Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.


# Supporting Information

# Triazole Linker-Based Trivalent Sialic Acid Inhibitors of Adenovirus Type 37 Infection of Human Corneal Epithelial Cells

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**Contents:**

**Methyl (2-bromoethoxy(5-N-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl))-onate (3a).** Compound **3a** was synthesized following the general method for the glycosylation reaction. Purification by column chromatography (gradient *n*-Heptane/EtOAc) afforded a mixture of compound **3a**, the corresponding reverse anomer and the glycal product (α/β/glycal (n.d.)). Compound **3a** was used in the next step without additional purification. ESI-MS *m/z* calcd for $C_{22}H_{33}BrNO_{13}$ $(M+H)^+$ 598.11 and $C_{22}H_{32}BrNNaO_{13}$ $(M+Na)^+$ 620.10; found 598.23 and 620.20, respectively.

**Methyl (3-bromo-propyloxy(5-N-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl))-onate (3b).** Compound **3b** was synthesized following the general method for the glycosylation reaction. Purification by column chromatography (gradient *n*-Heptane/EtOAc) afforded a mixture of compound **3b**, the corresponding reverse anomer and the glycal product (α/β/glycal (n.d.)). Compound **3b** was used in the next step without additional purification. ESI-MS *m/z* calcd for $C_{23}H_{35}BrNO_{13}$ $(M+H)^+$ 611.12 and $C_{23}H_{34}BrNNaO_{13}$ $(M+Na)^+$ 634.11; found 611.08 and 633.07, respectively.

**Methyl (2-bromoethoxy(5-N-propanoylamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl))-onate (4a).** Compound **4a** was synthesized following the general method for the glycosylation reaction. Purification by column chromatography (Toluene/EtOH, 10:1) afforded compound **4a** and the corresponding reverse anomer (α/β (6:1)). Compound **4a** was used in the next step without additional purification. HRMS *m/z* calcd for $C_{23}H_{34}BrNNaO_{13}$ $(M+Na)^+$ 634.1111; found 634.1083.

**Methyl (3-bromo-propyloxy(5-N-propanoylamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl))-onate (4b).** Compound **4b** was synthesized following the general method for the glycosylation reaction. Purification by column chromatography (Toluene/EtOH, 8:1) afforded compound **4b** and the corresponding reverse anomer (α/β (22:3)). Compound **4b** was used in the next step without additional purification. HRMS *m/z* calcd for $C_{24}H_{36}BrNNaO_{13}$ $(M+Na)^+$ 648.1268; found 648.1240.

**Methyl (2-azidoethoxy(5-N-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl))-onate (5a).** Compound **5a** was synthesized following the general method for the synthesis of azido derivatives. Purification by column chromatography ($CH_2Cl_2$/MeOH, 95:5) afforded compound **5a**, the corresponding reverse anomer and the glycal product (α/β/glycal (13:3:2)). Compound **5a** was used in the next step without additional purification. ESI-MS *m/z* calcd for $C_{22}H_{33}N_4O_{13}$ $(M+H)^+$ 561.20; found 561.26.

**Methyl (3-azido-propyloxy(5-N-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl))-onate (5b).** Compound **5b** was synthesized following the general method for the synthesis of azido derivatives. Purification by column chromatography ($CH_2Cl_2$/MeOH, 95:5) afforded compound **5b**, the corresponding reverse anomer and the glycal product (α/β/glycal (n.d.)). Compound **5b** was used in the next step without additional purification. ESI-MS *m/z* calcd for $C_{23}H_{35}N_4O_{13}$ $(M+H)^+$ 575.22; found 575.14.

**Methyl (2-azidoethoxy(5-N-propanoylamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl))-onate (6a).** Compound **6a** was synthesized following the

general method for the synthesis of azido derivatives. Purification by column chromatography (Toluene/$CH_2Cl_2$/MeOH, 10:2:0.5) afforded compound **6a** and the corresponding reverse anomer (α/β (6:1)). Compound **6a** was used in the next step without additional purification. HRMS *m/z* calcd for $C_{23}H_{34}N_4NaO_{13}$ $(M+Na)^+$ 597.2020; found 597.1985.

**Methyl (3-azido-propyloxy(5-N-propanoylamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl))-onate (6b).** Compound **6b** was synthesized following the general method for the synthesis of azido derivatives. Purification by column chromatography (Toluene/$CH_2Cl_2$/MeOH, 8:2:0.5) afforded compound **6b** and the corresponding reverse anomer (α/β (22:3)). Compound **6b** was used in the next step without additional purification. ESI-MS *m/z* calcd for $C_{24}H_{37}N_4O_{13}$ $(M+H)^+$ 589.24; found 588.97.

**Methyl (2-azidoethoxy(5-N-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl))-onate (7a).** Compound **7a** was synthesized following the general method for the *O*-deacylation of sialosides. Purification by column chromatography (EtOAc/EtOH/$H_2O$, 4:1:0.1) afforded compound **7a** (256 mg, 40% yield over three steps). $^1$H NMR (400 MHz, $CD_3OD$): δ 3.93-4.02 (m, 1H, -$OCH_{2b}$-), 3.85 (s, 3H, $OCH_3$), 3.80-3.84 (m, 2H, H-8, H-$9_b$), 3.77 (t, $J_{5,4} \approx J_{5,6}$ = 10.2 Hz, 1H, H-5), 3.60-3.73 (m, 3H, H-4, H-$9_a$, -$OCH_{2a}$-), 3.58 (dd, $J_{6,5}$ = 10.7 and $J_{6,7}$ = 1.8 Hz, 1H, H-6), 3.51 (dd, $J_{7,8}$ = 8.7 and $J_{6,7}$ = 1.8 Hz, 1H, H-7), 3.27-3.43 (m, 2H, -$CH_2N_3$), 2.72 (dd, $J_{3eq,3ax}$ = 12.8 and $J_{3eq,4}$ = 4.6 Hz, 1H, H-$3_{eq}$), 2.00 (s, 3H, $COCH_3$), 1.77 (dd, $J_{3eq,3ax}$ = 12.8 and $J_{3ax,4}$ = 11.8 Hz, 1H, H-$3_{ax}$). $^{13}$C NMR (100 MHz, $CD_3OD$): δ 175.22, 170.71, 100.20, 75.05, 72.37, 70.18, 68.49, 64.74, 64.47, 53.79, 53.42, 51.74, 41.57, 22.66. HRMS *m/z* calcd for $C_{14}H_{24}N_4NaO_9$ $(M+Na)^+$ 415.1441; found 415.1415.

**Methyl (3-azido-propyloxy(5-Nacetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl))-onate (7b).** Compound **7b** was synthesized following the general method for the *O*-deacylation of sialosides. Purification by column chromatography (EtOAc/EtOH/$H_2O$, 4:1:0.1) afforded compound **7b** (289 mg, 58% yield over three steps). $^1$H NMR (400 MHz, $CD_3OD$): δ 3.80-3.92 (m, 6H, -$OCH_{2b}$-, H-$9_b$, H-8, $OCH_3$), 3.77 (t, $J_{5,4} \approx J_{5,6}$ = 10.2 Hz, 1H, H-5), 3.61-3.69 (m, 2H, H-$9_a$, H-4), 3.59 (dd, $J_{6,5}$ = 10.3 and $J_{6,7}$ = 2.0 Hz, 1H, H-6), 3.51 (dd, $J_{7,8}$ = 9.2 and $J_{6,7}$ = 1.9 Hz, 1H, H-7), 3.43-3.50 (m, 1H, $OCH_{2a}$-), 3.38 (t, $J$ = 6.6 Hz, 2H, -$CH_2N_3$), 2.68 (dd, $J_{3eq,3ax}$ = 12.8 and $J_{3eq,4}$ = 4.7 Hz, 1H, H-$3_{eq}$), 2.00 (s, 3H, $COCH_3$), 1.75-1.85 (m, 2H, -$CH_2$-), 1.74 (dd, $J_{3eq,3ax}$ = 12.8 and $J_{3ax,4}$ = 11.8 Hz, 1H, H-$3_{ax}$). $^{13}$C NMR (100 MHz, $CD_3OD$): δ 175.22, 171.06, 100.21**,** 74.97, 72.45, 70.20, 68.52, 64.71, 62.09, 53.83, 53.38, 48.98, 41.69, 30.14**,** 22.65. HRMS *m/z* calcd for $C_{15}H_{26}N_4NaO_9$ $(M+Na)^+$ 429.1597; found 429.1572.

**Methyl (2-azidoethoxy(5-N-propanoylamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl))-onate (8a).** Compound **8a** was synthesized following the general method for the *O*-deacylation of sialosides. Purification by column chromatography (Toluene/$CH_2Cl_2$/MeOH, 3.5:6:0.5 to 3:6:1) afforded compound **8a** (120 mg, 46% yield over three steps). $^1$H NMR (400 MHz, $CD_3OD$): δ 3.94-4.00 (m, 1H, -$OCH_{2b}$-), 3.85 (s, 3H, $OCH_3$), 3.80-3.84 (m, 2H, H-8, H-$9_b$), 3.77 (t, $J_{5,4} \approx J_{5,6}$ = 10.4 Hz, 1H, H-5), 3.67-3.72 (ddd, $J_{4,3ax}$ = 11.6, $J_{5,4}$ = 10.4, $J_{4,3eq}$ = 4.6 Hz, 1H, H-4), 3.60-3.66 (m, 2H, H-$9_a$, -$OCH_{2a}$-), 3.57 (dd, $J_{6,5}$ = 10.2 and $J_{6,7}$ = 1.6 Hz, 1H, H-6), 3.49 (dd, $J_{7,8}$ = 8.9 and $J_{6,7}$ = 1.6 Hz, 1H, H-7), 3.32-3.35 (m, 1H, -$CH_{2b}N_3$), 2.72 (dd, $J_{3eq,3ax}$ = 12.8 and $J_{3eq,4}$ = 4.6 Hz, 1H, H-$3_{eq}$), 2.27 (q, $J$ = 7.6 Hz, 2H, -$COCH_2$-), 1.76 (dd, $J_{3eq,3ax}$ = 12.8 and $J_{3ax,4}$ = 11.6 Hz, 1H, H-$3_{ax}$), 1.14 (t, $J$ = 7.6 Hz, 3H, -$COCH_2$). $^{13}$C

NMR (100 MHz, $CD_3OD$): δ 179.01, 170.73, 100.19, 75.09, 72.36, 70.17, 68.40, 64.72, 64.47, 53.64, 53.43, 51.73, 41.64, 30.16, 10.28. HRMS *m/z* calcd for $C_{15}H_{26}N_4NaO_9$ $(M+Na)^+$ 429.1597; found 429.1578.

**Methyl (3-azido-propyloxy(5-N-propanoylamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl))-onate (8b).** Compound **8b** was synthesized following the general method for the *O*-deacylation of sialosides. Purification by column chromatography (Toluene/$CH_2Cl_2$/MeOH, 3.5:6:0.5 to 3:6:1) afforded compound **8b** (157 mg, 55% yield over three steps). $^1$H NMR (400 MHz, $CD_3OD$): δ 3.80-3.89 (m, 3H, -$OCH_{2b}$-, H-$9_b$, H-8), 3.84 (s, 3H, $OCH_3$), 3.76 (t, $J_{5,4} \approx J_{5,6}$ = 10.2 Hz, 1H, H-5), 3.60-3.69 (m, 2H, H-$9_a$, H-4), 3.58 (dd, $J_{6,5}$ = 10.2 and $J_{6,7}$ = 1.6 Hz, 1H, H-6), 3.49 (dd, $J_{7,8}$ = 8.9 and $J_{6,7}$ = 1.6 Hz, 1H, H-7), 3.43-3.50 (m, 1H, $OCH_{2a}$-), 3.38 (t, *J* = 6.6 Hz, 2H, -$CH_2N_3$), 2.68 (dd, $J_{3eq,3ax}$ = 12.8 and $J_{3eq,4}$ = 4.6 Hz, 1H, H-$3_{eq}$), 2.27 (q, *J* = 7.6 Hz, 2H, -$COCH_2$-), 1.75-1.83 (m, 2H, -$CH_2$-), 1.74 (dd, $J_{3eq,3ax}$ = 12.8 and $J_{3ax,4}$ = 11.8 Hz, 1H, H-$3_{ax}$), 1.13 (t, *J* = 7.6 Hz, 3H, $COCH_2\underline{CH_3}$). $^{13}$C NMR (100 MHz, $CD_3OD$): δ 178.99, 171.08, 100.20**,** 75.01, 72.43, 70.18, 68.42, 64.69, 62.07, 53.68, 53.39, 48.78, 41.76, 30.16**,** 30.13, 10.28. HRMS *m/z* calcd for $C_{16}H_{28}N_4NaO_9$ $(M+Na)^+$ 443.1754; found 443.1732.

**Tris ((1-(2-O-((methyl (5-N-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl)-onate))-2-oxoethyl-1H-1,2,3-triazol-4-yl)methyl)amine (9a).** Compound **9a** was synthesized following the general method for the synthesis of the first generation of trivalent sialic acid derivatives (40 mg, 51% yield). $^1$H NMR (400 MHz, $CD_3OD$): δ 8.08 (s, 3H, 3ArCH), 8.01 (d, $J_{5,NH}$ = 8.4 Hz, 3H, 3NH), 4.60 (t, *J* = 5.0 Hz, 6H, 3-$CH_2$-ArN), 4.15-4.27 (m, 3H, 3-$OCH_{2b}$), 3.70-3.97 (m, 27H, 3-$OCH_{2a}$, 3H-$9_b$,-$N(CH_2)_3$, 3H-8, 3H-5, 3-$OCH_3$), 3.55-3.67 (m, 9H, 3H-4, 3H-$9_b$, 3H-6), 3.48 (dd, $J_{7,8}$ = 8.8 and $J_{6,7}$ = 1.4 Hz, 3H, H-7), 2.58 (dd, $J_{3eq,3ax}$ = 12.8 and $J_{3eq,4}$ = 4.7 Hz, 3H, 3H-$3_{eq}$), 1.99 (s, 9H, 3-$COCH_3$), 1.71 (dd, $J_{3eq,3ax}$ = 12.8 and $J_{3ax,4}$ = 11.7 Hz, 3H, 3H-$3_{ax}$). $^{13}$C NMR (100 MHz, $CD_3OD$): δ 175.12, 170.47, 144.25, 126.94, 100.28, 75.07, 72.25, 70.16, 68.40, 64.84, 63.91, 53.68, 53.55, 51.47, 48.73, 41.44, 22.70. HRMS *m/z* calcd for $C_{51}H_{81}N_{13}NaO_{27}$ $(M+Na)^+$ 1330.5263; found 1330.5218.

**Tris ((1-(2-O-(methyl (5-N-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl)-onate))-3-oxopropyl-1H-1,2,3-triazol-4,1-yl)methyl)amine (9b).** Compound **9b** was synthesized following the general method for the synthesis of the first generation of trivalent sialic acid derivatives (60 mg, 45% yield). $^1$H NMR (400 MHz, $CD_3OD$): δ 8.01 (s, 3H, 3ArCH), 4.50 (t, *J* = 6.6 Hz, 6H, 3-$CH_2$-ArN), 3.70-3.88 (m, 27H, 3-$OCH_{2b}$-,3H-$9_b$, 3-$OCH_3$, -$N(CH_2)_3$, 3H-8, 3H-5), 3.54-3.69 (m, 9H, 3H-4, 3H-$9_b$, 3H-6), 3.49 (dd, $J_{7,8}$ = 9.0 and $J_{6,7}$ = 1.3 Hz, 3H, 3H-7), 3.35-3.43 (m, 3H, 3-$OCH_{2a}$), 2.68 (dd, $J_{3eq,3ax}$ = 12.8 and $J_{3eq,4}$ = 4.7 Hz, 3H, 3H-$3_{eq}$), 2.09-2.20 (m, 6H, 3-$CH_2$-), 2.00 (s, 9H, 3-$COCH_3$), 1.74 (dd, $J_{3eq,3ax}$ = 12.7 and $J_{3ax,4}$ = 11.8 Hz, 3H, 3H-$3_{ax}$). $^{13}$C NMR (100 MHz, $CD_3OD$): δ 175.20, 170.86, 145.26, 125.97, 100.12, 74.92, 72.43, 70.19, 68.50, 64.77, 61.77, 53.80, 53.54, 48.48, 48.10, 41.65, 31.29, 22.72. HRMS *m/z* calcd for $C_{54}H_{87}N_{13}NaO_{27}$ $(M+Na)^+$ 1372.5732; found 1372.5682.

**Tris ((1-(2-O-(methyl (5-N-propanoylamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl)-onate))-2-oxoethyl-1H-1,2,3-triazol-4-yl)methyl)amine (10a).** Compound **10a** was synthesized following the general method for the synthesis of the first generation of trivalent sialic acid derivatives (60 mg, 50% yield). $^1$H NMR (400 MHz, $CD_3OD$): δ 8.04 (s, 3H, 3ArCH), 4.50 (t, *J* = 5.1 Hz, 6H, 3-$CH_2$-ArN), 4.16-4.23 (m, 3H, 3-$OCH_{2b}$), 3.86-3.92 (m, 3H, 3-

$OCH_{2a}$), 3.74-3.84 (m, 15H, 3H-$9_b$,-$N(CH_2)_3$, 3H-8, 3H-5), 3.72 (s, 9H, 3-$OCH_3$), 3.59-3.67 (m, 6H, 3H-4, 3H-$9_b$), 3.57 (dd, $J_{6,5}$ = 10.4 and $J_{6,7}$ = 1.3 Hz, 3H, 3H-6), 3.47 (dd, $J_{7,8}$ = 8.9 and $J_{6,7}$ = 1.3 Hz, 3H, H-7), 2.58 (dd, $J_{3eq,3ax}$ = 12.4 and $J_{3eq,4}$ = 4.6 Hz, 3H, 3H-$3_{eq}$), 2.25 (q, $J$ = 7.6 Hz, 6H, 3-$COCH_2$-), 1.74 (appear as t, $J_{3eq,3ax} \approx J_{3ax,4}$ = 12.4 Hz, 3H, 3H-$3_{ax}$), 1.12 (t, $J$ = 7.6 Hz, 9H, 3-$COCH_2CH_3$). $^{13}$C NMR (100 MHz, $CD_3OD$): δ 178.88, 170.43, 145.08, 126.66, 100.23, 75.04, 72.15, 69.97, 68.29, 64.73, 63.95, 53.60, 53.45, 51.43, 49.07, 41.52, 31.15, 10.36. HRMS *m/z* calcd for $C_{54}H_{87}N_{13}NaO_{27}$ $(M+Na)^+$ 1372.5732; found 1372.5668.

**Tris ((1-(2-O-(methyl (5-N-propanoylamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl)-onate))-3-oxopropyl-1H-1,2,3-triazol-4-yl)methyl)amine (10b).** Compound **10b** was synthesized following the general method for the synthesis of the first generation of trivalent sialic acid derivatives (45 mg, 34% yield). $^1$H NMR (400 MHz, $CD_3OD$): δ 8.03 (s, 3H, 3ArCH), 7.89 (d, $J_{5,NH}$ = 8.5 Hz, 3H, 3NH), 4.50 (t, $J$ = 6.5 Hz, 6H, 3-$CH_2$-ArN), 3.73-3.86 (m, 18H, 3-$OCH_{2b}$-,3H-$9_b$,-$N(CH_2)_3$, 3H-8, 3H-5), 3.81 (s, 9H, 3-$OCH_3$), 3.58-3.70 (m, 6H, 3H-4, 3H-$9_b$), 3.56 (dd, $J_{6,5}$ = 10.4 and $J_{6,7}$ = 1.3 Hz, 3H, 3H-6), 3.47 (dd, $J_{7,8}$ = 8.9 and $J_{6,7}$ = 1.3 Hz, 3H, 3H-7), 3.35-3.43 (m, 3H, 3-$OCH_{2a}$), 2.68 (dd, $J_{3eq,3ax}$ = 12.4 and $J_{3eq,4}$ = 4.6 Hz, 3H, 3H-$3_{eq}$), 2.27 (q, $J$ = 7.6 Hz, 6H, 3-$COCH_2$-), 2.10-2.20 (m, 6H, 3-$CH_2$-), 1.74 (appear as t, $J_{3eq,3ax} \approx J_{3ax,4}$ = 12.4 Hz, 3H, 3H-$3_{ax}$), 1.13 (t, $J$ = 7.6 Hz, 9H, 3-$COCH_2\underline{CH_3}$). $^{13}$C NMR (100 MHz, $CD_3OD$): δ 178.92, 170.88, 143.82, 126.16, 100.12, 75.01, 72.44, 70.22, 68.44, 64.72, 61.73, 53.67, 53.52, 49.28, 49.07, 41.75, 31.28, 30.17, 10.32. HRMS *m/z* calcd for $C_{57}H_{93}N_{13}NaO_{27}$ $(M+Na)^+$ 1414.6202; found 1414.6137.

**Tris ((1-(2-O-(5-N-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosylonic acid))-2-oxoethyl-1H-1,2,3-triazol-4-yl)methyl)amine (11a).** Compound **11a** was synthesized following the general method for the saponification of methyl ester (22 mg, 76% yield). $[\alpha]_D^{20}$ -15.77 (c 4.8 mg/mL, $H_2O$). $^1$H NMR (400 MHz, $D_2O$): δ 8.40 (s, 3H, 3ArCH), 4.72 (t, $J$ = 5.2 Hz, 6H, 3-$CH_2$-ArN), 4.60 (bs, 6H, -$N(CH_2)_3$), 4.14-4.27 (m, 3H, 3-$OCH_{2b}$), 3.93-4.02 (m, 3H, 3-$OCH_{2a}$), 3.85 (dd, $J_{9a,9b}$ = 11.7 and $J_{9b,8}$ = 2.3 Hz 3H, 3H-$9_b$), 3.73-3.82 (m, 6H, 3H-8, 3H-5), 3.59-3.72 (m, 9H, 3H-4, 3H-6, 3H-$9_a$), 3.56 (dd, $J_{7,8}$ = 9.3 and $J_{6,7}$ = 1.7 Hz, 3H, H-7), 2.66 (dd, $J_{3eq,3ax}$ = 12.5 and $J_{3eq,4}$ = 4.6 Hz, 3H, 3H-$3_{eq}$), 2.04 (s, 9H, 3-$COCH_3$), 1.67 (appear as t, $J_{3eq,3ax} \approx J_{3ax,4}$ = 12.0 Hz, 3H, 3H-$3_{ax}$). $^{13}$C NMR (100 MHz, $D_2O$): δ 175.05, 172.55, 135.87, 128.64, 100.05, 72.72, 71.42, 68.10, 67.88, 62.68, 62.58, 51.73, 50.57, 46.49, 39.64, 22.0. HRMS *m/z* calcd for $C_{48}H_{76}N_{13}O_{27}$ $(M+H)^+$ 1266.4974 and $C_{48}H_{75}N_{13}NaO_{27}$ $(M+Na)^+$ 1288.4793; found 1266.4954 and 1288.4749, respectively.

**Tris ((1-(2-O-(5-N-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosylonic acid))-3-oxopropyl-1H-1,2,3-triazol-4-yl)methyl)amine (11b).** Compound **11b** was synthesized following the general method for the saponification of methyl ester (27 mg, 75% yield). $[\alpha]_D^{20}$ -3.83 (c 3.9 mg/mL, $H_2O$). $^1$H NMR (400 MHz, $D_2O$): δ 8.02 (s, 3H, 3ArCH), 4.45-4.64 (m, 6H, 3-$CH_2$-ArN), 3.92 (bs, 6H, $N(CH_2)_3$), 3.56-3.87 (m, 24H, 3H-$9_b$, 3-$OCH_2$-, 3H-8, 3H-5, 3H-4, 3H-6, 3H-$9_a$), 3.46-3.54 (m, 3H, 3H-7), 2.73 (dd, $J_{3eq,3ax}$ = 12.5 and $J_{3eq,4}$ = 4.7 Hz, 3H, 3H-$3_{eq}$), 2.15-2.25 (m, 6H, 3-$CH_2$-), 2.05 (s, 9H, $COCH_3$), 1.63 (appear as t, $J_{3eq,3ax} \approx J_{3ax,4}$ = 12.1 Hz, 3H, 3H-$3_{ax}$). $^{13}$C NMR (100 MHz, $D_2O$): δ 175.05, 173.52, 142.34, 125.79, 100.48, 72.56, 71.71, 68.28, 68.16, 62.51, 61.22, 51.89, 47.54, 47.30, 40.29, 29.64, 22.02. HRMS *m/z* calcd for $C_{51}H_{82}N_{13}O_{27}$ $(M+H)^+$ 1308.5443 and $C_{51}H_{81}N_{13}NaO_{27}$ $(M+Na)^+$ 1330.5263; found 1308.5391 and 1330.5211, respectively.

**Tris ((1-(2-O-(5-N-propanoylamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosylonic acid))-2-oxoethyl-1H-1,2,3-triazol-4-yl)methyl)amine (12a). 12a** was synthesized following the general method for the saponification of methyl ester (19 mg, 72% yield). $[\alpha]_D^{20}$ -14.34 (c 4.6 mg/mL, $H_2O$). $^1$H NMR (400 MHz, $D_2O$): δ 8.17 (s, 3H, 3ArCH), 4.64 (bs, 6H, 3-$CH_2$-ArN), 4.07-4.20 (m, 9H, 3-$OCH_{2b}$, -$N(CH_2)_3$), 3.87-3.93 (m, 3H, 3-$OCH_{2a}$), 3.82 (dd, $J_{9a,9b}$ = 11.7 and $J_{9b,8}$ = 2.4 Hz 3H, 3H-$9_b$), 3.75 (ddd, $J_{8,7}$ = 8.9, $J_{8,9a}$ = 5.6 Hz , $J_{9b,8}$ = 2.4 Hz 3H, 3H-8), 3.72 (t, $J_{5,6} \approx J_{5,4}$ = 10.1 Hz, 3H, 3H-5), 3.63-3.69 (m, 3H, 3H-4), 3.56-3.63 (m, 6H, 3H-6, 3H-$9_a$), 3.51 (dd, $J_{7,8}$ = 8.9 and $J_{6,7}$ = 1.6 Hz, 3H, H-7), 2.66 (dd, $J_{3eq,3ax}$ = 12.3 and $J_{3eq,4}$ = 4.6 Hz, 3H, 3H-$3_{eq}$), 2.28 (q, $J$ = 7.6 Hz, 6H, 3-$COCH_2$-), 1.74 (appear as t, $J_{3eq,3ax} \approx J_{3ax,4}$ = 12.3 Hz, 3H, 3H-$3_{ax}$), 1.10 (t, $J$ = 7.6 Hz, 9H, 3-$COCH_2CH_3$). $^{13}$C NMR (100 MHz, $D_2O$): δ 179.90, 174.12, 127.82, 101.35, 73.50, 72.50, 69.00, 68.91, 63.74, 63.40, 52.51, 51.39, 49.84, 40.87, 30.05, 10.36. HRMS *m/z* calcd for $C_{51}H_{82}N_{13}O_{27}$ $(M+H)^+$ 1308.5443 and $C_{51}H_{81}N_{13}NaO_{27}$ $(M+Na)^+$ 1330.5263; found 1308.5423 and 1330.5206, respectively.

**Tris ((1-(2-O-(5-N-propanoylamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosylonic acid)-3-oxopropyl-1H-1,2,3-triazol-4-yl)methyl)amine (12b). 12b** was synthesized following the general method for the saponification of methyl ester (27 mg, 75% yield). $[\alpha]_D^{20}$ -0.76 (c 4.8 mg/mL, $H_2O$). $^1$H NMR (400 MHz, $D_2O$): δ 8.31 (s, 3H, 3ArCH), 4.53-4.63 (m, 12H, 3-$CH_2$-ArN, $N(CH_2)_3$), 3.82 (dd, $J_{9b,9a}$ = 11.8 and $J_{9b,8}$ = 2.4 Hz, 3H, H-$9_b$), 3.74-3.83 (m, 12H, 3-$OCH_{2b}$-, 3H-8, 3H-5), 3.67-3.73 (m, 3H, 3H-4), 3.67 (dd, $J_{6,5}$ = 10.4 and $J_{6,7}$ = 1.5 Hz, 3H, 3H-6), 3.60 (dd, $J_{9a,9b}$ = 11.8 and $J_{9a,8}$ = 5.9 Hz, 3H, 3H-$9_a$), 3.53 (dd, $J_{7,8}$ = 8.9 and $J_{6,7}$ = 1.5 Hz, 3H, 3H-7), 3.42-3.50 (m, 3H, 3-$OCH_{2a}$), 2.69 (dd, $J_{3eq,3ax}$ = 12.4 and $J_{3eq,4}$ = 4.5 Hz, 3H, 3H-$3_{eq}$), 2.29 (q, $J$ = 7.6 Hz, 6H, 3-$COCH_2$-), 2.15-2.25 (m, 6H, 3-$CH_2$-), 1.65 (appear as t, $J_{3eq,3ax} \approx J_{3ax,4}$ = 12.4 Hz, 3H, 3H-$3_{ax}$), 1.11 (t, $J$ = 7.6 Hz, 9H, 3-$COCH_2\underline{CH_3}$). $^{13}$C NMR (100 MHz, $D_2O$): δ 178.87, 173.77, 136.66, 128.98, 100.86, 73.47, 72.34, 69.04, 68.66, 63.47, 61.82, 52.52, 48.52, 47.57, 40.83, 30.17, 30.01, 10.30. HRMS *m/z* calcd for $C_{54}H_{88}N_{13}O_{27}$ $(M+H)^+$ 1350.5913 and $C_{54}H_{87}N_{13}NaO_{27}$ $(M+Na)^+$ 1372.5732; found 1350.5834 and 1372.5627, respectively.

**Methyl 2-(but-3-ynyloxy(5-N-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl))-onate (13b).** Compound **13b** was synthesized following the general method for the glycosylation reaction. Purification by column chromatography (gradient *n*-Heptane/EtOAc) afforded compound **13b**, the corresponding reverse anomer and the glycal product (α/β/glycal (n.d.)) Compound **13b** was used in the next step without additional purification. ESI-MS *m/z* calcd for $C_{24}H_{34}NO_{13}$ $(M+H)^+$ 544.20; found 543.98.

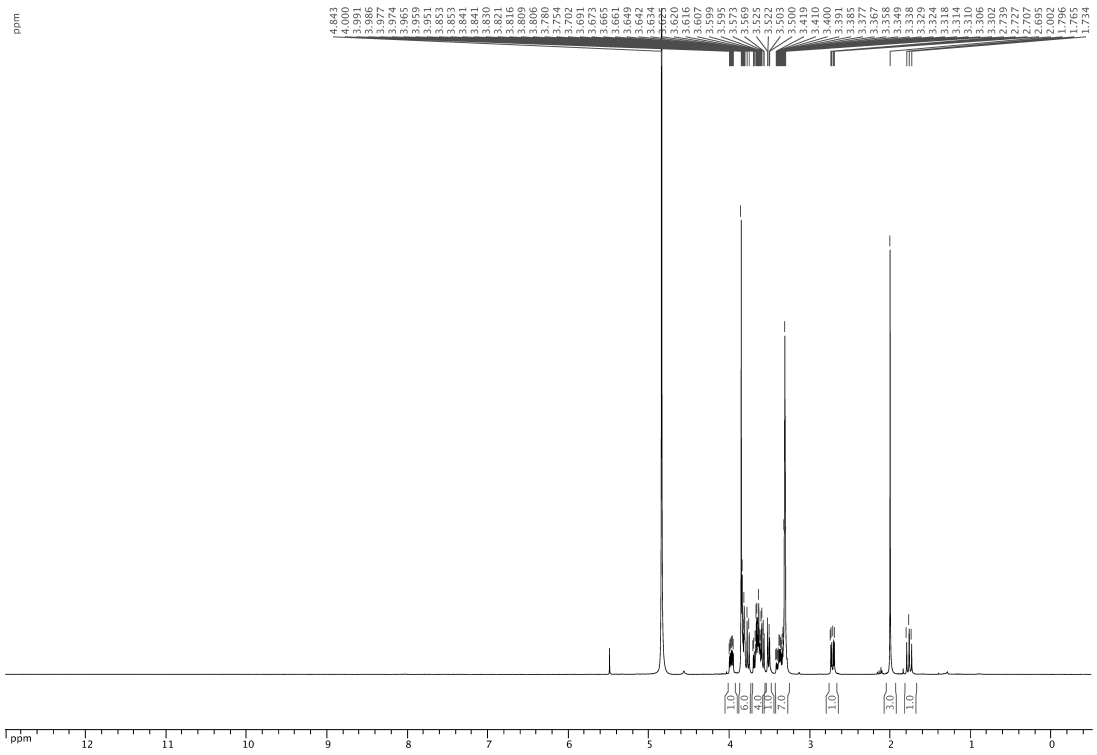
**Methyl 2-(but-3-ynyloxy(5-N-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl))-onate (14b).** Compound **14b** was synthesized following the general method for the *O*-deacylation of sialosides. Purification by column chromatography (EtOAc/EtOH/$H_2O$, 4:1:0.1) afforded compound 14b (218 mg, 31% yield over two steps). $^1$H NMR (360 MHz, $CD_3OD$): δ 3.72-3.95 (m, 7H), 3.61-3.69 (m, 2H), 3.45-3.60 (m, 3H), 2.69 (dd, $J_{3eq,4}$ =4.6 Hz, $J_{3eq,3ax}$ =12.8 Hz, 1H), 2.35-2.45 (m, 2H), 2.26 (t, $J$ = 2.7 Hz, 1H) 2.00 (s, 3H), 1.73 (dd, $J_{3eq,3ax}$ =12.3 Hz, $J_{3ax,4}$ = 12.0 Hz, 1H). $^{13}$C NMR (100 MHz, $CD_3OD$): δ 175.18, 170.81, 100.21, 81.49, 74.92, 72.38, 70.61, 70.17, 68.48, 64.71, 63.72, 53.74, 53.43, 41.61, 22.68, 20.62. HRMS *m/z* calcd for $C_{16}H_{25}NNaO_9$ $(M+Na)^+$ 398.1427; found 398.1422.

**Tris ((4-(2-O-(methyl (5-N-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)-onate))-2-oxoethyl-1H-1,2,3-triazol-1-yl)ethyl)amine (16b).** Compound **16b** was synthesized following the general method for the synthesis of the second generation of trivalent sialic acid derivatives (36 mg, 53% yield). $^{1}$H NMR (400 MHz, $CD_3OD$): δ 8.02 (d, *J*=8.5 Hz, 3H), 7.66 (s, 3H), 4.30 (t, *J*=5.9 Hz, 6H), 4.06 (dt, *J*=9.3 Hz, *J*=6.5 Hz, 3H), 3.74-3.87 (m, 18H), 3.57-3.72 (m, 12H), 3.50 (dd, *J*=1.2 Hz, *J*=8.7 Hz, 3H), 3.04 (t, *J*=5.7 Hz, 6H), 2.93 (t, *J*=6.1 Hz, 6H), 2.65 (dd, $J_{3eq,4}$ =4.6 Hz, $J_{3eq,3ax}$ =12.7 Hz, 3H), 2.00 (s, 9H), 1.72 (t, $J_{3eq,3ax}$ =12.3 Hz, 3H). $^{13}$C NMR (100 MHz, $CD_3OD$): δ 175.16, 170.90, 146.05, 124.81, 100.22, 74.94, 72.44, 70.25, 68.52, 64.86, 64.05, 54.85, 53.80, 53.48, 41.74, 27.27, 22.72. HRMS *m/z* calcd for $C_{54}H_{87}N_{13}NaO_{27}$ $(M+Na)^+$ 1372.5732; found 1372.5740.

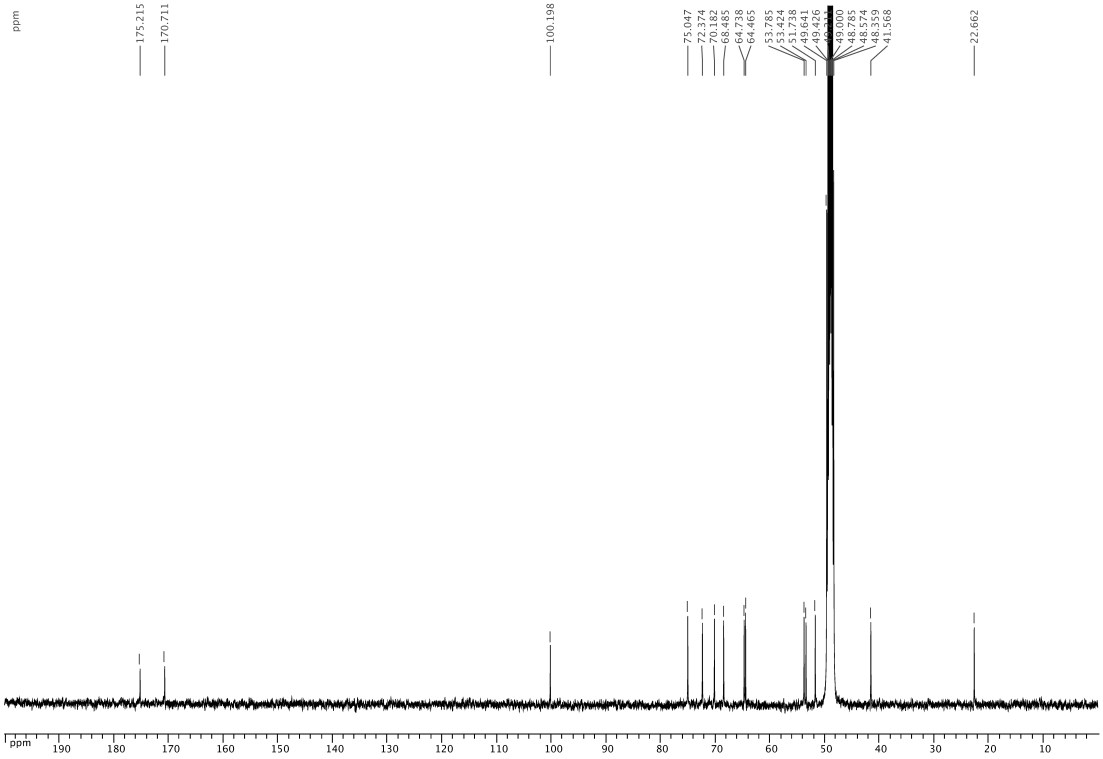
**Tris ((4-(2-O-(5-N-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid))-2-oxoethyl-1H-1,2,3-triazol-1-yl)ethyl)amine (17b). 17b** was synthesized following the general method for the saponification of methyl ester (22 mg, 88% yield). $[\alpha]_D^{20}$ -5.3 (c 1.0 mg/mL, $H_2O$). $^{1}$H NMR (400 MHz, $D_2O$): δ 7.68 (s, 3H), 4.47 (t, *J*=5.3 Hz, 6H), 3.96-4.10 (m, 3H), 3.66-3.87 (m, 18H), 3.59-3.66 (m, 3H), 3.52-3.58 (m, 3H), 3.30 (t, *J*=5.3 Hz, 6H), 2.99 (t, *J*=5.8 Hz, 6H), 2.63 (dd, $J_{3eq,4}$ =4.4 Hz, $J_{3eq,3ax}$ =12.7 Hz, 3H), 2.03 (s, 9H), 1.67 (t, $J_{3eq,3ax}$ =12.5 Hz, 3H). $^{13}$C NMR (100 MHz, $D_2O$): δ 175.01, 173.49, 144.82, 124.00, 100.60, 72.54, 71.68, 68.27, 68.16, 63.21, 62.55, 52.48, 51.85, 48.02, 40.20, 25.67, 22.00. HRMS *m/z* calcd for $C_{51}H_{82}N_{13}O_{27}$ $(M+H)^+$ 1308.5443 and $C_{51}H_{81}N_{13}NaO_{27}$ $(M+Na)^+$ 1330.5263; found 1308.5382 and 1330.5188, respectively.

Compound **7a**

$^{1}$H NMR (400 MHz, $CD_3OD$)

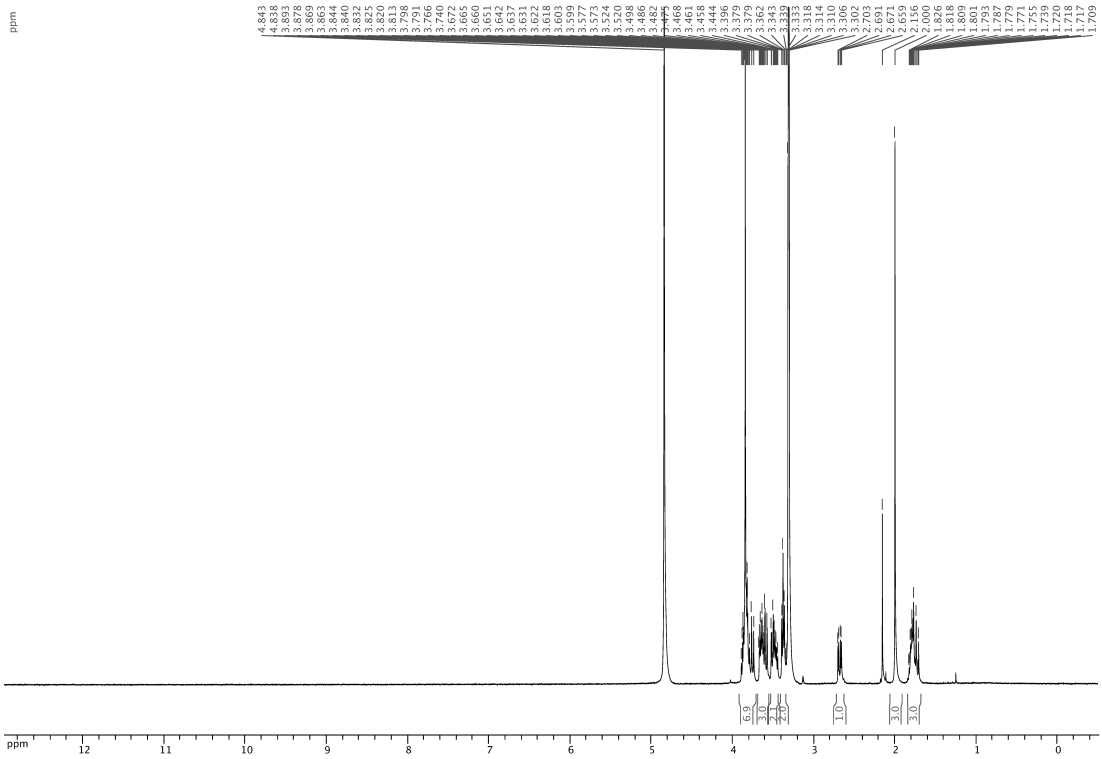


$^{13}$C NMR (100 MHz, $CD_3OD$)

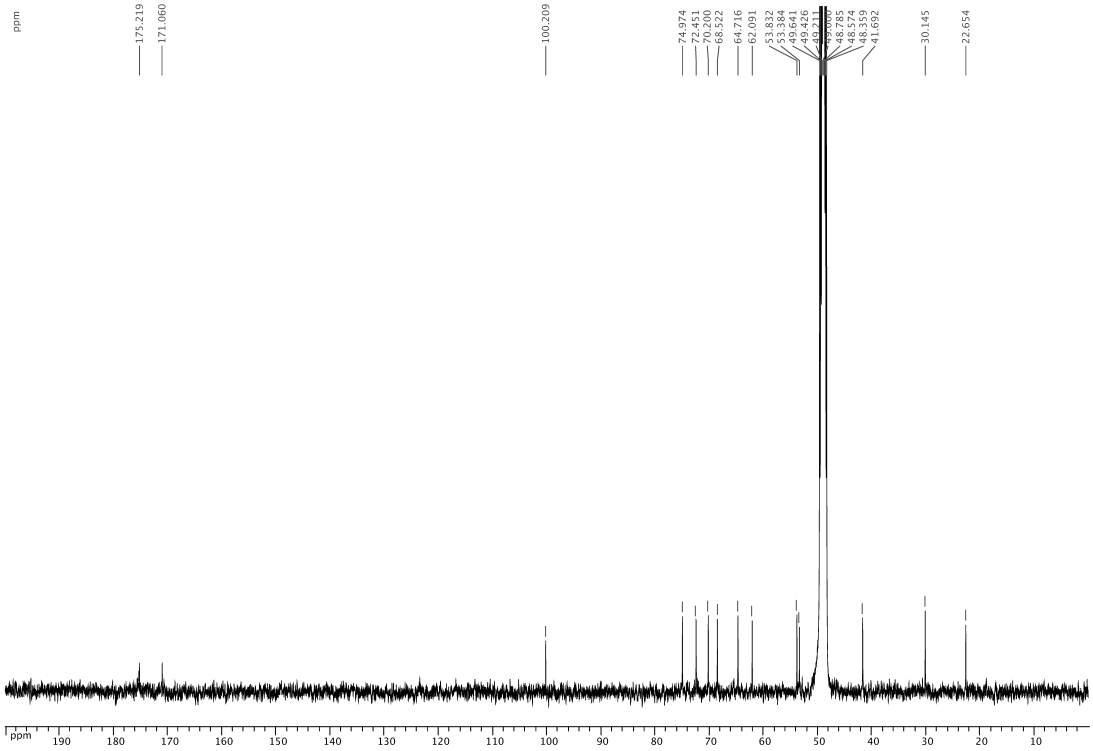


Compound **7b**

$^{1}$H NMR (400 MHz, $CD_3OD$)

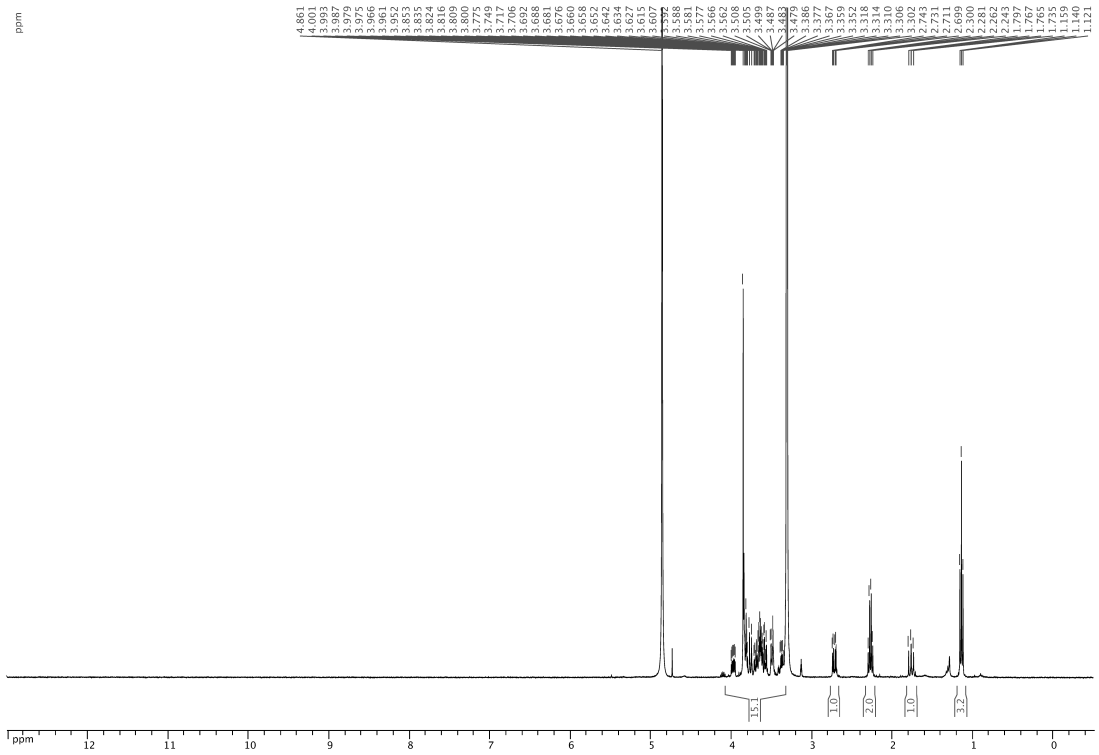


$^{13}$C NMR (100 MHz, $CD_3OD$)

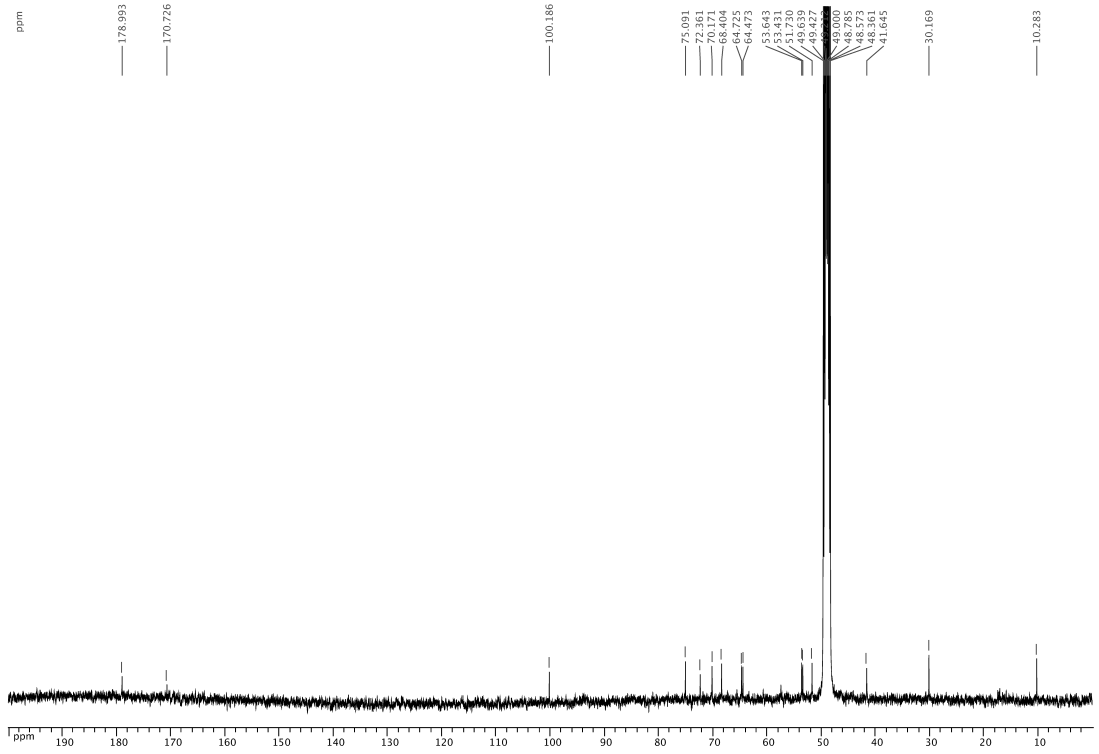


Compound **8a**

$^{1}$H NMR (400 MHz, $CD_3OD$)

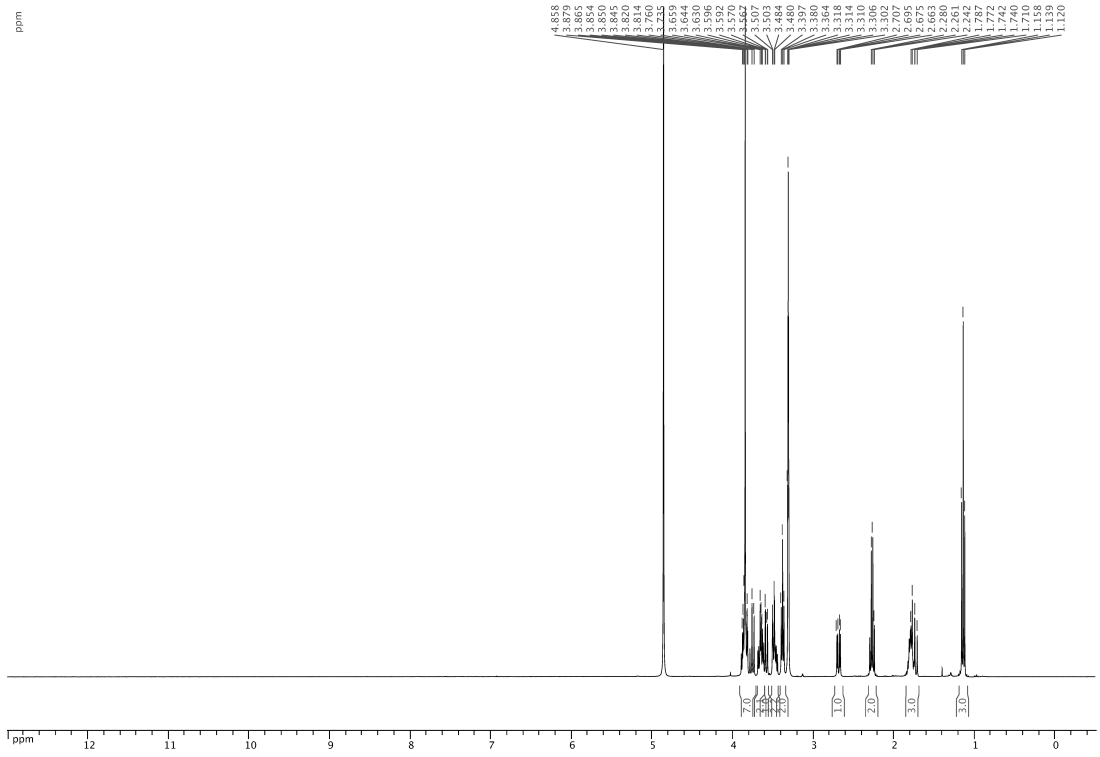


$^{13}$C NMR (100 MHz, $CD_3OD$)

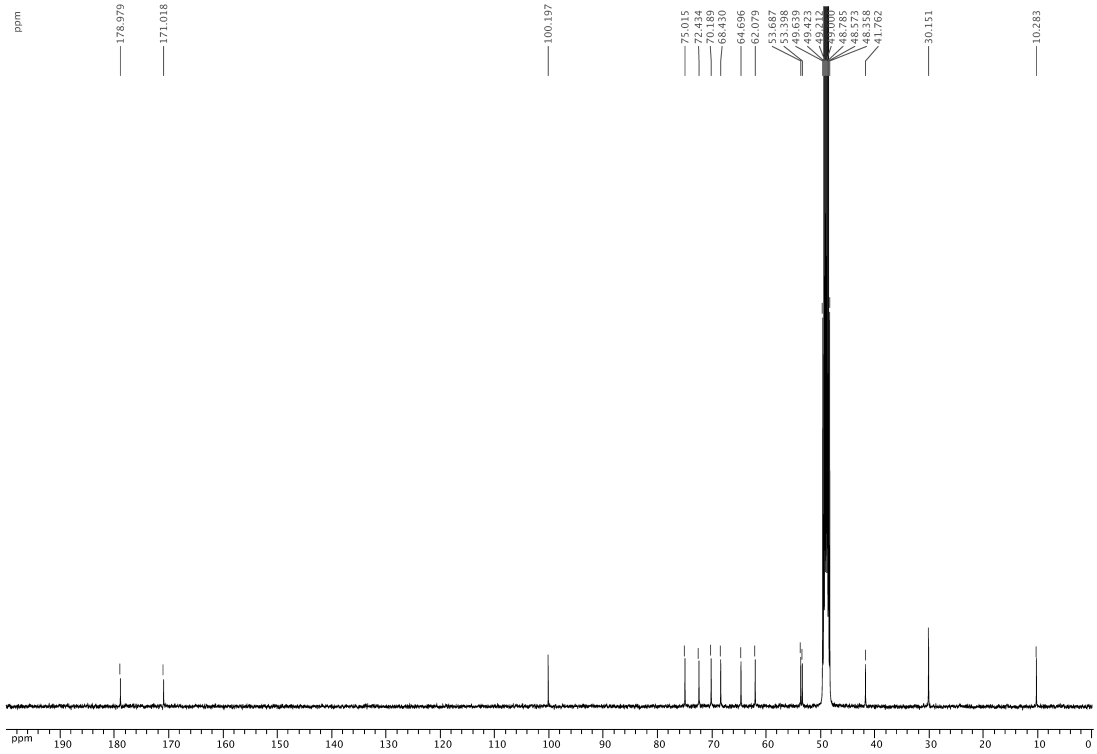


Compound **8b**

$^{1}$H NMR (400 MHz, $CD_3OD$)

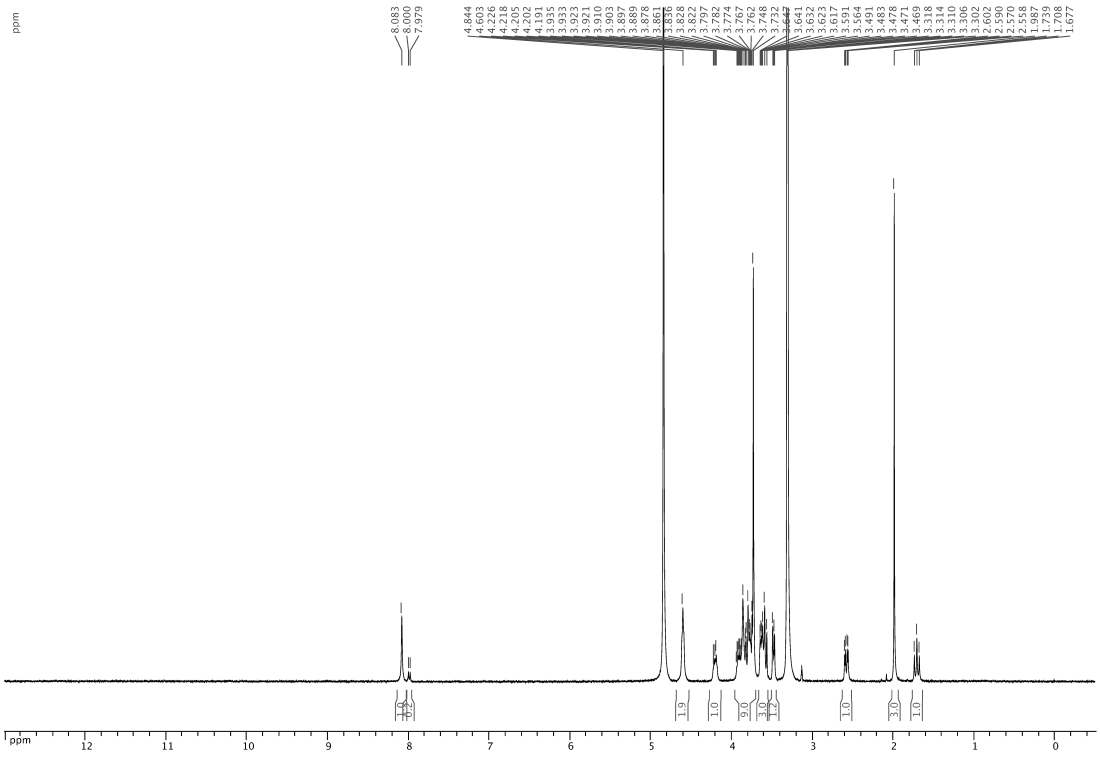


$^{13}$C NMR (100 MHz, $CD_3OD$)

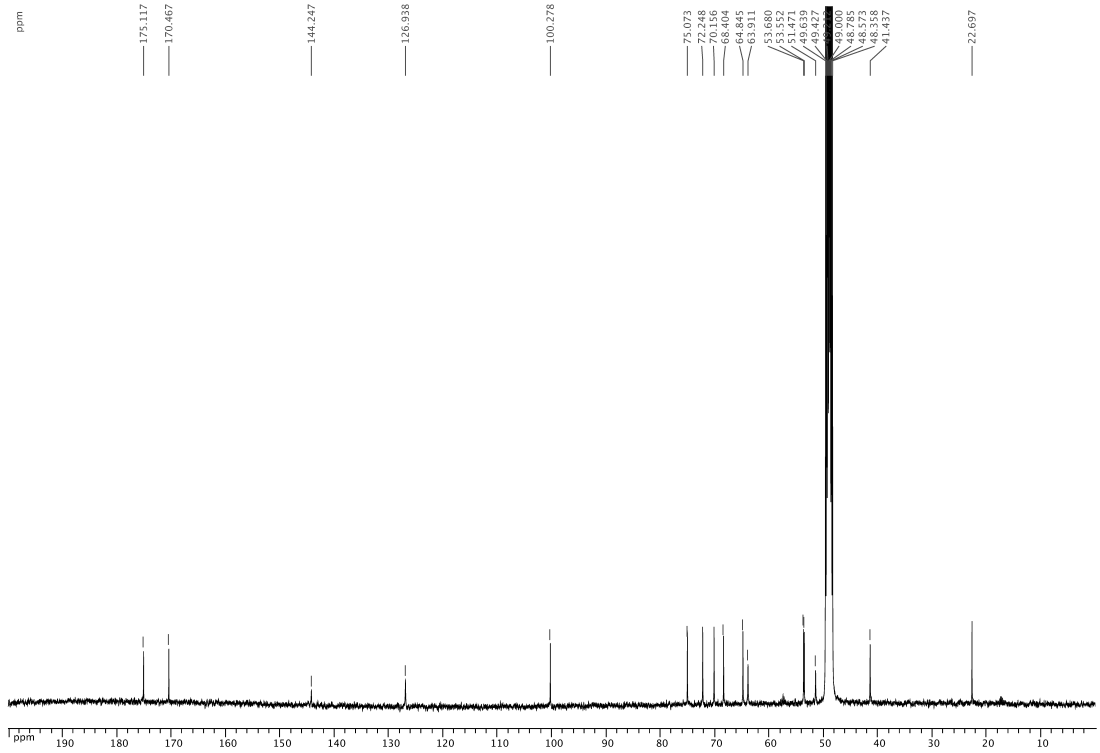


Compound **9a**

$^{1}$H NMR (400 MHz, $CD_3OD$)

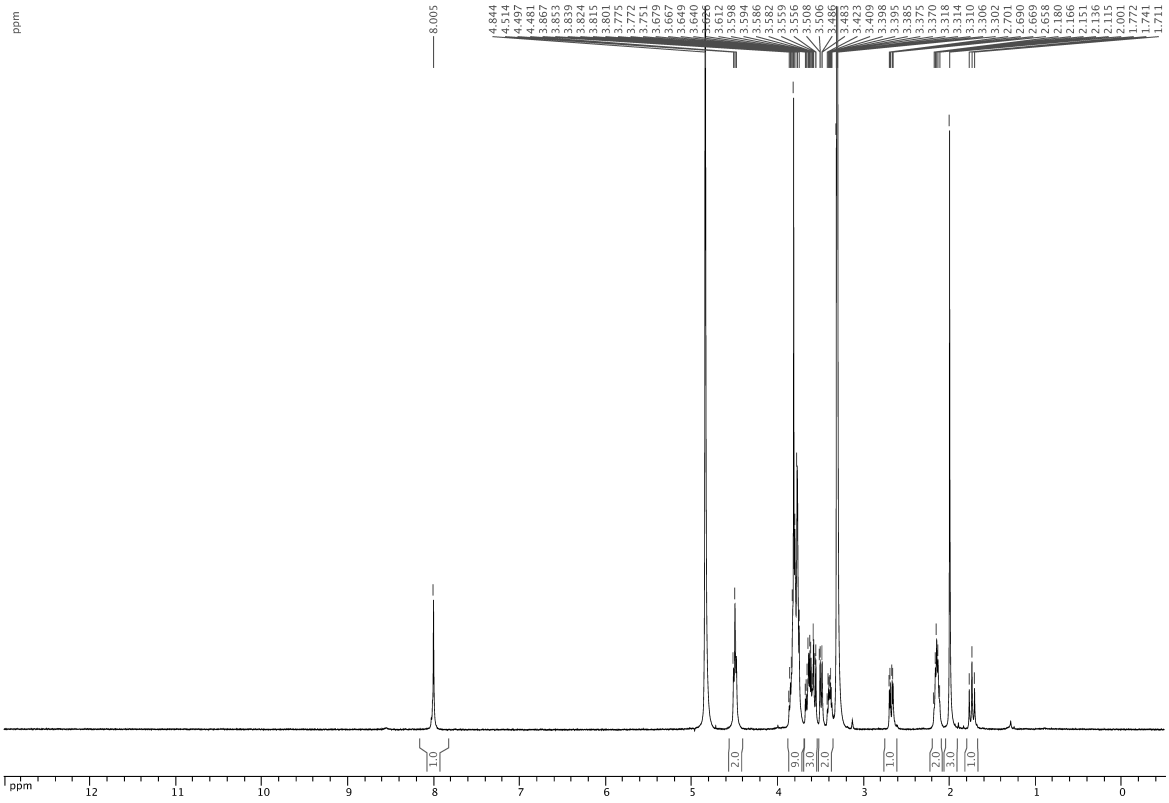


$^{13}$C NMR (100 MHz, $CD_3OD$)

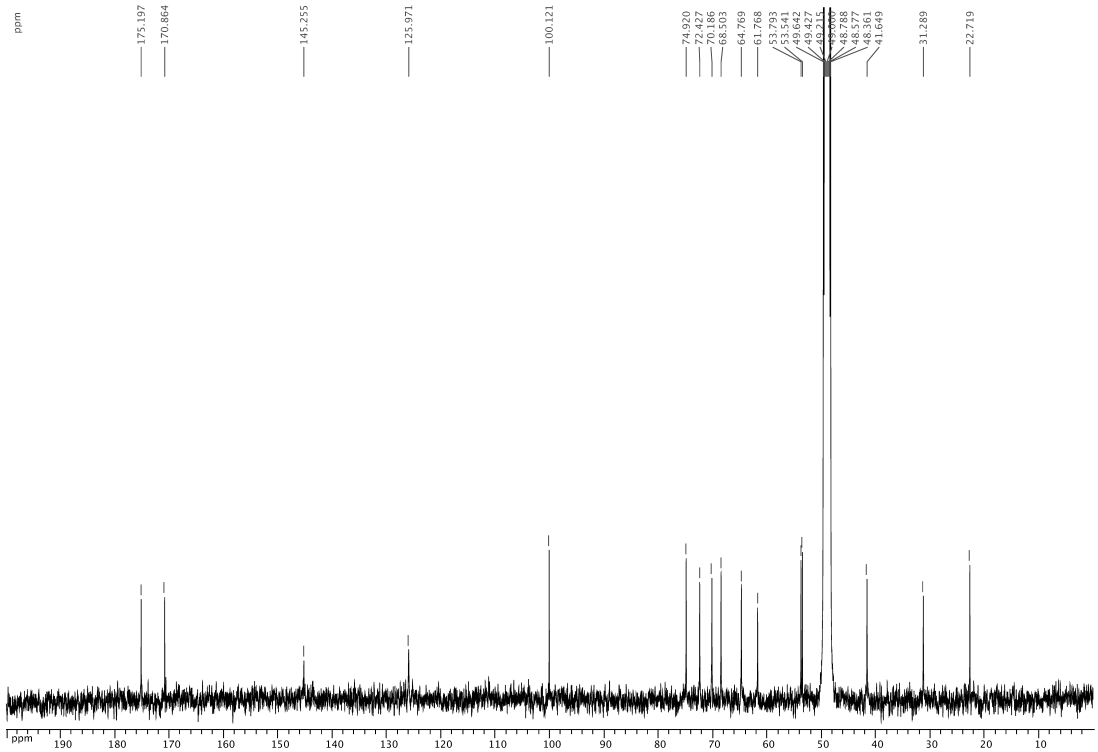


Compound **9b**

$^{1}$H NMR (400 MHz, $CD_3OD$)

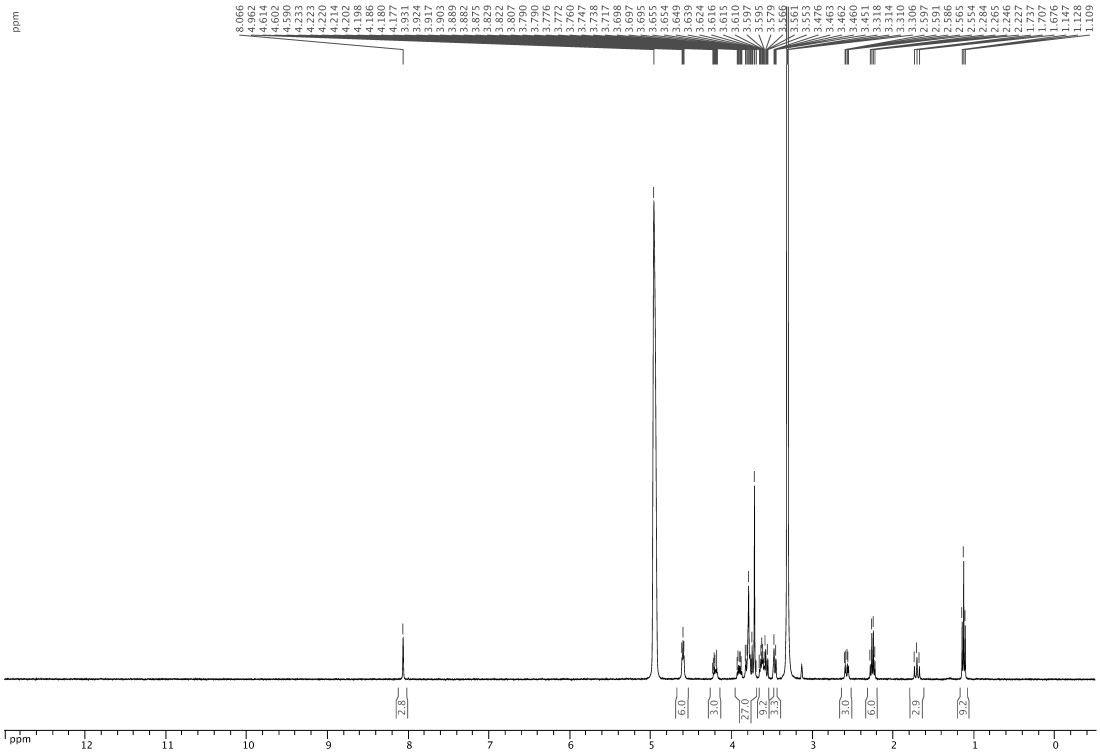


$^{13}$C NMR (100 MHz, $CD_3OD$)

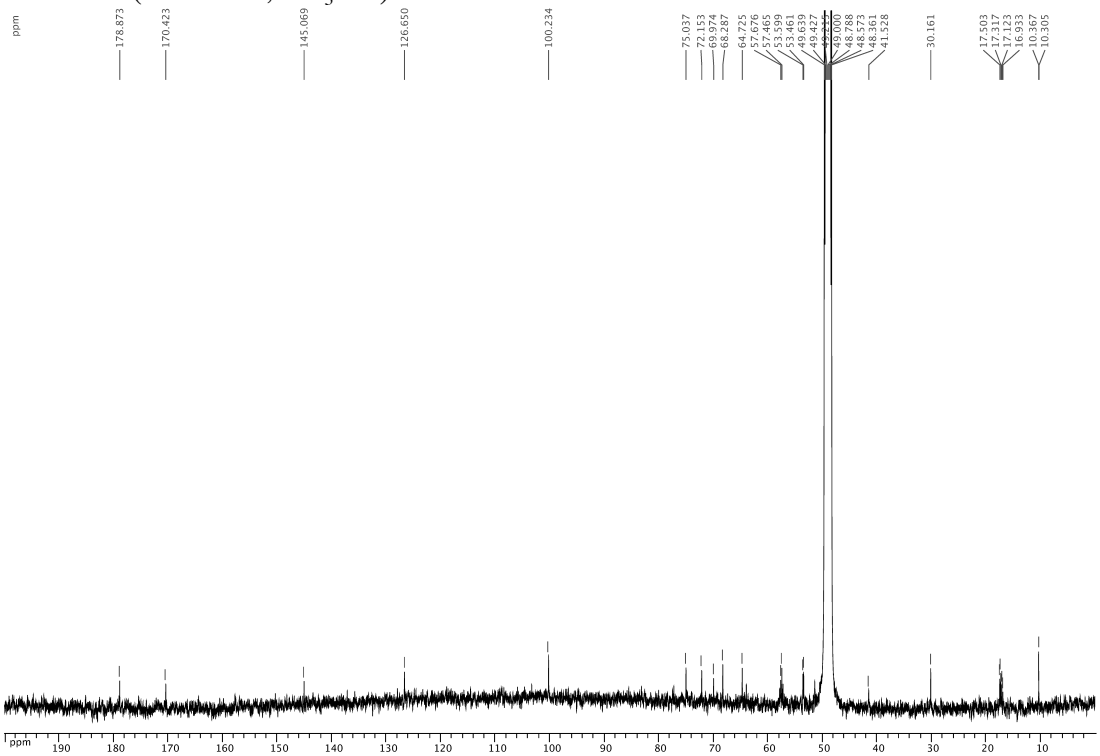


Compound **10a**

$^{1}$H NMR (400 MHz, $CD_3OD$)

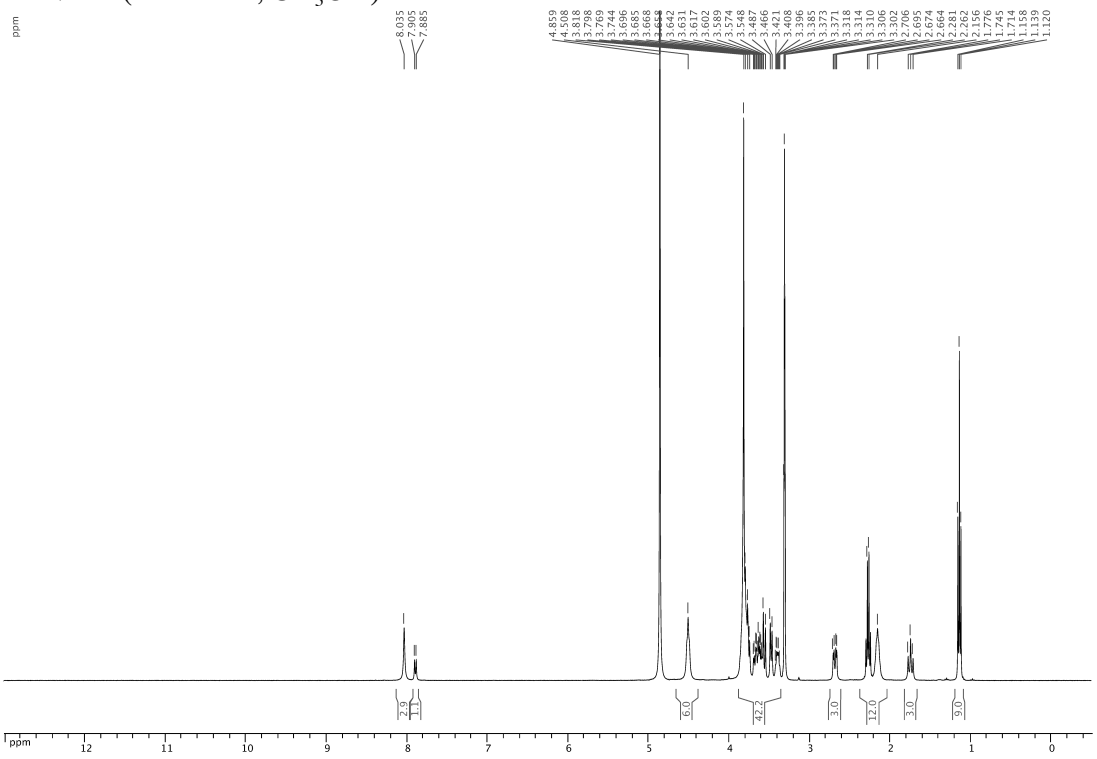


$^{13}$C NMR (100 MHz, $CD_3OD$)

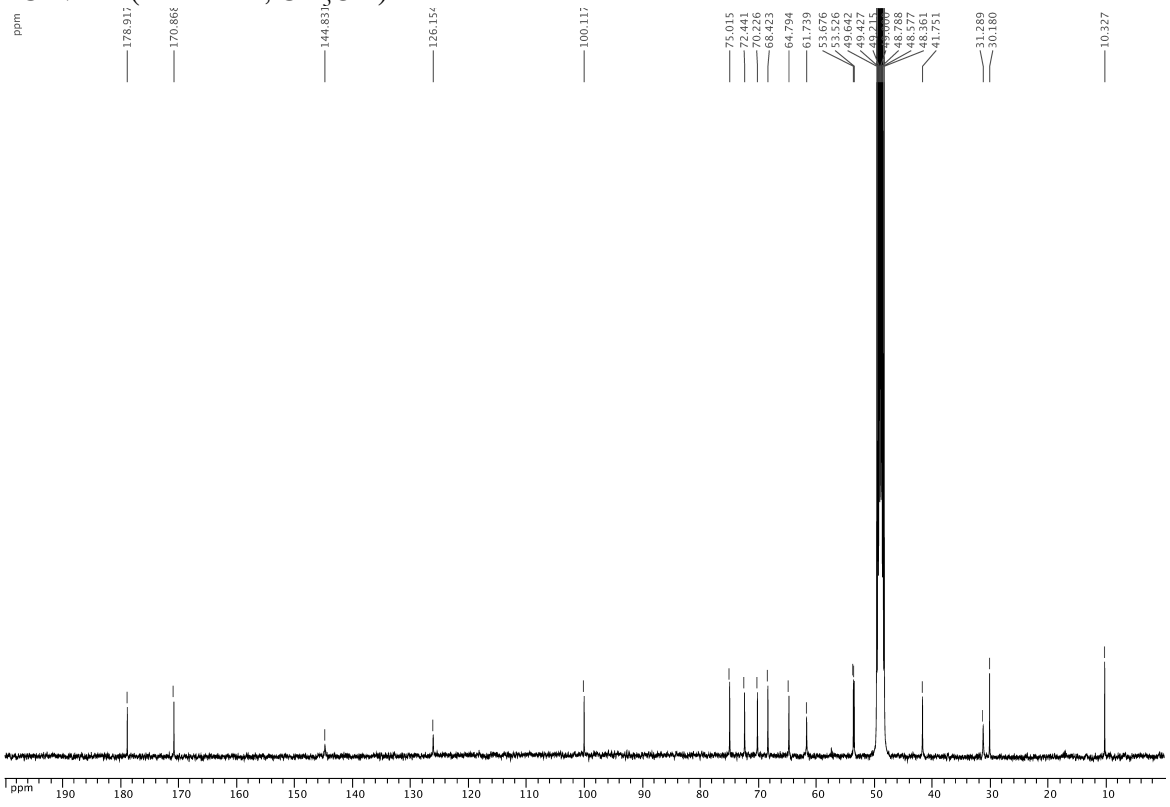


Compound **10b**

$^{1}$H NMR (400 MHz, $CD_3OD$)

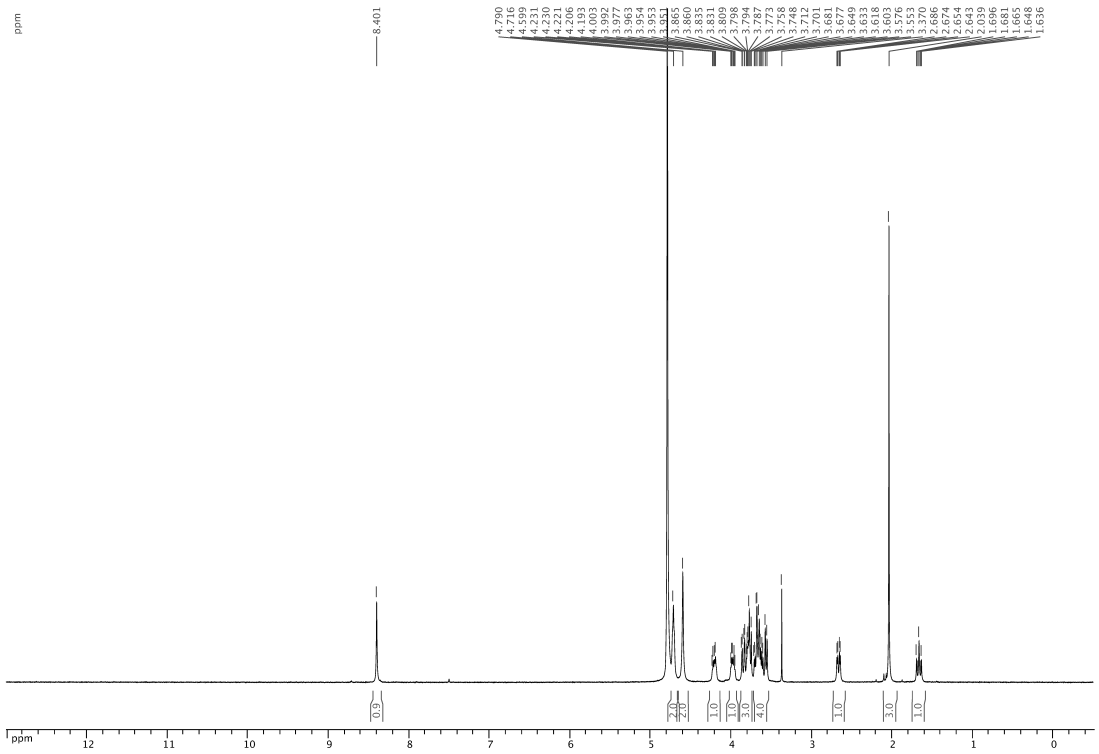


$^{13}$C NMR (100 MHz, $CD_3OD$)

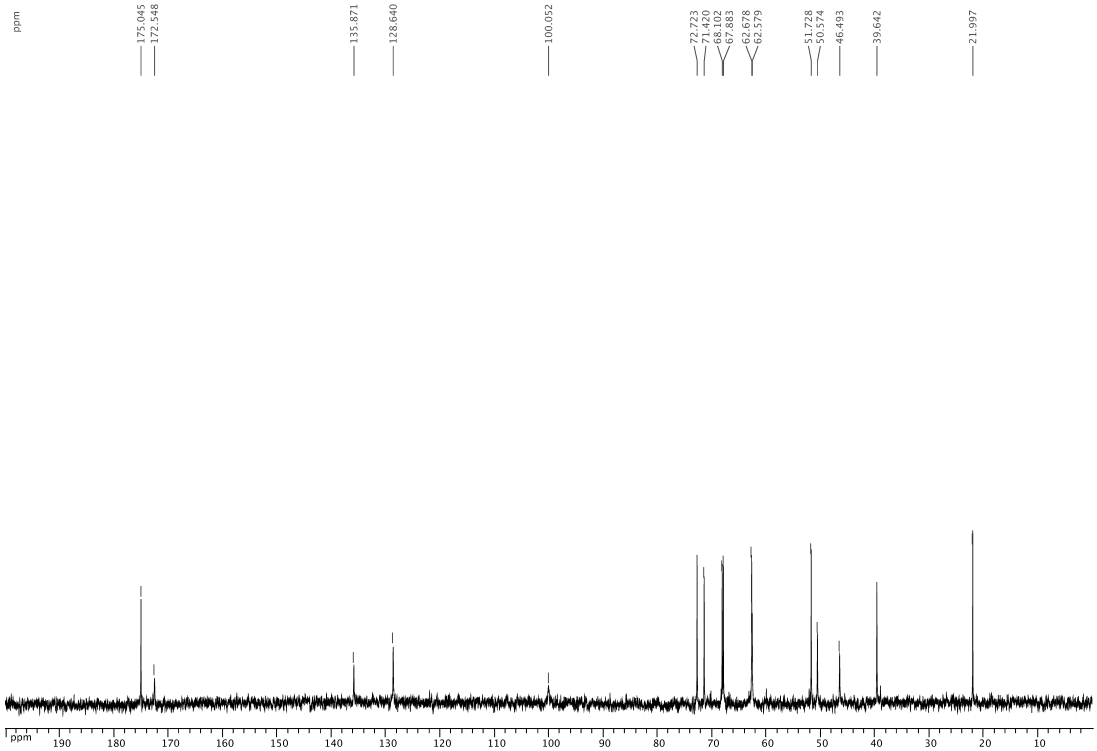


Compound **11a**

$^{1}$H NMR (400 MHz, $D_2O$)

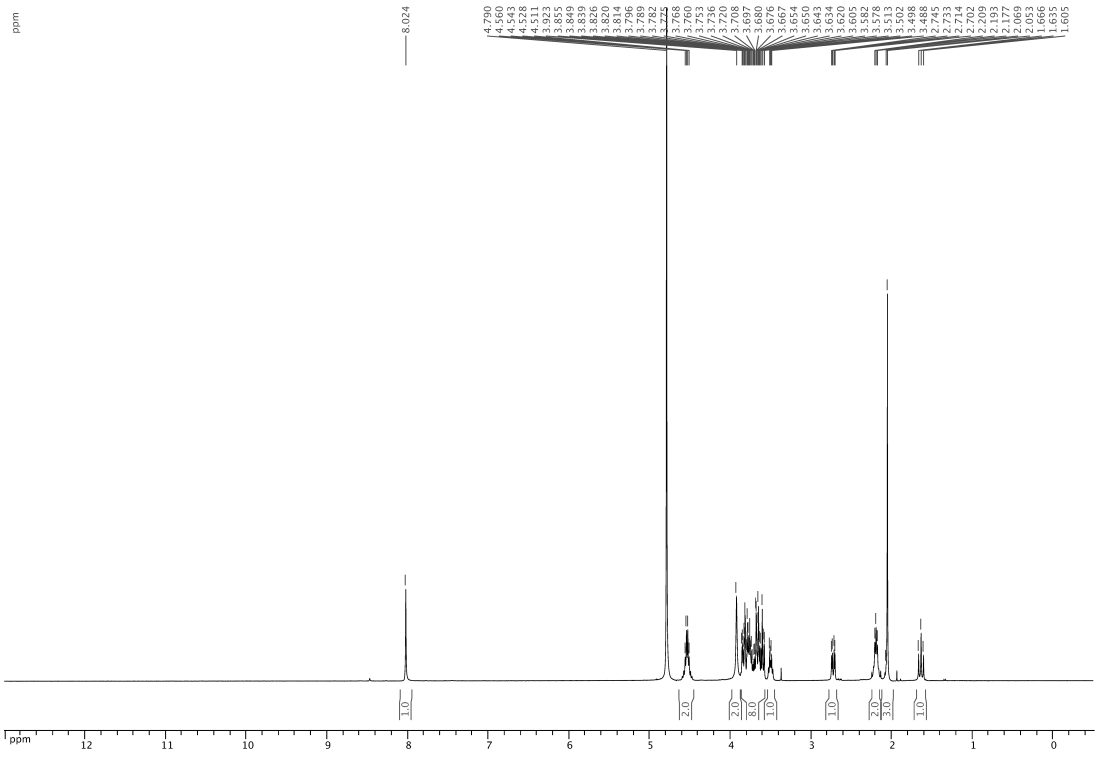


$^{13}$C NMR (100 MHz, $D_2O$)

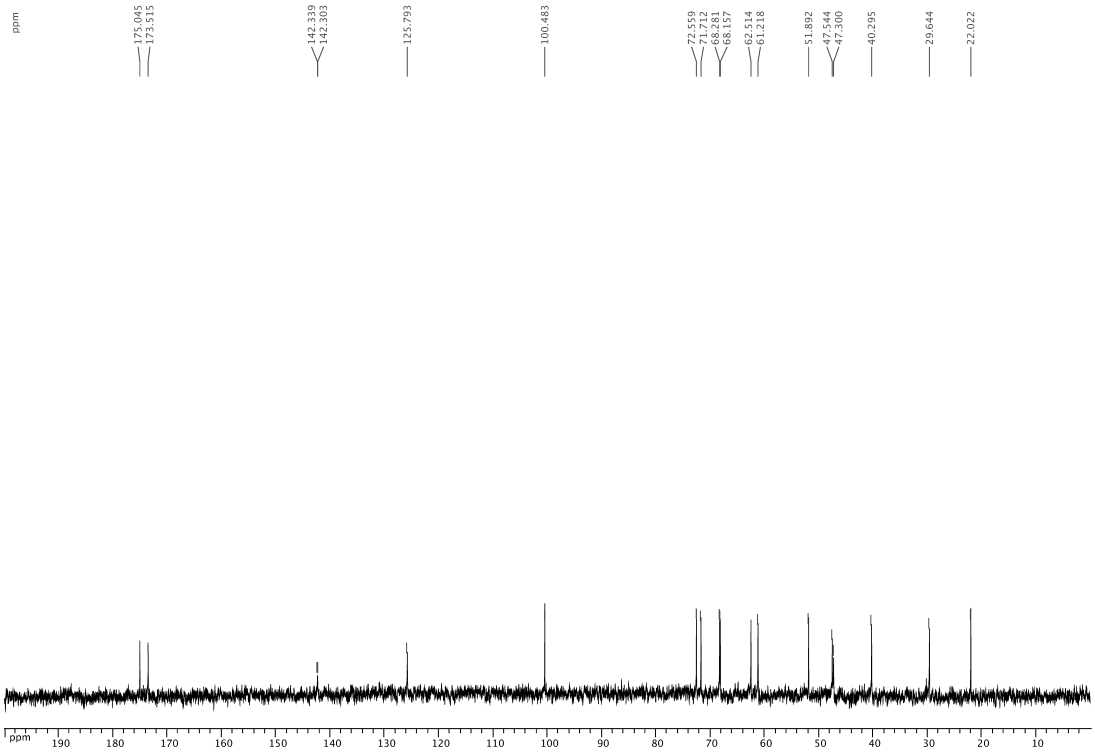


Compound **11b**

$^{1}$H NMR (400 MHz, $D_2O$)

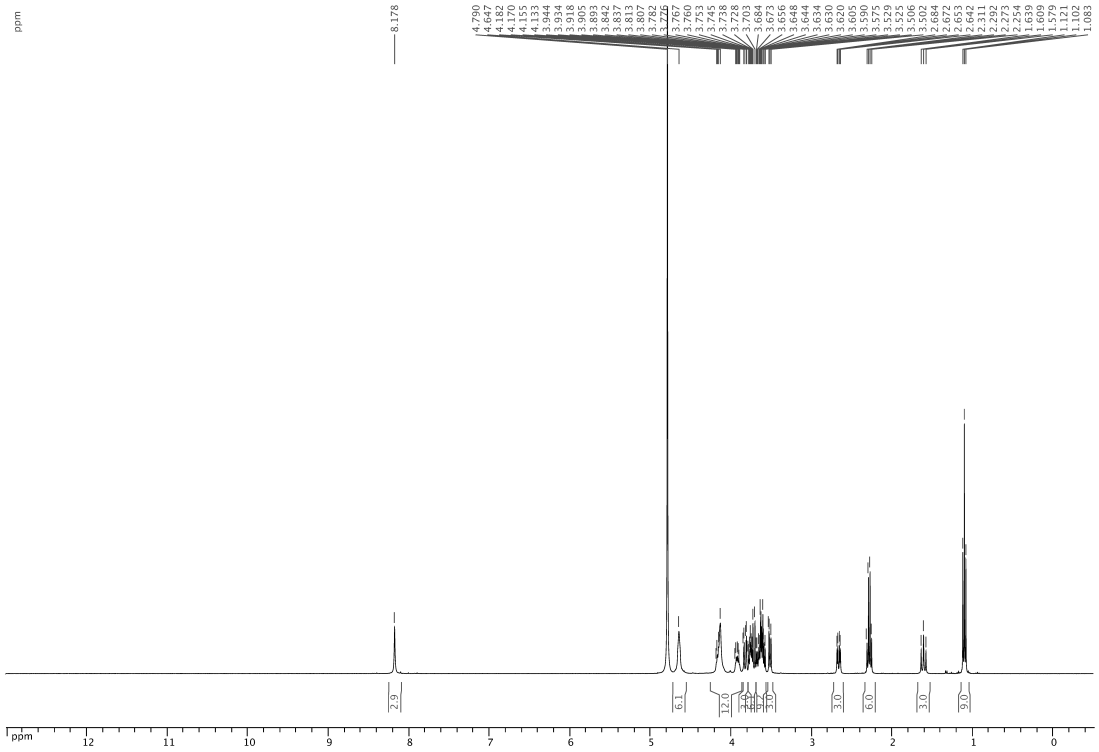



$^{13}$C NMR (100 MHz, $D_2O$)

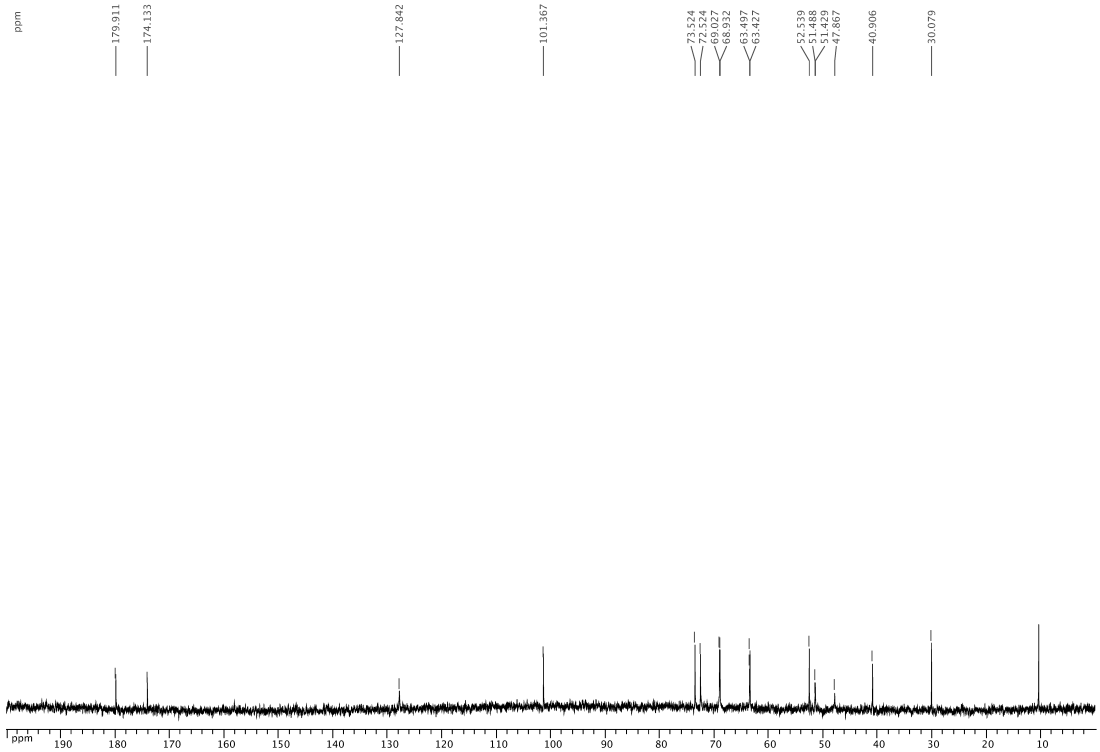


Compound **12a**

$^{1}$H NMR (400 MHz, $D_2O$)

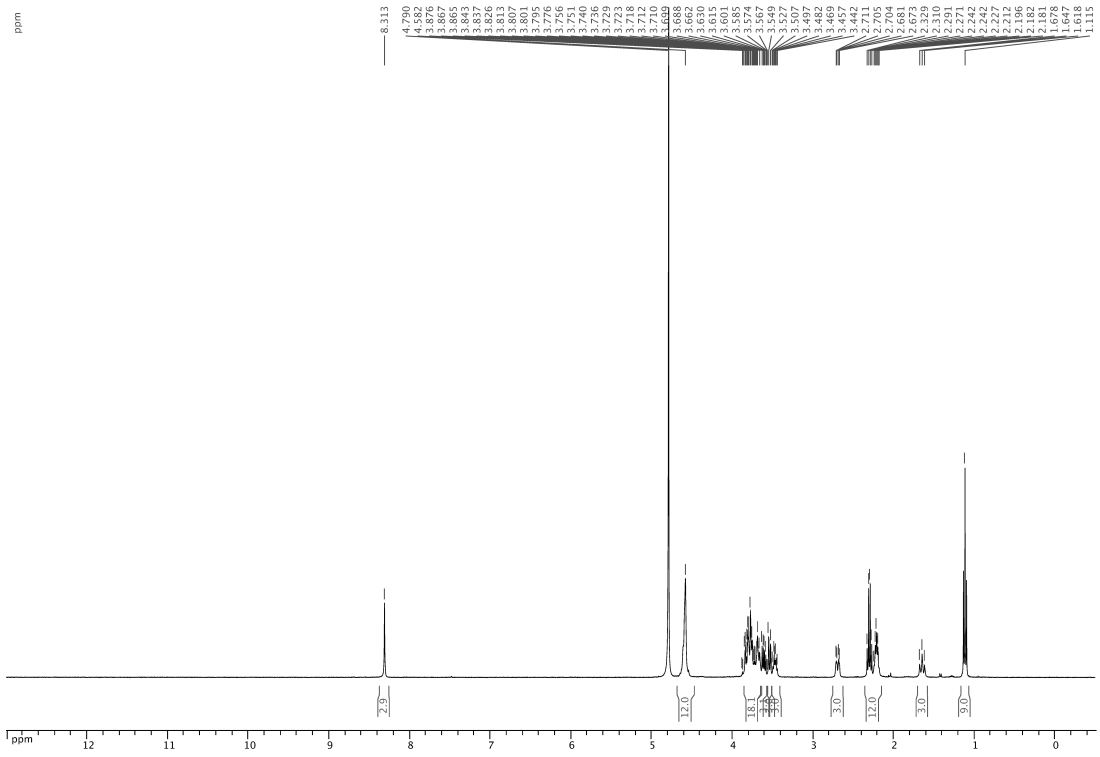


$^{13}$C NMR (100 MHz, $D_2O$)

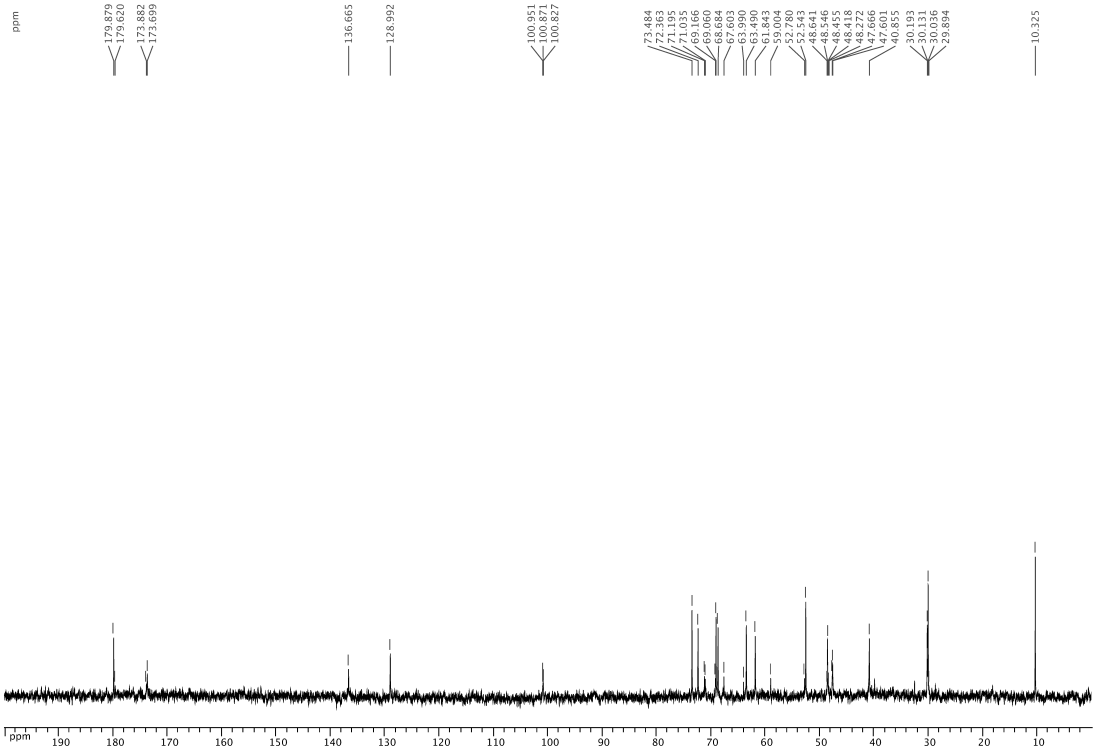


Compound **12b**

$^{1}$H NMR (400 MHz, $D_2O$)

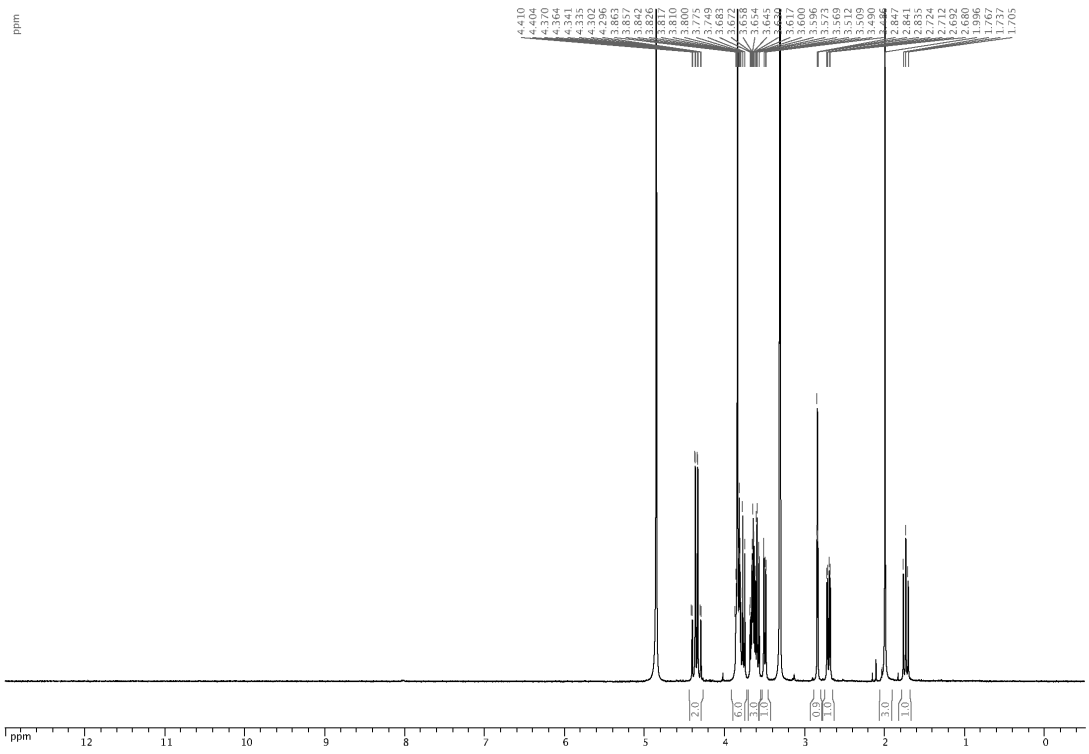


$^{13}$C NMR (100 MHz, $D_2O$)

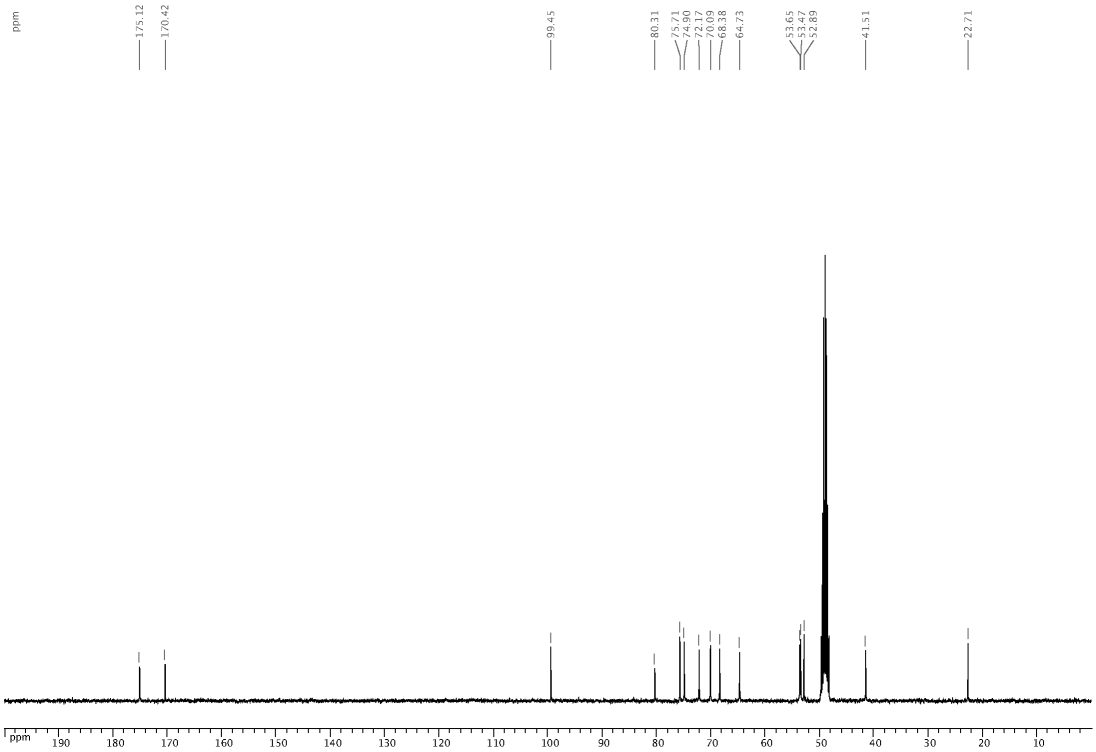


Compound **14a**

$^{1}$H NMR (400 MHz, $CD_3OD$)

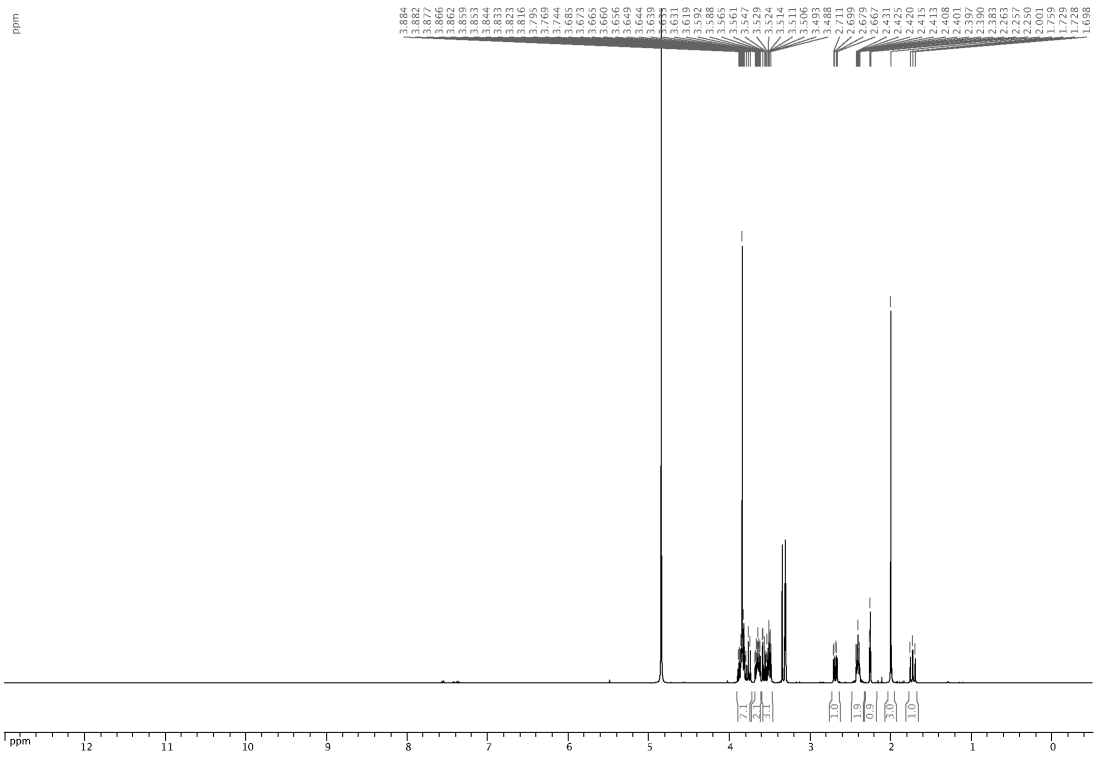


$^{13}$C NMR (100 MHz, $CD_3OD$)

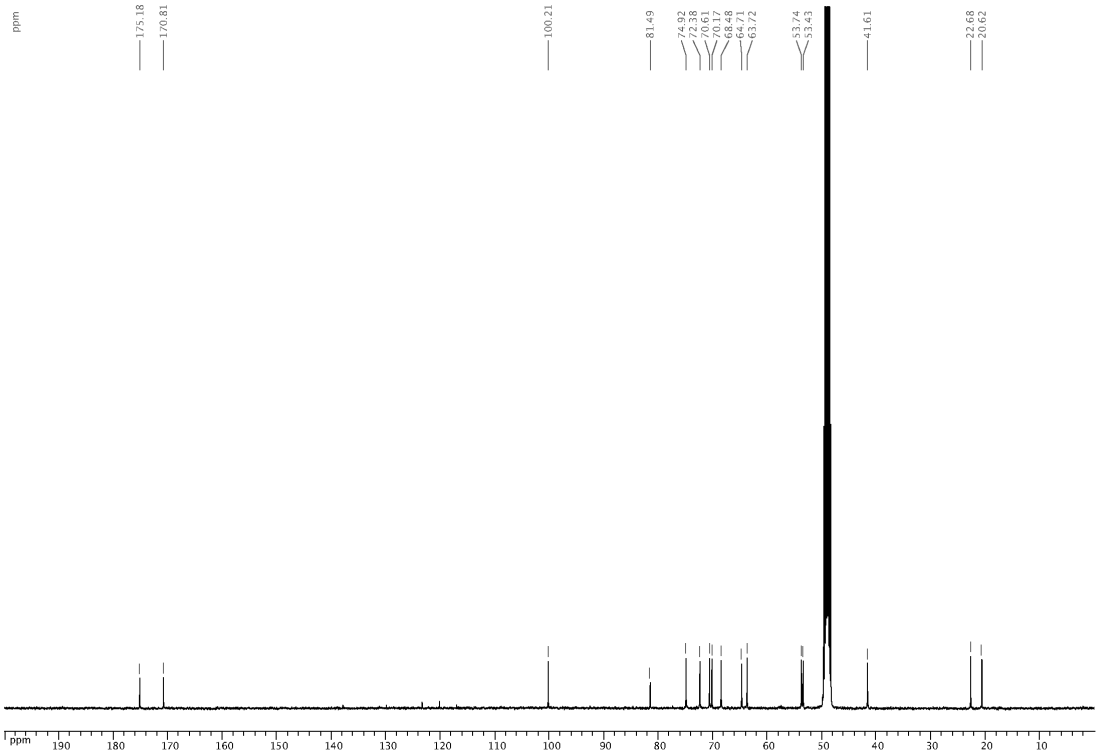


Compound **14b**

$^{1}$H NMR (400 MHz, $CD_3OD$)

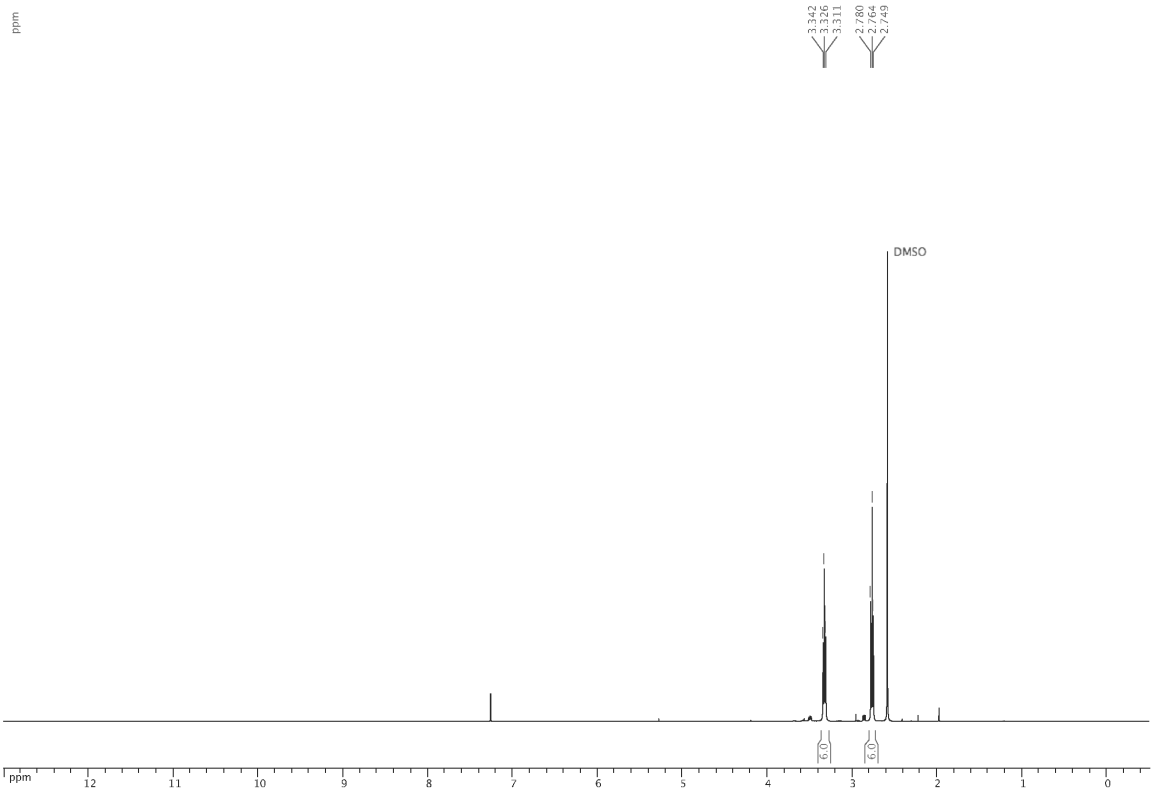


$^{13}$C NMR (100 MHz, $CD_3OD$)



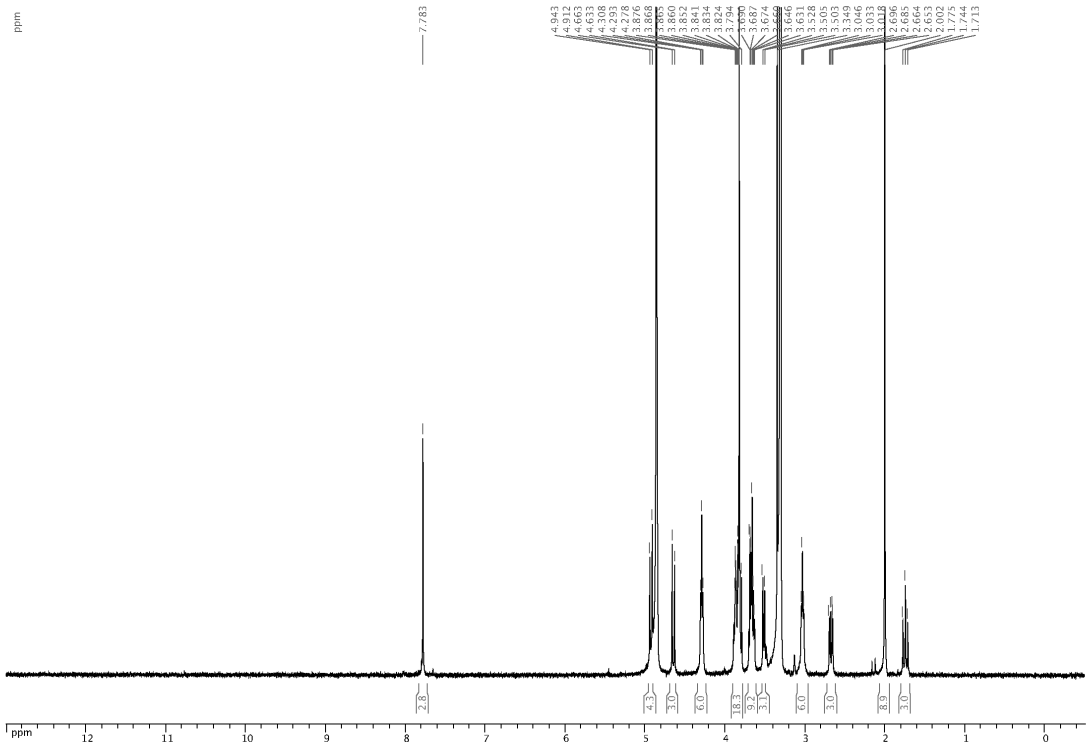
Compound **15**

$^{1}$H NMR (400 MHz, $CDCl_3$, crude spectra, directly from the reaction mixture)

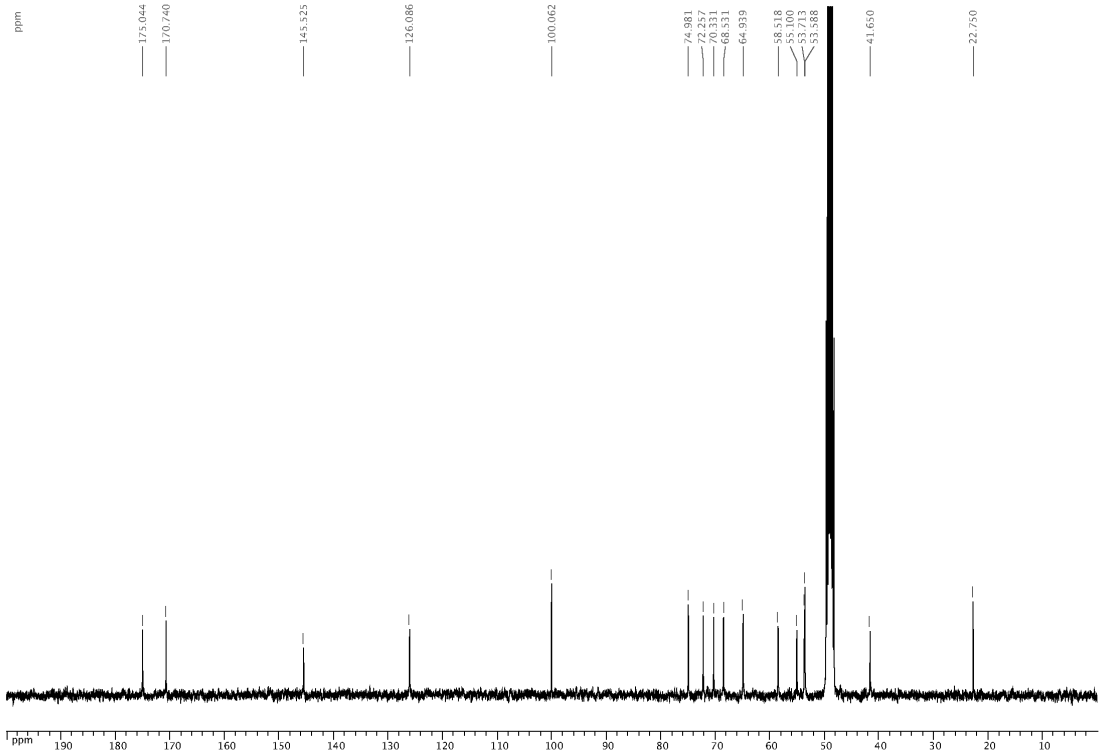


Compound **16a**

$^{1}$H NMR (400 MHz, $D_2O$)

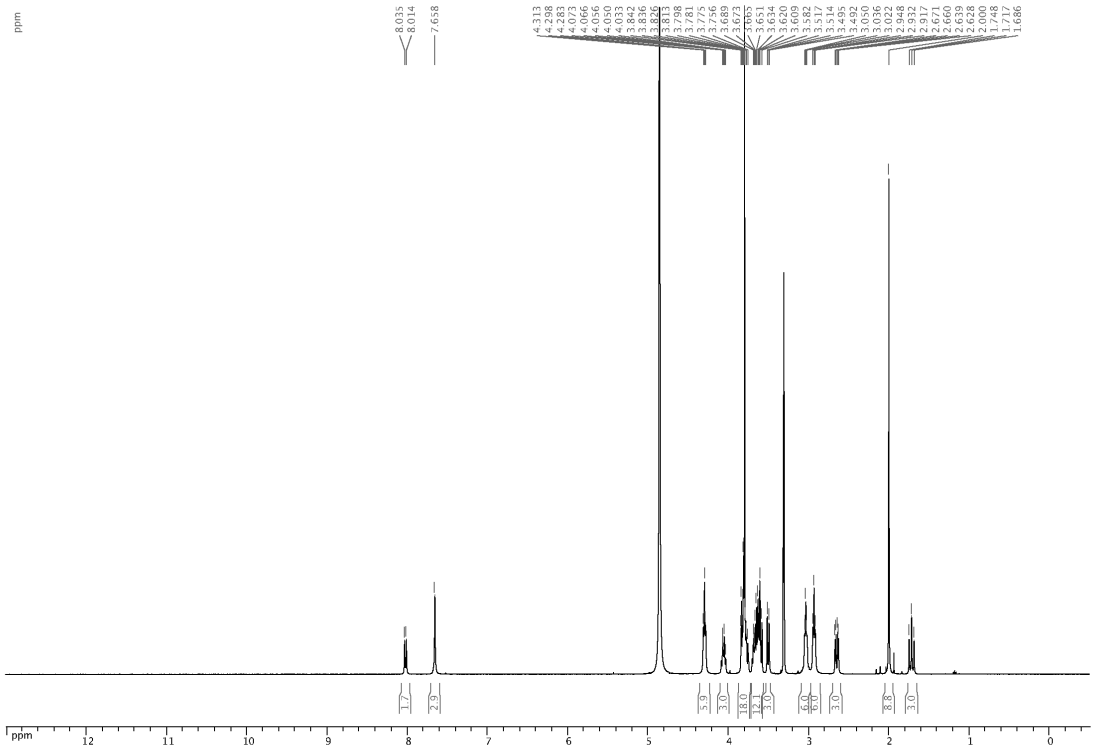


$^{13}$C NMR (100 MHz, $D_2O$)

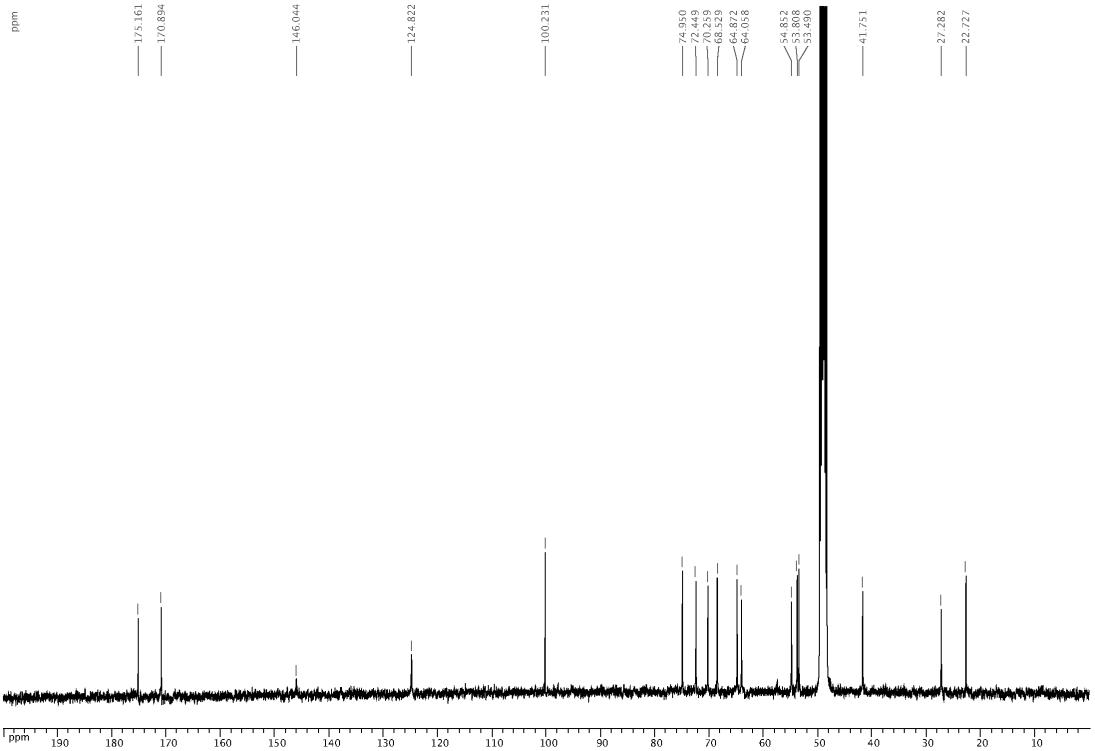


Compound **16b**

$^{1}$H NMR (400 MHz, $D_2O$)

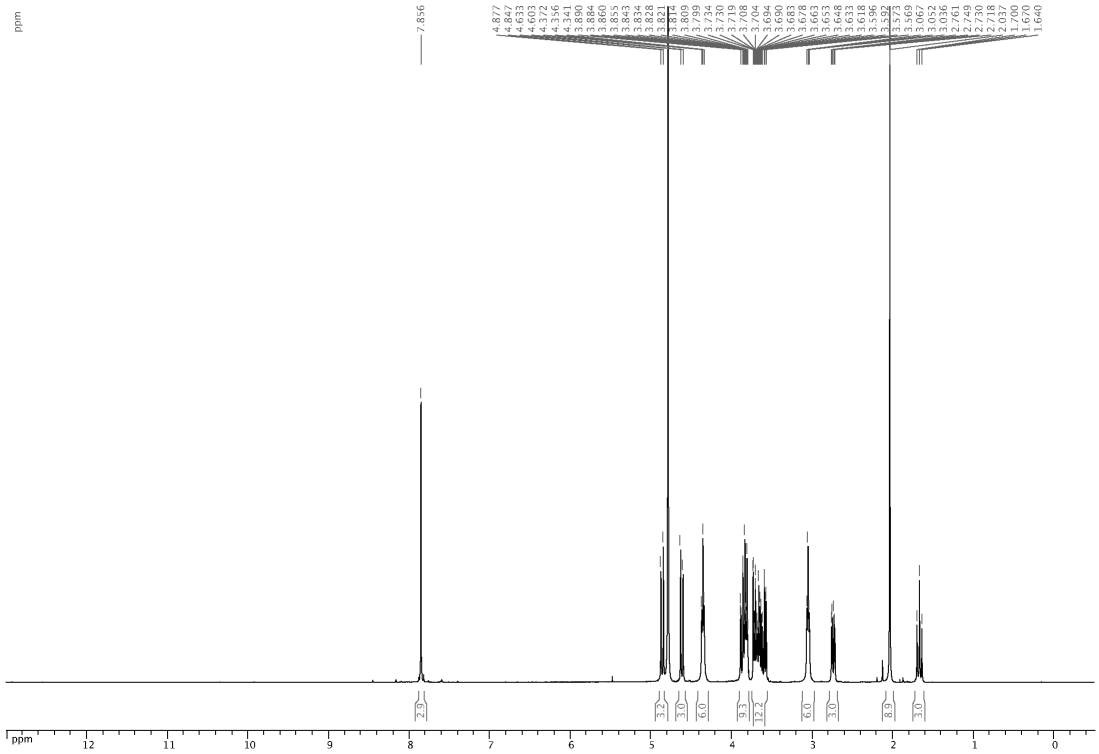


$^{13}$C NMR (100 MHz, $D_2O$)

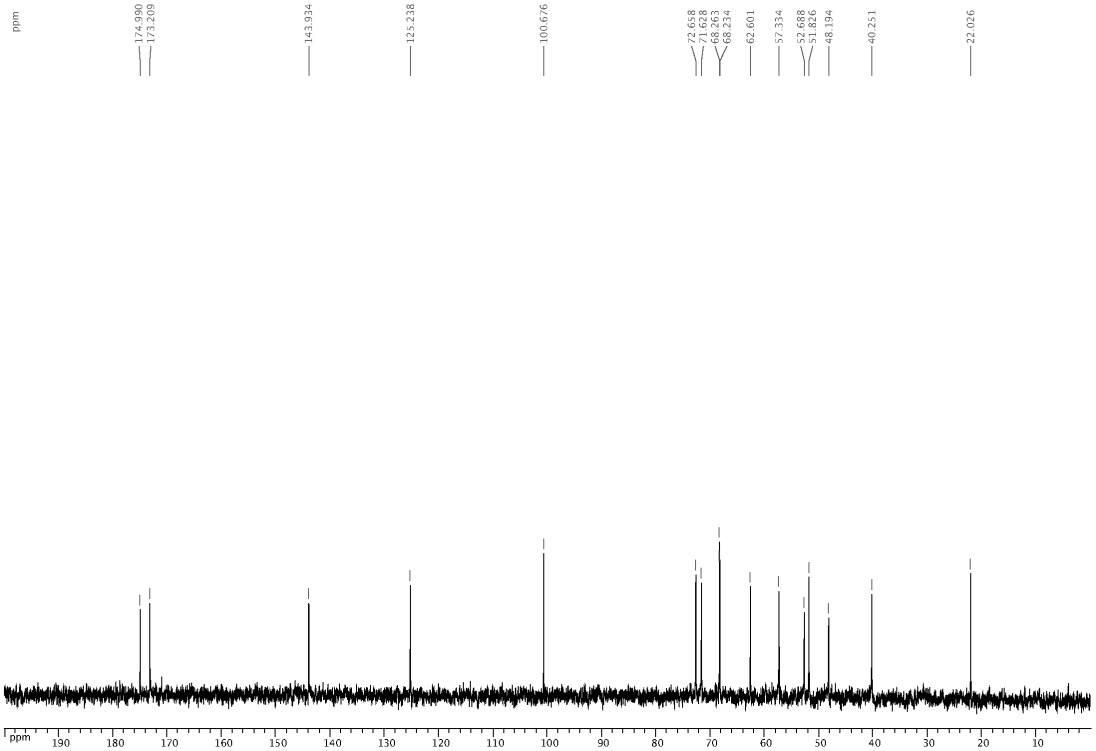


Compound **17a**

$^{1}$H NMR (400 MHz, $D_2O$)

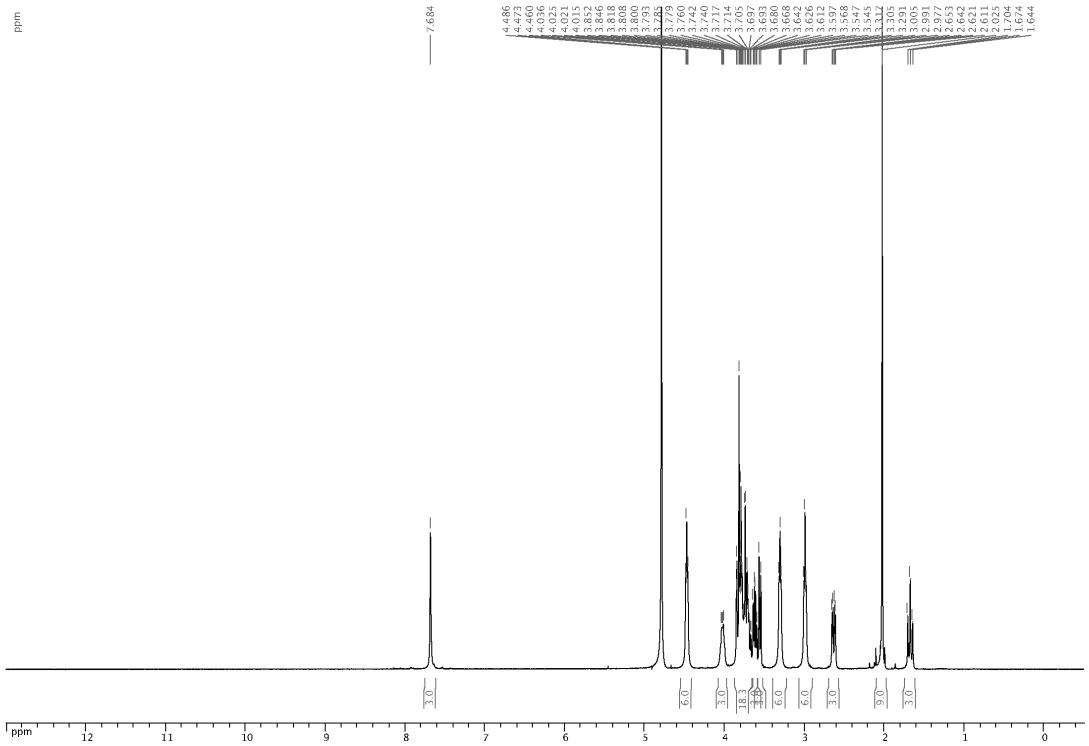


$^{13}$C NMR (100 MHz, $D_2O$)



Compound **17b**

$^{1}$H NMR (400 MHz, $D_2O$)



$^{13}$C NMR (100 MHz, $D_2O$)

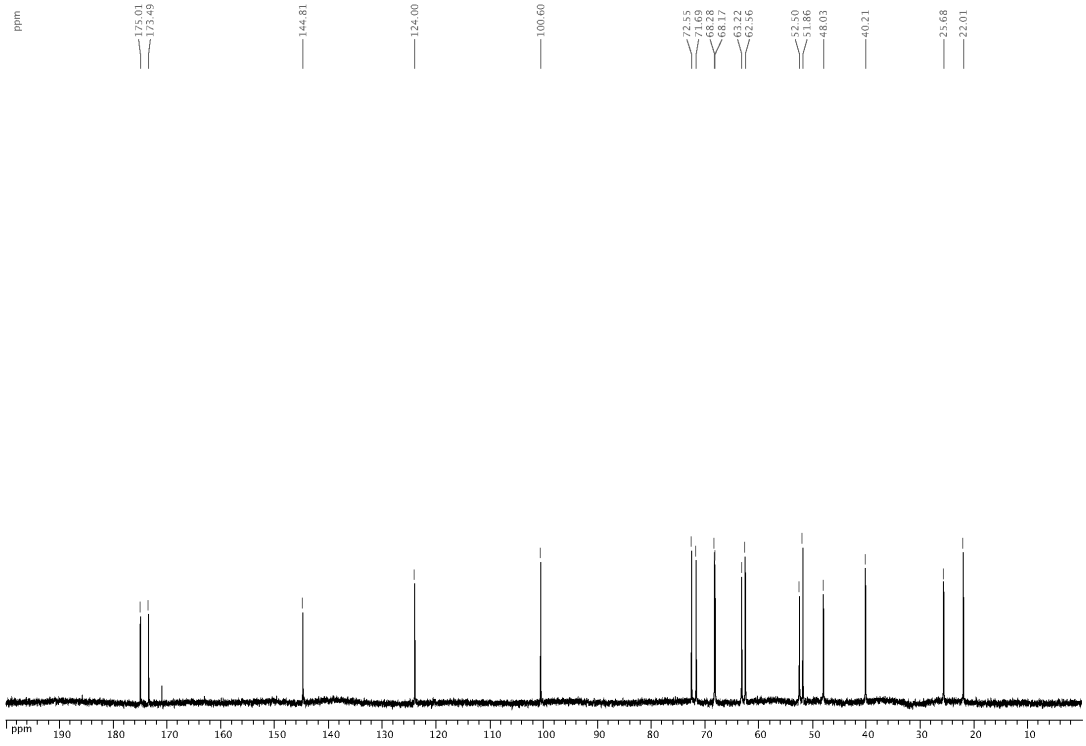


**Table S1.** Surface Plasmon Resonance summary of results.

| Compound | Binding affinity ($K_D$) | Theoretical $RU_{max}$ | Experimental $RU_{max}$ |
|---|---|---|---|
| **ME0322** | 124 μM | 350 | 273 |
| **11a** | 76 μM | 284 | 145 |
| **11b** | 69 μM | 293 | 161 |
| **17a** | 9.6 μM | 228 | 119.3 |
| **17b** | 175 μM | 235 | 79 |

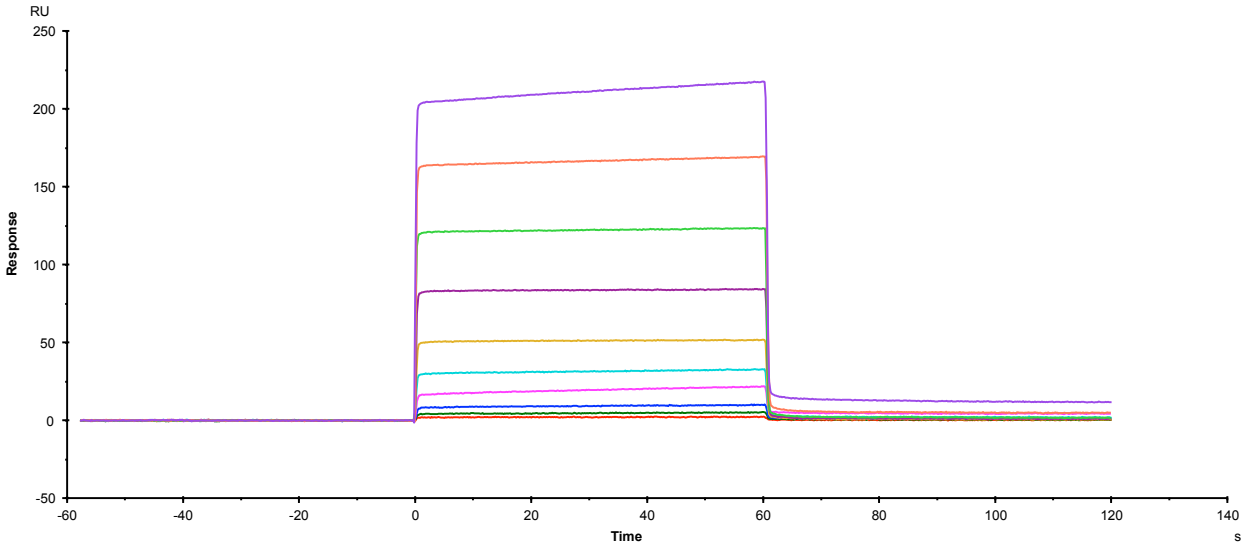



**Figure S1**. Surface Plasmon Resonance curve tracing for **ME0322**.

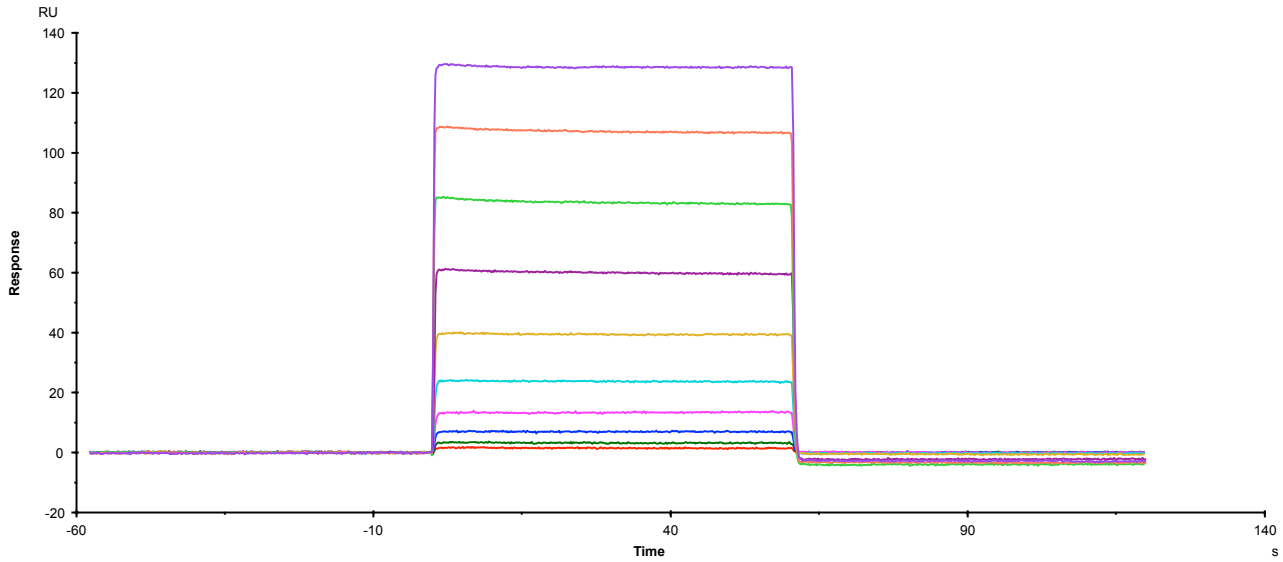



**Figure S2**. Surface Plasmon Resonance curve tracing for **11a**.

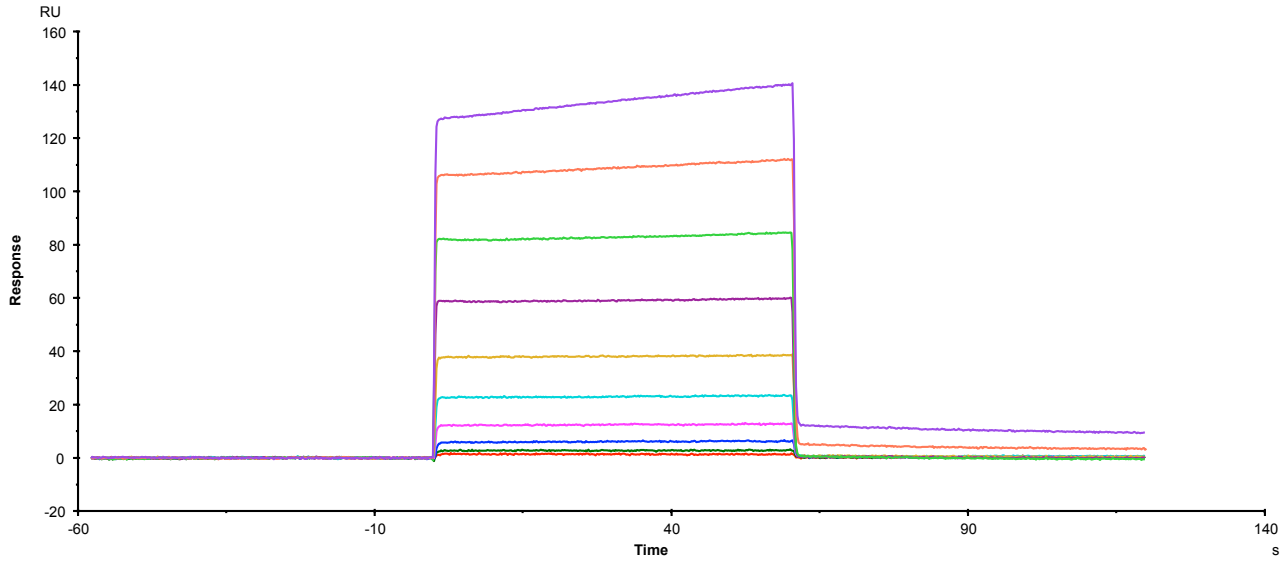



**Figure S3**. Surface Plasmon Resonance curve tracing for **11b**.

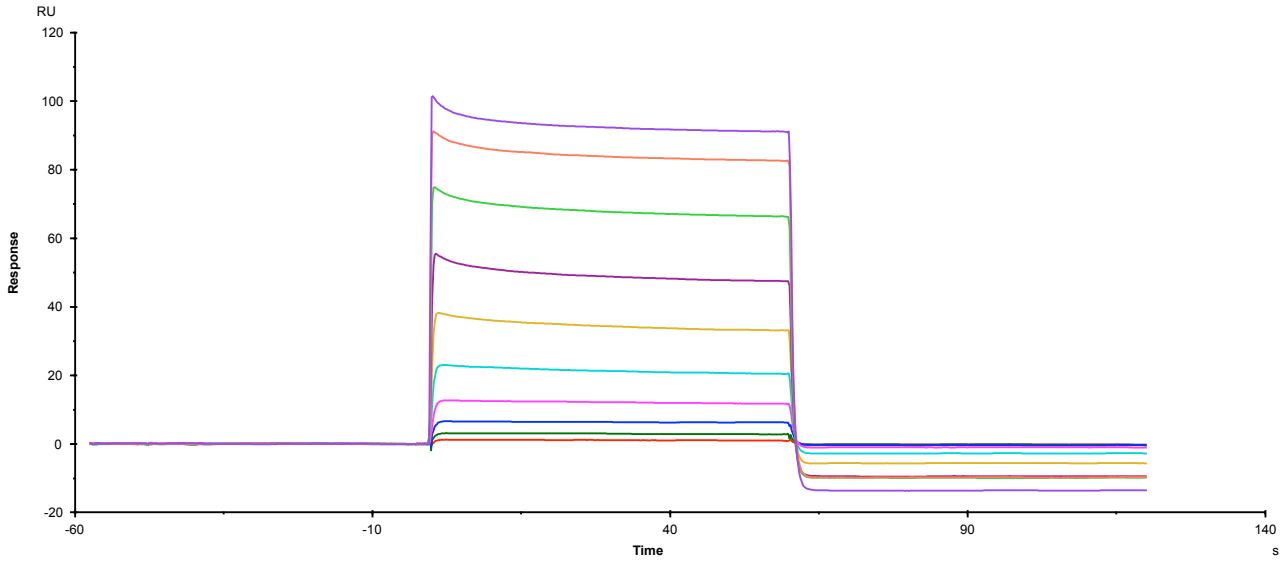



**Figure S4**. Surface Plasmon Resonance curve tracing for **17a**.

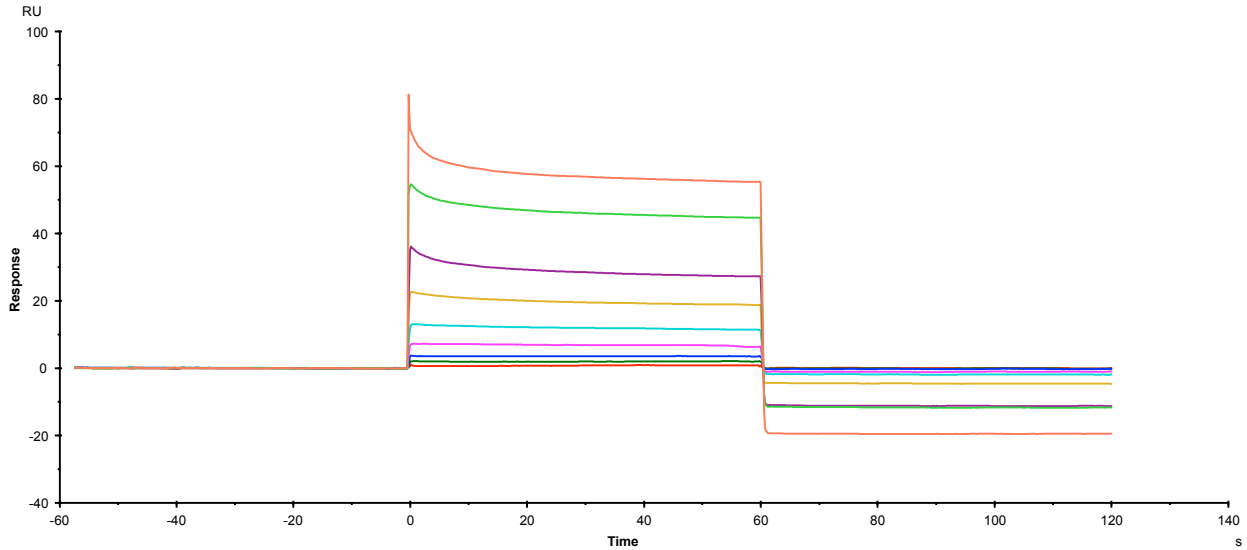



**Figure S5**. Surface Plasmon Resonance curve tracing for **17b**.

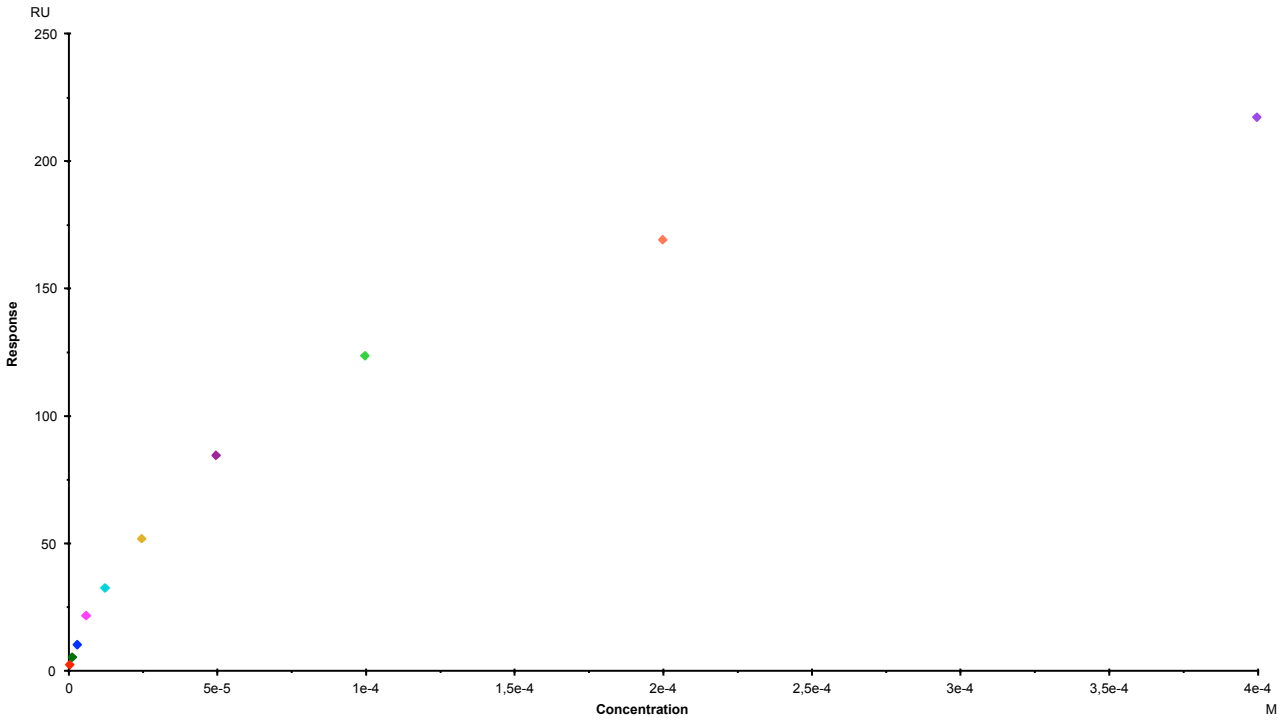



**Figure S6**. Surface Plasmon Resonance titration curve for **ME0322**.

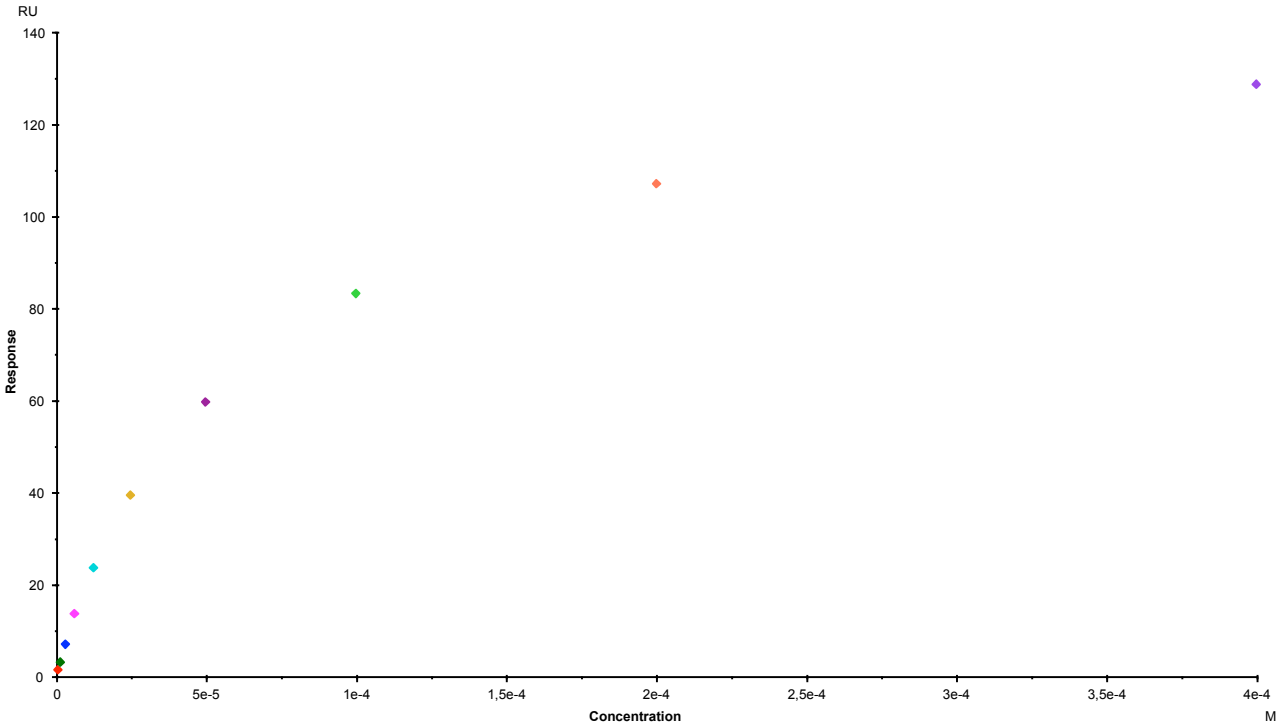



**Figure S7**. Surface Plasmon Resonance titration curve for **11a**

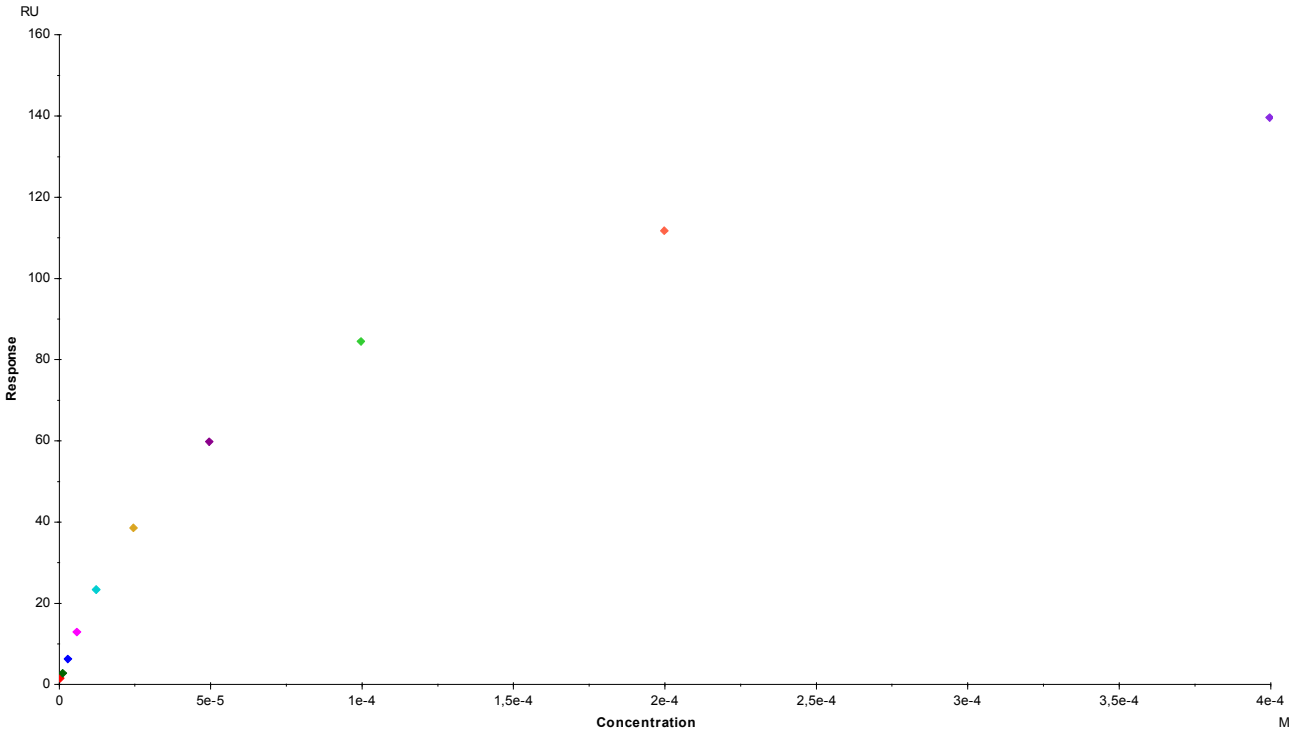



**Figure S8**. Surface Plasmon Resonance titration curve for **11b**.

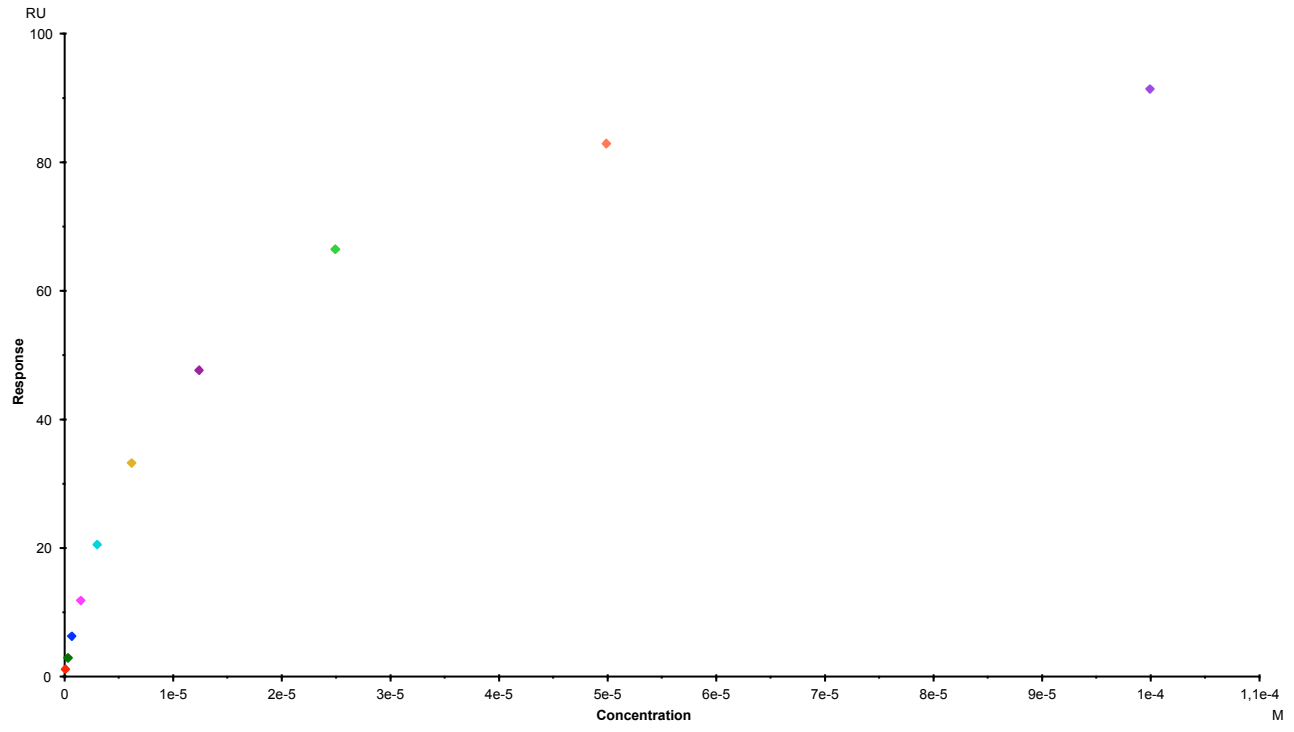



**Figure S9**. Surface Plasmon Resonance titration curve for **17a**

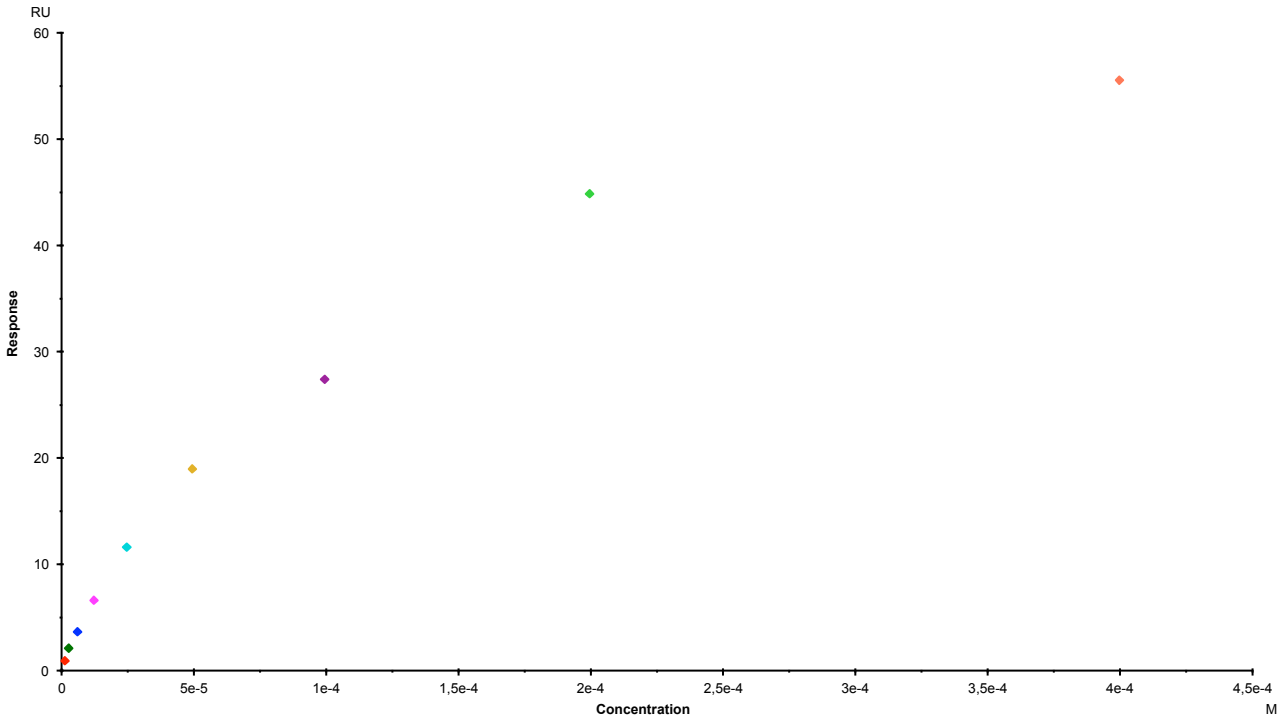



**Figure S10**. Surface Plasmon Resonance titration curve for **17b**

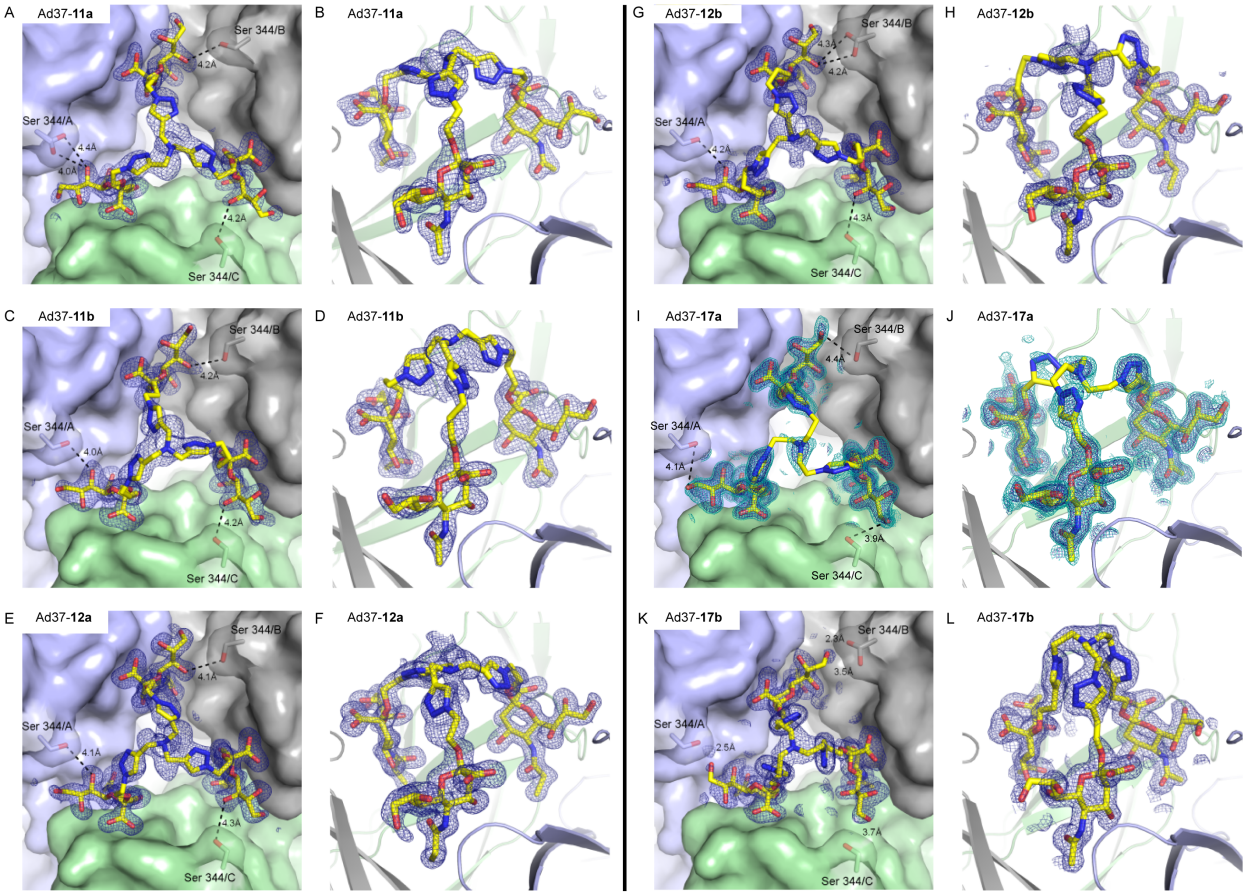



**Figure S11**. **Electron densities for the different inhibitors.** All simulated annealing Fo-Fc omit maps were calculated at 3 sigma and carved 2.4 Å around the ligand (dark blue). For **17a**, an additional map at 2 sigma shows there is ordered density for the ligand base (cyan). Serine 344 is within the van-der-Waals radius of the sialic acid moieties. Only **17b** is capable of forming hydrogen bonds with this residue.

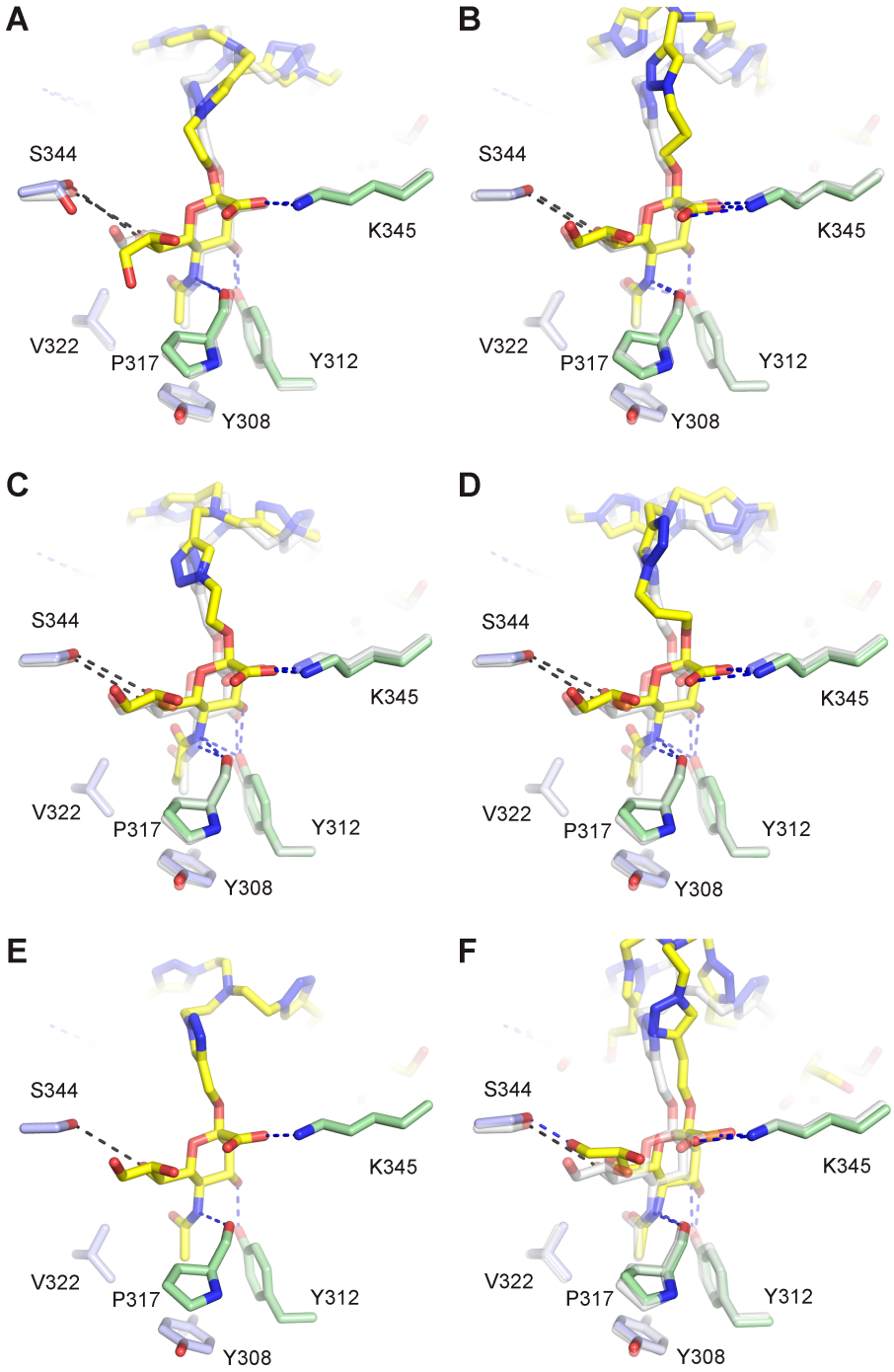



**Figure S12**. **Interactions involved in ligand binding.** All protein chains were superimposed with **17a** (grey ghost) in PyMOL. Most binding interactions are conserved for all ligands. A) **11a** B) **11b** C) **12a** D) **12b** E) **17a** F) **17b**.

**Table S2.** Data collection and refinement statistics.

| | Ad37-**11a** | Ad37-**11b** | Ad37-**12a** | Ad37-**12b** | Ad37-**17a** | Ad37-**17b** |
|---|---|---|---|---|---|---|
| **Data collection** | | | | | | |
| Beamline | X06SA (SLS) | X06SA (SLS) | X06DA (SLS) | X06DA (SLS) | X06SA (SLS) | MX-14-1 (BESSY) |
| Space group | P $2_1$ | P $2_1$ | P $2_1$ | P $2_1$ | P $2_1$ $2_1$ $2_1$ | P $2_1$ |
| Cell dimensions | | | | | | |
| *a*, *b*, *c* (Å) | 74.94, 67.18, 121.43 | 60.86, 69.91, 74.07 | 60.93, 69.28, 74.56 | 61.32, 69.68, 74.78 | 68.23, 75.62, 131.11 | 61.43, 70.32, 74.48 |
| α, β, γ (°) | 90.00, 96.81, 90.00 | 90.00, 94.62, 90.00 | 90.00, 94.92, 90.00 | 90.00, 94.43, 90.00 | 90.00, 90.00, 90.00 | 90.00, 94.47, 90.00 |
| Ad trimer / ASU | 2 | 1 | 1 | 1 | 1 | 1 |
| Resolution (Å) | 40-2.0 (2.05-2.00) | 40-1.90 (1.95-1.90) | 50-1.50 (1.54-1.50) | 50-1.50 (1.54-1.50) | 50-1.41 (1.49-1.41) | 50-1.60 (1.69-1.60) |
| $CC_{1/2}$ (%) | 98.6 (75.7) | 99.8 (85.1) | 99.9 (62.8) | 99.9 (57.9) | 99.8 (84.3) | 99.9 (71.5) |
| I / (σI) | 6.1 (2.1) | 9.9 (1.8) | 15.1 (1.7) | 14.6 (1.3) | 16.5 (1.7) | 14.9 (2.0) |
| Completeness (%) | 99.3 (99.1) | 98.6 (96.2) | 99.4 (96.0) | 99.5 (95.7) | 99.6 (98.2) | 99.4 (97.2) |
| Redundancy | 3.0 (3.0) | 2.9 (2.3) | 3.7 (3.5) | 3.7 (3.5) | 12.75 (12.63) | 3.73 (3.57) |
| Wilson *B*-factor (Å$^2$) | 30,4 | 36,7 | 27,3 | 27,5 | 30,7 | 25,9 |
| **Refinement** | | | | | | |
| Resolution (Å) | 37.26-2.00 | 37.91-1.90 | 45.66-1.50 | 45.96-1.50 | 47.3-1.41 | 45.54-1.60 |
| No. of unique reflections | 79104 | 47283 | 96426 | 97973 | 131319 | 83278 |
| $R_{work}$ / $R_{free}$ | 16.1 / 20.0 | 17.7 / 22.2 | 12.8 / 16.7 | 13.4 / 18.5 | 14.2 / 17.2 | 15.7 / 17.8 |
| No. of non-H atoms | | | | | | |
| Protein chain a / b / c | 1466 / 1463 / 1459 | 1464 / 1463 / 1476 | 1454 / 1461 / 1476 | 1471 / 1453 / 1474 | 1457 / 1463 / 1447 | 1465 / 1466 / 1470 |
| Protein chain e / f / g | 1458 / 1448 / 1472 | - | - | - | - | |
| Inhibitor | 88 | 91 | 91 | 94 | 88 | 91 |
| Water | 822 | 445 | 643 | 621 | 540 | 654 |
| *B*-factors (Å$^2$) | | | | | | |
| Protein chain a / b / c | 24.7 / 25.6 / 30.2 | 39.2 / 40.3 / 32.0 | 28.1 / 27.6 / 20.2 | 26.0 / 27.2 / 20.5 | 33.4 / 32.8 / 28.4 | 19.1 / 22.4 / 17.6 |
| Protein chain e / f / g | 26.3 / 28.9 / 23.9 | - | - | - | - | |
| Inhibitor | 37,5 | 46,6 | 24,8 | 30,9 | 34,3 | 19,4 |
| Water | 35,5 | 40,2 | 35,2 | 33,2 | 44,8 | 30,4 |
| R.m.s. deviations | | | | | | |
| Bond lengths (Å) | 0,011 | 0,014 | 0,013 | 0,015 | 0,015 | 0,009 |
| Bond angles (°) | 1,433 | 1,426 | 1,461 | 1,446 | 1,611 | 1,227 |