Synthesis, Oligonucleotide Incorporation and Base Pairing of 7-Methyl-8-oxo-2'-deoxyguanosine

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Electronic Supplementary Information

Experimental

All reagents were used as supplied by Aldrich or Acros Chemicals except where noted. Methylene chloride used in chromatography was passed through Alumina (Active Basic, Activity I), and Dowex (50 x 4-400) was washed with methanol prior to use. 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 California-Riverside Mass Spectrometry Facility or in-house on a Bruker BioTOF-Q Mass Spectrometer. 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 Beckman Ultrasphere ODS 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.

2-*N*,2',3',5'-*O*-tetraisobutyryl-7-methylguanosine (2)

A mixture of **1** (2.5 g, 4.4 mmol) and iodomethane (2.8 mL, 45 mmol) in DMF (20 mL) was stirred under argon at 35 ºC overnight. DMF was removed under reduced pressure and the resulting foam was purified by silica gel chromatography using 0-6% methanol in chloroform to yield 2.53 g (4.37 mmol; 99%) of **2** as a yellow foam. ^{1}H NMR ($CDCl_3$) δ: 12.81 (s, 1H), 10.81 (s, 1H), 10.37 (s, 1H), 6.51 (d, J = 4.7, 1H), 5.98 (t, J = 5.7, 1H), 5.80 (t, J = 5.8, 1H), 4.75 (q, 1H), 2.53 (m, 2H), 4.33 (s, 3H), 2.97 (m, 1H), 2.54-2.69 (m, 3H), 1.06-1.33 (d, 24H). ^{13}C NMR ($CDCl_3$) δ: 178.8, 177.7, 176.2, 175.8, 151.5, 150.3, 146.5, 142.6, 105.7, 84.6, 78.8, 72.1, 70.8, 62.9, 36.5, 33.9, 33.8, 33.7, 29.0, 18.9, 18.87, 18.84, 18.82, 18.78, 18.70. 18.7. HR-ESI (M^+) for $C_{27}H_{40}N_5O_9$. Calculated: 578.2826; Found: 578.2840.

2-*N*,2',3',5'-*O*-tetraisobutyryl-7-methyl-8-oxoguanosine (3)

To a solution of **2** (1.93 g, 3.34 mmol) in 20 mL of acetic acid was added 0.86 mL of 30% hydrogen peroxide. The solution was stirred at 37 ºC for 3.25 hours before 1.5 M sodium sulfite (13.5 mL) was added to quench the remaining hydrogen peroxide. The solution was concentrated under vacuum and extracted in water (100 mL). The aqueous layer was then washed twice with 100 mL of chloroform. The organic layers were combined, dried with sodium sulfate and concentrated. The resulting oil was purified by silica gel chromatography using 1% methanol in chloroform to yield 1.48 g (2.5 mmol;

75%) of **3** as a white foam. ^{1}H NMR ($CDCl_3$) δ: 11.99 (s, 1H), 9.05 (s, 1H), 6.84 (d, *J* = 4.1, 1H), 6.02 (t, *J* = 5.0, 1H), 5.93 (t, *J* = 5.0, 1H), 4.68 (dd, *J* = 11.5, 4.0, 1H), 4.44 (q, *J* = 5.4, 1H), 4.37 (m, 1H), 3.60 (s, 3H), 2.73 (m, 1H), 2.54-2.66 (m, 3H), 1.09-1.23 (24H). ^{13}C NMR ($CDCl_3$) δ: 178.8, 177.7, 176.2, 175.8, 151.5, 150.3, 146.6, 142.7, 105.7, 84.6, 78.8, 72.1, 70.8, 62.9, 36.5, 34.0, 33.9, 33.8, 29.1, 18.92, 18.90, 18.87, 18.85, 18.81 (2C), 18.73, 18.70. HR-ESI ($M+H^+$) for $C_{27}H_{40}N_5O_{10}$. Calculated: 594.2775; Found: 594.2787.

2-*N*-Isobutyryl-3',5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl)-7-methyl-8-oxoguanosine (4)

36 mL of 0.2 M sodium methoxide in 65:35 pyridine:methanol was added to compound **3** (0.9 g, 1.52 mmol) and stirred for 30 minutes on ice. The reaction was quenched with washed Dowex, and the solvents were evaporated under vacuum to yield 2-*N*-isobutyryl-7-methyl-8-oxoriboguanosine which was used directly in the next reaction without further purificaton.1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (0.65 mL, 2.03 mmol) and anhydrous pyridine (6.6 mL) were then added to 2-*N*-Isobutyryl-7-methyl-8-oxoriboguanosine from above and the reaction was stirred for 1 hour under argon. The solution was concentrated under vacuum and the resulting oil was purified by silica gel chromatography using 1% methanol in chloroform to yield 0.68 g (1.09 mmol; 71%) of **4** as a white foam. ^{1}H NMR ($CDCl_3$) δ: 11.94 (s, 1H), 8.11 (s, 1H), 5.79 (d, J = 1.7, 1H), 5.00 (t, J = 5.9, 1H), 4.83 (d, J = 5.9, 1H), 4.03 (m, 2H), 3.96 (m, 1H), 3.56 (s, 3H), 2.59 (m, J = 6.9, 1H), 1.30 (d, J = 6.9, 6H), 0.98-1.16 (28H). ^{13}C NMR ($CDCl_3$) δ: 178.3, 150.7, 150.0, 146.9, 142.9, 105.3, 87.9, 80.8, 75.1, 70.6, 60.9, 36.7, 28.8, 18.72, 18.68, 14.4, 17.33, 17.28, 17.1, 17.0, 13.4, 13.23, 13.19, 13.1, 13.0, 12.75, 12.69, 12.61. HR-ESI ($M+H^+$) for $C_{27}H_{48}N_5O_8Si_2$. Calculated: 626.3041; Found: 626.3023.

2-*N*-Isobutyryl-3',5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl)-7-methyl-8-oxo-2'-deoxyguanosine (5)

Phenylchlorothiocarbonate (0.49 mL; 3.6 mmol), compound **5** (1.49 g, 2.4 mmol), 4-dimethylaminopyridine (DMAP; 0.585 g; 4.8 mmol), methylene chloride (30 mL) and acetonitrile (5 mL) were combined and stirred for 2 hours at room temperature under argon. The solvents were removed under reduced pressure and the resulting oil was dissolved in methylene chloride (120 mL). The organic layer was washed twice with equal amounts of 1 N HCl and sat. $NaHCO_3$, dried with sodium sulfate and concentrated under vacuum. To the unpurified product from above 2,2'-azobis(2-methylpropionitrile) (AIBN; 100 mg; 0.6 mmol), tributyltin hydride (3.8 mL; 14.4 mmol) and toluene (75 mL) were added. The reaction was stirred for 1.5 hours at 70 ºC under argon before it was concentrated under vacuum and purified by silica gel chromatography using 0.25% methanol in chloroform to yield 0.74 g (1.21 mmol; 50%) of **5** a white foam. ^{1}H NMR ($CDCl_3$) δ: 11.94 (s, 1H), 7.96 (s, 1H), 6.10 (dd, 1H), 5.04 (q, 1H), 4.02 (dd, 1H), 3.93 (m, 1H), 3.82 (m, 1H), 3.58 (s, 3H), 3.10 (m, 1H), 2.57 (q, 1H), 2.40 (m, 1H), 1.30 (d, 6H), 1.06-1.15 (28H). ^{13}C NMR ($CDCl_3$) δ: 177.9, 151.0, 150.1, 146.0, 143.4, 105.4, 84.9, 79.9, 72.6, 63.5, 37.2, 36.7, 28.8, 19.0, 18.9, 17.52, 17.47, 17.47, 17.37, 17.27, 17.08, 17.06, 17.01, 13.4, 13.2, 13.0, 12.9. HR-ESI ($M+H^+$) for $C_{27}H_{48}N_5O_7Si_2$. Calculated: 610.3092; Found: 610.3073.

2-*N*-Isobutyryl-7-methyl-8-oxo-2'-deoxyguanosine (6)
A mixture of compound **5** (0.600 g; 0.98 mmol), 4 mL of 1 M tetrabutylammonium-fluoride in tetrahydrofuran, and tetrohydrofuran (4 mL) was stirred for 2.5 hours under argon. The mixture was then concentrated under vacuum and purified by silica gel chromatography using 0-4% methanol in chloroform to yield 0.322 g (0.877 mmol; 89%) of **6** as a white foam. ^{1}H NMR (DMSO-d_6) δ: 12.06 (s, 1H), 11.59 (s, 1H), 6.12 (t, 1H), 5.15 (d, 1H), 4.68 (t, 1H), 4.37 (m, 1H), 3.76 (m, 1H), 3.56 (m, 1H), 3.44 (m, 1H), 3.40 (s, 3H), 3.05 (m, 1H), 2.75 (m, 1H), 1.99 (ddd, 1H), 1.12 (d, 6H). ^{13}C NMR (DMSO-d_6) δ: 180.4, 151.3, 150.1, 147.7, 144.3, 104.3, 87.86, 81.9, 71.6, 62.7, 35.7, 35.2, 28.7, 19.3 (2C). HR-ESI (M+H$^+$) for $C_{15}H_{22}N_5O_6$. Calculated: 368.1570; Found: 368.1570.

2-*N*-Isobutyryl-5'-*O*-(4',4'-dimethoxytrityl)-7-methyl-8-oxo-2'-deoxyguanosine (7)
Compound **6** (0.750 g; 2.04 mmol) was coevaporated three times with pyridine to remove any associated water before 4',4'-Dimethoxytrityl chloride (DMTr-Cl; 1.04 g; 3.06 mmol) and 4-dimethylaminopyrdine (DMAP; 16 mg; 0.1 mmol) were added and the flask covered with argon. Triethylamine (0.5 ml; 3.6 mmol) and anhydrous pyridine (24 mL) were then added and the reaction was stirred for 1.25 hours. The reaction was concentrated under vacuum and purified by silica gel chromatography using 0-5% methanol in chloroform to yield 0.870 g (1.3 mmol; 64%) of **7** as a pale orange foam. ^{1}H NMR (DMSO-d_6) δ: 12.11 (s, IH), 11.44 (s, 1H), 7.15-7.34 (m, 9H), 7.7 (dd, J = 20, 8.7, 4H), 6.15 (dd, J = 7.5, 5.9, 1H), 5.15 (d, J = 4.8, 1H), 4.42 (m, 1H), 3.99 (m, 1H), 3.72 (s, 3H), 3.71 (m, 3H), 3.39 (s, 3H), 3.07 (dd, J = 10, 3.2, 1H), 2.98 (m, 1H), 2.72 (m, 1H), 2.12 (m, 1H), 1.12 (d, J = 3.1, 3H), 1.10 (d, J = 2.6, 1H). ^{13}C NMR (DMSO-d_6) δ: 206.9, 180.4, 158.4, 158.3, 151.2, 150.1, 147.4, 145.5, 144.2, 136.23, 136.17, 130.2, 130.1, 128.3, 128.0, 126.9, 113.3, 113.2, 104.5, 86.3, 85.6, 81.7, 79.6, 71.4, 65.3, 55.42, 55.38, 40.6, 40.5, 40.2, 36.5, 35.2, 31.1, 28.8, 19.4, 19.2. HR-ESI (M+Na$^+$) for $C_{36}H_{39}N_5O_8Na$. Calculated: 692.2691; Found: 692.2683.

2-*N*-Isobutyryl-5'*O*-(4',4'-dimethoxytrityl)-7-methyl-8-oxo-2'-deoxyguanosine 3'-*O*-[(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite] (8)
7 (250 mg; 0.373 mmol) was coevaporated three times with pyridine to remove any associated water before suspension in 7 mL of anhydrous methylene chloride. Redistilled diisopropylethylamine (0.163mL; 0.93 mmol), freshly distilled 1-methylimidazole (14.8μL; 0.19 mmol) and *N*,*N*-diisopropyl-2-cyanoethyl-phosphonamidic chlorine (141.5μL ; 0.63 mmol; Chemgenes) were added and the reaction was stirred continuously for 0.5 hour under argon. After concentrating under vacuum, the resulting oil was purified by silica gel chromatography using 3% acteone in CH_2Cl_2 with 0.1% triethylamine to yield 0.140 g (0.161 mmol; 43%) of **8** as a white foam.
^{31}P NMR ($CDCl_3$) δ: 147.8, 147.6. HR-ESI (M+Na$^+$) for $C_{45}H_{56}N_7O_9PNa$. Calculated: 892.3769; Found: 892.3736.

7-methyl-8-oxo-2'-deoxyguanosine (10)

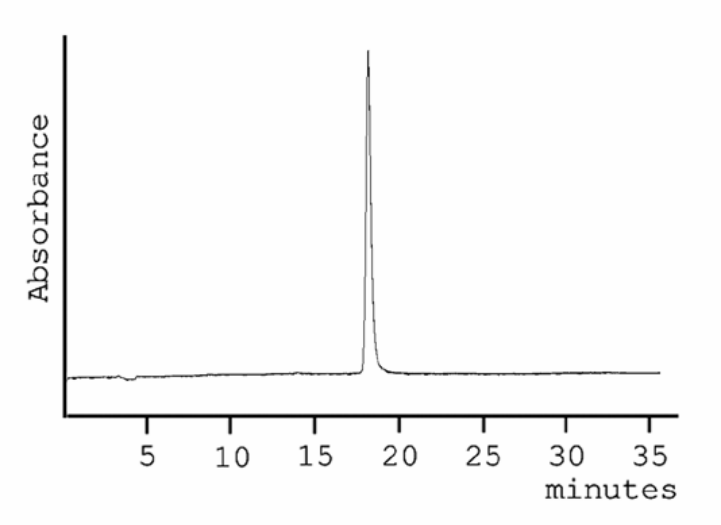
3.5 mL of 0.3 M sodium methoxide in methanol was added to 60 mg (0.17 mmol) of **6** and the reaction was stirred for 5 hours at 40 ºC. The reaction was then quenched with Dowex, filtered, evaporated to dryness and dissolved in 5 mL of water. After two 5 mL chloroform washes, the water layer was run through a 20cc C_{18} sep-pak column using 0-7.5% acetonitrile in water to yield **10** as a white powder. NMR (DMSO-d_6) δ: 6.70 (b, 2H), 6.07 (t, 1H), 5.11 (b, 1H), 4.99 (b, 1H), 4.34 (m, 1H), 3.76 (d, 1H), 3.57 (dd, 1H), 3.44 (dd, 1H), 3.17 (s, 3H), 2.96 (m, 1H), 1.93 (m, 1H). ^{13}C NMR (DMSO-d_6) δ: 154.6, 154.1, 150.9, 146.2, 99.3, 87.5, 81.4, 71.5, 62.5, 35.9, 28.2.HR-ESI ($M+Na^+$) for $C_{11}H_{15}N_5O_5Na$. Calculated: 320.0965; Found: 320.0963.

DNA Synthesis

Oligonucleotides **9b** and **9c** were purchased from Integrated DNA Technologies and Eurogenetec Inc., respectively. Synthesis of oligonucleotide **9a** 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 ºC for 18 hours.

DNA Purification

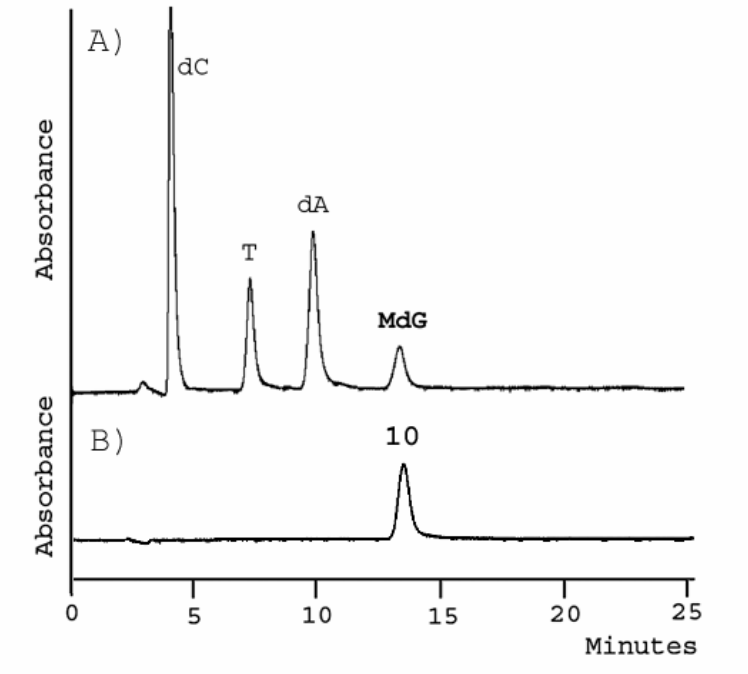
Oligonucleotide **9a** was purified by 20% denaturing PAGE before UV visualization. The slowest running band was excised and soaked twice in water, in the dark, for 24 hours. The resulting solutions were then concentrated, combined by resuspension in 1mL of water, and filtered. The oligonucleotide was then further purified by preparative RP-HPLC using a linear gradient of 5-20% solvent B in A over 30 min. **9a** MALDI-TOF (MH^+) for $C_{105}H_{138}N_{37}O_{65}P_{10}$. Calculated: 3266.6; Found: 3266.6.



HPLC trace of **9a** after purification.

Nuclease Digestion

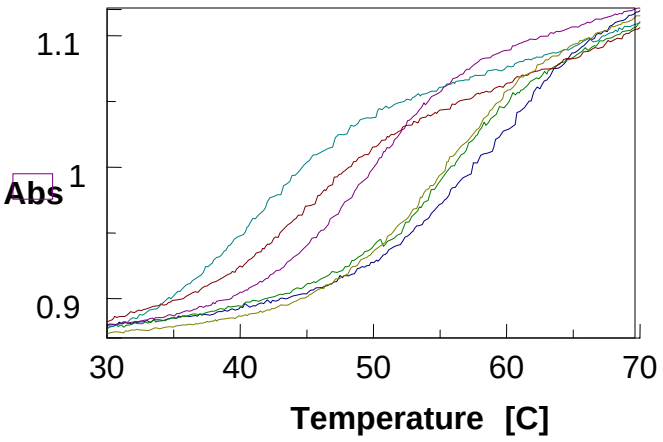
0.3 OD_{260} of **9a** and **10** were incubated for 16 hours at 37 ˚C with 12 μg units snake venom phosphodiesterase (*Crotalus adamanteus*), 2 units bacterial alkaline phosphatase, 32 mM Tris pH 7.5 and 15 mM $MgCl_2$ in a final volume of 80 μL. When the reaction was complete, 10 μL of 3 M sodium acetate 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 RP-HPLC using a linear gradient of 5-6.5% B in A over 20 min.



HPLC analyses of Nuclease Digested **9a** (A) and **10** (B).

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. The resulting melting curves were analyzed by least squares fitting to ΔH° and T_m for a two state stable transition.



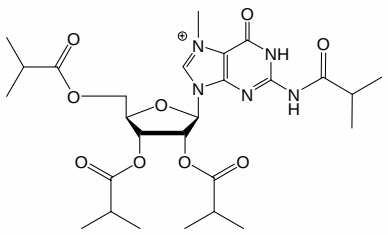
Raw melting temperature data. The data shown is for the base pairs **dG:dC** (navy), **OdG dC** (green), **MdG:dC** (yellow), **OdG:dA** (purple), **dG:dA** (red) and **MdG:dA** (aqua).

NMR Studies.
2'-Deoxyguanosine (dG) and 8-oxo-2'-deoxyguanosine (OdG) were purchased from Chemgenes and Berry Inc, respectively. 7-Methyl-8-oxo-2'-deoxyguanosine (MdG) was synthesized as described above. All spectra were collected using a Bruker AVANCE500 NMR spectrometer with 0.04 M nucleoside in DMSO-d_6 with 0.1 % tetramethylsilane (TMS). All chemical shifts are given relative to TMS.

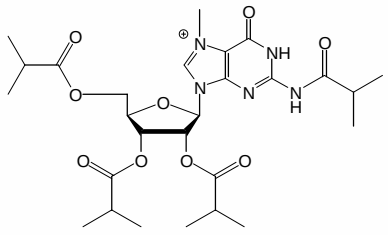
	N2-H	1'-H	3'-H	4'-H	5'-H*	5''-H*	2'-H	2''-H
dG	6.43	6.12	4.33	3.81	3.55	3.50	2.50	2.19
OdG	6.53	6.07	4.34	3.76	3.58	3.45	2.97	1.92
MdG	6.70	6.07	4.34	3.76	3.57	3.44	2.96	1.93

*Could be reversed

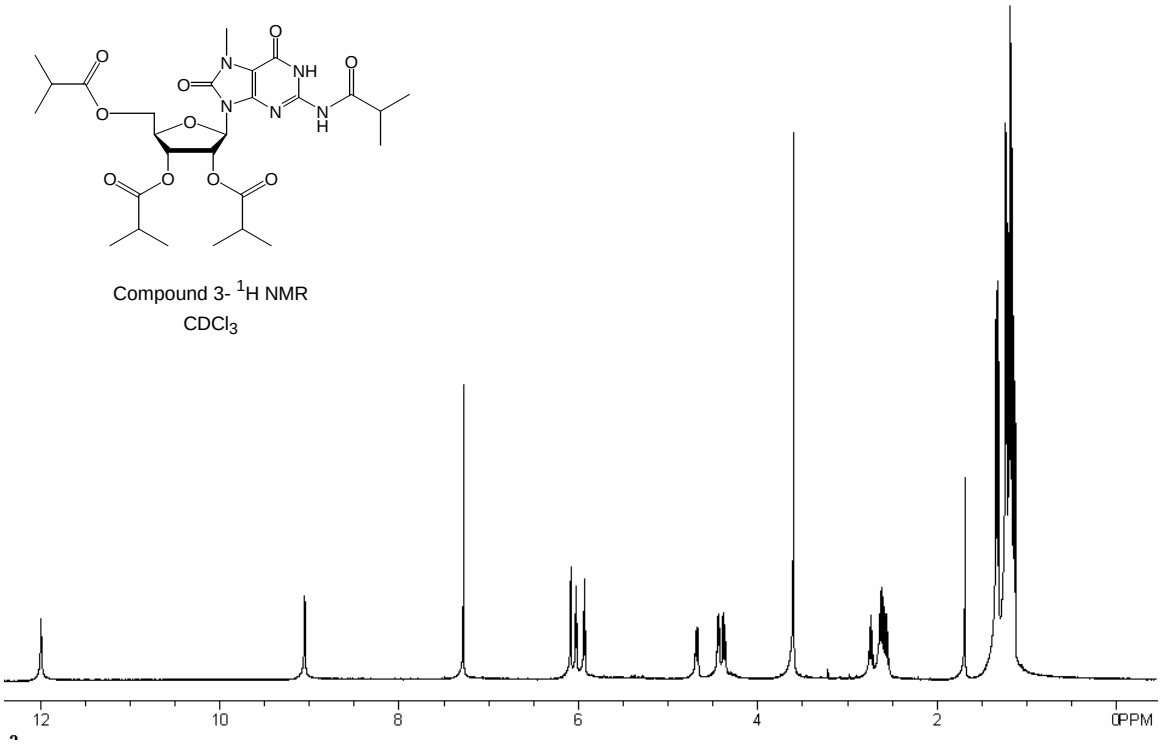
	C4'	C1'	C3'	C5'	C2'
dG	87.5	82.5	70.6	61.6	39.5
OdG	87.3	81.1	71.4	62.4	35.7
MdG	87.4	81.3	71.4	62.4	35.8



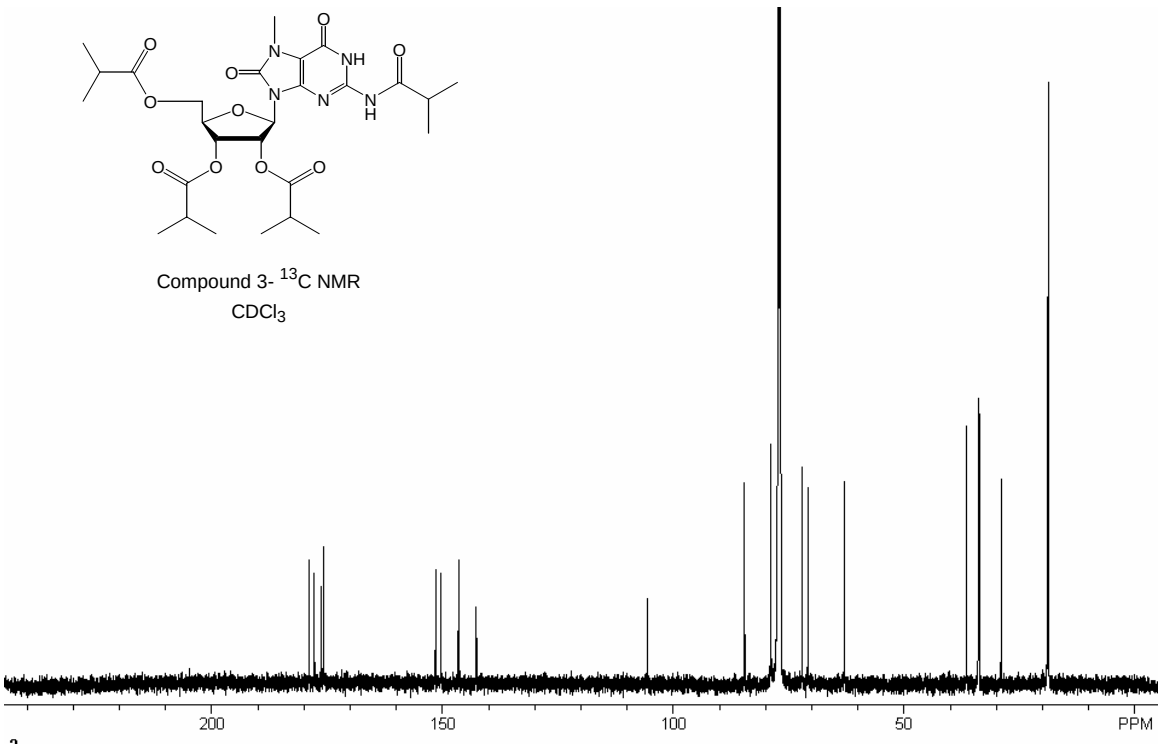
Compound 2- ^{1}H NMR
$CDCl_3$



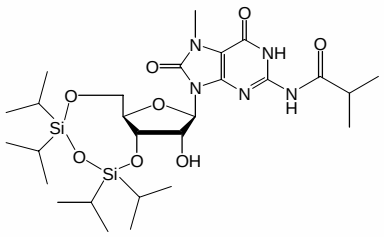
Compound 2- ^{13}C NMR
$CDCl_3$



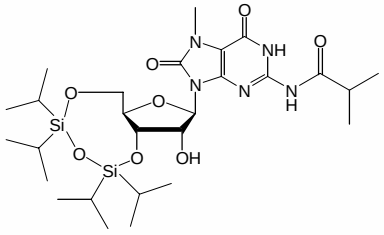
Compound 3- ^{1}H NMR
$CDCl_3$



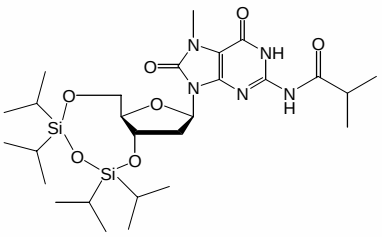
Compound 3- ^{13}C NMR
$CDCl_3$



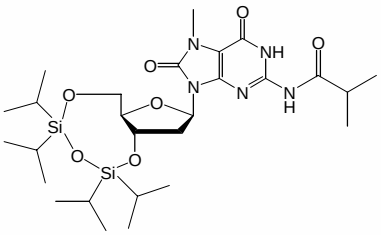
Compound 4- ^{1}H NMR
$CDCl_3$



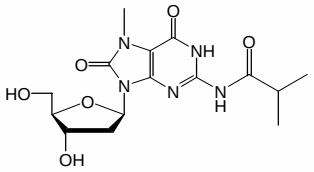
Compound 4- ^{13}C NMR
$CDCl_3$



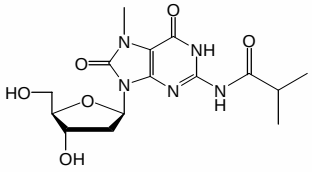
Compound 5- ^{1}H NMR
$CDCl_3$



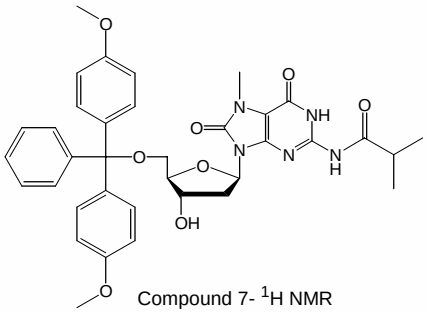
Compound 5- ^{13}C NMR
$CDCl_3$



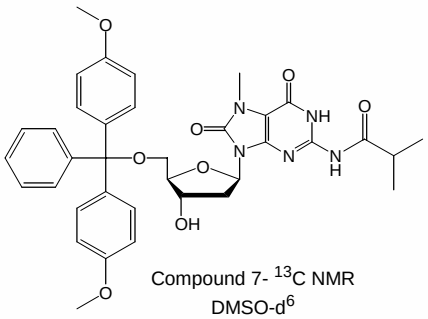
Compound 6- ^{1}H NMR
DMSO-d_6



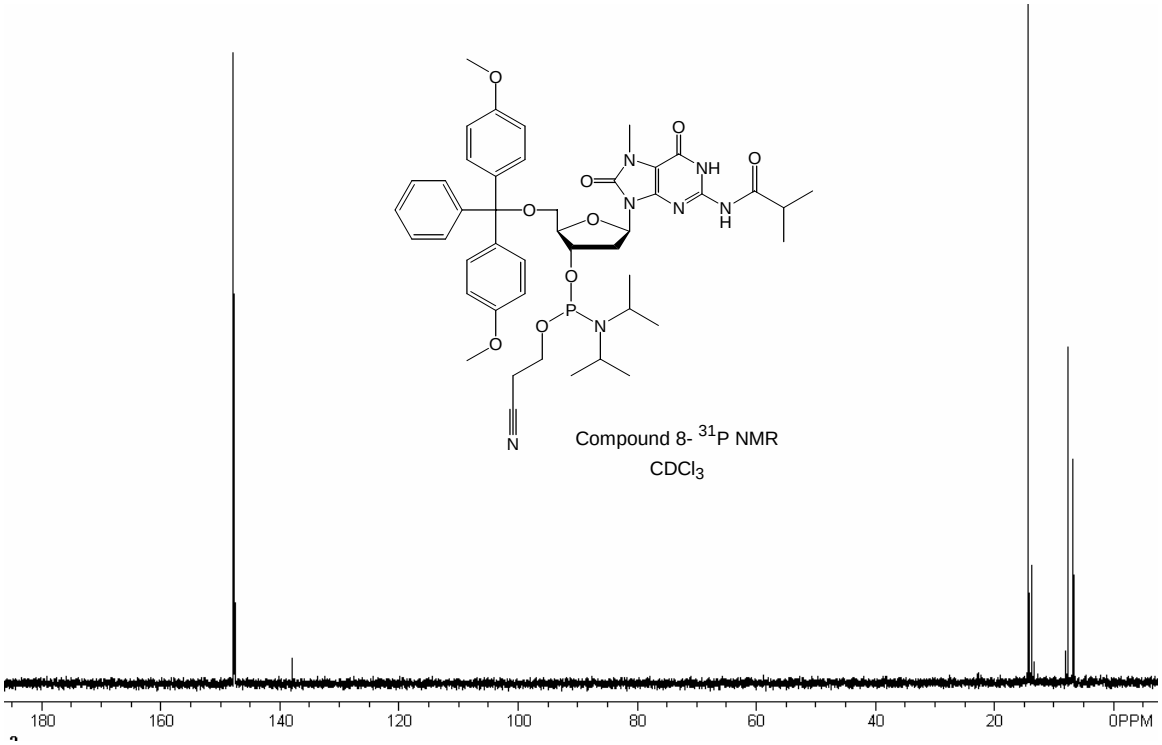
Compound 6- ^{13}C NMR
DMSO-d_6



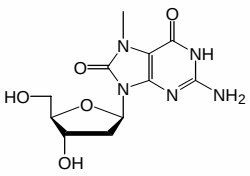
Compound 7- ^{1}H NMR
DMSO-d_6



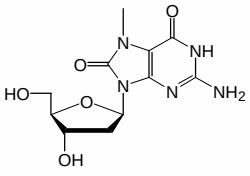
Compound 7- ^{13}C NMR
DMSO-d^6



Compound 8- ^{31}P NMR
$CDCl_3$



Compound 10- ^{1}H NMR
DMSO-d_6



Compound 10- ^{13}C NMR
DMSO-d_6