Supporting Information

The Bivalent Ligand Approach Leads to a Bioactive Inhibitor of MBNL1·CUG^{exp} Complex

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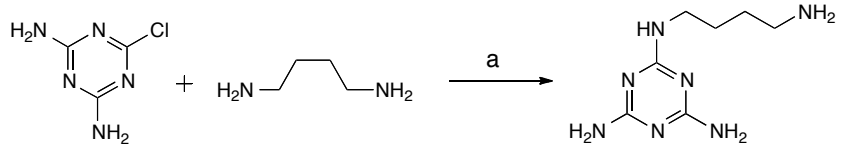
1. General Materials and Methods

All reagents were purchased from commercial suppliers and used without further purification. Anhydrous solvents were obtained from an anhydrous solvent dispensing system. For all reactions employing anhydrous solvents, glassware was oven-dried, cooled under vacuum, and then purged and conducted under dry nitrogen. Purified compounds were further dried under high vacuum (0.01–0.05 Torr) or lyophilized using a Labconco lyophilizer. NMR spectra were recorded at ambient temperature on Varian Unity 500 or Varian Unity Inova 500NB, operating at 500 MHz and 125 MHz for ^{1}H and ^{13}C acquisitions, respectively unless indicated. All other spectra recorded on a Unity 400 operating at 400 MHz and 100 MHz for ^{1}H and ^{13}C acquisitions. Unless specified the spectra were recorded in chloroform-*d*. Chemical shifts are reported in ppm and referenced to the corresponding residual proton signal: $CDCl_3$ (7.26 ppm ^{1}H, 77.16 ppm ^{13}C); DMSO (2.50 ppm ^{1}H, 39.52 ppm ^{13}C); D_2O (4.79 ppm ^{1}H); CD_3OD (3.31 ppm ^{1}H, 50.41 ppm ^{13}C). Multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), sext (sextet), dd (doublet of doublets), td (triplet of doublets), dt (doublet of triplets), m (multiplet), b (broad). Integration is provided and coupling constants, *J*, are reported in Hertz (Hz). ESI mass spectra were recorded using the Quattro or ZMD mass spectrometer. High resolution mass spectra (HRMS) were obtained at the University of Illinois mass spectrometry facility. Compounds described herein gave NMR and mass spectral data in accord with their structures. Elemental analysis was obtained at the Microanalysis Lab at University of Illinois.

2. Synthesis and characterization of intermediate compounds

Supplementary Scheme 1. Synthesis of **28**[a]

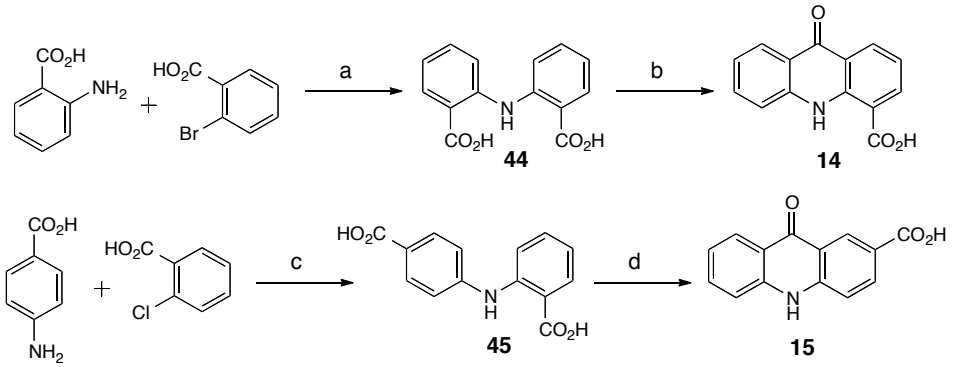


28

[a]Reagents and conditions: (a) 130 °C, 80%.

***N*^{2}-(4-Aminobutyl)-1,3,5-triazine-2,4,6-triamine (28).** Title compound was synthesized as described previously in 80% yield,[1] with minor changes in the work-up procedure. ^{1}H NMR (DMSO-d_6) δ 6.40 (t, *J* = 5.6, 1H), 6.05 (s, 2H), 5.88 (s, 2H), 3.14 (q, *J* = 6.3, 2H), 2.56–2.51 (m, 2H), 1.44 (p, *J* = 7.1, 2H), 1.37–1.31 (m, 2H); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 198.1; found 198.1.

Supplementary Scheme 2. Synthesis of compounds **14** and **15**[a]



[a]Reagents and conditions: (a) Cu, Cu_2O, K_2CO_3, DMF, Reflux, 24 h, 91%; (b) PPA, 120 °C, 95%; (c) Cu, Cu_2O, K_2CO_3, 2-ethoxyethanol, 130 °C, 70%; (d) PPA, 120 °C, 95%.

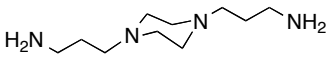
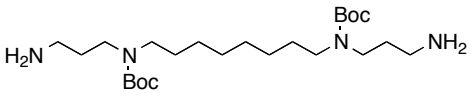
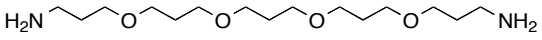
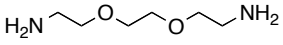
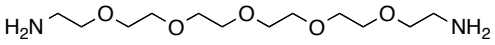
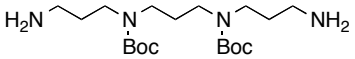
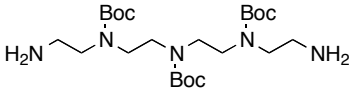
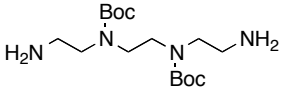
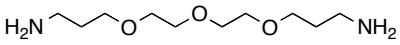
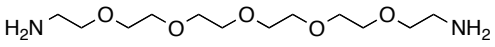
2,2'-Azanediyldibenzoic acid (44). Title compound was synthesized as described previously in 91% yield,[2] with minor changes in the work-up procedure. ^{1}H NMR (DMSO-d_6) δ 7.91 (dd, J = 7.9, 1.3, 2H), 7.50–7.40 (m, 4H), 6.92–6.97 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.41, 143.58, 133.38, 131.81, 119.99, 117.56, 113.56. *m/z* LRMS (ESI) calculated for $[M+H]^+$: 258.1; found 258.0.

9-Oxo-9,10-dihydroacridine-4-carboxylic acid (14). Title compound was synthesized as described previously in 95% yield,[2] with minor changes in the work-up procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.01 (s, 1H), 8.53 (dd, J = 8.0, 1.5, 1H), 8.45 (dd, J = 7.5, 1.6, 1H), 8.24 (d, J = 8.0, 1H), 7.83–7.73 (m, 3H), 7.41–7.29 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 176.53, 169.14, 141.20, 139.92, 136.90, 134.11, 132.41, 125.89, 122.32, 121.63, 120.60, 120.24, 118.63, 115.01; *m/z* LRMS (ESI) calculated for $[M-H]^-$: 238.1; found 238.2.

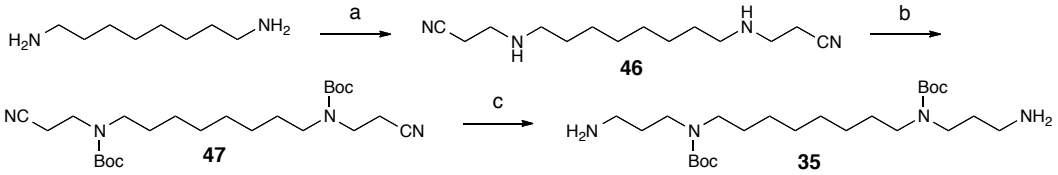
2-((4-Carboxyphenyl)amino)benzoic acid (45). Title compound was synthesized as described previously in 70% yield,[3] with minor changes in the work-up procedure. ^{1}H NMR (DMSO-d_6) δ 9.87 (s, 1H), 7.94 (d, J = 8.0, 1H), 7.87 (d, J = 8.7, 2H), 7.51–7.45 (m, 2H), 7.27 (d, J = 8.7, 2H), 6.96–6.91 (m, 1H); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 258.1; found 258.0.

9-Oxo-9,10-dihydroacridine-2-carboxylic acid (15). Title compound was synthesized as described previously in 95% yield,[4] with minor changes in the work-up procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.90 (s, 1H), 12.06 (s, 1H), 8.83 (d, J = 2.0, 1H), 8.26–8.18 (m, 2H), 7.75–7.82 (m, 1H), 7.56–7.61 (m, 2H), 7.36–7.27 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 177.00, 167.41, 143.13, 140.90, 134.12, 133.88, 133.62, 128.39, 126.10, 121.76, 120.85, 119.69, 117.69, 117.38.

Supplementary table 1. Summary of compounds **34-42**.

Compound	Structure
34	
35	
36	
37	
38	
39	
40	
41	
42	
43	

Supplementary Scheme 3. Synthesis of **35**[a]



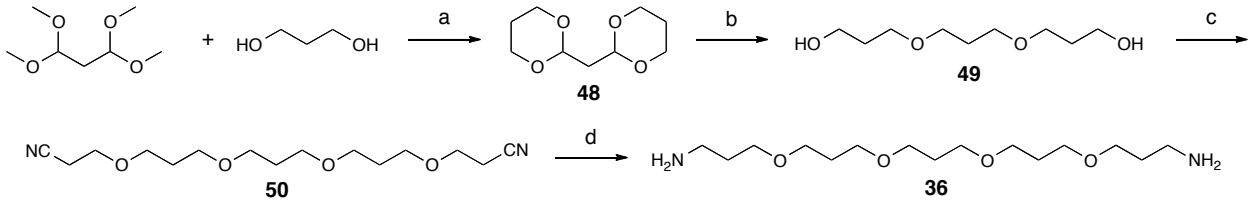
[a]Reagents and conditions: (a) Acrylonitrile (2 equiv.), EtOH, 0 °C to RT, 48 h, 83%; (b) Boc_2O, NEt_3, MeOH, 0 °C to RT, 24 h; (c) H_2, Ra-Ni, Pd/C, LiOH, dioxane, H_2O, 24 h, 65%.

***N,N'*-Bis(2-Cyanoethyl)-1,8-octanediamine (46).** 1,8-octanediamine (1.8 g, 12.5 mmol, 1 equiv.) was dissolved in ethanol. Acrylonitrile (1.33 g, 25 mmol, 2 equiv.) was added dropwise to the solution and stirred for 48 h at room temperature to yield the product in 83% yield. ^{1}H NMR δ 2.93 (td, *J* = 6.6, 2.2, 4H), 2.66–2.59 (m, 4H), 2.52 (td, *J* = 6.6, 2.2, 4H), 1.48 (s, 4H), 1.31 (s, 8H); ^{13}C NMR δ 118.87, 49.36, 45.27, 30.16, 29.54, 27.28, 18.90.

Di-*tert*-Butyl octane-1,8-diylbis((2-cyanoethyl)carbamate) (47). Compound **46** (3.13 g, 12.5 mmol, 1 equiv.) was dissolved in methanol (50 mL) and cooled to 0 °C. Di-*tert*-butyl dicarbonate (10.91 g, 50 mmol, 4 equiv.) and then triethylamine (5.06 g, 50 mmol, 4 equiv.) were added dropwise. The reaction was warmed to room temperature and stirred for 24 h to give **47** in 85% yield. *m/z* LRMS (ESI) calculated for $[M+Na]^+$: 473.3; found 473.1.

***N,N'*-Bis(3-Aminopropyl)-Bis(t-butylcarbamate)-1,8-octanediamine (35).** Compound **47** was hydrogenated in the presence of Pd and Raney nickel and LiOH in a water/ dioxane mixture for 24 h at 100 atm to give the product in 65% yield. *m/z* LRMS (ESI) calculated for $[M+H]^+$: 459.4; found 459.1.

Supplementary Scheme 4. Synthesis of **36**[a]



[a]Reagents and conditions: (a) TsOH (cat.), toluene, 110 °C, 24 h, 96%; (b) BH_3-THF, 90 °C, 4 days, 61%; (c) acrylonitrile, NaH, 15-crown-5, 0 °C, 30 min, 94%; (d) BH_3-THF, 90 °C 24 h, 90%.

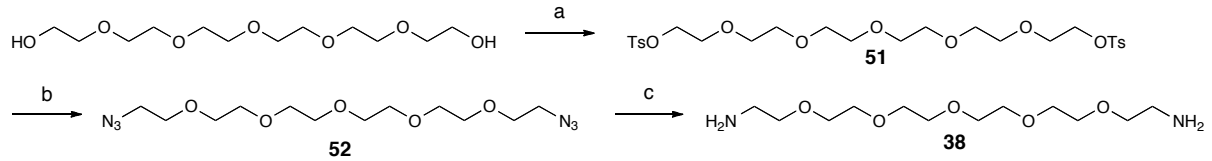
Di(1,3-dioxan-2-yl)methane (48). 3-Methoxybutyraldehyde dimethyl acetal (0.135 g, 0.82 mmol, 1 equiv.) and propanediol (0.218 g, 2.86 mmol, 1.74 equiv.) with catalytic TsOH (0.02 g, 0.1 mmol. 0.13 equiv.) were reacted in toluene (25 mL) at 110 °C for 24 h to give the product in 96% yield.[5] ^{1}H NMR (400 MHz) δ 4.75–4.60 (m, 2H), 4.17–4.01 (m, 4H), 3.83–3.71 (m, 4H), 2.15–2.01 (m, 2H), 1.96–1.88 (m, 2H), 1.36–1.34 (m, 1H), 1.33–1.31 (m, 1H).

Tripropylene glycol (49). Compound **48** (2.95 g, 15.7 mmol, 1 equiv.) was dissolved in THF (125 mL) and cooled to 0 °C. Borane (39.25 mL, 39.25 mmol, 2.5 equiv.) in THF (1M solution) was added dropwise and the reaction was refluxed for 4 days at 90 °C. The product was purified via flash chromatography (SiO_2; Acetone:EtOAc, 15:85) in 61% yield. ^{1}H NMR δ 3.65 (t, *J* = 5.8, 4H), 3.52 (t, *J* = 5.8, 4H), 3.47–3.43 (m, 2H), 1.79–1.70 (m, 6H); ^{13}C NMR δ 69.45, 68.05, 61.03, 31.98, 29.85.

4,8,12,16-Tetraoxanonadecane-1,19-dinitrile (50). Acrylonitrile (10 mL, 152 mmol, 15 equiv.), 15-crown-5 (44 mg, 0.2 mmol, 0.02 equiv.) and **49** (1.85 g, 9.6 mmol, 1 equiv.) were combined and cooled to 0 °C. NaH (8 mg, 0.2 mmol, 0.02 equiv.) was added and the reaction was stirred at 0 °C for 30 min to give the product in 94% yield. ^{1}H NMR δ 3.65 (t, *J* = 6.4, 4H), 3.57 (t, *J* = 6.3, 4H), 3.52–3.47 (m, 8H), 2.59 (t, *J* = 6.3, 4H), 1.90–1.78 (m, 6H); ^{13}C NMR δ 118.05, 68.40, 68.02, 67.51, 65.50, 30.18, 30.02, 19.02; *m/z* LRMS (ESI) calculated for $[M+H]^+$: 299.2; found 299.1.

4,8,12,16-Tetraoxanonadecane-1,19-diamine (36). A solution of **50** and THF was added dropwise to a solution of BH_3 in THF (1 M). This was stirred for 30 min at 0 °C and then refluxed at 90 °C overnight. The reaction mixture was quenched with methanol and then HCl (37%) was added slowly. The reaction mixture was stirred for 1 h and dried *in vacuo*. Trimethyl borane was removed by coevaporation with methanol (3 x 10 mL). To the viscous liquid, NaOH (15 mL, 1 M) was added and extracted with DCM (3 x 20 mL). Organic layers were combined, dried over sodium sulfate and then dried *in vacuo* to give the product in 90% yield. ^{1}H NMR δ 3.53–3.43 (m, 16H), 2.87–2.77 (m, 4H), 2.05 (bs, 4H), 1.89–1.79 (m, 6H), 1.74 (p, *J* = 6.4, 1H); ^{13}C NMR δ 70.91, 69.44, 68.41, 68.38, 68.13, 39.89, 32.87, 30.22; *m/z* LRMS (ESI) calculated for $[M+H]^+$: 307.3; found 307.2.

Supplementary Scheme 5. Synthesis of **38**[a]



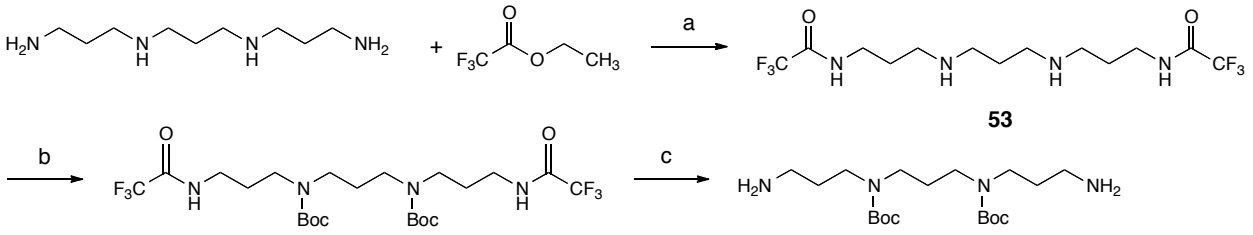
[a]Reagents and conditions: (a) TsCl, CH_2Cl_2, DMAP, NEt_3, 0 °C to RT, 24 h, 77%; (b) NaN_3, DMF, 80 °C, 24 h, 85%; (c) H_2, Pd/C, CH_3OH, 87%.

3,6,9,12,15-Pentaoxaheptadecane-1,17-diyl bis(4-methylbenzenesulfonate) (51). Hexaethylene glycol (0.705 g, 2.5 mmol, 1 equiv.), DMAP (60 mg, 0.5 mmol, 0.2 equiv.) and NEt_3 (3.5 mL, 24 mmol, 10 equiv.) were dissolved in anhydrous DCM (50 mL) and cooled to 0°C. Tosyl Chloride (1.605 g, 10 mmol, 4 equiv.) was added and the reaction was stirred for 24 h at room temperature to give the product in 77% yield.[6] ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 2H), 8.75 (s, 2H), 3.26 (q, *J* = 6.5, 4H), 2.98 (t, *J* = 7.5, 4H), 2.94–2.88 (m, 4H), 1.91 (p, *J* = 7.8, 2H), 1.82 (p, *J* = 7.0, 4H); ^{13}C NMR (DMSO-d_6) δ 156.40, 117.08, 44.63, 44 .02, 36.61, 25.22, 22.56.

1,17-Diazido-3,6,9,12,15-pentaoxaheptadecane (52). Excess sodium azide (663 mg, 10.2 mmol, 10.2 equiv.) was combined with **51** (1 g, 1.7 mmol, 1 equiv.), dissolved in anhydrous DMF (15 mL) and stirred at 80°C for 24 h. The crude mixture was purified via flash chromatography (SiO_2; CH_2Cl_2:MeOH, 98:2 to 95:5) to yield the product in 85% yield.[6] ^{1}H NMR (DMSO-d_6) δ 9.41 (bs, 2H), 3.18–3.08 (m, 12H), 1.72–1.62 (m, 6H), 1.40–1.35 (m, 18H); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 333.2; found 333.4.

3,6,9,12,15-Pentaoxaheptadecane-1,17-diamine (38). Compound **52** was dissolved in methanol and hydrogenated in the presence of 5% Pd/C for 24 h to yield hexaethylene glycol diamine. The crude mixture was purified via flash chromatography (SiO_2; CH_2Cl_2:MeOH:NH_4OH, 80:19:1 to 67:30:2) to give the product in 87% yield. ^{1}H NMR δ 3.28 (bs, 4H), 3.16 (bs, 4H), 2.69 (t, *J* = 6.4, 4H), 1.81–1.70 (m, 2H), 1.64 (p, *J* = 6.8, 4H), 1.45 (s, 18H); ^{13}C NMR δ 155.29, 79.06, 44.44, 43.68, 38.96, 32.34, 31.73, 28.17; *m/z* LRMS (ESI) calculated for $[M+H]^+$: 281.2; found 281.3.

Supplementary Scheme 6. Synthesis of **39**[a]



[a]Reagents and conditions: (a) H_2O, ACN, 80 °C, 24 h, 77%; (b) Boc_2O, NEt_3, 0 °C to RT, 24 h, 85%; (c) 0.6 M NaOH/MeOH, RT, 24 h, 87%.

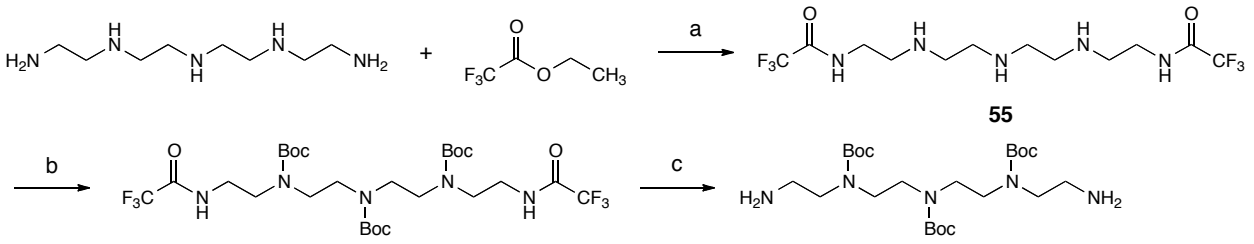
***N,N'*-((Propane-1,3-diylbis(azanediyl))bis(propane-3,1-diyl))bis(2,2,2-trifluoroacetamide) (53).** *N,N'*-Bis(3-aminopropyl)-1,3-propanediamine (0.5 g, 2.65 mmol, 1 equiv.) was dissolved in acetonitrile (10 mL). Ethyltrifluoroacetate (1.89 g, 13.27 mmol, 5 equiv.) and water (119.25 mg, 6.62 mmol, 2.5 equiv.) were added dropwise at room temperature. The reaction was stirred at 80 ºC for 24 h to give the product in 77% yield.[7, 8] ^{1}H NMR δ 7.79 (d, *J* = 8.3, 4H), 7.34 (d, *J* = 8.1, 4H), 4.15 (t, J = 5, 4H), 3.69–3.67 (m, 4H), 3.64–3.56 (m, 16H), 2.44 (s, 6H); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 381.2; found 381.0.

Di-*tert*-Butyl propane-1,3-diylbis((3-(2,2,2-trifluoroacetamido)propyl)carbamate) (54). Compound **53** (1.28 g, 2.04 mmol, 1 equiv.) was treated with NEt_3 (1.24 g, 12.24 mmol, 6 equiv.). Di-*tert*-butyl dicarbonate (1.34 g, 6.12 mmol, 3 equiv.) was added dropwise at 0 ºC. The solution was stirred at RT for 24 h and worked up as described previously,[7, 8] to yield the product in 85% yield. ^{1}H NMR δ 3.69–3.65 (m, 20H), 3.42–3.36 (m, 4H); ^{13}C NMR δ 70.85, 70.82, 70.78, 70.73, 70.18, 50.83; *m/z* LRMS (ESI) calculated for $[M+Na]^+$: 603.3; found 603.0.

Di-*tert*-Butyl propane-1,3-diylbis((3-aminopropyl)carbamate) (39). Compound **54** (1.0 g, 1.72 mmol, 1 equiv.) was stirred in a 0.6 M methanolic sodium hydroxide solution at RT for 24 hours to give the

product in 87% yield.[7, 8] ^{1}H NMR δ 3.67–3.60 (m, 20H), 3.50 (t, *J* = 5.2, 4H), 2.85 (t, *J* = 5.2, 4H); ^{13}C NMR δ 73.69, 70.77, 70.76, 70.73, 70.45, 42.01.

Supplementary Scheme 7. Synthesis of **40**[a]



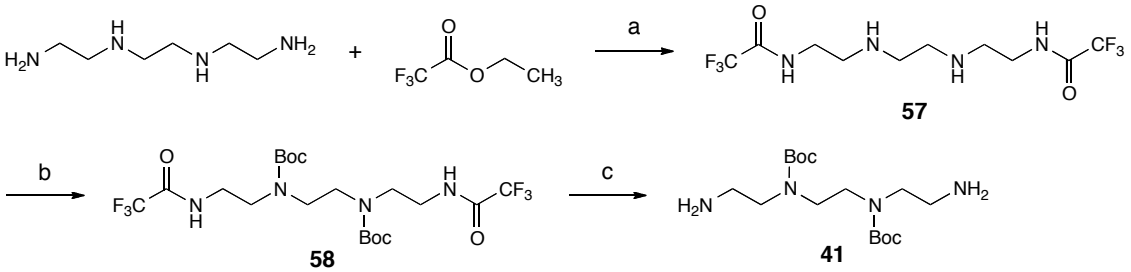
[a]Reagents and conditions: (a) DCM, 0 °C to RT, 24 h (b) Boc_2O, NEt_3, Boc_2O, 0 °C to RT, 48 h; (c) methanol, NaOH, RT, 48 h, 34% overall.

N,N'-(((Azanediylbis(ethane-2,1-diyl))bis(azanediyl))bis(ethane-2,1-diyl))bis(2,2,2-trifluoroacetamide) (55). Tetraethylenepentamine (5 g, 26.4 mmol, 1 equiv.) was dissolved in DCM (100 mL) and cooled to 0 °C. Ethyl trifluoroacetate (7.5 g, 52.8 mol, 2 equiv.) was added dropwise and the reaction was stirred for 30 min at 0 °C and then for 24 h at RT. The reaction was worked up as described previously.[9]

Di-*tert*-Butyl(((*tert*-butoxycarbonyl)azanediyl)bis(ethane-2,1-diyl))bis((2-(2,2,2-trifluoroacetamido)ethyl)carbamate) (56). Without isolation, TEA (14.6 mL, 105.6 mmol, 4 equiv.) was added to the previous reaction mixture containing **55**. A solution of Boc_2O (23 g, 105.6 mmol, 4 equiv.) in DCM (30 mL) was added dropwise and then the reaction mixture was stirred for 48 h. The mixture was washed with $NaHCO_3$ and water and dried over sodium sulfate. The solution was concentrated by rotary evaporation and worked up as described previously.[9]

Di-*tert*-Butyl(((*tert*-butoxycarbonyl)azanediyl)bis(ethane-2,1-diyl))bis((2-amino ethyl)carbamate) (40). Methanol (200 mL) and NaOH (8 g) were added to the previous reaction flask containing **55** and the mixture was stirred for 48 h. The reaction mixture was concentrated by rotary evaporation, dissolved in chloroform and NaOH and then filtered through celite. The filtrate was washed with water and the organic layers were dried over sodium sulfate and concentrated. The crude mixture was purified via flash chromatography (SiO_2; CH_2Cl_2:MeOH:NH_4OH, 90:10:0 to 90:10:1) to yield the product in 33.8% overall yield.

Supplementary Scheme 8. Synthesis of **41**[a]



[a]Reagents and conditions: (a) DCM, methanol, RT, 24 h (b) Boc_2O, DCM, NEt_3, 0 °C to RT, 3 h; (c) NaOH, methanol, RT, 24 h, 35.5% overall.

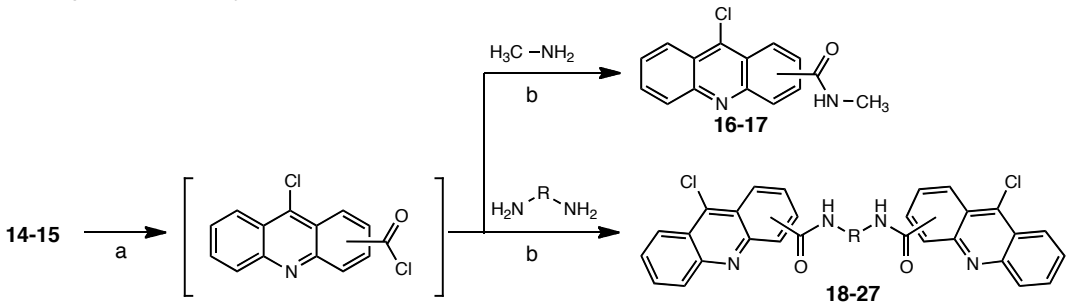
***N,N'*-((Ethane-1,2-diylbis(azanediyl))bis(ethane-2,1-diyl))bis(2,2,2-trifluoroacetamide) (57).** Triethylenepropylamine (5 g, 34.1 mmol, 1 equiv.) was dissolved in DCM (20 mL) and methanol (10 mL). A solution of ethyl trifluoroacetate (10.2 g, 71.8 mmol, 2.1 equiv.) in DCM (50 mL) was added dropwise over 1 h at 0 °C. This was stirred at RT for 3 h and worked up as described previously.[10]

Di-*tert*-Butylethane-1,2-diylbis((2-(2,2,2-trifluoroacetamido)ethyl)carbamate) (58). Without isolation, a solution of Boc_2O (25 g, 114.5 mmol, 3.36 equiv.) in DCM (20 mL) was added dropwise to the reaction mixture containing **57** over a half hour. Triethylamine (16.1 mL, 114. 5 mmol, 3.36 equiv.) was added and the reaction was stirred overnight. The solvent was then evaporated to 150 mL, washed with saturated $NaHCO_3$, sodium citrate (5%) and water. The organic layer was dried over sodium sulfate, filtered, and concentrated.

Di-*tert*-Butylethane-1,2-diylbis((2-aminoethyl)carbamate) (41). Without purification, **58** was dissolved in methanol. NaOH (1 M) was added to the solution and the reaction was stirred overnight. The reaction mixture was concentrated by rotary evaporation, dissolved in chloroform, dried over sodium sulfate, filtered through celite and concentrated by rotary evaporation. The crude mixture was purified via flash chromatography (SiO_2; CH_2Cl_2:MeOH 95:5) to yield the product in 35.5% yield overall. *m/z* LRMS (ESI) calculated for $[M+H]^+$: 347.3; found 347.2.

3. Synthesis and characterization of final compounds

Supplementary Scheme 9. Synthesis of compounds **16-27**[a]

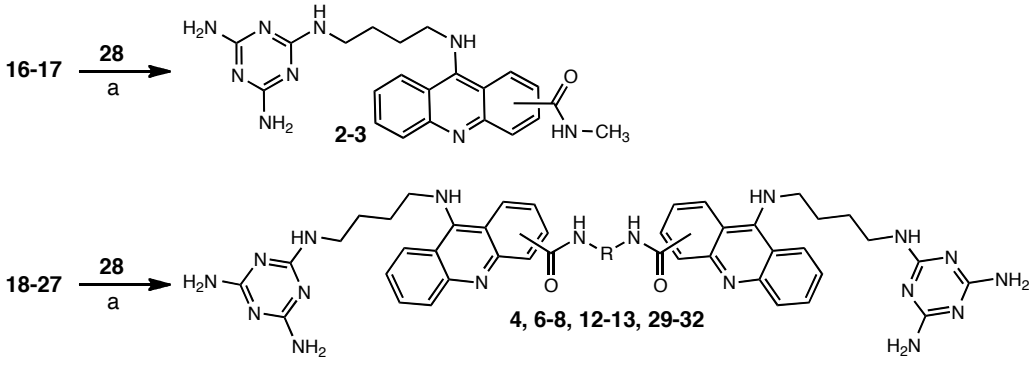


[a]Reagents and conditions: (a) $SOCl_2$, DMF (Cat.), 70 °C, 2 h; (b) DCM, NEt_3, 0 °C to RT, 15 h.

General synthetic procedure for compounds 16-27

A round-bottom flask, equipped with a stir bar, was charged with **14** or **15** (1 equiv.) and freshly distilled thionyl chloride (16.4 equiv.). A catalytic amount of DMF was added and heated gently under reflux at 70 °C, stirring until homogeneous and then for 2 h. The excess thionyl chloride was distilled off and the last traces of it were removed azeotropically via co-evaporation with DCM (3 x 50 mL). It was left under vacuum (minimally) for 1 h to afford the crude intermediate as a yellow powder. The crude intermediate was dissolved in anhydrous DCM. Anhydrous triethylamine was added to the solution until the pH was 11 and it was cooled to 0 °C. Corresponding diamines **34-43** (0.45 equiv.) or methylamine (1.1 equiv.) was added and the solution was stirred at 0 °C for 2 hours and slowly warmed to room temperature overnight. The solvent was removed by rotary evaporation and the crude mixture was purified via flash chromatography (SiO_2; CH_2Cl_2:MeOH, generally from 98:2 to 95:5) to yield **16-27** as a yellow solid.

Supplementary Scheme 10. Synthesis of compounds **2-4, 6-8, 12-13, and 29-32**[a]

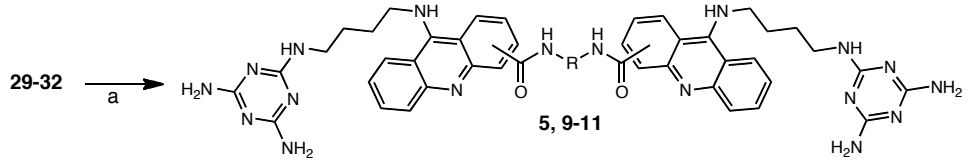


[a]Reagents and conditions: (a) DMF, DIPEA, 80 °C, 6 h.

General synthetic procedure for compounds 4, 6-8, 12,13, 29-32

A round-bottom flask, equipped with a stir bar, was charged with one of compounds **18-27** (0.5 equiv.) or **16-17** (1 equiv.) and **28** (1.1 equiv.). DIPEA (1.1 equiv.) and anhydrous DMF (25 mL) were added. The solution was heated at 80 °C for 6 hours. The solvent was removed by rotary evaporation and the product purified via flash chromatography (Basic Alumina; DCM:Methanol:NH_4OH, generally from 95:4.9:0.1 to 90:9.5:0.5) to yield the corresponding compound, **2-4**, **6-8**, **12-13**, or **29-32** as a yellow solid.

Supplementary Scheme 11. Synthesis of compounds **5, 9-11**[a]



[a]Reagents and conditions: (a) TFA, DCM, RT, 6 h.

General synthetic procedure for compounds 5, 9-11

A round-bottom flask, equipped with a stir bar, was charged with one of compounds **29-32** (1 equiv.). TFA (30 mL) and anhydrous DCM (70 mL) were added and stirred at room temperature for 6 h. The solvents were removed to yield compounds **5**, **9-11** as a yellow solid in quantitative yield.

Characterization of final compounds

9-Chloro-*N*-methylacridine-2-carboxamide (16). ^{1}H NMR δ 8.79 (d, J = 1.6, 1H), 8.40 (d, J = 8.6, 1H), 8.24–8.19 (m, 2H), 8.14 (dd, J = 9.0, 1.8, 1H), 7.86–7.83 (m, 1H), 7.69–7.62 (m, 1H), 6.56 (bs, 1H), 3.13 (d, J = 4.8, 3H); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 271.1; found 271.1; *m/z* LRMS (ESI) calculated for $[M+H]^+$: 271.1; found 271.3. Yield = 75%.

9-((4-((4,6-Diamino-1,3,5-triazin-2-yl)amino)butyl)amino)-*N*-methylacridine-2-carboxamide (2). ^{1}H NMR (methanol-d_4) δ 8.91 (s, 1H), 8.33 (d, J = 8.6, 1H), 8.02 (dd, J = 9.1, 1.9, 1H), 7.85 (t, J = 7.6, 2H), 7.75–7.68 (m, 1H), 7.42–7.35 (m, 1H), 4.03 (t, J = 7.2, 2H), 3.37–3.33 (m, 2H), 3.00 (s, 3H), 1.93 (p, J = 7.4, 2H), 1.70 (p, J = 7.2, 2H); *m/z* HRMS (ESI) calculated for $[M+H]^+$: 432.2; found 432.4. Yield = 70%.

9-Chloro-*N*-methylacridine-4-carboxamide (17). ^{1}H NMR δ 11.33 (s, 1H), 8.90 (d, *J* = 6.9, 1H), 8.41 (d, *J* = 8.3, 1H), 8.26 (d, *J* = 8.3, 1H), 8.02–7.95 (m, 1H), 7.76 (t, *J* = 7.4, 1H), 7.65–7.55 (m, 2H), 3.19 (d, *J* = 4.7, 3H). Yield = 78%.

9-((4-((4,6-Diamino-1,3,5-triazin-2-yl)amino)butyl)amino)-*N*-methylacridine-4-carboxamide (3). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 (d, *J* = 8.2, 2H), 8.41 (d, *J* = 8.3, 1H), 7.99 (d, *J* = 8.2, 1H), 7.79–7.73 (m, 1H), 7.42 (q, *J* = 8.8, 2H), 6.54 (t, *J* = 5.5, 1H), 6.14 (s, 2H), 6.00 (s, 2H), 3.89 (t, *J* = 6.5, 2H),

3.17 (q, *J* = 6.3, 2H), 3.01 (d, *J* = 4.2, 3H), 1.79 (p, *J* = 7.6, 2H), 1.52 (p, *J* = 7.2, 2H); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 432.2; found 432.4. Yield = 80%.

***N*,*N*'-(Piperazine-1,4-diylbis(propane-3,1-diyl))bis(9-chloroacridine-2-carboxamide) (18).** Yield= 65%.

***N*,*N*'-(Piperazine-1,4-diylbis(propane-3,1-diyl))bis(9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino)butyl)amino)acridine-2-carboxamide) (4).** *m/z* LRMS (ESI) calculated for $[M+H]^+$: 1001.6; found 1001.8. Yield = 61%.

Di-*tert*-Butyloctane-1,8-diylbis((3-(9-chloroacridine-2-carboxamido)propyl) carbamate) (19). ^{1}H NMR δ 9.08 (s, 2H), 8.52–8.42 (m, 4H), 8.35 (s, 4H), 7.87 (s, 2H), 7.68 (s, 2H), 3.53 (s, 4H), 3.44 (s, 4H), 3.18 (s, 4H), 1.81 (s, 4H), 1.56 (s, 4H), 1.52 (s, 18H), 1.32 (s, 8H); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 937.4; found 937.2. Yield = 65%.

Di-*tert*-Butyloctane-1,8-diylbis((3-(9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino) butyl)amino)acridine-2-carboxamido)propyl)carbamate) (29). ^{1}H NMR (Methanol-d_4) δ 8.90 (s, 2H), 8.29 (d, *J* = 8.6, 2H), 8.04 (d, *J* = 8.6, 2H), 7.84 (s, 4H), 7.73–7.66 (m, 2H), 7.40–7.33 (m, 2H), 4.01 (t, *J* = 7.0, 4H), 3.45 (t, *J* = 6.7, 4H), 3.35 (s, 8H), 3.17 (s, 4H), 1.94–1.84 (m, 8H), 1.68 (p, *J* = 7.0, 4H), 1.49 (s, 4H), 1.43 (s, 18H), 1.26 (s, 8H); *m/z* LRMS (ESI) calculated for $[M+2H]^{2+}$: 630.4; found 630.2. Yield = 61%.

***N*,*N*'-((Octane-1,8-diylbis(azanediyl))bis(propane-3,1-diyl))bis(9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino)butyl)amino)acridine-2-carboxamide) (5).** ^{1}H NMR (Deuterium Oxide) δ 8.62 (s, 2H), 8.10–8.07 (m, 4H), 7.90–7.85 (m, 2H), 7.51 (s, 2H), 7.50–7.43 (m, 4H), 3.98 (t, *J* = 6.2, 4H), 3.56 (t, *J* = 6.6, 4H), 3.19 (t, *J* = 6.1, 4H), 3.15–3.11 (m, 4H), 3.07–3.03 (m, 4H), 2.05 (q, *J* = 6.9, 4H), 1.91–1.85 (m, 4H), 1.73–1.68 (m, 4H), 1.66–1.61 (m, 4H), 1.41–1.34 (m, 8H); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 1059.6; found 1059.7. Yield = 100%.

***N*,*N*'-(4,8,12,16-Tetraoxanonadecane-1,19-diyl)bis(9-chloroacridine-4-carboxamide) (20).** ^{1}H NMR δ 9.00 (d, *J* = 6.9, 2H), 8.58 (dd, *J* = 8.7, 1.4, 2H), 8.41 (d, *J* = 8.7, 2H), 8.14 (d, *J* = 8.7, 2H), 7.89–7.84 (m, 2H), 7.76–7.71 (m, 2H), 7.71–7.65 (m, 2H), 3.76 (q, *J* = 6.8, 4H), 3.66 (t, *J* = 6.3, 4H), 3.55 (t, *J* = 6.4, 4H), 3.46 (t, *J* = 6.4, 4H), 3.41 (t, *J* = 6.4, 4H), 2.08 (p, *J* = 6.5, 4H), 1.83 (p, *J* = 6.4, 4H), 1.76 (p, *J* = 6.4, 2H). Yield = 62%.

***N*,*N*'-(4,8,12,16-Tetraoxanonadecane-1,19-diyl)bis(9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino)butyl)amino)acridine-4-carboxamide) (6).** ^{1}H NMR (DMSO-d_6) δ 8.59 (s, 2H), 8.52 (d, *J* = 7.6, 2H), 8.39 (d, *J* = 7.2, 2H), 7.87 (s, 2H), 7.71 (s, 2H), 7.55 (s, 2H), 7.38 (p, *J* = 7.6, 4H), 6.46 (t, *J* = 5.7, 2H), 6.08 (s, 4H), 5.94 (s, 4H), 3.85 (t, *J* = 6.3, 4H), 3.53 (t, *J* = 5.7, 8H), 2.46–3.38 (m, 3H), 3.31 (t, *J* = 6.4, 2H), 3.26 (t, *J* = 6.4, 2H), 3.16 (q, *J* = 6.4, 3H), 1.89 (p, *J* = 6.2, 4H), 1.81–1.73 (m, 4H), 1.67 (p, *J* =

6.4, 4H), 1.58 (p, *J* = 6.4, 2H), 1.55–1.46 (m, 4H); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 1007.6; found 1007.8. Yield = 60%.

***N*,*N*'-((Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(9-chloroacridine-2-carboxamide) (21).** ^{1}H NMR (DMSO-d_6) δ 9.03–8.96 (m, 2H), 8.84 (s, 2H), 8.34 (d, *J* = 8.7, 2H), 8.24 (dd, *J* = 9.1, 1.8, 2H), 8.20–8.12 (m, 4H), 7.95–7.90 (m, 2H), 7.79–7.72 (m, 2H), 3.65 (d, *J* = 8.6, 8H), 3.51 (q, *J* = 5.3, 4H); ^{13}C NMR (DMSO- d_6) δ 176.86, 165.62, 142.45, 140.77, 133.70, 132.02, 126.79, 126.03, 125.70, 121.55, 120.72, 119.54, 117.51, 117.27, 69.57, 68.94; *m/z* LRMS (ESI) calculated for $[M+H]^+$: 627.2; found 627.1. Yield = 66%.

***N*,*N*'-((Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino)butyl)amino)acridine-2-carboxamide) (7).** ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.73 (s, 2H), 8.11 (d, *J* = 8.6, 2H), 7.91 (dd, *J* = 9.1, 1.6, 2H), 7.69 (dd, *J* = 21.1, 8.7, 4H), 7.63–7.56 (m, 2H), 7.29–7.20 (m, 2H), 3.82 (t, *J* = 7.1, 4H), 3.74 (d, *J* = 5.5, 8H), 3.64 (t, *J* = 5.2, 4H), 3.28 (d, *J* = 6.7, 4H), 1.81 (p, *J* = 7.5, 4H), 1.66–1.59 (m, 4H); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 949.5; found 949.7. Yield = 61%.

***N*,*N*'-(3,6,9,12,15-Pentaoxaheptadecane-1,17-diyl)bis(9-chloroacridine-4-carboxamide) (22).** ^{1}H NMR δ 8.88 (dd, *J* = 9.5, 1.4, 2H), 8.41 (dd, *J* = 9.7, 1.6, 2H), 8.22 (d, *J* = 8.7, 2H), 8.05 (d, *J* = 8.6, 2H), 7.76 (t, *J* = 9.7, 2H), 7.62 (t, *J* = 7.1, 2H), 7.55 (t, *J* = 7.5, 2H), 3.85 (d, *J* = 4.9, 4H), 3.83–3.79 (m, 4H), 3.76–3.73 (m, 4H), 3.72–3.68 (m, 4H), 3.62–3.58 (m, 4H), 3.55–3.51 (m, 4H); ^{13}C NMR δ 165.36, 146.99, 146.02, 142.74, 135.50, 131.49, 129.35, 128.64, 128.42, 127.47, 126.46, 124.51, 124.26, 123.57, 70.80, 70.68, 70.58, 70.42, 70.22, 39.76. Yield = 70%.

N,N'-(3,6,9,12,15-Pentaoxaheptadecane-1,17-diyl)bis(9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino)butyl)amino)acridine-4-carboxamide) (8). ^{1}H NMR (DMSO-d_6) δ 8.60 (dd, *J* = 7.1, 1.1, 2H), 8.56–8.51 (m, 2H), 8.39 (d, *J* = 9.1, 2H), 7.92 (d, *J* = 8.6, 2H), 7.75–7.68 (m, 2H), 7.53 (t, *J* = 5.5, 2H), 7.41 (dd, *J* = 8.6, 7.3, 2H), 7.39–7.35 (m, 2H), 6.42 (t, *J* = 5.7, 2H), 6.02 (bs, 4H), 5.88 (bs, 4H), 3.85 (q, *J* = 6.5, 4H), 3.69–3.64 (m, 4H), 3.64–3.61 (m, 8H), 3.58–3.55 (m, 4H), 3.47–3.43 (m, 4H), 3.39–3.35 (m, 3H), 3.14 (q, *J* = 6.5, 4H), 1.76 (p, *J* = 7.5, 4H), 1.49 (p, *J* = 6.9, 4H). Yield = 65%.

Di-tert-Butylpropane-1,3-diylbis((3-(9-chloroacridine-4-carboxamido)propyl) carbamate) (23). ^{1}H NMR δ 9.00 (s, 2H), 8.36 (d, *J* = 14.0, 4H), 8.27 (s, 2H), 8.20 (d, *J* = 16.2, 4H), 7.80 (t, *J* = 7.4, 2H), 7.62 (t, *J* = 6.2, 2H), 3.54 (s, 4H), 3.45 (s, 4H), 3.23 (s, 4H), 1.89–1.81 (m, 6H), 1.52 (s, 18H); ^{13}C NMR δ 149.56, 149.23, 132.76, 131.12, 130.12, 129.75, 128.54, 127.13, 124.59, 124.33, 123.26, 44.94, 43.54, 36.14, 28.52, 27.87, 27.63; *m/z* LRMS (ESI) calculated for $[M+H]^+$: 867.3; found 867.1. Yield = 60%.

Di-*tert*-Butylpropane-1,3-diylbis((3-(9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino) butyl)amino)acridine-4-carboxamido)propyl)carbamate) (30). ^{1}H NMR (Methanol-d_4) δ 8.60 (s, 2H), 8.37 (d, *J* = 8.5, 2H), 8.20 (d, *J* = 8.5, 2H), 7.86 (d, *J* = 54.6, 2H), 7.63 (t, *J* = 7.1, 2H), 7.38–7.32 (m, 2H),

7.32–7.27 (m, 2H), 3.82 (s, 4H), 3.49 (t, *J* = 6.2, 4H), 3.35 (s, 4H), 3.27 (t, *J* = 6.8, 4H), 3.19 (s, 4H), 1.88 (s, 1H), 1.84–1.75 (m, 4H), 1.59 (p, *J* = 7.1, 6.7, 17H), 1.34 (s, 4H); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 1189.7; found 1189.6. Yield = 55%.

***N*,*N*'-((Propane-1,3-diylbis(azanediyl))bis(propane-3,1-diyl))bis(9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino)butyl)amino)acridine-4-carboxamide) (9).** ^{1}H NMR (Deuterium Oxide) δ 8.34 (d, *J* = 8.3, 1H), 8.17 (d, *J* = 8.3 , 1H), 8.11 (d, *J* = 7.4, 1H), 7.78 (t, *J* = 7.7, 1H), 7.52 (d, *J* = 8.3, 1H), 7.47–7.38 (m, 2H), 4.07 (t, *J* = 6.8, 2H), 3.58 (t, *J* = 6.7, 2H), 3.36–3.31 (m, 2H), 3.26 (t, *J* = 5.9, 6H), 2.31 (p, *J* = 7.9, 7.5 , 1H), 2.14–2.08 (m, 2H), 1.94 (p, *J* = 7.2, 3H), 1.68 (q, *J* = 6.5, 5.7, 3H); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 989.6; found 989.6. Elemental analysis, Calculated for $C_{51}H_{64}N_{20}O_2$. $6CF_3COOH$. $6H_2O$: C, 42.48 %; H, 4.64 %; F, 19.20 %; N, 15.73 %; Found: C, 42.50 %; H, 4.11 %; F, 19.94 %; N, 15.04 %, *m/z* HRMS (ESI) calculated for $[M+H]^+$: 989.6; Found 990.1. Yield = 100%.

Di-*tert*-Butyl(((*tert*-butoxycarbonyl)azanediyl)bis(ethane-2,1-diyl))bis((2-(9-chloroacridine-4-carboxamido)ethyl)carbamate) (24). Yield = 60%.

Di-*tert*-Butyl (((*tert*-butoxycarbonyl)azanediyl)bis(ethane-2,1-diyl))bis((2-(9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino)butyl)amino)acridine-4-carboxamido)ethyl) carbamate) (31). Yield = 58%.

***N*,*N*'-(((Azanediylbis(ethane-2,1-diyl))bis(azanediyl))bis(ethane-2,1-diyl))bis(9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino)butyl)amino)acridine-4-carboxamide) (10).** ^{1}H NMR (Deuterium Oxide) δ 8.23 (d, *J* = 8.5, 2H), 8.09 (d, *J* = 7.4, 2H), 7.99 (d, *J* = 8.6, 2H), 7.65 (t, *J* = 7.7, 2H), 7.43 (d, *J* = 8.4, 2H), 7.36 (t, *J* = 7.9, 2H), 7.28 (s, 2H), 3.88 (t, *J* = 6.7, 3H), 3.76 (t, *J* = 5.4, 3H), 3.52 (s, 4H), 3.37 (t, *J* = 5.5, 3H), 3.13 (t, *J* = 6.1, 3H), 1.79 (p, *J* = 6.9, 3H), 1.56–1.50 (m, 3H); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 990.6; found 990.7. Yield = 100%.

Di-*tert*-Butylethane-1,2-diylbis((2-(9-chloroacridine-4-carboxamido)ethyl) carbamate) (25). *m/z* LRMS (ESI) calculated for $[M+Na]^+$: 847.3; found 847.0. Yield = 62%.

Di-*tert*-Butylethane-1,2-diylbis((2-(9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino) butyl)amino)acridine-4-carboxamido)ethyl)carbamate) (32). Yield = 57%.

***N*,*N*'-((Ethane-1,2-diylbis(azanediyl))bis(ethane-2,1-diyl))bis(9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino)butyl)amino)acridine-4-carboxamide) (11).** ^{1}H NMR (Deuterium Oxide) δ 8.18 (d, *J* = 8.6, 2H), 8.07 (d, *J* = 7.2, 2H), 8.05–8.01 (m, 2H), 7.67 (t, *J* = 7.5, 2H), 7.38 (d, *J* = 8.4, 2H), 7.34 (s, 2H), 7.29 (t, *J* = 8.0, 2H), 3.95 (s, 4H), 3.83 (s, 4H), 3.73 (s, 4H), 3.50 (s, 4H), 3.21 (s, 4H), 1.86 (s, 4H), 1.61 (s, 4H); ^{13}C NMR (Deuterium Oxide) δ 170.11, 163.32, 163.03, 162.75, 162.47, 159.99, 159.26, 157.58, 156.52, 119.95, 118.19, 117.63, 115.30, 112.98, 48.85, 48.65, 43.81, 40.32, 36.56, 26.06, 24.93; *m/z* LRMS (ESI) calculated for $[M+H]^+$: 947.5; found 947.6. Yield = 100%.

***N*,*N*'-(((Oxybis(ethane-2,1-diyl))bis(oxy))bis(propane-3,1-diyl))bis(9-chloroacridine-2-carboxamide) (26).** ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.76 (s, 2H), 8.24 (d, *J* = 8.6, 2H), 8.15–8.09 (m, 4H), 8.07 (d, *J* = 9.1, 2H), 7.77–7.69 (m, 4H), 7.54 (t, *J* = 7.6, 2H), 3.65–3.56 (m, 12H), 3.52–3.48 (m, 4H), 1.89 (p, *J* = 5.7, 4H) . Yield = 70%.

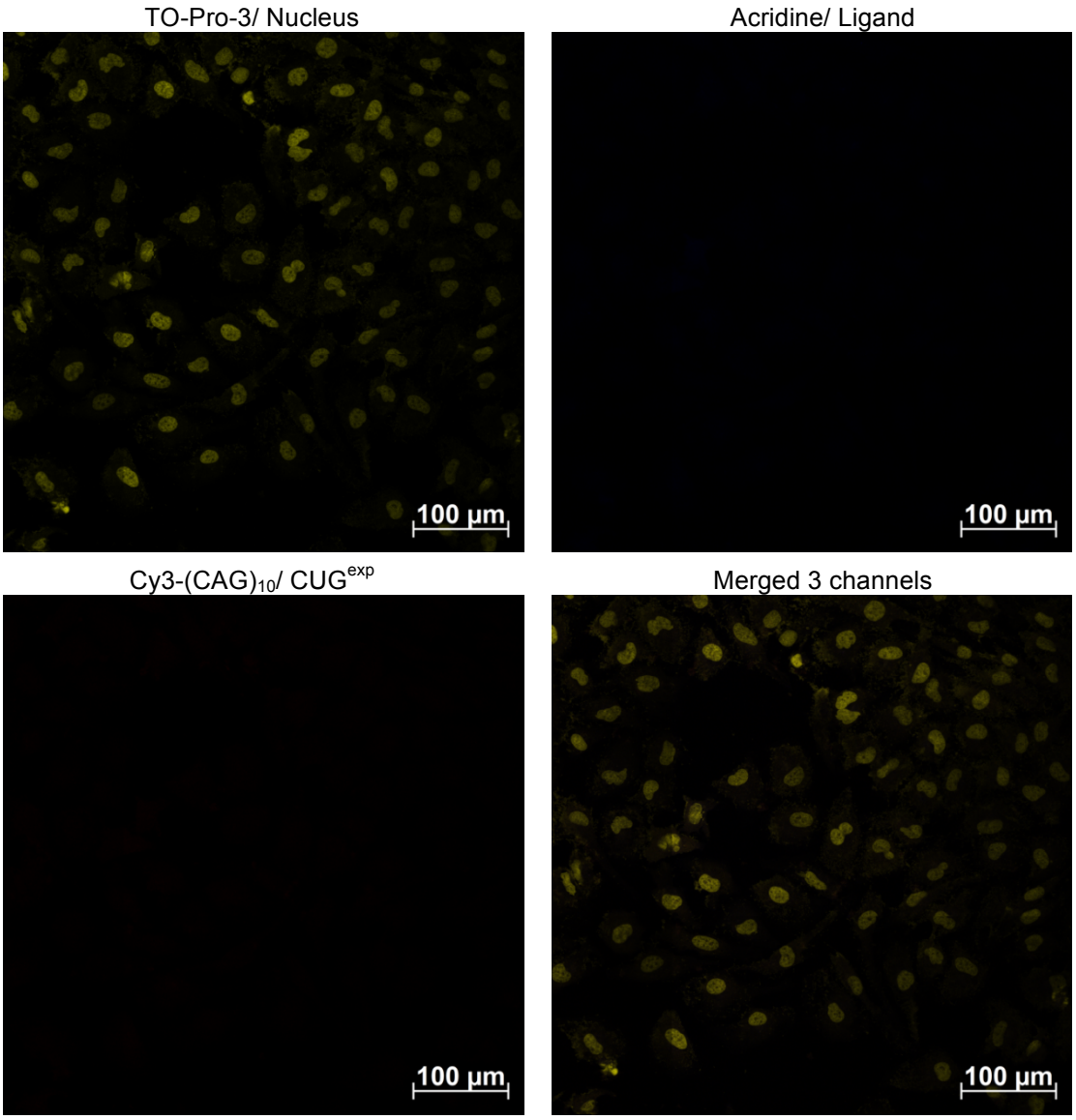
***N*,*N*'-(((Oxybis(ethane-2,1-diyl))bis(oxy))bis(propane-3,1-diyl))bis(9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino)butyl)amino)acridine-2-carboxamide) (12).** ^{1}H NMR (Methanol-d_4) δ 8.75 (s, 2H), 8.15 (d, *J* = 8.6, 2H), 7.97–7.91 (m, 2H), 7.79–7.70 (m, 4H), 7.65–7.58 (m, 2H), 7.31–7.24 (m, 2H), 3.86 (t, *J* = 7.1, 4H), 3.66–3.62 (m, 4H), 3.59–3.55 (m, 8H), 3.52 (t, *J* = 6.8, 4H), 3.32–3.27 (m, 4H), 1.91–1.86 (m, 4H), 1.86–1.80 (m, 4H), 1.64 (p, *J* = 6.9, 4H); *m/z* LRMS (ESI) calculated for $[M+H]^+$: 1021.5; found 1021.7. Yield = 66%.

***N*,*N*'-(3,6,9,12,15-Pentaoxaheptadecane-1,17-diyl)bis(9-chloroacridine-2-carboxamide) (27).** ^{1}H NMR δ 8.89 (s, 2H), 8.39–8.15 (m, 8H), 7.88–7.82 (m, 2H), 7.66–7.61 (m, 2H), 3.75 (s, 8H), 3.66 (d, *J* = 5.3, 4H), 3.63 (d, *J* = 5.4, 4H), 3.61–3.58 (m, 4H), 3.55–3.51 (m, 4H). Yield = 65%.

***N*,*N*'-(3,6,9,12,15-Pentaoxaheptadecane-1,17-diyl)bis(9-((4-((4,6-diamino-1,3,5-triazin-2-yl)amino)butyl)amino)acridine-2-carboxamide) (13).** ^{1}H NMR (Methanol-d_4) δ 8.82 (s, 2H), 8.22 (d, *J* = 8.7, 2H), 8.03–7.96 (m, 2H), 7.79 (s, 4H), 7.66 (q, *J* = 8.4, 7.7, 2H), 7.36–7.28 (m, 2H), 3.91 (t, *J* = 7.2, 4H), 3.66 (t, *J* = 5.0, 4H), 3.64–3.60 (m, 4H), 3.60–3.57 (m, 4H), 3.57–3.54 (m, 4H), 3.52–3.51 (m, 4H), 3.48–3.47 (m, 4H), 3.30–3.22 (m, 4H), 1.86 (p, *J* = 7.6, 4H), 1.65 (p, *J* = 7.0, 4H); *m/z* LRMS (ESI) calculated for $[M+2H]^{2+}$: 541.3; found 541.5. Yield = 60%.

4. Representative confocal microscopy images

A

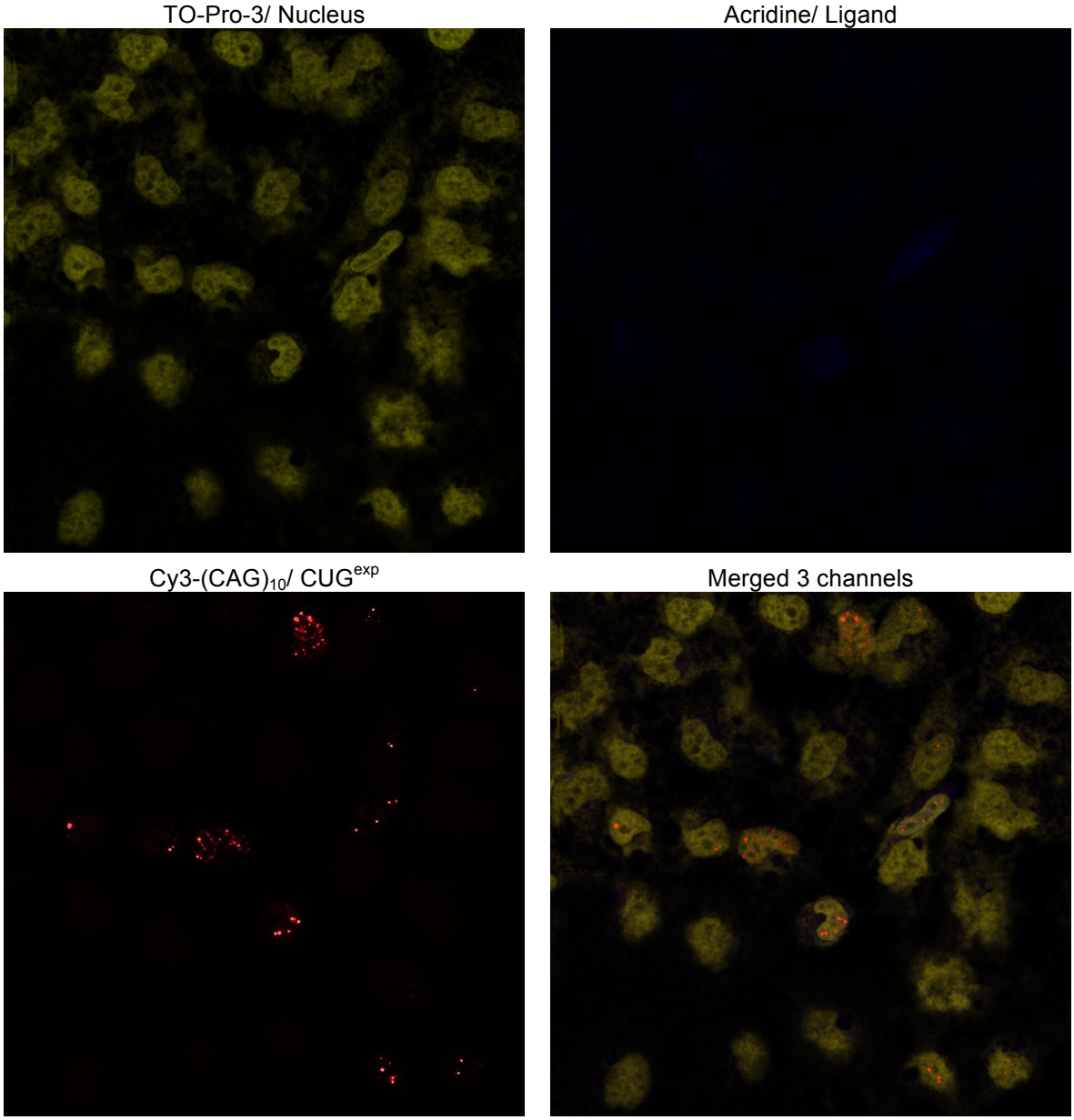


B

Total number of cells	Number foci-containing cells	Percentage of foci-containing cells
120	0	0

Supplementary Figure 1. HeLa cells transfected with CTG_0 served as a negative control cell.

A

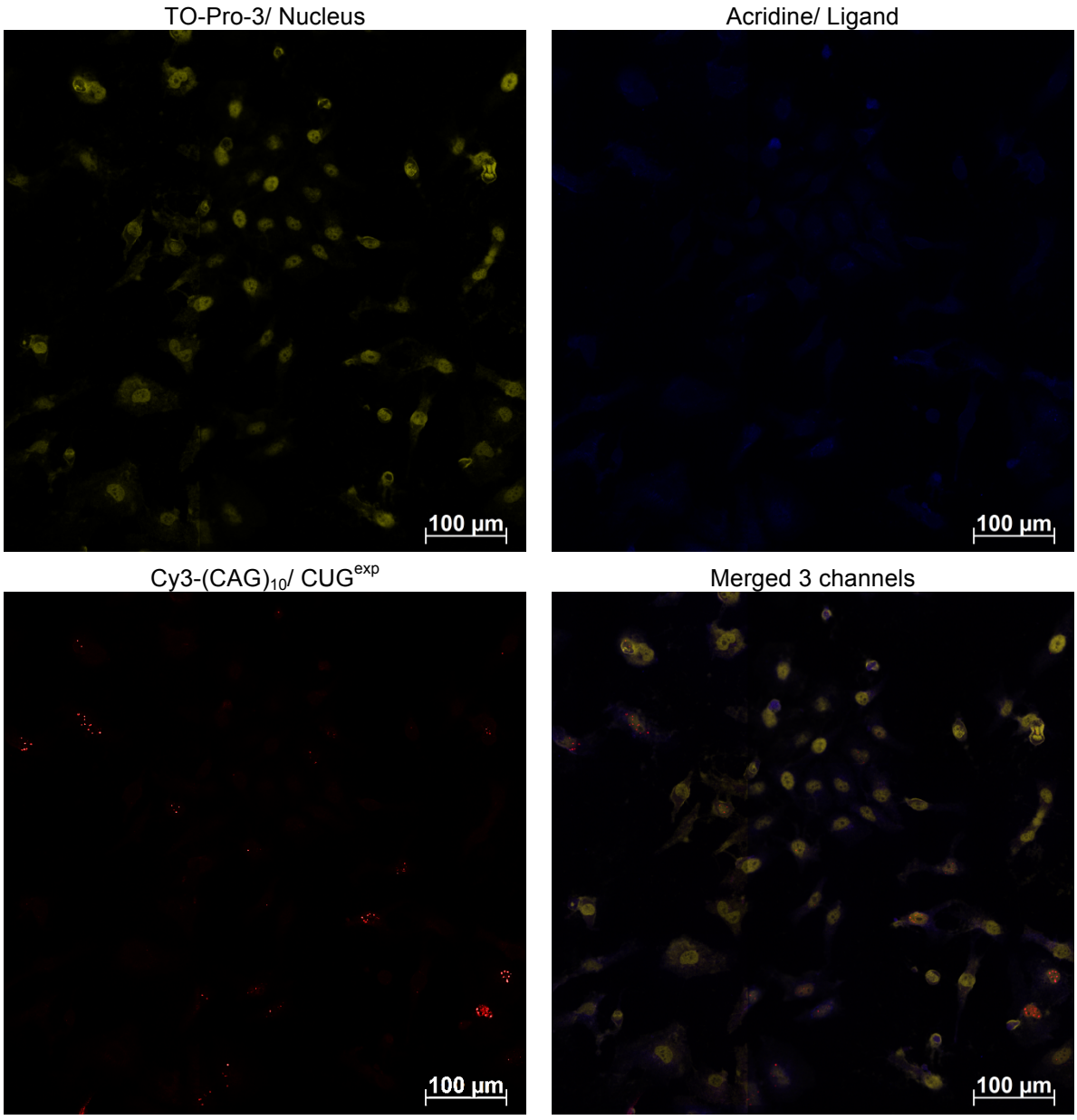


B

Total number of cells	Number foci-containing cells	Percentage of foci-containing cells
32	8	25

Supplementary Figure 2. HeLa cells transfected with CTG_{960} served as a DM1 cell model.

A

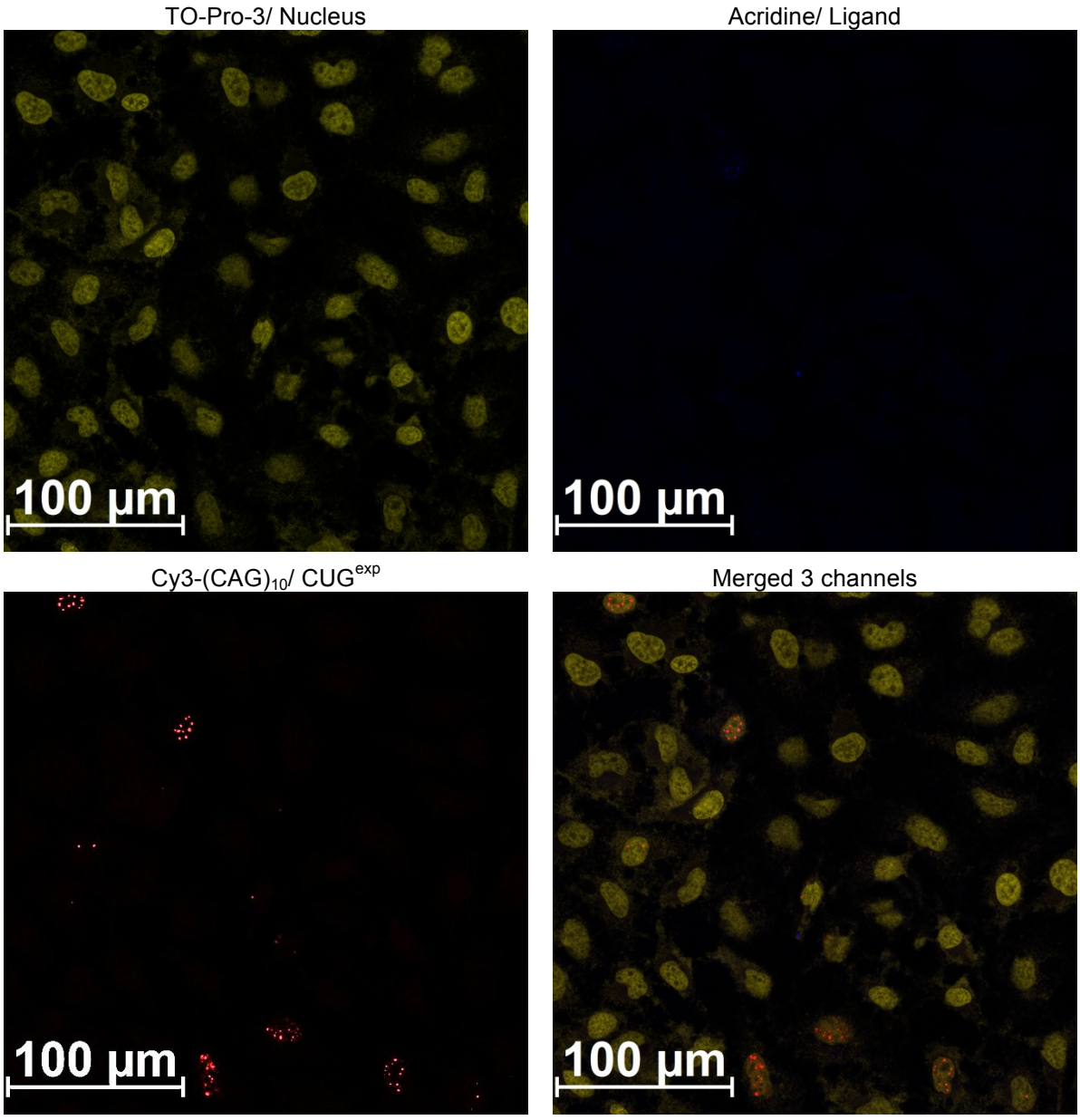


B

Total number of cells	Number foci-containing cells	Percentage of foci-containing cells
64	17	26.5

Supplementary Figure 3. DM1 cell model treated with Spermine, negative control compound, 50 µM.

A

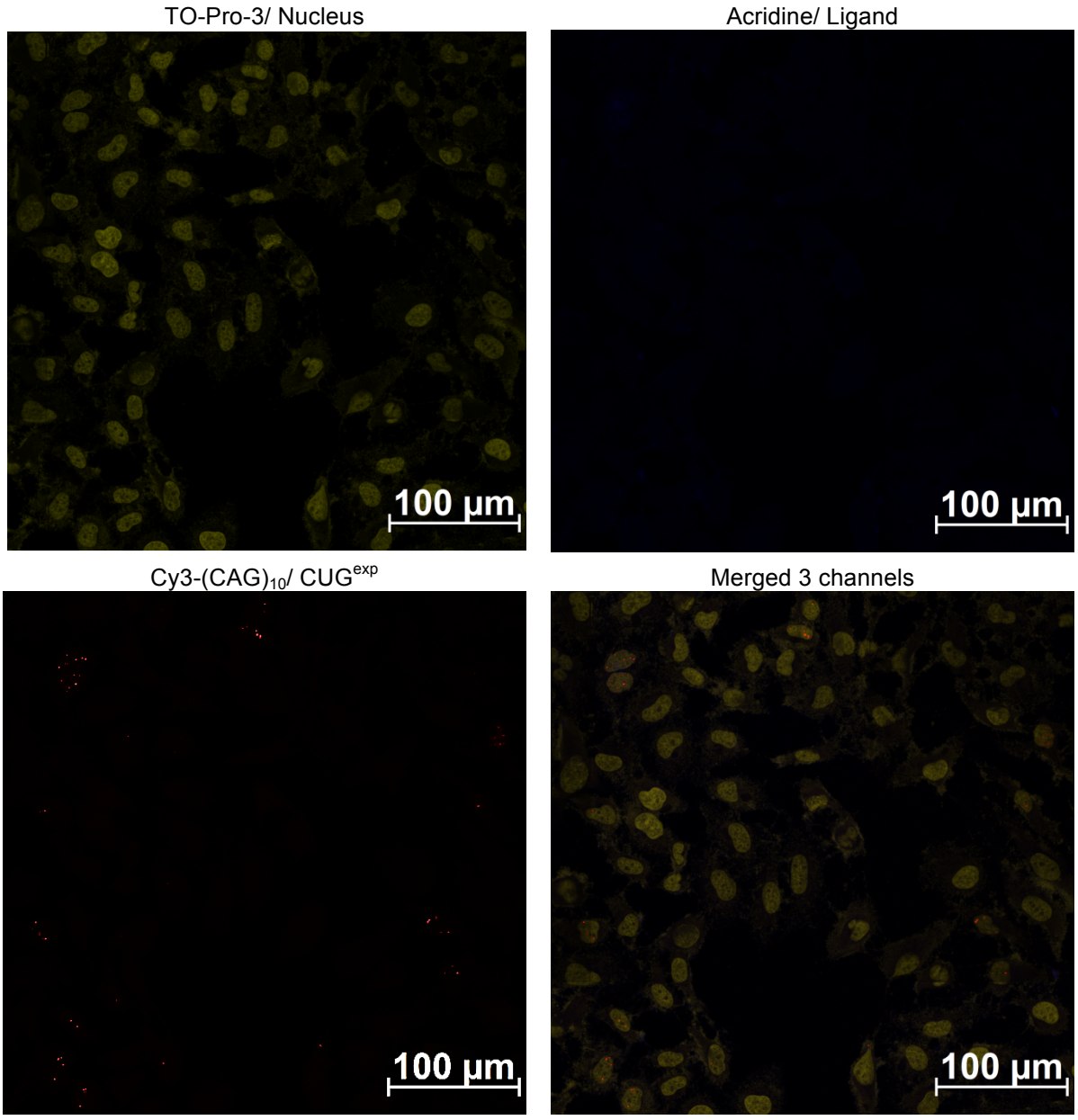


B

Total number of cells	Number of foci-containing cells	Percentage of foci-containing cells
51	8	15.7

Supplementary Figure 4. DM1 cell model treated with **2**, 20 µM.

A

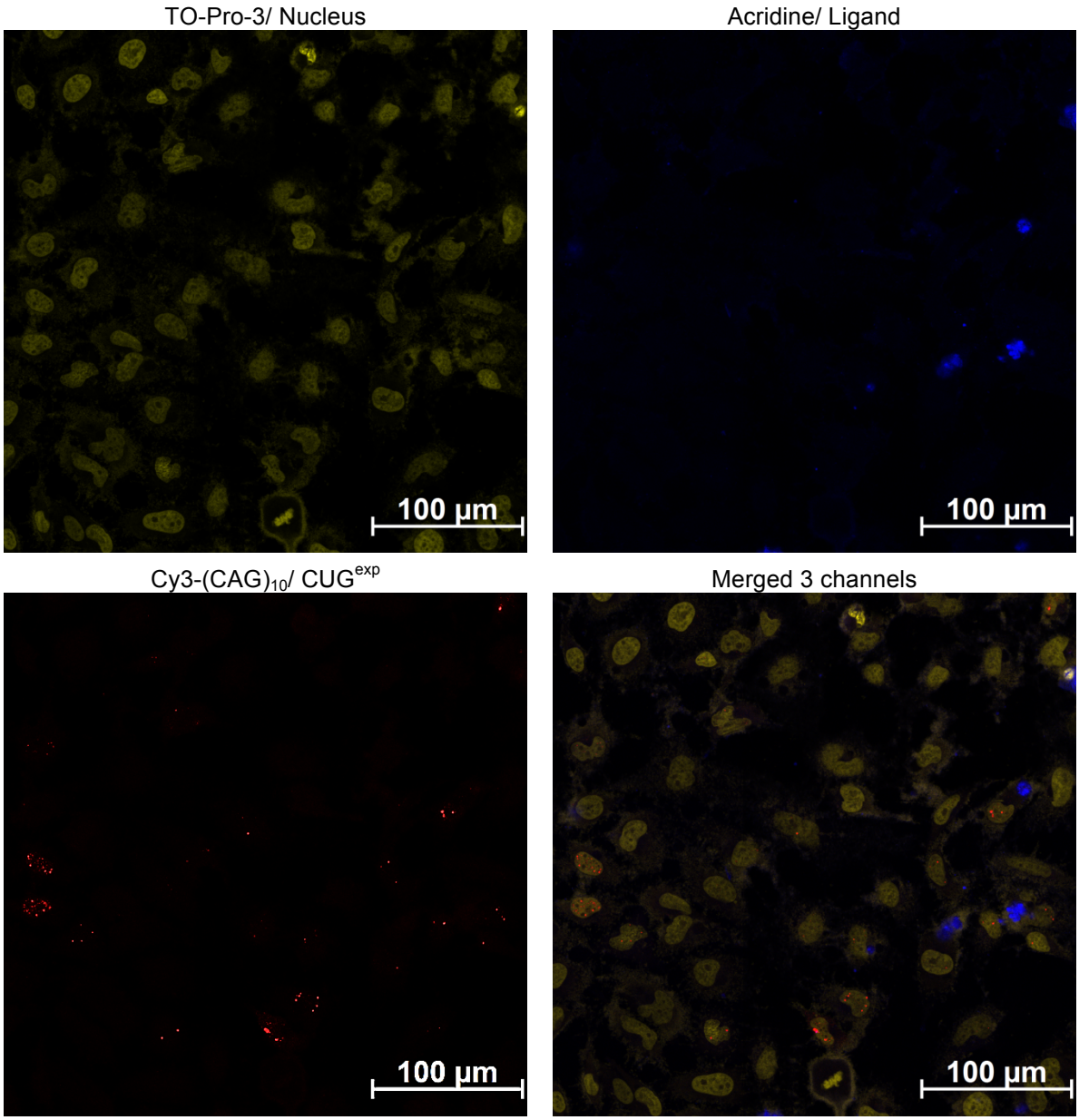


B

Total number of cells	Number of foci-containing cells	Percentage of foci-containing cells
67	11	16.4

Supplementary Figure 5. DM1 cell model treated with **2,** 35 µM.

A

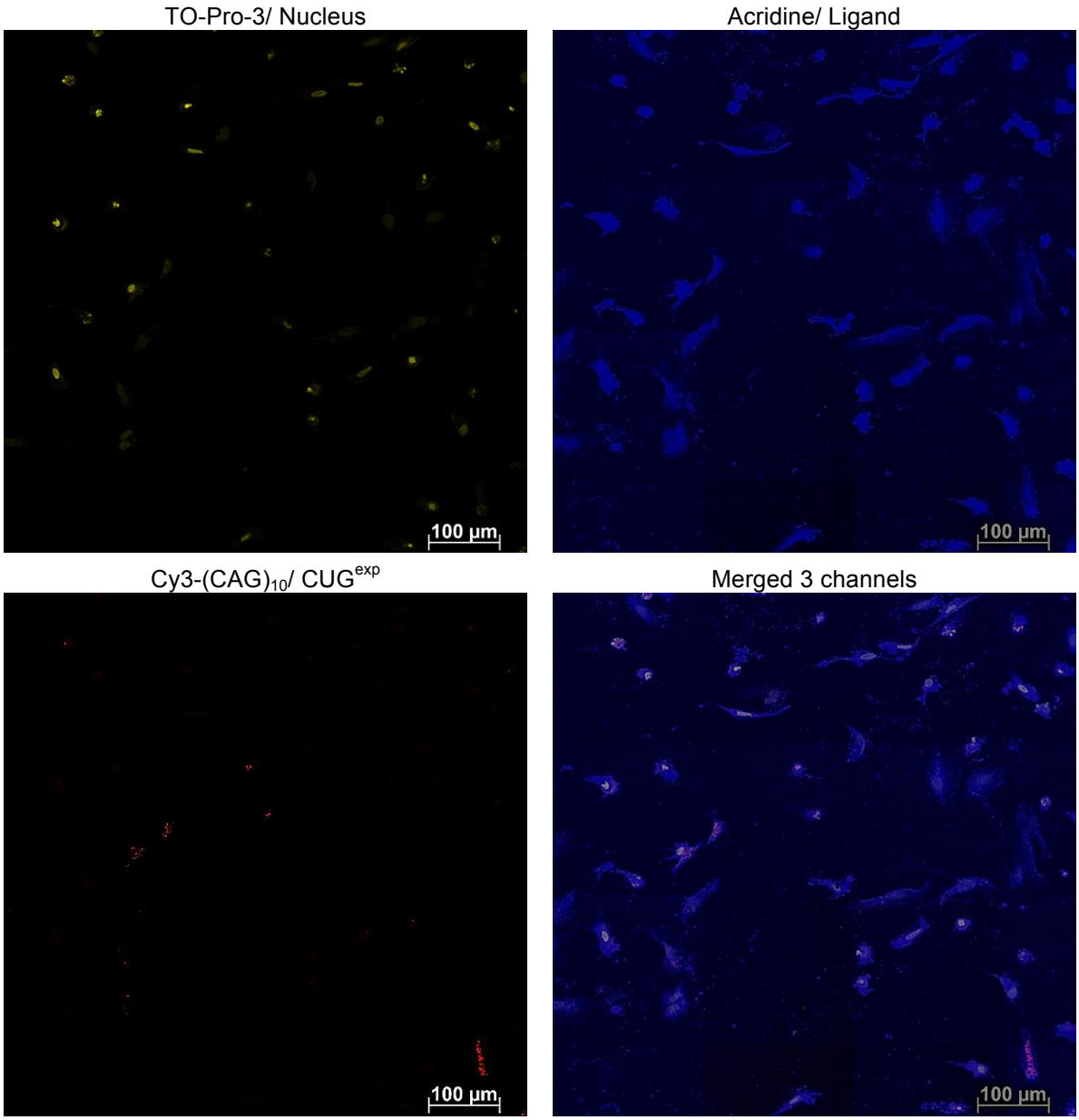


B

Total number of cells	Number foci-containing cells	Percentage of foci-containing cells
58	12	20.7

Supplementary Figure 6. DM1 cell model treated with **2**, 50 µM.

A

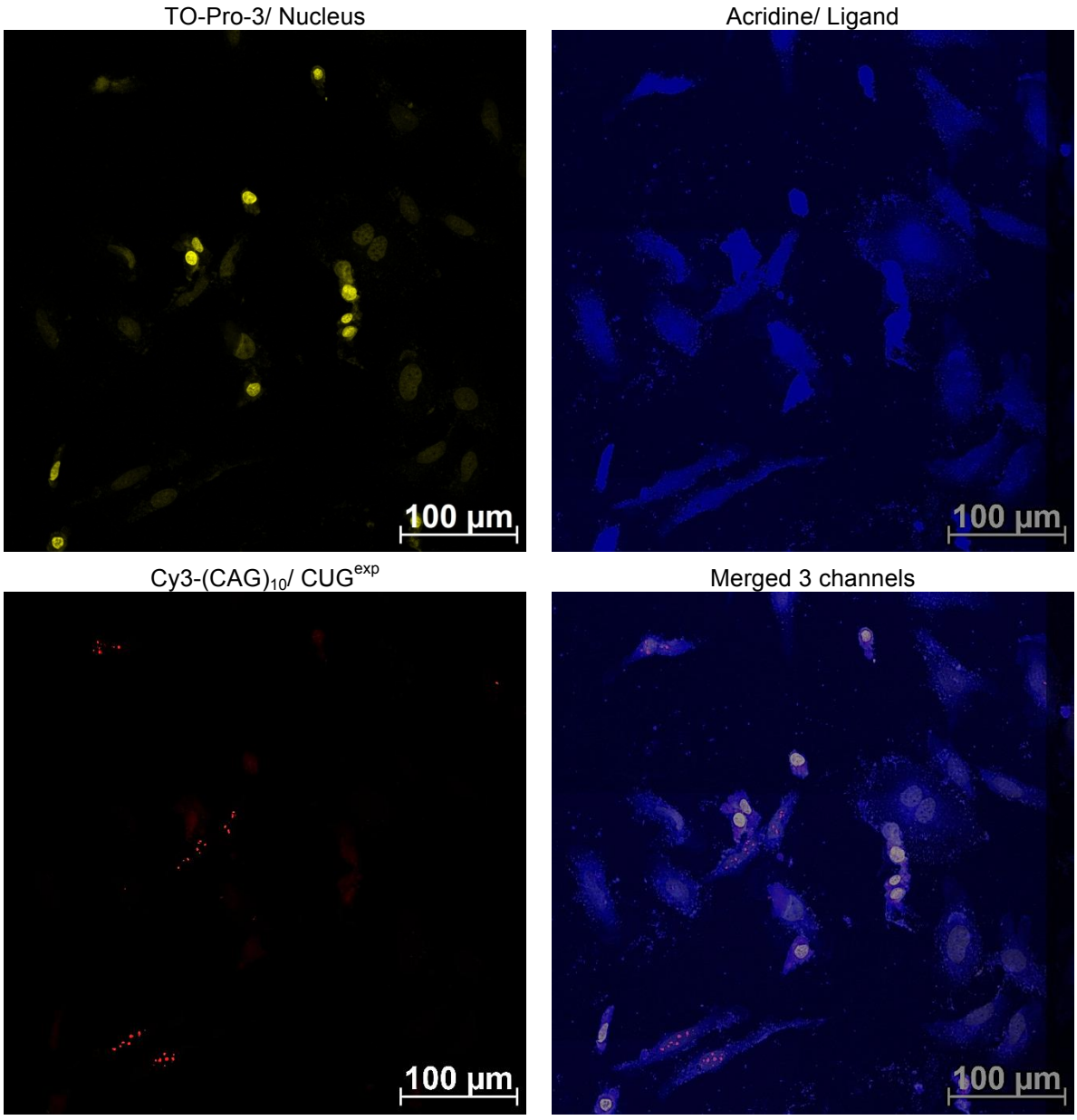


B

Total number of cells	Number foci-containing cells	Percentage of foci-containing cells
43	7	16.3

Supplementary Figure 7. DM1 cell model treated with **5**, 20 µM.

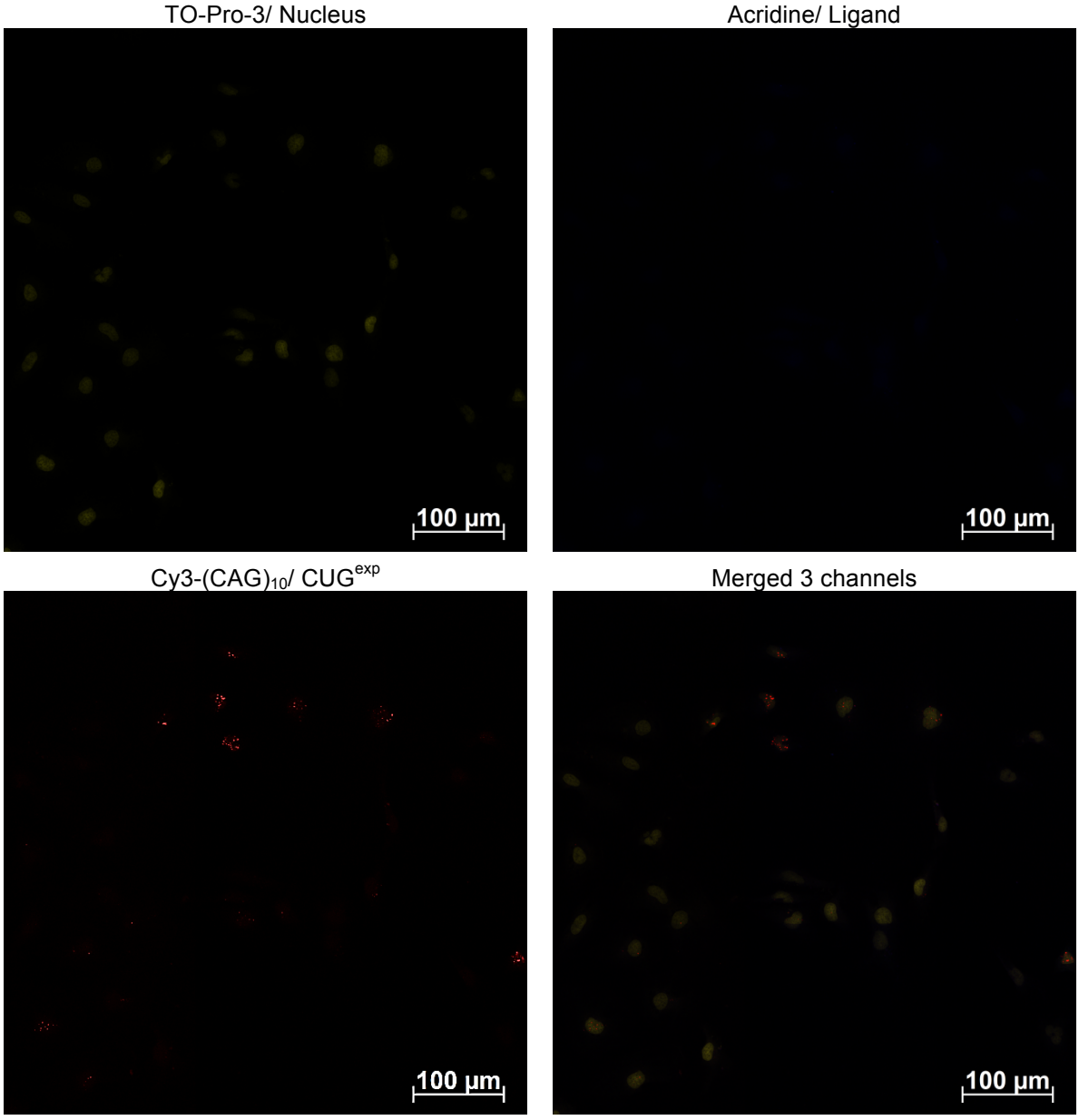
A



B

Total number of cells	Number foci-containing cells	Percentage of foci-containing cells
27	5	18.5

Supplementary Figure 8. DM1 cell model treated with **5**, 35 µM.

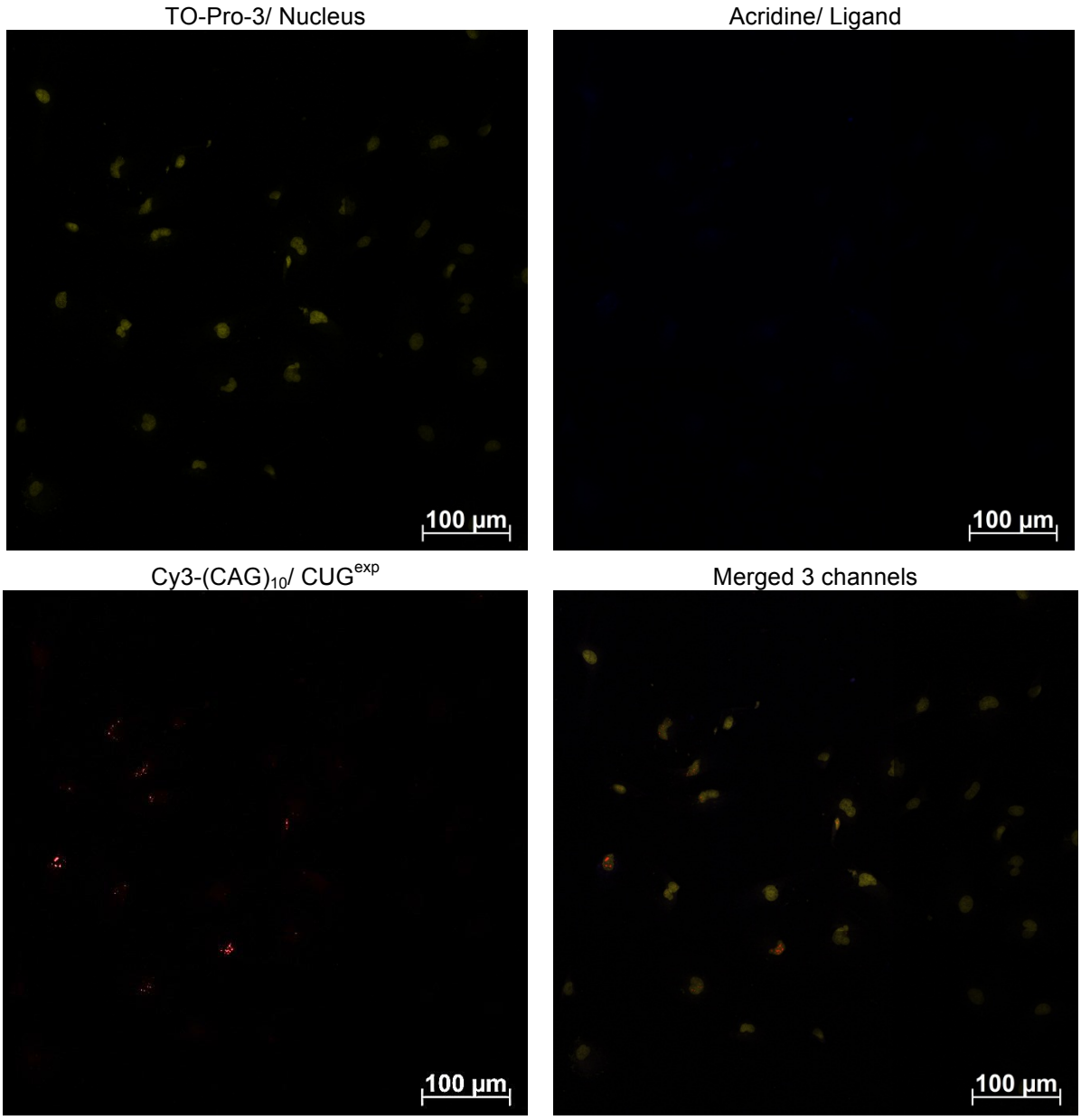


B

Total number of cells	Number foci-containing cells	Percentage of foci-containing cells
32	8	25

Supplementary Figure 9. DM1 cell model treated with **11**, 20 µM.

A

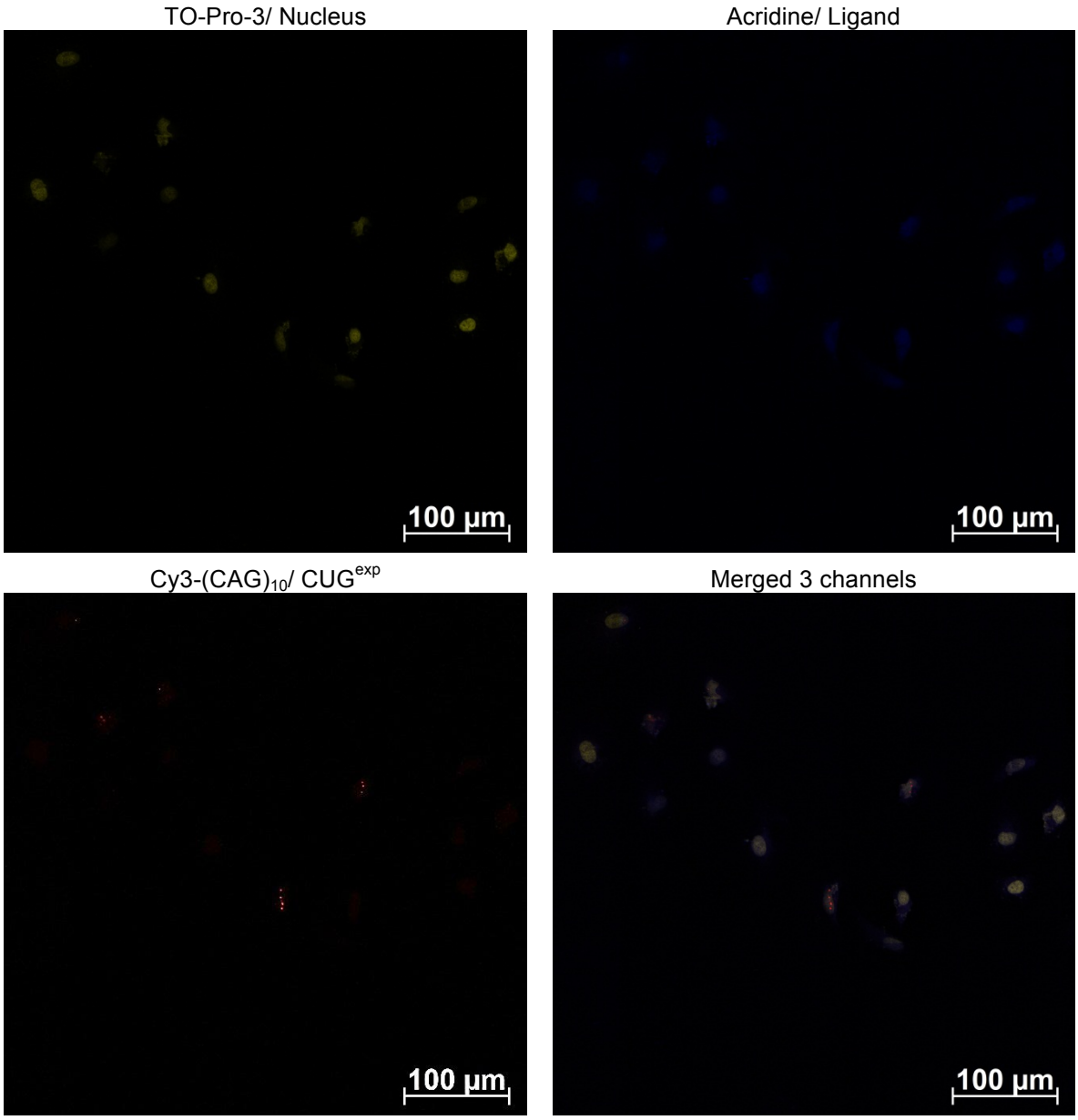


B

Total number of cells	Number foci-containing cells	Percentage of foci-containing cells
38	7	18.4

Supplementary Figure 10. DM1 cell model treated with **11**, 35 µM.

A

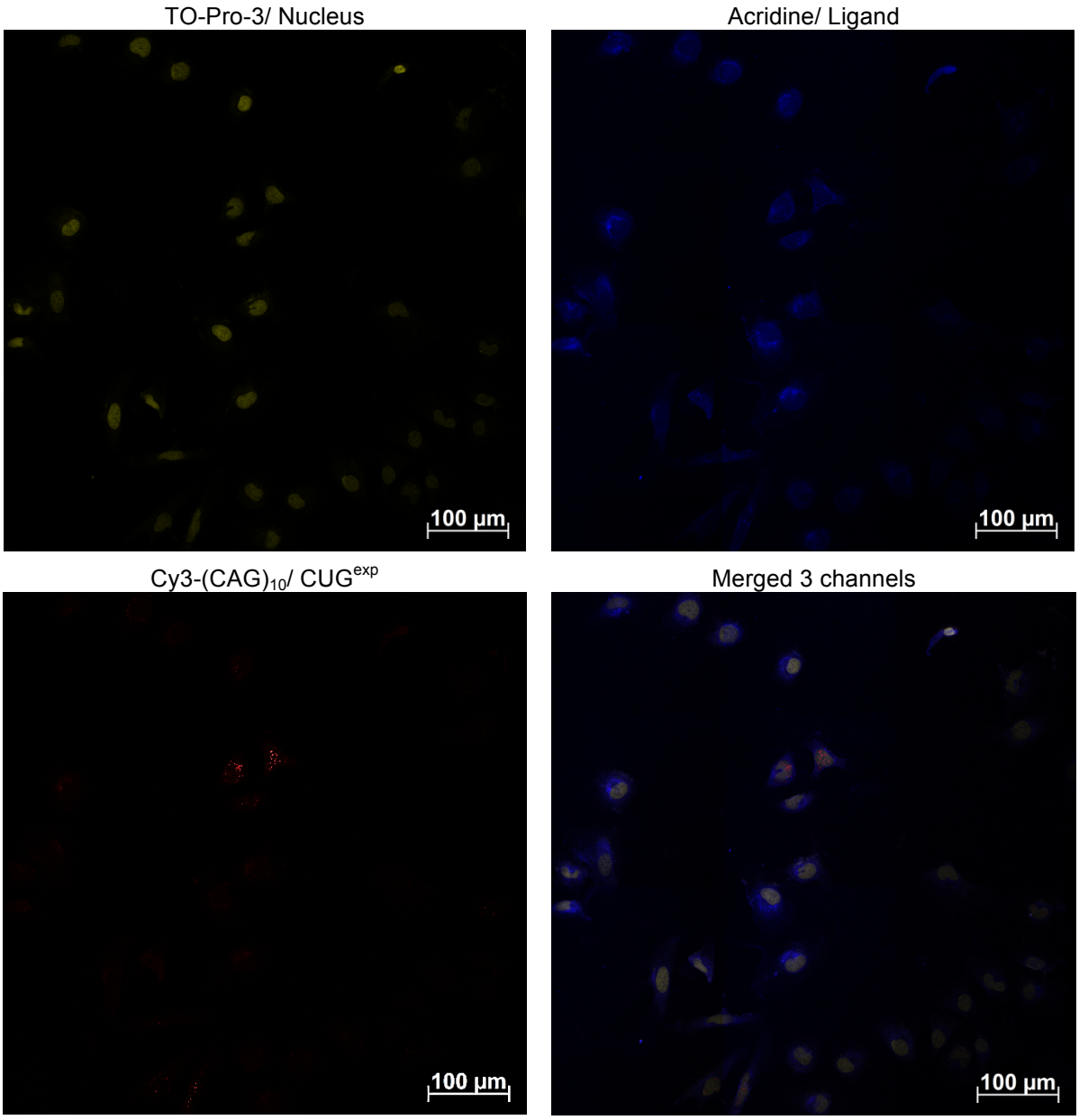


B

Total number of cells	Number foci-containing cells	Percentage of foci-containing cells
15	3	20

Supplementary Figure 11. DM1 cell model treated with **11**, 50 µM.

A

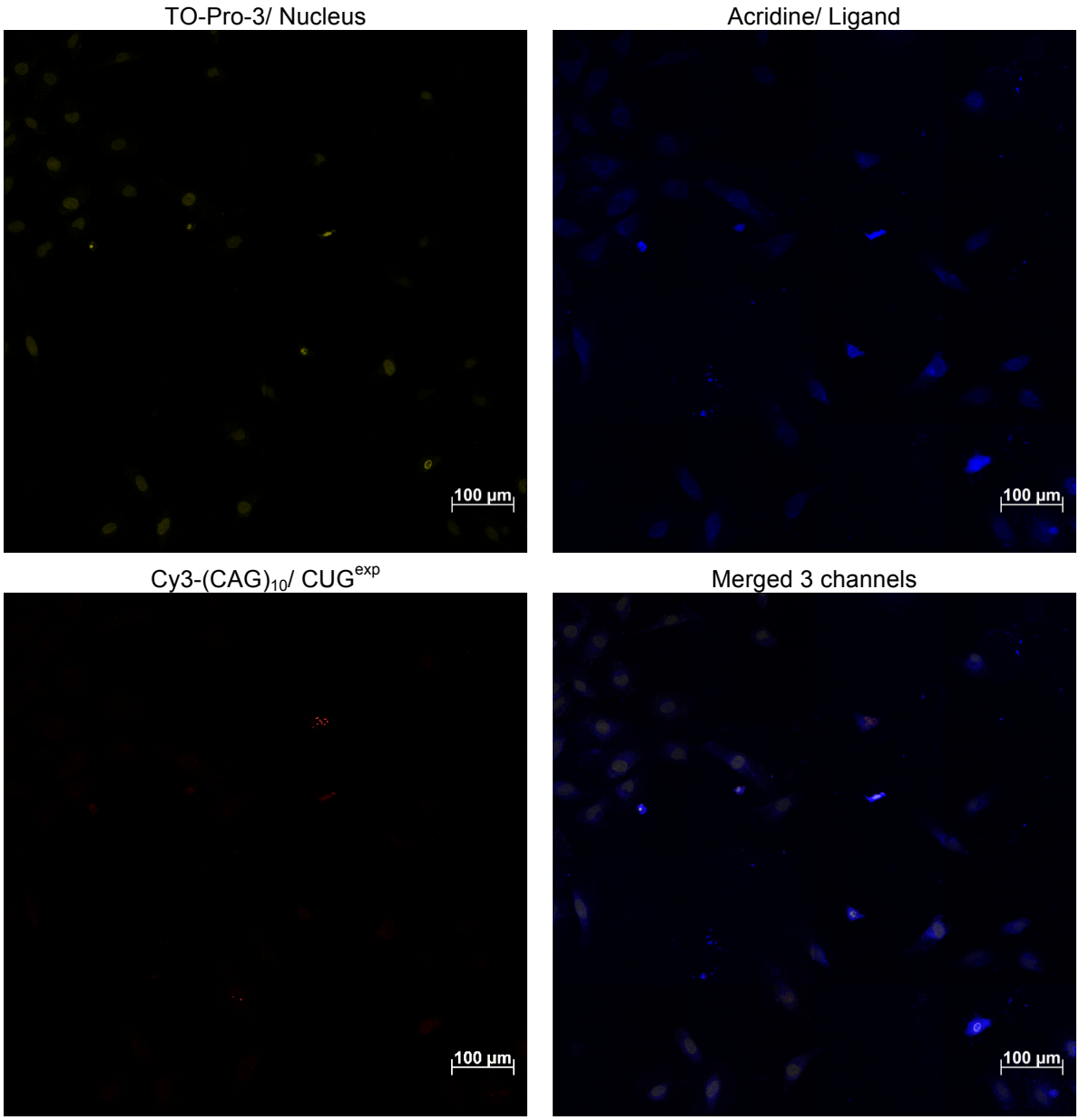


B

Total number of cells	Number foci-containing cells	Percentage of foci-containing cells
35	3	8.6

Supplementary Figure 12. DM1 cell model treated with **9**, 20 µM.

A

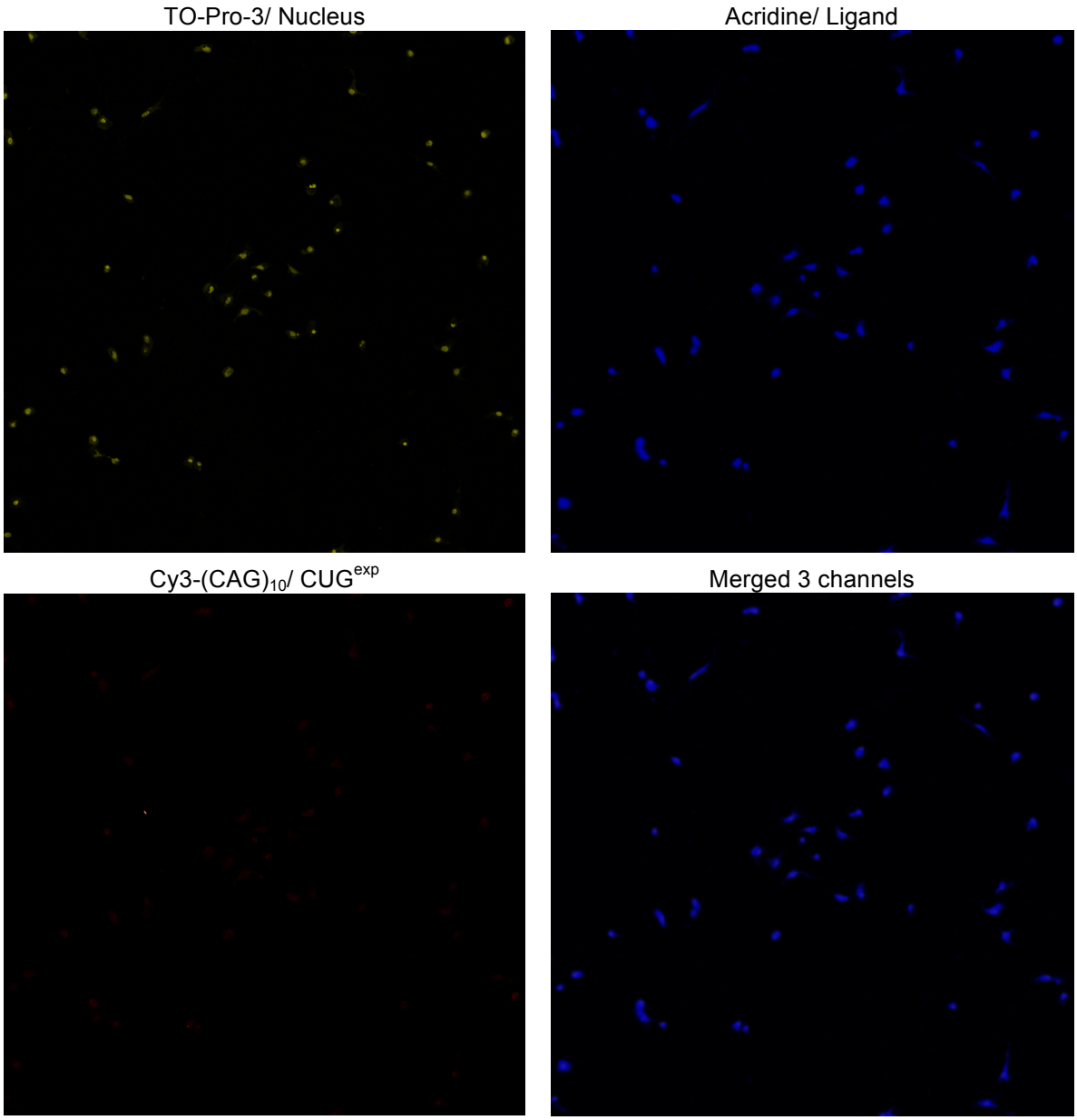


B

Total number of cells	Number foci-containing cells	Percentage of foci-containing cells
38	2	5.3

Supplementary Figure 13. DM1 cell model treated with **9**, 35 µM.

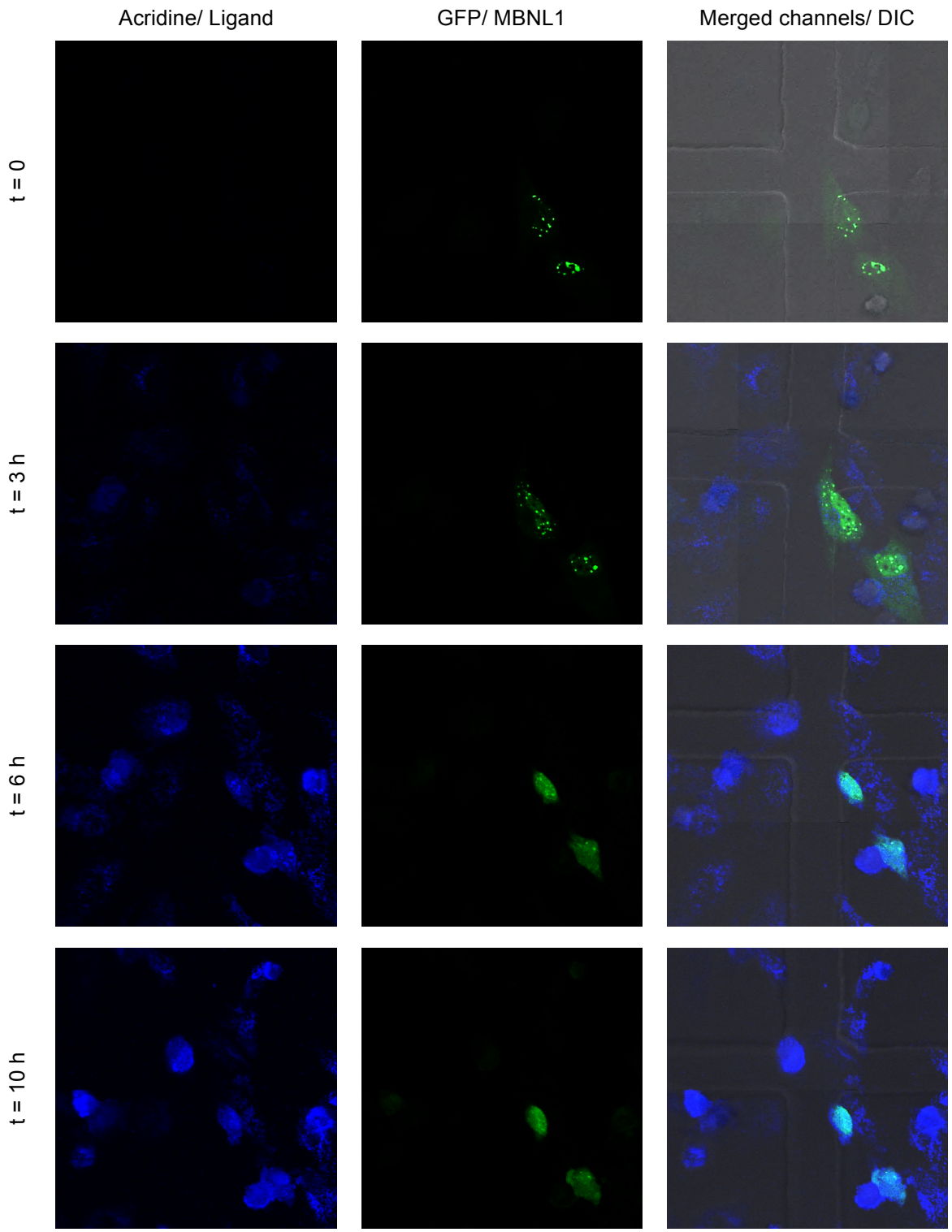
A



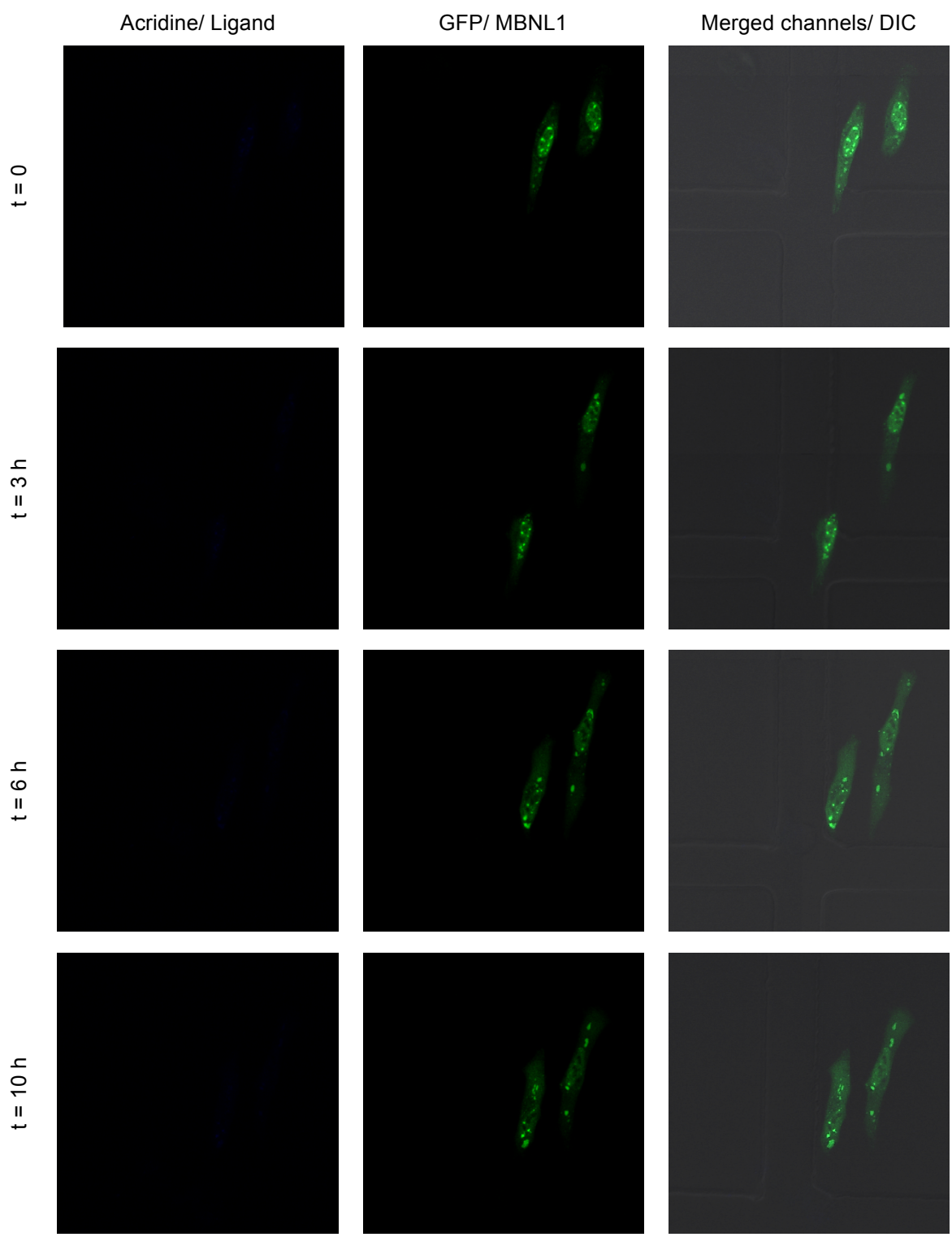
B

Total number of cells	Number foci-containing cells	Percentage of foci-containing cells
32	0	0

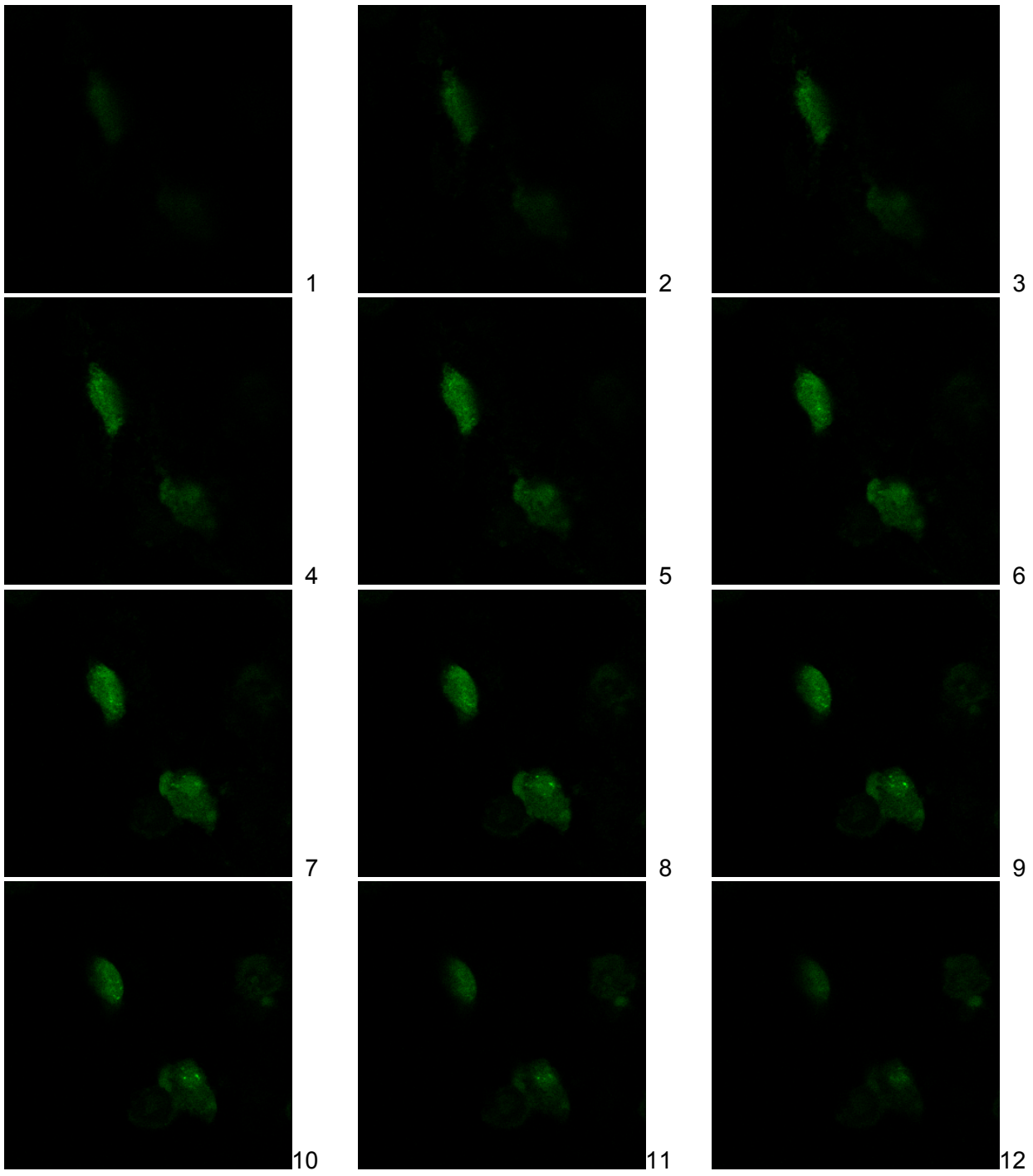
Supplementary Figure 14. DM1 cell model treated with **9**, 50 μM.



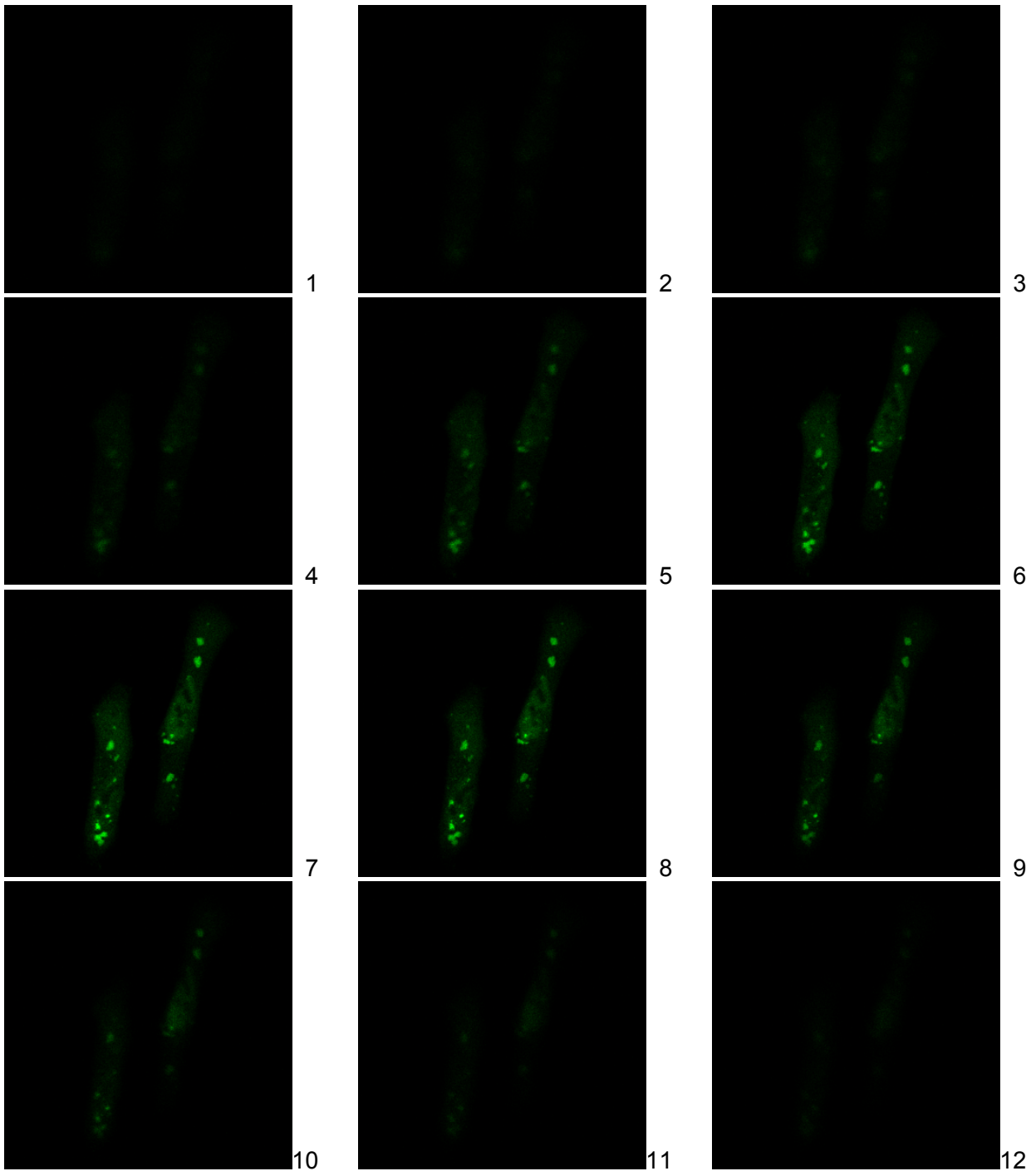
Supplementary Figure 15. Merged channels of two live DM1 model cells treated with **9** (50 μM), corresponding to two cells in Figure 7a are combined with the DIC channel to show the absolute position of each individual cell. Each box shows 200 μM X 200 μM



Supplementary Figure 16. Merged channels of two untreated live DM1 model cells, corresponding to Figure 7b are combined with the DIC channel to show the absolute position of each individual cell. Each box shows 200 μM X 200 μM.



Supplementary Figure 17. Z-stacked image of two live DM1 model cells treated with **9** (50 μM) at t = 10 h, corresponding to two cells in the last row of figure 7a. It contains 12 Slices, 1 μM apart from each other. Almost all MBNL1 foci are dispersed throughout the nuclei at t = 10 h. Each box shows 120 μM X 120 μM.



Supplementary Figure 18. Z-stacked image of two untreated live DM1 model cells at t = 10 h, corresponding to two cells in the last row of figure 7b. It contains 12 Slices, 1 μM apart from each other. Many MBNL1 foci are present in the nuclei at t = 10 h. Each box shows 120 μM X 120 μM.

5. References

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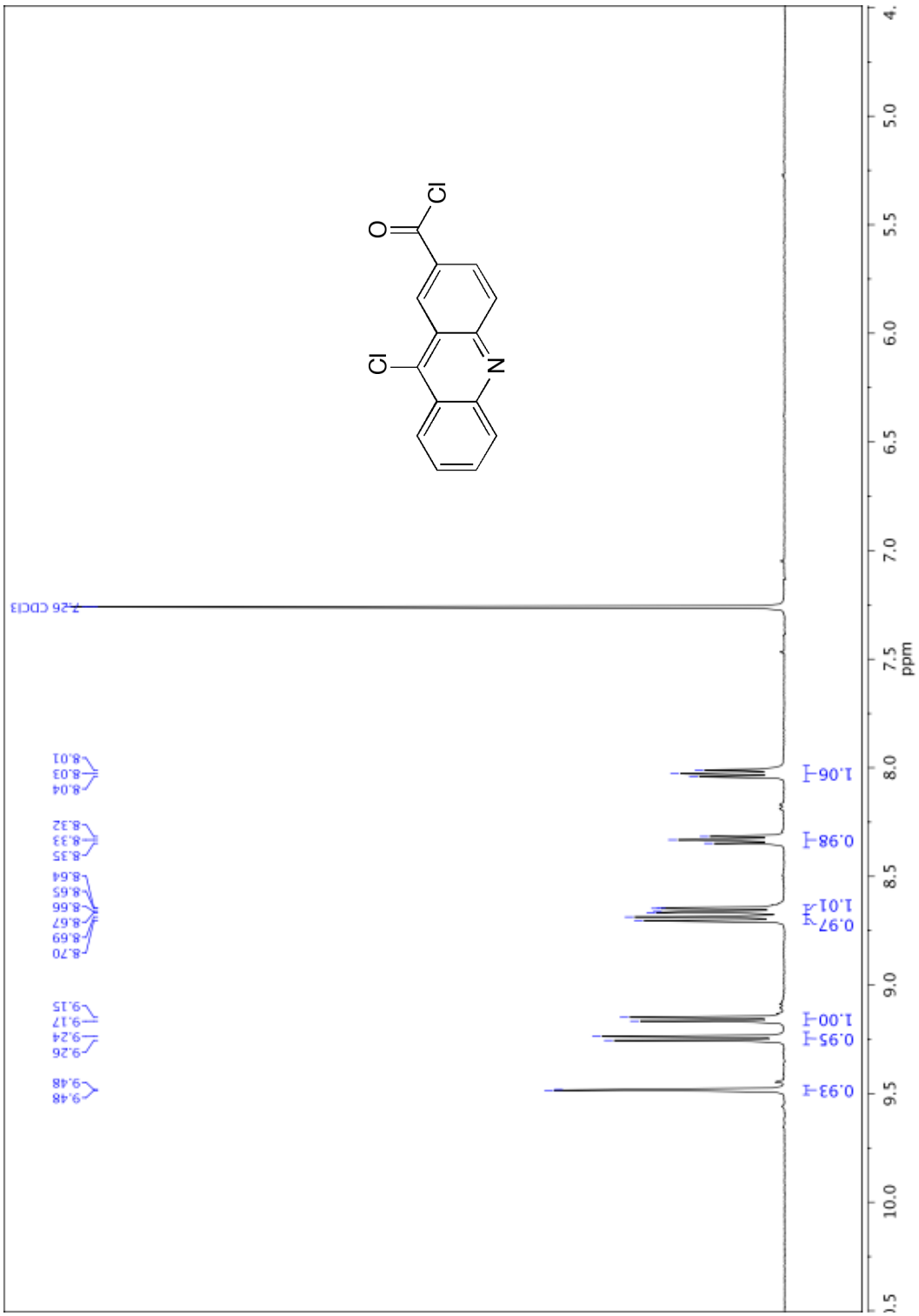
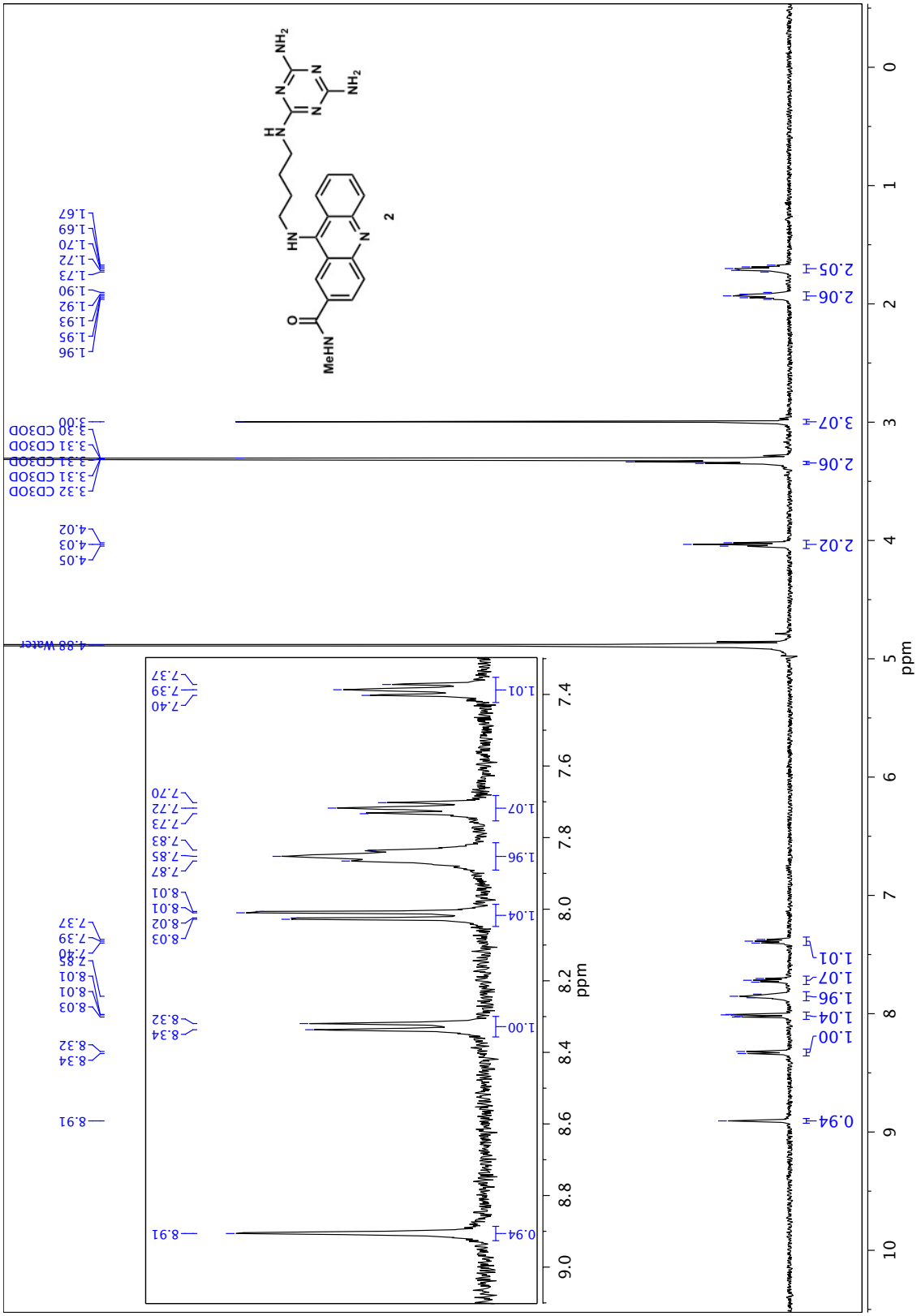
6. Supplementary NMR Spectra

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N
NH
N
N
N
NH2
NH2
2
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8.91
8.34
8.32
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8.01
7.85
7.80
7.39
7.37
3.32 CD3OD
3.31 CD3OD
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1.95
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1.92
1.90
1.73
1.72
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4.05
4.03
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2.06
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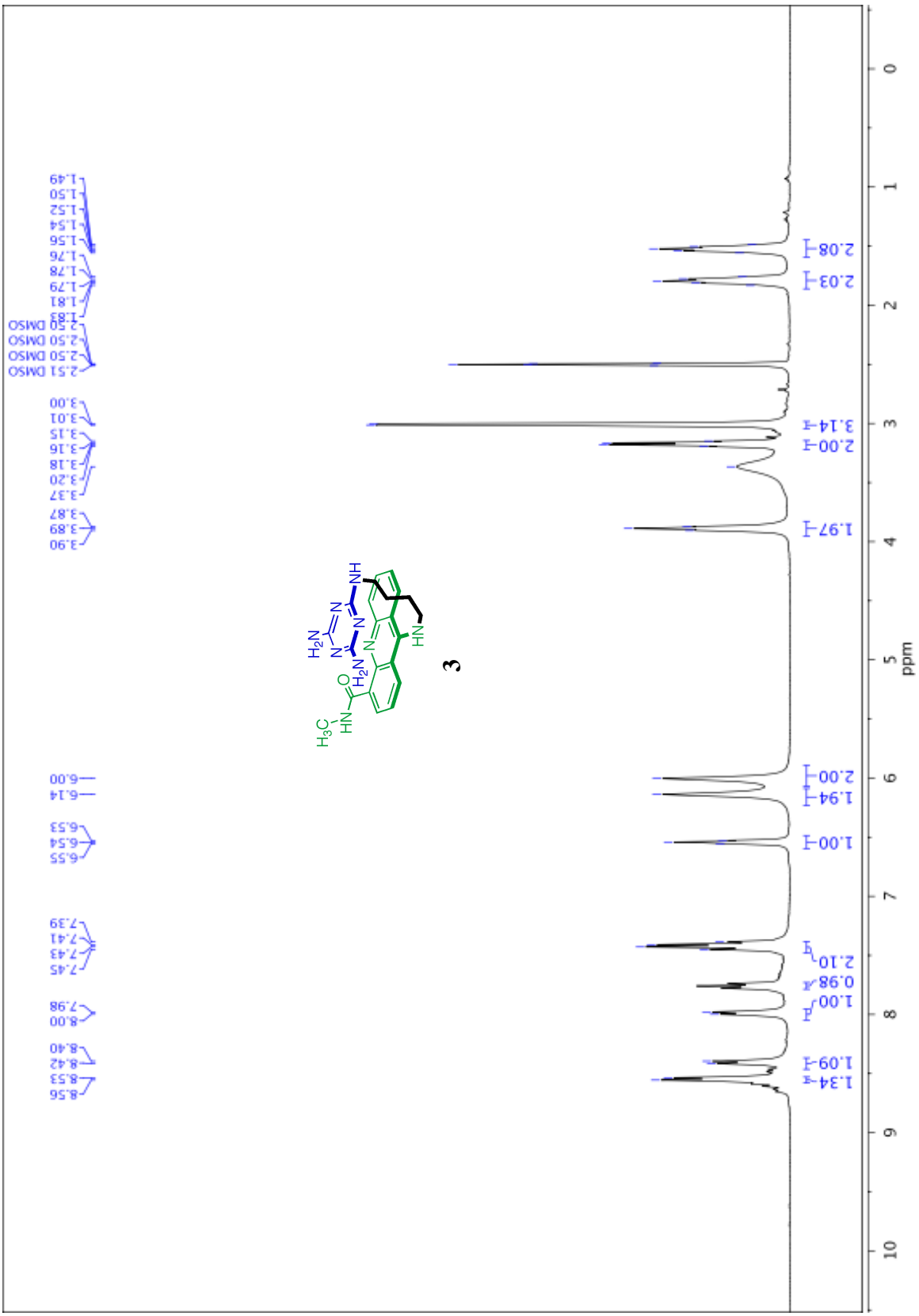
2.06
2.05
ppm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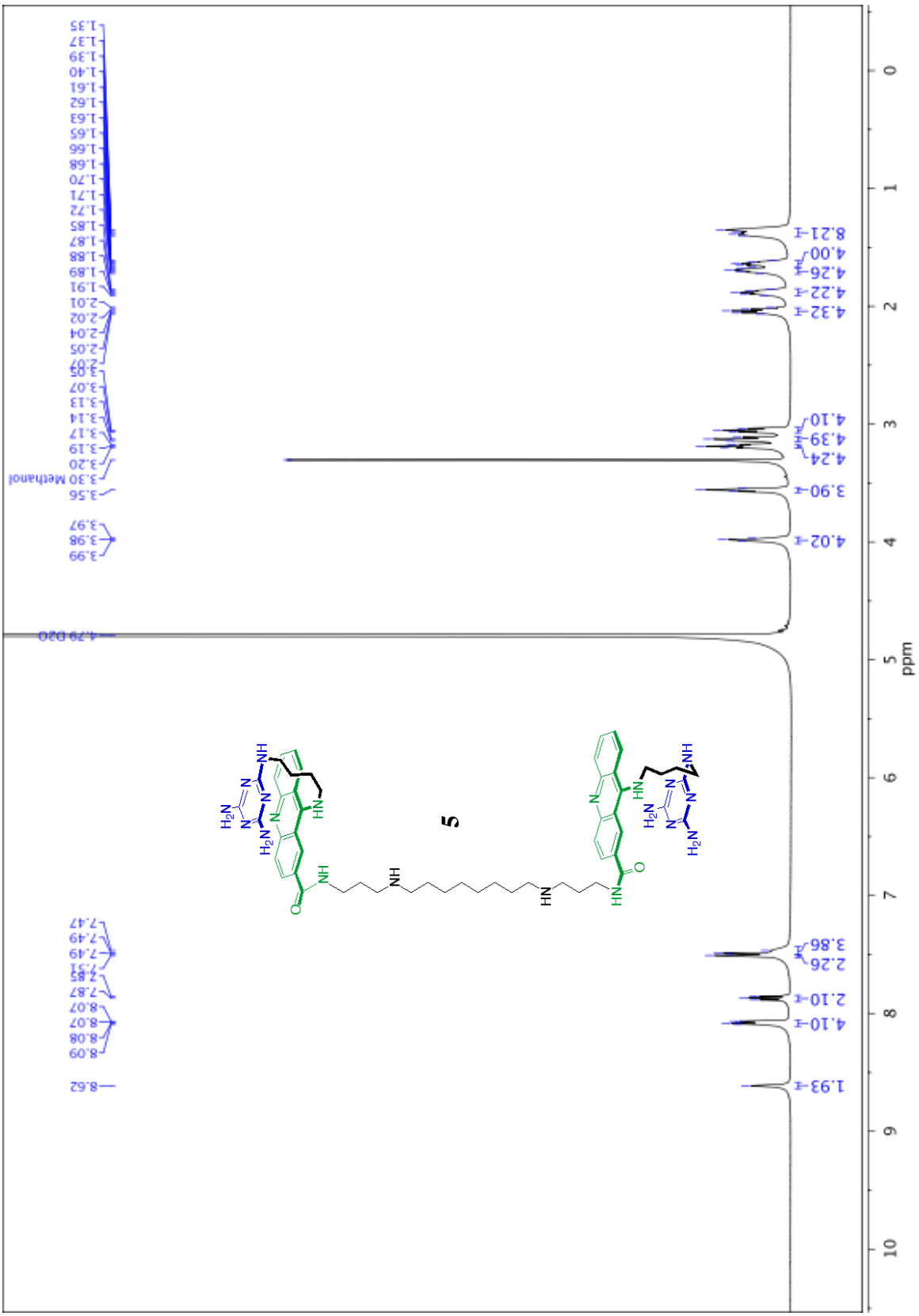
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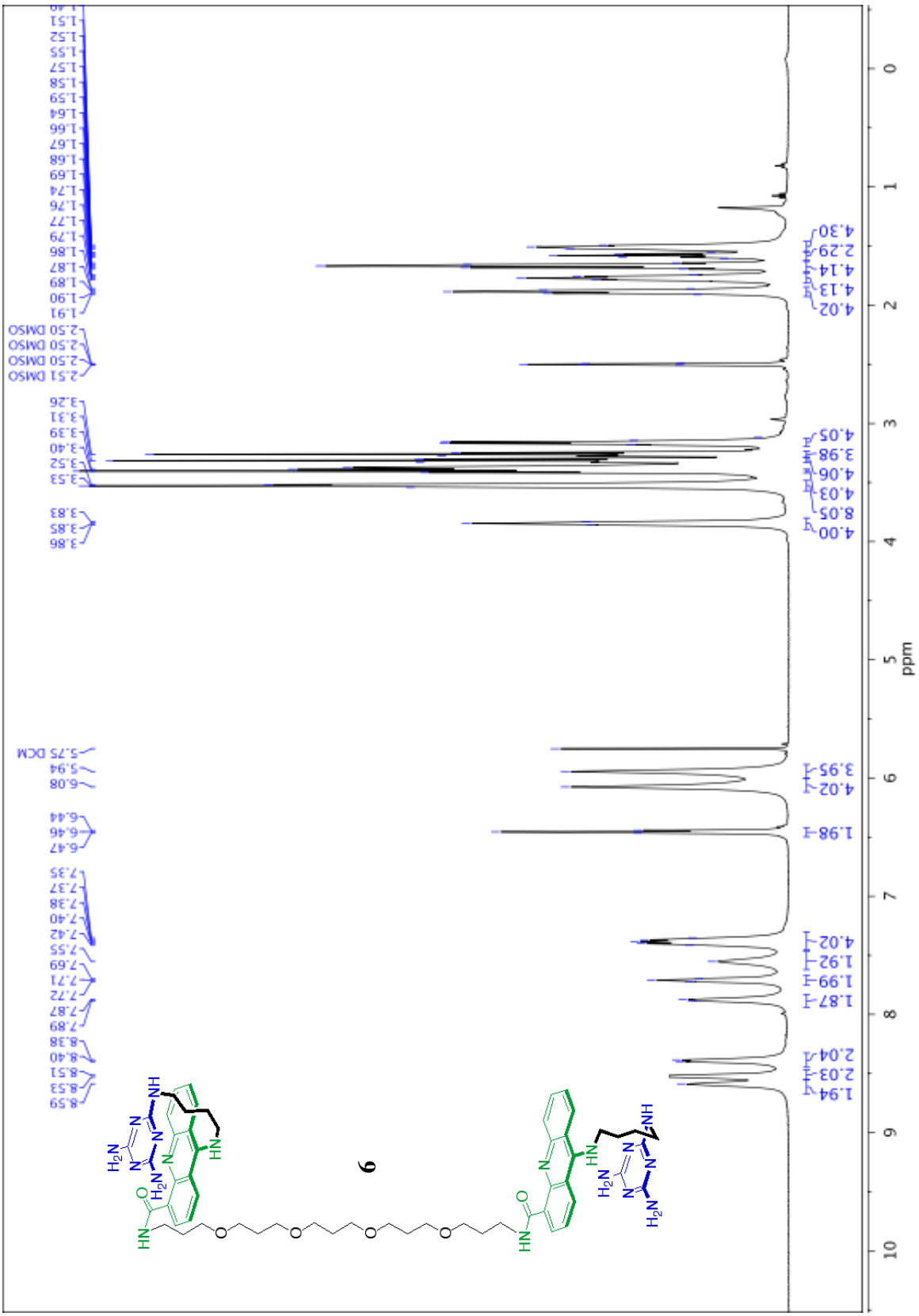
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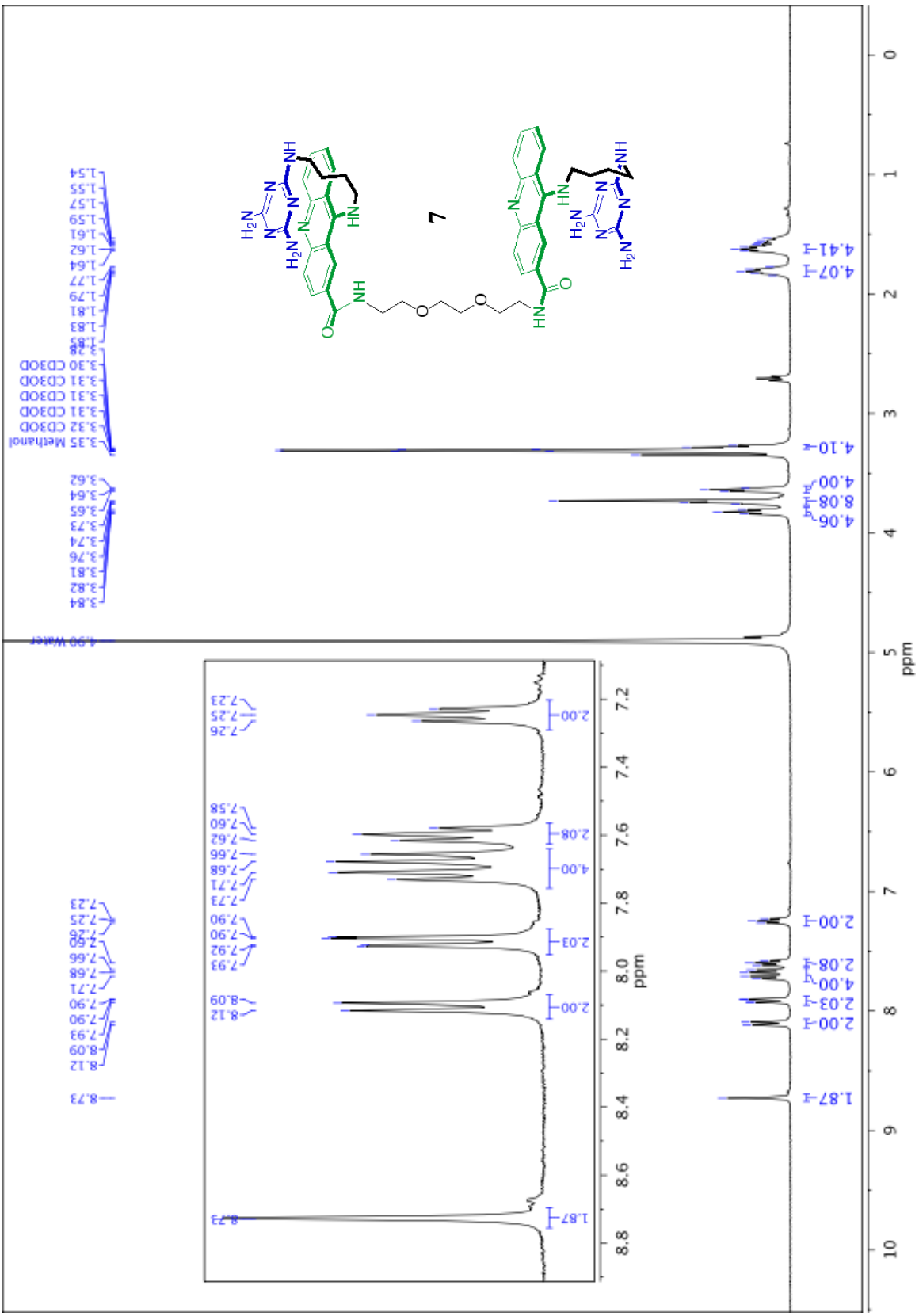
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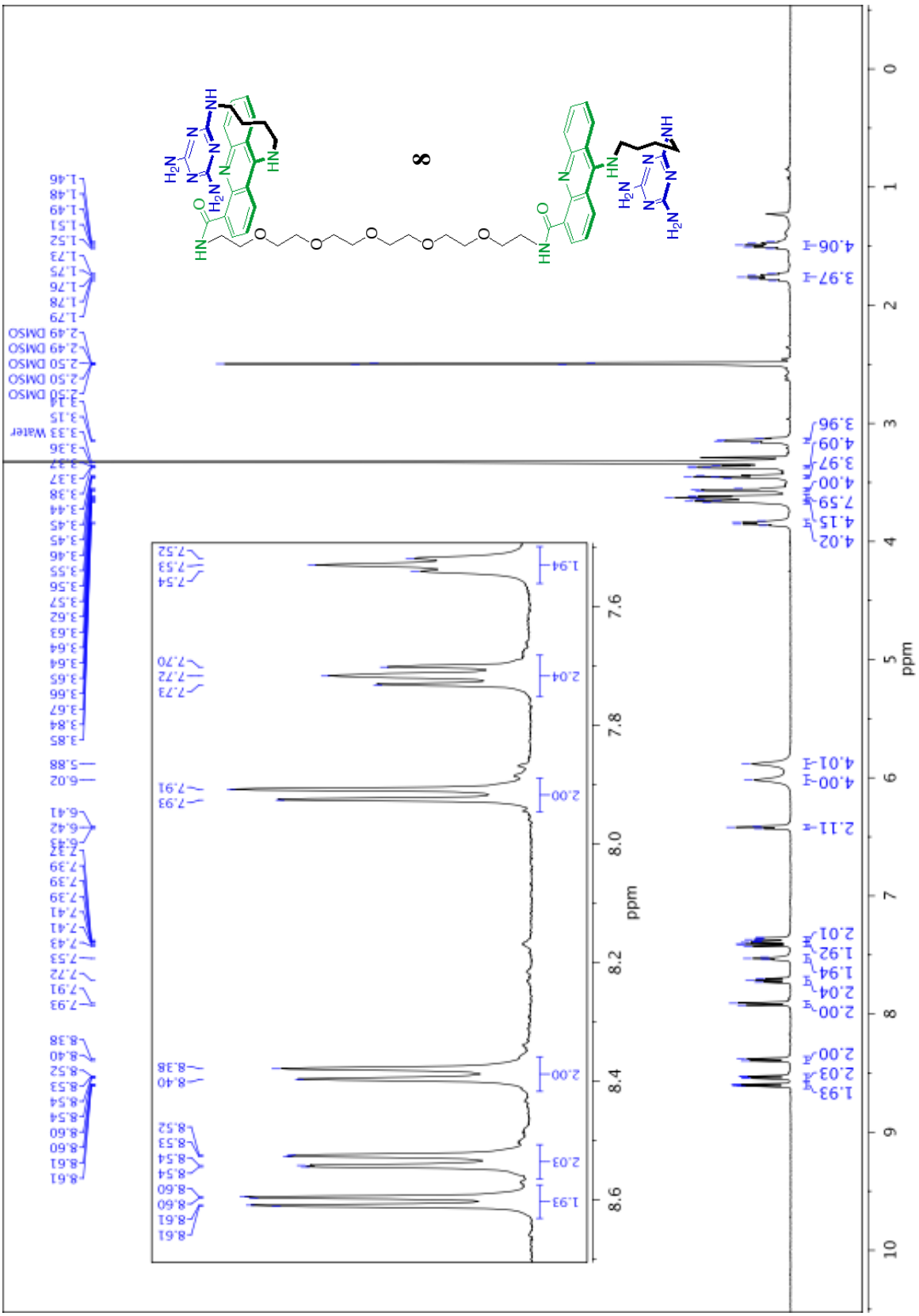

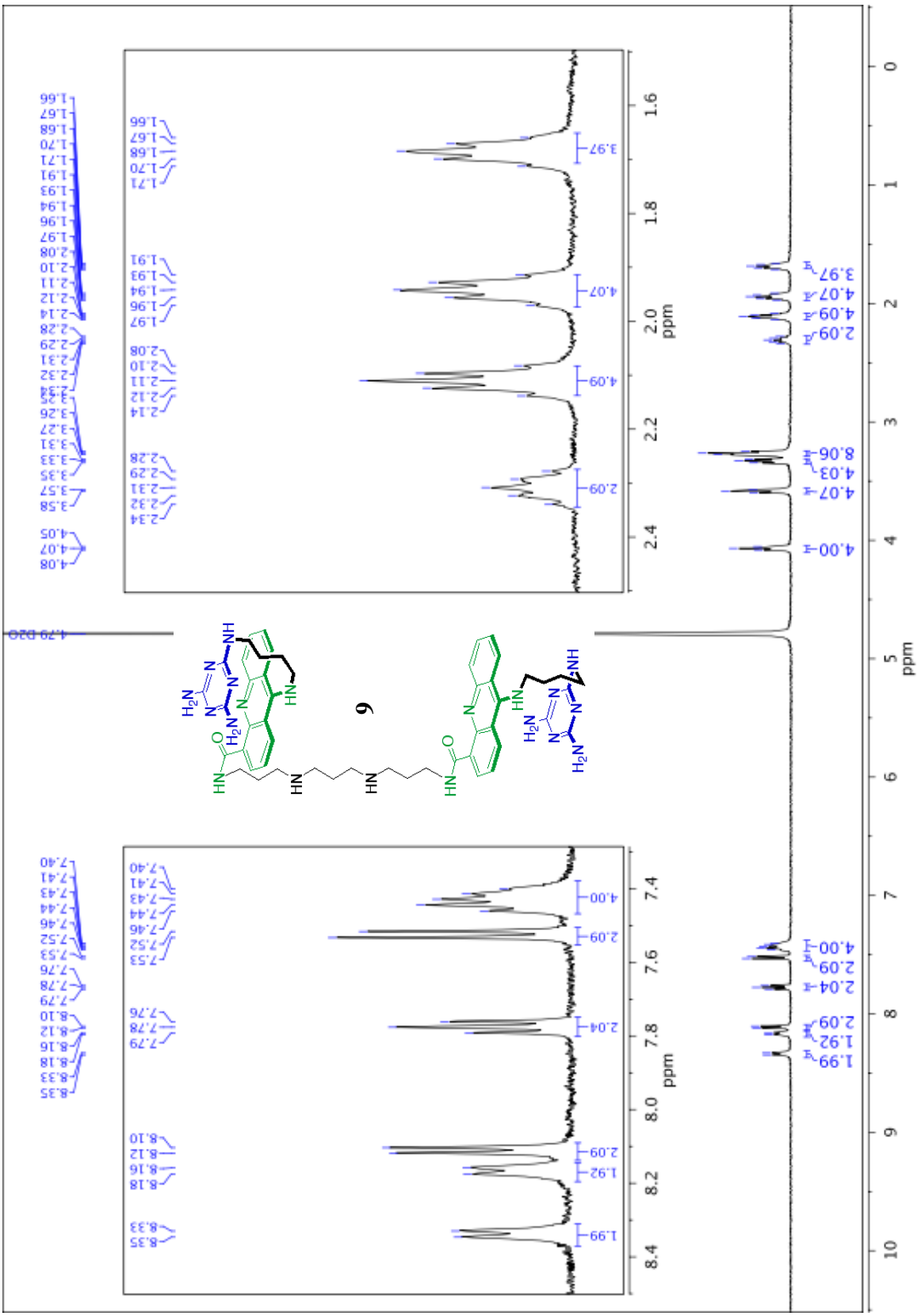
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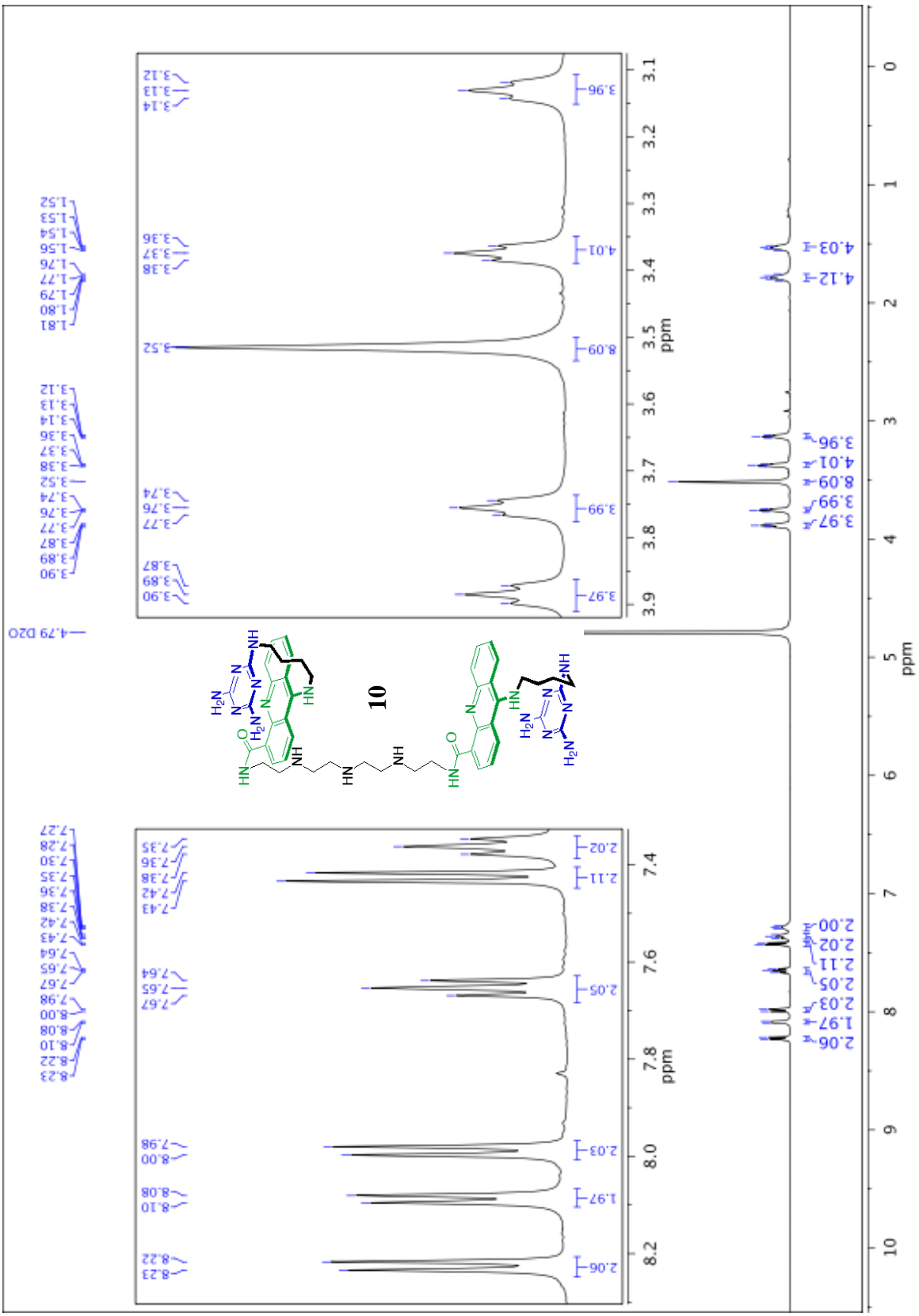
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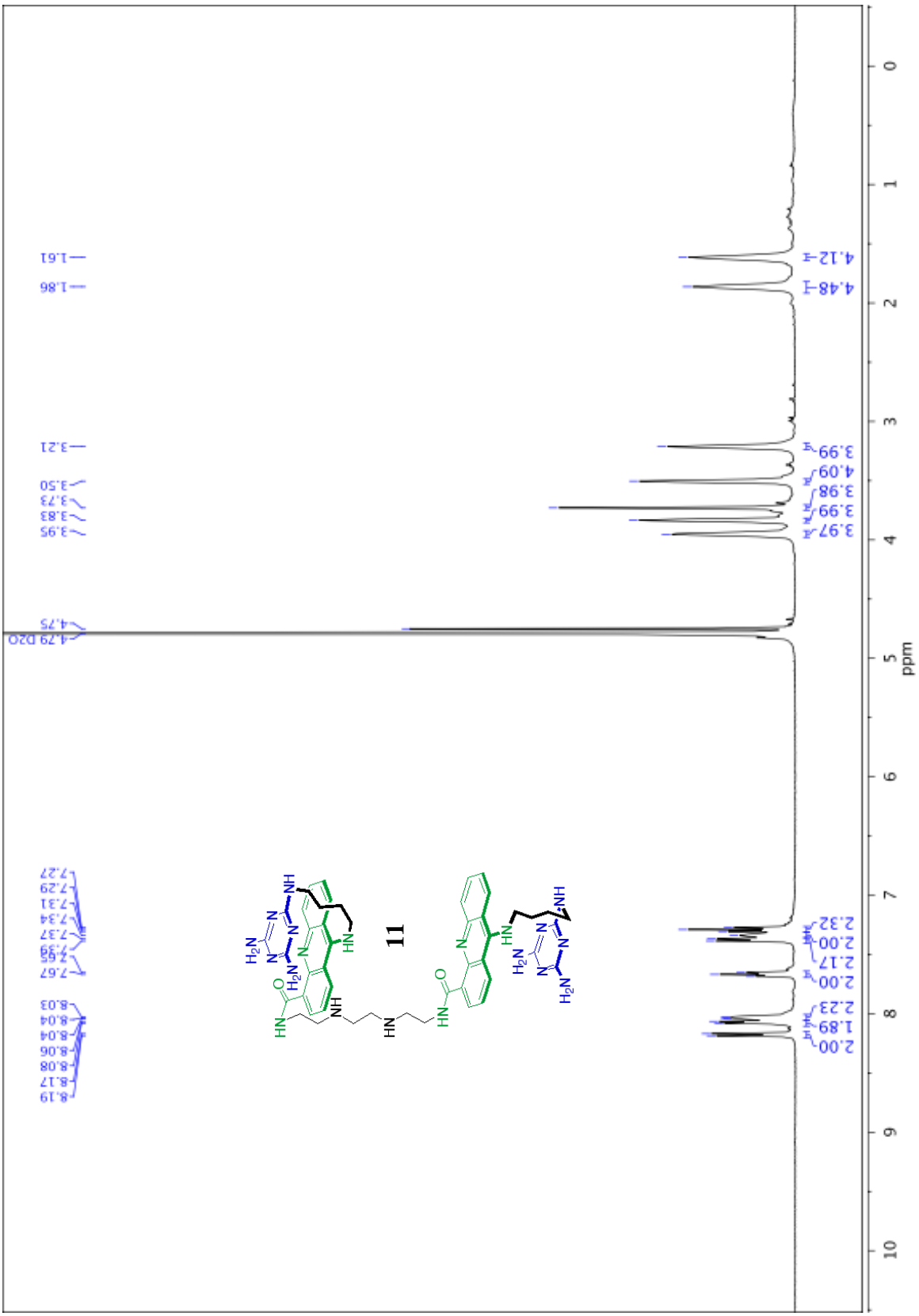
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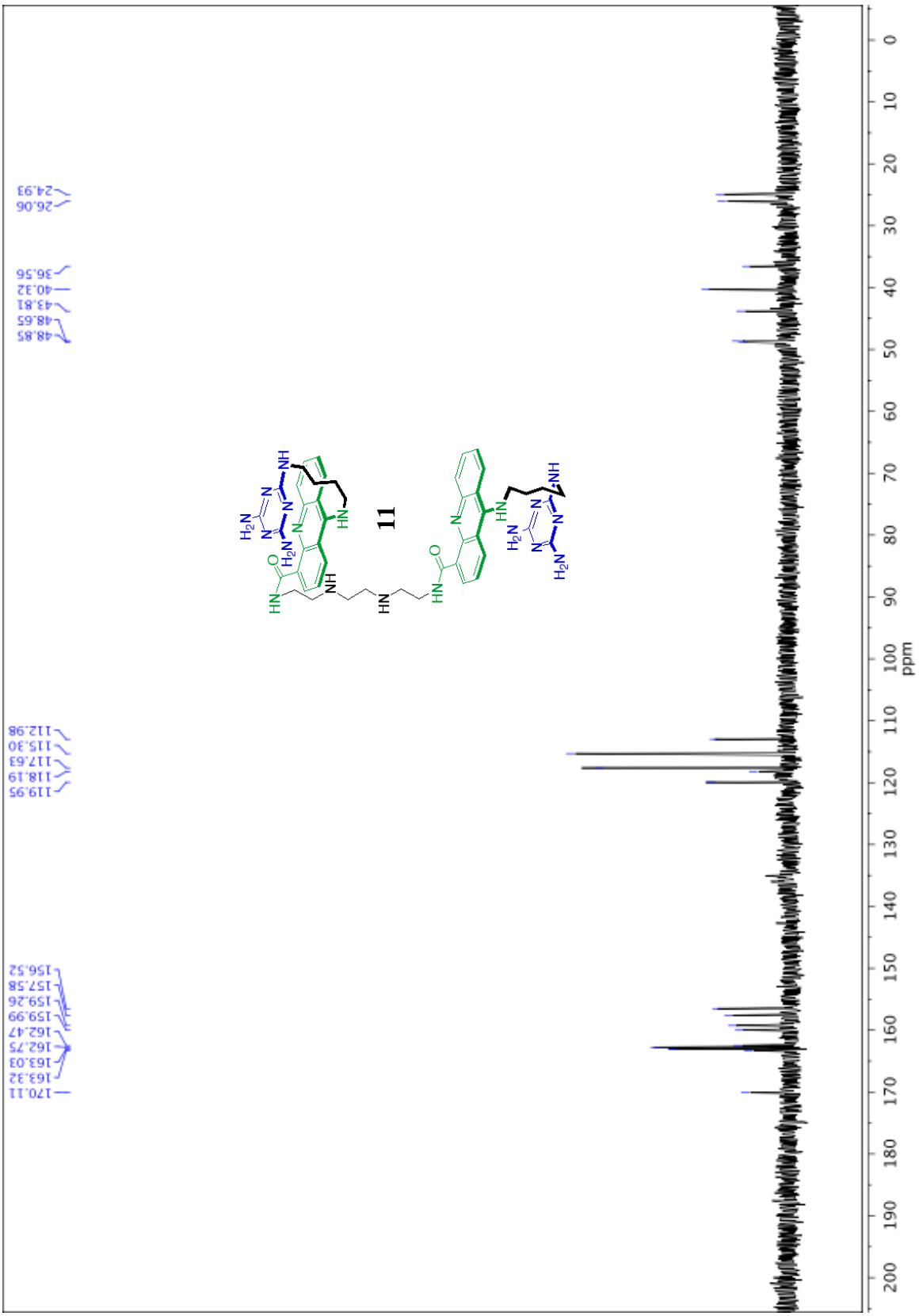
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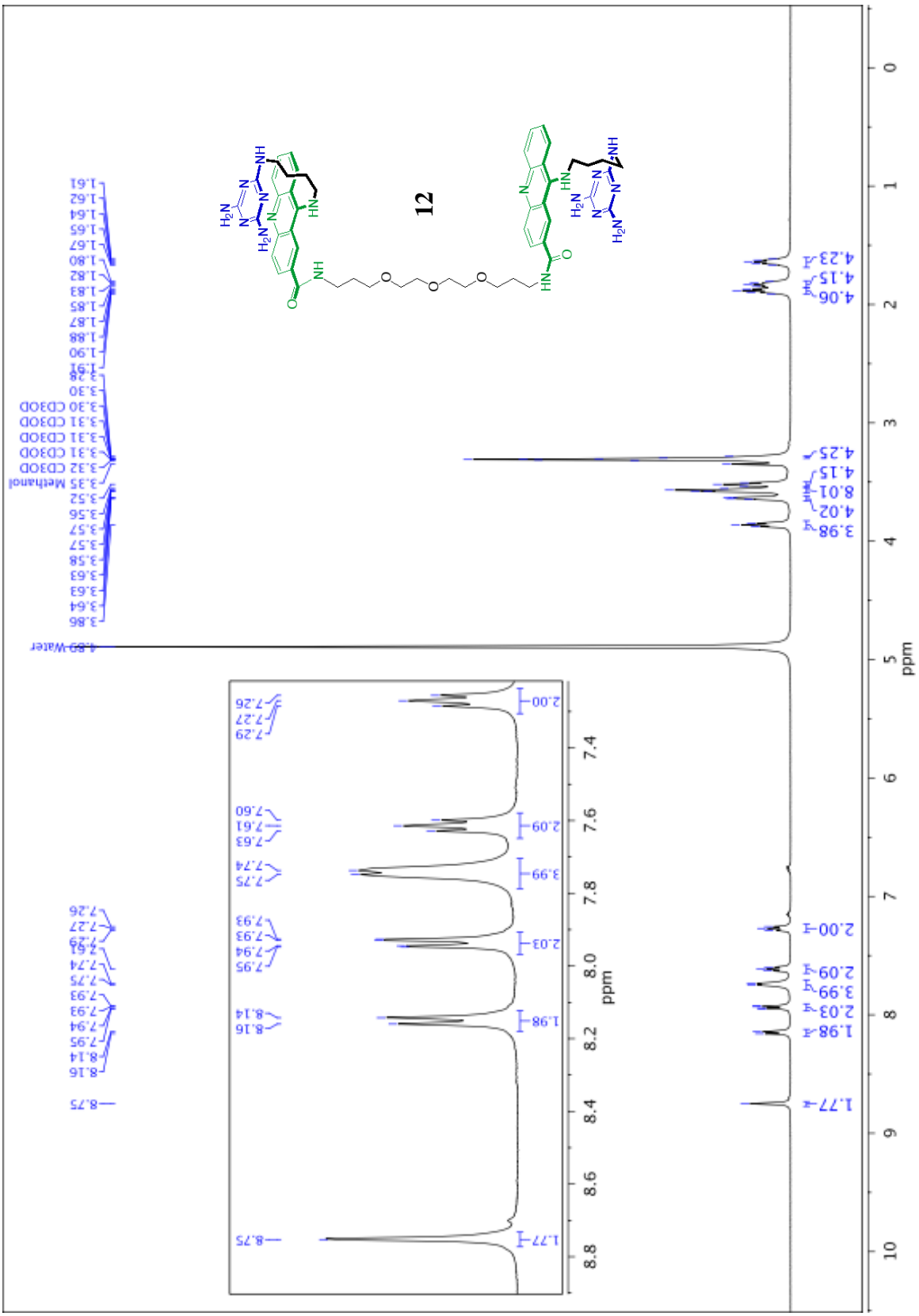
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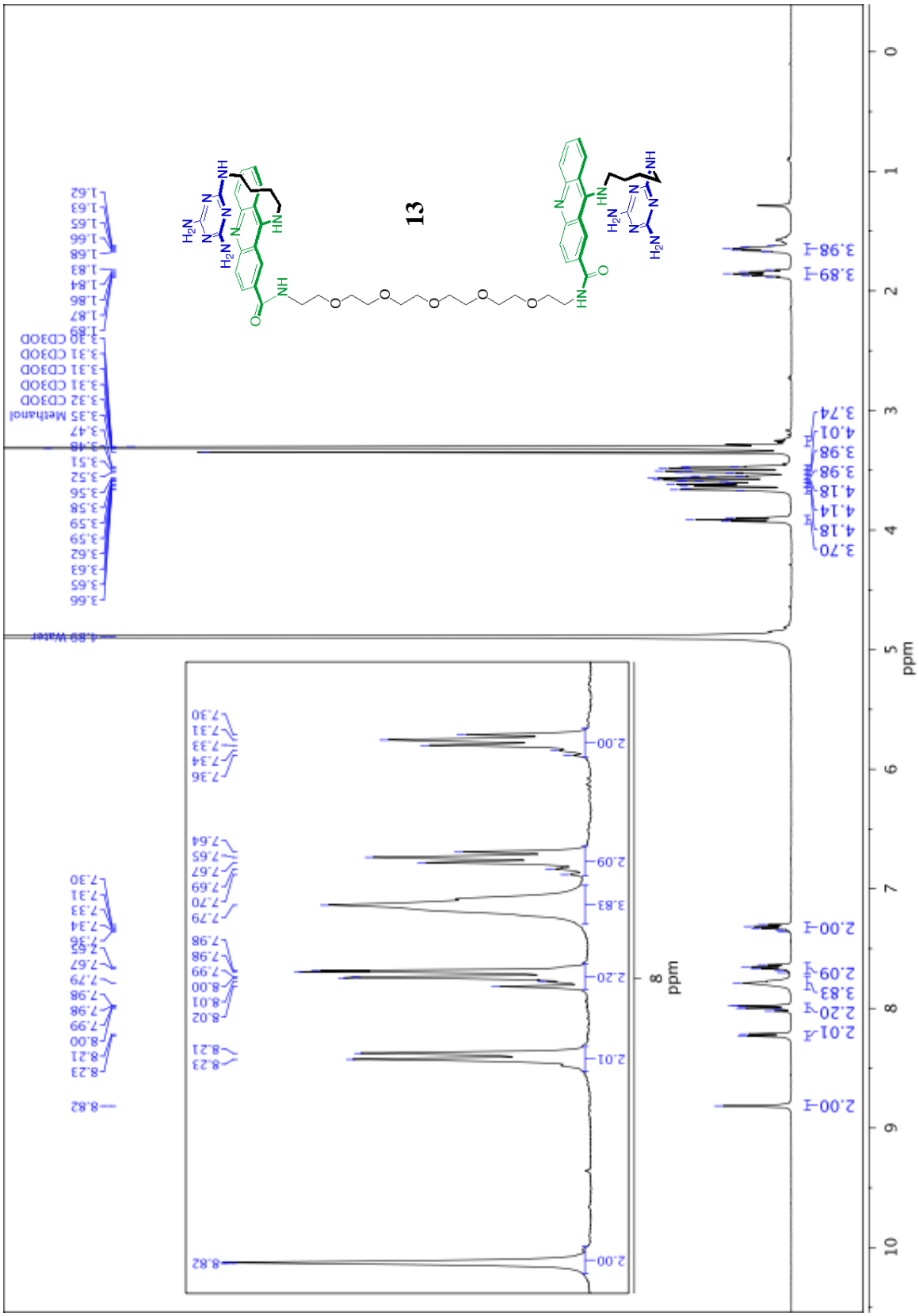
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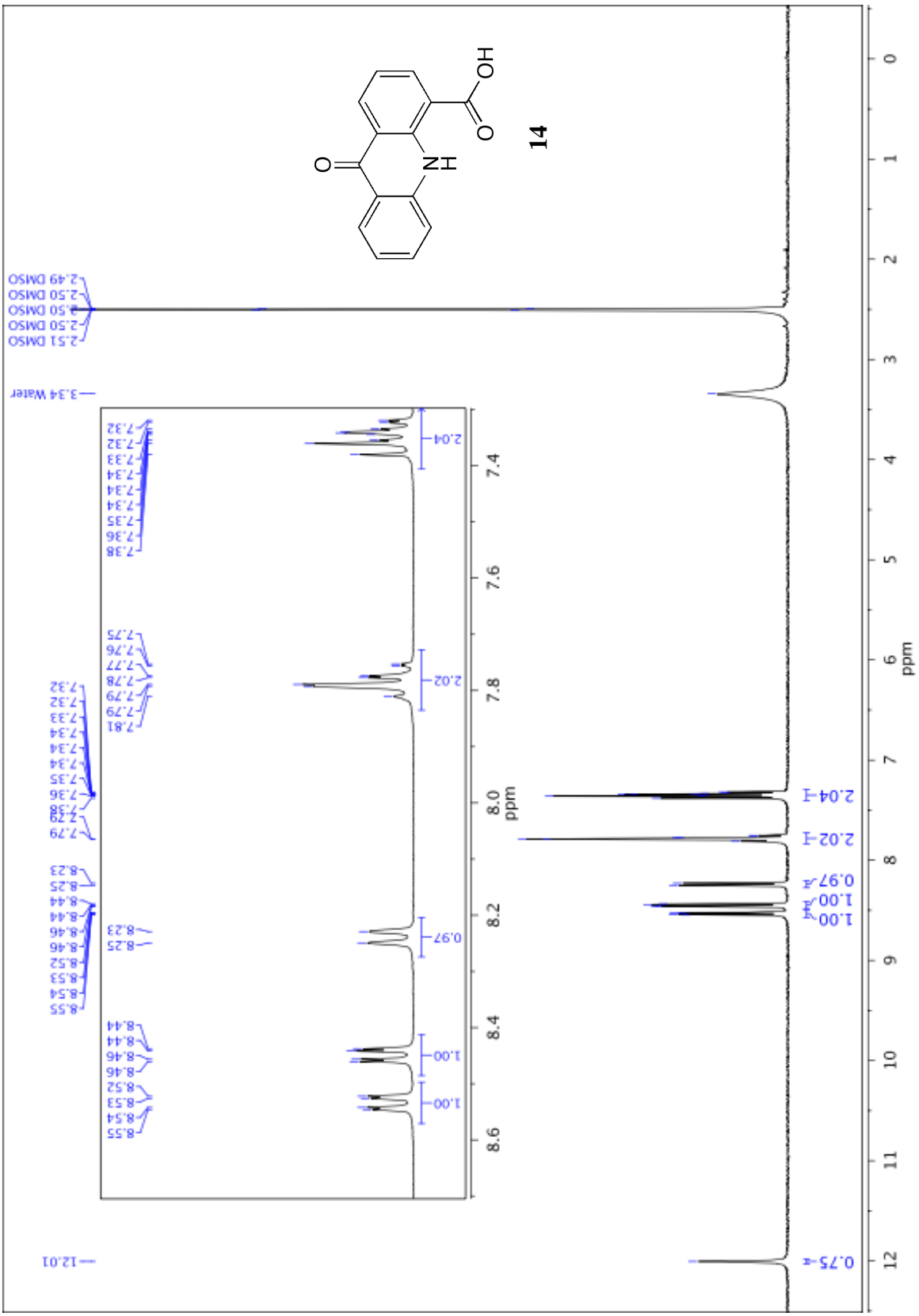
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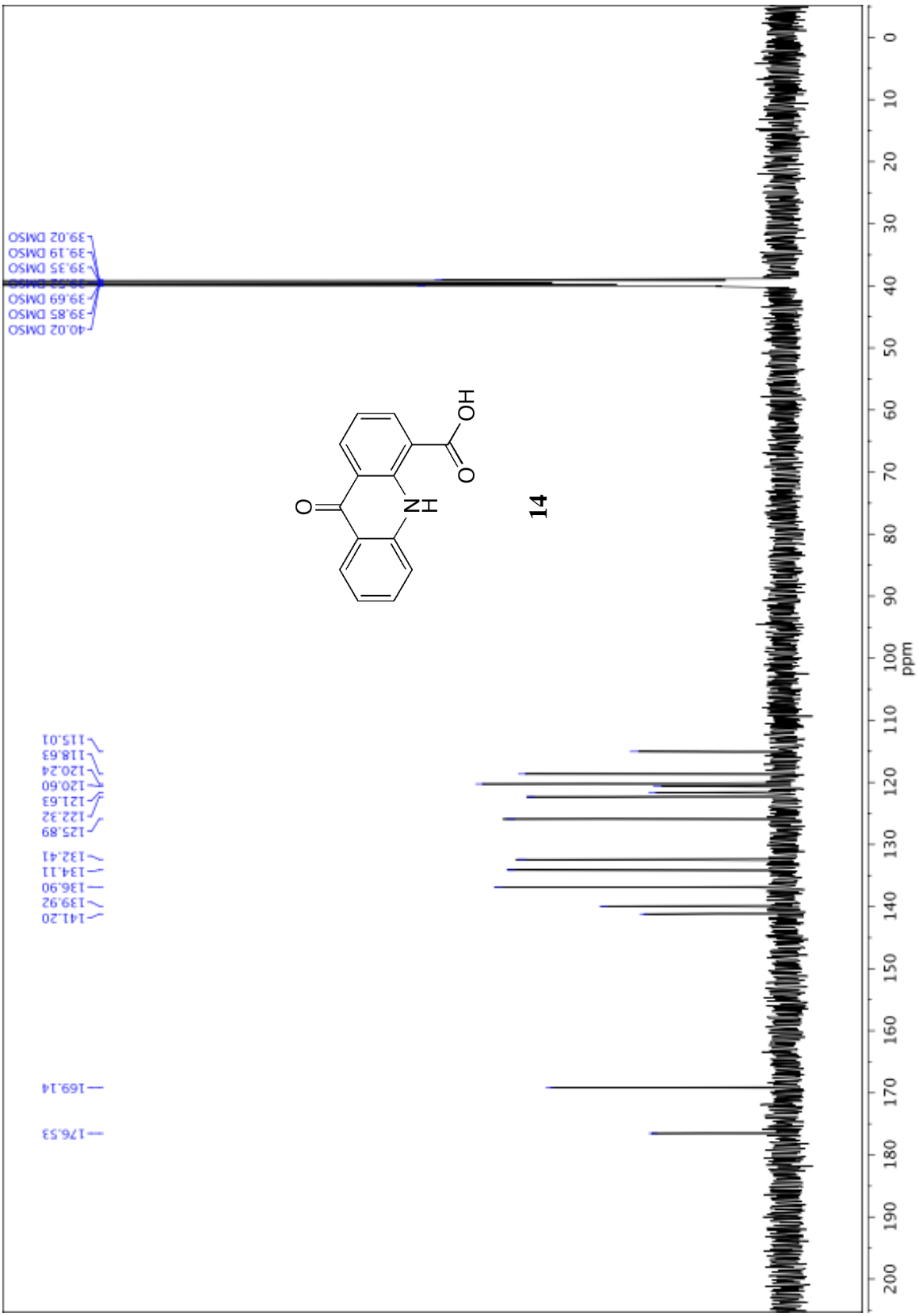
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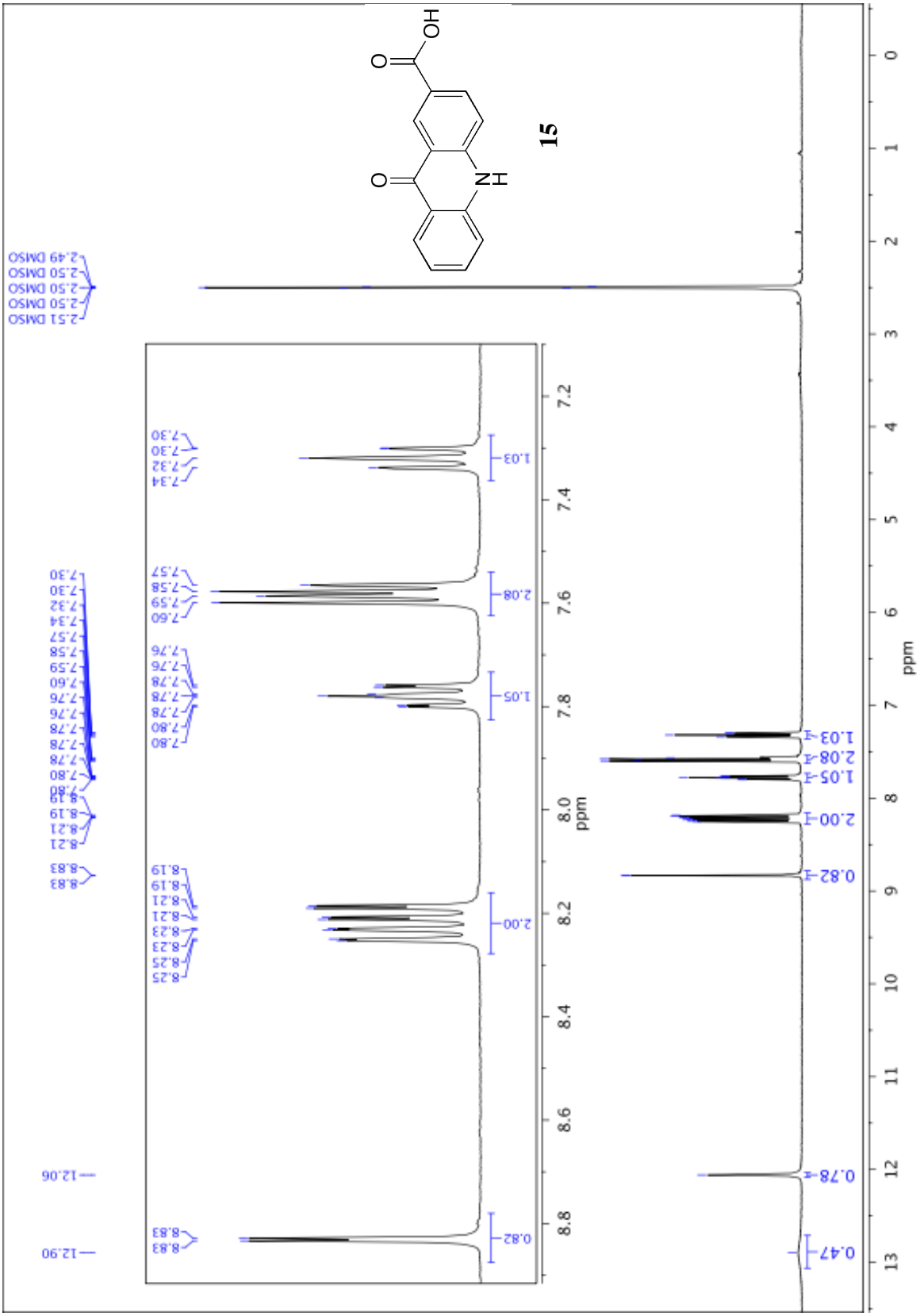
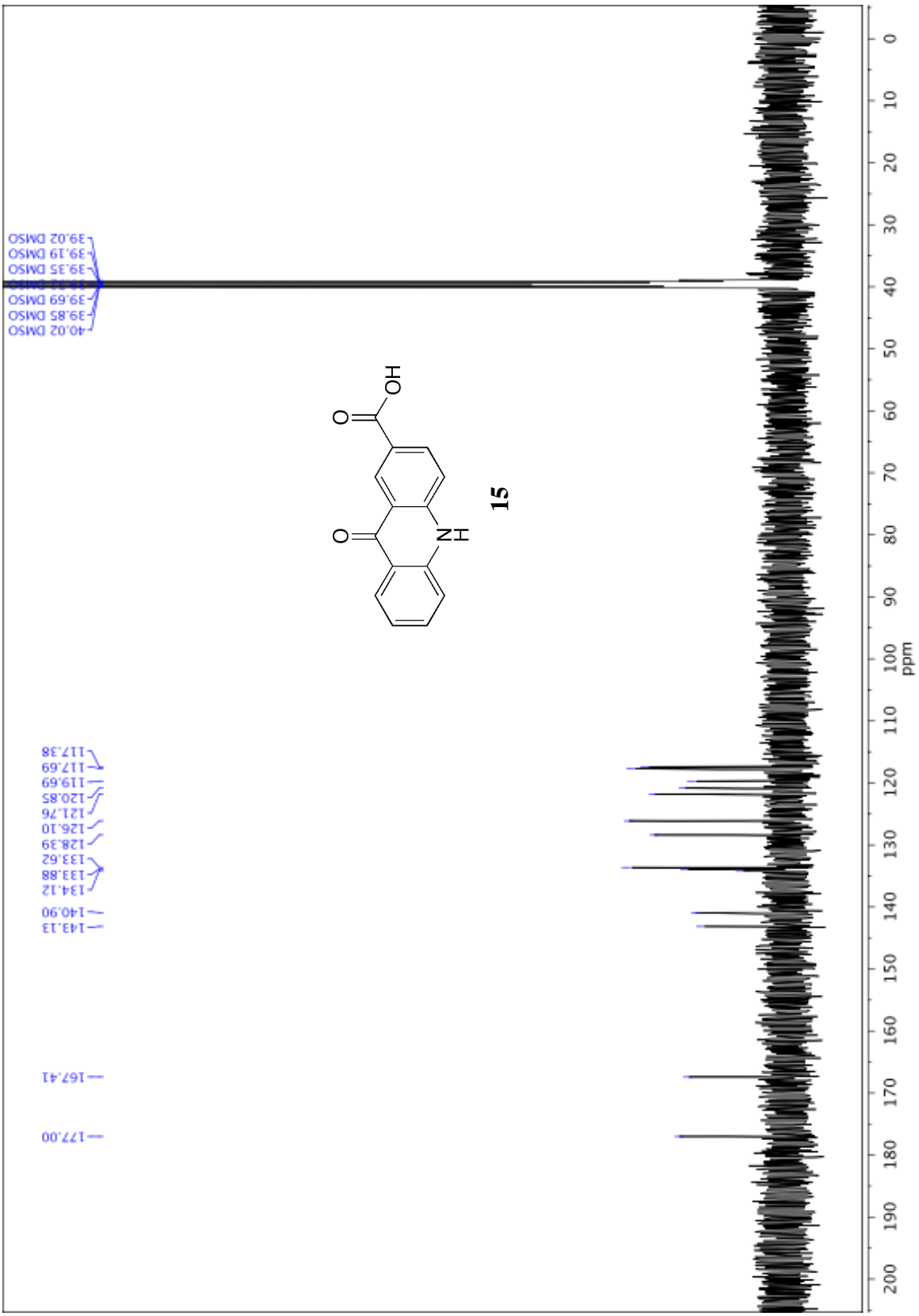
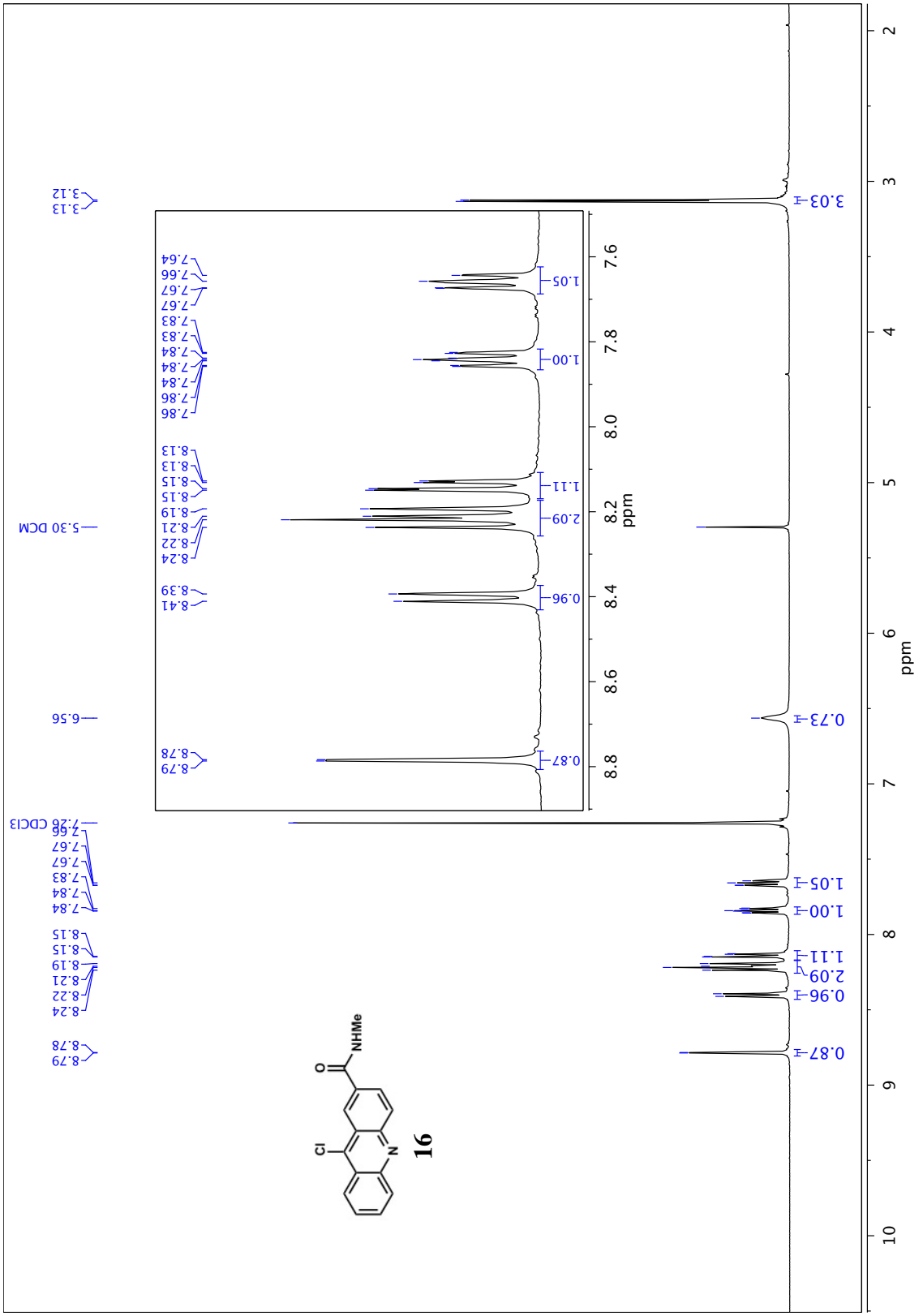
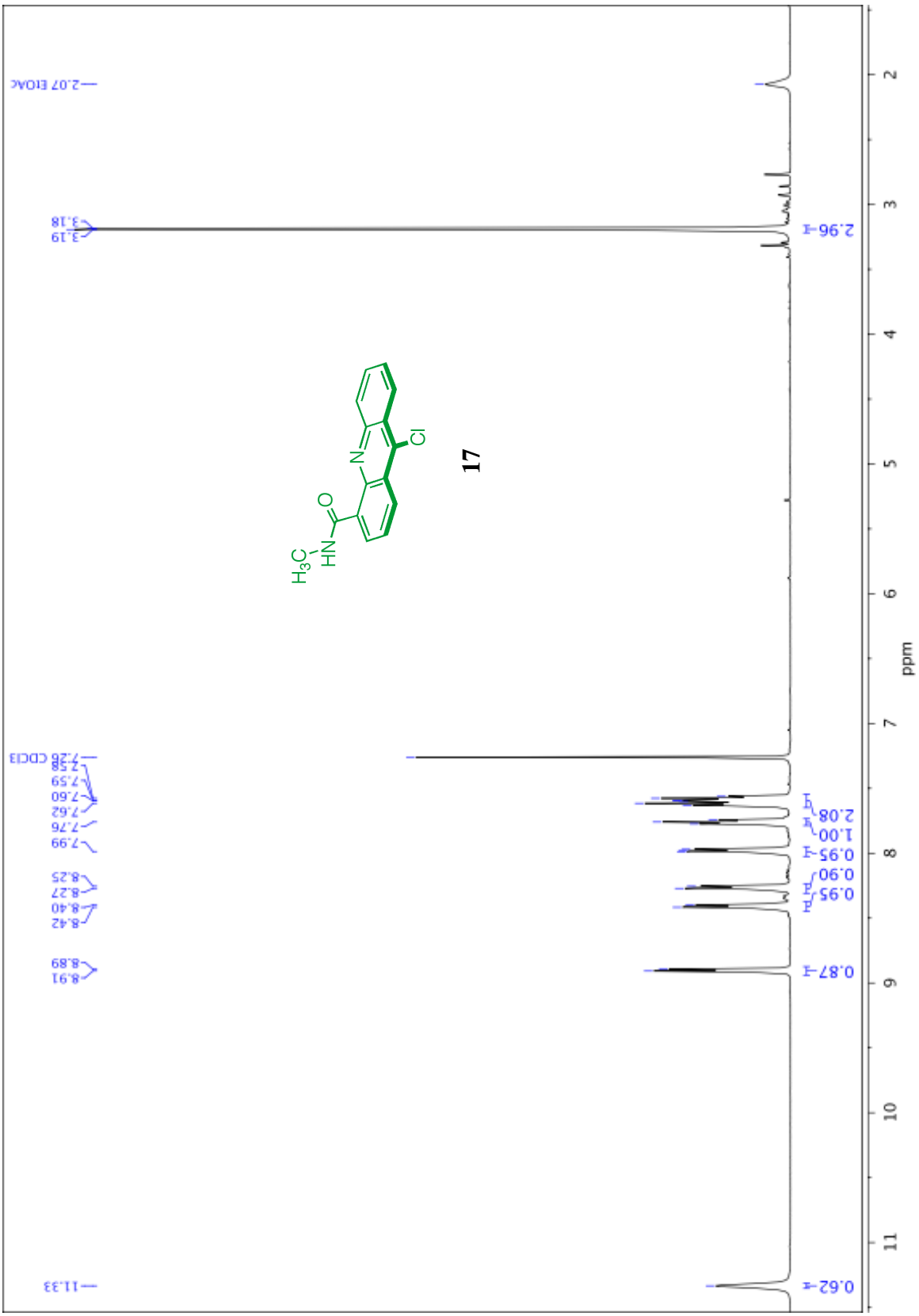
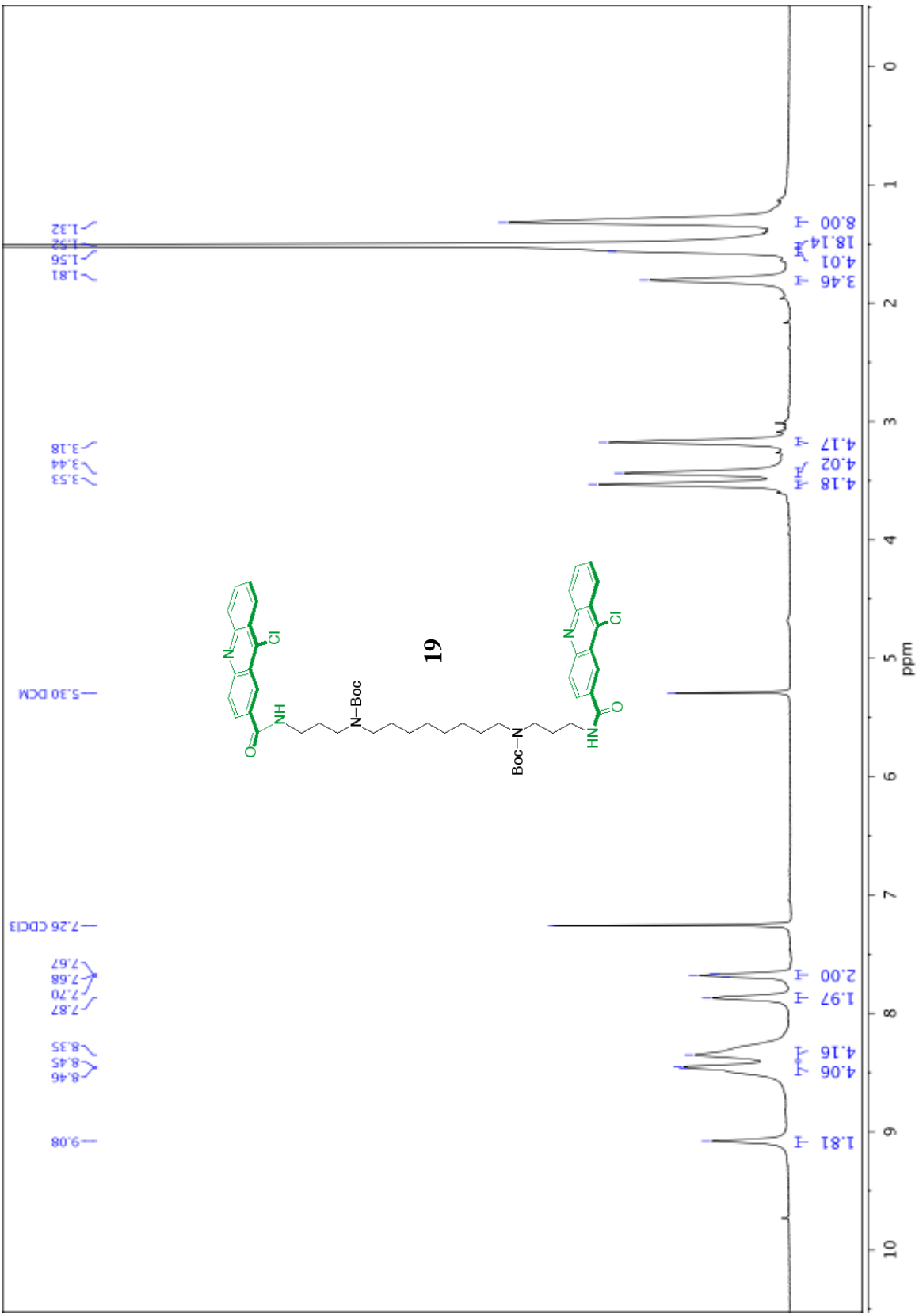
39.19 DMSO
39.35 DMSO
39.69 DMSO
39.85 DMSO
40.02 DMSO
115.01
118.63
120.24
120.60
121.63
122.32
125.89
132.41
134.11
136.90
139.92
141.20
169.14
176.53
O
NH
O
OH
14
ppm

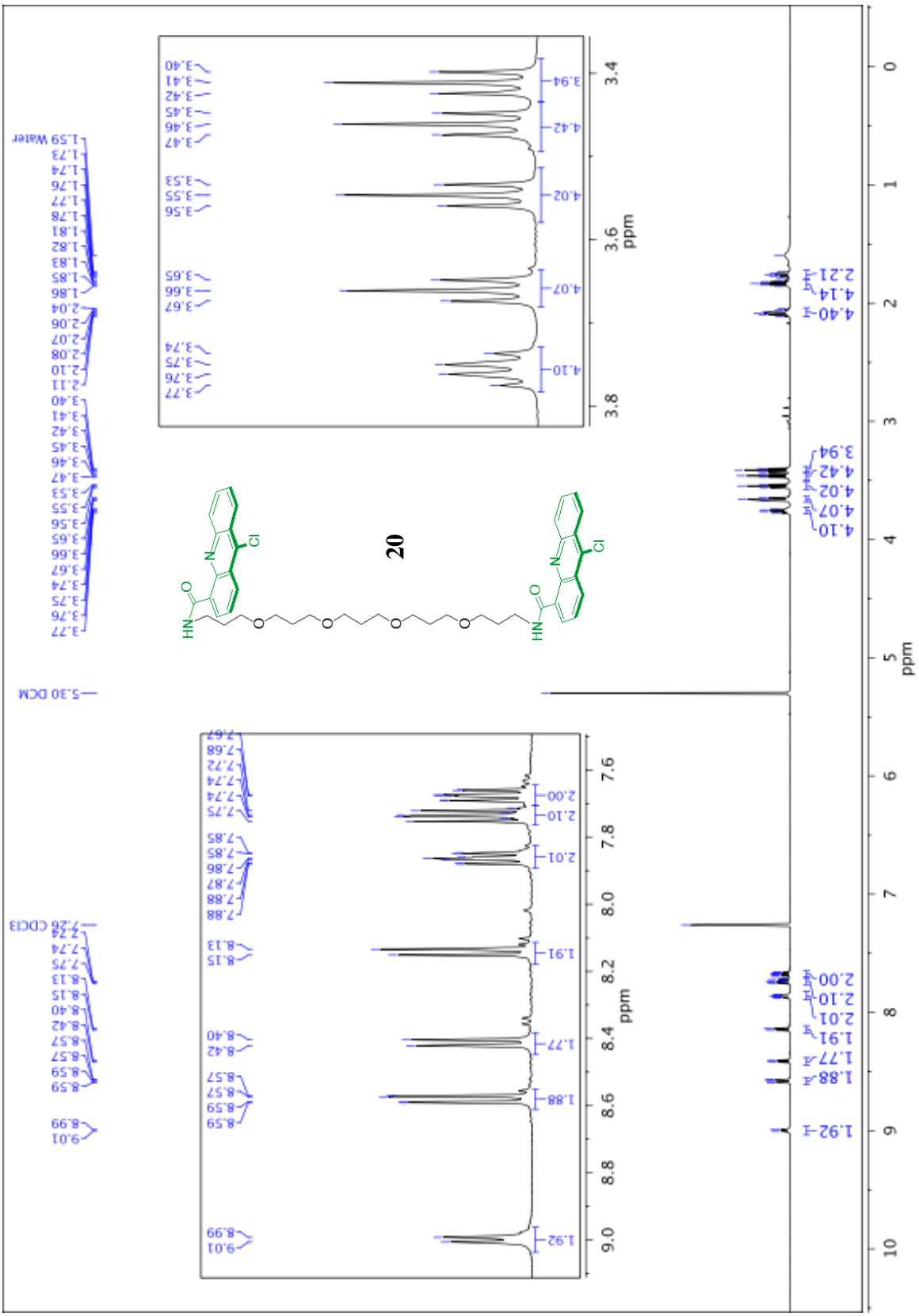

15
O
OH
O
N
H
ppm

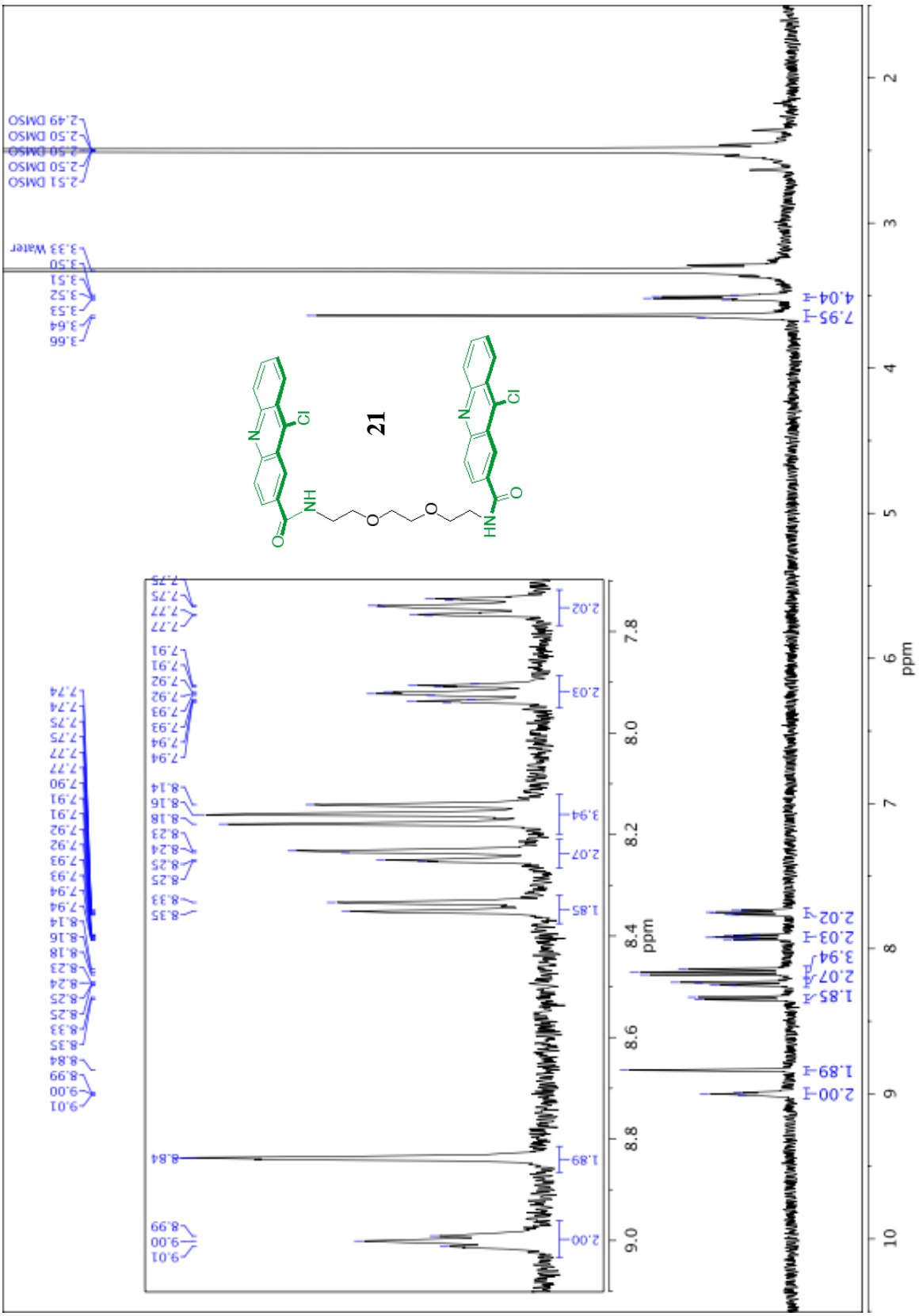

39.02 DMSO
39.19 DMSO
39.35 DMSO
39.69 DMSO
39.85 DMSO
40.02 DMSO
117.38
117.69
119.69
120.85
121.76
126.10
128.39
133.62
133.88
134.12
140.90
143.13
167.41
177.00
O
OH
O
NH
15
ppm

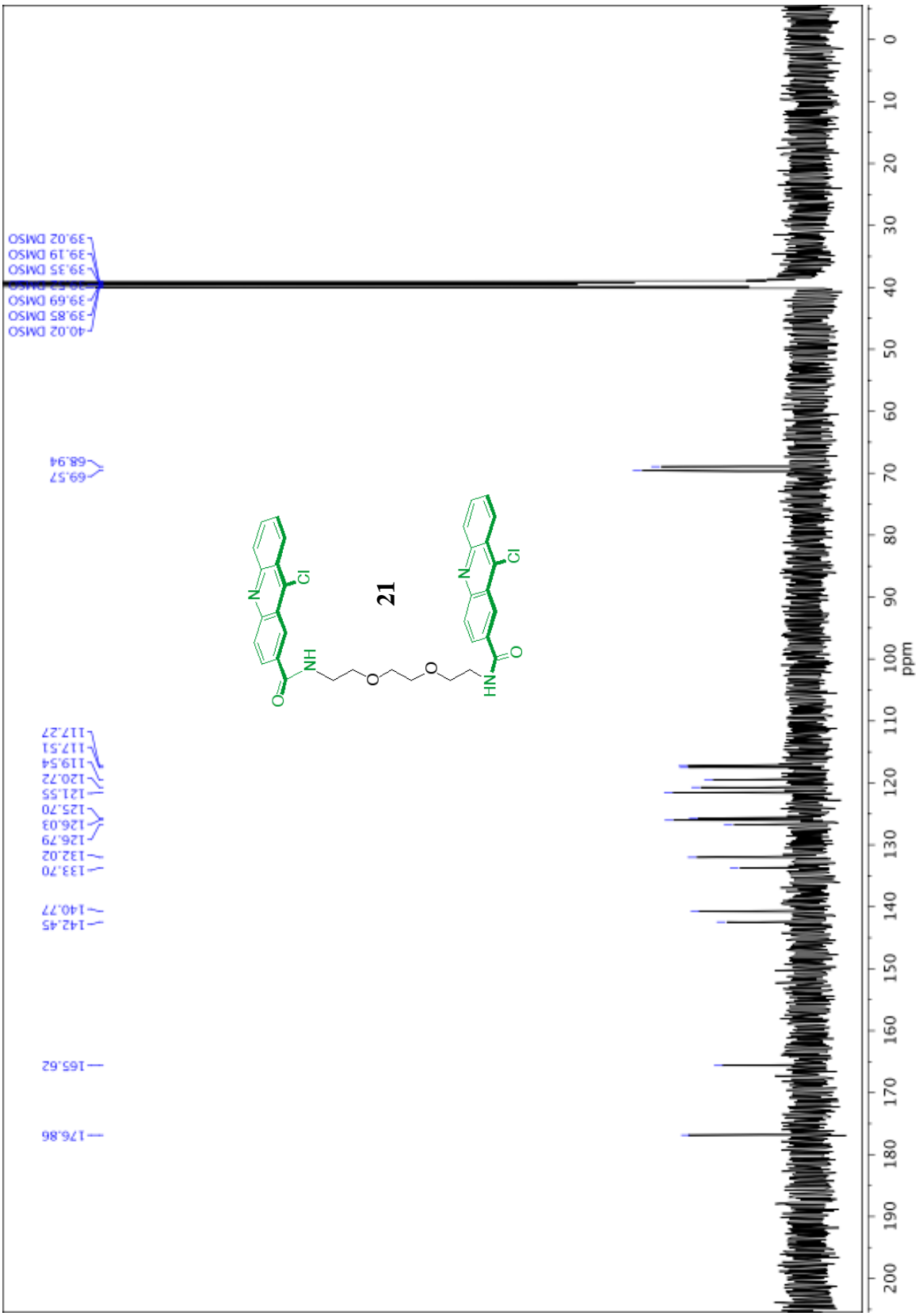

NHMe
O
Cl
N
16
ppm

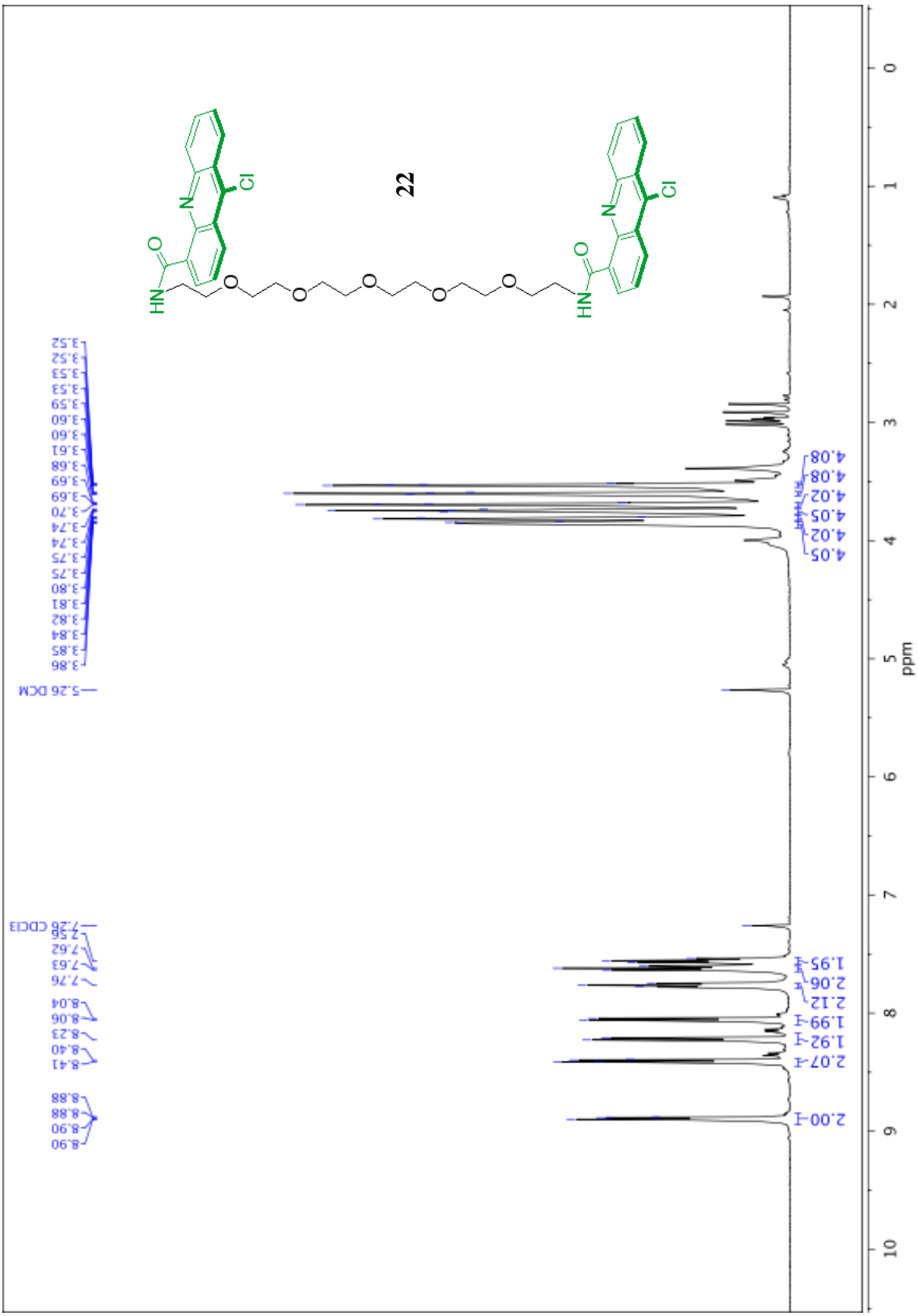

17
Cl
N
O
HN
H3C
ppm
2.07 EtOAc
3.19
3.18
2.96
7.26 CDCl3
7.58
7.59
7.60
7.62
7.76
7.99
8.25
8.27
8.40
8.42
8.89
8.91
11.33
0.62
0.87
0.95
0.90
0.95
0.95
1.00
2.08

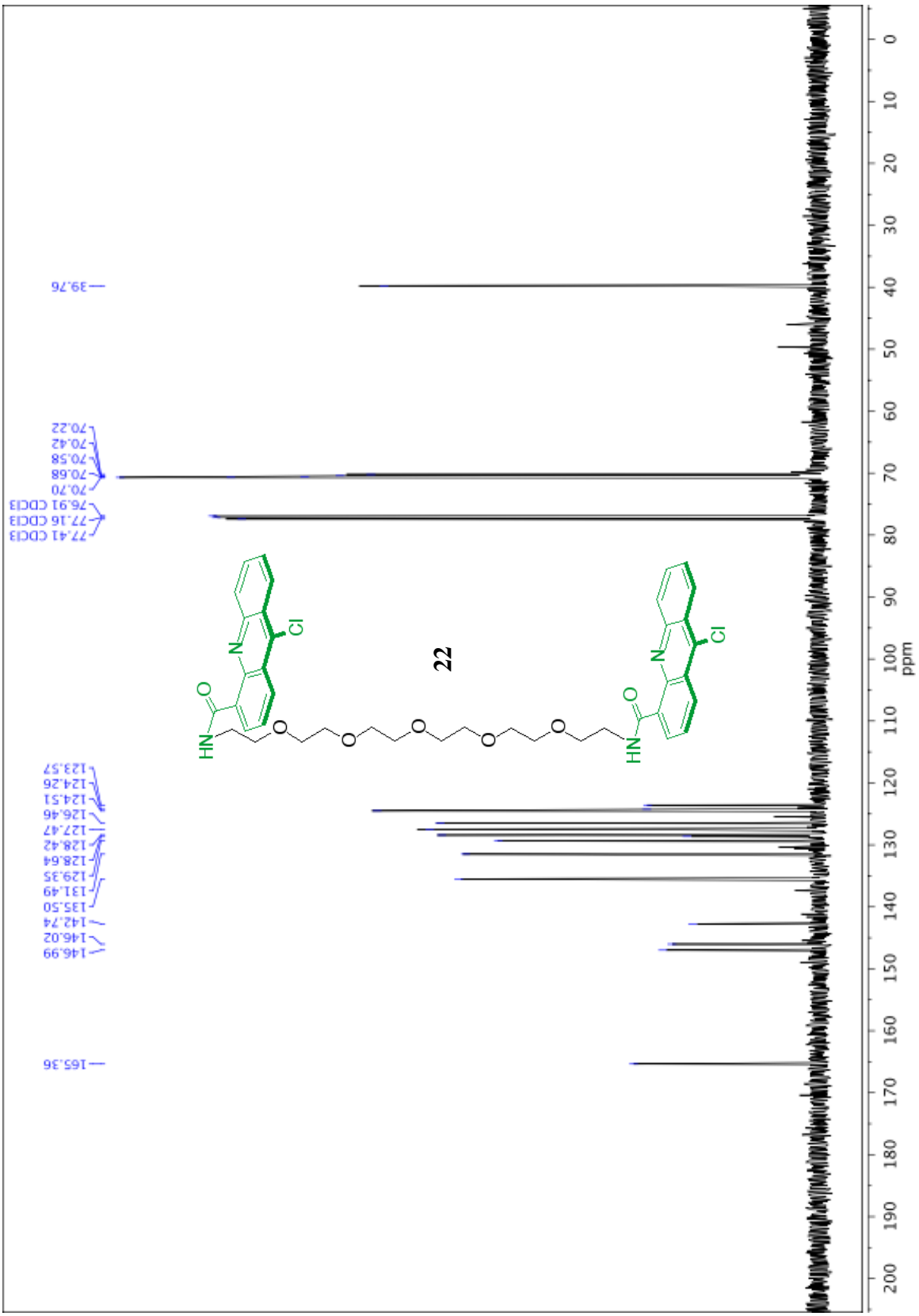

19
Cl
N
NH
O
N–Boc
Boc–N
HN
O
Cl
N
8.00
18.14
4.01
3.46
4.17
4.02
4.18
2.00
1.97
4.16
4.06
1.81
1.32
1.52
1.56
1.81
3.18
3.44
3.53
5.30 DCM
7.26 CDCl3
7.67
7.68
7.70
7.87
8.35
8.45
8.46
9.08
ppm

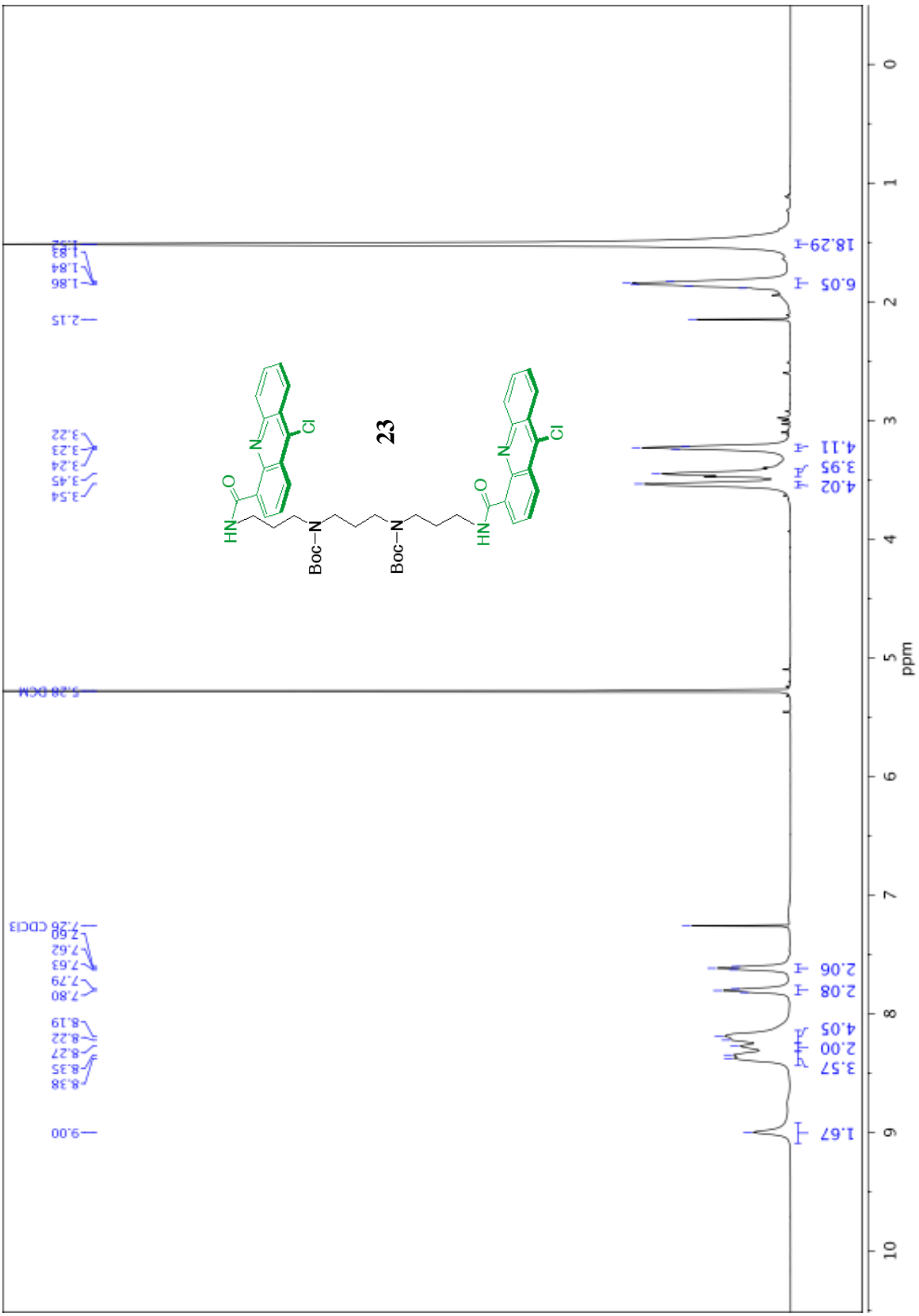

20
Cl
N
O
HN
O
O
O
O
O
Cl
N
O
HN
ppm
ppm
ppm
ppm
5.30 DCM
7.26 CDCl3
1.59 Water

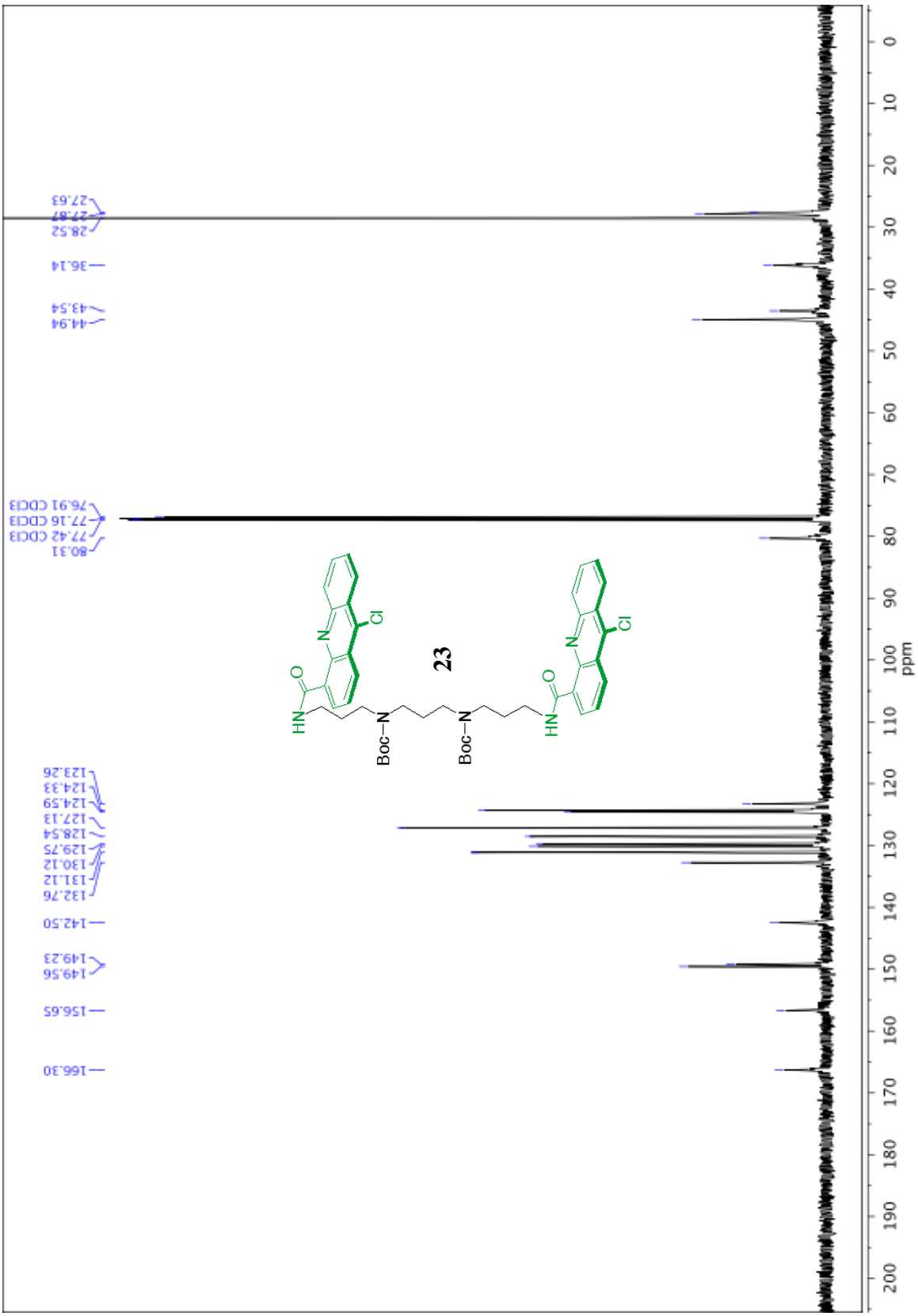

21
Cl
N
NH
O
O
O
NH
O
Cl
N
ppm

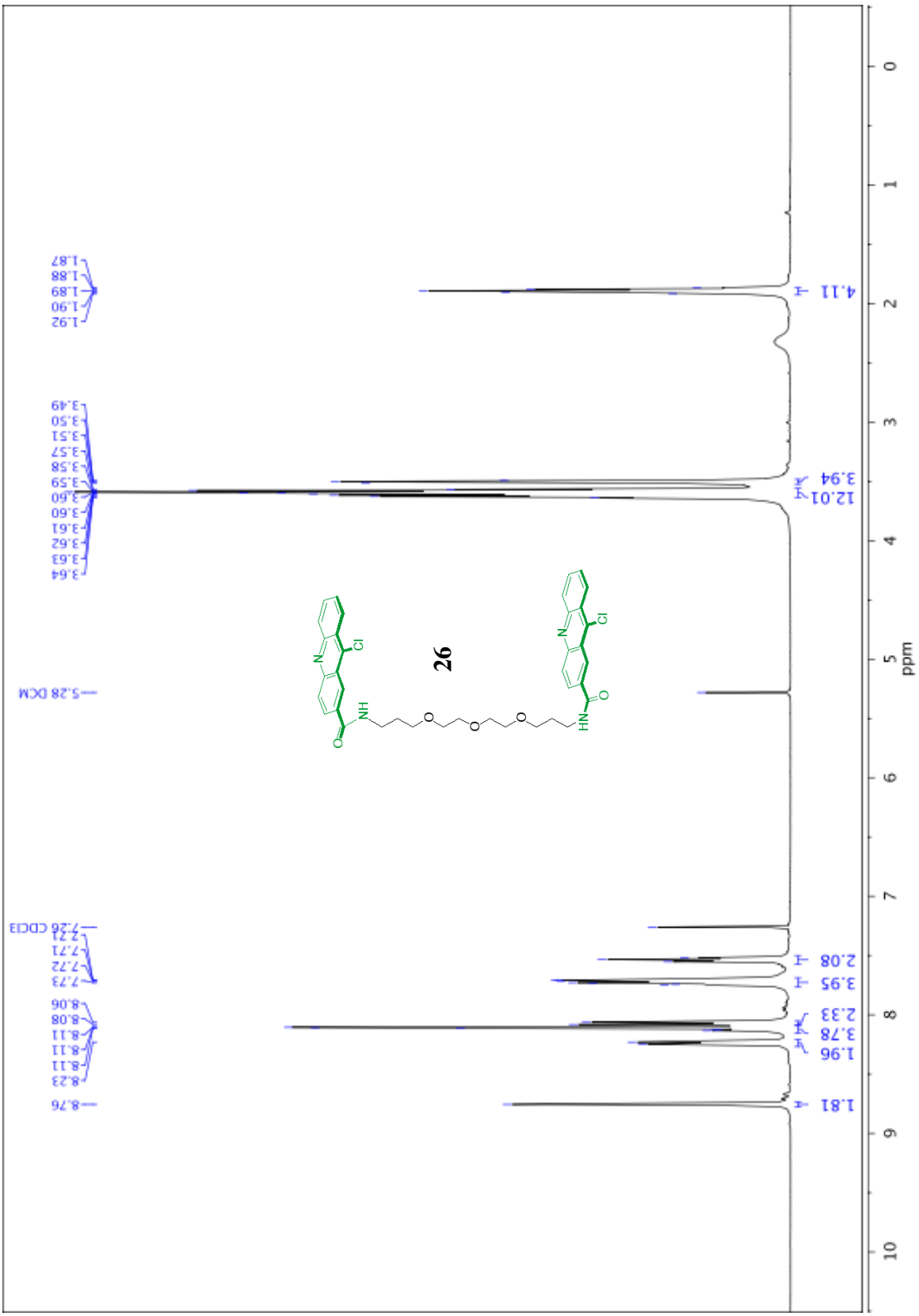

21
Cl
N
NH
O
O
O
HN
O
Cl
N
ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
39.02 DMSO
39.19 DMSO
39.35 DMSO
39.69 DMSO
39.85 DMSO
40.02 DMSO
68.94
69.57
117.27
117.51
119.54
120.72
121.55
125.70
126.03
126.79
132.02
133.70
140.77
142.45
165.62
176.86

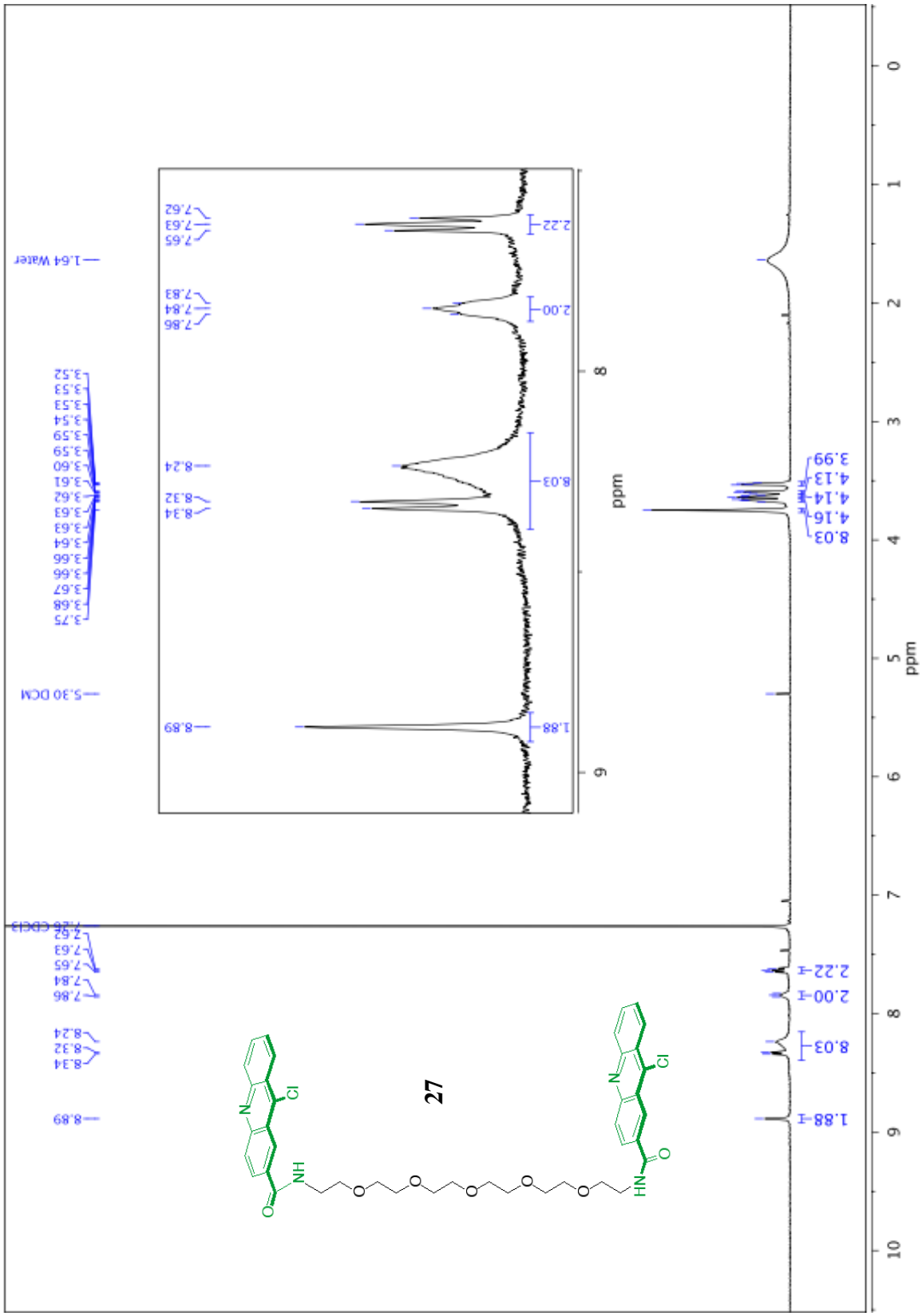

22
Cl
N
O
HN
O
O
O
O
O
Cl
N
O
HN
ppm
0
1
2
3
4
5
6
7
8
9
10
4.08
4.08
4.02
4.05
4.02
4.05
1.95
2.06
2.12
1.99
1.92
2.07
2.00
3.52
3.52
3.53
3.53
3.59
3.60
3.60
3.61
3.68
3.69
3.69
3.70
3.74
3.74
3.75
3.75
3.80
3.81
3.82
3.84
3.85
3.86
5.26 DCM
7.26 CDCl3
7.56
7.62
7.63
7.76
8.04
8.06
8.23
8.40
8.41
8.88
8.88
8.90
8.90

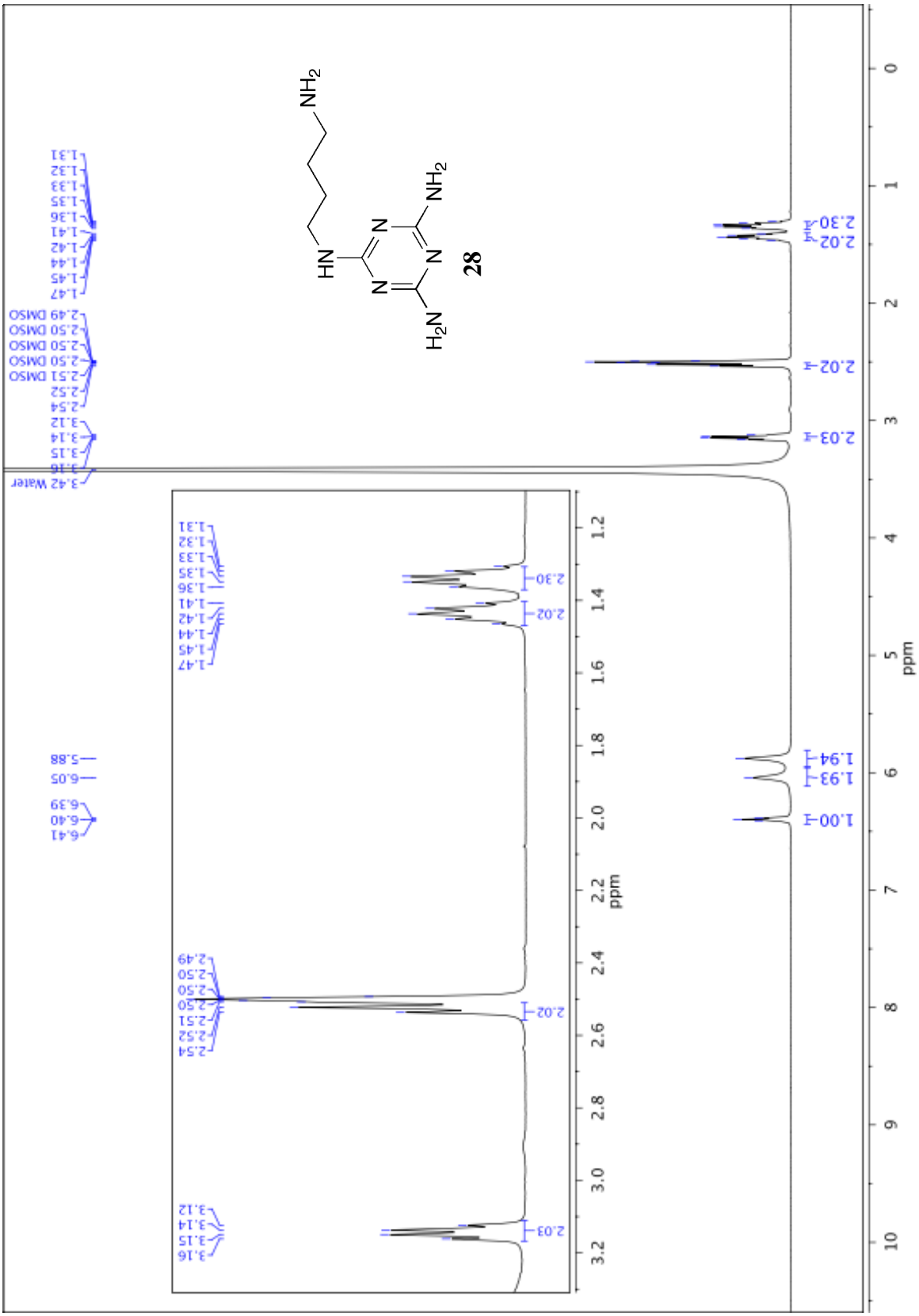

22
Cl
N
O
HN
ppm
39.76
70.70
70.68
70.58
70.42
70.22
76.91 CDCl3
77.16 CDCl3
77.41 CDCl3
123.57
124.26
124.51
126.46
127.47
128.42
128.64
129.35
131.49
135.50
142.74
146.02
146.99
165.36

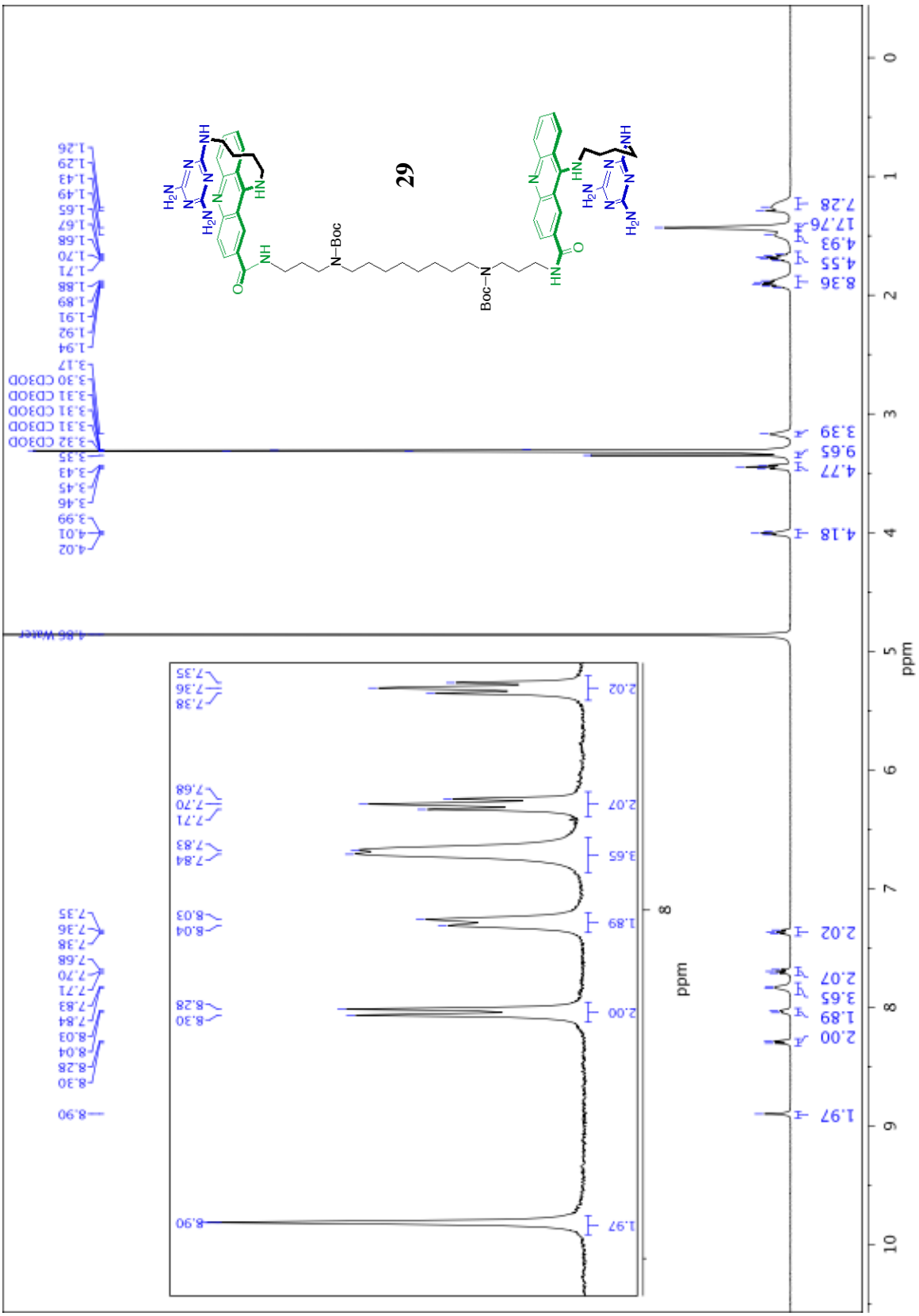

23
Boc—N
Boc—N
HN
O
N
Cl
ppm

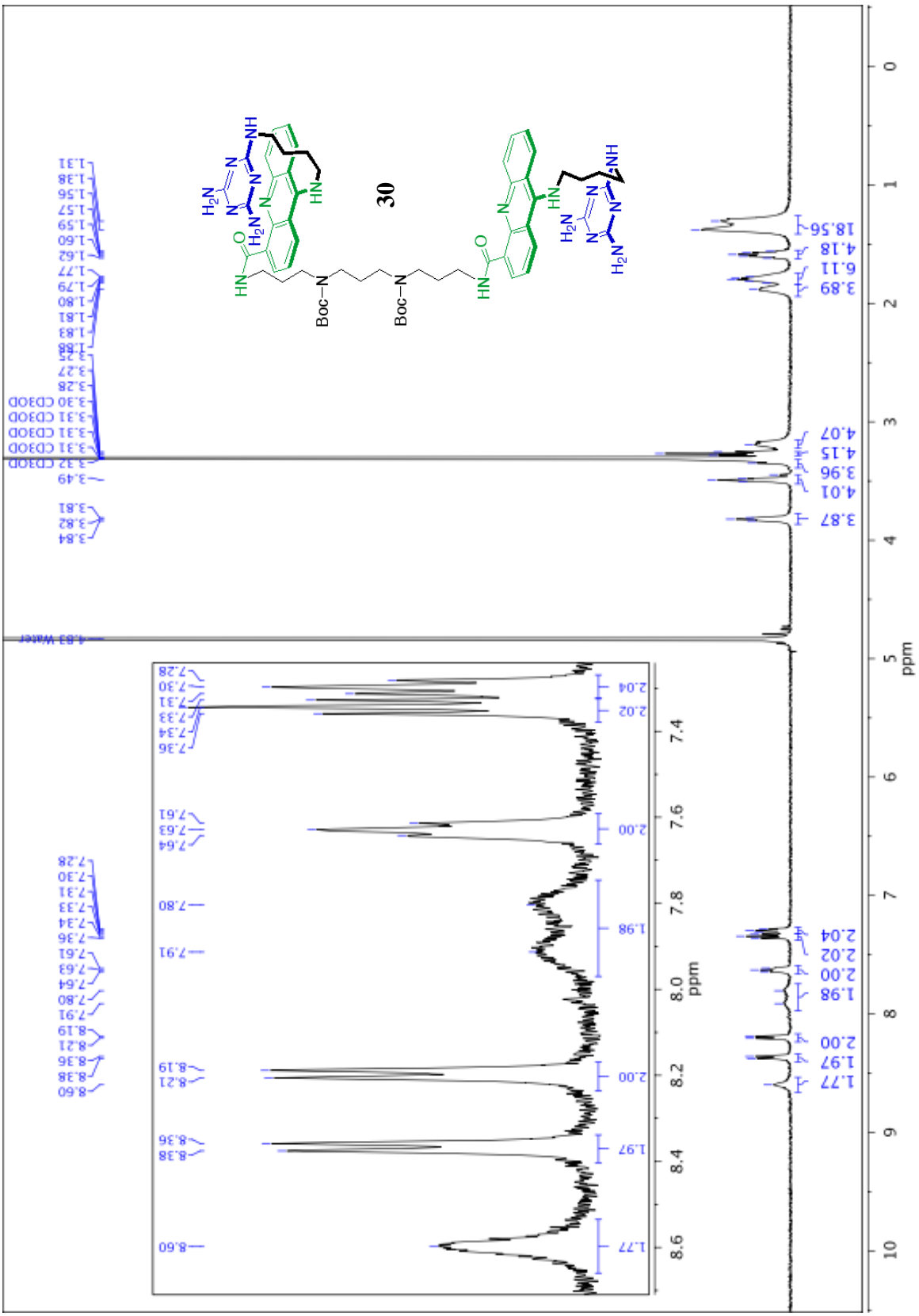

27.63
27.87
28.52
36.14
43.54
44.94
76.91 CDCl3
77.16 CDCl3
77.42 CDCl3
80.31
123.26
124.33
124.59
127.13
128.54
129.75
130.12
131.12
132.76
142.50
149.23
149.56
156.65
166.30
23
Boc—N
Boc—N
HN
HN
O
O
N
N
Cl
Cl
ppm

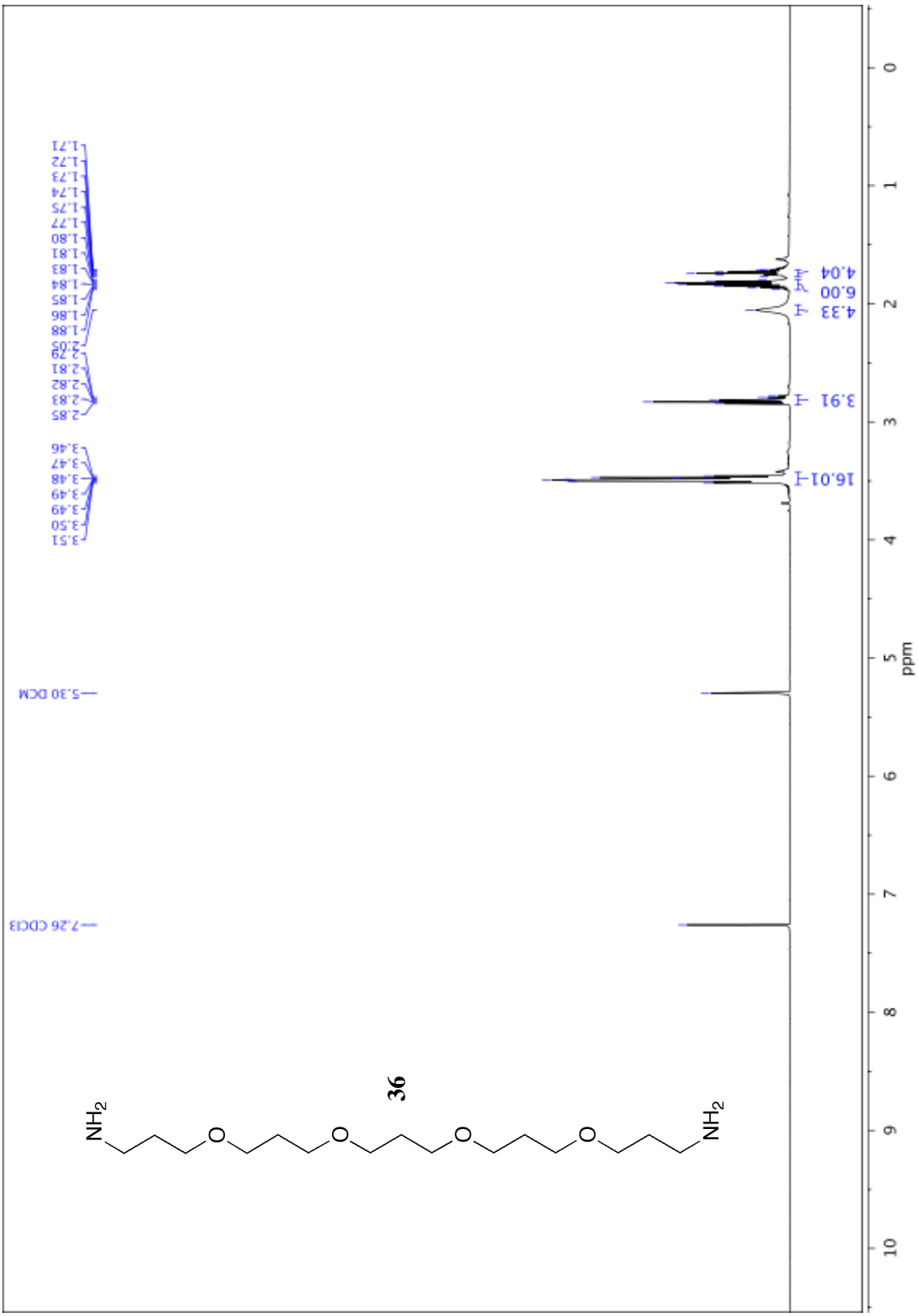

26
Cl
N
NH
O
HN
O
ppm
0
1
2
3
4
5
6
7
8
9
10
4.11
12.01
3.94
2.08
3.95
2.33
3.78
1.96
1.81
1.87
1.88
1.89
1.90
1.92
3.49
3.50
3.51
3.57
3.58
3.59
3.60
3.60
3.61
3.62
3.63
3.64
5.28 DCM
7.26 CDCl3
7.21
7.71
7.72
7.73
8.06
8.08
8.11
8.11
8.11
8.23
8.76

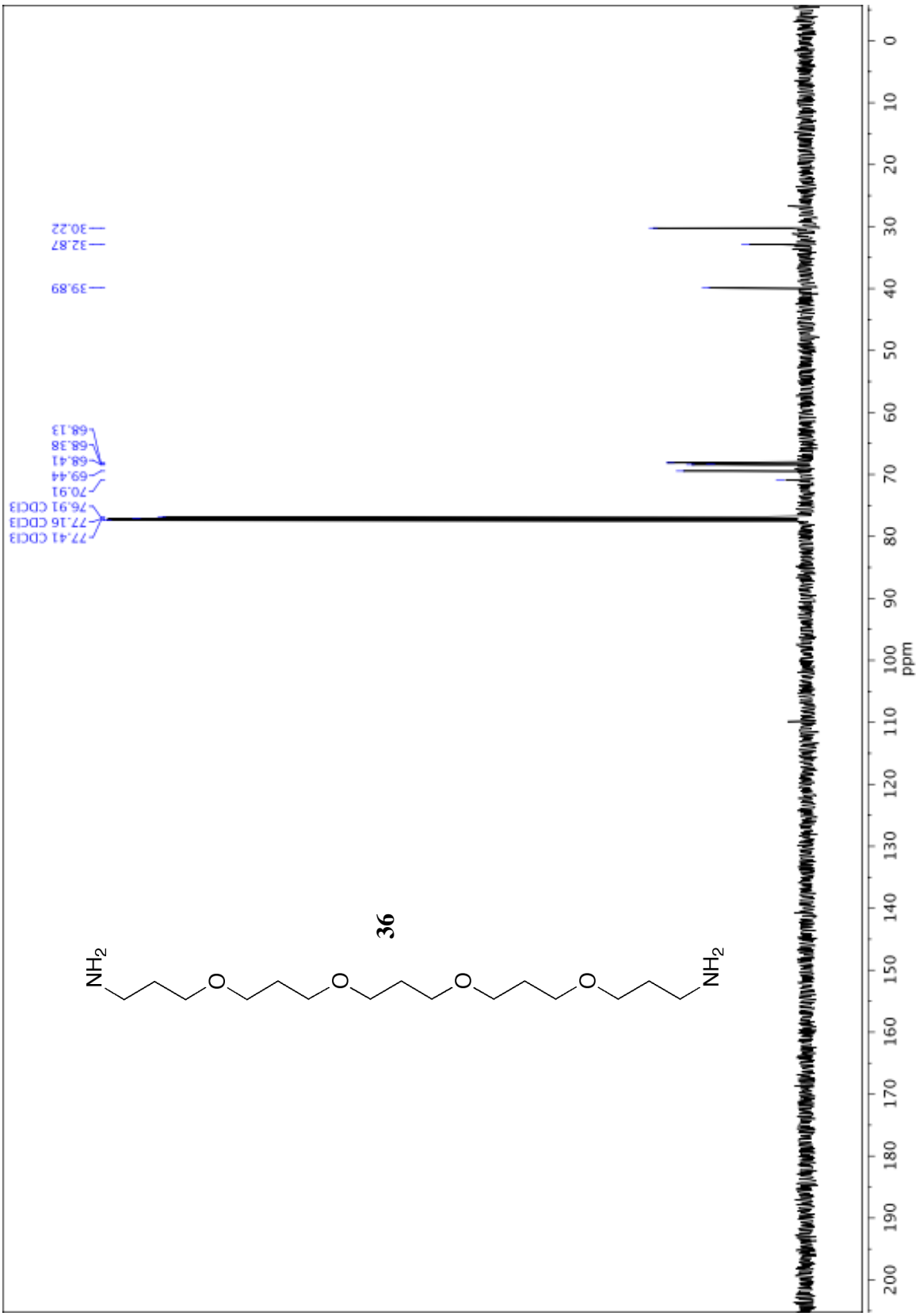

27
ppm

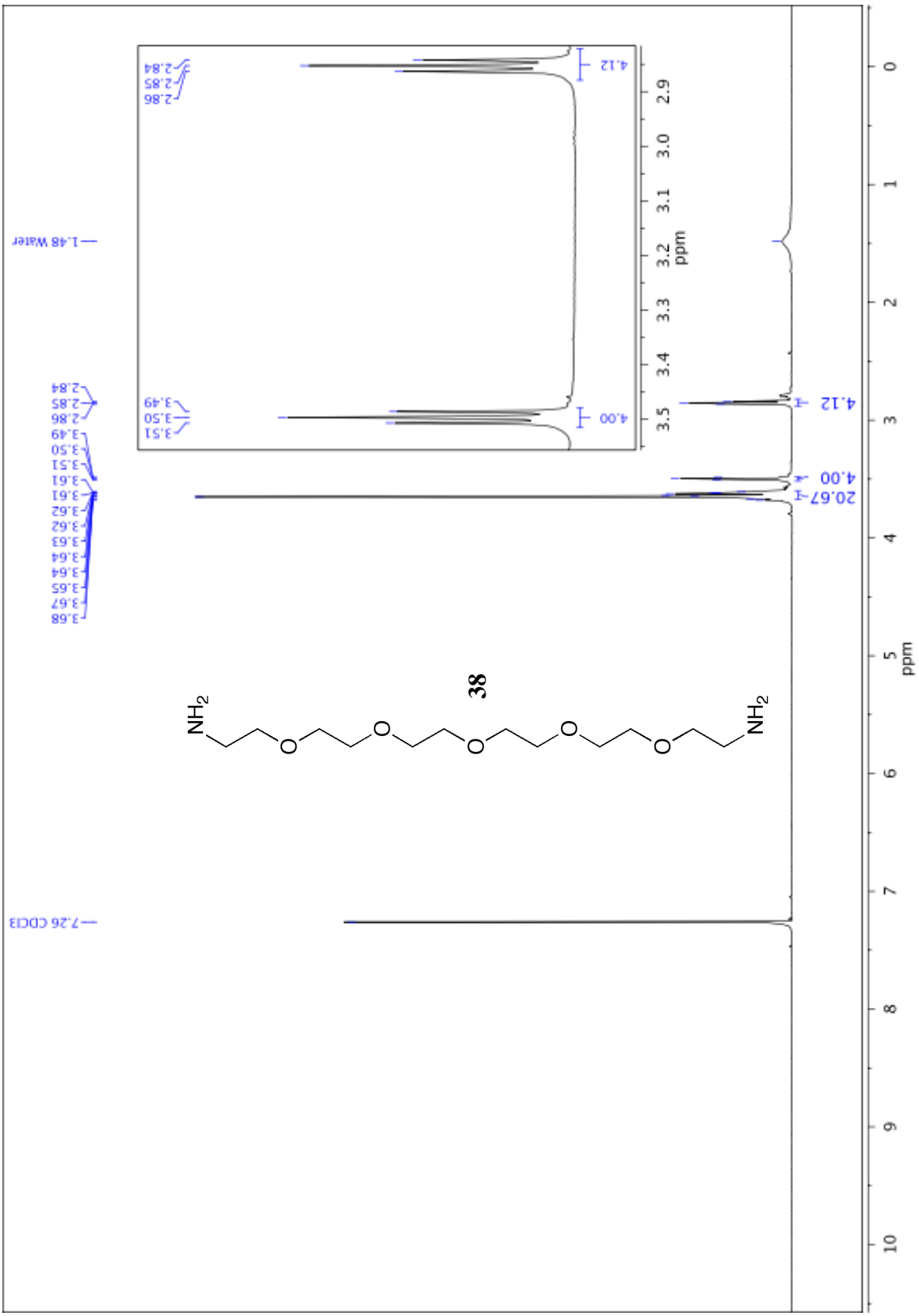

NH2
NH2
HN
N
N
N
H2N
28
ppm

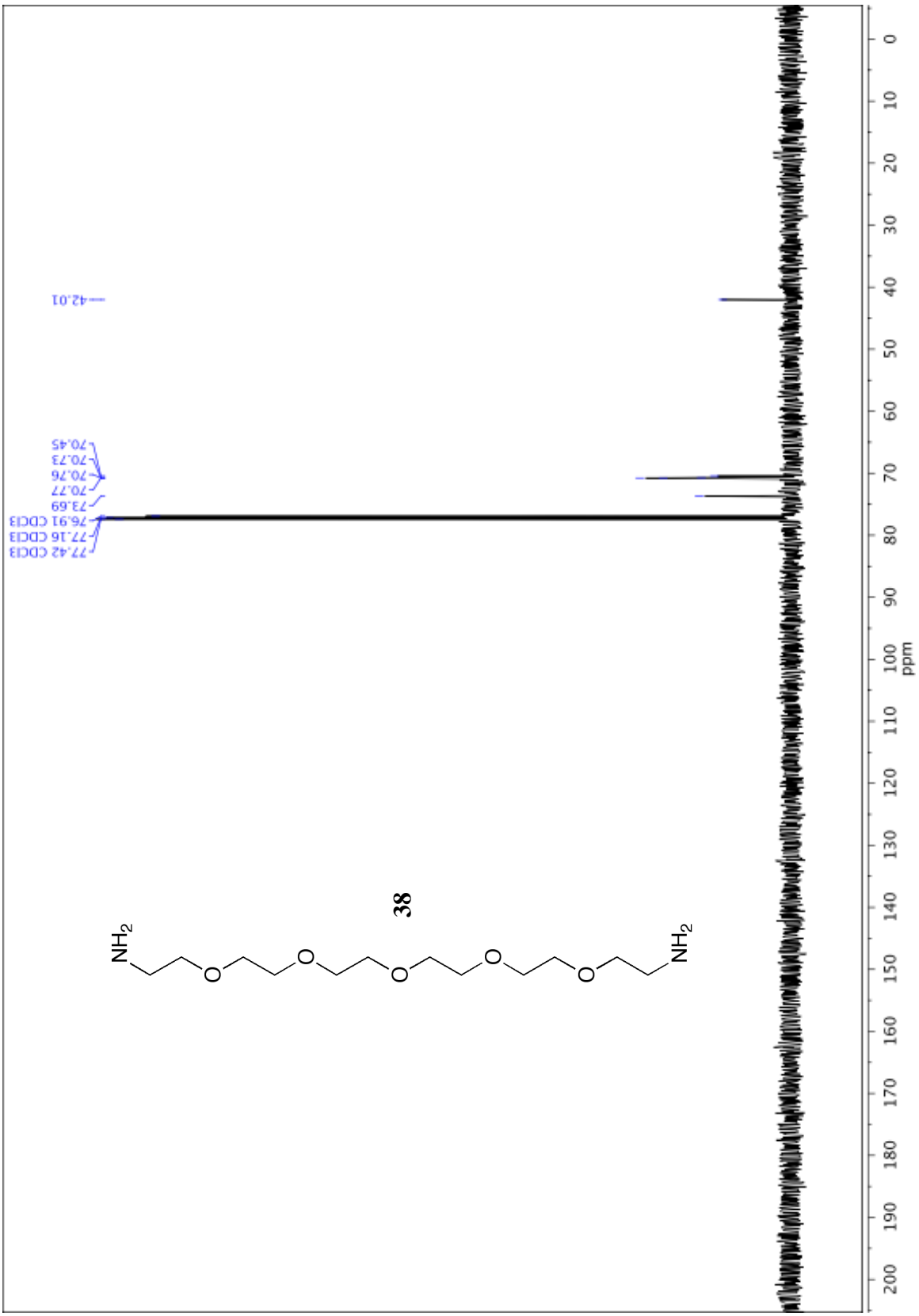

29
N–Boc
Boc–N
ppm

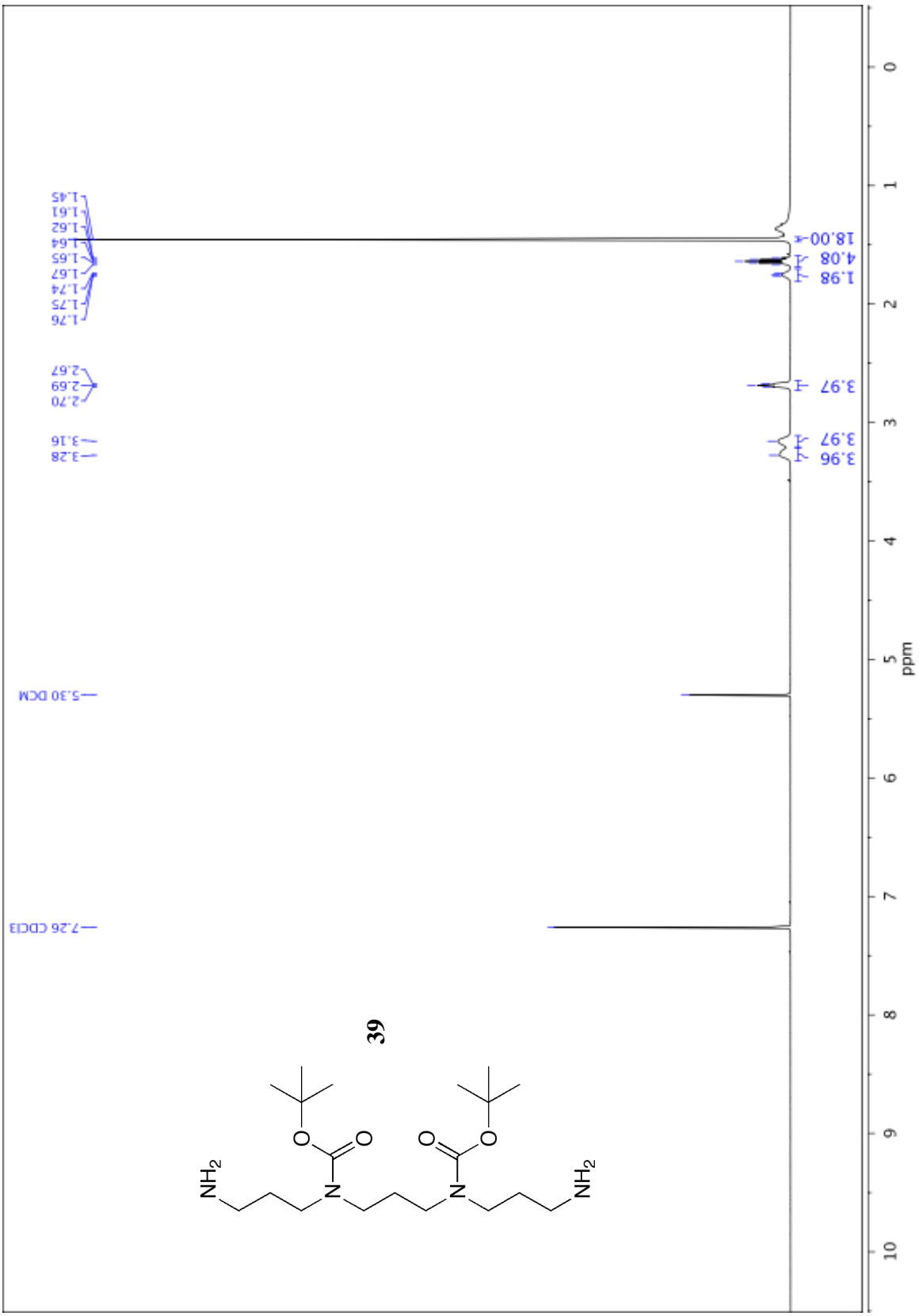

30
Boc–N
Boc–N
ppm


36
NH2
O
O
O
O
NH2
16.01
3.91
4.33
6.00
4.04
7.26 CDCl3
5.30 DCM
ppm

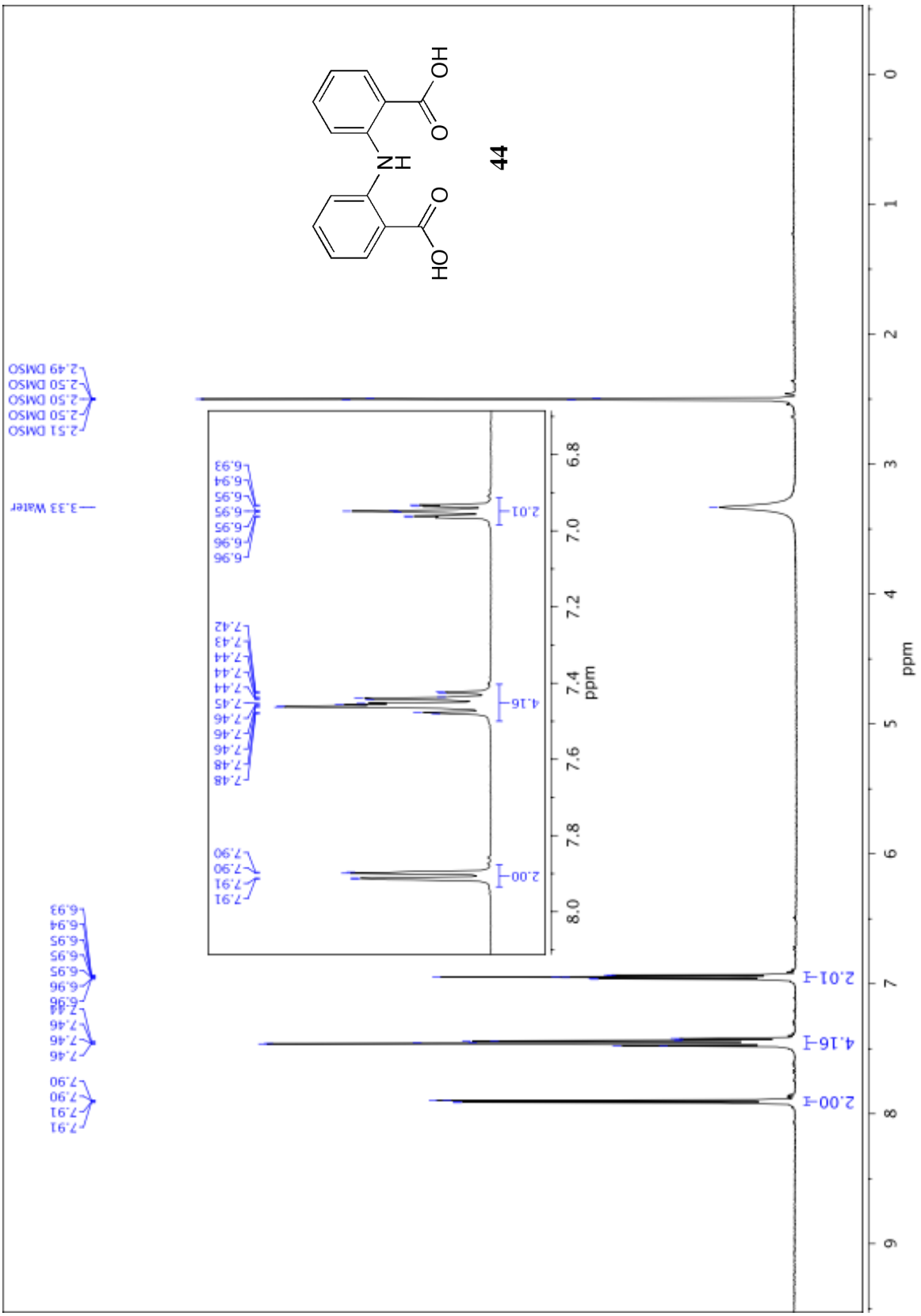

30.22
32.87
39.89
68.13
68.38
68.41
69.44
70.91
76.91 CDCl3
77.16 CDCl3
77.41 CDCl3
ppm
36
NH2
NH2
O
O
O
O

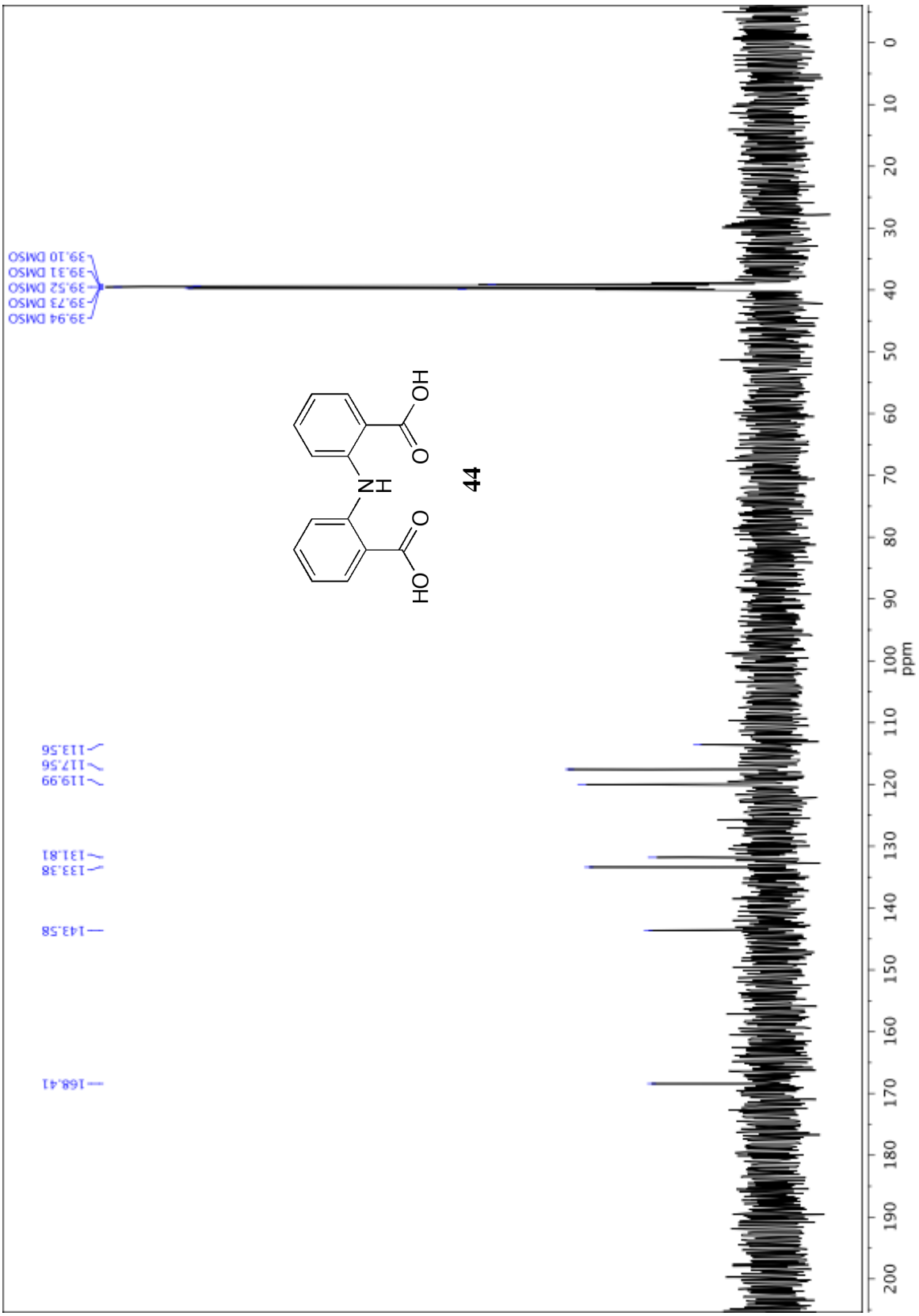

38
NH2
O
O
O
O
NH2
4.12
4.00
20.67
1.48 Water
7.26 CDCl3
ppm

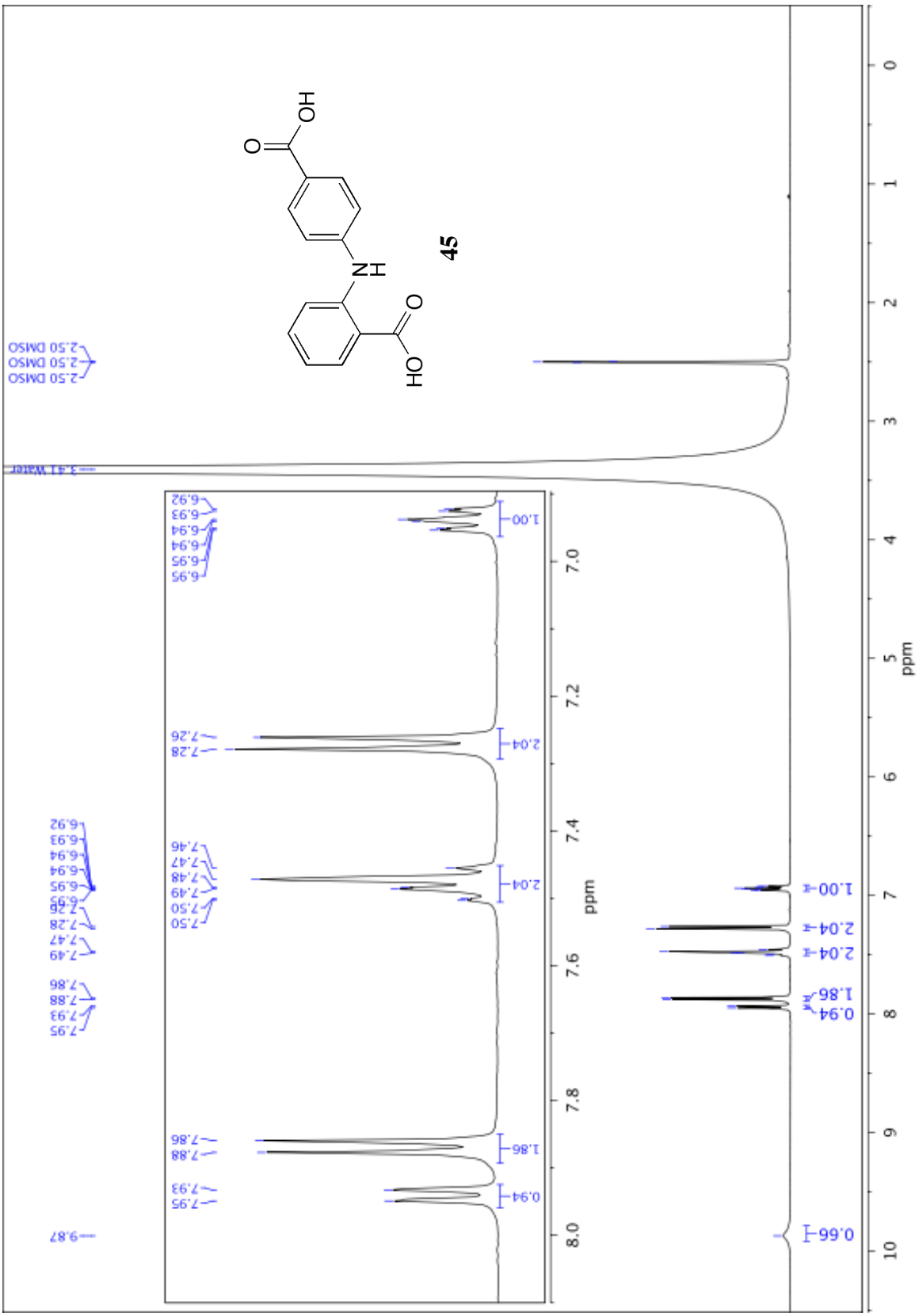

42.01
70.45
70.73
70.76
70.77
73.69
76.91 CDCl3
77.16 CDCl3
77.42 CDCl3
38
NH2
O
O
O
O
NH2
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
ppm

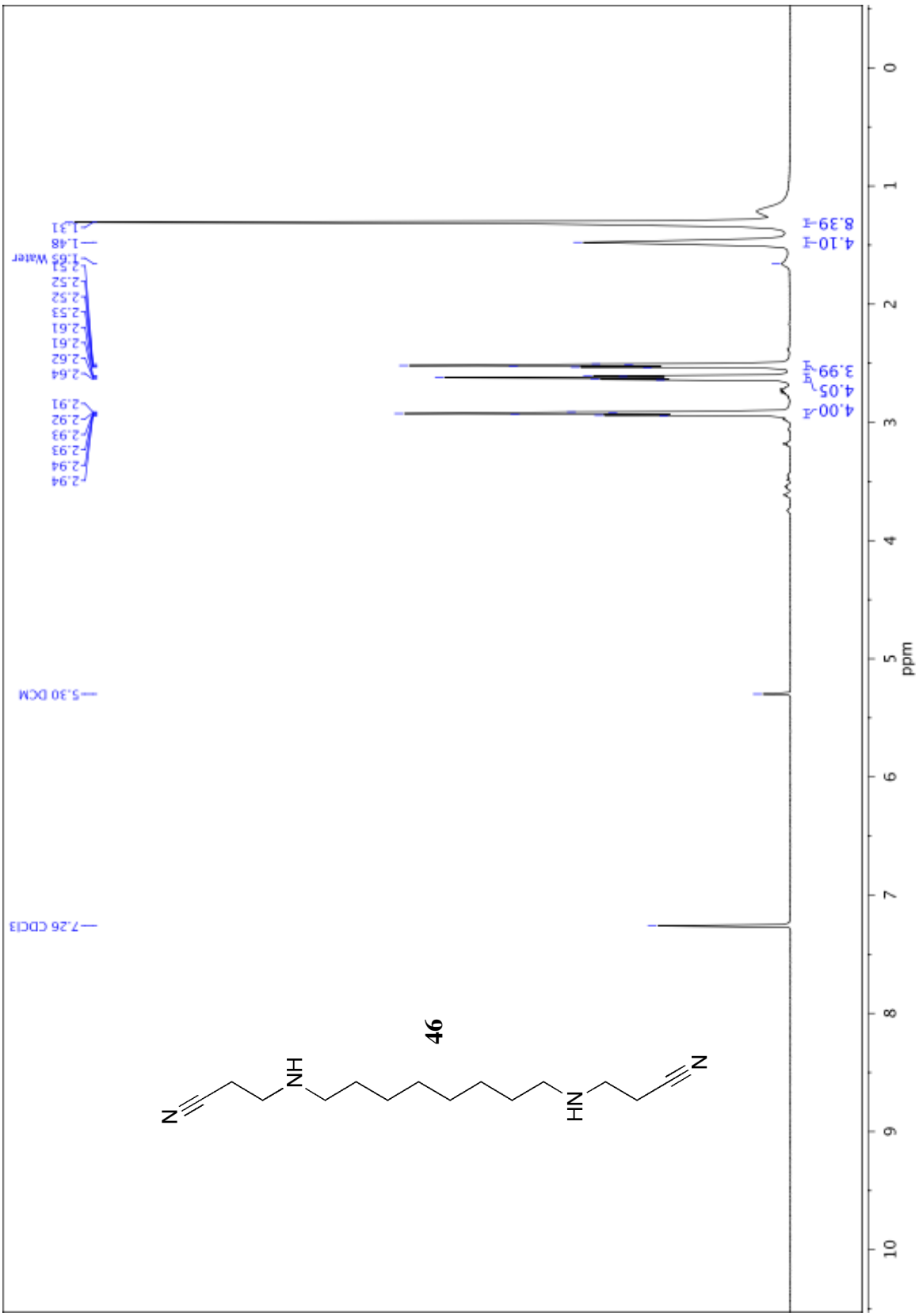

1.45
1.61
1.62
1.64
1.65
1.67
1.74
1.75
1.76
2.67
2.69
2.70
3.16
3.28
5.30 DCM
7.26 CDCl3
18.00
4.08
1.98
3.97
3.97
3.96
ppm
0
1
2
3
4
5
6
7
8
9
10

39

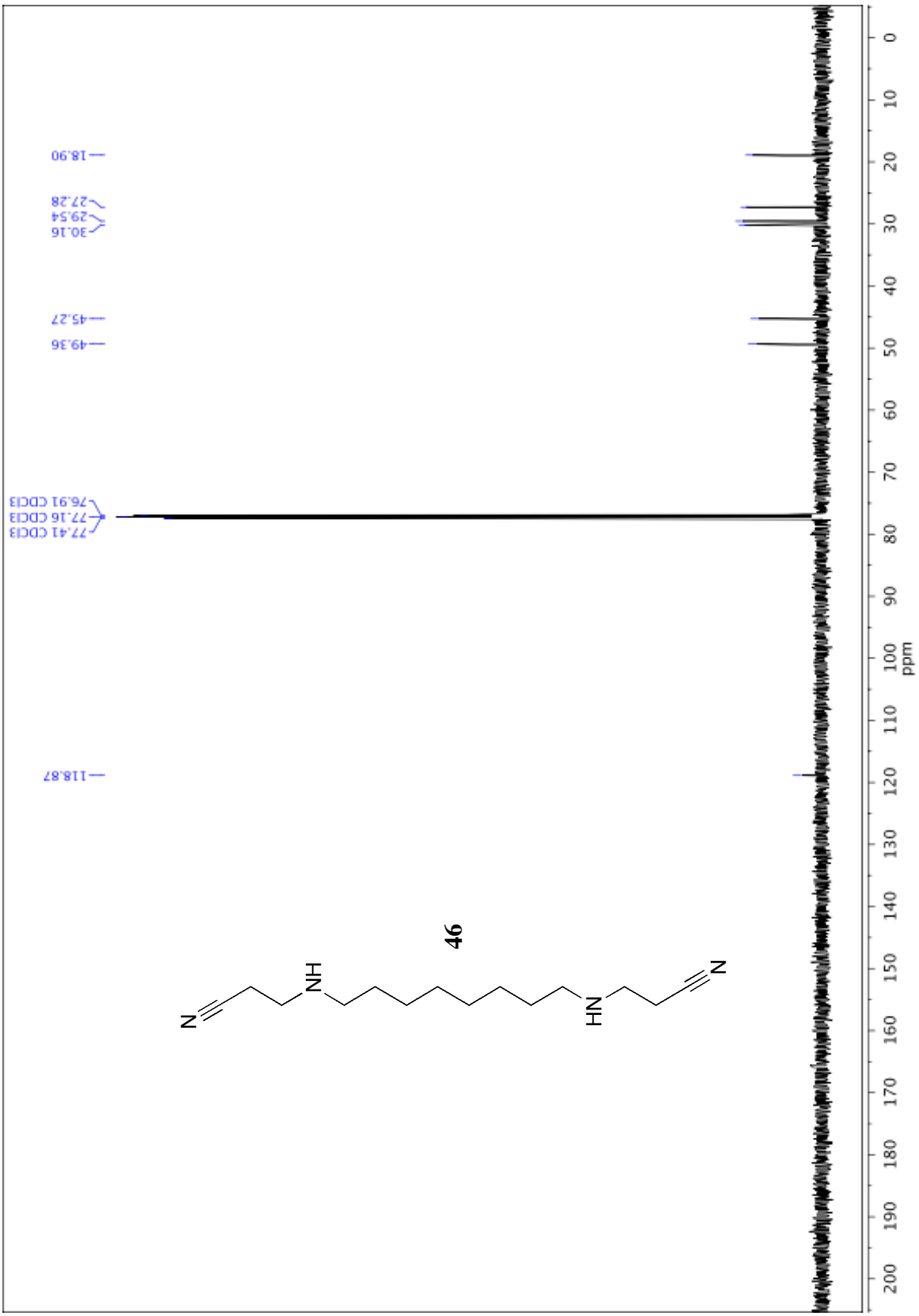

44
HO
O
N
H
O
HO
ppm
2.01
4.16
2.00
3.33 Water
2.49 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.51 DMSO


39.10 DMSO
39.31 DMSO
39.52 DMSO
39.73 DMSO
39.94 DMSO
113.56
117.56
119.99
131.81
133.38
143.58
168.41
44
HO
O
NH
O
HO
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
ppm

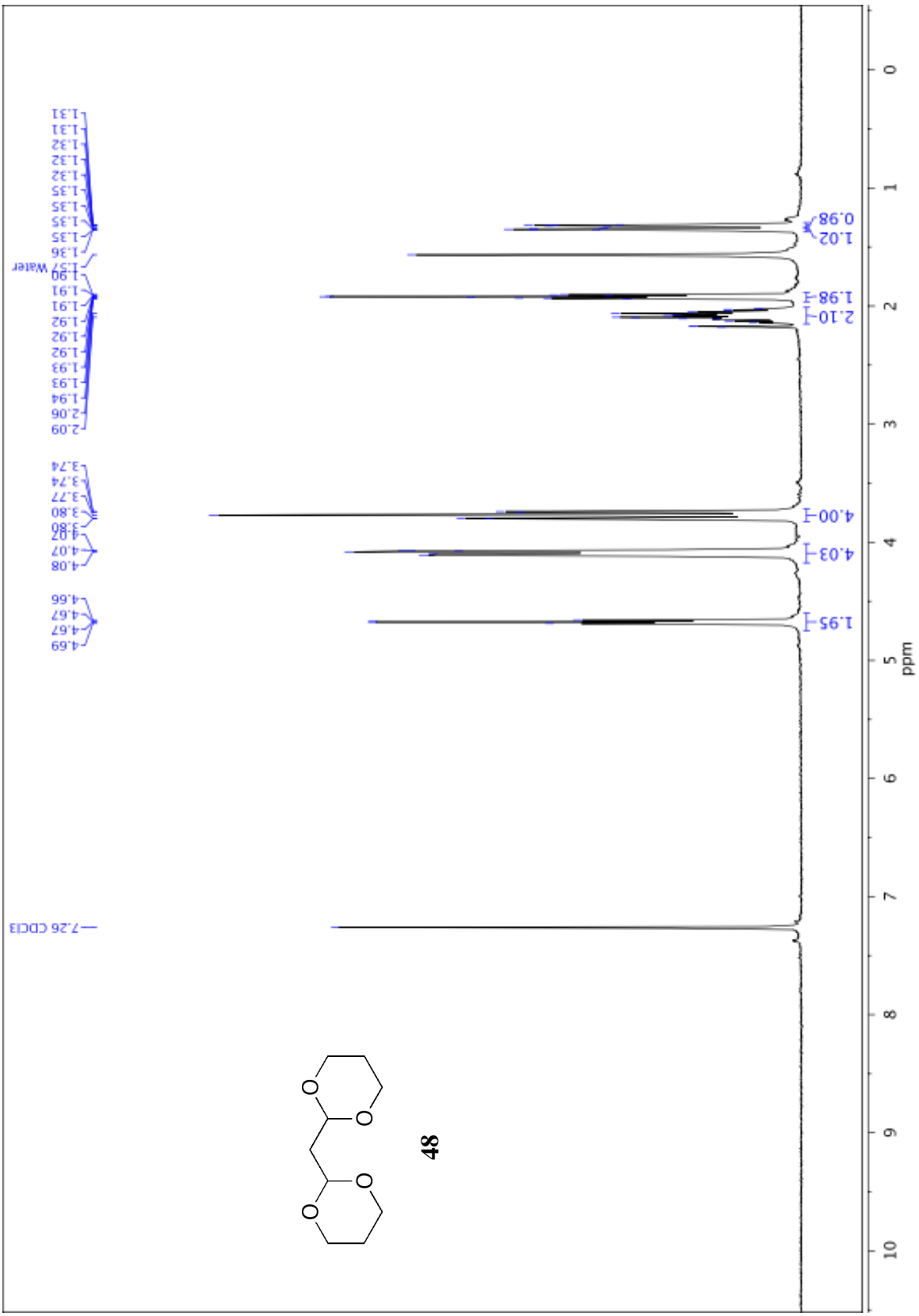

45
OH
O
NH
O
HO
2.50 DMSO
3.41 Water
9.87
7.95
7.93
7.88
7.86
7.50
7.49
7.48
7.47
7.46
7.28
7.26
6.95
6.94
6.93
6.92
1.00
2.04
2.04
1.86
0.94
0.66
ppm

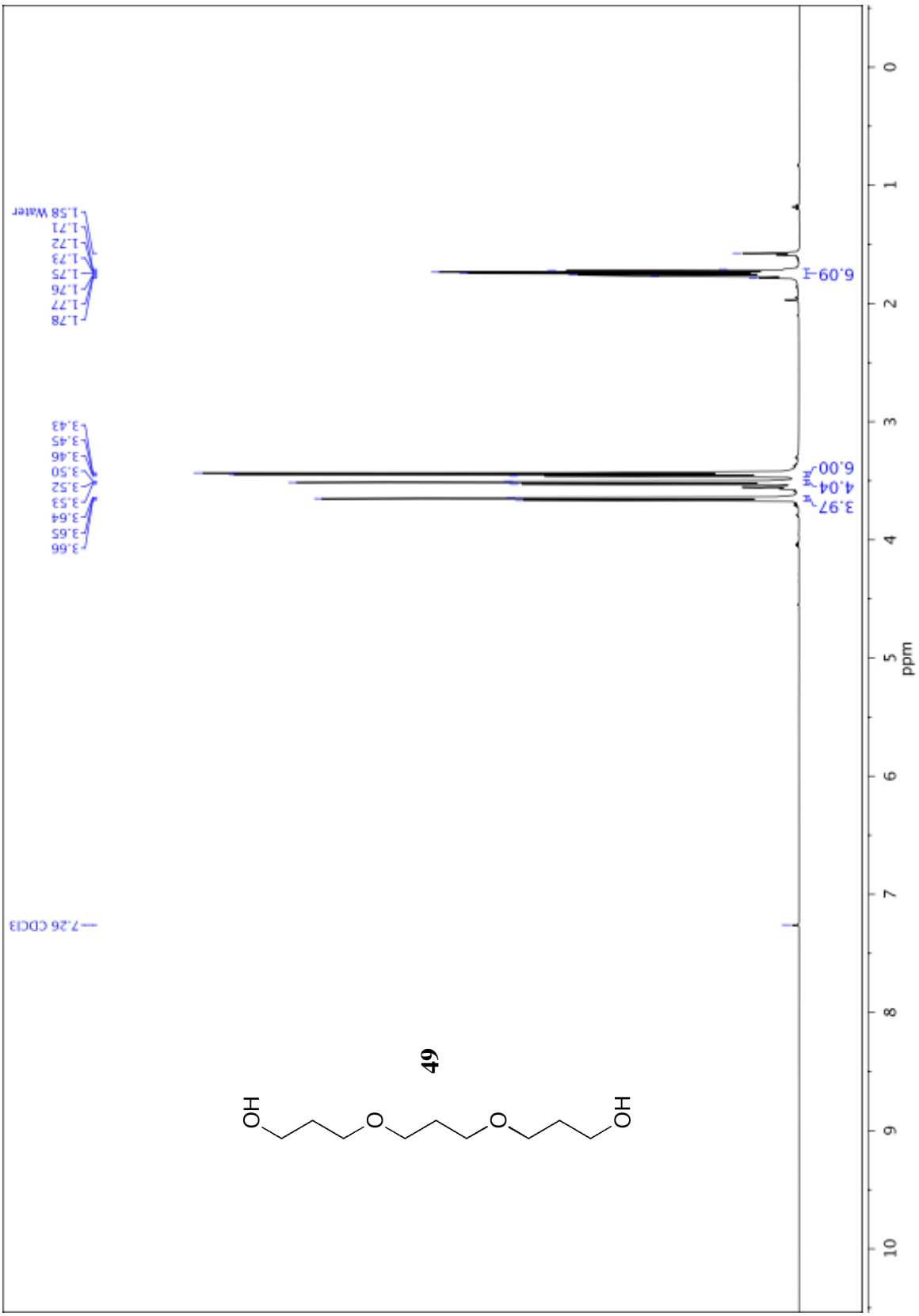

8.39
4.10
3.99
4.05
4.00
2.91
2.92
2.93
2.93
2.94
2.94
2.64
2.62
2.61
2.61
2.53
2.52
2.52
2.51
1.65 Water
1.48
1.31
5.30 DCM
7.26 CDCl3
ppm
0
1
2
3
4
5
6
7
8
9
10

46


18.90
27.28
29.54
30.16
45.27
49.36
76.91 CDCl3
77.16 CDCl3
77.41 CDCl3
118.87
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
ppm

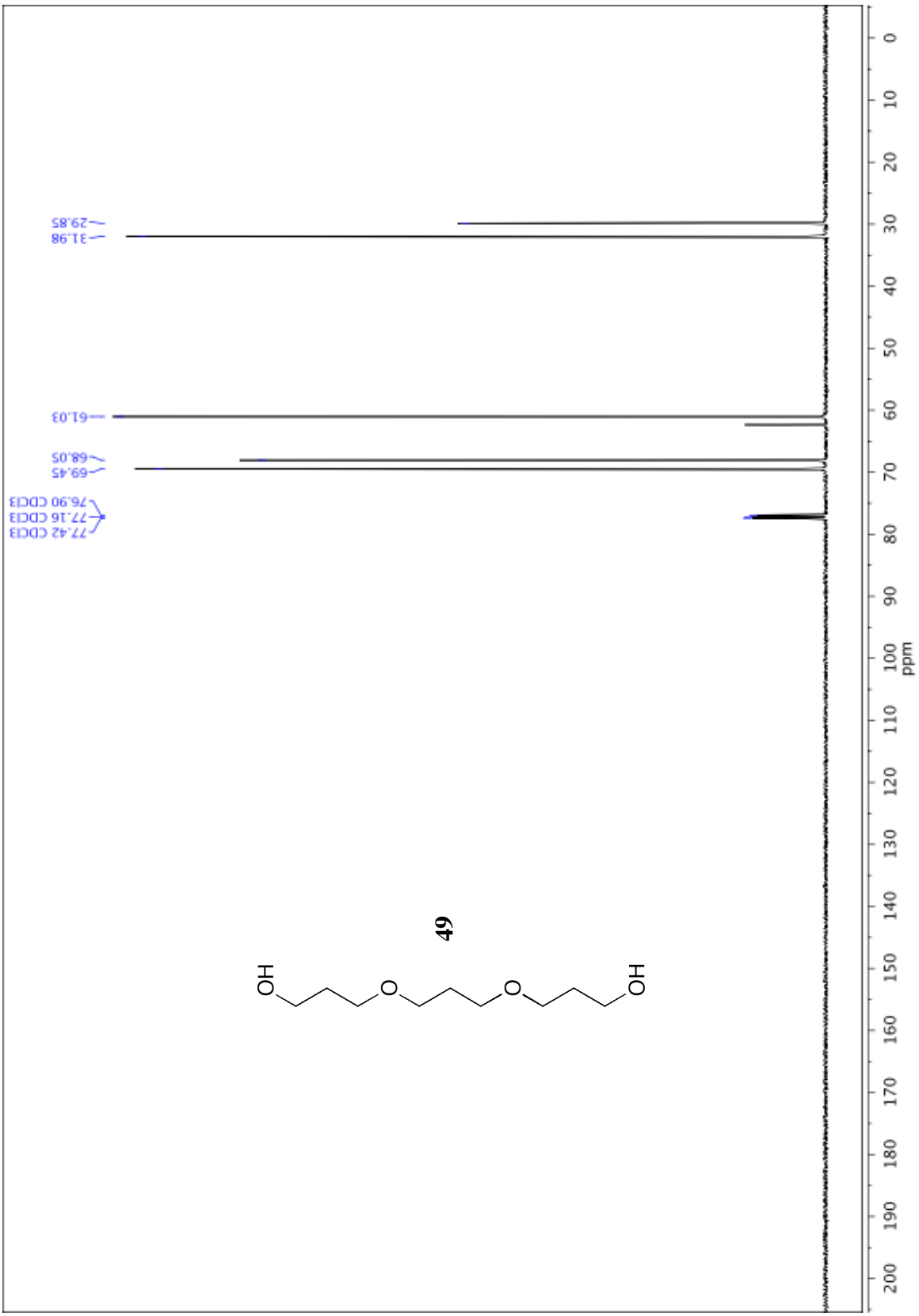
46

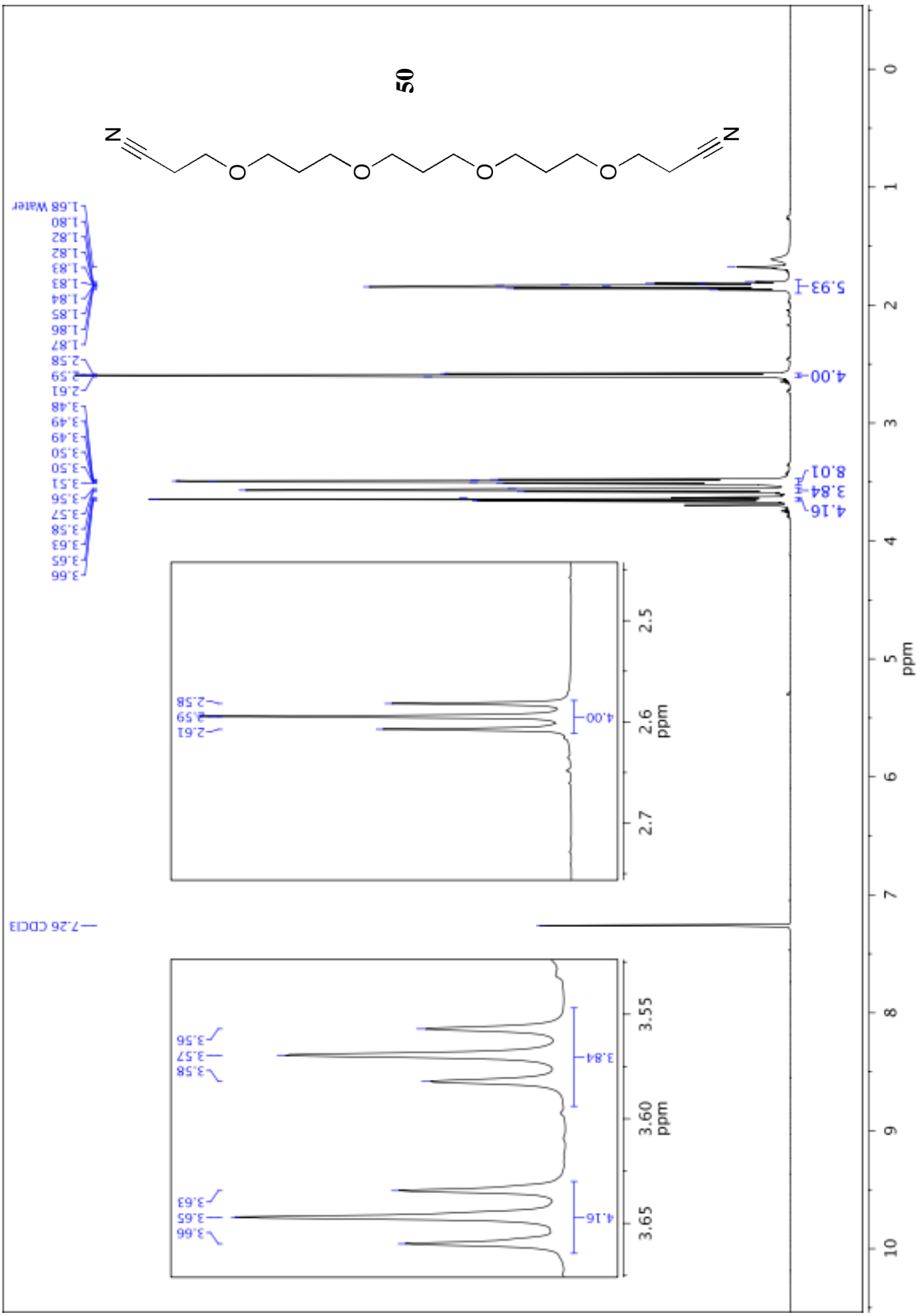

48
ppm
0.98
1.02
1.98
2.10
4.00
4.03
1.95
Water
7.26 CDCl3

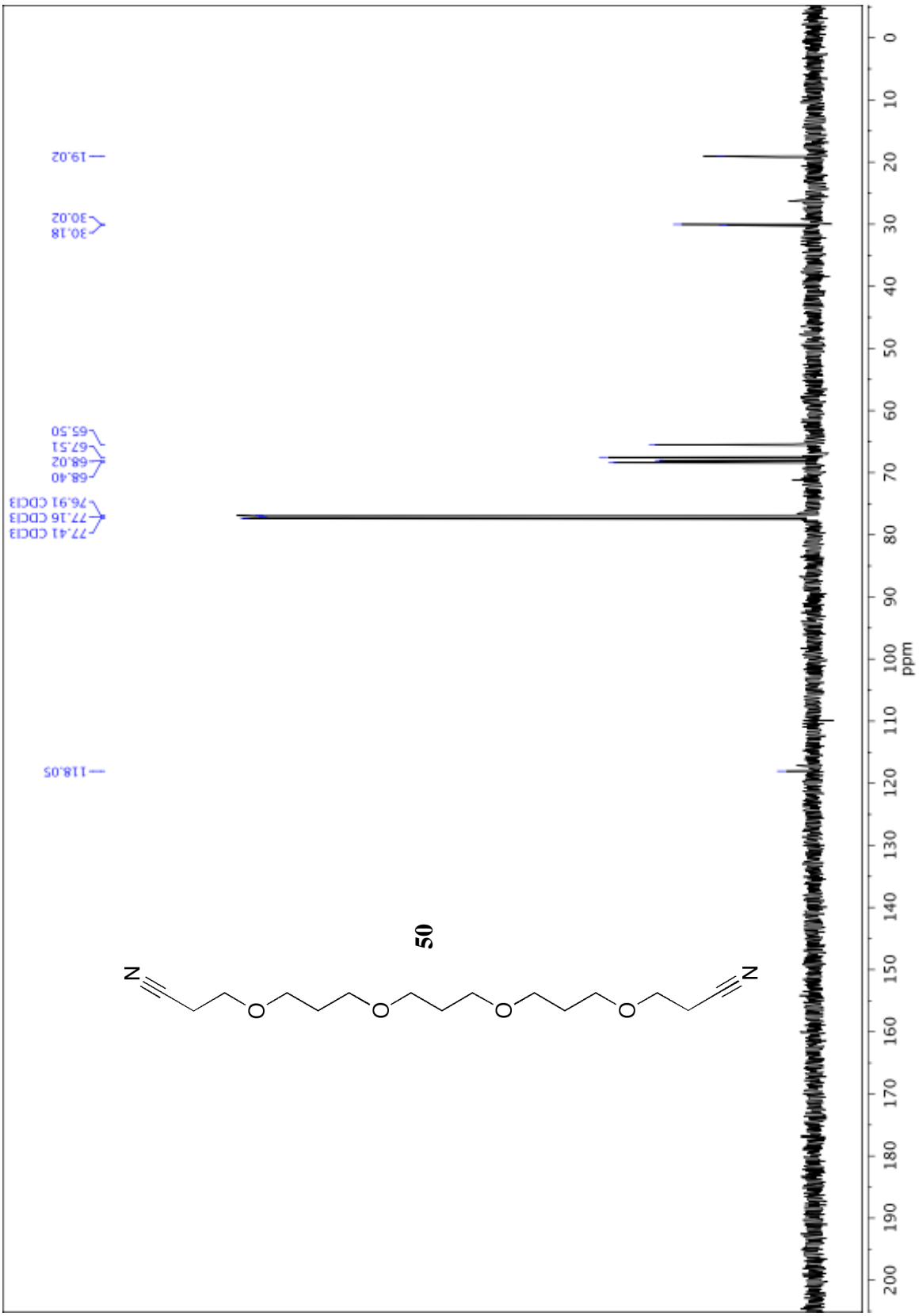

1.58 Water
1.71
1.72
1.73
1.75
1.76
1.77
1.78
3.43
3.45
3.46
3.50
3.52
3.53
3.64
3.65
3.66
6.09
6.00
4.04
3.97
7.26 CDCl3
ppm
0
1
2
3
4
5
6
7
8
9
10

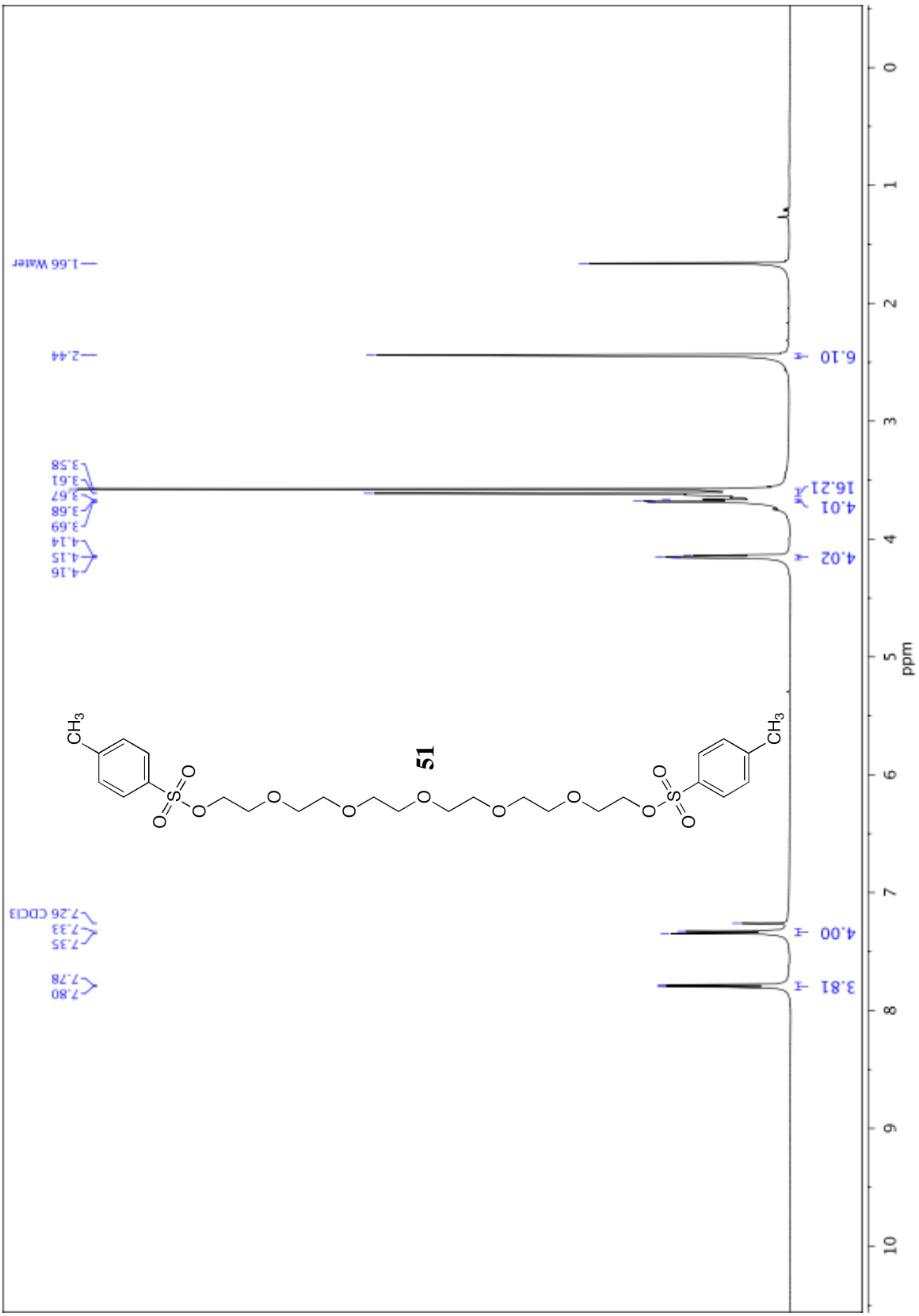
49

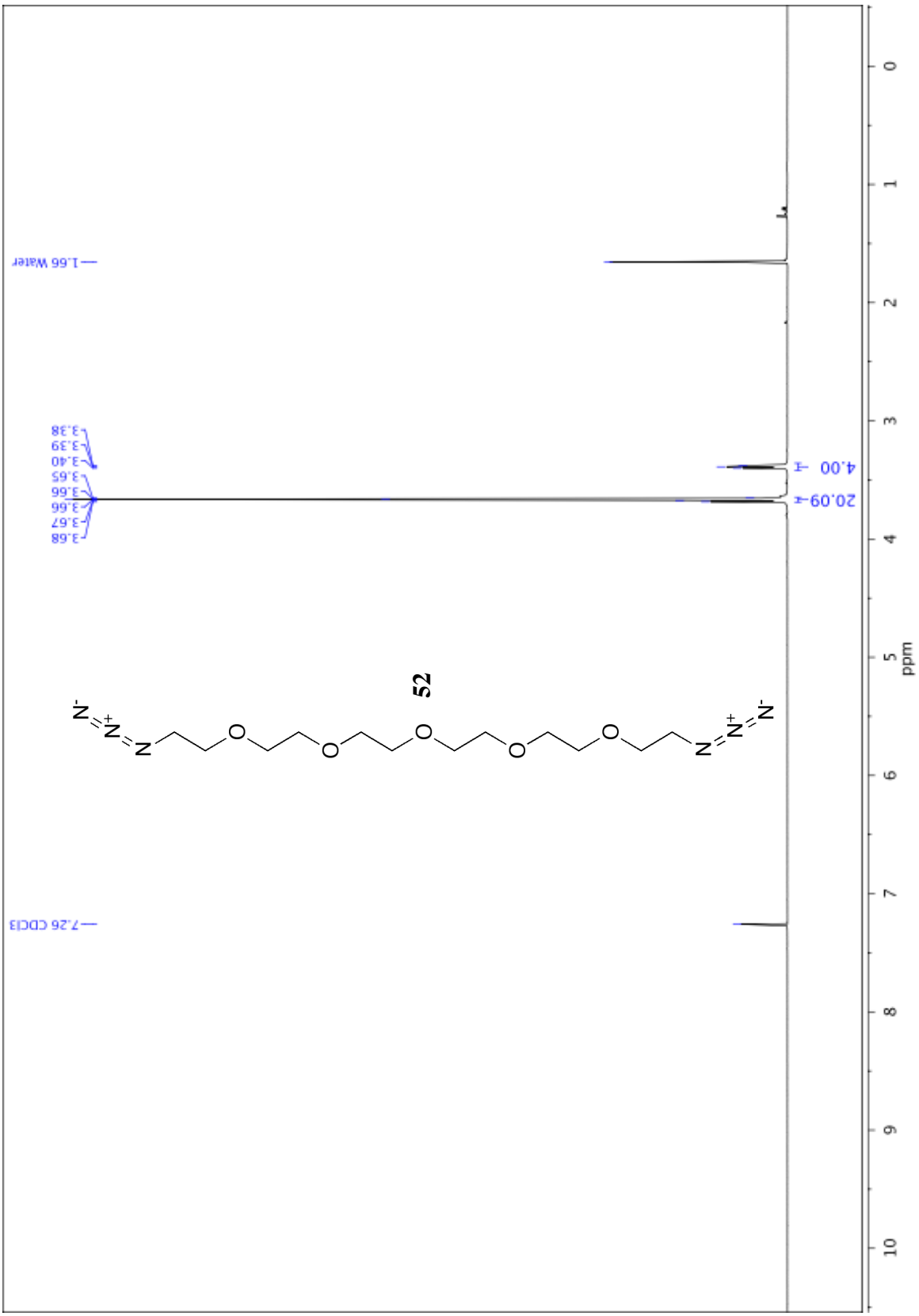
HO
O
O
OH


29.85
31.98
61.03
68.05
69.45
76.90 CDCl3
77.16 CDCl3
77.42 CDCl3
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
ppm
49
HO
O
O
OH

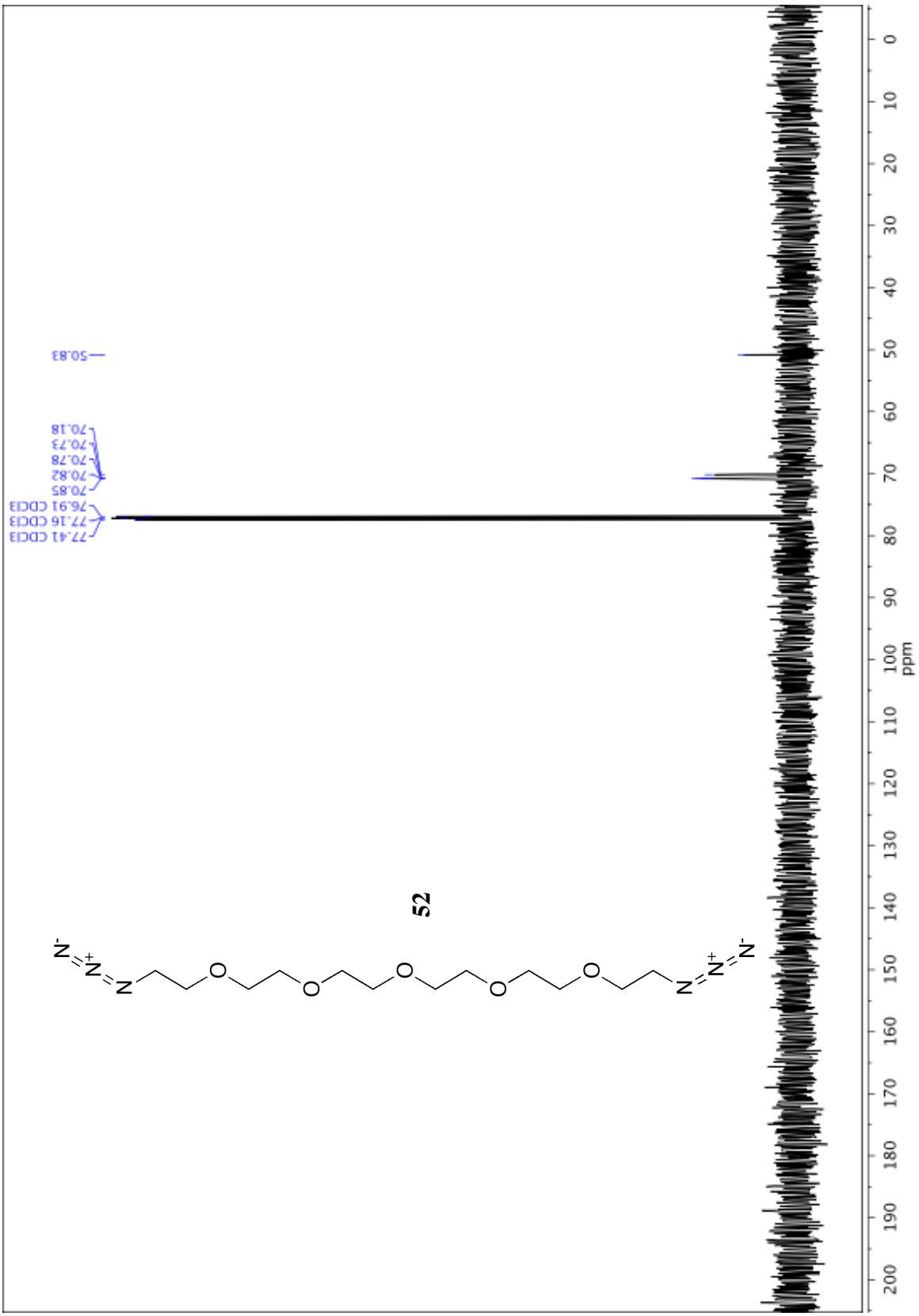

50
N
O
O
O
O
N
1.58 Water
1.80
1.82
1.82
1.83
1.83
1.84
1.85
1.86
1.87
2.58
2.59
2.61
3.48
3.49
3.49
3.50
3.50
3.51
3.56
3.57
3.58
3.63
3.65
3.66
7.26 CDCl3
5.93
4.00
8.01
3.84
4.16
2.5
2.6
2.7
ppm
3.55
3.60
3.65
ppm
0
1
2
3
4
5
6
7
8
9
10
ppm


19.02
30.18
30.02
65.50
67.51
68.02
68.40
76.91 CDCl3
77.16 CDCl3
77.41 CDCl3
118.05
ppm
50

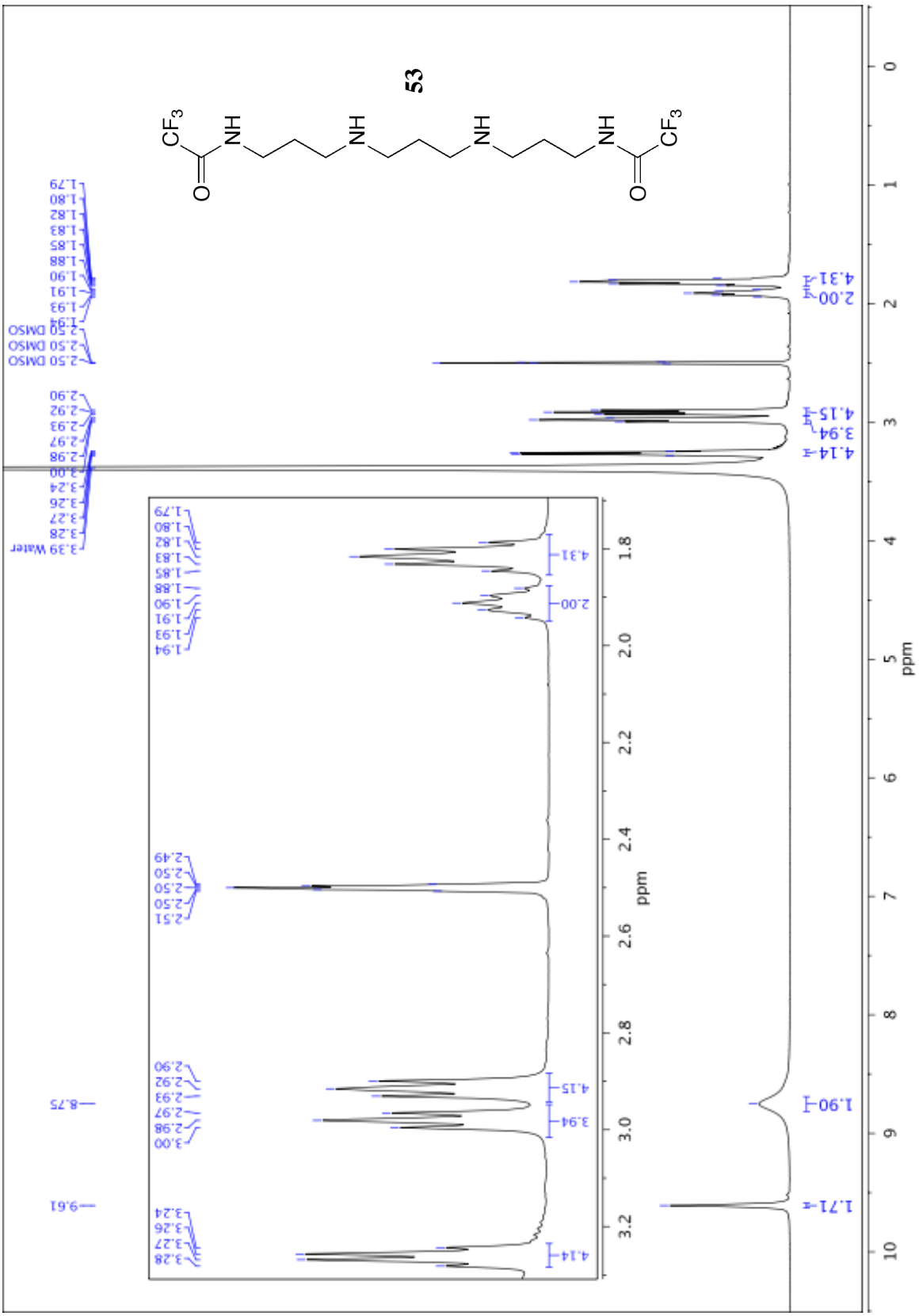

1.66 Water
2.44
3.58
3.61
3.67
3.68
3.69
4.14
4.15
4.16
7.26 CDCl3
7.33
7.35
7.78
7.80
6.10
16.21
4.01
4.02
4.00
3.81
ppm
51

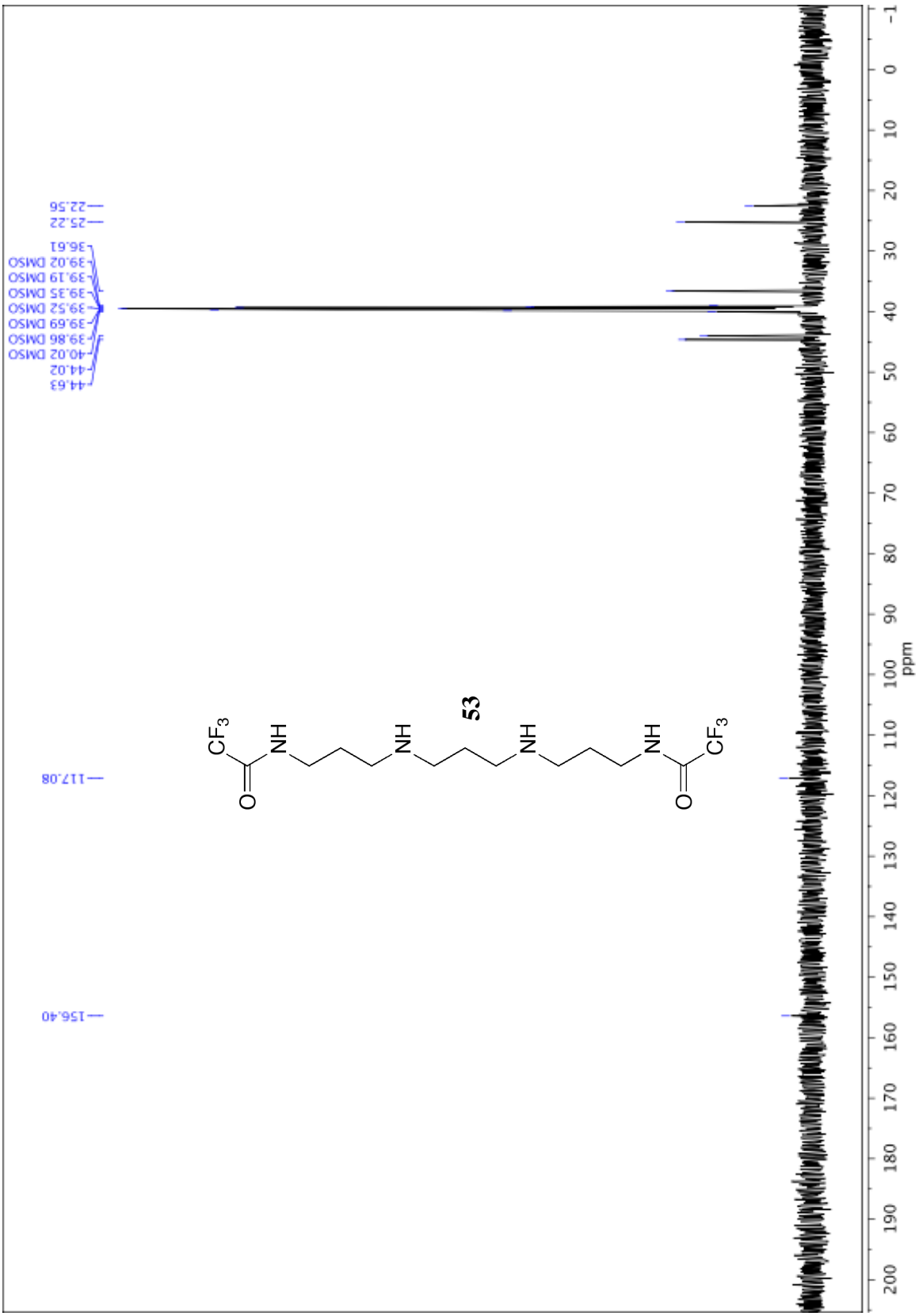

1.66 Water
7.26 CDCl3
3.38
3.39
3.40
3.65
3.66
3.66
3.67
3.68
4.00
20.09
ppm
0
1
2
3
4
5
6
7
8
9
10

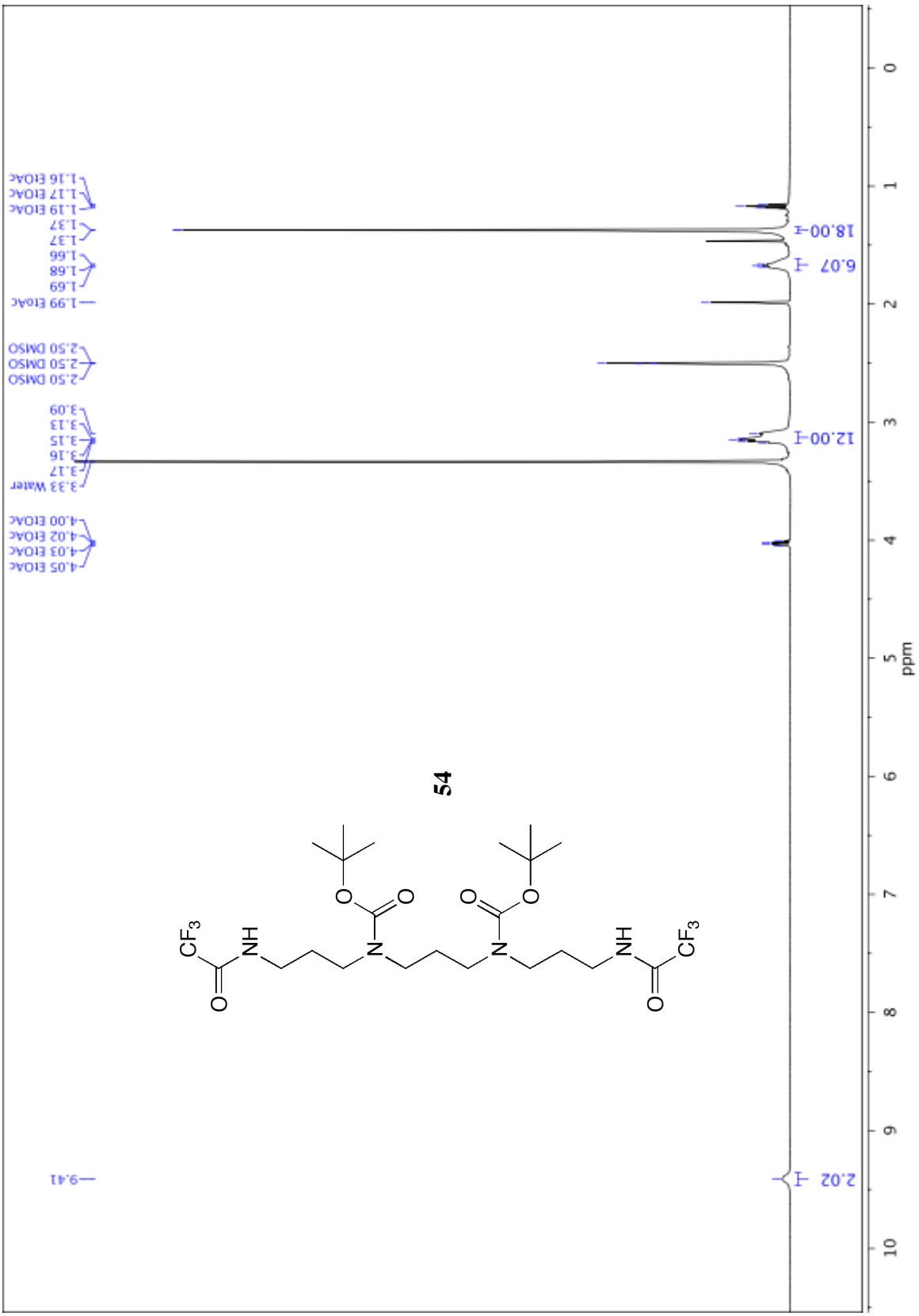
52


50.83
70.18
70.73
70.78
70.82
70.85
76.91 CDCl3
77.16 CDCl3
77.41 CDCl3
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
ppm

52


53
CF3
O
NH
NH
NH
NH
CF3
O
ppm

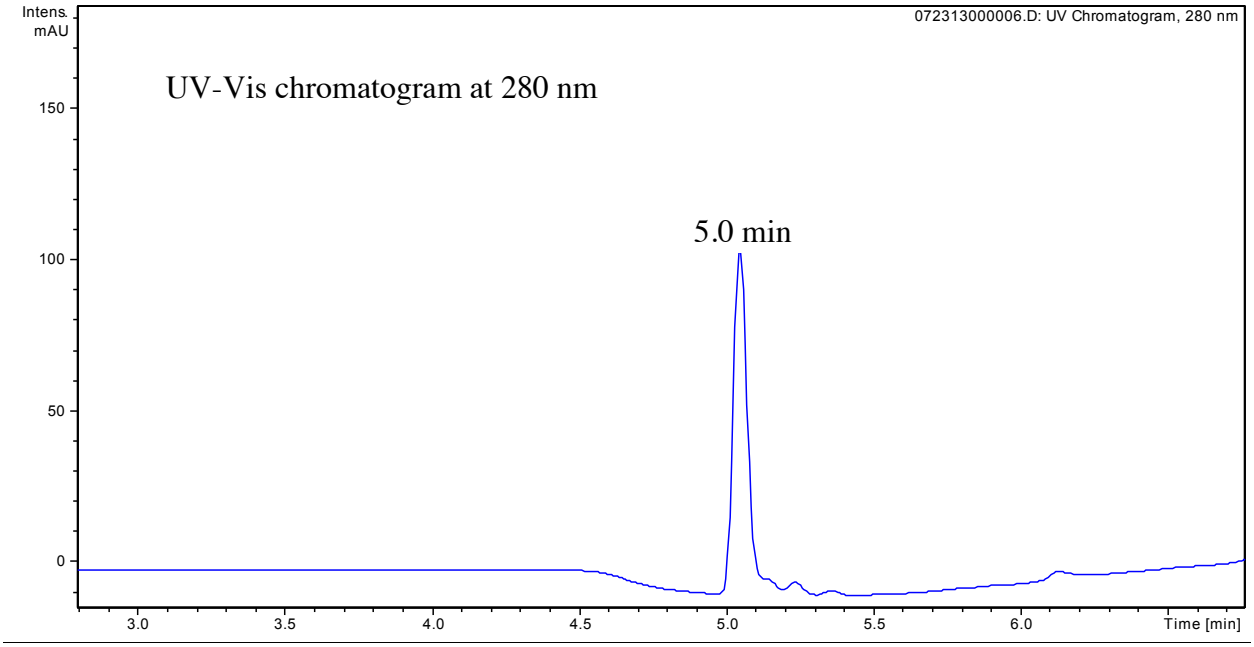

53
CF3
NH
NH
NH
NH
CF3
O
O
ppm
22.56
25.22
36.61
39.02 DMSO
39.19 DMSO
39.35 DMSO
39.52 DMSO
39.69 DMSO
39.86 DMSO
40.02 DMSO
44.02
44.63
117.08
156.40

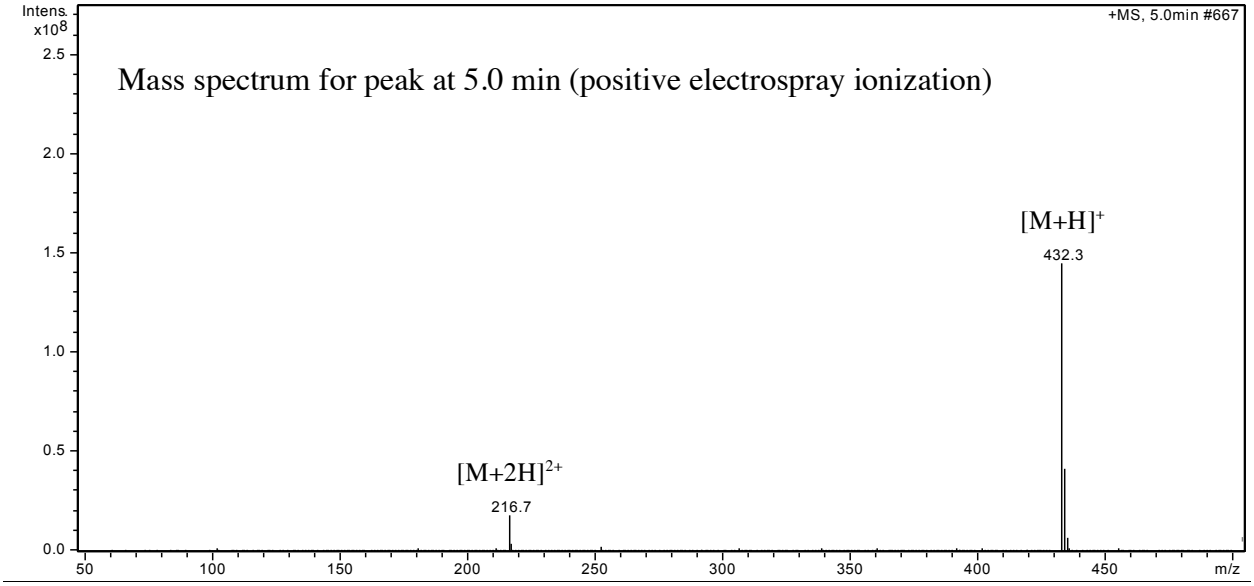

54
ppm
18.00
6.07
12.00
2.02
9.41
1.99 EtOAc
1.69
1.68
1.66
1.37
1.37
1.19 EtOAc
1.17 EtOAc
1.16 EtOAc
2.50 DMSO
2.50 DMSO
2.50 DMSO
3.33 Water
3.17
3.16
3.15
3.13
3.09
4.05 EtOAc
4.03 EtOAc
4.02 EtOAc
4.00 EtOAc

7. Supplementary LC(UV)/MS analysis

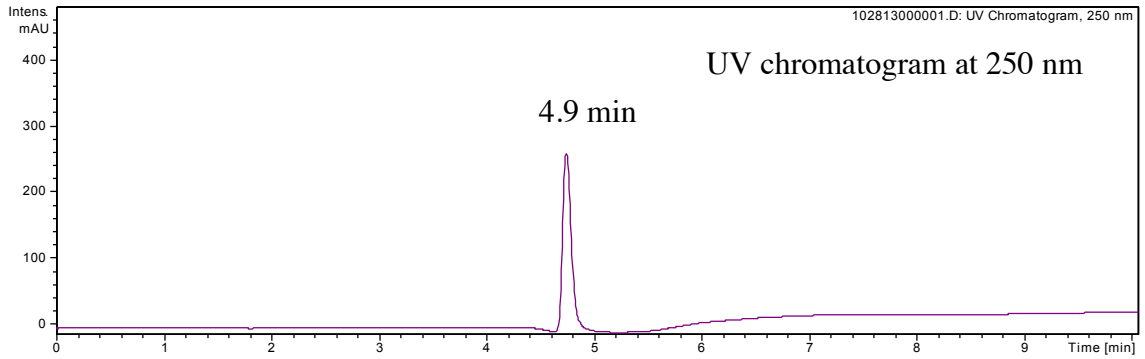
Ligands were analyzed with the Agilent LC/MSD Trap XCT plus system (Agilent Technologies, Santa Clara, CA) which has a 1100 series HPLC system including a multiple wavelength detector (MWD) set at 250 nm and 280 nm, a degasser, an autosampler, and a binary pump. The LC separation was performed on a Phenomenex Gemini 3u C6-phenyl 110A column (2 mm × 100 mm) (Torrance, CA) with mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile). The flow-rate was 0.25 mL/min. The linear gradient was as follows: 0-1 min, 100% A; 5-10 min, 0% A. The autosampler was set at 5ºC and the injection volume is 1 µL. Positive mass spectrometry was achieved with electrospray ionization (ESI) with the high voltage at 3500 V. The nebulizer, dry gas, and dry temperature were 35 psi, 8 L/min, and 350ºC, respectively. The relative peak area in the UV chromatogram was used to determine the purity of the compounds. The purity of all compounds was ≥95 %.

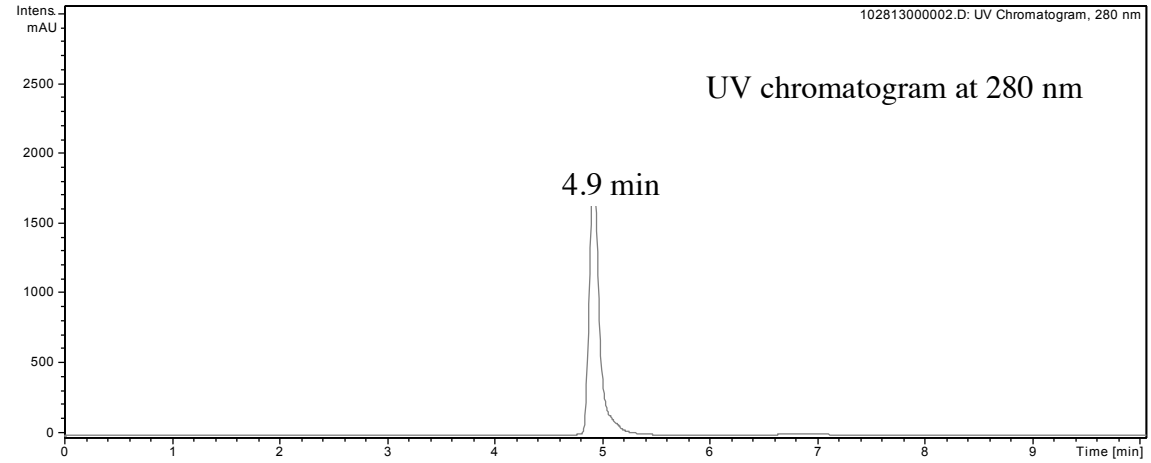
Ligand **2**: Purity is 97.3%.

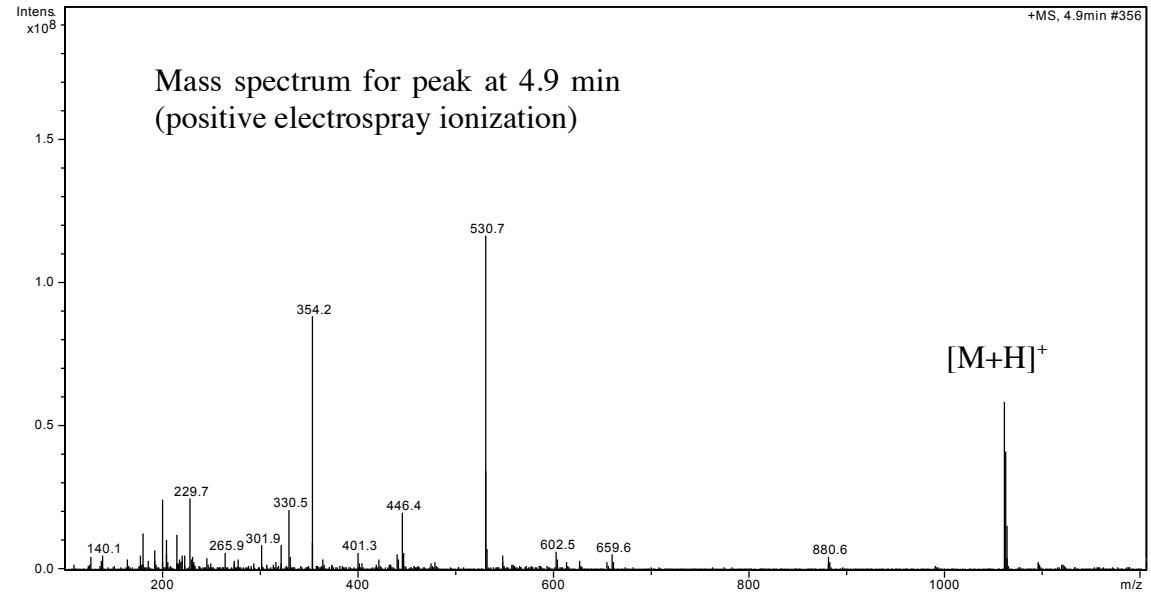




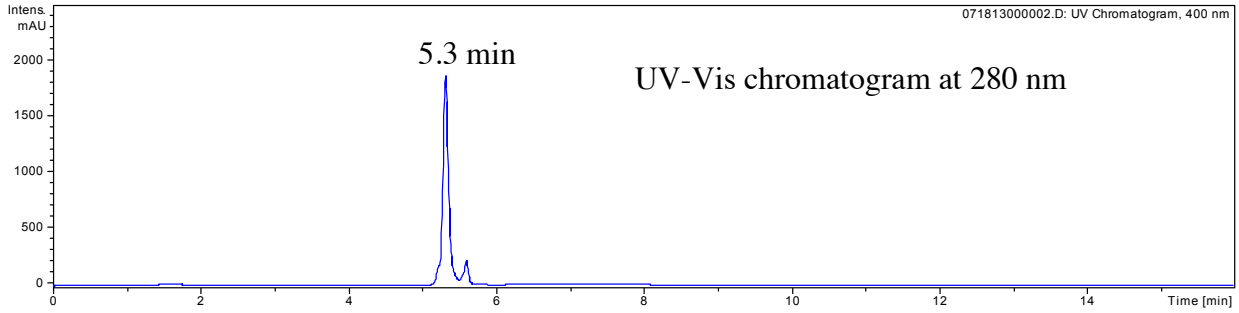
Ligand **5**: Purity is > 99.0%

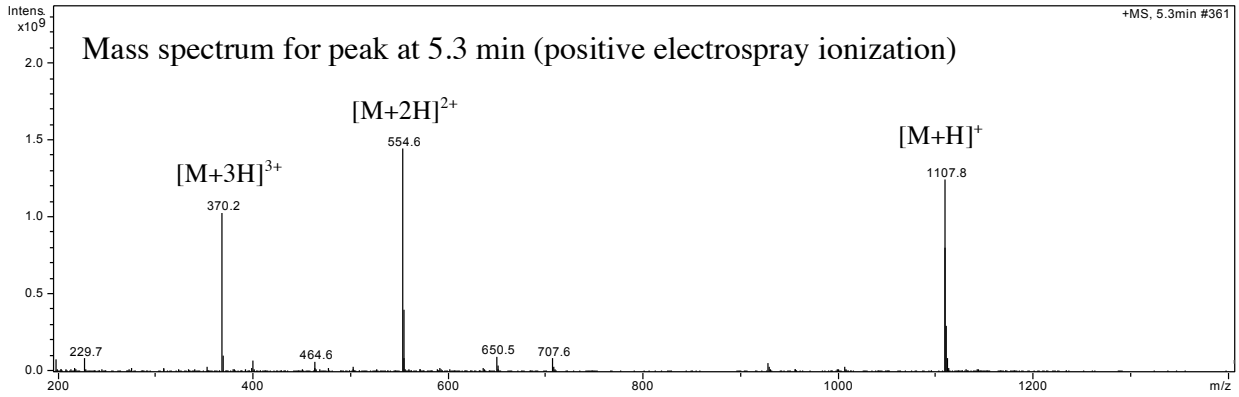




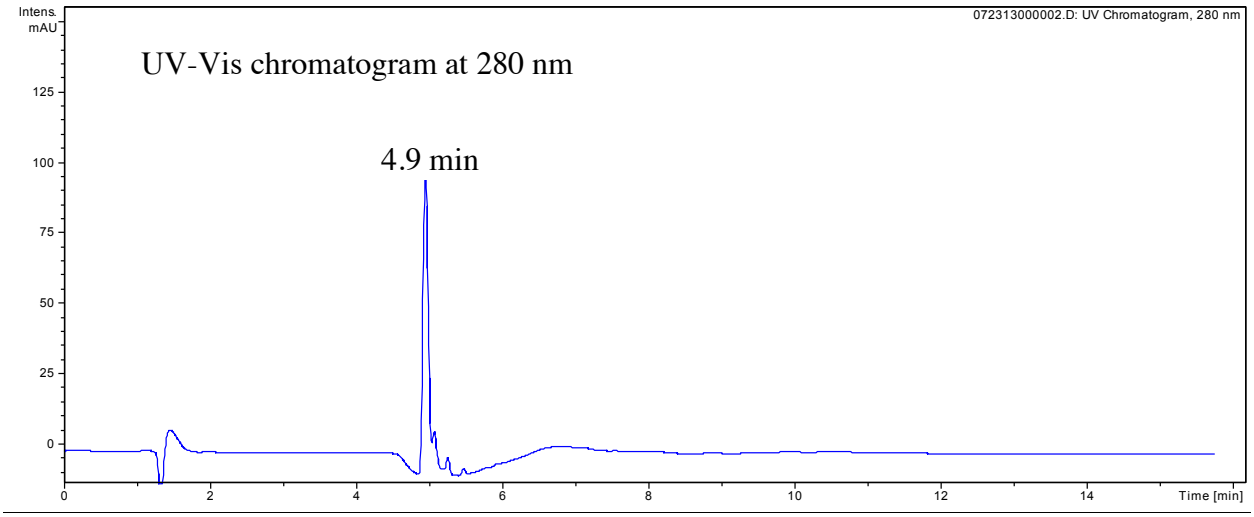


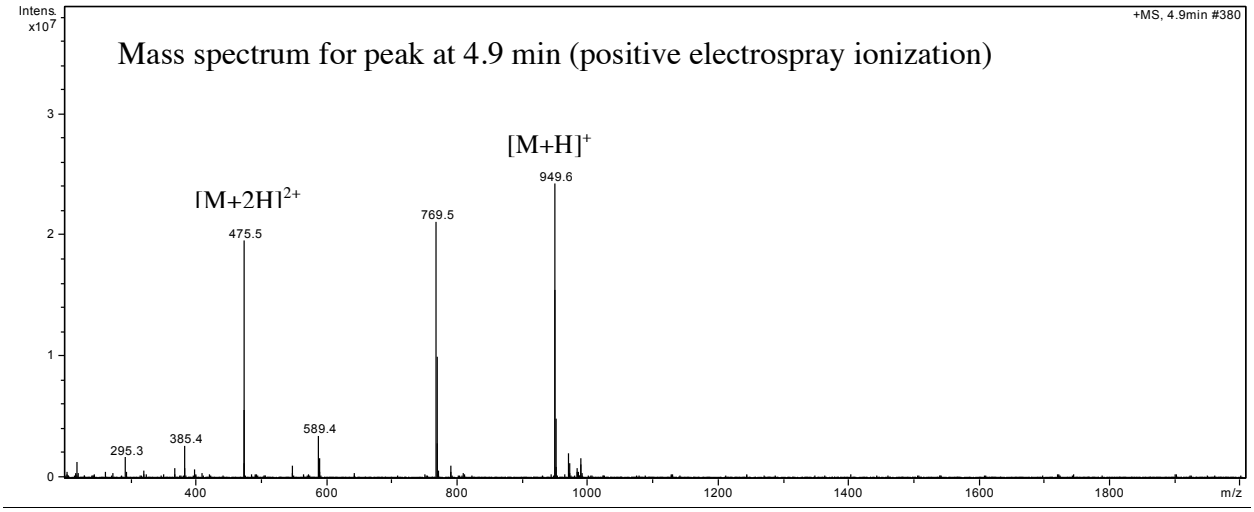
Ligand **6**: Purity is 95.1%.



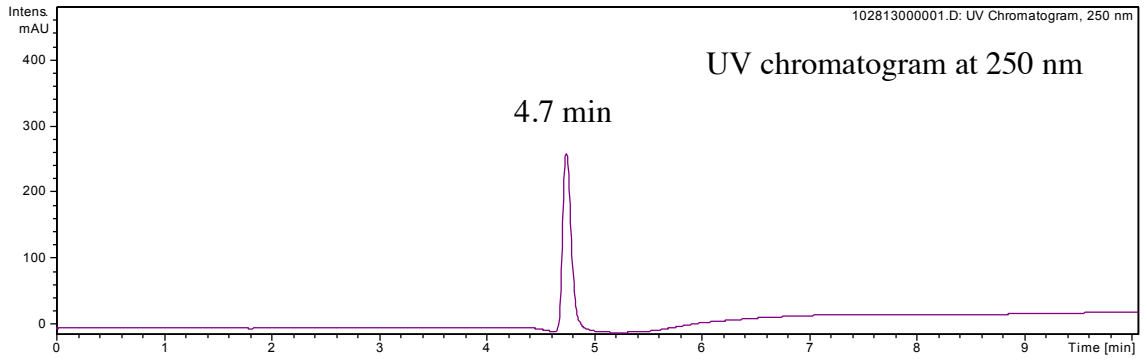


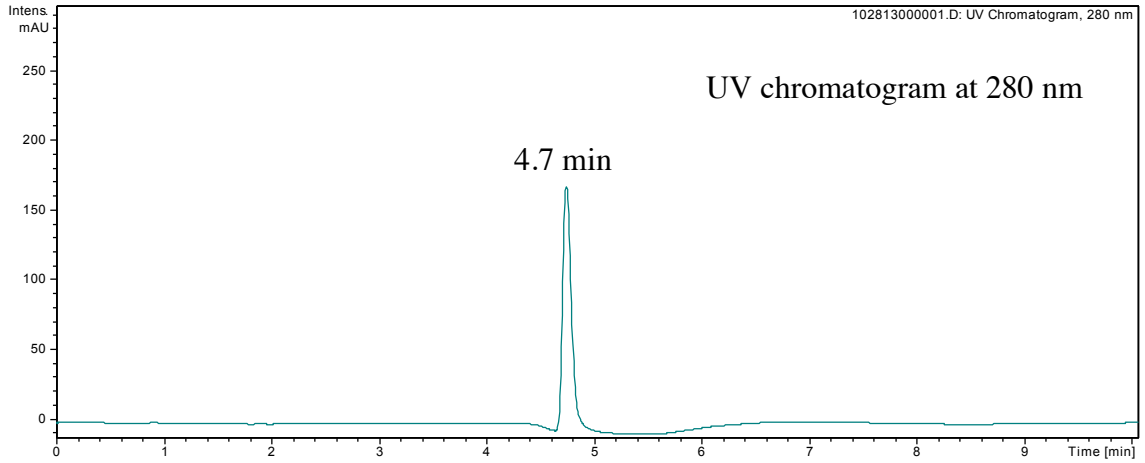
Ligand **7**: Purity is 96.7%.

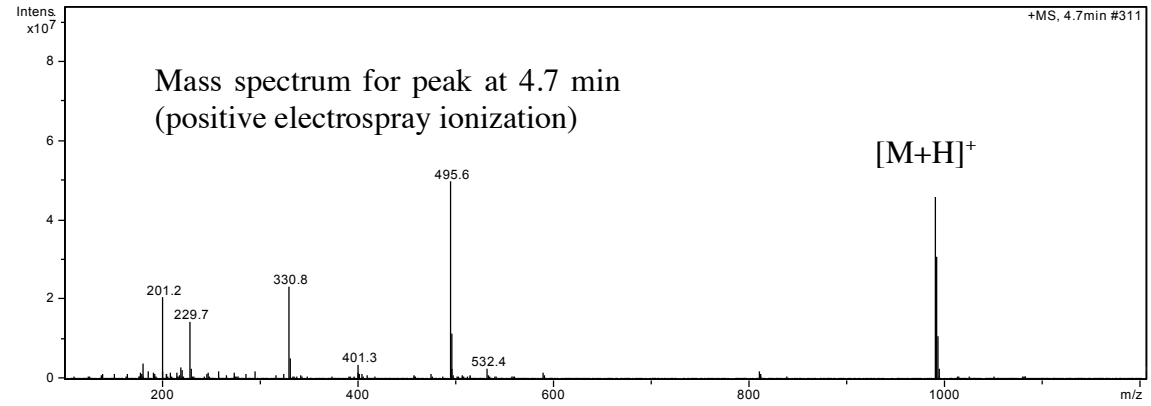




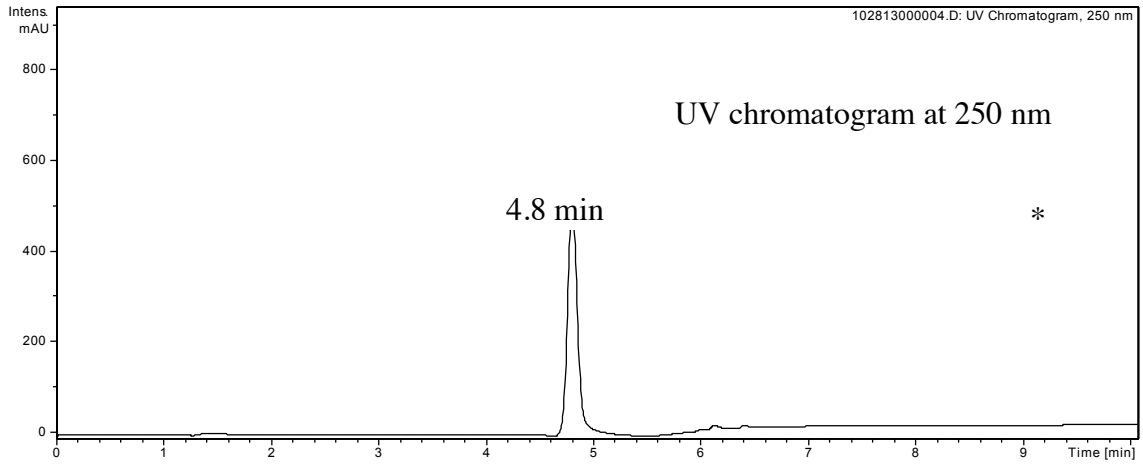
Ligand **9**: Purity is > 99.0%

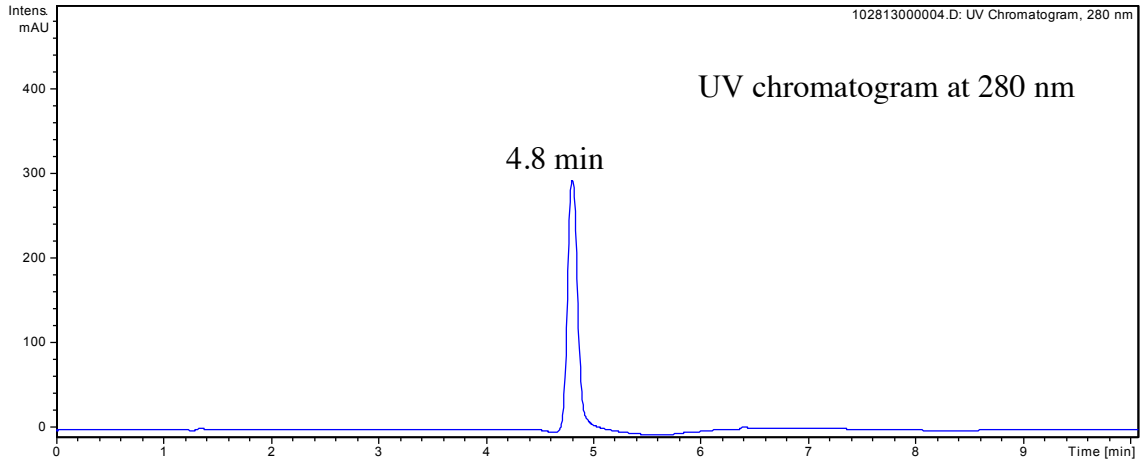


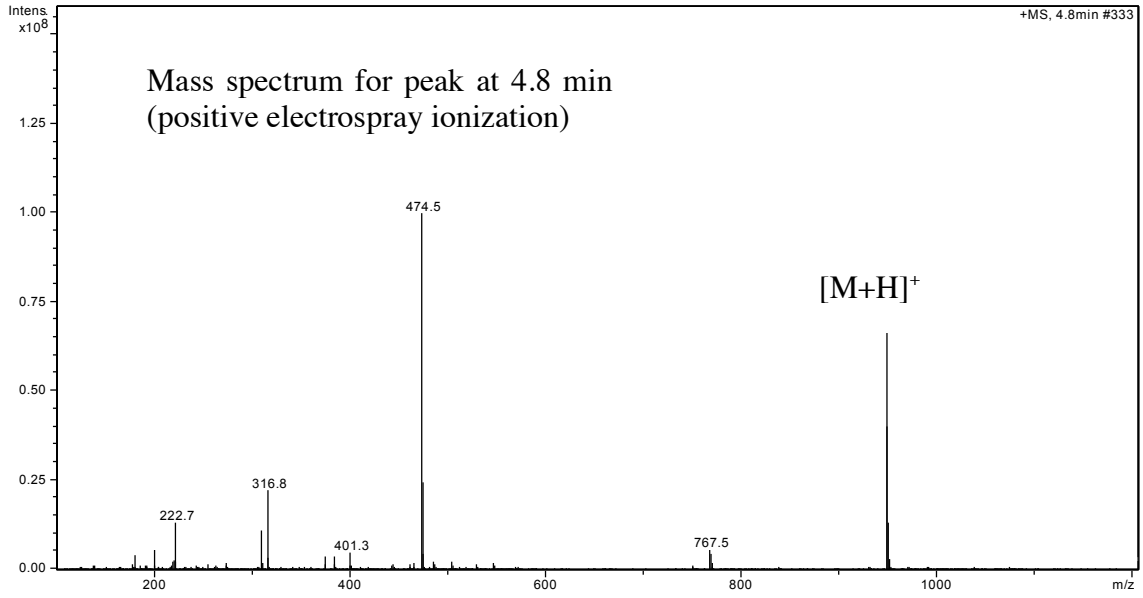




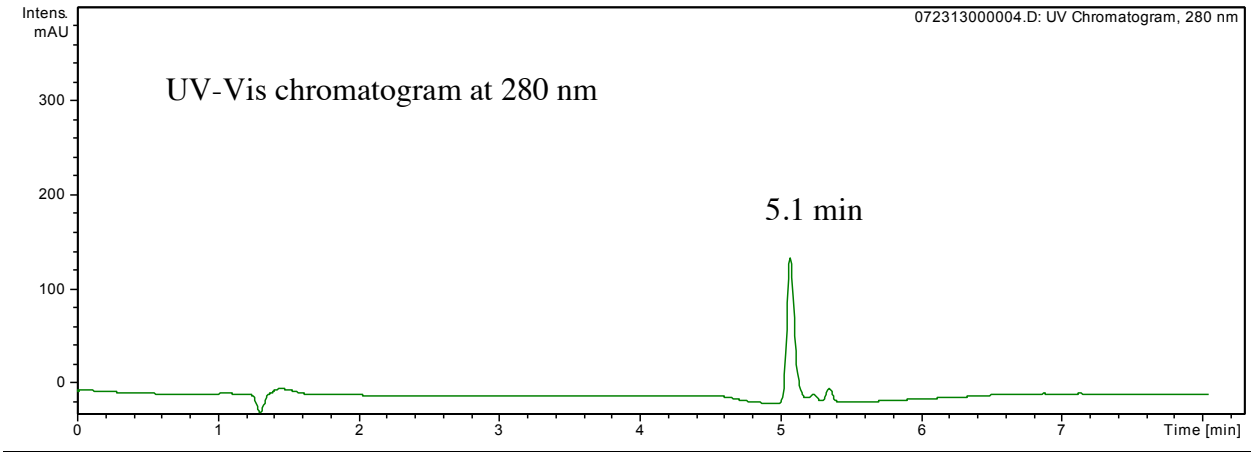
Ligand **11**: Purity is > 99%

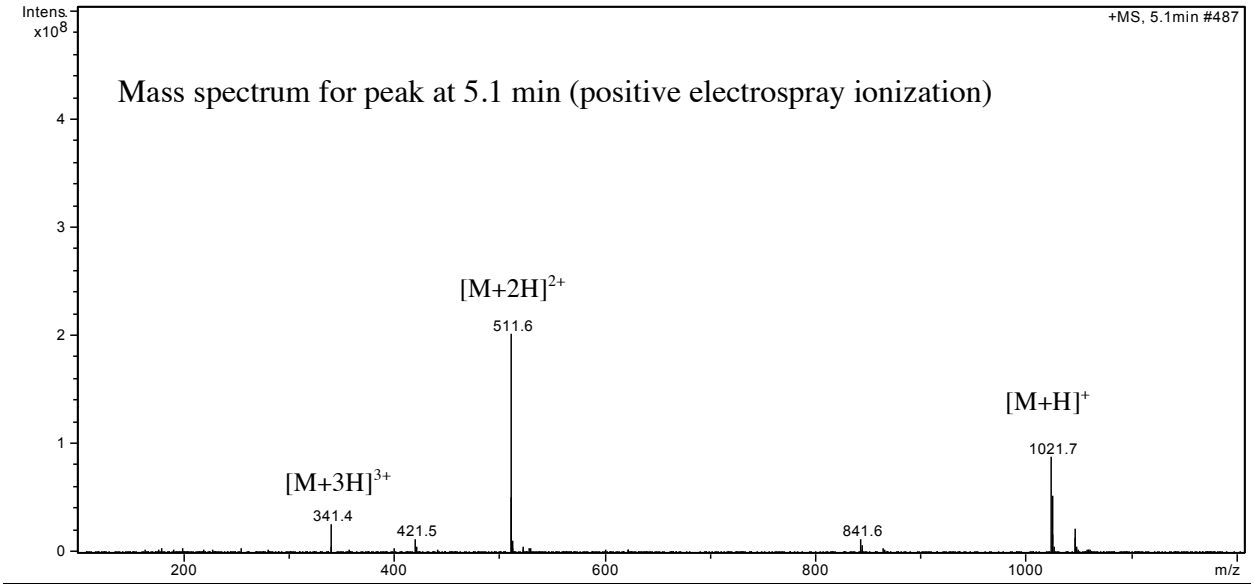






Ligand **12**: Purity is 95.7%.





Ligand **14**: Purity is > 99.0%.