Supporting Information

Tuning the hybridization properties of modified oligonucleotides: from flexible to conformationally constrained phosphonate internucleotide linkages.

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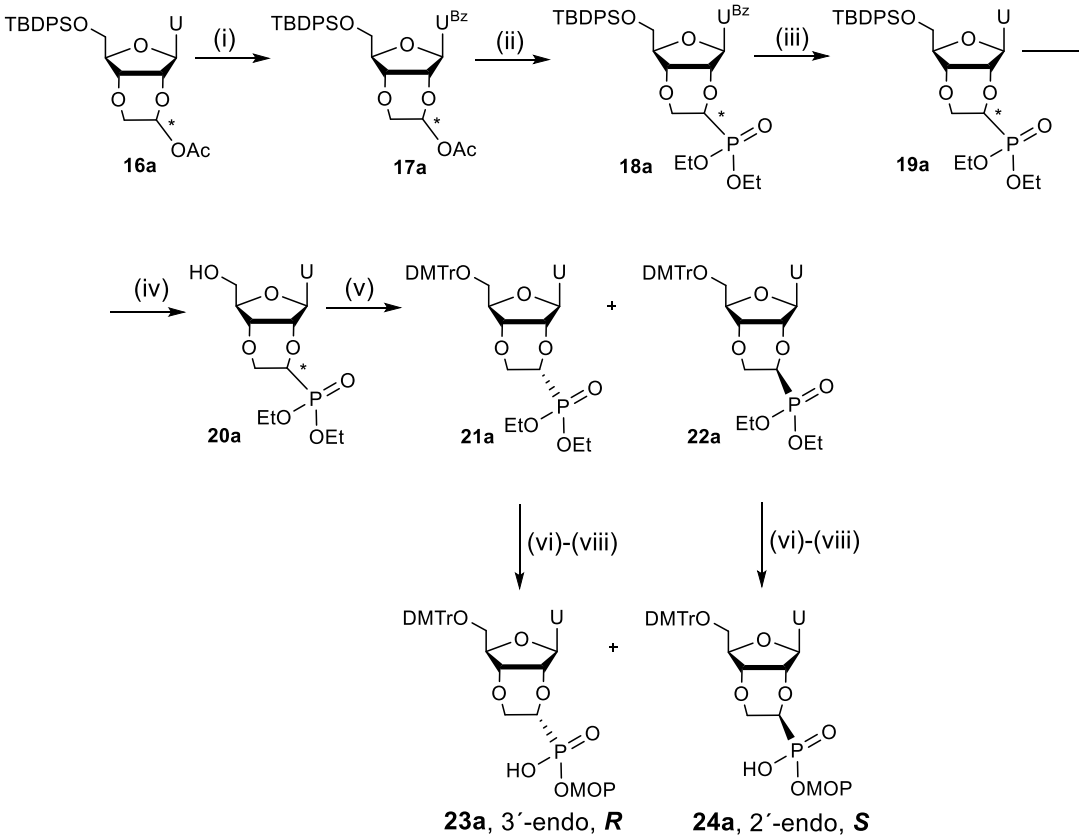
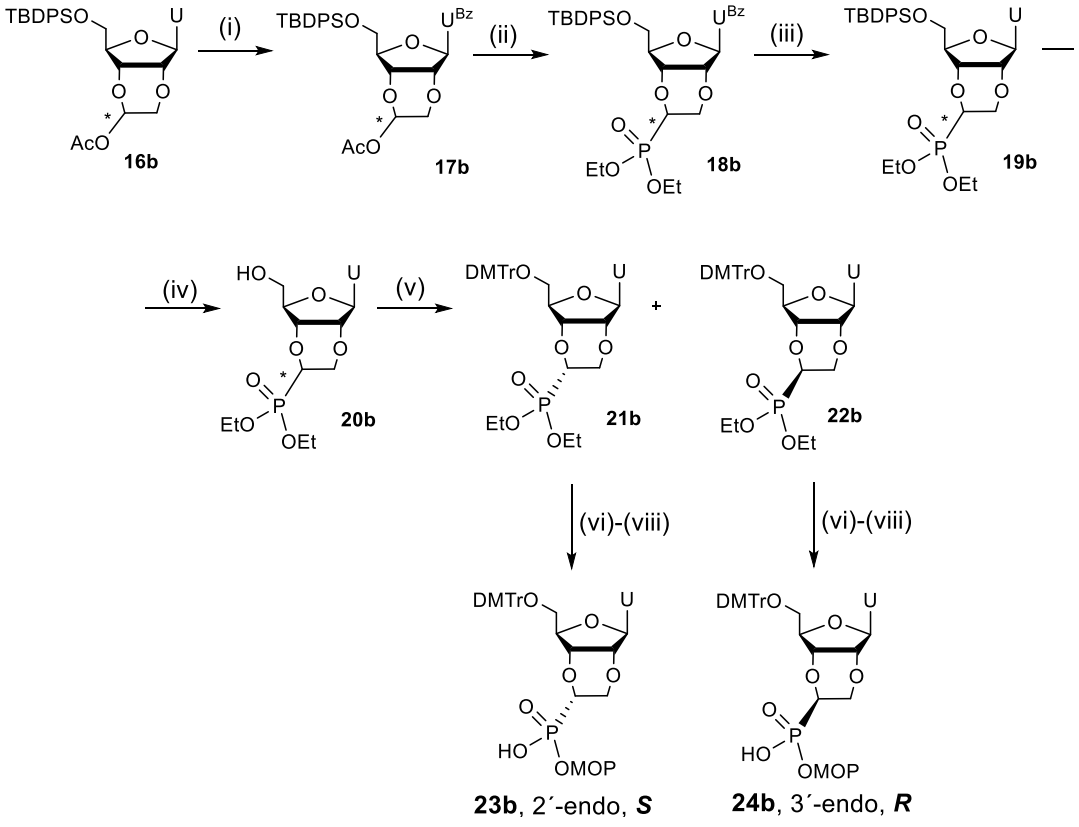
Experimental

Unless stated otherwise, all used solvents were anhydrous. Mass spectra were recorded on a ZAB-EQ (VG Analytical) instrument using FAB (ionization with Xe, accelerating voltage 8 kV; glycerol and thioglycerol as matrices) and on LTQ Orbitrap XL (Thermo Fisher Scientific) using ESI ionization. MALDI TOF MS was performed on Reflex IV (Bruker - Daltonics) instrument using 3-hydroxypyridine-2-carboxylic acid and pyridine-2-carboxylic acid as matrices. The NMR spectra were measured on Bruker AVANCE-600 instrument (^{1}H at 600.13 MHz and ^{13}C at 150.9 MHz) with a cryoprobe or Bruker AVANCE-500 instrument (^{1}H at 500.13 MHz and ^{13}C at 125.8 MHz) in [d6]DMSO or in $CDCl_3$. Spectra in DMSO were referenced to solvent peak (using δ_H(DMSO) = 2.50 ppm; δ_C(DMSO) = 39.7 ppm). Structural assignment of proton and carbon signals was achieved combining 1D-^{1}H and ^{13}C-spectra with homonuclear 2D-H,H-COSY, 2D-H,H-ROESY and heteronuclear 2D-H,C-HSQC and 2D-H,C-HMBC spectra. 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.

Synthesis of monomers

The synthesis of 1,4-dioxane phosphonates started from synthon **16** which was prepared as a 1:1 mixture of epimers according to our previously published protocol.[1] Direct phosphonylation of this hemiacetal using triethyl phosphite and trimethylsilyl trifluoromethanesulfonate afforded the desired phosphonate only in 15% yield. We reasoned that TMSOTf could serve as a silylating agent and emerging trifluoromethanesulfonic acid could be responsible for the decomposition of either starting compound or product. Neither the use of various Lewis acids such as tin(IV) chloride, boron trifluoride diethyl etherate, zinc trifluoromethanesulfonate nor the use of diethyl trimethylsilyl phosphite provided better results. Therefore, the uracil nucleobase was protected with 3-*N*-benzoyl group and precursor **17** was treated with triethyl phosphite and trimethylsilyl trifluoromethanesulfonate to afford desired phosphonate derivative **18** as a 1:1 mixture of epimers in significantly improved yield of 69%. After cleavage of 3-*N*-Bz and 5´-*O*-TBDPS groups, the 5´-hydroxyl was protected with DMTr group. At this point, the epimers were easily separated using chromatography on silica gel. Finally, phosphonate diester was converted to 4-methoxy-1-oxido-2-pyridylmethyl (MOP) phosphono ester (**23**, **24**) using our standard protocol (i) deprotection of diethyl ester groups using bromotrimethylsilane in the presence of 2,6-lutidine (ii) complete esterification of free phosphonic acid with 4-methoxy-1-oxido-2-pyridylmethanol (MOP-OH) in the presence of 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane 2-oxide (CDDO) and 4-methoxypyridine-*N*-oxide (MPNO) and (iii) deprotection of one MOP ester group with benzenethiol. Using this synthetic route, we prepared all four 1,4-dioxane-based phosphonates (**23a**, **23b, 24a, 24b**) in good total yields (**Scheme S1**).





Scheme S1. (i) BzCl, DIPEA, DCM, r.t. (ii) $(EtO)_3P$, TMSO-Tf, ACN, r.t. (iii) $MeNH_2$/EtOH (iv) TBAF, THF, r.t. (v) DMT-Cl, py, r.t. (vi) Me_3SiBr, 2,6-lutidine, ACN, r.t. (vii) MOP-OH, MPNO, CDDO, py, r.t. (viii) PhSH, TEA, dioxane, r.t.

Diethyl (1*RS*),2-[(5´-*O*-*tert*-butyldiphenylsilyluridin-2´,3´-di-*O*-yl)-[(2´-*O*)→1; (3´-*O*)→2]]ethanephosphonate (**19a**)

Benzoyl chloride (1.7 mL; 14.4 mmol) was added to a solution of (1RS),2-(5´-*O*-*tert*-butyldiphenylsilyluridin-2´,3´-di-*O*-yl)-[(2´-*O*)→1; (3´-*O*)→2]-ethyliden-1-ylacetate **16a** (4.0 g; 7.2 mmol) and TEA (4.8 mL; 28.8 mmol) in DCM (100 mL). The reaction mixture was stirred for 16 h at r.t., quenched by the addition of methanol (5 mL) and evaporated. The residue was dissolved in chloroform (0.5 L) and extracted with saturated solution of sodium hydrogencarbonate (3x100 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude benzoyl derivative **17a** was used without further purification.

LC-MS (ESI) calcd for $C_{36}H_{39}N_2O_9Si$ $(M+H)^+$ 671.2, found 671.2.

Trimethylsilyl trifluoromethanesulfonate (1.1 mL; 6.0 mmol) was added to a solution of benzoyl derivative **17a** (4.0 g; 6.0 mmol) and triethyl phosphite (3.1 mL; 18.0 mmol) in ACN (100 mL). The reaction mixture was stirred for 3 h at r.t. After that, the reaction mixture was treated 1 h at r.t. with 33% methylamine in ethanol (20 mL) and evaporated. The product was purified by chromatography on silica gel (elution with gradient of 0-50% acetone in toluene) to yield 2.8 g (72%) of white foam.

LC-MS (ESI) calcd for $C_{31}H_{42}N_2O_9PSi$ $(M+H)^+$ 645.2, found 645.3.

Diethyl (1*S*),2-[(5´-*O*-dimethoxytrityluridin-2´,3´-di-*O*-yl)-[(2´-*O*)→1; (3´-*O*)→2]]ethanephosphonate (**22a**) and diethyl (1*R*),2-[(5´-*O*-dimethoxytrityluridin-2´,3´-di-*O*-yl)-[(2´-*O*)→1; (3´-*O*)→2]]ethanephosphonate (**21a**)

Derivative **19a** (2.8 g; 4.3 mmol) was treated 2 h at r.t. with 0.5M TBAF in THF (20 mL). After that, the reaction mixture was evaporated. The product was purified by chromatography on silica gel (elution with gradient of 0-10% ethanol in chloroform) to yield 1.3 g (74%) of white foam.

LC-MS (ESI) calcd for $C_{15}H_{24}N_2O_9P$ $(M+H)^+$ 407.1, found 407.1.

Dimethoxytrityl chloride (1.6 g; 4.8 mmol) was added to a solution of derivative **20a** (1.3 g; 3.2 mmol) in pyridine (50 mL). The reaction mixture was stirred for 16 h at r.t., quenched by the addition of methanol (1 mL) and evaporated. The epimers were separated by chromatography on silica gel (elution with gradient of 0-2% ethanol in chloroform) as faster eluting *S* isomer **22a** (0.7 g; 31%; white foam) and slower eluting *R* isomer **21a** (0.9 g; 40%; white foam).

21a: HRMS (FAB) calcd for $C_{36}H_{42}N_2O_{11}P$ $(M+H)^+$ 709.2526, found 709.2528.

22a: HRMS (FAB) calcd for $C_{36}H_{42}N_2O_{11}P$ $(M+H)^+$ 709.2526, found 709.2527.

4-Methoxy-1-oxido-2-pyridylmethyl (1*R*),2-[(5´-*O*-dimethoxytrityluridin-2´,3´-di-*O*-yl)-[(2´-*O*)→1; (3´-*O*)→2]]ethanephosphonate (**23a**)

Bromotrimethylsilane (0.8 mL; 5.2 mmol) was added to a solution of diethyl phosphonate **21a** (0.9 g; 1.3 mmol) and 2,6-lutidine (1.2 mL; 10.4 mmol) in ACN (20 ml). The reaction mixture was stirred for 16 h at r.t. and evaporated. The residue was dissolved in chloroform (0.2 L) and extracted with 0.2 M TEAB (3 x 30 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude nucleoside phosphonic acid was used without further purification.

2-Chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane 2-oxide (1.1 g; 6.5 mmol) was added to a solution of nucleoside phosphonic acid, 4-methoxy-1-oxido-2-pyridylmethanol (0.7 g; 3.9 mmol) and 4-methoxy-1-oxido-2-pyridine (0.8 g; 6.5 mmol) in pyridine (20 ml). The reaction mixture was stirred for 16 h at r.t., quenched by the addition of 2M TEAB (5 mL) and evaporated. The residue was dissolved in chloroform (0.2 L) and extracted with 0.2 M TEAB (3 x 30 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated.

After that, diMOP phosphonate was treated 1 h at r.t. with benzenethiol (1 mL) and TEA (1.4 mL) in dioxane (10 mL) to cleave off one MOP group. The reation mixture was diluted with ethyl acetate and directly purified by chromatography on silica gel (elution with gradient of 0-100% ethyl

acetate/ethanol/acetone/water 4:1:1:1 in ethyl acetate). The product **23a** was lyophilized from dioxane. Yield 0.64 g (62%).
HRMS (FAB) calcd for $C_{39}H_{41}N_3O_{13}P$ $(M+H)^+$ 790.2377, found 790.2375.

4-Methoxy-1-oxido-2-pyridylmethyl (1*S*),2-[(5´-*O*-dimethoxytrityluridin-2´,3´-di-*O*-yl)-[(2´-*O*)→1; (3´-*O*)→2]]ethanephosphonate (**24a**)
Bromotrimethylsilane (0.5 mL; 4.0 mmol) was added to a solution of diethyl phosphonate **22a** (0.7 g; 1.0 mmol) and 2,6-lutidine (0.9 mL; 8.0 mmol) in ACN (10 ml). The reaction mixture was stirred for 16 h at r.t. and evaporated. The residue was dissolved in chloroform (0.2 L) and extracted with 0.2 M TEAB (3 x 30 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude nucleoside phosphonic acid was used without further purification.
2-Chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane 2-oxide (0.9 g; 5.0 mmol) was added to a solution of nucleoside phosphonic acid, 4-methoxy-1-oxido-2-pyridylmethanol (0.4 g; 3.0 mmol) and 4-methoxy-1-oxido-2-pyridine (0.6 g; 5.0 mmol) in pyridine (10 ml). The reaction mixture was stirred for 16 h at r.t., quenched by the addition of 2M TEAB (5 mL) and evaporated. The residue was dissolved in chloroform (0.2 L) and extracted with 0.2 M TEAB (3 x 30 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated.
After that, diMOP phosphonate was treated 1 h at r.t. with benzenethiol (1 mL) and TEA (1.4 mL) in dioxane (10 mL) to cleave off one MOP group. The reation mixture was diluted with ethyl acetate and directly purified by chromatography on silica gel (elution with gradient of 0-100% ethyl acetate/ethanol/acetone/water 4:1:1:1 in ethyl acetate). The product **24a** was lyophilized from dioxane. Yield 0.48 g (61%).
HRMS (FAB) calcd for $C_{39}H_{41}N_3O_{13}P$ $(M+H)^+$ 790.2377, found 790.2379.

Diethyl (1*RS*),2-[(5´-*O*-*tert*-butyldiphenylsilyluridin-2´,3´-di-*O*-yl)-[(2´-*O*)→2; (3´-*O*)→1]]ethanephosphonate (**19b**)
Benzoyl chloride (0.8 mL; 7.2 mmol) was added to a solution of (1RS),2-(5´-*O*-*tert*-butyldiphenylsilyluridin-2´,3´-di-*O*-yl)-[(2´-*O*)→2; (3´-*O*)→1]-ethyliden-1-ylacetate **16b** (2.0 g; 3.6 mmol) and TEA (2.4 mL; 14.4 mmol) in DCM (50 mL). The reaction mixture was stirred for 16 h at r.t., quenched by the addition of methanol (5 mL) and evaporated. The residue was dissolved in chloroform (0.5 L) and extracted with saturated solution of sodium hydrogencarbonate (3x100 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude benzoyl derivative **17b** was used without further purification.
LC-MS (ESI) calcd for $C_{36}H_{39}N_2O_9Si$ $(M+H)^+$ 671.2, found 671.3.
Trimethylsilyl trifluoromethanesulfonate (0.5 mL; 3.1 mmol) was added to a solution of benzoyl derivative **17b** (2.1 g; 3.1 mmol) and triethyl phosphite (1.6 mL; 9.3 mmol) in ACN (50 mL). The reaction mixture was stirred for 3 h at r.t. After that, the reaction mixture was treated 1 h at r.t. with 33% methylamine in ethanol (10 mL) and evaporated. The product was purified by chromatography on silica gel (elution with gradient of 0-50% acetone in toluene) to yield 1.6 g (81%) of white foam.
LC-MS (ESI) calcd for $C_{31}H_{42}N_2O_9PSi$ $(M+H)^+$ 645.2, found 645.1.

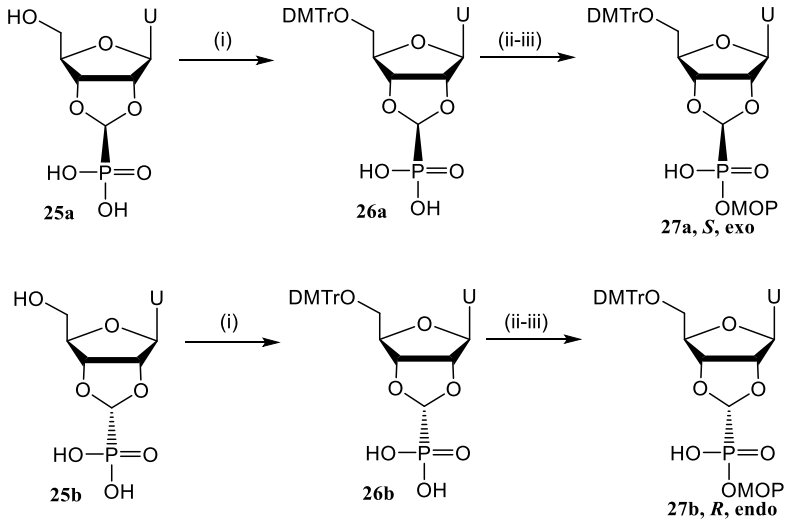
Diethyl (1*S*),2-[(5´-*O*-dimethoxytrityluridin-2´,3´-di-*O*-yl)-[(2´-*O*)→2; (3´-*O*)→1]]ethanephosphonate (**21b**) and diethyl (1*R*),2-[(5´-*O*-dimethoxytrityluridin-2´,3´-di-*O*-yl)-[(2´-*O*)→2; (3´-*O*)→1]]ethanephosphonate (**22b**)
Derivative **19b** (1.6 g; 2.5 mmol) was treated 2 h at r.t. with 0.5M TBAF in THF (10 mL). After that, the reaction mixture was evaporated. The 5´-hydroxy derivative **20b** was purified by chromatography on silica gel (elution with gradient of 0-10% ethanol in chloroform) to yield 0.8 g (79%) of white foam.

LC-MS (ESI) calcd for $C_{15}H_{24}N_2O_9P$ $(M+H)^+$ 407.1, found 407.1.
Dimethoxytrityl chloride (1.3 g; 3.9 mmol) was added to a solution of derivative **20b** (0.8 g; 2.0 mmol) in pyridine (30 mL). The reaction mixture was stirred for 16 h at r.t., quenched by the addition of methanol (1 mL) and evaporated. The epimers were separated by chromatography on silica gel (elution with gradient of 0-2% ethanol in chloroform) as faster eluting *S* isomer **21b** (0.6 g; 43%; white foam) and slower eluting *R* isomer **22b** (0.5 g; 36%; white foam).
21b: HRMS (FAB) calcd for $C_{36}H_{42}N_2O_{11}P$ $(M+H)^+$ 709.2526, found 709.2528.
22b: HRMS (FAB) calcd for $C_{36}H_{42}N_2O_{11}P$ $(M+H)^+$ 709.2526, found 709.2529.

4-Methoxy-1-oxido-2-pyridylmethyl (1*S*),2-[(5´-*O*-dimethoxytrityluridin-2´,3´-di-*O*-yl)-[(2´-*O*)→2;(3´-*O*)→1]]ethanephosphonate (**23b**)
Bromotrimethylsilane (0.5 mL; 3.2 mmol) was added to a solution of diethyl phosphonate **21b** (0.6 g; 0.8 mmol) and 2,6-lutidine (0.8 mL; 6.4 mmol) in ACN (10 ml). The reaction mixture was stirred for 16 h at r.t. and evaporated. The residue was dissolved in chloroform (0.2 L) and extracted with 0.2 M TEAB (3 x 30 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude nucleoside phosphonic acid was used without further purification.
2-Chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane 2-oxide (0.7 g; 4.0 mmol) was added to a solution of nucleoside phosphonic acid, 4-methoxy-1-oxido-2-pyridylmethanol (0.4 g; 2.4 mmol) and 4-methoxy-1-oxido-2-pyridine (0.5 g; 4.0 mmol) in pyridine (10 ml). The reaction mixture was stirred for 16 h at r.t., quenched by the addition of 2M TEAB (5 mL) and evaporated. The residue was dissolved in chloroform (0.2 L) and extracted with 0.2 M TEAB (3 x 30 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated.
After that, diMOP phosphonate was treated 1 h at r.t. with benzenethiol (1 mL) and TEA (1.4 mL) in dioxane (10 mL) to cleave off one MOP group. The reation mixture was diluted with ethyl acetate and directly purified by chromatography on silica gel (elution with gradient of 0-100% ethyl acetate/ethanol/acetone/water 4:1:1:1 in ethyl acetate). The product **23b** was lyophilized from dioxane. Yield 0.42 g (67%).
HRMS (FAB) calcd for $C_{39}H_{41}N_3O_{13}P$ $(M+H)^+$ 790.2377, found 790.2377.

4-Methoxy-1-oxido-2-pyridylmethyl (1*R*),2-[(5´-*O*-dimethoxytrityluridin-2´,3´-di-*O*-yl)-[(2´-*O*)→2;(3´-*O*)→1]]ethanephosphonate (**24b**)
Bromotrimethylsilane (0.4 mL; 2.8 mmol) was added to a solution of diethyl phosphonate **22b** (0.5 g; 0.7 mmol) and 2,6-lutidine (0.7 mL; 5.6 mmol) in ACN (10 ml). The reaction mixture was stirred for 16 h at r.t. and evaporated. The residue was dissolved in chloroform (0.2 L) and extracted with 0.2 M TEAB (3 x 30 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude nucleoside phosphonic acid was used without further purification.
2-Chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane 2-oxide (0.6 g; 3.5 mmol) was added to a solution of nucleoside phosphonic acid, 4-methoxy-1-oxido-2-pyridylmethanol (0.3 g; 2.1 mmol) and 4-methoxy-1-oxido-2-pyridine (0.4 g; 3.5 mmol) in pyridine (10 ml). The reaction mixture was stirred for 16 h at r.t., quenched by the addition of 2M TEAB (5 mL) and evaporated. The residue was dissolved in chloroform (0.2 L) and extracted with 0.2 M TEAB (3 x 30 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated.
After that, diMOP phosphonate was treated 1 h at r.t. with benzenethiol (1 mL) and TEA (1.4 mL) in dioxane (10 mL) to cleave off one MOP group. The reation mixture was diluted with ethyl acetate and directly purified by chromatography on silica gel (elution with gradient of 0-100% ethyl acetate/ethanol/acetone/water 4:1:1:1 in ethyl acetate). The product **24b** was lyophilized from dioxane. Yield 0.36 g (65%).
HRMS (FAB) calcd for $C_{39}H_{41}N_3O_{13}P$ $(M+H)^+$ 790.2377, found 790.2376.

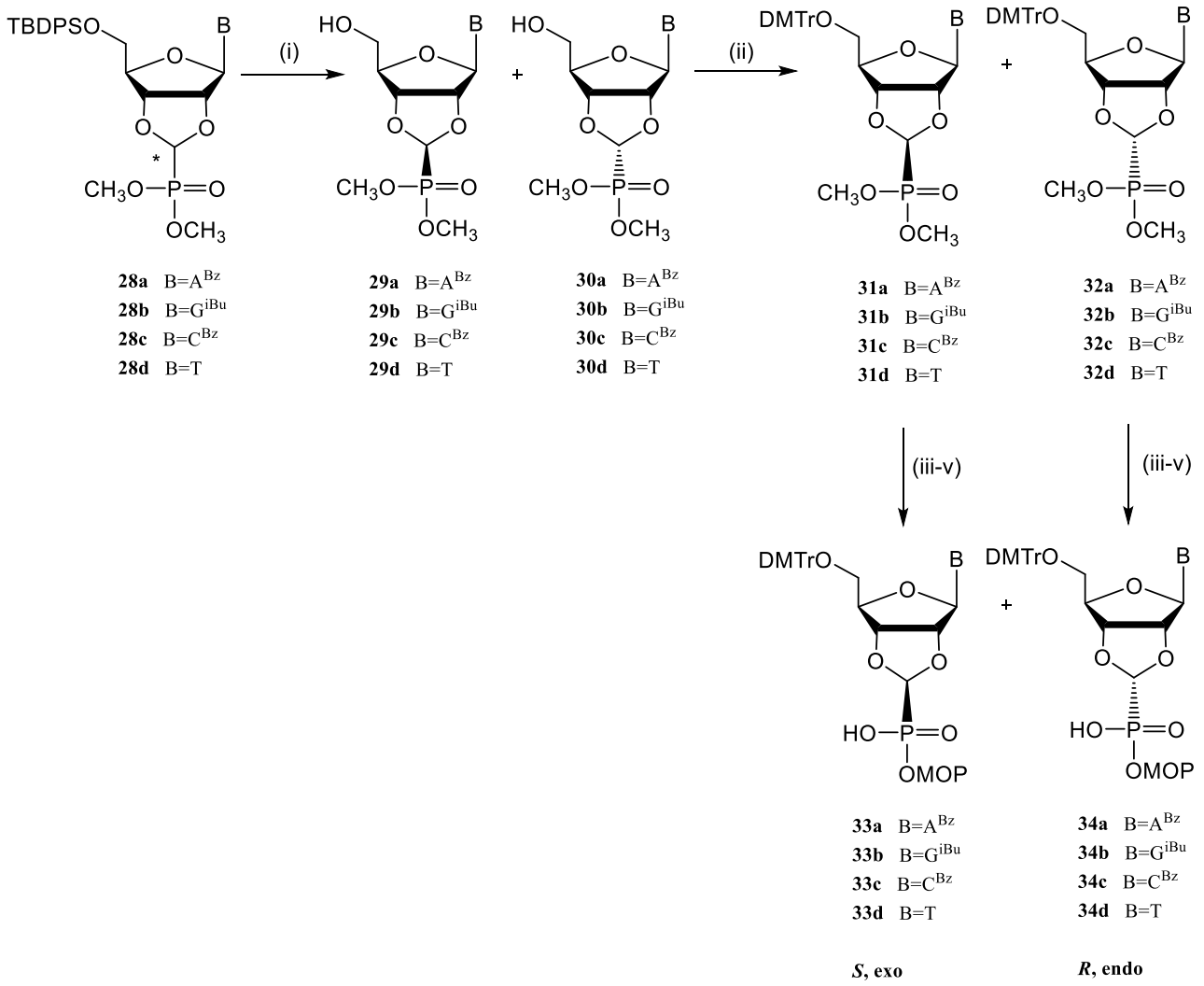
1,3-Dioxolane phosphonates were prepared from free phosphonic acids **25** which were prepared as described earlier.[1,2] Protection of the 5´-hydroxyl with DMTr group and monoprotection of phosphonate group with MOP group afforded respective monomers **27a** and **27b** in good yields (**Scheme S2**).



Scheme S2. (i) DMT-Cl, py, r.t. (ii) MOP-OH, MPNO, CDDO, py, r.t. (iii) PhSH, TEA, dioxane, r.t.

4-Methoxy-1-oxido-2-pyridylmethyl (*S*)-(5´-*O*-dimethoxytrityluridin-2´,3´-di-*O*-yl)-methanephosphonate (**27a**) and
4-Methoxy-1-oxido-2-pyridylmethyl (*R*)-(5´-*O*-dimethoxytrityluridin-2´,3´-di-*O*-yl)-methanephosphonate (**27b**)
Dimethoxytrityl chloride (0.68 g; 2.0 mmol) was added to a solution of the free phosphonic acid, (*S*)-epimer **25a** (0.48 g; 1.4 mmol) in pyridine (15 mL). The reaction mixture was stirred for 96 h at r.t., quenched by the addition of methanol (1 mL) and evaporated. The residue was dissolved in chloroform (100 mL) and extracted with 0.2 M TEAB (3 x 30 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude nucleoside phosphonic acid **26a** was used without further purification. 2-Chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane 2-oxide (1.2 g; 7.0 mmol) was added to a solution of nucleoside phosphonic acid **26a**, 4-methoxy-1-oxido-2-pyridylmethanol (0.48 g; 4.2 mmol) and 4-methoxy-1-oxido-2-pyridine (0.8 g; 7.0 mmol) in pyridine (15 ml). The reaction mixture was stirred for 16 h at r.t., quenched by the addition of 2M TEAB (5 mL) and evaporated. The residue was dissolved in chloroform (0.2 L) and extracted with 0.2 M TEAB (3 x 50 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated. After that, diMOP phosphonate was treated 1 h at r.t. with benzenethiol (1 mL) and TEA (1.4 mL) in dioxane (10 mL) to cleave off one MOP group. The reation mixture was diluted with ethyl acetate and directly purified by chromatography on silica gel (elution with gradient of 0-100% ethyl acetate/ethanol/acetone/water 4:1:1:1 in ethyl acetate). The product **27a** was lyophilized from 20% water in dioxane. Yield 0.64 g (58%) of (*S*)-epimer **27a**.
HRMS (FAB) calcd for $C_{38}H_{37}N_3O_{13}P$ $(M-H)^-$ 774.2064, found 774.2061.

(*R*)-epimer **27b** was prepared from free phosphonic acid, (*R*)-epimer **25b** (0.36 g; 1.1 mmol) using the protocol described for (*S*)-epimer **27a**. Yield 0.42 g (49 %) of (*R*)-epimer **27b**.
HRMS (FAB) calcd for $C_{38}H_{37}N_3O_{13}P$ $(M-H)^-$ 774.2064, found 774.2067.



Scheme S3. (i) TBAF, THF, r.t. (ii) DMT-Cl, py, r.t. (iii) 80% py/water, 60 °C (iv) MOP-OH, TIPS, MeIm, py, r.t. (v) 80% py/water, 60 °C.

4-Methoxy-1-oxido-2-pyridylmethyl (*S*)-(6-*N*-benzoyl-5´-*O*-dimethoxytrityladenosin-2',3'-di-*O*-yl)-methanephosphonate (**33a**) and
4-Methoxy-1-oxido-2-pyridylmethyl (*R*)-(6-*N*-benzoyl-5´-*O*-dimethoxytrityladenosin-2',3'-di-*O*-yl)-methanephosphonate (**34a**)
Mixture of epimers **28a** (1.5 g; 2.1 mmol) was treated 3 h at r.t. with 0.5M TBAF in THF (10 mL). After that, the reaction mixture was diluted with 50% MeOH in water (100 mL) and treated with Dowex 50 Et_3NH^+ to remove tetrabutylammonium ions. The epimers were separated by chromatography on silica gel (elution with gradient of 0-10% ethanol in chloroform) as faster eluting *S* isomer **29a** (0.56 g; 54%; white foam) and slower eluting *R* isomer **30a** (0.25 g; 24%; white foam). Dimethoxytrityl chloride (0.5 g; 1.5 mmol) was added to a solution of the dimethyl phosphonate, (*S*)-epimer **29a**, (0.56 g; 1.1 mmol) in pyridine (10 mL). The reaction mixture was stirred for 16 h at r.t., quenched by the addition of methanol (1 mL) and evaporated. The product was purified by chromatography on silica gel (elution with gradient of 0-10% ethanol in chloroform) to yield 0.7 g (80%) of white foam.
The phosphonate dimethylester **31a** was treated 4 h at 60 °C with 80% pyridine in water (20 mL) to afford phosphonate monomethylester, dried by co-evaporation with anhydrous dioxane and used without further purification.

The phosphonate monomethyl ester (0.6 g, 0.8 mmol) and 4-methoxy-1-oxido-2-pyridylmethanol (0.18 g, 1.2 mmol) were co-evaporated with anhydrous acetonitrile (20 mL) and diluted with acetonitrile (20 mL). 1-Methylimidazole (0.4 mL, 4.7 mmol), and 2,4,6-triisopropylbenzenesulfonyl chloride (0.7 g, 2.3 mmol) were added. The reaction mixture was stirred for 2 h at r.t., quenched by the addition of 2M TEAB (0.5 mL) and evaporated. The residue was purified by chromatography on silica gel (elution with gradient of 0-100% ethyl acetate/ethanol/acetone/water 4:1:1:1 in ethyl acetate). Finally, the mixed phosphonate diester was treated 16 h at r.t. with 80% pyridine in water (20 mL) to afford phosphonate monomer **33a**. The product was purified by chromatography on silica gel (elution with gradient of 0-100% ethyl acetate/ethanol/acetone/water 4:1:1:1 in ethyl acetate) and lyophilized from 20% water in dioxane. Yield 0.32 g (44%) of (*S*)-epimer **33a**.
HRMS (FAB) calcd for $C_{46}H_{42}N_6O_{12}P$ $(M-H)^-$ 901.2598, found 901.2598.

(*R*)-epimer **34a** was prepared from appropriate dimethyl phosphonate, (*R*)-epimer **30a** (0.25 g; 0.49 mmol) using the protocol described for (*S*)-epimer **33a**. Yield 0.18 g (40 %) of (*R*)-epimer **34a**.
HRMS (FAB) calcd for $C_{46}H_{42}N_6O_{12}P$ $(M-H)^-$ 901.2598, found 901.2569.

4-Methoxy-1-oxido-2-pyridylmethyl (*S*)-(5´-*O*-dimethoxytrityl-2-*N*-isobutyrylguanosin-2',3'-di-*O*-yl)-methanephosphonate (**33b**)
Guanine derivative of (*S*)-epimer **33b** was prepared from appropriate dimethyl phosphonate, (*S*)-epimer **29b** (0.82 g; 1.7 mmol) using the protocol described for adenosine derivative (*S*)-epimer **33a**. Yield 0.4 g (26 %) of (*S*)-epimer **33b**.
HRMS (FAB) calcd for $C_{43}H_{44}O_{13}N_6P$ $(M-H)^-$ 883.2704, found 883.2660.

Since the phosphonylation of 5´-*O*-*tert*-butyldiphenylsilyl-2´, 3´-*O*-ethoxymethylidene-2-*N*-isobutyrylguanosine afforded *S*-epimer as a major product, *R*-isomer **34b** was not prepared in guanine series.

4-Methoxy-1-oxido-2-pyridylmethyl (*S*)-(4-*N*-benzoyl-5´-*O*-dimethoxytritylcytidin-2',3'-di-*O*-yl)-methanephosphonate (**33c**) and
4-Methoxy-1-oxido-2-pyridylmethyl (*R*)-(4-*N*-benzoyl-5´-*O*-dimethoxytritylcytidin-2',3'-di-*O*-yl)-methanephosphonate (**34c**)
Mixture of epimers **28c** (1.5 g; 2.1 mmol) was treated 16 h at r.t. with 0.5M TBAF in THF (10 mL). After that, the reaction mixture was diluted with 50% MeOH in water (100 mL) and treated with Dowex 50 Et_3NH^+ to remove tetrabutylammonium ions. The mixture of epimers was purified by chromatography on silica gel (elution with gradient of 0-10% ethanol in chloroform) and treated with dimethoxytrityl chloride (1.1 g; 3.2 mmol) and pyridine (10 mL). The reaction mixture was stirred for 16 h at r.t., quenched by the addition of methanol (1 mL) and evaporated. The epimers were separated by chromatography on reverse phase (isocratic elution with 50% acetonitrile in water) as faster eluting *R* isomer **32c** (0.3 g; 19%; white foam) and slower eluting *S* isomer **31c** (0.9 g; 56%; white foam).
The conversion of phosphonate dimethylesters **31c** and **32c** to MOP monomers **33c** and **34c**, resp., was performed using the protocol described for adenosine derivative (*S*)-epimer **33a**.
Yield 0.5 g (49 %) of (*S*)-epimer **33c**.
HRMS (FAB) calcd for $C_{45}H_{42}O_{13}N_4P$ $(M-H)^-$ 877.2481, found 877.2461.
Yield 0.13 g (38 %) of (*R*)-epimer **34c**.
HRMS (FAB) calcd for $C_{45}H_{42}O_{13}N_4P$ $(M-H)^-$ 877.2481, found 877.2470.

4-Methoxy-1-oxido-2-pyridylmethyl (*S*)-(5´-*O*-dimethoxytrityl-5-methyluridin-2',3'-di-*O*-yl)-methanephosphonate **(33d)** and
4-Methoxy-1-oxido-2-pyridylmethyl (*R*)-(5´-*O*-dimethoxytrityl-5-methyluridin-2',3'-di-*O*-yl)-methanephosphonate **(34d)**
The epimers **28d** (1.7 g; 2.8 mmol) were separated by chromatography on silica gel (isocratic elution with 4% ethanol in chloroform) as faster eluting *S* isomer (1.1 g; 65%; white foam) and slower eluting *R* isomer (0.5 g; 29%; white foam).
5-Methyluracil derivative of (*S*)-epimer **33d** was prepared from appropriate dimethyl phosphonate, (*S*)-epimer (1.1 g; 1.8 mmol) using the protocol described for adenosine derivative (*S*)-epimer **33a**. Yield 0.55 g (39 %) of (*S*)-epimer **33d**.
HRMS (FAB) calcd for $C_{39}H_{39}O_{13}N_3P$ $(M-H)^-$ 788.2226, found 788.2219.

Since the phosphonylation of 5´-*O*-*tert*-butyldiphenylsilyl-2´, 3´-*O*-ethoxymethylidene-5-methylthymidine afforded *S*-epimer as a major product, *R*-isomer **34d** was not prepared in 5-methyluridine series.

Table S1
Proton NMR data of compounds **19a – 24b** in DMSO. Coupling constants are given in brackets.

Comp.	Conf.	H-1'	H-2'	H-3'	H-4'	H-5'	H-5''	O-CH_2-CH(P)-O	Base
19a [a] 63%	[R] C3'	6.38 d (7.9)	4.40 dd (7.9; 4.4)	4.15 dd (4.4; 1.4)	4.09 m (4.4; 3.9; 1.4)	3.88 dd (11.5; 4.4)	3.80 dd (11.5; 3.9)	3.98 ddd (12.0; 3.0; 1.5) 3.70 ddd (12.0; 10.7; 3.8) 4.30 td (10.7; 10.7; 3.0)	H-5: 5.47 d (8.1) H-6: 7.59 d (8.1) NH: 11.49
19a [b] 37%	[S] C2'	5.72 br s (<1)	4.35 br d (4.4; <1)	4.44 ddd (9.5; 4.4; 2.1)	4.47 ddd (9.5; 3.5; 2.0)	3.99 dd (12.0; 2.0)	3.85 dd (12.0; 3.5)	3.78 td (11.9; 11.9; 4.4) 3.66 ddd (11.9; 3.0; 1.3) 4.16 ddd (11.9; 10.7; 3.0)	H-5: 5.22 d (8.1) H-6: 7.74 d (8.1) 11.41 br s
21a [c]	[R] C3'	5.70 br s (<1)	4.35 br d (4.6; <1)	4.51 dd (9.4; 4.6)	4.46 ddd (9.4; 4.4; 2.3)	3.33 dd (11.3; 4.4)	3.29 dd (11.3; 2.3)	3.75 ddd (11.7; 10.7; 4.5) 3.63 ddd (11.7; 3.0; 1.4) 4.14 ddd (11.8; 10.7; 3.0)	H-5: 5.29 br d (8.1; <2) H-6: 7.81 d (8.1) NH: 11.43 br s (<2)
22a [d]	[S] C2'	6.34 d (7.7)	4.48 dd (7.7; 4.5)	4.19 dd (4.5; 1.4)	4.08 ddd (4.4; 3.9; 1.4)	3.33 dd (10.8; 4.4)	3.20 dd (10.8; 3.9)	3.96 ddd (11.7; 3.0; 2.6) 3.73 ddd (11.7; 10.6; 3.7) 4.30 ddd (10.6; 3.7; 23.0)	H-5: 5.49 br d (8.1; <2) H-6: 7.60 d (8.1) NH: 11.50 br s (<2)
23a [e] T = 50 deg	[R] C3'	5.66 br s (<1)	4.12 br d (4.6; <1)	4.37 ddd (9.2; 4.6; 2.0)	4.42 ddd (9.2; 4.4; 2.8)	3.34 dd (10.8; 2.8)	3.31dd (10.8; 4.4)	3.63 – 3.73 m (3H)	H-5: 5.26 dd (8.0; 1.9) H-6: 7.72 d (8.0) NH: 11.30 d (1.9)
24a [f]	[S] C2'	6.35 d (8.0)	4.44 dd (8.0; 4.3)	4.07 dd (4.3; 1.0)	4.01 td (4.2; 3.7; 1.0)	3.32 dd (10.8; 4.2)	3.15 dd (10.8; 3.7)	3.96 m, 1H 3.95 m, 1H 3.72 m, 1H	H-5: 5.40 dd (8.2; 2.2) H-6: 7.58 d (8.2) NH: 11.41 d (2.2)
19b [g] 51%	[S] C2'	6.42 d (7.9)	4.30 dd (7.9; 4.2)	4.28 dd (4.2; 1.1)	4.08 overlapped	3.88 dd (11.5; 4.5)	3.80 dd (11.5; 3.8)	3.86 ddd (11.4; 10.4; 4.5 3.75 ddd (11.4; 2.5; 1.0) 4.30 ddd (12.0; 10.4; 2.5)	H-5: 5.44 d (8.1) H-6: 7.57 d (8.1) NH: 11.49 br s
19b [h] 49%	[R] C3'	5.75 d (1.1)	4.27 dd (4.2; 1.1)	4.57	4.565	3.98 dd (12.0; 2.0)	3.79 dd (12.0; 3.0)	3.95 ddd (12.0; 2.0; <1) 3.64 ddd (12.0; 10.5; 3.8) 4.37 td (10.5; 10.5; 2.9)	H-5: 5.25 d (8.1) H-6: 7.75 d (8.1) NH: 11.41 br s
21b [i]	[S] C2'	6.39 d (8.1)	4.39 ddd (8.1; 4.4; 2.1)	4.32 br d (4.4; <2)	4.08 m (4.3; 4.0; <2)	3.35 dd (10.8; 4.3)	3.18 dd (10.8; 4.0)	3.85 ddd (11.8; 10.9; 4.7) 3.77 ddd (11.8; 3.2; 1.5) 4.30 ddd (12.0; 10.9; 3.2)	H-5: 5.47 dd (8.2; 2.2) H-6: 7.57 d (8.2) NH: 11.50 d (2.2)
22b [j]	[R] C3'	5.71 d (0.9)	4.26 dd (4.0; 0.9)	4.62 dd (9.2; 4.0)	4.60 ddd (9.2; 3.5; 2.5)	3.27 dd (11.0; 2.5)	3.25 dd (11.0; 3.5)	3.92 br dd (12.0; 2.9; <1) 3.62 ddd (12.0; 10.8; 3.6) 4.37 ddd (10.8; 10.4; 2.9)	H-5: 5.31 dd (8.1; 2.2) H-6: 7.80 d (8.1) NH: 11.43 d (2.2)
23b [k] T = 50 deg	[S] C2'	6.38 (8.2)	4.26 ddd (8.2; 4.5; 1.8)	4.05 br d (4.5; <1)	3.97 br dd (4.2; 3.5; <1)	3.32 dd (10.8; 4.2)	3.17 dd (10.8; 3.5)	3.84 m, 1H 3.83 m, 1H 3.73 m, 1H	H-5: 5.40 dd (8.2; 2.0) H-6: 7.54 d (8.2) NH: 11.43 br d (2.0)
24b [l] T = 50 deg	[R] C3'	5.71 br s (<1)	4.41 br d (4.3; <1)	4.53 dd (9.2; 4.3)	4.50 ddd (9.2; 4.0; 2.0)	3.34 dd (10.8; 4.0)	3.29 dd (10.8; 2.0)	3.92 m, 2H 3.60 m, 1H	H-5: 5.25 dd (8.1; 2.0) H-6: 7.76 d (8.1) NH: 11.28 br d (2.0)

Substituents:
[a] **5'-OTBDPS**: 7.62 m, 4H (*ortho*-ArH), 7.43 m, 4H (*meta*-ArH), 7.48 m, 2H (*para*-ArH), 1.03 s, 9H (*t*-Bu); **P(OCH_2CH_3)$_2$**: 4.05 – 4.11 m, 4H (P-OCH_2), 1.226 t and 1.217 t, *J* = 7.0 Hz, 6H (2x CH_3);
[b] **5'-OTBDPS**: 7.62 m, 4H (*ortho*-ArH), 7.43 m, 4H (*meta*-ArH), 7.48 m, 2H (*para*-ArH), 1.02 s, 9H (*t*-Bu); **P(OCH_2CH_3)$_2$**: 4.05 – 4.11 m, 4H (P-OCH_2), 1.260 t and 1.257 t, *J* = 7.0 Hz, 6H (2x CH_3).
[c] **5'-ODMTr**: 7.37 m, 2H (*ortho*-ArH), 7.31 m, 2H (*meta*-ArH), 7.25 m, 1H (*para*-ArH), 7.24 m, 4H (*ortho*-ArH), 6.89 m, 4H (*meta*-ArH), 3.738 s and 3.736 s (2x OCH_3); **P(OCH_2CH_3)$_2$**: 4.07 – 4.12 m, 4H (P-OCH_2), 1.256 t and 1.253 t, *J* = 7.1 Hz (2x CH_3).
[d] **5'-ODMTr**: 7.37 m, 2H (*ortho*-ArH), 7.34 m, 2H (*meta*-ArH), 7.25 m, 5H (*para*-ArH + 4x *ortho*-ArH), 6.92 m, 4H (*meta*-ArH), 3.744 s and 3.742 s (2x OCH_3); **P(OCH_2CH_3)$_2$**: 4.04 – 4.08 m, 4H (P-OCH_2), 1.23 t and 1.22 t, *J* = 7.1 Hz (2x CH_3);
[e] **5'-ODMTr**: 7.36 m, 2H (*ortho*-ArH), 7.30 m, 2H (*meta*-ArH), 7.23 m, 5H (*para*-ArH + 4x *ortho*-ArH), 6.87 m, 4H (*meta*-ArH), 3.74 s (2x OCH_3); **P-O-Py**: 4.92 dd, *J* = 16.7 and 8.4 Hz and 4.87 dd, *J* = 16.7 and 8.6 Hz (P-O-CH_2), 8.19 d, *J* = 7.2 Hz (ArH), 7.15 d, *J* = 3.6 Hz (ArH), 6.98 dd, *J* = 7.2 and 3.6 Hz (ArH), 3.80 s (OCH_3);

[f] **5'-ODMTr**: 7.35 m, 4H (*ortho*-ArH + *meta*-ArH), 7.25 m, 1H (*para*-ArH), 7.23 m, 4H (*ortho*-ArH), 6.92 m, 4H (*meta*-ArH), 3.740 s and 3.738 s (2x OCH_3); **P-O-Py**: 4.92 dd, *J* = 17.0 and 8.0 Hz and 4.87 dd, *J* = 17.0 and 8.3 Hz (P-O-CH_2), 8.12 d, *J* = 7.2 Hz (ArH), 7.15 d, *J* = 3.6 Hz (ArH), 6.93 dd, *J* = 7.2 and 3.6 Hz (ArH), 3.79 s (OCH_3);
[g] **5'-OTBDPS**: 7.62 m, 4H (*ortho*-ArH), 7.43 m, 4H (*meta*-ArH), 7.48 m, 2H (*para*-ArH), 1.03 s, 9H (*t*-Bu); **P(OCH_2CH_3)$_2$**: 4.03 – 4.12 m, 4H (P-OCH_2), 1.263 t and 1.260 t, *J* = 7.0 Hz, 6H (2x CH_3);
[h] **5'-OTBDPS**: 7.62 m, 4H (*ortho*-ArH), 7.43 m, 4H (*meta*-ArH), 7.48 m, 2H (*para*-ArH), 1.02 s, 9H (*t*-Bu); **P(OCH_2CH_3)$_2$**: 4.03 – 4.12 m, 4H (P-OCH_2), 1.213 t and 1.202 t, *J* = 7.0 Hz, 6H (2x CH_3);
[i] **5'-ODMTr**: 7.36 m, 2H (*ortho*-ArH), 7.34 m, 2H (*meta*-ArH), 7.25 m, 1H (*para*-ArH), 7.24 m, 4H (*ortho*-ArH), 6.92 m, 4H (*meta*-ArH), 3.743 s and 3.740 s (2x OCH_3); **P(OCH_2CH_3)$_2$**: 4.07 – 4.12 m, 4H (P-OCH_2), 1.255 t and 1.258 t, *J* = 7.1 Hz (2x CH_3);
[j] **5'-ODMTr**: 7.38 m, 2H (*ortho*-ArH), 7.31 m, 2H (*meta*-ArH), 7.25 m, 4H (*ortho*-ArH), 7.24 m, 1H (*para*-ArH), 6.89 m, 4H (*meta*-ArH), 3.736 s and 3.735 s (2x OCH_3); **P(OCH_2CH_3)$_2$**: 3.98 – 4.02 m, 4H (P-OCH_2), 1.156 t and 1.153 t, *J* = 7.1 Hz (2x CH_3);
[k] **5'-ODMTr**: 7.35 m, 2H (*ortho*-ArH), 7.33 m, 2H (*meta*-ArH), 7.25 m, 1H (*para*-ArH), 7.23 m, 4H (*ortho*-ArH), 6.92 m, 4H (*meta*-ArH), 3.751 s and 3.749 s (2x OCH_3); **P-O-Py**: 4.97 br d, *J* = 8.5 Hz (P-O-CH_2), 8.12 d, *J* = 7.2 Hz (ArH), 7.18 d, *J* = 3.6 Hz (ArH), 6.93 dd, *J* = 7.2 and 3.6 Hz (ArH), 3.79 s (OCH_3);
[l] **5'-ODMTr**: 7.36 m, 2H (*ortho*-ArH), 7.29 m, 2H (*meta*-ArH), 7.24 m, 4H (*ortho*-ArH), 7.20 m, 1H (*para*-ArH), 6.87 m, 4H (*meta*-ArH), 3.725 s and 3.719 s (2x OCH_3); **P-O-Py**: 4.94 dd, *J* = 17.0 and 8.4 Hz and 4.87 dd, *J* = 17.0 and 8.4 Hz (P-O-CH_2), 8.12 d, *J* = 7.1 Hz (ArH), 7.14 d, *J* = 3.6 Hz (ArH), 6.95 dd, *J* = 7.1 and 3.6 Hz (ArH), 3.797 s (OCH_3).

Table S2
Carbon-13 NMR data of compounds **19a – 24b** in DMSO. Coupling constants *J*(C,P) are given in brackets.

Comp.	Conf.	C-1'	C-2'	C-3'	C-4'	C-5'	O-CH_2-CH(P)-O	Base
19a [a] 63%	[R] C3'	80.74	74.27 (9.3)	72.91	82.96	64.05	63.71 (4.8) 65.52 (164.3)	C-2: 150.74; C-4: 162.83; C-5: 102.58; C-6: 139.90
19a [b] 37%	[S] C2'	88.92	76.64 (11.6)	69.14	75.70	62.72	59.52 (6.2) 69.61 (166.1)	C-2: 150.20; C-4: 163.18; C-5: 101.61; C-6: 140.89
21a [c]	[R] C3'	89.48	76.58 (11.5)	69.89	74.88	62.66	59.44 (5.9) 69.58 (166.0)	C-2: 150.24; C-4: 163.28; C-5: 101.63; C-6: 141.54
22a [d]	[S] C2'	81.04	74.31 (9.1)	72.88	81.94	63.62	63.47 (5.0) 65.53 (164.1)	C-2: 150.76; C-4: 162.90; C-5: 102.53; C-6: 140.18
23a [e] T = 50 deg	[R] C3'	89.20	76.49 (9.8)	69.70	74.27	62.62	61.23 (~5) 72.66 (153.6)	C-2: 150.07; C-4: 162.98; C-5: 101.52; C-6: 141.00
24a [f]	[S] C2'	80.61	73.88 (8.6)	73.12	81.80	63.72	65.43 (4.2) 67.62 (154.2)	C-2: 150.63; C-4: 162.61; C-5: 102.25; C-6: 140.05
19b [g] 51%	[S] C2'	79.60	73.91	74.03 (11.7)	83.01	64.06	60.03 (6.2) 69.51 (165.8)	C-2: 150.84; C-4: 162.78; C-5: 102.63; C-6: 139.61
19b [h] 49%	[R] C3'	88.06	75.68	69.20 (9.7)	76.09	62.45	63.62 (5.3) 65.08 (164.1)	C-2: 150.24; C-4: 163.15; C-5: 101.61; C-6: 140.55
21b [i]	[S] C2'	79.82	74.03	74.00 (11.2)	81.97	63.65	60.04 (5.8) 69.34 (165.9)	C-2: 150.83; C-4: 162.84; C-5: 102.57; C-6: 139.83
22b [j]	[R] C3'	88.89	75.64	70.05 (10.1)	74.94	62.43	63.75 (5.3) 64.81 (164.4)	C-2: 150.30; C-4: 163.31; C-5: 101.65; C-6: 141.30
23b [k]	[S] C2'	79.70	74.28	74.03 (10.2)	82.46	63.96	61.70 (6.0) 72.41 (154.6)	C-2: 150.88; C-4: 162.87; C-5: 102.47; C-6: 139.83
24b [l]	[R] C3'	88.91	75.80	69.98 (9.1)	75.16	62.58	65.57 (5.7) 67.37 (155.2)	C-2: 150.28; C-4: 163.28; C-5: 101.46; C-6: 141.17

Substituents:
[a] **5'-OTBDPS**: 135.37 and 135.20 (4x *ortho*-ArCH), 132.68 and 132.26 (2x *ipso*-ArC), 130.35 and 130.31 (2x *para*-ArCH), 128.26 (4x *meta*-ArCH), 26.89 (3x CH_3), 18.96 (>C<); **P(OCH_2CH_3)$_2$**: 62.45, *J* = 6.2 Hz (2x P-OCH_2), 16.37, *J* = 5.4 Hz and 16.36, *J* = 5.6 Hz (2x CH_3).
[b] **5'-OTBDPS**: 135.30 and 135.17 (4x *ortho*-ArCH), 132.87 and 132.40 (2x *ipso*-ArC), 130.23 and 130.19 (2x *para*-ArCH), 128.15 and 128.18 (4x *meta*-ArCH), 26.81 (3x CH_3), 19.01 (>C<); **P(OCH_2CH_3)$_2$**: 62.81, *J* = 6.3 Hz and 62.59, *J* = 6.3 Hz (2x P-OCH_2), 16.46, *J* = 5.2 Hz (2x CH_3).
[c] **5'-ODMTr**: 86.10 (>C<), 158.35 and 158.33 (2x *para*-ArCH), 144.81 (*ipso*-ArC), 135.52 and 135.30 (2x *ipso*-ArC), 129.97 and 129.95 (4x *ortho*-ArCH), 128.09 (2x *meta*-ArCH), 127.90 (2x *ortho*-ArCH), 127.00 (*para*-ArCH), 113.44 and 113.43 (4x *meta*-ArCH), 55.23 (2x OCH_3); **P(OCH_2CH_3)$_2$**: 62.80, *J* = 6.4 Hz (P-OCH_2), 62.58, *J* = 6.2 Hz (P-OCH_2), 16.48, *J* = 5.2 Hz (2x CH_3).
[d] **5'-ODMTr**: 86.47 (>C<), 158.38 (2x *para*-ArCH), 144.61 (*ipso*-ArC), 135.41 and 135.14 (2x *ipso*-ArC), 129.94 and 129.90 (4x *ortho*-ArCH), 128.20 (2x *meta*-ArCH), 127.85 (2x *ortho*-ArCH), 127.05 (*para*-ArCH), 113.55 and 113.54 (4x *meta*-ArCH), 55.25 (2x OCH_3); **P(OCH_2CH_3)$_2$**: 62.76, *J* = 6.5 Hz (P-OCH_2), 62.40, *J* = 6.1 Hz (P-OCH_2), 16.39, *J* = 5.3 Hz (2x CH_3);
[e] **5'-ODMTr**: 85.93 (>C<), 158.24 and 158.22 (2x *para*-ArCH), 144.63 (*ipso*-ArC), 135.43 and 135.28 (2x *ipso*-ArC), 129.79 and 129.76 (4x *ortho*-ArCH), 127.84 (2x *meta*-ArCH), 127.81 (2x *ortho*-ArCH), 126.79 (*para*-ArCH), 113.29 (4x *meta*-ArCH), 55.11 (2x OCH_3); **P-O-Py**: 156.61 (ArC), ……. (ArC), 139.33 (ArCH), 110.13 (ArCH), 108.94 (ArCH), 61.04 (P-O-CH_2), 56.04 (OCH_3);
[f] **5'-ODMTr**: 86.38 (>C<), 158.29 (2x *para*-ArCH), 144.43 (*ipso*-ArC), 135.32 and 135.10 (2x *ipso*-ArC), 129.74 (4x *ortho*-ArCH), 127.98 (2x *meta*-ArCH), 127.75 (2x *ortho*-ArCH), 126.88 (*para*-ArCH), 113.42 (4x *meta*-ArCH), 55.13 (2x OCH_3); **P-O-Py**: 157.05 (ArC), 150.41 (ArC), 139.44 (ArCH), 110.42 (ArCH), 109.00 (ArCH), 60.78, *J* = 4.4 Hz (P-O-CH_2), 56.13 (OCH_3);
[g] **5'-OTBDPS**: 135.16 (4x *ortho*-ArCH), 132.68 and 132.19 (2x *ipso*-ArC), 130.31 and 130.22 (2x *para*-ArCH), 128.26 (4x *meta*-ArCH), 26.87 (3x CH_3), 18.95 (>C<); **P(OCH_2CH_3)$_2$**: 62.87, *J* = 6.5 Hz and 62.57, *J* = 6.4 Hz (2x P-OCH_2), 16.40, *J* = 6.1 Hz and 16.27, *J* = 6.6 Hz (2x CH_3);

[h] **5'-OTBDPS**: 135.36 and 135.26 (4x *ortho*-ArCH), 132.71 and 132.27 (2x *ipso*-ArC), 130.36 and 130.20 (2x *para*-ArCH), 128.18 (4x *meta*-ArCH), 26.77 (3x CH_3), 18.98 (>C<); **P(OCH_2CH_3)$_2$**: 62.52, *J* = 6.2 Hz and 62.32, *J* = 6.2 Hz (2x P-OCH_2), 16.05, *J* = 6.5 Hz and 16.03, *J* = 6.5 Hz (2x CH_3).
[i] **5'-ODMTr**: 86.47 (>C<), 158.36 (2x *para*-ArCH), 144.58 (*ipso*-ArC), 135.30 and 135.02 (2x *ipso*-ArC), 129.91 (2x *ortho*-ArCH), 129.87 (2x *ortho*-ArCH), 128.17 (2x *meta*-ArCH), 127.77 (2x *ortho*-ArCH), 127.04 (*para*-ArCH), 113.52 and 113.50 (4x *meta*-ArCH), 55.21 (2x OCH_3); **P(OCH_2CH_3)$_2$**: 62.82, *J* = 6.5 Hz (P-OCH_2), 62.56, *J* = 6.3 Hz (P-OCH_2), 16.47 and 16.43 (2x CH_3);
[j] **5'-ODMTr**: 86.06 (>C<), 158.36 (2x *para*-ArCH), 144.78 (*ipso*-ArC), 135.48 and 135.25 (2x *ipso*-ArC), 129.98 (4x *ortho*-ArCH), 128.11 (2x *meta*-ArCH), 127.89 (2x *ortho*-ArCH), 127.01 (*para*-ArCH), 113.44 (4x *meta*-ArCH), 55.24 (2x OCH_3); **P(OCH_2CH_3)$_2$**: 62.75, *J* = 6.5 Hz (P-OCH_2), 62.33, *J* ~ 5 Hz (P-OCH_2), 16.42 and 16.39 (2x CH_3);
[k] **5'-ODMTr**: 86.53 (>C<), 158.38 (2x *para*-ArCH), 144.58 (*ipso*-ArC), 135.36 and 135.02 (2x *ipso*-ArC), 129.93 and 129.91 (4x *ortho*-ArCH), 128.22 (2x *meta*-ArCH), 127.82 (2x *ortho*-ArCH), 127.06 (*para*-ArCH), 113.58 and 113.55 (4x *meta*-ArCH), 55.25 (2x OCH_3); **P-O-Py**: 157.38 (ArC), 150.61 (ArC), 139.66 (ArCH), 110.60 (ArCH), 109.02 (ArCH), 61.35, *J* = 3.6 Hz (P-O-CH_2), 56.29 (OCH_3);
[l] **5'-ODMTr**: 86.06 (>C<), 158.31 and 151.28 (2x *para*-ArCH), 144.88 (*ipso*-ArC), 135.48 and 135.28 (2x *ipso*-ArC), 129.97 and 129.89 (4x *ortho*-ArCH), 128.07 (2x *meta*-ArCH), 127.88 (2x *ortho*-ArCH), 126.92 (*para*-ArCH), 113.45 and 113.43 (4x *meta*-ArCH), 55.17 and 55.15 (2x OCH_3); **P-O-Py**: 157.14 (ArC), 150.58 (ArC), 139.62 (ArCH), 110.49 (ArCH), 109.04 (ArCH), 61.35, *J* = 4.0 Hz (P-O-CH_2), 56.30 (OCH_3).

Table S3
Proton NMR data of compounds **27a – 34c**. Coupling constants are given in brackets.

Comp.	Conf.	Solv.	H-1'	H-2'	H-3'	H-4'	H-5'	H-5''	O-CH(P)-O	Base
27a [a]	[S]	DMSO	5.81 d (2.6)	4.84 dd (6.5; 2.6)	4.72 dd (6.5; 4.8)	4.11 ddd (6.8; 4.8; 3.7)	3.31 dd (10.1; 6.8)	3.07 dd (10.1; 3.7)	5.12 d (18.1)	H-5: 5.54 dd (8.0; 2.0) H-6: 7.71 d (8.0) NH: 11.24 d (2.0)
27b [b]	[R]	DMSO	5.92 d (1.9)	4.87 dd (6.5; 1.9)	4.70 dd (6.5; 4.5)	4.34 ddd (6.9; 4.5; 3.3)	3.25 dd (10.3; 6.9)	3.02 dd (10.3; 3.3)	4.97 d (19.5)	H-5: 5.47 dd (8.0; 2.1) H-6: 7.73 d (8.0) NH: 11.38 d (2.1)
31b [c]	[S]	DMSO	6.30 d (1.5)	5.20 dd (6.0; 1.5)	5.57 dd (6.0; 4.3)	4.40 ddd (9.0; 4.3; 3.3)	3.43 dd (10.5; 9.0)	3.06 dd (10.5; 3.3)	5.79 d (29.0)	H-8: 8.17 s NH: 11.99 s NHCO: 11.22 s
31c [d]	[S]	$CDCl_3$	5.99 d (1.2)	5.17 m	5.17 m	4.48 ddd (5.4; 3.2; 2.3)	3.47 dd (10.8; 3.2)	3.41 dd (10.8; 5.4)	5.64 d (29.0)	H-5: 7.28-7.25 H-6: 7.96 d (7.6) NH: 8.76 s
32c [e]	[R]	$CDCl_3$	6.08 d (2.0)	5.08 dd (6.1; 2.0)	4.95 dd (6.1; 3.4)	4.71 ddd (4.6; 3.5; 3.4)	3.47 dd (10.8; 3.5)	3.44 dd (10.8; 4.6)	5.36 d (28.5)	H-5: 7.27-7.23 H-6: 7.99 d (7.4) NH: 8.84 s
33a [f]	[S]	DMSO	6.31 d (2.6)	5.41 dd (6.2; 2.6)	5.06 dd (6.2; 4.0)	4.32 ddd (6.6; 4.8; 4.0)	3.21 dd (10.0; 6.6)	3.10 dd (10.0; 4.8)	5.33 d (19.5)	H-2: 8.62 s H-8: 8.58 s NH: 11.22 br s
33b [g]	[S]	DMSO	6.11 d (2.0)	5.07 dd (6.2; 2.0)	5.18 dd (6.2; 4.3)	4.23	3.34	3.02 dd (10.5; 3.5)	5.13 d (18.0)	H-8: 8.13 s NHCO: 11.54 br s NH: 12.03 br s
33c [h]	[S]	$CDCl_3$	5.94 d (1.7)	5.02 dd (6.1; 1.7)	5.11 dd (6.1; 3.7)	4.51 ddd (5.8; 3.7; 2.9)	3.46 dd (11.2; 2.9)	3.01 dd (11.2; 5.8)	5.53 d (21.5)	H-5: 7.21 d (7.4) H-6: 8.02 d (7.4)
33d [i]	[S]	DMSO	5.84 d (3.0)	4.84 dd (6.6; 3.0)	4.73 dd (6.6; 4.6)	4.10 ddd (6.6; 4.6; 3.6)	3.29 dd (10.2; 6.6)	3.10 dd (10.2; 3.6)	5.15 d (18.3)	H-6: 7.55 q (1.2) CH_3: 1.64 d (1.2) NH: 11.42 s
34a [j]	[R]	DMSO	6.44 d (2.5)	5.43 dd (6.4; 2.5)	5.00 dd (6.4; 3.5)	4.49 ddd (6.6; 4.7; 3.5)	3.18 dd (10.2; 6.6)	3.09 dd (10.2; 4.7)	5.10 d (20.3)	H-2: 8.61 s H-8: 8.56 s NH: 11.21 br s
34c [k]	[R]	$CDCl_3$	6.11 d (2.3)	4.94 dd (6.2; 2.3)	4.86 dd (6.2; 2.4)	4.78 td (3.5; 3.5; 2.4)	3.42 dd (11.0; 3.5)	3.39 dd (11.0; 3.5)	5.28 d (20.8)	H-5: 7.24 – 7.19 H-6: 8.01 d (7.4)

Substituents:

[a] **P=O(OH)O-Py**: 10.01 br s (P-OH), 4.88 d, *J* = 8.0 Hz (OCH_2), 8.10 d, *J* = 7.1 Hz (ArH), 7.08 d, *J* = 3.6 Hz (ArH), 6.93 dd, *J* = 7.1 and 3.6 Hz (ArH), 3.78 s (OCH_3), **5'-ODMTr**: 7.36 m, 2H (*ortho*-ArH), 7.29 m, 2H (*meta*-ArH), 7.24 m, 4H (*ortho*-ArH), 7.22 m, 1H (*para*-ArH), 6.88 m, 4H (*meta*-ArH), 3.734 s and 3.729 s (2x OCH_3);

[b] **P=O(OH)O-Py**: 10.12 br s (P-OH), 4.93 d, *J* = 7.7 Hz (OCH_2), 8.12 d, *J* = 7.2 Hz (ArH), 7.13 d, *J* = 3.6 Hz (ArH), 6.96 dd, *J* = 7.2 and 3.6 Hz (ArH), 3.80 s (OCH_3), **5'-ODMTr**: 7.35 m, 2H (*ortho*-ArH), 7.28 m, 2H (*meta*-ArH), 7.22 m, 4H (*ortho*-ArH), 7.21 m, 1H (*para*-ArH), 6.87 m, 4H (*meta*-ArH), 3.731 s and 3.726 s (2x OCH_3);

[c] **CO-CH(CH_3)$_2$**: 2.70 h, *J* = 6.9 Hz (–CH<); 1.129 d and 1.126 d, *J* = 6.9 Hz (2x CH_3); **P=O(OCH_3)$_2$**: 3.73 d, *J* = 10.4 Hz (2x OCH_3); **5'-ODMTr**: 7.26 m, 2H (*ortho*-ArH), 7.15 m, 2H (*meta*-ArH), 7.14 m, 1H (*para*-ArH), 7.13 m and 7.12 m, 4H (*ortho*-ArH), 6.74 and 6.69, 4H (*meta*-ArH), 3.705 s and 3.694 s (2x OCH_3);

[d] **P=O(OCH_3)$_2$**: 3.87 d and 3.84 d, *J* = 10.4 Hz (2x OCH_3); **N-Bz:** 7.90 m, 2H (*ortho*-ArH), 7.61 m, 1H (*para*-ArH), 7.52 m, 2H (*meta*-ArH); **5'-ODMTr**: 7.37 m, 2H (*ortho*-ArH), 7.29 m, 2H (*meta*-ArH), 7.28-7.25 m, 4H (*ortho*-ArH), 7.25 m (*para*-ArH), 6.84 m, 4H (*meta*-ArH), 3.772 s and 3.768 s (2x OCH_3);

[e] **P=O(OCH_3)$_2$**: 3.93 d and 3.89 d, *J* = 10.4 Hz (2x OCH_3); **N-Bz:** 7.90 m, 2H (*ortho*-ArH), 7.61 m, 1H (*para*-ArH), 7.52 m, 2H (*meta*-ArH); **5'-ODMTr**: 7.35 m, 2H (*ortho*-ArH), 7.29 m, 2H (*meta*-ArH), 7.27-7.23 m, 4H (*ortho*-ArH), 7.22 m (*para*-ArH), 6.83 m, 4H (*meta*-ArH), 3.77 s and 3.76 s (2x OCH_3);

[f] **N-Bz:** 8.05 m, 2H (*ortho*-ArH), 7.65 m, 1H (*para*-ArH), 7.56 m, 2H (*meta*-ArH); **P=O(OH)O-Py:** 9.87 br s (P-OH), 4.95 d, *J* = 8.2 Hz (OCH_2), 8.15 d, *J* = 7.2 Hz (ArH), 7.16 d, *J* = 3.6 Hz (ArH), 6.97 dd, *J* = 7.2 and 3.6 Hz (ArH), 3.78 s (OCH_3), **5'-ODMTr**: 7.30 m, 2H (*ortho*-ArH), 7.22 m, 2H (*meta*-ArH), 7.17 m, 2H (*ortho*-ArH), 7.16 m, 3H (1x *para*-ArH and 2x *ortho*-ArH), 6.81 m, 2H (*meta*-ArH), 6.79 m, 2H (*meta*-ArH), 3.712 s and 3.706 s (2x OCH_3);

[g] **CO-CH(CH_3)$_2$**: 2.73 h, *J* = 6.8 Hz (-CH<); 1.11 d, *J* = 6.8 Hz (2x CH_3); **P=O(OH)O-Py**: 4.87 d, *J* = 8.0 Hz (OCH_2), 8.08 d, *J* = 7.2 Hz (ArH), 7.08 d, *J* = 3.6 Hz (ArH), 6.92 dd, *J* = 7.2 and 3.6 Hz (ArH), 3.76 s (OCH_3); **5'-ODMTr**: 7.27 m, 2H (*ortho*-ArH), 7.18 m, 2H (*meta*-ArH), 7.17 m, 1H (*para*-ArH), 7.14 m and 7.13 m, 4H (*ortho*-ArH), 6.77 and 6.72, 4H (*meta*-ArH), 3.71 s and 3.70 s (2x OCH_3);

[h] **N-Bz:** 7.90 m, 2H (*ortho*-ArH), 7.59 m, 1H (*para*-ArH), 7.50 m, 2H (*meta*-ArH); **P=O(OH)O-Py**: 5.27 d, *J* = 7.2 Hz (OCH_2), 8.02 d, *J* = 7.2 Hz (ArH), 7.31 d, *J* = 3.4 Hz (ArH), 6.67 dd, *J* = 7.2 and 3.4 Hz (ArH), 3.79 s (OCH_3), **5'-ODMTr**: 7.36 m, 2H (*ortho*-ArH), 7.29 m, 2H (*meta*-ArH), 7.26 m, 2H (*ortho*-ArH), 7.25 m, 2H (*ortho*-ArH), 7.21 m, 1H (*para*-ArH), 6.84 m, 4H (*meta*-ArH), 3.77 s and 3.76 s (2x OCH_3);

[i] **P=O(OH)O-Py**: 10.23 br s (P-OH), 4.89 d, *J* = 8.1 Hz (OCH_2), 8.11 d, *J* = 7.2 Hz (ArH), 7.07 d, *J* = 3.6 Hz (ArH), 6.94 dd, *J* = 7.2 and 3.6 Hz (ArH), 3.78 s (OCH_3), **5'-ODMTr**: 7.37 m, 2H (*ortho*-ArH), 7.29 m, 2H (*meta*-ArH), 7.25 m, 2H (*ortho*-ArH), 7.23 m, 2H (*ortho*-ArH), 7.22 m, 1H (*para*-ArH), 6.88 m, 2H (*meta*-ArH), 6.86 m, 2H (*meta*-ArH), 3.731 s and 3.727 s (2x OCH_3);
[j] **N-Bz:** 8.05 m, 2H (*ortho*-ArH), 7.65 m, 1H (*para*-ArH), 7.55 m, 2H (*meta*-ArH); **P=O(OH)O-Py**: 9.78 br s (P-OH), 5.04 d, *J* = 8.2 Hz (OCH_2), 8.22 d, *J* = 7.2 Hz (ArH), 7.24 d, *J* = 3.6 Hz (ArH), 7.02 dd, *J* = 7.2 and 3.6 Hz (ArH), 3.83 s (OCH_3), **5'-ODMTr**: 7.29 m, 2H (*ortho*-ArH), 7.20 m, 2H (*meta*-ArH), 7.16 m, 3H (1x *para*-ArH and 2x *ortho*-ArH), 7.15 m, 2H (*ortho*-ArH), 6.80 m, 2H (*meta*-ArH), 6.77 m, 2H (*meta*-ArH), 3.71 s and 3.70 s (2x OCH_3);
[k] **N-Bz:** 7.90 m, 2H (*ortho*-ArH), 7.60 m, 1H (*para*-ArH), 7.50 m, 2H (*meta*-ArH); **P=O(OH)O-Py**: 5.35 dd and 5.30 dd, *J* = 17.2 and 7.5 Hz (OCH_2), 8.06 d, *J* = 7.1 Hz (ArH), 7.35 d, *J* = 3.5 Hz (ArH), 6.71 dd, *J* = 7.1 and 3.5 Hz (ArH), 3.85 s (OCH_3), **5'-ODMTr**: 7.33 – 7.26 m, 4H (2x *ortho*-ArH + 2x *meta*-ArH), 7.24 – 7.19 m, 5H (4x *ortho*-ArH + *para*-ArH), 6.83 m and 6.82 m, 4H (*meta*-ArH), 3.77 s and 3.75 s (2x OCH_3);

Table S4
Carbon-13 NMR data of compounds **27a – 34c**. Coupling constants *J*(C,P) are given in brackets.

Comp.	Conf.	Solvent	C-1'	C-2'	C-3'	C-4'	C-5'	O-CH(P)-O	Base
27a [a]	[S]	DMSO	91.20	83.42 (6.6)	81.02 (6.5)	82.74	64.24	102.48 (180.2)	C-2: 150.43; C-4: 163.33; C-5: 102.05; C-6: 142.86
27b [b]	[R]	DMSO	91.25	85.06 (8.2)	81.18 (6.5)	84.83	64.04	105.54 (179.9)	C-2: 150.38; C-4: 163.38; C-5: 101.79; C-6: 143.02
31b [c]	[S]	DMSO	88.32	84.71 (5.2)	82.40 (5.7)	83.94	64.74	100.25 (194.3)	C-2: 154.88; C4: 147.65; C-5: 121.11; C-6: 147.72; C-8: 139.51
31c [d]	[S]	$CDCl_3$	93.33	86.73 (2.6)	82.22 (2.7)	85.31	63.38	101.85 (199.0)	C-2: 154.78; C4: 162.49; C-5: 96.42; C-6: 145.42;
32c [e]	[R]	$CDCl_3$	93.50	87.16 (8.0)	82.48 (7.8)	85.77	63.23	102.52 (199.0)	C-2: 154.67; C4: 162.54; C-5: 96.25; C-6: 145.56;
33a [f]	[S]	DMSO	88.56	83.29	81.74	83.24	63.87	102.83 (181.5)	C-2: 151.7; C-4: 151.7; C-5: 125.86; C-6: 150.73; C-8: 144.08
33b [g]	[S]	DMSO	88.72	83.76 (6.1)	81.47 (6.2)	83.87	64.75	103.06 (180.5)	C-2: 154.97; C-4: 147.87; C-5: 120.91; C-6: 147.93; C-8: 139.13
33c [h]	[S]	$CDCl_3$	93.30	86.40 (4.0)	81.17 (4.0)	85.63	63.36	103.06 (187)	C-2: 154.95; C-4: 162.49; C-5: 96.43; C-6: 145.00
33d [i]	[S]	DMSO	89.88	83.25 (6.8)	80.83 (7.3)	82.12	64.15	102.39 (180.6)	C-2: 150.51; C-4: 163.96; C-5: 110.05; C-6: 137.86; CH_3: 12.06
34a [j]	[R]	DMSO	88.51	84.29	82.09	84.58	63.81	105.5 (180.0)	C-2: 152.20; C-4: 151.70; C-5: 125.88; C-6: 150.65; C-8: 143.88
34c [k]	[R]	$CDCl_3$	93.84	86.91 (8.0)	82.80 (9.0)	85.81	63.55	104.52 (184)	C-2: 154.93; C-4: 162.38; C-5: 96.40; C-6: 145.38

Substituents:
[a] **P=O(OH)O-Py:** 61.71, *J* = 4.4 Hz (OCH_2), 156.84 (ArC), 150.95 (ArC), 139.44 (ArCH), 110.38 (ArCH), 108.52 (ArCH), 56.20 (OCH_3); **5'-ODMTr**: 85.75 (>C<), 158.30 and 158.27 (2x *para*-ArC), 144.92 (*ipso*-ArC), 135.60 and 135.49 (2x *ipso*-ArC), 129.86 and 129.80 (4x *ortho*-ArCH), 128.02 (2x *meta*-ArCH), 127.83 (2x *ortho*-ArCH), 126.91 (*para*-ArCH), 113.38 (4x *meta*-ArCH), 55.20 and 55.18 (2x OCH_3);
[b] **P=O(OH)O-Py:** 61.73, *J* = 4.4 Hz (OCH_2), 156.72 (ArC), 150.82 (ArC), 139.51 (ArCH), 110.59 (ArCH), 108.50 (ArCH), 56.19 (OCH_3); **5'-ODMTr**: 85.76 (>C<), 158.28 and 158.25 (2x *para*-ArCH), 144.94 (*ipso*-ArC), 135.57 and 135.44 (2x *ipso*-ArC), 129.87 and 129.81 (4x *ortho*-ArCH), 128.01 (2x *meta*-ArCH), 127.82 (2x *ortho*-ArCH), 126.88 (*para*-ArCH), 113.38 and 113.37 (4x *meta*-ArCH), 55.19 and 55.17 (2x OCH_3);
[c] **CO-CH(CH_3)$_2$**: 180.05 (CO), 35.06 (-CH<), 19.17 and 18.86 (2x CH_3); **P=O(OCH_3)$_2$:** 53.81 d, *J* = 6.7 Hz and 53.79 d, *J* = 6.7 Hz; **5'-ODMTr**: 85.59 (>C<), 158.21 and 158.15 (2x *para*-ArC), 144.80 (*ipso*-ArC), 135.48 and 135.40 (2x *ipso*-ArC), 129.84 and 129.69 (4x *ortho*-ArCH), 127.82 (2x *ortho*-ArCH), 127.74 (2x *meta*-ArCH), 126.76 (*para*-ArCH), 113.06 and 112.98 (4x *meta*-ArCH), 55.16 and 55.11 (2x OCH_3);
[d] **P=O(OCH_3)$_2$:** 54.16 d, *J* = 7.0 Hz and 53.61 d, *J* = 7.0 Hz; **N-Bz**: 165.92 (C=O), 133.22 (*para*-ArCH); 132.93 (*ipso*-ArC), 129.04 (2x *meta*-ArCH), 127.46 (2x *ortho*-ArCH), **5'-ODMTr**: 86.94 (>C<), 158.60 and 158.59 (2x *para*-ArCH), 143.98 (*ipso*-ArC), 135.12 and 135.04 (2x *ipso*-ArC), 130.02 and 129.97 (4x *ortho*-ArCH), 127.98 (2x *meta*-ArCH and 2x *ortho*-ArCH), 127.06 (*para*-ArCH), 113.25 (4x *meta*-ArCH), 55.18 (2x OCH_3);
[e] **P=O(OCH_3)$_2$:** 54.46 d, *J* = 6.0 Hz and 54.00 d, *J* = 6.0 Hz; **N-Bz**: 165.88 (C=O), 133.21 (*para*-ArCH); 132.70 (*ipso*-ArC), 129.01 (2x *meta*-ArCH), 127.49 (2x *ortho*-ArCH), **5'-ODMTr**: 86.91 (>C<), 158.59 and 158.57 (2x *para*-ArCH), 144.01 (*ipso*-ArC), 135.15 and 135.06 (2x *ipso*-ArC), 130.01 and 129.95 (4x *ortho*-ArCH), 127.97 and 127.95 (2x *meta*-ArCH and 2x *ortho*-ArCH), 127.05 (*para*-ArCH), 113.21 (4x *meta*-ArCH), 55.17 (2x OCH_3);
[f] **N-Bz**: 165.9 (C=O), 133.56 (*ipso*-ArC), 128.72 (2x *ortho*-ArCH), 128.67 (2x *meta*-ArCH), 132.65 (*para*-ArCH); **P=O(OH)O-Py:** 61.73 (OCH_2), 157.4 (ArC), 150.73 (ArC), 139.71 (ArCH), 110.64 (ArCH), 109.02 (ArCH), 56.33 (OCH_3); **5'-ODMTr**: 85.66 (>C<), 158.24 and 158.23 (2x *para*-ArCH), 144.82 (*ipso*-ArC), 135.59 and 135.42 (2x *ipso*-ArC), 129.73 (4x *ortho*-ArCH), 127.94 (2x *meta*-ArCH), 127.72 (2x *ortho*-ArCH), 126.85 (*para*-ArCH), 113.33 and 113.31 (4x *meta*-ArCH), 55.19 and 55.17 (2x OCH_3);
[g] **CO-CH(CH_3)$_2$**: 180.34 (CO), 34.98 (-CH<), 19.18 and 18.98 (2x CH_3); **P=O(OH)O-Py:** 61.86, *J* = 4.5 Hz (OCH_2), 156.87 (ArC), 151.26, *J* = 4.7 Hz (ArC), 139.48 (ArCH), 110.35 (ArCH), 108.53 (ArCH), 56.24 (OCH_3); **5'-ODMTr**: 85.57 (>C<), 158.22 and 158.17 (2x *para*-ArC), 144.89 (*ipso*-ArC), 135.58 and 135.50 (2x *ipso*-ArC), 129.85 and 129.75 (4x *ortho*-ArCH), 127.57 (2x *meta*-ArCH), 127.84 (2x *ortho*-ArCH), 126.78 (*para*-ArCH), 113.15 and 113.09 (4x *meta*-ArCH), 55.19 and 55.15 (2x OCH_3);

[h] **N-Bz**: 166.21 (C=O), 133.08 (*para*-ArCH); 132.97 (*ipso*-ArC), 128.94 (2x *meta*-ArCH), 127.62 (2x *ortho*-ArCH), **P=O(OH)O-Py:** 61.86, *J* = 4.5 Hz (OCH_2), 158.49 (ArC), 151.07, J = 7 Hz (ArC), 139.54 (ArCH), 110.88 (ArCH), 108.16 (ArCH), 56.19 (OCH_3); **5'-ODMTr**: 86.84 (>C<), 158.62 and 158.61 (2x *para*-ArCH), 144.05 (*ipso*-ArC), 135.19 and 135.12 (2x *ipso*-ArC), 130.02 and 129.97 (4x *ortho*-ArCH), 128.00 (2x *meta*-ArCH), 127.99 (2x *ortho*-ArCH), 127.06 (*para*-ArCH), 113.30 (4x *meta*-ArCH), 55.19 (2x OCH_3);
[i] **P=O(OH)O-Py:** 61.76, *J* = 4.5 Hz (OCH_2), 156.73 (ArC), 150.85 (ArC), 139.49 (ArCH), 110.42 (ArCH), 108.52 (ArCH), 56.22 (OCH_3); **5'-ODMTr**: 85.76 (>C<), 158.33 and 158.30 (2x *para*-ArC), 144.94 (*ipso*-ArC), 135.58 and 135.50 (2x *ipso*-ArC), 129.88 and 129.83 (4x *ortho*-ArCH), 128.06 (2x *meta*-ArCH), 127.83 (2x *ortho*-ArCH), 126.95 (*para*-ArCH), 113.40 (4x *meta*-ArCH), 55.22 and 55.20 (2x OCH_3);
[j] **N-Bz**: 165.9 (C=O), 133.55 (*ipso*-ArC), 128.71 (2x *ortho*-ArCH), 128.66 (2x *meta*-ArCH), 132.66 (*para*-ArCH); **P=O(OH)O-Py:** 61.78 (OCH_2), 158.0 (ArC), 150.69 (ArC), 139.84 (ArCH), 110.91 (ArCH), 109.28 (ArCH), 56.41 (OCH_3); **5'-ODMTr**: 85.67 (>C<), 158.22 and 158.20 (2x *para*-ArCH), 144.85 (*ipso*-ArC), 135.60 and 135.42 (2x *ipso*-ArC), 129.73 (4x *ortho*-ArCH), 127.93 (2x *meta*-ArCH), 127.71 (2x *ortho*-ArCH), 126.82 (*para*-ArCH), 113.32 and 113.29 (4x *meta*-ArCH), 55.18 and 55.16 (2x OCH_3);
[k] **N-Bz**: 166.01 (C=O), 133.09 (*para*-ArCH); 133.09 (*ipso*-ArC), 128.96 (2x *meta*-ArCH), 127.57 (2x *ortho*-ArCH), **P=O(OH)O-Py:** 62.33, *J* = 4 Hz (OCH_2), 158.47 (ArC), 151.08, J = 7 Hz (ArC), 139.56 (ArCH), 110.96 (ArCH), 108.21 (ArCH), 56.23 (OCH_3); **5'-ODMTr**: 86.98 (>C<), 158.66 and 158.62 (2x *para*-ArCH), 143.95 (*ipso*-ArC), 135.17 and 134.99 (2x *ipso*-ArC), 130.05 and 129.92 (4x *ortho*-ArCH), 128.00 (2x *meta*-ArCH), 127.96 (2x *ortho*-ArCH), 127.08 (*para*-ArCH), 113.27 and 113.25 (4x *meta*-ArCH), 55.20 and 55.18 (2x OCH_3);

Synthesis of oligonucleotides

Oligonucleotides were synthesized using standard phosphoramidite and advanced phosphotriester protocols. Commercially available 2´-*O*-TBDMS-protected phosphoramidites (ChemGenes) were used for the incorporation of natural nucleotides. Synthesis was performed on a 1 μmol scale in DMTr OFF mode.

Phosphoramidite condensation method		
1. Detritylation	3% DCA in DCM	120 s
2. Condensation	0.1M phosphoramidite in ACN	180 s
	0.5 M ETT in ACN	
3. Capping	Ac_2O/Pyridine/THF 1:1:8	120 s
	1-MeIm/THF 1:9	
4. Oxidation	1.1M t-BuOOH in DCM	180s
Phosphotriester condensation method		
1. Detritylation	3% DCA in DCM	120 s
2. Condensation	0.1 M phosphonate in pyridine	300 s
	0.3 M TIPS-Cl in ACN	
3. Capping	Ac_2O/Pyridine/THF 1:1:8	120 s
	1-MeIm/THF 1:9	

Deprotection of DNA nonamers was achieved in two steps. First, the column was treated with benzenethiol/TEA/DMF (1/1.4/2 v/v/v) for 6 h to remove MOP protecting groups from the phosphonates 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 16 h to remove acyl protecting groups, and to release the final product from the solid support. Finally, the deprotected oligonucleotide was eluted from the column with 10% ACN in 20 mM NaCl, 20 mM NaOAc buffer (pH 7.5, 1 mL) and purified using ionic exchange chromatography (DNAPac PA-100, 250x4 mm, Dionex).

Deprotection of RNA nonamers was achieved in three steps. First, the column was treated with benzenethiol/TEA/DMF (1/1.4/2 v/v/v) for 6 h to remove MOP protecting groups from the phosphonates 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 16 h to remove acyl protecting groups, and to release the final product from the solid support. TBDMS-protected oligoribonucleotide was eluted from the column with 20% water in THF (1 mL), speedvac evaporated and coevaporated with isopropanol (3 x 0.5 mL). Third, TBDMS-protected oligoribonucleotide was treated 3 h at 65 ^{o}C with a mixture of triethylamine trihydrofluoride (75 μL) and TEA (60 μL) in DMSO (115 μL) to remove TBDMS protecting groups. Finally, the reaction mixture was diluted with 1M ammonium acetate (385 μL) and water (500 μL) and, the deprotected oligoribonucleotide was first desalted using reverse phase HPLC (Luna C_{18} 50x10 mm, 5μm, Phenomenex), then purified using ionic exchange chromatography (DNAPac PA-100, 250x4 mm, Dionex), and then finally desalted using reverse phase HPLC (Luna C_{18} 50x10 mm, 5μm, Phenomenex).

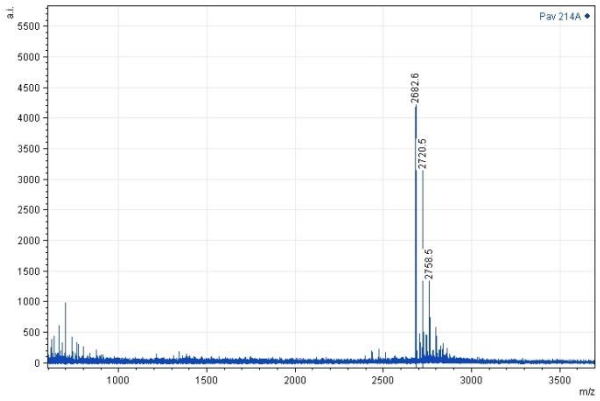
RP HPLC (desalting) – isocratically A 10 min, then A - B 15 min; A: 0.05M ammonium acetate, B: 50% ACN/0.05M ammonium acetate
IE chromatography (purification) – A - 20% B 20 min, 55 °C; A: 10% ACN in 20 mM NaCl, 20 mM NaOAc buffer (pH 7.5), B: 10% ACN in 1.5M NaCl, 20 mM NaOAc buffer (pH 7.3)

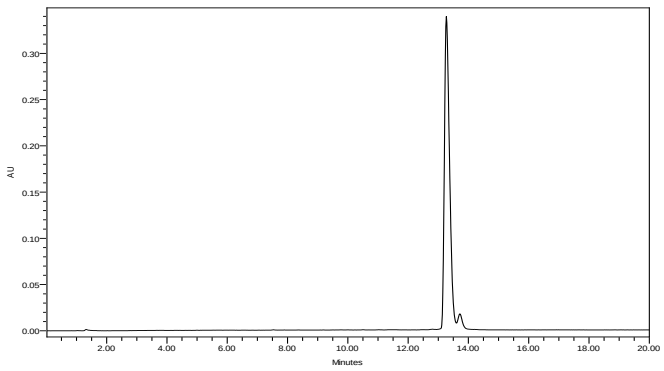
Maldi-MS and purity chromatograms of prepared oligonucleotides

Table S5. Maldi-MS of prepared oligonucleotides.

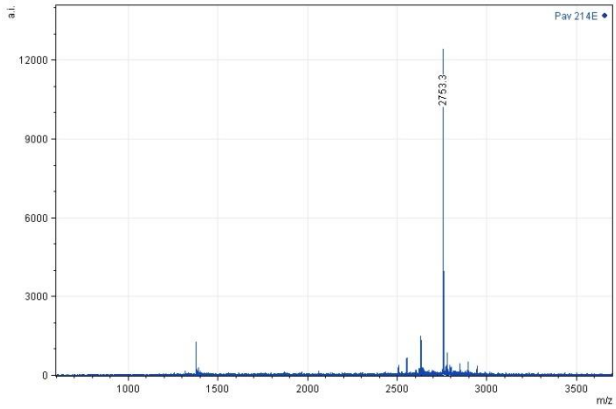
		Phosphonate unit U	$(M+H)^+$ calcd	$(M+H)^+$ found
	5'-d(GTG ATA TGC)-3'		2753.5	2753.3
	5'-d(GCA TAT CAC)-3'		2682.5	2682.6
	5'-r(GUG AUA UGC)-3'		2855.4	2855.6
	5'-r(GCA UAU CAC)-3'		2798.4	2798.5
D2	**5'-r(GUG AUA UGC)-3'**	**8**	2898.8	2898.8
D3		**9**	2898.8	2899.1
D4		**10**	2933.5	2933.5
D5		**11**	2933.5	2933.7
D6		**12**	2933.5	2933.4
D7		**13**	2933.5	2933.4
D8		**14**	2891.4	2891.3
D9		**15**	2891.4	2891.3
D11	**5'-d(GUG AUA UGC)-3'**	**10**	2837.5	2837.6
D12		**11**	2837.5	2837.6
D13		**12**	2837.5	2837.4
D14		**13**	2837.5	2837.5
D15		**14**	2795.4	2795.2
D16		**15**	2795.4	2795.3
D17	**5'-d(GTG AUA TGC)-3'**	**12**	2781.5	2782.3
D18		**14**	2767.5	2767.5

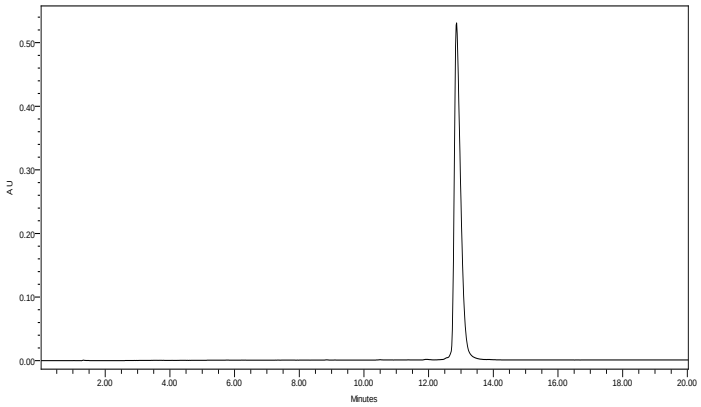
5'-d(GCA TAT CAC)-3'



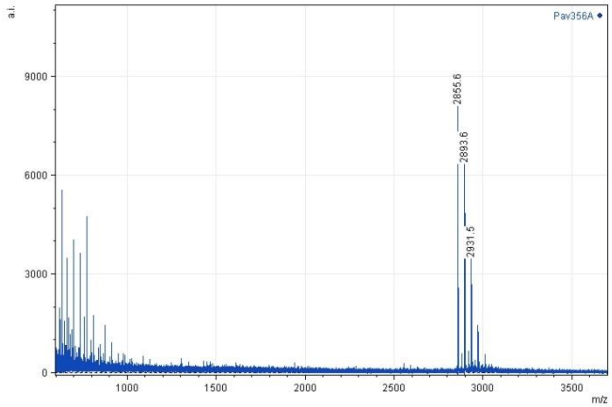


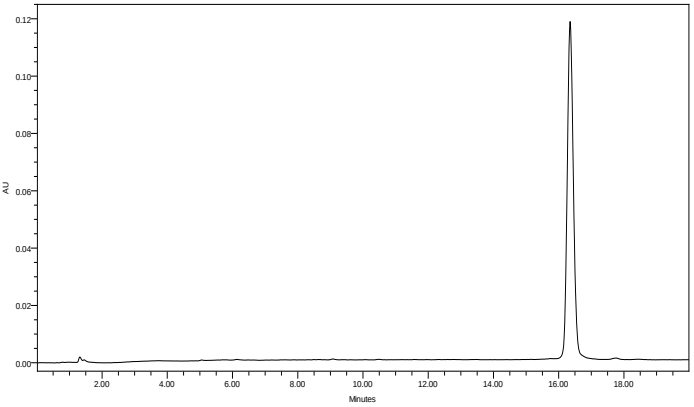
5'-d(GTG ATA TGC)-3'



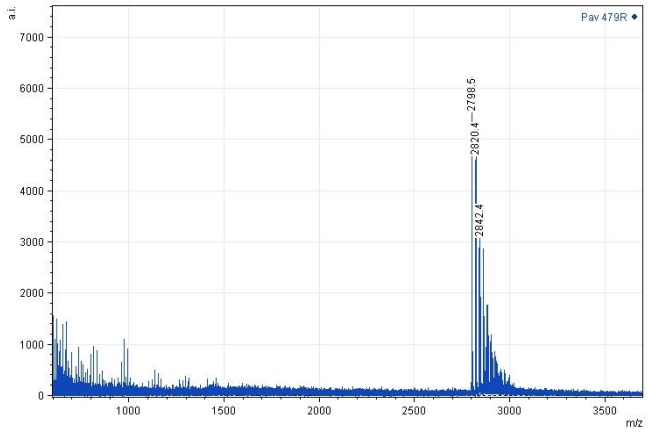


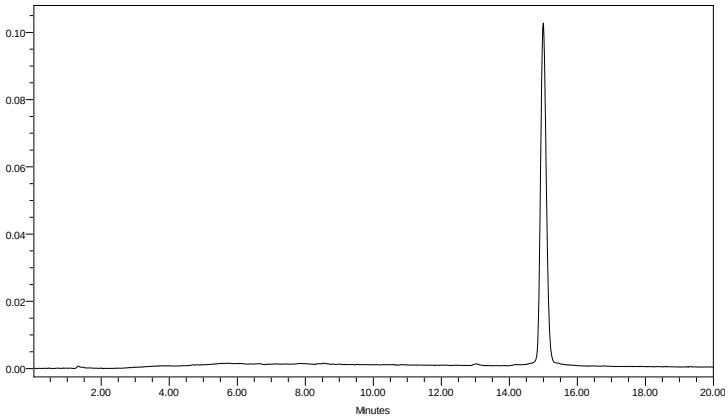
5'-r(GUG AUA UGC)-3'



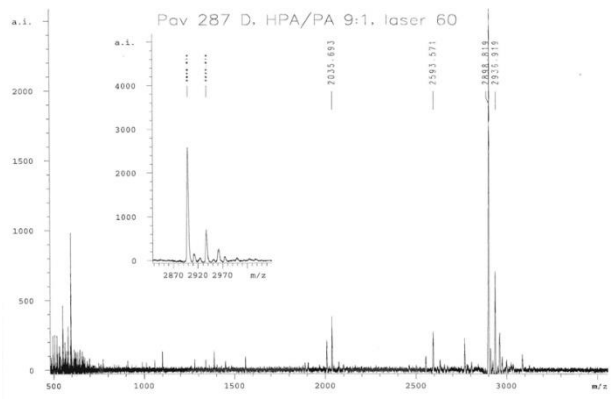


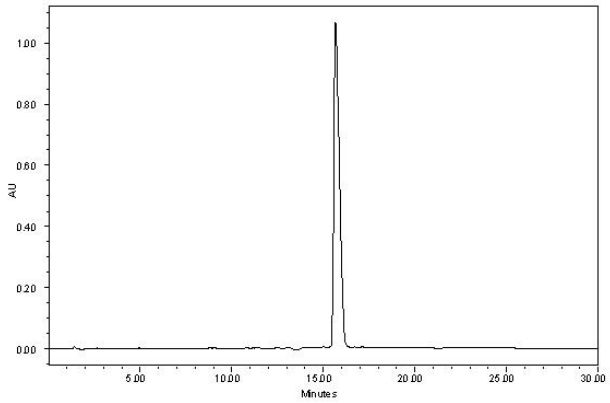
5'-r(GCA UAU CAC)-3'



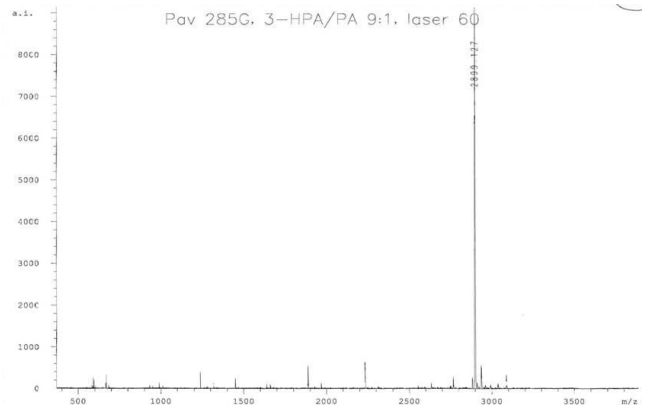


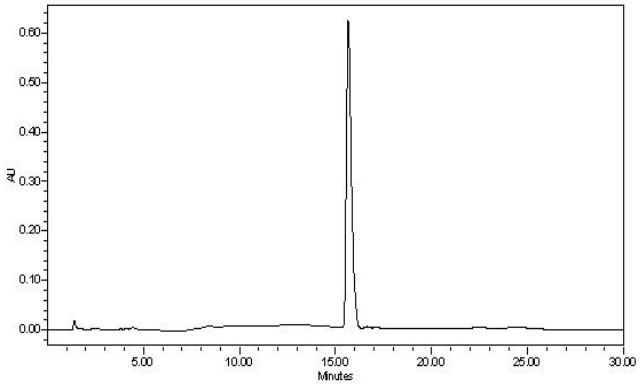
D2 5´-r(GUG AUA UGC)-3´ U phosphonate unit 8



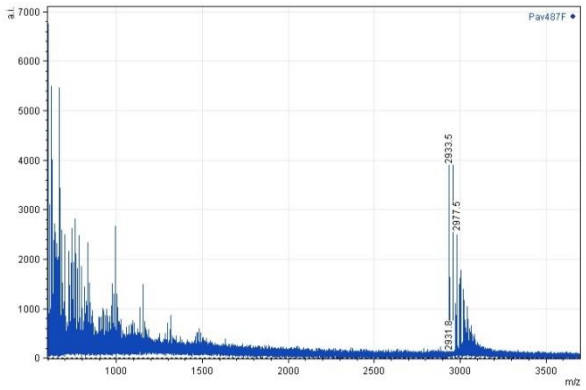


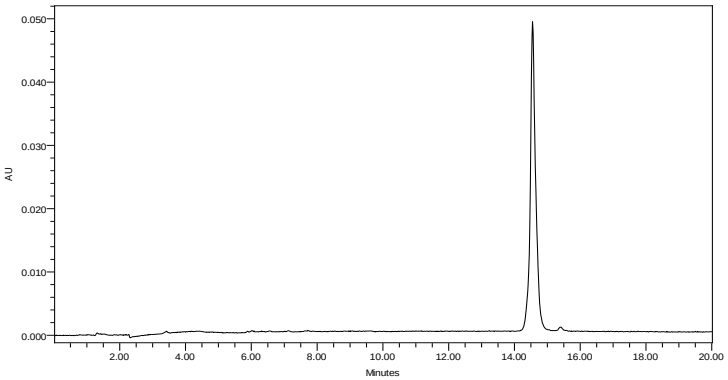
D3 5´-r(GUG AUA UGC)-3´ U phosphonate unit 9



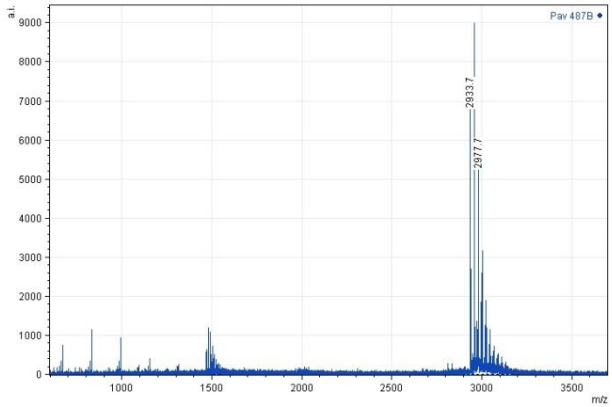


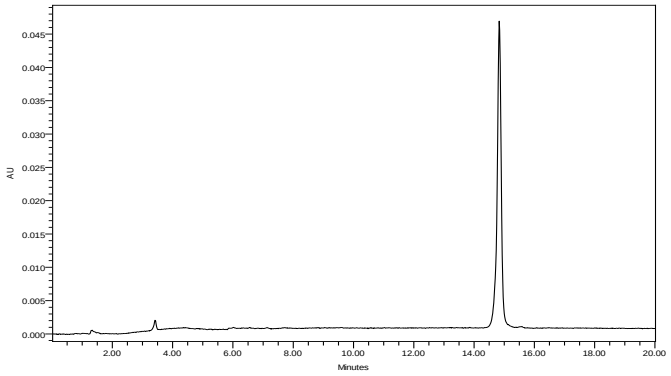
D4 5´-r(GUG AUA UGC)-3´ U phosphonate unit 10



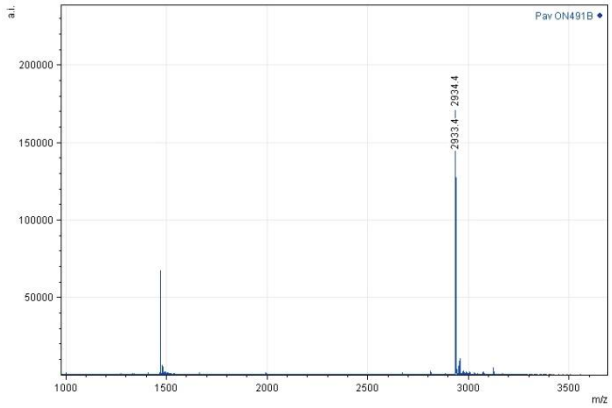


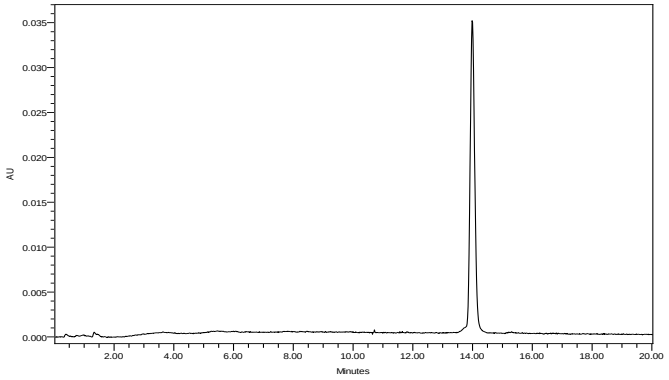
D5 5´-r(GUG AUA UGC)-3´ U phosphonate unit 11



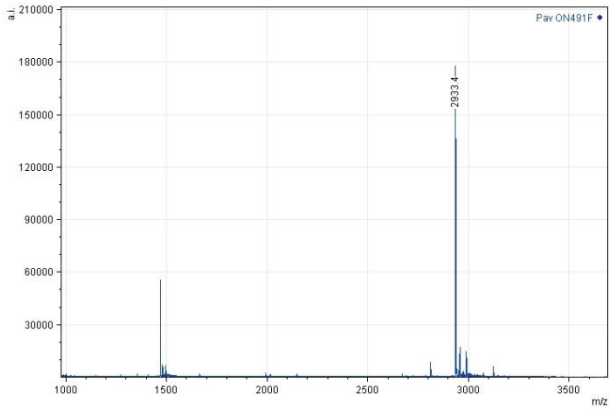


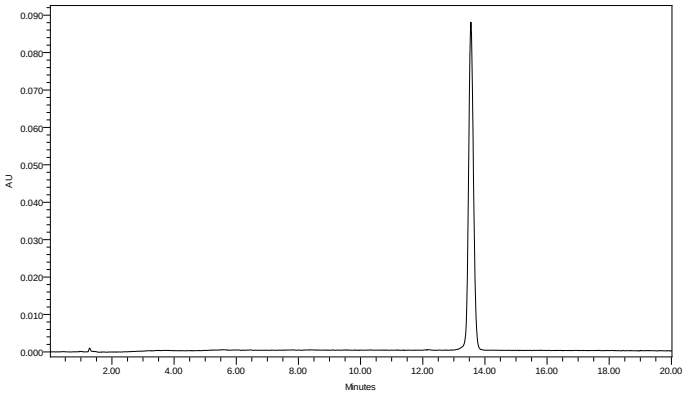
D6 5´-r(GUG AUA UGC)-3´ U phosphonate unit 12



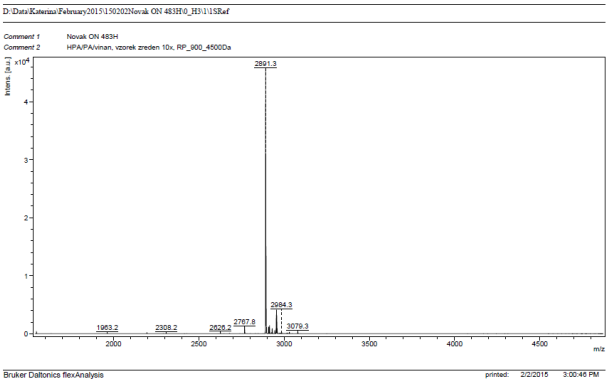


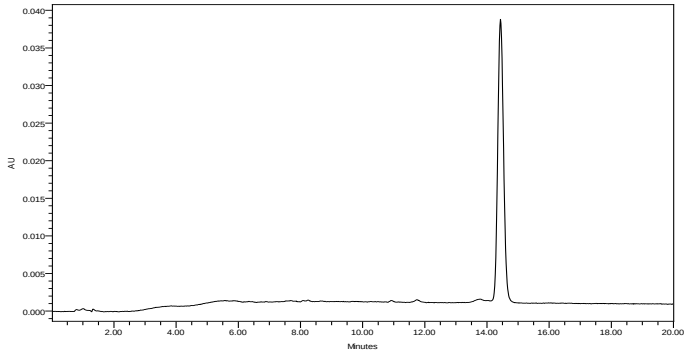
D7 5´-r(GUG AUA UGC)-3´ U phosphonate unit 13



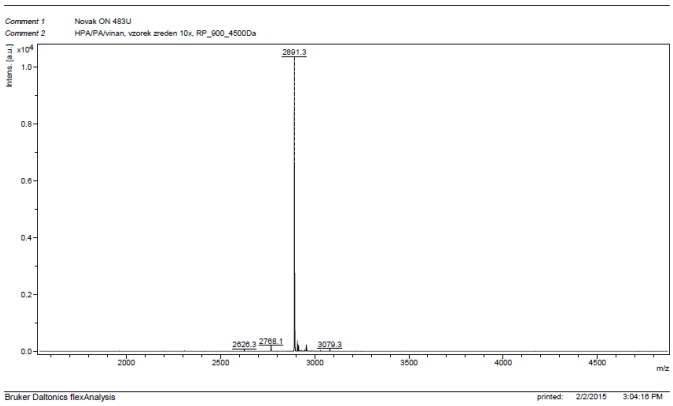


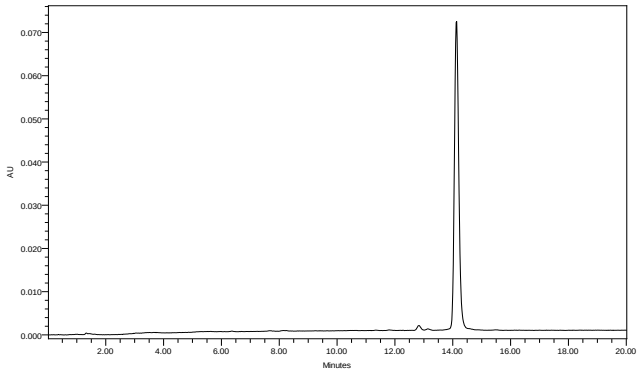
D8 5´-r(GUG AUA UGC)-3´ U phosphonate unit 14



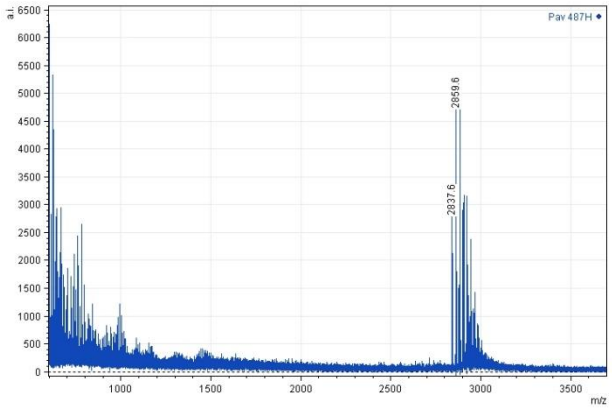


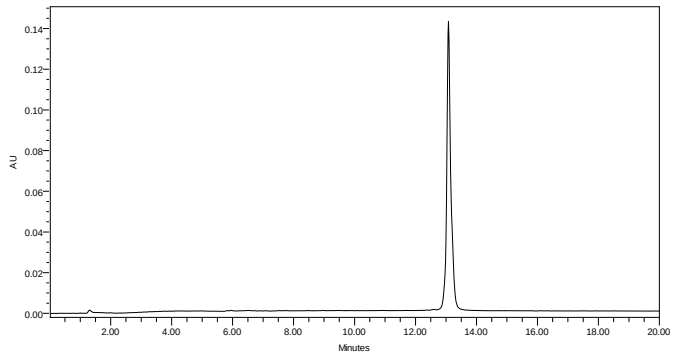
D9 5´-r(GUG AUA UGC)-3´ U phosphonate unit 15



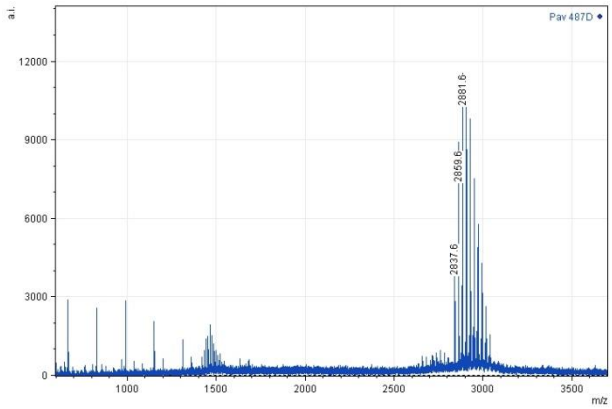


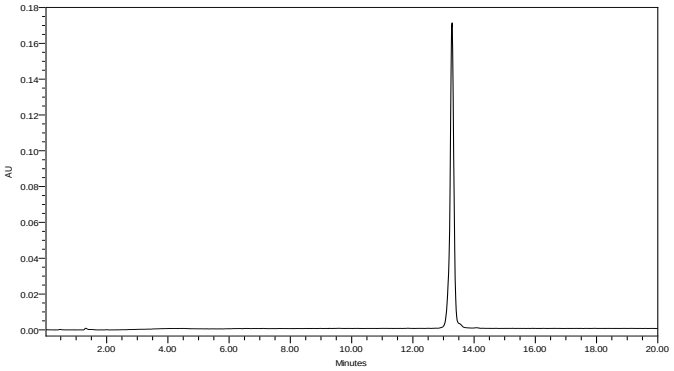
D11 5´-d(GUG AUA UGC)-3´ U phosphonate unit 10



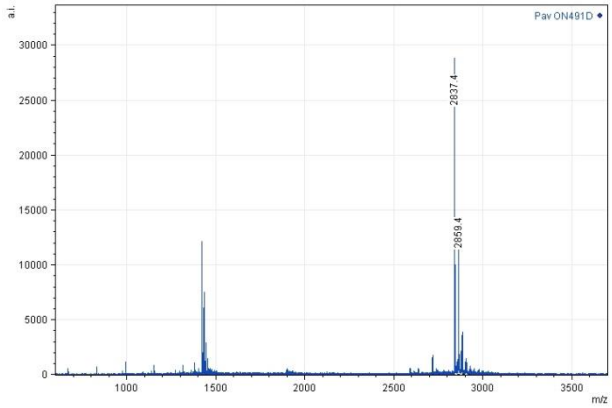


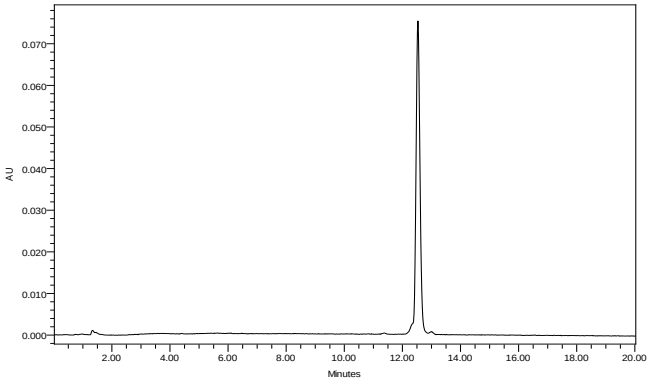
D12 5´-d(GUG AUA UGC)-3´ U phosphonate unit 11



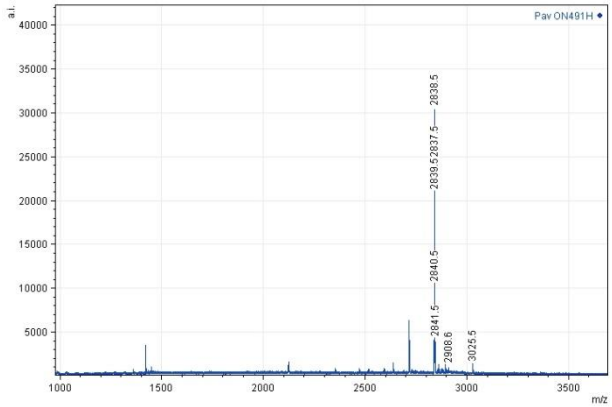


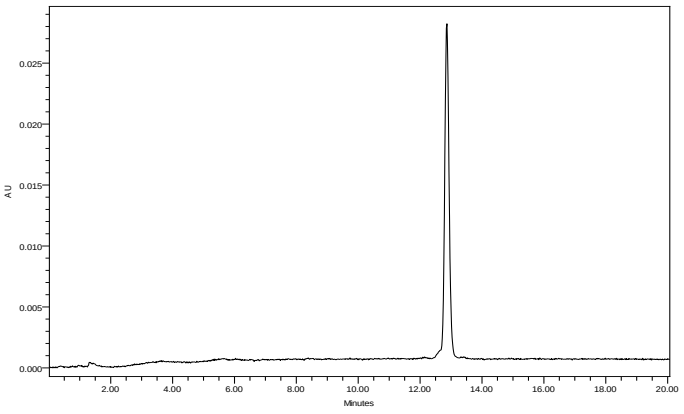
D13 5´-d(GUG AUA UGC)-3´ U phosphonate unit 12



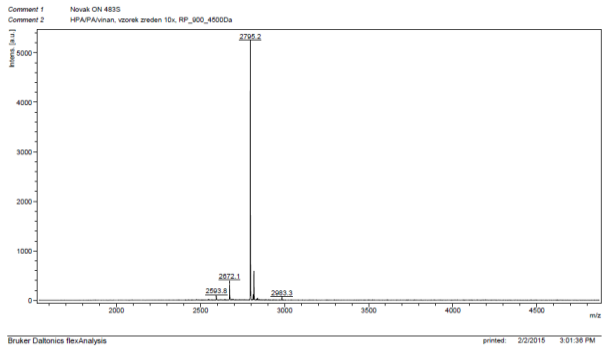


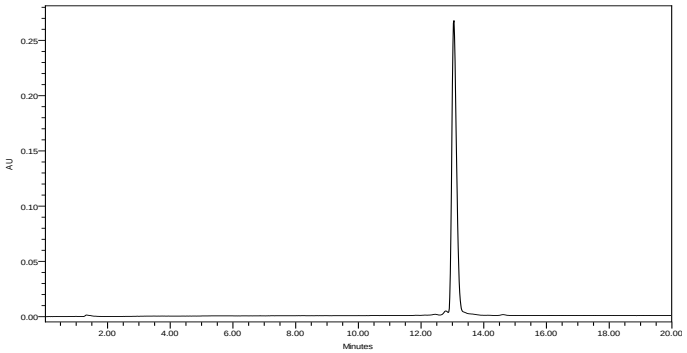
D14 5´-d(GUG AUA UGC)-3´ U phosphonate unit 13



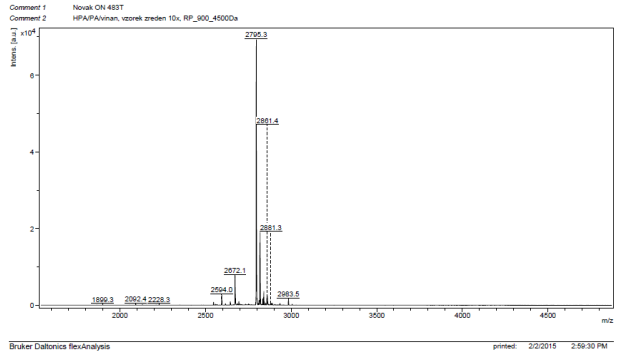


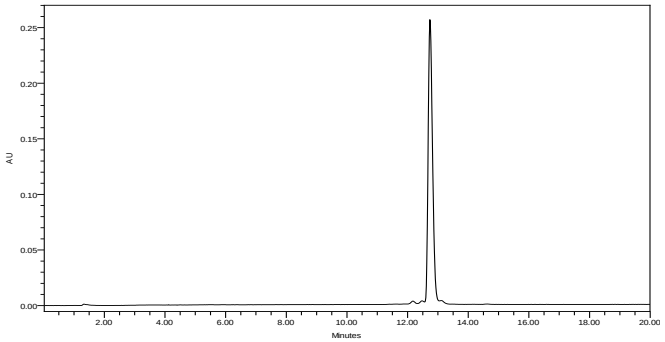
D15 5´-d(GUG AUA UGC)-3´ U phosphonate unit 14



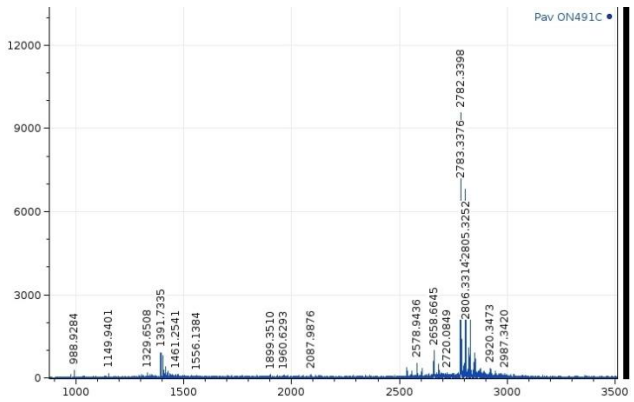


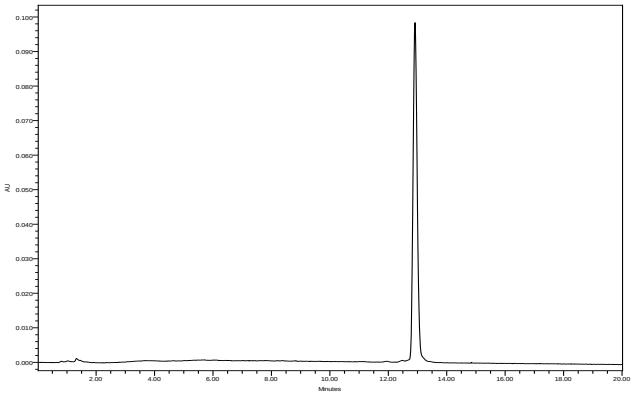
D16 5´-d(GUG AUA UGC)-3´ U phosphonate unit 15



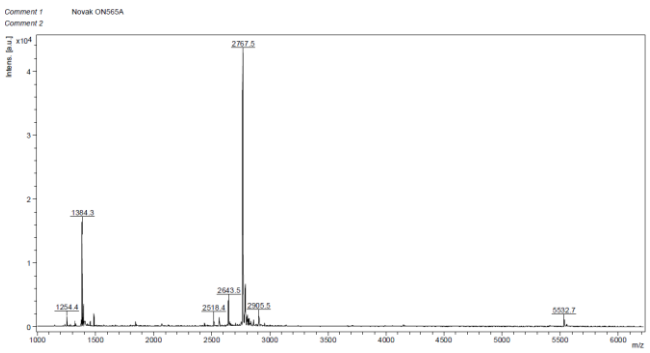


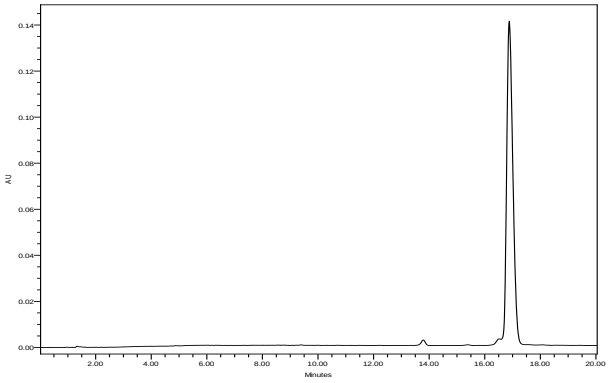
D17 5´-d(GTG AUA TGC)-3´ U phosphonate unit 12





D18 5´-d(GTG AUA TGC)-3´ U phosphonate unit 14





Hybridization properties

The measurements of thermal characteristics were performed on CARY 100 Bio UV Spectrophotometer (Varian Inc.) equipped with Peltier temperature controller and thermal analysis software. The samples were prepared by mixing together complementary strands in 100mM NaCl, 50mM NaH_2PO_4, 1mM EDTA, pH 7.2 to afford 4 μM final concentration. Temperature gradient of 1°C/min was applied. Tm values were determined from the maximum of the first derivative of the absorbance/temperature plots (Tm ± 0.5 °C).

Table S6. Hybridization affinities of modified nonamers bearing three phosphonate units towards complementary RNA and DNA counterpart.

		Phosphonate Unit U	**5'-r(GCA UAU CAC)-3'** T_m (ΔT_m/modif.) °C	**5'-d(GCA TAT CAC)-3'** T_m (ΔT_m/modif.) °C
D1	**5'-r(GUG AUA UGC)-3'**		46.1	34.9
D2	**5'-r(GUG AUA UGC)-3'**	**8**	31.4 (-4.9)	24.2 (-3.6)
D3		**9**	34.6 (-3.8)	< 15.0
D4		**10**	< 15.0	< 15.0
D5		**11**	< 15.0	< 15.0
D6		**12**	41.7 (-1.5)	< 15.0
D7		**13**	< 15.0	< 15.0
D8		**14**	45.7 (-0.1)	27.8 (-2.4)
D9		**15**	24.0 (-7.4)	18.0 (-5.6)
D10	**5'-d(GTG ATA TGC)-3'**		34.5	36.9
D11	**5'-d(GUG AUA UGC)-3'**	**10**	< 15.0	< 15.0
D12		**11**	15.2	< 15.0
D13		**12**	32.9 (-0.5)	22.6 (-4.8)
D14		**13**	< 15.0	< 15.0
D15		**14**	46.7 (+4.1)	38.1 (+0.4)
D16		**15**	30.2 (-1.4)	26.2 (-3.6)
D17	**5'-d(GTG AUA TGC)-3'**	**12**	36.0 (+1.5)	n.d.
D18		**14**	39.7 (+5.2)	n.d.

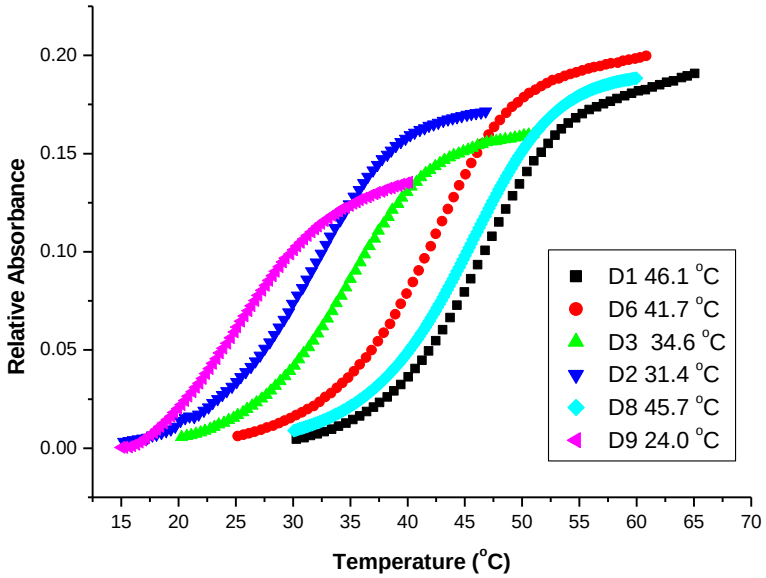


Figure S1. Hybridization affinities of modified RNA nonamers bearing three phosphonate units towards complementary RNA counterpart.

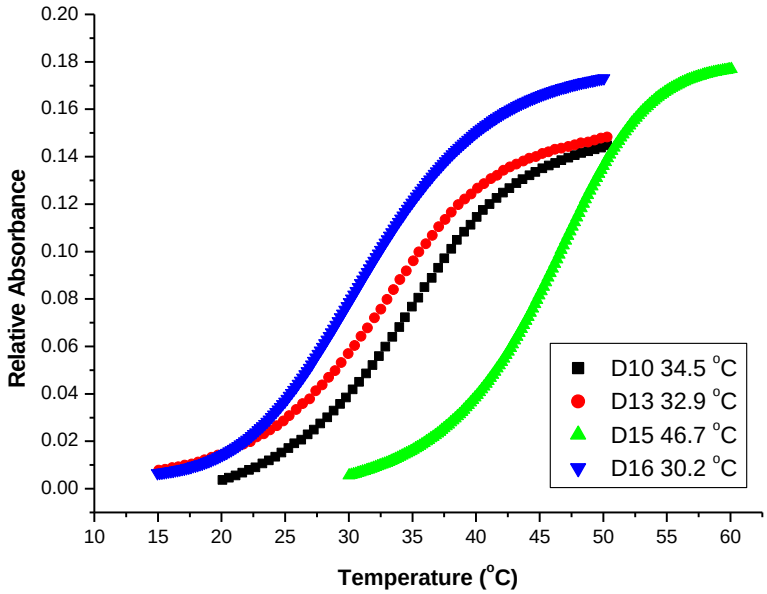


Figure S2. Hybridization affinities of modified DNA nonamers bearing three phosphonate units towards complementary RNA counterpart.

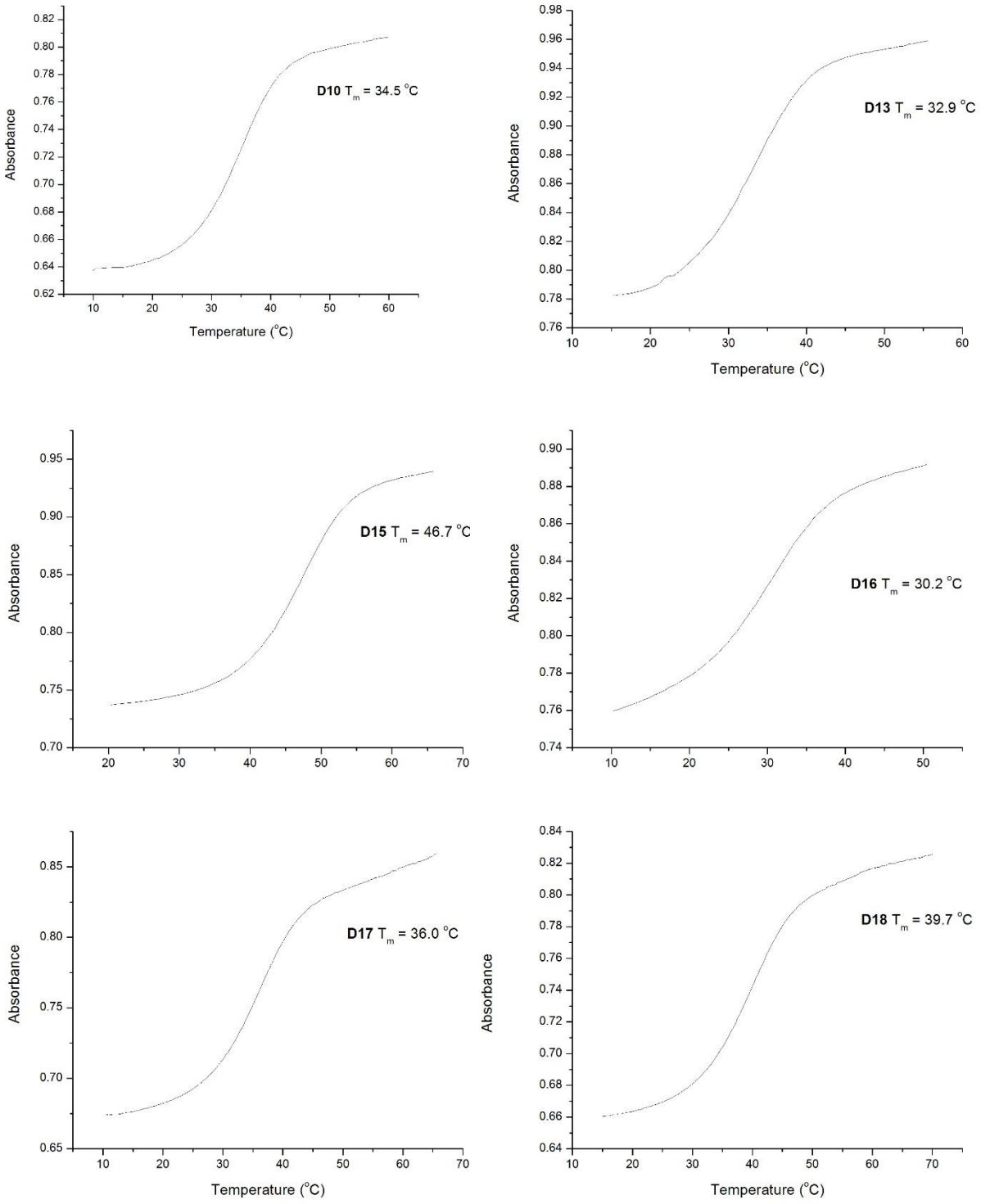


Figure S3. Melting curves of the most potent DNA/RNA duplexes.

MD simulations

First we constructed DNA/RNA nonamers using the AmberTools software package.[3-7] The base sequence was the same as the one used in our experiments - i.e. 5'-d (GTG ATA TGC)-3' on the DNA strand and 5'-r(GCA UAU CAC)-3 on the RNA strand. Various chemical phosphonate modifications – i.e. **15** (**D16**), **14** (**D15**), **13** (**D14**), **12** (**D13**), **11** (**D12**), **10** (**D11**) - were incorporated into the DNA strand by means of the Molefacture plugin from the software package VMD.[8] It led to the 5'-d(GUG AUA UGC)-3' oligonucleotides, wherein U indicates positioning of chemically modified internucleotide linkages.

Double helical structures were surrounded by water molecules which extended to a distance of approximately 10 Å (in each direction) from the nucleic acids atoms (this gives a periodic box size of ~68 Å, ~64 Å, ~77 Å). Resulting simulated systems consisted of ~10.000 atoms. New *.inpcrd (initial coordinates) and *.prmtop (molecular topology, force field)[9-13] files for the whole simulated systems, were created by means of the TLEAP module from the AMBER software package.[3-7] We used the AMBER force field[9-13] for nucleic acids and TIP3P[14] for water molecules. In the case of modified parts of nucleic acids the force constants obtained using ab initio calculations were used as in our previous studies of phosphonate oligonucleotides.[15-23]

MD trajectories lasting 130ns were computed with the aid of the NAMD 2.7 software package.[24] The Particle-mesh Ewald (PME) method was employed for long-range electrostatic forces.[25] The non-bonded cutoff was set to 9 Å. The SHAKE algorithm (tolerance 0.0005) was applied to constrain bonds where the hydrogen atoms were involved.[26] Simulated systems were energy minimized then the Langevin dynamics was used for a temperature control.[24] The simulated systems were heated from 0 K to 310/410 K. The Langevin piston method was applied to reach an efficient pressure control with target pressure set to 1 atm.[24] The integration timestep was set to 2 fs.

Data were recorded every 10 ps. MD trajectories were analyzed with the aid of the VMD 1.9,[8] CHIMERA 1.5.3,[27] CURVES+[28] and AMBER12/PTRAJ-CPPTRAJ[29] software packages. Figures were produced by means of the ICM Molsoft software package.

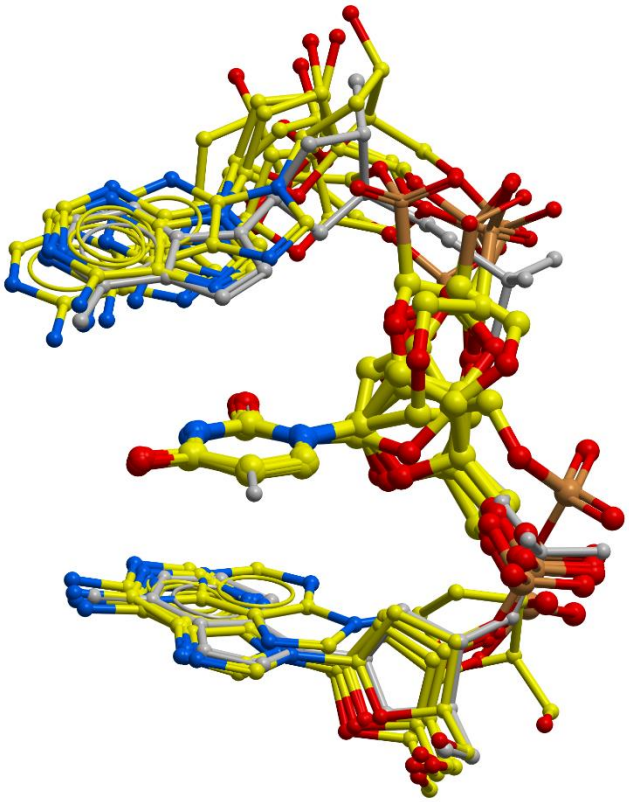
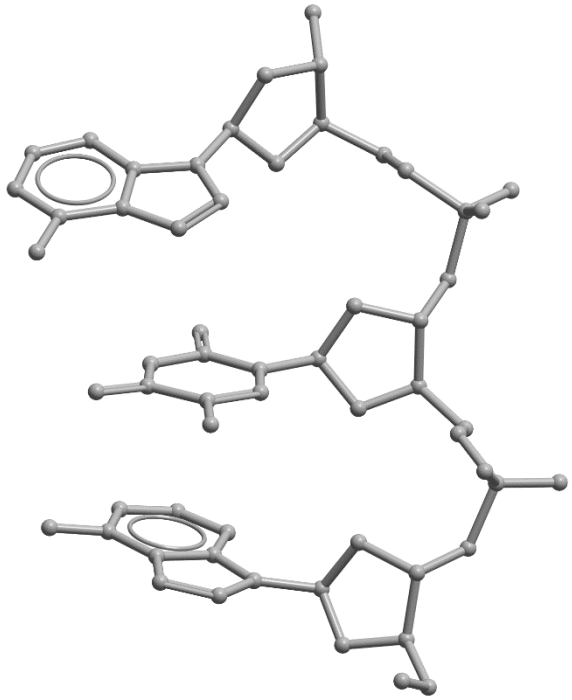
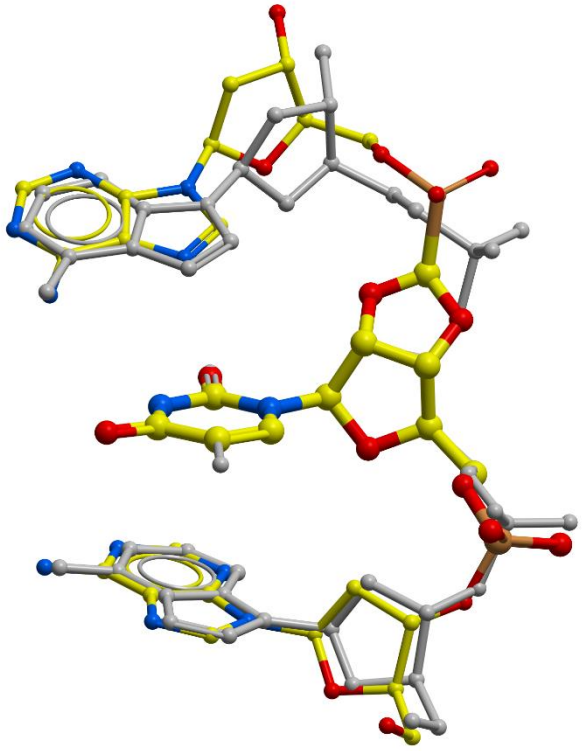


Figure S4. Structural alignment of central segments (5’-d(AUA)-3’ trimers) of studied DNA nonamers (oligonucleotides **D11-D16**). Structural alignments were made for a central base of nucleotide U. The natural 5’-d(ATA)-3’ trimer is displayed using gray balls and sticks. Atoms of chemically modified trimers are depicted with following color code: carbon (yellow), oxygen (red), nitrogen (blue) and phosphorus (orange).

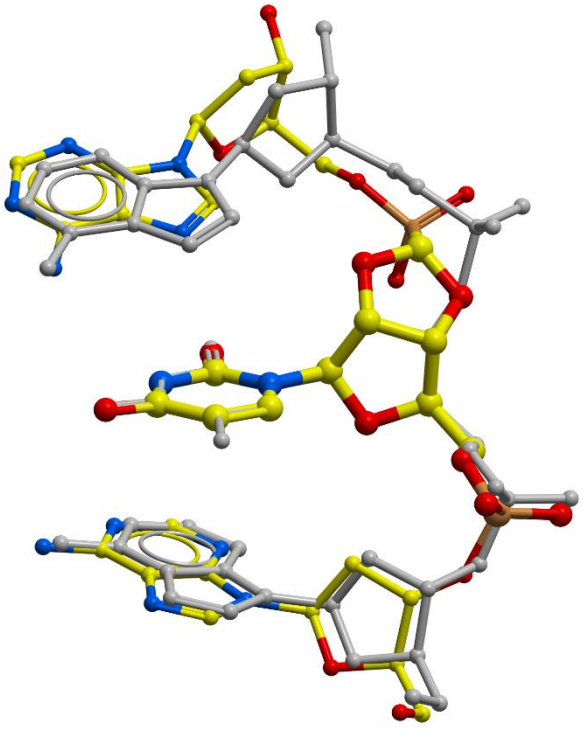


D10, T_m 34.5ºC (0.0ºC), BP 13

Figure S5. The natural 5’-d(ATA)-3’ trimer displayed as gray balls and sticks.

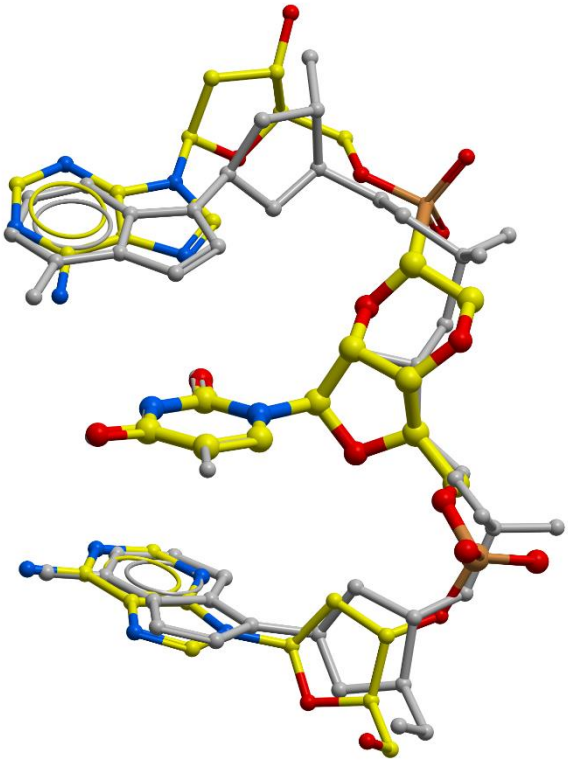


D15, Tm 46.7ºC (+4.1ºC), BP 32

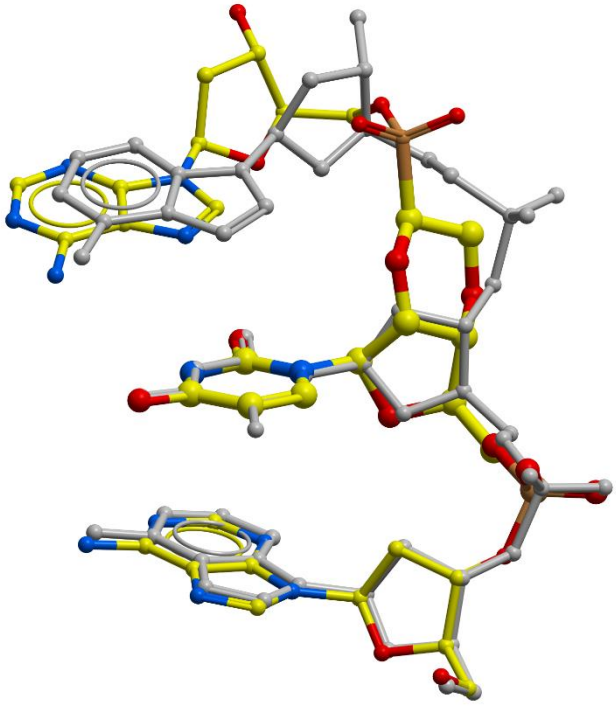


D16, Tm 30.2ºC (-1.4ºC), BP 11

Figure S6. Structural alignment of central parts (i.e. 5'-d(AUA)-3' trimers) of studied DNA nonamers (i.e. oligonucleotides **D15**, **D16**). Structural alignments were made for a central base U. The natural 5'-d(ATA)-3' trimer is displayed as gray balls and sticks. Atoms of chemically modified trimers are depicted with this color code: carbon (yellow), oxygen (red), nitrogen (blue), phosphorus (orange).

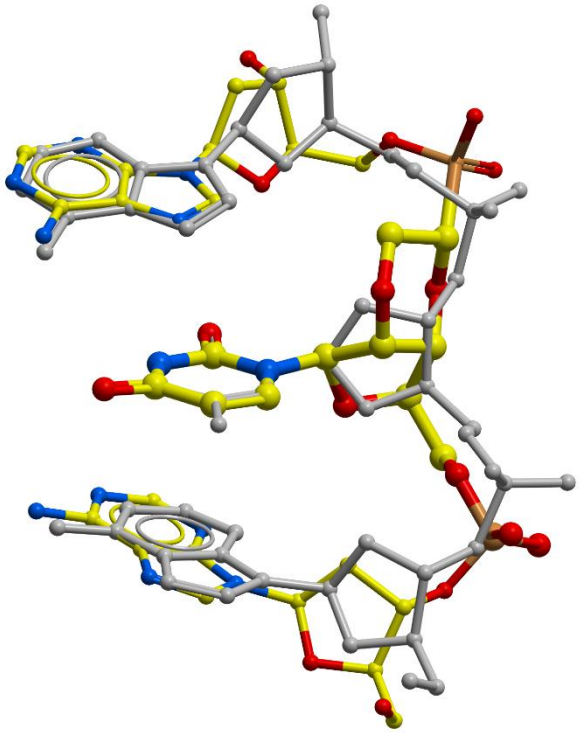


D13, Tm 32.9ºC (-0.5ºC), BP 16

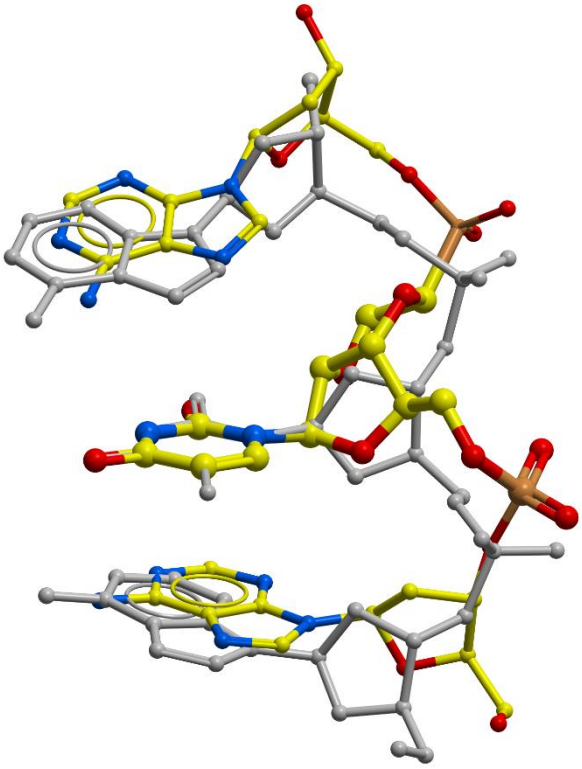


D14, Tm <15ºC, BP 2

Figure S7. Structural alignment of central parts (i.e. 5'-d(AUA)-3' trimers) of studied DNA nonamers (i.e. oligonucleotides **D13**, **D14**). Structural alignments were made for a central base U. The natural 5'-d(ATA)-3' trimer is displayed as gray balls and sticks. Atoms of chemically modified trimers are depicted with this color code: carbon (yellow), oxygen (red), nitrogen (blue), phosphorus (orange).



D12, Tm <15ºC, BP 5



D11, Tm <15ºC, BP 0

Figure S8. Structural alignment of central parts (i.e. 5'-d(AUA)-3' trimers) of studied DNA nonamers (i.e. oligonucleotides **D12**, **D11**). Structural alignments were made for a central base U. The natural 5'-d(ATA)-3' trimer is displayed as gray balls and sticks. Atoms of chemically modified trimers are depicted with this color code: carbon (yellow), oxygen (red), nitrogen (blue), phosphorus (orange).

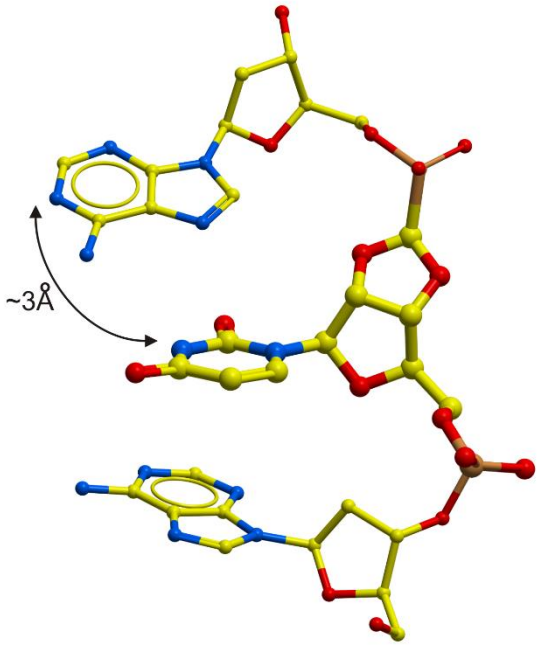


Figure S9. The 1-*N* ↔ 3-*N* distance indicating stacking of bases in subsequent **Figure S10**. 5'-d(AUA)-3' trimer is depicted with the following color code: carbon (yellow), oxygen (red), nitrogen (blue), phosphorus (orange).

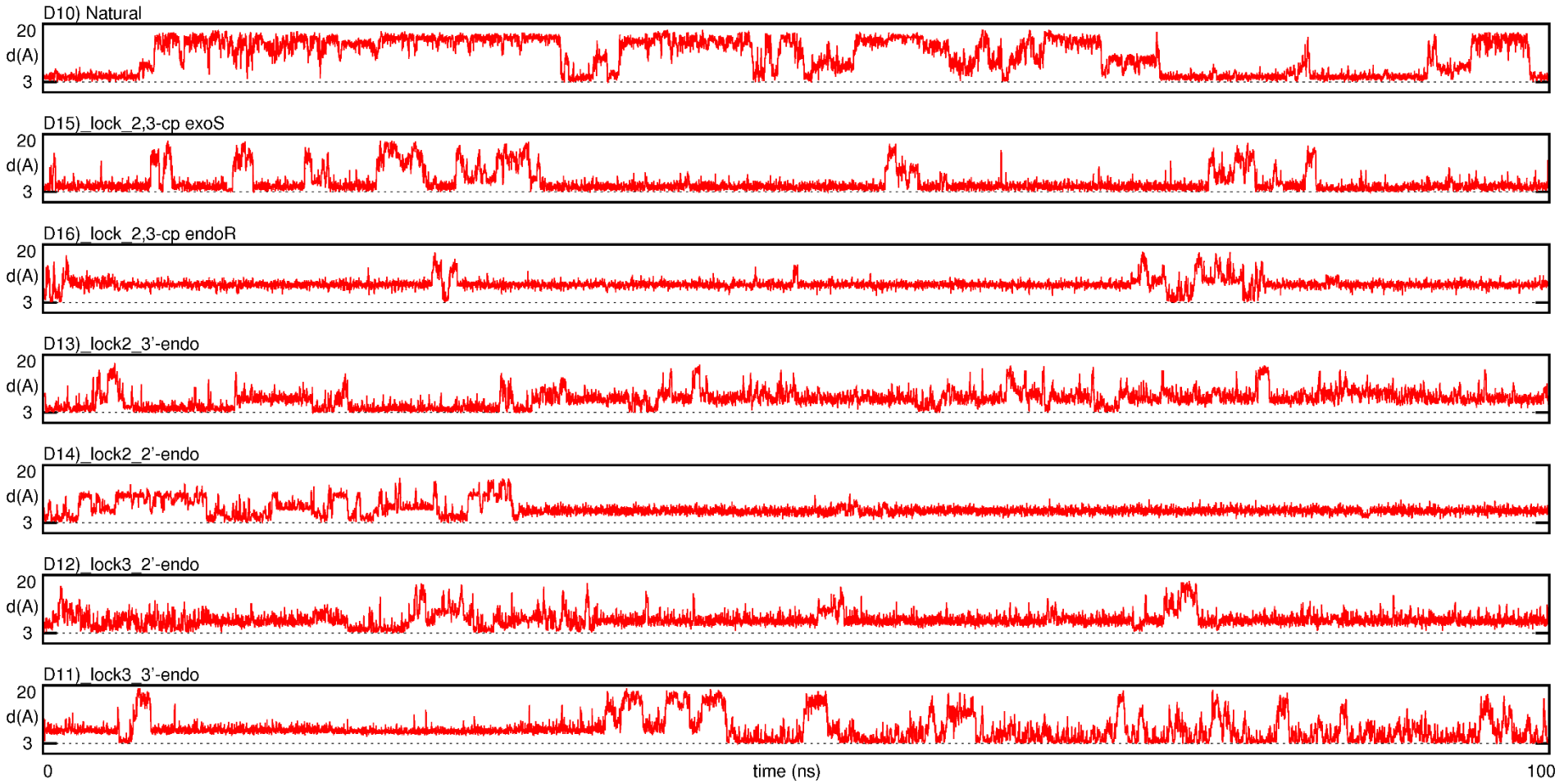


Figure S10. Time evolution of 1-*N* ↔ 3-*N* distances (see previous **Figure S9**) in MD simulations of 5'-d(AUA)-3' trimers representing central parts of oligonucleotides **D10-D16**.

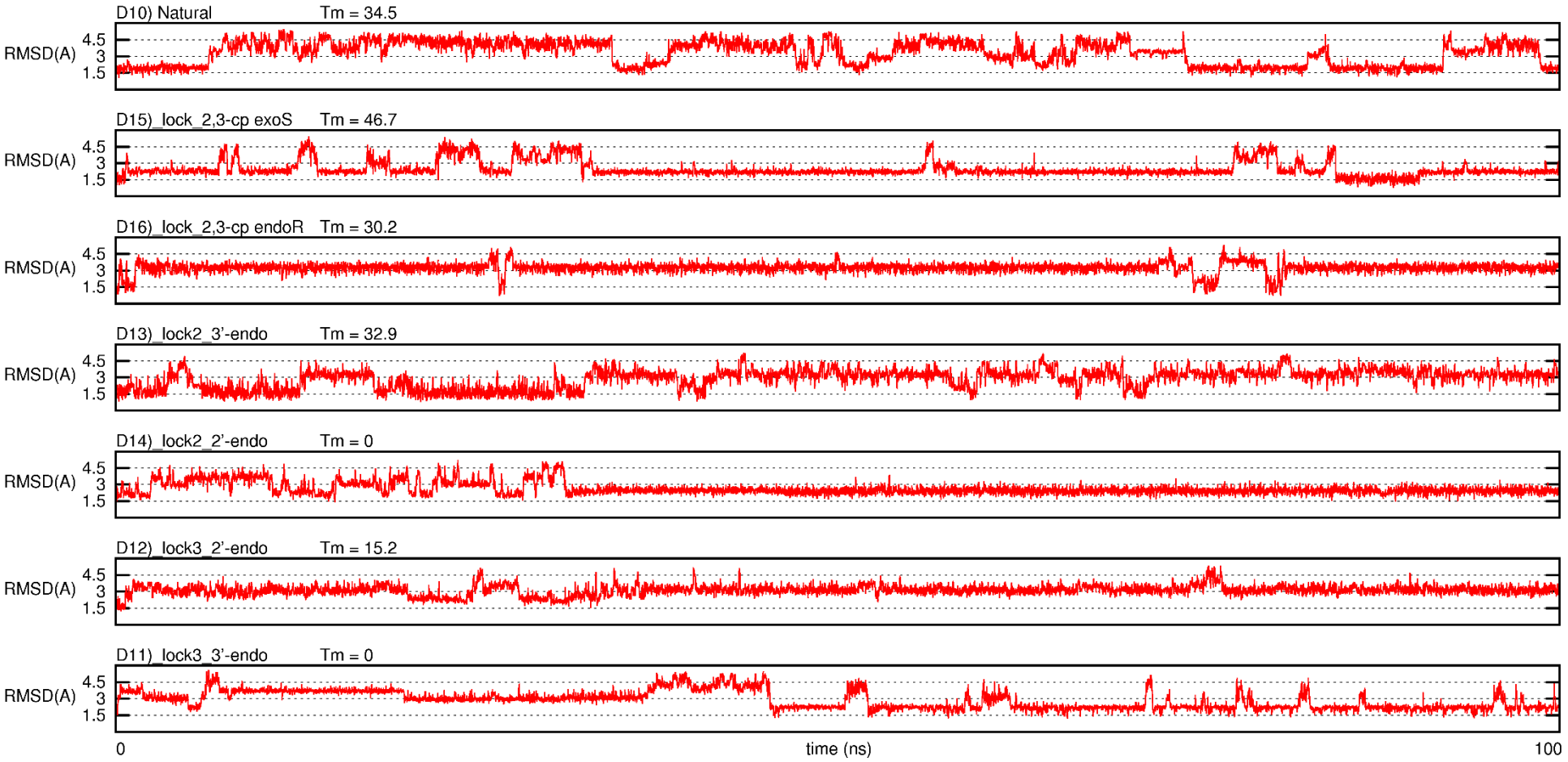


Figure S11. Time evolution of RMSD in MD simulations of 5'-d(AUA)-3' trimers representing central parts of oligonucleotides **D10-D16**.

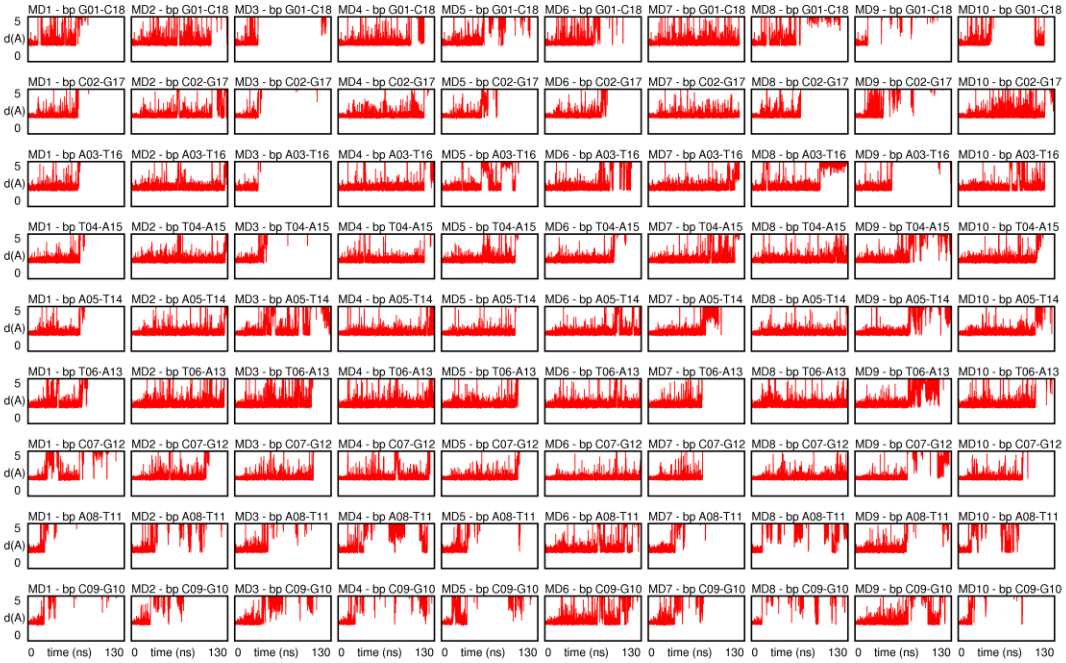


Figure S12. Time evolutions of distances between atoms forming central hydrogen bonds in all base pairs of natural **D10**/RNA duplex from ten independent MD runs. Values for each base pair along the duplex are grouped in rows. Values from the same MD run are grouped in columns.

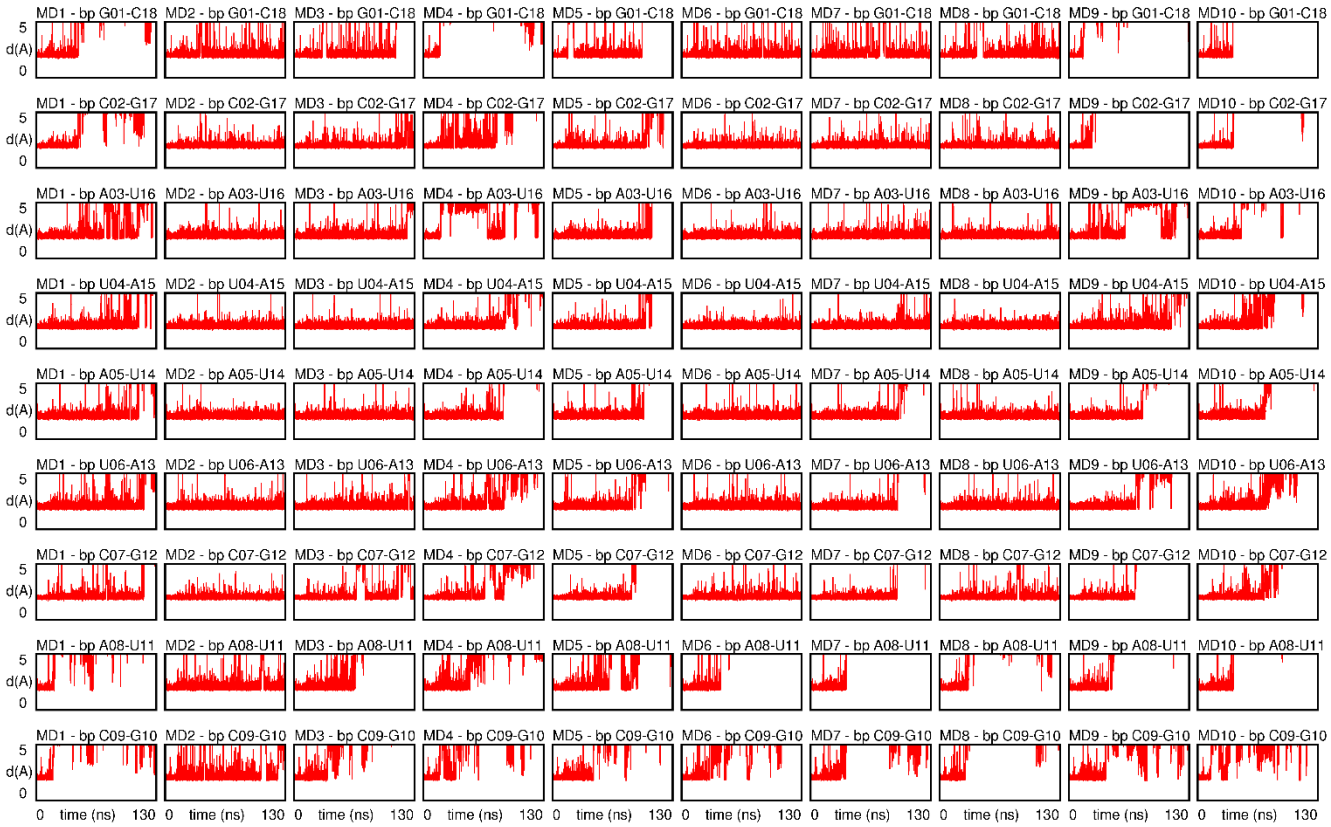


Figure S13. Time evolutions of distances between atoms forming central hydrogen bonds in all base pairs of **D15**/RNA duplex from ten independent MD runs. Values for each base pair along the duplex are grouped in rows. Values from the same MD run are grouped in columns.

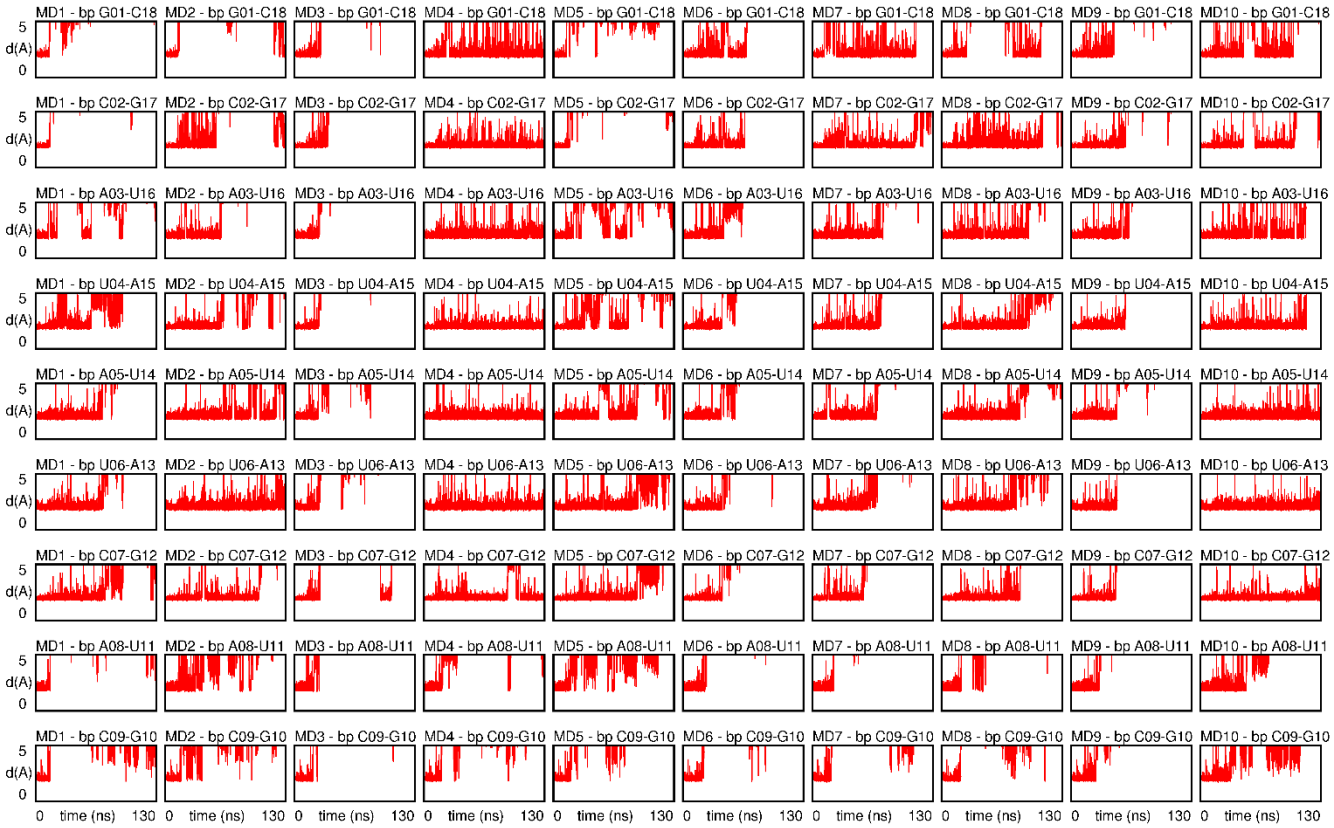


Figure S14. Time evolutions of distances between atoms forming central hydrogen bonds in all base pairs of **D16**/RNA duplex from ten independent MD runs. Values for each base pair along the duplex are grouped in rows. Values from the same MD run are grouped in columns.

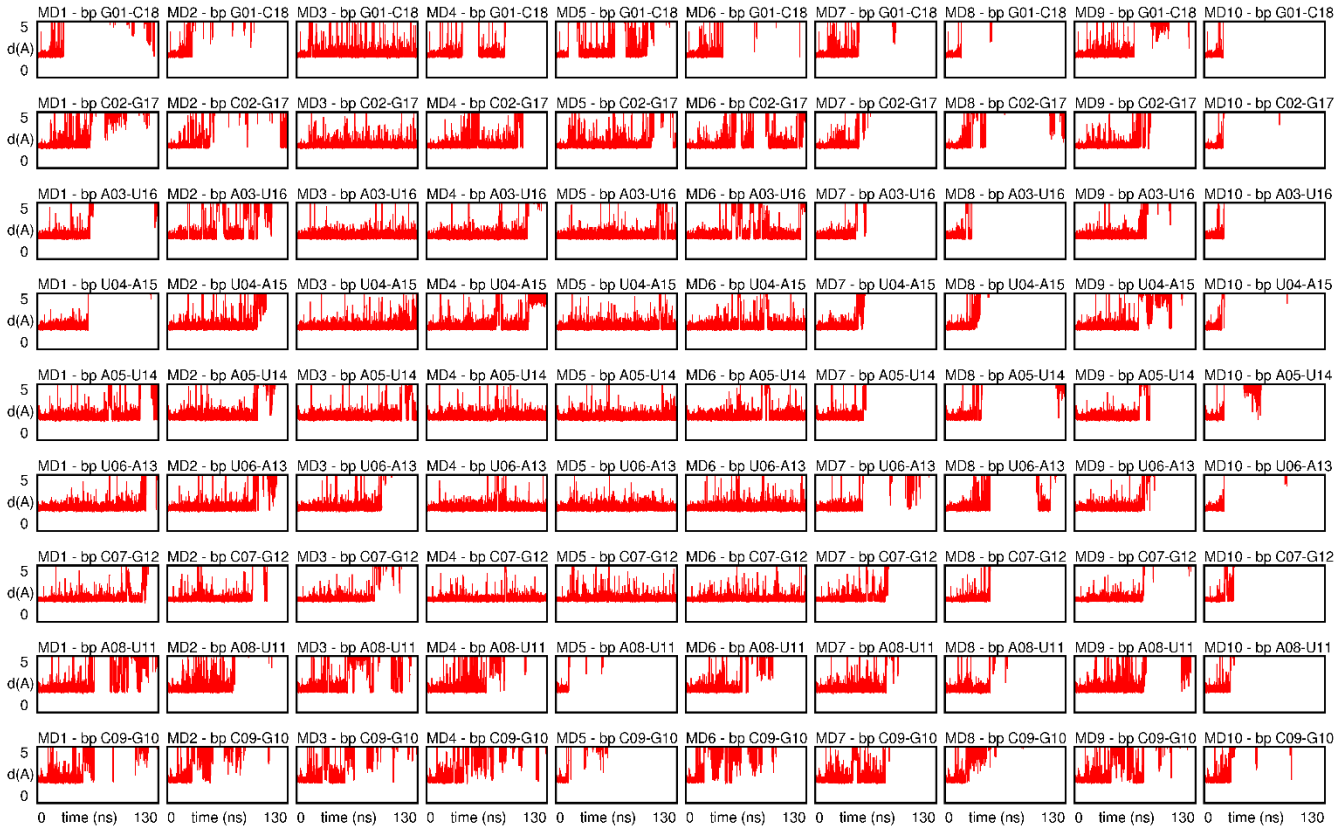


Figure S15. Time evolutions of distances between atoms forming central hydrogen bonds in all base pairs of **D13**/RNA duplex from ten independent MD runs. Values for each base pair along the duplex are grouped in rows. Values from the same MD run are grouped in columns.

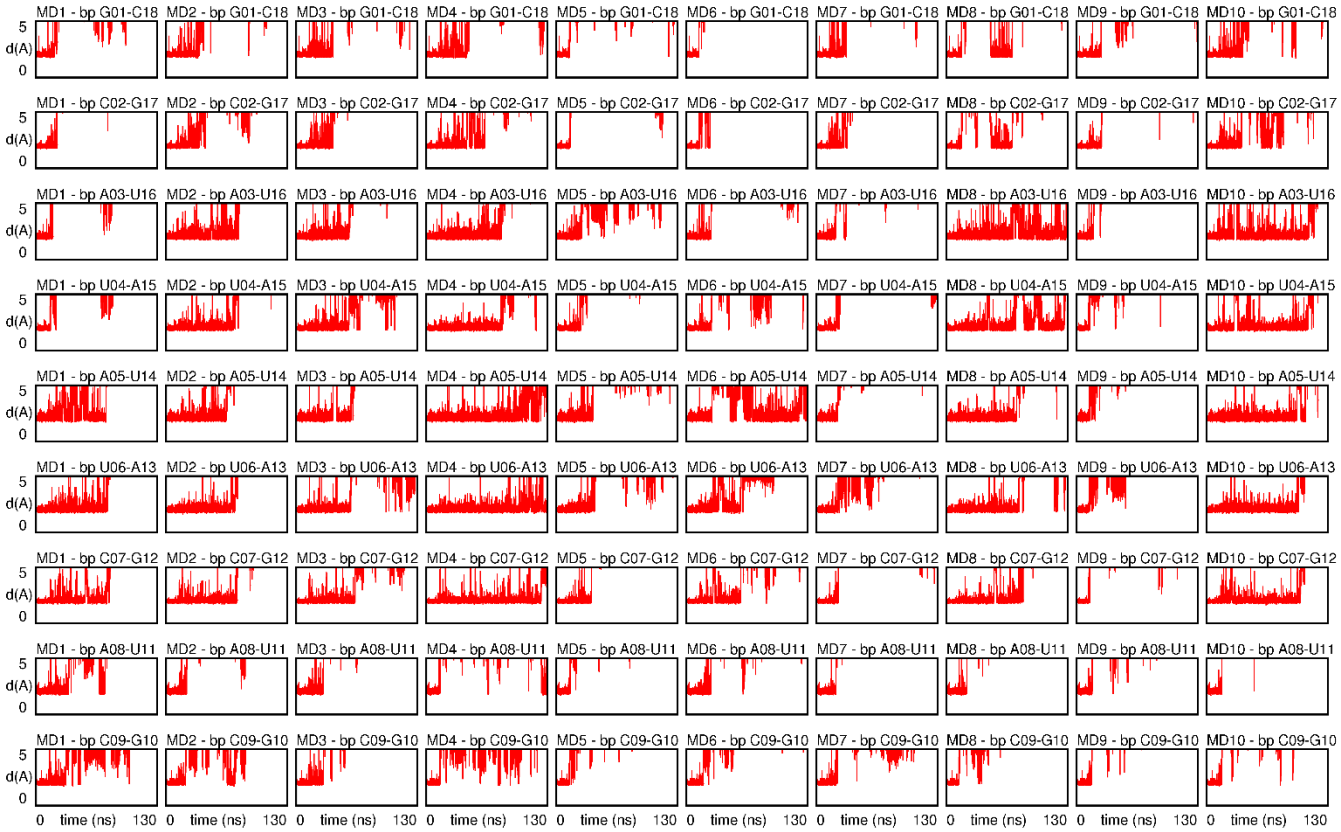


Figure S16. Time evolutions of distances between atoms forming central hydrogen bonds in all base pairs of **D14**/RNA duplex from ten independent MD runs. Values for each base pair along the duplex are grouped in rows. Values from the same MD run are grouped in columns.

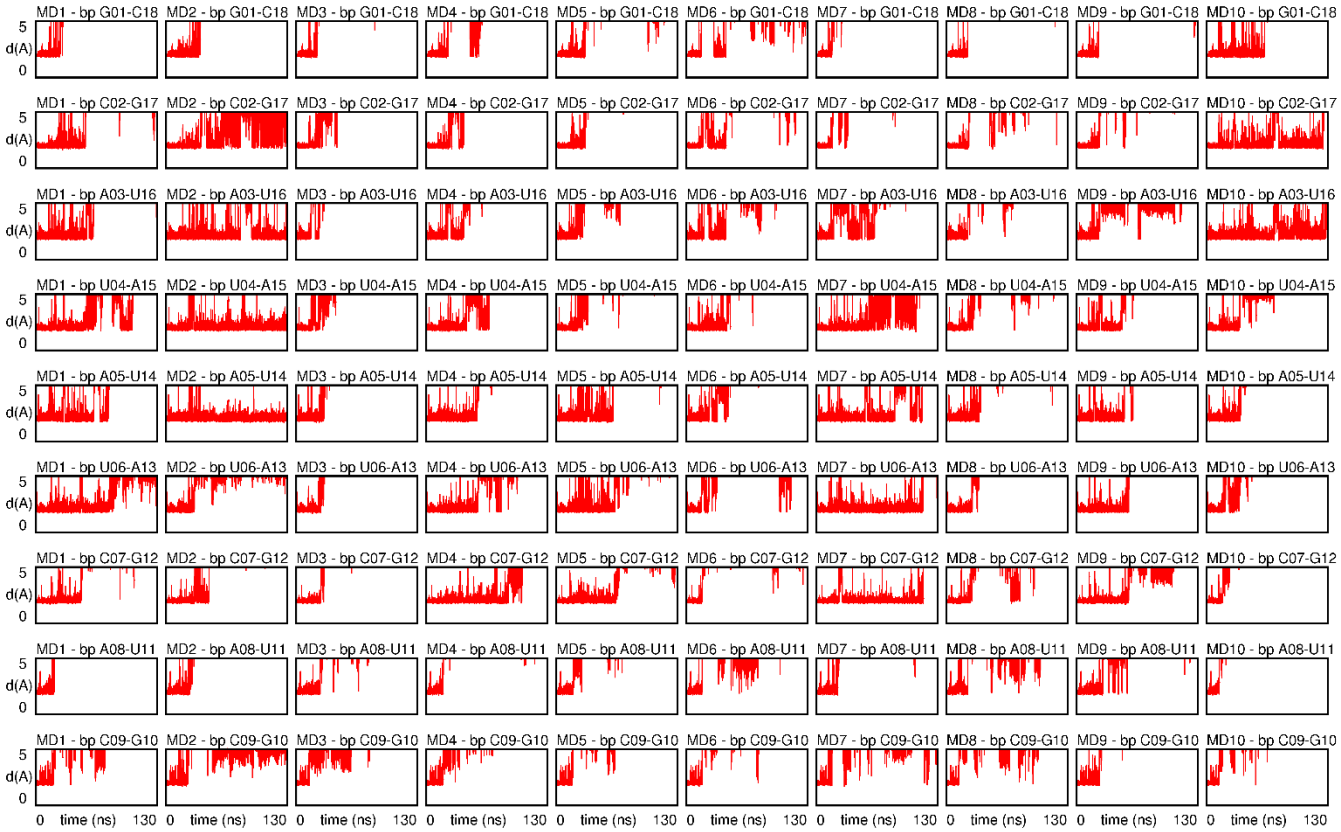


Figure S17. Time evolutions of distances between atoms forming central hydrogen bonds in all base pairs of **D12**/RNA duplex from ten independent MD runs. Values for each base pair along the duplex are grouped in rows. Values from the same MD run are grouped in columns.

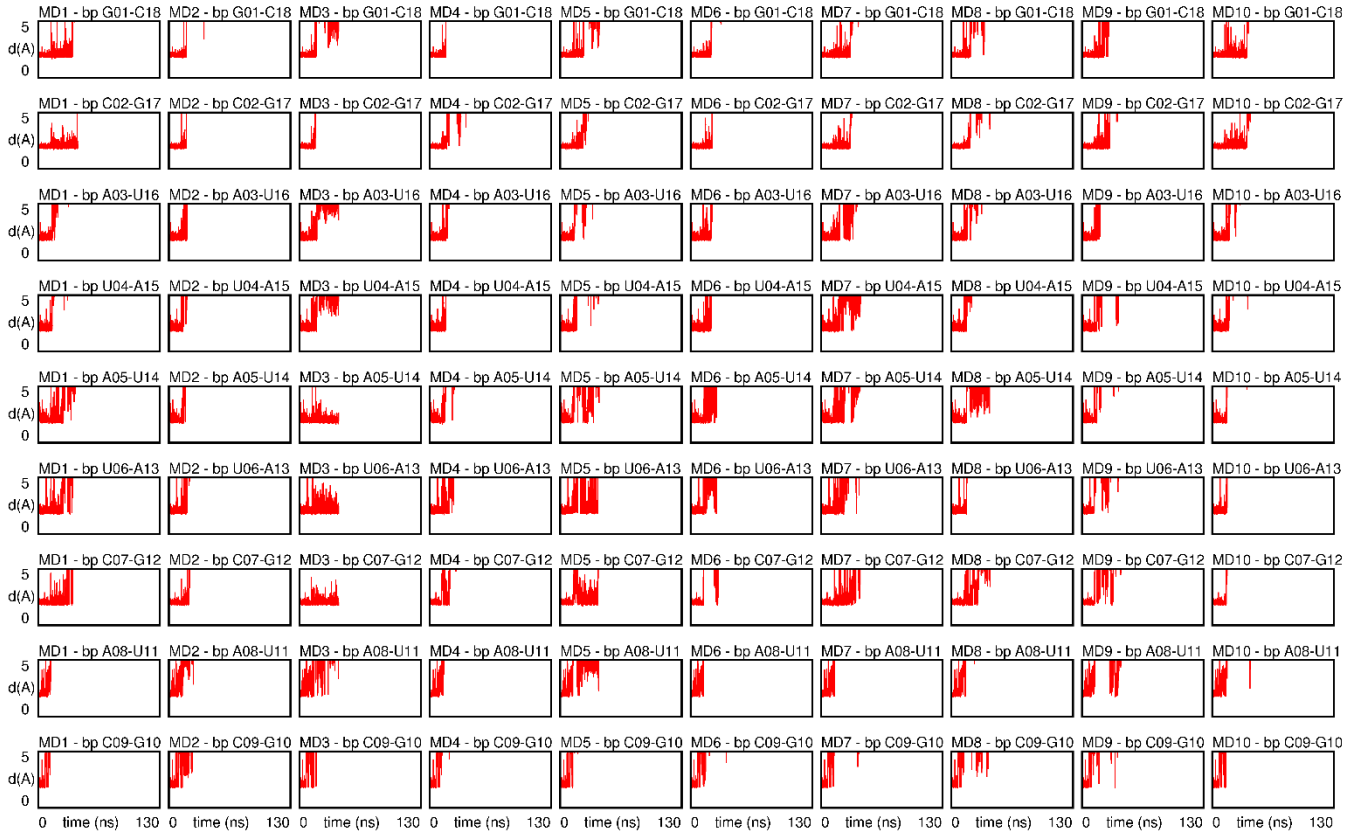


Figure S18. Time evolutions of distances between atoms forming central hydrogen bonds in all base pairs of **D11**/RNA duplex from ten independent MD runs. Values for each base pair along the duplex are grouped in rows. Values from the same MD run are grouped in columns.

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