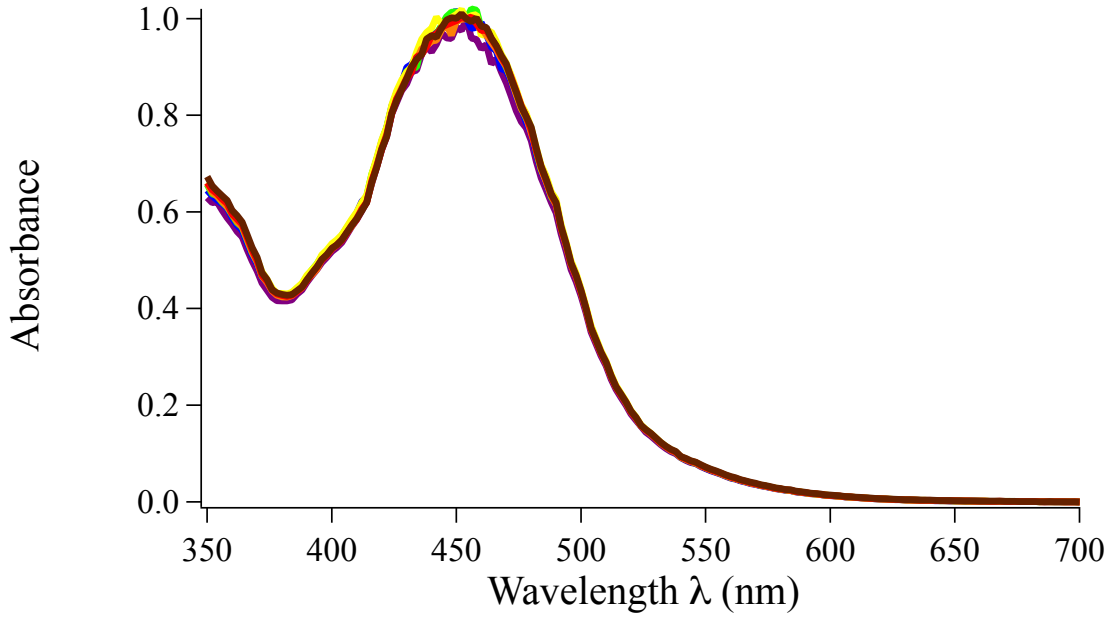

Figure S63. Absorbance scan for complex **3** at 37.0°C in Cell growth media. The spectra were taken at t = 0 (purple), 4 (blue), 8 (green), 12 (yellow), 16 (orange), 20 (red) and 24 (brown) h.

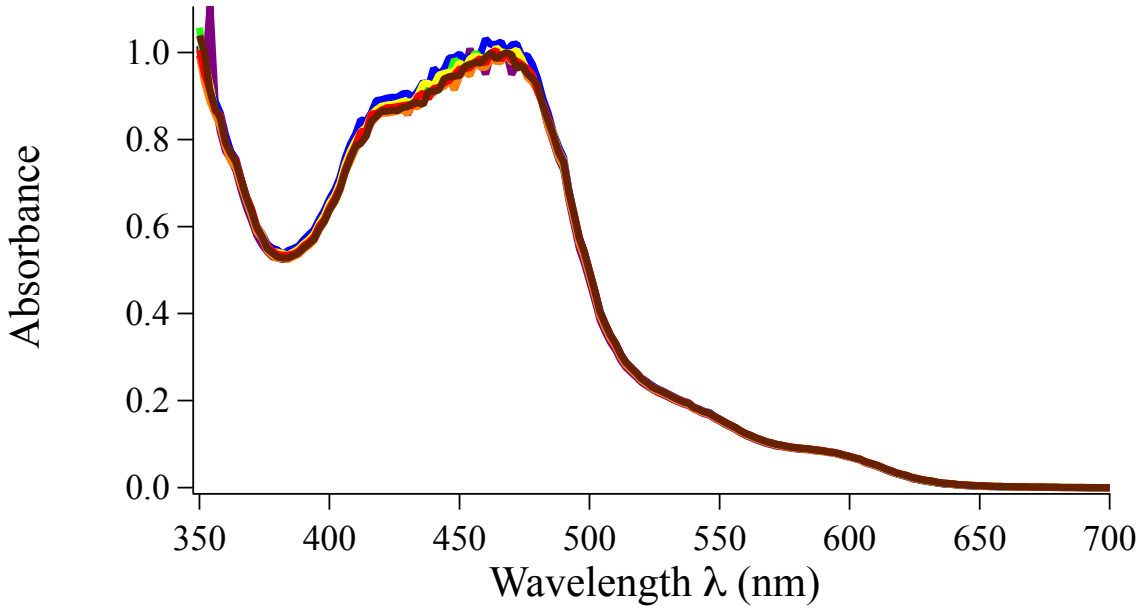

Figure S64. Absorbance scan for complex **4** at 37.0°C in Cell growth media. The spectra were taken at t = 0 (purple), 4 (blue), 8 (green), 12 (yellow), 16 (orange), 20 (red) and 24 (brown) h.

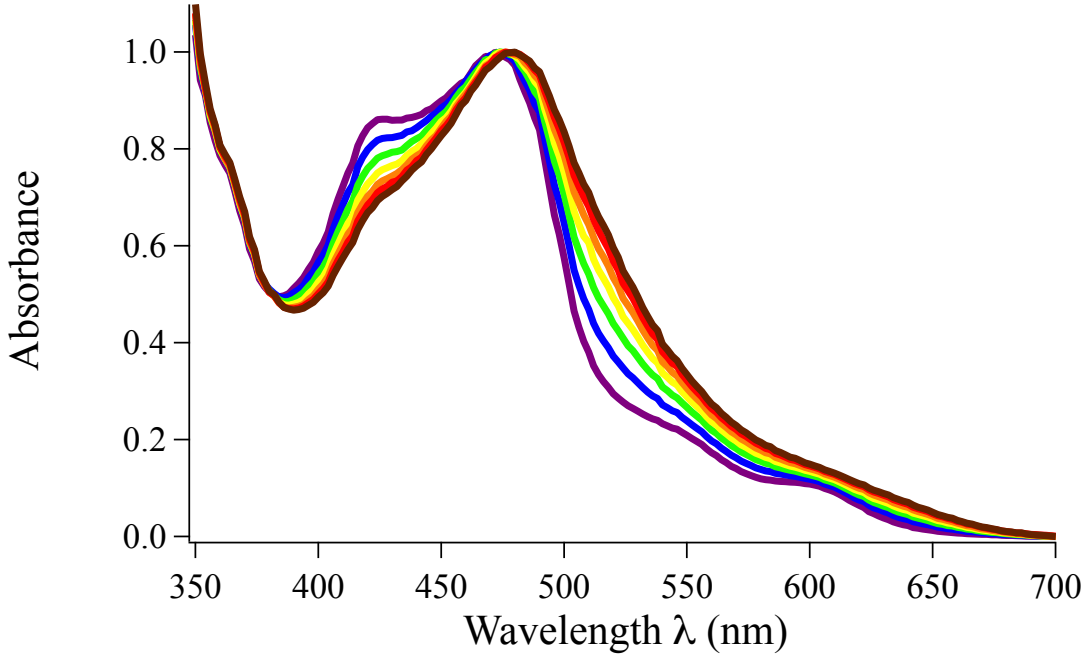

Figure S65. Absorbance scan for complex **5** at 37.0°C in Cell growth media. The spectra were taken at t = 0 (purple), 4 (blue), 8 (green), 12 (yellow), 16 (orange), 20 (red) and 24 (brown) h.

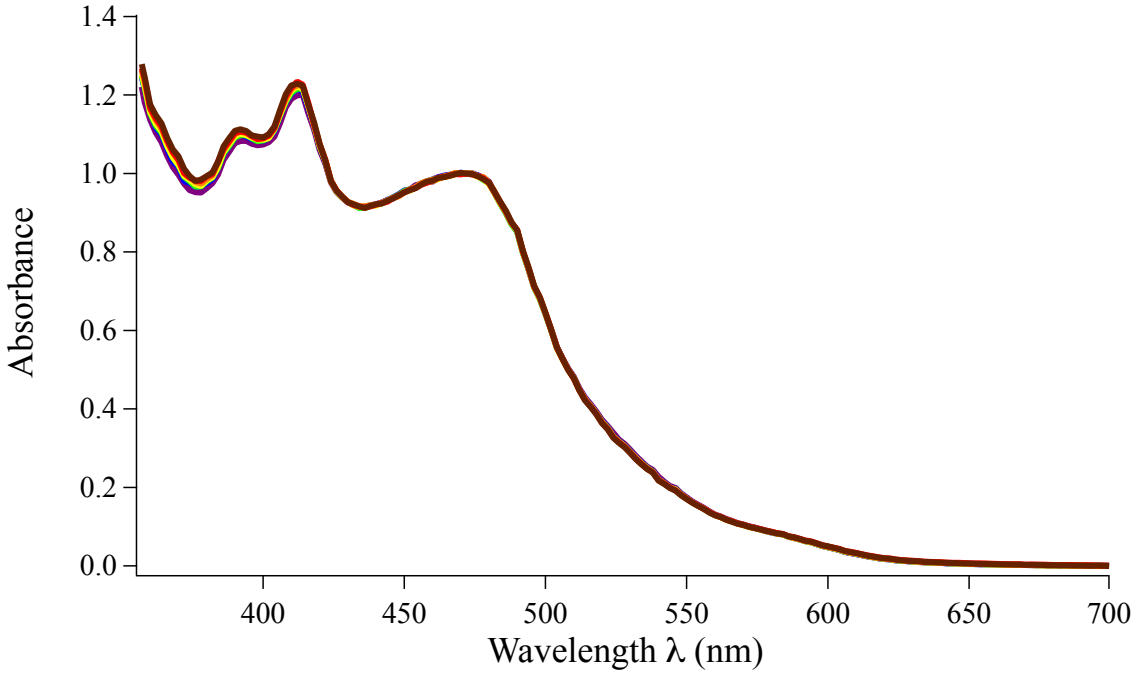

Figure S66. Absorbance scan for complex **6** at 37.0°C in Cell growth media. The spectra were taken at t = 0 (purple), 4 (blue), 8 (green), 12 (yellow), 16 (orange), 20 (red) and 24 (brown) h.

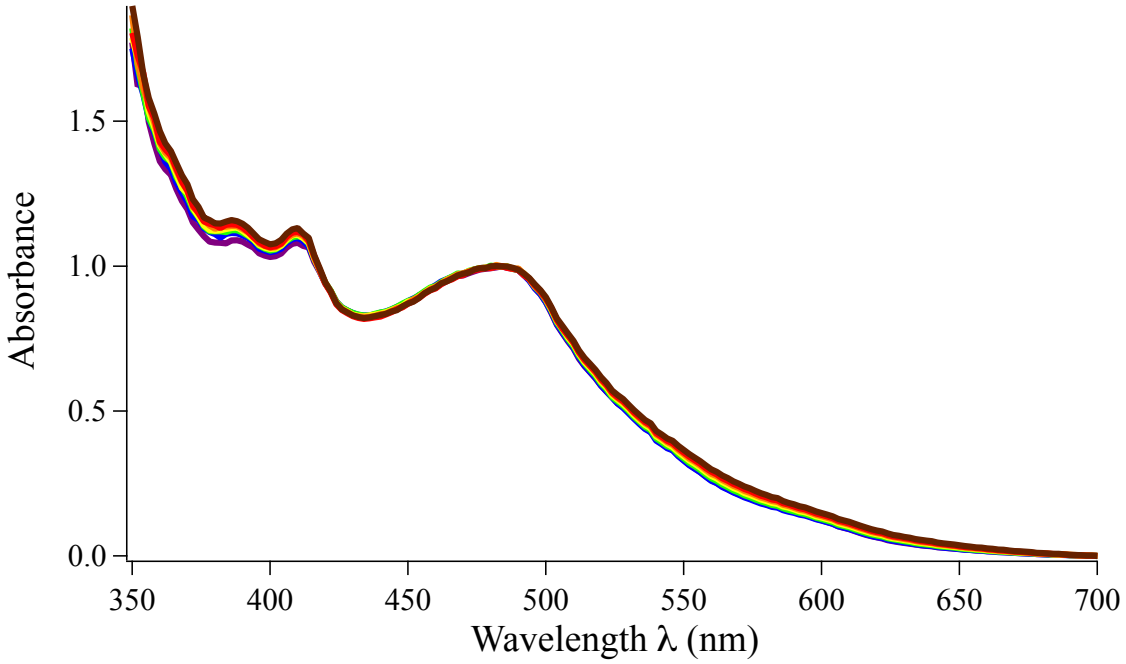

Figure S67. Absorbance scan for complex **7** at 37.0°C in Cell growth media. The spectra were taken at t = 0 (purple), 4 (blue), 8 (green), 12 (yellow), 16 (orange), 20 (red) and 24 (brown) h.

References:

(1) Huisman, M.; White, J. K.; Lewalski, V. G.; Podgorski, I.; Turro, C.; Kodanko, J. J. *Chem. Commun.* **2016**, *52*, 12590.