Potent Ligands for Prokaryotic UDP-Galactopyranose Mutase That Exploit an Enzyme Subsite

Emily C. Dykhuizen[1] and Laura L. Kiessling[1,2]

Departments of Chemistry[1] and Biochemistry[2], University of Wisconsin–Madison, Madison, Wisconsin, 53706

Kiessling@chem.wisc.edu

Supporting Information

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General Synthetic Procedures

All reagents were purchased from Sigma-Aldrich Co., except for octanolamine and decanolamine, which were purchased from TCI America. All compounds were used as received. Methanol (MeOH) was distilled from magnesium, methylene chloride (CH_2Cl_2) and diisopropylethylamine (DIEA) were distilled from calcium hydride, and dimethyl formamide (DMF) was used as biotech grade (Sigma-Aldrich Co.).

Flash chromatography was performed using silica gel 60, 230-450 mesh (Sorbent Technologies). Analytical thin-layer chromatography (TLC) was carried out on EM Science TLC plates precoated with silica gel 60 F254 (250-μm layer thickness). Visualization of TLC was accomplished using a UV lamp.

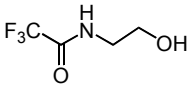
^{1}H NMR and ^{13}C NMR specta were obtained at 300 or 500 MHz and 75 or 125 MHz, respectively, using a or Varian MercuryPlus 300 or a Varian UNITY 500 spectrometer. Chemical shifts are reported relative to residual solvent signals ($CDCl_3$): ^{1}H: δ 7.27, ^{13}C: δ 77.23; (CD_3OD): ^{1}H: δ 3.31, ^{13}C: δ 49.15; (DMF-d_7): ^{1}H: δ 2.92, ^{13}C: δ 34.89; (D_2O): ^{1}H: δ 4.79. ^{1}H NMR data are assumed to be first order with apparent doublets and triplets reported as d and t, respectively. Multiplets are reported as m and resonances that appear broad are designated as br s.

High-resolution electrospray ionization mass spectra (HRESI-MS) were obtained on a Micromass LCT. LC-MS (ESI) were obtained using a Shimadzu LCMS-2010 (Columbia, MD) equipped with two pumps (LC-10Advp), controller (SCL-10Avp), autoinjector (SIL-10Advp), UV diode array detector (SPD- M10Avp), and single quadrupole analyzer (by electrospray ionization, ESI). The LC-MS is interfaced with a PC running the Shimadzu LCMS solution software package (Version 2.04 Su2-H2). A Supelco (Bellefonte, PA) 15 cm x 2.1 mm C-18 wide pore reverse phase column was used for all LC-MS analyses. Standard reverse phase HPLC conditions were used as follows: flow rate = 200 mL/min; mobile phase A = 0.1% formic acid; mobile phase B = 0.1% formic acid in acetonitrile, 50-95% B over 7 min. UV spectra were recorded using an HP-8452 UV-Vis spectrometer running UV Visible Chemstation software. High performance liquid chromatography (HPLC) was performed on a C18 reverse phase column using water (A) and acetonitrile (B) (both buffered with 0.02% trifluoroacetic acid) as the elution solvents at 10 mL/min. Compound elution was detected by UV absorbance at range 200-600 nm.

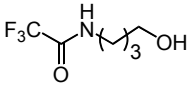
Cation-exchange resin Dowex 50WX8-200 (H+ form, strongly acidic) was purchased from Aldrich and converted to the appropriate salt form prior to use. Uridine 5'-monophosphate (5'-UMP) disodium salt was purchased from Sigma and converted to the triethylammonium salt (1.4 eq by ^{1}H NMR) prior to coupling reactions by stirring with Dowex 50WX8-200 (NEt_3H^+ form) overnight. The resin was removed by filtration and washed with H_2O. Combined filtrates were lyophilized to produce the UMP-Et_3NH^+ salt as a fluffy white solid.

General Procedure I: Formation of the trifluoracetamide.

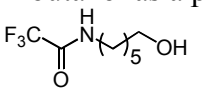
A solution of amino alcohol (1.0 eq) and triethylamine (2.5 eq) in MeOH was cooled to 0 °C. Trifluoroacetic anhydride (1.4 eq) was added dropwise under an argon atmosphere and the reaction was allowed to warm to room-temperature. The solution was stirred 4 h, concentrated, and the product was purified by silica gel chromatography to afford the trifluoroacetamide.



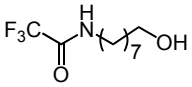
2-Trifluoroacetamido-1-ethanol: Following general procedure I, ethanolamine (2.0 g, 32.74 mmol) was combined with triethylamine (11.4 mL, 81.86 mmol) and trifluoroacetic anhydride (6.47 mL, 45.84 mmol) in MeOH (30 mL). Purification by silica gel chromatography (2:3 hexanes/EtOAc) yielded 4.86 g (94%) of 2-trifluoroacetamido-1-ethanol as a white solid.[1]



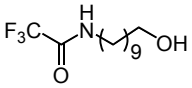
4-Trifluoroacetamido-1-butanol: Following general procedure I, butanolamine (2.0 g, 22.44 mmol) was combined with triethylamine (7.82 mL, 56.09 mmol) and trifluoroacetic anhydride (4.44 mL, 24.10 mmol) in MeOH (20 mL). Purification by silica gel chromatography (2:3 hexanes/EtOAc) yielded 4.03 g (97%) of 4-trifluoroacetamido-1-butanol as a pale yellow oil.[1]



6-Trifluoroacetamido-1-hexanol: Following general procedure I, hexanolamine (2.0 g, 17.06 mmol) was combined with triethylamine (5.95 mL, 42.67 mmol) and trifluoroacetic anhydride (3.37 mL, 23.89 mmol) in MeOH (15 mL). Purification by silica gel chromatography (2:3 hexanes/EtOAc) yielded 3.35 g (92%) of 6-trifluoroacetamido-1-hexanol as a white solid.[2]



8-Trifluoroacetamido-1-octanol: Following general procedure I, octanolamine (2.5 g, 17.21 mmol) was combined with triethylamine (6.0 mL, 43.04 mmol) and trifluoroacetic anhydride (3.4 mL, 24.10 mmol) in MeOH (15 mL). Purification by silica gel chromatography (2:3 hexanes/EtOAc) yielded 4.09 g (98%) of 8-trifluoroacetamido-1-octanol as a white solid. ^{1}H (300 MHz, CD_3OD): δ 3.61 (t, 2H, J = 6.6 Hz), 3.34 (t, 2H, J = 7.2), 1.63 (m, 4H), 1.42 (m, 8H); ^{13}C (75 mHz, CD_3OD): δ 158.61 (q, J = 81.5 Hz), 117.58 (q, J = 284.6 Hz), 63.02, 40.79, 33.88, 30.51, 30.48, 30.30, 29.85, 27.79, 28.88; ESI-MS calcd for $C_{10}H_{18}F_3NO_2$ $[M + Na]^+$: 264.1197 found 264.1186.



10-Trifluoroacetamido-1-decanol: Following general procedure I, decanolamine (0.50 g, 2.89 mmol) was combined with triethylamine (1.0 mL, 7.21 mmol) and trifluoroacetic anhydride (0.57 mL, 4.04 mmol) in MeOH (3 mL). Purification by silica gel chromatography (2:3 hexanes/EtOAc) yielded 0.722 g (99%) of 10-trifluoroacetamido-1-decanol as a white solid. ^{1}H (300 MHz, CD_3OD): δ 3.55 (t, 2H, J = 5.4 Hz), 3.28 (t, 2H, J = 7.2 Hz), 1.55 (m, 4H), 1.35 (m, 10H). ^{13}C (75 mHz, CD_3OD): δ 82.99, 40.74, 33.83, 30.62, 30.54, 30.25, 29.78, 27.77, 26.92; ESI-MS calcd for $C_{12}H_{22}F_3NO_2$ $[M + Na]^+$: 292.1500 found 292.1507.

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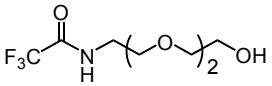
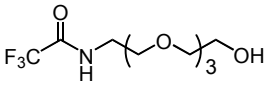
[1] Lokhov, S. G.; Podyminogin, M. A.; Sergeev, D. S.; Silnikov, V. N.; Kutyavin, I. V.; Shishkin, G. V.; Zarytova, V. P. *Bioconjugate Chem.* **1992**, *3*, 414-419.

[2] Vincent, S. P.; Gastinel, L. N. *Carbohydr. Res*. **2002**, *337*, 1039-1042.



8-Trifluoroacetamido-3,6-dioxa-1-octanol: 8-azido-3,6-dioxa-1-octanol[3] (0.7 g, 4.0 mmol) was synthesized from triethylene glycol following the reported procedure.[4] The azide was combined with Pd/C (150 mg) in MeOH (8.0 mL) and stirred 12 h under H_2 (1 atm). The suspension was filtered over celite, and the filtrate was concentrated to afford the amine (0.590 g, 4.0 mmol) in quantitative yields.[5] Following general procedure I, 2-[2-(2-aminoethoxy)ethoxy]ethanol (0.590 g, 3.95 mmol) was combined with triethylamine (1.378 mL, 9.89 mmol) and trifluoroacetic anhydride (0.782 mL, 5.54 mmol) in MeOH (5 mL). Purification by silica gel chromatography (EtOAc) yielded 0.576 g (60%) of 8-trifluoroacetamido-3,6-dioxa-1-octanol as a white solid. ^{1}H (300 MHz, CD_3OD): δ 7.87 (br s, 1H), 3.73 (m, 2H), 3.68-3.60 (m, 10H), 3.55 (t, 2H, J = 4.5 Hz), 3.32 (br s, 1H); ^{13}C (75 mHz, CD_3OD): δ 157.6 (q, J = 36.6 Hz), 116.1 (q, J = 286.1 Hz), 72.68, 70.35, 70.24, 68.90, 61.52, 39.84; ESI-MS calcd for $C_8H_{14}F_3NO_4$ $[M - H]^-$: 244.0797 found 244.0795.



11-Trifluoroacetamido-3,6,9-trioxa-1-undecanol: 11-azido-3,6,9-trioxa-1-undecane (0.6 g, 2.7 mmol) was synthesized from tetraethylene glycol following the reported procedure.[4] The azide was combined with Pd/C (150 mg) in MeOH (7.0 mL) and stirred 12 h under H_2 (1 atm). The suspension was filtered over celite and the filtrate was concentrated to afford the amine (0.520 g, 2.7 mmol) in quantitative yields.[6]{Xie, 2005 #12} Following general procedure I, 2-[2-(2-[2-aminoethoxy]ethoxy)ethoxy]ethanol (0.50 g, 2.59 mmol) was combined with triethylamine (0.902 mL, 6.48 mmol) and trifluoroacetic anhydride (0.51 mL, 3.62 mmol) in MeOH (3 mL). Purification by silica gel chromatography (EtOAc) yielded 0.343 g (46%) of 11-trifluoroacetamido-3,6,9-trioxa-1-undecanol as a white solid. ^{1}H (300 MHz, CD_3OD): δ 3.69 (t, 2H, J = 3.9 Hz), 3.64 (m, 12H), 3.57 (t, 2H, J = 3.5 Hz). ^{13}C (75 mHz, CD_3OD): δ 157.7 (q, J = 37.1 Hz), 118.1 (q, J = 285.8 Hz), 72.56. 70.78, 70.46, 70.15, 69.82, 69.56, 61.34, 39.95; ESI-MS calcd for $C_{10}H_{18}F_3NO_5$ $[M - H]^-$: 288.1059 found 288.1060.

General Procedure II: Coupling to dibenzyl phosphate.
Dibenzyl phosphate (1.5 eq) was dissolved in DMF and CH_2Cl_2, and the resulting solution was cooled to 0 ºC. Oxalyl chloride (3.0 eq) was added dropwise under an argon atmosphere. The solution was allowed to warm to room-temperature, and was stirred for 1 h. The solvent was removed in vacuo and the remaining material was azeotroped with toluene. The resulting viscous liquid was dissolved in CH_2Cl_2 and added dropwise to a flask containing the trifluoroacetamide and 4 Å molecular sieves (ca. 5-10) in pyridine at 0 ºC. The solution was stirred under an argon atmosphere for 1 h at 0 ºC and then for 3 h at room-temperature. The solvent was removed in vacuo and the product was purified by silica gel chromatography (1:1→1:2 Hexane/EtOAc) to afford the phosphotriester.

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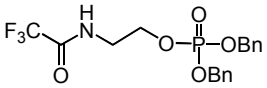
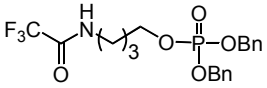
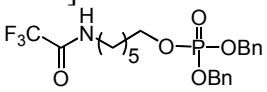
[3] Amvamzollo, P. H.; Sinay, P. *Carbohydr. Res*. **1986**, *150*, 199-212.
[4] Bertozzi, C. R.; Bednarski, M. D. *J. Org. Chem*. **1991**, *56*, 4326-4329.
[5] Sato, H.; Hayashi, E.; Yamada, N.; Yatagai, M.; Takahara, Y. *Bioconjugate Chem*. **2001**, *12*, 701-710.
[6] Xie, H. Z.; Braha, O.; Gu, L. Q.; Cheley, S.; Bayley, H. *Chem. Biol.* **2005**, *12*, 109-120.



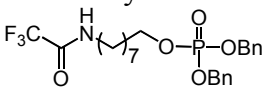
1-O-*(Dibenzyl phosphoryloxy)-2-trifluoroacetamido-1-ethanol*: Following general procedure II, dibenzyl phosphate (1.79 g, 6.45 mmol) was dissolved in DMF (10 µL) and CH_2Cl_2 (25 mL) and combined with oxalyl chloride (1.13 mL, 12.9 mmol). After concentration, the compound was redissolved in CH_2Cl_2 (5 mL) and added to 2-trifluoroacetamido-1-ethanol (675 mg, 4.3 mmol) in pyridine (10 mL) and 4 Å molecular sieves to yield 621 mg (35%) of the product as an off-white solid. ^{1}H (300 MHz, $CDCl_3$): δ 7.35 (m, 10H), 5.03 (ABX system, 4H, J_{AB} = 15 Hz, J_{AP} = J_{BP} = 11.7 Hz), 4.04 (pentet, 2H, J = 4.8 Hz), 3.50 (q, 2H, J = 5.1). ^{13}C (75 mHz, $CDCl_3$): δ 135.45, 129.00, 128.12, 70.02, 65.7, 40.49. ESI-MS calcd for $C_{18}H_{19}F_3NO_5P$ $[M + H]^+$: 418.1031 found 418.1019.



1-O-*(Dibenzyl phosphoryloxy)-4-trifluoroacetamido-1-butanol*: Following general procedure II, dibenzyl phosphate (1.79 g, 6.45 mmol) was dissolved in DMF (10 µL) and CH_2Cl_2 (30 mL) and combined with oxalyl chloride (1.13 mL, 12.9 mmol). After concentration, the compound was redissolved in CH_2Cl_2 (5 mL) and added to 4-trifluoroacetamido-1-butanol (796 mg, 4.3 mmol) in pyridine (10 mL) and 4 Å molecular sieves to yield 777 mg (41%) of product as a clear oil. ^{1}H (300 MHz, $CDCl_3$): δ 7.35 (m, 10H), 5.01 (ABX system, 4H, J_{AB} = 15 Hz, J_{AP} = J_{BP} = 11.7 Hz), 3.97 (q, 2H, J = 6 Hz), 3.28 (q, 2H, J = 6), 1.70 (m, 4H). ^{13}C (75 mHz, $CDCl_3$): δ 135.90, 128.84, 128.16, 116.54 (q, J = 284.6 Hz), 69.60, 67.42, 39.97, 27.49, 25.08; ESI-MS calcd for $C_{20}H_{23}F_3NO_5P$ $[M + Na]^+$: 468.1164 found 468.1160.

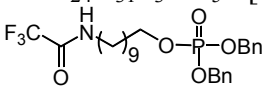


1-O-*(Dibenzyl phosphoryloxy)-6-trifluoroacetamido-1-hexanol*: Following general procedure II, dibenzyl phosphate (1.79 g, 6.45 mmol) was dissolved in DMF (10 µL) and CH_2Cl_2 (25 mL) and combined with oxalyl chloride (1.13 mL, 12.9 mmol). After concentration, the compound was redissolved in CH_2Cl_2 (5 mL) and added to 6-trifluoroacetamido-1-hexanol (917 mg, 4.3 mmol) in pyridine (10 mL) and 4 Å molecular sieves to yield 884 mg (43%) of product as a clear oil.[2]

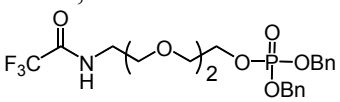


1-O-*(Dibenzyl phosphoryloxy)-8-trifluoroacetamido-1-octanol*: Following general procedure II, dibenzyl phosphate (1.73 g, 6.22 mmol) was dissolved in DMF (10 µL) and CH_2Cl_2 (25 mL) and combined with oxalyl chloride (1.08 mL, 12.4 mmol). After concentration, the compound was redissolved in CH_2Cl_2 (5 mL) and added to 8-trifluoroacetamido-1-octanol (1.00 g, 4.15 mmol) in pyridine (10 mL) and 4 Å molecular sieves to yield 966 mg (46%) of product as a clear oil. ^{1}H (300 MHz, $CDCl_3$): δ 7.31 (m, 10H), 5.00 (ABX system, 4H, J_{AB} = 15 Hz, J_{AP} = J_{BP} = 11.7 Hz), 3.96 (q, 2H, J = 6.6 Hz), 3.31 (q, 2H, J = 6.6 Hz), 1.55 (m, 4H), 1.27 (m, 8H). ^{13}C (75 mHz, $CDCl_3$): δ

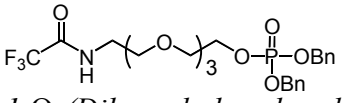
135.92, 128.63, 127.96, 69.33, 67.98, 39.99, 30.09, 28.91, 26.58, 25.29; ESI-MS calcd for $C_{24}H_{31}F_3NO_5P$ [M + Na] $^+$: 524.1790 found 524.1802.



1-O-*(Dibenzyl phosphoryloxy)-10-trifluoroacetamido-1-decanol*: Following general procedure II, dibenzyl phosphate (775 mg, 2.78 mmol) was dissolved in DMF (10 µL) and CH_2Cl_2 (15 mL) and combined with oxalyl chloride (0.50 mL, 5.57 mmol). After concentration, the compound was redissolved in CH_2Cl_2 (5 mL) and added to 10-trifluoroacetamido-1-decanol (500 mg, 1.86 mmol) in pyridine (5 mL) and 4 Å molecular sieves to yield 578 mg (59%) of product as a clear oil. ^{1}H (300 MHz, $CDCl_3$): δ 7.34 (m, 10H), 5.01 (ABX system, 4H, J_{AB} = 15 Hz, J_{AP} = J_{BP} = 11.7 Hz), 3.97 (q, 2H, J = 6 Hz), 3.33 (q, 2H, J = 6.6), 1.70 (m, 4H), 1.25 (m, 12H). ^{13}C (75 mHz, $CDCl_3$): δ 135.99, 128.62, 127.98, 69.33, 66.18, 40.09, 30.25, 29.44, 29.30, 29.19, 29.02, 28.97, 26.10, 25.37; ESI-MS calcd for $C_{26}H_{35}F_3NO_5P$ [M + Na] $^+$: 552.2103 found 552.2094.



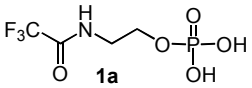
1-O-*(Dibenzyl phosphoryloxy)-8-trifluoroacetamido-3,6-dioxa-1-octanol*: Following general procedure II, dibenzyl phosphate (981 mg, 3.53 mmol) was dissolved in DMF (20 µL) and CH_2Cl_2 (15 mL) and combined with oxalyl chloride (0.62 mL, 7.05 mmol). After concentration, the compound was redissolved in CH_2Cl_2 (5 mL) and added to 8-trifluoroacetamido-3,6-dioxa-1-octanol (500 mg, 2.35 mmol) in pyridine (5 mL) and 4 Å molecular sieves to yield 623 mg (53%) of product as a clear oil. ^{1}H (300 MHz, $CDCl_3$): δ 7.34 (m, 10H), 5.04 (ABX system, 4H, J_{AB} = 15 Hz, J_{AP} = J_{BP} = 11.7 Hz), 4.12-4.09 (m, 2H), 3.64 (t, 2H, J = 5.1 Hz), 3.61-3.54 (m, 6H), 3.47 (q, 2H, J = 4.8 Hz); ^{13}C (75 mHz, $CDCl_3$): δ 157.5 (q, J = 36.5 Hz), 135.9 (d, J = 7.1 Hz), 135.8, 128.7, 128.0, 116.0 (q, J = 286.4 Hz), 70.7, 70.4, 70.0 (d, J = 6.5 Hz), 69.4 (d, J = 5.1 Hz), 68.8, 66.9 (d, J = 5.9 Hz), 39.94; ESI-MS calcd for $C_{22}H_{27}F_3NO_7P$ [M - H] $^-$: 504.1399 found 504.1420.



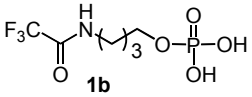
1-O-*(Dibenzyl phosphoryloxy)-11-trifluoroacetamido-3,6,9-trioxa-1-undecanol*: Following general procedure II, dibenzyl phosphate (433 mg, 1.56 mmol) was dissolved in DMF (10 µL) and CH_2Cl_2 (10 mL) and combined with oxalyl chloride (0.27 mL, 3.11 mmol). After concentration, the compound was redissolved in CH_2Cl_2 (5 mL) and added to 11-trifluoroacetamido-3,6,9-trioxa-1-undecanol (300 mg, 1.04 mmol) in pyridine (5 mL) and 4 Å molecular sieves to yield 499 mg (87%) of product as a clear oil. ^{1}H (300 MHz, $CDCl_3$): δ 7.34 (m, 10H), 5.04 (ABX system, 4H, J_{AB} = 15 Hz, J_{AP} = J_{BP} = 11.7 Hz), 4.14 (m, 2H), 3.64 (t, 2H, J = 5.0 Hz), 3.62-3.55 (m, 10H), 3.50 (q, 2H, J = 5.1 Hz). ^{13}C (75 mHz, $CDCl_3$): δ 157.5 (q, J = 37.2 Hz), 136.0 (d, J = 6.1 Hz), 128.7, 128.1, 116.1 (q, J = 286.3 Hz), 70.8, 70.7, 70.6, 70.4, 70.1 (d, J = 6.8 Hz), 68.8, 66.9 (d, J = 5.9 Hz), 39.9; ESI-MS calcd for $C_{24}H_{31}F_3NO_8P$ [M + Na] $^+$: 572.1637 found 572.1654.

General Procedure III: Hydrogenolysis of phosphotriester.
The phosphotriester (1 eq) was dissolved in 3:2 MeOH/EtOAc with triethylamine (1 eq). Pd/C was added and the suspension was stirred under H_2 (1 atm) for 12 h. The suspension

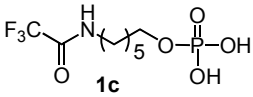
was filtered through celite and the filtrate was concentrated to yield phosphate **1** as the triethylammonium salt.



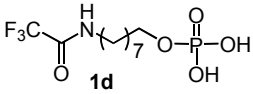
2-Trifluoroacetamido-ethanol-1-phosphate (**1a**): Following general procedure III, 1-*O*-(dibenzyl phosphoryloxy)-2-trifluoroacetamido-1-ethanol (367 mg, 0.88 mmol) was dissolved in 3:2 MeOH/EtOAc (15 mL) and triethylamine (0.12 mL), and combined with with Pd/C (184 mg) and H_2 to yield the triethylammonium salt of **1a** (290 mg, 97%) as a white solid. ^{1}H (300 MHz, CD_3OD): δ 3.98 (q, 2H, *J* = 6.9), 3.52 (t, 2H, *J* = 5.4 Hz), 3.18 (q, 6H, *J* = 7.2 Hz), 1.31 (t, 9H, *J* = 7.5 Hz); ^{13}C (75 mHz, CD_3OD): δ 63.78, 47.52, 41.93, 9.10; ESI-MS calcd for $C_4H_7F_3NO_5P$ [M - H]$^-$: 235.9936 found 235.9947.



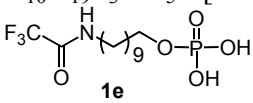
4-Trifluoroacetamido-butanol-1-phosphate (**1b**): Following general procedure III, 1-*O*-(dibenzyl phosphoryloxy)-4-trifluoroacetamido-1-butanol (392 mg, 0.88 mmol) was dissolved in 3:2 MeOH/EtOAc (15 mL) and triethylamine (0.12 mL), and combined with with Pd/C (196 mg) and H_2 to yield the triethylammonium salt of **1b** (339 mg, 97%) as a white solid. ^{1}H (300 MHz, CD_3OD): δ 3.85 (q, 2H, *J* = 6.3), 3.25-3.20 (m, 2H), 3.14 (q, 6H, *J* = 7.5 Hz), 1.74-1.54 (m, 4H), 1.27 (t, 9H, *J* = 7.2 Hz); ^{13}C (75 mHz, CD_3OD): δ 64.35, 46.31, 39.26, 27.76, 25.24, 7.89; ESI-MS calcd for $C_6H_{11}F_3NO_5P$ [M - H]$^-$: 264.0249 found 264.0240.



6-Trifluoroacetamido-hexanol-1-phosphate (**1c**): Following general procedure III, 1-*O*-(dibenzyl phosphoryloxy)-6-trifluoroacetamido-1-hexanol (441 mg, 0.93 mmol) was dissolved in 3:2 MeOH/EtOAc (15 mL) and triethylamine (0.13 mL), and combined with with Pd/C (220 mg) and H_2 to yield the triethylammonium salt of **1c** (351 mg, 96%) as a clear oil.[2]

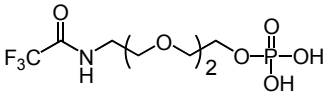


8-Trifluoroacetamido-octanol-1-phosphate (**1d**): Following general procedure III 1-*O*-(dibenzyl phosphoryloxy)-8-trifluoroacetamido-1-octanol (400 mg, 0.80 mmol) was dissolved in 3:2 MeOH/EtOAc (12 mL) and triethylamine (0.11 mL), and combined with with Pd/C (200 mg) and H_2 to yield the triethylammonium salt of **1d** (320 mg, 95%) as a white solid. ^{1}H (300 MHz, CD_3OD): δ 3.92 (q, 2H, *J* = 6.6), 3.26 (t, 2H, *J* = 7.5), 3.20 (q, 6H, *J* = 7.2 Hz), 1.70-1.50 (m, 4H), 1.40-1.29 (m, 17H); ^{13}C (75 mHz, CD_3OD): δ 67.23, 47.74, 40.70, 30.17, 29.79, 27.71, 26.64, 18.78, 17.30, 9.18; ESI-MS calcd for $C_{10}H_{19}F_3NO_5P$ [M - H]$^-$: 320.0875 found 320.0883.

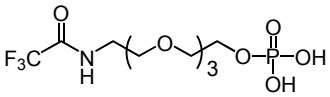


10-Trifluoroacetamido-decanol-1-phosphate (**1e**): Following general procedure III, 1-*O*-(dibenzyl phosphoryloxy)-10-trifluoroacetamido-1-decanol (554 mg, 1.02 mmol) was dissolved in 3:2 MeOH/EtOAc (15 mL) and triethylamine (0.15 mL), and combined with

with Pd/C (270 mg) and H_2 to yield the triethylammonium salt of **1e** (460 mg, 98%) as a white solid. ^{1}H (300 MHz, CD_3OD): δ 3.77 (q, 2H, *J* = 6.6), 3.19 (t, 2H, *J* = 7.2 Hz), 3.09 (q, 6H, *J* = 7.2 Hz), 1.59-1.43 (m, 4H), 1.33-1.20 (m, 21H); ^{13}C (75 mHz, CD_3OD): δ 66.22, 47.48, 40.74, 30.63, 30.53, 30.50, 30.45, 30.29, 29.80, 27.79, 26.94, 9.09; ESI-MS calcd for $C_{12}H_{22}F_3NO_5P$ [M - H]$^-$: 348.1188 found 348.1198.



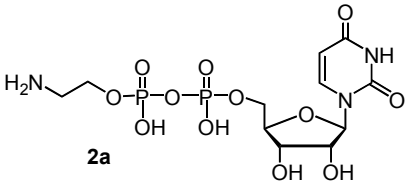
8-trifluoroacetamido-3,6-dioxa-octanol-1-phosphate: Following general procedure III, 1-*O*-(dibenzyl phosphoryloxy)-11-trifluoroacetamido-3,6,9-trioxa-1-undecanol (550 mg, 1.09 mmol) was dissolved in MeOH (10 mL) and triethylamine (0.15 mL), and combined with with Pd/C (275 mg) and H_2 to yield the triethylammonium salt (462 mg, 99%) as a white solid. ^{1}H (300 MHz, CD_3OD): δ 3.81 (td, 2H, J = 6.1, 4.4 Hz), 3.50-3.40 (m, 8H), 3.29 (t, 2H, J = 5.3 Hz), 3.00 (q, 6H, J = 7.0 Hz), 1.35 (t, 9H, J = 7.1 Hz); ^{13}C (75 mHz, CD_3OD): δ 159.2 (q, J= 36.2 Hz), 117.7 (q, J = 285.1 Hz), 72.2 (d, J = 7.2 Hz), 71.7, 71.5, 69.9, 65.6 (d, J = 5.6 Hz), 47.5, 40.9, 9.3; ESI-MS calcd for $C_8H_{15}F_3NO_7P$ [M - H]$^-$: 324.0460 found 324.0471.



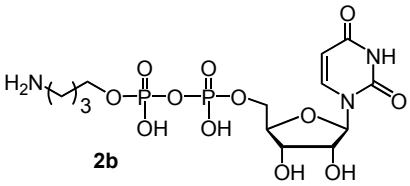
11-trifluoroacetamido-3,6,9-trioxa-undecanol-1-phosphate: Following general procedure III, 1-*O*-(dibenzyl phosphoryloxy)-11-trifluoroacetamido-3,6,9-trioxa-1-undecanol (450 mg, 0.82 mmol) was dissolved in MeOH (9 mL) and triethylamine (0.12 mL), and combined with with Pd/C (225 mg) and H_2 to yield the triethylammonium salt (397 mg, 99%) as a pasty, white solid. ^{1}H (300 MHz, CD_3OD): δ 3.92-2.90 (m, 2H), 3.60-3.47 (m, 12H), 3.37 (t, 2H, J = 5.1 Hz), 3.08 (q, 6H, J = 7.1 Hz), 1.21 (t, 9H, J = 6.8 Hz); ^{13}C (75 mHz, CD_3OD): δ 117.7 (q, J = 284.9 Hz), 72.0, 71.7, 71.6, 71.5, 69.9, 66.0, 47.8, 40.9, 9.3; ESI-MS calcd for $C_{10}H_{19}F_3NO_8P$ [M - H]$^-$: 368.0722 found 368.0721.

General Procedure IV: Coupling of phosphate to uridine 5'-monophosphate (UMP) and trifluoracetamide removal.

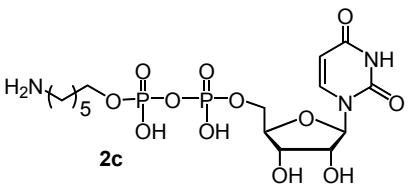
5'-UMP (triethylammonium salt, 1.0 eq) was suspended in acetonitrile (3 mL) and cooled to 0 °C. Dimethylaniline (4 eq) and triethylamine (2 eq) were added under an argon atmosphere. A pre-cooled solution of trifluoroacetic anhydride (6 eq) in acetonitrile (1 mL) was added dropwise over several minutes to yield a transparent, pink solution. The reaction was stirred at 0 °C for 15 min and the solvent was removed in vacuo. The residue was resuspended in acetonitrile (3 mL) and stirred at 0 °C with 4 Å molecular sieves (ca. 5-10) under an argon atmosphere. Triethylamine (5 eq) and 1-methyl imidazole (5.3 eq) were added dropwise and the solution turned bright yellow. Phosphate **1** (0.63 eq) was dissolved in acetonitrile (1 mL) and added dropwise to the solution. The reaction was stirred at 0 °C for 1 h, and room-temperature for 3 h. The reaction was quenched with 15 mL H_2O and extracted with CH_2Cl_2 (15 mL). The organic layer was extracted with H_2O (15 mL) and the combined aqueous layers were combined and concentrated, and the product was purified by silica gel chromatography. The resulting product was treated with 3 M ammonium hydroxide to deprotect the trifluoroacetamide moiety. The solvent was evaporated in vacuo and the product was lyophilized to yield diphosphate **2** as the ammonium salt.



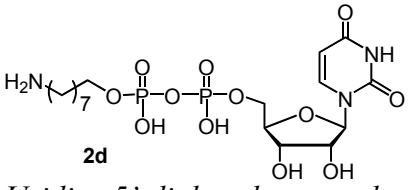
Uridine 5'-diphosphoethanolamine (**2a**): Following general procedure IV, UMP·Et_3NH^+ (102 mg, 0.238 mmol) was activated with dimethyl aniline (120 µL, 0.952 mmol), triethylamine (50 µL, 0.476 mmol) and trifluoroacetic anhydride (200 µL, 1.428 mmol). The activated UMP was then reacted with 1-methylimidazole (100 mL, 1.26 mmol), triethylamine (130 µL, 1.19 mmol) and phosphate **1a** (50 mg, 0.15 mmol). The product was purified by silica gel chromatography (5:4:1 $CHCl_3$/MeOH/1 M ammonium acetate) to yield the ammonium salt of the diphosphate as an off white solid. The diphosphate was stirred with 3 M ammonium hydroxide (10 mL) for 2 h under N_2, the solvent was removed *in vacuo* and the product was lyophilized to yield the the ammonium salt of uridine 5'-diphosphoethanolamine **2a** (33 mg, 45%) as an off-white solid. ^1H (300 MHz, D_2O): δ 7.98 (d, 1H, J = 8.1 Hz), 6.03 (d, 1H, J = 4.6 Hz), 6.01 (d, 1H, J = 8.5 Hz), 4.42 (m, 2H), 4.33 (br s, 1H), 4.30-4.22 (m, 5H), 3.35 (t, 2H, J = 6.9 Hz), 1.92 (s, 4H); ^{13}C (75 mHz, D_2O): δ 152.1, 141.7, 102.8, 88.8, 83.3 (d, J = 8.0 Hz), 73.9, 69.8, 65.1 (d, J = 7.7 Hz), 62.5, 36.8, 23.5 ; ESI-MS calcd for $C_{11}H_{19}N_3O_{12}P_2$ $[M - H]^-$: 446.0366 found 446.0348.



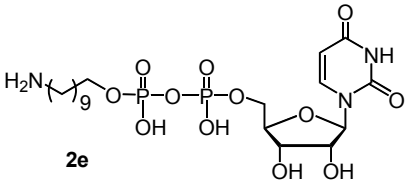
Uridine 5'-diphosphobutanolamine (**2b**): Following general procedure IV, UMP·Et_3NH^+ (102 mg, 0.238 mmol) was activated with dimethyl aniline (120 µL, 0.952 mmol), triethylamine (50 µL, 0.476 mmol) and trifluoroacetic anhydride (200 µL, 1.428 mmol). The activated UMP was then reacted with 1-methylimidazole (100 mL, 1.26 mmol), triethylamine (130 µL, 1.19 mmol) and phosphate **1b** (55 mg, 0.15 mmol). The product was purified by silica gel chromatography (5:4:1 $CHCl_3$/MeOH/1 M ammonium acetate) to yield the ammonium salt of the diphosphate as an off white solid. The diphosphate was stirred with 3 M ammonium hydroxide (10 mL) for 2 h under N_2, the solvent was removed *in vacuo* and the product was lyophilized to yield the the ammonium salt of uridine 5'-diphosphobutanolamine **2b** (46 mg, 58%) as an off-white solid. ^1H (300 MHz, D_2O): δ 7.96 (d, 1H, J = 8.1 Hz), 6.01 (d, 1H, J = 4.7 Hz), 6.00 (d, 1H, J = 8.4 Hz), 4.39 (br s, 2H), 4.31 (br s, 1H), 4.31-4.22 (m, 2H), 4.04-4.00 (m, 2H), 3.68 (br s, 2H), 1.96 (s, 4H), 1.78 (m, 4H); ^{13}C (75 mHz, D_2O): δ 165.1, 150.6, 140.1, 101.2, 87.1, 81.7, 72.2, 68.2, 63.5, 62.1, 37.8, 25.2 (d, J = 6.6 Hz), 22.2; ESI-MS calcd for $C_{13}H_{23}N_3O_{12}P_2$ $[M + Na]^+$: 498.0655 found 498.0648.



Uridine 5'-diphosphohexanolamine (**2c**): Following general procedure IV, UMP·Et_3NH^+ (102 mg, 0.238 mmol) was activated with dimethyl aniline (120 µL, 0.952 mmol), triethylamine (50 µL, 0.476 mmol) and trifluoroacetic anhydride (200 µL, 1.428 mmol). The activated UMP was then reacted with 1-methylimidazole (100 mL, 1.26 mmol), triethylamine (130 µL, 1.19 mmol) and phosphate **1c** (6 mg, 0.15 mmol). The product was purified by silica gel chromatography (5:4:1 $CHCl_3$/MeOH/H_2O) to yield the triethylammonium salt of the diphosphate as an off-white solid. The diphosphate was stirred with 3 M ammonium hydroxide (10 mL) for 2 h under N_2, the solvent was removed *in vacuo* and the product was lyophilized to yield the the ammonium salt of uridine 5'-diphosphohexanolamine **2c** (56 mg, 71%) as an off-white solid.[7]



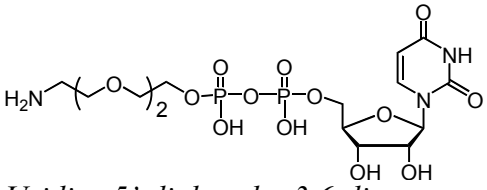
Uridine 5'-diphosphooctanolamine (**2d**): Following general procedure IV, UMP·Et_3NH^+ (102 mg, 0.238 mmol) was activated with dimethyl aniline (120 µL, 0.952 mmol), triethylamine (50 µL, 0.476 mmol) and trifluoroacetic anhydride (200 µL, 1.428 mmol). The activated UMP was then reacted with 1-methylimidazole (100 mL, 1.26 mmol), triethylamine (130 µL, 1.19 mmol) and phosphate **1d** (65 mg, 0.15 mmol). The product was purified by silica gel chromatography (5:4:1 $CHCl_3$/MeOH/H_2O) to yield the triethylammonium salt of the diphosphate as an off-white solid. The diphosphate was stirred with 3 M ammonium hydroxide (10 mL) for 2 h under N_2, the solvent was removed *in vacuo* and the product was lyophilized to yield the the ammonium salt of uridine 5'-diphosphooctanolamine **2d** (78 mg, 89%) as an off-white solid. ^1H (300 MHz, D_2O): δ 8.67 (s, 1H), 7.98 (d, 1H, J = 7.9 Hz), 7.44 (s, 1H), 6.0-5.97 (m, 2H), 4.37-4.29 (m, 2H), 4.37 (d, 1H, J = 3.9 Hz), 4.29-4.18 (m, 3H), 3.98-3.92 (m, 2H), 2.99 (t, 2H, J = 8.4 Hz), 2.75 (s, 4H), 1.68-1.61 (m, 4H), 1.38-1.30 (m, 8H); ^{13}C (75 mHz, D_2O): δ 168.8, 154.4, 144.3, 137.5, 125.5, 122.0, 105.3, 91.0, 85.8 (d, J = 5.4 Hz), 76.4, 72.1, 69.6 (d, J = 3 Hz), 67.5, 42.1, 41.3, 38.0, 32.2 (d, J = 4.0 Hz), 30.5, 29.2, 27.9, 27.2; ESI-MS calcd for $C_{17}H_{31}N_3O_{12}P_2$ $[M + Na]^+$: 554.1281 found 554.1257.



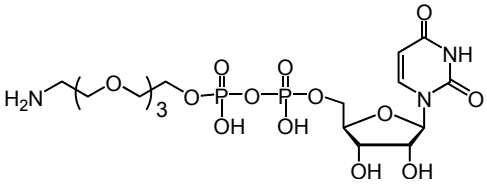
Uridine 5'-diphosphodecanolamine (**2e**): Following general procedure IV, UMP·Et_3NH^+ (102 mg, 0.238 mmol) was activated with dimethyl aniline (120 µL, 0.952 mmol), triethylamine (50 µL, 0.476 mmol) and trifluoroacetic anhydride (200 µL, 1.428 mmol). The activated UMP was then reacted with 1-methylimidazole (100 mL, 1.26 mmol), triethylamine (130 µL, 1.19 mmol) and phosphate **1e** (70 mg, 0.15 mmol). The product was purified by silica gel chromatography ($CHCl_3$/MeOH/H_2O 12/6/1) to yield the triethylammonium salt of the diphosphate as an off-white solid. The diphosphate was stirred with 3 M ammonium hydroxide (10 mL) for 2 h under N_2, the solvent was removed *in vacuo* and the product was lyophilized to yield the the ammonium salt of

[7] Barker, R.; Shaper, J. H.; Hill, R. L.; Olsen, K. W. *J. Biol. Chem*. **1972**, *247*, 7135.

uridine 5'-diphosphodecanolamine **2e** (66 mg, 76%) as an off-white solid. ^{1}H (300 MHz, D_2O): δ 8.77 (br s, 1H, 8.11, (d, 1H, J = 8.0 Hz), 7.57 (br s, 2H), 6.09-6.04 (m, 2H), 4.47-4.42 (m, 2H), 4.34 (br s, 3H), 4.04 (s, 4H), 4.02 (m, 2H), 3.11 (t, 2H, J = 7.6 Hz), 1.78 (t, 2H, J = 6.2 Hz), 1.69 (t, 2H, J = 6.7 Hz); ^{13}C (75 mHz, 10:1 D_2O/d_6-acetone): δ 165.9, 151.8, 142.1, 102.9, 88.8, 83.6 (d, J = 8.5 Hz), 74.3, 70.1, 66.9 (d, J = 5.3 Hz), 65.3, 39.9, 35.7, 29.2, 29.1, 28.8, 27.2, 26.2, 25.6; ESI-MS calcd for $C_{19}H_{35}N_3O_{12}P_2$ $[M + H]^+$: 560.1774 found 560.1777.



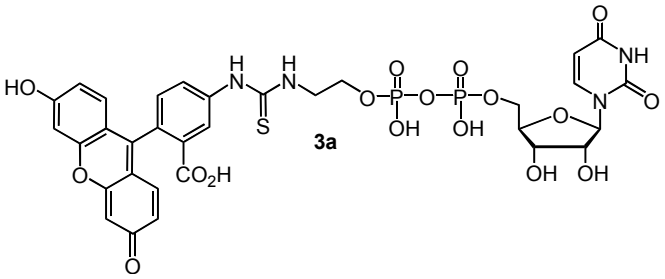
Uridine 5'-diphospho-3,6-dioxa-octanolamine: Following general procedure IV, UMP·Et_3NH^+ (150 mg, 0.35 mmol) was activated with dimethyl aniline (177 µL, 1.40 mmol), triethylamine (98 µL, 0.70 mmol) and trifluoroacetic anhydride (297 µL, 2.10 mmol). The activated UMP was then reacted with 1-methylimidazole (171 mL, 1.86 mmol), triethylamine (244 µL, 1.75 mmol) and 8-trifluoroacetamido-3,6-dioxa-octanol-1-phosphate (94 mg, 0.22 mmol). The product was purified by silica gel chromatography ($CHCl_3$/MeOH/H_2O 12/6/1) to yield the triethylammonium salt of the diphosphate as an off-white solid. The diphosphate was stirred with 3 M ammonium hydroxide (10 mL) for 2 h under N_2, the solvent was removed *in vacuo* and the product was lyophilized to yield the the ammonium salt of uridine 5'-diphospho-3,6-dioxa-octanolamine (98 mg, 78 %) as an off-white solid. ^{1}H (300 MHz, D_2O): δ 7.96 (d, 1H, J = 8.2 Hz), 6.01 (d, 1H, J = 4.2 Hz), 5.99 (d, 1H, J = 7.8 Hz), 4.39 (m, 2H), 4.30 (m, 1H), 4.15-4.09 (m, 2H), 3.80 (t, 2H, J = 6.0 Hz), 3.56 (br s, 8H), 3.25 (t, 2H, J = 5.1 Hz); ^{13}C (75 mHz, D_2O): δ 166.3, 151.9, 141.8, 102.8, 88.7, 83.3 (d, J = 8.2 Hz), 73.9, 70.2, 70.2, 69.8, 69.6, 66.6, 65.3, 65.1 (d, J = 7.7 Hz), 39.3; ESI-MS calcd for $C_{15}H_{28}N_3O_{14}P_2$ $[M + H]^+$: 536.1047 found 536.1060.



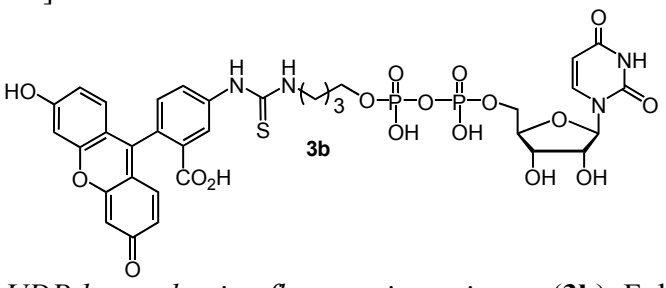
Uridine 5'-diphospho-3,6,9-trioxa-undecanolamine: Following general procedure IV, UMP·Et_3NH^+ (147 mg, 0.344 mmol) was activated with dimethyl aniline (174 µL, 1.376 mmol), triethylamine (96 µL, 0.688 mmol) and trifluoroacetic anhydride (292 µL, 2.064 mmol). The activated UMP was then reacted with 1-methylimidazole (168 mL, 1.823 mmol), triethylamine (240 µL, 1.770 mmol) and 11-trifluoroacetamido-3,6,9-trioxa-undecanol-1-phosphate (102 mg, 0.220 mmol). The product was purified by silica gel chromatography ($CHCl_3$/MeOH/H_2O 12/6/1) to yield the triethylammonium salt of the diphosphate as an off-white solid. The diphosphate was stirred with 3 M ammonium hydroxide (10 mL) for 2 h under N_2, the solvent was removed *in vacuo* and the product was lyophilized to yield the ammonium salt of uridine 5'-diphospho-3,6,9-trioxa-undecanolamine (95 mg, 70 %) as an off-white solid. ^{1}H (300 MHz, D_2O): δ 7.93 (d, 1H, J = 8.1 Hz), 5.97 (d, 1H, J = 4.2 Hz), 5.95 (d, 1H, J = 8.1 Hz), 4.39-4.33 (m, 2H), 4.28 (br s, 1H), 4.10 (br s, 2H), 3.78 (t, 2H, J = 5.4 Hz), 3.72 (br s, 12H), 3.23 (t, 2H, J = 5.3 Hz); ^{13}C (75 mHz, D_2O): δ 166.2, 151.8, 141.8, 102.7, 88.8, 73.8, 70.2, 69.7, 69.6, 69.5, 66.5,

65.3 (d, J = 7.5 Hz), 39.3; ESI-MS calcd for $C_{17}H_{31}N_3O_{15}P_2$ [M - H]$^-$: 578.1152 found 578.1130.

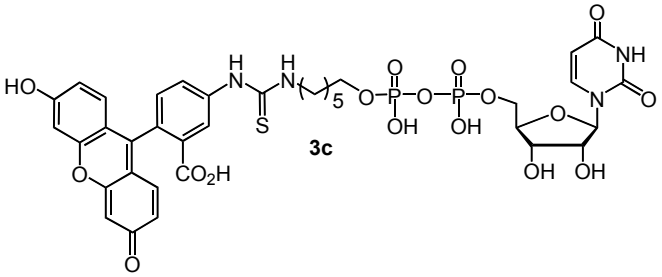
General Procedure V: Conjugation of uridine 5'-diphospho (UDP)-alcoholamine 2 to fluorescein-5-isothiocyanate (FITC).
UDP-alcoholamine **2** (1.0 eq) was was combined with FITC (1.5 eq) in 2:1 DMF/0.1 M $NaHCO_3$. The reaction mixture was stirred for 2 h and concentrated. The product was purified by silica gel chromatography (12:10:1 $CHCl_3$/MeOH/H_2O) to yield the sodium salt of UDP-fluorescein conjugate **3** as a yellow solid.



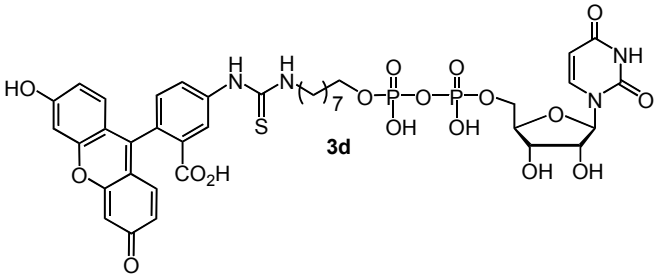
UDP-ethanolamine-fluorescein conjugate (**3a**): Following general procedure V, UDP-ethanolamine **2a** (5 mg, 9.6μmol) was combined with FITC (5.6 mg, 14.4 μg) in 2:1 DMF/0.1M $NaHCO_3$ (150 μL) to yield the sodium salt of UDP-ethanolamine-fluorescein conjugate **3a** (5.3 mg, 64%) as a yellow solid. ^{1}H (500 MHz, 5:1 D_2O:d_7-DMF): δ 7.94 (m, 2H), 7.73 (d, 1H, J = 6.4 Hz), 7.24-7.20 (m, 3H), 6.75-6.71 (m, 5H), 5.92-5.90 (m, 2H), 4.36-4.31 (m, 2H), 4.27-4.22 (m, 5H), 3.80 (br s, 2H); ^{13}C (125 MHz, 5:1 D_2O:d_7-DMF): δ 171.1, 164.4, 155.7, 150.1, 140.5, 140.2, 130.1, 118.7, 112.9, 101.8, 101.2, 87.3, 81.8, 72.5, 68.3, 63.7, 63.2, 53.1, 41.2; ESI-MS calcd for $C_{32}H_{30}N_4O_{17}P_2S$ [M - 2H]$^{-2}$: 417.0323 found 417.0313.



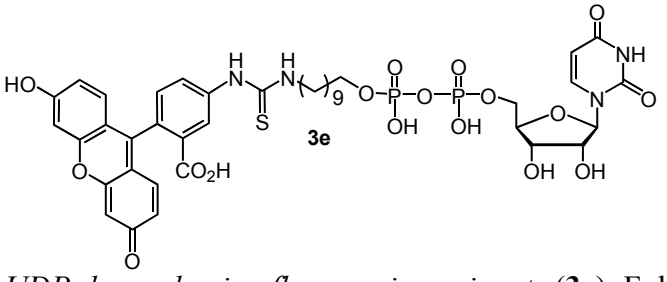
UDP-butanolamine-fluorescein conjugate (**3b**): Following general procedure V, UDP-butanolamine **2b** (10 mg, 20 μmol) was combined with FITC (12 mg, 30 μmol) in 2:1 DMF/0.1 M $NaHCO_3$ (200 μL) to yield the sodium salt of UDP-ethanolamine-fluorescein conjugate **3b** (11 mg, 62%) as a yellow solid. ^{1}H (500 MHz, 5:1 D_2O:d_7-DMF): δ 7.96 (br s, 1H), 7.88 (d, 1H, J = 8.6 Hz), 7.65 (m, 1H), 7.10 (m, 1H), 6.97 (d, 2H, J = 9.2 Hz), 6.63 (d, 2H, J = 1.8 Hz), 6.60 (d, 2H, J = 9.8 Hz), 5.87-5.82 (m, 2H), 4.25 (br s, 2H), 4.13 (br s, 3H), 3.93 (br s, 2H), 3.52 (br s, 2H), 1.63 (br s, 4H); ^{13}C (125 MHz, 5:1 D_2O:d_7-DMF): δ 182.8, 174.0, 168.0, 158.5, 154.0, 144.3, 133.5, 121.0, 115.7, 105.6, 105.1, 91.0, 86.0, 76.4, 72.8, 68.7, 67.5, 56.9, 45.1, 30.2, 27.5; ESI-MS calcd for $C_{34}H_{34}N_4O_{17}P_2S$ [M - 2H]$^{-2}$: 431.0479 found 459.0502.



UDP-hexanolamine-fluorescein conjugate (**3c**): Following general procedure V, UDP-hexanolamine **2c** (20 mg, 38 μmol) was combined with FITC (22 mg, 57 μmol) in 2:1 DMF/0.1 M $NaHCO_3$ (200 μL) to yield the sodium salt of UDP-hexanolamine-fluorescein conjugate **3c** (13.7 mg, 40%) as a yellow solid.[8]



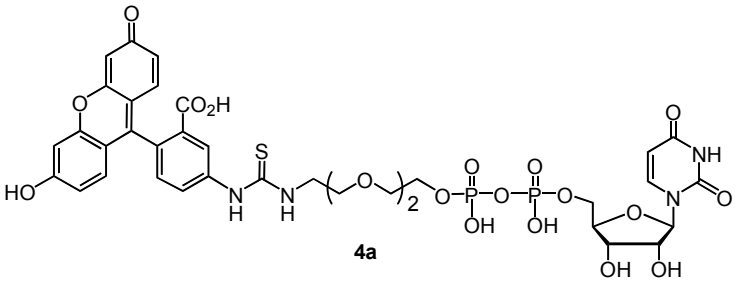
UDP-octanolamine-fluorescein conjugate (**3d**): Following general procedure V, UDP-octanolamine **2d** (3.0 mg, 5.4 μmol) was combined with FITC (3.2 mg, 8.1 μmol) in 2:1 DMF/0.1 M $NaHCO_3$ (200 μL) to yield the sodium salt of UDP-octanolamine-fluorescein conjugate **3d** (4.7 mg, 90%) as a yellow solid. ^{1}H (500 MHz, 5:1 D_2O:d_7-DMF): δ 8.14-8.10 (m, 1H), 7.88 (d, 1H, J = 7.9 Hz), 7.34 (d, 1H, J = 7.9 Hz), 7.23 (d, 2H, J = 9.8 Hz), 6.79 (m, 4H), 6.10-6.06 (m, 2H), 4.46-4.43 (m, 2H), 4.33 (br s, 3H), 4.04 (m, 2H), 3.70 (br s, 2H), 1.73-1.68 (m, 4H), 1.47 (br s, 8H); ^{13}C (125 MHz, 5:1 D_2O:d_7-DMF): δ 156.1, 150.6, 140.9, 130.3, 119.5, 112.1, 102.2, 101.7, 87.4, 82.7, 73.0, 69.1, 65.5, 64.1, 53.4, 41.6, 28.0, 27.6, 25.6, 24.4; ESI-MS calcd for $C_{38}H_{42}N_4O_{17}P_2S$ $[M - 2H]^{-2}$: 459.0792 found 459.0776.



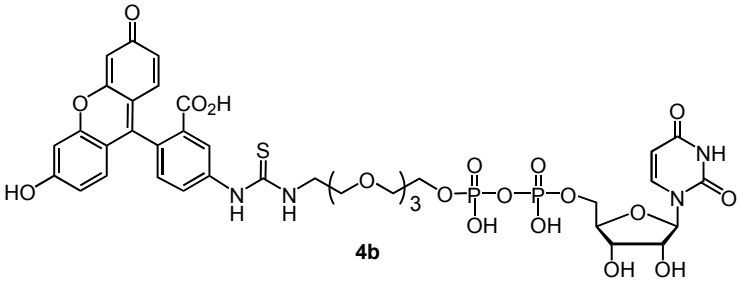
UDP-decanolamine-fluorescein conjugate (**3e**): Following general procedure V, UDP-decanolamine **2e** (5 mg, 8.4 μmol) was combined with FITC (5 mg, 12.6 μmol) in 2/1 DMF/0.1 M $NaHCO_3$ (200 μL) to yield the sodium salt of UDP-decanolamine-fluorescein conjugate **3e** (6.6 mg, 81%) as a yellow solid. ^{1}H (500 MHz, 5:1 D_2O:d_7-DMF): δ 8.13 (br s, 1H), 7.90 (d, 1H, J = 7.7 Hz), 7.63 (br s, 1H), 7.00 (br s, 1H), 6.73 (m, 2H), 6.66 (s, 2H), 6.54 (m, 2H), 5.88-5.84 (m, 2H), 4.25 (br s, 2H), 4.15 (br s, 3H), 3.83 (br s, 2H), 3.39 (br s, 2H), 1.44 (br s, 4H), 1.13 (br s, 4H), 1.03 (s, 8H); ^{13}C (125

[8] Soltero-Higgin, M.; Carlson, E. E.; Phillips, J. H.; Kiessling, L. L. *J. Am. Chem. Soc*. **2004**, *126*, 10532-10533.

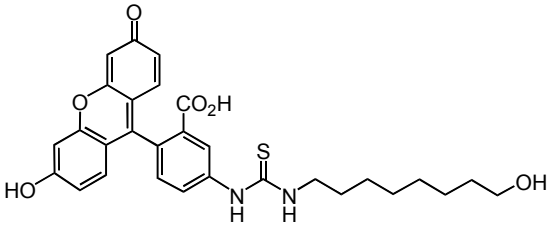
MHz, 5:1 D_2O:d_7-DMF): δ 179.2, 169.6, 164.3, 153.0, 150.4, 140.6, 128.9, 121.8, 116.9, 114.5, 110.6, 101.8, 101.5, 87.4, 82.4, 72.9, 68.8, 65.5, 64.0, 53.3, 43.3, 41.5, 28.2, 28.1, 27.9, 27.4, 25.5, 24.4; ESI-MS calcd for $C_{40}H_{46}N_4O_{17}P_2S$ [M - 2H]$^{-2}$: 473.0949 found 473.0965.



UDP-3,6-dioxa-octanolamine-fluorescein conjugate (**4a**): Following general procedure V, UDP-3,6-dioxa-octanolamine (5 mg, 8.8 μmol) was combined with FITC (5.1 mg, 13.2 μmol) in 2:1 DMF/0.1 M $NaHCO_3$ (200 μL) to yield the sodium salt of UDP-3,6-dioxa-octanolamine-fluorescein conjugate **4a** (5.6 mg, 66%) as a yellow solid. ^{1}H (500 MHz, 5:1 D_2O:d_7-DMF): δ 8.25 (br s, 1H), 7.81 (br s, 1H), 7.26 (d, 1H, J = 8.7 Hz), 6.91-6.66 (m, 9H), 5.90 (m, 2H), 4.34-4.26 (m, 2H), 4.18 (br s, 3H), 4.10 (br s, 2H), 3.80-3.66 (m, 10H); ^{13}C (125 MHz, 5:1 D_2O:d_7-DMF): 181.8, 168.7, 153.2, 151.4, 143.0, 141.0, 130.4, 127.8, 119.2, 116.9, 114.6, 102.6, 102.5, 88.7, 84.8, 83.6, 74.0, 70.6, 70.3, 69.1, 66.7, 53.9, 44.0, 42.1, 17.9, 16.6, 12.1 δ ESI-MS calcd for $C_{36}H_{38}N_4O_{19}P_2S$ [M - 2H]$^{-2}$: 461.0574 found 461.0590.

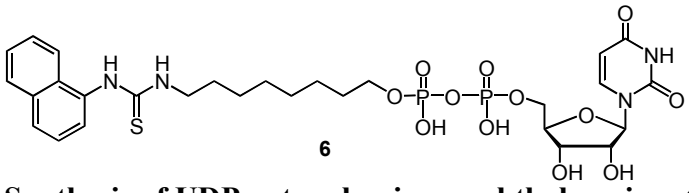


UDP-3,6,9-trioxa-undecanolamine-fluorescein conjugate (**4b**): Following general procedure V, UDP-3,6,9-trioxa-undecanolamine (2 mg, 3.3 μmol) was combined with FITC (2.0 mg, 5.0 μmol) in 2:1 DMF/0.1 M $NaHCO_3$ (200 μL) to yield the sodium salt of UDP-3,6,9-trioxa-undecanolamine-fluorescein conjugate **4b** (2.5 mg, 76%) as a yellow solid. ^{1}H (300 MHz, D_2O): δ 7.91 (d, 1H, J = 8.3 Hz), 7.86 (d, 1H, J = 2.0 Hz), 7.65 (dd, 1H, 8.3, 2.1 Hz), 7.32 (dd, 2H, J = 9.3, 4.0 Hz), 7.27 (d, 1H, J = 8.2 Hz), 6.78 (dt, 2H, J = 9.1, 2.2 Hz), 6.72 (s, 2H), 5.95-5.91 (m, 2H), 4.38-4.31 (m, 2H), 4.24 (br s, 3H), 4.15 (br s, 2H), 3.86-3.79 (m, 14H); ^{13}C (75 MHz, D_2O): δ 167.4, 160.4, 160.0, 154.1, 144.1, 143.0, 141.6, 134.3, 133.4, 123.6, 120.4, 117.4, 113.2, 105.8, 105.1, 91.1, 85.6 (d, J = 4.8 Hz), 76.4, 72.6, 72.5, 72.3, 72.1, 71.4, 68.8, 67.8, 67.5; ESI-MS calcd for $C_{38}H_{42}N_4O_{20}P_2S$ [M - 2H]$^{-2}$: 483.0705 found 483.0720.



5

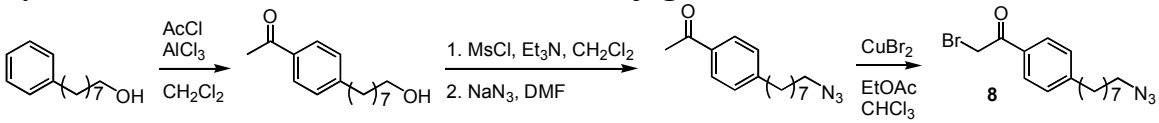
Synthesis of octanolamine-fluorescein conjugate 5: 8-amino-1-octanol (11.2 mg, 0.077 mmol) was combined with FITC (10 mg, 0.026 mmol) in MeOH (0.3 mL) and stirred at room-temperature for 1 h. The product was purified by silica gel chromatography (4:1 CH_2Cl_2/MeOH containing 1% H_2O) to yield compound **5** (13.6 mg, 98%) as an orange solid. ^{1}H (300 MHz, CD_3OD): δ 8.12 (d, 1H, J = 1.7 Hz), 7.74 (dd, 1H, J = 8.3, 1.6 Hz), 7.15 (d, 1H, J = 8.2 Hz), 6.77 (d, 2H, J = 8.8 Hz), 6.67 (d, 2H, J = 2.3 Hz), 6.56 (dd, 2H, J = 8.8, 2.2 Hz), 3.60-3.52 (m, 4H), 1.68-1.63 (m, 4H), 1.56-1.51 (m, 2H), 1.38 (m, 8H); ^{13}C (75 mHz, CD_3OD): δ 171.8, 155.2, 142.6, 131.0, 115.3, 112.6, 103.8, 63.2, 63.1, 33.8, 30.7, 30.6, 30.1, 28.2, 27.1); ESI-MS calcd for $C_{29}H_{30}N_2O_6S$ $[M - H]^-$: 533.1825 found 533.1846.



6

Synthesis of UDP-octanolamine-naphthyl conjugate 6: UDP-octanolamine **2d** (25 mg, 50 μmol) was combined with 2-naphthyl isothiocyanate (20 mg, 100 μmol) in 3:1 DMF/0.1 M $NaHCO_3$ (200 μL). The reaction was stirred 1 h and concentrated. The product was purified by silica gel chromatography (12:10:1 $CHCl_3$/MeOH/H_2O) to yield the sodium salt of UDP-octanolamine-naphthyl conjugate **6** (30.9 mg, 83%) as an off-white solid: ^{1}H (500 MHz, 5:1 D_2O:d_7-DMF): δ 8.05 (d, 1H, J = 8.4 Hz), 8.00-7.91 (m, 2H), 7.59-7.46 (m, 5H), 5.98-5.93 (m, 2H), 4.36-4.33 (m, 2H), 4.19 (br s, 3H), 3.91 (br s, 2H), 3.51 (t, 2H, J = 6.6 Hz), 1.54 (br s, 2H), 1.48 (br s, 2H), 1.24-1.15 (m, 8H); ^{13}C (125 mHz, 5:1 D_2O:d_7-DMF): δ 164.4, 160.4, 150.8, 141.3, 133.9, 128.0, 127.5, 126.5, 126.3, 125.5, 125.1, 112.8, 117.6, 115.1, 101.9, 87.4, 83.1, 73.3, 69.4, 65.4, 64.3, 44.1, 28.4, 28.3, 28.1, 25.8, 24.7; ESI-MS calcd for $C_{28}H_{38}N_4O_{12}P_2S$ $[M - 2H]^{-2}$: 357.0763 found 357.0776.

Synthesis of the aminothiazole-fluorescein conjugate 10:

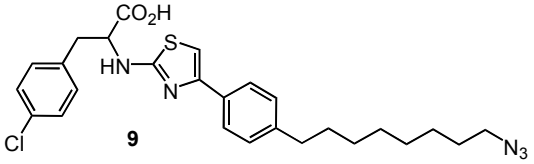


2-Bromo,4'-(8-azido-octyl) acetophenone (**8**): Aluminum chloride (3.25 g, 24 mmol) in CH_2Cl_2 (25 mL) was cooled to 0 ºC. To the pre-cooled solution, 8-phenyl-1-octanol (0.50 g, 2.4 mmol) in CH_2Cl_2 (2 mL) was added dropwise. Upon dropwise addition of acetyl chloride (0.343 mL, 4.8 mmol), the solution turned yellow. The solution was stirred for 12 h at room-temperature, after which it was poured into a mixture of ice and concentrated HCl. The mixture was stirred for 2 h until all salts were dissolved. The organic layer was separated, washed with saturated $NaHCO_3$ and brine, dried and

concentrated. The product was purified by silica gel chromatography (10%→30% ethyl acetate in hexanes) to yield the acetophenone (0.352 g, 1.42 mmol) as an off-white solid in 59% yield. ^{1}H (300 MHz, $CDCl_3$): δ 7.74 (d, 2H, J = 8.1 Hz), 7.12 (d, 2H, J = 8.1 Hz), 3.49 (t, 2H, J = 6.7 Hz), 3.06 (s, 1H), 2.51 (t, 2H, J = 7.5 Hz), 2.43 (s , 3H), 1.49-1.41 (m, 4H), 1.20 (m, 8H); ^{13}C (75 mHz, $CDCl_3$): δ 197.9, 148.6, 134.7, 128.4, 128.3, 62.4, 35.8, 32.5, 30.9, 29.3, 29.2, 29.0, 26.3, 25.7; EI-MS calcd for $C_{16}H_{24}O_2$ [M +•]: 248.1776 found 248.1777.

Triethylamine (0.296 mL, 2.12 mmol) was added to a solution of acetophenone (0.262 g, 1.06 mmol) in CH_2Cl_2 (10 mL). Mesyl chloride (0.165 mL, 2.12 mmol) was added dropwise, and the solution was stirred 1 h under N_2. The pink solution was washed with brine, dried, filtered and concentrated. The crude product was combined with sodium azide in DMF (8 mL) and stirred for 12 h at 80 °C. A solution of 10% EtOAc in hexanes (100 mL) was added to the reaction, which was washed twice with brine (50 mL), filtered and evaporated. The product was purified by silica gel chromatography (5%→10% ethyl acetate in hexanes) to yield the azide (0.243 g, 0.889 mmol) as an oil in 84% yield.^{1}H (300 MHz, $CDCl_3$): δ 7.77 (d, 2H, J = 8.1 Hz), 7.15 (d, 2H, J = 8.2 Hz), 3.13 (t, 2H, J = 6.8 Hz), 2.55 (t, 2H, J = 7.6 Hz), 2.46 (s, 3H), 1.55-1.43 (m, 4h), 1.22 (m, 8H); ^{13}C (75 mHz, $CDCl_3$): δ 197.6, 148.6, 135.0, 128.6, 128.5, 51.4, 39.9, 31.0, 29.3, 29.1, 29.0, 28.8, 26.7, 26.5; ESI-MS calcd for $C_{16}H_{23}N_3O$ [M + Na]$^+$: 296.1739 found 296.1753.

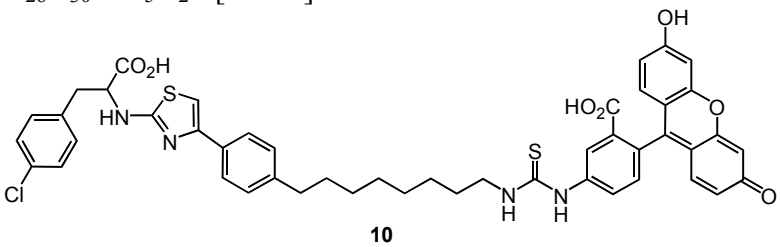
A solution of $CuBr_2$ (76.3 mg, 0.342 mmol) in EtOAc (1 mL) was heated to reflux. A solution of acetophenone in $CHCl_3$ (1 mL) was added dropwise. The solution was stirred at reflux until all $CuBr_2$ appeared to be consumed (precipitate turns white). The solution was filtered, concentrated, and the product purified by silica gel chromatography (2%→4% EtOAc in hexanes) to yield 2-bromo,4'-(8-azido-octyl) acetophenone **8** in 43% yield (32.7 mg, 0.093 mmol).^{1}H (300 MHz, $CDCl_3$): δ 7.89 (d, 2H, J = 8.4 Hz), 7.28 (d, 2H, J = 7.3 Hz), 4.43 (s, 2H), 3.25 (t, 2H, 4.8 Hz), 2.65 (m, 2H), 1.59 (m, 4H), 1.33 (m, 8H); ^{13}C (75 mHz, $CDCl_3$): δ 191.2, 150.0, 148.9, 129.1, 128.7, 77.1, 36.2, 31.2, 31.1, 29.5, 29.3, 23.2, 29.0, 26.9; ESI-MS calcd for $C_{16}H_{23}N_3O$ [M + Na]$^+$: 374.0844 found 374.0854.



3-(4-Chlorophenyl)-2-[4-(8-azido-octyl-phenyl)-thiazol-2-ylamino]-propionic acid (**9**): Thiourea[9] (26.4 mg, 0.102 mmol) and α-bromoketone **8** (32.7 mg, 0.093 mmol) were combined in DMF (300 μL). The reaction was stirred under nitrogen for 2 h and concentrated. The product was purified by silica gel chromatography (3:1 hexanes/EtOAc→2:1 hexanes/EtOAc containing 2% AcOH) to yield **9** (17 mg, 0.033 mmol) as a white solid in 36% yield.^{1}H (300 MHz, $CDCl_3$): δ 7.54 (d, 2H, J = 7.7 Hz), 7.17 (m, 6H), 6.45 (s, 1H), 4.24 (m, 1H) 3.35 (dd, 1H, J = 14.2, 5.3 Hz), 3.25 (t, 2H, J = 6.7 Hz), 3.15 (dd, 1H, J = 13.3, 6.2 Hz), 2.62 (t, 2H, J = 7.5 Hz), 1.59 (m, 4H), 1.32 (m, 8H); ^{13}C (75 mHz, $CDCl_3$): δ 174.3, 169.9, 143.8, 135.7, 132.9, 131.2, 129.0, 128.8,

[9] Dykhuizen, E. C.; May, J. F.; Tongpenyai, A.; Kiessling, L. L. *J. Am. Chem. Soc*. **2008**, XX, XXXX.

126.3, 99.4, 61.9, 51.7, 37.7, 35.9, 31.4, 29.5, 29.4, 29.3, 29.0, 26.9: ESI-MS calcd for $C_{26}H_{30}ClN_5O_2S$ $[M - H]^-$: 510.1730 found 510.1741.



10

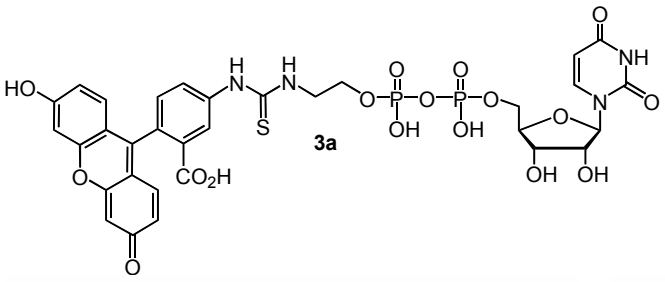
Aminothiazole-fluorescein conjugate (**10**): Azide **9** (12 mg, 23 µmol) was combined with $Pd(OH)_2/C$ (6 mg) in 4:1 MeOH/$CHCl_3$ (0.5 mL) and stirred 12 h under H_2 (1 atm). The suspension was filtered over celite and the filtrate was concentrated to yield the amine as a white solid (11.3 mg, 23 µmol) in quantitative yields. ^{1}H (300 MHz, CD_3OD): δ 7.47 (d, 2H, J = 7.9 Hz), 7.27 (m, 7H), 3.42 (dd, 1H, J = 14.3, 3.9 Hz), 3.11 (dd, 1H, J = 14.3, 8.7 Hz), 2.86 (t, 2H, J = 7.1 Hz), 2.62 (t, 2H, J = 7.7 Hz), 1.60 (m, 4H), 1.32 (m, 8H); ^{13}C (75 mHz, CD_3OD): δ 170.5, 170.3, 145.5, 140.2, 134.9, 133.2, 131.0, 129.1, 128.7, 126.4, 125.8, 60.4, 39.7, 39.6, 36.8, 35.4, 31.2, 29.1, 29.0, 27.4, 26.3; ESI-MS calcd for $C_{26}H_{32}ClN_3O_2S$ $[M + H]^+$: 486.1982 found 486.1969.

The amine (11.3 mg, 23 µmol) was combined with fluorescein isothiocyanate (10.4 mg, 35 µmol) and DIEA (12 µL, 69 µmol) in DMF (400 µL). The solution was stirred 1.5 h and concentrated. The product was purified using HPLC (gradient 50-70% ACN/H_2O) to yield **10** as an orange solid (16.2 mg, 18.5 µmol) in 80% yield. ^{1}H (500 MHz, CD_3OD): δ 8.11 (s, 1H), 7.70 (d, 1H, J = 8.1 Hz), 7.51 (d, 2H, J = 7.8 Hz), 7.20 (s, 4H), 7.15 (d, 2H, J = 8.5 Hz), 7.08 (d, 1H, J = 8.2 Hz), 6.71 (d, 2H, J = 8.5 Hz), 6.68 (d, 2H, J = 2.1 Hz), 6.54 (dd, 2H, J = 8.8, 2.4 Hz), 4.66 (dd, 1H, J = 8.4, 5.0 Hz), 3.53 (m, 2H), 3.30 (dd, 1H, J = 14.4, 5.0 Hz), 3.05 (dd, 1H, J = 14.0, 8.2 Hz), 2.56 (t, 2H, J = 7.8 Hz), 1.60-1.57 (m, 4H), 1.31 (br s, 8H); ^{13}C (125 mHz, CD_3OD): δ 182.5, 173.4, 170.4, 170.1, 154.6, 136.5, 133.6, 131.8, 130.5, 129.6, 129.3, 126.9, 126.0, 114.1, 144.0, 103.2, 60.4, 60.3, 37.7, 36.3, 32.1, 30.1, 30.0, 29.8, 29.6, 27.6; LC/MS (ESI) (m/z) $[M+H]^+$ calcd 875.3, found 875.3.

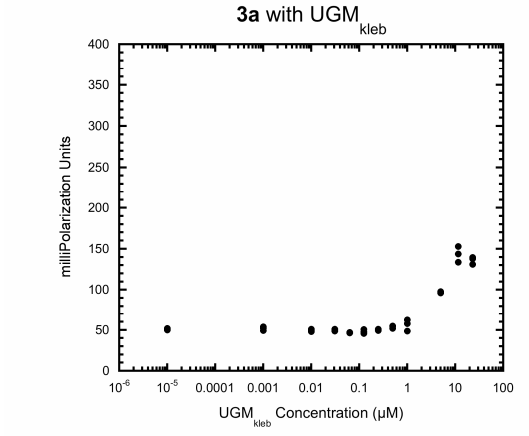
UGM Binding

Fluorescence polarization binding assay:
Serial dilutions of dialyzed UGM (maximum concentration was typically 30 μM) was incubated with 15 nM of fluorescent compounds **5a-5f**, **6** or **11** in 50 mM sodium phosphate buffer, pH 7.0 at 25 °C. Final volumes were 30 μL in 384 well black microtiter plates (Costar). Fluorescence polarization was analyzed using a Wallac EnVision plate reader. Data were fit to y = m1+((m2-m1)*x^m3)/(m4^m3+x^m3); m2 = maximum FP signal, m1 = minimum FP signal, m3 = slope, m4 = binding constant (KaleidaGraph, Synergy Software).

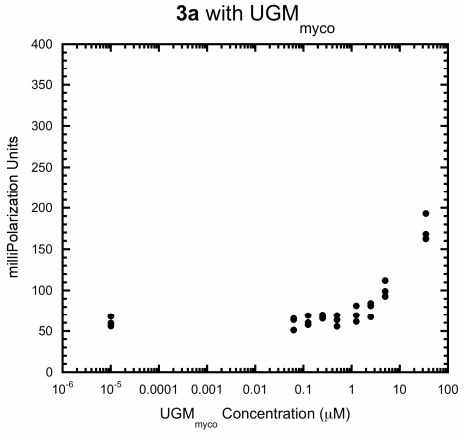
Fluorescence polarization inhibition assay:
The fluorescence polarization inhibition assay was performed as previously described.[8] Reactions contained 580 nM UGM_{myco} or 500 nM UGM_{kleb} and 15 nM of the fluorescent probe **3c** in 50 mM sodium phosphate buffer, pH 7.0 at 25 °C. Final volumes were 30 μL in 384 well black microtiter plates (Costar). Serial dilutions of UDP, **2d** or **6** were added to the wells. Fluorescence polarization was analyzed using a Wallac EnVision plate reader. Data were fit to y = m1+((m2-m1)*x^m3)/(m4^m3+x^m3); m2 = maximum FP signal, m1 = minimum FP signal, m3 = slope, m4 = apparent binding constant (KaleidaGraph, Synergy Software). To determine K_d values, the apparent binding constant was then subjected to $K_{app} = K_d(1+(I)/K_I)$ where I = concentration of the fluorescent probe and K_I = binding affinity of the fluorescent probe to UGM. For UGM_{myco} I = 15 nM and K_I = 160 nM. For UGM_{kleb} I = 15 nM and K_I = 100 nM.



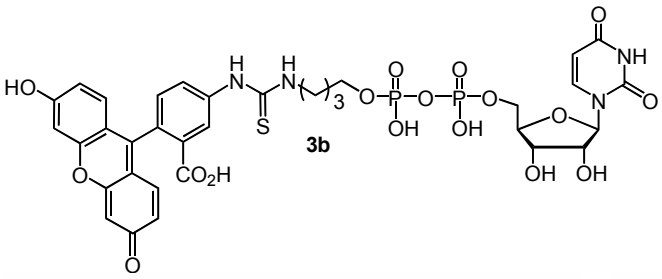
3a



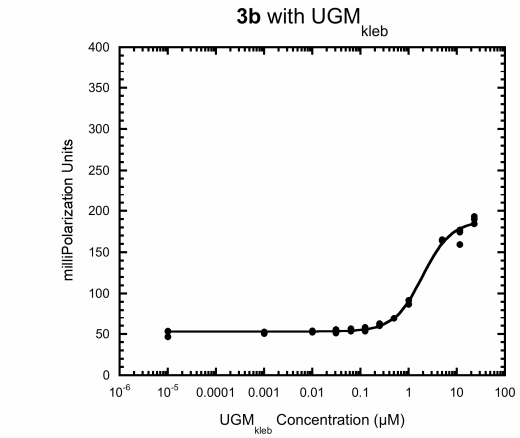
K_d > 30 μM



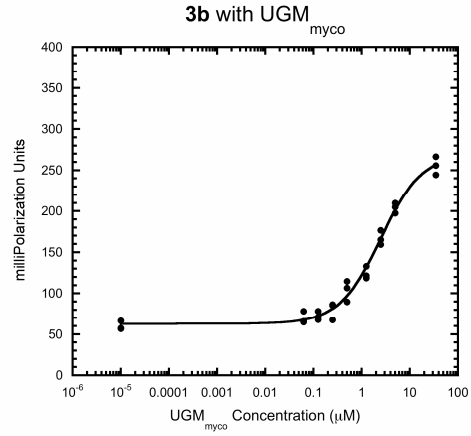
K_d > 30 μM



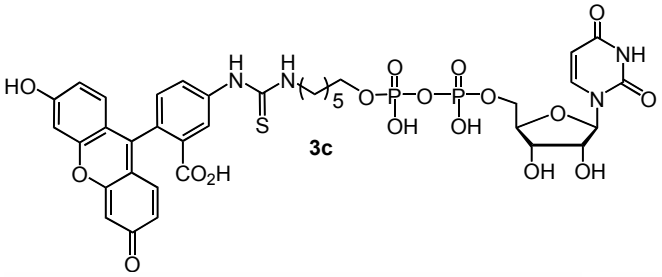
3b



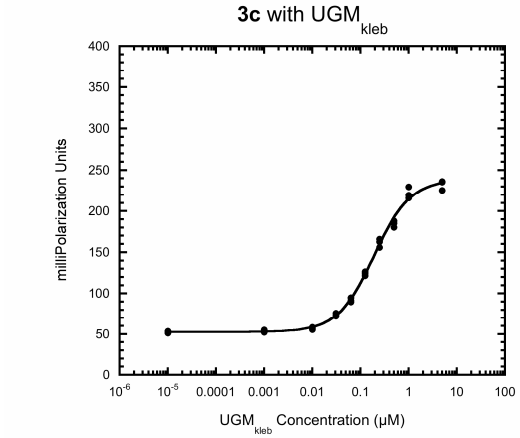
K_d = 1.9 ± 0.2 μM



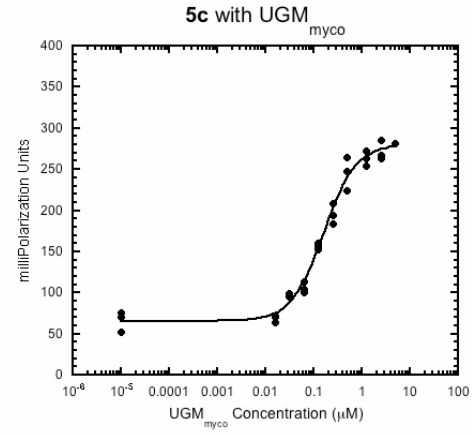
K_d = 2.5 ± 0.3 μM



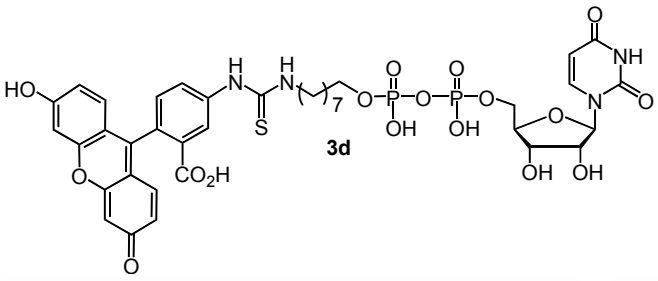
3c



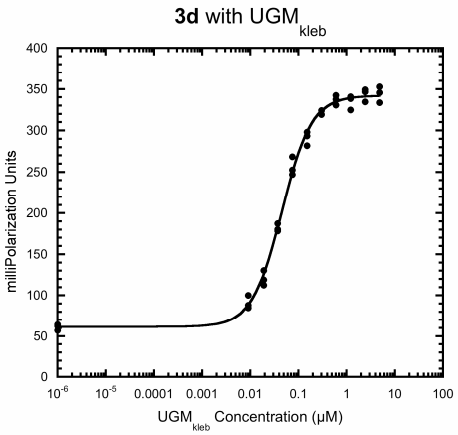
K_d = 0.19 ± 0.01 μM



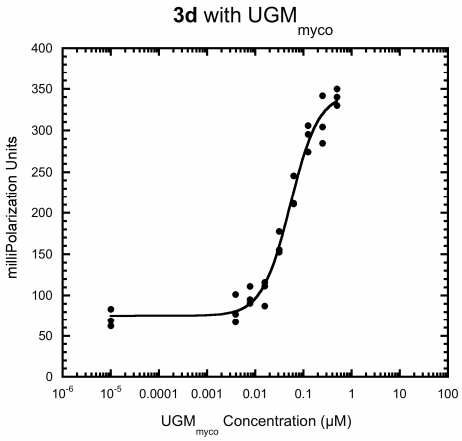
K_d = 0.17 ± 0.01 μM



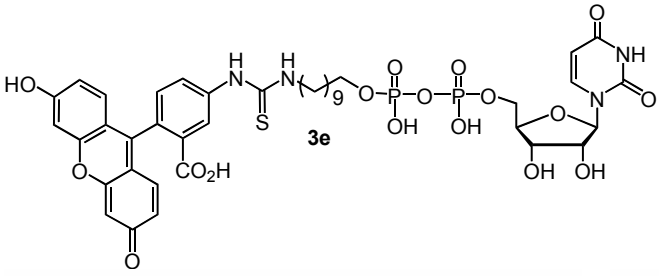
3d



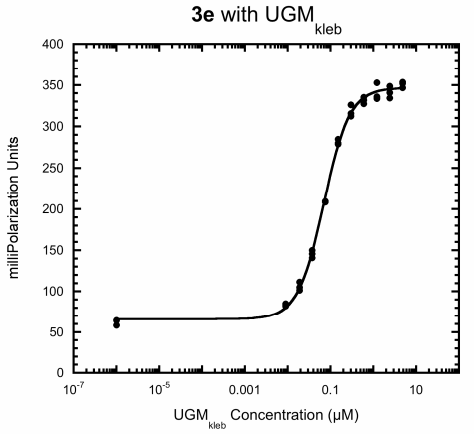
K_d = 0.045 ± 0.002 μM



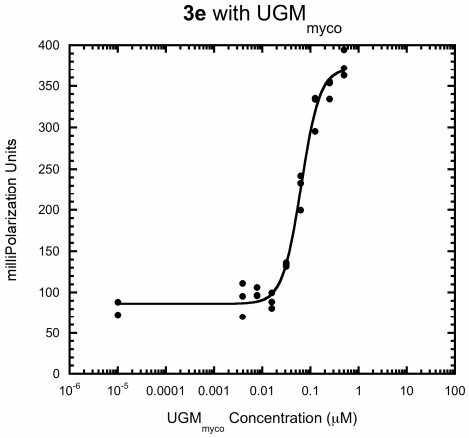
K_d = 0.054 ± 0.006 μM



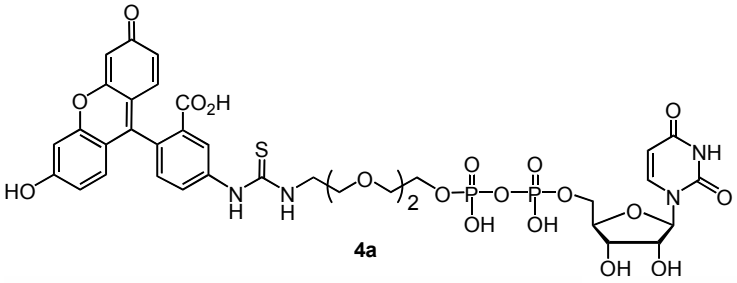
3e



K_d = 0.070 ± 0.002 μM



K_d = 0.064 ± 0.004 μM



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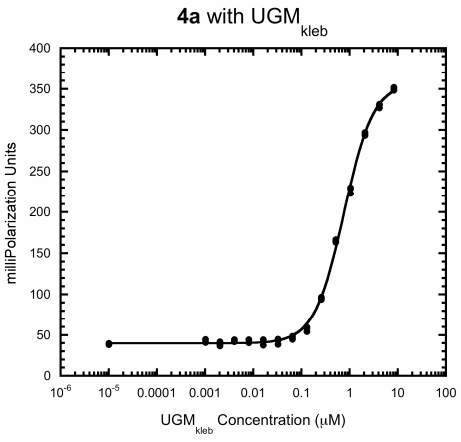
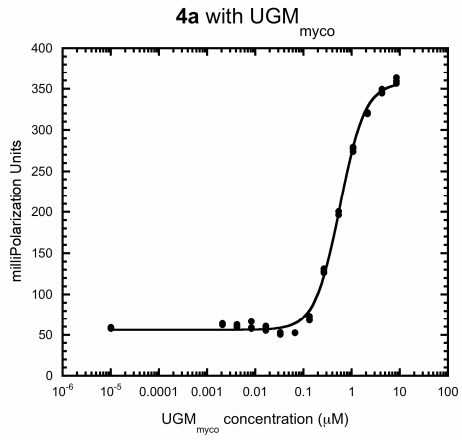
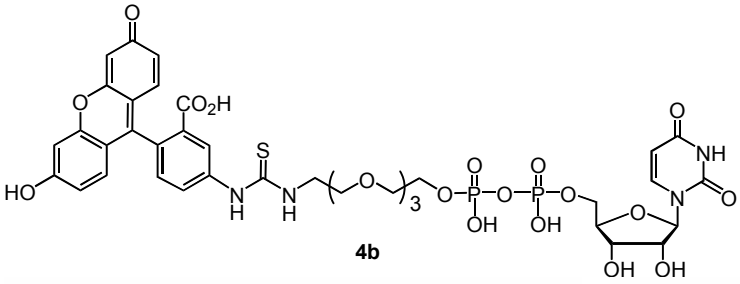


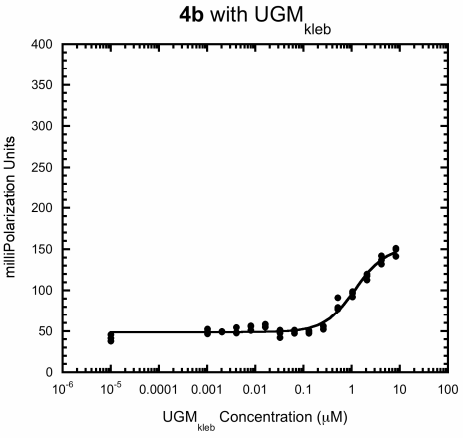
K_d = 0.77 ± 0.02 μM



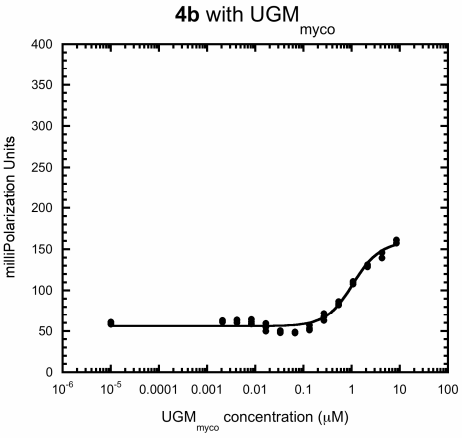
K_d = 0.58 ± 0.02 μM



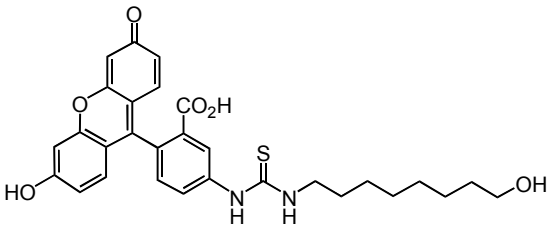
4b



K_d = 1.2 ± 0.1 μM



K_d = 1.1 ± 0.1 μM



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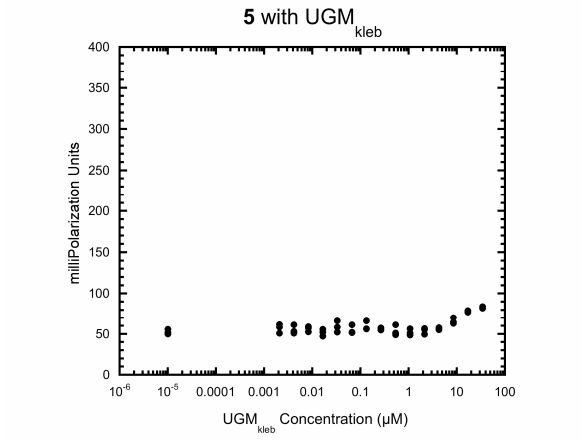
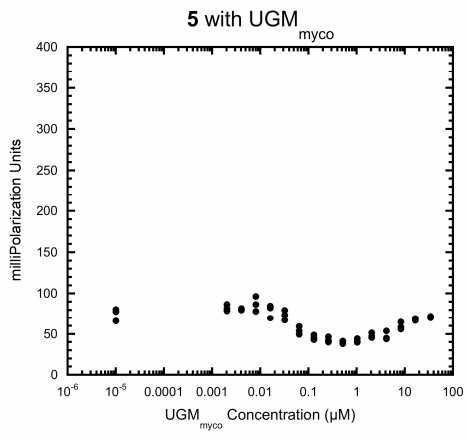
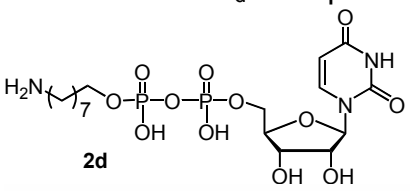


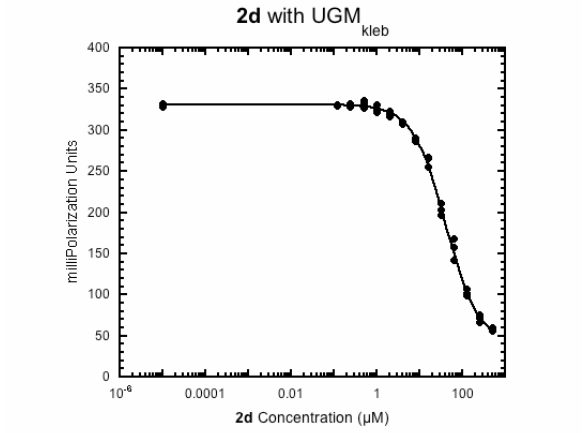
K_d > 30 μM



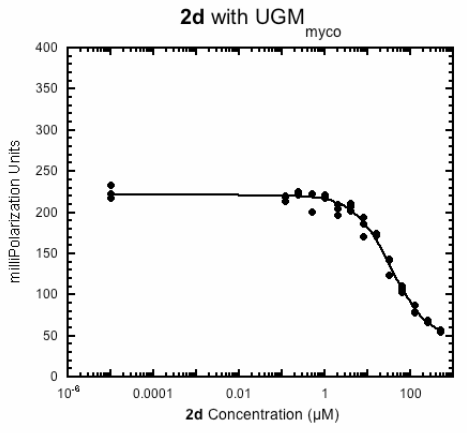
K_d > 30 μM



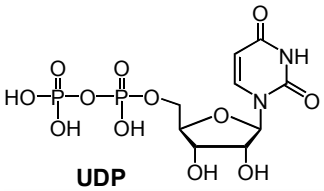
2d



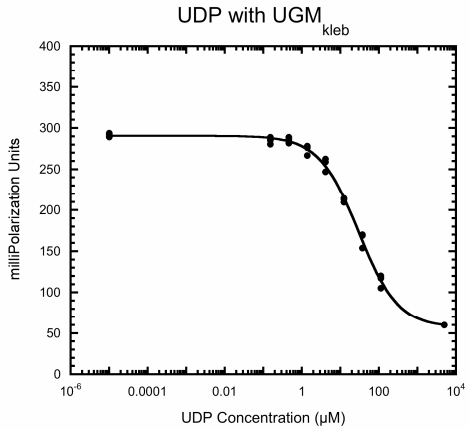
K_d = 38 ± 2 μM



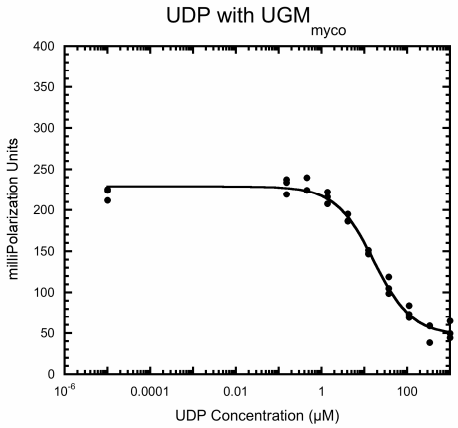
K_d = 32 ± 3 μM



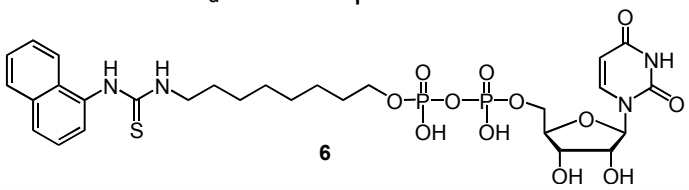
UDP



K_d = 26 ± 2 μM



K_d = 15 ± 2 μM



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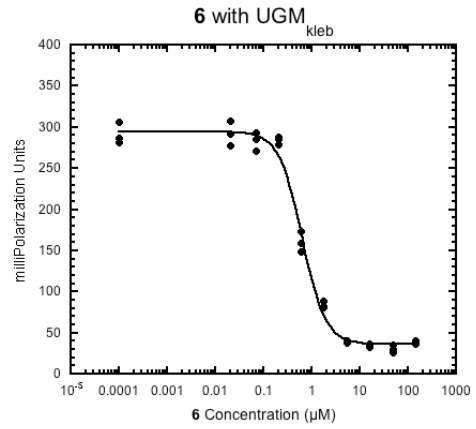
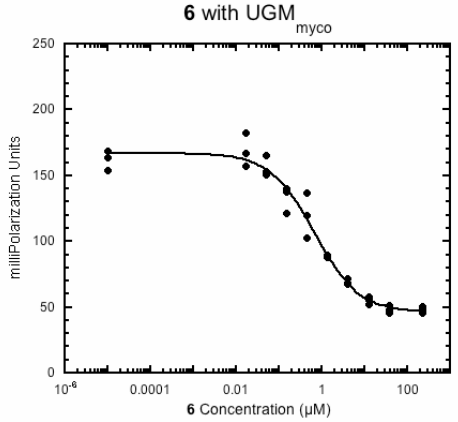
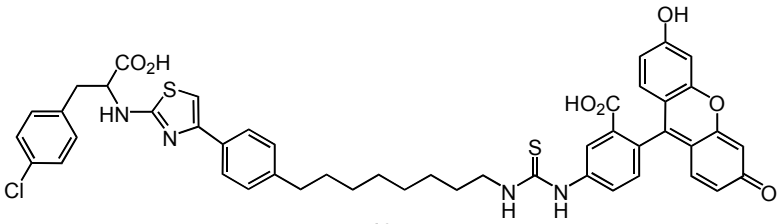


K_d = 0.58 ± 0.04 μM



K_d = 0.61 ± 0.09 μM



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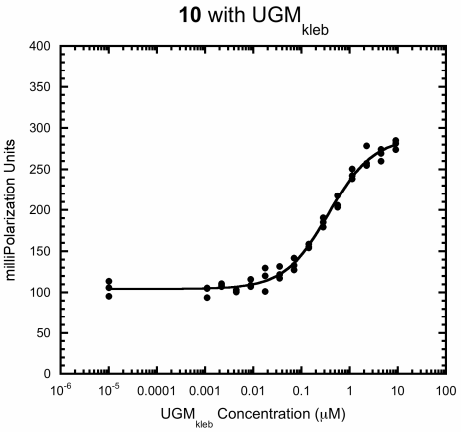
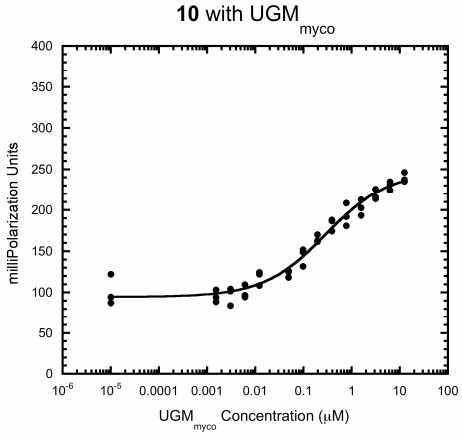


K_d = 0.39 ± 0.04 μM



K_d = 0.30 ± 0.07 μM

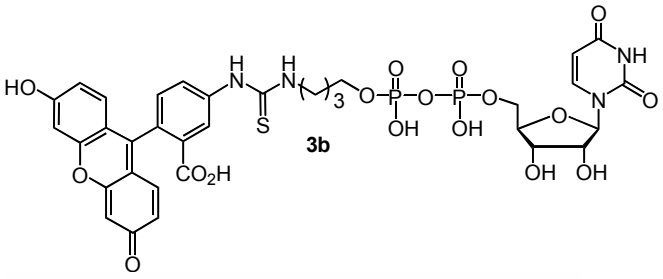
UGM Activity Assays:

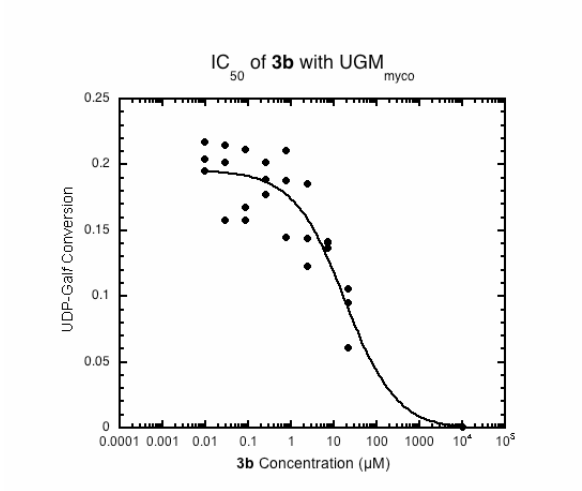
UGM_{myco} inhibition was assessed using a previously described HPLC assay to measure the extent of enzymatic conversion of UDP-galactofuranose to UDP-galactopyranose.[8] The relative activity of UGM_{myco} was compared to that in the presence of varying concentrations of the UDP-fluorescein conjugates **3b**, **3c**, and **3d**. Enzymatic reaction conditions follow: 20 µM UDP-Gal*f*, 20 nM UGM_{myco} and 20 mM sodium dithionite in 50 mM sodium phosphate buffer pH 7.0. Final reaction volumes were 60 µL. Reactions were performed at 37 °C for 30 s, and quenched with 60 µL of 1:1 $CHCl_3$/MeOH.

The relative activity of UGM_{myco} in the presence of DMSO alone was compared to that in the presence of aminothiazole-fluorescein conjugate **10**. Enzymatic reaction conditions follow: 20 µM UDP-Gal*f*, 20 nM UGM_{myco} and 20 mM sodium dithionite in 5% DMSO v/v in 50 mM sodium phosphate buffer, pH 7.0. Final reaction volumes were 60 µL. Reactions were performed at 37 °C for 40 s, and quenched with 60 µL of 1:1 $CHCl_3$/MeOH.

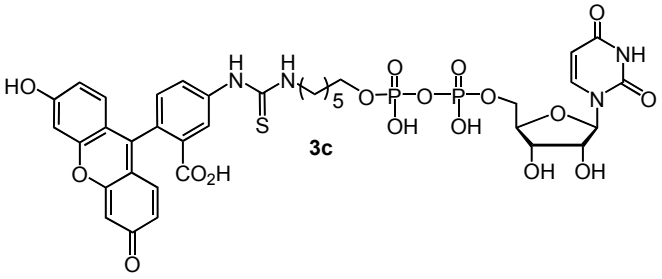
Conversion is the amount of UDP-Gal*p* formed divided by the amount of total UDP-Gal (UDP-Gal*p* + UDP-Gal*f*).

The data were fit to the equation as described.[8]

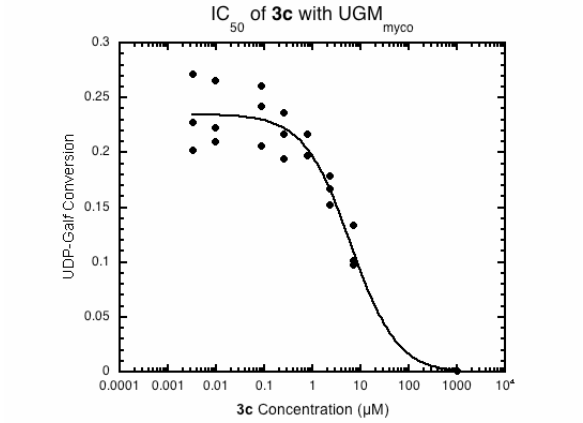




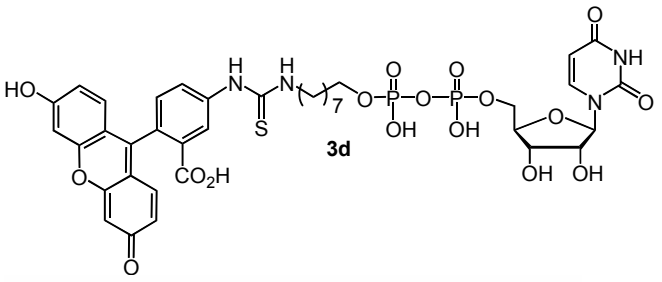
IC_{50} = 18 ± 7 µM



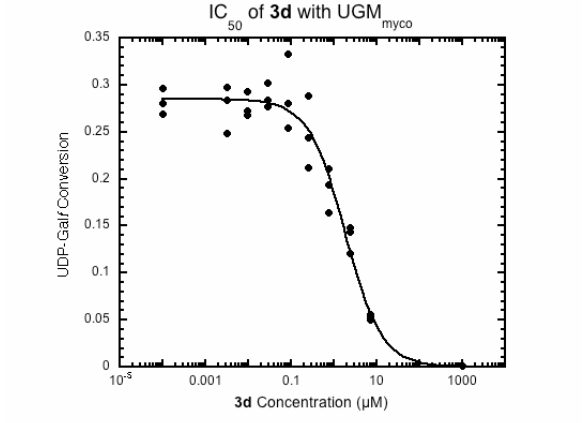
3c



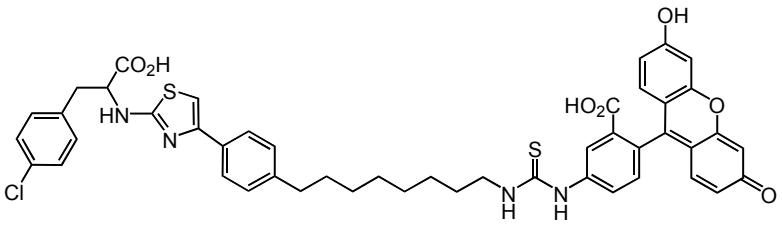
IC_{50} = 6 ± 1 μM



3d



IC_{50} = 1.9 ± 0.3 μM



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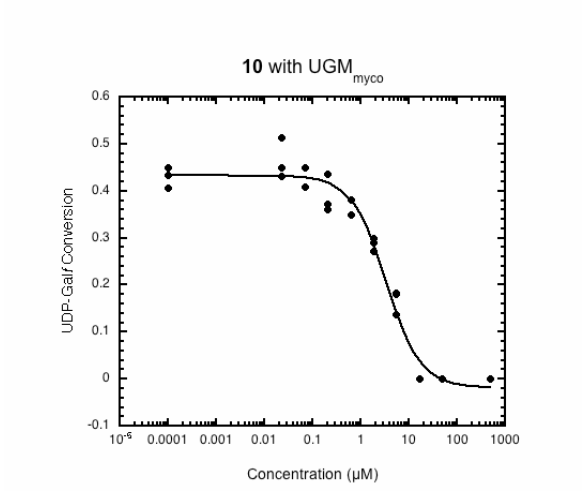
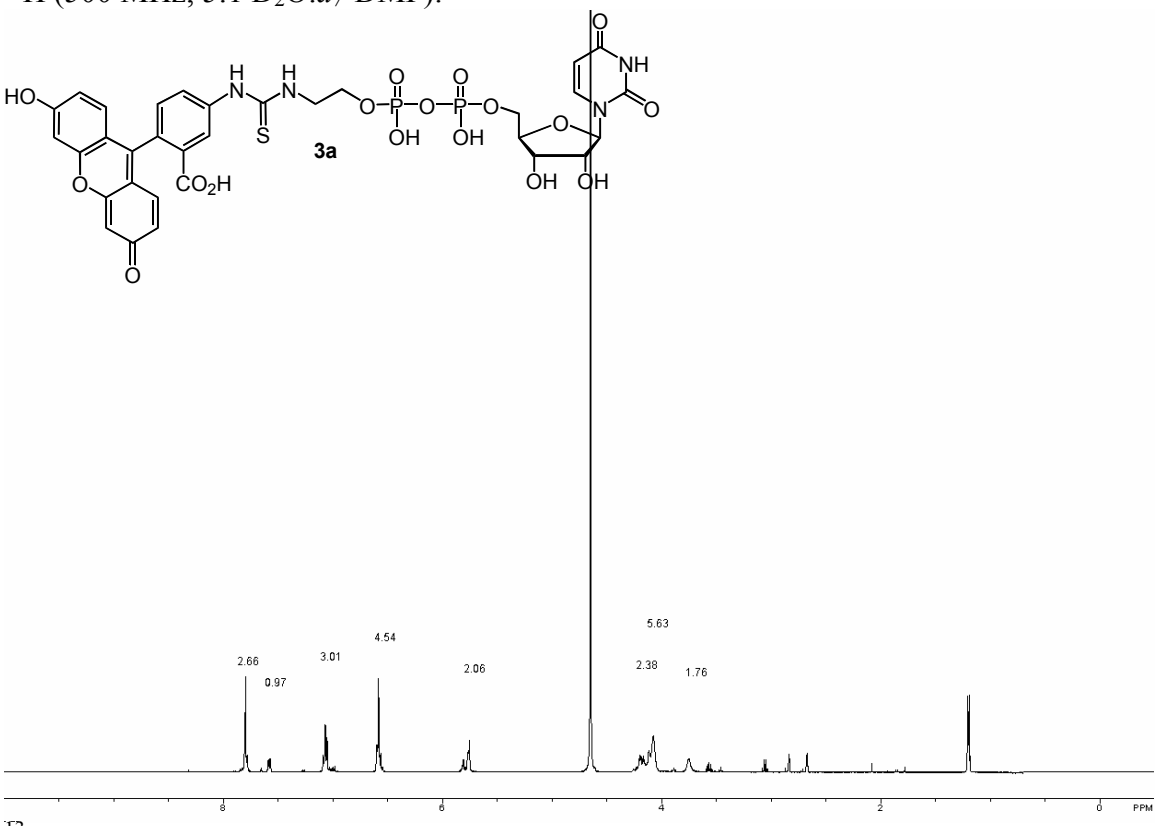
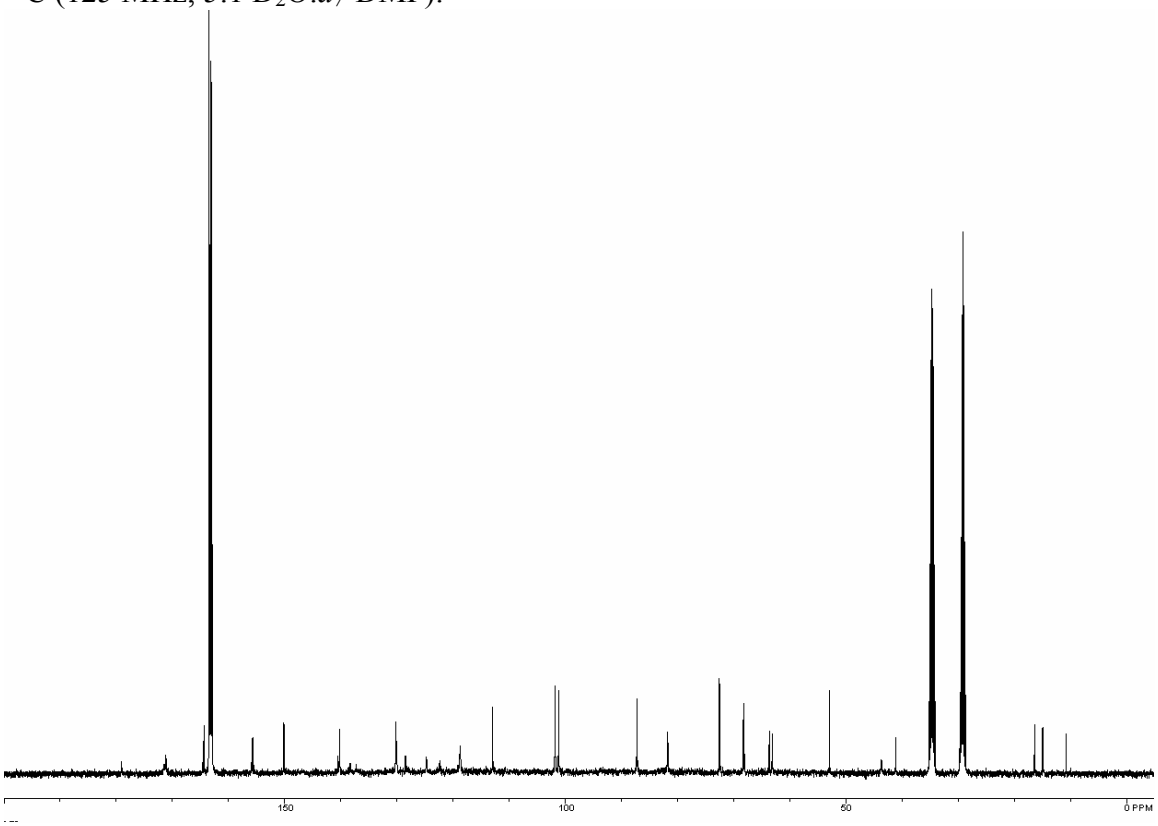


IC_{50} = 3.5 ± 0.5 μM

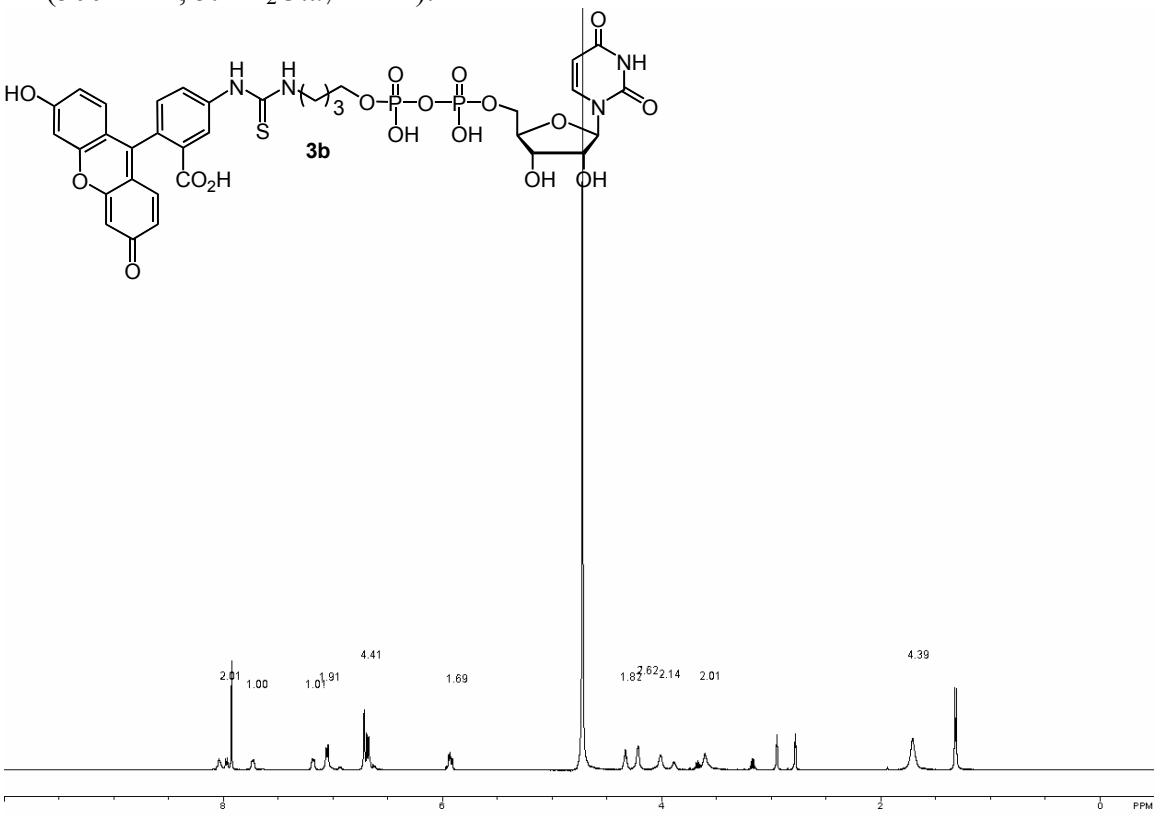
^{1}H (500 MHz, 5:1 D_2O:d_7-DMF):



^{13}C (125 MHz, 5:1 D_2O:d_7-DMF):



^{1}H (500 MHz, 5:1 D_2O:d_7-DMF):



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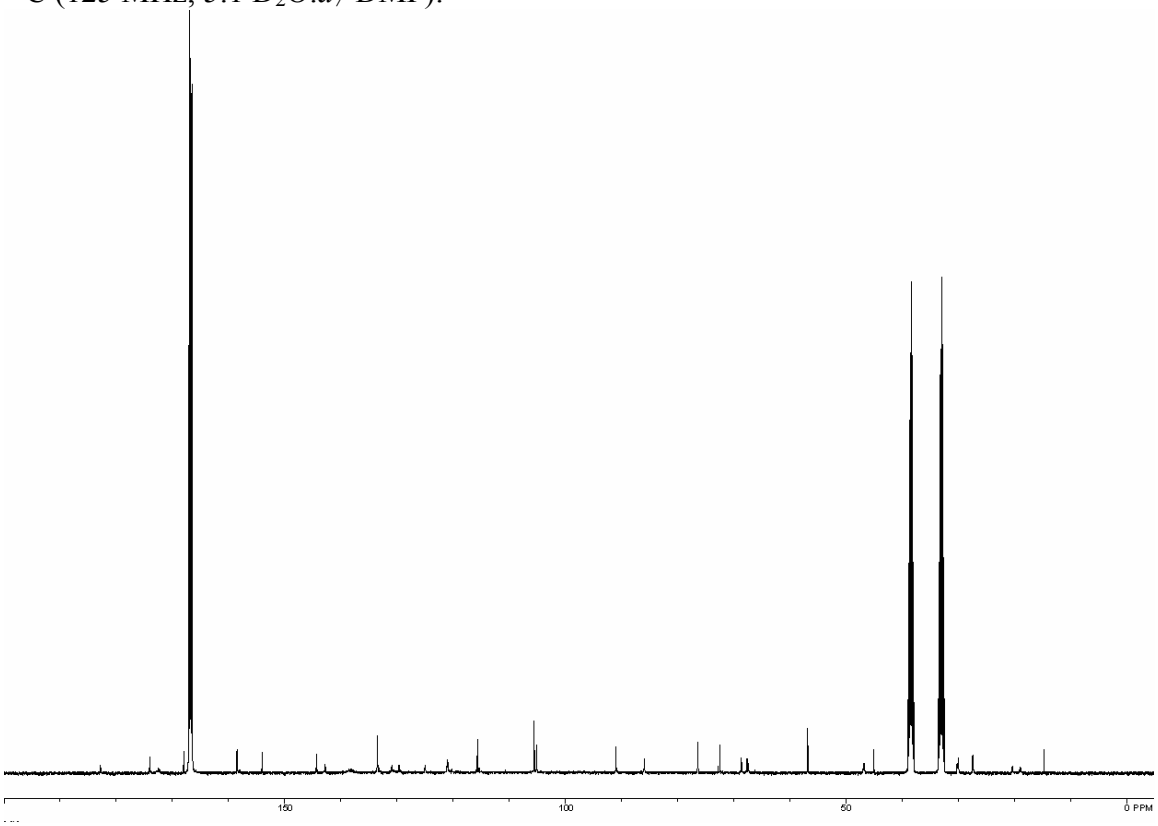
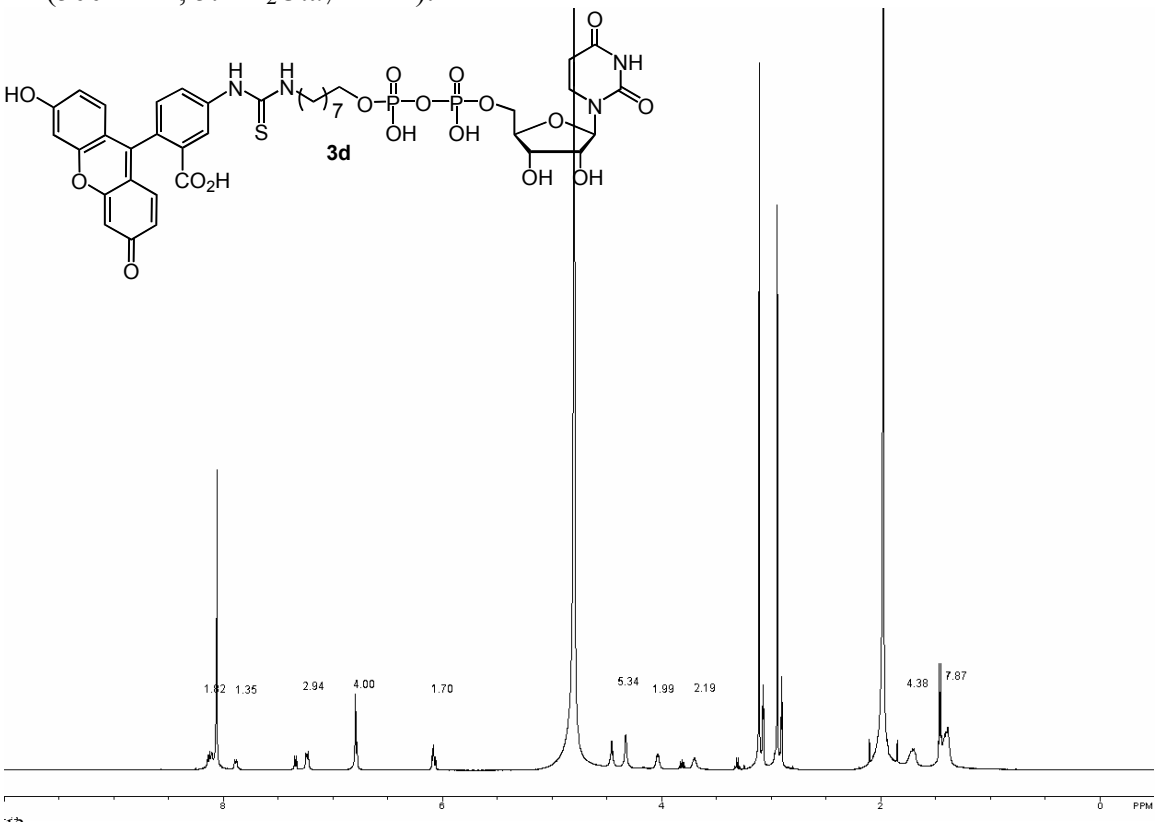


^{1}H (500 MHz, 5:1 D_2O:d_7-DMF):



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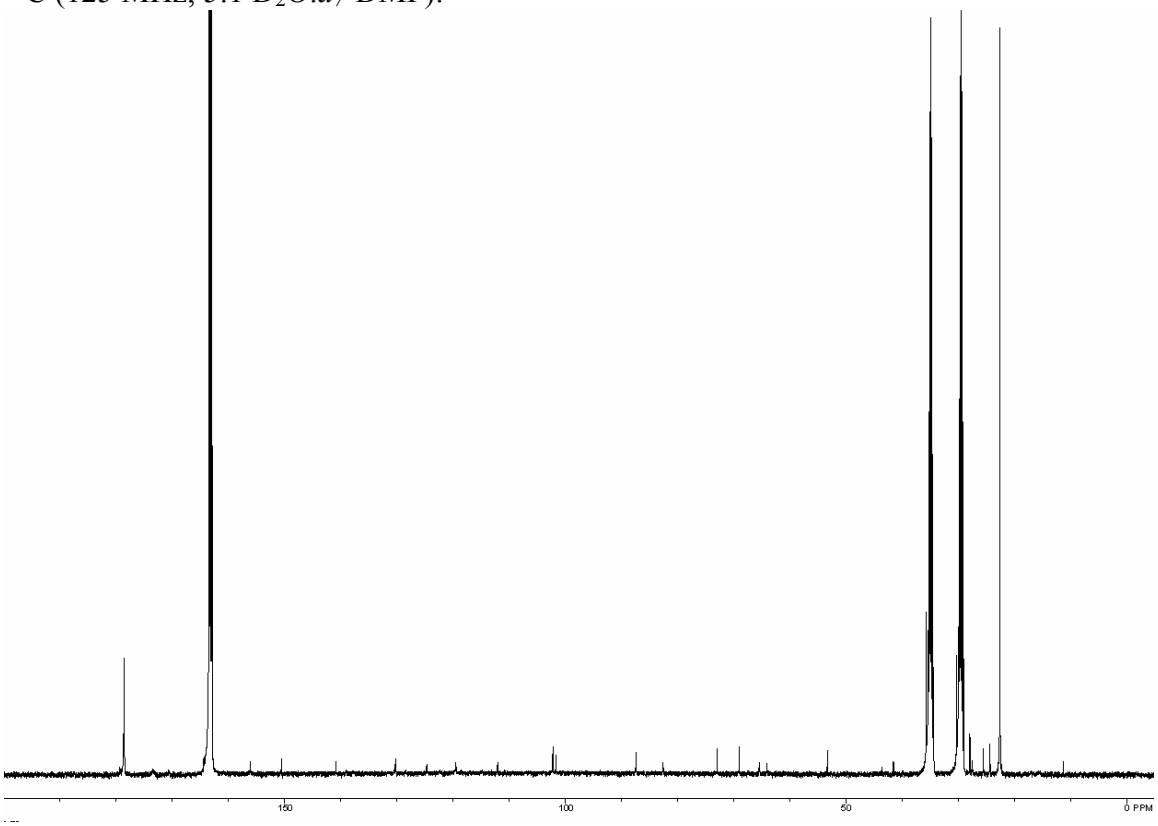

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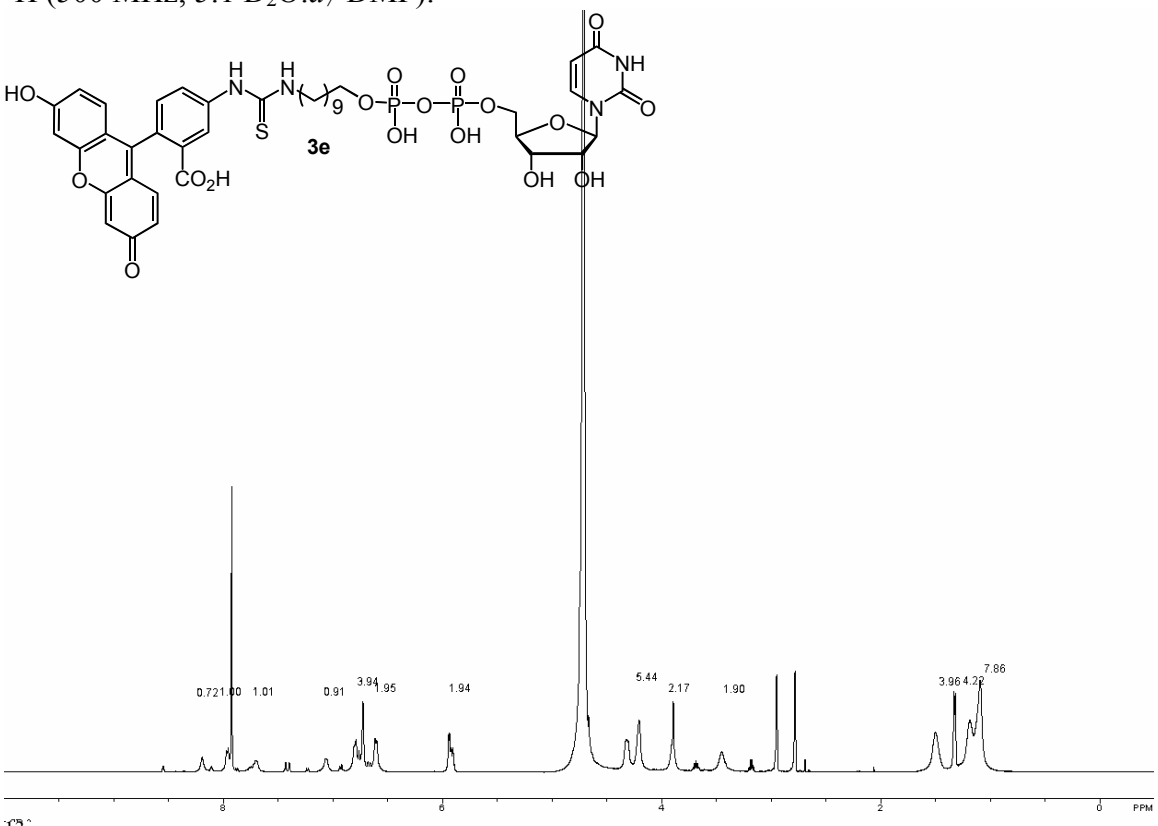
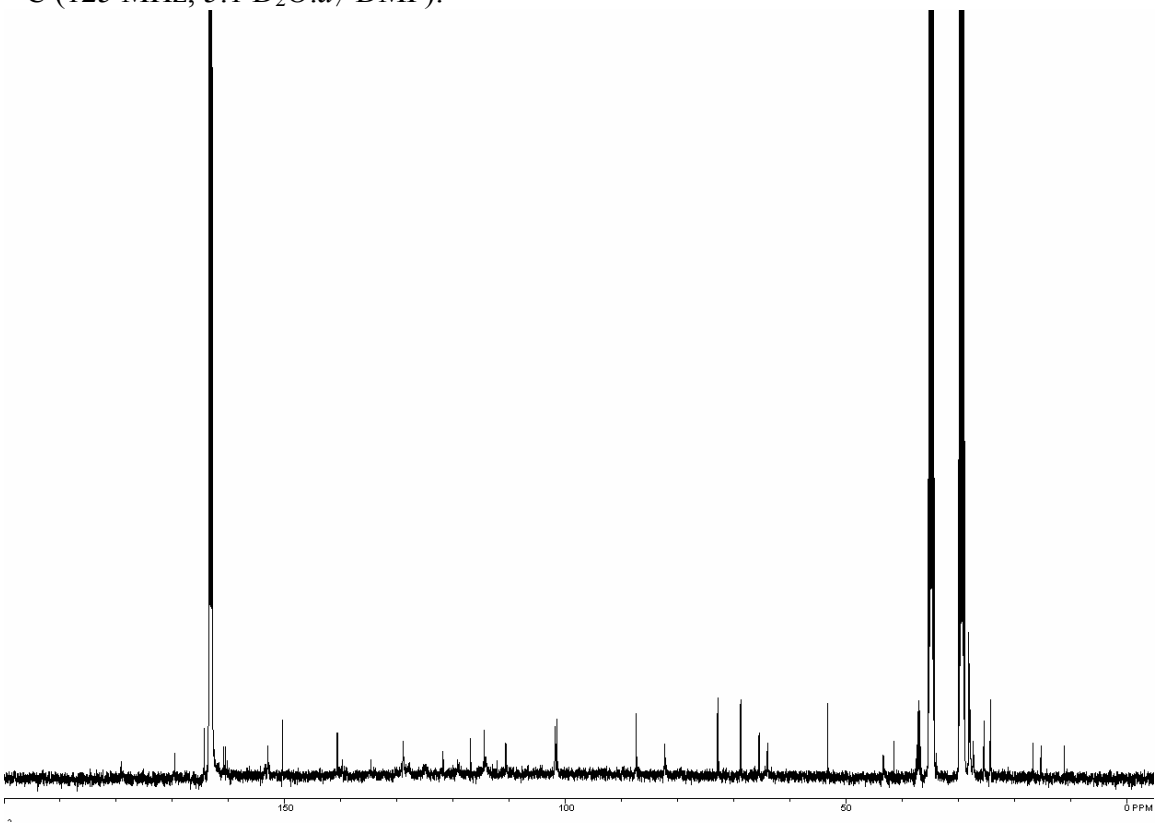
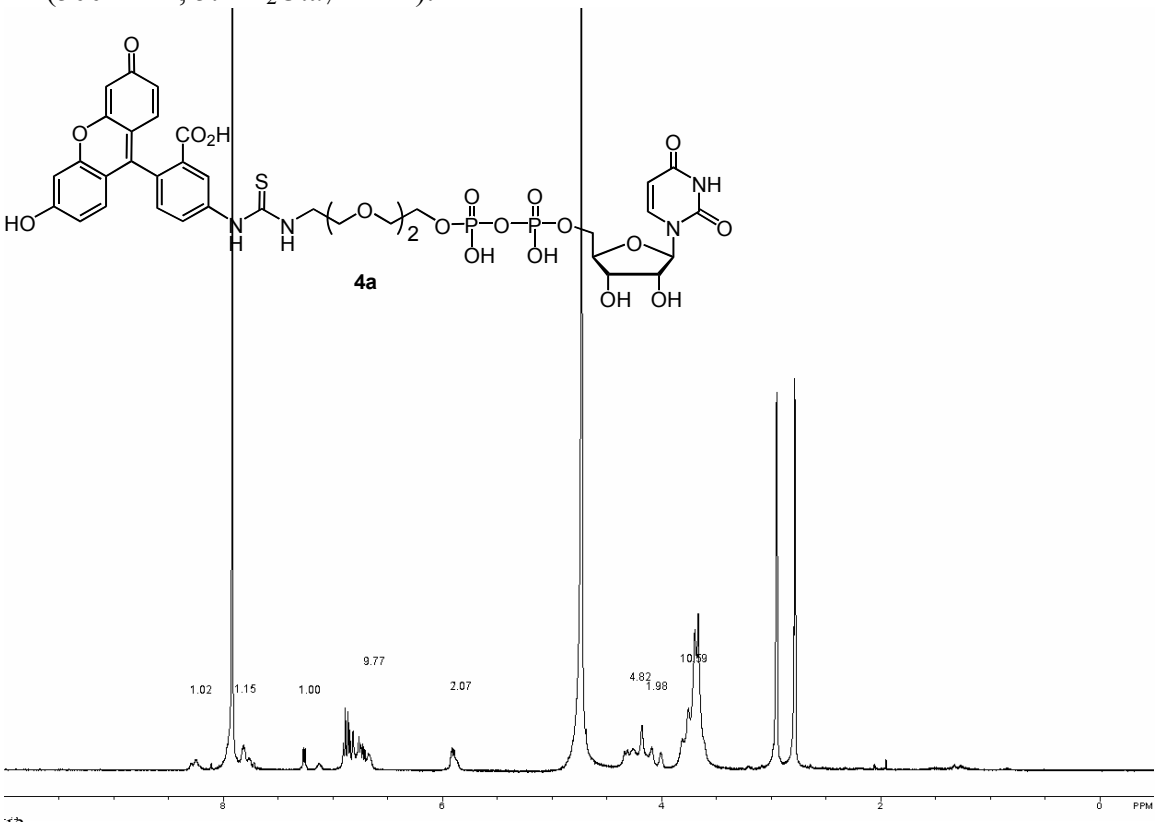


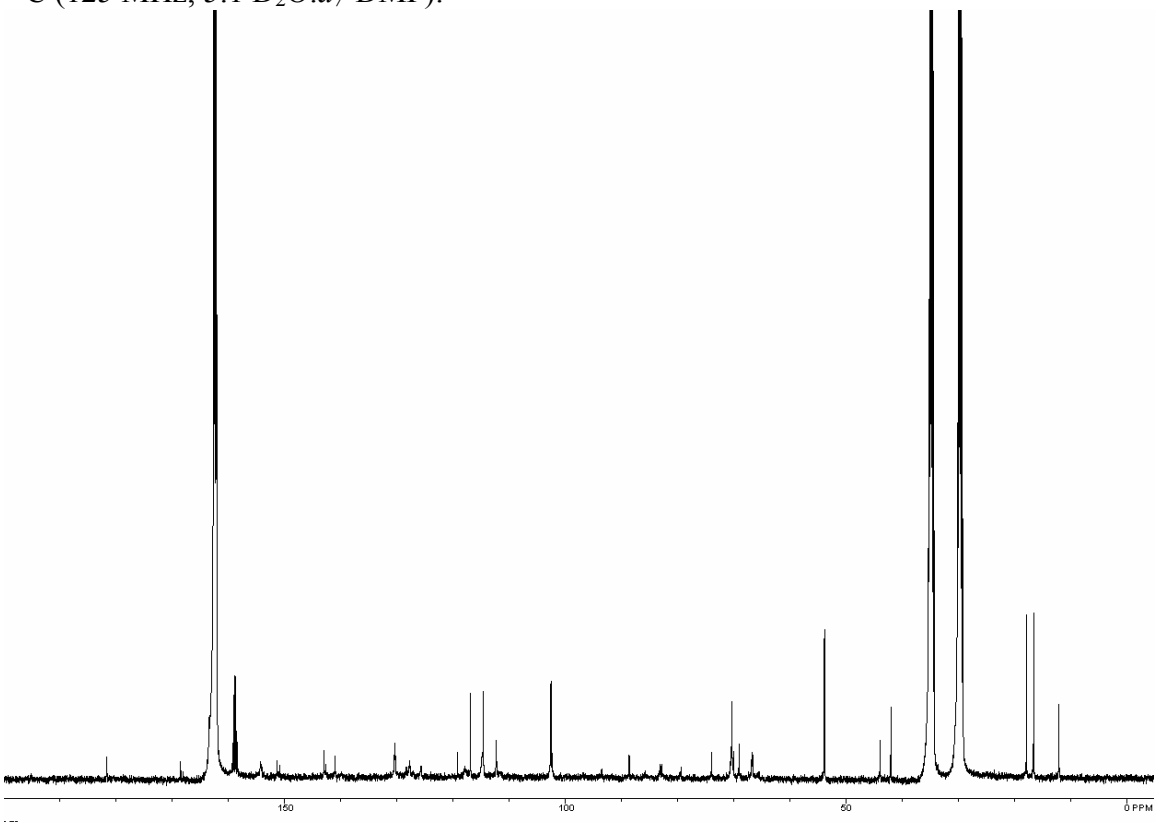
^{13}C (125 MHz, 5:1 D_2O:d_7-DMF):



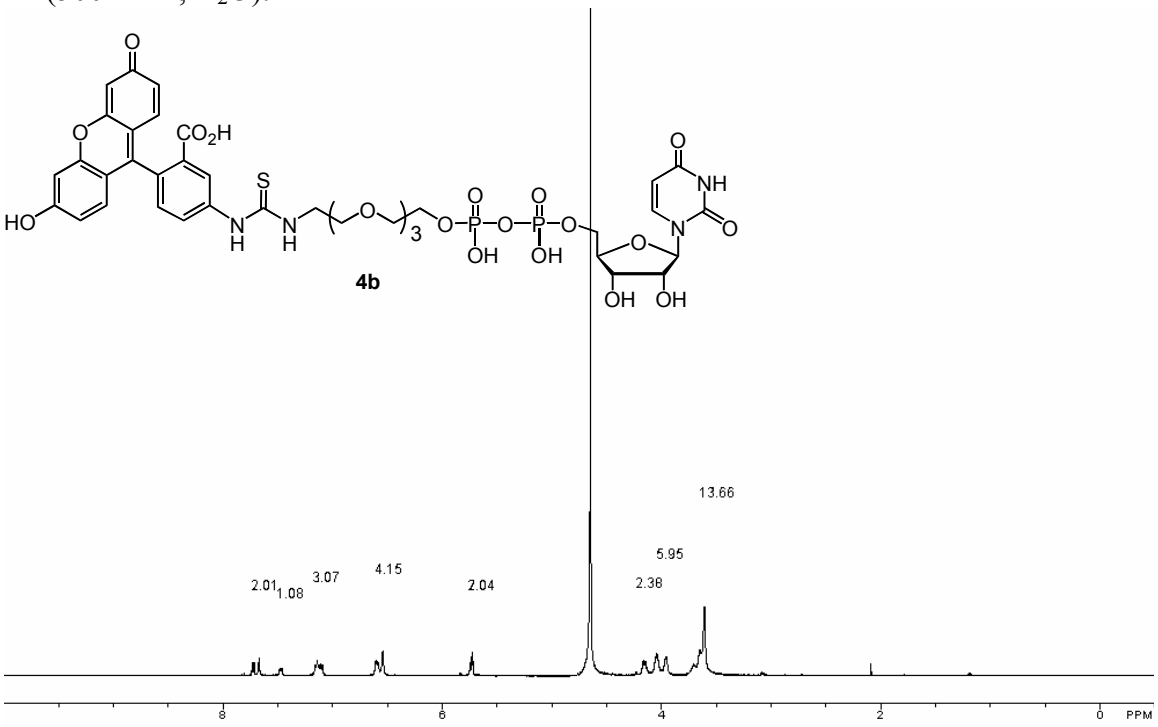
^{1}H (500 MHz, 5:1 D_2O:d_7-DMF):



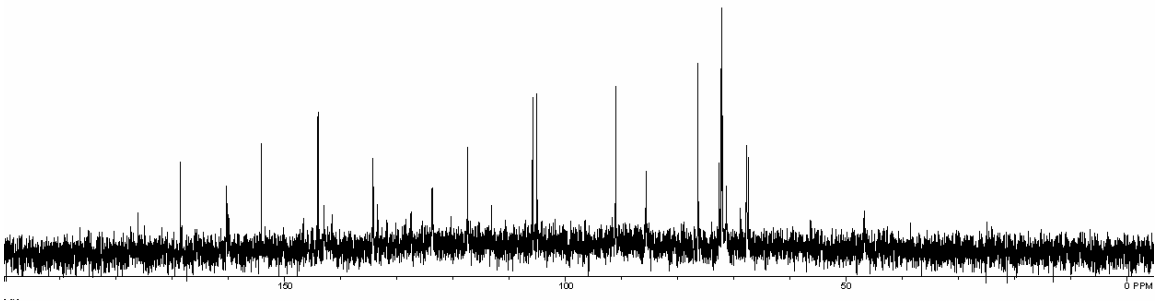
^{13}C (125 MHz, 5:1 D_2O:d_7-DMF):



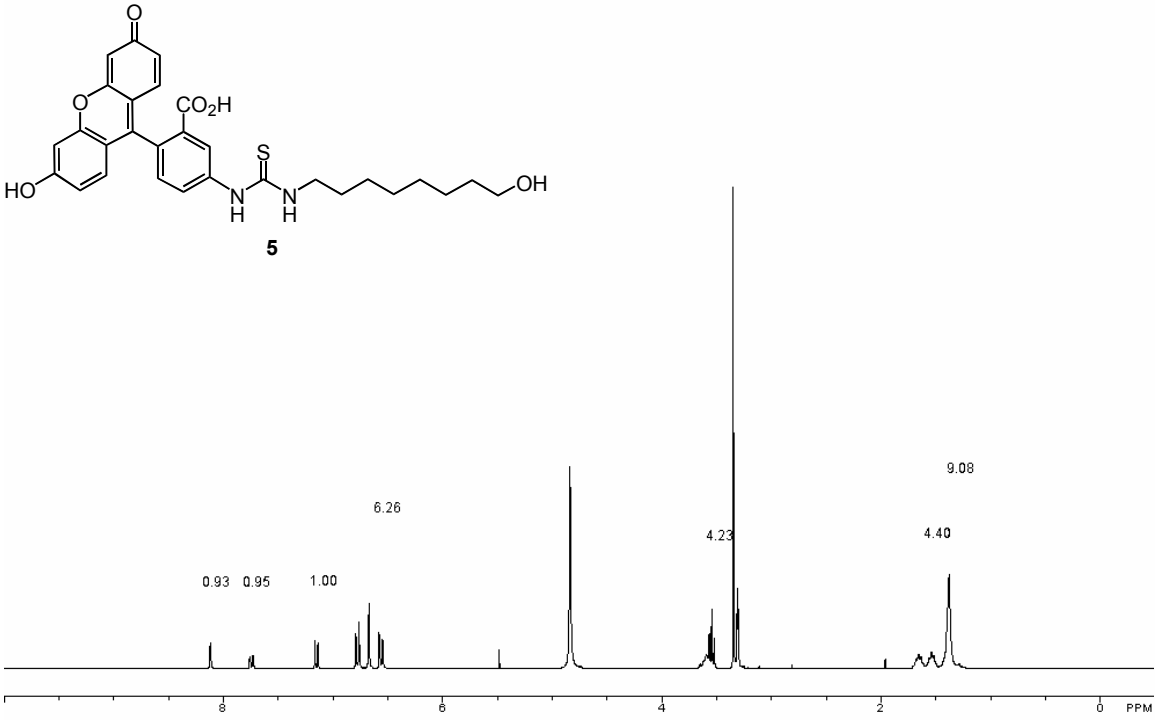
^{1}H (500 MHz, D_2O):



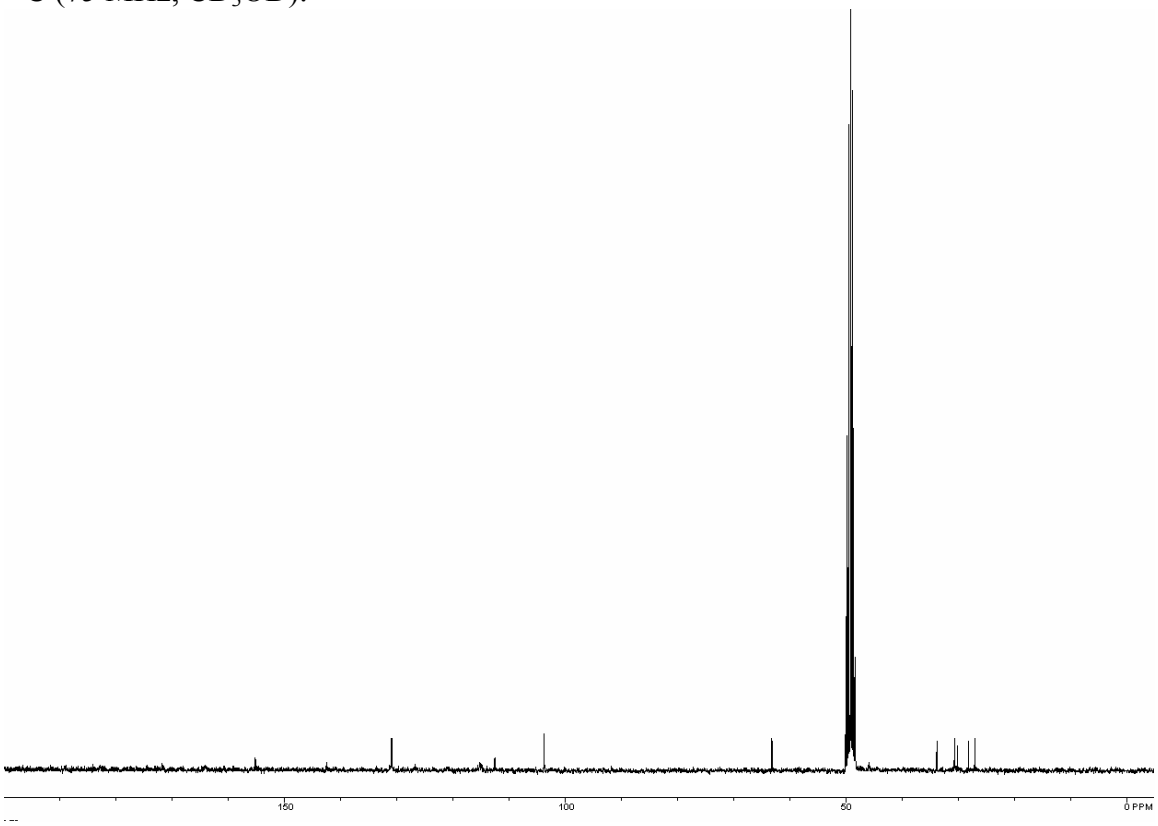
^{13}C (125 MHz, D_2O):



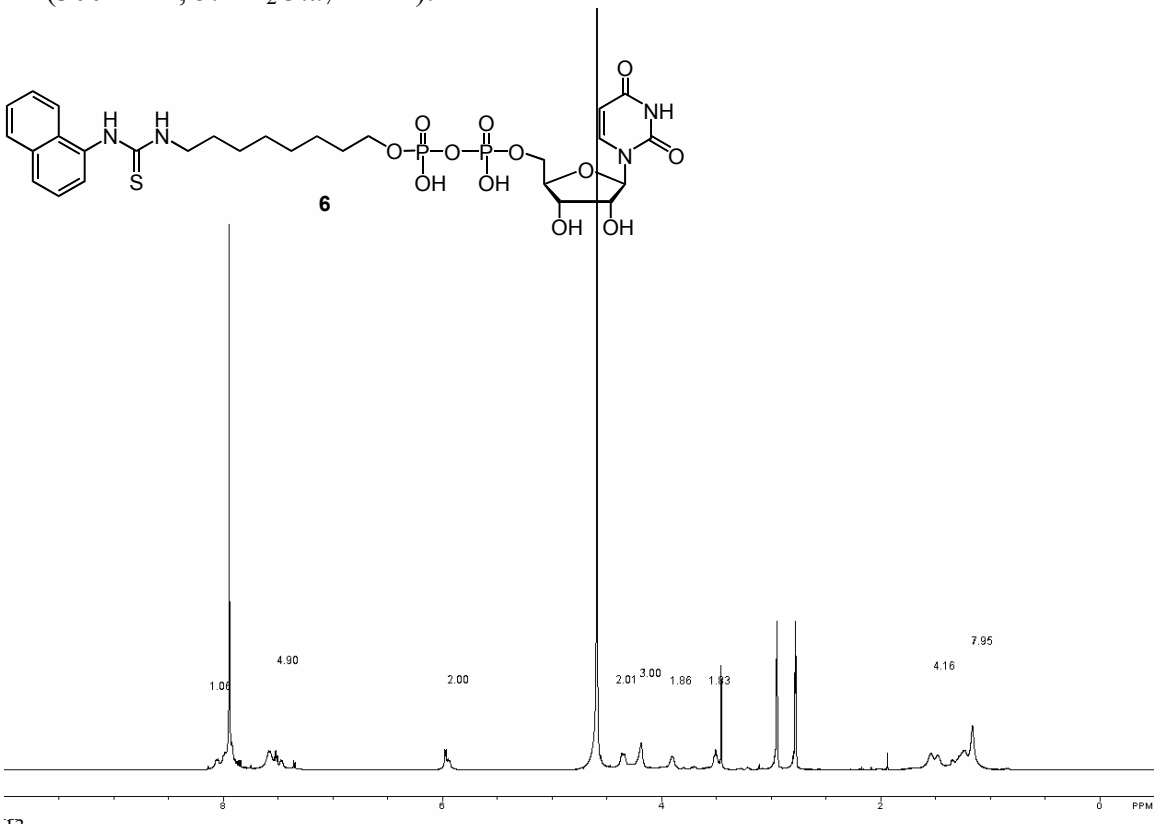
^{1}H (300 MHz, CD_3OD):



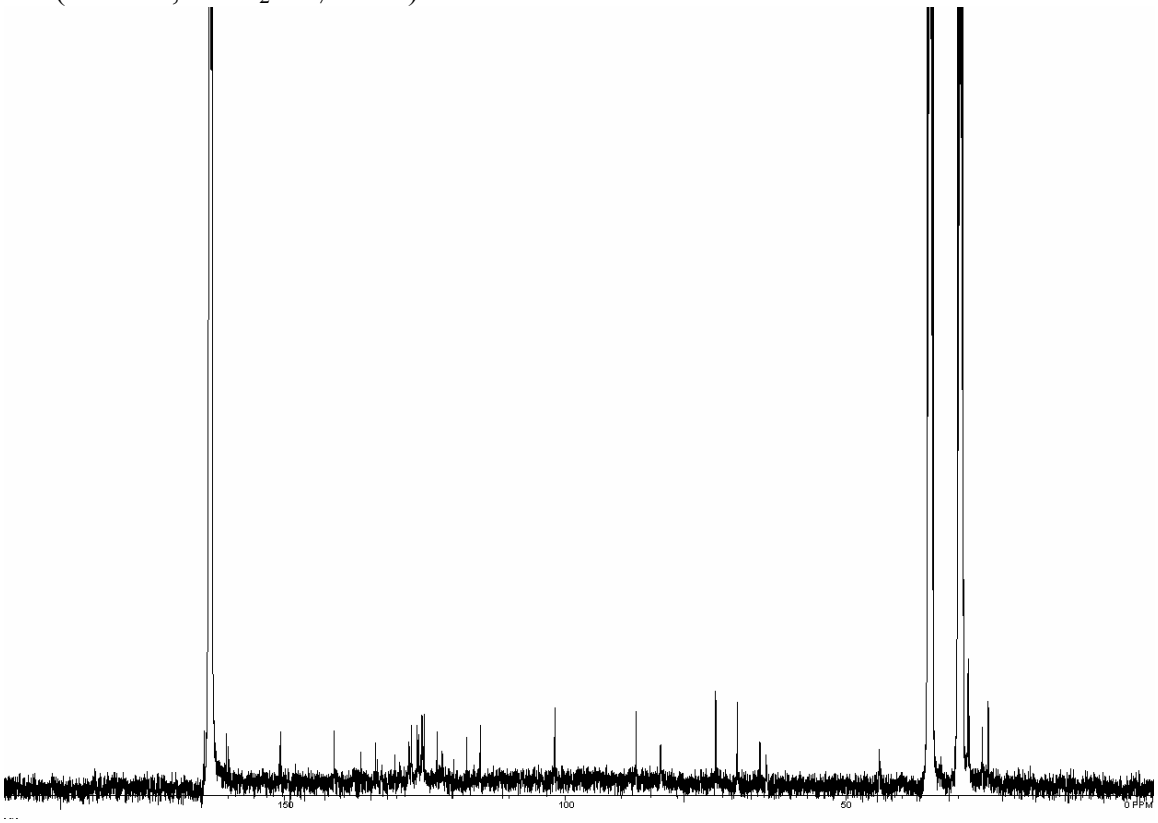
^{13}C (75 MHz, CD_3OD):



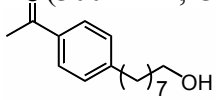
^{1}H (300 MHz, 5:1 D_2O:d_7-DMF):

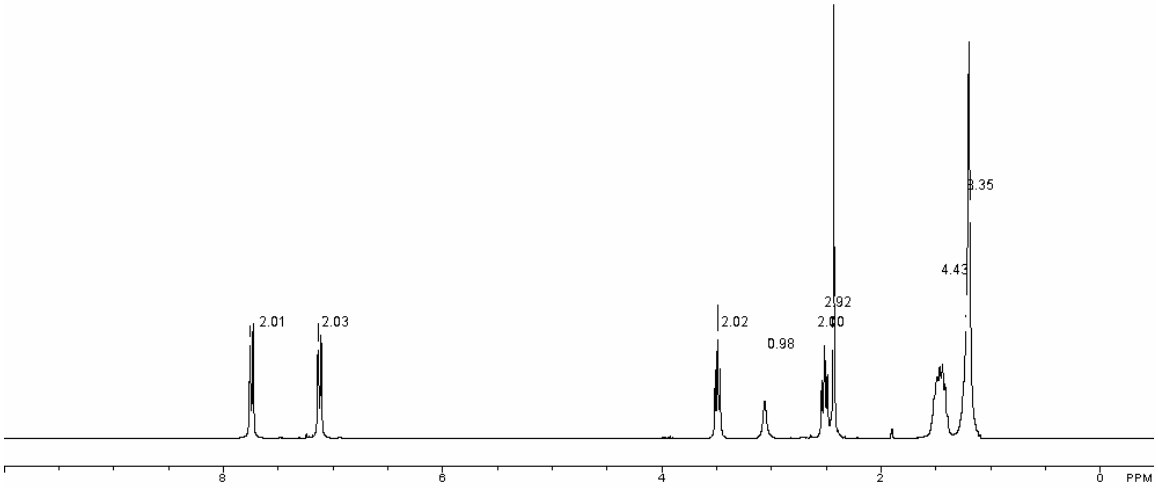


^{13}C (75 MHz, 5:1 D_2O:d_7-DMF):

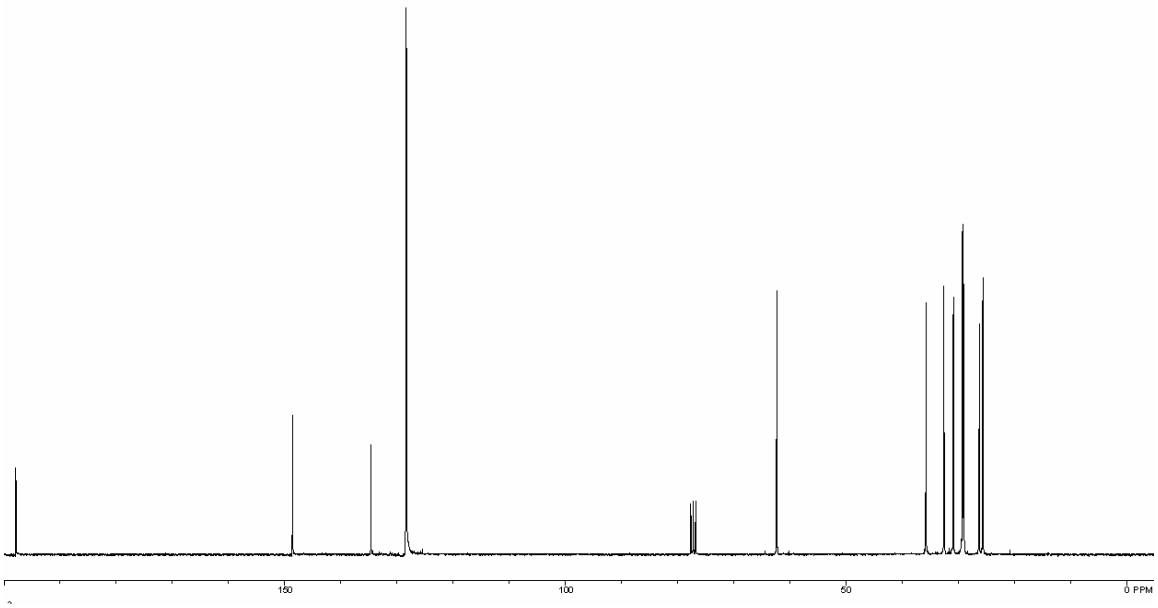


^{1}H (300 MHz, $CDCl_3$):

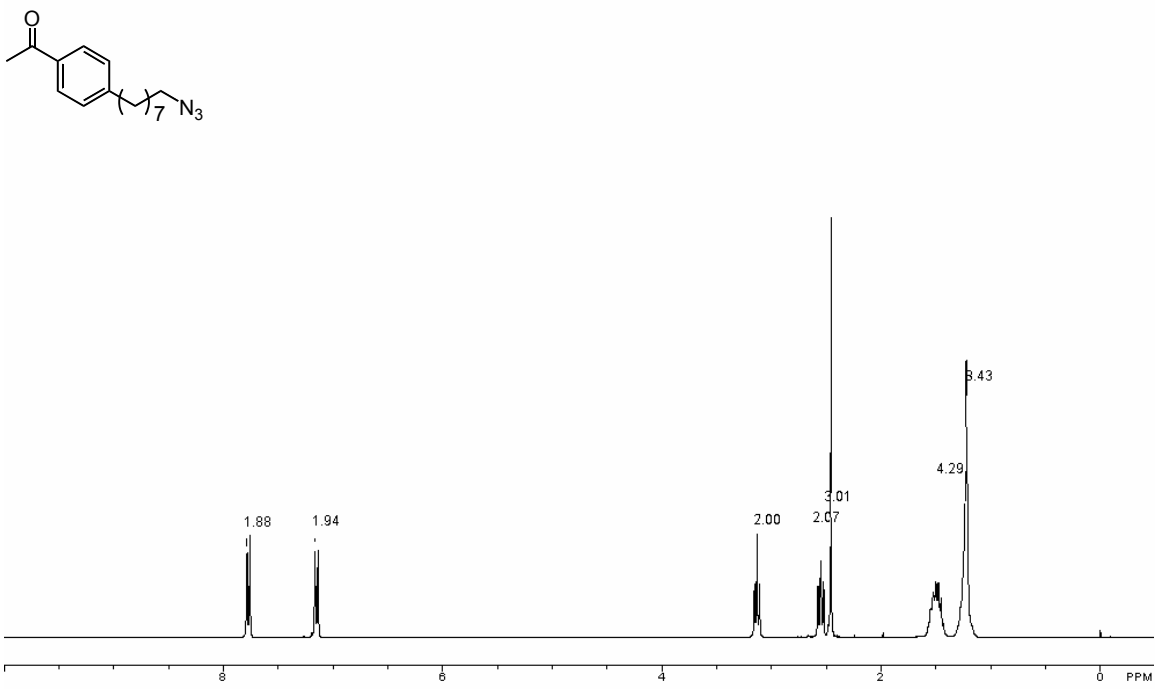




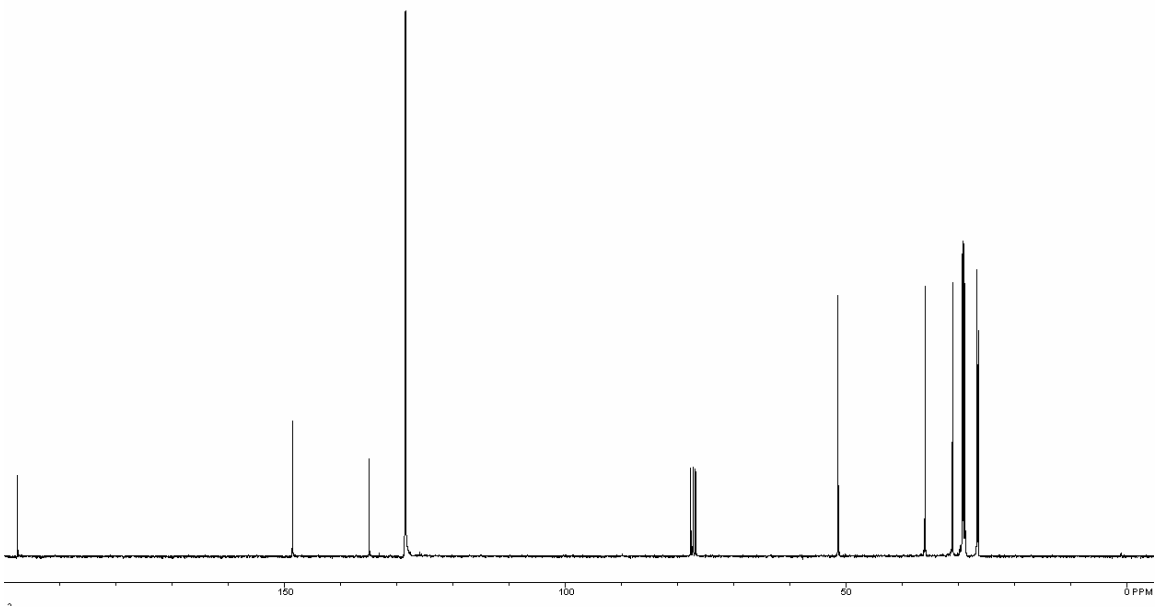
^{13}C (75 MHz, $CDCl_3$):



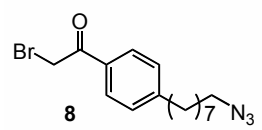
^{1}H (300 MHz, $CDCl_3$):



^{13}C (75 MHz, $CDCl_3$):



^{1}H (300 MHz, $CDCl_3$):



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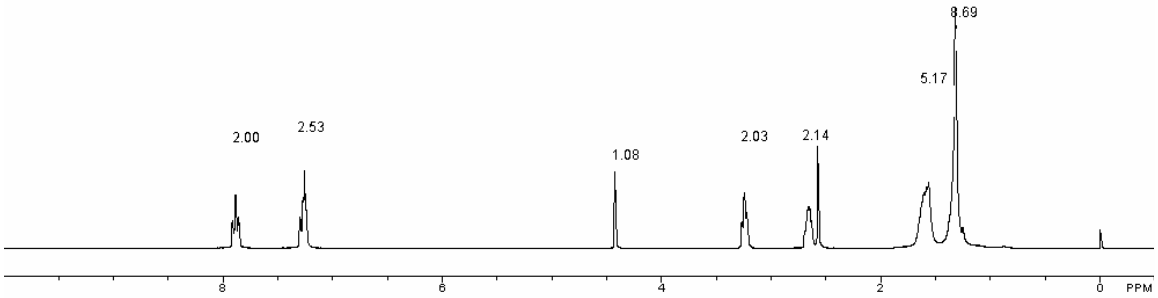
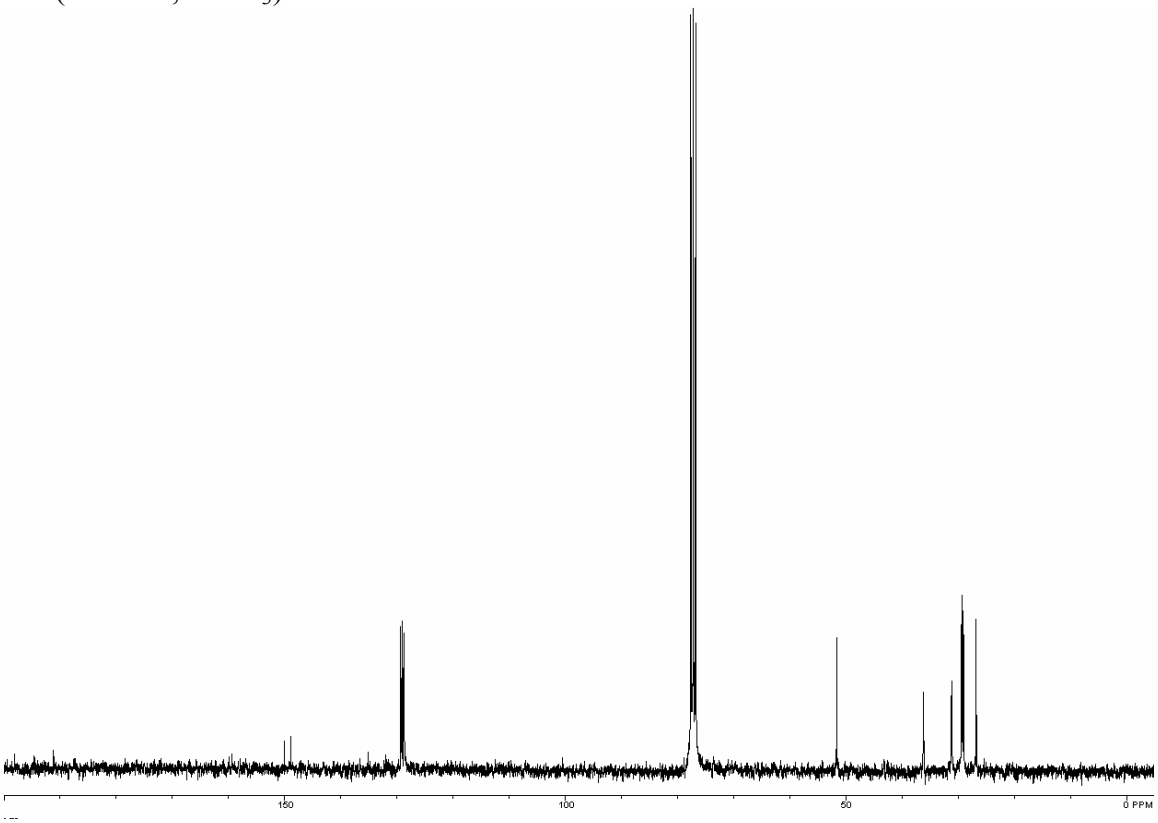
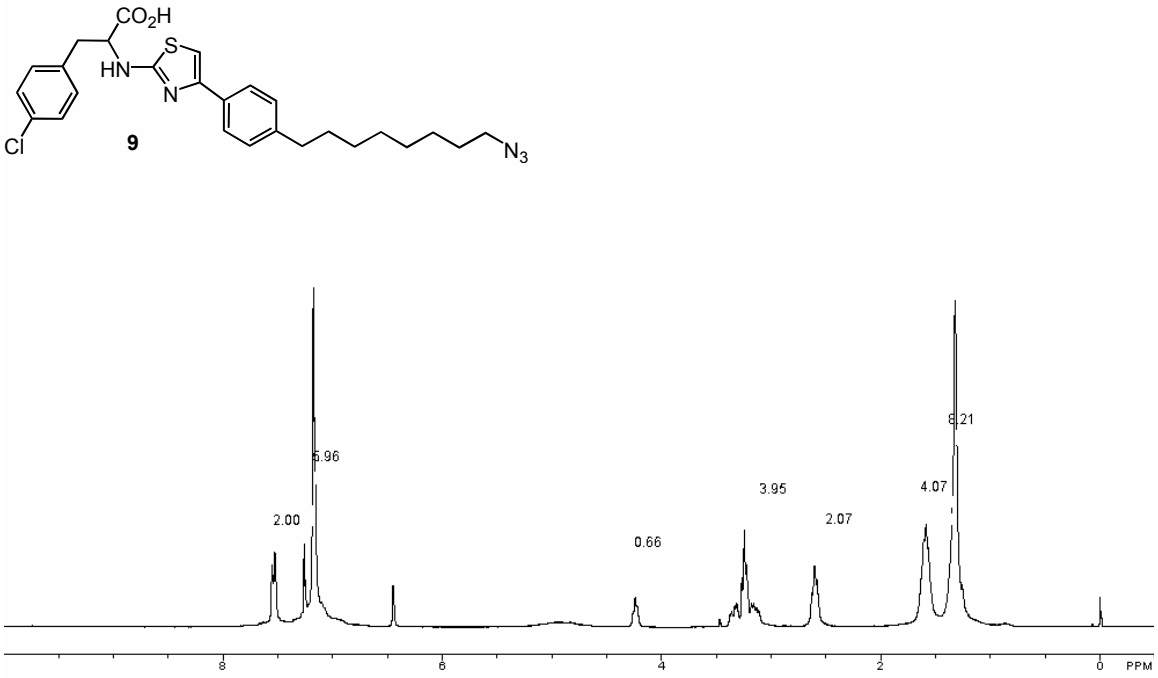


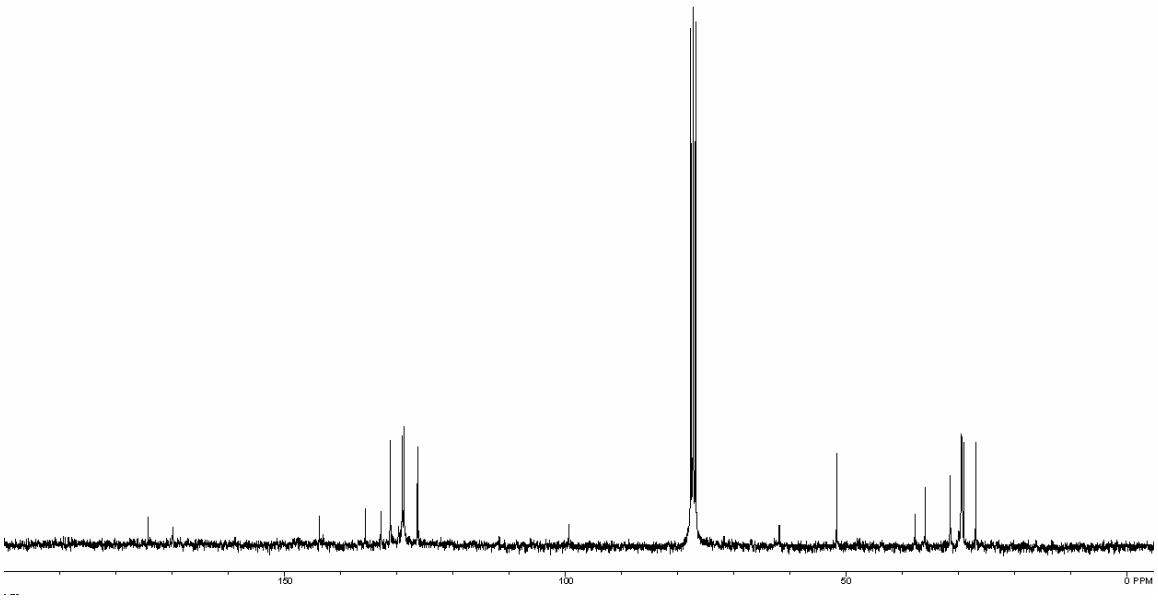
^{13}C (75 MHz, $CDCl_3$):



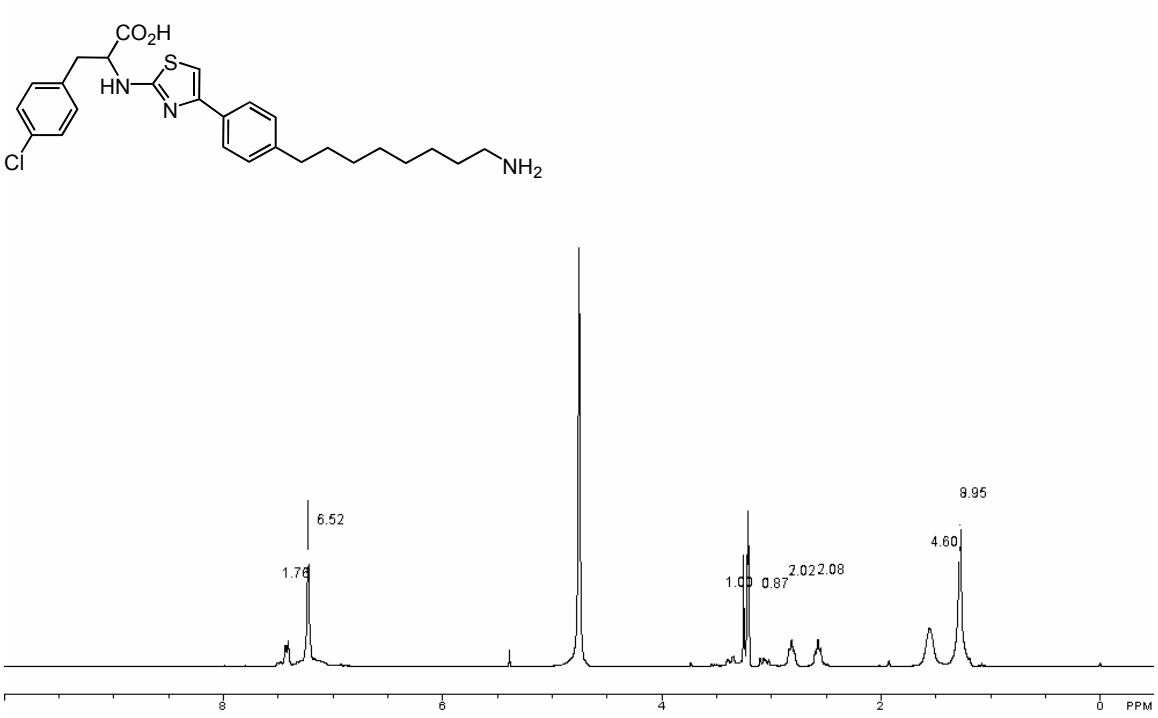
^{1}H (300 MHz, $CDCl_3$):



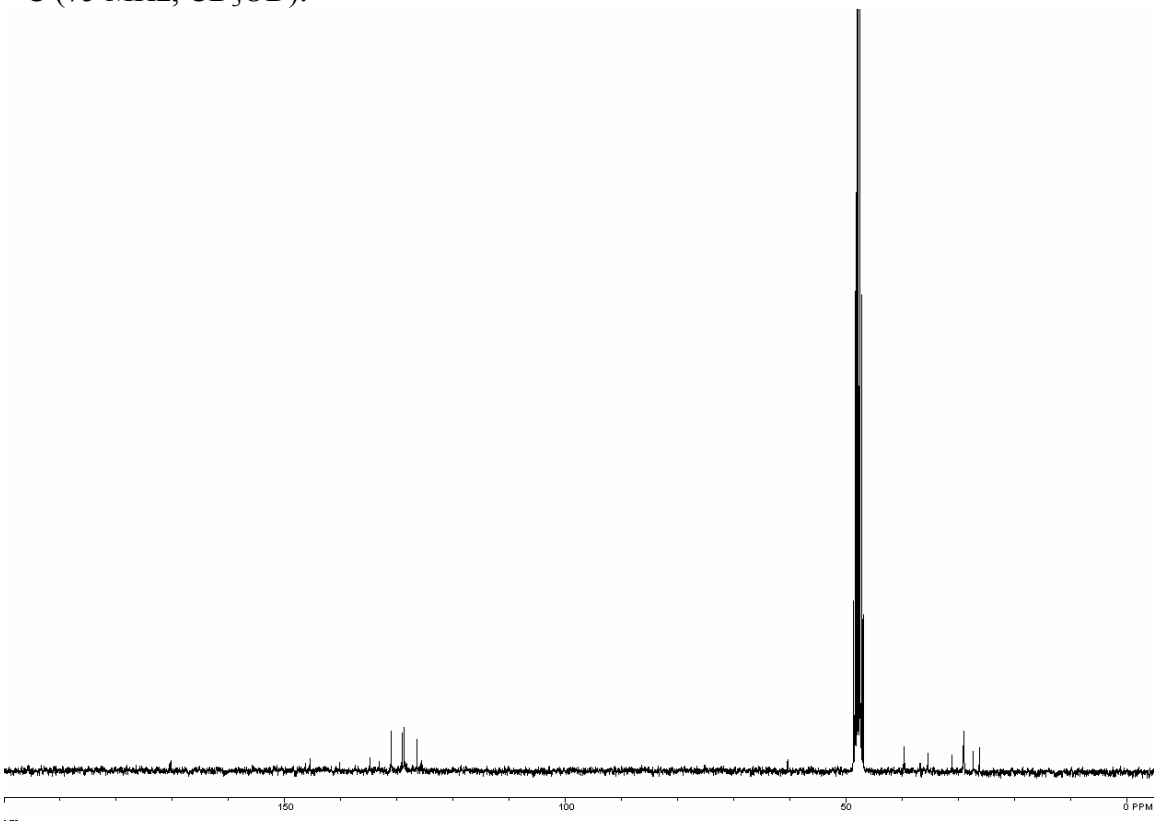
^{13}C (75 MHz, $CDCl_3$):



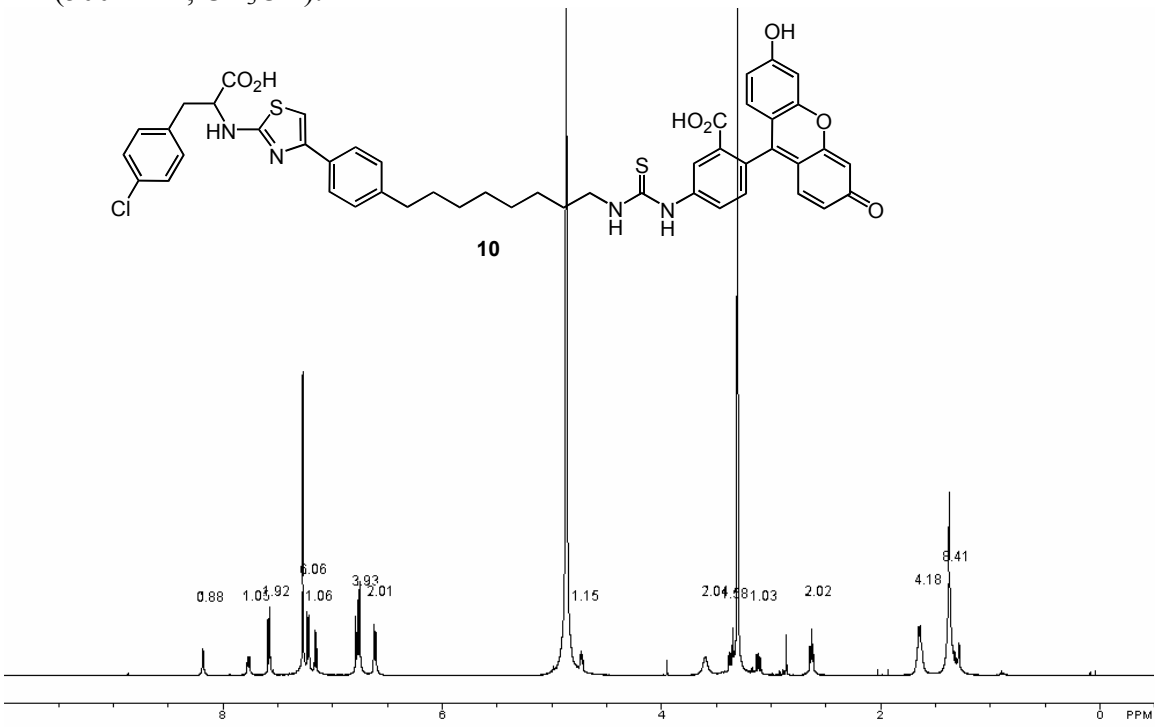
^{1}H (300 MHz, CD_3OD):



^{13}C (75 MHz, CD_3OD):



^{1}H (500 MHz, CD_3OD):



^{13}C (125 MHz, CD_3OD):