Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Supporting Information

Novel benzimidazole-based pseudo-irreversible butyrylcholinesterase inhibitors with neuroprotective activity in an Alzheimer´s disease mouse model

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Synthesis and characterisation

2-(4-(Benzyloxy)phenyl)acetic acid. 4-Hydroxyphenylacetic acid (2.5 g, 16.4 mmol, 1.0 eq.), benzyl bromide (2.04 mL, 2.94 g, 17.2 mmol, 1.05 eq.), potassium hydroxide (2.30 g, 41.0 mmol, 2.5 eq.) and sodium iodide (49.2 mg, 328 µmol, 0.02 eq.) were dissolved in ethanol (75 mL) and refluxed for 20 h. The solution was then cooled to rt, before hydrochloric acid (3.0 M, 75 mL) was added. The resulting precipitate was filtered, washed with water (50 mL) and dried in vacuo, yielding 2-(4-(benzyloxy)phenyl)acetic acid (1.84 g, 7.60 mmol, 46%) as a white solid (m.p. 121.9°C). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.44 – 7.36 (m, 4H), 7.36 – 7.29 (m, 1H), 7.22 – 7.18 (m, 2H), 6.97 – 6.92 (m, 2H), 5.05 (s, 2H), 3.59 (s, 2H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 176.88, 158.27, 137.10, 130.58, 128.74, 128.12, 127.60, 125.75, 115.19, 70.20, 40.10 ppm; ESI-MS: m/z = 243.10 $[M+H]^+$, 265.00 $[M+Na]^+$, calc. 243.10.

4-Nitrophenyl azetidine-1-carboxylate. Azetidine (100 µL , 84.7 mg, 1.48 mmol, 1.0 eq.) was dissolved in anhydrous DCM (11 mL) before TEA (614 µL, 448 mg, 4.44 mmol, 3.0 eq.) and 4-nitrophenyl chloroformate (329 mg, 1.63 mmol, 1.1 eq.) were added subsequently. The reaction mixture was stirred for two hours at rt before it was diluted with DCM (19 mL), washed with hydrochloric acid (2x25 mL) and brine (25 mL). After drying over sodium sulfate, subsequent column chromatography (petroleum ether:ethyl acetate, 2:1) yielded the product 4-nitrophenyl azetidine-1-carboxylate (259 mg, 1.17 mmol, 79%) as an off white solid (m.p. 70.7°C-71.5°C). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.22 (d, *J* = 9.2 Hz, 2H), 7.30 (d, *J* = 9.1 Hz, 2H), 4.19 (dt, *J* = 41.9, 7.7 Hz, 4H), 2.36 (p, 2H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 156.23 , 152.60 , 144.78 , 125.17 , 122.07, 55.03, 49.39, 15.89 ppm; ESI-MS: m/z = 223.05 $[M+H]^+$, calc. 223.07.

6-(3,4-Dihydroisoquinolin-2(1H)-yl)hexan-1-amine. 1,2,3,4-Tetrahydroisoquinoline (106 µL, 113 mg, 852 µmol, 1.2 eq.) and TEA (344 µL, 251 mg, 2.49 mmol, 3.5 eq.) were dissolved in DMF, before 2-(6-bromohexyl)isoindoline-1,3-dione (220 mg, 710 µmol, 1.0 eq.) was added in one portion. The solution was stirred at 100°C overnight. Afterwards, the solvent was evaporated under reduced pressure. The residue was taken up in ethanol, hydrazine monohydrate (153 µL, 156 mg, 3.12 mmol, 10 eq.) was added and the solution was refluxed overnight. Subsequent column chromatography (DCM:methanol:TEA, 8:2:0.1) yielded the product 6-(3,4-dihydroisoquinolin-2(1*H*)-yl)hexan-1-amine (39.0 mg, 168 µmol, 20% over two steps) as a yellowish oil. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 7.09 (dq, *J* = 6.9, 4.2, 3.0 Hz, 3H), 7.03 – 6.96 (m, 1H), 4.45 (s, 3H), 3.60 (s, 2H), 2.89 (t, *J* = 6.0 Hz, 2H), 2.71 (q, *J* = 6.0, 4.9 Hz, 4H), 2.48 (dd, *J* = 8.9, 6.3 Hz, 2H); **^{13}C-NMR** (101 MHz, $CDCl_3$): *δ* = 134.91, 134.41, 128.71, 126.68, 126.16, 125.65, 58.46, 56.30, 51.06, 41.22, 31.65, 29.16, 27.40, 27.19, 26.84 ppm; **ESI-MS**: *m/z* = 233.15 $[M+H]^+$, calc. 233.20.

N,N-Diethyl-4-fluoro-3-nitrobenzamide (**5**). 4-Fluoro-3-nitrobenzoic acid (5.00 g, 27.0 mmol, 1.0 eq.) was dissolved in DCM and treated with catalytic amounts of DMF before oxalyl chloride (2.55 mL, 3.77 g, 1.2 eq.) was added at 0°C. After stirring the solution for 1 h at 0°C, a mixture of diethylamine (3.09 ml, 2.17 g, 29.7 mmol, 1.1 eq) and triethylamine was added slowly at 0°C and stirred for 4 h at rt. The organic was washed with 1 M hydrochloric acid (2x) and 1 M $NaOH_{aq}$. Drying over sodium sulfate and evaporation of the solvent under reduced pressure yielded *N*,*N*-diethyl-4-fluoro-3-nitrobenzamide (**5**) (6.44 g, 26.8 mmol, 99%) as a light, brown oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.11 (dd, *J* = 7.0, 2.1 Hz, 1H), 7.79 – 7.53 (m, 1H), 7.34 (dd, *J* = 10.3, 8.7 Hz, 1H), 3.36 (dq, J = 64.0, 6.8 Hz), 1.19 (dt, *J* = 11.4, 7.1 Hz, 8H) ppm; ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 202.93, 167.78, 157.19, 154.52, 134.13, 133.99, 133.90, 124.70, 124.67, 119.11, 118.90, 77.16, 43.67, 39.91, 14.41, 14.12 ppm; ESI-MS: m/z = 241.15 $[M+H]^+$, calc. 241.09.

N,N-Diethyl-4-(isopentylamino)-3-nitrobenzamide (**6a**). *N*,*N*-Diethyl-4-fluoro-3-nitrobenzamide (**5**) (2.50 g, 10.4 mmol, 1.0 eq.) was dissolved in ethanol (20 mL) before isoamylamine (1.44 mL, 994 mg, 11.4 mmol, 1.1 eq.) and TEA (2.16 mL, 1.58 g, 15.6 mmol, 1.5 eq.) dissolved 35 mL ethanol were added. The solution was stirred for 3 h at 55°C, resulting in an orange solution that was then evaporated under reduced pressure. The residue was taken up in diethyl ether (150 mL) and washed with citric acid (2.5% in water, 100 mL) and water (100 mL). The combined aqueous phases are extracted with diethyl ether (100 mL). The combined organic layers were then dried over magnesium sulfate and the solvent was evaporated under reduced pressure to yield *N*,*N*-diethyl-4-(isopentylamino)-3-nitrobenzamide (**6a**) (2.82 g, 9.19 mmol, 88%) as an orange oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.27 (d, *J* = 2.0 Hz, 1H), 8.14 (bs, 1H, NH), 7.56 (dd, *J* = 8.9, 2.1 Hz, 1H), 6.88 (d, *J* = 8.9 Hz, 1H), 3.45 (dt, *J* = 7.7, 4.0 Hz, 4H), 3.34 (td, *J* = 7.4, 5.0 Hz, 2H), 1.78 (dq, *J* = 13.3, 6.7 Hz, 1H), 1.64 (q, *J* = 7.1 Hz, 2H), 1.22 (t, *J* = 7.1 Hz, 6H), 0.99 (d, *J* = 6.6 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 169.60, 146.14 , 135.36, 130.75, 125.78, 123.55, 114.09, 41.53, 37.88, 26.11, 22.60 ppm; ESI-MS: m/z = 308.25 $[M+H]^+$, 615.45 $[2M+H]^+$, calc. 308.19.

N,N-diethyl-3-nitro-4-((2-(piperidin-1-yl)ethyl)amino)benzamide (**6b**). *N*,*N*-Diethyl-4-fluoro-3-nitrobenzamide (**5**) (1.43 g, 5.96 mmol, 1.0 eq.) was dissolved in ethanol (4.5 mL) before 2-(piperidin-1-yl)ethan-1-amine (930 µL, 840 mg, 6.56 mmol, 1.1 eq.) and TEA (1.24 mL, 903 mg, 8.94 mmol, 1.5 eq.) dissolved ethanol (9 mL) were added. The solution was stirred for 3.5 h at 55°C, resulting in an orange solution. The solvent was then evaporated under reduced pressure. The residue was taken up in DCM (100 mL) and washed with ammonium chloride (saturated in water, 100 mL) and water (100 mL). The combined aqueous phases are extracted with DCM (100 mL). The combined organic layers were then dried over magnesium sulfate and the solvent was evaporated under reduced pressure to yield *N*,*N*-diethyl-3-nitro-4-((2-(piperidin-1-yl)ethyl)amino)benzamide (**6b**) (2.12 g, quant.) as an orange oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.64 (t, *J* = 4.7 Hz, 1H), 8.27 (t, *J* = 1.8 Hz, 1H), 7.54 (dt, *J* = 8.9, 1.9 Hz, 1H), 6.85 (dd, *J* = 8.8, 2.7 Hz, 1H), 3.54 – 3.33 (m, 6H), 2.67 (d, *J* = 6.3 Hz, 2H), 2.53 – 2.40 (m, 4H), 1.62 (p, *J* = 5.5 Hz, 4H), 1.53 – 1.41 (m, 2H), 1.21 (t, *J* = 7.1 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 169.61, 145.88, 135.21, 130.91, 125.74, 123.47, 114.39, 56.33, 54.37, 40.07, 26.11, 24.50 ppm; ESI-MS: m/z = 349.20 $[M+H]^+$, 387.10 $[M+K]^+$, 697.35 $[2M+H]^+$, 719.35 $[2M+Na]^+$, 735.30 $[2M+K]^+$, calc. 349.22.

N,N-Diethyl-3-nitro-4-((4-(piperidin-1-yl)butyl)amino)benzamide (**6c**). *N*,*N*-Diethyl-4-fluoro-3-nitrobenzamide (**5**) (782 mg, 3.26 mmol, 1.0 eq.) was dissolved in ethanol (9 mL). 4-(Piperidin-1-yl)butan-1-amine (615 µL, 494 mg, 4.89 mmol, 1.1 eq.) and triethylamine (677 µL, 494 mg, 4.98 mmol, 1.5 eq.) were added at rt. The solution was heated to 55°C and stirred overnight. The solvent was evaporated under reduced pressure, the residue was taken up in DCM (50 mL) and washed with ammonium chloride (2x 50 mL) and brine (50 mL) before the organic layer was dried over sodium sulfate and the solvent was evaporated under reduced pressure, yielding *N*,*N*-diethyl-3-nitro-4-((4-(piperidin-1-yl)butyl)amino)benzamide (**6c**) in quantitative yield as an orange oil.^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.25 (d, *J* = 2.1 Hz, 1H), 8.15 (t, *J* = 5.4 Hz, 1H), 7.54 (dd, *J* = 8.9, 2.1 Hz, 1H), 6.88 (d, *J* = 8.9 Hz, 1H), 3.56 – 3.32 (m, 6H), 2.84 – 2.53 (m, 7H), 1.91 – 1.69 (m, 8H), 1.62 – 1.45 (m, 2H), 1.20 (t, *J* = 7.1 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 169.41, 145.88, 135.30, 130.92, 125.76, 124.01, 114.05, 57.99, 54.14, 42.81, 26.82, 24.48, 23.48, 23.06 ppm; ESI-MS: m/z = 377.20 $[M+H]^+$, calc. 377.25.

3-Amino-N,N-diethyl-4-(isopentylamino)benzamide (**7a**). *N*,*N*-diethyl-4-(isopentylamino)-3-nitrobenzamide (**6a**) (1.50 g, 4.89 mmol, 1.0 eq.) was dissolved in ethanol (18 mL), before palladium on carbon (10 wt.%, 150 mg) was added. The solvent was purged with hydrogen, before the solution was stirred overnight under hydrogen atmosphere. After reaction control indicated consumption of the starting material, the suspension was filtrated over Celite. Evaporation of the starting material under reduced pressure yielded 3-amino-*N*,*N*-diethyl-4-(isopentylamino)benzamide (**7a**) (1.28 g, 4.62 mmol, 94%) as purple oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 6.86 (dd, *J* = 8.1, 1.9 Hz, 1H), 6.80 (d, *J* = 2.0 Hz, 1H), 6.60 (d, *J* = 8.0 Hz, 1H), 3.42 (q, *J* = 7.3 Hz, 4H), 3.13 (t, *J* = 7.4 Hz, 2H), 1.79 – 1.69 (m, 1H), 1.60 – 1.52 (m, 2H), 1.16 (t, *J* = 7.0 Hz, 6H), 0.96 (d, *J* = 6.6 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 172.02, 139.26, 133.40, 126.23, 119.40, 115.36, 109.94, 42.22, 38.48, 26.09, 22.63 ppm; ESI-MS: m/z = 278.15 $[M+H]^+$, 555.40 $[2M+H]^+$, calc. 278.22.

3-Amino-N,N-diethyl-4-((2-(piperidin-1-yl)ethyl)amino)benzamide (**7b**). *N*,*N*-Diethyl-3-nitro-4-((4-(piperidin-1-yl)ethyl)amino)benzamide (**6b**) (1.06 g, 3.05 mmol, 1.0 eq.) was dissolved in methanol (15 mL). Palladium on carbon (106 mg) was added and the solvent was flushed with hydrogen (3x) before the suspension was stirred for 1.5 h under hydrogen. The suspension was then filtrated by Celite and washed with methanol. Evaporation of the solvent yielded 3-amino-*N*,*N*-diethyl-4-((2-(piperidin-1-yl)ethyl)amino)benzamide (**7b**) (911 mg, 2.86 mmol, 94%) as a brown oil. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 6.83 (dd, *J* = 8.0, 1.9 Hz, 1H), 6.77 (d, *J* = 1.8 Hz, 1H), 6.56 (d, *J* = 8.0 Hz, 1H), 3.51 – 3.38 (m, 6H), 3.19 (t, *J* = 6.0 Hz, 2H), 2.66 (t, *J* = 9.0 Hz, 2H), 2.49 – 2.41 (m, 4H), 1.67 – 1.56 (m, 4H) 1.46 (p, *J* = 6.2 Hz, 2H), 1.16 (t, *J* = 7.1 Hz, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$): *δ* = 171.95, 138.66, 134.04, 126.85, 119.05, 114.79, 110.53, 57.21, 54.29, 40.47, 25.73, 24.21 ppm; **ESI-MS**: *m/z* = 160.10 $[M+2H]^+$, 319.15 $[M+H]^+$, calc. 319.25.

3-Amino-N,N-diethyl-4-((4-(piperidin-1-yl)butyl)amino)benzamide (**7c**). *N*,*N*-Diethyl-3-nitro-4-((4-(piperidin-1-yl)butyl)amino)benzamide (**6c**) (797 mg, 2.12 mmol, 1.0 eq.) was dissolved in methanol (11 mL). Palladium on carbon (79.7 mg) was added and the solvent was flushed with hydrogen (3x) before the suspension was stirred for 1.5 h under hydrogen. The suspension was then filtrated by Celite and washed with methanol. Evaporation of the solvent and subsequent column chromatography (DCM:methanol:ammonia (25% in water); 11:1:0.1) yielded 3-amino-*N*,*N*-diethyl-4-((2-(piperidin-1-yl)butyl)amino)benzamide (**7c**) (474 mg, 1.37 mmol, 65%) as a brown oil. **^{1}H-**

NMR (400 MHz, $CDCl_3$): δ = 6.85 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.78 (d, *J* = 2.1 Hz, 1H), 6.57 (dd, *J* = 8.1, 1.7 Hz, 1H), 3.73 – 3.28 (m, 7H), 3.13 (td, *J* = 6.6, 2.2 Hz, 2H), 2.44 – 2.32 (m, 6H), 1.70 – 1.57 (m, 8H), 1.43 (p, *J* = 5.9 Hz, 2H), 1.16 (t, *J* = 7.1 Hz, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$): *δ* = 172.04, 139.34, 133.44, 126.60, 119.69, 115.55, 110.19, 59.12, 54.73, 44.01, 27.65, 25.99, 24.78, 24.52 ppm; **ESI-MS**: *m/z* = 174.20 $[M+2H]^+$, 347.20 $[M+H]^+$, calc. 347.28.

3-(2-(4-(Benzyloxy)phenyl)acetamido)-N,N-diethyl-4-(isopentylamino)benzamide (**8a**). 2-(4-(Benzyloxy)phenyl)acetic acid (770 mg, 3.18 mmol, 1.1 eq.), HBTU (1.21 g, 3.18 mmol, 1.1 eq.) and TEA (600 µL, 438 mg, 4.34 mmol, 1.5 eq.) were dissolved in DMF (12 mL), before 3-amino-*N*,*N*-diethyl-4-(isopentylamino)benzamide (**7a**) (800 mg, 2.89 mmol, 1.0 eq.) in DMF (8mL) is added. The solution was then stirred for 3 h at rt and the solvent was evaporated under reduced pressure. Subsequent column chromatography (ethyl acetate/petroleum ether, 4:1) yielded 3-(2-(4-(benzyloxy)phenyl)acetamido)-*N*,*N*-diethyl-4-(isopentylamino)benzamide (**8a**) (993 mg, 1.98 mmol, 69%) as a purple oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.02 (s, 1H), 7.46 – 7.26 (m, 8H), 7.04 – 6.90 (m, 4H), 6.39 (d, *J* = 8.4 Hz, 1H), 5.02 (s, 2H), 3.61 (s, 2H), 3.48 – 3.32 (m, 4H), 2.91 (t, *J* = 7.3 Hz, 2H,), 2.01 (s, 1H,), 1.68 – 1.55 (m, 1H), 1.37 (td, *J* = 7.2 Hz, 2H), 1.18 – 1.09 (m, 6H), 0.92 (d, *J* = 6.8 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 172.03, 171.14, 157.63, 143.90, 136.87, 130.00, 128.34, 128.19, 127.70, 127.22, 125.22, 125.08, 123.23, 122.23, 114.78, 109.92, 69.76, 42.59, 41.43, 38.03, 25.71, 22.46 ppm; ESI-MS: m/z = 502.35 $[M+H]^+$, 1003.45 $[2M+H]^+$, calc. 502.30.

3-(2-(4-(Benzyloxy)phenyl)acetamido)-N,N-diethyl-4-((2-(piperidin-1-yl)ethyl)amino)benzamide (**8b**). 3-Amino-*N*,*N*-diethyl-4-((2-(piperidin-1-yl)ethyl)amino)benzamide (**7b**) (398 mg, 1.25 mmol, 1.0 eq.) in DMF (5 mL) was added to a mixture of 2-(4-(benzyloxy)phenyl)acetic acid (333 mg, 1.38 mmol, 1.1 eq.), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU) (524 mg, 1.38 mmol, 1.1 eq.) and triethylamine (260 µL, 190 mg, 1.88 mmol, 1.5 eq.) in DMF (5 mL). The solution was stirred for 6 h at rt before the solvent was evaporated under reduced pressure. The residue was treated with ethyl acetate (75 mL) and washed with ammonium chloride (saturated, 75 mL) and $NaHCO_3$ (saturated in water, 2x75 mL). The organic phase was dried over magnesium sulfate and the solvent was evaporated. Subsequent column chromatography (DCM:methanol, 12:1) yielded 3-(2-(4-(benzyloxy)phenyl)acetamido)-*N*,*N*-diethyl-4-((2-(piperidin-1-yl)ethyl)amino)benzamide (**8b**) (252 g, 462 µmol, 37%) as a brown oil. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 7.79 (s, 1H), 7.44 – 7.36 (m, 5H), 7.34 – 7.30 (m, 3H), 7.11 (dt, *J* = 8.3, 1.5 Hz, 1H), 6.99 – 6.94 (m, 2H), 6.55 (d, *J* = 8.4 Hz, 1H), 5.06 (s, 2H), 3.74 (s, 2H), 3.47 – 3.36 (m, 4H), 3.17 (t, *J* = 5.8 Hz, 2H), 2.69 (t, *J* = 5.8 Hz, 2H), 2.60 – 2.52 (m, 4H), 1.71 – 1.63 (m, 4H), 1.52 – 1.46 (m, 2H), 1.16 (t, *J* = 7.1 Hz, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$): *δ* = 171.29, 170.70, 158.10, 136.91, 130.67, 128.60, 127.99, 127.45, 127.38, 125.72, 125.67, 124.55, 123.16, 115.34, 111.14, 70.08, 56.67, 54.11, 43.10, 39.43, 25.02, 23.65 ppm; **ESI-MS**: *m/z* = 543.30 $[M+H]^+$, calc. 543.33.

3-(2-(4-(Benzyloxy)phenyl)acetamido)-N,N-diethyl-4-((4-(piperidin-1-yl)butyl)amino)benzamide (**8c**). 3-Amino-*N*,*N*-diethyl-4-((2-(piperidin-1-yl)butyl)amino)benzamide (**7c**) (439 mg, 1.27 mmol, 1.0 eq.) in DMF (10 mL) was added to a mixture of 2-(4-(benzyloxy)phenyl)acetic acid (322 mg, 1.33 mmol, 1.05 eq.), HBTU (505 mg, 1.33 mmol, 1.05 eq.) and triethylamine (264 µL, 193 mg, 1.88 mmol, 1.5 eq.) in DMF (5 mL). The solution was stirred for 6 h at rt before the solvent was evaporated under reduced pressure. The residue was treated with DCM (75 mL) and washed with ammonium chloride (saturated, 75 mL), $NaHCO_{3,\,aq}$ (saturated, 2x75 mL) and brine. The organic phase was dried over sodium sulfate and the solvent was evaporated. Subsequent column chromatography (DCM:methanol, 21:2) yielded 3-(2-(4-(benzyloxy)phenyl)acetamido)-*N*,*N*-diethyl-4-((4-(piperidin-1-yl)butyl)amino)benzamide (**8c**) (510 g, 893 µmol, 70%) as a yellowish oil. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 8.50 – 8.40 (m, 1H), 7.44 – 7.28 (m, 8H), 7.22 (d, *J* = 2.0 Hz, 1H), 7.06 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.93 (d, *J* = 8.6 Hz, 2H), 6.50 (d, *J* = 8.4 Hz, 1H), 5.04 (s, 2H), 3.71 (s, 2H), 3.46 – 3.28 (m, 4H), 3.00 (t, *J* = 6.6 Hz, 2H), 2.61 – 2.52 (m, 4H), 2.46 (t, *J* = 7.7 Hz, 2H), 1.70 – 1.43 (m, 10H, CH_2), 1.14 (t, *J* = 7.1 Hz, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$): *δ* = 171.74, 171.18, 158.05, 143.47, 137.08, 130.52, 128.67, 128.05, 127.55, 125.69, 125.00, 124.62, 122.81, 115.30, 114.75, 110.79, 70.16, 58.17, 54.09, 43.14, 43.03, 26.59, 24.77, 23.63, 23.32 ppm; **ESI-MS**: *m/z* = 286.25 $[M+2H]^+$, 571.30 $[M+H]^+$, calc. 571.36.

2-(4-(Benzyloxy)benzyl)-N,N-diethyl-1-isopentyl-1H-benzo[d]imidazole-5-carboxamide (**9a**). 3-(2-(4-(Benzyloxy)phenyl)acetamido)-*N*,*N*-diethyl-4-(isopentylamino)benzamide (**8a**) (970 mg, 1.93 mmol, 1.0 eq.) was dissolved in acetic acid (15 mL) and stirred for 3 h at 130°C. The reaction mixture was cooled to rt before ammonia (25% in water) was added until a pH > 10. Then the aqueous phase was extracted with DCM (3x75 mL), the combined

organic layers were dried over magnesium sulfate and the solvent was extracted under reduced pressure, yielding 2-(4-(benzyloxy)benzyl)-*N*,*N*-diethyl-1-isopentyl-1*H*-benzo[*d*]imidazole-5-carboxamide (**9a**) (665 mg, 1.37 mmol, 71%) as a brown oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 1.4 Hz, 1H), 7.42 – 7.28 (m, 7H), 7.16 (d, *J* = 8.6 Hz, 2H), 6.94 – 6.89 (m, 2H), 5.03 (s, 2H), 4.27 (s, 2H), 4.04 – 3.91 (m, 2H), 3.65 – 3.27 (m, 4H), 1.62 – 1.51 (m, 1H), 1.43 – 1.34 (m, 2H), 1.23 – 1.19 (m, 6H), 0.90 (d, *J* = 6.6 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 171.70, 157.91, 154.20, 136.88, 135.66, 131.20, 129.55, 128.57, 128.26, 127.97, 127.39, 121.47, 117.46, 115.27, 109.50, 70.07, 42.67, 38.12, 33.74, 26.14, 22.36 ppm; ESI-MS: m/z = 484.35 $[M+H]^+$, 967.70 $[2M+H]^+$, calc. 484.29.

2-(4-(Benzyloxy)benzyl)-N,N-diethyl-1-(2-(piperidin-1-yl)ethyl)-1H-benzo[d]imidazole-5-carboxamide (**9b**). 3-(2-(4-(Benzyloxy)phenyl)acetamido)-*N*,*N*-diethyl-4-((2-(piperidin-1-yl)ethyl)amino)benzamide (**8b**) (227 mg, 418 µmol, 1.0 eq.) was dissolved in acetic acid (5 mL) and stirred for 3 h at 135°C. The solution was allowed to cool down to rt before being basified (pH > 8) by ammonia (25% in water). The aqueous solution was then extracted with DCM (2x75 mL) and the combined organic phases were washed with brine (100 mL) and dried over magnesium sulfate. Subsequent column chromatography (DCM:methanol, 15:1) yielded 2-(4-(benzyloxy)benzyl)-*N*,*N*-diethyl-1-(2-(piperidin-1-yl)ethyl)-1*H*-benzo[*d*]imidazole-5-carboxamide (**9b**) (159 mg, 303 µmol, 72%) as a brown oil. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 7.75 (d, *J* = 1.1 Hz, 1H), 7.44 – 7.27 (m, 7H), 7.16 (d, *J* = 8.2 Hz, 2H), 6.95 – 6.88 (m, 2H), 5.03 (s, 2H), 4.31 (s, 2H), 4.23 – 4.05 (m, 2H), 3.66 – 3.29 (m, 4H), 2.43 – 2.29 (m, 6H), 1.66 – 1.52 (m, 4H), 1.45 – 1.36 (m, 2H), 1.29 – 1.12 (m, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$): *δ* = 171.91, 158.03, 154.93, 142.28, 137.04, 136.02, 135.96, 129.71, 128.73, 128.67, 128.13, 127.55, 121.57, 117.78, 115.43, 109.65, 70.22, 55.10, 53.56, 38.76, 33.86, 26.00, 24.18 ppm; **ESI-MS**: *m/z* = 263.20 $[M+2H]^+$, 525.20 $[M+H]^+$, calc. 525.32.

2-(4-(Benzyloxy)benzyl)-N,N-diethyl-1-(4-(piperidin-1-yl)butyl)-1H-benzo[d]imidazole-5-carboxamide (**9c**). 3-(2-(4-(Benzyloxy)phenyl)acetamido)-*N*,*N*-diethyl-4-((4-(piperidin-1-yl)butyl)amino)benzamide (**8c**) (486 mg, 851 µmol) was dissolved in acetic acid (4 mL) and stirred for 3 h at 135°C. The solution was allowed to cool down to rt before being basified (pH > 8) by ammonia (25% in water). The aqueous solution was then extracted with DCM (2x75 mL) and the combined organic phases were washed with brine (100 mL) and dried over magnesium sulfate. Subsequent column chromatography (DCM:methanol, 13:2) yielded 2-(4-(benzyloxy)benzyl)-*N*,*N*-diethyl-1-(4-(piperidin-1-yl)butyl)-1*H*-benzo[*d*]imidazole-5-carboxamide (**9c**) (415 mg, 750 µmol, 88%) as a light brown oil. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 7.71 (s, 1H), 7.37 – 7.23 (m, 7H), 7.12 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.4 Hz, 2H), 4.97 (s, 2H), 4.21 (s, 2H), 3.96 (t, *J* = 7.4 Hz, 2H), 3.53 – 3.29 (m, 4H), 2.41 – 2.28 (m, 4H), 2.21 (t, *J* = 7.3 Hz, 2H), 1.56 (p, J = 5.6 Hz, 4H), 1.52 – 1.37 (m, 4H), 1.23 – 1.06 (m, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$): *δ* = 171.74, 157.79, 154.33, 142.03, 136.81, 135.76, 131.02, 129.50, 128.50, 128.39, 127.90, 127.35, 121.20, 117.47, 115.17, 109.60, 69.98, 58.08, 54.27, 43.84, 33.68, 27.26, 25.35, 23.96, 23.56 ppm; **ESI-MS**: *m/z* = 277.45 $[M+2H]^+$, 553.35 $[M+H]^+$, calc. 553.35.

N,*N*-Diethyl-2-(4-hydroxybenzyl)-1-isopentyl-1*H*-benzo[*d*]imidazole-5-carboxamide (**10a**). 2-(4-(Benzyloxy)benzyl)-*N*,*N*-diethyl-1-isopentyl-1*H*-benzo[*d*]imidazole-5-carboxamide (**9a**) (290 mg, 599 µmol, 1.0 eq.) was dissolved in methanol (10 mL) before palladium on carbon (10 wt.%, 29.0 mg) was added. The solvent was purged with hydrogen, before the solution was stirred overnight under hydrogen atmosphere. After reaction control indicated consumption of the starting material, the suspension was filtrated over Celite. Evaporation of the starting material under reduced pressure yielded *N*,*N*-Diethyl-2-(4-hydroxybenzyl)-1-isopentyl-1*H*-benzo[*d*]imidazole-5-carboxamide (**10a**) (214 mg, 545 µmol, 91%) as a brown solid (m.p. 166.6°C). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.71 (d, *J* = 1.3 Hz, 1H), 7.31 – 7.24 (m, 2H), 6.94 – 6.88 (m, 2H), 6.74 – 6.66 (m, 2H), 4.16 (s, 2H), 3.97 (dd, *J* = 9.7, 6.8 Hz, 2H), 3.57 – 3.25 (m, 5H), 1.63 – 1.48 (m, 1H, CH), 1.40 – 1.32 (m, 2H), 1.26 – 1.03 (m, 6H), 0.88 (d, *J* = 6.6, 1.4 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 171.99, 156.71, 154.90, 140.93, 135.51, 131.15, 129.44, 125.90, 121.54, 116.99, 116.23, 109.88, 42.80, 38.12, 33.49, 26.21, 22.43 ppm; ESI-MS: m/z = 394.25 $[M+H]^+$, 787.45 $[2M+H]^+$, calc. 394.24.

N,N-diethyl-2-(4-hydroxybenzyl)-1-(2-(piperidin-1-yl)ethyl)-1H-benzo[d]imidazole-5-carboxamide (**10b**). 2-(4-(Benzyloxy)benzyl)-*N*,*N*-diethyl-1-(2-(piperidin-1-yl)ethyl)-1*H*-benzo[*d*]imidazole-5-carboxamide (**9b**) (159 mg, 303 µmol, 1.0 eq.) was dissolved in methanol (6 mL). Palladium on carbon (15.9 mg) was added and the solvent was flushed with hydrogen (3x) before the suspension was stirred for 3 h under hydrogen. The suspension was then filtrated by Celite and washed with methanol. Evaporation of the solvent yielded *N*,*N*-diethyl-2-(4-hydroxybenzyl)-1-(2-(piperidin-1-yl)ethyl)-1*H*-benzo[*d*]imidazole-5-carboxamide (**10b**) (114 mg, 262 µmol, 86%) as a off-white oil. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 7.69 (d, *J* = 1.3 Hz, 1H), 7.32 – 7.22 (m, 2H), 6.92 (d, *J* = 8.2 Hz, 2H), 6.69 – 6.63 (m, 2H), 4.19 (s, 2H), 4.11 (t, *J* = 7.3 Hz, 2H), 3.56 – 3.23 (m, 5H), 2.40 – 2.32 (m, 6H), 1.56 – 1.49 (m, 4H), 1.38 (p, *J* = 5.9

Hz, 2H), 1.24 – 1.08 (m, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$): *δ* = 171.95, 156.62, 155.39, 141.41, 135.66, 131.10, 129.47, 126.13, 121.44, 117.16, 116.19, 109.78, 54.91, 50.32, 38.68, 33.45, 25.69, 23.96 ppm; **ESI-MS**: *m/z* = 218.25 $[M+2H]^+$, 435.20 $[M+H]^+$, calc. 435.27.

N,N-Diethyl-2-(4-hydroxybenzyl)-1-(4-(piperidin-1-yl)butyl)-1H-benzo[d]imidazole-5-carboxamide (**10c**). 2-(4-(Benzyloxy)benzyl)-*N*,*N*-diethyl-1-(4-(piperidin-1-yl)butyl)-1*H*-benzo[*d*]imidazole-5-carboxamide (**9c**) (390 mg, 705 µmol, 1.0 eq.) was dissolved in methanol (10 mL). Palladium on carbon (39.0 mg) was added and the solvent was flushed with hydrogen (3x) before the suspension was stirred overnight under light hydrogen pressure (balloon). The suspension was then filtrated by Celite and washed with methanol. Evaporation of the solvent yielded *N*,*N*-diethyl-2-(4-hydroxybenzyl)-1-(4-(piperidin-1-yl)butyl)-1*H*-benzo[*d*]imidazole-5-carboxamide (**10c**) (250 mg, 540 µmol, 77%) as a off-white solid (m.p. 153.2°C). **^{1}H-NMR** (400 MHz, methanol-d^4): δ = 7.84 – 7.81 (m, 1H), 7.66 (s, 1H), 7.41 (d, *J* = 8.3 Hz, 1H), 7.16 (d, *J* = 8.0 Hz, 2H), 6.76 (d, *J* = 8.1 Hz, 2H), 4.42 (s, 2H), 4.35 (t, *J* = 7.3 Hz, 2H), 3.56 – 3.21 (m, 6H), 2.92 (t, *J* = 7.8 Hz, 2H), 2.82 (t, *J* = 12.4 Hz, 3H), 1.90 – 1.38 (m, 10H), 1.28 – 0.98 (m, 6H); **^{13}C-NMR** (101 MHz, methanol-d^4): *δ* = 172.50, 158.27, 156.08, 136.16, 135.12, 134.58, 131.31, 125.47, 124.11, 117.07, 115.13, 113.34, 57.41, 54.16, 45.30, 43.01 (d, *J* = 403.7 Hz, 2C), 32.72, 27.14, 24.08, 22.60, 13.78 (d, *J* = 129.9 Hz, 2C) ppm; **ESI-MS**: *m/z* = 232.40 $[M+2H]^+$, 463.25 $[M+H]^+$, calc. 463.30.

4-((5-(Diethylcarbamoyl)-1-isopentyl-1H-benzo[d]imidazol-2-yl)methyl)phenyl heptylcarbamate (**11a**). *N*,*N*-Diethyl-2-(4-hydroxybenzyl)-1-isopentyl-1*H*-benzo[*d*]imidazole-5-carboxamide (**10a**) (80.0 mg, 203 µmol, 1.0 eq.) was dissolved in anhydrous DCM (2 mL) before TEA (42.2 µL, 30.8 mg, 305 µmol, 1.5 eq.) was added. After addition of heptyl isocyanate (49.1 µL, 43.0 mg, 305 µmol, 1.5 eq.), the solution was stirred for 12 h at rt. Then, the solution was diluted with DCM (18 mL) and washed with water (20 mL). The aqueous phase was extracted with DCM (2x20 mL) and the combined organic layers were dried over magnesium sulfate. Subsequent column chromatography (DCM:methanol, 24:1) yielded *4*-((5-(diethylcarbamoyl)-1-isopentyl-1*H*-benzo[*d*]imidazol-2-yl)methyl)phenyl heptylcarbamate (**11a**) (75.9 mg, 142 µmol, 70%) as a purple oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.79 (d, *J* = 1.3 Hz, 1H), 7.37 – 7.30 (m, 2H), 7.25 (d, *J* = 8.3 Hz, 2H), 7.09 (d, *J* = 8.3 Hz, 2H), 5.46 (t, *J* = 6.0 Hz, 1H), 4.33 (s, 2H), 4.05 – 3.93 (m, 2H), 3.63 – 3.32 (m, 4H), 3.25 (td, *J* = 6.7 Hz, 2H), 1.65 – 1.53 (m, 3H), 1.50 – 1.43 (m, 2H), 1.38 – 1.20 (m, 14H), 1.03 – 0.88 (m, 9H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 171.84 , 154.54, 153.90, 150.26, 142.08, 135.78, 132.81, 131.06, 129.24, 122.04, 121.38, 117.59, 109.52, 42.65, 41.28, 38.25, 33.95, 31.74, 29.81, 28.93, 26.72, 26.12, 22.59, 22.38, 14.08 ppm; ESI-MS: m/z = 535.45 $[M+H]^+$, calc. 535.36.

4-((5-(Diethylcarbamoyl)-1-isopentyl-1H-benzo[d]imidazol-2-yl)methyl)phenyl (6-(3,4-dihydroisoquinolin-2(1H)-yl)hexyl)carbamate (**11b**). 4-Nitrophenylchloroformate (50.9 mg, 252 µmol, 1.5 eq.) was dissolved in anhydrous DCM (3 mL) and cooled to 0°C before TEA (34.9 µL, 25.5 mg, 252 µmol, 1.5 eq.) was added. Then, (3,4-dihydroisoquinolin-2(1*H*)-yl)hexan-1-amine (39.0 mg, 168 µmol, 1.0 eq) dissolved in anhydrous DCM (3.5 mL) was added and the solution is stirred at rt for 4.5 h. The reaction mixture was then slowly added to a solution of *N*,*N*-diethyl-2-(4-hydroxybenzyl)-1-isopentyl-1*H*-benzo[*d*]imidazole-5-carboxamide (**10a**) (66.2 mg, 168 µmol, 1.0 eq.) and TEA dissolved in anhydrous DCM (2 mL) and stirred overnight. Subsequent column chromatography (DCM:methanol, 16:1) yielded the product 4-((5-(diethylcarbamoyl)-1-isopentyl-1*H*-benzo[*d*]imidazol-2-yl)methyl)phenyl (6-(3,4-dihydro-isoquinolin-2(1*H*)-yl)hexyl)carbamate (**11b**) (10.0 mg, 15.3 µmol, 9%) as a brown, highly viscous oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.75 (d, *J* = 1.3 Hz, 1H), 7.30 (dd, *J* = 7.8, 6.3 Hz, 2H), 7.21 (d, *J* = 8.2 Hz, 2H), 7.15 – 6.99 (m, 6H), 5.12 (t, *J* = 5.9 Hz, 1H), 4.29 (s, 2H), 4.04 – 3.92 (m, 2H), 3.70 (s, 2H), 3.61 – 3.32 (m, 4H), 3.24 (q, *J* = 6.6 Hz, 2H), 2.94 (t, *J* = 6.0 Hz, 2H), 2.81 (t, *J* = 6.0 Hz, 2H), 2.57 (t, *J* = 7.7 Hz, 2H), 1.66 (d, *J* = 8.0 Hz, 2H), 1.61 – 1.54 (m, 2H), 1.47 – 1.36 (m, 6H), 1.31 – 1.14 (s, 6H), 0.91 (d, *J* = 6.6 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 171.93, 154.59, 153.97, 150.30, 142.22, 135.90, 133.90, 133.05, 131.22, 129.39, 128.80, 126.76, 126.54, 125.96, 122.18, 122.15, 121.53, 117.75, 109.64, 57.88, 55.76, 50.78, 42.78, 41.26, 38.39, 34.10, 29.83, 28.48, 27.16, 26.75, 26.67, 26.25, 22.82, 22.50, 14.25 ppm; ESI-MS: m/z = 327.00 $[M+2H]^+$, 652.35 $[M+H]^+$, calc. 652.42.

4-((5-(Diethylcarbamoyl)-1-isopentyl-1H-benzo[d]imidazol-2-yl)methyl)phenyl pyrrolidine-1-carboxylate (**11c**). *N*,*N*-Diethyl-2-(4-hydroxybenzyl)-1-isopentyl-1*H*-benzo[*d*]imidazole-5-carboxamide (**10a**) (80.0 mg, 203 µmol, 1.0 eq.) was dissolved in anhydrous THF (3 mL) before sodium hydride (60% in paraffin oil, 9.77 mg, 244 µmol, 1.2 eq.) was added and the solution was stirred for 5 min. Then, pyrrolidine-1-carbonyl chloride (27.0 µL, 32.7 mg, 244 µmol, 1.2 eq.) was added. The solution was stirred overnight at rt, diluted with DCM (15 mL) and washed with citric acid (2.5% in water, 15 ml) and brine (15 mL). Subsequent column chromatography (DCM:methanol, 8:1) yielded 4-((5-

(diethylcarbamoyl)-1-isopentyl-1*H*-benzo[*d*]imidazol-2-yl)methyl)phenyl pyrrolidine-1-carboxylate (**11c**) (77.0 mg, 157 µmol, 77%) as a off-white oil. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 7.72 (d, *J* = 1.2 Hz, 1H), 7.29 – 7.22 (m, 2H), 7.18 (d, *J* = 8.4 Hz, 2H), 7.04 (d, J = 8.5 Hz, 2H), 4.26 (s, 2H,), 3.94 (t, 2H), 3.59 – 3.28 (m, 8H), 1.93 – 1.82 (m, 4H), 1.59 – 1.49 (m, 1H,), 1.44 – 1.36 (m, 2H), 1.19 (t, J = 12.2 Hz, 6H), 0.88 (d, J = 6.6 Hz, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$): *δ* = 171.78, 153.89, 152.97, 150.54, 142.03, 135.77, 132.71, 131.10, 129.19, 122.16, 121.38, 117.57, 109.53, 46.45, 46.35, 42.65, 38.23, 33.98, 26.11, 25.80, 24.97, 22.38 ppm; **ESI-MS**: *m/z* = 491.30 $[M+H]^+$, calc. 491.30.

4-((5-(Diethylcarbamoyl)-1-isopentyl-1H-benzo[d]imidazol-2-yl)methyl)phenyl azetidine-1-carboxylate (**11d**). *N*,*N*-Diethyl-2-(4-hydroxybenzyl)-1-isopentyl-1*H*-benzo[*d*]imidazole-5-carboxamide (**10a**) (45.0 mg, 114 µmol, 1.0 eq.) was dissolved in anhydrous THF (2.5 mL) before potassium *tert*-butoxide (16.6 mg, 148 µmol, 1.3 eq.) was added and the solution was stirred for 5 min. Then, 4-nitrophenyl azetidine-1-carboxylate (30.4 mg, 137 µmol, 1.2 eq.) was added. The solution was stirred overnight at 55°C, then diluted in DCM (30 mL), washed with water (25 mL), ammonium chloride (saturated, 25 mL) and brine (25 mL). Subsequent column chromatography (DCM:methanol, 24:1) yielded 4-((5-(diethylcarbamoyl)-1-isopentyl-1*H*-benzo[*d*]imidazol-2-yl)methyl)phenyl azetidine-1-carboxylate (**11d**) (41.1 mg, 86.2 µmol, 76%) as a off-white oil. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 7.74 (s, 1H), 7.33 – 7.25 (m, 2H, arom.), 7.20 (d, *J* = 8.3 Hz, 2H), 7.04 (d, *J* = 8.4 Hz, 2H), 4.28 (s, 2H), 4.20 – 4.05 (m, 4H), 3.99 – 3.92 (m, 2H), 3.61 – 3.27 (m, 4H), 2.30 (p, 2H), 1.62 – 1.50 (m, 1H), 1.45 – 1.38 (m, 2H), 1.26 – 1.15 (m, 6H), 0.90 (d, *J* = 6.6 Hz, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$): *δ* = 171.84, 154.15, 153.90, 150.31, 142.03, 135.81, 132.89, 131.22, 129.31, 122.09, 121.51, 117.64, 109.62, 50.32, 49.30, 42.75, 38.30, 34.03, 26.20, 22.44, 15.84 ppm; **ESI-MS**: *m/z* = 477.30 $[M+H]^+$, calc. 477.28.

4-((5-(Diethylcarbamoyl)-1-(2-(piperidin-1-yl)ethyl)-1H-benzo[d]imidazol-2-yl)methyl)phenyl heptylcarbamate (**11e**). *N,N*-diethyl-2-(4-hydroxybenzyl)-1-(2-(piperidin-1-yl)ethyl)-1*H*-benzo[*d*]imidazole-5-carboxamide (**10b**) (75.0 mg, 172 µmol, 1.0 eq.) was dissolved in anhydrous DCM (2 mL) before TEA (35.8 µL, 26.1 mg, 258 µmol, 1.5 eq.) was added. After addition of heptyl isocyanate (38.8 µL, 34.0 mg, 241 µmol, 1.4 eq.), the solution was stirred for 12 h at rt. Then, the solution was diluted with DCM (10 mL) and washed with water (10 mL). The aqueous phase was extracted with DCM (2x15 mL) and the combined organic layers were washed with brine (30 ml) and dried over magnesium sulfate. Subsequent column chromatography (DCM:methanol, 17:1) yielded 4-((5-(diethylcarbamoyl)-1-(2-(piperidin-1-yl)ethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)phenyl heptylcarbamate (**11e**) (73.5 mg, 128 µmol, 74%) as a yellow oil. **^{1}H-NMR** (400 MHz, $CDCl_3$): *δ* = 7.73 (s, 1H), 7.29 (s, 2H), 7.19 (d, *J* = 8.3 Hz, 2H,), 7.03 (d, *J* = 8.3 Hz, 2H), 5.23 (bs, 1H), 4.34 (s, 2H), 4.09 (t, *J* = 7.2 Hz, 2H), 3.59 – 3.28 (m, 4H), 3.20 (td, *J* = 6.7 Hz, 2H), 2.45 – 2.27 (m, 6H), 1.57 – 1.48 (m, 6H), 1.43 – 1.37 (m, 2H), 1.33 – 1.13 (m, 14H), 0.86 (t, *J* = 6.7 Hz, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$): *δ* = 171.86, 154.56, 154.51, 150.25, 142.20, 135.90, 133.11, 131.22, 129.32, 122.11, 121.48, 117.68, 109.63, 57.74, 55.09, 42.30, 41.35, 33.93, 31.79, 29.88, 28.99, 26.78, 26.00, 24.11, 22.65, 14.13 ppm; **ESI-MS**: *m/z* = 288.90 $[M+2H]^+$, 576.40 $[M+H]^+$, calc. 576.39.

4-((5-(Diethylcarbamoyl)-1-(4-(piperidin-1-yl)butyl)-1H-benzo[d]imidazol-2-yl)methyl)phenyl heptylcarbamate (**11f**). *N*,*N*-diethyl-2-(4-hydroxybenzyl)-1-(4-(piperidin-1-yl)butyl)-1*H*-benzo[*d*]imidazole-5-carboxamide (**10c**) (60.0 mg, 130 µmol, 1.0 eq.) was dissolved in anhydrous DCM (2 mL) before TEA (27.0 µL, 19.7 mg, 195 µmol, 1.5 eq.) was added. After addition of heptyl isocyanate (31.4 µL, 27.5 mg, 169 µmol, 1.3 eq.), the solution was stirred for 12 h at rt. Then, the solution was diluted with DCM (10 mL) and washed with water (10 mL). The aqueous phase was extracted with DCM (2x15 mL) and the combined organic layers were washed with brine (30 ml) and dried over magnesium sulfate. Subsequent column chromatography (DCM:methanol, 19:1 to 6:1) yielded 4-((5-(diethylcarbamoyl)-1-(4-(piperidin-1-yl)butyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)phenyl heptylcarbamate (**11f**) (67.7 mg, 112 µmol, 86%) as a yellow oil. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 7.72 (s, 1H, arom.), 7.31 – 7.26 (m, 2H), 7.20 (d, *J* = 8.2 Hz, 2H), 7.01 (d, *J* = 8.3 Hz, 2H), 5.32 (t, *J* = 5.5 Hz, 1H,), 4.28 (s, 2H), 3.98 (t, *J* = 7.5 Hz, 2H), 3.51 – 3.30 (m, 4H), 3.18 (td, *J* = 6.7 Hz, 2H), 2.58 – 2.42 (m, 4H), 2.32 (t, *J* = 7.6 Hz, 2H), 1.71 – 1.62 (m, 4H), 1.54 – 1.37 (m, 8H), 1.32 – 1.13 (m, 14H), 0.85 (t, *J* = 6.7 Hz, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$): *δ* =171.88, 154.64, 153.93, 150.24, 142.03, 135.84, 133.14, 131.23, 129.39, 122.22, 121.48, 117.62, 109.75, 57.61, 53.94, 43.96, 41.38, 34.06, 31.79, 29.86, 28.98, 27.19, 26.80, 24.84, 23.68, 23.06, 22.64, 14.12.13 ppm; **ESI-MS**: *m/z* = 604.40 $[M+H]^+$, calc. 604.42.

Ethyl 4-(isopentylamino)-3-nitrobenzoate (**12**). Ethyl 4-Fluoro-3-nitrobenzoate (3.50 g, 16.4 mmol, 1.0 eq.) was dissolved in ethanol (35 mL). Isoamylamin (2.09 mL, 1.57 g, 18.0 mmol, 1.1 eq.) and triethylamine (3.40 mL, 2.48 g, 24.6 mmol, 1.5 eq.) were added at rt. The solution was then heated to 55°C for 75 min. The solvent was evaporated under reduced pressure, the residue was taken up in ethyl acetate (150 mL) and washed with citric acid (2.5% in

water, 100 mL), ammonium chloride (saturated, 100 mL) and brine (100 mL) before the organic layer was dried over sodium sulfate and the solvent was evaporated under reduced pressure yielding ethyl 4-(isopentylamino)-3-nitrobenzoate (**12**) (4.54 g, 16.2 mmol, 99%) as an orange oil. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 8.85 (d, *J* = 1.9 Hz, 1H), 8.29 (bs, 1H), 8.03 (dd, *J* = 9.1, 2.1 Hz, 1H, arom.), 6.85 (d, *J* = 9.1 Hz, 1H), 4.34 (q, *J* = 7.1, 2H), 3.35 (td, *J* = 7.4, 5.1 Hz, 2H), 1.83 – 1.70 (m, 1H), 1.64 (q, *J* = 7.1 Hz, 2H), 1.38 (t, *J* = 7.1, 3H), 0.98 (dd, *J* = 6.6, 0.9 Hz, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$): δ = 165.28, 147.81, 136.44, 131.25, 129.54, 117.44, 113.54, 61.09, 41.60, 37.75, 26.08, 22.54, 14.49 ppm; **ESI-MS**: m/z = 281.10 $[M+H]^+$, calc. 291.15.

Ethyl 3-amino-4-(isopentylamino)benzoate (**13**). Ethyl 4-(isopentylamino)-3-nitrobenzoate (**12**) (3.50 g, 12.5 mmol, 1.0 eq.) was dissolved in methanol (160 mL). After adding palladium on carbon (10wt. %, 350 mg), the flask was flushed three times with hydrogen. After stirring for 2.5 h at rt, the mixture was filtrated over celite and the solvent was evaporated under reduced pressure, yielding ethyl 3-amino-4-(isopentylamino)benzoate (**13**) (3.08 g, 12.3 mmol, 98%) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.60 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.41 (d, *J* = 2.0 Hz, 1H), 6.59 (d, *J* = 8.3 Hz, 1H), 4.31 (q, *J* = 7.1 Hz, 2H), 3.51 (s, 2H), 3.21 – 3.14 (m, 2H), 1.79 – 1.69 (m, 1H), 1.57 (td, *J* = 7.1 Hz, 2H), 1.36 (t, *J* = 7.1 Hz, 3H), 0.96 (d, J = 6.6 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 167.20, 143.20, 132.07, 124.43, 119.16, 118.25, 109.45, 60.34, 42.06, 38.50, 26.20, 22.73, 14.59 ppm; ESI-MS: m/z = 251.15 $[M+H]^+$, calc. 251.17.

Ethyl 3-(2-(4-(benzyloxy)phenyl)acetamido)-4-(isopentylamino)benzoate (**14**). 2-(4-(Benzyloxy)phenyl)acetic acid (2.98 g, 12.3 mmol, 1.1 eq.), HBTU (4.67 g, 12.3 mmol, 1.1 eq.) and TEA (2.33 mL, 1.70 g, 16.8 mmol, 1.5 eq.) were dissolved in DMF (50 mL), before ethyl 3-amino-4-(isopentylamino)benzoate (**13**) (2.80 g, 11.2 mmol, 1.0 eq.) in DMF (25 mL) was added. The solution was then stirred for 3 h at rt and the solvent was evaporated under reduced pressure. The residue was taken up in ethyl acetate and washed with ammonium chloride (saturated 120 mL), citric acid (2.5% in water, 120 mL), sodium bicarbonate (saturated, 120 mL) and brine (120 mL). Subsequent column chromatography (ethyl acetate:petroleum ether, 2:3) yielded ethyl 3-(2-(4-(benzyloxy)phenyl)acetamido)-4-(isopentylamino)benzoate (**14**) (1.81 g, 3.81 mmol, 34%) as a brown oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.84 (dd, *J* = 8.6, 2.0 Hz, 1H), 7.77 (s, 1H), 7.48 – 7.39 (m, 5H), 7.38 – 7.31 (m, 3H), 7.04 (d, *J* = 8.6 Hz, 2H), 5.10 (s, 2H), 4.33 (q, *J* = 7.1 Hz, 2H), 3.75 (s, 2H), 3.12 (t, *J* = 7.5 Hz, 2H), 1.71 – 1.61 (m, 1H), 1.50 (td, *J* = 7.2 Hz, 2H), 1.37 (t, *J* = 7.1 Hz, 3H), 0.94 (d, *J* = 6.6 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 170.89, 166.54, 158.60, 147.34, 136.84, 130.67, 130.28, 128.78, 128.23, 128.03, 127.61, 127.03, 121.69, 118.62, 115.90, 110.90, 70.28, 43.45, 41.80, 38.28, 26.11, 22.71 ppm; ESI-MS: m/z = 475.25 $[M+H]^+$, calc. 475.26.

2-(4-(Benzyloxy)benzyl)-1-isopentyl-1H-benzo[d]imidazole-5-carboxylic acid (**15**). 3-(2-(4-(Benzyloxy)phenyl)acetamido)-4-(isopentylamino)benzoate (**14**) (200 mg, 421 µmol, 1.0 eq.) was dissolved in acetic acid (8 mL) and stirred for 1.5 h at 135°C. The solution was then basified (pH 12) with lithium hydroxide (0.5 M in water/MeOH, 1:1), the pH was adjusted to 12 with aqueous sodium hydroxide (30%) and the solution was refluxed overnight. After evaporation of THF under reduced pressure, the solution was acidified (pH 2) by aqueous hydrochloric acid (6 M) and extracted with DCM (3x150 mL), yielding 2-(4-(benzyloxy)benzyl)-1-isopentyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid (**15**) (119 mg, 277 µmol, 66% global yield) as a white solid (m.p. 211.0°C). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.69 (s, 1H), 8.09 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.36 (ddd, *J* = 15.6, 9.1, 4.9 Hz, 7H), 7.21 (d, *J* = 8.4 Hz, 2H), 6.92 (d, *J* = 8.4 Hz, 2H), 5.02 (s, 2H), 4.45 (s, 2H), 4.05 – 4.00 (m, 2H), 1.62 – 1.56 (m, 1H), 1.37 – 1.31 (m, 2H,), 0.91 (d, *J* = 6.6 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 192.53, 170.76, 158.16, 143.35, 137.00, 134.60, 129.89, 129.80, 129.14, 128.71, 128.10, 127.53, 125.00, 122.02, 115.53, 109.42, 70.21, 43.06, 38.13, 29.57, 26.33, 22.48 ppm; ESI-MS: m/z = 429.20 $[M+H]^+$, calc. 429.21

(2-(4-(Benzyloxy)benzyl)-1-isopentyl-1H-benzo[d]imidazol-5-yl)(piperidin-1-yl)methanone (**16a**). 2-(4-(Benzyloxy)benzyl)-1-isopentyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid (**15**) (73.0 mg, 170 µmol, 1.0 eq.), HBTU (77.3 mg, 204 µmol, 1.2 eq.) and TEA (70.5 µL, 51.5 mg, 510 µmol, 3.0 eq.) were dissolved in DMF (6 mL), before piperidine (20.2 µL, 17.4 mg, 204 µmol, 1.2 eq.) was added. The solution was then stirred for 3 h at rt and the solvent was evaporated under reduced pressure. The residue was taken up in ethyl acetate and washed with citric acid (2.5% in water, 2x90 mL), sodium bicarbonate (saturated, 2x90 mL) and brine (90 mL). Subsequent column chromatography (DCM:MeOH, 11:1) yielded ethyl (2-(4-(benzyloxy)benzyl)-1-isopentyl-1*H*-benzo[*d*]imidazol-5-yl)(piperidin-1-yl)methanone (**16a**) (99.0 mg, 200 µmol, quant.) as a brown oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.77 (s, 1H, arom.), 7.41 – 7.23 (m, 7H), 7.13 (d, *J* = 8.2 Hz, 2H), 6.86 (d, *J* = 8.2 Hz, 2H), 4.97 (s, 2H), 4.29 (s, 2H), 4.04 – 3.92 (m, 2H), 3.76 – 3.31 (m, 4H), 1.66 – 1.48 (m, 7H), 1.32 – 1.27 (m, 2H), 0.85 (d, *J* = 6.6 Hz, 6H); ^{13}C-NMR (101

MHz, $CDCl_3$): δ = 170.38, 158.08, 154.08, 136.84, 134.89, 131.27, 129.75, 128.59, 127.99, 127.41, 127.21, 122.69, 117.08, 115.40, 110.22, 70.06, 43.06, 37.86, 32.84, 29.47, 26.15, 24.60, 22.34 ppm; ESI-MS: m/z = 496.25 $[M+H]^+$, calc. 496.26.

2-(4-(Benzyloxy)benzyl)-N,1-diisopentyl-1H-benzo[d]imidazole-5-carboxamide (**16b**). 2-(4-(Benzyloxy)benzyl)-1-isopentyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid (**15**) (56.0 mg, 131 µmol, 1.0 eq.), HBTU (59.5 mg, 157 µmol, 1.2 eq.) and TEA (54.4 µL, 39.7 mg, 393 µmol, 3.0 eq.) were dissolved in DMF (3 mL), before isopentyl amine (18.3 µL, 13.7 mg, 157 µmol, 1.2 eq.) was added. The solution was then stirred overnight at rt and the solvent was evaporated under reduced pressure. The residue was taken up in ethyl acetate (25 mL) and washed with citric acid (2.5% in water, 2x20 mL), potassium carbonate (saturated, 20 mL) and brine (20 mL). Subsequent column chromatography (DCM:MeOH, 34:1) yielded 2-(4-(benzyloxy)benzyl)-*N*,1-diisopentyl-1*H*-benzo[*d*]imidazole-5-carboxamide (**16b**) (55.0 mg, 110 µmol, 84%) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.09 (s, 1H), 7.78 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.41 – 7.24 (m, 6H), 7.17 – 7.10 (m, 2H), 6.92 – 6.86 (m, 2H), 6.32 (t, *J* = 5.7 Hz, 1H), 5.01 (s, 2H), 4.24 (s, 2H), 4.01 – 3.90 (m, 2H), 3.54 – 3.44 (m, 2H), 1.75 – 1.64 (m, 1H), 1.59 – 1.48 (m, 3H), 1.41 – 1.33 (m, 2H), 0.95 (d, *J* = 6.6 Hz, 6H), 0.88 (d, *J* = 6.7 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 168.01, 157.98, 154.84, 142.14, 137.40, 136.93, 129.61, 129.14, 128.63, 128.24, 128.03, 127.44, 122.10, 117.85, 115.34, 109.41, 70.12, 42.78, 38.65, 38.52, 38.18, 33.81, 26.19, 26.08, 22.60, 22.42 ppm; ESI-MS: m/z = 498.30 $[M+H]^+$, calc. 498.31.

2-(4-(Benzyloxy)benzyl)-1-isopentyl-N-phenyl-1H-benzo[d]imidazole-5-carboxamide (**16c**). 2-(4-(Benzyloxy)benzyl)-1-isopentyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid (**15**) (23.0 mg, 53.6 µmol, 1.0 eq.), HBTU (24.4 mg, 204 µmol, 1.2 eq.) and TEA (22.3 µL, 16.3 mg, 161 µmol, 3.0 eq.) were dissolved in DMF (2 mL), before aniline (5.87 µL, 5.99 mg, 64.3 µmol, 1.2 eq.) was added. The solution was then stirred overnight at rt and the solvent was evaporated under reduced pressure. The residue was taken up in ethyl acetate (25 mL) and washed with citric acid (2.5% in water, 2x20 mL), potassium carbonate (saturated, 20 mL) and brine (20 mL). Subsequent column chromatography (DCM:MeOH, 12:1) yielded ethyl 2-(4-(benzyloxy)benzyl)-1-isopentyl-N-phenyl-1*H*-benzo[*d*]imidazole-5-carboxamide (**16c**) (37.7 mg, 37.7 µmol, 70%) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.25 (s, 1H), 7.88 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.73 – 7.68 (m, 2H), 7.42 – 7.31 (m, 8H), 7.17 – 7.10 (m, 3H), 6.90 (d, *J* = 8.6 Hz, 2H), 5.03 (s, 2H), 4.25 (s, 2H), 4.03 – 3.96 (m, 2H), 1.62 – 1.52 (m, 1H), 1.45 – 1.34 (m, 2H), 0.90 (d, *J* = 6.6 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 166.39, 158.08, 155.19, 141.97, 138.55, 137.72, 136.97, 129.68, 129.37, 129.16, 128.71, 128.11, 128.09, 127.52, 124.35, 122.49, 120.26, 118.17, 115.43, 109.80, 70.20, 42.93, 38.24, 33.80, 26.29, 22.48 ppm; ESI-MS: m/z = 504.25 $[M+H]^+$, calc. 504.26.

(2-(4-Hydroxybenzyl)-1-isopentyl-1H-benzo[d]imidazol-5-yl)(piperidin-1-yl)methanone (**17a**). 2-(4-(Benzyloxy)benzyl)-1-isopentyl-1*H*-benzo[*d*]imidazol-5-yl)(piperidin-1-yl)methanone (**16a**) (99.0 mg, 200 µmol, 1.0 eq) was dissolved in ethanol (12 mL). After adding palladium on carbon (10wt. %, 9.90 mg), the flask was flushed three times with hydrogen. After stirring overnight at rt, the mixture was filtrated over celite and the solvent was evaporated under reduced pressure, yielding (2-(4-hydroxybenzyl)-1-isopentyl-1*H*-benzo[*d*]imidazol-5-yl)(piperidin-1-yl)methanone (**17a**) (49.0 mg, 121 µmol, 61%) as a yellow oil. ^{1}H-NMR (400 MHz, methanol-d^4): δ = 7.77 – 7.71 (m, 2H), 7.50 (dd, *J* = 8.6, 1.3 Hz, 1H), 7.13 (d, *J* = 8.2 Hz, 2H), 6.78 (d, *J* = 8.3 Hz, 2H), 4.44 (s, 2H), 4.35 – 4.28 (m, 2H), 3.79 – 3.66 (m, 2H), 3.42 – 3.34 (m, 2H), 1.70 – 1.51 (m, 7H), 1.45 – 1.39 (m, 2H,), 0.92 (d, *J* = 6.7 Hz, 6H); ^{13}C-NMR (101 MHz, methanol-d^4): δ = 171.37, 158.50, 155.91, 135.46, 135.15, 134.18, 131.20, 125.08, 124.79, 117.09, 115.55, 113.18, 44.81, 38.49, 32.63, 27.32, 25.38, 22.65 ppm; ESI-MS: m/z = 406.25 $[M+H]^+$, 811.45 $[2M+H]^+$, calc. 406.24.

2-(4-Hydroxybenzyl)-N,1-diisopentyl-1H-benzo[d]imidazole-5-carboxamide (**17b**). 2-(4-(Benzyloxy)benzyl)-N,1-diisopentyl-1*H*-benzo[*d*]imidazole-5-carboxamide (**16b**) (55.0 mg, 110 µmol, 1.0 eq) was dissolved in methanol (5 mL). After adding palladium on carbon (10wt. %, 5.50 mg), the flask was flushed three times with hydrogen. After stirring overnight at rt, the mixture was filtrated over celite and the solvent was evaporated under reduced pressure, yielding 2-(4-hydroxybenzyl)-*N*,1-diisopentyl-1*H*-benzo[*d*]imidazole-5-carboxamide (**17b**) (44.0 mg, 108 µmol, 98%) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.07 (s, 1H), 7.81 (d, *J* = 8.5 Hz, 1H), 7.31 – 7.25 (m, 1H), 6.87 (t, *J* = 15.5 Hz, 3H), 6.71 (d, *J* = 8.0 Hz, 2H), 4.18 (s, 2H), 4.00 (t, *J* = 8.4 Hz, 2H), 3.43 (td, *J* = 6.6 Hz, 2H), 1.65 – 1.45 (m, 4H), 1.42 – 1.33 (m, 2H), 0.90 – 0.85 (m, 12H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 167.88, 156.79, 154.93, 139.10, 136.34, 130.06, 129.59, 125.16, 123.10, 116.82, 116.35, 110.08, 43.10, 38.74, 38.53, 38.07, 32.98, 26.20, 26.08, 22.58, 22.41 ppm; ESI-MS: m/z = 408.24 $[M+H]^+$, calc. 408.26.

2-(4-Hydroxybenzyl)-1-isopentyl-N-phenyl-1H-benzo[d]imidazole-5-carboxamide (**17c**). *2*-(4-(Benzyloxy)benzyl)-*N*,1-diisopentyl-1*H*-benzo[*d*]imidazole-5-carboxamide (**16c**) (19.0 mg, 37.7 µmol, 1.0 eq) was dissolved in methanol (2.5 mL). After adding palladium on carbon (10wt. %, 1.9 mg), the flask was flushed three times with hydrogen. After stirring overnight at rt, the mixture was filtrated over celite and the solvent was evaporated under reduced pressure, yielding 2-(4-hydroxybenzyl)-1-isopentyl-*N*-phenyl-1*H*-benzo[*d*]imidazole-5-carboxamide (**17c**) (15.1 mg, 36.5 µmol, 97%) as a yellow oil. **^{1}H-NMR** (400 MHz, methanol-d^{4}): δ = 8.36 (s, 1H), 8.21 (d, *J* = 8.6 Hz, 1H) 1H), 7.97 (d, *J* = 8.7 Hz, 1H), 7.74 – 7.68 (m, 2H), 7.38 (t, *J* = 7.8 Hz, 2H), 7.23 (d, *J* = 8.3 Hz, 2H), 7.17 (t, *J* = 7.4 Hz, 2H), 6.87 (d, *J* = 8.2 Hz, 2H), 4.59 (s, 2H), 4.52 – 4.44 (m, 2H), 1.78 – 1.68 (m, 1H), 1.54 (dt, *J* = 12.1, 6.7 Hz, 2H), 0.99 (d, *J* = 6.6 Hz, 6H); **^{13}C-NMR** (101 MHz, methanol-d^{4}): δ = 167.06, 159.04, 155.88, 142.12, 139.64, 135.37, 135.05, 131.53, 129.87, 126.84, 125.90, 123.47, 122.35, 117.41, 115.24, 113.89, 45.50, 38.29, 31.97, 27.38, 22.61 ppm; **ESI-MS**: *m/z* = 414.20 $[M+H]^{+}$, calc. 414.21.

4-((1-Isopentyl-5-(piperidine-1-carbonyl)-1H-benzo[d]imidazol-2-yl)methyl)phenyl heptylcarbamate (**18a**). (2-(4-Hydroxybenzyl)-1-isopentyl-1*H*-benzo[*d*]imidazol-5-yl)(piperidin-1-yl)methanone (**17a**) (49.0 mg, 121 µmol, 1.0 eq.) was dissolved in anhydrous DCM (3 mL) before TEA (25.1 µL, 18.4 mg, 182 µmol, 1.5 eq.) was added. After addition of heptyl isocyanate (27.2 µL, 23.8 mg, 169 µmol, 1.4 eq.), the solution was stirred for 36 h at rt. Then, the solution was diluted with DCM (18 mL), washed with water (20 mL) and ammonium chloride (saturated, 20 mL). The combined aqueous layers was extracted with DCM (2x20 mL) and the combined organic layers were washed with brine (80 mL) and dried over magnesium sulfate. Subsequent column chromatography (DCM:methanol, 24:1) yielded 4-((1-isopentyl-5-(piperidine-1-carbonyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)phenyl heptylcarbamate (**18a**) (29.5 mg, 53.9 µmol, 45%) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.79 (s, 1H), 7.36 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.23 (d, *J* = 8.3 Hz, 2H), 7.07 (d, *J* = 8.3 Hz, 2H), 5.17 (t, *J* = 5.9 Hz, 1H), 4.31 (s, 2H), 4.03 – 3.94 (m, 2H), 3.80 – 3.42 (m, 4H), 3.30 – 3.15 (m, 2H), 1.71 – 1.52 (m, 9H), 1.48 – 1.40 (m, 2H), 1.35 – 1.26 (m, 8H), 0.95 – 0.86 (m, 9H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 170.94, 154.53, 153.97, 150.33, 141.88, 135.98, 132.85, 130.42, 129.35, 122.13, 122.08, 118.29, 109.64, 42.78, 41.38, 38.33, 34.00, 31.82, 29.90, 29.01, 26.80, 26.20, 24.80, 22.67, 22.46, 14.15 ppm; ESI-MS: m/z = 547.35 $[M+H]^{+}$, calc. 547.36.

4-((1-Isopentyl-5-(isopentylcarbamoyl)-1H-benzo[d]imidazol-2-yl)methyl)phenyl heptylcarbamate (**18b**). 2-(4-Hydroxybenzyl)-*N*,1-diisopentyl-1*H*-benzo[*d*]imidazole-5-carboxamide (**17b**) (44.0 mg, 108 µmol, 1.0 eq.) was dissolved in anhydrous DCM (2.5 mL) before TEA (25.5 µL, 18.6 mg, 184 µmol, 1.7 eq.) was added. After addition of heptyl isocyanate (26.0 µL, 22.8 mg, 162 µmol, 1.5 eq.), the solution was stirred for 36 h at rt. Then, the solution was diluted with DCM (18 mL), washed with water (20 mL) and ammonium chloride (saturated, 20 mL). The combined aqueous layers was extracted with DCM (2x20 mL) and the combined organic layers were washed with brine (80 mL) and dried over magnesium sulfate. Subsequent column chromatography (DCM:methanol, 30:1) yielded 4-((1-isopentyl-5-(isopentylcarbamoyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)phenyl heptylcarbamate (**18b**) (45.0 mg, 82.0 µmol, 76%) as an off-white oil. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 8.07 (d, *J* = 1.5 Hz, 1H), 7.76 (dd, *J* = 8.5, 1.6 Hz, 1H.), 7.25 (dd, *J* = 8.1, 2.0 Hz, 1H), 7.18 (d, *J* = 8.5 Hz, 2H), 7.03 (d, *J* = 8.2 Hz, 2H), 6.30 (bs, 1H), 5.24 (t, *J* = 8.0 Hz, 1H), 4.27 (s, 2H), 3.95 (t, *J* = 8.2 Hz, 2H), 3.53 – 3.32 (m, 2H), 3.21 (td, *J* = 6.8 Hz, 2H), 1.72 – 1.62 (m, 1H), 1.58 – 1.47 (m, 5H), 1.40 (q, *J* = 7.5 Hz, 2H), 1.33 – 1.24 (m, 8H), 0.95 – 0.83 (m, 15H); **^{13}C-NMR** (101 MHz, $CDCl_3$): δ = 167.99, 154.53, 154.37, 150.33, 142.05, 137.33, 132.68, 129.31, 129.22, 122.20, 122.12, 117.88, 109.47, 42.80, 41.36, 38.65, 38.53, 38.28, 33.92, 31.79, 29.87, 28.98, 26.78, 26.18, 26.07, 22.64, 22.60, 22.41, 14.13 ppm; **ESI-MS**: *m/z* = 549.35 $[M+H]^{+}$, calc. 549.38.

4-((1-Isopentyl-5-(phenylcarbamoyl)-1H-benzo[d]imidazol-2-yl)methyl)phenyl heptylcarbamate (**18c**). 2-(4-Hydroxybenzyl)-1-isopentyl-*N*-phenyl-1*H*-benzo[*d*]imidazole-5-carboxamide (**17c**) (15.1 mg, 36.5 µmol, 1.0 eq.) was dissolved in anhydrous DCM (1.5 mL) before TEA (10.1 µL, 7.37 mg, 73.0 µmol, 2.0 eq.) was added. After addition of heptyl isocyanate (9.39 µL, 8.23 mg, 58.4 µmol, 1.6 eq.), the solution was stirred for 36 h at rt. Then, the solution was diluted with DCM (18 mL), washed with water (20 mL) and ammonium chloride (saturated, 20 mL). The combined aqueous layers was extracted with DCM (2x20 mL) and the combined organic layers were washed with brine (80 mL) and dried over magnesium sulfate. Subsequent column chromatography (DCM:ethyl acetate, 2:1) yielded 4-((1-isopentyl-5-(phenylcarbamoyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)phenyl heptylcarbamate (**18c**) (6.2 mg, 11.2 µmol, 31%) as an off-white oil. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 8.24 (d, *J* = 1.5 Hz, 1H), 8.17 (s, 1H), 7.89 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.69 (d, *J* = 7.7 Hz, 3H), 7.41 – 7.33 (m, 3H), 7.21 (d, *J* = 8.3 Hz, 2H), 7.14 (t, *J* = 7.4 Hz, 1H), 7.07 (d, *J* = 8.5 Hz, 2H), 5.02 (t, *J* = 5.9 Hz, 1H), 4.31 (s, 2H), 4.08 – 3.97 (m, 2H), 3.24 (td, *J* = 6.7 Hz, 2H), 1.64 – 1.52

(m, 3H), 1.49 – 1.42 (m, 2H), 0.93 (d, *J* = 6.6 Hz, 6H), 0.90 – 0.86 (m, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$): δ = 166.23, 154.67, 154.51, 150.45, 141.61, 138.49, 137.56, 132.49, 129.57, 129.46, 129.19, 124.42, 122.70, 122.26, 120.27, 118.05, 109.96, 43.03, 41.45, 38.36, 33.90, 31.87, 29.95, 29.06, 26.84, 26.29, 22.72, 22.49, 14.20 ppm; **ESI-MS**: *m/z* = 555.35 $[M+H]^+$, calc. 555.33.

Melting points

Melting point were measured with OptiMelt MPA-100, an automated melting point system from Stanford Research Systems. The device records a sigmoidal melting curve, wherein the inflection point is reported as the melting point.

Radioligand binding of 11d: competition assay against [^{3}H]CP55940

Cannabinoid receptors

Radioligand binding studies. Radioligand binding studies were performed as previously described by our group. *h*CB_2-HEK cells were a kind gift from AbbVie Laboratories (Chicago, U.S.). Cells were grown in Dulbecco's modified Eagle's medium containing high glucose supplemented with 8% fetal calve serum and 25 µg/mL zeocin in a 37 °C incubator in the presence of 5% CO_2. The respective *h*CB_2R membranes were prepared as described in the literature.[1]

Saturation and competition assays were done according to the protocol previously established in M. Decker's research group.[1]

Competition assays were done with 5–10 concentrations (0.1 nM to 0.4 mM) of target compound (previously isomerized to their cis or trans states with light of 366 or 454 nm, respectively) and 0.6 nM [^{3}H]CP55940. The positive controls for the assays over CB_2R were respectively the selective ligands rimonabant and compound **4**.Reactions were started by adding membrane (25 µg/well for rCB1 or 8 µg/well for *h*CB_2R) of a 96 well Multiscreen filter plate (Millipore) containing the radioligand in assay buffer (50 mM Tris-HCl, pH = 7.4; 5 mM MgCl2·6H2O; 2.5 mM EDTA; 2 mg/mL BSA). After 3 h incubation at room temperature, the reaction was stopped by vacuum filtration and each well was washed 4 times with cold binding buffer (50 mM Tris-HCl, pH = 7.4; 5 mM $MgCl_2$·6H2O; 2.5 mM EDTA). The filter plate was dried at 45 °C. Lately, 20 µL of IRGA Safe plus-scintillation cocktail (PerkinElmer) was added to each well. The activity was counted in a Micro Beta Trilux counter (Wallac). The stock solutions of all tested compounds (5 mM) were prepared by dissolving in DMSO. The dilution series of all stock solutions were prepared diluting with binding buffer. Statistical analyses and sigmoidal dose–response curve fittings were performed with GraphPad Prism 6 for Windows (version 6.01, September 21, 2012).

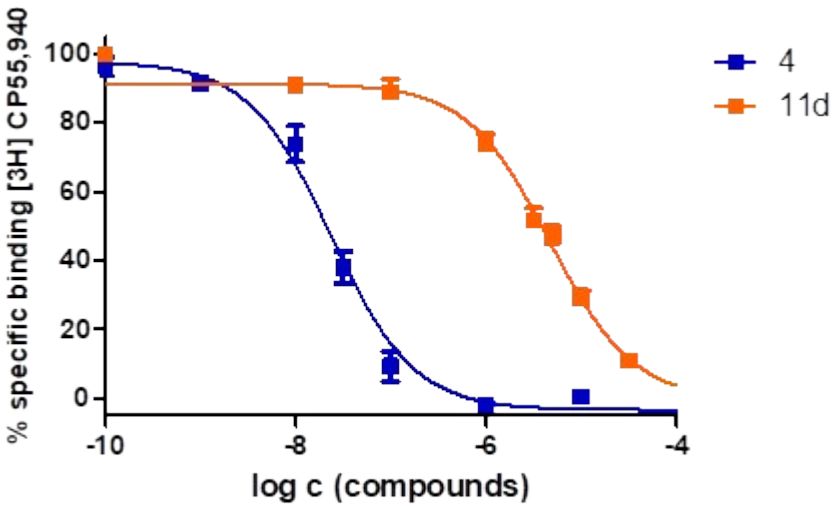

Figure S 1. Radioligand binding of reference **4** and **11d**: competition assay against [^{3}H]CP55940 using *h*CB2-HEK cells.

Molecular docking studies on AChE

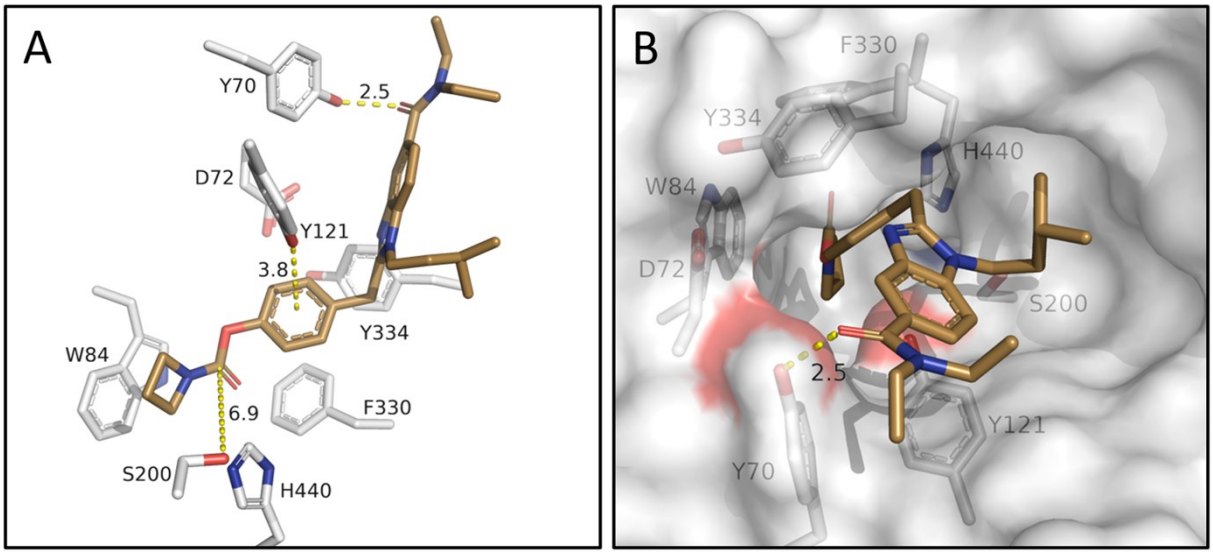

Figure S 2. Binding pose of **11d** at the AChE (PDB: 1GQR) suggested by a docking approach according to the procedure explained under Molecular Docking Studies. The crystal structure of the AChE belongs to the *Tetronarce californica* and was chosen because of the high resolution of 2.20 Å and the complex formed with pseudoirreversible inhibitor rivastigmine.[2] (A) Display of repulsive effects between Tyr121 and the benzene ring system of compound **11d** (brown). (B) Interaction of the amide of inhibitor **11d** (brown) with Tyr70 and the spatial demand of this compound in comparison with the surface of the enzyme.

The rate of carbamylation of the active serine of both *h*BChE and *h*AChE is closely related to the entrance of the carbamate moiety into the gorge of the esterases. It will be determined by the space available for the binding of the inhibitors and the interactions with the amino acids of the enzymes. Selectivity will thereby be mainly a result of the differences between the two active pockets of the two respective serine-hydrolases.[2]
Several previous studies have investigated exactly these differences between the *h*AChE and the *h*BChE.[3-5] The overall active-site gorge of *h*BChE is about 200 Å^3 larger than that of *h*AChE.[5] Fang *et al.* have calculated differences of the radii at the main doors available at each tetramer of the hydrolases and have thereby shown, that the entrance at the main door of the *h*AChE has only a maximum radius of 2.30 Å (*h*BChE: 4.00 Å).[3] Herein, we present a series of benzimidazoles, which are substituted with different aliphatic, spatially challenging moieties (Table **1**). Even with the isopentyl residue, as the least sterically demanding scaffold at this position, the entry of the inhibitors at the larger main door of the *h*BChE should be highly preferred. (Fig. S 2) The described selectivity over the *h*AChE might consequently be driven additionally by the spatial demand of the tested inhibitors. This assumption is even strengthened by **11e**, which resulted in a drastic loss of absolute activity at *h*AChE.

NMR spectra of non-target compounds

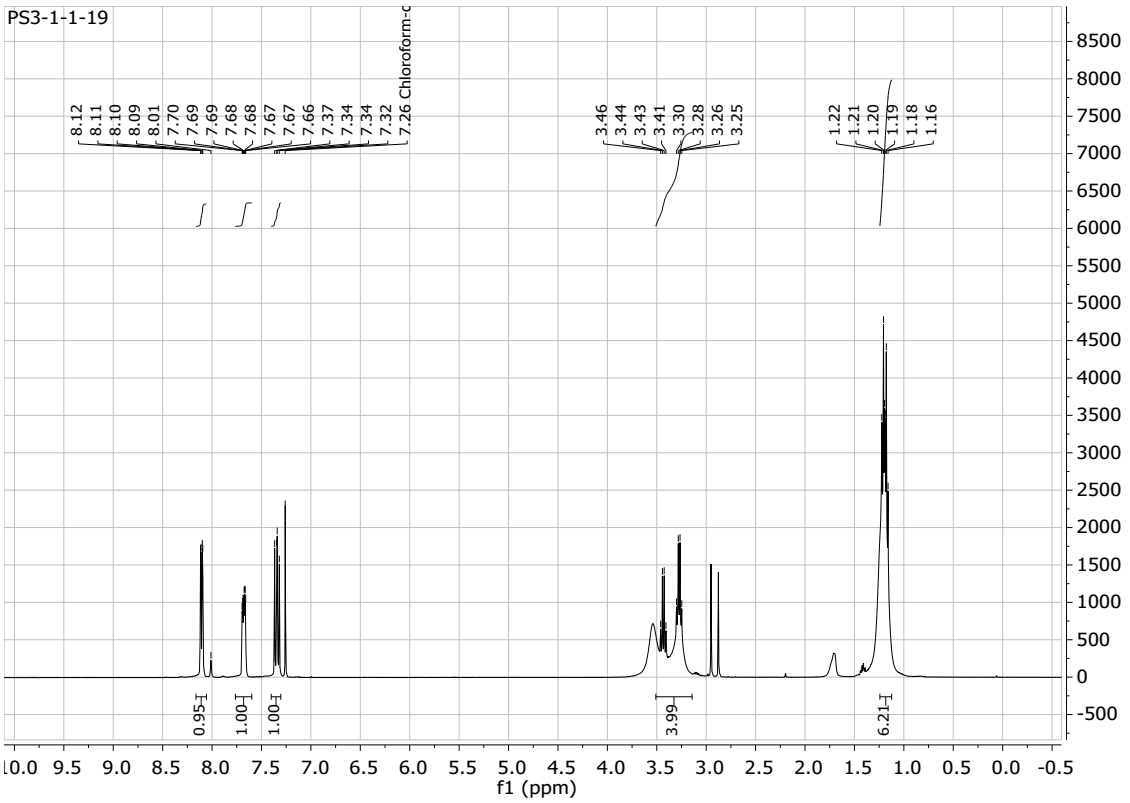

Figure S 3. Proton-NMR of **5** in deutero-chloroform.

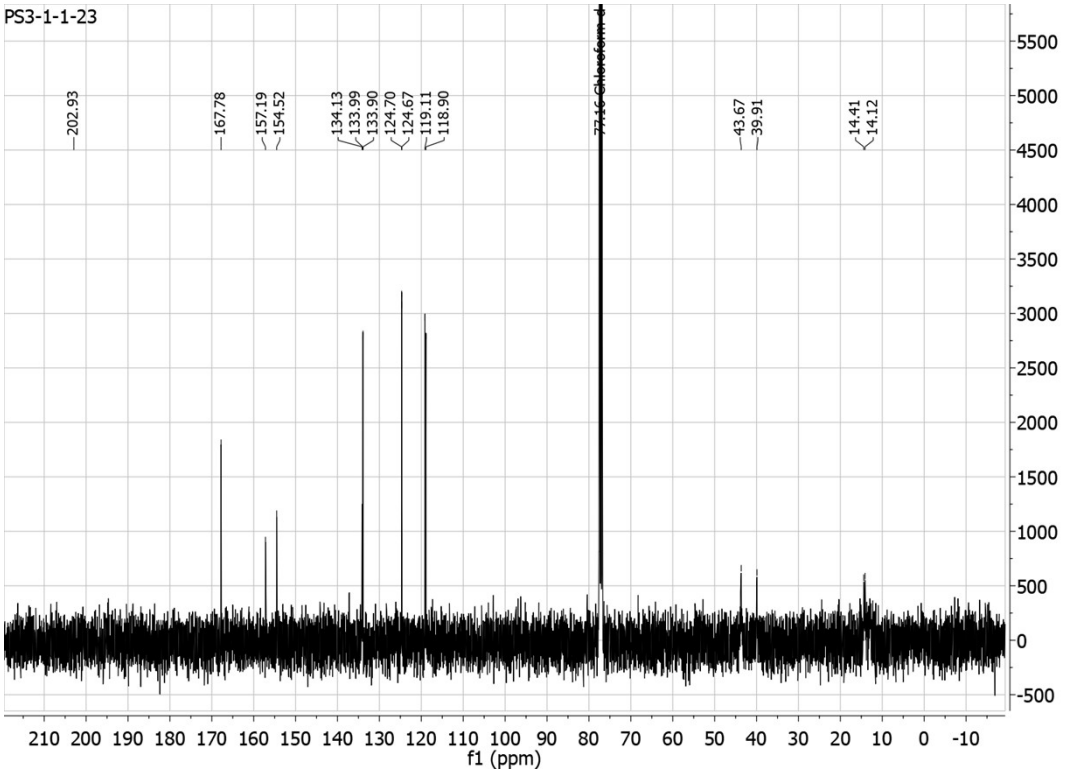

Figure S 4. ^{13}C-NMR spectrum of **5** in deutero-chloroform

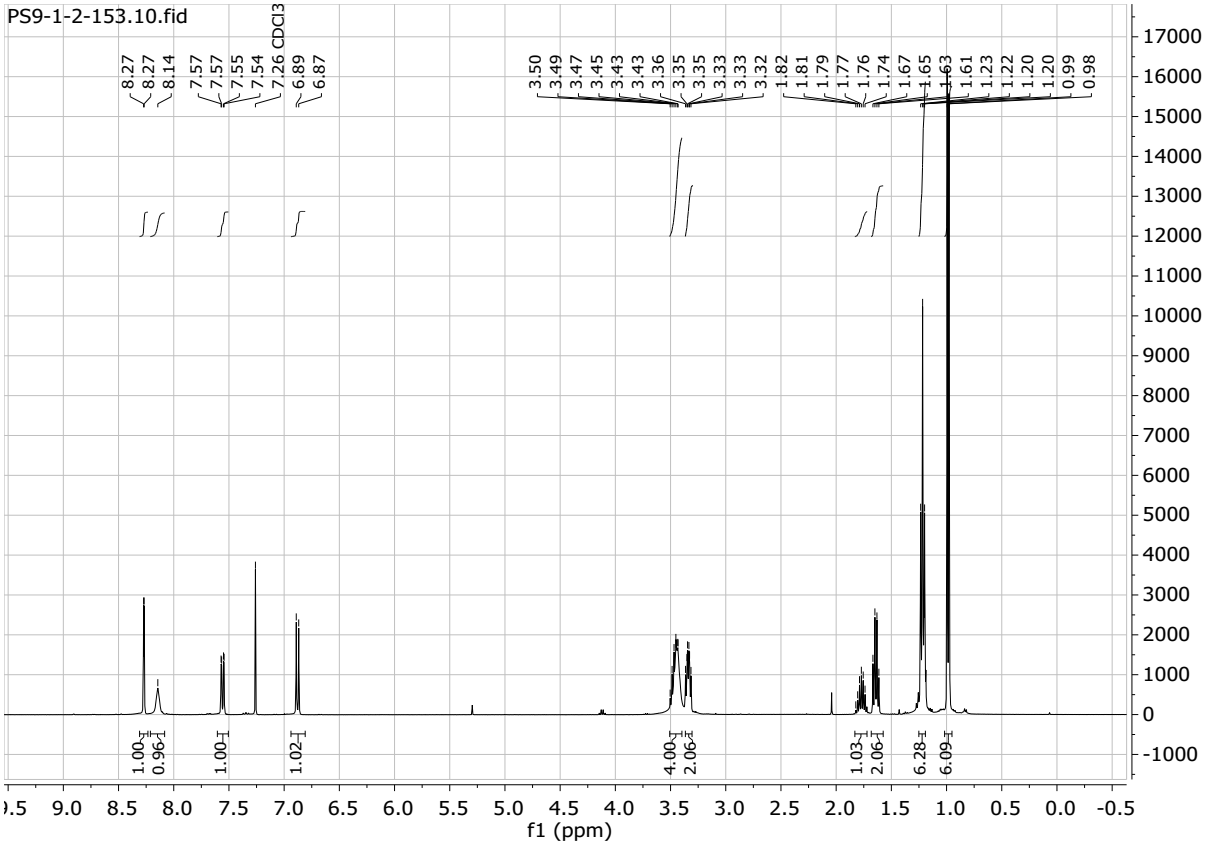

Figure S 5. Proton-NMR of **6a** in deutero-chloroform.

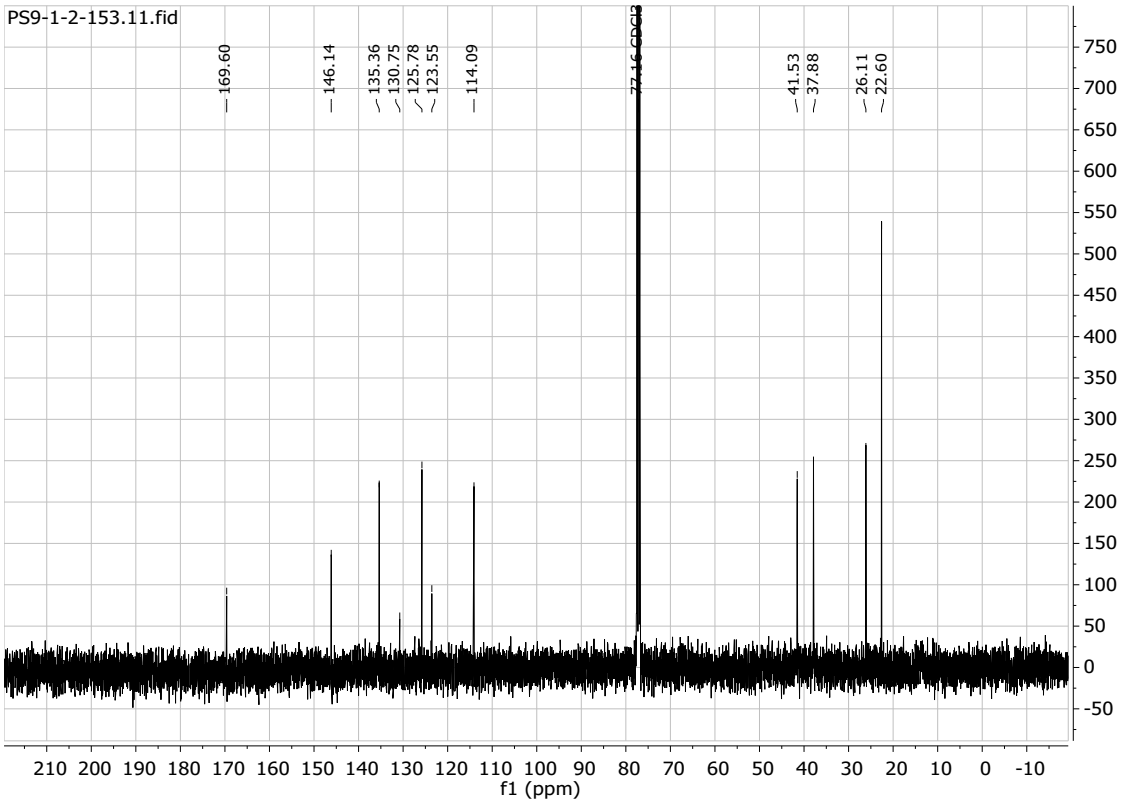

Figure S 6. ^{13}C-NMR spectrum of **6a** in deutero-chloroform.

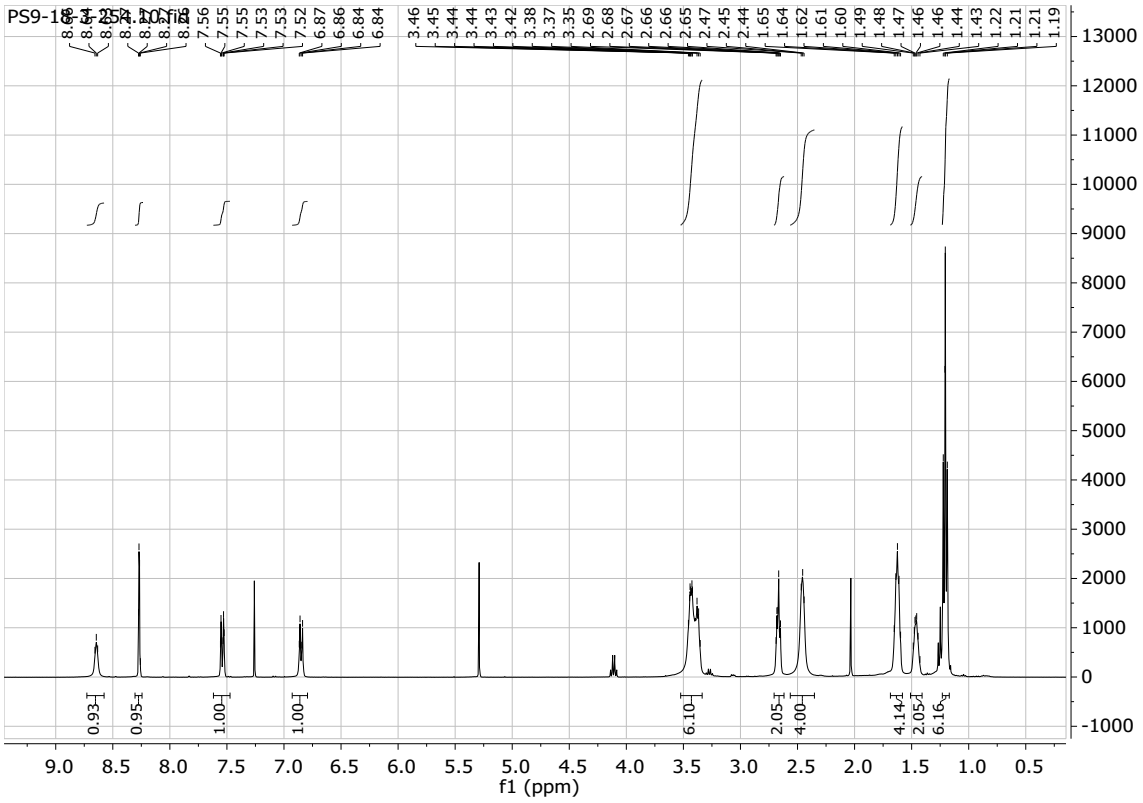

Figure S 7. Proton-NMR of **6b** in deutero-chloroform.

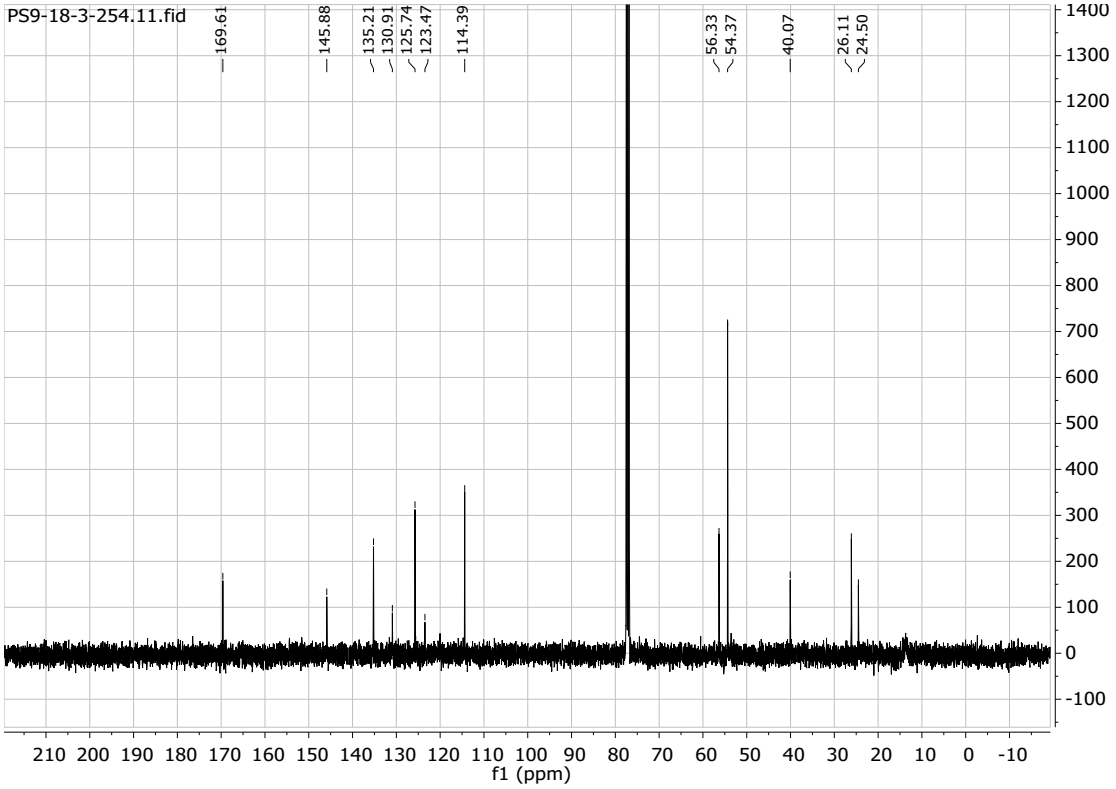

Figure S 8. ^{13}C-NMR spectrum of **6b** in deutero-chloroform.

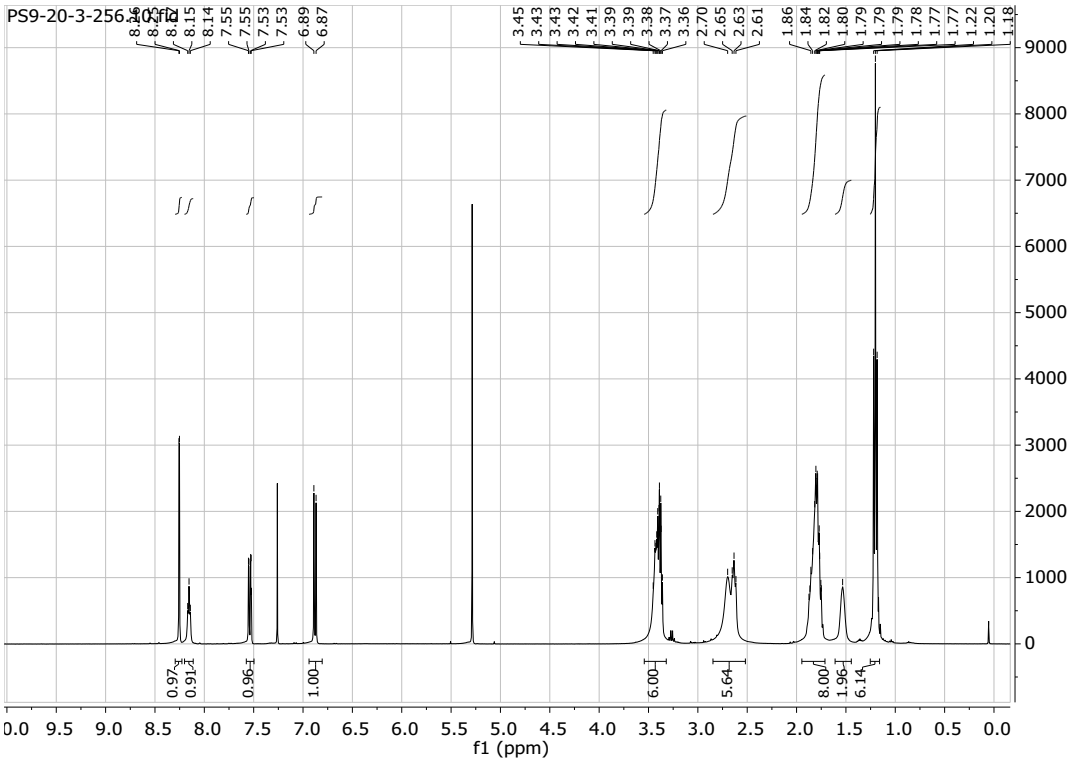

Figure S 9. Proton-NMR of **6c** in deutero-chloroform.

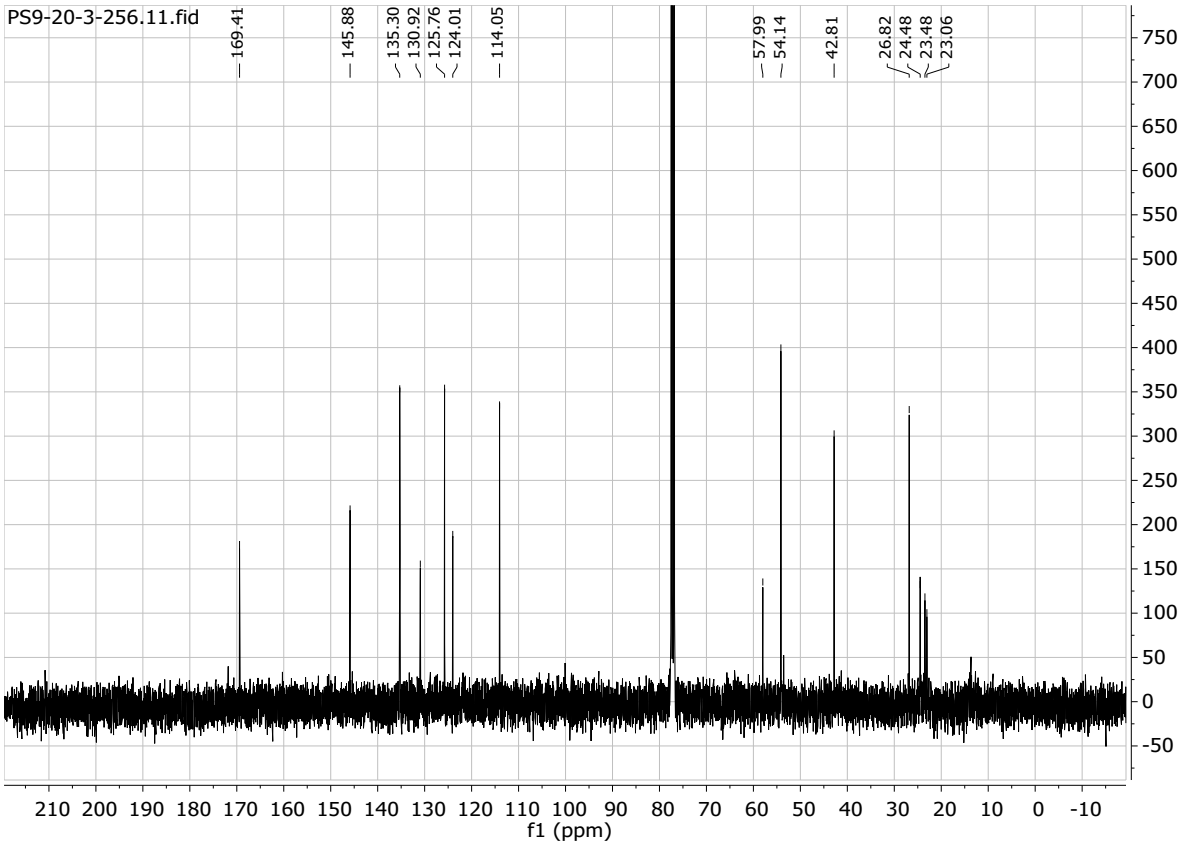

Figure S 10. ^{13}C-NMR spectrum of **6c** in deutero-chloroform.

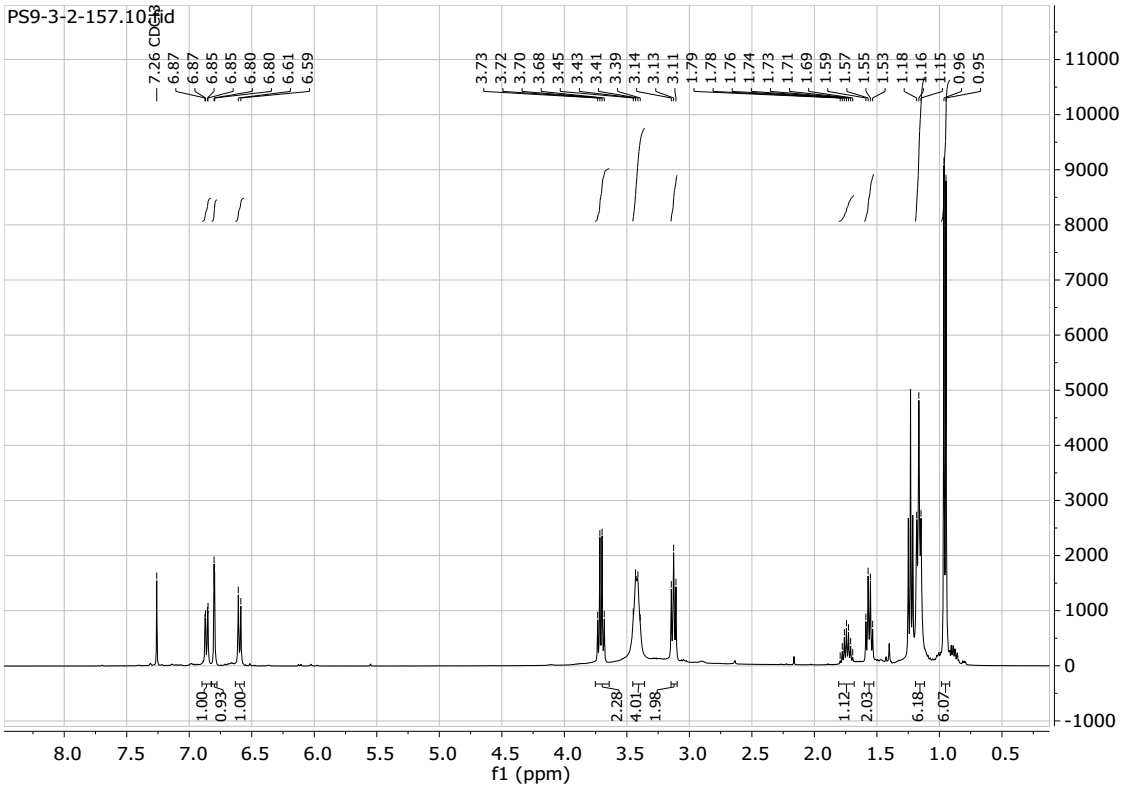

Figure S 11. Proton-NMR of **7a** in deutero-chloroform.

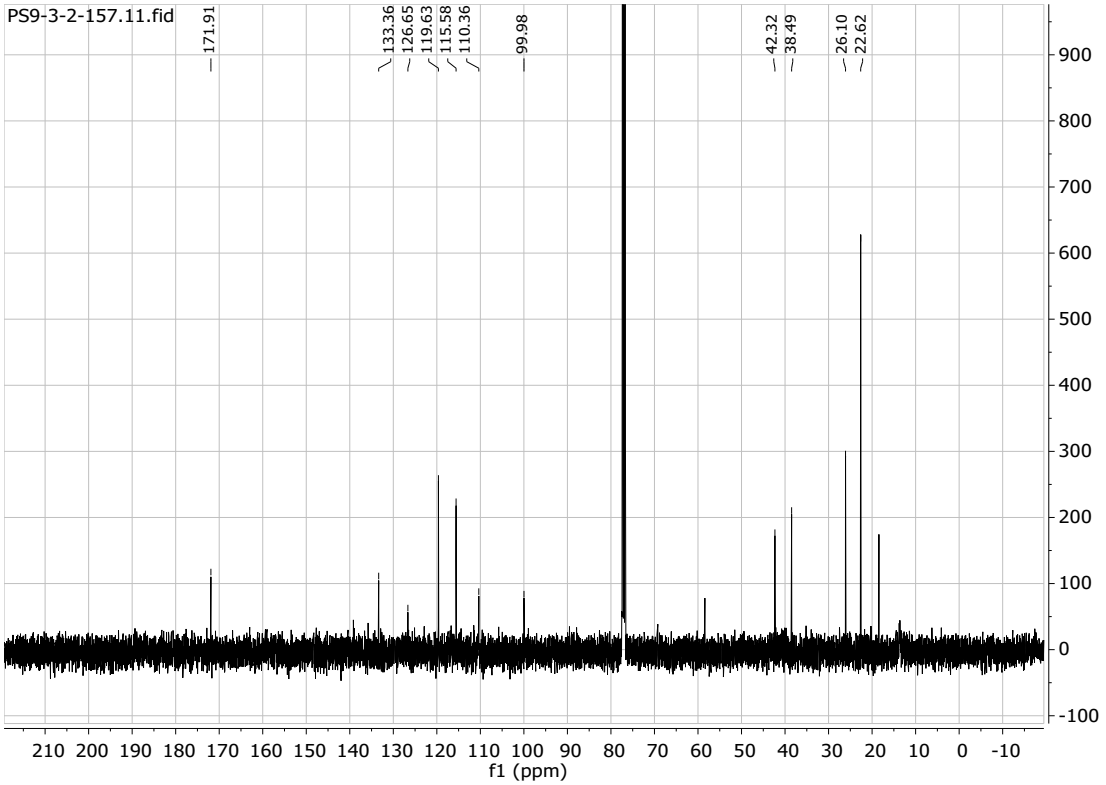

Figure S 12. ^{13}C-NMR spectrum of **7a** in deutero-chloroform.

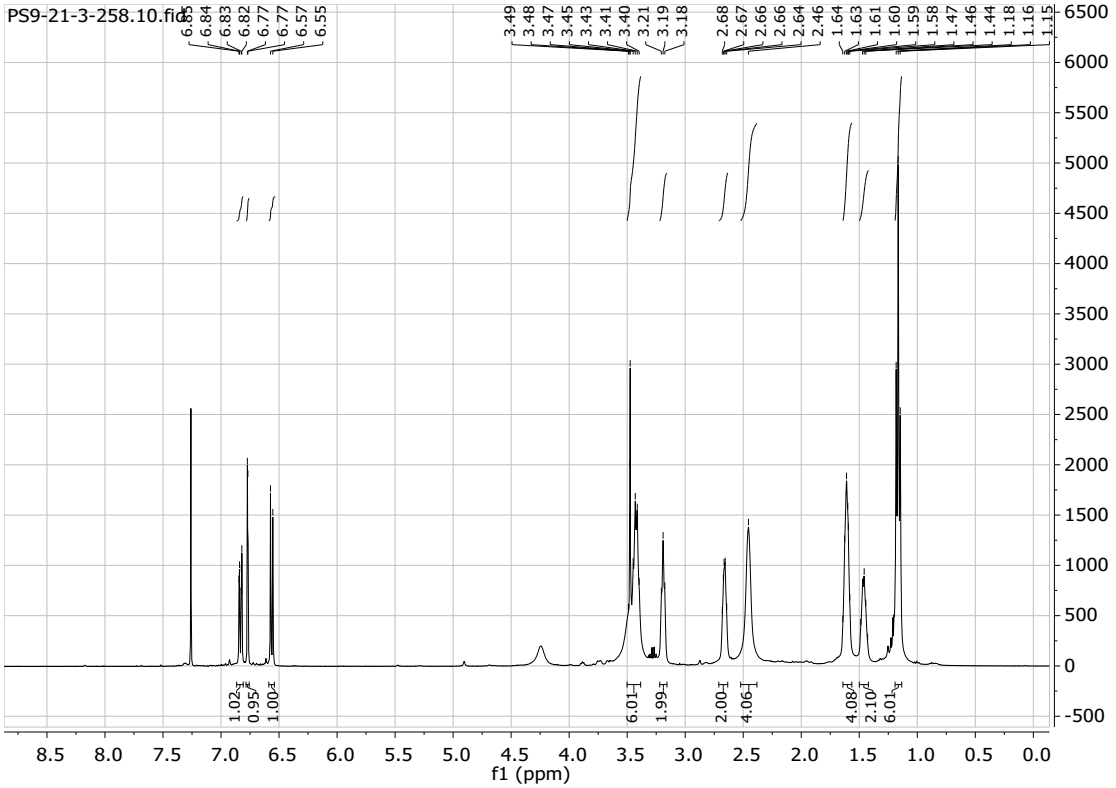

Figure S 13. Proton-NMR of **7b** in deutero-chloroform.

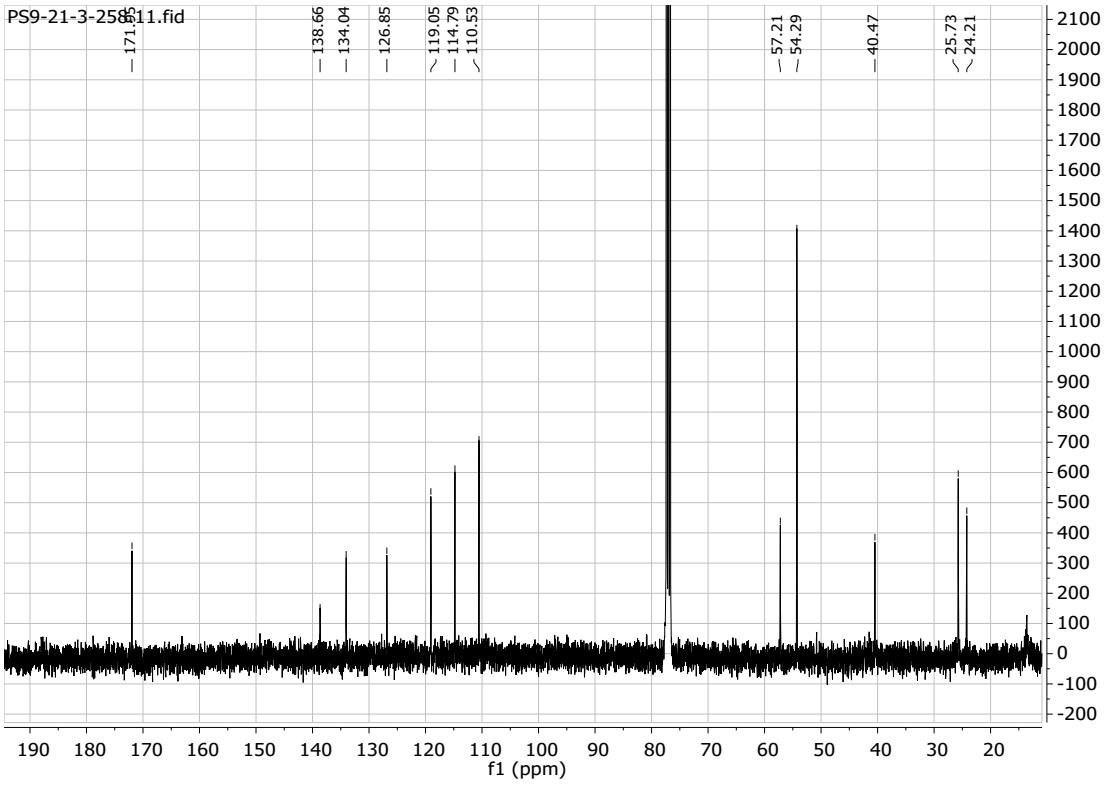

Figure S 14. ^{13}C-NMR spectrum of **7b** in deutero-chloroform.

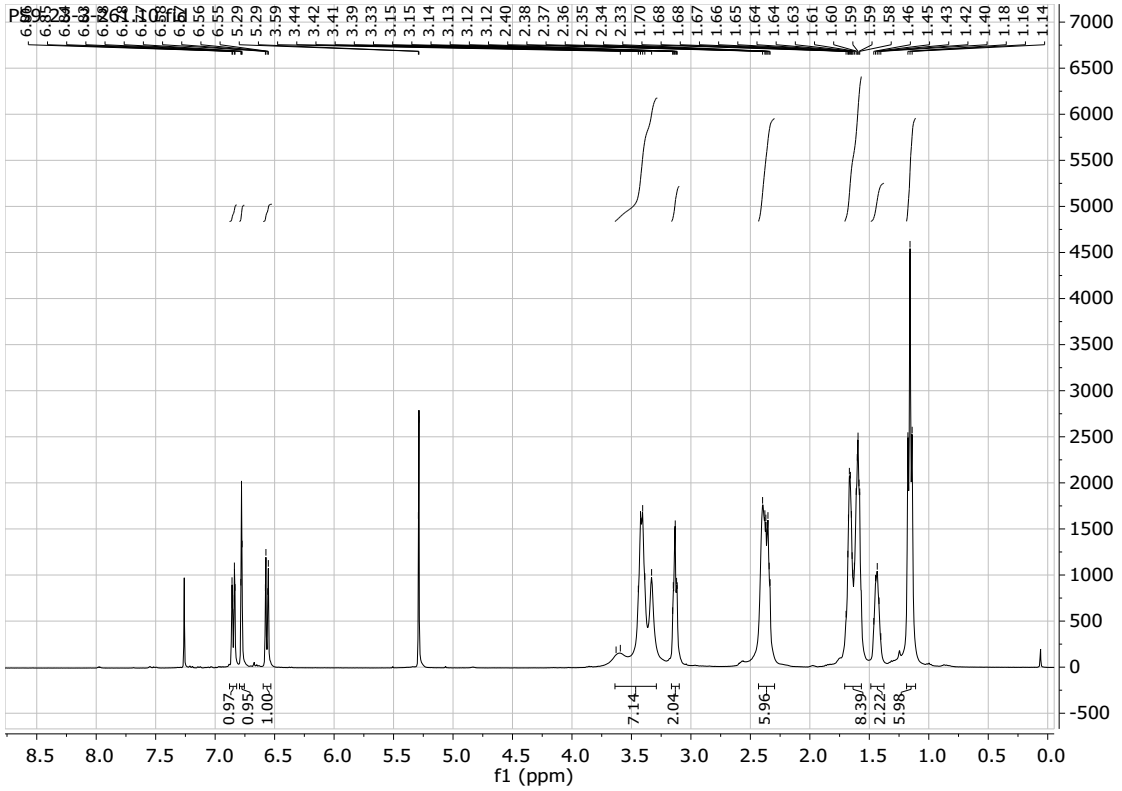

Figure S 15. Proton-NMR of **7c** in deutero-chloroform.

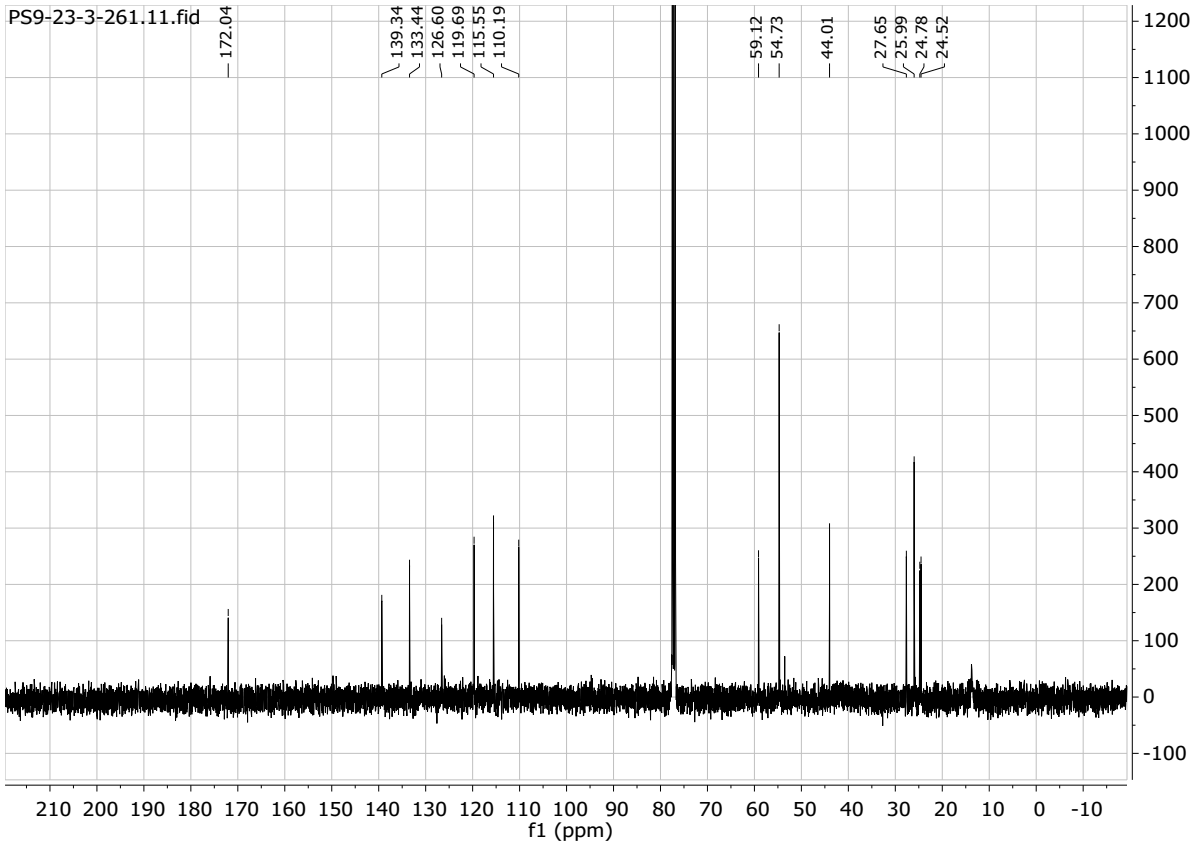

Figure S 16. 13C-NMR spectrum of **7c** in deutero-chloroform.

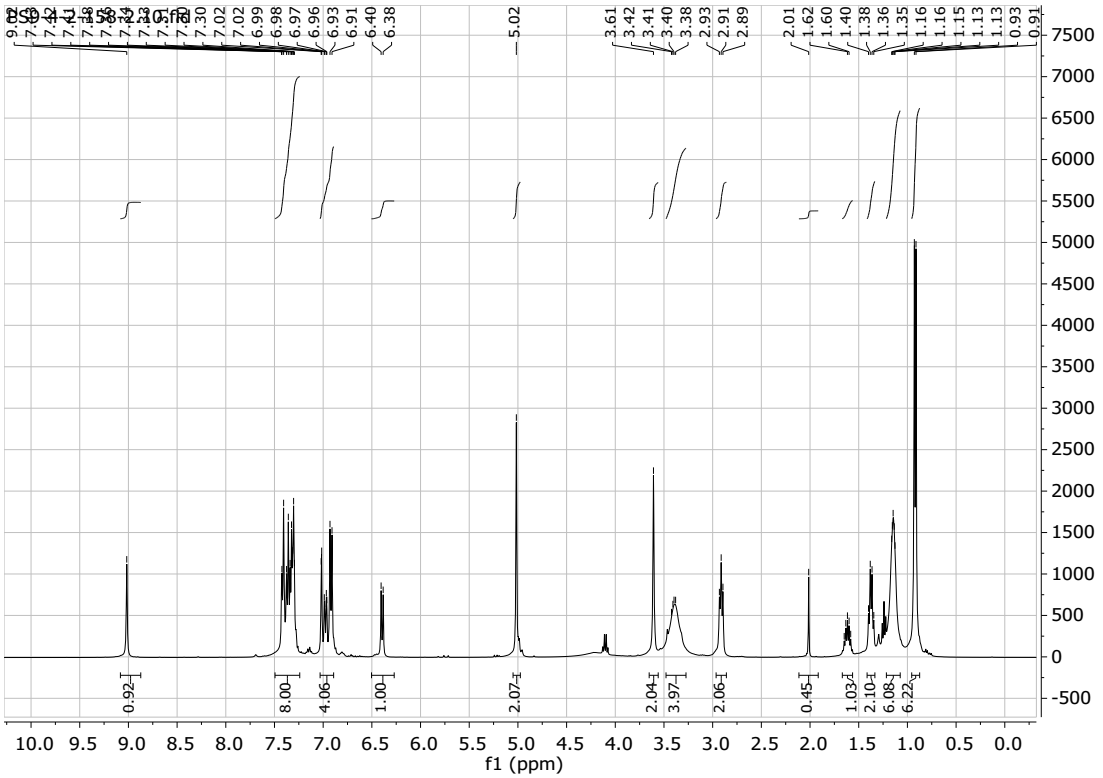

Figure S 17. Proton-NMR of **8a** in deutero-chloroform.

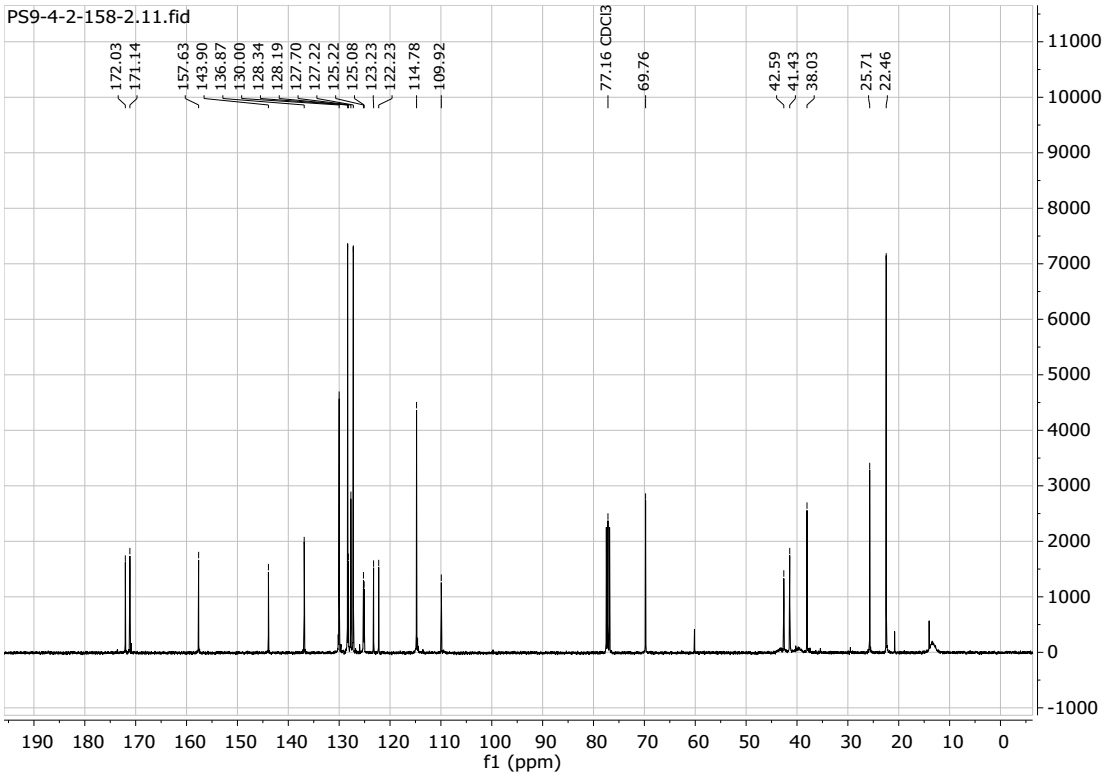

Figure S 18. ^{13}C-NMR spectrum of **8a** in deutero-chloroform.

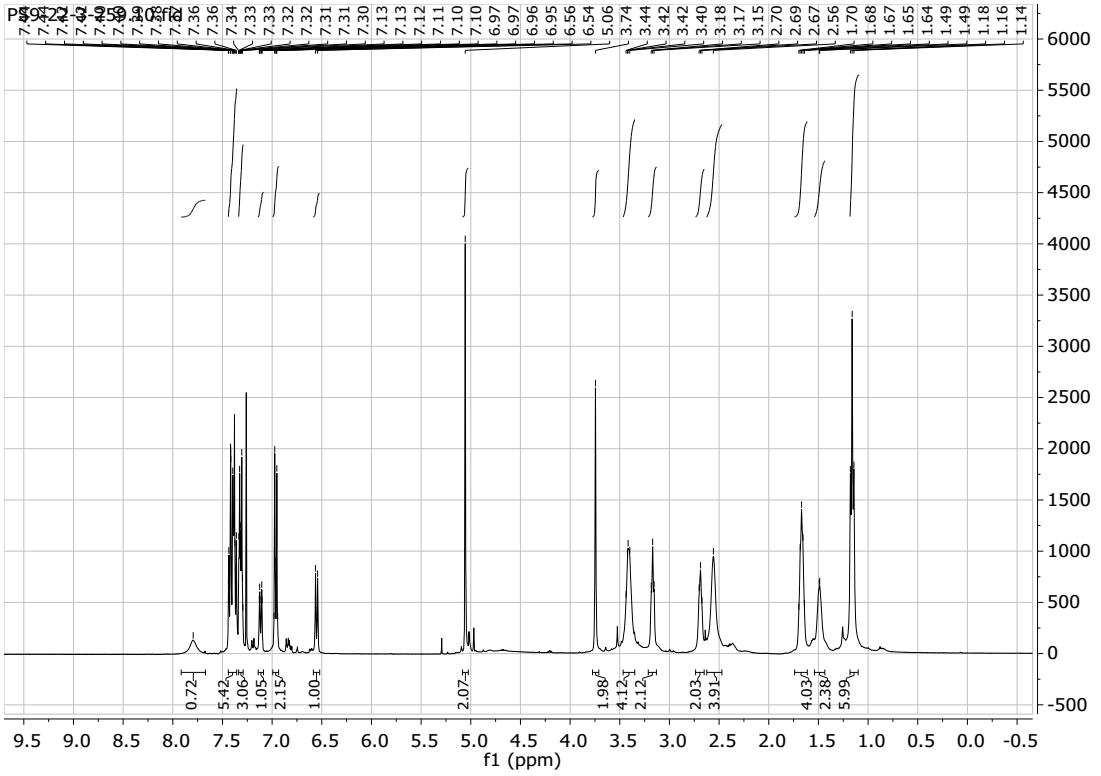

Figure S 19. Proton-NMR of **8b** in deutero-chloroform.

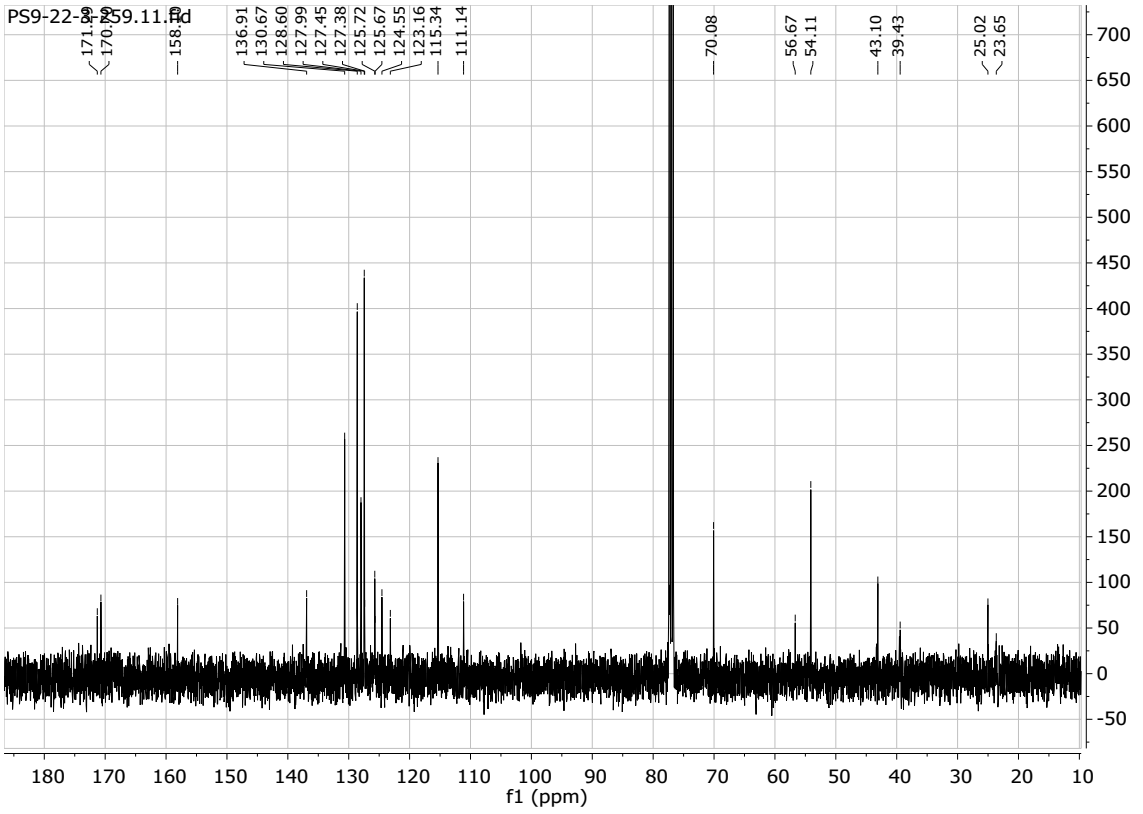

Figure S 20. ^{13}C-NMR spectrum of **8b** in deutero-chloroform.

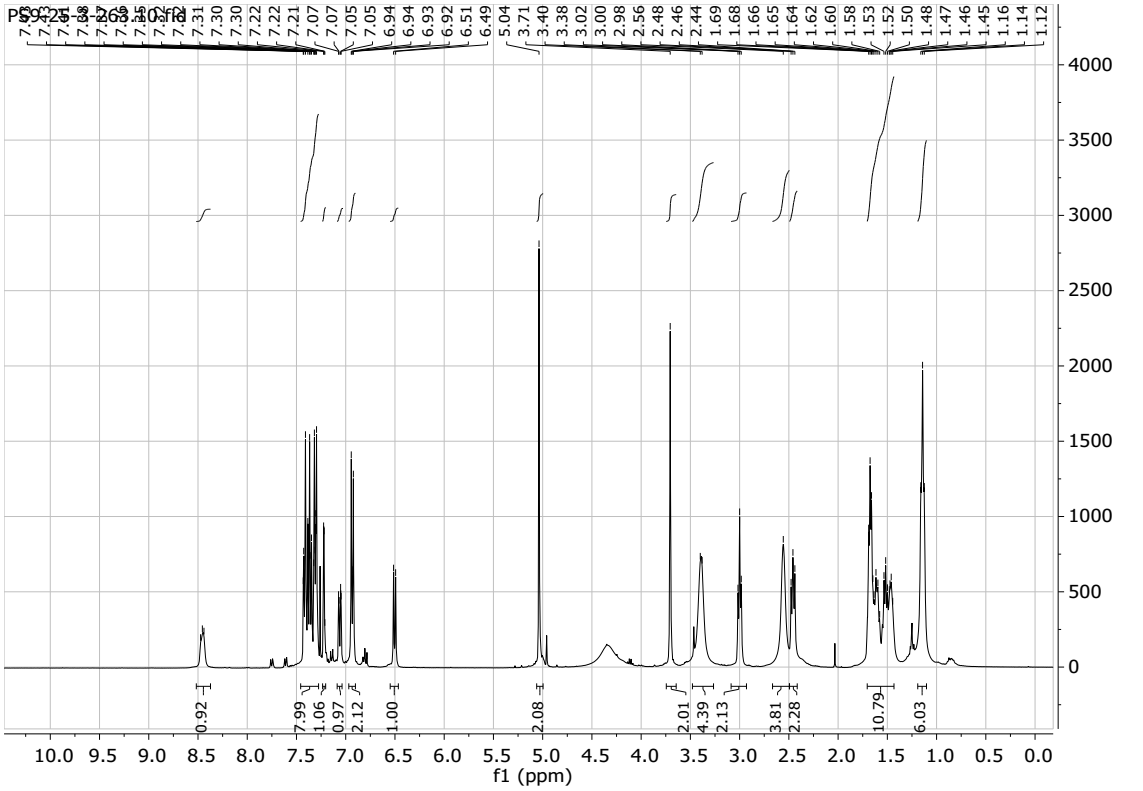

Figure S 21. Proton-NMR of **8c** in deutero-chloroform.

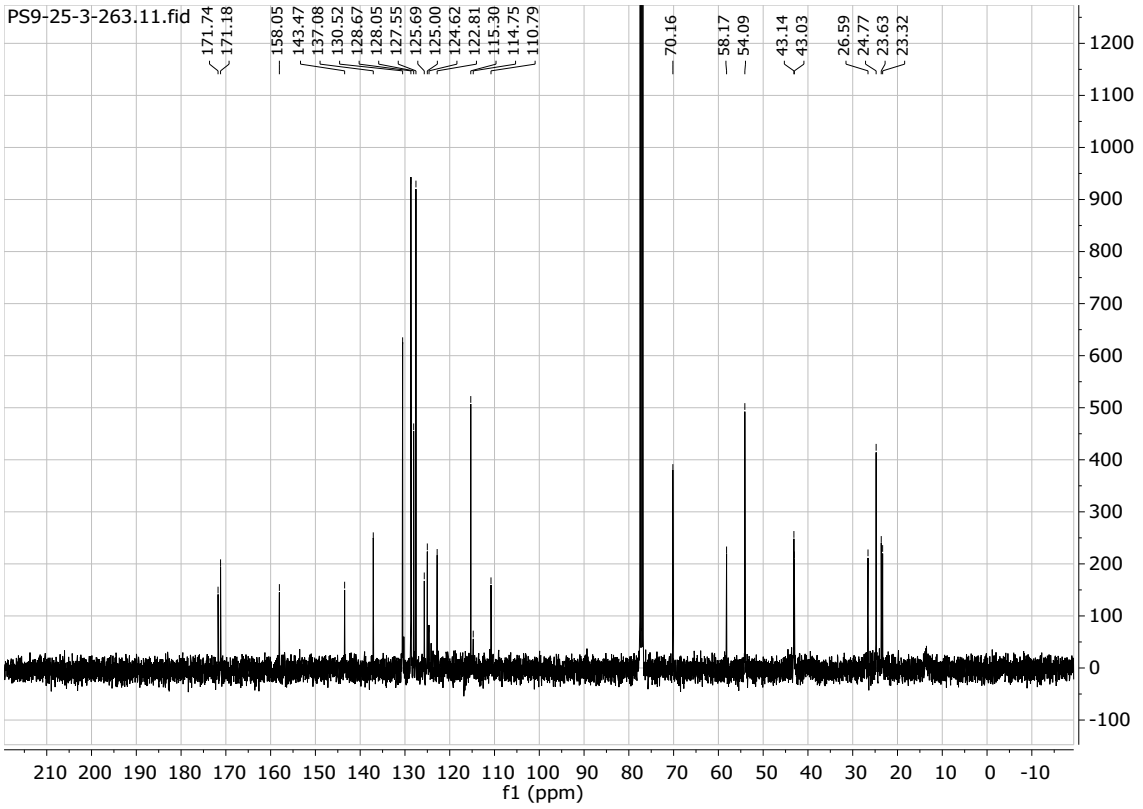

Figure S 22. ^{13}C-NMR spectrum of **8c** in deutero-chloroform.

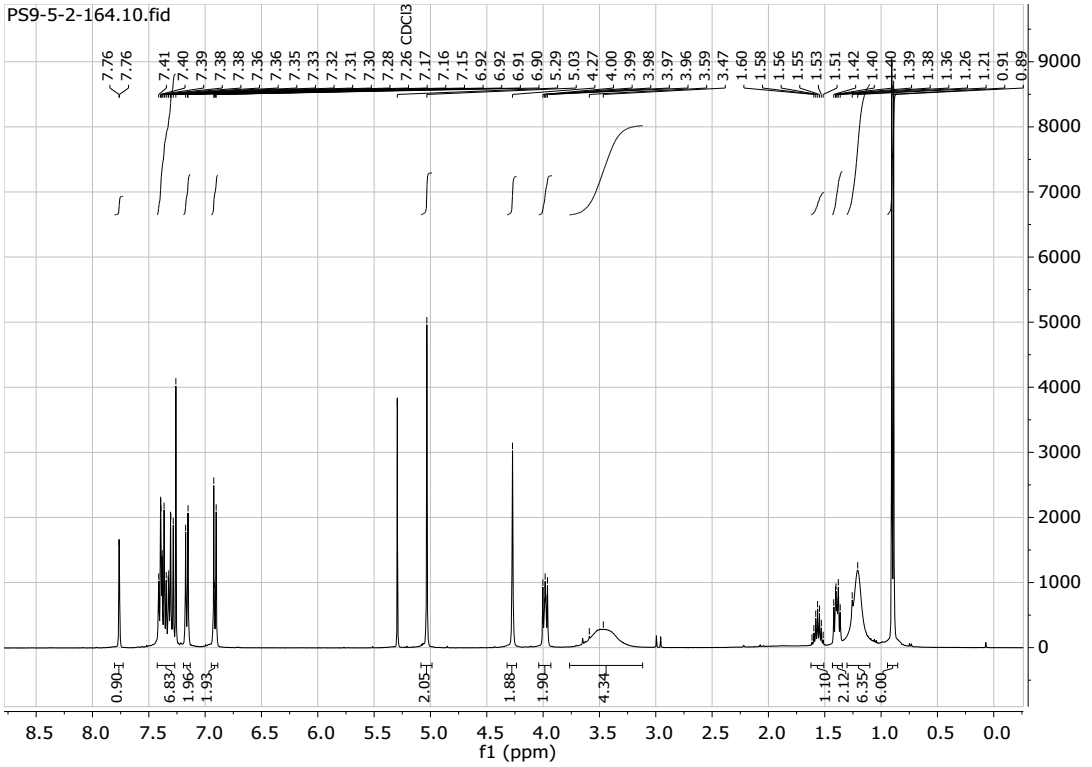

Figure S 23. Proton-NMR of **9a** in deutero-chloroform.

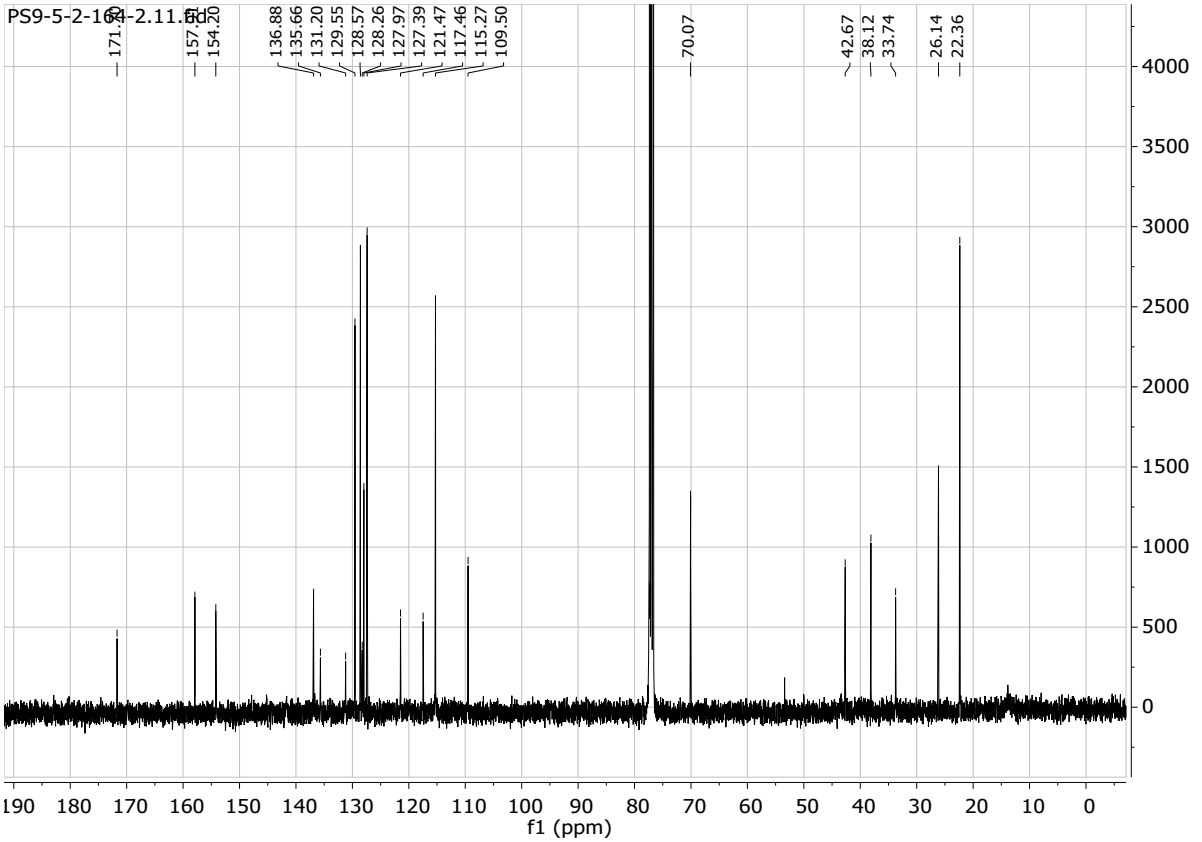

Figure S 24. 13C-NMR spectrum of **9a** in deutero-chloroform.

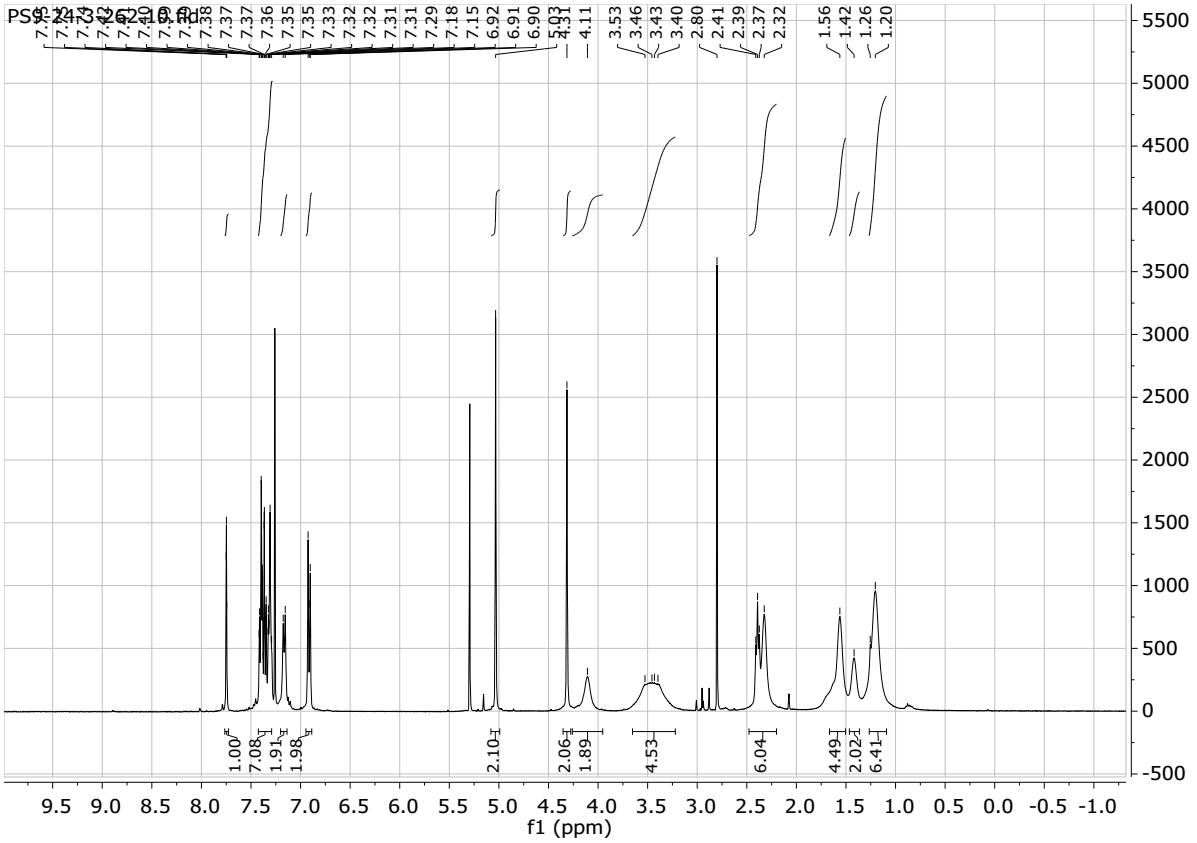

Figure S 25. Proton-NMR of **9b** in deutero-chloroform.

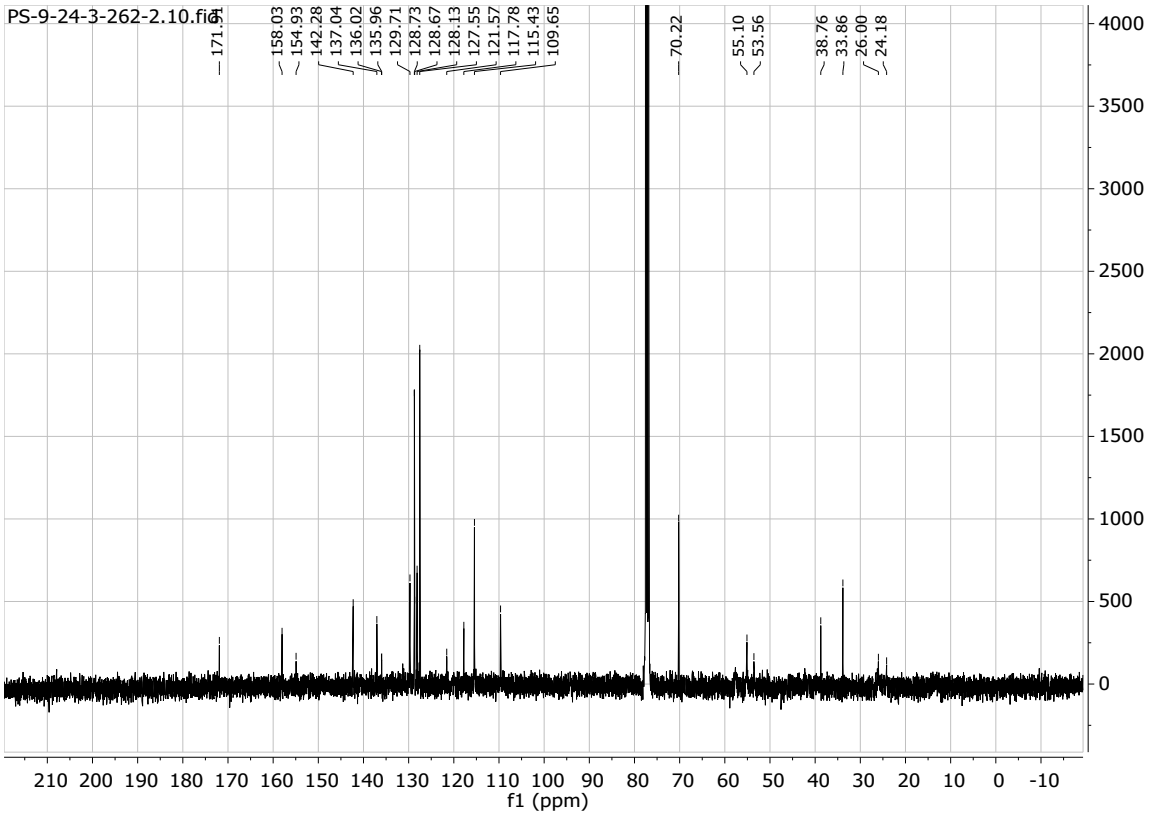

Figure S 26. ^{13}C-NMR spectrum of **9b** in deutero-chloroform.

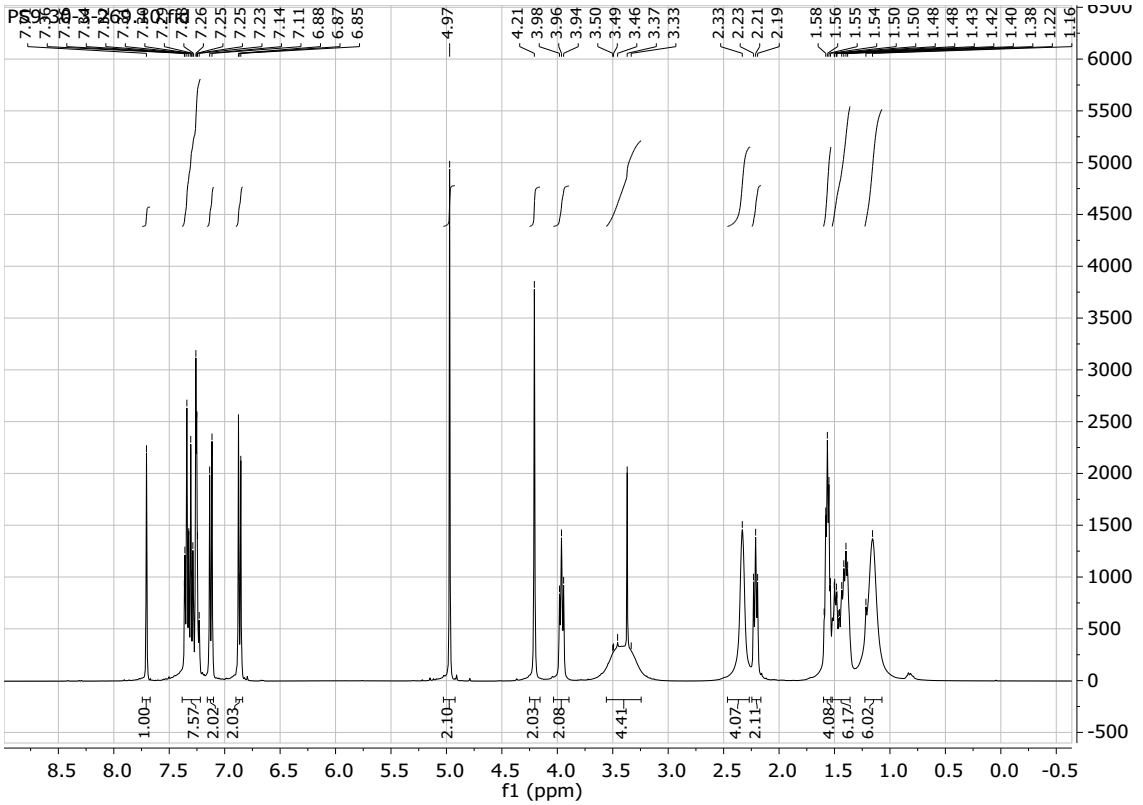

Figure S 27. Proton-NMR of **9c** in deutero-chloroform.

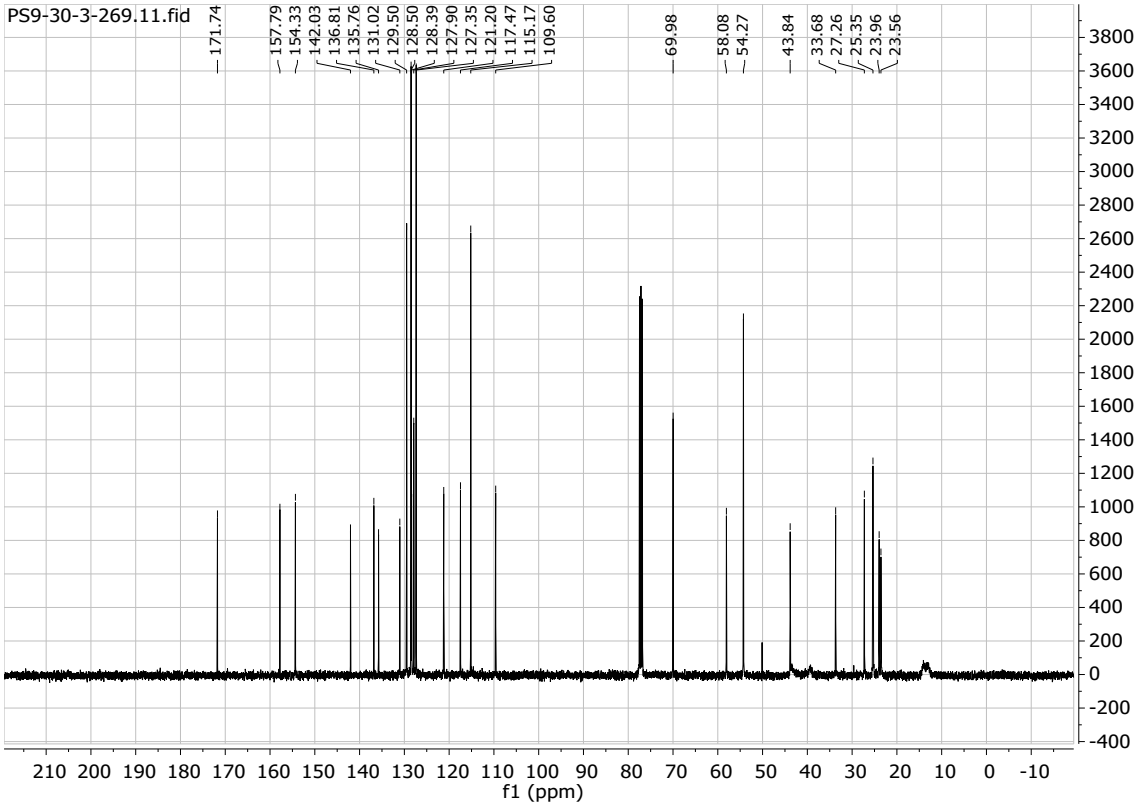

Figure S 28. ^{13}C-NMR spectrum of **9c** in deutero-chloroform.

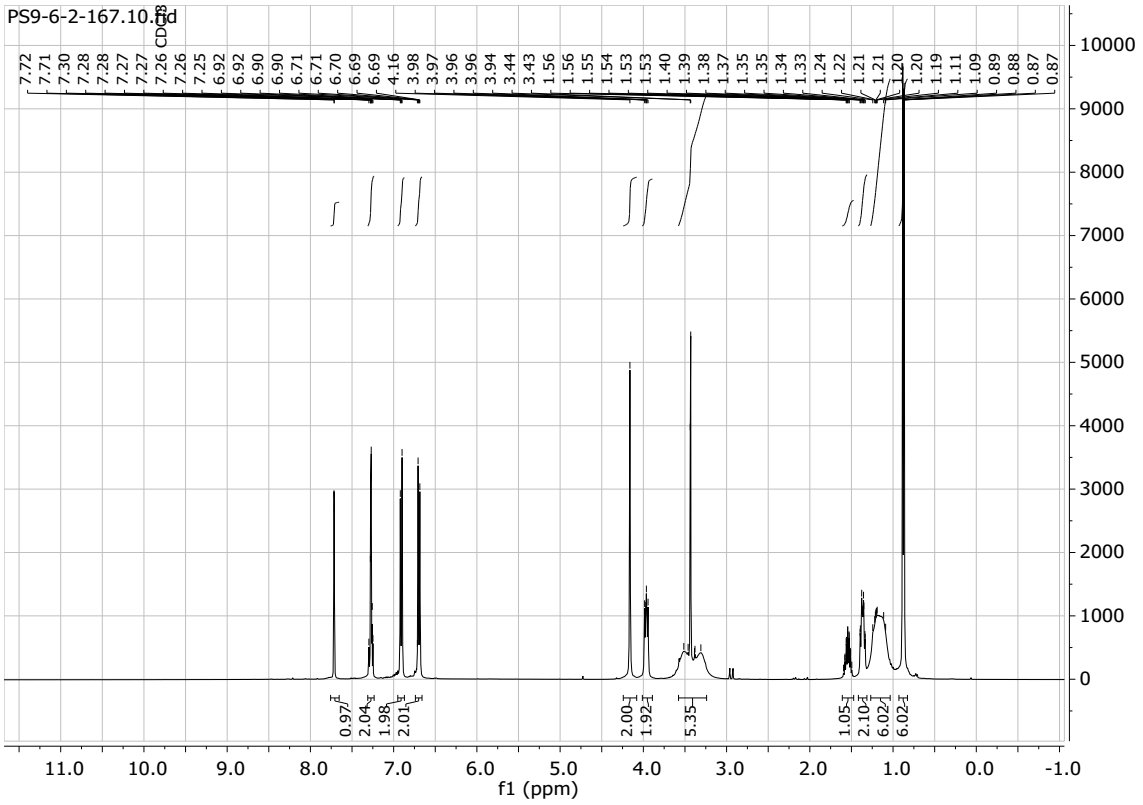

Figure S 29. Proton-NMR of **10a** in deutero-chloroform.

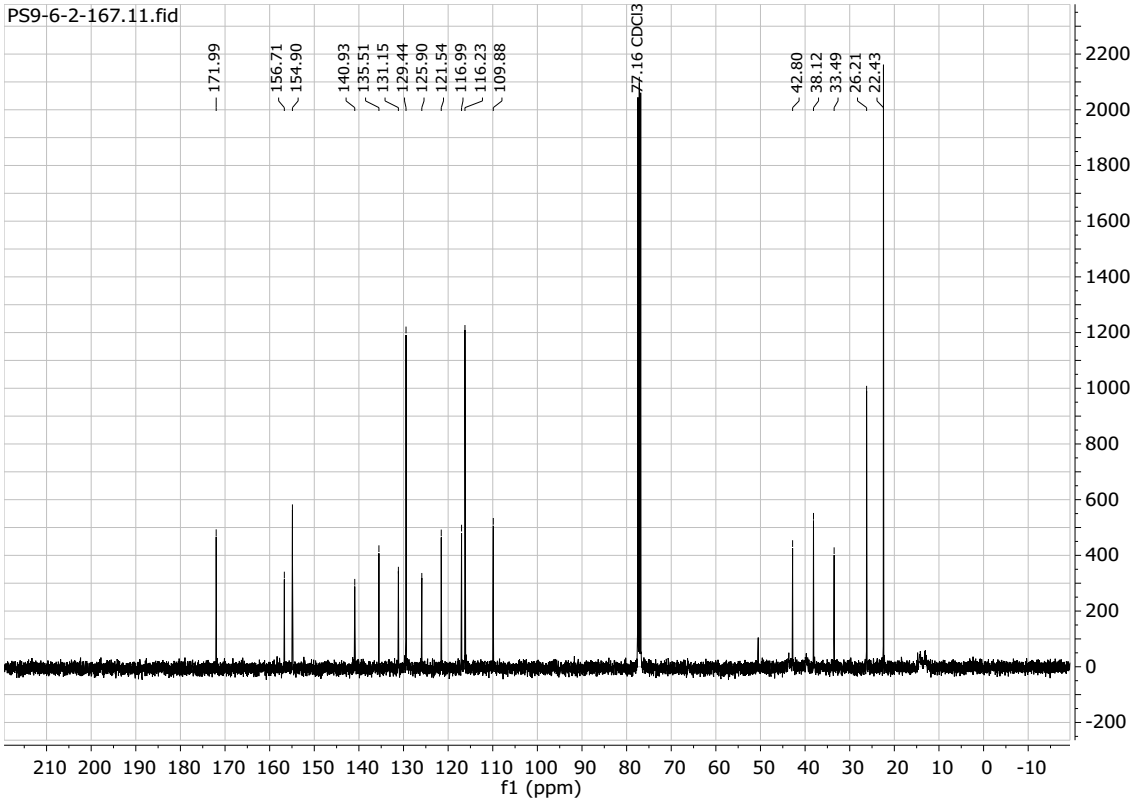

Figure S 30. ^{13}C-NMR spectrum of **10a** in deutero-chloroform.

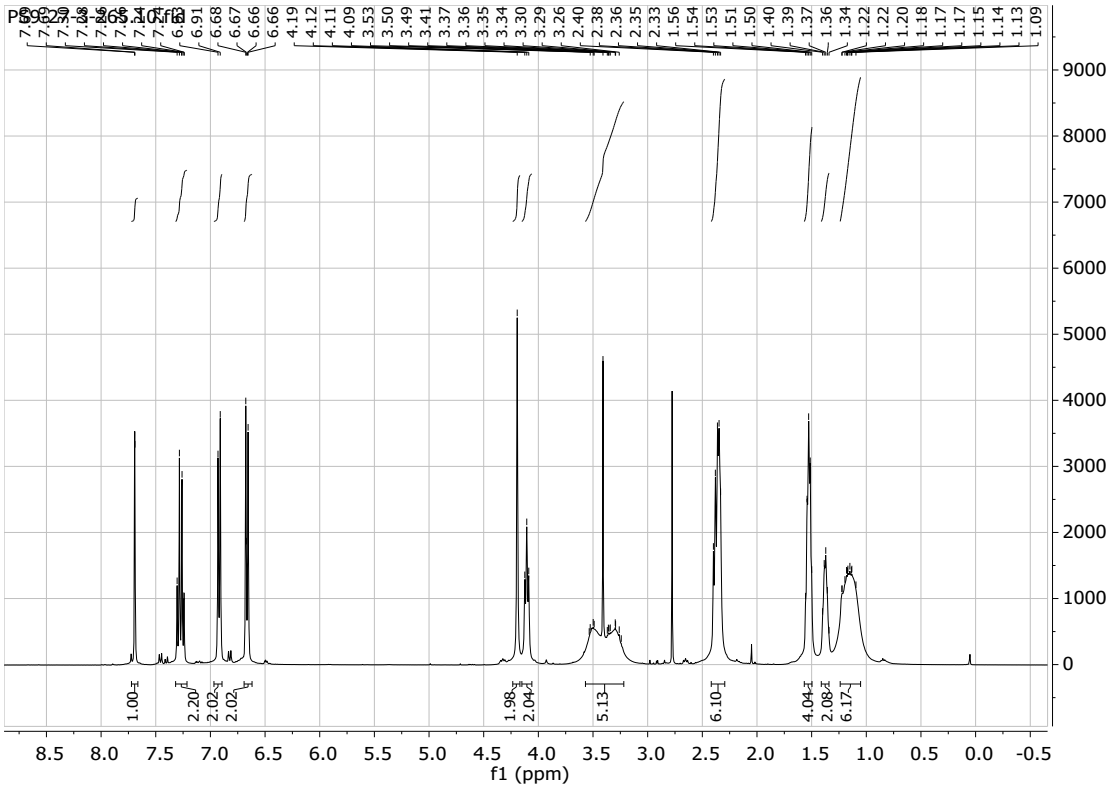

Figure S 31. Proton-NMR of **10b** in deutero-chloroform.

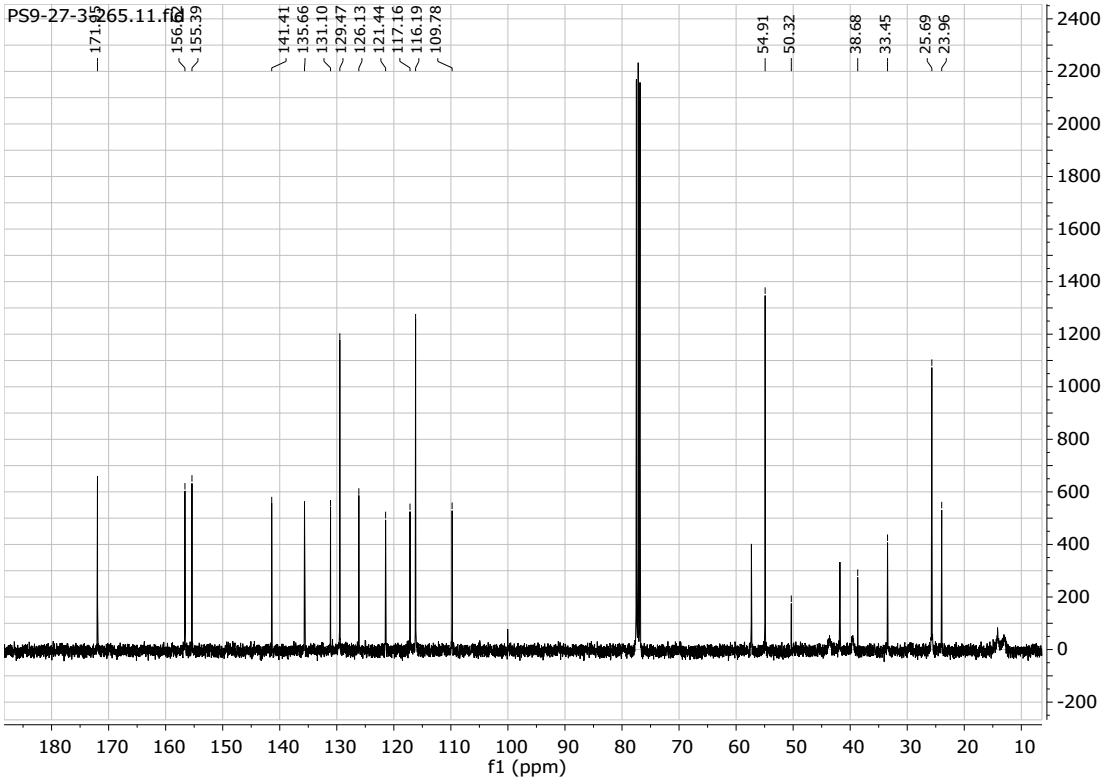

Figure S 32. ^{13}C-NMR spectrum of **10b** in deutero-chloroform.

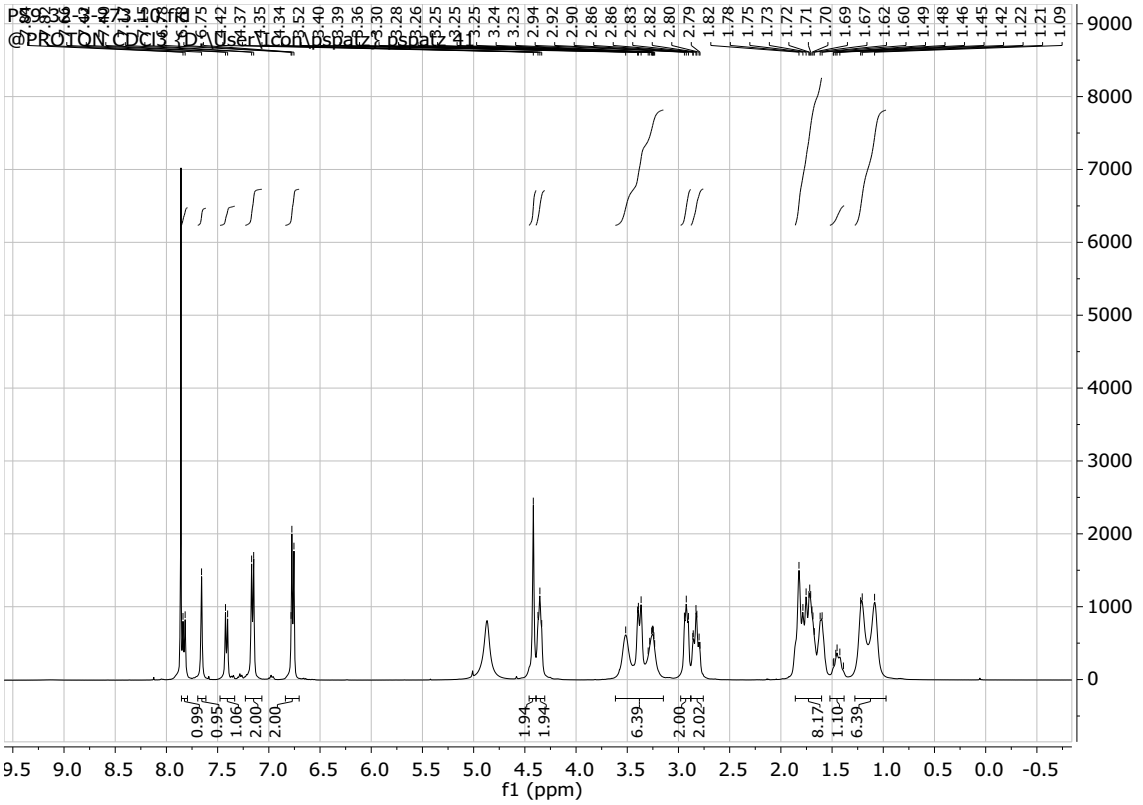

Figure S 33. Proton-NMR of **10c** in deutero-methanol.

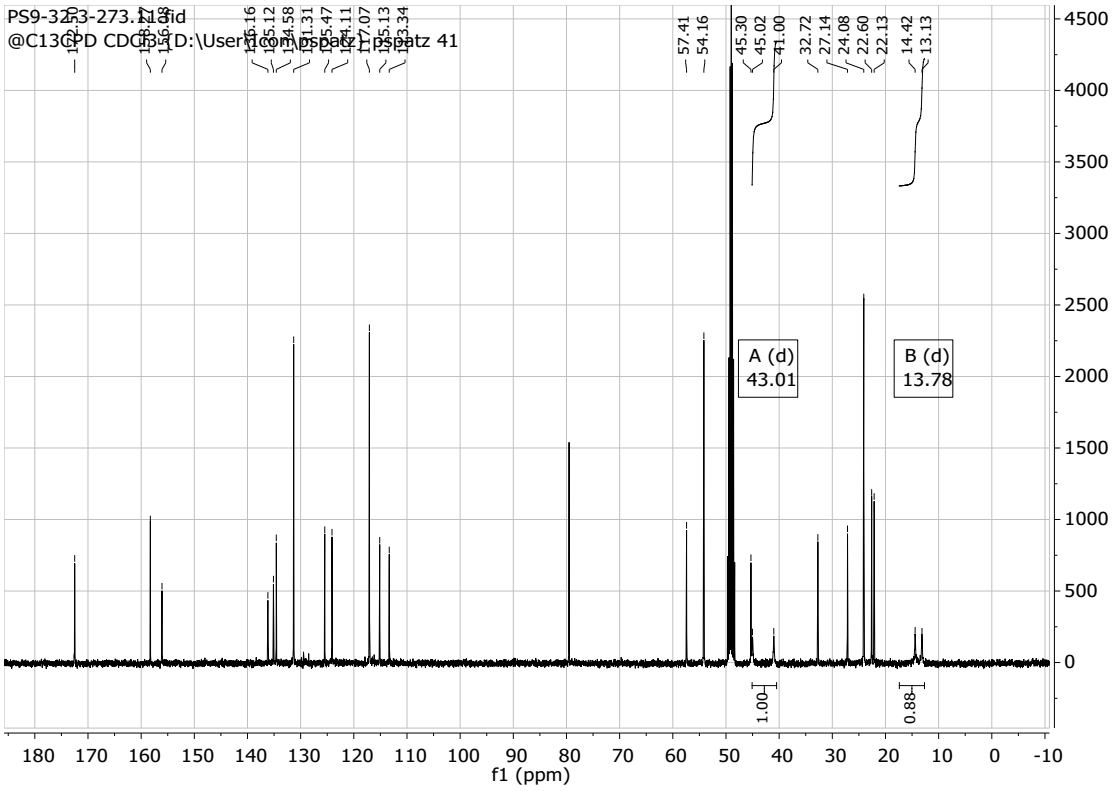

Figure S 34. ^{13}C-NMR spectrum of **10c** in deutero-methanol.

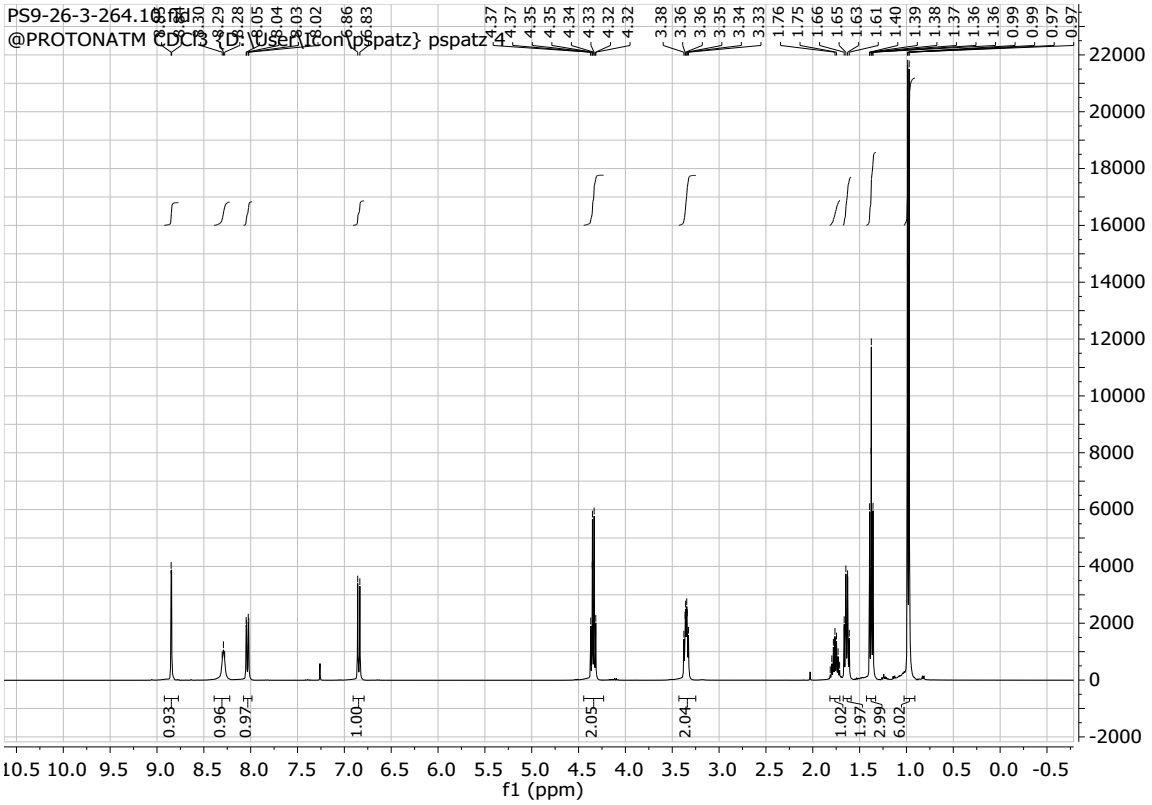

Figure S 35. Proton-NMR of **12** in deutero-chloroform.

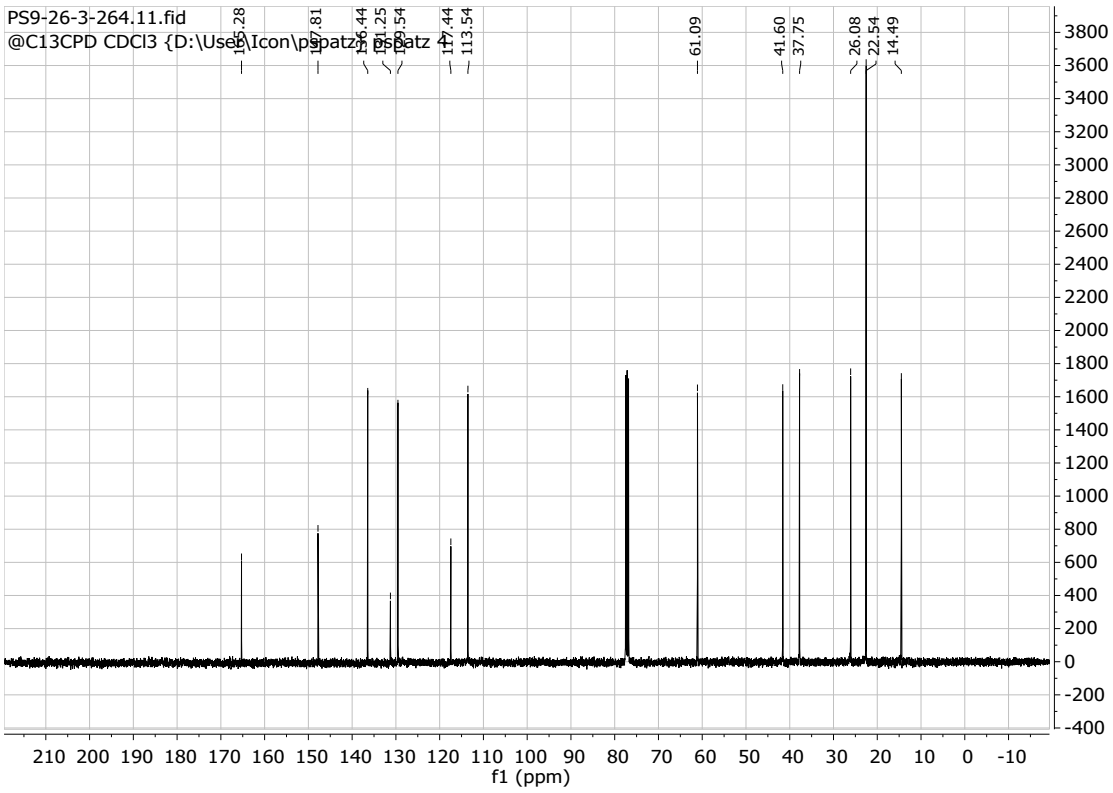

Figure S 36. ^{13}C-NMR spectrum of **12** in deutero-chloroform.

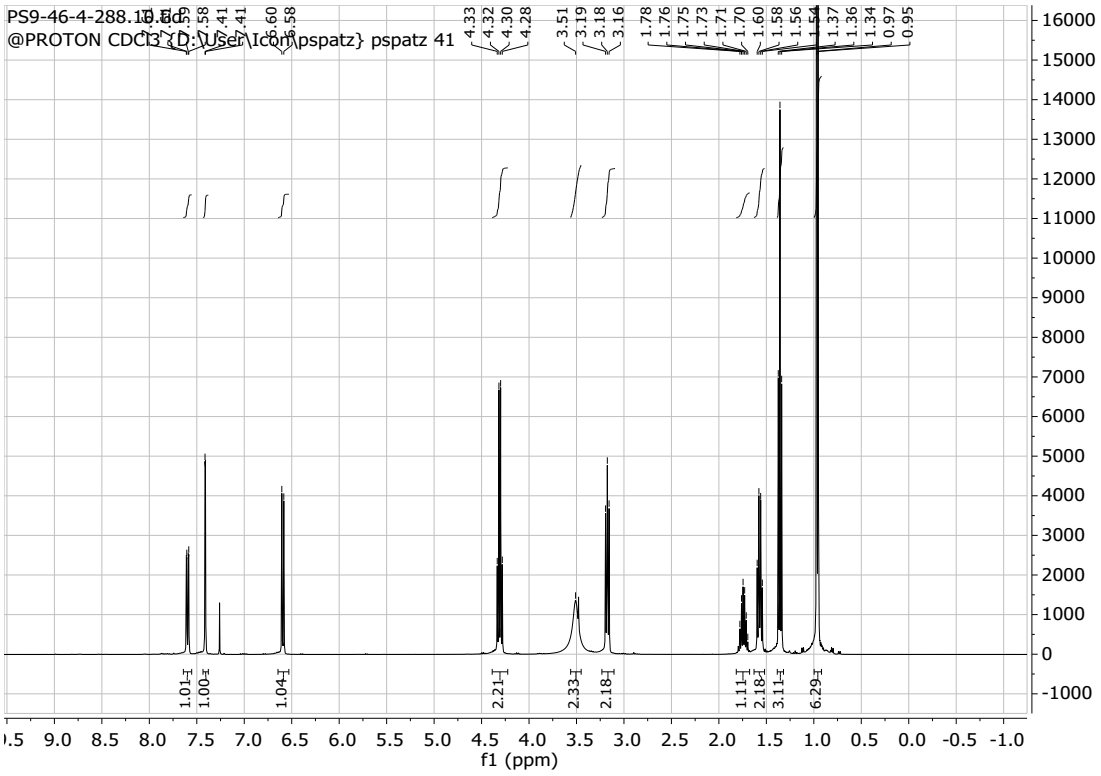

Figure S 37. Proton-NMR of **13** in deutero-chloroform.

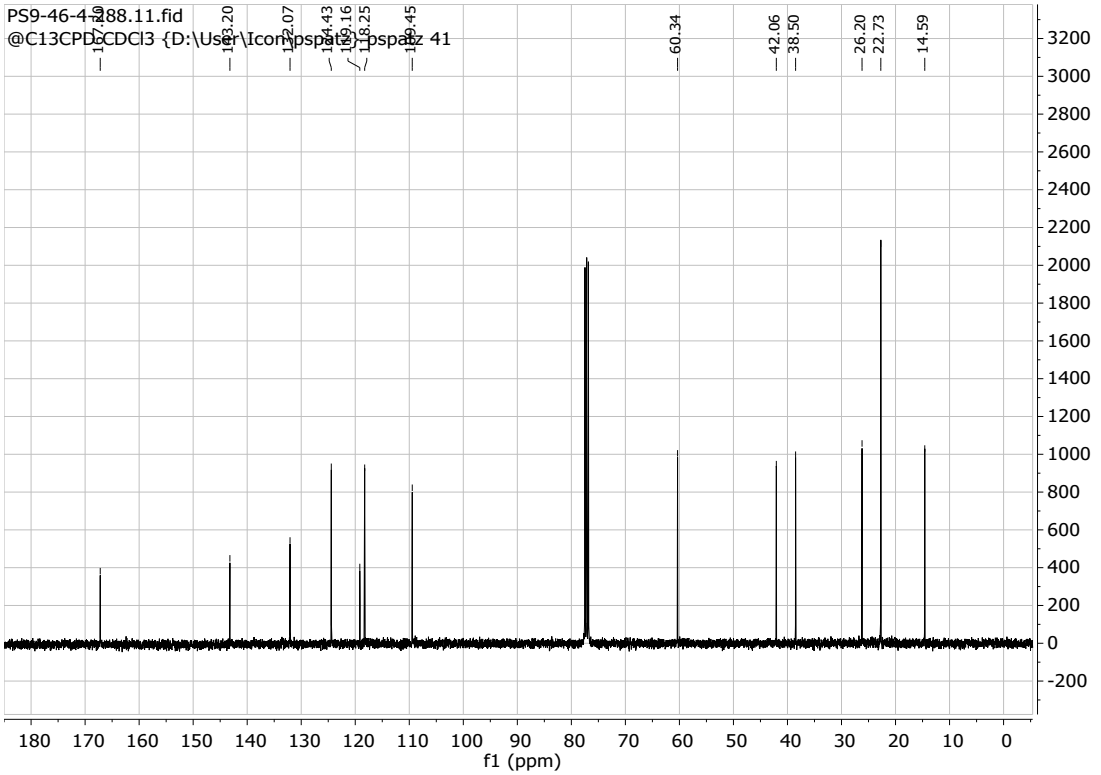

Figure S 38. ^{13}C-NMR spectrum of **13** in deutero-chloroform.

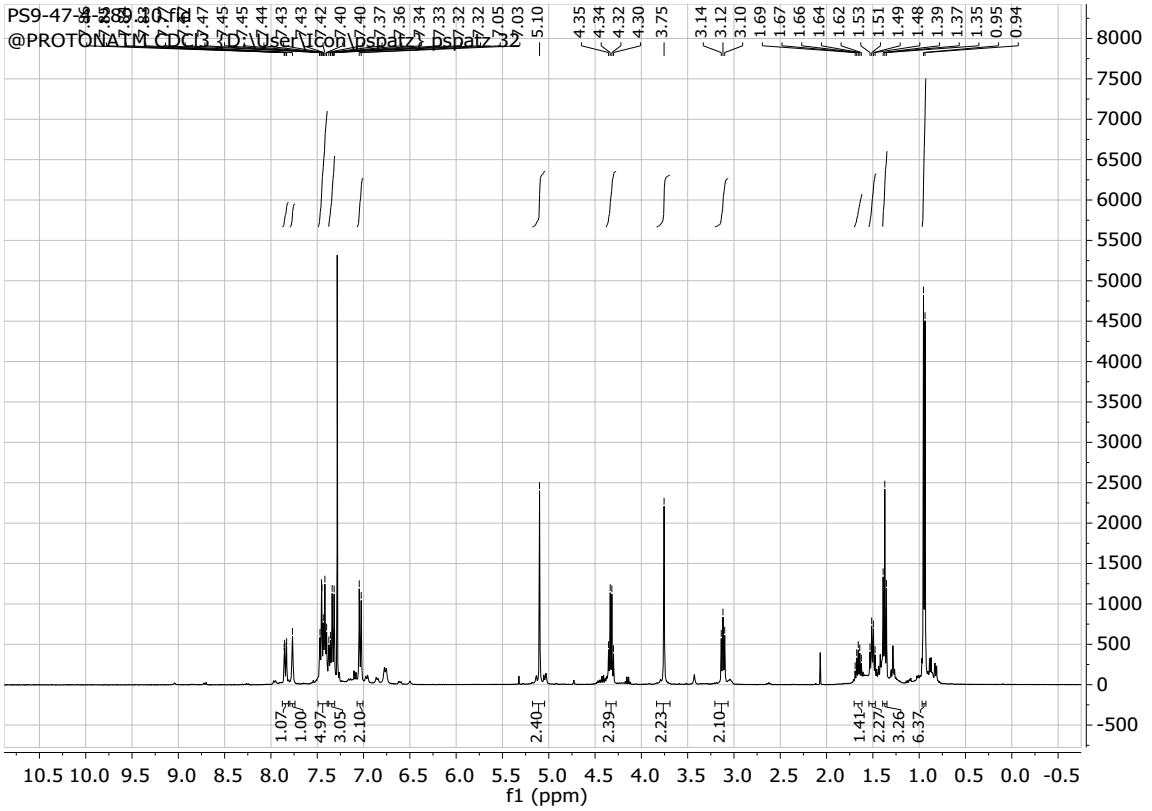

Figure S 39. Proton-NMR of **14** in deutero-chloroform.

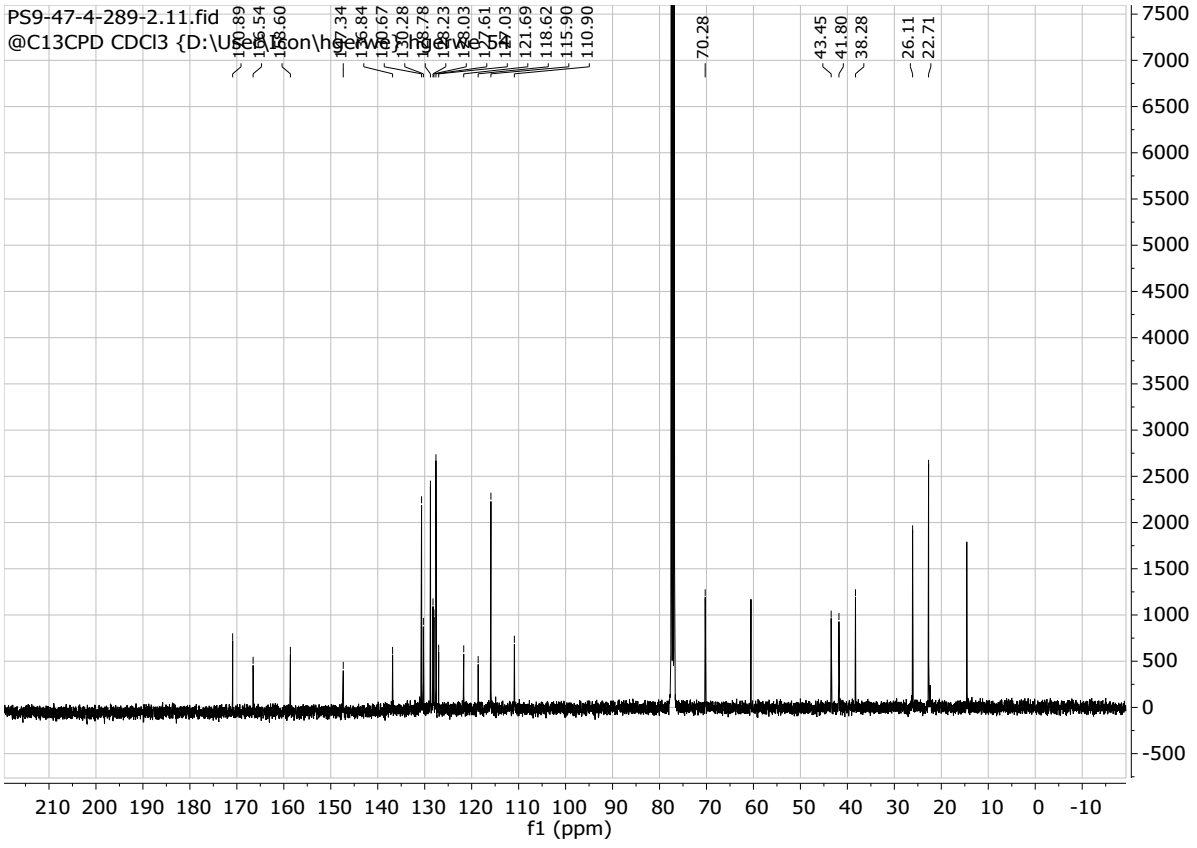

Figure S 40. ^{13}C-NMR spectrum of **14** in deutero-chloroform.

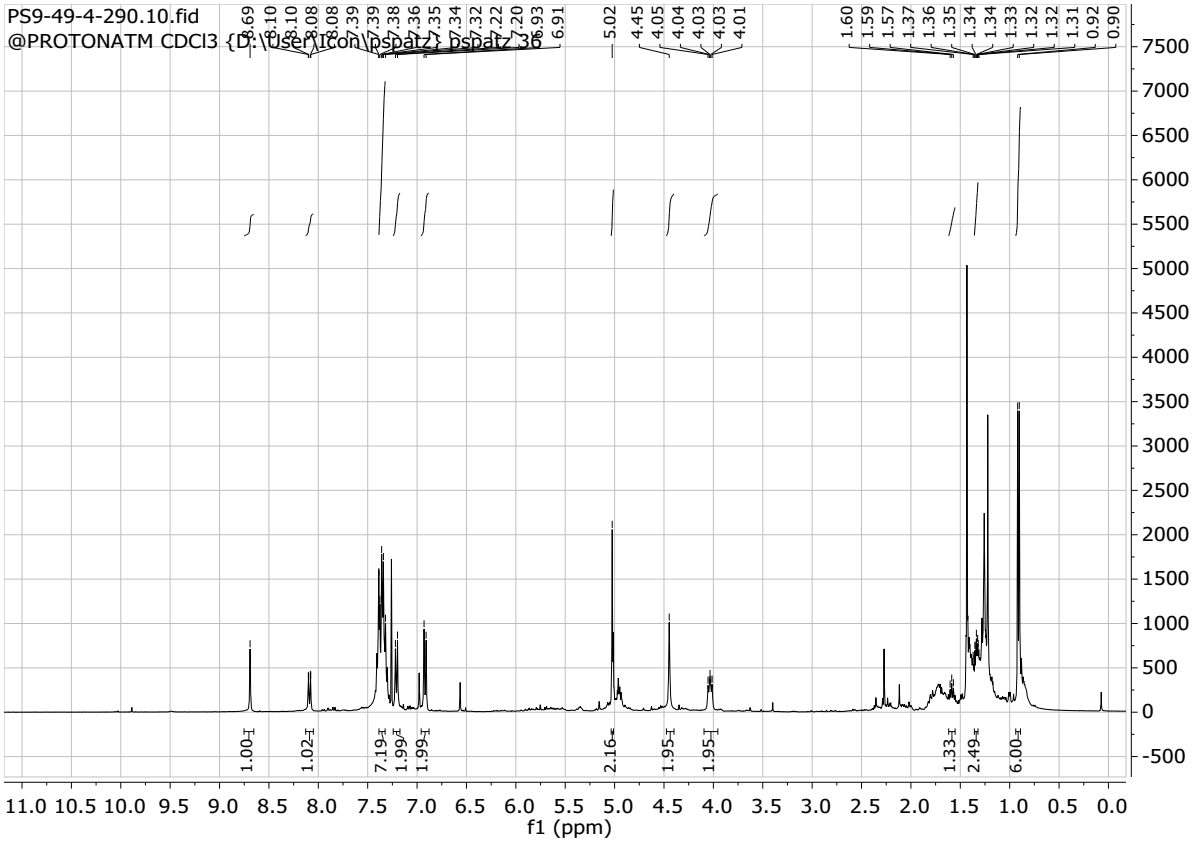

Figure S 41. Proton-NMR of **15** in deutero-chloroform.

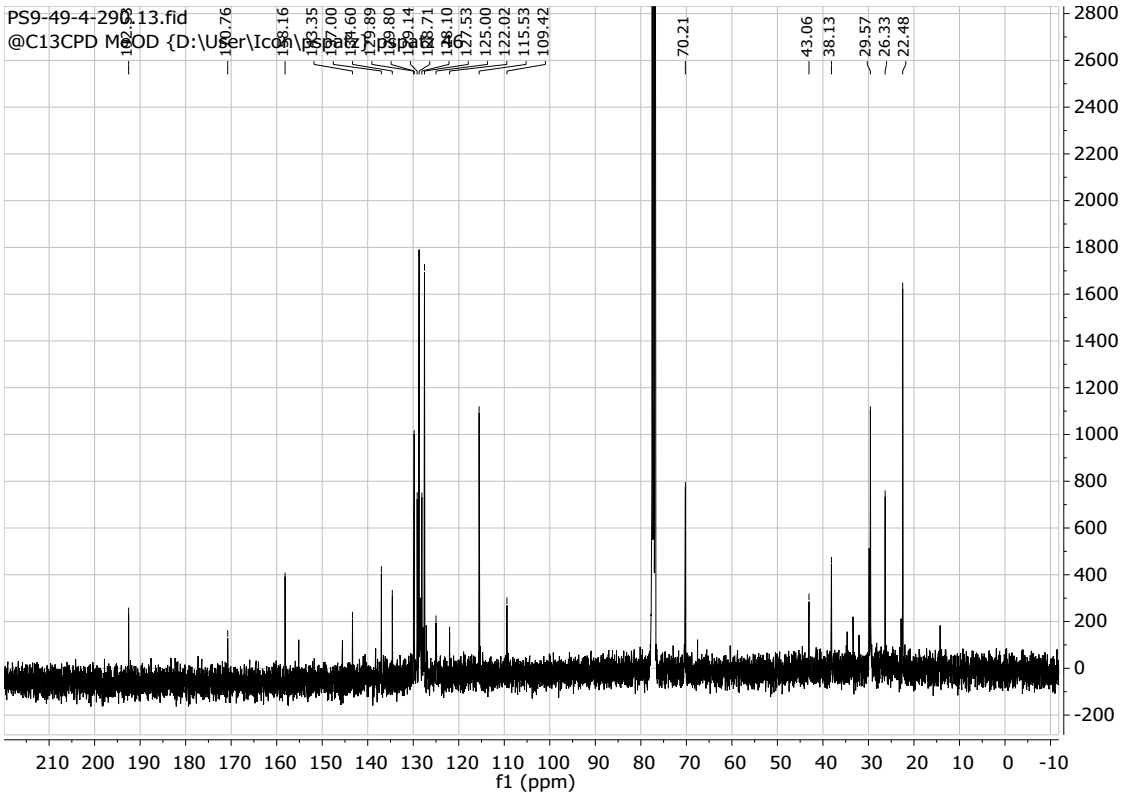

Figure S 42. ^{13}C-NMR spectrum of **15** in deutero-chloroform.

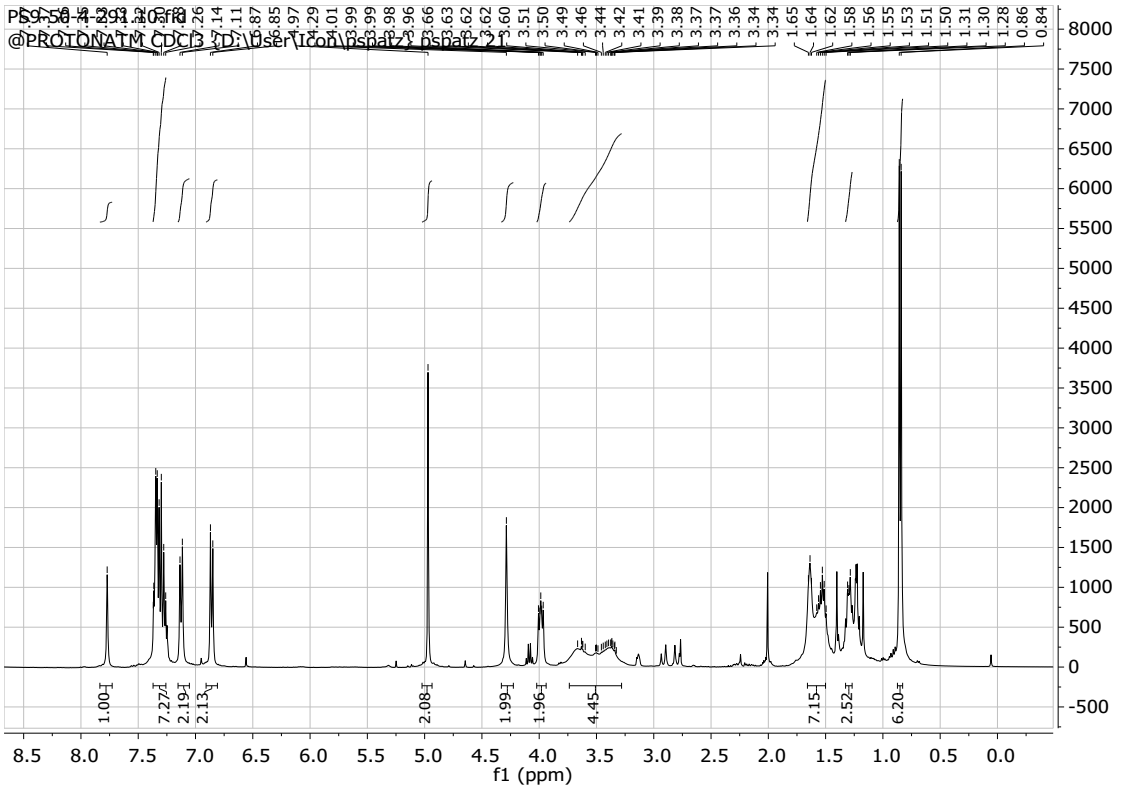

Figure S 43. Proton-NMR of **16a** in deutero-chloroform.

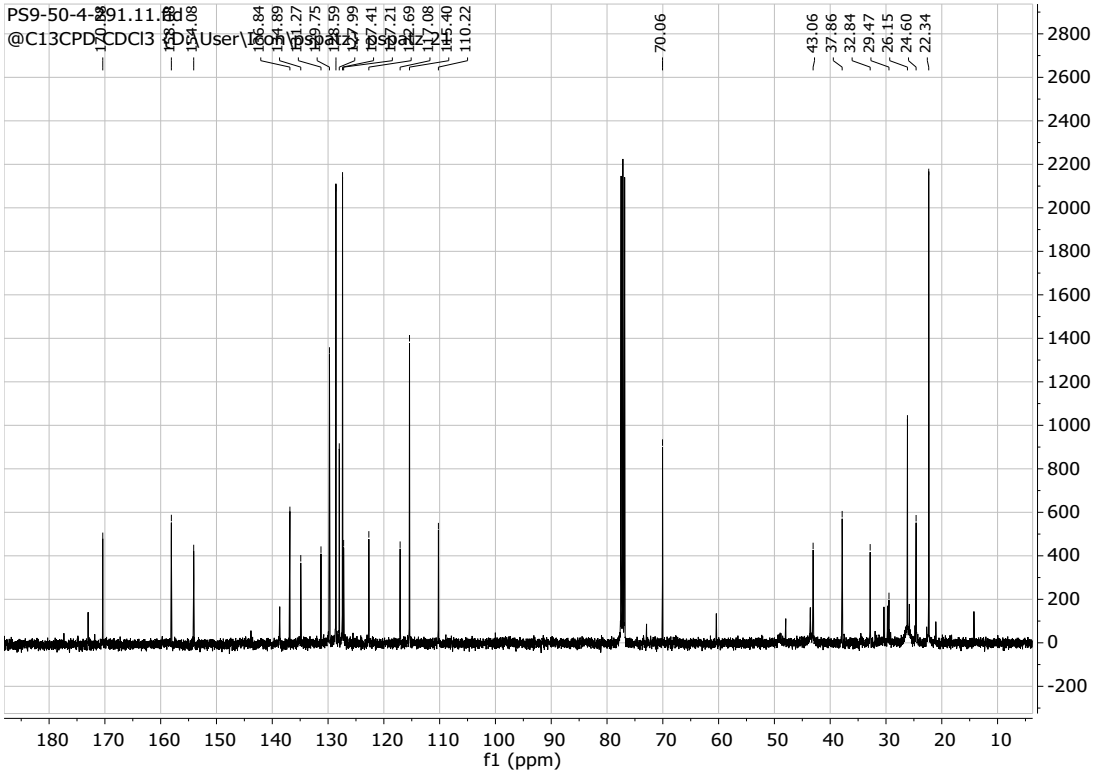

Figure S 44. ^{13}C-NMR spectrum of **16a** in deutero-chloroform.

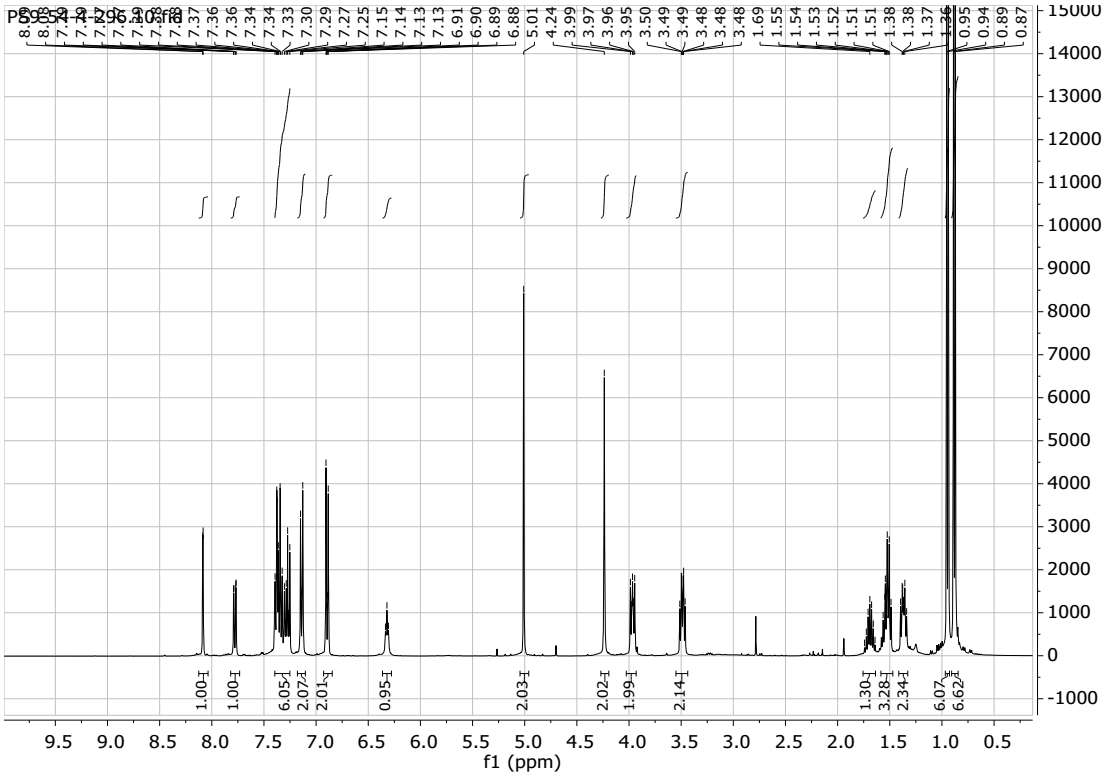

Figure S 45. Proton-NMR of **16b** in deutero-chloroform.

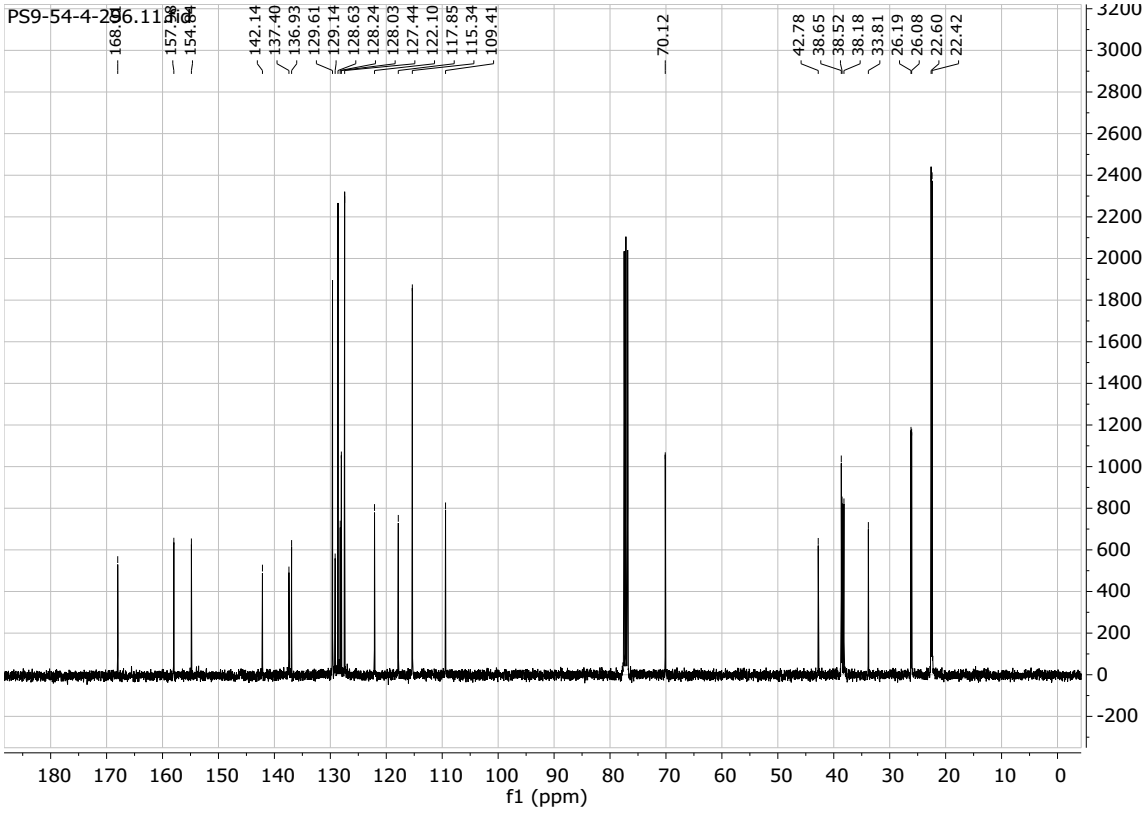

Figure S 46. ^{13}C-NMR spectrum of **16b** in deutero-chloroform.

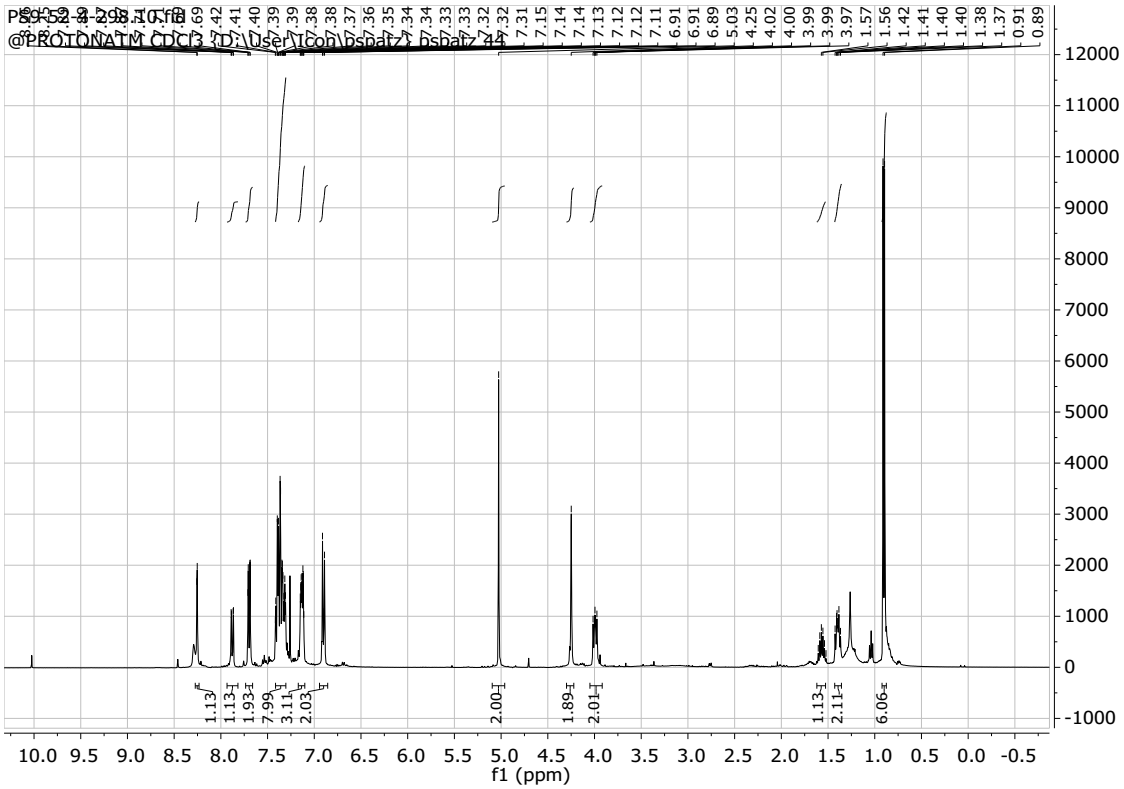

Figure S 47. Proton-NMR of **16c** in deutero-chloroform.

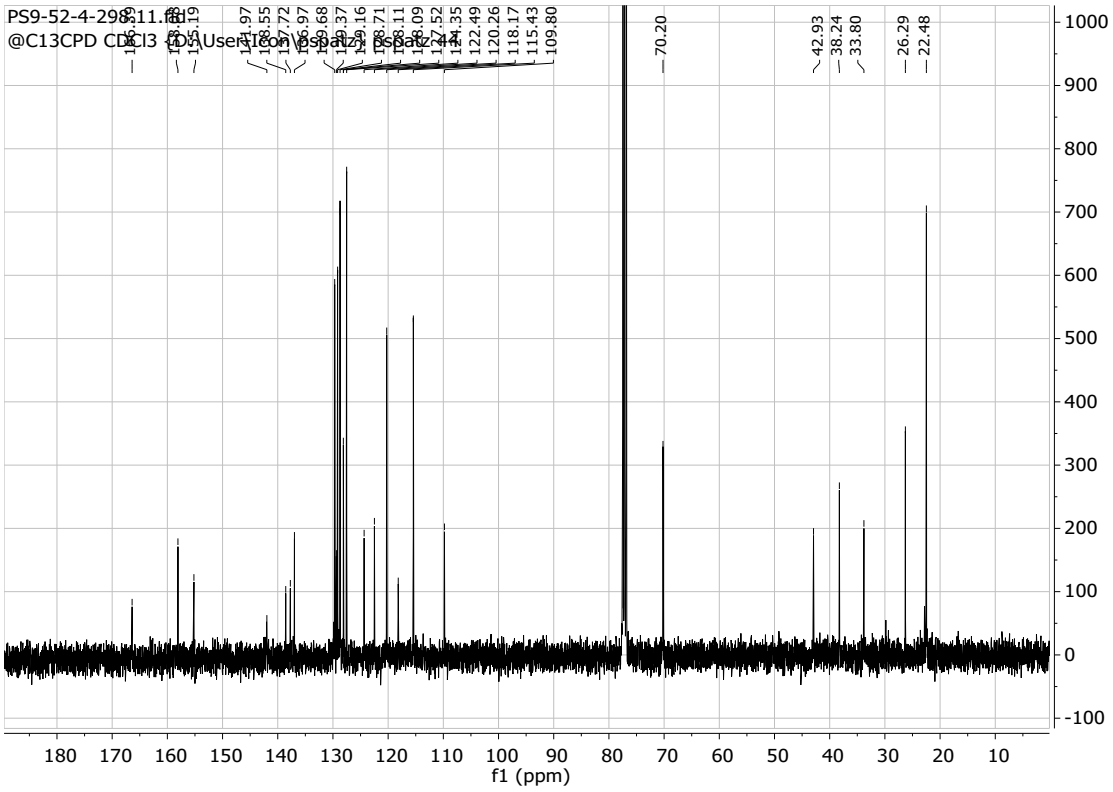

Figure S 48. ^{13}C-NMR spectrum of **16c** in deutero-chloroform.

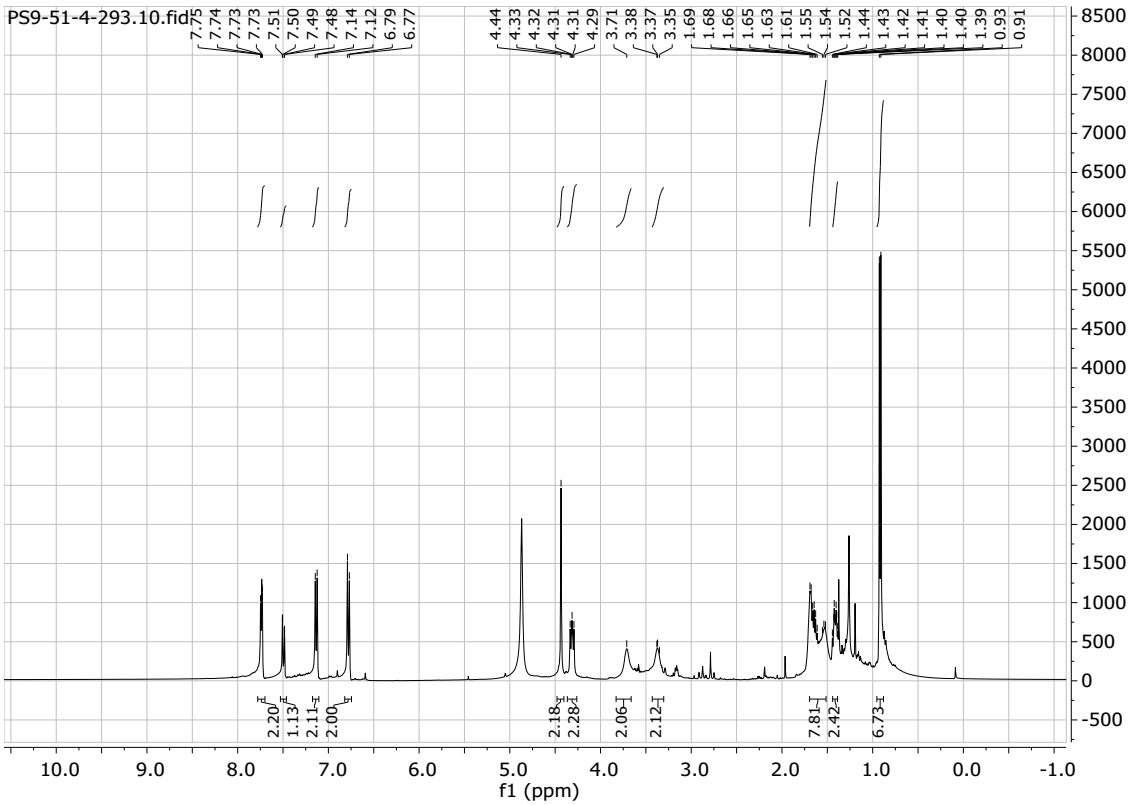

Figure S 49. Proton-NMR of **17a** in deutero-methanol.

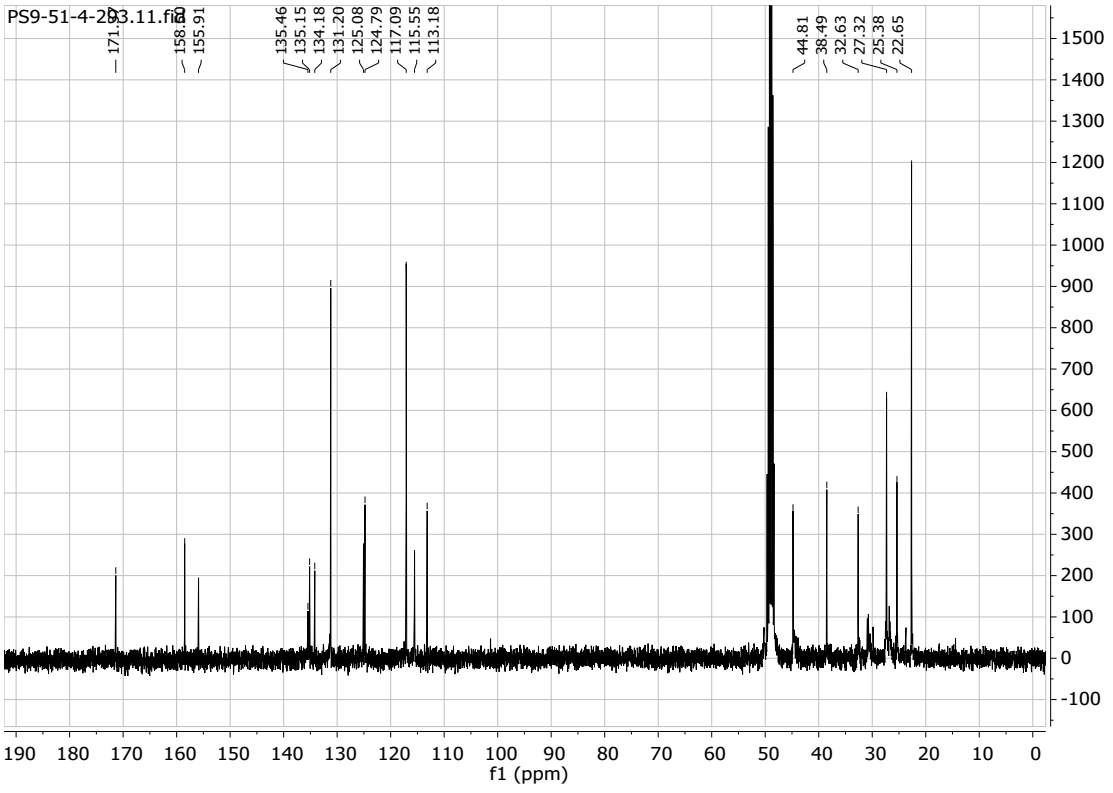

Figure S 50. ^{13}C-NMR spectrum of **17a** in deutero-methanol.

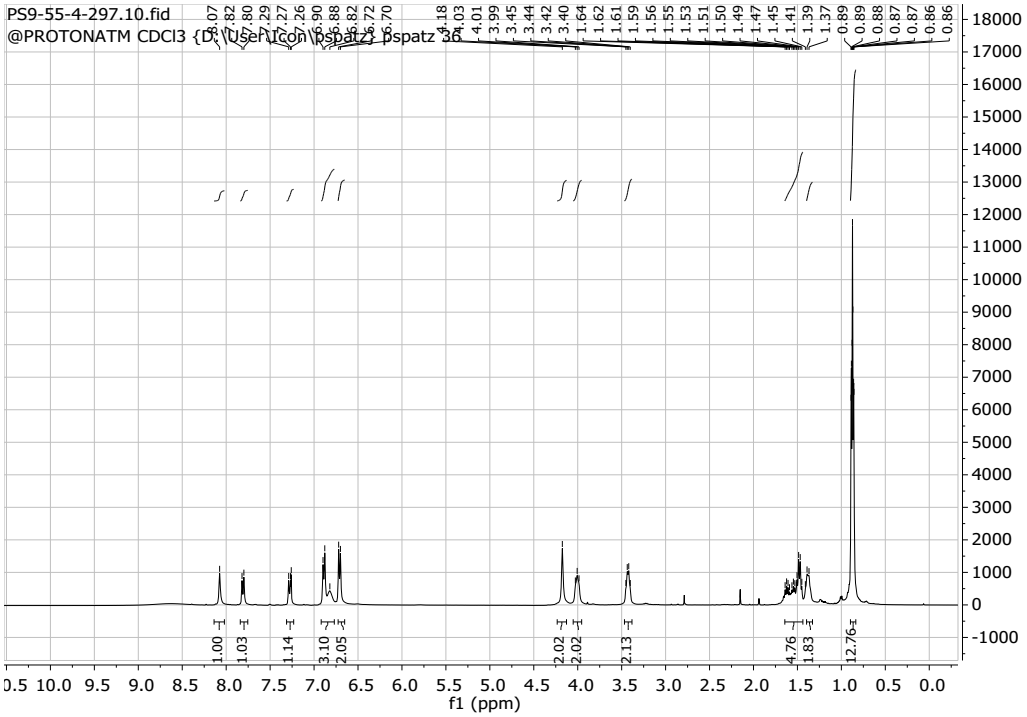

Figure S 51. Proton-NMR of **17b** in deutero-chloroform.

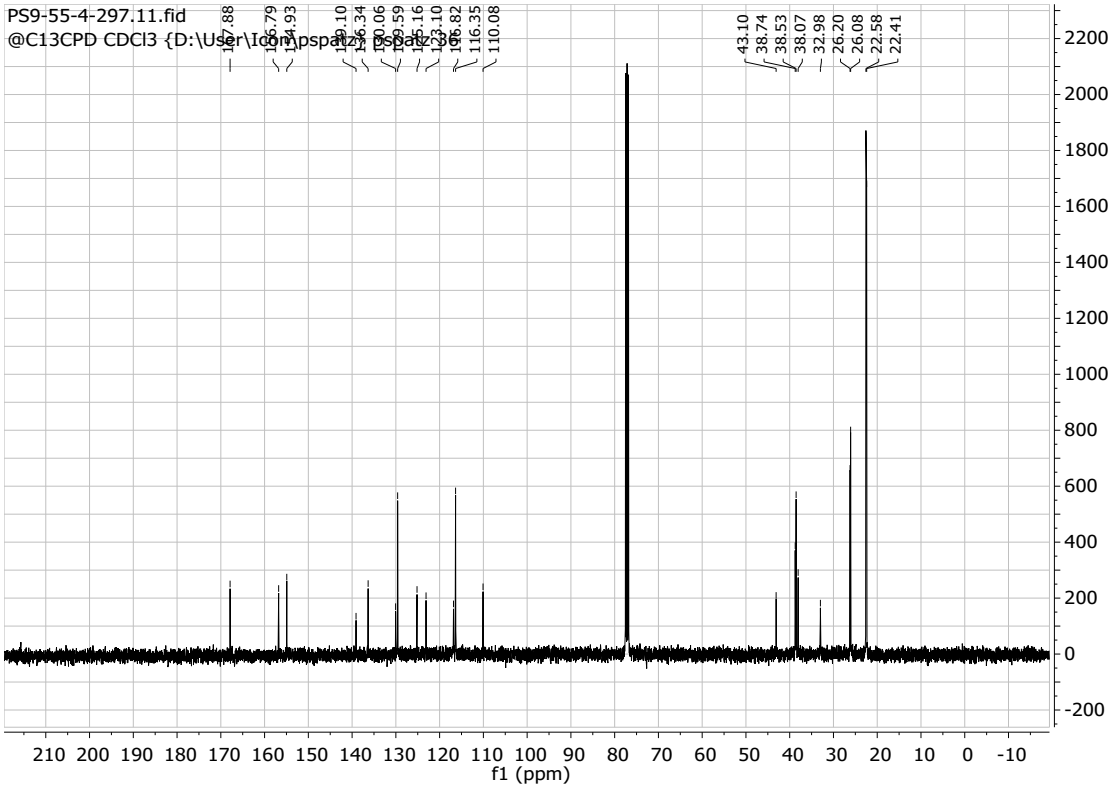

Figure S 52. 13C-NMR spectrum of **17b** in deutero-chloroform.

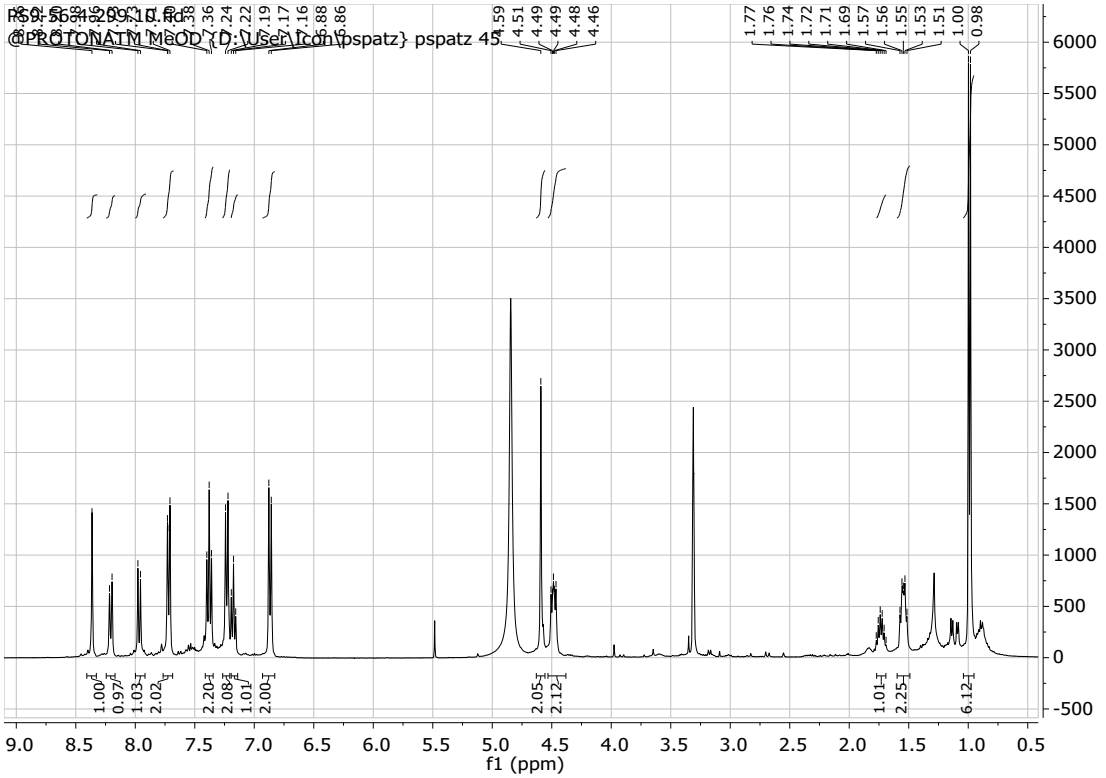

Figure S 53. Proton-NMR of **17c** in deutero-methanol.

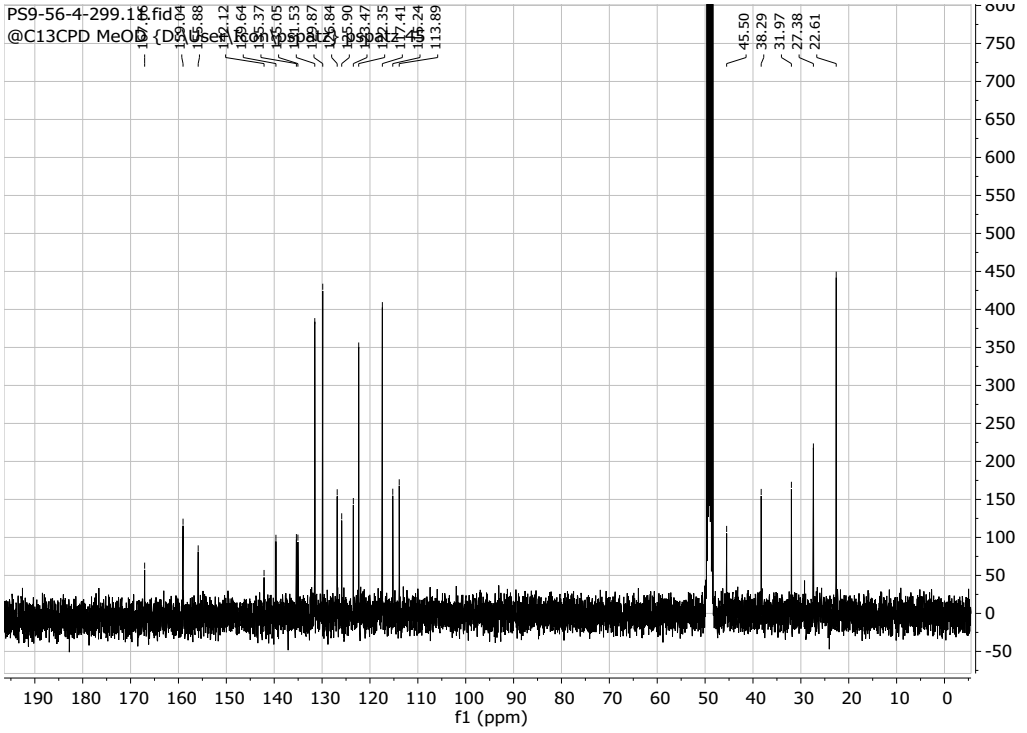

Figure S 54. ^{13}C-NMR spectrum of **17c** in deutero-methanol.

NMR spectra of target compounds

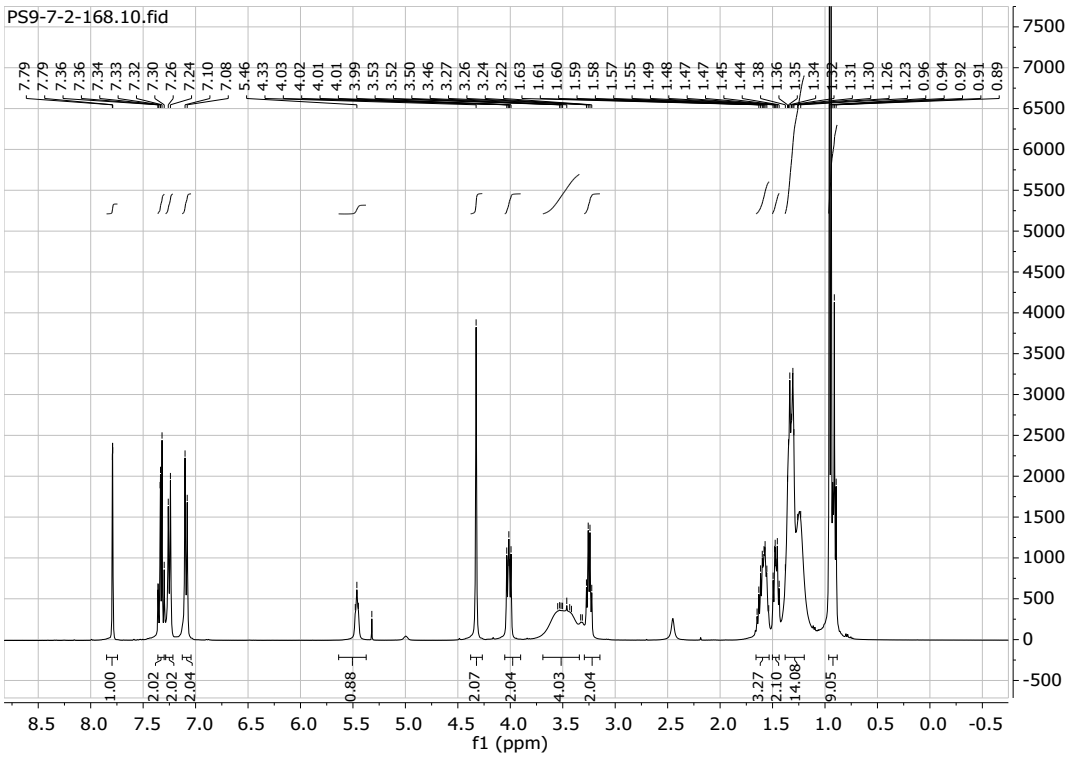

Figure S 55. Proton-NMR of **11a** in deutero-chloroform.

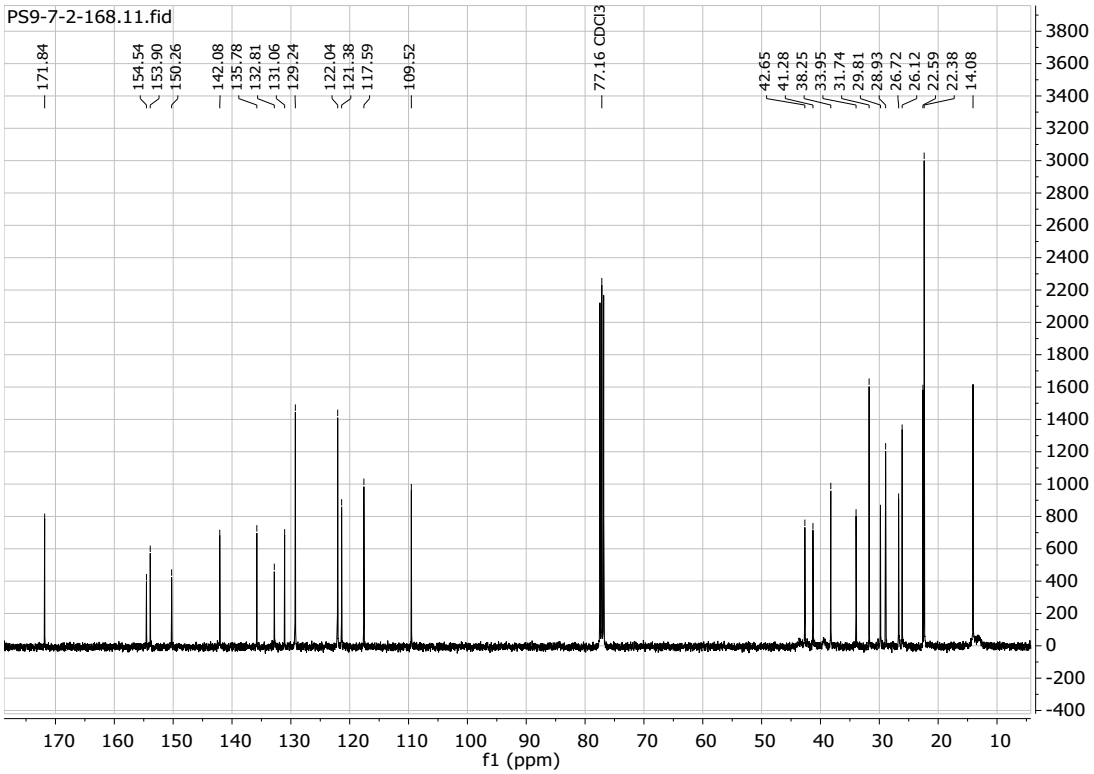

Figure S 56. ^{13}C-NMR spectrum of **11a** in deutero-chloroform.

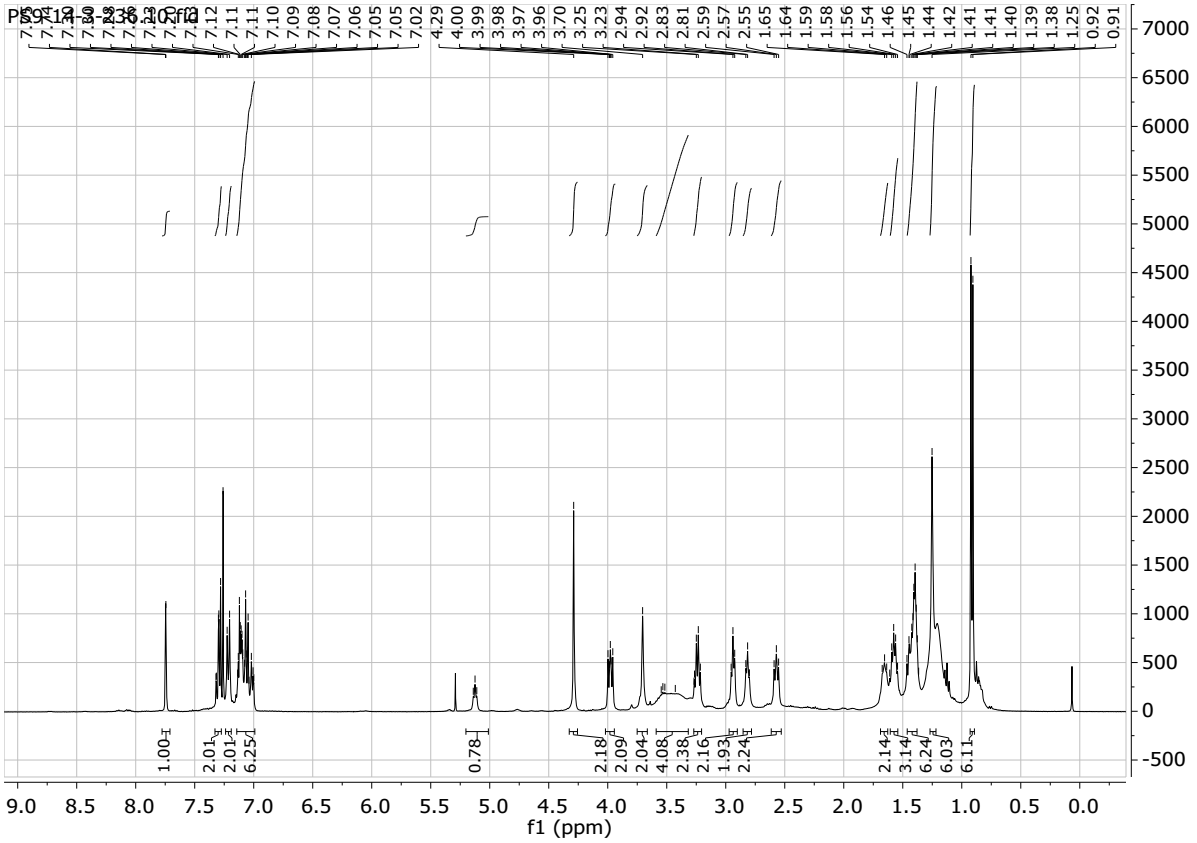

Figure S 57. Proton-NMR of **11b** in deutero-chloroform.

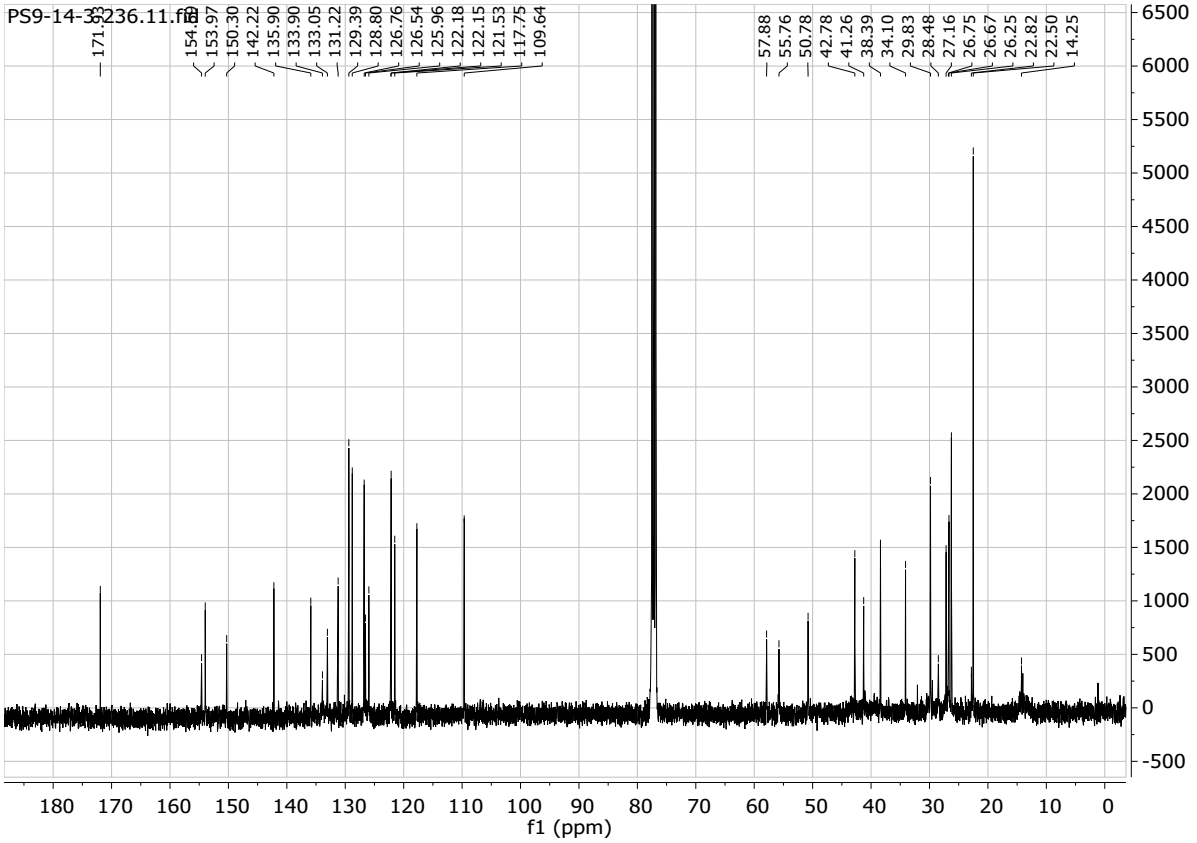

Figure S 58. ^{13}C-NMR spectrum of **11b** in deutero-chloroform.

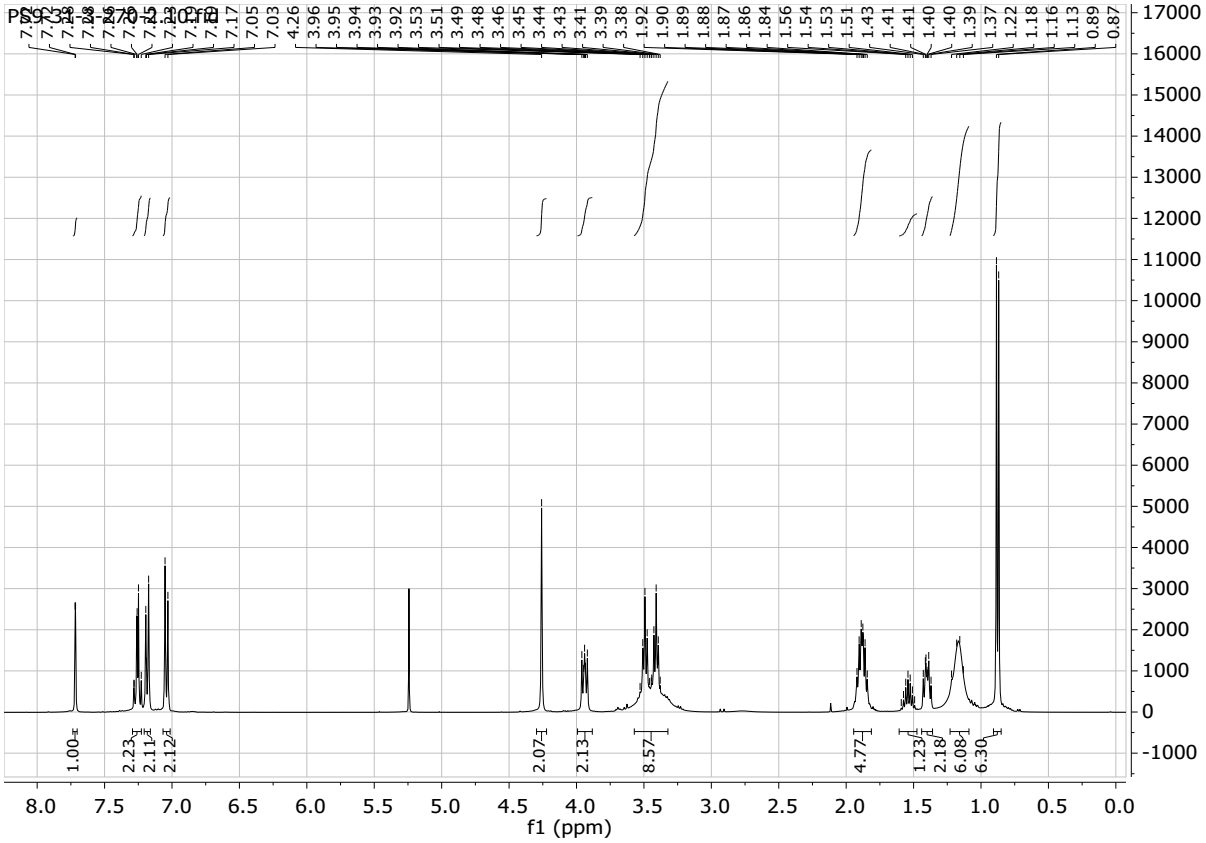

Figure S 59. Proton-NMR of **11c** in deutero-chloroform.

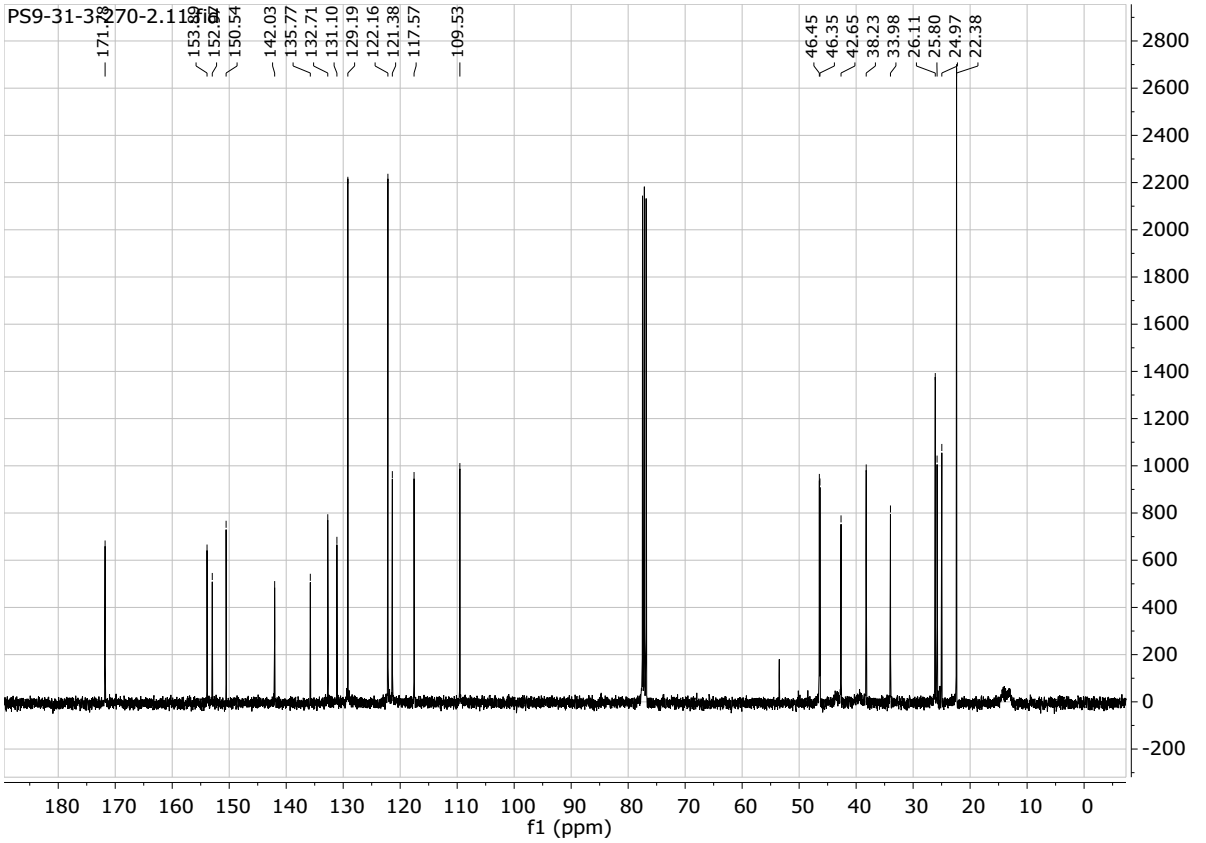

Figure S 60. ^{13}C-NMR spectrum of **11c** in deutero-chloroform.

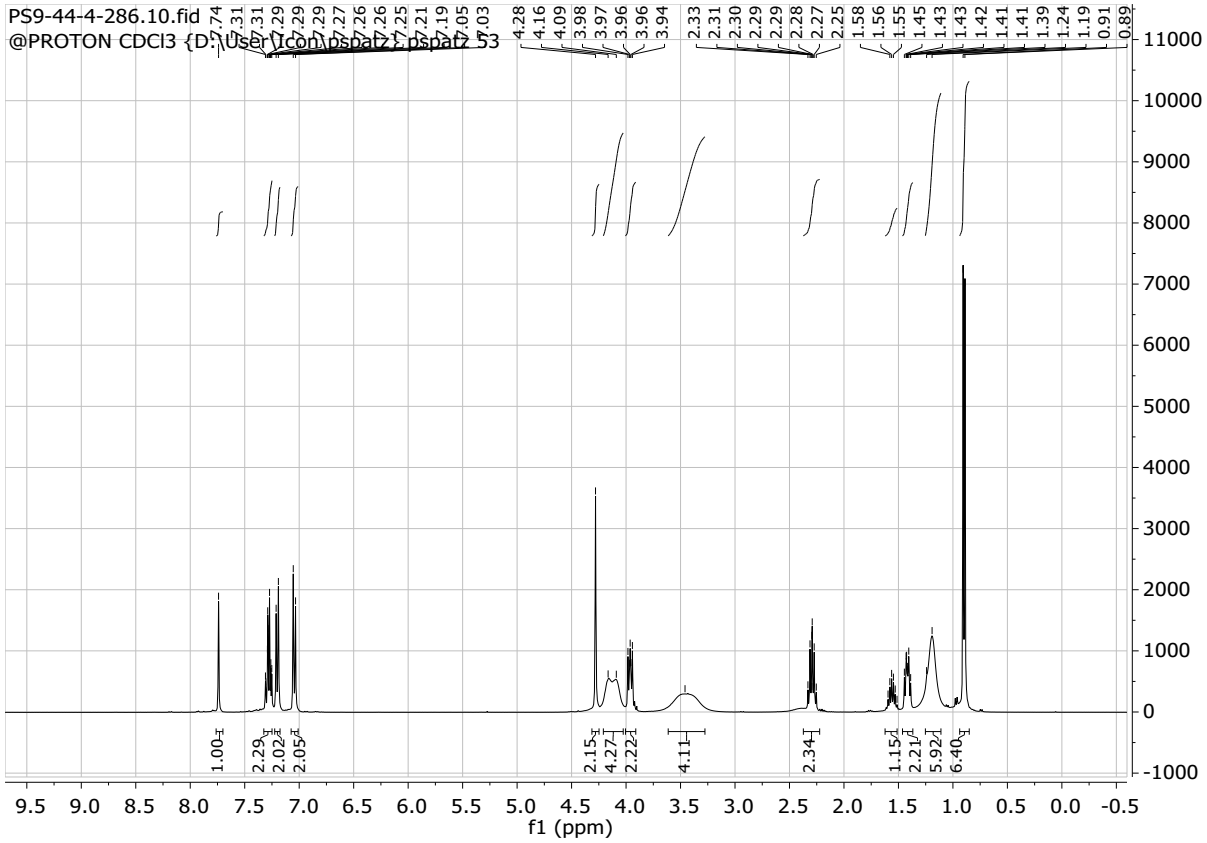

Figure S 61. Proton-NMR of **11d** in deutero-chloroform.

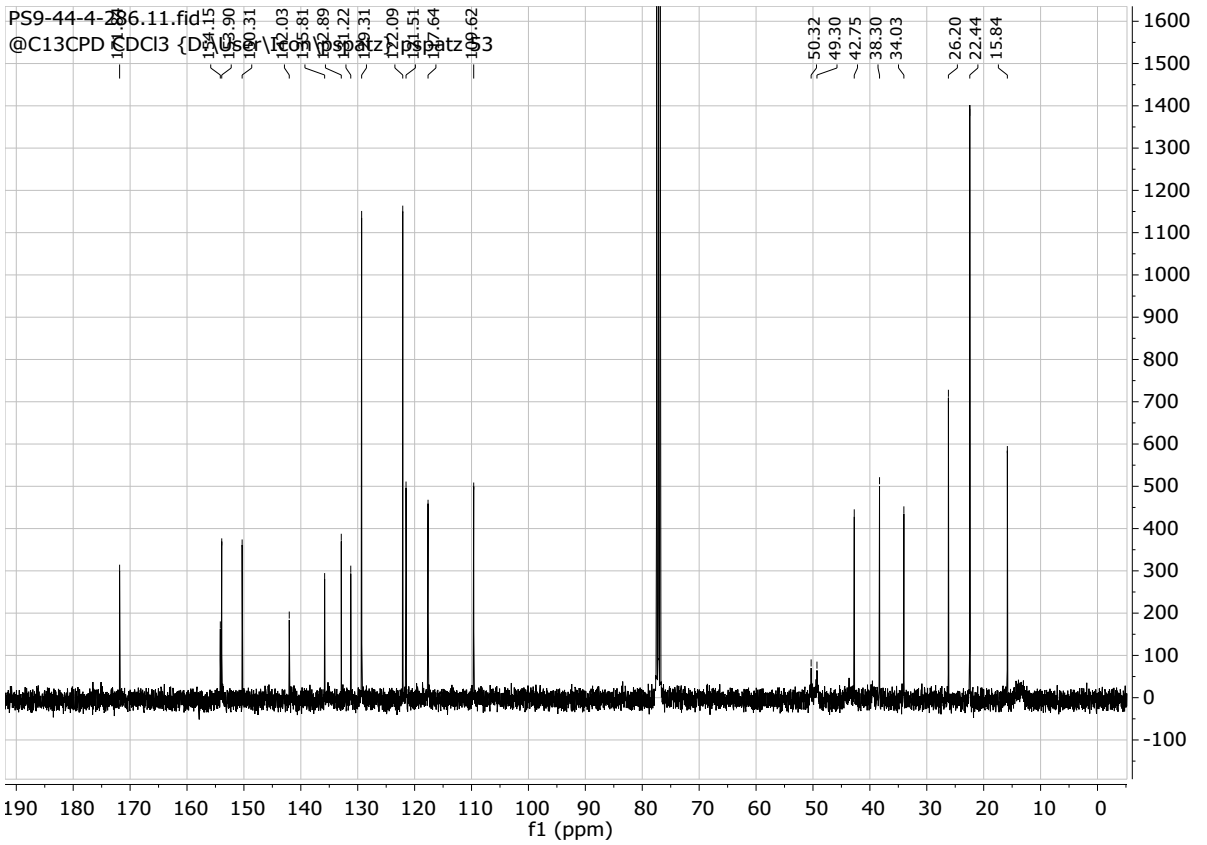

Figure S 62. ^{13}C-NMR spectrum of **11d** in deutero-chloroform.

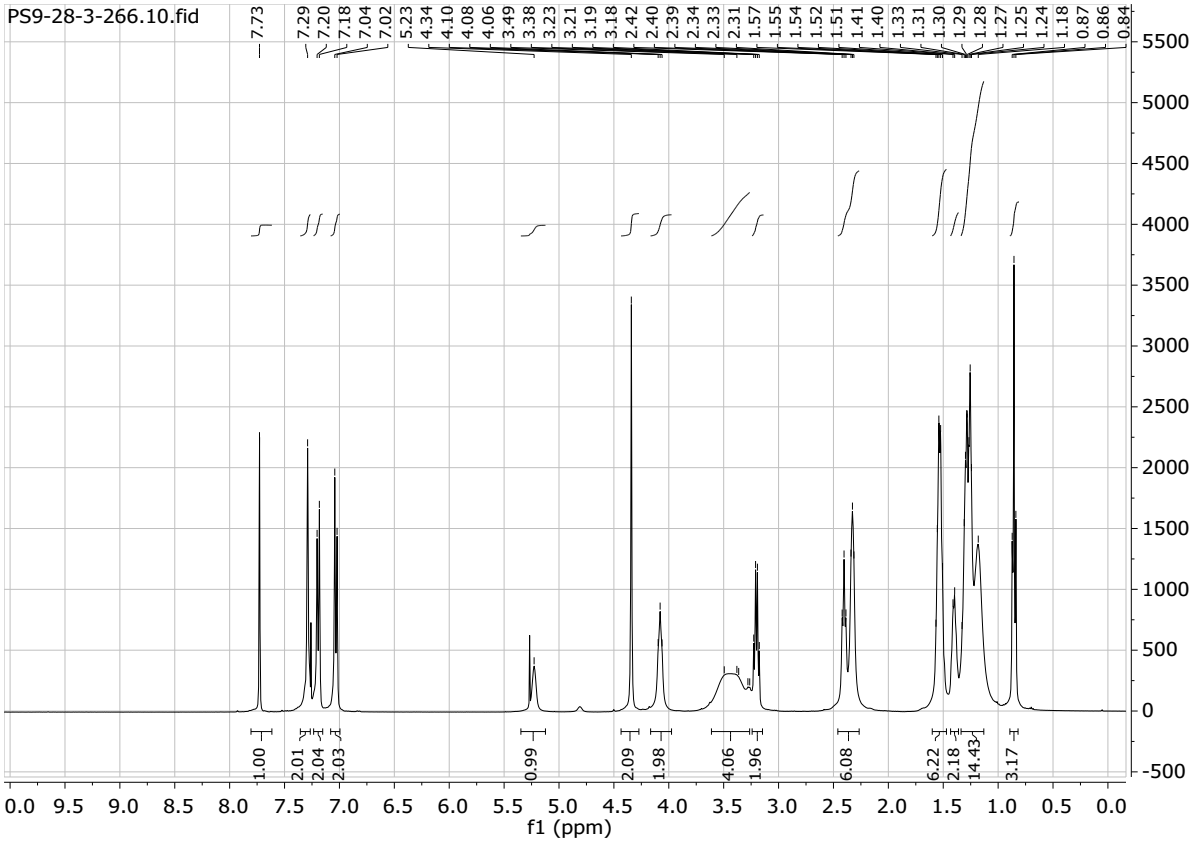

Figure S 63. Proton-NMR of **11e** in deutero-chloroform.

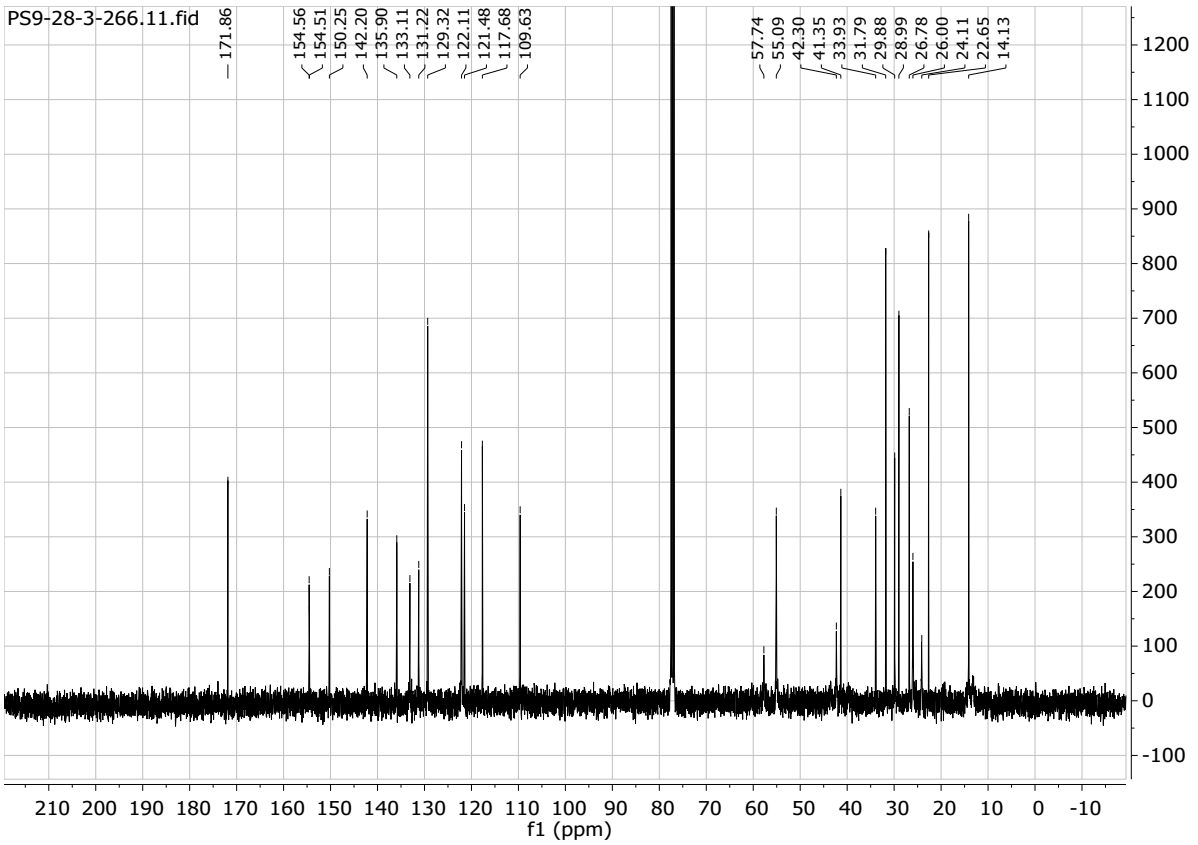

Figure S 64. ^{13}C-NMR spectrum of **11e** in deutero-chloroform.

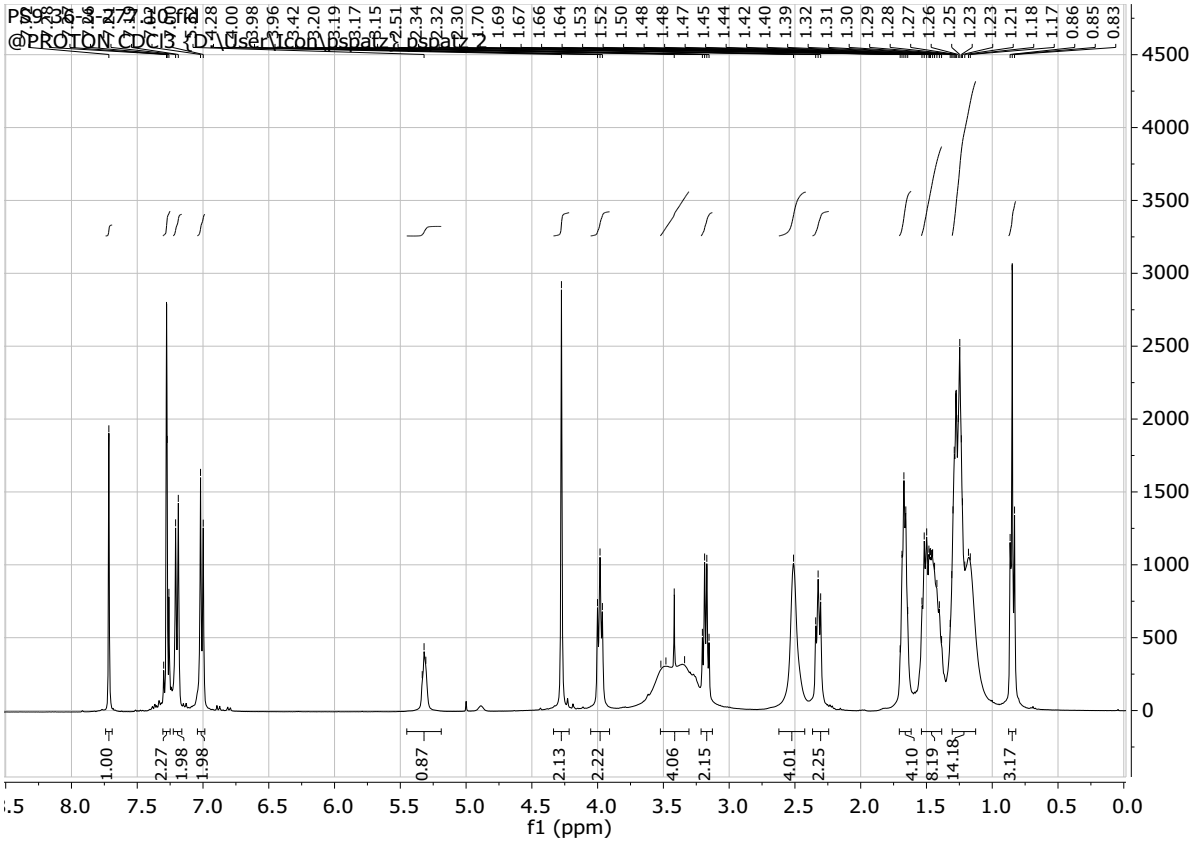

Figure S 65. Proton-NMR of **11f** in deutero-chloroform.

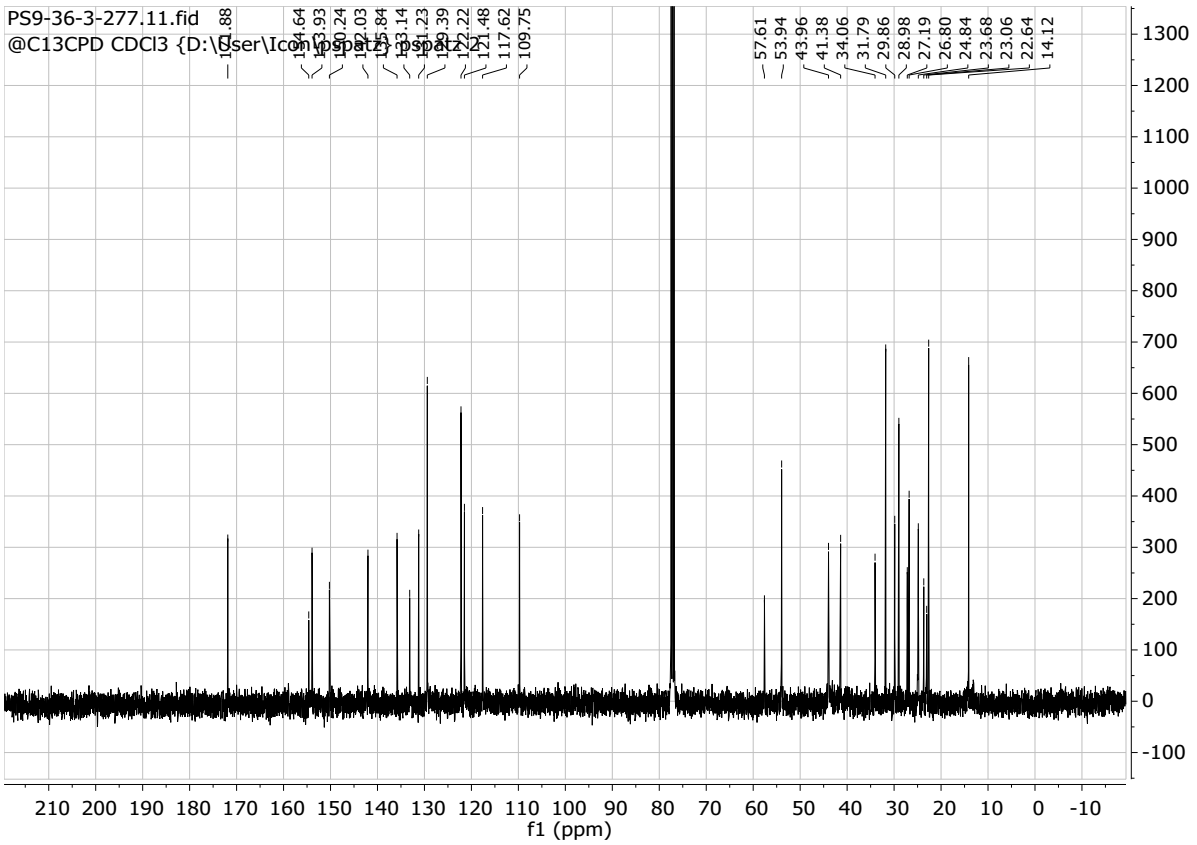

Figure S 66. ^{13}C-NMR spectrum of **11f** in deutero-chloroform.

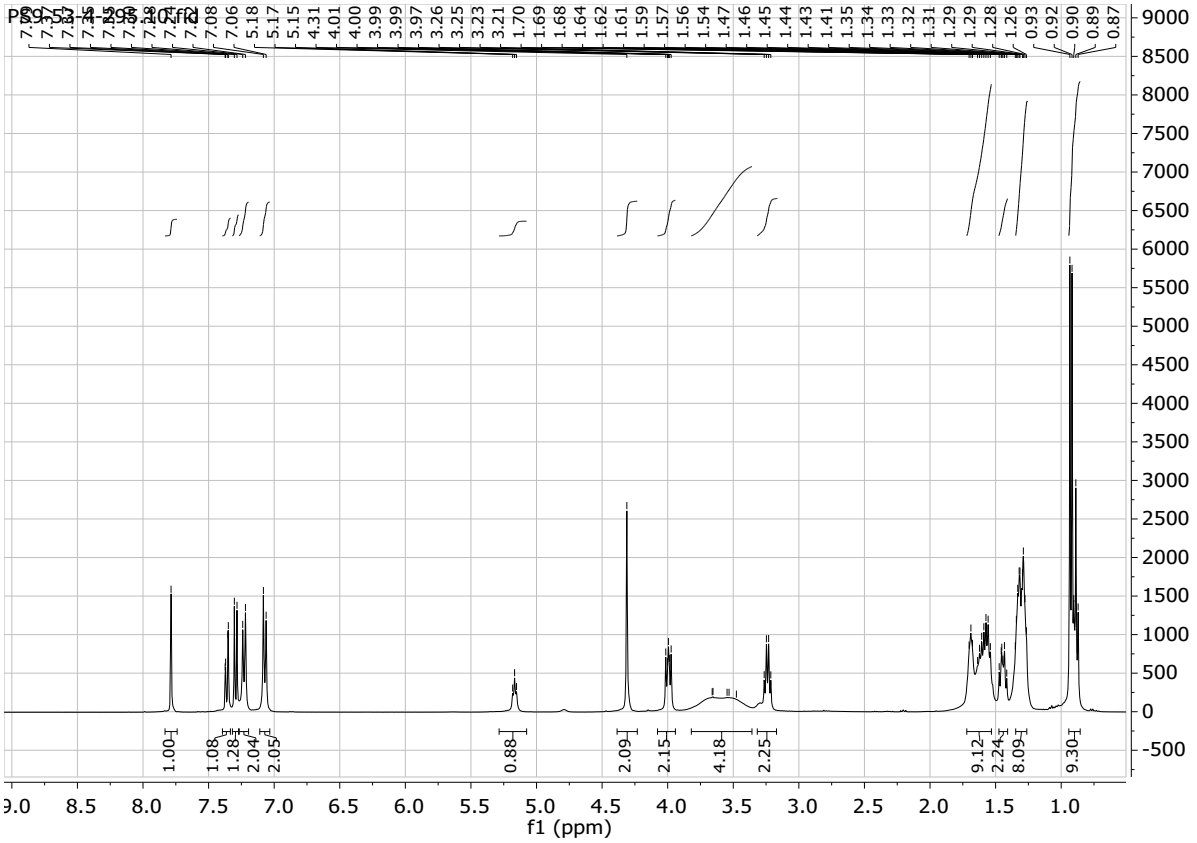

Figure S 67. Proton-NMR of **18a** in deutero-chloroform.

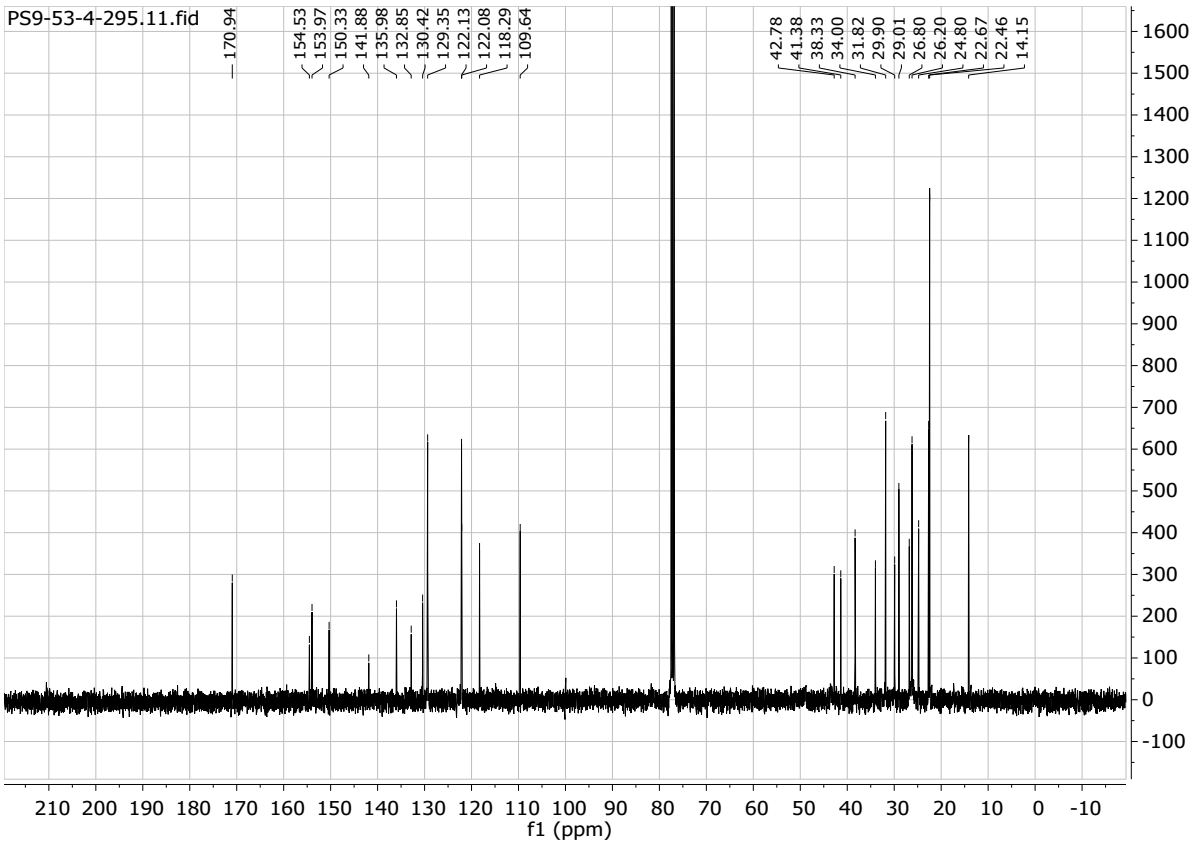

Figure S 68. ^{13}C-NMR spectrum of **18a** in deutero-chloroform.

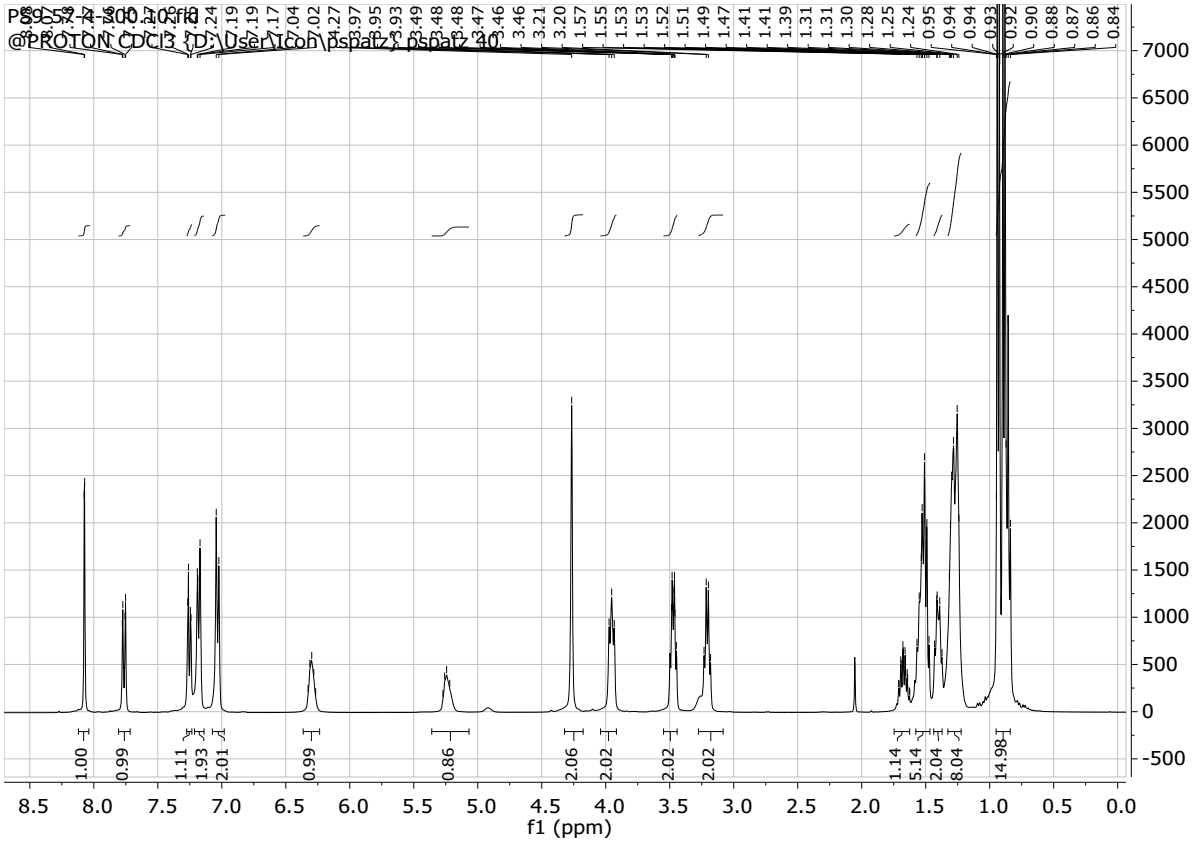

Figure S 69. Proton-NMR of **18b** in deutero-chloroform.

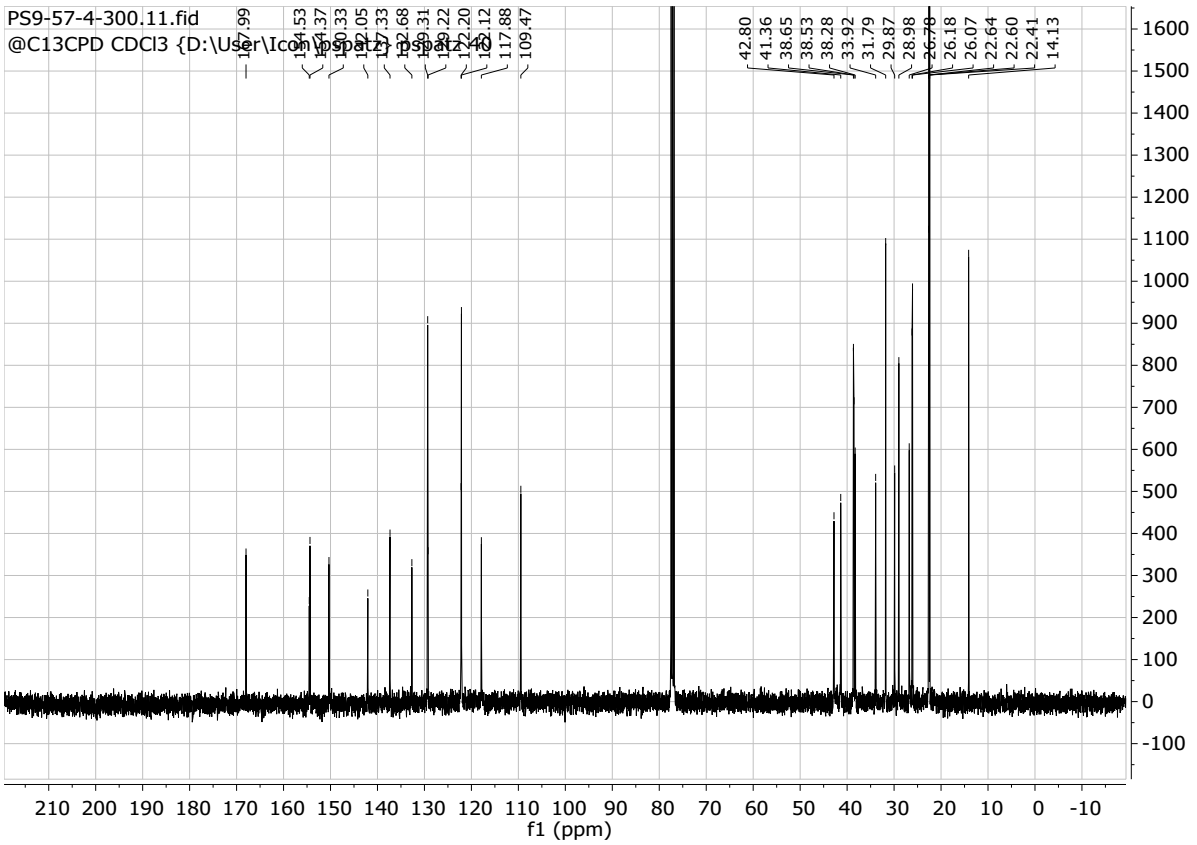

Figure S 70. ^{13}C-NMR spectrum of **18b** in deutero-chloroform.

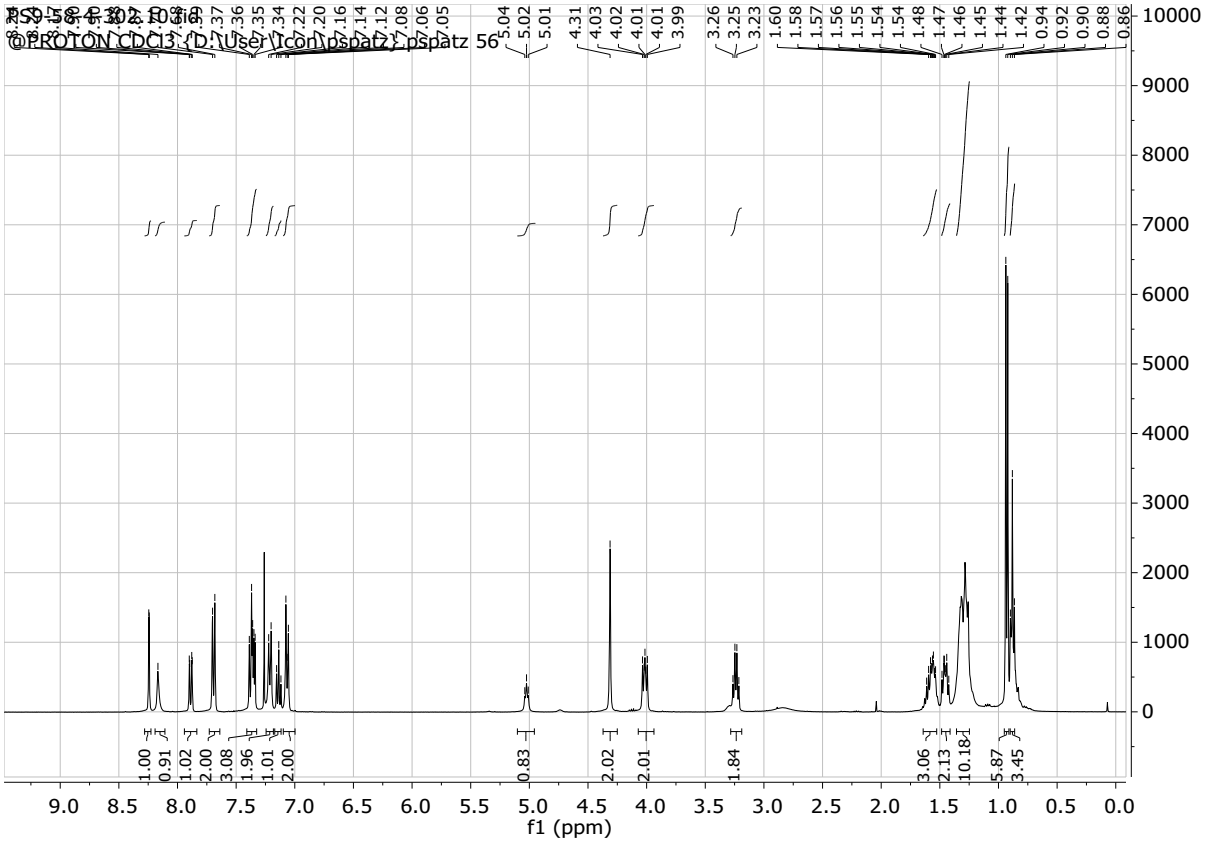

Figure S 71. Proton-NMR of **18c** in deutero-chloroform.

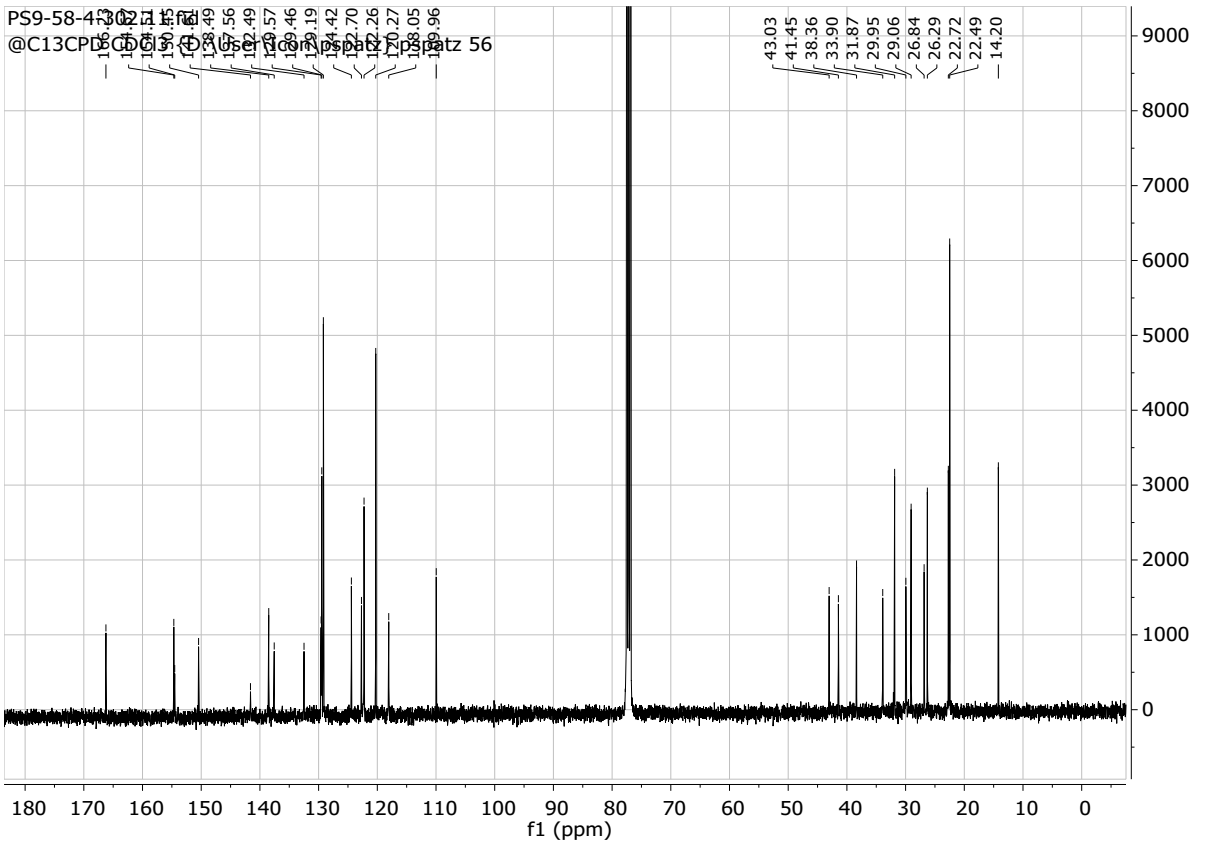

Figure S 72. 13C-NMR spectrum of **18c** in deutero-chloroform.

LC chromatograms of target molecules

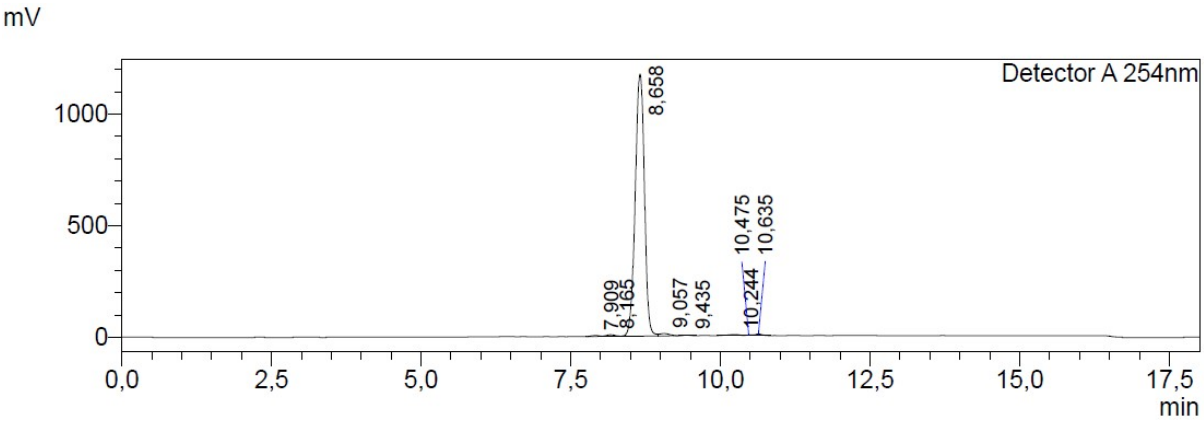

Figure S 73. LC chromatogram of **10a**.

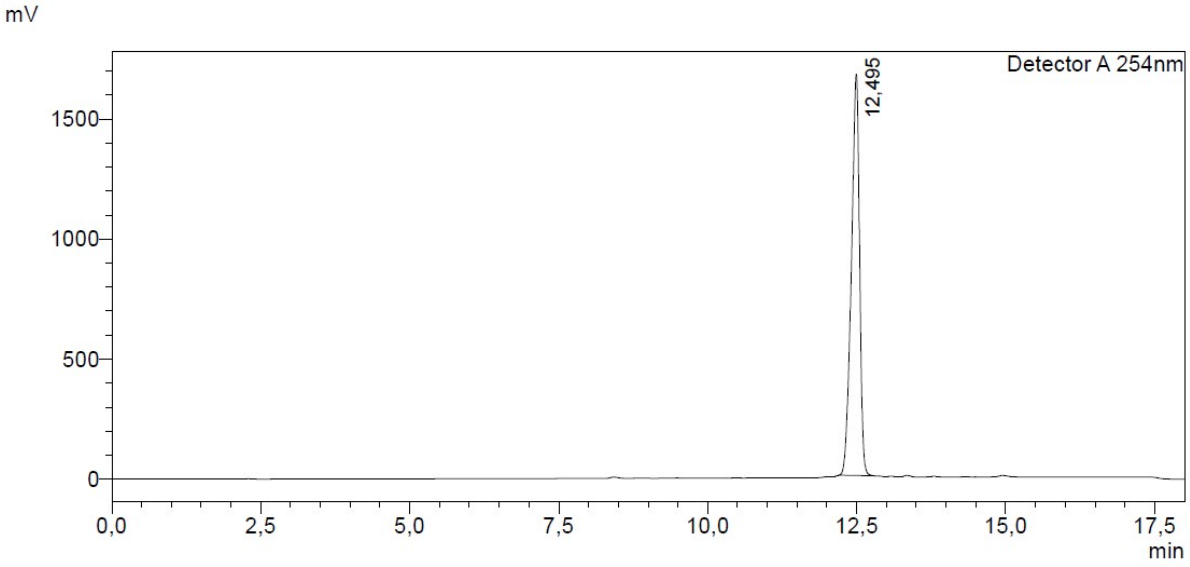

Figure S 74. LC chromatogram of **11a**.

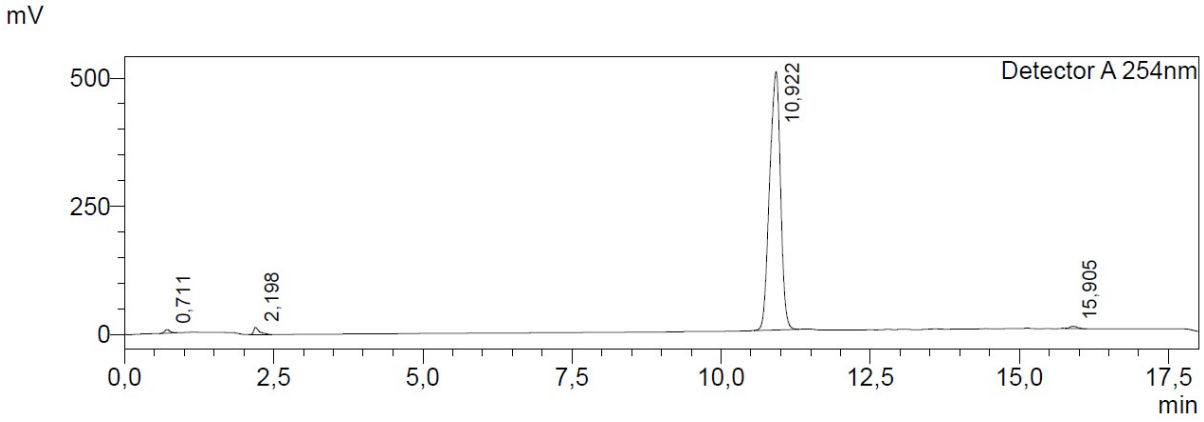

Figure S 75. LC chromatogram of **11b**.

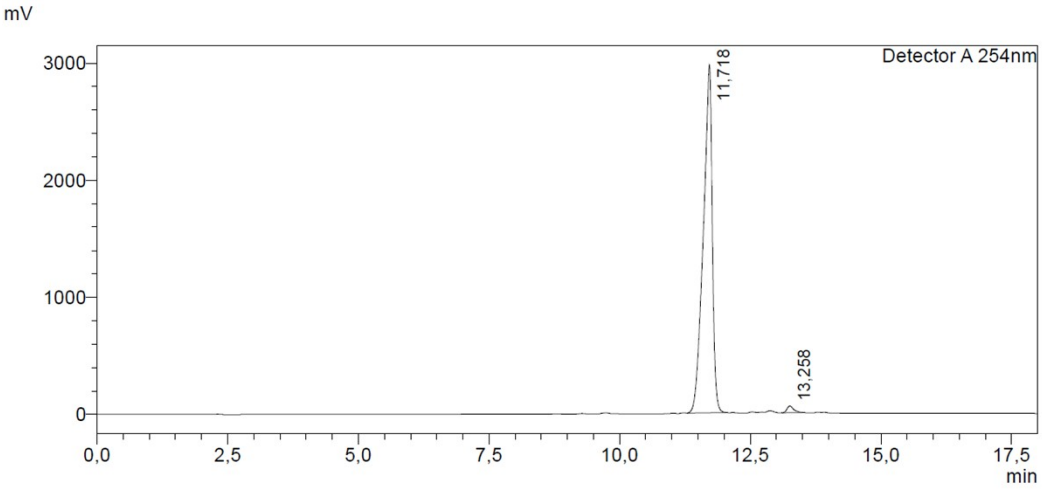

Figure S 76. LC chromatogram of **11c**.

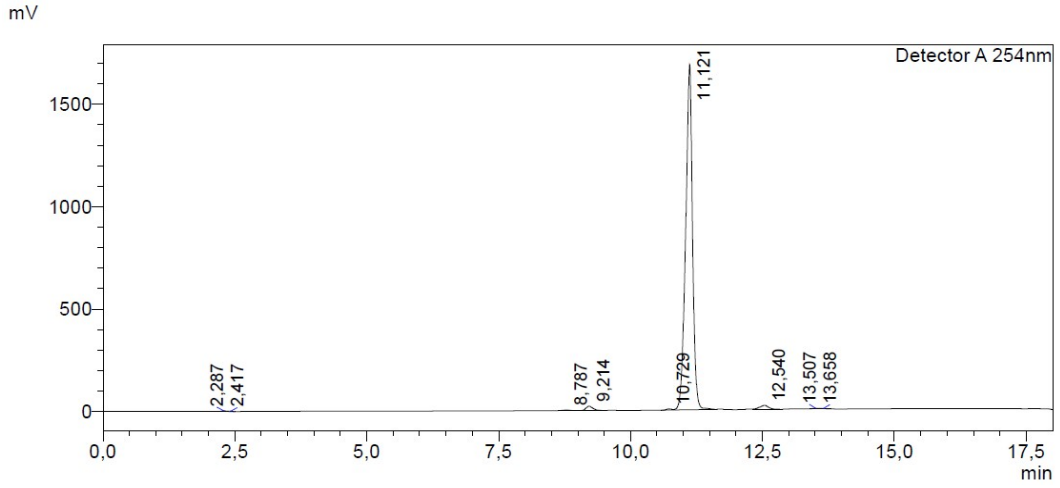

Figure S 77. LC chromatogram of **11d**.

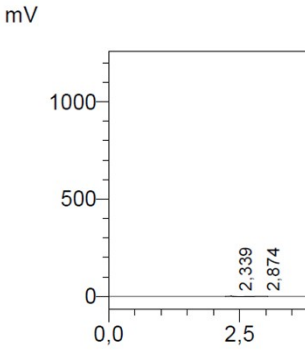

Figure S 78. LC chromatogram of **11e**.

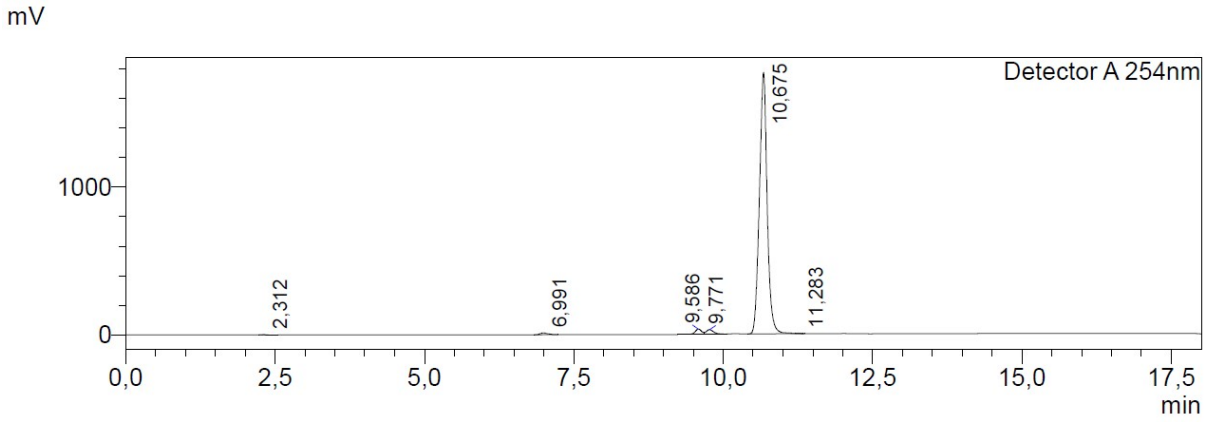

Figure S 79. LC chromatogram of **11f**.

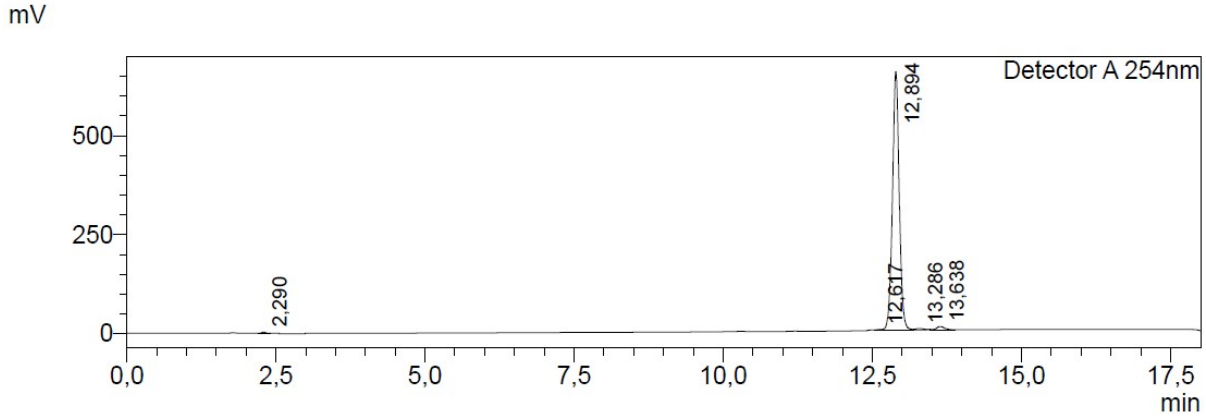

Figure S 80. LC chromatogram of **18a**.

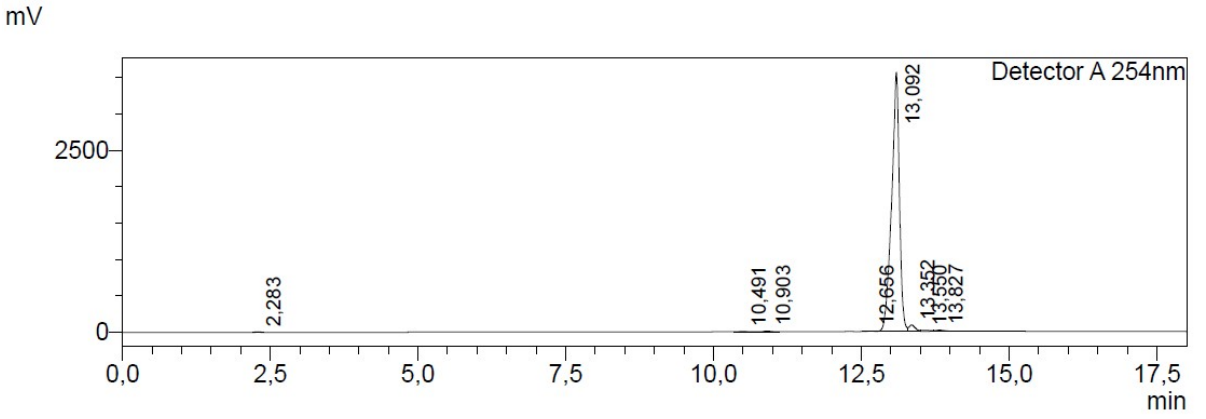

Figure S 81. LC chromatogram of **18b**.

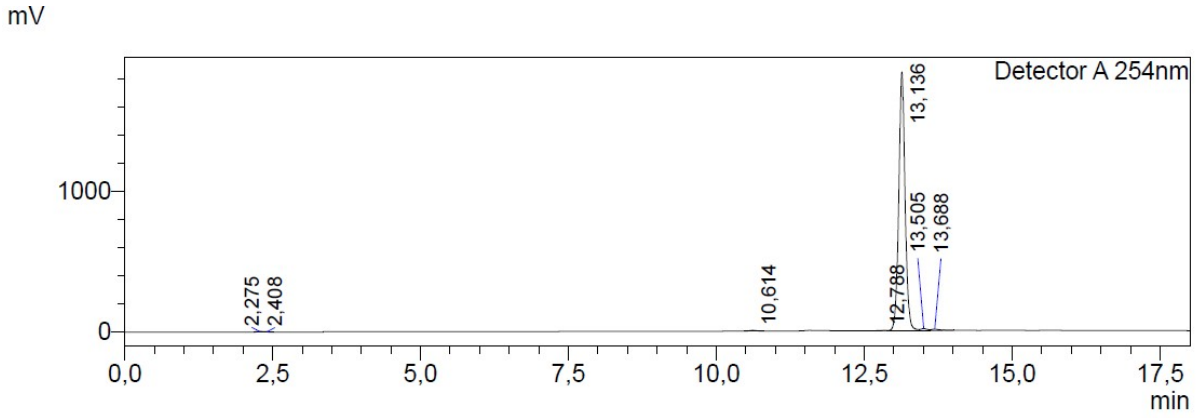

Figure S 82. LC chromatogram of **18c**.

Statement regarding Pan Assay Interference Compounds (PAINS)

None of biological tested compounds contain any PAIN-related structural motifs.

Functional groups of tested compounds are limited to benzimidazole, 1,2,3,4-Tetrahydroisochinolin, simple amides, piperidine and carbamate structures.

Additional references

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