

## Supporting Information for

### **2'-Azido RNA – A Versatile Tool for Chemical Biology: Synthesis, X-ray Structure, siRNA Applications, Click Labeling**

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## CONTENTS

### Supporting Methods

Synthesis of 2'-azido cytidine and 2'-azido guanosine nucleosides building blocks ( <b>2 to 7</b> and <b>9 to 17</b> )	2
NMR spectra of 2'-azido cytidine building block <b>7</b> and precursor compound <b>6</b>	11
NMR spectra of 2'-azido guanosine building block <b>17</b> and precursor compound <b>16</b>	13
RNA solid phase synthesis	15
Deprotection of 2'-azido modified RNA	15
Purification of 2'-azido modified RNA	16
Mass spectrometry of 2'-azido modified RNA	16
NMR spectroscopy of 2'-azido modified RNA	16
RNA interference and Northern analysis	17

### Supporting Figures

Supporting Figure 1	18
Supporting Figure 2	19
Supporting Figure 3	20
Supporting Figure 4	21
Supporting Figure 5	22
Supporting Figure 6	23
Supporting Figure 7	24
Supporting Figure 8	25
Supporting Figure 9	26

### Supporting Tables

Supporting Table 1	27
Supporting Table 2	28

References	29
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## Synthesis of 2'-azido cytidine and 2'-azido guanosine nucleoside building blocks (7 and 17).

**General.**  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{31}\text{P}$  NMR spectra were recorded on a Bruker DRX 300 MHz or Avance II+ 600 MHz instrument. The chemical shifts are referenced to the residual proton signal of the deuterated solvents:  $\text{CDCl}_3$  (7.26 ppm),  $\text{d}_6\text{-DMSO}$  (2.49 ppm) for  $^1\text{H}$  NMR spectra;  $\text{CDCl}_3$  (77.0 ppm) or  $\text{d}_6\text{-DMSO}$  (39.5 ppm) for  $^{13}\text{C}$  NMR spectra.  $^{31}\text{P}$ -shifts are relative to external 85% phosphoric acid.  $^1\text{H}$ - and  $^{13}\text{C}$ -assignments were based on COSY and HSQC experiments. MS experiments were performed on a Finnigan LCQ Advantage MAX ion trap instrument. Analytical thin-layer chromatography (TLC) was carried out on Marchery-Nagel Polygram SIL G/UV254 plates. Flash column chromatography was carried out on silica gel 60 (70-230 mesh). All reactions were carried out under argon atmosphere. 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).

**2'-Azido-3'-O-(*tert*-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine (2).** A suspension of compound **1** (500 mg, 0.87 mmol) (**1**) in anhydrous DMF (10 ml) was treated with imidazole (238 mg, 3.5 mmol) and *tert*-butyldimethylsilyl chloride (264 mg, 1.75 mmol). The solution was kept under argon and stirred for 16 h at room temperature. The solvent was evaporated under high vacuum. The product was precipitated from the oily yellow residue by the addition of water, isolated by filtration, washed with water and hexane and dried under high vacuum. The crude product as brown foam was used for the next step without further purification. An analytical sample was prepared by column chromatography on silica gel ( $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ , 100/0 – 97/3 v/v). Yield: 570 mg of **2** as white foam (95%). TLC ( $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ , 95/5):  $R_f$  = 0.57;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  -0.03 (s, 3H,  $\text{SiCH}_3$ ); 0.10 (s, 3H,  $\text{SiCH}_3$ ); 0.84 (s, 9H,  $\text{SiC}(\text{CH}_3)_3$ ); 3.35 (dd,  $J_1$  = 2.1 Hz,  $J_2$  = 11.1 Hz, 1H, H(a)-C(5')); 3.67 (dd,  $J_1$  = 2.4 Hz,  $J_2$  = 11.1 Hz, 1H, H(b)-C(5')); 3.80 (s, 6H, 2 x O-CH<sub>3</sub>); 3.82-3.84 (m, 1H, H-C(2')); 4.09 (m, 1H, H-C(4')); 4.45 (t,  $J$  = 5.3 Hz, 1H, H-C(3')); 5.38 (d,  $J$  = 8.2 Hz, 1H, H-C(5)); 6.05 (d,  $J$  = 4.2 Hz, 1H, H-C(1')); 6.85 (dd,  $J_1$  = 1.5 Hz,  $J_2$  = 8.7 Hz, 4H, H-C(ar) DMT); 7.22-7.33 (m, 9H, H-C(ar) DMT); 7.95 (d,  $J$  = 8.2 Hz, 1H, H-C(6)); 9.02 (s, br, 1H, H-N(3)) ppm;  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  -4.57 ( $\text{SiCH}_3$ ); -5.06 ( $\text{SiCH}_3$ ); 17.99 ( $\text{SiC}(\text{CH}_3)_3$ ); 25.64 ( $\text{SiC}(\text{CH}_3)_3$ ); 55.31 (2 x OCH<sub>3</sub>); 61.51 (C(5')); 66.15 (C(2')); 71.97 (C(3')); 84.20 (C(4')); 86.99 (tert.C-DMT); 87.51 (C(1')); 102.64 (C(5)); 113.37 (C(ar)-DMT); 127.37, 128.07, 128.27, 130.21 (C(ar)-DMT); 134.87; 134.96; 139.57 (C(6)); 143.91; 150.14; 158.90; 163.14 ppm; ESI-MS ( $m/z$ ):  $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{36}\text{H}_{34}\text{N}_5\text{O}_7\text{Si}$ , 708.74; found 708.56.

**2'-Azido-3'-O-(*tert*-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)-O<sup>6</sup>-(2,4,6-triisopropyl-benzenesulfonyl)-2'-deoxyuridine (3).** A solution of compound **2** (210 mg, 0.31 mmol) in dry dichloromethane

(2.5 ml) was treated under argon with 4-(dimethylamino)pyridine (5 mg, 0.037 mmol) and triethylamine (440  $\mu$ l, 0.47 mmol). 2,4,6-Triisopropylbenzenesulfonyl chloride (142 mg, 0.47 mmol) was slowly added and the solution stirred for 1 h at room temperature. The mixture was diluted with dichloromethane, extracted with saturated sodium bicarbonate solution, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The crude product as brown foam was used for the next step without further purification. An analytical sample was prepared by column chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH, 100/0 – 98/2 v/v). Yield: 175 mg of **3** as white foam (60%). TLC (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH, 99/1): R<sub>f</sub> = 0.83; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  -0.16 (s, 3H, SiCH<sub>3</sub>); 0.01 (s, 3H, SiCH<sub>3</sub>); 0.72 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>); 1.21 (d,  $J$  = 6.8 Hz, 12H, 3 x CH(CH<sub>3</sub>)<sub>2</sub>); 1.28 (d,  $J$  = 6.7 Hz, 6H, 3 x CH(CH<sub>3</sub>)<sub>2</sub>); 2.86 (q,  $J$  = 6.9 Hz, 1H, 3 x CH(CH<sub>3</sub>)<sub>2</sub>); 3.29 (d,  $J$  = 11.1 Hz, 1H, H(a)-C(5')); 3.76 (s, 6H, 2 x O-CH<sub>3</sub>); 3.79 (m, 1H, H(b)-C(5')); 3.98 (d,  $J$  = 4.9 Hz, 1H, H-C(2')); 4.08 (d,  $J$  = 8.3 Hz, 1H, H-C(4')); 4.20 (q,  $J$  = 6.6 Hz, 2H, 3 x CH(CH<sub>3</sub>)<sub>2</sub>); 4.40 (dd,  $J_1$  = 5.1 Hz,  $J_2$  = 8.3 Hz, 1H, H-C(3')); 5.55 (d,  $J$  = 7.3 Hz, 1H, H-C(5)); 5.71 (s, 1H, H-C(1')); 6.80 (dd,  $J_1$  = 2.4 Hz,  $J_2$  = 8.7 Hz, 4H, H-C(ar)); 7.13-7.18 (m, 11H, H-C(ar)); 7.95 (d,  $J$  = 8.2 Hz, 1H, H-C(6)) ppm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  5.19 (SiCH<sub>3</sub>); -4.39 (SiCH<sub>3</sub>); 17.86 ((SiC(CH<sub>3</sub>)<sub>3</sub>)); 23.49, 24.42, 24.73, (CH(CH<sub>3</sub>)<sub>2</sub>); 25.52 ((SiC(CH<sub>3</sub>)<sub>3</sub>)); 29.73, 34.31 (CH(CH<sub>3</sub>)<sub>2</sub>); 55.32 (OCH<sub>3</sub>); 59.98 (C(5')); 66.50 (C(2')); 69.79 (C(3')); 82.74 (C(4')); 87.33 (tert.C-DMT); 89.61 (C(1')); 95.27 (C(5)); 113.31 (C(ar)); 124.12, 127.51, 128.04, 128.50, 130.37 (C(ar)); 134.69; 134.74; 143.71; 145.92 (C(6)); 151.29; 153.74; 154.64; 158.99; 167.34 ppm; ESI-MS (m/z): [M+Na]<sup>+</sup> calcd for C<sub>51</sub>H<sub>65</sub>N<sub>5</sub>O<sub>9</sub>SSi, 975.23; found 975.47.

**2'-Azido-3'-O-(tert-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidine (4).** The crude compound **3** (0.5 g, 0.53 mmol) which was dried under vacuum, was dissolved in THF (10 ml) and treated with aqueous ammonia (15 ml, 32%). The solution was stirred at room temperature overnight. The solvents were evaporated, the mixture was diluted with dichloromethane, extracted with water, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The crude product as yellow foam was purified by column chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH, 99/1 – 96/4 v/v). Yield: 340 mg of **4** as white foam (95%). TLC (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH, 95/5): R<sub>f</sub> = 0.28; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  -0.09 (s, 3H, SiCH<sub>3</sub>); 0.05 (s, 3H, SiCH<sub>3</sub>); 0.79 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>); 3.29 (dd,  $J_1$  = 2.4 Hz,  $J_2$  = 11.0 Hz, 1H, H(a)-C(5')); 3.71 (dd,  $J_1$  = 2.0 Hz,  $J_2$  = 11.0 Hz, 1H, H(b)-C(5')); 3.79 (s, 6H, 2 x O-CH<sub>3</sub>); 3.82-3.97 (dd,  $J_1$  = 2.0 Hz,  $J_2$  = 5.0 Hz, 1H, H-C(2')); 4.10 (m, 1H, H-C(4')); 4.41 (dd,  $J_1$  = 5.2 Hz,  $J_2$  = 7.6 Hz, 1H, H-C(3')); 5.40 (d,  $J$  = 7.4 Hz, 1H, H-C(5)); 5.94 (d,  $J$  = 1.9 Hz, 1H, H-C(1')); 6.84 (dd,  $J_1$  = 2.1 Hz,  $J_2$  = 8.9 Hz, 4H, H-C(ar) DMT); 7.24-7.39 (m, 9H, H-C(ar) DMT); 8.09 (d,  $J$  = 7.5 Hz, 1H, H-C(6)) ppm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  -5.15 (SiCH<sub>3</sub>); -4.50 (SiCH<sub>3</sub>); 17.91 ((SiC(CH<sub>3</sub>)<sub>3</sub>)); 25.59 ((SiC(CH<sub>3</sub>)<sub>3</sub>)); 55.29 (2 x OCH<sub>3</sub>); 61.03 (C(5')); 66.80 (C(2')); 70.60 (C(3')); 82.65 (C(4')); 87.01 (tert.C-DMT); 88.59 (C(1')); 94.68 (C(5)); 113.24, 113.28 (C(ar)-DMT); 127.22, 127.96, 128.41, 130.28, 130.32 (C(ar)-DMT); 135.13; 135.19;

140.75 (C(6)); 144.08; 155.71; 158.80; 166.03 ppm; ESI-MS (m/z): [M+Na]<sup>+</sup> calcd for C<sub>36</sub>H<sub>44</sub>N<sub>6</sub>O<sub>6</sub>Si, 707.85; found 707.39.

**N<sup>4</sup>-Acetyl-2'-azido-3'-O-(*tert*-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidine (5).** A solution of **4** (3.0 g, 4.90 mmol) in dry pyridine (8 ml) was cooled to 0°C under argon, treated with acetic anhydride (1 ml, 10.9 mmol), allowed to warm to room temperature and stirred for 90 minutes. The reaction was quenched by the addition of methanol (1 ml) and the solvents were evaporated. The oily residue was diluted with dichloromethane, extracted with 5% citric acid, water and saturated sodium bicarbonate solution, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The crude product as brown foam was purified by column chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH, 99/1 – 95/5 v/v). Yield: 3.18 g of **5** as white foam (97%). TLC (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH, 97/3): R<sub>f</sub> = 0.29; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ -0.10 (s, 3 H, SiCH<sub>3</sub>); 0.04 (s, 3H, SiCH<sub>3</sub>); 0.78 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>); 2.27 (s, 3H, COCH<sub>3</sub>); 3.34 (dd, *J*<sub>1</sub> = 2.0 Hz, *J*<sub>2</sub> = 11.1 Hz, 1H, H(a)-C(5')); 3.76-3.80 (m, 1H, H(b)-C(5')); 3.82 (s, 6H, 2 x O-CH<sub>3</sub>); 3.97 (dd, *J*<sub>1</sub> = 1.6 Hz, *J*<sub>2</sub> = 5.0 Hz, 1H, H-C(2')); 4.16 (m, 1H, H-C(4')); 4.42 (dd, *J*<sub>1</sub> = 5.1 Hz, *J*<sub>2</sub> = 7.8 Hz, 1H, H-C(3')); 5.95 (d, *J* = 7.9 Hz, 1H, H-C(1')); 6.86 (d, *J* = 7.9 Hz, 4H, H-C(ar) DMT); 7.09 (d, *J* = 7.4 Hz, 1H, H-C(5)); 7.23-7.37 (m, 9H, H-C(ar) DMT); 8.51 (d, *J* = 7.5 Hz, 1H, H-C(6)) ppm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ -5.21 (SiCH<sub>3</sub>); -4.50 (SiCH<sub>3</sub>); 17.90 ((SiC(CH<sub>3</sub>)<sub>3</sub>); 24.92 (COCH<sub>3</sub>); 25.55 ((SiC(CH<sub>3</sub>)<sub>3</sub>); 55.30 (2 x OCH<sub>3</sub>); 60.44 (C(5')); 66.82 (C(2')); 70.28 (C(3')); 82.94 (C(4')); 87.28 (tert.C-DMT); 88.89 (C(1')); 97.15 (C(5)); 113.31, 113.33 (C(ar)-DMT); 127.43, 128.06, 128.46, 130.28 (C(ar)-DMT); 134.87; 134.90; 143.65; 144.36 (C(6)); 155.02; 158.88; 163.25; 170.78 ppm; ESI-MS (m/z): [M+Na]<sup>+</sup> calcd for C<sub>38</sub>H<sub>46</sub>N<sub>6</sub>O<sub>7</sub>Si, 749.88; found 749.51.

**N<sup>4</sup>-Acetyl-2'-azido-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidine (6).** Compound **5** (0.4 g, 0.55 mmol) was treated with 1 ml of 1M TBAF/0.5M AcOH in THF. The solution was stirred at room temperature for 2.5 h. The solvents were evaporated and the residue was coevaporated twice with dichloromethane. The crude product as with foam was purified by column chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH, 99/1 – 96/4 v/v). Yield: 0.38 g of **6** as white foam (100%). TLC (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH, 97/3): R<sub>f</sub> = 0.20; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 2.11 (s, 3H, COCH<sub>3</sub>); 3.52 (2dd, *J*<sub>1</sub> = 2.4 Hz, *J*<sub>2</sub> = 11.5 Hz, 2H, H<sub>2</sub>C(5')); 3.72-3.76 (m, 7H, HO-C(3'), 2 x O-CH<sub>3</sub>); 4.10 (m, 1H, H-C(4')); 4.50-4.56 (m, 2H, H-C(2'), H-C(3')); 5.83 (s, 1H, H-C(1')); 6.81 (d, *J* = 8.8 Hz, 4H, H-C(ar) DMT); 7.12 (d, *J* = 7.5 Hz, 1H, H-C(5)); 7.22-7.38 (m, 9H, H-C(ar) DMT); 8.42 (d, *J* = 7.5 Hz, 1H, H-C(6)); 9.23 (s, br, 1H, NH) ppm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 24.86 (COCH<sub>3</sub>); 55.28 (2 x OCH<sub>3</sub>); 60.72 (C(5')); 67.04 (C(3')); 68.48 (C(2')); 82.71 (C(4')); 87.11 (tert.C-DMT); 89.68 (C(1')); 97.03 (C(5)); 113.39, 127.22, 128.12, 130.08 (C(ar)-DMT); 135.22; 135.50; 144.29; 144.54 (C(6)); 155.58; 158.73; 162.82; 170.50 ppm; ESI-MS (m/z): [M+Na]<sup>+</sup> calcd for C<sub>32</sub>H<sub>32</sub>N<sub>6</sub>O<sub>7</sub>, 635.62; found 635.39.

**N<sup>4</sup>-Acetyl-2'-azido-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidin****3'-(2-chlorophenylphosphate)**

**triethylammonium salt (7).** A solution of 1,2,4-triazole (248 mg, 3.59 mmol) in dry THF (10 ml) was mixed with triethylamine (0.46 ml, 3.27 mmol) and 2-chlorophenyl phosphorodichloridate (0.27 ml, 1.63 mmol), which soon led to a white precipitate. The suspension was stirred at room temperature under argon for 15 minutes, then a solution of compound **6** (0.40 g, 0.65 mmol) in dry THF (10 ml) and 1-methylimidazole (0.21 ml, 2.61 mmol) were added. The mixture was stirred for 45 minutes at room temperature, then quenched by the addition of water (0.18 ml) and triethylamine (1.2 ml). The solvents were evaporated, the residue was dissolved with dichloromethane and extracted with 10% sodium bicarbonate solution and saturated sodium chloride solution, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The oily residue was purified by column chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH/Et<sub>3</sub>N, 98/1/1 – 92/7/1 v/v/v). Yield: 485 mg of **7** as white foam (82%). TLC (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH, 95/5): R<sub>f</sub> = 0.12; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.23 (t, *J* = 7.3 Hz, 9H, ((CH<sub>3</sub>CH<sub>2</sub>)<sub>3</sub>NH)<sup>+</sup>); 2.26 (s, 3H, COCH<sub>3</sub>); 2.93 (q, *J* = 7.3 Hz, 6H, ((CH<sub>3</sub>CH<sub>2</sub>)<sub>3</sub>NH)<sup>+</sup>); 3.52 (m, 2H, H<sub>2</sub>C(5')); 3.80 (d, *J* = 3.5 Hz, 6H, 2 x O-CH<sub>3</sub>); 4.41 (m, 1H, H-C(4')); 4.59 (dd, *J*<sub>1</sub> = 1.4 Hz, *J*<sub>2</sub> = 5.0 Hz, 1H, H-C(2')); 5.12 (m, 1H, H-C(3')); 5.98 (s, 1H, H-C(1')); 6.82 (dd, *J*<sub>1</sub> = 3.5 Hz, *J*<sub>2</sub> = 8.8 Hz, 4H, H-C(ar) DMT); 6.94 (t, *J* = 7.5 Hz, 1H, H-C(ar) Phos); 7.02 (d, *J* = 7.5 Hz, 1H, H-C(5)); 7.09 (t, *J* = 7.8 Hz, 1H, H-C(ar) Phos); 7.32-7.42 (m, 10H, H-C(ar) DMT and Phos); 7.58 (d, *J* = 8.1 Hz, 6H, H-C(ar) DMT); 8.22 (d, *J* = 7.5 Hz, 1H, H-C(6)); 9.88 (s, br, 1H, NH) ppm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 8.88 (((CH<sub>3</sub>CH<sub>2</sub>)<sub>3</sub>NH)<sup>+</sup>); 25.01 (COCH<sub>3</sub>); 45.80 (((CH<sub>3</sub>CH<sub>2</sub>)<sub>3</sub>NH)<sup>+</sup>); 55.28 (2 x OCH<sub>3</sub>); 61.29 (C(5')); 66.30 (C(2')); 72.19 (C(3')); 81.60 (C(4')); 87.08 (tert.C-DMT); 89.31 (C(1')); 96.59 (C(5)); 113.25 (C(ar)-DMT); 121.65, 123.76 (C(ar)-Phos); 125.16; 125.21; 127.00, 127.52 (C(ar)-Phos); 127.95, 128.29, 130.19, 130.26 (C(ar)-DMT); 135.25; 135.38; 144.07; 144.30 (C(6)); 155.08; 158.56; 162.80; 170.78 ppm; <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>): δ -5.91 ppm; ESI-MS (*m/z*): [M+NEt<sub>3</sub>]<sup>+</sup> calcd for C<sub>38</sub>H<sub>35</sub>ClN<sub>6</sub>O<sub>10</sub>P, 1005.53; found 1005.35.

**N<sup>2</sup>-[(Dimethylamino)methylene]-[3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)]-guanosine (9).** A suspension of compound **8** (1.86 g, 3.53 mmol) (2) in anhydrous DMF (25 ml) was treated with *N,N*-dimethylformamide dimethyl acetal (1.05 ml, 8.84 mmol) and stirred under argon for 6 h at room temperature. After evaporation of the solvent under high vacuum the crude product (yellow oil) was purified by column chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH, 97/3 – 94/6 v/v). Yield: 1.73 g of **9** as white foam (84%). TLC (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH, 96/4): R<sub>f</sub> = 0.45; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 1.00-1.05 (m, 28H, 2 x (Si(CH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>)); 3.04 (s, 3H, N(CH<sub>3</sub>)); 3.16 (s, 3H, N(CH<sub>3</sub>)); 3.97-4.06 (m, 3H, H<sub>2</sub>-C(5'), H-C(4')); 4.38 (m, 2H, H-C(3'), H-C(2')); 5.67 (s, 1H, HO-C(2')); 5.81 (s, 1H, H-C(1')); 7.86 (s, 1H, H-C(8)); 8.55 (s, 1H, N(2)=CH-); 11.34 (s, 1H, H-N(1)) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ 12.02, 12.27, 12.42, 12.77, 16.77, 16.84, 16.86, 16.93, 17.09,

17.13, 17.29 (2 x (Si(CH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>)); 34.63 (N(CH<sub>3</sub>)); 40.59 (N(CH<sub>3</sub>)); 60.82 (C(5')); 69.76 (C(3')); 73.86 (C(2')); 80.99 (C(4')); 88.12 (C(1')); 116.69; 135.40 (C(8)); 149.35; 157.41; 157.54; 157.94 (N(2)=CH-) ppm; ESI-MS (m/z): [M+Na]<sup>+</sup> calcd for C<sub>25</sub>H<sub>44</sub>N<sub>6</sub>O<sub>6</sub>Si<sub>2</sub>, 548.74; found 548.33.

**N<sup>2</sup>-[(Dimethylamino)methylene]-O<sup>6</sup>-[2-(4-nitrophenyl)ethyl]-[3',5'-O-(1,1,3,3-tetraisopropyl-disiloxane-1,3-diyl)]guanosine (10).** Compound **9** (0.86 g, 1.48 mmol), triphenylphosphine (0.54 g, 2.07 mmol) and 2-(4-nitrophenyl)ethanol were coevaporated with dry dioxane and then suspended in dioxane (30 ml). After 30 minutes of stirring under argon at room temperature diisopropyl azodicarboxylate (0.38 ml, 1.92 mmol) was added and stirring continued for 2 h. The solvents were removed and the residue was diluted with dichloromethane, extracted with a saturated sodium bicarbonate solution, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The crude product as yellow oil was purified by column chromatography on silica gel (EtOAc/hexanes, 6/4 – 8/2 v/v). Yield: 0.43 g of **10** as yellow foam (40%). TLC (EtOAc/hexanes, 6/4): R<sub>f</sub> = 0.38; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ 1.01-1.08 (m, 28H, 2 x (Si(CH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>)); 3.08 (s, 3H, N(CH<sub>3</sub>), shoulder NP-CH<sub>2</sub>); 3.16 (s, 3H, N(CH<sub>3</sub>)); 4.04-4.11 (m, 3H, H<sub>2</sub>-C(5'), H-C(4')); 4.31 (d, *J* = 4.5 Hz, 1 H, H-C(2')); 4.54-4.56 (m, 3H, H-C(3'), O(6)-CH<sub>2</sub>); 6.00 (s, 1H, H-C(1')); 7.37 (d, *J* = 8.2 Hz, 2H, 4-NP H-C(2) and H-C(6)); 7.80 (s, 1H, H-C(8)); 8.09 (d, *J* = 8.2 Hz, 2H, 4-NP H-C(3) and H-C(5)); 8.42 (s, 1H, N(2)=CH-) ppm; <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>): δ 13.84, 17.09, 17.16, 17.21, 17.29, 17.49, 17.51, 17.57, 17.64 (2 x (Si(CH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>)); 34.85 (NP-CH<sub>2</sub>); 35.39 (N(CH<sub>3</sub>)); 41.46 (N(CH<sub>3</sub>)); 43.38 (O(6)-CH<sub>2</sub>); 61.46 (C(5')); 70.57 (C(3')); 75.67 (C(2')); 82.08 (C(4')); 87.83 (C(1')); 123.74 (4-NP C(2) and C(6)); 130.06 (4-NP C(3) and C(5)); 135.68 (C(8)); 146.81; 147.63; 147.69; 157.16; 157.80; 157.98 (N(2)=CH-) ppm; ESI-MS (m/z): [M+H]<sup>+</sup> calcd for C<sub>33</sub>H<sub>51</sub>N<sub>7</sub>O<sub>8</sub>Si<sub>2</sub>, 730.98; found 730.46.

**N<sup>2</sup>-[(Dimethylamino)methylene]-O<sup>6</sup>-[2-(4-nitrophenyl)ethyl]-3',5'-O-(1,1,3,3-tetraisopropyl-disiloxane-1,3-diyl)-2'-O-[(trifluoromethyl)sulfonyl]guanosine (11).** Compound **10** (0.35 g, 0.48 mmol) and 4-(dimethylamino)pyridine (176 mg, 1.44 mmol) were dissolved in dry dichloromethane (12 ml) and the reaction mixture was cooled to 0°C. Then, trifluoromethanesulfonyl chloride (82 µl, 0.72 mmol) was added drop wise and the solution was stirred for 15 minutes at 0°C. The reaction mixture was diluted with dichloromethane, washed with 5% sodium bicarbonate solution, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The crude product was used for the next step without purification. For analysis the oily residue was purified by column chromatography on silica gel (EtOAc/hexanes, 5/5 v/v). Yield: 182 mg of **11** as yellow foam (44%). TLC (EtOAc/hexanes, 6/4): R<sub>f</sub> = 0.49; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 0.97-1.09 (m, 28H, 2 x (Si(CH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>)); 3.11 (s, 3H, N(CH<sub>3</sub>), shoulder NP-CH<sub>2</sub>); 3.15 (s, 3H, N(CH<sub>3</sub>)); 4.04 (dd, *J*<sub>1</sub> = 4.5 Hz, *J*<sub>2</sub> = 13.5 Hz, 1H, H(a)-C(5')); 4.15 (d, *J* = 9.4 Hz, 1H, H-C(4')); 4.29 (d, *J*<sub>1</sub> = 13.5 Hz, 1H, H(b)-C(5')); 4.56 (t, *J* = 7.6 Hz, 2H, O(6)-CH<sub>2</sub>); 4.64 (dd, *J*<sub>1</sub> = 4.1 Hz, *J*<sub>2</sub> = 9.3

Hz, 1H, H-C(3')); 5.53 (d,  $J = 4.0$  Hz, 1H, H-C(2')); 6.12 (s, 1H, H-C(1')); 7.39 (d,  $J = 8.6$  Hz, 2H, 4-NP H-C(2) and H-C(6)); 7.89 (s, 1H, H-C(8)); 8.10 (d,  $J = 8.6$  Hz, 2H, 4-NP H-C(3) and H-C(5)); 8.52 (s, 1H, N(2)=CH-) ppm;  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.92, 12.98, 13.02, 13.44, 16.77, 16.81, 16.82, 16.84, 17.25, 17.34, 17.51 (2 x (Si(CH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>)); 34.74 (NP-CH<sub>2</sub>); 35.26 (N(CH<sub>3</sub>)); 41.07 (N(CH<sub>3</sub>)); 43.21 (O(6)-CH<sub>2</sub>); 59.44 (C(5')); 67.48 (C(3')); 81.73 (C(4')); 86.65 (C(1')); 88.42 (C(2')); 120.45; 123.65 (4-NP C(2) and C(6)); 129.92 (4-NP C(3) and C(5)); 134.73 (C(8)); 146.77; 147.87; 147.44; 157.39; 157.79; 158.29 (N(2)=CH-) ppm; ESI-MS ( $m/z$ ):  $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{34}\text{H}_{50}\text{F}_3\text{N}_7\text{O}_{10}\text{SSi}_2$ , 730.98; found 730.46.

**9- $\{N^2$ -[(Dimethylamino)methylene]- $O^6$ -[2-(4-nitrophenyl)ethyl]-3',5'- $O$ -(1,1,3,3-tetraisopropyl-disiloxane-1,3-diyl)- $b$ - $D$ -arabinofuranosyl}guanine (12).** To a solution of compound **11** (0.5 g, 0.58 mmol) in dry toluene (20 ml) was mixed with potassium trifluoroacetate (0.44 mg, 2.9 mmol), 18-crown-6 (307 mg, 1.16 mmol) and  $N$ -ethyldiisopropylamine (149  $\mu\text{l}$ , 0.87 mmol) and the suspension was stirred for 16 h at 80°C. The reaction mixture then filtrated over celite and the solvents were evaporated. The residue was diluted with dichloromethane and filtrated again over celite, washed with 5% sodium bicarbonate solution, dried over  $\text{Na}_2\text{SO}_4$  and evaporated. The crude product as brown oil was purified by column chromatography on silica gel ( $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ , 99/1 – 97/3 v/v). Yield: 420 mg of **12** as yellow foam (99%). TLC ( $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ , 98/2):  $R_f = 0.22$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.06-1.13 (m, 28H, 2 x (Si(CH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>)); 2.95 (s, 3H, N(CH<sub>3</sub>), shoulder NP-CH<sub>2</sub>); 3.05 (s, 3H, N(CH<sub>3</sub>)); 3.81-3.87 (m, 1H, H-C(4')); 3.94-4.00 (m, 1H, H(a)-C(5')); 4.05-4.10 (dd,  $J_1 = 3.5$  Hz,  $J_2 = 11.6$  Hz, 1H, H(b)-C(5')); 4.19-4.30 (m, 2H, O(6)-CH<sub>2</sub>); 4.47-4.50 (dd,  $J_1 = 3.5$  Hz,  $J_2 = 5.6$  Hz, 1H, H-C(3')); 4.77 (q,  $J = 1.7$  Hz, 1H, H-C(2')); 5.85 (s, 1H, HO-C(2')); 6.10 (d,  $J = 4.5$  Hz, 1H, H-C(1')); 7.28 (d,  $J = 8.6$  Hz, 2H, 4-NP H-C(2) and H-C(6)); 7.78 (s, 1H, H-C(8)); 8.08 (d,  $J = 8.6$  Hz, 2H, 4-NP H-C(3) and H-C(5)); 8.31 (s, 1H, N(2)=CH-) ppm;  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.68, 13.12, 13.47, 13.70, 17.15, 17.22, 17.24, 17.31, 17.52, 17.54, 17.69 (2 x (Si(CH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>)); 34.72 (NP-CH<sub>2</sub>); 35.02 (N(CH<sub>3</sub>)); 41.27 (N(CH<sub>3</sub>)); 43.27 (O(6)-CH<sub>2</sub>); 63.62 (C(5')); 77.20 (C(2')); 79.71 (C(3')); 82.74 (C(4')); 83.61 (C(1')); 118.02; 123.61 (4-NP C(2) and C(6)); 129.83 (4-NP C(3) and C(5)); 139.17 (C(8)); 146.76; 147.17; 147.46; 156.23; 156.97; 157.71 (N(2)=CH-) ppm; ESI-MS ( $m/z$ ):  $[\text{2M}]^+$  calcd for  $\text{C}_{33}\text{H}_{51}\text{N}_7\text{O}_8\text{Si}_2$ , 1459.94; found 1459.35.

**9- $\{N^2$ -[(Dimethylamino)methylene]- $O^6$ -[2-(4-nitrophenyl)ethyl]-3',5'- $O$ -(1,1,3,3-tetraisopropyl-disiloxane-1,3-diyl)-2'- $O$ -[(trifluoromethyl)sulfonyl]- $b$ - $D$ -arabinofuranosyl}guanine (13).** Compound **12** (1.38 g, 1.89 mmol) was coevaporated with dry pyridine and dissolved in dry dichloromethane (80 ml). 4-(Dimethylamino)pyridine (346 mg, 2.84 mmol) and triethylamine (0.66 ml, 4.73 mmol) were added and the reaction mixture was cooled to 0°C. Then, trifluoromethanesulfonyl chloride (302  $\mu\text{l}$ , 2.84 mmol) was added drop wise and the solution was stirred for 15 minutes at 0°C. The reaction mixture was diluted with

dichloromethane, washed with saturated sodium bicarbonate solution, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The crude product was purified by column chromatography on silica gel (EtOAc/hexanes, 5/5 v/v). Yield: 1.33 g of **13** as white foam (82%). TLC (EtOAc/hexanes, 7/3): R<sub>f</sub> = 0.56; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ 0.99-1.16 (m, 28H, 2 x (Si(CH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>)); 3.03 (s, 3H, N(CH<sub>3</sub>)); 3.09 (s, 3H, N(CH<sub>3</sub>), shoulder NP-CH<sub>2</sub>); 3.88-3.89 (m, 1H, H-C(4')); 4.07-4.10 (dd, *J*<sub>1</sub> = 2.8 Hz, *J*<sub>2</sub> = 13.3 Hz, 1H, H(a)-C(5')); 4.12-4.14 (dd, *J*<sub>1</sub> = 2.7 Hz, *J*<sub>2</sub> = 13.3 Hz, 1H, H(b)-C(5')); 4.58 (t, *J* = 7.3 Hz, 2H, O(6)-CH<sub>2</sub>); 4.80 (t, *J* = 8.2 Hz, 1H, H-C(3')); 5.39-5.41 (m, 1H, H-C(2')); 6.31 (d, *J* = 5.9 Hz, 1H, H-C(1')); 7.31 (d, *J* = 8.6 Hz, 2H, 4-NP H-C(2) and H-C(6)); 7.92 (s, 1H, H-C(8)); 8.05 (d, *J* = 8.6 Hz, 2H, 4-NP H-C(3) and H-C(5)); 8.36 (s, 1H, N(2)=CH-) ppm; <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>): δ 12.74, 13.04, 13.24, 13.36, 16.56, 16.63, 16.79, 16.88, 17.29, 17.40, 17.43, 17.55 (2 x (Si(CH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>)); 34.79 (NP-CH<sub>2</sub>); 35.06 (N(CH<sub>3</sub>)); 41.21 (N(CH<sub>3</sub>)); 43.15 (O(6)-CH<sub>2</sub>); 60.75 (C(5')); 71.20 (C(3')); 79.51 (C(1')); 80.56 (C(4')); 87.58 (C(2')); 119.64; 123.54 (4-NP C(2) and C(6)); 130.00 (4-NP C(3) and C(5)); 135.63 (C(8)); 146.63; 147.65; 147.96; 157.37; 157.87; 158.04 (N(2)=CH-) ppm; ESI-MS (m/z): [M]<sup>+</sup> calcd for C<sub>34</sub>H<sub>50</sub>F<sub>3</sub>N<sub>7</sub>O<sub>10</sub>SSi<sub>2</sub>, 802.03; found 802.13.

**2'-Azido-2'-deoxy-N<sup>2</sup>-[(dimethylamino)methylene]-O<sup>6</sup>-[2-(4-nitrophenyl)ethyl]-[3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)]guanosine (14).** Compound **13** (0.5 g, 0.58 mmol) was dissolved in dry DMF (11 ml) and treated with LiN<sub>3</sub> (142 mg, 2.9 mmol). The suspension was stirred for 16 h under argon at room temperature. The solvent was evaporated under high vacuum, the residue was dissolved in ethyl acetate and washed with 5% citric acid, water and saturated sodium bicarbonate solution, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The crude product was purified by column chromatography on silica gel (EtOAc/hexanes, 6/4 - 8/2 v/v). Yield: 311 mg of **14** as yellow foam (71%). TLC (EtOAc/hexanes, 7/3): R<sub>f</sub> = 0.44; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ 1.00-1.09 (m, 28H, 2 x (Si(CH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>)); 3.10 (s, 3H, N(CH<sub>3</sub>), shoulder NP-CH<sub>2</sub>); 3.17 (s, 3H, N(CH<sub>3</sub>)); 4.01-4.04 (dd, *J*<sub>1</sub> = 2.7 Hz, *J*<sub>2</sub> = 13.2 Hz, 1H, H(a)-C(5')); 4.08-4.10 (tt, *J*<sub>1</sub> = 2.3 Hz, *J*<sub>2</sub> = 8.8 Hz, 1H, H-C(4')); 4.12-4.14 (m, 2H, H(b)-C(5') and H-C(2')); 4.55 (t, *J* = 7.7 Hz, 2H, O(6)-CH<sub>2</sub>); 4.68-4.71 (dd, *J*<sub>1</sub> = 5.4 Hz, *J*<sub>2</sub> = 8.8 Hz, 1H, H-C(3')); 5.86 (s, 1H, H-C(1')); 7.38 (d, *J* = 8.6 Hz, 2H, 4-NP H-C(2) and H-C(6)); 7.84 (s, 1H, H-C(8)); 8.09 (d, *J* = 8.6 Hz, 2H, 4-NP H-C(3) and H-C(5)); 8.44 (s, 1H, N(2)=CH-) ppm; <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>): δ 12.83, 13.14, 13.16, 13.67, 17.02, 17.12, 17.19, 17.35, 17.38, 17.49, 17.45, 17.65 (2 x (Si(CH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>)); 34.86 (NP-CH<sub>2</sub>); 35.42 (N(CH<sub>3</sub>)); 41.47 (N(CH<sub>3</sub>)); 43.35 (O(6)-CH<sub>2</sub>); 60.15 (C(5')); 66.98 (C(2')); 70.35 (C(3')); 82.09 (C(4')); 86.38 (C(1')); 120.30; 123.75 (4-NP C(2) and C(6)); 130.04 (4-NP C(3) and C(5)); 135.14 (C(8)); 146.83; 147.25; 147.59; 157.21; 157.83; 157.94 (N(2)=CH-) ppm; ESI-MS (m/z): [M]<sup>+</sup> calcd for C<sub>33</sub>H<sub>50</sub>N<sub>10</sub>O<sub>7</sub>Si<sub>2</sub>, 754.98; found 754.98.



**2'-Azido-2'-deoxy- $N^2$ -[(dimethylamino)methylene]- $O^6$ -[2-(4-nitrophenyl)ethyl]guanosine (15).**

Compound **14** (176 mg, 0.23 mmol) was treated with 0.9 ml of 1M TBAF/0.5M AcOH in THF. The solution was stirred at room temperature for 2.5 h. The solvents were evaporated and the residue was coevaporated twice with dichloromethane. The crude product as yellow foam was purified by column chromatography on silica gel ( $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ , 97/3 – 94/6 v/v). Yield: 111 mg of **15** as white foam (93%). TLC ( $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ , 97/3):  $R_f$  = 0.29;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.05-3.10 (m, 2H, NP- $\text{CH}_2$ ); 3.13 (s, 3H,  $\text{N}(\text{CH}_3)$ ); 3.19 (s, 3H,  $\text{N}(\text{CH}_3)$ ); 4.06 (s, 1H,  $\text{H}_2\text{-C}(5')$ ); 4.19-4.21 (m, 1H,  $\text{H-C}(4')$ ); 4.33-4.35 (dd,  $J_1 = 3.3$  Hz,  $J_2 = 4.8$  Hz, 1H,  $\text{H-C}(2')$ ); 4.52 (t,  $J = 15.3$  Hz, 2H,  $\text{O}(6)\text{-CH}_2$ ); 4.77 (t,  $J = 11.5$  Hz, 1H,  $\text{H-C}(3')$ ); 5.30 ( $\text{CH}_2\text{Cl}_2$ ); 6.00 (d,  $J = 3.1$  Hz, 1H,  $\text{H-C}(1')$ ); 7.38 (d,  $J = 8.6$  Hz, 2H, 4-NP  $\text{H-C}(2)$  und  $\text{H-C}(6)$ ); 8.12 (d,  $J = 8.6$  Hz, 2H, 4-NP  $\text{H-C}(3)$  and  $\text{H-C}(5)$ ); 8.44-8.45 (m, 2H,  $\text{H-C}(8)$  and  $\text{N}(2)=\text{CH-}$ ) ppm;  $^{13}\text{C}$  NMR (150 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  34.01 (NP- $\text{CH}_2$ ); 34.80 ( $\text{N}(\text{CH}_3)$ ); 40.68 ( $\text{N}(\text{CH}_3)$ ); 42.40 ( $\text{O}(6)\text{-CH}_2$ ); 60.90 ( $\text{C}(5')$ ); 64.64 ( $\text{C}(2')$ ); 71.08 ( $\text{C}(3')$ ); 84.35 ( $\text{C}(4')$ ); 85.99 ( $\text{C}(1')$ ); 118.78; 123.39 (4-NP  $\text{C}(2)$  and  $\text{C}(6)$ ); 130.04 (4-NP  $\text{C}(3)$  and  $\text{C}(5)$ ); 136.80 ( $\text{C}(8)$ ); 146.12; 147.40; 147.82; 156.73; 156.86; 157.57 ( $\text{N}(2)=\text{CH-}$ ) ppm; ESI-MS ( $m/z$ ):  $[\text{2M}+\text{Na}]^+$  calcd for  $\text{C}_{21}\text{H}_{24}\text{N}_{10}\text{O}_6$ , 1047.94; found 1047.26.

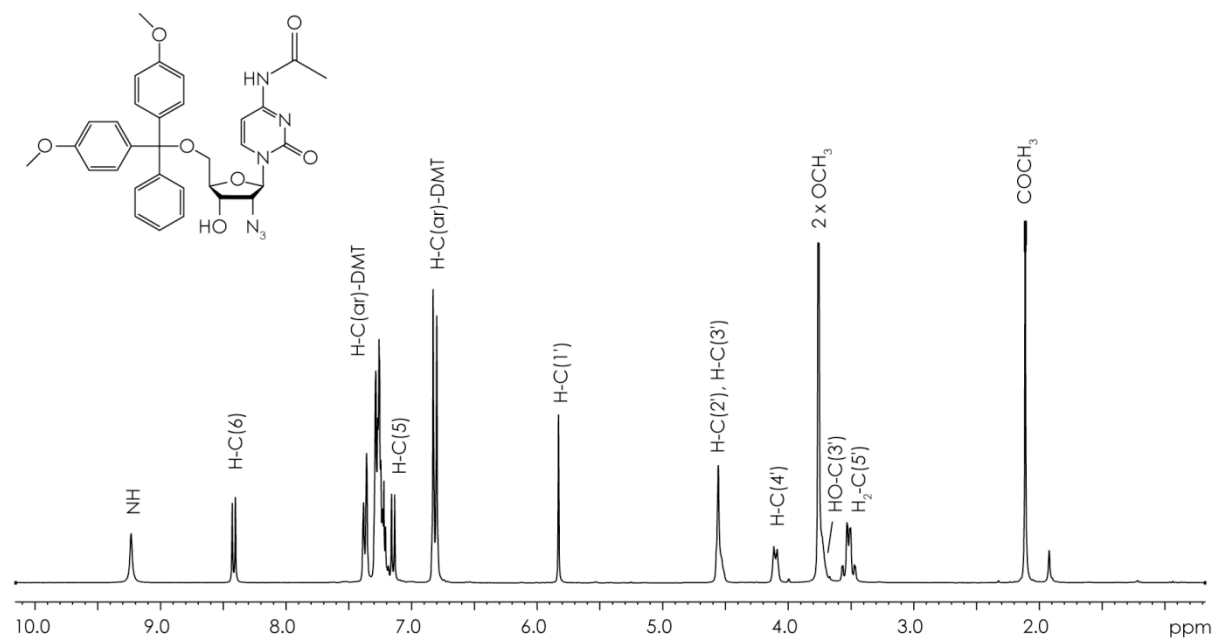
**2'-Azido-2'-deoxy-5'- $O$ -(4,4'-dimethoxytrityl)- $N^2$ -[(dimethylamino)methylene]- $O^6$ -[2-(4-nitrophenyl)ethyl]guanosine (16).** Compound **15** (66 mg, 0.129 mmol) was coevaporated twice with dry pyridine and then dissolved in dry pyridine (1 ml). The solution was treated with 4,4'-dimethoxytrityl chloride (61 mg, 0.18 mmol) in three portions over 1 h, then stirring was for 16 h. The reaction was quenched with a few drops of methanol and the solvents were evaporated. The residue was dissolved in dichloromethane and washed with 5% citric acid, water and saturated sodium bicarbonate solution, dried over  $\text{Na}_2\text{SO}_4$  and evaporated. The crude product was purified by column chromatography on silica gel ( $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NEt}_3$ , 98/1/1 – 94/5/1 v/v/v). Yield: 95 mg of **16** as yellow foam (91%). TLC ( $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ , 96/4):  $R_f$  = 0.35;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.08 (m, 8H, NP- $\text{CH}_2$  und 2 x  $\text{N}(\text{CH}_3)$ ); 3.39-3.49 (m, 1H,  $\text{H}_2\text{-C}(5')$ ); 3.74 (s, 6H, 2 x DMT- $\text{OCH}_3$ ); 4.23-4.25 (m, 1H,  $\text{H-C}(4')$ ); 4.36 (t,  $J = 9.5$  Hz, 1H,  $\text{H-C}(2')$ ); 4.54 (t,  $J = 15.0$  Hz, 2H,  $\text{O}(6)\text{-CH}_2$ ); 4.76 (t,  $J = 5.5$  Hz, 1H,  $\text{H-C}(3')$ ); 5.28 ( $\text{CH}_2\text{Cl}_2$ ); 6.09 (d,  $J = 4.2$  Hz, 1H,  $\text{H-C}(1')$ ); 6.78 (d,  $J = 8.5$  Hz, 4H, DMT  $\text{H-C}(\text{ar})$ ); 7.15-7.42 (m, 11H, DMT  $\text{H-C}(\text{ar})$ , 4-NP  $\text{H-C}(2)$  and  $\text{H-C}(6)$ ); 7.79 (s, 1H,  $\text{H-C}(8)$ ); 8.07 (d,  $J = 8.6$  Hz, 2H, 4-NP  $\text{H-C}(3)$  and  $\text{H-C}(5)$ ); 8.47 (s, 1H,  $\text{N}(2)=\text{CH-}$ ) ppm;  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  34.76 (NP- $\text{CH}_2$ ); 35.30 ( $\text{N}(\text{CH}_3)$ ); 41.25 ( $\text{N}(\text{CH}_3)$ ); 43.34 ( $\text{O}(6)\text{-CH}_2$ ); 55.36 (2 x DMT- $\text{OCH}_3$ ); 63.41 ( $\text{C}(5')$ ); 67.36 ( $\text{C}(2')$ ); 71.74 ( $\text{C}(3')$ ); 83.51 ( $\text{C}(4')$ ); 85.87 ( $\text{C}(1')$ ); 86.87 (tert.C-DMT); 113.35 (DMT- $\text{C}(\text{ar})$ ); 119.89; 123.68 (4-NP  $\text{C}(2)$  and  $\text{C}(6)$ ); 127.12, 128.09, 128.27 (DMT- $\text{C}(\text{ar})$ ); 129.97 (4-NP  $\text{C}(3)$  and  $\text{C}(5)$ ); 130.18, 130.22 (DMT- $\text{C}(\text{ar})$ ); 135.68; 135.79

(C(8)); 144.62; 146.76; 147.42; 147.96; 157.18; 157.91; 158.00 (N(2)=CH-); 158.74 ppm; ESI-MS (m/z): [M]<sup>+</sup> calcd for C<sub>21</sub>H<sub>24</sub>N<sub>10</sub>O<sub>6</sub>, 814.85; found 814.97.

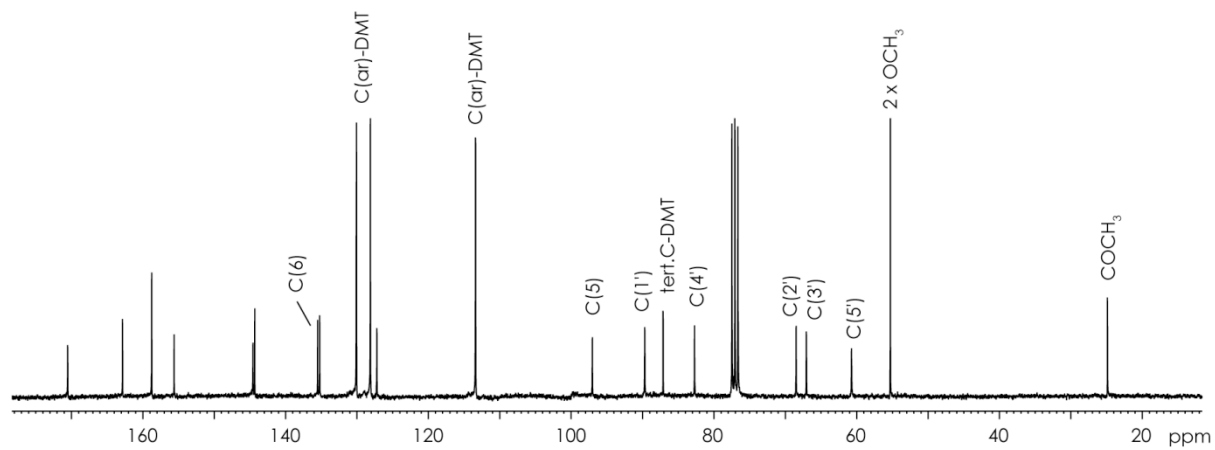
**2'-Azido-2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-N<sup>2</sup>-[(dimethylamino)methylene]-O<sup>6</sup>-[2-(4-nitro-phenyl)ethyl]guanosine 3'-(2-chlorophenylphosphate) triethylammonium salt (17).** A solution of 1,2,4-triazole (42 mg, 0.60 mmol) in dry THF (2 ml) was mixed with triethylamine (76 µl, 0.55 mmol) and 2-chlorophenyl phosphorodichloridate (45 µl, 0.27 mmol), which soon led to a white precipitate. The suspension was stirred for 15 minutes at room temperature under argon, then a solution of compound 16 (89 mg, 0.11 mmol) in dry THF (1.5 ml) and 1-methylimidazole (35 µl, 0.44 mmol) were added. The mixture was stirred for 45 minutes at room temperature, then quenched by the addition of water (0.30 ml) and triethylamine (0.15 ml). The solvents were evaporated; the residue was dissolved with dichloromethane and extracted with 10% sodium bicarbonate solution and saturated sodium chloride solution, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The oily residue was purified by column chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH/Et<sub>3</sub>N, 98/1/1 – 95/4/1 v/v/v). Yield: 84 mg of **17** as white foam (70%). TLC (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH, 94/6): R<sub>f</sub> = 0.35; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.22 (t, *J* = 7.3 Hz, 9H, ((CH<sub>3</sub>CH<sub>2</sub>)<sub>3</sub>NH)<sup>+</sup>); 2.95 (q, *J* = 7.3 Hz, 6H, ((CH<sub>3</sub>CH<sub>2</sub>)<sub>3</sub>NH)<sup>+</sup>); 3.06 (2, 3H, N(CH<sub>3</sub>)); 3.09-3.12 (m, 2H, NP-CH<sub>2</sub>); 3.16 (2, 3H, N(CH<sub>3</sub>)); 3.32-3.44 (m, 1H, H<sub>2</sub>-C(5')); 3.73 (s, 6H, 2 x DMT-OCH<sub>3</sub>); 4.38-4.40 (m, 1H, H-C(4')); 4.57 (t, *J* = 7.2 Hz, 2H, O(6)-CH<sub>2</sub>); 4.73 (t, *J* = 5.2 Hz, 1H, H-C(2')); 5.21-5.27 (m, 1H, H-C(3')); 5.27 (CH<sub>2</sub>Cl<sub>2</sub>); 6.03 (d, *J* = 5.0 Hz, 1H, H-C(1')); 6.72 (d, *J* = 8.9 Hz, 4H, DMT H-C(ar)); 6.82-6.92 (m, 1H, H-C(ar)Phos); 7.00-7.06 (m, 1H, H-C(ar)Phos); 7.14-7.34 (m, 10H, H-C(ar)Phos and DMT H-C(ar)); 7.37 (d, *J* = 8.6 Hz, 2H, 4-NP H-C(2) and H-C(6)); 7.56 (d, *J* = 8.2 Hz, 1H, H-C(ar)Phos); 7.64 (s, 1H, H-C(8)); 8.08 (d, *J* = 8.6 Hz, 2H, 4-NP H-C(3) und H-C(5)); 8.60 (s, 1H, N(2)=CH-) ppm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 8.70 ((CH<sub>3</sub>CH<sub>2</sub>)<sub>3</sub>NH)<sup>+</sup>; 34.81 (NP-CH<sub>2</sub>); 35.33 (N(CH<sub>3</sub>)); 41.28 (N(CH<sub>3</sub>)); 43.17 (O(6)-CH<sub>2</sub>); 45.93 ((CH<sub>3</sub>CH<sub>2</sub>)<sub>3</sub>NH)<sup>+</sup>; 55.34 (2 x DMT-OCH<sub>3</sub>); 63.59 (C(5')); 65.39 (C(2')); 75.02 (C(3')); 82.99 (C(4')); 86.12 (C(1')); 86.72 (tert.C-DMT); 113.27 (DMT-C(ar)); 120.25; 121.54 (Phos-C(ar)); 123.66 (4-NP C(2) and C(6)); 123.80 (Phos-C(ar)); 125.04; 125.14; 126.95; 127.63 (Phos-C(ar)); 127.95; 128.33 (DMT-C(ar)); 129.97 (4-NP C(3) and C(5)); 130.23 (DMT-C(ar)); 135.70; 135.78; 136.25 (C(8)); 144.62; 146.73; 148.08; 149.26; 149.34; 157.04; 158.01; 158.29; 158.61 (N(2)=CH-) ppm; <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>): δ -5.54 ppm; ESI-MS (m/z): [M+NEt<sub>3</sub>]<sup>+</sup> calcd for C<sub>54</sub>H<sub>61</sub>ClN<sub>11</sub>O<sub>11</sub>P, 1106.56; found 1106.30.

# NMR spectra of 2'-azido cytidine building block **7** and precursor compound **6**

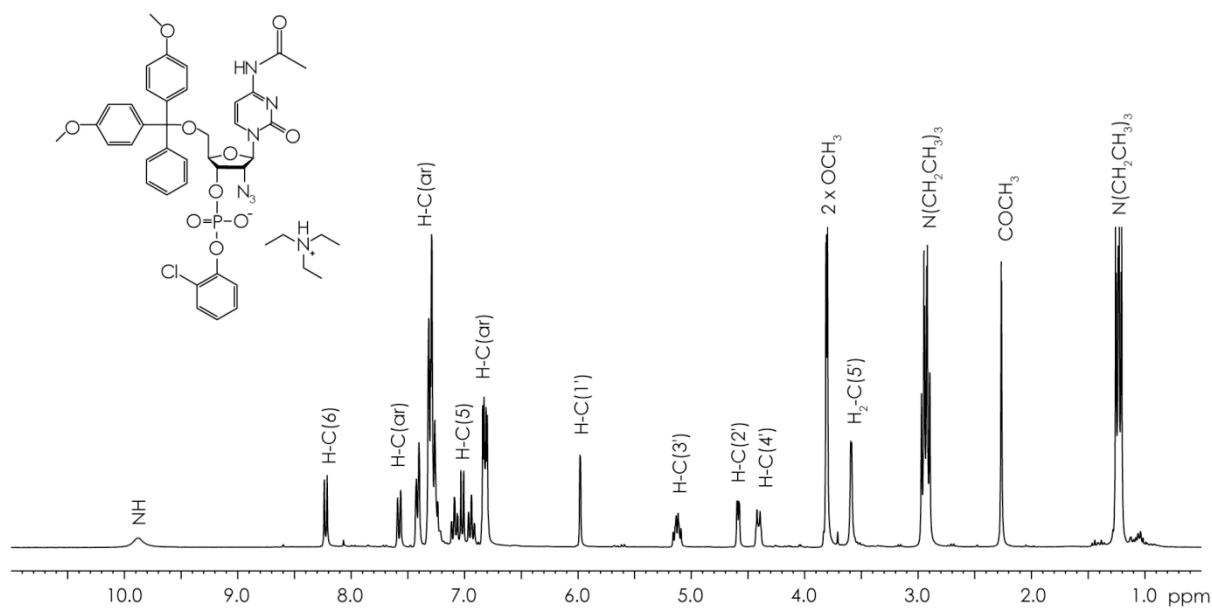
$^1\text{H}$ -NMR (300 MHz,  $\text{CDCl}_3$ ) of compound **6**:



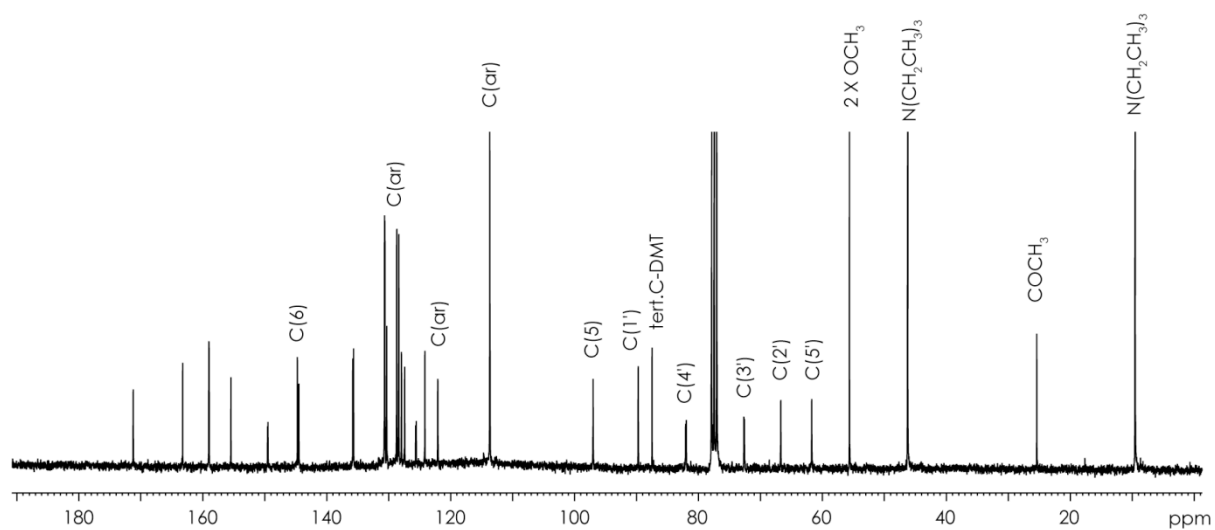
$^{13}\text{C}$ -NMR (75 MHz,  $\text{CDCl}_3$ ) of compound **6**:



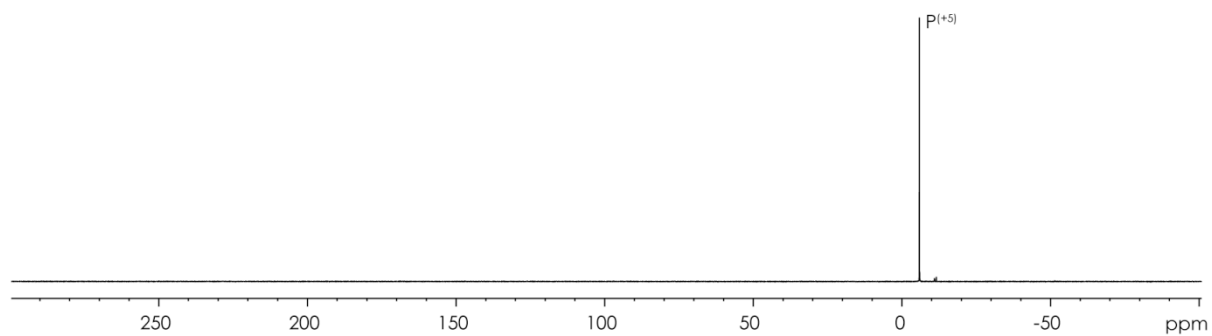
$^1\text{H}$ -NMR (300 MHz,  $\text{CDCl}_3$ ) of compound 7:



$^{13}\text{C}$ -NMR (75 MHz,  $\text{CDCl}_3$ ) of compound 7:

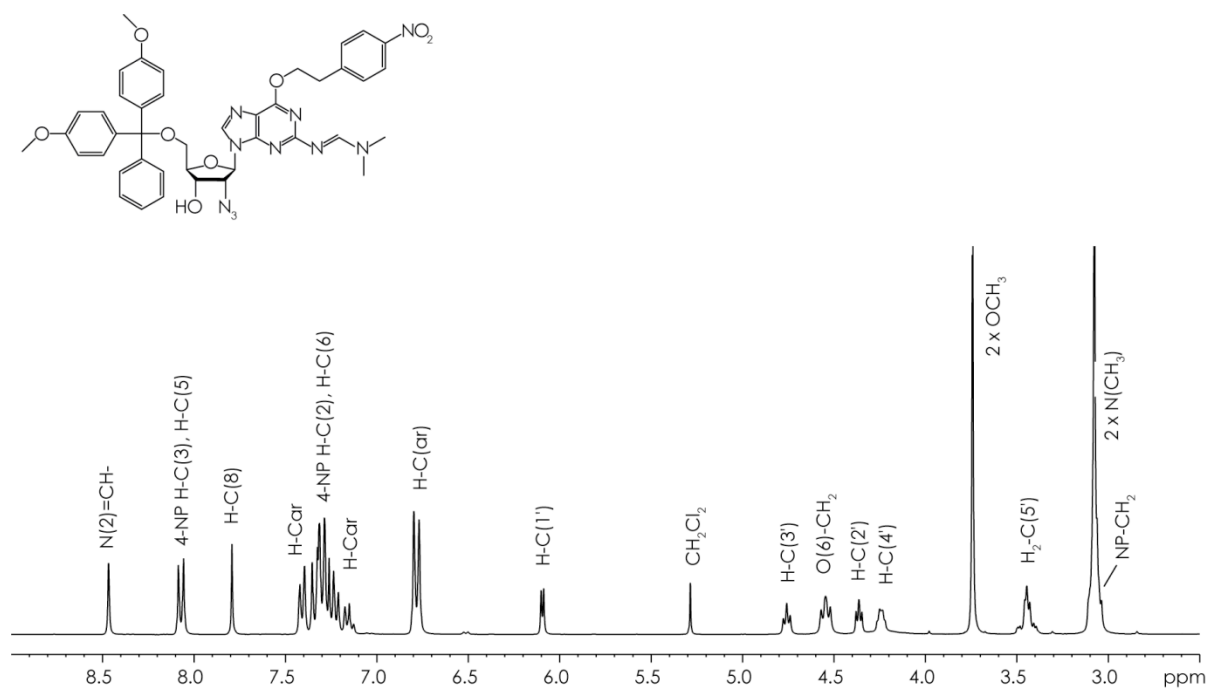


$^{31}\text{P}$ -NMR (121 MHz,  $\text{CDCl}_3$ ) of compound 7:

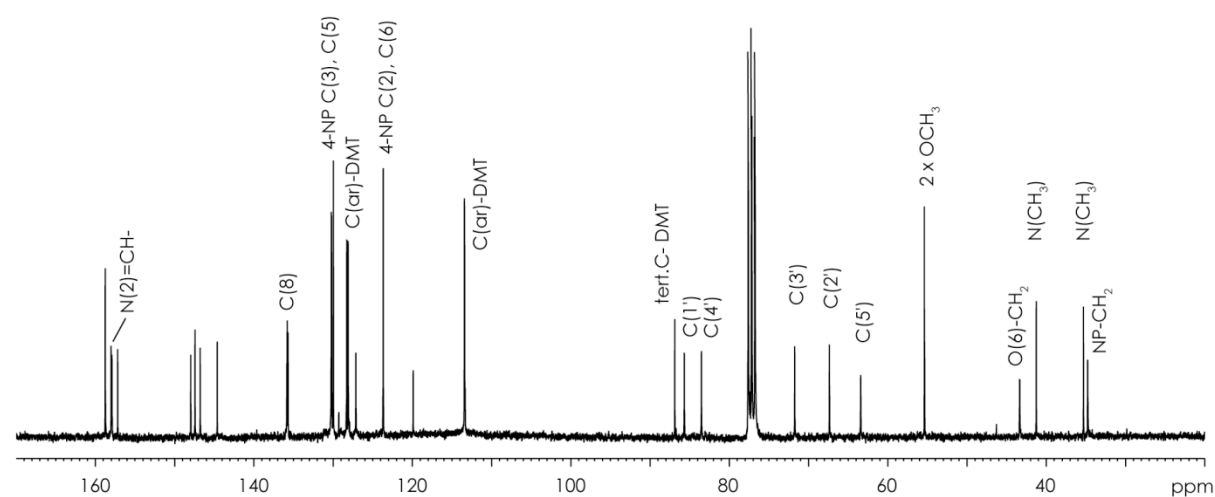


# **NMR spectra of 2'-azido guanosine building block **17** and precursor compound **16****

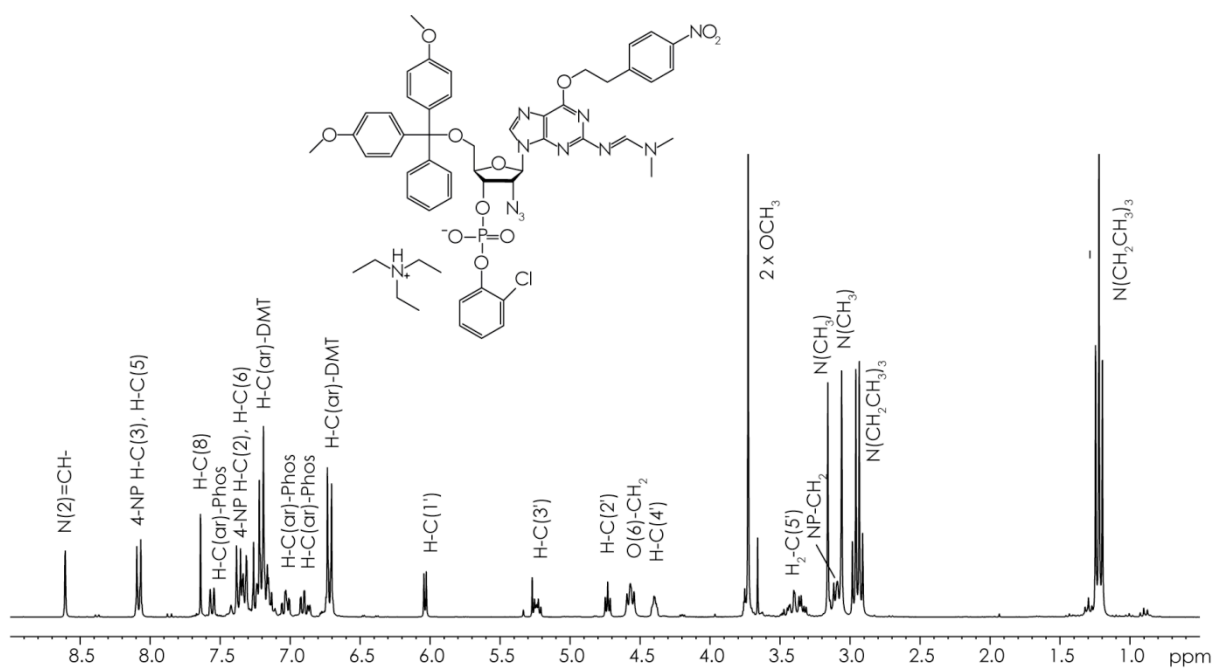
<sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) of compound **16**:



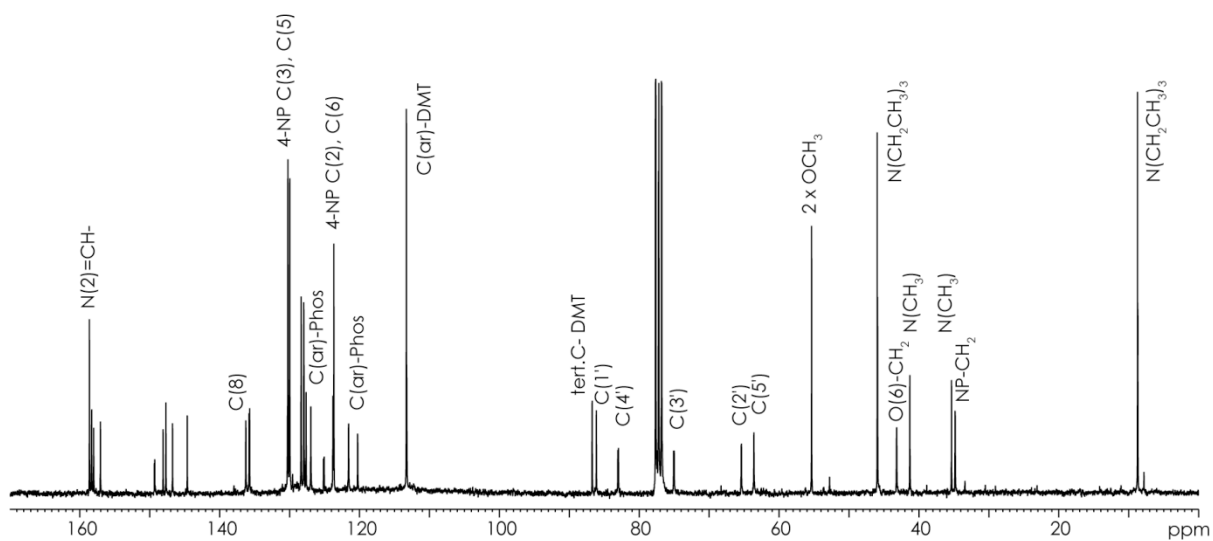
<sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>) of compound **16**:



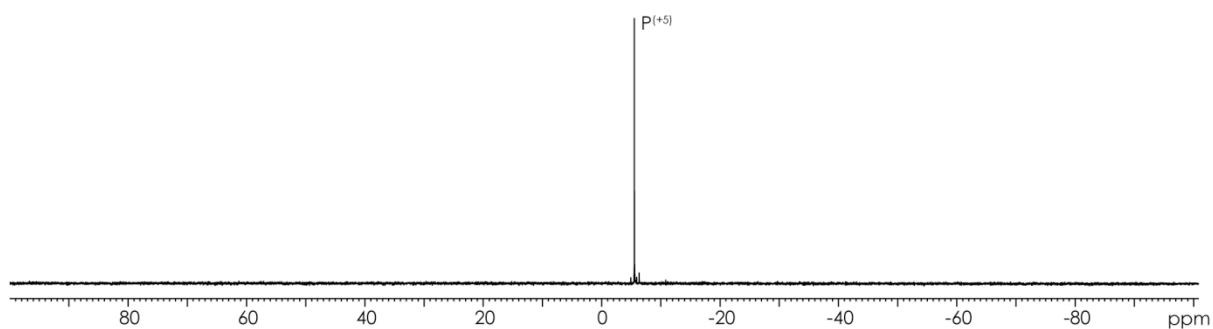
$^1\text{H}$ -NMR (300 MHz,  $\text{CDCl}_3$ ) of compound **17**:



$^{13}\text{C}$ -NMR (75 MHz,  $\text{CDCl}_3$ ) of compound **17**:



$^{31}\text{P}$ -NMR (121 MHz,  $\text{CDCl}_3$ ) of compound **17**:



**RNA solid-phase synthesis.** Standard phosphoramidite chemistry was applied for RNA strand elongation up to the position of the 2'-azido nucleoside incorporation: for the synthesis 2'-O-TOM standard RNA nucleoside phosphoramidite building blocks were purchased from GlenResearch and ChemGenes, the polystyrene support from GE Healthcare (Custom Primer Support<sup>TM</sup>, 80  $\mu$ mol/g; PS 200). All oligonucleotides were synthesized on a ABI 392 Nucleic Acid Synthesizer following standard methods: detritylation (80 sec) with dichloroacetic acid/1,2-dichloroethane (4/96); coupling (2.0 min) with phosphoramidites/acetonitrile (0.1 M x 130  $\mu$ L) and benzylthiotetrazole/acetonitrile (0.3 M x 360  $\mu$ L); capping (3 x 0.4 min, Cap A/Cap B =1/1) with Cap A: 4-(dimethylamino)pyridine in acetonitrile (0.5 M) and Cap B: Ac<sub>2</sub>O/*sym*-collidine/acetonitrile (2/3/5); oxidation (1.0 min) with I<sub>2</sub> (20 mM) in THF/pyridine/H<sub>2</sub>O (35/10/5). The solutions of amidites and tetrazole, and acetonitrile were dried over activated molecular sieves (4 Å) overnight.

The incorporation of the 2'-azido nucleosides **7** or **17** was performed manually according to phosphotriester chemistry. The respective building block **7** or **17** was coupled to the 5'-OH of the standard synthesized RNA chain on the solid support. For this the solid support was dried at first under high vacuum. For coupling a 0.3 M solution of **7** or **17** in dry pyridine was made, which was activated with 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT; 0.9 M) 10 min before coupling. For the coupling the dry solid support and the activated coupling solution were transferred in a 1 ml tube and shaken for 1 h at room temperature.

The solid support was washed with pyridine (1 mL) and acetonitrile (2 x 1 mL). The capping step was performed manually subsequently with Cap A and Cap B from standard synthesis reagents, (Cap A/Cap B = 1/1, 1 ml, 10 min at room temperature). After capping, the solid support was washed with dry acetonitrile (2 x 1 mL), dried under high vacuum and transferred again in the synthesis column. RNA strand elongation was continued by automated standard phosphoramidite chemistry starting with the detritylation step to remove the 5'-trityl group of the manually coupled building block.

**Deprotection of 2'-azido modified RNA.** The first group cleaved was the 2-chlorophenyl group using a solution of *syn*-2-pyridinealdoxime/tetramethylguanidine (0.1 M for each) in dioxane/water (2:1, 1 mL). After 16 h at room temperature, the supernatant was removed and the solid support was washed three times with dioxane/water (2/1, v/v). The solutions were combined and evaporated to dryness. The solid support and the evaporated residue were treated each with MeNH<sub>2</sub> in EtOH (33%, 0.5 mL) and MeNH<sub>2</sub> in water (40%, 0.5 mL) for 7 h at room temperature. The supernatant was removed from and the solid support was washed 3 x with ethanol/water (1/1, v/v). The supernatant and the washings were combined with the deprotection solution of the residue and the whole mixture was evaporated to dryness. To remove the 2'-silyl protecting groups the resulting residue was treated with tetrabutylammonium fluoride trihydrate (TBAF·3H<sub>2</sub>O) in THF (1 M, 1 mL) at 37°C

overnight. The reaction was quenched by the addition of triethylammonium acetate (TEAA) (1 M, pH 7.4, 1 mL). The volume of the solution was reduced and the solution was desalted with a size exclusion column (GE Healthcare, HiPrep™ 26/10 Desalting; 2.6 x 10 cm; Sephadex G25) eluting with H<sub>2</sub>O, the collected fraction was evaporated to dryness and dissolved in 1 mL H<sub>2</sub>O. Analysis of the crude RNA after deprotection was performed by anion-exchange chromatography on a Dionex DNAPac® PA-100 column (4 mm x 250 mm) at 80°C. Flow rate: 1 mL/min, eluant A: 25mM Tris·HCl (pH 8.0), 6 M urea; eluant B: 25 mM Tris·HCl (pH 8.0), 0.5 M NaClO<sub>4</sub>, 6 M urea; gradient: 0-60 % B in A within 45 min or 0-40 % B in 30 min for short sequences up to 15 nucleotides, UV-detection at 260 nm.

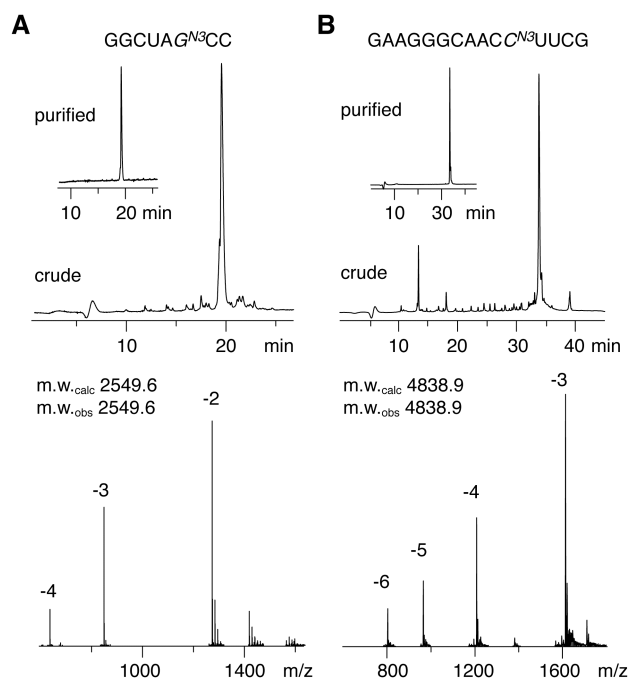
**Purification of 2'-azido modified RNA.** Crude RNA products were purified on a semipreparative Dionex DNAPac® PA-100 column (9 mm x 250 mm) at 80°C with flow rate 2 mL/min. Fractions containing RNA were loaded on a C18 SepPak Plus® cartridge (Waters/Millipore), washed with 0.1-0.15 M (Et<sub>3</sub>NH)<sup>+</sup>HCO<sub>3</sub><sup>-</sup>, H<sub>2</sub>O and eluted with H<sub>2</sub>O/CH<sub>3</sub>CN (1/1). RNA containing fractions were lyophilized. Analysis of the quality of purified RNA was performed by anion-exchange chromatography with same conditions as for crude RNA; the molecular weight was confirmed by LC-ESI mass spectrometry. Yield determination was performed by UV photometrical analysis of oligonucleotide solutions.

**Mass spectrometry of 2'-azido 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<sub>2</sub>O (pH 8.0); eluant B: methanol; gradient: 0-100 % B in A within 30 min; UV-detection at 254 nm.

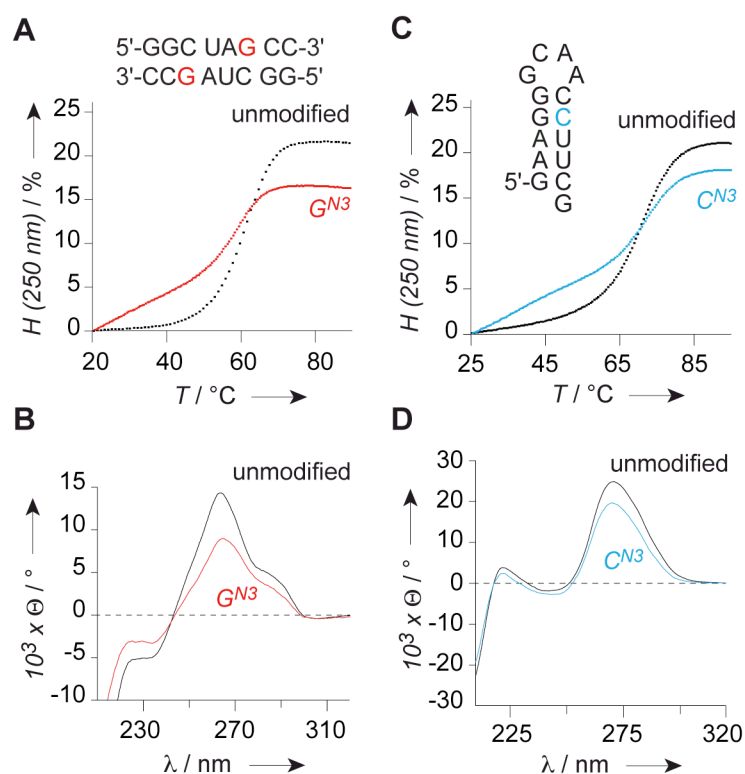
**NMR spectroscopy of 2'-azido modified RNA.** <sup>1</sup>H NMR imino proton spectra were recorded on a Varian Unity 500 MHz instrument and applied a selective excitation refocusing sequence employing selective pulses shaped according to the G4 (excitation; 2.62 ms, RF amplitude 1.74 kHz) and REBURP (refocusing; 1.4 ms, RF amplitude 4.47 kHz) profile, respectively (7) Both shaped pulses were centered at 13 ppm. Typical NMR sample preparation: The RNA probe (triethyl ammonium salts) was lyophilized and dissolved in water, the corresponding amount of arsenate buffer stock solution (pH 6.5) and D<sub>2</sub>O was added to a total volume of 500 µL. Final 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.



**RNA interference and Northern analysis.** Lyophilized synthetic siRNA duplexes consisting of sense r(GGUCUCUGCCAAUAAGACA)dTdT and antisense r(UGUCUUAUUGGCAGAGACC)dTdG strands targeted against the chicken *BASPI* mRNA sequence 5'-CAGGUCUCUGCCAAUAAGACA-3', or chemically modified derivatives thereof were dissolved in a buffer containing 100 mM potassium acetate, 30 mM Hepes-KOH (pH 7.4), and 2 mM magnesium acetate to obtain a 40  $\mu$ M siRNA stock solution. The solution was heated at 90°C for 1 min, incubated at 37°C for 1 h, and then stored in 10- $\mu$ l aliquots at -80°C. Delivery of siRNAs into the chicken cell line DF-1, RNA isolation, and Northern analysis were done as described previously (6).



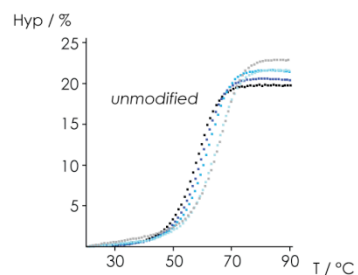
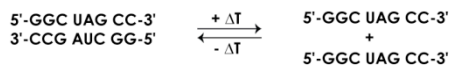
**Supporting Figure 1.** Characterization of 2'-azido-modified oligoribonucleotides by anion-exchange HPLC and LC-ESI mass spectrometry. A) 8 nucleotide (nt) RNA containing 2'-azido-2'-deoxyguanosine. B) 15 nt RNA containing 2'-azido-2'-deoxycytidine. For conditions see SI, page 16. C<sup>N3</sup> 2'-N<sub>3</sub> cytidine, G<sup>N3</sup> 2'-N<sub>3</sub> guanosine.



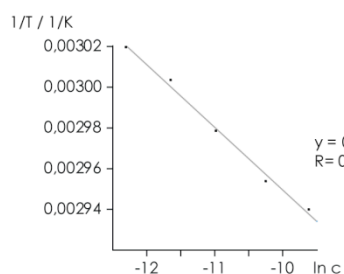
**Supporting Figure 2.** Spectroscopic characterization of 2'-azido-modified oligoribonucleotides. **A-B)** GGCUAGCC and GGCUAG<sup>N3</sup>CC; overlay of UV melting profiles,  $c_{\text{RNA}} = 16 \mu\text{M}$  (A); overlay of CD spectra,  $c_{\text{RNA}} = 5 \mu\text{M}$  (B); conditions for (A) and (B): 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 150 mM NaCl, pH 7.0. **C-D)** GAAGGGCAACCUUCG and GAAGGGCAACC<sup>N3</sup>UUCG; overlay of UV melting profiles,  $c_{\text{RNA}} = 8 \mu\text{M}$  (C); overlay of CD spectra,  $c_{\text{RNA}} = 3 \mu\text{M}$  (D); conditions for (C) and (D): same as (A) and (B).  $H$  = hyperchromicity.  $C^{N3}$  2'-N<sub>3</sub> cytidine,  $G^{N3}$  2'-N<sub>3</sub> guanosine.

**A**

unmodified:



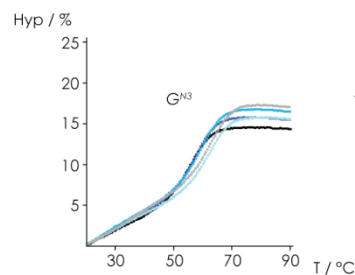
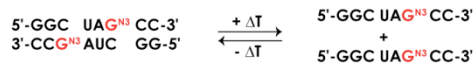
c [μM]	T <sub>m</sub> [°C]
4.5	58.0
8.7	59.8
17.0	62.6
35.3	65.4
66.7	67.0



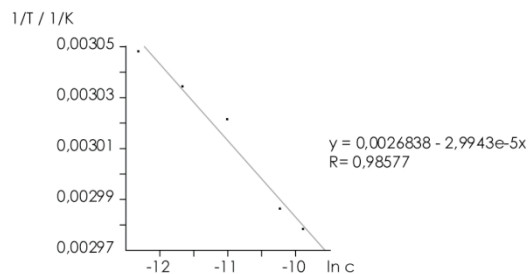
$$\Delta H^{\circ} = -64.5 \text{ kcal mol}^{-1}$$

$$\Delta S^{\circ} = -170 \text{ cal mol}^{-1} \text{ K}^{-1}$$

$$\Delta G^{\circ} = -13.7 \text{ kcal mol}^{-1}$$

**B**2'-N<sub>3</sub>-G (G<sup>N3</sup>) modified:

c [μM]	T <sub>m</sub> [°C]
4.5	54.9
8.6	56.4
16.6	57.8
35.8	61.7
50.7	62.6

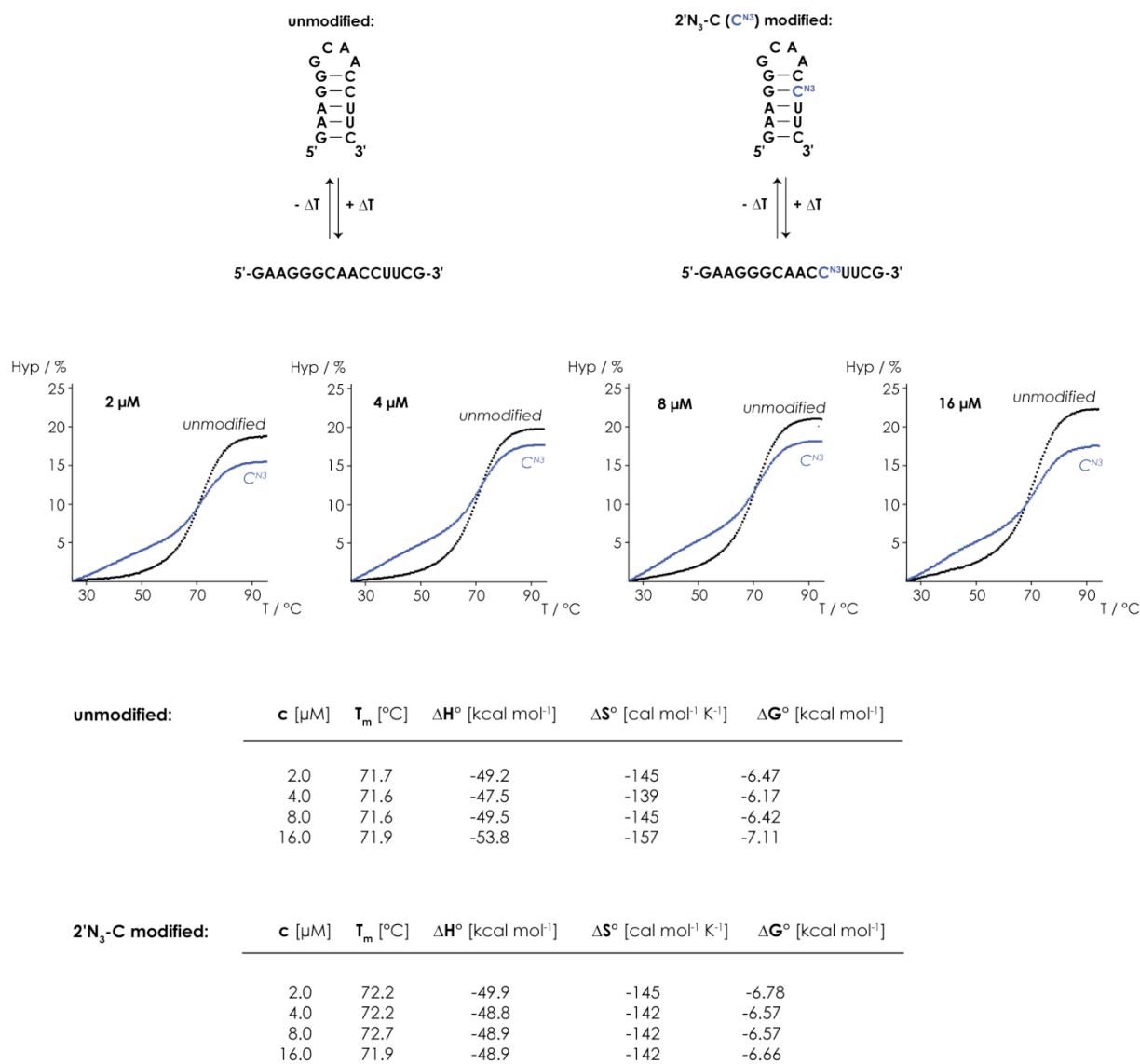


$$\Delta H^{\circ} = -66.4 \text{ kcal mol}^{-1}$$

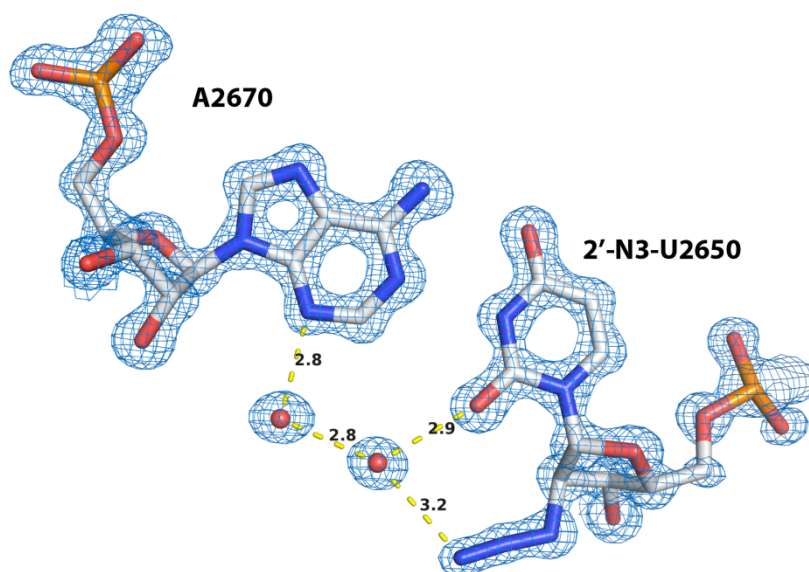
$$\Delta S^{\circ} = -178 \text{ cal mol}^{-1} \text{ K}^{-1}$$

$$\Delta G^{\circ} = -13.3 \text{ kcal mol}^{-1}$$

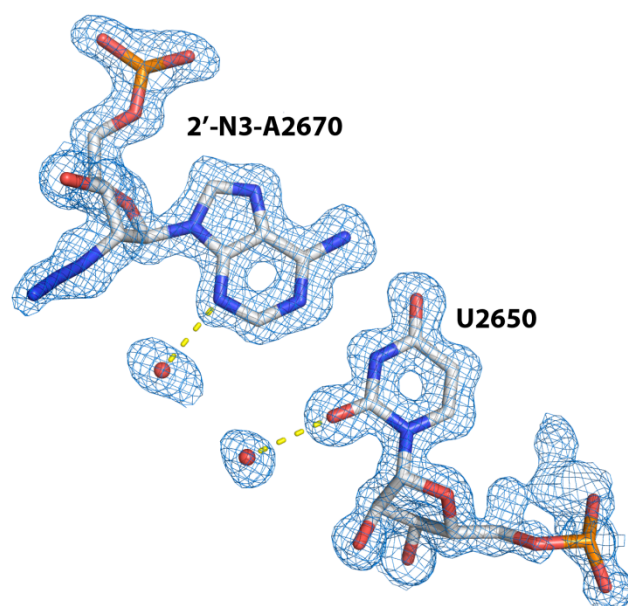
**Supporting Figure 3.** Comparison of melting profiles and thermodynamic analysis of unmodified 5'-GGCUAGCC-3' (A) and 2'-N<sub>3</sub> guanosine modified 5'-GGCUAG<sup>N3</sup>CC-3' (B) self-complementary RNAs. Values of  $\Delta H^{\circ}$  and  $\Delta S^{\circ}$  for the bimolecular melting transition were derived from 1/T versus ln c plots as described in the literature (3,4). Melting data were fitted with polynomial functions, T<sub>m</sub> values were obtained from the first derivative. Conditions: c<sub>RNA</sub> as indicated, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 150 mM NaCl, pH 7.0.



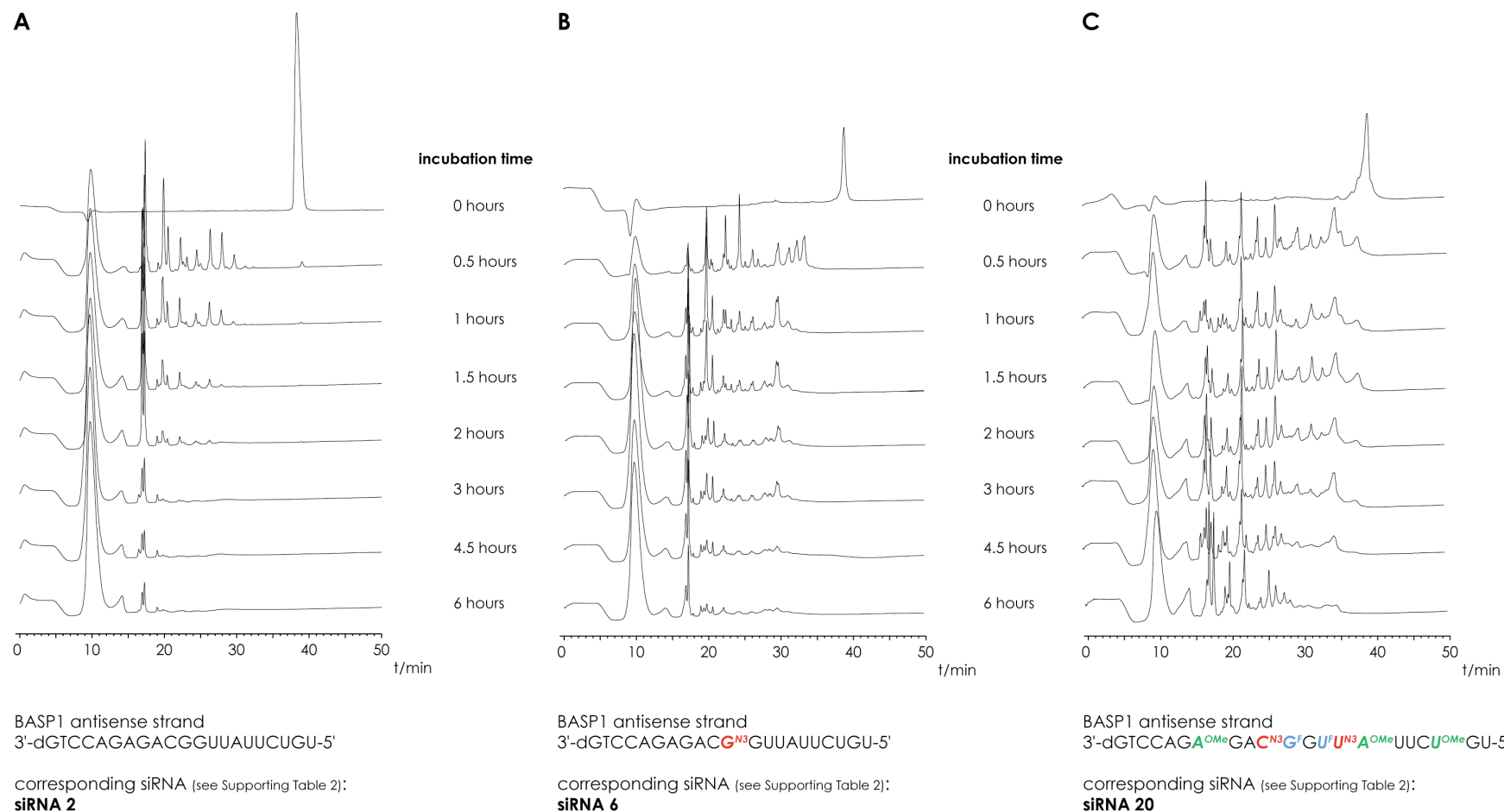
**Supporting Figure 4.** Comparison of thermal denaturation of unmodified 5'-GAAGGGCAACCUUCG-3' and 2'-N<sub>3</sub> cytidine modified 5'-GAAGGGCAACC<sup>N3</sup>UUCG-3' hairpin forming RNA sequences. The thermodynamic properties for monomolecular melting transitions were derived from a two-state van't Hoff analysis by fitting the shape of the individual a versus temperature curve as described in the literature (3,4).



**Supporting Figure 5.** Crystal structure of 2'-N<sub>3</sub> uridine modified sarcin-ricin-loop (SRL) RNA.  $2F_{\text{obs}}-F_{\text{calc}}$  electron density map showing the 2'-N<sub>3</sub>-U2650/A2670 base pair. Water molecules are shown as red spheres.



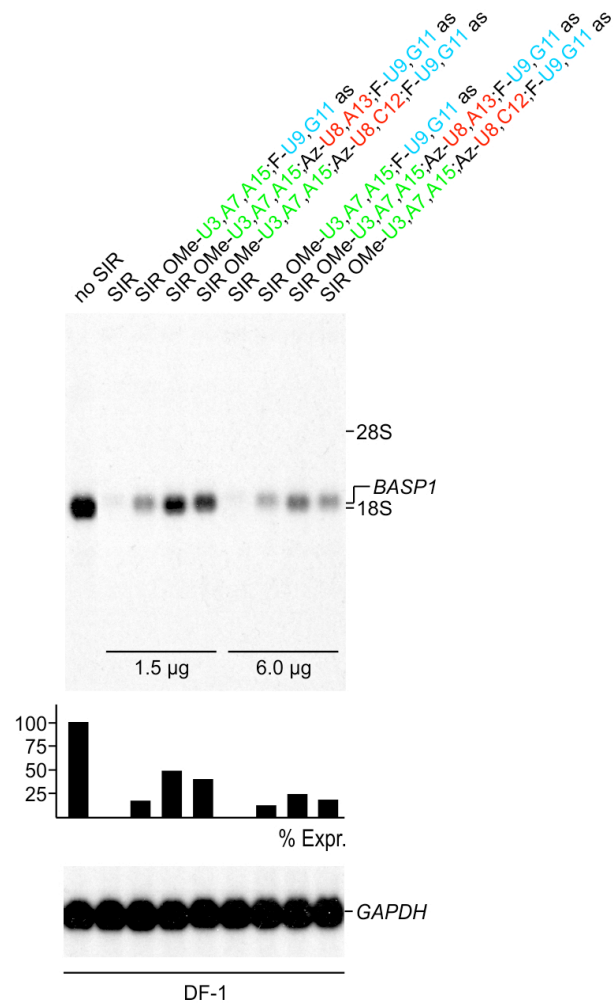
**Supporting Figure 6.** Crystal structure of 2'-N<sub>3</sub> adenosine modified sarcin-ricin-loop (SRL) RNA.  $2F_{\text{obs}} - F_{\text{calc}}$  electron density map showing the U2650/2'-N<sub>3</sub>-A2670 base pair.



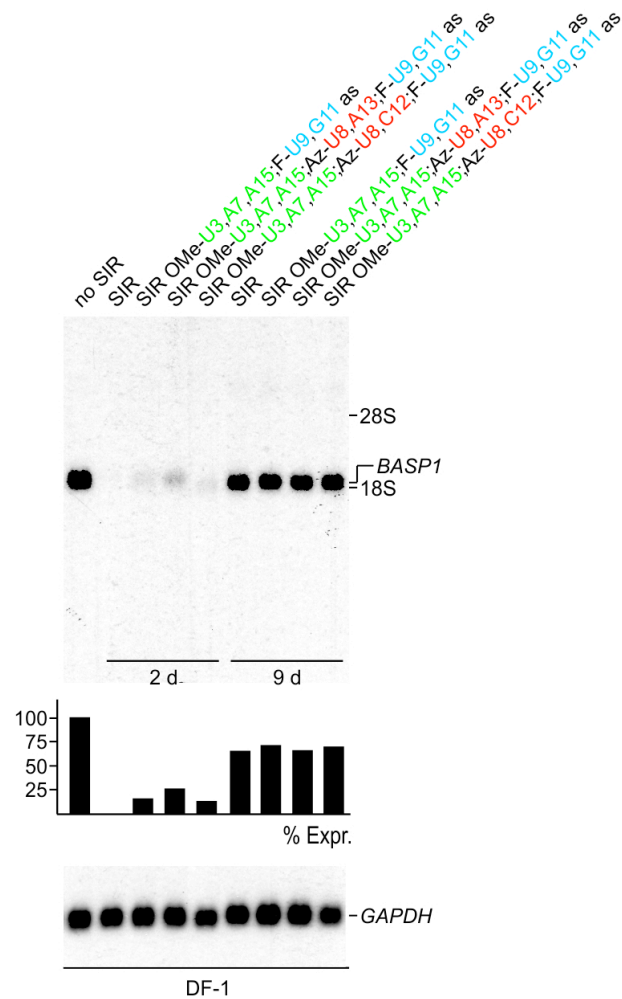
**Supporting Figure 7.** Analytical HPLC traces of unmodified, 2'-azido-G modified and multiple modified BASP1 siRNA antisense strands incubated with phosphodiesterase type II (5). Conditions: 2.5 nmol of BASP1 antisense strand; 6 µl of phosphodiesterase type II (Calf Spleen, 10U solution, 7.22 U/ml, Merck); incubation time as indicated; HPLC gradient 0 to 43% B in 45min (buffer A: 25 mM Tris·HCl, 6M urea, pH 8.0, buffer B: 25 mM Tris·HCl, 0.5 M NaClO<sub>4</sub>, 6M urea, pH 8.0). The experiment provided evidence for a decrease in degradation if the strands were highly modified. The multiple modified antisense strand corresponding to siRNA **20** showed highest resistance.

<sup>C<sup>N3</sup></sup> = 2'-azido-2'-deoxy cytidine, <sup>G<sup>N3</sup></sup> = 2'-azido-2'-deoxy guanosine, <sup>U<sup>N3</sup></sup> = 2'-azido-2'-deoxy uridine; <sup>A<sup>OMe</sup></sup> = 2'-methoxy-2'-deoxy adenosine, <sup>U<sup>OMe</sup></sup> = 2'-methoxy-2'-deoxy uridine; <sup>G<sup>F</sup></sup> = 2'-fluoro-2'-deoxy guanosine, <sup>U<sup>F</sup></sup> = 2'-fluoro-2'-deoxy uridine.





**Supporting Figure 8.** Dose-dependent experiment of gene silencing of the *BASP1* mRNA with highly modified siRNAs. The siRNAs used in the experiment are (from left to right, for exact sequence see Supporting Table 2): no siRNA, siRNA2, siRNA18, siRNA19, siRNA20; amounts: 0.12 nmol (1.5  $\mu$ g) or 0.48 nmol (6.0  $\mu$ g). The experiment showed stronger mRNA suppression if higher initial amounts of siRNA were used (6).



**Supporting Figure 9.** Time-course experiment of gene silencing of the *BASP1* mRNA with highly modified siRNAs. The siRNAs used in the experiment are (from left to right, for exact sequence see Supporting Table 2): no siRNA, siRNA2, siRNA18, siRNA19, siRNA20. The experiment showed significant suppression of *BASP1* mRNA still after two days of incubation for both the unmodified and the highly modified siRNA duplexes. After nine days of incubation, high *BASP1* expression levels recurred for all four duplexes tested (6).

**Supporting Table 1.** X-ray crystallographic data collection and refinement statistics of 2'-N<sub>3</sub> modified sarcin-ricin-loop (SRL) RNAs.

	SRL RNA <b>A2670-N<sub>3</sub></b>	SRL RNA <b>U2650-N<sub>3</sub></b>
PDB ID	3DU8	3S7C
Space group	P4 <sub>3</sub>	P4 <sub>3</sub>
a = b (Å)	29.57	29.61
c (Å)	76.34	76.18
Beamline	PX I - X06SA	PX I - X06SA
Resolution range (Å)	30 – 1.2	30 – 1.1
Number of frames	480	1600
Oscillation angle	0.25°	0.25°
Wavelength	0.91361	0.91361
Average redundancy	3.9	13.1
Completeness <sup>1</sup>	97.5% (91.1%)	99.6% (94.3%)
R <sub>merged</sub> <sup>1</sup>	11.4% (54.5%)	6.0% (45.4%)
Average I/σ <sup>1</sup>	9.0 (2.7)	16.8 (2.9)
R/R <sub>free</sub>	14.3 / 17.8	11.7 / 15.6
Coordinate error (Å)	0.13	0.11
Twinning fraction	not detected	0.205
Twinning operator	n.a.	h, -k, -l

<sup>1</sup> Values for last resolution shell are shown in parenthesis

**Supporting Table 2.** Unmodified, 2'-azido modified, and multiple modified *BASPI* siRNAs used for gene silencing experiments.

N°	Synonym	siRNA duplex	m.w. calc [g/mol]	m.w. found [g/mol]
1	SIR random	U CUG GGU CUA AGC CAA ACA UT dGU A GAC CCA GAU UCG GUU UGU	6674.03 6655.06	6674.39 6655.01
2	SIR unmod	G GUC UCU GCC AAU AAG ACA TT dGT C CAG AGA CGG UUA UUC UGU	6653.08 6672.05	6652.60 6672.07
3	SIR Az-C10 s	G GUC UCU G <sup>Az</sup> C AAU AAG ACA TT dGT C CAG AGA CG G UUA UUC UGU	6678.09 6672.05	6677.75 6672.07
4	SIR Az-C9 s	G GUC UCU GC <sup>Az</sup> AAU AAG ACA TT dGT C CAG AGA CGG UUA UUC UGU	6678.09 6672.05	6677.41 6672.07
5	SIR Az-C12 as	G GUC UCU G CC AAU AAG ACA TT dGT C CAG AGA C <sup>Az</sup> GG UUA UUC UGU	6653.08 6672.05	6652.60 6672.07
6	SIR Az-G11 as	G GUC UCU GC C AAU AAG ACA TT dGT C CAG AGA C <sup>Az</sup> G UUA UUC UGU	6653.08 6697.07	6652.60 6696.50
7	SIR Az-G14 as	G GUC UC U GCC AAU AAG ACA TT dGT C CAG AG <sup>Az</sup> A CGG UUA UUC UGU	6653.08 6697.07	6652.60 6696.57
8	SIR Az- G10 as	G GUC UCU GCC AAU AAG ACA TT dGT C CAG AGA CG <sup>Az</sup> UUA UUC UGU	6653.08 6697.07	6652.60 6696.50
9	SIR Ome- G10 as	G GUC UCU GCC AAU AAG ACA TT dGT C CAG AGA CG <sup>Ome</sup> UUA UUC UGU	6653.08 6685.05	6652.60 6685.84
10	SIR F- G10 as	G GUC UCU GCC AAU AAG ACA TT dGT C CAG AGA CG <sup>F</sup> UUA UUC UGU	6653.08 6674.04	6652.60 6673.78
11	SIR Ome-G11 as	G GUC UCU GCC AAU AAG ACA TT dGT C CAG AGA C <sup>Ome</sup> G UUA UUC UGU	6653.08 6686.05	6652.60 6685.98
12	SIR F-G11 as	G GUC UCU GCC AAU AAG ACA TT dGT C CAG AGA C <sup>F</sup> G UUA UUC UGU	6653.08 6674.04	6652.60 6673.84
13	SIR Ome-G10, G11 as	G GUC UCU GCC AAU AAG ACA TT dGT C CAG AGA C <sup>Ome</sup> <sup>Ome</sup> UUA UUC UGU	6653.08 6700.05	6652.60 6700.06
14	SIR OMe-G10, G14; F-G11 as	G GUC UCU GCC AAU AAG ACA TT dGT C CAG AG <sup>Ome</sup> A C <sup>F</sup> <sup>Ome</sup> UUA UUC UGU	6653.08 6702.04	6652.60 6702.30
15	SIR F-G10, G12, G14 as	G GUC UCU GCC AAU AAG ACA TT dGT C CAG AG <sup>F</sup> C <sup>F</sup> <sup>F</sup> UUA UUC UGU	6653.08 6679.01	6652.60 6678.56
16	SIR OMe-A7, A13, A15; F-U9, G11 as	G GUC UCU GCC AAU AAG ACA TT dGT C CAG A <sup>Ome</sup> GA <sup>Ome</sup> C <sup>F</sup> G U <sup>F</sup> UA <sup>Ome</sup> UUC UGU	6653.08 6718.12	6652.60 6718.92
17	SIR F-C4, A7, A15, A17, U9, G11 as	G GUC UCU GCC AAU AAG ACA TT dGT C C <sup>F</sup> G A <sup>F</sup> GA C <sup>F</sup> G U <sup>F</sup> UA <sup>F</sup> UUC <sup>F</sup> UGU	6653.08 6683.99	6652.60 6684.47
18	SIR OMe-U3, A7, A15; F-U9, G11 as	G GUC UCU GCC AAU AAG ACA TT dGT C CAG A <sup>Ome</sup> GA C <sup>F</sup> G U <sup>F</sup> UA <sup>Ome</sup> UUC U <sup>Ome</sup> GU	6653.08 6718.09	6652.60 6718.48
19	SIR OMe-U3, A7, A15; Az-U8, A13; F-U9, G11 as	G GUC UCU GCC AAU AAG ACA TT dGT C CAG A <sup>Ome</sup> GA <sup>Az</sup> C <sup>F</sup> G U <sup>F</sup> U <sup>Az</sup> A <sup>Ome</sup> UUC U <sup>Ome</sup> GU	6653.08 6768.11	6652.60 6768.52
20	SIR OMe-U3, A7, A15; Az-U8, C12; F-U9, G11 as	G GUC UCU GCC AAU AAG ACA TT dGT C CAG A <sup>Ome</sup> GA C <sup>Az</sup> <sup>F</sup> G U <sup>F</sup> U <sup>Az</sup> A <sup>Ome</sup> UUC U <sup>Ome</sup> GU	6653.08 6768.11	6652.60 6767.54

<sup>Az</sup> = 2'-azido-2'-deoxy adenosine, <sup>Cz</sup> = 2'-azido-2'-deoxy cytidine, <sup>Gz</sup> = 2'-azido-2'-deoxy guanosine, <sup>Uz</sup> = 2'-azido-2'-deoxy uridine; <sup>Ome</sup> = 2'-methoxy-2'-deoxy adenosine, <sup>Gme</sup> = 2'-methoxy-2'-deoxy guanosine, <sup>Ume</sup> = 2'-methoxy-2'-deoxy uridine; <sup>F</sup> = 2'-fluoro-2'-deoxy adenosine, <sup>C</sup> = 2'-fluoro-2'-deoxy cytidine, <sup>G</sup> = 2'-fluoro-2'-deoxy guanosine, <sup>U</sup> = 2'-fluoro-2'-deoxy uridine. All siRNA duplexes contain 2'-deoxynucleosides overhangs; in each row: first lane = sense strand in 5' -> 3' direction. second lane = antisense strand in 3' -> 5' direction.

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