Supporting Information

Short, synthetic and selectively ^{13}C-labeled RNA sequences for the NMR structure determination of protein–RNA complexes

Philipp Wenter, Luc Reymond, Sigrid D. Auweter, Frédéric H.-T. Allain* and Stefan Pitsch*

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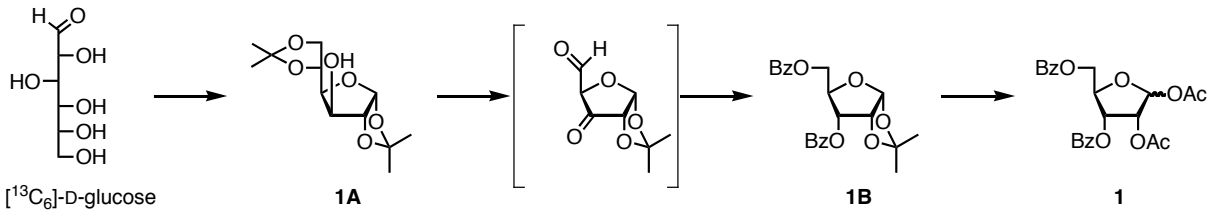
1. Synthesis of Sugar Building Block 1



Scheme 1: Synthesis of the [$^{13}C_5$]-D-ribose derivative **1**; Bz = benzoyl, Ac = acetyl

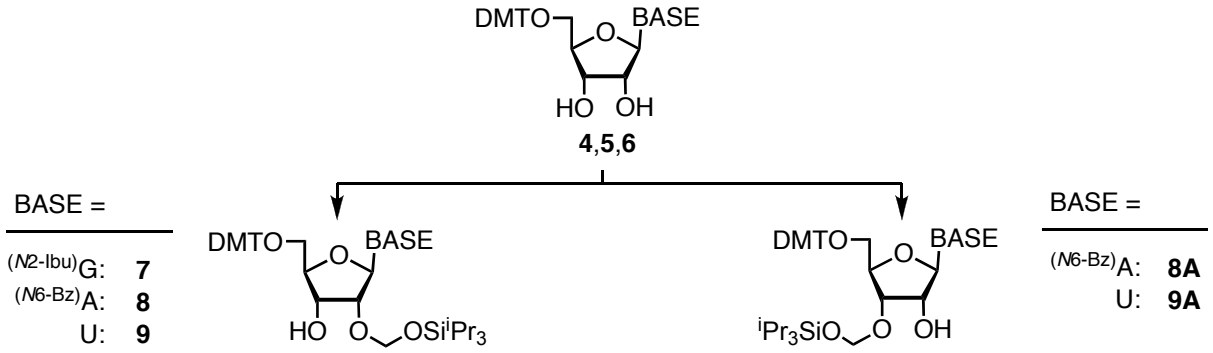
[1,2,3,4,5,6–$^{13}C_6$]-1,2:5,6-Di-O-*isopropylidene-α-D-glucofuranose - crude product* (**1A**). [$^{13}C_6$]-D-glucose (9.85 g, 52.9 mmol) and anhydrous $MgSO_4$ (80 g, 667 mmol) were suspended in dry acetone (550 ml). Anhydrous $FeCl_3$ (1.80 g, 11.0 mmol) was added and the mixture was stirred for 24 h at 20°. Then a 1M $Et_3N{\cdot}H_2CO_3$ solution in MeOH (obtained by bubbling CO_2 through 1M Et_3N in MeOH) was added (60 ml). After 15 min the reaction mixture was filtered and the solid was washed with CH_2Cl_2 (2 x 200 ml). The filtrate and the washings were evaporated to dryness. The residue was dissolved in CH_2Cl_2 (300 ml), extracted with H_2O/satd. aq. $NaHCO_3$ 3:1 and the aqueous phase was washed with CH_2Cl_2 (2 x 100 ml). The organic phase was dried over $MgSO_4$, filtered and the solvent was evaporated: 13.9 g of crude **1A** as light yellow oil which crystallized upon standing. TLC (AcOEt/hexane 4:1) R_f 0.57.

[1,2,3,4,5–$^{13}C_5$]-3,5-Di-O-*benzoyl-1,2-isopropylidene-α-D-ribofuranose* (**1B**). Crude **1A** (13.8 g, 51.9 mmol) was dissolved in CH_2Cl_2 (110 ml), and then pyridinium dichromate (11.9 g, 31.6 mmol) and Ac_2O (14.9 ml, 158.4 mmol) were added. The reaction was stirred for 1.5 h at reflux. The solvents were evaporated and the brown residue was coevaporated with toluene (3 x 50 ml), suspended in EtOAc (100 ml) and filtered through SiO_2 (150 g, elution with AcOEt). The product containing fractions were evaporated and dried overnight under vacuum (0.01 mbar) yielding 12.5 g of a light green oil, which was dissolved in THF (140 ml), cooled to 0° and treated with H_5IO_6 (14.05 g, 61.6 mmol). The mixture was stirred for 2 h at 20° and the precipitate was filtered off. The filtrate was concentrated to 90 ml, diluted with EtOH (90 ml), cooled to 0° and treated with solid $NaBH_4$ (3.6 g, 95.2 mmol) in small portions. After 1 h, AcOH was carefully added until the pH was neutral. Then 50 g of SiO_2 were directly added to the reaction mixture and the solvents were evaporated. The residue was filtered through SiO_2 (50 g, MeOH/AcOEt 1:9 as eluent). The resulting yellow oil was dissolved in pyridine (150 ml) and treated with benzoyl chloride (10.0 ml, 86.5 mmol). Afer 2 h, the pyridine was evaporated, the residue was dissolved in CH_2Cl_2 (250 ml), extracted with 10% aq. citric acid, satd. aq. $NaHCO_3$, dried over $MgSO_4$ and evaporated. Purification by CC (150 g SiO_2, AcOEt/hexane 1:19 → 1:4) yielded **1B** (10.6 g, 50% from [$^{13}C_6$]-D-glucose) as white solid. TLC (AcOEt/hexane 3:7) R_f 0.48. ^{1}H-NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$): 1.38, 1.61 (2*s*, CMe_2); 4.51 (*dd*, *J* = 4.5, 12.2, H–C(5)); 4.65 (*m*, H–C(4)); 4.71 (*dd*, *J* = 2.9, 12.2, H'–C(5)); 5.03–5.07 (*m*, 2 H, H–C(2), H–C(3)); 5.96 (*d*, *J* = 3.2, H–C(1)); 7.39–7.48 (*m*, 4 arom. H); 7.54–7.62 (*m*, 2 arom. H); 8.03–8.09 (*m*, 4 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 26.7 (*Me*$_2$C); 63.2 (*d*, *J* = 45, C(5)); [73.3 (*t*, *J* = 42), 75.7 (*t*, *J* = 43), 77.4 (*dd*, *J* = 33, 40): C(2), C(3), C(4)]; 104.5 (*d*, *J* = 33, C(1)); 113.3 (Me$_2$*C*); 128.4, 128.5, 129.8, 129.9, 133.2, 133.5 (6 arom. CH); 165.8, 166.2 (2 CO). MALDI-MS: 426.5 (100, [*M* + Na]$^+$).

[1,2,3,4,5–$^{13}C_5$]-1,2-Di-O-*acetyl-3,5-di*-O-*benzoyl-α,β-D-ribofuranose* (**1**). A solution of **1B** (10.3 g, 25.5 mmol) in AcOH (12 ml) and Ac_2O (14.6 ml) was treated with H_2SO_4 (0.44 ml, 7.8 mmol) and stirred for 1.5 h at 20°. The mixture was then cooled to 0°, Et_3N (2.1 ml, 15.7 mmol) was added, and half of the solvents were evaporated. The residue was poured in a well stirred mixture of H_2O (400 ml) and CH_2Cl_2 (400 ml), the organic

phase was washed with satd. $NaHCO_3$ and dried over $MgSO_4$. Purification by CC (100 g SiO_2, AcOEt/hexane 1:19 → 1:3) yielded **1** (9.2 g, 81%) as white solid (α/β 1:9). TLC (AcOEt/hexane 3:7) R_f 0.32. ^{1}H-NMR (^{13}C decoupled, 400 MHz, $CDCl_3$, only the signals of the β-isomer are shown): 1.99, 2.11 (2*s*, *Me*$_2$C); 4.49 (*m*, H–C(5)); 4.68–4.76 (*m*, H–C(4), H'–C(5)); 5.58 (*m*, H–C(2)); 5.79 (*m*, H–C(3)); 6.28 (*s*, H–C(1)); 7.39–7.49 (*m*, 4 arom. H); 7.55–7.62 (*m*, 2 arom. H); 8.01–8.09 (*m*, 4 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$, only the signals of the β-isomer are shown): 20.5, 20.7 (2 *Me*CO); 63.6 (*d*, *J* = 44, C(5)); [71.4 (*t*, *J* = 41), 74.4 (*dd*, *J* = 39, 47), 79.8 (*dd*, *J* = 41, 43): C(2), C(3), C(4)]; 98.3 (*d*, *J* = 47, C(1)); 128.4, 128.6, 129.7, 133.3, 133.7 (5 arom. CH); 165.3, 165.9 (*2* Ph*C*O); 169.1, 169.4 (2 Me*C*O). MALDI-MS: 470.6 (100, $[M + Na]^+$).

Synthesis of the Intermediates 7 - 9



Scheme 3: Synthesis of the 2'-*O*- and 3'-*O*-TOM-protected [$^{13}C_5$]-D-ribonucleosides **7**, **8**, **9** and **8A**, **9A**, respectively.

[1',2',3',4',5'–$^{13}C_5$]-5'-O-*(4,4'-Dimethoxytrityl)*-N^2-*isobutyryl-2'*-O-*{[(triisopropylsilyl)oxy]methyl}guanosine* (**7**). A solution of **4** (2.80 g, 4.23 mmol) and ^{i}Pr$_2$NEt (2.53 ml, 14.81 mmol) in $(CH_2Cl)_2$ (17 ml) was treated with Bu_2SnCl_2 (1.54 g, 5.08 mmol), stirred for 30 min, heated to 80°, treated with TOM-Cl (0.94g, 4.23 mmol) and stirred for 20 minutes at 80°. Usual workup and CC (40 g SiO_2, AcOEt/hexane 1:1 → AcOEt) gave **14** (1.80 g, 50%). TLC (AcOEt/hexane 4:1) R_f 0.57. ^{1}H-NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$): 0.67, 0.88 (2*d*, *J* = 6.4, *Me*$_2$CHCO); 0.97–1.15 (*m*, ^{i}Pr$_3$Si); 1.52 (*m*, Me$_2$C*H*CO); 3.00 (br. *s*, HO–C(3')); 3.11 (br. *d*, *J* = 10.9, H–C(5')); 3.54 (br. *d*, *J* = 9.9, H'–C(5')); 3.76, 3.77 (2*s*, 2 MeO); 4.22 (*m*, H–C(4')); 4.56 (*m*, H–C(3')); 4.96 (*d*, *J* = 4.6, OCH_2O); 5.09 (*m*, H–C(2')); 5.15 (*d*, *J* = 4.6, OCH_2O); 5.89 (*d*, *J* = 5.1, H–C(1')); 6.77–6.83 (*m*, 4 arom. H); 7.19–7.56 (*m*, 9 arom. H); 7.65 (br. *s*, HN–C(2)); 7.79 (*s*, H–C(8)); 12.02 (br. *s*, H-N(1)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.8 (Me$_2$*C*H); 17.8 (*Me*$_2$CH); 18.4, 18.5 (*Me*$_2$CHCO); 36.0 (Me$_2$*C*HCO); 55.2 (MeO); 63.7 (*d*, *J* = 43, C(5')); [70.6 (*t*, *J* = 37), 81.2 (*dd*, *J* = 37, 44), 84.2 (*t*, *J* = 41): C(2'), C(3'), C(4')]; 86.5 (*d*, *J* = 44, C(1')); 90.7 (OCH_2O); 113.3 (arom. CH); 127.1, 128.0, 130.0 (arom. CH); 135.7, 136.1 (2 arom. C); 139.1 (C(8)); 145.0 (arom. C); 147.0 (C(4)); 148.2 (C(2)); 155.6 (C(6)); 158.7 (arom. C); 177.9 (Me$_2$CH*C*O). ESI-MS: 847.34 (100, $[M + H]^+$).

[1',2',3',4',5'–$^{13}C_5$]-N^6-*Benzoyl-5'*-O-*(4,4'-dimethoxytrityl)-2'*-O-*{[(triisopropylsilyl)oxy]methyl}adenosine* (**8**) *and* N^6-*benzoyl-5'*-O-*(4,4'-dimethoxytrityl)-3'*-O-*{[(triisopropylsilyl)oxy]methyl}adenosine* (**8A**). A solution of **5** (3.54 g, 5.22 mmol) and ^{i}Pr$_2$NEt (3.12 ml, 18.27 mmol) in $(CH_2Cl)_2$ (21 ml) was treated with Bu_2SnCl_2 (1.76 g, 5.78 mmol), stirred for 30 min at 20°, heated to 80°, treated with [(triisopropylsilyl)oxy]methyl chloride (= TOM-Cl, 1.39g, 6.3 mmol) and stirred for 20 min at 80°. Usual workup and CC (70 g SiO_2, AcOEt/hexane 1:4 → AcOEt) gave **8** (1.16 g). The other products (**5**, **8A** and other side products) were combined and treated with 1M Et_4NF in MeCN (15 ml) for 5 min at 20°. Usual workup and CC (20 g SiO_2, CH_2Cl_2 (+ 2% Et_3N) →

CH_2Cl_2/MeOH 24:1 (+ 2% Et_3N)) gave **5** (1.90 g), which was again treated with TOM-Cl according to the above mentioned procedure. Final yield: 1.91g **8** (42%) and 0.21g **8A** (5%).

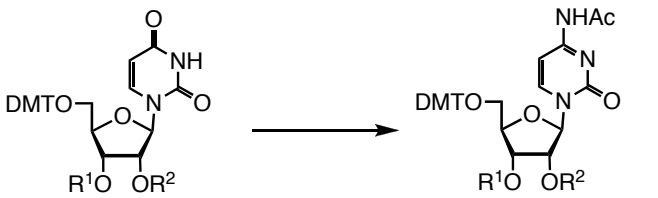
Data of **8**: TLC (AcOEt/hexane 3:2) R_f 0.56. ^{1}H-NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$): 1.02–1.16 (*m*, ^{i}Pr$_3$Si); 3.12 (br. *s* HO–C(3)); 3.43 (*dd*, *J* = 9.8, 3.0, H–C(5')); 3.54 (*dd*, *J* = 10.2, 2.0, H'–C(5')); 3.80 (*s*, 2 MeO); 4.33 (*m*, H–C(4')); 4.60 (*m*, H–C(3')); 4.95–5.05 (*m*, 2 H, H–C(2'), OCH_2O); 5.18 (*d*, *J* = 4.7, OCH_2O); 6.26 (*d*, *J* = 4.2, H–C(1')); 6.81–6.85 (*m*, 4 arom. H); 7.21–7.65 (*m*, 12 arom. H); 8.05 (*d*, *J* = 7.5, 2 arom. H); 8.23 (*s*, H–C(8)); 8.74 (*s*, H–C(2)); 9.05 (br. *s*, HN–C(6)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.8 (Me_2CH); 17.7 (Me_2CH); 55.2 (MeO); 63.3 (*d*, *J* = 44, C(5')); [70.9 (*t*, *J* = 39), 82.1 (*dd*, *J* = 36, 44), 84.3 (*dd*, *J* = 39, 43): C(2'), C(3'), C(4')]; 86.6 (arom. C); 90.0 (*d*, *J* = 44, C(1')); 90.9 (OCH_2O); 113.1 (arom. CH); 123.4 (C(5)); 126.9, 127.8, 128.1, 128.8, 130.0, 132.8 (6 arom. CH); 133.7, 135.62, 135.66 (3 arom. C); 141.9 (C(8)); 144.5 (arom. C); 149.4 (C(6)); 151.7 (C(4)); 152.7 (C(2)); 158.5 (arom. C), 164.5 (Ph*C*O). ESI-MS: 865.37 (100, $[M + H]^+$).

Data of **8A**: TLC (AcOEt/hexane 3:2) R_f 0.41. ^{1}H-NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$): 1.06–1.16 (*m*, ^{i}Pr$_3$Si); 3.36 (*dd*, *J* = 9.7, 3.0, H–C(5')); 3.53 (*dd*, *J* = 7.5, 2.0, H'–C(5')); 3.77 (br. *s*, OH–C(2')); 3.79 (*s*, 2 MeO); 4.41 (*m*, H–C(4')); 4.56 (*m*, H–C(3')); 4.96 (*m*, H–C(2')); 4.99 and 5.14 (2*d*, *J* = 4.5, OCH_2O); 6.09 (*d*, *J* ≈ 4, H–C(1')); 6.77–6.84 (*m*, 4 arom. H); 7.21–7.64 (*m*, 12 arom. H); 8.05 (*d*, *J* = 7.3, 2 arom. H); 8.23 (*s*, H–C(8)); 8.77 (*s*, H–C(2)); 9.09 (br. *s*, HN–C(6)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.7 (Me_2CH); 17.6 (Me_2CH); 55.2 (MeO); 63.0 (*d*, *J* = 43, C(5')); [74.1 (*dd*, *J* = 37, 42), 79.2 (*t*, *J* = 39), 82.9 (*dd*, *J* = 40, 43): C(2'), C(3'), C(4')]; 86.8 (arom. C); 89.4 (*d*, *J* = 42, C(1')); 90.6 (OCH_2O); 113.1 (arom. CH); 126.9, 127.8, 128.1, 128.8, 130.0, 132.7 (6 arom. CH); 133.7, 135.5, 135.7 (3 arom. C); 142.0 (C(8)); 144.4 (arom. C); 146.4 (C(4)); 149.4 (C(6)); 152.7 (C(2)); 158.4 (arom. C), 164.4 (Ph*C*O). ESI-MS: 865.37 (100, $[M + H]^+$).

[1',2',3',4',5'–$^{13}C_5$]-5'-O-*(4,4'-Dimethoxytrityl)-2'*-O-*{[(triisopropylsilyl)oxy]methyl}uridine* (**9**) *and 5'*-O-*(4,4'-Dimethoxytrityl)-3'*-O-*{[(triisopropylsilyl)oxy]methyl}uridine* (**9A**). A solution of **6** (2.06 g, 3.63 mmol) and ^{i}Pr$_2$NEt (2.17 ml, 12.7 mmol) in $(CH_2Cl)_2$ (12 ml) was treated with Bu_2SnCl_2 (1.32 g, 4.36 mmol), stirred for 30 min, heated to 80°, treated with TOM-Cl (0.97 g, 4.4 mmol) and stirred for 20 minutes at 80°. Usual workup and CC (50 g SiO_2, AcOEt/hexane 1:4 → AcOEt) gave **9** (690 mg, 27%). The other products (**6**, **9A** and other side products) were combined and treated with 1M Et_4NF in MeCN (10 ml) for 5 min at 20°. Usual workup and CC (10 g SiO_2, CH_2Cl_2 (+ 2% Et_3N) → CH_2Cl_2/MeOH 24:1 (+ 2% Et_3N)) gave **6** (1.0 g), which was again treated with TOM-Cl according to the above mentioned procedure. Final yield: 1.25 g **9** (47%) and 0.15g **9A** (6%).

Data of **9**: TLC (AcOEt/hexane 11:9) R_f 0.57. ^{1}H-NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$): 1.09–1.20 (*m*, ^{i}Pr$_3$Si); 3.19 (br. *d*, *J* ≈ 3, OH–C(2')); 3.53 (*m*, 2 H, H–C(5')); 3.82 (*s*, 2 MeO); 4.13 (*d*, *J* = 3.9, H–C(4')); 4.29 (*m*, H–C(2')); 4.48 (br. *q*, *J* ≈ 5, H–C(3')); 5.06 and 5.25 (2*d*, *J* = 4.7, OCH_2O); 5.32 (*d*, *J* = 7.9, H–C(5)); 6.05 (br. *s*, H–C(1')); 6.84–6.89 (*m*, 4 arom. H); 7.24–7.42 (*m*, 9 arom. H); 7.96 (*d*, *J* = 8.1, H–C(6)); 8.74 (br. *s*, H –N(3)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.8 (Me_2CH); 17.8 (Me_2CH); 55.2 (MeO); 62.1 (*d*, *J* = 43, C(5')); [69.3 (*t*, *J* = 69), 82.8 (*dd*, *J* = 44, 36), 83.6 (*dd*, *J* = 39, 43): C(2'), C(3'), C(4')]; 87.3 (arom. C); 87.8 (*d*, *J* = 44), C(1')); 90.63 (OCH_2O); 102.2 (C(5)); 113.2 (arom. C); 127.1, 128.0, 128.1, 133.0, 130.1 (5 arom. CH); 135.0, 135.3 (2 arom. C); 140.1 (C(6)); 144.3 (arom. C); 150.1 (C(2)); 158.6 (arom. C); 163.0 (C(4)). ESI-MS: 760.33 (100, $[M + Na]^+$).

Data of **9A**: TLC (AcOEt/hexane 11:9) R_f 0.36. ^{1}H-NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$): 1.03–1.19 (*m*, ^{i}Pr$_3$Si); 3.41 (*d*, *J* = 9.7, H–C(5')); 3.43 (br. *s*, OH–C(2')); 3.58 (*d*, *J* = 10.5, H–C(5')); 3.82 (*s*, 2 MeO); 4.25–4.38 (*m*, 3 H, H–C(2'), H–C(3'), H–C(4')); 4.93 and 5.08 (2*d*, *J* = 4.8, OCH_2O); 5.43 (*d*, *J* = 8.1, H–C(5)); 5.97 (br. *s*, H–C(1')); 6.83–6.88 (*m*, 4 arom. H); 7.25–7.41 (*m*, 9 arom. H); 7.79 (*d*, *J* = 8.1, H–C(6)); 8.57 (br. *s*, H–N(3)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.8 (Me_2CH); 17.8 (Me_2CH); 55.2 (MeO); 62.4 (*d*, *J*(C,C)= 43, C(5')); [74.5 (*dd*, *J* = 43, 37), 78.2 (*t*, *J* = 37), 82.1 (*t*, *J* = 41): C(2'), C(3'), C(4')]; 87.0 (arom. C); 89.2 (*d*, *J* = 43, C(1')); 90.5 (OCH_2O); 102.3 (C(5)); 113.2 (arom. CH); 127.1, 128.0, 128.1, 130.1 (4 arom. CH); 135.2, 135.3 (2 arom. C); 140.4 (C(6)); 144.2 (arom. C); 150.3 (C(2)); 158.7 (arom. C); 162.9 (C(4)). ESI-MS: 760.33 (100, $[M + Na]^+$).



R^1 = H, R^2 = TOM: **9**
R^1 = TOM, R^2 = H: **9A**

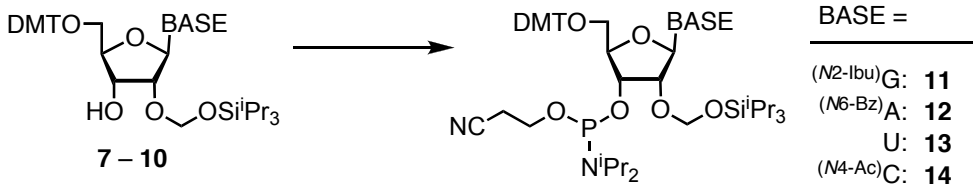
R^1 = H, R^2 = TOM: **10**
R^1 = TOM, R^2 = H: **10A**

Scheme 4: Base transformation and preparation of the 2'-*O*- and 3'-*O*-TOM-protected [$^{13}C_5$]-cytidines **10** and **10A**.

[1',2',3',4',5'–$^{13}C_5$]-N^4*-Acetyl-5'*-O-*(4,4'-dimethoxytrityl)-2'*-O-*{[(triisopropylsilyl)oxy]methyl}cytidine* (**10**). To a solution of **9** (1.04g, 1.41 mmol) in pyridine (5.64 ml) were added DMAP (34 mg, 0.28 mmol) and Ac_2O (0.28 ml, 2.82 mmol). After 2 h at 20°, MeOH (0.2 ml) was added and the solvent was evaporated and coevaporated with toluene (5 ml). The residue was dissolved in CH_2Cl_2 (50 ml) and extracted with 10% aq. citric acid (50 ml) and satd. aq. $NaHCO_3$ (50 ml). The organic phase was dried over $MgSO_4$ and the solvent was evaporated. The resulting foam was dried overnight under vacuum (0.05 mbar). Separately, $(ClC_6H_4)P(O)Cl_2$ (0.69 ml, 4.2 mmol) was added at 4° dropwise to a suspension of dry and finely powdered 1,2,4-triazole (1.75 g, 25.4 mmol) in dry MeCN (9.6 ml). The mixture was stirred 15 min at 4°C and $^iPr_2NEt$ (3.61 ml, 21.2 mmol) was added. The mixture was stirred 30 min at 20°, cooled again to 4°C and treated with a solution of the crude 3'-*O*-acetylated **9** in MeCN (4.8 ml). The mixture was stirred 4 h at 20°, then diluted with dioxane (14.4 ml) and treated with 25% aq. NH_3 (21 ml). After 3 h at 20°, the reaction mixture was concentrated to half of the volume, poured in CH_2Cl_2 (100 ml) and extracted with 10% aq. citric acid (100 ml) and satd. aq. $NaHCO_3$ (100 ml). The organic phase was dried over $MgSO_4$ and the solvents were evaporated. The resulting foam was dissolved in MeOH/THF 4:5 (54 ml) and treated with aq. 2N NaOH (5.8 ml). After 45 min, AcOH (0.68 ml) was added, the solution was concentrated (to ca. 20 ml) and usual work up was performed, followed by filtration on 12 g SiO_2 (CH_2Cl_2 (+ 1% Et_3N) → CH_2Cl_2/MeOH 19:1 (+ 1% Et_3N). The resulting foam (760 mg, 1.03 mmol) was dissolved in DMF (4.9 ml), treated with Ac_2O (0.14 ml, 1.44 mmol) and stirred for 8 h at 20°. The reaction mixture was diluted with AcOEt/hexane 1:1 (100 ml) and extracted with satd. aq. $NaHCO_3$ (100 ml). The organic phase was dried over $MgSO_4$ and the solvents were evaporated. CC (8 g SiO_2, AcOEt/hexane 1:4 (+ 1% Et_3N) → AcOEt/hexane 4:1 (+ 1% Et_3N) gave **10** (699 mg, 65%, based on **9**). TLC (AcOEt/hexane 7:3): R_f 0.40. ^{1}H-NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$): 1.03–1.19 (*m*, $^iPr_3Si$); 2.25 (*s*, MeCO); 3.32 (br. *s*, HO–C(3')); 3.56 (br. *d*, *J* = 10.8, H–C(5')); 3.62 (br. *d*, *J* = 11.3, H'–C(5')); 3.84 (*s*, 2 MeO); 4.12 (br. *d*, *J* = 7.3, H–C(4')); 4.25 (*m*, H–C(2')); 4.40 (*m*, H–C(3')); 5.18, 5.31 (2*d*, *J* ≈ 3, OCH_2O); 6.00 (br. *s*, H–C(1')); 6.87–6.89 (*m*, 4 arom. H); 7.09 (*d*, *J* = 7.1, C(5)); 7.24–7.45 (*m*, 9 arom. H); 8.48 (*d*, *J* = 7.2, H–C(6)); 8.97 (br. s, NH – C(4)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.80 (Me_2CH); 17.76 (Me_2CH); 24.88 (Me_2CO); 55.2 (*q*, MeO); 61.1 (*d*, *J* = 44, C(5')); [67.6 (*t*, *J* = 39), 83.1 (*dd*, *J* = 43, 15), 83.6 (*dd*, *J* = 43, 11): C(2'), C(3'), C(4')]; 87.0 (arom. C); 90.0 (*d*, *J* = 43, C(1')); 90.7 (OCH_2O); 96.6 (arom. C); 113.2 (arom. CH); 127.1, 128.0, 128.2, (3 arom. C); 130.1 (C(5)); 135.2, 135.5, 144.3 (arom. C); 144.8 (C(6)); 154.9 (C(2)); 158.6 (arom. C); 162.8 (C(4)); 170.4 (CO). ESI-MS: 779.30 (100, $[M + H]^+$).

[1',2',3',4',5'–$^{13}C_5$]-N^4*-Acetyl-5'*-O-*(4,4'-dimethoxytrityl)-3'*-O-*{[(triisopropylsilyl)oxy]methyl}cytidine* (**10A**). To a solution of **9A** (280 mg, 0.38 mmol) in pyridine (1.52 ml) were added DMAP (10 mg, 0.08 mmol) and Ac_2O (0.08 ml, 0.76 mmol). After 2 h, MeOH (0.1 ml) was added and the solvent was evaporated and coevaporated with toluene (2 ml). The residue was dissolved in CH_2Cl_2 (25 ml) and extracted with 10% aq. citric acid (25 ml) and satd. aq. $NaHCO_3$ (25 ml). The organic phase was dried over $MgSO_4$ and the solvent was evaporated. The resulting foam was dried overnight under vacuum (0.05 mbar). Separately, (p-ClC_6H_4)$P(O)Cl_2$ (0.19 ml, 1.14 mmol) was added at 4° dropwise to a suspension of dry and finely powdered 1,2,4-triazole (0.47

g, 6.84 mmol) in dry MeCN (2.6 ml). The mixture was stirred 15 min at 4°C and $^{i}Pr_2NEt$ (0.97 ml, 5.70 mmol) was added. The mixture was stirred 30 min at 20°, cooled again to 4°C and treated with a solution of the crude 2'-*O*-acetylated **9A** in MeCN (1.3 ml). The mixture was stirred 4 h at 20°, then diluted with dioxane (3.9 ml) and treated with 25% aq. NH_3 (5.8 ml). After 3 h at 20°, the reaction mixture was concentrated to half of the volume, diluted with CH_2Cl_2 (50 ml) and extracted with 10% aq. citric acid (50 ml) and satd. aq. $NaHCO_3$ (50 ml). The organic phase was dried over $MgSO_4$ and the solvents were evaporated. The resulting foam was dissolved in MeOH/THF 4:5 (14 ml) and treated with 2N NaOH (1.56 ml). After 45 min, AcOH (0.18 ml) was added, the solution was concentrated (to ca. 5 ml) and usual workup was performed, followed by filtration on 4 g SiO_2 (CH_2Cl_2 (+ 1% Et_3N) → CH_2Cl_2/MeOH 19:1 (+ 1% Et_3N). The resulting foam (212 mg, 0.29 mmol) was dissolved in DMF (1.4 ml), treated with Ac_2O (0.04 ml, 0.40 mmol) and stirred for 8 h at 20°. The reaction mixture was diluted with AcOEt/hexane 1:1 (50 ml) and extracted with satd. aq. $NaHCO_3$ (50 ml). The organic phase was dried over $MgSO_4$ and the solvents were evaporated. CC (8 g SiO_2, AcOEt/hexane 1:4 (+ 1% Et_3N) → AcOEt/hexane 4:1 (+ 1% Et_3N) gave **10A** (182 mg, 61%). TLC (AcOEt/hexane 7:3): R_f 0.16. ^{1}H-NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$): 1.04–1.14 (*m*, $^{i}Pr_3Si$); 2.27 (*s*, MeCO); 3.42 (br. *d*, *J* = 10.4, H–C(5')); 3.60–3.65 (*m*, H'–C(5'), HO–C(2')); 3.83 (*s*, 2 MeO); 4.28–4.44 (*m*, 3 H, H–C(2'), H–C(3'), H–C(4')); 4.92, 5.04 (2*d*, *J* = 4.7, OCH_2O); 6.00 (br. *s*, H–C(1')); 6.86–6.90 (*m*, 4 arom. H); 7.20 (*d*, *J* = 7.4, C(5)); 7.27–7.41 (*m*, 9 arom. H); 8.31 (*d*, *J* = 7.4, H–C(6)); 9.54 (br. s, NH – C(4)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.80 (Me_2CH); 17.77 (Me_2CH); 24.9 (*Me*CO); 55.2 (MeO); 61.9 (*d*, *J* = 44, C(5')); [75.5 (*t*, *J* = 39), 76.7 (*t*, *J* = 38), 82.3 (*t*, *J* = 42): C(2'), C(3'), C(4')]; 87.0 (arom. C); 90.7 (OCH_2O); 91.6 (*d*, *J* = 41, C(1')); 96.6 (arom. C); 113.2 (arom. CH); 127.1, 128.0, 128.1, 130.0 (4 arom. CH); 130.1 (C(5)); 135.2, 135.4, (2 arom. C); 144.2 (C(6)); 144.8 (arom. C); 155.4 (C(2)); 158.7 (arom. C); 162.7 (C(4)); 170.5 (CO). ESI-MS: 779.30 (100, $[M + H]^+$).



Scheme 5: Preparation of the [$^{13}C_5$]-D-ribonucleoside phosphoramidite building blocks **11** – **14**.

[1',2',3',4',5'–$^{13}C_5$]-5'-O-*(4,4'-Dimethoxytrityl)*-N^2-*isobutyryl-2'*-O-*{[(triisopropylsilyl)oxy]methyl}guanosine 3'-(2-Cyanoethyl Diisopropylphosphoramidite)* (**11**). A solution of **7** (647 mg, 0.76 mmol) in CH_2Cl_2 (3.1 ml) was treated with $^{i}Pr_2NEt$ (0.33 ml, 1.91 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (0.20 g, 0.84 mmol). After stirring for 14 h at 20° the mixture was subjected to CC (10 g SiO_2, AcOEt/hexane 2:3 (+ 4% Et_3N) → AcOEt (+ 4% Et_3N)): **11** (555 mg, 70%) as colorless foam (2 diastereoisomers). TLC (AcOEt/hexane 7:3): R_f 0.53. ^{1}H-NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$): 0.80 (br. *d*, *J* = 6.7, Me_2CHCO); 0.91–0.98 (*m*, $^{i}Pr_3Si$); 1.03, 1.04, 1.17, 1.18, 1.20, 1.29, 1.30 (7*d*, *J* ≈ 6 ($Me_2CH)_2N$); 1.76, 1.97 (2*m* Me_2CHCO); 2.29 (*t*, *J* = 6.3, 1 H, CH_2CN); 2.76 (*td*, *J* = 7.1, 2.0, 1 H, CH_2CN); 3.28 (*m*, 1 H, H–C(5')); 3.45–3.70 (*m*, 4 H, $(Me_2CH)_2N$, H'–C(5'), $POCH_2$); 3.777, 3.779, 3.785, 3.789 (4*s*, 2 MeO); 3.94, 4.06 (2*m*, 1 H, $POCH_2$); 4.25, 4.34 (2*m*, H–C(4')); 4.56 (br. *d*, *J* = 11.5, 0.5 H, H–C(3')); 4.63 (*m*, 0.5 H, H–C(3')); 4.93 (*d*, *J* = 5.2, 0.5 H, OCH_2O); 4.95 (*s*, 1 H, OCH_2O); 5.00 (*d*, *J* = 5.2, 0.5 H, OCH_2O); 5.09 (*m*, H–C(2')); 5.15 (*d*, *J* = 4.6, OCH_2O); 5.09, 5.18 (2*m*, H–C(1')); 6.79–6.84 (*m*, 4 arom. H); 7.20–7.57 (*m*, 9 arom. H); 7.77, 7.82 (2*s*, H–C(8)); 8.01, 8.40 (2 br. *s*, HN–C(2)); 12.04 (br. *s*, H–N(1)). ^{13}C–NMR (100 MHz, $CDCl_3$): 11.8 (($Me_2CH)_2Si$)); 17.60, 17.62, 17.69, 17.73, 17.75 ($Me_2CH)_2Si$)); 18.5, 18.6, 18.7 (Me_2CHCO); 20.1 (*t*, *J*(C,P) = 6, CH_2CN); 20.2 (*t*, *J*(C,P) = 5, CH_2CN);

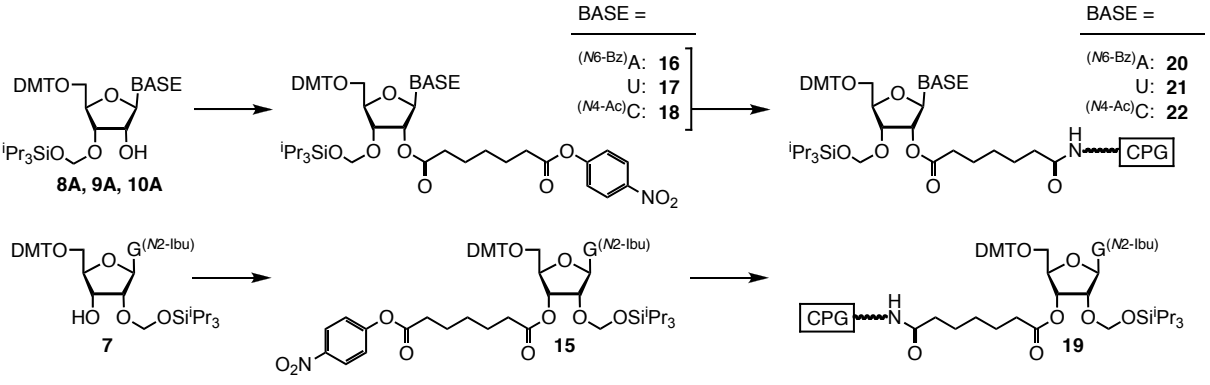
24.49, 24.55, 24.57, 24.60 ((*Me*$_2$CH)$_2$N); 36.8, 36.9 (Me$_2$*C*HCO); 43.0, 43.3 (2*d*, *J*(C,P) = 13, (Me$_2$*C*H)$_2$N); 55.2 (MeO); 57.1, (*t*, *J*(C,P) = 19, POCH$_2$); 58.9, (*t*, *J*(C,P) = 14, POCH$_2$); 63.4, 63.6 (*d*, *J*(C,C) = 43, C(5')); [70.5 (*td*, *J*(C,C) = 39, *J*(C,P) = 17), 71.4 (*td*, *J*(C,C) = 39, *J*(C,P) = 14), 76.3 (*t*, *J* = 39), 78.0 (*t*, *J* = 39), 84.3 (*td*, *J*(C,C) = 40, *J*(C,P) = 8), 86.0 (*t*, *J* = 44): C(2'), C(3'), C(4')]; 88.2, 89.9 (2*d*, *J* = 44, C(1')); 90.1, 90.6 (OCH$_2$O); 113.1, 113.2 (2 arom. CH); 117.3, 117.9 (2 CN); 127.0, 127.1 (2 C(5')); 127.9, 128.0, 128.1, 128.2, 129.9, 130.0, 130.1 (7 arom. CH); 135.6, 135.8, 136.0, 136.3 (4 arom. C); 137.8, 139.1 (2 C(8)); 144.5, 144.9 (2 arom. C); 147.0, 147.3 (2 C(4)); 148.1, 148.5 (2 C(2)); 155.58, 155.62 (2 C(6)); 158.6, 158.7 (2 arom. C); 177.9 (Me$_2$CH*C*O). ESI-MS: 1047.30 (100, [*M* + H]$^+$).

[1',2',3',4',5'–$^{13}C_5$]-N^6*-Benzoyl-5'*-O-*(4,4'-dimethoxytrityl)-2'*-O-*{[(triisopropylsilyl)oxy]methyl}adenosine 3'-(2-Cyanoethyl Diisopropylphosphoramidite)* (**12**). A solution of **8** (1.27g, 1.46 mmol) in CH_2Cl_2 (5.9 ml) was treated with ^{i}Pr$_2$NEt (0.97 ml, 5.70 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (0.41 g, 1.76 mmol). After stirring for 14 h at 20°, the mixture was subjected to CC (25g SiO_2, AcOEt/hexane 1:4 (+ 4% Et$_3$N) → AcOEt/hexane 4:1 (+ 4% Et$_3$N)): **12** (1.21 g, 78%) as colorless foam (2 diastereoisomers). TLC (AcOEt/hexane 1:1): R_f: 0.42. ^{1}H-NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$): 0.91–1.01 (*m*, ^{i}Pr$_3$Si); 1.11, 1.21, 1.23 (3*d*, *J* = 6.8, (*Me*$_2$CH)$_2$N); 2.41 (*t*, *J* = 6.4, 1 H, CH$_2$CN); 2.68 (*td*, *J* = 6.3, 2.7, 1 H, CH$_2$CN); 3.38 (*m*, H–C(5')); 3.54–3.70 (*m*, 1 H of POCH$_2$, 2 H of (Me$_2$C*H*)$_2$N, 1 H of H'–C(5')); 3.79, 3.80 (2*s*, 2 MeO); 3.87–4.02 (*m*, 1 H, POCH$_2$); 4.40, 4.45 (2*m*, H–C(4')); 4.73 (*m*, H–C(3')); 4.97, 5.00, 5.03 (3*d*, *J* = 5.0, OCH$_2$O); 5.24 (*m*, H–C(2')); 6.23, 6.25 (2*d*, *J* = 5.2, H–C(1')); 6.79–6.83 (*m*, 4 arom. H); 7.20–7.65 (*m*, 12 arom. H); 8.05 (*d*, *J* = 7.3, 2 arom. H); 8.20, 8.22 (2*s*, H–C(8)); 8.71, 8.74 (2*s*, H–C(2)); 9.07 (br. *s*, HN–C(6)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.8 ((Me$_2$*C*H)$_3$Si); 17.63, 17.66 ((*Me*$_2$CH)$_3$Si); 20.1 (*d*, *J*(C,P) = 7.2, *C*H$_2$CN); 20.4 (*d, J*(C,P) = 5.6, *C*H$_2$CN); 24.53, 24.56, 24.60, 24.64 (4 (*Me*$_2$CH)$_2$N); 43.2, 43.4 (2*d*, *J*(C,P) = 12, (Me$_2$*C*H)$_2$N); 55.18, 55.21 (2 MeO); 58.0, (*d*, *J*(C,P) = 20, POCH$_2$); 58.9, (*d*, *J*(C,P) = 17, POCH$_2$); 62.8, 63.2 (2*d*, *J* ≈ 40, C(5')); [71.2 (*td*, *J*(C,C) = 39, *J*(C,P) = 17), 69.6 (*td*, *J*(C,C) = 39, *J*(C,P) = 15), 76.9 (*t*, *J* = 44), 77.6 (*t*, *J* = 41), 84.0 (*td, J(C,C)* = 42, *J*(C,P) = 3), 84.1 (*td, J(C,C)* = 41, *J*(C,P) = 5): C(2'), C(3'), C(4')]; 86.6, 87.1 (2 arom. C); 87.4, 87.9 (2*d*, *J* = 41, C(1')); 89.3, 89.7 (2 OCH$_2$O); 96.5, 96.6 (2 C(5)); 113.1 (arom. CH); 117.3, 117.6 (2 CN); 123.3 (C(5)); 126.8, 126.9, 127.8, 128.2, 128.3, 128.8, 130.01, 130.03, 130.07, 132.7 (10 arom. CH); 133.75, 133.78, 135.63, 135.68, 135.76, (5 arom. C); 142.3, 142.4 (2 C(8)); 144.4, 144.5 (2 arom. C); 149.4 (C(4)); 151.51, 151.53 (2 C(6)); 152.64, 152.67 (2 C(2)); 158.5 (arom. C); 164.4 (Ph*C*O). ^{31}P-NMR (162 MHz, $CDCl_3$): 151.3, 151.4 (2*td*, *J*(C,P) = 3, 17). ESI-MS: 1065.29 (100, [*M* + H]$^+$).

[1',2',3',4',5'–$^{13}C_5$]-5'-O-*(4,4'-Dimethoxytrityl)-2'*-O-*{[(triisopropylsilyl)oxy]methyl}uridine 3'-(2-Cyanoethyl Diisopropylphosphoramidite)* (**13**). A solution of **9** (606 mg, 0.82 mmol) in CH_2Cl_2 (3.3 ml) was treated with ^{i}Pr$_2$NEt (0.35 ml, 2.05 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (0.23 g, 0.98 mmol). After stirring for 14 h at 20°, the mixture was subjected to CC (15 g SiO_2, AcOEt/hexane 2:3 (+ 4% Et$_3$N) → AcOEt/hexane 3:2 (+ 4% Et$_3$N)); **13** (697 mg, 90%) as colorless foam (2 diastereoisomers). TLC (AcOEt/hexane 1:1): R_f 0.53. ^{1}H-NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$): 1.02–1.09 (*m*, ^{i}Pr$_3$Si); 1.17–1.20 (*m*, (*Me*$_2$CH)$_2$N); 2.41 (*t*, *J* = 6.4, 1 H, CH$_2$CN); 2.66 (*dt*, *J* ≈ 2.7, 6.5, 1 H, CH$_2$CN); 3.41 (br. *t*, *J* ≈ 9.5, H–C(5')); 3.51–3.65 (*m*, 4 H, (Me$_2$C*H*)$_2$N, H'–C(5'), POC*H*$_2$); 3.81, 3.82 (2*s*, 2 MeO); 3.82–3.99 (*m*, 1 H, POC*H*$_2$); 4.21, 4.28 (2*m*, H–C(4')); 4.40–4.52 (*m*, H–C(2'), H–C(3')); 5.00–5.08 (*m*, OCH$_2$O); 5.34, 5.38 (2*d*, *J* = 8.1, H–C(5)); 6.14 (*d*, *J* = 4.4, 0.5 H, H–C(1')); 6.15 (*d*, *J* = 3.6, 0.5 H, H–C(1')); 6.83–6.87 (*m*, 4 arom. H); 7.24–7.43 (*m*, 9 arom. H); 7.82, 7.89 (2*d*, *J* = 8.1, H–C(6)); 8.43 (br. *s*, H–N(3)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.9 (Me$_2$*C*H)$_3$Si); 17.7, 17.8 (2 (*Me*$_2$CH)$_3$Si); 20.2 (*d*, *J*(C,P) = 7.1, *C*H$_2$CN); 20.4 (*d*, *J*(C,P) = 5.6, *C*H$_2$CN); 24.47, 24.50, 24.54, 24.59 (4 (*Me*$_2$CH)$_2$N); 43.1, 43.3 (2*d*, *J*(C,P) = 12, (Me$_2$*C*H)$_2$N); 55.19, 55.22 (2 MeO); 57.8, (*d*, *J*(C,P) = 19, POCH$_2$); 58.8, (*d*, *J*(C,P) = 17, POCH$_2$); 62.1, 62.5 (2*d*, *J* = 41, C(5')); [70.3 (*td*, *J*(C,C) = 40, *J*(C,P) = 16), 70.8 (*td*, *J*(C,C) = 40, *J*(C, P) = 14), 77.4 (*td*, *J*(C,C) = 40, *J*(C,P) = 4), 78.0 (*t*, *J* = 42), 83.3 (*td, J(C,C)* = 39, *J*(C,P) = 5),

83.4 (*t, J* = 41): (C(2'), C(3'), C(4')]; 87.0, 87.5 (2*d*, *J* = 41, C(1')); 89.21, 89.24 (2 OCH_2O); 102.2, 102.4 (2 C(5)); 113.20, 113.23 (2 arom. CH); 117.3, 117.7 (2 CN); 127.1, 127.9, 128.17, 128.23, 130.1, 130.18 (6 arom. CH); 135.0, 135.20, 135.23, 135.3 (4 arom. C); 140.33, 140.38 (2 C(6)); 144.1, 144.3 (2 arom. C); 149.9, 150.0 (2 C(2)); 158.7 (arom. C); 162.8, 162.9 (2 C(4)). ^{31}P-NMR (162 MHz, $CDCl_3$): 150.8, 151.3 (2 *dt*, *J*(C,P) = 15, 3). ESI-MS: 938.33 (100, $[M + H]^+$).

[1',2',3',4',5'–$^{13}C_5$]-N^4*-Acetyl-5'*-O-*(4,4'-dimethoxytrityl)-2'*-O-*{[(triisopropylsilyl)oxy]methyl}cytidine 3'-(2-Cyanoethyl Diisopropylphosphoramidite)* (**14**). A solution of **10** (665 mg, 0.90 mmol) in CH_2Cl_2 (3.6 ml) was treated with $^iPr_2NEt$ (0.39 ml, 2.25 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (0.26 g, 1.08 mmol). After stirring for 14 h at 20°, the mixture was subjected to CC (16 g SiO_2, AcOEt/hexane 2:3 (+ 4% Et_3N) → AcOEt/hexane 7:3 (+ 4% Et_3N): **14** (767 mg, 86%) as colorless foam (2 diastereoisomers). TLC (AcOEt/hexane 7:3): R_f 0.54. ^{1}H-NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$): 1.03–1.12 (*m*, $^iPr_3Si$); 1.16, 1.18 (2*d*, *J* = 6.8, $(Me_2CH)_2N$); 2.25, 2.26 (2*s*, MeCO); 2.41 (*t*, *J* = 6.4, 1 H, CH_2CN); 2.63 (br. *q*, *J* ≈ 6, 1 H, CH_2CN); 3.44–3.70 (*m*, 1 H of $POCH_2$, $(Me_2CH)_2N$, H–C(5')); 3.830, 3.837, 3.839 (3*s*, 2 MeO); 3.90–3.98 (*m*, 1 H, $POCH_2$); 4.27, 4.40 (*m*, H–C(2'), H–C(4')); 4.52 (*m*, H–C(3')); 5.18 (*t*, *J* = 4.4, 1 H of OCH_2O); 5.23 (*dd*, *J* = 4.3, 7.2, 1 H of OCH_2O); 6.17 (br. *s*, 0.5 H, H–C(1')); 6.18 (br. *s*, 0.5 H, H–C(1')); 6.85–6.89 (*m*, 4 arom. H); 6.98, 7.05 (2*d*, *J* = 7.3, H–C(5)); 7.28–7.47 (*m*, 9 arom. H); 8.38, 8.50 (2*d*, *J* = 7.5, H–C(6)); 9.67, 9.74 (2br. *s*, NH–C(4)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.9 $(Me_2CH)_3Si$); 17.8, 17.9 (2 $Me_2CH)_3Si$); 20.1 (*d*, *J*(C,P) = 7.0, CH_2CN); 20.3 (*d*, *J*(C,P) = 6.7, CH_2CN); 24.44, 24.51, 24.54, 24.57, 24.61, 24.64 (6 $(Me_2CH)_2N$); 24.9 (*Me*CO); 43.1, 43.3 (2*d*, *J*(C,P) = 6, $(Me_2CH)_2N$); 55.2, 55.3 (2 MeO); 58.1, (*d*, *J*(C,P) = 20, $POCH_2$); 58.7, (*d*, *J*(C,P) = 17, $POCH_2$); 60.8, 61.5 (2*d*, *J* = 42, C(5')); [69.2 (*td*, *J*(C,C) = 39, *J*(C,P) = 14), 69.6 (*td*, *J*(C,C) = 39, *J*(C,P) = 14), 78.6 (*t*, *J* = 40), 78.9 (*t*, *J* = 40), 82.2 (*td, J*(C,C) = 43, *J*(C,P) = 3), 82.4 (*td, J*(C,C) = 42, *J*(C,P) = 5): C(2'), C(3'), C(4')]; 87.0, 87.1 (2 arom. C); 89.3, 89.7 (2*d*, *J* = 40, C(1')) 90.0 (OCH_2O); 96.5, 96.6 (2 C(5)); 113.20, 113.22 (2 arom. CH); 117.3, 117.6 (2 CN); 127.1, 127.9, 128.3, 130.25, 130.26 (5 arom. CH); 135.13, 135.20, 135.26, 135.36, 144.1, 144.2 (6 arom. C); 144.9, 145.0 (2 C(6)); 149.9, 150.0 (2 C(2)); 158.7 (arom. C); 162.66, 162.71 (2 C(4)). ^{31}P-NMR (162 MHz, $CDCl_3$): 150.7, 151.0 (2*d*, *J*(C,P) = 14). ESI-MS: 979.35 (100, $[M + H]^+$).



Scheme 6: Preparation of the [$^{13}C_5$]-D-ribonucleoside activated ester derivatives **15** – **18** and the immobilized [$^{13}C_5$]-D-ribonucleosides (= solid supports) **19** – **22**

[1',2',3',4',5'–$^{13}C_5$]-5'-O-*(4,4'-Dimethoxytrityl)*-N^2*-isobutyryl-2'*-O-*{[(triisopropylsilyl)oxy]methyl}guanosine 3'-(4-Nitrophenyl Heptanedioate)* (**15**). To a soln. of **7** (125 mg, 0.15 mmol) in pyridine (1.5 ml), bis(4-nitrophenyl)heptanedioate (358 mg, 0.90 mmol) and DMAP (10 mg, 0.08 mmol) were added. After stirring for 14 h at 20°, the mixture was evaporated and co-evaporated with toluene (2 x 5 ml). CC (3 g SiO_2, AcOEt/hexane 1:4 → AcOEt) gave **15** (94 mg, 56%) as colorless foam. TLC (AcOEt/hexane 7:3) R_f 0.53. ^{1}H-NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$): 0.68, 0.88 (2*d*, *J* = 6.8, Me_2CHCO); 0.91–0.98 (*m*, $^iPr_3Si$); 1.40–1.55 (*m*, 3H,

CH_2, Me_2CHCO); 1.73 (*m*, CH_2); 1.81 (*m*, CH_2); 2.44 (*dt*, *J* = 7.4, 2.7, CH_2); 2.64 (*t*, *J* = 7.4, CH_2); 3.15 (br. *d*, *J* = 9.6, H–C(5')); 3.60 (br. *d*, *J* = 10.1, H'–C(5')); 3.77, 3.78 (2*s*, 2 MeO); 4.25 (*m*, H–C(4')); 4.89, 4.94 (2*d*, *J* = 5.3, OCH_2O); 5.51 (*m*, H–C(2')); 4.57 (*m*, H–C(3')); 5.87 (*d*, *J* = 6.7, H–C(1')); 6.79–6.83 (*m*, 4 arom. H); 7.21–7.57 (*m*, 11 arom., H, HN–C(2)); 7.80 (*s*, H–C(8)); 8.25–8.30 (*m*, 2 arom. H); 11.97 (br. *s*, H–N(1)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.8 (Me_2CH); 17.6 (Me_2CH); 18.3, 18.5 (2 Me_2CHCO); 24.3, 24.4, 28.4, 33.8, 34.0 (5 CH_2); 36.0 (Me_2CHCO); 55.2 (MeO); 63.2 (*d*, *J* = 43, C(5')); [71.5 (*t*, *J* = 39), 76.0 (*dd*, *J* = 39, 44), 83.5 (*dd*, *J* = 39, 42)): C(2'), C(3'), C(4')]; 86.7 (*d*, *J* = 45, C(1')); 89.8 (OCH_2O); 113.3 (arom. CH); 115.9 (C(5)); 122.4, 125.2, 126.1, 127.2, 128.1, 129.9, 130.0, (7 arom. CH); 135.5, 136.1 (2 arom. C); 139.3 (C(8)); 144.8 (arom. C); 146.9 (C(4)); 148.2 (C(2)); 155.4 (C(6)); 158.7 (arom. C); 171.1, 172.6 (2 CO); 178.1 (Me_2CHCO). ESI-MS: 1110.82 (100, $[M + H]^+$).

*[1',2',3',4',5'–$^{13}C_5$]-*N^6*-Benzoyl-5'-*O*-(4,4'-dimethoxytrityl)-3'-*O*-{[(triisopropylsilyl)oxy]methyl}adenosine 2'-(4-Nitrophenyl Heptanedioate)* (**16**). To a soln. of **8A** (126 mg, 0.15 mmol) in pyridine (1.5 ml), bis(4-nitrophenyl) heptanedioate (358 mg, 0.90 mmol) and DMAP (10 mg, 0.08 mmol) were added. After stirring for 14 h at 20°, the mixture was evaporated and co-evaporated with toluene (2 x 5 ml). CC (3 g SiO_2, AcOEt/hexane 1:4 → AcOEt/hexane 7:3): **16** (118 mg, 70%) as colorless foam. TLC (AcOEt/hexane 7:3) R_f 0.67. ^{1}H-NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$): 0.87–1.14 (*m*, $^iPr_3Si$); 1.43 (*m*, CH_2); 1.65–1.80 (*m*, 2 CH_2); 2.44 (*t*, *J* = 7.4, CH_2); 2.59 (*t*, *J* = 7.4, CH_2); 3.39 (*dd*, *J* = 9.7, 3 H–C(5')); 3.61 (*dd*, *J* = 7.5, 2 H'–C(5')); 3.78 (*s*, 2 MeO); 4.32 (*m*, H–C(4')); 4.64 (*m*, H–C(3')); 4.87 and 5.01 (2*d*, *J* ≈ 5, OCH_2O); 6.14 (*m*, H–C(2')); 6.56 (*m*, H–C(1')); 6.78–6.82 (*m*, 4 arom. H); 7.18–7.64 (*m*, 14 arom. H); 8.04 (*d*, *J* = 8.2, 2 arom. H); 8.21 (*s*, H–C(2)); 8.24–8.27 (*m*, 2 arom. H); 8.75 (*s*, H–C(8)); 9.11 (br. *s*, HN–C(6)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.7 (Me_2CH); 17.6 (Me_2CH); 55.2 (MeO); 63.0 (*d*, *J* = 43, C(5')); [74.1 (*dd*, *J* = 37, 42), 79.2 (*t*, *J* = 39), 82.9 (*dd*, *J* = 40, 43): C(2'), C(3'), C(4')]; 86.8 (arom. C); 89.4 (*d*, *J* = 42, C(1')); 90.6 (OCH_2O); 113.1 (arom. CH); 126.9, 127.8, 128.1, 128.8, 130.0, 132.7 (6 arom. CH); 133.7, 135.5, 135.7 (3 arom. C); 142.0 (C(8)); 144.4 (arom. C); 146.4 (C(4)); 149.4 (C(6)); 152.7 (C(2)); 158.4 (arom. C), 164.4 (Ph*C*O). ESI-MS: 1128.79 (100, $[M + H]^+$).

*[1',2',3',4',5'–$^{13}C_5$]-5'-*O*-(4,4'-Dimethoxytrityl)-3'-*O*-{[(triisopropylsilyl)oxy]methyl}uridine 2'-(4-Nitrophenyl Heptanedioate)* (**17**). To a soln. of **9A** (138 mg, 0.19 mmol) in pyridine (1.9 ml), bis(4-nitrophenyl) heptanedioate (454 mg, 1.14 mmol) and DMAP (12 mg, 0.1 mmol) were added. After stirring for 14 h at 20°, the mixture was evaporated and co-evaporated toluene (2 x 5 ml). CC (4 g SiO_2, AcOEt/hexane 1:4 → AcOEt): **17** (156 mg, 82%) as colorless foam. TLC (AcOEt/hexane 7:3): R_f 0.77 ^{1}H-NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$): 0.95–1.10 (*m*, $^iPr_3Si$); 1.48 (*m*, CH_2); 1.67–1.84 (*m*, 2 CH_2); 2.45 (*td*, *J* = 7.3, 1.5, CH_2); 2.62 (*t*, *J* = 7.4, CH_2); 3.47 (*d*, *J* = 10.3, H–C(5')); 3.57 (*d*, *J* = 10.3, H–C(5')); 3.80 (*s*, 2 MeO); 4.33 (*m*, H–C(4')); 4.58 (*m*, H–C(3')), 4.84 and 4.97 (2*d*, *J* = 4.7, OCH_2O); 5.32 (*d*, *J* = 8.1, H–C(5)); 5.43 (br. *t*, *J* = 4.3, H–C(2')); 6.18 (*d*, *J* = 4.8, H–C(1')); 6.81–6.87 (*m*, 4 arom. H); 7.23–7.42 (*m*, 11 arom. H); 7.74 (*d*, *J* = 8.1, H–C(6)); 8.24–8.27 (*m*, 2 arom. H); 8.45 (br. *s*, H–N(3)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.8 (Me_2CH); 17.8 (Me_2CH); 24.27, 24.32, 28.3, 33.6, 34.0 (5 CH_2); 55.2 (MeO); 62.6 (*d*, *J* = 43, C(5')); [74.0 (*t*, *J* = 40), 74.2 (*t*, *J* = 40), 83.1 (*t*, *J* = 41): (C(2'), C(3'), C(4')]; 86.4 (*d*, *J* = 48), C(1')); 89.6 (OCH_2O); 102.5 (C(5)); 113.3 (arom. CH); 115.6, 122.4, 125.2, 126.1, 127.2, 128.0, 128.6, 130.1, 130.2 (9 arom. CH); 135.0, 135.1 (2 arom. C); 140.0 (C(6)); 144.0, 145.3 (2 arom. C); 150.1 (C(2)); 155.4 (arom. C); 158.8 (arom. C); 162.9 (C(4)); 171.0, 172.3 (2 CO). ESI-MS: 1023.35 (100, $[M + Na]^+$).

*[1',2',3',4',5'–$^{13}C_5$]-*N^4*-Acetyl-5'-*O*-(4,4'-dimethoxytrityl)-3'-*O*-{[(triisopropylsilyl)oxy]methyl}cytidine 2'-(4-Nitrophenyl Heptanedioate)* (**18**). To a soln. of **10A** (148 mg, 0.19 mmol) in pyridine, (1.9 ml) bis(4-nitrophenyl) heptanedioate (454 mg, 1.14 mmol) and DMAP (12 mg, 0.1 mmol) were added. After stirring for 14 h at 20°, the mixture was evaporated and co-evaporated with toluene (2 x 5 ml). CC (4 g SiO_2, AcOEt/hexane 1:4 → AcOEt):

18 (122 mg, 63%) as colorless foam. TLC (AcOEt/hexane 7:3): R_f 0.30. ^{1}H-NMR (^{13}C-decoupled, 400 MHz, $CDCl_3$): 0.92–1.05 (*m*, ^{i}Pr$_3$Si); 1.47 (*m*, CH_2); 1.67–1.82 (*m*, 2 CH_2); 2.21 (*s*, MeCO); 2.44 (*td*, 4 H, *J* = 7.3, 2.0, 2 CH_2); 2.60 (*t*, *J* = 7.4, CH_2); 3.49 (br. *d*, *J* = 10.6, H–C(5')); 3.69 (br. *d*, *J* = 10.6, H'–C(5')); 3.81 (*s*, 2 MeO); 4.35 (*m*, H–C(4')) 4.51 (*m*, H–C(3'); 4.82 and 4.89 (2*d*, *J* = 4.1, OCH_2O); 5.49 (*m*, H–C(2')); 6.18 (br. *s*, H–C(1')); 6.86 (*m*, 4 arom. H); 7.06 (*d*, *J* = 7.4, C(5)); 7.23–7.43 (*m*, 11 arom. H); 8.12 (*d*, *J* = 8.8, H–C(6)); 8.21–8.28 (*m*, 2 arom. H); 8.86 (br. s, NH–C(4)). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.8 (Me_2CH); 17.8 (Me_2CH); 24.3, 24.4, 24.9, 33.7, 34.0 (5 CH_2); 24.9 (*Me*CO); 55.2 (MeO); 61.9 (*d*, *J* = 43, C(5')); [72.4 (*t*, *J* = 38), 74.5 (*t*, *J* = 40), 82.3 (*t*, *J* = 42): C(2'), C(3'), C(4')]; 87.2 (arom. C); 88.7 (*d*, *J* = 43, C(1')); 89.6 (OCH_2O); 96.9 (arom. C); 113.3, 115.8 (2 arom. CH); 122.4, 125.2, 126.1, 127.2, 128.0, 128.3 (6 arom. CH); 130.2 (C(5)); 135.1 (arom. C); 135.2 (C(6')); 144.0, 144.6, 145.2 (3 arom. C); 155.4 (C(2)); 155.8 (arom. C); 158.7 (arom. C); 162.5 (C(4)); 170.0, 171.0, 171.9 (3 CO). ESI-MS 1042.31 (100, $[M + H]^+$), 1064.81 (90, $[M + Na]^+$).

Preparation of solid supports **19–22** *by immobilization of nucleoside derivatives* ***15–18*** *on LCAA-CPG.* A suspension of LCAA-CPG (1.0 g, 500Å, *Millipore*), (4-nitrophenyl heptanedioate)-modified nucleosides **15-18** (0.1 mmol) and ^{i}Pr$_2$NEt (1 ml) in DMF (5 ml) was shaken for 16 h at 25°. After filtration, the solid was washed with DMF and CH_2Cl_2, suspended in pyridine (1.2 ml) and Ac_2O (0.8 ml), and shaken for 2 h at 25°. After filtration, the solid was washed with DMF and CH_2Cl_2, and dried to give the solid supports **19-22** with loadings of 30 – 35 μmol/g.