Synthesis and Incorporation into $\alpha$-DNA of a Novel Conformationally Constrained $\alpha$-Nucleoside Analogue

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Abstract: The synthesis and incorporation into $\alpha$-DNA of a novel conformationally constrained $\alpha$-nucleoside analogue is described. The carbohydrate part of this analogue was prepared in 4 steps from the known bicyclic precursor 1 via a stereospecific, intramolecular, Et3B-mediated radical addition to a keto function as the key step. The thus obtained intermediate 4 was transformed stereoselectively into the corresponding $\alpha$-nucleoside analogues 7 and 8 containing the bases adenine and thymine, and were further elaborated into the phosphoramidite building blocks 11 and 12. Both building blocks were incorporated into $\alpha$-oligodeoxynucleotides and their pairing behavior to parallel complementary DNA was studied by UV-melting experiments. Single substitutions of $\alpha$-deoxynucleobases units by the new analogues in the center of duplexes were found to be thermally destabilizing by only $-0.8$ to $-3.1 \, ^\circ C$.

Key words: radical reactions, DNA, nucleosides, antisense agents, bioorganic chemistry

Introduction

Oligonucleotide analogues continue to be an attractive goal in medicinal chemistry as specific inhibitors (antisense agents) for the expression of disease related genes.$^{1-3}$ While a first generation phosphorothioate oligodeoxynucleotide 21-mer (ISIS 2922, Vitravene®), acting against cytomegalovirus (CMV) induced retinitis, is available on the market since 1998, a number of first and second generation (2'-O-modified-RNA) oligonucleotides are currently in clinical trials. A prerequisite for efficient inhibition of the expression of the genetic message is high affinity of an oligonucleotide analogue to its target RNA, as well as high biological stability in a cellular environment. Within this context others and we have successfully applied the principle of oligonucleotide single strand preorganization via conformational constriction of nucleosides as one way to improve the binding properties of oligonucleotides to their RNA target.$^4$

The $\alpha$-anomeric isomer of DNA ($\alpha$-DNA, Figure 1) was shown earlier to bind to complementary DNA and RNA in a parallel fashion with similar affinity as DNA.$^{5,6}$ Furthermore, $\alpha$-oligodeoxynucleotides are more stable against degradation by nucleases.$^7$ NMR structural analyses on $\alpha$-DNA/DNA duplexes showed that these are fully Watson–Crick base-paired and adopt a right-handed helical structure with an overall geometry that is close to the B-conformation of DNA.$^8$ We have investigated earlier the conformationally constrained $\alpha$-DNA analogue $\alpha$-bicyclo-DNA and found it to pair to complementary DNA and RNA in a parallel fashion with similar affinity as DNA.$^9$ Given the fact that in $\alpha$-bicyclo-DNA one of the backbone torsion angles ($\gamma$) deviates intrinsically ($anti$) from that observed in DNA and $\alpha$-DNA/DNA duplexes ($syn$) we wished to correct for this local structural inconsistency and further constrit conformational flexibility, and designed the fixed nucleic acid analogue FNA (Figure 1).

In this communication we report on the synthesis of the underlying nucleoside analogues 7 and 8, as well as their building blocks for oligodeoxynucleotide synthesis. Furthermore, we disclose first pairing properties of $\alpha$-oligodexoxyxynucleotides carrying single $\alpha$-FNA nucleotide residues with complementary DNA, as determined by UV-melting curve analysis and CD-spectroscopy.

**Figure 1** Chemical structures of $\alpha$-bicyclo-DNA, $\alpha$-DNA and $\alpha$-FNA. The red part of the structures conformationally constrain the $C3'$–$C4'$ and the $C4'$–$C5'$ bonds (cf. torsion angles $\gamma$ and $\delta$) of the deoxynucleobase unit. In $\alpha$-DNA, the torsion angle $\gamma$ is locked in the $syn$-conformation, as observed in $\alpha$-DNA/DNA duplexes, while it is preferentially $anti$ in $\alpha$-bicyclo-DNA. The conformation of the furanose units is in the south ($S$) conformational range in all cases.

Results

**Synthesis of Monomers**

The synthesis of the nucleosides 7 and 8 started from the diol 1 for which we had already elaborated a productive synthetic access.$^{10}$ In order to obtain the tricyclic sugar surrogate 4 as the first key intermediate, we planned to introduce a suitably functionalized C2 synthon into ketone 2, which was easily obtained in high yield by Dess–Martin oxidation. First experiments with the allyl Grignard reagent not unexpectedly yielded exclusively the addition
product with the undesired relative configuration at C-6. In order to control the relative stereochemistry at this center, an intramolecular approach using the tertiary hydroxy function in 2 as an anchor was envisaged. Exploratory experiments into this direction with the corresponding α-bromoacetate (→ intramolecular Reformatsky reaction) failed due to the unreliable and low yielding formation of the sterically hindered ester. Encouraged by a recent report on intramolecular addition of alkyl radicals to aldehydes and ketones by Malacria, we decided to explore a similar approach. To this end the necessary iodoacetal 3 was conveniently prepared as a ca. 1:1 mixture of diastereoisomers by treatment of 2 with ethyl vinyl ether in the presence of N-iodosuccinimide (NIS). The following intramolecular radical addition with Et₃B as the radical initiator and terminator lead to 4 in an appreciable yield (55%) for this type of reaction (Scheme 1). The enhanced electrophilicity of the keto group in 4, due to the neighboring electronegative substituents, might certainly have contributed to the efficiency of this reaction. Interestingly, 4 was isolated as a α:β = 7:1 mixture with respect to the configuration at the acetal center. This can only be explained by epimerization during or after the ring closing reaction, most probably induced by Et₃B acting as a Lewis acid. This observation was important with respect to the stereochemical outcome of the following nucleosidation reactions.

![Scheme 1](image1)

Scheme 1 Reagents and conditions: (a) Dess–Martin reagent, CH₂Cl₂, r.t., 30 min; (b) NIS (2.5 equiv), ethyl vinyl ether (2.5 equiv), CH₂Cl₂, –78 °C, 12 h; (c) 15% Et₃B in hexane, –20 °C, air, 72 h, 55%

With 4 in our hands we approached the nucleoside synthesis via the classical Vorbrüggen one-pot procedure (Scheme 2).

![Scheme 2](image2)

Scheme 2 Reagents and conditions: (a) i) thymine or 6-benzoyladenine (2–3 equiv), BSA (6–8 equiv), MeCN (for 5), ClCH₂CH₂Cl (for 6), r.t., –60 °C, 30 min, ii) Me₃SiOTf (1.5–2.0 equiv), 60–80 °C, 2–7 h

Bu₄NF in THF, the nucleoside analogues 7 and 8 (Scheme 3) bearing free OH groups became available. The α-(3S)-configuration at the anomeric center in 7 and 8 could unambiguously be assigned by ¹H NMR difference NOE spectroscopy (see experimental section).

![Scheme 3](image3)

Scheme 3 Reagents and conditions: (a) Bu₄NF (2.0–2.5 equiv), THF, 2–4 h, r.t.–40 °C; (b) 9-chloro-9-phenylxanthene (4–8 equiv), pyridine, r.t., 18 h; (c) (NCCH₂CH₂O)(i-Pr₂)₂PCl (1.5 equiv), i-Pr₂NEt (6 equiv), MeCN, r.t.

Selective introduction of the 9-phenyl-9-xanthenyl (pixyl) group into the secondary hydroxyl function could be achieved with the corresponding chloride in absolute pyridine, yielding 9 and 10 in very good yields. The pixyl group has been shown in the past to be a valuable alternative for the commonly used, sterically more demanding dimethoxy trityl group (DMT) in DNA or RNA synthesis for the protection of less accessible, secondary hydroxy groups. The phosphoramidite building blocks 11 and
12 were obtained by reaction of 9 and 10 with the commonly used phosphitylation reagent (Scheme 3). As expected, 11 and 12 appear as diastereoisomeric mixtures at phosphorus (1H NMR and 31P NMR).

## Oligonucleotide Synthesis

The synthesis of oligonucleotides 13–18 (Table 1) was accomplished on a DNA synthesizer on the 1.3 μmol scale by standard cyanoethyl phosphoramidite chemistry. The chain extension cycles were essentially identical to those for natural oligodeoxynucleotide synthesis with coupling times increased to 15–20 min for the building blocks 11 and 12 and replacement of the usual activator 1H-T-tetrazole by the more powerful (5-alkyl)-1H-tetrazoles. Coupling yields of >95% per step (trityl assay) were obtained for DNA or α-DNA building blocks. The incorporation of the α-FNA building blocks, however, proceeded only with yields of ≤ 60% even at increased phosphoramidite concentration. This inefficiency in the coupling step is most likely due to steric hindrance around the reacting groups in 11 and 12 and clearly needs to be addressed in the future. This precluded the synthesis of multiply or fully modified α-FNA oligonucleotides. The oligonucleotides were deprotected and cleaved from solid support in a standard manner. Crude oligonucleotides were purified to homogeneity by DEAE ion-exchange HPLC, controlled by RP-HPLC, and analyzed by ESI-MS. Table 2 gives an overview of the α-oligodeoxynucleotides prepared and used for the following investigations.

### Table 1

<table>
<thead>
<tr>
<th>Product</th>
<th>Oligonucleotides (5′→3′)</th>
<th>[M–H]+ calc</th>
<th>[M–H]+ found</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>α-d(TTTTT-4-TTTT)</td>
<td>3048.2</td>
<td>3047.8</td>
</tr>
</tbody>
</table>
| 14      | α-d(TTTT-4-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
residue (entry 8). Again, no antiparallel duplex formation could be observed, neither in the modified nor in the unmodified duplex (entry 9, 10). From these data we conclude that within the duplex the FNA residues are fully base-paired, recognize complementary bases with high selectivity and do not perturb the preferred parallel strand orientation in duplexes of α-DNA with DNA.

**Structural Investigations by CD-Spectroscopy**

In order to evaluate structural differences between modified and unmodified duplexes we recorded CD spectra of the duplexes corresponding to entries 1, 2 and 4 of Table 2 (Figure 2). Comparison of the CD spectra at low temperatures reveals a distinct negative maximum around 270 nm in the case of the modified duplexes (Figure 2b and c) which dissipates upon melting of the duplex (at higher temperatures). This behavior is essentially absent in the case of the unmodified duplex (Figure 2a). Thus, the CD spectra indicate subtle structural variations upon introduction of a single α-T-FNA unit. This is not unusual and shows local, probably cooperative conformational alterations around the modified unit. These structural variations could be the reason that substitutions near to the strand-terminus are more destabilizing than those in the center. An extrapolation of these observations would point to a different conformation of a fully modified α-FNA/DNA duplex as compared to a α-DNA/DNA duplex. This does not preclude, however, that the thermal stability of such a fully modified α-FNA/DNA duplex could not be higher.

![Figure 2](image-url) CD-spectra of selected α-DNA/DNA duplexes in 10 mM Na-cacodylate, 1 M NaCl, pH 7.0 (c = 2 μM).

**Discussion and Conclusions**

Within the growing family of conformationally constrained DNA analogues there are two members that are of particular interest here, namely the α-bicyclo-DNA\(^9,16\) (Figure 1) and α-d-LNA\(^7,18\). A direct comparison of properties with α-bicyclo-DNA is difficult due to the fact that no data on single substitutions of α-DNA with α-bicyclo-DNA exists. The comparison of homogeneous backbone (fully modified) oligonucleotides with that of non-homogeneous (partially modified) ones is difficult and can be misleading as has been demonstrated before in the case of the pairing of α-d-LNA to RNA.\(^8\) Data of α-DNA sequences containing single α-d-LNA residues, however, are available. These data show that α-LNA residues destabilize pairing to complementary DNA and RNA thermally by −6.5 to −8.0 °C/mod. This is distinctly more than observed for α-FNA units. The main structural difference between α-LNA and α-FNA, presented here, concerns the conformation of the furanose ring that is N in the former and S in the latter case, and the torsion angle γ which is unrestricted in α-d-LNA. The overall B-structural features of parallel α-β-DNA duplexes,\(^8\) are thus better accommodated in the FNA units, showing a more appropriate geometric preorganization than α-d-LNA. In fact, the latter analogue fails to form duplexes with complementary DNA but forms stable duplexes with RNA as a complement.

In conclusion, we have presented a concise synthesis of the adenine and thymine containing building blocks of the new conformationally constrained DNA-analogue α-FNA. As determined from single incorporations into α-
DNA, this analogue diminishes affinity to complementary DNA only slightly. The limiting factor of a more detailed study of this new DNA-analogue is the coupling yield upon oligomerization, which precludes at this point the study of fully modified α-FNA. Experiments to improve the coupling chemistry, as well as towards the synthesis of β-FNA are currently on our plans.

Solvents for extraction: technical grade, distilled. Solvents for reactions: reagent grade, distilled over CaH₂ (MeCN, CH₂Cl₂, pyridine), LiAlH₄ (Et₂O) or Na (THF). All reactions were performed under anhydrous conditions in an argon atmosphere. Reagents were, unless otherwise stated, from Fluka, highest quality available. Optical rotation: Perkin-Elmer-241 polarimeter. IR: Perkin-Elmer FTIR 1600. NMR: Bruker AC-300, DRX500, 10 mm sample. MPM multiplicity from DEPT spectra, J in Hz. TLC: Merck Si G 25 UV 254. Flash column chromatography (FC): silica gel (30–60 micron) from Fluka.

CD-spectra were recorded on a Jasco J-710 spectropolarimeter. Complementary oligodeoxynucleotides were mixed to 1:1 stoichiometry with a 2:1 (molar) mixture of T3 and T15. Curves were determined at 260 nm on a Varian Cary 3E spectrophotometer. CD-spectra were recorded on a Jasco J-715 spectropolarimeter with a Jasco PFO-350S temperature controller. The temperature was varied using a Varian heating-cooling-heating cycle (0°C–90°C) and a Varian Cary 3E spectrophotometer that was equipped with a Peltier block using the Varian WinUV software. Complementary oligodeoxynucleotides were synthesized by the solid-phase method using a 96-well plate synthesizer (2002, No. 6, 789–796 ISSN 0039-7881 © Thieme Stuttgart · New York).

HRMS (ESI-TOF, [M + H]+): C₇₇H₅₄O₂₅Si: m/z calcd 2487.2892, found: 2487.1012.

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SiO<sub>2</sub>-hexane–EtOAc, 1:1) afforded 5 (425mg, 74%) as a mixture of αβ: 3S/3R = 9:1; white foam.

**α-3S Isomer**

TLC (hexane–EtOAc, 1:1): R<sub>f</sub> 0.60.

IR (CH<sub>3</sub>C{l}): 3392w, 3027w, 2957w, 1688s, 1471m, 1361w, 1256m, 1109m, 908m, 853m cm<sup>−1</sup>.

1H NMR (300 MHz, CDCl<sub>3</sub>): δ = 8.25 (br s, 1 H, NH), 7.09 [d, J = 1.21 Hz, 1 H, H-6(C6)], 5.84 [dd, J = 9.27, 2.82 Hz, 1 H, H-C(3′)], 4.40 (s, 1 H, OH), 4.26–4.19 [m, 1 H, H-9(C′)], 4.04 [dd, J = 5.23, 1.47 Hz, 1 H, H-C(7′)], 3.82–3.77 [m, 1 H, H-C(9′)], 3.67 [s, 1 H, H-C(11′)], 2.9 [dd, J = 14.31, 9.27 Hz, 1 H, H-C(4′)], 2.50–2.44 [m, 2 H, H-C(4′)], 2.40–1.95 [m, 4 H, 2H-C(10′)], H-C(6′)], 1.91 (s, 3 H, CH<sub>3</sub>), 0.92 [s, 9 H, (CH<sub>3</sub>)<sub>3</sub>Si], 0.15, 0.12 [2 s, 6 H, (CH<sub>3</sub>)<sub>3</sub>Si].

13C NMR (75 MHz, CDCl<sub>3</sub>): δ = 163.76 (s, C-4′), 150.0 (s, C-2′), 136.7 (d, C-6), 109.7 (s, C-5′), 96.0 (s, C-1′), 88.49 (d, C-3′), 83.48 (s, C-5′), 80.7 (d, C-11′), 74.6 (d, C-7′), 59.37 (t, C-9′), 50.54 (t, C-6′), 41.2 (t, C-4′), 32.5 (t, C-10′), 25.98, 25.81, 25.67 [3 (CH<sub>3</sub>), C(3′)], 18.06 [s, (CH<sub>3</sub>)<sub>3</sub>Si], 12.55 (q, CH<sub>3</sub>-thymine), −4.9, −5.1 [2 q, (CH<sub>3</sub>)<sub>3</sub>Si].


**1(15R,15S,7S,11R)-N-[9-(11-tert-Butyl-dimethyl-silyloxy)-5-trimethylsiloxy-2,8-dioxatricyclo[5.3.1.0<sub>1,5</sub>]undec-3-yl]-5-methyl-1H-purin-6-yl-benzamide (8)**

To a suspension of N<sub>2</sub>-benzoyladenine (40 mg, 0.324 mmol) in CICH<sub>2</sub>Cl<sub>2</sub> (1 mL) was added BSA (0.12 mL, 0.65 mmol). Upon stirring for 2 h at 80 °C a clear solution was formed. To this solution was added 4 [37 mg, 0.108 mmol] in CICH<sub>2</sub>Cl<sub>2</sub> (1 mL), followed by TMSOTf (0.05 mL, 0.21 mmol). This mixture was kept at 80 °C for 7 h. The solution was diluted with EtOAc (30 mL) and washed with NaHCO<sub>3</sub> (20 mL). The aqueous phase was extracted with EtOAc, (2 × 30 mL), the combined organic layers were dried (MgSO<sub>4</sub>) and evaporated. FC (8 g SiO<sub>2</sub>—EtOAc—hexane—MeOH) to give 6 (410 mg, 0.67 mmol) in THF (10 mL) was added BuNF-3H<sub>O</sub> (11 g, 2.2 mmol). After stirring for 4 at 40 °C, the mixture was evaporated and the residue purified by FC (40 g SiO<sub>2</sub>, EtOAc—hexane—MeOH). HRMS (ESI-TOF, [M + H] <sup>+</sup>, C<sub>14</sub>H<sub>17</sub>N<sub>2</sub>O<sub>6</sub>): m/z calced 309.1068, found: 309.1062.

**1(15R,15S,7S,11R)-1-[5-Hydroxy-11-(9-phenyl-9H-xanthen-9- yloxy)-2,8-dioxatricyclo[5.3.1.0<sub>1,5</sub>]undec-3-yl]-5-methyl-1H-pyrimidine-2,4-dione (9)**

To a solution of 7 (210 mg, 0.68 mmol) in anhyd pyridine (16 mL) was added argon 9-chloro-9-phenylxanthenethione (800 mg, 2.72 mmol) and the mixture was stirred at r.t. for 18 h. After that time the mixture was diluted with EtOAc (20 mL) and extracted with aq sat NaHCO<sub>3</sub> (30 mL). The aqueous phase was washed with EtOAc (40 mL) and the combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated. FC (10 g SiO<sub>2</sub>, EtOAc–hexane, 3:1 + 1% Et<sub>2</sub>N) gave 9 (308 mg, 80%) as a white foam. TLC (EtOAc–hexane, 3:1 + 1% Et<sub>2</sub>N): R<sub>f</sub> 0.21.

IR (CH<sub>3</sub>C{l}): 3391w, 3026w, 1688s, 1603w, 1575w, 1478m, 1448m, 1320w, 1279w, 1125w, 1071m, 908m, 786s cm<sup>−1</sup>.

1H NMR (300 MHz, CDCl<sub>3</sub>): δ = 8.77 (s, 1 H, NH), 7.40–7.00 (m, 13 H), 5.60 [dd, J = 6.8, 2.4 Hz, 1 H, H-C(3′)], 4.55 (s, 1 H, OH), 4.0 [m, 1 H, H-C(9′)], 3.57 [dd, J = 6.76, 11.0 Hz, 1 H, H-C(9′)].
A Novel Conformationally Constrained α-Nucleoside Analogue

3.34 [1, H, C(7')], 3.34-3.13 [m, 2, H, C(4')], C(11')], 2.63 [d, J = 14.7 Hz, 1, H, C(4')]], 2.45 [d, J = 15.4, 5.2 Hz, 1, H, C(10')], 1.96-1.91 [m, 5, H, C(6')], (CH3), 1.70 [m, 1, H, C(6']).

13C NMR (75 MHz, CDCl3); δ = 163.81 (s, C-4), 151.85 (C arom), 150.7 (s, C-2), 150.25, 151.75 (C arom), 139.71 (d, C-6), 130.31, 130.11, 129.97, 128.11, 127.15, 126.85, 126.30, 123.50, 123.31, 122.48, 116.87, 116.49 (C arom), 110.81 (s, C-9), 93.64 (s, C-1'), 94.35 (d, C-3'), 83.11 (d, C-7'), 82.87 (s, C-11'), 78.61 (s, C-5'), 76.59 (s, C arom, 60.21 (t, C-6'), 48.84 (t, C-4'), 41.67 (t, C-3'), 33.09 (t, C-10'), 12.35 (q, CH3).

1H NMR-difference NOE (500 MHz, CDCl3); δ = 2.63 [H{H(4')}] → 4.55 (OH), 3.13 [H{H-C(4')}] → 6.00 [H{H-C(3')}], 6.00 [H{H-C(3')}] → 7.2 H arene.

HRMS (ESI-TOF, [M – H]–, C 40 H 32 N 5 O 6 ); found: 576.2182 (100).
enosine was from R.I. Chemicals. α-Deoxythymidine as well as the α-nucleoside phosphoramidite building blocks were prepared as described. The assembly of oligonucleotides was performed according to the standard phosphoramidite protocol (trityl off mode) with the exception of a prolonged coupling time (15–20 min), and an increased concentration of the phosphoramidite solutions (0.2 M in MeCN, 20-fold excess per coupling) for the modified building blocks 11 and 12. In the coupling step, 1H-tetrazole was replaced by the more active (S-benzylthio)-1H-tetrazole (0.25 M in MeCN) or 5-(ethylthio)-1H-tetrazole (0.25 M in MeCN). As solid support the universal solid support from CT Gen was used. After synthesis, the solid support was suspended in conc. NH₄OH (ca. 1 mL) and left at 65 °C for 72–96 h. The crude oligonucleotides were purified by anion-exchange HPLC (Macherey-Nagel, nucleogen DEAE 60/7) and desalted over SEP-PAK C-18 cartridges (Waters).

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