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Supporting Information

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Triggering of RNA secondary structures by a functionalized nucleobase

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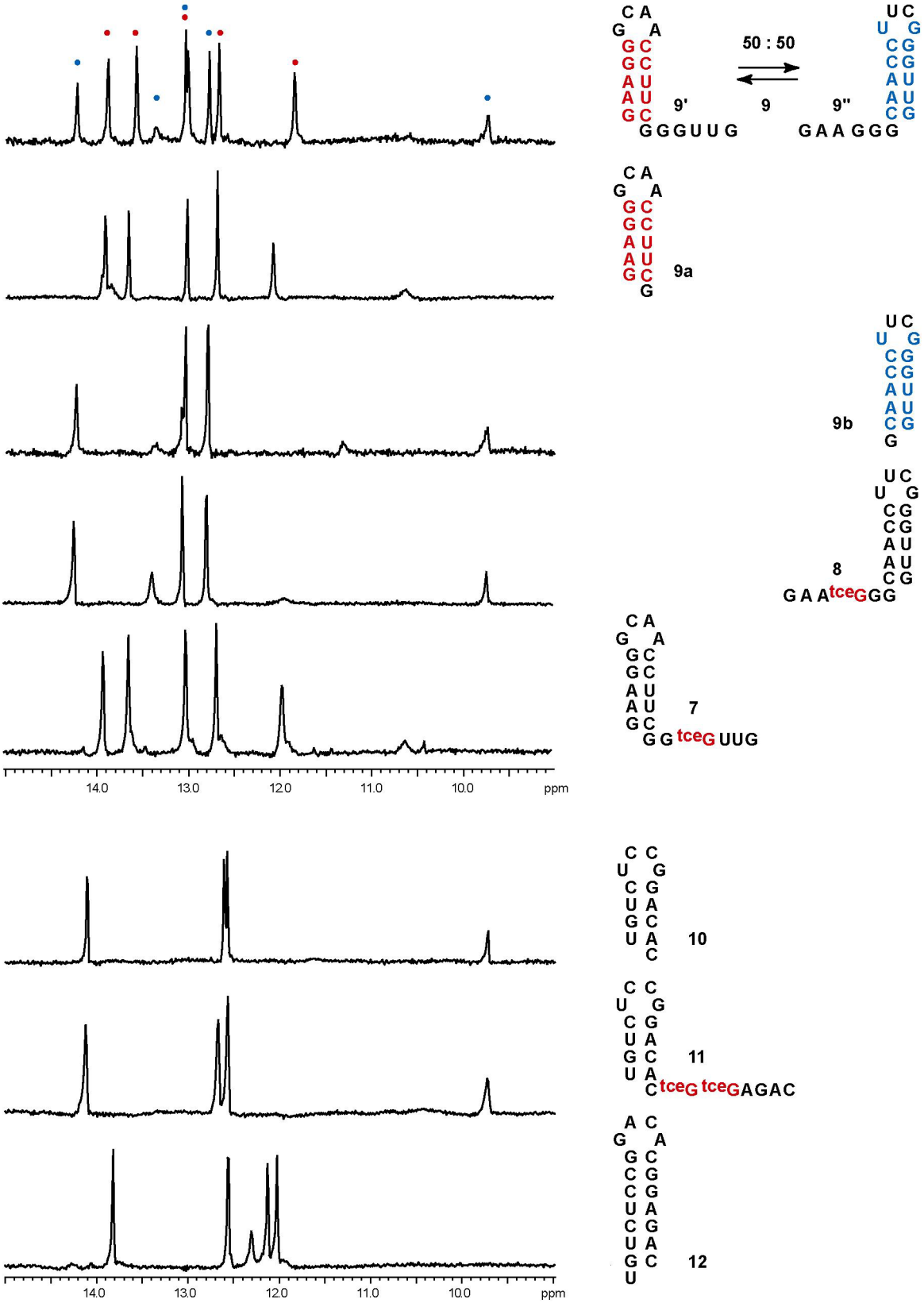
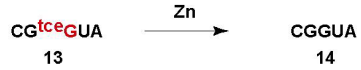
^{1}H-NMR spectroscopy for probing of ^{tce}G induced RNA secondary structure modulation



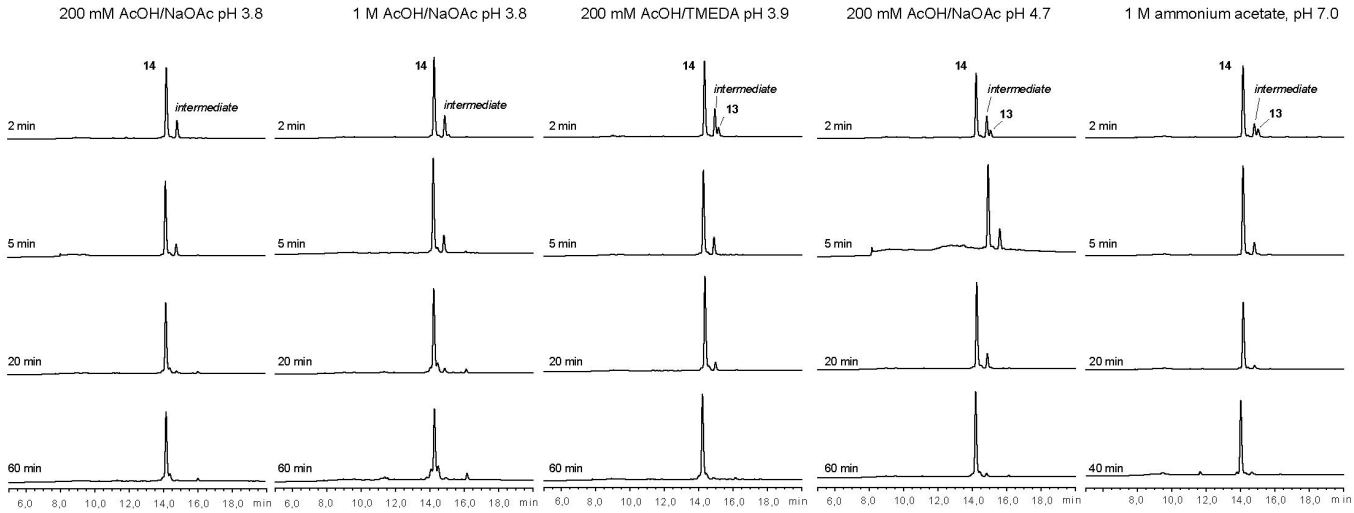
c = 0.2 -0.3 mM oligonucleotide, 25 mM sodium arsenate buffer, pH 7.4, H_2O/D_2O 9/1, 26°C.

At 26°C only 5 out of 7 possible resonances were detected for sequence **12**; the terminal imino proton resonances and the imino proton resonance of the loop closing base pair are not detected due to base pair fraying. (The loop in **12** is not an extrastable GNRA-tetraloop!)

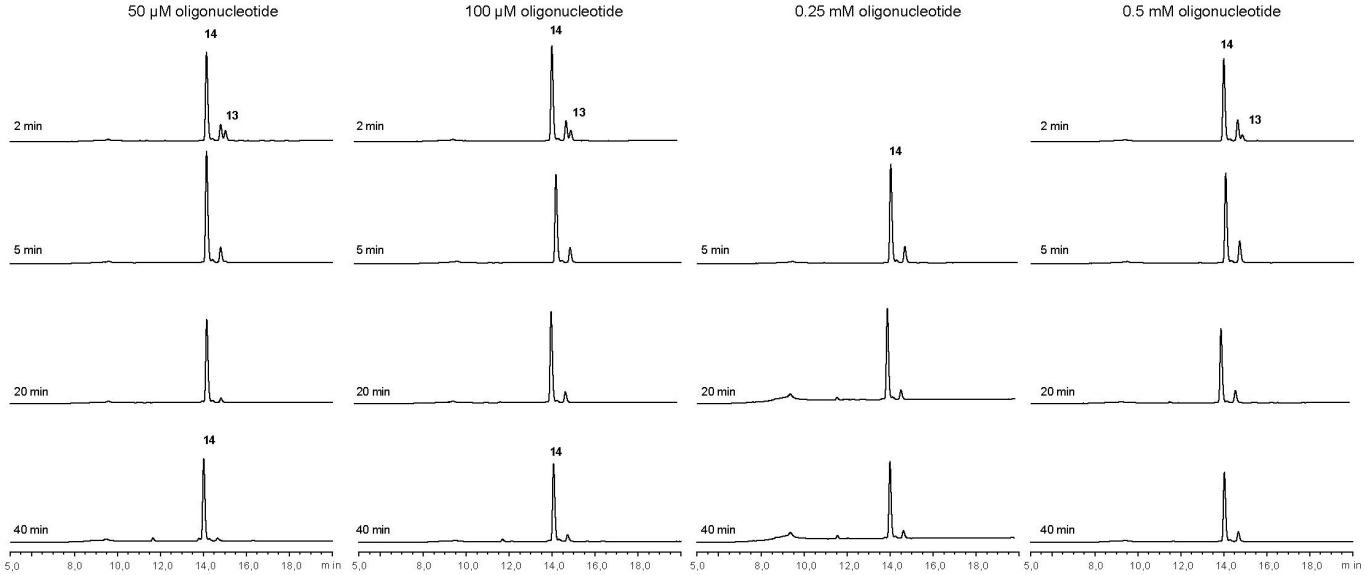
Release of the *O*^{6}-tce group from rCG^{tce}GUA under various conditons



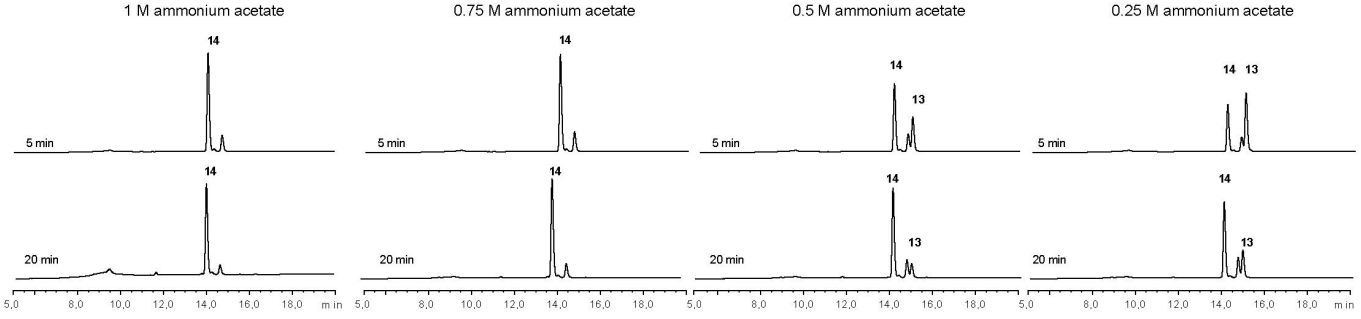
1) constant oligonucleotide concentration 50 µM, variation of pH



2) constant buffer conditions 1M ammonium acetate, pH 7, variation of oligonucleotide concentration

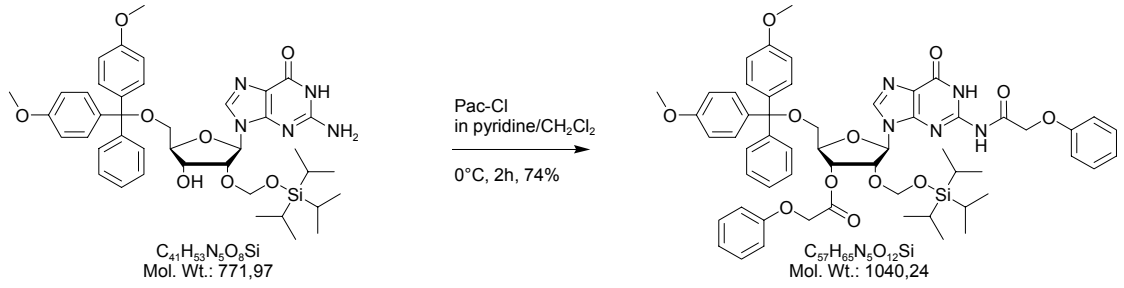


3) constant oligonucleotide concentration 0.25 mM, constant pH, variation of buffer concentration



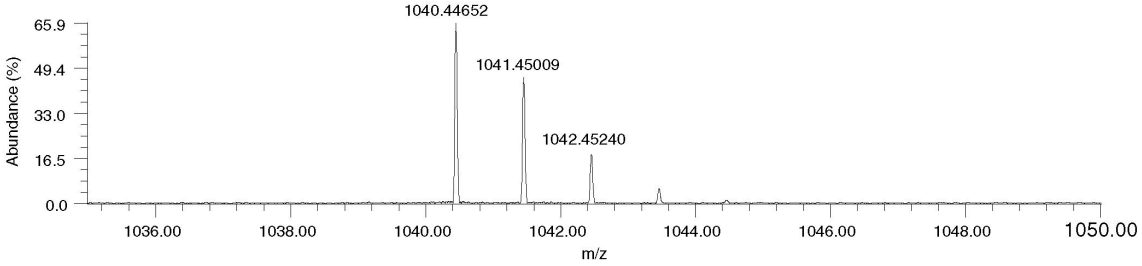
Anion exchange HPLC on Dionex DNAPAc PA 100 column, 4x250 mm, detection at 260 nm, 0-40% B in 30 min, 1 ml/min, 60°C,
A: 25 mM Tris.HCl, 6 M urea, pH 8.0, B: 25 mM Tris.HCl, 0.5 M $NaClO_4$, 6 M urea, pH 8.0

N^2,3′-*O*-Bis(phenoxyacetyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[[(triisopropyl)silyl-oxy]methyl] guanosine (2)



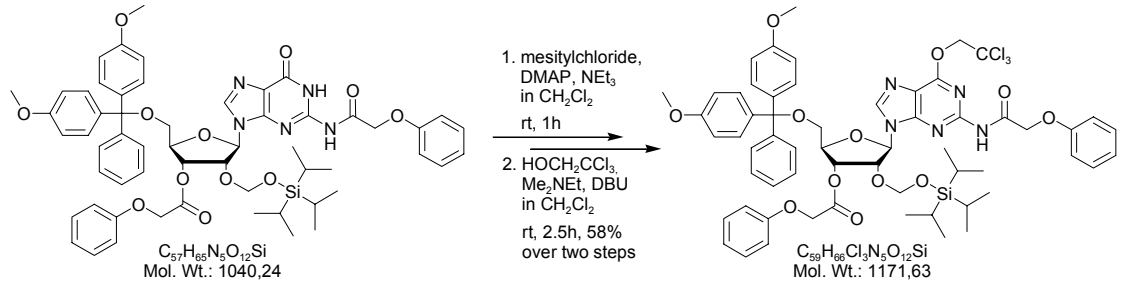
Compound **1** (200 mg, 0.26 mmol) was dissolved in dry pyridine (10 ml) and cooled to 0°C. Phenoxyacetyl chloride (200 µl, 1.45 mmol) was dissolved in dry dichloromethane (1.5 ml) and slowly added to the cooled solution. Stirring was continued for 2 h and the temperature was kept at 0°C. After addition of methanol (0.25 ml) the solvents were evaporated, the residue was dissolved in dichloromethane, extracted with 5% citric acid, water and semi-saturated sodium bicarbonate solution, dried over Na_2SO_4 and evaporated. The crude product was purified by chromatography on SiO_2 with dichloromethane + 0% to 2% methanol.

Yield: 200 mg of **2** as yellow foam (74 %);
TLC (dichloromethane/methanol 98/2): Rf 0.60;
UV (MeOH): $\lambda(\varepsilon)$ 260 (18000), 275 (16100) l.mol^{-1}.cm^{-1};
^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.92-0.96 (m, 21H, iPr_3Si); 3.36 (dd, *J* = 10.5, 3.8 Hz, 1H, H^1-C(5′)); 3.47 (dd, *J* = 10.5, 3.0 Hz, 1H, H^2-C(5′)); 3.74 (s, 6H, 2 OCH_3); 4.31 (quartettoid, 1H, H-C(4')); 4.58 (s, 2H, $COCH_2O$); 4.68, 4.72 (2d, *J* = 15.8 Hz, 2H, $COCH_2O$); 4.83, 4.87 (2d, *J* = 5.3 Hz, 2H, OCH_2O); 5.13 (triplettoid, 1H, H-C(2′)); 5.75 (triplettoid, 1H, H-C(3′)); 5.98 (d, *J* = 6.0 Hz, 1H, H-C(1′)); 6.77 (m, 4H, H-C(ar)); 6.91-7.11 (m, 6H, H-C(ar)); 7.18-7.39 (m, 13H, H-C(ar)); 7.83 (s, 1H, H-C(8)); 8.92 (s, br., 1H, NH); 11.77 (s, br., NH) ppm;
^{13}C-NMR (75 MHz, $CDCl_3$): δ 11.73 ($\underline{C}H(CH_3)_2$); 17.60 ($CH(\underline{C}H_3)_2$); 55.16 (OCH_3); 62.64 (C(5′)); 65.11 (CH_2O); 66.85 (CH_2O); 72.33 (C(3′)); 77.16 (C(2′)); 81.65 (C(4′)); 86.44; 86.82 (C(1′)); 90.05 (OCH_2O); 113.21, 114.70, 114.98, 121.95 (4 C(ar)); 122.40; 123.00, 127.01, 127.89, 128.03, 129.55, 129.94, 129.99 (7 C(ar)); 135.32, 135.46; 138.10 (C(8)); 144.29, 146.08, 147.88, 155.19, 156.40; 157.68; 158.64; 168.22, 169.36 ($\underline{C}OCH_2$) ppm;
FT-ICR ESI-MS: m/z calculated for $C_{57}H_{65}N_5O_{12}Si$ $[M+H]^+$ 1040.44765, found 1040.44652 (Δm 0.00113, error 1.1 ppm).



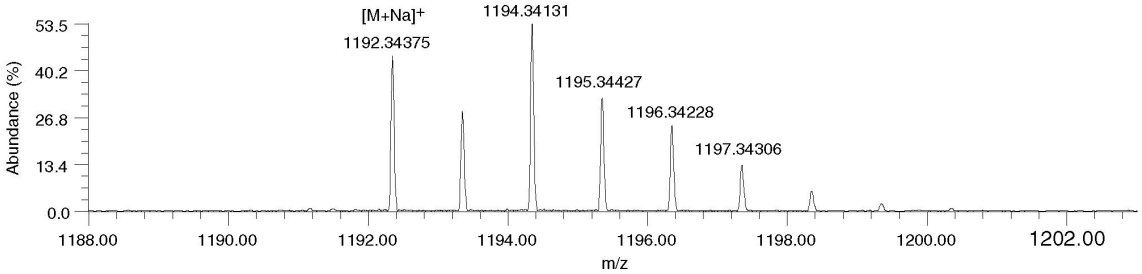
FT-ICR ESI-MS of **2**.

N^2,3′-*O*-Bis(phenoxyacetyl)-*O*^{6}-(2,2,2-trichloroethyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[[(triisopropyl)silyloxy]methyl] guanosine (4)



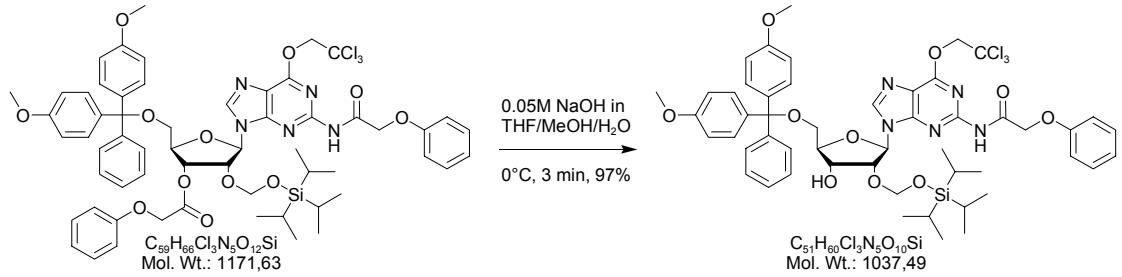
Compound **2** (207 mg, 0.19 mmol) and DMAP (2.0 mg, 16 µmol) were dissolved in dry dichloromethane (3 ml), triethylamine (140 µl, 1.06 mmol) and mesitylensulfonyl chloride (56 mg, 0.25 mmol) were added successively and the mixture was stirred at rt for 1 h. The mixture was diluted with dichloromethane, washed with water and saturated sodium bicarbonate solution and dried over Na_2SO_4. After evaporation of dichloromethane, the yellow foamy *O* ^{6}-sulfonylated intermediate **3** was dried under vacuum. The intermediate was dissolved in dry dichloromethane (2 ml), *N*-ethyldimethylamine (110 µl, 1.01 mmol) and 2,2,2-trichloroethanol (78 mg, 0.52 mmol) were added at rt with stirring. After 5 min, DBU (29 mg, 0.19 mmol) was added and stirring was continued for 2 h at rt. The mixture was concentrated and the crude product was purified by chromatography on SiO_2 with dichloromethane + 0% to 1% methanol.

Yield: 129 mg of **4** as colorless foam (58 %);
TLC (dichloromethane/methanol 98/2): Rf intermediate **3** 0.70, product **4** 0.73;
UV (MeOH): λ(ε) 260 (17800), 270 (max, 19500), 275 (18600) l.mol^{-1}.cm^{-1};
^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.84-0.89 (m, 21H, iPr_3Si); 3.49 (dd, *J* = 10.5, 3.0 Hz, 1H, H^1-C(5′)); 3.55 (dd, *J* = 10.5, 4.5 Hz, 1H, H^2-C(5′)); 3.75 (s, 6H, 2 OCH_3); 4.35 (quartettoid, 1H, H-C(4')); 4.63, 4.64 (2br.d, 2H, $COCH_2O$); 4.73, 4.74 (2br.d, 2H, $COCH_2O$); 4.80, 4.84 (2d, *J* = 5.3 Hz, 2H, OCH_2O); 5.27 (triplettoid, 1H, H-C(2′)); 5.35 (2br.d, 2H, CH_2CCl_3); 5.74 (quartettoid, 1H, H-C(3′)); 6.11 (d, *J* = 6.0 Hz, 1H, H-C(1′)); 6.76 (m, 4H, H-C(ar)); 6.92-7.09 (m, 6H, H-C(ar)); 7.16-7.41 (m, 13H, H-C(ar)); 8.03 (s, 1H, H-C(8)); 8.70 (s, br., 1H, NH) ppm;
^{13}C-NMR (75 MHz, $CDCl_3$): δ 11.70 ($\underline{C}H(CH_3)_2$); 17.56 ($CH(\underline{C}H_3)_2$); 55.13 (OCH_3); 63.47 (C(5′)); 65.04 (CH_2O); 67.91 (CH_2O); 72.88 (C(3′)); 76.32 ($\underline{C}H_2CCl_3$); 77.17 (C(2′)); 82.31 (C(4′)); 86.76 (C(1′)); 86.95; 90.10 (OCH_2O); 94.95 ($CH_2\underline{C}Cl_3$); 113.13, 113.16, 114.73, 115.00 (4 C(ar)); 118.65; 121.85, 122.40, 126.90, 127.82, 128.08, 129.51, 129.80, 129.97, 130.02 (9 C(ar)); 135.50, 135.57; 141.64 (C(8)); 144.40, 150.73, 153.60, 157.11, 158.55, 159.37; 165.45, 168.10 ($\underline{C}OCH_2$) ppm;
FT-ICR ESI-MS: m/z calculated for $C_{59}H_{66}Cl_3N_5O_{12}Si$ $[M+Na]^+$ 1192.34400, found 1192.34375 (Δm 0.00025, error 0.2 ppm).



FT-ICR ESI-MS of **4**.

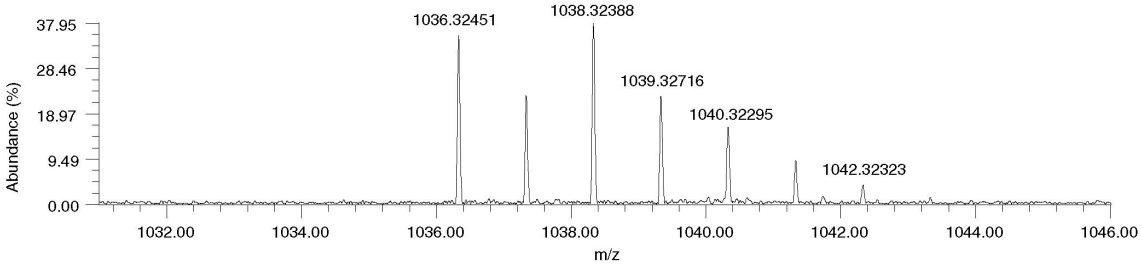
O^6-(2,2,2-Trichloroethyl)- 5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[[(triisopropyl)silyl-oxy]methyl]-*N*^{2}-phenoxyacetyl guanosine (5)



4 **5**

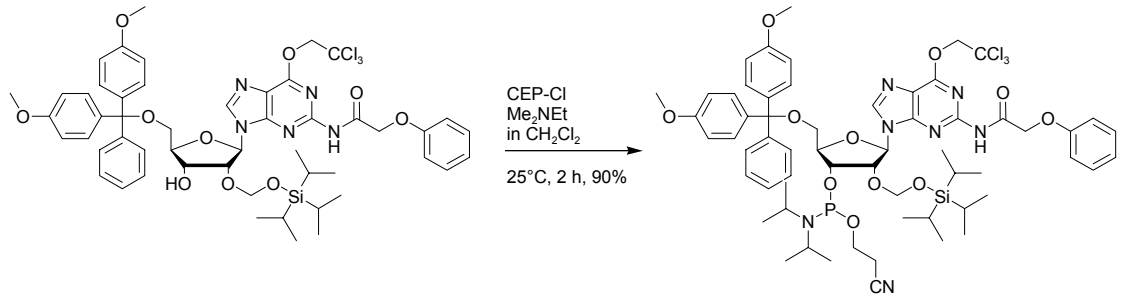
Compound **4** (120 mg, 0.10 mmol) was dissolved in THF/methanol 5/4 (3 ml) and cooled in an ice-water bath. 2 M NaOH (75 µl) was added at 0°C and the mixture was stirred for 3 minutes before it was neutralized by the addition of 2 M acetic acid (75 µl). The solvents were evaporated and the residue was dissolved in dichloromethane and extracted with water, the organic solution was dried over Na_2SO_4 and the solvent was evaporated. The crude product was purified by chromatography on SiO_2 with dichloromethane + 0% to 1% methanol.

Yield: 103 mg of **5** as slightly yellow foam (97%);
TLC (dichloromethane/methanol 98/2): Rf 0.70;
UV (MeOH): $\lambda(\varepsilon)$ 260 (16800), 270 (18300), 275 (17400) l.mol^{-1}.cm^{-1};
^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.00-1.08 (m, 21H, iPr_3Si); 3.07 (d, *J* = 3.8 Hz, 1H, HO-C(3')); 3.45 (m, 2H, H_2-C(5′)); 3.78 (s, 6H, 2 OCH_3); 4.28 (quartettoid, 1H, H-C(4')); 4.62 (quartettoid, 1H, H-C(3')); 4.69 (s, 2H, $COCH_2O$); 4.89 (triplettoid, 1H, H-C(2')); 5.05, 5.16 (2d, *J* = 4.5 Hz, 2H, OCH_2O); 5.31, 5.34 (2d, J = 11.7 Hz, 2H, CH_2CCl_3); 6.21 (d, *J* = 5.3 Hz, 1H, H-C(1′)); 6.78 (m, 4H, H-C(ar)); 7.01 (d, *J* = 7.5 Hz, 2H, H-C(ar)); 7.09 (t, *J* = 7.5 Hz, 1H, H-C(ar)); 7.19-7.42 (m, 11H, H-C(ar)); 8.09 (s, 1H, H-C(8)); 8.70 (s, br., 1H, NH) ppm;
^{13}C-NMR (75 MHz, $CDCl_3$): δ 11.83 ($\underline{C}H(CH_3)_2$); 17.73 ($CH(\underline{C}H_3)_2$); 55.17 (OCH_3); 63.70 (C(5′)); 67.99 (CH_2O); 70.89 (C(3′)); 76.37 ($\underline{C}H_2CCl_3$); 82.49 (C(2′)); 84.42 (C(4′)); 86.62 (C(1′)); 87.07; 91.05 (OCH_2O); 94.96 ($CH_2\underline{C}Cl_3$); 113.17, 114.97 (2 C(ar)); 118.60; 122.40, 126.90, 127.83, 128.15, 129.82, 130.05, 130.08 (7 C(ar)); 135.64, 135.74; 141.40 (C(8)); 144.55, 150.75, 153.52, 157.14, 158.57, 159.36; 165.78 ($\underline{C}OCH_2$) ppm;
FT-ICR ESI-MS: m/z calculated for $C_{51}H_{60}Cl_3N_5O_{10}Si$ $[M+H]^+$ 1036.32525, found 1036.32451 (Δm 0.00074, error 0.7 ppm).



FT-ICR ESI-MS of **5**.

O ^{6}**-(2,2,2-Trichloroethyl)- 5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[[(triisopropyl)silyl-oxy]methyl]-*N*** ^{2}**-phenoxyacetyl guanosine 3′-(2-cyanoethyl *N,N*-diisopropyl phosphoramidite) (6)**



$C_{51}H_{60}Cl_3N_5O_{10}Si$
Mol. Wt.: 1037,49

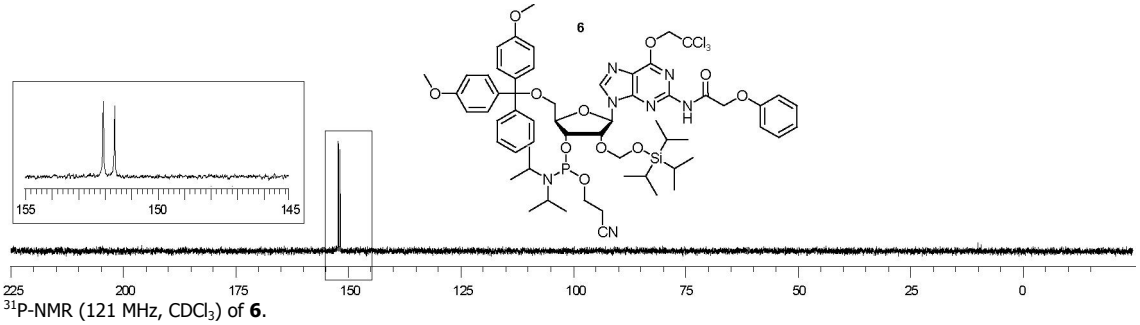
5

$C_{60}H_{77}Cl_3N_7O_{11}PSi$
Mol. Wt.: 1237,71

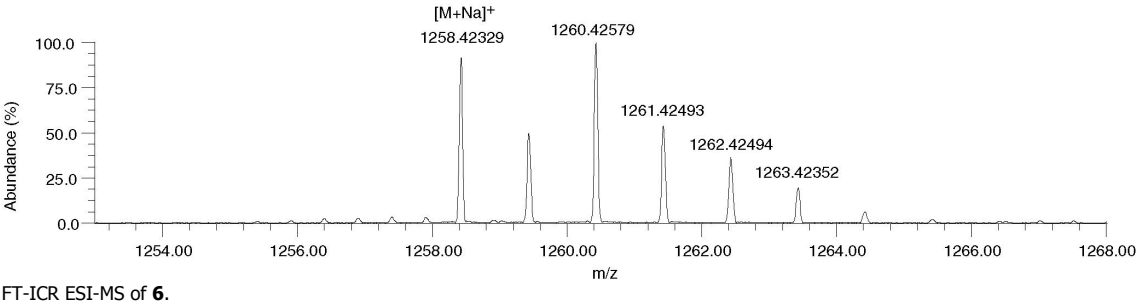
6

A solution of compound **5** (120 mg, 115 µmol) in dry dichloromethane (3.0 ml) under argon atmosphere was treated consecutively with *N*-ethyldimethylamine (125 µl, 1.1 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (41 mg, 172 µmol). After stirring for 2 h at rt the mixture was quenched with methanol (100 µl), diluted with dichloromethane and extracted with semi-saturated sodium bicarbonate solution, dried over Na_2SO_4 and evaporated. The crude product was subjected to chromatography on SiO_2 with ethyl acetate/hexanes 1/9 to 1/1 (+ 1 % NEt_3).

Yield: 129 mg of **6** as colorless foam (1:1 mixture of diastereoisomers, 90 %);
TLC (ethyl acetate/hexanes 3/7): Rf 0.7;
UV (MeOH): $\lambda(\varepsilon)$ 260 (17900), 270 (max, 19600), 275 (18500) $l.mol^{-1}.cm^{-1}$;
^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.86-0.90 (m, 42H, iPr_3Si); 1.04-1.18 (m 24H, $((C\underline{H}_3)_2CH)_2N$); 2.33 (m, 2H, CH_2CN); 2.64 (m, 2H, CH_2CN); 3.42-3.52 (m, 4H, H_2-C(5′)); 3.58-3.70 (m, 6H, $POCH_2$, $((CH_3)_2C\underline{H})_2N$); 3.76 (2s, 12H, OCH_3); 3.86, 3.95 (2m, 2H, $POCH_2$); 4.32, 4.38 (2m, 2H, H-C(4′)); 4.60 (m, 2H, H-C(3′)); 4.69 (br.s, 4H, $COCH_2O$); 4.91, 4.97 (2m, 4H, OCH_2O); 5.04 (m, 2H, H-C(2')); 5.30-5.36 (m, 4H, CH_2CCl_3); 6.16, 6.21 (2d, J = 5.8 Hz, 2H, H-C(1′)); 6.76 (m, 8H, H-C(ar)); 6.99 (m, 4H, H-C(ar)); 7.05 (m, 2H, H-C(ar); 7.18-7.42 (m, 22H, H-C(ar)); 8.09 (s, 2H, H-C(8)); 8.60, 8.65 (2 br. s, 2H, NH) ppm;
^{31}P-NMR (121 MHz, $CDCl_3$): δ 151.62, 152.06 ppm;
FT-ICR ESI-MS: m/z calculated for $C_{60}H_{77}Cl_3N_7O_{11}PSi$ $[M+Na]^+$ 1258.41507, found 1258.42329 (Δm 0.00822, error 6.5 ppm).



^{31}P-NMR (121 MHz, $CDCl_3$) of **6**.



FT-ICR ESI-MS of **6**.