# Oligonucleotide Incorporation of 8-Thio-2'-deoxyguanosine

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## Supporting Material

### Experimental

All reagents were from Aldrich or Acros Chemicals except where noted. NMR spectra were obtained on Bruker AVANCE300 and 500 NMR spectrometers. MALDI- TOF analyses were performed at the University of California-Riverside Mass Spectrometry Facility. HR-ESI was performed at either the University of Nebraska-Lincoln Mass Spectrometry Facility or University of California-Riverside Mass Spectrometry Facility. Preparative and analytical HPLC were performed using a Beckman Ultrasphere ODS $C_{18}$ column (10 x 250 mm) run at 3 mL/min and a Varian Microsorb-mv $C_{18}$ column (4.6 x 250 mm) run at 1 mL/min, respectively. HPLC solvents A and B were 0.1 M triethylammonium acetate (TEAAC) pH 7 and acetonitrile, respectively. Merck silica gel, 200-400 mesh, 60 Å was used for column chromatography.

**8-(2-(Trimethylsilyl)ethyl)thio-2'-deoxyguanosine (2).**
1.0 g (2.9 mmol) of 8-bromo-2'-deoxyguanosine (**1**) and 1.6 g of $K_2CO_3$ (35 mmol) were suspended in 14 mL of dry *N*,*N*-dimethylformamide (DMF) before the addition of 1.83 mL (11.6 mmol) of trimethylsilylethanethiol. The reaction was stirred under argon at 60 ˚C for 36 hours before concentration *in vacuo*. The resulting residue was then washed twice with hexane, dried, and recrystallized with water. The solid was filtered, washed with water and dried to give 670 mg (1.63 mmol) of **2** as yellow crystals. The filtrate was concentrated and cooled. White crystals appeared and after filtration yielded an additional 80 mg (0.2 mmol) of **2** for a combined yield of 63%. $^1$H NMR (DMSO-$d_6$) δ: 10.71 (s, 1H), 6.37 (s, 2H), 6.15 (m, 1H), 5.23 (d, J= 4.0, 1H), 4.91 (t, J=5.6,1H), 4.36 (d, J=2.9, 1H), 3.79 (m, 1H), 3.62 (m, 1H), 3.51 (m, 1H), 3.16 (m, 2H), 3.03 (m, 1H), 2.02 (m, 1H), 0.96 (m, 2H), 0.05 (s, 8H) $^{13}$C NMR (DMSO-$d_6$) δ: 156.3, 153.5, 152.7, 143.1, 117.7, 88.2, 84.2, 71.7, 62.6, 37.3, 29.5, 17.2, 1.2. HR-ESI ($M+H^+$) for $C_{15}H_{26}N_5O_4SSi$. Expected: 400.1475; Found: 400.1470.

**8-Thio-2'-deoxyguanosine (3)**.
6 mg (0.017 mmol) of **2** was covered with argon and 0.5 mL of 1.0 M tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) was added. The reaction sat for 30 minutes before 0.5 mL of 1.0 M TEACC was added to quench the reaction. The reaction mixture was then purified twice by preparative HPLC using first a linear gradient of 5-8% B in A over 30 min to remove the TBAF, and second, a linear gradient of 5-8% B in water over 30 min to remove the TEAAC. After concentration, 4.6 mg of **3**

(0.015 mmol; 88%) was obtained as a white powder. $^{1}$H NMR (DMSO-$d_6$) δ: 6.69 (m, 1H), 6.47 (s, 2H), 5.13 (d, J= 3.8, 1H), 4.85 (m, 1H), 4.41 (m, 1H), 3.78 (m, 1H), 3.66 (m, 1H), 3.52 (m, 1H), 3.02 (m, 1H), 1.99 (m, 1H). $^{13}$C NMR (DMSO-$d_6$) δ:165.4, 153.9, 151.5, 149.7, 104.8, 88.1, 84.6, 71.8, 62.8, 36.7. HR-ESI ($MH^+$) for $C_{10}H_{14}N_5O_4S$. Expected: 300.0767; Found: 322.0757.

***N*-Isobutyryl-8-(2-(trimethylsilyl)ethyl)thio-2'-deoxyguanosine (4).**
1.0 g (2.5 mmol) of **2** was coevaporated three times with pyridine to remove any associated water before suspension in 13 mL of anhydrous pyridine. 1.6 mL of trimethylsilylchloride (12.6 mmol) was added and the reaction stirred for 30 min under argon before the addition of 2.14 mL of isobutyryl anhydride (12.6 mmol). After 4 hours at room temperature, the reaction was cooled in an ice bath and 2.5 mL water was added. After 10 min, 2.5 mL of 29.7% ammonium hydroxide was added the reaction stirred for 30 min before concentration to near dryness. The resulting solution was suspended in 50 mL of water and extracted once with 50 mL of chloroform. The organic layer was dried with $Na_2SO_4$ and concentrated. The resulting solid was purified by silica gel chromatography using 5% methanol in chloroform to yield 690 mg of **4** (1.5 mmol; 60%) as a white powder. $^{1}$H NMR (DMSO-$d_6$) δ: 12.01 (s, 1H), 11.48 (s, 1H), 6.21 (m, 1H), 5.27 (d, J=4.2, 1H), 4.75 (t, J=5.8, 1H), 4.39 (m, 1H), 3.79 (m, 1H), 3.61 (m, 1H), 3.53 (m, 1H), 3.26 (m, 2H), 3.06 (m, 1H), 2.78 (m, 1H), 2.09 (m, 1H), 1.13 (d, J=7.0, 6H), 1.00 (m, 2H), 0.06 (s, 9H). $^{13}$C NMR (DMSO-$d_6$) δ: 180.5, 154.1, 150.4, 147.5, 121.0, 88.1, 83.9, 71.3, 62.4, 36.9, 35.2, 29.1, 19.4, 19.3, 17.1, -1.2. HR-ESI ($M+H^+$) for $C_{19}H_{32}N_5O_5SSi$; Expected: 470.1893; Found: 470.1883.

***N*-Isobutyryl -5'-*O*-dimethoxytrityl-8-(2-(trimethylsilyl)ethyl)thio-2'-deoxyguanosine (5).** 620 mg (1.3 mmol) of **4** was coevaporated three times with pyridine to remove any associated water before addition of 660 mg (1.95 mmol) of dimethoxytritylchloride and 8 mg (0.065 mmol) of 2-(dimethylamino)pyridine. The flask was covered with argon and 5 mL of anhydrous pyridine and 0.25 mL of triethylamine were added. After stirring at room temperature for 4 hours, the reaction was concentrated and the resulting oil purified by silica gel chromatography using 1% methanol in chloroform to yield 780 mg of **5** (1.0 mmol; 77%) as a yellow foam. $^{1}$H NMR (DMSO-$d_6$) δ: 12.04 (s, 1H), 11.33 (s, 1H), 7.33 (m, 2H), 7.15-7.2 (m, 7H), 6.75 (m, 4H), 6.29 (t, J=7.1, 1H), 5.27 (d, J=4.8, 1H), 4.45 (m, 1H), 4.00 (m, 1H), 3.72 (s, 3H), 3.70 (s, 3H), 3.39 (m, 1H), 3.24 (m, 2H), 3.11 (m, 1H), 3.03 (m, 1H), 2.73 (m, 1H), 2.17 (m, 1H), 1.12 (d, J=6.7, 6H), 0.93 (m, 2H), 0.03 (s, 9H).$^{13}$C NMR (DMSO-$d_6$) δ: 180.4, 158.4, 158.3, 154.1, 150.1, 147.3, 146.7, 145.3, 136.1, 130.2, 130.1, 128.3, 128.0, 126.9, 121.1, 113.3, 113.2, 86.8, 85.7, 84.3, 79.6, 71.4, 64.9, 55.4, 55.3, 37.5, 35.2, 29.3, 20.0, 19.4, 19.2, 17.1, -1.3. HR-ESI ($M+H^+$) for $C_{40}H_{50}N_5O_7SSi$; Expected: 772.3200; Found: 772.3164.

**2-*N*-Isobutyryl-5'-*O*-dimethoxytrityl-8-(2-(trimethylsilyl)ethyl)thio-2'-deoxyguanosin-3'-yl β-cyanoethyl-*N*,*N*-diisopropylphosphoramidite (6).** 300 mg of **5** (0.39 mmol) was coevaporated three times with pyridine to remove any associated water before suspension in 5 mL of anhydrous methylene chloride. The flask was covered with argon and 170 μL (0.97 mmol) of redistilled diisopropylethylamine, 20.5 μL of (0.97 mmol) freshly distilled 1-methylimidazole and 150 μL (0.66 mmol) of *N*,*N*-diisopropyl-

2-cyanoethyl-phosphonamidic chlorine (Chemgenes) were added. The reaction was stirred for 30 min at room temperature and concentrated. The resulting oil was purified by silica gel chromatography using 3% acetone and 0.1% triethylamine in methylene chloride that had been run through alumina to yield 230 mg of **6** (0.24 mmol; 62%) as a light yellow foam. $^{31}$P NMR ($CDCl_3$) δ: 148.1, 147.7. HR-MS ($M+H^+$) for $C_{49}H_{67}N_7O_8PSSi$; Expected: 972.4279; Found: 972.4249.

**DNA Synthesis**

DNA Synthesis was performed at the University of Virginia Biomolecular Research Facility using all standard procedures. Oligonucleotides were deprotected and cleaved from the column using 29.7% ammonium hydroxide incubated at 55 ˚C for 12 hours.

**DNA Purification**

The DNA dimer **7a** was purified by preparative HPLC using a linear gradient of 5-40% solvent B in A over 30 min. Oligonucleotides **7b** and **7c** were purified by 20% denaturing PAGE before UV visualization. The slowest running band was excised and soaked twice in water, in the dark, for 24 hours. The resulting solutions were then concentrated, combined by resuspension in 1mL of water, and filtered. The oligonucleotides were then further purified by preparative HPLC using a linear gradient of 5-60% solvent B in A over 30 min. **7a** MALDI-TOF ($M^-$): Expected: 702; Found: 702. **7b** MALDI-TOF ($MH^+$): Expected: 2212; Found: 2212. **7c** MALDI-TOF ($MH^+$): Expected: 7636; Found: 7636.

**Deprotection of the Thiocarbonyl Group**

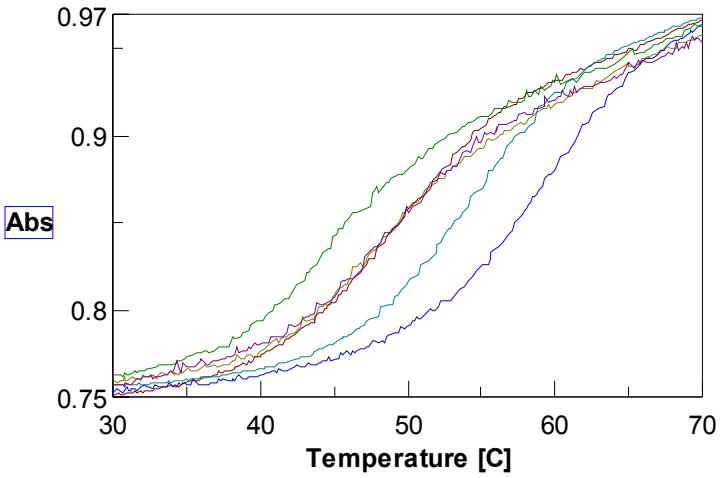
To a dry sample of oligonucleotide (**7a-c**) was added 0.2-0.5 mL of 1.0 M TBAF in THF. The solution was covered with argon and wrapped in foil. After 30 min, the reaction was quenched with an equal amount of 1.0 M TEAAC and the THF removed by concentration. The samples were then purified either twice by preparative HPLC (to remove the last traces of tetrabutylamine) using a linear gradient of 5-40% solvent B in A over 30 min (**8a-b**) or by NAP-5 column (Amersham; **8c**). **8a** MALDI-TOF ($M^-$): Expected: 602; Found: 602. **8b** MALDI-TOF ($MH^+$): Expected: 2112; Found: 2112. **8c** MALDI-TOF ($MH^+$): Expected: 7536; Found: 7536.

**Nuclease Digestion**

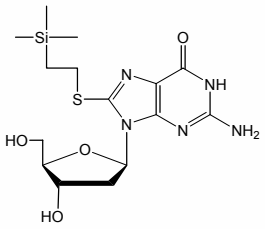
0.2 $OD_{260}$ of **8b** or **3** was incubated for 16 hours at 37 ˚C with 12 μg units snake venom phosphodiesterase (*Crotalus adamanteus*), 2 units bacterial alkaline phosphatase, 32 mM Tris pH 7.5 and 15 mM $MgCl_2$ in a final volume of 80 μL. When the reaction was complete, 10 μL of 3 M sodium acetate pH 7 and 250 μL of ethanol were added and the solution incubated for 30 min at –78 ˚C before centrifugation for 20 min at 12,000 rpm. The supernatant was isolated, dried *in vacuo*, and resuspended in 150 μL of water before analysis by analytical HPLC using a linear gradient of 5-6.5% B in A over 20 min.

**Melting Studies**

5 μM of each oligonucleotide, 1 M NaCl, 0.1 mM EDTA and 100 mM phosphate buffer pH 7 in a total volume of 1 mL was heated for 5 minutes at 90 ˚C. The solution was then allowed to cool at room temperature for at least 30 min and at 4 ˚C for at least 30 min. Melting temperatures were determined on a Jasco 560 Spectrophotometer with Peltier temperature controller. The absorbance at 260 nm was monitored from 20 – 80 ˚C with the temperature increased at a rate of 0.5 ˚C/minute.

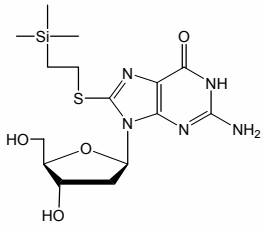



Representative melting curves for helices containing dG:dC (blue; $T_m$ = 57.6 ˚C), dG:dA (green; $T_m$ = 43.0 ˚C ), OdG:dC (aqua; $T_m$ = 52.6 ˚C), OdG:dA (red; $T_m$ = 48.0 ˚C), SdG:dC (brown; $T_m$ = 46.5 ˚C) and SdG:dA (purple; $T_m$ = 47.7 ˚C) base pairs.

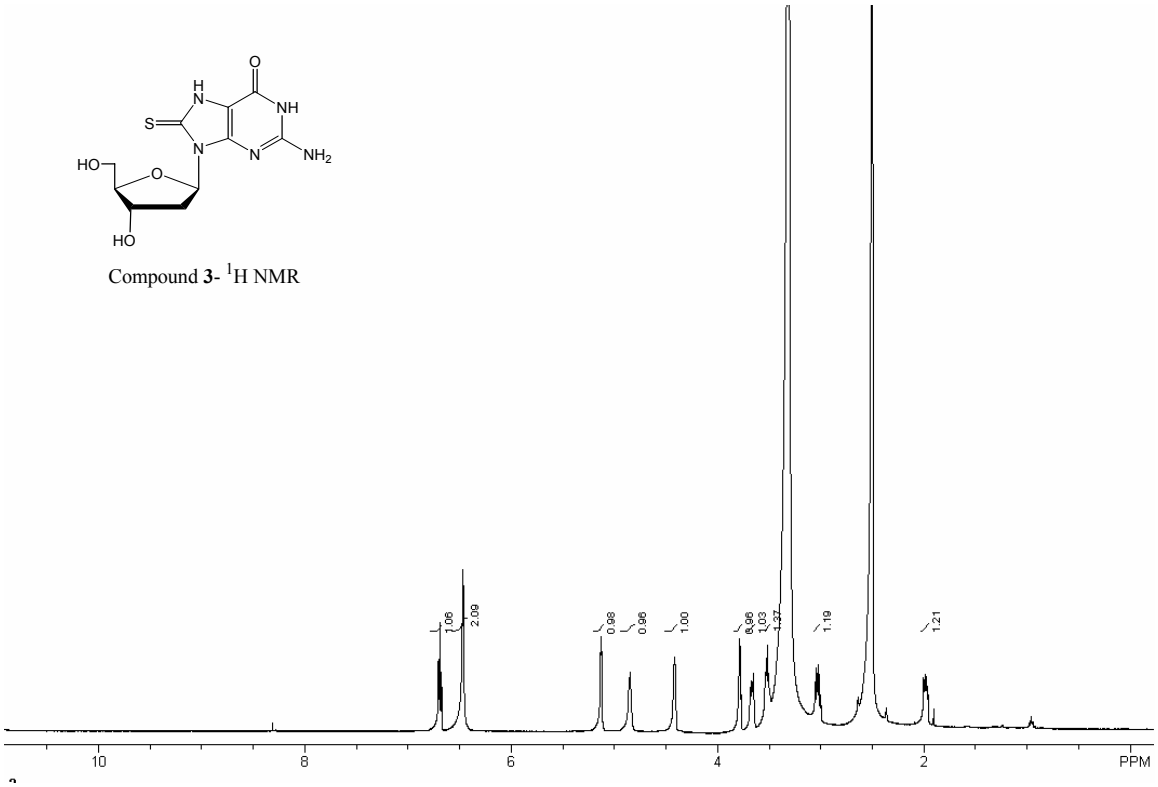


Compound **2**- $^{1}$H NMR

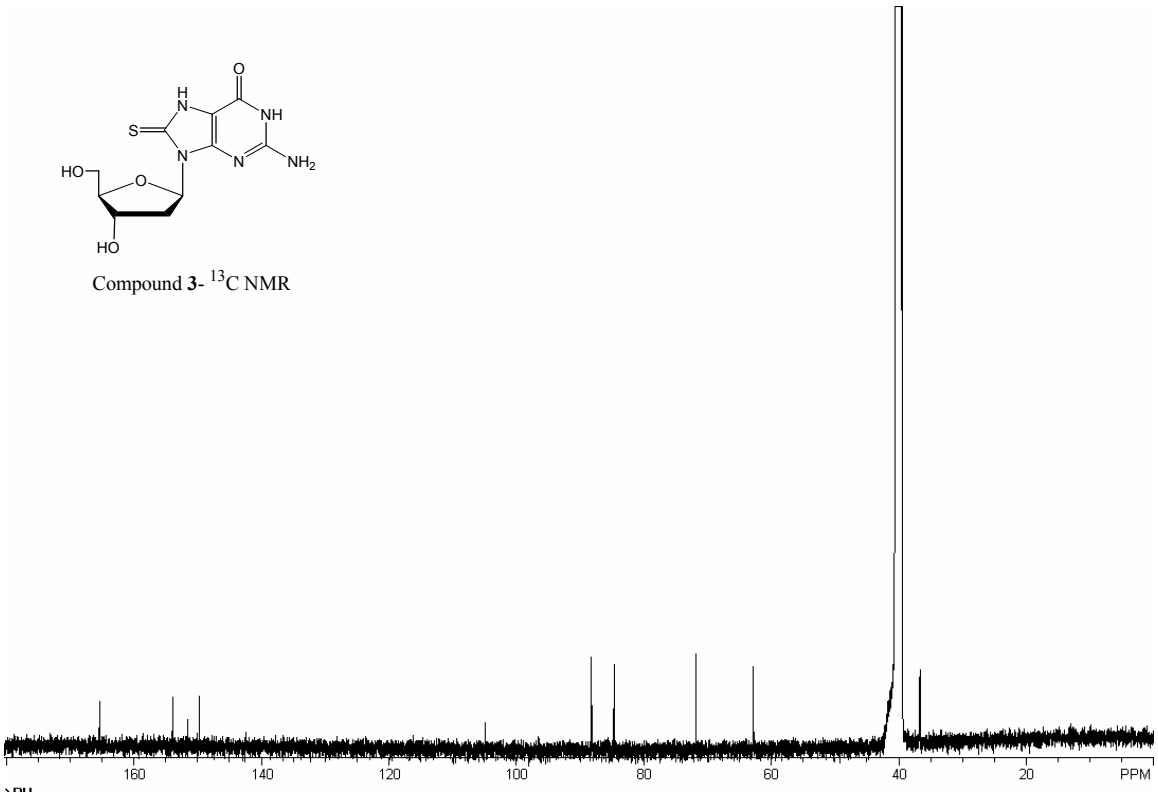


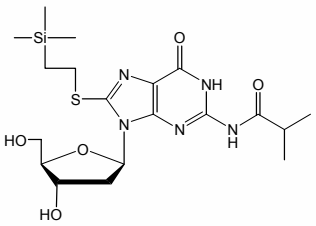
Compound **2**- $^{13}$C NMR




Compound **3**- $^{1}$H NMR

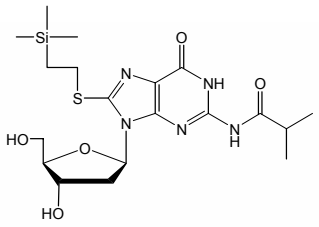



Compound **3**- $^{13}$C NMR

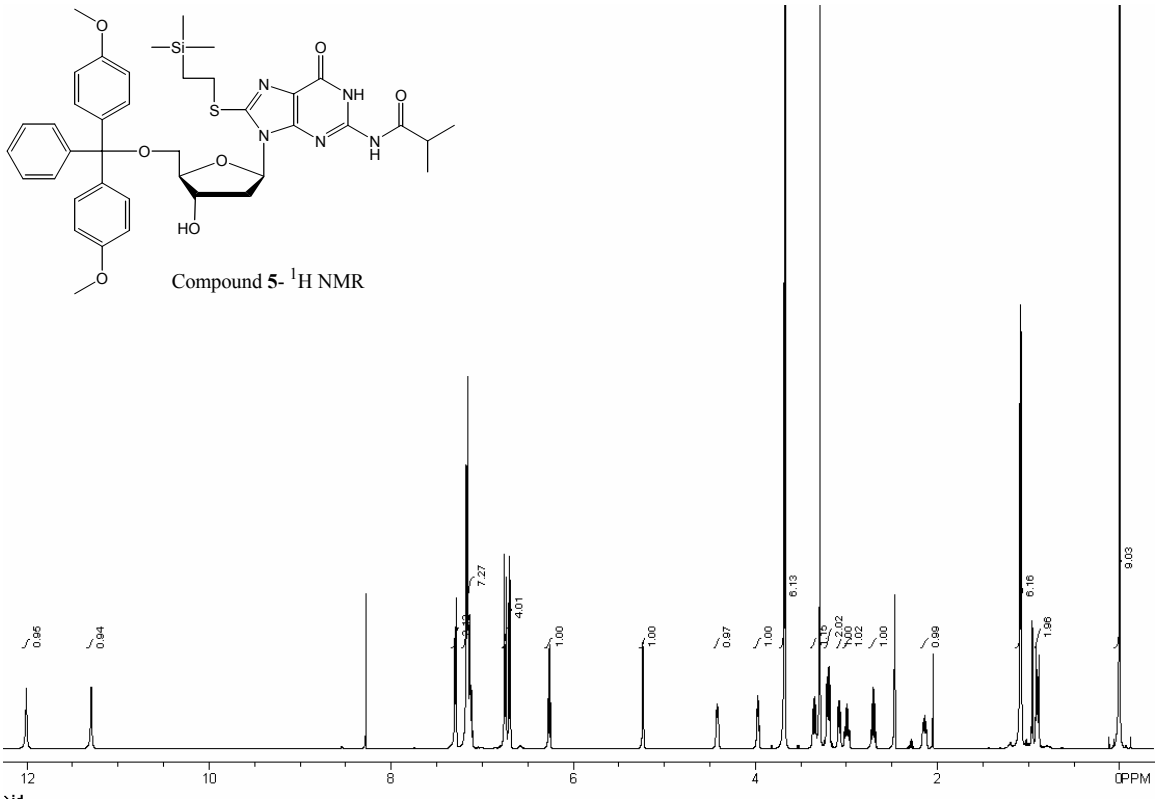



Compound **4**- $^{1}$H NMR

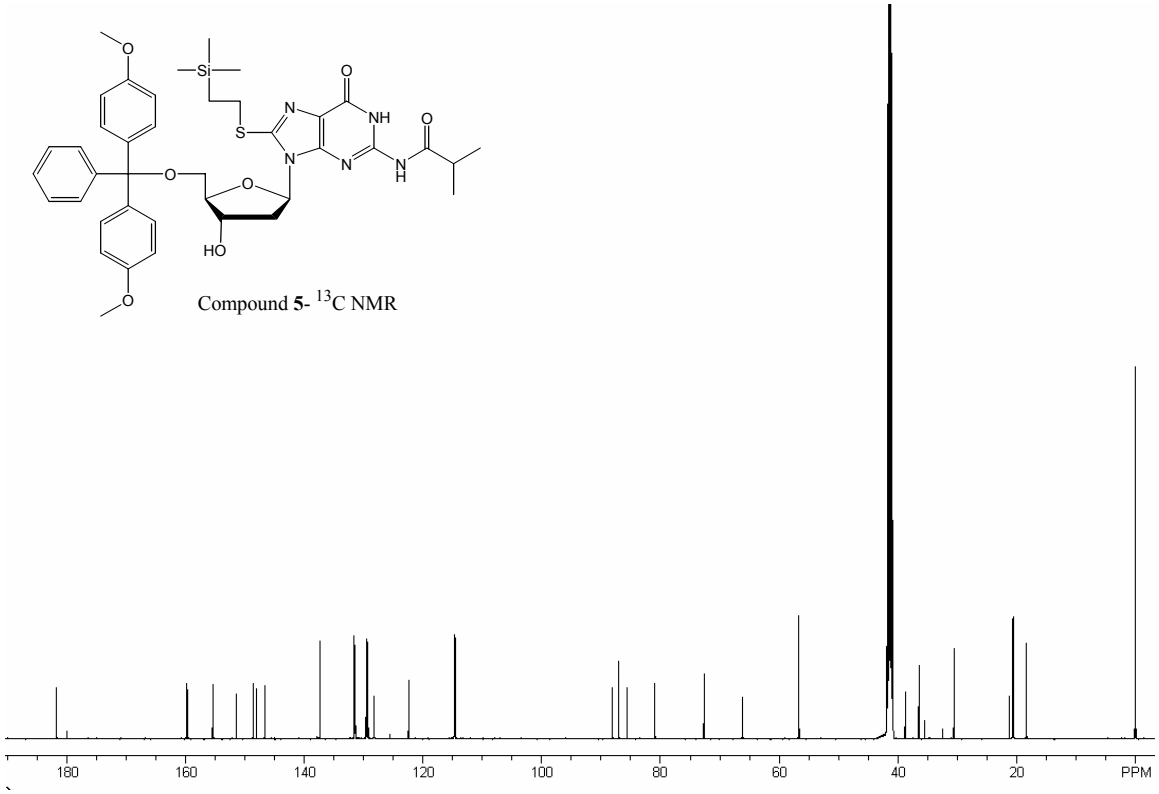


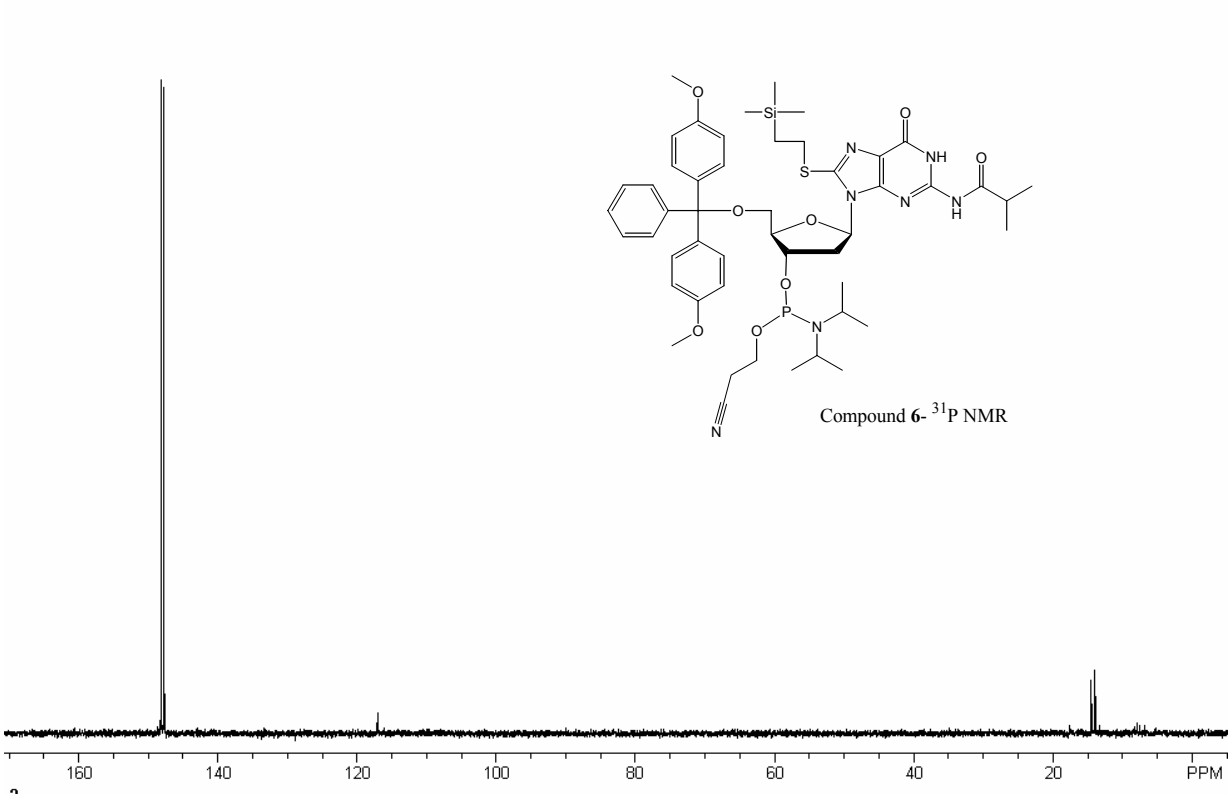

Compound **4**- $^{13}$C NMR



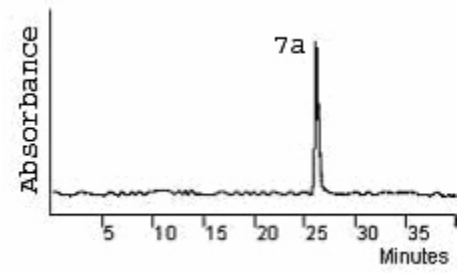

Compound **5**- $^{1}$H NMR



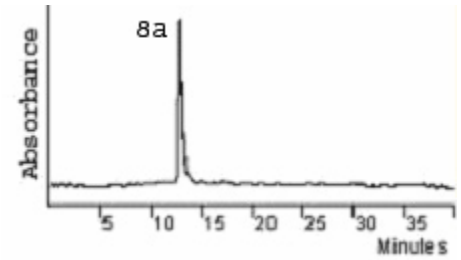

Compound **5**- $^{13}$C NMR



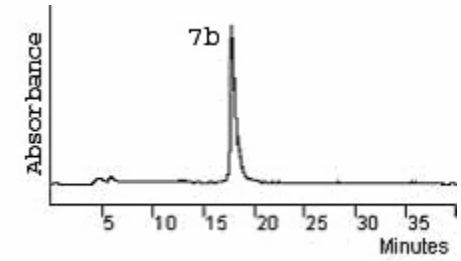

Compound **6**- $^{31}$P NMR



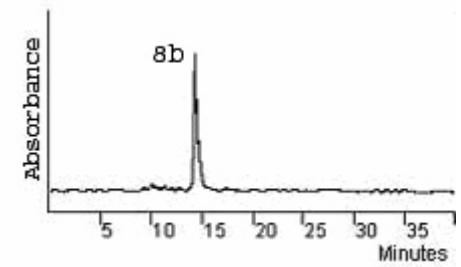

HPLC trace of 5'-d$^{SiS}$GT-3' (**7a**) using a linear gradient of 5-40% B in A over 30 min.



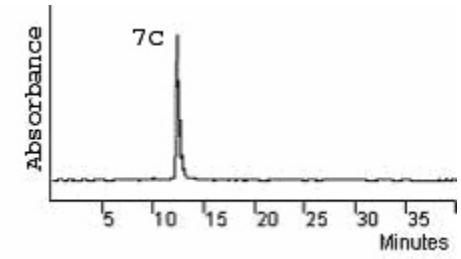

HPLC trace of 5'-d$^{S}$GT-3' (**8a**) using a linear gradient of 5-40% B in A over 30 min.



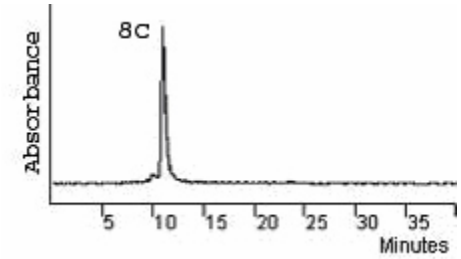

HPLC trace of 5'-dACT$^{SiS}$GTCA-3' (**7b**) using a linear gradient of 5-40% B in A over 30 min.




HPLC trace of 5'-dACT$^{S}$GTCA-3' (**8b**) using a linear gradient of 5-60% B in A over 30 min.




HPLC trace of 5'-dCATCGATACGATCT$^{SiS}$GCCTCTCTCTC-3' (**7c**) using a linear gradient of 5-60% B in A over 30 min.




HPLC trace of 5'-dCATCGATACGATCT$^{S}$GCCTCTCTCTC-3' (**8c**) using a linear gradient of 5-60% B in A over 30 min.