Supporting Information

Light-Activated Deoxyguanosine: Photochemical Regulation of Peroxidase Activity

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1. Synthesis of the Caged Deoxyguanosine Phosphoramidite 6:

2'-Deoxy-3',5'-diacetate-N^2-dimethylaminomethylene-guanosine (2). To 2'-deoxyguanosine (2g, 7.5mmol) suspension in dry MeOH (30mL) was added N,N-Dimethylformamide dimethyl acetal (4mL, 30mmol). The reaction was refluxed for 5h and the solvent subsequently evaporated. The residue was redisolved in pyridine (20mL) and cat. DMAP and AcOAc (2.8mL, 30mmol) were added to the solution. The reaction was stirred at room temperature for 8h. The solvents were evaporated and the residue purified by silica gel chromatography using $CHCl_3$:MeOH (90:10) with 2% TEA, affording 1.86g of **2** as a white foam (61% yield).
^{1}H NMR ($CDCl_3$): δ = 2.02 (s, 3H), 2.10 (s, 3H), 2.50 (m, 1H). 2.95-3.05 (m, 4H), 3.17 (s, 3H), 2.95-3.20 (m, 3H), 5.30 (m, 1H), 6.21 (t, *J* = 6.9 Hz, 1H), 7.66 (s, 1H), 8.60 (s, 1H), 9.91 (s, 1H). ^{13}C NMR (CDCl3): δ = 20.9, 21.1, 35.5, 37.0, 41.5, 63.9, 74.3, 82.2, 84.3, 121.3, 136.9, 150.1, 157.2, 158.3, 158.4, 170.6, 170.8. HRMS-LC: m/z $[M+H]^+$ calcd for $C_{13}H_{19}N_3O_6$: 314.1352; found: 314.1347.

2'-Deoxy-3',5'-diacetate-N^2-dimethylaminomethylene-N^1-(6-nitropiperonyloxymethylene)-guanosine (3). To compound **2** (100mg, 0.25mmol) in DMF (1mL) was added DBU (55µL, 0.38mmol) and the solution was stirred for 30 minutes at room temperature. To the solution was then added 6-nitropiperonylchloromethyl ether (NPOM-Cl,[1] 97µL, 0.38mmol) dissolved in DMF (0.5mL) and the reaction was stirred at room temperature for 8h. The reaction was diluted with EtOAc (10mL) and washed with sat. $NaHCO_3$, H_2O and brine (10mL of each). The organic layer was dried over Na_2SO_4, filtered and the solvent was evaporated. Purification by silica gel chromatography using $CHCl_3$ with 2% TEA afforded 124mg of compound **3** as a yellow foam (79% yield).
^{1}H NMR ($CDCl_3$): δ = 1.43 (d, *J* = 6.3 Hz, 3H), 2.05 (s, 3H), 2.11 (s, 3H), 2.42-2.51 (m, 1H), 2.95-3.07 (m, 4H), 3.20 (s, 3H), 4.16-4.38 (m, 3H), 5.33-5.49 (m, 2H), 5.68 (m, 1H), 5.82-5.94 (m, 3H), 6.14 (m, 1H), 7.14 (m,1H), 7.29 (s, 1H), 7.60 (m, 1H), 8.36 (m, 1H). ^{13}C NMR ($CDCl_3$): δ = 20.9, 21.2, 24.1, 35.5, 36.8, 37.2, 41.5, 71.7, 73.9, 74.4, 82.1, 84.1, 103.0, 104.6,

[1] H. Lusic and A. Deiters, *Synthesis*, **2006**, 2147-2150.

107.2, 120.8, 136.5, 136.7, 139.3, 141.1, 146.8, 147.8, 152.5, 157.1, 157.8, 170.5, 170.8. HRMS-LC: m/z $[M+H]^+$ calcd for $C_{27}H_{31}N_7O_{11}$: 630.2160; found: 630.2280.

2'-Deoxy-N^2-dimethylaminomethylene-N^1-(6-nitropiperonyloxymethylene)-guanosine (4). Compound **3** (500mg, 0.79mmol) was dissolved in MeOH (5mL) and to the solution was added K_2CO_3 (510mg, 3.95mmol). The reaction progress was monitored by TLC. After disappearance of the starting material, the reaction was filtered and solvent evaporated. Purification by silica gel chromatography using $CHCl_3$:MeOH (85:15) with 2% TEA afforded 385mg of **4** as a yellow foam (92% yield).
^{1}H NMR ($CDCl_3$): δ = 1.44 (m, 3H), 2.48 (m, 1H), 2.66 (m, 1H), 3.03 (m, 3H), 3.19 (m, 3H), 3.92 (m, 2H), 4.13 (m, 1H), 4.82 (m, 1H), 5.37 (m, 1H), 5.69-5.94 (m, 4H), 6.32 (m, 1H), 7.06 (m, 1H), 7.26 (m, 1H), 8.15-8.28 (m, 2H). ^{13}C NMR ($CDCl_3$): δ = 24.1, 35.4, 40.8, 41.6, 62.2, 70.9, 71.1, 72.1, 74.5, 85.2, 88.5, 103.2, 104.6, 106.8, 120.0, 138.3, 139.1, 141.1, 146.8, 147.9, 152.3, 156.9, 158.2. HRMS-LC: m/z $[M+H]^+$ calcd for $C_{23}H_{27}N_7O_9$: 546.1948; found: 546.2047.

5'-O-DMT-2'-Deoxy-N^2-dimethylaminomethylene-N^1-(6-nitropiperonyloxymethylene)-guanosine (5). To a solution of **4** (200mg, 0.37mmol) and cat. DMAP in pyridine (3mL), at 0ºC, was added dimethoxytrityl chloride (150mg, 0.44mmol). The solution was allowed to stir for 8h. The reaction was quenched with addition of 1mL of MeOH and the solvent was subsequently evaporated. Purification by silica gel chromatography $CHCl_3$:MeOH (98:2) with 2% TEA afforded 240mg of **5** as a yellow foam (77% yield).
^{1}H NMR ($CDCl_3$): δ = 1.43 (m, 3H), 2.50 (br. s, 2H), 3.00 (m, 3H), 3.15 (m, 3H), 3.28-3.41 (m, 2H), 3.75 (s, 6H), 4.08 (m, 1H), 4.61 (br. s, 1H), 5.39 (m, 1H), 5.65-5.88 (m, 4H), 6.30 (m, 1H), 6.80 (m, 4H), 7.18-7.41 (m, 11H), 7.61 (m, 1H), 8.15-8.20 (m, 1H). ^{13}C NMR ($CDCl_3$): δ = 24.1, 35.4, 41.2, 41.6, 55.5, 64.5, 71.5, 72.8, 73.8, 82.8, 86.0, 86.7, 103.0, 104.5, 107.0, 113.4, 119.7, 127.2, 128.2, 128.3, 130.3, 135.6, 135.9, 139.4, 141.0, 144.8, 146.7, 148.1, 152.6, 157.1, 157.9, 158.0, 158.8. HRMS-LC: m/z $[M+H]^+$ calcd for $C_{44}H_{45}N_7O_{11}$: 848.3255; found: 848.3251.

5'-O-DMT-2'-Deoxy-N^2-dimethylaminomethylene-N^1-(6-nitropiperonyloxymethylene)-guanosine-3'-[(2-cyanoethyl)-N,N-diisopropylaminophosphoramidite] (6). Compound **5** (200mg, 0.24mmol) was dissolved in DCM (2mL). To the solution was added DIEA (165µL, 0.96mmol), followed by 2-cyanoethyl-N,N-diisopropyl-chlorophosphoramidite (80µL, 0.36mmol) and the reaction was allowed to proceed for 2h. The solvent was subsequently evaporated and the residue purified by silica gel chromatography using $CHCl_3$:hexanes (70:30) with 2% TEA. The chromatographed product was additionally purified by precipitation from DCM:pentane, affording 215mg of **6** as a yellow foam (82% yield).
^{1}H NMR ($CDCl_3$): δ = 1.05-1.23 (m, 12H), 1.42 (m, 3H), 2.42-2.62 (m, 4H), 2.98 (m, 3H), 3.17 (m, 3H), 3.26 (m, 2H), 3.51-3.83 (m, 10H), 4.21 (m, 1H), 4.61 (m, 1H), 5.39 (m, 1H), 5.63-5.91 (m, 4H), 6.22 (m, 1H), 6.78 (m, 4H), 7.15-7.41 (m, 11H), 7.62 (m, 1H), 8.25-8.40 (m, 1H). ^{13}C NMR ($CDCl_3$): δ = 20.5, 24.1, 24.8, 35.5, 40.5, 41.5, 43.4, 55.5, 58.6, 63.9, 71.4, 73.5, 74.5, 82.9, 85.6, 86.7, 103.1, 104.5, 107.0, 113.4, 117.8, 120.0, 127.2, 128.1,128.3, 130.2, 135.5, 135.8, 139.4, 141.1, 144.7, 146.7, 148.1, 152.7, 157.0, 157.9, 158.1, 158.8. ^{31}P NMR ($CDCl_3$): δ = 148.8, 148.9, 149.0.

2. Preparation of DNAzyme samples for reaction rate measurements:

A DNAzyme solution (0.01mM) in TE buffer (10mM TRIS/HCl, 0.1mM EDTA, pH=7.5) was heated to 90ºC for 5 min and allowed to slowly cool to room temperature. The DNAzyme was further diluted (0.1μM) into 20KH buffer (50mM HEPES/NH_4OH, 20mM KCl, 200mM NaCl, 0.05% Triton X-100, 1% DMSO, pH=8.0) containing hemin (0.1μM) and allowed to incubate at 25ºC for 40 min. To the solution was then added ABTS (5mM final conc.) and the reaction initialized by addition of H_2O_2 (0.6mM final conc.). The kinetic spectra were taken at 25ºC on a Molecular Devices SpectraMax Plus 384 spectrophotometer.

3. CD measurements:

Following the heating/cooling period, DNAzymes were diluted to 1μM in 20KH buffer and allowed to equilibrate for 40 min in the absence or presence of hemin (1μM). Spectra were taken at 25ºC using Jasco J-600 Circular Dichroism Spectrophotometer.

4. UV/VIS measurements:

Following the heating/cooling period, DNAzymes were diluted to 5μM in 20KH buffer and allowed to equilibrate for 40 min in the presence of hemin (5μM). Spectra were taken at 25ºC, using Nanodrop ND1000 Spectrophotometer.

The potential to induce decaging during CD and UV measurements was assessed by exposing a 0.1mM solution of NPOM caged deoxyguanosine in water to UV light in the Jasco CD Spectrophotometer and the Molecular Devices UV plate reader for >2 hours. The analysis of the irradiated samples by HPLC/MS showed no decaging or any other form of decomposition.

5. Dynamic Light Scattering:

Following the heating/cooling period, DNAzymes were diluted to 5μM in 20KH buffer and allowed to equilibrate for 40 min in the presence of hemin (5μM). Spectra were taken at 25ºC. DLS measurements were performed using Malvern Zetasizer Nano ZS (Model ZEN 3600) with a 633 nm laser. The dispersant refractive index was set as 1.330, the material absorption was set as 0.01, the solvent approximated as water with a viscosity of 0.8872. The size and the distribution of the particles were calculated by the Malvern Dispersion Technology Software (DTS) version 5.03. The aggregation state of the "wildtype" DNAzyme **D1** in solution was compared to the aggregation state of the double-caged DNAzyme **D5**. An obvious difference in particle size between the monomeric caged DNAzyme **D5** and the aggregated, G-wire forming DNAzyme **D1** was noticed (Figure S1).

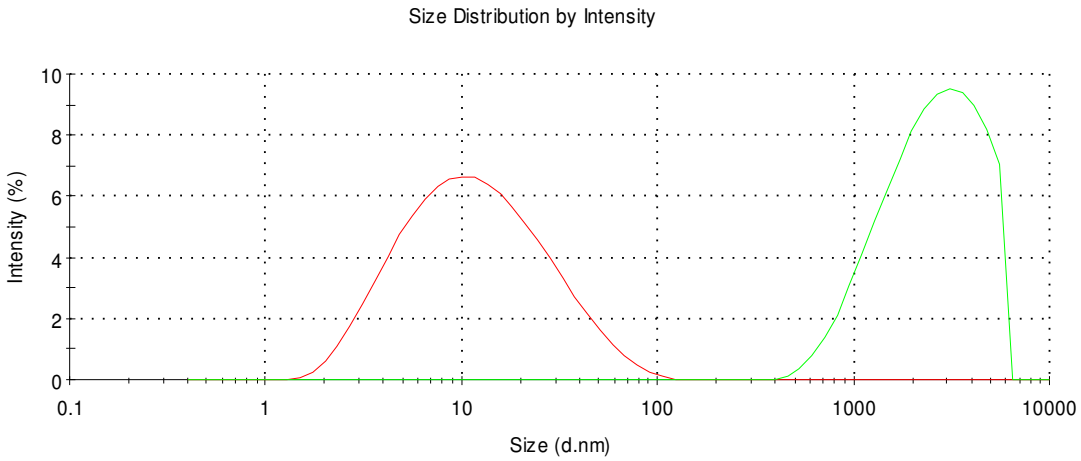


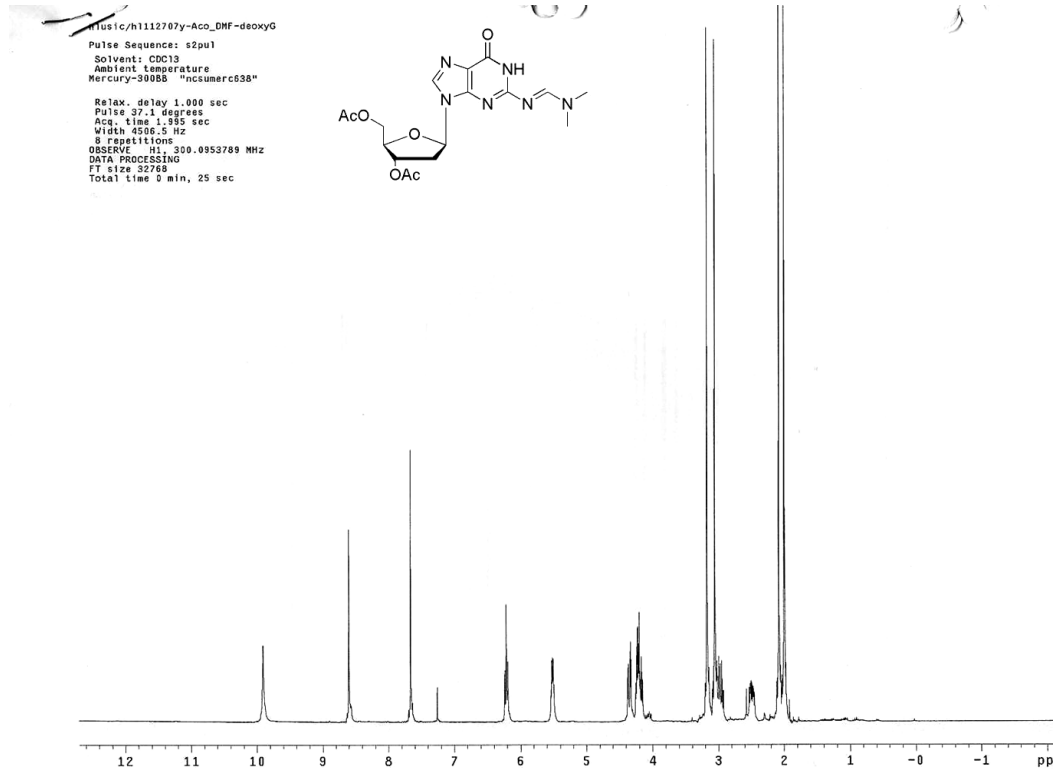
Figure S1. Results for the oligo **D1** (green line) and the caged oligo **D5** (red line).

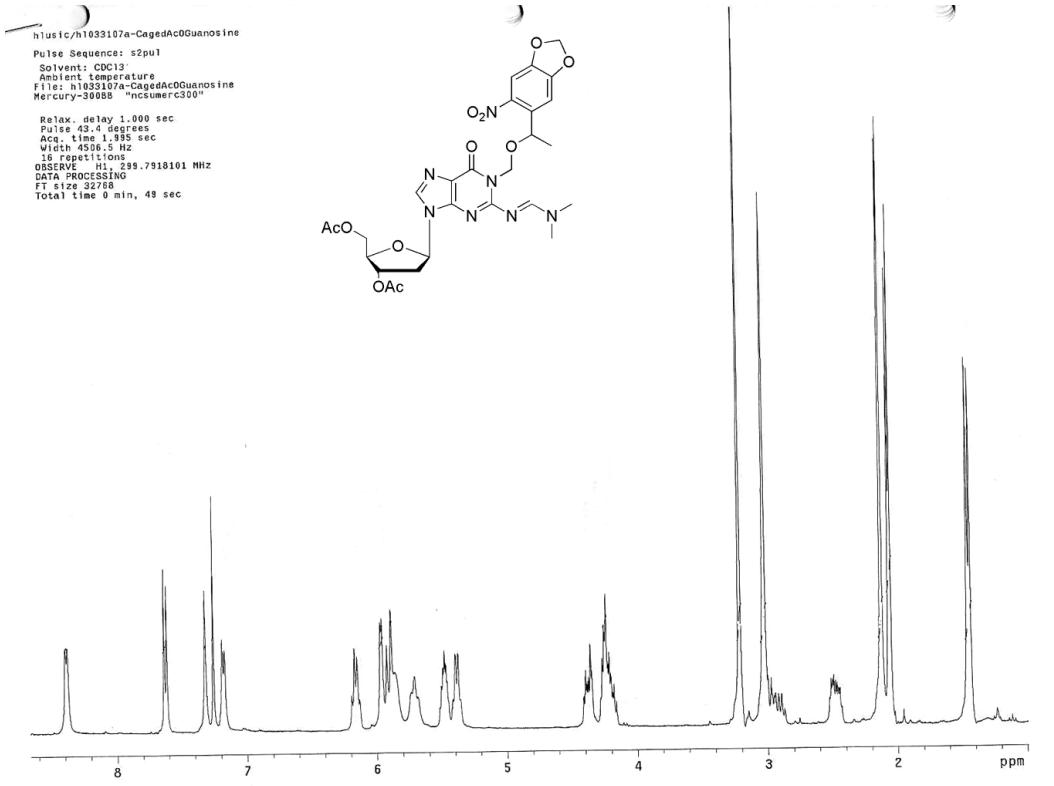
6. DNAzyme Decaging:

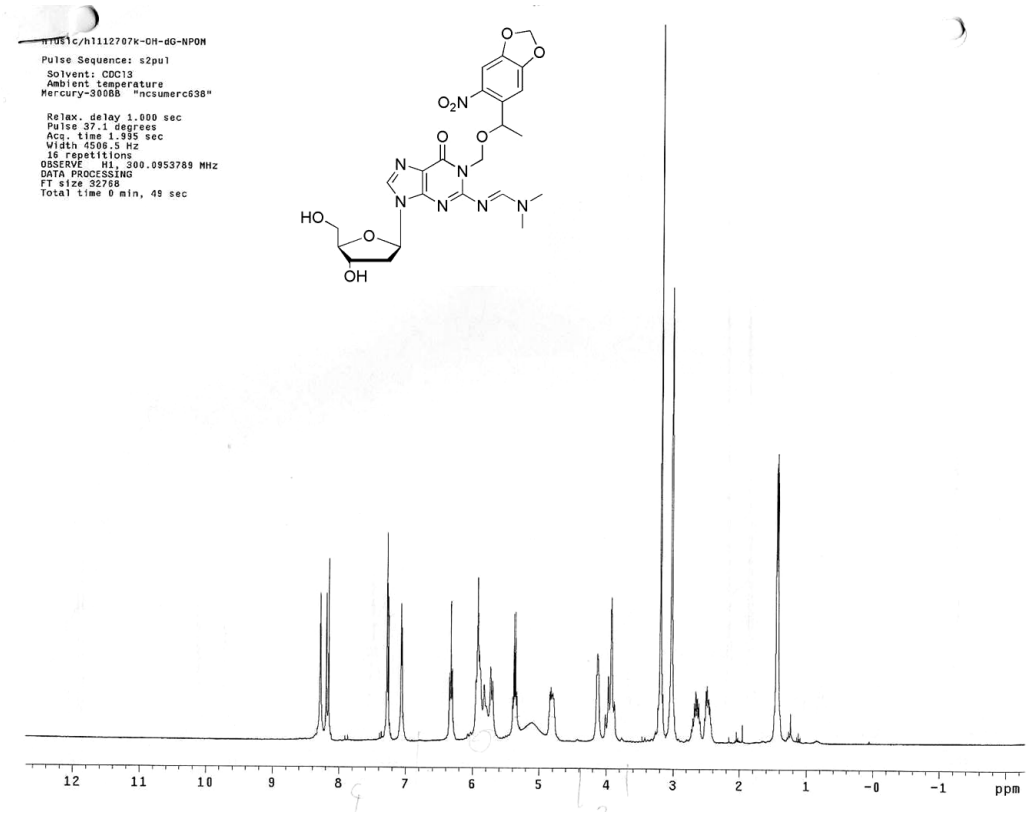
Decaging was done using a hand-held Spectroline ENF-280C UV lamp at 365 nm setting for 5 min (23W) in TE buffer (0.01mM) after the heating/cooling period, but before dilution into 20KH buffer.

7. ^{1}H NMR spectra of compounds (2-6):

All spectra were measured in $CDCl_3$ on a Varian Mercury 300MHz NMR instrument.



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Pulse 37.1 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
16 repetitions
OBSERVE H1, 300.0953789 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 49 sec
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O
OH
O_2N
O
N
N
N
N
N
O
O
O
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11
10
9
8
7
6
5
4
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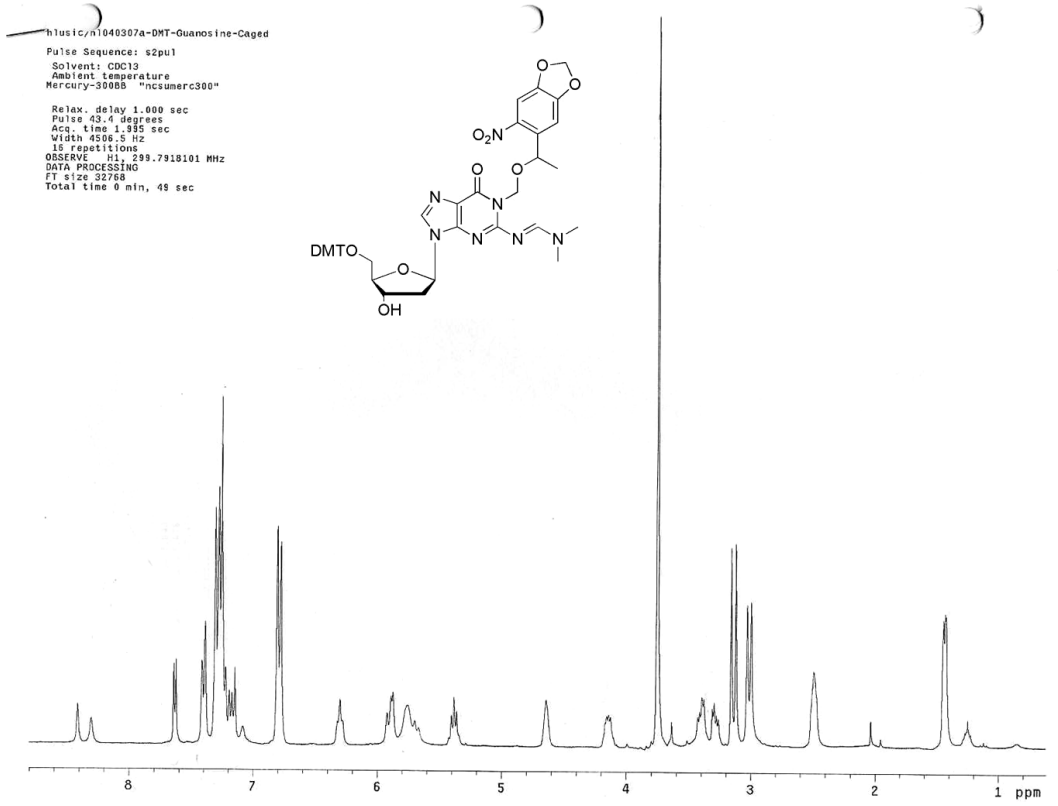
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Ambient temperature
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Pulse 43.4 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
16 repetitions
OBSERVE H1, 299.7918101 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 49 sec
DMTO
O
OH
O_2N
O
N
N
N
N
N
O
O
O
8
7
6
5
4
3
2
1 ppm