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Synthesis and Properties of Isobicyclo-DNA

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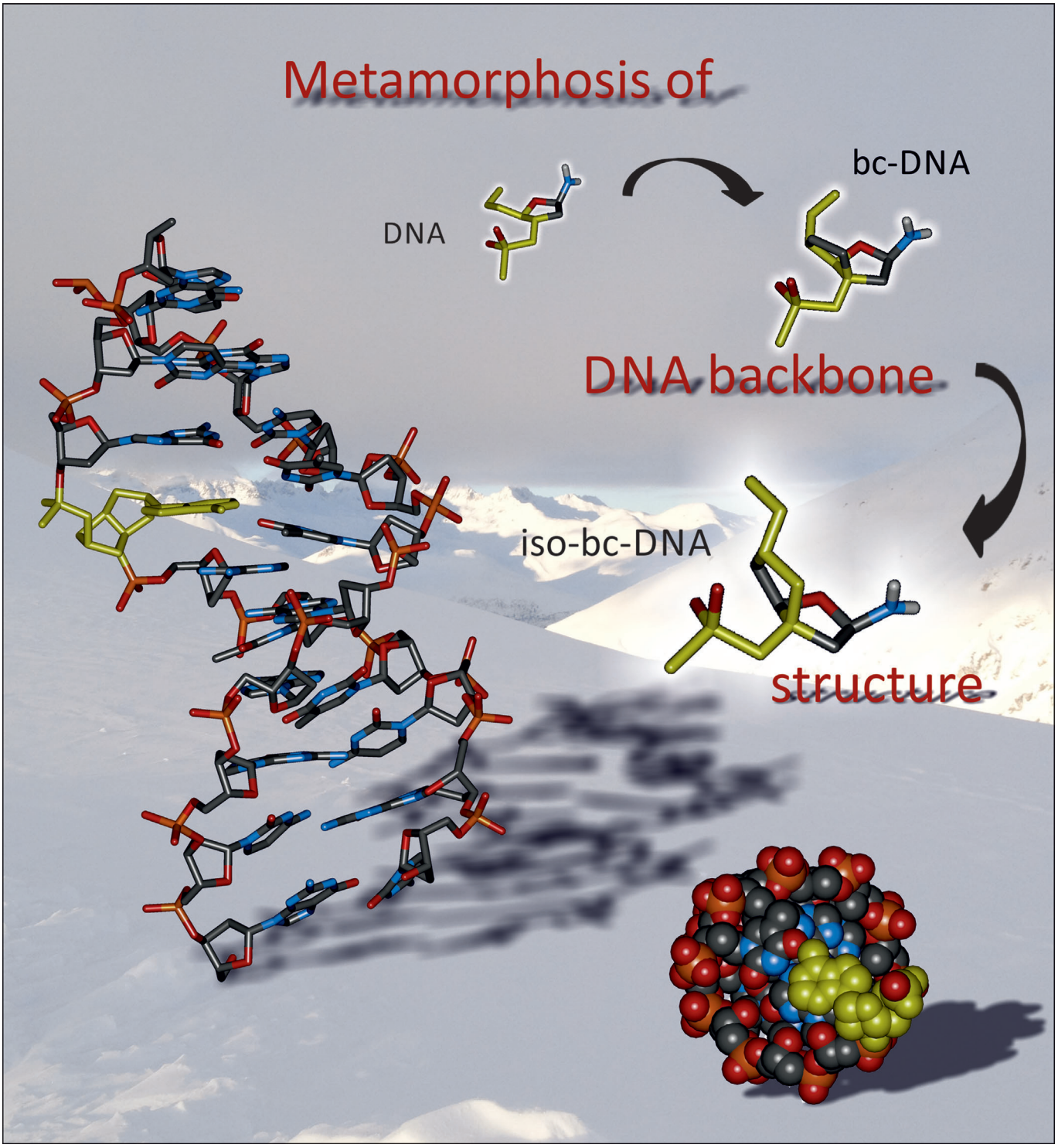

Abstract: We present the synthesis of the isobicyclo-DNA building blocks with the nucleobases A, C, G and T, as well as biophysical and biological properties of oligonucleotides derived thereof. The synthesis of the sugar part was achieved in 5 steps starting from a known intermediate of the tricyclo-DNA synthesis. Dodecamers containing single isobicyclo-thymidine incorporations, fully modified A- and T-containing sequences, and fully modified oligonucleotides containing all four bases were synthesized and characterized. Isobicyclo-DNA forms stable duplexes with natural nucleic acids with a pronounced preference for DNA over RNA as complements. The most stable duplexes, however, arise by self-pairing. Isobicyclo-DNA forms preferentially B-DNA-like duplexes with DNA and A-like duplexes with complementary RNA as determined by circular dichroism (CD) spectroscopy. Self-paired duplexes show a yet unknown structure, as judged from CD spectroscopy. Biochemical tests revealed that isobicyclo-DNA is stable in fetal bovine serum and does not elicit RNaseH activity.

Keywords: DNA · nucleosides · oligonucleotides · RNA · serum stability

Introduction

Oligonucleotides are a class of compounds with considerable therapeutic potential. Unlike small molecule drugs that typically target proteins, therapeutic oligonucleotides act through Watson–Crick hybridization to a matched sequence tract on an RNA of interest, modulating its biological function.[1] Originally, mRNAs of disease-related proteins have been the target of classical antisense oligonucleotides that either sterically block or induce RNaseH degradation of the mRNA in vivo.[2] The project ENCODE[3] recently revealed that 80% of the human genome is transcribed into RNA. Of these RNAs only 2% encode for proteins whereas a considerable subset, such as micro-RNAs (miRNAs) and generally non-coding RNAs, are involved in gene regulation. Thus, they play a pivotal role in the onset of disease, which greatly expands the palette of RNA sequences as therapeutic targets in the future.

The basic requirements for therapeutic oligonucleotides are 1) high affinity towards a target RNA, 2) biostability, and 3) cellular availability. A large number of chemical modifications on the DNA bases, sugar, and internucleoside linkage have been reported over the last two decades, but only a handful of those have been used in extensive pre-clinical or clinical tests. Amongst those are the class of 2′O-alkylated RNA,[4] the morpholino phosphorodiamidates,[5] the peptide nucleic acids (PNA),[6] and the locked nucleic acids (LNA)[7].

In our laboratory, we have been involved in the design and synthesis of conformationally constrained oligonucleotides over the last two decades. Offsprings of these efforts were the bicyclo-[8] and tricyclo-DNA (tc-DNA)[9] molecular platform (Figure 1). Unique to both families is the carbocyclic 5-membered ring connecting C(5′) and C(3′) and thereby rigidifying the rotational freedom around the C(3′)-C(4′) and the C(4′)-C(5′) bonds (Figure 1, A and C).

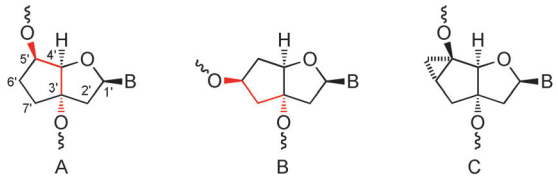

Figure 1. A) bicyclo-DNA, B) isobicyclo-DNA, C) tricyclo-DNA.

Besides conformational restriction, this carbocyclic ring offers the unique advantage to change the geometry of the repeating backbone unit in a single strand by moving the hydroxyl groups involved in the internucleotidic linkages to other carbon atoms within the cycle. In this context we became interested in exploring the properties of isobicyclo-DNA (Figure 1B) in which the C(5′) hydroxyl group has been moved to C(6′). Interestingly, this isobicyclo-DNA shows the same number of bonds within the repeating backbone unit as DNA. In this article we report on the synthesis of the corresponding isobicyclo-DNA building blocks with all four natural bases, on their incorporation into oligonucleotides, their RNA and DNA recognition properties, as well as on their structure as determined by circular dichroism (CD) spectroscopy and their biological properties (serum stability and RNaseH activation).

Results

Molecular modeling of monomers: To investigate the influence of the C(5′)→C(6′) hydroxyl shift on the structure of the nucleosides, a conformational search was performed by using the MM+ force field as implemented in the software package HyperChem. The C(3′), C(5′) dimethylated isobicyclo thymidine was initially built in a 2′-endo conformation and then energy minimized. In a conformational search experiment all endocyclic torsion angles were then varied, resulting in two different low-energy conformational families

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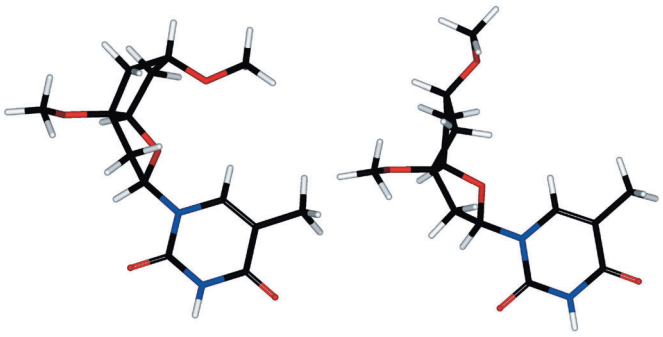


Figure 2. Lowest energy conformers of 3′,5′-dimethylated isobicyclo-thymidine: a) O(6′) in axial position, furanose in C(1′)-exo conformation; b) O(6′) in equatorial position, furanose in C(4′)-exo conformation.

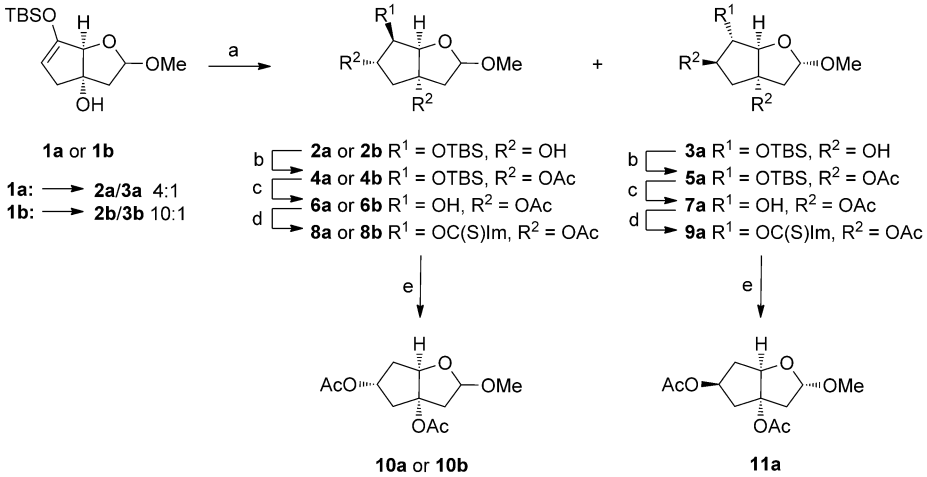
(Figure 2). Table 1 summarizes the endocyclic torsion angles of the furanose unit and the corresponding energies of two representative structures of each family. The lowest energy conformer turned out to be that with the 6′ substituent in an axial position (Figure 2a). This structure fits well into a B-DNA double helix as judged from simple model building.

Table 1. Results of the conformational search experiment for an isobicyclic thymidine. ω is the angle between the phosphate oxygen and the C6′ atom.

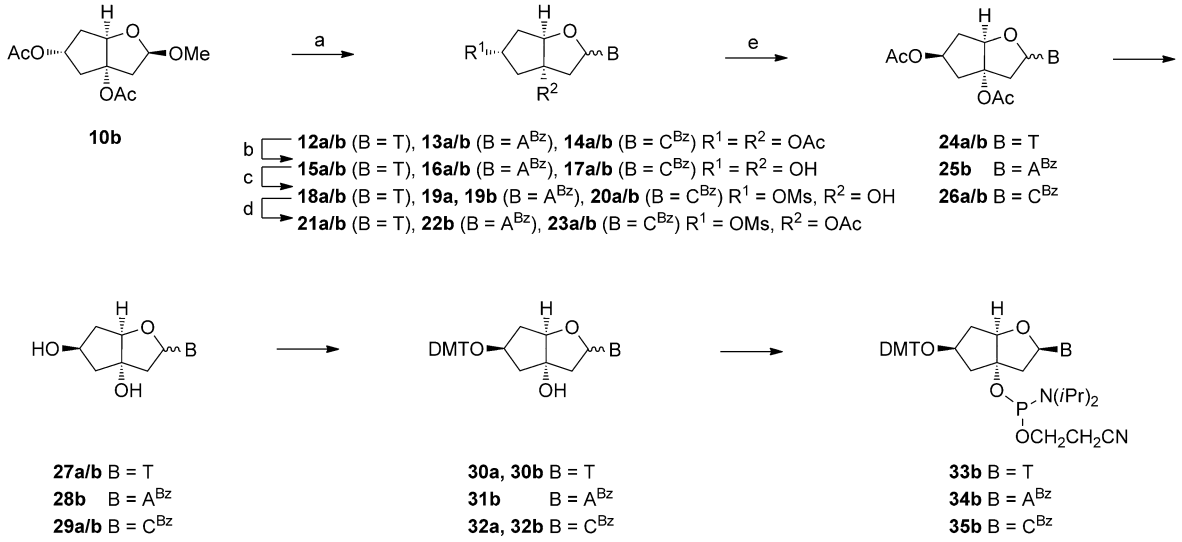
	ν0	ν1	ν2 [°]	ν3	ν4	ω	Furanose pucker	Energy [kcal mol^{-1}]
a)	−41	35	−17	−6	30	82	C(1′)-exo	29.22
b)	−36	15	8	−30	41	170	C(4′)-exo	30.26

Synthesis of the isobicyclo sugar: The bicyclic silyl enol ethers **1a** (C(1′)-α), **1b** (C(1′)-β) used in the synthesis of the tricyclo sugar[10] were considered to be the starting materials of choice for the synthesis of the isobicyclo-DNA scaffold. Hydroboration of **1a** or **1b** yielded the compounds **2a**, **2b** and **3a**, **3b** (Scheme 1) in diastereomeric ratios of 4:1 in the α-series and 10:1 in the β-series.[11] The lowest abundant isomer **3b** could not be isolated as a pure compound and was not used in further transformations. All other isomers **2a**, **2b**, and **3a** were taken forward in the following nucleoside synthesis. Acetylation of **2a**, **2b**, and **3a** was performed under standard conditions by using Ac_2O/ 4-dimethylaminopyridine (DMAP) in pyridine at room temperature yielding **4a**, **4b**, and **5a**. Desilylation with HF/Et_3N (37% HF) in THF yielded compounds **6a**, **6b**, and **7a**. To defunctionalize C(5′), the alcohols **6a**, **6b**, and **7a** were converted into the thiocarbamates **8a**, **8b**, and **9a** in good yields. The subsequent Barton–McCombie reduction was performed with azobisisobutyronitrile (AIBN) as the radical initiator and tris(trimethylsilyl)silane (TTMSS) as the H-donor to yield **10a**, **10b**, and **11a**, again in good yields (Scheme 1).

Synthesis of the phosphoramidite building blocks: The nucleosidation of building block **10b** (for nucleosidation of **10a** see the Supporting Information) with the bases T, A^{Bz}, and C^{Bz} was performed under Vorbrüggen conditions leading to almost 1:1 mixtures of anomers **12a/b**, **13a/b**, and **14a/b** in 75–85% yields that were not separable by standard chromatography techniques (Scheme 2). Subsequent deacetylation under mild conditions yielded **15a/b**, **16a/b**, and **17a/b**. We decided to invert the configuration at C(6′) at this stage. Therefore, the free nucleosides **15a/b**, **16a/b**, and **17a/b** were converted into the corresponding C(6′) mesylates **21a/b**, **22a**, **22b**, and **23a/b**. At this point it was possible to separate the α- and β-anomers of **22** by using column chromatography, whereas **21** and **23** had unfortunately still to be taken forward as mixtures of anomers. Subsequent acetylation of O(3′) followed by displacement of the 6′-mesyl function by CsOAc in DMSO yielded **24a/b**, **25b**, and **26a/b**. Saponification (→**27a/b**, **28b**, **29a/b**) and subsequent tritylation gave compounds **30a**, **30b**, **31b**, **32a**, and **32b**. As already observed in other cases, the tritylated nucleosides **30** and **32** were now separable by chromatography and isolated as pure isomers. The relative configuration at the centers C(1′) in **30b**, **31b**, and **32b** was ascertained by using ^{1}H NMR-ROESY (the Supporting Information). The treatment of the β-nucleosides **30b**, **31b**, and **32b** with 2′-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (CEP-Cl) under standard conditions finally yielded the desired phosphoramidites **33b**, **34b**, and **35b**.



Scheme 1. a) 1) BH_3·THF, THF, −78°C→RT, 22 h, 2) Oxone in sat. $NaHCO_3$, RT, 2 h, 80%; b) Ac_2O, DMAP, pyridine, RT, 95%; c) HF/Et_3N, THF, RT, 85%; d) TCDI, THF, heat at reflux, 82%; e) AIBN, TTMSS, toluene, heat at reflux, 82%.



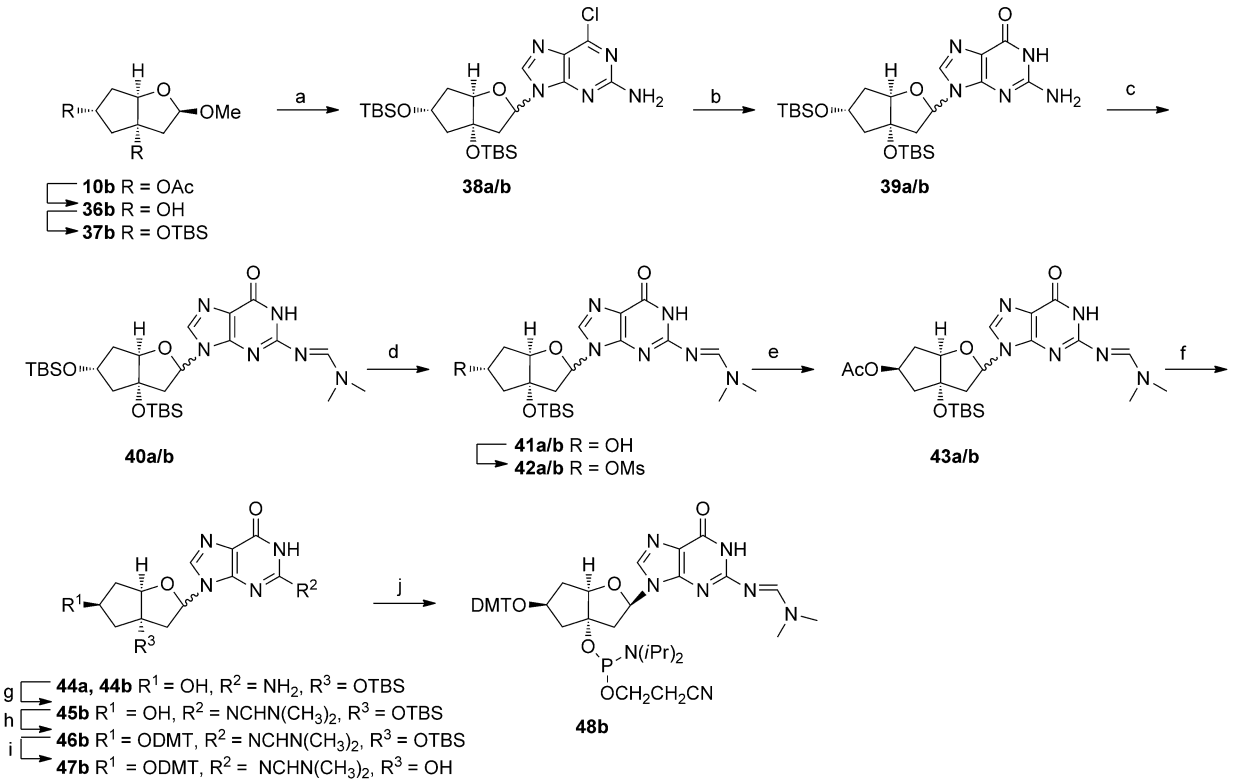
Scheme 2. a) Thymine (2 equiv), BSA (5 equiv), TMSOTf (3 equiv), MeCN, 0 °C to RT, 85 %/benzoyl-adenine (2 equiv), BSA (4 equiv), TMSOTf (0.3 equiv), MeCN, 85 °C, 75 %/benzoyl-cytosine (2 equiv), BSA (5 equiv), TMSOTf (3 equiv), 0 °C to RT, 75 %; b) 0.2 M NaOH in 5:4:1 THF/MeOH H_2O, 0 °C, 75–90 %; c) MsCl (1 equiv), pyridine, 0 °C→RT, 60–70 %; d) Ac_2O, DMAP, pyridine, RT, 80–91 %; e) CsOAc, DMSO, 90 °C, 81–93 %; f) 0.2 M NaOH in 5:4:1 THF/MeOH, H_2O, 0 °C, 60-84 %; g) DMT-Cl, pyridine, RT, 48 % (**30a**), 40 % (**30b**), 90 % (**31b**), 62 % (**32a**), 30 % (**32b**); h) Hünig's base, CEP-Cl, MeCN, RT, 72–87 %.

For the synthesis of the isobicyclo guanosine, a slightly different protecting-group strategy had to be employed due to the notoriously high degree of insolubility imparted by the guanine base. Thus the O(3′) and O(5′) acetyl groups in **10b** were replaced by TBS groups, leading to **37b**, which was subjected to nucleosidation with 2-amino-6-chloropurine under classical Vorbrüggen conditions giving nucleosides **38a/b** in an anomeric ratio of 1:1 in acceptable yields (Scheme 3). Treatment of **38a/b** with sodium hydride and 3-hydroxypropionitrile exchanged the C(6′)-chloro substituent by oxygen (→**39a/b**). Further protection of the exo amino function of the guanine base yielded amidine **40a/b**. To invert the configuration at C(6′), selective desilylation at this position was desirable. This goal was achieved by the use of HF/Et_3N (→**41a/b**). Although desilylation with fluoride ions is known to be unspecific,[12] we observed high selectivity towards deprotection of the sterically less-hindered O(6′). The free hydroxyl group in **41a/b** was then mesylated (→**42a/b**), inverted (→**43a/b**), and the acetyl function removed with concomitant removal of the amidine protecting group, leading to the chromatographically separable anomers **44a** and **44b**. Re-protection of the N(2) amino function in the β-anomer (→**45b**) and dimethoxytrityl (DMT) protection by using standard conditions yielded **46b** in good yields. Subsequent deprotection with TBAF (→**47b**) followed by phosphitylation finally concluded the synthesis of the isobicyclo-G building block **48b**. Again, the relative configuration at C(1′) was confirmed by using ^{1}H NMR-ROESY on compound **47b** (the Supporting Information).

Oligonucleotide synthesis: Modified oligonucleotides were synthesized by using standard phosphoramidite chemistry on a DNA synthesizer on the 1.3 μmol scale. Either natural deoxynucleoside-derived controlled pore glass (CPG) solid support was used or, for the fully modified strands, a universal solid support was used. For the incorporation of the isobicyclo-nucleoside building blocks the coupling time was increased to 9 min. All other steps remained unchanged. After the chain-assembly the oligonucleotides were cleaved from the solid support and deprotected by aminolysis at 70 °C overnight, then purified by using reverse phase or ion exchange HPLC. The expected masses were confirmed by ESI^- mass spectrometry.

Biophysical properties of oligonucleotides containing isobicyclo-thymidines: Oligonucleotides **S1**–**S4** containing single and consecutive isobicyclo-thymidine modifications were synthesized and their pairing properties with complementary DNA and RNA investigated by UV melting curves at 260 nm. (Table 2 and Figures S1 and S2, the Supporting Information). Analysis of the T_m data revealed a stabilization of 1 °C per modification with complementary DNA. This occurs in all four duplexes independently of the position or the number of modifications in the strand. In the case of RNA, the isobc-T modification seems to be stabilizing for single incorporations but destabilizing for consecutive incorporations. As expected, the CD-spectra of **S1**–**S4** with complementary DNA and RNA do not deviate significantly in shape from that of **S5**, indicating minor structural perturbations imparted by the isobc-T residues (Figures S5 and S6, the Supporting Information).

Synthesis and properties of fully modified oligonucleotides containing isobc-T and isobc-A: As for the synthesis with



Scheme 3. a) 2-Amino-6-chloropurine, BSA, TMSOTf, MeCN, 60 °C, 5 h, 64 %; b) NaH, 3-hydroxypropionitrile, THF, 0 °C to RT, 3 h; c) *N,N*-DMF-dimethylacetal, DMF, 55 °C, 3 h, 71 % over two steps; d) HF/Et_3N, THF, RT, 55 %; e) MsCl, pyridine, RT, 92 %; c) CsOAc, DMSO, 85 °C, 16 h, 85 %; f) 1 M KOH in 5:3 MeOH/H_2O, 60 °C, 42 % (**53b**), 20 % (**53a**); g) *N,N*-DMF-dimethylacetal, DMF, 55 °C, 3 h, 64 %; h) DMT-Cl, pyridine, RT, 16 h, 94 %; i) TBAF, THF, RT, 5 h, 70 %; j) Hünig's base, CEP-Cl, MeCN, RT, 1 h, 88 %.

Table 2. T_m values derived from UV-melting-temperature experiments at 260 nm. Conditions: NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0, c = 1.2 μM strand concentration.

	Sequence[a]	Complementary DNA	Complementary RNA
S1	5′-d(GGA**t**GTTCTCGA)-3′	48.0	49.0
S2	5′-d(GGATG**tt**CTCGA)-3′	49.0	47.5
S3	5′-d(GGATGTTC**t**CGA)-3′	48.0	50.0
S4	5′-d(GGA**t**G**tt**C**t**CGA)-3′	51.0	49.5
S5	5′-d(GGATGTTCTCGA)-3′	47.0	49.0

[a] Upper case letters: natural nucleotides; lower case letters: isobc-nucleotides.

Table 3. T_m values derived from UV-melting-temperature experiments at 260 nm. Conditions: NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0, c = 1.2 μM strand concentration.

	Sequence	T_m vs. DNA antiparallel	T_m vs. RNA antiparallel	T_m vs. DNA parallel
S6	6′-d(ataatttaataa)-3′	25.1	<10	21.9
S7	6′-d(ttattaaattat)-3	25.9	<10	28.1
S8	5′-d(ATAATTTAA-TAA)-3′	23.2	n.m.[a]	19.8
S9	5′-d(TTATTAAAT-TAT)-3′	23.2	11.2	n.m.[a]

[a] Not measured.

single isobc-T incorporations, a non-self-complementary sequence motif was chosen. **S6** and **S7** (Table 3) are complementary and can therefore give insight into the self-pairing of isobicyclo-DNA. The melting curves of **S6** and **S7** with complementary DNA and RNA partially confirm the results obtained with the thymidine-modified oligonucleotides. All duplex melting curves reflect cooperative and reversible melting (Figure 3). The isobc modifications stabilize duplexes with DNA, but not as predicted from single incorporations with 1 °C per modification. The overall stabilization is +2 (**S6**) and +3 °C (**S7**) per duplex, respectively (Table 3).

In the case of RNA as a complement, duplex destabilization with a T_m value below 10 °C is observed. This was predicted from the earlier experiments in which we have shown that single incorporations stabilize the duplex and consecutive modifications destabilize it. Thermal denaturation experiments were also performed for the fully modified duplex **S6**/**S7**. The T_m in this case is remarkably high with a value of 41.9 °C, which is almost twice as high as that for the natural DNA duplex. Furthermore, to check the specificity for the antiparallel orientation in the duplex, melting temperatures for the parallel duplexes with DNA were measured (Figure S3, the Supporting Information). Both **S6** and **S7** showed T_m values that are comparable to the ones measured for the antiparallel duplexes (−3 for **S6** and +2 °C for **S7**, Table 3). The same is true for the natural duplex, which

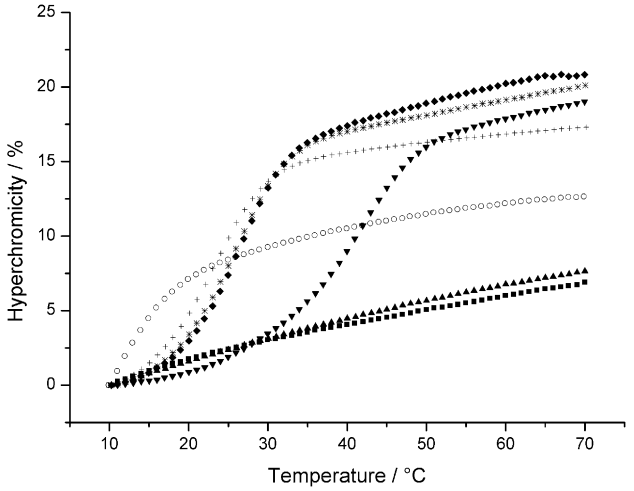


Figure 3. UV-melting curves (260 nm) of the AT-duplexes. Conditions: NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0, $c = 1.2$ μM strand concentration. ■ **S6**:RNA, ▲ **S7**:RNA, ▼ **S6**:**S7**, ○ DNA:RNA, + DNA:DNA, * **S6**:DNA, ◆ **S7**:DNA.

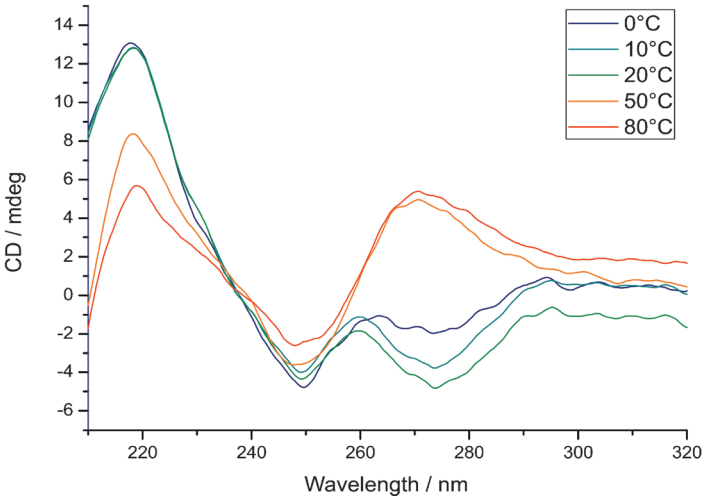


Figure 4. CD spectrum of the duplex **S6**:**S7** at different temperatures. Conditions: 10 mM NaH_2PO_4, 150 mM NaCl, pH 7, $c = 1.2$ μM strand concentration.

was slightly destabilized by −3 °C compared with the antiparallel duplex.

It thus appears that, much like natural DNA,[13] also isobc-AT sequences have the potential to form parallel reverse Watson–Crick duplexes. It also becomes clear that isobc-AT-oligonucleotides do not have a considerable stabilizing effect on duplexes with DNA but have a significant destabilizing effect on duplexes with RNA as complement. In contrary, the fully unnatural duplex is significantly more stable compared with pure DNA or hybrid (isobicyclo-DNA/DNA) duplexes. This is not uncommon for sugar-modified oligonucleotide analogues, such as hexitol nucleic acids (HNA)[14] or tricycloDNA, and reflects most likely a more perfect geometric match of the repeating backbone units in homo-backbone duplexes as compared with hetero-backbone duplexes.

To gain an insight into the structural properties of these AT duplexes, CD spectra were measured. The CD spectra for the natural duplex **S8**:**S9** as well as for the hybrid duplex **S6**:**S9** (Figure S7 and S8, the Supporting Information) showed a B-like conformation and, in the case of the fully modified duplex **S6**:**S7**, the CD spectrum showed a signature that is significantly different from A-, B-, or Z-DNA (Figure 4). The CD traces of the single strands (at high temperature) are similar to the unmodified ones and the hybrid duplexes. In the paired state, however, the CD shows an intense negative band between 290 and 260 nm, which mirrors the band of the unmodified duplex in this part of the spectrum (Figure 4). For the parallel duplexes in all three cases a B-type conformation is observed (Figure S9, the Supporting Information).

Reported CD spectra of natural, parallel AT-duplexes were found to be sequence specific: A parallel duplex with alternating AT base pairs shows a negative band at 290 nm[15] whereas a $d(A)_{10}$·$d(T)_{10}$ hairpin shows a spectrum close to the one obtained in our case.[13] However, the negative band between 260 and 290 nm in the **S6**:**S7** duplex is most likely not the result of a partial parallel isobc-AT duplex structure because achieving a melting temperature of 41.9 °C with a minimum of 6 mismatches in a dodecamer seems very unlikely. Hence we hypothesize that there exists a different, yet undetermined duplex conformation for pure isobc-AT duplexes.

Thermodynamics of duplex formation: By using standard curve-fitting procedures to experimental melting curves[16] we calculated the thermodynamic data of duplex formation of the fully modified duplex **S6**:**S7** as well as the hybrid duplex **S6**:**S9** and compared the data to that of the natural duplex **S8**:**S9** (Table 4). From there it appears that the natural duplex **S8**:**S9** is enthalpically favored over the fully modified duplex. The free energy ΔG at 25 °C is, as expected from the T_m data, in favor of the fully modified duplex **S6**:**S7**. Thus, the fully modified duplex is entropically favored, which is in agreement with its reduced conformational flexibility. The hybrid duplex shows a somewhat unusually high enthalpy ΔH, which is counterbalanced by the entropy term, indicating enthalpy/entropy compensation to be operative.

Table 4. Thermodynamic data of duplex formation from curve fitting to the experimental melting curve. Conditions: NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0, $c = 1.2$ μM strand concentration.

Duplex	ΔH [kcal mol^{-1}]	ΔS [cal mol^{-1} K^{-1}]	ΔG at 25 °C [kcal mol^{-1}]
S6:**S7**	−73.0	−202.8	−12.6
S6:**S9**	−84.7	−254.0	−8.9
S8:**S9**	−76.6	−228.9	−8.3

We also investigated the influence of salt concentration on duplex stability by varying the NaCl concentration of the buffer in a range from 0.05–1.5 M. The uptake of sodium ions upon duplex formation helps to stabilize the duplex by reducing the repulsion between the phosphate groups. Since the negative charge density is higher in a duplex than in single strands, counterions are screened upon duplex formation. As expected, an increase of the T_m values with increasing NaCl concentration was observed (Figure 5). The duplexes **S8**:**S9** and **S6**:**S9** both doubled their T_m values within the 30-fold increase of salt concentration. The fully modified duplex **S6**:**S7** increased its T_m by 35 °C, which is slightly more than twice the starting value at 0.05 M NaCl. We further determined the relative counterion uptake Δn (Table 5) according to classical polyelectrolyte theory,[17] by using the transition enthalpy (ΔH) data from Table 4.

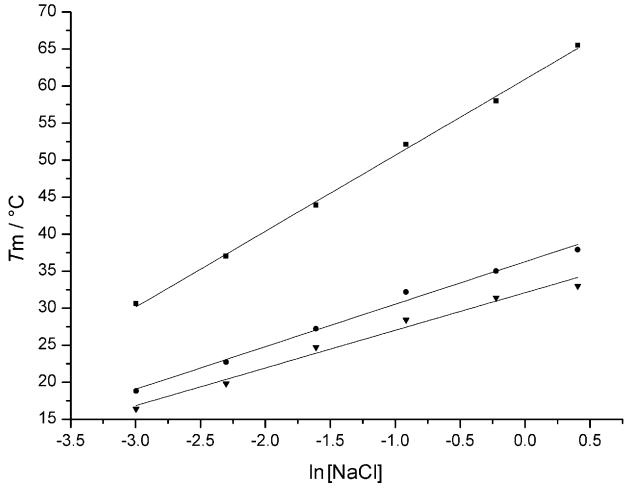


Figure 5. Plot of the T_m versus ln[NaCl]; 10 mM NaH_2PO_4, 50 mM–1.5 M NaCl, pH = 7.0, c = 1.2 μM strand concentration. ▼ DNA:DNA, ■ **S6**:**S7**, ● **S6**:DNA.

Table 5. Calculated counterion uptake. Conditions: NaH_2PO_4 (10 mM), NaCl (200 mM), pH 7.

	S8:**S9**	**S6**:**S9**	**S6**:**S7**
Δn	1.1	1.6	2.1
$\delta T_m/\delta(\ln[NaCl])$	5.3	7.2	11.8

$$\Delta n = -2\Delta H/RT_m^2 \times \delta T_m/\delta(\ln[NaCl])$$

The fully modified isobc system **S6**:**S7** screens about twice as much counterions upon duplex formation than the corresponding unmodified duplex and about a third more than the hybrid duplex. This is in agreement with a higher degree of spatial compression of the phosphate groups upon transition from single strands to the duplex. Whether this is due to a more compressed duplex structure or a more relaxed single strand structure remains unknown at this point.

Table 6. T_m data for fully modified isobc-oligonucleotides containing all four bases. Conditions: NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0, strand concentration for all dodecamers: c = 1.2 μM. Total strand concentration for the natural hexamers: 2.4 μM and for **S11**: 4.8 μM.

	Sequence	DNA antiparallel	RNA antiparallel	isobc duplex
S10	6′-d(cctactagagct)-3′	51.0 (+4)	30.0 (−11.5)	–[a]
S11	6′-d(cctagg)-3′[b]	< 10	34.5	40.7

[a] Not detected. [b] Self-complementary duplex: T_m data refer to the respective backbone type.

Synthesis and properties of oligonucleotides containing all four isobc-nucleosides: Two fully modified sequences containing isobc-A, -T, -G, and -C residues were synthesized: a dodecamer (**S10**) for the investigation of the pairing properties with complementary DNA and RNA and a self-complementary hexamer (**S11**) to get further insight into the structure of fully modified duplexes (Table 6). Thermal melting of **S10** with complementary DNA shows, as all modified DNA sequences, a cooperative and reversible melting behavior (Figure 6). Again, a stabilization of the duplex was observed but, even including the more stable CG base pairs, the difference in melting temperature is small (+4 °C/duplex) and comparable to the one observed in the AT-series (Table 6). In addition, the melting behavior of **S10** with a parallel DNA complement was investigated. No cooperative transition was observed revealing that isobc-DNA, as DNA, strongly prefers antiparallel Watson–Crick pairing in sequences containing all four base-pairs. For the hexamer **S11** a very stable duplex was found with for GC base-pairs usually low hyperchromicities at 260 nm (Figure 6). This again confirms that fully modified isobc-duplexes are very stable, irrespective of the base composition.

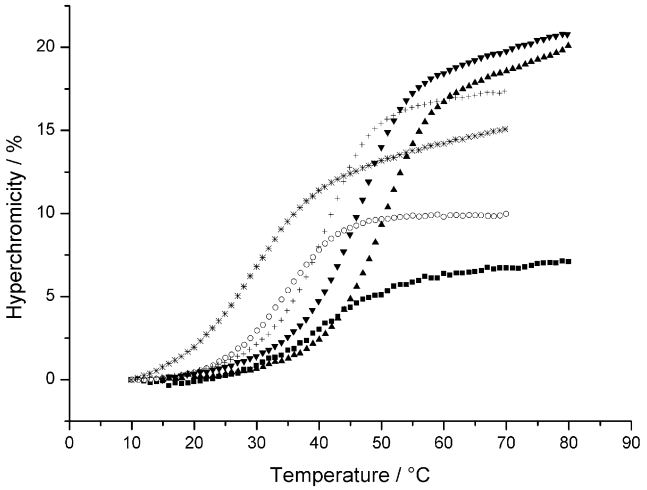


Figure 6. UV melting curves (260 nm) for the modified sequence S7 with DNA (left) and RNA (right). Conditions: NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0, c = 1.2 μM strand concentration. ■ **S11**, ○ RNA hexamer, ▲ **S10**:DNA, ▼ DNA:DNA, * **S10**:RNA, + DNA:RNA.

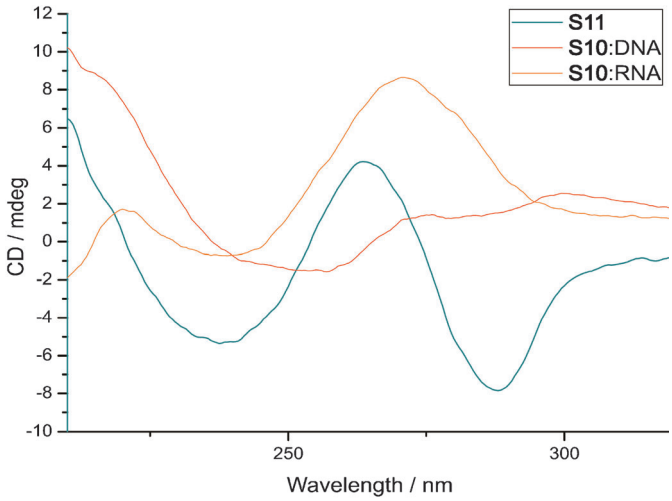


Figure 7. CD spectra of **S11**:**S11**, **S10**:DNA, and **S10**:RNA. Conditions: NaH_2PO_4 (10 mM), NaCl (150 mm), pH 7, $c = 1.2$ μM strand concentration.

The CD spectra of the hybrid duplex (**S10**:**DNA**, Figure 7) revealed a completely different curve shape compared with the unmodified duplex (Figure S13, the Supporting Information), indicating substantial deviation from classical A- or B-type structures. In contrast, in the case of the RNA complement, a typical A-form is observed with comparable spectra for the unmodified and the modified duplex. The CD signature of the **S11**:**S11** duplex, however, shows an intense negative cotton effect at 290 nm and a positive cotton effect at 260 nm (Figure 7). It thus deviates significantly from that of the self-complementary RNA hexamer (A-type conformation, data not shown) and also to some extent from the fully modified isobc-AT duplex **S6**:**S7** (Figure 3). It thus shows some similarities to that of Z-DNA[18] which, given the high GC content, could be a possible structure for **S11**. However, more high resolution structural work is needed to get a reliable picture of the 3D structure.

Mismatch discrimination: To test the base-pairing selectivity of the isobc-DNA system, the melting temperatures of different mismatched duplexes complementary to **S10** were measured. A thymidine residue in the complementary DNA sequence was replaced by an A, C or G, and the corresponding T_m values were determined (Table 7).

In all cases thermal destabilizations in the mismatched duplexes were observed. The A–C mismatch was the strongest discriminating followed by the A–A and the A–G mismatches. The decrease is generally of about the same extent as in DNA, indicating an equal selectivity of the two Watson–Crick pairing systems.

Serum stability: The serum stability of **S10** was measured in heat deactivated fetal bovine serum (FBS) in which the predominant nucleases are the 3′-exophosphodiesterases.[19] As a control the stability of the corresponding natural DNA complement was also tested. After incubation at 37 °C in medium containing 10 % FBS, aliquots were taken in regular intervals and after ethanol precipitation analyzed by PAGE. The gel was visualized with stains-all solution (Figure 8).

Table 7. Mismatch discrimination. The ΔT_m values relative to the matched duplex are given in parentheses. Conditions: NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0, $c = 1.2$ μM strand concentration.

Duplex	X = A	X = G	X = C	X = T (match)
6′-d(cctactagagct)-3′ (**S10**)	37	43	33	51
5′-d(ACG**X**CTTGTAGG)-3′	(−14)	(−8)	(−18)	
5′-d(CCTACAAGAGCT)-3′	34	39	30	47
5′-d(ACG**X**CTTGTAGG)-3′	(−13)	(−8)	(−17)	

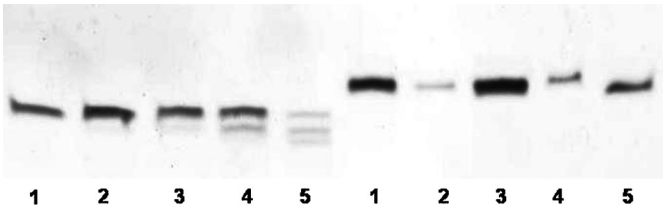


Figure 8. PAGE analysis of the serum stability of DNA (left) and isobicyclo-DNA (right). 1: 0 h, 2: 0.5 h, 3: 2 h, 4: 6.5 h, 5: 21 h.

The resulting gel shows that **S10** is stable over a period of at least 21 h without signs of degradation. In contrast, the DNA complement is substantially hydrolyzed after this period of time, indicating a significantly higher degree of biostability of isobc-DNA.

RNaseH activity: Due to its ability to cleave DNA/RNA heteroduplexes, it is desirable for chemically modified oligonucleotides designed to ablate an RNA of interest, to be able to activate the endogenous enzyme RNaseH. This is, however, difficult to achieve since only a small number of DNA analogues are known to be compatible with RNaseH activity.[20] To investigate this, the RNA complement of **S10** was 5′-labeled with [γ-^{32}P]-ATP and T4 polynucleotide kinase (T4 PNK) and annealed to **S10**. The natural RNA/RNA and the hybrid DNA/RNA duplexes served as negative and positive control. As expected, the RNA/RNA duplex did not show any RNaseH-mediated degradation, whereas the DNA/RNA hybrid turned out to be a good substrate. The **S10**/RNA duplex showed no signs of RNA cleavage after treatment with RNaseH, clearly showing that isobc-DNA does not elicit RNaseH activity (Figure 9).

Discussion

We synthesized all four isobicyclo nucleosides and incorporated them successfully into DNA. Single isobicyclo-T incor-

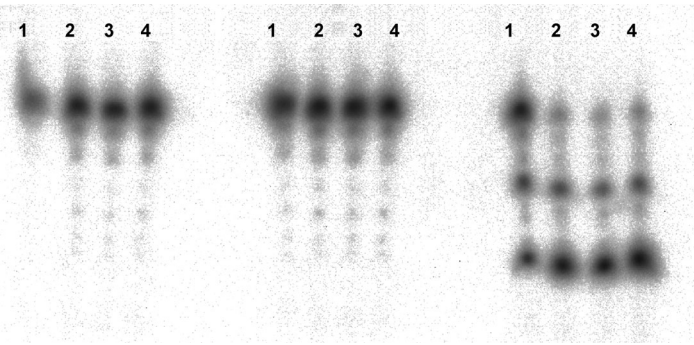


Figure 9. PAGE analysis of the RNaseH acitvity of the duplexes: RNA:32P-RNA (C12:32P-C9), isobicyclo-DNA:32P-RNA (S7:32P-C9), DNA:32P-RNA (C8:32P-C9). 1: 5 min, 2: 45 min, 3: 120 min, 4: 300 min.

porations in natural DNA showed stable duplexes with complementary DNA and RNA. Consecutive incorporations destabilize the RNA hybrid. The same result was obtained for the fully modified AT duplexes where a stabilized duplex with DNA and a significantly destabilized duplex with complementary RNA was found. An interesting finding in the AT series was the very stable self-duplex with a T_m of 41.9 °C. The CD spectrum of this fully modified isobicyclo duplex (Figure 3) revealed a structure different from A- or B-form duplex.

It is known that the duplex conformation as revealed by CD-spectroscopy is dependent on the base sequence and on the salt concentration of the buffer. Increasing the salt concentration typically leads to a decrease of the long wavelength band between 290 and 260 nm corresponding to variants of B-DNA structures differing in the number of base pairs per helix turn.[21] Vorlíčková and Kypr investigated the conformational changes of poly(A–T) sequences at different CsF concentrations and found that the long wavelength part of the CD spectra falls off, inverts and becomes negative at high salt concentrations.[22] They called that structure X-DNA and suggested that this X-DNA is a right handed antiparallel double helix with Hoogsteen base-pairs that were found by Abrescia et al. some years ago.[21,23] In this Hoogsteen DNA[23] the overall helical parameters are similar to that of B-DNA with the adenines in a *syn*-conformation. Therefore one hypothesis is that the fully modified isobicyclo-AT duplex **S6**:**S7** forms such an X-DNA structure.

Further evidence for such a X-DNA structure comes from CD-experiments with **S6**:**S7** as well as the hybrid DNA duplexes of **S6** at different salt concentrations. While no major changes occur in the case of the unmodified duplex **S8**:**S9** and the hybrid duplex **S6**:**S9** (Figures S7 and S8, the Supporting Information), the CD of the fully modified duplex **S6**:**S7** shows a high dependence on the sodium chloride concentration (Figure S10, the Supporting Information). The negative band at 275 nm increases in intensity upon increasing the NaCl concentration. This could indeed be an indication of a partial structural transition from the more compact X- towards a B-type conformation when changing from high to low electrolyte concentration, which supports the preference of an X-type conformation of isobc-DNA duplexes at physiological salt concentration.

The fully modified isobc-AT oligonucleotides form also stable parallel duplexes. Parallel duplex formation in poly-(dAT) sequences is well known and investigated.[15,24] They require reversed Watson–Crick base-pairs and their thermal stability is highly sequence dependent. This explains the variability in the T_m values for the two duplexes **S6** and **S7** with parallel complements. However, no melting transition was found for the fully modified **S10**, containing all four bases, with a parallel DNA complement, revealing that isobc-DNA prefers strictly antiparallel pairing in sequences containing also G–C base-pairs. These preferences are identical to that of natural DNA where parallel reversed Watson Crick is also restricted to AT sequences.

Isobc-DNA was found to be unable to activate RNaseH in isobc-DNA/RNA hybrids. RNaseH is known to preferably bind to A-form rather than B-form duplexes. Hence, as a consequence the enzyme binds RNA/RNA duplexes and DNA/RNA hybrids much tighter than a DNA/DNA duplex.[25] The RNA in dsRNA duplexes is not cleaved since the 2′-OH groups affect the hydration pattern in the minor groove.[26] Since the CD spectrum of the isobicyclo-DNA/RNA duplex shows an A-like structure and is very similar to that of the DNA/RNA duplex, an activation of RNaseH could have been expected. However, it has also been shown that the enzyme is very sensitive to structural changes within the minor groove. This is the reason why all 2′ modified oligonucleotides (e.g., 2′-OMe[27]) as well as the sterically constrained (e.g., LNA[28]; tcDNA[9a]) do not activate RNaseH.[26] From this point of view it is not surprising that isobc-DNA is unable to do so.

Conclusion

We have achieved the synthesis of all four isobc-DNA building blocks starting from the silyl enol ethers **1a** or **1b**. These phosphoramidites were successfully incorporated into natural deoxyoligonucleotides or were used for the synthesis of fully modified isobc-DNA by using standard solid-phase DNA synthesis. Oligodeoxynucleotides containing single incorporations as well as fully modified isobc-DNA show stable duplexes with DNA complements with a slightly enhanced stability (ca., 0.3 °C per mod). RNA is discriminated as a complement in all the investigated duplexes by approximately 1 °C per modification. All hybrid duplexes show B-conformation with DNA and A-conformation with RNA as judged from CD-experiments. Base mismatches are discriminated in the same way as in natural DNA. Fully modified isobicyclo oligonucleotides form very stable duplexes within their own backbone series that are of higher thermodynamic stability than the corresponding natural duplexes. Fully modified duplexes containing only AT base-pairs show a CD-signature that is significantly different from that of canonical DNA conformations. Based on the available biophysi-

cal data we propose a double helical structure with Hoogsteen base-pairs. Furthermore, isobc-DNA is stable in bovine serum and does not activate RNaseH.

Thus it appears that the phosphate shift from C(5′) to C(6′) in the bicyclic system is well tolerated in duplexes with DNA and less well in duplexes with RNA. Pure isobc-DNA duplexes, however, display considerable structural differences of a yet unknown type. The feature of isobc-DNA to stablize DNA duplexes and discriminate RNA is interesting and could find possible applications in diagnostics, as for example, in primers for the polymerase chain reaction (PCR).

Experimental Section

General: All reactions were performed under Ar and in dried glassware. Anhydrous solvents for reactions were obtained by filtration through activated alumina or by storage over molecular sieves (4 Å). Column chromatography (CC) was performed on silica gel with an average particle size of 40 μm. All solvents for column chromatography were of technical grade and distilled prior to use. Thin-layer chromatography was performed on silica gel plates. Visualization was achieved either under UV light or by dipping in staining solution (CerIV-sulfate (10.5 g), phosphormolybdenic acid (21 g), conc. H_2SO_4 (60 mL), H_2O (900 mL) or *p*-anisaldehyde (10 mL), conc. H_2SO_4 (10 mL), glacial acetic acid (2 mL), ethanol (180 mL)) followed by heating with a heat gun. NMR spectra were recorded at 300 or 400 MHz field width (^{1}H) in either $CDCl_3$ or CD_3OD. Chemical shifts (δ) are reported in ppm relative to residual undeuterated solvent $CHCl_3$: 7.26 ppm (^{1}H) and 77.16 ppm (^{13}C); CHD_2OD: 3.31 ppm (^{1}H) and 49.0 ppm (^{13}C), J in Hz. Signal assignments are based on DEPT and on ^{1}H-^{1}H and ^{1}H-^{13}C correlation experiments (COSY/HMSC). High-resolution mass spectra were recorded on an Applied Biosystems Sciex QSTAR Pulsar.

*(3R,5S,8R,7S)-8-[(tert-Butyldimethylsilyl)oxy]-3-methoxyhexahydro-2H-cyclopenta[b]furan-3a,6-diyl diacetate (**4b**)*: A solution of **2b** (4.3 g, 14.2 mmol) in pyridine (35 mL) was cooled to 0 °C and DMAP (171 mg, 1.4 mmol) and Ac_2O (4.0 mL, 42.7 mmol) were added. The mixture was stirred for 16 h at RT and, after cooling to 0 °C, was carefully quenched and washed with sat. $NaHCO_3$. The aqueous layers were extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and evaporated. The product was separated by flash chromatography (FC) (ethyl acetate/hexane, 1:3) yielded **4b** (4.9 g, 12.6 mmol, 90 %) as a white solid. R_f = 0.9 (hexane/ethyl acetate 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.24 (*m*, 1H), 5.06 (*d*, 1H, *J* = 5.3), 4.36 (*d*, 1H, *J* = 6.4), 4.20 (dd, 1H, J = 8.6, 6.4), 3.36 (s, 3H), 2.86 (dd, 1H, J = 13.9, 7.6), 2.55 (d, 1H, J = 13.8), 2.36 (ddd, 1H, *J* = 13.8, 5.7, 1.8), 2.02 (*s*, 6H), 1.76 (ddd, 1H, *J* = 13.8, 9.7, 1.6), 0.90 (s, 9H), 0.11 (s, 3H), 0.08 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.55, 170.17, 105.42, 89.29, 85.33, 77.97, 75.80, 54.62, 47.69, 40.15, 25.89, 21.62, 21.16, −4.60, −4.71 ppm; HRMS (NSI$^+$, MeCN): *m/z* calcd for $C_{18}H_{32}O_7Si$: 411.1813; found: 411.1810 [$M+Na^+$].

*(3R,5S,8R,7S)-8-Hydroxy-3-methoxyhexahydro-2H-cyclopenta[b]furan-3a,6-diyl diacetate (**6b**)*: A solution of **4b** (10.4 g, 26.8 mmol) in THF (80 mL) was cooled to 0 °C. HF/Et_3N (37 % HF, 23 mL, 53.6 mmol) was added and the solution was allowed to warm to RT. The reaction mixture was stirred for 60 h at RT and subsequently quenched with silica. The solvent was evaporated and the crude mixture purified by using FC (ethyl acetate/hexane 1:1). Compound **6b** (5.9 g, 21.5 mmol, 80 %) was obtained as a colorless oil. R_f = 0.4 (ethyl acetate/hexane 1:1), ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.10–5.13 (m, 2H), 4.53 (*d*, *J* = 6.4, 1H), 4.10 (m, 1H), 3.39 (*s*, 3H); 2.78–2.80 (m, 2H), 2.48 (m, 2H), 2.03 (s, 3H); 2.00 (s, 3H), 1.95–1.86 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.06, 170.09, 106.93, 89.23, 85.93, 78.71, 74.79, 55.79, 47.14, 39.93, 21.51, 21.14 ppm; HRMS (NSI$^+$, MeCN/H_2O, 1 % HF): *m/z* calcd for $C_{12}H_{18}O_7$: 279.09447; found: 297.09538 [$M+Na^+$].

*(3R,5S,8R,7S)-8-[(1H-Imidazole-1-carbonthioyl)oxy]-3-methyoxyhexahydro-2H-cyclopenta[b]furan-3a,6-diyl diacetate (**8b**)*: TCDI (4.2 g, 23.3 mmol) was added to a solution of **6b** (3.2 g, 11.7 mmol) in THF (60 mL). The yellow solution was heated at reflux at 75 °C for 3 h. The THF was then evaporated and the crude mixture was purified by FC (ethyl acetate/hexane 1:1) to yield **8b** (4.2 g, 10.9 mmol, 93 %) as a yellow foam. R_f = 0.40 (ethyl acetate/hexane 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.39 (m, 1H) 7.67 (m, 1H), 7.04 (m, 1H) 5.92–5.85 (m, 1H) 5.70–5.67 (m, 1H), 5.08, 5.06 (2m, 2H) 3.34 (s, 3H), 2.94 (dd, 1H, *J* = 13.9, 8.1), 2.58 (d, 1H, *J* = 14.0), 2.49 (ddd, 1H, *J* = 14.1, 5.7, 1.8), 2.14–2.10 (m, 1H) 2.08, 2.05 ppm (2s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 183.54, 170.40, 170.29, 137.40, 131.24, 118.26, 106.14, 89.25, 84.34, 82.33, 73.85, 55.33, 47.82, 39.38, 21.55, 21.15 ppm; HRMS (NSI$^+$, EtOAc): *m/z* calcd for $C_{16}H_{20}N_2O_7S$: 385.1064; found: 385.1051 [$M+H^+$].

*(3R,5S,7S)-3-Methoxyhexahydro-2H-cyclopenta[b]furan-3a,6-diyl diacetate (**10b**)*: The sugar **8b** (4.2 g, 10.9 mmol), AIBN (270 mg, 2.2 mmol), and TTMSS (6.7 mL, 22 mmol) were dissolved in toluene (87 mL) and stirred for 2 h at 85 °C. The toluene was then evaporated and the crude mixture purified by FC (ethyl acetate/hexane 1:3). Compound **10b** (2.6 g, 10.1 mmol, 92 %) was obtained as a colorless oil. R_f = 0.6 (ethyl acetate/hexane 1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.39–5.31 (*p*, 1H, *J* = 6.3), 5.04 (dd, 1H, *J* = 5.6, 0.7), 4.71 (dd, 1H, *J* = 7.0, 3.5) 3.33 (*s*, 3H), 2.78–2.71 (dd, 1H, *J* = 14.6, 6.2), 2.60 (d, 1H, *J* = 14.0), 2.33 (ddd, 1H, *J* = 14.2, 5.7, 0.8), 2.29–2.07 (m, 3H) 2.02, 2.00 ppm (2s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.58, 170.37, 106.61, 92.63, 87.86, 75.03, 54.99, 46.55, 44.15, 39.02, 21.65, 21.28 ppm; HRMS (NSI$^+$, MeOH): *m/z* calcd for $C_{12}H_{18}O_6$: 281.0996, found: 281.1002 [$M+Na^+$].

*(3′S,6′S(6′R))-1-[3′,6′-Di-O-acetyl-2′-deoxy-3′,5′-ethano-α- and -β-D-ribofuranosyl] thymine (**12a/b**)*: Thymine (293 mg, 2.3 mmol) was suspended in MeCN (12 mL) and BSA (1.4 mL, 5.8 mmol) was added. The suspension was stirred until the solution became clear. The solution was then cooled to 0 °C and **10b** (300 mg, 1.2 mmol, dissolved in 6 mL MeCN) and TMSOTf (0.63 mL, 3.5 mmol) were added. After 1 h at 0 °C the mixture was allowed to warm to r.t. and the stirring was continued for another 16 h. It was then diluted with ethyl acetate and washed with sat. $NaHCO_3$. Purification by using FC (ethyl acetate/hexane 4:1) yielded the desired nucleoside **12a/b** (355 mg, 1.0 mmol, 87 %) in a anomeric ratio (α/β) of approximately 1:1 as a white foam. R_f = 0.49 (ethyl acetate/hexane 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.29 (*m*, 2H), 7.27 (s, 1H), 7.11 (*s*, 1H), 6.12 (m, 2H), 5.23 (*m*, 2H), 4.93 (dd, 1H, *J* = 6.4, 3.1), 4.56 (d, 1H, *J* = 5.6), 2.86 (m, 2H), 2.79 (ddd, 1H, *J* = 13.8, 6.3, 1.3), 2.66 (m, 1H), 2.54 (m, 1H), 2.36 (m, 1H), 2.25 (m, 2H), 2.16 (m, 1H), 2.10 (m, 2H), 2.08 (s, 3H), 2.06 (s, 3H), 2.04 (2s, 6H), 2.00 (m, 1H)1.96 (m, 3H), 1.95 ppm (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.69, 170.49, 170.35, 170.15, 163.73, 163.44, 150.30, 150.28, 135.31, 134.44, 112.04, 111.11, 91.21, 90.17, 87.69, 86.78, 85.33, 83.74, 73.65, 73.23, 44.46, 44.35, 43.64, 43.37, 37.84, 36.47, 21.73, 21.63, 21.19, 21.16, 12.86, −1.73 ppm; HRMS (NSI$^+$, MeCN): *m/z* calcd for $C_{16}H_{20}N_2O_7$: 375.1163; found: 375.1145 [$M+Na^+$].

*(3′S,6′S)-N6-Benzoyl-9-[3′,6′-di-O-acetyl-2′-deoxy-3′,5′-ethano-α- and -β-D-ribofuranosyl] adenine (**13a/b**)*: BSA (1.9 mL, 7.6 mmol) was added to a suspension of *N*6-benzoyl-adenine (926 mg, 3.9 mmol) in MeCN (15 mL). The mixture was stirred at RT until the solution was clear. Compound **10b** (500 mg, 1.9 mmol, solved in 4 mL MeCN) and TMSOTf (0.1 mL, 0.6 mmol) were added and the solution was stirred for 2 h at 85 °C. After cooling to RT the mixture was quenched with sat. $NaHCO_3$ and extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$ and evaporated. FC (3 % methanol in CH_2Cl_2) yielded **13a/b** (654 mg, 1.4 mmol, 74 %) in an anomeric ration of ≈1:1 as a white foam. R_f = 0.65 (10 % methanol in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.81, 8.79 (2s, 2H) 8.24, 8.15, (2s, 2H) 8.03 (m, 4H), 7.64–7.59 (m, 2H), 7.55–7.50 (m, 4H), 6.45 (dd, 1H, *J* = 6.9, 2.7), 6.34 (dd, 1H, *J* = 8.4, 6.4), 5.55–5.46 (m, 1H, H-C(6′), β), 5.31–5.21 (m, 1H), 4.89 (dd, 1H *J* = 6.1, 1.0), 4.73 (dd, 1H, *J* = 6.5, 1.7), 3.44 (dd, 1H, *J* = 15.4, 2.7), 3.03–2.90 (m, 4H), 2.88–2.81 (m, 1H) 2.45–2.36 (m, 2H), 2.22–2.12 (m, 4H), 2.12 (s, 3H), 2.06, 2.05 (2s, 6H), 1.95 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.60, 170.55, 170.47, 170.21, 164.72, 153.09, 152.97, 149.89, 149.72, 141.43, 141.25, 133.85, 133.07, 133.03, 129.13, 128.07, 123.67, 91.03, 90.99,

87.65, 86.25, 85.85, 84.84, 77.55, 77.23, 76.91, 73.46, 73.26, 44.25, 44.17, 43.77, 37.02, 36.88, 21.81, 21.52, 21.23, 21.18 ppm; HRMS (NSI$^+$, MeOH): *m/z* calcd for $C_{23}H_{23}N_5O_6$: 466.1721; found: 466.1724 [$M+H^+$].

*(3′S,6′S)-N6-Benzoyl-1-[3′,6′-di-O-acetyl-2′-deoxy-3′,5′-ethano-α- and -β-*D*-ribofuranosyl] cytosine (**14a/b**)*: *N*-Benzoylcytosine (833 mg, 3.9 mmol) was suspended in MeCN (20 mL), BSA (2.3 mL, 9.5 mmol) was added and the suspension was stirred until the solution became clear. The solution was then cooled to 0 °C and **10b** (500 mg, 1.9 mmol, dissolved in 20 mL MeCN) and TMSOTf (1 mL, 3.9 mmol) were added. After 1 h at 0 °C the mixture was allowed to warm to RT and the stirring was continued for another 16 h. It was diluted with ethyl acetate and washed with sat. $NaHCO_3$. Purification by FC (ethyl acetate/hexane 4:1) yielded the desired nucleoside **14a/b** (638 mg, 1.5 mmol, 76 %) in an anomeric ratio (α/β) of 1.5:1 as a white foam. R_f=0.26 (ethyl acetate/hexane 4:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.87 (br, 2H), 7.92 (m, 4H), 7.63–7.47 (m, 8H), 6.10 (m, 2H), 5.20 (m, 2H), 5.03 (dd, 1H, *J*=6.8, 3.2), 4.66 (d, 1H, *J*=5.7), 3.19 (dd, 1H, *J*=14.8, 5.4), 2.91 (dd, 1H, *J*=15.4, 6.5), 2.70 (m, 3H), 2.30 (m, 1H), 2.25–2.11 (m, 5H), 2.07 (s, 3H), 2.04, 2.03 (2s, 6H), 1.99 (m, 1H), 1.93 ppm (s, 3H); ^{13}C NMR (101 MHz, MeOD): δ= 172.40, 172.25, 172.03, 171.74, 164.98, 146.02, 145.77, 134.66, 134.14, 134.12, 130.47, 129.84, 129.57, 129.17, 129.14, 123.36, 120.19, 98.89, 98.11, 92.31, 92.07, 91.40, 90.17, 89.95, 89.44, 88.06, 87.65, 75.13, 61.55, 45.90, 45.44, 44.12, 43.44, 38.32, 37.57, 21.47, 21.30, 20.90, 20.88, 20.86, 14.45 ppm; HRMS (NSI$^+$, MeCN): *m/z* calcd for $C_{22}H_{23}N_3O_7$: 442.1609; found: 442.1617 [$M+H^+$].

*(3′S,6′S)-1-[2′-Deoxy-3′,5′-ethano-α- and -β-*D*-ribofuranosyl] thymine (**15a/b**)*: The nucleoside **12a/b** (480 mg, 1.36 mmol) was dissolved in 0.2 M NaOH in 5:4:1 THF/methanol/H_2O (65 mL) at 0 °C: The solution was stirred for one hour at 0 °C and quenched by the addition of ammonium chloride. The solvents were then evaporated and the remains absorbed on silica gel. Purification by FC (10 % methanol in CH_2Cl_2) yielded compound **15a/b** (294 mg, 1.1 mmol, 81 %) in an anomeric ratio of α/β ≈1.25:1 as a white foam. R_f=0.19 (10 % methanol in CH_2Cl_2), ^{1}H NMR (400 MHz, MeOD): δ=7.74 (*s,* 1H), 7.40 (s, 1H), 6.10 (m, 2H), 4.55 (dd, 1H, *J*=6.6, 1.9), 4.38 (m, 1H,), 4.28 (m, 1H) 4.18 (dd, 1H, *J*=6.7, 1.0), 2.52 (dd, 1H, *J*=14.7, 7.1), 2.34 (*m,* 4H), 2.14 (m, 1H), 2.05 (m, 2H), H-C(7′)), 1.90 (m, 3H), 1.89 (m, 3H), 1.88 (m, 2H), 1.80 (m, 1H), 1.71 ppm (m, 1H); ^{13}C NMR (101 MHz, MeOD): δ=166.55, 166.22, 152.52, 152.23, 138.60 (C-6), 137.07 (C-6), 111.98, 111.06, 91.65, 89.49 88.58, 86.24, 85.50, 84.89, 71.98, 71.69, 49.33, 47.57, 46.94, 42.08, 41.15, 20.84, 12.51, 12.41 ppm; HRMS (NSI$^+$, MeCN): *m/z* calcd for $C_{12}H_{16}N_2O_5$: 269.1132; found: 268.1141 [$M+H^+$].

*(3′S,6′S)-N6-Benzoyl-9-[2′-deoxy-3′,5′-ethano-α- and -β-*D*-ribofuranosyl] adenine (**16a/b**)*: The nucleosides **13a/b** (606 mg, 1.3 mmol) were dissolved in 0.2 M NaOH in 5:4:1 THF/methanol/H_2O (90 mL) at 0 °C: The solution was stirred for one hour at 0 °C and quenched by the addition of ammonium chloride. The solvents were then evaporated and the remains absorbed on silica gel. Purification by FC (10 % methanol in CH_2Cl_2) yielded compound **16a/b** (437 mg, 1.14 mmol, 88 %) in anomeric ratio of α/β≈1:1 as a white foam. R_f=0.2 (10 % methanol in CH_2Cl_2); ^{1}H NMR (300 MHz, MeOD): δ=8.76 (s, 1H), 8.70 (2s, 2H), 8.54 (s, 1H), 8.07 (m, 4H), 7.67–7.61 (m, 2H), 7.57–7.52 (m, 4H), 6.51 (dd, 1H *J*=7.2, 1.8), 6.33 (dd, 1H, *J*=9.7, 5.3), 4.64–4.58 (m, 1H), 4.49 (*d*, *J*=5.9), 4.37–4.26 (m, 2H), 2.91–2.83 (m, 2H), 2.72 (dd, 1H *J*=14.9, 7.3), 2.59 (dd, 1H, *J*= 13.5, 5.3), 2.48–2.37 (m, 2H), 2.21–2.07 (m, 2H), 1.92–1.74 ppm (m, 4H); ^{13}C NMR (75 MHz, MeOD): δ=153.36, 153.18, 152.95, 151.12, 151.08, 144.82, 144.29, 134.95, 133.88, 129.74, 129.41, 91.51, 90.22, 86.86, 86.06, 85.82, 85.41, 71.74, 71.54, 47.85, 47.03, 41.50, 41.32 ppm; HRMS (NSI+, MeOH): *m/z* calcd for $C_{19}H_{19}N_5O_4$: 382.1510; found: 382.1512 [$M+H^+$].

*(3′S,6′S)-N6-Benzoyl-1-[2′-deoxy-3′,5′-ethano-α- and -β-*D*-ribofuranosyl] cytosine (**17a/b**)*: The nucleoside **14a/b** (316 mg, 0.72 mmol) was dissolved in 0.2 M NaOH in 5:4:1 THF/methanol/H_2O (50 mL) at 0 °C. The solution was stirred for one hour at 0 °C and quenched by the addition of ammonium chloride. The solvents were then evaporated and the remains absorbed on silica gel. Purification by FC (10 % methanol in CH_2Cl_2) yielded compound **17a/b** (191 mg, 0.53 mmol, 74 %) in an anomeric ratio of α/β≈1:1 as a white foam. R_f=0.36 (10 % methanol in CH_2Cl_2); ^{1}H NMR (300 MHz, MeOD): δ=8.26 (d, 1H, *J*=7.5), 8.18 (d, 1H, *J*= 7.5), 7.99 (m, 2H), 7.96 (m, 2H), 7.63 (m, 4H), 7.55 (m, 4H), 6.10 (m, 2H), 4.72 (dd, 1H, *J*=7.2, 2.1), 4.40–4.28 (m, 3H), 2.69 (dd, 1H, *J*=13.7, 5.2), 2.59 (dd, 1H, *J*=14.8, 6.7), 2.45 (dd, 1H, *J*=14.8, 2.3), 2.38–2.29 (m, 2H), 2.23 (m, 1H), 2.14 (m, 1H), 1.96–1.85 (m, 3H), 1.81–1.69 ppm (m, 2H); ^{13}C NMR (101 MHz, MeOD): δ=164.81, 146.56, 146.10, 145.61, 134.74, 134.69, 134.10, 134.04, 129.83, 129.82, 129.16, 129.11, 98.80, 97.96, 92.69, 91.59, 90.35, 88.28, 87.99, 87.32, 85.96, 85.89, 82.54, 80.66, 79.66, 72.15, 71.89, 71.53, 58.32, 57.06, 57.01, 48.88, 48.31, 46.79, 45.89, 43.79, 42.06, 41.63, 41.24, 18.36 ppm; HRMS (NSI+, MeCN) *m/z* calcd for $C_{18}H_{19}N_3O_7$: 358.1397; found: 358.1387 [$M+H^+$].

*(3′S,6′S)-1-[2′-Deoxy-6′-O-(methylsulfonyl)-3′,5′-ethano-α- and -β-*D*-ribofuranosyl] thymine (**18a/b**)*: A solution of **15a/b** (294 mg, 1.09 mmol) was dissolved in pyridine (15 mL) and cooled to 0 °C. MsCl (85 μL, 1.09 mmol) was added and the mixture was stirred at 0 °C and overnight at 4 °C. After quenching by the addition of sat. $NaHCO_3$ and washing with ethyl acetate the combined organic layers were dried over $MgSO_4$ and purified by FC (5 % methanol in CH_2Cl_2) to yield **18a/b** (250 mg, 0.72 mmol, 66 %) in an anomeric ratio of α/β≈1.25:1 as a white solid. R_f=0.45 (10 % methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO): δ= 11.35 (s, 1H), 11.27 (s, 1H), 7.68(s, 1H), 7.55(*s,* 1H), 6.08 (m, 2H), 5.18 (m, 1H), 5.07 (m, 1H), 4.50 (dd, 1H, *J*=6.2, 2.0), 4.10 (dd, 1H, *J*=7.1, 2.2), 3.22 (s, 3H), 3.20 (s, 3H), 2.52 (m, 3H), 2.20 (m, 5H), 2.08 (m, 2H), 1.99 (m, 1H), 1.90 (m, 1H), 1.81 (m, 3H), 1.79 ppm (m, 3H); ^{13}C NMR (101 MHz, DMSO): δ=163.80, 163.61, 150.45, 150.43, 136.44, 135.96, 109.70, 109.11, 88.09 (C4′), 86.61 (C4′), 85.51 (C1′), 84.10 (C1′), 83.45, 83.11, 80.49, 80.23, 45.40, 45.21, 45.05, 44.06, 38.32, 37.70, 37.55, 37.42, 12.2, 12.11 ppm; HRMS (NSI+, MeOH): *m/z* calcd for $C_{13}H_{18}N_2O_7S$: 347.0907; found 347.0902 [$M+H^+$].

*(3′S,6′S)-N6-Benzoyl-9-[2′-deoxy-6′-O-(methylsulfonyl)-3′,5′-ethano-α- and -β-*D*-ribofuranosyl] adenine (**19a/b**)*: A solution of **16a/b** (153 mg, 0.4 mmol) was dissolved in pyridine (6 mL) and cooled to 0 °C. MsCl (0.03 mL, 0.4 mmol) was added and the mixture was stirred for 2 h at RT. Silica was then added and the pyridine was evaporated and shortly high vac dried. FC (7 % methanol in CH_2Cl_2) yielded the corresponding anomers **19a** (105 mg. 0.23 mmol, 57 %) and **19b** (73 mg, 0.16 mmol, 39 %) as white foams. Compound **19a**: R_f=0.5 (10 % methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, MeOD): δ=8.74 (2 *s*, 2H), 8.09 (m, 2H), 7.65 (m, 1H), 7.57 (m, 2H), 6.56 (dd, 1H, *J*=7.1, 2.6), 5.21–5.14 (*m*, 1H), 4.59 (dd, 1H, *J*=6.3, 1.5), 3.12 (*s*, 3H), 2.92 (dd, 1H, *J*=14.9, 2.6), 2.84 (dd, 1H, *J*=14.8, 7.2), 2.67 (ddd, 1H, *J*=13.4, 6.5, 1.3), 2.40–2.35 (m, 1H), 2.26 –2.14 ppm (m, 2H); ^{13}C NMR (101 MHz, MeOD): δ=168.16, 153.11, 151.22, 144.78, 138.41, 134.99, 133.92, 129.78, 129.43, 125.29, 90.39, 86.96, 85.87, 81.13, 47.38, 47.26, 39.44, 38.11 ppm; HRMS (NSI+, MeOH): *m/z* calcd for $C_{20}H_{21}N_5O_6S$: 460.1285; found: 460.1272 [$M+H^+$]; Compound **19b**: R_f=0.4 (10 % methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, MeOD): δ=8.73 (*s*, 1H), 8.54 (s, 1H), 8.08 (m, 2H), 7.63 (m, 1H), 7.55 (m, 2H), 6.37 (dd, 1H, *J*=9.3, 5.9), 5.66–5.58 (m, 1H), 4.38 (dd, 1H, *J*=6.9, 1.8), 3.13 (*s*, 3H), 3.05 (m, 1H), 2.75 (ddd, 1H, *J*=13.0, 6.2, 1.3), 2.63 (dd, 1H, *J*=13.8, 5.9), 2.43 (m, 1H), 2.20 (ddd, 1H, *J*= 14.1, 8.5, 7.0), 2.10 ppm (dd, 1H, *J*=13.0, 8.9); ^{13}C NMR (101 MHz, MeOD): δ=168.15, 153.20, 151.22, 144.75, 138.43, 134.96, 133.89, 129.79, 129.75, 129.41, 125.56, 89.26, 86.34, 85.54, 81.11, 46.85, 46.41, 39.32, 38.16, 18.39 ppm; HRMS (NSI+, MeOH): *m/z* calcd for $C_{20}H_{21}N_5O_6S$: 460.1285; found: 460.1273 [$M+H^+$].

*(3′S,6′S)-N6-Benzoyl-1-[2′-deoxy-6′-O-(methylsulfonyl)-3′,5′-ethano-α- and -β-*D*-ribofuranosyl] cytosine (**20a/b**)*: A solution of **17a/b** (415 mg, 1.16 mmol) in pyridine (16 mL) was cooled to 0 °C and MsCl (90 μL, 1.16 mmol) was added. The mixture was stirred overnight at 4 °C. Sat. $NaHCO_3$ was added and the aqueous phase was extracted with ethyl acetate. The crude product **20a/b** precipitated in the organic phase and was used in the next experiment without further purification. R_f=0.49 (10 % methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO): δ=11.21 (br, 2H), 8.23 (m, 2H), 8.00 (m, 4H), 7.62 (m, 2H), 7.51 (m, 4H), 7.37 (m, 2H), 6.11 (dd, 1H, *J*=9.3, 5.1), 6.04 (dd, 1H, *J*=6.8, 2.7), 5.68 (s, 1H), 5.53 (s, 1H), 5.20 (m, 1H), 5.10 (m, 1H), 4.67 (dd, 1H, *J*=6.9, 2.3), 4.23 (dd, 1H, *J*=7.1, 1.9), 3.23 (s, 3H), 3.21 (s, 3H), 2.63 (dd, 1H, *J*=14.5, 6.9), 2.47 (m, 2H), 2.31 (m, 2H), 2.24 (m, 2H), 2.14 (*m*, 2H), 2.06 (*m*, 1H) 1.96 ppm (*m*, 2H); ^{13}C NMR (101 MHz, DMSO): δ=162.99, 145.29,

133.20, 132.74, 132.65, 128.45, 128.40, 95.63, 89.25, 88.90, 87.29, 85.21, 83.76, 80.22, 79.86, 46.10, 45.99, 45.16, 45.10, 38.38, 37.71, 37.40, 21.01 ppm; HRMS (NSI+, THF): *m/z* calcd for $C_{19}H_{21}N_3O_7S$: 436.1173; found: 436.1172 $[M+H^+]$.

*(3′S,6′S)-1-[3′-O-Acetyl-2′-deoxy-6′-O-(methylsulfonyl)-3′,5′-ethano-α- and -β-*D*-ribofuranosyl] thymine (**21a/b**)*: A solution of **18a/b** (250 mg, 0.72 mmol) in pyridine (7 mL) was cooled to 0°C and DMAP (9 mg, 0.07 mmol) and Ac_2O (0.08 mL, 0.8 mmol) were added. The mixture was stirred over night at RT and, after cooling to 0°C, carefully quenched and washed with sat. $NaHCO_3$. The aqueous layers were extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and evaporated. FC (3% methanol in CH_2Cl_2) yielded **21a/b** (218 mg, 0.56 mmol, 78%) an anomeric ratio of α/β≈1.25:1 as a white foam. R_f=0.49 (10% methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.24 (m, 1H), 7.06 (m, 1H), 6.11 (t, 1H, *J*=6.2), 6.03 (dd, 1H, *J*=9.2, 5.6), 5.24–5.15 (m, 2H), 4.95 (dd, 1H, *J*=6.2, 3.3), 4.58 (dd, 1H, *J*=6.4, 1.4), 3.05, 3.03 (2s, 6H), 2.92 (dd, 1H, *J*=14.8, 6.6), 2.84 (m, 2H), 2.68 (dd, 1H, *J*=15.3, 6.0), 2.54 (m, 2H), 2.48–2.29 (m, 5H), 2.14 (m, 1H), 2.09, 2.07 (2s, 6H), 1.97 (m, 3H), 1.96 ppm (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ= 170.02, 163.14, 162.93, 149.91, 149.81, 134.92, 134.66, 111.82, 111.20, 90.48, 89.56, 86.93, 86.34, 84.73, 84.62, 79.91, 78.92, 77.33, 77.01, 76.69, 44.27, 43.96, 43.89, 43.79, 38.55, 38.46, 37.24, 21.05, 12.68, 12.63 ppm; HRMS (NSI+, MeCN): *m/z* calcd for $C_{15}H_{20}N_2O_8S$: 389.1013; found 389.1019 $[M+H^+]$.

*(3′S,6′S)-N6-Acetyl-9-[3′-O-acetyl-2′-deoxy-6′-O-(methylsulfonyl)-3′,5′-ethano-β-*D*-ribofuranosyl]-N6-benzoyladenine (**22b**)*: A solution of **19b** (195 mg, 0.42 mmol) in pyridine (12 mL) was cooled to 0°C and DMAP (5 mg, 42 μmol) and Ac_2O (0.12 mL, 1.3 mmol) were added. The mixture was stirred for 16 h at RT and, after cooling to 0°C, carefully quenched and washed with sat. $NaHCO_3$. The aqueous layers were extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and evaporated. FC (5% methanol in CH_2Cl_2) yielded **22b** (192 mg, 0.38 mmol, 91%) as a white foam. R_f=0.8 (10% methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.69 (s, 1H), 8.31 (s, 1H), 7.71 (m, 2H), 7.42 (m, 1H), 7.32 (m, 2H), 6.42 (dd, 1H, *J*=6.9, 2.9), 5.18 (m, 1H) 4.90 (dd, 1H, *J*=6.1, 1.7), 3.41 (m, 1H), 3.03 (s, 3H), 2.89 (m, 2H), 2.44 (m, 2H), 2.34 (m, 1H), 2.09 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ=175.38, 172.86, 172.05, 170.24, 152.94, 152.53, 152.43, 151.16, 134.41, 133.07, 129.44, 129.01, 128.78, 90.34, 87.23, 79.21, 44.41, 43.96, 38.65, 37.68, 25.61, 21.37 ppm; HRMS (NSI+, EtOAc): *m/z* calcd for $C_{22}H_{23}N_5O_4S$: 502.1391; found: 502.11385 $[M+H^+]$.

*(3′S,6′S)-N6-Acetyl-1-[3′-O-acetyl-2′-deoxy-6′-O-(methylsulfonyl)-3′,5′-ethano-α- and β-*D*-ribofuranosyl]-N6-benzoylcytosine (**23a/b**)*: A solution of **20a/b** (769 mg, 1.76 mmol) in pyridine (35 mL) was cooled to 0°C and DMAP (22 mg, 0.18 mmol) and Ac_2O (0.5 mL, 5.28 mmol) were added. The mixture was stirred for 20 h at RT and, after cooling to 0°C, carefully quenched and washed with sat. $NaHCO_3$. The aqueous layers were extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and evaporated. FC (5% methanol in CH_2Cl_2) yielded **23a/b** (628 mg, 1.3 mmol, 75% over two steps) in an anomeric ratio of α/β≈2:1 as a white foam. R_f=0.9 (10% methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.93 (m, 6H), 7.61 (m, 4H), 7.55 (m, 4H), 6.10 (dd, 1H, *J*=6.4, 4.1), 6.04 (dd, 1H, *J*=8.5, 5.6), 5.19 (m, 2H), 5.04 (dd, 1H, *J*=5.9, 3.7), 4.69 (d, 1H), 3.15 (m, 1H), 3.04 (s, 3H), 3.03 (s, 3H), 2.99 (m, 1H), 2.83–2.67 (m, 3H), 2.50 (m, 2H), 2.35 (m, 4H), 2.08 (m, 6H), 2.04 ppm (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ=176.17, 170.32, 170.09, 163.25, 163.18, 154.73, 149.28, 144.01, 136.97, 133.47, 132.99, 129.09, 128.19, 97.45, 96.72, 90.54, 90.08, 89.53 (C(1′)), 88.09, 86.89, 85.77, 79.77, 79.31, 45.55, 45.11, 44.25, 43.74, 38.64, 38.61, 38.58, 37.66, 29.87, 29.53, 21.66, 21.53, 21.15 ppm; HRMS (NSI+, EtOAc): *m/z* calcd for $C_{21}H_{23}N_3O_8S$: 478.1279; found: 478.1268 $[M+H^+]$.

*(3′S,6′R)-1-[3′,6′-Di-O-acetyl-2′-deoxy-3′,5′-ethano-α- and -β-*D*-ribofuranosyl] thymine **24a/b***: A solution containing **21a/b** (301 mg, 0.77 mmol) and CsOAc (2.1 g, 10.7 mmol) in DMSO (15 mL) was heated to 85°C and stirred for 16 h. After cooling to RT the mixture was diluted with ethyl acetate and washed with sat. $NaHCO_3$. The combined organic layers were washed with H_2O and dried over $MgSO_4$. By using FC (5% methanol in CH_2Cl_2) **24a/b** (254 mg, 0.72 mmol, 93%) an anomeric ratio of α/β≈1.25:1 was obtained as a white foam. R_f=0.88 (10% methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.36 (m, 1H), 7.30 (m, 1H,), 6.41 (t, 1H, *J*=6.4), 6.20 (dd, 1H, *J*=9.7, 5.2), 5.43 (m, 1H), 5.27 (m, 1H), 4.94 (dd, 1H, *J*=6.8, 1.4), 4.62 (d, 1H, *J*=6.8), 2.98 (*m*, 1H,), 2.84 (dd, 1H, *J*=14.3, 5.2), 2.55 (m, 2H), 2.43 (m, 1H), 2.38 (m, 3H), 2.30 (m, 1H), 2.15 (m, 2H), 2.10, 2.09 (2s, 6H), 2.06–2.01 (m, 1H), 2.04, 2.03 (2s, 6H), 1.95 (m, 3H), 1.93 ppm (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ= 170.51, 170.27, 169.74, 163.97, 163.70, 150.64, 150.52, 135.17, 134.65, 111.64, 111.37, 93.65, 93.53, 88.38, 87.53, 86.04, 83.88, 77.43, 77.28, 75.10, 45.15, 45.04, 44.65, 44.36, 42.85, 38.75, 37.50, 21.76, 21.64, 21.40, 12.86, 12.81 ppm; HRMS (NSI+, MeOH): *m/z* calcd for $C_{16}H_{20}N_2O_7$: 353.1343; found: 353.1341 $[M+H^+]$.

*(3′S,6′R)-N6-Benzoyl-9-[3′,6′-di-O-acetyl-2′-deoxy-3′,5′-ethano-β-*D*-ribofuranosyl] adenine (**25b**)*: A solution containing **22b** (219 mg, 0.41 mmol) and CsOAc (1.1 g, 6 mmol) in DMSO (8 mL) was heated to 85°C and stirred for 16 h. After cooling to RT the mixture was diluted with ethyl acetate and washed with sat. $NaHCO_3$. The combined organic layers were washed with H_2O and dried over $MgSO_4$. FC purification (5% methanol in CH_2Cl_2) yielded **25b** (155 mg, 0.33 mmol, 81%) as a white foam. R_f=0.75 (10% methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=9.11 (br, 1H), 8.77 (s, 1H), 8.28 (s, 1H), 8.03 (m, 2H), 7.60 (m, 1H), 7.52 (m, 2H), 6.61 (dd, 1H, *J*=6.8, 3.1), 5.35 (m, 1H), 4.96 (d, 1H), 3.33 (m, 1H), 3.14 (m, 1H), 2.40 (m, 4H), 2.11 (s, 3H), 1.96 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ=170.38, 170.30, 152.85, 141.23, 141.15, 136.16, 133.04, 130.49, 129.06, 128.15, 94.17, 89.59, 86.63, 86.10, 85.24, 76.29, 45.24, 44.58, 38.61, 37.99, 29.90, 21.58, 21.48 ppm; HRMS (NSI+, MeCN): *m/z* calcd for $C_{23}H_{23}N_5O_6$: 466.1721; found: 466.1727 $[M+H^+]$.

*(3′S,6′R)-N6-Benzoyl-1-[3′,6′-Di-O-acetyl-2′-deoxy-3′,5′-ethano-α- and -β-*D*-ribofuranosyl] cytosine (**26a/b**)*: A solution containing **23a/b** (523 mg, 1.09 mmol) and CsOAc (2.9 g, 15.1 mmol) in DMSO (20 mL) was heated to 85°C and stirred for 16 h. After cooling to RT the mixture was diluted with ethyl acetate and washed with sat. $NaHCO_3$. The combined organic layers were washed with H_2O and dried over $MgSO_4$. The crude product **26a/b** was used for the following step without purification. R_f=0.82 (10% methanol in CH_2Cl_2); HRMS (NSI+, EtOAc): *m/z* calcd for $C_{22}H_{23}N_3O_7$: 442.1609; found: 442.1601 $[M+H^+]$.

*(3′S,6′R)-1-[2′-Deoxy-3′,5′-ethano-α- and -β-*D*-ribofuranosyl] thymine (**27a/b**)*: The nucleoside **24a/b** (254 mg, 0.72 mmol) was dissolved in 0.2 M NaOH in a solution of THF/methanol/H_2O (5:4:1, 40 mL) at 0°C. The solution was stirred for one hour at 0°C and quenched by the addition of ammonium chloride. The solvents were then evaporated and the remains absorbed on silica gel. Purification by FC (10% methanol in CH_2Cl_2) yielded compound **27a/b** (123 mg, 0.45 mmol, 64%) in anomeric ratio of α/β≈1.25:1 as a white foam. R_f=0.2 (10% methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO): δ=11.25 (br, 2H), 7.76 (s, 1H), 7.66 (s, 1H), 6.20 (m, 2H), 5.31 (s, 1H), 5.26 (s, 1H), 4.94 (d, 1H, *J*=2.7), 4.71 (d, 1H, *J*=3.8), 4.39 (dd, 1H, *J*=7.1, 3.0), 4.31 (m, 1H), 4.23 (m, 1H), 4.08 (m, 1H), 2.62 (dd, 1H, *J*=13.7, 6.9), 2.31 (m, 1H), 2.22 (m, 1H), 2.15 (m, 1H), 2.09 (m, 1H), 2.02 (m, 2H), 1.95 (m, 1H), 1.85 (m, 1H), 1.80 (m, 3H), 1.76 (m, 3H), 1.77 (m, 2H), 1.55 ppm (m, 1H); ^{13}C NMR (101 MHz, DMSO): δ=163.79, 163.63, 150.40, 150.36, 136.50, 109.57, 109.13, 90.11, 89.98, 86.81, 86.10, 85.59, 83.66, 72.36, 70.90, 48.33, 47.92, 46.80, 46.02, 41.32, 40.66, 12.34, 12.25 ppm; HRMS (NSI+, MeOH) for $C_{12}H_{16}N_2O_5$: *m/z* calcd for 268.0951; found: 268.0955 $[M^+]$.

*(3′S,6′R)-N6-Benzoyl-9-[2′-deoxy-3′,5′-ethano-β-*D*-ribofuranosyl] adenine (**28b**)*: The nucleoside **25b** (115 mg, 0.25 mmol) was dissolved in 0.2 M NaOH in THF/methanol/H_2O (5:4:1, 17 mL) at 0°C. The solution was stirred for one hour at 0°C and quenched by the addition of ammonium chloride. The solvents were then evaporated and the remains absorbed on silica gel. Purification by FC (10% methanol in CH_2Cl_2) yielded compound **28b** (80 mg, 0.21 mmol, 84%) as a white foam. R_f=0.3 (10% methanol in CH_2Cl_2); ^{1}H NMR (300 MHz, MeOD): δ=8.71 (s, 1H), 8.65 (s, 1H), 8.09 (m, 2H), 7.65 (m, 1H), 7.55 (m, 1H), 6.55 (dd, 1H, *J*=9.3, 5.9), 4.50 (m, 1H), 4.38 (dd, *J*=6.9, 1.4, 1H), 3.01 (dd, 1H, *J*=13.5, 9.4), 2.70 (dd, 1H, *J*=13.6, 5.9), 2.33–2.24 (m, 2H), 2.03 ppm (m, 2H); ^{13}C NMR (75 MHz, MeOD) δ=153.22, 153.09, 151.14, 144.27, 134.98, 133.90, 129.76, 129.42, 125.05, 92.42, 88.68, 85.90, 74.93, 54.79, 49.46,

41.83 ppm; HRMS (NSI+, MeOH): *m/z* calcd for $C_{19}H_{19}N_5O_4$: 382.1510; found: 382.1505 $[M+H^+]$.

*(3′S,6′R)-N6-Benzoyl-1-[2′-deoxy-3′,5′-ethano-α- and -β-D-ribofuranosyl] cytosine (**29a/b**)*: The nucleoside **26a/b** (386 mg, 0.87 mmol) was dissolved in 0.2 M NaOH in THF/methanol/H_2O (5:4:1, 50 mL) at 0 °C. The solution was stirred for one hour at 0 °C and quenched by the addition of ammonium chloride. The solvents were then evaporated and the remains absorbed on silica gel. Purification by FC (5% methanol in CH_2Cl_2) yielded compound **29a/b** (183 mg, 0.51 mmol, 59% over two steps) in an anomeric ratio of α/β≈2:1as a white foam. R_f=0.43 (10% methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, MeOD): δ=8.46 (*d*, 1H, *J*=7.5), 8.28 (d, 1H, *J*=7.5), 7.98 (m, 4H), 7.63 (*m*, 4H), 7.54 (m, 4H), 6.33 (dd, 1H, *J*=8.9, 5.8), 6.21 (dd, 1H, *J*=6.8, 3.2), 4.68 (dd, 1H, *J*=7.2, 3.1), 4.49 (*m*, 1H), 4.43 (m, 1H), 4.35 (d, 1H), 3.03 (dd, 1H, *J*=14.4, 6.8), 2.67 (dd, 1H, *J*=13.6, 5.8), 2.37 (m, 3H), 2.24 (m, 1H), 2.15 (m, 1H), 2.09 (m, 2H), 2.04 (m, 1H), 1.94 (m, 1H), 1.85 ppm (m, 1H); ^{13}C NMR (101 MHz, MeOD): δ=169.13, 164.79, 157.92, 146.88, 146.56, 134.76, 134.72, 134.10, 134.06, 129.83, 129.17, 129.13, 98.76, 98.06, 93.64, 92.52, 92.07, 88.92, 88.10, 87.97, 74.88, 73.43, 50.01, 48.88, 42.27, 41.63 ppm; HRMS (NSI+, MeOH): *m/z* calcd for $C_{18}H_{19}N_3O_5$: 380.1217; found: 380.1220 $[M+Na^+]$.

*(3′S,6′R)-1-[2′-Deoxy-6′-O-[(4,4′-dimethoxytriphenyl)methyl]-3′,5′-ethano-α- and -β-D-ribofuranosyl] thymine (**30a/b**)*: To a solution of nucleoside **27a/b** (456 mg, 1.7 mmol) in pyridine (8 mL) was added DMT-Cl (1.7 g 5.1 mmol) in three portions and it was stirred for 24 h at RT. The solution was then diluted with ethyl acetate, washed with sat. $NaHCO_3$ and dried over $MgSO_4$. Purification by FC (2.5% methanol in CH_2Cl_2) yielded the corresponding anomers α and β, compound **30a** (470 mg, 0.82 mmol, 48%) and **30b** (384 mg, 0.67 mmol, 40%), respectively, as pale-yellow foams. Compound **30a**: R_f=0.23 (3% methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.47 (m, 2H), 7.35 (m, 4H), 7.29 (m, 2H), 7.22 (m, 2H), 6.83 (m, 4H), 5.95 (dd, 1H, *J*=7.8, 3.7), 4.40 (dd, 1H, *J*=7.0, 3.1), 4.24 (m, 1H), 3.79 (s, 6H), 2.89 (dd, 1H, *J*=14.5, 7.8), 2.30 (dd, 1H, *J*=14.5, 3.7), 1.95 (m, 1H), 1.91 (m, 3H), 1.67 (m, 1H), 1.53 ppm (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ=163.87, 158.81, 150.59, 145.86, 138.45, 137.10, 136.98, 130.28, 130.25, 128.28, 128.16, 127.04, 113.49, 110.80, 91.87, 91.50, 87.46, 87.22, 75.18, 55.45, 47.22, 46.53, 46.39, 40.46, 12.63, 11.67 ppm; HRMS (NSI+, MeCN): *m/z* calcd for $C_{33}H_{34}N_2O_7$: 593.2258; found: 593.2250 $[M+Na^+]$; Compound **30b**: R_f=0.17 (3% methanol in CH_2Cl_2), ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.69 (br, 1H), 7.45 (m, 2H), 7.35 (m, 4H), 7.32 (m, 1H), 7.27 (m, 2H), 7.21 (m, 1H), 6.82 (m, 4H), 6.27 (dd, 1H, *J*=8.8, 5.9), 4.33 (m, 1H), 4.05 (dd, 1H, *J*=7.5, 2.1), 3.78 (s, 6H), 2.49 (m, 2H), 2.10 (m, 1H), 1.77 (m, 2H), 1.50 (m, 3H), 1.18 ppm (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ=163.72, 158.91, 158.89, 150.54, 145.65, 136.86, 136.66, 135.61, 130.22, 130.15, 128.34, 128.18, 127.18, 113.67, 111.57, 89.85, 88.10, 87.68, 85.01, 76.40, 55.47, 46.99, 46.62, 46.08, 39.32, 12.31 ppm; HRMS (NSI+, MeCN): *m/z* calcd for $C_{33}H_{34}N_2O_7$: 593.2258; found: 593.2251 $[M+Na^+]$.

*(3′S,6′R)-N6-Benzoyl-9-[2′-Deoxy-6′-O-[4,4′-(dimethoxytriphenyl)methyl]-3′,5′-ethano-β-D-ribofuranosyl] adenine (**31b**)*: To a solution of nucleoside **28b** (80 mg, 0.21 mmol) in pyridine (2 mL) was added DMT-Cl (213 mg 0.63 mmol) in three portions and the mixture was stirred for 24 h at RT. The solution was then diluted with ethyl acetate, washed with sat. $NaHCO_3$ and dried over $MgSO_4$. Purification by FC (5% methanol in CH_2Cl_2) yielded compound **31b** (129 mg, 0.19 mmol, 90%) as a white foam. R_f=0.42 (10% methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.81 (s, 1H), 8.27 (s, 1H), 8.04 (m, 2H), 7.61 (m, 1H,), 7.53 (m, 2H), 7.45 (m, 2H), 7.35 (m, 4H), 7.28 (m, 2H), 7.22 (m, 1H), 6.81 (m, 4H), 6.46 (dd, 1H, *J*=9.1, 5.4), 4.35 (m, 1H), 4.21 (dd, 1H, *J*=7.4, 3.5), 3.78 (s, 6H), 2.92 (dd, 1H, *J*=13.2, 9.2), 2.64 (dd, 1H, *J*=13.2, 5.5), 2.02 (m, 1H), 1.83–1.58 ppm (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ=171.37, 171.24, 158.84, 153.04, 150.05, 149.74, 145.65, 141.30, 136.90, 136.87, 136.18, 132.97, 130.25, 129.09, 128.28, 128.23, 128.11, 127.14, 123.95, 113.57, 90.02, 87.58, 87.17, 85.18, 77.43, 75.85, 55.46, 47.26, 46.60, 46.08, 39.91 ppm; HRMS (NSI+, MeOH): *m/z* calcd for $C_{40}H_{37}N_5O_6$: 684.2817; found: 684.2830 $[M+H^+]$.

*(3′S,6′R)-N6-Benzoyl-1-[2′-Deoxy-6′-O-[4,4′-(dimethoxytriphenyl)methyl]-3′,5′-ethano-α and -β-D-ribofuranosyl] cytosine (**32a/b**)*: DMT-Cl (1.1 g 3.3 mmol) was added in three portions to a solution of nucleoside **29a/b** (394 mg, 1.1 mmol) in pyridine (6 mL) and it was stirred for 24 h at RT. The solution was then diluted with ethyl acetate, washed with sat. $NaHCO_3$ and dried over $MgSO_4$. Purification by FC (ethyl acetate/hexane, 4:1 to 5% methanol in CH_2Cl_2) yielded the corresponding anomers α and β, **32a** (450 mg, 0.68 mmol, 62%) and **32b** (217 mg, 0.33 mmol, 30%) as white foams. Compound **32a**: R_f=0.22 (ethyl acetate/hexane, 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=9.00 (br, 1H), 7.87 (m, 3H), 7.55 (m, 1H), 7.46 (m, 5H), 7.36 (m, 4H), 7.26 (m, 2H), 7.21 (m, 1H), 6.83 (m, 4H), 6.05 (dd, 1H, *J*=7.1, 2.1), 4.48 (dd, 1H, *J*=7.3, 3.8), 4.25 (m, 1H), 3.79 (s, 6H), 3.03 (dd, 1H), 2.64 (dd, 1H, *J*=14.8, 2.0), 1.64 ppm (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ=162.60, 158.72, 155.27, 146.01, 145.82, 137.07, 137.03, 133.27, 133.14, 130.26, 129.08, 128.32, 128.10, 127.87, 126.98, 113.43, 96.20, 93.05, 92.81, 87.17, 86.83, 75.04, 60.59, 55.40, 47.40, 46.27, 40.51, 21.22, 14.37 ppm; HRMS (NSI+, MeCN): *m/z* calcd for $C_{39}H_{37}N_3O_7$: 660.2704, found: 660.2724 $[M+H^+]$; Compound **43b**: R_f=0.05 (ethyl acetate/hexane, 4:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.77 (br, 1H), 8.23 (m, 1H), 7.89 (m, 2H), 7.60 (m, 1H), 7.51 (m, 4H), 7.37–7.19 (m, 8H), 6.84 (m, 4H), 6.45 (dd, 1H, *J*=8.6, 5.5), 4.37 (m, 1H), 4.23 (d, 1H, *J*=5.8), 3.79, 3.77 (2 s, 6H), 2.84 (dd, 1H, *J*=13.5, 5.4), 2.36 (dd, 1H, *J*=13.5, 8.7), 1.88- 1.73 (m, 3H), 1.42 ppm (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ=158.87, 158.37, 145.45, 136.83, 136.71, 133.38, 130.28, 130.20, 129.26, 128.35, 128.31, 127.76, 127.17, 113.62, 113.51, 90.84, 89.31, 88.06, 87.68, 87.12, 76.79, 55.43, 55.42, 47.10, 39.46 ppm; HRMS (NSI+, MeCN): *m/z* calcd for $C_{39}H_{37}N_3O_7$: 660.2704; found: 660.2704 $[M+H^+]$.

*(3′S,6′R)-1-[6′-O-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-2′-deoxy-6′-O-[(4,4′-dimethoxytriphenyl)methyl]-3′,5′-ethano-β-D-ribofuranosyl} thymine (**33b**)*: Compound **30b** (163 mg, 0.29 mmol) was dissolved in MeCN (2.5 mL) and Hünig's base (0.24 mL, 1.43 mmol) and CEP-Cl (0.19 mL, 0.87 mmol) were added. The solution was stirred at RT for 1 h, diluted with ethyl acetate and washed with sat. $NaHCO_3$. The aqueous layer is extracted with ethyl acetate and dried over $MgSO_4$. Purification by using FC (ethyl acetate/hexane 1:1) yielded phosphoramidite **33b** (214 mg, 0.24 mmol, 72%) as a white foam. ^{31}P NMR showed no phosphonate peak, therefore no further purification was necessary. R_f=0.63 (ethyl acetate/hexane 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.11 (br, 1H), 7.45 (m, 2H), 7.35 (m, 4H), 7.27 (m, 4H), 7.20 (m, 1H), 6.82 (m, 4H), 6.23 (dd, 1H, *J*=9.6, 4.9), 4.33 (m, 1H), 4.27 (dd, 1H, *J*=14.8, 7.5), 3.79 (m, 6H), 3.80–3.55 (m, 4H), 2.85 (m, 1H), 2.64 (m, 1H), 2.58 (t, 1H, *J*=6.3), 2.52 (m, 1H), 2.19 (m, 2H), 1.70 (m, 1H), 1.50 (m, 3H), 1.16 (m, 9H), 1.10 (m, 3H), 1.05 ppm (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ=163.59, 158.89, 150.19, 145.61, 136.87, 136.59, 135.70, 130.24, 130.14, 128.35, 128.19, 127.18, 113.69, 111.30, 91.69, 89.46, 88.19, 85.18, 77.44, 76.32, 76.20, 60.62, 58.17, 57.99, 55.47, 43.66, 43.51, 38.94, 38.85, 24.76, 24.53, 24.45, 20.56, 14.41, 12.30, 12.26 ppm; ^{31}P NMR (122 MHz, $CDCl_3$): δ=142.13, 141.81; HRMS (NSI+, MeCN): *m/z* calcd for $C_{42}H_{51}N_4O_8P$: 771.3517; found: 771.3531 $[M+H^+]$

*(3′S,6′R)-N6-Benzoyl-9-{3′-O-[(2-cyanoethoxy)(diisopropylamino)phosphino]-2′-deoxy-6′-O-[4,4′-dimethoxytriphenyl)methyl]-3′,5′-ethano-β-D-ribofuranosyl} adenine (**34b**)*: Compound **31b** (290 ng, 0.42 mmol) was dissolved in MeCN (4 mL) and Hünig's base (0.36 mL, 2.1 mmol) and CEP-Cl (0.28 mL, 1.3 mmol) were added. The solution was stirred at RT for 1 h, diluted with ethyl acetate and washed with sat. $NaHCO_3$. The aqueous layer was extracted with ethyl acetate and the organic layers dried over $MgSO_4$. Purification by FC (ethyl acetate/hexane 1:1) yielded phosphoramidite **34b** (310 mg, 0.35 mmol, 83%) as a white foam. ^{31}P NMR showed a H-phosphonate peak. Compound **34b** had therefore to be further purified and was dissolved in CH_2Cl_2 (1 mL) and added dropwise to ice-cold hexane. The filter cake was collected by washing the filter with CH_2Cl_2 and evaporating the solvent. ^{31}P NMR showed no more phosphonate so the pure phosphoramidite **34b** (325 mg, 0.37 mmol, 87%) could be collected. R_f=0.48 (ethyl acetate/hexane 2:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=9.07 (s, 1H), 8.83 (d, 1H, *J*=3.2), 8.26 (s, 1H), 8.04 (m, 2H), 7.61 (m, 1H), 7.55 (m, 2H)), 7.46 (m, 2H), 7.36 (m, 4H), 7.32 (m, 2H), 6.82 (m, 4H), 6.45 (m, 1H), 4.36 (m, 2H), 3.78 (s, 6H), 3.74–3.54 (m, 4H), 3.05 (m, 2H), 2.63 (t, 1H, *J*=6.3), 2.55 (t, 1H, *J*=6.3), 2.08 (m, 1H), 1.90 (m, 2H), 1.50 (m, 1H), 1.16 (m, 9H,), 1.10 ppm (d, *J*=6.8, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ=164.71, 158.73, 153.02, 151.71,

145.59, 141.41, 141.28, 136.85, 136.80, 134.02, 132.86, 130.21, 130.16, 129.02, 128.22, 128.15, 128.00, 127.02, 123.21, 117.78, 117.72, 113.49, 91.16, 91.07, 91.04, 90.95, 89.89, 89.71, 89.68, 89.64, 87.60, 87.55, 85.26, 85.11, 77.36, 75.67, 75.53, 58.11, 58.03, 57.93, 57.85, 55.38, 45.48, 45.40, 45.12, 43.60, 43.56, 43.48, 43.43, 39.35, 39.32, 24.73, 24.67, 24.50, 24.47, 24.39, 20.56, 20.48, 20.40 ppm; ^{31}P NMR (122 MHz, $CDCl_3$): $\delta = 142.13$, 142.07; HRMS (NSI+, MeCN): *m/z* calcd for $C_{49}H_{54}N_7O_7P$: 884.3901; found: 884.3902 $[M+H^+]$.

*(3′S,6′R)-N6-Benzoyl-1-{3′-O-[(2-cyanoethoxy)(diisopropylamino)phosphino]-2′-deoxy-6′-O-[4,4′-dimethoxytriphenyl)methyl]-3′,5′-ethano-β-*D*-ribofuranosyl} cytosine (**35b**)*: Compound **32b** (216 mg, 0.33 mmol) was dissolved in MeCN (3 mL) and Hünig's base (0.28 mL, 1.6 mmol) and CEP-Cl (0.22 mL, 0.99 mmol) were added. The solution was stirred at RT for 2 h, diluted with ethyl acetate and washed with sat. $NaHCO_3$. The aqueous layer was extracted with ethyl acetate and dried over $MgSO_4$. Purification using by FC (ethyl acetate/hexane 1:1) yielded phosphoramidite **35b** (214 mg, 0.24 mmol, 72%) as a white foam. ^{31}P NMR showed a phosphonate peak. Compound **35b** had therefore to be further purified; it was dissolved in of CH_2Cl_2 (1 mL) and added dropwise to ice-cold hexane. The filter cake was collected by washing the filter with CH_2Cl_2 and evaporating the CH_2Cl_2. After three cycles and another FC the ^{31}P NMR spectra showed no more phosphonate so the pure phosphoramidite **35b** could be collected. $R_f = 0.4$ (ethyl acetate/hexane, 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.70$ (br, 1H), 8.20 (m, 1H), 7.90 (m, 2H), 7.62 (m, 1H), 7.52 (m, 2H), 7.44 (m, 2H), 7.33 (m, 5H), 7.25 (m, 3H), 6.84 (m, 4H), 6.24 (m, 1H), 4.40 (m, 2H), 3.78 (2 s, 6H), 3.77–3.53 (m, 4H), 3.11 (dd, 1H, 13.9, 4.7), 2.65 (t, 1H, $J = 6.3$), 2.57 (t, 1H, $J = 6.4$), 2.38 (m, 1H), 2.09 (m, 1H), 1.85 (m, 1H), 1.81 (m, 1H), 1.44 (m, 1H) 1.15 (m, 9H), 1.08 ppm (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta =$ 162.21, 158.87, 158.84, 145.37, 136.79, 136.63, 133.33, 130.26, 130.16, 129.25, 128.32, 127.75, 127.17, 113.63, 91.68, 91.59, 91.52, 91.43, 90.40, 90.34, 90.17, 90.10, 88.12, 88.09, 87.38, 87.34, 77.43, 76.44, 76.43, 58.25, 58.08, 58.00, 55.48, 55.42, 46.98, 46.96, 46.74, 46.64, 46.56, 46.33, 46.25, 45.79, 45.68, 43.64, 43.51, 39.09, 24.79, 24.72, 24.53, 24.47, 24.40, 24.21, 24.13, 24.10, 22.54, 20.54, 20.50, 20.46, 20.42, 14.41, 14.33 ppm; ^{31}P NMR (122 MHz, $CDCl_3$): $\delta = 149.96$, 149.68 ppm; HRMS (NSI+, THF): *m/z* calcd for $C_{48}H_{54}N_5O_8P$: 860.3788; found: 860.3789 $[M+H^+]$.

*(3R,5S,7S)-3-Methoxyoctahydropentalene-7,5-diol (**36b**)*: The sugar **10b** (1.0 g, 3.6 mmol) was dissolved in 0.2 M NaOH in THF/methanol/H_2O (5:4:1, 150 mL) at 0 °C. The solution was stirred for one hour at 0 °C and quenched by the addition of ammonium chloride. The solvents were then evaporated and the remains absorbed on silica gel. Purification by using FC (5% methanol in CH_2Cl_2) yielded compound **36b** (618 mg, 3.5 mmol, 97%) as a colorless oil. $R_f = 0.14$ (ethyl acetate/hexane 1:1); ^{1}H NMR (300 MHz, MeOD): $\delta = 4.98$ (dd, 1H, $J = 5.8$, 2.0), 4.40 (m, 1H), 4.23 (dd, 1H, = 6.7, 2.3), 3.27 (s, 3H), 2.27 (m, 2H), 2.00 (m, 1H), 1.94 (m, 1H), 1.85 (m, 1H), 1.74 ppm (dd, 1H, $J = 13.0$, 7.9); ^{13}C NMR (75 MHz, MeOD): $\delta = 107.38$, 90.61, 86.83, 72.44, 55.27, 49.70, 48.90, 42.88 ppm; HRMS (NSI+, MeOH): *m/z* calcd for $C_8H_{14}O_4$: 197.0784; found: 197.0788 $[M+Na^+]$.

*tert-Butyl-(((3R,5S,7S)-3-methoxy-7-(((2,3,3-trimethylbutan-2-yl)silyl)oxy)-octahydropentalen-3a-yl)oxy)dimethylsilane (**37b**)*: Compound **36b** (1.7 g, 10.0 mmol) was dissolved in DMF (40 mL). TBSCl (3.8 g, 25.0 mmol) and imidazole (2.1 g, 30.0 mmol) were added and the mixture was stirred at 85 °C for 2 days. The solution was then diluted with ethyl acetate, washed with brine and H_2O and dried over $MgSO_4$. Purification by using FC (ethyl acetate/hexane, 3:1) yielded **46** (3.3 g, 8.2 mmol, 82%) as a colorless oil. $R_f = 0.9$ (ethyl acetate/hexane 1:1); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 4.99$ (dd, 1H, $J = 5.7$, 2.2), 4.49 (m, 1H), 4.25 (d, 1H, $J = 6.4$), 3.33 (s, 3H), 2.33 (m, 2H), 2.00 (m, 2H), 1.77 (m, 2H), 0.88 (m, 18H), 0.08 ppm (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 105.87$ (C(3)), 89.93 ((C1)), 71.85 (C(7)), 55.13 (OMe), 50.60 (C(4)), 49.47 (C(6)), 41.98 (C(8)), 26.08 (TBS), 25.89 (TBS), 18.42 (TBS), 17.99 (TBS), −2.50 (TBS), −2.55 ppm (TBS); HRMS (NSI+, THF): *m/z* calcd for $C_{20}H_{42}O_4Si_2$: 403.2694; found: 403.2693 $[M+H^+]$.

*(3′S,6′S)-2-Amino-6-chloro-9-{3′,6′-di-O-[(tert-butyl)-dimethylsilyl]-2′-deoxy-3′,5′-ethano-α- and -β-*D*-ribofuranosyl} purine (**38a/b**)*: To a suspension of 2′-amino-6′-chloropurine (814 mg, 4.8 mmol) in MeCN (11 mL) was added BSA (2.3 mL. 9.6 mmol). The mixture was stirred at r.t. until a clear solution appeared. Compound **46** (858 mg, 2.1 mmol, in 5 mL MeCN) and TMSOTf (0.13 mL, 0.72 mmol) was then added and the mixture was stirred for 6 h at 55 °C. The brownish solution was diluted by the addition of ethyl acetate, washed with sat. $NaHCO_3$ and dried over $MgSO_4$. Purification by FC (ethyl acetate/hexane, 1:3) yielded **38b** (411 mg, 0.76 mmol, 36%) and **38a** (316 mg, 0.58 mmol, 28%) as white foams. Compound **38b**: $R_f = 0.4$ (hexane/ethyl acetate, 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.87$ (s, 1H), 6.00 (dd, 1H, $J = 9.1$, 5.6), 5.13 (br, 2H), 4.40 (m, 1H), 4.31 (d, 1H, $J = 6.3$), 2.54 (m, 2H), 2.34 (m, 1H), 2.12 (m, 1H), 1.84 (m, 2H), 0.90 (m, 18H), 0.16 (m, 6H), 0.08 ppm (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 159.18$, 153.72, 151.77, 140.60, 126.19, 89.88, 86.42, 83.86, 71.80, 48.95, 47.24, 40.71, 26.05, 25.87, 18.33, 18.03, −2.37, −2.45, −4.42, −4.48 ppm; HR-MS (NSI+, EtOAc): *m/z* calcd for $C_{24}H_{43}N_5O_3ClSi_2$: 540.2587; found: 540.2608 $[M+H^+]$; Compound **38a**: $R_f = 0.26$ (hexane/ethyl acetate 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 8.19 (s, 1H), 6.24 (dd, 1H, $J = 7.1$, 1.6), 5.11 (br, 2H), 4.45 (d, 1H, $J =$ 6.1), 4.25 (m, 1H), 2.85 (dd, 1H, $J = 14.6$, 1.5), 2.48 (dd, 1H, = 14.6, 1.5), 2.30 (m, 1H), 2.12 (m, 1H), 1.90 (m, 1H), 1.81 (m, 1H), 0.89, 0.80 (2 s, 18H_3), 0.12 (s, 3H), 0.07 (s, 6H), 0.02 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 159.15$, 153.63, 151.52, 141.11, 125.92, 91.16, 86.93, 84.80, 71.49, 50.35, 48.48, 40.79, 26.04, 25.79, 18.31, 17.97, −2.26, −2.55, −4.47, −4.56 ppm; HRMS (NSI+, EtOAc): *m/z* calcd for $C_{24}H_{43}N_5O_3ClSi_2$: 540.2587; found: 540.2607 $[M+H^+]$.

*(3′S,6′S)-)-9-{3′,6′-Di-O-[(tert-butyl)-dimethylsilyl]-2′-deoxy-3′,5′-ethano-α- and -β-*D*-ribofuranosyl} guanine (**39a/b**)*: NaH (60%, 285 mg, 7.1 mmol) was carefully added to a solution of 3-hydroxypropionitrile (0.4 mL, 5.9 mmol) in THF (8 mL) at 0 °C and the suspension was stirred for 1 h at RT. The anomeric compound **38a/b** (700 mg, 1.3 mmol in 8 mL THF) was added and the brown mixture was stirred for another 3 h at RT. Addition of silica, evaporation of the solvents and filtration (10% methanol in CH_2Cl_2) over a patch of silica yielded the crude product **39a/b**. The brown foam was used without further purification in the next step. $R_f =$ 0.5 (10% methanol in CH_2Cl_2); HRMS (NSI+, MeCN) *m/z* calcd for $C_{24}H_{43}N_5O_4Si_2$: 521.81; found 522.29 $[M+H^+]$.

*(3′S,6′S)-N2-(N,N-Dimethylformamidino)-9-{3′,6′-di-O-[(tert-butyl)-dimethylsilyl]-2′-deoxy-3′,5′-ethano-α- and -β-*D*-ribofuranosyl} guanine (**40a/b**)*: The crude compound **39a/b** (750 mg, ≈1.4 mmol) was dissolved in DMF (12 mL) and *N,N*-dimethylformamide dimethyl acetate (0.37 mL, 2.8 mmol) was added. The clear yellow solution was stirred for 2 h at 55 °C. The solvent was evaporated through Kugelrohr distillation and purified by using FC (5% methanol in CH_2Cl_2) to obtain nucleoside **40a/b** (715 mg, 1.2 mmol, 71% over 2 steps) in an anomeric ratio of α/β≈2:1as yellow oil. $R_f = 0.5$ (10% methanol in CH_2Cl_2); ^{1}H NMR (300 MHz, MeOD): $\delta = 8.66$ (s, 1H), 8.62 (s, 1H), 8.01 (2 s, 2H), 6.25 (dd, 1H, $J =$ 6.8, 1.7), 6.14 (dd, 1H, $J = 9.6$, 5.1), 4.57 (d, 1H, $J = 6.1$), 4.45 (m, 1H), 4.34 (m, 2H), 3.20 (m, 6H), 3.12 (m, 6H), 2.99 (s, 1H), 2.86 (m, 2H), 2.64–2.36 (m, 5H), 2.12 (m, 2H), 1.85 (m, 4H), 0.96 (s, 18H), 0.92 (2 s, 18H), 0.77 (m, 12H), 0.21 ppm (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 158.38$, 158.28, 158.22, 157.10, 156.90, 150.37, 135.72, 118.34, 120.54, 90.85, 89.89, 87.00, 86.54, 84.23, 82.88, 77.43, 71.95, 71.65, 58.07, 48.82, 48.72, 41.56, 40.80, 35.43, 26.02, 25.90, 25.83, 21.70, 18.27, 18.06, 18.00, −2.29, −2.46, −2.52, −4.47, −4.54 ppm; HRMS (NSI+, EtOAc): *m/z* calcd for $C_{27}H_{49}N_6O_4Si_2$: 577.3348; found: 577.3339 $[M+H^+]$.

*(3′S,6′S)-N2-(N,N-Dimethylformamidino)-9-{3′-O-[(tert-butyl)-dimethylsilyl]-2′-deoxy-3′,5′-ethano-α- and -β-*D*-ribofuranosyl} guanine (**41a/b**)*: A solution of **40a/b** (1.5 g, 2.6 mmol) in THF (20 mL) was cooled to 0 °C. HF/Et_3N (37% HF, 1.7 mL, 3.9 mmol) was added and allowed to warm to RT. The reaction mixture was stirred at RT overnight and then quenched with silica. The solvent was evaporated and the crude mixture purified by using FC (5% methanol in CH_2Cl_2). **41a/b** (658 mg, 1.4 mmol, 55%) in an anomeric ratio of α/β≈2:1was obtained as a white foam. $R_f = 0.5$ (10% methanol in CH_2Cl_2), ^{1}H NMR (400 MHz, MeOD): $\delta = 8.66$ (s, 1H), 8.62 (s, 1H), 8.01 (s, 1H), 7.97 (s, 1H), 6.24 (dd, 1H, $J =$ 6.7, 1.6), 6.09 (dd, 1H, $J = 10.0$, 5.1), 4.59 (m, 1H), 4.53 (m, 1H), 4.30 (m, 1H), 4.25 (m, 1H), 3.21 (m, 6H), 3.12 (m, 6H), 2.92 (dd, 1H, $J = 14.6$, 1.6), 2.71 (m, 1H), 2.60 (m, 2H), 2.49 (m, 1H), 2.41 (m, 1H), 2.15 (m, 2H), 1.89–1.79 (m, 4H), 0.95 (s, 9H), 0.76 (s, 9H), 0.22 (m, 6H), 0.12 (s,

3H), −0.02 ppm (s, 3H); ^{13}C NMR (101 MHz, MeOD): δ=160.16, 159.69, 159.62, 159.00, 158.85, 151.96, 151.43, 138.82, 138.31, 121.07, 120.95, 92.76, 91.03, 88.52, 88.12, 87.23, 84.98, 71.63, 71.39, 50.21, 47.74, 41.62, 41.45, 41.18, 40.91, 35.34, 26.24, 26.07, 18.73, 18.56, −2.41, −2.45, −2.48, −2.75 ppm; HRMS (NSI+, MeCN): *m/z* calcd for $C_{21}H_{34}N_6O_4Si$: 463.2484; found: 463.2472 [$M+H^+$].

*(3′S,6′S)-N2-(N,N-Dimethylformamidino)-9-{6′-O-(methylsulfonyl)-3′-O-[(tert-butyl)-dimethylsilyl]-2′-deoxy-3′,5′-ethano-α- and -β-*D*-ribofuranosyl} guanine (**42a/b**)*: A solution of **41a/b** (243 mg, 0.53 mmol) in pyridine (5.5 mL) was cooled to 0 °C and MsCl (40 μL, 0.58 mmol) was added. The mixture was stirred for 4 h at RT. After the addition of silica the pyridine was evaporated and the crude product purified by using FC (5% methanol in CH_2Cl_2). Compound **42a/b** (263 mg, 0.49 mmol, 92%) in an anomeric ratio of α/β≈2:1was obtained as a white solid. R_f=0.4 (10% methanol in CH_2Cl_2), ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.62 (s, 1H, N=CHN(CH_3)$_2$), 8.53 (s, 1H), 7.93 (s, 1H), 7.79 (s, 1H), 6.24 (dd, 1H, *J*=7.1, 3.7), 6.04 (dd, 1H, *J*=9.4, 5.4), 5.24–5.13 (m, 2H), 4.54 (d, 1H, *J*=5.4), 4.35 (dd, 1H, *J*=6.9, 1.6), 3.20 (s, 3H), 3.18 (s, 3H), 3.11 (s, 6H), 3.05 (s, 3H), 3.04 (s, 3H), 2.72 (m, 2H), 2.61 (m, 4H), 2.42 (m, 2H), 2.26–2.12 (m, 4H), 0.92 (s, 9H), 0.87 (s, 9H), 0.19 (s, 3H), 0.18 (s, 3H), 0.15 (s, 3H), 0.08 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ=158.06, 157.99, 156.93, 150.34, 136.75, 136.43, 121.20, 89.19, 88.54, 86.97, 86.46, 83.87, 79.69, 79.22, 47.39, 47.30, 46.53, 46.39, 41.70, 38.59, 38.00, 37.84, 35.50, 25.83, 25.80, 18.04, −2.46, −2.48 ppm; HRMS (NSI+, EtOAc): *m/z* calcd for $C_{22}H_{36}N_6O_6SSi$: 540.2186; found: 541.2183 [M^+].

*(3′S,6′R)-N2-{N,N-Dimethylformamidino}-9-{6′-O-acetyl-3′-O-[(tert-butyl)-dimethylsilyl]-2′-deoxy-3′,5′-ethano-α- and -β-*D*-ribofuranosyl} guanine (**43a/b**)*: A solution containing **42a/b** (620 mg, 1.15 mmol) and CsOAc (3.0 g, 15.9 mmol) in DMSO (16 mL) was heated to 85 °C and stirred for 16 h. After cooling to RT the mixture was diluted with ethyl acetate and washed with sat. $NaHCO_3$. The combined organic layers were washed with H_2O and dried over $MgSO_4$. By using FC purification (5% methanol in CH_2Cl_2) **43a/b** (491 mg, 0.97 mmol, 85%) was obtained in an anomeric ratio of α/β≈2:1 as a white foam. The ^{1}H NMR spectra still showed a considerable amount of DMSO. R_f=0.5 (10% methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=9.72 (br, 2H), 8.60 (s, 1H), 8.58 (s, 1H), 7.95 (s, 1H), 7.89 (s, 1H), 6.32 (m, 1H), 6.22 (m, 1H), 5.34 (m, 2H), 4.59 (m, 1H), 4.36 (dd, 1H, *J*=6.9, 1.3), 3.17 (m, 6H), 3.10 (m, 6H), 2.70–2.53 (m, 4H), 2.41- 2.28 (m, 6H) 2.16 (m, 2H), 2.06 (s, 3H), 2.05 (s, 3H), 0.90 (s, 9H), 0.87 ppm (s, 9H,), 0.14 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$): δ=170.49, 158.39, 158.13, 157.17, 156.95, 150.16, 136.83, 135.83, 132.06, 120.35, 95.82, 91.14, 91.03, 89.43, 89.36, 84.92, 83.83, 76.72, 76.30, 48.04, 46.85, 46.22, 45.88, 41.57, 37.76, 37.59, 35.40, 25.85, 25.80, 25.74, 21.52, 21.40, 17.99, −2.56, −2.63, −2.73 ppm; HRMS (NSI+, EtOAc) calcd for $C_{23}H_{36}N_6O_5Si$: 505.2589; found: 505.2582 [$M+H^+$].

*(3′S,6′R)-9-{3′-O-[(tert-Butyl)-dimethylsilyl]-2′-deoxy-3′,5′-ethano-α- and -β-*D*-ribofuranosyl} guanine (**44a/b**)*: The anomeric mixture **43a/b** (512 mg, 1.0 mmol) was dissolved in 1 M KOH in methanol/H_2O (5:3, 12 mL). The solution was stirred for 6 h at 60 °C and quenched by the addition of ammonium chloride. The solvents were then evaporated and the remains absorbed on silica gel. FC (5% methanol in CH_2Cl_2) yielded the anomers **44b** (170 mg, 0.42 mmol, 42%) and **44a** (78 mg, 0.2 mmol, 20%) as white solids. Compound **44a**: R_f=0.27 (10% methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, MeOD): δ=7.96 (s, 1H), 6.19 (dd, 1H, *J*=9.5, 5.7), 4.48 (m, 1H), 4.34 (dd, 1H, *J*=7.0, 1.7), 2.93 (dd, 1H, *J*=13.4, 9.6), 2.59 (dd, 1H, *J*=13.4, 5.7), 2.26 (m, 2H), 2.08 (dd, 1H, *J*=13.7, 5.4), 1.95 (m, 1H), 0.93 (s, 9H), 0.19 (s, 3H), 0.17 ppm (s, 3H); ^{13}C NMR (101 MHz, MeOD): δ=159.29, 155.44, 152.66 137.90, 117.66, 93.15, 91.18, 85.41, 74.81, 49.30, 41.34, 26.18, 18.69, −2.53, −2.59 ppm; HRMS (NSI+, MeOH): *m/z* calcd for $C_{18}H_{29}N_5O_4Si$: 408.2062; found: 408.2056 [$M+H^+$]; Compound **44b**: TLC R_f=0.27 (10% methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, MeOD): δ=7.89 (s, 1H), 6.26 (dd, 1H, *J*=6.7, 3.0), 4.66 (dd, 1H, *J*=7.0, 2.0), 4.45 (m, 1H), 2.95 (dd, 1H, *J*=13.9, 6.7), 2.75 (dd, 1H, *J*=13.9, 3.0), 2.31 (m, 1H), 2.14 (m, 2H), 1.84 (m, 1H), 0.78 (s, 9H), 0.11 (s, 3H), −0.02 ppm (s, 3H); ^{13}C NMR (101 MHz, MeOD): δ=159.33, 155.17, 152.30, 137.30, 118.09, 94.21, 91.23, 88.09, 74.20, 49.66, 48.90, 41.35, 26.06, 18.57, −2.56, −2.89 ppm; HRMS (NSI+, MeOH): *m/z* calcd for $C_{18}H_{29}N_5O_4Si$: 408.2062; found: 408.2057 [$M+H^+$].

*(3′S,6′R)-N2-(N,N-Dimethylformamidino)-9-{3′-O-[(tert-butyl)-dimethylsilyl]-2′-deoxy-3′,5′-ethano-β-*D*-ribofuranosyl} guanine (**45b**)*: The nucleoside **44b** (170 mg, 0.41 mmol) was dissolved in DMF (4.5 mL) and *N,N*-dimethylformamide dimethyl acetate (0.11 mL, 0.82 mmol) was added. The solution was stirred for 3 h at 55 °C. The DMF was then evaporated by Kugelrohr distillation and the crude product purified by FC (10% methanol in CH_2Cl_2). Compound **45b** (122 mg, 0.26 mmol, 64%) was obtained as a white foam. R_f=0.3 (10% methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=9.13 (s, 1H), 8.68 (s, 1H), 7.87 (s, 1H), 6.04 (dd, 1H, *J*=10.0, 5.5), 4.53 (m, 1H), 4.35 (d, 1H, *J*=6.3), 3.22 (dd, 1H, *J*=13.4, 10.1), 3.17 (s, 3H), 3.07 (s, 3H), 2.53 (dd, 1H, *J*=13.4, 5.5), 2.40 (m, 1H), 2.18 (m, 1H) 2.10 (m, 1H), 2.01 (m, 1H), 0.90 (m, 9H), 0.15 (s, 3H), 0.13 ppm (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=81.93, 80.54, 79.99, 72.95, 60.80, 44.42, 15.14, 12.56, 7.82, 0.20, −1.84, −27.06, −29.22, −35.90, −36.81, −42.05, −51.34, −59.19, −79.57, −79.66; HRMS (NSI+, MeCN): *m/z* calcd for $C_21H_{34}N_6O_4Si$: 463.2484; found: 463.2475 [$M+H^+$].

*(3′S,6′R)-N2-(N,N-Dimethylformamidino)-9-{5′-O-[4,4′-(dimethoxytriphenyl)methyl]-3′-O-[(tert-butyl)-dimethylsilyl]-2′-deoxy-3′,5′-ethano-β-*D*-ribofuranosyl} guanine (**46b**)*: DMT-Cl (154 mg 0.45 mmol) was added in three portions to a solution of nucleoside **45b** (70 mg, 0.15 mmol) in pyridine (0.7 mL) and it was stirred for 24 h at RT. The solution was then diluted with ethyl acetate, washed with sat. $NaHCO_3$ and dried over $MgS0_4$. Purification by FC (5% methanol in CH_2Cl_2) yielded the nucleoside **46b** (108 mg, 0.14, mmol, 94%) as a white foam. R_f=0.66 (10% methanol in CH_2Cl_2), ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.94 (br, 1H), 8.61 (s, 1H), 7.88 (m, 1H), 7.47 (m, 2H), 7.36 (m, 4H), 7.30 (m, 1H), 7.20 (m, 1H), 6.83 (m, 4H), 6.19 (dd, 1H, *J*=9.6, 5.1), 4.27 (m, 1H), 4.12 (dd, 1H, *J*=7.5, 3.9), 3.79 (s, 6H), 3.13 (s, 3H), 3.10 (s, 3H), 2.73 (dd, 1H, *J*=13.0, 9.6), 2.42 (dd, 1H, *J*=13.0, 5.1), 1.98 (m, 1H), 1.68 (m, 1H), 1.60 (m, 2H), 0.83 (s, 9H,), 0.04 (s, 3H), −0.04 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=158.86, 158.29, 157.96, 156.81, 150.17, 145.69, 136.96, 136.21, 130.28, 128.28, 128.22, 127.11, 113.55, 90.76, 88.62, 87.44, 84.73, 75.66, 55.47, 46.96, 45.56, 41.51, 39.70, 35.37, 25.88, 18.02, −2.63 ppm; HRMS (NSI+, MeCN): *m/z* calcd for $C_{42}H_{52}N_6O_6Si$: 765.3790; found: 765.3779 [$M+H^+$].

*(3′S,6′R)-N2-(N,N-Dimethylformamidino)-9-{6′-O-[4,4′-(dimethoxytriphenyl)methyl]-2′-deoxy-3′,5′-ethano-β-*D*-ribofuranosyl} guanine (**47b**)*: Nucleoside **46b** (74 mg, 0.1 mmol) was dissolved in THF (2.2 mL) and TBAF (1 M in THF, 0.1 mL, 0.1 mmol) was added. The clear solution was stirred for 5 h at RT and then quenched by the addition of silica. Purification by FC (5% methanol in CH_2Cl_2) yielded **47b** (44 mg, 70%) as a white foam. R_f=0.4 (10% methanol in CH_2Cl_2); ^{1}H NMR (400 MHz, MeOD): δ=8.68 (s, 1H), 7.97 (s, 1H), 7.46 (m, 2H), 7.34 (m, 4H), 7.27 (m, 2H), 7.21 (m, 1H), 6.84 (m, 4H), 6.24 (dd, 1H, *J*=9.4, 5.4), 4.33 (m, 1H), 4.08 (dd, 1H, *J*=7.4, 3.4), 3.78 (s, 6H), 3.16 (s, 3H), 3.12 (s, 3H), 2.91 (dd, 1H, *J*=13.1, 9.5), 2.49 (dd, 1H, *J*=13.2, 5.4), 1.95 (m, 1H), 1.82 (m, 1H), 1.67 (dd, 1H, *J*=14.0, 6.1), 1.57 ppm (m, 1H); ^{13}C NMR (101 MHz, MeOD): δ=160.25, 159.82, 159.17, 151.84, 147.10, 138.25, 138.09, 138.06, 131.32, 129.33, 128.92, 127.86, 120.65, 114.27, 90.89, 88.62, 87.54, 85.98, 77.02, 55.73, 47.44, 46.93, 41.52, 40.74, 35.33 ppm; HRMS (NSI+, MeCN, 0.1% HCOOH): *m/z* calcd for $C_{36}H_{38}N_6O_6$: 651.2926; found: 651.2925 [$M+H^+$].

*(3′S,6′R)-N2-(N,N-Dimethylformamidino)-9-{3′-O-[(2-cyanoethoxy)(diisopropylamino)phosphino]-6′-O-[4,4′-(dimethoxytriphenyl)methyl]-3′-O-[(tert-butyl)-dimethylsilyl]-2′-deoxy-3′,5′-ethano-β-*D*-ribofuranosyl} guanine (**48b**)*: Hünig's base (0.06 mL, 0.34 mmol) and CEP-Cl (0.04 mL, 0.2 mmol) was added to a solution of **47b** (44 mg, 0.07 mmol) in MeCN (1 mL). The mixture was stirred at RT for 1 h then diluted with ethyl acetate and washed with sat. $NaHCO_3$. Purification by FC (3% methanol in CH_2Cl_2) yielded the desired phosphoramidite **48b** (50 mg, 0.06 mmol, 88%) as a white foam. R_f=0.7 (10% methanol in CH_2Cl_2), ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.95 (br, 1H), 8.60 (m, 1H), 7.85 (m, 1H), 7.45 (m, 2H), 7.35 (m, 4H), 7.28 (m, 2H), 7.21 (m, 1H), 6.83 (m, 4H), 6.22 (m, 1H), 4.32 (m, 2H), 3.79 (m, 6H), 3.77–3.54 (m, 4H), 3.13 (s, 3H), 3.09 (m, 3H), 2.84 (m, 2H), 2.57 (t, 1H, *J*=6.3), 2.51 (t, 1H, *J*=6.3), 2.09

(m, 1H), 1.86 (m, 2H), 1.60 (m, 1H), 1.15 (m, 9H), 1.09 ppm (d, 3H, J= 6.8); ^{13}C NMR (101 MHz, $CDCl_3$): δ=158.80, 158.33, 158.00, 156.94, 156.88, 150.38, 150.30, 145.66, 136.87, 136.83, 136.38, 130.30, 130.25, 128.24, 127.08, 120.90, 117.75, 113.53, 90.91, 90.82, 90.76, 89.12, 87.55, 87.52, 84.33, 75.62, 75.51, 58.20, 58.02, 57.92, 55.46, 44.92, 44.60, 43.59, 43.47, 41.50, 39.41, 35.36, 29.89, 24.75, 24.50, 20.53 ppm; ^{31}P NMR (122 MHz, $CDCl_3$): δ=141.88 ppm; HRMS (NSI+, MeCN) for $C_{45}H_{55}N_8O_7P$: *m/z* calcd for: 851.4010; found: 851.4009 [$M+H^+$].

RNaseH activity: A solution (0.2 μM) of the oligonucleotide **S10** and of the corresponding DNA and RNA strands was solved in a buffer system (containing 75 mM KCl, 50 mM Tris-HCl, 3 mM $MgCl_2$ and10 mM dithiothreitol) and was then hybridized with 0.1 μM of the ^{32}P labeled complementary RNA strand to give a total volume of 60 μL. After heating to 80°C the solution was incubated for 15 min at 37°C. The enzyme (2.5 U) was then added and the mixture was kept at 25°C. Aliquots were taken at 5, 45, 120, and 300 min and were quenched by the addition of a loading buffer (100% formamide, bromphenol blue, and xylencyanol). The samples were stored at 4°C overnight, analyzed on a 20% denaturing polyacrylamide gel and the bands visualized by autoradiography.

Serum stability: The sequence **S10** (202 μL) and the corresponding DNA sequence 108 μL, ≈30 μg each) were speed vac dried. The remains were taken up in Dulbecco's Modified Eagle Medium DMEM/F12 (386 μL), incubated at 37°C and FBS (40 μL) was added to give a total concentration of 10% serum. Aliquots of 84 μL were taken after 0, 0.5, 2, 6.5, and 21 h and quenched by the addition of 9 M urea (80 μL) in 2x TBE buffer. H_2O (60 μL) were then added to the solutions and they were precipitated in 3 M NaOAc (25 μL) and cold ethanol (1 mL). After 1 h at −80°C the tubes were centrifuged for 1 h at 2°C. The remains were taken up in loading buffer (15 μL, formamide/TBE 9:1) and separated on a 20% polyacrylamide gel. To visualize the bands the gel was stained with stains-all.

Isobicyclo-oligonucleotide synthesis: The chemical synthesis of oligonucleotides was performed on the 1.3 μmol scale on a Pharmacia LKB Gene Assembler Special DNA-synthesizer by using standard phosphoramidite chemistry. The phosphoramidite building blocks of the natural nucleosides and the nucleosides bound to CPG solid support were purchased from Glen Research. Universal solid support was purchased from Glen Research or CTGen. Natural oligonucleotides were purchased from Microsynth. Solvents and reagents used for the synthesis were prepared according to the manufacturer's indications. 5-(Ethylthio)-1H-tetrazole (ETT) was used as activator and dichloroacetic acid in dichloroethane was used for the detritylation step. The concentrations of the phosphoramidite solutions were 0.1 M. The coupling times for natural phosphoramidites were 1.5 min and for the modified phosphoramidite was 9 min. The oligonucleotides **S1**–**S4** were cleaved from the resin and deprotected in 33% NH_3 solution at 55°C overnight. Also the fully modified sequences **S6**, **S7**, **S10**, and **S11** were cleaved and deprotected in 33% NH_3 but at 70°C overnight. After evaporation the crude samples were filtered, taken up in water and purified by reversed phase (Source 15 RPC ST 100/4.6 Polystyrene-15 column from Pharmacia Biotech) or DEAE-HPLC (DNAPac-200 4×250 mm column with pre-column both from Dionex). All samples were then desalted over a Sep-Pak C-18 cartridge (Waters) according to the manufacturer's protocol. The integrity of all oligonucleotides was confirmed by ESI-MS spectrometry. Concentrations of the oligonucleotide solutions were determined by UV absorption at 260 nm. The extinction coefficients ε of the oligonucleotides were supposed to be identical to the natural oligonucleotides and were calculated using the oligocalculator (www.ambion.com).

UV/Vis spectroscopy: Thermal denaturation experiments were measured on a Cary 100 Bio, UV/Visible Spectrophotometer. The samples were measured at 260 nm and the heating rate was set to 0.5°C min^{-1}. The first derivatives of the curves were calculated with the Origin Pro 8 program. To avoid evaporation of the solution, a layer of dimethylpolysiloxane was added over the samples within the cell. The OD values of the oligonucleotides were measured with a NanoDrop ND-1000 Spectrophotometer at 260 nm.

Circular dichroism spectroscopy (CD): Circular dichroism spectra were recorded on a Jasco J-715 spectropolarimeter equipped with a Jasco PFO-350S temperature controller. The temperature was measured from the heating block. The graphs were subsequently smoothed with a noise filter. The phosphate buffer was used as blank. Spectra are given in mdeg from 210 to 320 nm.

Molecular modeling: Molecular modeling was carried out with the Amber force field as incorporated in the software package HyperChem Release 8.0.4 for Windows of Hypercube, Inc. Only original Amber parameters were used and no explicit water or counterions were included.

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DNA

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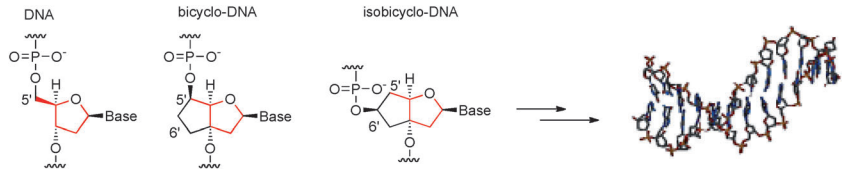
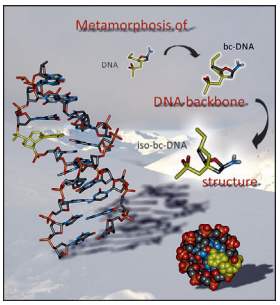
Synthesis and Properties of Isobicyclo-DNA



A twist to DNA structure: Moving the phosphodiester backbone from the natural C(5′) position into the C(6′) position, which is only available on the bicyclo-DNA skeleton, leads to isobicyclo-DNA (Isobc-DNA; see scheme). Isobc-DNA forms stable duplexes with DNA and discriminates RNA as a complement. In addition, it forms very stable antiparallel self-duplexes with a structure that is different from A-, B-, or Z-DNA.



DNA

The synthesis of isobicyclo-DNA (iso-bc-DNA) building blocks with all four natural bases is presented, as well as their incorporation into oligonucleotides and their RNA and DNA recognition properties. Their structure was determined by circular dichroism (CD) spectroscopy and their biological properties through serum stability and RNaseH activation. Slight changes in the DNA backbone geometry lead to remarkable consequences on the duplex structure, as can be seen from the properties of isobicyclo-DNA. For more details see the Full Paper by C. J. Leumann and A.-B. Gerber on page ■■ ff.