Nucleic-Acid Analogs with Restricted Conformational Flexibility in the Sugar-Phosphate Backbone ('Bicyclo-DNA')

Part 7[1])

Synthesis and Properties of Oligodeoxynucleotides Containing [(3′*S*,5′*S*,6′*R*)-6′-Amino-2′-deoxy-3′,5′-ethano-β-D-ribofuranosyl]thymine (=(6′*R*)-6′-Amino-bicyclo-thymidine)

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Dedicated to Prof. Dr. *Frank Seela* on the occasion of his 60th birthday

We describe the synthesis of the acetamido- and trifluoroacetamido-functionalized bicyclo-thymidines **11** and **12**, starting from the silyl enol ether **1**, in 6 steps. These nucleosides were converted to the corresponding cyanoethyl phosphoramidite building blocks **16** and **17** and subsequently incorporated into the homo-thymidylate decamers **18**–**22**. Upon deprotection of the oligomers, the trifluoroacetamido functions were cleaved, leaving behind a free amino function in the sugar-phosphate backbone that is protonated at neutral pH, giving rise to partially zwitterionic oligonucleotides. Pairing properties with the complementary DNA oligomer $d(A_{10})$, as determined by UV/melting curves, revealed a slightly increased stability of the duplex $d(A_{10})\cdot$**20**, in which the decathymidylate sequence shows an alternating arrangement of natural thymidine and amino-bicyclo-thymidine residues, relative to the natural reference duplex. The dependence of T_m on the salt concentration of the medium is reduced in this case. Duplex destabilization occurs if the amino-bicyclo-thymidine residues are replaced by the charge-neutral acetamido-bicyclo-nucleosides (*e.g.*, $d(A_{10})\cdot$**22**), most probably due to steric interference of the acetamido substituent with the backbone P–O(5′) bond.

1. Introduction. – The design and synthesis of new oligonucleotide analogs with improved pairing properties, enhanced biostability and bioavailability relative to natural DNA and RNA, is of importance with respect to potential applications as antisense agents in human therapy and biotechnology [2], in DNA or RNA diagnostics [3], and in materials and computer science [4]. Furthermore, such oligonucleotides serve as tools to chemically rationalize the supramolecular assembly of oligonucleotide single strands, thus contributing to the understanding of the interrelation between monomeric nucleoside structure and the association properties of oligomers thereof.

In this context, we recently prepared the conformationally constrained DNA analog bicyclo-DNA and explored its pairing properties in detail (*Fig. 1*) [5–8]. As the backbone torsion angle γ in bicyclo-DNA, in contrast to natural A- and B-DNA, is preferentially located in the antiperiplanar (*ap*) and not in the synclinal (+*sc*) arrangement, this provided a unique opportunity to explore the effect of a change of

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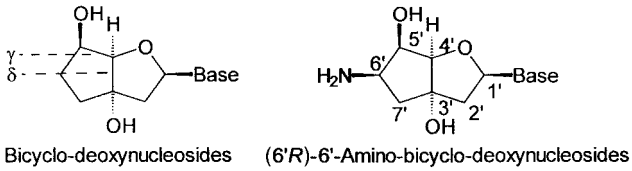


Fig. 1. *Structure of the bicyclo-deoxynucleosides and the (6′*R*)-6′-amino-bicyclo-deoxynucleosides*

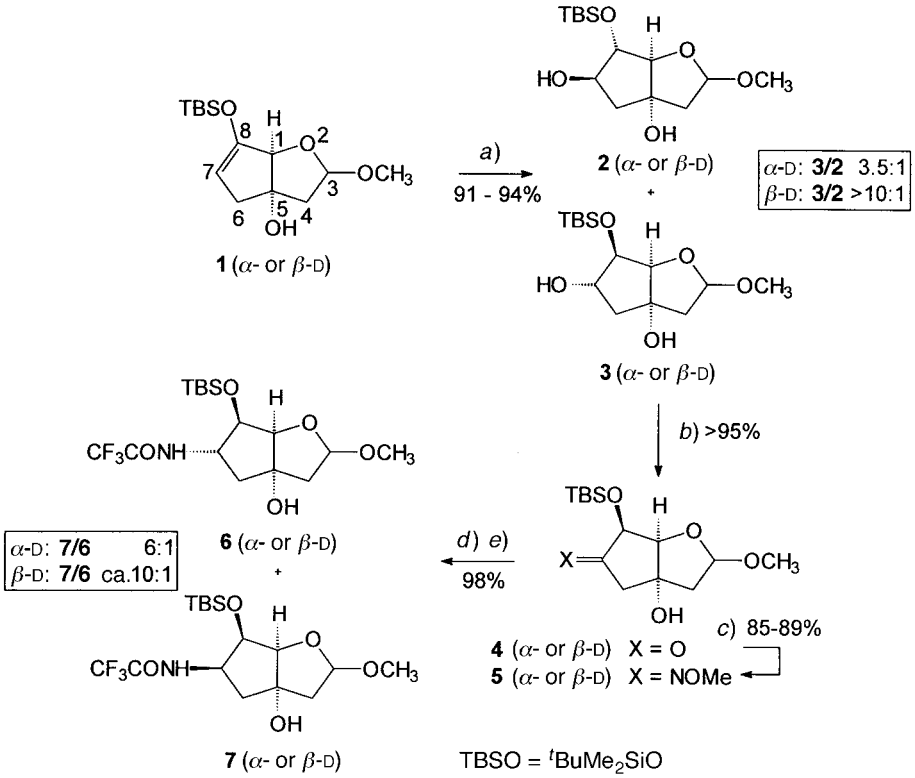
this particular torsion angle on the association mode of duplex formation [9]. This conformational study was further extended to oligodeoxynucleotides containing 5′-epi-bicyclo-deoxynucleosides, in which γ is restricted to the $-ac/-sc$ conformational space [1].

In addition to probing the effect of structural preorganization on duplex formation, the carbocyclic ring in the bicyclo-deoxynucleosides is ideally suited for introducing functional groups into well-defined positions at the sugar-phosphate backbone. Such functional groups may serve various purposes, *e.g.*, the adjustment of torsion angle γ by changing the preferred conformation of the carbocyclic ring or the change of the electrostatic environment of the sugar-phosphate backbone. Furthermore, they may be useful as anchor points for the introduction of reporter molecules or chemically reactive groups, or to enhance the catalytic power of DNA enzymes [10]. Here we report on the synthesis of (6′*R*)-6′-amino-bicyclo-thymidine, its incorporation into oligonucleotides, and a first assessment of the base-pairing properties of accordingly modified oligonucleotides.

2. Synthesis of the (6′*R*)-6′-Amino-bicyclo-deoxysugar Unit. – Of primary interest to us were the (6′*R*)-6′-amino-bicylo-deoxynucleosides, in which the amino function is attached to the concave (β) side of the bicyclic ring system. The (*R*)-configuration was chosen for two reasons. From the *cis* relationship of the substituents at C(5′) and C(6′), we expected a conformational change of the carbocyclic ring, relative to the unsubstituted bicyclo-nucleosides, towards a conformation in which the 5′-OH group is located in the pseudoaxial position. This orients torsion angle γ into the $(+sc)$ range and thus mimicks closely the orientation observed in DNA duplexes of the A and B type. Furthermore, from model building, it appeared that attachment to the β face orients the functional group into the direction of the major groove of a DNA duplex, while attachement to the convex α face would orient it straight away from the duplex into the solvent.

The synthesis started from the silyl enol ether **1** (*Scheme 1*), prepared and used previously in the synthesis of tricyclo-DNA [11]. Although the relative configuration at the anomeric center of **1** seemed to be of minor importance with respect to the stereochemical outcome of the envisaged transformations in the carbocyclic part of the molecule, both, the α-D- and β-D-anomers were used separately and in parallel. While we anticipated that direct methods for the introduction of the amino substituent, *e.g. via* aziridination [12] of **1** would yield predominantly products with the amino substituent in α-position, we preferred a classical procedure *via* hydroboration followed by exchange of the resulting OH substituent at C(7) by an NH_2 substituent under inversion of configuration.

Scheme 1. *Synthesis of the Amino-Functionalized Bicyclo-sugar* **7**



a) $BH_3 \cdot THF$ (3 equiv.), THF, $-78° \rightarrow$ r.t., 31 h. *b*) *Dess-Martin* periodinane (2 equiv.), CH_2Cl_2, r.t., 2 h. *c*) $(MeONH_3)Cl$ (2 equiv.), NaOAc (1 equiv.), 90% EtOH, r.t., 20 min. *d*) *Raney*-Ni, $EtOH/H_2O$/conc. NH_3 soln. 10 : 4 : 0.8, H_2 (10 bar), r.t., 12 h. *e*) CF_3COOEt, Et_3N (0.2 equiv.), r.t., 1 h.

Hydroboration of **1** proceeded smoothly and produced the two diastereoisomeric diols **2** and **3** in yields $>90\%$ and in ratios **3/2** of 3.5 : 1 in the α-D series and $>10:1$ in the β-D series[2]). The higher selectivity in the β-D series is most likely due to the MeO substituent that sterically obstructs the attack of the borane reagent from the β-face, thus pronouncing the already inherent preference for attack at the convex α face of the bicyclic system. While direct conversion of the secondary-alcohol function to an amino function in **3** *via Mitsunobu* reaction [13] failed in our hands, we had to adopt a redox procedure to introduce the desired functionality. Oxidation of **3** ($\rightarrow$**4**) followed by treatment with *O*-methylhydroxylamine gave the corresponding (*E*)/(*Z*)-oximes **5** in high yields in both the α-D and β-D series. Subsequent hydrogenolysis with *Raney*-Ni

[2]) The relative configurations at the centers C(7) and C(8) of all intermediates **2**–**7** were assigned on the basis of ^{1}H-NMR NOE experiments and are discussed in detail in [14].

then produced in almost quantitative yield[3]) the corresponding amines, which were isolated as the corresponding trifluoracetates **6**/**7**. As expected, the reaction proceeded with considerable stereoselectivity with ratios of **6**/**7** > 6 : 1 in both the α-D and β-D series.

3. X-Ray Analysis and Solution Structure of α-7[4]). – Suitable crystals of α-**7** were subjected to X-ray analysis (*Fig. 2*)[5]). The structure unequivocally supports the assignment of the relative configuration at the centers C(7) and C(8) and, furthermore, provides insight into the preferred conformational properties of the bicyclic sugar component. As in the unsubstituted bicyclo-deoxynucleosides, the furanose unit adopts an almost perfect 1′-*exo* conformation with a pseudorotation phase angle *P* of 144.3°. Importantly, the carbocyclic ring exists in a conformation with the silyloxy substituent at C(8) in a pseudoaxial and the trifluoroacetamido substituent at C(7) in a pseudoequatorial position. Its conformation thus deviates substantially from that of the unsubstituted bicyclo-deoxynucleosides in which the corresponding OH substituents were shown to exist invariably in the pseudoequatorial orientation.

Translated into the structural description of the DNA backbone, the introduction of the amido substituent corrects torsion angle γ from the (unnatural) *ap*-orientation (*ca.* 150°) to the (in A- and B-DNA naturally occurring) *sc*-orientation (77.2°), while torsion angle δ (135.5°) remains largely unaffected with values as observed in B-DNA helices [15]. Analysis of the conformation of α-**7** in solution by NMR coupling constant analysis essentially confirms that occurring in the solid state [14].

4. Synthesis of Nucleosides and Building Blocks for DNA Synthesis. – With both anomers α- and β-**7** in hand, we then approached the nucleosidation reaction (*Scheme 2*). Interestingly, the stereochemical outcome of the nucleosidation according to the *Vorbrüggen* procedure [16] was strongly dependent upon the configuration of the anomeric center of **7** and upon the temperature. In the α-D series, ratios of nucleosides α/β-**8**[4]) varied from > 6 : 1 at high temperature to 1 : 1.7 at low temperature. In the β-D series, no reaction took place at low temperatures, while mixtures α/β-**8** 4 : 1 were obtained upon heating. The higher reactivity of α-**7** over β-**7** is in accord with a

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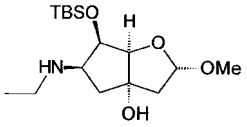
[3]) During preliminary experiments for the reduction of **5** with *Raney*-Ni, we isolated amine **12** in yields of up to 25%. This product most likely arises from reduction of the imine formed between acetaldehyde and the already formed amine. The source of the acetaldehyde is unknown but most probably arises from the solvent EtOH, from which it is produced in minor amounts by the catalyst. The formation of this by-product could be completely suppressed by addition of ammonia as a competing amine.



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[4]) The descriptor α or β preceding a key number refers to the configuration at the anomeric center.

[5]) Crystallographic data (excluding structure factors) for α-**7** have been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC-132022. Copies of the data can be obtained, free of charge, on application to the *CCDC*, 12 Union Road, Cambridge CB2 1EZ, UK (fax: + 44 (1223) 336033; e-mail: deposit@ccdc.can.ac.uk).

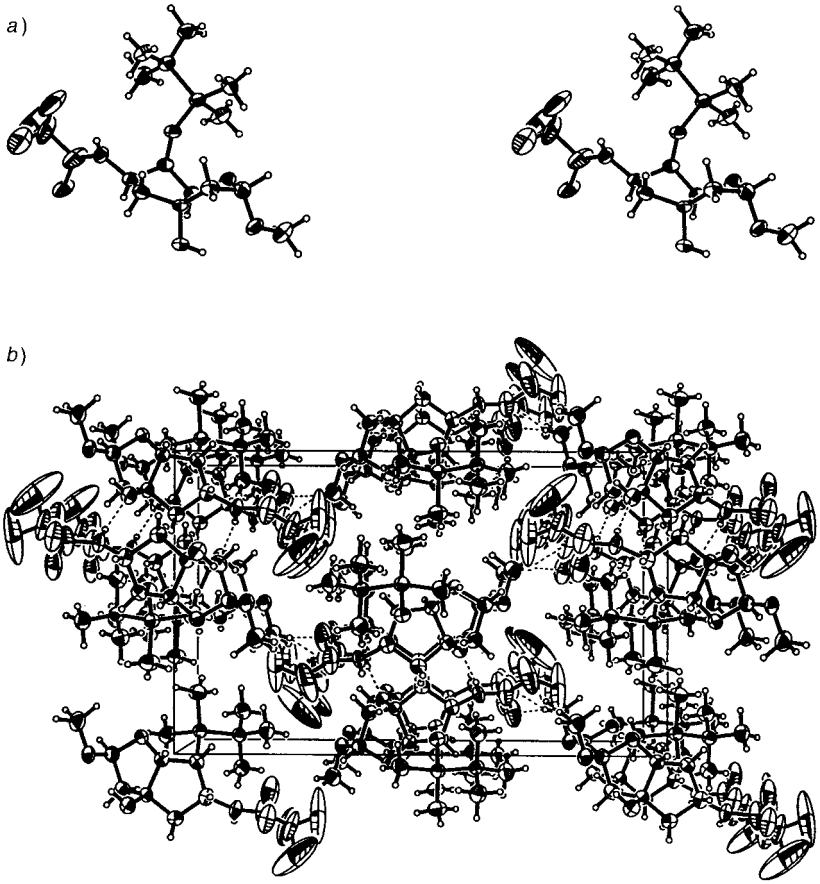


Fig. 2. *ORTEP Plot of crystals of* α-**7**: a) *stereoscopic view* (25% probability thermal ellipsoids) *and* b) *view along the crystallographic* a-*axis*

pronounced kinetic anomeric effect. The preferred formation of the β-D-nucleoside at low temperature is most likely the result of a kinetically controlled reaction, while formation of the α-D-nucleoside at high temperature seems to occur under thermodynamic control[6]).

The further synthetic transformations into the building blocks for oligonucleotide synthesis were straightforward and are summarized in *Scheme 3*. Desilylation of the trifluoroacetyl-protected amine β-**8** afforded **12** in high yield, which could be tritylated to **14** and converted into the phosphoramidite **16** according to typical protocols in nucleotide chemistry. Moreover, the trifluoroacetyl group of **12** could be easily

[6]) Support for this interpretation comes from additional equilibration experiments. When β-**8** was subjected to the reaction conditions for nucleosidation at room temperature, equilibration to a mixture α/β-**8** 1.5 : 1 was observed after 23 h. No such equilibration was observed when pure α-**8** was used.

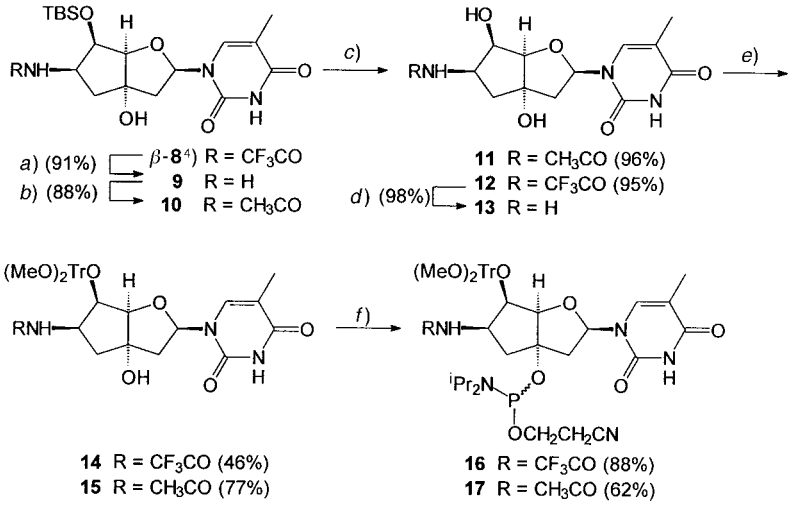
Scheme 2. *One-Pot Nucleosidation Reaction of Thymine with* **7**

TBSO = ^{t}BuMe$_2$SiO

7[4])	Temp. [°C]	α/β-**8**[4])	Yield [%]
α	<10	1:1.7	84
α	60	6:1	77
β	10	n.r.[a])	n.r.[a])
β	60	4:1	63

[a]) n. r. = no reaction

Scheme 3. *Preparation of Building Blocks* **16** *and* **17** *for Oligonucleotide Synthesis*



TBSO = ^{t}BuMe$_2$Si, (MeO)$_2$Tr = 4,4'-dimethoxytrityl

a) MeOH, conc. NH_3, r.t., 4 h. *b*) Ac_2O (1 equiv.), C_5H_5N, 0°, 2 h. *c*) $Et_3N \cdot 3HF$ (1.3 equiv.), C_5H_5N, r.t., 24 h (**11**); $Bu_4NF \cdot 3H_2O$ (8 equiv.), MeCN, AcOH (10 equiv.), r.t., 2 h (**12**). *d*) Conc. NH_3 soln., MeOH, r.t. 45 min. *e*) $(MeO)_2TrOSO_2CF_3$ (1.5–2.0 equiv.), C_5H_5N, 50°, 16 h. *f*) ^{i}Pr$_2$NEt (4 equiv.), Cl[P(OCH$_2$CH$_2$CN)(N^{i}Pr$_2$)] (2 equiv.), MeCN, r.t., 2 h.

removed (→**13**). A similar deprotection of the amino function in β-**8** (→**9**) and subsequent transformation into the acetamide **10** provided access to the acetamido series. The corresponding phosphoramidite building block **17** was obtained, in analogy to the trifluoroacetyl series, from **10** *via* desilylation (→**11**), tritylation (→**15**), and phosphitylation. While the trifluoroacetyl group will be lost during standard oligonucleotide deprotection to produce a free, protonatable amino function, the acetyl group is stable under these conditions and thus leads to a largely isosteric but charge-neutral residue.

5. Synthesis of Oligonucleotides. – The synthesis of the decamers **18** – **22** containing (6′*R*)-6′-amino- and (6′*R*)-6′-acetamido-bicyclo-deoxythymidine (see *Fig. 3*) were carried out on a *Pharmacia-Gene-Assembler-Plus*® DNA synthesizer on the 1.3-μmol scale in the 'trityl-off' mode (for details, see *Exper. Part*). Commercially available, thymidine-loaded controlled-pore glass (CPG) was used as the starting unit. Coupling yields, as monitored by the on-line trityl assay, amounted on average to 90 – 98%, when the modified building blocks **16** and **17** were coupled to a natural thymidine unit. The same yields were observed for coupling of a natural thymidine unit to a 6′-amino-bicyclo-thymidine unit. The coupling of two consecutive amino-bicyclo-thymidine building blocks, however, was less efficient and proceeded with coupling yields per cycle of only *ca*. 60%. This low yield, most likely arising from steric interference of the 6′-substituents, precluded the synthesis of completely modified decamers. After completion of chain assembly, the detachment from the solid support and deprotection was carried out by standard treatment with conc. NH_3 solution.

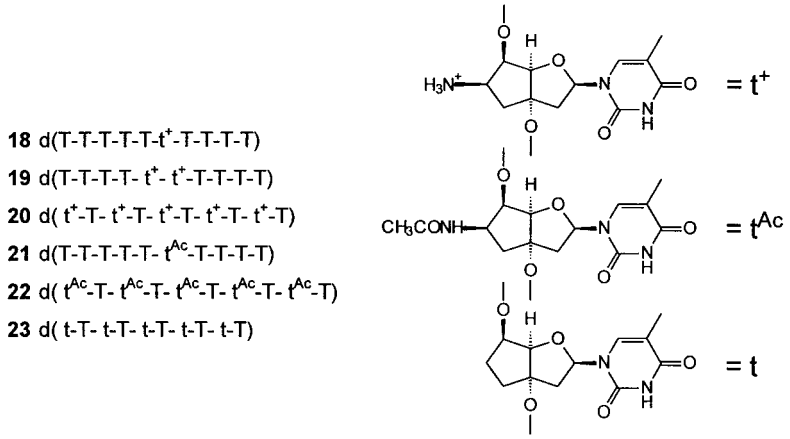


Fig. 3. *Sequences of the oligonucleotides* **18** – **23**

The crude oligomers were purified by anion-exchange HPLC and the purity of the collected fractions controlled by reversed-phase HPLC. As expected, the retention times during DEAE-HPLC of the decamers decreased with increasing content of amino-bicyclo-deoxynucleotide units, due to the reduced overall negative charge of the oligomers. The integrity of the oligomers was confirmed by matrix-assisted laser-desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (*Table 2*, *Exper. Part*). The synthesis of sequence **23**, containing unsubstituted bicyclo-deoxythymidine units was described previously [6].

All sequences **18** – **22** were chemically stable (HPLC and MALDI-ToF-MS control) under the conditions used for recording UV/melting curves (NaCl-containing aqueous buffer, pH 6 – 10, 0 – 100°). No enhanced instability of the oligomers towards hydrolysis, due to the presence of the 6′-amino function was observed.

6. Pairing Properties. – Initial pairing properties with the DNA complement d(A_{10}) were obtained from UV/melting-curve analysis in buffer solutions containing 150 mM

Table 1. T_m *Values for Duplex Formation of Sequences* **18**–**23** *with* $d(A_{10})$. Buffer: 10 mM Na-cacodylate (pH 6.0/7.0), 10 mM Na_2HPO_4 (pH 8.0/9.0), 150 mM NaCl; *c*(duplex) 3.7–4.0 μM.

	T_m [°][a])			
	pH 6.0	pH 7.0	pH 8.0	pH 9.0
18, d(T-T-T-T-T-t$^+$-T-T-T-T)	22.4	22.2	21.7	21.3
19 d(T-T-T-T-t$^+$-t$^+$-T-T-T-T)	18.9	19.0	18.4	18.2
20 d(t$^+$-T-t$^+$-T-t$^+$-T-t$^+$-T-t$^+$-T)	27.5	27.3	26.1	24.6
21 d(T-T-T-T-T-t^{Ac}-T-T-T-T)	20.0	19.5	20.1	19.6
22 d(t^{Ac}-T-t^{Ac}-T-t^{Ac}-T-t^{Ac}-T-t^{Ac}-T)	13.5	13.5	13.2	13.1
23 d(t-T-t-T-t-T-t-T-t-T)	–	21.0[b])	–	–

[a]) T_m 23° for $d(A_{10}) \cdot dT_{(10)}$ under identical conditions at pH 7.0. [b]) Taken from [6].

NaCl, and in the pH range 6.0–9.0. T_m Data were extracted from the melting curves and are summarized in *Table 1*.

While none of the oligonucleotides **18**–**23** showed any transition without the DNA complement, which excludes self-aggregation of the pyrimidine strands, all formed stable duplexes with $d(A_{10})$[7]). Compared to the natural reference duplex $d(A_{10}) \cdot d(T_{10})$, which under the given conditions melts with a T_m of 23°, the duplex **20** $\cdot d(A_{10})$, with five charged residues (t^+) spaced by five natural thymidine residues, displayed slightly enhanced T_m values. As expected, the T_m is dependent on pH and slightly decreases with increasing pH. Replacement of the positively charged t^+ by its acetylated, charge-neutral derivative t^{Ac}, as in the duplex **22** $\cdot d(A_{10})$, leads to a noticeable decrease of T_m compared to both, the all-DNA duplex $d(A_{10}) \cdot d(T_{10})$ and the duplex $d(A_{10}) \cdot$ **23**, containing the underivatized bicyclo-thymidine unit t. As expected, duplex melting in the case of **22** $\cdot d(A_{10})$ is pH insensitive in the pH-range investigated. Duplexes containing the mono- and disubstituted pyrimidine sequences **18**, **19**, and **21** essentially follow the described overall properties.

Duplex formation in the case of **20** $\cdot d(A_{10})$ is less dependent on the salt concentration of the medium, compared to the duplex $d(T_{10}) \cdot d(A_{10})$. In the range of 25–600 mM NaCl, a linear dependence of T_m from ln[NaCl] was measured with slopes $\delta T_m/\delta \ln[\text{NaCl}]$ of 4.32 K · M^{-1} for **20** $\cdot d(A_{10})$ and 5.24 K · M^{-1} for $d(T_{10}) \cdot d(A_{10})$. The lower dependence of T_m from the electrolyte concentration is again in agreement with the partially zwitterionic nature of the backbone of **20**.

7. Discussion and Conclusions. – Zwitterionic oligonucleotides were prepared in the past either by derivatization of the pyrimidine C(5) position with a flexible aminohexyl chain [17][18] or by introducing a basic 2-(dimethylamino)ethylphosphoramidate group as the linking unit between nucleoside residues [19]. In both cases, a negligible salt-concentration dependence of duplex formation was reported. However, no benefit in terms of strength of duplex formation arose as a result of the modifications.

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7) All complexes formed between the oligonucleotides **18**–**23** and $d(A_{10})$ at 1:1 stoichiometry of single strands were duplexes and not triple helices. This is deduced from the fact that only monophasic and not biphasic melting curves were observed at 260 nm, and that no transitions at 284 nm occurred. At the latter wavelength, the *Hoogsteen*-strand melting in dT · dA · dT triple helices is visible.

From the experiments presented here, we conclude that, indeed, the positive charge associated with the amino-bicyclo-nucleosides exhibits a slightly stabilizing effect at neutral pH, most probably by neutralizing intrastrand repulsion of two neighboring negatively charged phosphate units. However, this stabilizing electrostatic effect seems in part to be compromised by a repulsive steric interaction between the substituent at position C(6′) of the bicyclo-nucleosides and the 5′-phosphate residues. This interpretation is corroborated by the relatively low T_m of duplexes containing the residues t^{Ac} (*e.g.*, **22**·d(A_{10})), in which charge contributions to stability are disentangled from structural contributions. Thus, geometrically constrained and suitably positioned cationic units in the DNA backbone can stabilize DNA duplexes and may do so even more effectively, if the charge-carrying group does not sterically interfere with the preferred backbone conformation.

Although the 6′-substituted (6′*R*)-bicyclo-deoxynucleosides seem to prefer a conformation favoring the $+sc$ orientation of torsion angle γ as deduced from the X-ray structure of the precursor α-**7**, not enhanced but reduced affinity to the DNA target, compared to unmodified bicyclo-DNA is the result. This is evident from comparison of the T_m data of the duplexes **22**·d(A_{10}) and **23**·d(A_{10}). Model building suggests that this is due to repulsive nonbonding interactions between the substituent at C(6′) and the adjacent O(5′)–P ester bond, perturbing the preferred conformation of the latter with respect to the B-DNA backbone structure (too many atoms in the sugar-phosphate backbone(!), see [20]).

Given the relatively weak destabilizing effect upon replacement of one (6′*R*)-6′-acetamido-bicyclo-deoxynucleoside residue for a thymidine residue within a decamer duplex, we envision the use of this scaffold, as well as of its (6′*S*)-isomer, in the future for the site-specific introduction of functional units into DNA double and triple helices.

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Experimental Part

General. Solvents for extraction: technical grade, distilled. Solvents for reactions: reagent grade, distilled over CaH_2 (MeCN, CH_2Cl_2, pyridine) or Na (THF). Reagents: if not otherwise stated, from *Fluka*, highest quality available. TLC: *Merck SiL G-25 UV$_{254}$*; non-UV-visible compounds were stained by dipping the plate in a mixture of EtOH (180 ml), 4-methoxybenzaldehyde (10 ml), conc. H_2SO_4 soln. (10 ml), and AcOH (2 ml), followed by heating with a heat gun. Flash column chromatography (FC): silica gel (30–60 μm) from *Baker*. HPLC: *Pharmacia-LKB-2249* gradient pump attached to an *ABI-Kratos-Spectroflow-757*-UV/VIS detector and a *Tarkan-W+W* recorder *600*; t_R in min. UV/Melting curves: *Varian-Cary-3E*-UV/VIS spectrometer equipped with a temp. controller unit and connected to a *Compaq-ProLinea-3/25-zs* personal computer, temp. gradient 0.5°/min; data-point collection in intervals of *ca.* 0.3°; at <20°, the cell compartment was flushed with N_2 to avoid condensation of H_2O on the UV cells; the transition temperature T_m was determined as the maximum of the first derivative of the melting curve using the software package *Origin*™ *V5.0*. M.p.: *Büchi 510*; uncorrected. Optical rotations: *Perkin-Elmer-241* polarimeter; 10-mm cell. IR: *Perkin Elmer FTIR 1600*; $\tilde{\nu}$ in cm^{-1}. NMR: *Bruker AC-300*, *DRX500*; δ in ppm, ^{13}C multiplicities from DEPT spectra, *J* in Hz. EI-MS: *Varian MAT CH-7A*; ionizing voltage 70 eV; *m*/*z* (intensity in %). Liquid secondary ion mass spectrometry (LSI-MS): *Micromass Autospec Q*, primary ions Cs^+ (25 keV); matrix: dithioerythrol/dithio-DL-threitol. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) of oligonucleotides was performed as described in [21].

*(1*R,*3*S,*5*S,*7*R,*8*S*)-8-{[(*tert*-*Butyl*)*dimethylsilyl*]*oxy*}-3-*methoxy*-2-*oxabicyclo*[3.3.0]*octan*-5,7-*diol* (α-**2**) *and (1*R,*3*S,*5*S,*7*S,*8*R*)-8-{[(*tert*-*Butyl*)*dimethylsilyl*]*oxy*}-3-*methoxy*-2-*oxabicyclo*[3.3.0]*octan*-5,7-*diol* (α-**3**). A soln. of α-**1** (1.462 g, 5.11 mmol) in THF (abs.) was treated with $BH_3 \cdot THF$ (1M, 3 equiv.) at −78°, allowed to warm up to r.t., and quenched after 31 h with sat. $NaHCO_3$ soln. (26 ml). A soln. of $KHSO_5$ triple salt (12.55 g, 20.4 mmol) in sat. $NaHCO_3$ soln. (125 ml) was added, and the mixture stirred for an additional 2 h. Extraction with Et_2O (4 × 200 ml) followed by drying the org. phase (Na_2SO_4) and evaporation afforded the crude products, which were separated by FC (hexane/AcOEt 5 : 1 → 0 : 1): 0.320 g (21%) of α-**2** and 1.14 g (73%) of α-**3**, both as white crystals.

Data of α-**2**: M.p. 87.0–87.5°. TLC (CH_2Cl_2, 7.5% MeOH): R_f 0.39. IR (CCl_4): 3620*w*, 3561*w*, 2954*s*, 2930*s*, 2858*m*, 1472*w*, 1463*w*, 1447*w*, 1389*w*, 1362*w*, 1316*w*, 1293*w*, 1253*m*, 1197*m*, 1145*m*, 1125*m*, 1100*s*, 1070*s*, 1028v*s*, 1006*w*, 983*w*, 940*m*, 863*m*, 838*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.09, 0.11 (2*s*, Me_2Si); 0.88 (*s*, Me_3CSi); 1.87 (*dd*, *J* = 14.0, 6.6, 1 H−C(6)); 1.92 (*d*, *J* = 4.4, OH); 2.12 (*dd*, *J* = 13.6, 0.7, 1 H−C(4)); 2.19 (*dd*, *J* = 13.8, 3.9, 1 H−C(4)); 2.29 (*dd*, *J* = 14.0, 6.6, 1 H−C(6)); 2.62 (*s*, OH); 3.33 (*s*, MeO); 3.78 (*dd*, *J* = 5.2, 4.0, H−C(8)); 4.02 (*d*, *J* = 3.7, H−C(1)); 4.13–4.21 (*m*, H−C(7)); 5.07 (*dd*, *J* = 3.9, 0.9, H−C(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): −4.88, −4.70 (2*q*, Me_2Si); 18.03 (*s*, Me_3CSi); 25.77 (*q*, Me_3CSi); 43.64, 48.63 (2*t*, C(4), C(6)); 54.49 (*q*, MeO); 78.29, 82.69 (2*d*, C(7), C(8)); 84.34 (*s*, C(5)); 94.66 (*d*, C(1)); 106.79 (*d*, C(3)). EI-MS: 273 (11, $[M - OMe]^+$), 131 (*100*).

Data of α-**3**: M.p. 95–97°. TLC (CH_2Cl_2 + 7.5% MeOH): R_f 0.32. IR ($CHCl_3$): 3601*w*, 3566*w* (br.), 3003*m*, 2954*s*, 2931*s*, 2858*m*, 1472*m*, 1464*m*, 1442*w*, 1434*w*, 1390*m*, 1362*m*, 1344*w*, 1314*w*, 1300*m*, 1257*s*, 1224v*s*, 1206v*s*, 1142*s*, 1098*s*, 1080*s*, 1050*m*, 1020*m*, 986*w*, 949*m*, 919*m*, 882*m*, 850*s*, 839*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.09 (*s*, Me_2Si); 0.88 (*s*, Me_3CSi); 1.70 (*dd*, *J* = 13.4, 7.2, 1 H−C(6)); 1.94 (*dd*, *J* = 13.8, 4.2, 1 H−C(4)); 2.11 (*d*, *J* = 13.6, 1 H−C(4)); 2.21 (*dd*, *J* = 13.6, 1 H−C(6)); 2.26 (*s*, OH); 2.91 (*s*, OH); 3.32 (*s*, MeO); 3.87–3.98 (*m*, H−C(7), H−C(8)); 4.16 (*d*, *J* = 5.5, H−C(1)); 5.06 (*d*, *J* = 4.4, H−C(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): −4.90, −4.73 (2*q*, Me_2Si); 18.27 (*s*, Me_3CSi); 25.81 (*q*, Me_3CSi); 41.85, 48.74 (2*t*, C(4), C(6)); 54.34 (*q*, MeO); 76.85, 77.80 (2*d*, C(7), C(8)); 83.75 (*s*, C(5)); 88.48 (*d*, C(1)); 106.51 (*d*, C(3)). EI-MS: 273 (5, $[M - OMe]^+$), 215 (*100*).

*(1*R,*3*R,*5*S,*7*S,*8*R*)-8-{[(*tert*-*Butyl*)*dimethylsilyl*]*oxy*}-3-*methoxy*-2-*oxabicyclo*[3.3.0]*octan*-5,7-*diol* (β-**3**). From β-**1** (588 mg, 2.05 mmol), as described above. FC (CH_2Cl_2, 2.5% MeOH) yielded β-**3** (566 mg, 91%). White crystals. M.p. 124–125°. TLC (CH_2Cl_2, 5% MeOH): R_f 0.18. IR ($CHCl_3$): 3602*m*, 3423*w* (br.), 2955*s*, 2931*s*, 2900*m*, 2858*m*, 1558*w*, 1472*m*, 1463*m*, 1450*w*, 1389*w*, 1362*w*, 1310*m*, 1252*s*, 1159*s*, 1109*s*, 1068*s*, 1034*m*, 1007*w*, 988*m*, 956*m*, 877*m*, 840*s*, 800v*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.09 (*s*, Me_2Si); 0.90 (*s*, Me_3CSi); 1.66 (*ddd*, *J* = 13.2, 9.1, 1.1, 1 H−C(6)); 1.93 (br. *s*, OH); 2.09 (*dd*, *J* = 13.4, 1.7, 1 H−C(4)); 2.21 (br. *s*, OH); 2.31 (*ddd*, *J* = 13.6, 5.8, 1.6, 1 H−C(4)); 2.41 (*dd*, *J* = 13.2, 7.0, 1 H−C(6)); 3.35 (*s*, MeO); 3.83 (*dd*, *J* = 7.7, 5.9, H−C(8)); 4.04 (*d*, *J* = 5.5, H−C(1)); 4.23 (*dd*, *J* = 16.4, 7.5, H−C(7)); 5.05 (*dd*, *J* = 5.7, 1.6, H−C(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): −4.76, −4.62 (2*q*, Me_2Si); 18.22 (*s*, Me_3CSi); 25.84 (*q*, Me_3CSi); 44.18, 49.15 (2*t*, C(4), C(6)); 54.56 (*q*, MeO); 76.04, 79.05 (2*d*, C(7), C(8)); 83.75 (*s*, C(5)); 88.40 (*d*, C(1)); 105.69 (*d*, C(3)). EI-MS: 273 ($[M - OMe]^+$, 3), 215 (*100*).

*(1*R,*3*S,*5*R,*8*S*)-8-{[(*tert*-*Butyl*)*dimethylsilyl*]*oxy*}-5-*hydroxy*-3-*methoxy*-2-*oxabicyclo*[3.3.0]*octan*-7-*one* (α-**4**). To a soln. of α-**3** (2.376 g, 7.81 mmol) in CH_2Cl_2 (36 ml), a soln. of *Dess-Martin* periodinane [22] (6.622 g, 15.58 mmol) in CH_2Cl_2 (32 ml) was added at r.t. After 2 h, the mixture was diluted with ^{t}BuOMe (200 ml) and extracted with sat. $NaHCO_3$/20% $Na_2S_2O_3$ soln. 1 : 1 (100 ml) and the org. phase dried ($MgSO_4$) and evaporated: crude α-**4** (2.515 g, 90% pure by ^{1}H-NMR). Colorless oil that was directly introduced into the next step. Anal. data of a FC-purified sample (AcOEt/hexane 1 : 4): TLC (5% MeOH/CH_2Cl_2): IR ($CHCl_3$): 3673*w*, 3584*w*, 3509*w*, 3425*w*(sh), 3002*w*, 2933*s*, 2858*m*, 1764*s*, 1605*w*, 1467*m*, 1394*m*, 1364*m*, 1297*m*, 1253*s*, 1218*s*, 1190*m*, 1145*m*, 1076*s*, 1019*m*, 949*s*, 842*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.10, 0.13 (2*s*, Me_2Si); 0.89 (*s*, Me_3CSi); 2.15 (*dd*, *J* = 13.8, 1.7, 1 H−C(4)); 2.25 (*dd*, *J* = 14.0, 4.8, 1 H−C(4)); 2.50 (*d*, *J* = 18.8, 1 H−C(6)); 2.59 (*dd*, *J* = 18.8, 1.5, 1 H−C(6)); 2.94 (br. *s*, OH); 3.38 (*s*, MeO); 4.27 (*d*, *J* = 5.9, H−C(1) or H−C(8)); 4.37 (*dd*, *J* = 6.1, 1.7, H−C(1) or H−C(8)); 5.04 (*dd*, *J* = 5.0, 1.7, H−C(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): −5.15, −4.76 (2*q*, Me_2Si); 18.45 (*s*, Me_3CSi); 25.71 (*q*, Me_3CSi); 46.69 (*t*, C(6)); 48.06 (*t*, C(4)); 55.01 (*q*, MeO); 76.60, 84.28 (2*d*, C(1), C(8)); 80.38 (*s*, C(5)); 105.28 (*d*, C(3)); 210.57 (*s*, C(7)). EI-MS: 271 (2, $[M - OMe]^+$), 213 (*100*).

*(1*R,*3*R,*5*R,*8*S*)-8-{[(*tert*-*Butyl*)*dimethylsilyl*]*oxy*}-5-*hydroxy*-3-*methoxy*-2-*oxabicyclo*[3.3.0]*octan*-7-*one* (β-**4**). From β-**3** (566 mg, 1.86 mmol), as described above: crude β-**4** (740 mg). White solid that was used in the next step without further purification. Anal. data of a FC-purified sample (7.5% MeOH/CH_2Cl_2): TLC (7.5% MeOH/CH_2Cl_2): R_f 0.54. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.08, 0.13 (2*s*, Me_2Si); 0.91 (*s*, Me_3CSi); 2.21 (*d*, *J* = 12.5, 1 H−C(4)); 2.29 (*ddd*, *J* = 12.5, 4.6, 1.3, 1 H−C(4)); 2.59 (*d*, *J* = 18.4, 1 H−C(6)); 2.72 (*d*, *J* = 18.4, 1 H−C(6)); 3.17 (*s*, MeO); 4.45 (*d*, *J* = 1.8, H−C(1) or H−C(8)); 4.48 (*d*, *J* = 1.8, H−C(1) or H−C(8)); 5.00 (*d*, *J* = 4.4,

H–C(3)). ^{13}C-NMR (300 MHz, $CDCl_3$): –5.15, –4.69 (2*q*, Me_2Si); 18.36 (*s*, Me_3CSi); 25.75 (*q*, *Me*$_3$CSi); 48.26, 49.10 (2*t*, C(4), C(6)); 54.19 (*q*, MeO); 78.13, 85.21 (2*d*, C(1), C(8)); 80.23 (*s*, C(5)); 104.40 (*d*, C(3)); 210.65 (*s*, C(7)).

*(1*R*,3*S*,5*S*,7*E/Z*,8*R*)-8-{[(*tert*-*Butyl*)*dimethylsilyl*]*oxy*}-5-hydroxy-3-methoxy-2-oxabicyclo[3.3.0]octan-6-one* O-*Methyloxime* (α-**5**). A soln. of crude α-**4** (5.03 g, *ca.* 15 mmol) in abs. EtOH (92 ml) was treated with a ($MeONH_3$)Cl soln. (2.609 g, 31.24 mmol) and anh. NaOAc (1.281 g, 15.62 mmol) in H_2O (11 ml) (pH *ca.* 4), and the mixture was stirred for 20 min at r.t. Dilution with ^{t}BuOMe (400 ml) followed by extraction with sat. $NaHCO_3$ soln. (400 ml), drying of the org. phase ($MgSO_4$), evaporation, and FC of the residual yellowish oil (AcOEt/hexane 4.5 : 1 → AcOEt) afforded α-**5** (4.626 g, 89%; (*E*)/(*Z*) 5 : 1 (tentative assignment) by ^{1}H-NMR) as a yellow solid. Anal. data are from separated isomers.

Data of α-**5** (apolar (*E*)-isomer (tentative)): TLC (2.5% MeOH/CH_2Cl_2): R_f 0.51. IR ($CHCl_3$): 3516*w* (br.), 3001*w*, 2935*m*, 2858*w*, 1657*w*, 1467*w*, 1421*w*, 1394*w*, 1358*w*, 1307*w*, 1253*m*, 1217v*s*, 1126*m*, 1082*s*, 1042*s*, 945*m*, 901*m*, 868*m*, 837*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.06, 0.07 (2*s*, Me_2Si); 0.85 (*s*, Me_3CSi); 2.06 (*d*, *J* = 13.2, 1 H–C(4)); 2.27 (*dd*, *J* = 13.2, 4.8, 1 H–C(4)); 2.61 (*d*, *J* = 19.1, 1 H–C(6)); 2.78 (*d*, *J* = 19.5, 1 H–C(6)); 3.27 (*s*, OH); 3.37 (*s*, MeO); 3.82 (*s*, MeON=C); 4.25 (*d*, *J* = 5.1, H–C(1) or H–C(8)); 4.36 (*d*, *J* = 5.5, H–C(1) or H–C(8)); 5.11 (*d*, *J* = 4.8, H–C(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): 4.97, –4.75 (*q*, Me_2Si); 18.19 (*s*, Me_3CSi); 25.70 (*q*, *Me*$_3$CSi); 35.19, 46.16 (2*t*, C(4), C(6)); 54.91 (*q*, MeO); 61.81, 72.66, 89.93 (2*d*, *q*, C(1), C(8), *Me*ON=C); 84.48 (*s*, C(5)); 108.18 (*d*, C(3)); 160.37 (*s*, C(7)). EI-MS: 331 (1, M^+), 242 (*100*).

Data of α-**5** (polar (*Z*)-isomer (tentative)): TLC (2.5% MeOH/CH_2Cl_2): R_f 0.35. IR ($CHCl_3$): 3516*w*, 3001*w*, 2934*m*, 2858*w*, 1466*w*, 1428*w*, 1393*w*, 1358*w*, 1311*w*, 1250*m*, 1217v*s*, 1133*m*, 1082*s*, 1046*s*, 1008*w*, 939*m*, 879*m*, 839*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.03, 0.06 (2*s*, Me_2Si); 0.83 (*s*, Me_3CSi); 2.09 (*d*, *J* = 13.2, 1 H–C(4)); 2.23 (*dd*, *J* = 13.2, 4.4, 1 H–C(4)); 2.52 (*d*, *J* = 18.4, 1 H–C(6)); 2.88 (*d*, *J* = 18.0, 1 H–C(6)); 3.35 (*s*, OH); 3.37 (*s*, MeO); 3.80 (*s*, MeON=C); 4.20 (*d*, *J* = 5.5, H–C(1) or H–C(8)); 4.75 (*dt*, *J* = 0.7, 5.5, H–C(1) or H–C(8)); 5.12 (*d*, *J* = 4.4, H–C(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): *ca.* –5 (*q*, Me_2Si); 18.17 (*s*, Me_3CSi); 25.65 (*Me*$_3$CSi); 37.47, 45.73 (2*t*, C(4), C(6)); 54.90 (*q*, MeO); 61.38, 66.60 (2*d*, C(1), C(8)); 83.98 (*s*, C(5)); 90.66 (*q*, *Me*ON=C); 108.29 (*d*, C(3)); 160.60 (*s*, C(7)). EI-MS: 331 (38, M^+), 210 (*100*).

*(1*R*,3*R*,5*S*,7*E/Z*,8*R*)-8-{[(*tert*-*Butyl*)*dimethylsilyl*]*oxy*}-5-hydroxy-3-methoxy-2-oxabicyclo[3.3.0]octan-6-one* O-*Methyloxime* (β-**5**). From crude β-**4** (566 mg, 1.86 mmol), as described above. FC (AcOEt/hexane 5 : 1) gave β-**5** (523 mg, 85%; (*E*)/(*Z*) 6 : 1 (tentative assignment) by ^{1}H-NMR) as a colorless oil. Anal. data are from pure isomers:

Data of β-**5** (apolar (*E*)-isomer (tentative)): TLC (5% MeOH/CH_2Cl_2): R_f 0.54. IR ($CHCl_3$): 3600*w*, 3418*w* (br.), 3000*w*, 2956*m*, 2933*s*, 2893*m*, 2853*m*, 1479*w*, 1468*w*, 1448*w*, 1414*w*, 1395*w*, 1368*w*, 1312*w*, 1257*m*, 1168*s*, 1110*m*, 1052*s*, 1024*w*, 985*w*, 971*w*, 944*m*, 888*m*, 864*s*, 844*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.11, 0.12 (2*s*, Me_2Si); 0.91 (*s*, Me_3CSi); 1.75 (*s*, OH); 2.16–2.27 (*m*, 2 H–C(4)); 2.41 (*d*, *J* = 18.8, 1 H–C(6)); 3.15 (*dd*, *J* = 18.8, 1.1, 1 H–C(6)); 3.29 (*s*, MeO); 3.83 (*s*, MeON=C); 4.09 (*d*, *J* = 5.5, H–C(1) or H–C(8)); 4.62 (*dd*, *J* = 5.5, 1.1, H–C(1) or H–C(8)); 5.13 (*dd*, *J* = 4.6, 2.8, H–C(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): –4.93, –4.62 (2*q*, Me_2Si); 18.44 (*s*, Me_3CSi); 25.82 (*q*, *Me*$_3$CSi); 38.43, 47.73 (2*t*, C(4), C(6)); 54.83 (*q*, MeO); 61.75, 73.68 (2*d*, C(1), C(8)); 88.18 (*q*, *Me*ON=C); 83.25 (*s*, C(5)); 105.82 (*d*, C(3)); 160.30 (*s*, C(7)). EI-MS: 332 (2, $[M+H]^+$), 331 (3, M^+), 242 (*100*).

Data of β-**5** (polar (*Z*)-isomer (tentative)): TLC (5% MeOH/CH_2Cl_2): R_f 0.43. IR ($CHCl_3$): 3600*w*, 3422*w* (br.), 3000*w*, 2967*s*, 2933*s*, 2904*m*, 2862*m*, 1476*m*, 1463*m*, 1445*w*, 1390*w*, 1362*w*, 1312*w*, 1296*w*, 1257*m*, 1129*s*, 1090*m*, 1057*s*, 1028*m*, 985*w*, 943*m*, 887*m*, 863*m*, 844*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.09, 0.11 (2*s*, Me_2Si); 0.89 (*s*, Me_3CSi); 1.69 (*s*, OH); 2.18 (*dd*, *J* = 12.9, 4.4, H–C(4)); 2.28 (*dd*, *J* = 12.9, 5.1, H–C(4)); 2.41 (*d*, *J* = 17.3, 1 H–C(6)); 3.18 (*d*, *J* = 17.7, 1 H–C(6)); 3.38 (*s*, MeO)); 3.81 (*s*, MeON=C); 4.06 (*d*, *J* = 5.5, H–C(1) or H–C(8)); 4.85 (*d*, *J* = 5.5, H–C(1) or H–C(8)); 5.24 (*dd*, *J* = 5.5, 4.4, H–C(3)). EI-MS: 331 (3, M^+), 89 (*100*).

N-*{(1*R*,3*S*,5*S*,7*S*,8*R*)-8-{[(*tert*-*Butyl*)*dimethylsilyl*]*oxy*}-5-hydroxy-3-methoxy-2-oxabicyclo[3.3.0]oct-7-yl}-2,2,2-trifluoroacetamide* (α-**6**) *and* N-*{(1*R*,3*S*,5*S*,7*R*,8*R*)-8-{[(*tert*-*Butyl*)*dimethylsilyl*]*oxy*}-5-hydroxy-3-methoxy-2-oxabicyclo[3.3.0]oct-7-yl}-2,2,2-trifluoroacetamide* (α-**7**). In an autoclave, *Raney*-Ni (1.03 g) was suspended in H_2O (4 ml) and pretreated with H_2 (10 bar) under heavy stirring. After 10 min, a degassed soln. of α-**5** ((*E*)/(*Z*); 873 mg, 2.64 mmol) in EtOH (10 ml), containing 0.8 ml of conc. NH_3 soln. was carefully transferred to the autoclave, and the mixture was hydrogenated for 12 h at 10 bar. The mixture was filtered over *Celite* and the filtrate evaporated to give 796 mg (99%) of the crude amines as a colorless oil. This oil was dissolved in CF_3COOEt (1.55 ml) containing 70 µl (0.2 equiv.) of Et_3N, the soln. stirred for 1 h at r.t. and evaporated, and the residue separated by FC (AcOEt/hexane 2.5 : 1 → 1 : 1): α-**6** (144 mg, 14%) as a colorless oil and α-**7** (888 mg, 84%) as a white solid.

Data of α-**6**: TLC (8% MeOH/CH_2Cl_2): R_f 0.50. IR ($CHCl_3$): 3553*w*, 3426*m*, 2995*w*, 2955*s*, 2932*s*, 2858*m*, 1723v*s*, 1541*m*, 1471*m*, 1442*w*, 1386*w*, 1347*w*, 1296*w*, 1280*w*, 1252*s*, 1189*s*, 1170v*s*, 1131*s*, 1105*s*, 1055*m*, 1021*m*, 1005*w*, 947*m*, 927*s*, 886*m*, 872*m*, 842*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.07, 0.09 (2*s*, Me_2Si); 0.86 (*s*, Me_3CSi); 1.73 (*dd*, *J* = 13.6, 6.6, 1 H–C(6)); 2.01 (*dd*, *J* = 13.8, 4.2, 1 H–C(4)); 2.14 (*d*, *J* = 13.6, 1 H–C(4)); 2.34 (*dd*, *J* = 14.0, 5.9, 1 H–C(6)); 3.06 (*s*, OH); 3.34 (*s*, MeO); 4.04–4.16 (*m*, H–C(1), H–C(7), H–C(8)); 5.13 (*d*, *J* = 4.4, H–C(3)); 6.62 (*d*, *J* = 6.7, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): −5.18, −4.82 (2*q*, Me_2Si); 18.12 (*s*, Me_3CSi); 25.62 (*q*, Me_3CSi); 39.22, 48.12 (*t*, C(4), C(6)); 54.49 (*q*, MeO); 57.47, 75.28, 88.84 (3*d*, C(1), C(7), C(8)); 85.16 (*s*, C(5)); 107.34 (*d*, C(3)); 115.68 (*q*, *J*(C,F) = 288.1, CF_3); 156.89 (*q*, *J*(C,F) = 37.2, $COCF_3$). EI-MS: 368 (14, $[M-OMe]^+$), 310 (*100*).

Data of α-**7**: M.p. 84–85°. TLC (8% MeOH/CH_2Cl_2): R_f 0.61. IR ($CHCl_3$): 3531*w*, 3427*m*, 2998*w*, 2956*m*, 2931*m*, 2859*m*, 1724*s*, 1534*m*, 1472*m*, 1464*m*, 1443*m*, 1427*w*, 1390*w*, 1373*w*, 1362*w*, 1344*w*, 1322*w*, 1303*w*, 1261*s*, 1173*s*, 1092*s*, 1043*s*, 966*m*, 948*m*, 875*m*, 838*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.05, 0.07 (2*s*, Me_2Si); 0.87 (*s*, Me_3CSi); 1.89–2.01 (*m*, 1 H–C(4), 1 H–C(6)); 2.15 (*d*, *J* = 13.6, 1 H–C(4)); 2.23 (*dd*, *J* = 13.8, 7.6, 1 H–C(6)); 2.96 (*s*, OH); 3.34 (*s*, MeO); 4.14 (*t*, *J* = 4.0, H–C(8)); 4.19 (*d*, *J* = 4.4, H–C(1)); 4.53 (*ddd*, *J* = 16.8, 8.4, 3.8, H–C(7)); 5.10 (*d*, *J* = 4.4, H–C(3)); 6.66 (*d*, *J* = 7.7, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): −5.45, −4.43 (2*q*, Me_2Si); 18.10 (*s*, Me_3CSi); 25.66 (*q*, Me_3CSi); 41.14, 48.24 (2*t*, C(4), C(6)); 53.34 (*d*, C(7)); 54.65 (*q*, MeO); 73.34 (*d*, C(8)); 84.95 (*s*, C(5)); 91.05 (*d*, C(1)); 107.96 (*d*, C(3)); 111.93 (*q*, *J*(C,F) = 286, CF_3); 156.24 (*q*, *J*(C,F) = 37, CF_3CO). EI-MS: 368 (11, $[M-OMe]^+$), 312 (*100*).

X-Ray Analysis of α-**7**: Suitable crystals were obtained from 0.02M α-**7** in hexane at −24°. Colorless transparent needles (0.57 × 0.27 × 0.19 mm); $C_{16}H_{28}F_3NO_5Si$; orthorhombic space group *P*212121. Intensities were measured with a *Stoe-AED*-4-circle diffractometer (MoK_α, λ 0.71073 Å). Of the 2265 independent reflections (θ = 2.03–25.47°), 1645 with $F > 4\sigma(F)$ were used in the refinement. The structure was solved using direct methods with SHELX-86 [23] and refined by full-matrix least-square procedures SHELXL-97. Non-H-atoms were refined anisotropically. The positions of all H-atoms were calculated and adjusted after every least-squares cycle. The refinement converged at *R* = 0.0627, *Rw* = 0.1755.

N-*{(1*R*,3*R*,5*S*,7*S*,8*R*)-8-{[(*tert*-Butyl)dimethylsilyl]oxy}-5-hydroxy-3-methoxy-2-oxabicyclo[3.3.0]oct-7-yl}-2,2,2-trifluoroacetamide* (β-**6**) *and* N-*{(1*R*,3*R*,5*S*,7*R*,8*R*)-8-{[(*tert*-Butyl)dimethylsilyl]oxy}-5-hydroxy-3-methoxy-2-oxabicyclo[3.3.0]oct-7-yl}-2,2,2-trifluoroacetamide* (β-**7**). Procedure not optimized. As described above, from β-**5** ((*E*)/(*Z*); 495 mg, 1.50 mmol) by hydrogenation (H_2/20 bar) over 10% Pd/C (495 mg) in MeOH, followed by trifluoroacetylation. FC (2% MeOH/CH_2Cl_2) yielded β-**6**/β-**7** (104 mg, 17%; ratio 1:5 by ^{1}H-NMR) besides 33% of nonconverted β-**5**.

Data of β-**7**: TLC (10% MeOH/CH_2Cl_2): R_f 0.64. IR ($CHCl_3$): 3597*w*, 3416*w*, 3265*w*(sh), 3101*w*, 2954*m*, 2931*s*, 2901*m*, 2858*m*, 1720v*s*, 1548*m*, 1472*m*, 1464*m*, 1448*m*, 1385*m*, 1362*m*, 1314*m*, 1282*m*, 1256*s*, 1166v*s*, 1118*m*, 1103*m*, 1088*m*, 1071*m*, 1031*m*, 1006*w*, 978*m*, 959*m*, 940*m*, 922*m*, 865*m*, 839*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.06, 0.10 (2*s*, Me_2Si); 0.87 (*s*, Me_3CSi); 2.13 (*s*, OH); 2.21–2.26 (*m*, 3 H, $CH_2(4)$, $CH_2(6)$); 2.45 (*dd*, *J* = 14.0, 6.3, 1 H, $CH_2(4)$, $CH_2(6)$); 3.41 (*s*, MeO); 4.05 (*d*, *J* = 4.8, H–C(1)); 4.30 (*dd*, *J* = 6.4, 5.0, H–C(8)); 4.56 (*m*, H–C(7)); 5.17 (*dd*, *J* = 6.6, 2.2, H–C(3)); 8.08 (*d*, *J* = 8.1, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): −5.27, −5.05 (2*q*, Me_2Si); 18.13 (*s*, Me_3CSi); 25.61 (*q*, Me_3CSi); 43.87, 49.03 (2*t*, C(4), C(6)); 53.03, 56.22 (*d* + *q*, C(7), MeO)); 73.09, 90.11 (2*d*, C(1), C(8)); 84.31 (*s*, C(5)); 107.39 (*d*, C(3)); 117.95 (*q*, CF_3); 156.75 (*q*, CF_3CO).

*(3′*S*,5′*R*,6′*R*)-1-{5′-*O*-[(*tert*-Butyl)dimethylsilyl]-2′-deoxy-6′-(trifluoroacetamido)-3′,5′-ethano-α/β-*D*-ribofuranosyl}thymine* (α/β-**8**). A mixture of α-**7** (1.000 g, 2.51 mmol) and dry thymine (631 mg, 5.00 mmol) in abs. MeCN (25 ml) was cooled to 3° and treated with *N*,*O*-bis(trimethylsilyl)acetamide (BSA; 3.06 ml, 1.25 mmol) followed by Me_3SiCl (158 μl, 1.25 mmol). After 90 min (→ clear soln.), $Me_3SiOSO_2CF_3$ (1.82 ml, 10.03 mmol) was added and the mixture stirred for 20 h at 3°. Then the mixture was treated with 1M HCl (25 ml) for 5 min, diluted with CH_2Cl_2 (150 ml), and extracted with sat. $NaHCO_3$ soln. The org. phases were dried ($MgSO_4$), and evaporated and the crude nucleosides purified by FC (0–3% MeOH/CH_2Cl_2) to give α/β-**8** (1.110 g, 90%; ratio 1:1.5 by ^{1}H-NMR) as a white foam. Separation of the anomers was effected by prep. HPLC (*LiChrosorb SI 60*, 7 μ, 23 × 250 mm; hexane/^{i}PrOH 8:2; t_R 22.5 (β-**8**), 27.5 (α-**8**)) to give anal. pure β-**8** (577 mg, 47%) and α-**8** (332 mg, 27%), both as white foams.

Data of α-**8**: TLC (hexane/^{i}PrOH 8:2): R_f 0.35. IR ($CHCl_3$): 3426*m*, 2956*m*, 2932*m*, 2900*w*, 2860*m*, 1691v*s*, 1535*m*, 1472*m*, 1443*m*, 1427*w*, 1411*w*, 1364*m*, 1264*s*, 1237*s*, 1229*s*, 1212*s*, 1200*s*, 1173*s*, 1104*s*, 1051*m*, 1006*m*, 967*m*, 872*m*, 837*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.07, 0.12 (2*s*, Me_2Si); 0.90 (*s*, Me_3CSi); 1.84 (*s*, Me–C(5)); 2.06 (*dd J* = 13.6, 9.9, 1 H–C(7′)); 2.38 (*dd*, *J* = 13.6, 7.7, 1 H–C(7′)); 2.49–2.63 (*m*, 2 H–C(2′)); 4.18 (*t*, *J* = 3.9, H–C(5′)); 4.33 (br. *s*, OH); 4.53–4.63 (*m*, H–C(6′)); 4.58 (*d*, *J* = 4.0, H–C(4′)); 6.06 (*dd*, *J* = 6.8, 2.8, H–C(1′)); 6.66 (*d*, *J* = 8.1, CF_3CONH); 7.32 (*s*, H–C(6)); 9.76 (br. *s*, H–N(3)). ^{13}C-NMR (75 MHz, $CDCl_3$):

−5.44, −4.47 (2*q*, Me_2Si); 12.40 (*q*, *Me*−C(5)); 18.00 (*s*, Me_3CSi); 25.68 (*q*, *Me*$_3$CSi); 43.40, 47.99 (2*t*, C(2′), C(7′)); 52.05 (*d*, C(6′)); 73.47 (*d*, C(5′)); 84.34 (*s*, C(3′)); 91.40 (C(1′)); 93.32 (*d*, C(4′)); 109.95 (*s*, C(5)); 115.67 (*q*, *J*(C,F) = 288, CF_3); 137.58 (*s*, C(6)); 150.56 (*s*, C(2)); 156.52 (*q*, *J*(C,F) = 37, *C*OCF_3); 164.53 (*s*, C(4)). LSI-MS: 646.2 (2, $[M + \text{matrix}]^+$), 532.1 (3, $[M + K]^+$), 516.2 (2, $[M + Na]^+$), 494.2 (39, $[M + 1]^+$), 368.2 (*100*).

*Data of β-***8**: TLC (hexane/^{i}PrOH 8 : 2): R_f 0.27. IR ($CHCl_3$): 3596*w*, 3397*m*, 2957*m*, 2932*m*, 2899*w*, 2860*w*, 1693v*s*, 1532*m*, 1472*m*, 1442*w*, 1427*w*, 1414*w*, 1371*m*, 1318*w*, 1281*m*, 1261*s*, 1238*s*, 1213*s*, 1199*s*, 1175*s*, 1101*s*, 1056*m*, 1012*m*, 977*m*, 931*m*, 910*m*, 839*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.07 (*s*, Me_2Si); 0.89 (*s*, Me_3CSi); 1.87 (*s*, Me−C(5)); 1.96 (*dd*, *J* = 13.2, 9.9, 1 H−C(2′)); 2.16–2.30 (*m*, 2 H−C(7′)); 2.55 (*dd*, *J* = 13.6, 5.2, 1 H−C(2′)); 4.10 (*d*, *J* = 5.2, H−C(4′)); 4.38 (*t*, *J* = 5.0, H−C(5′)); 4.44–4.50 (*m*, H−C(6′) OH); 6.32 (*dd*, *J* = 9.6, 5.5, H−C(1′)); 6.98 (*d*, *J* = 6.6, CF_3CONH); 7.21 (*s*, H−C(6)); 10.19 (*s*, H−N(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): −5.40, −4.52 (2*q*, Me_2Si); 12.14 (*q*, Me−C(5)); 18.13 (*s*, *Me*$_3$CSi); 25.72 (*q*, *Me*$_3$CSi); 42.36, 46.58 (2*t*, C(2′), C(7′)); 54.20 (*d*, C(6′)); 71.80 (*d*, C(5′)); 83.75 (*s*, C(3′)); 85.49 (*d*, C(1′)); 88.99 (*d*, C(4′)); 111.82 (*s*, C(5)); 115.72 (*q*, *J*(C,F) = 288, CF_3); 134.91 (*d*, C(6)); 150.87 (*s*, C(2)); 156.74 (*q*, *J*(C,F) = 37, *C*OCF_3); 163.87 (*s*, C(4)). LSI-MS: 646.1 (1, $[M + \text{matrix}]^+$), 532.1 (4, $[M + K]^+$), 516.1 (2, $[M + Na]^+$), 494.1 (39, $[M + 1]^+$), 368.1 (*100*).

*(3′*S*,5′*R*,6′*R*)-1-{6′-*Amino-5′-O-*[(*tert*-butyl)dimethylsilyl]-2′-*deoxy-3′,5′-*ethano-α/β-*D*-ribofuranosyl}thymine* (**9**). To a soln. of *β*-**8** (640 mg, 1.3 mmol) in MeOH (1.3 ml), conc. NH_3 soln. (26 ml) was added. The resulting mixture was stirred for 4 h at r.t. and subsequently evaporated. CC (CH_2Cl_2/MeOH/NH_3 soln. 50 : 5 : 3) gave **9** (472 mg, 91%). Slightly yellow oil. TLC (CH_2Cl_2/MeOH/NH_3 soln. 18 : 2 : 1): R_f 0.43. UV (MeOH): 264 (9930). IR (KBr): 3419*s* (br.), 3198*s* (br), 3062*s* (br.), 2954*s*, 2930*s*, 2898*s*, 2857*s*, 1698v*s*, 1686v*s*, 1472*m*, 1287*w*, 1262*w*, 1152*w*, 836*w*, 780*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.07, 0.10 (2*s*, Me_2Si); 0.91 (*s*, Me_3CSi); 1.84–1.99 (*m*, 2 H−C(7′)); 1.88 (*s*, Me−C(5)); 2.33 (*dd*, *J* = 13.1, 9.6, 1 H−C(2′)); 2.41 (*dd*, *J* = 13.2, 5.5, 1 H−C(2′)); 3.62 (*dd*, *J* = 10.3, 4.3, H−C(6′)); 4.03 (*d*, *J* = 5.7, H−C(4′)); 4.11–4.14 (*m*, H−C(5′)); 6.36 (*dd*, *J* = 9.6, 5.3, H−C(1′)); 7.79 (*d*, *J* = 1.1, H−C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): −5.00, −4.45 (2*q*, Me_2Si); 12.21 (*q*, Me−C(5)); 18.26 (*s*, *Me*$_3$CSi); 25.91 (*q*, *Me*$_3$CSi); 43.74, 46.72 (2*t*, C(2′), C(7′)); 55.70 (*d*, C(6′)); 73.60 (*d*, C(5′)); 84.55 (*s*, C(3′)); 87.03 (*d*, C(1′)); 89.21 (*d*, C(4′)); 110.92 (*s*, C(5)); 137.79 (*d*, C(6)); 151.13 (*s*, C(2)); 164.27 (*s*, C(4)). HR-LSI-MS ($C_{18}H_{32}N_3O_5Si$): 398.2111 (calc. 398.2109).

*(3′*S*,5′*R*,6′*R*)-1-{6′-Acetamido-5′-*O-*[(*tert*-butyl)dimethylsilyl]-2′-deoxy-3′,5′-ethano-β-*D*-ribofuranosyl}thymine* (**10**). To a soln. of **9** (396 mg, 0.99 mmol) in pyridine (3.5 ml), Ac_2O (102 µl, 1 mmol) was added at 0°, and the mixture was stirred for 2 h. Dilution with CH_2Cl_2 (50 ml), extraction with sat. $NaHCO_3$ soln. (50 ml), and evaporation of the dried ($MgSO_4$) org. phase, followed by FC (CH_2Cl_2/MeOH 100 : 8) yielded **10** (387 mg, 88%). White foam. TLC (CH_2Cl_2/MeOH 100 : 8): R_f 0.48. UV (MeOH): 262 (9480). IR (KBr): 3368*m* (br.), 3068*w* (br.), 2954*m*, 2930*m*, 2892*w*, 2856*m*, 1700*s*, 1540*w*, 1472*m*, 1374*m*, 1284*m*, 1260*m*, 1154*m*, 1046*m*, 980*w*, 883*w*, 836*m*, 779*m*. ^{1}H-NMR (300 MHz, CD_3OD): 0.11, 0.13 (*s*, Me_2Si); 0.98 (*s*, Me_3CSi); 1.93 (*s*, Me−C(5)); 2.01 (*s*, MeCO); 2.01–2.07 (*m*, 1 H−C(7′)); 2.16–2.26 (*m*, 1 H−C(2′), 1 H−C(7′)); 2.36 (*dd*, *J* = 13.1, 5.2, 1 H−C(2′)); 4.06 (*d*, *J* = 4.8, H−C(4′)); 4.40 (*t*, *J* = 4.4, H−C(5′)); 4.44–4.49 (*m*, H−C(6′)); 6.23 (*dd*, *J* = 10.0, 5.2, H−C(1′)); 7.53 (*d*, *J* = 1.1, H−C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): −4.48, −3.75 (2*q*, Me_2Si); 12.77 (*q*, *Me*−C(5)); 19.78 (*s*, Me_3*C*Si); 23.09 (*q*, *Me*CO); 26.91 (*q*, *Me*$_3$CSi); 43.42, 46.47 (2*t*, C(2′), C(7′)); 55.13 (*d*, C(6′)); 73.72 (*d*, C(5′)); 84.27 (*s*, C(3′)); 88.63 (*d*, C(1′)); 91.42 (*d*, C(4′)); 111.78 (*s*, C(5)); 138.75 (*d*, C(6)); 152.52 (*s*, C(2)); 166.45 (*s*, C(4)); 173.06 (*s*, Me*C*O). HR-LSI-MS ($C_{20}H_{34}N_3O_6Si$): 440.2217 (calc. 440.2201).

*(3′*S*,5′*R*,6′*R*)-1-(6′-Acetamido-2′-deoxy-3′,5′-ethano-β-*D*-ribofuranosyl)thymine* (**11**). To a soln. of **10** (305 mg, 0.69 mmol) in pyridine (1.5 ml), $Et_3N \cdot 3$ HF (160 µl, 0.98 mmol) was added and the mixture stirred. After 24 h at r.t., excess solid $(NH_4)_2CO_2$ was added, the mixture was filtered, and the filtrate evaporated. FC (CH_2Cl_2/MeOH 100 : 8) gave **11** (215 mg, 96%). White foam. TLC (CH_2Cl_2/MeOH 100 : 8): R_f 0.11. UV (H_2O): 264 (9370). IR (KBr): 3384*s* (br.), 3066*m*, 2930*m*, 2820*w*, 1700*s*, 1684*s*, 1654*m*, 1540*m*, 1472*m*, 1374*m*, 1288*m*, 1264*m*, 1144*m*, 1056*m*, 940*w*, 814*w*, 782*w*, 668*w*, 610*w*, 560*w*. ^{1}H-NMR (300 MHz, CD_3OD): 1.92 (*d*, *J* = 1.3, Me−C(5)); 2.02 (*s*, MeCO); 2.07–2.23 (*m*, 1 H−C(2′), 2 H−C(7′)); 2.40 (*dd*, *J* = 13.0, 5.2, 1 H−C(2′)); 4.15 (*t*, *J* = 3.8, H−C(5′)); 4.21 (*d*, *J* = 4.2, H−C(4′)); 4.52 (*ddd*, *J* = 11.0, 8.6, 3.3, H−C(6′)); 6.44 (*dd*, *J* = 10.1, 5.2, H−C(1′)); 8.20 (*d*, *J* = 1.1, H−C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 12.81 (*q*, *Me*−C(5)); 22.84 (*q*, *Me*CO); 43.21, 47.76 (2*t*, C(2′), C(7′)); 54.05 (*d*, C(6′)); 84.27 (*s*, C(3′)); 89.30, 92.72 (2*d*, C(1′), C(4′)); 111.76 (*s*, C(5)); 139.07 (*d*, C(6)); 152.60 (*s*, C(2)); 166.67 (*s*, C(4)); 173.22 (*s*, Me*C*O). HR-LSI-MS ($C_{14}H_{20}N_3O_6$): 326.1352 (calc. 326.1358).

*(3′*S*,5′*R*,6′*R*)-1-[2′-Deoxy-6′-(trifluoroacetamido)-3′,5′-ethano-β-*D*-ribofuranosyl]thymine* (**12**). To a soln. of *β*-**8** (993 mg, 2.00 mmol) in MeCN (10 ml), a soln. of $Bu_4NF \cdot 3H_2O$ (5.05 g, 16.02 mmol) in MeCN (10 ml) and AcOH (1.15 ml, 20.01 mmol) was added, and the resulting mixture was stirred for 2 h at r.t. Dilution with AcOEt (100 ml), extraction with sat. $NaHCO_3$ soln., followed by drying of the org. phase ($MgSO_4$) gave crude

product that was purified by FC (AcOEt/hexane 3:1): **12** (719 mg, 95%). White foam. TLC (8% MeOH/CH_2Cl_2): R_f 0.19. UV (H_2O): 265 (8910). IR (KBr): 3408*s* (br.), 3000*s* (sh), 3072*m*, 2954*w*, 2830*w*, 1698*vs*, 1562*m*, 1552*m*, 1536*w*, 1475*m*, 1408*w*, 1376*m*, 1289*s*, 1266*s*, 1215*s*, 1188*s*, 1158*s*, 1107*m*, 1054*m*, 1010*w*, 968*w*, 944*m*, 882*w*, 852*w*, 814*w*, 784*w*, 756*w*, 668*w*, 644*w*. ^{1}H-NMR (500 MHz, CD_3OD): 1.92 (*d*, *J* = 1.3, Me–C(5)); 2.16 (*d*, *J* = 13.5, 8.0, 1 H–C(7′)); 2.17 (*dd*, *J* = 13.0, 10.0, 1 H–C(2′)); 2.34 (*dd*, *J* = 13.4, 11.8, 1 H–C(7′)); 2.42 (*dd*, *J* = 13.0, 5.2, 1 H–C(2′)); 4.23–4.25 (*m*, H–C(4′), H–C(5′)); 4.55 (*ddd*, *J* = 11.4, 8.4, 2.6, H–C(6′)); 6.47 (*dd*, *J* = 10.1, 5.2, H–C(1′)); 8.19 (*d*, *J* = 1.3, H–C(6)). ^{13}C-NMR (125 MHz, CD_3OD): 12.79 (*q*, *Me*–C(5)); 42.43, 47.65 (2*t*, C(2′), C(7′)); 54.65 (*d*, C(6′)); 72.86 (*d*, C(4′) or C(5′)); 85.71 (*s*, C(3′)); 89.37 (*d*, C(1′)); 92.52 (*d*, C(4′) or C(5′)); 111.83 (*s*, C(5)); 117.69 (*q*, *J*(C,F) = 287, CF_3); 138.93 (*d*, C(6)); 152.60 (*s*, C(2)); 158.77 (*q*, *J*(C,F) = 37, *C*OCF_3); 166.66 (*s*, C(4)). LSI-MS: 380.0 (18, $[M+H]^+$), 127 (*100*).

*(3′S,5′R,6′R)-1-(6′-Amino-2′-deoxy-3′,5′-ethano-β-*D*-ribofuranosyl)thymine* (**13**). A soln. of **12** (20 mg, 53 μmol) in MeOH (0.1 ml) was treated with conc. NH_3 soln. (1.5 ml). After 45 min at r.t., the mixture was lyophilized and the residue adsorbed on silica gel (MeOH) and purified by FC (CH_2Cl_2/MeOH 5:1 + 5% conc. NH_3 soln.): **13** (15 mg, 98%). Colorless film. TLC ($MeCl_2$/MeOH 4:1 + 5% conc. NH_3): R_f 0.25. UV (MeOH): 267 (9770). IR (KBr): 3072*m* (br.), 2963*m*, 1684*s*, 1472*m*, 1436*w*, 1292*m*, 1264*m*, 1203*s*, 1132*s*, 1046*m*, 934*w*. ^{1}H-NMR (300 MHz, CD_3OD): 1.92 (*d*, *J* = 1.1, Me–C(5)); 2.18–2.37 (*m*, 1 H–C(2′), 2 H–C(7′)); 2.44 (*dd*, *J* = 13.1, 5.4, 1 H–C(2′)); 3.86 (*ddd*, *J* = 9.8, 8.7, 3.7, H–C(6′)); 4.21 (*d*, *J* = 4.2, H–C(4′)); 4.31 (*t*, *J* = 4.0, H–C(5′)); 6.40 (*dd*, *J* = 9.9, 5.1, H–C(1′)); 7.96 (*d*, *J* = 1.1, H–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 12.8 (*q*, *Me*–C(5)); 42.5, 47.4, (2*t*, C(2′), C(7′)); 54.6 (*d*, C(6′)); 72.0 (*d*, C(5′)); 85.9 (*s*, C(3′)); 90.0, 92.2 (2*d*, C(1′), C(4′)); 112.1 (*s*, C(5)); 139.0 (*d*, C(6)); 152.7 (*s*, C(2)); 166.6 (*s*, C(4)). HR-LSI-MS ($C_{11}H_{18}N_3O_5$): 284.1245 (calc. 284.1246).

*(3′S,5′R,6′R)-1-{2′-*Deoxy-5′-O-*[(4,4′-dimethoxytriphenyl)methyl]-6′-(trifluoroacetamido)-3′,5′-ethano-β-*D*-ribofuranosyl}thymine* (**14**). A soln. of **12** (173 mg, 0.46 mmol) in dry pyridine (0.5 ml), containing activated molecular sieves (3 Å), was treated with $(MeO)_2TrOSO_2CF_3$ (312 mg, 0.69 mmol) and heated to 50°. After 9 h, another portion of $(MeO)_2TrOSO_2CF_3$ (103 mg, 0.23 mmol) was added. After a total of 16 h, the mixture was diluted with AcOEt (30 ml) and extracted with sat. $NaHCO_3$ soln. (30 ml), the org. phase dried ($MgSO_4$) and evaporated, and the residual oil purified by FC (2.5–10% MeOH/CH_2Cl_2 + 1% Et_3N): **14** (144 mg, 46%), besides 40% of recovered starting material. TLC (8% MeOH/CH_2Cl_2): R_f 0.46. IR ($CHCl_3$): 3595*w*, 3393*w*, 2961*w*, 2935*w*, 2912*w*, 2839*w*, 1694*s*, 1628*w*, 1608*m*, 1580*w*, 1528*w*, 1512*m*, 1467*m*, 1443*w*, 1426*w*, 1413*w*, 1370*w*, 1320*w*, 1303*m*, 1281*m*, 1257*s*, 1237*s*, 1216*s*, 1201*vs*, 1180*s*, 1097*m*, 1057*m*, 1035*m*, 1014*m*, 910*w*, 872*w*, 825*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.76 (*dd*, *J* = 15.3, 6.4, 1 H–C(7′)); 1.93 (*s*, Me–C(5)); 2.04 (*dd*, *J* = 13.6, 9.9, 1 H–C(2′)); 2.35 (*d*, *J* = 15.1, 1 H–C(7′)); 2.49 (*dd*, *J* = 13.8, 5.3, 1 H–C(2′)); 3.00 (*dd*, *J* = 10.5, 5.7, H–C(6′)); 3.4 (br. *s*, OH); 3.75 (*s*, MeO); 3.89 (*d*, *J* = 5.5, H–C(4′)); 4.22 (*t*, *J* = 6.1, H–C(5′)); 6.19 (*dd*, *J* = 9.6, 5.5, H–C(1′)); 6.79–6.83 (*m*, 4 arom. H); 7.16–7.50 (*m*, 14 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 12.16 (*q*, *Me*–C(5)); 42.13, 47.92 (2*t*, C(2′), C(7′)); 53.89 (*d*, C(6′)); 55.29 (*q*, MeO); 72.29 (*d*, C(5′)); 83.47, 88.10 (2*s*, C(3′), Ar_2*C*Ph); 84.85 (*d*, C(1′)); 88.69 (*d*, C(4′)); 115.72 (*q*, *J*(C,F) = 289, CF_3); 112.36 (*s*, C(5)); 113.57, 113.61, 127.41, 127.53, 128.23, 129.56, 129.67 (7*d*, arom. C); 134.56 (*d*, C(6)); 135.30, 135.59, 144.47 (3*s*, arom. C); 150.52 (*s*, C(2)); 156.76 (*q*, *J*(C,F) = 37, *C*OCF_3); 159.04, 159.07 (2*s*, arom. C); 163.39 (*s*, C(4)). LSI-MS: 720.1 (2, $[M+K]^+$), 681.1 (2, M^+), 303.1 (*100*).

*(3′S,5′R,6′R)-1-{6′-Acetamido-2′-deoxy-5′-*O*-[(4,4′-dimethoxytriphenyl)methyl]-3′,5′-ethano-β-*D*-ribofuranosyl}thymine* (**15**). As described for **14**, from **11** (186 mg, 0.57 mmol) and $(MeO)_2TrOSO_2CF_3$ (395 mg, 0.87 mmol) in pyridine (0.6 ml). FC (CH_2Cl_2/MeOH 50:1) gave **15** (277 mg, 77%). Slightly brownish foam. TLC (CH_2Cl_2/MeOH 50:4): R_f 0.53. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.70 (*dd*, *J* = 14.8, 6.3, 1 H–C(7′)); 1.90 (*d*, *J* = 0.9, Me–C(5)); 1.94 (*s*, MeCO); 2.21–2.32 (*m*, 1 H–C(2′), 1 H–C(7′)); 2.41 (*dd*, *J* = 13.7, 5.8, 1 H–C(2′)); 3.26 (br. *s*, OH–C(3′)); 3.32 (*dd*, *J* = 11.0, 6.0, H–C(6′)); 3.65 (*d*, *J* = 5.5, H–C(4′)); 3.74 (*s*, MeO); 4.08–4.14 (*m*, H–C(5′)); 6.01 (*dd*, *J* = 9.4, 5.9, H–C(1′)); 6.55 (*d*, *J* = 4.6, CONH); 6.79 (*d*, *J* = 8.6, 4 arom. H); 7.21 (*d*, *J* = 0.9, H–C(6)); 7.16–7.50 (3*m*, 9 arom. H); 9.10 (*s*, H–N(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): 12.61 (*q*, *Me*–C(5)); 23.40 (*q*, *Me*CO); 43.33, 47.41 (2*t*, C(2′), C(7′)); 53.66 (*d*, C(6′)); 55.25 (*q*, MeO); 72.94 (*d*, C(5′)); 83.35, 87.94 (2*s*, C(3′), Ar_2*C*Ph); 85.64 (*d*, C(1′)); 88.78 (*d*, C(4′)); 111.40 (*s*, C(5)); 113.40, 127.21, 127.85, 128.06, 129.84 (5*d*, arom. C); 135.82 (*d*, C(6)); 135.94, 144.84 (2*s*, arom. C); 150.41 (*s*, C(2)); 158.83 (*s*, arom. C); 163.57 (*s*, C(4)); 170.41 (*s*, CO). LSI-MS: 628 (3, $[M+1]^+$), 303 (*100*). Anal. calc. for $C_{35}H_{37}N_3O_8 \cdot 0.5H_2O$: C 66.03, H 6.02, N 6.60; found: C 66.24, H 6.25, N 6.56.

*(3′S,5′R,6′R)-1-{2′-Deoxy-5′-*O*-[(4,4′-dimethoxytriphenyl)methyl]-6′-(trifluoroacetamido)-3′,5′-ethano-β-*D*-ribofuranosyl}thymine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**16**). A soln. of **14** (73 mg, 0.11 mmol) in MeCN (1 ml) was treated with ^{i}Pr$_2$NEt (73.5 μl, 0.43 mmol) and [P(OCH$_2$CH$_2$CN)(N^{i}Pr$_2$)]Cl (48 μl, 0.22 mmol) and the resulting mixture stirred for 2 h at r.t. Then additional ^{i}Pr$_2$NEt (37 μl, 0.22 mmol) and

Cl[P(OCH$_2$CH$_2$CN)(N^{i}Pr$_2$)] (24 µl, 0.11 mmol) were added, and the mixture was worked up after 7 h by dilution with AcOEt and extraction with sat. NaHCO$_3$ soln. After drying and evaporation of the org. phase, the residual gum was purified by FC (hexane/AcOEt 2:1 → 0:1): **16** (83 mg, 88%). White foam. TLC (hexane/AcOEt 1:1): R_f 0.31, 0.26. ^{1}H-NMR (300 MHz, CDCl$_3$): 1.05–1.13 (*m*, 2 *Me*$_2$CH); 1.93, 1.94 (2*d*, *J* = 0.7, Me–C(5)); 2.02–2.20 (*m*, 2 H, CH$_2$(2′) or CH$_2$(7′)); 2.34–2.39 (*m*, 1 H, CH$_2$(2′) or CH$_2$(7′)); 2.50–2.59 (*m*, OCH$_2$C*H*$_2$CN); 2.84–2.92 (*m*, 1 H, CH$_2$(2′) or CH$_2$(7′)); 3.17–3.27 (*m*, Me$_2$C*H*); 3.44–3.65 (*m*, OC*H*$_2$CH$_2$CN); 3.65–3.75 (*m*, H–C(5′)); 3.76 (*s*, MeO); 3.82, 3.99 (2*d*, *J* = 5.3, H–C(4′)); 4.11–4.19 (*m*, H–C(6′)); 5.96–6.04 (*m*, H–C(1′)); 6.80–6.84 (*m*, 4 arom. H); 7.12–7.50 (*m*, H–C(6), 9 arom. H); 9.21 (br. *s*, H–N(3)). ^{13}C-NMR (75 MHz, CDCl$_3$): 12.18 (*q*, *Me*–C(5)); 20.10, 20.13, 20.20, 20.23 (4*t*, *J*(C,P) = 2.4, NCCH$_2$CH$_2$O); 24.16, 24.19, 24.27, 24.29, 24.37, 24.40, 24.46, 24.48 (8*q*, *Me*$_2$CH); 41.56, 41.917 (2*t*, *J*(C,P) = 8.9, C(2′), C(7′)); 43.27, 43.43 (2*d*, Me$_2$*C*H); 44.97, 45.81 (2*t*, *J*(C,P) = 9.0, C(2′), C(7′)); 53.20, 53.43 (2*d*, C(6′)); 55.22 (*q*, MeO); 57.66, 57.89 (2*t*, *J*(C,P) = 9.5, NCCH$_2$*C*H$_2$O); 72.12, 72.30 (2*d*, C(5′)); 85.55, 86.15 (2*d*, *J*(C,P) = 1.2, C(1′)); 86.99, 87.23 (2*s*, *J*(C,P) = 7.9, 8.5, C(3′)); 87.46, 87.69 (2*d*, *J*(C,P) = 5.5, 7.3, C(4′)); 88.06 (*s*, C(3′), Ar$_2$*C*Ph); 111.90, 111.91 (2*s*, C(5)); 113.47, 113.50, 113.53 (3*d*, arom. C); 113.81, 117.64 (2*q*, *J*(C,P) = 288, CF$_3$); 117.52, 117.55 (2*s*, CN); 127.26, 127.29, 127.54, 127.57, 128.14, 128.17, 129.59, 129.63, 129.69, 129.71 (10*d*, arom. C); 135.04, 135.06, 135.55, 135.58 (4*d*, C(6), arom. C); 135.34, 135.42, 144.49, 144.52 (4*s*, arom. C); 150.29, 150.38 (2*s*, C(2)); 156.72 (*q*, *J*(C,P) = 37, *C*OCF$_3$); 158.93, 158.95, 158.97 (3*s*, arom. C); 163.50 (*s*, C(4)). ^{31}P-NMR (81 MHz, CDCl$_3$): 141.6, 142.6.

*(3′S,5′*R*,6′*R*)-1-[(6′-Acetamido-2′-deoxy-5′*-O*-[(4,4′-dimethoxytriphenyl)methyl]-3′,5′-ethano-β-*D*-ribofuranosyl]thymine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**17**). As described for **16**, from **15** (213 mg, 0.34 mmol), ^{i}Pr$_2$NEt (235 µl, 1.37 mmol), and [P(OCH$_2$CH$_2$CN)(N^{i}Pr$_2$)]Cl (155 µl, 0.69 mmol) in MeCN (3 ml). FC (CH$_2$Cl$_2$/MeOH 50:1) gave **17** (176 mg, 62%; *ca.* 1:1 diastereoisomer mixture). White foam. TLC (CH$_2$Cl$_2$/MeOH 25:2): R_f 0.48, 0.65. ^{1}H-NMR (300 MHz, CDCl$_3$): 1.04–1.12 (*m*, 2 *Me*$_2$CH); 1.92, 1.93 (2*s*, Me–C(5)); 1.95, 1.96 (2*s*, 3 MeCO); 1.96–2.02 (*m*, 1 H, CH$_2$(2′) or CH$_2$(7′)); 2.34–2.39 (*m*, 2 H, CH$_2$(2′) or CH$_2$(7′)); 2.49–2.57 (*m*, OCH$_2$C*H*$_2$CN); 2.77–2.83 (*m*, 1 H, CH$_2$(2′) or CH$_2$(7′)); 3.41–3.75 (*m*, H–C(4′), H–C(5′), OC*H*$_2$CH$_2$CN, 2 Me$_2$C*H*$_2$); 3.77 (*s*, 2 MeO); 4.01–4.11 (*m*, H–C(6′)); 5.83–5.93 (*m*, H–C(1′)); 6.57, 6.71 (2*d*, *J* = 4.8, 5.3, CONH); 6.79–6.83 (*m*, 4 H arom.); 7.18–7.50 (*m*, H–C(6), 9 arom. H); 8.41 (br. *s*, H–N(3)). ^{31}P-NMR (81 MHz, CDCl$_3$): 141.8, 142.4. LSI-MS: 828 (2, $[M+1]^+$), 303 (*100*). Anal. calc. for C$_{44}$H$_{54}$N$_5$O$_9$P: C 63.83, H 6.57, N 8.46; found: C 63.53, H 6.76, N 8.37.

Oligonucleotide Synthesis. Oligonucleotide synthesis was performed on a *Pharmacia Gene Assembler Special* connected to a *Compaq-Pro-Linea-3/25-zs* personal computer. All syntheses were performed using the 1.3-µmol cycle with coupling times of 6–9 min and detritylation times of 60–90 s per unnatural building block. Solvents and solns. were prepared according to the manufacturer's protocol. Phosphoramidite (0.1M in MeCN) and 1*H*-tetrazole (0.45M in MeCN) solns. were equal in concentration to those used for the synthesis of natural oligodeoxynucleotides. For the synthesis of **21** and **22**, the activator 1*H*-tetrazole was replaced by 5-

Table 2. *Synthesis and Analytical Data of Oligonucleotides* **18**–**22**

Sequence (1.3 µmol)	HPLC	Isolated yield *OD*(260 nm) ([%])	MALDI-TOF-MS $[M-H]^-$	
			m/z (calc.)	*m/z* (found)
18 d(TTTTT t$^+$TTTT)	DEAE[a]): 20–35% *B* in 20 min; t_R 16.5 *RP*[b]): 15–22% *B* in 30 min; t_R 11	37 (40)	3020.1	3018.6
19 d(TTTTt$^+$t$^+$TTTT)	DEAE[a]): 20–35% *B* in 20 min; t_R 13 *RP*[b]): 15–22% *B* in 30 min; t_R 9.5	15 (17)	3061.1	3059.7
20 d(t$^+$T t$^+$T t$^+$T t$^+$T t$^+$T)	DEAE[a]): 10–18% *B* in 13 min; t_R 9 *RP*[b]): 10–12% *B* in 30 min; t_R 11.8	31 (33)	3184.3	3185.9
21 d(TTTTTt^{Ac}TTTT)	DEAE[a]): 25–45% *B* in 13 min; t_R 18.1 *RP*[b]): 10–30% *B* in 30 min; t_R 12.5	45 (48)	3061.9	3061.1
22 d(t^{Ac}Tt^{Ac}Tt^{Ac}Tt^{Ac}Tt^{Ac}T)	DEAE[a]): 25–55% *B* in 30 min; t_R 17.8 *RP*[b]): 5–20% *B* in 30 min; t_R 21.5	48 (51)	3394.2	3394.1

[a]) *Nucleogen* DEAE *60-7*, 125 × 4.0 mm (*Macherey & Nagel*); *A:* 20 mM KH$_2$PO$_4$ in H$_2$O/MeCN 4:1, pH 6.0; *B:* *A* + 1M KCl; flow 1 ml/min; detection at 260 nm. [b]) *Aquapore Rp-300* 220 × 4.6 mm, 7 µm (*Brownlee Labs*); *A:* 0.1M (Et$_3$NH)OAc in H$_2$O, pH 7.0; *B:* 0.1M (Et$_3$NH)OAc in H$_2$O/MeCN 1:4, pH 7.0; flow 1 ml/min; detection at 260 nm.

(benzylthio)-1*H*-tetrazole (0.25M in MeCN) [24]. Average coupling yields, monitored by the on-line trityl assay, were in the range of 90–98% for **18** and **20**–**22**. For sequence **19**, the coupling step between the two adjacent trifluoroacetamido-bicyclo-thymidine residues proceeded with only 59% yield. All syntheses were run in the trityl-off mode.

Deprotection and Purification of Oligonucleotides. Removal of the protecting groups and detachment from the solid support was effected in conc. NH_3 soln. (1–2 ml) at r.t. for 13–18 h. The crude oligomers were purified by DEAE ion-exchange HPLC, desalted over *Sep-Pak* (*Waters*), and their purity controlled by reversed-phase chromatography. *Table 2* contains synthetic and anal. data of the oligonucleotides described here. All natural DNA sequences used in this study were prepared according to standard CED- or PAC-phosphoramidite chemistry and purified by HPLC.

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