SUPPORTING INFORMATION

Synthesis of oligoribonucleotides with phosphonate-modified linkages.

Authors: Ondřej Páv,[a] Ivana Košiová,[a] Ivan Barvík,[b] Radek Pohl,[a] Miloš Buděšínský[a] and Ivan Rosenberg*[a]

[a] *Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic*

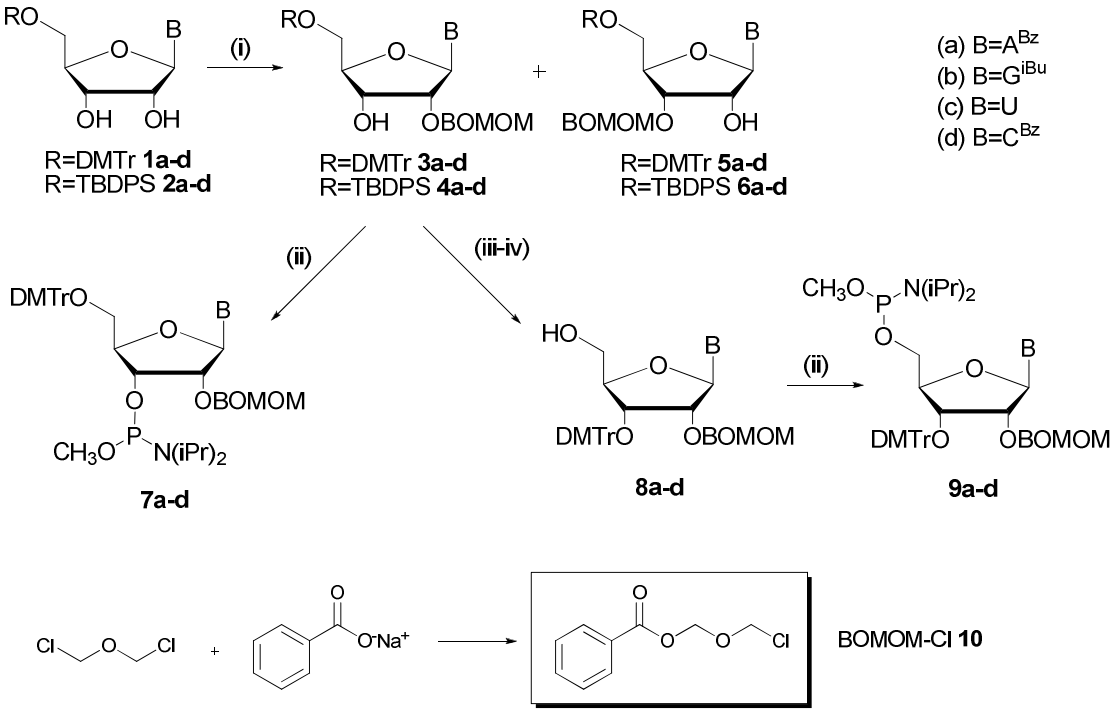
[b] *Institute of Physics, Faculty of Mathematics and Physics, Charles University, Ke Karlovu 5, 121 16 Prague 2, Czech Republic*

[*] *ivan@uochb.cas.cz*

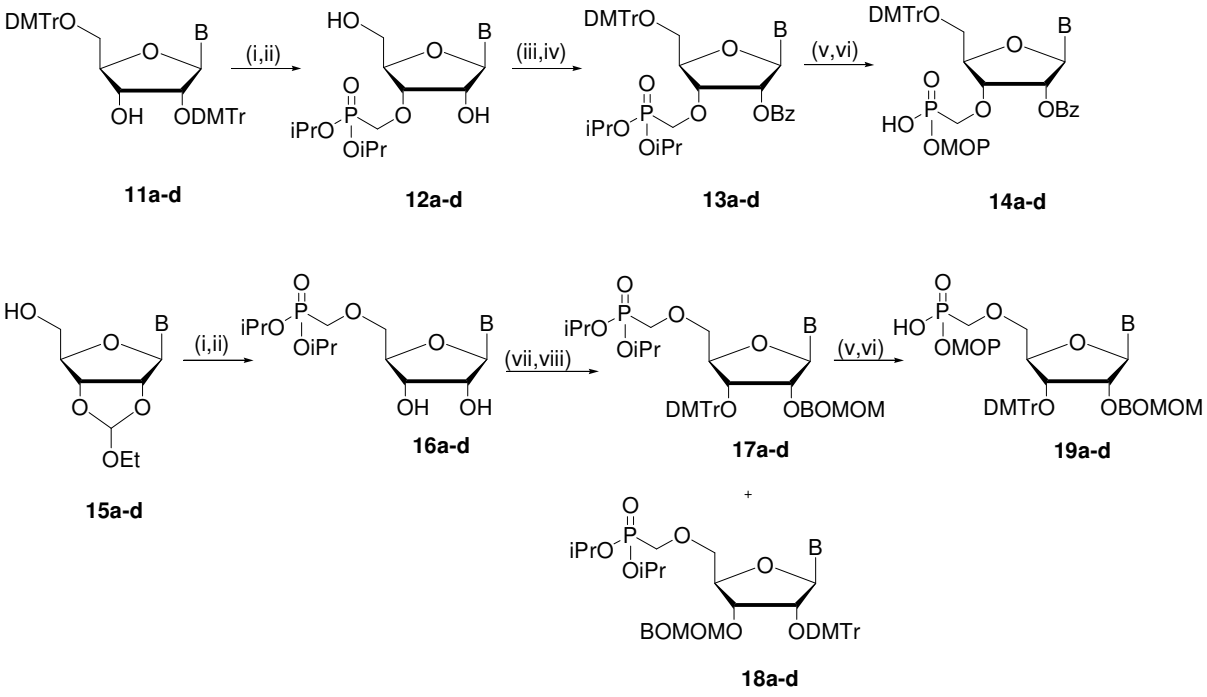
Content:

NMR spectra were measured on a Varian UNITY-500 or Bruker Avance-500 spectrometer. Mass spectra were recorded on a ZAB – EQ (VG Analytical) instrument using FAB (ionisation by Xe, accelerating voltage 8 kV) technique with glycerol and thioglycerol as matrices. MALDI TOF MS was performed on Reflex IV (Bruker - Daltonics) instrument using 3-hydroxypyridine-2-carboxylic acid and pyridine-2-carboxylic acid as matrices. Oligoribonucleotides were synthesized on a 1 μmol scale using GenSyn V02 DNA/RNA synthesizer. Thermal stabilities of duplexes were measured on spectrophotometer Varian Cary 100 Bio.

1. Preparation of monomers



Scheme 1. Preparation of phosphoramidites **7** and **9**, and benzoyloxymethoxymethyl chloride **10**.



Scheme 2. Preparation of phosphonates **14** and **19**.

General Methods

Method A - Preparation of nucleosides 3, 4, 5 and 6.
Dibutyltin dichloride (1.1 mmol) was added to a solution of 5´-protected derivative **1**, **2** (1.0 mmol) and DIPEA (2.2 mmol) in DCE (10 ml). The mixture was stirred 1 h at r. t. After that, reaction mixture was warmed up to 85 °C and benzoyloxymethoxymethyl chloride **10** (1.1 mmol) was added. The reaction mixture was heated 3 h at 85 °C to afford mixture of 2´-BOMOM a 3´-BOMOM derivatives. The regioisomers were separated by silica gel chromatography (elution with gradient of 0 - 50% acetone in toluene, guanosine derivatives 0 - 100% acetone in toluene) as TLC faster eluted isomer (2´-BOMOM derivative **3**, **4**) and TLC slower eluted isomer (3´-BOMOM derivative **5**, **6**).

Method B – Preparation of nucleosides 8.
BOMOM derivative **4**, **6** (1.0 mmol) in pyridin (10 ml) was added to a 30 min pre-stirred mixture of silver triflate (2.0 mmol) and dimethoxytrityl chloride (2.0 mmol) in pyridine (10 ml). The reaction mixture was stirred 16 h at r.t. Reaction was quenched by the addition of methanol (5 ml), silver chloride was filtered off and the mixture was concentrated under reduced pressure. The product was purified by chromatography on silica gel (elution with gradient of 0 – 50% ethylacetate in toluene).
To cleave off 5'-TBDPS group, fully protected nucleoside (1.0 mmol) was dissolved in 0.5M TBAF in THF (10 ml), and reaction mixture was stirred 2 h at r.t. After that, mixture was diluted with toluene and concentrated under reduced pressure. The product was purified by chromatography on silica gel (elution with gradient of 0 - 50% acetone in toluene, guanosine derivatives 0 - 100% acetone in toluene).

Method C – Preparation of phosphoramidites 7 and 9.
Tetrazol (1.1 mmol) was added to a solution of derivative **3**, **8** (1.0 mmol) and methyl *N*,*N*,*N´*,*N´*-tetraisopropylphosphordiamidite (2.0 mmol) in DCM (10 mL). The reaction mixture was stirred 2 h at r.t. After that, TEA (1 mmol) was added and the reaction mixture was evaporated. The product was purified by chromatography on silica gel (elution with a gradient of 0–50% ethyl acetate in toluene, guanosine derivatives used 0–50% acetone in toluene). In addition, the guanosine derivative were dissolved in DCM and precipitated from hexane. Lastly, phosphoramidites were freeze-dried from benzene.

Method D – Preparation of phosphonates 12 and 16.
Sodium hydride (3.0 mmol) was added at 0 °C to a stirred solution of protected nucleoside **11**, **15** (1.0 mmol) and diisopropyl tosyloxymethylphosphonate (2.0 mmol) in DMF (10 ml), and the reaction mixture was left to warm up gradually to r.t., and then stirred 16 h at r.t. The reaction was quenched by the addition of glacial acetic acid (1 ml) at 0 °C and the mixture was concentrated under reduced pressure. After that, nucleoside-phosphonate was treated with 80% acetic acid (10 ml) 16h at r.t., and concentrated under reduced presure. The product was purified by chromatography on silica gel (elution with gradient of 0 – 10% ethanol in chloroform).

Method E – Preparation of phosphonates 13.
Dimethoxytrityl chloride (1.1 mmol) was added under stirring to a solution of phosphonate **12** (1.0 mmol) in pyridine (10 ml), and the mixture was stirred 16 h at r.t. The reaction was quenched by the addition of triethylamine (1 ml) and methanol (5 ml), and the mixture was concentrated under reduced pressure. The residue was dissolved in chloroform (50 ml) and extracted with saturated solution of sodium hydrogencarbonate (3 x 20 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated. After that, benzoyl cyanide (1.5 mmol) was added under stirring to a solution of 5´-dimethoxytrityl phosphonate derivative (1.0 mmol) and triethylamine (0.2 mmol) in acetonitrile (10 ml), and the mixture was stirred 16 h at r.t. The reaction was quenched by the addition of methanol (5 ml) and the mixture was concentrated under reduced pressure. The product was purified by chromatography on silica gel (elution with gradient of 0 – 50% ethyl acetate in toluene).

Method F – Preparation of MOP phosphonates 14 and 19.
Bromotrimethylsilane (4.0 mmol) was added to a solution of diisopropyl phosphonate **13**, **17** (1.0 mmol) and 2,6-lutidine (8.0 mmol) in acetonitrile (10 ml). The reaction mixture was stirred 16 h at r.t., and then concentrated under reduced presure. The residue was treated with 2M TEAB (5 ml) and methanol (5 ml). The solution was evaporated, the residue was dissolved in chloroform (50 ml) and extracted with 0.2 M TEAB (3 x 20 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude nucleoside phosphonic acid was used for the further steps without purification. DCC (5.0 mmol) was added to a solution of nucleoside phosphonic acid (1.0 mmol) and 4-methoxy-1-oxido-2-pyridylmethanol (3.0 mmol) in pyridine (6 ml), and the reaction mixture was stirred for 3 days at r.t. After that, the reaction mixture was diluted with water (4 ml) to achieve 60% solution of pyridine in water, heated 16 h at 50 °C to cleave off one ester group and concentrated under reduced pressure. The product was purified by chromatography on silica gel (elution with gradient of 0 - 100% of mixture ethyl acetate/ethanol/acetone/water 4:1:1:1 in ethyl acetate) and lyophilized from dioxane.

Method G – Preparation of phosphonates 17 and 18.

Dibutyltin dichloride (1.1 mmol) was added to a solution of 5´-phophonomethyl derivative **16** (1.0 mmol) and DIPEA (2.2 mmol) in DCE (10 ml). The mixture was stirred 1 h at r. t. After that, reaction mixture was warmed up to 85 °C and benzoyloxymethoxymethyl chloride **10** (1.1 mmol) was added. The reaction mixture was heated 3 h at 85 °C to afford mixture of 2´-BOMOM a 3´-BOMOM derivatives. The regioisomers were purified by chromatography on silica gel (elution with gradient of 0 - 50% acetone in toluene, guanosine derivatives 0 - 100% acetone in toluene) as an unseparable mixture of regioisomers, and used for further step without separation. Mixture of BOMOM derivatives (1.0 mmol) in pyridin (10 ml) was added to a 30 min pre-stirred mixture of silver triflate (2.0 mmol) and dimethoxytrityl chloride (2.0 mmol) in pyridine (10 ml). The reaction mixture was stirred 16 h at r.t. Reaction was quenched by the addition of methanol (5 ml), silver chloride was filtered off and the mixture was concentrated under reduced pressure. The regioisomers were purified by chromatography on silica gel (elution with gradient of 0 - 50% acetone in toluene, guanosine derivatives 0 - 100% acetone in toluene) as an unseparable mixture of regioisomers, and after that separated by preparative HPLC (elution with gradient of 50 - 100% methanol in water) as reverse phase faster eluted isomer (3´-BOMOM derivative **18**) and reverse phase slower eluted isomer (2´-BOMOM derivative **17**).

6-*N*-Benzoyl-9-(2-*O*-benzoyloxymethoxymethyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)adenine (**3a**)
6-*N*-Benzoyl-9-(3-*O*-benzoyloxymethoxymethyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)adenine (**5a**)
Prepared by Method A from 6-*N*-benzoyl-9-(5-*O*-dimethoxytrityl-β-D-ribofuranosyl)adenine **1a**. Yield 32% of TLC faster eluted isomer **3a** and 25% of TLC slower eluted isomer **5a**.
3a: ^{1}H NMR (500 MHz, d_6-DMSO): 11.25 s, 1 H (NH); 8.68 s, 1 H (H-2); 8.61 s, 1 H (H-8); 8.06 d, 2 H, 7.93 d, 2 H, 7.64 t, 1 H, 7.63 t, 1 H, 7.55 t, 2 H, 7.48 t, 2 H, 7.35 d, 2 H, 7.22 m, 7 H, 6.83 m, 4 H; 6.27 d, 1 H, *J*(1´,2´) = 4.4 (H-1´); 5.53 d, 1 H, *J*(OH,3´) = 6.0 (3´-OH); 5.50 s, 2 H (OCH_2O); 5.07 bt, 1 H, *J*(2´,3´) = 5.0 (H-2´); 5.04 d, 1 H a 5.02 d, 1 H, *J* = 7.0 (OCH_2O); 4.59 bq, 1 H (H-3´); 4.15 bq, 1H, *J*(4´,3´) = 5.5 (H-4´); 3.72 s a 3.71 s, 2 x 3 H (2 x OCH_3); 3.26 d, 2 H, *J*(5´,4´) = 4.4 (H-5´). ^{13}C NMR (125.7 MHz, d_6-DMSO): 165.83, 165.31, 133.84, 133.57, 132.65, 129.48 (2), 129.28, 128.93 (2), 127.97 (2) a 127.87 (2) (N-CO-C_6H_5 a O-CO-C_6H_5); 158.25 (2), 144.96, 135.74, 135.63, 129.89 (4), 128.71 (2), 128.65 (2), 126.86, 113.33 (4), 85.83 a 55.18 (2) (DMTr); 152.17 (C-6); 151.89 (C-2); 150.71 (C-4); 143.46 (C-8); 126.01 (C-5); 93.29 a 85.73 (OCH_2O); 86.76 (C-1´); 83.70 (C-4´); 78.64 (C-2´); 69.58 (C-3´); 63.56 (C-5´). HR FAB calcd for $C_{47}H_{44}N_5O_{10}$ $(M+H)^+$ 838.3088, found 838.3079.
5a: ^{1}H NMR (d_6-DMSO): 11.23 s, 1 H (NH); 8.66 s, 1 H (H-2); 8.60 s, 1 H (H-8); 8.05 d, 2 H, 7.98 d, 2 H, 7.67 t, 1 H, 7.65 t, 1 H, 7.55 t, 2 H, 7.52 t, 2 H, 7.35 d, 2 H, 7.26 t, 2 H, 7.22 d, 4 H, 7.20 t, 1 H, 6.85 d, 2 H, 6.84 d, 2 H; 6.06 d, 1 H, *J*(1´,2´) = 5.7 (H-1´); 5.84 d, 1 H, *J*(OH,2´) = 6.0 (2´-OH); 5.57 d, 1 H a 5.55 d, 1 H, *J* = 6.4 (OCH_2O); 5.04 btd, 1 H, *J*(2´,3´) = 4.0, *J*(2´,1´) = *J*(2´,OH) = 5.9 (H-2´); 5.03 s, 2 H (OCH_2O); 4.49 dd, 1 H, *J*(3´,2´) = 4.0, *J*(3´,4´) = 4.8 (H-3´); 4.23 bq, 1H (H-4´); 3.72 s a 3.71 s, 2 x 3 H (2 x OCH_3); 3.28 dd, 1 H, *J*(5´b,5´a) = 10.5, *J*(5´a,4´) = 4.3 (H-5´a); 3.22 dd, 1 H, *J*(5´b,5´a) = 10.5, *J*(5´b,4´) = 5.0 (H-5´b). ^{13}C NMR (125.7 MHz, d_6-DMSO): 165.82, 165.33, 133.89, 133.51, 132.66, 129.55 (2), 129.40, 129.00 (2), 128.00 (2) a 127.83 (2) (N-CO-C_6H_5 a O-CO-C_6H_5); 158.27 (2), 144.89, 135.64, 135.58, 129.85 (4), 128.68 (2), 128.66 (2), 126.90, 113.35 (4), 86.04 a 55.18 (2) (DMTr); 152.36 (C-6); 151.81 (C-2); 150.66 (C-4); 143.50 (C-8); 126.15 (C-5); 93.31 a 85.84 (OCH_2O); 88.00 (C-1´); 81.82 (C-4´); 76.41 (C-2´); 71.99 (C-3´); 63.29 (C-5´). HR FAB calcd for $C_{47}H_{44}N_5O_{10}$ $(M+H)^+$ 838.3088, found 838.3052.

9-(2-*O*-Benzoyloxymethoxymethyl-5-*O*-dimethoxytrityl-2-*N*-isobutyryl-β-D-ribofuranosyl)guanine (**3b**)
Prepared by Method A from 9-(5-*O*-dimethoxytrityl-β-D-ribofuranosyl)-2-*N*-isobutyrylguanine **1b**. Yield 40%.
^{1}H NMR (500 MHz, d_6-DMSO): 12.08 s, 1 H (NH); 11.59 s, 1 H (NH); 8.13 s, 1 H (H-8); 7.90 d, 2 H, 7.62 t, 1 H, 7.46 t, 2 H, 7.33 d, 2 H, 7.25 t, 2 H, 7.21 m, 5 H, 6.83 d, 2 H, 6.82 d, 2 H; 6.02 d, 1 H, *J*(1´,2´) = 5.1 (H-1´); 5.49 d, 1 H a 5.47 d, 1 H, *J* = 6.5 (OCH_2O); 5.44 d, 1 H, *J*(OH,3´) = 5.7 (5´-OH); 5.01 s, 2 H (OCH_2O); 4.82 t, 1 H, *J*(2´,1´) = 5.0 (H-2´); 4.42 bq, 1 H (H-3´); 4.09 ddd, 1H, *J*(4´,3´) = 4.6, *J*(4´,5´a) = 6.0, *J*(4´,5´b) = 2.8 (H-4´); 3.72 s a 3.71 s, 2 x 3 H (2 x OCH_3); 3.28 dd, 1 H, *J*(5´b,5´a) = 10.4, *J*(5´a,4´) = 6.0 (H-5´a); 3.18 dd, 1 H, *J*(5´b,5´a) = 10.4, *J*(5´b,4´) = 2.8 (H-5´b); 2.75 septet, 1 H, *J*(CH,CH_3) = 6.8 (CH); 1.12 d, 6 H, *J*(CH_3,CH) = 6.8 (CH_3). ^{13}C NMR (125.7 MHz, d_6-DMSO): 165.29, 133.80, 129.42 (2), 129.25 a 128.90 (2) (O-CO-C_6H_5); 180.32, 34.96, 19.09 a 18.98 (iBu); 158.27, 158.25, 144.94, 135.66, 135.59, 129.94 (2), 129.90 (2), 127.96 (2), 127.92 (2), 126.87, 113.30 (4), 85.97, 55.18 a 55.17 (DMTr); 154.99 (C-6); 148.92 (C-2); 148.33 (C-4); 137.72 (C-8); 120.66 (C-5); 93.29 a 85.79 (OCH_2O); 85.66 (C-1´); 84.08 (C-4´); 78.93 (C-2´); 69.63 (C-3´); 63.95 (C-5´). HR FAB calcd for $C_{44}H_{46}N_5O_{11}$ $(M+H)^+$ 820.3194, found 820.3170.

1-(2-*O*-Benzoyloxymethoxymethyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)uracil (**3c**)
1-(3-*O*-Benzoyloxymethoxymethyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)uracil (**5c**)
Prepared by Method A from 1-(5-*O*-dimethoxytrityl-β-D-ribofuranosyl)uracil **1c**. Yield 35% of TLC faster eluted isomer **3c** and 28% of TLC slower eluted isomer **5c**.
3c: ^{1}H NMR (500 MHz, d_6-DMSO): 11.40 bs, 1 H (NH); 7.72 d, 1 H, *J*(6,5) = 8.1 (H-6); 5.28 dd, 1 H, *J*(5,6) = 8.1, *J*(5,NH) = 2.2 (H-5); 8.00 d, 2 H, 7.68 t, 1 H, 7.53 t, 2 H, 7.38 d, 2 H, 7.33 t, 2 H, 7.23 t, 1 H, 7.25 d, 2 H, 7.24 d, 2 H, 6.91 d, 2 H, 6.90 d, 2 H; 5.87 d, 1 H, *J*(1´,2´) = 3.5 (H-1´); 5.58 d, 1 H a 5.55 d, 1 H, *J* = 6.5 (OCH_2O); 5.41 d, 1 H, *J*(OH,3´) = 6.4 (3´-OH); 5.02 d, 1 H a 4.99 d, 1 H, *J* = 6.7 (OCH_2O); 4.32 dd, 1 H, *J*(2´,1´) = 3.5, *J*(2´,3´) = 5.3 (H-2´);

4.28 td, 1 H, *J*(3´,2´) = 5.3, *J*(3´,4´) = *J*(3´,OH) = 6.2 (H-3´); 3.99 ddd, 1H, *J*(4´,3´) = 6.0, *J*(4´,5´a) = 4.5, *J*(4´,5´b) = 2.8 (H-4´); 3.74 s, 6 H (2 x OCH_3); 3.30 dd, 1 H, *J*(5´b,5´a) = 10.6, *J*(5´a,4´) = 4.5 (H-5´a); 3.22 dd, 1 H, *J*(5´b,5´a) = 10.6, *J*(5´b,4´) = 2.8 (H-5´b). ^{13}C NMR (125.7 MHz, d_6-DMSO): 165.34, 133.90, 129.55 (2), 129.38 a 128.99 (2) (O-CO-C_6H_5); 158.32 (2), 144.78, 135.53, 135.24, 129.96 (4), 128.10 (2), 127.89 (2), 126.99, 113.46 (4), 85.50 a 55.23 (2) (DMTr); 163.16 (C-4); 150.54 (C-2); 140.36 (C-6); 101.72 (C-5); 92.58 a 86.11 (OCH_2O); 87.66 (C-1´); 82.73 (C-4´); 78.92 (C-2´); 68.69 (C-3´); 62.74 (C-5´). HR FAB calcd for $C_{39}H_{39}N_2O_{11}$ $(M+H)^+$ 711.2254, found 711.2259.
5c: ^{1}H NMR (500 MHz, d_6-DMSO): 11.40 bs, 1 H (NH); 7.71 d, 1 H, *J*(6,5) = 8.0 (H-6); 5.31 dd, 1 H, *J*(5,6) = 8.0, *J*(5,NH) = 2.2 (H-5); 7.96 d, 2 H, 7.67 t, 1 H, 7.51 t, 2 H, 7.35 t, 2 H, 7.31 d, 2 H, 7.24 t, 1 H, 7.21 d, 4 H, 6.89 d, 4 H; 5.75 d, 1 H, *J*(1´,2´) = 4.9 (H-1´); 5.68 d, 1 H, *J*(OH,2´) = 5.7 (2´-OH); 5.50 d, 1 H a 5.47 d, 1 H, *J* = 6.3 (OCH_2O); 4.95 s, 2 H (OCH_2O); 4.31 bq, 1 H (H-2´); 4.25 t, 1 H, *J*(3´,2´) = *J*(3´,4´) = 5.0 (H-3´); 4.06 bq, 1H (H-4´); 3.74 s a 3.73 s, 2 x 3 H (2 x OCH_3); 3.24 dd, 1 H, *J*(5´b,5´a) = 10.8, *J*(5´a,4´) = 3.3 (H-5´a); 3.20 dd, 1 H, *J*(5´b,5´a) = 10.8, *J*(5´b,4´) = 4.0 (H-5´b). ^{13}C NMR (125.7 MHz, d_6-DMSO): 165.29, 133.89, 129.52 (2), 129.35 a 128.97 (O-CO-C_6H_5); 158.35 (2), 144.70, 135.36, 135.22, 129.95 (2), 129.91 (2), 128.11 (2), 127.86 (2), 127.02, 113.45 (4), 85.90 a 55.22 (2) (DMTr); 163.14 (C-4); 150.71 (C-2); 140.52 (C-6); 101.76 (C-5); 93.06 a 86.23 (OCH_2O); 88.60 (C-1´); 80.93 (C-4´); 75.47 (C-2´); 72.43 (C-3´); 62.69 (C-5´). HR FAB calcd for $C_{39}H_{39}N_2O_{11}$ $(M+H)^+$ 711.2254, found 711.2258.

4-*N*-Benzoyl-1-(2-*O*-benzoyloxymethoxymethyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)cytosine (**3d**)
4-*N*-Benzoyl-1-(3-*O*-benzoyloxymethoxymethyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)cytosine (**5d**)
Prepared by Method A from 4-*N*-benzoyl-1-(5-*O*-dimethoxytrityl-β-D-ribofuranosyl)cytosine **1d**. Yield 48% of TLC faster eluted isomer **3d** and 30% of TLC slower eluted isomer **5d**.
3d: ^{1}H NMR (500 MHz, d_6-DMSO): 11.30 s, 1 H (NH); 8.38 d, 1 H (H-6); 7.17 d, 1 H, *J*(5,6) = 7.4 (H-5); 8.01 d, 4 H, 7.66 t, 1 H, 7.62 t, 1 H, 7.52 t, 4 H, 7.41 d, 2 H, 7.35 t, 2 H, 7.27 t, 1 H, 7.29 d, 4 H, 6.93 d, 4 H; 5.91 d, 1 H, *J*(1´,2´) = 1.2 (H-1´); 5.64 d, 1 H a 5.63 d, 1 H, *J* = 6.5 (OCH_2O); 5.49 d, 1 H a 5.10 d, 1 H, *J* = 6.7 (OCH_2O); 5.43 d, 1 H, *J*(OH,3´) = 7.3 (3´-OH); 4.39 ddd, 1 H, *J*(3´,2´) = 5.0, *J*(3´,4´) = 8.4, *J*(3´,OH) = 7.3 (H-3´); 4.27 dd, 1 H, *J*(2´,1´) = 1.2, *J*(2´,3´) = 5.0 (H-2´); 4.08 ddd, 1H, *J*(4´,3´) = 8.4, *J*(4´,5´a) = 3.8, *J*(4´,5´b) = 2.1 (H-4´); 3.76 s, 6 H (2 x OCH_3); 3.38 dd, 1 H, *J*(5´b,5´a) = 11.0, *J*(5´a,4´) = 3.8 (H-5´a); 3.31 dd, 1 H, *J*(5´b,5´a) = 11.0, *J*(5´b,4´) = 2.1 (H-5´b). ^{13}C NMR (125.7 MHz, d_6-DMSO): 167.50, 165.39, 133.85, 133.29, 132.94, 129.58 (2), 129.48, 128.99 (2), 128.17 (2) a 128.02 (2) (N-CO-C_6H_5 a O-CO-C_6H_5); 158.40, 158.37, 144.52, 135.75, 135.35, 130.00 (2), 129.85 (2), 128.65 (4), 127.09, 113.51 (4), 86.22 a 55.19 (2) (DMTr); 163.43 (C-4); 154.49 (C-2); 144.57 (C-6); 96.31 (C-5); 92.18 a 85.54 (OCH_2O); 89.57 (C-1´); 82.07 (C-4´); 79.56 (C-2´); 67.78 (C-3´); 61.61 (C-5´). HR FAB calcd for $C_{46}H_{44}N_3O_{11}$ $(M+H)^+$ 814.2976, found 814.3003.
5d: ^{1}H NMR (500 MHz, d_6-DMSO): 11.28 s, 1 H (NH); 8.41 d, 1 H (H-6); 7.14 d, 1 H, *J*(5,6) = 7.2 (H-5); 8.00 d, 2 H, 7.96 d, 2 H, 7.66 t, 1 H, 7.63 t, 1 H, 7.59 d, 2 H, 7.52 t, 2 H, 7.51 t, 2 H, 7.35 t, 2 H, 7.24 t, 1 H, 7.26 d, 4 H, 6.91 d, 4 H; 5.83 d, 1 H, *J*(1´,2´) = 2.3 (H-1´); 5.82 d, 1 H, *J*(OH,2´) = 5.0 (2´-OH); 5.45 s, 2 H (OCH_2O); 4.95 d, 1 H a 4.91 d, 1 H, *J* = 6.8 (OCH_2O); 4.37 dd, 1 H, *J*(3´,2´) = 4.6, *J*(3´,4´) = 7.7 (H-3´); 4.33 ddd, 1 H, *J*(2´,1´) = 2.3, *J*(2´,3´) = 4.6, *J*(2´,OH) = 5.0 (H-2´); 4.21 bdt, 1H, *J*(4´,3´) = 7.7, *J*(4´,5´) = 2.9 (H-4´); 3.74 s a 3.73 s, 2 x 3 H (2 x OCH_3); 3.42 dd, 1 H, *J*(5´b,5´a) = 11.1, *J*(5´a,4´) = 2.2 (H-5´a); 3.30 dd, 1 H, *J*(5´b,5´a) = 11.1, *J*(5´b,4´) = 3.3 (H-5´b). ^{13}C NMR (125.7 MHz, d_6-DMSO): 167.45, 165.32, 133.90, 133.27, 132.94, 129.53 (2), 129.35, 128.99 (2), 128.17 (2) a 128.01 (2) (N-CO-C_6H_5 a O-CO-C_6H_5); 158.41 (2), 144.37, 135.46, 135.28, 129.95 (2), 129.90 (2), 128.66 (2), 128.61 (2), 127.12, 113.50 (4), 86.34 a 55.17 (2) (DMTr); 163.34 (C-4); 154.60 (C-2); 144.77 (C-6); 96.25 (C-5); 92.75 a 85.66 (OCH_2O); 91.29 (C-1´); 80.42 (C-4´); 74.41 (C-2´); 73.02 (C-3´); 61.58 (C-5´). HR FAB calcd for $C_{46}H_{44}N_3O_{11}$ $(M+H)^+$ 814.2976, found 814.2960.

6-*N*-Benzoyl-9-(2-*O*-benzoyloxymethoxymethyl-5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)adenine (**4a**)
6-*N*-Benzoyl-9-(3-*O*-benzoyloxymethoxymethyl-5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)adenine (**6a**)
Prepared by Method A from 6-*N*-benzoyl-9-(5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)adenine **2a**. Yield 32% of TLC faster eluted isomer **4a** and 27% of TLC slower eluted isomer **6a**.
4a: ^{1}H NMR (500 MHz, d_6-DMSO): 11.22 s, 1 H (NH); 8.64 s, 1 H (H-2); 8.60 s, 1 H (H-8); 8.03 d, 2 H, 7.90 d, 2 H, 7.70 – 7.30 m, 16 H; 6.26 d, 1 H, *J*(1´,2´) = 4.6 (H-1´); 5.56 d, 1 H, *J*(OH,3´) = 5.9 (3´-OH); 5.48 d, 1 H a 5.46 d, 1 H, *J* = 6.5 (OCH_2O); 5.47 dd, 1 H, *J*(2´,1´) = 4.6, *J*(2´,3´) = 5.5 (H-2´); 5.02 d, 1 H a 4.99 d, 1 H, *J* = 6.8 (OCH_2O); 4.60 bq, 1 H, *J*(3´,2´) = 5.5, *J*(3´,4´) = 5.4 (H-3´); 4.11 td, 1H, *J*(4´,3´) = 4.8, *J*(4´,5´a) = 3.6, *J*(4´,5´b) = 4.8 (H-4´); 3.95 dd, 1 H, *J*(5´b,5´a) = 11.4, *J*(5´a,4´) = 3.6 (H-5´a); 3.82 dd, 1 H, *J*(5´b,5´a) = 11.4, *J*(5´b,4´) = 5.0 (H-5´b); 0.96 s, 9H (tBu). HR FAB calcd for $C_{42}H_{44}N_5O_8Si$ $(M+H)^+$ 774.2959, found 774.2995.
6a: ^{1}H NMR (500 MHz, d_6-DMSO): 11.22 s, 1 H (NH); 8.65 s, 1 H (H-2); 8.59 s, 1 H (H-8); 8.05 d, 4 H, 7.70 – 7.30 m, 16 H; 6.07 d, 1 H, *J*(1´,2´) = 6.0 (H-1´); 5.85 d, 1 H, *J*(OH,2´) = 6.0 (2´-OH); 5.63 s, 2 H a 5.07 s, 2 H (2 x OCH_2O); 4.99 td, 1 H, *J*(2´,1´) = *J*(2´,OH) = 6.0, *J*(2´,3´) = 5.0 (H-2´); 4.52 dd, 1 H, *J*(3´,2´) = 5.0, *J*(3´,4´) = 3.7 (H-3´); 4.22 td, 1H, *J*(4´,3´) = 3.7, *J*(4´,5´a) = *J*(4´,5´b) = 4.7 (H-4´); 3.93 dd, 1 H, *J*(5´b,5´a) = 11.2, *J*(5´a,4´) = 4.6 (H-5´a); 3.79 dd, 1 H, *J*(5´b,5´a) = 11.2, *J*(5´b,4´) = 4.8 (H-5´b); 0.97 s, 9H (tBu). HR FAB calcd for $C_{42}H_{44}N_5O_8Si$ $(M+H)^+$ 774.2959, found 774.2995.

9-(2-*O*-Benzoyloxymethoxymethyl-5-*O*-*tert*-butyldiphenylsilyl-2-*N*-isobutyryl-β-D-ribofuranosyl)guanine (**4b**)
Prepared by Method A from 9-(5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)-2-*N*-isobutyrylguanine **2b**. Yield 46%.
^{1}H NMR (500 MHz, d_6-DMSO): 12.06 bs, 1 H (NH); 11.59 bs, 1 H (NH); 8.12 s, 1 H (H-8); 7.90 d, 2 H, 7.63 t, 1 H, 7.59 d, 4 H, 7.47 t, 2 H, 7.44 t, 2 H, 7.39 t, 2 H, 7.36 t, 2 H; 6.00 d, 1 H, *J*(1´,2´) = 5.2 (H-1´); 5.49 d, 1 H, *J*(OH,3´) = 5.5 (3´-OH); 5.46 d, 1 H a 5.44 d, 1 H, *J* = 6.5 (OCH_2O); 4.98 s, 2 H (OCH_2O); 4.73 t, 1 H, *J*(2´,1´) = 5.2, *J*(2´,3´) = 5.0 (H-2´); 4.47 bq, 1 H, *J*(3´,2´) = 5.0, *J*(3´,4´) = 4.2 (H-3´); 4.05 td, 1H, *J*(4´,3´) = 4.1, *J*(4´,5´a) = 3.5, *J*(4´,5´b) = 4.5 (H-4´); 3.90 dd, 1 H, *J*(5´b,5´a) = 11.5, *J*(5´a,4´) = 3.5 (H-5´a); 3.79 dd, 1 H, *J*(5´b,5´a) = 11.5, *J*(5´b,4´) = 4.8 (H-5´b); 2.74 septet, 1 H, *J*(CH,CH_3) = 6.8 (CH); 1.11 d a 1.10 d, 2 x 3 H, *J*(CH_3,CH) = 6.8 (CH_3); 0.97 s, 9H (tBu). HR FAB calcd for $C_{39}H_{46}N_5O_9Si$ $(M+H)^+$ 756.3065, found 756.3077.

1-(2-*O*-Benzoyloxymethoxymethyl-5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)uracil (**4c**)
1-(3-*O*-Benzoyloxymethoxymethyl-5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)uracil (**6c**)
Prepared by Method A from 1-(5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)uracil **2c**. Yield 45% of TLC faster eluted isomer **4c** and 32% of TLC slower eluted isomer **6c**.
4c: ^{1}H NMR (500 MHz, d_6-DMSO): 11.40 s, 1 H (NH); 7.72 d, 1 H, *J*(6,5) = 8.1 (H-6); 5.24 dd, 1 H, *J*(5,6) = 8.1, *J*(5,N4) = 2.1 (H-5); 8.00 d, 2 H, 7.65 t, 1 H, 7.64 d, 2 H, 7.62 d, 2 H, 7.53 t, 2 H, 7.46 m, 5 H; 5.92 d, 1 H, *J*(1´,2´) = 3.8 (H-1´); 5.57 d, 1 H a 5.55 d, 1 H, *J* = 6.5 (OCH_2O); 5.44 d, 1 H, *J*(OH,3´) = 6.1 (3´-OH); 5.02 d, 1 H a 4.98 d, 1 H, *J* = 6.8 (OCH_2O); 4.30 dd, 1 H, *J*(2´,1´) = 3.8, *J*(2´,3´) = 5.3 (H-2´); 4.26 bq, 1 H, *J*(3´,2´) = 5.3, *J*(3´,4´) = 5.6 (H-3´); 3.78 dt, 1H, *J*(4´,3´) = 5.6, *J*(4´,5´a) = *J*(4´,5´b) = 3.0 (H-4´); 3.95 dd, 1 H, *J*(5´b,5´a) = 11.6, *J*(5´a,4´) = 2.7 (H-5´a); 3.80 dd, 1 H, *J*(5´b,5´a) = 11.6, *J*(5´b,4´) = 3.3 (H-5´b); 1.03 s, 9H (tBu). HR FAB calcd for $C_{34}H_{39}N_2O_9Si$ $(M+H)^+$ 647.2425, found 647.2426.
6c: ^{1}H NMR (d_6-DMSO): 11.40 s, 1 H (NH); 7.44 d, 1 H, *J*(6,5) = 8.1 (H-6); 5.28 dd, 1 H, *J*(5,6) = 8.1, *J*(5,N4) = 2.0 (H-5); 7.97 d, 2 H, 7.61 m, 5 H, 7.50 m, 8 H; 5.81 d, 1 H, *J*(1´,2´) = 5.4 (H-1´); 5.71 d, 1 H, *J*(OH,2´) = 5.6 (2´-OH); 5.58 d, 1 H a 5.56 d, 1 H, *J* = 6.6 (OCH_2O); 5.01 d, 1 H a 4.99 d, 1 H, *J* = 6.8 (OCH_2O); 4.28 bq, 1 H, *J*(2´,1´) = 5.5, *J*(2´,3´) = 5.2 (H-2´); 4.24 dd, 1 H, *J*(3´,2´) = 5.2, *J*(3´,4´) = 4.2 (H-3´); 4.08 bq, 1H, *J*(4´,3´) = 4.2, *J*(4´,5´a) = *J*(4´,5´b) = 3.6 (H-4´); 3.87 dd, 1 H, *J*(5´b,5´a) = 11.5, *J*(5´a,4´) = 3.8 (H-5´a); 3.74 dd, 1 H, *J*(5´b,5´a) = 11.5, *J*(5´b,4´) = 3.4 (H-5´b); 1.01 s, 9H (tBu). HR FAB calcd for $C_{34}H_{39}N_2O_9Si$ $(M+H)^+$ 647.2425, found 647.2416.

4-*N*-Benzoyl-1-(2-*O*-benzoyloxymethoxymethyl-5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)cytosine (**4d**)
4-*N*-Benzoyl-1-(3-*O*-benzoyloxymethoxymethyl-5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)cytosine (**6d**)
Prepared by Method A from 4-*N*-benzoyl-1-(5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)cytosine **2d**. Yield 32% of TLC faster eluted isomer **4d** and 19% of TLC slower eluted isomer **6d**.
4d: ^{1}H NMR (500 MHz, d_6-DMSO): 11.30 bs, 1 H (NH); 8.33 d, 1 H, (H-6); 7.22 d, 1 H, *J*(5,6) = 7.1 (H-5); 8.00 m, 4 H, 7.67 m, 8 H, 7.55 – 7.45 m, 8 H; 5.95 d, 1 H, *J*(1´,2´) = 1.7 (H-1´); 5.64 d, 1 H a 5.62 d, 1 H, *J* = 6.5 (OCH_2O); 5.45 d, 1 H, *J*(OH,3´) = 6.8 (3´-OH); 5.18 d, 1 H a 5.09 d, 1 H, *J* = 6.7 (OCH_2O); 4.31 ddd, 1 H, *J*(3´,2´) = 4.9, *J*(3´,4´) = 8.0, *J*(3´,OH) = 6.8 (H-3´); 4.27 dd, 1 H, *J*(2´,1´) = 1.7, *J*(2´,3´) = 4.9 (H-2´); 4.06 dt, 1H, *J*(4´,3´) = 8.0, *J*(4´,5´a) = *J*(4´,5´b) = 2.6 (H-4´); 4.04 dd, 1 H, *J*(5´b,5´a) = 12.0, *J*(5´a,4´) = 2.2 (H-5´a); 3.84 dd, 1 H, *J*(5´b,5´a) = 12.0, *J*(5´b,4´) = 3.1 (H-5´b); 1.05 s, 9H (tBu). HR FAB calcd for $C_{41}H_{44}N_3O_9Si$ $(M+H)^+$ 750.2847, found 750.2877.
6d: ^{1}H NMR (500 MHz, d_6-DMSO): 11.30 bs, 1 H (NH); 8.25 d, 1 H, (H-6); 7.16 d, 1 H, *J*(5,6) = 7.1 (H-5); 7.98 m, 4 H, 7.65 m, 8 H, 7.50 – 7.45 m, 8 H; 5.88 d, 1 H, *J*(1´,2´) = 2.9 (H-1´); 5.82 d, 1 H, *J*(OH,2´) = 5.4 (2´-OH); 5.56 s, 2 H (OCH_2O); 4.98 d, 1 H a 4.94 d, 1 H, *J* = 6.8 (OCH_2O); 4.31 ddd, 1 H, *J*(2´,1´) = 2.9, *J*(2´,3´) = 4.6, *J*(2´,OH) = 5.4 (H-2´); 4.27 dd, 1 H, *J*(3´,2´) = 4.6, *J*(3´,4´) = 6.7 (H-3´); 4.18 dt, 1H, *J*(4´,3´) = 6.7, *J*(4´,5´a) = *J*(4´,5´b) = 2.9 (H-4´); 4.01 dd, 1 H, *J*(5´b,5´a) = 11.8, *J*(5´a,4´) = 2.8 (H-5´a); 3.79 dd, 1 H, *J*(5´b,5´a) = 11.8, *J*(5´b,4´) = 3.0 (H-5´b); 1.04 s, 9H (tBu). HR FAB calcd for $C_{41}H_{44}N_3O_9Si$ $(M+H)^+$ 750.2847, found 750.2848.

N,*N*-Diisopropyl-methyl-[1-(6-*N*-benzoyladenin-9-yl)-2-*O*-benzoyloxymethoxymethyl-5-*O*-dimethoxytrityl-β-D-ribofuranos-3-*O*-yl]phosphoramidite (**7a**)
Prepared by Method C from 6-*N*-benzoyl-9-(2-*O*-benzoyloxymethoxymethyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)adenine **3a**. Yield 78%. Mixture of isomers 3:2.
^{1}H NMR (499.8 MHz, C_6D_6): 1.09, 1.118, 1.120, 1.21 (4 × d, 4 × 6H, J_{vic} = 6.8, $(C\mathbf{H_3})_2$CH-A,B); 3.18 (d, 3H, $J_{H,P}$ = 13.2, CH_3OP-B); 3.32 (d, 3H, $J_{H,P}$ = 13.2, CH_3OP-A); 3.329, 3.334 (2 × s, 2 × 3H, CH_3O-DMTr-B); 3.339, 3.344 (2 × s, 2 × 3H, CH_3O-DMTr-A); 3.52-3.60 (m, 4H, $(CH_3)_2C\mathbf{H}$); 3.62 (dd, 1H, J_{gem} = 10.7, $J_{5'b,4'}$ = 4.5, H-5'b-A); 3.64 (dd, 1H, J_{gem} = 10.7, $J_{5'b,4'}$ = 4.5, H-5'b-B); 3.77 (dd, 1H, J_{gem} = 10.7, $J_{5'a,4'}$ = 3.5, H-5'a-A); 3.80 (dd, 1H, J_{gem} = 10.7, $J_{5'a,4'}$ = 4.0, H-5'a-B); 4.64 (bddd, 1H, $J_{4',3'}$ = 4.8, $J_{4',5'}$ = 4.5, 3.5, H-4'-A); 4.71 (ddd, 1H, $J_{4',5'}$ = 4.5, 4.0, $J_{4',3'}$ = 3.4, H-4'-B); 4.79 (d, 1H, J_{gem} = 6.8, $OCH_a\mathbf{H_b}OCH_2OBz$-B); 5.01-5.07 (m, 3H, H-3'-A,B, $OC\mathbf{H_a}H_bOCH_2OBz$-B); 5.10 (s, 2H, $OC\mathbf{H_2}OCH_2OBz$-A); 5.41 (dd, 1H, $J_{2',3'}$ = 4.8, $J_{2',1'}$ = 4.2, H-2'-A); 5.45 (d, 1H, J_{gem} = 6.3, $OCH_2OCH_a\mathbf{H_b}OBz$-B); 5.46 (dd, 1H, $J_{2',3'}$ = 5.3, $J_{2',1'}$ = 4.7, H-2'-B); 5.49 (d, 1H, J_{gem} = 6.3, $OCH_2OC\mathbf{H_a}H_bOBz$-B); 5.51, 5.53 (2 × d, 2 × 1H, J_{gem} = 6.3, $OCH_2OC\mathbf{H_2}OBz$-A); 6.31 (d, 1H, $J_{1',2'}$ = 4.7, H-1'-B); 6.32 (d, 1H, $J_{1',2'}$ = 4.2, H-1'-A); 6.76-6.80 (m, 8H, H-*m*-C_6H_4-DMTr-A,B); 6.94-7.22 (m, 18H, H-*m*,*p*-BzN-A,B, H-*m*,*p*-BzO-A,B, H-m,*p*-C_6H_5-DMTr-A,B); 7.48-7.52 (m, 8H, H-*o*-C_6H_4-DMTr-A,B); 7.67 (m, 4H, H-*o*-C_6H_5-DMTr-A,B); 7.77 (m, 4H, H-*o*-BzN-A,B); 7.97 (s, 1H, H-8-B); 8.00 (s,

1H, H-8-A); 8.01 (m, 2H, H-*o*-BzO-B); 8.02 (m, 2H, H-*o*-BzO-A); 8.73 (s, 1H, H-2-A); 8.74 (s, 1H, H-2-B); 9.20 (bs, 2H, NH-A,B). ^{13}C NMR (125.7 MHz, C_6D_6): 24.65, 24.72 (d, $J_{C,P}$ = 6.5, (**C**H$_3$)$_2$CH-A,B); 24.73, 24.78 (d, $J_{C,P}$ = 7.5, (**C**H$_3$)$_2$CH-A,B); 43.21 (d, $J_{C,P}$ = 12.5, (CH$_3$)$_2$**C**H-A); 43.33 (d, $J_{C,P}$ = 12.7, (CH$_3$)$_2$**C**H-B); 50.24 (d, $J_{C,P}$ = 17.9, CH$_3$OP-B); 50.81 (d, $J_{C,P}$ = 16.8, CH$_3$OP-A); 54.78 (CH$_3$O-DMTr-A,B); 63.19 (CH$_2$-5'-B); 63.34 (CH$_2$-5'-A); 71.80 (d, $J_{C,P}$ = 15.8, CH-3'-A); 72.11 (d, $J_{C,P}$ = 13.8, CH-3'-B); 78.43 (d, $J_{C,P}$ = 4.4, CH-2'-B); 78.86 (CH-2'-A); 83.69 (d, $J_{C,P}$ = 5.2, CH-4'-A); 84.21 (d, CH-4'-B); 85.97 (OCH$_2$O**C**H$_2$OBz-A,B) 87.06, 87.11 (C-DMTr-A,B); 88.130 (CH-1'-B); 88.55 (CH-1'-A); 93.75 (O**C**H$_2$OCH$_2$OBz-A); 93.84 (O**C**H$_2$OCH$_2$OBz-B); 113.55, 113.58 (CH-*m*-C$_6$H$_4$-DMTr-A,B); 124.35 (C-5-A,B); 127.15, 127.17 (CH-*p*-C$_6$H$_5$-DMTr-A,B); 128.29 (CH-*m*-C$_6$H$_5$-DMTr-A,B); 128.55, 128.56, 128.62, 128.75, 128.78 (CH-*m*-BzO-A,B, CH-*m*-BzN-A,B, CH-*o*-C$_6$H$_5$-A,B); 129.99 (CH-*o*-BzO-A,B); 130.10, 130.13 (C-*i*-BzO-A,B); 130.64, 130.67 (CH-*o*-BzN-A,B, CH-*o*-C$_6$H$_4$-DMTr-A,B); 131.97 (CH-*p*-BzN-A,B); 133.26 (CH-*p*-BzO-A,B); 134.63 (C-*i*-BzN-A,B); 136.08, 136.17 (C-*i*-C$_6$H$_4$-DMTr-A); 136.22, 136.25 (C-*i*-C$_6$H$_4$-DMTr-B); 142.27 (CH-8-A); 142.43 (CH-8-B); 145.34 (C-*i*-C$_6$H$_5$-DMTr-A); 145.44 (C-*i*-C$_6$H$_5$-DMTr-B); 150.53 (C-6-A,B); 151.76 (C-4-A); 151.82 (C-4-B); 152.82 (C-2-A,B); 159.20 (C-*p*-C$_6$H$_4$-DMTr-A,B); 164.28 (NCOPh-A,B); 165.58 (OCOPh-B); 165.65 (OCOPh-A). ^{31}P{^{1}H} NMR (202.3 MHz, C_6D_6): 151.49, 151.65. HR FAB calcd for $C_{54}H_{60}N_6O_{11}P$ (M+H)$^+$ 999.4058, found 999.4053.

N,*N*-Diisopropyl-methyl-[2-*O*-benzoyloxymethoxymethyl-5-*O*-dimethoxytrityl-1-(2-*N*-isobutyrylguanin-9-yl)-β-D-ribofuranos-3-*O*-yl]phosphoramidite (**7b**)

Prepared by Method C from 9-(2-*O*-benzoyloxymethoxymethyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)-2-*N*-isobutyrylguanine **3b**. Yield 71%. Mixture of isomers 1:1.

^{1}H NMR (600.1 MHz, C_6D_6): 0.80, 0.84, 0.95, 0.98 (4 × d, 4 × 3H, J_{vic} = 6.8, (C**H$_3$**)$_2$CHC); 0.99, 1.07, 1.08, 1.16 (4 × d, 4 × 6H, J_{vic} = 6.8, (C**H$_3$**)$_2$CHN); 1.96, 2.08 (2 × m, 2 × 1H, J_{vic} = 6.8, (CH$_3$)$_2$C**H**C); 3.07, 3.25 (2 × d, 2 × 3H, $J_{H,P}$ = 13.1, CH$_3$OP); 3.35, 3.36, 3.38 (3 × s, 12H, CH$_3$O-DMTr); 3.44-3.53 (m, 6H, H-5'b, (CH$_3$)$_2$C**H**N); 3.76, 3.80 (2 × dd, 2 × 1H, J_{gem} = 10.6, $J_{5'a,4'}$ = 1.9, H-5'a); 4.58 (ddd, 1H, $J_{4',3'}$ = 5.5, $J_{4',5'}$ = 4.6, 1.9, H-4'); 4.61 (ddd, 1H, $J_{4',3'}$ = 5.5, $J_{4',5'}$ = 3.6, 1.9, H-4'); 4.85 (ddd, 1H, $J_{H,P}$ = 10.4, $J_{3',4'}$ = 5.5, $J_{3',2'}$ = 4.6, H-3'); 4.92 (dt, 1H, $J_{H,P}$ = 9.9, $J_{3',2'}$ = $J_{3',4'}$ = 5.5, H-3'); 5.11 (d, 1H, J_{gem} = 6.8, OCH$_a$**H$_b$**OCH$_2$OBz); 5.12 (d, 1H, J_{gem} = 6.7, OCH$_a$**H$_b$**OCH$_2$OBz); 5.19 (d, 1H, J_{gem} = 6.8, OC**H$_a$**H$_b$OCH$_2$OBz); 5.27 (dd, 1H, $J_{2',3'}$ = 4.6, $J_{2',1'}$ = 3.9, H-2'); 5.30 (d, 1H, J_{gem} = 6.7, OC**H$_a$**H$_b$OCH$_2$OBz); 5.35 (dd, 1H, $J_{2',3'}$ = 5.5, $J_{2',1'}$ = 4.5, H-2'); 5.46, 5.53 (2 × d, 2 × 1H, J_{gem} = 6.4, OCH$_2$OCH$_a$**H$_b$**OBz); 5.71, 5.73 (2 × d, 2 × 1H, J_{gem} = 6.4, OCH$_2$OC**H$_a$**H$_b$OBz); 6.13 (d, 1H, $J_{1',2'}$ = 4.5, H-1'); 6.14 (d, 1H, $J_{1',2'}$ = 3.9, H-1'); 6.808, 6.812, 6.823, 6.828 (4 × m, 4 × 2H, H-*m*-C$_6$H$_4$-DMTr); 7.01-7.08 (m, 6H, H-*m*,*p*-Bz); 7.11, 7.12 (2 × m, 2 × 1H, H-*p*-C$_6$H$_5$-DMTr); 7.20, 7.21 (2 × m, 2 × 2H, H-*m*-C$_6$H$_5$-DMTr); 7.54, 7.55, 7.56, 7.57 (4 × m, 4 × 2H, H-*o*-C$_6$H$_4$-DMTr); 7.71 (m, 4H, H-*o*-C$_6$H$_5$-DMTr); 7.91, 7.97 (2 × s, 2 × 1H, H-8); 7.99, 8.01 (2 × m, 2 × 2H, H-*o*-Bz); 8.64, 8.77 (2 × bs, 2 × 1H, NH). ^{13}C NMR (150.9 MHz, C_6D_6): 18.70, 18.75, 18.93, 18.94 ((**C**H$_3$)$_2$CHC); 24.56, 24.64 (d, $J_{C,P}$ = 6.6, (**C**H$_3$)$_2$CH); 24.67, 24.77 (d, $J_{C,P}$ = 7.5, (**C**H$_3$)$_2$CHN); 36.04, 36.07 ((CH$_3$)$_2$**C**HC); 43.10, 43.27 (d, $J_{C,P}$ = 12.2, (CH$_3$)$_2$**C**H); 50.06 (d, $J_{C,P}$ = 18.1, CH$_3$OP); 50.66 (d, $J_{C,P}$ = 16.7, CH$_3$OP); 54.84, 54.85, 54.86 (CH$_3$O-DMTr); 63.20, 63.42 (CH$_2$-5'); 71.38 (d, $J_{C,P}$ = 16.5, CH-3'); 71.42 (d, $J_{C,P}$ = 13.7, CH-3'); 77.72 (d, $J_{C,P}$ = 3.8, CH-2'); 78.42 (CH-2'); 83.16 (d, $J_{C,P}$ = 5.0, CH-4'); 83.78 (d, CH-4'); 86.84, 86.88, 86.95 (OCH$_2$O**C**H$_2$OBz, C-DMTr); 88.13, 88.40 (CH-1'); 93.91, 94.01 (O**C**H$_2$OCH$_2$OBz); 113.62, 113.65, 113.70 (CH-*m*-C$_6$H$_4$-DMTr); 123.06, 123.14 (C-5); 127.30, 127.32 (CH-*p*-C$_6$H$_5$-DMTr); 128.29 (CH-*m*-C$_6$H$_5$-DMTr); 128.69 (CH-*m*-Bz); 128.75, 128.79 (CH-*o*-C$_6$H$_5$); 129.85, 129.87 (CH-*o*-Bz); 129.88, 129.93 (C-*i*-Bz); 130.53, 130.64 (CH-*o*-C$_6$H$_4$-DMTr); 133.61 (CH-*p*-Bz); 136.13, 136.22, 136.28, 136.40 (C-*i*-C$_6$H$_4$-DMTr); 137.55, 137.85 (CH-8); 145.27, 145.43 (C-*i*-C$_6$H$_5$-DMTr); 147.89, 147.96, 148.09, 148.21 (C-4,6); 155.34 (C-2); 159.30, 159.32 (C-*p*-C$_6$H$_4$-DMTr); 166.39, 166.54 (COPh); 178.57, 178.63 (COiPr). ^{31}P{^{1}H} NMR (202.3 MHz, C_6D_6): 151.45, 151.77. HR FAB calcd for $C_{51}H_{62}N_6O_{12}P$ (M+H)$^+$ 981.4163, found 981.4159.

N,*N*-Diisopropyl-methyl-[2-*O*-benzoyloxymethoxymethyl-5-*O*-dimethoxytrityl-1-(uracil-1-yl)-β-D-ribofuranos-3-*O*-yl]phosphoramidite (**7c**)

Prepared by Method C from 1-(2-*O*-benzoyloxymethoxymethyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)uracil **3c**. Yield 82%. Mixture of isomers 1:1.

^{1}H NMR (600.1 MHz, C_6D_6): 1.00, 1.04, 1.06, 1.12 (4 × d, 4 × 6H, J_{vic} = 6.9, (C**H$_3$**)$_2$CH); 3.11, 3.21 (2 × d, 2 × 3H, $J_{H,P}$ = 13.1, CH$_3$OP); 3.325, 3.327, 3.332, 3.336 (4 × s, 4 × 3H, CH$_3$O-DMTr); 3.40-3.50 (m, 4H, (CH$_3$)$_2$C**H**); 3.57, 3.62 (2 × dd, 2 × 1H, J_{gem} = 10.9, $J_{5'b,4'}$ = 2.8, H-5'b); 3.72, 3.75 (2 × dd, 2 × 1H, J_{gem} = 10.9, $J_{5'a,4'}$ = 2.3, H-5'a); 4.37, 4.43 (2 × ddd, 2 × 1H, $J_{4',3'}$ = 7.8, $J_{4',5'}$ = 2.8, 2.3, H-4'); 4.56, 4.61 (2 × dd, 2 × 1H, $J_{2',3'}$ = 4.8, $J_{2',1'}$ = 2.0, H-2'); 4.75, 4.78 (2 × ddd, 2 × 1H, $J_{3',4'}$ = 7.8, $J_{H,P}$ = 5.8, $J_{3',2'}$ = 4.8, H-3'); 5.15 (d, 1H, J_{gem} = 6.7, OCH$_a$**H$_b$**OCH$_2$OBz); 5.21, 5.23 (2 × d, 2 × 1H, $J_{5,6}$ = 8.1, H-5); 5.27 (d, 1H, J_{gem} = 6.7, OCH$_a$**H$_b$**OCH$_2$OBz); 5.31, 5.36 (2 × d, 2 × 1H, J_{gem} = 6.7, OC**H$_a$**H$_b$OCH$_2$OBz); 5.84, 5.87 (2 × d, 2 × 1H, J_{gem} = 6.5, OCH$_2$OCH$_a$**H$_b$**OBz); 5.88, 5.98 (2 × d, 2 × 1H, J_{gem} = 6.5, OCH$_2$OC**H$_a$**H$_b$OBz); 6.19, 6.20 (2 × d, 2 × 1H, $J_{1',2'}$ = 2.0, H-1'); 6.79, 6.80, 6.81, 6.82 (4 × m, 4 × 2H, H-*m*-C$_6$H$_4$-DMTr); 7.01-7.05 (m, 4H, H-*m*-Bz); 7.06-7.12 (m, 4H, H-*p*-Bz, H-*p*-C$_6$H$_5$-DMTr); 7.21 (m, 4H, H-*m*-C$_6$H$_5$-DMTr); 7.43, 7.44, 7.46 (3 × m, 8H, H-*o*-C$_6$H$_4$-DMTr); 7.60, 7.62 (2 × m, 2 × 2H, H-*o*-C$_6$H$_5$-DMTr); 7.99, 8.03 (2 × d, 2 × 1H, $J_{6,5}$ = 8.1, H-6); 8.10, 8.12 (2 × m, 2 × 2H, H-*o*-Bz); 9.69 (bs, 2H, NH). ^{13}C NMR (150.9 MHz, C_6D_6): 24.47, 24.65 (d,

$J_{C,P}$ = 6.1, (**C**H$_3$)$_2$CH); 24.66, 24.73 (d, $J_{C,P}$ = 7.5, (**C**H$_3$)$_2$CH); 43.08 (d, $J_{C,P}$ = 12.2, (CH$_3$)$_2$**C**H); 43.19 (d, $J_{C,P}$ = 12.5, (CH$_3$)$_2$**C**H); 49.99 (d, $J_{C,P}$ = 17.1, CH$_3$OP); 50.20 (d, $J_{C,P}$ = 15.9, CH$_3$OP); 54.80 (CH$_3$O-DMTr); 61.26, 61.39 (CH$_2$-5'); 70.31 (d, $J_{C,P}$ = 12.6, CH-3'); 70.75 (d, $J_{C,P}$ = 15.5, CH-3'); 78.81 (d, $J_{C,P}$ = 2.2, CH-2'); 79.25 (CH-2'); 82.24 (d, $J_{C,P}$ = 5.4, CH-4'); 82.56 (d, CH-4'); 85.79, 85.90 (OCH$_2$O**C**H$_2$OBz); 87.44, 87.49 (C-DMTr); 89.14, 89.51 (CH-1'); 92.56, 92.90 (O**C**H$_2$OCH$_2$OBz); 102.32, 102.34 (CH-5); 113.56, 113.59, 113.61, 113.64 (CH-*m*-C$_6$H$_4$-DMTr); 127.38, 127.40 (CH-*p*-C$_6$H$_5$-DMTr); 128.26, 128.29 (CH-*m*-C$_6$H$_5$-DMTr); 128.50 (CH-*m*-Bz); 128.84 (CH-*o*-C$_6$H$_5$); 130.14 (CH-*o*-Bz); 130.47, 130.52 (C-*i*-Bz); 130.84, 130.86, 130.88 (CH-*o*-C$_6$H$_4$-DMTr); 133.05, 133.07 (CH-*p*-Bz); 135.50, 135.62, 135.67, 135.78 (C-*i*-C$_6$H$_4$-DMTr); 139.41, 139.55 (CH-6); 145.05, 145.20 (C-*i*-C$_6$H$_5$-DMTr); 150.53, 150.56 (C-2); 159.30, 159.32, 159.36 (C-*p*-C$_6$H$_4$-DMTr); 163.09, 163.14 (C-4); 165.71, 165.80 (COPh). ^{31}P{^{1}H} NMR (202.3 MHz, C$_6$D$_6$): 150.59, 151.67. HR FAB calcd for C$_{46}$H$_{55}$N$_3$O$_{12}$P (M+H)$^+$ 872.3523, found 872.3529.

N,*N*-Diisopropyl-methyl-[1-(4-*N*-benzoylcytosin-1-yl)-2-*O*-benzoyloxymethoxymethyl-5-*O*-dimethoxytrityl-β-D-ribofuranos-3-*O*-yl]phosphoramidite (**7d**)
Prepared by Method C from 4-*N*-benzoyl-1-(2-*O*-benzoyloxymethoxymethyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)cytosine **3d**. Yield 79%. Mixture of isomers 1:1.
^{1}H NMR (499.8 MHz, C$_6$D$_6$): 1.03, 1.05, 1.07, 1.12 (4 × d, 4 × 6H, J_{vic} = 6.8, (C**H$_3$**)$_2$CH); 3.12, 3.18 (2 × d, 2 × 3H, $J_{H,P}$ = 13.2, CH$_3$OP); 3.455, 3.462, 3.468 (3 × s, 12H, CH$_3$O-DMTr); 3.44-3.54 (m, 4H, (CH$_3$)$_2$C**H**); 3.64, 3.70 (2 × dd, 2 × 1H, J_{gem} = 11.0, $J_{5'b,4'}$ = 2.5, H-5'b); 3.81, 3.84 (2 × dd, 2 × 1H, J_{gem} = 11.0, $J_{5'a,4'}$ = 2.2, H-5'a); 4.47, 4.50 (2 × ddd, 2 × 1H, $J_{4',3'}$ = 9.0, $J_{4',5'}$ = 2.5, 2.5, H-4'); 4.71 (d, 1H, $J_{2',3'}$ = 4.5, H-2'); 4.74 (d, 1H, $J_{2',3'}$ = 4.8, H-2'); 4.81-4.88 (m, 2H, H-3'); 5.32, 5.40 (2 × d, 2 × 1H, J_{gem} = 6.5, OCH$_a$**H$_b$**OCH$_2$OBz); 5.53, 5.59 (2 × d, 2 × 1H, J_{gem} = 6.5, OC**H$_a$**H$_b$OCH$_2$OBz); 5.92, 5.96, 5.99, 6.04 (4 × d, 4 × 1H, J_{gem} = 6.4, OCH$_2$OC**H$_2$**OBz); 6.28, 6.29 (2 × s, 2 × 1H, H-1'); 6.85-6.90 (m, 8H, H-*m*-C$_6$H$_4$-DMTr); 7.03 (m, 4H, H-*m*-BzO); 7.07-7.20 (m, 12H, H-5, H-*m,p*-BzN, H-*p*-BzO, H-*p*-C$_6$H$_5$-DMTr); 7.30 (m, 4H, H-*m*-C$_6$H$_5$-DMTr); 7.50, 7.53 (2 × m, 2 × 4H, H-*o*-C$_6$H$_4$-DMTr); 7.66, 7.68 (2 × m, 2 × 2H, H-*o*-C$_6$H$_5$-DMTr); 7.88 (bm, 4H, H-*o*-BzN); 8.09-8.12 (m, 4H, H-*o*-Bz); 8.67, 8.72 (2 × bm, 2 × 1H, H-6); 9.48 (bs, 2H, NH). ^{13}C NMR (125.7 MHz, C$_6$D$_6$): 24.49, 24.70 (d, $J_{C,P}$ = 6.1, (**C**H$_3$)$_2$CH); 24.71, 24.78 (d, $J_{C,P}$ = 7.5, (**C**H$_3$)$_2$CH); 43.21 (d, $J_{C,P}$ = 12.2, (CH$_3$)$_2$**C**H); 43.23 (d, $J_{C,P}$ = 12.6, (CH$_3$)$_2$**C**H); 50.06 (d, $J_{C,P}$ = 16.3, CH$_3$OP); 50.17 (d, $J_{C,P}$ = 17.2, CH$_3$OP); 54.87 (CH$_3$O-DMTr); 60.85, 61.02 (CH$_2$-5'); 69.56 (d, $J_{C,P}$ = 12.1, CH-3'); 70.52 (d, $J_{C,P}$ = 15.0, CH-3'); 79.22 (d, $J_{C,P}$ = 2.6, CH-2'); 79.62 (CH-2'); 82.08 (d, $J_{C,P}$ = 5.8, CH-4'); 82.34 (d, CH-4'); 86.24, 86.33 (OCH$_2$O**C**H$_2$OBz); 87.59, 87.65 (C-DMTr); 90.67, 90.95 (CH-1'); 92.68, 93.10 (O**C**H$_2$OCH$_2$OBz); 113.71, 113.76 (CH-*m*-C$_6$H$_4$-DMTr); 127.46, 127.48 (CH-*p*-C$_6$H$_5$-DMTr); 128.29, 128.35 (CH-*m*-C$_6$H$_5$-DMTr); 128.47 (CH-*m*-BzO); 128.69 (CH-*m*-BzN); 129.07, 129.11 (CH-*o*-C$_6$H$_5$-DMTr); 130.16, 130.18 (CH-*o*-BzO); 130.63, 130.67 (C-*i*-Bz); 130.81, 130.85 (CH-*o*-C$_6$H$_4$-DMTr); 132.45 (CH-*p*-BzN); 132.97 (CH-*p*-BzO); 135.81, 135.90, 135.97, 136.13 (C-*i*-C$_6$H$_4$-DMTr); 144.84, 144.94 (C-*i*-C$_6$H$_5$-DMTr); 159.42 (C-*p*-C$_6$H$_4$-DMTr); 165.73, 165.84 (OCOPh); (C-2,4,5,6, C-*i*,*o*-BzN and NCOPh not observed). ^{31}P{^{1}H} NMR (202.3 MHz, C$_6$D$_6$): 150.16, 152.00. HR FAB calcd for C$_{53}$H$_{60}$N$_4$O$_{12}$P (M+H)$^+$ 975.3945, found 975.3939.

6-*N*-Benzoyl-9-(2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-β-D-ribofuranosyl)adenine (**8a**)
Prepared by Method B from 6-*N*-benzoyl-9-(2-*O*-benzoyloxymethoxymethyl-5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)adenine **4a**. Yield 52%.
^{1}H NMR (500 MHz, d$_6$-DMSO): 11.25 s, 1 H (NH); 8.75 s, 1 H (H-2); 8.70 s, 1 H (H-8); 8.05 d, 2 H, 7.92 d, 2 H, 7.65 m, 2 H, 7.52 m, 6 H, 7.40 d, 2 H, 7.35 m, 4 H, 7.25 t, 1 H, 6.91 d, 4 H; 6.45 d, 1 H, *J*(1´,2´) = 6.7 (H-1´); 5.17 t, 1 H, *J*(OH,5´) = 5.5 (5´-OH); 5.21 d, 1 H a 5.07 d, 1 H, *J* = 6.4 (OCH$_2$O); 4.95 d, 1 H a 4.70 d, 1 H, *J* = 6.8 (OCH$_2$O); 4.64 dd, 1 H, *J*(2´,1´) = 6.7, *J*(2´,3´) = 4.9 (H-2´); 4.37 dd, 1 H, *J*(3´,2´) = 4.9, *J*(3´,4´) = 2.2 (H-3´); 3.74 s a 3.73 s, 2 x 3 H (2 x OCH$_3$); 3.30 m, 1H (H-4´); 3.27 ddd, 1 H, *J*(5´b,5´a) = 12.2, *J*(5´a,4´) = 2.3, *J*(5´a,OH) = 4.9 (H-5´a); 3.12 ddd, 1 H, *J*(5´b,5´a) = 12.2, *J*(5´b,4´) = 3.1, *J*(5´b,OH) = 6.1 (H-5´b). ^{13}C NMR (125.7 MHz, d$_6$-DMSO): 165.80, 165.15, 133.83, 133.51, 132.65, 129.54 (2), 129.24, 128.89 (2), 128.70 (2) a 128.64 (2) (N-CO-C$_6$H$_5$ a O-CO-C$_6$H$_5$); 158.59, 158.54, 145.55, 136.08, 135.98, 130.32 (2), 130.30 (2), 128.16 (2), 128.09 (2), 127.14, 113.50 (4), 85.80, 55.27 a 55.25 (DMTr); 152.18 (C-6); 151.90 (C-2); 150.75 (C-4); 142.92 (C-8); 125.89 (C-5); 93.78 a 86.54 (OCH$_2$O); 86.23 (C-1´); 84.96 (C-4´); 79.58 (C-2´); 72.74 (C-3´); 61.03 (C-5´). HR FAB calcd for C$_{47}$H$_{44}$N$_5$O$_{10}$ (M+H)$^+$ 838.3088, found 838.3079.

9-(2-*O*-Benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-2-*N*-isobutyryl-β-D-ribofuranosyl)guanine (**8b**)
Prepared by Method B from 9-(2-*O*-benzoyloxymethoxymethyl-5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)-2-*N*-isobutyrylguanine **4b**. Yield 47%.
^{1}H NMR (500 MHz, d$_6$-DMSO): 12.10 bs, 1 H (NH); 11.65 bs, 1 H (NH); 8.22 s, 1 H (H-8); 7.89 d, 2 H, 7.66 t, 1 H, 7.51 m, 4 H, 7.39 d, 2 H, 7.34 d, 2 H, 7.26 t, 1 H, 6.93 d, 4 H; 6.20 d, 1 H, *J*(1´,2´) = 7.8 (H-1´); 5.20 d, 1 H a 5.10 d, 1 H, *J* = 6.4 (OCH$_2$O); 5.06 bt, 1 H, *J*(OH,5´a) = *J*(OH,5´b) = 5.6 (5´-OH); 4.95 d, 1 H a 4.76 d, 1 H, *J* = 6.8 (OCH$_2$O); 4.71 dd, 1 H, *J*(2´,1´) = 7.8, *J*(2´,3´) = 4.9 (H-2´); 4.28 bd, 1 H, *J*(3´,2´) = 4.9, *J*(3´,4´) < 1.0 (H-3´); 3.75 s a 3.74 s, 2 x 3 H (2 x OCH$_3$); 3.16 m, 1H (H-4´); 3.12 ddd, 1 H, *J*(5´b,5´a) = 11.8, *J*(5´a,4´) = 2.7, *J*(5´a,OH) = 6.0 (H-5´a); 2.99 ddd, 1 H, *J*(5´b,5´a) = 11.8, *J*(5´b,4´) = 2.7, *J*(5´b,OH) = 5.2 (H-5´b); 2.78 septet, 1 H, *J*(CH,CH$_3$) = 6.8 (CH); 1.14 d a

1.13 d, 2 x 3 H, $J(CH_3,CH)$ = 6.8 (CH_3). ^{13}C NMR (125.7 MHz, d_6-DMSO): 165.13, 133.81, 129.47 (2), 129.20 a 128.88 (2) (O-CO-C_6H_5); 180.35, 34.96, 19.06 a 19.03 (iBu); 158.60, 158.55, 145.61, 136.10, 135.96, 130.27 (2), 130.25 (2), 128.14 (2), 128.09 (2), 113.55 (4), 86.54, 55.30 a 55.28 (DMTr); 154.96 (C-6); 149.18 (C-2); 148.58 (C-4); 137.18 (C-8); 120.10 (C-5); 93.38 a 85.66 (OCH_2O); 85.66 (C-1´); 84.83 (C-4´); 79.83 (C-2´); 73.17 (C-3´); 61.05 (C-5´). HR FAB calcd for $C_{44}H_{45}N_5O_{11}Na$ $(M+H)^+$ 842.3013, found 842.3027.

1-(2-*O*-Benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-β-D-ribofuranosyl)uracil (**8c**)
Prepared by Method B from 1-(2-*O*-benzoyloxymethoxymethyl-5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)uracil **4c**. Yield 59%.
^{1}H NMR (500 MHz, d_6-DMSO): 11.42 s, 1 H (NH); 7.89 d, 1 H, J(6,5) = 8.2 (H-6); 5.69 d, 1 H, J(5,6) = 8.2 (H-5); 7.98 d, 2 H, 7.69 t, 1 H, 7.53 t, 2 H, 7.48 d, 2 H, 7.37 t, 2 H, 7.24 t, 1 H, 7.32 d, 4 H, 6.91 d, 4 H; 6.23 d, 1 H, J(1´,2´) = 7.3 (H-1´); 5.44 d, 1 H a 5.42 d, 1 H, J = 6.4 (OCH_2O); 5.09 bt, 1 H, J(OH,5´a) = J(OH,5´b) = 4.6 (5´-OH); 5.06 d, 1 H a 4.75 d, 1 H, J = 6.8 (OCH_2O); 4.24 bd, 1 H, J(3´,2´) = 5.0, J(3´,4´) = 1.6 (H-3´); 4.16 dd, 1 H, J(2´,1´) = 7.3, J(2´,3´) = 5.0 (H-2´); 3.74 s a 3.73 s, 2 x 3 H (2 x OCH_3); 3.13 ddd, 1 H, J(5´b,5´a) = 12.0, J(5´a,4´) = 2.2, J(5´a,OH) = 4.8 (H-5´a); 3.04 bq, 1H, J(4´,3´) = 1.6, J(4´,5´) = 2.2 (H-4´); 2.95 ddd, 1 H, J(5´b,5´a) = 12.0, J(5´b,4´) = 2.2, J(5´b,OH) = 4.4 (H-5´b). ^{13}C NMR (125.7 MHz, d_6-DMSO): 165.29, 133.91, 129.63 (4) a 129.32 (O-CO-C_6H_5); 158.59, 158.55, 145.54, 136.14, 135.00, 130.27 (4), 128.17 (4), 127.15, 113.68 (4), 85.65, 55.29 a 55.28 (DMTr); 163.08 (C-4); 151.08 (C-2); 140.16 (C-6); 102.68 (C-5); 93.36 a 86.45 (OCH_2O); 85.40 (C-1´); 84.38 (C-4´); 78.85 (C-2´); 72.63 (C-3´); 60.83 (C-5´). HR FAB cacld for $C_{39}H_{39}N_2O_{11}$ $(M+H)^+$ 711.2254, found 711.2259.

4-*N*-Benzoyl-1-(2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-β-D-ribofuranosyl)cytosine (**8d**)
Prepared by Method B from 4-*N*-benzoyl-1-(2-*O*-benzoyloxymethoxymethyl-5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)cytosine **4d**. Yield 55%.
^{1}H NMR (500 MHz, d_6-DMSO): 11.30 bs, 1 H (NH); 8.38 d, 1 H (H-6); 7.30 d, 1 H, J(5,6) = 7.3 (H-5); 8.01 d, 2 H, 7.97 d, 2 H, 7.67 t, 1 H, 7.62 t, 1 H, 7.49 m, 6 H, 7.31 m, 6 H, 7.24 t, 1 H, 6.89 d, 4 H; 6.23 d, 1 H, J(1´,2´) = 5.4 (H-1´); 5.46 d, 1 H a 5.42 d, 1 H, J = 6.4 (OCH_2O); 5.10 t, 1 H, J(OH,5´a) = J(OH,5´b) = 4.8 (5´-OH); 5.02 d, 1 H a 4.77 d, 1 H, J = 6.8 (OCH_2O); 4.16 t, 1 H, J(3´,2´) = J(3´,4´) = 4.2 (H-3´); 3.75 bt, 1 H, J(2´,1´) = J(2´,3´) = 5.4 (H-2´); 3.73 s a 3.72 s, 2 x 3 H (2 x OCH_3); 3.40 m, 1H (H-4´); 3.38 ddd, 1 H, J(5´b,5´a) = 12.0, J(5´a,4´) = 2.0, J(5´a,OH) = 4.8 (H-5´a); 3.17 ddd, 1 H, J(5´b,5´a) = 12.0, J(5´b,4´) = 2.0, J(5´b,OH) = 4.8 (H-5´b). ^{13}C NMR (125.7 MHz, d_6-DMSO): 167.52, 165.26, 133.84, 133.22, 132.96, 129.59 (4), 129.37, 128.11 (2) a 128.03 (2) (N-CO-C_6H_5 a O-CO-C_6H_5); 158.61, 158.58, 145.51, 135.97, 135.87, 130.32 (2), 128.63 (4), 127.13, 113.42 (4), 86.15, 55.27 a 55.26 (DMTr); 163.46 (C-4); 154.89 (C-2); 145.04 (C-6); 96.73 (C-5); 93.49 a 86.54 (OCH_2O); 87.27 (C-1´); 83.99 (C-4´); 79.88 (C-2´); 71.74 (C-3´); 60.21 (C-5´). HR FAB calcd for $C_{46}H_{44}N_3O_{11}$ $(M+H)^+$ 814.2976, found 814.2976.

N,*N*-Diisopropyl-methyl-[1-(6-*N*-benzoyladenine-9-yl)-2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-β-D-ribofuranos-5-*O*-yl]phosphoramidite (**9a**)
Prepared by Method C from 6-*N*-benzoyl-9-(2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-β-D-ribofuranosyl)adenine **8a**. Yield 82%. Mixture of isomers 5:2.
^{1}H NMR (600.1 MHz, C_6D_6): 1.01, 1.06 (2 × d, 2 × 6H, J_{vic} = 6.7, $(C\mathbf{H_3})_2$CH-A); 1.08, 1.16 (2 × d, 2 × 6H, J_{vic} = 6.7, $(C\mathbf{H_3})_2$CH-B); 3.16 (d, 3H, $J_{H,P}$ = 13.0, CH_3OP-B); 3.28 (d, 3H, $J_{H,P}$ = 13.6, CH_3OP-A); 3.29, 3.305, 3.311 (3 × s, 12H, CH_3O-DMTr-A,B); 3.38-3.50 (m, 5H, H-5'b-A, $(CH_3)_2C\mathbf{H}$-A,B); 3.59 (dd, 1H, $J_{2',3'}$ = 4.2, $J_{2',1'}$ = 3.0, H-2'-B); 3.74 (ddd, 1H, J_{gem} = 11.5, $J_{H,P}$ = 4.5, $J_{5'b,4'}$ = 2.9, H-5'b-B); 3.78 (ddd, 1H, J_{gem} = 11.4, $J_{H,P}$ = 6.5, $J_{5'a,4'}$ = 2.3, H-5'a-A); 3.84 (ddd, 1H, J_{gem} = 11.5, $J_{H,P}$ = 4.9, $J_{5'a,4'}$ = 2.1, H-5'a-B); 4.18 (q, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 2.3, H-4'-A); 4.36 (dt, 1H, $J_{4',3'}$ = 6.5, $J_{4',5'}$ = 2.1, H-4'-B); 4.56-4.59 (m, 2H, H-2',3'-A); 4.67 (dd, 1H, $J_{3',4'}$ = 6.5, $J_{3',2'}$ = 4.2, H-3'-B); 4.69 (d, 1H, J_{gem} = 6.8, $OCH_a\mathbf{H_b}OCH_2OBz$-A); 4.75 (d, 1H, J_{gem} = 6.7, $OCH_a\mathbf{H_b}OCH_2OBz$-B); 4.97 (d, 1H, J_{gem} = 6.8, $OC\mathbf{H_a}H_bOCH_2OBz$-A); 5.06 (d, 1H, J_{gem} = 6.7, $OC\mathbf{H_a}H_bOCH_2OBz$-B); 5.29, 5.32 (2 × d, 2 × 1H, J_{gem} = 6.4, $OCH_2OC\mathbf{H_2}OBz$-A); 5.47, 5.49 (d, 1H, J_{gem} = 6.4, $OCH_2OCH_a\mathbf{H_b}OBz$-B); 6.62 (d, 1H, $J_{1',2'}$ = 3.0, H-1'-B); 6.67, 6.69 (2 × m, 4H, H-*m*-C_6H_4-DMTr-B); 6.73 (m, 4H, H-*m*-C_6H_4-DMTr-A); 6.88 (d, 1H, $J_{1',2'}$ = 4.8, H-1'-A); 6.93-7.18 (m, 18H, H-*m,p*-BzO-A,B, H-*m,p*-BzN-A,B, H-*p,m*-C_6H_5-DMTr-A,B); 7.48, 7.50 (2 × m, 2 × 2H, H-*o*-C_6H_4-DMTr-B); 7.56, 7.58 (2 × m, 2 × 2H, H-*o*-C_6H_4-DMTr-A); 7.70 (m, 2H, H-*o*-C_6H_5-DMTr-B); 7.77 (m, 6H, H-*o*-BzN-A,B, H-*o*-C_6H_5-DMTr-A); 8.02 (m, 2H, H-*o*-Bz-A); 8.10 (m, 2H, H-*o*-Bz-B); 8.73 (s, 1H, H-8-A); 8.78 (d, 1H, J = 1.3, H-2-B); 8.79 (s, 1H, H-8-B); 8.84 (d, 1H, J = 1.6, H-2-A); 9.15-9.25 (bm, 2H, NH-A,B). ^{13}C NMR (150.9 MHz, C_6D_6): 24.64, 24.79, 24.81, 24.84 (d, $J_{C,P}$ = 7.1, $(\mathbf{C}H_3)_2$CH-A,B); 43.00 (d, $J_{C,P}$ = 12.4, $(CH_3)_2\mathbf{C}$H-A); 43.09 (d, $J_{C,P}$ = 12.4, $(CH_3)_2\mathbf{C}$H-B); 50.48 (d, $J_{C,P}$ = 18.8, CH_3OP-B); 50.60 (d, $J_{C,P}$ = 18.8, CH_3OP-A); 54.78, 54.79, 54.82 (CH_3O-DMTr-A,B); 61.80 (d, $J_{C,P}$ = 15.4, CH_2-5'-B); 63.43 (d, $J_{C,P}$ = 18.4, CH_2-5'-B); 71.56 (CH-3'-B); 73.16 (CH-3'-A); 80.87 (CH-2'-A); 81.13 (CH-2'-B); 82.83 (d, $J_{C,P}$ = 9.8, CH-4'-B); 83.81 (d, $J_{C,P}$ = 9.1, CH-4'-A); 86.11 ($OCH_2O\mathbf{C}H_2OBz$-A); 86.69 ($OCH_2O\mathbf{C}H_2OBz$-B); 87.36 (CH-1'-A); 87.52 (C-DMTr-B); 87.55 (C-DMTr-A); 87.79 (CH-1'-B); 94.50 ($O\mathbf{C}H_2OCH_2OBz$-A); 95.19 ($O\mathbf{C}H_2OCH_2OBz$-B); 113.47, 113.57, 113.61 (CH-*m*-C_6H_4-DMTr-A,B); 124.25 (C-5-A); 124.39 (C-5-B); 127.28 (CH-*p*-C_6H_5-DMTr-B); 127.32 (CH-*p*-C_6H_5-DMTr-A); 128.29 (CH-*m*-C_6H_5-DMTr-A,B); 128.51, 128.53, 128.60 (CH-*m*-BzO-A,B, CH-*m*-BzN-A,B); 128.86 (CH-*o*-C_6H_5-DMTr-A,B); 130.06 (CH-*o*-BzO-A);

130.11 (CH-*o*-BzO-B); 130.29 (C-*i*-Bz-A); 130.36 (C-*i*-Bz-B); 130.38 (CH-*o*-BzN-A,B); 130.99, 131.05, 131.16, 131.22 (CH-*o*-C_6H_4-DMTr-A,B); 131.92 (CH-*p*-BzN-A); 132.02 (CH-*p*-BzN-B); 133.23 (CH-*p*-BzO-A); 133.29 (CH-*p*-BzO-B); 134.58 (C-*i*-BzN-B); 134.64 (C-*i*-BzN-A); 136.10, 136.30 (C-*i*-C_6H_4-DMTr-B); 136.41, 136.68 (C-*i*-C_6H_4-DMTr-A); 141.57 (CH-8-B); 142.07 (CH-8-A); 145.98 (C-*i*-C_6H_5-DMTr-B); 146.28 (C-*i*-C_6H_5-DMTr-A); 150.25 (C-6-B); 150.38 (C-6-A); 151.81 (C-4-B); 152.41 (C-4-A); 159.44, 159.47, 159.53 (C-*p*-C_6H_4-DMTr-A,B); 164.32 (NCOPh-A,B); 165.57 (OCOPh-A); 165.67 (OCOPh-B). $^{31}P\{^1H\}$ NMR (202.3 MHz, C_6D_6): 150.67 (P-B); 150.90 (P-A). HR FAB calcd for $C_{54}H_{60}N_6O_{11}P$ $(M+H)^+$ 999.4058, found 999.4065.

N,*N*-Diisopropyl-methyl-[2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-1-(2-*N*-isobutyrylguanine-9-yl)-β-D-ribofuranos-5-*O*-yl]phosphoramidite (**9b**)
Prepared by Method C from 9-(2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-β-D-ribofuranosyl)-2-*N*-isobutyrylguanine **8b**. Yield 75%. Mixture of isomers 3:2.
^1H NMR (499.8 MHz, C_6D_6): 1.05, 1.065 (2 × d, 2 × 3H, J_{vic} = 6.7, $(CH_3)_2$CHC-B); 1.07, 1.09 (2 × d, 2 × 6H, J_{vic} = 6.7, $(CH_3)_2$CHN-B); 1.10 (d, 6H, J_{vic} = 6.7, $(CH_3)_2$CHN-A); 1.13, 1.16 (2 × d, 2 × 3H, J_{vic} = 6.7, $(CH_3)_2$CHC-A); 1.23 (d, 6H, J_{vic} = 6.7, $(CH_3)_2$CHN-A); 2.49 (h, 1H, J_{vic} = 6.7, $(CH_3)_2CH$C-B); 2.73 (h, 1H, J_{vic} = 6.7, $(CH_3)_2CH$C-A); 2.85 (bdd, 1H, $J_{2',3'}$ = 3.7, $J_{2',1'}$ = 1.5, H-2'-A); 3.20 (d, 3H, $J_{H,P}$ = 13.1, CH_3OP-A); 3.31(d, 3H, $J_{H,P}$ = 13.3, CH_3OP-B); 3.32, 3.34, 3.35 (3 × s, 12H, CH_3O-DMTr-A,B); 3.32-3.42, 3.45-3.52 (2 × m, 4H, $(CH_3)_2CH$-A,B); 3.56 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 5.0, $J_{5'b,4'}$ = 2.3, H-5'b-B); 3.85 (m, 1H, H-2'-B); 3.87 (bm, 1H, H-5'b-A); 3.93 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 6.7, $J_{5'a,4'}$ = 2.3, H-5'a-B); 4.00 (ddd, 1H, J_{gem} = 11.9, $J_{H,P}$ = 4.9, $J_{5'a,4'}$ = 1.7, H-5'a-A); 4.31 (dt, 1H, $J_{4',3'}$ = 5.5, $J_{4',5'}$ = 2.3, H-4'-B); 4.47 (dt, 1H, $J_{4',3'}$ = 8.1, $J_{4',5'}$ = 1.7, H-4'-A); 4.51 (dd, 1H, $J_{3',4'}$ = 5.5, $J_{3',2'}$ = 4.6, H-3'-B); 4.62 (dd, 1H, $J_{3',4'}$ = 8.1, $J_{3',2'}$ = 3.7, H-3'-A); 4.75 (d, 1H, J_{gem} = 6.8, $OCH_aH_bOCH_2OBz$-A); 4.79 (d, 1H, J_{gem} = 6.8, $OCH_aH_bOCH_2OBz$-B); 5.04 (d, 1H, J_{gem} = 6.8, $OCH_aH_bOCH_2OBz$-A); 5.06 (d, 1H, J_{gem} = 6.8, $OCH_aH_bOCH_2OBz$-B); 5.52, 5.53 (2 × d, 2 × 1H, J_{gem} = 6.4, OCH_2OCH_2OBz-B); 5.59, 5.66 (2 × d, 2 × 1H, J_{gem} = 6.5, $OCH_2OCH_aH_bOBz$-A); 6.14 (d, 1H, $J_{1',2'}$ = 1.5, H-1'-A); 6.41 (d, 1H, $J_{1',2'}$ = 3.7, H-1'-B); 6.63, 6.70, 6.71 (3 × m, 8H, H-*m*-C_6H_4-DMTr-A,B); 7.00-7.18 (m, 12H, H-*m*,*p*-Bz-A,B, H-m,*p*-C_6H_5-DMTr-A,B); 7.43 (m, 4H, H-*o*-C_6H_4-DMTr-A); 7.49, 7.51 (2 × m, 2 × 2H, H-*o*-C_6H_4-DMTr-B); 7.66 (m, 2H, H-*o*-C_6H_5-DMTr-A); 7.71 (m, 2H, H-*o*-C_6H_5-DMTr-B); 8.04 (m, 2H, H-*o*-Bz-B); 8.08 (m, 2H, H-*o*-Bz-A); 8.43 (s, 1H, H-8-B); 8.50 (s, 1H, H-8-B); 9.36 (bs, 1H, NH-B); 9.87 (bs, 1H, NH-A). ^{13}C NMR (125.7 MHz, C_6D_6): 18.97, 19.04 ($(CH_3)_2$CHC-B); 19.09, 19.14 ($(CH_3)_2$CHC-A); 24.71, 24.81, 24.84, 24.93 (d, $J_{C,P}$ = 7.2, $(CH_3)_2$CH-A,B); 36.10 ($(CH_3)_2$CHC-A); 36.15 ($(CH_3)_2$CHC-B); 43.09 (d, $J_{C,P}$ = 12.2, $(CH_3)_2$CH-B); 43.18 (d, $J_{C,P}$ = 12.3, $(CH_3)_2$CH-A); 50.23 (d, $J_{C,P}$ = 18.5, CH_3OP-A); 50.59 (d, $J_{C,P}$ = 18.7, CH_3OP-B); 54.83, 54.87, 54.89 (CH_3O-DMTr-A,B); 61.55 (d, $J_{C,P}$ = 14.7, CH_2-5'-A); 62.86 (d, $J_{C,P}$ = 17.6, CH_2-5'-B); 70.65 (CH-3'-A); 72.35 (CH-3'-B); 81.12 (CH-2'-B); 81.32 (CH-2'-A); 81.94 (d, $J_{C,P}$ = 9.9, CH-4'-A); 82.99 (d, $J_{C,P}$ = 9.0, CH-4'-B); 87.02 (OCH_2OCH_2OBz-B); 87.32 (CH-1'-B); 87.50 (C-DMTr-B); 87.59 (C-DMTr-A); 87.62 (CH-1'-A); 87.64 (OCH_2OCH_2OBz-A); 95.10 (OCH_2OCH_2OBz-B); 95.69 (OCH_2OCH_2OBz-A); 113.41, 113.52, 113.59 (CH-*m*-C_6H_4-DMTr-A,B); 122.15 (C-5-B); 122.23 (C-5-A); 127.21, 127.29 (CH-*p*-C_6H_5-DMTr-A,B); 128.14, 128.29 (CH-*m*-C_6H_5-DMTr-A,B); 128.65, 128.67, 128.72, 128.75 (CH-*m*-Bz-A,B, CH-*o*-C_6H_5-DMTr-A,B); 130.03 (CH-*o*-Bz-A,B); 130.14 (C-*i*-Bz-A,B); 131.04, 131.18, 131.29 (CH-*o*-C_6H_4-DMTr-A,B); 133.50 (CH-*p*-Bz-B); 133.56 (CH-*p*-Bz-A); 135.87, 136.09 (C-*i*-C_6H_4-DMTr-A); 136.18, 136.42 (C-*i*-C_6H_4-DMTr-B); 136.88 (CH-8-A); 137.16 (CH-8-B); 146.03 (C-*i*-C_6H_5-DMTr-A); 146.29 (C-*i*-C_6H_5-DMTr-B); 148.00, 148.17, 148.51 (C-4,6-A,B); 155.87 (C-2-B); 155.96 (C-2-A); 159.46, 159.50, 159.52, 159.56 (C-*p*-C_6H_4-DMTr-A,B); 166.32 (COPh-B); 166.60 (COPh-A); 179.01 (COiPr-B); 179.42 (COiPr-A). $^{31}P\{^1H\}$ NMR (202.3 MHz, C_6D_6): 150.67 (P-B); 150.86 (P-B). HR FAB calcd for $C_{51}H_{62}N_6O_{12}P$ $(M+H)^+$ 981.4163, found 981.4171.

N,*N*-Diisopropyl-methyl-[2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-1-(uracil-1-yl)-β-D-ribofuranos-5-*O*-yl]phosphoramidite (**9c**)
Prepared by Method C from 1-(2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-β-D-ribofuranosyl)uracil **8c**. Yield 84%. Mixture of isomers 3:2.
^1H NMR (600.1 MHz, C_6D_6): 0.95, 1.02 (2 × d, 2 × 6H, J_{vic} = 6.8, $(CH_3)_2$CH-B); 1.03, 1.06 (2 × d, 2 × 6H, J_{vic} = 6.8, $(CH_3)_2$CH-A); 3.03 (d, 3H, $J_{H,P}$ = 13.2, CH_3OP-A); 3.10 (d, 3H, $J_{H,P}$ = 13.2, CH_3OP-B); 3.22 (ddd, 1H, J_{gem} = 11.4, $J_{H,P}$ = 4.8, $J_{5'b,4'}$ = 2.2, H-5'b-B); 3.24 (m, 1H, H-2'-A); 3.30-3.40 (m, 4H, $(CH_3)_2CH$-A,B); 3.320, 3.323 (2 × s, 2 × 3H, CH_3O-DMTr-B); 3.36 (s, 6H, CH_3O-DMTr-A); 3.56 (ddd, 1H, J_{gem} = 11.6, $J_{H,P}$ = 4.9, $J_{5'b,4'}$ = 1.6, H-5'b-A); 3.61-3.66 (m, 2H, H-5'a-A,B); 3.97 (dt, 1H, $J_{4',3'}$ = 3.3, $J_{4',5'}$ = 2.2, H-4'-B); 4.03 (dd, 1H, $J_{2',1'}$ = 6.0, $J_{2',3'}$ = 5.0, H-2'-B); 4.15 (dt, 1H, $J_{4',3'}$ = 6.2, $J_{4',5'}$ = 1.6, H-4'-A); 4.33 (dd, 1H, $J_{3',2'}$ = 5.0, $J_{3',4'}$ = 3.3, H-3'-B); 4.39 (dd, 1H, $J_{3',4'}$ = 6.2, $J_{3',2'}$ = 4.3, H-3'-A); 4.75 (d, 1H, J_{gem} = 6.8, $OCH_aH_bOCH_2OBz$-B); 4.82 (d, 1H, J_{gem} = 6.6, $OCH_aH_bOCH_2OBz$-A); 5.07 (d, 1H, J_{gem} = 6.8, $OCH_aH_bOCH_2OBz$-B); 5.10 (d, 1H, J_{gem} = 6.6, $OCH_aH_bOCH_2OBz$-A); 5.54, 5.58 (2 × d, 2 × 1H, J_{gem} = 6.3, OCH_2OCH_2OBz-B); 5.62 (d, 1H, J_{gem} = 6.3, $OCH_2OCH_aH_bOBz$-A); 5.63 (d, 1H, $J_{5,6}$ = 8.1, H-5-B); 5.64 (d, 1H, J_{gem} = 6.3, $OCH_2OCH_aH_bOBz$-A); 5.70 (d, 1H, $J_{5,6}$ = 8.1, H-5-A); 6.39 (d, 1H, $J_{1',2'}$ = 3.4, H-1'-A); 6.72, 6.73, 6.74 (3 × m, 8H, H-*m*-C_6H_4-DMTr-A,B); 6.81 (d, 1H, $J_{1',2'}$ = 6.0, H-1'-B); 7.00-7.07 (m, 6H, H-*m*-Bz-A,B, H-*p*-C_6H_5-DMTr-A,B); 7.09-7.17 (m, 6H, H-*p*-Bz-A,B, H-*m*-C_6H_5-DMTr-A,B); 7.49, 7.52, 7.53, 7.55 (4 × m, 4 × 2H, H-*o*-C_6H_4-DMTr-A,B); 7.69 (m, 2H, H-*o*-C_6H_5-DMTr-A); 7.73 (m, 2H, H-*o*-C_6H_5-DMTr-B); 7.96 (d, 1H, $J_{6,5}$ = 8.1, H-6-B); 8.08 (d, 1H, $J_{6,5}$ =

8.1, H-6-A); 8.09 (m, 2H, H-*o*-Bz-B); 8.13 (m, 2H, H-*o*-Bz-A); 9.33 (bs, 1H, NH-B); 9.48 (bs, 1H, NH-A). ^{13}C NMR (150.9 MHz, C_6D_6): 24.57, 24.67, 24.72, 24.74 (d, $J_{C,P}$ = 7.5, (**C**H_3)$_2$CH-A,B); 42.97, 43.00 (d, $J_{C,P}$ = 12.4, (CH_3)$_2$**C**H-A,B); 50.41, 50.53 (d, $J_{C,P}$ = 17.8, CH_3OP-A,B); 54.81, 54.85, 54.89 (CH_3O-DMTr-A,B); 61.48 (d, $J_{C,P}$ = 15.3, CH_2-5'-A); 63.48 (d, $J_{C,P}$ = 19.4, CH_2-5'-B); 71.22 (CH-3'-A); 72.75 (CH-3'-B); 79.80 (CH-2'-B); 80.08 (CH-2'-A); 82.55 (d, $J_{C,P}$ = 9.9, CH-4'-A); 83.25 (d, $J_{C,P}$ = 9.7, CH-4'-B); 86.14 (OCH$_2$O**C**H$_2$OBz-B); 86.64 (OCH$_2$O**C**H$_2$OBz-A); 87.32 (CH-1'-B); 87.53 (C-DMTr-B); 87.56 (C-DMTr-A); 87.77 (CH-1'-A); 93.99 (O**C**H$_2$OCH$_2$OBz-B); 94.42 (O**C**H$_2$OCH$_2$OBz-A); 101.76 (CH-5-A); 102.51 (CH-5-B); 113.53, 113.57, 113.60 (CH-*m*-C_6H_4-DMTr-A,B); 127.28, 127.33 (CH-*p*-C_6H_5-DMTr-A,B); 128.29 (CH-*m*-C_6H_5-DMTr-A,B); 128.52 (CH-*m*-Bz-A); 128.55 (CH-*m*-Bz-B); 128.86 (CH-*o*-C_6H_5-DMTr-B); 128.93 (CH-*o*-C_6H_5-DMTr-A); 130.14 (CH-*o*-Bz-B); 130.17 (CH-*o*-Bz-A); 130.29 (C-*i*-Bz-B); 130.47 (C-*i*-Bz-A); 130.94, 131.06, 131.11, 131.19 (CH-*o*-C_6H_4-DMTr-A,B); 133.11 (CH-*p*-Bz-A); 133.20 (CH-*p*-Bz-B); 136.21, 136.42 (C-*i*-C_6H_4-DMTr-A); 136.47, 136.75 (C-*i*-C_6H_4-DMTr-B); 139.96 (CH-6-A); 140.33 (CH-6-B); 145.87 (C-*i*-C_6H_5-DMTr-A); 146.18 (C-*i*-C_6H_5-DMTr-B); 150.59 (C-2-A); 150.89 (C-2-B); 159.40, 159.45 (C-*p*-C_6H_4-DMTr-B); 159.48, 159.57 (C-*p*-C_6H_4-DMTr-A); 163.01 (C-4-B); 163.16 (C-4-A); 165.63 (COPh-B); 165.70 (COPh-A). ^{31}P{^{1}H} NMR (202.3 MHz, C_6D_6): 150.51 (P-A); 151.10 (P-B). HR FAB calcd for $C_{46}H_{55}N_3O_{12}P$ $(M+H)^+$ 872.3523, found 872.3513.

N,*N*-Diisopropyl-methyl-[1-(4-*N*-benzoylcytosin-1-yl)-2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-β-D-ribofuranos-5-*O*-yl]phosphoramidite (**9d**)
Prepared by Method C from 4-*N*-benzoyl-1-(2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-β-D-ribofuranosyl)cytosine **8d**. Yield 79%. Mixture of isomers 1:1.
^{1}H NMR (600.1 MHz, C_6D_6): 1.01, 1.09, 1.11, 1.15 (4 × d, 4 × 6H, J_{vic} = 6.7, (C**H$_3$**)$_2$CH); 2.95 (bm, 1H, H-2'); 3.13, 3.25 (2 × d, 2 × 3H, $J_{H,P}$ = 13.1, CH_3OP); 3.35-3.48 (m, 4H, (CH_3)$_2$C**H**); 3.355, 3.360, 3.39, 3.40 (4 × s, 4 × 3H, CH_3O-DMTr); 3.54 (bm, 1H, H-5'b); 3.65 (bm, 1H, H-2'); 3.78 (bm, 1H, H-5'b); 3.85, 3.88 (2 × bm, 2 × 1H, H-5'a); 4.28 (bm, 1H, H-4'); 4.33 (dd, 1H, $J_{3',2'}$ = 6.0, $J_{3',4'}$ = 4.6, H-3'); 4.39 (dd, 1H, $J_{3',4'}$ = 8.3, $J_{3',2'}$ = 4.2, H-3); 4.44 (bm, 1H, H-4'); 4.98, 5.03 (2 × d, 2 × 1H, J_{gem} = 6.0, OCH$_a$**H$_b$**OCH$_2$OBz); 5.20-5.23 (d, 1H, J_{gem} = 6.8, OC**H$_a$**H$_b$OCH$_2$OBz); 5.65, 5.69 (2 × d, 2 × 1H, J_{gem} = 6.4, OCH$_2$OC**H$_2$**OBz); 5.71, 7.73 (2 × d, 2 × 1H, J_{gem} = 6.6, OCH$_2$OC**H$_2$**OBz); 6.28 (d, 1H, $J_{1',2'}$ = 1.8, H-1'); 6.81 (d, 1H, $J_{1',2'}$ = 3.4, H-1'); 6.72-6.77 (m, 8H, H-*m*-C_6H_4-DMTr); 6.98-7.20 (m, 20H, H-5, H-*m,p*-BzN, H-*m,p*-BzO, H-m,*p*-C_6H_5-DMTr); 7.50, 7.52, 7.54, 7.57 (4 × m, 4 × 2H, H-*o*-C_6H_4-DMTr); 7.70, 7.74 (2 × m, 2 × 2H, H-*o*-C_6H_5-DMTr); 7.80 (bm, 4H, H-*o*-BzN); 8.11, 8.15 (2 × m, 2 × 2H, H-*o*-BzO); 8.48, 8.68 (2 × bm, 2 × 1H, H-6); 9.10 (bs, 2H, NH). ^{13}C NMR (150.9 MHz, C_6D_6): 24.65, 24.78, 24.82 (d, $J_{C,P}$ = 6.8, (**C**H_3)$_2$CH); 43.07, 43.08 (d, $J_{C,P}$ = 12.4, (CH_3)$_2$**C**H); 50.46, 50.57 (d, $J_{C,P}$ = 18.3, CH_3OP); 54.85, 54.89, 54.96 (CH_3O-DMTr); 60.91 (d, $J_{C,P}$ = 14.8, CH_2-5'); 62.81 (d, $J_{C,P}$ = 19.1, CH_2-5'); 70.36, 71.83 (CH-3'); 80.25, 80.47 (CH-2'); 82.14 (d, $J_{C,P}$ = 1.1, CH-4'); 82.82 (d, $J_{C,P}$ = 8.8, CH-4'); 86.81, 87.11 (OCH$_2$O**C**H$_2$OBz); 87.46, 87.68 (C-DMTr); 89.15, 89.30 (CH-1'); 94.41, 94.68 (O**C**H$_2$OCH$_2$OBz); 113.53, 113.54, 113.57, 113.63 (CH-*m*-C_6H_4-DMTr); 127.33, 127.37 (CH-*p*-C_6H_5-DMTr); 128.29 (CH-*m*-C_6H_5-DMTr); 128.49 (CH-*m*-BzO); 128.66 (CH-*m*-BzN); 128.91, 128.95 (CH-*o*-C_6H_5-DMTr); 130.17, 130.20 (CH-*o*-BzO); 130.46, 130.60 (C-*i*-BzO); 131.06, 131.13, 131.24, 131.27 (CH-*o*-C_6H_4-DMTr); 132.45, 132.47 (CH-*p*-BzN); 133.04, 133.08 (CH-*p*-BzO); 136.05, 136.24, 136.30, 136.59 (C-*i*-C_6H_4-DMTr); 145.83, 146.16 (C-*i*-C_6H_5-DMTr); 159.51, 159.58, 159.70 (C-*p*-C_6H_4-DMTr); 165.75, 165.70 (OCOPh); (C-2,4,5,6; C-*i,o*-BzN and NCOPh not observed). ^{31}P{^{1}H} NMR (202.3 MHz, C_6D_6): 150.43, 151.06. HR FAB calcd for $C_{53}H_{60}N_4O_{12}P$ $(M+H)^+$ 975.3945, found 975.3953.

Benzoyloxymethoxymethyl chloride (**10**).
CAUTION: Bis(chloromethyl)ether is known carcinogene, all reaction should be performed in the hood and with caution.
The following reaction can be strongly exothermic. Bis(chloromethyl)ether. Zinc bromide (27.5 g; 0.12 mol) was added to a mixture of paraformaldehyde (300.0 g; 9.9 mol) and thionyl chloride (378.0 ml; 5.1 mol), and the mixture was stirred 24 h at 80°C. The product was isolated by destillation (b.p. 103 – 104°C) to afford di(chloromethyl)ether in 80% yield (470.6 g). ^{1}H NMR (200 MHz, $CDCl_3$): 5.57 s.
A mixture of di(chloromethyl)ether (200 ml; 2.3 mol) and sodium benzoate (105.0 g; 0.75 mol) was stirred 16 h at 80°C. The reaction mixture was diluted with DCM (200 ml), filtered and evaporated. The product was isolated by vacuum destillation (b.p.(13 Pa) 118 – 123°C) to afford benzoyloxymethoxymethyl chloride in 62% yield (93.5 g). ^{1}H NMR (500 MHz, $CDCl_3$): 8.07 d, 2H, 7.60 m, 1 H, 7.47 m 2 H (C_6H_5); 5.68 s and 5.59 s, 2 x 2 H (2 x CH_2). ^{13}C NMR (d_6-DMSO): 165.55, 133.72, 129.95, 129.09, 128.57, 85.47, 78.33. Elemental analysis calcd for $C_9H_9ClO_3$ C 53.88, H 4.52, Cl 17.67, found C 53.62, H 4.71, Cl 17.01.

Diisopropyl-[1-(6-*N*-benzoyladenin-9-yl)-β-D-ribofuranos-3-*O*-yl]methylphosphonate (**12a**)
Prepared by Method D from 6-*N*-benzoyl-9-(2,5-*O*-bis-dimethoxytrityl-β-D-ribofuranosyl)adenine **11a**. Yield 72%. ^{1}H NMR (500 MHz, d_6-DMSO): 11.21 bs, 1 H (NH); 8.71 s, 1 H (H-2); 8.62 s, 1 H (H-8); 8.05 m, 2H, 7.66 m, 1H and 7.56 m, 2H (C_6H_5); 6.35 d, 1 H, *J*(1´,2´) = 4.9 (H-1´); 5.94 d, 1 H, *J*(OH,2´) = 5.3 (2´-OH); 5.17 d, 1 H, *J*(OH,5´a) = 5.5, *J*(OH,5´b) = 5.3 (5´-OH); 4.67 m, 1 H and 4.66 m, 1 H, *J*(H,P) = 7.2, *J*(vic) = 6.1 (2 x O-C<u>H</u>(CH_3)$_2$); 4.44 m, 1 H,

J(2´,1´) = 4.9, *J*(2´,3´) = 4.0, *J*(2´,OH) = 5.3 (H-2´); 4.18 dd, 1 H, *J*(3´,2´) = 4.0, *J*(3´,4´) = 4.3, (H-3´); 4.02 dd, 1 H, *J*(gem) = 11.8, *J*(Ha,P) = 8.0 (O-CHaHb-P); 3.97 m, 1H, *J*(4´,3´) = 4.3, *J*(4´,5´a) = 4.6, *J*(4´,5´b) = 5.1 (H-4´); 3.93 dd, 1 H, *J*(gem) = 11.8, *J*(Hb,P) = 8.2 (O-CHaHb-P); 3.72 ddd, 1 H, *J*(5´a,5´b) = 11.8, *J*(5´a,4´) = 4.6, *J*(5´a,OH) = 5.5 (H-5´a); 3.56 ddd, 1 H, *J*(5´b,5´a) = 11.8, *J*(5´b,4´) = 5.1, *J*(5´b,OH) = 5.3 (H-5´b); 1.28 d and 1.26 d, 2 x 6 H, *J*(vic) = 6.2 (2 x O-CH(CH$_3$)$_2$). HR FAB calcd for $C_{24}H_{33}N_5O_8P$ 550.2067 $(M+H)^+$, found 550.2045.

Diisopropyl-[1-(2-*N*-isobutyrylguanin-9-yl)-β-D-ribofuranos-3-*O*-yl]methylphosphonate (**12b**)
Prepared by Method D from 9-(2,5-*O*-bis-dimethoxytrityl-β-D-ribofuranosyl)-2-*N*-isobutyrylguanine **11b**. Yield 67%. ^{1}H NMR (500 MHz, d$_6$-DMSO): 12.10 bs, 1 H (NH); 11.75 bs, 1 H (NH); 8.26 s, 1 H (H-8); 5.82 d, 1 H, *J*(1´,2´) = 6.2 (H-1´); 5.67 d, 1 H, *J*(OH,2´) = 5.5 (2´-OH); 5.13 t, 1 H, *J*(OH,5´) = 5.0 (5´-OH); 4.63 m, 2 H (2 x O-CH(CH$_3$)$_2$); 4.60 m, 1 H (H-2´); 4.12 dd, 1 H, *J*(3´,2´) = 4.6, *J*(3´,4´) = 3.3 (H-3´); 4.05 dd, 1 H, *J*(gem) = 13.9, *J*(Ha,P) = 8.0 (O-CHaHb-P); 4.01 td, 1H, *J*(4´,3´) = 3.3, *J*(4´,5´a) = 3.9, *J*(4´,5´b) = 3.9 (H-4´); 3.93 dd, 1 H, *J*(gem) = 13.9, *J*(Hb,P) = 8.9 (O-CHaHb-P); 3.64 dt, 1 H, *J*(5´a,5´b) = 12.0, *J*(5´a,4´) = 4.5, *J*(5´a,OH) = 4.5 (H-5´a); 3.54 dt, 1 H, *J*(5´b,5´a) = 12.0, *J*(5´b,4´) = 4.5, *J*(5´b,OH) = 4.5 (H-5´b); 2.76 septet, 1 H, *J*(CH,CH$_3$) = 6.8, (CH); 1.27 d, 12 H, *J*(vic) = 6.2 (2 x O-CH(CH$_3$)$_2$); 1.12 d, 6 H, *J*(CH$_3$,CH) = 6.8, (CH$_3$). HR FAB calcd for $C_{21}H_{35}N_5O_9P$ 532.2172 $(M+H)^+$, found 532.2131.

Diisopropyl-[1-(uracil-1-yl)-β-D-ribofuranos-3-*O*-yl]methylphosphonate (**12c**)
Prepared by Method D from 1-(2,5-*O*-bis-dimethoxytrityl-β-D-ribofuranosyl)uracil **11c**. Yield 75%. ^{1}H NMR (500 MHz, CDCl$_3$): ^{1}H NMR (400 MHz, CDCl$_3$): 11.42 bs, 1 H (NH); 7.78 d, 1 H, *J*(6,5) = 7.9 (H-6); 5.57 d, 1 H, *J*(5,6) = 7.9 (H-5); 5.86 d, 1 H, *J*(1´,2´) = 5.5 (H-1´); 5.45 bs, 1 H, (2´-OH); 5.21 bs, 1 H, (5´-OH); 4.65 m, 2 H (2 x O-CH(CH$_3$)$_2$); 4.15 dd, 1 H, *J*(2´,1´) = 5.2, *J*(2´,3´) = 5.2 (H-2´); 4.08 dt, 1 H, *J*(4´,3´) = 3.8, *J*(4´,5´a) = 3.2, *J*(4´,5´b) = 3.2 (H-4´); 4.02 dd, 1 H, *J*(3´,2´) = 4.4, *J*(3´,4´) = 4.4 (H-3´); 3.95 dd, 1 H, *J*(gem) = 13.9, *J*(Ha,P) = 7.9 (O-CHaHb-P); 3.83 dd, 1 H, *J*(gem) = 13.9, *J*(Hb,P) = 7.9 (O-CHaHb-P); 3.67 dd, 1 H, *J*(5´a,5´b) = 11.8, *J*(5´a,4´) = 2.2 (H-5´a); 3.54 dd, 1 H, *J*(5´b,5´a) = 11.8, *J*(5´b,4´) = 2.2 (H-5´b); 1.25 d, 1.24 d, 1.22 d and 1.20 d, 4 x 3 H, *J*(vic) = 6.2 (2 x O-CH(CH$_3$)$_2$). HR FAB calcd for $C_{16}H_{28}N_2O_9P$ 423.1532 $(M+H)^+$, found 423.1523.

Diisopropyl-[1-(4-*N*-benzoylcytosin-1-yl)-β-D-ribofuranos-3-*O*-yl]methylphosphonate (**12d**)
Prepared by Method D from 4-*N*-benzoyl-1-(2,5-*O*-bis-dimethoxytrityl-β-D-ribofuranosyl)cytosine **11d**. Yield 86%. ^{1}H NMR (500 MHz, d$_6$-DMSO): 11.35 bs, 1 H (NH); 8.48 d, 1 H, (H-6); 7.33 bd, 1 H, *J*(5,6) = 7.6 (H-5); 8.01 d, 2H, 7.62 t, 1 H, 7.51 t, 2 H; 5.82 d, 1 H, *J*(1´,2´) = 3.3 (H-1´); 5.65 d, 1 H, *J*(OH,2´) = 5.4 (2´-OH); 5.27 t, 1 H, *J*(OH,5´) = 5.0 (5´-OH); 4.62 m, 2 H (2 x O-CH(CH$_3$)$_2$); 4.30 ddd, 1 H, *J*(2´,1´) = 3.3, *J*(2´,3´) = 4.4, *J*(2´,OH) = 5.4 (H-2´); 4.05 dt, 1 H, *J*(4´,3´) = 6.5, *J*(4´,5´a) = 2.8, *J*(4´,5´b) = 2.8 (H-4´); 4.00 dd, 1 H, *J*(3´,2´) = 4.4, *J*(3´,4´) = 6.5 (H-3´); 3.99 dd, 1 H, *J*(gem) = 14.0, *J*(Ha,P) = 7.9 (O-CHaHb-P); 3.85 dd, 1 H, *J*(gem) = 14.0, *J*(Hb,P) = 8.4 (O-CHaHb-P); 3.79 ddd, 1 H, *J*(5´a,5´b) = 12.2, *J*(5´a,4´) = 2.7, *J*(5´a,OH) = 5.0 (H-5´a); 3.61 ddd, 1 H, *J*(5´b,5´a) = 12.2, *J*(5´b,4´) = 2.9, *J*(5´b,OH) = 5.0 (H-5´b); 1.25 d, 1.25 d, 1.24 d and 1.23 d, 4 x 3 H, *J*(vic) = 6.2 (2 x O-CH(CH$_3$)$_2$). HR FAB calcd for $C_{23}H_{33}N_3O_9P$ 526.1954 $(M+H)^+$, found 526.1941.

Diisopropyl-[2-*O*-benzoyl-1-(6-*N*-benzoyladenin-9-yl)-5-*O*-dimethoxytrityl-β-D-ribofuranos-3-*O*-yl]methylphosphonate (**13a**)
Prepared by Method E from diisopropyl-[1-(6-*N*-benzoyladenine-9-yl)-β-D-ribofuranos-3-*O*-yl]methylphosphonate **12a**. Yield 70%. ^{1}H NMR (500 MHz, d$_6$-DMSO): 11.30 bs, 1 H (NH); 8.68 s, 1 H (H-2); 8.65 s, 1 H (H-8); 8.04 m, 4 H, 7.70 t, 1 H, 7.65 t, 1 H, 7.56 t, 4 H, 7.37 d, 2 H, 7.23 m, 7H, 6.83 d, 2 H, 6.82 d, 2 H; 6.51 d, 1 H, *J*(1´,2´) = 3.8 (H-1´); 6.31 dd, 1 H, *J*(2´,1´) = 3.8, *J*(2´,3´) = 5.2 (H-2´); 4.84 dd, 1 H, *J*(3´,2´) = 5.2, *J*(3´,4´) = 6.0 (H-3´); 4.56 m, 2 H (2 x O-CH(CH$_3$)$_2$); 4.40 btd, 1 H, *J*(4´,3´) = 5.7, *J*(4´,5´a) = 3.4, *J*(4´,5´b) = 5.7 (H-4´); 3.90 dd, 1 H, *J*(gem) = 13.9, *J*(Ha,P) = 9.1 (O-CHaHb-P); 3.87 dd, 1 H, *J*(gem) = 13.9, *J*(Hb,P) = 8.9 (O-CHaHb-P); 3.71 s, 6 H (2 x OCH$_3$); 3.41 dd, 1 H, *J*(5´a,5´b) = 10.9, *J*(5´a,4´) = 3.4 (H-5´a); 3.37 dd, 1 H, *J*(5´b,5´a) = 10.9, *J*(5´b,4´) = 5.4 (H-5´b); 1.07 d and 1.05 d, 2 x 6 H, *J*(vic) = 6.1 (2 x O-CH(CH$_3$)$_2$). HR FAB calcd for $C_{52}H_{55}N_5O_{11}P$ 956.3636 $(M+H)^+$, found 956.3645.

Diisopropyl-[2-*O*-benzoyl-1-(2-*N*-isobutyrylguanin-9-yl)-5-*O*-dimethoxytrityl-β-D-ribofuranos-3-*O*-yl]methylphosphonate (**13b**)
Prepared by Method E from diisopropyl-[1-(2-*N*-isobutyrylguanine-9-yl)-β-D-ribofuranos-3-*O*-yl]methylphosphonate **12b**. Yield 63%. ^{1}H NMR (500 MHz, d$_6$-DMSO): 12.05 bs, 1 H (NH); 11.52 bs, 1 H (NH); 8.19 s, 1 H (H-8); 8.05 m, 2 H, 7.60 m, 5 H, 7.25 m, 7 H, 6.80 d, 4 H; 6.28 d, 1 H, *J*(1´,2´) = 3.7 (H-1´); 6.10 dd, 1 H, *J*(2´,1´) = 3.7, *J*(2´,3´) = 5.0 (H-2´); 4.99 dd, 1 H, *J*(3´,2´) = 5.0, *J*(3´,4´) = 6.1 (H-3´); 4.49 m, 1 H and 4.42 m, 1 H (2 x O-CH(CH$_3$)$_2$); 4.31 dt, 1H, *J*(4´,3´) = 6.1, *J*(4´,5´a) = 4.5, *J*(4´,5´b) = 4.5 (H-4´); 3.91 dd, 1 H, *J*(gem) = 14.0, *J*(Ha,P) = 7.9 (O-CHaHb-P); 3.84 dd, 1 H, *J*(gem) = 14.0, *J*(Hb,P) = 8.7 (O-CHaHb-P); 3.72 s, 6 H (2 x OCH$_3$); 3.37 d, 2 H, *J*(5´,4´) = 4.5 (H-5´); 2.73 septet, 1 H, *J*(CH,CH$_3$) = 6.8, (CH); 1.13 d and 1.12 d, 2 x 3 H, *J*(CH$_3$,CH) = 6.8, (CH$_3$); 1.11 d, 1.09 d, 1.07 d and 0.98 d, 4 x 3 H, *J*(vic) = 6.1 (2 x O-CH(CH$_3$)$_2$). HR FAB calcd for $C_{49}H_{57}N_5O_{12}P$ 938.3741 $(M+H)^+$, found 938.3761.

Diisopropyl-[2-*O*-benzoyl-1-(uracil-1-yl)-5-*O*-dimethoxytrityl-β-D-ribofuranos-3-*O*-yl]methylphosphonate (**13c**)
Prepared by Method E from diisopropyl-[1-(uracil-1-yl)-β-D-ribofuranos-3-*O*-yl]methylphosphonate **12c**. Yield 76%. ^{1}H NMR (400 MHz, $CDCl_3$): 11.40 s, 1 H (NH); 7.68 d, 1 H (H-6); 5.31 d, 1 H, *J*(5,6) = 8.1 (H-5); 8.08 m, 2 H, 7.37 d, 2 H, 7.32 t, 2H, 7.24 m, 4 H, 6.90 d, 4 H, 6.81 m, 2 H, 6.78 m, 2 H; 5.79 d, 1 H, *J*(1´,2´) = 4.4 (H-1´); 4.33 td, 1 H, *J*(2´,1´) = 4.4, *J*(2´,3´) = 5.5 (H-2´); 4.56 m, 2 H (2 x O-C<u>H</u>$(CH_3)_2$); 4.09 bt, 1 H, *J*(3´,4´) = 5.5, *J*(3´,2´) = 5.5 (H-3´); 4.07 m, 1 H (H-4´); 4.01 dd, 1 H, *J*(gem) = 14.0, *J*(Ha,P) = 7.9 (O-C<u>Ha</u>Hb-P); 3.84 dd, 1 H, *J*(gem) = 14.0, *J*(Hb,P) = 8.6 (O-CHa<u>Hb</u>-P); 3.74 s, 6 H (2 x OCH_3); 3.29 dd, 1 H, *J*(5´a,5´b) = 10.6, *J*(5´a,4´) = 4.3 (H-5´a); 3.25 dd, 1 H, *J*(5´b,5´a) = 10.6, *J*(5´b,4´) = 2.6 (H-5´b); 1.21 d, 1.19 d, 1.18 d and 1.15 d, 4 x 3 H, *J*(vic) = 6.2 (2 x O-CH(C<u>H$_3$</u>)$_2$). HR FAB calcd for $C_{44}H_{50}N_2O_{12}P$ 829.3101 $(M+H)^+$, found 829.3105.

Diisopropyl-[2-*O*-benzoyl-1-(4-*N*-benzoylcytosin-1-yl)-5-*O*-dimethoxytrityl-β-D-ribofuranos-3-*O*-yl]methylphosphonate (**13d**)
Prepared by Method E from diisopropyl-[1-(4-*N*-benzoylcytosine-1-yl)-β-D-ribofuranos-3-*O*-yl]methylphosphonate **12d**. Yield 79%. ^{1}H NMR (500 MHz, d_6-DMSO): 11.38 bs, 1 H (NH); 8.31 bd, 1 H, *J*(6,5) = 7.6 (H-6); 7.20 bd, 1 H (H-5); 8.07 m, 2 H, 8.02 d, 2 H, 7.70 t, 1 H, 7.69 t, 1 H, 7.58 t, 2 H, 7.50 t, 2 H, 7.44 d, 2 H, 7.36 t, 2 H, 7.32 d, 4 H, 7.25 t, 1 H, 6.93 d, 4 H; 6.09 d, 1 H, *J*(1´,2´) = 2.0 (H-1´); 5.85 dd, 1 H, *J*(2´,3´) = 4.9, *J*(2´,1´) = 2.0 (H-2´); 4.58 dd, 1 H, *J*(3´,4´) = 8.2, *J*(3´,2´) = 4.9 (H-3´); 4.42 m, 2 H (2 x O-C<u>H</u>$(CH_3)_2$); 4.35 ddd, 1 H, *J*(4´,3´) = 8.2, *J*(4´,5´a) = 3.0, *J*(4´,5´b) = 4.0 (H-4´); 3.85 dd, 1 H, *J*(gem) = 13.6, *J*(Ha,P) = 9.1 (O-C<u>Ha</u>Hb-P); 3.80 dd, 1 H, *J*(gem) = 13.6, *J*(Hb,P) = 9.4 (O-CHa<u>Hb</u>-P); 3.76 s, 6 H (2 x OCH_3); 3.47 dd, 1 H, *J*(gem) = 11.2, *J*(5´a,4´) = 3.0 (H-5´a); 3.35 dd, 1 H, *J*(gem) = 11.2, *J*(5´b,4´) = 4.0 (H-5´b); 1.06 d, 6 H, 1.02 d, 3 H, 0.95 d, 3 H, *J*(vic) = 6.2 (2 x O-CH(C<u>H$_3$</u>)$_2$). HR FAB calcd for $C_{51}H_{55}N_3O_{12}P$ 932.3523 $(M+H)^+$, found 932.3450.

4-Methoxy-1-oxido-2-pyridylmethyl-[2-*O*-benzoyl-1-(6-*N*-benzoyladenin-9-yl)-5-*O*-dimethoxytrityl-β-D-ribofuranos-3-*O*-yl]methylphosphonate (**14a**)
Prepared by Method F from diisopropyl-[2-*O*-benzoyl-1-(6-*N*-benzoyladenine-9-yl)-5-*O*-dimethoxytrityl-β-D-ribofuranos-3-*O*-yl]methylphosphonate **13a**. Yield 62%. ^{1}H NMR (500 MHz, d_6-DMSO): 11.25 bs, 1 H (NH); 10.40 bs, 1 H (P-OH); 8.68 s, 1 H (H-2); 8.67 s, 1 H (H-8); 8.04 d, 2 H, 7.96 d, 2 H, 7.64 t, 1 H, 7.60 t, 1 H, 7.55 t, 2 H, 7.43 t, 2 H, 7.34 d, 2 H, 7.22 t, 2 H, 7.21 d, 2 H, 7.16 t, 1 H, 6.81 d, 2 H, 6.79 d, 2 H; 8.09 d, 1 H, 6.97 d, 1 H, 6.90 dd, 1 H; 6.45 d, 1 H, *J*(1´,2´) = 4.0 (H-1´); 5.33 bt, 1 H (H-2´); 4.89 bt, 1 H (H-3´); 4.79 d, 2 H, *J*(H,P) = 8.0 ($POCH_2$); 4.39 btd, 1H, *J*(4´,3´) = 5.1, *J*(4´,5´a) = 5.1, *J*(4´,5´b) = 3.7 (H-4´); 3.72 s, 3 H (OCH_3); 3.69 s, 6 H (2 x OCH_3); 3.63 dd, 1 H, *J*(gem) = 12.9, *J*(Ha,P) = 8.4 (O-C<u>Ha</u>Hb-P); 3.58 dd, 1 H, *J*(gem) = 12.9, *J*(Hb,P) = 8.4 (O-CHa<u>Hb</u>-P); 3.39 dd, 1 H, *J*(5´a,5´b) = 10.5, *J*(5´a,4´) = 5.0 (H-5´a); 3.35 dd, 1 H, *J*(5´b,5´a) = 10.5, *J*(5´b,4´) = 3.7 (H-5´b). ^{13}C NMR (d_6-DMSO): 166.12, 165.22, 140.66, 133.87, 129.31 (2), 129.08, 128.89 (2), 128.12 (2), 127.86 (2) and 126.89 (N-CO-C_6H_5 and O-CO-C_6H_5); 158.32 (2), 135.94, 135.89, 129.40 (4), 113.20 (4), 86.17, 55.37 and 55.32 (DMTr); 158.95, 151.08 d, *J*(C,P) = 3.0, 140.23, 111.12, 108.61, 61.20 d, *J*(C,P) = 3.0 and 56.56 (MOP); 152.24 (C-6); 152.11 (C-2); 150.75 (C-4); 143.72 (C-8); 126.08 (C-5); 86.76 (C-1´); 83.81 (C-4´); 78.04 d, *J*(C,P) = 8.3 (C-3´); 74.98 (C-2´); 66.80 (C-5´); 66.45 d, *J*(C,P) = 153.0 (OCH_2P). ^{31}P{^{1}H} NMR (202.3 MHz, d_6-DMSO): 13.31. HR FAB calcd for $C_{53}H_{50}N_6O_{13}P$ 1009.3174 $(M+H)^+$, found 1009.3171.

4-Methoxy-1-oxido-2-pyridylmethyl-[2-*O*-benzoyl-1-(2-*N*-isobutyrylguanin-9-yl)-5-*O*-dimethoxytrityl-β-D-ribofuranos-3-*O*-yl]methylphosphonate (**14b**)
Prepared by Method F from diisopropyl-[2-*O*-benzoyl-1-(2-*N*-isobutyrylguanine-9-yl)-5-*O*-dimethoxytrityl-β-D-ribofuranos-3-*O*-yl]methylphosphonate **13b**. Yield 46%. ^{1}H NMR (500 MHz, d_6-DMSO): 13.20 bs, 1 H (NH); 11.90 bs, 1 H (NH); 8.11 s, 1 H (H-8); 8.08 d, 1 H, *J* = 7.2, 7.00 d, 1 H, *J* = 3.5, 6.89 dd, 1 H, *J* = 7.2, *J* = 3.5 (MOP); 8.01 d, 2 H, 7.68 t, 1 H, 7.54 t, 2 H, 7.24 d, 2 H, 7.13 d, 4 H, 7.10 t, 2 H, 7.09 t, 1 H, 6.70 d, 2 H, 6.68 d, 2 H; 6.26 d, 1 H, *J*(1´,2´) = 1.0 (H-1´); 6.19 bdd, 1 H, *J*(3´,2´) = 5.2, *J*(3´,4´) = 8.2 (H-3´); 6.03 dd, 1 H, *J*(2´,1´) = 1.0, *J*(2´,3´) = 5.2 (H-2´); 4.78 dd, 1 H and 4.73 dd, 1 H, *J*(H,P) = 8.9, *J*(gem) = 17.6 ($POCH_2$); 4.39 ddd, 1H, *J*(4´,3´) = 8.2, *J*(4´,5´a) = 2.4, *J*(4´,5´b) = 6.8 (H-4´); 3.69 m, 2 H (O-<u>CHaHb</u>-P); 3.66 s, 3 H (OCH_3); 3.65 s, 6 H (2 x OCH_3); 3.48 dd, 1 H, *J*(5´a,5´b) = 10.6, *J*(5´a,4´) = 2.4 (H-5´a); 3.32 dd, 1 H, *J*(5´b,5´a) = 10.6, *J*(5´b,4´) = 6.8 (H-5´b); 2.73 septet, 1 H, *J*(CH,CH_3) = 6.8, (CH); 1.07 d and 1.04 d, 2 x 3 H, *J*(CH_3,CH) = 6.8, (CH_3). ^{13}C NMR (d_6-DMSO): 165.02, 133.90, 129.67 (2), 129.34, 127.95 (2) (O-CO-C_6H_5); 158.05, 158.03, 144.98, 135.72, 135.65, 129.86 (4), 128.99 (2), 127.66 (2), 126.58, 113.00 (4), 85.56 and 55.04 (2) (DMTr); 156.61, 150.68 d, *J*(C,P) = 4.9, 139.40, 110.23, 108.78, 60.92 d, *J*(C,P) = 4.5 and 56.04 (MOP); 155.14 (C-6); 148.30 (C-2); 147.99 (C-4); 140.40 (C-8); 120.66 (C-5); 87.58 (C-1´); 81.14 (C-4´); 75.27 d, *J*(C,P) = 3.0 (C-3´); 74.68 (C-2´); 63.85 (C-5´); 65.99 d, *J*(C,P) = 154.3 (OCH_2P); 180.57, 34.93, 19.03 and 18.79 (iBu). ^{31}P{^{1}H} NMR (202.3 MHz, d_6-DMSO): 12.21. HR FAB calcd for $C_{50}H_{51}N_6O_{14}PNa$ 1013.3099 $(M+H)^+$, found 1013.3089.

4-Methoxy-1-oxido-2-pyridylmethyl-[2-*O*-benzoyl-1-(uracil-1-yl)-5-*O*-dimethoxytrityl-β-D-ribofuranos-3-*O*-yl]methylphosphonate (**14c**)
Prepared by Method F from diisopropyl-[2-*O*-benzoyl-1-(uracil-1-yl)-5-*O*-dimethoxytrityl-β-D-ribofuranos-3-*O*-

yl]methylphosphonate **13c**. Yield 65%. ^{1}H NMR (500 MHz, d_6-DMSO): 11.42 d, 1 H, *J*(NH,5) = 2.2 (NH); 8.10 d, 1 H, J(6,5) = 7.2 (H-6 (MOP)); 7.97 m, 2H, 7.59 m, 1 H and 7.43 m, 2 H (C_6H_5 (Bz)); 7.73 d, 1 H, *J*(6,5) = 8.1 (H-6); 7.40 m, 2 H, 7.31 m, 2 H and 7.21 m, 1 H (C_6H_5 (DMTr); 7.27 m, 2 H, 7.26 m, 2 H and 6.89 m, 4 H (2x C_6H_4 (DMTr)); 6.96 bd, 1 H, *J*(3,5) = 3.6 (H-3 (MOP)); 6.91 dd, 1 H, *J*(5,3) = 3.6, *J*(5,6) = 7.2 (H-5 (MOP)); 6.02 d, 1 H, *J*(1´,2´) = 4.3 (H-1´); 5.72 dd, 1 H, *J*(2´,1´) = 4.3, *J*(2´,3´) = 5.1 (H-2´); 5.35 ddm 1 H, *J*(5,6) = 8.1, *J*(5,NH) = 2.2 (H-5); 4.78 bd, 2 H, *J*(H,P) = 8.2 (PO-CH_2); 4.58 dd, 1 H, *J*(3´,2´) = 5.1, *J*(3´,4´) = 5.8 (H-3´); 4.25 m, 1H, *J*(4´,3´) = 5.8, *J*(4´,5´a) = 4.2, *J*(4´,5´b) = 2.6 (H-4´); 3.72 s, 6 H (2x OMe); 3.58 dd, 1 H and 3.78 dd, 1 H, *J*(gem) = 13.0, *J*(Ha,P) = *J*(Hb,P) = 8.5 (O-CHaHb-P); 3.42 dd, 1 H, *J*(5´a,5´b) = 10.8, *J*(5´a,4´) = 4.2 (H-5´a); 3.30 dd, 1 H, *J*(5´b,5´a) = 10.8, *J*(5´b,4´) = 2.6 (H-5´b). ^{13}C NMR (d_6-DMSO): 165.01 (C=O (Bz); 163.22 (C-4 (U)); 158.32, 158.29, 135.46, 135.25, 130.02(2), 129.95(2), 113.45(2) and 113.49(2) (2x C_6H_5 (DMTr)); 157.01, 150.47, 139.48, 110.41 and 108.78 (MOP); 144.81, 128.12(2), 127.94(2) and 126.93 (C_6H_5 (DMTr)); 140.73 (C-6 (U)); 133.75, 129.65(2), 129.03 and 128.79(2) (C_6H_5 (Bz)); 101.91 (C-5 (U)); 87.44 (C-1´); 86.31 (>C< (DMTr)); 81.54 (C-4´); 78.01 (C-3´); 74.31 (C-2´); 67.32 d, *J*(C,P) = 157 (OCH_2P); 62.99 (C-5´); 60.90 (CH_2-O-P); 55.20 and 55.19 (2x OMe). ^{31}P{^{1}H} NMR (202.3 MHz, d_6-DMSO): 13.76. HR FAB calcd for $C_{45}H_{44}N_3O_{14}$PNa 904.2459 $(M+H)^+$, found 904.2404.

4-Methoxy-1-oxido-2-pyridylmethyl-[2-*O*-benzoyl-1-(4-*N*-benzoylcytosin-1-yl)-5-*O*-dimethoxytrityl-β-D-ribofuranos-3-*O*-yl]methylphosphonate (**14d**)
Prepared by Method F from diisopropyl-[2-*O*-benzoyl-1-(4-*N*-benzoylcytosine-1-yl)-5-*O*-dimethoxytrityl-β-D-ribofuranos-3-*O*-yl]methylphosphonate **13d**. Yield 72%. ^{1}H NMR (500 MHz, d_6-DMSO): 11.40 s, 1 H (NH); 11.00 bs, 1 H (P-OH); 8.32 d, 1 H, *J*(6,5) = 7.6 (H-6); 7.97 d, 1 H (H-5); 8.01 d, 2 H, 7.63 t, 2 H, 7.58 t, 1 H, 7.53 t, 2 H, 7.25 m, 3 H, 7.34 t, 2 H, 7.32 m, 6 H, 7.21 t, 2 H, 7.21 m, 1 H, 7.07 m, 1 H, 6.90 m, 5 H; 6.06 d, 1 H, *J*(1´,2´) = 2.0 (H-1´); 5.83 dd, 1 H, *J*(2´,3´) = 4.8, *J*(2´,1´) = 2.0 (H-2´); 4.73 d, 2 H, *J*(H,P) = 7.6 ($POCH_2$); 4.68 dd, 1 H, *J*(3´,4´) = 7.2, *J*(3´,2´) = 4.8 (H-3´); 4.33 dt, 1H, *J*(4´,3´) = 7.2, *J*(4´,5´a) = 3.3, *J*(4´,5´b) = 3.3 (H-4´); 3.72 s and 3.70, 2 x 3 H (2 x OCH_3); 3.70 m, 2 H (O-CHaHb-P); 3.56 s, 3 H (OCH_3); 3.30 m, 2 H (H-5´a, H-5´b). ^{13}C NMR (d_6-DMSO): 167.64, 164.61, 133.66, 133.31, 132.96, 129.14, 129,62 (2), 128.65 (2), 128.74 (2), 128.65 (2) (N-CO-C_6H_5 and O-CO-C_6H_5); 158.29 (2), 144.58, 135.64, 135.35, 129.98 (2), 129.91 (2), 128.17 (2), 128.02 (2), 126.94, 113.51 (4), 86.31, 55.13 (2) (DMTr); 156.51, 150.72 d, *J*(C,P) = 5.4, 139.31, 110.13, 108.49, 60.88 d, *J*(C,P) = 4.5, 56.04 (MOP); 163.47 (C-4); 154.32 (C-2); 144.93 (C-6); 96.51 (C-5); 89.35 (C-1´); 81.27 (C-4´); 77.00 d, *J*(C,P) = 12.2 (C-3´); 74.34 (C-2´); 62.16 (C-5´); 67.65 d, *J*(C,P) = 153.8 (OCH_2P). ^{31}P{^{1}H} NMR (202.3 MHz, d_6-DMSO): 12.62. HR FAB calcd for $C_{52}H_{49}N_4O_{14}$PNa 1007.2881 $(M+H)^+$, found 1007.2829.

Diisopropyl-[1-(6-*N*-benzoyladenin-9-yl)-β-D-ribofuranos-5-*O*-yl]methylphosphonate (**16a**)
Prepared by Method D from 6-*N*-benzoyl-9-(2,3-*O*- ethoxymethylidene-β-D-ribofuranosyl)adenine **15a**. Yield 64%. ^{1}H NMR (500 MHz, d_6-DMSO): 11.25bs, 1 H (NH); 8.77s, 1 H (H-2); 8.71, 1 H (H-8); 8.05 d, 2H, 7.65t, 1H a 7.55 t, 2H (C_6H_5); 6.07 d, 1 H, *J*(1´,2´) = 5.9 (H-1´); 5.60bs, 1 H (2´-OH); 5.38 bs, 1 H (3´-OH); 4.66 bt, 1 H (H-2´); 4.60 m, 2 H (2 x O-C<u>H</u>$(CH_3)_2$); 4.18 bt, 1 H (H-3´); 4.01 bq, 1H, *J*(4´,3´) = 3.7, *J*(4´,5´a) = 3.7, *J*(4´,5´b) = 4.6 (H-4´); 3.82 d, 2 H, *J*(Ha,P) = *J*(Hb,P) = 8.3 (O-CH_2-P); 3.79 dd, 1 H, *J*(5´a,5´b) = 10.0, *J*(5´a,4´) = 3.4 (H-5´a); 3.74 dd, 1 H, *J*(5´b,5´a) = 10.0, *J*(5´b,4´) = 4.6 (H-5´b); 1.24 d, 1.23 d, 1.22 d a 1.21 d, 4 x 3 H, *J*(vic) = 6.1 (2 x O-CH(C<u>H</u>$_3$)$_2$). HR FAB calcd for $C_{24}H_{33}N_5O_8$P $(M+H)^+$ 550.2067, found 550,2049.

Diisopropyl-[1-(2-*N*-isobutyrylguanin-9-yl)-β-D-ribofuranos-5-*O*-yl]methylphosphonate (**16b**)
Prepared by Method D from 2-*N*-isobutyryl-9-(2,3-*O*-ethoxymethylidene-β-D-ribofuranosyl)guanine **15b**. Yield 71%. ^{1}H NMR (500 MHz, d_6-DMSO): 12.10 bs, 1 H (NH); 11.65 bs, 1 H (NH); 8.20 s, 1 H (H-8); 5.82 d, 1 H, *J*(1´,2´) = 6.2 (H-1´); 5.55 d, 1 H, *J*(OH,2´) = 6.0 (2´-OH); 5.28 d, 1 H, *J*(OH,3´) = 4.4 (3´-OH); 4.51 bq, 1 H, *J*(2´,1´) = 6.1, *J*(2´,3´) = 4.8, *J*(2´,OH) = 6.1 (H-2´); 4.11 td, 1 H, *J*(3´,2´) = 4.5, *J*(3´,4´) = 3.3, *J*(3´,OH) = 4.5 (H-3´); 4.03 bq, 1H, *J*(4´,3´) = 3.3, *J*(4´,5´a) = 3.5, *J*(4´,5´b) = 3.7 (H-4´); 3.79 dd, 1 H, *J*(5´a,5´b) = 10.7, *J*(5´a,4´) = 3.5 (H-5´a); 3.83 dd, 1 H, *J*(gem) = 13.8, *J*(Ha,P) = 8.3 (O-C<u>H</u>aHb-P); 3.80 dd, 1 H, *J*(gem) = 13.8, *J*(Hb,P) = 8.3 (O-CHa<u>H</u>b-P); 3.68 dd, 1 H, *J*(5´b,5´a) = 10.7, *J*(5´b,4´) = 4.3 (H-5´b); 2.78 septet, 1 H, *J*(CH,CH_3) = 6.8 (CH); 1.27 d, 1.25 d, 1.24 d a 1.23 d, 4 x 3 H, *J*(vic) = 6.1 (2 x O-CH(C<u>H</u>$_3$)$_2$); 1.13 d a 1.12 d, 2 x 3 H, *J*(CH_3,CH) = 6.8 (CH_3). HR FAB calcd for $C_{21}H_{35}N_5O_9$P $(M+H)^+$ 532.2172, found 532.2146.

Diisopropyl-[1-(uracil-1-yl)-β-D-ribofuranos-5-*O*-yl]methylphosphonate (**16c**)
Prepared by Method D from 1-(2,3-*O*-ethoxymethylidene-β-D-ribofuranosyl)uracil **15c**. Yield 79%. ^{1}H NMR (500 MHz, $CDCl_3$): 11.30 bs, 1 H (NH); 7.83 d, 1 H, *J*(6,5) = 8.2 (H-6); 5.61 d, 1 H, *J*(5,6) = 8.2 (H-5); 5.79 d, 1 H, *J*(1´,2´) = 5.4 (H-1´); 5.45 bs, 2 H (OH); 4.64 m, 2 H (2 x O-C<u>H</u>$(CH_3)_2$); 4.02 dd, 1 H, *J*(2´,1´) = 5.4, *J*(2´,3´) = 4.6 (H-2´); 3.95 m, 2 H (H-3´, H-4´); 3.82 d, 1 H, *J*(H,P) = 8.7 (O-CH_2-P); 3.74 dd, 1 H, *J*(5´a,5´b) = 10.8, *J*(5´a,4´) = 2.4 (H-5´a); 3.67 dd, 1 H, *J*(5´b,5´a) = 10.8, *J*(5´b,4´) = 3.0 (H-5´b); 1.26 d a 1.25 d, 2 x 3 H, 1.24 d, 6 H, *J*(vic) = 6.2 (2 x O-CH(C<u>H</u>$_3$)$_2$). HR FAB calcd for $C_{16}H_{28}N_2O_9$P $(M+H)^+$ 423.1532, found 423.1542.

Diisopropyl-[1-(4-*N*-benzoylcytosin-1-yl)-β-D-ribofuranos-5-*O*-yl]methylphosphonate (**16d**)

Prepared by Method D from 4-*N*-benzoyl-1-(2,3-*O*-ethoxymethylidene-β-D-ribofuranosyl)cytosine **15d**. Yield 66%. ^{1}H NMR (500 MHz, d_6-DMSO): 11.30 bs, 1 H (NH); 8.38 d, 1 H, (H-6); 7.44 d, 1 H, *J*(5,6) = 7.4 (H-5); 8.02 d, 2H, 7.61 t, 1 H, 7.50 t, 2 H; 5.86 d, 1 H, *J*(1´,2´) = 3.1 (H-1´); 5.80 bs, 2 H (OH); 4.64 m, 2 H (2 x O-C<u>H</u>$(CH_3)_2$); 4.05 bdt, 1 H, *J*(4´,3´) = 6.3, *J*(4´,5´a) = 2.6, *J*(4´,5´b) = 2.6 (H-4´); 4.02 dd, 1 H, *J*(2´,1´) = 3.1, *J*(2´,3´) = 4.8 (H-2´); 3.98 dd, 1 H, *J*(3´,2´) = 4.8, *J*(3´,4´) = 6.3 (H-3´); 3.88 dd, 1 H, *J*(5´a,5´b) = 11.0, *J*(5´a,4´) = 2.4 (H-5´a); 3.87 d, 2 H, *J*(Ha,P) = 8.7 (O-CH_2-P); 3.74 dd, 1 H, *J*(5´b,5´a) = 11.0, *J*(5´b,4´) = 2.9 (H-5´b); 1.28 d a 1.26 d, 2 x 3 H, 1.25 d, 6 H, *J*(vic) = 6.2 (2 x O-CH(C<u>H</u>$_3$)$_2$). HR FAB calcd for $C_{23}H_{33}N_3O_9P$ $(M+H)^+$ 526.1954, found 526.1961.

Diisopropyl-[1-(6-*N*-benzoyladenin-9-yl)-2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-β-D-ribofuranos-5-*O*-yl]methylphosphonate (**17a**) and
diisopropyl-[1-(6-*N*-benzoyladenin-9-yl)-3-*O*-benzoyloxymethoxymethyl-2-*O*-dimethoxytrityl-β-D-ribofuranos-5-*O*-yl]methylphosphonate (**18a**)
Prepared by Method G from diisopropyl-[1-(6-*N*-benzoyladenin-9-yl)-β-D-ribofuranos-5-*O*-yl]methylphosphonate **16a**. Yield 24% of reverse phase faster eluted isomer **18a** and 36% of reverse phase slower eluted isomer **17a**.
18a: ^{1}H NMR (500 MHz, d_6-DMSO): 11.25 bs, 1 H (NH); 8.66 s, 1 H (H-2); 8.67 s, 1 H (H-8); 8.05 d, 2 H, 8.01 d, 2 H, 7.69 t, 1 H, 7.65 t, 1 H, 7.57 t, 2 H, 7.56 t, 2 H, 7.24 – 7.10 m, 5 H, 7.10 d, 2 H, 6.98 d, 2 H, 6.77 d, 2 H, 6.64 d, 2 H; 6.26 d, 1 H, *J*(1´,2´) = 7.4 (H-1´); 5.54 s, 2 H (OCH_2O); 5.06 dd, 1 H, *J*(2´,1´) = 7.4, *J*(2´,3´) = 4.5 (H-2´); 4.99 d, 1 H a 4.38 d, 1 H, *J* = 6.8 (OCH_2O); 4.70 dd, 1 H, *J*(gem) = 14.2, *J*(Ha,P) = 8.2 (O-C<u>Ha</u>Hb-P); 4.69 dd, 1 H, *J*(gem) = 14.2, *J*(Hb,P) = 8.2 (O-CHa<u>Hb</u>-P); 4.51 m, 2 H (2 x O-C<u>H</u>$(CH_3)_2$); 4.25 bt, 1 H, *J*(3´,2´) = 4.5, *J*(3´,4´) = 4.5 (H-3´); 3.70 s a 3.66 s, 2 x 3 H (2 x OCH_3); 3.72 bq, 1 H, *J*(4´,3´) = 4.5, *J*(4´,5´a) = 4.5, *J*(4´,5´b) = 4.5 (H-4´); 3.61 dd, 1 H, *J*(5´a,5´b) = 10.8, *J*(5´a,4´) = 4.4 (H-5´a); 3.56 dd, 1 H, *J*(5´b,5´a) = 10.8, *J*(5´b,4´) = 4.7 (H-5´b); 1.18 d, 1.16 d, 1.13 d a 1.10 d, 4 x 3 H, *J*(vic) = 6.2 (2 x O-CH(C<u>H</u>$_3$)$_2$). HR FAB calcd for $C_{54}H_{59}N_5O_{13}P$ $(M+H)^+$ 1016.3847, found 1016.3805.
17a: ^{1}H NMR (500MHz, d_6-DMSO): 11.25 bs, 1 H (NH); 8.73 s, 1 H (H-2); 8.67 s, 1 H (H-8); 8.05 d, 2 H, 7.92 d, 2 H, 7.67 t, 1 H, 7.64 t, 1 H, 7.53 m, 6 H, 7.36 m, 6 H, 7.25 t, 1 H, 6.92 m, 4 H; 6.43 d, 1 H, *J*(1´,2´) = 6.5 (H-1´); 5.20 d, 1 H a 5.08 d, 1 H, *J* = 6.4 (OCH_2O); 5.00 d, 1 H a 4.67 d, 1 H, *J* = 6.8 (OCH_2O); 4.56 dd, 1 H, *J*(2´,1´) = 6.5, *J*(2´,3´) = 4.9 (H-2´); 4.50 m, 2 H (2 x O-C<u>H</u>$(CH_3)_2$); 4.36 dd, 1 H, *J*(3´,2´) = 4.9, *J*(3´,4´) = 2.2 (H-3´); 3.72 m, 1 H (H-4´); 3.74 s a 3.72 s, 2 x 3 H (2 x OCH_3); 3.69 dd, 1 H, *J*(gem) = 13.8, *J*(Ha,P) = 8.4 (O-C<u>Ha</u>Hb-P); 3.59 dd, 1 H, *J*(gem) = 13.8, *J*(Hb,P) = 8.4 (O-CHa<u>Hb</u>-P); 3.36 dd, 1 H, *J*(5´a,5´b) = 11.2, *J*(5´a,4´) = 5.4 (H-5´a); 3.33 dd, 1 H, *J*(5´b,5´a) = 11.2, *J*(5´b,4´) = 5.7 (H-5´b); 1.17 d, 1.15 d, 1.11 d a 1.08 d, 4 x 3 H, *J*(vic) = 6.2 (2 x O-CH(C<u>H</u>$_3$)$_2$). HR FAB calcd for $C_{54}H_{59}N_5O_{13}P$ $(M+H)^+$ 1016.3847, found 1016.3841.

Diisopropyl-[2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-1-(2-*N*-isobutyrylguanin-9-yl)-β-D-ribofuranos-5-*O*-yl]methylphosphonate (**17b**)
Prepared by Method G from diisopropyl-[1-(2-*N*-isobutyrylguanin-9-yl)-β-D-ribofuranos-5-*O*-yl]methylphosphonate **16b**. Yield 59%. ^{1}H NMR (500 MHz, d_6-DMSO): 12.10 bs, 1 H (NH); 11.80 bs, 1 H (NH); 8.20 s, 1 H (H-8); 7.90 d, 2 H, 7.66 t, 1 H, 7.51 m, 4 H, 7.39 t, 2 H, 7.34 d, 4 H, 7.26 t, 1 H, 6.93 d, 4 H; 6.21 bd, 1 H, *J*(1´,2´) = 5.0 (H-1´); 5.16 m, 1 H a 5.06 m, 1 H (OCH_2O); 4.99 d, 1 H a 4.71 d, 1 H, *J* = 6.9 (OCH_2O); 4.77 m, 1 H, 4.27 m, 1 H, 3.08 m, 1 H (H-2´, H-3´, H-4´); 4.54 m, 2 H (2 x O-C<u>H</u>$(CH_3)_2$); 3.74 s a 3.73 s, 2 x 3 H (2 x OCH_3); 3.66 dd, 1 H, *J*(gem) = 13.7, *J*(Ha,P) = 8.7 (O-C<u>Ha</u>Hb-P); 3.62 dd, 1 H, *J*(gem) = 13.7, *J*(Hb,P) = 8.7 (O-CHa<u>Hb</u>-P); 3.28 dd, 1 H, *J*(5´a,5´b) = 10.6, *J*(5´a,4´) = 2.2 (H-5´a); 3.15 dd, 1 H, *J*(5´b,5´a) = 10.6, *J*(5´b,4´) = 3.5 (H-5´b); 2.79 m, 1 H (CH); 1.21 d, 1.17 d, 1.16 d a 1.10 d, 4 x 3 H, *J*(vic) = 6.2 (2 x O-CH(C<u>H</u>$_3$)$_2$); 1.13 d a 1.12 d, 2 x 3 H, *J*(CH_3,CH) = 6.8 (CH_3). HR FAB calcd for $C_{51}H_{61}N_5O_{14}P$ $(M+H)^+$ 998.3953, found 998.3946.

Diisopropyl-[2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-1-(uracil-1-yl)-β-D-ribofuranos-5-*O*-yl]methylphosphonate (**17c**) and
diisopropyl-[3-*O*-benzoyloxymethoxymethyl-2-*O*-dimethoxytrityl-1-(uracil-1-yl)-β-D-ribofuranos-5-*O*-yl]methylphosphonate (**18c**)
Prepared by Method G from diisopropyl-[1-(uracil-1-yl)-β-D-ribofuranos-5-*O*-yl]methylphosphonate **16c**. Yield 26% of reverse phase faster eluted isomer **18c** and 39% of reverse phase slower eluted isomer **17c**.
18c: ^{1}H NMR (500 MHz, d_6-DMSO): 11.50 bs, 1 H (NH); 7.42 d, 1 H, *J*(6,5) = 8.2 (H-6); 5.39 d, 1 H, *J*(5,6) = 8.2 (H-5); 7.99 d, 2 H, 7.68 t, 1 H, 7.55 t, 2 H, 7.32 d, 2 H, 7.25 t, 2 H, 7.20 m, 5 H, 6.85 d, 2 H, 6.80 d, 2 H; 6.22 d, 1 H, *J*(1´,2´) = 7.9 (H-1´); 5.48 s, 2 H (OCH_2O); 4.93 d, 1 H a 4.26 d, 1 H, *J* = 6.8 (OCH_2O); 4.55 m, 2 H (2 x O-C<u>H</u>$(CH_3)_2$); 4.23 dd, 1 H, *J*(2´,1´) = 7.9, *J*(2´,3´) = 4.9 (H-2´); 4.12 bt, 1 H, *J*(4´,3´) = *J*(4´,5´) = 2.6 (H-4´); 3.77 dd, 1 H, *J*(gem) = 14.2, *J*(Ha,P) = 7.3 (O-C<u>Ha</u>Hb-P); 3.73 bd, 1 H, *J*(3´,2´) = 4.9, *J*(3´,4´) < 1.0 (H-3´); 3.71 s a 3.70 s, 2 x 3 H (2 x OCH_3); 3.65 dd, 1 H, *J*(gem) = 14.2, *J*(Hb,P) = 9.0 (O-CHa<u>Hb</u>-P); 3.54 dd, 1 H, *J*(5´a,5´b) = 10.6, *J*(5´a,4´) = 2.8 (H-5´a); 3.37 dd, 1 H, *J*(5´b,5´a) = 10.6, *J*(5´b,4´) = 2.5 (H-5´b); 1.20 d, 1.15 d, 1.14 d a 1.12 d, 4 x 3 H, *J*(vic) = 6.1 (2 x O-CH(C<u>H</u>$_3$)$_2$). HR FAB calcd for $C_{46}H_{53}N_2O_{14}NaP$ $(M+H)^+$ 911.3132, found 911.3167. **17c**: ^{1}H NMR (500 MHz, d_6-DMSO): 11.45 bs, 1 H (NH); 7.80 d, 1 H, *J*(6,5) = 8.2 (H-6); 5.62 d, 1 H, *J*(5,6) = 8.2 (H-5); 7.99 d, 2 H, 7.69 t, 1 H, 7.54 t, 2 H, 7.47 d, 2 H, 7.32 m, 6 H, 7.24 t, 1 H, 6.91 d, 4 H; 6.13 d, 1 H, *J*(1´,2´) = 6.2 (H-1´); 5.45 d, 1 H a

5.43 d, 1 H, *J* = 6.4 (OCH_2O); 5.06 d, 1 H a 4.68 d, 1 H, *J* = 6.8 (OCH_2O); 4.51 m, 2 H (2 x O-C<u>H</u>$(CH_3)_2$); 4.17 dd, 1 H, *J*(3´,2´) = 4.9, *J*(3´,4´) = 2.9 (H-3´); 3.76 dd, 1 H, *J*(2´,1´) = 6.2, *J*(2´,3´) = 4.9 (H-2´); 3.74 s a 3.73 s, 2 x 3 H (2 x OCH_3); 3.67 dd, 1 H, *J*(gem) = 13.7, *J*(Ha,P) = 9.3 (O-C<u>Ha</u>Hb-P); 3.61 dd, 1 H, *J*(gem) = 13.7, *J*(Hb,P) = 8.2 (O-CHa<u>Hb</u>-P); 3.35 dd, 1 H, *J*(5´a,5´b) = 10.6, *J*(5´a,4´) = 2.1 (H-5´a); 3.27 bq, 1 H (H-4´); 3.24 dd, 1 H, *J*(5´b,5´a) = 10.6, *J*(5´b,4´) = 2.8 (H-5´b); 1.18 d, 1.17 d, 1.13 d a 1.11 d, 4 x 3 H, *J*(vic) = 6.1 (2 x O-CH(C<u>H</u>$_3)_2$). HR FAB calcd for $C_{46}H_{53}N_2O_{14}NaP$ $(M+H)^+$ 911.3132, found 911.3109.

Diisopropyl-[1-(4-*N*-benzoylcytosin-1-yl)-2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-β-D-ribofuranos-5-*O*-yl]methylphosphonate (**17d**) and
diisopropyl-[1-(4-*N*-benzoylcytosin-1-yl)-3-*O*-benzoyloxymethoxymethyl-2-*O*-dimethoxytrityl-β-D-ribofuranos-5-*O*-yl]methylphosphonate (**18d**)
Prepared by Method G from diisopropyl-[1-(4-*N*-benzoylcytosin-1-yl)-β-D-ribofuranos-5-*O*-yl]methylphosphonate **16d**. Yield 22% of reverse phase faster eluted isomer **18d** and 35% of reverse phase slower eluted isomer **17d**.
18d: ^{1}H NMR (500 MHz, d_6-DMSO): 11.40 bs, 1 H (NH); 7.57 d, 1 H, (H-6); 7.20 d, 1 H, *J*(5,6) = 7.4 (H-5); 8.04 d, 2 H, 8.00 d, 1 H, 7.69 t, 1 H, 7.64 t, 1 H, 7.56 t, 2 H, 7.54 t, 2 H, 7.32 d, 2 H, 7.25 t, 2 H, 7.19 m, 5 H, 6.84 d, 2 H, 6.76 d, 2 H; 6.48 d, 1 H, *J*(1´,2´) = 7.8 (H-1´); 5.49 s, 2 H (OCH_2O); 4.96 d, 1 H a 4.26 d, 1 H, *J* = 6.8 (OCH_2O); 4.58 m, 2 H (2 x O-C<u>H</u>$(CH_3)_2$); 4.29 dd, 1 H, *J*(2´,1´) = 7.8, *J*(2´,3´) = 4.9 (H-2´); 4.16 bt, 1 H, *J*(4´,5´) = 2.6 (H-4´); 3.83 dd, 1 H, *J*(gem) = 14.2, *J*(Ha,P) = 7.2 (O-C<u>Ha</u>Hb-P); 3.72 bd, 1 H, *J*(3´,2´) = 4.9, *J*(3´,4´) < 1.0 (H-3´); 3.68 dd, 1 H, *J*(gem) = 14.2, *J*(Hb,P) = 9.0 (O-CHa<u>Hb</u>-P); 3.68 s a 3.63 s, 2 x 3 H (2 x OCH_3); 3.60 dd, 1 H, *J*(5´a,5´b) = 10.6, *J*(5´a,4´) = 2.1 (H-5´a); 3.36 dd, 1 H, *J*(5´b,5´a) = 10.6, *J*(5´b,4´) = 2.9 (H-5´b); 1.22 d, 3 H, 1.17 d, 6 H a 1.14 d, 3 H, *J*(vic) = 6.1 (2 x O-CH(C<u>H</u>$_3)_2$). HR FAB calcd for $C_{53}H_{58}N_3O_{14}NaP$ $(M+H)^+$ 1014.3554, found 1014.3515.
17d: ^{1}H NMR (500 MHz, d_6-DMSO): 11.30 bs, 1 H (NH); 8.31 bd, 1 H, (H-6); 7.46 bd, 1 H, *J*(5,6) = 7.4 (H-5); 8.00 m, 4 H, 7.68 t, 1 H, 7.62 t, 1 H, 7.52 m, 4 H, 7.45 d, 2 H, 7.35 - 7.20 m, 8 H, 6.88 d, 2 H, 6.86 d, 2 H; 6.06 d, 1 H, *J*(1´,2´) = 3.8 (H-1´); 5.48 d, 1 H a 5.46 d, 1 H, *J* = 6.4 (OCH_2O); 5.00 d, 1 H a 4.74 d, 1 H, *J* = 6.7 (OCH_2O); 4.57 m, 1 H a 4.51 m, 1 H (2 x O-C<u>H</u>$(CH_3)_2$); 4.17 m, 1 H (H-4´); 4.11 dd, 1 H, *J*(3´,2´) = 4.4, *J*(3´,4´) = 5.6 (H-3´); 3.73 s a 3.72 s, 2 x 3 H (2 x OCH_3); 3.71 dd, 1 H, *J*(gem) = 13.6, *J*(Ha,P) = 9.0 (O-C<u>Ha</u>Hb-P); 3.63 dd, 1 H, *J*(gem) = 13.6, *J*(Hb,P) = 8.0 (O-CHa<u>Hb</u>-P); 3.62 dd, 1 H, *J*(5´a,5´b) = 10.6, *J*(5´a,4´) = 2.5 (H-5´a); 3.49 dd, 1 H, *J*(5´b,5´a) = 10.6, *J*(5´b,4´) = 2.0 (H-5´b); 3.12 bt, 1 H, *J*(2´,1´) = *J*(2´,3´) = 4.0 (H-2´); 1.21 d, 1.20 d, 1.13 d a 1.12 d, 4 x 3 H, *J*(vic) = 6.1 (2 x O-CH(C<u>H</u>$_3)_2$). HR FAB calcd for $C_{53}H_{58}N_3O_{14}NaP$ $(M+H)^+$ 1014.3554, found 1014.3570.

4-Methoxy-1-oxido-2-pyridylmethyl-[1-(6-*N*-benzoyladenin-9-yl)-2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-β-D-ribofuranos-5-*O*-yl]methylphosphonate (**19a**)
Prepared by Method F from diisopropyl-[1-(6-*N*-benzoyladenin-9-yl)-2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-β-D-ribofuranos-5-*O*-yl]methylphosphonate **17a**. Yield 62%. ^{1}H NMR (500 MHz, d_6-DMSO): 11.19 bs, 1 H (NH); 10.62 bs, 1 H (P-OH); 9.16 s, 1 H (H-2); 8.72 s, 1 H (H-8); 8.05 – 6.80 m, 26 H; 6.42 d, 1 H, *J*(1´,2´) = 5.0 (H-1´); 5.13 d, 5.08 d, 4.96 d a 4.80 d, 4 x 1 H, *J* = 6.4 (2 x OCH_2O); 4.84 d, 2 H, *J*(H,P) = 8.6 ($POCH_2$); 4.77 m, 1 H (H-2´); 4.30 m, 1 H (H-3´); 3.71 m, 5 H (H-4´, H-5´, PCH_2); 3.70 s, 3 H a 3.57 s, 6 H (3 x OCH_3). ^{13}C NMR (125.7 MHz, d_6-DMSO): 165.81, 165.13, 133.79, 133.56, 132.60, 129.52 (4), 129.28 a 128.88 (4) (N-CO-C_6H_5 a O-CO-C_6H_5); 158.57, 158.52, 145.39, 135.90, 135.88, 130.28 (4), 128.50 (2), 128.15 (2), 128.15, 113.49 (4), 86.55 a 55.22 (2) (DMTr); 157.13, 150.50 d, *J*(C,P) = 3.0, 139.43, 110.45, 108.77, 60.97 d, *J*(C,P) = 3.5 a 56.13 (MOP); 152.68 (C-6); 151.88 (C-2); 150.34 (C-4); 143.61 (C-8); 125.41 (C-5); 94.30 a 86.08 (OCH_2O); 85.42 (C-1´); 83.08 (C-4´); 79.85 (C-2´); 73.11 (C-3´); 71.95 d, *J*(C,P) = 10.0 (C-5´); 66.40 d, *J*(C,P) = 156.0 (OCH_2P). ^{31}P{^{1}H} NMR (202.3 MHz, d_6-DMSO): 14.35. HR FAB calcd for $C_{55}H_{54}N_6O_{15}P$ $(M+H)^+$ 1069.3385, found 1069.3379.

4-Methoxy-1-oxido-2-pyridylmethyl-[2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-1-(2-*N*-isobutyrylguanin-9-yl)-β-D-ribofuranos-5-*O*-yl]methylphosphonate (**19b**)
Prepared by Method F from diisopropyl-[2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-1-(2-*N*-isobutyrylguanin-9-yl)-β-D-ribofuranos-5-*O*-yl]methylphosphonate **17b**. Yield 58%. ^{1}H NMR (500 MHz, d_6-DMSO): 12.50 bs, 1 H (NH); 12.15 bs, 1 H (NH); 8.44 s, 1 H (H-8); 7.88 d, 2 H, 7.69 t, 1 H, 7.49 m, 4 H, 7.37 d, 2 H, 7.32 m, 4 H, 7.22 t, 1 H, 6.86 d, 4 H; 8.02 d, 1 H, *J* = 7.1, 7.06 m, 1 H, 6.85 m, 1 H (MOP); 6.18 d, 1 H, *J*(1´,2´) = 7.9 (H-1´); 5.20 d a 5.15 m, 2 x 1 H, *J* = 6.4 (OCH_2O); 5.03 d a 4.91 d, 2 x 1 H, *J* = 6.8 (OCH_2O); 4.86 d, 2 H, *J*(H,P) = 7.0 ($POCH_2$); 4.78 m, 1 H (H-2´); 4.25 m, 1 H (H-3´); 3.72 s, 3.71 s a 3.56 s, 3 x 3 H (3 x OCH_3); 3.38 m, 4 H (H-5´, PCH_2); 3.09 m, 1 H (H-4´); 2.90 m, 1 H (CH); 1.08 d a 1.06 d, 2 x 3 H, *J*(CH_3,CH) = 6.8 (CH_3). ^{13}C NMR (125.7 MHz, d_6-DMSO): 180.85, 34.57, 19.22 a 18.96 (iBu); 165.12, 133.78, 129.48 (2), 129.24, 128,89 (2) (O-CO-C_6H_5); 158.54, 158.46, 145.52, 136.10, 135.99, 130.30 (2), 130.20 (2), 128.16 (2), 128.07 (2), 127.09, 113.48 (4), 86.52, 55.24 a 55.22 (DMTr); 156.91, 150.40, 139.59, 110.28, 108.81, 60.86 a 56.14 (MOP); 155.01 (C-6); 149.02 (C-2); 148.28 (C-4); 139.42 (C-8); 120.00 (C-5); 93.68 a 85.64 (OCH_2O); 89.48 (C-1´); 83.52 (C-4´); 77.03 (C-2´); 72.71 (C-3´); 71.71 d, *J*(C,P) = 11.2 (C-5´). ^{31}P{^{1}H} NMR (202.3 MHz, d_6-DMSO): 12.44. HR FAB calcd for $C_{52}H_{56}N_6O_{16}P$ $(M+H)^+$ 1051.3490, found 1051.3518.

4-Methoxy-1-oxido-2-pyridylmethyl-[2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-1-(uracil-1-yl)-β-D-ribofuranos-5-*O*-yl]methylphosphonate (**19c**)
Prepared by Method F from diisopropyl-[2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-1-(uracil-1-yl)-β-D-ribofuranos-5-*O*-yl]methylphosphonate **17c**. Yield 59%. ^{1}H NMR (500 MHz, d_6-DMSO): 11.30 bs, 1 H (NH); 7.98 d, 1 H, *J*(6,5) = 8.2 (H-6); 5.71 d, 1 H, *J*(5,6) = 8.2 (H-5); 7.96 d, 2 H, 7.68 t, 1 H, 7.52 t, 2 H, 7.44 d, 2 H, 7.33 t, 2 H, 7.28 m, 4 H, 7.21 t, 1 H, 6.86 d, 4 H; 8.26 m, 1 H, 7.04 m, 1 H, 6.81 dd, 1 H, *J* = 3.3, *J* = 7.0 (MOP); 6.14 bd, 1 H, *J*(1´,2´) = 4.5 (H-1´); 5.46 d, 1 H a 5.41 d, 1 H, *J* = 6.5 (OCH_2O); 5.00 d, 1 H a 4.75 d, 1 H, *J* = 6.5 (OCH_2O); 4.79 dd, 1 H, *J*(gem) = 16.0, *J*(Ha,P) = 9.0 (O-CHaHb-P); 4.72 dd, 1 H, *J*(gem) = 16.0, *J*(Hb,P) = 9.0 (O-CHaHb-P); 4.12 dd, 1 H, *J*(3´,2´) = 4.5, *J*(3´,4´) = 2.3 (H-3´); 3.74 s a 3.71 s, 2 x 3 H (2 x OCH_3); 3.70 m, 1 H (H-2´); 3.56 m, 2 H ($POCH_2$); 3.56 s, 3 H (OCH_3); 3.38 m, 1 H (H-4´); 3.27 m a 3.20 m, 2 x 1 H (H5´). ^{13}C NMR (125.7 MHz, d_6-DMSO): 165.29, 133.85, 129.61 (2), 129.39 a 129.95 (2) (O-CO-C_6H_5); 158.58, 158.53, 145.40, 135.95 (2), 130.31 (2), 130.27 (2), 128.18 (2), 128.08 (2), 127.15, 113.47 (4), 86.47, 55.27 a 55.26 (DMTr); 156.68, 110.15, 108.63, 60.97 a 56.13 (MOP); 163.46 (C-4); 151.31 (C-2); 139.34 (C-6); 103.06 (C-5); 93.97 a 86.18 (OCH_2O); 85.76 (C-1´); 82.45 (C-4´); 79.10 (C-2´); 72.68 (C-3´); 71.42 d, *J*(C,P) = 11.2 (C-5´). ^{31}P{^{1}H} NMR (202.3 MHz, d_6-DMSO): 12.63. HR FAB calcd for $C_{47}H_{48}N_3O_{16}NaP$ $(M+H)^+$ 964.2670, found 964.2673.

4-Methoxy-1-oxido-2-pyridylmethyl-[1-(4-*N*-benzoylcytosin-1-yl)-2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-β-D-ribofuranos-5-*O*-yl]methylphosphonate (**19d**)
Prepared by Method F from diisopropyl-[1-(4-*N*-benzoylcytosin-1-yl)-2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-β-D-ribofuranos-5-*O*-yl]methylphosphonate **17d**. Yield 58%. ^{1}H NMR (500 MHz, d_6-DMSO): 11.30 bs, 1 H (NH); 10.65 bs, 1 H (P-OH); 8.15 m, 1 H (H-6); 7.20 m, 1 H (H-5); 8.00 m, 5 H, 7.68 t, 1 H, 7.63 t, 1 H, 7.54 t, 2 H, 7.52 t, 2 H, 7.30 m, 2 H, 7.25 – 7.10 m, 6 H, 6.80 d, 2 H, 6.72 d, 2 H; 8.00 m, 1 H, 7.10 m, 1 H, 6.95 m, 1 H (MOP); 6.46 m, 1 H (H-1´); 5.48 s, 2 H (OCH_2O); 4.97 d, 1 H a 4.84 d, 1 H, *J* = 6.7 (OCH_2O); 4.92 dd, 1 H, *J*(gem) = 16.0, *J*(Ha,P) = 9.0 (O-CHaHb-P); 4.86 dd, 1 H, *J*(gem) = 16.0, *J*(Hb,P) = 9.0 (O-CHaHb-P); 4.48 m, 1 H (H-2´); 4.34 m, 1 H (H-3´); 4.12 m, 1 H (H-4´); 3.71 m, 2 H ($POCH_2$); 3.63 s, 6 H a 3.56 s, 3 H (3 x OCH_3); 3.44 m a 3.32 m, 2 H (H5´). ^{13}C NMR (125.7 MHz, d_6-DMSO): 168.71, 165.30, 133.88, 133.67, 132.85, 129.54 (2), 129.37, 128.99 (2), 128.15 (2) a 127.83 (2) (N-CO-C_6H_5 a O-CO-C_6H_5); 158.52, 158.29, 144.98, 135.68, 134.90, 130.62 (2), 130.00 (2), 128.62 (2), 128.57 (2), 127.01, 113.27 (2), 113.20 (2), 86.46, 55.05 a 54.94 (DMTr); 157.67, 150.37, 139.60, 110.53, 109.14, 60.79 a 56.13 (MOP); 163.05 (C-4); 154.67 (C-2); 146.40 (C-6); 94.39 a 86.52 (OCH_2O); 88.28 (C-1´); 83.00 (C-4´); 78.97 (C-2´); 76.16 (C-3´); 72.39 d, *J*(C,P) = 11.7 (C-5´). ^{31}P{^{1}H} NMR (202.3 MHz, d_6-DMSO): 12.55. HR FAB calcd for $C_{54}H_{55}N_4O_{16}NaP$ $(M+H)^+$ 1067.3092, found 1067.3106.

[2(3)-*O*-benzoyl-5-*O*-dimethoxytrityl-1-(4-*N*-benzoylcytosin-1-yl)-β-D-ribofuranos-3(2)-*O*-yl]succinate (**36**)
Benzoyl cyanide (1.5 mmol) was added under stirring to a solution of 4-*N*-benzoyl-1-(5-*O*-dimethoxytrityl-β-D-ribofuranosyl)cytosine **1d** (1.0 mmol) and triethylamine (0.2 mmol) in acetonitrile (10 ml), and the mixture was stirred 16 h at r.t. The reaction was quenched by the addition of methanol (5 ml) and the mixture was concentrated under reduced pressure. After that, the mixture of 2'- and 3'-benzoyl derivatives was added to a solution of DMAP (2.0 mmol) and succinic anhydride (5.0 mmol) in pyridine, and the reaction mixture was stirred 16 h at r.t. The reaction was quenched by the addition of 2M TEAB (1 ml), and the mixture was concentrated under reduced pressure. The residue was dissolved in chloroform (50 ml) and extracted with 10% citric acid (3 x 20 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated. The product was purified as a mixture of two regioisomers by chromatography on silica gel (elution with gradient of 0 – 20% methanol in chloroform). Yield 65%.
^{1}H NMR (500 MHz, d_6-DMSO): 11.40 bs, 1 H (NH); 8.28 d a 8.27 d, 1 H, *J*(6,5) = 7.6 (H-6); 7.35 d a 7.33 d, 1 H, *J*(6,5) = 7.6 (H-5); 8.01 m, 4 H, 7.71 t, 1 H, 7.64 t, 1 H, 7.57 t, 2 H, 7.53 t, 2 H, 7.41 t, 2 H, 7.30 – 7.20 m, 7 H, 6.92 d, 2 H, 6.83 d, 2 H; 6.14 d a 6.00 d, 1 H, *J*(1´,2´) = 2.9, 3.4 (H-1´); 5.82 dd a 5.70 dd, 1 H, *J*(2´,3´) = 5.7, 5.9 (H-2´); 5.80 dd a 5.64 dd, 1 H, *J*(3´,4´) = 6.7, 7.3 (H-3´); 4.46 dt a 4.38 ddd, 1H, *J*(4´,3´) = 6.7, 7.3, *J*(4´,5´) = 3.2, 3.9, 4.1 (H-4´); 3.74 s a 3.69 s, 6 H (2 x OCH_3); 3.44 dd a 3.42 dd, 1 H, *J*(5´b,5´a) = 11.0, *J*(5´a,4´) = 4.1, 4.2 (H-5´a); 3.41 dd a 3.38 dd, 1 H, *J*(5´b,5´a) = 11.0, *J*(5´b,4´) = 3.2, 3.6 (H-5´b); 2.54 m a 2.39 m, 4 H (CH_2CH_2). ^{13}C NMR (125.7 MHz, d_6-DMSO): 173.37, 173.34, 171.37, 171.33, 28.80 (2) a 28.73 (2) (CO-CH_2CH_2-CO); 164.86, 164.75, 134.13, 134.04, 129.09 (2), 129.05 (2), 128.81, 128.76 a 128.69 (2) (O-CO-C_6H_5); 167.77, 133.27, 133.21, 133.12, 133.00, 128.15 (2), 128.07 (2), 127.88 (2) a 127.83 (2) (N-CO-C_6H_5); 158.43 (2), 158.33 (2), 144.60, 144.53, 135.45 (2), 135.31 (2), 129.92 (2), 129.85 (2), 129.69 (2), 129.59 (2), 128.64 (4), 127.04, 127.01, 113.49 (4), 113.41 (4), 86.37, 86.34, 55.25, 55.20, 55.22 a 55.13 (DMTr); 163.84, 163.77 (C-4); 154.31, 154.25 (C-2); 146.27, 146.07 (C-6); 97.59, 96.86 (C-5); 90.70, 90.15 (C-1´); 80.79, 80.34 (C-4´); 74.08, 73.63 (C-2´); 70.18, 70.10 (C-3´); 62.39, 62.32 (C-5´). HR FAB calcd for $C_{48}H_{44}N_3O_{12}$ $(M+H)^+$ 854.2925, found 854.2946.

[2-*O*-Benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-1-(2-*N*-isobutyrylguanin-9-yl)-β-D-ribofuranos-5-*O*-yl]succinate (**37**)
9-(2-*O*-Benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-2-*N*-isobutyryl-β-D-ribofuranosyl)guanine **8b** was added to a solution of DMAP (2.0 mmol) and succinic anhydride (5.0 mmol) in pyridine, and the reaction mixture was stirred 16

h at r.t. The reaction was quenched by the addition of 2M TEAB (1 ml), and the mixture was concentrated under reduced pressure. The residue was dissolved in chloroform (50 ml) and extracted with 10% citric acid (3 x 20 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated. The product was purified as a mixture of two regioisomers by chromatography on silica gel (elution with gradient of 0 – 20% methanol in chloroform). Yield 71%. ^{1}H NMR (500 MHz, d_6-DMSO): 12.00 bs, 3 H (2 x NH, OH); 8.09 s, 1 H (H-8); 7.89 d, 2 H, 7.66 t, 1 H, 7.50 m, 4 H, 7.38 d, 2 H, 7.34 m, 4 H, 7.25 t, 1 H, 6.90 d, 4 H; 6.15 d, 1 H, *J*(1´,2´) = 6.8 (H-1´); 5.27 d, 1 H a 5.21 d, 1 H, *J* = 6.4 (OCH_2O); 4.98 d, 1 H a 4.76 d, 1 H, *J* = 6.4 (OCH_2O); 4.49 bt, 1 H (H-2´); 4.29 dd, 1 H, *J*(3´,2´) = 5,0, *J*(3´,4´) = 2,6 (H-3´); 3.80 dd, 1 H, *J*(5´b,5´a) = 12,2, *J*(5´a,4´) = 5.7 (H-5´a); 3.73 s a 3.72 s, 2 x 3 H (2 x OCH_3); 3.71 dd, 1 H, *J*(5´b,5´a) = 12.2, *J*(5´b,4´) = 5.1 (H-5´b); 3.43 btd, 1H, *J*(4´,3´) = 2,6, *J*(4´,5´) = 5.4 (H-4´); 2.77 septet, 1 H, *J*(CH,CH_3) = 6.8 (CH); 2.36 m a 2.30 m, 2 x 2 H (CH_2CH_2); 1.22 d, 6 H, *J*(CH_3,CH) = 6.8 (CH_3). ^{13}C NMR (125.7 MHz, d_6-DMSO): 172.20 (2) a 29.47 (2) (CO-CH_2CH_2-CO); 165.18, 133.87, 129.47 (2), 129.24 a 128.93 (2) (O-CO-C_6H_5); 180.00, 35.00 a 19.11 (2) (iBu); 158.70, 158.64, 145.38, 135.82, 135.68, 130.34 (2), 130.30 (2), 128.18 (2), 128.08 (2), 127.25, 113.57 (4), 86.85, 55.30 a 55.28 (DMTr); 155.00 (C-6); 149.00 (C-2, C-4); 137.50 (C-8); 120.39 (C-5); 93.97 a 85.98 (OCH_2O); 85.14 (C-1´); 81.26 (C-4´); 78.68 (C-2´); 72.24 (C-3´); 63.65 (C-5´). HR FAB calcd for $C_{48}H_{50}N_5O_{14}$ $(M+H)^+$ 920.3354, found 920.3364.

2. Attachment of Nucleosides to Solid Support

TOTU (6.6 mg) was added to a suspension of succinate **36**, **37** (0.02 mmol), DIPEA (10.5 μl) and LCAA CPG 500 Å, 50 mesh (0.5 g) in DMF (1.5 ml), and the suspension was shaken 2.5 h at r.t. The solid support was washed with methanol and diethylether. After that, it was treated 20 min with Cap A and Cap B capping solutions (1:1), washed with methanol and diethylether, and dried. Loading of the solid support was determined from the absorbtion of DMTr cation at the peak maximum at 504 nm.
By this method, we prepared solid supports with loading 40 – 50 μmol/g.

3. Synthesis of oligoribonucleotides

Oligonucleotides **20-33** were synthesized using the protocol shown in **Table 1**. Synthesis was performed on a 1 μmol scale, in 3'→5' direction on [2(3)-*O*-benzoyl-5-*O*-dimethoxytrityl-1-(4-*N*-benzoylcytosin-1-yl)-β-D-ribofuranos-3(2)-*O*-succinyl]LCAA-CPG **36** and in 5'→3' directions on [2-*O*-benzoyloxymethoxymethyl-3-*O*-dimethoxytrityl-1-(2-N-isobutyrylguanin-9-yl)-β-D-ribofuranos-5-*O*-succinyl]LCAA-CPG **37** using GenSyn V02 DNA/RNA synthesizer.

Table 1 Solid phase synthesis protocol

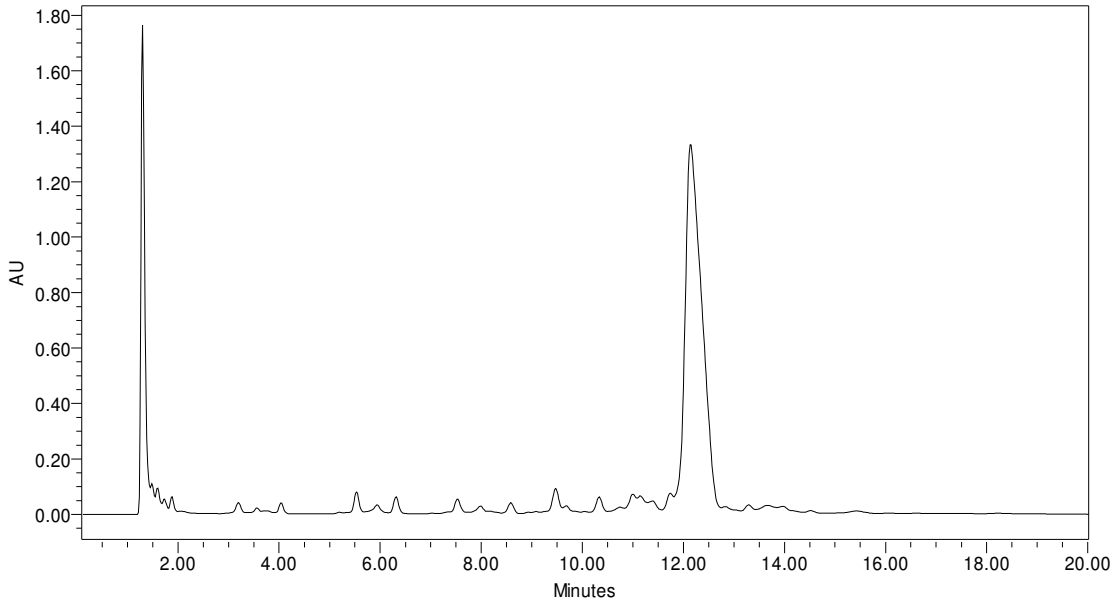
Phosphoramidite condensation method		
1. Detritylation	3% DCA in DCM	120s
2. Condensation	0.1M phosphoramidite in ACN 0.5M ETT in ACN	180s
3. Capping	Ac_2O/2,6-lutidine/THF 1:1:8 6.5% DMAP in THF	60s
4. Oxidation	10% tBuOOH in DCM	90s
Phosphotriester condensation method		
1. Detritylation	3% DCA in DCM	120s
2. Condenzation	0.1M phosphonate in pyridine 0.3M TIPS-Cl in ACN	600s
3. Capping	Ac_2O/2,6-lutidine/THF 1:1:8 6.5% DMAP in THF	60s

Deprotection of nonamers was achieved in two steps. First, the column with oligoribonucleotide anchored to the LCAA-CPG was treated with benzenethiol/TEA/DMF (1:1.4:2 v:v:v) mixture for 8 h to remove methyl and MOP protecting groups from the phosphate and phosphonate groups, respectively, and then washed with DMF, ACN and dried. Second, the column was inserted into the pressure vessel and treated with gaseous ammonia (0.7 MPa) for 8 h to remove BOMOM and acyl protecting groups, and to release the final product from the solid support. In the end, deprotected RNA was washed out from the column by 0.1M TEAA buffer and purified using Poly-Pak purification protocol (GlenResearch, DMTr ON mode).

Ionic exchange chromatograms (elution with linear gradient 0-20%B in 20 min, 65°C; A 0.025 M TRIS.HCl, 10% ACN, pH 6.5; B 0.5 M $LiClO_4$, 0.025 M TRIS.HCl, 10% ACN, pH 6.5) of crude nonamers 20, 21, 25 and 29.

5'-r(GCA UAU CAC)-3' (**20**) 5'→3' synthesis

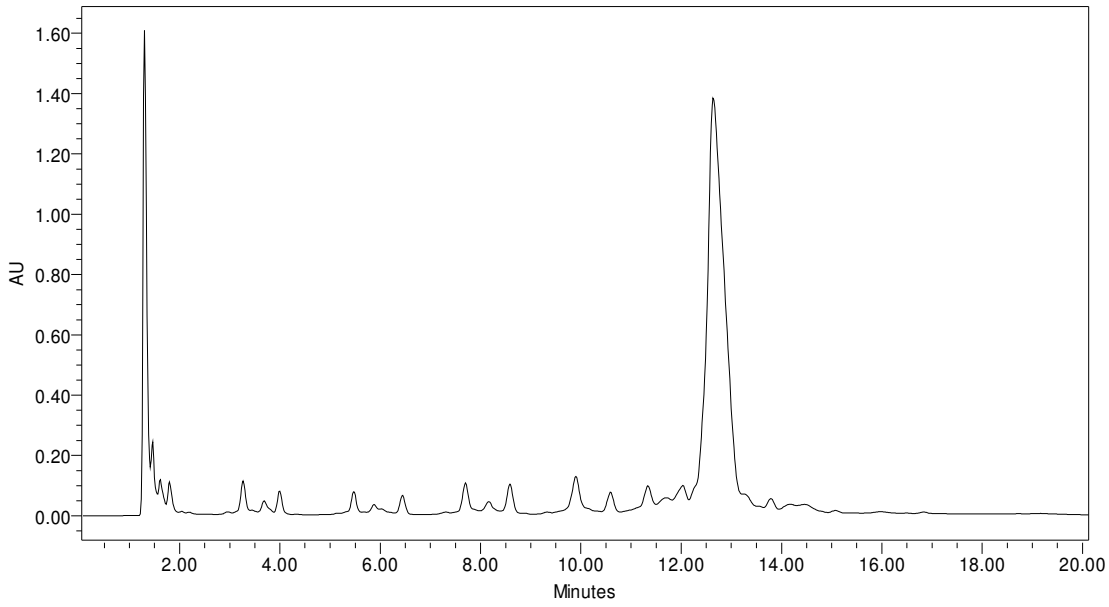
Overall coupling yield = 83.7%
Average stepwise coupling yield = 97.8%



	Retention Time	Area	% Area	Height
1	1.477	66048	0.19	21202
2	1.594	138334	0.41	38614
3	1.729	86105	0.25	22144
4	1.879	196285	0.58	45767
5	3.195	234149	0.69	35606
6	3.562	134315	0.39	15115
7	4.039	217443	0.64	36965
8	5.195	16593	0.05	3316
9	5.532	422982	1.24	70118
10	5.939	177419	0.52	23595
11	6.314	397767	1.17	58543
12	7.531	326386	0.96	45711
13	7.985	150884	0.44	19749
14	8.587	291778	0.86	37349
15	9.472	822994	2.42	82052
16	10.333	419952	1.23	52932
17	10.745	51558	0.15	7098
18	11.002	1004734	2.95	49735
19	11.742	187665	0.55	28854
20	12.143	27973093	82.18	1279730
21	12.827	41768	0.12	6230
22	13.293	137035	0.40	18366
23	13.665	382964	1.13	16013
24	14.518	53068	0.16	6443
25	15.430	107188	0.31	6792

5'-r(GUG AUA UGC)-3' (**21**) 5'→3' synthesis

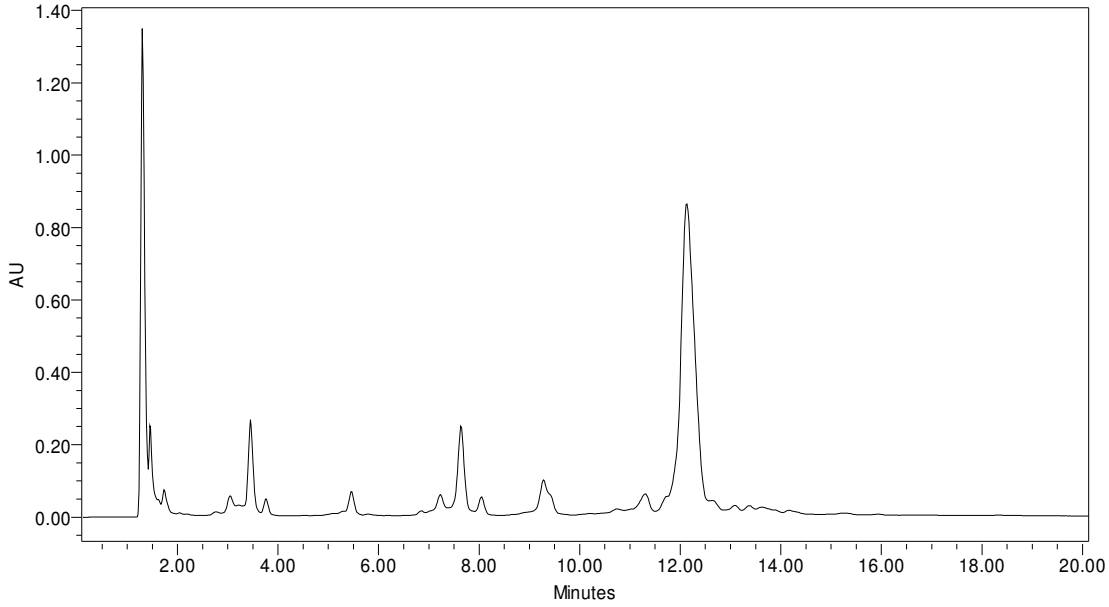
Overall coupling yield = 81.7%
Average stepwise coupling yield = 97.5%



	Retention Time	Area	% Area	Height
1	1.460	348563	0.93	108632
2	1.614	196725	0.52	51955
3	1.799	373152	0.99	75614
4	3.264	643159	1.71	100074
5	3.685	248136	0.66	34311
6	3.993	410607	1.09	69127
7	5.472	443643	1.18	67459
8	5.877	227466	0.61	22407
9	6.443	386515	1.03	57074
10	7.698	753543	2.01	93823
11	8.585	1099825	2.93	94358
12	9.898	1236264	3.29	112882
13	10.589	428991	1.14	56668
14	11.334	545391	1.45	64815
15	12.029	420592	1.12	45874
16	12.640	29043321	77.38	1296726
17	13.788	247245	0.66	31068
18	14.465	482600	1.29	17566

5'-r(GUG AUA UGC)-3' (**29**) 5'→3' synthesis

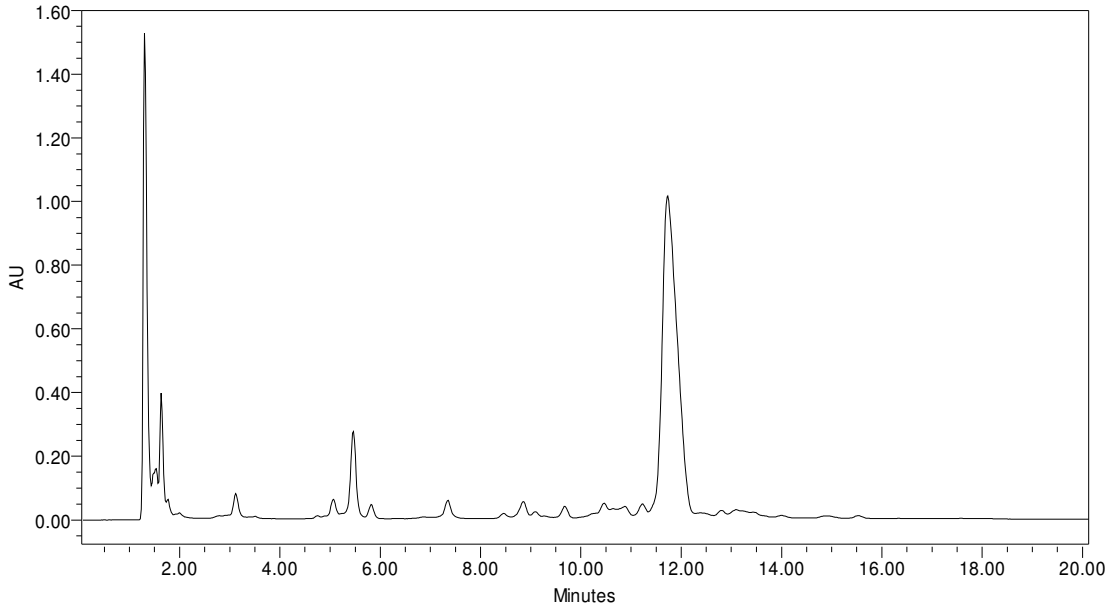
Overall coupling yield = 69.8%
Average stepwise coupling yield = 95.6%
Phosphonate average stepwise coupling yield = 94.1%



	Retention Time	Area	% Area	Height
1	1.461	408483	1.93	131884
2	1.737	227821	1.07	46460
3	3.047	224938	1.06	35697
4	3.452	1438331	6.78	237683
5	3.760	211228	1.00	38290
6	5.460	323102	1.52	53590
7	7.228	241910	1.14	36337
8	7.638	1819753	8.58	221931
9	8.046	275334	1.30	41153
10	9.282	930905	4.39	80362
11	11.300	414685	1.96	40614
12	12.128	14531551	68.51	812166
13	13.088	91351	0.43	12102
14	13.374	71533	0.34	9593

5'-r(GCAUAU CAC)-3' (**25**) 5'→3' synthesis

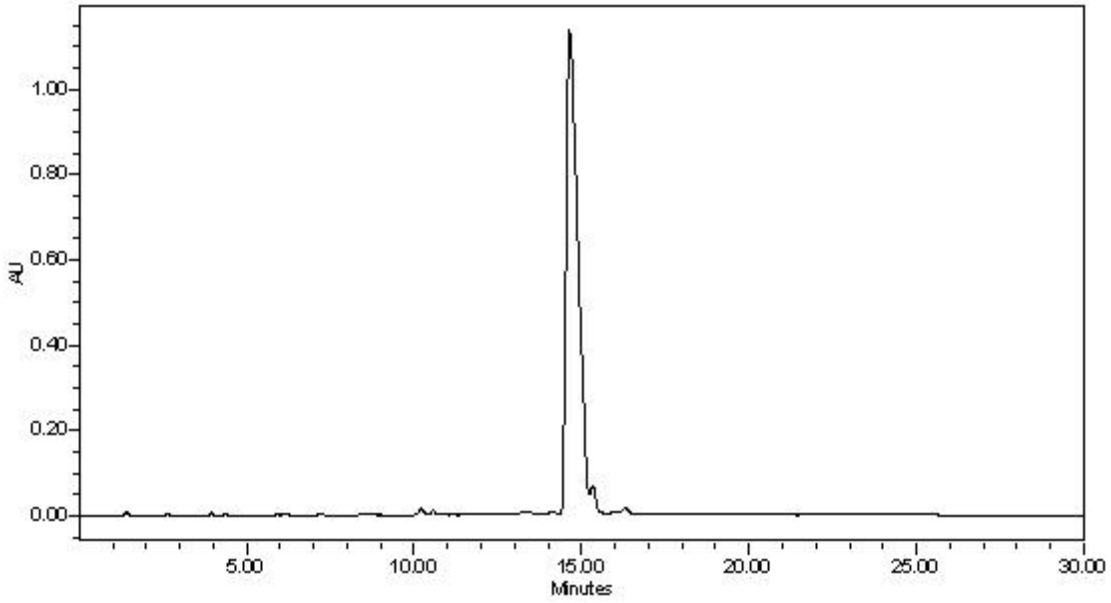
Overall coupling yield = 75.1%
Average stepwise coupling yield = 96.5%
Phosphonate average stepwise coupling yield = 95.0%

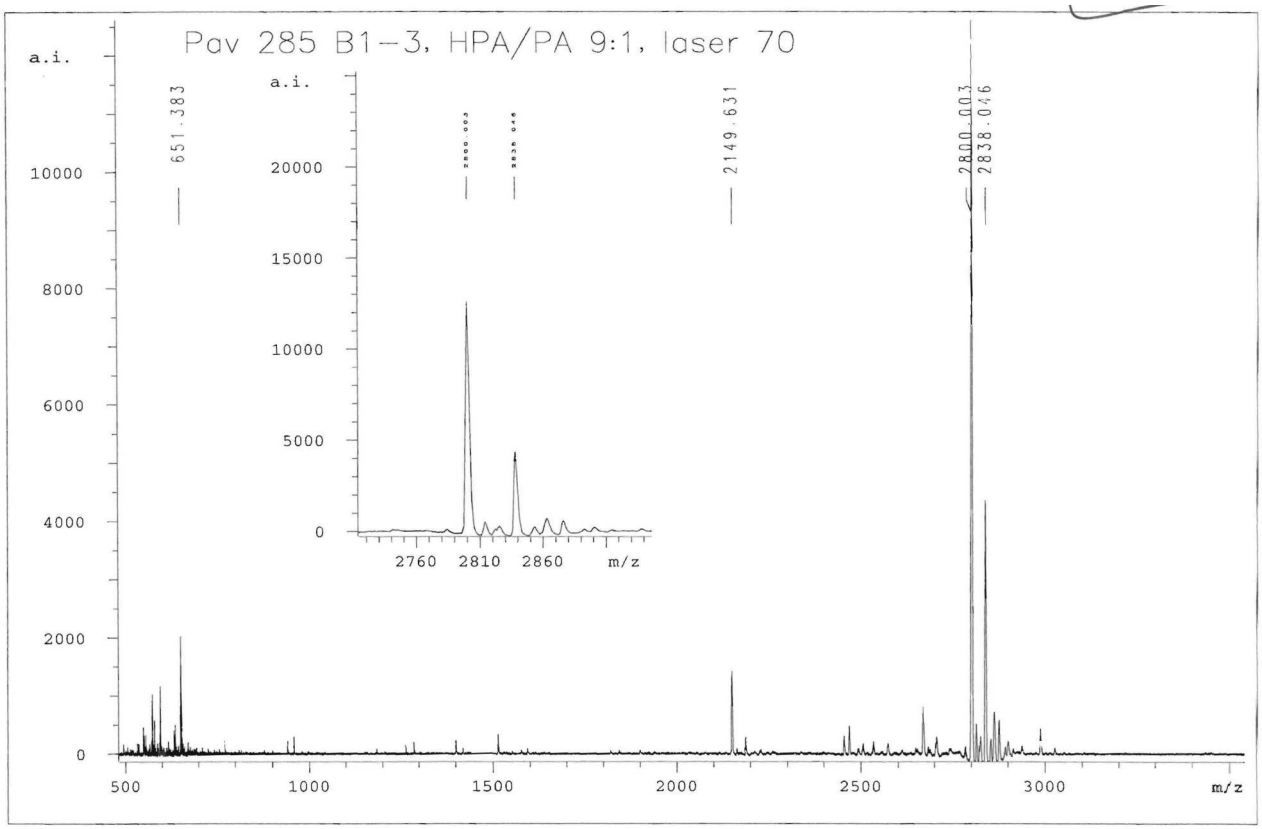


	Retention Time	Area	% Area	Height
1	1.635	1484815	5.65	301799
2	3.119	335788	1.28	60668
3	5.064	286324	1.09	47205
4	5.462	1729190	6.58	251149
5	5.818	228310	0.87	37481
6	7.346	317817	1.21	46141
7	8.459	60261	0.23	9614
8	8.851	355452	1.35	43422
9	9.091	20190	0.08	3454
10	9.679	239010	0.91	31721
11	10.463	626394	2.38	29891
12	11.229	171098	0.65	25084
13	11.729	20064968	76.34	989272
14	12.802	105403	0.40	13434
15	13.094	133514	0.51	10630
16	14.892	75118	0.29	5277
17	15.534	50652	0.19	6092

Ionic exchange chromatograms (elution with linear gradient 0-25%B in 20 min, 65°C; A 0.025 M TRIS.HCl, 10% ACN, pH 7.0; B 0.5 M $NaClO_4$, 0.025 M TRIS.HCl, 10% ACN, pH 7.0) and MALDI spectra of purified nonamers **20-33.**

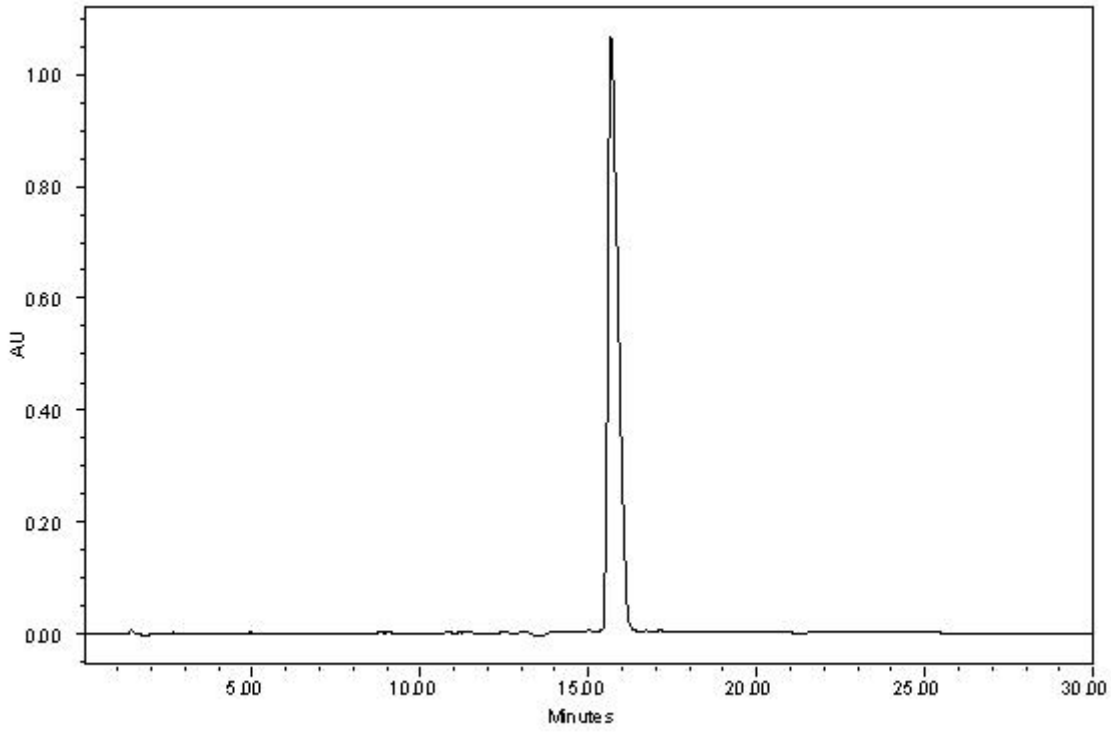
5'-r(GCA UAU CAC) -3' **20**

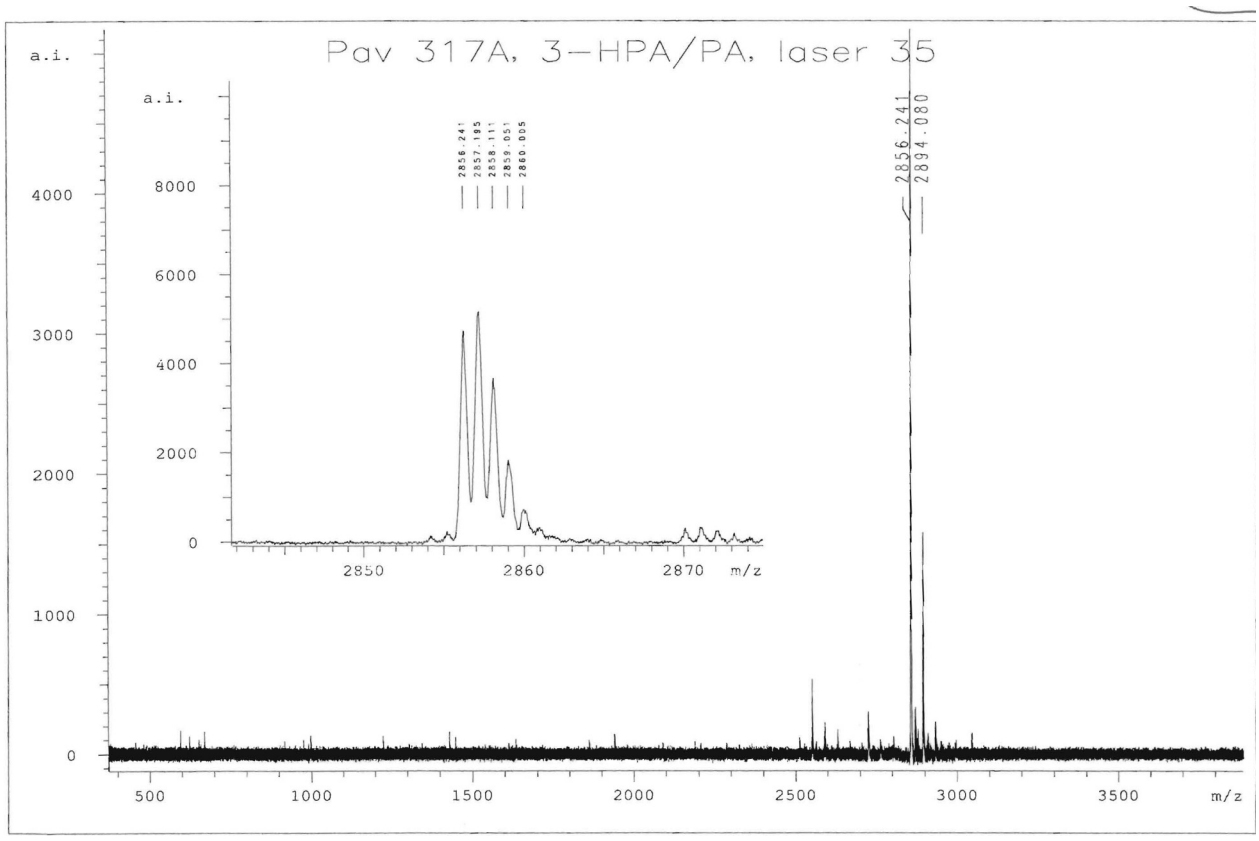




Calcd $[M+H]^+$ 2799.742; Found 2800.003
Isolated yield OD_{260} = 66.0 (75%).

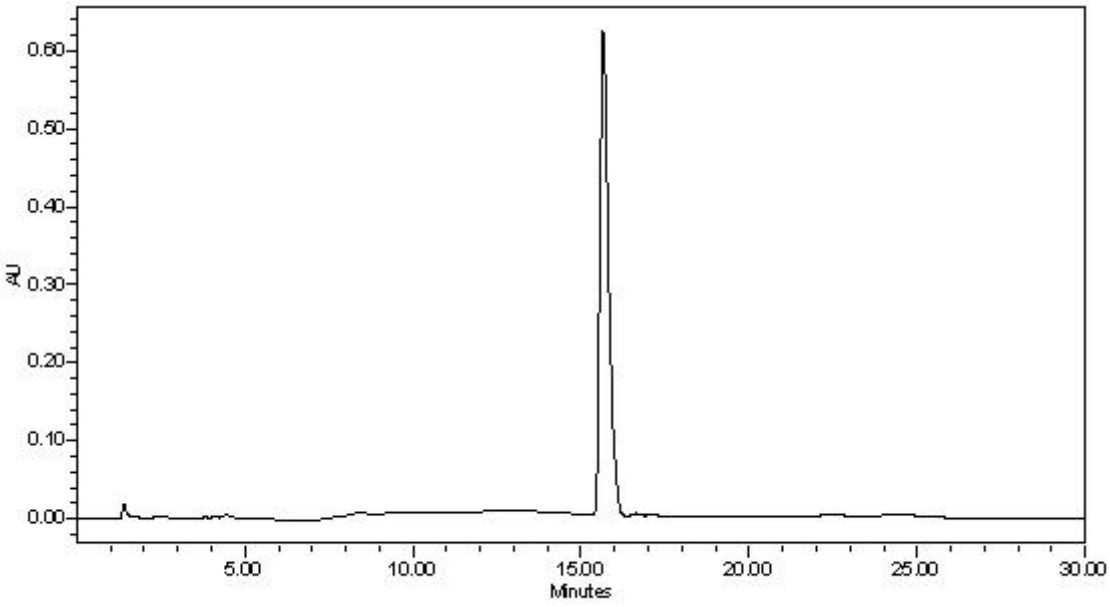
5'-r(GUG AUA UGC)-3' **21**

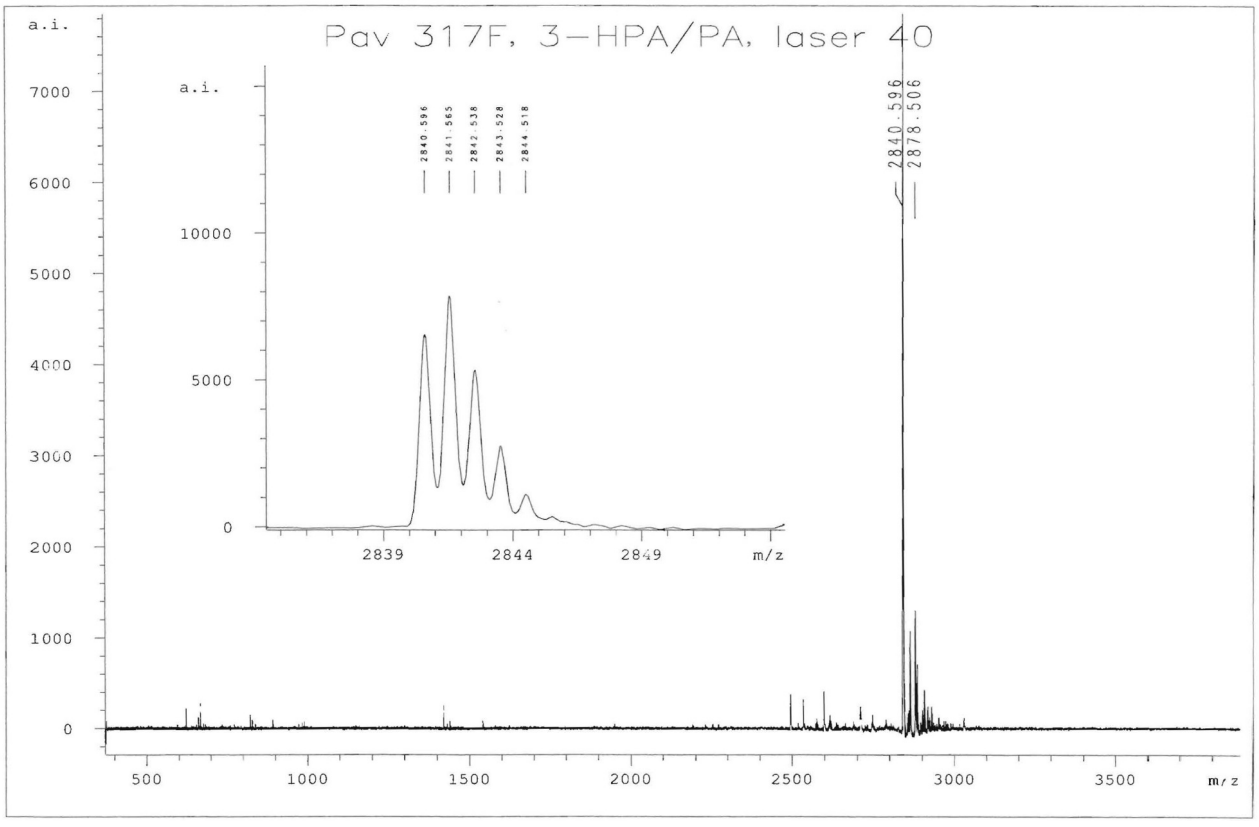




Calcd $[M+H]^+$ 2855.417; Found 2856.241
Isolated yield OD_{260} = 66.6 (74%).

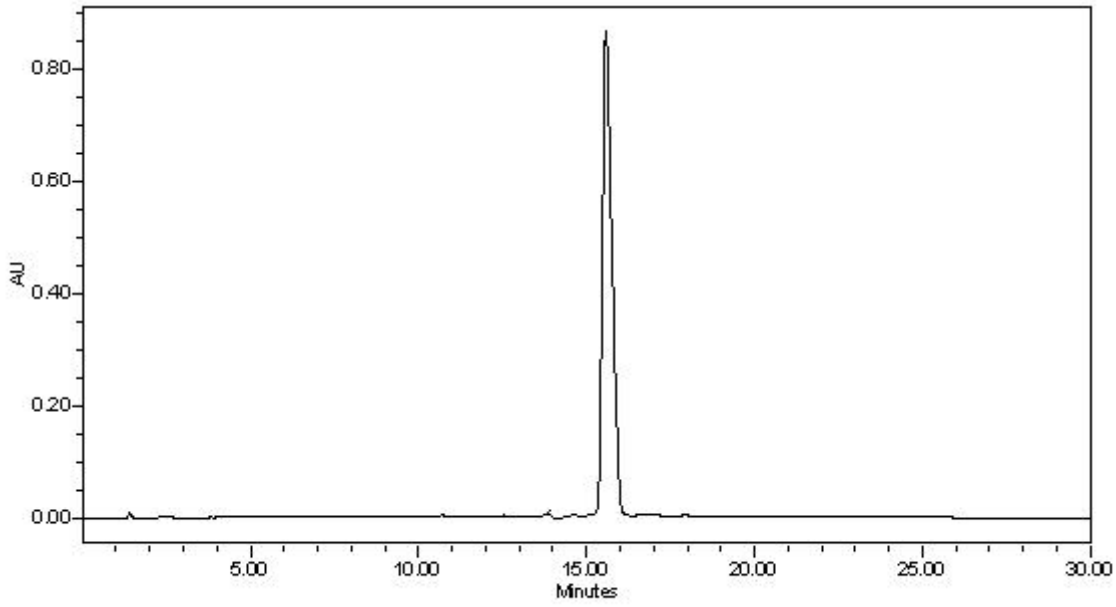
5'-r(GCA UAU CAC)-3' **22**

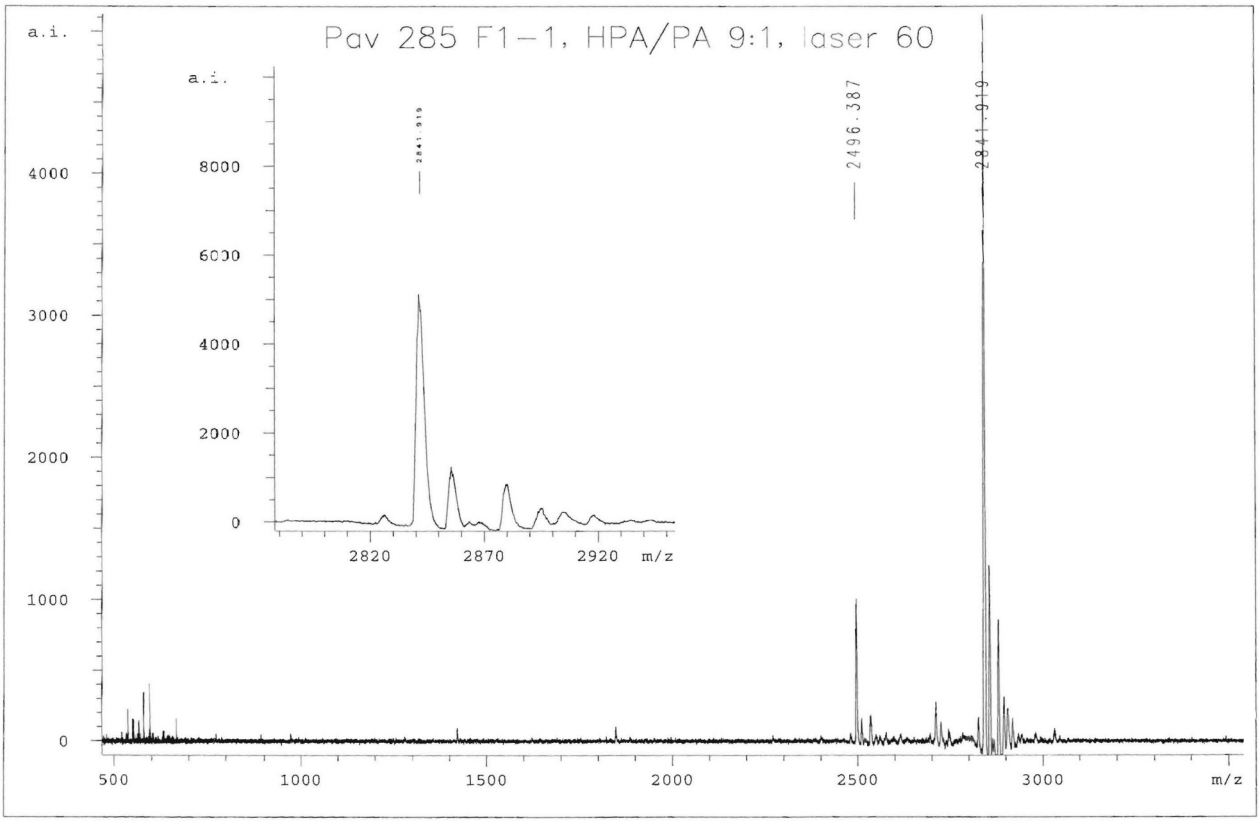




Calcd $[M+H]^+$ 2841.822; Found 2840.596
Isolated yield OD_{260} = 57.2 (65%).

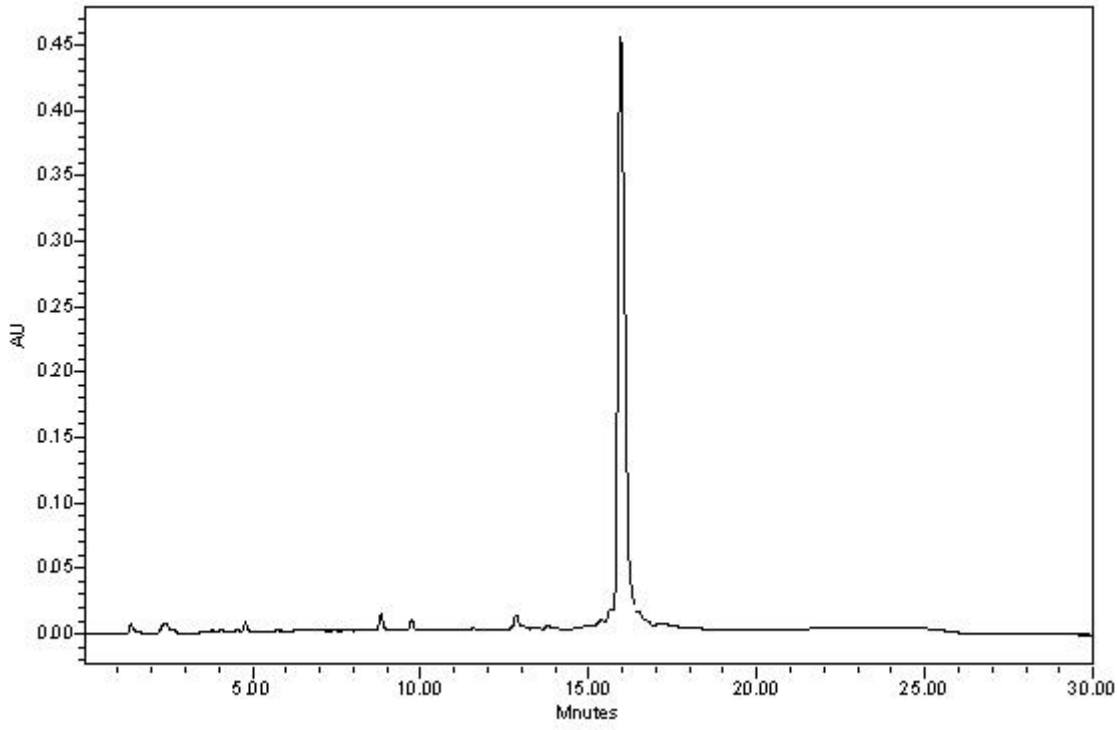
5'-r(GCA UAU CAC)-3' **23**

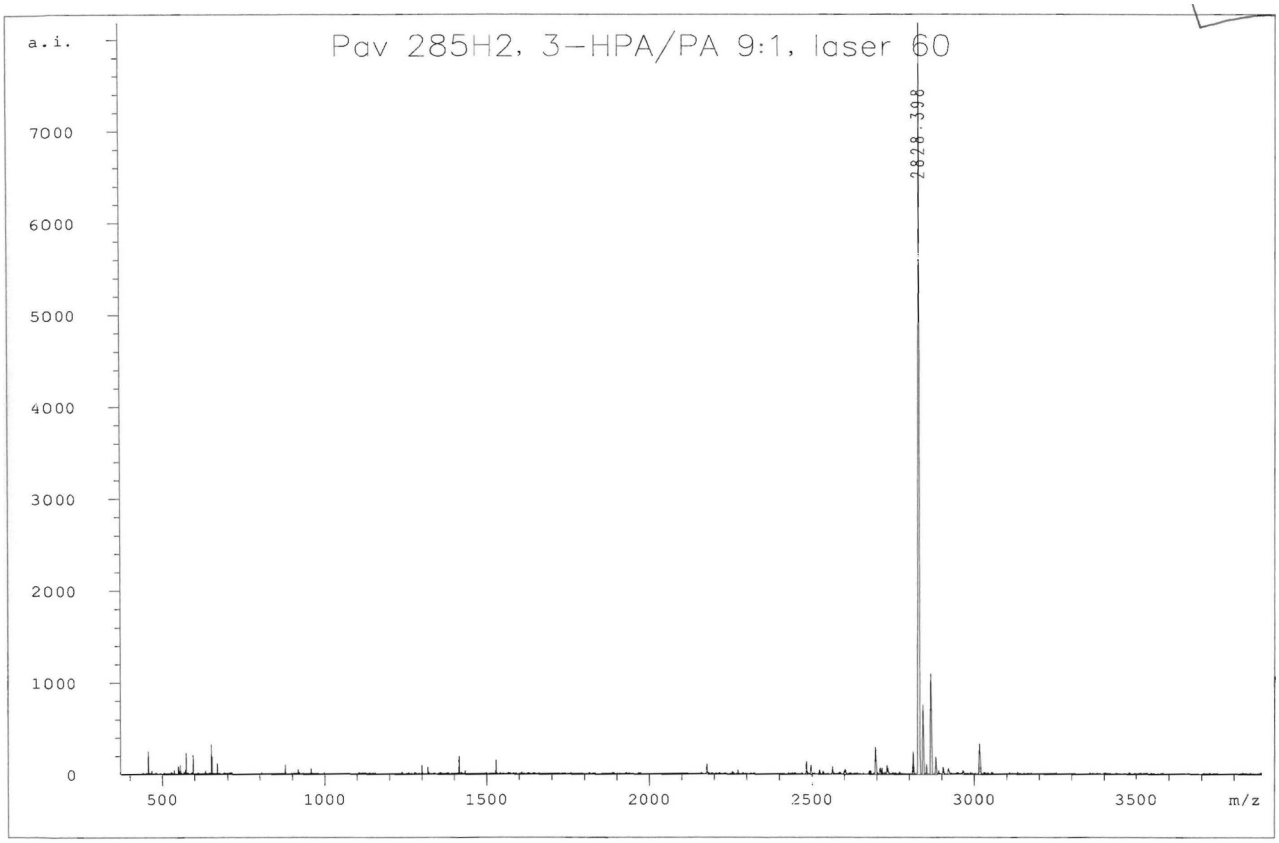




Calcd $[M+H]^+$ 2841.822; Found 2841.919
Isolated yield OD_{260} = 56.3 (64%).

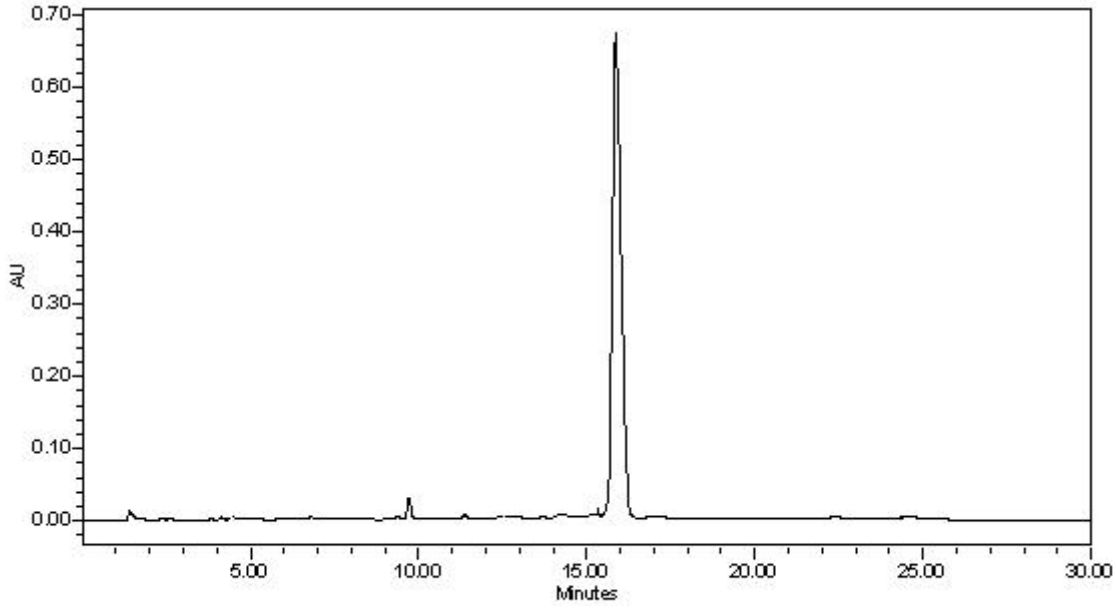
5'-r(GCA UAU CAC)-3' **24**

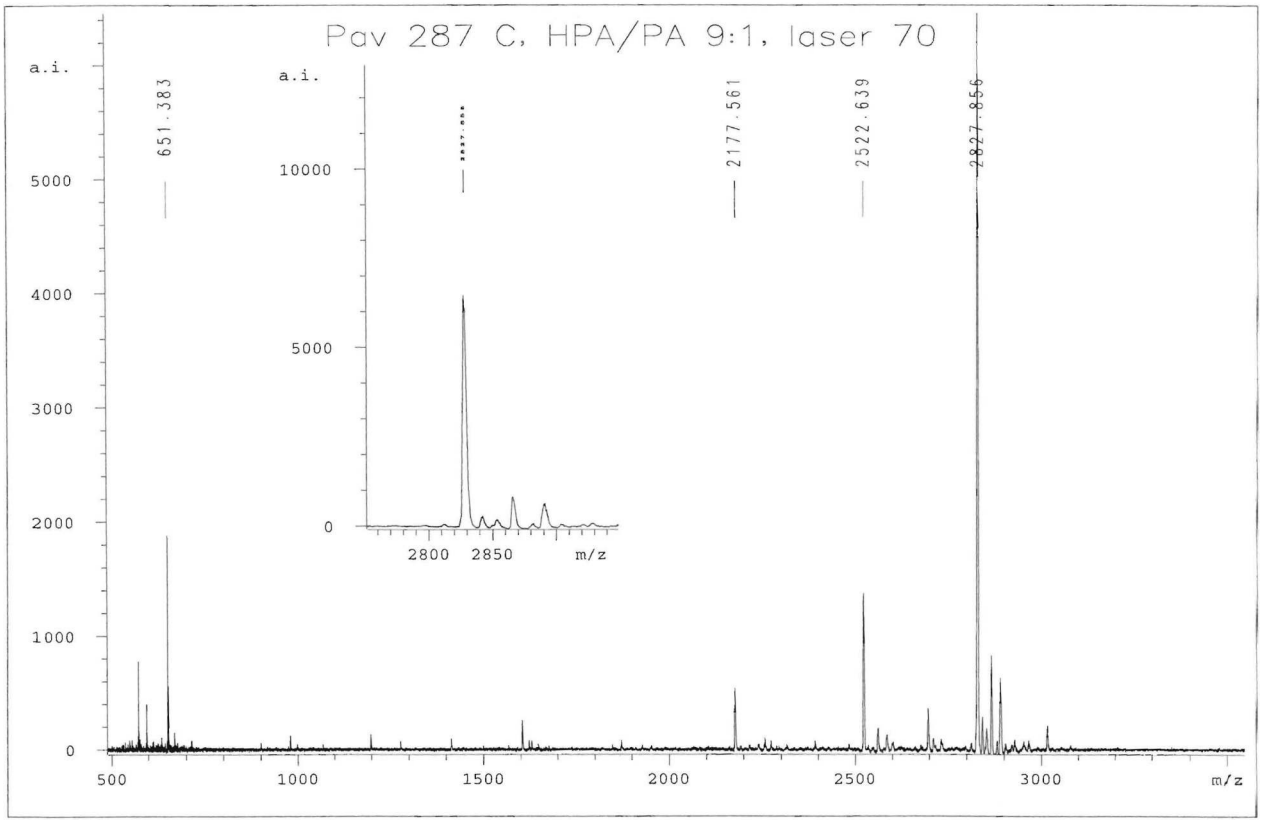




Calcd $[M+H]^+$ 2827.795; Found 2828.398
Isolated yield OD_{260} = 62.5 (71%).

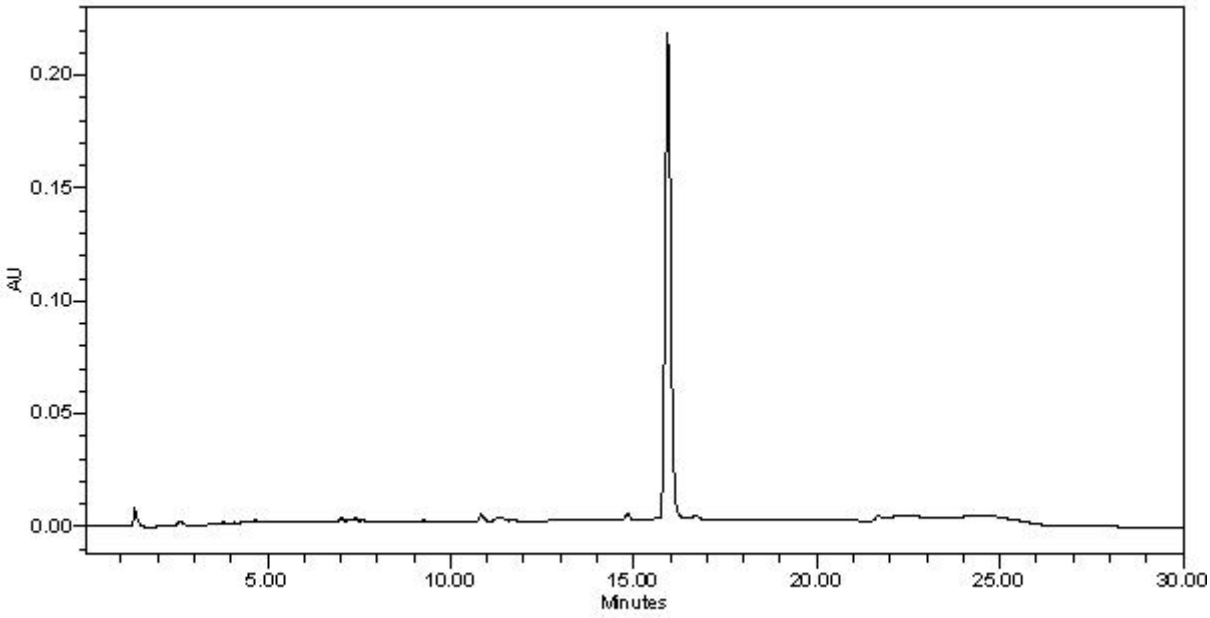
5'-r(GCA UAU CAC)-3' **25**

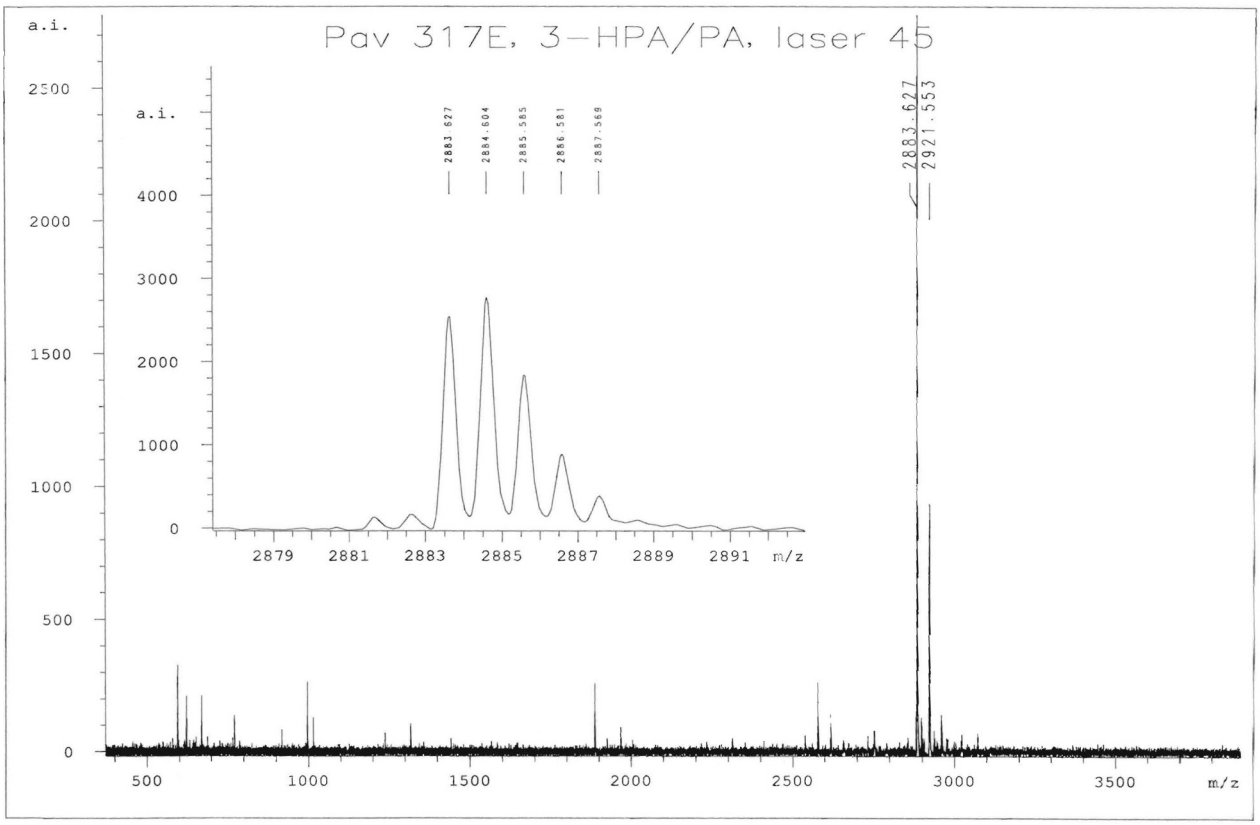




Calcd $[M+H]^+$ 2827.795; Found 2827.856
Isolated yield OD_{260} = 60.7 (69%).

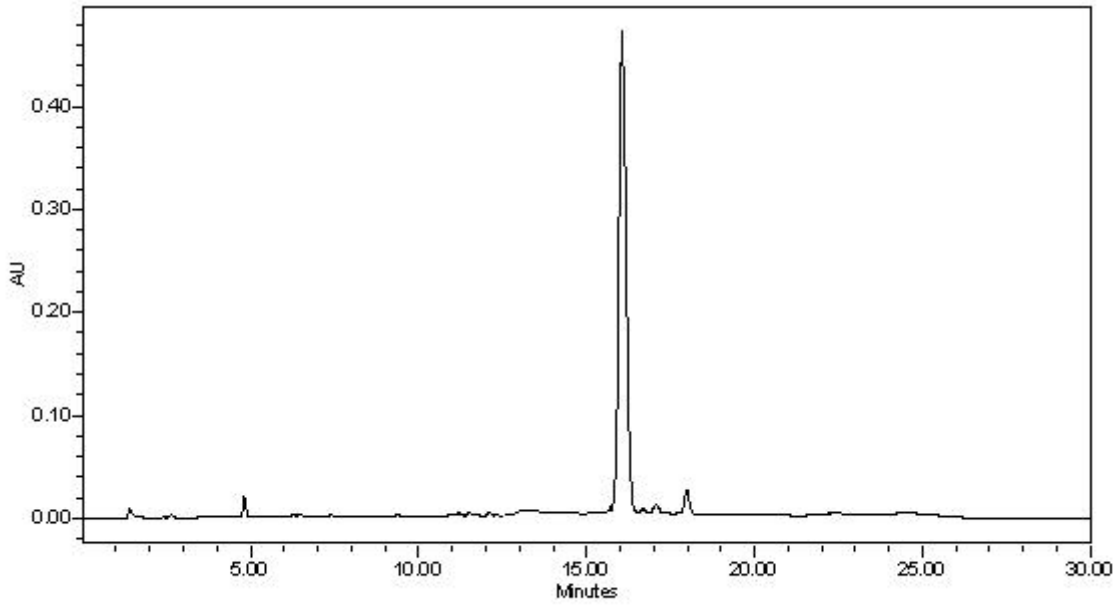
5'-r(GUG AUA UGC)-3' **26**

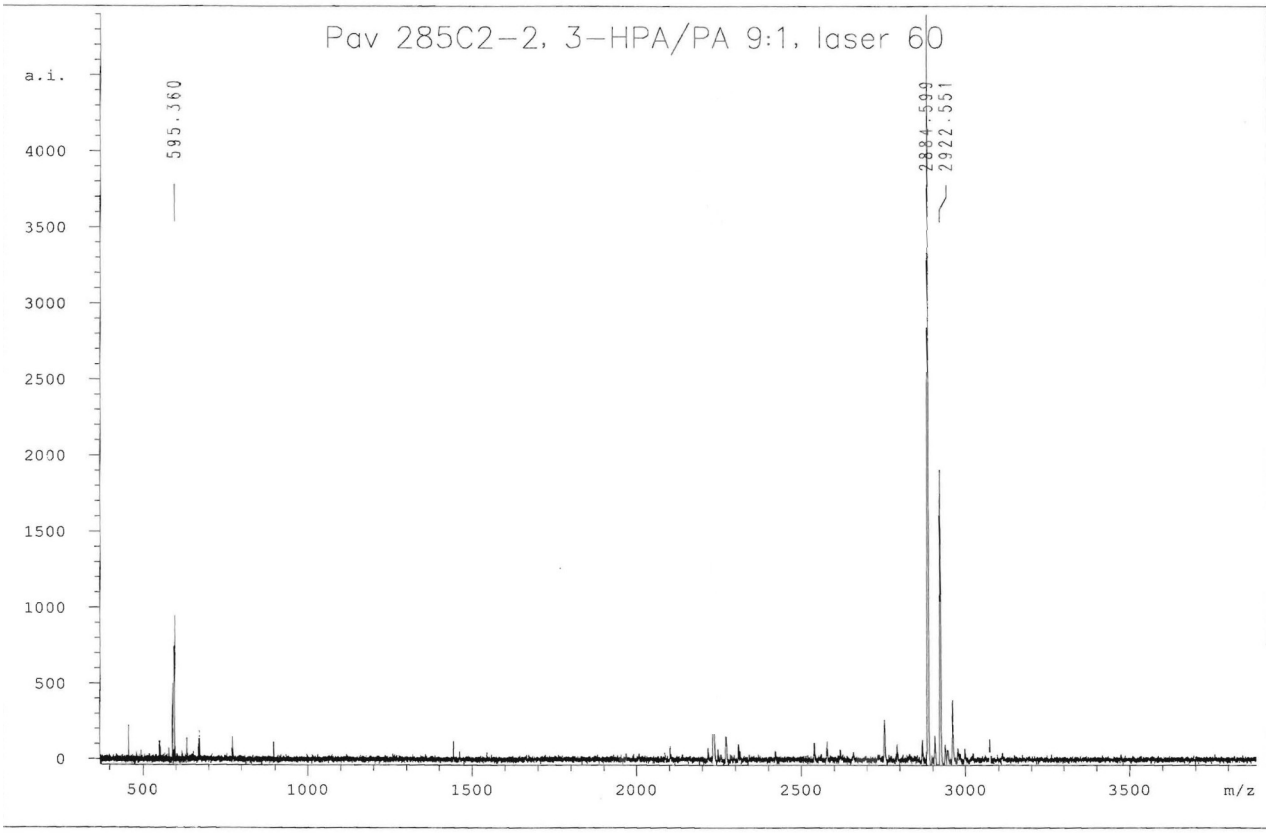




Calcd $[M+H]^+$ 2884.804; Found 2883.627
Isolated yield OD_{260} = 58.5 (65%).

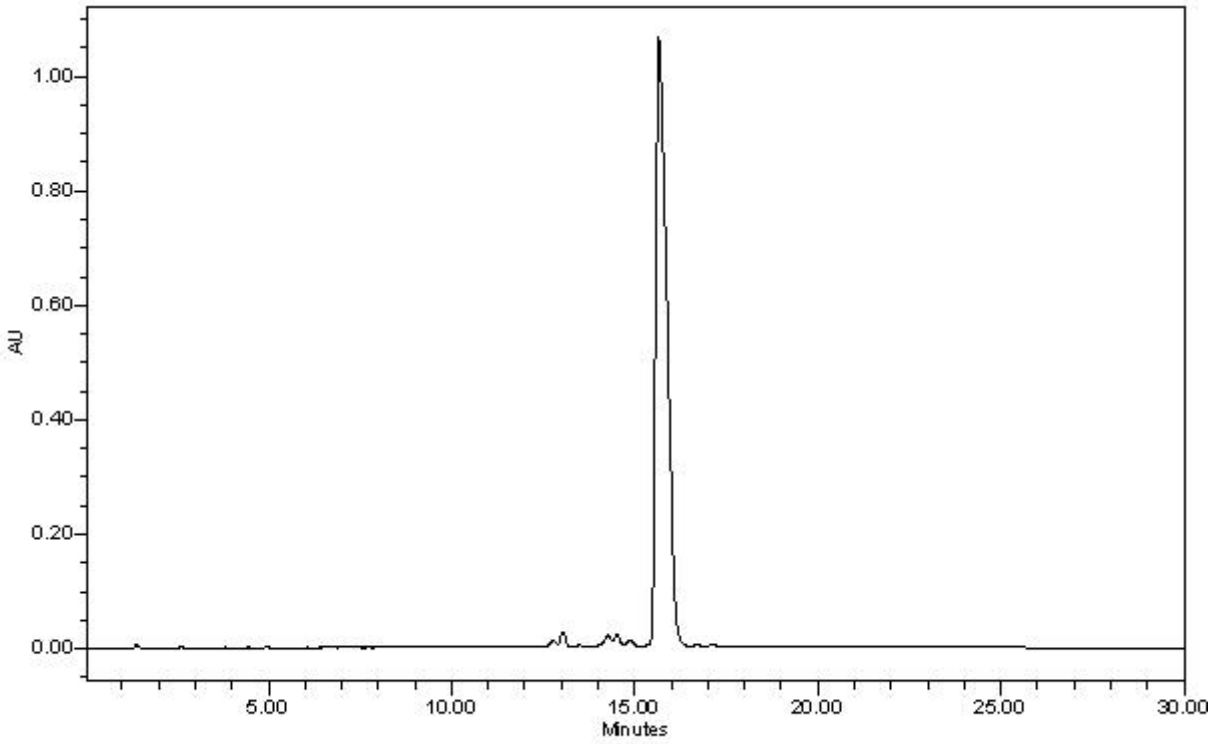
5'-r(GUG AUA UGC)-3' **27**

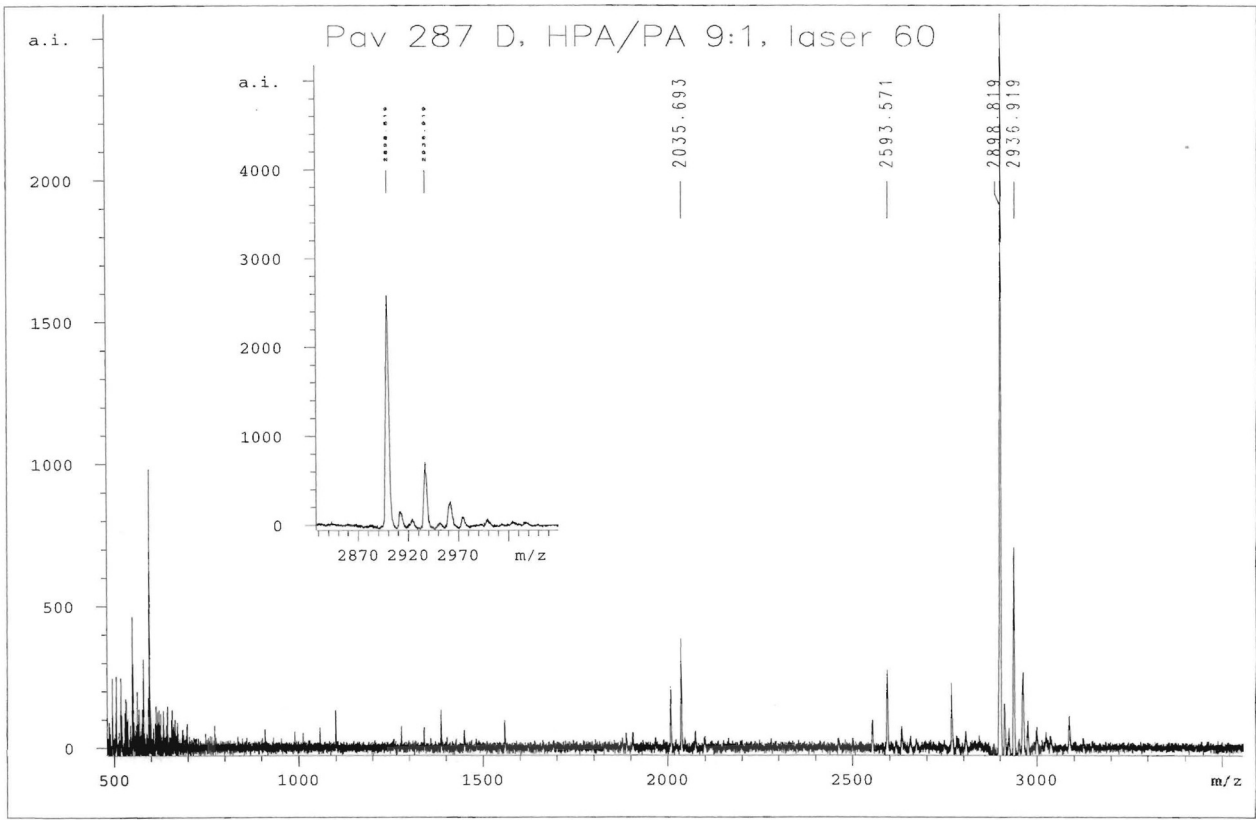




Calcd $[M+H]^+$ 2884.804; Found 2884.599
Isolated yield OD_{260} = 57.6 (64%).

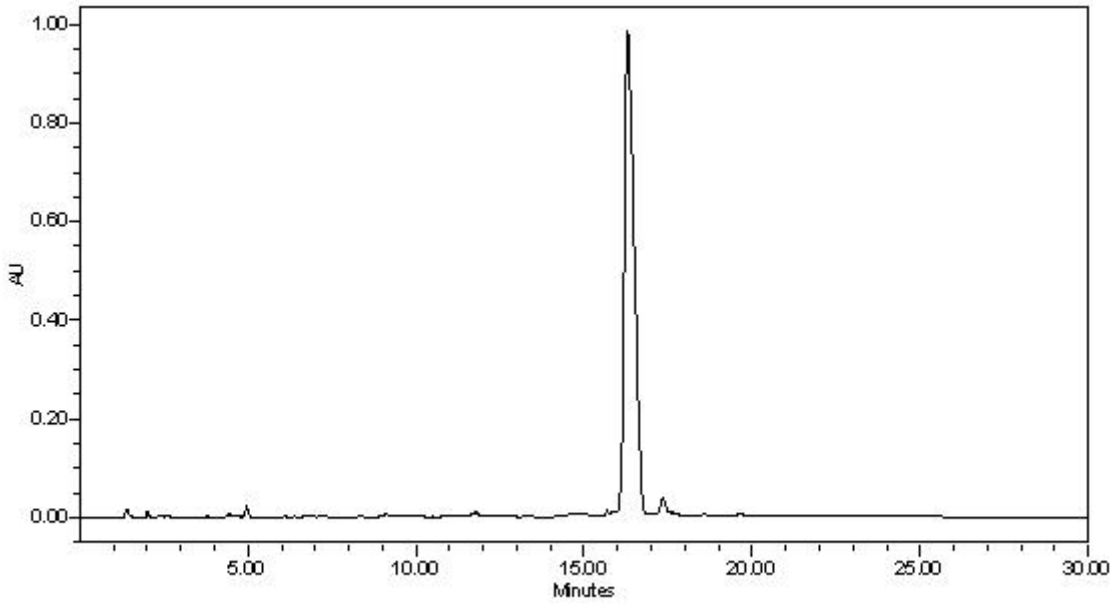
5'-r(GUG AUA UGC)-3' **28**

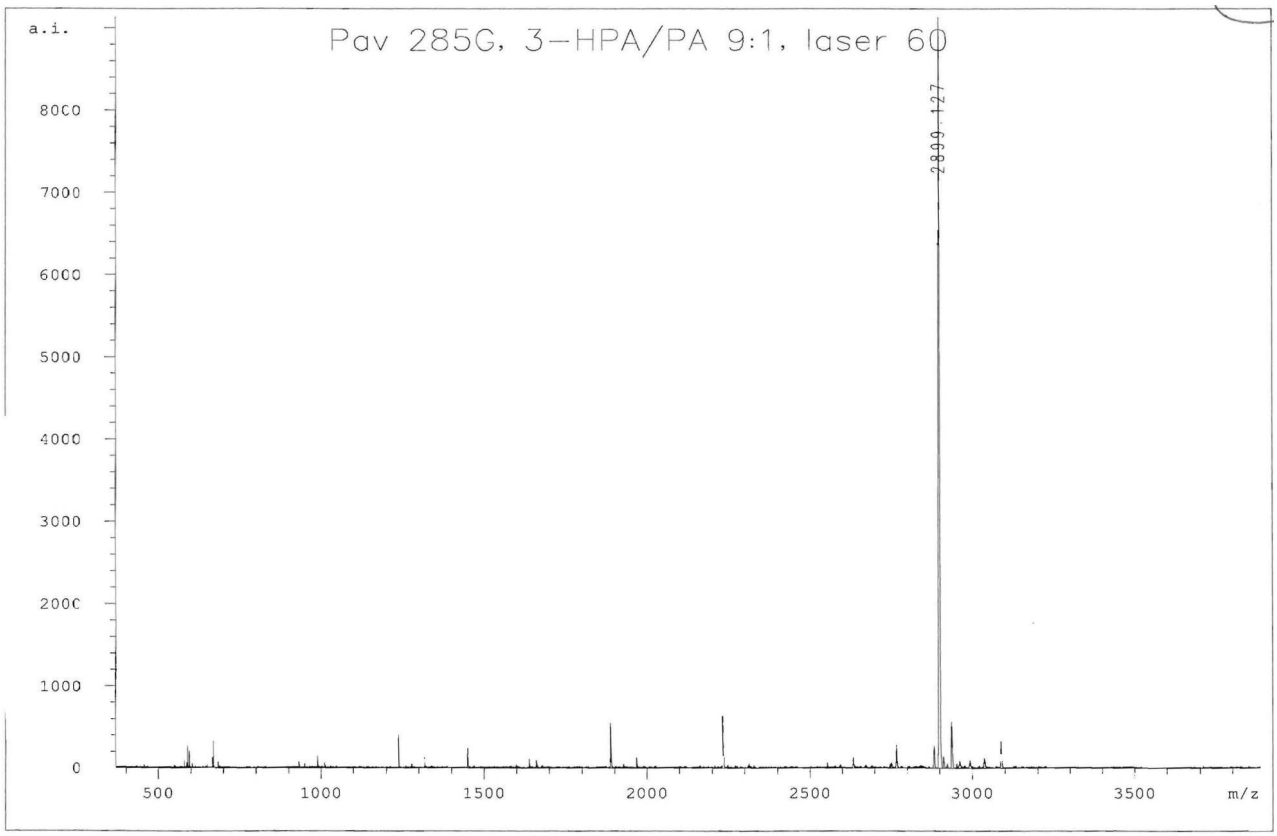




Calcd $[M+H]^+$ 2898.830; Found 2898.819
Isolated yield OD_{260} = 55.8 (62%).

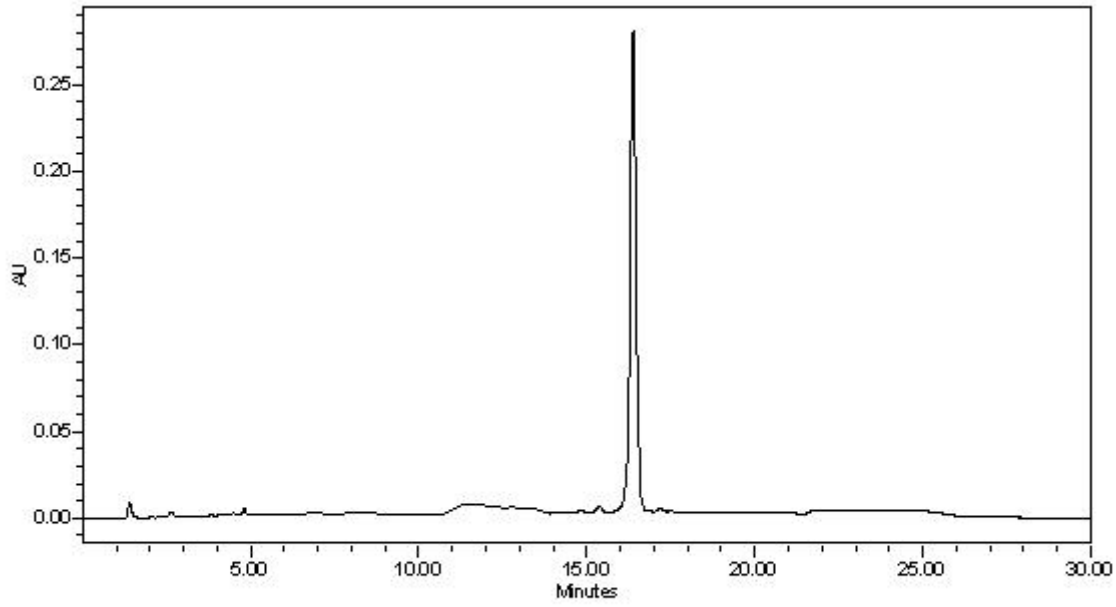
5'-r(GUG AUA UGC)-3' **29**

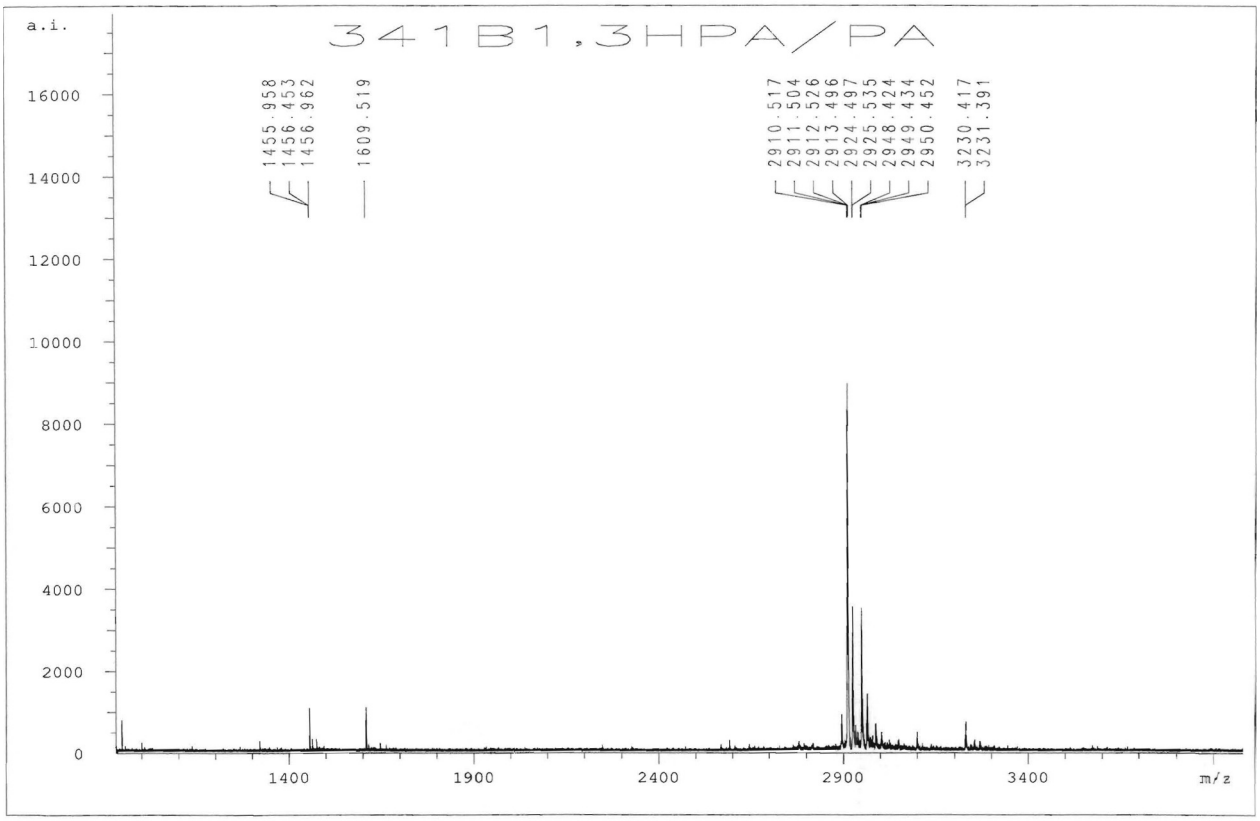




Calcd $[M+H]^+$ 2898.830; Found 2899.127
Isolated yield OD_{260} = 54.9 (61%).

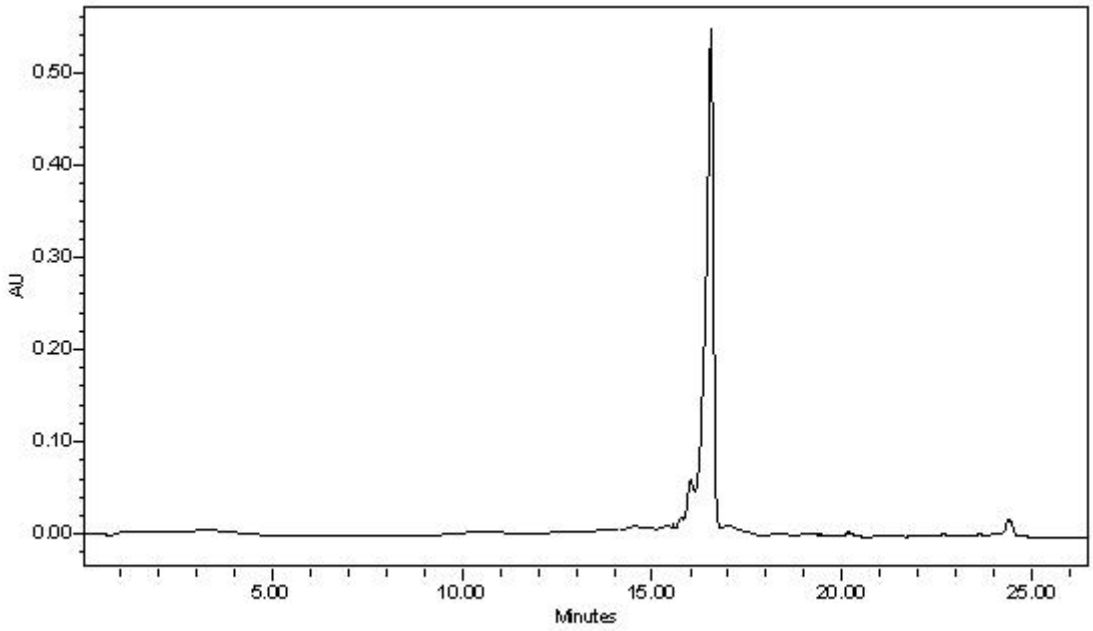
5'-r(GCA UAU CAC) -3' **30**

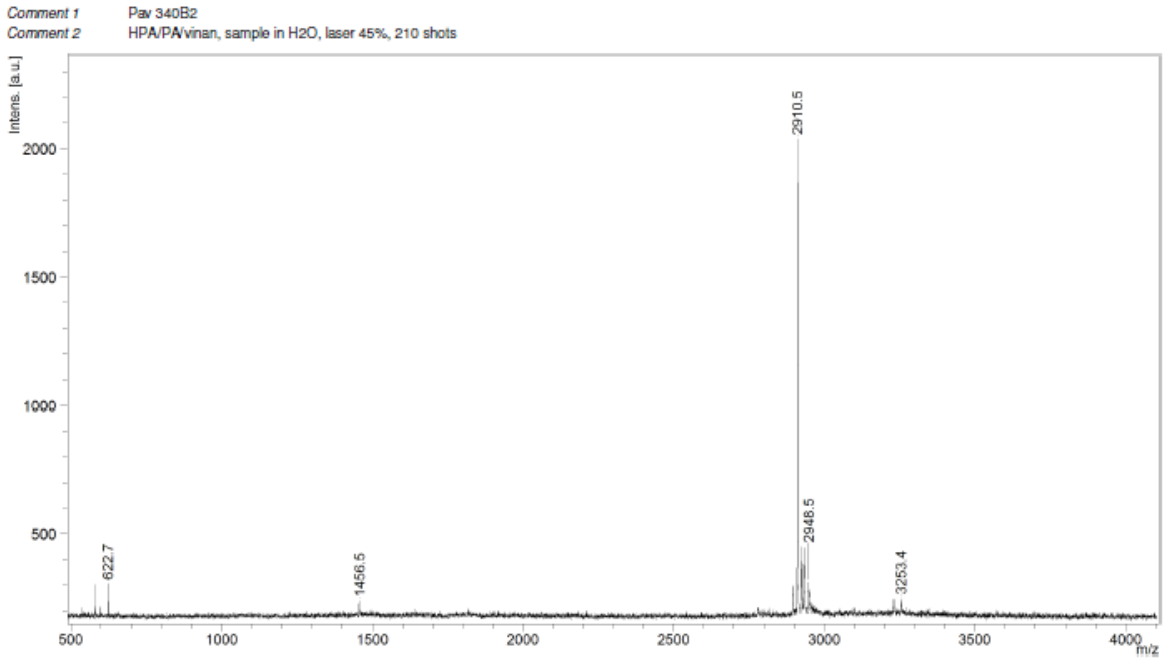




Calcd $[M+H]^+$ 2910.557; Found 2910.517
Isolated yield OD_{260} = 53.7 (61%).

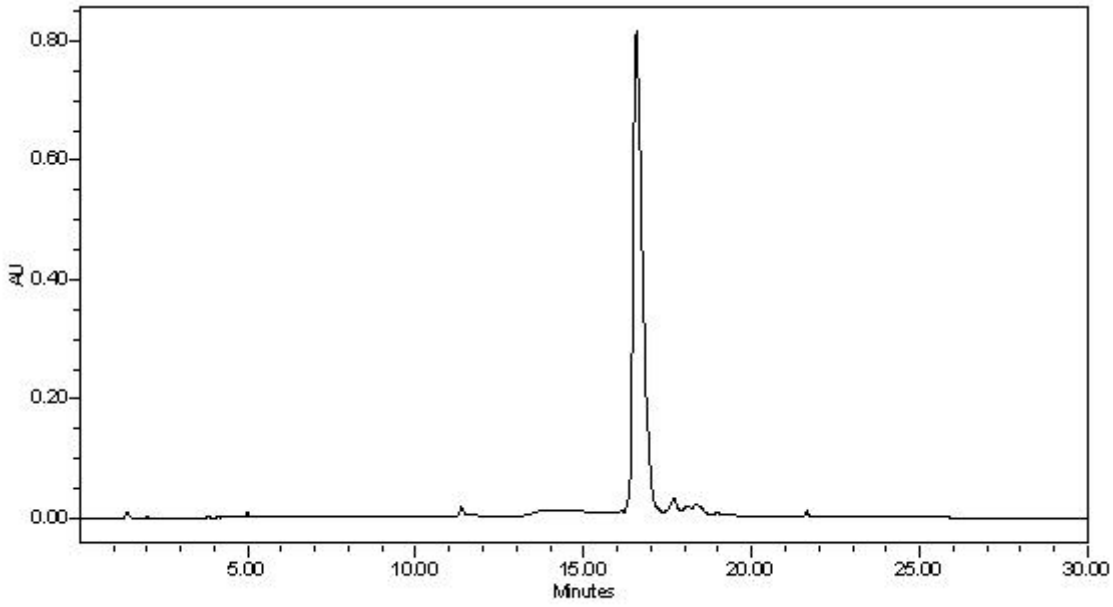
5'-r(GCA UAU CAC) -3' **31**

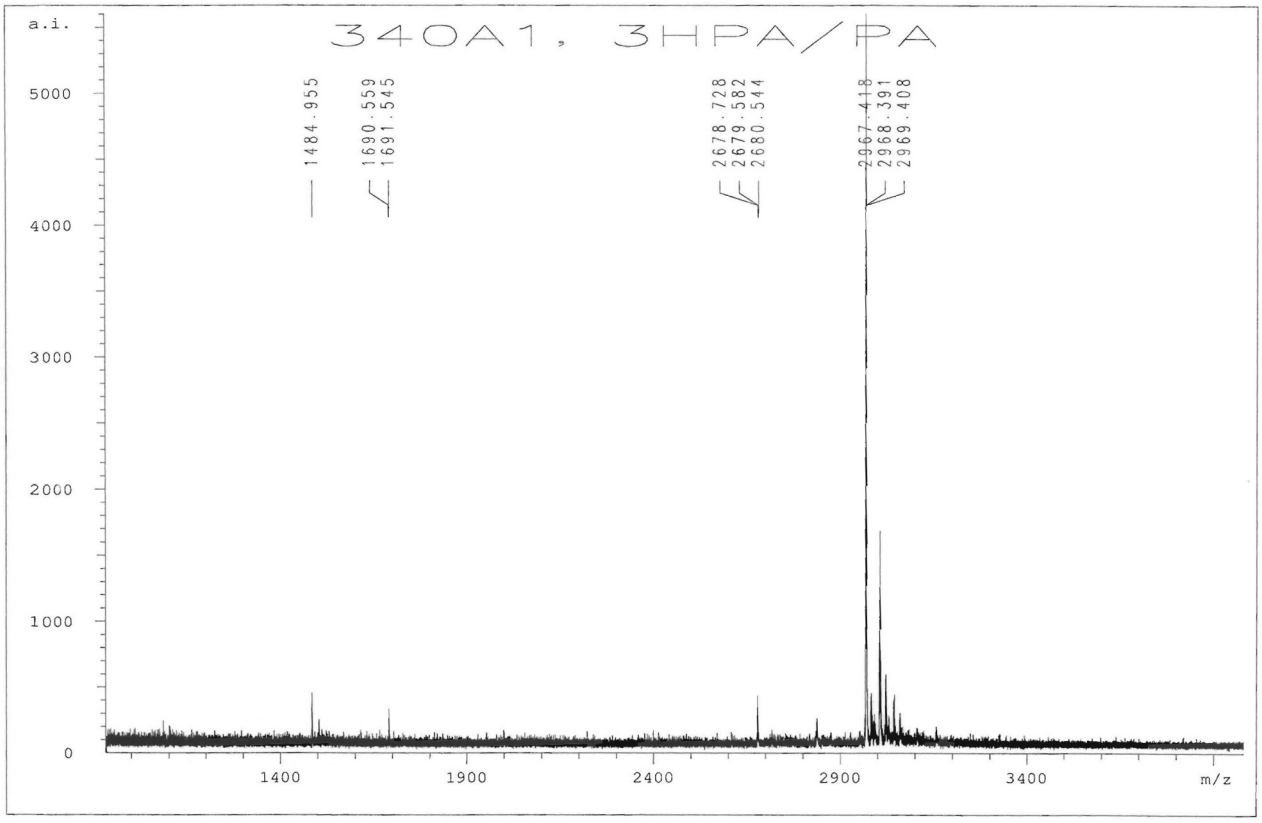




Calcd $[M+H]^+$ 2910.557; Found 2910.5
Isolated yield OD_{260} = 51.9 (59%).

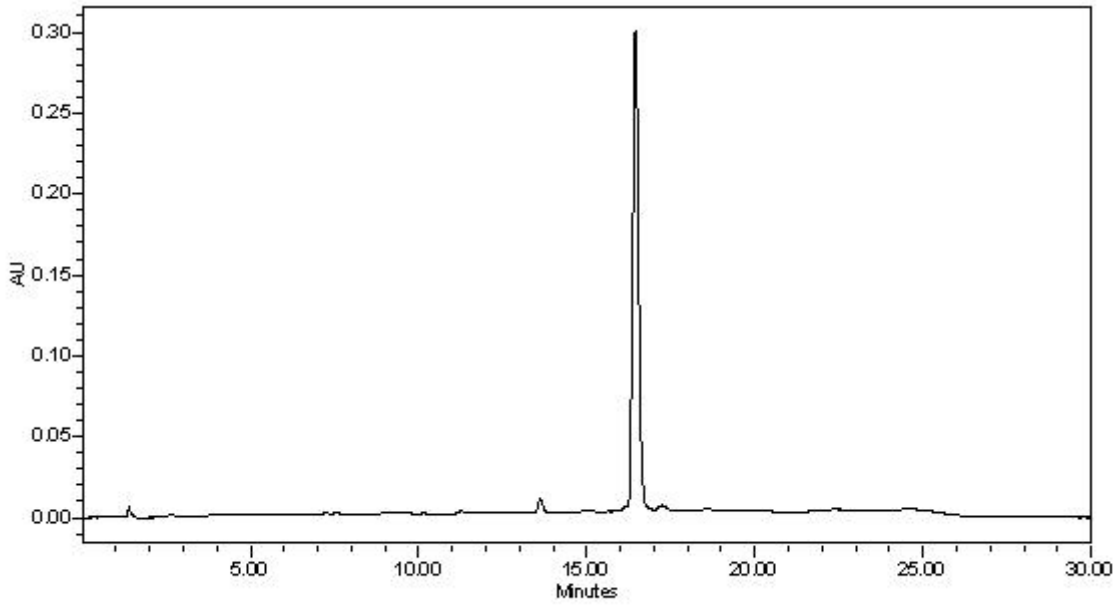
5'-r(GUG AUA UGC)-3' **32**

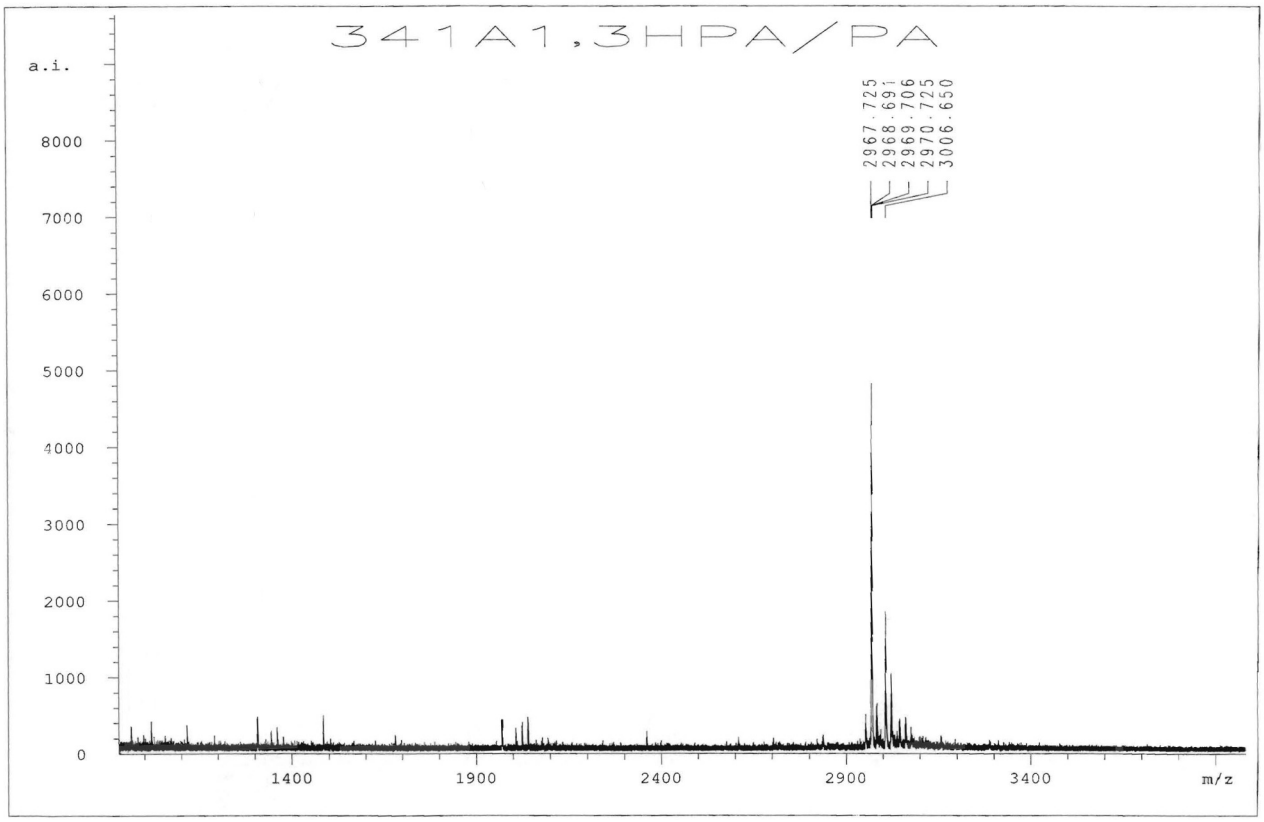




Calcd $[M+H]^+$ 2967.542; Found 2967.418
Isolated yield OD_{260} = 52.2 (58%).

5'-r(GUG AUA UGC)-3' **33**





Calcd $[M+H]^+$ 2967.542; Found 2967.725
Isolated yield OD_{260} = 49.5 (55%).

4. Measurement of thermal characteristics and T_m values

Measurement of thermal characteristics was performed on a CARY 100 Bio UV Spectrophotometer (Varian Inc.) equipped with a Peltier temperature controller and a thermal analysis software. The samples were prepared by mixing complementary strands together to give 4 μM final concentration in 50mM TRIS/HCl pH 7.2, 1mM EDTA, 100mM Na^+. A heating-cooling cycle in the 5-60°C temperature range with a gradient of 0.2°C/min was applied. T_m values were determined from the maxima of the first derivative plots of absorbance versus temperature ($T_m \pm 0.5$ °C).

Table 2 Hybridization properties of phosphonate-modified oligoribonucleotides.

3´-Phosphonate	Strands No.		Strands No.	**5´-Phosphonate**
T_m (°C) (ΔT_m/mod.)				T_m (°C) (ΔT_m/mod.)
37.1	**20/21**	5'-r(GCA UAU CAC)-3' / 5'-r(GUG AUA UGC)-3'	**20/21**	37.1
18.1 (-6.3)	**22/21**	5'-r(GC<u>A</u> U<u>A</u>U C<u>A</u>C)-3' / 5'-r(GUG AUA UGC)-3'	**23/21**	31.3 (-1.9)
26.2 (-5.5)	**24/21**	5'-r(GCA <u>UAU</u> CAC)-3' / 5'-r(GUG AUA UGC)-3'	**25/21**	34.2 (-1.5)
24.2 (-6.5)	**20/26**	5'-r(GCA UAU CAC)-3' / 5'-r(GUG <u>AUA</u> UGC)-3'	**20/27**	34.4 (-1.4)
23.3 (-4.6)	**20/28**	5'-r(GCA UAU CAC)-3' / 5'-r(G<u>UG</u> A<u>UA</u> <u>UGC</u>)-3'	**20/29**	32.3 (-1.6)
no T_m	**30/21**	5'-r(<u>GCA UAU CAC</u>)-3' / 5'-r(GUG AUA UGC)-3'	**31/21**	19.3 (-2.2)
no T_m	**20/32**	5'-r(GCA UAU CAC)-3' / 5'-r(<u>GUG AUA UGC</u>)-3'	**20/33**	19.9 (-2.2)

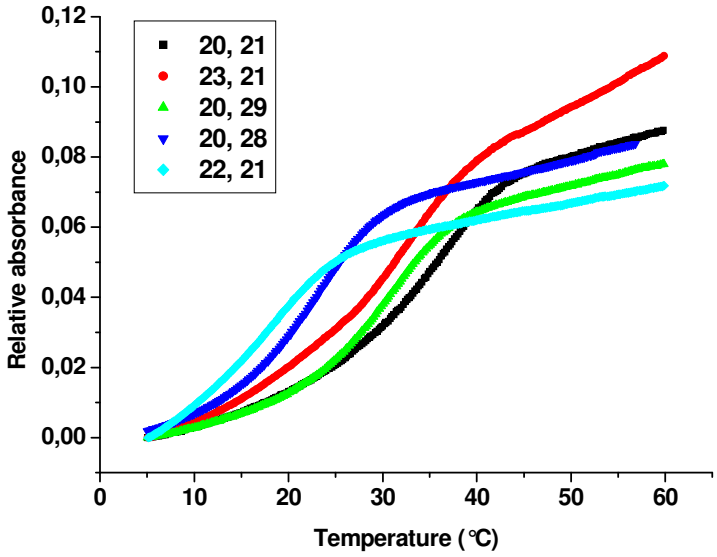


Figure 1 Preview of melting curves of phosphonate-modified duplexes.

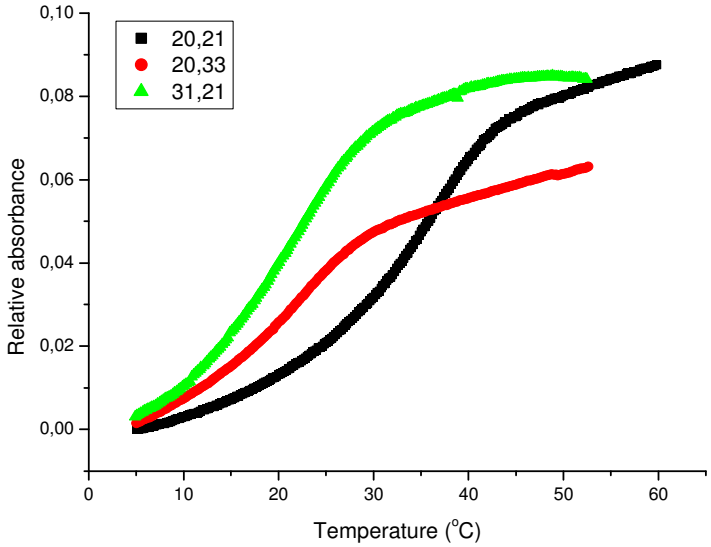


Figure 2 Preview of melting curves of fully phosphonate-modified duplexes.

5. Enzymatic digestion

Dimers **34**, **35** and nonamers **26-29** (25 μM) in 50mM TRIS/HCl pH 7.6, 10mM Mg^{2+} were incubated 2 h at r.t. with the appropriate nuclease (0.3 unit); ribonuclease T2 (EC 3.1.27.1), ribonuclease A (EC 3.1.27.5), phosphodiesterase I (EC 3.1.4.1), and phosphodiesterase II (EC 3.1.16.1). The reaction mixture was then analyzed by LC-MS at 260 nm.

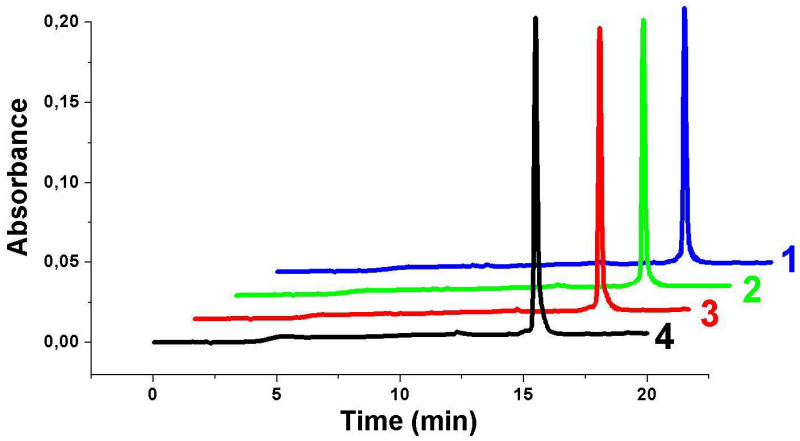


Figure 3 Stability of the 5'-phosphonate dimer A-p_cA **35** in the presence of (**1**) ribonuclease T2, (**2**) phosphodiesterase I, and (**3**) phosphodiesterase II. Record (**4**) is the A-p_cA blank after 2 hours of incubation in the cleavage buffer. Under experimental conditions, the half-life of the cleavage of phosphodiester dimer ApA was less than 1 minute (data not shown).

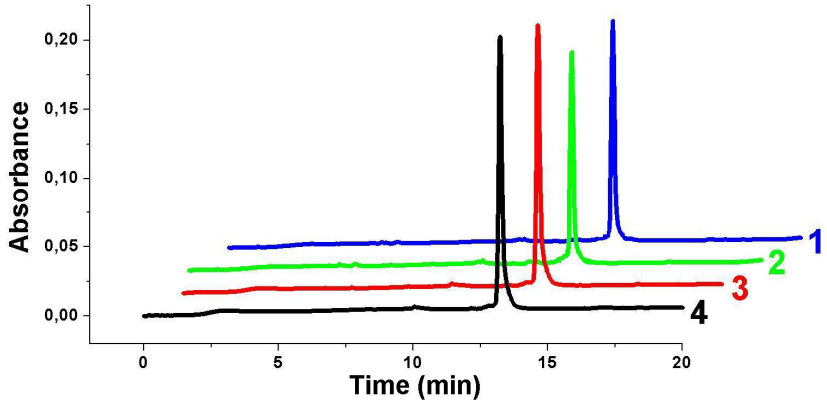


Figure 4 Stability of the 3'-phosphonate dimer Ap_c-A **34** in the presence of (**1**) ribonuclease T2, (**2**) phosphodiesterase I, and (**3**) phosphodiesterase II. Record (**4**) is the Ap_c-A blank after 2 hours of incubation in the cleavage buffer. Under experimental conditions, the half-life of the cleavage of phosphodiester dimer ApA was less than 1 minute (data not shown).

Stability of phosphonate modified nonamers **26-29** in the presence of ribonuclease A (EC 3.1.27.5), phosphodiesterase I (EC 3.1.4.1) and phosphodiesterase II (EC 3.1.16.1) has been examined. The course of the cleavage reaction has been monitored by LC-MS.
Ribonuclease A is an endoribonuclease which cleaves preferentialy 3'-end of uridine residues, leaving a 3'-phosphorylated product, via a 2',3'-cyclic monophosphate. Phosphodiesterase I and II are exonucleases. Phosphodiesterase I cleaves from 3'-end releasing 5'-nucleotide units. Phosphodiesterase II cleaves from 5'-end releasing 3'-nucleotide units.
Cleavage products of modified nonamers are shown in **Table 3**. Cleavage of natural nonamer **21** shows the expected cleavage products.

Table 3 Cleavage profile of modified nonamers.

	Oligonucleotide	Ribonuclease A	Phosphodiesterase I	Phosphodiesterase II
21	5'-r(GUGAUAUGC)-3'	GUp, GAUp, AUp, GC	pC, pG, pU, pA	Gp, Up, Ap, Cp
28	5'-r(GU*GAU*AU*GC)-3'	no cleavage	pC, pU*G, pU*A, pA, GU*G	Gp, U*GAU*AU*GC
29	5'-r(G*UGA*UA*UGC)-3'	G*Up, GA*Up, A*Up, GC	pC, pG, pA*U, G*UGA*U	G*Up, Gp, A*Up, A*UGC
26	5'-r(GUGA*UA*UGC)-3'	GUp, GA*Up, A*Up, GC	pC, pG, pA*U, GUGA*U	Gp, Up, A*Up, A*UGC
27	5'-r(GUG*AU*AUGC)-3'	GUp, G*AU*AUp, GC	pC, pG, pU, pU*A, GUG*A	Gp, Up, G*AU*AUGC

Modified units are underlined. A* and U* mean 3'-phosphonates; *A and *U mean 5'-phosphonates

Neither 3'-phosphonate (**28**) nor 5'-phosphonate (**27**) linkages were cleaved by ribonuclease A. We observed only complete cleavage of phosphodiester linkages. In the case of phosphodiesterase I and II, we observed an interesting cleavage pattern. The one-by-one cleavage of units occurred until the modified linkage was met. After that, the modified unit was omitted and the phosphonate dimer was cleaved, but neither the 3´-phosphonate nor 5´-phosphonate linkages were cleaved. Moreover, after this unexpected cleavage step, further cleavage did not continue in most cases (**26**-**29**).

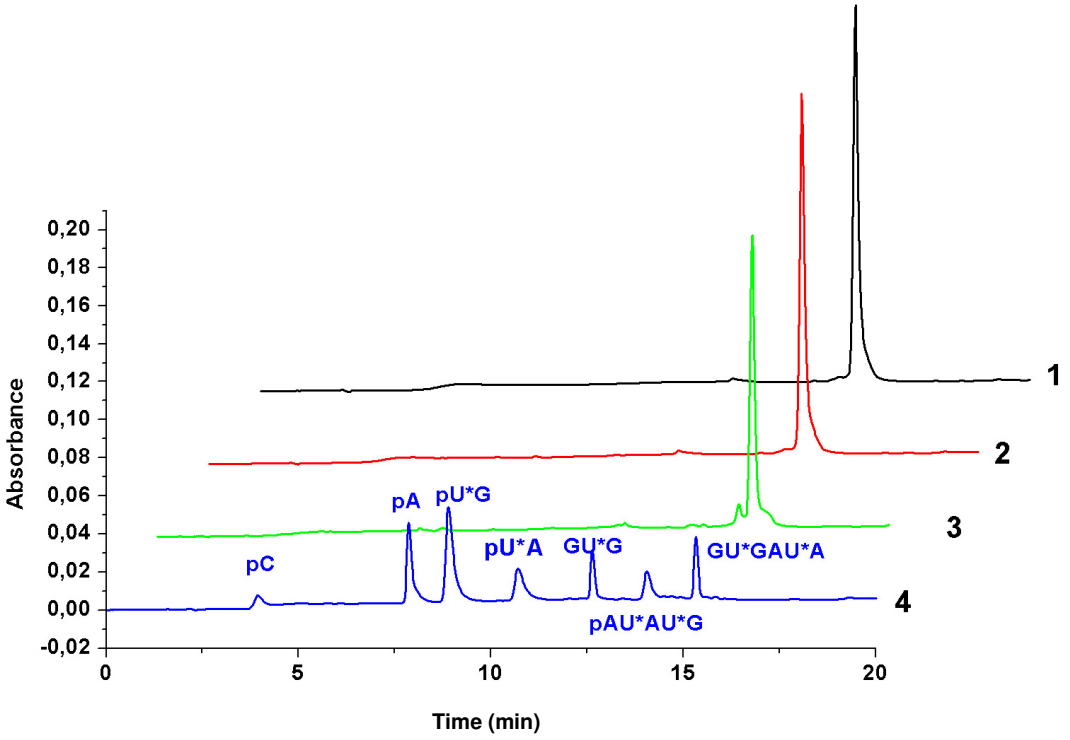


Figure 5 Cleavage profile (2 h) of nonamer 5'-r(GU*GAU*AU*GC)-3' **28;** (1) blank of **28**, (2) ribonuclease A, (3) phosphodiesterase II, and (4) phosphodiesterase I. [A→30%B 20 min (A 0.1M TEAB; B 50% CH_3CN/0.1M TEAB)].

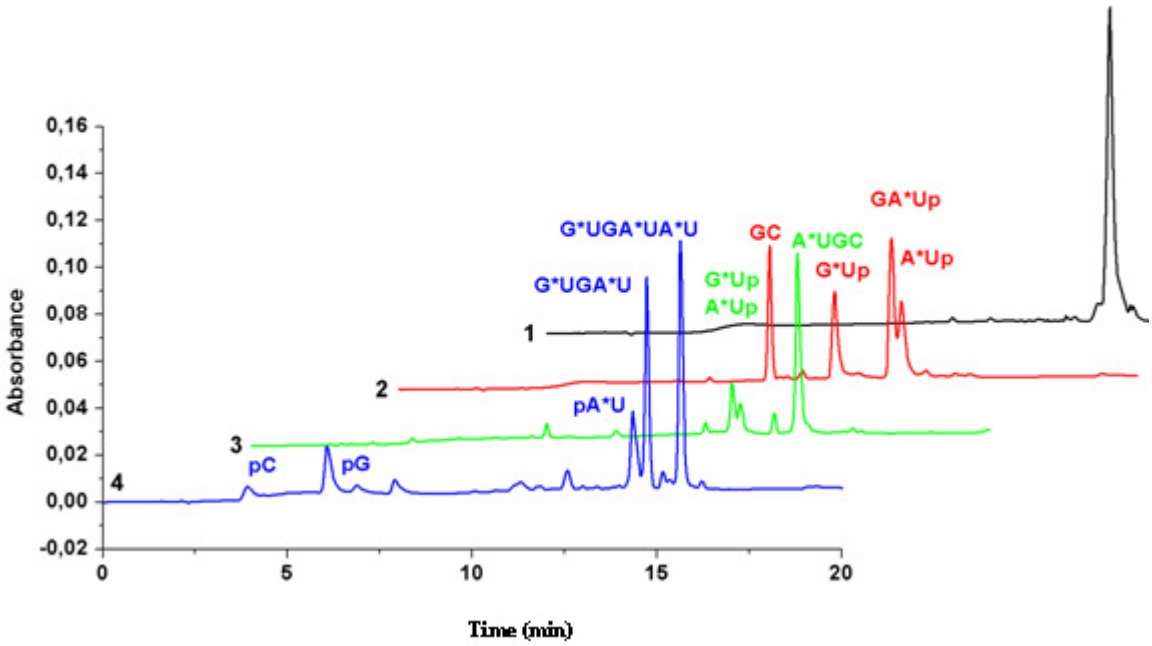


Figure 6 Cleavage profile (2 h) of nonamer 5'-r(G*UGA*UA*UGC)-3' **29**; (1) blank of **29**, (2) ribonuclease A, (3) phosphodiesterase II, and (4) phosphodiesterase I. [A→30%B 20 min (A 0.1M TEAB; B 50% CH_3CN/0.1M TEAB)].

6. NMR spectra and conformation of dinucleoside phosphonates

3´-Phosphonate dimer (Apc-A, **34**) and 5´-phosphonate dimer (A-pcA, **35**) were prepared as described in literature.[1] The ^{1}H and ^{13}C NMR parameters of compounds ApA(3´,5´), A-pcA(3´,5´) and Apc-A(3´,5´) are given in **Tables 4 - 6**.

The observed intraresidue NOE contacts between adenine H-8 and ribose H-1´, H-2´ and H-3´ protons indicate a preferred *anti* orientation of adenine of both 3´- and 5´-nucleoside units in all studied compounds. Interresidue NOE contacts were observed between adenine H-8 proton of 5´-residue and ribose H-1´, H-2´ protons of 3´-residue.

The ^{3}J(H,H)s and program PSEUROT[2,3] based on the concept of pseudorotation[4] were used to obtain the information on conformation of furanose ring. The NMR conformational studies show that with nucleotides in solution, mostly two preferred conformers (N-type and S-type) of the furanose ring are present in a fast equilibrium and can be fully described by five parameters (phase angles P(N), P(S), puckering amplitudes (ϕ_m(N), ϕ_m(S)) and the molar concentration of one conformer x(N) or x(S)).

Table 4 Proton NMR chemical shifts of ApA (3´-5´), A-pcA(3´-5´) and Apc-A(3´-5´) in D_2O.

Compound	Unit	H-1´	H-2´	H-3´	H-4´	H-5´	H-5''	H-2	H-8	P-CHaHb
ApA(3´-5´)	A	5.81	4.65	4.63	4.34	3.88	3.81	7.87	8.15	--
	A	5.93	4.54	4.48	4.36	4.35	4.15	8.02	8.21	--
A - p_cA(3´-5´)	A	5.83	4.71	4.84	~4.31	3.82	3.78	7.83	8.24	--
	p_cA	5.88	4.52	4.34	~4.31	3.95	3.88	7.95	8.08	3.88; 3.82
Ap$_c$ - A(3´-5´)	Ap$_c$	5.67	4.65	4.07	4.20	3.72	3.67	7.86	8.10	3.91; 3.79
	A	6.00	4.67	4.47	4.37	4.25	4.22	7.93	8.34	--

Table 5 Coupling constants J(H,H) and J(H,P) of ApA(3´-5´), A-pcA(3´-5´) and Apc-A(3´-5´) in D_2O.

		Proton-Proton							Proton-Phosphorus				
Compound	**Unit**	**1´,2´**	**2´,3´**	**3´,4´**	**4´,5´**	**4´,5''**	**5´,5''**	**Ha,Hb**	**3´,P**	**5´,P**	**5'',P**	**Ha,P**	**Hb,P**
ApA(3´-5´)	A	3.7	~5.0	~5.0	2.5	3.6	13.0	--	~9.0			--	--
	A	4.1	5.1	5.5	~3.0	~3.8	12.2	--		3.9	3.8	--	--
A - p_cA(3´-5´)	A	5.0	5.0	4.6	~2.6	3.4	13.1	--	8.6			--	--
	p_cA	4.4	5.0	5.7	2.4	4.9	11.3	13.4				9.6	8.3
Ap$_c$ - A(3´-5´)	Ap$_c$	6.8	5.0	2.6	3.1	3.5	12.9	13.3				9.5	9.2
	A	5.15	5.3	4.55	2.8	3.6	11.7	--		4.3	3.6	--	--

Table 6 Carbon-13 chemical shifts and coupling constants J(C,P) of ApA(3´-5´), A-pcA(3´-5´) and Apc-A(3´-5´) in D_2O.

Compound	Unit	C-1´	C-2´ (JC2´,P)	C-3´ (JC3´,P)	C-4´ (JC4´,P)	C-5´ (JC5´,P)	P-C (J(C,P)	C-2	C-4	C-5	C-6	C-8
ApA(3´-5´)	A	87.23	72.55 (3.7)	73.09 (5.4)	83.24 (5.3)	60.23	--	151.55	147.76	118.26	154.58	138.51
	A	88.77	73.88	68.98	82.37 (9.4)	64.04 (5.4)	--	152.12	147.13	117.78	154.45	139.47
A - p_CA(3´-5´)	A	87.78	72.38 (~3.5)	73.04 (~5.0)	83.96 (~2.0)	60.33	--	151.75	147.62	117.97	154.47	138.96
	p_CA	87.24	73.85	69.26	82.02	71.68 (13.2)	66.16 (161.0)	151.99	147.33	117.68	154.20	139.38
Ap_C - A(3´-5´)	Ap_C	86.92	72.69	79.85 (13.2)	82.61	61.16	64.10 (158.2)	151.78	148.08	118.22	154.76	139.10
	A	87.01	73.93	69.72	83.21 (7.8)	63.73 (5.8)	--	152.05	147.71	117.74	154.55	139.62

Since there are only three ^3J(H,H)s in the furanose ring in this case, we made our PSEUROT calculation supposing that $\phi_m(N) = \phi_m(S)$ and changing their values in 2° steps over the range 30° to 46° calculating P(N), P(S) and x(N) to obtain best fit between the calculated and observed ^3J(H,H) values (rms < 0.001). The results are summarized in **Table 7**. All calculated conformers have ϕ_m = 34° to 40° and both N- and S-type are comparably populated in a fast equilibrium. In some cases (both residues of ApA(3´,5´) and 3´-residue of A-pcA(3´,5´)) calculations give two rather different possible combinations of N- and S-conformers. The detailed study of ApA(3´,5´) in our paper[5] showed that conformers with negative P(N) give better agreement with temperature dependence of *J*-values.

Table 7 The calculated pseudorotation parameters for ApA(3´,5´), A-pcA(3´,5´) and Apc-A(3´,5´)

Compound	3´-end sugar					5´-end sugar				
	Φm(N) Φm(S)	P(N)	x(N)	P(S)	x(S)	Φm(N) Φm(S)	P(N)	x(N)	P(S)	x(S)
ApA(3´-5´)	36	26 (^{3}E)	0.52	206 ($^4T_3$)	0.48	36	37 ($^3T_4$)	0.58	193 ($_3$E)	0.42
	38	-26 ($_2$E)	0.68	138 ($^2T_1$)	0.32	40	-25 ($_2$E)	0.62	123 ($_1$E)	0.38
A - p_CA(3´-5´)	38	-18 (^{3}E)	0.50	138 ($^2T_1$)	0.50	38	41 ($^3T_4$)	0.59	188 ($^2T_3$)	0.41
	40	49 ($_4$E)	0.45	202 ($_3$E)	0.55	40	46 ($^3T_4$)	0.58	198 ($_3$E)	0.42
Ap_C - A(3´-5´)	36	-22 ($_2$E)	0.27	153 ($^2T_1$)	0.73	36	-19 ($_2$E)	0.47	133 ($_1$E)	0.53
	40	-38 ($^1T_2$)	0.32	145 ($^2T_1$)	0.68	38	-26 ($_2$E)	0.48	125 ($_1$E)	0.52

There are rather limited information about conformation of the internucleotide linkage from vicinal coupling constants *J*(H,H), *J*(H,P) and *J*(C,P). For each studied dinucleotide there are always only tree torsion angles related to observable vicinal couplings of internucleotide linkage (from five torsion angles in ApA(3´,5´) or six torsion angles in A-pcA(3´,5´) and Apc-A(3´,5´)). Depending on the dinucleotide structure there are torsion angles ε and β in ApA(3´-5´), angles ε and α´ in A-pcA(3´-5´), angles ζ´ and β in Apc-A(3´-5´) and angle γ in all three dinucleotides. The torsion angles are shown together with corresponding *J*-values in **Figure 7**. For estimation of torsion angles or rotamer populations the Karplus type relations given in a review paper of Wijmenga and van Buuren[6] have been used. Small values of ^3J(H4´/H5´) and ^3J(H4´/H5´´) in C5´-residues indicate a preference of *gauche*$^+$ rotamer (67%, 62% and 71%) with angle γ about +60°. The values of *J*(P,C4´), *J*(P,H5´ and *J*(PH5´´) lead to a high preference (> 80%) of *trans*-conformation around O-C5´ bond (angle β) in both ApA(3´,5´) and Apc-A(3´,5´) dinucleotides.

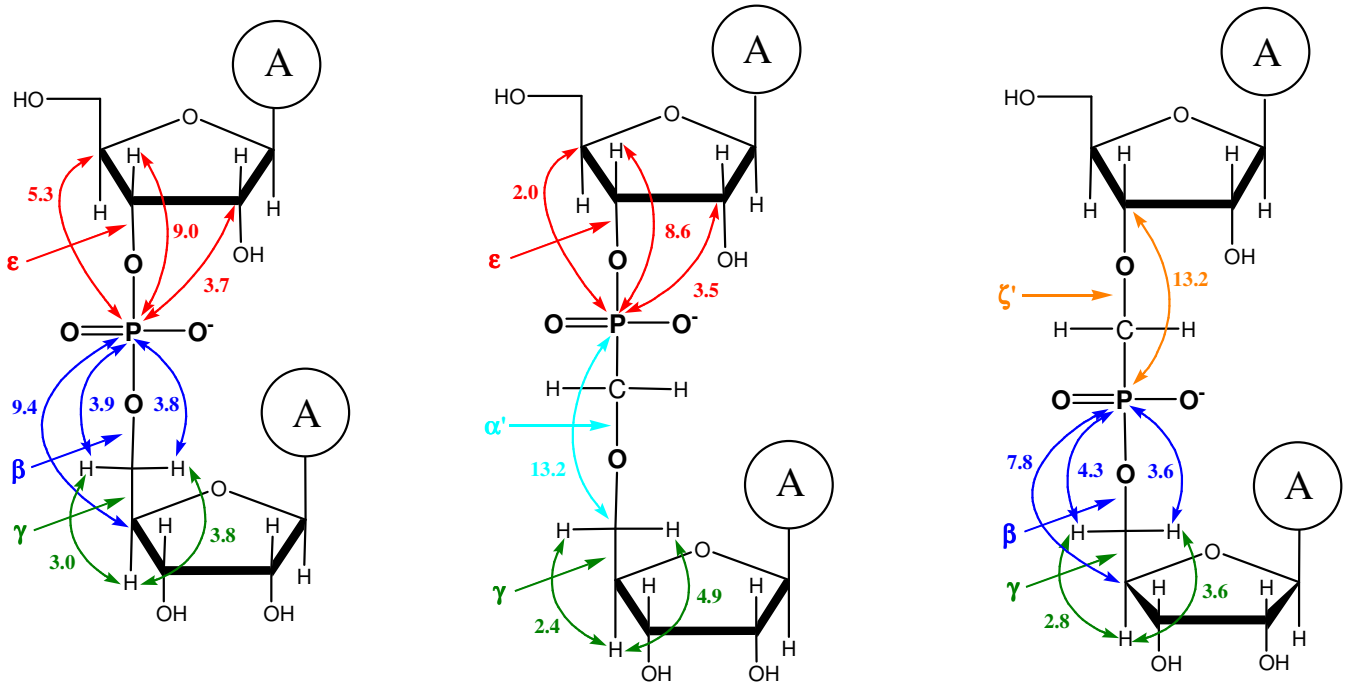


Figure 7 Schematic structures of dinucleotides ApA(3´,5´), A-pcA(3´,5´) and Apc-A(3´,5´). Torsion angles related to vicinal couplings in the internucleotide linkage are defined and distinguished by colors.

Multiple torsion angles ε can be derived from periodical Karplus type relations for *J*(P,H4´), *J*(P,C2´) and *J*(P,C4´) with best agreement on the angle ε around -120. For torsion angles α´ and ζ´ (that do not appear in "normal" nucleotides) a very high value of corresponding couplings *J*(P,C5´) and *J*(P,C3´) = 13.2 Hz indicate *trans*-arrangement (α´ and ζ´ around 180°). Comparison of internucleotide torsion angles and base orientation derived from vicinal couplings with those obtained by molecular modeling of analogous fragment in duplex oligonucleotide structures is given in **Table 8**. It can be seen that for 3CP5 dinucleotide (3´-phosphonate) there is a good semiquantitative agreement of all comparable

torsion angles but for 3PC5 (5´-phosphonate) dinucleotide torsion angles α´ and γ are significantly different.

Table 8 Internucleotide torsion angles and base orientation. Comparison of values estimated from accessible coupling constants with those obtained by molecular modeling of analogous fragment in duplex oligonucleotide structures (given *in italics*)

Compound	**ε**	**ζ**	**ζ´**	**α**	**α´**	**β**	**γ**	**χ1/χ2**
A-pcA(3´,5´) = 3PC5	~ -120	*	--	*	~180	*	~ 60	anti / anti
C-pcA(3´-5´)	*-166*	*-119*	--	*58*	*97*	*-166*	*-167*	*-142 / -169*
Apc-A(3´,5´) = 3CP5	*	--	~180	*	*	~ 180	~ 60	anti / anti
Apc-C(3´-5´)	*-100*	--	*-160*	*55*	*-80*	*154*	*53*	*179 / -136*

* can not be estimated from obtained vicinal couplings

EXPERIMENTAL

The NMR spectra were measured on Varian UNITY-500 (^{1}H at 500 MHz and ^{13}C at 125.7 MHz) in D_2O at 25 °C. Proton chemical shifts are referenced to residual solvent peak using δ(HDO) = 4.80 and carbon-13 chemical are referenced to DSS. Structural assignment of proton and carbon signals was done using the 2D-H,H-COSY, 2D-H,C-HMQC and 2D-H,C-HMBC spectra.

REFERENCES

[1] M. Presova, M. Endova, Z. Tocik, R. Liboska, I. Rosenberg, *Bioorg. Med. Chem. Lett.*, 1998, 8, 1225-30.
[2] J. van Wijk, C. A. G. Haasnoot, F. A. A. M. de Leeuw, B. D. Huckriede, A. Westra Hoekzema, C. Altona, *PSEUROT 6.2 1993, PSEUROT 6.3 1999*. Leiden Institute of Chemistry, Leiden University.
[3] F. A. A. M. de Leeuw, C. Altona, *J. Comput. Chem.,* 1983, *4*, 428.
[4] C. Altona, M. Sundaralingam, *J. Am. Chem. Soc.,* 1972, *94*, 8205.
[5] Z. Vokáčová, M**.** Buděšínský, I. Rosenberg, B. Schneider, J. Šponer, V. Sychrovský, *J.Phys.Chem. B,* 2009*, 113*, 1182.
[6] S.S. Wijmenga, B.N.M. van Buuren, *Progr. NMR Spectr.,* 1998, *32*, 287.

7. MD simulations

Methodology

Initial duplex structures were constructed using the BIOPOLYMER module from the BIOSYM software package. The CH2 groups were inserted into the backbone manually. Produced coordinates were used as input files for the AMBER software package. Explicit net neutralizing Na^+ counterions were placed at the phosphates of the oligonucleotide by the EDIT module of AMBER. The nucleic acid with the Na+ counterions were surrounded by a periodic box of ~8.500 TIP3P water atoms, which extended approximately 10 A˚ (in each direction) from the nucleic acid atoms. This leads to a periodic box size of ~60 A˚ by ~ 40 A˚ by ~40 A˚ .

The AMBER force fields parameters described by Cornell et al.[1] do not contain explicit force constants needed to describe the modified parts of the 3CP5 and 3PC5 analogs. The completion was made as in previous MD simulation[2] on the basis of ab initio calculations on the MMP (methyl methoxymethyl phosphonate) molecule and using recommended standard procedures[3]. MMP stretching and bending force constants as well as corresponding equilibrium values of bond lengths and angles and partial charges were presented earlier[4,5]. Missing torsion force constants were gained by the same way as for the phosphodiester linkage in the original AMBER forcefield[1]. The total energy of MMP was computed (ab initio MP2/6-31 G*) in the whole interval of the OPCO, COPC, and PCOC backbone torsion angles and the resulting energy profile was fitted on the AMBER formula for torsion energy terms[2] .

Fully solvated trajectories were computed by using the SANDER module of the AMBER software package. The implemented Particle Mesh Ewald (PME) summation method of electrostatic interactions enabled unrestrained MD simulations of fully solvated nucleic acids to be reached in a nanosecond regime[6]. Usual computational procedures and equilibration protocol were used: periodic boundary conditions, cut off distances of 9A^o for the nonbonded interactions, MD time step = 0.002 ps. Initially, for 10 ps, the system was heated up to 300/350K. MD simulations were then continued at constant T and constant P for 10 ns.

Figures were produced using the CHIMERA software package[7].

Results

Systematically higher melting temperatures were determined for oligonucleotides with the 5´-phosphonate 3PC5 internucleotide linkages (comparing to 3´-phoshonate 3CP5). In order to shed some light on the above mentioned experimental findings, we performed molecular dynamic simulations at the atomic level with the explicit inclusion of water molecules into the simulated system. Overall, four different duplexes were studied (always at temperatures of 300 K and 350 K). The length of each molecular dynamics simulation was 10 ns (a total of 80 ns were accumulated).

MD simulations produced at elevated temperature of 350K helped to speed up transitions between different conformers of modified internucleotide linkages what lead to a better and faster sampling of conformational spaces for internucleotide linkages. This enabled

to identify structural factors (interstrand hydrogen bonds) contributing to higher melting temperatures of the 3PC5 oligonucleotides.

It was not our ambition to follow the entire process of melting or even to make a quantitative comparison of *in silico* detected melting temperatures. This would require population of trajectories with a length of several hundreds of nanoseconds as Watson-Crick base pairs were quite stable even at elevated 350 K temperature here. We observed only the first signs of the melting process (see MD4 in **Figure 10** – **20/26** duplex bearing the 3CP5 internucleotide linkages and showing weak hybridization in experiment).

Detail views of preferred conformers of the 3PC5/3CP5 internucleotide linkages are depicted in **Figure 8**. Pictures of whole duplexes are shown in **Figures 9**, **10**. Graphs illustrating conformational transitions observed within MD simulations and discussed in following paragraphs are shown in **Figures 11, 12, 13**. Conformational behavior of modified internucleotide linkages was captured through the conformational preferences of C3´-O3´-ζ´-C-α-P-α´-O5´-C5´ and/or C3´-O3´-ζ-P-α-C-α´-O5´-C5´ (**Figure 12**, for notation see **Figure 7** in part NMR spectra and conformation of dinucleoside phosphonates). In addition, there are graphs illustrating the time evolution of the Watson-Crick base pairing, where the hydrogen bonds occassionally reversibly break and back together (**Figure 11**). Distances between atoms involved in the **2´OH..O5´** intrastrand hydrogen bonds are measured in **Figure 13**. These hydrogen bonds were found to be extremely vital in the case of the 3PC5 internucleotide linkages.

MD1 - 3CP5 (3´-phosphonate) - 12 duplex 22/21 - 300K - A3-U4 A5-U6 A8-C9

The **C9:G10** (terminal base pair) Watson-Crick hydrogen bonding has been observed to interrupt repeatedly (**Figure 11**). This was not observed in any other 300K trajectory presented here. On the other hand, it should be noted that the chain no. **22** is a little bit disadvantaged comparing to others. The modified internucleotide linkage is located between the terminal (**C9**) and penultimate (**A8**) base. In other oligonucleotides, modified internucleotide linkages were located either between the second and third bases or in the central part of oligonucleotides.

The **A3-U4, A5-U6, A8-C9** modified internucleotide linkages preferred various conformations including **tg-g** depicted on **Figure 8**. Although the **t-g-g** conformation (the most vital one at 350K – see below) was temporarily realized here as well, it survived only shortly at 300 K.

As one of the factors obstructing completion of the **t-g-g** transition was found the **2´OH..O5´** intrastrand hydrogen bond. The **2´OH..O5´** atoms were repeatedly getting into the mutual distances <2A^{o}. However, in the case of the 3PC5 internucleotide linkages the hydrogen bonds were much more stable (see below).

MD2 - 3CP5 (3´-phosphonate) - 14 duplex 20/26 - 300K - A13-U14 A15-U16

The Watson-Crick hydrogen bond net was completely stable (**Figure 11**). The modified internucleotide linkages adopted the **tg-g** conformation (**Figure 8**)

MD3 - 3CP5 (3´-phosphonate) - 12 duplex 22/21 - 350K - A3-U4 A5-U6 A8-C9

The C9:G10 terminal base pair (which showed signs of instability already in MD1 - at 300 K) was disintegrated for a significant part of MD3.

Apparently this was related to the initial suboptimal tg-g conformation of the A8-C9 internucleotide linkage. After the transition to the t-g-g conformation, the C9:G10 Watson-Crick base pair was restored again. Certain signs of instability appeared at the very end of the MD3 simulation - again in coincidence with transient occurrences of the **tg-g** conformer. Remaining modified internucleotide linkages (**A3-U4, A5-U6**) showed similar conformational variability - ie oscillations between the **t-g-g** (major) and **tg-g** (minor) conformers (**Figure 10, 11**).

If the **t-g-g** conformer was realized then the intrastrand **2´OH..O5´** hydrogen bond was not observed.

MD4 - 3CP5 (3´-phosphonate) - 14 duplex 20/26 - 350K - A13-U14 A15-U16

At the very end of the MD4 simulation, synchronized disruption of the Watson-Crick hydrogen bonds was observed in four consecutive base pairs (**U4:A15, A5:U14, U6:A13, C7:G12**) in the central part of the duplex, where two modified internucleotide linkages **A13-U14** and **A15-U16** were located. **Figure 10** clearly shows that the sugar phosphate backbone is severly deformed after this episode.

The same, what was stated in the case of MD3, is true regarding the conformational preferences of the modified internucleotide linkages and the **2´OH..O5´** intrastrand hydrogen bond.

MD5 - 3PC5 (5´-phosphonate) - 12 duplex 23/21 - 300K - C2-A3 U4-A5 C7-A8

In this and all subsequent 3PC5 MD simulations, transitions of modified internucleotide linkages to the **–ggg** conformation were observed sooner or later (**Figure 8**). It was accompanied by remarkable stabilization of intrastrand **2´OH..O5´** hydrogen bonds. Only the C2-A3 internucleotide linkage remained in the **-g-gt** conformation with a destabilized intrastrand 2´OH..O5´ hydrogen bond.

MD6 - 3PC5 (5´-phosphonate) - 14 duplex 20/27 - 300K - G12-A13 U14-A15

The Watson-Crick hydrogen bond net was stable (**Figure 11**), the **G12-A13, U14-A15** modified internucleotide linkages preferred the **-ggg** conformation stabilized by the **2´OH..O5´** intrastrand hydrogen bonds. The **U14-A15** internucleotide linkage underwent transition to **-ggg** a little bit later, thus it was uncovered that the intermediate **-ggt** conformation is compatible with the intrastrand **2´OH..O5´** hydrogen bond as well.

MD7 - 3PC5 (5´-phosphonate) - 12 duplex 23/21 - 350K - C2-A3 U4-A5 C7-A8

The Watson-Crick hydrogen bond net was stable (**Figure 11**), the modified internucleotide linkages **C2-A3, U4-A5** and **C7-A8** passed into the **-ggg** conformation which was stabilized by intrastrand **2´OH..O5´** hydrogen bonds. Some oscillations into the short-living **–ggt** and **-g-gt** conformations were observed too.

MD8 - 3PC5 (5´-phosphonate) - 14 duplex 20/27 - 350K - G12-A13 U14-A15

The Watson-Crick hydrogen bond net was stable (**Figure 11**), modified internucleotide linkages **G12-A13**, **U14-A15** adopted the **-ggg** conformation stabilized by the **2´OH-O5´** intrastrand hydrogen bonds. The conformational change of the **U14-A15** internucleotide linkage to **-ggg** caused switch of the **G12-A13** internucleotide linkage into an atypical **-gt-g** conformation (accompanied by a temporary interruption of the intrastrand **2´OH..O5´** hydrogen bonding), but shortly afterthat the **U14-A15** internucleotide linkage relaxed into the ususal **-ggg** conformation.

Summary

In conclusion, the intrastrand **2´OH..O5´** hydrogen bonds were very vital in the case of the **3PC5** (5´-phosphonate) internucleotide linkages leading to prevalence of the **–ggg** conformers and causing higher melting temperatures. In contrast, the **3CP5** (3´-phosphonate) internucleotide linkages preferred conformations in which the intrastrand hydrogen bonds were not realized.

References

[1] W. D. Cornell, P. Cieplak, Ch. I. Bayly, I. R. Gould, K. M. Merz, Jr., D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell, and P. A. Kollman, J. Am. Chem. Soc. **1995**, 117, 5179-5197.
[2] Barvík Jr. I., Štěpánek J., Bok J., Journal of Biomolecular Structure & Dynamics**, 2002**, vol. 19, p. 863-875. Barvík Jr. I., Štěpánek J., Bok J., Computer Physics Communications**, 2002**, vol. 147, 158-161. Hanuš J., Barvík Jr. I., Štěpánek J., Turpin P.–Y., Bok J., Rosenberg I., Petrová M., Nucleic Acid Research, **2001**, vol. 29, 5182-5194. Barvík Jr. I., Štěpánek J., Bok J., Czechoslovak Journal of Physics**, 1998**, vol. 48, 409-415. Z. Točík, M. Buděšínský, I. Barvík, I. Rosenberg, Biopolymers **2009**, 91, 514-29.
[3] J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman, D. A. Case, J. Comput. Chem. **2004**, 25, 1157-1174
[4] Florian, J.; Strajbl, M.; Warshel, A., J Am Chem Soc **1998**, 120 (31), 7959–7966.
[5] Strajbl, M.; Florian, J., J Biomol Struct Dyn **1996**, 13 (4), 687–694.
[6] T. Darden, D. York and L. Pedersen, J. Chem. Phys. **1993**, 98 (12), 10089-10092
[7] E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, T. E. Ferrin, J. Comput. Chem. **2004**, 25(13), 1605-1612

Table 9: Summary of simulated systems

MD1)	3CP5	12: **22**/21	300K 10ns
MD2)	3CP5	14: 20/**26**	300K 10ns
MD3)	3CP5	12: **22**/21	350K 10ns
MD4)	3CP5	14: 20/**26**	350K 10ns
MD5)	3PC5	12: **23**/21	300K 10ns
MD6)	3PC5	14: 20/**27**	300K 10ns
MD7)	3PC5	12: **23**/21	350K 10ns
MD8)	3PC5	14: 20/**27**	350K 10ns

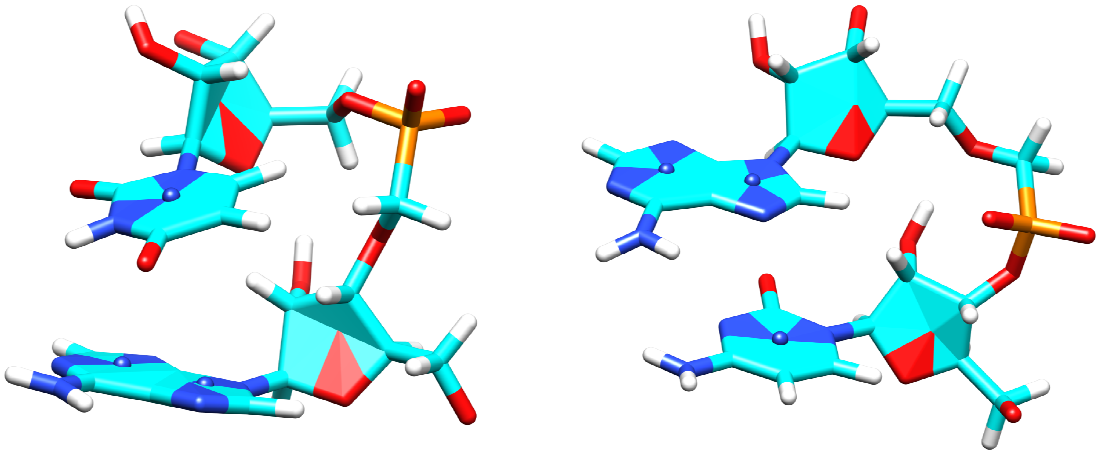


Figure 8: Conformational preferences of the 3CP5 (3´-phosphonate, left, tg-g) and 3PC5 (5´-phosphonate, right, -ggg) internucleotide linkages.

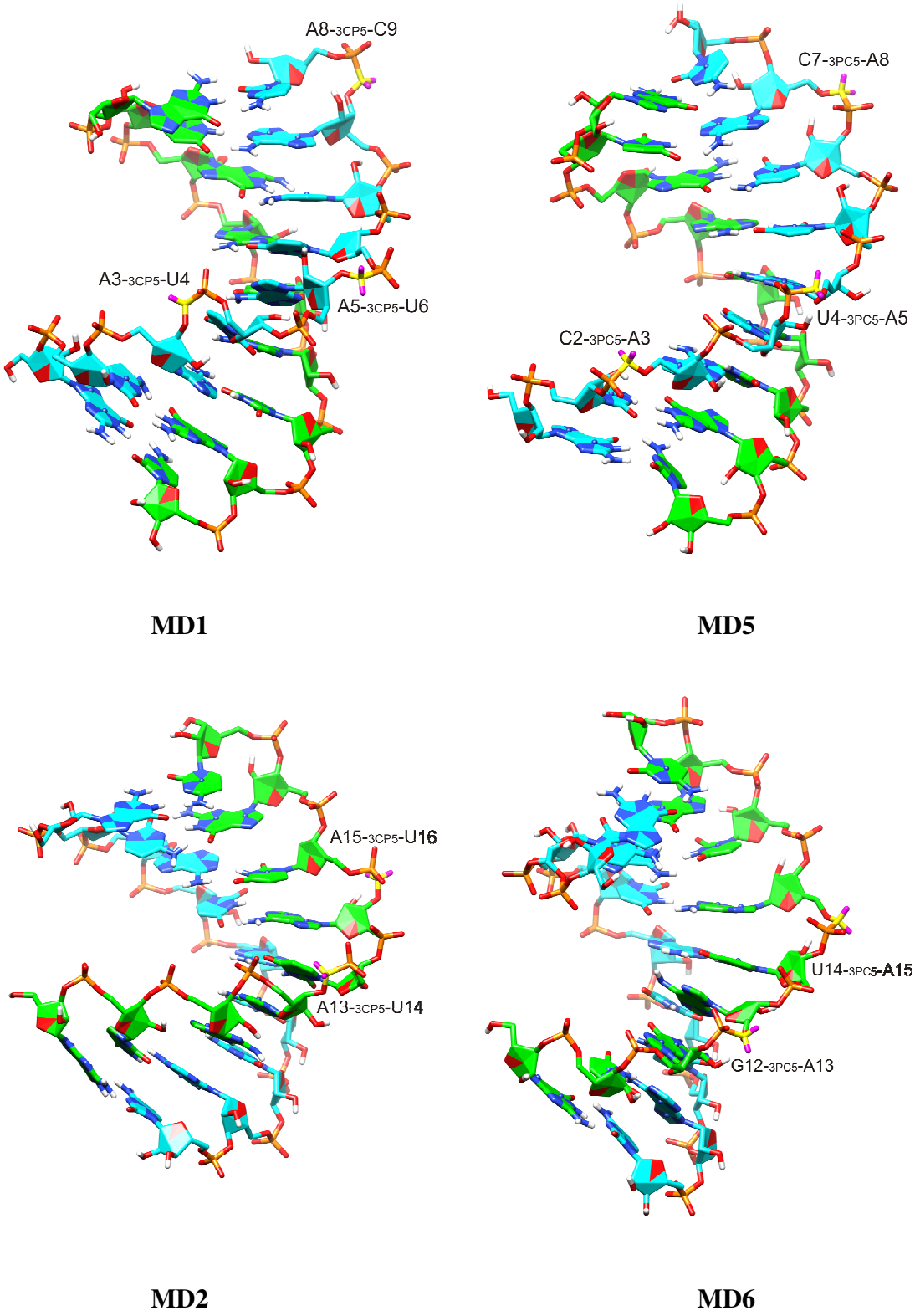


Figure 9: Final conformations of the duplex structures from MD runs produced at 300K.

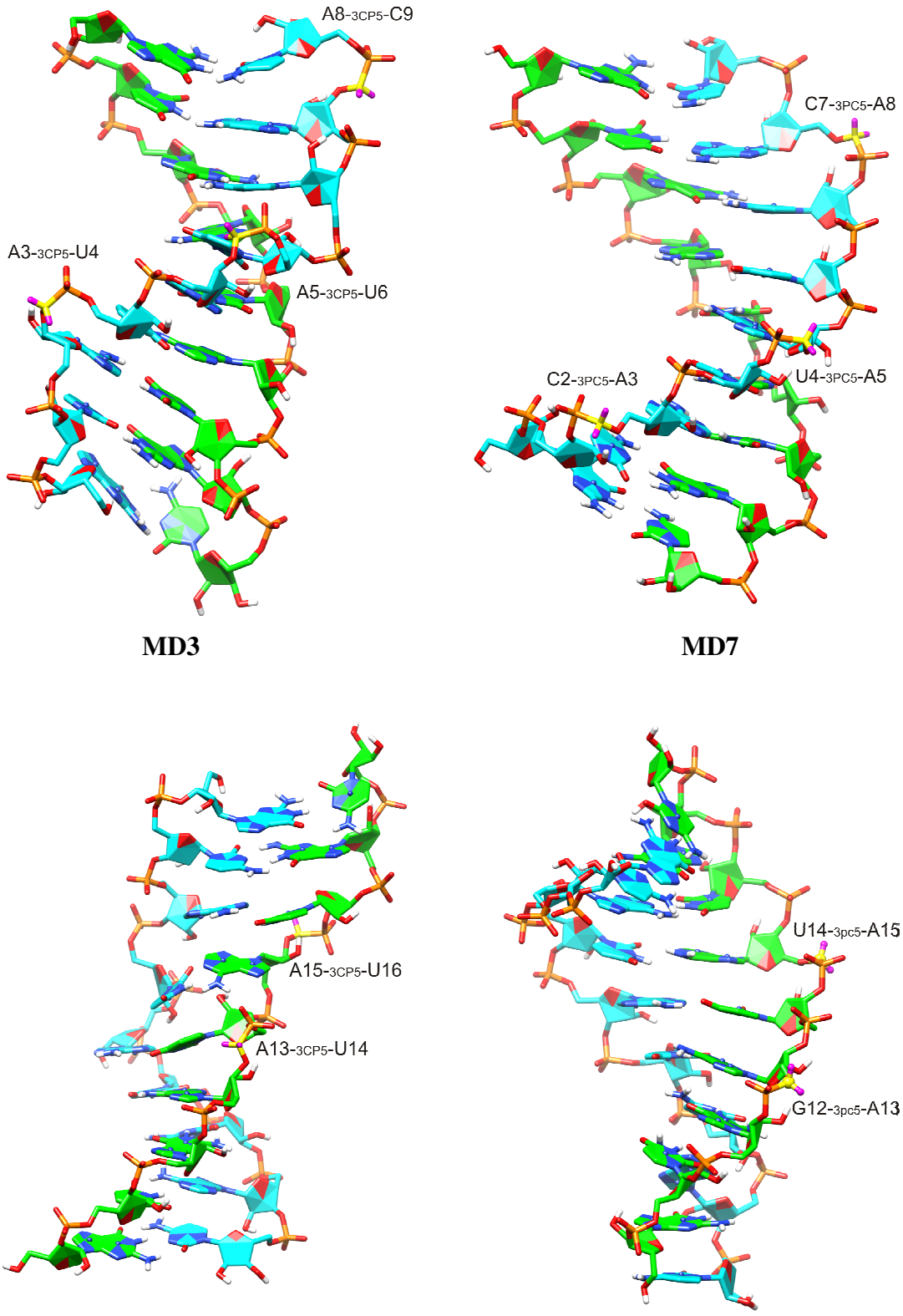


Figure 10: Final conformations of the duplex structures from MD runs produced at 350K.

Figure 11: Time evolution of the Watson-Crick base pairing. X-axis: time (ns), Y-axis: distance (A)

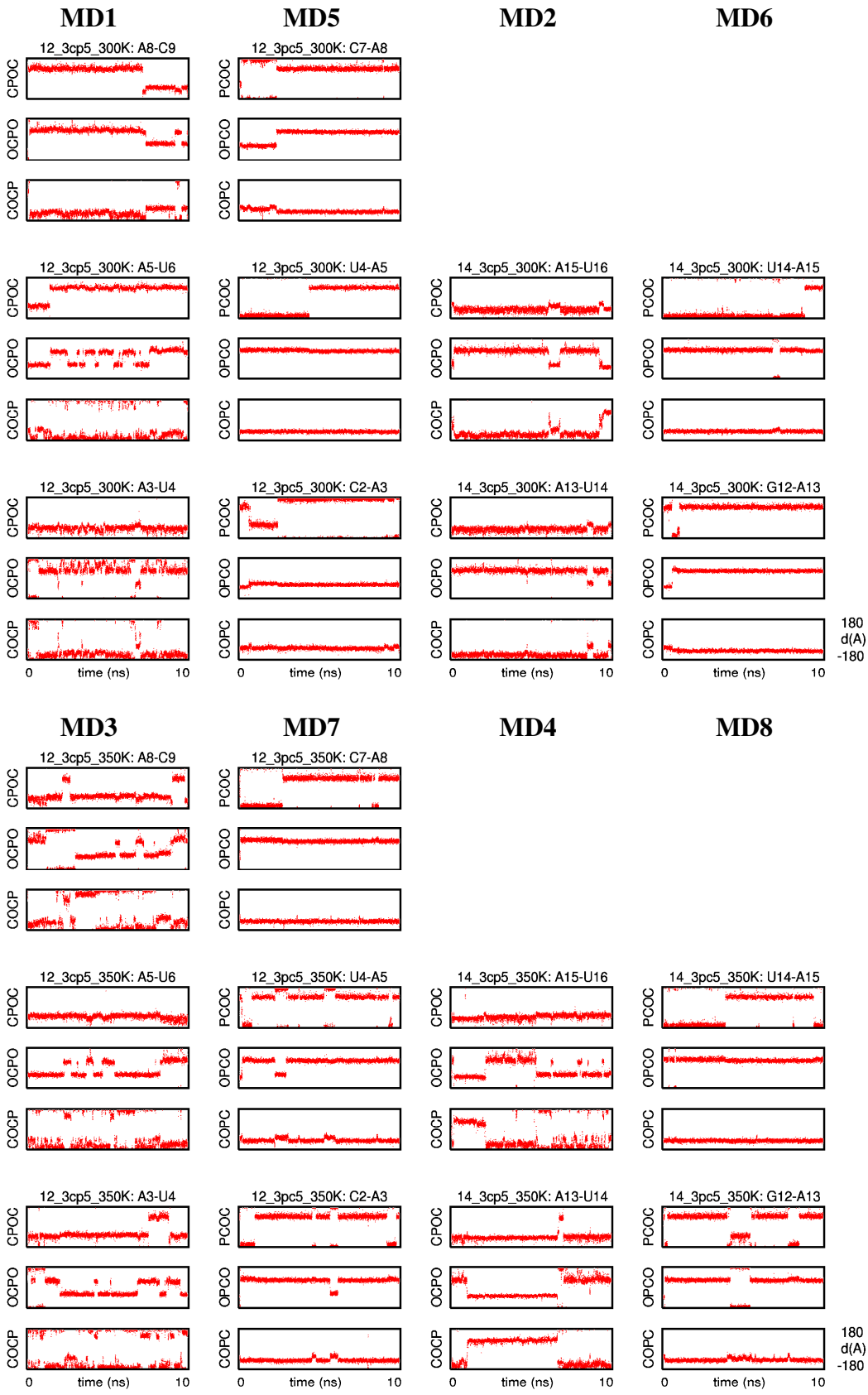


Figure 12: Time evolution of conformational preferences of the 3CP5 / 3PC5 linkages within MD runs monitored using C3´-O3´-ζ´-C-α-P-α´-O5´-C5´ or C3´-O3´-ζ-P-α-C-α´-O5´-C5´ torsion angles. X-axis: time (ns), Y-axis: angle (deg)

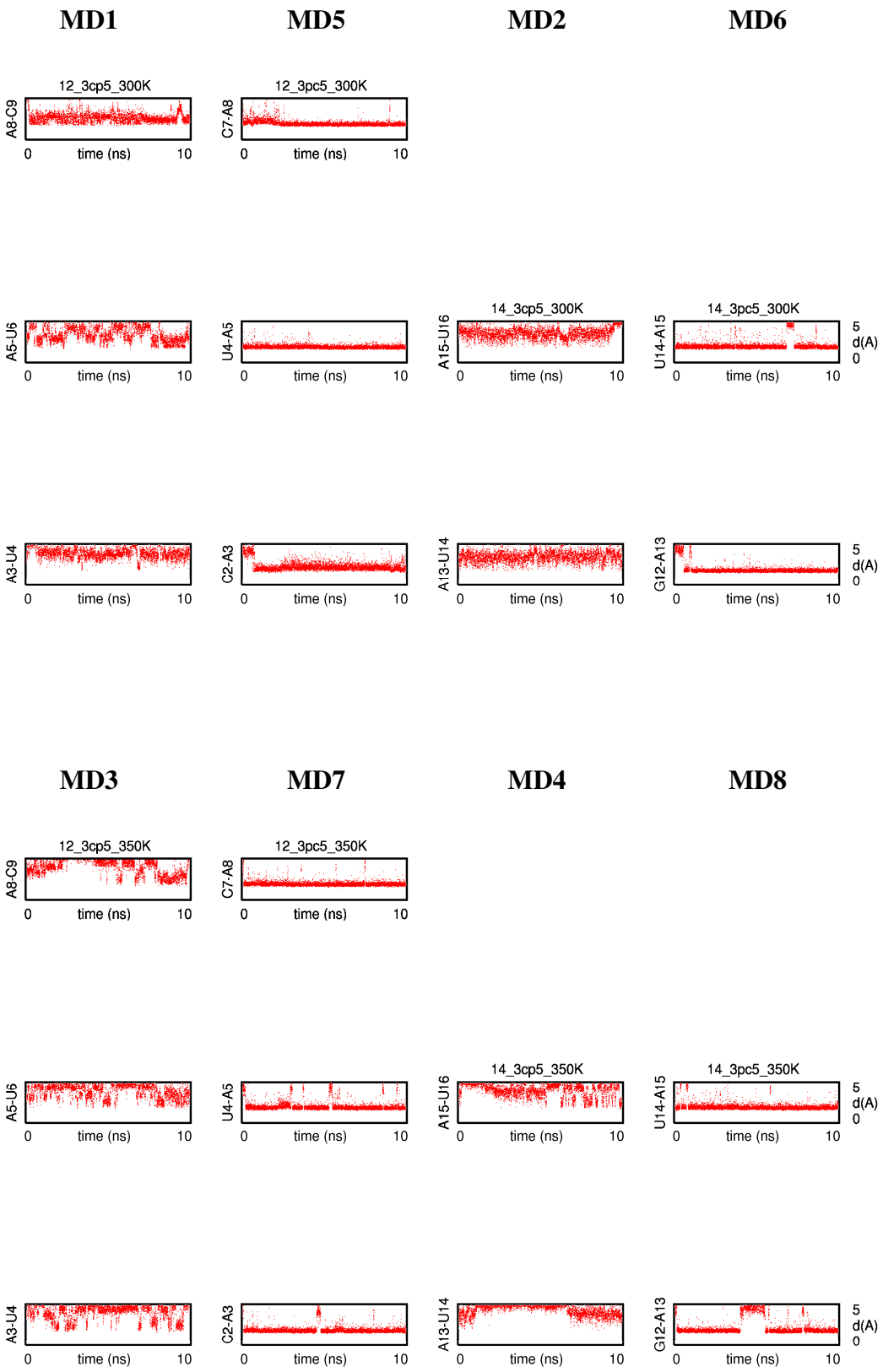


Figure 13: Time evolution of the 2´OH..O5´ intrastrand hydrogen bonding. X-axis: time (ns), Y-axis: distance (A)