Synthesis and Incorporation into α-DNA of a Novel Conformationally Constrained α-Nucleoside Analogue

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Abstract: The synthesis and incorporation into α-DNA of a novel conformationally constrained α-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, Et_3B-mediated radical addition to a keto function as the key step. The thus obtained intermediate **4** was transformed stereoselectively into the corresponding α-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 α-oligodeoxynucleotides and their pairing behavior to parallel complementary DNA was studied by UV-melting experiments. Single substitutions of α-deoxyribnucleoside units by the new analogues in the center of duplexes were found to be thermally destabilizing by only –0.8 to –3.1 °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 α-anomeric isomer of DNA (α-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, α-oligodeoxynucleotides are more stable against degradation by nucleases.[7] NMR structural analyses on α-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 α-DNA analogue α-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 α-bicyclo-DNA one of the backbone torsion angles (γ) deviates intrinsically (*anti*, pseudoequatorial orientation of the oxy-substituent at C-5′) from that observed in DNA and α-DNA/DNA duplexes (*syn*) we wished to correct for this local structural inconsistency and further constrict 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 α-oligodeoxynucleotides carrying single α-FNA nucleotide residues with complementary DNA, as determined by UV-melting curve analysis and CD-spectroscopy.

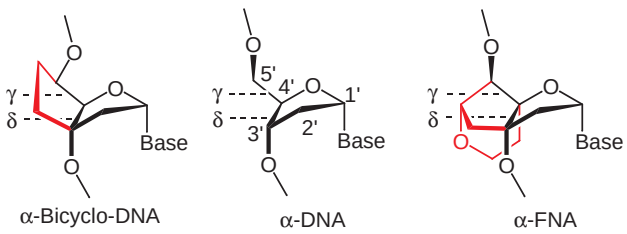

Figure 1 Chemical structures of α-bicyclo-DNA, α-DNA and α-FNA. The red part of the structures conformationally constrict the C3′–C4′ and the C4′–C5′ bonds (cf. torsion angles γ and δ) of the deoxyribose unit. In α-FNA, the torsion angle γ is locked in the *syn*-conformation, as observed in α-DNA/DNA duplexes, while it is preferentially *anti* in α-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

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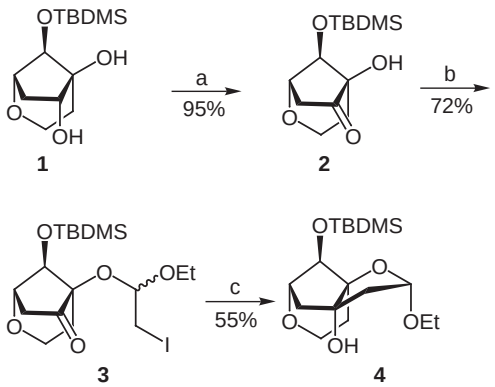
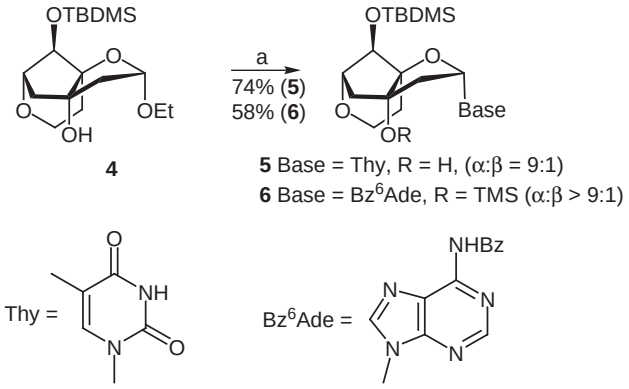
product with the undesired relative configuration at C-6. In order to control the relative stereochemistry at this center, an intramolecular approach using the tertiary hydroxy function in **2** as an anchor was envisaged. Exploratory experiments into this direction with the corresponding α-bromoacetate (→ 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 [11] 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).[12] The following intramolecular radical addition with Et_3B 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 **3**, 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 Et_3B acting as a Lewis acid. This observation was important with respect to the stereochemical outcome of the following nucleosidation reactions.



Scheme 1 *Reagents and conditions*: (a) Dess–Martin reagent, CH_2Cl_2, r.t., 30 min; (b) NIS (2.5 equiv), ethyl vinyl ether (2.5 equiv), CH_2Cl_2, –78 °C, 12 h; (c) 15% Et_3B in hexane, –20 °C, air, 72 h, 55%

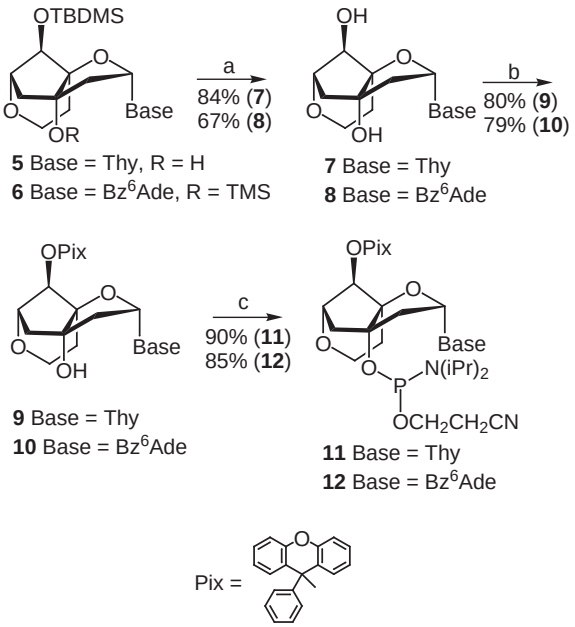
With **4** in our hands we approached the nucleoside synthesis via the classical Vorbrüggen one-pot procedure (Scheme 2).[13]

Me_3SiOTf-mediated condensation of **4** with in situ persilylated thymine in MeCN afforded **5** as an α:β (3*S*:3*R* = 9:1) mixture (^{1}H NMR) in 74% yield. Variations in temperature, solvent and Lewis acid, had no effect on the ratio of anomers. In the case of the purine base, the condensation of **4** with persilylated N^6-benzoyladenine was best performed in 1,2-dichloroethane and yielded the bis-silyl nucleoside **6** in 58%. Only small amounts of the corresponding β-(3*R*)-isomer (<10% relative to α-isomer) could be detected by ^{1}H NMR. After deprotection with



Scheme 2 *Reagents and conditions*: (a) i) thymine or N^6-benzoyladenine (2–3 equiv), BSA (6–8 equiv), MeCN (for **5**), $ClCH_2CH_2Cl$ (for **6**), r.t., –60 °C, 30 min, ii) Me_3SiOTf (1.5–2.0 equiv), 60–80 °C, 2–7 h

Bu_4NF in THF, the nucleoside analogues **7** and **8** (Scheme 3) bearing free OH groups became available. The α-(3*S*)-configuration at the anomeric center in **7** and **8** could unambiguously be assigned by ^{1}H NMR difference NOE spectroscopy (see experimental section).



Scheme 3 *Reagents and conditions*: (a) Bu_4NF (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) $(NCCH_2CH_2O)(i\text{-}Pr_2N)PCl$ (1.5 equiv), $i\text{-}Pr_2NEt$ (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.[14,15] 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 phosphorous (^{1}H NMR and ^{31}P 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 1*H*-tetrazole by the more powerful (*S*-alkyl)-1*H*-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 Sequence Information and MS Data of α-Oligonucleotides Prepared for the Present Study[a]

Product	Oligonucleotides (5′→3′)	$[M–H]^-$ calcd	$[M–H]^-$ found
13	α-d(TTTTT-**t**-TTTT)	3048.2	3047.8
14	α-d(TTT-**t**-TTTTTT)	3048.2	3047.7
15	α-d(T-**t**-TT TTTTTT)	3048.2	3048.5
16	α-d(TTTTTTTTTT)	2980.0	2979.9
17	α-d(TAAA-**a**-TATAA)	3111.2	3110.8
18	α-d(TAAAATATAA)	3043.0	3043.6

[a] Capital letters denote 2′-α-deoxyribonucleosides, small letters between hyphens FNA units.

Pairing Properties of Modified α-Oligonucleotides

In order to assess the influence of the backbone modification on duplex stability we recorded UV-melting curves of the modified α-oligonucleotides **13**–**15** and **17** with their β-DNA complements in parallel, antiparallel and base-mismatch arrangements and compared their properties with those of the unmodified α-oligonucleotides **16** and **18**. All measurements were performed in standard buffer at pH 7.0. The corresponding Tm-data are summarized in Table 2.

Table 2 Tm-Data (°C) from UV-Melting Curves (260 nm) in 10 mM Na-Cacodylate, pH 7.0 and 1 M NaCl (duplex concd = 2 μM)

Entry	Duplex	Tm	ΔTm/mod.
1	α-d(TTTTTTTTTT) β-d(AAAAAAAAAA)	40.1	-
2	α-d(TTTTT-**t**-TTTT) β-d(AAAAA-A-AAAA)	37.6	–2.5
3	α-d(TTT-**t**-TTTTTT) β-d(AAA-A-AAAAAA)	37.0	–3.1
4	α-d(T-**t**-TTTTTTTT) β-d(A-A-AAAAAAAA)	28.5	–11.5
5	α-d(TAAAATATAA) β-d(ATTTTATATT)	16.5	-
6	α-d(TAAA-**a**-TATAA) β-d(ATTT-T-ATATT)	15.7	–0.8
7	α-d(TAAAATATAA) β-d(ATTTCATATT)	n.d.[a]	-
8	α-d(TAAA-**a**-TATAA) β-d(ATTT-**C**-ATATT)	n.d.[a]	-
9	α-d(TAAAATATAA) (ATTTTATATT)d-β	n.d.[a]	-
10	α-d(TAAA-**a**-TATAA) (ATTT-T-ATATT)d-β	n.d.[a]	-

[a] n.d.= not detected.

In a first series of experiments, we focused on the pairing properties of the homobasic α-decanucleotides **13**–**15**, containing one modified α-T-FNA unit at various positions, with their DNA complement. By comparing the Tm-data (Table 2) it becomes obvious, that incorporation of a α-T-FNA unit in the center of the sequence leads to a decrease in Tm by ca. 2.5 to 3.1 °C (entries 2 and 3). From these experiments we conclude that complementary base-pairing occurs at the site of modification, albeit on an overall lower affinity-level compared to unmodified α-DNA. Surprisingly, incorporation near to the end of the sequence leads to a decrease in Tm of 11.5 °C (entry 4). This points to a negative cooperative effect of the α-T-FNA, probably disrupting base-pairing of the 5′-terminal nucleotide unit in **15**.

In order to further explore effects on preferred strand orientation and base-pairing selectivity we investigated the asymmetric, modified decamer **17** and compared its pairing properties to that of the unmodified α-oligonucleotide **18**. These experiments also allowed for the detection of eventual effects arising from the bases, as the α-A-FNA unit was used here. Also in this case, a stable duplex was formed with the parallel β-DNA complement (entry 6) with a ΔTm-value of only –0.8 °C. As expected, base-pairing is selective. No duplex formation could be observed if cytosine was introduced opposite the α-A-FNA

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 dissapears 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.

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.[17,18] 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.[18] 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-

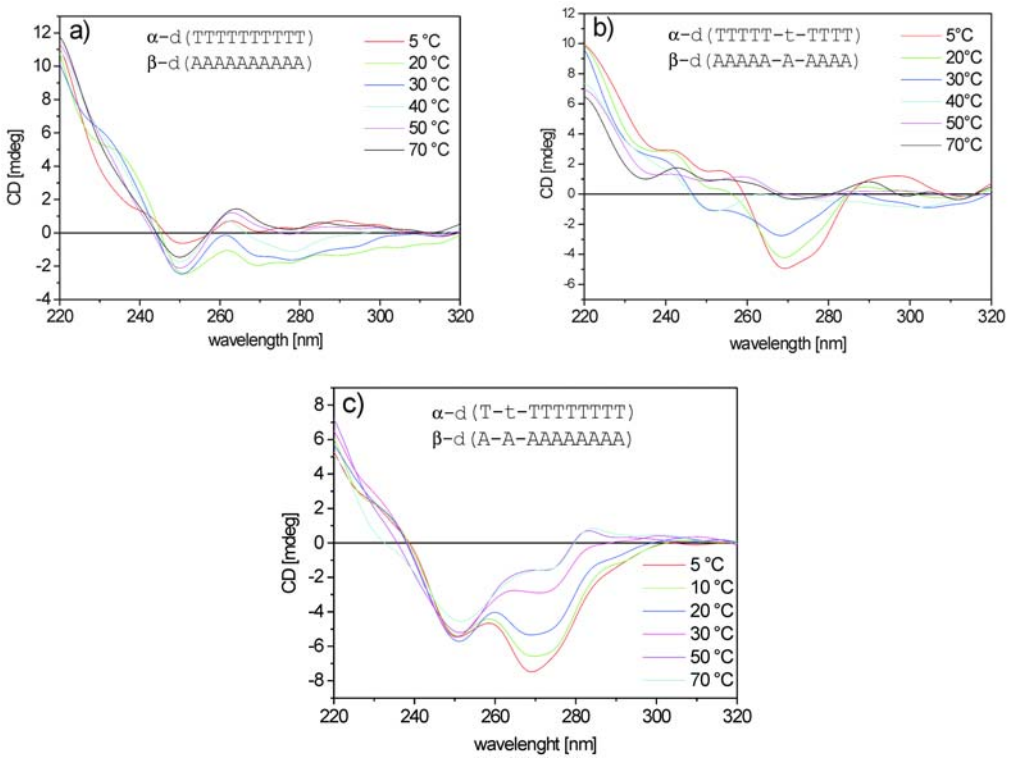


Figure 2 CD-spectra of selected α-DNA/DNA duplexes in 10 mM Na-cacodylate, 1 M NaCl, pH 7.0 (c = 2 μM).

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 CaH_2 (MeCN, CH_2Cl_2, pyridine), $LiAlH_4$ (Et_2O) 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, δ in ppm, ^{13}C multiplicities from DEPT spectra, *J* in Hz. TLC: Merck SiL G-25 UV_{254}. Flash column chromatography (FC): silica gel (30–60 micron) from Fluka.

CD-spectra were recorded on a Jasco J-715 spectropolarimeter with a Jasco PFO-350S temperature controller. The temperature was measured directly in the cell (path length 10 mm). UV-melting curves were determined at 260 nm on a Varian Cary 3E spectrophotometer that was equipped with a Peltier block using the Varian WinUV software. Complementary oligodeoxynucleotides were mixed to 1:1 stoichiometry with a 2 μM single strand oligonucleotide concentration. A heating-cooling-heating cycle (0→90 °C) was applied with a temperature gradient of 0.5 °C/min. Tm values were defined as the maximum of the first derivative of the melting curve using the software package Origin™v5.0.

(1*S*,5*R*,8*R*)-8-{[*tert*-Butyl(dimethyl)silyl]oxy}-5-hydroxy-2-oxabicyclo[3.2.1]octan-6-one (2)

To a solution of **1**[10] (1.00 g, 3.6 mmol) in CH_2Cl_2 (45 mL) was added 1,1,1-tris(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one (Dess–Martin reagent,[19] 1.55 g, 7.2 mmol) and the mixture was stirred at r.t. After 30 min, the solution was washed with aq 20% $Na_2S_2O_3$ (50 mL) and aq sat. $NaHCO_3$ (50 mL) and the aqueous phase was extracted with CH_2Cl_2 (2 × 40 mL). The combined organic layers were dried ($MgSO_4$) and evaporated. FC (40 g SiO_2, hexane–EtOAc, 1:2) afforded **2** (960 mg, 95%) as a colorless oil. TLC (EtOAc–hexane, 2:1): R_f 0.67.

IR ($CHCl_3$): 2956s, 2931s, 2859m, 1756s, 1471w, 1379w, 1258m, 1098s, 838s cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 4.36 [dd, *J* = 5.7, 2.2 Hz, 1 H, H-C(1)], 3.86 [s, 1 H, H-C(8)], 3.83 [dd, *J* = 12.8, 4.0 Hz, 1 H, H-C(3)], 3.65 [dd, *J* = 12.8, 4.0 Hz, 1 H, H-C(3)], 2.80 [br s, 1 H, OH), 2.55 [dd, *J* = 19.1, 5.7 Hz, 1 H, H-C(7)], 2.40 [dd, *J* = 19.1, 1.5 Hz, 1 H, H-C(7)], 2.05 [dt, *J* = 12.9, 7.3 Hz, 1 H, H-C(4)], 1.71 [dd, *J* = 12.5, 4.0 Hz, 1 H, H-C(4)], 0.82 [s, 9 H, $(CH_3)_3CSi$], 0.08, 0.02 [2 s, 6 H, $(CH_3)_2Si$].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 199.4 (s, C-6), 78.67 (s, C-5), 74.11 (d, C-8), 65.95 (d, C-1), 59.48 (t, C-3), 43.7 (t, C-7), 39.7 (t, C-4), 25.66 [q, $(CH_3)_3Csi$], 18.2 [s, $(CH_3)_3CSi$], –4.8, –5.31 [2 q, $(CH_3)_2Si$].

HRMS (ESI-TOF, $[M - H]^-$, $C_{13}H_{23}O_4Si$): *m*/*z* calcd 271.1354, found: 271.1365.

(1*S*,5*R*,8*R*)-8-{[*tert*-Butyl(dimethyl)silyl]oxy}-5-(1-ethoxy-2-iodoethoxy)-2-oxabicyclo[3.2.1]octan-6-one (3)

To a solution of **2** (1.75 g, 6.43 mmol) in CH_2Cl_2 (20 mL) were added *N*-iodosuccinimide (3.50 g, 16 mmol) and ethyl vinyl ether (2.1 mL, 16 mmol) at –78 °C. The mixture was then allowed to warm up to r.t. After the reaction was complete, the solution was diluted with EtOAc (150 mL) and extracted with aq sat. $NaHCO_3$ (100 mL). The aqueous phase was washed with EtOAc (2 × 50mL), the combined organic layers were dried ($MgSO_4$) and evaporated. FC (100 g SiO_2, hexane–EtOAc, 9:1) afforded **3** (2.20 g, 72%) as a colorless oil. TLC (EtOAc–hexane, 2:1): R_f 0.77.

IR ($CHCl_3$): 3504w(br), 2957s, 2930s, 1755m, 1471m, 1431m, 1256m, 1109s, 973m, 838s cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 5.48, 5.2 (2 dd, *J* = 8.09, 3.31 Hz, 1 H, OCHO), 4.39–4.30 [2 dd, *J* = 5.89, 2.21 Hz, 1 H, H-C(1)], 4.06, 3.89 [2 s, 1 H, H-C(8)], 3.9–3.1 [m, 6 H, 2 H-C(3)], 2.70, 2.64 [2 dd, *J* = 5.9, 2.2 Hz, 1 H, H-C(7)], 2.39, 2.32 [2 dd, *J* = 5.5, 1.8 Hz, 1 H, H-C(7)], 2.28–1.92 [m, 1 H, H-C(4)], 1.86 [dd, *J* = 12.5, 3.7 Hz, 1 H, H-C(4)], 1.20 (m, 3 H, OCH_2CH_3), 0.86, 0.85 [2 s, 9 H, $(CH_3)_3CSi$], 0.14, 0.10 [2 s, 6 H, $(CH_3)_2Si$].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 214.2, 214 (2 s, C-6), 97.04, 96.6 (2 d, OCHO), 84.8, 84.3 (2 s, C-5), 78.8 (d, C-8), 74.51, 74.37 (2 d, C-1), 59.3, 59.24 (2 t, C-3), 59.2, 59.08 (2 t OCH_2CH_3), 38.55, 38.25 (2 t, C-7), 35.58, 34.36 (2 t, C-4), 25.68, 25.62 [2 q, $(CH_3)_3CSi$], 18.06, 17.92 [2 s, $(CH_3)_3CSi$], 15.2, 14.9 (2 q, OCH_2CH_3), 6.12, 5.99 (2 t, CH_2I), –4.40, –4.52, –4.68, –4.96 [4 q, $(CH_3)_2Si$].

HRMS (ESI-TOF, $[(M + H_2O)– H]^-$, $C_{17}H_{32}O_6Si$): *m*/*z* calcd 487.2892, found: 487.1012.

(1*R*,3*S*,5*S*,7*S*,11*R*)-11-{[*tert*-Butyl(dimethyl)silyl]oxy}-3-ethoxy-2,8-dioxatricyclo[5.3.1.0^{1,5}]undecan-5-ol (4)

A suspension of **3** (820 mg, 1.74 mmol) in a solution of 15% Et_3B in hexane was treated at –20 °C with air (20 mL). The mixture was kept at –20 °C for 72 h. The solution was then diluted with EtOAc (80 mL) and washed with aq sat. $NaHCO_3$ (50 mL). The aqueous phase was extracted with EtOAc (2 × 50 mL) and the combined organic layers were dried ($MgSO_4$) and concentrated. FC (60 g SiO_2, hexane→hexane–EtOAc, 5:1) afforded **4** (325 mg, 55%, 3*S*:3*R* = 7:1), 15% starting material **3** and 15% of **2**. TLC (5% Et_2O in CH_2Cl_2): R_f 0.34.

IR ($CHCl_3$): 3503w (br), 3007m, 2957s, 2930s, 2858m, 1471w, 1430w, 1375w, 1335w, 1307w, 1258m, 1228w, 1130s, 1109s, 968m, 929m, 854s cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 5.48 [dd, *J* = 5.7, 3.68 Hz, 0.12 H, β H-C(3)], 5.19 [d, *J* = 4.78 Hz, 0.88 H, α H-C(3)], 4.34–4.25 [m, H-C(9)], 4.06 [dd, *J* = 5.14, 1.47 Hz, 1 H, H-C(7)], 3.82–3.61 [m, 2 H, H-C(9), OCH_2], 3.55 [d, *J* = 1.84 Hz, 1 H, H-C(11)], 3.46–3.41 (m, OCH_2), 3.32 (s, 1 H, OH), 2.59 [dd, *J* = 13.23, 4.78 Hz, 1 H, H-C(4)], 2.37 [dd, *J* = 15.07, 5.15 Hz, 1 H, H-C(6)], 2.1–1.75 [m, 4 H, H-C(4), 2 × H-C(10), H-C(6)], 1.19 [t, *J* = 6.9 Hz, 3 H, CH_3), 0.86 [s, 9 H, $(CH_3)_3CSi$], 0.07 [s, 6 H, $(CH_3)_2Si$].

^{13}C NMR (75 MHz, $CDCl_3$): 107.52 (d, C-3), 83.98 (s, C-1), 82.18 (d, C-11), 80.99 (d, C-7), 76.58 (s, C-5), 62.7 (t, OCH_2), 61.11 (t, C-9), 47.15 (t, C-6), 38.51 (t, C-4), 34.57 (t, C-10), 25.72, 25.65 [2 q, $(CH_3)_3CSi$], 18.01 [s, $SiC(CH_3)_3$], 15.33 (q, CH_3CH_2), –4.8, –5.02 (2 q, $[CH_3)_2Si$].

HRMS (ESI-TOF, $[M – H]^-$, $C_{17}H_{31}O_5Si$): *m*/*z* calcd 343.1938, found: 343.1940.

(1*R*,3*S*,5*S*,7*S*,11*R*)-1-{11-[*tert*-Butyl(dimethyl)silyl]oxy}-5-hydroxy-2,8-dioxatricyclo[5.3.1.01,5]undec-3-yl]-5-methyl-1*H*-pyrimidine-2,4-dione (5)

A suspension of thymine (300 mg, 2.37 mmol) and **4** (400 mg, 1.16 mmol) in MeCN (20 mL) was treated with *N*,*O*-bis(trimethylsilyl)acetamide (BSA, 2.8 mL, 11.8 mmol) at 60 °C until a clear solution was formed. After addition of TMSOTf (0.4 mL, 1.75 mmol) the reaction was kept at 60 °C for 2 h. The solution was then diluted with EtOAc (60 mL) and washed with aq sat. $NaHCO_3$ (40 mL). The aqueous phase was extracted with EtOAc (2 × 40 mL), the combined organic layers dried ($MgSO_4$) and evaporated. FC (40 g

SiO_2, hexane–EtOAc, 1:1) afforded **5** (425mg, 74%) as a mixture of α:β (3*S*/3*R* = 9:1); white foam.

α-(3*S*) Isomer

TLC (hexane–EtOAc, 1:1): R_f 0.60.

IR ($CHCl_3$): 3392w, 3027w, 2957w, 1688s, 1471m, 1361w, 1256m, 1109m, 908m, 853m cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 8.25 (br s, 1 H, NH), 7.09 [d, *J* = 1.21 Hz, 1 H, H-C(6)], 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-C(9′)], 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′), H-C(6′)], 2.10–1.95 [m, 3 H, 2 H-C(10′), H-C(6′)], 1.91 (s, 3 H, CH_3), 0.92 [s, 9 H, $(CH_3)_3CSi$], 0.15, 0.12 [2 s, 6 H, $(CH_3)_2Si$].

^{13}C NMR (75 MHz, $CDCl_3$): 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′), 83.84 (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 q, $(CH_3)_3CSi$], 18.06 [s, $(CH_3)_3CSi$], 12.55 (q, CH_3-thymine), –4.9, –5.1 [2 q, $(CH_3)_2Si$].

HRMS (ESI-TOF, $[M - H]^-$, $C_{20}H_{32}N_2O_6Si$): *m*/*z* calcd 423.1971, found: 423.1985.

(1*R*,3*S*,5*S*,7*S*,11*R*)-*N*-{9-[11-(*tert*-Butyl{dimethyl}silyl)oxy]-5-trimethylsilyloxy-2,8-dioxatricyclo[5.3.1.0^{1,5}]undec-3-yl]-9*H*-purin-6-yl}benzamide (6)

To a suspension of N^6-benzoyladenine (40 mg, 0.324 mmol) in $ClCH_2CH_2Cl$ (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 a solution of **4** (37 mg, 0.108 mmol) in $ClCH_2CH_2Cl$ (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 aq sat. $NaHCO_3$ (20 mL). The aqueous phase was extracted with EtOAc, (2 × 30 mL), the combined organic layers were dried ($MgSO_4$) and evaporated. FC (8 g SiO_2, hexane–EtOAc, 1:3) provided the α-(3*S*) nucleoside **6** (38 mg, 58%) as a white foam together with the corresponding de-trimethylsilyl product (10 mg, 17%). TLC (hexane–EtOAc, 1:3): R_f 0.39.

IR ($CHCl_3$): 3401w, 2956m, 2360w, 1700s, 1613s, 1583m, 1454s, 1297w, 1256s, 1229s, 1122s, 908m, 853s cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 8.82 [s, 1 H, H-C(2)], 8.38 [s, 1 H, H-C(8)], 8.05 (d, *J* = 7.3 Hz, 2 H_{arom}), 7.61–7.53 (m, 3 H_{arom}), 6.62 [d, *J* = 7.0 Hz, 1 H, H-C(3′)], 4.14 [dd, *J* = 1.1, 5.2 Hz, 1 H, H-C(7′)], 4.06–3.8 [2 m, 2 H, 2 H-C(9′)], 3.69 [s, 1 H, H-C(11′)], 3.15 [dd, *J* = 7.7, 14.0 Hz, 1 H, H-C(4′)], 2.57–2.5 [m, 2 H, H-C(4′), H-C(6′)], 2.24–2.00 [m, 3 H, 2 H-C(10′), H-C(6′)], 0.98 [s, 9 H, $(CH_3)_3CSi$], 0.20, 0.17 [2 s, 6 H, $(CH_3)_2Si$], 0.04 [s, 9 H, $(CH_3)_3Si$].

^1H NMR-difference-NOE (300 MHz, $CDCl_3$): δ = 8.38 H-C(8) → 6.62 H-C(3′), 2.20 H-C(10′).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 152.58 (s, C-2), 151.32 (s, C-4), 149.12 (s, C-6), 141.81 (d, C-8), 132.68, 128.86, 128.66, 127.76, 127.20 (C_{arom}), 120.15 (s, C-5), 96.79 (s, C-1′), 88.71 (d, C-3′), 85.30 (s, C-5′), 81.31 (d, C-7′), 80.38 (d, C-11′), 60.90 (t, C-9′), 49.89 (t, C-6′), 41.03 (t, C-4′), 32.84 (t, C-10′), 25.86, 18.08 [2 q, $(CH_3)_3CSi$], 1.82 [q, $(CH_3)_2Si$], 1.48 [q, $(CH_3)_3Si$].

MS (–ESI-TOF): *m*/*z* (%) = 608.27 (100, $[M - H]^-$), 423.19 (30), 262.07 (80).

HRMS (ESI-TOF, $[M - H]^-$, $C_{30}H_{42}N_5O_5Si_2$): *m*/*z* calcd 608.2725, found 608.2724.

(1*R*,3*S*,5*S*,7*S*,11*R*)-1-(5,11-Dihydroxy-2,8-dioxatricyclo-[5.3.1.0^{1,5}]undec-3-yl)-5-methyl-1*H*-pyrimidine-2,4-dione (7)

A solution of **5** (200 mg, 0.47 mmol) in THF (4 mL) was treated with $Bu_4NF{\cdot}3H_2O$ (380 mg, 1.2 mmol) at r.t. After 2 h, SiO_2 (1 g) was added, the mixture evaporated and the residue was subjected to FC (8 g SiO_2, EtOAc → EtOAc + 2% MeOH) to give **7** (104 mg, 84%) as a white foam. TLC (EtOAc–hexane, 3:1): R_f 0.10; $[\alpha]_D$ +51.2 (*c* = 1.0, MeOH).

IR ($CHCl_3$): 3392w, 3027w, 2957w, 1688s, 1471m, 1361w, 1256m, 1109m, 908m, 852m cm^{-1}.

^1H NMR (300 MHz, CD_3OD): δ = 7.79 [d, *J* = 1.47 Hz, 1 H, H-C(6)], 6.28 [dd, *J* = 8.83, 2.21 Hz, 1 H, H-C(3′)], 4.13–3.98 [m, 2 H, H-C(9′), H-C(7′)], 3.7 [dd, *J* = 11.4, 6.25 Hz, 1 H, H-C(9′)], 3.48 (s, 1 H, OH), 3.21 [t, *J* = 1.47 Hz, 1 H, H-C(11′)], 2.9 [dd, *J* = 14.3, 8.83 Hz, 1 H, H-C(4′)], 2.42 [dd, *J* = 15.4, 5.52 Hz, 1 H, H-C(4′)], 2.14–1.87 [m, 3 H, H-C(6′), 2 H-C(10′)], 1.77 (s, 3 H, CH_3).

^{13}C NMR (75 MHz, CD_3OD): δ = 164.8 (s, C-4), 150.9 (s, C-2), 138.51 (d, C-6), 109.65 (s, C-5), 94.8 (s, C-1′), 88.98 (d, C-3′), 81.03 (s, C-5′), 80.32 (d, C-7′), 80.07 (d, C-11′), 57.98 (t, C-9′), 49.49 (t, C-6′), 41.21 (t, C-4′), 31.79 (t, C-10′), 12.4 (q, CH_3).

HRMS (ESI-TOF, $[M - H]^-$, $C_{14}H_{17}N_2O_6$): *m*/*z* calcd 309.1086, found: 309.1092.

(1*S*,5*S*,7*S*,11*R*)-*N*-[9-(5,11-Dihydroxy-2,8-dioxatricyclo-[5.3.1.0^{1,5}]undec-3-yl)-9*H*-purin-6-yl]benzamide (8)

To a solution of **6** (410 mg, 0.67 mmol) in THF (10 mL) was added $Bu_4NF{\cdot}3H_2O$ (1.1 g, 2.2 mmol). After sirring for 4 h at 40 °C, the mixture was evaporated and the residue purified by FC (40 g SiO_2, CH_2Cl_2 + 10% MeOH) to give **8** (190 mg, 67%) as a white foam. TLC (5% MeOH in CH_2Cl_2): R_f 0.22; $[\alpha]_D$ +26.5 (*c* = 0.5, MeOH).

IR ($CHCl_3$): 3027s, 2673m, 2359m, 2071m, 1699s, 1653s, 1588m, 1540m, 1506m, 1456m, 1117s, 973s, 893s cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 9.13 (s, 1 H, NH), 8.80 [d, *J* = 4.4 Hz, 1 H, H-C(2)], 8.12 [s, 1 H, H-C(8)], 8.1–7.9 (m, 2 H_{arom}), 7.7–7.5 (m, 3 H_{arom}), 6.52 [d, *J* = 9.19 Hz, 1 H, H-C(3′)], 4.43–4.35 [m, 1 H, H-C(9′)], 4.23 [d, *J* = 5.15 Hz, 1 H, H-C(7′)], 3.94–3.75 [m, 2 H, H-C(9′), H-C(11′)], 3.24 [m, 2 H, 2 H-C(4′)], 3.0–2.5 [m, 3 H, OH, H-C(10′), H-C(6′)], 2.25–1.94 [2 H, H-C(10′), H-C(6′)].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 151.31 (s, C-4), 150.99 (s, C-2), 144.08 (d, C-8), 143.49 (s, C-6), 132.45, 128.26, 127.95 (C_{arom}), 129.15 (s, C-5), 94.98 (s, C-1′), 87.84 (d, C-3′), 81.90 (s, C-5′), 80.34 (d, C-7′), 80.27 (d, C-11′), 60.29 (t, C-9′), 49.19 (t, C-6′), 40.81 (t, C-4′), 32.00 (t, C-10′).

HRMS (ESI-TOF, $[M - H]^-$, $C_{21}H_{20}N_5O_5$): *m*/*z* calcd 422.1465, found: 422.1469.

(1*R*,3*S*,5*S*,7*S*,11*R*)-1-[5-Hydroxy-11-(9-phenyl-9*H*-xanthen-9-yloxy)-2,8-dioxatricyclo[5.3.1.0^{1,5}]undec-3-yl]-5-methyl-1*H*-pyrimidine-2,4-dione (9)

To a solution of **7** (210 mg, 0.68 mmol) in anhyd pyridine (16 mL) was added under argon 9-chloro-9-phenylxanthene (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. $NaHCO_3$ (30 mL). The aqueous phase was washed with EtOAc (40 mL) and the combined organic layers were dried (Na_2SO_4) and evaporated. FC (10 g SiO_2, EtOAc–hexane, 3:1 + 1% Et_3N) gave **9** (308 mg, 80%) as a white foam. TLC (EtOAc–hexane, 3:1 + 1% Et_3N): R_f 0.21.

IR ($CHCl_3$): 3391w, 3026w, 1688s, 1603w, 1575w, 1478m, 1448m, 1320w, 1279w, 1125m, 1071m, 908m, 786s cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 8.77 (s, 1 H, NH), 7.40–7.00 (m, 13 H_{arom}), 6.00 [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′)],

3.34 [s, 1 H, H-C(7′)], 3.34–3.13 [m, 2 H, H-C(4′), H-C(11′)], 2.63 [d, *J* = 14.7 Hz, 1 H, H-C(4′)], 2.45 [dd, *J* = 15.4, 5.2 Hz, 1 H, H-C(10′)], 1.96–1.91 [m, 5 H, H-C(6′), H-C(10′), CH_3], 1.70 [m, 1 H, H-C(6′)].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.81 (s, C-4), 151.85 (C_{arom}), 150.7 (s, C-2), 150.25, 147.51 (C_{arom}), 139.71 (d, C-6), 130.31, 130.11, 129.97, 129.82, 128.11, 127.15, 126.96, 123.60, 123.50, 123.31, 122.48, 116.87, 116.49 (C_{arom}), 110.81 (s, C-5), 93.64 (s, C-1′), 93.45 (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-9′), 48.84 (t, C-6′), 41.67 (t, C-4′), 33.09 (t, C-10′), 12.35 (q, CH_3).

^{1}H NMR-difference-NOE (500 MHz, $CDCl_3$): δ = 2.63 [αH-C(4′)] → 4.55 (OH), 3.13 [βH-C(4′)] → 6.00 [H-C(3′)], 6.00 [H-C(3′)] → 7.2 H_{arom}.

HRMS (ESI-TOF, [M – H]$^-$, $C_{33}H_{29}N_2O_7$): *m*/*z* calcd 565.1957, found: 565.1974.

(1*R*,3*S*,5*S*,7*S*,11*R*)-*N*-{9-[5-Hydroxy-11-(9-phenyl-9*H*-xanthen-9-yloxy)-2,8-dioxatricyclo[5.3.1.0^{1,5}]undec-3-yl]-9*H*-purin-6-yl}benzamide (10)

To a solution of **8** (60 mg, 0.14 mmol) in pyridine (0.7 mL) was added 9-chloro-9-phenylxanthene (420 mg, 1.4 mmol), and the mixture was stirred at r.t for 18 h. After dilution with EtOAc (20 mL), the mixture was washed with aq sat. $NaHCO_3$ (30 mL) and the aqueous phase was extracted with EtOAc (2 × 40 mL). The combined organic layers were dried (Na_2SO_4) and evaporated. FC (8 g SiO_2, EtOAc–hexane, 3:1 + 1% Et_3N) provided **10** (75 mg, 79%) as a white foam. TLC (EtOAc–hexane, 3:1 + 1% Et_3N): R_f 0.26.

IR ($CHCl_3$): 3675w, 3026w, 2916w, 1700w, 1615m, 1478m, 1456s, 1319m, 1229m, 1118m, 1072w cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.11 (s, 1 H, NH), 8.77 [s, 1 H, H-C(2)], 8.02 [d, *J* = 7.0 Hz, 2 H_{arom}], 7.98 [s, 1 H, H-C(8)], 7.6–6.9 (m, 16 H_{arom}), 6.42 [dd, *J* = 2.9, 9.5 Hz, 1 H, H-C(3′)], 4.21 [dd, *J* = 4.0, 11.4 Hz, 1 H, H-C(9′)], 3.60 (dd, *J* = 11.4, 6.6 Hz, 1 H, H-C(9′)], 3.51–3.28 [m, 2 H, H-C(4′), H-C(11′)], 3.22 [d, *J* = 5.2 Hz, 1 H, H-C(7′)], 2.91 [dd, *J* = 2.6, 14.7 Hz, 1 H, H-C(4′)], 2.56 [dd, *J* = 5.5, 15.4 Hz, 1 H, H-C(10′)], 2.11–2.05 [m, 2 H, H-C(10′), H-C(6′)], 1.65 [dd, *J* = 7.0, 11.7 Hz, 1 H, H-C(6′)].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.72 (s, CO), 152.02 (s, C-4), 151.55 (s, C-2), 147.47 (d, C-8), 143.30 (s, C-6), 133.38, 132.98, 130.18, 130.07, 129.98, 129.87, 129.40, 129.03, 128.88, 128.84, 128.62, 128.03, 127.99, 127.92, 127.73, 127.25, 127.04, 126.51, 126.45, 126.40, 126.38, 126.34, 125.29, 123.53, 125.43, 116.94, 116.58, 116.18 (C_{arom}, s, C-5), 94.02 (d, C-3′), 88.26 (s, C-1′), 83.19 (s, C-5′), 82.97 (d, C-11′), 78.73 (d, C-7′), 77.26 (s, C_{arom}), 60.17 (t, C-9′), 49.21 (t, C-6′), 41.57 (t, C(4′), 29.70 (t, C-10′).

HRMS (ESI-TOF, [M – H]$^-$, $C_{40}H_{32}N_5O_6$): *m*/*z* calcd 678.2378, found: 678.2358.

(1*R*,3*S*,5*S*,7*S*,11*R*)-1-{5-[(2′cyanoethoxy)(diisopropylamino)-phosphinoxy]-11-(9-phenyl-9*H*-xanthen-9-yloxy)-2,8-dioxatricyclo[5.3.1.0^{1,5}]undec-3-yl}-5-methyl-1*H*-pyrimidine-2,4-dione (11)

A stirred solution of **9** (170 mg, 0.3 mmol) in MeCN (2 mL) was treated at r.t. with *i*-Pr_2NEt (0.4 mL, 2.4 mmol) and chloro(diisopropylamino)-β-cyanoethoxyphosphine (0.26 mL, 1.2 mmol). After 3 h, the mixture was diluted with EtOAc (40 mL) and washed with aq sat. $NaHCO_3$ (30 mL). The aqueous phase was extracted with EtOAc (2 × 20 mL), the combined organic layers dried (Na_2SO_4) and evaporated. FC (10 g SiO_2, EtOAc–hexane, 3:1 + 1% Et_3N) followed by precipitation from hexane (4 °C) afforded **11** (210 mg, 90%, mixture of isomers) as a white solid. TLC (EtOAc–hexane 3:1 + 1% Et_3N): R_f 0.44/0.49.

IR ($CHCl_3$): 3686w, 3624m, 3024s, 2977m, 2435w, 1401s, 1685w, 1580, 1523s, 1424s, 1220s, 1047s, 799s cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.93 (s, 1 H, NH), 7.58 [s, 1 H, H-C(6)], 7.40–7.06 (m, 13 H_{arom}), 6.66 [dd, *J* = 8.1, 1.8 Hz, 0.5 H, H-C(3′)], 6.51 [d, *J* = 6.3, 0.5 H, H-C(3′)], 3.9–3.5 [m, 6 H, 2 H-C(9), 2 CH, OC*H*$_2$CH$_2$CN], 3.3–2.9 [m, 3 H, H-C(7′), H-C(11′), H-C(4′)], 2.6–2.3 [m, 4 H, H-C(10′), H-C(4′), OCH$_2$C*H*$_2$CN], 1.9–1.75 [m, 5 H, 2 H-C(6), CH_3], 1.2–1.0 [m, 12 H, 2 × (C*H*$_3$)$_2$CH].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.18, 163.93 (2 s, C-4), 151.55, 150.7 (s, C-2), 147.53–147.23 (4 C_{arom}), 137.77 (d, C-6), 135.92, 135.89, 131.03, 130.97, 130.82, 130.41, 130.37, 130.32, 129.79, 129.76, 129.74, 129.69, 128.02, 128.00, 127.02, 126.95, 126.85, 126.79, 123.74, 123.61, 123.60, 123.37, 123.,8, 123.19, 122.26, 122.3, 116.64, 116.15 (C_{arom}), 116.09, 116.05 (2 s, CN), 109.55, 109.32 (2 d), 108.90 (d), 95.06 (s), 89.29, 88.74 (2 d), 88.66, 88.00 (2 s), 81.85, 81.72 (2 d), 79.32, 79.19 (2 d), 78.60, 78.33 (2 d), 60.17, 60.05 (2 t), 57.93, 57.51 (2 t), 46.66, 46.26 (2 t), 43.43, 43.26 (2 d), 39.68, 39.55 (2 t), 33.13, 33.05 (2 t), 24.59, 24.39, 24.22, 23.84 (4 q), 20.54, 20.36 (2 t), 12.35, 12.28 (2 q).

^{31}P NMR (162 MHz, $CDCl_3$): δ = 148.20, 145.62.

MS (–ESI-TOF): 765.26 (30, [M – H]$^-$), 728.26 (50), 514.28 (45), 380.18 (100).

HRMS (ESI-TOF, [M – H]$^-$, $C_{42}H_{47}N_4O_8P$): *m*/*z* calcd 765.3074, found: 765.3053.

(1*R*,3*S*,5*S*,7*S*,11*R*)-*N*-{9-[5-(2-Cyanoethoxydiisopropylamino-phosphinoxy)-11-(9-phenyl-9*H*-xanthen-9-yloxy)-2,8-dioxatricyclo[5.3.1.0^{1,5}]undec-3-yl]-9*H*-purin-6-yl}benzamide (12)

To a solution of **10** (70 mg, 0.1 mmol) in THF (2 mL) was added *i*-Pr_2NEt (0.1 mL, 0.6 mmol) and chloro(diisopropylamino)-β-cyanoethoxyphosphine (0.07 mL, 0.3 mmol). After stirring at r.t. for 8 h, the mixture was diluted with EtOAc (20 mL) and washed with aq sat. $NaHCO_3$ (15 mL). The aqueous phase was extracted with EtOAc (2 × 20 mL). The combined organic phases were dried (Na_2SO_4) and evaporated. FC (14 g SiO_2, EtOAc–hexane, 2:1 + 1% Et_3N) followed by precipitation from hexane (4 °C) afforded **12** (75 mg, 85%) as a mixture of isomers in the form of a white solid; TLC (EtOAc–hexane, 3:1 + 1% Et_3N): R_f 0.59/0.52.

IR ($CHCl_3$): 3407w, 3020w, 2971m, 1707m, 1613s, 1585m, 1479w, 1455s, 1321w, 1297m, 1200s, 1124m, 1077m, 941w, 909s, 823w cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.04 (s, 1 H, NH), 8.85 [s, 1 H, H-C(2)], 8.28 [s, 1 H, H-C(8)], 8.02 (d, *J* = 7.0 Hz, 2 H_{arom}), 7.7–7.0 (m, 16 H_{arom}), 6.85 [d, *J* = 6.25 Hz, 1 H, H-C(3′)], 4.1–3.9 [m, 1 H, H-C(9′)], 3.9–3.75 [m, 1 H, H-C(9′)], 3.74–3.20 [m, 7 H, H-C(4′), H-C(11′), H-C(7′), 2 (CH$_3$)$_2$C*H*, OCH$_2$], 2.65–2.4 [m, 4 H, H-C(4′), H-C(10′), CH_2CN], 2.05–1.7 [m, 3 H, H-C(10′), 2 H-C(6′)], 1.1–0.84 [m, 12 H, (C*H*$_3$)$_2$CH)].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 152.66 (s, C-2), 151.59 (s, C-4), 147.62 (s, C-6), 141.57 (d, C-8), 132.65, 130.97, 130.48, 129.86, 128.85, 128.11, 127.78, 127.03, 123.70, 123.44, 123.36 (C_{arom}), 122.32 (s, C-5), 116.79, 116.19 (2 s), 96.05 (s), 88.05, 87.97 (2 d), 81.66 (d), 79.57 (d), 78.43 (s), 76.58 (s), 60.34, 57.86, 57.57, 57.55, 57.45 (5 t), 43.52, 43.34 (2 d), 39.87, 39.78, 33.43 (3 t), 24.74, 24.65, 24.29, 24.19 (4 q), 20.48, 20.37 (2 t).

^{31}P NMR (162 MHz, $CDCl_3$): δ = 147.34, 145.60.

HRMS (ESI-TOF, [M – H]$^-$, $C_{49}H_{50}N_7O_7P$): *m*/*z* calcd 878.3428, found: 878.3431.

Synthesis of Oligonucleotides 13–18

Oligonucleotides **13**–**18** were prepared on a Pharmacia Gene Assembler Special DNA synthesizer. All unmodified phosphoramidite building blocks were from Glen Research. *N*^{6}-Benzoyl-α-deoxyad-

enosine was from R.I. Chemicals. α-Deoxythymidine as well as the α-nucleoside phosphoramidite building blocks were prepared as described.[20] 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, 1*H*-tetrazole was replaced by the more active (*S*-benzylthio)-1*H*-tetrazole (0.25 M in MeCN) or 5-(ethylthio)-1*H*-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. NH_4OH (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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