SUPPORTING INFORMATION

Synthesis and incorporation of ^{13}C-labeled DNA building blocks to probe structural dynamics of DNA by NMR

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Synthetic procedures for $^{13}C/^{2}H$ labeled DNA phosphoramidites

Synthesis of (6-^{13}C)-thymidine 5´-DMT protected building block 29

2-(3-^{13}C)Cyanopropionic Acid (1)

2-Bromopropionic acid (7.69 g, 50.2 mmol) was dissolved in 40 mL water. Then sodium carbonate (3.4 g, 32.1 mmol) was added in portions to reach pH 9. ^{13}C-labeled potassium cyanide was added at once and the reaction mixture was heated to 60°C for 3h with stirring. After cooling to room temperature, the reaction was stopped by addition of an excess of concentrated hydrochloric acid to reach pH 1. The solvent was evaporated and the residue dried in high vacuum for 30 minutes. Then, 2-(3-^{13}C)-cyanopropionic acid was extracted from the salt cake with 500 mL diethyl ether. The ether was evaporated and the residue dried in high vacuum to obtain **1** as a yellow oil.

Yield: 4.80 g (97%)

^{1}H-NMR (300 MHz, DMSO-d_6, 25°C): δ 4.03 (dq, 1H, $^{3}J_{HH}$ = 7.4 Hz, $^{2}J_{CH}$ = 10.8 Hz, *H*C^{13}C); 1.41 (dd, 3H, $^{3}J_{HH}$ = 7.4 Hz, $^{3}J_{CH}$ = 6.1 Hz, C*H*$_3$) ppm.

^{13}C-NMR (75 MHz, DMSO-d_6, 25°C): δ 168.5 (s, COOH); 119.1 (s, ^{13}CN); 31.14 (d, $^{1}J_{CC}$ = 59.4 Hz, CH); 14.90 (d,$^{2}J_{CC}$ = 2.0 Hz, CH_3) ppm.

(3-^{13}C)N-Carbamoyl-2-cyanacetamid (2)

Compound **1** (2.34 g, 23.6 mmol), urea (1.43 g, 23.6 mmol) and acetic anhydride (2.36 g, 25.8 mmol) were heated to 90°C for 2h. After 1h a white precipitate was formed. After cooling to room temperature, 200 mL of diethyl ether was added and the formation of a suspension was completed by treating the reaction mixture in an ultrasonic bath. Solid **2** was filtered off and dried in high vacuum.

Yield: 2.23g (67 %) as a pale yellow solid.

^{1}H-NMR (300 MHz, DMSO-d_6, 25°C): δ 4.03 (dq, 1H, $^{3}J_{HH}$ = 7.4 Hz, $^{2}J_{CH}$ = 10.8 Hz, *H*C^{13}C); 1.41 (dd, 3H, $^{3}J_{HH}$ = 7.4 Hz, $^{3}J_{CH}$ = 6.1 Hz, C*H*$_3$) ppm.

^{13}C-NMR (75 MHz, DMSO-d_6, 25°C): δ 168.5 (s, COOH); 119.1 (s, ^{13}CN); 31.14 (d, $^{1}J_{CC}$ = 59.4 Hz, CH); 14.90 (d,$^{2}J_{CC}$ = 2.0 Hz, CH_3) ppm.

(6-^{13}C)Thymine (3)

Pd/$BaSO_4$ (5%, 800 mg) was suspended in 10 mL 50% aqueous acetic acid in a 500 mL three-necked round bottom flask. The palladium catalyst was treated several times with hydrogen by evacuating the flask followed by spilling with hydrogen. Simultaneously, compound **2** (1.76g, 12.4 mmol) was dissolved in 40 mL of boiling 50% acetic acid and then added to the reduced palladium catalyst. The reaction

mixture was stirred at room temperature under a hydrogen atmosphere for 16h. Before being filtered through a bed of celite, the mixture was heated to 70°C for 1h. The solvent was evaporated until a white precipitate was formed. Precipitation of **3** was completed by storing the suspension at 4°C overnight. Compound **3** was filtered off and dried in high vacuum overnight.

Yield: 1.10 g (70 %) as a pale yellow solid.

^{1}H-NMR (300 MHz, DMSO-d_6, 25°C): δ 10.64(bs, 2H, N*H*); 7.24(d, 1H, $^{1}J_{CH}$ = 178.3 Hz, *H*^{13}C); 1.72 (d, 3H, $^{2}J_{CH}$ = 5.8Hz, CH_3) ppm.

^{13}C-NMR (75 MHz, DMSO-d_6, 25°C): δ 165.8 (C4); 152.4 (C2); 138.6 (^{13}C6); 108.5 (d, $^{1}J_{CC}$ = 70.5 Hz, C5); 12.6 (d, $^{2}J_{CC}$ = 4.9 Hz) ppm.

3´,5´-O-Bis toloyl-(6-^{13}C)-2´-deoxythymidine (4)

Compound **3** (1.0 g, 7.93 mmol) together with hexamethyldisilazane (30 mL) and trimethylsilylchloride (3.0 mL) was refluxed under an argon atmosphere at 120°C overnight. After cooling to room temperature, the mixture was evaporated to an oily residue, which was co-evaporated with a small amount of dry chloroform and then dried in high vacuum for 30min. The oil was dissolved in 40 mL of dry chloroform, Hoffer´s α-chlorosugar (3.06 g, 7.89 mmol, 1-chloro-2-deoxy-3,5-di-*O*-toluoyl-α-*D*-ribofuranose) was added at once and the mixture stirred for 4h at 40°C. After cooling to room temperature, the mixture was diluted with methylene chloride and washed twice with saturated sodium bicarbonate solution. The organic phase was dried over sodium sulfate and evaporated to dryness. The solid residue was re-crystallized from boiling ethanol and dried in high vacuum to give **4**.

Yield: 2.05 g (54%) as a white, crystalline solid.

TLC: (CH_2Cl_2/MeOH = 9/1) R_f = 0.7

^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 8.66 (d, 1H, $^{1}J_{CH}$ 180.0 Hz, C6*H*); 7.97 - 7.30 (m, 8H, C*H*(ar)); 6.49 (m, 1H, C1'*H*); 5.66 (d, 1H; C3'*H*); 4.75 (m, 2H, C5'*H*); 4.55(bs, 1H, C4'*H*); 2.72, 2.33 (m, 2H, C2'H_2); 2.45(s, 6H, 2 x ar-CH_3); 1.65(s, 3H, C5-CH_3) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$, 25°C): δ 166.22 (C q); 166.16 (C q); 163.59 (C4 q); 150.42 (C2 q); 144.69 (C q); 134.53 (^{13}C6); 129.96 (CH ar); 129.64 (CH ar); 129.59 (CH ar); 129.41 (CH ar); 126.68 (C q); 126.40 (C q); 111.78 (d, $^{1}J_{CC}$ = 77.1 Hz, C5H); 85.06 (C1'); 82.92 (C4'); 75.02 (C3'); 64.30 (C5'); 38.18 (C2'); 21.84 (C(ar)-CH_3); 21.80 (C(ar)-CH_3; 12.23 (C5-CH_3) ppm.

(6-^{13}C)-2´-Deoxythymidine (5)

Compound **4** (2.0 g, 4.18 mmol) was treated with 50 mL methylamine solution (33 wt % in absolute ethanol) and stirred at room temperature overnight. The reaction mixture was evaporated to an oily residue which was dissolved in a minimum (about 5 mL) of hot methanol. The methanolic solution was added with a pipette to 150 mL of a mixture of methylene chloride/diethyl ether/n-hexane (1/1/1) with stirring. A white precipitate was formed and precipitation was completed by storing the mixture 3h at 4°C. The suspension was filtered with suction, washed with methylene chloride and solid compound **5** was dried in high vacuum.

Yield: 920 mg (91 %) of a white solid.

TLC: (CH_2Cl_2/MeOH = 9/1) R_f = 0.2

^{1}H-NMR (300 MHz, DMSO-d_6, 25°C): δ 11.25 (bs, 1H, NH); 7.69 (d, 1H, $^1J_{CH}$ = 180.1 Hz, C6H); 6.17 (m, 1H, C1'H); 5.21 (d, 1H, C3'OH); 4.99 (t, 1H, C5'OH); 4.23 (m, 1H, C3'H); 3.76 (dt, 1H, C4'H); 3.56 (m, 2H, C5'H_2); 2.06 (m, 2H, C2'H_2); 1.77 (d, 3H, $^3J_{CH}$ = 6.3 Hz, CH_3) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$, 25°C): δ 163.71 (C q); 150.44 (C q); 136.08 (^{13}C6); 109.32 (C5); 87.24 (C4'); 83.74 (C1'); 70.42 (C3'); 61.33 (C5'); 39.40 (C2'); 12.22 (CH_3) ppm.

5'-O-(4,4-Dimethoxytrityl)(6-^{13}C)-2´-deoxythymidine (6)

Compound **5** (0.80 g, 3.29 mmol) together with one spatula (catalytic amount) of 4-(dimethylamino)pyridine was co-evaporated twice with anhydrous pyridine and then dissolved in 15 mL of anhydrous pyridine. Then 4,4'-dimethoxytrityl chloride (1.23 g, 3.62 mmol) was added in three portions within one hour and the mixture was stirred 3 h at room temperature or until TLC showed complete conversion. The mixture was quenched with 2 mL of methanol, evaporated to an oily residue and two times co-evaporated with toluene. The orange foam was dried in high vacuum for 30 min and dissolved in methylene chloride. The organic phase was washed two times with 5 % citric acid, once with saturated sodium bicarbonate solution, dried over sodium sulfate and evaporate to dryness. The crude product was applied onto a silica gel column with methylene chloride and eluted with a gradient from 0% to 5% methanol in methylene chloride.

Yield: 1.39 g (77 %) as a yellowish foam.

TLC: (CH_2Cl_2/MeOH = 9/1) R_f = 0.4

^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 9.27 (bs, 1H, N*H*); 7.61 (d, 1H, $^1J_{CH}$ = 181.0 Hz, ^{13}C6*H*) 7.45 – 7.29 (m, C*H*ar); 6.88 – 6.85 (m, C*H*ar); 6.45 (m, 1H, C1'*H*); 4.60 (m,

1H, C3'*H*); 4.09 (m, 1H, C4'*H*); 3.81(s, 6H, 2 x O-CH_3); 3.47 (m, 2H, C5'H_2); 2.97 (d, 1H, C3'O*H*); 2.38 (m, 2H, C2'H_2); 1.50 (d, 3H, $^3J_{CH}$ = 6.3 Hz, CH_3) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$, 25°C): δ 163.93 (C q); 158.86 (C q); 150.60; 144.46; 135.77 (s, ^{13}C6); 135.55 (C q); 135.50 (C q); 130.21 (CH ar); 128.26 (CH ar); 128.12 (CH ar); 127.27 (C q); 113.42 (CH ar); 111.42 (C5); 86.34 (C4'); 84.89 (C1'); 72.64 (C3'); 63.73 (C5'); 55.38 (2 x OCH_3); 41.08 (C2'); 11.95 (-CH_3) ppm.

5'-O-(4,4'-dimethoxytrityl)-2'-deoxythymidine-3'-O-[O-(2-cyanoethyl)-N,N'-diisopropylphosphoramidite] (29)

Compound **29** was synthesized according to the general procedure for the synthesis of phosphoramidites described below, using 6 (1.30 g, 2.39 mmol) and CEP-Cl (675 mg, 2.86 mmol). The product was eluted from the silica gel column using a gradient from 40 to 20 % n-hexane in ethyl acetate (+ 3% triethylamine).

Yield: 1.38 g (78 %) of a colorless foam.

TLC: (ethylacetate / n-hexane = 1/1 + 3 % NEt_3) R_f = 0.7

^{1}H-NMR (300 MHz, DMSO-d_6, 25°C): δ 8.53 (bs, 1H, N*H*); 7.60 (dd, 1H, $^1J_{CH}$ = 179.8 Hz, ^{13}C6*H*); 7.4 – 7.2 (m, C*H* ar); 6.85 – 8.81 (m, C*H* ar); 6.40 (m, 1H, C1'*H*); 4.64 (m, 1H, C3'*H*); 4.16(m, 1H, C4'*H*); 3.66 (m, 2H, -CH_2OP); 3.46 (m, 2H, C5'H_2); 2.62 – 2.42 (m, 2H, -CH_2CN); 2.44 (m, 2H, C2'H_2); 1.45 (d, 3H, $^3J_{CH}$ = 6.3 Hz, CH_3); 1.17 (d, 14H, iPr-C*H*, iPr-CH_3) ppm.

^{31}P-NMR (121 MHz, $CDCl_3$, 25°C): δ 149.62; 149.19 ppm.

ESI-MS: $[M+H]^+$ 746.3373 (calc. 746.3394)

Synthesis of (5-D-6-^{13}C)-2'-deoxy-N^4-Ac-cytidine 5´-DMT protected building block 27

3´,5´-O-Bis-toloyl-(5-D-6-^{13}C)-2´-deoxy uridine (8)

Compound **7** (1.0 g, 8.77 mmol) together with hexamethyldisilazane (30 mL) and trimethylsilylchloride (3.0 mL) was refluxed under an argon atmosphere at 120°C overnight. After cooling to room temperature, the mixture was evaporated to an oily residue, which was co-evaporated with a small amount of dry chloroform and then dried in high vacuum for 30min. The oil was dissolved in 40 mL of dry chloroform, Hoffer´s α-chlorosugar (3.06 g, 7.89 mmol, 1-chloro-2-deoxy-3,5-di-*O*-toluoyl-α-*D*-ribofuranose) was added at once and the mixture stirred for 4h at 40°C. After cooling to room temperature, the mixture was diluted with methylene chloride and washed twice with saturated sodium bicarbonate solution. The organic phase was dried over

sodium sulfate and evaporated to dryness. The solid residue was re-crystallized from boiling ethanol and dried in high vacuum to give **8**.

Yield: 1.96 g (48 %) as a white, crystalline solid.

TLC: (CH_2Cl_2/MeOH = 9/1) R_f = 0.4

^{1}H-NMR (300 MHz, DMSO-d_6, 25°C): δ 11.38 (s, 1H, N*H*); 7.92 - 7.31 (m, 8H, C*H* ar); 7.70 (d, 1H, $^1J_{CH}$ = 181.10 Hz, C6*H*); 6.27 (m, 1H, C1'*H*); 5.58 (m, 1H, C3'*H*); 4.57 (m, 2H, C5'*H*); 4.49 (m, 1H, C4'H); 2.58 (m, 2H, C2'*H*); 2.38 (2 x s, 6H, 2 x ar-CH_3) ppm.

^{13}C-NMR (75 MHz, DMSO-d_6, 25°C): δ 165.45 (C q); 165.18 (C q); 162.91 (C4 q); 150.32 (C2 q); 144.03 (C q); 143.86 (C q); 140.42 (^{13}C6); 129.42 (CH ar); 129.29 (CH ar); 126.52; 126.43; 84.83 (C1'); 81.21 (C4'); 74.44 (C3'); 64.13 (C5'); 36.04 (C2'); 21.14 (2 x CH_3) ppm.

(5-D-6-^{13}C)-2´-deoxyuridine (9)

Compound **8** (1.90 g, 4.07 mmol) was treated with 50 mL methylamine solution (33 wt% in absolute ethanol) and stirred at room temperature overnight. The reaction mixture was evaporated to an oily residue which was dissolved in a minimum (about 5 mL) of hot methanol. The methanolic solution was added with a pipette to 150 mL of a mixture of methylene chloride / diethyl ether / hexane (1:1:1) with stirring. A white precipitate was formed and precipitation was completed by storing the mixture 3h at 4°C. The suspension was filtered with suction, washed with methylene chloride and solid compound **9** was dried in high vacuum.

Yield: 937 mg (93 %) of a white solid.

TLC: (CH_2Cl_2/MeOH = 8/2) R_f = 0.3

^{1}H-NMR (300 MHz, DMSO-d_6, 25°C): δ 11.26 (bs, 1H, NH); 7.86 (d, 1H, $^1J_{CH}$ = 182.2 Hz, C6H); 6.15 (m, 1H, C1'H); 5.22 (d, 1H, C3'OH); 4.98 (t, 1H, C5'OH); 4.23 (m, 1H, C3'H); 3.78 (dt, 1H, C4'H); 3.56 (m, 2H, C5'H_2); 2.08 (m, 2H, C2'H_2) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$, 25°C): δ 163.13 (C q); 150.48 (C q); 141.33 (C5) 140.47 (^{13}C6); 87.39 (C4'); 84.13 (C1'); 70.44 (C3'); 61.29 (C5'); 39.67 (C2') ppm.

5'-O-(4,4'-dimethoxytrityl)-(5-D-6-^{13}C)-2´-deoxy uridine (10)

Compound **9** (0.90 g, 3.91 mmol) together with one spatula tip (catalytic amount) of 4-(dimethylamino)pyridine was co-evaporated twice with anhydrous pyridine and then dissolved in 15 mL of anhydrous pyridine. Then 4,4'-dimethoxytrityl chloride (1.45 g, 4.30 mmol) was added in three portions within one hour and the mixture was stirred 3 h at room temperature or until TLC showed complete conversion. The mixture was quenched with 2 mL of methanol, evaporated to an oily residue and two

times co-evaporated with toluene. The orange foam was dried in high vacuum for 30 min and dissolved in methylene chloride. The organic phase was washed two times with 5 % citric acid, once with saturated sodium bicarbonate solution, dried over sodium sulfate and evaporate to dryness. The crude product was applied onto a silica gel column with methylene chloride and eluted with a gradient from 0% to 5% methanol in methylene chloride.

Yield: 1.71 g (82 %) as a yellowish foam.

TLC: (CH_2Cl_2/MeOH = 9/1) R_f = 0.4

^{1}H-NMR (300 MHz, DMSO-d_6, 25°C): δ 11.38 (s, 1H, N*H*); 7.63 (d, 1H, $^1J_{CH}$ = 181.0 Hz, ^{13}C6*H*) 7.37 – 7.23 (m, C*H*ar); 6.90 – 6.89 (m, C*H*ar); 6.15 (m, 1H, C1'*H*); 5.24 (m, 1H, C3'*H*); 4.07 (m, 1H, C4'*H*); 3.74(s, 6H, 2 x O-CH_3); 3.34, 3.22 (m, 2H, C5'H_2); 2.42, 2.32 (m, 2H, C2'H_2) ppm.

^{13}C-NMR (75 MHz, DMSO-d_6, 25°C): δ 169.94(C q); 162.93 (C q); 158.14 (C q); 150.32 (C q); 144.58 (C q); 140.26 (^{13}C6); 135.32 (C q); 135.14 (C q); 129.71 - 126.80 (CH ar); 113.25 (CH ar); 85.97 (C5); 84.31 (C1'); 82.89 (C4'); 73.92 (C3'); 63.45 (C5'); 55.03 (2 x O*C*H_3); 36.39 (C2') ppm.

5'-O-(4,4'-dimethoxytrityl)-N4-(acetyl)-(5-D-6-^{13}C)-2´-deoxy cytidine (13)

Step 1:

Compound **10** (1.65 g, 3.10 mmol) together with one spatula tip (catalytic amount) of 4-(dimethylamino)pyridine was dissolved in 10 mL of dry pyridine. While stirring acetic anhydride (350 mg, 3.41 mmol) was added at 0°C. After stirring for 2h at room temperature TLC showed complete conversion. The mixture was evaporated to an oily residue, coevaporated twice with toluene and dried in high vacuum for 30 minutes. The oil was dissolved in methylene chloride, washed with saturated sodium bicarbonate solution, 5 % citric acid solution and water. The organic phase was dried over sodium sulfate, filtered and evaporated to dryness. The crude product was dried in high vacuum and used without further purification for the next step.

TLC: (CH_2Cl_2/MeOH = 9/1) R_f = 0.9

Step 2:

The intermediate from step 1, triethylamine (3.13 g, 31.0 mmol) and 4-(dimethylamino)pyridine (75 mg, 0.62 mmol) were dissolved in 25 mL of dry methylene chloride. 2,4,6-Triisopropylbenzenesulfonyl chloride (T*i*PS-Cl, 1.40 g, 4.65 mmol) was added in three portions within 20 minutes at room temperature. After stirring for 3h at room temperature TLC showed complete conversion. The mixture was diluted with methylene chloride, washed with saturated sodium bicarbonate

solution, the organic phase was dried over sodium sulfate, filtered and evaporated to dryness. The crude product was dried in high vacuum and used without further purification for the next step.

TLC: (CH_2Cl_2/MeOH = 9.5/0.5) R_f = 0.9

Step 3

The crude product from the previous step was dissolved in a mixture 25 mL of tetrahydrofurane and 35 mL of 30 % ammonium hydroxide solution. After stirring at room temperature overnight the mixture was evaporated to dryness and the brown foam dried in high vacuum for 30 minutes. The residue was dissolved in 30 mL of methylamine solution (33 wt% in absolute ethanol) and stirred at room temperature overnight. The mixture was evaporated to an oily residue which was dissolved in methylene chloride. The organic phase was washed with saturated sodium bicarbonate solution, dried over sodium sulfate, evaporated to dryness and dried in high vacuum. The crude product was applied to a silica gel column with methylene chloride and eluted using a gradient of 0 to 10% methanol in methylene chloride to give compound **12** as a yellowish foam with 2,4,6-triisopropylbenzenesulfonic acid (T*i*PS-OH) as impurity.

TLC: (CH_2Cl_2/MeOH = 9/1) R_f = 0.2

Step 4

To a solution of compound **12** in 15 mL of dry dimethylformamide was added acetic anhydride (253 mg, 2.48 mmol) at once and the mixture was stirred overnight at room temperature. TLC showed complete conversion, the reaction was quenched with a small amount of methanol and evaporated to dryness. The oily residue was dissolved in methylene chloride, the organic phase washed one time with saturated sodium bicarbonate solution, twice with saturated sodium chloride solution, dried over sodium sulfate, filtered and evaporated to dryness. The yellow foam was dried in high vacuum, applied to a silica gel column with methylene chloride and eluted using a gradient of 0 to 5 % methanol in methylene chloride.

Yield: 853 mg (48 % referred to compound **10**) as a yellowish foam.

TLC: (CH_2Cl_2/MeOH = 9/1) R_f = 0.4

^{1}H-NMR (300 MHz, DMSO-d_6, 25°C): δ 10.86 (s, 1H, N*H*); 8.12 (d, 1H, $^1J_{CH}$ = 182.3 Hz, ^{13}C6*H*) 6.90 – 6.87 (m, C*H*ar); 6.12 (m, 1H, C1'*H*); 4.28 (m, 1H, C3'*H*); 3.95 (m, 1H, C4'*H*); 3.74(s, 6H, 2 x O-CH_3); 3.26 (m, 2H, C5'H_2); 2.32, 2.14 (m, 2H, C2'H_2); 2.09 (s, 3H, Ac-CH_3) ppm.

^{13}C-NMR (75 MHz, DMSO-d_6, 25°C): δ 170.93 (C q); 162.20 (C q); 158.15 (C q); 154.31 (C q); 145.54 (C q) 144.44 (^{13}C6); 135.37 (C q); 135.26 (C q); 129.74 - 126.79 (CH ar); 113.25 (CH ar); 85.91, 85.8, 85.69 (C5, C1' and C4'); 69.33 (C3'); 62.90 (C5'); 54.99 (2 x O*C*H$_3$); 40.76 (C2'); 24.31 (Ac-CH$_3$) ppm.

5'-O-(4,4'-dimethoxytrityl)-N-acetyl-2'-deoxycytidine 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]phosphoramidite (27)

Compound **27** was synthesized according to the general procedure for the synthesis of phosphoramidites described below, using **13** (2.0 g, 3.49 mmol) and CEP-Cl (988 mg, 4.19 mmol). The product was eluted from the silica gel column using a gradient from 30 to 0 % n-hexane in ethyl acetate (+ 3% triethylamine).

Yield: 1.90 g (70 %) of a colorless foam.

TLC: (ethylacetate / n-hexane = 3/7 + 3 % NEt$_3$) R_f = 0.3

^{1}H-NMR (300 MHz, DMSO-d_6, 25°C): δ 9.86 (b, 1H, N*H*); 8.27 (2 x d, $^1J_{CH}$ = 182.1 Hz, ^{13}C6*H*); 7.38 – 7.26 (m, C*H* ar); 6.85 – 6.81 (m, C*H* ar); 6.29 (m, 1H, C1'*H*); 4.59 (m, 1H, C3'*H*); 4.20 (b, 1H, C4'*H*); 3.60 (m, 2H, C*H*$_2$OP); 3.56, 3.44 (m, 2H, C5'*H*$_2$); 2.74, 2.32 (m, 2H, C2'*H*$_2$); 2.59, 2.43 (2 x t, 2H, C*H*$_2$CN); 2.26 (s, 3H, Ac-C*H*$_3$); 1.15 – 1.06 (m, 14H, iPr-C*H*, iPr-C*H*$_3$) ppm.

^{31}P-NMR (121 MHz, $CDCl_3$, 25°C): δ 150.67; 150.10 ppm.

ESI-MS: $[M+H]^+$ 774.3542 (calc. 774.3566)

Synthesis of (8-^{13}C)-2'-deoxy-N^2-Ac-guanosine 5´-DMT protected building block 19

3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-(8-^{13}C)-N^2-iso-butyryl-guanosine (15)

Compound **14** (3.0 g, 8.47 mmol) was co-evaporated twice with dry pyridine and dissolved in 40 mL of dry pyridine. Then 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (T*i*PDSiCl_2, 2,20 g, 3,69 mmol) was added in three portions and the mixture was stirred for 3h at room temperature. As TLC showed complete conversion, the reaction was quenched with 1 mL of methanol, all solvents were evaporated and co-evaporated three times with toluene. After drying the crude product in high vacuum, it was dissolved in methylene chloride, washed twice with 5% citric acid, once with saturated sodium bicarbonate solution, before the organic phase was dried over sodium sulfate. After filtration and evaporation of the solvents, the crude product was purified by column chromatography. The crude product was applied to a silica gel column with methylene chloride and eluted with a gradient from 0 to 7 % methanol in methylene chloride.

Yield: 2.2 g (44 %) as a slightly yellowish foam.

TLC: (CH_2Cl_2/MeOH = 9.5/0.5) R_f = 0.5

^{1}H-NMR (700 MHz, DMSO, 25°C): δ 12.11 (s, 1H, NH); 11.74 (s, 1H, NHCO); 8.07 (d, 1H, $^1J_{CH}$ = 214.32 Hz, ^{13}C8H); 5.79 (d, 1H, C1'H); 5.68 (m, 1H, C2'OH); 4.36 (dd, 1H, C3'H); 4.31 (m, 1H, C2'H_2); 4.13 (dd, 1H, C5'H_2); 4.04 (m, 1H, C4'H); 3.95 (dd, 1H, C5'H_2); 2.78 (m, 6H, $CH(CH_3)_2$); 1.23 (d, 6H, $CH(CH_3)_2$); 1.08-0.95 (m, 28H, $CH(CH_3)_2$) ppm.

^{13}C-NMR (75 MHz, DMSO-d_6, 25°C): δ 136.54 (C8); 88.59 (C1'); 81.92 (C4'); 74.42 (C2'); 69.91 (C3'); 61.12 (C5'); 35.70 (iPr C); 19.27 – 19.35 (iPr C); 17.89 - 17.17 (iPr C); 13.46 - 12.32 (iPr C) ppm.

3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-2'-O-(1-imidazole-carbothiolate)-(8-^{13}C)-N^2-iso-butyryl-guanosine (16)

Compound **15** (2.00 g; 3.35 mmol) was dissolved in 20 mL of dry DMF and 1,1'-thiocarbonyldiimidazole (0.776 g; 4.36 mmol) was added. The mixture was stirred for 6 h at room temperature under argon atmosphere. After the reaction, DMF was removed at reduced pressure. The oily residue has been dissolved in methylene chloride, was washed with saturated sodium chloride solution three times, dried over sodium sulfate, filtered and evaporated to dryness. The obtained crude product was used in the next step without further purification.

Yield: 2.47 g (quantitative) as a yellowish foam.

TLC: (CH_2Cl_2/MeOH = 9.5/0.5) R_f = 0.55

3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-(8-^{13}C)-2'-deoxy-N^2-iso-butyryl-guanosine (17)

Compound **16** (1.97 g, 2.79 mmol) was dissolved in 30m dry toluene and the mixture was degassed with a continuous stream of argon for 20 minutes. Next 2,2'-azobis-(2-methylpropionitrile) (AIBN, 91,52 mg, 0.557 mmol) and tributyltin hydride (1.22 g, 4.18 mmol) was added and degassing was continued for another 20 minutes. The degassed mixture was heated to 75 °C for 2h until TLC showed complete conversion. The mixture was evaporated to an oily residue, dissolved in methylene chloride, was washed with saturated sodium bicarbonate and saturated sodium chloride solution, dried over sodium sulfate, evaporated, filtered and dried in high vacuum. Crude compound 17 was purified by column chromatography using a gradient of 0% to 3% methanol in methylene chloride.

Yield: 1.50 g (93 %) as a yellow foam.

TLC: (CH_2Cl_2/MeOH = 9/1) R_f = 0.65

^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 12.18 (s, 1H, NH); 9.26 (s, 1H, NHCO); 8.25 (d, 1H, $^{1}J_{CH}$ = 213.39 Hz, ^{13}C8H); 6.11 (m, 1H, C1'H); 4.79 (dd, 1H, C3'H); 4.02 (d, 1H, C5'H_2); 3.83 (m, 1H, C4'H); 2.77 (m, 6H, $CH(CH_3)_2$); 2.55 (m, 1H, C2'H_2); 1.27 (m, 6H, $CH(CH_3)_2$); 1.24-0.96 (m, 28H, $CH(CH_3)_2$) ppm.
^{13}C-NMR (75 MHz, DMSO, 25°C): δ 136.72 (C8); 84.50 (C4'); 81.32 (C1'); 70.81 (C3'); 61.92 (C5'); 34.72 (OCH_3); 38.76 (C2'); 19.08 - 16.58 ($CH(CH_3$); 12.88 - 11.75 ($CH(CH_3)_2$) ppm.

(8-^{13}C)-2'-deoxy-N^2-iso-butyryl-guanosine (18)

Compound **17** (2.69 g, 2.69 mmol) was dissolved in 30 mL of dry tetrahydrofuran and triethylamine trihydrofluoride (1,49 g, 9.26 mmol) was added. The solution was stirred 4h at room temperature. Next, the solvent was evaporated and the crude compound co-evaporated with methanol. After drying the crude product in high vacuum it was purified by column chromatography using a gradient of 2% to 20% methanol in methylene chloride.

Yield: 1.55 g (99 %) of a pale yellow solid.

TLC: (CH_2Cl_2/MeOH = 9/1) R_f = 0.14

^{1}H-NMR (300 MHz, DMsolventO-d_6, 25°C): δ 12.07 (s, 1H, NH); 11.66 (s, 1H, NHCO); 8.22 (d, 1H, $^{1}J_{CH}$ = 214.66 Hz, ^{13}C8H); 6.20 (m, 1H, C1'H); 4.36 (s, 1H, C3'H); 3.83 (m, 1H, C4'H); 3.53 (m, 1H, C5'+5''H); 2.76 (m, 6H, $CH(CH_3)_2$); 2.56 + 2.27 (m, 2H, C2'H + C2''H); 1.14 - 1.07 (m, 6H, $CH(CH_3)_2$) ppm.
^{13}C-NMR (75 MHz, DMSO-d_6, 25°C): δ 137.36 (C8); 87.71 (C4'); 82.92 (C1'); 70.31 (C3'); 61.23 (C5'); 40.95 (C2'); 34.73 ($CH(CH_3$); 18.84 ($CH(CH_3$) ppm.

5'-O-(4,4-Dimethoxytrityl)(8-^{13}C)-2'-deoxy-N^2-iso-butyryl-guanosine (19)

Compound **18** (1.55 g, 4.58 mmol) together with a catalytic amount of 4-(dimethylamino)pyridine was co-evaporated twice with anhydrous pyridine and then dissolved in 20 mL of anhydrous pyridine. 4,4'-Dimethoxytrityl chloride (1.86 g, 5.50 mmol) was dissolved in 10 ml of dry pyridine and added dropwise over a dropping funnel within 15 minutes. After the addition stirring was continued for 2.5h until TLC confirmed the complete conversion. The obtained mixture was directly washed with saturated sodium bicarbonate solution, dried over sodium sulfate and evaporates to dryness. The crude product was purified by column chromatography using a gradient of 0% to 8% methanol in methylene chloride

Yield: 2.00 g (68 %) of a yellow foam.

TLC: (CH_2Cl_2/MeOH = 9/1) R_f = 0.6

^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 12.30 (s, 1H, NH); 9.80 (s, 1H, NHCO); 7.90 (d, 1H, $^{1}J_{CH}$ = 212.88 Hz, ^{13}C8H); 7.44-7.10 (m, 9H, C(arom)H); 6.80-6.70 (m, 4H, C(arom)H); 6.21 (m, 1H, C1'H); 4.75 (s, 1H, C3'H); 4.20 (m, 1H, C4'H); 3.72 (s, 6H, C(arom)OCH_3); 3.35-3.27 (m, 1H, C5'+5"H); 2.53 (m, 1H, $CH(CH_3)_2$); 2.50 (2d, 1H, C2"H); 1.17 - 1.07 (m, 6H, $CH(CH_3)_2$) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$, 25°C): δ 138.5 (C8); 129.51 (ar CH); 127.63 (ar CH); 127.63 (ar CH); 126.45 (ar CH); 112.65 (ar CH); 86.03 (C4'); 84.82 (C1'); 71.51 (C3'); 64.10 (C5'); 51.40 (OCH_3); 40.72 (C2'); 36.71 ($CH(CH_3$); 19.3 ($CH(CH_3)_2$) ppm.

N^2-iso-butyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine-3'-O-[O-(2-cyanoethyl)-N,N'-diisopropylphosphoramidite] (28)

Compound **28** was synthesized according to the general procedure for the synthesis of phosphoramidites described below, using compound 19 (2 g, 3.12 mmol) and CEP-Cl (1.48 g, 6.24 mmol). The crude product was purified twice by column chromatography using a gradient of 20% to 50% ethyl acetate in hexane (+ 3% triethylamine).

Yield: 1.20 g (46 %) of a colorless foam.

TLC: (ethylacetate / n-hexane = 8/2 + 3 % NEt_3) R_f = 0.4

^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 11.95 (s, 1H, NH); 8.51 (s, 1H, NHCO); 7.79 + 7.74 (d, 1H, $^{1}J_{CH}$ = 214.40 Hz; ^{13}C8H); 7.48 - 7.15 (m, CH ar); 6.82 – 6.73 (m, CH ar); 6.18 (m, 1H, C1'H); 4.85-4.64 (m, 1H, C3'H); 4.32 - 4.20 (m, 1H, C4'H); 3.77 (s, 6H, C(arom)OCH_3); 3.59 (m, 2H, CH_2OP); 3.36 - 3.21 (m, 2H, C5'H_2); 2.99 - 2.73 (m, 2H, C2'H_2); 2.29 - 2.03 (m, 1H, $CH(CH_3)_2$); 1.19 – 1.10 (m, 6H, $CH(CH_3)_2$); 2,77 – 2.35 (2 x m, 2H, CH_2CN); 1.09 – 0.95 (m, 6H, $CH(CH_3)_2$) ppm.

^{31}P-NMR (121 MHz, $CDCl_3$, 25°C): δ 149.34, 148.62 ppm.

ESI-MS: $[M+H]^+$ 841.3859 (calc. 841.3878)

Synthesis of (8-^{13}C)-2'-deoxy-N^6-Bz-adenosine 5´-DMT protected building block 26

3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-(8-^{13}C)-N^6-Bz-adenosine (21)

Compound **20** (2.0 g, 5.39 mmol) was co-evaporated two times with dry pyridine and dissolved in 16 mL of dry pyridine. Then 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (T*i*PDSiCl_2, 1.87 g, 5.93 mmol) was added and the mixture was stirred for 3 h at room temperature. The reaction was quenched with 1 mL of methanol, evaporated to an oily residue and co-evaporated twice with toluene. The yellow foam was dried in high vacuum for 30 minutes, dissolved in methylene chloride, washed two times with 5% citric acid and once with saturated sodium bicarbonate solution. The organic

phase was dried over sodium sulfate, evaporated and dried in high vacuum. The crude product was applied to a silica gel column with methylene chloride and eluted with a gradient from 0 to 7 % methanol in methylene chloride.

Yield: 2.54 g (77 %) as a yellowish foam.

TLC: (CH_2Cl_2/MeOH = 9/1) R_f = 0.4

^{1}H-NMR (300 MHz, DMSO-d_6, 25°C): δ 11.21 (bs, 1H, N*H*); 8.68 (s, 1H, C2*H*); 8.55 (d, 1H, $^1J_{CH}$ = 214.60 Hz, ^{13}C8*H*); 8.05 (d, 2H, ar-C*H*); 7.66 - 7.55 (m, 3H, ar-C*H*); 6.02 (m, 1H, C1'*H*); 5.67 (m, 1H, C2'O*H*); 4.84 (m, 1H, C3'*H*); 4.67 (m, 1H, C2'*H*); 4.06 (m, 1H, C4'*H*) 4.06 - 3.96 (m, 2H, C5'H_2), 1.06 (m, 28H, 4 x iPr) ppm.

^{13}C-NMR (75 MHz, DMSO-d_6, 25°C): δ 165.57 (C q); 151.44 (C2); 150.42 (C q); 143.08 (^{13}C8); 133.30 (C q); 132.42 (CH ar); 128.46 (CH ar); 128.42 (CH ar); 125.96 (C q); 89.51 (C1'); 80.95 (C4'); 73.37 (C2'); 69.89 (C3'); 60.75 (C5'); 17.31 - 17.00 (iPr C); 12.73 - 12.05 (iPr C) ppm.

3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-2'-O-(1-imidazole-carbothiolate)-(8-^{13}C)-N^6-Bz-adenosine (22)

Compound **21** (2.50 g; 4.08 mmol) together with 1,1'-thiocarbonyldiimidazole (0.871 g; 4.89 mmol) was dissolved in 15 mL of dry DMF and stirred for 4 h at room temperature. The solvent was removed at reduced pressure and the oily residue dissolved in methylene chloride. The organic phase was washed with saturated sodium chloride solution three times, dried over sodium sulfate and evaporated to dryness. The crude product was applied to a silica gel column with methylene chloride and eluted with a gradient from 0 to 5 % methanol in methylene chloride.

Yield: 2.60 g (88 %) as a yellowish foam.

TLC: (CH_2Cl_2/MeOH = 9/1) R_f = 0.6

^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 9.04 (bs, 1H, N*H*); 8.74 (s, 1H, C2*H*); 8.39 (s, 1H, imidazole *H*); 8.13 (d, 1H, $^1J_{CH}$ = 213.90 Hz, ^{13}C8*H*); 8.02 (m, 2H, ar-C*H*); 7.68 (s, 1H, imidazole *H*); 7.62 - 7.51 (m, 3H, ar-C*H*); 7.09 (s, 1H, imidazole *H*); 6.45 (m, 1H, C2'*H*); 6.21 (m, 1H, C1'*H*); 5.51 (m, 1H, C3'*H*); 4.14 (m, 1H, C4'*H*); 4.21 - 4.08 (m, 2H, C5'H_2); 1.13 - 0.97 (m, 28H, 4 x iPr) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$, 25°C): δ 183.13 (C q); 164.63 (C q); 153.02 (C2); 151.23 (d, C q); 149.95 (d, C q); 142.26 (^{13}C8); 136.98 (C imidazole); 133.69 (C q); 133.00(CH ar); 131.46 (C imidazole); 129.05 (CH ar); 127.99 (CH ar); 123.80 (C q); 123.78 (C q); 118.23 (C imidazole); 87.64 (C1'); 83.63 (C2'); 82.66 (C4'); 69.75 (C3'); 60.63 (C5'H_2); 17.52 - 17.04 (iPr C); 13.33 - 12.79 (iPr C) ppm.

3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-(8-^{13}C)-2'-deoxy -N^6-Bz-adenosine (23*)*

Compound **22** (2.50 g, 3.45 mmol) together with 2,2'-azobis(2-methylpropionitrile) (AIBN, 113 mg, 0.690 mmol) and tributyltin hydride (1.51 g, 5.18 mmol) was dissolved in 80 mL of dry toluene. After degassing the solution by passing a continuous stream of argon through the mixture for 10 minutes, the mixture was heated to 75 °C for 1.5h with stirring. The mixture was evaporated to an oily residue which was dissolved in methylene chloride. The organic phase was washed with saturated sodium bicarbonate solution, dried over sodium sulfate, evaporated and the yellowish oil dried in high vacuum for 30 minutes. The crude product was applied to a silica gel column with methylene chloride and eluted with a gradient from 0 to 5 % methanol in methylene chloride.

Yield: 1.87 g (91 %) as a yellow foam.

TLC: (CH_2Cl_2/MeOH = 9/1) R_f = 0.7

^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 9.02 (bs, 1H, N*H*); 8.77 (s, 1H, C2*H*); 8.21(d, $^1J_{CH}$ = 213.30 Hz, 1H, ^{13}C8*H*); 8.01 (m, 2H, ar-C*H*); 7.60 - 7.51 (m, 3H, ar-C*H*); 6.35 (d, 1H, C1'*H)*; 4.99 (dd, 1H, C3'*H*); 4.05 (d, 1H, C5'H_2); 3.92 (m, 1H, C4'*H*); 2.80 - 2.66 (m, 1H, C2'H_2); 1.11 - 1.04 (m, 28H, 4 x iPr) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$, 25°C): δ 164.68 (C q); 152.71 (C2); 151.06 (C q); 149.64 (C q); 142.16 (C q); 141.66 (^{13}C8); 140.94 (C q); 133.86 (C q); 132.89 (ar CH); 129.01 (ar CH); 127.96 (ar CH); 85.47 (C4'); 83.58 (C1'); 70.10 (C3'); 61.95 (C5'); 40.15 (C5'); 17.54 - 17.03 (iPr C); 13.51 - 12.68 (iPr C) ppm.

(8-^{13}C)-2'-deoxy-N^6-Bz-adenosine (24)

Compound **23** (1.80 g, 3.02 mmol) was dissolved in 20 mL of dry tetrahydrofuran, triethylamine trihydrofluoride (631 mg, 3.92 mmol) was added at once and the mixture was heated for 2h at 45 °C. The mixture was evaporated to an oily residue, dried in high vacuum for 30 minutes and dissolved in a minimum of hot methanol. The methanolic solution was added with a pipette to 150 mL of a mixture of methylene chloride / diethyl ether / hexane (1:1:1) with stirring. A white precipitate was formed and precipitation was completed by storing the mixture for 3h at 4°C. The suspension was filtered with suction, washed with a small amount of methylene chloride and solid compound 24 was dried in high vacuum.

Yield: 772 mg (72 %) of a pale yellow solid.

TLC: (CH_2Cl_2/MeOH = 9/1) R_f = 0.2

^{1}H-NMR (300 MHz, DMSO-d_6, 25°C):

δ 11.17 (s, 1H, N*H*); 8.75 (s, 1H, C2*H*); 8.68 (d, 1H, $^1J_{CH}$ = 215.30 Hz, ^{13}C8*H*); 8.05 (m, 2H, CH ar); 7.66 - 7.54 (m, 3H, CH ar); 6.49 (m, 1H, C1'*H*) 5.35 (d, 1H, C3'O*H*);

5.00 (t, 1H, C5'O*H*); 4.46 (m, 1H, C3'*H*); 3.91 (m, 1H, C4'*H*); 3.66 - 3.53 (m, 2H, C5'H_2); 2.80, 2.37 (m, 2H, C2'H_2) ppm.

^{13}C-NMR (75 MHz, DMSO-d_6, 25°C): δ 165.56 (C q); 151.51 (C2); 143.02 (^{13}C8); 133.33 (C q); 132.39 (ar CH); 128.43 (ar CH); 125.89 (C q); 87.96 (C4'); 83.70 (C1'); 70.66 (C3'); 61.58 (C5'); 40.06 (C2') ppm.

5'-O-(4,4-Dimethoxytrityl)-(8-^{13}C)-2'-deoxy-N^6-Bz-adenosine (25)

Compound **24** (700 mg, 1.96 mmol) together with one spatula tip (catalytic amount) of 4-(dimethylamino)pyridine was co-evaporated twice with anhydrous pyridine and then dissolved in 15 mL of anhydrous pyridine. 4,4'-Dimethoxytrityl chloride (800 mg, 2.36 mmol) was added in three portions within one hour and the mixture was stirred 3 h at room temperature or until TLC showed complete conversion. The mixture was quenched with 2 mL of methanol, evaporated to an oily residue and two times co-evaporated with toluene. The orange foam was dried in high vacuum for 30 min and dissolved in methylene chloride. The organic phase was washed two times with 5 % citric acid, once with saturated sodium bicarbonate solution, dried over sodium sulfate and evaporate to dryness. The crude product was applied onto a silica gel column with methylene chloride and eluted with a gradient from 0% to 5% methanol in methylene chloride.

Yield: 1.02 g (79 %) of a pale yellow foam.

TLC: (CH_2Cl_2/MeOH = 9/1) R_f = 0.6

^{1}H-NMR (300 MHz, DMSO-d_6, 25°C): δ 11.17 (s, 1H, N*H*); 8.66 (s, 1H, C2*H*); 8.57 (d, 1H, $^1J_{CH}$ = 213.65 Hz, ^{13}C8*H*); 8.05 (m, CH ar); 7.66 - 7.54 (m, CH ar); 7.33 - 7.19 (m, CH ar); 6.83 - 6.79 (m, CH ar); 5.42; 4.51 (m, 1H, C3'*H*); 4.03 (m, 1H, C4'*H*); 3.71 (s, 6H, 2 x OCH_3) 3.20 (m, 2H, C5'H_2); 2.95, 2.41 (m, 2H, C2'H_2) ppm.

^{13}C-NMR (75 MHz, DMSO-d_6, 25°C): δ 158.00 (C q); 151.39 (C2); 145.91 (C q); 143.23 (C8); 135.61 (C q); 135.49 (C q); 129.63 (ar CH); 128.46 (ar CH); 128.43 (ar CH); 127.70 (ar CH); 113.08 (ar CH); 85.95 (C4'); 83.76 (C1'); 70.61 (C3'); 64.01 (C5'); 54.97 (OCH_3); 38.48 (C2') ppm.

N^6-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenosine-3'-O-[O-(2-cyanoethyl)-N,N'-diisopropylphosphoramidite] (26)

Compound **26** was synthesized according to the general procedure for the synthesis of phosphoramidites described below, using 25 (900 mg, 1.37 mmol) and CEP-Cl (485 mg, 2.05 mmol). The product was eluted from the silica gel column using a gradient from 50 to 20 % n-hexane in ethyl acetate (+ 3% triethylamine).

Yield: 845 mg (72 %) of a colorless foam.

TLC: (ethylacetate / n-hexane = 8/2 + 3 % NEt_3) R_f = 0.3

^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 8.93; 8.75; 8.19 (dd, 1H, $^1J_{CH}$ = 212.30 Hz; ^{13}C8*H*); 8.02 (m, CH ar); 7.63 - 7.21 (m, CH ar); 6.81 - 6.77 (m, CH ar); 6.51 (m, 1H, C1'*H*); 4.79 (m, 1H, C3'*H*); 4.33(m, 1H, C4'*H*); 3.88, 3.71 (m, 2H, CH_2OP); 3.77 (s, 6H, 2 x OCH_3); 3.66 (m, 2H, 2 x iPr CH); 3.41 (m, 2H, C5'H_2); 2.96, 2.75 (m, 2H, C2'H_2); 2.62, 2.47 (2 x t, 2H, CH_2CN); 1.29 - 1.12 (m, 12H, 2 x iPr CH_3) ppm.

^{31}P-NMR (121 MHz, $CDCl_3$, 25°C): δ 149.62, 149.46 ppm.

ESI-MS: $[M+H]^+$ 859.3751 (calc. 859.3772)

General procedure for the synthesis of 3'-O-[O-(2-cyanoethyl)-N,N'-diisopropylphosphoramidites]

The corresponding 5'-DMT protected 2'-deoxynucleoside (1.0 eq, 1.00 mmol) together with *N*,*N*-diisopropylethylamine (10 eq, 10.0 mmol, 1.29 g) was dissolved in 10 mL of dry methylene chloride and sealed with a septum under an argon atmosphere. Then, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (CEP-Cl, 1.2 to 2.0 eq.) was added with a syringe and the mixture was stirred at room temperature for 3h or until TLC showed complete conversion. The reaction was quenched by adding 2 mL of dry methanol and stirring was continued for 10 minutes. The mixture was diluted with methylene chloride, washed with saturated sodium bicarbonate solution, the organic phase was dried over sodium sulfate, evaporated to dryness and dried in high vacuum for 30 minutes. The crude product was applied onto a silica gel column and eluted with a mixture of hexane/ethylacetate (+ 3% triethylamine) as described for the respective compound.

Expression of NCp7 peptide

For the protein expression, the plasmid was transformed into BL21(DE3) E. coli cells and 20 mL of LB medium (1% (w/v) tryptone/peptone, 0.5% (w/v) yeast extract; 1% (v/v) glycerol; 2 mM $MgSO_4$; 15 mM $(NH_4)_2SO_4$; 10 mM NaP, pH 8.0) supplemented with 100 µg/ml ampicillin were inoculated with a single colony from a LB-agar plate overnight culture. The LB culture was grown at 37°C for 16 hours. Then, 20 mL of the LB starter culture were added to 2 L of M9 minimal medium supplemented with 0.1 mM $ZnCl_2$ containing $^{15}NH_4Cl$ and/or $^{13}C_6$-glucose. The M9 culture was grown at 37 °C until an OD^{600} of 0.5 to 0.6 was reached. Over-expression was then induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside. After induction, the bacteria were allowed to grow for another 3 hours and were then harvested.

For cell lysis, the pellets were re-suspended in 30 mL of lysis buffer (50 mM Tris-HCl (pH 8.0), 10% (v/v) glycerol, 0.1 M NaCl, 0.1 mM $ZnCl_2$, 5 mM dithiothreitol, 2 mM

EDTA) and 170 µL of 10 mM PMSF (phenylmethylsulfonyl fluoride) and 2 mL of 1% (w/v) sodium deoxycholate were added. Cell lysis was completed via ultrasonic treatment and 4% (w/v) polyethyleneimine (pH 7.9) was added dropwise till the concentration reached a final value of 0.4%. The lysis suspension was then shaken for 20 minutes, followed by centrifugation (9600g, 40 minutes, 4°C) to separate insoluble components. The supernatant was filtered through a 45 µm syringe filter and loaded onto a strong anion exchanger column (2 x 5mL HiTrap Q FF, GE Healthcare) connected in series with a strong cation exchanger column (2 x 5mL HiTrap SP XL, GE Healthcare). Before loading the supernatant with a flowrate of 1 mL min^{-1} the columns were equilibrated with 300 mL of bufferA [50 mM Tris-HCl (pH 8.0), 10% glycerol, 0.1 M NaCl, 0.1 mM $ZnCl_2$, 10 mM BME (β-mercaptoethanol)]. After the sample application, the columns were washed with 60 mL of buffer A. Subsequently, the HiTrap Q FF columns were detached and the HiTrap SP XL columns were washed with another 40 mL of buffer A. To elute the NCp7 protein, a gradient from 0% to 50% buffer B (50 mM Tris-HCl (pH 8.0), 10% glycerol, 1.0 M NaCl,0.1 mM $ZnCl_2$, 10 mM BME) in 20 minutes with a flowrate of 5 mL min^{-1} was run. The $^{13}C/^{15}N$ or ^{15}N labeled NCp7 was collected by fractionation (fraction volume 5 mL). The three fractions containing NCp7 were identified by SDS PAGE and pooled. In the final purification step the peptide was loaded on a HiLoad 26/600 Superdex 75 pg size exclusion column (GE Healthcare) and exchanged into buffer C (50 mM Tris-HCl, pH 7.0, 10% glycerol, 0.1 M NaCl, 0.1 mM $ZnCl_2$, 10 mM BME). Finally, the NCp7 peptide was concentrated in a 3 kDa cutoff centrifugation unit from Millipore.

For NMR measurements, buffer C was replaced with NMR buffer (15 mM NaP, 25 mM NaCl, 0.1 mM $ZnCl_2$, 10% D_2O, pH 6.5) via multiple cycles of concentration and dilution using a 3 kDa cutoff centrifugator (Millipore).

NMR sample preparation

DNA samples were lyophilized as the sodium salts and dissolved in the respective buffer.

HIV-1 PPT DNA 30, 31 and 32

0.6 mM, 15 mM sodium phosphate buffer, 25 mM NaCl, pH 6.5, 9/1 H_2O/D_2O.

mini-cTAR DNA 33

1 mM DNA, 15 mM sodium phosphate buffer, 25 mM NaCl, 0.1 mM $ZnCl_2$, pH 6.5, 9/1 H_2O/D_2O.

1 mM DNA, 1 eq. NCp7, 15 mM sodium phosphate buffer, 25 mM NaCl, 0.1 mM $ZnCl_2$, pH 6.5, 9/1 H_2O/D_2O.

Full-length cTAR DNA 34

0.4 mM, 15 mM sodium phosphate buffer, 25 mM NaCl, pH 6.5, 9/1 H_2O/D_2O.

G-quadruplex DNAs 35 and 36

0.6 (35) and 0.7 (36) mM, 20 mM potassium phosphate buffer, 70 mM KCl, pH 7, 9/1 H_2O/D_2O.

4-way junction J9a 37 and 38

0.8 mM, 15 mM sodium cacodylate buffer, 25 mM NaCl, pH 6.5, 9/1 H_2O/D_2O.

For the T_1/ZZ-exchange experiments the sample was lyophilized together with the buffer salts and re-dissolved in pure D_2O.

NMR spectroscopy

NMR experiments on ^{13}C-modifed DNA sequences were conducted either on a Bruker 600 MHz Avance II+ with a Prodigy TCI™ probe or on an Agilent DD2 500 MHz with a room temperature triple resonance probe instrument.

The 2D ^{1}H-^{13}C correlation spectra were acquired using either a ^{1}H-^{13}C-BEST TROSY pulse sequence (provided by Bernhard Brutscher, IBS Grenoble) or a standard Bruker pulse program (*hsqcetgpsisp2.2*). The HMBC spectrum of DNA **32** was acquired using the standard Bruker pulse sequence including a water pre-saturation element (*hmbcgplpndprqf*). For the ^{13}C-filtered NOESY experiment the standard pulse program from the Bruker library was used (*noesyhsqcetgpsi3d*).

^{13}C-CPMG relaxation dispersion experiments of the mini-cTAR DNA **33** were acquired at 11.7 and 14.1 T using a previously published pulse sequence with CPMG field strengths of 100, 200, 300, 300, 400, 500, 500, 600, 700, 800, 800, 900 and 1000 Hz. The relaxation delay τ_{CP} was set to 20 ms. 1024*96 (1024*96) complex data points were recorded at 11.7 (14.1) T. The number of transients was set to 32, and the inter-scan delay was set to 1.5 s yielding total experimental times of 16h at each field strength.

A preliminary ^{13}C-CPMG relaxation dispersion experiment of the full-length cTAR DNA **34** was acquired at 14.1 T with CPMG field strengths of 100, 200, 300, 300, 400, 500, 500, 600, 700, 800, 800, 900 and 1000 Hz. The relaxation delay τ_{CP} was set to 20 ms. 1024*96 complex data points were recorded at 14.1 T. The number of transients was set to 64, and the inter-scan delay was set to 1.5 s yielding total experimental time of 32h.

Real-time NMR data of the G-quadruplex sequence **35** were acquired using a ^{1}H-^{13}C-BEST TROSY pulse sequence. 1024*32 complex data points were recorded at 14.1

T. The number of transients was set to 8, and the inter-scan delay was set to 0.2 s yielding total experimental time of 88 s. A total of 200 spectra were recorded.

Exchange data for J9a-DNA **37** were determined using an alternative approach conducting a combination of a T_1 and a longitudinal exchange NMR experiment. Arrays of T_1 and ZZ exchange spectra were recorded at 14.1 T at 25°C with mixing times of 8, 48, 98, 148, 198, 298 and 398 ms. The size of the data matrices for each spectrum was 1024*96 complex data points, the number of scans was 128 and the inter-scan delay was 1.0 s to yield a total measuring time of 72 h for seven T_1 experiments (36h) and ZZ exchange (36h) experiment, respectively.

For data processing Topspin 3.5 pl6™ or the *nmrpipe*/*nmrDraw* software package were used (1).

NMR data evaluation

Processing of NMR data sets, peak picking and integration were performed using *nmrPipe* and the *nmrDraw* software package. All subsequent steps were performed using in-house written software written in *Matlab* (The MathWorks, www.mathworks.com).

Processing and analysis of ^{13}C-CPMG RD data of mini cTAR DNA 33

The peak intensities at the various CPMG fields were obtained by summing over 2x2 (f_1xf_2) data points using the serT-script implemented in the nmrPipe package and used to calculate effective transverse relaxation rates $R_{2,eff}$ according to equation **(1)**:

$$R_{2,eff} = -\frac{1}{\tau_{CP}} ln \frac{I_{\nu_{CPMG}}}{I_o} \quad \textbf{(1)}$$

with τ_{CP} relaxation delay, $I_{\nu CPMG}$ peak intensity at the CPMG frequency ν_{CPMG} and I_0 peak intensity from the experiment without relaxation delay.

The transverse relaxation rates were used as the experimental input for fitting the dispersion profiles according to a general expression for the intermediate exchange regime (Carver-Richards equation (2)):

$$R_{2,eff} = R_{2,0} + \frac{k_{ex}}{2} - \nu_{CPMG} cosh^{-1}[D_+ \cosh(\eta_+) - \mathrm{D}_- \cosh(\eta_-) \quad \textbf{(2)}$$

with

$$D_\pm = \frac{1}{2}\left[\pm 1 + \frac{\Psi + 2\delta\omega^2}{(\Psi^2 + \xi^2)^{1/2}}\right], \eta_\pm = \frac{\left[\pm\Psi + (\Psi + \xi^2)^{1/2}\right]^{1/2}}{2\sqrt{2}\nu_{CPMG}}$$

$\Psi = {k_{ex}}^2 - \delta\omega^2,\ \xi = -2\delta\omega(p_a k_{ex} - p_b k_{ex})$

where $\delta\omega$ is the chemical shift difference between ground and excited state, k_{ex} (= $k_{forward}$ + $k_{backward}$) exchange rate, p_a population of ground state and p_b population of excited state. Errors for the exchange parameters were obtained from 1000 runs of Monte Carlo analysis. Results from the fitting procedure for mini-cTAR DNA **33** in the absence and presence of NCp7 are given in the main text (**Table 1**).

Fitting of real-time NMR data of DNA 35

Peak intensities for the real-time experimental data were obtained by summing over 2x2 (f_1xf_2) data points as described above. Mono-exponential least squares fitting was used to determine the refolding rate for the kinetic transition between parallel quadruplex fold 2 and the (3+1) quadruplex fold 1, along with the effective lag time of the real-time experiment. In this procedure, peak intensities for the build-ups of the fold 1 signals and the decays of the fold 2 signals were fitted simultaneously, normalized by the populations obtained from the peak intensities in a fully relaxed ^{1}H-^{13}C-BEST TROSY spectrum (taken 16 hours after initiation of the refolding reaction). The data were analyzed separately for the 8-^{13}C-labeled guanosine (G10 and G17) spin labels in **35**. To obtain confidence intervals of the refolding rate, a Monte Carlo approach was employed by using the standard deviation of the fitting residuals to simulate 500 data sets with random scatter around the best fit. The standard deviation of the refolding rate in this procedure values is reported in the publication.

Processing and analysis of longitudinal exchange data of J9a-DNA 37

Peak intensities at the various mixing periods were obtained by summing over 2x2 (f_1xf_2) data points as above. Then, a least square fitting procedure was applied to determine $k_{A/D \rightarrow A/B}$, $k_{A/B \rightarrow A/D}$ and $R_1^{A/D}$ and $R_1^{A/B}$ by fitting the expressions **(3)** and **(4)**:

$$M_{AA}(t)/M_{AA}(0) = \frac{1}{(\lambda_1-\lambda_2)}[(a_{22}+\lambda_1)e^{\lambda_1 t} - (a_{11}+\lambda_2)e^{\lambda_2 t}]$$

$$M_{BB}(t)/M_{BB}(0) = \frac{1}{(\lambda_1-\lambda_2)}[(a_{11}+\lambda_1)e^{\lambda_1 t} - (a_{22}+\lambda_2)e^{\lambda_2 t}] \quad \textbf{(3)}$$

and

$$M_A(t)/M_A(0) = \frac{1}{(\lambda_1-\lambda_2)}[(a_{22}-a_{21}+\lambda_1)e^{\lambda_1 t} - (a_{22}-a_{21}+\lambda_2)e^{\lambda_2 t}]$$

$$M_B(t)/M_B(0) = \frac{1}{(\lambda_1-\lambda_2)}[(a_{11}-a_{12}+\lambda_1)e^{\lambda_1 t} - (a_{11}-a_{12}+\lambda_2)e^{\lambda_2 t}] \quad \textbf{(4)}$$

with M_{AA} and M_{BB} are the intensities of the correlation peaks from the ZZ exchange experiment. These intensities are normalized by the peak volumes at zero mixing time (M_{AA}^0 and M_{BB}^0). $M_A(0)/M_B(0)$ and $M_A(t)/M_B(t)$ are the peak intensities of fold A and B at zero mixing time or the mixing time t from the T_1 experiment. The

eigenvalues of the spin density matrix with magnetization transfer effects from chemical exchange are given by

$$\lambda_{1/2} = \frac{1}{2}[-(a_{11} + a_{22}) \pm [(a_{11} - a_{22})^2 + 4a_{12}a_{21}]^{1/2}$$

with the elements a_{ij} defined as:

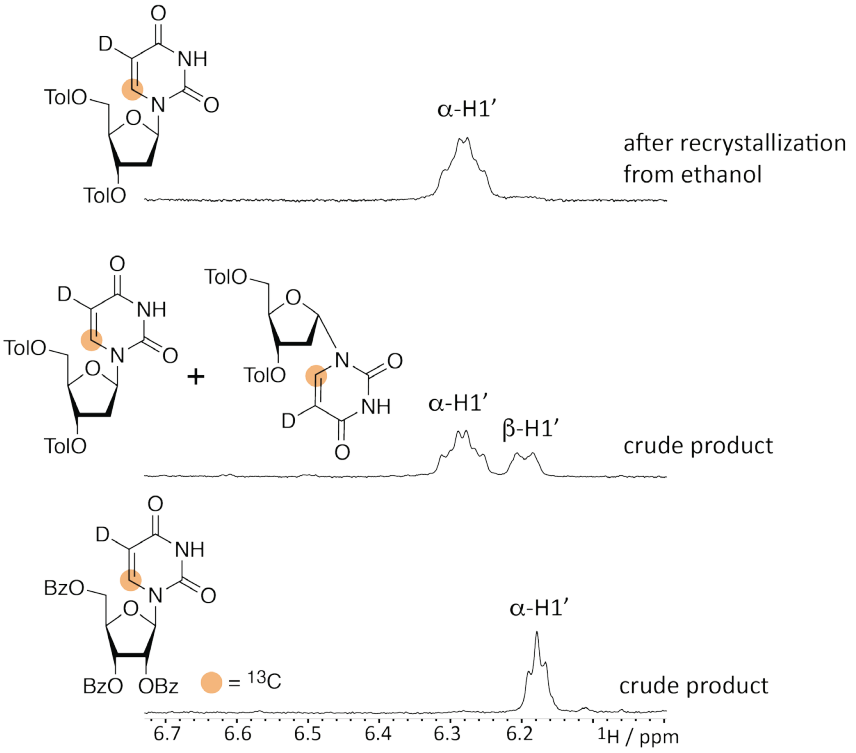
$$a_{11} = k_{A/D \to A/B} + R_1^{A/D}, a_{22} = k_{A/B \to A/D} + R_1^{A/B}, a_{12} = -k_{A/B \to A/D}, a_{11} = k_{A/D \to A/B}$$

Parameters $k_{A/D \to A/B}$ and $k_{A/B \to A/D}$ describe the refolding rate constants describing the interconversion between the two folding states (A/D and A/B conformer) of DNA 37, while $R_1^{A/D}$ and $R_1^{A/B}$ are the longitudinal relaxation rates in conformation A and B, respectively. Experimental uncertainties were estimated from 1000 Monte Carlo runs in which synthetic data sets were generated from the best-fit values of $k_{A/D \to A/B}$, $k_{A/B \to A/DA}$ and $R_1^{A/D}$, $R_1^{A/B}$ by adding random errors based on the signal-to-noise ratio to the best-fit curves. For further details please refer to (2).

Isothermal Titration Calorimetry

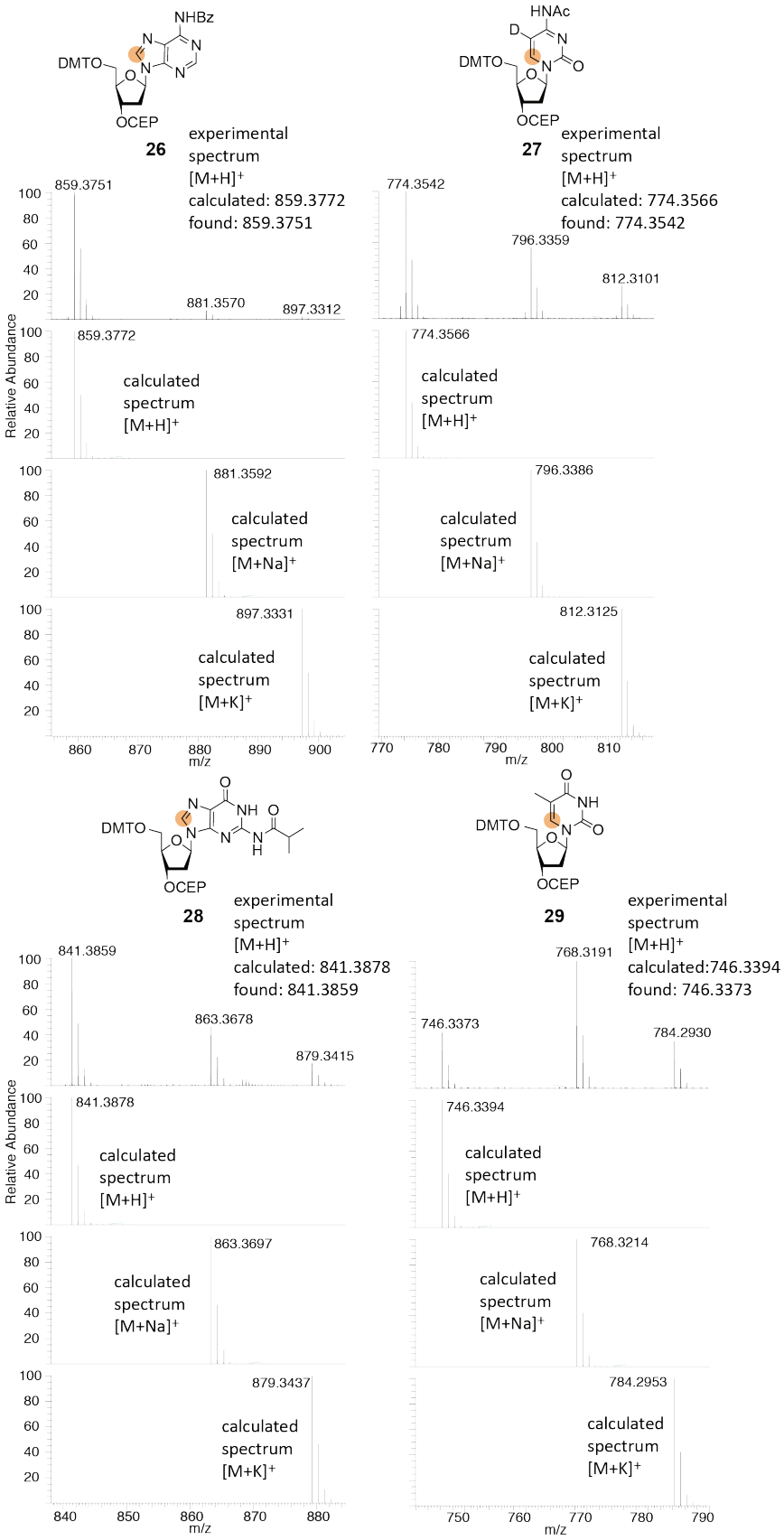
ITC experiments were performed on a Microcal ITC200 calorimeter at 20°C. The cTAR wildtype DNA and mutants were ordered from IDT (Integrated DNA technologies, HPLC purified, sodium salt form) and dissolved in 300 µL NMR buffer (15 mM sodium phosphate buffer, 25 mM NaCl, 0.1 mM $ZnCl_2$, pH 6.5, 9/1 H_2O/D_2O) to give a final concentration of 3.7 µM. The NCp 7 peptide was exchanged into NMR buffer via an ultracentrifugation unit (Vivaspin 500 3000 MWCO PES, 5x with 200 µl) and the final concentration was 150 µM. This NCp 7 solution was titrated into the DNA solution in the sample cell (V = 207 µl; stirring speed 1000) by 19 serial injections of 2 µl each, with 150 second intervals for the wt cTAR DNA and 120 second intervals for the mutants between injections, and a reference power of 10 µcal/s. The thermograms were integrated and analyzed using Origin 7.0 software (Microcal). We used the “one set of sites” model to estimate the binding constants and thermodynamic values are included in **Supporting Figure 4**.

Supporting Figure 1. NMR spectroscopic comparison (anomeric proton region) of nucleosidation reactions using the *Silyl-Hilbert-Johnson* method (lower spectrum) and the direct access to 2'-deoxyuridine using Hoffer's α-chlorosugar (middle and upper spectrum).

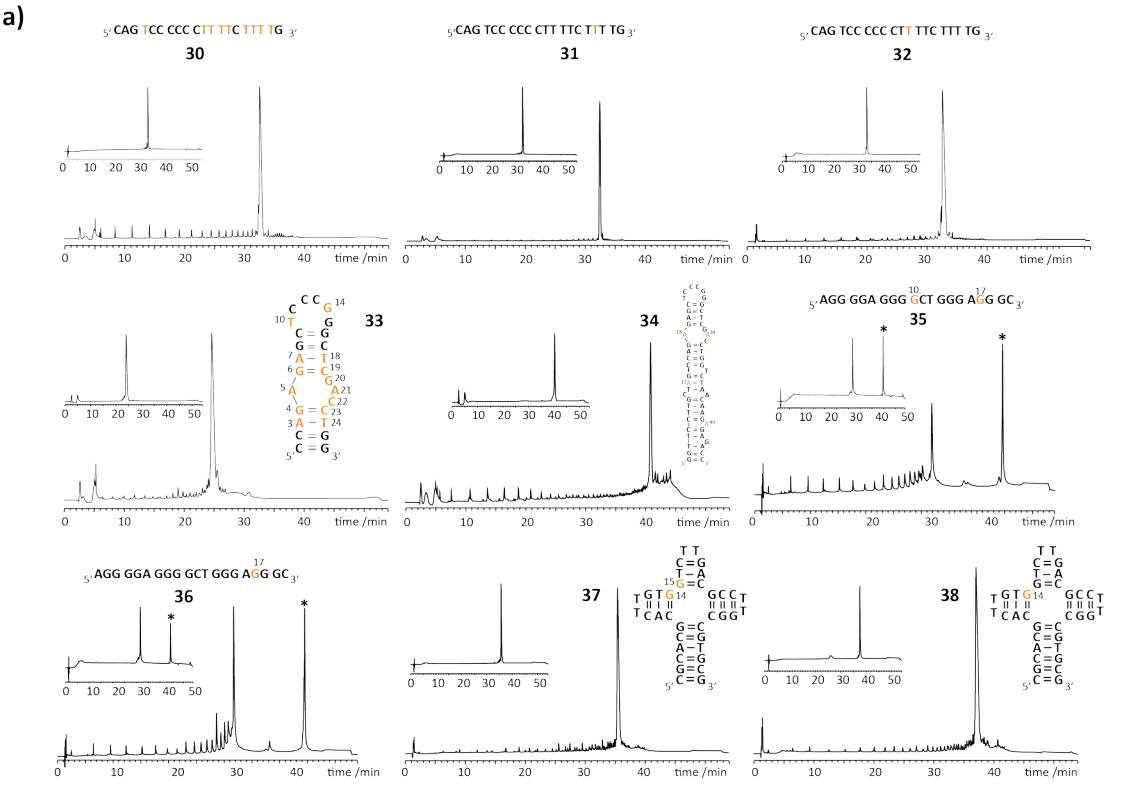


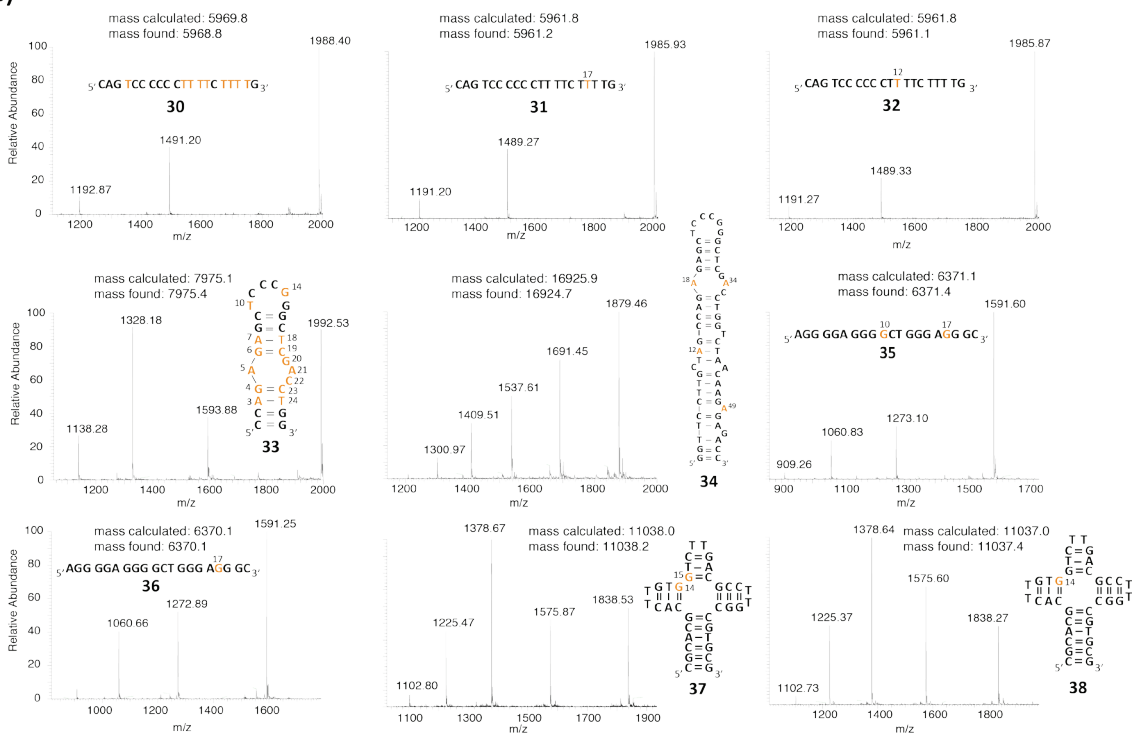
300 MHz NMR, 25°C, $CDCl_3$

Supporting Figure 2. High-resolution mass spectrometric analysis of the DNA phosphoramidites **26**, **27**, **28** and **29**.

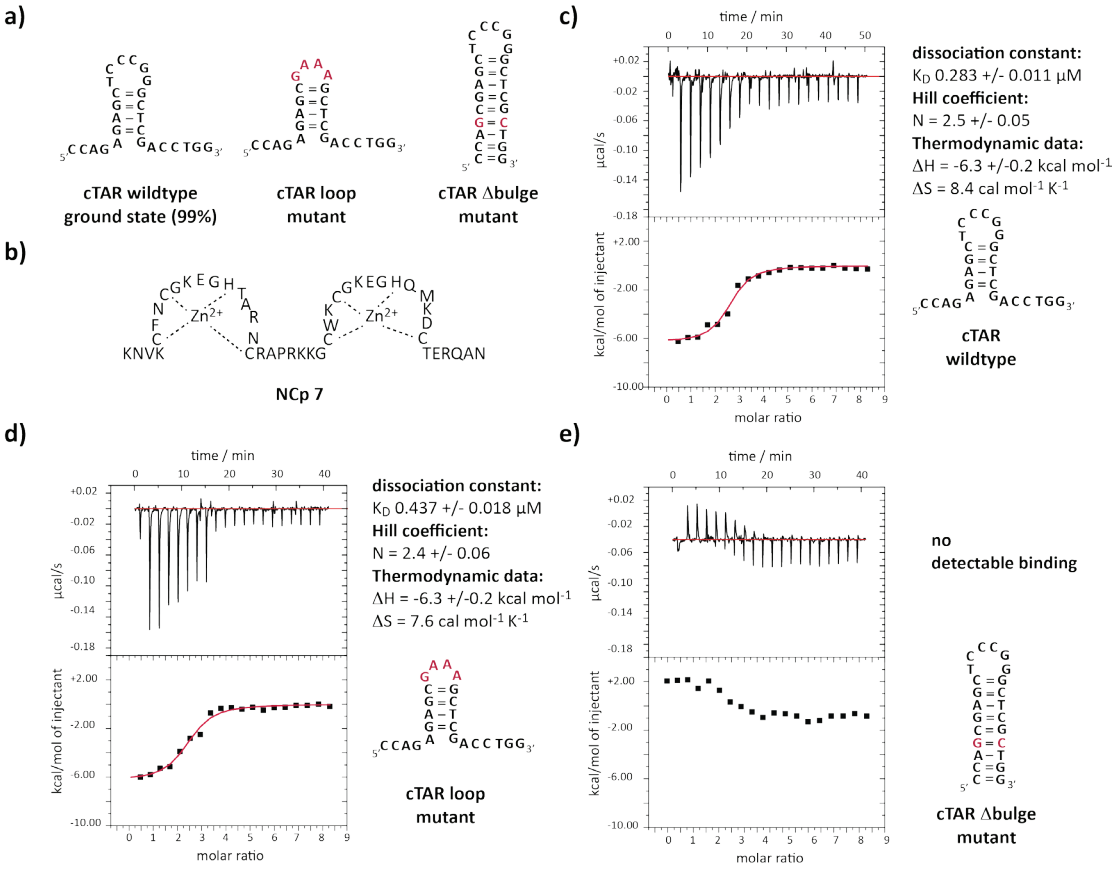


Supporting Figure 3. a) Anion-exchange HPLC traces of crude and purified DNAs. **b)** Mass spectrometric analysis of the purified DNAs.

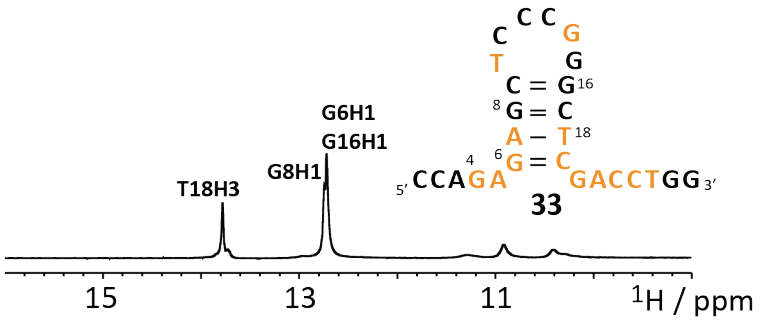




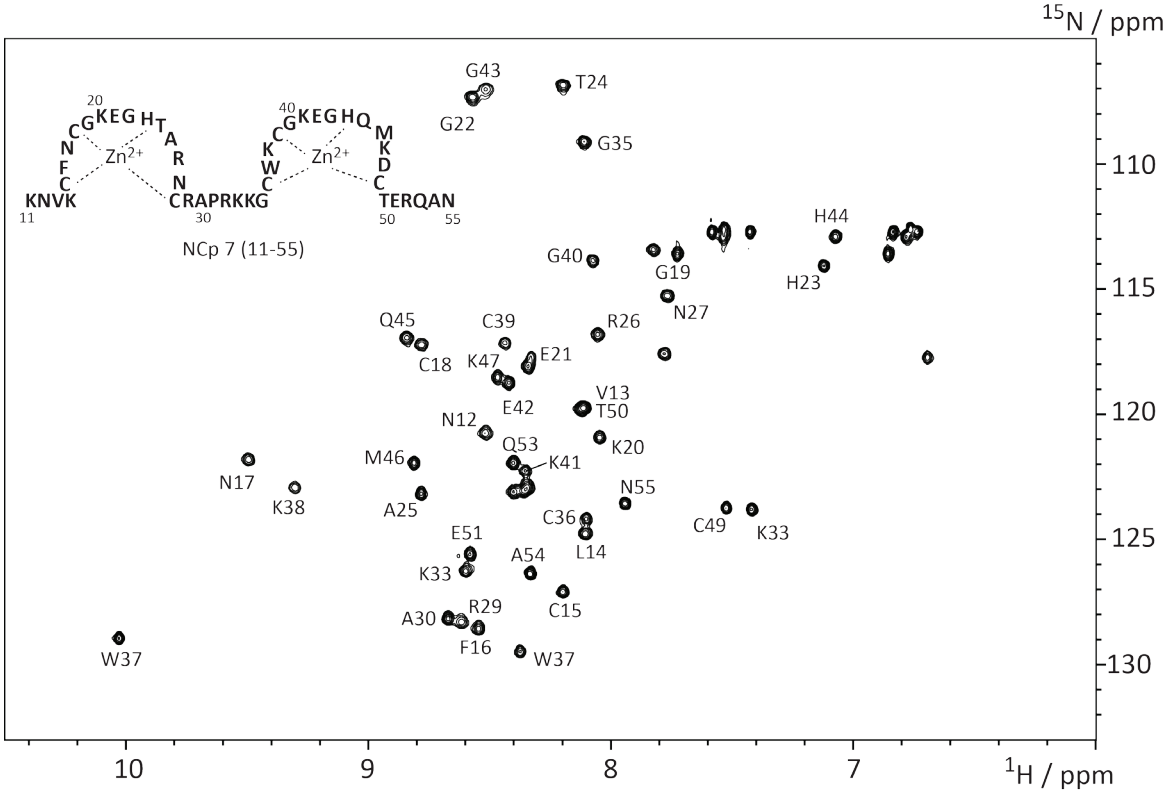
Supporting Figure 4. Isothermal titration calorimetry study of the wildtype and mutant mini-cTAR DNAs and the NCp 7 peptide. **a)** Secondary structures of the wildtype cTAR DNA, the cTAR loop mutant and the cTAR Δbulge mutant. **b)** Schematic representation of the NCp7 peptide. **c)** ITC experiment and data of the cTAR wt DNA and NCp 7. **d)** ITC experiment and data of the cTAR loop mutant DNA and NCp 7. **e)** ITC experiment and data of the cTAR Δbulge mutant DNA and NCp 7.



Supporting Figure 5. Imino proton spectrum of $^{13}C/^{2}H$-labeled mini cTAR DNA **33**. Assignments are shown. No NMR base evidence for the formation of the lower stem was found.



Supporting Figure 6. ^{1}H-^{15}N-HSQC spectrum of NCp7 peptide with assignments.



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