

Supplementary Information

Glycodendron-Rhenium complexes as luminescent probes for lectins sensing

Alessandro Palmioli^{*a‡}, Monica Panigati^a and Anna Bernardi^a

^a Department of Chemistry, University of Milano, via Golgi 19, 20133 Milano, Italy.

[‡] Present address: Department of Biotechnology and Biosciences, University of Milano-Bicocca, piazza della Scienza, 2, 20126 Milano, Italy. Email alessandro.palmioli@unimib.it

Content

Synthesis of multivalent glycoconjugates	pg. 2
Compound 7-17	pg. 2-4
Compounds characterization: NMR and Mass spectra	pg. 5-20
PNA sensing	pg. 21

Synthesis of multivalent glycoconjugates

Compound 7. Penta-O-acetyl-D-mannose (**4**) (1.25 g, 3.2 mmol, 1 eq) were added to the solution of (**6**) (4 mmol, 1.25 eq) in DCM (25 mL), then the mixture was treated with $\text{BF}_3\text{-Et}_2\text{O}$ (633 μL , 5 mmol, 1.5 eq) at 0°C under nitrogen atmosphere. Then mixture was slowly allowed to warm and stirred at room temperature (TLC Hex:AcOEt 1:1 $R_{f,\text{prod.}} = 0.32$). After 5h the reaction was quenched by addition of Et_3N (1,5 mL) stirred for additional 15 min and concentrated under reduced pressure. The crude was purified by flash chromatography (Hex:AcOEt gradient elution) affording pure compound (**7**) (822 mg, 56 %, α -anomer). ^1H NMR (400 MHz, CDCl_3) δ 5.37 (dd, $J_{3,4} = 10.0$, $J_{2,3} = 3.2$ Hz, 1H, H-3), 5.29 (t, $J_{3,4} = 10.0$ Hz, 2H, H-4), 5.28 (dd, $J_{2,3} = 3.2$, $J_{1,2} = 1.6$ Hz, 1H, H-2), 4.88 (d, $J_{1,2} = 1.6$ Hz, 1H, H-1), 4.29 (dd, $J = 12.4$, 5.2 Hz, 1H, H-7a), 4.13 – 4.04 (m, 2H, H-7b, H-5), 3.89 – 3.79 (m, 1H, H-6a), 3.70 – 3.64 (m, 5H, H-6b, H-8, H-9), 3.42 – 3.35 (m, 2H, H-10), 2.15 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.99 (s, 3H, OAc). ^{13}C NMR (100 MHz, CDCl_3) δ 170.83, 170.20, 170.07, 169.91 (CO), 97.87 (C1), 70.34, 70.20 (C8, C9), 69.66 (C2), 69.21 (C3), 68.55 (C5), 67.45 (C6), 66.26 (C4), 62.57 (C7), 50.87 (C10), 21.05, 20.90, 20.85, 20.85 (OAc). MS (ESI) calculated for $[\text{C}_{18}\text{H}_{27}\text{N}_3\text{O}_{11}\text{Na}]^+$: 484.15 found: 484.3 $[\text{M}+\text{Na}]^+$; $[\alpha]_D^{25}$: +37.77 ($C = 0.705$, CHCl_3).

Compound 8. Penta-O-acetyl-D-galactose (**5**) (1.25 g, 3.2 mmol, 1 eq) were added to the solution of (**6**) (4 mmol, 1.25 eq) in DCM (25 mL), then the mixture was treated with $\text{BF}_3\text{-Et}_2\text{O}$ (609 μL , 4.8 mmol, 1.5 eq) at 0°C under nitrogen atmosphere. Then mixture was slowly allowed to warm and stirred at room temperature (TLC Hex:AcOEt 1:1 $R_{f,\text{prod.}} = 0.36$). After 6h the reaction was quenched by addition of Et_3N (1 mL) stirred for additional 15 min and concentrated under reduced pressure. The crude was purified by flash chromatography (Hex:AcOEt gradient elution) affording pure compound (**8**) (1.03 mg, 70 %, β -anomer). ^1H NMR (400 MHz, CDCl_3) δ 5.38 (dd, $J_{3,4} = 3.4$, $J_{4,5} = 0.9$ Hz, 1H, H-4), 5.21 (dd, $J_{2,3} = 10.5$, $J_{1,2} = 8.0$ Hz, 1H, H-2), 5.02 (dd, $J_{2,3} = 10.5$, $J_{3,4} = 3.4$ Hz, 1H, H-3), 4.58 (d, $J_{1,2} = 8.0$ Hz, 1H, H-1), 4.15 (qd, $J = 11.2$, 6.7 Hz, 2H, H-6), 3.96 (dt, $J = 11.1$, 4.0 Hz, 1H, H-7a), 3.90 (td, $J_{5,6} = 6.7$, $J_{4,5} = 0.9$ Hz, 1H, H-5), 3.80 – 3.72 (m, 1H, H-7b), 3.69 – 3.62 (m, 4H, H-8, H-9), 3.39 – 3.33 (m, 2H, H-10), 2.14 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.98 (s, 3H, OAc). ^{13}C NMR (100 MHz, CDCl_3) δ 170.54, 170.39, 170.28, 169.64 (CO), 101.48 (C1), 71.03 (C3), 70.80 (C5), 70.58, 70.34 (C8, C9), 69.18 (C7), 68.95 (C2), 67.18 (C4), 61.41 (C6), 50.92 (C10), 20.91, 20.82, 20.81, 20.73 (OAc). MS (ESI) calculated for $[\text{C}_{18}\text{H}_{27}\text{N}_3\text{O}_{11}\text{Na}]^+$: 484.15 found: 484.3 $[\text{M}+\text{Na}]^+$.

Compound 9. A solution of compound (**7**) (790 mg, 1.71 mmol) in freshly distilled MeOH (75 mL) was treated with a solution of NaOMe (7,5 mL, 1 M in MeOH). The reaction was stirred at room temperature under nitrogen atmosphere. After 2 h the reaction was complete. The mixture was diluted with MeOH and neutralized by addition of Amberlite IR 120- H^+ resin, then the beads was filtered off and washed with MeOH. Finally the solvent was removed under reduced pressure and the crude was purified by flash chromatography (DCM: MeOH 9:1) affording pure compound (**9**) (427 mg, 85 %). ^1H NMR (400 MHz, MeOD) δ 4.80 (d, $J_{1,2} = 1.6$ Hz, 1H, H-1), 3.90 – 3.79 (m, 3H, H-2, H-7a, H-6a), 3.75 – 3.54 (m, 9H, H-8, H-9, H-3, H-4, H-5, H-6b, H-7b), 3.39 – 3.35 (m, 2H, H-10). ^{13}C NMR (100 MHz, MeOD) δ 101.82 (C1), 74.62 (C4), 72.55 (C3), 72.11 (C2), 71.26, 71.19 (C8, C9), 68.57 (C5), 67.86 (C7), 62.91 (C6), 51.74 (C10). MS (ESI) calculated for $[\text{C}_{10}\text{H}_{19}\text{N}_3\text{O}_7\text{Na}]^+$: 316.11, found: 316.06 $[\text{M}+\text{Na}]^+$; $[\alpha]_D^{25}$: + 52.27 ($C = 0.465$, MeOH).

Compound 10. A solution of compound (**8**) (1.0 g, 2.16 mmol) in freshly distilled MeOH (100 mL) was treated with a solution of NaOMe (10 mL, 1 M in MeOH). The reaction was stirred at room temperature under nitrogen atmosphere. After 2 h the reaction was complete. The mixture was diluted with MeOH and neutralized by addition of Amberlite IR 120- H^+ resin, then the beads was filtered off and washed with MeOH. Finally the solvent was removed under reduced pressure and the crude was purified by flash chromatography (DCM: MeOH 9:1) affording pure compound (**10**) (590 mg, 93 %). ^1H NMR (400 MHz, MeOD) δ 4.27 (d, $J_{1,2} = 8.0$ Hz, 1H, H-1), 4.08 – 3.98 (m, 1H, H-7a), 3.82 (dd, $J_{3,4} = 3.4$, $J_{4,5} = 0.9$ Hz, 1H, H-4), 3.79 – 3.65 (m, 7H, H-7b, H-6, H-8, H-9), 3.58 – 3.44 (m, 3H, H-2, H-3, H-5), 3.43 – 3.37 (m, 2H, H-10). ^{13}C NMR (100 MHz, MeOD) δ 105.13 (C1), 76.70 (C3), 74.90 (C5), 72.53 (C2), 71.47, 71.01 (C8, C9), 70.29 (C4), 69.70 (C7), 62.50 (C6), 51.78 (C10). MS (ESI) calculated for $[\text{C}_{10}\text{H}_{19}\text{N}_3\text{O}_7\text{Na}]^+$: 316.11, found: 316.2 $[\text{M}+\text{Na}]^+$.

Compound 12. To a reaction vessel containing compound (**11**) (55 mg, 154 μmol , 1 eq), TBTA (16.3 mg, 31 μmol , 0.2 eq), CuSO_4 (3.8 mg, 15 μmol , 0.1 eq), sodium ascorbate (12 mg, 61 μmol , 0.4 eq), compound (**9**) (150 mg, 509 μmol , 3.3 eq) were added and dissolved in a mixture of H_2O :THF 1:1 (8 mL), then the reaction was stirred under nitrogen at room temperature for 16h. The crude mixture was directly loaded in a C-18 column and purified by RP-18 chromatography (H_2O :MeOH gradient elution) obtaining 187 mg of pure compound (**12**) (yield 98%). ^1H NMR (400 MHz, MeOD) δ 7.98 (s, 3H, H-11), 4.75 (d, $J_{1,2} = 1.6$ Hz, 3H, H-1), 4.58 (t, $J_{9,10} = 5.0$ Hz, 6H, H-10), 4.55 (s, 6H, H-13), 3.89 (t, $J_{9,10} = 5.0$ Hz, 6H, H-9), 3.85 – 3.79 (m, 6H, H-7a, H-6a), 3.78 (dd, $J_{2,3} = 3.2$, $J_{1,2} = 1.6$ Hz, 3H, H-2), 3.74 – 3.49 (m, 29H, H-3, H-4, H-5, H-6b, H-7b, H-8, H-17, H-18, H-19, H-20), 3.47 (s, 6H, H-14), 3.42 (s, 2H, H-16). ^{13}C NMR (100 MHz, MeOD) δ 146.07 (C12), 125.80 (C11), 101.64 (C11), 74.65 (C5), 72.55 (C3), 72.48 (C19), 72.09 (C17), 72.06 (C2), 71.37 (C18), 71.20 (C8), 70.79 (C14), 70.39 (C9), 70.07 (C16), 68.65 (C4), 67.63 (C7), 65.31 (C13), 62.96 (C6), 51.43 (C10), 46.53 (C15), 44.05 (C20). MS (MALDI, DHB matrix) calculated for $[\text{C}_{48}\text{H}_{82}\text{ClN}_9\text{O}_{26}]$: 1235.50, found: 1237.1 $[\text{M}+\text{H}]^+$, 1259.1 $[\text{M}+\text{Na}]^+$, $[\alpha]_D^{25}$: +28.20 (C = 0.395, MeOH).

Compound 13. To a reaction vessel containing compound (**11**) (55 mg, 154 μmol , 1 eq), TBTA (16.3 mg, 31 μmol , 0.2 eq), CuSO_4 (3.8 mg, 15 μmol , 0.1 eq), sodium ascorbate (12 mg, 61 μmol , 0.4 eq), compound (**10**) (150 mg, 509 μmol , 3.3 eq) were added and dissolved in a mixture of H_2O :THF 1:1 (8 mL), then the reaction was stirred under nitrogen at room temperature for 16h. The crude mixture was directly loaded in a C-18 column and purified by RP-18 chromatography (H_2O :MeOH gradient elution) obtaining 173 mg of pure compound (**13**) (yield 91 %). ^1H NMR (400 MHz, MeOD) δ 8.04 (s, 3H, H-11), 4.63 – 4.57 (m, 6H, H-10), 4.54 (s, 6H, H-13), 4.25 (d, $J_{1,2} = 8.0$ Hz, 3H, H-1), 4.01 – 3.94 (m, 3H, H-7a), 3.92 (td, $J = 5.5, 0.8$ Hz, 6H, H-9), 3.83 (dd, $J_{3,4} = 3.4$, $J_{4,5} = 0.9$ Hz, 3H, H-4), 3.80 – 3.65 (m, 18H, H-6, H-7b, H-8, H-17, H-19), 3.65 – 3.58 (m, 4H, , H-20, H-18), 3.57 – 3.45 (m, 15H, H-2, H-3, H-5, H-14), 3.42 (s, 2H, H-16). ^{13}C NMR (100 MHz, MeOD) δ 146.06 (C12), 125.99 (C11), 105.09 (C11), 76.72 (C3), 74.97 (C5), 72.54 (C2), 72.48 (C19), 72.11 (C17), 71.43 (C8), 71.36 (C18), 70.80 (C16), 70.37 (C9), 70.29 (C4), 70.04 (C14), 69.76 (C7), 65.29 (C13), 62.56 (C6), 51.37 (C10), 46.53 (C15), 44.06 (C20). MS (MALDI, DHB matrix) calculated for $[\text{C}_{48}\text{H}_{82}\text{ClN}_9\text{O}_{26}]$: 1235.50, found: 1237.0 $[\text{M}+\text{H}]^+$, 1259.3 $[\text{M}+\text{Na}]^+$, $[\alpha]_D^{25}$: - 8.66 (C = 0.350, MeOH).

Compound 14. To a solution of compound (**12**) (180 mg, 145 μmol , 1 eq) in anhydrous DMF (2 mL), NaN_3 (76 mg, 1.17 mmol, 8 eq) and NaI (2.2mg, 14.5 μmol , 0.1 eq) were added. The reaction mixture was stirred at 60°C under nitrogen overnight. The crude mixture was directly loaded in a C-18 column and purified by RP-18 chromatography (H_2O :MeOH gradient elution) obtaining 168 mg of pure compound (**14**) (yield 93%). ^1H NMR (400 MHz, MeOD) δ 7.98 (s, 3H, H-11), 4.75 (d, $J_{1,2} = 1.6$ Hz, 3H, H-1), 4.58 (t, $J_{9,10} = 5.0$ Hz, 6H, H-10), 4.55 (s, 6H, H-13), 3.89 (t, $J_{9,10} = 5.0$ Hz, 6H, H-9), 3.86 – 3.79 (m, 6H, H-6a, H-7a), 3.78 (dd, $J_{2,3} = 3.2$, $J_{1,2} = 1.6$ Hz, 3H, H-2), 3.74 – 3.50 (m, 27H, H-3, H-4, H-4, H-5, H-6b, H-7b, H-8, H-17, H-18, H18, H-19), 3.47 (s, 6H, H-14), 3.43 (s, 2H, H-16), 3.36 – 3.32 (m, 2H, H-20). ^{13}C NMR (100 MHz, MeOD) δ 146.08 (C12), 125.79 (C11), 101.64 (C1), 74.65 (C5), 72.55 (C3), 72.16 (C19), 72.06 (C2), 71.42 (C17), 71.19 (C8), 71.17 (C18), 70.82 (C16), 70.38 (C9), 70.08 (C14), 68.65 (C4), 67.62 (C7), 65.31 (C13), 62.96 (C6), 51.82 (C20), 51.42 (C10), 46.53 (C15). MS (ESI) calculated for $[\text{C}_{48}\text{H}_{82}\text{N}_{12}\text{O}_{26}\text{Na}]^+$:1265.54, found: 1266.1 $[\text{M}+\text{Na}]^+$; $[\alpha]_D^{25}$: +28.5 (C = 0.48, MeOH).

Compound 15. To a solution of compound (**13**) (165 mg, 133 μmol , 1 eq) in anhydrous DMF (2 mL), NaN_3 (69 mg, 1 mmol, 8 eq) and NaI (2.0 mg, 13 μmol , 0.1 eq) were added. The reaction mixture was stirred at 60°C under nitrogen overnight. The crude mixture was directly loaded in a C-18 column and purified by RP-18 chromatography (H_2O :MeOH gradient elution) obtaining 158 mg of pure compound (**15**) (yield 96%). ^1H NMR (400 MHz, MeOD) δ 8.03 (s, 3H, H-11), 4.62 – 4.56 (m, 6H, H-10), 4.54 (s, 6H, H-13), 4.25 (d, $J_{1,2} = 8.0$ Hz, 3H, H-1), 4.00 – 3.95 (m, 3H, H-7), 3.91 (bt, $J_{9,10} = 5.1$ Hz, 6H, H-9), 3.83 (dd, $J_{3,4} = 3.4$, $J_{4,5} = 0.9$ Hz, 3H, H-4), 3.79 – 3.62 (m, 18H, H-6a, H-7b, H-8, H-17, H-18, H-19), 3.61 – 3.55 (m, 3H, H-6b), 3.54 – 3.45 (m, 16H, H-2, H-3, H-5, H-14), 3.43 (s, 2H, H-16), 3.37 – 3.32 (m, 2H, H-20). ^{13}C NMR (100 MHz, MeOD) δ 146.07 (C12), 125.97 (C11), 105.09 (C1), 76.72 (C3), 74.97 (C5), 72.54 (C2), 72.17 (C17), 71.43 (C8, C18), 71.17 (C19), 70.83 (C16), 70.37 (C9), 70.29 (C4), 70.05 (C14), 69.76 (C7), 65.29 (C13), 62.56 (C6), 51.83 (C20), 51.36 (C10), 46.54

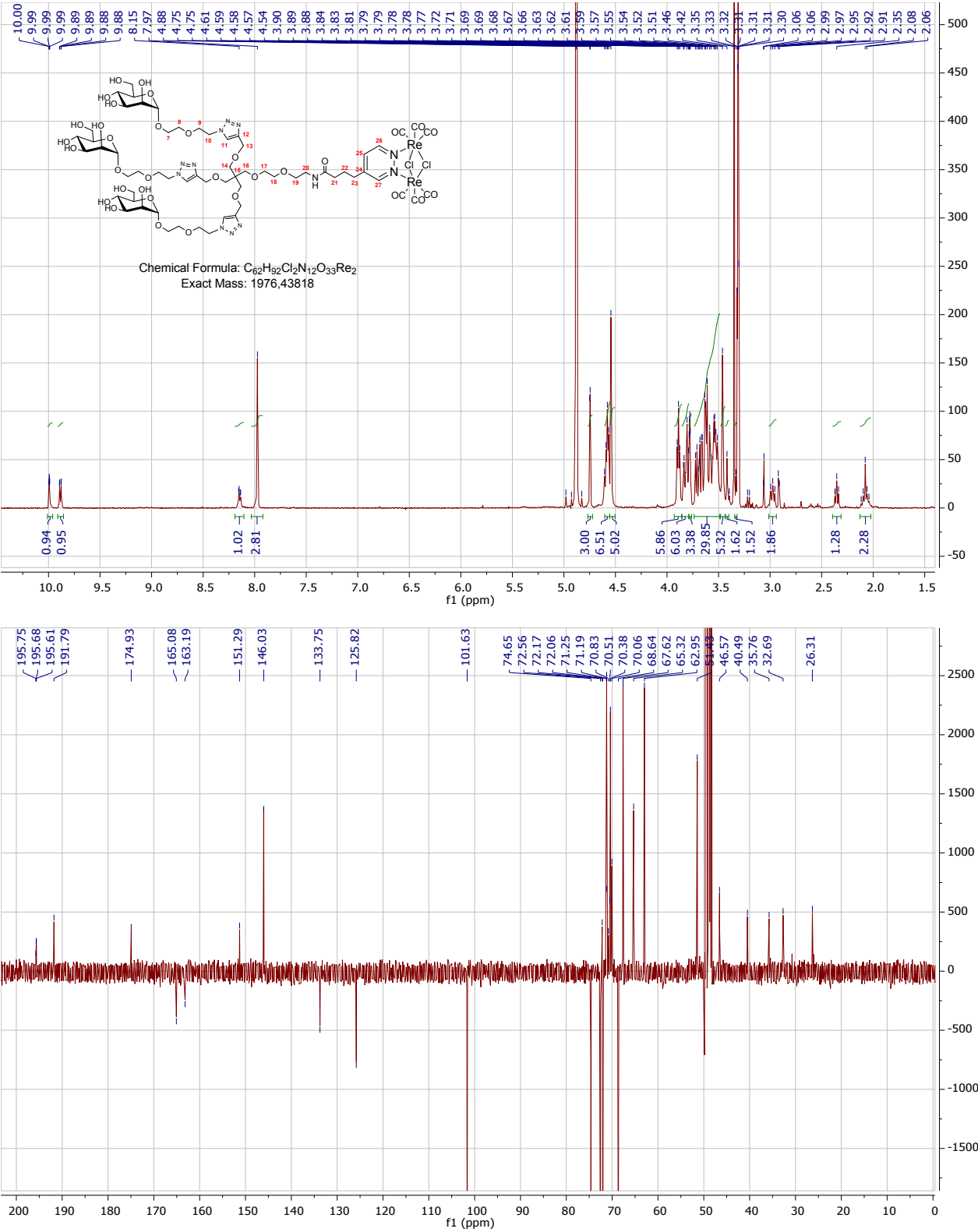
(C15). MS (ESI) calculated for $[C_{48}H_{82}N_{12}O_{26}Na]^+$: 1265.54, found: 1265.8 $[M+Na]^+$; $[\alpha]_D^{25}$: -10.1 (C = 0.29, MeOH).

Compound 16. To a solution of compound (**14**) (26 mg, 15 μ mol, 1 eq) in freshly distilled MeOH (1 mL), HCl (21 μ L, 1.25 M in MeOH) was slowly added under nitrogen atmosphere. After 10 min a catalytic amount of Pd/C was added, then the mixture was stirred under H_2 atmosphere at r.t. for 2h. The crude was diluted with MeOH and the catalyst was filtered off through a celite pad. Removal of the solvent under reduced pressure afforded pure compound (**16**) (21.5 mg, 83 % yield) as chlorohydrate salt. 1H NMR (400 MHz, MeOD) δ 7.98 (s, 3H, H-11), 4.75 (d, $J_{1,2}$ = 1.6 Hz, 3H, H-1), 4.59 (t, $J_{9,10}$ = 5.0 Hz, 6H, H-10), 4.55 (s, 6H, H-13), 3.89 (t, $J_{9,10}$ = 5.0 Hz, 6H, H-9), 3.84 – 3.79 (m, 6H, H-6a, H-7a), 3.78 (dd, $J_{2,3}$ = 3.2, $J_{1,2}$ = 1.7 Hz, 3H, H-2), 3.75 – 3.50 (m, 27H, H-3, H-4, H-5, H-6b, H-7b, H-8, H-17, H-18, H-19), 3.47 (s, 6H, H-14), 3.43 (s, 2H, H-16), 3.08 – 3.01 (m, 2H, H-20). ^{13}C NMR (100 MHz, MeOD) δ 145.95 (C12), 125.86 (C11), 101.62 (C1), 74.62 (C5), 72.54 (C3), 72.19 (C19), 72.05 (C2), 71.35 (C17), 71.17 (C18, C8), 70.91 (C16), 70.35 (C9), 70.07 (C14), 69.45, 68.59 (C4), 67.61 (C7), 65.25 (C13), 62.86 (C6), 51.43 (C10), 46.52 (C15), 41.16 (C20). MS (ESI) calculated for $[C_{48}H_{85}N_{10}O_{26}]^+$: 1218.24, found: 1217.8 $[M+H]^+$, 1238.9 $[M+Na]^+$, 1253.8 $[M+K]^+$; $[\alpha]_D^{25}$: +28.4 (C = 0.404, MeOH).

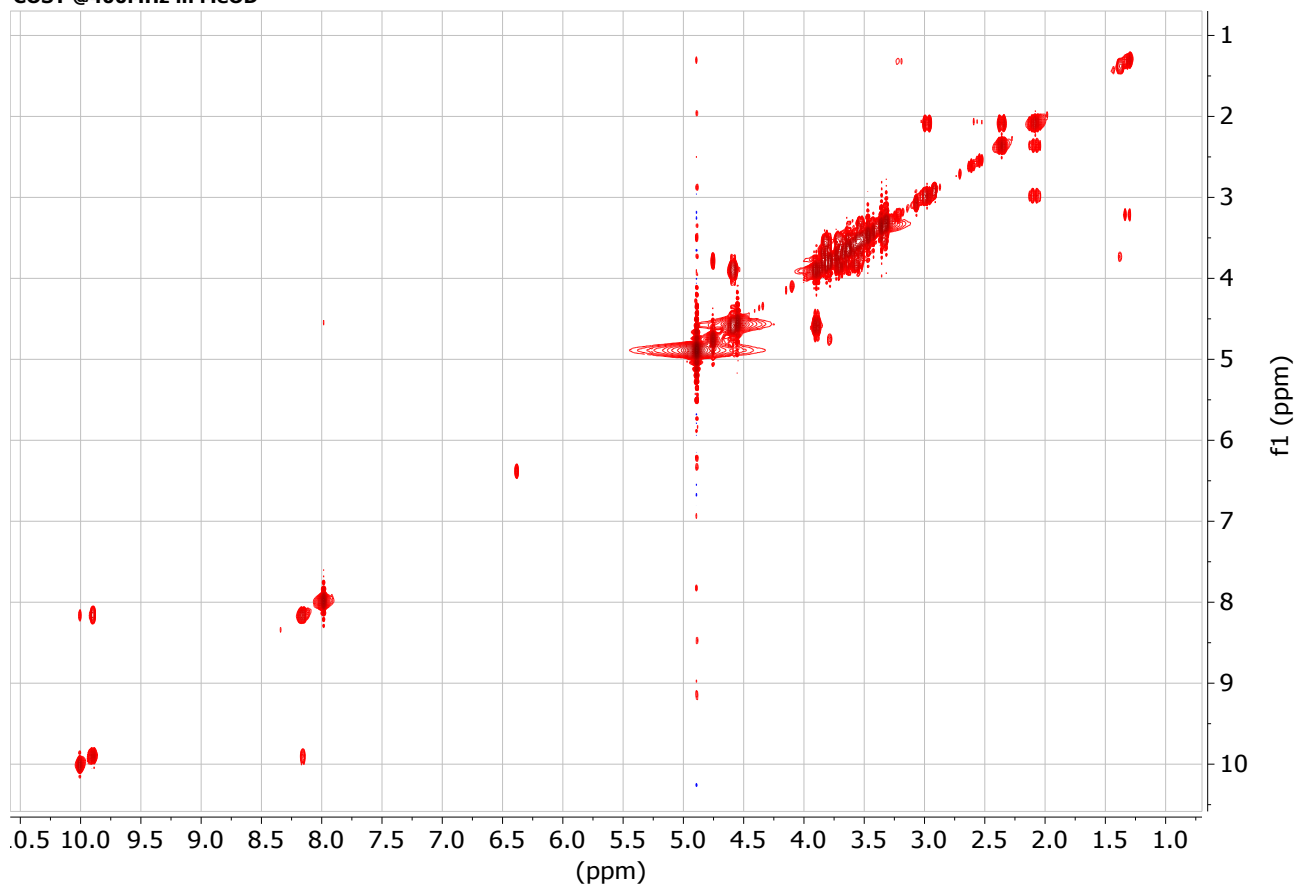
Compound 17. To a solution of compound (**15**) (29 mg, 23 μ mol, 1 eq) in freshly distilled MeOH (1.2 mL), HCl (24 μ L, 1.25 M in MeOH) was slowly added under nitrogen atmosphere. After 10 min a catalytic amount of Pd/C was added, then the mixture was stirred under H_2 atmosphere at r.t. for 2h. The crude was diluted with MeOH and the catalyst was filtered off through a celite pad. Removal of the solvent under reduced pressure afforded pure compound (**17**) (28.2 mg, 96 % yield) as chlorohydrate salt. 1H NMR (400 MHz, MeOD) δ 8.06 (s, 3H, H-11), 4.64 – 4.57 (m, 6H, H-10), 4.54 (s, 6H, H-13), 4.26 (d, $J_{1,2}$ = 8.0 Hz, 3H, H-1), 4.02 – 3.95 (m, 3H, H-7a), 3.92 (bt, $J_{9,10}$ = 5.0 Hz, 6H, H-9), 3.84 (bm, 3H, H-4), 3.79 – 3.65 (m, 18H, H-6a, H-7b, H-8, H-17, H-18, H-19), 3.63 – 3.48 (m, 12H, H-2, H-3, H-5, H-6b), 3.46 (s, 6H, H-14), 3.42 (s, 2H, H-16), 3.16 – 3.11 (m, 2H, H-20). ^{13}C NMR (100 MHz, MeOD) δ 145.92 (C12), 126.10 (C11), 105.06 (C1), 76.71 (C3), 74.94 (C5), 72.55 (C2), 72.21 (C17), 71.38 (C8, C18), 70.92 (C16), 70.33 (C9), 70.29 (C4), 70.04 (C14), 69.77 (C7), 67.89 (C19), 65.18 (C13), 62.54 (C6), 51.34 (C10), 46.47 (C15), 40.83 (C20). MS (ESI) calculated for $[C_{48}H_{85}N_{10}O_{26}]^+$: 1218.24, found: 1217.6 $[M+H]^+$, 1239.7 $[M+Na]^+$; $[\alpha]_D^{25}$: -6.83 (C = 0.615, MeOH).

Compounds characterization: NMR and Mass spectra

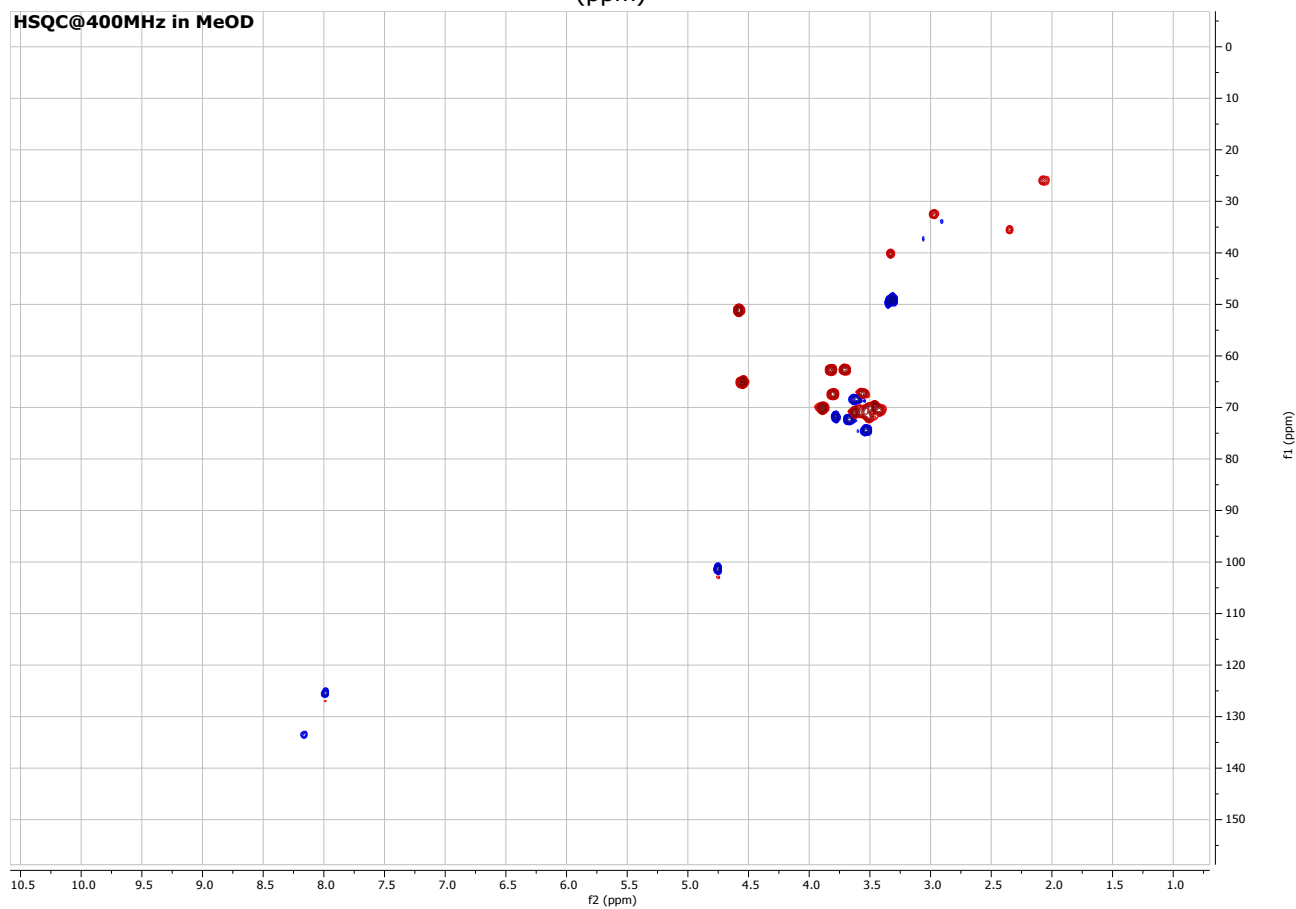
Compound 2

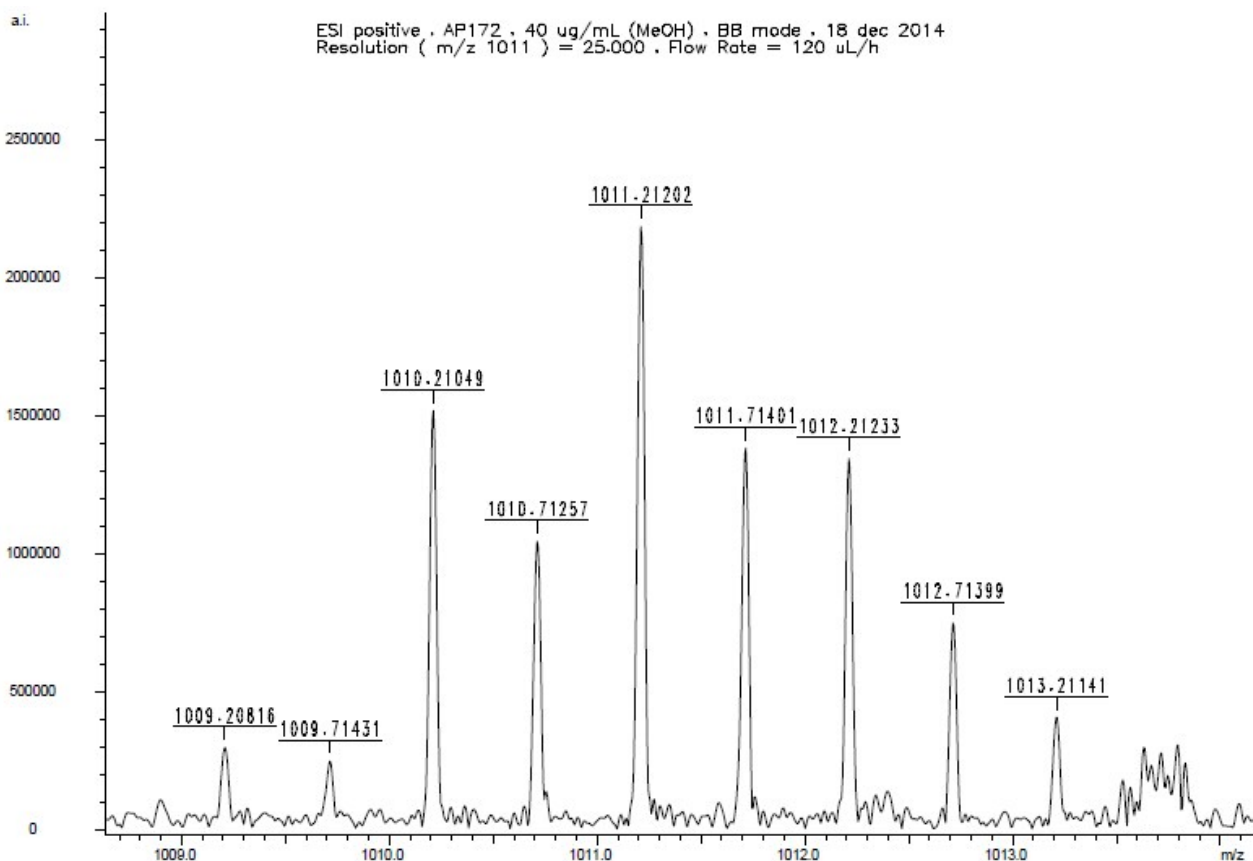


COSY @400MHz in MeOD

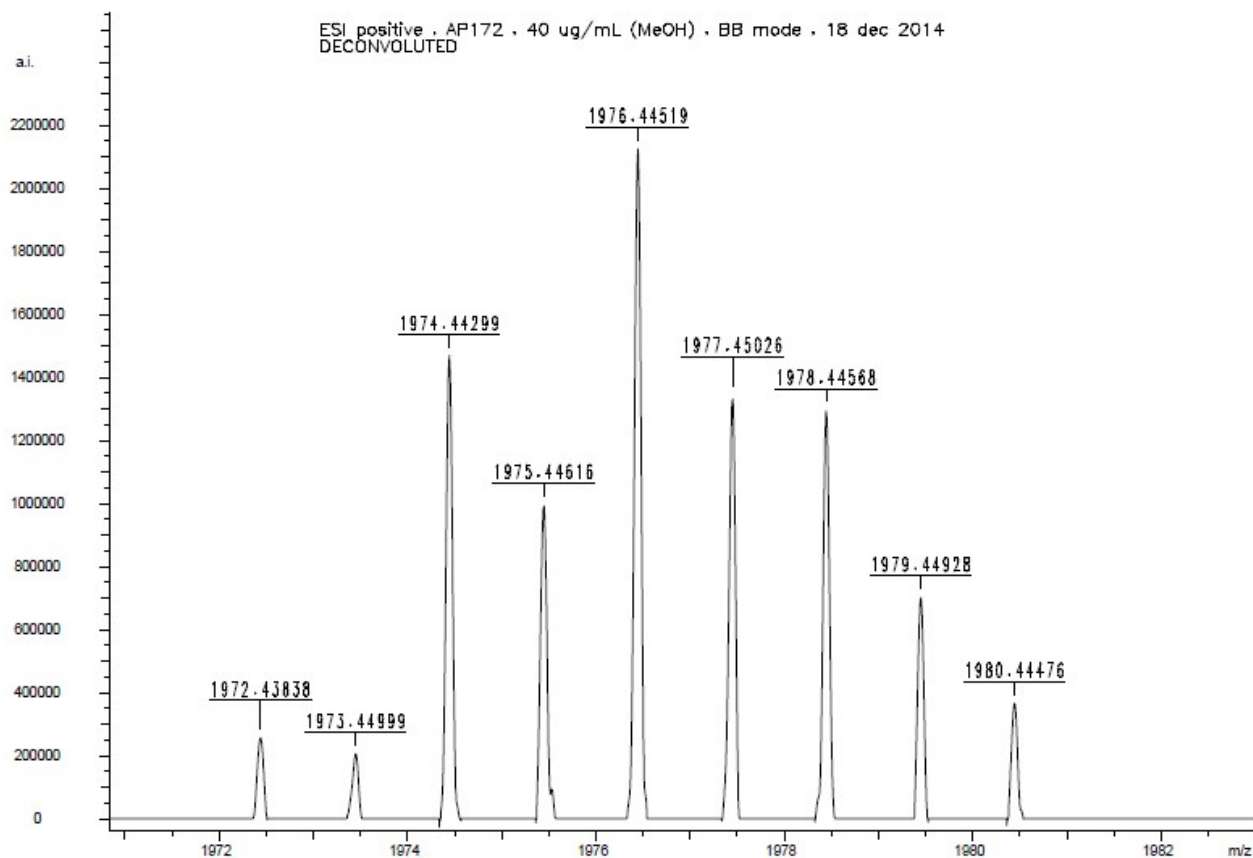


HSQC@400MHz in MeOD

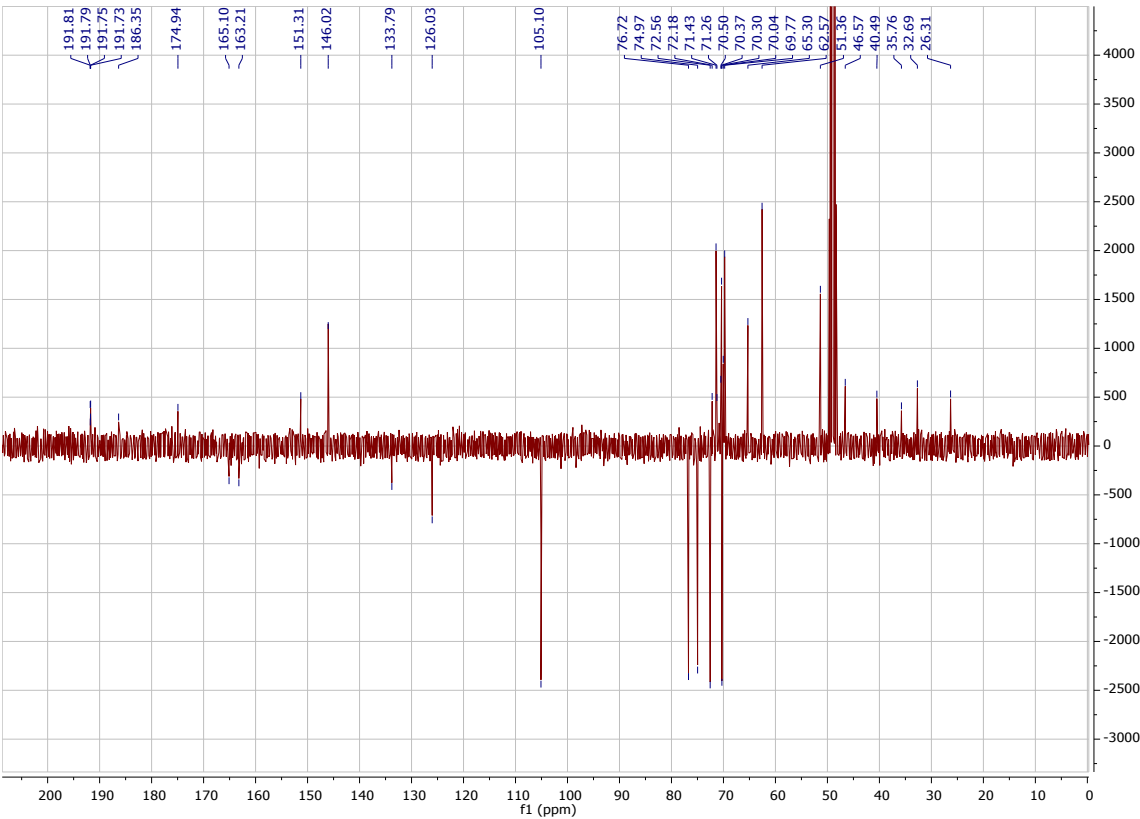
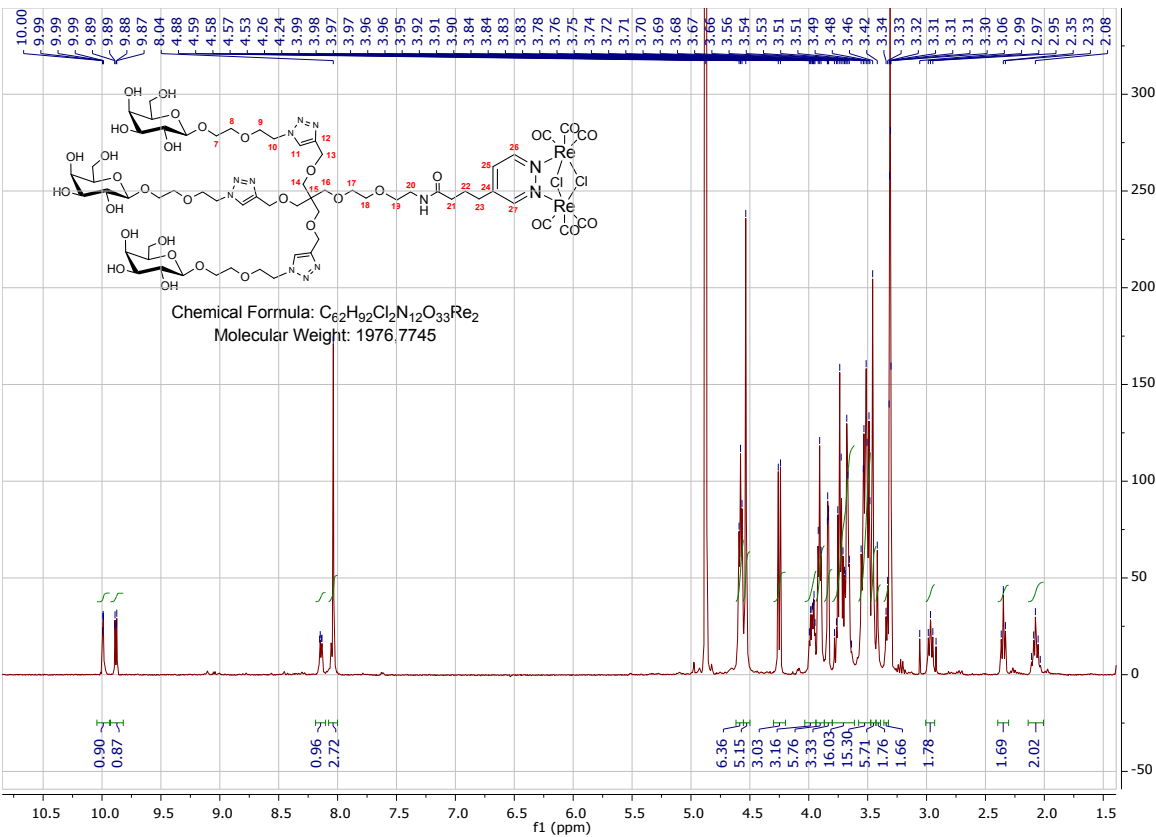




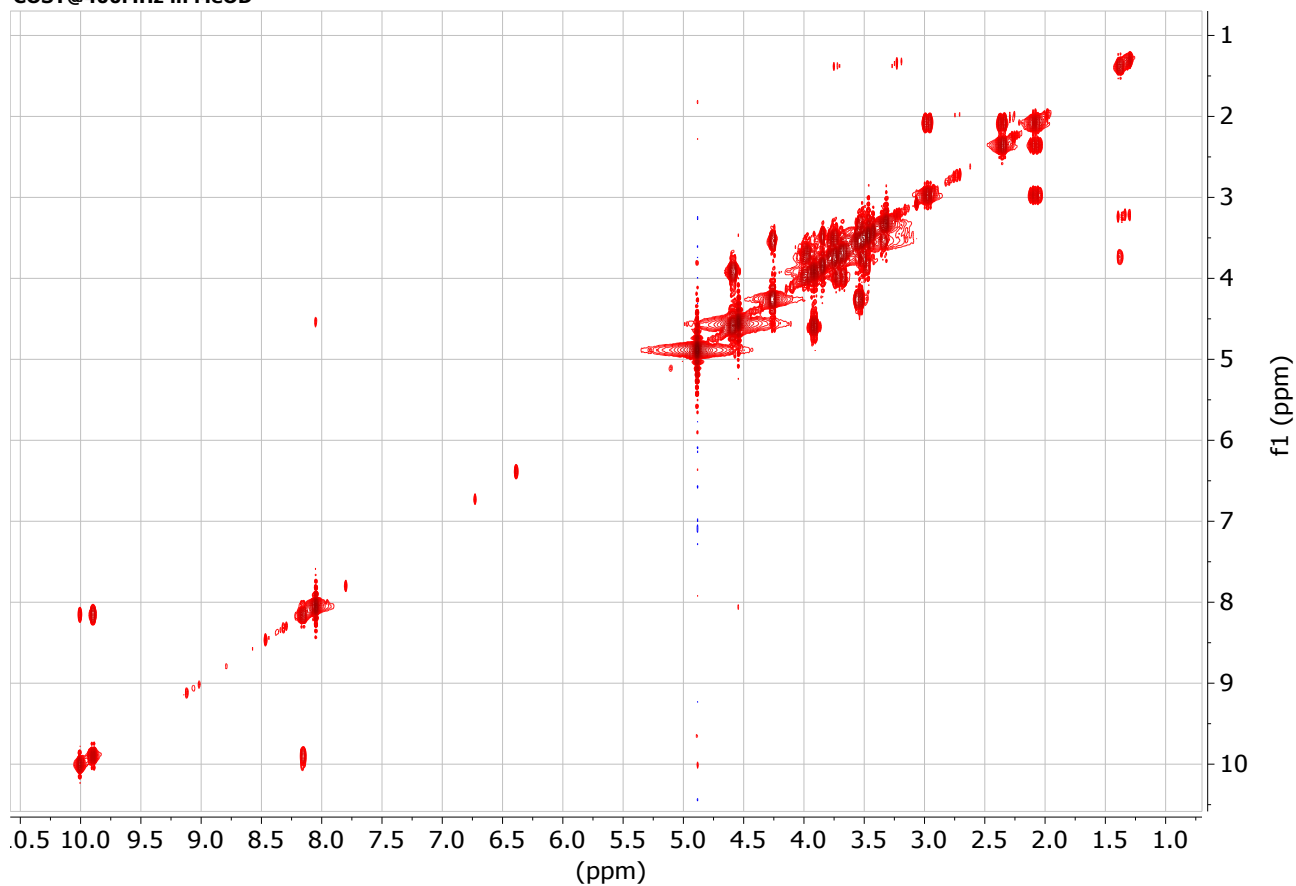
/Data/CIGA/CHIM/2014/Bernardi/AP172/4/odata/1 Administrator Thu Dec 18 16:57:49 2014



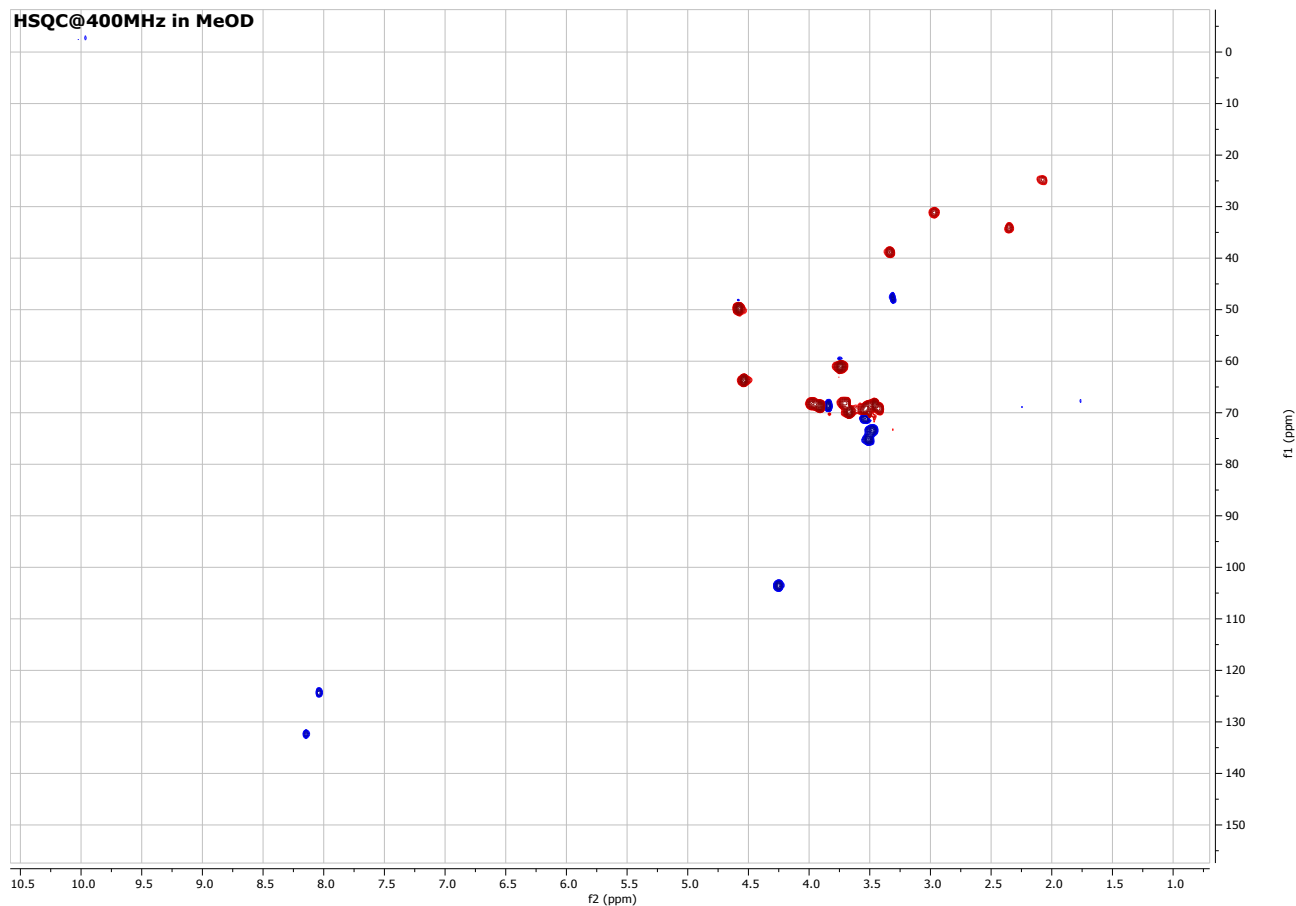
Compound 3

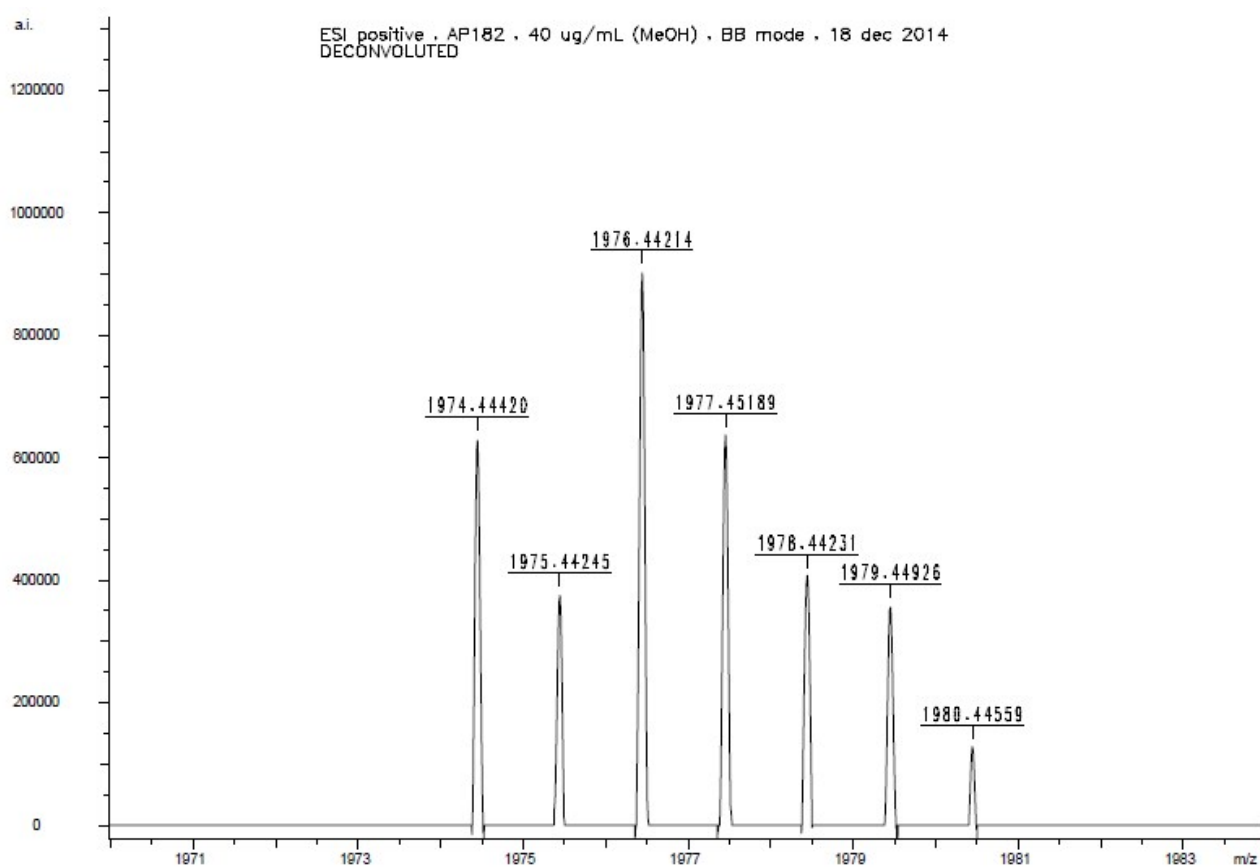
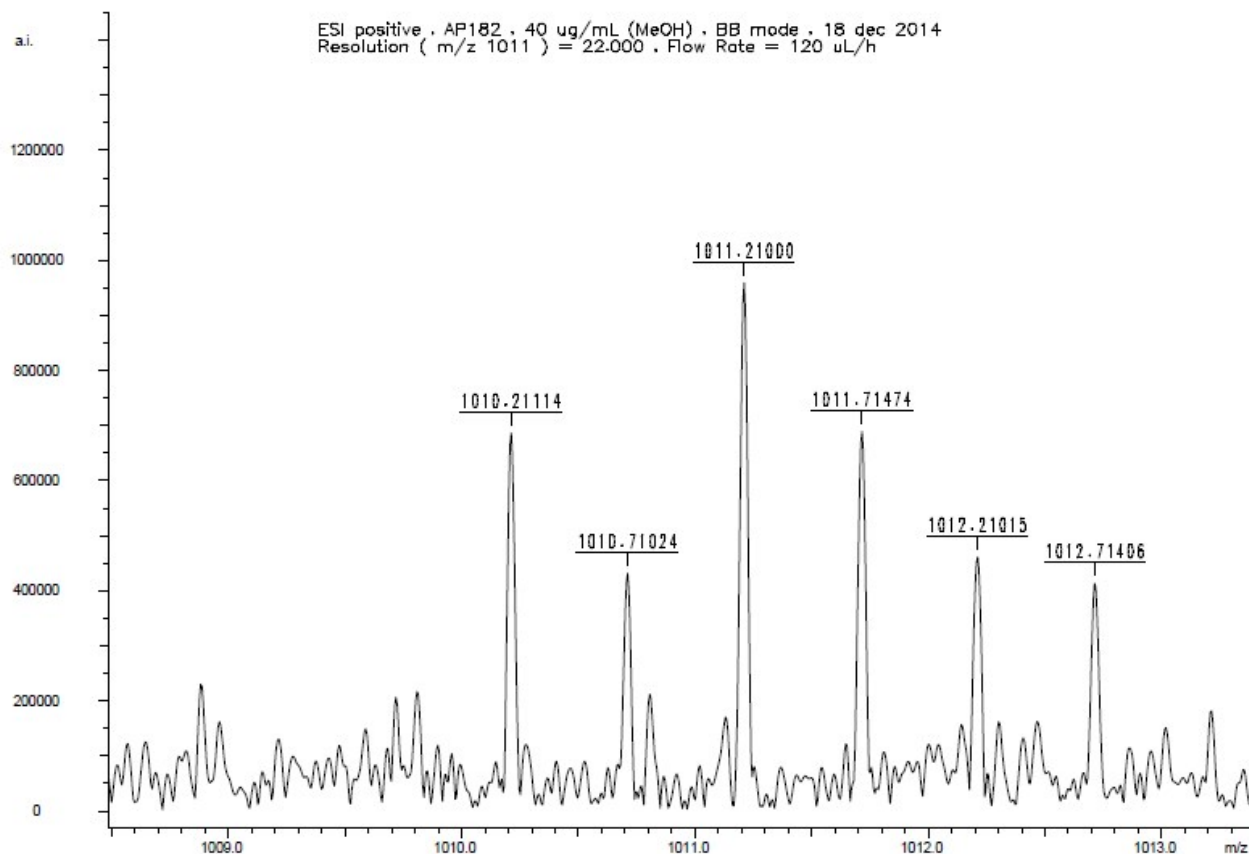


COSY@400MHz in MeOD



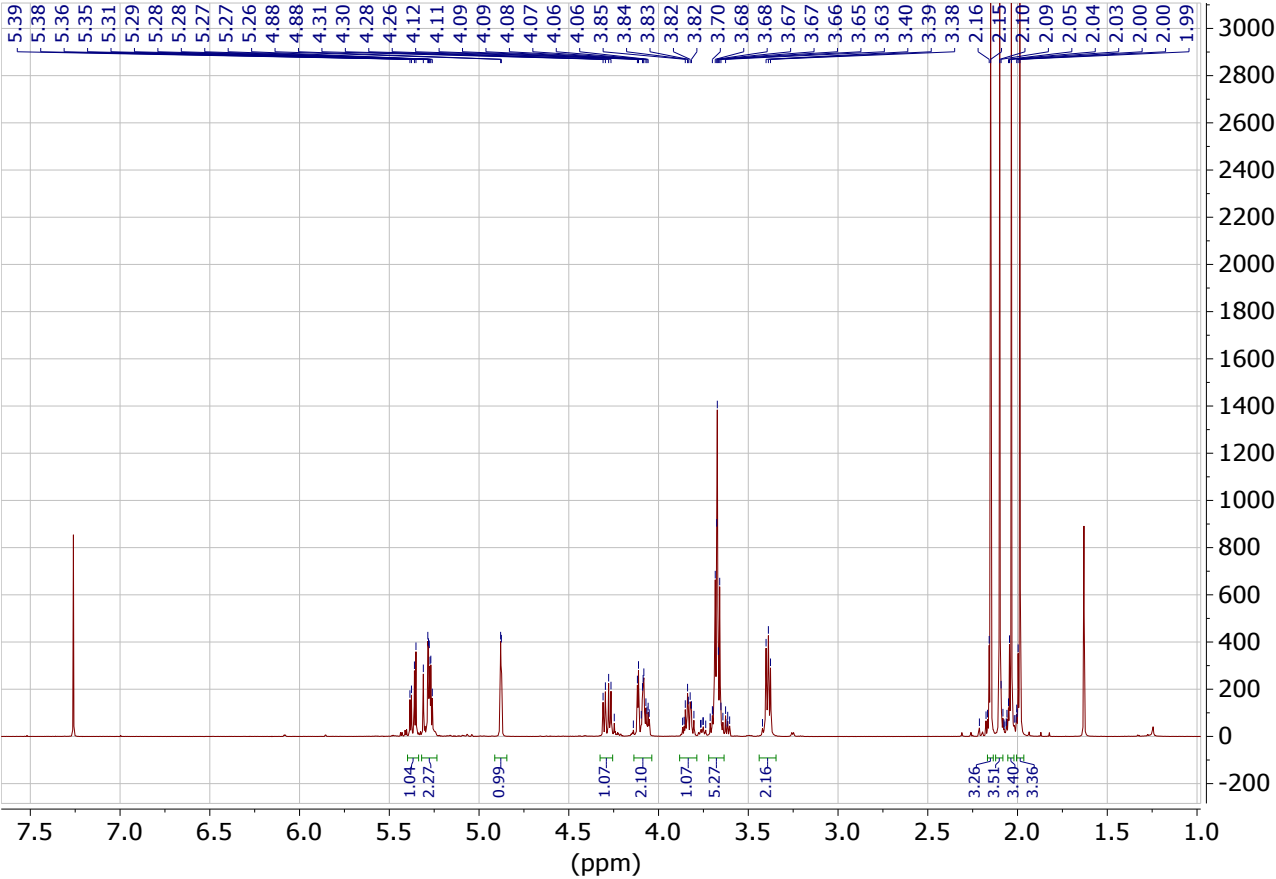
HSQC@400MHz in MeOD



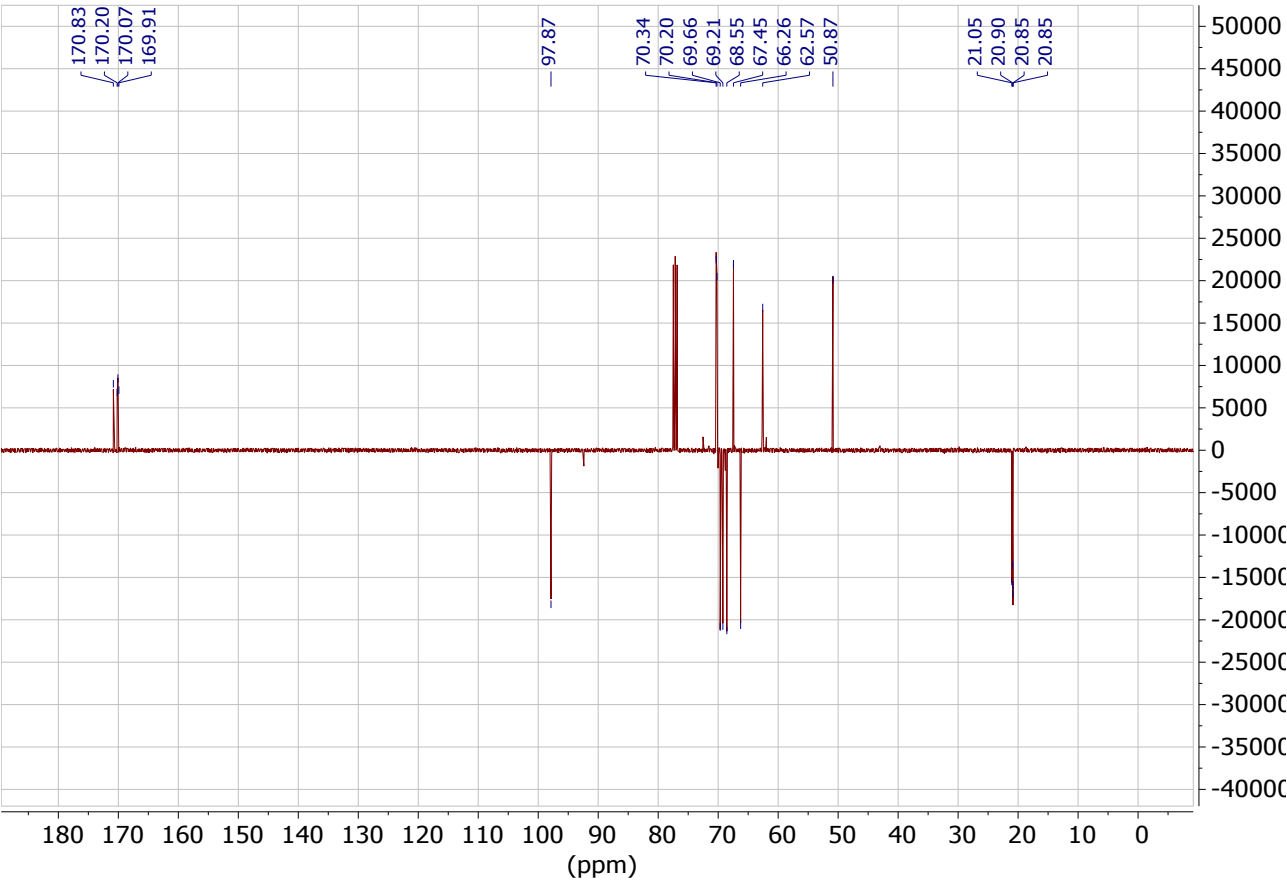


Compound 7

¹H-NMR @400MHz in CDCl₃

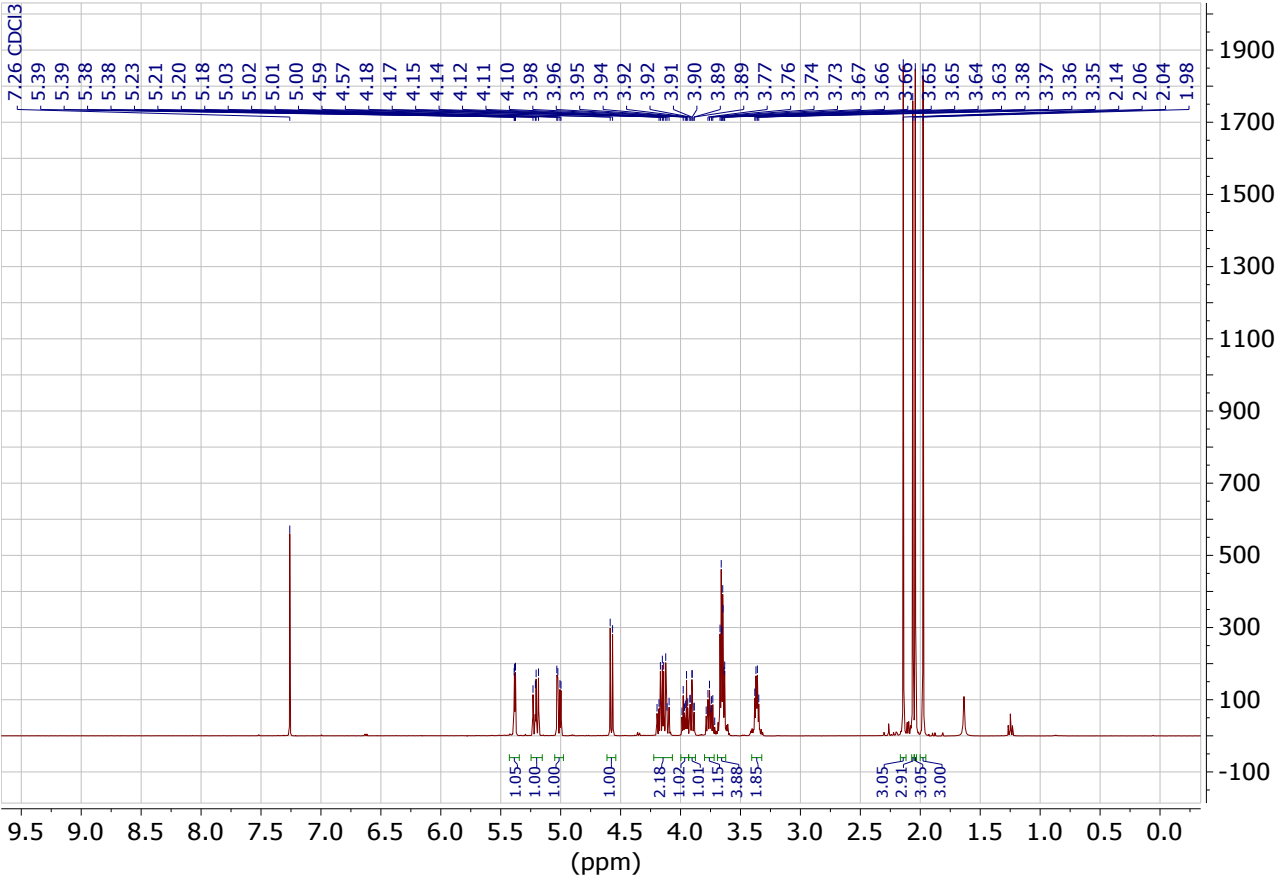


¹³C-NMR @400MHz in CDCl₃

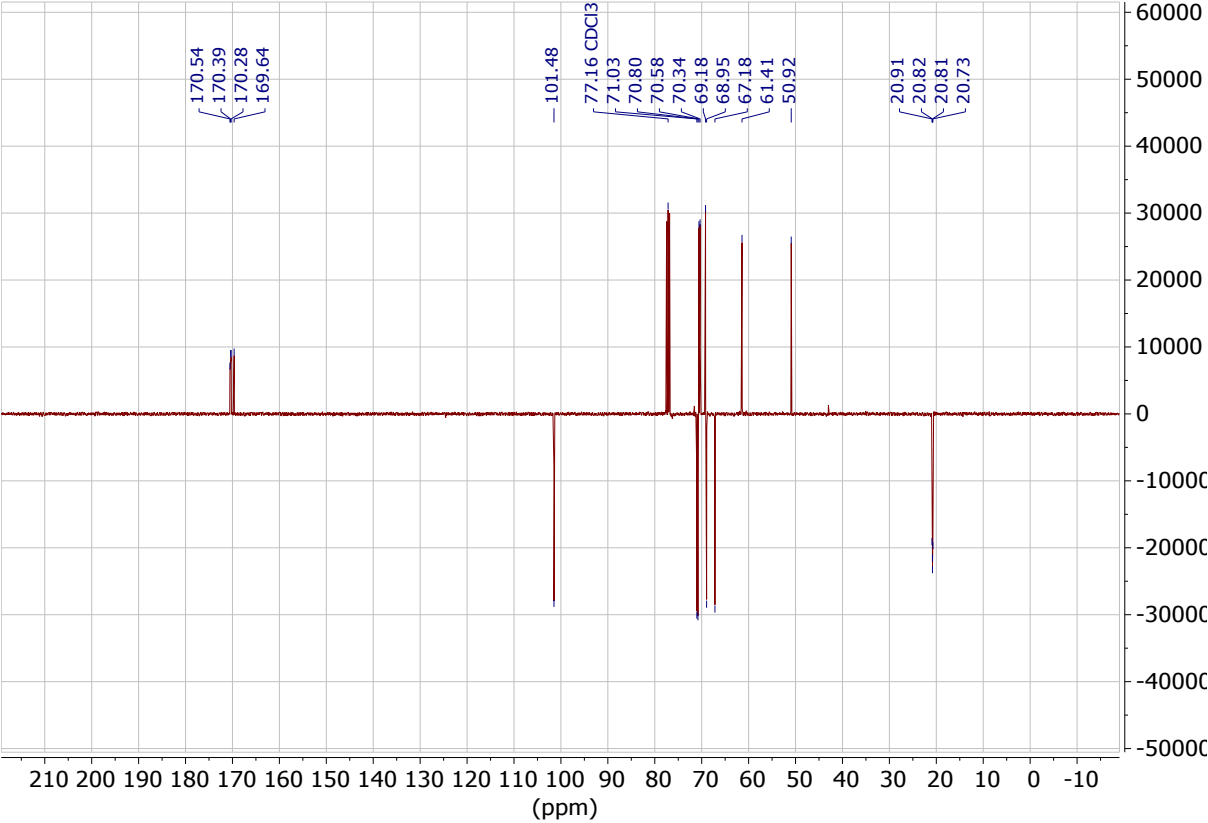


Compound 8

¹H-NMR @400MHz in CDCl₃

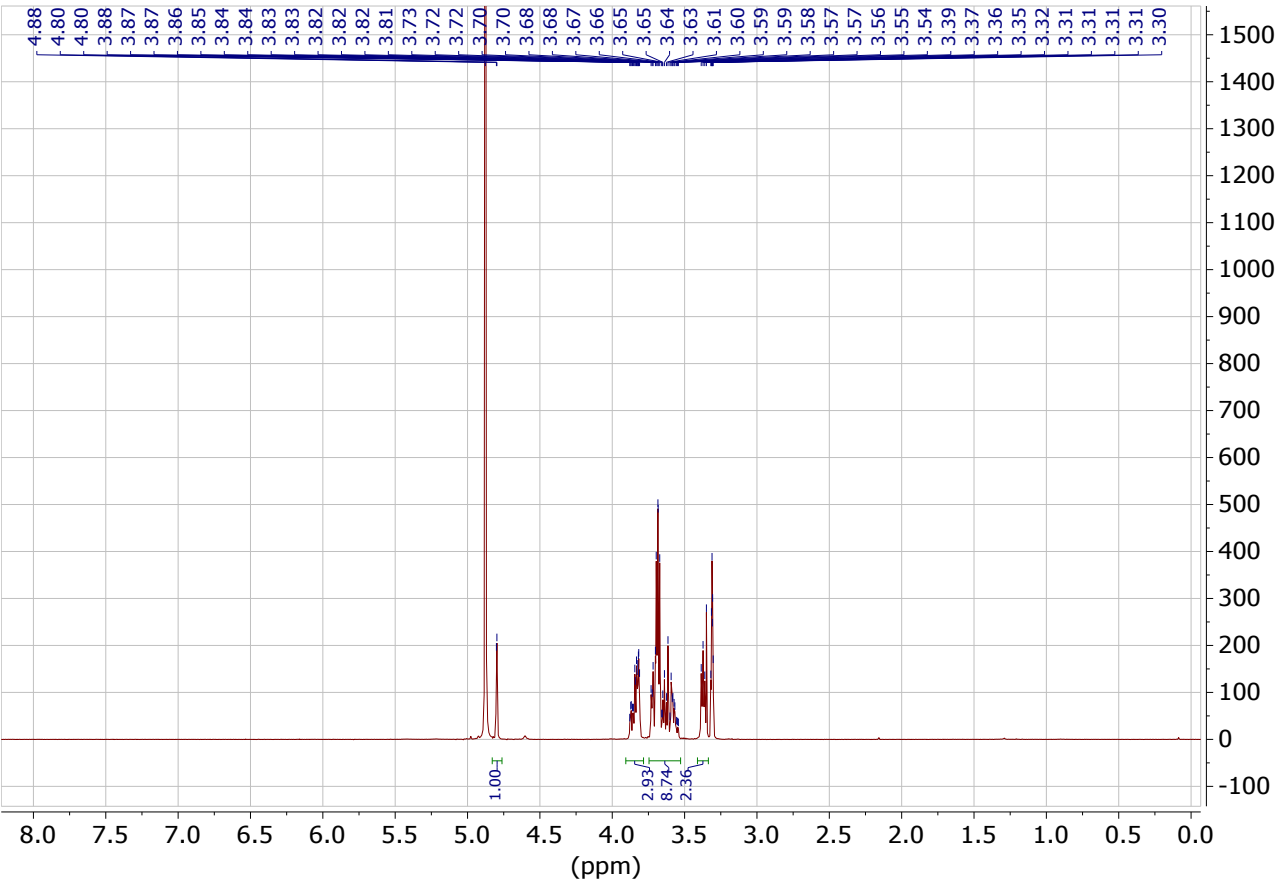


¹³C-NMR @400MHz in CDCl₃

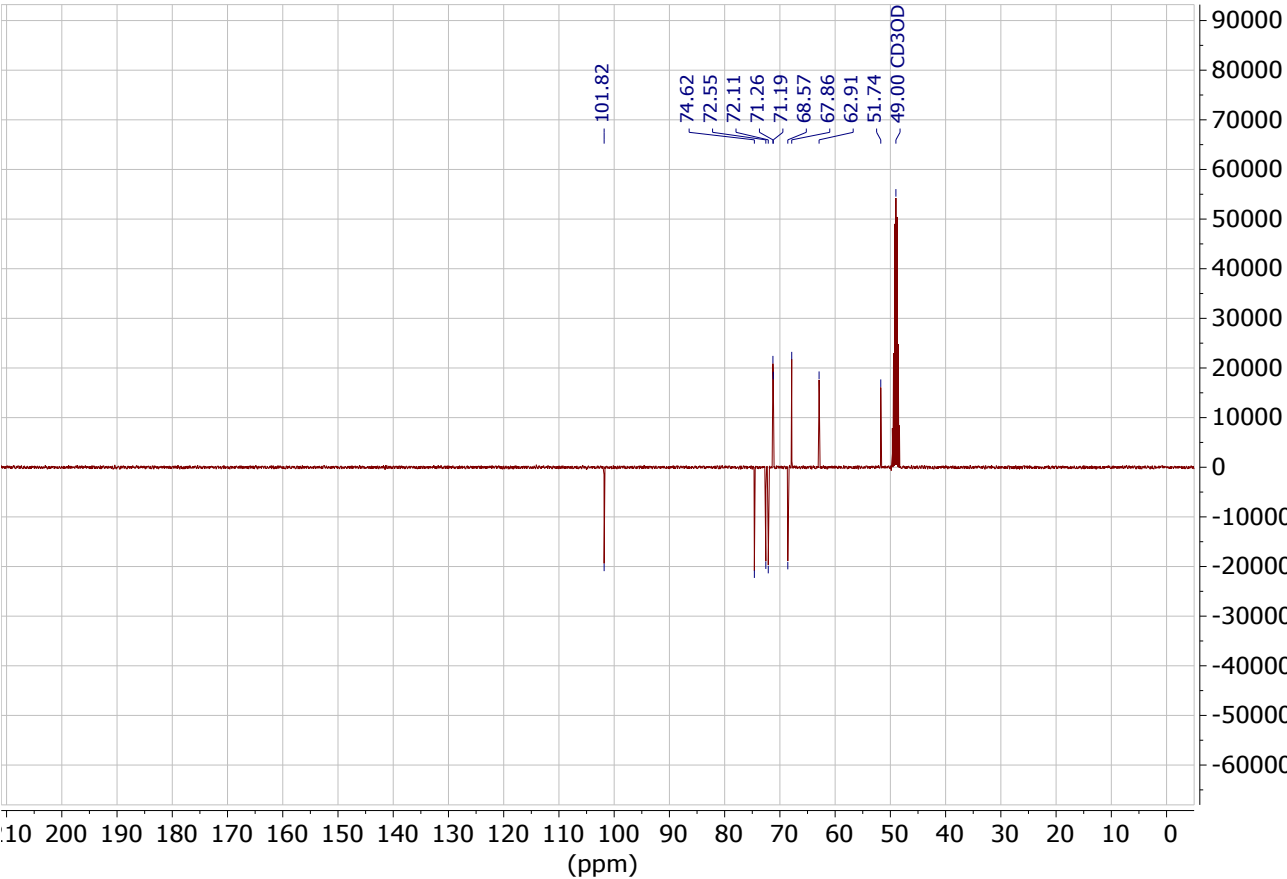


Compound 9

¹H-NMR @400MHz in MeOD

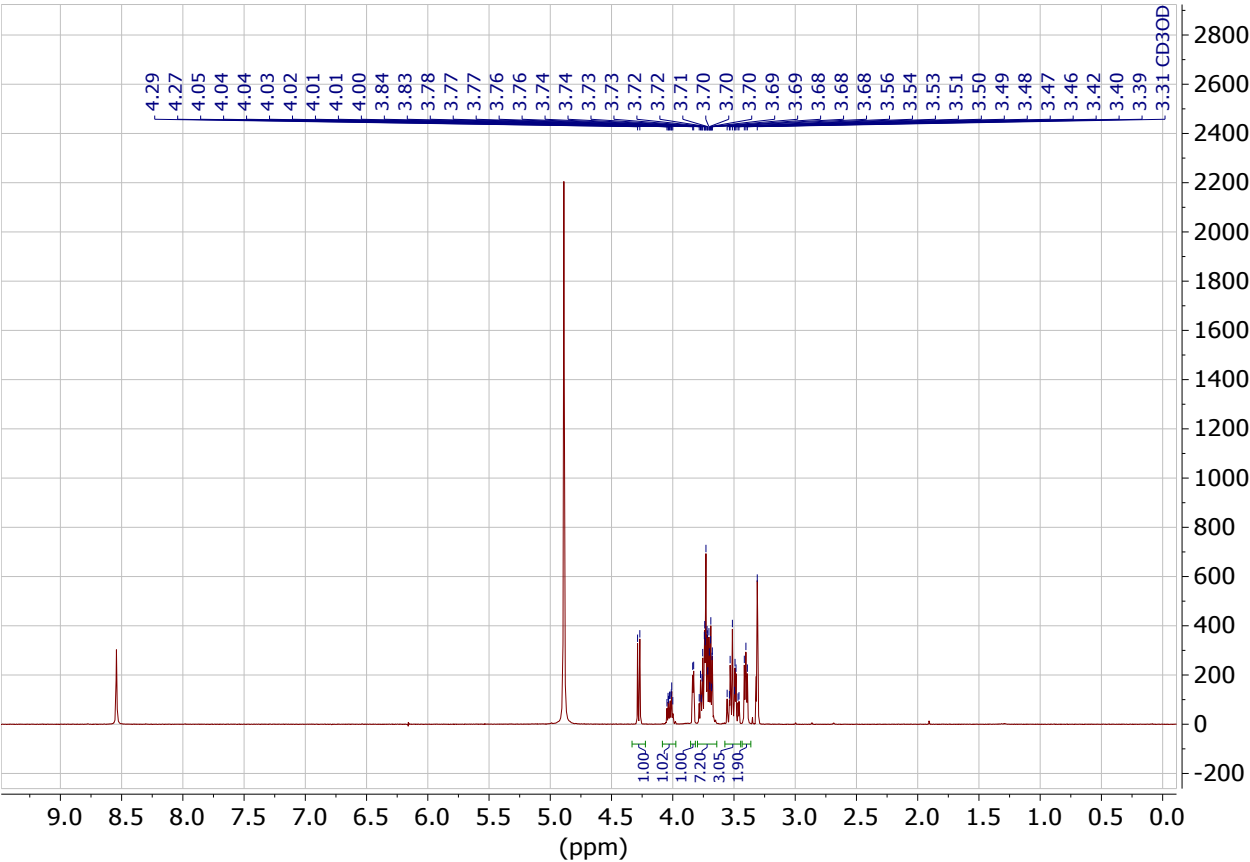


¹³C-NMR @400MHz in MeOD

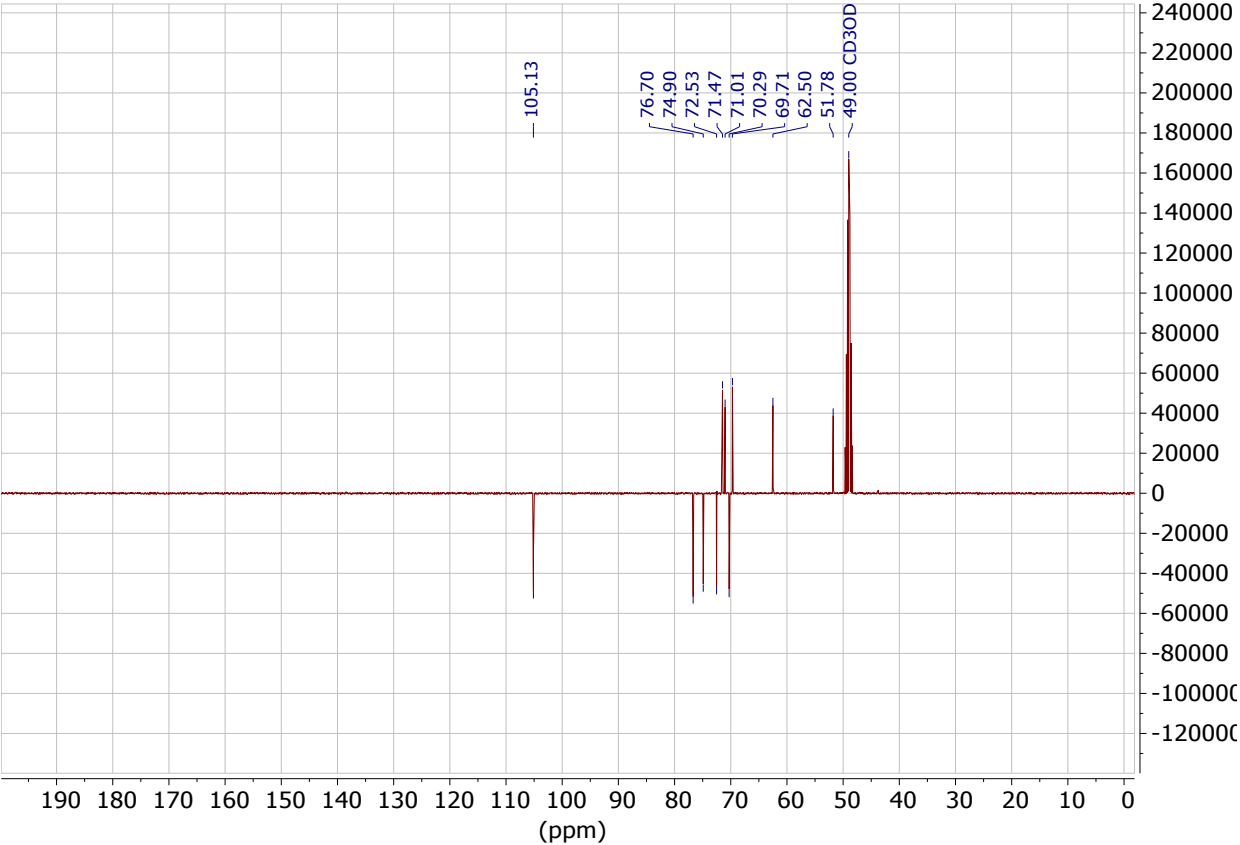


Compound 10

¹H-NMR @400MHz in MeOD

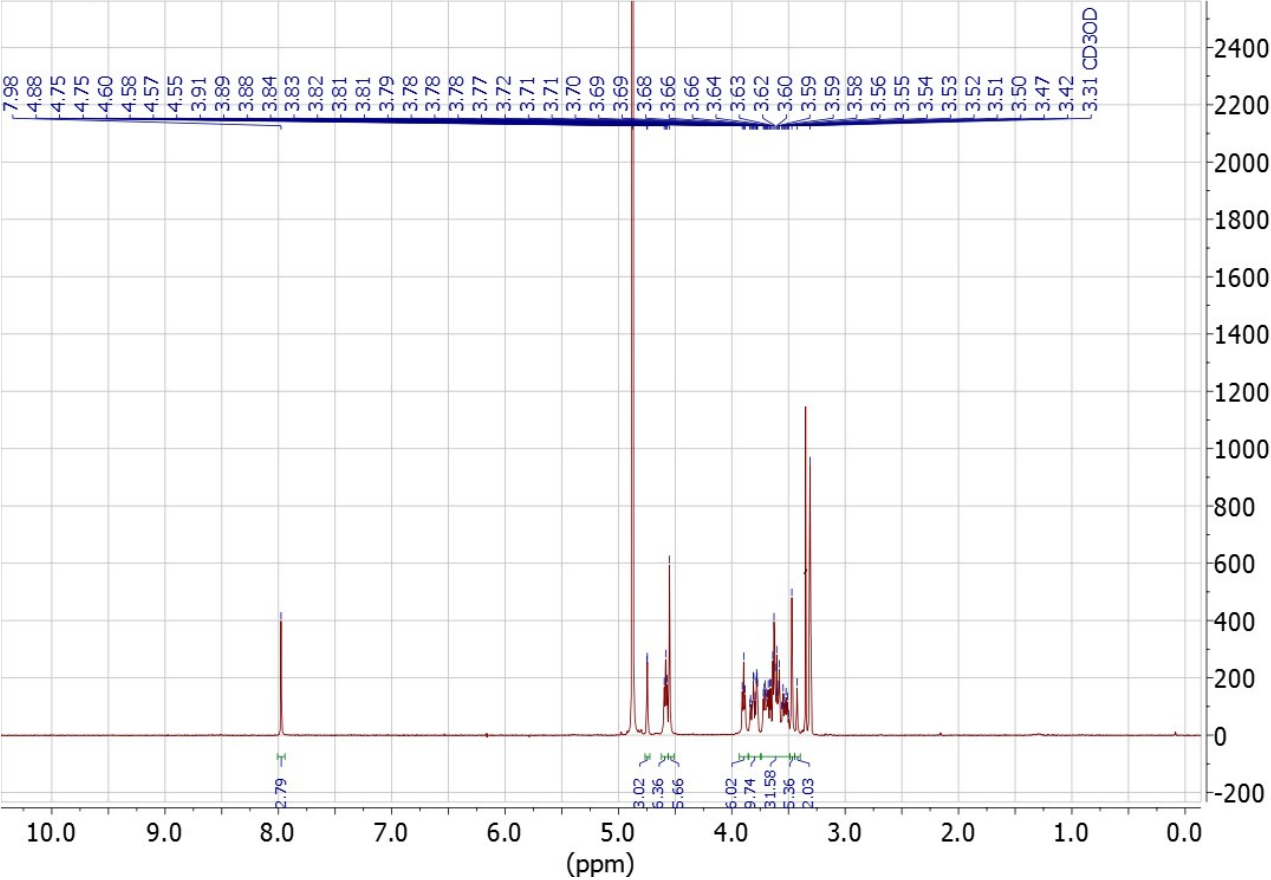


¹³C-NMR @400MHz in MeOD

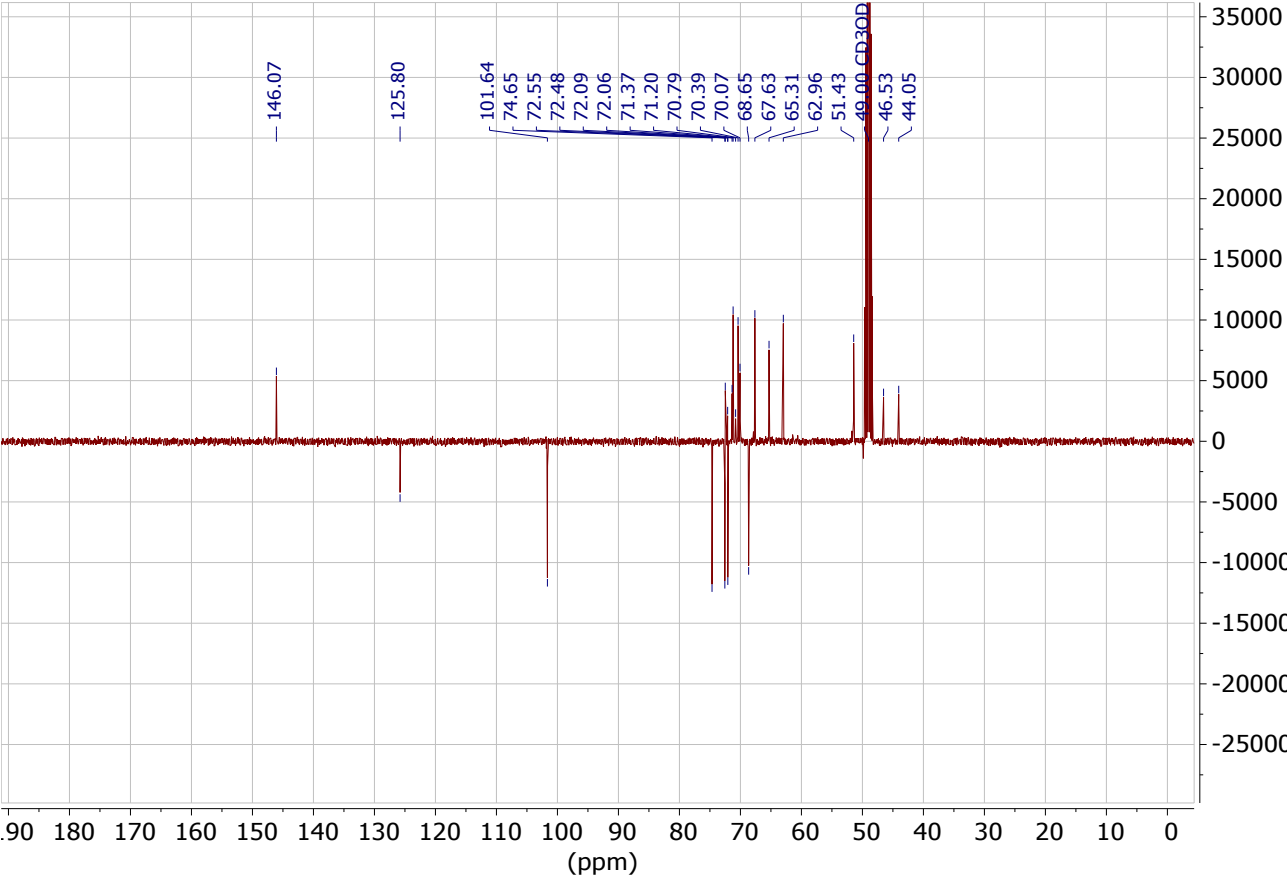


Compound 12

¹H-NMR @400MHz in MeOD

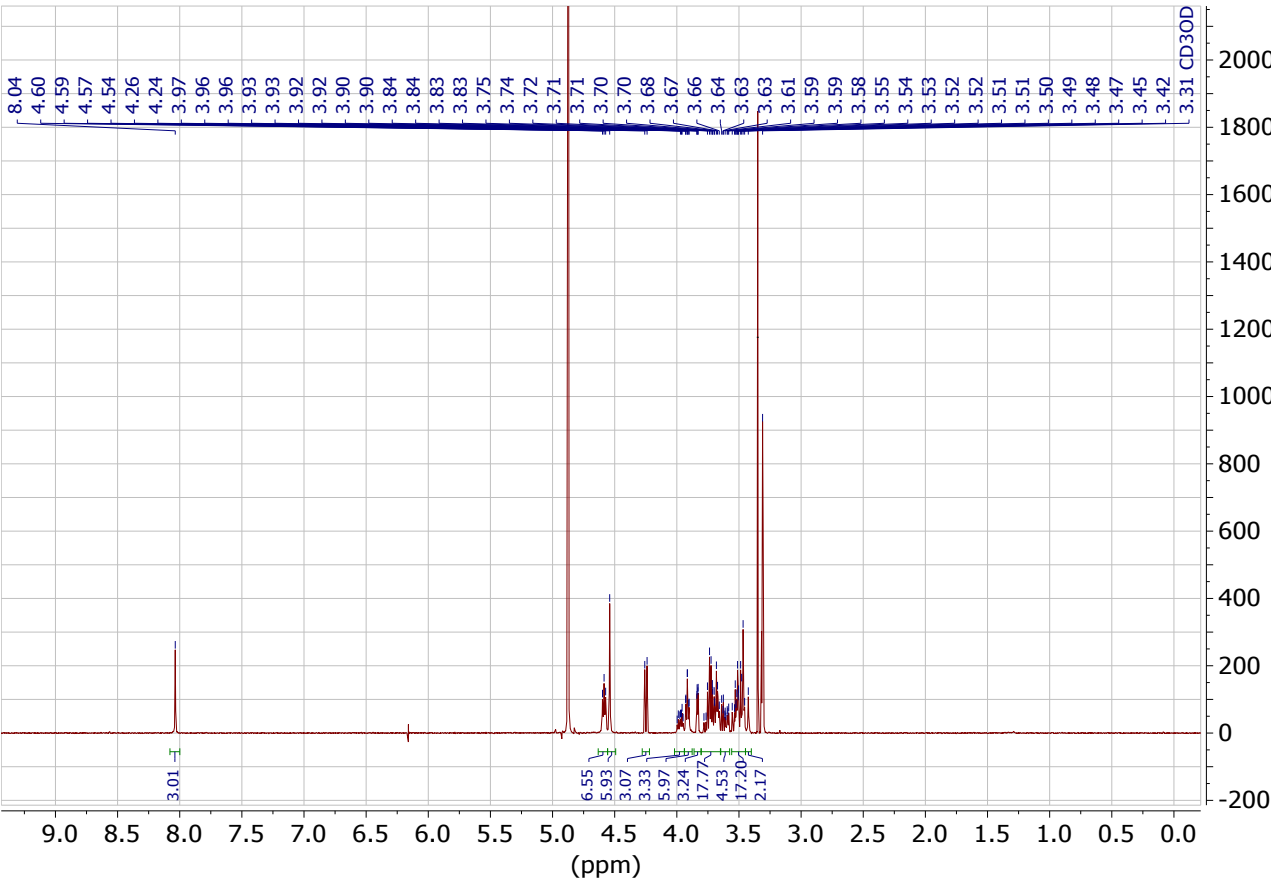


¹³C-NMR @400MHz in MeOD

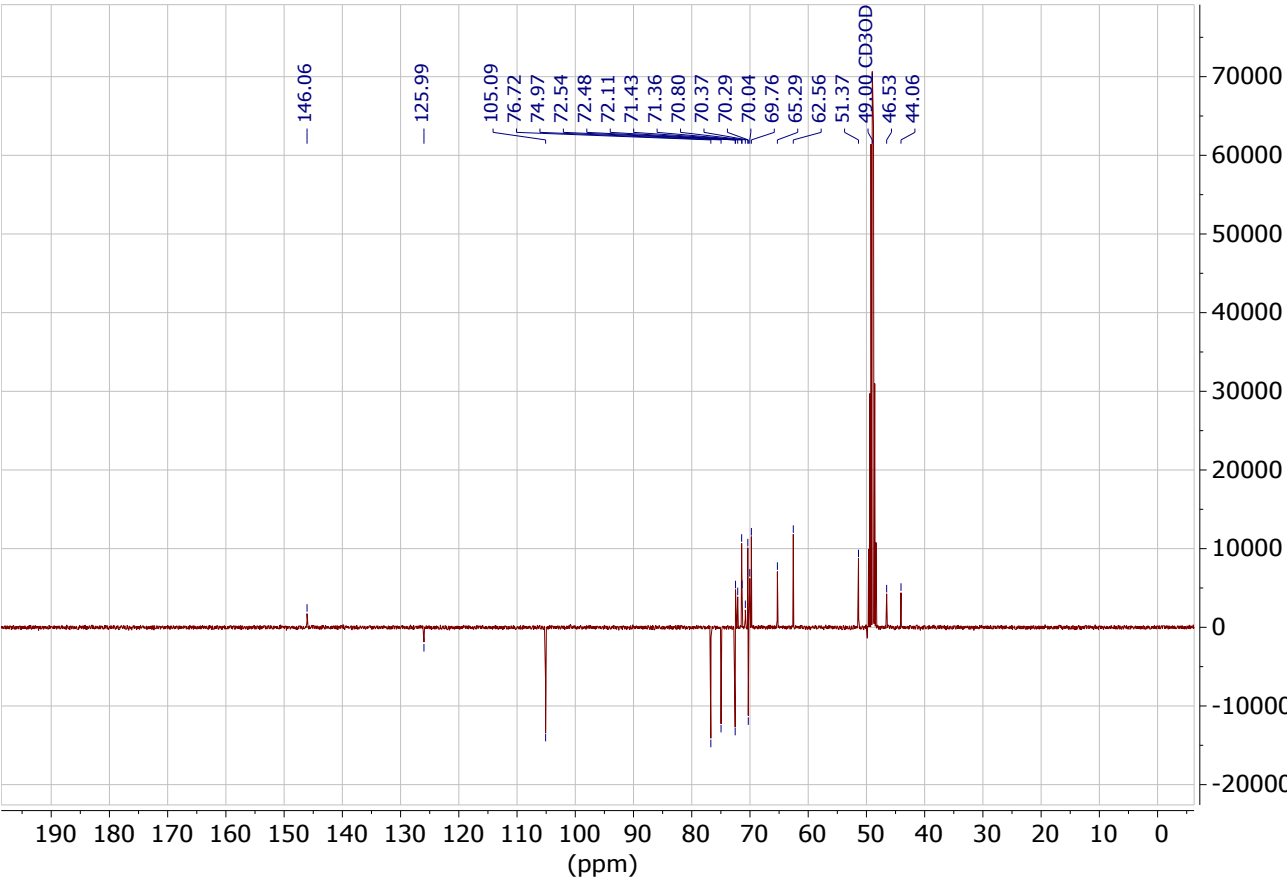


Compound 13

¹H-NMR @400MHz in MeOD

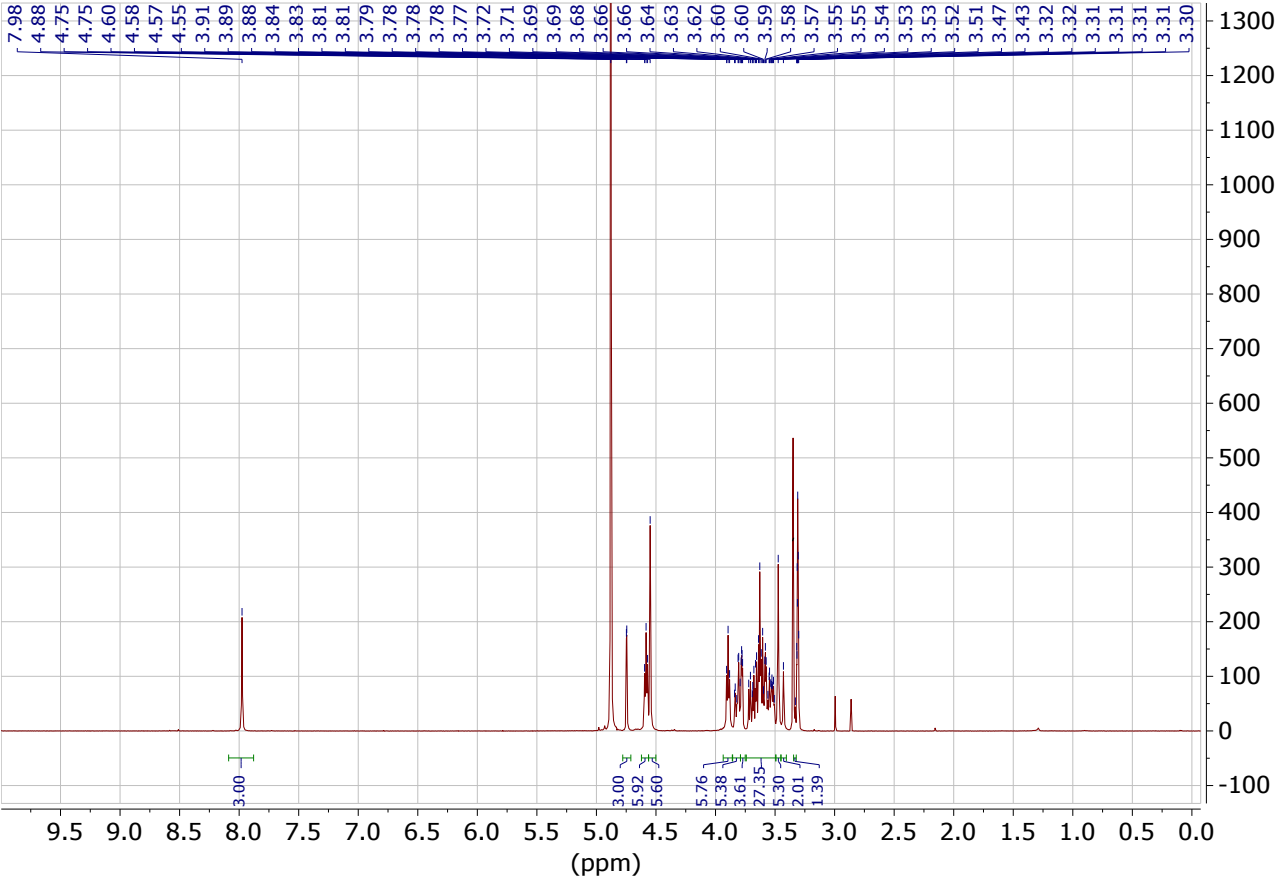


¹³C-NMR @400MHz in MeOD

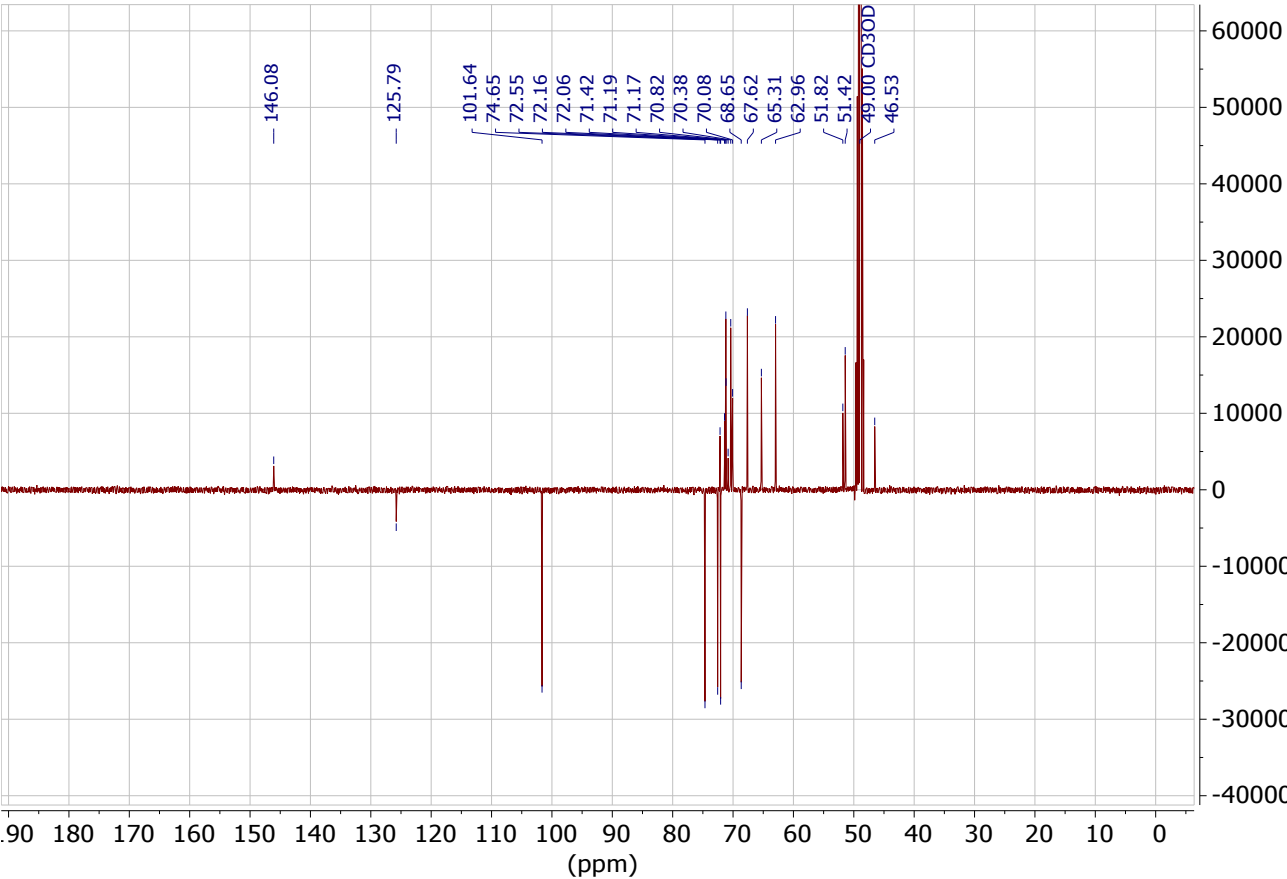


Compound 14

¹H-NMR @400MHz in MeOD

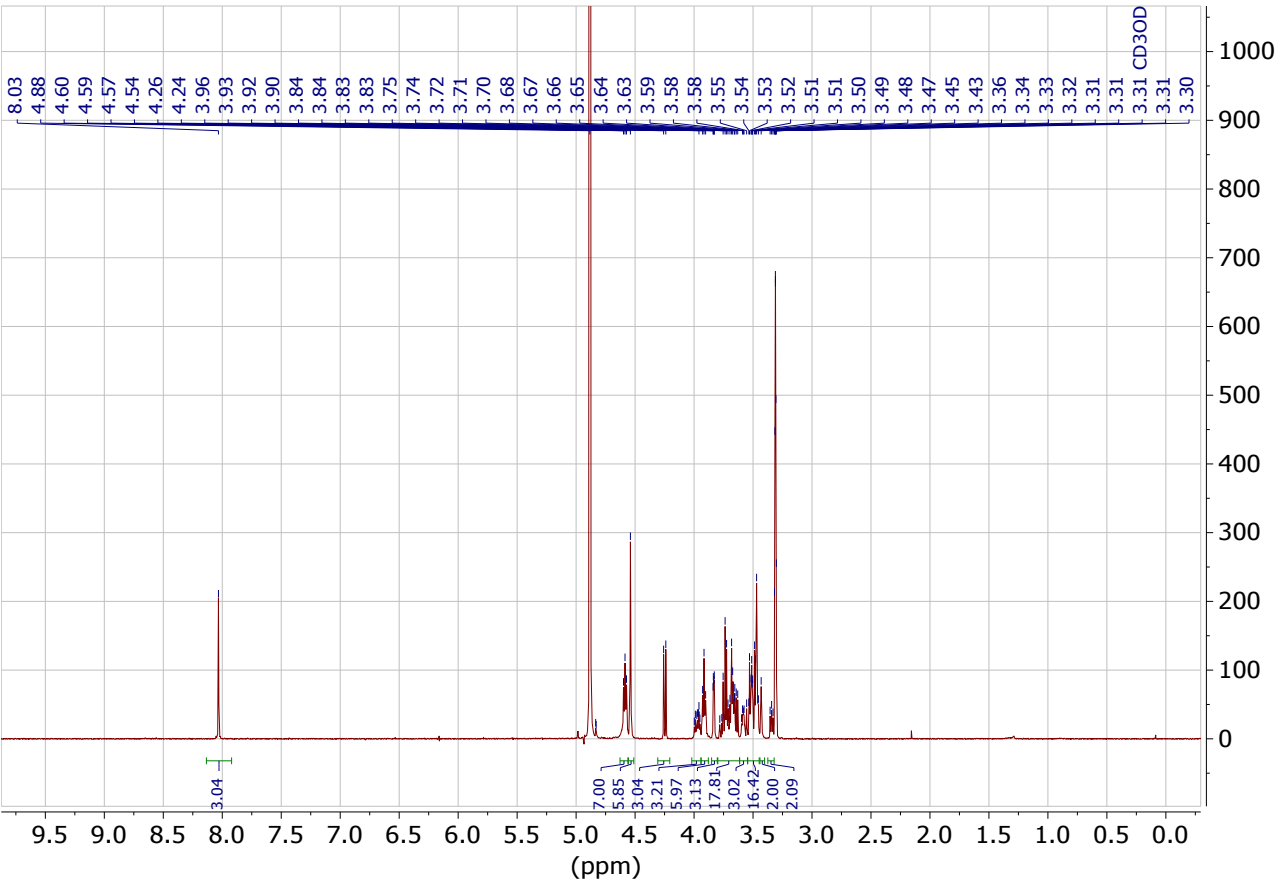


¹³C-NMR @400MHz in MeOD

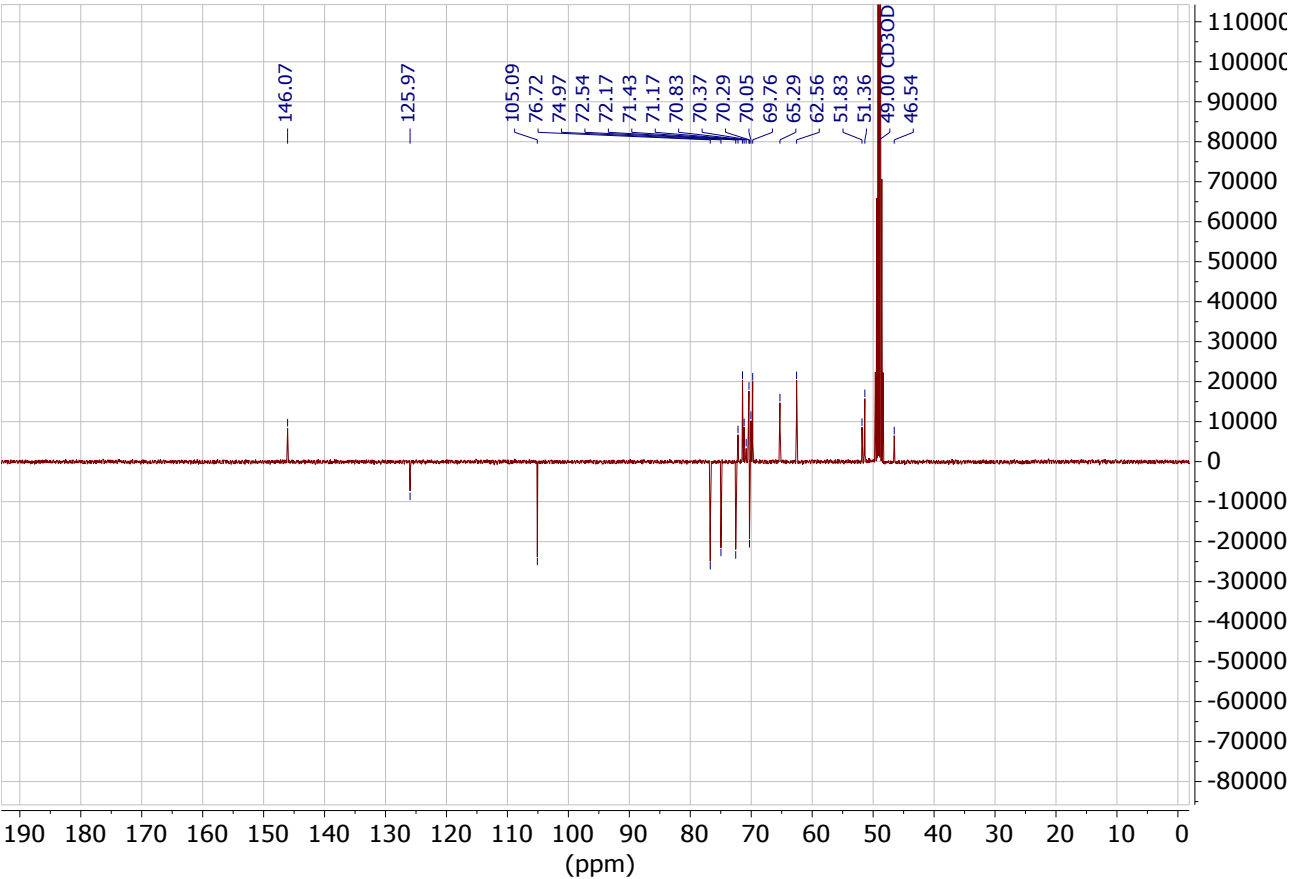


Compound 15

¹H-NMR @400MHz in MeOD

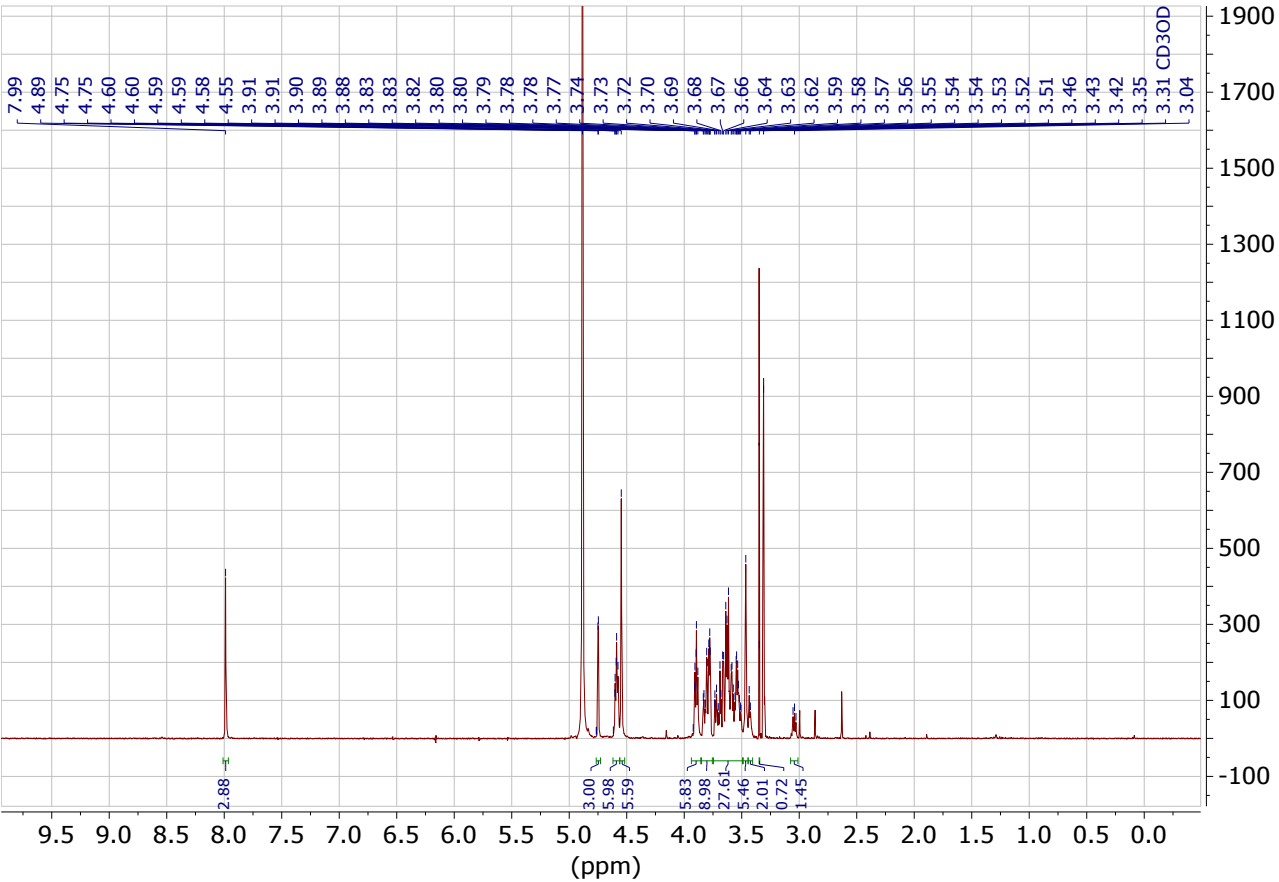


¹³C-NMR @400MHz in MeOD

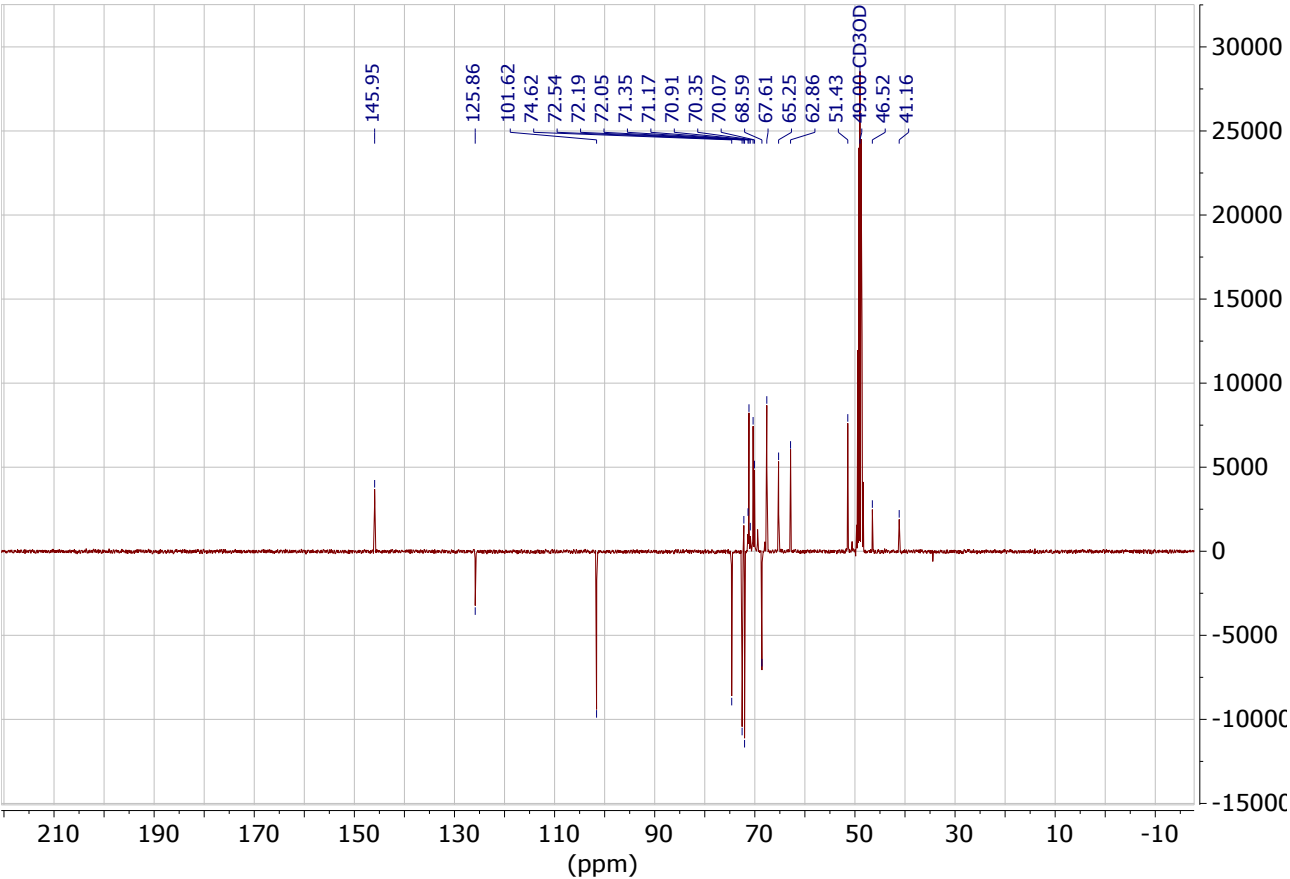


Compound 16

¹H-NMR @400MHz in MeOD

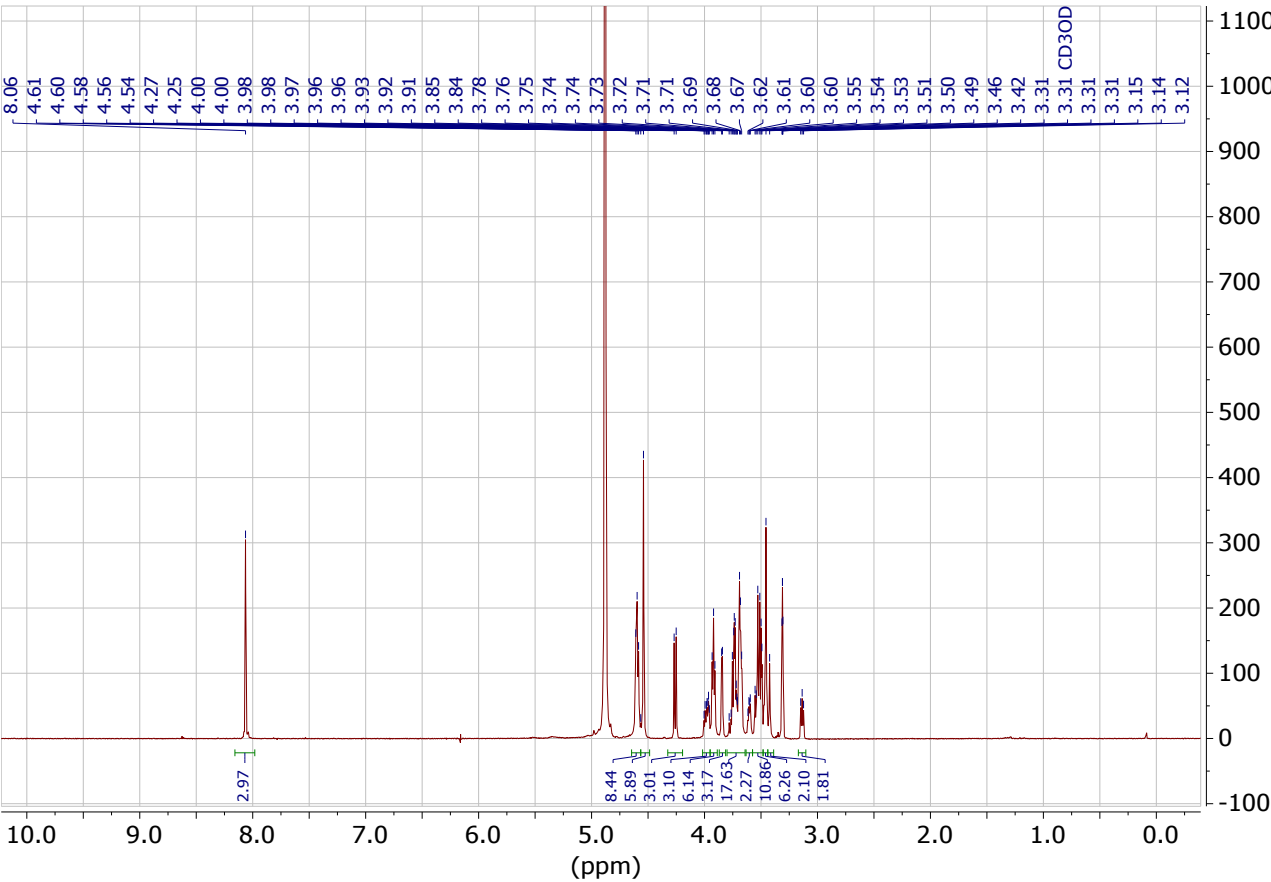


¹³C-NMR @400MHz in MeOD

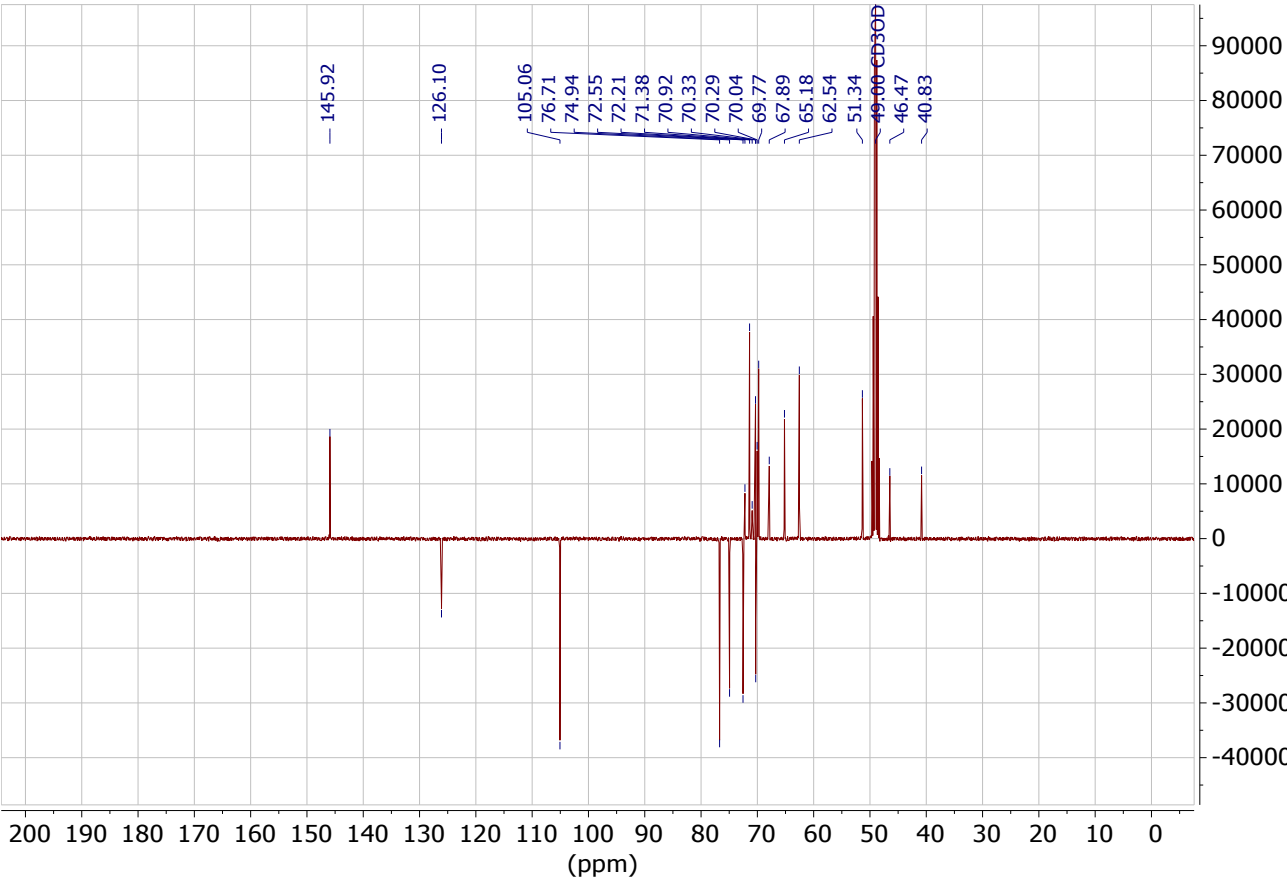


Compound 17

¹H-NMR @400MHz in MeOD



¹³C-NMR @400MHz in MeOD



PNA sensing

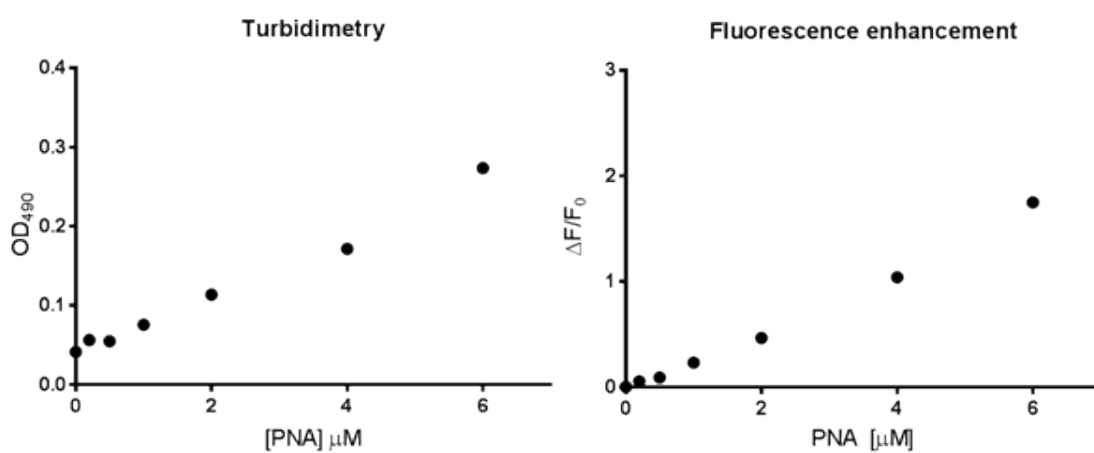


Figure S1: Turbidimetric and Fluorescence Binding assay of compound 3 (5μM) in presence of increasing amount of PNA in 10 mM TRIS buffer pH 7.4 CaCl₂ 0.1mM and MnCl₂ 0.1mM.