

Oligonucleotide Analogues with a Nucleobase-Including Backbone:

Part 6

2-Deoxy-D-erythrose-Derived Phosphoramidites: Synthesis and Incorporation into 14-Mer DNA Strands

by Werngard Czechtizky and Andrea Vasella*

Laboratorium für Organische Chemie, ETH-Zentrum, Universitätstrasse 16, CH-8092 Zürich

Two modified DNA 14-mers have been prepared, containing either a 2-deoxy-D-erythrose-derived adenosine analogue carrying a C(8)–CH₂O group (dA*), or a 2-deoxy-D-erythrose-derived uridine analogue, possessing a C(6)–CH₂O group (dU*). These nucleosides are linked *via* a phosphinato group between O–C(3') (dA* and dU*) and O–C(5') of one neighbouring nucleotide, and between C(8)–CH₂O (dA*), or C(6)–CH₂O (dU*) and O–C(3') of the second neighbour. *N*⁶-Benzoyl-9-(β-D-erythrofuransyl)adenine (**3**) and 1-(β-D-erythrofuransyl)uracil (**4**) were prepared from D-glucose, deoxygenated at C(2'), and converted into the required phosphoramidites **1** and **2**. The modified tetradecamers **31** and **32** were prepared by solid-phase synthesis. Pairing studies show a decrease in the melting temperature of 7 to 8 degrees for the duplexes **31**·**30** and **32**·**29**, as compared to the unmodified DNA duplex **29**·**30**. A comparison with the pairing properties of tetradecamers similarly incorporating deoxyribose- instead of the deoxyerythrose-derived nucleotides evidences that the CH₂OH substituent at C(4') has no significant effect on the pairing.

Introduction. – Oligonucleotide analogues with a nucleobase-including backbone should allow to answer the question whether the structural differentiation between nucleobase and backbone in DNA, RNA, and their analogues is a necessary prerequisite for the formation of stable homo- and/or heteroduplexes [1–5]. We have already described oligonucleotide analogues with a phosphinato group between O–C(3') and either a C(8)–CH₂O group of 2'-deoxyadenosine (dA_n^{*1}); *Fig. 1*), or a C(6)–CH₂O group of 2'-deoxyuridine (dU_n^{*1}); *Fig. 1*) [5]. Force-field calculations [6] and *Maruzen* model studies suggested that these analogues may form autonomous pairing systems, and that the incorporation of single modified dA* or dU* units into DNA 14-mers is compatible with duplex formation. Pairing studies of singly modified tetradecamers, however, showed that the introduction of dA* or dU* units into a 14-mer DNA duplex lowered the melting temperature by 6–7°, as compared to the unmodified DNA duplex [5]. This destabilization may be due to a distortion of the DNA duplex caused by the *syn*-conformation [7] of the dA* or dU* units, as enforced by an unfavourable interaction between the C(8)- or C(6)-substituent and the carbohydrate moiety – presumably the O–C(5') group – of dA* and dU*. To reduce this steric interaction, we decided to remove the CH₂OH substituent at C(4') of dA*

¹) The repeating units of B-DNA are denoted dA, dT, dG, and dC. 2-Deoxyribose-derived units linked *via* O–C(3') and O(10), or O(7) are denoted dA* and dU*, respectively, and 2-deoxy-D-erythrose-derived units linked *via* O–C(3') and O(10), or O(7), dA* and dU*, respectively (*Fig. 1*).

and dU*, and to introduce the 2-deoxyerythrose-derived units deA*¹) and deU*¹) (Fig. 1) into a DNA duplex.

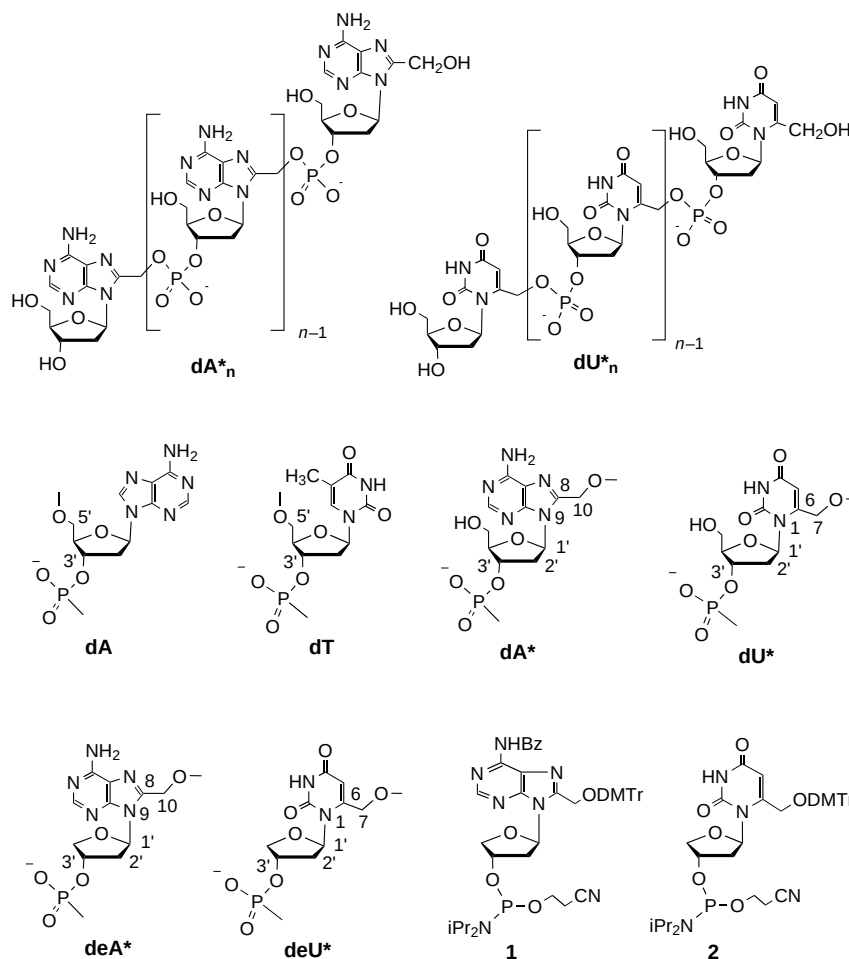


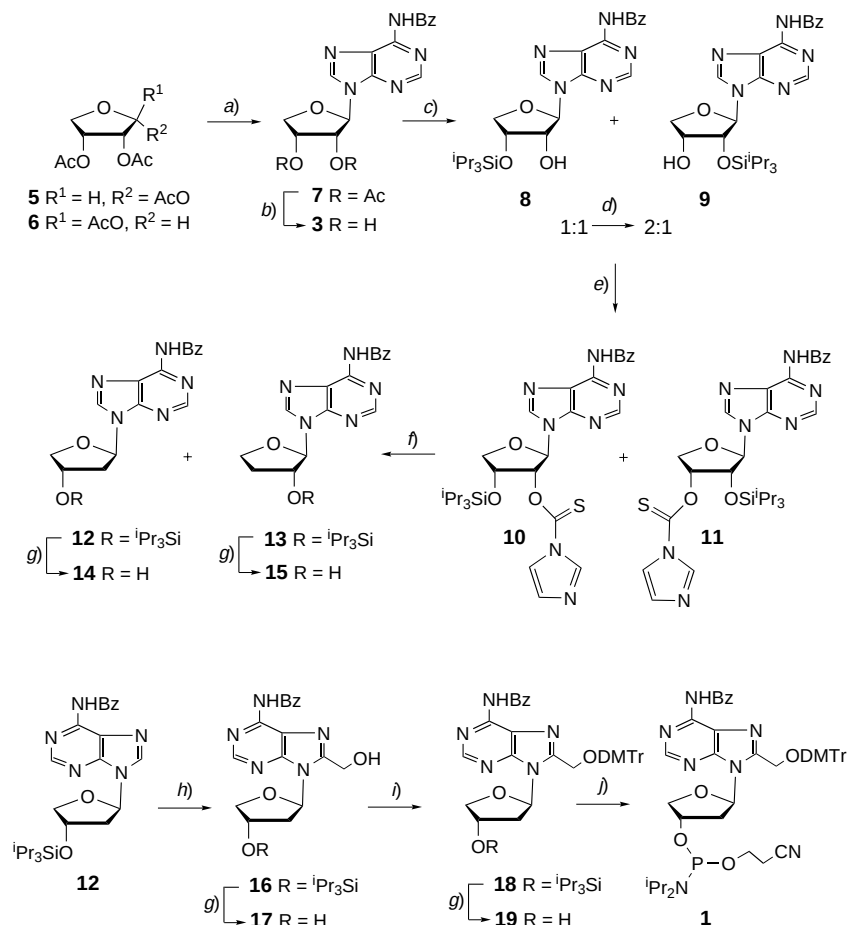
Fig. 1. 2-Deoxyribose-derived oligonucleotide analogues dA_n* and dU_n*, DNA units dA and dT, 2-deoxyribose-derived units dA* and dU*, 2-deoxyerythrose-derived units deA* and deU*, and the phosphoramidites **1** and **2**

We report on the syntheses of the deA*- and deU*-derived phosphoramidites **1** and **2** (Fig. 1), the incorporation of a single deA*, or deU* unit into a tetradecamer, and on the pairing of these tetradecamers with an unmodified complementary strand.

Results and Discussion. – 1. *Synthesis of the Phosphoramidites 1 and 2.* Nucleosidation of 2-deoxy-D-erythrose derivatives with thymine [8] and uracil [9] proceeds with a low selectivity, affording 2:1 to 1:1 mixtures of anomers, while nucleosidation of anomeric mixtures of triacetyl erythrofuranoose with *N*⁶-benzoyladenine or uracil afforded diastereoselectively the β-D-nucleosides **3** and **4** (Schemes 1 and 2) after deprotection [10]. For this reason, we decided to prepare the phosphoramidites

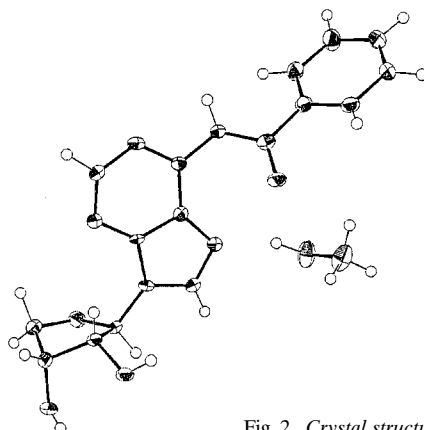
1 and **2** from the 1,2,3-tri-*O*-acetyl- α,β -D-erythroses **5/6** (Scheme 1) rather than from 2-deoxy-D-erythrose, and to subsequently remove the OH group at C(2'). Triacetyl-erythrose is readily accessible by degradation of D-glucose with Pb(OAc)₄ [11][12] and acetylation [13]²⁾. Deoxygenation of **3** and **4**, followed by hydroxymethylation, protection, and phosphorylation, should lead to the phosphoramidites **1** and **2**.

Scheme 1



a) 1. *N*⁶-Benzoyladenine, *N,O*-bis(trimethylsilyl)acetamide (BSA); 2. SnCl₄; 80%. *b*) 1. NaOH, H₂O/MeOH/THF 5:4:1; 2. NH₄Cl; 80%. *c*) ⁱPr₃SiOTf, pyridine, DMF; 92%. *d*) SiO₂, Et₃N. *e*) *N,N*-(thiocarbonyl)diimidazole, CH₂Cl₂. *f*) Bu₃SnH, 2,2'-azobis(isobutyronitrile) (AIBN), toluene; 44% of **12** and 20% of **13** (from **8/9** 2:1). *g*) Bu₄NF · 3 H₂O, THF; **14** (74%); **15** (89%); **19** (76%). *h*) 1. LDA/THF; 2. DMF; 3. AcOH; 4. NaBH₄/EtOH; 88%. *i*) Dimethoxytrityl chloride (= bis(4-methoxyphenyl)(phenyl)methyl chloride; DMTrCl), Et₃N/Pr₂, 4-(dimethylamino)pyridine (DMAP), CH₂Cl₂; 89%. *j*) (NCCH₂CH₂O)P(ⁱPr₂N)Cl, CH₂Cl₂, Et₃N/Pr₂; 80% of 2 diastereoisomers (1:1).

2) 2-Deoxy-D-erythrose has been prepared from 3-deoxy-D-erythropentose [14], dimethyl (*S*)-malate [15], D-isoascorbic acid [16], and (*S*)-solketal [16].

Fig. 2. Crystal structure of **3**·MeOH

Nucleosidation of the anomeric mixture **5/6** with *N*⁶-benzoyladenine in the presence of SnCl₄ and *N,O*-bis(trimethylsilyl)acetamide (BSA) at 60° (*cf.* [17][18]) gave the β-D-configured nucleoside **7** (80%), which was deacetylated with 2M NaOH to yield 80% of the nucleoside **3**. Stannylation of **3** with Bu₃SnO in toluene [19], followed by treatment of the crude Sn-acetal with Et₃SiCl, led to a 1:1 mixture of regioisomeric silyl ethers. Regioselective silylation of **3** at HO–C(3') with various trialkylchlorosilanes in the presence of Ag salts and DABCO as reported for ribonucleosides [20] led again to unsatisfactory results. Finally, we treated **3** with a 2.5-fold excess of ⁱPr₃SiOTf in DMF/pyridine 16:1 at 23° to obtain 92% of a 1:1 mixture of the 3'- and 2'-O-ⁱPr₃Si-protected nucleosides **8** and **9**. Treatment of this mixture with silica gel in Et₃N led to partial migration of the ⁱPr₃Si group from O(2') to O(3'), resulting in a 2:1 mixture **8/9** after 24 h. This mixture was converted to the thiocarbamates **10/11** (2:1). Treatment of the crude mixture with Bu₃SnH and 2,2'-azobis(isobutyronitrile) (AIBN) in refluxing toluene yielded a mixture **12/13** (2:1; 64% from **8/9**). The nucleosides **12/13** were readily separated by FC. Desilylation of **12** and **13** yielded **14** and **15** (80% each), respectively. Deprotonation of **12** by LDA [21], addition of DMF, and reduction with NaBH₄ gave 88% of the C(8)-CH₂OH nucleoside **16** that was desilylated to **17** (80%). Dimethoxytritylation of **16** gave **18** (89%) that was desilylated to **19** (80%). The alcohol **19** was converted to the phosphoramidite **1** in the usual way [22]; **1** is stable at –15° for several months.

The configuration of **3** was established by X-ray crystal-structure analysis of **3**·MeOH³⁾ (Fig. 2). The erythrofuransyl moiety adopts the ²T₃ conformation, similarly to the conformation deduced for **3** in D₂O solution [10]. HO–C(3') forms a weak intramolecular H-bond to O–C(2') (distance O(2')...O(3') 2.77 Å); intermolecular H-bonds are found between HO–C(3') and N(1), H–N(6) and O–C(2'), MeOH and

³⁾ The crystallographic data have been deposited with the *Cambridge Crystallographic Data Centre* (deposition No. CCDC-158435 (**3**), CCDC-158436 (**4**), and CCDC-158437 (**25**)). 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.cam.ac.uk).

N(7), and between HO–C(2') and MeOH. The nucleobase adopts the *syn*-conformation.

The structures of **8** and **9** were assigned on the basis of the ¹H-NMR spectra of **8/9** (2:1). The signals of H–C(2') and H–C(3') of **8/9** were assigned on the basis of their multiplicity and coupling constants (see Table 3). In CDCl₃, H–C(2') of the major compound appears as a *dt* with $J(2',3')=5.0$, $J(1',2')=4.1$, and $J(\text{H},\text{OH})=5.3$ Hz. Upon addition of D₂O, this signal collapsed to a *dd*, while the H–C(3') *q* remained unchanged, evidencing that the major compound possesses structure **8**. The broad H–C(3') signal of the minor compound (**9**) collapsed to a *ddd* upon addition of D₂O, while the H–C(2') *t* remained unchanged. The structure determination was corroborated by the ¹H-NMR spectra of a 2:1 mixture of the thiocarbamates **10/11**. The H–C(2') and H–C(3') signals of **10/11** were assigned on the basis of their multiplicity and coupling constants (see Table 3). Carbamoylation of O–C(2') of the major compound **8**, leading to **10**, induced a 1.6-ppm downfield shift for H–C(2'), while carbamoylation of O–C(3') of the minor compound **9**, leading to **11**, resulted in a 1.67-ppm downfield shift for H–C(3'). As expected, H–C(1') of **12** appears as a *t* at 6.46 ppm with $J(1',2')=6.6$ Hz (see Table 3 in *Exper. Part*). The H_{pro-S}–C(2') of **12** gives rise to a *ddd* at 2.97 ppm, while H_{pro-R}–C(2') appears at 2.60 ppm as a *dddd* (W coupling to H–C(4'), $J(2',4')=1.0$ Hz). The signals of H–C(1'), H–C(2'), H–C(3'), and H–C(4') of **13** were assigned on the basis of irradiation experiments. H–C(1') of **13** resonates at 6.00 ppm as a *d*, $J(1',2')=1.6$ Hz, H_{pro-S}–C(3') of **13** at 2.32 ppm as a *dt*, and H_{pro-R}–C(3') at 2.12 ppm as a *dddd*. As a rule, the shift from the *anti*- to the *syn*-conformation is indicated by a downfield shift for H_{pro-S}–C(2') and an upfield shift for C(2') [23]. The significant downfield shifts for H_{pro-S}–C(2') ($\Delta\delta=0.18\text{--}0.30$ ppm) and the upfield shifts for C(2') ($\Delta\delta=-1.1$ to -2.6 ppm) of the nucleosides **16**, **18**, **19**, and the phosphoramidite **1**, as compared to the corresponding signals of **12**, evidence that all C(8)-substituted nucleosides adopt predominantly the *syn*-conformation (Table 1).

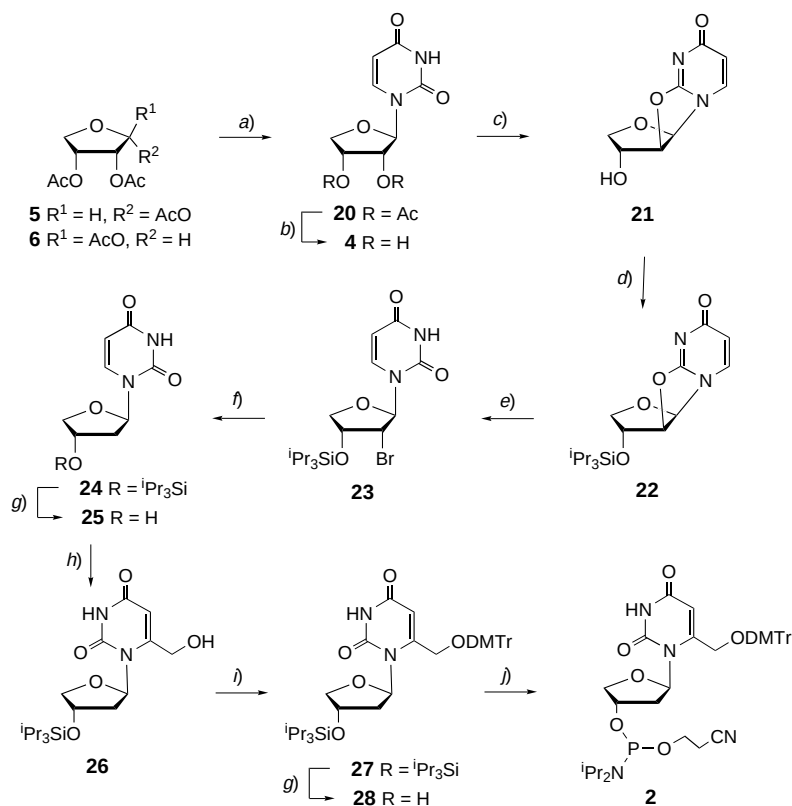
The ratio $J(1',2'_{\text{pro-S}})/J(3'_{\text{pro-R}},4')$ is considered a measure of the position of the equilibrium between the *S*- (sugar-pucker ${}_3E-{}^2T_1$) and *N*-conformers (sugar-pucker ${}^3T_2-{}^3T_4$) [24]. The $J(1',2'_{\text{pro-S}})$ (6.6 Hz) and $J(3',4')$ (≤ 2.1 Hz) values (Table 1) evidence a predominance of the *S*-conformer of the nucleosides **1**, **12**, **14**, **16**, **18**, and **19**.

Table 1. ¹H- and ¹³C-NMR Shift Differences $\Delta\delta(\text{H}-\text{C}(2'))$, and $\Delta\delta(\text{C}(2'))$ [ppm] for the Nucleosides **16**, **18**, **19**, and **1** as Compared to **12**, and for **24**, **26**–**28**, and for **2** as Compared to **24**, and Ratio of *S*/*N*-Conformers of these Nucleosides, as Deduced from $J(1',2')/J(3',4')$ [Hz]

Nucleoside	$\Delta\delta(\text{H}-\text{C}(2'_{\text{pro-S}}))$	$\Delta\delta(\text{C}(2'))$	$J(1',2')$	$J(3',4')$	Ratio <i>S</i> / <i>N</i>
12	–	–	6.6	2.1	76/24
16	0.18	– 1.1	6.6	< 1.0	> 87/13
18	0.23	– 1.1	6.6	< 1.0	> 87/13
19	0.23	– 1.8	6.6	< 1.0	> 87/13
1	0.30	– 2.6	6.6	< 1.0	> 87/13
24	–	–	6.5	2.2	75/25
26	0.79	– 2.0	6.6	< 1.0	> 87/13
27	0.74	– 2.1	7.0	< 1.0	> 88/12
28	0.71	– 3.1	7.0	< 1.0	> 88/12
2	0.77	– 4.1	6.5	< 1.0	> 87/13

To prepare the phosphoramidite **2** (Scheme 2), the anomeric mixture **5/6** was treated with uracil in the presence of BSA and SnCl_4 in MeCN at 60° (cf. [17][18]). We obtained 80% of the β -D-configured nucleoside **20**, which was deacetylated with 2M NaOH to yield 80% of **4**. To deoxygenate **4** at C(2'), we planned to convert **4** to the corresponding 2,2'-anhydronucleoside [25], to cleave the C(2')–O bond with LiBr and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ [26], and to reductively debrominate the resulting 2'-bromo-2'-deoxynucleoside. The 2,2'-anhydro-1-(β -D-threofuranosyl)uracil (**21**) was prepared from **4** by treatment with diphenyl carbonate and NaHCO_3 in DMF [25]. However, all attempts to convert **21** to the 2'-deoxynucleoside **25** failed. Silylation of the 2,2'-anhydronucleoside **21** with a 5-fold excess of $i\text{Pr}_3\text{SiOTf}$ yielded **22** (61% from **4**). Treatment of **22** with LiBr and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ gave the 2'-bromo-2'-deoxynucleoside **23** (88%) that was debrominated in 97% yield with Bu_3SnH and AIBN. The resulting 2'-deoxynucleoside

Scheme 2



a) 1. Uracil, *N,O*-bis(trimethylsilyl)acetamide (BSA); 2. SnCl_4 ; 80%. b) 1. NaOH, $\text{H}_2\text{O}/\text{MeOH}/\text{THF}$ 5:4:1; 2. NH_4Cl ; 80%. c) 1. Diphenyl carbonate, DMF; 2. NaHCO_3 . d) $i\text{Pr}_3\text{SiOTf}$, pyridine, DMF; 62% (from **4**). e) LiBr, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, 1,4-dioxane; 88%. f) Bu_3SnH , AIBN, toluene; > 97%. g) $\text{Bu}_4\text{NF} \cdot 3 \text{H}_2\text{O}$, THF; **25** (88%), **28** (96%). h) 1. LDA/THF; 2. DMF; 3. AcOH; 4. $\text{NaBH}_4/\text{EtOH}$; 51%. i) DMTrCl, $i\text{Pr}_2\text{NEt}$, DMAP, CH_2Cl_2 ; 77%. j) $(\text{NCCCH}_2\text{CH}_2\text{O})\text{P}(i\text{Pr}_2\text{N})\text{Cl}$, CH_2Cl_2 , $i\text{Pr}_2\text{NEt}$; 69% of 2 diastereoisomers (2:1).

24 was desilylated to **25** (80%). Hydroxymethylation of **24** to **26** was accomplished in 52% yield similarly as described above for **12**. Dimethoxytritylation of **26** to **27** and desilylation of **27** yielded 73% of the alcohol **28**, which was converted to the phosphoramidite **2** in the usual way (70%); at -15° , **2** is stable for several months.

The β -D-configuration of 1-(β -D-erythrofuransyl)uracil (**4**) was established by X-ray crystal-structure analysis (Fig. 3,a).

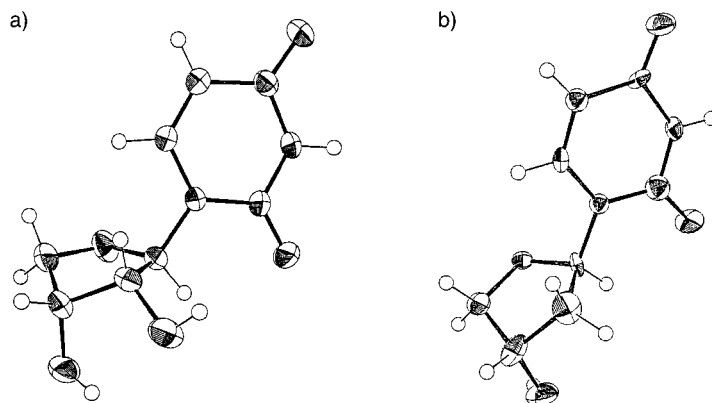


Fig. 3. Crystal structures of a) **4** and b) **25**

Similarly to *N*⁶-benzoyl-9-(β -D-erythrofuransyl)adenine (**3**), the erythrofuransyl moiety of **4** adopts the $_3E$ -conformation. There is a (weak?) intramolecular H-bond between HO–C(3') and O–C(2') (distance O(2') $\cdots$ O(3') 2.69 Å), while intermolecular H-bonds are found between HO–C(2') and O–C(2'), HO–C(3') and O–C(4'), and H–N(3) and O–C(4'). Unlike **3**, the uracil derivative **4** adopts the *anti*-conformation.

The H-atoms H–C(1'), H–C(2'), H–C(3'), H–C(5), and H–C(6) of the 2,2'-anhydronucleoside **21** (Table 5 in *Exper. Part*) resonate at almost the same field as the corresponding signals of 2,2'-anhydrouridine ($\Delta\delta = \pm 0.04$ ppm) [27]. As observed for 5'-protected 2,2'-anhydrouridines [28] [29], C(2), C(4), C(5), C(1'), and C(2') of **21** are shifted significantly downfield, as compared to **4**: C(4) and C(2) give rise to two *s* at 178.7 and 164.2 ppm (**4**: two *s* at 163.5 and 151.0 ppm), C(5) resonates as a *d* at 112.1 ppm (**4**: *d* at 102 ppm), and C(1') and C(2') as two *d* at 93.4 and 90.6 ppm (**4**: two *d* at 88.8 and 74.1 ppm). Bromination of **22** to **23** is indicated by a strong upfield shift for C(2') of **23** ($\delta = 52.4$ as compared to $\delta = 74.1$ ppm in **4**), and the $[M+H]^+$ peak in the FAB-MS of **23** shows the typical isotope pattern for Br compounds.

As expected, H–C(1') of **24** appears at 6.15 ppm as a *t* with $J(1',2') = 6.5$ Hz. The H_{pro-R} –C(2') of **24** gives rise to a *ddd* at 2.50, and H_{pro-S} –C(2') to a *dt* at 2.11 ppm. The ratio $J(1',2')/J(3',4')$ indicates that the 2-deoxy-D-erythrofuransyl moiety of **24** adopts predominantly the *S*-conformation (Table 1).

The structure of **25** was established by X-ray crystal-structure analysis (Fig. 3,b). Two symmetrically independent molecules in the elementary cell form a dimer with intermolecular H-bonds between H–N(3) and O–C(4), and between H–N(3) and O–C(2). Both independent molecules adopt the 2T_3 - and the *anti*-conformations.

Similarly to the adenine series, the downfield shift for $H_{pro-S}-C(2')$ ($\Delta\delta = 0.71 - 0.79$ ppm) and the upfield shift for $C(2')$ of **2** and **26–28**, as compared to **24** ($\Delta\delta = -2.0$ to -4.1 ppm), show the predominance of the *syn*-conformation for the $C(6)$ -substituted nucleosides (Table 1). The equilibrium between the *S*- and the *N*-conformers of **2** and **26–28** is clearly in favour of the *S*-conformer ($> 88/12$), as indicated by $J(1',2'_{pro-S})$ and $J(3',4')$ values of **2** and **26–28** (Table 1).

2. *Solid-Phase Synthesis of 14-Mer DNA Strands Containing deA* or deU*, and of the Complementary DNA Strands.* Table 2 shows the 14-mer DNA strands **29** and **30** possessing a random sequence with an internal dA or dT unit flanked by dG or dC units. Replacement of the internal dA of **29** by deA* (derived from **1**) led to the modified 14-mer **31**, while replacement of the internal dT of **30** by deU* (from **2**) led to the modified 14-mer **32**. The solid-phase synthesis of the oligomers **29–32** was carried out on a DNA-synthesizer using essentially the protocol for the synthesis of pRNA [30]. The coupling time for the attachment of **1**, **2**, and the DNA phosphoramidites following the incorporation of **1** and **2** was arbitrarily set to 30 min; it led to coupling yields of 82%⁴⁾ for **1** and *ca.* 80–90% for the DNA-phosphoramidites following **1**, and of *ca.* 90% for **2** (99% for all other couplings). Longer coupling times did not improve yields. The DNA 14-mers **29** and **30** were deprotected and cleaved off the solid support by ammonolysis with saturated aqueous $NH_3/MeOH$ 1:1 at 50° within 20 h, while **31** was deprotected under the same conditions within 3.5 h. Prolonged heating of the modified DNA strand **32** (incorporating deU*) at 50° led to degradation; this strand was deprotected at 35°. The crude products were analyzed by RP-HPLC. The DNA strands **29** and **30** were pure, the modified tetradecamer **32** was *ca.* 70–80% pure, while crude **31** contained three major products A, B, and C in a *ca.* 1:2:1 ratio. The products were purified by RP-HPLC, desalted, and analyzed by RP-HPLC and MALDI-TOF mass spectroscopy; only fraction C (from crude **31**) exhibited a molecular ion in agreement with structure **31**⁵⁾. The yield of the oligomers, as determined from the detritylation assay, and their molecular masses are shown in Table 2.

Table 2. Total Yields, and Calculated and Experimental Molecular Masses of the Oligodeoxynucleotides **29–32**

Sequence	Total yield [%] ^{a)}	Calc. mass	Exper. mass (MALDI-TOF)
29 5'-d(CGTAAGCTCGATAG)-3'	94	4287.8	4287.4
30 5'-d(CTATCGAGCTTACG)-3'	99	4238.8	4239.1
31 5'-d(CTATCGeA*GCTTACG)-3'	27	4238.8	4237.7
32 5'-d(CGTAAGCeU*CGATAG)-3'	75	4273.8	4276.6

^{a)} Calculated from the yield of the coupling steps, as determined by the detritylation assay.

3. *Pairing Studies.* The modified 14-mer **31** (Table 2) was hybridized with the complementary 14-mer **30** at a concentration of (2+2) μM and at pH 7 (10 mM NaH_2PO_4/Na_2HPO_4 in H_2O) in 0.1M aqueous NaCl. The modified duplex **31·30**

⁴⁾ Each chain elongation cycle began with an acid promoted cleavage of the dimethoxytrityl group. The efficiency of each coupling step was calculated by UV-determination of the amount of DMTr⁺ ($\epsilon = 70000 \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$).

⁵⁾ In agreement with this assignment, the fractions A and B did not pair with **30**.

showed a decrease of the melting temperature by 8°, as compared to the non-modified duplex **29**·**30** (see Fig. 4 for the melting curves).

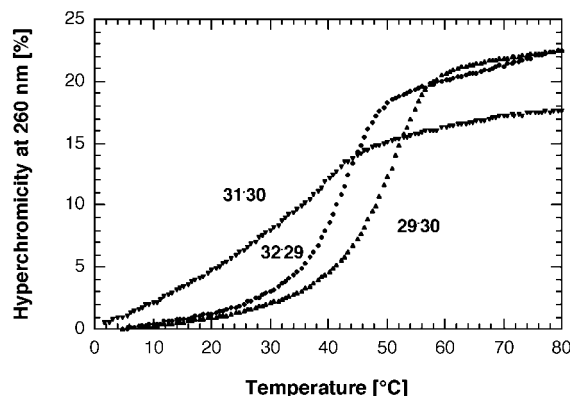


Fig. 4. Temperature-dependent UV spectra ('melting curves') of the duplexes **29**·**30**, **31**·**30**, and **32**·**29**

Similarly, the modified 14-mer **32** (Table 2) incorporating one deU* unit was hybridized with the complementary 14-mer **29** at a concentration of (2 + 2) μM and at pH 7 (10 mM $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ in H_2O) in 0.1M aqueous NaCl. Again, the modified duplex **32**·**29** melted 7° lower than the non-modified DNA duplex **29**·**30** (see Fig. 4 for the melting curves). Since the modified 14-mer **32** is degraded during prolonged heating, the melting process for the duplex **32**·**29** was irreversible.

Similarly as reported for the incorporation of the 2-deoxyribose-derived units dA* and dU* [5], the introduction of the 2-deoxy-D-erythrose-derived units deA* and deU* into a DNA duplex causes a decrease in melting temperature by 7–8°, as compared to the unmodified duplex. A comparison of the duplex destabilization imposed by dA*, dU*, deA*, and deU* shows that this destabilization is independent of the CH_2OH substituent at C(4').

We thank Dr. B. Bernet for checking the experimental part, and the Swiss National Science Foundation and F. Hoffmann-La Roche AG, Basel for generous support.

Experimental Part

General. Solvents were distilled before use: THF from K/benzophenone, toluene from Na, CH_2Cl_2 , DMF, pyridine, 1,4-dioxane, and $\text{Et}_3\text{N}/\text{Pr}_2$ from CaH_2 . Reactions were run under Ar. Qual. TLC: precoated silica-gel plates (Merck silica gel 60 F_{254}); detection by spraying with 'mostain' (400 ml of 10% aq. H_2SO_4 , 20 g of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot \text{H}_2\text{O}$, 0.4 g of $\text{Ce}(\text{SO}_4)_2$) and heating. Flash chromatography (FC): silica gel Merck 60 (0.04–0.063 mm). Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC): analytical RP column: Macherey-Nagel; LiChroCart®250-4 HPLC Cartridge filled with LiChrospher®100, RP-18e (5 μm); prep. RP column: Macherey-Nagel, Hibar® RT250-10, customized packing: LiChrospher®100, RP-18e (5 μm), eluents: 0.1M aq. Et_3NHOAc , MeCN, H_2O . Optical rotations: 1-dm cell at 25° and 589 nm. FT-IR: 1–2% soln. in the indicated solvent. ^1H -, ^{13}C -, and ^{31}P -NMR: at 200, 300, or 400 MHz, 50 or 75 MHz, or 121 MHz, resp. MS: fast-atom bombardment (FAB), matrix-assisted laser-desorption ionization (MALDI), high-resolution (HR). NOBA: 3-nitrobenzyl alcohol.

N⁶-Benzoyl-9-(2',3'-di-O-acetyl- β -D-erythrofuranosyl)adenine (7). A suspension of **5/6** (1:4) [11–13] (4.16 g, 16.9 mmol) and N⁶-benzoyladenine (4.4 g, 18.4 mmol) in MeCN (18 ml) was treated dropwise with N,O-bis(trimethylsilyl)acetamide (10 ml, 41 mmol) at 60°. The mixture was stirred for 30 min, treated with SnCl_4

(8 ml, 68 mmol), stirred for 20 min, poured into a stirred mixture of AcOEt (300 ml) and sat. aq. NaHCO₃ (300 ml), and stirred for 15 min. The org. layer was dried (Na₂SO₄) and evaporated. FC (AcOEt/MeOH 100:1) gave **7** (5.7 g, 80%). Colourless powder. *R*_f(AcOEt/hexane 6:1) 0.40. $[\alpha]_D^{25} = -90.1$ (*c* = 0.75, CHCl₃). IR (CHCl₃): 3375w (br.), 3007m, 1750s, 1709s, 1612s, 1587s, 1504m, 1480s, 1469s, 1374s, 1072s. ¹H-NMR (200 MHz, CDCl₃): see Table 3; additionally, 9.22 (br. s, NH); 8.05–7.95 (*m*, 2 arom. H); 7.63–7.40 (*m*, 3 arom. H); 2.16 (*s*, AcO); 2.05 (*s*, AcO). ¹³C-NMR (50 MHz, CDCl₃): see Table 4; additionally, 170.0, 169.5 (2*s*, 2 O–C=O); 164.9 (*s*, N–C=O); 133.0 (*s*); 132.9 (*d*); 128.9 (*d*, 2 C); 128.0 (*d*, 2 C); 20.7 (*q*, Me); 20.4 (*q*, Me). FAB-MS (NOBA): 426 (69, *M*⁺), 427 (25, [*M* + H]⁺). HR-MALDI-MS: 448.123 (C₂₀H₁₉N₅NaO₆, [*M* + Na]⁺; calc. 448.123).

Table 3. Selected ¹H-NMR Chemical Shifts [ppm] and Coupling Constants [Hz] for the Nucleosides **7**–**13**, **16**–**19**, and **1** in CDCl₃, for **3** in (D₆)DMSO, and for **14** and **15** in CD₃OD

	7	3	8	9	10	11	12	13
H–C(1')	6.1–6.2	5.97	5.94	5.90	6.33	6.00	6.46	6.00
H–C(2' _{pro-S})	6.1–6.2	4.86	4.83	5.56	6.42	5.90	2.97	5.10
H–C(2' _{pro-R})	–	–	–	–	–	–	2.60	–
H _a –C(3')	5.74	4.28	5.00	4.45	5.47	6.12	4.93	2.32
H _b –C(3')	–	–	–	–	–	–	–	2.12
H _a –C(4')	4.73	4.40	4.50	4.58	4.62	4.88	4.43	4.42
H _b –C(4')	4.17	3.84	4.00	4.15	4.00	4.37	4.03	4.32
H–C(2)	8.75	8.75	8.76	8.80	8.71	8.76	8.82	8.80
H–C(8)	8.10	8.68	8.08	8.00	8.35	8.45	8.13	8.05
<i>J</i> (1',2')	^{a)}	6.6	4.2	5.3	4.1	6.2	6.6	1.6
<i>J</i> (2',2')	–	–	–	–	–	–	13.7	–
<i>J</i> (2' _{pro-S} ,3')	5.1	4.1	5.0	5.0	4.4	4.4	6.6	4.4
<i>J</i> (2' _{pro-R} ,3')	–	–	–	–	–	–	2.5	2.4
<i>J</i> (3',3')	–	–	–	–	–	–	–	13.0
<i>J</i> (3',4'a)	4.1	3.7	5.0	3.4	5.0	3.0	4.0	8.4, 4.0
<i>J</i> (3',4'b)	2.1	1.2	4.1	< 1.0	5.0	< 1.0	2.1	8.7, 6.2
<i>J</i> (4'a,4'b)	10.4	9.1	9.3	10.0	9.1	11.5	9.1	8.4
	14	15	16	17	18	19	1	
H–C(1')	6.57	6.10	6.50	6.68	6.47	6.37	6.46	
H–C(2' _{pro-S})	2.95	4.84	3.15	3.25	3.20	3.20	3.27	
H–C(2' _{pro-R})	2.58	–	2.39	2.50	2.43	2.35	2.60, 2.51	
H _a –C(3')	4.77	2.40	4.95	4.82	4.94	4.87	4.85	
H _b –C(3')	–	2.10	–	–	–	–	–	
H _a –C(4')	4.42	4.42	4.36	4.57	4.55	4.50	4.53	
H _b –C(4')	3.96	4.28	3.89	3.96	3.92	3.92	4.08, 4.04	
H _a –C(10)	–	–	4.90	4.95	4.55	4.52	4.53, 4.50	
H _b –C(10)	–	–	4.90	4.90	4.40	4.45	4.45, 4.42	
H–C(2)	8.70	8.70	8.68	8.66	8.80	8.76	8.78	
H–C(8)	8.50	8.42	–	–	–	–	–	
<i>J</i> (1',2')	6.6	1.9	6.6	6.8	6.6	6.6	6.6	
<i>J</i> (2',2')	14.0	–	13.7	14.0	13.3	14.0	13.3	
<i>J</i> (2' _{pro-S} ,3')	5.6	5.6	6.8	5.6	6.6	6.0	6.4	
<i>J</i> (2' _{pro-R} ,3')	1.2	3.1	1.0	1.3	< 1.0	< 1.0	< 1.0	
<i>J</i> (3'a,3'b)	–	14.0	–	–	–	–	–	
<i>J</i> (3',4'a)	3.7	8.4, 4.3	4.0	3.7	3.3	3.7	3.3	
<i>J</i> (3',4'b)	1.2	8.4, 7.1	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	
<i>J</i> (4'a,4'b)	9.3	8.4	8.7	9.0	8.7	9.3	9.3	
<i>J</i> (10 _a ,10 _b)	–	–	^{a)}	14.3	11.8	11.8	12.1	

^{a)} Not assigned.

Table 4. Selected ^{13}C -NMR Chemical Shifts [ppm] for **7**–**13**, **16**–**19**, and **1** in CDCl_3 , for **14** and **15** in CD_3OD , and for **3** in $(D_6)\text{DMSO}$

	C(1')	C(2')	C(3')	C(4')	C(10)	C(2)	C(4)	C(5)	C(6)	C(8)
7	87.3	74.2	71.6	72.7	–	151.8	149.9	124.1	152.9	142.1
3	88.0	74.5	70.4	74.0	–	151.9	150.6	126.3	152.6	144.0
8	91.4	75.2	71.7	74.7	–	152.9	150.2	124.1	151.5	142.5
9	91.2	75.9	71.9	74.7	–	153.2	150.2	124.1	151.5	143.1
10	87.7	83.4	70.4	74.4	–	152.6	150.3	124.2	151.7	142.3
11	91.0	74.3	82.4	72.0	–	152.7	150.3	124.2	151.7	143.0
12	86.2	41.7	72.6	77.3	–	152.8	149.9	124.2	151.8	142.2
13	92.9	76.4	33.8	69.3	–	152.5	149.6	123.8	151.2	141.3
14	87.2	41.7	73.0	78.0	–	153.0	151.3	125.6	153.1	144.8
15	93.8	76.7	33.8	70.5	–	153.0	151.0	125.6	152.8	144.0
16	85.6	40.6	73.0	77.6	57.8	154.8	149.3	122.0	153.0 ^{a)}	152.4 ^{a)}
17	87.2	40.7	73.3	78.2	58.3	156.5	150.5	124.2	154.5 ^{a)}	152.9 ^{a)}
18	86.1	40.6	73.2	77.8	59.7	152.0	149.5	122.5	152.7 ^{a)}	152.6 ^{a)}
19	85.7	39.9	72.7	77.4	59.5	152.0	149.4	122.5	152.6 ^{a)}	152.5 ^{a)}
1	85.9	39.2	74.4	76.5	59.9	152.2	149.7	122.7	152.7 ^{a)}	152.6 ^{a)}
	85.8	39.0	74.2	76.2		152.1	149.6			

^{a)} Assignment may be interchanged.

N^6 -Benzoyl-9-(β -D-erythrofuranosyl)adenine $\cdot$ MeOH (**3**). At 23°, a soln. of **7** (5.2 g, 12.2 mmol) in THF (190 ml), MeOH (152 ml), and H_2O (38 ml) was treated with 2M NaOH in H_2O (25 ml, 50 mmol), stirred at 23° for 10 min, treated with NH_4Cl (5.26 g, 98.3 mmol), and evaporated after addition of SiO_2 (46 g). The resulting powder was dried at 0.1 mbar for 14 h, filled in a column, and eluted with $\text{CHCl}_3/\text{MeOH}$ 10:1. The colourless crystals obtained from a few pooled fractions were submitted to X-ray analysis. Evaporation of the residual fractions gave **3** (3.2 g, 78%). Colourless powder. R_f ($\text{CHCl}_3/\text{MeOH}$ 10:1) 0.20. IR (KBr): 3407s (br.), 3237s (br.), 1685s, 1608m, 1572s, 1518s, 1466m, 1428m, 1396m, 1325m, 1294m, 1248m, 1194m, 1146m, 1107m, 1070s, 1025s. ^1H -NMR (200 MHz, $(D_6)\text{DMSO}$): see Table 3; additionally, 11.17 (br. s, NH); 8.06–7.97 (m, 2 arom. H); 7.69–7.47 (m, 3 arom. H); 5.56 (d , $J=6.2$, $\text{HO}-\text{C}(2')$); 5.29 (d , $J=3.7$, $\text{HO}-\text{C}(3')$); 4.01 (q , $J=5.5$, MeOH); 3.16 (d , $J=5.5$, MeOH). ^{13}C -NMR (50 MHz, $(D_6)\text{DMSO}$): see Table 4; additionally, 165.9 (s, $\text{C}=\text{O}$); 133.6 (s); 132.6 (d); 128.7 (d , 4 C); 48.6 (q , MeOH). FAB-MS (NOBA): 342 (100, $[M+H]^+$).

N^6 -Benzoyl-9-(3'-O-(triisopropylsilyl)- β -D-erythrofuranosyl)adenine (**8**) and N^6 -Benzoyl-9-(2'-O-(triisopropylsilyl)- β -D-erythrofuranosyl)adenine (**9**). A soln. of **3** (3.2 g, 9.4 mmol) in pyridine (4.1 ml, distilled from CaH_2) and DMF (64 ml, distilled from CaH_2) was treated with $^i\text{Pr}_3\text{SiOTf}$ (3.2 ml, 12 mmol) at 23°, stirred for 4 h, treated with $^i\text{Pr}_3\text{SiOTf}$ (3 ml, 11 mmol), stirred for 4 h at 23°, and evaporated. FC (AcOEt/hexane 6:1) gave **8/9** 1:1 (4.3 g, 92%). A soln. of this mixture in 150 ml of Et_3N was treated with SiO_2 (60 g), stirred at 23° for 16 h, and evaporated. FC ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 10:1) gave **8/9** 2:1. Colourless foam.

Data of **8/9**: R_f (AcOEt/hexane 6:1) 0.41. $[\alpha]_D^{25} = -58.4$ ($c=1.135$, CHCl_3 , **8/9** 2:1). IR (CHCl_3 , **8/9** 2:1): 3411w (br.), 3003m, 2947s, 2893m, 2869s, 1708s, 1671m, 1612s, 1585s, 1503m, 1457s, 1387m, 1354m, 1328m, 1141m, 1112m. ^1H -NMR (300 MHz, CDCl_3 , **8/9** 2:1): see Table 3; additionally, 9.13 (br. s, 0.33 H), 9.10 (br. s, 0.67 H) (NH); 8.05–7.95 (m, 2 arom. H); 7.65–7.40 (m, 3 arom. H); 3.45 (d , $J=5.6$, 0.67 H, exch., $\text{HO}-\text{C}(2')$ of **8**); 3.02–2.96 (m, 0.33 H, exch., $\text{HO}-\text{C}(3')$ von **9**); 1.20–0.80 (m, $(\text{Me}_2\text{CH})_3\text{Si}$). ^{13}C -NMR (75 MHz, CDCl_3 , **8/9** 2:1): see Table 4; additionally, 165.9 (s, $\text{C}=\text{O}$); 134.0 (s); 133.1 (d); 129.0 (d , 2 C); 128.4 (d , 2 C); 17.9–17.5 ($4q$, $(\text{Me}_2\text{CH})_3\text{Si}$); 12.4–12.0 ($3d$, $(\text{Me}_2\text{CH})_3\text{Si}$). FAB-MS (NOBA, **8/9** 2:1): 498 (100, $[M+H]^+$). HR-MALDI-MS (**8/9** 2:1): 498.254 ($\text{C}_{25}\text{H}_{36}\text{N}_5\text{O}_4\text{Si}$, $[M+H]^+$; calc. 498.254); 520.236 ($\text{C}_{25}\text{H}_{35}\text{N}_5\text{NaO}_4\text{Si}$, $[M+\text{Na}]^+$; calc. 520.236).

N^6 -Benzoyl-9-[2'-O-(imidazol-1-yl)thiocarbonyl]-3'-O-(triisopropylsilyl)- β -D-erythrofuranosyladenine (**10**) and N^6 -Benzoyl-9-(3'-O-(imidazol-1-yl)thiocarbonyl)-2'-O-(triisopropylsilyl)- β -D-erythrofuranosyladenine (**11**). A soln. of **8/9** 2:1 (2 g, 4 mmol) in CH_2Cl_2 (20 ml) was treated with 1,1'-(thiocarbonyl)diimidazole (4.6 g, 26 mmol), stirred at 23° for 3 d, extracted once with H_2O and brine, and dried (Na_2SO_4). Evaporation gave a yellow oil (3.8 g), consisting of **10/11** (2:1) and 1,1'-(thiocarbonyl)diimidazole. It was used without

further purification. R_f (AcOEt/hexane 6:1, **10/11** 2:1) 0.33. $^1\text{H-NMR}$ (300 MHz, CDCl_3 , **10/11** 2:1): see Table 3; additionally, 9.40 (br. s, 0.67 H), 9.37 (br. s, 0.33 H) (NH); 8.20–7.80 (*m*, 3 arom. H); 7.80–7.30 (*m*, 3 arom. H); 7.20–6.95 (*m*, 2 arom. H); 1.20–0.80 (*m*, $(\text{Me}_2\text{CH})_3\text{Si}$). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): see Table 4; additionally, 183.0, 182.9 (2s, C=S); 165.3, 165.1 (2s, C=O); 137.0 (*d*); 133.7 (*s*); 133.6 (*s*); 132.8–128.0 (several *d*); 118.1 (*d*); 17.8–17.4 (several *q*, $(\text{Me}_2\text{CH})_3\text{Si}$), 12.1, 11.9 (2*d*, $(\text{Me}_2\text{CH})_3\text{Si}$). FAB-MS (NOBA, **10/11** 2:1): 608 (63, $[M+H]^+$).

N^6 -Benzoyl-9-[2'-deoxy-3'-O-(triisopropylsilyl)- β -D-glycero-tetrofuranosyl]adenine (**12**) and N^6 -Benzoyl-9-[3'-deoxy-2'-O-(triisopropylsilyl)- β -D-glycero-tetrofuranosyl]adenine (**13**). A suspension of **10/11** 2:1 (3.8 g) in toluene (20 ml) and a mixture of Bu_3SnH (6 ml, 22.6 mmol) and AIBN (3.5 g, 21 mmol) in toluene (10 ml) were each degassed. The suspension of **10/11** was heated to 110°, treated with the soln. of AIBN and Bu_3SnH in 6 portions over 40 min, and stirred at 110° for 2 h. The soln. was diluted with CHCl_3 (200 ml), extracted once with H_2O and brine, dried (Na_2SO_4), and evaporated. FC (AcOEt/hexane 1:1) gave **12** (850 mg, 44% from **8/9**) and **13** (385 mg, 20% from **8/9**) as colourless foams.

Data of **12**: R_f (AcOEt/hexane 6:1) 0.51. $[\alpha]_D^{25} = -40.8$ ($c = 1.07$, CHCl_3). IR (CHCl_3): 3406w (br.), 3003*m*, 2946*m*, 2892w, 2868*m*, 1708*m*, 1612s, 1583s, 1503*m*, 1457s, 1386w, 1346w, 1328*m*, 1071s. $^1\text{H-NMR}$ (200 MHz, CDCl_3): see Table 3; additionally, 9.00 (br. s, NH); 8.10–8.00 (*m*, 2 arom. H); 7.65–7.50 (*m*, 3 arom. H); 1.20–1.00 (*m*, $(\text{Me}_2\text{CH})_3\text{Si}$). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): see Table 4; additionally, 165.2 (*s*, C=O); 134.0 (*s*); 133.1 (*d*); 129.2 (*d*, 2 C); 128.3 (*d*, 2 C); 17.9 (*q*, $(\text{Me}_2\text{CH})_3\text{Si}$); 12.0 (*d*, $(\text{Me}_2\text{CH})_3\text{Si}$). FAB-MS (NOBA): 482 (77, $[M+H]^+$). HR-FAB-MS: 482.2585 ($\text{C}_{25}\text{H}_{36}\text{N}_5\text{O}_3\text{Si}$, $[M+H]^+$; calc. 482.2587).

Data of **13**: R_f (AcOEt/hexane 6:1) 0.57. $[\alpha]_D^{25} = -51.1$ ($c = 1.065$, CHCl_3). IR (CHCl_3): 3403w (br.), 3002*m*, 2946*m*, 2892w, 2868*m*, 1707*m*, 1612s, 1584*m*, 1501*m*, 1455s, 1327w. $^1\text{H-NMR}$ (300 MHz, CDCl_3): see Table 3; additionally, 9.00 (br. s, NH); 8.07–7.98 (*m*, 2 arom. H); 7.65–7.45 (*m*, 3 arom. H); 1.20–1.00 (*m*, $(\text{Me}_2\text{CH})_3\text{Si}$). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): see Table 4; additionally, 165.0 (*s*, C=O); 133.7 (*s*); 132.7 (*d*); 128.7 (*d*, 2 C); 128.0 (*d*, 2 C); 17.9 (*q*, $(\text{Me}_2\text{CH})_3\text{Si}$); 12.0 (*d*, $(\text{Me}_2\text{CH})_3\text{Si}$). FAB-MS (NOBA): 482 (55, $[M+H]^+$). HR-MALDI-MS: 504.240 ($\text{C}_{25}\text{H}_{35}\text{N}_5\text{NaO}_3\text{Si}$, $[M+Na]^+$; calc. 504.240).

N^6 -Benzoyl-9-(2'-deoxy- β -D-glycero-tetrofuranosyl)adenine (**14**). A soln. of **12** (20 mg, 0.042 mmol) in THF (2 ml) was treated with 1*M* Bu_4NF in THF (0.24 ml, 0.24 mmol) at 23° for 3 h and evaporated after addition of SiO_2 (0.5 g). The resulting powder was dried at 0.1 mbar for 14 h and filled in a column. Elution ($\text{CHCl}_3/\text{MeOH}$ 20:1) gave **14** (10 mg, 74%) as colourless powder. R_f ($\text{CHCl}_3/\text{MeOH}$ 10:1) 0.29. $^1\text{H-NMR}$ (300 MHz, CD_3OD): see Table 3; additionally, 8.10–8.00 (*m*, 2 arom. H); 7.70–7.50 (*m*, 3 arom. H). $^{13}\text{C-NMR}$ (75 MHz, CD_3OD): see Table 4; additionally, 168.0 (*s*, C=O); 135.0 (*s*); 134.0 (*d*); 130.0 (*d*, 2 C); 129.8 (*d*, 2 C). FAB-MS (NOBA): 326 (56, $[M+H]^+$), 348 (16, $[M+Na]^+$).

N^6 -Benzoyl-9-(3'-deoxy- β -D-glycero-tetrofuranosyl)adenine (**15**). A soln. of **13** (100 mg, 0.208 mmol) in THF (2 ml) was treated with 1*M* Bu_4NF in THF (0.62 ml, 0.62 mmol) at 23° for 3 h and evaporated after addition of SiO_2 (0.5 g). The resulting powder was dried at 0.1 mbar for 14 h and filled in a column. Elution ($\text{CHCl}_3/\text{MeOH}$ 20:1) gave **15** (60 mg, 89%) as colourless powder. R_f ($\text{CHCl}_3/\text{MeOH}$ 10:1) 0.42. $^1\text{H-NMR}$ (300 MHz, CD_3OD): see Table 3; additionally, 8.10–8.00 (*m*, 2 arom. H); 7.70–7.50 (*m*, 3 arom. H). $^{13}\text{C-NMR}$ (75 MHz, CD_3OD): see Table 4; additionally, 168.0 (*s*, C=O); 135.0 (*s*); 134.0 (*d*); 129.9 (*d*, 2 C); 129.5 (*d*, 2 C). FAB-MS (NOBA): 326 (65, $[M+H]^+$).

N^6 -Benzoyl-9-(2-deoxy-3-O-(triisopropylsilyl)- β -D-erythrofuranosyl)-8-(hydroxymethyl)adenine (**16**). At –70°, a soln. of **12** (800 mg, 1.663 mmol) in THF (20 ml) was treated dropwise with 2*M* LDA in THF (4.2 ml, 8.4 mmol), stirred at –70° for 2 h, treated with DMF (3.3 ml, 42.8 mmol), stirred at –70° for 2.5 h, treated with AcOH (1.4 ml), allowed to warm to 23°, and diluted with EtOH (30 ml). The resulting soln. was treated with NaBH_4 (220 mg, 5.8 mmol) for 25 min and evaporated. The residue was taken up in CH_2Cl_2 (100 ml), washed once with H_2O (50 ml) and brine (50 ml), dried (Na_2SO_4), and evaporated. FC (AcOEt/hexane 6:1) gave **16** (748 mg, 88%). Colourless foam. R_f (AcOEt/hexane 6:1) 0.34. $[\alpha]_D^{25} = -40.4$ ($c = 1.095$, CHCl_3). IR (CHCl_3): 3404*m* (br.), 3006*m*, 2945s, 2892*m*, 2868s, 1706s, 1655*m*, 1614s, 1585s, 1462s, 1436s, 1384*m*, 1336s, 1072s. $^1\text{H-NMR}$ (300 MHz, CDCl_3): see Table 3; additionally, 9.60 (br. s, NH); 8.10–7.90 (*m*, 2 arom. H); 7.60–7.40 (*m*, 3 arom. H); 4.15 (br. s, exch., OH); 1.20–0.80 (*m*, $(\text{Me}_2\text{CH})_3\text{Si}$). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): see Table 4; additionally, 165.5 (*s*, C=O); 134.2 (*s*); 132.8 (*d*); 129.0 (*d*, 2 C); 128.3 (*d*, 2 C); 17.9 (*q*, $(\text{Me}_2\text{CH})_3\text{Si}$); 12.0 (*d*, $(\text{Me}_2\text{CH})_3\text{Si}$). FAB-MS (NOBA): 512 (35, $[M+H]^+$). HR-MALDI-MS: 512.268 ($\text{C}_{26}\text{H}_{38}\text{N}_5\text{O}_4\text{Si}$, $[M+H]^+$; calc. 512.269), 534.251 ($\text{C}_{26}\text{H}_{37}\text{N}_5\text{NaO}_4\text{Si}$, $[M+Na]^+$; calc. 534.251).

N^6 -Benzoyl-9-(2-deoxy- β -D-erythrofuranosyl)-8-(hydroxymethyl)adenine (**17**). A soln. of **16** (20 mg, 0.039 mmol) in THF (1 ml) was treated with 1*M* Bu_4NF in THF (0.12 ml, 0.12 mmol) at 23° for 3 h and evaporated after addition of SiO_2 (0.4 g). The resulting powder was dried at 0.1 mbar for 14 h and filled in a column. Elution ($\text{CHCl}_3/\text{MeOH}$ 20:1 to 10:1) gave **17** (11 mg, 80%) as colourless powder. R_f ($\text{CHCl}_3/\text{MeOH}$

6:1) 0.51. ¹H-NMR (300 MHz, CD₃OD): see Table 3; additionally, 8.10–8.00 (*m*, 2 arom. H); 7.70–7.50 (*m*, 3 arom. H). ¹³C-NMR (75 MHz, CD₃OD): see Table 4; additionally, 168.3 (*s*, C=O); 135.2 (*s*); 134.0 (*d*); 129.8 (*d*, 2 C); 129.5 (*d*, 2 C). FAB-MS (NOBA): 356 (78, [*M* + H]⁺), 378 (41, [*M* + Na]⁺).

N⁶-Benzoyl-9-[2'-deoxy-3'-O-(triisopropylsilyl)-β-D-glycero-tetrofuranosyl]-8-[4,4'-dimethoxytrityloxy]-methyladenine (**18**). A soln. of **16** (748 mg, 1.46 mmol), EtN(i-Pr)₂ (1.03 ml, 6 mmol) and DMAP (50 mg, 0.4 mmol) in CH₂Cl₂ (15 ml) was treated with DMTrCl (2 g, 5.91 mmol) at 0°. The mixture was stirred at 23° for 16 h, and evaporated. The residue was diluted with CH₂Cl₂, washed once with H₂O and brine, dried (Na₂SO₄), and evaporated. FC (AcOEt/hexane 1:2) gave **18** (1056 mg, 89%). Colourless foam. *R*_f(AcOEt/hexane 2:1) 0.61. [*α*]_D²⁵ = –15.2 (*c* = 2.32, CHCl₃). IR (CHCl₃): 3409*m* (br.), 3007*m*, 2960*m*, 2867*w*, 1706*m*, 1610*s*, 1584*m*, 1509*s*, 1463*m*, 1447*m*, 1428*m*, 1070*m*, 1034*m*. ¹H-NMR (200 MHz, CDCl₃): see Table 3; additionally, 9.06 (br. *s*, NH); 8.10–8.00 (*m*, 2 arom. H); 7.69–7.20 (*m*, 12 arom. H); 6.92–6.80 (*m*, 4 arom. H); 3.78 (*s*, 2 MeO); 1.20–1.00 (*m*, (Me₂CH)₃Si). ¹³C-NMR (75 MHz, CDCl₃): see Table 4; additionally, 165.0 (*s*, C=O); 159.2 (*s*, 2 C); 144.4 (*s*); 135.6 (*s*); 135.3 (*s*); 134.4 (*s*); 132.8 (*d*); 130.2–127.2 (several *d*); 113.3 (*d*, 4 C); 87.9 (*s*, Ar₃C); 55.3 (*q*, 2 MeO); 18.0 (*q*, (Me₂CH)₃Si), 12.0 (*d*, (Me₂CH)₃Si). FAB-MS (NOBA): 814 (38, [*M* + H]⁺), 303 (100, DMTr⁺). HR-MALDI-MS: 836.382 (C₄₇H₅₅N₅NaO₆Si, [*M* + Na]⁺; calc. 836.381).

N⁶-Benzoyl-9-[2'-deoxy-β-D-glycero-tetrofuranosyl]-8-[4,4'-dimethoxytrityloxy)methyladenine (**19**). A soln. of **18** (976 mg, 1.2 mmol) in THF (15 ml) was treated dropwise with 1*M* Bu₄NF in THF (3.55 ml, 3.55 mmol) at 23° for 1.5 h and evaporated. FC (AcOEt/hexane 2:1) gave **19** (600 mg, 76%). Colourless foam. *R*_f(AcOEt/hexane 2:1) 0.13. [*α*]_D²⁵ = –12.5 (*c* = 1.69, CHCl₃). IR (CHCl₃): 3405*m* (br.), 3007*s*, 2839*w*, 1707*m*, 1613*s*, 1585*m*, 1509*s*, 1462*s*, 1448*s*, 1428*s*, 1409*m*, 1336*s*, 1302*s*, 1065*s*, 1036*s*. ¹H-NMR (300 MHz, CDCl₃): see Table 3; additionally, 9.20 (br. *s*, NH); 8.05–7.95 (*m*, 2 arom. H); 7.60–7.10 (*m*, 12 arom. H); 6.85–6.75 (*m*, 4 arom. H); 3.75 (*s*, 2 MeO); 2.60–2.40 (br. *s*, exch., HO–C(3')). ¹³C-NMR (50 MHz, CDCl₃): see Table 4; additionally, 164.9 (*s*, C=O); 159.0 (*s*, 2 C); 144.3 (*s*); 135.3 (*s*); 135.2 (*s*); 134.2 (*s*); 132.9 (*d*); 130.2–127.3 (several *d*); 113.6 (*d*, 4 C); 88.0 (*s*, Ar₃C); 55.3 (*q*, 2 MeO). FAB-MS (NOBA): 659 (2, [*M* + H]⁺), 303 (100, DMTr⁺). HR-FAB-MS (NOBA): 658.2663 (C₃₈H₃₆N₅O₆, [*M* + H]⁺; calc. 658.2666).

N⁶-Benzoyl-9-(2'-deoxy-β-D-glycero-tetrofuranosyl)-8-[4,4'-dimethoxytrityloxy)methyladenine 3'-[(2-Cyanoethyl)diisopropylphosphoramidite] (**1**). A soln. of **19** (395 mg, 0.601 mmol) and EtN(i-Pr)₂ (2 ml) in CH₂Cl₂ (20 ml) was treated with 2-cyanoethyl diisopropylchlorophosphoramidite (184 mg, 0.77 mmol) at 23°, stirred for 3 h, and evaporated. FC (AcOEt/hexane 2:1) gave **1** (413 mg, 80%, 2 diastereoisomers (1:1)). Colourless foam. *R*_f(AcOEt/hexane 2:1, 2 diastereoisomers (1:1)) 0.34; 0.45. IR (CHCl₃, 2 diastereoisomers (1:1)): 3404*w* (br.), 3007*m*, 2970*m*, 2935*m*, 1707*m*, 1613*s*, 1585*m*, 1509*s*, 1462*m*, 1448*m*, 1428*w*, 1408*m*, 1365*m*, 1337*m*, 1063*m*, 1036*m*. ¹H-NMR (300 MHz, CDCl₃, 2 diastereoisomers (1:1)): see Table 3; additionally, 9.00 (br. *s*, NH); 8.10–7.95 (*m*, 2 arom. H); 7.70–7.20 (*m*, 12 arom. H); 6.90–6.80 (*m*, 4 arom. H); 3.90–3.50 (*m*, OCH₂CH₂CN, (Me₂CH)₂N); 3.78 (*s*, 2 MeO); 2.63 (*t*, *J* = 6.3), 2.55 (*t*, *J* = 6.1) (CH₂CN); 1.30–1.10 (*m*, (Me₂CH)₂N). ¹³C-NMR (75 MHz, CDCl₃, 2 diastereoisomers (1:1)): see Table 4; additionally, 165.1 (*s*, N–C=O); 159.2 (*s*, 2 C); 144.5, 144.4 (2*s*, 1 C); 135.6, 135.5, 135.4, 134.4 (several *s*, 3 C); 132.9–127.5 (several *d*); 117.8 (*s*, CN); 113.7 (*d*, 4 C); 88.1 (*s*, Ar₃C); 58.5 (*dt*, ²*J*(C,P) = 19.0, OCH₂CH₂CN); 55.4 (*q*, 2 MeO); 43.4 (*dd*, ²*J*(C,P) = 12.0, (Me₂CH)₂N); 24.8–24.5 (several *q*, (Me₂CH)₂N); 20.5–20.3 (*m*, OCH₂CH₂CN). ³¹P-NMR (121.5 MHz, CDCl₃): 148.9; 148.6. FAB-MS (NOBA): 858 (49, [*M* + H]⁺), 303 (100, DMTr⁺). HR-MALDI-MS: 880.356 (C₄₇H₅₂N₇NaO₇P, [*M* + Na]⁺; calc. 880.356).

1-(2',3'-Di-O-acetyl-β-D-erythrofuransyl)uracil (**20**). A suspension of **5/6** 1:4 (1.8 g, 7.3 mmol) and uracil (0.92 g, 8.2 mmol) in MeCN (18 ml) was treated dropwise with *N,O*-bis(trimethylsilyl)acetamide (4.32 ml, 17.7 mmol) at 60°, stirred at 60° for 30 min, and treated with SnCl₄ (3.47 ml, 29.5 mmol). The mixture was stirred at 60° for 20 min, poured into a stirred mixture of AcOEt (300 ml) and sat. aq. NaHCO₃ (300 ml), and stirred for 15 min. The org. layer was dried (Na₂SO₄) and evaporated. FC (AcOEt/hexane 10:1) gave **20** (1.74 g, 80%). Colourless powder. *R*_f(AcOEt/hexane 6:1) 0.38. ¹H-NMR (200 MHz, CDCl₃): see Table 5; additionally, 9.48 (br. *s*, NH); 2.14 (*s*, AcO); 2.09 (*s*, AcO). ¹³C-NMR (75 MHz, CDCl₃): see Table 6; additionally, 170.4, 170.2 (2*s*, 2 O–C=O); 20.7, 20.5 (2*q*, 2 Me).

1-(β-D-Erythrofuransyl)uracil (**4**). At 23°, a soln. of **20** (1.1 g, 3.7 mmol) in a mixture of THF (94 ml), MeOH (75 ml), and H₂O (19 ml) was treated with 2*M* NaOH in H₂O (12.4 ml, 25 mmol), stirred at 23° for 8 min, treated with NH₄Cl (2.6 g, 48.6 mmol), treated with SiO₂ (22.8 g), and evaporated. The resulting colourless powder was dried at 0.1 mbar for 14 h, filled in a column, and eluted (CHCl₃/MeOH 6:1). The colourless crystals obtained from a few pooled fractions were submitted to X-ray analysis. Evaporation of the residual fractions gave **4** (0.63 g, 80%). Colourless crystals. *R*_f(CHCl₃/MeOH 6:1) 0.16. [*α*]_D²⁵ = –41.2 (*c* = 0.9, H₂O). IR (KBr): 3385*s* (br.), 3186*s*, 1693*s*, 1470*m*, 1404*s*, 1262*m*, 1130*m*, 1065*m*. ¹H-NMR (300 MHz, (D₆)DMSO): see

Table 5. *Selected ^1H -NMR Chemical Shifts [ppm] and Coupling Constants [Hz] for **20**, **22**–**24**, **26**–**28**, and **2** in CDCl_3 , for **4** in $(D_6)\text{DMSO}$, and for **21** and **25** in D_2O*

	2	4	20	22	23	24	26	27	28	21	25
H–C(1')	5.87	5.70	5.86	6.27	5.90	6.15	6.11	5.90	5.85	6.56	6.18
H–C(2' _{pro-S})	2.88	4.18	5.5–5.6	5.13	4.64	2.11	2.90	2.85	2.82	5.45	2.19
H–C(2' _{pro-R})	2.32, 2.25	–	–	–	–	2.50	2.23	2.18	2.12	–	2.40
H–C(3')	4.72	4.08	5.4–5.5	4.64	4.53	4.65	4.83	4.73	4.65	4.70	4.53
H _a –C(4')	4.34	4.21	4.52	4.04	4.35	4.20	4.42	4.38	4.34	4.16	4.25
H _b –C(4')	3.4–3.9	3.67	4.06	3.80	4.02	3.87	3.83	3.73	3.73	3.93	3.88
H _a –C(7)	3.98, 3.97	–	–	–	–	–	4.52	3.96	4.02	–	–
H _b –C(7)	3.98, 3.97	–	–	–	–	–	4.45	3.96	3.95	–	–
H–C(5)	5.76, 5.73	5.60	5.77	6.06	5.76	5.73	5.80	5.80	5.80	6.22	5.69
H–C(6)	–	7.65	7.22	7.38	7.20	7.32	–	–	–	7.94	7.61
$J(1', 2')$	6.5	6.2	5.3	5.0	5.6	6.5	6.6	7.0	7.0	5.3	7.0
$J(2', 2')$	13.7	–	–	–	–	14.0	12.8	13.0	13.4	–	14.0
$J(2'_{\text{pro-S}}, 3')$	6.5	4.4	^{a)}	< 1.0	4.7	6.2	6.5	6.5	6.5	< 1.0	5.3
$J(2'_{\text{pro-R}}, 3')$	^{a)}	–	–	–	–	2.3	< 1.0	< 1.0	< 1.0	–	1.6
$J(3', 4'a)$	4.4	4.0	4.5	< 1.0	4.4	4.2	4.4	4.1	4.0	< 1.0	3.7
$J(3', 4'b)$	< 1.0	2.2	2.0	2.5	3.7	2.2	< 1.0	< 1.0	< 1.0	2.8	1.5
$J(4'a, 4'b)$	8.7	9.1	10.3	10.6	9.0	9.0	8.5	8.4	9.0	11.2	9.3
$J(5, 6)$	–	8.1	8.1	7.5	8.1	8.1	–	–	–	7.5	8.1
$J(7_a, 7_b)$	^{a)}	–	–	–	–	–	14.7	^{a)}	13.4	–	–

^{a)} Not assigned.Table 6. *Selected ^{13}C -NMR Chemical Shifts [ppm] for **20**, **22**–**24**, **26**–**28**, and **2** in CDCl_3 , for **4** in $(D_6)\text{DMSO}$, and for **21** and **25** in D_2O*

	C(1')	C(2')	C(3')	C(4')	C(7)	C(6)	C(2)	C(4)	C(5)
20	89.9	73.8	71.1	72.4	–	140.7	150.7	163.4	103.4
4	88.8	74.1	70.0	73.6	–	141.8	151.0	163.5	102.0
21	93.4 ^{a)}	90.6 ^{a)}	75.7 ^{b)}	75.7 ^{b)}	–	141.7	164.2	178.7	112.1
22	90.3 ^{a)}	87.7 ^{a)}	75.0 ^{b)}	74.0 ^{b)}	–	135.2	160.3	172.0	110.8
23	95.4	52.4	71.7	74.8	–	141.4	150.1	163.8	102.9
24	88.0	42.4	72.0	77.2	–	140.0	150.8	164.0	102.6
25	88.4	41.9	72.3	77.5	–	142.3	152.1	166.0	102.6
26	87.7	40.4	73.6	78.7	61.2	156.0	151.2	164.0	101.6
27	88.1	40.3	73.5	78.5	62.8	153.5	151.2	163.8	102.5
28	88.1	39.3	73.0	78.0	62.5	153.3	151.2	163.6	102.6
2	88.2	38.4	74.5	79.8	62.8	153.3	150.6	162.6	102.6
		38.3	74.3	79.6			150.5		102.5

^{a)} ^{b)} Assignments may be interchanged.

Table 5; additionally, 11.22 (br. s, NH); 5.34 (*d*, $J = 6.2$, exch., HO–C(2')); 5.07 (*d*, $J = 3.7$, exch., HO–C(3')). ^{13}C -NMR (50 MHz, $(D_6)\text{DMSO}$): see Table 6. FAB-MS (NOBA): 215 (100, $[M + H]^+$).

2,2'-Anhydro-1-(β -D-threofuranosyl)uracil (**21**). A suspension of **4** (1 g, 4.67 mmol) and diphenyl carbonate (1.29 g, 0.61 mmol) in DMF (25 ml) was stirred at 80° for 30 min, treated with NaHCO_3 (390 mg) at 80°, stirred at 150° for 2 h, and evaporated. The residue was diluted with AcOEt and H_2O . Evaporation of the aq. layer gave 998 mg of a colourless powder containing **21** and Na salts. $R_f(\text{AcOEt}/\text{MeOH } 3:2)$ 0.57. $[\alpha]_D^{25} = -44$ ($c = 1.05$, H_2O). IR (KBr): 2923*m*, 1662*s*, 1620*m*, 1533*s*, 1482*s*, 1272*w*, 1241*w*, 1190*w*, 1113*w*, 1062*m*, 1041*m*. ^1H -NMR (300 MHz, D_2O): see Table 5. ^{13}C -NMR (75 MHz, D_2O): see Table 6. FAB-MS (NOBA): 219 (100, $[M + \text{Na}]^+$), 197 (24, $[M + H]^+$). HR-MALDI-MS: 219.037 ($\text{C}_8\text{H}_8\text{N}_2\text{NaO}_4$, $[M + \text{Na}]^+$; calc. 219.038).

2,2'-Anhydro-1-[3'-O-(triisopropylsilyl)- β -D-threofuranosyl]uracil (22). A soln. of **21** (1 g, 5.1 mmol) in DMF (16 ml) and pyridine (12.5 ml) was treated with Pr_3SiOTf (7 ml, 26 mmol), stirred at 23° for 24 h, and evaporated. FC (AcOEt/MeOH 30:1) gave **22** (790 mg, 61.5% from **4**). Colourless foam. R_f (AcOEt/MeOH 10:1) 0.37. $[\alpha]_D^{25} = -40.6$ ($c = 0.9$, CHCl_3). IR (CHCl_3): 2944s, 1672s, 1644s, 1544m, 1468s, 1387w, 1134m, 1072m, 1042m. $^1\text{H-NMR}$ (200 MHz, CDCl_3): see Table 5; additionally, 1.20–0.90 (m , $(\text{Me}_2\text{CH})_3\text{Si}$). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): see Table 6; additionally, 18.0 (q , $(\text{Me}_2\text{CH})_3\text{Si}$); 12.0 (d , $(\text{Me}_2\text{CH})_3\text{Si}$). FAB-MS (NOBA): 353 (100, $[M + H]^+$), 705 (8, $[2M + H]^+$). HR-MALDI-MS: 353.189 ($\text{C}_{17}\text{H}_{29}\text{N}_2\text{O}_4\text{Si}$, $[M + H]^+$; 353.189).

1-[2'-Bromo-2'-deoxy-3'-O-(triisopropylsilyl)- β -D-erythrofuransyl]uracil (23). A suspension of **22** (670 mg, 1.9 mmol) and LiBr (215 mg, 2.47 mmol) in 1,4-dioxane (20 ml) was heated to 60° and treated dropwise with $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (0.62 ml, 4.9 mmol). The mixture was stirred at 60° for 6 h, and taken to dryness. The residue was diluted with CHCl_3 (50 ml), washed once with H_2O (20 ml) and brine (20 ml), dried (Na_2SO_4), and evaporated. FC (AcOEt/hexane 1:1) gave **23** (720 mg, 88%). Colourless foam. R_f (AcOEt/hexane 1:1) 0.34. $[\alpha]_D^{25} = -29.9$ ($c = 1.15$, CHCl_3). IR (CHCl_3): 3391w (br.), 2940m, 2868m, 1695s, 1633w, 1457m, 1384m, 1143m, 1108m, 1068m, 1017m. $^1\text{H-NMR}$ (300 MHz, CDCl_3): see Table 5; additionally, 8.60 (br. s, NH); 1.15–1.03 (m , $(\text{Me}_2\text{CH})_3\text{Si}$). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): see Table 6; additionally, 18.0 (q , $(\text{Me}_2\text{CH})_3\text{Si}$); 12.0 (d , $(\text{Me}_2\text{CH})_3\text{Si}$). FAB-MS (NOBA): 433, 435 (23, 24; $[M + H]^+$). HR-MALDI-MS: 375.173 ($\text{C}_{17}\text{H}_{28}\text{N}_2\text{NaO}_4\text{Si}$, $[M - \text{HBr} + \text{Na}]^+$; calc. 375.172).

1-[2'-Deoxy-3'-O-(triisopropylsilyl)- β -D-glycero-tetrofuransyl]uracil (24). A suspension of **23** (696 mg, 1.61 mmol) in toluene (10 ml) and a mixture of Bu_3SnH (0.43 ml, 1.62 mmol) and AIBN (100 mg, 0.61 mmol) in toluene (5 ml) were each degassed. The suspension of **23** was heated to 80° and treated with the soln. of AIBN and Bu_3SnH over 20 min. The mixture was heated to 110°, stirred for 30 min, diluted with CHCl_3 (200 ml), washed with H_2O and brine, dried (Na_2SO_4), and evaporated. FC (AcOEt/hexane 1:1) gave **24** (556 mg, 97%). Colourless foam. R_f (AcOEt/hexane 1:1) 0.30. IR (CHCl_3): 3391w (br.), 2946m, 1690s, 1633w, 1461m, 1390m, 1135m, 1073m. $^1\text{H-NMR}$ (300 MHz, CDCl_3): see Table 5; additionally, 9.67 (br. s, NH); 1.17–0.94 (m , $(\text{Me}_2\text{CH})_3\text{Si}$). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): see Table 6; additionally, 18.0 (q , $(\text{Me}_2\text{CH})_3\text{Si}$); 12.0 (d , $(\text{Me}_2\text{CH})_3\text{Si}$). FAB-MS (NOBA): 355 (18, $[M + H]^+$). HR-FAB-MS: 355.2052 ($\text{C}_{17}\text{H}_{31}\text{N}_2\text{O}_4\text{Si}$, $[M + H]^+$; calc. 355.2053).

1-(2'-Deoxy- β -D-glycero-tetrofuransyl)uracil (25). A soln. of **24** (91 mg, 0.258 mmol) in THF (2 ml) was treated with 1M $\text{Bu}_4\text{NF} \cdot 3 \text{H}_2\text{O}$ in THF (0.76 ml, 0.76 mmol) at 23° for 3 h, and evaporated after addition of SiO_2 (0.5 g). The resulting powder was dried at 0.1 mbar for 14 h, filled in a column, and eluted ($\text{CHCl}_3/\text{MeOH}$ 20:1). Evaporation gave **25** (45 mg, 88%) as white powder. Recrystallization in MeOH gave colourless crystals used for X-ray analysis. R_f ($\text{CHCl}_3/\text{MeOH}$ 10:1) 0.28. $^1\text{H-NMR}$ (300 MHz, CD_3OD): see Table 5. $^{13}\text{C-NMR}$ (75 MHz, CD_3OD): see Table 6. FAB-MS (NOBA): 199 (28, $[M + H]^+$).

1-[2'-Deoxy-3'-O-(triisopropylsilyl)- β -D-glycero-tetrofuransyl]-6-(hydroxymethyl)uracil (26). At -70° , 2M LDA in THF/heptane/ethylbenzene (FLUKA; 1.63 ml, 3.26 mmol) was treated with a soln. of **24** (230 mg, 0.652 mmol) in THF (4 ml). The mixture was stirred at -70° for 30 min, treated with DMF (1.7 ml, 16.5 mmol), stirred at -70° for 2.5 h, and treated with AcOH (0.55 ml). The mixture was allowed to warm to 23°, diluted with EtOH (4 ml), and treated with NaBH_4 (83 mg, 2.17 mmol) for 25 min. After evaporation, a soln. of the residue in CH_2Cl_2 was washed with H_2O and brine, dried (Na_2SO_4), and evaporated. FC (AcOEt/hexane 4:1) gave **26** (128 mg, 51%). Colourless foam. R_f (AcOEt/hexane 1:1) 0.15. $[\alpha]_D^{25} = -82.2$ ($c = 0.4$, CHCl_3). IR (CHCl_3): 3390m (br.), 2946s, 2867s, 1697s, 1602m, 1462m, 1377m, 1094s, 1015s. $^1\text{H-NMR}$ (300 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$): see Table 5; additionally, 1.16–0.94 (m , $(\text{Me}_2\text{CH})_3\text{Si}$). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): see Table 6; additionally, 18.0 (q , $(\text{Me}_2\text{CH})_3\text{Si}$); 12.0 (d , $(\text{Me}_2\text{CH})_3\text{Si}$). FAB-MS (NOBA): 385 (21, $[M + H]^+$).

1-[2'-Deoxy-3'-O-(triisopropylsilyl)- β -D-glycero-tetrofuransyl]-6-[4,4'-dimethoxytrityloxy)methyl]uracil (27). A soln. of **26** (117 mg, 0.305 mmol), EtN(i-Pr)₂ (0.24 ml, 1.39 mmol) and DMAP (10 mg, 0.4 mmol) in CH_2Cl_2 (5 ml) was treated with DMTrCl (413 mg, 1.26 mmol) at 0°, stirred at 23° for 16 h, and evaporated. A soln. of the residue in CH_2Cl_2 (50 ml) was washed once with H_2O (20 ml) and brine (20 ml), dried (Na_2SO_4), and evaporated. FC (AcOEt/hexane/Et₃N 1:1:0.02) gave **27** (161 mg, 77%). Colourless foam. R_f (AcOEt/hexane 1:1) 0.36. $[\alpha]_D^{25} = -25$ ($c = 0.1$, CHCl_3). IR (CHCl_3): 3389m (br.), 2975m, 1695s, 1607m, 1509s, 1457w, 1045m. $^1\text{H-NMR}$ (300 MHz, CDCl_3): see Table 5; additionally, 9.75 (br. s, NH); 7.50–7.10 (m , 9 arom. H); 6.93–6.70 (m , 4 arom. H); 3.80 (s , 2 MeO); 1.15–0.75 (m , $(\text{Me}_2\text{CH})_3\text{Si}$). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): see Table 6; additionally, 159.4 (s , 2 C); 144.3 (s); 135.5 (s); 135.1 (s); 130.1–127.6 (several d); 113.6, 113.5 (2d, 4 C); 88.6 (s , Ar₃C); 55.4 (q , 2 MeO); 18.0 (q , $(\text{Me}_2\text{CH})_3\text{Si}$); 12.0 (d , $(\text{Me}_2\text{CH})_3\text{Si}$). FAB-MS (NOBA): 686 (7, $[M + H]^+$); 303 (100, DMTr⁺).

1-(2'-Deoxy- β -D-glycero-tetrofuransyl)-6-[4,4'-dimethoxytrityloxy)methyl]uracil (28). A soln. of **27** (161 mg, 0.235 mmol) in THF (6 ml) was treated dropwise at 23° with 1M Bu_4NF in THF (0.7 ml, 0.7 mmol)

for 1.5 h, and evaporated. FC (AcOEt/hexane/Et₃N 10:1:0.02) gave **28** (120 mg, 96%). Colourless foam. R_f (AcOEt/hexane 10:1) 0.24. $[\alpha]_D^{25} = -17.9$ ($c = 0.9$, CHCl₃). IR (CHCl₃): 3390m (br.), 2961m, 1692s, 1608m, 1584w, 1509s, 1464s, 1410m, 1378m, 1302m, 1066s, 1035s. ¹H-NMR (300 MHz, CDCl₃): see Table 5; additionally, 9.60 (br. s, NH); 7.50–7.20 (m, 9 arom. H); 6.90–6.70 (m, 4 arom. H); 3.75 (s, 2 MeO). ¹³C-NMR (75 MHz, CDCl₃): see Table 6; additionally, 159.2 (s, 2 C); 144.2 (s); 135.2 (s, 2 C); 130.1–127.7 (several d); 114.0 (d, 4 C); 88.2 (s, Ar₃C); 55.3 (q, 2 MeO). FAB-MS (NOBA): 530 (12, [M + H]⁺, 303 (100, DMTr⁺). HR-FAB-MS: 530.2053 (C₃₀H₃₀N₂O₇, M⁺; calc. 530.2053).

1-(2'-Deoxy-β-D-glycero-tetrofuranosyl)-6-[(4,4'-dimethoxytrityloxy)methyl]uracil 3'-[(2-Cyanoethyl)diisopropylphosphoramidite] (**2**). A soln. of **28** (108 mg, 0.204 mmol) and EtN(i-Pr)₂ (0.12 ml, 0.69 mmol, distilled from CaH₂) in CH₂Cl₂ (2 ml) was treated with 2-cyanoethyl diisopropylchlorophosphoramidite (46 μl, 0.206 mmol) at 23°, stirred for 3 h, and evaporated. FC (AcOEt/hexane 2:1) gave **2** (102 mg, 69%, 2 diastereoisomers (2:1)). Colourless foam. R_f (AcOEt/hexane 1:1, 2 diastereoisomers (2:1)) 0.26. IR (CHCl₃, 2 diastereoisomers (2:1)): 3391w (br.), 2967s, 2929s, 1693s, 1608s, 1509s, 1463s, 1378s, 1064m, 1035m. ¹H-NMR (300 MHz, CDCl₃, 2 diastereoisomers (2:1)): see Table 5; additionally, 8.30–8.20 (br. s, NH); 7.45–7.20 (m, 9 arom. H); 6.90–6.80 (m, 4 arom. H); 3.90–3.45 (m, Me₂CH)₂N, OCH₂CH₂CN, H–C(4''); 3.80 (s, 2 MeO); 2.62 (t, $J = 6.5$, 0.67 H), 2.52 (t, $J = 6.5$, 1.33 H) (CH₂CN); 1.36–1.00 (m, (Me₂CH)₂N). ¹³C-NMR (75 MHz, CDCl₃, 2 diastereoisomers (2:1)): see Table 6; additionally, 159.3 (s, 2 C); 144.2, 144.1 (2s, 1 C); 135.2, 135.0 (2s, 2 C); 130.2–127.7 (several d); 113.9 (d, 4 C); 111.3 (s, CN); 88.4 (s, Ar₃C); 58.5 (t, ²J(C,P) = 18, OCH₂CH₂CN); 55.5 (q, 2 MeO); 43.3 (dd, ²J(C,P) = 13.0, (Me₂CH)₂N); 24.8–24.4 (several q, (Me₂CH)₂N); 20.5, 20.4 (2t, OCH₂CH₂CN). ³¹P-NMR (121.5 MHz, CDCl₃, 2 diastereoisomers (2:1)): 148.7, 148.2. FAB-MS (NOBA, 2 diastereoisomers (2:1)): 513 (40, [M – OPR₂]⁺), 303 (100, DMTr⁺). HR-MALDI-MS: 535.184 (C₃₀H₂₈N₂NaO₆, [M – HOP(OCH₂CH₂CN)NⁱPr₂ + Na]⁺; calc. 535.185), 449.156 (C₁₈H₂₇N₄NaO₆P, [M – DMTrH + Na]⁺; calc. 449.157), 303.138 (C₂₁H₁₉O₂, DMTr⁺; calc. 303.139), 241.108 (C₉H₁₉N₂NaO₂P, [HOP(OCH₂CH₂CN)NⁱPr₂ + Na]⁺; calc. 241.108).

Oligonucleotide Synthesis. Oligonucleotide syntheses were performed on a *Pharmacia Gene Assembler* on a 1.3-μmol scale. The commercial phosphoramidites and the CPG solid-supports were from *Glen Research*. Solvents and reagents were prepared according to the protocol for the synthesis of pRNA [30]. Detritylation was accomplished within 2 min with 3% Cl₂CHCOOH in (CH₂Cl)₂. Couplings (0.16 ml of 0.1M phosphoramidite soln. + 0.36 ml of 0.25M 1-(benzylthio)-1H-tetrazole soln. in MeCN) were performed within 6–10 min (DNA-phosphoramidites coupled on unmodified nucleotides), or 30 min (modified phosphoramidites, and DNA-phosphoramidites coupled on modified nucleotides, resp.). Capping and oxidation were accomplished under standard conditions [31].

Deprotection and Purification of Oligonucleotides. Removal of the protecting groups and detachment from the solid support was effected in conc. aq. NH₃ soln./MeOH 1:1 (2.5 ml) at 50° within 20 h (oligomers **29** and **30**), or 3.5 h (oligomer **31**), or at 35° within 3.5 h (oligomer **32** containing dU*, see *Results and Discussion*). After filtration and evaporation of the resulting mixture, the crude oligomers were dissolved in aq. buffer soln. (0.1M AcOH, 0.1M Et₃N), purified by RP-HPLC, and desalted by RP-HPLC. Their compositions were confirmed by MALDI-TOF mass spectroscopy.

REFERENCES

- [1] S. Eppacher, N. Solladié, B. Bernet, A. Vasella, *Helv. Chim. Acta* **2000**, 83, 1311.
- [2] H. Gunji, A. Vasella, *Helv. Chim. Acta* **2000**, 83, 1331.
- [3] H. Gunji, A. Vasella, *Helv. Chim. Acta* **2000**, 83, 2975.
- [4] H. Gunji, A. Vasella, *Helv. Chim. Acta* **2000**, 83, 3229.
- [5] W. Czechtizky, A. Vasella, *Helv. Chim. Acta* **2001**, 84, 594.
- [6] F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, 11, 440.
- [7] W. Saenger, 'Principles of Nucleic Acid Structure', Springer Verlag, 1984, pp. 69–78.
- [8] C. Chu, L. Teng, T. Yang, L. Kotra, F. Naguib, Q. Teng, J. Sommadossi, M. Kouni, *Tetrahedron Lett.* **1995**, 36, 983.
- [9] J. Vastra, A. Vasella, unpublished results.
- [10] A. Serianni, P. Kline, *J. Org. Chem.* **1992**, 57, 1772.
- [11] A. Perlin, C. Brice, *Can. J. Chem.* **1955**, 33, 1216.
- [12] T. Storz, B. Bernet, A. Vasella, *Helv. Chim. Acta* **1999**, 82, 2380.

- [13] E. Westerlund, R. Andersson, O. Theander, *Carbohydr. Res.* **1978**, *61*, 501.
- [14] A. Serianni, J. Snyder, *Carbohydr. Res.* **1991**, *210*, 21.
- [15] D. Critcher, S. Conolly, M. Mahon, M. Wills, *J. Chem. Soc., Chem. Commun.* **1995**, 139.
- [16] C. Demuynck, C. Andre, J. Bolte, *Tetrahedron: Asymmetry* **1998**, *9*, 1359.
- [17] B. Vorbrüggen, B. Bennua, *Chem. Ber.* **1981**, *114*, 1279.
- [18] H. Vorbrüggen, K. Krolukiewicz, B. Bennua, *Chem. Ber.* **1981**, *114*, 1234.
- [19] J. Wagner, P. H. Verheyden, J. G. Moffatt, *J. Org. Chem.* **1974**, *39*, 24.
- [20] K. Ogilvie, G. Hakimelahi, Z. Proba, *Can. J. Chem.* **1982**, *60*, 1106.
- [21] H. Hayakawa, K. Haraguchi, H. Tanaka, T. Miyasaka, *Chem. Pharm. Bull.* **1987**, *35*, 72.
- [22] S. Pitsch, *Helv. Chim. Acta* **1997**, *80*, 2286.
- [23] L. Dudycz, R. Stolarski, R. Pless, D. Shugar, *Z. Naturforsch.* **1978**, *34c*, 359.
- [24] D. B. Davies, 'Conformations of Nucleosides and Nucleotides', in 'Progress in NMR Spectroscopy', 1978, *12*, p. 135.
- [25] A. Miah, C. B. Reese, Q. Song, Z. Sturdy, S. Neidle, I. J. Simpson, M. Read, E. Rayner, *J. Chem. Soc., Perkin Trans. 1* **1998**, 3277.
- [26] E. Kawashima, Y. Ishido, Y. Aoyama, T. Sekine, Y. Iwamoto, *Nucleosides Nucleotides* **1996**, *15*, 733.
- [27] B. P. Cross, T. Schleich, *Biopolymers* **1973**, *12*, 2381.
- [28] F. Moris, V. Gotor, *Tetrahedron* **1993**, *49*, 10089.
- [29] L. F. Garcia-Alles, J. Magdalena, V. Gotor, *J. Org. Chem.* **1996**, *61*, 6980.
- [30] S. Pitsch, S. Wendeborn, B. Jaun, A. Eschenmoser, *Helv. Chim. Acta* **1993**, *76*, 2161.
- [31] Pharmacia, 'User Manual for Gene Assembler Plus'.

Received February 23, 2001