Supporting Information

for

Solution phase synthesis of short oligoribonucleotides on a precipitative tetrapodal support

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3-Benzoyl-2'-O-(2-cyanoethyl)-3',5'-O-(1,1,3,3-tetraisopropylsiloxane-1,3-diyl)uridine (1a) was prepared as described in literature [1]. The $^1$H and $^{13}$C NMR spectra were identical with those reported earlier [1]. Positive ion ESI-MS: $m/z$ obsd. 644.30 [M+H]$^+$, 666.28 [M+Na]$^+$; calcd. 644.28 [M+H]$^+$, 666.26 [M+Na]$^+$.

2'-O-(2-Cyanoethyl)-N^2-(dimethylaminomethylene)-3',5'-O-(1,1,3,3-tetraisopropylsiloxane-1,3-diyl)guanosine (1b) was prepared as described in literature [1]. The $^1$H and $^{13}$C NMR spectra were identical with those reported earlier [1]. Positive ion ESI-MS: $m/z$ obsd. 900.44 [M+H]$^+$; calcd. 900.45 [M+H]$^+$.

2'-O-(2-Cyanoethyl)-N^4-(dimethylaminomethylene)-3',5'-O-(tetraisopropylsiloxane-1,3-diyl)cytidine (1c) was prepared as described in literature [1]. The $^1$H and $^{13}$C NMR spectra were identical with those reported earlier [1]. Positive ion ESI-MS: $m/z$ obsd. 594.34 [M+H]$^+$; calcd. 594.31 [M+H]$^+$.

2'-O-(2-Cyanoethyl)-N^6-(dimethylaminomethylene)-3',5'-O-(1,1,3,3-tetraisopropylsiloxane-1,3-diyl)adenosine (1d) was prepared as described in literature [1]. The $^1$H and $^{13}$C NMR spectra were identical with those reported earlier [1]. Positive ion ESI-MS: $m/z$ obsd. 618.35 [M+H]$^+$; calcd. 618.33 [M+H]$^+$.

3-Benzoyl-2'-O-(2-cyanoethyl)-3'-O-(1,1,3,3-tetraisopropylsiloxane-1-yl)uridine (2a). Compound 1a (2.35g, 3.65mmol) was dissolved in THF (47 mL), 1:1 mixture (v/v) of TFA and water (23.5 mL) was added dropwise on an ice-bath and the mixture was left with stirring for 3.5 h at 0 $^\circ$C. The solution was extracted with EtOAc (150 mL), and the organic phase was washed with aq NaHCO$_3$ (2 x 300 mL) and dried over Na$_2$SO$_4$. Purification by column chromatography on silica gel using a gradient of 1-10% MeOH in DCM gave compound 2a in 89% yield as white foam (2.15 g, 3.25 mmol). $^1$H-NMR (400 MHz, CDCl$_3$): $\delta = 0.86$-1.20 (m, 28H), 2.61 (t, $J = 5.9$ Hz, 2H), 3.80-4.21 (m, 8H), 4.49-4.52 (m, 1H), 5.76 (d, $J = 8.2$ Hz, 1H), 5.78 (s, 1H), 7.50 (t, $J = 7.4$ Hz, 2H), 7.67 (t, $J = 7.4$ Hz, 1H), 7.91 (d, $J = 7.4$ Hz, 2H), 8.43(d, $J = 8.2$ Hz, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 12.8, 13.1, 13.4, 13.6, 17.0, 17.1, 17.2, 18.9, 58.6, 64.9, 66.8, 83.0, 83.5, 88.6, 101.0, 117.5, 129.3, 130.5, 131.2, 135.4, 140.7, 149.2, 162.6, 168.5. For the spectra, see page S11. Positive ion ESI-HRMS: $m/z$ obsd. 684.2734 [M+Na]$^+$; calcd. 684.2749 [M+Na]$^+$.

2'-O-(2-Cyanoethyl)-N^2-(dimethylaminomethylene)-3'-O-(tetraisopropylsiloxane-1-yl)guanosine (2b). Compound 1b (2.30 g, 3.63 mmol) was dissolved in THF (40 mL) and 1:1 mixture (v/v) of TFA and water (6.6 mL) was added dropwise on an ice-bath. The reaction mixture was allowed to stand 3h on the ice-bath and then another portion (6.6 mL) of aq TFA was added. The mixture was stirred for 4.5 h on the ice-bath and extracted with EtOAc (150 mL). The organic phase
was washed with aq. NaHCO$_3$ (2 x 300 mL) and dried over Na$_2$SO$_4$. Purification by column chromatography on silica gel using a gradient of 1-10% MeOH in DCM gave compound 2b in 82% yield as white foam (2.53 g; 2.98 mmol). $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 0.95-1.13$ (m, 28H), 2.69 (t, $J = 5.9$ Hz, 2H), 3.09 (s, 3H), 3.19 (s, 3H), 3.85-4.16 (m, 4H), 4.22-4.30 (m, 2H), 4.50-4.53 (m, 1H), 6.03 (s, 1H), 7.94 (s, 1H), 8.66 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 12.6, 12.9, 13.0, 13.4, 16.9, 17.1, 17.3, 17.5, 19.3, 35.2, 41.3, 60.4, 66.4, 69.7, 81.1, 83.7, 87.6, 117.6, 122.2, 135.4, 149.3, 156.9, 157.8, 158.2. For the spectra, see page S12. Positive ion ESI-HRMS: m/z obsd. 652.3333 [M+H]$^+$; calcd. 652.3310 [M+H]$^+$.

2'-O-(2-Cyanoethyl)-N$^4$-(dimethylaminomethylene)-3'-O-(1,1,3,3-tetraisopropyldisiloxane-1-yl)cytidine (2c). Compound 1c (3.10 g, 5.22 mmol) was hydrolyzed to 2c as described above for the hydrolysis of 1b to 2b. Purification by column chromatography on silica gel using a gradient of 1-10% MeOH in DCM gave compound 2c as white foam (1.31 g; 2.14 mmol). Additionally, 2'-O-(2-cyanoethyl)-3'-O-(1,1,3,3-tetraisopropyldisiloxane-1-yl)cytidine (1.21 g, 2.17 mmol) was obtained. To introduce the base moiety protection lost during the hydrolysis, the compound was dissolved in dry MeOH (140 mL) and 3.35 equiv. of N,N-dimethylformamide dimethylacetal (1.03 mL; 7.75 mmol) was added. After 4 h at room temperature, the solvent was removed and 2c (1.25 g; 2.04 mmol) formed was isolated by column chromatography on silica using a gradient of 1-10% MeOH in DCM. Accordingly, the overall yield of 2c was 80% (2.56 g; 4.19 mmol). $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 0.87-1.12$ (m, 28H), 2.45 (m, 2H), 3.23 (s, 3H), 3.28 (s, 3H), 3.40-3.48 (m, 1H), 3.72-3.93 (m, 3H), 3.96-4.04 (m, 1H), 4.29-4.31 (br s, 1H), 4.76-4.84 (m, 2H), 5.99 (s, 1H), 8.04 (s, 1H), 8.49 (s, 1H), 8.98 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 13.1, 13.3, 13.4, 13.5, 17.1, 17.2, 17.3, 17.4, 18.8, 35.2, 41.4, 62.4, 65.5, 71.5, 81.4, 88.5, 89.4, 117.0, 127.6, 141.7, 150.0, 152.1, 158.4, 160.4. For the spectra, see page S13. Positive ion ESI-HRMS: m/z obsd. 612.3259 [M+H]$^+$; calcd. 612.3249 [M+H]$^+$.

2'-O-(2-Cyanoethyl)-N$^4$-dimethylaminomethylene-3'-O-(1,1,3,3-tetraisopropyldisiloxane-1-yl)adenosine (2d). Compound 1d (310 mg, 0.502 mmol) was hydrolyzed to 2d as described above for the hydrolysis of 1b to 2b. Purification by column chromatography on silica gel using a gradient of 1-10% MeOH in DCM gave compound 2d in 89% yield as white foam (284 mg; 0.447 mmol). $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 0.97-1.08$ (m, 28H), 2.45 (m, 2H), 3.23 (s, 3H), 3.28 (s, 3H), 3.40-3.48 (m, 1H), 3.72-3.35 (m, 3H), 3.96-4.04 (m, 1H), 4.29-4.31 (br s, 1H), 4.76-4.84 (m, 2H), 5.99 (s, 1H), 8.04 (s, 1H), 8.49 (s, 1H), 8.98 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 13.1, 13.3, 13.4, 13.5, 17.1, 17.2, 17.3, 17.4, 18.8, 35.2, 41.4, 62.4, 65.5, 71.5, 81.4, 88.5, 89.4, 117.0, 127.6, 141.7, 150.0, 152.1, 158.4, 160.4. For the spectra, see page S14. Positive ion ESI-HRMS: m/z obsd. 636.3353 [M+H]$^+$; calcd. 636.3361 [M+H]$^+$.
3-Benzoyl-2'-O-(2-cyanoethyl)-5'-O-(1-methoxy-1-methylethyl)-3'-O-(1,1,3,3-tetraisopropyldisiloxane-1-yl)uridine (3a). Compound 2a (2.53 g, 3.88 mmol) was dissolved in dry THF (60 mL) and 10 equiv. of 2-methoxypropene (3.90 mL, 40.7 mmol) was added. A catalytic amount of p-toluenesulfonic acid monohydrate (15.0 mg, 0.077 mmol) dissolved in dry THF (0.5 mmol) was added. The progress of acetalization was monitored by TLC and p-toluenesulfonic acid monohydrate was added portionwise until the starting material had disappeared. The crude mixture was extracted with EtOAc (100 mL). The organic phase was washed with aq. NaHCO₃ (200 mL) and dried over Na₂SO₄. Compound 3a was obtained in 96% yield as white foam (2.70 g, 3.73 mmol). ¹H NMR (400 MHz, CDCl₃): δ = 0.98-1.19 (m, 28H), 1.16 (s, 3H), 1.66 (s, 3H), 2.79 (t, J = 6.1 Hz, 2H), 3.25 (s, 3H), 3.59-3.92 (m, 2H), 3.97-4.07 (m, 2H), 4.20-4.30 (m, 2H), 4.68-4.72 (m, 1H), 5.78 (d, J = 8.2 Hz, 1H), 5.79 (s, 1H), 7.58 (t, J = 7.4 Hz, 2H), 7.75 (t, J = 7.4 Hz, 1H), 8.02 (d, J = 7.4 Hz, 2H), 8.36 (d, J = 8.2 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃): δ = 13.1, 13.3, 13.4, 13.5, 16.7, 16.9, 17.0, 17.2, 18.4, 23.5, 26.4, 48.1, 57.6, 66.0, 67.7, 81.4, 82.7, 89.2, 96.0, 100.3, 117.9, 129.2, 130.2, 135.0, 140.1, 149.2, 161.9, 169.1. For the spectra, see page S15. Positive ion ESI-HRMS: m/z obsd. 756.3299 [M+Na]+; calcd. 756.3324 [M+Na]+.

2'-O-(2-Cyanoethyl)-N¹-(dimethylaminomethylene)-5'-O-(1-methoxy-1-methylethyl)-3'-O-(1,1,3,3-tetraisopropyldisiloxane-1-yl)guanosine (3b). Compound 2b (2.53 g, 3.88 mmol) was transformed to 3b as described above for the transformation of 2a to 3a. dissolved in dry THF (60 mL) and to the slightly yellowish solution were added 10 equiv of 2-methoxypropene (3.90 mL, 40.7 mmol). Compound 3b was obtained in 96% yield as white foam (2.70 g, 3.73 mmol). ¹H NMR (400 MHz, CDCl₃): δ = 0.90-1.08 (m, 28H), 1.40 (s, 6H), 2.65 (dd, J = 12.3 and 6.2 Hz, 2H), 3.10 (s, 3H), 3.18 (s, 3H), 3.19 (s, 3H), 3.60-3.68 (m, 2H), 3.70-3.76 (m, 1H), 3.80-3.90 (m, 3H), 4.24-4.33 (m, 2H), 4.72-4.73 (m, 1H), 6.16 (s, 1H), 8.03 (s, 1H), 8.64 (s, 1H). ¹³C NMR (100 MHz, CDCl₃): δ = 13.2, 13.3, 13.4, 13.5, 17.1, 17.2, 17.3, 18.9, 24.1, 24.4, 35.2, 41.3, 48.8, 59.5, 65.5, 70.6, 82.9, 83.4, 85.8, 100.6, 117.6, 120.3, 136.1, 149.7, 156.9, 157.6, 158.2. For the spectra, see page S16. Positive ion ESI-HRMS: m/z obsd. 724.3885 [M+H]+; calcd. 724.3885 [M+H]+.

2'-O-(2-Cyanoethyl)-N¹-(dimethylaminomethylene)-5'-O-(1-methoxy-1-methylethyl)-3'-O-(1,1,3,3-tetraisopropyldisiloxane-1-yl)cytidine (3c). Compound 2c (2.00 g, 3.27 mmol) was transformed to 3c as described above for the transformation of 2a to 3a. Purification by column chromatography on silica gel using a stepwise gradient of 1-5% MeOH in DCM containing 1% triethylamine gave 3c in 91% yield as white foam (2.03 g, 2.97 mmol). ¹H NMR (400 MHz, CDCl₃): δ = 0.95-1.05 (m, 28H), 1.40 (s, 6H), 2.75 (t, J = 6.3 Hz, 3H), 3.13 (s, 3H), 3.16 (s, 3H), 3.23 (s, 3H), 3.60 (d, J = 11.4 Hz, 1H), 3.91-4.00 (m, 3H), 4.26-4.38 (m, 2H), 4.39-4.43 (m, 1H), 5.95 (s, 1H), 6.06 (d, J = 7.5 Hz, 1H), 8.39 (d, J = 7.5 Hz, 1H), 8.82 (s, 1H). ¹³C NMR (100 MHz, CDCl₃): δ = 13.1, 13.3, 13.4, 13.5, 17.0, 17.1, 17.2, 17.3, 18.9, 23.8, 24.4, 35.1, 41.5, 48.8, 57.8, 64.6, 67.9, 81.2, 82.9, 88.6, 100.6, 101.9, 117.8,
2′-O-(2-Cyanoethyl)-N4-(dimethylaminomethylene)-5′-O-(1-methoxy-1-methylethyl)-3′-O-(1,1,3,3-tetraisopropyldisiloxane-1-yl)adenosine (3d). Compound 2d (284 mg, 0.447 mmol) was transformed to 3d as described above for the transformation of 2a to 3a. Compound 3d was obtained in 94% yield as white foam (300 mg; 0.424 mmol). 1H NMR (400 MHz, CDCl3): δ = 0.94-1.07 (m, 28H), 1.37 (s, 3H), 1.38 (s, 3H), 2.64 (br s, 2H), 3.16 (s, 3H), 3.18 (s, 3H), 3.23 (s, 3H), 3.60 (dd, J = 10.9 and 1.0 Hz, 1H), 3.77-3.92 (m, 3H), 4.26-4.28 (m, 1H), 4.47-4.49 (m, 1H), 4.72-4.73 (m, 1H), 6.18 (s, 1H), 8.31 (s, 1H), 8.48 (s, 1H), 8.88 (s, 1H). 13C NMR (100 MHz, CDCl3): δ = 13.2, 13.3, 13.4, 13.5, 17.1, 17.2, 17.2, 17.3, 18.9, 24.2, 24.4, 35.1, 41.3, 48.7, 59.4, 65.2, 70.4, 82.8, 83.0, 86.7, 100.6, 117.4, 126.5, 140.3, 150.9, 152.6, 158.1, 159.6. For the spectra, see page S18. Positive ion ESI-HRMS: m/z obsd. 706.3642 [M+H]+; calcd. 706.3643 [M+H]+.

3-Benzoyl-2′-O-(2-cyanoethyl)-5′-O-(1-methoxy-1-methylethyl)uridine (4a). Compound 3a (0.90 g, 1.22 mmol) was dissolved in dry MeOH (48 mL) and 3 equiv. of NH4F (140 mg, 3.78 mmol) was added. The mixture was agitated for 30h at room temperature. The solution was then extracted with DCM (30 mL) and the organic phase was washed with aq. NaHCO3 (100 mL). The aqueous phase was back-extracted twice with DCM (2 x 30mL) and the combined organic phase was dried over Na2SO4. Purification by column chromatography on silica gel using a gradient of 1-10% MeOH in DCM containing 1% pyridine afforded 4a in 83% yield as white foam (0.48 g, 1.01 mmol). 1H NMR (400 MHz, CDCl3): δ = 1.40 (s, 3H), 1.41 (s, 3H), 1.52 (s, 3H), 2.62 (t, J = 6.1 Hz, 2H), 3.23 (s, 3H), 3.64-3.72 (m, 1H), 3.78-3.94 (3H), 3.95-4.15 (m, 3H), 4.19-4.24 (1H), 4.29-4.33 (m, 1H), 5.79 (d, J = 8.2 Hz, 1H), 5.86 (s, 1H), 7.50 (t, J = 7.4 Hz, 2H), 7.66 (t, J = 7.4 Hz, 1H), 7.93 (d, J = 7.4 Hz, 2H), 8.31 (d, J = 8.2 Hz, 1H). 13C NMR (100 MHz, CDCl3): δ = 18.9, 24.2, 24.3, 48.7, 57.9, 65.3, 67.8, 82.8, 83.0, 88.0, 100.4, 101.3, 117.7, 129.3, 130.5, 131.2, 135.4, 139.9, 149.3, 162.2, 168.7. For the spectra, see page S19. Positive ion ESI-HRMS: m/z obsd. 496.1692 [M+Na]+; calcd. 496.1696 [M+Na]+.

2′-O-(2-Cyanoethyl)-5′-O-(1-methoxy-1-methylethyl)uridine (4a′). Compound 3a (4.69 g, 6.39 mmoles) was dissolved in dry MeOH (220 mL) and 5 equiv. of NH4F (1.18 g, 31.9 mmol) was added. The mixture was agitated for 96 h at room temperature. The solvent was removed under reduced pressure and the residue was subjected to column chromatography on silica gel using a gradient of 1-5% MeOH in DCM containing 1% pyridine to afford 4a′ in 84% yield as white foam (1.98 g, 5.36 mmol). 1H NMR (400 MHz, CDCl3): δ = 1.40 (s, 6H), 2.73-2.77 (m, 2H), 3.22 (s, 3H), 3.70 (dd, J = 11.4 and 2.0 Hz, 1H), 3.88 (dd, J = 11.4 and 2.0 Hz, 1H), 3.92-3.97 (m, 2H), 4.10-4.14 (m, 1H), 4.18-4.24 (m, 1H), 4.27-4.31 (m, 1H), 5.69 (d, J = 8.2 Hz, 1H), 5.89 (s, 1H), 8.17 (d, J = 8.2 Hz, 1H), 10.2 (br s, 1H). 13C NMR (100 MHz, CDCl3): δ = 19.0, 24.2, 24.3, 48.7, 57.9, 65.3, 67.9, 82.8, 83.0, 87.7, 100.4, 101.7, 117.6, 118.2, 130.4, 132.1, 135.4, 139.9, 149.3, 162.2, 168.7.
2'-O-(2-Cyanoethyl)-N2-(dimethylaminomethylene)-5'-O-(1-methoxy-1-methylethyl)guanosine (4b). Compound 3b (1.52 g, 2.10 mmol) was dissolved in dry MeOH (83 mL), 5 equiv. of NH₄F (390 mg, 10.52 mmol) was added and the mixture was agitated for 20 h at room temperature. Purification by column chromatography on silica gel using a gradient of 1-10% MeOH in DCM containing 1% triethylamine afforded 4b in 85% yield as white foam (0.83 g, 1.79 mmol). 

1H NMR (400 MHz, CDCl₃): δ = 1.41 (s, 6H), 2.71 (t, J = 6.0 Hz, 2H), 3.09 (s, sH), 3.19 (s, 3H), 3.20 (s, 3H), 3.67 (dd, J = 11.0 and 3.3 Hz, 1H), 3.78-3.84 (m, 2H), 3.93-4.00 (m, 2H), 4.17-4.19 (m, 1H), 4.22-4.24 (m, 1H), 4.62-4.66 (m, 1H), 6.12 (s, 1H), 8.00 (s, 1H), 8.63 (s, 1H).

13C NMR (100 MHz, CDCl₃): δ = 19.0, 24.3, 35.2, 41.5, 48.7, 59.7, 65.9, 69.9, 82.7, 83.6, 86.3, 100.4, 117.7, 120.1, 136.1, 149.9, 157.0, 158.1, 158.3.

For the spectra, see page S21.

2'-O-(2-Cyanoethyl)-5'-O-(1-methoxy-1-methylethyl)cytidine (4c'). Compound 3c (1.15 g, 1.68 mmol) was desilylated as described above for the desilylation of 3b. Purification by column chromatography on silica gel using a stepwise gradient of 1-14% MeOH in DCM containing 1% triethylamine gave 4c' in 80% yield (0.50 g, white foam). 

1H NMR (400 MHz, CDCl₃): δ = 1.40 (s, 3H), 1.41 (s, 3H), 2.75 (t, J = 6.1 Hz, 2H), 3.22 (s, 3H), 3.69 (dd, J = 11.3 and 2.0 Hz, 1H), 3.88 (dd, J = 11.3 and 2.0 Hz, 1H), 4.21-4.30 (m, 2H), 5.75 (d, J = 7.5 Hz, 1H), 5.88 (s, 1H), 8.19 (d, J = 7.5 Hz, 1H). 

13C NMR (100 MHz, CDCl₃): δ = 19.0, 24.3, 48.7, 58.0, 65.1, 67.7, 82.4, 82.8, 88.4, 93.2, 100.3, 117.8, 141.2, 155.7, 165.8. For the spectra, see page S22.

N4-Benzoyl-2'-O-(2-cyanoethyl)-5'-O-(1-methoxy-1-methylethyl)cytidine (4c''). Compound 4c' (0.35 g, 0.95 mmol) was dissolved in dry pyridine (45 mL) and 1.1 equiv. of benzoyl chloride (121 µL, 1.04 mmol) was added. The mixture was agitated for 20 h at room temperature and subjected then to DCM/aq. NaHCO₃ workup. Purification by column chromatography on silica gel using a stepwise gradient of 1-6% MeOH in DCM containing 1% triethylamine gave 4c'' in 91% yield (0.41 g, white foam).

1H NMR (400 MHz, CDCl₃): δ = 1.43 (s, 3H), 1.44 (s, 3H), 2.77 (t, J = 5.6 Hz, 2H), 3.24 (s, 3H), 3.73 (d, J = 10.9 Hz, 1H), 3.93 (d, J = 10.9 Hz, 1H), 3.98-4.06 (m, 2H), 4.16-4.19 (m, 1H), 4.27-4.30 (m, 1H), 4.33-4.37 (m, 1H), 5.93 (s, 1H), 7.50-7.57 (m, 3H), 7.62 (t, J = 7.5 Hz, 1H), 7.92 (d, J = 7.5 Hz, 2H), 8.42 (d, J = 7.4 Hz, 1H), 9.05 (br s, 1H). 

13C NMR (100 MHz, CDCl₃): δ = 19.0, 24.3, 24.5, 48.7, 57.5, 65.2, 67.3, 82.6, 82.8, 88.8, 96.0, 100.4, 117.7, 127.6, 129.0, 132.9, 133.2, 144.9, 154.9, 162.7, 166.6. For the spectra, see page S23.

For Negative ion ESI-HRMS: m/z obsd. 471.1862 [M-H]⁻; calcd. 471.1881 [M-H]⁻.

S7
2'-O-(2-Cyanoethyl)-N4-(dimethylaminomethylene)-5'-O-(1-methoxy-1-methylethyl)adenosine (4d). Compound 3d (1.20 g, 1.69 mmol) was desilylated as described above for the desilylation of 3b to 4b. Purification by column chromatography on silica gel using a gradient of 1-5% MeOH in DCM containing 1% triethylamine afforded 4d as white foam in 63% yield (0.48 g; 1.07 mmol). In addition, 2'-O-(2-cyanoethyl)-5'-O-(1-methoxy-1-methylethyl)adenosine (0.14 g, 0.35 mmol) was obtained. Treatment of this compound in MeOH (10 mL) with N,N-dimethylformamide dimethyl acetal (151 µL, 0.86 mmol) for 72 h at room temperature yielded additional 0.33 mmol of 4d. Accordingly, 4b was obtained in 83% overall yield. H NMR (400 MHz, CDCl3): δ = 1.42 (s, 3H), 1.43 (s, 3H), 2.75 (m, J = 5.6 Hz, 2H), 3.21 (s, 6H), 3.26 (s, 3H), 3.70 (d, J = 5.6 Hz, 1H), 3.87 (d, J = 10.6 Hz, 1H), 3.86-3.95 (m, 1H), 4.10-4.14 (m, 1H), 4.22-4.24 (m, 1H), 4.29-4.30 (m, 1H), 4.52-4.53 (m, 1H), 6.24 (s, 1H), 8.39 (s, 1H), 8.51 (s, 1H), 8.95 (s, 1H). 13C NMR (100 MHz, CDCl3): δ = 19.0, 24.3, 35.2, 41.3, 48.7, 59.1, 65.9, 69.3, 83.0, 83.1, 86.8, 100.4, 117.3, 126.4, 139.7, 150.8, 152.6, 158.1, 159.6. For the spectra, see page S24. Positive ion ESI-HRMS: m/z obsd. 592.2511 [M+Na]+; calcd. 592.2512 [M+Na]+.

2'-O-(2-Cyanoethyl)-5'-O-(1-methoxy-1-methylethyl)uridine 3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (5a). Compound 4a (1.40 g, 3.79 mmol) was dissolved in dry DCM (15 mL) under N2. N,N-Diisopropylethylamine (0.95 mL, 5.45 mmol) and 1-chloro-1-(2-cyanoethoxy)-N,N-diisopropylphosphonamine (0.93 mL, 4.16 mmol) were added and the mixture was stirred for 3 h at room temperature. The solution was passed through a short silica gel column, which was first eluted with DCM containing 1% triethylamine and then with a 84:15:1 mixture (v/v/v) EtOAc, petroleum ether and triethylamine. 5a was obtained in 95% yield as white foam (2.05 g, 3.60 mmol). 1H NMR (400 MHz, CDCl3): δ = 1.17-1.28 (m, 14H), 1.39 (s, 6H), 2.62-2.75 (m, 4H), 3.22 (s, 3H), 3.52-4.35 (m, 9H), 5.66 (d, J = 8.1 Hz, 1H), 5.88 (s, 0.6H), 5.90 (s, 0.4H), 8.16 (m, 1H). 13C NMR (100 MHz, CDCl3): δ = 19.0, 24.1, 24.6, 48.9, 58.1, 65.5, 69.3, 81.4, 82.5, 87.7, 100.4, 101.7, 117.9, 139.8, 150.6, 163.6. 31P NMR (CDCl3): 149.0 (40%), 150.2 (60%). For the 31P NMR spectrum, see page S25. Positive ion ESI-HRMS: m/z obsd. 592.2511 [M+Na]+; calcd. 592.2512 [M+Na]+.

2'-O-(2-Cyanoethyl)-N2-(dimethylaminomethylene)-5'-O-(1-methoxy-1-methylethyl)guanosine 3'-cyanoethyl-N,N-diisopropyl)phosphoramidite (5b). Compound 4b (0.49 g, 1.05 mmol) was phosphitylated to 5b as described above for the phosphitylation of 4a to 5a. The product was purified by passing the mixture through a short silica gel using acetone that contained 1% N,N-diisopropylethylamine as an eluent. 5b was obtained as white foam in 88% yield (0.62 g; 0.92 mmol). 1H NMR (400 MHz, CDCl3): δ = 1.20-1.27 (m, 14H), 1.42 (s, 6H), 2.60-2.67 (m, 4H), 3.10 (s, 3H), 3.18 (s, 3H), 3.20 (s, 3H), 3.57-4.40 (m, 8H), 4.53-4.58 (m, 1H), 6.12 (s, 1H), 8.05 (s, 0.4H), 8.07 (s, 0.6H), 8.65 (s, 0.4H), 8.87 (s, 0.6H), 8.85 (br s, 1H). 13C NMR (100 MHz, CDCl3): δ = 19.0, 24.5, 35.1, 41.3, 48.8, 58.1, 66.0, 69.5, 81.9, 83.2, 86.9, 100.6, 117.8, 120.6, 135.8, 149.6, 156.9, 157.6, 158.3. 31P NMR (CDCl3):
N\(^4\)-Benzoyl-2\(^'-\)O-(2-cyanoethyl)-5\(^'-\)O-(1-methoxy-1-methylethyl)cytidine 3\(^'-\)(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (5c\(^'-\)). Compound 4c\(^'-\) (0.41 g, 0.87 mmol) was phosphorylated to 5c as described above for the phosphitylation of 4a to 5a. The crude mixture was passed through a short silica gel column by using acetone that contained 1% N,N-diisopropylethylamine as eluent. 5c\(^'-\) was obtained as white foam in 91% yield (0.53 g; 0.79 mmol). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 1.14-1.30\) (m, 1H), 1.43 (s, 3H), 1.46 (s, 3H), 2.60-2.74 (m, 4H), 3.25 (s, 3H), 3.72-4.35 (m, 9H), 5.94 (s, 1H), 7.49-7.54 (3H, m), 7.61 (m, 1H), 7.91 (m, 2H), 8.72 (m, 1H), 8.81 (br s, 1H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta = 19.0, 24.3, 48.9, 57.5, 65.3, 69.5, 81.8, 82.2, 89.8, 95.9, 100.4, 117.4, 117.9, 127.5, 129.1, 133.0, 133.2, 144.9, 154.8, 162.4, 166.6. \(^{31}\)P NMR (CDCl\(_3\)) 149.0 (40%), 150.0 (60%). For the \(^{31}\)P NMR spectrum, see page S26. Negative ion ESI-HRMS: \([m/z]\) obsd. 671.2964 [M-H]\(^-\); calcld. 671.2958 [M-H]\(^-\).

2\(^'-\)O-(2-Cyanoethyl)-N\(^4\)-(dimethylaminomethylene)-5\(^'-\)O-(1-methoxy-1-methylethyl)adenosine 3\(^'-\)(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (5d). Compound 4d (0.22 g, 0.49 mmol) was phosphorylated to 5d as described above for the phosphitylation of 4a to 5, dissolved in dry DCM (10 mL). The crude product mixture was passed through a short silica gel column eluting first with EtOAc containing 1% N,N-diisopropylethylamine and then with acetone also containing as 1% N,N-diisopropylethylamine. Later a constant gradient using 1% N,N-diisopropylethylamine. 5d was obtained as white foam in 92% yield (0.293 g; 0.45 mmol). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 1.19-1.26\) (m, 14H), 1.39 (s, 3H), 1.41 (s, 3H), 2.62-2.70 (m, 4H), 2.75 (s, 3H), 3.21 (s, 3H), 3.26 (s, 3H), 3.60-4.00 (m, 6H), 4.36-4.64 (m, 3H), 6.21 (br s, 1H), 8.35 (s, 0.5H), 8.37 (s, 0.5H), 8.53 (s, 1H), 8.94 (s, 1H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta = 19.1, 24.5, 35.2, 41.3, 48.7, 59.1, 65.6, 69.5, 82.3, 82.6, 87.5, 100.5, 117.4, 126.6, 140.1, 151.1, 152.7, 157.9, 159.7. \(^{31}\)P NMR (CDCl\(_3\)) : 149.6 (45%), 149.9 (55%). For the \(^{31}\)P NMR spectrum, see page S26. Positive ion ESI-HRMS: \([m/z]\) obsd. 648.3388 [M+H]\(^+\); calcld. 648.3387 [M+H]\(^+\).

N\(^3\)-Benzoyl-2\(^'-\)O-(2-cyanoethyl)-3\(^'-\)O-(pent-4-ynoyl)-5\(^'-\)O-(1-methoxy-1-methylethyl)uridine (6a). 4-Pentynoic acid (0.32 g, 3.56 mmol) was dissolved in dry dioxane (5 mL) and the solution obtained was added dropwise to a solution of DCC (0.36 g, 1.78 mmol) in dioxane (10 mL) on an ice-bath. The mixture was stirred for 2 h at room temperature, filtered and concentrated by evaporation. 4-Pentynoic anhydride obtained was then added to a solution of 4a (0.49 g, 1.03 mmol) in pyridine (20 mL) on an ice-bath. A catalytic amount of DMAP was added and the mixture was stirred for 2 h at room temperature. After completion, the solvent was removed by evaporation and the yellowish oil was subjected to chromatographic purification on a silica gel column using a gradient of 1-2% MeOH in DCM containing 1% TEA as an eluent. 6a was obtained in 95% yield (0.544 g, 0.983 mmol). \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta = 1.19\) (s, 3H), 1.22 (s, 3H), 2.29-2.53 (m, 4H), 2.77 (m, 2H), 2.90 (s, 1H), 3.00 (s,
3H), 3.44 (dd, $J = 11.4$ and 1.4 Hz, 1H), 3.51-3.55 (m, 1H), 3.70 (dd, $J = 11.4$ and 1.9 Hz, 1H), 3.74-3.78 (m, 1H), 4.10-4.14 (m, 1H), 4.26 (d, $J = 8.0$ Hz, 1H), 4.88-4.91 (m, 1H), 5.60 (d, $J = 8.2$ Hz, 1H), 5.72 (s, 1H), 7.31 (t, $J = 7.4$ Hz, 2H), 7.46 (t, $J = 7.4$ Hz, 1H), 7.75 (d, $J = 7.4$ Hz, 2H), 8.04 (d, $J = 8.2$ Hz, 1H), 8.65 (br s, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta = 14.2$, 18.8, 24.3, 32.8, 48.8, 58.0, 65.5, 69.3, 69.7, 80.7, 81.0, 82.2, 88.6, 100.6, 101.8, 117.5, 128.3, 129.3, 130.5, 135.3, 149.3, 161.9, 168.5, 171.3. For the spectra, see page S27. Positive ion ESI-HRMS: m/z obsd. 576.1965 [M+Na]$^+$; calcd. 576.1958 [M+Na]$^+$. 
$^1$H NMR. Compound 2a

$^{13}$C NMR. Compound 2a
$^1$H NMR. Compound 2b

$^{13}$C NMR. Compound 2b
$^1$H NMR. Compound 2c

$^{13}$C NMR. Compound 2c
$^1$H NMR. Compound 2d

$^{13}$C NMR. Compound 2d
$^1$H NMR. Compound 3a

$^{13}$C NMR. Compound 3a
$^1$H NMR. Compound 3b

$^{13}$C NMR. Compound 3b
$^1$H NMR. Compound 3c

$^{13}$C NMR. Compound 3c
$^1$H NMR. Compound 3d

$^{13}$C NMR. Compound 3d
$^1$H NMR. Compound 4a

$^{13}$C NMR. Compound 4a
$^1$H NMR. Compound 4a'

$^{13}$C NMR. Compound 4a'
$^1$H NMR. Compound 4b

$^{13}$C NMR. Compound 4b
$^1$H NMR. Compound 4c'

$^{13}$C NMR. Compound 4c'
$^1$H NMR. Compound 4c''

$^{13}$C NMR. Compound 4c''
$^1$H NMR. Compound 4d

$^{13}$C NMR. Compound 4d
$^{31}$P NMR. Compound 5a'
$^{31}$P NMR. Compound 5c''

$^{31}$P NMR. Compound 5d
$^1$H NMR. Compound 6a

$^{13}$C NMR. Compound 6a
A Thermo ODS Hypersil C18 (250 x 4.6 mm, 5µm) column eluted with a mixture of MeCN and aq Et₃N (0.1 mol L⁻¹) at flow rate 1mL min⁻¹. A linear gradient from MeCN 25% at t = 0 min to MeCN 100% at t = 25min.
Negative ion ESI-MS of pentamer 3’-UUGCA-5’
References:

[1] Saneyoshi, H.; Seio, K.; Sekine, M. *J. Org. Chem.* **2005**, *70*, 10453-10460.