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Supporting Information

Expanding the Scope of 2′-SCF_3 Modified RNA

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Contents

1. Synthesis of 2'-deoxy-2'-trifluoromethylthioadenosine building block A9.

General. ^{1}H, ^{13}C, ^{31}P and ^{19}F NMR spectra were recorded on a Bruker DRX 300 MHz or Avance II+ 600 MHz instrument. Chemical shifts are referenced to the deuterated solvents ($CDCl_3$ (7.26 ppm - ^{1}H; 77.1 ppm - ^{13}C); d_6-DMSO (2.5 ppm - ^{1}H; 39.5 ppm - ^{13}C); ^{31}P shifts are relative to external 85 % phosphoric acid, ^{19}F shifts are relative to external CCl_3F). Assignment of ^{1}H, ^{13}C spectra were carried out using COSY and HSQC experiments. MS experiments were performed on a Finnigan LCQ Advantage MAX ion trap instrument. Unless specified, all reactions were carried out at room temperature and under argon atmosphere. POLYGRAM® SIL G/UV_{254} pre-coated polyester sheets (0.2 mm silica gel with fluorescent indicator) were used for thin layer chromatography. The nucleosides were visualized at 254 nm. Column chromatography was executed on silica gel purchased from Fluka (pore size 60 Å, 70 – 230 mesh, 63 – 200 µm). 1 % Triethylamine was added packing silica gel columns. Chemical reagents and solvents were purchased from commercial suppliers and used without further purification. Organic solvents for reactions were dried overnight over freshly activated molecular sieves (4 Å).

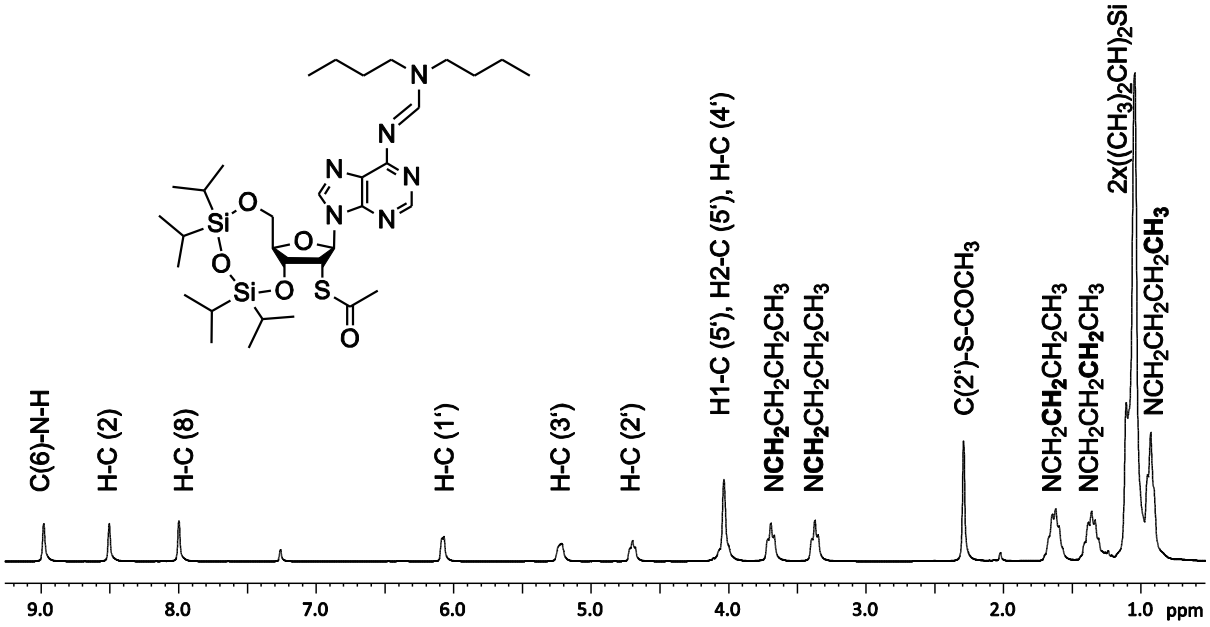
***N*^{6}-[(Dibutylamino)methylene]-3',5'-*O*-(1,1,3,3-tetraisopropylsiloxane-1,3-diyl) (β-D-arabino-furanosyl)adenine (A2).** β-D-Arabinofuranosyladenine (2.35 g, 8.79 mmol) was coevaporated three times with pyridine. DMF/pyridine (1/1, 50 mL) was added resulting a suspension to which 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (3.48 mL, 10.87 mmol) was added dropwise. The suspension turned into a transparent solution during stirring for 24 h. The solvents were removed under reduced pressure, the residue dissolved in dichloromethane and sequentially washed with aqueous $NaHCO_3$ (5%) and brine, dried over Na_2SO_4, evaporated, and dried under vacuum yielding **A1** as colorless oil which was used for the next step without further purification. TLC (6% CH_3OH in dichloromethane) R_f = 0.45. Nucleoside **A1** (4.85 g, 9.51 mmol) was dissolved in DMF (53 mL), *N*,*N*-dibutylformamid-dimethylacetal (4.57 g, 22.5 mmol) was added and the solution was stirred for 26 h. After that time, the DMF was removed under reduced pressure yielding a yellow oil, which was purified by column chromatographic purification on SiO_2 (0.5–3.5% CH_3OH in dichloromethane) yielding **A2** (5.20 g, 96% over two steps) as a colorless foam. TLC (6% CH_3OH in dichloromethane) R_f = 0.24. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.95 (m, 6H, $N(CH_2CH_2CH_2CH_3)_2$); 1.04 - 1.09 (m, 28H, 2x $((CH_3)_2CH)_2Si$); 1.34 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 1.63 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 3.37 (t, *J* = 6.95 Hz, 2H, $N(CH_2CH_2CH_2CH_3)_2$); 3.57 (m, 1H, H-C(3')); 3.94 (m, 2H, $N(CH_2CH_2CH_2CH_3)_2$; 3.99 (m, 3H, H1-C(5'), H2-C(5'), H-C(4')); 4.64 (m, 1H, H-C(2')); 5.46 (s, br, HO-C(2')); 6.16 ((d, *J* = 4.89 Hz, H-C(1')); 8.02 (s, 1H, H-C(8)); 8.30 (s, 1H, H-C(2)); 8.79 (s, 1H, H-C=N-C(6)) ppm. ESI-MS (m/z): $[M+H]^+$ calcd for $C_{31}H_{57}N_6O_5Si_2$ 649.38, found 649.42.

***N*^{6}-[(Dibutylamino)methylene]-3',5'-*O*-(1,1,3,3-tetraisopropylsiloxane-1,3-diyl)-2'-*O*-(trifluoro-methanesulfonyl) (β-D-arabinofuranosyl)adenine (A3).** Compound **A2** (5.43 g, 8.37 mmol) was dissolved in dry dichloromethane (100 mL) and the solution was cooled to 0°C. Then, 4-(*N*,*N*-dimethylamino)pyridine (3.08 g, 25.2 mmol) followed by trifluoromethanesulfonyl chloride (1.33 mL; 12.5 mmol) were added, and the solution was stirred for 30 min. After that time, the yellow solution was washed with 5% aqueous $NaHCO_3$ solution, dried over Na_2SO_4, evaporated, and dried under vacuum. The resulting compound **A3** was used for the next step without further purification. TLC (6% CH_3OH in dichloromethane) R_f = 0.53.

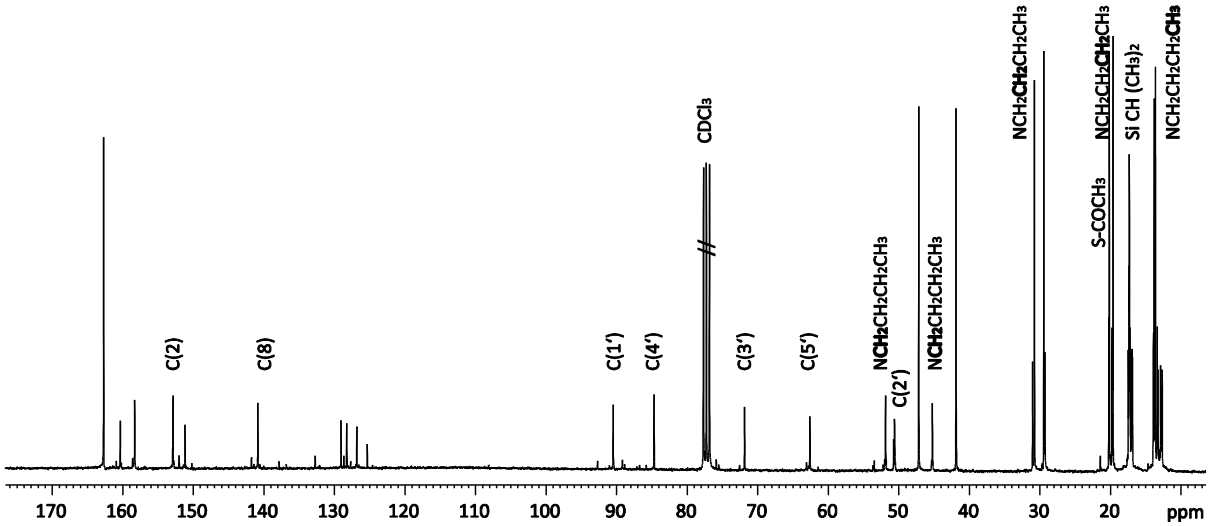
***N*^{6}-[(Dibutylamino)methylene]-3',5'-*O*-(1,1,3,3-tetraisopropylsiloxane-1,3-diyl)-2'-acetylthio-2'-deoxyadenosine (A4).** Nucleoside **A3** (6.53 g, 8.36 mmol) was dissolved in toluene (190 mL). Potassium thioacetate (1.43 g, 12.6 mmol) and 18-crown-6 (3.32 g, 12.5 mmol) were added, and the sudpension stirred at 45°C for 17 h, yielding a yellow solution. The solvents were removed under reduced pressure and the crude product purified by column chromatography on SiO_2 (3.5/6.5–8/2 hexan/ethylacetate v/v) yielding **A4** as yellow oil (5.49 g, 7.764 mmol, 93% over two steps). TLC (6% CH_3OH in dichloromethane) R_f = 0.51. ^{1}H-NMR (300 MHz, $CDCl_3$): *δ* 0.93 (t, 6H, $N(CH_2CH_2CH_2CH_3)_2$); 1.05–1.11 (m, 28H, 2x $((CH_3)_2CH)_2Si$); 1.36 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 1.62 (m, 4H,

N($CH_2CH_2CH_2CH_3$)$_2$); 2.29 (s, 3H, $SCOCH_3$); 3.37 (t, 4H, N($CH_2CH_2CH_2CH_3$)$_2$); 3.69 (t, 4H, N($CH_2CH_2CH_2CH_3$)$_2$); 4.04 (m, 3H, H1-C(5'),H2-C(5'), H-C(4')); 4.70 (triplettoid, 1H, H-C(2')); 5.22 (m, 1H, H-C(3')); 6.07 (dd, 1H, H-C(1')); 8.00 (s, 1H, H-C(8)); 8.51 (s,1H, H-C(2)); 8.98 (s, 1H, C(6)N=C-H) ppm. ^{13}C- NMR (75 MHz, $CDCl_3$): δ 13.65, 13.82 (N($CH_2CH_2CH_2CH_3$)$_2$); 17.34 (2x ((CH_3)$_2$CH)$_2$Si); 19.67, 20.20 (N($CH_2CH_2CH_2CH_3$)$_2$); 29.46 (N($CH_2CH_2CH_2CH_3$)$_2$), 45.26, 51.90 (N($CH_2CH_2CH_2CH_3$)$_2$); 50.62 (C(2')); 62.62 (C(5')); 71.88 (C(3')); 84.71 (C(4')); 90.50 (C(1')); 140.84 (C(8)); 152,87 (C(2)) ppm. ESI-MS (m/z): $[M+H]^+$ calcd $C_{33}H_{58}N_6O_5SSi_2$ 707.37, found 707.42.

^{1}H-NMR (300 MHz, $CDCl_3$) of **A4**



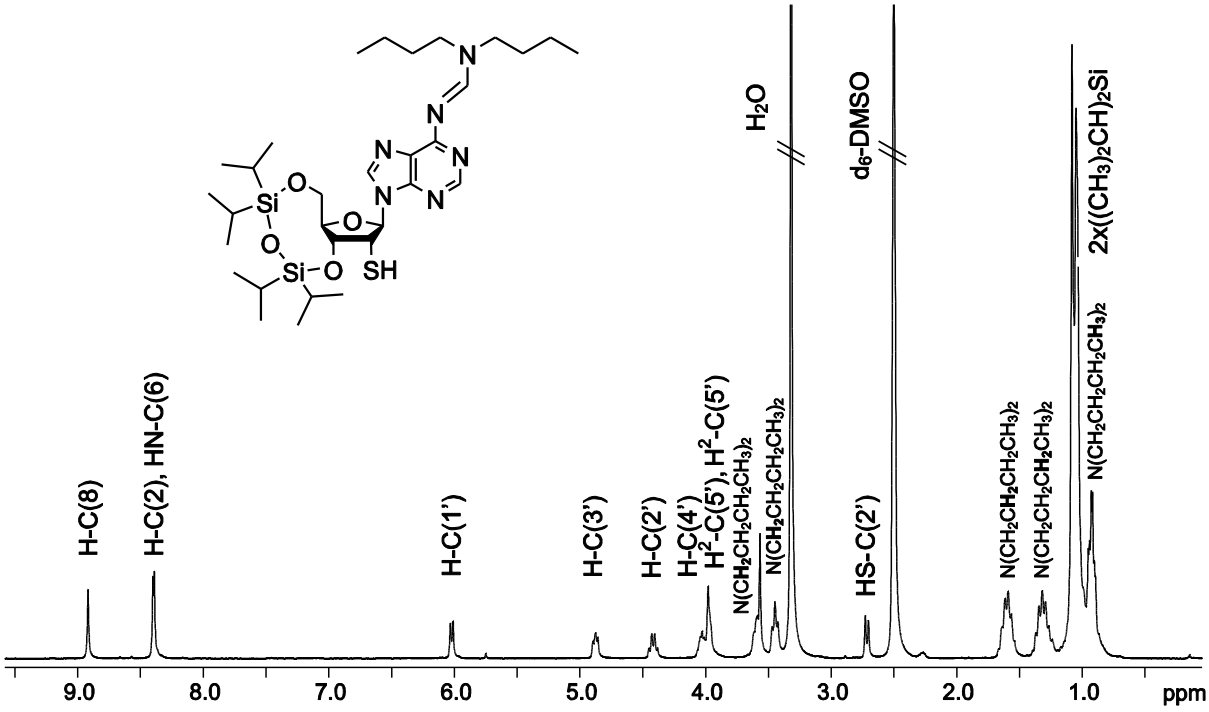
^{13}C-NMR (75 MHz, $CDCl_3$) compound **A4**



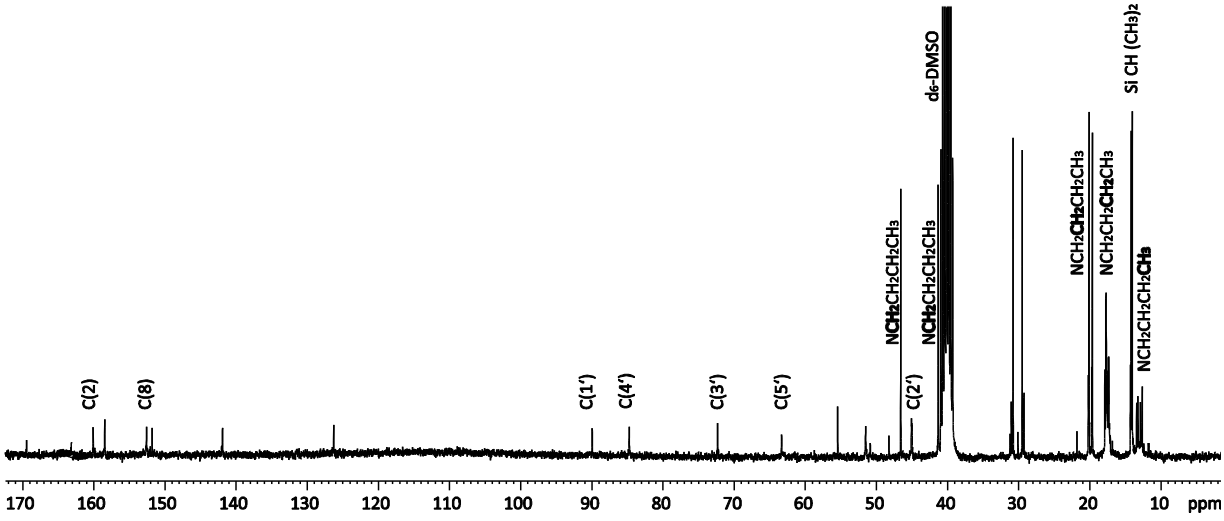
***N*^{6}-[(Dibutylamino)methylene]-3',5'-*O*-(1,1,3,3-tetraisopropylsiloxane-1,3-diyl)-2'-sulfanyl-2'-deoxyadenosine (A5).** Nucleoside **A4** (0.20 g, 0.28 mmol) was dissolved in ethanol/pyridine (1/1, 20 mL) and the solution was cooled to 0°C. A solution of 1 M NaOH in Ethanol/H_2O (1/1, 2.86 mL) was added. After 10 min, DOWEX® was added until the pH value of the solution became neutral. DOWEX® was removed by filtration, the solvents were evaporated, and the crude product purified by column chromatography on SiO_2 (0.5–2.5% CH_3OH in dichloromethane) yielding **A5** as colorless oil (140 mg, 0.211 mmol, 75%). TLC (6% CH_3OH in dichloromethane) R_f = 0.42. ^{1}H NMR (300 MHz, d_6-DMSO): δ

0.93 (t, 6H, $N(CH_2CH_2CH_2CH_3)_2$); 1.08 (m, 28H, 2x $((CH_3)_2CH)_2Si$); 1.30–1.32 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 1.60 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 2.72 (d, 1H, C(2')SH); 3.45 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 3.57 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 3.98(m, 2H, H1-C(5'),H2-C(5')); 4.03 (m, 1H, H-C(4')); 4.41–4.43 (dd, 1H, H-C(2')); 4.88 (triplettoid, 1H, H-C(3')); 6.03 (dd, 1H, H-C(1')); 8.40 (s, 2H, H-C(8), C(6)N-H); 8.92(s, 1H, H-C(2)) ppm. ^{13}C NMR (75 MHz, d_6-DMSO): δ 12.67 $(N(CH_2CH_2CH_2CH_3)_2)$; 14.06 (2x $((CH_3)_2CH)_2Si$); 17.77 $(N(CH_2CH_2CH_2CH_3)_2)$; 20.15 $(N(CH_2CH_2CH_2CH_3)_2)$, 40.26, 46.50 $(N(CH_2CH_2CH_2CH_3)_2)$; 45.11 (C(2')); 63.68 (C(5')); 72.36 (C(3')); 84.91 (C(4')); 90.30 (C(1')); 153.30 (C(8)); 160.73 (C(2)) ppm. ESI-MS (m/z): $[M+H]^+$ calcd for $C_{31}H_{57}N_6O_4SSi_2$ 665.36, found 665.44.

^{1}H NMR (300 MHz, d_6-DMSO) of **A5**



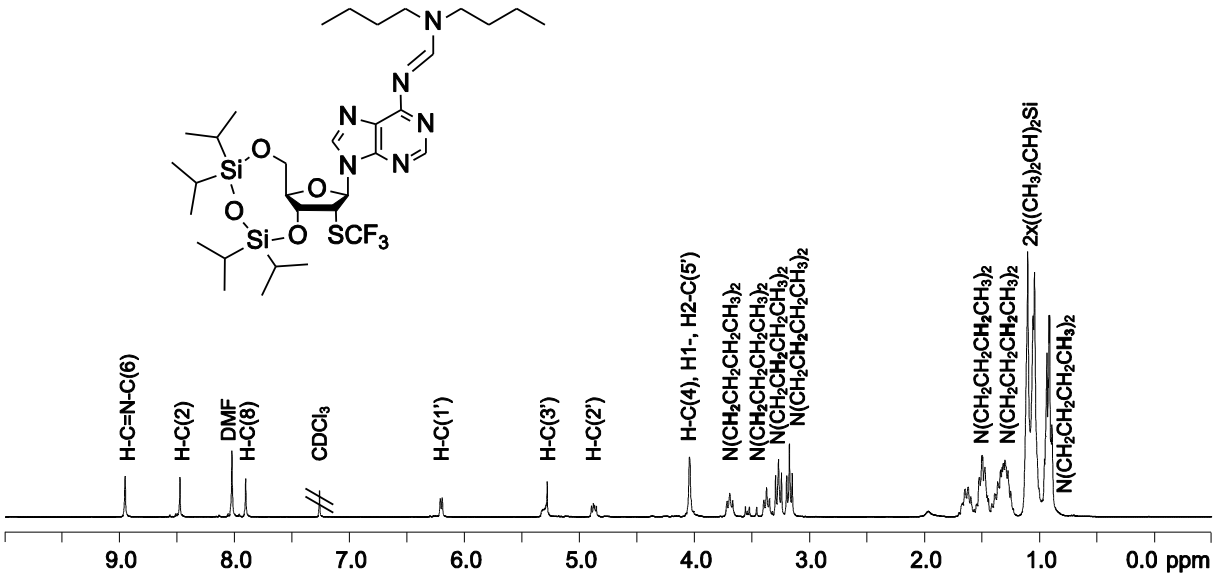
^{13}C NMR (75 MHz, d_6-DMSO) of **A5**



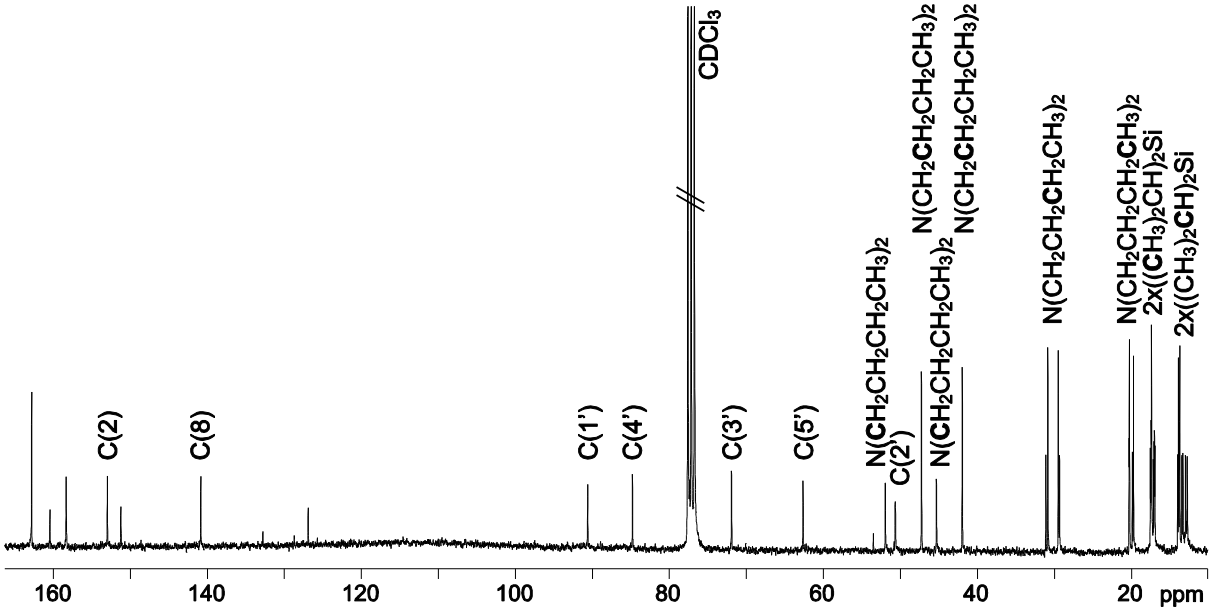
***N*^{6}-[(Dibutylamino)methylene]-3',5'-*O*-(1,1,3,3-tetraisopropylsiloxane-1,3-diyl)-2'-trifluoro-methylthio-2'-deoxyadenosine (A6).** Compound **A5** (1.50 g, 2.26 mmol) was dissolved in dichloromethane (45 mL) and the solution was cooled to –78°C. 3,3-Dimethyl-1-(trifluoromethyl)-1,2-benziodoxole (Togni's reagent; 0.89 g, 2.70 mmol) was added as solid. The stirred solution was

allowed to warm to room temperature and stirring continued for 16 h. The solvents were evaporated and the crude product purified by column chromatography on SiO_2 (0.5–2.0% CH_3OH in dichloromethane) yielding **A6** (1.48 g, 85%) as slightly yellow oil. TLC (6% CH_3OH in dichloromethane v/v) R_f = 0.67. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.91–0.92 (m, 6H, $N(CH_2CH_2CH_2CH_3)_2$); 1.05–1.10 (m, 28H, 2x $((CH_3)_2CH)_2Si$); 1.31 (m, 2H, $N(CH_2CH_2CH_2CH_3)_2$); 1.50 (m, 2H, $N(CH_2CH_2CH_2CH_3)_2$); 3.17 (t, *J* = 7.11 Hz, 2H, $N(CH_2CH_2CH_2CH_3)_2$); 3.27 (t, *J* = 7.44, 2H, $N(CH_2CH_2CH_2CH_3)_2$); 3.37 (t, *J* = 7.28 Hz, 2H, $N(CH_2CH_2CH_2CH_3)_2$); 3.69 (t, *J* = 7.47 Hz, 2H, $N(CH_2CH_2CH_2CH_3)_2$); 4.04 (s, 3H, H1-C(5'), H2-C(5'), H-C(4')); 4.88 (dd, *J* = 7.79, 4.94 Hz, 1H, H-C(2')); 5.28 (m, 1H, H-C(3')); 6.20 (d, *J* = 4.89 Hz, 1H, H-C(1')); 7.90 (s, 1H, H-C(8)); 8.48 (s, 1H, H-C(2)); 8.95 (s, 1H, H-C=N-C(6)) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.76–17.57, (2x $((CH_3)_2CH)_2Si$); 13.91, 20.29 $(N(CH_2CH_2CH_2CH_3)_2)$; 29.53, 30.88 $(N(CH_2CH_2CH_2CH_3)_2)$; 41.99, 47.29 $(N(CH_2CH_2CH_2CH_3)_2)$; 45.35, 51.99 $(N(CH_2CH_2CH_2CH_3)_2)$; 50.69 (C(2')); 62.66 (C(5')); 71.92 (C(3')); 84.78 (C(4')); 90.60 (C(1')); 126.90 (C(5)); 140.38 (C(8)); 152.97 (C(2)) ppm. ^{19}F NMR (565 MHz, $CDCl_3$): δ –39.30 ppm. ESI-MS (m/z): $[M+H]^+$ calcd for $C_{32}H_{56}F_3N_6O_4SSi_2$ 733.35, found 733.37.

^{1}H NMR (300 MHz, $CDCl_3$) compound **A6**

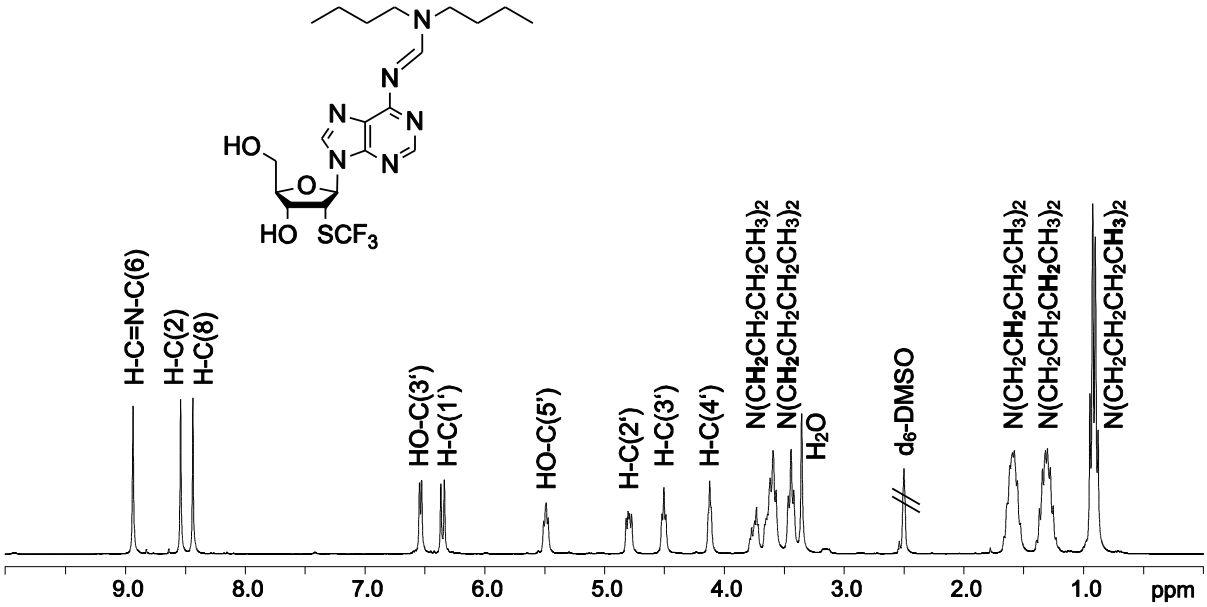


^{13}C NMR (75 MHz, $CDCl_3$) compound **A6**

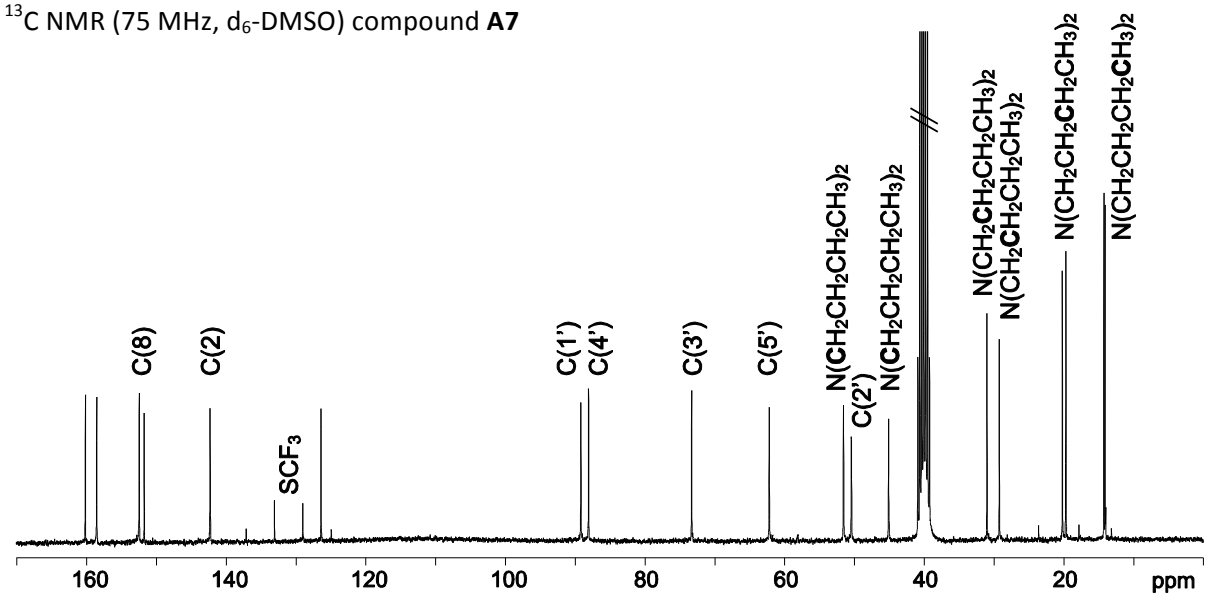


***N*^{6}-[(Dibutylamino)methylene]-2'-trifluoromethylthio-2'-deoxy-adenosine (A7).** Compound **A6** (2.80 g, 3.82 mmol) was dissolved in a solution of tetrabutylammoniumfluoride trihydrate (1 M in THF, 8 mL) and acetic acid (1 M in water, 8 mL) and stirred for one hour. Then, the solvents were removed under reduced pressure. The remaining residue was coevaporated with dichloromethane twice to give a yellow oil. The crude product was purified by column chromatography on SiO_2 (1–4% CH_3OH in dichloromethane v/v) yielding **A7** as white foam (1.26 g, 72%). TLC (10% CH_3OH in dichloromethane v/v) R_f = 0.44. ^{1}H NMR (300 MHz, d_6-DMSO): δ 0.91 (m, 6H, $N(CH_2CH_2CH_2CH_3)_2$); 1.31 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 1.58 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 3.35 (t, J = 6.95 Hz, 2H, $N(CH_2CH_2CH_2CH_3)_2$); 3.59 (t, J = 7.37 Hz, 2H, $N(CH_2CH_2CH_2CH_3)_2$, H1-C(5')); 3.73 (m, 1H, H2-C(5')); 4.12 (m, 1H, H-C(4')); 4.50 (m, 1H, H-C(3')); 4.80 (dd, J = 8.66, 5.03 Hz, 1H, H-C(2')); 5.49 (m, 1H, HO-C(5')); 6.35 (d, J = 8.86 Hz, H-C(1')); 6.54 (d, J = 5.10 Hz, 1H, HO-C(3')); 8.44 (s, 1H, H-C(8)); 8.54 (s, 1H, H-C(2)); 8.94 (s, 1H, H-C=N-C(6)) ppm. ^{13}C NMR (75 MHz, d_6-DMSO): δ 14.11, 14.30 (2x $N(CH_2CH_2CH_2CH_3)_2$); 19.73, 20.24 (2x $N(CH_2CH_2CH_2CH_3)_2$); 29.27, 31.05 (2x $N(CH_2CH_2CH_2CH_3)_2$); 45.12, 51.59 (2x $N(CH_2CH_2CH_2CH_3)_2$); 50.46 (C(2')); 62.23 (C(5')); 73.34 (C(3')); 88.10 (C(4')); 89.21 (C(1')); 131.08 (q, J = 306.4 Hz, CF_3); 142.32 (C(2)); 152.49 (C(8)) ppm. ^{19}F NMR (565 MHz, $CDCl_3$) δ –40.09 ppm. ESI-MS (m/z): $[M+H]^+$ calcd for $C_{20}H_{30}F_3N_6O_3S$ 491.20, found 491.20.

^{1}H NMR (300 MHz, d_6-DMSO) compound **A7**

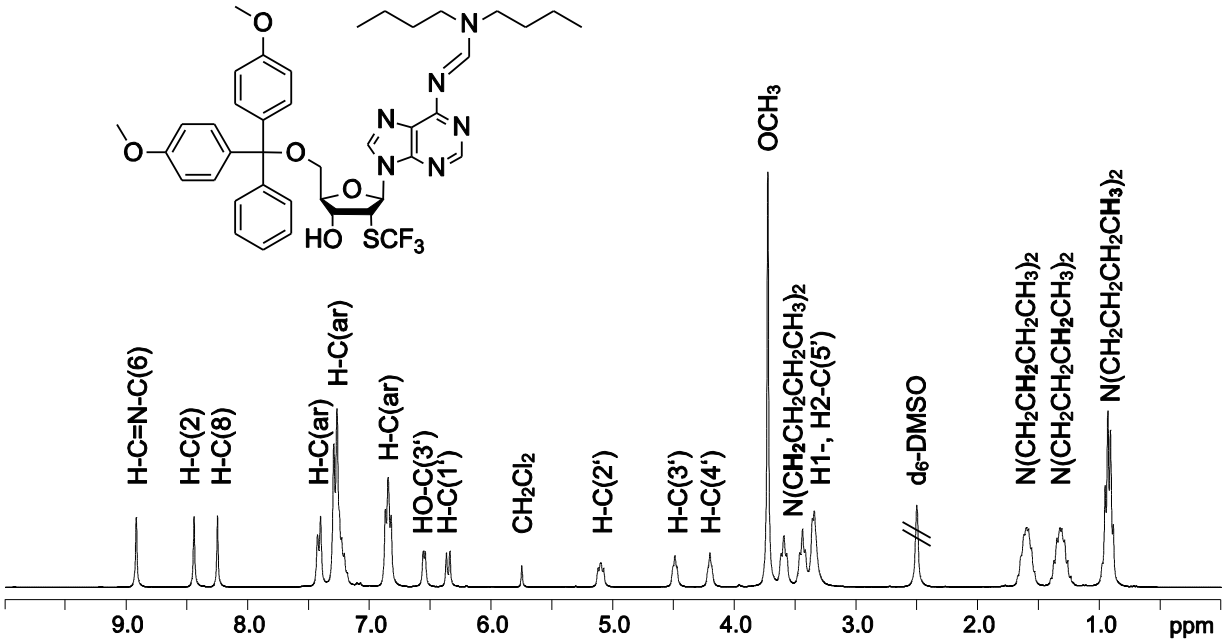


^{13}C NMR (75 MHz, d_6-DMSO) compound **A7**

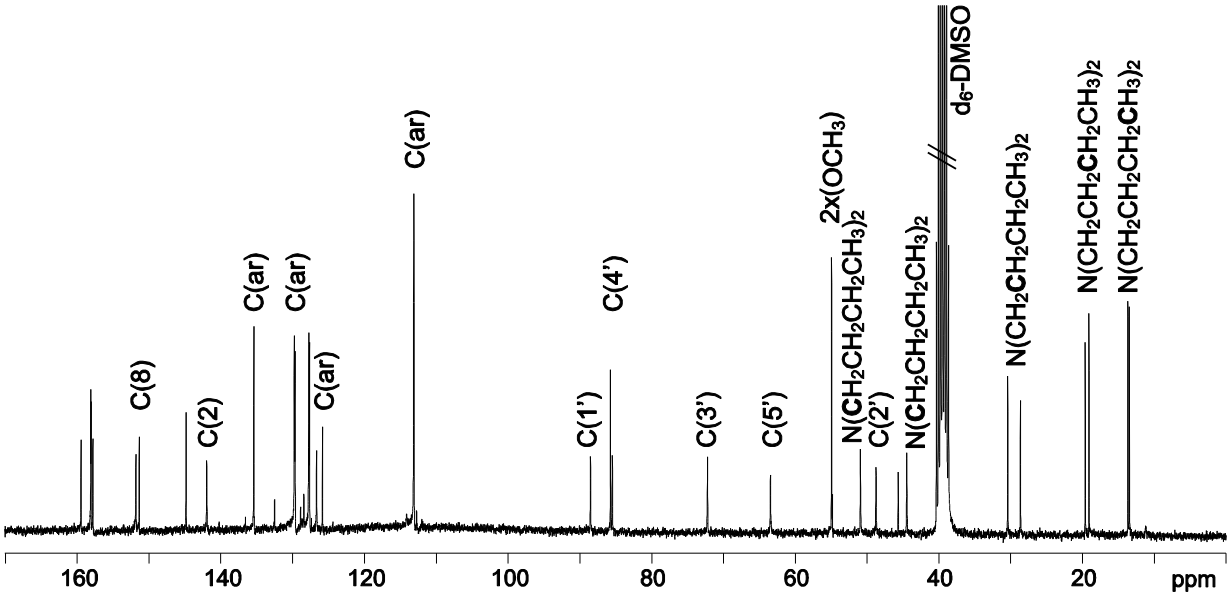


***N*^{6}-[(Dibutylamino)methylene]-2'-trifluoromethylthio-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyadenosine (A8).** Compound **A7** (240 mg, 0.489 mmol) was coevaporated three times with dry pyridine (5 mL) and then dissolved in dry pyridine (2.5 mL). 4-(*N*,*N*-Dimethylamino)pyridine (8 mg, 0.049 mmol) was added. The mixture was treated with 4,4'-dimethoxytritylchloride (185 mg, 0.547 mmol; added in two portions over a period of one hour). Stirring of the yellow solution was continued for 14 h. Methanol (1 mL) was added before the solvents were evaporated, and the remaining residue coevaporated with methanol once more. The yellow foam was dissolved in dichloromethane, washed with aqueous 5% citric acid and saturated aqueous sodium bicarbonate solution, dried over sodium sulfate, and evaporated to give a white foam. The crude product was purified by column chromatography on SiO_2 (1.0–2.5% CH_3OH in dichloromethane v/v) yielding **A8** as white foam (336 mg, 0.424 mmol, 87%). TLC (6% CH_3OH in dichloromethane v/v) R_f = 0.38. ^{1}H NMR (300 MHz, d_6-DMSO): δ 0.91 (m, 6H, N($CH_2CH_2CH_2CH_3$)$_2$); 1.31, 1.32 (m, 4H, N($CH_2CH_2CH_2CH_3$)$_2$); 1.58, 1.60 (m, 4H, N($CH_2CH_2CH_2CH_3$)$_2$); 3.34 (m, 2H, H_2C(5')); 3.44 (t, *J* = 6.68 Hz, 2H, N($CH_2CH_2CH_2CH_3$)$_2$); 3.59 (t, *J* = 7.07 Hz, 2H, N($CH_2CH_2CH_2CH_3$)$_2$); 3.723 (s, 6H, 2x O-CH_3); 4.02 (m, 1H, H-C(4')); 4.49 (triplettoid, 1H, H-C(3')); 5.01 (triplettoid, 1H, H-C(2')); 6.35 (d, *J* = 8.52 Hz, 1H, H-C(1')); 6.55 (d, *J* = 4.71, 1H, HO-C(3')); 6.85 (triplettoid, 4H, H-C(ar)); 7.27, 7.29 (m, 6H, H-C(ar)); 7.40 (m, 3H, 2x H-C(ar), H-C(4ar)); 8.25 (s, 1H, H-C(8)); 8.44 (s, 1H, H-C(2)); 8.92 (s, 1H, H-C=N-C(6)) ppm. ^{13}C NMR (75 MHz, d_6-DMSO): δ 13.05, 13.69 (2x N($CH_2CH_2CH_2CH_3$)$_2$); 19.11, 19.63 (2x N($CH_2CH_2CH_2CH_3$)$_2$); 28.66, 30.43 (2x N($CH_2CH_2CH_2CH_3$)$_2$); 44.49, 50.94 (N($CH_2CH_2CH_2CH_3$)$_2$); 48.77 (C(2')); 54.97 (2x O-CH_3); 63.47 (C(5')); 72.22 (C(3')); 85.72 (C(4')); 88.50 (C(1')); 113.11–135.39 (C(ar)); 130.46 (q, *J* = 306.6 Hz, SCF_3); 141.95 (C(2)); 151.82 (C(8)) ppm. ^{19}F NMR (565 MHz, d_6-DMSO): δ –38.98 ppm. ESI-MS (m/z): $[M+H]^+$ calcd for $C_{41}H_{48}F_3N_6O_5S$ 793.38, found 793.33.

^{1}H NMR (300 MHz, d_6-DMSO) compound **A8**

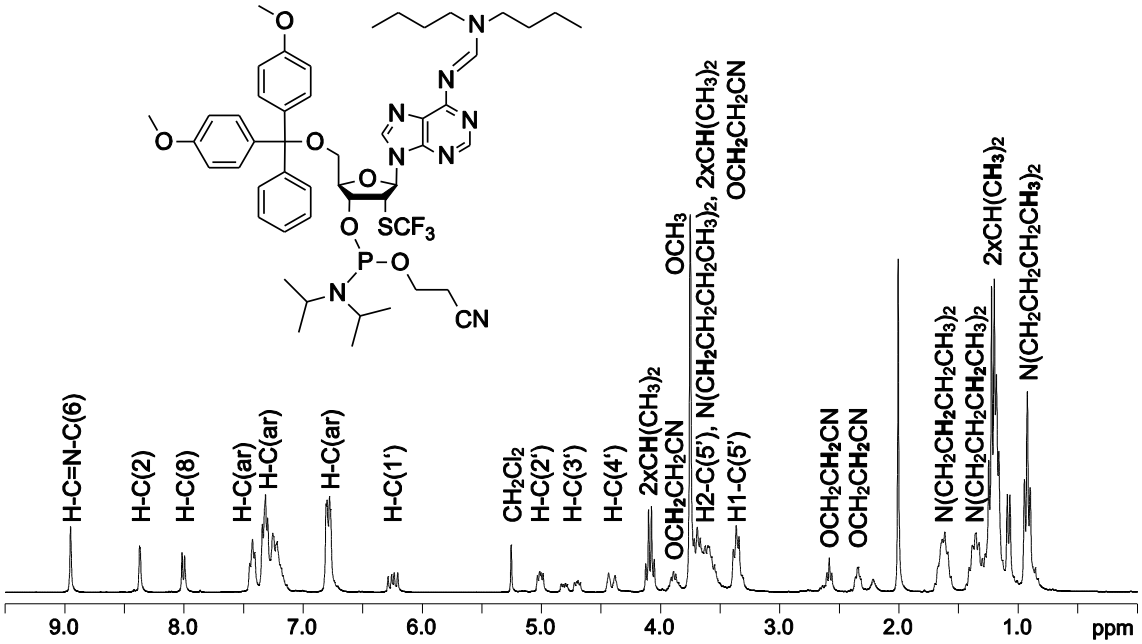


^{13}C NMR (75 MHz, d_6-DMSO) compound **A8**

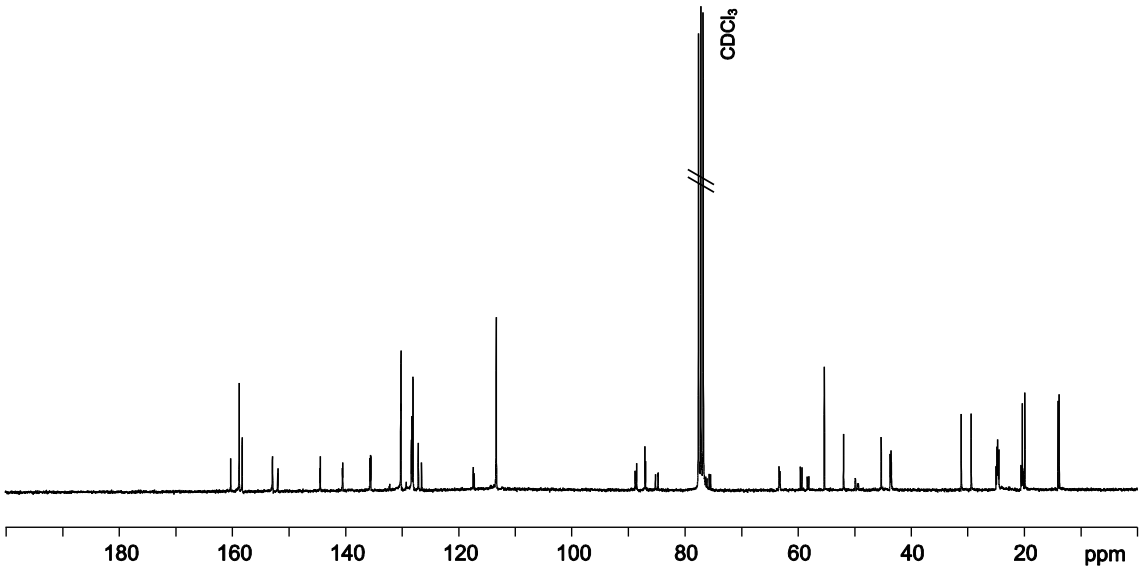


N^6-[(Di-*n*-butylamino)methylene]-2'-trifluoromethylthio-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyadenosine 3'-O-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (A9). Compound **A8** (243 mg, 0.306 mmol) was dissolved in 2 mL dichloromethane, N,N-ethyldimethylamine (330 µL, 3.05 mmol) was added and the solution was stirred for 0.5 hours. The mixture was treated with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (109 mg, 0.461 mmol) for 3.5 hours. After that time the solvents were removed and the crude product purified by column chromatography on SiO_2 (5/5–6/4 ethylacetate/hexane v/v) yielding **A9** as white foam (222 mg, 73%). TLC (5/5 ethylacetate/hexane v/v) R_f = 0.38. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.92 (m, 6H, 2x $N(CH_2CH_2CH_2CH_3)_2$); 1.20, 1.22 (m, 6H, 2x $CH(CH_3)_2$); 1.35 (m, 4H, 2x $N(CH_2CH_2CH_2CH_3)_2$); 1.61 (m, 4H, 2x $N(CH_2CH_2CH_2CH_3)_2$); 2.34, 2.58 (t, J = 6.24, 2H, 2x $CH(CH_3)_2$); 3.37 (m, 3H, H1-C(5'), $N(CH_2CH_2CH_2CH_3)_2$); 3.55–3.69 (m, 6H, OCH_2CH_2CN, $N(CH_2CH_2CH_2CH_3)_2$, 2x $CH(CH_3)_2$); 3.75 (s, 6H, 2x OCH_3); 3.89 (m, OCH_2CH_2CN); 4.09 (m, 2x $CH(CH_3)_2$); 4.38, 4.43 (s, 1H, H-C(4')); 4.69, 4.82 (m, 1H, H-C(3')); 5.00, 5.01 (m, 1H, H-C(2')); 6.22, 6.27 (2x d, J = 9.00, 8.88 Hz, 1H, H-C(1')); 6.80 (m, 4H, H-C(ar)); 7.31 (m, 6H, H-C(ar)); 7.42 (m, 3H, H-C(ar)); 7.99, 8.01 (s, 1H, H-C(8)); 8.37 (s, 1H, H-C(2)) ppm. ^{31}P NMR (121 MHz, d_6-$CDCl_3$): δ 151.19, 152.64 ppm. ^{19}F NMR (565 MHz, $CDCl_3$): δ –39.74, –39.82 ppm. ESI-MS (m/z): $[M+H]^+$ calcd for $C_{50}H_{65}F_3N_8O_6PS$ 993.44, found 993.38.

^{1}H-NMR (300 MHz, $CDCl_3$) compound **A9**



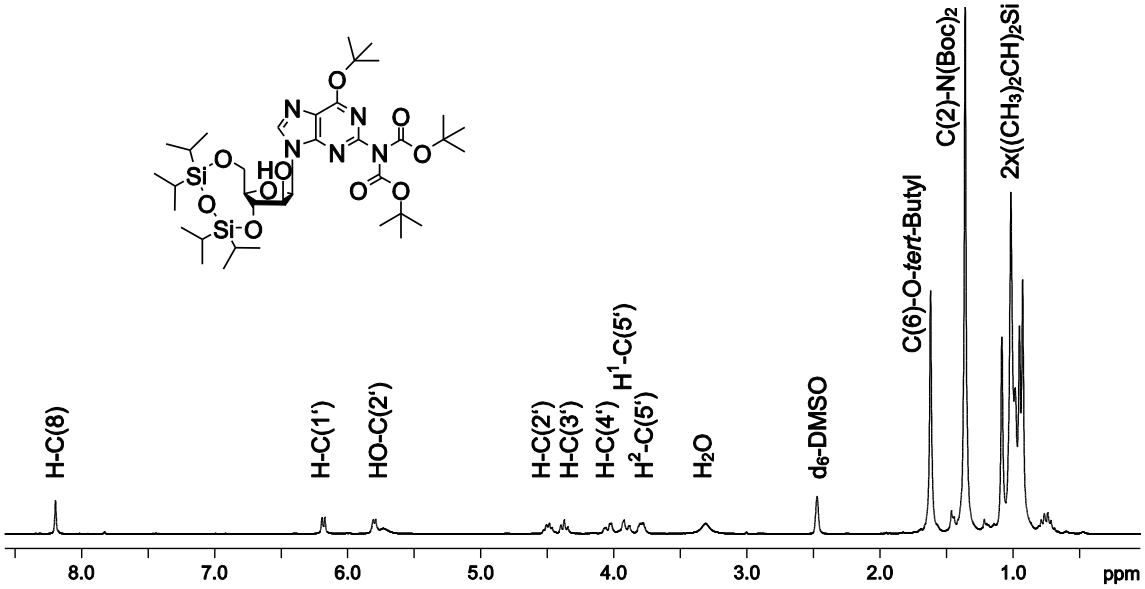
^{13}C-NMR (75 MHz, $CDCl_3$) compound **A9**



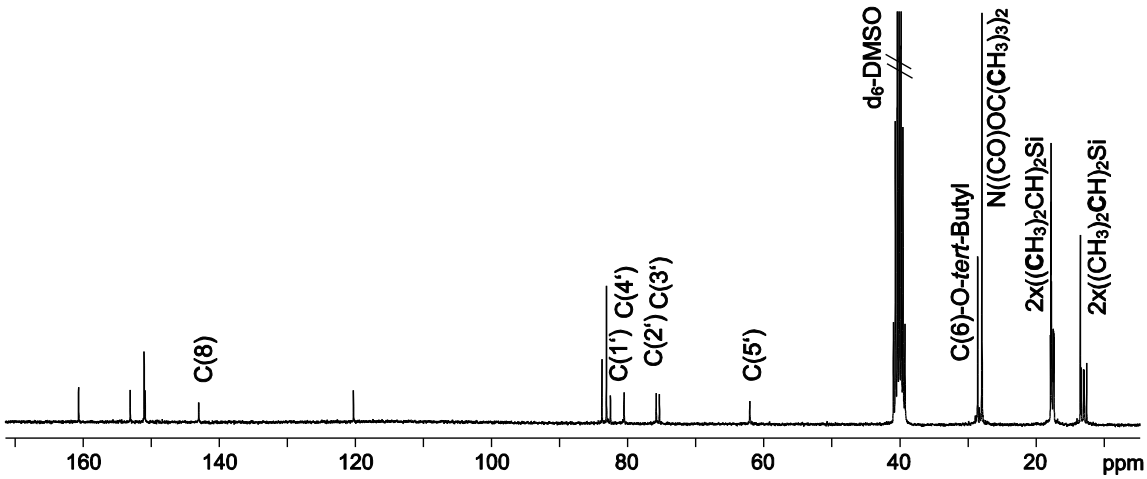
2. NMR spectra of 2'-SCF_3 guanosine building block G7 and the precursors G1 to G6.

***O^6*-*tert*-Butyl-*N,N*-bis(*tert*-butyloxycarbonyl)-3',5'-*O*-(1,1,3,3-tetra*iso*propylsiloxane-1,3-diyl)-arabinoguanosine (G1).**

^{1}H NMR (300 MHz, d_6-DMSO) of compound **G1**

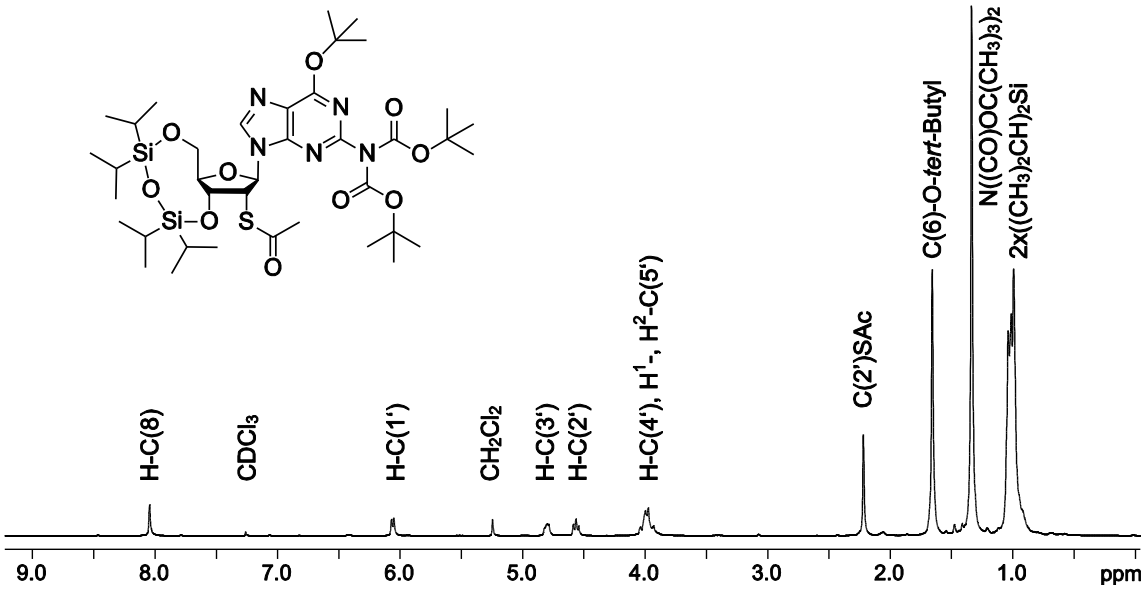


^{13}C NMR (75 MHz, d_6-DMSO) of compound **G1**

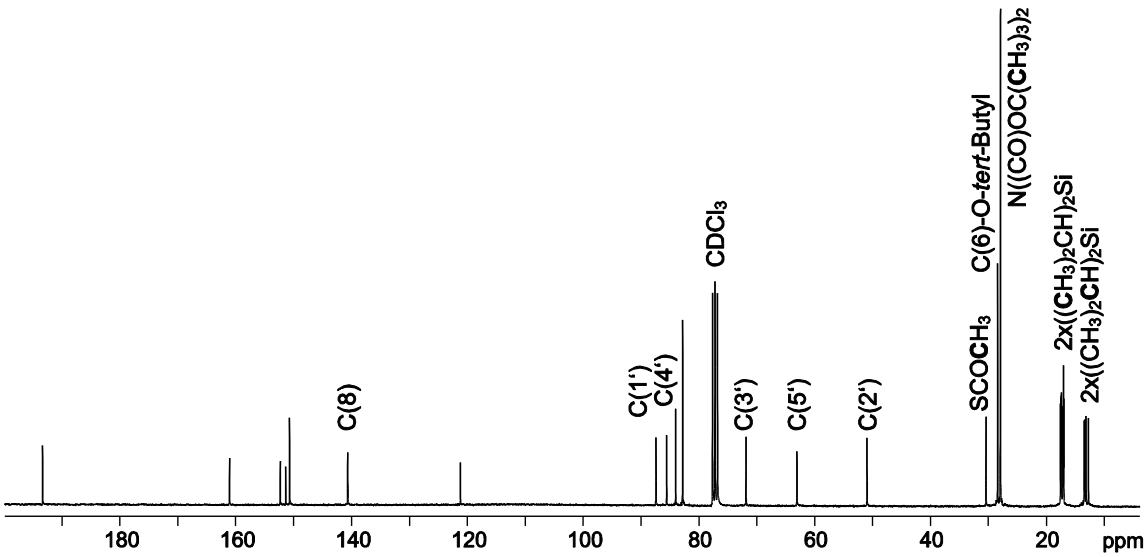


***O*^{6}-*tert*-Butyl-*N,N*-bis(*tert*-butyloxycarbonyl)-3',5'-*O*-(1,1,3,3-tetra*iso*propylsiloxane-1,3-diyl)-2'-acetylthio-2'-deoxyguanosine (G2).**

^{1}H NMR (300 MHz, $CDCl_3$) of **G2**

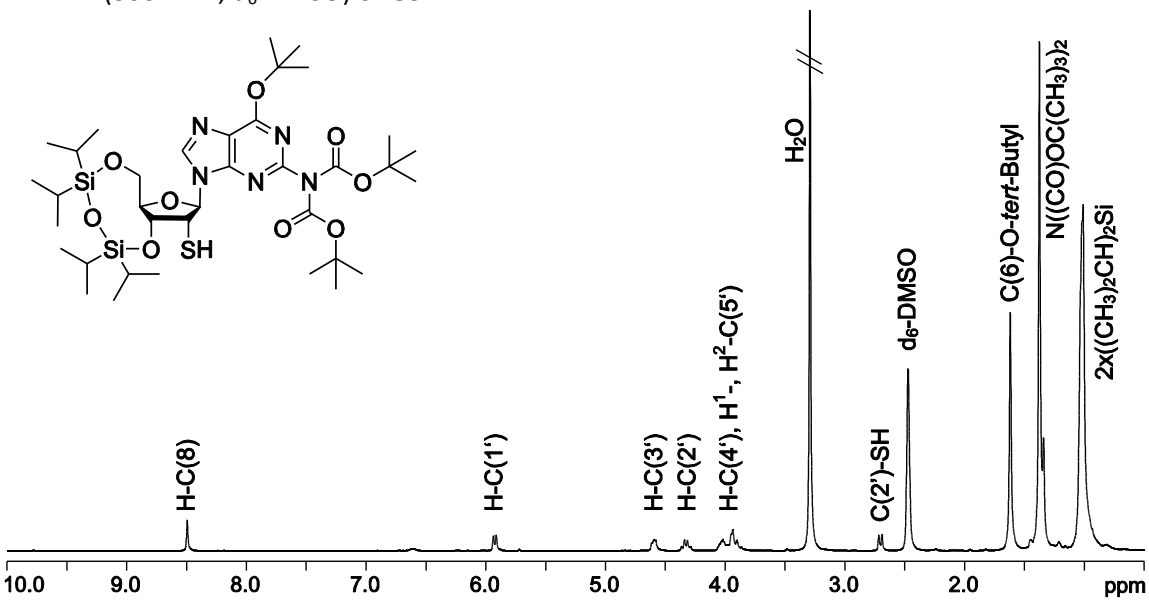


^{13}C NMR (75 MHz, $CDCl_3$) compound **G2**

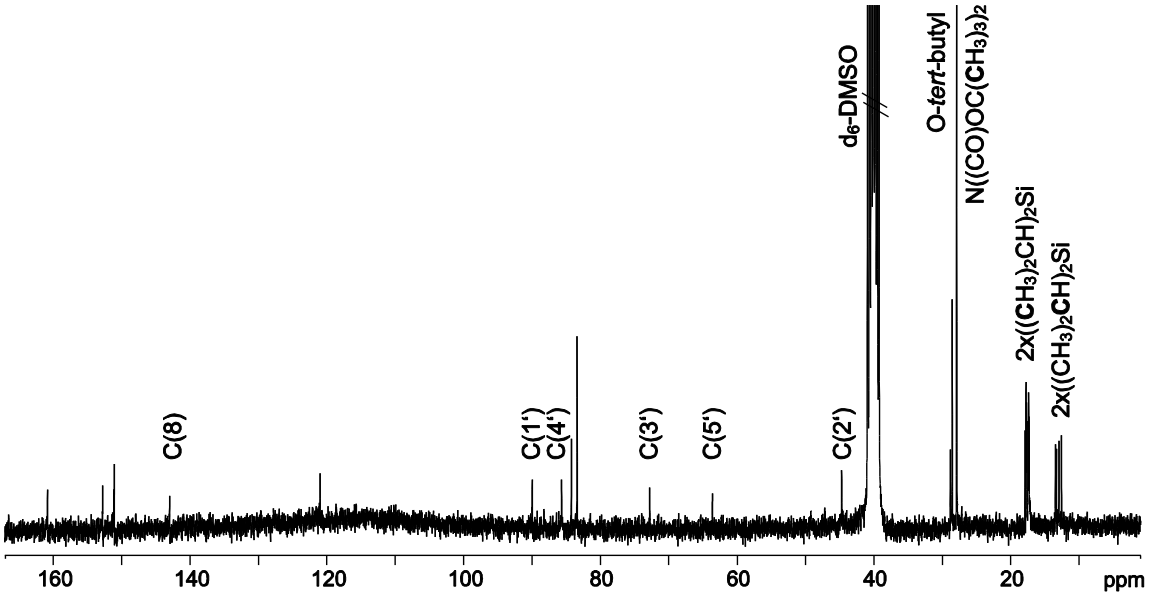


***O*^{6}-*tert*-Butyl-*N,N*-bis(*tert*-butyloxycarbonyl)-3',5'-*O*-(1,1,3,3-tetra*iso*propylsiloxane-1,3-diyl)-2'-sulfanyl-2'-deoxyguanosine (G3)**.

^{1}H NMR (300 MHz, d_6-DMSO) of **G3**

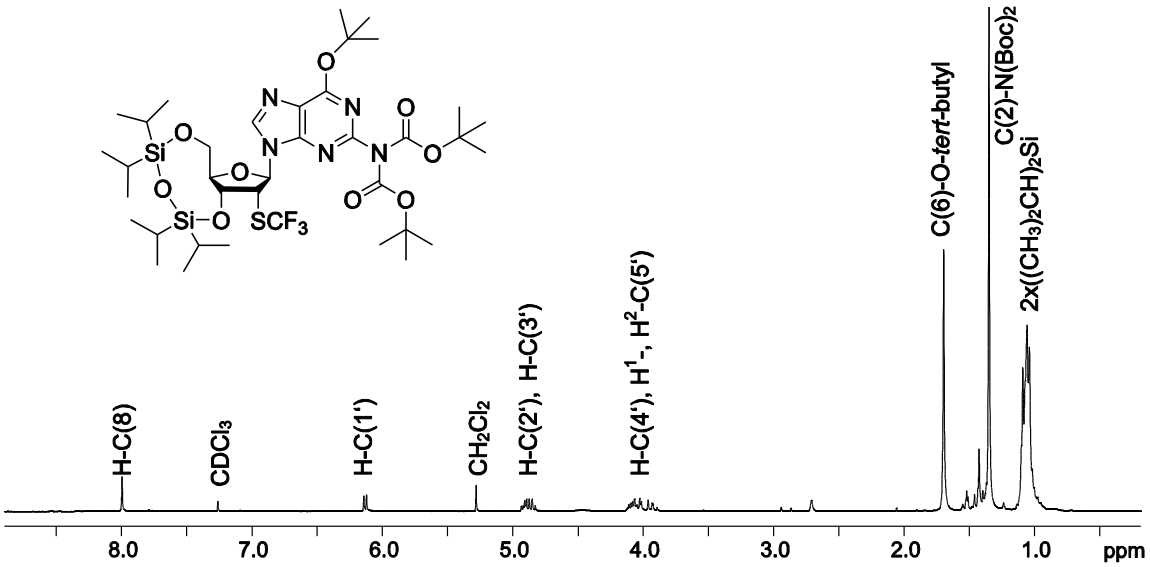


^{13}C NMR (75 MHz, d_6-DMSO) compound **G3**

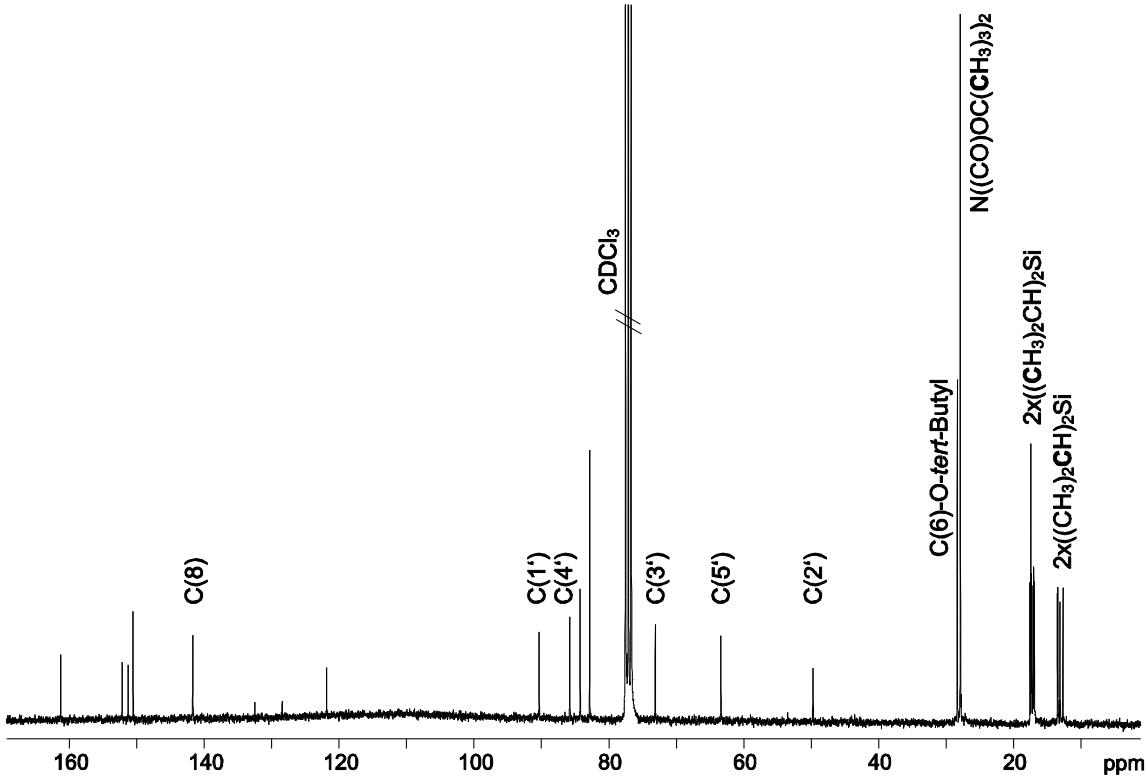


***O*^{6}-*tert*-Butyl-*N,N*-bis(*tert*-butyloxycarbonyl)-3',5'-*O*-(1,1,3,3-tetra*iso*propylsiloxane-1,3-diyl)-2'-trifluoromethylthio-2'-deoxyguanosine (G4)**.

^{1}H NMR (300 MHz, $CDCl_3$) of **G4**

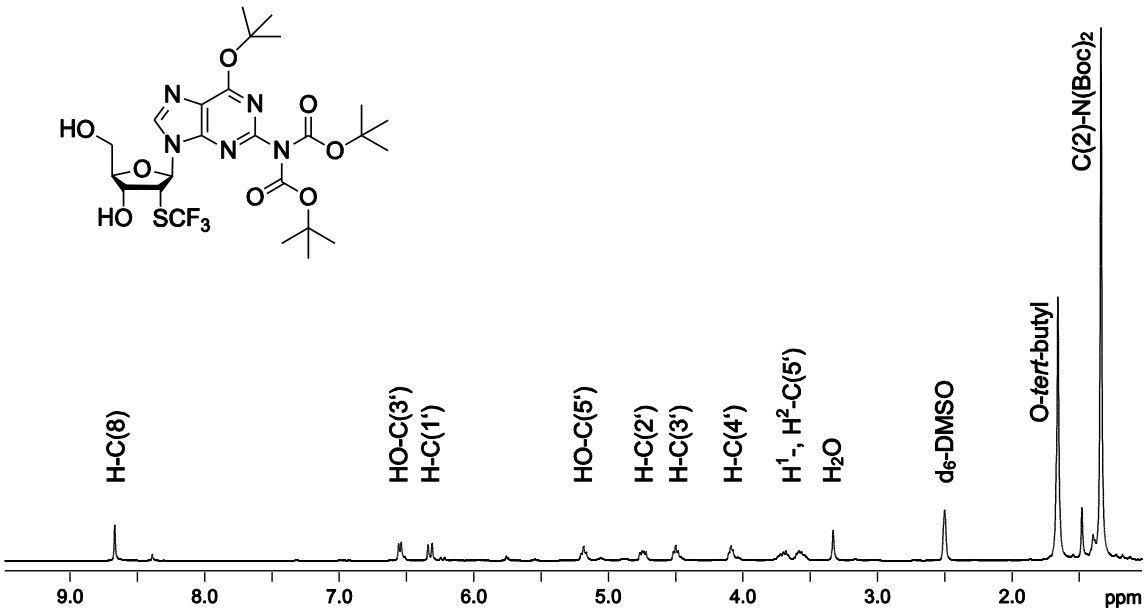


^{13}C NMR (75 MHz, $CDCl_3$) compound **G4**

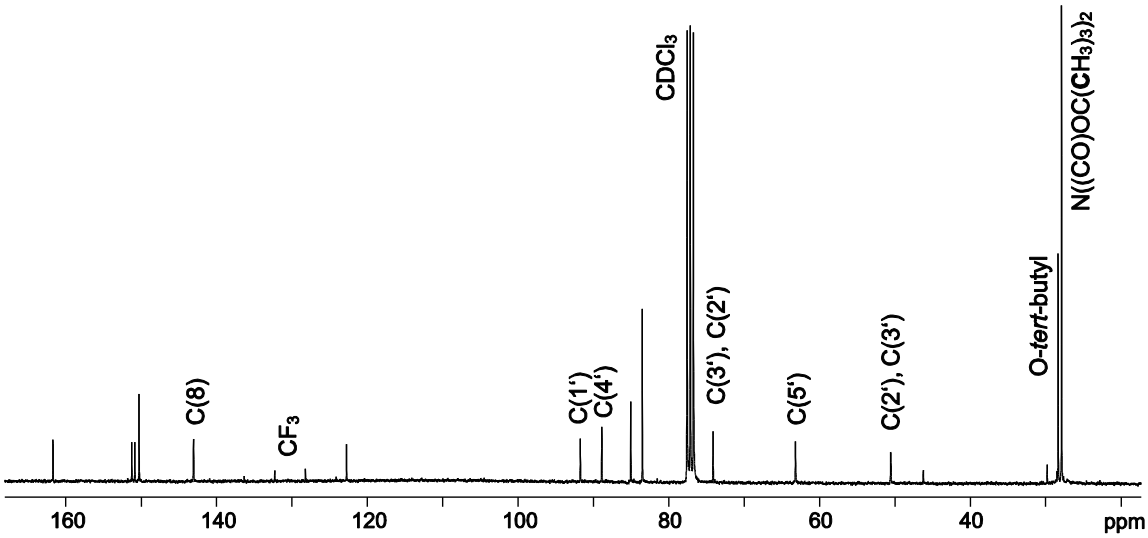


***O*^{6}-*tert*-Butyl-*N,N*-bis(*tert*-butyloxycarbonyl)-2'-trifluoromethylthio-2'-deoxyguanosine (G5)**.

^{1}H NMR (300 MHz, d_6-DMSO) of **G5**

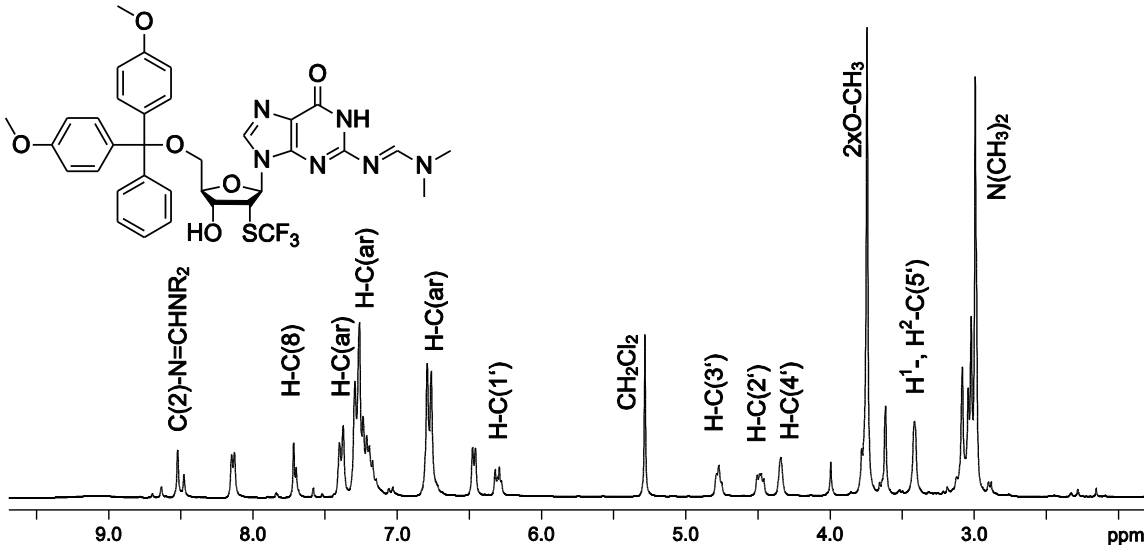


^{13}C NMR (75 MHz, $CDCl_3$) compound **G5**

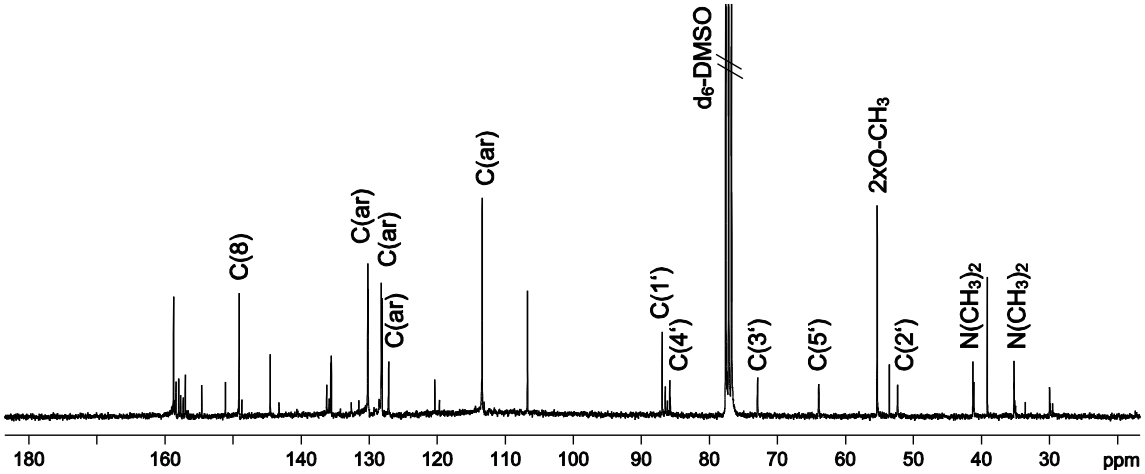


***N*^{2}-[(Dimethylamino)methylene]-2'-trifluoromethylthio-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyguanosine (G6).**

^{1}H NMR (300 MHz, $CDCl_3$) of **G6**

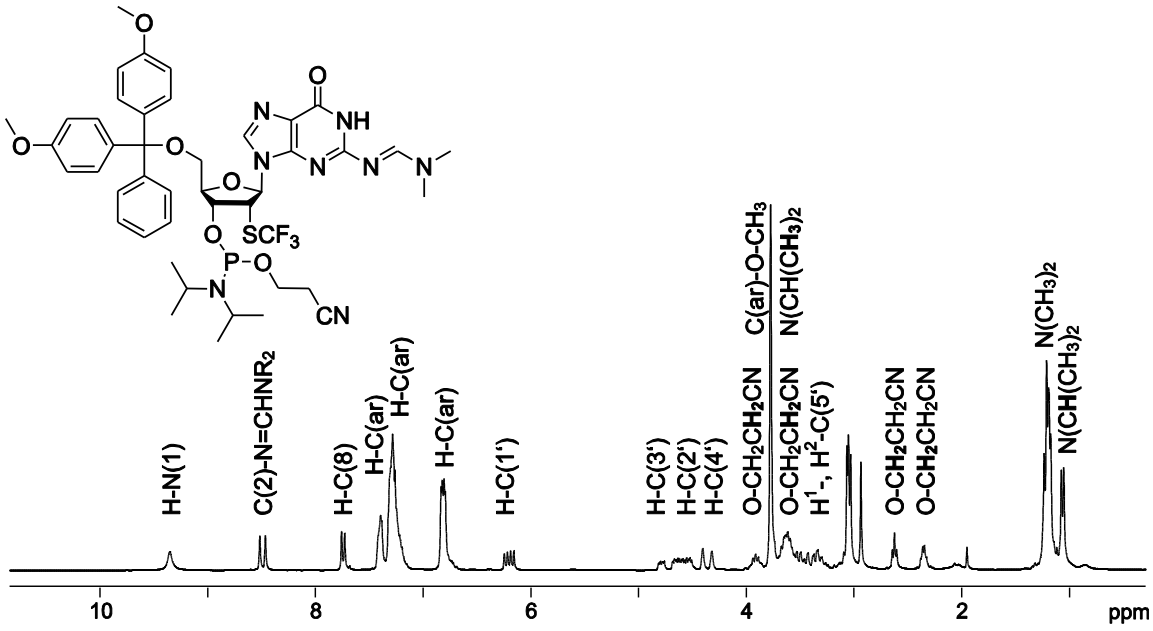


^{13}C NMR (75 MHz, $CDCl_3$) compound **G6**

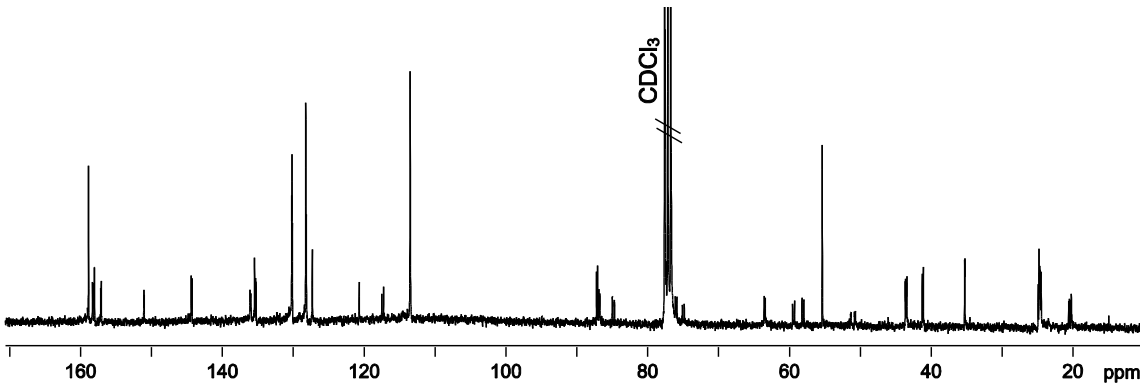


***N*^{2}-[(Dimethylamino)methylene]-2'-trifluoromethylthio-5'-*O*-(4,4'-dimethoxytriphenylmethyl)-2'-deoxyguanosine 3'-*O*-((2-cyanoethyl) *N*,*N*-diisopropylphosphoramidite) (G7).**

^{1}H NMR (300 MHz, $CDCl_3$) of **G7**



^{13}C NMR (75 MHz, $CDCl_3$) compound **G7**



3. Mass spectrometry of 2'-SCF_3 modified RNA

All experiments were performed on a Finnigan LCQ Advantage MAX ion trap instrumentation connected to an Amersham Ettan micro LC system. RNA sequences were analyzed in the negative-ion mode with a potential of -4 kV applied to the spray needle. LC: Sample (200 pmol RNA dissolved in 30 µL of 20 mM EDTA solution; average injection volume: 30 µL); column (Waters XTerra® MS, C18 2.5 µm; 1.0 x 50 mm) at 21°C; flow rate: 30 µL/min; eluant A: 8.6 mM TEA, 100 mM 1,1,1,3,3,3-hexafluoroisopropanol in H_2O (pH 8.0); eluant B: methanol; gradient: 0-100 % B in A within 30 min; UV-detection at 254 nm.

4. ^{19}F and ^{1}H NMR experiments of 2'-SCF_3 modified RNA

The RNA sample was lyophilized and dissolved in 25 mM sodium cacodylate buffer pH 7.0 (450 µL) and D_2O (50 µL) was added to a total volume of 500 µL. RNA concentrations were as indicated in the corresponding figure captions. All samples were heated to 90 °C for 1 min, then rapidly cooled in an ice bath and equilibrated to room temperature for 15 min before measurements. The ^{1}H 1D-NMR spectra were acquired using a double-pulsed field gradient spin-echo pulse sequence. ^{19}F NMR spectra were recorded at a frequency of 564.6 MHz on a Bruker Avance II+ 600 MHz spectrometer equipped with a 5 mm QNP probe with Z-gradient ($^{2}H/^{1}H/^{19}F$). Typical experimental parameters were chosen as follows: spectral width 20 ppm, ^{19}F 90°-pulse 13 µs, acquisition time 1.2 s, relaxation delay 1.5 to 2.0 s. Prior to Fourier transformation all time domain data was processed with an exponential window function using a line broadening factor of 2-4 Hz. ^{19}F resonances are reported relative to external CCl_3F.

5. UV-melting profile experiments and analysis

Absorbance versus temperature profiles were recorded at 250, 260, 270, and 280 nm on a Cary-1 spectrophotometer equipped with a Peltier temperature control device. RNAs were measured at various concentrations ranging from ~1 to 60 µM in buffer solutions of 10 mM Na_2HPO_4, pH 7.0, containing 150 mM NaCl. Data were collected after a complete cooling and heating cycle at a rate of 0.7°C min^{-1}. Melting transitions were reversible and essentially the same with respect to the four different wavelengths. For sample preparation, oligonucleotides were lyophylized to dryness, dissolved in the corresponding buffer from stock solutions and subsequently degassed. A layer of silicon oil was placed on the surface of the solution. ΔH^{vH} and ΔS^{vH} values for biomolecular melting transitions were obtained from plots of T_m^{-1} versus (ln c) plots where ΔH^{vH} and ΔS^{vH} are extracted from the slope and intercept of linear fits to the data. For monomolecular transitions, ΔH^{vH} and ΔS^{vH} were obtained from a two-state van't Hoff analysis by fitting the shape of the individual α versus temperature curve according to the literature (Marky, L. A., Breslauer, K. J. Calculating Thermodynamic Data for Transitions of Any Molecularity from Equilibrium Melting Curves. *Biopolymers* 1987, *26* (9), 1601–1620; Xia, T., McDowell, J. A., Turner, D. H. Thermodynamics of Nonsymmetric Tandem Mismatches Adjacent to G·C Base Pairs in RNA. *Biochemistry* 1997, *36* (41), 12486–12497.

Table S1. Selection of 2'-SCF_3 modified RNAs synthesized in the course of this study.

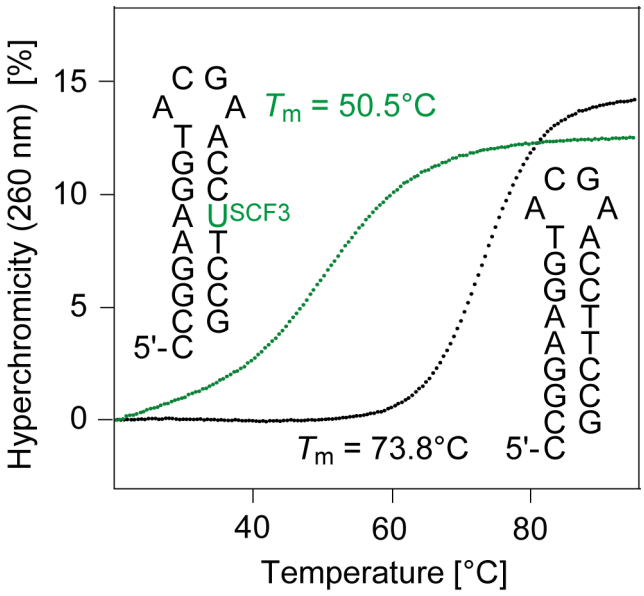
RNA sequence (5'-3')	nt	Isolated total amount [nmol]	M_r calc [amu]	M_r found [amu][1]
UU$A_{2'SCF3}$ GCG	6	505	1959.24	1959.02
UU$A_{2'SCF3}$ GCG	6	393	1959.25	1958.64
UU$G_{2'SCF3}$ UUU	6	778	1898.16	1897.83
GGU CG$A_{2'SCF3}$ CC	8	70	2608.63	2608.69
GGU C$G_{2'SCF3}$A CC	8	286	2608.65	2608.55
CGG AGU $G_{2'SCF3}$AU CCG	12	55	3934.45	3934.26
GAA $G_{2'SCF3}$GG CAA CCU UCG	15	157	4898.05	4898.13
GA$A_{2'SCF3}$ GGG CAA CCU UCG	15	169	4898.05	4897.69
GGU CUC UGC CAA UA $A_{2'SCF3}$ GAC AUU	21	94	6741.12	6740.82
GGU CUC U $G_{2'SCF3}$C CAA UAA GAC AUU	21	87	6741.12	6741.52
UGU CUU AUU $G_{2'SCF3}$GC AGA GAC CUG	21	98	6774.11	6774.52
U$G_{2'SCF3}$U CUU AUU GGC $A_{2'SCF3}$GA GAC CUG	21	135	6858.17	6858.25
U$G_{2'SCF3}$U CUU AUU GGC AGA GAC CUG	21	94	6774.11	6774.55
UGC UCC UAG UAC GAG $A_{2'SCF3}$GG ACC GGA GUG	27	152	8811.39	8811.88
UGC UCC UAG UAC GAG AGG ACC GG$A_{2'SCF3}$ GUG	27	165	8811.39	8811.41
UGC UCC U$G_{2'SCF3}$G UAC GAG AGG ACC GGA GUG	27	73	8811.39	8811.23
UGC UCC UA$G_{2'SCF3}$ UAC GAG AGG ACC GGA GUG	27	159	8811.39	8811.75
UGC UCC UAG UAC GAG AGG ACC $G_{2'SCF3}$GA GUG	27	191	8811.39	8811.76
CUG GGU CGC AGU AAC CCC AGU UAA CAA AAC AAG $G_{2'SCF3}$G	35	101	11356.98	11357.23

[1] determined by LC-ESI mass spectrometry

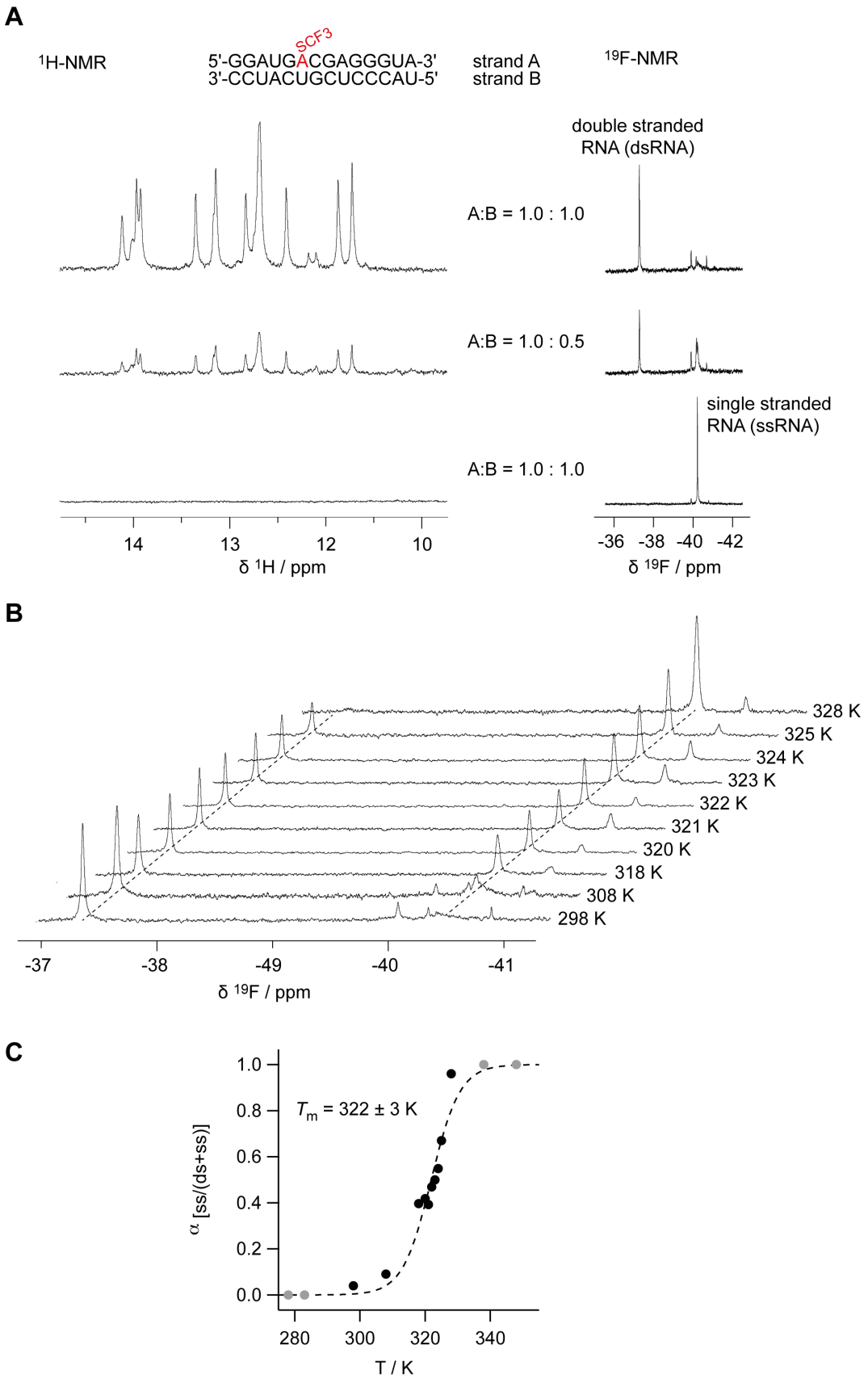
Table S2. Selection of 2'-SCF_3 modified siRNAs investigated in this study.

Synonym	siRNA duplex	Mr calcd [g/mol]	Mr found [g/mol]
SIR3 unmod	5'- G G U C U C U G C C A A U A A G A C A U U -3' 3'- G U C C A G A G A C G G U U A U U C U G U -5'	6657.06 6690.05	6657.39 6690.39
SIR3 2'-SCF3-A15 s	5'- G G U C U C U G C C A A U A A G A C A U U -3' 3'- G U C C A G A G A C G G U U A U U C U G U -5'	6741.12 6690.05	6740.82 6690.39
SIR3 2'-SCF3-G8 s	5'- G G U C U C U G C C A A U A A G A C A U U -3' 3'- G U C C A G A G A C G G U U A U U C U G U -5'	6741.12 6690.05	6741.52 6690.39
SIR3 2'-SCF3-G10 as	5'- G G U C U C U G C C A A U A A G A C A U U -3' 3'- G U C C A G A G A C G G U U A U U C U G U -5'	6657.06 6774.11	6657.39 6774.52
SIR3 2'-SCF3-G2 as	5'- G G U C U C U G C C A A U A A G A C A U U -3' 3'- G U C C A G A G A C G G U U A U U C U G U -5'	6657.06 6774.11	6657.39 6774.55
SIR3 2'-SCF3-G2/A13 as	5'- G G U C U C U G C C A A U A A G A C A U U -3' 3'- G U C C A G A G A C G G U U A U U C U G U -5'	6657.06 6858.17	6657.39 6858.25
SIR3 random	5'- U C U G G G U C U A A G C C A A A C A U T -3' 3'- dG U A G A C C C A G A U U C G G U U U G U -5'	6674.03 6655.06	6674.39 6655.01

A=2'-SCF3 Adenosine, G=2'-SCF3 Guanosine

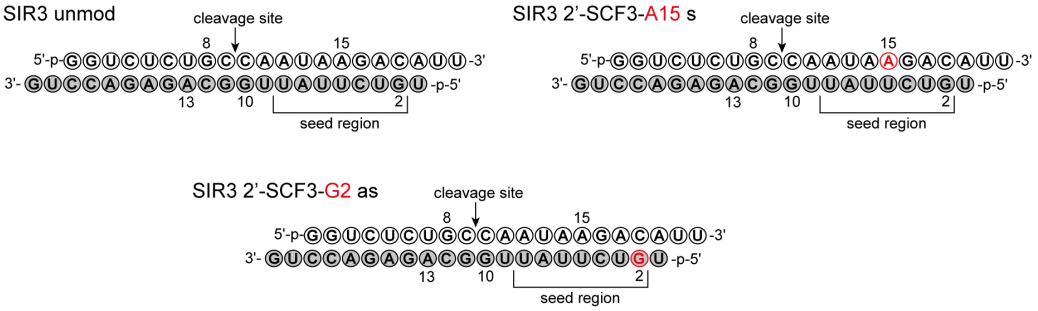


Supporting Figure S1. Thermal stabilities of a 2'-SCF_3 uridine modified DNA hairpin. Overlay of UV melting profiles of modified and unmodified DNA hairpins. Conditions: c_{DNA} = 4 µM; 10 mM Na_2HPO_4, 150 mM NaCl, pH 7.0.

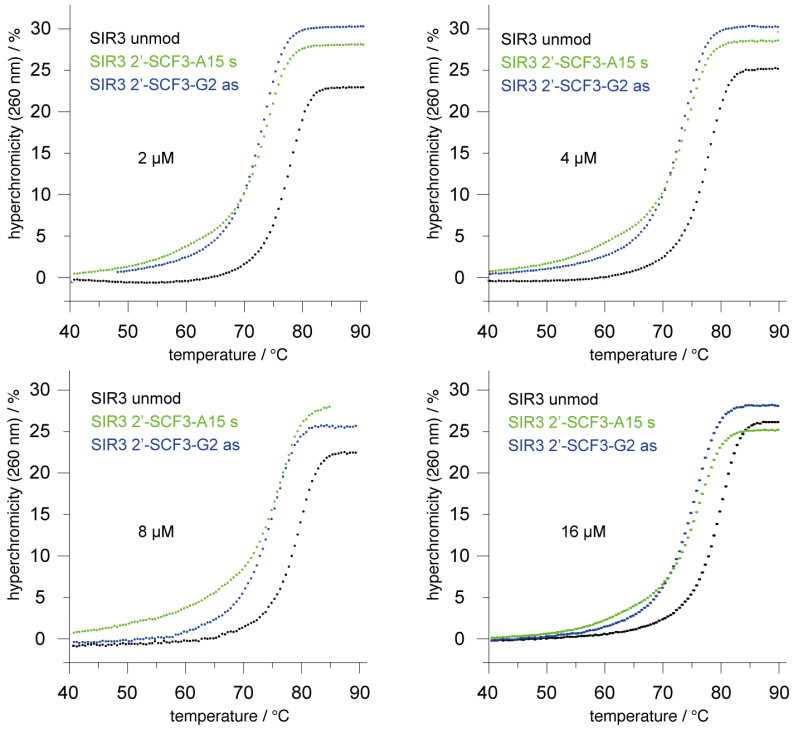


Supporting Figure S2. Duplex formation of 2'-SCF_3 adenosine modified RNA. **A**) Titration experiment followed by 1D ^{1}H and ^{19}F NMR spectroscopy; conditions: c_{RNA} = 0.23 mM, 50 mM Na_2HPO_4, pH 6.5, 298 K; **B**) ^{19}F NMR spectra at different temperatures; **C**) Melting profile and estimated T_m value derived from α/T graph of ∂F.

A



B



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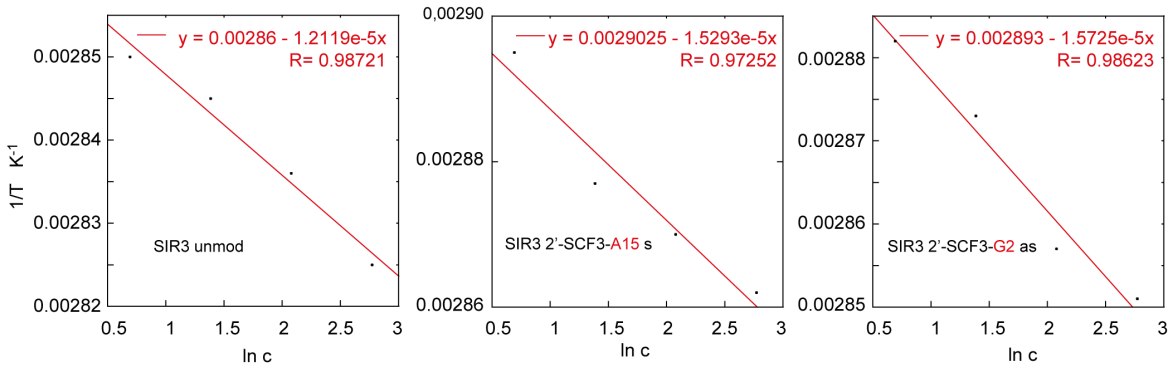

D

siRNA	ΔH°	ΔS°	ΔG°
SIR3 unmod	-164.0	-0.469	-24.2
SIR3 2'-SCF3-A15 s	-129.9	-0.377	-17.5
SIR3 2'-SCF3-G2 as	-126.4	-0.366	-17.4

Supporting Figure S3. Thermodynamic analysis of siRNA duplexes with single 2'-SCF_3 modifications. **A**) RNA sequences; position of 2'-SCF_3 indicated in red. **B**) UV melting profiles of the three siRNA duplexes; concentrations as indicated. **C**) 1/T *versus* ln *c* graphs; **D**) Thermodynamic parameters derived from 1/T *versus* ln *c* graphs. Conditions: c_{RNA} as indicated; 10 mM Na_2HPO_4, 150 mM NaCl, pH 7.0.