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Screening the Structural and Functional Properties of Bicyclo-DNA: bc^{ox}-DNA

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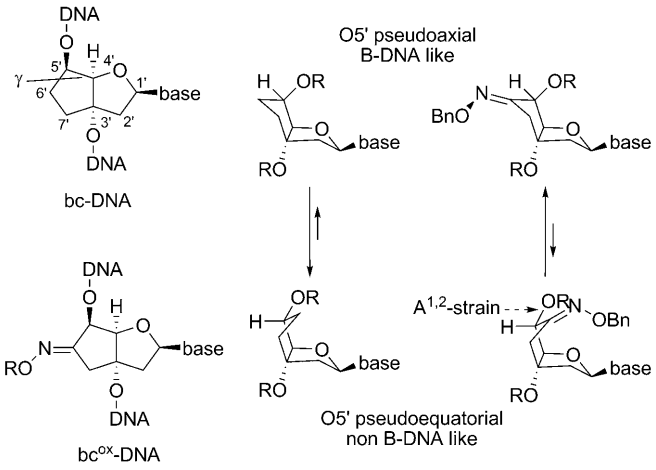
The synthesis of two novel pyrimidine bicyclonucleosides (bc^{ox}-nucleosides) has been accomplished. These bicyclonucleosides each carry a lipophilic benzyloxime substituent on the carbocyclic ring and show improved conformational similarity to 2′-deoxyribonucleosides as shown by their X-ray structures. The thymine-containing bc^{ox}-nucleoside was converted into the corresponding phosphoramidite building block and incorporated into oligodeoxyribonucleotides by standard phosphoramidite chemistry. T_m data with complementary RNA and DNA were measured and compared to corresponding cases of natural and unfunctionalized bc-DNA. It was found that single incorporations of bc^{ox} residues destabilize duplexes by roughly 5 °C per modification. The destabilization was found to be due to the oxime substituent and not to the bicyclic scaffold itself. No significant alteration of the base-pairing selectivity as a function of the modification was observed. With RNA (but not with DNA) as a complement the relative thermal destabilization of bc^{ox}-oligothymidylates was gradually reduced and converted into a stabilizing interaction with increasing numbers of consecutive modifications. While no cellular uptake of bc^{ox}-oligonucleotides into HeLa cells occurred without transfecting agents, a significant increase in the transfection rate relative to unmodified DNA was observed in complexation with lipofectamine.

Introduction

The concept of conformational restriction[1] has been successfully applied in the past in nucleic acid chemistry and has produced analogues such as, for example, the family of the locked nucleic acids (LNA, BNA, etc.),[2] the hexitol nucleic acids,[3] or tricyclo-DNA (tc-DNA),[4] all of which show increased affinity towards complementary RNA without base-pairing selectivity being compromised. These analogues are currently regarded as advanced generation antisense agents and are expected to replace the phosphorothioate DNA and some of the simpler 2′-O-alkyl-RNA analogues in therapy.[5] Besides this, some of these analogues have also proven to increase siRNA efficacy.[6] While chemistry has provided solutions for increasing duplex stability with target RNA and for enhancement of resistance towards nuclease-induced degradation, there are still a series of largely unsolved problems on the pathway to effective oligonucleotide drugs, the most prominent ones being cellular uptake and distribution,[7] as well as—depending on the mechanism of action—off-target effects.[8]

Our first-generation, conformationally restricted oligonucleotide analogue bicyclo-DNA (bc-DNA; Scheme 1)[9] shows no significantly improved RNA affinity relative to DNA, probably due to misalignment of one out of the six repetitive backbone torsion angles (γ, C4′–C5′ bond) in relation to standard duplex DNA and RNA. This is a consequence of the preferred conformation of the carbocyclic ring in bc-DNA, in which the 5′-O substituent occupies a pseudoequatorial position.

We reasoned that one way of correcting γ could be through the introduction of an sp^2 substituent at C(6′), causing an alteration of the conformation of the underlying five-membered ring in order to avoid $A^{1,2}$ strain. Substituents in this position would be expected to be sufficiently remote from the base-pairing region to avoid interference with duplex formation (Figure 1). Furthermore, they might allow for addition of further functionalities to oligonucleotides, which might in turn aid in improving biological properties such as, for example, facilitating cellular uptake.



Scheme 1. Chemical structures and conformational preferences of bicyclo (bc) DNA and bcox-DNA.

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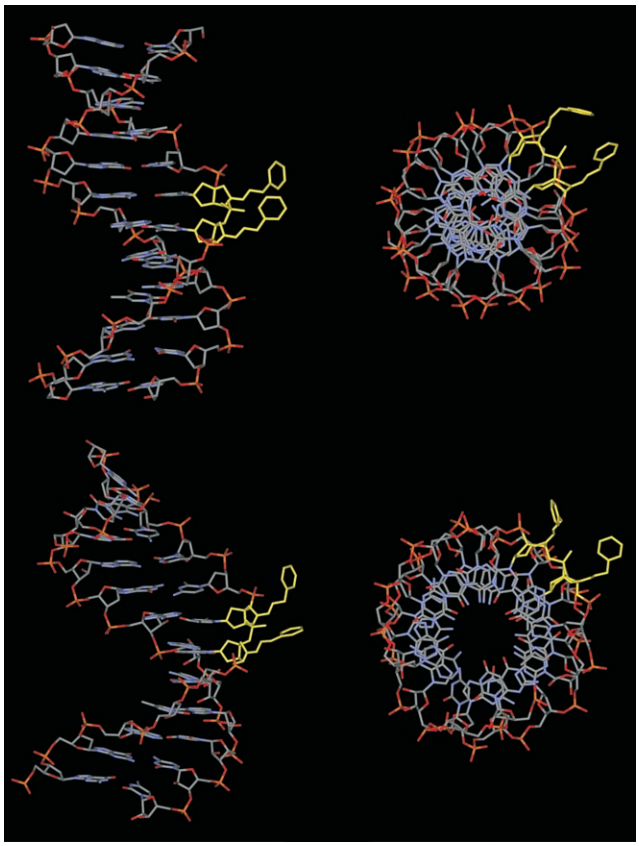


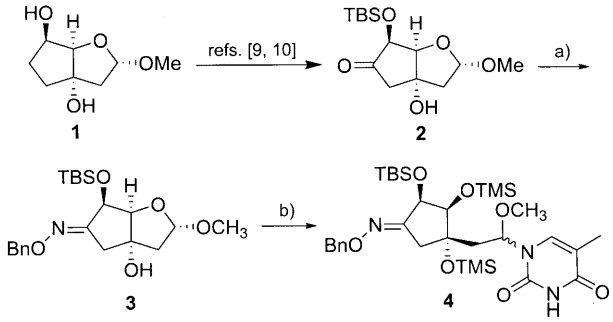
Figure 1. Energy-minimized molecular model of a DNA duplex containing two consecutive bc^{ox} modifications (colored in yellow) in one strand in either the B (top) or the A (bottom) conformation. Left: side view; right: view along the helical axis. The hydrophobic benzyl residues are located on the rim of the backbone and point out towards the solvent.

Here we report on the synthesis of bc^{ox}-T and bc^{ox}-C nucleosides carrying a benzyloxime group at C6′ (Scheme 1), on their conformational preferences as determined by X-ray crystallography, on their incorporation into oligodeoxyribonucleotides by solid-phase DNA synthesis, and on their base-pairing properties with DNA and RNA and their uptake into HeLa cells.

Results

Synthesis of nucleosides

The synthesis of nucleosides **8** and **13** started with the already known ketone **2** (Scheme 2), which we had used previously for the synthesis of amino-bc-DNA.[10] Ketone **2** is in turn easily accessible from the bicyclo sugar **1**, a central intermediate in bicyclo- and tricyclo-DNA synthesis.[11] Addition of *O*-benzylhydroxylamine in buffered EtOH/H_2O led to the corresponding oxime **3** in 77% yield. Nucleoside synthesis was first attempted by the Vorbrüggen one-pot procedure.[12] Treatment of in situ persilylated thymine with **3** and TMSOTf as Lewis acid at room temperature surprisingly led to the *seco*-nucleoside **4**, most likely originating from a TMS-trapped ring-opened intermediate, in 64% yield. The nucleoside **7**, together with its α-anomer, was only observed in traces. Clearly, another route for



Scheme 2. Synthesis of bc^{ox}-nucleosides via acetal **3**. Reagents and conditions: A) NaOAc, *O*-benzyl hydroxylamine·HCl, EtOH/H_2O 1:1, RT, 2 h, 77%; B) thymine, BSA, TMS-OTf, $ClCH_2CH_2Cl$, 0 °C→RT, 14 h, 64%.

reliable production of sufficient quantities of the β-bc^{ox}-nucleosides would have to be found.

An alternative strategy for constructing the nucleosidic bond consists of the addition of a silylated base to a furanose glycal in the presence of *N*-iodosuccinimide (NIS) as an electrophilic activator.[13] The intermediate iodonucleoside can then be converted into the parent nucleoside by radical reduction. We have utilized this two-step procedure before in the synthesis of the tc-DNA pyrimidine building blocks and found it to yield β-nucleosides exclusively.[14] On the basis of this experience we next prepared glycal **5** and explored its use for β-nucleoside synthesis (Scheme 3).

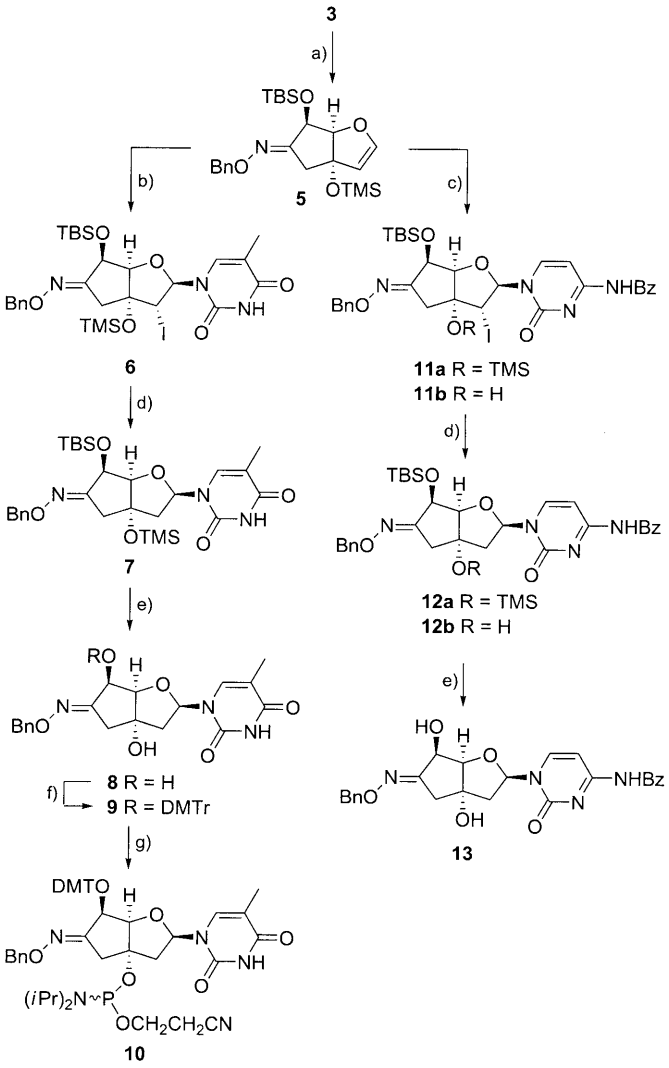
Glycal **5** was obtained from oxime **3** by treatment with TMSOTf at 0 °C. Under these conditions, concomitant silylation of the 3′-OH function occurred. Because of the somewhat labile TMS group, glycal **5** was used directly without purification. NIS-mediated addition of the in situ silylated bases thymine and N^4-benzoylcytosine at temperatures at or below 0 °C led selectively to the iodo-β-nucleosides **7** and **12a** and **12b** in yields of 40–50%. No traces of the corresponding α-nucleosides could be isolated. Removal of the iodine atom with Bu_3SnH, followed by desilylation with HF·pyridine, then gave the sugar-deprotected β-bc^{ox} nucleosides **8** and **13**. The configurations at their anomeric centers were unambiguously assigned both by ^{1}H NMR-NOE spectroscopy and by X-ray crystallography (Figure 2).

The phosphoramidite building block **10** for oligonucleotide synthesis was then prepared by standard DNA chemistry protocols. Selective tritylation of the sterically less hindered tertiary 5′-OH function in **8** was accomplished with 4,4′-dimethoxytrityl triflate[15] (→**9**), and subsequent phosphitylation with the suitably protected chlorophosphine proceeded smoothly to give **10** in 87% yield.

X-ray structures of nucleosides 8 and 13

Crystals of both nucleosides **8** and **13** were subjected to X-ray analysis, not only for the purpose of establishing the anomeric configuration, but mainly to map the conformational preferences of the bicyclic core structure (Figure 2, Table 1).

The oxime double bonds in **8** and **13** are in both cases *E*-configured and point away from the 5′-OH group. The bicyclic



Scheme 3. Synthesis of bc^{ox}-nucleosides via glycal **5**. Reagents and conditions: A) TMSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C→RT, 4 h; B) thymine, BSA, NIS, CH_2Cl_2, 0 °C, 4 h, yield over two steps, 54%; C) N^4-benzoylcytosine, BSA, NIS, CH_2Cl_2, −20 °C, 4 h, yields over two steps: **11a**+**11b** (41%); D) AIBN, Bu_3SnH, toluene, 100 °C, 1 h, **7** (96%), **12a** (53%), **12b** (47%); E) HF·pyridine, pyridine, 0 °C→RT, 12 h, **8** (72%), **13** (46%); F) DMTrOTf, py, RT, 6 h, **9** (68%); G) (iPr$_2$N)P(Cl)OCH$_2$CH$_2$CN, CH_3CN, (iPr)$_2$NEt, 0 °C→RT, 1 h, **10** (87%).

core conformations in the two nucleosides are almost identical, although the nucleosides crystallized in different space groups. The carbocyclic ring in each case shows an envelope conformation with C-5′ out of plane. As a consequence the torsion angle γ is in a $-g$ orientation, as is also observed in A- and B-DNA (Table 1). In both nucleosides **8** and **13** the furanose rings are in 2′-*endo* (south) conformations, with pseudorotation phase angles (*P*) of 163° and 180°, respectively (Table 1). A comparison with the X-ray structures of the parent bc-T and bc-C nucleosides shows that the introduction of the oxime substituent resulted in a shift of the conformation of the furanose ring from the 1′-*exo* to the 2′-*endo* form and to a change in the torsion angle γ from the anticlinal to the $-g$ range. We have interpreted this change in conformation in terms of the 5′-O substituent escaping $A^{1,2}$ strain with the C=N double bond of the oxime function.

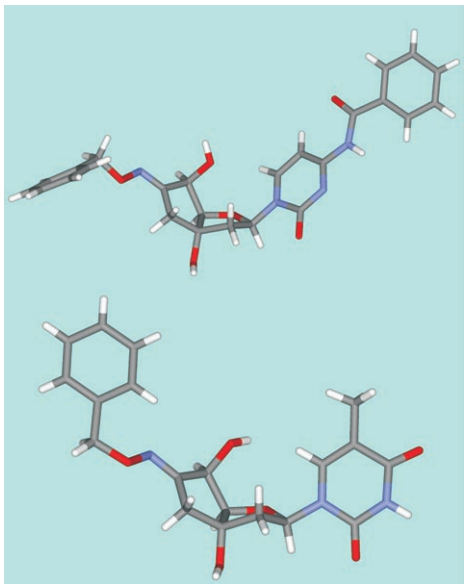


Figure 2. X-ray structures of nucleosides **8** (bottom) and **13** (top).

Table 1. Pseudorotation phase angles and selected nucleoside torsion angles of bc^{ox}-nucleosides **8** and **13** in relation to bc-nucleosides and natural deoxyribonucleosides in the B conformation.

Nucleoside	P[a]	γ	δ	χ
8	163°	87.6	148.3	−118.1
13	180°	86.3	146.2	−167.7
bc-T[b]	128°	149.3	126.5	−112.8
bc-C[c]	114°	156.5	133.5	−107.5
dN[d]	144°	57	122	−119

[a] Pseudorotation phase angle. [b] Taken from ref. [9a]. [c] 5′-Terminal bc-C nucleotide from a parallel dimer duplex (ref. [16]). [d] Average deoxynucleotide conformation in B-DNA (ref. [17]).

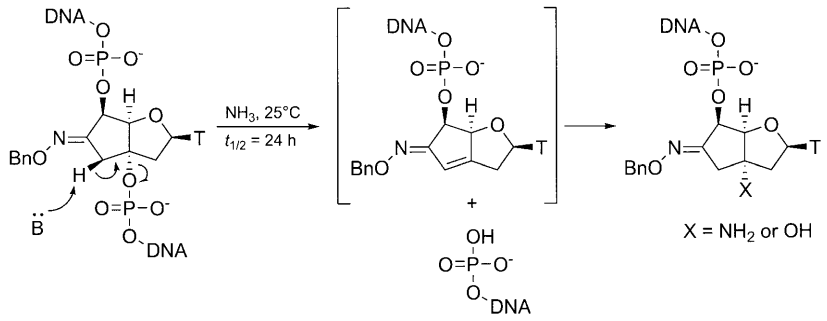
Oligonucleotide synthesis

A series of mixed-base oligonucleotides containing single or double bc^{ox}-T mutations (**ox1–3**; Table 2) were synthesized on a 0.5–1 μmol scale by standard automated phosphoramidite chemistry (Table 2). For incorporation of the modified building blocks the standard coupling step was extended to 6–15 min. No further changes were necessary, and the coupling yields for

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

Code	Oligonucleotide[a]	Mod X	T_m vs. DNA [°C][b]	T_m vs. RNA [°C][b]
ox1	d(GGATGXXCTCGA)	X = bc^{ox}-T	42.0 (−2.8)	46.5 (−1.5)
bc1		X = bc-T	48.7 (+0.6)	48.2 (−0.6)
ox2	d(GGATGTTCXCGA)	X = bc^{ox}-T	43.0 (−4.5)	44.0 (−5.5)
bc2		X = bc-T	49.0 (+1.5)	49.0 (−0.5)
ox3	d(GGAXGTTCXCGA)	X = bc^{ox}-T	38.5 (−4.5)	39.5 (−5.0)
bc3		X = bc-T	47.9 (+0.2)	48.0 (−0.7)

[a] T_m of unmodified oligodeoxynucleotide: 47.5 °C (vs. DNA); 49.5 °C (vs. RNA). [b] ΔT_m per modification in parenthesis.



Scheme 4. Proposed β-elimination chemistry at the 3′-side of bc^{ox}-nucleotides during oligonucleotide deprotection.

the modified building blocks as judged from the trityl assay were $>90\%$.

The oligonucleotides were then deprotected and detached from the solid support. Initial concerns that β-elimination 3′ to the bc^{ox}-T residues, and thus strand cleavage (Scheme 4), could occur during standard ammonia deprotection (conc aq. NH_3, 55 °C, 16 h) proved to be justified. Indeed, under these conditions quantitative cleavage was observed, as determined by HPLC. Corroboration of the β-elimination chemistry was provided by mass analysis of the fragments of a synthesized nonamer (**ox6**, Supporting Information) containing one modification, which were in agreement with a 5′-phosphorylated 3′-oligonucleotide fragment and a 5′-fragment containing a 3′-terminal bc^{ox} unit with either an OH or a NH_2 group at C3′. Clearly a milder procedure had to be found. A series of less basic but more nucleophilic alternatives to ammonia, including hydroxylamine and hydrazine (10% in H_2O or MeOH) at temperatures from 0–50 °C were tested with limited success. The best conditions turned out to be conc. NH_3 at room temperature. Under these conditions a half-life time of 24 h was experimentally determined (Supporting Information). The best results were obtained when modified oligonucleotides were deprotected with conc. ammonia at room temperature for 4 h. Under these conditions, cleavage at the site of modification could be limited to approximately 10% as judged by HPLC. To determine the effect of the oxime substituent on the pairing properties, oligonucleotides containing unmodified bc-T residues were also synthesized as controls. Their synthesis required no special care during deprotection. All oligonucleotides were purified by standard HPLC methods and analyzed by ESI$^-$-MS (Supporting Information).

T_m measurements

UV-melting curve analysis was performed at 260 nm with a cooling–heating–cooling cycle at a rate of 0.5 °C min^{-1} in standard saline buffer at pH 7.0. All curves within a cycle, especially those involving bc^{ox}-modified oligonucleotides, were superimposable, thus ruling out strand cleavage at bc^{ox} modifications under neutral conditions. Analysis of the T_m data revealed a reduction in the thermal stability of a duplex containing a single bc^{ox} modification (**ox2**) by 4.5 °C against DNA and 5.5 °C against RNA (Table 2). The same level of destabilization per modification is observed in a duplex containing two bc^{ox} units, spaced by natural deoxynucleotides (**ox3**). On the other hand, in a sequence context with two adjacent bc^{ox} units (**ox1**) the relative destabilization per modification is considerably reduced, especially with RNA as the complement. This behavior is not observed with the unsubstituted bicyclo-nucleosides (**bc1**–**3**), with which neither significant stabilization nor destabilization relative to DNA occurs. It is therefore evident that the destabilization and its sequence dependence are due to the substituent at the carbocyclic ring and not to the bicyclic structural scaffold itself.

To determine the relative effect of the modifications on pairing selectivity we measured the T_m data for the singly modified oligonucleotides with complementary DNA carrying a mismatched base opposite the modification (Table 3).

Table 3. T_m data [°C] from UV-melting curves (260 nm) of duplexes of d(GGATGTTCXCGA) with DNA complements carrying a mismatched base opposite X.

	T_m [°C]		
Mismatch[a,b]	X = dT	X = bc-T (**bc2**)	X = bc^{ox}-T (**ox2**)
G–T	39.7 (−7.8)	37.0 (−12.0)	31.9 (−11.1)
C–T	36.0 (−11.5)	35.0 (−14.0)	32.6 (−10.4)
T–T	38.0 (−9.5)	32.0 (−17.0)	34.9 (−8.1)

[a] T_m values for matched duplexes; see Table 2. [b] Values in parentheses are ΔT_m values relative to the matched duplex.

As expected, considerable thermal destabilization of the mismatched duplexes was observed in all cases. There are some differences in the special case of the G–T mismatch (wobble pair), which is more strongly destabilized by the bc oligonucleotide family. Interestingly, the high mismatch discrimination observed for bc-DNA is slightly reduced in the bc^{ox} series, which closely matches that of unmodified DNA. From these results we conclude that no major perturbation of the pairing selectivity is induced by the bc^{ox} modification.

Motivated by the reduced destabilization in duplexes containing two consecutive bc^{ox} residues, we set out to investigate the trend in thermal stability with extension from a single modification within a DNA backbone to a uniformly modified bc^{ox} backbone. We therefore prepared the two decathymidylates containing five and nine consecutive bc^{ox}-T residues (**ox4** and **ox5**, respectively). The corresponding T_m values are summarized in Table 4. The data clearly show that the trend to destabilization of duplexes with complementary DNA is not changed with an increasing number of modifications. In contrast, complexes with complementary RNA become gradually more stable as the number of modifications increases. While

Table 4. T_m data [°C] from UV-melting curves (260 nm) for modified decathymidylates with complementary DNA and RNA

	Oligonucleotide[a]	Mod. X	T_m [°C][b] vs. DNA	vs. RNA
ox4	d(TTXXXXXTTT)	X = bc^{ox}-T	ca. 5 (−3.3)	22.6 (−0.3)
ox5	d(XXXXXXXXXt)[c]	X = bc^{ox}-T	< 5 (−3.3)	31.0 (+0.7)

[a] T_m of unmodified decathymidylate: 21.7 °C (vs. DNA); 24.3 °C (vs. RNA). [b] ΔT_m per modification in parentheses. [c] t denotes a tricyclo-T residue (ref. [4a]).

ox4 shows greatly reduced destabilization in RNA duplexes (only −0.3 °C per modification), **ox5**—which can be considered almost a fully modified bc^{ox}-oligonucleotide—shows stabilization by +0.7 °C per modification. Whether this result can be generalized to any sequence, however, remains to be shown.

The effect of bc^{ox} modifications on duplex structure was investigated by CD spectroscopy in the cases of **ox1**, **ox3**, and **ox4** in complexation with RNA and DNA (Supporting Information). As anticipated, no major deviations in the CD spectra from those of the unmodified DNA/DNA and DNA/RNA duplexes were found, indicating no major structural changes. To determine local structural perturbations near the sites of modification, high-resolution structural analysis needs to be performed.

Cellular uptake of bc^{ox} oligonucleotides

To test whether the more hydrophobic natures of the bc^{ox}-T nucleotides would favor cellular uptake we synthesized **ox4** containing a fluorescein (FAM) label at its 3′-end (**ox4-FAM**). As a control, the corresponding unmodified decamer **d(T$_{10}$)-FAM** was prepared. These oligonucleotides were transfected into HeLa cells in the presence and absence of lipofectamine as transfecting agent, and cellular uptake was followed by fluorescence microscopy 24 h after transfection.

In the absence of a transfecting agent no uptake of fluorescently labeled oligonucleotides could be detected by fluorescence imaging in either case (data not shown), indicating that the bc^{ox} modification does not permit passive transport through the cell membrane at a significant level. In complexation with lipofectamine, however, a clear advantage for uptake of **ox4-FAM** relative to the control **d(T$_{10}$)-FAM** was observed (Figure 3). Fluorescence was detected both in the cytosol and in the nucleus. From these experiments we conclude that the bc^{ox} modification helps cellular uptake by permitting a more efficient packaging into cationic lipofectamine particles, most likely due to the more hydrophobic nature of the corresponding oligonucleotides.

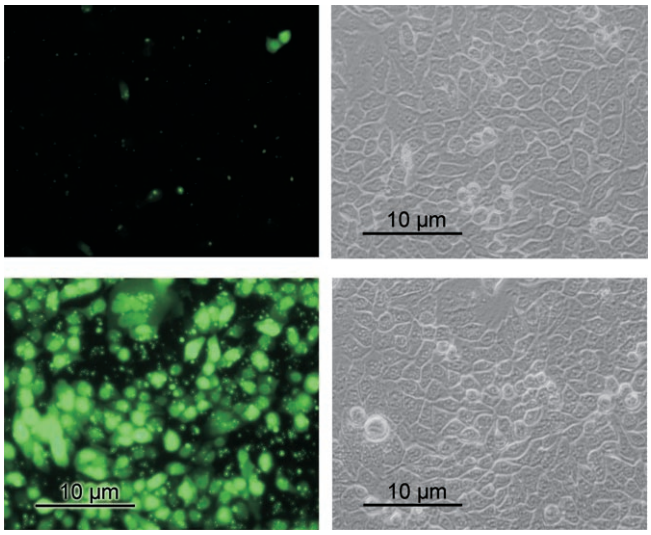


Figure 3. Fluorescence microscope images (left) and corresponding contour images (right) of HeLa cells 24 h after lipofectamine-mediated transfection of **d(T$_{10}$)-FAM** (top) and **ox4-FAM** (bottom).

Discussion and Conclusions

We have successfully prepared a novel bc nucleoside modification containing a hydrophobic chemical entity in the carbocyclic ring. The purpose of this modification was twofold. Firstly, it was designed to bring about a correction of the misaligned torsion angle γ in bc-DNA to increase DNA/RNA affinity, and secondly, the increased hydrophobicity of the corresponding oligonucleotides was expected to improve cellular uptake. As can be inferred from the X-ray structures of the two pyrimidine nucleosides **8** and **13**, the first goal—namely, changing the torsion angle γ to the *−g* arrangement as observed in DNA and RNA—is indeed fulfilled. We explain this structural change in terms of the system escaping A^{1,2} strain between the oxime moiety and the 5′-oxygen.

Synthesis of the monomers proceeded smoothly, yielding only the β-anomeric nucleosides. This highlights the advantage in stereoselectivity of the NIS-induced glycal nucleosidation over alternative nucleosidation methods such as, for example, the Vorbrüggen procedure,[12] in the bi- or tricyclo-DNA series. Oligonucleotide synthesis with **10** proceeded with coupling yields that were acceptable (90–93 %), but significantly lower than those obtained with natural or bc building blocks. This is certainly an obstacle for the synthesis of longer oligonucleotides and must be addressed in the future. A more serious problem, however, is posed by the relative instability of bc^{ox} oligonucleotides towards standard deprotection conditions, due to elimination chemistry at the 3′-side of the modification (Scheme 3). This is largely responsible for the fact that no longer (> 10 nt.) fully modified oligonucleotides could be prepared and also discouraged the use of the bc^{ox}-C^{Bz} nucleoside building block. Synthetically, this problem might be solved by changing to the alloc/allyl protecting group regime, as previously introduced by Hayakawa et al.,[18] for bases and phosphates.

More importantly, however, we were able to verify that under neutral, near physiological conditions in the 0–80 °C range, no strand cleavage of bc^{ox} oligonucleotides occurred, thus establishing that the chemical stability is sufficiently high for biophysical and biological measurements and applications. Furthermore, the oxime function is compatible with oligonu-

cleotide synthesis and deprotection chemistry, as we were not able to isolate side products arising from oxime hydrolysis or aminolysis.

While the structural similarity to an unmodified 2′-deoxyribonucleoside was increased with this modification, this did not materialize in increased thermal stabilities of correspondingly modified oligodeoxynucleotides in duplexes with DNA. In addition, less destabilization, or even stabilization, was observed in duplexes with RNA, despite the fact that the furanose conformation of the bc^{ox} monomers had been found to be of the S-(DNA) and not of the N-(RNA) type. From a direct comparison with bc-modified oligonucleotides it becomes very clear that the changes in DNA and RNA affinity are a consequence of the substituent at C6′ and not of the bicyclic core structure. From the molecular model (Figure 1) one can exclude interference with Watson–Crick pairing as a likely source of the destabilizing effect of the benzyloxime groups. Our current explanation points towards the change in hydration of the duplex induced by the hydrophobic benzyl groups. Such a perturbation of the hydration shell is especially pronounced in the cases in which the benzyl groups are isolated, and is less pronounced where they are consecutively aligned with the possibility of forming a "benzyl-zipper". To uphold or to disprove this hypothesis, rigorous thermodynamic data on duplex formation (also in organic/aqueous solvent mixtures), as well as high-resolution structural data relating to the modifications clearly need to be made available.

A very interesting fact concerns the cellular uptake behavior of the modified oligonucleotides. Although no spontaneous penetration in the absence of a transfecting agent could be observed, it clearly emerges that in the presence of lipofectamine the bc^{ox} oligonucleotides have a considerable advantage over their unmodified counterparts. We explain this as a direct consequence of improved interaction of the more hydrophobic oligonucleotide with the lipofectamine. Whether this is a general feature or dependent on oligonucleotide sequence, transfecting agent, and cell type cannot yet be stated and requires more experimentation. We believe, though, that increasing the lipophilicities of oligonucleotides to improve complex stabilities with cationic lipids, and thus transfection rates, is a promising concept for further pursuit.

In comparison with natural DNA, the bc-DNA scaffold offers additional potential for the introduction of functional elements. Here we have reported on the consequences of adding a hydrophobic residue to bc-oligonucleotides and its effect on the thermochemical and cellular uptake behavior. With the modular synthesis given in Schemes 1 and 2 it seems feasible to screen a variety of other substituents with the goal of improving the chemistry and biology of potentially therapeutic oligonucleotides.

Experimental Section

General: All reactions were performed under Ar 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 (Fluka) with an average particle size of 40 μm. All solvents for CC were of technical grade and distilled prior to use. Thin-layer chromatography (TLC) was performed on silica gel plates (Macherey–Nagel, 0.25 mm, UV254). Visualization was achieved either under UV or by staining in dip solution [Ce^{IV} sulfate (10.5 g), phosphomolybdenic acid (21 g), sulfuric acid (conc., 60 mL), H_2O (900 mL)], followed by heating with a heat gun. NMR spectra were recorded on a Bruker DRX 400 or a Bruker AC 300 spectrometer at 400 MHz or 300 MHz (^{1}H NMR) or 100 MHz (^{13}C NMR) in either $CDCl_3$ or CD_3OD. δ in ppm relative to residual undeuterated solvent [$CHCl_3$: 7.26 ppm (^{1}H) and 77.0 ppm (^{13}C); CHD_2OD: 3.35 ppm (^{1}H) and 49.3 ppm (^{13}C)], J in Hz. ^{13}C-multiplicities were determined from DEPT spectra, and signal assignments are based on ^{13}C/^{1}H-HMBC spectra. Proton signal assignments were based on COSY and HMBC. ^{1}H NMR difference-NOE spectra were recorded on a Bruker DRX 500 instrument at 500 MHz. High-resolution electrospray ionization (ESI) mass spectra (MS, *m/z*) were recorded on an Applied Biosystems Sciex QSTAR Pulsar instrument. IR spectra ($\tilde{\nu}$ in cm^{-1}) were recorded on a Perkin–Elmer Spectrum 1 machine. UV spectra were measured on a Varian Cary 3E UV/Vis spectrophotometer.

6-Benzyloximino-8-*tert*-butyldimethylsilyloxy-5-hydroxy-3-methoxy-2-oxabicyclo[3.3.0]octane (3): A solution of sodium acetate (410 mg, 5 mmol) and *O*-benzylhydroxylamine hydrochloride (1.74 g, 11.6 mmol) in hot EtOH/H_2O (2:1, 10 mL) was added to a solution of ketone **2** (1.84 g, 4.5 mmol) in EtOH (5 mL). After stirring for 2 h at RT, the mixture was diluted with AcOEt (10 mL) and washed with sat. $NaHCO_3$ (3×20 mL), and the aqueous phase was extracted with AcOEt (3×40 mL). The combined organic phases were dried over $MgSO_4$ and concentrated. CC (hexane/AcOEt 4:1) yielded the title compound **3** (1.42 g, 77%) as a colorless oil. R_f (hexane/AcOEt 4:1) 0.61; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.33–7.29 (m, 4H), 5.14 (d, J = 4.8 Hz, 1H), 5.08 (s, 2H), 4.4 (d, J = 5.3 Hz, 1H), 4.28 (d, J = 5.3 Hz, 1H), 3.40 (s, 3H), 2.86 (d, J = 19.2 Hz, 1H), 2.71 (d, J = 19.3 Hz, 1H), 2.29 (dd, J_1 = 13.2, J_2 = 4.88 Hz, 1H), 2.09 (d, J = 13.2 Hz, 1H), 0.85 (s, 9H), 0.07 (s, 3H); 0.01 ppm (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 161.26 (s), 138.27 (s), 128.60 (d), 128.43 (d), 128.04 (d), 108.59 (d), 90.42 (d), 85.03 (s), 76.37 (t), 72.97 (d), 55.26 (q), 46.40 (t), 35.69 (t), 26.036 (q), 18.53 (s), −4.54 ppm (q); ESI$^+$-HRMS calcd for $C_{21}H_{33}NO_5NaSi$: 430.2025 $[M+Na]^+$; found: 430.2033.

***seco*-Nucleosides 4**: Bis(*N,O*-trimethylsilyl)acetamide (BSA, 0.35 mL, 1.47 mmol) was added to a suspension of thymine (143 mg, 1.15 mmol) in 1,2-dichloroethane (1.5 mL), and the mixture was stirred until a clear solution appeared. A solution of oxime **3** (61 mg, 0.15 mmol) in dichloromethane (1 mL) was then added, the mixture was cooled to 0 °C, and TMSOTf (0.1 mL, 0.45 mmol) was added. The reaction mixture was stirred for 4 h at 0 °C and then for 10 h at RT. Workup was initiated by dilution with EtOAc (10 mL), followed by washing with sat. $NaHCO_3$ (3×15 mL) and extraction with EtOAc (3×15 mL). The combined organic layers were evaporated, and the residue was purified by CC (hexane/EtOAc 2:1) to give a 4:1 mixture of isomers of *seco*-nucleosides **4** (65.3 mg, 64%). R_f (hexane/EtOAc 3:1) 0.41; ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.96 (br s, 1.1H), 7.47–7.40 (m, 5H), 7.11 (d, J = 1.24 Hz, 0.8H), 7.09 (d, J = 1.2 Hz, 0.2H), 6.02–5.94 (m, 1H), 5.28–5.18 (m, 2H), 4.91 (dd, J_1 = 0.73, J_2 = 0.31 Hz, 0.8H), 4.71 (dd, J_1 = 0.88, J_2 = 0.24 Hz, 0.2H), 4.07 (dd, J_1 = 0.32, J_2 = 0.23 Hz, 0.8H), 3.95 (d, J = 1.05 Hz, 0.2H), 3.44 (s, 2.4H), 3.28 (s, 0.6H), 2.78–2.60 (m, 2H), 2.44 (dd, J_1 = 5.79, J_2 = 3.78 Hz, 0.8H), 2.08 (s, 3H), 1.80 (dd, J_1 = 7.81, J_2 = 1.78 Hz, 0.8H), 1.06, 0.87 (2 × s, 9H), 0.15–0.8 ppm (5 × s, 24H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 163.66 (s), 160.06 (s), 150.57 (s), 138.71 (s), 135.28 (d), 135.04 (d), 128.17 (d), 128.11 (d),

128.02 (d), 127.54 (d), 127.46 (d), 111.48 (s), 84.32 (d), 80.82 (d), 79.29 (d), 75.70 (t), 75.16 (d), 73.73 (d), 56.19 (q), 55.99 (q), 41.47 (t), 39.89 (t), 37.98 (t), 37.50 (t), 26.09 (q), 25.90 (q), 18.66 (s), 12.64 (q), 12.54 (q), 2.09 (q), 1.55 (q), 0.79 (q), 0.57 (q), −4.29 (q), −4.92 ppm (q); ESI$^+$-HRMS calcd for $C_{32}H_{55}N_3O_7NaSi_3$: 700.32455 $[M+Na]^+$; found: 700.3243.

6-Benzyloximino-8-*tert*-butyldimethylsilyloxy-5-hydroxy-2-oxabicyclo[3.3.0]oct-3-ene (5): TMSOTf (1.9 mL, 10 mmol) was added dropwise at 0 °C to a solution of **3** (1.42 g, 3.48 mmol) and 2,6-lutidine (2.1 mL, 18 mmol) in DCM (7.5 mL). The mixture was allowed to warm to RT. After stirring for 4 h the reaction mixture was diluted with AcOEt (20 mL) and washed with sat. $NaHCO_3$ (3×20 mL), and the aqueous phase was extracted with AcOEt (3×40 mL). The combined organic phases were dried over $MgSO_4$ and concentrated, and the crude title compound **5** (colorless oil) was used in the next reaction without further purification. R_f (hexane/AcOEt 4:1) = 0.91; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.34–7.32 (m, 5H), 6.44 (d, J = 2.7 Hz, 1H), 5.09 (m, 3H), 4.65 (d, J = 6.2 Hz, 1H), 4.46 (d, J = 6.3 Hz, 1H), 3.03 (d, J = 18.0 Hz, 1H), 2.74 (d, J = 18.1 Hz, 1H), 0.86 (s, 9H), 0.12 (s, 9H), 0.07 (s, 3H), 0.05 ppm (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 161.04 (s), 149.92 (d), 138.42 (s), 128.55 (d), 128.50 (d) 127.96 (d), 107.89 (d), 90.16 (d), 89.31 (s), 76.32 (t), 73.56 (d), 39.76 (t), 26.01 (q), 18.72 (s), 2.16 (q), −4.59 (q), −4.74 ppm (q); ESI$^+$-HRMS: calcd for $C_{23}H_{37}NO_4NaSi_2$: 470.2158 $[M+Na]^+$; found: 470.2145.

(6′-Benzyloximino-5′-*O*-*tert*-butyldimethylsilyl-3′-*O*-trimethylsilyl-2′-deoxy-3′,5′-ethano-2′-iodo-β-D-ribofuranosyl)thymine (6): BSA (2.1 mL, 8.7 mmol) was added to a suspension of thymine (880 mg, 7 mmol) in DCM (4 mL), and the mixture was stirred at RT until a homogeneous solution was formed (1 h). A solution of glycal **5** (784 mg, 1.75 mmol) in DCM (3 mL) was then added, and the solution was cooled to 0 °C. Solid *N*-iodosuccinimide (NIS, 590 mg, 2.6 mmol) was added, and the mixture was stirred for 4 h at 0 °C. The crude reaction mixture was then diluted with AcOEt (10 mL) and washed with sat. Na_2CO_3 (2×30 mL) and with sat. $NaHCO_3$ (1× 30 mL). The aqueous phases were extracted with AcOEt (3× 50 mL), and the combined organic phases were dried over $MgSO_4$ and concentrated. CC (hexane/AcOEt 7:3) yielded the title compound **6** (660 mg, 0.94 mmol, 54% over two steps) as a colorless oil. R_f (hexane/AcOEt 7:3) 0.50. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.23 (s, 1H; H-N(3)), 7.31–7.27 (m, 5H; arom.H), 7.27 (d, J = 1.2 Hz, 1H; H-C(6)), 6.41 (d, J = 9.4 Hz, 1H; H-C(1′)), 5.10 (s, 2H; CH_2Ph), 4.62 (dd, J_1 = 0.6, J_2 = 6.4 Hz, 1H; H-C(5′)), 4.32 (d, J = 6.4 Hz, 1H; H-C(4′)), 3.93 (d, J = 9.3 Hz, 1H; H-C(2′)), 2.80 (d, J = 18.5 Hz, 1H; H-C(7′)), 2.64 (d, J = 18.5 Hz, 1H; H-C(7′)), 1.89 (d, J = 1.2 Hz, 3H; Me-C(5)), 0.87 (s, 9H; TBDMS), 0.20 (s, 9H; TMS), 0.12 (s, 3H; TBDMS), 0.10 ppm (s, 3H; TBDMS); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 163.44 (s, C(6′)), 159.13 (s, C(2)), 137.88 (s, arom.C), 134.87 (d, C(6)), 128.71 (d, arom.C), 128.68 (d, arom.C), 128.35 (d, arom.C), 111.81 (s, C(5)), 90.49 (d, C(1′)), 86.00 (d, C(4′)), 85.70 (s, C(3′)), 76.85 (t, CH_2Ph), 72.36 (d, C(5′)), 35.80 (d, C(2′)), 34.71 (t, C(7′)), 26.18 (q, TBDMS), 19.02 (s, TBDMS), 12.83 (q, Me-C(5)), 2.05 (q, TMS), −4.09 (q, TBDMS), −4.25 ppm (q, TBDMS); ESI$^+$-HRMS: calcd for $C_{28}H_{43}N_3O_6Si_2I$: 700.1735 $[M+H]^+$; found: 700.1758.

(6′-Benzyloximino-5′-O-*tert*-butyldimethylsilyl-3′-O-trimethylsilyl-2′-deoxy-3′,5′-ethano-β-D-ribofuranosyl)thymine (7): Azoisobutyronitrile (AIBN, 130 mg, 0.78 mmol) was added at RT to a solution of nucleoside **6** (785 mg, 1.12 mmol) and Bu_3SnH (0.48 mL, 1.70 mmol) in toluene (5 mL). After the system had been at reflux for 1 h, the solvent was evaporated, and the residue was purified by CC (hexane/AcOEt 3:1) to give the title compound **7** (615 mg, 96%) as a white foam. R_f (hexane/AcOEt 3:1) 0.45; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.19 (s, 1H; H-N(3), 7.56 (d, J = 1.1 Hz, 1H; H-C(6)), 7.33–7.29 (m, 5H; arom.H), 6.19 (dd, J_1 = 5.8, J_2 = 7.0 Hz, 1H; H-C(1′)), 5.09 (d, J = 12.1 Hz, 1H; CH_2Ph), 5.05 (d, J = 12.1 Hz, 1H; CH_2Ph), 4.66 (dd, J_1 = 6.0, J_2 = 1.1 Hz, 1H; H-C(5′)), 4.23 (d, J = 6.0 Hz, 1H; H-C(4′)), 2.88 (dd, J_1 = 18.3, J_2 = 0.7 Hz, 1H; H-C(7′)), 2.62–2.54 (m, 2H; H-C(2′), H-C(7′)), 1.98 (dd, J_1 = 13.32, J_2 = 7.12 Hz, 1H; H-C(2′)), 1.91 (d, J = 1.2 Hz, 3H; Me-C(5)), 0.90 (s, 9H; TBS), 0.17 (s, 9H; TMS), 0.14 (s, 3H; TBS), 0.11 ppm (s, 3H; TBS); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 163.824 (s, C(6′)), 160.35 (s, C(4)), 150.29 (s, C(2)), 138.04 (s, arom.C), 136.02 (d, C(6)), 128.75 (d, arom.C), 128.64 (d, arom.C), 128.28 (d, arom.C), 114.98 (s, C(5)), 89.32 (d, C(1′)), 87.13 (d, C(4′)), 85.25 (s, C(3′)), 76.75 (t, CH_2Ph), 72.97 (d, C(5′)), 46.51 (t, C(2′)), 37.99 (t, C(7′)), 26.10 (q, TBS), 18.84 (s, TBS), 12.79 (q, Me-C(5)), 2.10 (q, TMS), −4.27 (q, TBS), −4.41 ppm (q, TBS); ESI$^+$-HRMS: calcd for $C_{28}H_{44}N_3O_6Si_2$: 574.2768 $[M+Na^+]$; found: 574.2756.

(6′-Benzyloximino-2′-deoxy-3′,5′-ethano-β-D-ribofuranosyl)thymine (8): HF·pyridine (0.25 mL, 9.62 mmol) was added at 0 °C to a solution of nucleoside **7** (354 mg, 0.62 mmol) in pyridine (5 mL). After the solution had been stirred at RT for 12 h, silica gel (about 1 g) was added, and the mixture was stirred for another 15 min. The slurry was filtered over Celite and washed with AcOEt/MeOH (10:1), the solvents were evaporated, and the residue was crystallized from MeOH (slow evaporation), to yield the title compound **8** (172 mg, 72%) as colorless needles. R_f (AcOEt): 0.46; m.p.: 204 °C (decomp); ^{1}H NMR (CD_3OD, 400 MHz): δ = 7.96 (d, J = 1.0 Hz, 1H; H-C(6)), 7.29–7.21 (m, 5H; arom.H), 6.28 (dd, J_1 = 8.2, J_2 = 5.8 Hz, 1H; H-C(1′)), 5.06 (s, 2H; CH_2Ph), 4.53 (d, J = 5.5 Hz, 1H; H-C(5′)), 4.13 (d, J = 5.7 Hz, 1H; H-C(4′)), 2.96 (d, J = 19.3 Hz, 1H; H-C(7′)), 2.55 (d, J = 19.2 Hz, 1H; H-C(7′)), 2.39 (dd, J_1 = 13.3, J_2 = 5.8 Hz, 1H; H-C(2′)), 2.12 (dd, J_1 = 13.3, J_2 = 8.20 Hz, 1H; H-C(2′)), 1.78 ppm (s, 3H; Me-C(5)); ^{13}C NMR (CD_3OD, 100 MHz): δ = 166.72 (s, C(6′)), 163.26 (s, C(4)), 152.63 (s, C(2)), 139.52 (s, arom.C), 138.62 (d, C(6)), 129.70 (d, arom.C), 129.47 (d, arom.C), 111.46 (s, C(5)), 89.91 (d, C(1′)), 88.36 (d, C(4′)), 84.63 (s, C(3′)), 77.54 (t, CH_2Ph), 73.18 (d, C(5′)), 46.70 (d, C(2′)), 38.87 (t, C(7′)), 12.86 ppm (q, Me-C(5)); ^{1}H NMR difference-NOE (CD_3OD, 400 MHz): δ = 6.28→7.96 (1.9%), 4.13 (3.2%), 2.39 (6.5%); 4.53→4.13 (13.1%); 4.13→6.28 (3.0%), 4.53 (12.6%); 2.96→2.55 (29.9%), 2.12 (4.2%); 2.55→7.28 (2.2%), 4.53 (1.4%), 4.13 (2.4%), 2.96 (27.0%), 1.78 (1.0%); 2.39→7.28 (3.1%), 6.28 (14.0%), 5.06 (2.9%), 4.13 (2.8%), 2.12 (24.2%), 1.78 (1.1%); 2.12→ 7.96 (10.8%), 7.28 (3.2%), 6.3 (3.2%), 5.06 (2.1%), 4.13 (1.4%), 2.96 (5.6%), 2.42 ppm (25.2%); IR ($CHCl_3$): $\tilde{\nu}$ = 3316, 3227, 3075, 2921, 1671, 1649 cm^{-1}; UV: λ_{max} (ε) = 264 nm (6400 mol^{-1} dm^3 cm^{-1}); ESI$^+$-HRMS: calcd for $C_{19}H_{22}N_3O_6$: 388.1508 $[M+H]^+$; found: 388.1507.

[6′-Benzyloximino-5′-*O*-(4,4′-dimethoxytriphenyl)methyl-2′-deoxy-3′,5′-ethano-β-D-ribofuranosyl]thymine (9): (4,4′-Dimethoxytriphenyl)methyl triflate (DMT-OTf, 953 mg, 2.1 mmol) was added at RT in three portions to a solution of nucleoside **8** (272 mg, 0.70 mmol) in pyridine (2 mL). After stirring for 6 h, the mixture was diluted with AcOEt (10 mL) and washed with sat. $NaHCO_3$ (3× 15 mL), and the aqueous phases were extracted with AcOEt (3× 20 mL). The combined organic layers were concentrated, and the crude product was purified by CC (AcOEt, 1% NEt_3) to give the title compound **9** (326 mg, 68%) as a slightly yellow foam. R_f (AcOEt): 0.66; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.05 (s, 1H; H-N(3)), 7.55–7.20 (m, 16H; H-C(6), arom.H), 6.80–6.74 (m, 4H; arom.H), 5.82 (dd, J_1 = 6.68, J_2 = 4.24 Hz, 1H; H-C(1′)), 5.17 (dd, J = 25.44, J_2 = 12.32 Hz, 2H; CH_2Ph), 4.67 (d, J = 5.16 Hz, 1H; H-C(4′)), 3.79, 3.78 (2×s, 6H; OMe), 2.88 (dd, J_1 = 18.88, J_2 = 0.92 Hz , 1H; H-C(7′)), 2.68 (d, J = 5.20 Hz, 1H; H-C(5′)), 2.47 (dd, J_1 = 14.00, J_2 = 6.84 Hz, 1H; H-C(2′)), 2.38 (d, J = 18.76 Hz, 1H; H-C(7′)), 2.16 (dd, J_1 = 14.16,

J_2 = 4.24 Hz, 1H; H-C(2′)), 1.89 ppm (d, J = 1.04 Hz, 3H; Me-C(5)); ^{13}C NMR (CD_3OD, 100 MHz): δ = 163.45 (s, C(4)), 159.12 (s, C(6′)), 158.81 (s, arom.C), 158.73 (s, arom.C), 149.84 (s, C(2)), 145.15 (s, arom.C), 138.07 (d, arom.C), 136.26 (s, arom.C), 135.95 (s, arom.C), 135.35 (d, C(6)), 130.43 (d, arom.C), 130.22 (d, arom.C), 128.43 (d, arom.C), 128.17 (d, arom.C), 127.92 (d, arom.C), 127.87 (d, arom.C), 127.01 (s, arom.C), 113.20 (d, arom.C), 113.14 (d, arom.C), 110.10 (s, C(5)), 87.78 (s, CPh_3), 86.90 (d, C(1′)), 85.93 (d, C(4′)), 81.53 (s, C(3′)), 76.50 (t, CH_2Ph), 74.79 (d, C(5′)), 55.25 (q, OMe), 47.77 (t, C(2′)), 38.11 (t, C(7′)), 12.43 ppm (q, Me-C(5)); ESI$^+$-HRMS: calcd for $C_{40}H_{39}N_3O_8Na$: 712.2634 $[M+Na]^+$; found: 712.2638.

{6′-Benzyloximino-3′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-5′-*O*-(4,4′-dimethoxytriphenyl)methyl-2′-deoxy-3′,5′-ethano-β-D-ribofuranosyl)}thymine (10): 2-Cyanoethoxy diisopropylamino chlorophosphine (CEP-Cl, 0.28 mL, 1.3 mmol) was added at RT to a solution of nucleoside **9** (312 mg, 0.45 mmol) and diisopropylethylamine (DIPEA, 0.45 mL, 2.63 mmol) in acetonitrile (2 mL). After stirring for 1 h, the mixture was diluted with AcOEt (10 mL) and washed with sat. $NaHCO_3$ (2×10 mL) and brine (5 mL). The aqueous phases were extracted with AcOEt (3×20 mL), and the combined organic phases were dried over $MgSO_4$ and concentrated. CC (hexane/AcOEt 1:2 + 1% TEA) gave the title compound **10** (350 mg, 87%) as a white foam. R_f (hexane/AcOEt 1:2): 0.68; ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.92 (br s, 1H; H-N(3)), 7.63–7.45 (m, 7H; arom.H), 7.41–7.34 (m, 5H; arom.H), 7.24–7.22 (m, 3H; arom.H, H-C(6)), 6.79–6.73 (m, 4H; arom.H), 5.90–5.82 (m, 1H; H-C(1′)), 5.17–5.13 (m, 2H; CH_2Ph), 4.66–4.59 (m, 1H; H-C(4′)), 3.79, 3,78 (2 × s, 6H; OMe), 3.60–3.46 (m, 5H; H-C(*i*-Pr), -OCH_2-), 2.85–2.73 (m, 4H; H-C(2′), H-C(7′), H-C(5′)), 2.45 (m, 2H; -CH_2-CN), 2.18–2.04 (m, 1H; H-C(2′)), 1.88, 1.83 (2 × d, J = 0.6 Hz, 3H; Me-C(5)), 1.21–1.02 ppm (m, 12H; $(CH_3)_2CH$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 163.73 (s), 159.49 (s), 159.11 (s), 159.05 (s), 150.11 (s), 145.50 (s), 138.50 (d), 135.74 (d), 130.77 (d), 130.56 (d), 128.73 (d), 128.55 (d), 128.22 (d), 128.18 (d), 113.51 (d), 113.48 (d), 110.35 (s), 77.58 (t), 76.84 (d), 69.93 (t), 58.05 (s), 55.54 (q), 43.75, 43.63 (2 × t), 24.81, 24.74, 24.58, 24.50 (4 × q), 20.31 (t), 12.75, 12.67 ppm (q); ^{31}P NMR ($CDCl_3$, 162 MHz): δ = 144.26, 143.70 ppm; ESI$^+$-HRMS: calcd for $C_{49}H_{56}N_5O_9NaP$: 912.3713 $[M+Na]^+$; found: 912.3705.

N^4-Benzoyl-6′-benzyloximino-(5′-O-*tert*-butyldimethylsilyl-2′-iodo-2′-deoxy-3′,5′-ethano-β-D-ribofuranosyl)cytosine (11a + 11b): BSA (0.85 mL, 3.47 mmol) was added to a suspension of N^4-benzoylcytosine (600 mg, 2.80 mmol) in DCM (1 mL). After 1 h of stirring at RT the reaction mixture had become clear, and a solution of glycal **5** (314 mg, 0.7 mmol) in DCM (1 mL) was added. The mixture was then cooled to −20 °C, and *N*-iodosuccinimide (240 mg, 1.07 mmol) was added portionwise. After the system had been stirred for 4 h at −20 °C the reaction was quenched by addition of AcOEt (10 mL), followed by washing with sat. Na_2CO_3 (2×15 mL) and sat. $NaHCO_3$ (20 mL). The aqueous phases were extracted with AcOEt (3×50 mL), and the combined organic layers were concentrated. CC (hexane/AcOEt 7:3) yielded the title compound **11a** (88 mg ,15% over two steps) together with the 3′-desilylated product **11b** (126 mg, 26% over two steps).

Data for 11a. R_f (hexane/AcOEt 4:1): 0.33; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.27 (d, J = 7.0 Hz, 1H; H-C(6)), 7.98 (s, 2H; H-C(5), arom.H), 7.65–7.51 (m, 4H; arom.H), 7.32 (m, 5H; arom.H), 6.62 (d, J = 7.8 Hz, 1H; H-C(1′)), 5.10 (s, 2H; CH_2Ph), 4.68 (d, J = 5.9 Hz, 1H; H-C(5′)), 4.42 (d, J = 5.9 Hz, 1H; H-C(4′)), 4.09 (d, J = 8.2 Hz, 1H; H-C(2′)), 2.71 (d, J = 5.1 Hz, 2H; H-C(7′)), 0.90 (s, 9H; TBS), 0.21 (s, 9H; TMS), 0.16 (s, 3H; TBS), 0.14 ppm (s, 3H; TBS). ^{13}C NMR ($CDCl_3$, 400 MHz): δ = 137.73 (s, arom.C), 133.83 (s, arom.C), 129.42 (d, arom.C), 128.84 (d, arom.C),128.70 (d, arom.C), 128.42 (d, arom.C), 87.35 (d, C(1′)), 85.89 (d, C(4′)), 76.96 (t, CH_2Ph), 72.82 (d, C(5′)), 35.04 (t, C(7′)), 26.28 (q, TBS), 18.91 (s, TBS), 2.04 (q, TMS), −3.90 (q, TBS), −4.33 ppm (q, TBS); ESI$^+$-HRMS: calcd for $C_{34}H_{45}N_4O_6Si_2I$: 811.1820 $[M+Na]^+$; found: 811.1811.

Data for 11b: R_f (AcOEt/hexane 4:1): 0.60; ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.99 (br s, 1H; H-N(4)), 8.26 (d, J = 7.3 Hz, 1H; H-C(6)), 7.95–7.93 (m, 2H; H-C(5), arom.H), 7.63–7.50 (m, 4H; arom.H), 7.31–7.29 (m, 5H; arom.H), 6.67 (d, J = 6.9 Hz, 1H; H-C(1′)), 5.08 (d, J = 2.0 Hz, 2H; CH_2Ph), 4.76 (d, J = 5.5 Hz, 1H; H-C(5′)), 4.51 (d, J = 5.1 Hz, 1H; H-C(4′)), 4.38 (d, J = 6.9 Hz, 1H; H-C(2′)), 2.71 (s, 2H; H-C(7′)), 0.91 (s, 9H; TBS), 0.17 (s, 3H; TBS), 0.15 ppm (s, 3H; TBS). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 176.94 (s, Bz), 158.65 (s, C(2)), 137.36 (s, arom.C), 133.33 (d, arom.C), 129.05 (d, arom.C), 128.46 (d, arom.C), 128.36 (d, arom.C), 128.04 (d, arom.C), 127.27 (s, arom.C), 86.10 (d, C(1′)), 83.02 (d, C(4′)), 77.20 (d, C(5′)), 76.56 (t, CH_2Ph), 35.78 (t, C(7′)), 29.56 (t, C(2′)), 25.95 (q, TBS), 18.55 (s, TBS), −4.25(q, TBS), −4.63 ppm (q, TBS); ESI$^+$-HRMS: calcd for $C_{31}H_{37}N_4O_6SiI$: 739.1424 $[M+Na]^+$; found: 739.1396.

N^4-Benzoyl-(6′-benzyloximino-5′-O-*tert*-butyldimethylsilyl-3′-O-trimethylsilyl-2′-deoxy-3′,5′-ethano-β-D-ribofuranosyl]cytosine (12a): Bu_3SnH (0.05 mL, 0.19 mmol) was added at RT to a solution of iodonucleoside **11a** (88 mg, 0.11 mmol) in toluene (1 mL). AIBN (9.3 mg, 0.06 mmol) was added, and the solution was heated at reflux for 1 h. The solvents were evaporated, and the crude product was purified by CC (hexane/AcOEt 1:2) to yield nucleoside **12a** (39 mg, 53%) as a white foam, together with traces of the 3′-desilylated nucleoside **12b**. R_f (AcOEt): 0.54; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.68 (s, 1H; H-N(4)), 8.39 (d, J = 7.5 Hz, 1H; H-C(6)), 7.90 (d, J = 7.3 Hz, 2H; arom.H), 7.64–7.59 (m, 4H; arom.H, H-C(5)), 7.33–7.30 (m, 5H; arom.H), 6.23 (t, J = 5.8 Hz, 1H; H-C(1′)), 5.08 (s, 2H; CH_2Ph), 4.71 (dd, J_1 = 5.5, J_2 = 0.8 Hz, 1H; H-C(5′)), 4.31 (d, J = 5.5 Hz, 1H; H-C(4′)), 2.86 (dd, J_1 = 13.6, J_2 = 6.1 Hz, 1H; H-C(2′)), 2.73 (d, J = 18.8 Hz, 1H; H-C(7′)), 2.60 (d, J = 18.7 Hz, 1H; H-C(7′)), 2.33 (dd, J_1 = 13.4, J_2 = 5.8 Hz, 1H; H-C(2′)), 0.90 (s, 9H; TBS), 0.17 (s, 9H; TMS), 0.16 (s, 3H; TBS), 0.14 ppm (s, 3H; TBS); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 162.46 (s, C(6′)), 160.21 (s, C(4)), 137.94 (s, arom.C), 133.47 (d, arom.C), 129.37 (d, arom.C), 128.83 (d, arom.C), 128.67 (d, arom.C), 128.62 (d, arom.C), 128.36 (d, arom.C) 128.26 (d, arom.C), 127.83 (s, arom.C), 90.27 (d, C(1′)), 89.35 (d, C(5)), 85.04 (s, C(3′)), 76.75 (t, CH_2Ph), 73.34 (d, C(5′)), 47.26 (t, C(2′)), 38.46 (t, C(7′)), 26.19 (q, TBS), 18.76 (s, TBS), 2.09 (q, TMS), −4.21 (q, TBS), −4.27 ppm (q, TBS); ESI$^+$-HRMS: calcd for $C_{34}H_{47}N_4O_6Si_2$: 663.3034 $[M+Na]^+$; found: 663.3021.

N^4-Benzoyl-(6′-benzyloximino-5′-O-*tert*-butyldimethylsilyl-2′-deoxy-3′,5′-ethano-β-D-ribofuranosyl]cytosine (12b): AIBN (15 mg, 0.09 mmol) was added at RT to a solution of Bu_3SnH (0.08 mL, 0.27 mmol, 1.5 equiv) and iodonucleoside **11b** (126 mg, 0.18 mmol) in toluene (1 mL). The mixture was heated at reflux for 1 h, and then the solvent was evaporated. Purification by CC (hexane/AcOEt 1:2) yielded the title compound **12b** (50 mg, 47%) as a white foam. R_f (AcOEt): 0.36; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.46 (d, J = 7.4 Hz, 1H; H-C(6)), 7.90 (d, J = 7.66 Hz, 2H; arom.H), 7.54–7.50 (m, 4H; arom.H, H-C(5)), 7.32–7.30 (m, 5H; arom.H), 6.38 (t, J = 5.9 Hz, 1H; H-C(1′)), 5.07 (d, J = 2.0 Hz, 2H; CH_2Ph), 4.73 (dd, J_1 = 5.6, J_2 = 0.7 Hz, 1H; H-C(5′)), 4.35 (d, J = 5.7 Hz, 1H; H-C(4′)), 2.92 (dd, J_1 = 13.6, J_2 = 5.7 Hz, 1H; H-C(2′)), 2.86 (d, J = 18.8 Hz, 1H; H-C(7′)), 2.66 (d, J = 18.8 Hz, 1H; H-C(7′)), 2.12 (dd, J_1 = 13.5, J_2 = 7.0 Hz, 1H; H-C(2′)), 2.04 (s, 1H; C(3′)–OH), 0.89 (s, 9H; TBS), 0.15 (s, 3H; TBS), 0.14 ppm (s, 3H; TBS); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 162.60 (s, C(6′)), 160.23 (s, C(4)), 137.95 (s, arom.C),133.53 (d, arom.C), 129.39 (d, arom.C), 128.75 (d, arom.C), 128.63 (d, arom.C), 128.24 (d, arom.C), 127.82 (s, arom.C), 89.49 (d, C(1′)), 89.38 (d,

C(5)), 84.03 (s, C(3′)), 76.69 (t, CH_2Ph), 73.61 (d, C(5′)), 47.68 (t, C(2′)), 38.01 (t, C(7′)), 26.22 (q, TBS), 18.78 (s, TBS), −4.05 (q, TBS), −4.29 ppm (q, TBS); ESI^+-HRMS: calcd for $C_{31}H_{38}N_4O_6NaSi_2$: 613.2458 $[M+Na]^+$; found: 613.2441.

N^4-Benzoyl-(6′-benzyloximino-2′-deoxy-3′,5′-ethano-β-D-ribofuranosyl)cytosine (13): HF·Py (0.2 mL, 7.7 mmol) was added at 0 °C to a solution of nucleoside **12a** (57 mg) and **12b** (43 mg, 0.16 mmol in total) in Py (2 mL). After the system had been stirred for 12 h at RT, silica gel (ca 0.5 g) was added, and after another 15 min the mixture was filtered over Celite and washed with AcOEt (10 mL) and DCM/MeOH (5:1, 10 mL). The solvents were evaporated, and the crude product was purified by CC (CH_2Cl_2/MeOH 10:1) to give the title compound **13** (54 mg, 46%) as colorless needles after crystallization from MeOH (slow evaporation). R_f (DCM/MeOH 10:1): 0.38; m.p.: 135 °C; ^1H NMR (CD_3OD, 400 MHz): $\delta = 8.53$ (d, $J = 7.6$ Hz, 1H; H-C(6)), 7.94–7.91 (m, 2H; arom.H), 7.60–7.47 (m, 4H; arom.H, H-C(5)), 7.30–7.19 (m, 5H; arom.H), 6.19 (t, $J = 6.1$ Hz, 1H; H-C(1′)), 5.06 (s, 2H; CH_2Ph), 4.65 (dd, $J_1 = 5.2$, $J_2 = 1.1$ Hz, 1H; H-C(5′)), 4.50 (s, 1H; OH), 4.27 (d, $J = 5.2$ Hz, 1H; H-C(4′)), 2.83 (dd, $J_1 = 19.6$, $J_2 = 1.0$ Hz, 1H; H-C(7′)), 2.72 (dd, $J_1 = 13.6$, $J_2 = 6.4$ Hz, 1H; H-C(2′)), 2.54 (d, $J = 19.5$ Hz, 1H; H-C(7′)), 2.21 ppm (dd, $J_1 = 13.7$, $J_2 = 6.0$ Hz, 1H; H-C(2′)); ^{13}C NMR (CD_3OD, 100 MHz): $\delta = 162.74$ (s, C(6′)), 146.37 (s, C(4)), 139.09 (s, arom.C), 134.10 (d, arom.C), 129.85 (d, arom.C), 129.39 (d, arom.C), 129.28 (d, arom.C), 129.15 (d, arom.C), 128.93 (s, arom.C), 97.98 (s, C(5)), 90.28 (d, C(1′)), 83.37 (d, C(5)), 77.28 (t, CH_2Ph), 73.30 (d, C(5′)), 47.99 (t, C(2′)), 38.99 ppm (t, C(7′)); ESI^+-HRMS: calcd for $C_{25}H_{24}N_4O_6Na$: 499.1593 $[M+Na]^+$; found: 499.1579.

Oligonucleotide synthesis and purification: Oligonucleotides were synthesized by standard solid-phase phosphoramidite methodology either on the small slider of a Polygen DNA-synthesizer (**ox1**–**3**, **bc1**–**3**, and all natural oligonucleotides) or on the 1 μmol scale on a Pharmacia LKB Gene Assembler Special DNA synthesizer (**ox4**–**5** and all fluoresceine-labeled (FAM-labeled) oligonucleotides). Ethyl thiotetrazole (0.25 M in acetonitrile) was used as activator in the coupling step. The coupling time was extended to 6–15 min for all modified phosphoramidites. Oligonucleotides **ox1**–**4** and **bc1**–**3** were synthesized on commercial DNA solid support, whereas for **ox5** tcT-solid support was used. The fluorescein-labeled oligonucleotides were synthesized on FAM-solid support (Roche Diagnostics). The coupling efficiencies for bc^{ox}-T were in the range of 90 to 93%. Deprotection and detachment of **ox1**–**3** was performed in conc. NH_3 (1 mL) for 4 h at 25 °C, and for **ox4**–**5** and the FAM-labeled oligonucleotides for 1 h at 4 °C. The crude oligonucleotides were purified by ion-exchange HPLC with an Äkta™ basic 10/100 system (Amersham Pharmacia Biotech) and a DNAPAC PA200, 4×250 mm analytical column (Dionex). Mobile phases: A) NaH_2PO_4 (20 mM), H_2O, pH 7.0; B) NaH_2PO_4 (20 mM), NaCl (1.25 M), H_2O, pH 7.0. Gradient: 0–50% B in 30 min (**ox1**–**3**, **bc1**–**3**) and 0–100% B for **ox4**–**5** and FAM-labeled oligonucleotides. All oligonucleotides were desalted over Sep-Pak Classic C18 Cartridges (Waters), and were routinely analyzed by ESI^- mass spectrometry (Supporting Information).

Melting curves: UV-melting curves were recorded on a Varian Cary 3E UV/Vis spectrophotometer. Absorbances were monitored at 260 nm, and the heating rate was set to 0.5 °C min^{-1}. A cooling–heating–cooling cycle in a temperature range of 15–80 °C or 4–70 °C was applied. T_m values were obtained from the derivative curves with the aid of the Varian WinUV software. For temperatures below 15 °C, N_2 was flushed through the cuvette compartment to avoid condensation of water. To avoid evaporation of the solution, the sample solutions were covered with a layer of dimethylpolysiloxane. All measurements were carried out in NaCl (150 mM)/Na_2HPO_4 (10 mM) at pH 7.0 at a total oligonucleotide concentration of 4 μM, except for **ox5** versus RNA, which was measured at 0.8 μM.

Cell culture and transfection analysis: HeLa cells were maintained in an incubator under CO_2 (5%) at 37 °C in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with fetal calf serum (FBS, 10%), penicillin (100 U mL^{-1}), and streptomycin (Invitrogen, 100 μg mL^{-1}). For transfection, 5×10^5 HeLa cells were seeded onto a six-well plate for 12 h. Oligonucleotides (20 μM final conc.) were complexed with Lipofectamine (Invitrogen/Life Technologies, 10 μL) and diluted to 200 μL with Optimem (Invitrogen/Life Technologies) at room temperature. After 30 min the complex was diluted to a final volume of 1 mL and added to the cells for further incubation. The medium was replaced by normal growth medium after 5 h. The cells were analyzed after 24 h by fluorescence microscopy (Zeiss Axiovert 135, filter 450–490 nm).

CCDC 686081 (**8**) and 686080 (**13**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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