Supporting Information for:

One-flask Syntheses of c-di-GMP and the [R_p,R_p] and [R_p,S_p] Thiophosphate Analogs

Barbara L. Gaffney, Elizabeth Veliath, Jianwei Zhao, and Roger A Jones *

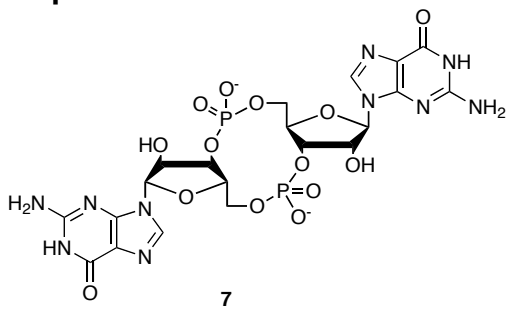
Department of Chemistry and Chemical Biology, 610 Taylor Road, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08854

Table of Contents:

A. General Methods

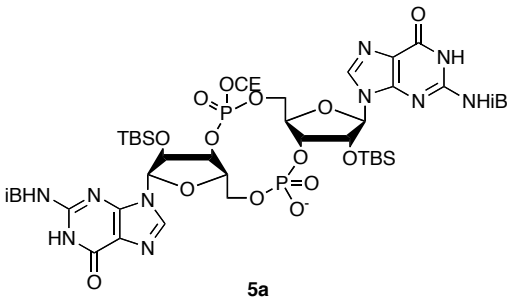
The amidite coupling reactions and cyclizations were carried out in anhydrous CH_3CN or pyridine, respectively, that had been dried using a silica solvent purification system. Anhydrous reactions were carried out under nitrogen. Preparative silica gel chromatography was carried out on pre-packed silica gel Super Flash columns from Varian using gradients of CH_3OH in CH_2Cl_2. Analytical reversed phase HPLC was carried out on a Waters 2960 system with a photodiode array detector, with an Atlantis C18 column, 100Å, 4.6 mm × 50 mm, 3.0 μm. Gradients of CH_3CN and 0.1 M triethylammonium acetate buffer (pH 6.8) were used with a flow rate of 1.0 mL/min. Chromatograms in Section D are shown at 250 nm. Low resolution ESI-MS was routinely acquired using a Waters Micromass single quadrupole LCZ system. High resolution ESI-MS for products was obtained from the Washington University Resource for Biomedical and Bio-organic Mass Spectrometry in St. Louis MO. The ^1H, ^{13}C, and ^{31}P NMR spectra were acquired on a Varian Inova 500 MHz spectrometer. The ^1H and ^{13}C spectra acquired in DMSO-d_6 or D_2O were referenced indirectly to 3-(trimethylsilyl)-1-propane-sulfonic acid, sodium salt, in DMSO-d_6 or D_2O, respectively. The ^{31}P NMR spectra acquired in DMSO-d_6 were referenced indirectly to neat H_3PO_4 while those acquired in D_2O were referenced indirectly to 10% phosphoric acid in D_2O.

B. Preparation of 7 on a 5 mmol scale



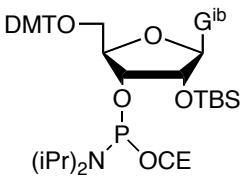
7

1. One-flask synthesis of 5a



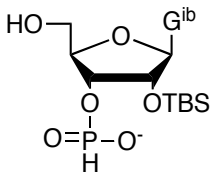
5a

a. Preparation of a dry solution of 1 in CH_3CN.
One portion of guanosine phosphoramidite, **1** (6.31 g, 6.5 mmol, 1.3 equiv), was dried three times by concentration from 40 mL portions of CH_3CN, the last time leaving 20 mL. Ten 3Å molecular sieves were added.



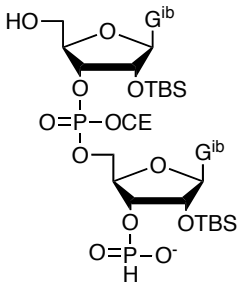
1

b. Hydrolysis, β-elimination, and detritylation.



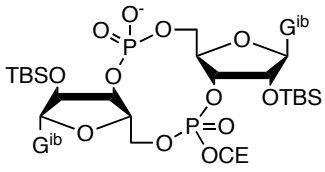
To a second portion of **1** (4.85 g, 5.0 mmol) dissolved in CH_3CN (25 mL) and H_2O (0.18 mL, 10 mmol, 2 equiv) was added pyridinium trifluoroacetate (1.16 g, 6.0 mmol, 1.2 equiv). After 1 min a 25 mL portion of *t*-$BuNH_2$ was added. After 10 min the mixture was concentrated to a foam, the residue was dissolved in a 50 mL portion of CH_3CN, and concentrated again to a foam. This addition of CH_3CN and concentration was repeated one more time. To the residue dissolved in a 60 mL portion of CH_2Cl_2 was added H_2O (0.90 mL, 50 mmol, 10 equiv), followed by a 60 mL portion of 6% dichloroacetic acid in CH_2Cl_2 (44 mmol). After 10 min the reaction was quenched by addition of pyridine (7.0 mL, 87 mmol, 2 equiv rel to DCA). The mixture was then concentrated, and the residue was dissolved in a 40 mL portion of dry CH_3CN and concentrated again. This process was repeated two more times, the last time leaving 12 mL.

c. Linear coupling, oxidation, and detritylation.



To the above solution was added the dried solution of **1** (from step **1a**) using a double-tipped needle and nitrogen pressure, followed by two 1 mL rinses of dry CH_3CN. After 2 minutes, anhydrous *t*-butyl hydroperoxide 5.5 M in decane (2.73 mL, 15 mmol, 3 equiv) was added. After 30 min the solution was cooled in an ice bath, and 1.25 g $NaHSO_3$ dissolved in 2.5 mL H_2O was added. The ice bath was removed, the mixture was stirred 5 min, and then concentrated to a small volume. The residual oil was dissolved in a 80 mL portion of CH_2Cl_2, followed by H_2O (0.90 mL, 50 mmol, 10 equiv) and then 80 mL 6% dichloroacetic acid in CH_2Cl_2 (58 mmol). After 10 min the reaction was quenched with a 50 mL portion of pyridine. The mixture was concentrated to a small volume, a 150 mL portion of pyridine was added, and the solution was concentrated to 100 mL.

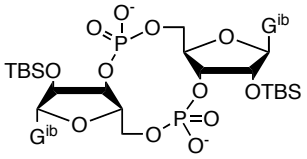
d. Cyclization and oxidation.



To the above solution was added 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane-2-oxide (DMOCP, 3.40 g of 95% reagent, 17.5 mmol, 3.5 equiv). After 10 min, the reaction was quenched by addition of H_2O (3.2 mL, 175 mmol, 10 equiv rel to DMOCP), and I_2 (1.65 g, 6.5 mmol, 1.3 equiv) was added immediately. After 5 min the mixture was poured into 700 mL H_2O containing 1.0 g $NaHSO_3$. After 5 minutes of stirring, 20 g of $NaHCO_3$ was slowly added.

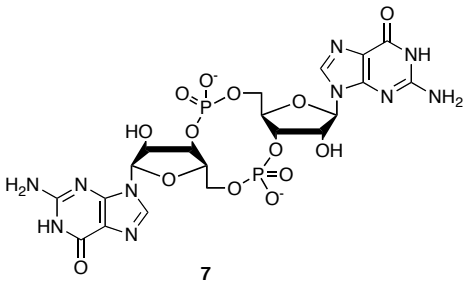
e. Isolation. After 5 min of stirring, the above aqueous solution was partitioned with 800 mL 1:1 EtOAc:Et_2O. Residual gum in the flask was dissolved in 5 mL pyridine and included. The separated aqueous layer was then partitioned with an additional 200 mL of 1:1 EtOAc:Et_2O. The organic layers were combined and concentrated to an oil. Excess pyridine was removed by concentration with three 10 mL portions of toluene.

2. **Conversion of 5a to 6a and crystallization of 6a.**



To the above **5a** dissolved in a 25 mL portion of CH_3CN was added a 25 mL portion of *t*-$BuNH_2$. After 10 min the mixture was concentrated to a foam, the residue was dissolved in a 25 mL portion of CH_3CN, and concentrated again to a foam. This addition of CH_3CN and concentration was repeated one more time. The residue was then dissolved in a 25 mL portion of CH_3OH for analysis, then filtered, and the filtrate was concentrated to a foam. This foam was dissolved in a 30 mL portion of CH_2Cl_2. Partial crystallization occurred overnight, and full crystallization then took place within minutes upon agitation. **6a** was collected by filtration and washed with minimal CH_2Cl_2. The crystals were dried in a dessicator over KOH overnight, giving 2.18 g (1.81 mmol, 36% from **1**) of **6a** as the *t*-$BuNH_3^+$ salt ($C_{48}H_{86}N_{12}O_{16}P_2Si_2$). A sample was recrystallized from CH_3OH/CH_2Cl_2, dried, and characterized: **mp** 191-193°C dec; the mass of **6a** was confirmed by HRMS in negative mode as *m/z* (M-H) 1057.3393 (calculated for $C_{40}H_{63}N_{10}O_{16}P_2Si_2^-$: 1057.3443); **UV** ($CH_3OH$) λ_{max} 258 nm, sh 281 nm; ^{1}H NMR (DMSO) 55°C δ 8.24 (s, 2H), 5.88 (d, *J* = 3 Hz, 2H), 4.93 (br, 2H), 4.38 (br, 2H), 4.14 (br, 2H), 4.10 - 4.03 (m, 2H), 3.86 – 3.79 (m, 2H), 2.83 (sep, *J* = 7 Hz, 2H), 1.22 (s, 22H), 1.06 (d, *J* = 7 Hz, 6H), 0.91 (d, *J* = 7 Hz, 6H), 0.84 (s, 18H), 0.14 (s, 6H), 0.07 (s, 6H); ^{13}C NMR (DMSO) 55°C δ (all resonances are singlets) 182.0, 156.4, 149.9, 149.5, 139.5, 122.3, 91.0, 82.4, 77.6, 72.7, 63.3, 52.3, 28.7, 27.4, 20.4, 20.1, 19.4, -2.9, -3.8; ^{31}P NMR (DMSO) 55°C δ -1.87.

3. **Deprotection of 6a and crystallization of 7.**

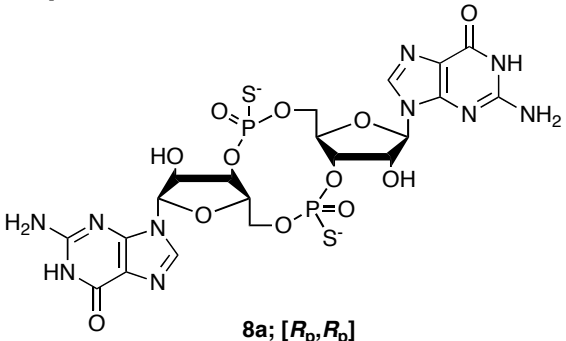


7

To 2.16 g, 1.79 mmol of the above **6a** was added 179 mL (1.44 mol, 400 equiv rel to each isobutyryl) CH_3NH_2 in anhydrous ethanol (33% by weight). After 90 min at room temp, the mixture was concentrated to an oil to which a 5 mL portion of pyridine and a 2 mL portion of Et_3N were added. The mixture was concentrated to an oil, and this process was repeated two more times. To the oil was added 4 mL pyridine and the flask with a vent needle was placed in an oil bath at 50°C. Et_3N (25 mL, 180 mmol) and Et_3N•3HF (14.8 mL, 272 mmol F^-, 75 equiv F^- rel to each TBS) were added simultaneously through syringes. The mixture was stirred at 50°, with occasional rotation of the flask at an angle to dissolve all material on the sides. ***Caution, HF:*** *Rinse all needles, syringes, septa, etc with aq K_2CO_3 before discarding.* After 1 h, the flask was removed from the oil bath. HPLC grade acetone (200 mL) was immediately added in a slow, steady stream to the stirring mixture. After 10 min of stirring, the crystals were collected by filtration and washed thoroughly 5X with 5 mL portions of acetone. The crystals were dried in a desiccator overnight over KOH, giving 1.32 g (1.48 mmol, 30% from **1**) of **7** as the Et_3NH^+ salt ($C_{32}H_{54}N_{12}O_{14}P_2$), which was characterized as follows: mp 193-196°C dec; the mass of 7 was confirmed by HRMS in negative mode as *m/z* (M-H) 689.0853 (calculated for $C_{20}H_{23}N_{10}O_{14}P_2^-$: 689.0876); UV ($H_2O$) λ_{max} 253 nm; ^{1}H NMR (D_2O) 55°C δ 8.03 (s, 2H), 5.81 (s, 2H), 5.07 (br, 2H), 4.80 (br, 2H), 4.07 – 3.96 (m, 2H), 3.13 (q, *J* = 7 Hz, 12H), 1.22 (t, *J* = 7 Hz, 18H); ^{13}C NMR (D_2O) 55°C δ (all resonances are singlets)

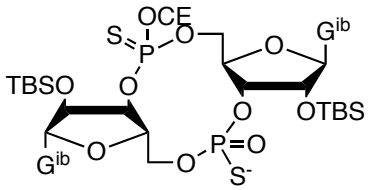
159.3 (br), 156.4, 152.1 (br), 139.1, 117.5 (br), 92.8, 83.0, 75.8, 73.0, 65.0, 49.4, 10.8; ^{31}P NMR (D_2O) 55°C δ -0.20.

C. Preparation of 8a and 8b on a 3mmol scale

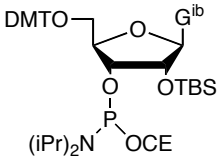


8a; [R_p,R_p]
8b; [R_p,S_p]

1. One-flask synthesis of 5b and 5c

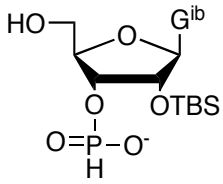


a. Preparation of a dry solution of 1 in CH_3CN.
One portion of guanosine phosphoramidite, **1** (3.78 g, 3.9 mmol, 1.3 equiv), was dried three times by concentration from 20 mL portions of CH_3CN, the last time leaving 12 mL. Six 3Å molecular sieves were added.

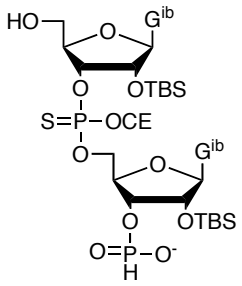


1

b. Hydrolysis, β-elimination, and detritylation.
To a second portion of **1** (2.91 g, 3.0 mmol) dissolved in CH_3CN (15 mL) and H_2O (0.108 mL, 6 mmol, 2 equiv) was added pyridinium trifluoroacetate (0.695 g, 3.6 mmol, 1.2 equiv). After 1 min a 15 mL portion of *t*-BuNH_2 was added. After 10 min the mixture was concentrated to a foam, the residue was dissolved in a 30 mL portion of CH_3CN, and concentrated again to a foam. This addition of CH_3CN and concentration was repeated one more time. To the residue dissolved in a 36 mL portion of CH_2Cl_2 was added H_2O (0.54 mL, 30 mmol, 10 equiv), followed by a 36 mL portion of 6% dichloroacetic acid in CH_2Cl_2 (26.2 mmol). After 10 min the reaction was quenched by addition of pyridine (4.2 mL, 52 mmol, 2 equiv rel to DCA). The mixture was then concentrated, and the residue was dissolved in a 20 mL portion of dry CH_3CN and concentrated again. This process was repeated two more times, the last time leaving 8 mL.

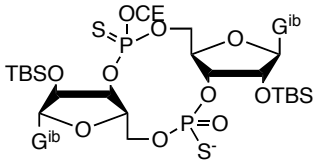


c. Linear coupling, sulfurization, and detritylation.



To the above solution was added the dried solution of **1** (from step C1a above) using a dry syringe. After 2 minutes, 3-((*N*,*N*-dimethylaminomethylidene)amino)-3H-1,2,4-dithiazole-5-thione (0.677 g, 3.3 mmol, 1.1 equiv) was added. After 30 min the solution was concentrated to a small volume, and the residual oil was dissolved in a 72 mL portion of CH_2Cl_2, followed by H_2O (0.36 mL, 20 mmol, 10 equiv) and 72 mL 6% dichloroacetic acid in CH_2Cl_2 (52.4 mmol). After 10 min the reaction was quenched with a 30 mL portion of pyridine. The mixture was concentrated to a small volume, a 90 mL portion of pyridine was added, and the solution was concentrated to 60 mL.

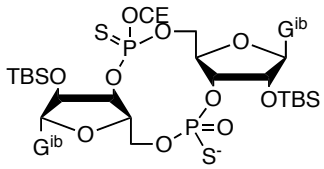
d. Cyclization, and sulfurization.



To the above solution was added 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane-2-oxide (DMOCP, 1.75 g of 95% reagent, 9 mmol, 3 equiv). After 3 min, the reaction was quenched by addition of H_2O (1.6 mL, 90 mmol, 10 equiv rel to DMOCP), and 3*H*-1,2-benzodithiol-3-one (0.779 g of 97% reagent, 4.5 mmol, 1.5 equiv) was added immediately. After 5 min the mixture was poured into 450 mL H_2O containing $NaHCO_3$ (12.6 g, 150 mmol).

e. Isolation. After 5 min of stirring, the above aqueous solution was partitioned with 450 mL 1:1 EtOAc:Et_2O. The separated aqueous layer was then partitioned with another 150 mL of 1:1 EtOAc:Et_2O. The organic layers were combined and concentrated to an oil. Excess pyridine was removed by concentration with three 10 mL portions of toluene.

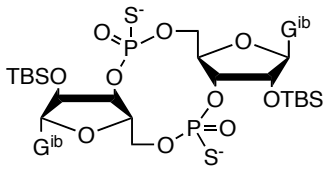
2. Separation of 5b and 5c.



The above oil was dissolved in CH_2Cl_2 and applied to a 150 g silica column using a gradient of 0 to 15% CH_3OH in CH_2Cl_2 over 50 min at 10 mL/min. The pure fractions of each diastereomer were combined, concentrated to a foam, and dried in a desiccator over KOH overnight, giving 0.977 g (0.803 mmol, 27% from **1**) **5b** as the *t*-$BuNH_3^+$ salt (**$C_{47}H_{78}N_{12}O_{14}P_2S_2Si_2$**) and 0.853 g (0.700 mmol, 23% from **1**) **5c** as the *t*-$BuNH_3^+$ salt (**$C_{47}H_{78}N_{12}O_{14}P_2S_2Si_2$**). **5b** was characterized as follows: the mass of **5b** [R_p,R_p] was confirmed by HRMS in negative mode as *m/z* (M-H) 1142.3206 (calculated for $C_{43}H_{66}N_{11}O_{14}P_2S_2Si_2^-$: 1142.3251); UV ($CH_3OH$) λ_{max} 256 nm, sh 280 nm; ^{1}H NMR (DMSO) 25°C δ 12.08 (s, 1H), 12.03 (s, 2H), 11.96 (s, 1H), 8.29 (s, 1H), 8.25 (s, 1H), 6.00 (d, *J* = 7 Hz, 1H), 5.95 (d, *J* = 7 Hz, 1H), 5.41 – 5.31 (m, 2H), 5.02 – 4.95 (m, 1H), 4.85 – 4.79 (m, 1H), 4.60 – 4.39 (m, 2H), 4.25 – 4.10 (m, 1H), 3.71 – 3.64 (m, 1H), 2.99 – 2.85 m, 2H), 2.72 (sep, *J* = 7 Hz, 1 H), 1.20 (s, 3H), 1.14 – 1.07 (m, 12 H), 0.75 (s, 9H), 0.68 (s, 9H), 0.098 (s, 3H), -0.023 (s, 6H), -0.14 (s, 3H); ^{13}C NMR (DMSO) 55°C δ (all resonances are singlets) 181.3, 180.8, 155.5, 150.0, 149.2, 148.6, 139.5, 137.1, 126.6, 125.9, 121.6, 120.6,

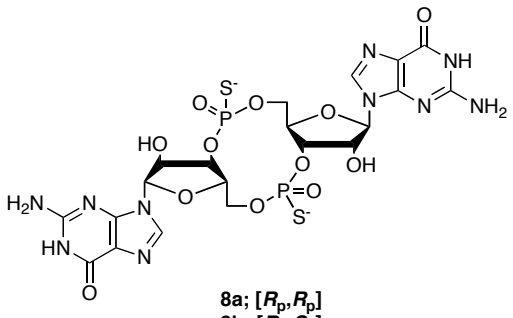
118.3, 89.2, 85.8, 82.2, 81.2, 78.7, 74.5, 73.0, 66.7, 64.1, 63.8, 61.9 , 47.3, 26.5, 26.1, 19.6, 19.5, 18.2, -2.5, -4.0, -4.4, -4.7, -5.1; ^{31}P NMR (DMSO) 25°C δ 64.86, 57.68. **5c** was characterized as follows: the mass of **5c** [R_p,S_p] was confirmed by HRMS in negative mode as *m/z* (M-H 1142.3202 (calculated for $C_{43}H_{66}N_{11}O_{14}P_2S_2Si_2^-$: 1142.3251); UV ($CH_3OH$) λ_{max} 256 nm, sh 279 nm; ^{1}H NMR (DMSO) 25°C δ 12.10 (s, 1H), 12.03 (s, 1H), 12.02 (s, 1H), 11.97 (s, 1H), 8.49 (s, 1H), 8.32 (s, 1H), 6.12 (d, *J* = 8 Hz, 1H), 5.98 (d, *J* = 7 Hz, 1H), 5.37 – 5.31 (m, 1H), 5.09 – 5.05 (m, 1H), 4.67 – 4.52 (m, 3H), 4.33 – 4.22 (m, 2H), 4.12 – 4.03 (m, 1H), 3.76 – 3.68 (m, 1H), 3.00 (t, *J* = 6 Hz, 2H), 2.88 (sep, *J* = 7 Hz, 1H), 2.72 (sep, *J* = 7 Hz, 1H), 1.24 (s, 5H), 1.20 (d, *J* = 7 Hz, 6H), 1.11 (d, *J* = 7 Hz, 6H), 0.70 (s, 8H), 0.65 (s, 9H), 0.067 (s, 3H), 0.059 (s, 3H), -0.028 (s, 3H), -0.19 (s, 3H); ^{13}C NMR (DMSO) 55°C δ (all resonances are singlets) 180.8, 180.8, 155.5, 155.5, 150.1, 149.5, 149.2, 148.8, 139.1, 137.9, 121.5, 120.6, 118.5, 87.5, 85.6, 81.8, 81.2, 78.0, 74.5, 72.7, 72.4, 67.5, 64.6, 64.1, 47.3, 27.9 26.5, 26.1, 19.6, 19.5, 19.4, 19.4,18.2, -2.5, -4.1, -4.7; ^{31}P NMR (DMSO) 25°C δ 70.92, 56.29.

3. Conversion of 5b/c to 6b/c and crystallization of 6b/c.



Samples of **5b** and **5c** were individually treated with *t*-$BuNH_2$ as described for **5a**. The CH_3OH solutions (10 mL) were filtered, and the filtrates were then partially concentrated along with 10 mL H_2O, giving crystalline products as the *t*-$BuNH_3^+$ salts that were dried in a desiccator over KOH for several days. **6b** was characterized as follows: mp 205-208°C dec; the mass of **6b** [R_p,R_p] was confirmed by HRMS in negative mode as *m/z* (M-H) 1089.2943 (calculated for $C_{40}H_{63}N_{10}O_{14}P_2S_2Si_2^-$: 1089.2986); UV ($CH_3OH$) λ_{max} 256 nm, sh 278 nm; ^{1}H NMR (DMSO) 25°C δ 8.24 (s, 2H), 5.90 (d, *J* = 4 Hz, 2H), 5.05 (br, 2H), 4.57 (br, 2H), 4.32 (br, 2H), 4.23 - 4.13 (m, 2H), 3.90 – 3.81 (m, 2H), 2.82 (sep, *J* = 7 Hz, 2H), 1.22 (s, 6H), 1.08 (d, *J* = 7 Hz, 6H), 0.99 (br, 6H), 0.76 (s, 18H), 0.14 (s, 6H), 0.04 (s, 6H); ^{13}C NMR (DMSO) 55°C δ (all resonances are singlets unless noted otherwise) 181.9 (br), 156.5 (br), 150.3 (br), 149.7 (br), 139.4, 122.0, 89.7, 82.6, 76.7, 73.5 (d, J_{CP} = 6 Hz), 64.4, 52.5, 28.8, 27.3, 20.4, 20.3, 19.3, -2.8, -3.9; ^{31}P NMR (DMSO) 25°C δ 54.65. 6c was characterized as follows: mp 203-206°C dec; the mass of **6c** [R_p,S_p] was confirmed by HRMS in negative mode as *m/z* (M-H) 1089.2941 (calculated for $C_{40}H_{63}N_{10}O_{14}P_2S_2Si_2^-$: 1089.2986); UV ($CH_3OH$) λ_{max} 255 nm, sh 280 nm; ^{1}H NMR (DMSO) 25°C δ 11.97 (br, 2H), 11.87 (br, 2H), 8.31 (s, 1H), 8.28 (s, 1H), 5.98 (d, *J* = 7 Hz, 1H), 5.95 (d, *J* = 7 Hz, 1H), 5.10 – 5.05 (m, 1H), 5.05 – 5.00 (m, 1H), 4.75 – 4.70 (m, 1H), 4.58 – 4.52 (m, 2H), 4.38 – 4.34 (m, 1H), 4.34 – 4.26 (m, 1 H), 4.22 – 4.14 (m, 1H), 3.86 – 3.79 (m, 1H), 3.74 – 3.65 (m, 1H), 2.82 – 2.71 (m, 2H), 1.24 (s, 18H), 1.13 – 1.08 (m, 13H), 0.68 (s, 18H), 0.12 (s, 3H), 0.09 (s, 3H), -0.01 (s, 3H), -0.02 (s, 3H); ^{13}C NMR (DMSO) 55°C δ (all resonances are singlets unless noted otherwise) 181.8, 181.6, 156.4, 150.8 (d, J_{CP} = 26 Hz), 149.7 (d, J_{CP} = 13 Hz), 139.3, 138.6, 121.8, 121.6, 88.4, 87.4, 83.66 (d, J_{CP} = 7 Hz), 83.1 (d, J_{CP} = 7 Hz), 76.3 (d, J_{CP} = 5 Hz), 75.8 (d, J_{CP} = 5 Hz), 75.5 (d, J_{CP} = 5 Hz), 74.1 (d, J_{CP} = 8 Hz), 64.1, 52.6, 28.8, 27.2, 27.1, 20.4, 20.4, 20.3, 20.3, 19.3, -2.6, -2.9, -3.9, -4.0; ^{31}P NMR (DMSO) 25°C δ 57.28, 53.81.

4. **Deprotection of 5b and crystallization of 8a.**



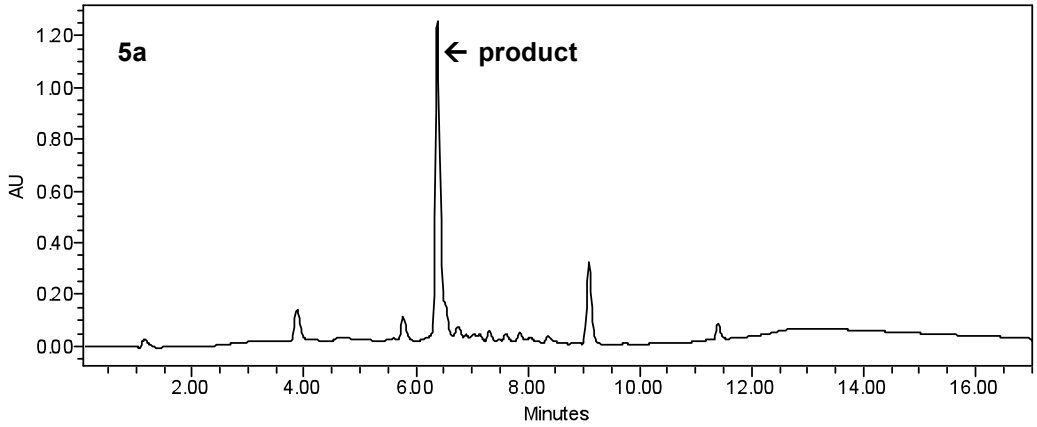
8a; [R_p,R_p]
8b; [R_p,S_p]

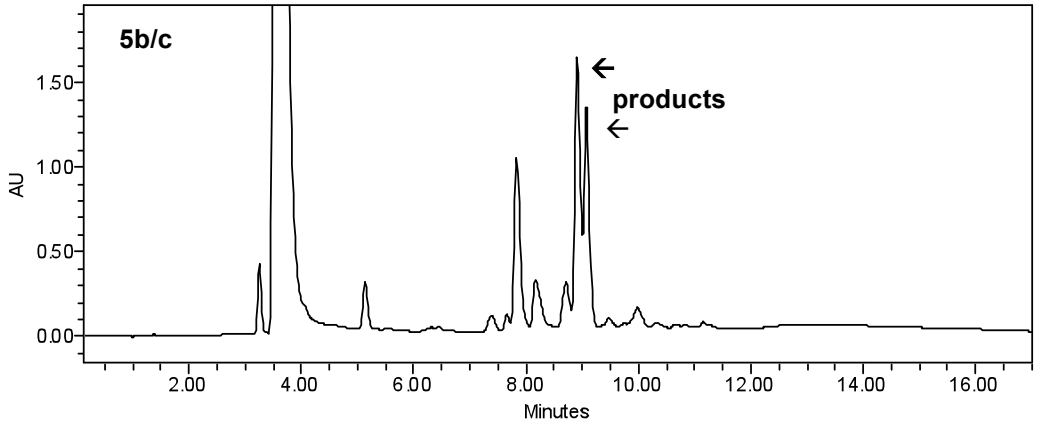
After separation on the column (section C2 above), **5b** (0.872 g, 0.717 mmol) was dissolved in 36 mL CH_3NH_2 in anhydrous ethanol (33% by weight) (290 mmol, 200 equiv rel to each isobutyryl). After 90 min at room temp, the mixture was concentrated to an oil, to which a 5 mL portion of pyridine and a 2 mL portion of Et_3N were added. The mixture was concentrated to an oil, and this process was repeated two more times. To the oil was added 2 mL pyridine, and the flask was placed in an oil bath at 50°C with a vent needle. Et_3N (10 mL, 72 mmol) and Et_3N•3HF (5.9 mL, 108 mmol F^-, 75 equiv F^- rel to each TBS) were added simultaneously through syringes. The mixture was stirred at 50°, with occasional rotation of the flask at an angle to dissolve all material on the sides. ***Caution, HF:*** *Rinse all needles, syringes, septa, etc with aq K_2CO_3 before discarding.* After 1 h, the flask was removed from the oil bath. HPLC grade acetone (90 mL) was immediately added in a slow, steady stream to the stirring mixture. After 10 min of stirring, the crystals were collected by filtration and washed thoroughly 5X with 3 mL portions of acetone. The crystals were dried in a desiccator overnight over KOH, giving 0.457 g (0.494 mmol) **8a** as the Et_3NH^+ salt ($C_{32}H_{54}N_{12}O_{12}P_2S_2$), which was characterized as follows: **mp** 193-195°C dec; the mass of **8a** [R_p,R_p] was confirmed by HRMS in negative mode as *m/z* (M-H) 721.0370 (calculated for $C_{20}H_{23}N_{10}O_{12}P_2S_2^-$: 721.0419); UV ($H_2O$) λ_{max} 253 nm; ^{1}H NMR (D_2O) 55°C δ 7.97 (s, 2H), 5.87 (s, 2H), 5.05 (br, 2H), 4.74 (br, 2H), 4.05 – 3.95 (m, 2H), 3.12 (q, J = 7 Hz, 12H), 1.21 (t, J = 7 Hz, 18H); ^{13}C NMR (D_2O) 55°C δ (all resonances are singlets unless noted otherwise) 160.7, 156.3, 153.1, 139.7, 118.8, 91.7, 82.6 (t_{app}, J_{CP} = 10 Hz), 75.9, 73.8 (d, J_{CP} = 8 Hz), 66.2 (d, J_{CP} = 4 Hz), 49.4, 10.8; ^{31}P NMR (D_2O) 55°C δ 54.47.

5. **Deprotection of 5c and crystallization of 8b.** After separation on the column (section C2 above), **5c** (0.777 g, 0.639 mmol) was dissolved in 32 mL CH_3NH_2 in anhydrous ethanol (33% by weight) (258 mmol, 200 equiv relative to each isobutyryl). After 90 min at room temp, the mixture was concentrated to an oil. To the oil was added a 5 mL portion of pyridine and a 2 mL portion of Et_3N, and the mixture was concentrated to an oil. This process was repeated two more times. To the oil was added 2 mL pyridine and the flask was placed in an oil bath at 50°C with a vent needle. Et_3N (9 mL, 65 mmol) and Et_3N•3HF (5.2 mL, 96 mmol F^-, 75 equiv F^- rel to each TBS) were added simultaneously through syringes. The mixture was stirred at 50°, with occasional rotation of the flask at an angle to dissolve all material on the sides. ***Caution, HF:*** *Rinse all needles, syringes, septa, etc with aq K_2CO_3 before discarding.* After 1 h, the flask was removed from the oil bath. HPLC grade acetone (90 mL) was immediately added in a slow, steady stream to the stirring mixture. After 10 min of stirring, the crystals were collected by filtration and washed thoroughly 5X with 3 mL portions of acetone. The crystals were dried in a desiccator overnight over KOH, giving 0.426 g (0.460 mmol) **8b** as the Et_3NH^+ salt ($C_{32}H_{54}N_{12}O_{12}P_2S_2$), which was characterized as follow: mp 193-196°C dec; the mass of **8b** [R_p,S_p] was confirmed by HRMS in negative mode as *m/z*

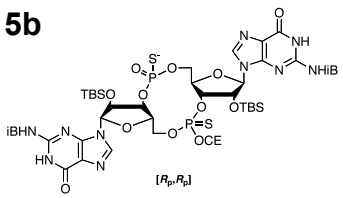
(M-H) 721.0377 (calculated for $C_{20}H_{23}N_{10}O_{12}P_2S_2^-$: 721.0419); UV ($H_2O$) λ_{max} 253 nm; ^1H NMR (D_2O) 55°C δ 8.09 (s, 1H), 7.98 (s, 1H), 5.92 (s, 2H), 5.01 (s, 2H), 4.92 (s, 1H), 4.75 (s, 1H), 4.04 - 3.96 (m, 2H), 3.12 (q, J = 7 Hz, 12H), 1.21 (t, J = 7Hz, 18H); ^{13}C NMR (D_2O) 55°C δ (all resonances are singlets unless noted otherwise) 161.3,156.4, 153.8 (br), 140.4 (br), 119.2 (br), 91.4, 91.3, 82.8 (t_{app}, J_{CP} = 9 Hz), 82.4 (t_{app}, J_{CP} = 9 Hz), 75.8, 75.4, 75.0 (d, J_{CP} = 4 Hz), 74.2 (d, J_{CP} = 8 Hz), 66.1 (d, J_{CP} = 3 Hz), 64.8 (d, J_{CP} = 6 Hz), 49.4, 10.8; ^{31}P NMR (D_2O) 55°C δ 55.93, 54.65.

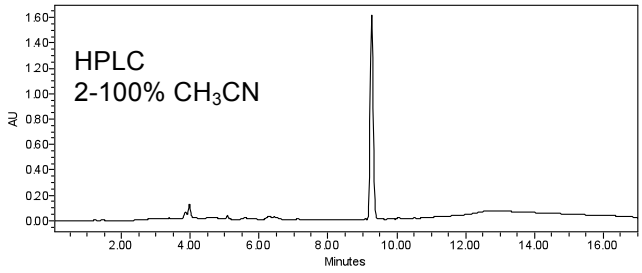
Chromatograms for Crude **5a** and **5b/c**

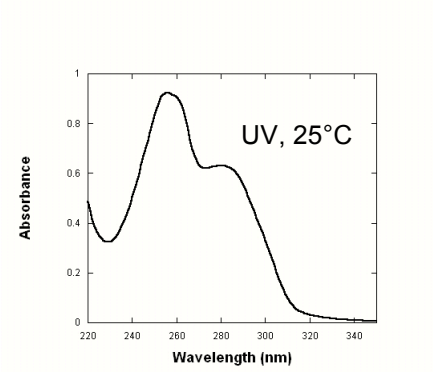




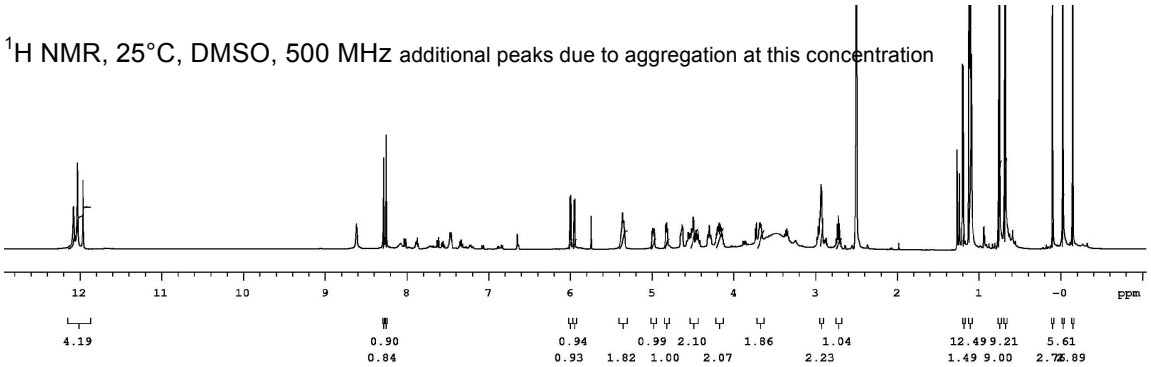
5b



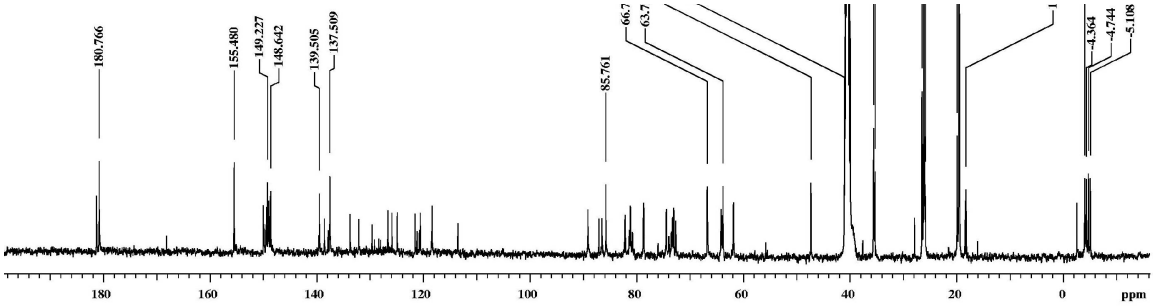




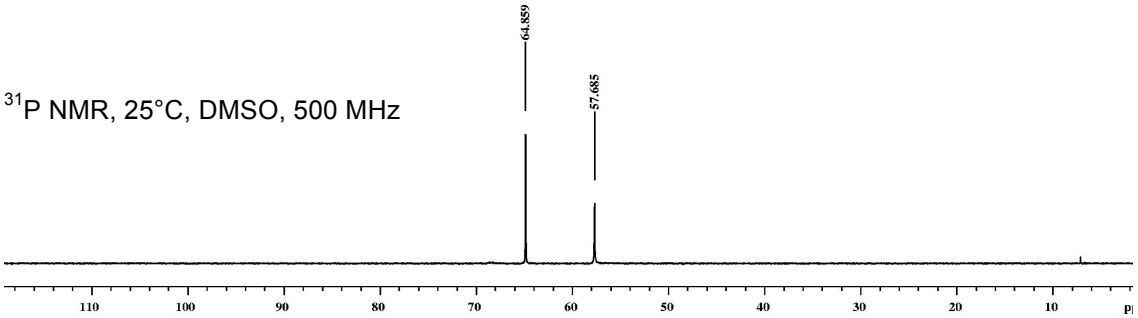
^{1}H NMR, 25°C, DMSO, 500 MHz additional peaks due to aggregation at this concentration



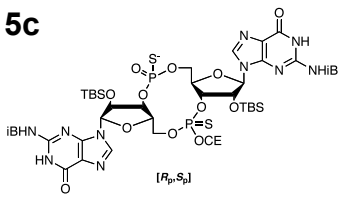
^{13}C NMR, 55°C, DMSO, 500 MHz additional peaks due to aggregation at this concentration

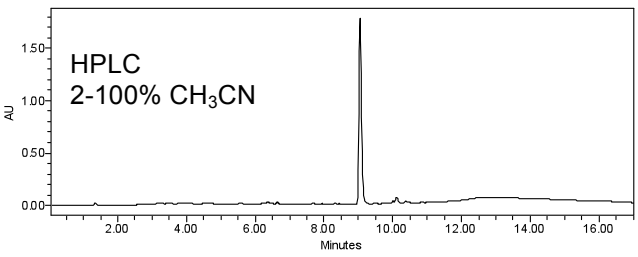


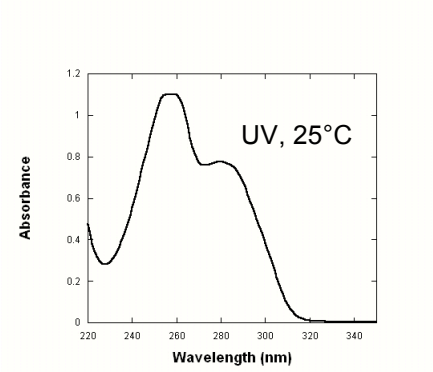
^{31}P NMR, 25°C, DMSO, 500 MHz



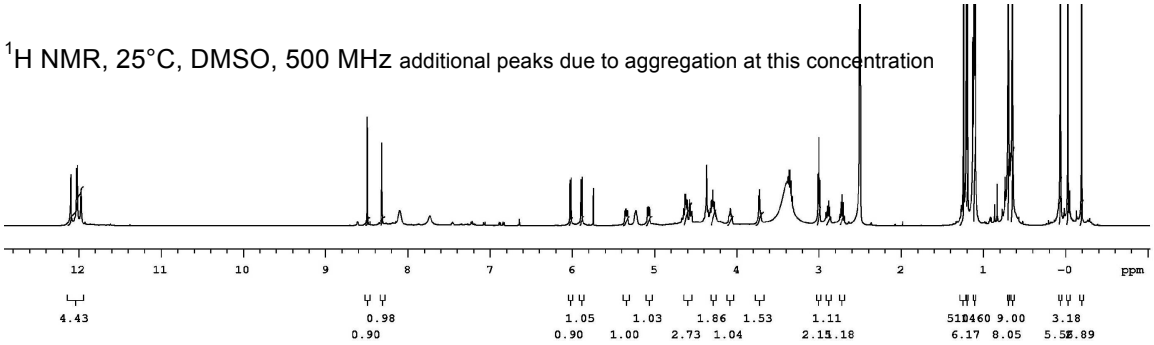
5c



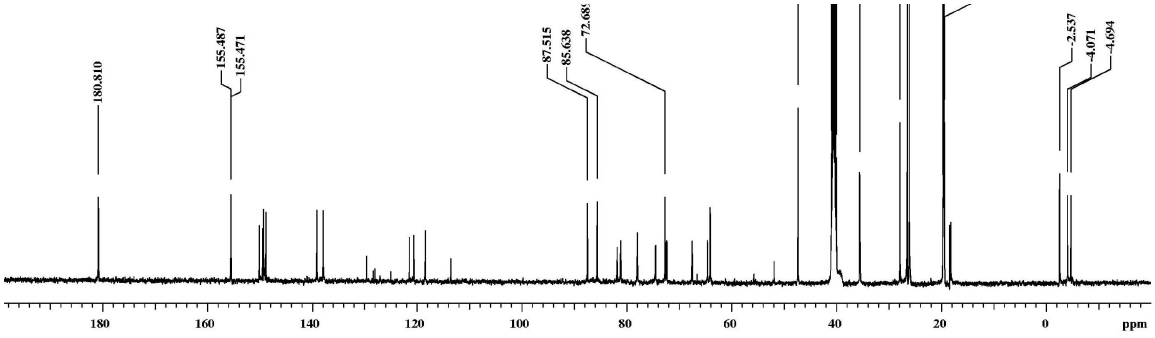




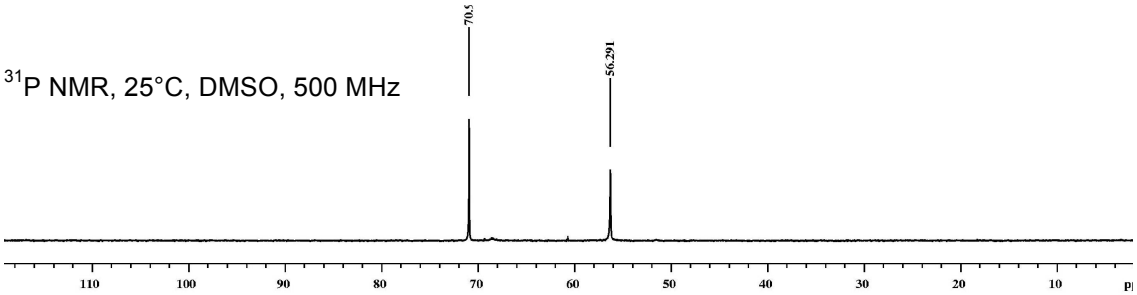
^{1}H NMR, 25°C, DMSO, 500 MHz additional peaks due to aggregation at this concentration



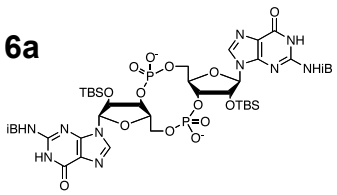
^{13}C NMR, 55°C, DMSO, 500 MHz additional peaks due to aggregation at this concentration

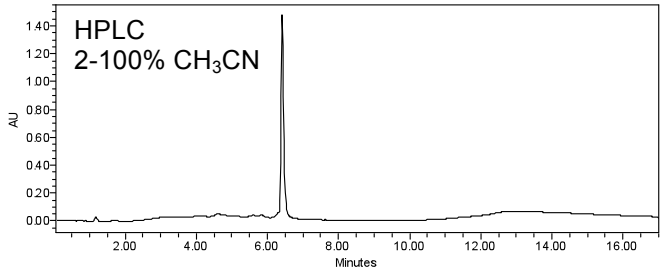


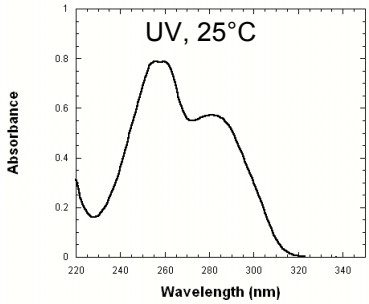
^{31}P NMR, 25°C, DMSO, 500 MHz



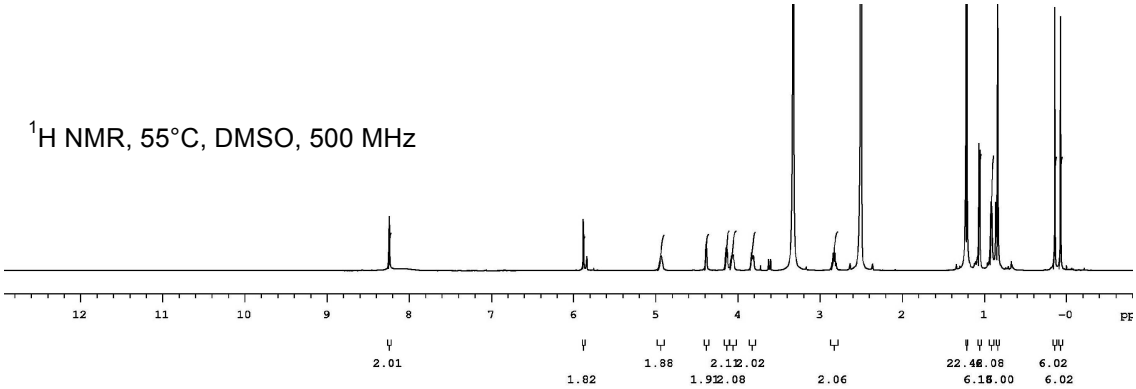
6a



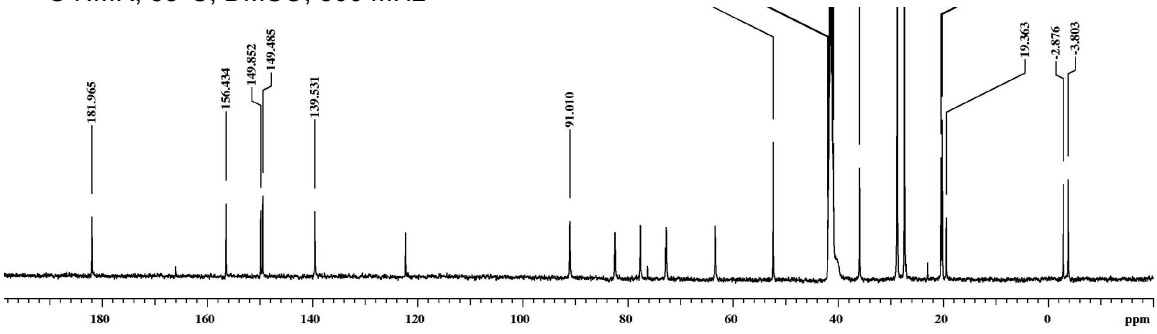




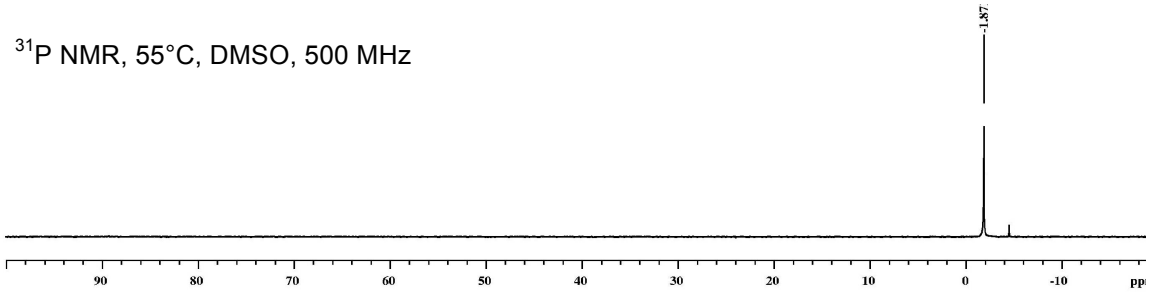
^{1}H NMR, 55°C, DMSO, 500 MHz



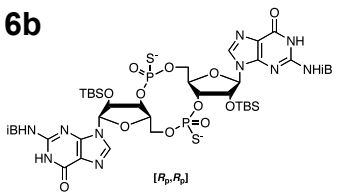
^{13}C NMR, 55°C, DMSO, 500 MHz

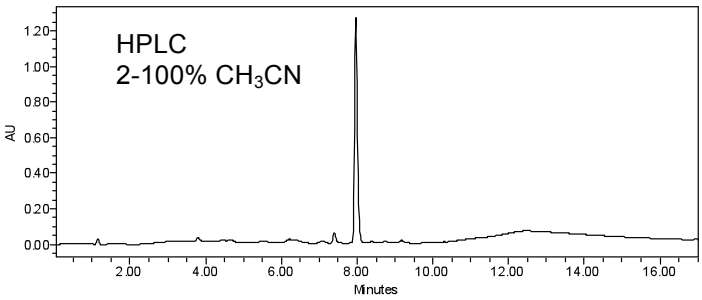


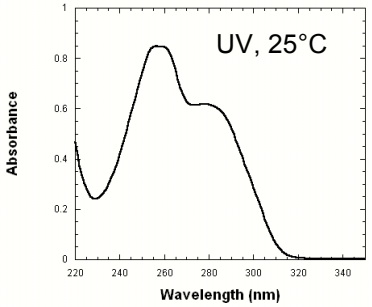
^{31}P NMR, 55°C, DMSO, 500 MHz



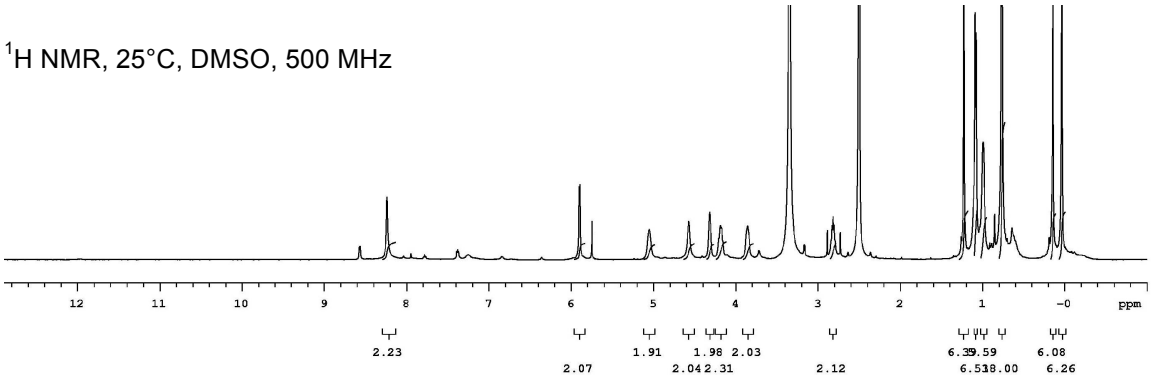
6b



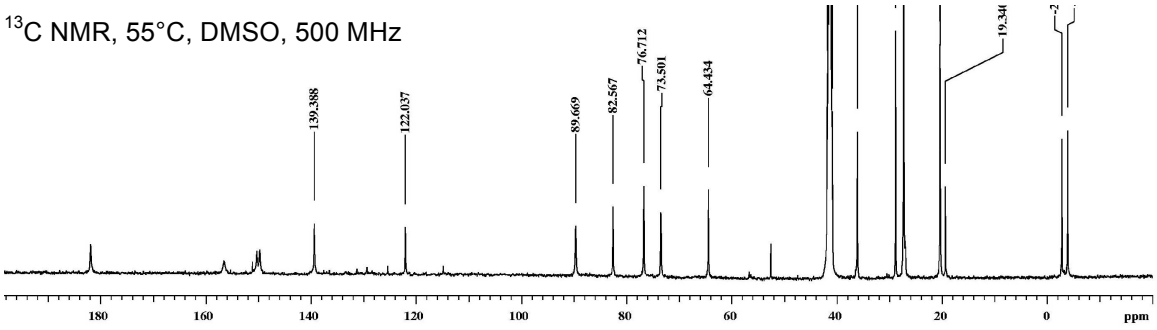




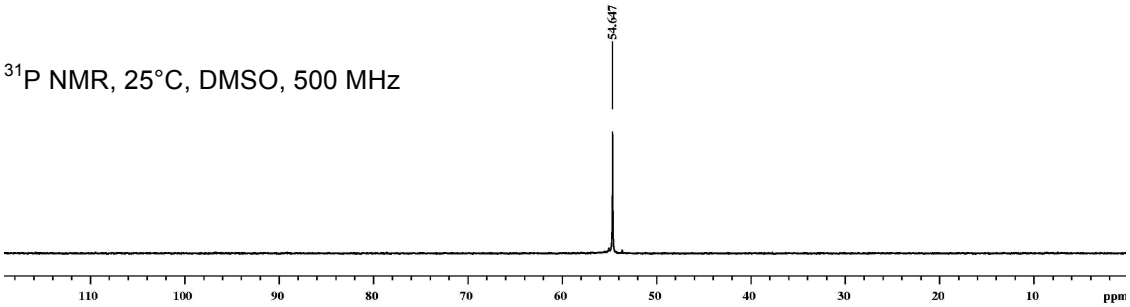
^{1}H NMR, 25°C, DMSO, 500 MHz



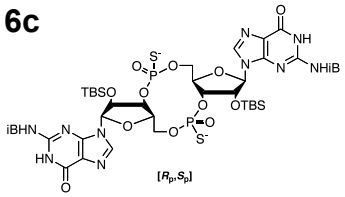
^{13}C NMR, 55°C, DMSO, 500 MHz

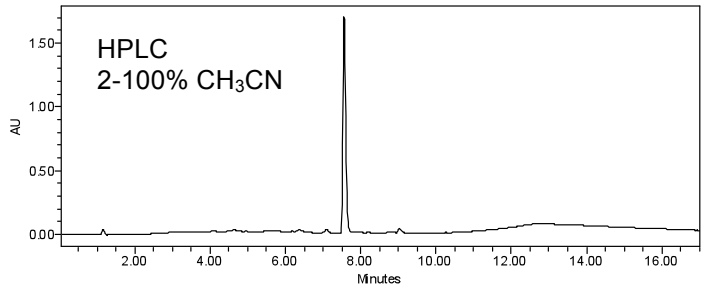


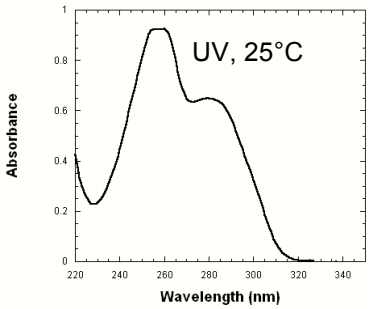
^{31}P NMR, 25°C, DMSO, 500 MHz



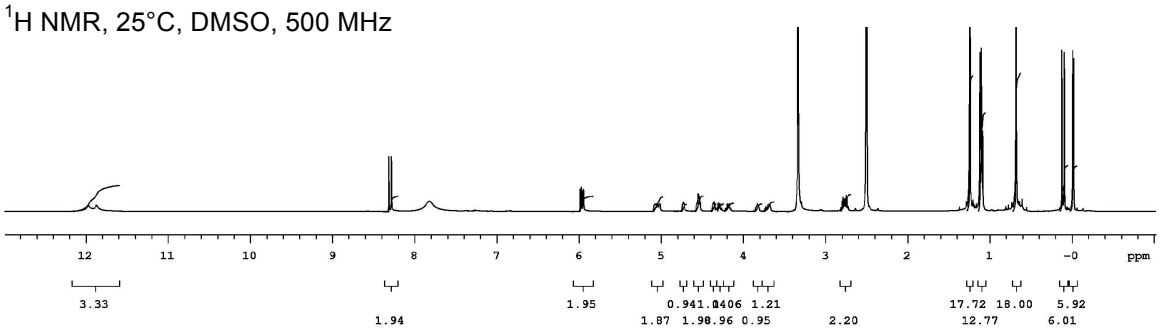
6c



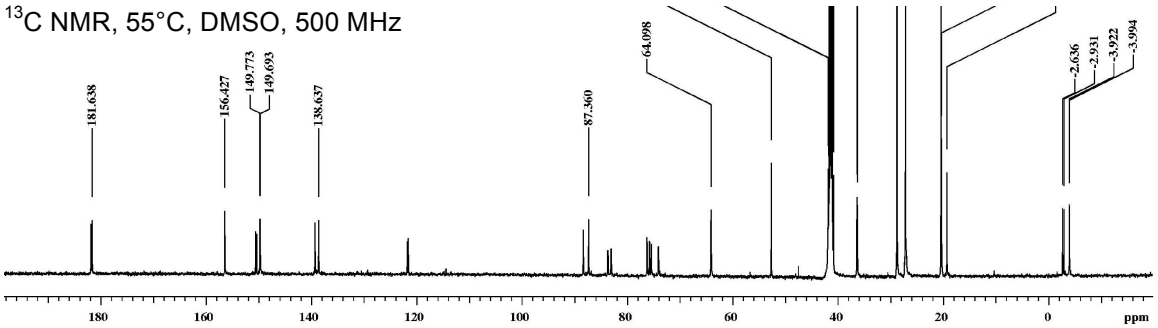




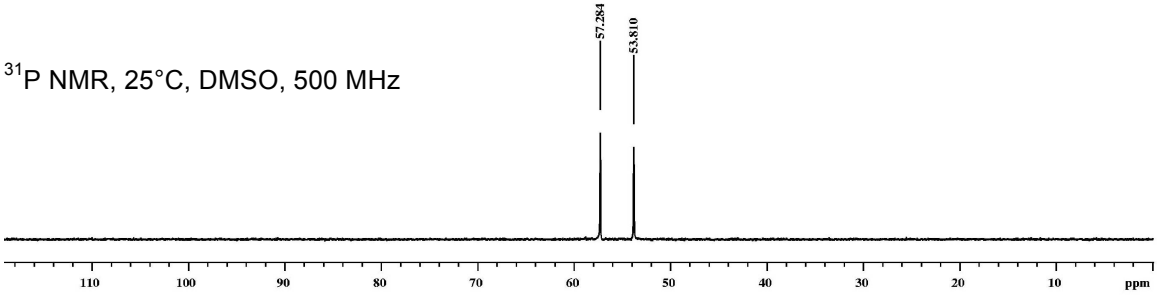
^{1}H NMR, 25°C, DMSO, 500 MHz



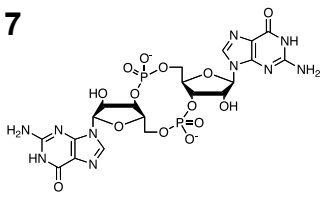
^{13}C NMR, 55°C, DMSO, 500 MHz

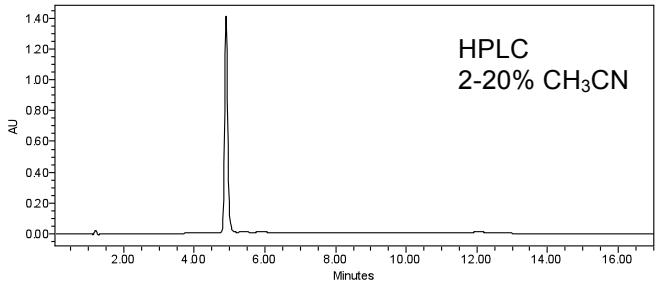


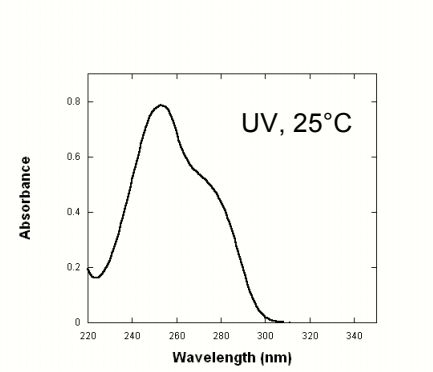
^{31}P NMR, 25°C, DMSO, 500 MHz



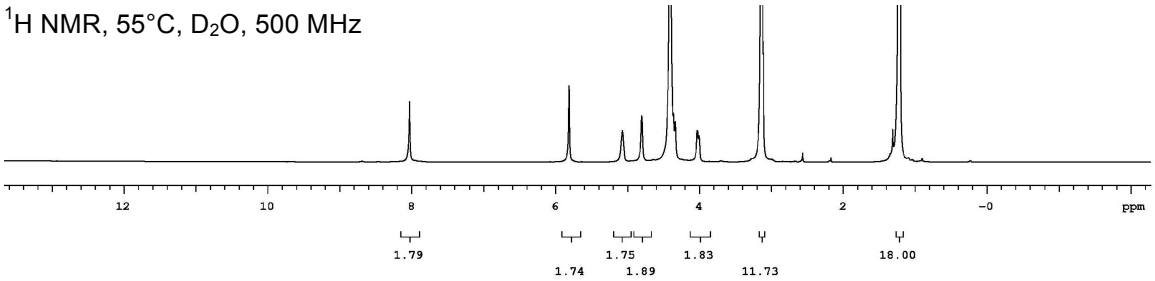
7



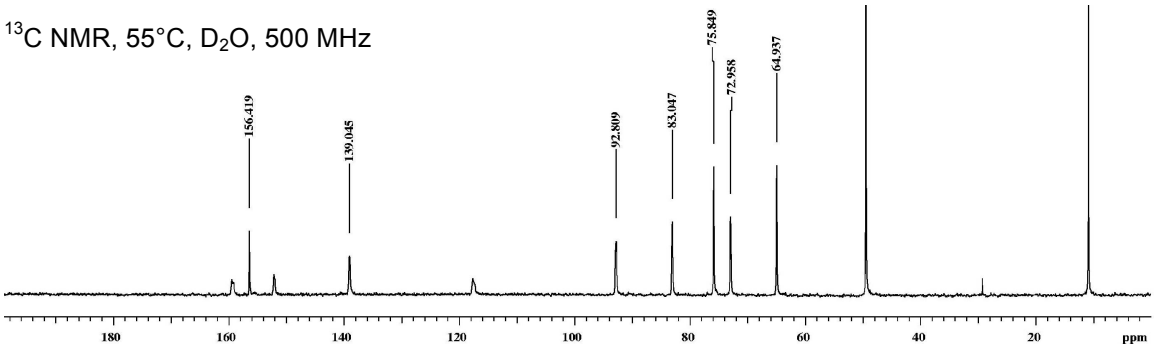




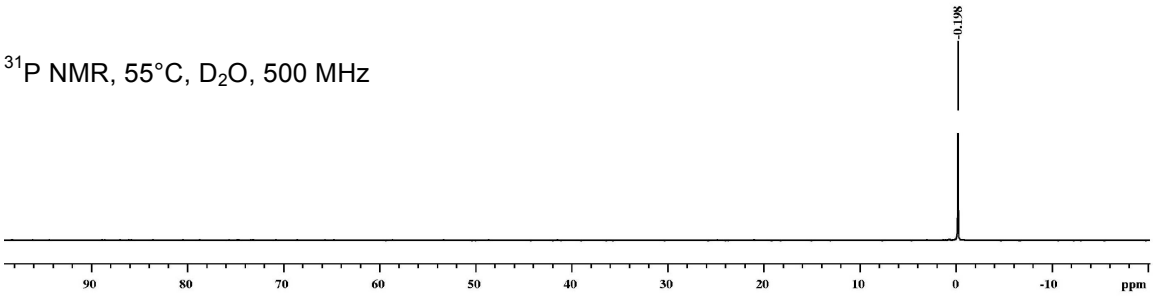
^{1}H NMR, 55°C, D_2O, 500 MHz



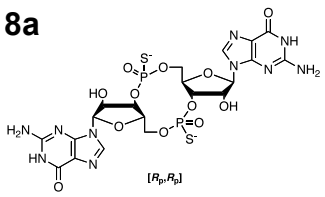
^{13}C NMR, 55°C, D_2O, 500 MHz

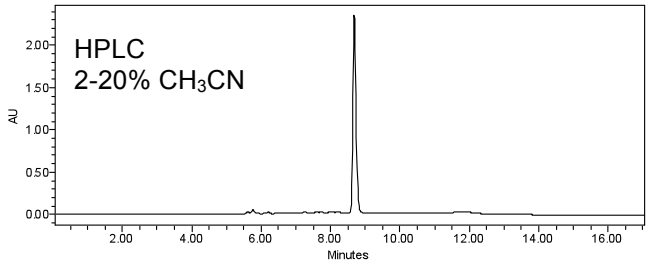


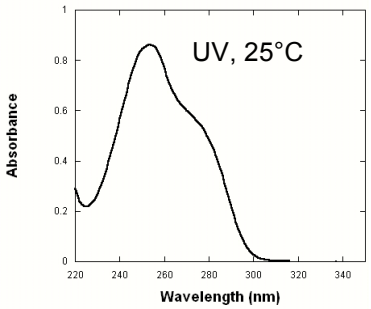
^{31}P NMR, 55°C, D_2O, 500 MHz



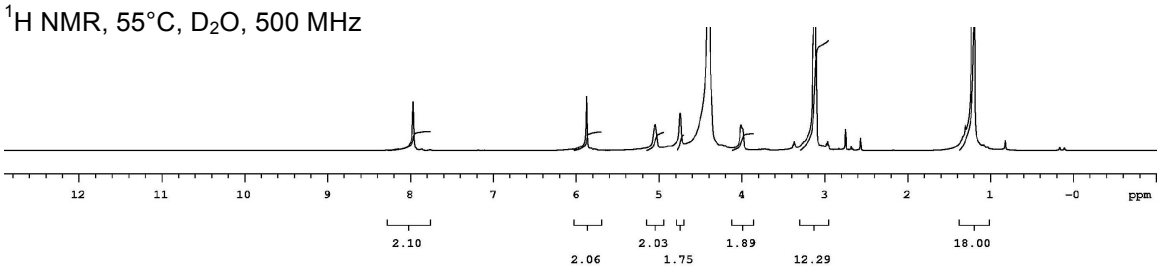
8a



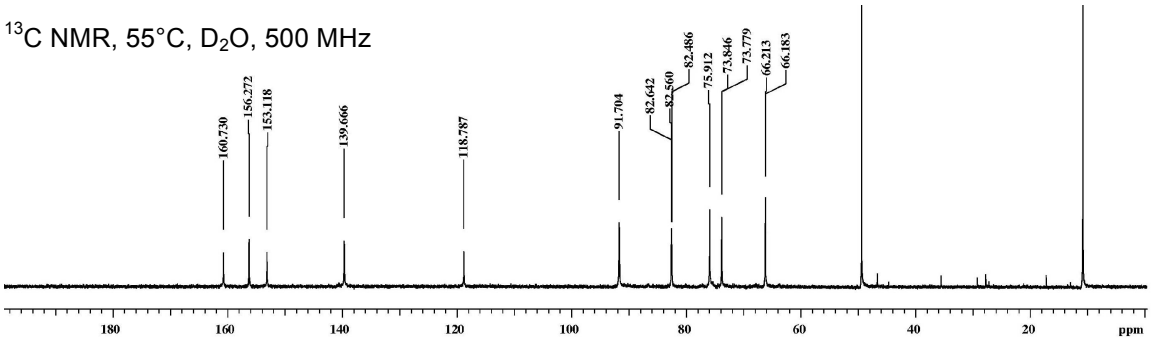




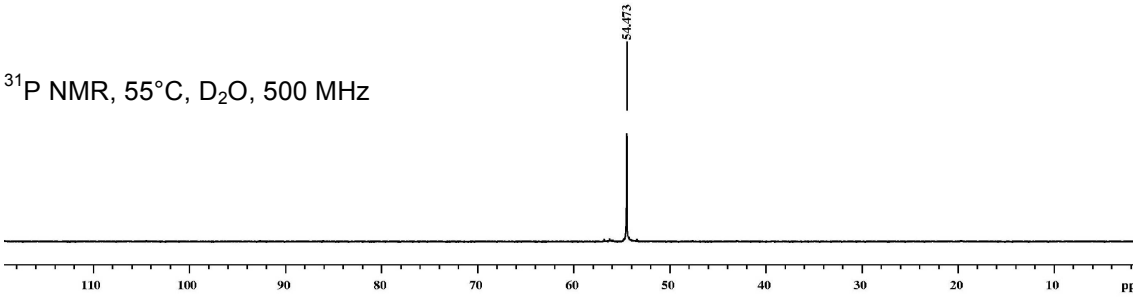
^{1}H NMR, 55°C, D_2O, 500 MHz



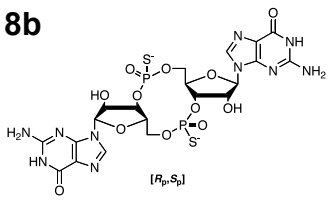
^{13}C NMR, 55°C, D_2O, 500 MHz

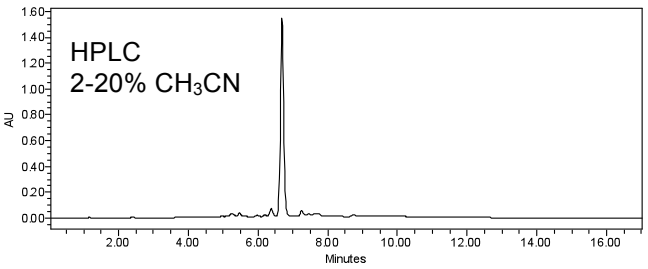


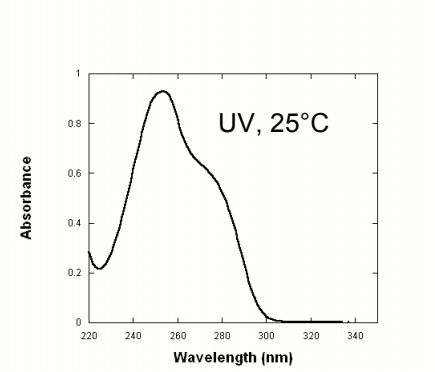
^{31}P NMR, 55°C, D_2O, 500 MHz



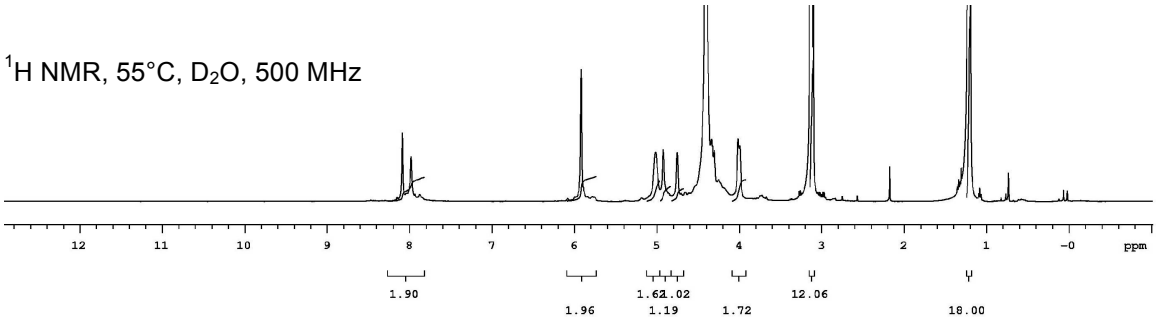
8b



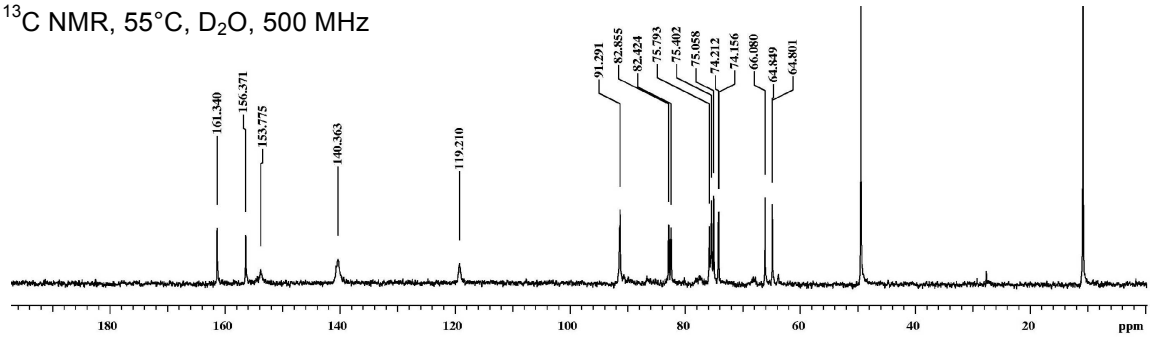




^{1}H NMR, 55°C, D_2O, 500 MHz



^{13}C NMR, 55°C, D_2O, 500 MHz



^{31}P NMR, 55°C, D_2O, 500 MHz