Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

RNA duplexes with biphenyl-substituents as base replacements are less stable than DNA duplexes

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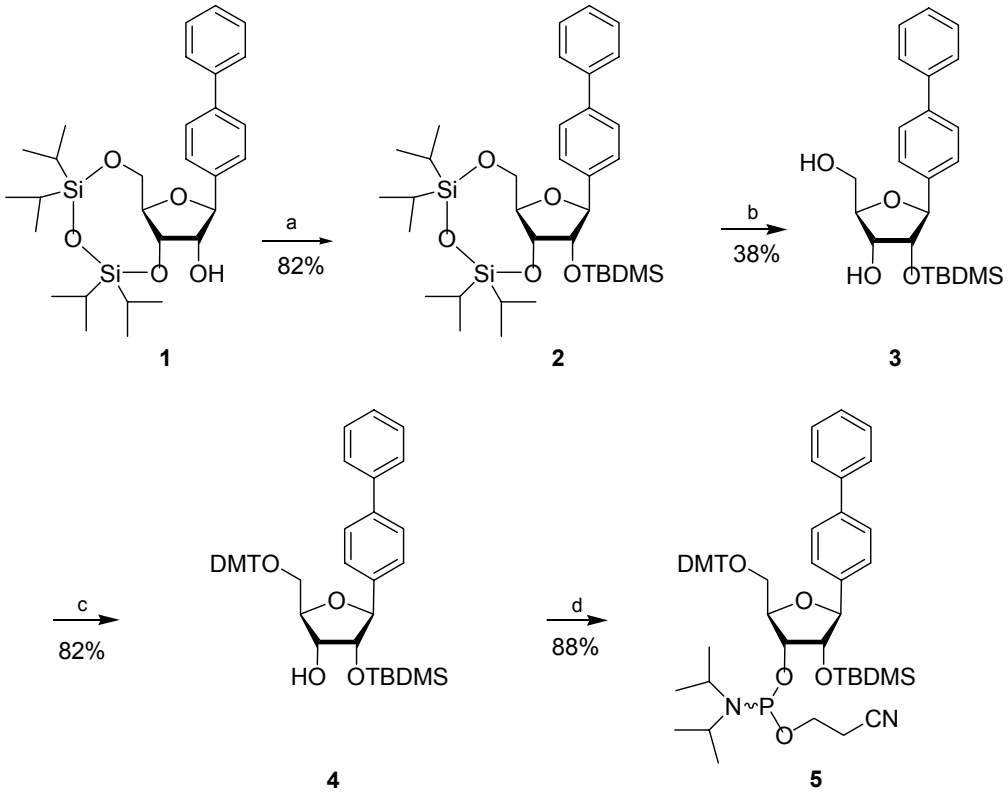
Outline of the synthesis of the rBph nuclesoide phosphoramidite 5 for RNA synthesis



Scheme S1: Reagents and conditions: a) **1** (1 equiv.), TBDMS-Cl (1.1 equiv.), DMF, 60°C; b) AcOH (1.5 equiv.), TBAF (2 equiv.), THF, 0 °C; c) 4,4'dimethoxytrityl chloride (1.6 equiv.), pyridine, RT; d) iPr$_2$NEt (3 equiv.), [(iPr$_2$N)(NCCH$_2$CH$_2$O)P]Cl (1.5 equiv.), THF, RT.

ABBREVIATIONS

ETOAc: Ethylacetate; TBAF: *tert*-Butylammonium fluoride; TEA: triethylamine; TBDMS-Cl: *tert*- Butyldimethylsilyl chloride; TIPDS: 1,3-dichloro-1,1,3,3-tetraisopropyl disiloxane; DMT: 4,4'-dimethoxytrityl.

EXPERIMENTAL PART

General: Reactions were performed under argon in distilled, anhydrous solvents. All chemicals were reagent grade from *Fluka* or *Aldrich*. ^{1}H NMR (300 MHz, 500 MHz) spectra were recorded on a Bruker AC-300 or Bruker DRX-500 spectrometer; the chemical shifts δ=

ppm were referenced to residual undeuterated solvent ($CDCl_3$ = 7.27), *J* = Hz. ^{13}C NMR (75 MHz) were recorded on a Bruker AC-300; the chemical shifts δ= ppm were referenced to residual undeuterated solvent $CDCl_3$= 77.00). Carbon multiplicity (s,d,t,q) from DEPT spectra. ^{31}P NMR spectra (162 MHz) were recorded on a Bruker DRX-400 spectrometer; the chemical shift δ= ppm was referenced to 85% H_3PO_4 as external standard. LSIMS and EI mass spectra were recorded on a Auto Speq Q VG at 70eV, ESI-MS mass spectra on a Fisons Instrument VG Platform. For TLC, pre-coated plates SIL-G UV254 (*Macherey Nagel*) have been used and visualized by UV and/or dipping into a solution of $Ce(SO_4)_2$ (10.5 g), phosphormolybdic acid (21 g), H_2SO_4 (60 mL), and H_2O (900 mL). Flash chromatography (FC) was performed with silica Gel 60 (230-400 mesh).

4-(3',5'-*O*-(1,1,3,3-Tetraisopropyldisiloxan-1,3-diyl)-2'-*O*-TBDMS-*β*-D-ribofuranosyl)-biphenyl (2). A solution of **1**[1] (985 mg, 1.86 mmol) in dry DMF (3.7 mL) was treated with TBDMS-Cl (308 mg, 2.05 mmol) and stirred for 2 h at 60°C. The mixture was taken up in $NaHCO_3$ (100 mL) and extracted with EtOAc (3 × 80 mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo*. Purification by FC (silica gel; hexane/EtOAc 11:1) yielded 982 mg (82%) of **2** as white solid. R_f 0.74 (hexane/EtOAc 10:1). ^1H NMR (300 MHz, $CDCl_3$, δ (ppm)): 7.59 (m, 9H, ArH); 4.98 (s, 1H, C1'H); 4.35 (m, 2H, C3'H, C4'H); 4.14-4.12 (m, 1H, C5'H; 4.06-4.03 (m, 2H, C4'H, C5'H); 1.16-0.99 (m, 37H, (TIPDS), Si-C$(CH_3)_3$); 0.15 (s, 3H, Si-$(CH_3)_2$); 0.12 (s, 3H, Si-$(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)): 141.92, 140.39, 140.17 (3s, ArC); 128.74, 127.20, 127.06, 127.04, 126,12 (5d, ArC); 87.39 (d, C2'); 80.28 (d, C4'); 79.53 (d, C1'); 69.38 (d, C3'); 60.39 (t, C5'); 25.81 (q, Si-C$(CH_3)_3$); 18,20, 17.59, 17.45, 17.44, 17.33, 17.12, 17.02, 16.97 (8q, CH_3-(TIPDS); 13.43, 13.09, 13.04, 12.89 (4d, CH-*i*Pr); -4.344, -4.60 34 (2q, Si-$(CH_3)_2$). MS (70eV, EI): m/z (%): 643.53 (28), 511.25 (100).

4-(2'-*O*-TBDMS-*β*-D-ribofuranosyl)-biphenyl (3).[2] To a stirred solution of **2** (500 mg, 0.78 mmol) in THF (9.0 mL) was added acidic acid (98µl, 1.71 mmol) and TBAF (1 M in THF, 1.54 mL) at 0°C. The reaction was followed by TLC, and as soon as the deprotection of the TBDMS-group at the C2' position occurred the reaction was quenched (EtOAc/H_2O, reaction time between 2 to 8 h). The separated organic layer was washed with water, followed by brine. The organic phase was dried ($MgSO_4$), concentrated *in vacuo* and purified by FC (silica gel; hexane/EtOAc: 3:2) yielding 76 mg (25 %) of **3** as a white solid. The starting material together with the compound in which the TIPDS group was only cleaved at the O5'-position was recovered and submitted again to the same reaction conditions yielding another 43 mg of **3,** which led to an overall yield of 38%. R_f 0.40 (hexane/EtOAc 1:1). ^{1}H NMR (300 MHz, $CDCl_3$, δ(ppm)): 7.62-7.60 (m, 4H, ArH); 7.49-7.33 (m, 5H, ArH); 4.75 (d, J = 6.42 Hz, 1H, C1'H); 4.15-4.09 (m, 1H, C2'H, C3'H); 4.05-3.96 (m, 2H, C4'H, C5'H); 3.85 (dd, J = 4.0, 11.9 Hz, 1H, C5'H); 0.89 (s, 9H, Si-C$(CH_3)_3$); -0.05 (s, 3H, Si-CH_3); -0.13 (s, 3H, Si-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, δ(ppm)): 141.16, 140.66, 138.13 (3s, ArC); 128.77, 127.20, 127.04, 127.01 (4d, ArC) 84.92 (d, C2'); 84.45 (d, C1'); 79.01 (d, C4'); 71.86 (d, C3'); 63.12 (t, C5'); 25.64 (q, Si-C(CH$_3$)$_3$); 17.95 (s, Si-C(CH$_3$)$_3$); -4.85, -5.19 (2q, Si-(CH$_3$)$_2$). HRMS (ESI$^+$): calcd for $C_{23}H_{32}O_4NaSi$: 423.1967, found 423.1957.

4-(5'-*O*-dimethoxytrityl)-2'-*O*-TBDMS-*β*-D-ribofuranosyl)-biphenyl (4)

To a stirred solution of **3** (117 mg, 0.29 mmol) in dry pyridine (4.1 mL) was added 4,4'-dimethoxytrityl chloride (157 mg, 0.46 mmol) in portions. After 2 h the reaction mixture was dissolved in EtOAc (50 mL) and washed with $NaHCO_3$ (3 × 20 mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo*. Purification by FC (silica gel was conditioned with 1-2% TEA in hexane/EtOAc 3:2 prior to loading of the reaction mixture) yielded 169 mg

(82%) of **4** as a white foam. R_f 0.74 (hexane/EtOAc 1:1). ^{1}H NMR (300 MHz, $CDCl_3$, δ(ppm)): 7.44-7.17 (m, 18H, ArH); 6.84 (d, 4H, ArH); 4.81 (d, *J* = 7.4 Hz, 1H, C1‘H); 4.24-4.20 (m, 2H, C2’H, C3‘H); 4.15-4.12 (m, 1H, C4‘H); 3.80 (s, 6H, 2 CH_3O-(DMT)); 3.56 (dd, *J* = 2.9, 10.3 Hz, 1H, C5‘H); 3.20 (dd, *J* = 3.3, 9.9 Hz, 1H, C5‘H); 0.88 (s, 9H, Si-C$(CH_3)_3$), -0.08 (s, 3H, Si-CH_3); -0.15 (s, 3H, Si-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)): 158.60, 158.43 144.97, 140.87, 140.83, 138.84, 136.09, 135.96 (8s, ArC); 130.15, 130.17, 129.11, 128.75, 128.19, 127.81, 127.74, 127.26, 127.11, 127.04, 127.00, 126.75 (12d, ArC); 113.11 (d, ArC); 86.29 (s, ArC); 84.18 (d, C4‘); 79.52 (d, C1‘); 72.91 (d, C3‘); 64.09 (t, C5‘); 25.65 (q, Si-C(CH_3)$_3$); 17.97 (s, Si-*C*$(CH_3)_3$); -4.96, -5.23 (2q, Si-$(CH_3)_2$). HRMS (ESI^+): calcd for $C_{44}H_{50}O_6NaSi$: 725.3274, found 725.3286.

4-(5’-*O*-dimethoxytrityl-*β*-D-ribofuranosyl)-3’-*O*-(2-cyanoethyl-*N*,*N*’-diisopropyl)-biphenyl-phosphoramidite (5). To a stirred solution of **4** (155 mg, 0.22 mmol) in dry THF (5.7 mL) was added *N*,*N*-diisopropylethylamine (113 µl, 0.66 mmol) followed by 2-cyanoethyl diisopropylchlorophosphoramidite (74 µl, 0.33 mmol) at room temperature. After 2 h the reaction mixture was concentrated *in vacuo*. Purification by FC (silica gel was conditioned with 1-2% TEA in hexane/EtOAc 9:1 prior to the load of the reaction mixture) hexane/EtOAc 9:1 yielded 175 mg (88 %) of **5** as a white foam. R_f 0.24 (hexane/EtOAc 9:1). ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)): 7.64-7.19 (m, 18H; ArH); 6.88-6.82 (m, 4H; ArH); 4.85 (m, 1H, C1’H); 4.37-4.15 (m, 2.7 H, C3‘H, C2‘H, CH_2); 4.04-3.90 (m, 1.3H, CH_2); 3.79 (s, 6H, CH_3O-(DMT)); 3.66-3.54 (m, 2H, C5‘H, C2‘H); 3.24-3.17 (m, 1H, C5‘H); 2.70 (td, *J*= 3.7, 7.0 Hz, 1H, CH_2CN); 2.24 (td, *J* = 2.2, 7.0 Hz, 1H, CH_2CN); 1.19-1.17 (m, 12H, CH_3-*i*Pr); 0.99 (d, 2H, CH-*i*Pr); 0.81 (s, 9H, Si-C$(CH_3)_3$); -0.11 (m, 3H, Si-$(CH_3)_2$); -0.25 (s, 3H, Si-$(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)): 158.45, 158.43, 144.92, 144.78, 141.00, 140.82, 139.20, 138.89, 136.15, 136.00, 135.92, 135.77 (11s, ArC); 130.19, 130.09, 128.72,

128.28, 128.15, 127.84, 127.35, 127.21, 127.04, 126.90, 126.84, 126.76 (12d, ArC); 117.68, 117.28 (2s, CN); 113.03, 113.12, 113.09 (3d, ArC); 86.33, 86.15 (2s, ArC); 84.29, 83.92, 83.03, 82.78 (4d, C4‘); 79.74, 79.70 (2d, C1‘); 79.21, 79.14 (2d, C2’); 75.19, 75.07, 754.16, 73.97 (4d, C3‘); 63.91, 63.74, 59.04, 58.84, 57.58, 55.18 (6t, C5‘, CH_2); 55.18 (1q, CH_3O-(DMT)); 43.47, 43.29, 42.82, 42.66 (4d, C2‘, CH-*i*Pr); 25.85, 25.82, 25.78, 24.70, 24.58, 24.52 (4q, CH_3-*i*Pr); 20.40, 20.33, 19.98, 19.89 (4t, CH_2CN); -4.72, -4.77, -5.24, -5,28 (2q, Si-$(CH_3)_2$). ^{31}P NMR (202 MHz, $CDCl_3$, δ (ppm)): 150.73, 148.29. HRMS (ESI^+): calcd for $C_{53}H_{68}N_2O_7SiP$: 903.4533, found 903.4528.

Synthesis, deprotection and purification of oligoribonucleotides.

The RNA oligonucleotides were synthesized on a 1 μmol scale on an Applied Biosystems Expedite Nucleic Acid Synthesizer (8909) using standard phosphoramidite chemistry in the trityl-off mode. The 2’O-TBDMS protected RNA-phosphoramidites of the natural nucleosides were from *Link Technologies* and the CPG solid supports were from *Glen Research*. The solvents and reagents used for the synthesis were prepared according to the manufacturer’s indications. The coupling time for the natural and modified phosphoramidites was 3 min, and 2-ethylthio-1*H*-tetrazole (0.25 M in CH_3CN) was used as activator. After synthesis, the oligonucleotides were detached and deprotected in 1 mL ethanolic ammonia (EtOH/conc.NH_3 1:3) at 55°C for 12h. From this point on work continued under sterile conditions. The ethanolic solution was filtered through Titan filters (Teflon, 0.45 μm, *Infochroma AB*) and rinsed two times with 1 mL of the following mixture (EtOH/H_2O/CH_3CN 3:1:1). The solution was evaporated to dryness. Then, 1 mL of a TBAF solution (1M in THF) was added to the residue and allowed to stand overnight at rt. Again the sample was evaporated and dissolved in 1 mL H_2O. The crude oligonucleotides were purified by RP-HPLC (15RPC ST 4.6/100 (*Pharmacia Biotech*); solvent A = 0.1 M triethylammonium

acetate in H_2O. pH 7.0; solvent B= 0.1 M triethylammonium acetate in H_2O/CH_3CN 1:4, pH 7.0. All oligonucleotides were routinely characterized by ESI^--mass spectrometry (see Table S1). Concentrations of oligonucleotides were determined by UV absorption using the Schepartz Lab Biopolymer Calculator.[3] For the Bph-nucleosides, an extinction coefficient of ε(260nm) = 19`000 (biph) was used.

UV-melting curve analysis and van't Hoff plots. Thermal denaturation experiments were carried out on a *Varian Cary 100* UV /Vis spectrometer. Absorbance was monitored at 260nm and the heating rate was set to 0.5 °C min^{-1}. A heating-cooling-heating cycle in the temperature range 10-90 °C was performed. T_m-values at different duplex concentrations (0.6, 1.2, 5.0, 10.0, 20.0, 30.0 μM) in 10mM NaH_2PO_4, 150mM NaCl, pH 7.0 were determined and the calculated values used for the determination of the thermodynamic data of duplex formation. The corresponding $1/T_m$ *vs*. ln(c) plots are reproduced in Figure S1 and the calculated thermodynamic data (non-self complementary duplex) are depicted in Table S2.

Table S1: Purification and mass spectrometric data of RNA-oligonucleotides.

Entry	Sequence	RP	R_t	ε_{260nm} [M^{-1} cm^{-1}]	ESI^-MS calc	ESI^- MS found
1	5'-rCUAGC**Bph**GUCAUC-3'	5-35%B	14.1	122 700	3774.26	3773.75
2	3'-rGAUCG**Bph**CAGUAG-5'	5-35%B	14.5	134 000	3877.36	3876.38
3	5'-rCUAGC**(Bph)$_2$**GUCAUC-3'	5-35%B	22.4	141 700	4122.54	4122.13
4	3'-rGAUCG**(Bph)$_2$**CAGUAG-5'	5-35%B	21.2	153 000	4225.63	4225.00
5	5'-rCUAGC**(Bph)$_3$**GUCAUC-3'	5-40%B	24.2	160 700	4470.82	4470.00
6	3'-rGAUCG**(Bph)$_3$**CAGUAG-5'	5-40%B	24.1	172 000	4573.91	4573.00
7	5'-rCUAGCGUCAUC-3'	5-35%B	11.0	103 700	3425.98	3425.25
8	3'-rGAUCGCAGUAG-5'	0-20%B	20.8	115 000	3529.08	3528.13

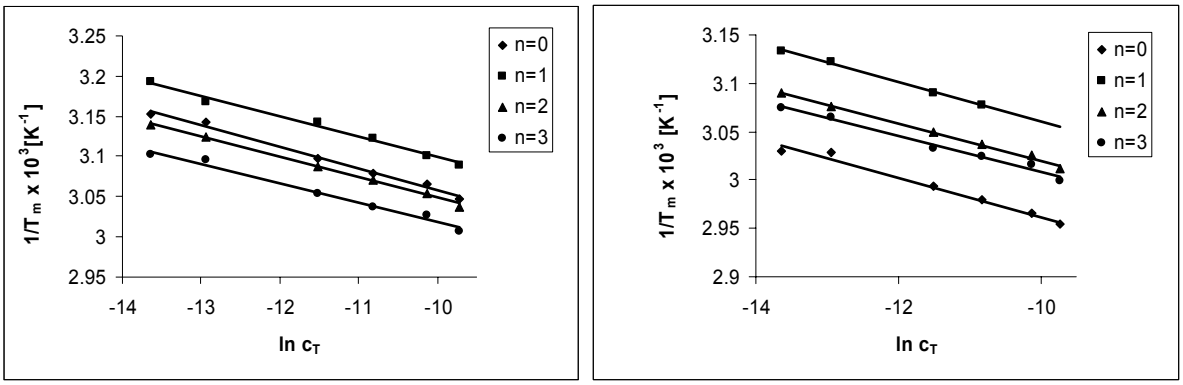


Figure S1: Thermodynamics from DNA- (left) and RNA-duplexes (right) with n=0-3; Linear regression of DNA- duplexes with R^2=0.9912 (n=0), 0.9910 (n=1), 0.9964 (n=2), 0.9852 (n=3), and RNA-duplexes R^2= 0.9848 (n=0), 0.9967 (n=1), 0.9968 (n=2), 0.9884 (n=3).

Table S2: Thermodynamic data of DNA- and RNA-duplexes with n= 0-3.

5'-GAT GAC (Bph)$_n$ GC TAG-3'
3'-CTA CTG (Bph)$_n$ CG ATC-5'

	-ΔH [kcal mol^{-1}]		-ΔS [cal K^{-1} mol^{-1}]		-ΔG$^{25°C}$ [kcal mol^{-1}]	
n	DNA	RNA	DNA	RNA	DNA	RNA
0	73	98	200	266	13.2	18.1
1	78	97	220	273	12.7	15.6
2	78	103	216	286	13.9	16.9
3	82	107	223	299	14.9	17.7

References

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2 S. Hoshika, N. Minakawa, A. Matsuda, *Nucleic Acids Res.,* 2004, **32**, 3815-3825

3 http://paris.chem.yale.edu