Bioorganic & Medicinal Chemistry 18 (2010) 7786–7793

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Parallel synthesis and nucleic acid binding properties of C(6′)-α-functionalized bicyclo-DNA

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ARTICLE INFO

Article history:
Received 23 August 2010
Revised 20 September 2010
Accepted 24 September 2010
Available online 1 October 2010

Keywords:
Oligonucleotides
Nucleosides
Antisense
DNA diagnostics
Bicyclo-DNA

ABSTRACT

Two novel bicyclo-T nucleosides carrying a hydroxyl or a carboxymethyl substituent in C(6′)-α-position were prepared and incorporated into oligodeoxynucleotides. During oligonucleotide deprotection the carboxymethyl substituent was converted into different amide substituents in a parallel way. T_m-measurements showed no dramatic differences in both, thermal affinity and mismatch discrimination, compared to unmodified oligonucleotides. The post-synthetic modification of the carboxymethyl substituent allows in principle for a parallel preparation of a library of oligonucleotides carrying diverse substituents at C(6′). In addition, functional groups can be placed into unique positions in a DNA double helix.

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1. Introduction

Oligonucleotide based gene silencing was discovered in the late seventies of the last century,[1,2] and has evolved over the years into oligonucleotide based therapeutic strategies, the two most important ones being antisense and RNA interference.[3,4] In the therapeutic context it is believed that chemically modified oligonucleotides have superior properties compared to natural DNA or RNA as they allow to address and improve on critical parameters as RNA target affinity, nuclease resistance and cellular uptake and distribution. Over the years a large number of chemically modified oligonucleotide analogs have been synthesized and their biological properties evaluated. The field has been reviewed on several occasions before, and so also very recently.[5] One successful concept applied to the design of oligonucleotide analogs is that of conformational restriction, resulting in structures such as locked-nucleic acids (LNA),[6,7] hexitol nucleic acids (HNA),[8] and tricyclo-DNA (tc-DNA, Fig. 1).[9,10] These analogs typically exhibit increased affinity to RNA and feature higher nuclease resistance. Due to their properties, some of these modified nucleic acids are now considered to become next generation oligonucleotide drugs.[4,11] While the challenges linked to target affinity and nuclease resistance have largely been met in the past, several drawbacks, the most prominent ones being cellular uptake and distribution, largely remained unsolved. A promising strategy for the latter problem may be bioconjugation of oligonucleotides to specific or unspecific cell targeting molecular entities.[12]

Some years ago we developed bicyclo-DNA (bc-DNA) as a first generation conformationally constrained oligonucleotide analog.[13] It turned out that bc-DNA base-pairs to natural nucleic acids with about equal stability as DNA itself.[14] One of the unique structural features of bc-DNA is the ethylene bridge between the centers C(3′) and C(5′) of the ribose unit which lends itself for further chemical substitution. We reasoned that additional substituents

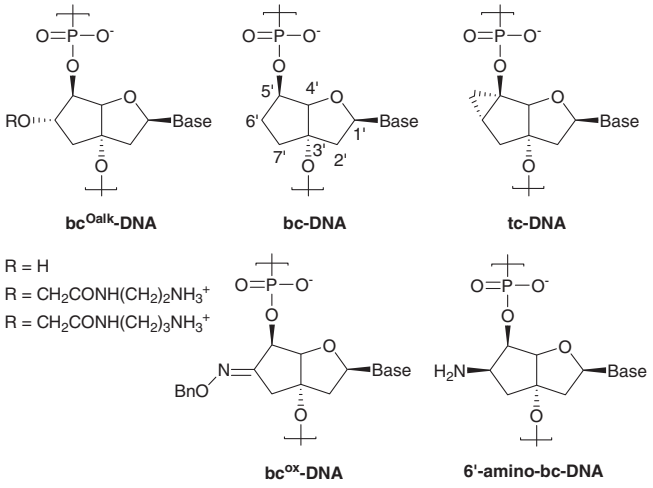

Figure 1. Conformationally constrained oligonucleotide analogs of the bicyclo-DNA type: left 6′-α-*O*-derivatized bicyclo-DNA (bc^{Oalk}-DNA), center bicyclo-DNA (bc-DNA), right: tricyclo-DNA (tc-DNA).

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doi:10.1016/j.bmc.2010.09.064

at position C(6′) could be interesting, for example, for controlling the conformation of the carbocyclic ring, thus contributing to the structure activity profile of the bc-DNA molecular platform. In addition, incorporation of a post-synthetically transformable group could be used either to attach cell targeting or reporter groups, or to improve the lipophilic character of bc-DNA, thus improving its cellular uptake properties. Earlier work on C(6′)-oxime modified bc-DNA (bc^{ox}-DNA) turned out to be encouraging in this context.[15] Another candidate of interest, particularly in the latter context, is bc^{Oalk}-DNA (Fig. 1). Here we report on the synthesis of the bc^{Oalk}-T nucleoside carrying an alkoxy substituent in α-position at C(6′), on its conformational preference as determined by X-ray crystallography, on its incorporation into oligonucleotides and on the base-pairing properties with DNA and RNA.

2. Results and discussion

2.1. Synthesis of nucleosides and building blocks

The synthesis of the building block **8**, leading to bc-DNA containing a hydroxyl group at C(6′), started from the already known diol **1** (Scheme 1) that was used previously in the synthesis of 6′-amino-bc-DNA.[16] The secondary hydroxyl group of diol **1** was selectively acetylated with Ac_2O to give compound **2**. Looking for a β-selective nucleosidation reaction we focused on a two step procedure involving NIS mediated regio- and stereoselective addition of persilylated thymine to glycals,[17,18] followed by radical removal of the iodo-substituent. This reaction sequence proved to be very useful for the β-selective synthesis of tricyclo-pyrimidine nucleosides.[19,20] Therefore, intermediate **2** was transformed to the furanose glycal **3** with TMS-OTf in the presence of 2,6-lutidine. Under these conditions the free 3′-OH group was simultaneously silylated. NIS mediated addition of persilylated thymine to glycal **3** then proceeded smoothly and yielded **4** in an anomeric ratio β/α of 85:15 (^{1}H NMR). The inseparable mixture of nucleosides **4** was deiodinated via radical reduction and the anomeric mixture resolved by chromatography (→**5**). Nucleoside **6** was then obtained by desilylation with HF in pyridine.

Suitable crystals of nucleoside **6** were subjected to X-ray analysis in order to prove the anomeric configuration as well as to map the conformational preferences of the bicyclic core structure (Fig. 2). In the crystal the furanose ring shows a perfect $^0_1$T conformation (twisted O(4′)-endo/C(1′)-exo) with a pseudorotation phase angle *P* of 108° and thus adopts S-type conformation as also the natural 2′-deoxynucleosides (Table 1). The carbocyclic ring

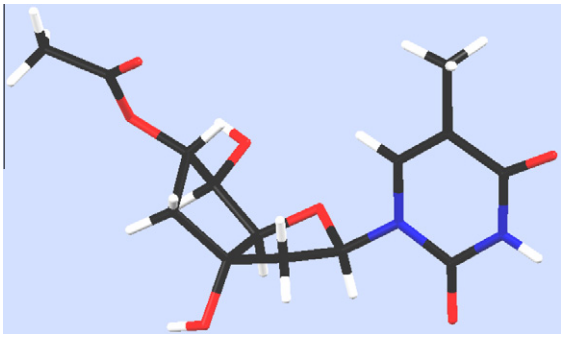


Figure 2. X-ray structure of nucleoside **6**.

Table 1
Pseudorotation phase angles (*P*) and selected nucleoside torsion angles

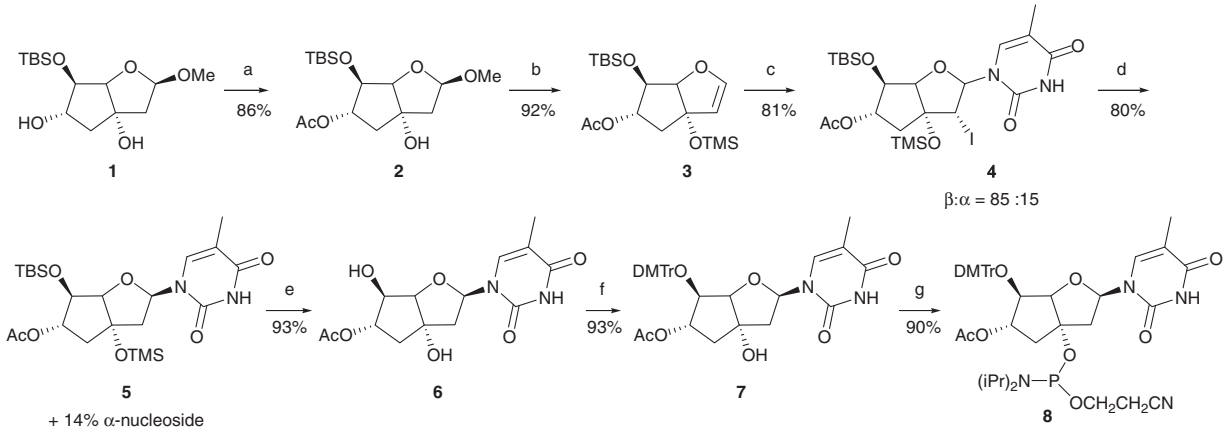
	P (°)	γ (°)	δ (°)	χ (°)
6	108	158.2	112.6	−133.5
bc-T[a]	128	149.3	126.5	−112.8
dN[b]	144	57	122	−119

[a] Taken from (Ref. 21).
[b] Average deoxynucleotide conformation in B-DNA (Ref. 22).

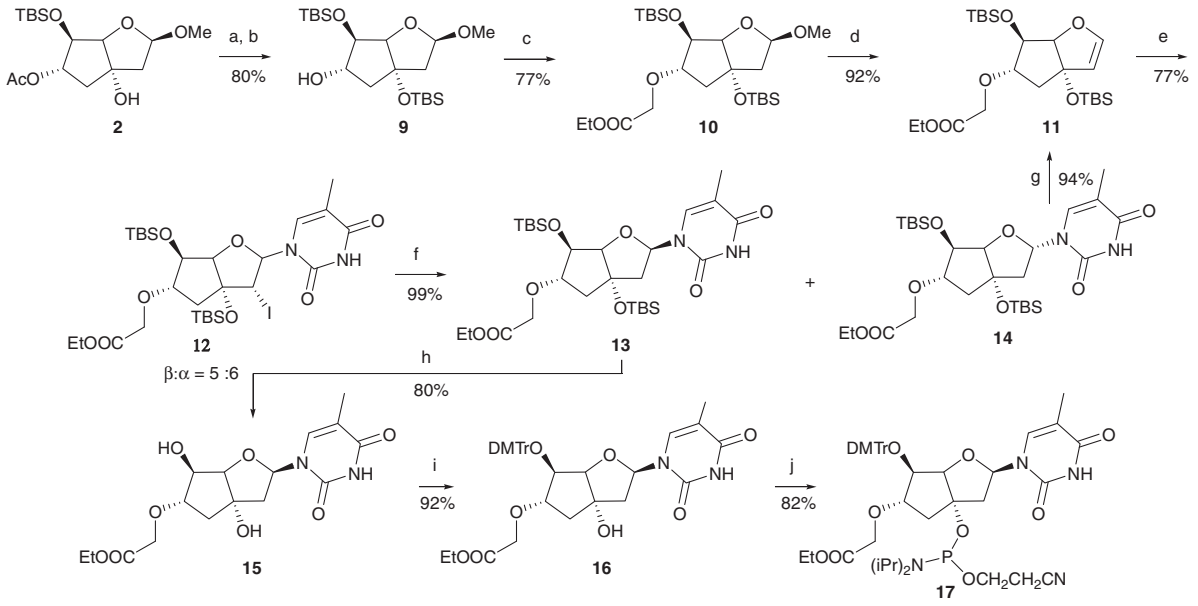
appears in an 6′-endo conformation with the 5′- and 6′-subsituents pseudoequatorially aligned. A comparison with the X-ray structures of the **bc-T** nucleoside shows a high degree of similarity of the torsion angles γ, δ and χ, indicating that a 6′-α-substituent does not cause any significant changes to the bicyclic core conformation.

The building block **8** for oligonucleotide synthesis was then prepared by standard protocols in DNA chemistry. The secondary OH function in **6** was selectively tritylated with 4,4′-dimethoxytrityl chloride (→**7**) followed by phosphitylation of the 3′-OH function leading to phosphoramidite **8**.

The synthesis of the alkoxy substituted building block **17** was first tried by alkylation of **1** using ethyl bromoacetate and a variety of bases such as ^{i}Pr$_2$EtN, DBU, pyridine, K_2CO_3. None of these attempts proved to be successful and typically ended with unconverted starting material **1** or decomposition. In situ addition of NaI or AgOTf did not solve the problem either, as it lead to only low yields of the desired alkylation product, or to double (O(5), O(7)) alkylation of the oxabicyclic ring system. The finally



Scheme 1. Synthesis of nucleoside building block **8** (a) Ac_2O (1.5 equiv), pyridine (2 equiv), $ClCH_2CH_2Cl$, 55 °C, 20 h. (b) TMS-OTf (2.5 equiv), 2,6-lutidine (5 equiv), CH_2Cl_2, rt, 3 h. (c) thymine (1.5 equiv), BSA (1.6 equiv), CH_2Cl_2, rt, 1 h, NIS (1.2 equiv), rt, 8 h. (d) Bu_3SnH (1.2 equiv), AIBN (0.3 equiv), toluene, 110 °C, 2 h. (e) HF.py, pyridine/DCM, rt, 16 h. (f) DMTr-Cl (1.2 equiv), pyridine (2 equiv), CH_2Cl_2, rt, 12 h. (g) iPr$_2$NP(Cl)CH$_2$CH$_2$CN (2.5 equiv), iPr$_2$NEt (4 equiv), MeCN, rt, 2 h.



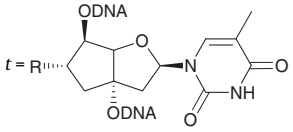
Scheme 2. Synthesis of bc-DNA building block **17** (a) TBDMS-Cl (2 equiv), imidazole (3 equiv), DMF, 75 °C, 2 days. (b) K_2CO_3 (2 equiv), EtOH, rt, 8 h. (c) $N_2CHCOOEt$ (2 equiv), $Cu(acac)_2$ (5 mol %), CH_2Cl_2, 70 °C, 5 h. (d) TMS-OTf (2 equiv), 2,6-lutidine (6 equiv), CH_2Cl_2, rt, 3 h. (e) thymine (3 equiv), BSA (2.5 equiv), CH_2Cl_2, 40 °C, 2 h, *N*-Iodosuccinimide (1.3 equiv), rt, 7 h. (f) Bu_3SnH (1.5 equiv), AIBN (0.4 equiv), toluene, 110 °C, 2 h. (g) TMS-OTf (2.5 equiv), 2,6-lutidine (7 equiv), $ClCH_2CH_2Cl$, 85 °C, 3 h. (h) HF.py, pyridine/THF, 50 °C, 2 days. (i) DMTr-Cl (1.5 equiv), pyridine (3 equiv), CH_2Cl_2, rt, 12 h. (*J*=) $(iPr)_2NP(Cl)OCH_2CH_2CN$ (2.5 equiv), iPr_2NEt (4 equiv), MeCN, rt, 2 h.

successful synthesis started with intermediate **2** and is depicted in Scheme 2. To prevent alkylation of the O(5) function, it was TBS-protected to give after deacetylation compound **9**. O(7)-alkylation was then accomplished by Cu(II) catalyzed carbene insertion of ethyl diazoacetate yielding **10**. Applying the same strategy as before, glycal **11**, obtained from **10** by elimination, was subjected to NIS mediated nucleosidation and produced the iodonucleosides **12**, however in a disappointing anomeric ratio β/α = 5:6 (^{1}H NMR). We believe that the increase in steric demand of the TBS group compared to the TMS group (as in **3**) is primarily responsible for the loss of selectivity. The anomeric mixture of nucleosides **12** were deiodinated as before and the nucleosides **13** and **14** could be isolated in pure form after chromatographic separation. The undesired α-nucleoside **14** could be recycled to glycal **11** while the β-nucleoside **13**, the configuration at the anomeric center of which was confirmed by ^{1}H NMR-NOE spectroscopy, was further converted to the phosphoramidite building block **17** following analogous procedures as for amidite **8**.

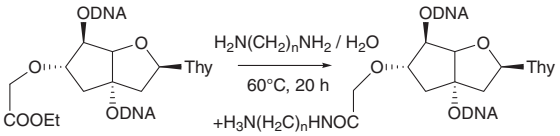
2.2. Oligonucleotide synthesis and deprotection

A series of oligodeoxyribonucleotides (Table 2) containing single or double substitutions were synthesized on a 1.3 μmol scale by standard automated phosphoramidite chemistry. For incorporation of the modified building blocks the coupling time was extended to 6 min for **8** and 12 min for **17**. No further changes to the synthesis cycle were necessary. Coupling yields, as monitored by the trityl assay, were in all cases in the range of 97–99 %. After completion of chain assembly, the detachment from the solid support and deprotection was carried out by standard treatment with conc. NH_3 (55 °C, 16 h 70 °C) for oligonucleotides that included building block **8**. Solid supported oligonucleotides constructed

Table 2
T_m data from UV-melting curves (260 nm) of modified dodecamer duplexes with complementary DNA and RNA

	Sequence	*t* = 	ESI$^-$-MS *m/z* calc	ESI$^-$-MS *m/z* found	T_m (°C) vs DNA[a,b] (ΔT_m/mod)	T_m (°C) vs RNA[a,c] (ΔT_m/mod)
ON1	*d*(GGA TGT TCt CGA)	R = H	3701.2	3701.0	49.1 (+1.5)	49.2 (−0.5)
ON2		R = OH	3718.4	3718.8	45.3 (−2.3)	48.3 (−1.4)
ON3		R = $OCH_2CONH(CH_2)_2NH_3^+$	3818.6	3817.9	48.0 (+0.4)	47.6 (−2.1)
ON4		R = $OCH_2CONH(CH_2)_3NH_3^+$	3832.6	3832.6	47.0 (−0.6)	47.2 (−2.5)
ON5	*d*(GGA tGT TCt CGA)	R = H	3727.1	3728.4	48.0 (+0.2)	51.1 (+0.7)
ON6		R = OH	3760.5	3761.2	45.6 (−1.0)	46.9 (−1.4)
ON7		R = $OCH_2CONH(CH_2)_2NH_3^+$	3960.7	3960.5	47.3 (−0.2)	46.2 (−1.7)
ON8		R = $OCH_2CONH(CH_2)_3NH_3^+$	3988.8	3988.4	47.3 (−0.2)	46.2 (−1.7)
ON9	*d*(GGA TGt tCT CGA)	R = H	3727.1	3728.6	48.8 (+0.6)	48.5 (−0.6)
ON10		R = OH	3760.5	3760.9	47.0 (−0.3)	47.9 (−0.9)
ON11		R = $OCH_2CONH(CH_2)_2NH_3^+$	3960.7	3960.9	47.0 (−0.3)	46.6 (−1.5)
ON12		R = $OCH_2CONH(CH_2)_3NH_3^+$	3988.8	3988.5	46.7 (−0.5)	46.6 (−1.5)

[a] Total strand conc 2 μM in 10 mM NaH_2PO_4, 150 mM NaCl, pH 7.0. Estimated error in T_m = ±0.5 °C.
[b] T_m of unmodified duplex: 47.6 °C.
[c] T_m of unmodified duplex: 49.7 °C.



Scheme 3. Conditions for detachment/deprotection with concomitant transformation of ester into amide functions of oligonucleotides built from phosphoramidite **17**.

from building block **17** were split into two parts and were treated in a parallel fashion either with 40% aqueous 1,2-diaminoethane or 1,3-diaminopropane at 60 °C for 20 h. Under these conditions, not only deprotection and detachment from solid support, but also aminolysis of the unique ester functions occurred (Scheme 3). With this procedure an aminoalkyl chain is covalently introduced which is expected to stabilize duplexes by electrostatic means and which could also beneficially influence cellular uptake of oligonucleotides.[23] The crude oligonucleotides were then purified by ion-exchange HPLC. Table 2 gives an overview on the sequences **ON1–12** obtained in this way as well as a confirmation of their structure by mass spectrometry. In addition the thermal melting data (T_m) with complementary DNA and RNA are summarized.

2.3. T_m measurements

UV-melting curves were measured at 260 nm with a cooling–heating–cooling cycle at a rate of 0.5 °C/min in standard saline buffer (10 mM Na-phosphate, 150 mM NaCl, pH 7.0). Analysis of the T_m data (Table 2) revealed that oligonucleotides containing single (**ON2–4**) or double substitutions in a discontinuous (**ON6–8**) or continuous (**ON10–12**) fashion either slightly destabilized duplexes with complementary DNA or were T_m neutral. ΔT_m per modification range from −2.3 to +0.4 °C. The 6′-OH-bc-T modification tends to destabilize more than the diaminoethyl- or diaminopropyl-bc-T units. This may be a consequence of the additional positive charge in the latter modifications, counterbalancing the repulsive effect of the two phosphodiester backbones in the duplex to some extent. Interestingly, the difference in the linker length had no significant effect on T_m. In the case of two consecutive modifications (**ON10–12**) almost no destabilization was observed against complementary DNA. This indicates that reducing the number of junctions between natural and bc-units in the backbone has a positive effect on T_m. The same trend, but somewhat more pronounced, also occurs with RNA as complement. All modifications show also slightly reduced affinity compared to duplexes containing unmodified bc-T units (**ON1, 5, 9**). The substitution of a 6′-α-hydrogen atom by a hydroxyl function thus destabilizes presumably less due to conformational changes but more due to the change in hydrophobicity. Structural models of 6′-α-bc^{Oalk}-DNA shows that the substituents point away in a perpendicular way from the helix axis, directly into the solvent. We thus exclude that interference of these substituents with base-pairing or with the phosphodiester backbone cause the slight drop in T_m.

Compared with 6′-amino-bicyclo-DNA (Fig. 1) which carries an amino function in β-position,[16] the C(6′)-α-OR modifications are slightly more destabilizing. This could be a consequence of the missing or differentially aligned positive charges in the latter modifications which might shield the negative charges of the phosphodiester backbone in a less efficient way. Alternatively, it could also be a conformational effect of the carbocyclic ring. In the case of C(5′,6′)-*cis*-substitution, as in 6′-amino-bc-DNA, the 5′-OH group can be expected to have a higher tendency to occur in a pseudoaxial position matching more closely the natural +*sc* conformation of torsion angle γ compared to C(5′,6′)-*trans*-substitution, as in 6′-α-bc^{Oalk}-DNA, where γ is more constrained to the *ap*-conformation. It needs, however, to be pointed out that direct comparison is difficult as the studies on 6′-amino-bc-DNA have been conducted on oligothymidylate sequences exclusively which may behave differently as the more general sequences chosen for this study.

Table 3
T_m data (°C) from UV-melting curves (260 nm) of **ON1** and **ON2** with DNA complements carrying a mismatched base opposite the modification

Mismatch[b]	ON2	ON1	Unmodified DNA[a]
G–T	37.6 (−7.7)	37.0 (−12.0)	39.7 (−7.8)
C–T	36.7 (−8.6)	35.0 (−14.0)	36.0 (−11.5)
T–T	32.3 (−13.0)	32.0 (−17.0)	38.0 (−9.5)

[a] 2 μM concn of strands, 150 mM NaCl, 10 mM phosphate buffer (pH 7.0).
[b] T_m of matched duplexes see Table 2. Values in parenthesis are ΔT_m relative to the matched duplex.

2.4. Mismatch discrimination

For the case of **ON2** as an example we determined T_m data with complementary DNA carrying a mismatched base opposite the modification to determine the relative effect of the modification on pairing selectivity (Table 3). Generally, the OH function on the bc-T unit leads to less discrimination of a mismatch situation compared with an unmodified bc-T unit. On the other hand, an unmodified bc-T unit shows higher discriminative power compared with a natural dT unit. Differences are particularly manifest in the case of the wobble (G–T) mismatch. The molecular origin of these thermochemical variations remains elusive at this point.

3. Conclusions

We successfully prepared the two phosphoramidite building blocks **8** and **17** containing 6′-alkoxy bc-T modifications and incorporated them into oligodeoxynucleotides. While building block **8** led to 6′-hydroxylated bc-T-units, **17** is versatile and can post-synthetically be transformed by ester aminolysis into a variety of structurally diverse oligonucleotides, depending on the deprotection conditions. We found that neither of the investigated 6′-modifications had a dramatic effect on T_m with complementary DNA and RNA. Thus, the synthetic strategy with building block **17** opens the door for rapid, parallel synthesis of oligonucleotides with varying functional groups that can be beneficial for bioanalytical or therapeutic purposes. In addition, the bc-DNA scaffold offers possibilities for introducing unique functional entities in positions of the DNA double helix without significantly compromising with DNA or RNA affinity that are otherwise not accessible. This may be exploited in the future for post-synthetic conjugation to improve the performance of diagnostic or therapeutic oligonucleotides.

4. Experimental

4.1. General

All reactions were performed under Ar in dried glassware. Anhydrous solvents for reactions were obtained by filtration through activated aluminum oxide, or by storage over 4 Å molecular sieves. Column chromatography (CC) was performed on silica gel (Fluka) with an average particle size of 40 μm. All solvents for CC were of technical grade and distilled prior to use. Thin layer chromatography (TLC) was performed on silica gel plates (Macherey-Nagel, 0.25 mm, UV254). Visualization was performed either by UV or by staining in dip solution (10.5 g Cer(IV)-sulfate, 21 g phophormolybdenic acid, 60 ml conc. sulfuric acid, 900 ml H_2O) followed by heating with a heat gun. NMR spectra were recorded on a Bruker DRX-400 or a Bruker AC-300 spectrometer at 400 or 300 MHz (^{1}H NMR) or 100 MHz (^{13}C NMR) in either $CDCl_3$

or CD_3OD. δ in ppm relative to residual undeuterated solvent ($CHCl_3$: 7.26 ppm (^1H) and 77.0 ppm (^{13}C). CHD_2OD: 3.35 ppm (^1H) and 49.3 ppm (^{13}C)), J in Hz. ^{13}C-multiplicities were determined from DEPT-spectra and signal assignments are based on $^{13}C/^1H$-HMBC spectra. Proton signal assignments were based on COSY and HMBC. ^1H NMR difference-NOE spectra were recorded on a Bruker DRX-500 instrument at 500 MHz. High resolution electrospray ionization (ESI) mass spectra (MS, *m*/*z*) were recorded on an Applied Biosystems Sciex QSTAR Pulsar instrument.

4.1.1. (1*R*,3*R*,5*S*,7*S*,8*R*)-7-[(Acetyl)oxy]-8-[(*tert*-butyldimethyl silyl)oxy]-3-methoxy-2-oxabicyclo[3.3.0]octan-5-ol (2)

Acetic anhydride (0.25 ml, 2.56 mmol) was added to a solution of **1** (520 mg, 1.708 mmol) and pyridine (0.28 ml, 3.42 mmol) in 1,2-dichloroethane (10 ml) at rt. The reaction mixture was stirred and heated to 55 °C for 20 h. After quenching with satd $NaHCO_3$ (30 ml) and extraction with CH_2Cl_2 (3 × 30 ml) the organic phases were combined, dried ($MgSO_4$) and evaporated. Column chromatography (hexane/EtOAc 3:1→1/1) afforded the monoacetylated product **2** (510 mg, 86 %) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.09, 0.11 (6H, 2 × s, Me_2Si), 0.91 (9H, s, Me_3CSi), 1.65 (1H, ddd *J* = 13.6, 9.0, 1.3 Hz, H–C(6)), 2.04 (3H, s, Ac), 2.21 (1H, dd, *J* = 13.4, 1.5 Hz, H–C(4)), 2.31 (1H, ddd, *J* = 13.4, 5.5, 1.5 Hz, H–C(4)), 2.67 (1H, dd, *J* = 13.6, 7.3 Hz, H–C(6)), 3.37 (3H, s, OMe), 4.06 (1H, d, *J* = 6.0 Hz, H–C(1)), 4.11 (1H, dd, *J* = 7.9, 6.0 Hz, H–C(8)), 5.09 (1H, dd, *J* = 5.5, 1.5 Hz, H–C(3)), 5.20 (1H, dt, *J* = 9.0, 7.7 Hz, H–C(7)). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −4.92, −4.78 (Me_2Si), 18.19 (Me_3CSi), 21.03 (Ac), 25.69 (Me_3CSi), 42.20 (C6), 49.43 (C4), 54.49 (MeO), 76.16 (C8), 78.27 (C-7), 83.52 (C5), 87.44 (C1), 105.56 (C3), 170.50 (Ac). ESI^+-HRMS: calcd for $C_{16}H_{30}O_6NaSi$ ($[M+Na]^+$) 369.1709. Found: 369.1708.

4.1.2. (1*R*,5*S*,7*S*,8*R*)-7-[(Acetyl)oxy]-8-[(tert-butyldimethylsilyl) oxy]-5-[(trimethylsilyl)oxy]-2-oxabicyclo[3.3.0]oct-3-ene (3)

To a solution of **2** (1.92 g, 5.54 mmol) and 2,6-lutidine (3.3 ml, 28.3 mmol) in dry CH_2Cl_2 (10 ml) was added dropwise at rt TMSOTf (2.5 ml, 13.85 mmol). After stirring for 4 h at rt the reaction mixture was diluted with AcOEt (50 ml), washed with satd $NaHCO_3$ (2 × 25 ml) and the aqueous phases extracted with AcOEt (2 × 25 ml). The combined organic phases were dried over $MgSO_4$ evaporated and the crude product purified by flash chromatography (hexane/^{t}BuOMe 10:1→5:1) to give **3** (1.97 g, 92%) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.10, 0.11 (15H, 2 × s, Me_3Si, Me_2Si), 0.90 (9H, s, Me_3CSi), 1.79 (1H, dd, *J* = 12.6, 9.8 Hz, H–C(6)), 2.04 (3H, s, Ac), 2.42 (1H, dd, *J* = 12.6, 6.2 Hz, H–C(6)), 4.22 (1H, dd, *J* = 8.1, 6.8 Hz, H–C(8)), 4.36 (1H, d, *J* = 6.8, H–C(1)), 4.75 (1H, ddd, *J* = 9.8, 8.1, 6.2 Hz, H–C(7)), 4.99 (1H, d, *J* = 2.6 Hz, H–C(4)), 6.46 (1H, d, *J* = 2.6 Hz, H–C(3)). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −4.98, −4.82 (Me_2Si), 1.83 (Me_3Si), 18.24 (Me_3CSi), 20.94 (Ac), 25.66 (Me_3CSi), 41.31 (C6), 76.28 (C8), 77.04 (C7), 87.20 (C5), 88.00 (C1), 106.99 (C4), 149.31 (C3), 170.34 (Ac). ESI^+-HRMS: calcd for $C_{18}H_{34}O_5NaSi_2$ ($[M+Na]^+$) 409.1842. Found: 409.1827.

4.1.3. 1-[(2′*R*,3′*S*,5′*R*,6′*S*)-6′-*O*-Acetyl-5′-*O*-(*tert*-butyldimethyl silyl)-3′-*O*-(trimethylsilyl)-2′-deoxy-2′-iodo-3′,5′-ethano-β-D-ribofuranosyl]thymine (4)

To a suspension of thymine (190 mg, 1.5 mmol) and glycal **3** (390 mg, 1.0 mmol) in CH_2Cl_2 (5 ml) was added BSA (0.37 ml, 1.5 mmol) and the mixture was stirred at rt for 1 h. Then solid *N*-iodsuccinimide (270 mg, 1.2 mmol) was added and the mixture stirred for 8 h at rt. The reaction was quenched with satd $NaHCO_3$ (15 ml) and $Na_2S_2O_3$ (10 %, 5 ml). The aqueous phase was extracted with AcOEt (3 × 25 ml) and the combined organic phases were dried over $MgSO_4$ and evaporated. Column chromatography (hexane/EtOAc 5:1→3:1) afforded the unseparable mixture of α- and β-anomers **4** (524 mg, 81 %, α/β 15:85). Data for the β-anomer: ^1H NMR (300 MHz, $CDCl_3$) δ: 0.09, 0.12 (6H, 2 × s, Me_2Si), 0.25 (9H, s, Me_3Si), 0.89 (9H, s, Me_3CSi), 1.64 (1H, dd, *J* = 13.8, 8.7 Hz, H–C(7′)), 1.94 (3H, d, *J* = 1.3 Hz, Me–C(5)), 2.10 (3H, s, Ac), 2.38 (1H, dd, *J* = 13.8, 6.4 Hz, H–C(7′)), 3.84 (1H, d, *J* = 9.8 Hz, H–C(2′)), 4.14 (1H, t, *J* = 6.8 Hz, H–C(5′)), 4.27 (1H, d, *J* = 7.2, H–C(4′)), 4.94 (1H dt, *J* = 8.7, 6.4 Hz, H–C(6′)), 6.34 (1H, d, *J* = 9.8 Hz, 1H–C(1′)), 7.22 (1H, d, *J* = 1.3, 1H–C(6)), 8.17 (1H, s, br, H–N(3)). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.12, −4.74 (Me_2Si), 2.02 (Me_3Si), 12.54 (Me–C(5)), 18.29 (Me_3CSi), 20.87 (Ac), 25.66 (Me_3CSi), 36.59 (C2′), 36.72 (C7′), 74.38 (C5′), 78.79 (C6′), 84.36 (C4′), 84.51 (C3′), 88.70 (C1′), 111.92 (C5), 134.19 (C6), 150.52 (C2), 163.46 (C4), 170.14 (Ac). ESI^+-HRMS: calcd for $C_{23}H_{39}N_2O_7NaSi_2I$ ($[M+Na]^+$) 661.1238. Found: 661.1226.

4.1.4. 1-[(3′*S*,5′*R*,6′*S*)-6′-*O*-Acetyl-5′-*O*-(tert-butyldimethylsilyl)-3′-*O*-(trimethylsilyl)-2′-deoxy-3′,5′-ethano-β-D-ribofuranosyl]thymine (5)

Azoisobutyronitril (AIBN, 40 mg, 0.24 mmol) was added at rt to a solution of the mixture of anomers **4** (500 mg, 0.78 mmol) and Bu_3SnH (0.25 ml, 0.93 mmol) in toluene (10 ml). After heating at reflux for 1 h the solvent was evaporated and the residue purified by column chromatography (hexane/EtOAc 5:1→3:1) to give α-anomer of **5** (58 mg, 14 %) and β-anomer of **5** (320 mg, 80 %) both as a white solid. Data for β-anomer: ^1H NMR (300 MHz, $CDCl_3$) δ: 0.10, 0.12 (6H, 2 × s, Me_2Si), 0.17 (9H, s, Me_3Si), 0.90 (9H, s, Me_3C-Si), 1.67 (1H, dd, *J* = 12.8, 10.2 Hz, H–C(7′)), 1.72 (1H, dd, *J* = 13.8, 9.2 Hz, H–C(2′)), 1.92 (3H, d, *J* = 1.1 Hz, Me–C(5)), 2.08 (3H, s, Ac), 2.47 (1H, dd, *J* = 12.8, 6.4 Hz, H–C(7′)), 2.65 (1H, dd, *J* = 13.8, 5.1 Hz, H–C(2′)) 4.09–4.14 (2H, m, H–C(4′), H–C(5′)), 4.86 (1H, m, H–C(6′)), 6.23 (1H, dd, *J* = 9.2, 5.1 Hz, H–C(1′)), 7.53 (1H, d, *J* = 1.1 Hz, H–C(6)), 8.23 (1H, s, br, H–N(3)). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.09, −4.81 (Me_2Si), 1.87 (Me_3Si), 12.56 (Me–C(5)), 18.27 (Me_3CSi), 20.91 (Ac), 25.65 (Me_3CSi), 39.14, 47.64 (C2′, C7′), 74.41 (C5′), 78.41 (C6′), 85.10 (C3′), 85.33 (C4′), 86.93 (C1′), 111.00 (C5), 135.15 (C6), 149.97 (C2), 163.48 (C4, Ac). ESI^+-HRMS: calcd for $C_{23}H_{40}N_2O_7NaSi_2$ ($[M+Na]^+$) 535.2271. Found: 535.2266.

4.1.5. 1-[(3′*S*,5′*R*,6′*S*)-6′-*O*-Acetyl-2′-deoxy-3′,5′-ethano-β-D-ribofuranosyl]thymine (6)

To a solution of nucleoside **5** (260 mg, 0.51 mmol) and pyridine (1 ml) in CH_2Cl_2 (5 ml) was added HF-pyridine (0.25 ml, 10.1 mmol) at 0 °C. After stirring for 15 h at rt, silica gel (ca. 1 g) was added and the mixture stirred for another 15 min. After evaporation the adsorbed product was purified by column chromatography (EtOAc/EtOH 10:1) to yield the title compound **6** (148 mg, 90%) as white crystals. Mp 210–211 °C. ^1H NMR (300 MHz, DMSO-d_6) δ: 1.58 (1H, dd, *J* = 13.6, 7.1 Hz, H–C(7′)), 1.78 (3H, d, *J* = 1.1 Hz, Me–C(5)), 1.95 (1H, dd, *J* = 13.2, 9.9 Hz, H–C(2′)), 2.03 (3H, s, Ac), 2.25 (1H, dd, *J* = 13.2, 5.3 Hz, H–C(2′)), 2.40 (1H, dd, *J* = 13.6, 6.4 Hz, H–C(7′)), 3.91–3.99 (2H, m, H–C(4′), H–C(5′)), 4.90 (1H, m, H–C(6′)), 5.43 (1H, d, *J* = 5.8 Hz, H–O(5′)), 5.54 (1H, s, H–O(3′)), 6.16 (1H, dd, *J* = 9.9, 5.3 Hz, H–C(1′)), 7.78 (1H, d, *J* = 1.1, H–C(6)), 11.34 (1H, s, H–N(3)). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.19 (Me–C(5)), 20.86 (Ac), 40.68, 46.11 (C2′, C7′), 73.09 (C5′), 78.46 (C6′), 82.33 (C3′), 84.75 (C4′), 87.18 (C1′), 109.33 (C5), 136.11 (C6), 150.31 (C2), 163.64 (C4), 169.91 (Ac). ESI^+-HRMS: calcd for $C_{14}H_{18}N_2O_7Na$ ($[M+Na]^+$) 349.1011. Found: 349.1012.

4.1.6. 1-[(3′*S*,5′*R*,6′*S*)-6′-*O*-Acetyl-5′-*O*-[(4,4′-dimethoxytriphenyl)methyl)-2′-deoxy-3′,5′-ethano-β-D-ribofuranosyl]thymine (7)

To a solution of nucleoside **6** (630 mg, 1.9 mmol) and pyridine (0.3 ml, 3.8 mmol) in CH_2Cl_2 (10 ml) was added (4,4′-dimethoxytriphenyl)methyl chloride (DMT-Cl, 773 mg, 2.28 mmol) and the mixture stirred at rt overnight. The reaction was quenched with satd

$NaHCO_3$ (20 ml) and the aqueous phase was extracted with EtOAc (3 × 20 ml). The combined organic phases were dried ($MgSO_4$), evaporated and the crude product purified by column chromatography (hexane/EtOAc 1:3, 1% NEt_3) to give the title compound **7** (1.19 g, 98%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.57 (3H, d, J = 1.1 Hz, Me–C(5)), 1.59 (1H, dd, J = 13.8, 7.3 Hz, H–C(7′)), 1.92 (1H, dd, J = 13.6, 9.6 Hz, 1H–C(2′)), 1.99 (3H, s, Ac), 2.43 (1H, dd, J = 13.8, 5.9 Hz, H–C(7′)), 2.55 (1H, dd, J = 13.6, 5.1 Hz, H–C(2′)), 3.40 (1H, d, J = 6.0, H–C(4′)), 3.78, 3.79 (6H, 2 × s, MeO), 4.17 (1H, m, H–C(5′)), 4.58 (1H, m, H–C(6′)), 6.21 (1H, dd, J = 9.6, 5.1, H–C(1′)), 6.80–6.82 (4H, m, H-arom), 7.24–7.30, 7.35–7.39, 7.47–7.50 (3H, 4H, 2H, 3 × m, H-arom), 7.60 (1H, d, J = 1.1 Hz, 1H–C(6)). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.09 (*Me*–C(5)), 21.09 (Ac), 40.59, 47.69 (C2′, C7′), 55.36 (MeO), 75.88 (C5′), 77.81 (C6′), 83.65 (C3′), 85.46 (C4′), 87.10 (C1′), 111.63 (C5), 113.20, 127.37, 127.87, 128.69, 130.70, 130.80 (HC-arom), 135.24 (C6), 135.81 135.99, 144.88 (C-arom), 150.41 (C2), 159.03, 159.08 (C-arom), 163.62 (C4), 170.07 (Ac). ESI$^+$-HRMS: calcd for $C_{35}H_{36}N_2O_9Na$ ([M+Na]$^+$) 651.2318. Found: 651.2311.

4.1.7. 1-[(3′*S*,5′*R*,6′*S*)-6′-*O*-Acetyl-5′-*O*-[(4,4′-dimethoxytriphenyl)methyl)-3′-*O*-(2-cyanoethoxy diisopropylaminophosphanyl)-2′-deoxy-3′,5′-ethano-β-D-ribofuranosyl]thymine-) (8)

2-Cyanoethoxy diisopropylamino chlorophosphine (CEP-Cl, 1.0 ml, 4.47 mmol) was added at rt to a solution of nuleoside **7** (1.18 g, 1.88 mmol) and diisopropylethylamine (1.3 ml, 7.48 mmol) in MeCN (8 ml). After stirring for 2 h at rt, the mixture was diluted with EtOAc (50 ml), washed with satd $NaHCO_3$ (2 × 20 ml) and brine (5 ml). The aqueous phases were extracted with EtOAc (3 × 25 ml). The combined organic phases were dried ($MgSO_4$), evaporated and the crude product purified by column chromatography (hexane/EtOAc 1:2 + 1% NEt_3) to give the title compound **8** (1.39 g, 90 %) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.08–1.14 (12H, m, $(Me_2CH)_2N$), 1.45, 1.60 (3H, 2 × d, J = 1.1 Hz, Me–C(5)), 1.80–1.95 (2H, m, C(2′), C(7′)), 1.96, 2.00 (3H, 2 × s, Ac), 2.40–2.50, 2.55–2.61 (2H, 2 × m, C(2′), C(7′)), 2.90–2.98 (1H, m, $(Me_2CH)_2N$), 3.50–3.77 (5H, m, C(4′), OCH_2CH_2CN), 3.79 (6H, s, MeO), 4.12–4.17 (1H, m, C(5′)), 4.41, 4.59 (1H, 2 × m, C(6′)), 6.13, 6.24 (1H, 2 × dd, J = 9.8, 4.8 Hz, C(1′)), 6.81 (4H, m, H-arom), 7.28, 7.38, 7.48 (9H, 3 × m, H-arom), 7.59 (1H, d, J = 1.1 Hz, C(6)), 8.22 (1H, s, br, H–N(3)). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.76, 12.03 (*Me*–C(5)), 20.15, 20.23 (d, $J_{C,P}$ = 8.3 Hz, OCH_2CH_2CN), 20.89, 20.97 (Ac), 24.14, 24.17, 24.27, 24.38, 24.46, 24.53 $(Me_2CH)_2N$), 39.20, 39.37 d, ($J_{C,P}$ = 11.5 Hz, C-2′ or C-7′), 43.31, 43.48 (d, $J_{C,P}$ = 8.3 Hz, $Me_2CH)_2N$), 45.99, 46.27 (d, $J_{C,P}$ = 10.7 Hz, C-2′ or C-7′), 55.26 (MeO), 57.74, 57.97 (d, $J_{C,P}$ = 7.0 Hz, OCH_2CH_2CN), 75.40, 75.45 (C5′), 77.84 (C6′), 85.28, 86.16 (2 × d, $J_{C,P}$ = 9.0, 4.1 Hz, C4′), 87.01 ($OC(Ar)_3$), 87.08, 87.16 (C1′), 87.63, 87.79 2 × d, ($J_{C,P}$ = 18.1, 9.9 Hz, C3′), 111.30 (C5), 117.55, 117.81 (CN), 113.10, 127.28, 127.39, 127.77, 127.82, 128.61, 128.67, 130.64, 130.70, 130.76 (HC-arom), 135.13, 135.36 (C6), 135.60 135.67, 135.72, 135.88, 144.58, 144.78 (C-arom), 150.00, 150.10 (C2), 158.95, 159.02, 159.06 (C-arom), 163.42, 163.45 (C4), 169.72, 170.07 (Ac). ^{31}P NMR (162 MHz, $CDCl_3$) δ: 142.08, 142.53. ESI$^+$-HRMS: calcd for $C_{44}H_{53}N_4O_{10}NaP$ ([M+Na]$^+$) 851.3397. Found: 851.3406.

4.1.8. (1*R*,5*S*,7*S*,8*R*)-5,8-[Di-(*tert*-butyldimethylsilyl)oxy]-3-methoxy-2-oxabicyclo[3.3.0]octan-7-ol (9)

To a solution of **2** (2.20 g, 6.35 mmol) and imidazole (1.29 g, 19 mmol) in DMF (25 ml) was added TBDMS-chloride (1.92 g, 12.7 mmol) at rt. The reaction mixture was stirred at 80 °C for 2 days. The reaction mixture was then diluted with EtOH (2 ml) and evaporated. The residue was dissolved in abs EtOH (30 ml) and K_2CO_3 (2.0 g, 14.5 mmol) was added. The suspension was stirred at rt for 8 h. The reaction mixture was filtered through a pad of silica gel, washed with EtOAc/EtOH 1:1 and the filtrate adsorbed on silica gel. Column chromatography (silica gel, hexane/EtOAc 8:1→1:1) afforded product **9** (2.13 g, 80%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.11, 0.15 (12H, 2 × s, 2 × Me_2Si), 0.86, 0.94 (18H, 2 × s, 2 × Me_3CSi), 1.73 (1H, dd, J = 12.9, 9.5 Hz, H–C(6)), 1.96 (1H, d, J = 3.8 Hz, OH), 2.03 (1H, dd, J = 13.7, 5.2 Hz, H–C(4)), 2.18–2.25 (2H, m, H–C(4), H–C(6)), 3.33 (3H, s, OMe), 3.86 (1H, dd, J = 8.0, 5.0 Hz, H–C(8)), 3.97 (1H, m, H–C(7)), 4.06 (1H, d, J = 5.0 Hz, H–C(1)), 5.02 (1H, dd, J = 5.2, 1.6 Hz, H–C(3)). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −4.65 (Me_2Si), 18.20 (Me_3CSi), 25.61, 25.93 (*Me*$_3$CSi), 45.25 (C6), 49.71 (C4), 54.51 (MeO), 75.36 (C8), 78.97 (C7), 83.62 (C5), 86.76 (C1), 105.34 (C3). ESI$^+$-HRMS: calcd for $C_{20}H_{42}O_5NaSi_2$ ([M+Na]$^+$) 441.2468. Found: 441.2463.

4.1.9. (1*R*,5*S*,7*S*,8*R*)-5,8-[Di-(*tert*-butyldimethylsilyl)oxy]-7-[(ethoxycarbonyl)methyl]oxy-3-methoxy-2-oxabicyclo[3.3.0]octane (10)

A solution of ethyl diazoacetate (1.5 ml, 13.0 mmol) in 1,2-dichloroethane (8 ml) was added dropwise within 4 h at 70 °C to a solution of **9** (2.1 g, 5.01 mmol) and $Cu(acac)_2$ (66 mg, 5 mol %) in 1,2-dichloroethane (10 ml). The reaction mixture was stirred for 3 h at 70 °C. After the reaction was complete, the mixture was evaporated and the crude product was purified by column chromatography (hexane/EtOAc 10:1→4:1) to give the title compound **10** (2.37 g, 77% yield) as a yellowish oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.09, 0.15, 0.16 (12H, 3 × s, 2 × Me_2Si), 0.85, 0.94 (18H, 2 × s, 2 × Me_3CSi), 1.27 (3H, t, J = 7.2 Hz, CH_3-Et), 1.81 (1H, dd, J = 13.1, 9.9 Hz, H–C(6)), 2.07 (1H, dd, J = 13.8, 5.3 Hz, H–C(4)), 2.22 (1H, dd, J = 13.8, 1.5 Hz, H–C(4), 2.31 (1H, dd, J = 13.1, 7.0 Hz, H–C(6)), 3.32 (3H, s, OMe), 3.78 (1H, ddd, J = 9.9, 8.0, 7.0 Hz, H–C(7)), 3.99–4.07 (2H, m, H–C(1), H–C(8)), 4.26 (2H, q, J = 7.2, CH_2-Et), 4.28 (2H, s, OCH_2CO), 5.01 (1H, dd, J = 5.3, 1.5 Hz, 1H–C(3)). ESI$^+$-HRMS: calcd for $C_{24}H_{48}O_7NaSi_2$ ([M+Na])$^+$ 527.2836. Found: 527.2834.

4.1.10. (1*R*,5*S*,7*S*,8*R*)-5,8-[Di-(*tert*-butyldimethylsilyl)oxy]-7-[(ethoxycarbonyl)methyl]oxy-2-oxabicyclo[3.3.0]oct-3-ene (11)

To a solution of **10** (1.13 g, 2.24 mmol) and 2,6-lutidine (1.6 ml, 13.44 mmol) in dry CH_2Cl_2 (15 ml) was added dropwise TMS-OTf (0.81 ml, 4.48 mmol) at rt. After stirring for 3 h the reaction mixture was quenched with satd $NaHCO_3$ (25 ml) and extracted with CH_2Cl_2 (3 × 25 ml). The combined organic phases were dried over $MgSO_4$, evaporated and the crude product purified by column chromatography (hexane/EtOAc 10:1→4:1) to give the title compound **11** as a colorless oil (0.98 g, 92 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.06, 0.07, 0.13 (12H, 4 × s, 2 × Me_2Si), 0.84, 0.92 (18H, 2 × s, 2 × Me_3CSi), 1.27 (3H, t, J = 7.2 Hz, CH_3-Et), 1.90 (1H, dd, J = 12.6, 10.2 Hz, H–C(6)), 2.30 (1H, dd, J = 12.6, 6.1 Hz, H–C(6)), 3.62 (1H, ddd, J = 10.2, 7.7, 6.1 Hz, H–C(7)), 4.16 (1H, dd, J = 7.7, 6.6 Hz, H–C(8)), 4.22 (2H, s, OCH_2CO), 4.26 (2H, q, J = 7.2 Hz, CH_2-Et), 4.30 (1H, d, J = 6.6 Hz, H–C(1)), 4.94 (1H, d, J = 2.6 Hz, H–C(4)), 6.41 (1H, d, J = 2.6, H–C(3)). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −4.93, −4.68, −3.13, −2.58 (2 × Me_2Si), 14.19 (CH_3-Et), 17.76, 18.22 (2 × Me_3CSi), 25.64, 25.79 (2 × *Me*$_3$CSi), 41.86 (C6), 60.73 (CH_2-Et), 67.91 (OCH_2CO), 78.66 (C8), 83.34 (C7), 86.53 (C5), 88.86 (C1), 107.42 (C4), 148.86 (C3), 170.47 (C=O). ESI$^+$-HRMS: calcd for $C_{23}H_{44}O_6NaSi_2$ ([M+Na])$^+$ 495.2574. Found: 495.2573.

4.1.11. 1-[(3′*S*,5′*R*,6′*S*)-3′,5′-*O*-[Di-(*tert*-butyldimethylsilyl)]-6′-*O*-(ethoxycarbonyl)methyl-2′-deoxy-2′-iodo-3′,5′-ethano-α,β-D-ribofuranosyl]thymine (12)

To a suspension of thymine (670 mg, 5.27 mmol) and glycal **11** (830 mg, 1.755 mmol) in CH_2Cl_2 (30 ml) was added BSA (0.9 ml, 3.5 mmol) and the mixture was stirred at 40 °C for 2 h. Then solid *N*-iodsuccinimide (515 mg, 2.29 mmol) was added at 0 °C and the

mixture was stirred for 7 h at rt. After quenching with satd $NaHCO_3$ (15 ml) and aq $Na_2S_2O_3$ (10%, 5 ml) the phases were separated and the aqueous phase extracted with AcOEt (3 × 25 ml). The combined organic phases were dried over $MgSO_4$, evaporated and the crude product purified by column chromatography (hexane/EtOAc 8:1→2:1) to give the unseparable mixture of α- and β-anomers **12** (980 mg, 77 %, α/β = 6/5) as a colorless oil. Data for α + β-anomers: ^{1}H NMR (300 MHz, $CDCl_3$): 0.10, 0.12, 0.15, 0.20, 0.21, 0.22, 0.24 (24H, 7 × s, 4 × Me_2Si), 0.89, 0.91, 0.95 (36H, 3 × s, 4 × Me_3CSi), 1.30 (6H, t, *J* = 7.2 Hz, 2 × CH_3-Et), 1.74 (1H, dd, *J* = 13.8, 8.7 Hz, 1H–C(7′)), 1.93, 1.96 (6H, 2 × d, *J* = 1.1 Hz, 2 × Me–C(5)), 1.99 1H, (m, H–C(7′)), 2.27 (1H, dd, *J* = 13.8, 6.1 Hz, 1H–C(7′)), 2.61 (1H, dd, *J* = 14.9, 7.3 Hz, H–C(7′)), 3.78 (1H, d, *J* = 9.8 Hz, H–C(2′)), 3.75–3.88 (2H, m, 2 × H–C(6′)), 4.10–4.16 (2H, m, 2 × H–C(5′)), 4.19, 4.21 (4H, 2 × s, 2 × OCH_2CO), 4.22, 4.23 (4H, 2 × q, *J* = 7.2 Hz, 2 × CH_2-Et), 4.28 (1H, m, H–C(4′)), 4.35 (1H, d, *J* = 4.7, H–C(4′)), 4.48 (1H, d, *J* = 9.1, H–C(2′)), 5.92 (1H, d, *J* = 9.1 Hz, H–C(1′α)), 6.31 (1H, d, *J* = 9.8 Hz, H–C(1′β)), 7.02, 7.20 (2H, 2 × d, *J* = 1.1 Hz, 2 × H–C(6)), 8.05, 8.09 (2H, 2 × s, br, 2 × H–N(3)). ^{13}C NMR (100 MHz, $CDCl_3$): −5.02, −4.92, −4.71, −4.55, −2.71, −2.48, −2.41, −2.10 (4 × Me_2Si), 12.59 (Me–C(5)), 14.20 (CH_3-Et), 17.87, 18.24, 18.27, 18.41 (4 × Me_3CSi), 25.55, 25.77, 25.80, 25.89 (4 × Me_3CSi), 36.50, 36.77 (2 × C2′), 37.25, 43.04 (2 × C7′), 60.90, 61.03 (2 × CH_2-Et), 67.86, 67.88 (2 × OCH_2CO), 76.08, 78.88 (2 × C5′), 83.97, 85.69 (2 × C3′), 84.27, 84.59 (2 × C6′), 85.88, 86.63 (2 × C4′), 88.59, 92.36 (2 × C1′), 111.72, 111.90 (2 × C5), 134.36, 135.37 (2 × C6), 149.77, 150.19 (2 × C2), 163.10, 163.17 (2 × C4), 169.92, 170.16 (2 × C=O). ESI$^+$-MS: 725.2 ([M+H])$^+$.

4.1.12. 1-[(3′*S*,5′*R*,6′*S*)-3′,5′-O-[Di-(*tert*-butyldimethylsilyl)]-6′-*O*-(ethoxycarbonyl)methyl-2′-deoxy-3′,5′-ethano-α,β-D-ribofuranosyl]thymine (14/13)

A solution of azoisobutyronitril (AIBN, 60 mg, 0.366 mmol) in toluene (2 ml) was added at 100 °C in four portions within 2 h to a solution of the mixture of anomers **12** (535 mg, 0.738 mmol) and Bu_3SnH (0.35 ml, 1.18 mmol) in dry toluene (15 ml). The reaction was complete after 2 h. The solvents were evaporated and the anomers separated by column chromatography (hexane/EtOAc 7:1→2:1) to give α-anomer **14** (240 mg, 54%) and β-anomer **13** (200 mg, 45 %), both as a white foam. Data of 14. ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: −0.02, 0.11, 0.12 (12H, 3 × s, 2 × Me_2Si), 0.83, 0.91 (18H, 2 × s, 2 × Me_3CSi), 1.29 (3H, t, *J* = 7.1 Hz, CH_3-Et), 1.92 (3H, d, *J* = 1.1 Hz, Me–C(5)), 1.98 (1H, dd, *J* = 13.9, 4.9 Hz, H–C(7′)), 2.28 (1H, dd, *J* = 14.3, 2.8 Hz, H–C(2′)), 2.38 (1H, dd, *J* = 13.9, 5.8 Hz, H–C(7′)), 2.47 (1H, dd, *J* = 14.3, 7.0 Hz, H–C(2′)), 3.77 (1H, m, H–C(6′)), 4.09 (1H, m, H–C(5′)), 4.10, 4.16 (2H, 2 × d, *J* = 16.4 Hz, OCH_2CO), 4.22 (2H, q, *J* = 7.2 Hz, CH_2-Et), 4.58 (1H, d, *J* = 5.5 Hz, H–C(4′)), 6.17 (1H, dd, *J* = 7.0, 2.8 Hz, H–C(1′)), 7.36 (1H, d, *J* = 1.1, H–C(6)), 8.15 (1H, s, br, H–N(3)). ^{1}H NMR difference NOE: H(1′)→H(4′) 0.5 %, H(6′) 1.1%. ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: −5.12, −4.71, −2.98, −2.82 (2 × Me_2Si), 12.61 (Me–C(5)), 14.20 (CH_3-Et), 17.74, 18.11 (2 × $Me_3\underline{C}Si$), 25.46, 25.76 (2 × Me_3CSi), 41.64 (C7′), 49.17 (C2′), 60.96 (CH_2-Et), 67.36 (OCH_2CO), 75.71 (C5′), 85.81 (C6′), 86.07 (C3′), 89.55 (C1′), 93.12 (C4′), 109.88 (C5), 136.23 (C6), 149.87 (C2), 163.58 (C4), 170.08 (C=O). ESI$^+$-MS: 599.3 ([M+H])$^+$. Data for **13**: ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 0.12, 0.15 (12H, 2 × s, 2 × Me_2Si), 0.88, 0.93 (18H, 2 × s, 2 × Me_3CSi), 1.29 (3H, t, *J* = 7.2 Hz, CH_3-Et), 1.63 (1H, dd, *J* = 13.6, 9.2 Hz, H–C(2′)), 1.79 (1H, dd, *J* = 12.7, 10.2 Hz, H–C(7′)), 1.92 (3H, d, *J* = 1.1, Me–C(5)), 2.37 (1H, dd, *J* = 12.7, 6.2 Hz, H–C(7′)), 2.62 (1H, dd, *J* = 13.6, 5.1 Hz, H–C(2′)), 3.73 (1H, m, H–C(6′)), 4.02–4.10 (2H, m, H–C(4′), H–C(5′)), 4.22 (2H, q, *J* = 7.2, CH_2-Et), 4.25 (2H, s, OCH_2CO), 6.16 (1H, dd, *J* = 9.2, 5.1 Hz, H–C(1′)), 7.52 (1H, d, *J* = 1.1 Hz, H–C(6)), 8.19 (1H, s, br, H–N(3)). ^{1}H NMR difference NOE: H(1′)→H(4′) 2.1%. H(6′)→H(6) 4.3%. ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: −5.03, −4.62, −2.77, −2.74 (2 × Me_2Si), 12.54 (Me–C(5)), 14.20 (CH_3-Et), 17.74, 18.11 (2 × Me_3CSi), 25.57, 25.75 (2 × Me_3CSi), 40.02 (C7′), 48.30 (C2′), 60.92 (CH_2-Et), 67.97 (OCH_2-CO), 76.65 (C5′), 84.40 (C3′), 84.98 (C6′), 85.34 (C1′), 87.90 (C4′), 110.70 (C5), 135.20 (C6), 149.82 (C2), 163.40 (C4), 170.17 (C=O). ESI$^+$-MS: 599 ([M+H])$^+$.

4.1.13. 1-[(3′*S*,5′*R*,6′*S*)-6′-*O*-(Ethoxycarbonyl)methyl-2′-deoxy-3′,5′-ethano-β-D-ribofuranosyl]thymine (15)

To a solution of nucleoside **13** (330 mg, 0.55 mmol) and pyridine (2.2 ml) in THF (10 ml) was added HF-pyridine (0.47 ml, 16.5 mmol) at 0 °C and the mixture stirred at 50 °C for 2 days. After complete conversion of **13**, silica gel (ca. 2 g) was added and the mixture stirred for another 15 min. Then the solvents were evaporated and the residue purified by column chromatography (EtOAc/EtOH 10:0→10:1) to give nucleoside **15** (160 mg, 80%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 1.29 (3H, t, *J* = 7.2 Hz, CH_3-Et), 1.91 (3H, d, *J* = 1.1 Hz, Me–C(5)), 1.99 (1H, dd, *J* = 14.4, 3.1 Hz, H–C(7′)), 2.32 (1H, dd, *J* = 13.3, 9.6 Hz, H–C(2′)), 2.40 (1H, dd, *J* = 14.4, 4.5 Hz, H–C(7′)), 2.45 (1H, dd, *J* = 13.3, 5.7 Hz, H–C(2′)), 3.62 (1H, d, *J* = 3.0 Hz, H–O(5′)), 3.87 (1H, s, br, H–O(3′)), 4.05 (1H, m, H–C(6′)), 4.07, 4.30 (2H, 2 × d, *J* = 17.0 Hz, OCH_2CO), 4.22 (1H, m, H–C(5′)), 4.23 (2H, q, *J* = 7.2 Hz, CH_2-Et), 4.42 (1H, d, *J* = 5.5 Hz, 1H–C(4′)), 6.17 (1H, dd, *J* = 9.6, 5.7 Hz, H–C(1′)), 7.21 (1H, d, *J* = 1.1 Hz, 1H–C(6)), 8.64 (1H s, H–N(3)). ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 12.43 (Me–C(5)), 14.11 (CH_3-Et), 41.63, 44.50 (C7′, C2′), 61.51 (CH_2-Et) 67.14 (OCH_2CO), 74.11 (C5′), 85.40 (C6′), 88.03 (C3′), 90.93, 91.34 (C1′, C4′), 111.40 (C5), 137.34 (C6), 150.27 (C2), 163.24 (C4), 171.55 (C=O). ESI$^+$-HRMS: calcd for $C_{16}H_{22}N_2O_8Na$ ([M+Na])$^+$, 393.1274. Found: 393.1257.

4.1.14. 1-[(3′*S*,5′*R*,6′*S*)-5′-*O*-[(4,4′-Dimethoxytriphenyl)methyl)-6′-*O*-(ethoxycarbonyl)methyl-2′-deoxy-3′,5′-ethano-β-D-ribofuranosyl]thymine (16)

To a solution of nucleoside **15** (150 mg, 0.405 mmol) and pyridine (0.1 ml, 1.25 mmol) in CH_2Cl_2 (10 ml) was added (4,4′-dimethoxytriphenyl)methyl chloride (DMT-Cl, 210 mg, 0.62 mmol) and the mixture was stirred at rt for 12 h. The reaction was diluted with satd $NaHCO_3$ (20 ml) and the aqueous phase extracted with CH_2Cl_2 (3 × 20 ml). The combined organic phases were dried ($MgSO_4$), evaporated and the crude product purified by column chromatography (hexane/EtOAc 1:1→0:1, 1% NEt_3) to give the title compound **16** (250 mg, 92%) as white foam. ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 0.99 (3H, d, *J* = 1.3 Hz, Me–C(5)), 1.23 (3H, t, *J* = 7.2 Hz, CH_3-Et), 1.85 (1H, d, *J* = 14.9 Hz, H–C(7′)), 2.00 (1H, dd, *J* = 12.8, 9.9 Hz, H–C(2′)), 2.20 (1H, dd, *J* = 14.9, 3.6 Hz, H–C(7′)), 2.43 (1H, m, H–C(6′)), 2.59 (1H, dd, *J* = 12.8, 4.9 Hz, H–C(2′)), 3.41, 3.86 (2H, 2 × d, *J* = 16.8 Hz, OCH_2CO), 3.61 (1H, s, br, H–O(3′)), 3.80, 3.81 (6H, 2 × s, MeO-DMTr), 4.12 (2H, q, *J* = 7.2 Hz, CH_2-Et), 4.27 (1H, m, H–C(5′)), 4.45 (1H, d, *J* = 5.1, H–C(4′)), 6.62 (1H, dd, *J* = 9.9, 4.9 Hz, H–C(1′)), 6.82–6.87 (4H, m, H-arom), 7.29–7.39, 7.44–7.47 (9H, 2 × m, H-arom), 7.68 (1H, d, *J* = 1.3 Hz, H–C(6)). ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 10.97 (Me–C(5)), 14.05 (CH_3-Et), 41.79 (C-7′), 45.65 (C-2′), 55.30 (*Me*O-DMTr), 61.40 (CH_2-Et), 67.01 (OCH_2CO), 75.74 (C5′), 86.72 (C3′), 87.95 (C6′), 88.17 (C1′), 91.77 (C4′), 111.21 (C5), 113.32, 127.57, 128.06, 128.75, 130.51 (HC-arom), 135.57, 135.72 (C-arom), 135.87 (C6), 149.89 (C2), 159.11, 159.13 (C-arom), 163.41 (C4), 171.20 (C=O). ESI$^+$-MS: 695.2 ([M+Na])$^+$.

4.1.15. 1-[(3′*S*,5′*R*,6′*S*)-5′-*O*-[(4,4′-Dimethoxytriphenyl)methyl)-6′-*O*-(ethoxycarbonyl)methyl]-3′-*O*-(2-cyanoethoxy diisopropylaminophosphanyl-2′-deoxy-3′,5′-ethano-β-D-ribofuranosyl] thymine) (17)

To a solution of nucleoside **16** (240 mg, 0.357 mmol) and diisopropylethylamine (0.25 ml, 1.44 mmol) in MeCN (8 ml) was added at rt 2-cyanoethoxy diisopropylamino chlorophosphine (CEP-Cl,

0.17 ml, 0.71 mmol). After stirring for 2 h the mixture was diluted with EtOAc (50 ml) and washed with satd $NaHCO_3$ (2 × 20 ml) and brine (5 ml). The aqueous phases were extracted with EtOAc (3 × 25 ml). The combined organic phases were dried ($MgSO_4$), evaporated and the crude product purified by column chromatography (hexane/EtOAc 3:1→1:2, 0.5% NEt_3) to give the title compound **17** (255 mg, 82%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.11–1.18 (12H, m, $(Me_2CH)_2N$), 1.20, 1.25 (3H, 2 × d, J = 1.3 Hz, Me–C(5)), 1.25, 1.26 (3H, 2 × t, J = 7.2 Hz, CH_3-Et), 1.91 (1H, m, H–C(2′)), 2.12–2.17 (1H, m, H–C(7′)), 2.28 (1H, m, H–C(7′)), 2.58–2.68 (2H, m, OCH_2CH_2CN), 2.90–2.95 (1H, m, H–C(2′)), 3.06–3.13 (1H, m, H–C(6′)), 3.54–3.70 (4H, m, $(Me_2CH)_2N$, OCH_2CH_2CN), 3.70, 3.74, 3.86, 3.88 (2H, 4 × d, J = 16.0 Hz, OCH_2-CO)), 3.79 (6H, s, MeO), 3.97, 4.06 (1H, 2 × d, J = 4.8 Hz, H–C(4′)), 4.16 (2H, q, J = 7.2 Hz, CH_2-Et), 4.16–4.20 (1H, m, H–C(5′)), 6.31 (1H, m, H–C(1′)), 6.81–6.84 (4H, m, H-arom), 7.27–7.31, 7.37–7.42, 7.50–7.53 (9H, 3 × m, H-arom), 7.60, 7.61 (1H, 2 × d, J = 1.3 Hz, C(6)), 8.05 (1H, s, br, H–N(3)). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 11.26, 11.35 (*Me*–C(5)), 14.16 (CH_3-Et), 20.15, 20.29 ($J_{C,P}$ = 9 Hz, OCH_2CH_2CN), 24.05, 24.15, 24.25, 24.45, 24.53 ($Me_2CH)_2N$), 39.00, 39.75 ($J_{C,P}$ = 10 Hz, C7′), 43.35, 43.52 ($J_{C,P}$ = 13 Hz, $Me_2CH)_2N$), 46.01, 46.12 (C2′), 55.23 (MeO), 57.73, 58.02 ($J_{C,P}$ = 16 Hz, OCH_2CH_2CN), 60.76, 60.78 (CH_2-Et), 67.37, 67.40 (OCH_2CO), 75.68, 75.70 (C5′), 85.15, 85.38 (C6′), 86.45, 86.68 (C1′), 87.85, 88.62 (Ar_2CPh), 88.75, 88.82 (C4′), 88.67, 88.86, (C3′), 111.07, 111.10 (C5), 117.83 (CN), 113.14, 113.20, 127.36, 127.89, 127.92, 128.76, 130.69, 130.76, (HC-arom), 135.74 (C6), 135.71, 135.78, 144.55, 144.58 (C-arom), 149.86, 149.94 (C2), 159.00, 159.03 (C-arom), 163.35, 163.36 (C4), 169.65, 169.76 (C=O). ^{31}P NMR (121 MHz, $CDCl_3$) δ: 141.43, 141.81. ESI$^+$-MS: 895.4 ($[M+Na]^+$).

4.2. Oligonucleotides synthesis and purification

Oligonucleotides were synthesized by standard solid phase phosphoramidite methodology on the 1.3 μmol scale on a Pharmacia LKB Gene Assembler Special DNA Synthesizer. Solvents and solutions were prepared according to the manufacturer's protocol. Ethylthiotetrazole (ETT, 0.25 M in MeCN) was used as activator in the coupling step. The coupling time was prolonged to 6–12 min for modified phosphoramidites **8** and **17**. Average coupling yields, as monitored by the on-line trityl assay, were in the range of 97–99%. Deprotection and detachment of the oligonucleotides built from **17** was effected either in aqueous 1,2-diaminoethane or 1,3-diaminopropane (1 ml, 40% aq) at 60 °C for 20 h. All other oligonucleotides were deprotected using standard conditions (33% aq NH, 55 °C, 16 h). The crude oligomers were purified by ion-exchange HPLC using a *DNAPAC PA200*, 4 × 250 mm analytical column (*Dionex*). Mobile phases *A*: 25 mM TRIZMA in H_2O, pH 8.0. *B*: 25 mM TRIZMA, 1.25 M NaCl in H_2O, pH 8.0, flow 1 ml/min detection at 260 nm. Purified oligonucleotides were desalted over *Sep-Pak Classic C18* Cartridges (*Waters*) and were analyzed by ESI$^-$ mass spectrometry (Table 2).

4.3. UV-melting curves

UV-melting curves were recorded on a *Varian Cary 100 Bio* UV–VIS spectrophotometer. Absorbances were monitored at 260 nm and the heating rate was set to 0.5 °C/min. A cooling–heating–cooling cycle in the temperature range 20–80 °C was applied. T_m values were obtained from the derivative curves using the *Varian WinUV* software. To avoid evaporation of the solution, the sample solutions were covered with a layer of dimethylpolysiloxane. All measurements were carried out in 150 mM NaCl, 10 mM Na-phosphate, pH 7.0 with duplex concentration of 2 μM.

4.4. X-ray structure of 6

Suitable crystals of **6** were obtained as colourless rods byrecrystallization from EtOH/EtOAc/toluene (2:1:2). The intensity data were collected at 173 K on a Stoe Image Plate Diffraction System (*Stoe & Cie, 2000, IPDS Software, Stoe & Cie GmbH, Darmstadt, Germany*) using MoKα graphite monochromated radiation. Image plate distance 70 mm, ϕ oscillation scans 0–150°, step $\Delta\phi$ = 1.0°, exposures of 6 min per image, 2θ range 3.27–52.1°, $d_{min} - d_{max}$ = 12.45–0.81 Å. The structure was solved by Direct methods using the programme SHELXS-97.[24] The refinement and all further calculations were carried out using SHELXL-97.[24] The H-atoms could all be located in Fourier difference maps but were included in calculated positions and treated as riding atoms using SHELXL default parameters. The non-H atoms were refined anisotropically, using weighted full-matrix least-squares on F^2. Crystallographic data (excluding structure factors) for compound **6** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 782500. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

Acknowledgments

This work was supported by the Swiss National Science Foundation (Grant No.: 200020-115913). We thank the BENEFRI small molecule crystallography service (Professor H. Stöckli-Evans) for solving the X-ray structure of **6**.

References and notes

1. Stephenson, M. L.; Zamecnik, P. C. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 285.
2. Zamecnik, P. C.; Stephenson, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 280.
3. Sibley, C. R.; Seow, Y.; Wood, M. J. A. *Mol. Ther.* **2010**, *18*, 466.
4. Bennett, C. F.; Swayze, E. E. *Annu. Rev. Pharmacol. Toxicol.* **2010**, *50*, 259.
5. Bell, N. M.; Micklefield, J. *ChemBioChem* **2009**, *10*, 2691.
6. Koshkin, A. A.; Nielsen, P.; Meldgaard, M.; Rajwanshi, V. K.; Singh, S. K.; Wengel, J. *J. Am. Chem. Soc.* **1998**, *120*, 13252.
7. Obika, S.; Nanbu, D.; Hari, Y.; Andoh, J. I.; Morio, K. I.; Doi, T.; Imanishi, T. *Tetrahedron Lett.* **1998**, *39*, 5401.
8. Hendrix, C.; Rosemeyer, H.; Verheggen, I.; Seela, F.; Van Aerschot, A.; Herdewijn, P. *Chem. Eur. J.* **1997**, *3*, 110.
9. Steffens, R.; Leumann, C. J. *J. Am. Chem. Soc.* **1997**, *119*, 11548.
10. Renneberg, D.; Leumann, C. J. *J. Am. Chem. Soc.* **2002**, *124*, 5993.
11. Tiemann, K.; Rossi, J. J. E. M. B. O. *Molecular Medicine* **2009**, *1*, 142.
12. Lönnberg, H. *Bioconjugate Chem.* **2009**, *20*, 1065.
13. Tarköy, M.; Bolli, M.; Leumann, C. *Helv. Chim. Acta* **1994**, *77*, 716.
14. Bolli, M.; Trafelet, H. U.; Leumann, C. *Nucleic Acids Res.* **1996**, *24*, 4660.
15. Luisier, S.; Leumann, C. J. *ChemBioChem* **2008**, *9*, 2244.
16. Meier, R.; Grüschow, S.; Leumann, C. J. *Helv. Chim. Acta* **1999**, *82*, 1813.
17. Mota, A. J.; Castellanos, E.; de Cienfuegos, L. A.; Robles, R. *Tetrahedron: Asymmetry* **2005**, *16*, 1615.
18. Kim, C. U.; Misco, P. F. *Tetrahedron Lett.* **1992**, *33*, 5733.
19. Luisier, S.; Silhar, P.; Leumann, C. J. *Nucleic Acids Symp. Ser.* **2008**, 581.
20. Ittig, D.; Renneberg, D.; Vonlanthen, D.; Luisier, S.; Leumann, C. J.; Coll. Symp. Series, Hocek, M., Ed., Academy of Sciences of the Czech Republic: Prague, 2005, pp 21–26.
21. Tarköy, M.; Bolli, M.; Schweizer, B.; Leumann, C. *Helv. Chim. Acta* **1993**, *76*, 481.
22. Saenger, W. *Principles of Nucleic Acid Structure*; Springer: New York, 1984.
23. Debart, F.; Abes, S.; Deglane, G.; Moulton, H. M.; Clair, P.; Gait, M. J.; Vasseur, J. J.; Lebleu, B. *Curr. Top. Med. Chem.* **2007**, *7*, 727.
24. Sheldrick, G. M. *Acta Crystallogr., Sect. A* **1990**, *46*, 467.