Supplementary information

7-(Benzofuran-2-yl)-7-deazadeoxyguanosine as a fluorescence turn-ON probe for single-strand DNA binding protein

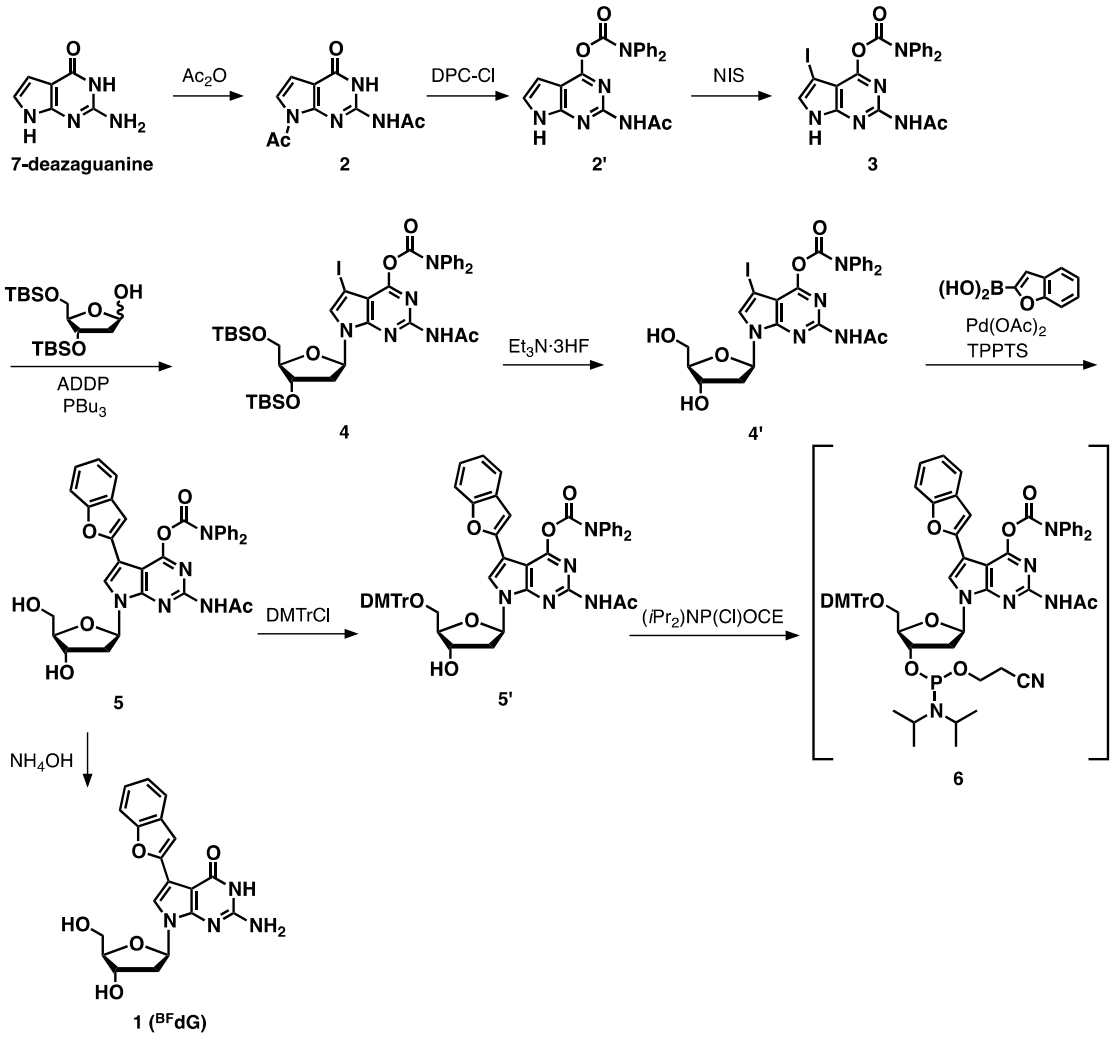
Munefumi Tokugawa[a], Yoshiaki Masaki[a], Jan Christian Canggadibrata[a], Kazuhei Kaneko[a], Takashi Shiozawa[a], Takashi Kanamori[a], Morten Grøtli[b], L. Marcus Wilhelmsson[c], Mitsuo Sekine[a] and Kohji Seio[a] *

[a] Department of Life Science, Tokyo Institute of Technology, 4259 J2-16 Nagatsuta-cho, Midori-ku, Yokohama, Japan.

[b]Department of Chemistry and Molecular Biology, University of Gothenburg, S-41296 Gothenburg, Sweden.

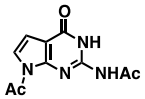
[c]Department of Chemistry and Chemical Engineering/Chemistry and Biochemistry, Chalmers University of Technology, S-41296 Gothenburg, Sweden.

Corresponding author's email address: kseio@bio.titech.ac.jp



Scheme S1 Synthetic scheme of 7-(Benzofuran-2-yl)-7-deazadeoxyguanosine.

2-*N*-*N*^{9}-Diacetyl-7-deazaguanine (2)

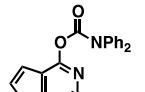


2

7-Deazaguanine (3.0 g, 20 mmol) was dried by co-evaporation with anhydrous pyridine (5 mL x 3) under reduced pressure. The resulting mixture was suspended in anhydrous pyridine (68 mL) and acetic anhydride (60 mL, 635 mmol) was added under argon. The mixture was stirred under reflux for 3 h. The reaction solution was then cooled on ice-bath and H_2O (120 mL) was slowly added over 20 min. White precipitate was obtained within 15 min. The mixture was stirred at this temperature for 90 min, filtered off, washed with chilled water, and dried over P_2O_5 at 40 °C under reduced pressure to afford 1.62 g as pale colored powder (55%).

^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.00-12.10 (1H, br), 11.51-11.63 (1H, br), 7.50 (1H, d, J = 4.0 Hz), 6.63 (1H, d, J = 4.0 Hz), 2.86 (3H, s), 2.21 (3H, s); ^{13}C NMR (DMSO-d_6, 126 MHz): δ 173.6, 168.6, 156.4, 148.2, 147.3, 119.4, 107.1, 105.4, 25.3, 23.9; ESI-TOF mass: calcd. for $C_{10}H_{10}N_4NaO_3$ $[M+Na]^+$ 257.0645, found 257.0647.

2-*N*-Acetyl-7-deaza-6-*O*-(*N*,*N*-diphenylcarbamoyl)guanine (2')

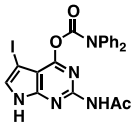


2'

Compound **2** (5.74 g, 24.5 mmol) was dissolved in anhydrous pyridine (88 mL) under argon. *N*,*N*-Diisopropylethylamine (9.4 mL, 53.2 mmol) and *N*,*N*-diphenylcarbamoyl chloride (6.26 g, 27.0 mmol) were added to the solution. The reaction mixture was stirred at r.t. under argon and dark red solution was obtained within 30 min. After stirred for 1 h, the mixture was cooled to 0 °C on ice bath. Addition of H_2O (34 mL) was followed by addition of aq. sodium hydroxide (1.0 M, 54 mL, 54.0 mmol, 2.2 eq). The mixture was stirred at r.t. for 30 min and the solvent was then removed by evaporation under reduced pressure. The residue was suspended in 260 mL of EtOH and H_2O (1:1, v/v), filtered off, washed with EtOH, and dried over P_2O_5 under reduced pressure to afford 9.17 g (23.7 mmol) as light tan powder (97%).

^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.21 (1H, br), 10.48 (1H, br), 7.47-7.58 (4H, m), 7.39-7.47 (5H, m), 7.27-7.33 (2H, m), 6.53 (1H, dd, J = 1.2 Hz, 3.5 Hz), 2.18 (3H, s); ^{13}C NMR (DMSO-d_6, 101 MHz): δ 168.5, 157.0, 155.2, 151.3, 150.5, 141.7, 129.3, 127.2, 127.1, 125.9, 104.7, 98.3, 24.4; ESI-TOF mass: calcd. for $C_{21}H_{17}N_5NaO_3$ $[M+Na]^+$ 410.1224, found 410.1223.

2-*N*-Acetyl-7-deaza-7-iodo-6-*O*-(*N*,*N*-diphenylcarbamoyl)guanine (3)

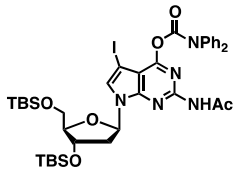


3

Compound **2'** (3.87 g, 10.0 mmol,) was dissolved in anhydrous DMF (70 mL) under argon and *N*-iodosuccinimide (2.36 g, 10.5 mmol) was added. The reaction solution was stirred at r.t. for 1 h. The solvent was evaporated under reduced pressure. The resulting residue was suspended in 80 mL of EtOH and H_2O (1:1, v/v), filtered off, washed with EtOH (5 mL x 4), and dried over P_2O_5 under reduced pressure to afford 4.96 g as pale brown powder (97%).

^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.57 (1H, s), 10.60 (1H, s), 7.18-7.79 (11H, m), 2.15 (3H, s); ^{13}C NMR (DMSO-d_6, 101 MHz): δ 168.1, 156.7, 154.7, 151.3, 150.2, 141.4, 130.4, 128.7, 126.8, 126.7, 106.4, 49.1, 23.9; ESI-TOF mass: calcd. for $C_{21}H_{16}IN_5NaO_3$ $[M+Na]^+$ 536.0190, found 536.0182.

2-*N*-Acetyl-7-deaza-2´-deoxy-7-iodo-6-*O*-(*N*,*N*-diphenylcarbamoyl)-3´,5´-bis-*O*-(*tert*-butyldimethylsilyl)-guanosine (4)



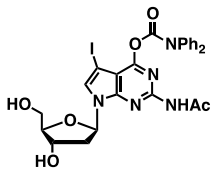
4

2-Deoxy-3,5-*bis*-*O*-(tert-butylmethylsilyl)-D-ribose (2.435 g, 6.71 mmol, 3.0 eq) and compound **3** (1.15 g, 2.24 mmol) were dissolved in anhydrous DMF (60 mL) and the solution was cooled to 0 °C. Tri-*n*-butylphosphine (1.66 mL, 6.71 mmol) and 1,1'-(azodicarbonyl)dipiperidine (4.70 g, 6.71 mmol) were added at 0 °C under argon, sequentially. The reaction solution was stirred at 0 °C for 4 h and H_2O (4 mL) was then added. The solvent was removed under reduced pressure. The resulting residue was suspended in Et_2O. Filtration of the suspension was followed by concentration of the filtrated under reduced pressure. The residue was purified by column chromatography on a silica gel (60N, 200 g) column, eluting with gradient 28% to 30% EtOAc in *n*-hexane to afford 1.18 g as yellowish foamy solid (61%).

^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.68 (1H, s), 7.81 (1H, s). 7.20-7.73 (10H, m), 6,46 (1H, t, J = 7.0 Hz, 7.0 Hz), 4.49-4.55 (1H, m), 3.80-3.87 (1H, m), 3.76 (1H, dd, J = 10.9, 5.3 Hz), 3.66 (1H, dd, J = 10.8, 3.7 Hz), 2.67-2.57 (1H, m), 2.19-2.28 (1H, m), 2.17 (3H, s), 0.83-0.95 (18H,

m), 0.02-0.14 (12H, m); ^{13}C NMR (DMSO-d_6, 126 MHz): δ 168.5, 157.0, 153.9, 151.7, 150.6, 141.6, 130.5, 129.3, 128.2, 126.6, 107.7, 87.1, 83.3, 72.3, 62.8, 52.2, 25.8, 25.7, 24.5, 18.0, 17.7, -4.8, -4.9, -5.5, -5.5; ESI-TOF mass: calcd. for $C_{38}H_{52}IN_5NaO_6Si_2$ $[M+Na]^+$ 880.2393, found 880.2382.

2-*N*-Acetyl-7-deaza-2´-deoxy-7-iodo-6-*O*-(*N*,*N*-diphenylcarbamoyl)guanosine (4')

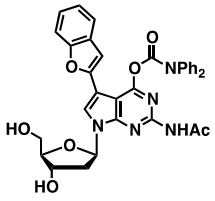


4'

Compound **4** (973 mg, 1.13 mmol) was dissolved in anhydrous THF (13 mL) under argon. Triethylamine (284 µL, 2.04 mmol) and $Et_3N{\cdot}3HF$ (646 µL, 3.97 mmol) were added to the solution and the reaction solution was stirred at r.t. for 24 h under argon. Ethoxytrimethylsilane (1.77 mL, 11.3 mmol) was then added and the mixture was further stirred for 90 min. The mixture was concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel (60N, 30 g) column, eluting with gradient 6% to 8% in MeOH in CH_2Cl_2 to afford 630 mg as pale yellowish powder (88%).

^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.66 (1H, s), 7.89 (1H, s). 7.24-7.74 (10H, m), 6.50 (1H, dd, J = 6.5 Hz, 7.5 Hz), 5.28 (1H, d, J = 3.5 Hz), 4.92 (1H, t, J = 5.5 Hz), 4.30-4.39 (1H, m), 3.78-3.87 (1H, m), 3.47-3.60 (2H, m), 2.20-2.28 (1H, m), 2.18 (3H, s); ^{13}C NMR (DMSO-d_6, 126 MHz): δ 168.6, 157.0, 154.0, 151.7, 150.7, 141.6, 130.7, 129.3, 128.3, 126.6, 107.6, 87.6, 70.9, 61.7, 52.1, 39.5, 24.5; ESI-TOF mass: calcd. for $C_{26}H_{24}IN_5NaO_6$ $[M+Na]^+$ 652.0663, found 652.0662.

2-*N*-Acetyl-7-(benzofuran-2-yl)-7-deaza-2´-deoxy-6-*O*-(*N*,*N*,-diphenylcarbamoyl)guanosine (5)

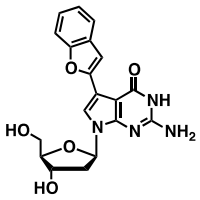


5

Compound **4'** (1.98 g, 3.15 mmol), benzofuran-2-boronic acid (1.02 g, 6.29 mmol), and Na_2CO_3 (733 mg, 6.9 mmol) were suspended in 40 mL of degassed solvent (MeCN-H_2O-DMF, 1:2:2, v/v/v). tris(3-sulfophenyl)phosphine (215 mg, 0.38 mmol) and $Pd(OAc)_2$ (42 mg, 0.19 mmol) were added to the suspension sequentially. The reaction mixture was stirred at 45 °C for 2 h under argon. The mixture was filtered and the residue was washed with MeCN. The residue was collected and purified by column chromatography on a silica gel (60N, 100 g) column, eluting with 5% MeOH in EtOAc-CH_2Cl_2 (4:5, v/v) to afford 1.36 g as white powder (70%).

^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.72 (1H, s), 8.18 (1H, s), 7.71 (1H, d, J = 7.5 Hz), 7.57 (1H, d, J = 8.0 Hz), 7.21-7.56 (12H, m), 6.92 (1H, s), 6.60 (1H, dd J = 8.0 Hz, 6.1 Hz), 5.34 (1H, d, J = 3.6 Hz), 5.00 (1H, t, J = 5.0 Hz), 4.37-4.44 (1H, m), 3.86 (1H, dd, J = 3.9 Hz, 3.7 Hz) 3.51-3.66 (2H, m), 2.62 (1H, ddd, J = 13.5 Hz, 8.0 Hz, 5.7 Hz), 2.25-2.30 (1H, m), 2.24 (1H, s); ^{13}C NMR (DMSO-d_6, 126 MHz): δ 168.9, 157.5, 154.7, 153.8, 152.2, 149.9, 149.8, 141.5, 129.4, 128.9, 127.7, 126.4, 124.6, 124.3, 123.3, 120.9, 110.8, 105.8, 102.8, 101.2, 87.7, 83.2, 71.0, 61.8, 24.7; ESI-TOF mass: calcd. for $C_{34}H_{29}N_5NaO_7$ $[M+Na]^+$ 642.1959, found 642.1960.

7-(Benzofuran-2-yl)-7-deaza-2´-deoxyguanosine (^{BF}dG, 1)



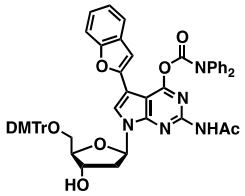
1 (^{BF}dG)

First, 28% aqueous NH_3 (7.5 mL) was added to a solution of compound **5** (500 mg, 0.807 mmol, 1.0 eq) in pyridine (7.5 mL). The flask was sealed and the reaction mixture was stirred at 55 °C for 5 h and the mixture was then cooled to room temperature. The mixture was concentrated under reduced pressure and the residue was purified by column chromatography on a silica gel (60N, 100 g) column, eluting with a gradient from 8% to 10% MeOH in CH_2Cl_2 to afford 289

mg as a white powder (94%).

^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.61-10.87 (1H, br), 7.84 (1H, s), 7.59 (1H, d, J = 7.6 Hz), 7.46-7.52 (2H, m), 7.15-7.25 (2H, m), 6.40-6.54 (2H, br), 6.37 (1H, dd, J = 8.3 Hz, 6.0 Hz), 5.25 (1H, d, J = 3.3 Hz), 4.98 (1H, t, J = 5.2 Hz), 4.30-4.36 (1H, m), 3.78-3.82 (1H, m), 3.48-3.61 (2H, m), 2.39-2.46 (1H, m), 2.08-2.16 (1H, m); ^{13}C NMR (DMSO-d_6, 126 MHz): δ 158.7, 153.5, 153.3, 152.1, 151.7, 129.4, 123.6, 122.8, 120.6, 115.1, 110.4, 110.0, 103.5, 96.3, 87.2, 82.3, 71.1, 62.0; ESI-TOF mass: calcd. for $C_{19}H_{18}N_4NaO_5$ $[M+Na]^+$ 405.1169, found 405.1173.

2-*N*-Acetyl-7-(benzofuran-2-yl)-7-deaza-2´-deoxy-5´-*O*-dimethoxytrityl-6-*O*-(*N*,*N*-diphenylcarbamoyl)-guanosine (5')



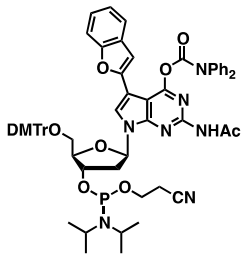
5'

Compound **5** (100 mg, 0.16 mmol) was co-evaporated with anhydrous pyridine (1 mL x 5) under reduced pressure and the residue was dissolved in anhydrous pyridine (1.6 mL). DMTrCl (60 mg, 0.177 mmol) was added and the reaction solution was stirred at r.t. for 1 h under argon. Additional DMTrCl (60 mg, 0.18 mmol) was then added and the solution was further stirred for 2 h, before another addition of DMTrCl (60 mg, 0.18 mmol). After 1h stirring at r.t. MeOH (1.5 mL) was added and the solution was concentrated under reduced pressure. The residue was diluted with EtOAc (10 mL) and the organic layer was washed with sat. $NaHCO_3$ (5 mL x 2) and brine (5 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography on a silica gel (C-200, 5.1 g) column, eluting with 75% EtOAc in *n*-hexane in presence of pyridine (0.5%) to afford 96 mg as yellowish formy solid (65%).

^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.72 (1H, s), 8.04 (1H, s), 7.82 (1H, s), 7.65-7.71 (1H, m), 7.45-7.59 (1H, br), 7.38-7.45 (5H, br), 7.33-7.38 (3H, br), 7.26-7.33 (4H, br), 7.23-7.26 (4H, m), 7.15-7.22 (3H, m), 7.11 (1H, t, J = 7.0 Hz), 6.79 (1H, s), 6.73-6.78 (4H, m), 6.60 (1H, t, J = 6.7 Hz), 5.36-5.42 (1H, m), 4.44-4.53 (1H, m), 3.96-4,03 (1H, m), 3.60 (3H, s), 3.59 (3H, s), 3.27 (1H, dd, J = 10.4 Hz, 5.7 Hz), 3.12 (1H, dd, J -10.6 Hz, 3.1 Hz), 2.72-2.82 (1H, m), 2.32-2.41 (1H, m), 2.23 (3H, s); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 158.6, 158.6, 158.3, 155.0, 154.4, 151.8, 150.3, 150.0, 144.6, 141.7, 135.9, 135.7, 130.2, 130.1, 129.4, 128.2, 128.0, 127.0, 124.0, 123.4,

122.8, 120.5, 113.3, 111.0, 107.5, 103.2, 102.3, 86.8, 86.0, 83.9, 72.7, 64.1, 55.2, 41.1, 25.4; ESI-TOF mass: calcd. for $C_{55}H_{47}N_5NaO_9$ $[M+Na]^+$ 944.3266, found 944.3246.

2-*N*-Acetyl-7-(benzofuran-2-yl)-7-deaza-2´-deoxy-5´-*O*-(4,4´-dimethoxytrityl)-6-*O*-(*N*,*N*-diphenylcarbamoyl)guanosine 3´-(2-cyanoethyl *N*,*N*-diisopropyl-phosphoramidite) (6)



6

Compound **5'** (74 mg, 0.08 mmol) was co-evaporated with anhydrous pyridine (1 mL x 3), anhydrous toluene (1 mL x 4), and anhydrous CH_2Cl_2 (1 mL) under reduced pressure. The residue was dissolved in anhydrous CH_2Cl_2 (1 mL) and *N*,*N*-diisopropylethylamine (21 μL, 0.12 mmol) was then added. 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (21 μL, 0.096 mmol) was slowly added and the reaction mixture was stirred at r.t. for 1 h under argon. The mixture was then diluted in CH_2Cl_2 (10 mL) and the organic layer was washed with sat. $NaHCO_3$ (5 mL x 2) and brine (5 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford yellow syrup. The crude mixture was used for synthesis of ODN without further purification.

Synthesis of oligodeoxynucleotides containing ^{BF}dG (ODN 1)

The synthesis of oligodeoxynucleotides 5′-d(CGCAAT **^{BF}G** TAACGC)-3′ (**ODN1**) and 5′-d(CGCAAC **^{BF}G** CAACGC)-3′ (**ODN2**) were performed in an ABI 392 DNA synthesizer using the standard 1.0 μmol-scale DNA phosphoramidite approach, which consists of detritylation, coupling, capping, and iodine oxidation steps. The synthesis was carried out using a DNA synthesis protocol installed in the synthesizer except for the coupling time for **6** were extended to 10 min. The synthesized oligomer were released from CPG supports by treatment with a solution of aq. NH_3, at r.t. for 2 h, then heated at 55 °C for 6 h. The solution was evaporated under reduced pressure at r.t. to remove NH_3, and the residue was diluted with 0.1 M NH_4OAc. The solution was placed on a C18 cartridge column, and the failure sequences were eluted using 10% MeCN/0.1 M NH_4OAc as an eluent. After washing with 0.1 M NH_4OAc and water, the column was treated with aq. 2% TFA to remove the DMTr group and further washed with water. The target oligodeoxynucleotide was eluted using 20% MeCN/water, and the fractions containing the target were concentrated. The residue was purified by RP HPLC to afford the pure material in 27% yield. The structures were confirmed by MALDI-TOF mass spectroscopy.

MALDI-TOF mass (**ODN1**): calcd. for $C_{135}H_{165}N_{50}O_{75}P_{12}$ $[M+H]^+$ 4059.8, found 4058.7.

MALDI-TOF mass (**ODN2**): calcd. for $C_{133}H_{163}N_{52}O_{73}P_{12}$ $[M+H]^+$ 4029.7, found 4029.0.

Procedure of the photophysical measurements and photophysical spectra for ^{BF}dG in high viscosity solvents

Compound **1** was dissolved in solvents of different viscosity (75%, 50%, 25%, 0% glycerol in MeOH, 0.01% DMSO). The final concentration of ^{BF}dG was 0.1 μM. The fluorescence measurements were performed at 25 °C with a FP-8300 Fluorescence Spectrometer (JASCO). The excitation wavelength was set to 318 nm.

Procedure of the fluorescence measurements for oligodeoxynucleotides containing ^{BF}dG

ODN1 was dissolved in 50 mM sodium phosphate buffer (pH 7.0, containing 100 mM NaCl) to adjust the final concentration of each oligodeoxynucleotide to 2.5 μM. Absorption spectra were measured with a U-2810 spectrophotometer (HITACHI). The fluorescence measurements were performed at 10 °C with a FP-8300 Fluorescence Spectrometer (JASCO). The excitation wavelength was set to 318 nm.

Procedure of the fluorescence measurements for ^{BF}dG in various solvents

A stock solution (1 mL) of **1** was prepared in DMSO (1 mM). First, a 25 μL aliquots of the stock solution was diluted with 975 μL of MeOH, MeCN, EtOAc, or H_2O, respectively, for use in UV-Vis absorption measurements. For fluorescence emission measurements, the 25 μM solution was further diluted 250 times to reach a 0.10 μM concentration. The resulting concentration of DMSO was 0.01%. Absorption spectra were measured with a U-2810 spectrophotometer (Hitachi). Steady-state fluorescent measurements were performed with a FP-8300 Fluorescence Spectrometer (JASCO); samples were excited at 303 nm. 2-Aminopurine ribonucleoside in H_2O (λ_{Abs} = 303 nm, Φ_F = 0.68) was used as a standard for evaluating the relative Φ_F of ^{BF}dG.

Procedure of the fluorescence measurements for oligodeoxynucleotides containing ^{BF}dG in the presence of Single-Stranded DNA Binding Protein

Each oligodeoxynucleotide and Single-Stranded DNA Binding Protein (Promega) were dissolved in 50 mM sodium phosphate buffer (pH 7.0, 1% glycerol, containing 100 mM NaCl) to adjust the final concentration of each oligodeoxynucleotide to 2.5 μM and Single-stranded DNA Binding Protein to each concentration. The fluorescence measurements were performed at 10 °C with a FP-8300 Fluorescence Spectrometer (JASCO). The excitation wavelength was set to 318 nm.

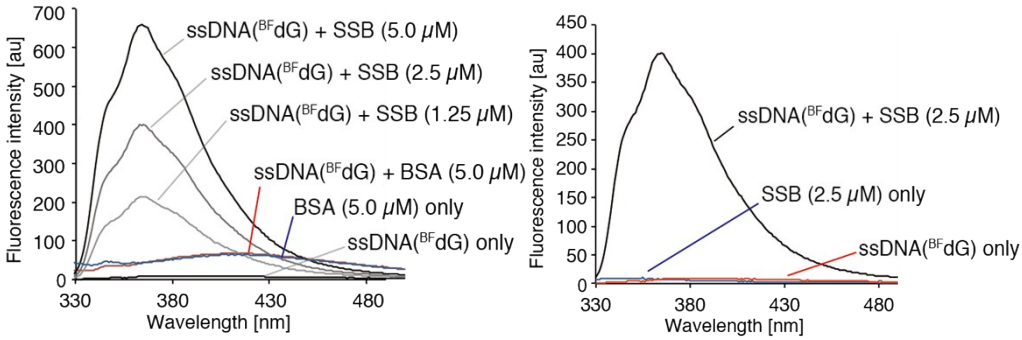


Figure S1. Fluorescent spectra of ssDNA(5′-d(CGCAAT **^{BF}G** TAACGC)-3′, 2.5 μM), BSA(left) and SSB (right). BSA showed weak fluorescence around 430 nm, whereas SSB showed no obvious fluorescence.

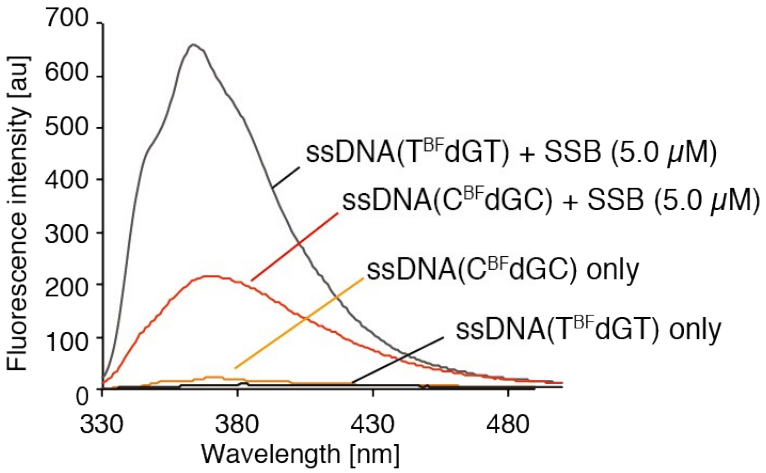
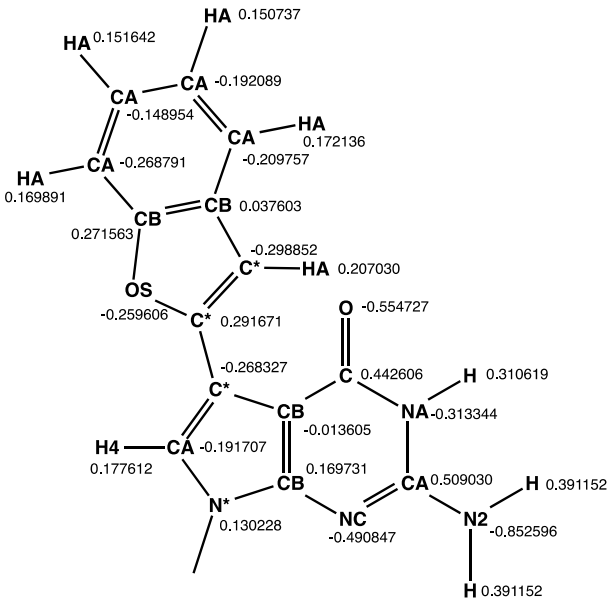


Figure S2 Fluorescent spectra of 5′-d(CGCAAC **^{BF}G** CAACGC)-3′ (ssDNA(C^{BF}dGC)) and 5′-d(CGCAAT **^{BF}G** TAACGC)-3′ (ssDNA(T^{BF}dGT)).

Computational method

Molecular dynamic simulations were carried out using AMBER 14 program package.[30,31] The sequence was 5′-d(CGCAAT^{BF}**G**TAACGC)-3′/5′-d(GCGTTACATTGCT)-3′. The charge of non-canonical modified residues was determined by the RESP charge fitting (HF/6-31G(d), iop(6/33=2)).[32] The ff14SB AMBER force fields were used for nucleic acids. The additional force field parameters were taken from GAFF.[33] The initial duplex was solvated in a periodic box with a 10 Å of water molecules, explicitly described by the TIP3P water model. Sodium and chloride ions were added to make 0.1 M NaCl solution. The minimization and equilibration (10.3 ns) were run accordingly to the protocol used in previous work.[34,35] The unrestrained extended simulations (10 ns) were performed with the Berendsen algorithm to maintain the temperature (300 K).[36] The 5000 steps of energy minimization were performed from the last snapshot of MD simulation. The obtained structure was used as computer-aided modeled structure in Fig. 2. During the MD simulation, hydrogen vibrations were removed using SHAKE bond constraints, allowing a longer time step of 2 fs. Long-range electrostatic interactions were treated using the Particle Mesh Ewald approach and a 10 Å cutoff.[37] All figures were produced by using Pymol.

RESP charges and atom types for modified nucleosides



Additional force fields taken from GAFF

```
remark goes here
MASS

BOND
N*-CA  411.10   1.391       same as c2-na
C*-OS  392.60   1.357       same as c2-os
C*-C*  418.30   1.429       same as cc-cc
C*-HA  344.30   1.087       same as c2-hc
C*-CA  411.70   1.434       same as ca-cc
OS-CB  392.60   1.357       same as c2-os

ANGLE
C*-C*-C*   67.880     110.700   same as cc-cc-cc
C*-C*-HA   50.300     119.700   same as c2-c2-hc
N*-CA-C*   69.830     121.380   same as c2-c2-na
OS-CB-CB   71.040     121.890   same as c2-c2-os
C -CB-C*   67.930     120.700   same as c -c2-c2
CA-C*-C*   67.660     111.040   same as ca-cc-cc
CT-N*-CA   64.230     117.200   same as c2-na-c3
CB-C*-HA   50.300     119.700   same as c2-c2-hc
C*-CA-H4   50.040     120.940   same as c2-c2-ha
CB-CB-C*   67.880     110.700   same as cc-cc-cc
CB-N*-CA   67.800     110.370   same as c2-na-c2
CB-C*-CA   67.660     111.040   same as ca-cc-cc
C*-OS-CB   65.950     113.140   same as c2-os-c2
C*-C*-OS   71.040     121.890   same as c2-c2-os
N*-CA-H4   51.180     112.420   same as ha-c2-na
CB-C*-C*   67.880     110.700   same as cc-cc-cc
OS-CB-CA   71.040     121.890   same as c2-c2-os

DIHE
CT-N*-CA-H4   1    0.625       180.000          2.000      same as X -c2-na-X
CB-C*-C*-OS   1    6.650       180.000          2.000      same as X -c2-c2-X
H4-CA-C*-C*   1    4.000       180.000          2.000      same as X -cc-cc-X
N*-CA-C*-CB   1    6.650       180.000          2.000      same as X -c2-c2-X
CB-N*-CA-H4   1    0.625       180.000          2.000      same as X -c2-na-X
CB-C*-CA-H4   1    4.000       180.000          2.000      same as X -cc-cc-X
CA-C*-C*-OS   1    6.650       180.000          2.000      same as X -c2-c2-X
C*-C*-C*-HA   1    6.650       180.000          2.000      same as X -c2-c2-X
N*-CA-C*-C*   1    6.650       180.000          2.000      same as X -c2-c2-X
CB-N*-CA-C*   1    0.625       180.000          2.000      same as X -c2-na-X
C*-OS-CB-CA   1    1.050       180.000          2.000      same as X -c2-os-X
CA-C*-C*-C*   1    4.000       180.000          2.000      same as X -cc-cc-X
OS-C*-C*-HA   1    6.650       180.000          2.000      same as X -c2-c2-X
C*-C*-OS-CB   1    1.050       180.000          2.000      same as X -c2-os-X
CB-C*-C*-C*   1    4.000       180.000          2.000      same as X -cc-cc-X
C*-OS-CB-CB   1    1.050       180.000          2.000      same as X -c2-os-X
CT-N*-CA-C*   1    0.625       180.000          2.000      same as X -c2-na-X
```

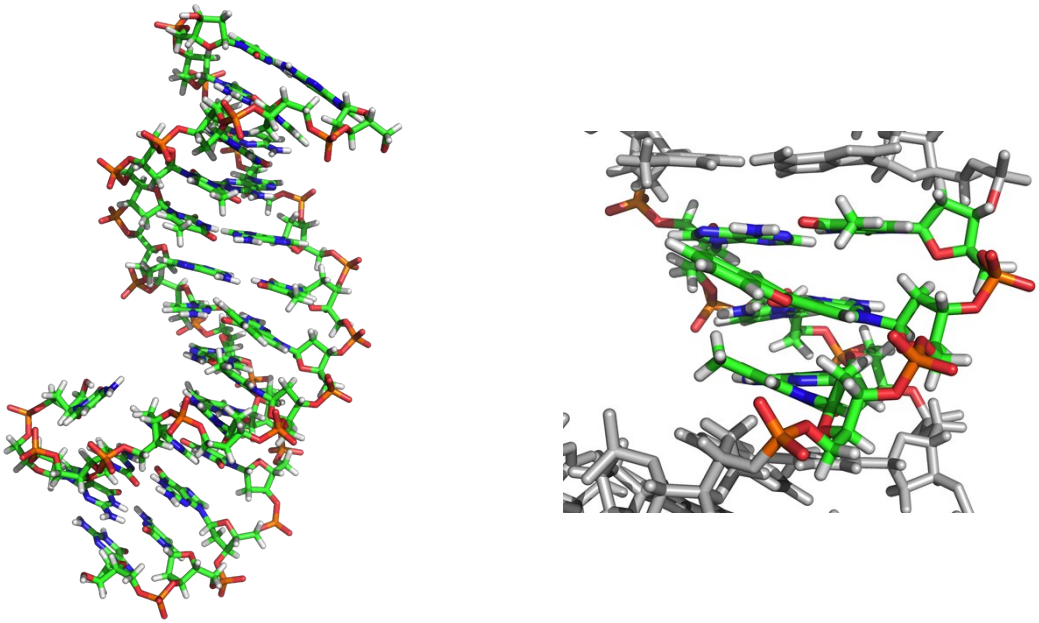
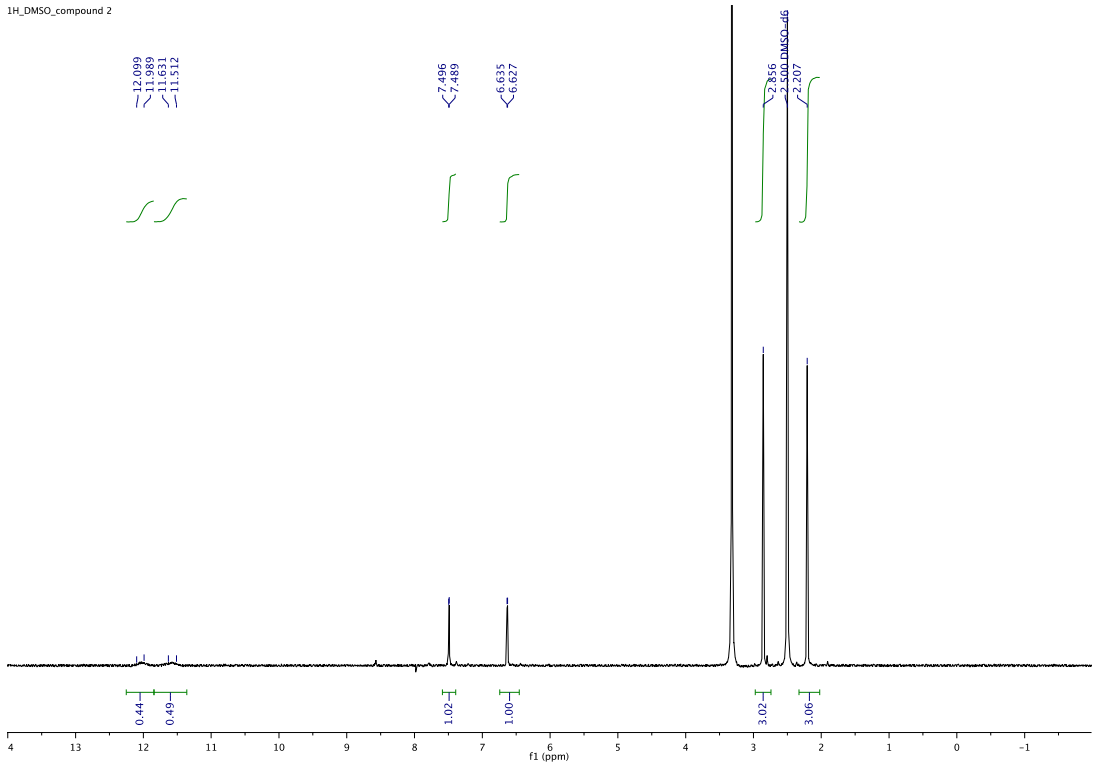


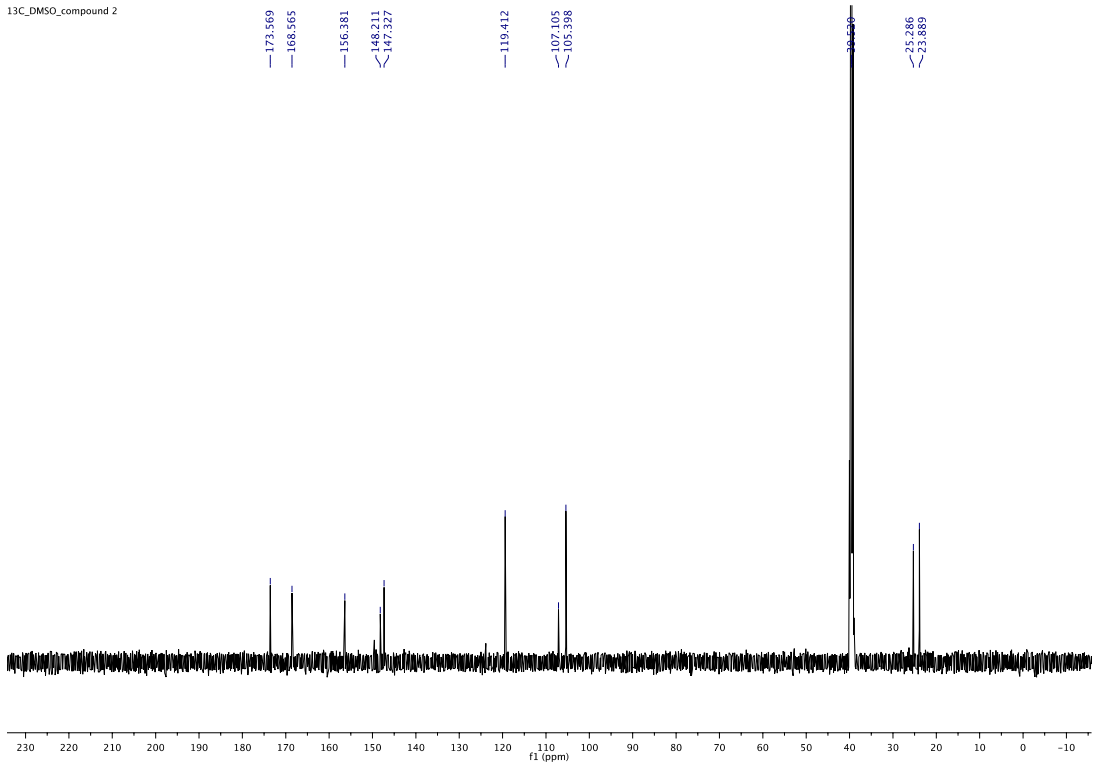
Figure S3 energy minimized structure from the last snapshot of 20.3 ns MD simulation.

Full citation for AMBER 14 and AmberTools 15

D.A. Case, J.T. Berryman, R.M. Betz, D.S. Cerutti, T.E. Cheatham, III, T.A. Darden, R.E. Duke, T.J. Giese, H. Gohlke, A.W. Goetz, N. Homeyer, S. Izadi, P. Janowski, J. Kaus, A. Kovalenko, T.S. Lee, S. LeGrand, P. Li, T. Luchko, R. Luo, B. Madej, K.M. Merz, G. Monard, P. Needham, H. Nguyen, H.T. Nguyen, I. Omelyan, A. Onufriev, D.R. Roe, A. Roitberg, R. Salomon-Ferrer, C.L. Simmerling, W. Smith, J. Swails, R.C. Walker, J. Wang, R.M. Wolf, X. Wu, D.M. York and P.A. Kollman (2015), AMBER 2015, University of California, San Francisco.

2-*N*-*N*^{9}-Diacetyl-7-deazaguanine (2)





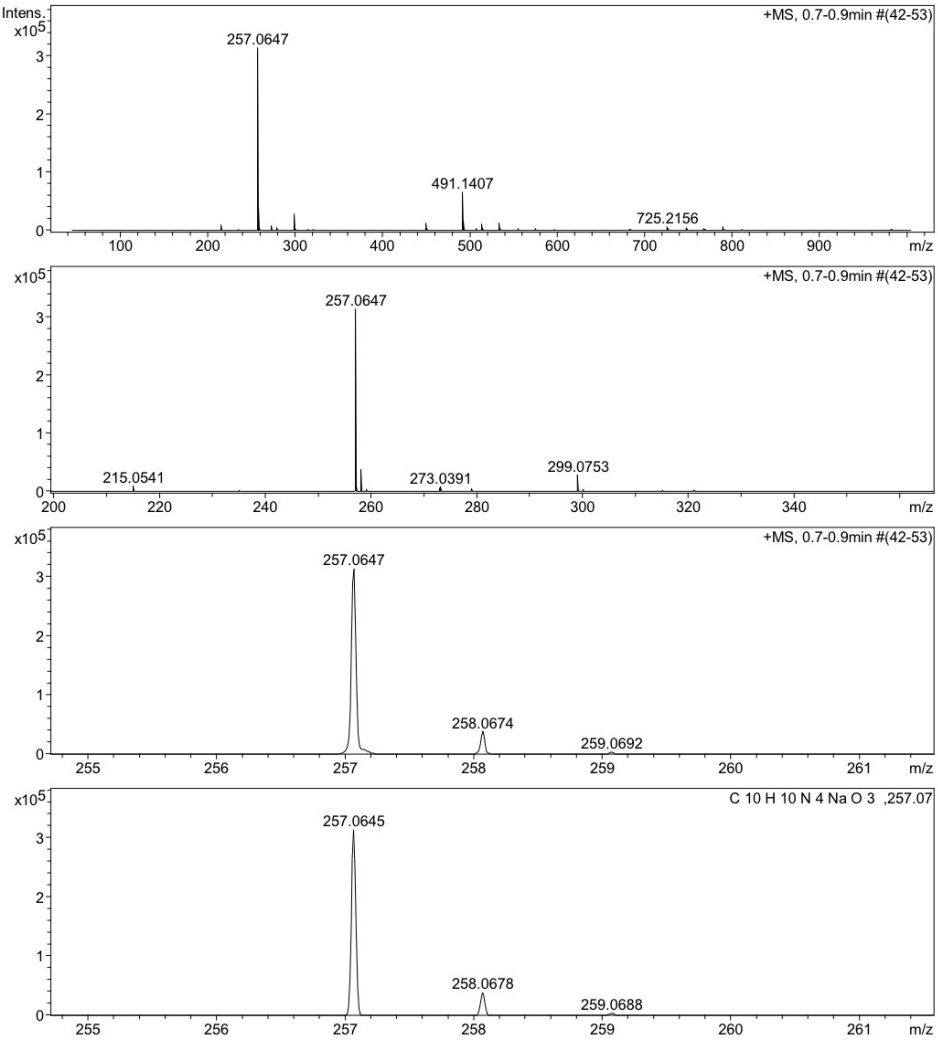
TDCMAS ESI-TOF

Analysis Info

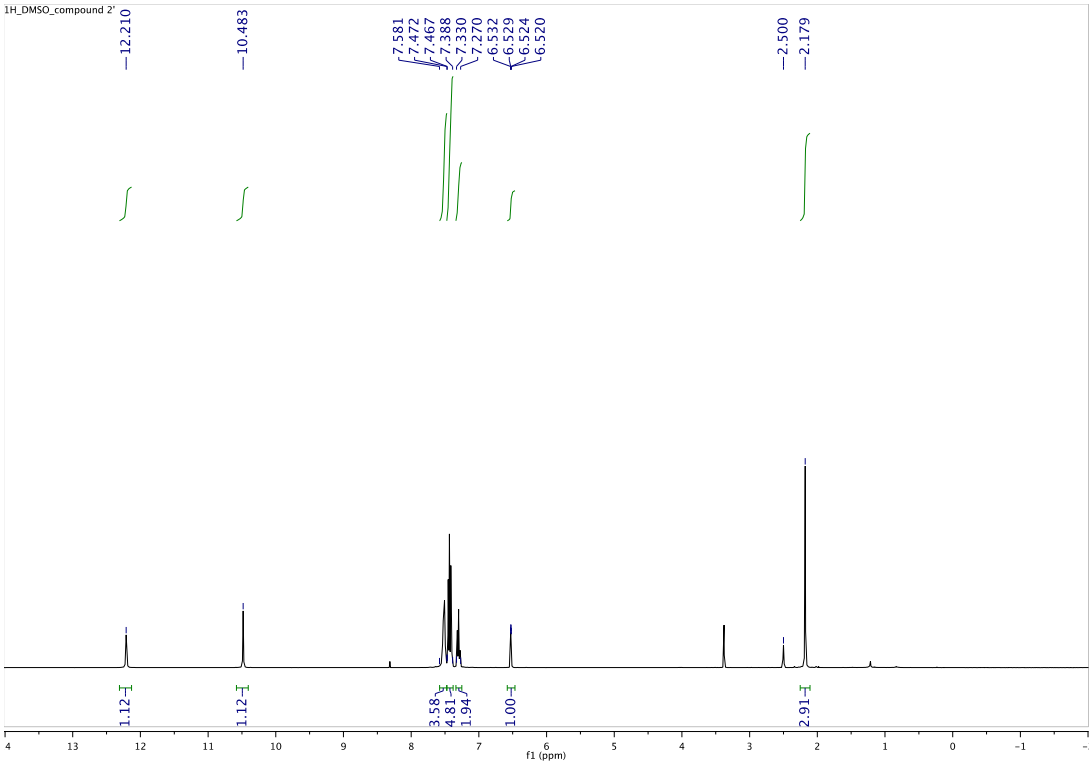
Acquisition Date	11/28/2014 11:22:22 AM		
Analysis Name	D:\Data\sekine_lab\tokugawa\141128\Munefumi-254-A-000002.d		
Method	esi_pos_low.m	Operator	BDAL@DE
Sample Name	Munefumi-254-A-	Instrument / Ser#	micrOTOF 10321
Comment			

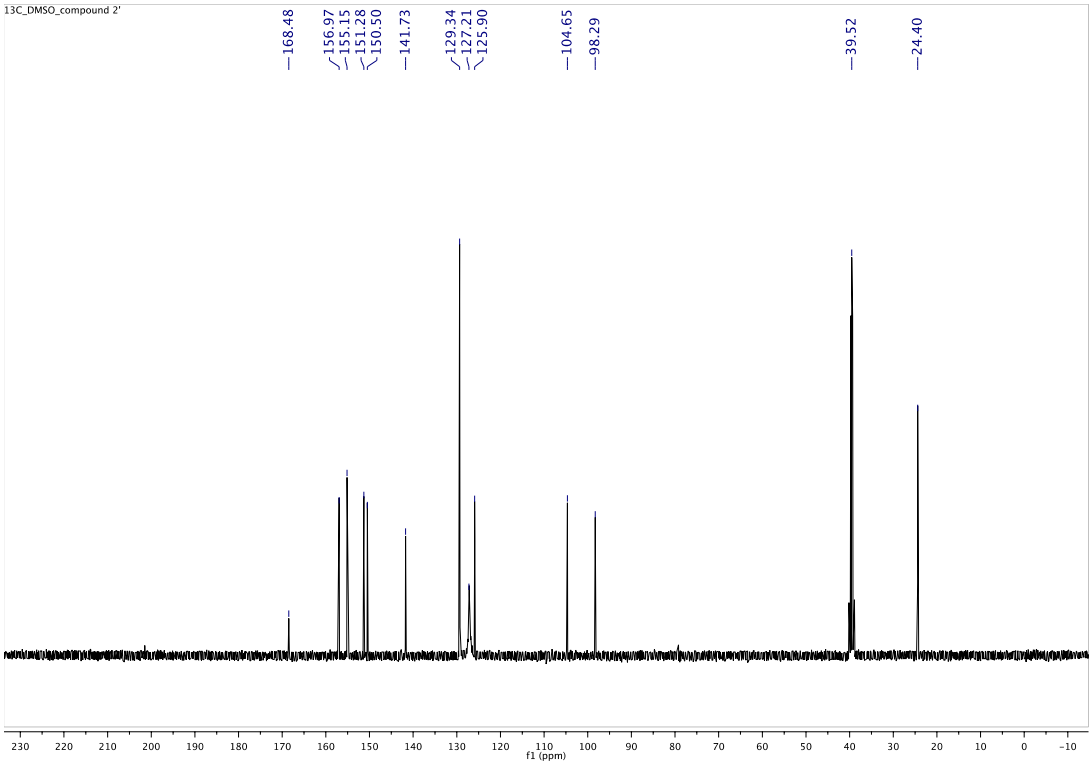
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	160 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	1000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



2-*N*-Acetyl-7-deaza-6-*O*-(*N*,*N*-diphenylcarbamoyl)guanine (2')





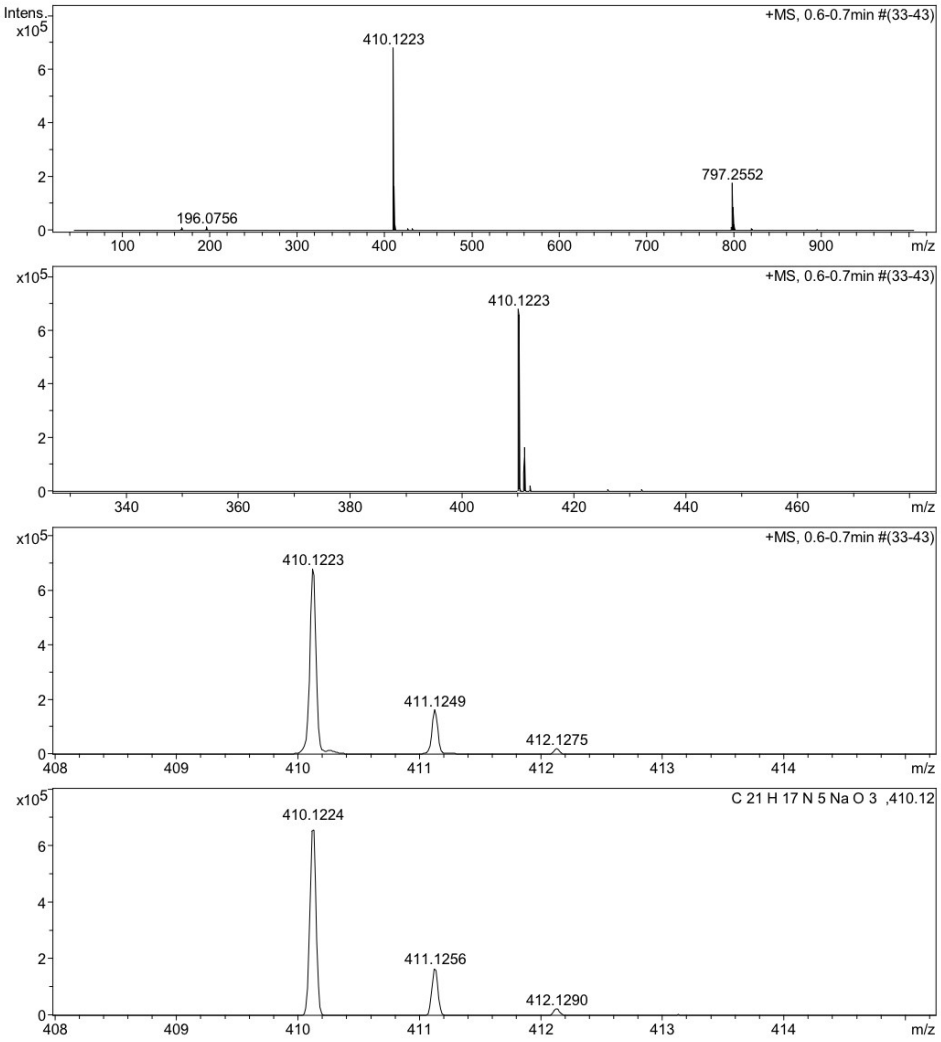
TDCMAS ESI-TOF

Analysis Info

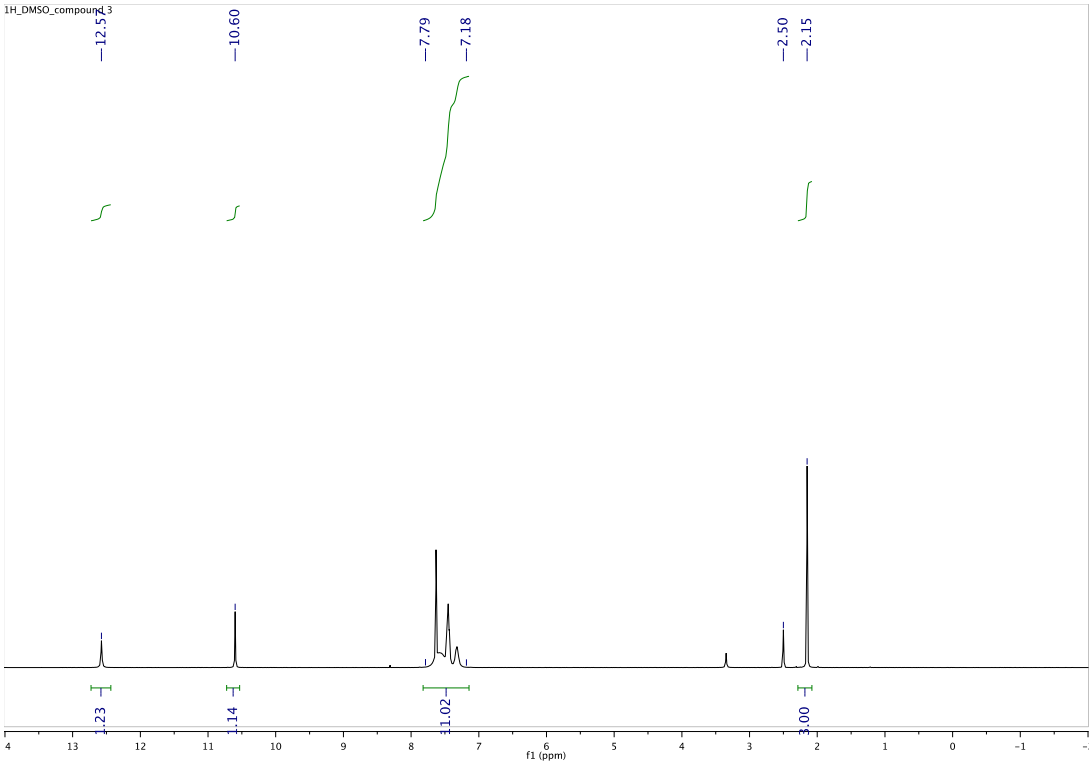
Analysis Name	D:\Data\sekine_lab\tokugawa\141128\Munefumi-256-A-000002.d	Acquisition Date	11/28/2014 11:37:37 AM
Method	esi_pos_low.m	Operator	BDAL@DE
Sample Name	Munefumi-256-A-	Instrument / Ser#	micrOTOF 10321
Comment			

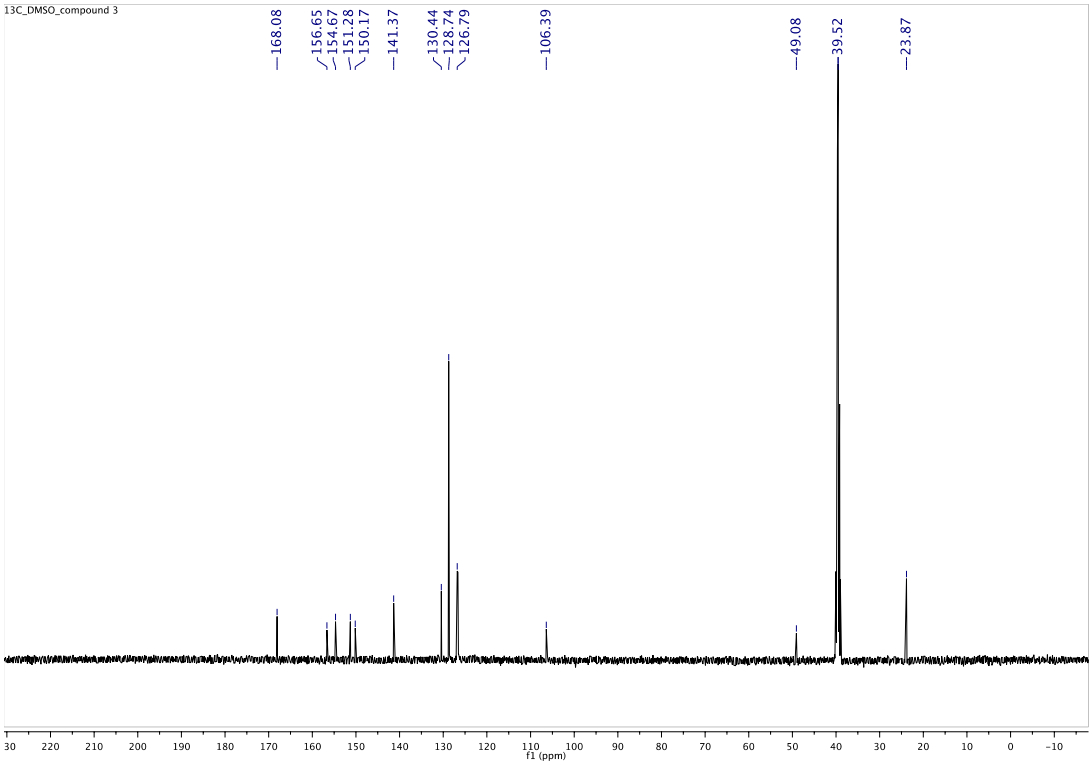
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	160 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	1000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



2-*N*-Acetyl-7-deaza-7-iodo-6-*O*-(*N*,*N*-diphenylcarbamoyl)guanine (3)





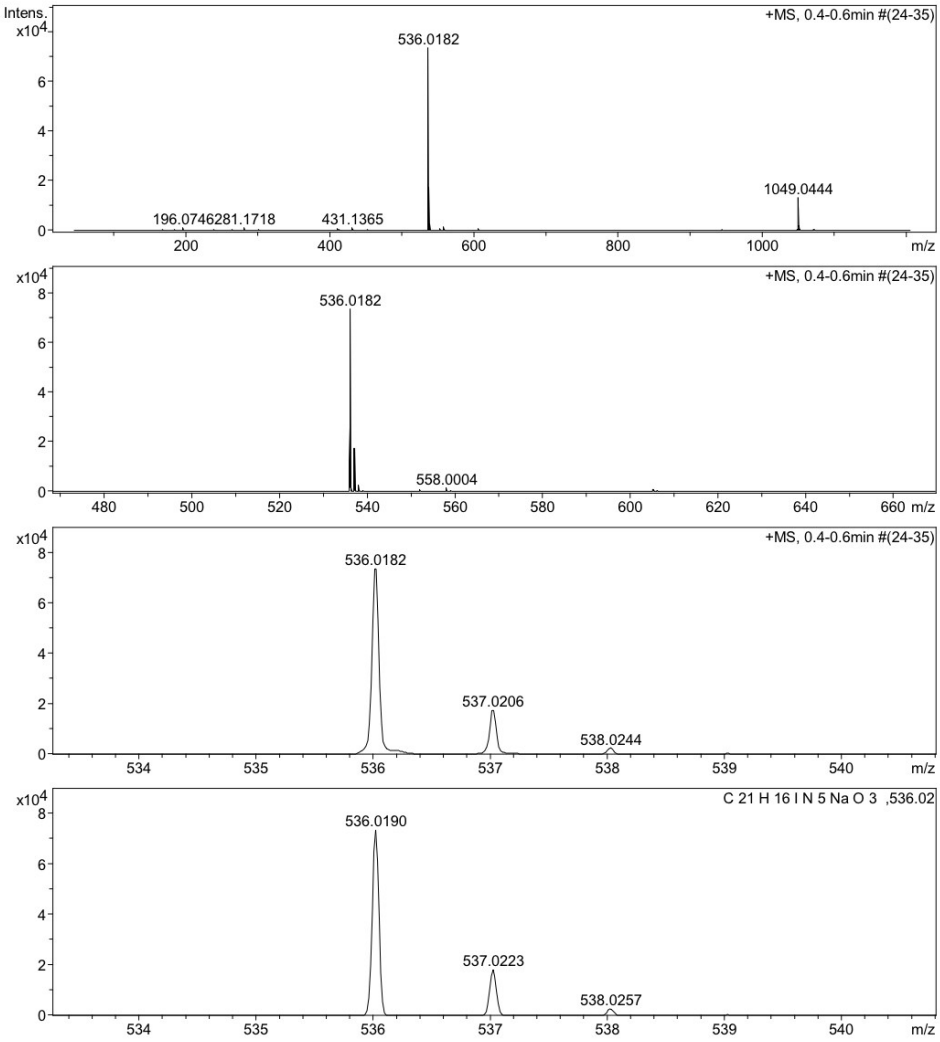
TDCMAS ESI-TOF

Analysis Info

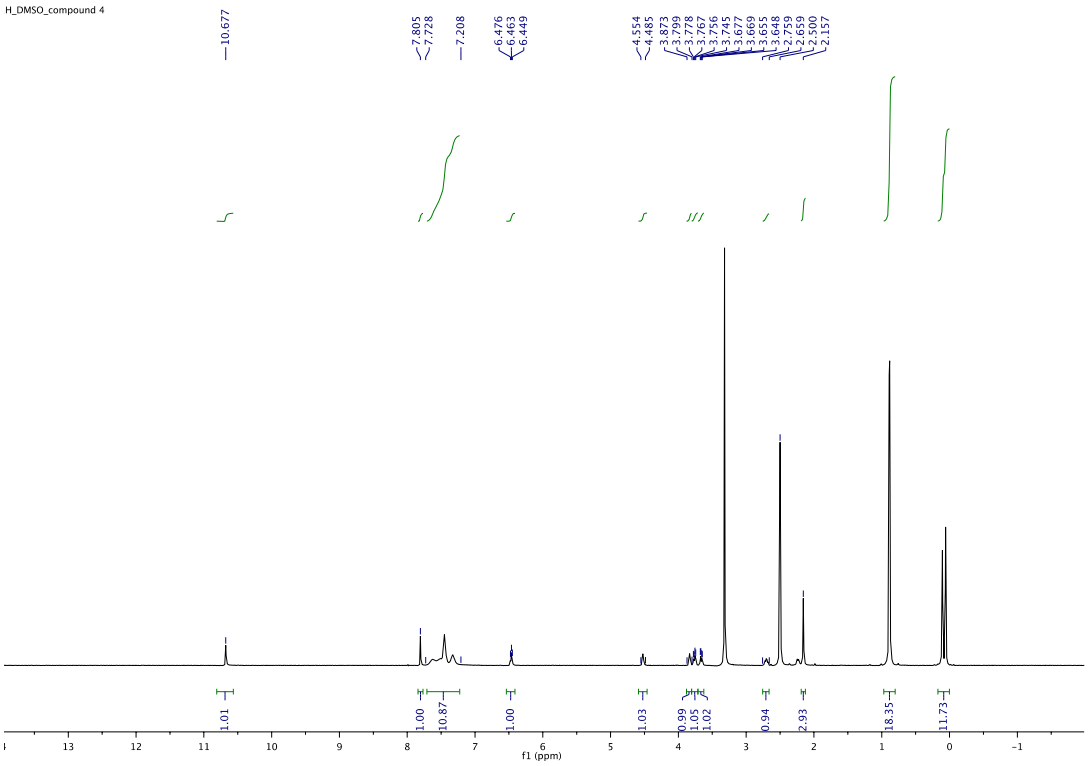
Analysis Name	D:\Data\sekine_lab\tokugawa\141128\Munefumi-258-A-000002.d	Acquisition Date	11/28/2014 11:53:22 AM
Method	esi_pos_low.m	Operator	BDAL@DE
Sample Name	Munefumi-258-A-	Instrument / Ser#	micrOTOF 10321
Comment			

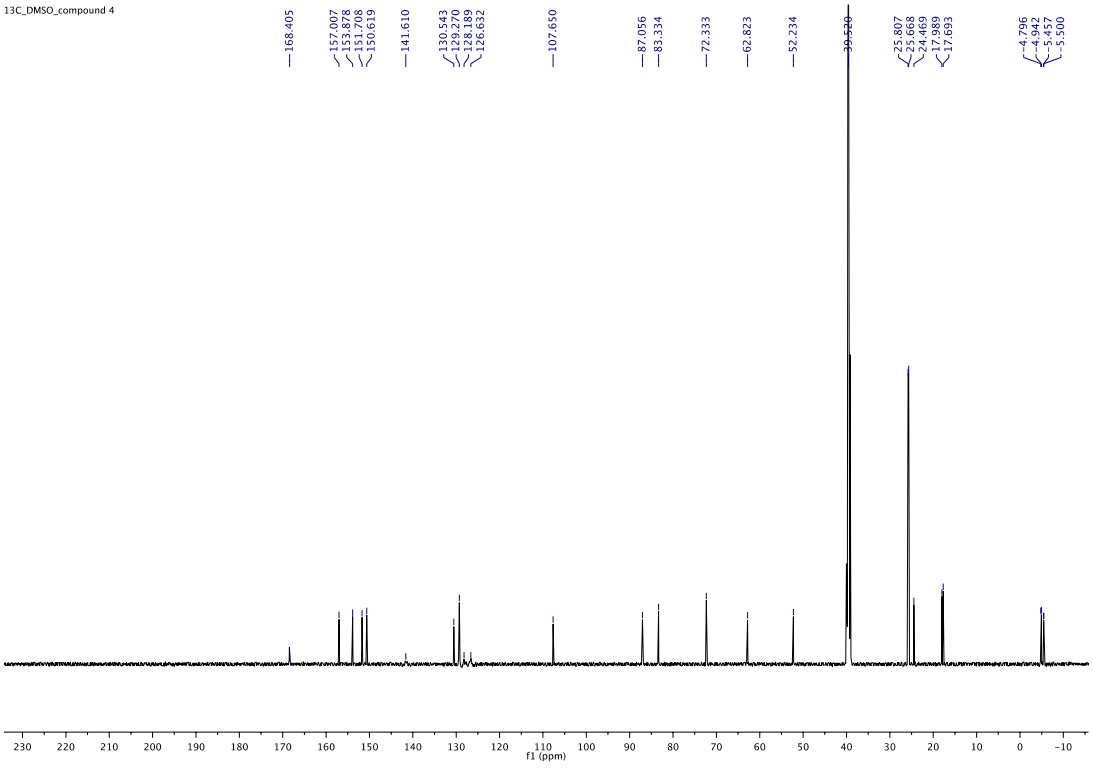
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	160 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	1200 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



2-*N*-Acetyl-7-deaza-2´-deoxy-7-iodo-6-*O*-(*N*,*N*-diphenylcarbamoyl)-3´,5´-bis-*O*-(*tert*-butyldimethylsilyl)-guanosine (4)





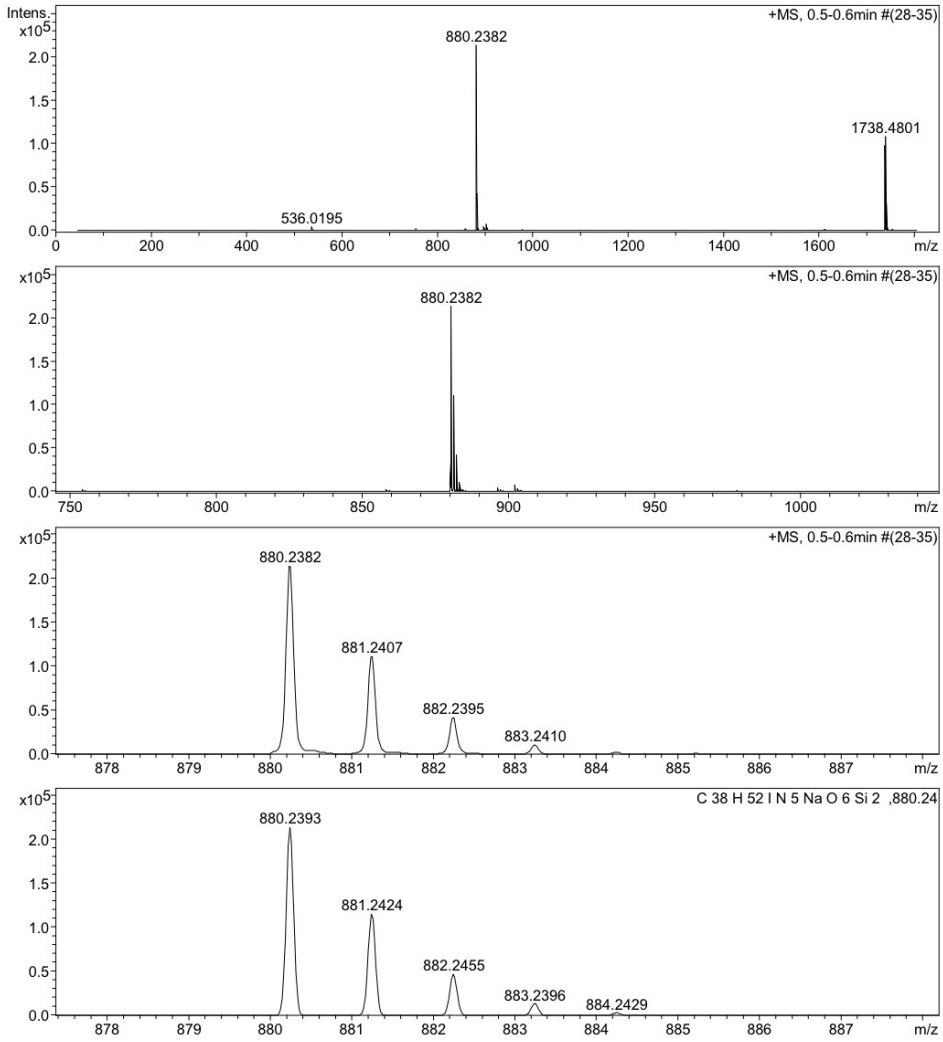
TDCMAS ESI-TOF

Analysis Info

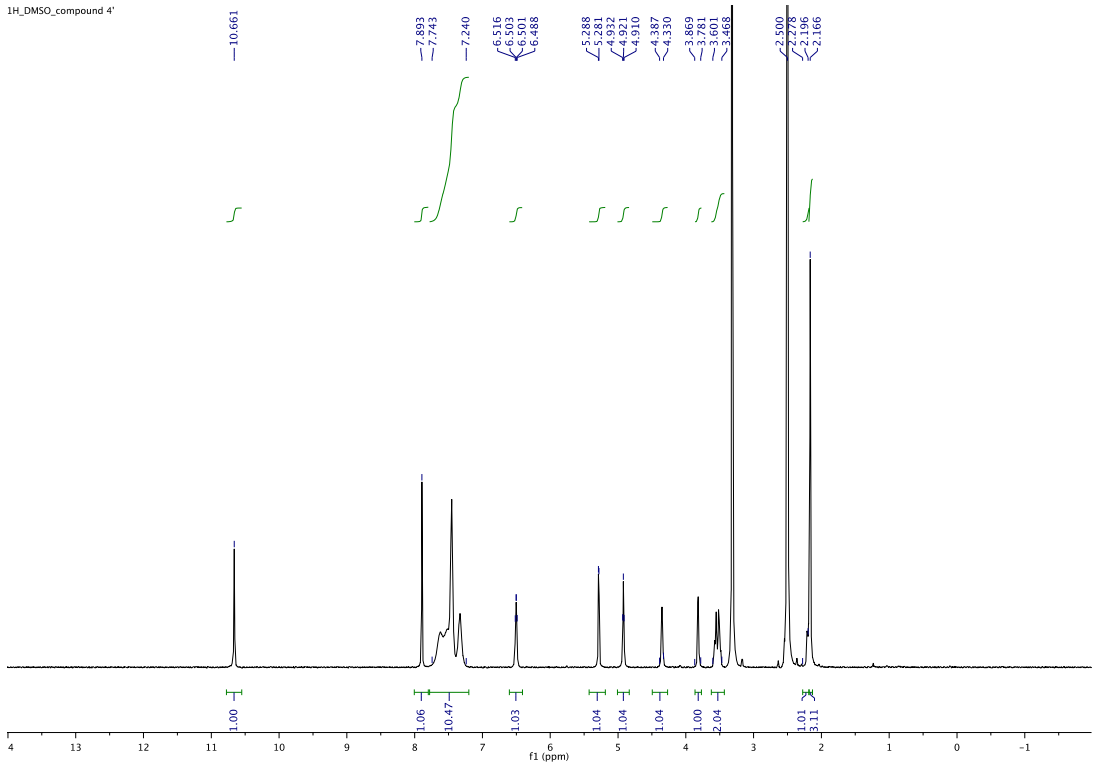
Analysis Name	D:\Data\sekine_lab\tokugawa\141216\Munefumi-167-5-000002.d	Acquisition Date	12/16/2014 10:03:18 AM
Method	esi_pos_low.m	Operator	BDAL@DE
Sample Name	Munefumi-167-5-	Instrument / Ser#	micrOTOF 10321
Comment			

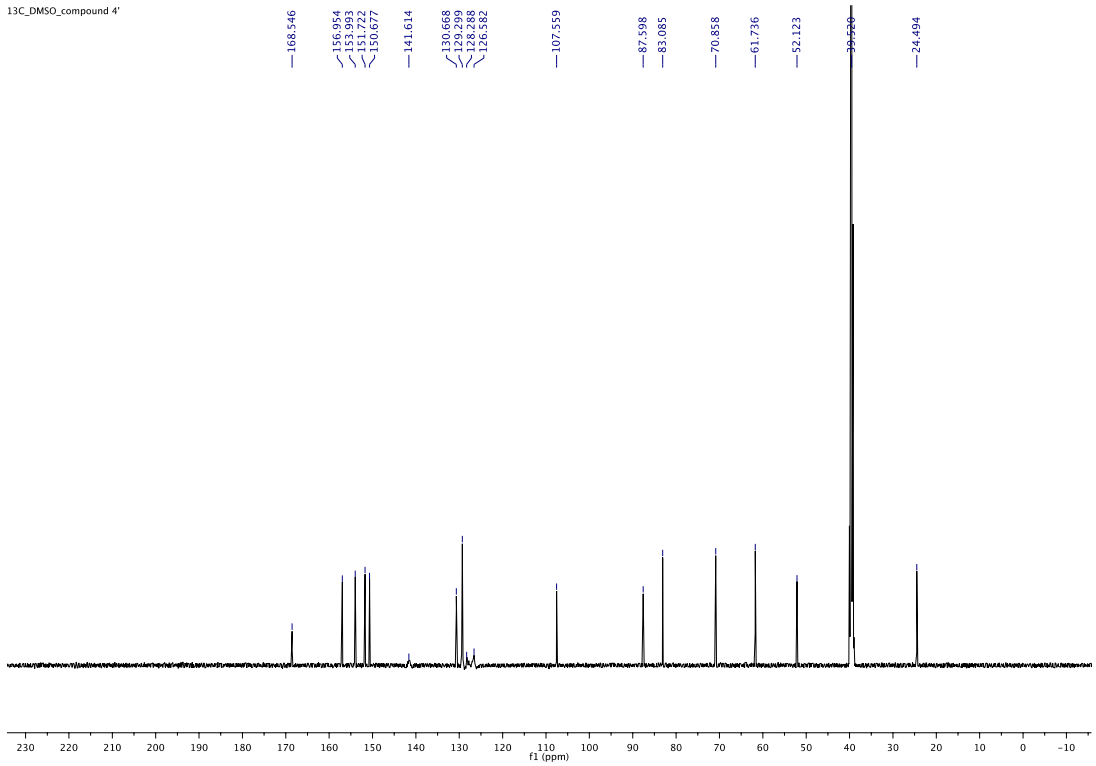
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.6 Bar
Focus	Not active			Set Dry Heater	160 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	1800 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



2-*N*-Acetyl-7-deaza-2´-deoxy-7-iodo-6-*O*-(*N*,*N*-diphenylcarbamoyl)guanosine (4')





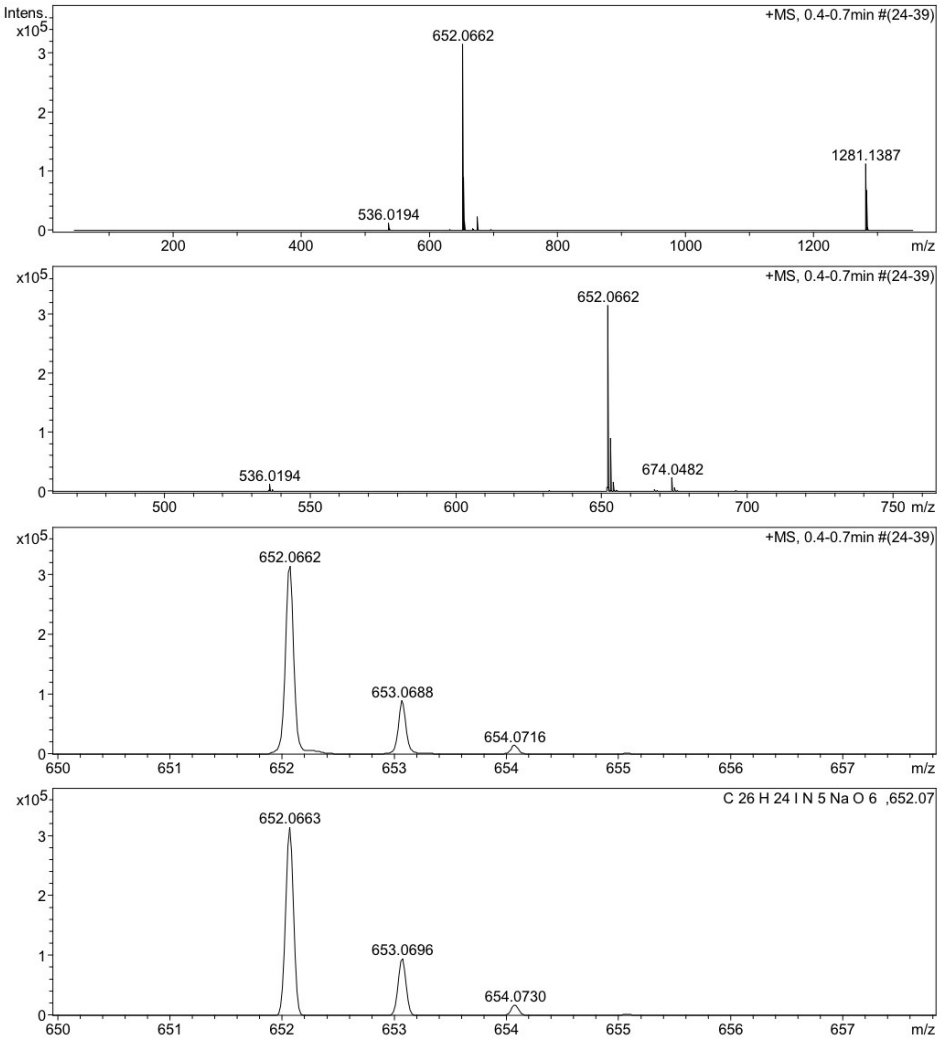
TDCMAS ESI-TOF

Analysis Info

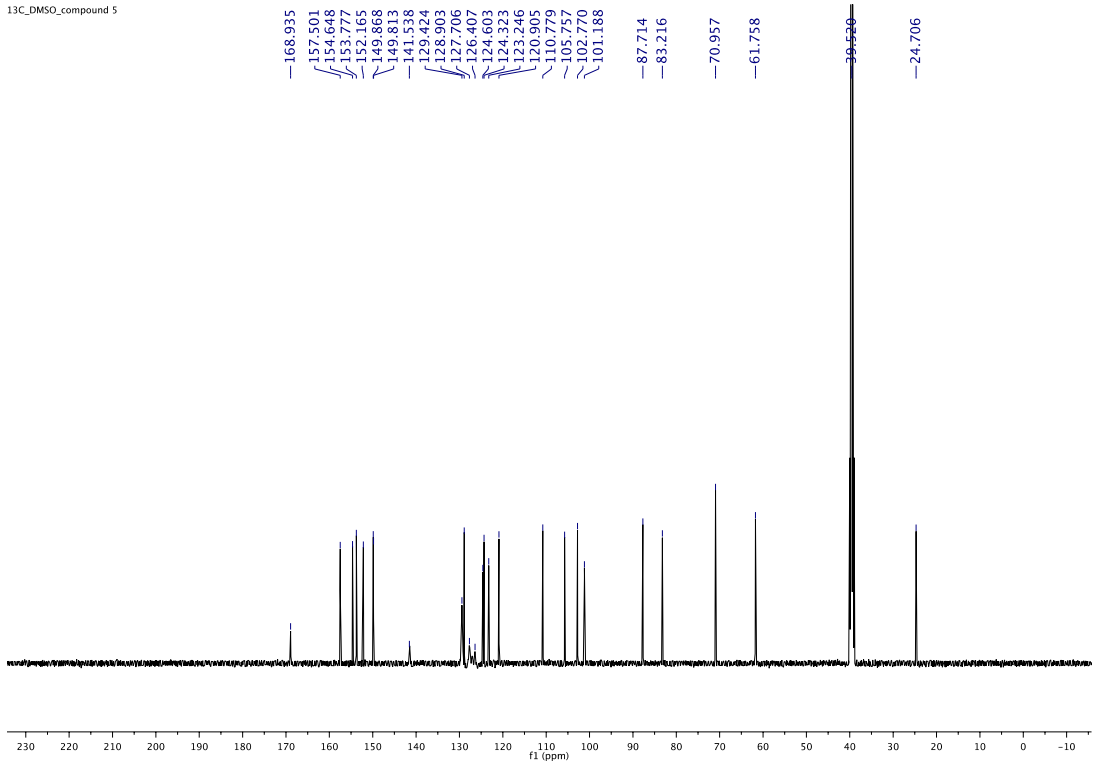
Analysis Name	D:\Data\sekine_lab\jan\141218\JAN-1-126B-000003.d	Acquisition Date	12/18/2014 5:19:20 PM
Method	esi_pos_low.m	Operator	BDAL@DE
Sample Name	JAN-1-126B-	Instrument / Ser#	micrOTOF 10321
Comment			

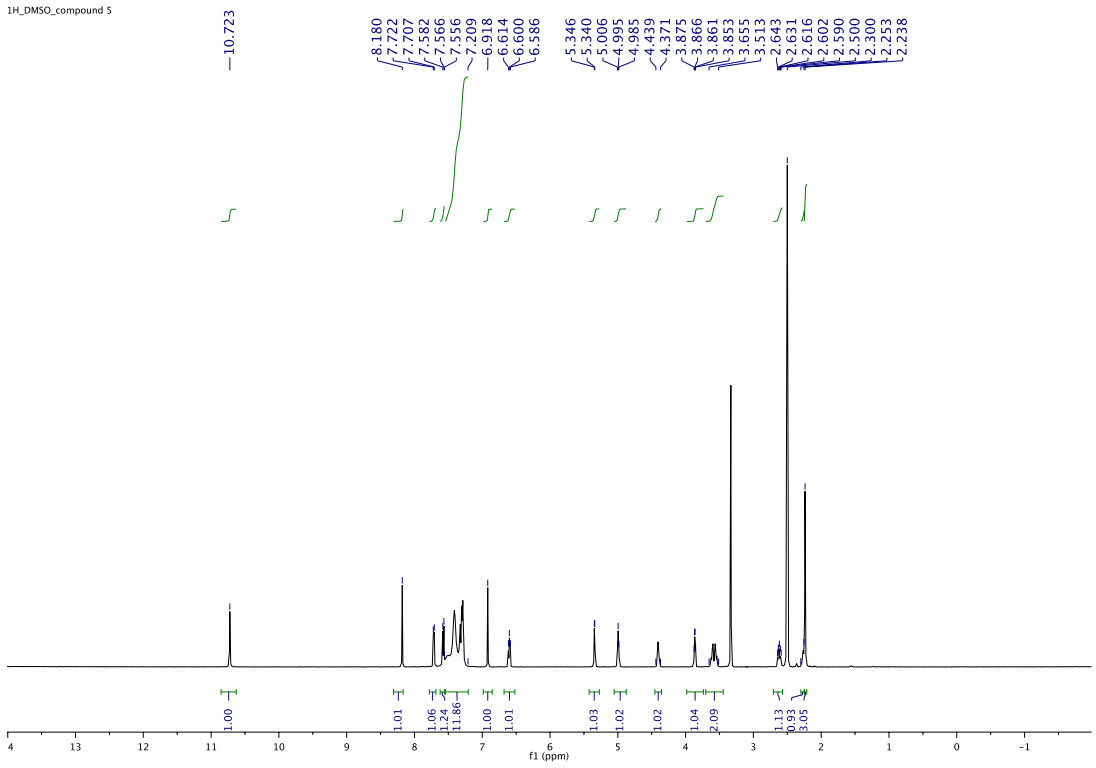
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.6 Bar
Focus	Not active			Set Dry Heater	160 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	1350 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



2-*N*-Acetyl-7-(benzofuran-2-yl)-7-deaza-2´-deoxy-6-*O*-(*N*,*N*,-diphenyl carbamoyl)guanosine (5)





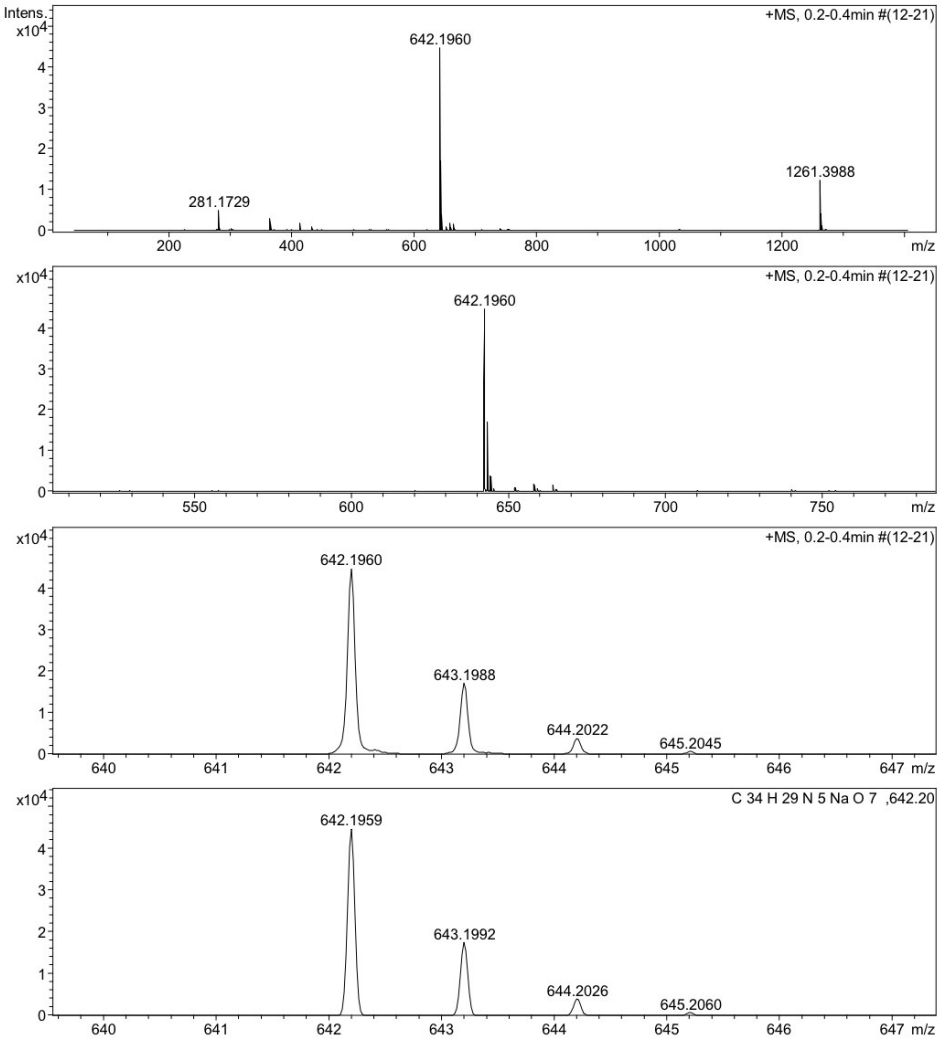
TDCMAS ESI-TOF

Analysis Info

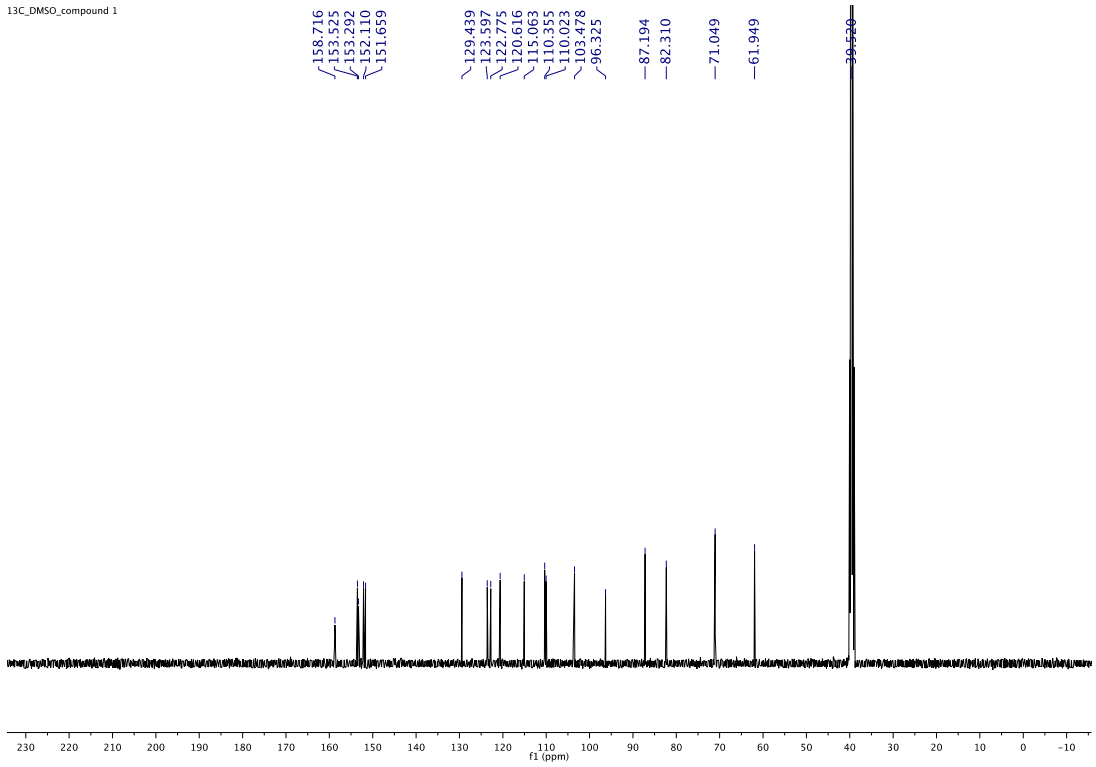
Analysis Name	D:\Data\sekine_lab\can\141201\JAN_1_70A-000001.d	Acquisition Date	12/1/2014 1:58:54 PM
Method	esi_pos_low.m	Operator	BDAL@DE
Sample Name	JAN_1_70A-	Instrument / Ser#	micrOTOF 10321
Comment			

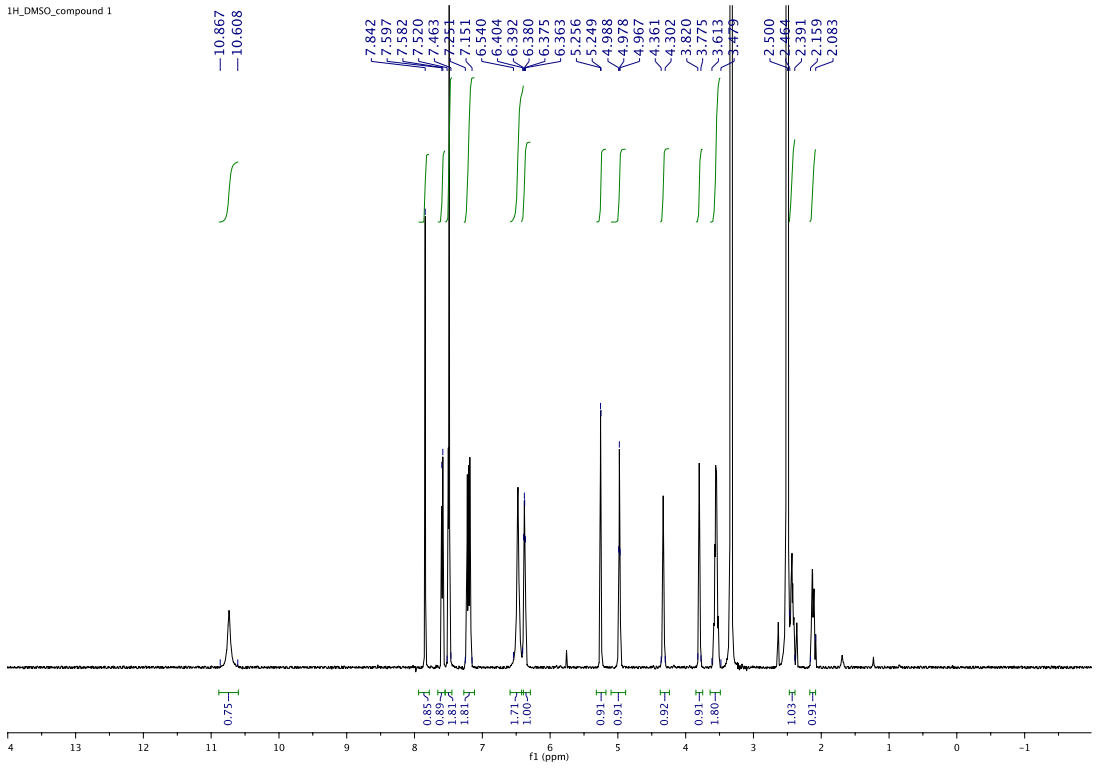
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	160 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	1400 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



7-(Benzofuran-2-yl)-7-deaza-2´-deoxyguanosine (^{BF}dG, 1)





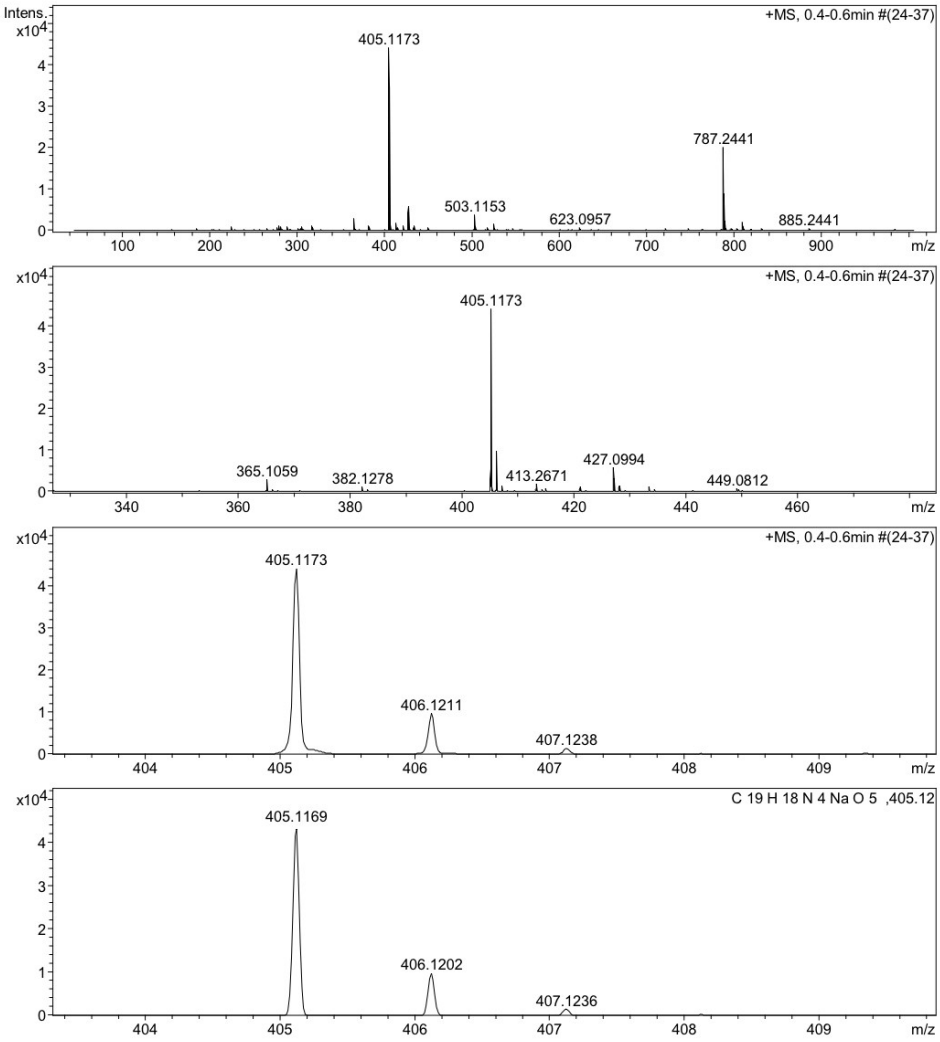
TDCMAS ESI-TOF

Analysis Info

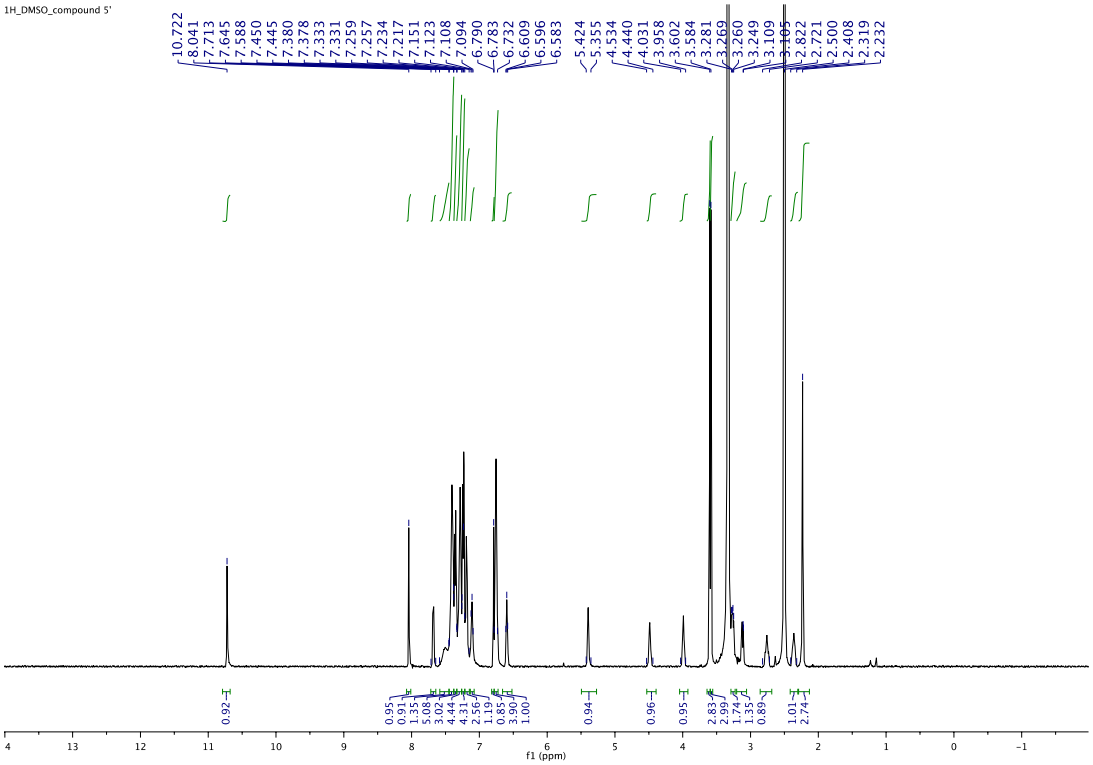
Analysis Name	D:\Data\sekine_lab\can\141201\JAN_1_72A-000002.d	Acquisition Date	12/1/2014 1:45:15 PM
Method	esi_pos_low.m	Operator	BDAL@DE
Sample Name	JAN_1_72A-	Instrument / Ser#	micrOTOF 10321
Comment			

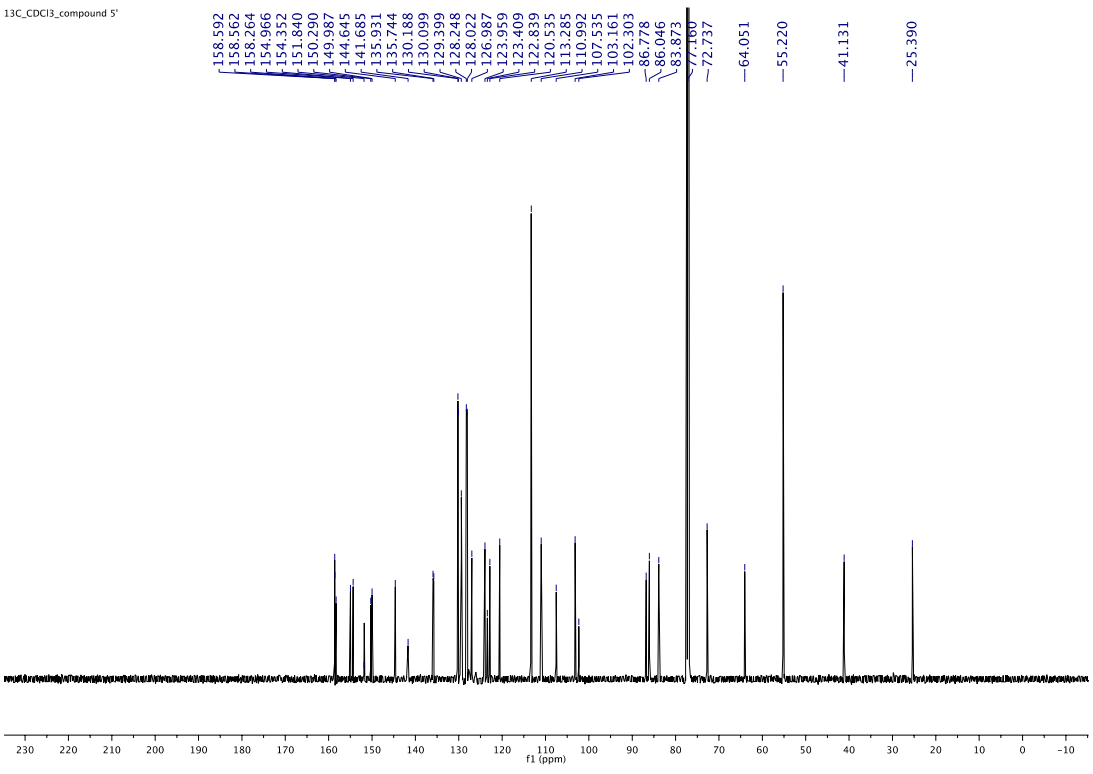
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	160 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	1000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



2-*N*-Acetyl-7-(benzofuran-2-yl)-7-deaza-2´-deoxy-5´-*O*-dimethoxytrityl -6-*O*-(*N*,*N*-diphenylcarbamoyl)-guanosine (5')





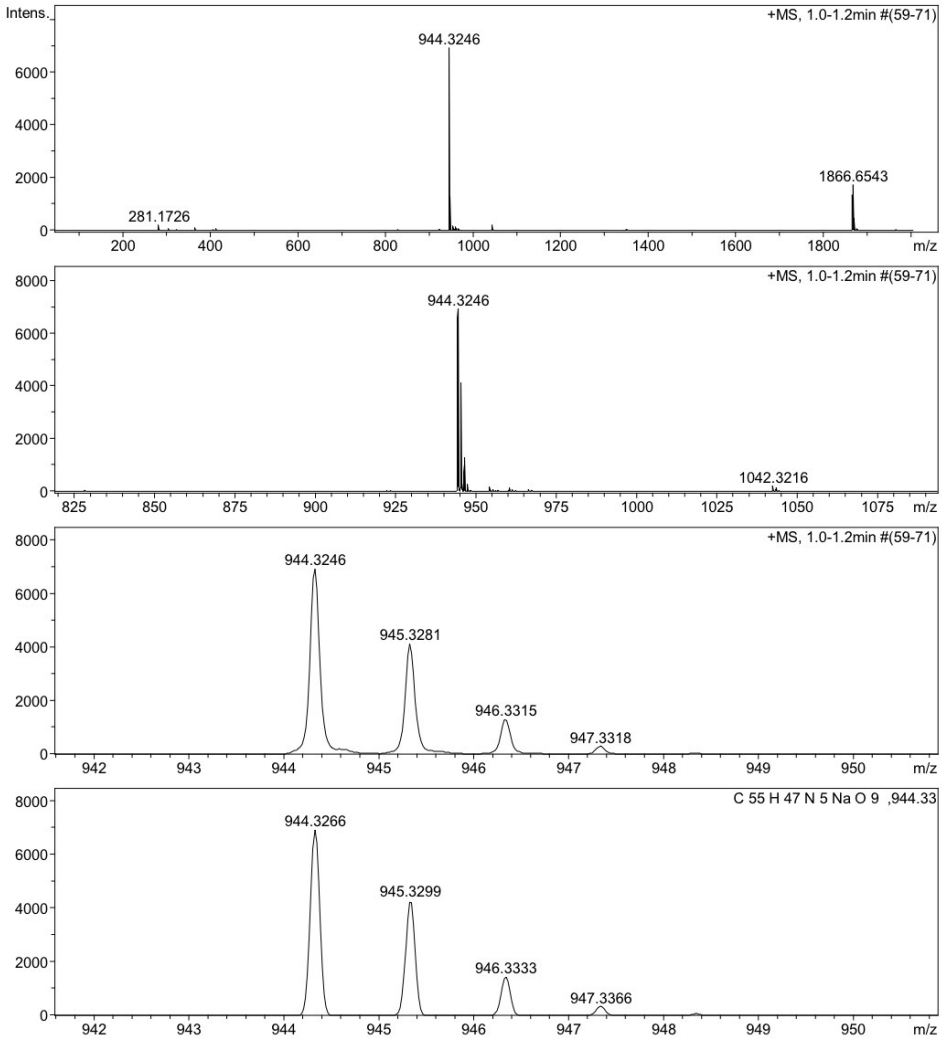
TDCMAS ESI-TOF

Analysis Info

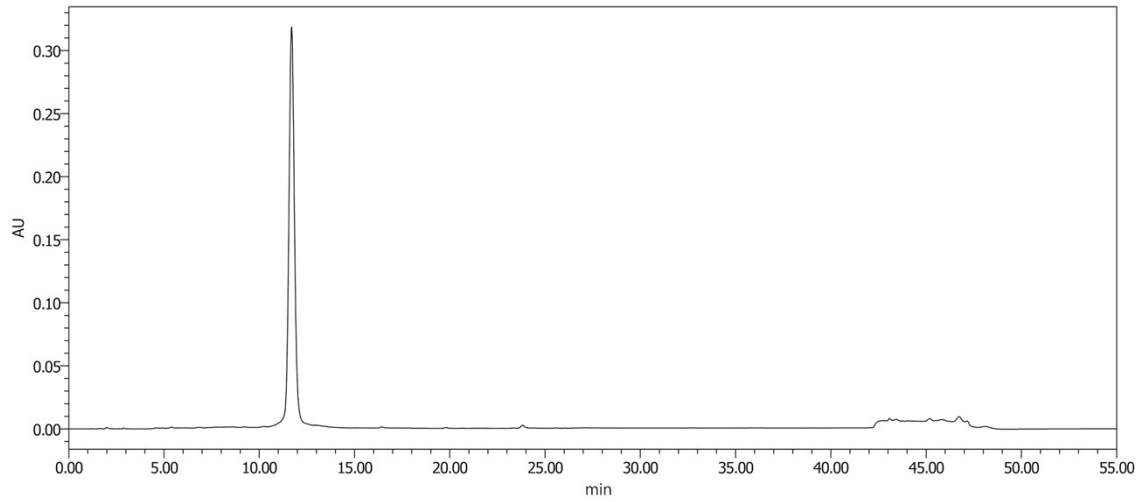
Analysis Name	D:\Data\sekine_lab\can\141201\JAN_1_74B-000002.d	Acquisition Date	12/1/2014 2:10:59 PM
Method	esi_pos_low.m	Operator	BDAL@DE
Sample Name	JAN_1_74B-	Instrument / Ser#	micrOTOF 10321
Comment			

Acquisition Parameter

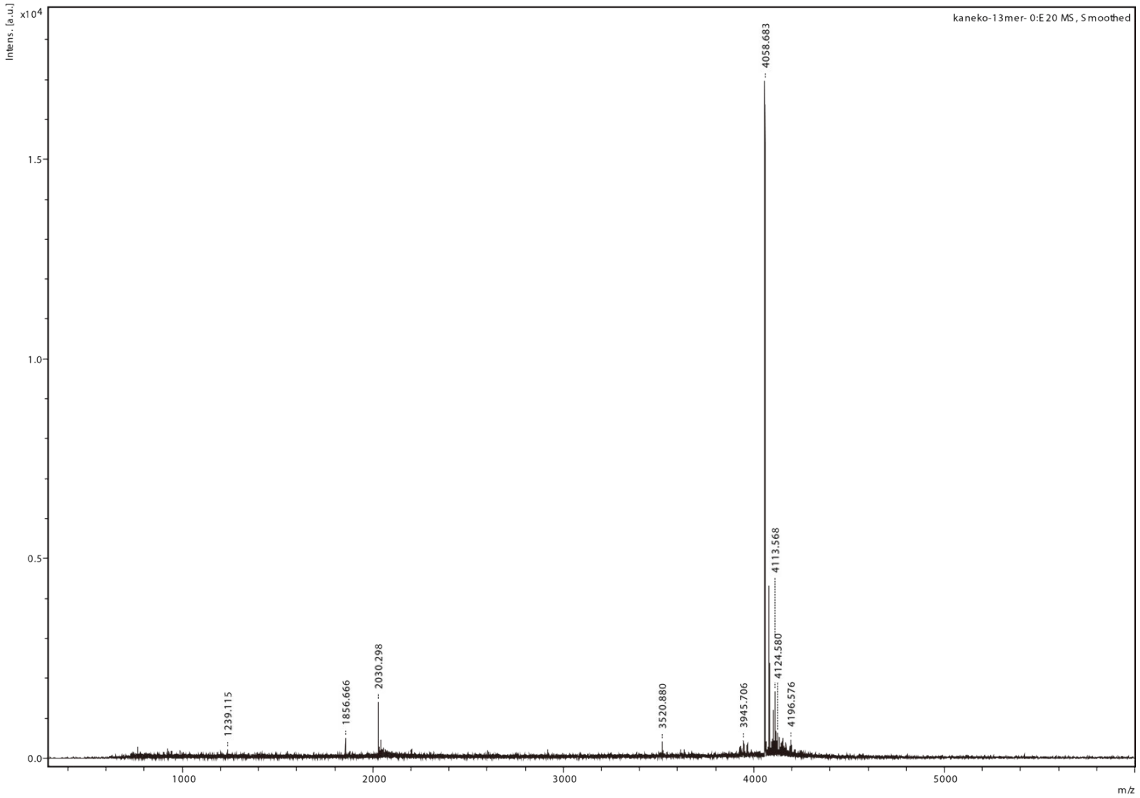
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	160 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



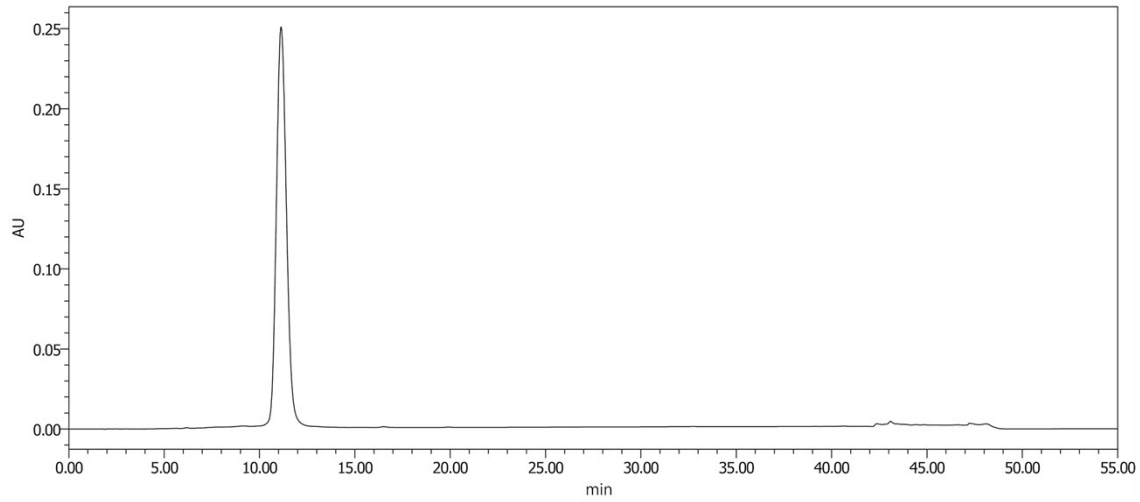
Reverse Phase HPLC chart of 5′-d(CGCAAT ^{BF}**G** TAACGC)-3′ (**ODN1**)



MALDI-TOF-Mass spectrum of **ODN1**



Reverse Phase HPLC chart of 5′-d(CGCAAC ^{BF}**G** CAACGC)-3′ (**ODN2**)



MALDI-TOF-Mass spectrum of **ODN2**