Aromatic *vs.* Carbohydrate Residues in the Major Groove: Synthesis of 5-[(Benzyloxy)methyl]pyrimidine Nucleosides and Their Incorporation into Oligonucleotides

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Dedicated to Prof. Dr. *Albert Eschenmoser* on the occasion of his 75th birthday

The synthesis of 5-[(benzyloxy)methyl]-substituted pyrimidine 2′-deoxynucleosides **14** and **15** starting from the uracil derivative **6** and tetra-*O*-acetyl-D-ribose is described (*Schemes 1–3*). These nucleosides were converted to the corresponding cyanoethyl phosphoramidites **18** and **19**, respectively, and incorporated into oligodeoxynucleotide decamers. The 5-[(benzyloxy)methyl]-nucleoside building blocks $^{bo}T_d$ and $^{bom}C_d$ (bo = benzyloxy, bom = (benzyloxy)methyl) – shape analogs of the naturally occurring glucosylated nucleosides **1** and **2** (see *Fig. 1*) – lead to weaker binding affinities of oligodeoxynucleotides pairing to DNA as well as RNA complements. The modification is more destabilizing in the case of $^{bo}T_d$ than $^{bom}C_d$. Analysis of the thermodynamics of duplex formation shows that $^{bo}T_d$ and $^{bom}C_d$ incorporation leads to a smaller entropy change in duplex formation that is, however, overcompensated by a less favorable enthalpy term. Molecular-modeling studies suggest that the benzyl groups reside in the major groove which would explain the improved pairing entropy as a result of the exclusion of ordered H_2O.

Introduction. – The development of structural analogs of oligonucleotides has been persued in the last three decades for various reasons [1]. Rarely, however, have such modifications been precedented by nature. This is not too surprising, given the main purpose of nucleic acids to conserve or relay information. As a consequence, the majority of modified nucleosides are found in tRNAs or as secondary metabolites of bacteria and fungi, and only a few unusual components of DNA have been reported [2].

An unusual nucleoside component of genomic DNA has recently been identified in *Trypanosoma brucei*, a single-cell parasite that causes African sleeping sickness [3][4]. About 10% of all thymidines are replaced by β-D-glucosylated 5-(hydroxymethyl)-deoxyuridine **1** (*Fig. 1*). The analogous 2′-deoxycytidine nucleoside **2** has earlier been reported to replace all C_d residues in the DNA of *E. coli* bacteriophages of the T-even series [2].

The glucose moiety of **1** (and presumably **2**) can easily be accommodated within the major groove of double-helical DNA [5]. The glucose shielding the major groove makes oligonucleotides containing **1** or **2** less prone to nuclease degradation [2][6]. This interesting property led us to investigate glycoside-modified nucleosides as constituents of antisense oligonucleotides. We recently found that oligonucleotides containing **1** display decreased affinity towards complementary DNA but a slightly increased affinity towards complementary RNA [7]. Encouraged by these results, we wanted to investigate the possibility of substituting the glucose portion in **1** with

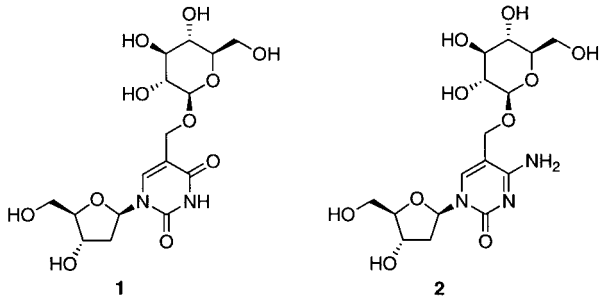


Fig. 1. *Structures of naturally occurring carbohydrate-modified pyrimidine nucleosides* **1** *and* **2**

residues similar in size that are chemically more stable and synthetically more readily accessible.

Pyrimidine nucleosides modified at the 5-position have been evaluated in antisense oligonucleotides in the past. The most notable examples are 5-propynyl-nucleosides such as **3** [8] (*Fig. 2*) or 5-aryl-substituted deoxyuridines such as **4** [9]. Oligodeoxynucleotides containing **3** or **4** display strongly increased affinity towards complementary RNA [9][10]. This has been interpreted in terms of improved π-stacking interactions due to an extended surface of the modified nucleobase. In contrast to these conjugated residues, we wanted to introduce an aromatic ring separated from the pyrimidine nucleus by two or three σ-bonds. For the ease of synthetic accessibility and chemical stability under oligonucleotide-synthesis conditions, we chose to incorporate 5-[(benzyloxy)methyl]substituted pyrimidine nucleosides $^{bo}T_d$ (*Fig. 2*) and its 2′-deoxycytidine analog $^{bom}C_d$ (bo = benzyloxy, bom = (benzyloxy)methyl). Molecular-model building studies suggested that the phenyl ring of $^{bo}T_d$ and $^{bom}C_d$ incorporated in an oligonucleotide duplex would reside flat on the floor of the major groove as does the glucose moiety in the case of **1** [5].

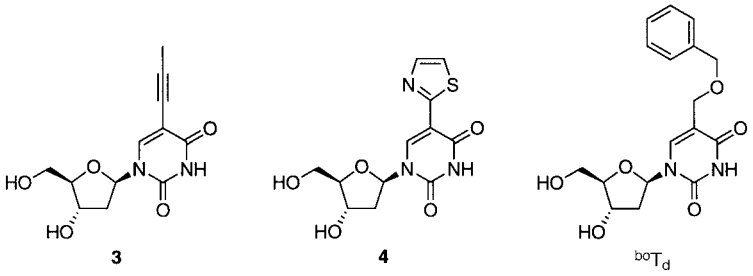


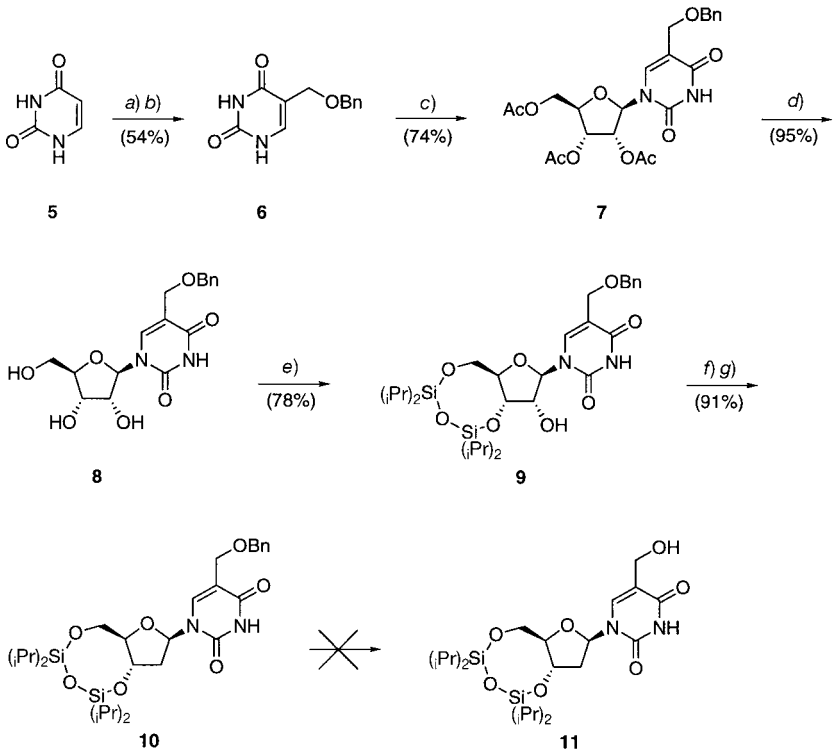
Fig. 2. *Pyrimidine nucleosides modified at the 5-position, which increase the pairing affinity*

Results and Discussion. – *Nucleoside Synthesis.* Most syntheses of 5-(hydroxymethyl)pyrimidine nucleosides and derivatives thereof start with the hydroxymethylation of 2′-deoxyuridine [11]. This reaction is cumbersome because of the low yield and, furthermore, the nucleoside starting material is rather expensive. Hence, for the synthesis of 5-[(benzyloxy)methyl]-modified pyrimidine nucleosides, a strategy was chosen in which the modification was introduced as early as possible, *i.e.*, in the

nucleobase itself. We felt that the 5-[(benzyloxy)methyl] group would be stable under the conditions used in later steps.

Uracil (**5**) was hydroxymethylated according to a known procedure, as was the ensuing benzylation under acid catalysis (*Scheme 1*) [12]. For nucleoside formation, we

Scheme 1. *Synthesis of the 5-[(Benzyloxy)methyl]-2′-deoxyuridine Derivative* **10**



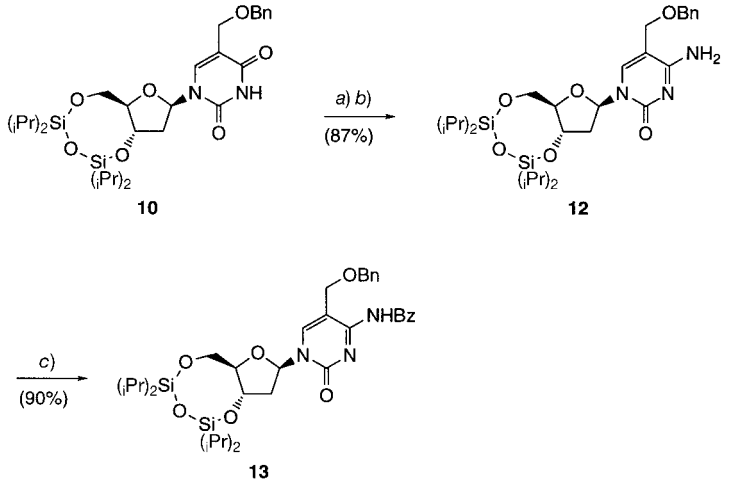
a) Paraformaldehyde, KOH, H_2O, 50°, 3 d. *b*) BnOH, HCl, toluene, 80 → 130°, 3 h. *c*) 1,2,3,5-Tetra-*O*-acetyl-D-ribose, *N*,*O*-bis(trimethylsilyl)acetamide, $CF_3SO_3SiMe_3$, MeCN, r.t., 18 h. *d*) NaOH, THF, MeOH, H_2O, 0°, 35 min. *e*) $(^iPr_2SiCl)_2O$, pyridine, r.t., 18 h. *f*) TolOC(=S)Cl, *N*,*N*-dimethylpyridin-4-amine (DMAP), MeCN, r.t., 18 h. *g*) Bu_3SnH, 2,2′-azobis[isobutyronitrile] (AIBN), toluene, 80°, 2.5 h.

relied on the directing effect of the 2-(acyloxy) group of a D-ribofuranose derivative. Thus, 5-[(benzyloxy)methyl]uracil (**6**) was reacted with tetra-*O*-acetyl-D-ribose under *Vorbrüggen* conditions [13] to give the protected β-configured nucleoside **7** in good yield. After hydrolysis of the ester groups (→ **8**), the 3′- and 5′-OH functions were protected as silyl ethers with *Markiewicz*' reagent to give **9** [14]. The 2′-OH group was then removed by *Barton-McCombie* reduction by a two-step protocol [15]. First, **9** was esterified with *p*-tolyl carbonochloridothioate (= *p*-tolyl chlorothionoformate) to give the corresponding carbonothioate, which was then reacted with tributyltin hydride to give 2′-deoxynucleoside **10** in excellent yield. Attempts to remove the benzyl group in **10** to obtain 2′-deoxy-5-(hydroxymethyl)uridine derivative **11**, which could be used

as a glycoside acceptor in the synthesis of glycosylated nucleoside **1**, were not successful[1]).

Deoxyuridine derivative **10** was subsequently converted to the corresponding cytidine derivative **12** (*Scheme 2*) by adaptation of the method of *Reese* [16]. Treatment of **10** with phosphoryltris(1,2,4-triazolide) generated *in situ* resulted in the formation of a 4-triazolyl-pyrimidine intermediate, which was directly subjected to ammonolysis to give **12** in 87% yield. The amino group of **12** was then protected as a benzamide (→ **13**), the standard protecting group for cytosine nucleosides in oligonucleotide synthesis.

Scheme 2. *Synthesis of Deoxycytidine Derivative* **13**



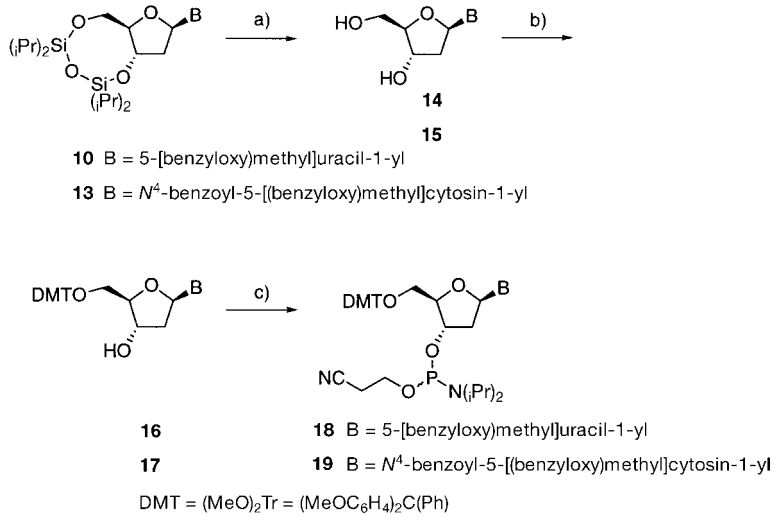
a) 1,2,4-1*H*-Triazole, $POCl_3$, Et_3N, MeCN, r.t., 40 min. *b*) Aq. NH_3 soln., dioxane, r.t., 30 min. *c*) Benzoyl chloride, pyridine, 0° → r.t., 15 h.

The synthetic transformations of (protected) nucleosides **10** and **13** to the corresponding phosphoramidite derivatives **18** and **19** suitable for automated oligonucleotide synthesis are summarized in *Scheme 3*. After desilylation of **10** and **13** by treatment with Bu_4NF, tritylation (→ **16** and **17**, resp.) and phosphitylation to **18** and **19**, respectively, were achieved by standard procedures [18].

Oligonucleotide Synthesis. With phosphoramidites **18** and **19**, the decamers **20**–**24** (*Fig. 3*) containing 5-[(benzyloxy)methyl]-2′-deoxyuridine and 2′-deoxycytidine were synthesized with an automated DNA synthesizer on a 1.3-μmol scale (for details, see *Exper. Part*). Changes to the standard synthesis protocol included the use of 5-(benzylthio)-1*H*-tetrazole instead of 1*H*-tetrazole [19] and a longer reaction time for **18** and **19**. Coupling yields were identical to phosphoramidites of the natural deoxynucleosides as judged from the automated trityl assay. The crude oligonucleo-

[1]) Several hydrogenation catalysts under varying conditions were used in the attempted cleavage of the benzyl ether. In almost all cases, the $C(5)CH_2$–O bond was cleaved, resulting in the formation of a thymidine derivative. Removal of the benzyl group by electrophilic reagents most likely would not succeed due to the lability of the glycosidic bond towards these reagents. The synthesis of **11** was achieved, however, by replacing the benzyl group with the acid-labile dimethoxytrityl group by an otherwise similar strategy [17].

Scheme 3. *Synthesis of 5-[(Benzyloxy)methyl]pyrimidine Phosphoramidites*



a) Bu_4NF, THF, r.t., 30 min. *b*) $(MeO)_2TrCl$, DMAP, pyridine, r.t., 3 h. *c*) $^iPr_2NP(Cl)OCH_2CH_2CN$, $^iPr_2EtN$, THF, r.t., 1 h.

tides **20**–**24** were purified by reversed-phase followed by anion-exchange FPLC (fast protein liquid chromatography). Their purity and identity were subsequently checked by MALDI-TOF mass spectrometry (see *Table 3* in the *Exper. Part*).

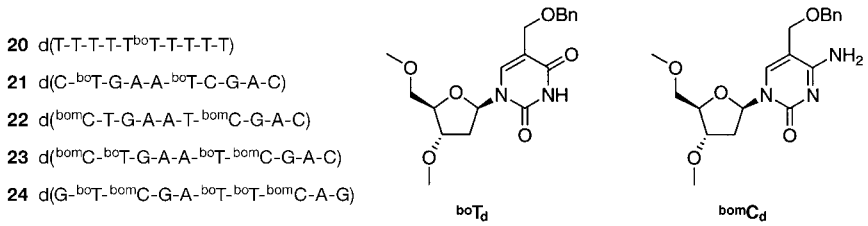


Fig. 3. *Sequences of oligonucleotides* **20**–**24**

Pairing Properties. Pairing properties of oligonucleotides **20**–**24** were obtained from analysis of UV/melting curves in buffer solutions containing 1M NaCl at pH 7.0. T_m data are summarized in *Table 1*.

Introducing a single 5-[(benzyloxy)methyl]-2′-deoxyuridine (= C^5-(benzyloxy)thymidine; $^{bo}T_d$) residue at a central position in the reference duplex $d(T_{10})\cdot d(A_{10})$ (**25**·**26**) leads to a drastic decrease in thermal stability ($\Delta T_m = -7°$; see *Table 1*, **20**·**26**). A decrease is also observed in a mixed sequence context. However, two $^{bo}T_d$ residues within the duplex d(CTGAATCGAC)·d(GTCGATTCAG) (**27**·**28**) decrease the T_m by only 3°/modification (see **21**·**28**). This difference might reflect the peculiar structure of oligo-d(A)·oligo-d(T) duplexes with their wide and flat major groove [21]. Substituting $^{bom}C_d$ for C_d in the mixed sequence (see **22**·**28**) is still better tolerated than the corresponding thymidine modification and diminishes the T_m by 1.8°/modification. Introducing both, two $^{bom}C_d$ and two $^{bo}T_d$, at the same time (see **23**·**28**) results in a ΔT_m

Table 1. T_m *Values for Duplex Formation of Sequences* **20**–**24**[a])[b])

	T_m [°][c])	ΔT_m/ Modification [°]
25·**26** d(T_{10})·d(A_{10})	33.0	–
20·**26** d(T-T-T-T-^{bo}T-T-T-T-T-T)·d(A_{10})	26.2	−6.8
27·**28** d(C-T-G-A-A-T-C-G-A-C)·d(G-T-C-G-A-T-T-C-A-G)	50.8[d])	–
21·**28** d(C-^{bo}T-G-A-G-^{bo}T-C-G-A-C)·d(G-T-C-G-A-T-T-C-A-G)	44.9	−3.0
22·**28** d(^{bom}C-T-G-A-A-T-^{bom}C-G-A-C)·d(G-T-C-G-A-T-T-C-A-G)	47.2	−1.8
23·**28** d(^{bom}C-^{bo}T-G-A-A-^{bo}T-^{bom}C-G-A-C)·d(G-T-C-G-A-T-T-C-A-G)	41.3	−2.4
27·**24** d(C-T-G-A-A-T-C-G-A-C)·d(G-^{bo}T-^{bom}C-G-A-^{bo}T-^{bo}T-^{bom}C-A-G)	33.6	−3.4
23·**24** d(^{bom}C-^{bo}T-G-A-A-^{bo}T-^{bom}C-G-A-C)·d(G-^{bo}T-^{bom}C-G-A-^{bo}T-^{bo}T-^{bom}C-A-G)	29.6	−2.4
27·**29** d(C-T-G-A-A-T-C-G-A-C)·r(G-U-C-G-A-U-U-C-A-G)	41.0[d])	–
23·**29** d(^{bom}C-^{bo}T-G-A-A-^{bo}T-^{bom}C-G-A-C)·r(G-U-C-G-A-U-U-C-A-G)	32.6	−2.1

[a]) $^{bo}T_d$ = C^5-(Benzyloxy)thymidine = 5-[(benzyloxy)methyl]-2′-deoxyuridine; $^{bom}C_d$ = 5-[(benzyloxy)methyl]-2′-deoxycytidine. [b]) Melting profile at 260 nm. Duplex concentration, 4.0 μM; buffer, 1M NaCl, 10 mM Na_2HPO_4, pH 7.0. [c]) T_m Data represent mean values of three melting curves. [d]) Taken from [20].

of −2.4°/modification – exactly the mean value of the individual substitutions. The same value is observed for a duplex with a total of nine modifications in both strands (see **23**·**24**). Finally, a slightly smaller depression of T_m is seen when d(^{bom}C^{bo}T GAA^{bo}T^{bom}C GAC) (**23**) is complexed with a complementary RNA strand (ΔT_m = −2.1°/modification).

These results show – not unexpectedly – that the covalent attachment of a residue similar in size to a monosaccharide also leads to a destabilization of a DNA duplex[2]). One might have expected that such modifications that cover the surface of the major groove would have a stabilizing effect. The displacement of (ordered) H_2O molecules should decrease the loss in entropy upon duplex formation. This is indeed observed when extracting the thermodynamic parameters of duplex formation in the system **23**·**28** (*Table 2*). A linear regression of the *van't Hoff* plot $1/T_m$ *vs.* ln(c) yields a pairing entropy of −160 cal/K·mol, much less than in the unmodified case **27**·**28** (−214 cal/K·mol). However, the more favorable entropic term is accompanied by a decrease in pairing enthalpy in the 5-[(benzyloxy)methyl] system resulting in an overall thermodynamically less stable duplex.

Table 2. *Thermodynamic Parameters for Duplex Formation of Oligonucleotides d(CTGAATCGAC)* (**27**) *and d(*^{bom}*C*^{Bo}*T GAA*^{bo}*T*^{bom}*CGAC)* (**23**) *with d(GTCGATTCAG)* (**28**)[a])[b])

	ΔH^0 [kcal/mol]	ΔS^0 [cal/K·mol]	ΔG^0 (25°) [kcal/mol]
27·**28** d(CTGAATCGAC)·d(GTCGATTCAG)	−77.6[c])	−214[c])	−13.8
23·**28** d(^{bom}C^{bo}TGAA^{bo}T^{bom}CGAC)·d(GTCGATTCAG)	−58.3	−160	−10.6

[a]) For $^{bo}T_d$ and $^{bom}C_d$, see *Table 1*. [b]) Duplex concentration, 1.0–40 μM; buffer, 1M NaCl, 10 mM Na_2HPO_4, pH 7.0. [c]) Taken from [20].

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[2]) Introduction of glucosylated hydroxymethyl-U_d **1** in the reference duplex **27**·**28** at the same positions as the $^{bo}T_d$ modification in **21** leads to a ΔT_m of −1.0°/modification [7]. The fact that the destabilization in the case of nucleoside **1** is less severe than with $^{bo}T_d$ or $^{bom}C_d$ indicates that the glucose residue is capable of forming additional stabilizing H-bonding contacts (most likely to the *Hoogsteen* face of the nucleobases).

Molecular-Dynamics Simulation. To better understand the structural factors leading to the observed destabilizing effect of the 5-[(benzyloxy)methyl]residue, molecular-dynamics calculations of the duplex **21**·**28** in comparison with the corresponding unmodified duplex **27**·**28** were carried out (for details, see *Exper. Part*). The average structure of the last 50 ps of a 200-ps dynamics run of **21**·**28** are shown in *Fig. 4*. According to the simulation, the benzyl groups reside in the major groove shielding the central base pairs. Overall, the structures of **21**·**28** and the parent **27**·**28** resemble each other very closely. They are characterized by a narrow minor groove with concomitant widening of the major groove in the region of the GAA tract which is a known characteristic of oligo-d(A)·oligo-d(T) sequences [21]. The major groove of the modified duplex is widened even more than the unmodified one – a likely consequence of dangling motions of the phenyl moieties. This structural distortion could account for the decreased enthalpic stability since an unwinding of the duplex (due to the widening of the major groove) will affect the H-bonding and stacking geometries.

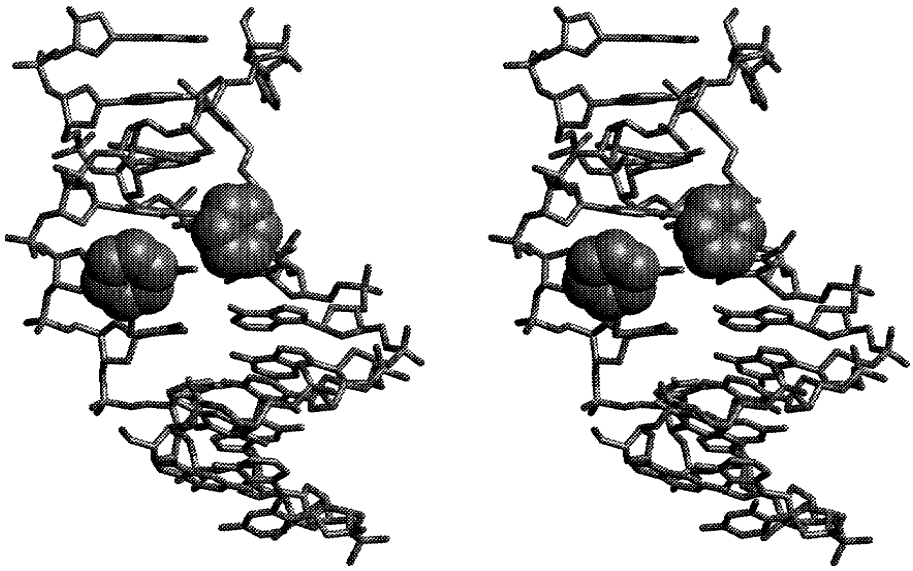

Fig. 4. *Stereoview of the duplex* **21**·**28** *in the molecular-dynamics simulation* (average over last 50 ps)

An interesting observation from the dynamics calculation is the observed trend of the (benzyloxy)methyl group of the deoxyuridine in position 6 of **21** to fold towards the middle of the duplex. In the initial structure, the benzyl group was pointing towards the 3′-end of **21** in the duplex. During the simulation, it readily adopted a different conformation as can be seen from the evolution of the corresponding torsion angle over time (*Fig. 5*). This behavior might actually reflect part of the observed thermodynamic data. It is likely that the H_2O molecules in the middle of the duplex are better ordered than towards the ends and hence a greater entropic gain could be achieved.

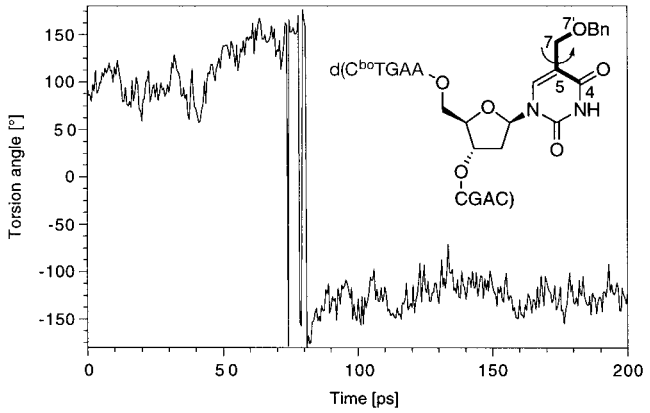

Fig. 5. *Time evolution of the torsion angle indicated in the molecular-dynamics simulation of duplex* **21** · **28**

The presumed widening of the major groove due to the presence of the 5-(benzyloxy)methyl groups seems to stand in contrast to the observation that the modification of a DNA · RNA duplex **23** · **29** is less destabilizing than in the DNA case (ΔT_m/modification = 2.1 *vs.* 2.4°). However, this might be related to the fact that RNA or DNA · RNA duplexes are conformationally more rigid than an all DNA duplex [21].

Conclusion. – The above results highlight the importance of several factors for the development of antisense agents with modified pyrimidine bases. The introduction of uncharged entities covering the major groove *via* flexible linkers leads to a decrease in pairing affinity. The anticipated beneficial effect on the pairing entropy by the displacement of ordered H_2O is overcompensated for by a negative influence on the pairing enthalpy. Most likely, the dangling motions of such residues weaken the H-bonding strength of the base pairs to which they are attached. This is evident from the fact that the modification of thymidine (two H-bonds) is less favorable than in that of 2′-deoxycytine (three H-bonds).

The negative effect of dangling substituents in the 5-position of pyrimidine bases on the enthalpy of duplex formation might be counterbalanced by anchoring these residues by additional attractive forces. For example, positively charged groups might lead to salt bridges between such residues and the negatively charged phosphodiester groups lined up along the rim of the major groove. In fact, we are currently investigating the pairing properties of oligonucleotides containing an analog of **1** in which the glucose OH groups in positions 2 and 6 are replaced with ammonium functions [17]. This modification strongly enhances duplex stability (ΔT_m/modification = 3–4°). A change in the electronic properties of aromatic residues positioned in the major groove might also be beneficial. The phenyl moieties in *Fig. 4* are covering two A · T base pairs from the *Hoogsteen* face. It is likely that such an interaction is stabilized by H-bonding between the exocyclic amino group of adenine (or cytosine) and the π-electron face of the aromatic rings. This interaction might be enhanced by substituting the phenyl moiety with electron-donating groups. On the other hand, extending the surface of such aromatic residues could also lead to intercalation.

Nevertheless, a combination of aromatic residues and anchoring groups such as aminoglycosides might lead to much improved binding and eventually to the development of unprecedented major-groove binders.

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Experimental Part

General. All reactions were carried out under Ar. Solvents for extraction: technical grade, distilled. Solvents for reactions: reagent grade, distilled from CaH_2 (MeCN, pyridine) or Na (THF). All reagents were purchased from *Fluka AG*, highest quality available, except 2-cyanoethyl diisopropylphosphoramidochloridite, which was purchased from *Aldrich Co*. Flash chromatography (FC): silica gel, particle size 40–63 μm (*Fluka*). TLC: *Macherey-Nagel SIL G-25 UV_{254}* plates. Optical rotation: *Perkin-Elmer-241* polarimeter, 10-cm cell. UV Spectra: *Perkin-Elmer Lambda Bio*; λ_{max} in nm (ε). IR Spectra: *Perkin-Elmer FTIR 1600*, $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker AC-300*, *DRX-400*, or *DRX-500*; δ in ppm, J in Hz; calibration to residual solvent peak; ^{13}C multiplicities from DEPT spectra; ^{31}P calibration to external H_3PO_4 ($\delta = 0$ ppm). MS: *Micromass Autospec O*, Cs^+-beam, 25 keV; matrix dithiothreitol/dithioerythritol 5:1. HR-MS: LSI-MS peak-matching with PEG-600 as internal standard.

2′,3′,5′-Tri-O-*acetyl-5-[(benzyloxy)methyl]uridine* (**7**). To a suspension of **6** [12] (3.20 g, 13.8 mmol) and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (5.70 g, 17.9 mmol) in abs. MeCN (68 ml), *N*,*O*-bis(trimethylsilyl)acetamide (6.74 ml, 27.6 mmol) was added under Ar. After stirring for 2 h at r.t., trimethylsilyl trifluoromethanesulfonate (4.98 ml, 27.6 mmol) was added. The soln. was stirred for another 18 h at r.t., then diluted with $CHCl_3$ (250 ml), and washed with H_2O (140 ml) and sat. $NaHCO_3$ soln. (2 × 140 ml). The aq. layers were re-extracted with $CHCl_3$ (140 ml). The combined org. phase was dried (Na_2SO_4) and evaporated and the remaining oil submitted to FC (silica gel (340 g), hexane/AcOEt 1:2): **7** (5.00 g, 74%). Colorless foam. R_f 0.29 (hexane/AcOEt 1:2). $[\alpha]_D^{25} = -32.1$ ($c = 0.92$, $CHCl_3$). UV (EtOH). 262 (7980). IR ($CHCl_3$): 3387*w*, 2955*w*, 2866*w*, 1751*s*, 1716*s*, 1694*s*, 1497*w*, 1468*m*, 1373*m*, 1097*m*, 1073*m*, 1049*m*, 941*w*, 903*w*, 868*w*. ^1H-NMR (500 MHz, $CDCl_3$): 9.55 (*s*, H–N(3)); 7.46 (*s*, H–C(6)); 7.24–7.33 (*m*, 5 arom. H); 6.08–6.11 (*m*, H–C(1′)); 5.30–5.33 (*m*, H–C(2′), H–C(3′)); 4.56 (*s*, $PhCH_2$); 4.24–4.33 (*m*, 2 H–C(5′), H–C(4′), CH_2–C(5)); 2.10, 2.06, 1.96 (3*s*, 3 Ac). ^{13}C-NMR (125 MHz, $CDCl_3$): 170.18, 169.59, 169.56 (3*s*, 3 Me*C*O); 162.18 (*s*, C(4)); 150.26 (*s*, C(2)); 137.40 (*s*, arom. C); 136.20 (*d*, C(6)); 128.46, 127.90, 127.81 (3*d*, arom. C); 113.10 (*s*, C(5)); 86.88 (*d*, C(1′)); 80.08 (*d*, C(4′)); 73.13 (*t*, Ph*C*H_2); 72.50 (*d*, C(2′)); 70.48 (*d*, C(3′)); 64.24 (*t*, *C*H_2–C(5)); 63.19 (*t*, C(5′)); 20.46, 20.38, 20.33 (3*q*, 3 *Me*CO). LSI-MS: 491 (9, M^+), 383 (21), 260 (12), *259* (100), 139 (17). HR-MS: 491.1666 ($C_{23}H_{27}N_2O_{10}^+$; calc. 491.1620).

5-[(Benzyloxy)methyl]uridine (**8**). To a soln. of **7** (5.01 g, 10.2 mmol) in THF/MeOH/H_2O 5:4:1 (409 ml) at 0°, 2M aq. NaOH (41 ml) was added. After stirring for 35 min at 0°, the reaction was quenched by addition of NH_4Cl (5.25 g). The suspension was stirred for another 20 min and then evaporated. The residue was purified by FC (silica gel (220 g), CH_2Cl_2/MeOH 9:1): **8** (3.52 g,95%). Colorless foam. R_f 0.22 (CH_2Cl_2/MeOH 9:1). $[\alpha]_D^{25} = -13.3$ ($c = 1.01$, H_2O). UV (EtOH): 264 (9990). IR (KBr): 3398*s*, 3061*m*, 2926*m*, 2867*m*, 1682*s*, 1471*s*, 1399*m*, 1275*m*, 1209*m*, 1177*w*, 1099*s*, 1061*s*, 910*w*, 863*w*, 785*w*, 743*m*, 700*m*, 668*w*, 585*m*. ^1H-NMR (300 MHz, (D_6)DMSO): 11.40 (*s*, H–N(3)); 8.00 (*s*, H–C(6)); 7.24–7.36 (*m*, 5 arom. H); 5.79 (*d*, $J = 5.5$, H–C(1′)); 5.39 (*d*, $J = 5.5$, OH–C(2′)); 5.08–5.14 (*m*, OH–C(3′), OH–C(5′)); 4.48 (*s*, $PhCH_2$); 4.18 (*d*, $J = 11.8$, 1 H, CH_2–C(5)); 4.13 (*d*, $J = 11.8$, 1 H, CH_2–C(5)); 4.04 (*dd*, $J = 5.0$, 10.1, H–C(2′)); 3.98 (*m*, H–C(3′)); 3.85 (*dd*, $J = 3.3$, 7.0, H–C(4′)); 3.61–3.68 (*m*, 1 H–C(5′)); 3.52–3.58 (*m*, 1 H–C(5′)). ^{13}C-NMR (75 MHz, (D_6)DMSO): 162.92 (*s*, C(4)); 150.83 (*s*, C(2)); 139.65 (*s*, C(6)); 138.61 (*s*, arom. C); 128.43, 127.65, 127.57 (3*d*, arom. C); 110.72 (*s*, C(5)); 87.95 (*d*, C(1′)); 85.05 (*d*, C(4′)); 73.75 (*d*, C(2′)); 71.62 (*t*, Ph*C*H_2); 70.04 (*d*, C(3′)); 64.59 (*t*, *C*H_2–C(5)); 61.05 (*t*, C(5′)). LSI-MS: 365 (35, MH^+), 307 (24), 289 (11), 287 (15), 257 (22), 149 (29), 139 (12), 138 (24), 137 (71), 125 (22), 124 (15), 120 (13), 115 (10), 107 (21). HR-MS: 365.1348 ($C_{17}H_{21}N_2O_7^+$; calc. 365.1348).

5-[(Benzyloxy)methyl]-3′,5′-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine* (**9**). At r.t., 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (2.95 ml, 9.43 mmol) was added to a soln. of **8** (2.86 g, 7.86 mmol) in abs. pyridine (78.6 ml). After 16 h stirring, additional 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (0.24 ml, 0.79 mmol) was added, and stirring was continued for 2 h. The soln. was diluted with CH_2Cl_2 (300 ml) and washed with H_2O (150 ml) and sat. $NaHCO_3$ soln. (150 ml). The aq. layer was extracted again with CH_2Cl_2

(150 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the remaining oil purified by FC (silica gel (250 g), hexane/AcOEt 1:1): **9** (3.72 g, 78%). Colorless foam. R_f 0.24 (hexane/AcOEt 1:1). $[\alpha]_D^{25} = -24.0$ ($c = 0.93$, $CHCl_3$). UV (EtOH): 268 (6400). IR (KBr): 3429*w*, 3192*w*, 3064*w*, 2945*s*, 2867*s*, 1694*s*, 1464*s*, 1387*m*, 1334*w*, 1270*m*, 1209*m*, 1161*m*, 1122*s*, 1061*s*, 1038*s*, 995*m*, 904*m*, 885*m*, 863*m*, 777*m*, 735*w*, 696*m*, 587*m*. ^{1}H-NMR (500 MHz, $CDCl_3$): 9.61 (*s*, H–N(3)); 7.61 (*s*, H–C(6)); 7.24–7.34 (*m*, 5 arom. H); 5.64 (*s*, H–C(1′)); 4.56 (*s*, PhCH_2); 4.40 (*dd*, $J = 5.3$, 8.2, H–C(3′)); 4.30 (*d*, $J = 12.4$, 1 H, CH_2–C(5)); 4.22 (*m*, H–C(2′)); 4.23 (*d*, $J =$ 12.6, 1 H, CH_2–C(5)); 4.12 (*dd*, $J = 3.2$, 12.8, 1 H, H–C(5′)); 4.06 (*dt*, $J = 3.1$, 8.2, H–C(4′)); 3.99 (*dd*, $J = 2.8$, 12.7, 1 H, H–C(5′)); 3.21 (br. *s*, OH–C(2′)); 0.90–1.09 (*m*, 28 H, ^{i}Pr). ^{13}C-NMR (125 MHz, $CDCl_3$): 162.77 (*s*, C(4)); 150.00 (*s*, C(2)); 138.45 (*d*, C(6)); 137.81 (*s*, arom. C); 128.32, 127.69, 127.63 (3*d*, arom. C); 111.61 (*s*, C(5)); 91.91 (*d*, C(1′)); 82.11 (*d*, C(4′)); 74.66 (*d*, C(2′)); 72.93 (*t*, Ph*C*H_2); 69.62 (*d*, C(3′)); 64.38 (*t*, *C*H_2–C(5)); 60.89 (*t*, C(5′)); 17.36, 17.21, 17.20, 17.15, 17.05, 16.94, 16.92, 16.82 (8*q*, 4 *Me*$_2$CH); 13.31, 12.92, 12.58, 12.46 (4*d*, 4 Me$_2$C*H*). LSI-MS: 607 (10, *M*H$^+$), 499 (62), 375 (17), 357 (10), 315 (14), 287 (20), *261* (100), 233 (16), 217 (12), 191 (10), 175 (15), 161 (12), 147 (18), 133 (28), 119 (28). Anal. calc. for $C_{29}H_{46}N_2O_8Si_2$ (606.87): C 57.40, H 7.64, N 4.62; found: C 57.43, H 7.37, N 4.61.

5-[(Benzyloxy)methyl]-2′-deoxy-3′,5′-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine* (**10**). *p*-Tolyl carbonochloridothioate (0.79 ml, 5.10 mmol) was added to a soln. of **9** (2.81 g, 4.64 mmol) and DMAP (1.13 g, 9.28 mmol) in dry MeCN (46.4 ml). After stirring for 18 h at r.t., the mixture was diluted with CH_2Cl_2 (240 ml) and washed with cold 1M aq. HCl (120 ml) and sat. $NaHCO_3$ soln. (2×120 ml). The aq. layers were re-extracted with CH_2Cl_2 (120 ml). The combined org. phase was dried (Na_2SO_4) and evaporated, and the resulting foam dried *in vacuo*. A soln. of the residue, AIBN (38 mg), and Bu_3SnH (1.84 ml, 6.96 mmol) in toluene (46.4 ml) was then heated for 2.5 h at 80°. After evaporation, the residue was purified by FC (silica gel (250 g), hexane/AcOEt 3:2): **10** (2.50 g, 91%). Colorless foam. R_f 0.31 (hexane/AcOEt 3:2). $[\alpha]_D^{25} = -12.2$ ($c = 1.07$, $CHCl_3$). UV (EtOH): 264 (10150). IR ($CHCl_3$): 3391*w*, 2948*m*, 2894*m*, 2869*m*, 1690*s*, 1496*w*, 1465*s*, 1388*w*, 1365*w*, 1333*w*, 1314*w*, 1271*m*, 1176*w*, 1146*m*, 1117*s*, 1093*m*, 1040*s*, 1012*m*, 965*w*, 919*w*, 887*m*, 864*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.90 (*s*, H–N(3)); 7.59 (*s*, H–C(6)); 7.25–7.35 (*m*, 5 arom. H); 6.05 (*dd*, $J = 2.9$, 7.4, H–C(1′)); 4.58 (*s*, 2 PhCH_2); 4.47 (*q*, $J = 7.7$, 16.2, H–C(3′)); 4.29 (*d*, $J = 12.5$, 1 H, CH_2–C(5)); 4.24 (*d*, $J = 12.1$, 1 H, CH_2–C(5)); 4.02 (*m*, 2 H–C(5′)); 3.76 (*dt*, $J = 3.4$, 7.5, H–C(4′)); 2.47 (*dt*, $J = 6.8$, 15.7, 1 H–C(2′)); 2.26 (*ddd*, $J = 2.9$, 7.7, 13.6, 1 H–C(2′)); 0.97–1.06 (*m*, 28 H, ^{i}Pr). ^{13}C-NMR (75 MHz, $CDCl_3$): 162.62 (*s*, C(4)); 149.98 (*s*, C(2)); 137.88 (*s*, arom. C); 137.69 (*d*, C(6)); 128.36, 127.72, 127.67 (3*d*, arom. C); 111.66 (*s*, C(5)); 85.11 (*d*, C(1′)); 84.20 (*d*, C(4′)); 72.99 (*t*, Ph*C*H_2); 68.88 (*d*, C(3′)); 64.51 (*t*, *C*H_2–C(5)); 61.07 (*t*, C(5′)); 39.88 (*t*, C(2′)); 17.43, 17.32, 17.26, 17.13, 17.00, 16.93, 16.84 (7*q*, 4 *Me*$_2$CH); 14.00, 13.03, 12.73, 12.47 (4*d*, 4 Me$_2$C*H*). LSI-MS: 591 (8, *M*H$^+$), 483 (20), 360 (11), 359 (65), 315 (23), 289 (13), 288 (10), 287 (70), 262 (13), *261* (1009), 235 (14), 233 (14), 217 (13), 205 (40), 191 (10), 175 (31), 161 (11), 147 (34), 135 (29), 133 (22), 125 (13), 121 (11), 119 (44), 105 (18). HR-MS: 591.2922 ($C_{29}H_{47}N_2O_7Si_2^+$; calc. 591.2910).

5-[(Benzyloxy)methyl]-2′-deoxy-3′,5′-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine* (**12**). $POCl_3$ (1.39 ml, 2.32 g, 15.13 mmol) and Et_3N (10.3 ml, 7.44 g, 73.51 mmol) were added dropwise to an ice-cooled suspension of 1,2,4-1*H*-triazole (4.48 g, 64.9 mmol) in MeCN (52 ml). After stirring for 1 h at 0°, a soln. of **10** (2.56 g, 4.32 mmol) in MeCN (17 ml) was added. The ice bath was removed, and stirring was continued for 40 min at r.t. The mixture was then diluted with AcOEt (200 ml) and washed with H_2O (100 ml) and sat. aq. $NaHCO_3$ soln. (2×100 ml). The aq. layers were reextracted with AcOEt (100 ml). The combined org. layer was dried (Na_2SO_4) and evaporated and the residue dried under high vacuum. To the resulting yellowish foam in dioxane (43 ml), conc. aq. ammonia (8.6 ml) was added, the soln. stirred for 30 min at r.t. and then evaporated, and the remaining oil purified by FC (180 g, SiO_2, CH_2Cl_2/MeOH 94:6): 2.23 g (87%) **12**. Colorless foam. R_f 0.38 (CH_2Cl_2/MeOH 94:6). $[\alpha]_D^{20} = +20.1$ ($c = 0.95$, $CHCl_3$). UV (EtOH): 240 (sh, 6750), 276 (7380). IR (KBr): 3444*m* (br.), 3338*m* (br.), 3176*m* (br.), 3065*m*, 3031*m*, 2945*s*, 2893*s*, 2867*s*, 2726*w*, 1674*s*, 1514*s*, 1494*s*, 1464*s*, 1410*m*, 1387*m*, 1362*m*, 1313*m*, 1294*m*, 1248*m*, 1174*m*, 1144*s*, 1117*s*, 1090*s*, 1039*s*, 991*s*, 960*m*, 919*m*, 887*s*, 788*m*, 736*m*, 697*s*, 600*m*, 548*m*, 521*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.73 (*s*, H–C(6)); 7.30–7.41 (*m*, 5 arom. H); 6.06 (*dd*, $J = 1.7$, 7.2, H–C(1′)); 6.03 (br. *s*, NH_2–C(4)); 4.52 (*d*, $J = 11.4$, 1 H, PhCH_2); 4.47 (*d*, $J = 11.4$, 1 H, PhCH_2); 4.33–4.42 (*m*, H–C(3′)); 4.37 (*d*, $J = 12.1$, 1 H, CH_2–C(5)); 4.28 (*d*, $J = 12.1$, 1 H, CH_2–C(5)); 4.16 (*dd*, $J = 1.8$, 13.2, 1 H–C(5′)); 4.02 (*dd*, $J = 2.9$, 13.2, 1 H–C(5′)); 3.79 (*dt*, $J = 2.4$, 8.5, H–C(4′)); 2.53 (*ddd*, $J =$ 7.3, 10.3, 13.2, 1 H–C(2′)); 2.33 (*ddd*, $J = 1.7$, 7.2, 13.2, 1 H–C(2′)); 0.90–1.11 (*m*, 28 H, ^{i}Pr). ^{13}C-NMR (75 MHz, $CDCl_3$): 165.41 (*s*, C(4)); 155.54 (*s*, C(2)); 139.68 (*d*, C(6)); 136.95 (*s*, arom. C); 128.63, 128.13, 128.05 (3*d*, arom. C); 101.62 (*s*, C(5)); 84.92, 84.84 (2*d*, C(1′), C(4′)); 71.88 (*t*, Ph*C*H_2); 67.30 (*t*, *C*H_2–C(5)); 66.96 (*d*, C(3′)); 60.02 (*t*, C(5′)); 40.00 (*t*, C(2′)); 17.49, 17.45, 17.39, 17.28, 17.06, 16.98, 16.85 (7*q*, 4 *Me*$_2$CH); 13.43, 12.97, 12.73, 12.40 (4*d*, 4 Me$_2$C*H*). LSI-MS: 590 (5, *M*H$^+$), 287 (5), 261 (5), 233 (16), *232* (100), 147 (5), 135 (5), 124 (17).

N⁴-*Benzoyl-5-[(benzyloxy)methyl]-2′-deoxy-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine* (**13**). To a stirred ice-cold soln. of **12** (2.04 g, 3.45 mmol) in pyridine (17 ml), 0.48 ml (582 mg, 4.14 mmol) benzoyl chloride was added. Stirring was continued for 3 h at 0° and then 12 h at r.t. The mixture was diluted with CH_2Cl_2 (200 ml) and extracted with H_2O (100 ml) and sat. aq. $NaHCO_3$ soln. (2 × 100 ml). After washing the aq. phases with 100 ml CH_2Cl_2, the combined org. layer was dried (Na_2SO_4) and evaporated and the remaining oil twice co-evaporated with toluene (50 ml). FC (75 g SiO_2, hexane/AcOEt 3:1) yielded 2.14 g (90%) of **13**. Colorless gum. R_f 0.36 (hexane/AcOEt 3:1). $[\alpha]_D^{20} = +42.5$ ($c = 1.15$, $CHCl_3$). UV (EtOH): 258 (10600), 325 (21090). IR ($CHCl_3$): 3068*w*, 2948*m*, 2894*w*, 2869*m*, 1706*s*, 1646*m*, 1598*m*, 1570*s*, 1488*m*, 1465*m*, 1449*m*, 1388*w*, 1366*m*, 1334*m*, 1312*m*, 1276*s*, 1172*m*, 1146*m*, 1117*s*, 1084*m*, 1068*m*, 1042*s*, 1013*m*, 992*m*, 963*w*, 919*w*, 886*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 13.39 (br. *s*, NH–C(4)); 8.20–8.25 (*m*, 2 arom. H); 7.90 (*s*, H–C(6)); 7.51–7.57 (*m*, 1 arom. H); 7.29–7.46 (*m*, 7 arom. H); 6.07 (*dd*, $J = 2.6$, 7.4, H–C(1′)); 4.70 (*s*, PhCH_2); 4.47–4.61 (*m*, H–C(3′), CH_2–C(5)); 4.12 (*dd*, $J = 3.7$, 12.7, 1 H–C(5′)); 4.06 (*dd*, $J = 3.1$, 12.7, 1 H–C(5′)); 3.84 (*dt*, $J = 3.4$, 7.4, H–C(4′)); 2.56 (*ddd*, $J = 7.4$, 9.2, 13.6, 1 H–C(2′)); 2.36 (*ddd*, $J = 2.6$, 7.7, 13.6, 1 H–C(2)); 0.95–1.13 (*m*, 28 H, ^{i}Pr). ^{13}C-NMR (75 MHz, $CDCl_3$): 158.67 (*s*, C(4)); 146.71 (*s*, C(2)); 138.70 (*d*, C(6)); 137.87, 136.87 (2*s*, arom. C); 132.47, 129.79, 128.47, 128.14, 127.80, 127.74 (6*d*, arom. C); 111.73 (*s*, C(5)); 85.39, 85.09 (2*d*, C(1′), C(4′)); 73.18 (*t*, Ph*C*H$_2$); 68.40 (*d*, C(3′)); 65.16 (*t*, *C*H$_2$–C(5)); 60.83 (*t*, C(5′)); 39.99 (*t*, C(2′)); 17.49, 17.37, 17.32, 17.15, 17.02, 16.98, 16.88 (7*q*, 4 *Me*$_2$CH); 13.43, 13.05, 12.75, 12.47 (4*d*, 4 Me$_2$*C*H). LSI-MS: 694 (7, *M*H$^+$), 337 (25), *336* (100), 287 (14), 261 (12), 232 (11), 229 (15), 228 (60), 175 (15), 147 (19), 135 (15), 119 (20), 105 (82). Anal. calc. for $C_{36}H_{51}N_3O_7Si_2$ (693.99): C 62.31, H 7.41, N 6.05; found: C 62.05, H 7.50, N 5.98.

General Procedure for the Deprotection of Nucleosides **10** *and* **13**. To a stirred 0.1M soln. of **10** or **13** in THF, solid $Bu_4NF \cdot 3\ H_2O$ was added. After stirring for 30 min at r.t., the solvent was evaporated and the remaining oil subjected to FC with the solvent system indicated: **14** (95%) as colorless foam and **15** (91%, after recrystallization from MeOH), resp.

5-[(Benzyloxy)methyl]-2′-deoxyuridine (**14**). R_f 0.23 (CH_2Cl_2/MeOH 37:3). $[\alpha]_D^{20} = +11.7$ ($c = 1.17$, EtOH). UV (EtOH): 265 (9570). IR (KBr): 3416*s* (br.), 3060*m*, 2930*m*, 2868*m*, 1682*s*, 1473*s*, 1404*m*, 1370*m*, 1277*s*, 1200*m*, 1094*s*, 1057*s*, 995*m*, 955*m*, 918*m*, 874*m*, 786*m*, 742*m*, 699*m*, 608*m*, 574*m*. ^{1}H-NMR (300 MHz, (D_6)DMSO): 11.38 (br. *s*, H–N(3)); 7.92 (*s*, H–C(6)); 7.22–7.36 (*m*, 5 arom. H); 6.15 (*t*, $J = 6.8$, H–C(1′)); 5.29 (*d*, $J = 4.4$, OH–C(3′)); 5.06 (*t*, $J = 5.0$, OH–C(5′)); 4.48 (*t*, $J = 12.7$, PhCH_2); 4.21–4.26 (*m*, H–C(3′)); 4.16 (*s*, CH_2–C(5)); 3.78 (*q*, $J = 3.6$, H–C(4′)); 3.50–3.62 (*m*, 2 H–C(5′)); 2.05–2.11 (*m*, 2 H–C(2′)). ^{13}C-NMR (75 MHz, (D_6)DMSO): 163.02 (*s*, C(4)); 150.59 (*s*, C(2)); 139.39 (*d*, C(6)); 138.69 (*s*, arom. C); 128.51, 127.70, 127.65 (3*d*, arom. C); 110.82 (*s*, C(5)); 87.69 (*d*, C(4′)); 84.50 (*d*, C(1′)); 71.66 (*t*, Ph*C*H$_2$); 70.66 (*d*, C(3′)); 64.67 (*t*, *C*H$_2$–C(5)); 61.56 (*t*, C(5′)); 39.98 (*t*, C(2′)). LSI-MS: 349 (6, *M*H$^+$), 242 (33), 228 (34), 187 (13), *186* (100), 125 (19), 117 (28). Anal. calc. for $C_{17}H_{20}N_2O_6$ (348.36): C 58.61, H 5.79, N 8.04; found: C 58.31, H 5.96, N 7.84.

N⁴-*Benzoyl-5-[(benzyloxy)methyl]-2′-deoxycytidine* (**15**). M.p. 181°. R_f 0.14 (CH_2Cl_2/MeOH 19:1). $[\alpha]_D^{25} = +59.2$ ($c = 1.08$, DMSO): UV (EtOH): 258 (10940), 326 (24180). IR (KBr): 3519*m*, 3407*m*, 3057*m*, 3007*w*, 2880*w*, 1690*s*, 1644*m*, 1591*m*, 1564*s*, 1487*m*, 1448*m*, 1386*m*, 1354*m*, 1332*m*, 1311*m*, 1281*s*, 1242*m*, 1228*m*, 1195*m*, 1171*m*, 1120*m*, 1092*s*, 1067*m*, 1042*m*, 1026*m*, 1000*w*, 959*m*, 938*w*, 868*w*, 856*w*, 827*w*, 810*w*, 796*m*, 758*m*, 741*m*, 716*m*, 698*m*, 683*w*, 678*m*, 668*w*, 594*w*, 574*w*, 532*w*. ^{1}H-NMR (300 MHz, (D_6)DMSO): 12.95 (br. *s*, NH–C(4)); 8.27 (*s*, H–C(6)); 8.07 (br. *s*, 2 arom. H); 7.54–7.60 (*m*, 1 arom. H); 7.43–7.48 (*m*, 2 arom. H); 7.23–7.33 (*m*, 5 arom. H); 6.15 (*t*, $J = 6.4$, H–C(1′)); 5.35 (*d*, $J = 4.4$, OH–C(3′)); 5.15 (*t*, $J = 5.0$, OH–C(5′)); 4.56 (*s*, PhCH_2); 4.43 (*s*, CH_2–C(5)); 4.24–4.30 (*m*, H–C(3′)); 3.86 (*q*, $J = 3.6$, H–C(4′)); 3.56–3.71 (*m*, 2 H–C(5′)); 2.11–2.29 (*m*, 2 H–C(2′)). ^{13}C-NMR (100 MHz, (D_6)DMSO): 178.21 (br. *s*, Ph*C*O); 158.20 (br. *s*, C(4)); 147.57 (br. *s*, C(2)); 141.08 (br. *d*, C(6)); 138.54 (*s*, arom. C); 136.70 (br. *s*, arom. C); 132.75, 129.46, 128.52, 128.42, 128.22, 127.75, 127.63 (7*d*, arom. C); 110.92 (br. *s*, C(5)); 88.07 (*d*, C(4′)); 85.69 (*d*, C(1′)); 71.87 (*t*, Ph*C*H$_2$); 70.21 (*d*, C(3′)); 64.77 (*t*, *C*H$_2$–C(5)); 61.19 (*t*, C(5′)); 40.34 (*t*, C(2′)). LSI-MS: 470 (11), 469 (47), 468 (23), *467* (100), 452 (5, *M*H$^+$), 336 (4), 265 (25), 203 (53). Anal. calc. for $C_{24}H_{25}N_3O_6$ (451.48): C 63.85, H 5.58, N 9.31; found: C 64.08, H 5.55, N 9.21.

(MeO)$_2$*Tr-Protected Nucleosides* **16** *and* **17**: *General Procedure.* To a 0.1M soln. of nucleoside **14** or **15** in pyridine, DMAP (10 mol-%) was added followed by 4,4′-dimethoxytrityl chloride (1.2 mol-equiv.). The soln. was stirred for 3 h at r.t. The mixture was then diluted with CH_2Cl_2 (100 ml) and washed with sat. aq. $NaHCO_3$ soln. (2 × 50 ml). The aq. layers were reextracted with CH_2Cl_2 (50 ml). The combined org. phase was dried (Na_2SO_4) and evaporated and the residue co-evaporated twice with toluene (25 ml) and then purified by FC with the solvent systems indicated: **16** (93%) and **17** (88%), resp., both as yellowish foam.

*5-[(Benzyloxy)methyl]-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)uridine* (**16**). R_f 0.13 (hexane/AcOEt 1:2). $[\alpha]_D^{20} = +11.5$ ($c = 1.10$, $CHCl_3$). UV (EtOH): 235 (21440), 266 (11590). IR (KBr): 3435*m* (br.), 3192*m* (br.),

3060*m*, 2931*m*, 2868*m*, 2836*m*, 1686*s*, 1608*m*, 1582*m*, 1508*s*, 1464*s*, 1446*m*, 1412*m*, 1363*m*, 1275*m*, 1252*s*, 1177*s*, 1155*m*, 1092*s*, 1070*m*, 1033*s*, 914*m*, 874*w*, 828*m*, 791*m*, 754*m*, 727*m*, 700*m*, 658*w*, 598*m*, 584*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.72 (*s*, H–N(3)); 7.80 (*s*, H–C(6)); 7.41–7.44 (*m*, 2 arom. H); 7.17–7.34 (*m*, 12 arom. H); 6.79–6.84 (*m*, 4 arom. H); 6.36 (*dd*, *J* = 6.1, 7.5, H–C(1′)); 4.50–4.54 (*m*, H–C(3′)); 4.28 (*s*, PhCH_2); 4.03 (*q*, *J* = 3.4, H–C(4′)); 3.98 (*d*, *J* = 11.8, 1 H, CH_2–C(5)); 3.77 (*d*, *J* = 11.8, 1 H, CH_2–C(5)); 3.74, 3.75 (2*s*, 2 MeO); 3.44 (*dd*, *J* = 3.7, 10.7, 1 H–C(5′)); 3.37 (*dd*, *J* = 3.5, 10.7, 1 H–C(5′)); 2.42 (*ddd*, *J* = 3.3, 5.9, 13.6, 1 H–C(2′)); 2.24–2.33 (*m*, 1 H–C(2′), OH–C(3′)). ^{13}C-NMR (75 MHz, $CDCl_3$): 162.55 (*s*, C(4)); 158.64 (*s*, arom. C); 150.25 (*s*, C(2)); 144.51 (*s*, arom. C); 138.52 (*d*, C(6)); 137.88, 135.52, 135.41 (3*s*, arom. C); 130.10, 128.29, 128.10, 128.01, 127.75, 127.58, 127.06, 113.29 (8*d*, arom. C); 112.21 (*s*, C(5)); 86.84 (*s*, *C*–O–C(5′)); 86.02 (*d*, C(4′)); 84.98 (*d*, C(1′)); 72.88 (*t*, Ph*C*H_2); 72.33 (*d*, C(3′)); 64.26 (*t*, *C*H_2–C(5)); 63.51 (*t*, C(5′)); 55.21 (*q*, MeO); 40.89 (*t*, C(2′)). LSI-MS: 651 (0.3, *M*H$^+$), 305 (3), 304 (26), *303* (100), 207 (4), 135 (4), 125 (2).

N^4-*Benzoyl-5-[(benzyloxy)methyl]-2′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)cytidine* (**17**). R_f 0.38 (hexane/AcOEt 1:3). $[\alpha]_D^{25} = +53.0$ (*c* = 0.92, $CHCl_3$). UV (EtOH): 234 (27260), 262 (sh, 11820), 326 (21050). IR (KBr): 3435*m* (br.), 3062*w*, 3030*w*, 3000*w*, 2930*m*, 2868*w*, 2835*w*, 1707*s*, 1648*m*, 1606*s*, 1566*s*, 1508*s*, 1487*m*, 1463*m*, 1447*m*, 1412*w*, 1365*m*, 1334*m*, 1310*m*, 1276*s*, 1251*s*, 1175*s*, 1092*s*, 1067*m*, 1034*m*, 1002*m*, 914*w*, 828*m*, 791*m*, 754*m*, 714*m*, 700*m*, 680*m*, 584*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 13.37 (*s*, NH–C(4)); 8.21 (br. *s*, 2 arom. H); 8.07 (br. *s*, H–C(6)); 7.22–7.53 (*m*, 17 arom. H); 6.82–6.85 (*m*, 4 arom. H); 6.37 (*t*, *J* = 6.5, H–C(1′)); 4.37 (br. *d*, 1 H, PhCH_2); 4.32 (br. *d*, 1 H, PhCH_2); 4.24 (br. *d*, 1 H, CH_2–C(5)); 4.10 (br. *d*, 1 H, CH_2–C(5)); 3.87–3.93 (br. *m*, H–C(4′)); 3.753, 3.748 (2*s*, 2 MeO); 3.48 (br. *dd*, 1 H–C(5′)); 3.40 (*dd*, *J* = 3.3, 10.7, 1 H–C(5′)); 2.71 (br. *s*, OH–C(3′)); 2.49–2.56 (br. *m*, 1 H–C(2′)); 2.34 (*pent*, *J* = 6.7, 1 H–C(2′)). ^{13}C-NMR (100 MHz, $CDCl_3$): 179.76 (*s*, Ph*CO*); 158.70 (*s*, arom. C, C(4)); 147.78 (*s*, C(2)); 144.55 (*s*, arom. C); 139.69 (*d*, C(6)); 137.93, 137.01, 135.55, 135.45 (4*s*, arom. C); 132.51, 130.17, 130.15, 129.93, 128.43, 128.16, 128.07, 127.86, 127.73, 127.12, 113.34 (11*d*, arom. C); 112.67 (*s*, C(5)); 86.94 (*s*, *C*–O–C(5′)); 86.24 (*d*, C(4′)); 85.69 (*d*, C(1′)); 73.04 (*t*, Ph*C*H_2); 72.12 (*d*, C(3′)); 64.56 (*t*, *C*H_2–C(5)); 63.42 (*t*, C(5′)); 55.25 (*q*, MeO); 41.36 (*t*, C(2′)). LSI-MS: 754 (2, *M*H$^+$), 337 (5), 336 (17), 304 (22), *303* (100), 228 (13), 105 (32).

Phosphoramidites **18** *and* **19**: *General Procedure.* The 2-cyanoethyl diisopropylphosphoramidochloridite (1.50 mmol) was added to a soln. of **16** or **17** (1 mmol) and ^{i}Pr$_2$NEt (3.00 mmol) in THF (10 ml). After stirring for 1 h at r.t., the reaction was quenched by addition of sat. aq. $NaHCO_3$ soln., the mixture extracted twice with CH_2Cl_2, the org. layer dried (Na_2SO_4) and evaporated, and the remaining crude product purified by FC with the solvent indicated. For prep. chromatography, 1‰ Et_3N was added to the solvent to prevent detritylation and hydrolysis of the phosphoramidite: **18** (92%) and **19** (91%), resp., both as brittle colorless foams.

5-[(Benzyloxy)methyl]-2′-deoxy-5′-O-*(4,4′-dimethoxytrityl)uridine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**18**). R_f 0.54, 0.44 (hexane/AcOEt 1:2). UV (EtOH): 235 (21980), 266 (11790). IR (KBr): 3185*m* (br.), 3061*m*, 3034*m*, 2966*s*, 2931*m*, 2870*m*, 2837*m*, 2252*w*, 1694*s*, 1608*m*, 1583*m*, 1510*s*, 1463*s*, 1397*m*, 1364*m*, 1274*s*, 1252*s*, 1200*m*, 1179*s*, 1155*m*, 1086*s*, 1033*s*, 978*s*, 900*m*, 828*s*, 791*m*, 754*m*, 727*m*, 700*m*, 634*w*, 598*m*, 584*m*, 523*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.86 (*s*, H–N(3)); 7.88, 7.84 (2*s*, H–C(6)); 7.42–7.45 (*m*, 2 arom. H); 7.15–7.35 (*m*, 12 arom. H); 6.80–6.84 (*m*, 4 arom. H); 6.38 (*t*, *J* = 7.7, 0.5 H, H–C(1′)); 6.37 (*t*, *J* = 7.8, 0.5 H, H–C(1′)); 4.61–4.67 (*m*, H–C(3′)); 4.24, 4.22 (2*s*, PhCH_2); 4.13–4.19 (*m*, H–C(4′)); 3.96 (*d*, *J* = 12.3, 0.5 H, CH_2–C(5)); 3.94 (*d*, *J* = 11.7, 0.5 H, CH_2–C(5)); 3.753, 3.745, 3.738 (3*s*, 2 MeO); 3.53–3.88 (*m*, 5 H, 2 Me_2CH, OCH_2CH_2CN, 1 H of CH_2–C(5)); 3.50 (*dd*, *J* = 3.0, 10.6, 0.5 H, H–C(5′)); 3.45 (*dd*, *J* = 3.1, 10.6, 0.5 H, H–C(5′)); 3.31, 3.33 (2*dd*, *J* = 3.3, 10.6, 1 H, H–C(5′)); 2.63 (*t*, *J* = 6.2, 1 H, OCH_2CH_2CN); 2.56 (*ddd*, *J* = 2.4, 5.7, 13.7, 0.5 H, H–C(2′)); 2.49 (*ddd*, *J* = 3.0, 5.8, 13.5, 0.5 H, H–C(2′)); 2.43 (*t*, *J* = 6.4, 1 H, OCH_2CH_2CN); 2.28–2.35 (*m*, 1 H, H–C(2′)); 1.17–1.19 (*m*, 9 H, 2 *Me*$_2$CH); 1.06 (*d*, *J* = 6.8, 3 H, 2 *Me*$_2$CH). ^{13}C-NMR (100 MHz, $CDCl_3$): 162.43 (*s*, C(4)); 158.61 (*s*, arom. C); 150.11, 150.05 (2*s*, C(2)); 144.40 (*s*, arom. C); 138.47 (*d*, C(6)); 137.86, 135.44, 135.32, 135.27 (4*s*, arom. C); 130.14, 130.10, 128.22, 128.18, 128.10, 127.94, 127.70, 127.48, 127.03, 127.00 (10*d*, arom. C); 117.51, 117.32 (2*s*, CN); 113.20 (*d*, arom. C); 112.22, 112.16 (2*s*, C(5)); 86.75 (*s*, *C*–O–C(5′)); 85.56, 85.34, 84.88, 84.84 (4*d*, C(1′), C(4′)); 73.65 (*dd*, *J*(C,P) = 17.5, C(3′)); 73.19 (*dd*, *J*(C,P) = 16.8, C(3′)); 72.81 (*t*, Ph*C*H_2); 64.19 (*t*, *C*H_2–C(5)); 63.08, 62.87 (2*t*, C(5′)); 58.26, 58.15 (2*dt*, *J*(C,P) = 18.9, O*C*H_2CH_2CN); 55.17, 55.16 (2*q*, MeO); 43.24 (*dd*, *J*(C,P) = 12.1, Me_2CH); 43.13 (*dd*, *J*(C,P) = 9.1, Me_2CH); 40.05 (*d*, C(2′)); 24.60, 24.53, 24.48, 24.40 (4*q*, *Me*$_2$CH); 20.34, 20.13 (2*dt*, *J*(C,P) = 7.1, OCH_2CH_2CN). ^{31}P-NMR (162 MHz, $CDCl_3$): 157.93, 157.45. LSI-MS: no *M*H$^+$, 336 (8), 304 (24), *303* (100), 232 (5), 228 (7), 105 (6).

N^4-*Benzoyl-5-[(benzyloxy)methyl]-2′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)cytidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**19**). R_f 0.54, 0.46 (hexane/AcOEt 1:1). UV (EtOH): 234 (28780), 263 sh (12490), 327 (22660). IR (KBr): 3436*w* (br.), 3064*w*, 3031*w*, 2966*m*, 2930*m*, 2869*m*, 2837*w*, 2252*w*, 1711*s*, 1648*m*, 1599*m*, 1570*s*, 1508*s*, 1488*m*, 1463*m*, 1448*m*, 1396*w*, 1364*m*, 1334*m*, 1310*m*, 1276*s*, 1251*s*, 1200*m*, 1179*s*, 1156*w*, 1084*m*,

1034*m*, 1002*m*, 978*m*, 901*w*, 828*m*, 810*w*, 791*w*, 715*m*, 700*m*, 681*w*, 636*w*, 583*w*, 521*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 13.33 (*s*, NH–C(4)); 8.17–8.21 (*m*, 2 arom. H); 8.10, 8.05 (2*s*, H–C(6)); 7.18–7.52 (*m*, 17 arom. H); 6.80–6.84 (*m*, 4 arom. H); 6.33–6.39 (*m*, H–C(1′)); 4.60–4.65 (*m*, H–C(3′)); 4.16–4.32 (*m*, CH_2–C(5), PhCH_2); 3.70–3.86 (*m*, 2 H, H–C(4′), O$CH_2$$CH_2$CN); 3.741, 3.735, 3.733, 3.727 (4*s*, 2 MeO); 3.45–3.64 (*m*, 4 H, 2 Me_2C*H*, O$CH_2$$CH_2$CN, H–C(5′)); 3.34, 3.32 (2*dd*, *J* = 3.2, 10.6, 1 H, H–C(5′)); 2.52–2.65 (*m*, 1 H, H–C(2′)); 2.61 (*t*, *J* = 6.3, 1 H, O$CH_2$$CH_2$CN); 2.41 (*t*, *J* = 6.4, 1 H, O$CH_2$$CH_2$CN); 2.33 (*quint.*, *J* = 6.9, 1 H, H–C(2′)); 1.15–1.18 (*m*, 9 H, 2 *Me*$_2$CH); 1.05 (*d*, *J* = 6.7, 3 H, 2 *Me*CH). ^{13}C-NMR (100 MHz, $CDCl_3$): 179.72 (*s*, Ph*C*O); 158.71 (*s*, arom. C, C(4)); 147.71 (*s*, C(2)); 144.48 (*s*, arom. C); 139.62 (*d*, C(6)); 137.95, 137.08, 135.50, 135.39, 135.35 (5*s*, arom. C); 132.44, 130.25, 130.20, 129.93, 128.38, 128.28, 128.20, 128.12, 128.03, 127.65, 127.13, 127.10 (12*d*, arom. C); 117.54, 117.37 (2*s*, CN); 113.30 (*d*, arom. C); 112.67 (*s*, C(5)); 86.88 (*s*, *C*–O–C(5′)); 85.90 (*d*, C(1′)); 85.65, 85.59 (2*d*, C(4′)); 73.69 (*dd*, *J*(C,P) = 17.1, C(3′)); 73.22 (*dd*, *J*(C,P) = 16.5, C(3′)); 73.04 (*t*, Ph*C*H$_2$); 64.50 (*t*, *C*H$_2$–C(5)); 63.07, 62.85 (2*t*, C(5′)); 58.30, 58.20 (2*dt*, *J*(C,P) = 19.2, O$CH_2$$CH_2$CN); 55.26, 55.23 (2*q*, 2 MeO); 43.33 (*dd*, *J*(C,P) = 12.2, Me_2*C*H); 43.24 (*dd*, *J*(C,P) = 12.5, Me_2*C*H); 40.43 (*d*, C(2′)); 24.67, 24.61, 24.55, 24.28 (4*q*, *Me*$_2$CH); 20.42, 20.20 (2*dt*, *J*(C,P) = 7.3, OCH_2*C*H$_2$CN). ^{31}P-NMR (162 MHz, $CDCl_3$): 158.09, 157.53. LSI-MS: 954 (3, MH^+), 336 (17), 304 (23), *303* (100), 273 (6), 228 (7), 187 (5), 147 (6), 105 (13).

Oligonucleotide Synthesis, Purification, and Characterization. Oligodeoxynucleotides **20**–**24** (*Table 3*) were prepared from phosphoramidites **18** and **19** and commercially available A_d, C_d, G_d, and T_d phosphoramidites (*Glen Research*) and deoxynucleoside-CPG (1.3 μmol, *Glen Research*) on a *Pharmacia Gene Assembler Special* automated DNA synthesizer by standard solid-phase phosphoramidite chemistry [18] with slight modifications. As coupling catalyst, 5-(benzylthio)-1*H*-tetrazole [19] was used instead of 1*H*-tetrazole, and the coupling time for phosphoramidites **18** and **19** was prolonged to 6 min. Coupling efficiencies were >99%. After chain elongation and final detritylation, the oligonucleotides were cleaved from the resin and deprotected by treatment with 1 ml of conc. aq. NH_3 soln. at 55° overnight. The crude oligonucleotides were purified by reversed-phase FPLC (fast protein liquid chromatography) with a *Pharmacia PepRPC HR 10/10* column followed by ion-exchange FPLC with a *Pharmacia Mono Q HR 10/10* column on a *Pharmacia FPLC* system. For solvent systems, see *Table 3*. The isolated oligonucleotides were desalted over *Sep-Pak C-18* cartridges (*Waters*) according to the manufacturer's protocol. Incorporation of intact 5-[(benzyloxy)methyl]nucleosides and integrity of oligodeoxynucleotides **20**–**24** were confirmed by MALDI-TOF mass spectometry (linear MALDI-TOF-MS, 20 keV, N_2 laser 337 nm), matrix conditions as described previously [22]. The observed single-product ions were all within 0.1% of the calculated mass (*Table 3*).

UV/Melting Experiments. UV/Melting experiments were performed on a *Cary 3E* UV/VIS spectrophotometer (*Varian*) equipped with a temperature controller. Data were collected with a generic *Pentium II*^{TM} PC running with the *Cary* WinUV Thermal software. Melting curves were recorded at 260 and 284 nm in a consecutive heating-cooling-heating cycle (0–90°) with a temp. gradient of 0.5°/min. All measurements were conducted in a buffer consisting of 10 mM NaH_2PO_4 and 1M NaCl (pH 7.0), at the oligonucleotide concentrations indicated. T_m values were determined from the first derivative of the melting curve with the software package *Origin*^{TM} V5.0. Thermodynamic data were calculated from *van't Hoff* plots according to [23].

Molecular Modeling. Molecular-dynamics calculations were performed by means of the software package InsightII from *Molecular Simulations Inc.*, San Diego, running on an *Octane* workstation from *SGI*. Starting duplex structures for the simulations were built with the canonical B-DNA templates from the *Biopolymer* module of InsightII. All calculations were performed with the AMBER forcefield [24] as implemented in the Discover3 of InsightII without the explicit inclusion of H_2O molecules or counterions. A distance-dependent dielectric constant of $4 \cdot r$ was used instead as a screening function [25]. The 1,4-nonbonded interactions were scaled by 0.5 [24a]. No cut-offs on nonbonded interactions were applied. Prior to molecular-dynamics calculations, duplex structures were energy-minimized. First, a steepest-decent algorithm was used until the energy gradient dropped below 10 kcal/mol · Å. Then, the conjugate-gradient method was used until the energy gradient reached 0.05 kcal/mol · Å. For molecular dynamics, a timestep of 1 fs was used during all simulations. The energy-minimized structures were first heated stepwise from 0 to 300 K (velocity scaling method): 1 ps at 50 K, 1 ps at 100 K, 2 ps at 150 K, 2 ps at 200 K, 4 ps at 250 K, and 10 ps at 300 K. The system was then kept at 300 K for 200 ps (coupling to an external bath [26]). Coordinates and energy terms were stored every 0.5 ps. The trajectories of the molecular dynamics runs were analyzed by means of the 'Analysis' and 'Decipher' modules of 'InsightII'. For structure representation, the trajectories were averaged over the last 50 ps of the simulation and the corresponding data was visualized by *MSI*s 'WebLab ViewerPro 3.5'.

Table 3. *Purification Conditions and MALDI-TOF-MS Analysis of Oligonucleotides* **20**–**24**

	FPLC	MALDI-TOF-MS ($[M-H]^-$)		
	Conditions	t_R [min]	*m/z* (calc.)	*m/z* (found)
20 d(T-T-T-T-^{bo}T-T-T-T-T-T)	reversed phase[a]): 0–30% *B* in 15 min	13.4	3085.1	3082.6
	ion exchange[b]): 0–50% *B* in 20 min	18.0		
21 d(C-^{bo}T-G-A-A-^{bo}T-C-G-A-C)	reversed phase[a]): 0–30% *B* in 15 min	14.4	3223.3	3222.1
	ion exchange[b]): 0–30% *B* in 5 min, 30–50% *B* in 15 min	15.9		
22 d(^{bom}C-T-G-A-A-T-^{bom}C-G-A-C)	reversed phase[a]): 0–30% *B* in 15 min	15.2	3251.3	3248.5
	ion exchange[b]): 0–30% *B* in 5 min, 30–50% *B* in 15 min	17.7		
23 d(^{bom}C-^{bo}T-G-A-A-^{bo}T-^{bom}C-G-A-C)	reversed phase[a]): 0–40% *B* in 15 min	15.1	3463.6	3463.6
	ion exchange[b]): 0–50% *B* in 5 min, 50–70% *B* in 15 min	13.8		
24 d(G-^{bo}T-^{bom}C-G-A-^{bo}T-^{bo}T-^{bom}C-A-G)	reversed phase[a]): 0–50% *B* in 15 min	12.8	3600.7	3598.8
	ion exchange[c]): 0–25% *B* in 5 min, 25–45% *B* in 15 min	15.5		

[a]) Solvent *A*, 0.1M (Et_3NH)OAc in H_2O (pH 7.0); solvent *B*, 0.1M (Et_3NH)OAc in H_2O/MeCN 1 : 4 (pH 7.0); flow 3.0 ml/min; detection at 254 nm. [b]) Solvent *A*, 10 mM Na_2HPO_4 in H_2O (pH 7.0); solvent *B*, 10 mM Na_2HPO_4 and 1M NaCl in H_2O (pH 7.0); flow 3.0 ml/min; detection at 254 nm. [c]) Solvent *A*, 10 mM Na_2HPO_4 in H_2O/MeCN 4 : 1 (pH 7.0); solvent *B*; 10 mM Na_2HPO_4 and 1M NaCl in H_2O/MeCN 4 : 1 (pH 7.0); flow 3.0 ml/min; detection at 254 nm.

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