The α-L-Threofuranosyl-(3′ → 2′)-oligonucleotide System ('TNA'): Synthesis and Pairing Properties[1])

by **Kai-Uwe Schöning**[2a]), **Peter Scholz**[2b]), **Xiaolin Wu**[2c]), **Sreenivasulu Guntha**[2d]), **Guillermo Delgado**[3]), **Ramanarayanan Krishnamurthy***[4]), and **Albert Eschenmoser***[5])

The Skaggs Institute for Chemical Biology at The Scripps Research Institute (TSRI), 10550 North Torrey Pines Road, La Jolla, CA-92037, USA

and

Laboratory of Organic Chemistry, Swiss Federal Institute of Technology, Hönggerberg, HCI-H309, CH-8093 Zürich

Dieter Seebach zum 65. Geburtstag gewidmet

Our studies of α-L-Threofuranosyl-(3′ → 2′)-oligonucleotides ('TNA') are part of a systematic experimental inquiry into the base-pairing properties of potentially natural nucleic acid alternatives taken from RNA's close structural neighborhood. TNA is an efficient *Watson-Crick* base-pairing system and has the capability of informational cross-pairing with both RNA and DNA. This property, together with the system's constitutional and (presumed) generational simplicity, warrants special scrutiny of TNA in the context of the search for chemical clues to RNA's origin.

1. Introduction. – Our systematic experimental studies on the base-pairing properties of potentially natural nucleic acid alternatives selected from the structural neighborhood of RNA [2] (*Fig. 1*) had started with hexapyranosyl-(6′ → 4′)-oligonucleotide systems ('*Why Pentose and Not Hexose Nucleic Acids?*' [3]), proceeded then to the pyranosyl isomer of RNA ('p-RNA' [4]), a comprehensive study of which was followed by an investigation of the whole family of pentopyranosyl-(4′ → 2′)-oligonucleotides ('*Chemical Etiology of Nucleic Acid Structure: Comparing Pentopyranosyl-(2′ → 4′)-oligonucleotides with RNA*') [5] (*Scheme 1*), and, more recently, finally arrived at α-L-threofuranosyl-(3′ → 2′)-oligonucleotides ('TNA'), an oligonucleotide system that contains a backbone built of sugar units with only four C-atoms. We reported on this latest nucleic acid alternative in preliminary communications [6][7][1] and provide here a description of the experimental details on the synthesis and properties of TNA oligonucleotides as studied in our laboratories thus far.

---

[1]) Communication No. 37 in the series 'Chemistry of α-Aminonitriles'. For No. 36, see [1].

[2]) Postdoctorates: a) ETH/TSRI June 1998 – Dec. 1999; b) ETH/TSRI Aug. 1998 – May 2000; c) TSRI April 2000 – Dec. 2001; d) ETH/TSRI Jan. 1998 – Jan. 2001.

[3]) On sabbatical leave from la Universidad Nacional Autónoma de México: TSRI Oct. 2000 – Sep. 2001.

[4]) E-mail: rkrishna@scripps.edu; fax: ++1-858-784-9573.

[5]) E-mail: eschenmoser@org.chem.ethz.ch; fax: ++41-1-632-1043.

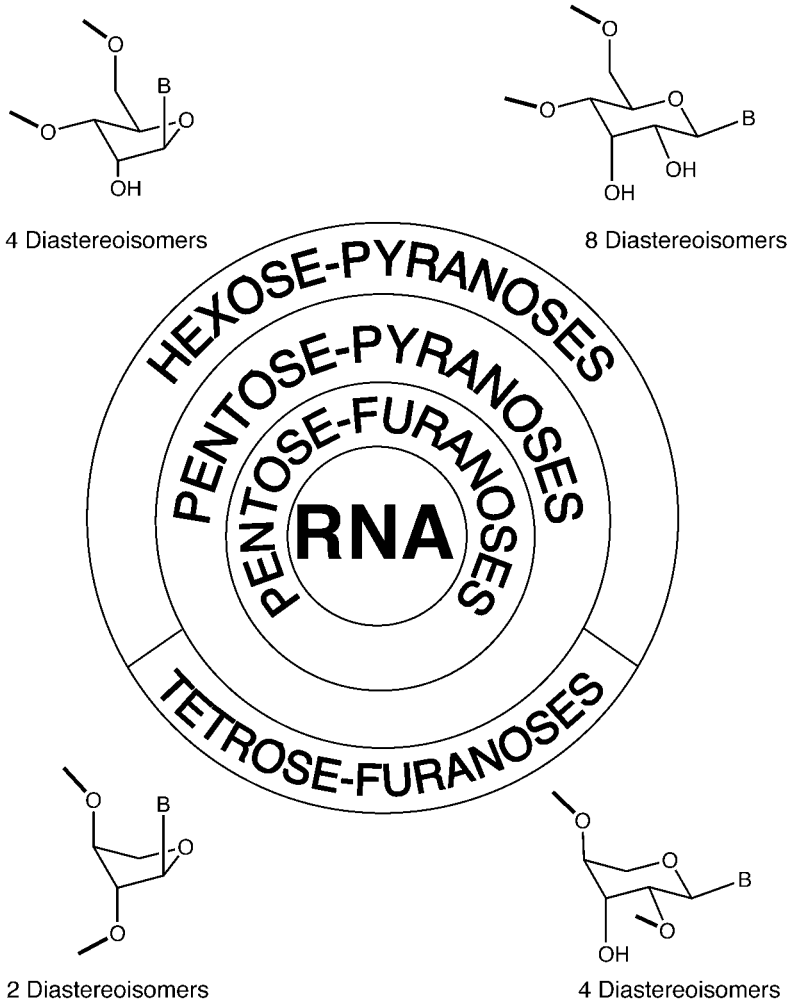
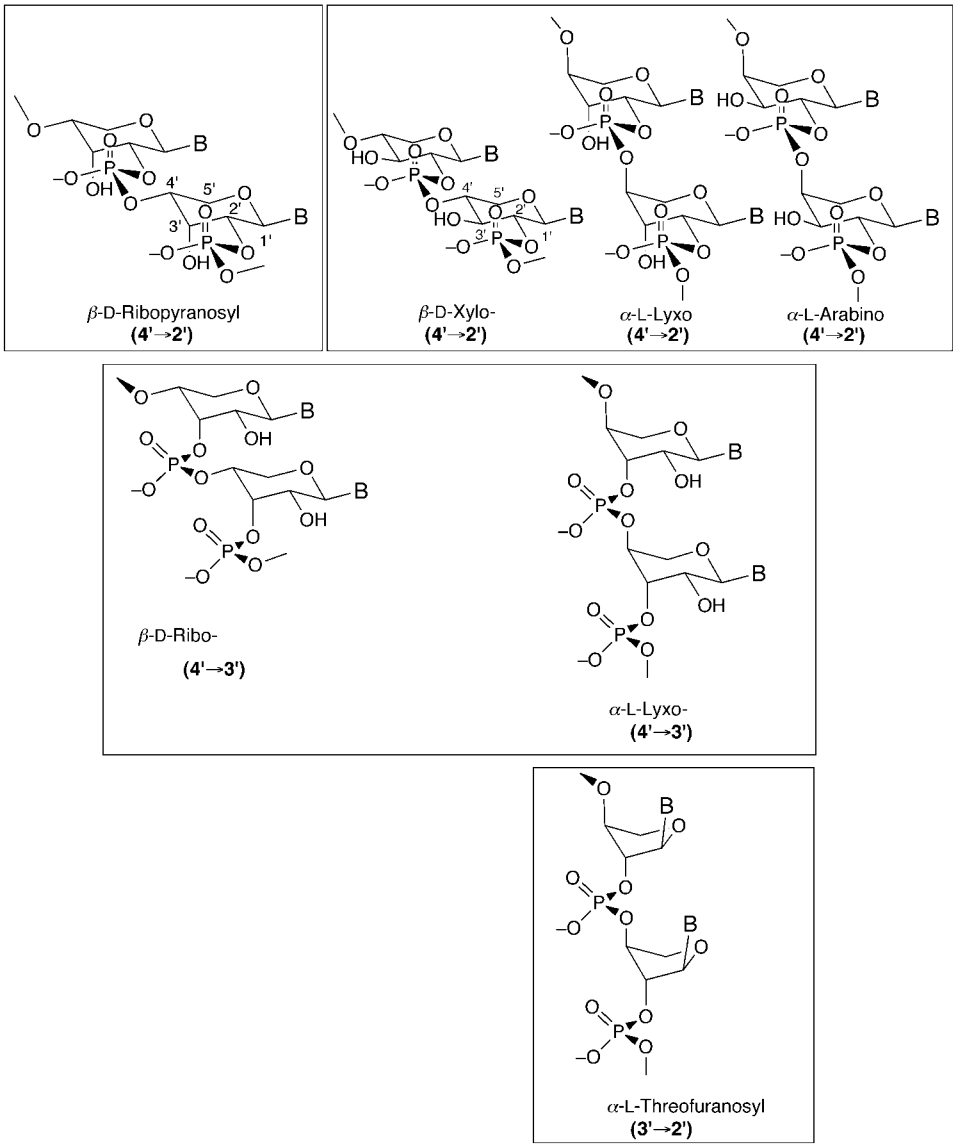


Fig. 1. *The strategy followed in our work toward a chemical etiology of nucleic acid structure:* select from the close structural neighborhood of RNA oligonucleotide systems that, according to chemical criteria, could have been chosen by Nature as alternatives of the contemporary nucleic acids, and check experimentally whether such systems have the capabilities of informational base-pairing.

The decision to investigate the threofuranosyl-($3' \rightarrow 2'$)-oligonucleotide system within the context of our studies toward a chemical etiology of nucleic acid structure was, by no means, reached in a straightforward manner. From the very outset of the work on nucleic acid alternatives [8], we applied two criteria for deciding whether a given alternative oligonucleotide system should be chosen for experimental study: first, whether the alternative is deemed to be a potentially natural structure (*i.e.*, derivable from sugar units and nucleobases by the same type of potentially natural chemistry as RNA is derivable from ribose and the nucleobases), and second, whether the system to be chosen could be expected to represent a *Watson-Crick*-pairing system as judged by

Scheme 1. *Idealized Conformational Formulae of the Structure of Nucleic Acid Alternatives, the Systematic Study of which Eventually Led to the Investigation of the α-L-Threofuranosyl-(3′→2′)-oligonucleotide System*



qualitative reasoning based on qualitative conformational criteria specifically defined for that purpose [3a]. The application of these conformational criteria forcefully supported the belief that an oligonucleotide system, in order to possess the capacity of base pairing, should, in its spacing of backbone units, correspond to that of the natural systems, implying that its repetitive units should be constitutionally separated by six covalent bonds [9]. Strict adherence to this proposition in the design of the constitution

of pyranosyl RNA [5a] proved very successful indeed and, at the same time, reinforced our belief that there is no need in our research project to be concerned about nucleic acid alternatives that would be derived from a tetrose sugar, simply because it is not possible to construct an oligonucleotide backbone that has a six-bond spacing of repeating units from a sugar that contains only four C-atoms. However, our comprehensive study on pairing properties within the family of pentopyranosyl-oligonucleotides led to an observation that challenged this kind of reasoning. That observation was the discovery of a drastic difference between the pairing behavior of the β-D-ribopyranosyl-(4′ → 3′)- and α-L-lyxopyranosyl-(4′ → 3′)-oligonucleotides, both systems containing in their backbone only five bonds per repeating unit as compared to the six bonds in the backbones of the corresponding (4′ → 2′)-oligonucleotide systems [9][10][6]). Whereas, as expected, β-D-ribopyranosyl-(4′ → 3′)-oligonucleotide sequences were observed to be devoid of the capacity of base pairing, we found that corresponding (4′ → 3′)-oligonucleotide sequences in the α-L-lyxopyranosyl series do, in fact, possess this capability [9][10]. It seemed clear that this difference in the pairing behavior of the two diastereoisomeric systems, which both have a shortened backbone, must be a reflection of the conformational difference between the two systems, namely, that the phosphodiester junctions are equatorial-axial in the ribopyranosyl-(4′ → 3′)-system and may be axial-axial in lyxopyranosyl-(4′ → 3′)-oligonucleotides. From this, we tentatively concluded that phosphodiester-type backbones with five bonds per repetitive unit are compatible with base pairing, provided that the vicinally bonded phosphodiester groups can assume an *antiperiplanar* conformation. This hypothesis led straightforwardly to the decision that the tetrose-sugar threose should no longer be excluded from the list of possible building blocks for potentially natural nucleic acid alternatives. After all, threose and erythrose derivatives had been observed as prominent products in our earlier studies on the aldolization of glycolaldehyde phosphates [11].

2. Synthesis. – 2.1. *Preparation of* L-*Threose.* For the purpose of our studies, we required access to large amounts of the tetrose sugar, L-threose. While there are numerous scattered reports about the synthesis of L-threose [12], none of them seemed amenable to large-scale preparation, either due to expensive starting materials, tedious workup, or poor yields. Therefore, a modification of the published procedures for the preparation of L-threose starting from the cheaply available starting material L-ascorbic acid was developed (*Scheme 2*). It had been reported that the oxidative degradation of L-ascorbic acid affords mainly the L-threonic acid [13]. Following the procedures of *Isbell* and *Frush* [14], and *Wei et al.* [15], we were able to isolate the Ca salt of threonic acid **2** in 65% yield after oxidation of L-ascorbic acid with aqueous H_2O_2 in the presence of $CaCO_3$. A one-pot lactonization by treatment with a *Dowex* resin (H^+ form) yielded threonolactone [13], which was benzoylated *in situ* with BzCl to give the 2,3-di-*O*-benzoyl-L-threonolactone (**3**) [16] in 60% yield[7]). Its reduction with DIBAL-H at −78° afforded the 2′,3′-di-*O*-benzoylated lactol as a mixture of α/β

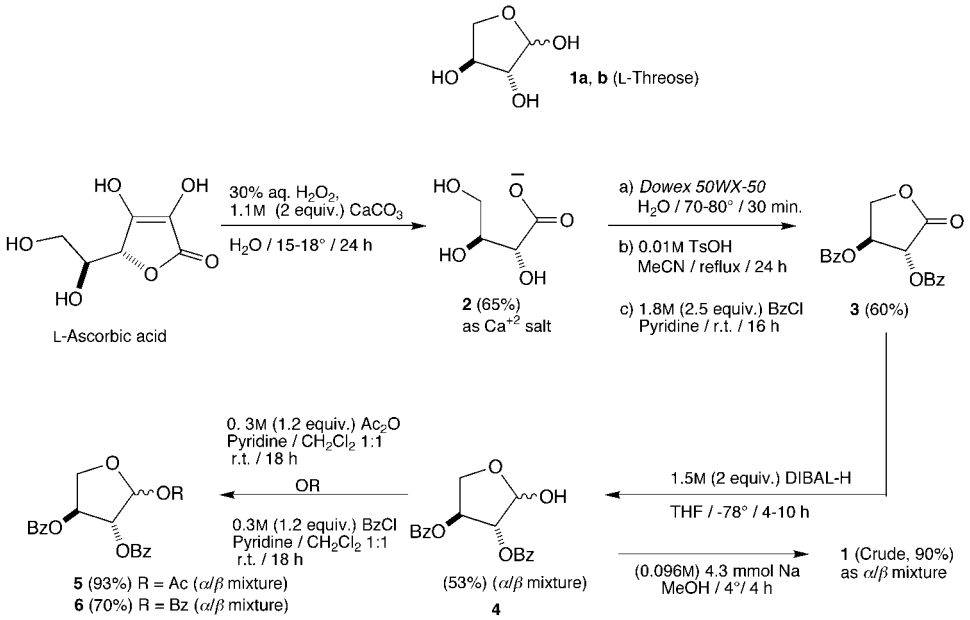
---

[6]) For the question why these two five-bond-per-unit systems have been studied and a more detailed description of the background of that work, see [10].

[7]) Benzoylation was preferred over acetylation, since the benzoylated material is less volatile.

anomers (colorless oil) in 53% yield[8]). Since, all of these steps can be carried out in large scale with relative ease, the epimeric mixture of the dibenzoylated lactol **4** was produced in multigram quantities without problems. Acetylation of 2′,3′-di-*O*-benzoyllactol **4** under standard conditions was straightforward and afforded the 1′-*O*-acetyl-2′,3′-di-*O*-benzoyl derivative **5** as a colorless oil (α/β mixture), while benzoylation of **4** provided both anomers of the known tri-*O*-benzoyl derivative **6** [18], the α-isomer in crystallized form. The triacylated α/β mixtures of **5** and **6** were suitable starting materials for the preparation of the L-threofuranosyl nucleosides required as the building blocks of TNA.

Scheme 2. *Preparation of the 1′,2′,3′-Triacylated* L-*Threose Derivatives* **5** *and* **6** *Starting from* L-*Ascorbic Acid*

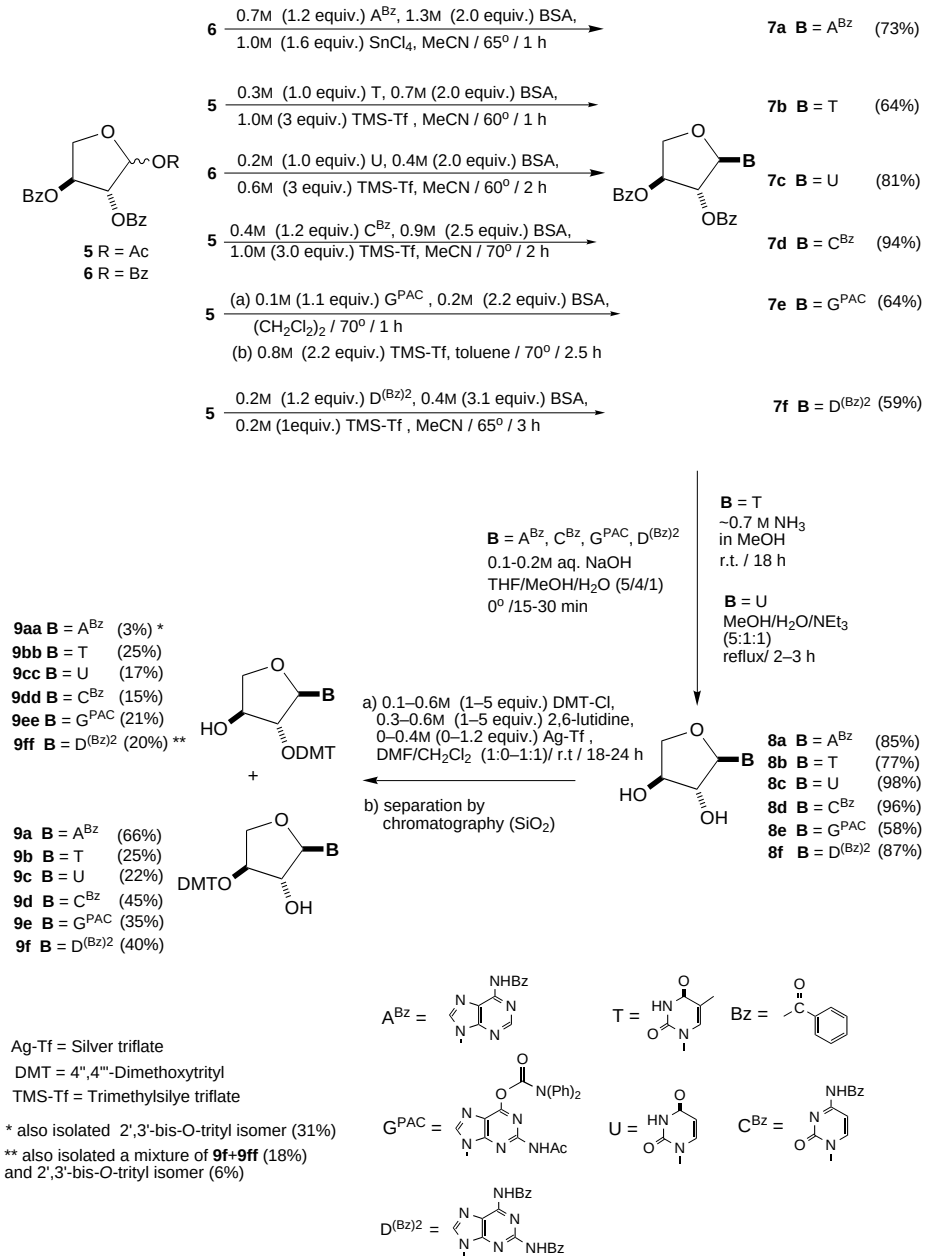


2.2. *Preparation of* α-L-*Threofuranosyl Nucleosides Containing Adenine (A), Thymine (T), Uracil (U), Cytosine (C), Guanine (G), and 2,6-Diaminopurine (D). Vorbrüggen-Hilbert-Johnson* nucleosidations [19] starting from the α/β-anomeric mixtures of the tribenzoyl derivative **5** or **6** and the appropriately protected nucleobases A^{Bz}, T, C^{Bz}, G^{PAC}, and $D^{(Bz)2}$ (see *Scheme 3*) afforded the nucleosides **7a**–**7f** in 73–59% yields[9]). For making of the guanine nucleoside **7e**, the method

[8]) Reduction of the threonolactone **3** with disiamylborane to afford L-threose has been reported [17], but, in our hands, reduction of **3** with this reagent was not easily reproducible, and, therefore, DIBAL-H was chosen as the reducing agent. The reaction time in this reduction step depended the rate of addition of DIBAL-H, yet neither a faster addition nor an excess of the reducing reagent were observed to give rise to over-reduction. In only one out of numerous successful experiments, the reduction of one of the two Bz groups was observed.

[9]) Threofuranosyl nucleoside **7a** has been previously described in the literature in a different context [20].

Scheme 3. *Preparation of the A-, T-, U-, C-, G- and D-containing Nucleoside Building Blocks in the α-L-Threofuranosyl Series*



described by *Robins et al.* [21] *via* the N^2-acetyl-O^6-diphenyl-carbamoyl-guanine (G^{PAC}) was used to give the nucleoside **7e** in fairly good yields (64%), with only the N^9-regioisomer being formed[10]). In the preparation of the N^2,N^6-dibenzoyl-diaminopurine-nucleoside **7f** from N^2, N^6-dibenzoyl-2,6-diaminopurine [22], the outcome depended on the nature and the number of equiv. of the *Lewis* acid used[11]). For example, when $SnCl_4$ (1.2 equiv. with respect to **5**) was used, the reaction led to a mixture of the desired α-anomer **7f** in 24% yield, along with 8% of the corresponding β-anomer and 6% of a product, which was the α-anomer further glycosylated at N^2 of N^2,N^6-dibenzoyl-diaminopurine[12]). Increasing the number of equiv. of $SnCl_4$ was counterproductive and resulted in the increase of the unwanted β-anomer of **7f**. Switching to TMS-Tf as the *Lewis* acid and with only 1 equiv. of it (with respect to **5**) led to the isolation of the desired N^2,N^6-dibenzoyl-diaminopurine-nucleoside **7f** in 59% yield with no side products observed. The constitution and the α-configuration of all α-L-threofuranosyl-nucleosides **7a**–**7f** were established by X-ray analysis, **7c** and **7e** as the dibenzoates, **7a**, **7b**, **7d**, and **7f** after hydrolysis to the corresponding diols **8a**, **8b**, **8d**, and **8f** (*Fig. 2*)[13]). The hydrolysis of the 2′,3′-dibenzoates **7a**–**7f** under basic conditions gave the 2′,3′-diols **8a**–**8f**[14]), respectively, and could be achieved without touching the protection of the nucleobases.

The next step, tritylation at the 3′-*O*-position of diols **8a**–**8f**, not unexpectedly, confronted us with selectivity problems. Tritylation under various conditions (see *Table 1*) always gave a mixture of 2′-*O*-DMT (**9a**–**9f**) and 3′-*O*-DMT (**9aa**–**9ff**) regioisomers, which had to be separated by chromatography on silica gel. In some cases (**8a** → **9a** + **9aa** and **8f** → **9f** + **9ff**), minor amounts of the 2′,3′-*O*-bis-tritylated derivatives were also isolated. The constitutional assignments of the 2′- and 3′-*O*-DMT isomers were uniformly based on homo decoupling and ^{1}H,^{1}H-COSY-NMR spectroscopy by correlating the H–C(2′) or H–C(3′) to the OH H-atom. *Fig. 3* gives as an example the case of the 3′-*O*-DMT derivative of guanine **9e**. Selected ^{1}H-NMR shifts of the 2′-*O*- and 3′-*O*-DMT nucleosides **9a**–**9f** and **9aa**–**9ff** are given in *Table 2*.

Although, for the nucleosides containing the nucleobases A, C, G, and D, the yields of the desired 3′-*O*-DMT regioisomers **9a** (55–65%), **9d** (40–45%), **9e** (20–27%) and **9f** (40%) were acceptable, they were uncomfortably low for the T- and U-nucleosides (**9b** (25–30%) and **9c** (22–25%), resp.). Therefore, an alternative method was used for

---

10) The use of N^2-acetylguanine gave *ca.* 3:1 mixture of N^9/N^7-guanine nucleoside, which was difficult to purify.

11) For another case where the amount of $SnCl_4$ plays an important role in the N^9/N^7-regioselectivity, see [23].

12) The assignment of structures was based on extensive ^{1}H- and ^{13}C-NMR (DEPT, COSY, HMBC, HMQC and NOESY) experiments. The details of these studies will be reported in a separate publication [24].

13) Carried out by Dr. *Bernd Schweizer*, ETH, and *Raj K. Chadha*, TSRI. Crystallographic data for the structure has been deposited with the *Cambridge Crystallographic Data Centre* (*CCDC*) as deposition Nos. CCDC 183997 (**7c**); 183842 (**7e**); 183994 (**8a**); 183996 (**8b**); 183995 (**8d**); 175169 (**8f**). Copies of the data can be obtained, free of charge, on application to the CCDC, 12 union Road, Cambridge CB121EZ UK (fax: + 44 (1233) 336 0333; e-mail: deposit@ccdc.cam.ac.uk).

14) In the case of nucleosides **8b** and **8c** it was found to be advantageous, from the point of view of workup, to use NH_3 or $MeNH_2$ instead of aqueous hydrolysis. The aqueous hydrolysis method suffers from the drawback that the polar products **8b** and **8c** were not easily separable from the salts.

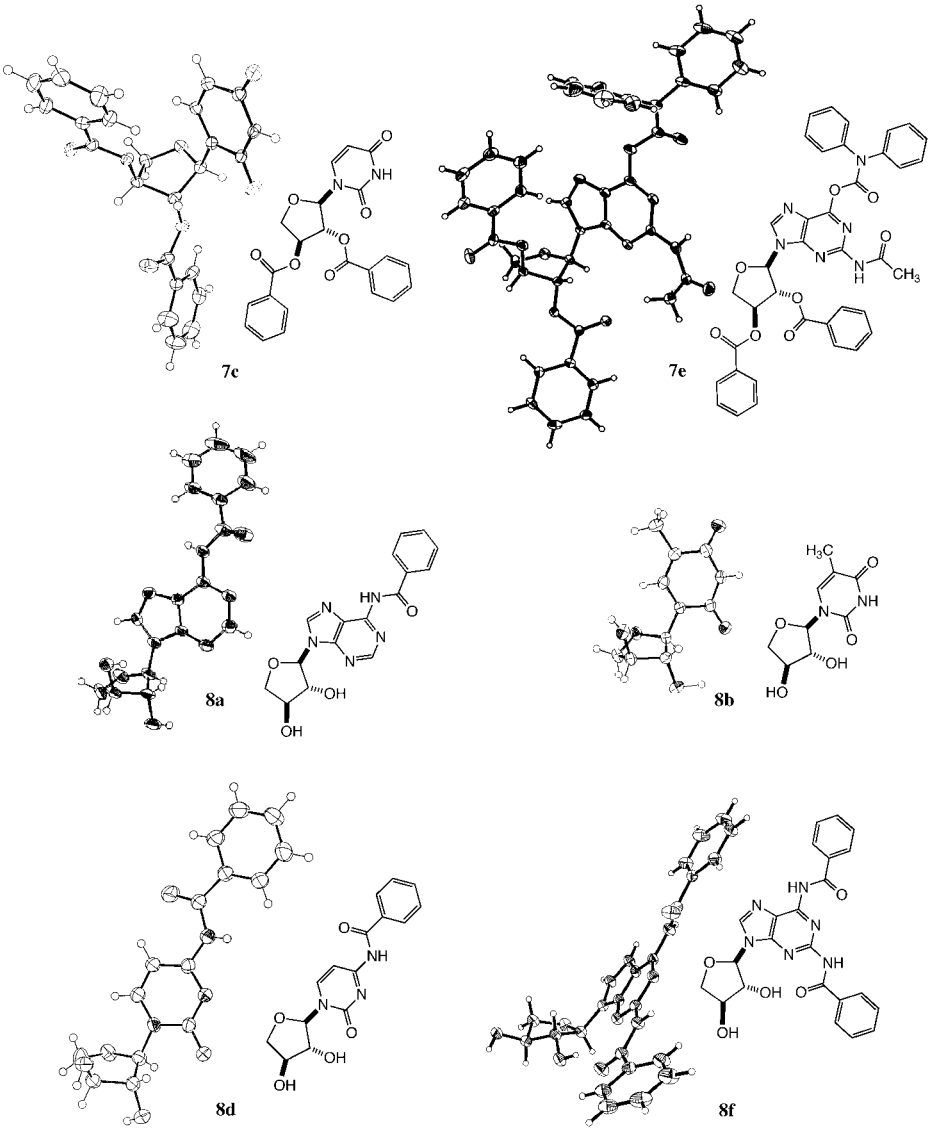


Fig. 2. *X-Ray-structure analyses of* **7c**, **7e**, **8a**, **8b**, **8d**, *and* **8f**. Torsion angles (max ± 0.5° are as follows): O–C(2′)–C(3′)–O: (**7c**) 165.7°; (**7e**) 160.2°; (**8a**) 164.9°; (**8b**) 158.6°; (**8d**) 131.5°; (**8f**) 77.7°; O–C(1′)–N(1)–C(2): (**7c**) –166.4°; (**8b**) –147.3°; (**8d**) –169.28°. O–C(1′)–N(9)–C(4): (**7e**) –176.7°; (**8a**) –169.3°; (**8f**) 166.8° (see *Footnote 13*).

these nucleosides, based on a procedure developed in the ribofuranosyl series by *Moffat* and co-workers [25][26]. Nucleosides **8b** and **8c** were heated for 1–2 h with diphenyl carbonate in HMPA as solvent in the presence of a catalytic amount of

Table 1. *Tritylation of Nucleosides* **8a–8f**

Entry	Nucleoside	Tritylation *Method*[a])	Yields [%] 3′-*O*-DMT	Yields [%] 2′-*O*-DMT
1	A (**8a**)	*1*	55–65 (**9a**)	< 5% (**9aa**)[b])
2	T (**8b**)	*1*	25–30 (**9b**)	25–30 (**9bb**)
3	T (**8b**)	*2*	< 5 (**9b**)	15–20 (**9bb**)
4	U (**8c**)	*1*	20–25 (**9c**)	20–25 (**9cc**)
5	U (**8c**)	*2*	< 5 (**9c**)	65–83 (**9cc**)
6	C (**8d**)	*1*	40–55 (**9d**)	10–20 (**9dd**)
7	C (**8d**)	*2*	< 5 (**9d**)	40–50 (**9dd**)
8	G (**8e**)	*3*	20–27 (**9e**)	20–28 (**9ee**)
9	G (**8e**)	*2*	5–7 (**9e**)	1–2 (**9ee**)
10	2,6-D (**8f**)	*1*	40 (**9f**)	20 (**9ff**)[c])

[a]) *Method 1:* 0.1–0.6M (1–5 equiv.) DMT-Cl, 0.3–0.6M (1–5 equiv.) 2,6-lutidine, 0.4M (1.2 equiv.) Ag-Tf, CH_2Cl_2/DMF, r.t. *Method 2:* 0.1–0.6M (1–5 equiv.) DMT-Cl, 0.3–0.6M (1–5 equiv.) 1,5,7-triazabicyclo[4.4.0]-dec-5-ene, 0.4M (1.2 equiv.) $(t\text{-Bu})_4NClO_4$, MeCN, reflux. *Method 3:* 0.1–0.6M (1–5 equiv.) DMT-Cl, 0.3–0.6M (1–5 equiv.) 2,6-lutidine, CH_2Cl_2/DMF, r.t. [b]) Also isolated 31% of the 2′,3′-bis-*O*-tritylated derivative. [c]) Also isolated 18% of a mixture of **9f** + **9ff** and 6% of 2′,3′-bis-*O*-tritylated derivative.

Table 2. *Selected ^{1}H-NMR Chemical Shifts of Nucleosides* **9a–f** *and* **9aa–ff**[a])

Entry	Nucleoside		H–C(1′)	H–C(2′)	H–C(3′)	H–C(4′)
	3′-*O*-DMT	2′-*O*-DMT				
1	**9a**		5.92	4.21	4.33	3.80
2		**9aa**	5.47	4.76	4.37	4.26, 4.10
3	**9b**		5.70	4.15	4.22	3.92–3.31
4		**9bb**	5.54	4.38	3.75	4.00–3.95
5	**9c**		5.64	4.06	4.22	3.90, 3.42
6		**9cc**	5.79	4.25	3.56	4.02, 3.95
7	**9d**		5.64	4.26	4.26	3.61, 3.38
8		**9dd**	5.43	4.64	3.94	4.12, 4.06
9	**9e**		5.68	5.09	3.67	4.35
10		**9ee**	5.28	4.81	4.32	4.09
11	**9f**		5.83	4.73	4.40	3.37, 3.14
12		**9ff**	5.32	5.34	4.64	4.29, 4.21

[a]) δ in ppm, measured in CDl_3.

$NaHCO_3$ (0.3 equiv.) converting them to the corresponding 2′-anhydro nucleosides **10b**[15]) and **10c** in 66 and 92% yields, respectively (*Scheme 4*)[16]). Heating the

[15]) Constitution and configuration confirmed by X-ray analysis carried out by *Raj K. Chadha*, TSRI. Crystallographic data for the structure have been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC 172142. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 union Road, Cambridge CB12 1EZ UK (fax: + 44 (1233) 336 0333; e-mail: deposit@ccdc.cam.ac.uk).

[16]) Since the reaction is carried out at high temperature, it is important to monitor the reaction by TLC. The 2′-anhydro product is unstable under prolonged heating (see *Exper. Part* for **10b**). The mechanism by which these 2′-anhydro nucleosides are produced is not necessarily obvious, considering the fact that the 2′- and 3′-OH groups are *trans* to each other, and, therefore, a cyclic 2′,3′′-carbonate intermediate as proposed for the ribofuranosyl system [25] may be questionable.

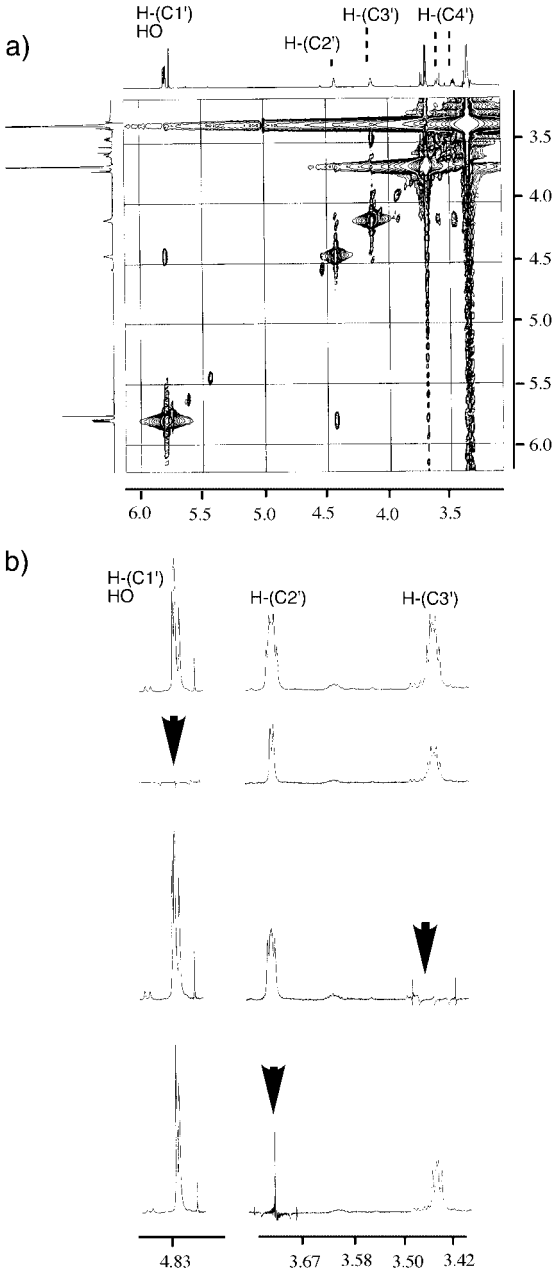
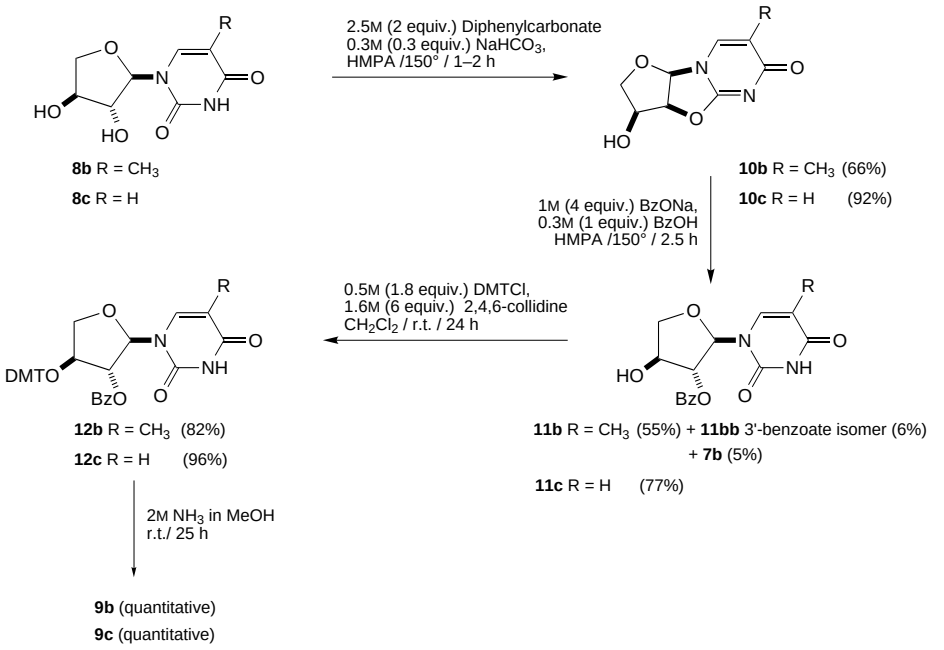


Fig. 3. *^{1}H-NMR Experiments* (in $CDCl_3$) *for the constitutional assignment of the 3′*-O-*DMT derivative* **9e** *of guanine*, *a*) ^{1}H,^{1}H-COSY Spectrum showing the correlation between the H−O and H−C(2′) protons and *b*) ^{1}H-homonuclear decoupling experiments showing the connectivity between H−O and H−C(2′) protons and the lack of connectivity between the H−O and H−C(3′) protons. The assignment of H−C(2′) is based on ^{1}H,^{1}H-COSY correlation CH_2(4′) → H−C(3′) → H−C(2′).

Scheme 4. *Preparatively Preferable Alternative Route to the 3′-*O*-DMT Derivatives of Nucleosides of T* (**9b**) *and U* (**9c**)



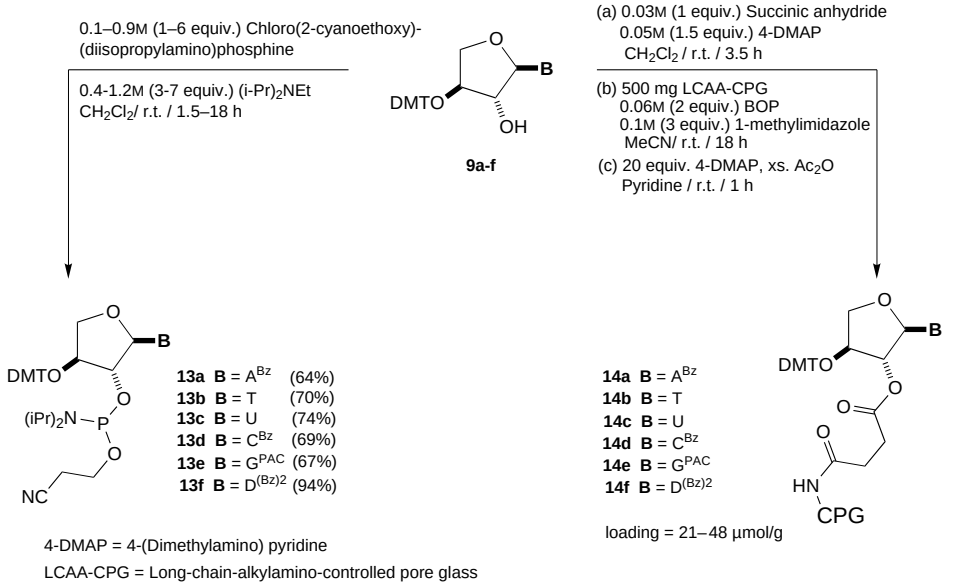
anhydronucleosides **10b** and **10c** to 150° in HMPA in the presence of excess sodium benzoate in the presence of 1 mol-equiv. BzOH as catalyst to afford, by S_N2 opening of the anhydro ring the 2′-*O*-benzoates, **11b**[17]) and **11c** in 55 and 77% yield, respectively. Tritylation of **11b** and **11c** in CH_2Cl_2 with 2,4,6-collidine as the base gave the 2′-*O*-benzoylated-3′-*O*-tritylated nucleosides **12b** and **12c** in high yields, from which ammonolysis in MeOH led to the 3′-*O*-tritylated derivatives **9b** and **9c**. Their spectroscopic identity with samples of the 3′-*O*-tritylated nucleosides **9b** and **9c** obtained by the direct-tritylation route (*Scheme 3*) corroborated the correctness of the not necessarily unambiguous course of the anhydro-nucleoside formation **8b** (**8c**) → **10b** (**10c**).

With the tritylated derivatives in hand, the building blocks, *i.e.*, the phosphoramidites **13a**–**13f** and the CPG-solid supports **14a**–**14f**, necessary for the oligonucleotide synthesis *via* the gene synthesizer, were accessed as outlined in *Scheme 5*. Phosphitylation of **9a**–**9f** with chloro(2-cyanoethoxy)(diisopropylamino)phosphine in the presence of *Hünig*'s base afforded the phosphoroamidites **13a**–**13f** in 64–94% yield, while treatment of **9a**–**9f** first with succinic anhydride in the presence of 4-DMAP, followed by LCAA-CPG with BOP as the activator [27] and finally capping with Ac_2O, provided the suitably derivatized CPG-solid supports **14a**–**14f**, the loading capacities of which were determined [4] to range from 21–48 μmol/g.

---

[17]) In the case of **11b**, minor amounts of the 3′-*O*-benzoate **11bb** (6%) and 2′,3′-dibenzoate **7b** (5%) were also isolated.

Scheme 5. *Preparation of the Phosphoramidite and CPG Derivatives for the Automated Synthesis of α-L-Threofuranosyl-(3′→2′)-oligonucleotides*



2.3. *Synthesis of α-L-Threofuranosyl-(3′→2′)-oligonucleotides (TNAs)*. The synthesis of TNA oligonucleotide sequences was carried out on 1-μM scale either with the *Gene Synthesizer Plus* (*Pharmacia*) or *Expedite 8909 Gene Assembler* (*PE Biosystems*), utilizing the phosphoramidites **13a**–**13f** and the suitably derivatized CPG-solid supports **14a**–**14f**, by well-established protocols that were developed earlier for the p-RNA oligonucleotide synthesis [4][10][28][29]. Certain modifications were necessary to optimize the overall coupling yields. These included *a*) the use of 0.1M solution of phosphoramidites **13a**–**13f** in MeCN, *b*) use of a 0.35M soln. of (ethylthio)-1*H*-tetrazole for the coupling step, *c*) increasing the coupling time to 26 min, and *d*) the use of a 6% Cl_2CHCO_2H solution in $Cl(CH_2)_2Cl$ for detritylation over a 10-min period. Average coupling efficiencies were $>95\%$ (by detritylation assay). While the majority of the oligonucleotides were synthesized in the 'Trityl-off' mode, a minor number of sequences were synthesized in the 'Trityl-on' mode[18]).

In the workup procedure, the CPG-solid supports containing the TNA sequences were dried and treated with Et_3N/dry pyridine to effect the removal of the β-cyanoethyl protection of the labile phosphotriester group, converting it to the more stable phosphodiester moiety [30], with the sequences still attached to the solid support. The choice of the subsequent deprotection steps depended on the specific base sequence (*cf. Table 3*). The concomitant deprotection of the nucleobase protecting groups and detachment of the oligonucleotide strands from the CPG-solid support was straightfor-

[18]) The sequences that were prepared in the 'Trityl-on' mode were conveniently handled in the purification step, when the base-deprotection step involved treatment with hydrazine hydrate (see *Exper. Part*, *5.2.1*).

Table 3. *HPLC and MS Data of α-L-Threofuranosyl-(3′ → 2′)-oligonucleotides*

Entry	TNA Oligonucleotide system	Deprotection[a]) *Method*	O.D. 260 nm (yield)	Analytical HPLC ion exchange[b]) 0–100% in 30 min retention time [min]	MALDI-TOF-MS[c]) $[M+H]^+$	
					obs.	calc.
1	**t**(A_8)	*A*	38.8 (25%)	12.8	2332	2332
2	**t**(T_8)	*A*	96.0 (92%)	11.5	2258	2258
3	**t**(U_8)	*B*	56.0 (54%)	17.5	2148	2148
4	**t**(A_{12})	*A*	61.7 (26%)	14.3	3529	3528
5	**t**(T_{12})	*B*	84.7 (54%)	16.9	3419	3418
6	**t**(U_{12})	*B*	53.4 (34%)	24.1	3253	3252
7	**t**(A_{16})	*A*	103.7 (33%)	15.5	4725	4725
8	**t**(T_{16})	*B*	89.6 (43%)	21.6	4580	4581
9	**t**(U_{16})	*B*	8.5 (4%)	27.1	4356	4354
10	**t**(A_{20})	*A*	77.7 (20%)	16.4	5920	5919
11	**t**(T_{20})	*A*	88.0 (34%)	22.7	5740	5739
12	**t**(U_{20})	*B*	1.5 (6%)	23.5	5561	5461
13	**t**(A_8T_8)	*A*	35.6 (14%)	17.6	4652	4651
14	**t**(T_8A_8)	*A*	43.8 (17%)	19.6	4652	4651
15	**t**(A_8U_8)	*A*	48.2 (19%)	24.8	4548	4539
16	**t**($(A_4T_4)_2$)	*A*	32.2 (16%)	21.4	4653	4651
17	**t**($(A_4U_4)_2$)	*A*	26.0 (13%)	22.5	4540	4539
18	**t**($(ATAU)_4$)	*A*	53.6 (21%)	18.3	4596	4595
19	**t**($(A_2T_2)_4$)	*A*	13.6 (7%)	20.8	4652	4651
20	**t**($(A_2U_2)_4$)	*A*	32.4 (16%)	21.9	4538	4539
21	**t**($(AT)_8$)	*A*	53.6 (17%)	22.9	4652	4651
22	**t**($(TA)_8$)	*A*	37.2 (22%)	22.9	4655	4651
23	**t**($(AU)_8$)	*A*	31.2 (12%)	23.4	4546	4539
24	**t**($A_{10}U_{10}$)	*A*	26.6 (13%)	25.8	5704	5689
25	**t**($A_4T_3ATAT_2AT_2A$)	*A*	53.0 (20%)	22.0	4651	4651
26	**t**($TA_2TA_2TATA_3T_4$)	*A*	40.6 (20%)	20.9	4657	4651
27	**t**($T_4A_3TATA_2TA_2T$)	*A*	43.6 (17%)	23.6	4661	4651
28	**t**(AT_2AT_2A)	*B*	14.5 (22%)	18.2	1996	1997
29	**t**(A_4T_3AT)-PO_3^{2-}-2′	*B*	12.3 (18%)	14.5	2674	2674
30	**t**(CGCGAATTCGCG)	*C*	14.0 (11%)	22.8	3478	3478
31	**t**(ATTCAGCG)	*C*	14.9 (16%)	20.5	2297	2297
32	**t**(CGCTGAAT)	*C*	20.0 (22%)	20.1	2298	2297
33	**t**($T(ATGC)_3ATA$)	*C*	30.1 (16%)	24.2	4658	4656
34	**t**($(TA)_3TGC(AT_3A$)	*C*	0.8 (0.4%)	22.7	4654	4654
35	**t**(C_8)	*D*	12.2 (18%)	15.1	2140	2139
36	**t**(CGA_2T_2CG)	*C*	17.9 (19%)	19.9	2298	2297
37	**t**$(CG)_4$	*C*	11.1 (11%)	21.2	2301	2299
38	**t**($AT_2AT_2ATAT_3A_4$)	*D*	66.0 (31%)	21.4	4653	4653
39	**t**($CGCGT_4CGCG$)	$C^{\pm}$	12.1 (16%)	23.9	3460	3460
40	**t**($CGCGA_2U_2CGCG$)	$C^{\pm}$	14.3 (17%)	25.6	3448	3447
41	**t**$((GA)_4)$	*B*	18.2 (24%)	18.2	2395	2398
42	**t**($C_2A_2C_3ATCTCTCTC$)	*B*	14.5 (16%)	21.7	4476	4475
43	**t**$(GA)_4$-PO_3^{2-}-2′	*B*	10.9 (14%)	17.9	2476	2477
44	**t**($TG_3T_2G_2$)	*B*	12.9 (15%)	25.2	2384	2385
45	**t**$((D)_{12})$	*E*	14.6 (19%)	15.1	$3730^{(Na+)}$	$3730^{(Na+)}$
46	**t**(DT_2DT_2D)	*E*	11.2 (16%)	20.9*	2040	2041
47	**t**(D_4T_3DT)-PO_3^{2-}-2′	*E*	12.2 (18%)	15.8	2479	2751
48	**t**($TD_2TD_2TDTD_3T_4$)	*E*	16.3 (22%)	19.4	4772	4473
49	**t**($D_4T_3DTDT_2DT_2D$)	*E*	15.4 (19%)	20.1	4772	4773

Table 3 (cont.)

Entry	TNA Oligonucleotide system	Deprotection[a]) *Method*	O.D. 260 nm (yield)	Analytical HPLC ion exchange[b]) 0–100% in 30 min. retention time [min.]	MALDI-TOF-MS[c]) $[M+H]^+$	
					obs.	calc.
50	**t**(DT_5D)	*E*	10.7 (13%)	21.5*	2016	2019
51	**t**(DT_2D_2TD)	*E*	8.9 (14%)	20.0*	2065	2065
52	**t**(D_3T_4DT)-PO_3^{2-}-2′	*E*	14.8 (17%)	20.4*	2725	2726
53	**t,r**(**r**(A_8)**t**(T_8))	$B^{\pm}$	24.4 (9%)	22.4	4890	4891
54	**t,r**((**rAtT**)$_8$)	$B^{\pm}$	16.0 (6%)	25.0	4890	4891
54	**t,r**(**t**(A_8)**r**(T_8))	$A^{\pm}$	32.8 (13%)	24.2	4895	4891
55	**t,r**((**tArT**)$_8$)	$A^{\pm}$	19.8 (8%)	25.4	4896	4891

[a]) *Method A:* 30% aq. NH_3, 50°, 4–12 h; *Method B:* 40% aq. $MeNH_2$/33% $MeNH_2$ in EtOH 1:1, r.t., 1.5–4 h; *Method C:* 2M (*Z*)-pyridine-2-carbaldehyde oxime in dioxane/H_2O 7:1, r.t., 3.5 min, followed by 24% aq. NH_2NH_2 hydrate, 4°, 18 h; *Method D:* 24% aq. NH_2NH_2 hydrate, 4°, 18 h. *Method E:* 40% aq. $MeNH_2$/33% $MeNH_2$ in EtOH 1:1, 50°, 18 h; *Method C*$^{\pm}$: same as *Method C* except NH_2NH_2 hydrate treatment was replaced by treatment with 40% aq. $MeNH_2$/33% $MeNH_2$ in EtOH 1:1, r.t., 4 h; *Method B*$^{\pm}$: 1M TBAF in THF, followed by *Method B*; *Method A*$^{\pm}$: 1M TBAF in THF, followed by *Method A*. [b]) All oligonucleotides were purified by ion-exchange chromatography on *Mono Q HR 5/5* (58 × 6.0 mm, *Pharmacia*) or on *SAX 1000-8* column (*Macherey & Nagel*); elution with 10 mM Na_2HPO_4 in H_2O and a linear gradient of 1M NaCl with a flow of 1 ml/min; peak purity (260 nm) 95–99%; followed by desalting on *Sep-Pak* cartridges. * 0–70% in 30 min. c) Matrix-assisted laser-desorption ionization time-of-flight mass spectrometry; matrix: 2,4,6-trihydroxyacetophenone and ammonium citrate buffer.

ward for the sequences that contained A, T, and U by treatment with either 14M aqueous NH_3, or a mixture of 8M $MeNH_2$ in EtOH/H_2O 1:1 at room temperature for 1.5–12 h. For G-containing sequences, such base treatments resulted, as observed by HPLC, in sequence-degradation problems. Therefore, a two-step procedure was used, in which the first step consisted of the removal of the O^6-PAC-protecting group at the guanine nucleus by treatment with 2M (*Z*)-pyridine-2-carbaldehyde oxime in dioxane/H_2O 7:1 at room temperature for 3.5 min [31], and the second step in the concomitant deprotection of the N^2-Bz protecting group and detachment of the oligomer strand from the solid support by treatment with 24% aqueous hydrazine hydrate treatment at 4° for 15 h (18 h for C-containing sequences).

In later experiments, it was observed that an initial separate step for removing the β-cyanoethyl protecting group conferred no advantage, and that the one-step-basic deprotection and detachment works equally well. Thus, treatment of CPG-solid support containing A, T, G, and C sequences with a mixture of 1 ml of 8M $MeNH_2$ solution in EtOH and 12M $MeNH_2$ solution in H_2O 1:1 for 2 h at room temperature was found to give the completely deprotected TNA. In the case of the 2,6-diaminopurine (D)-containing sequences, additional heating to 50° for 18 h was found to be necessary for complete removal of the N^2-Bz protecting group[19]). All deprotection results were monitored by ion-exchange HPLC for optimal deprotection. The sequences that were synthesized in the 'Trityl-on' mode were, after these deprotections, detritylated by treatment with 80% aqueous HCOOH.

[19]) TNA Sequences are found to be stable under such conditions (HPLC).

The completely deprotected sequences were purified by ion-exchange HPLC to a target purity of $>95\%$ and desalted by *C-18 Sep-Pak* cartridges to afford the salt free oligonucleotides (as Et_3NH^+ salts). The analytical purity of each sequence was checked by HPLC analysis and, for the correct mass, by MALDI-TOF spectroscopy. *Table 3* lists the various TNA sequences synthesized, the method of purification, and the MALDI-TOF data. The TNA sequences were stored as aqueous solutions in the freezer, and the concentrations of oligonucleotide solutions were calculated from the UV absorbance of the aqueous solutions at 260 nm (pH 7) at *ca.* 80° with the following molar extinction coefficients: ε (t(A)) = 15000, ε (t(D)) = 10000, ε (t(C)) = 7600, ε (t(G)) = 11700, ε (t(T)) = 10000, ε (t(U)) = 10000.

3. Base-Pairing Properties. – The pairing properties of TNAs were studied by the standard methods of *a*) T_m (melting temperature) determination by temperature-dependent UV spectroscopy [32], *b*) determination of base-pairing stoichiometry by UV mixing curve in selected cases [33], *c*) thermodynamic data measurement by T_m determination at various concentration [34], and *d*) temperature-dependent CD spectroscopy. *Tables 4–6* give a summary of the T_m and thermodynamic data of the various TNA sequences studied under the standard conditions of 1M NaCl in 0.01M NaH_2PO_4 buffer with 0.1 mM Na_2EDTA at pH 7.0 unless indicated otherwise. *Table 4* provides the summary of observations on TNA base-pairing in terms of T_m values and thermodynamic data of duplexes.

Initial studies conducted with TNA octamers t(A_8), t(T_8), and t(U_8) had ended in disappointment, since no pairing whatsoever between t(A_8) and either t(U_8) or t(T_8) could be observed, even under 'high-salt' conditions. This was in sharp contrast to what we were used to experience in our earlier studies, especially in the pentopyranosyl-oligonucleotide series. After more or less fruitlessly screening the effects of divalent metal ions (Mg, Cd, Co, Hg), we, fortunately, extended our search for indications of TNA base pairing to longer base-sequences. The dodecamer t(A_{12}) indeed exhibited a weak pairing with t(T_{12}) ($T_m \approx 14°$, 5 + 5 μM, 1M NaCl), while still no pairing was observed between t(A_{12}) and t(U_{12}). Eventually, it was gratifying to observe a consistent increase in the stability of homo-oligomeric duplexes t(A_n)·t(T_n) in proceeding from the 12-mers to the 16-mer ($T_m = 40°$, *Fig. 4, a*) and, finally the 20-mers (53°).

In the further course of our investigations, it became clear that, in the TNA series, duplexes of homo-oligomeric strands display exceptional behavior in that their stability is at the lower end in comparison to duplexes that have the same sequence-length but consist of hetero-oligomeric strands (*Table 4*, *Entries 1–8*, and *Fig. 4*). On the other end, maximal duplex stabilities in the TNA series are observed with sequences of regularly alternating purinic and pyrimidinic nucleobases (*Table 4*, *Entries 17–20*, *23*, *26–29*). Block co-oligomer sequences, such as those of *Entries 17–25* in *Table 4*, behave like interpolations between the two extremes with respect to duplex stability. The finding that there is hardly a difference in pairing strengths between TNA duplexes that contain self-complementary mirror sequences, *e.g.*, of type t(A_8T_8) as compared to t(T_8A_8) (*Entries 17* and *18*) is in stark contrast to the behavior of such mirror sequences in the pentopyranosyl-(4′ → 2′)-oligonucleotide series and in homo-DNA where the degree and the sign of such differences have been interpreted to correlate with the

Table 4. T_m *Values and Thermodynamic Data of TNA Duplexes*

Entry	Oligonucleotide system	T_m [°][a]) TNA Duplexes		ΔG (25°)	ΔH	$T\Delta S$ (25°)	Ref.
		1M NaCl, $c \approx 10$ μM		1M NaCl[a])[b])			
1	**t**(A_8) + **t**(T_8)	–*					
2	**t**(A_8) + **t**(U_8)	–*					
3	**t**(A_{12}) + **t**(T_{12})	14.6		– 6.7	– 28.1	– 21.4	
4	**t**(A_{12}) + **t**(U_{12})	< 0*					
5	**t**(A_{16}) + **t**(T_{16})	39.8		– 10.2	– 53.6	– 43.4	
6	**t**(A_{16}) + **t**(U_{16})	12.0		– 5.6	– 40.8	– 35.2	[6]
7	**t**(A_{20}) + **t**(T_{20})	52.6		– 14.2	– 75.1	– 60.9	[6]
8	**t**(A_{20}) + **t**(U_{20})	25.7		– 7.9	– 55.5	– 47.6	[6]
9	**t**($A_4T_3ATAT_2AT_2A$) + **t**($TA_2TA_2TATA_3T_4$)	56.2		– 14.5	– 73.7	– 59.2	[6]
10	**t**($T_4A_3TATA_2TA_2T$) + **t**($AT_2AT_2ATAT_3A_4$)	52.7		– 13.3	– 66.4	– 53.1	[6]
11	**t**($A_4T_3ATAT_2AT_2A$) + **t**($T_4A_3TATA_2TA_2T$)	–		– 6.2	– 24.3	– 18.1	
12	**t**(ATTCAGCG) + **t**(CGCTGAAT)	31.4		– 8.8	– 42.2	– 33.4	[6]
13	**t**(D_{12}) + t(T_{12})	45.7		– 11.4	– 53.1	– 41.7	[1]
14	**t**($D_4T_3DTDT_2DT_2D$) + **t**($TA_2TA_2TATA_3T_4$)	66.6		– 20.3	– 98.9	– 78.6	[1]
15	**t**($A_4T_3ATAT_2AT_2A$) + **t**($TD_2TD_2TDTD_3T_4$)	66.9		– 17.8	– 79.0	– 61.2	[1]
16	**t**($D_4T_3DTDT_2DT_2D$) + **t**($TD_2TD_2TDTD_3T_4$)	78.8		– 24.6	– 107.3	– 82.7	[1]
Self-complementary sequences							
17	**t**(A_8T_8)	38.3		– 9.2	– 56.2	– 47.0	[6]
18	**t**(T_8A_8)	36.3		– 8.6	– 50.8	– 42.2	[6]
19	**t**(A_8U_8)	18.2		– 5.6	– 53.8	– 48.3	[6]
20	**t**($A_{10}U_{10}$)	27.8		– 7.6	– 66.5	– 58.9	[6]
21	**t**($(A_2T_2)_4$)	54.9		– 13.7	– 75.1	– 61.3	[6]
22	**t**($(A_2U_2)_4$)	44.4		– 12.0	– 83.3	– 71.3	[6]
23	**t**($(ATAU)_4$)	72.4		– 21.8	– 109.6	– 87.8	[6]
24	**t**($(A_4T_4)_2$)	45.1		– 12.8	– 92.2	– 79.4	[6]
25	**t**($(A_4U_4)_2$)	29.5		– 7.8	– 58.6	– 50.8	[6]
26	**t**($(AT)_8$)	74.5		– 15.8	– 65.7	– 49.9	[6]
27	**t**($(TA)_8$)	71.4		– 17.4	– 78.5	– 61.1	[6]
			58.5***	– 16.0**	– 84.3**	– 68.3**	
28	**t**($(AU)_8$)	68.6		– 20.8	– 109.2	– 88.4	[6]
			61.9**	– 17.2**	– 93.8**	– 76.6**	
29	**t**($(CG)_4$)	67.0		– 14.6	– 62.9	– 48.3	[6]
30	**t**(CGAATTCG)	29.8		– 7.6	– 41.0	– 33.4	[6]
31	**t**(CGCGAATTCGCG)	72.5		– 18.3	– 79.3	– 61.0	[6]
			70.5**	– 18.1**	– 82.8**	– 64.7**	
32	**t**(CGCGAAUUCGCG)	69.5		– 18.8	– 92.3	– 73.5	
			68.2**	– 15.9**	– 73.2**	– 57.3**	[6]
33	**t**($(TA)_3TGC(AT)_3A$)		66.8***				
34	**t**($T(ATGC)_3ATA$)	> 90	84.3**				
35	**t**($(CG)_2T_4(AT)_2$) (hairpin)	67.4		– 5.0[c])	– 41.9[c])	– 36.9[c])	[6]

[a]) Measurements were made in 0.01M NaH_2PO_4, 0.1 mM Na_2(EDTA) buffer, pH 7.0 unless otherwise indicated. Error of T_m determination estimated ± 0.5°. Values with asterisk (*) were measured in 0.01M *Tris* · HCl buffer, pH 7.0; **: in 150 mM NaCl. ***: $c \approx 5$ μM in 150 mM NaCl. [b]) Thermodynamic data from plots of T_m^{-1} *vs.* ln c; experimental error estimated in ΔH values ± 5%. [c]) Thermodynamic data calculated for hairpin by T_m curve differentiation at $c \approx 5$ μM according to [34] (p. 1610).

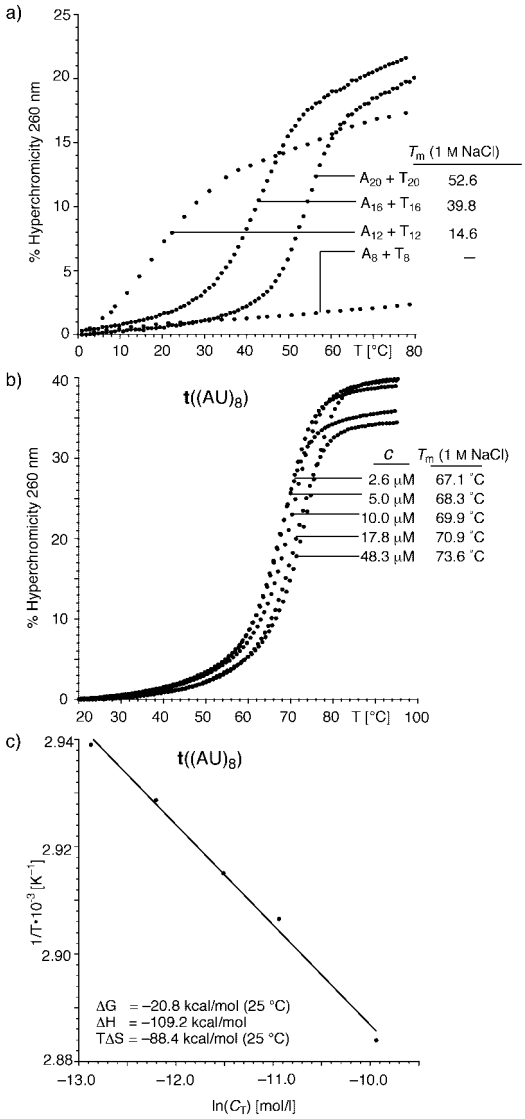


Fig. 4. *UV-Spectroscopic data documenting the pairing behavior of A,T- and A,U-containing (3′→2′)-α-*L*-threofuranosyl sequences. a*) UV-Spectroscopic T_m curves (heating) of selected duplexes formed between homo-basic sequences showing the effect of sequence length on T_m. *b*) UV-Spectroscopic T_m curves of the duplex formed by the self complementary sequence t(AU)$_8$ at various concentrations. *c*) Determination of thermodynamic data of indicated duplex formation from the concentration dependence of T_m values. For method, see [34]. All measurements were made in 10 mM aq. NaH_2PO_4 containing 0.1 mM Na_2(EDTA), 1M NaCl at pH 7.0. Total oligonucleotide concentrations in all measurements were ca. 10 μM unless otherwise indicated. All T_m curves were fully reversible (no hysteresis). T_m Values are calculated from the maxima of the first derivative curve with Kaleidagraph software program.

degree and sense of the backbone/base-pair axis inclination [35]. Hexadecamer duplexes of sequences with irregularly mixed purine and pyrimidine bases (*e.g.*, *Entries 9* and *10*) show representative average stabilities and demonstrate the antiparallel strand orientation in TNA duplexes. This latter preference is very pronounced, as the absence of base pairing between the parallel-complementary hexadecamer A,T sequences of *Entry 11* illustrates (see Fig. 2D in [6]). There is a consistent drop in duplex stability when T is exchanged by U, the difference being most pronounced in homo-oligomeric sequences (see, *e.g.*, *Entry 7 vs. 8*) and distinctly less in alternating sequences (see, *e.g.*, *Entry 26 vs. 28*). The stoichiometry of duplex formations has been tested UV-spectroscopically for the combination of $t(A_{16})$ and $t(T_{16})$, and found to be 1:1 (see Fig. 2B in [6]). No self-pairing of $t(A_{16})$ or $t(T_{16})$ single strands is observed. $t(A_{16})$ exhibits an UV-extinction/temperature behavior that is reminiscent of that of $r(A_{16})$ (Fig. 2A in [6]).

In sequences consisting of G and C nucleobases, strong pairing can be observed already at the octamer level (*Entry 29*). Mixed-base sequences containing G, C, A, and T (or U) were found to behave in the manner expected concerning duplex stability (*Entries 30–35*). *Fig. 5* documents corresponding data for the duplex from the two sequences t(ATTCAGCG) and t(CGCTGAAT), as well as of that of the self-complementary sequence t(CGCGAAUUCGCG). The ease by which hairpins are formed in the TNA series is exemplified by the sequence t(CGCGTTTTCGCG) (*Entry 35*) as shown in *Fig. 6*.

Replacement of A (adenine) with D (2,6-diaminopurine) has a dramatic effect on duplex stability as shown by the increase in T_m of nearly 30°, when $t(D_{12})$, instead of $t(A_{12})$ is paired with $t(T_{12})$ (*Entry 3 vs. 13*). Similar enhancements of thermal and thermodynamic stabilities by A → D replacement are observed in the mixed-base sequences as shown by *Entries 14–16* and partially documented in Fig. 7[20]).

Probably, the most important property of TNA is its capacity to undergo informational cross-pairing with RNA and DNA. To observe this was not necessarily a complete surprise, because cross-pairing with DNA was encountered before in the α-L-lyxopyranosyl-(4′ → 3′)-oligonucleotide series, the pairing system that had induced us to check on the properties of α-L-threofuranosyl-(3′ → 2′)-oligonucleotides in the first place. The efficiency of TNA's cross-pairing capacity is documented by T_m and thermodynamic data in *Tables 5* and *6*, and by the UV and CD curves in *Fig. 8* (see also the data presented in [1]). Inter- and intrasystem cross-pairing was extensively studied for the sequences t(3′-AAAATTTATATTATTA-2′) and t(3′-TAATAATATAAATTTT-2′), and the corresponding complementary sequences of the RNA and DNA series. The close similarity of the CD curves of the TNA/RNA self- and cross-pairing is indicative of the ability of the two systems to adopt a common type of duplex structure (*Fig. 8, c–f*); this is further exemplified by the stability of duplexes formed from chimeric TNA-RNA strands containing self-complementary sequences (*Fig. 8, b*, *Table 5*, *Entries 31–34*). As expected from previous observations, A → D replacements in TNA drastically

---

[20]) For further documentation of the effect of A → D replacement in the TNA series, as well as for literature references regarding the effect of A → D replacement in other oligonucleotide system, see our preliminary communication [1].

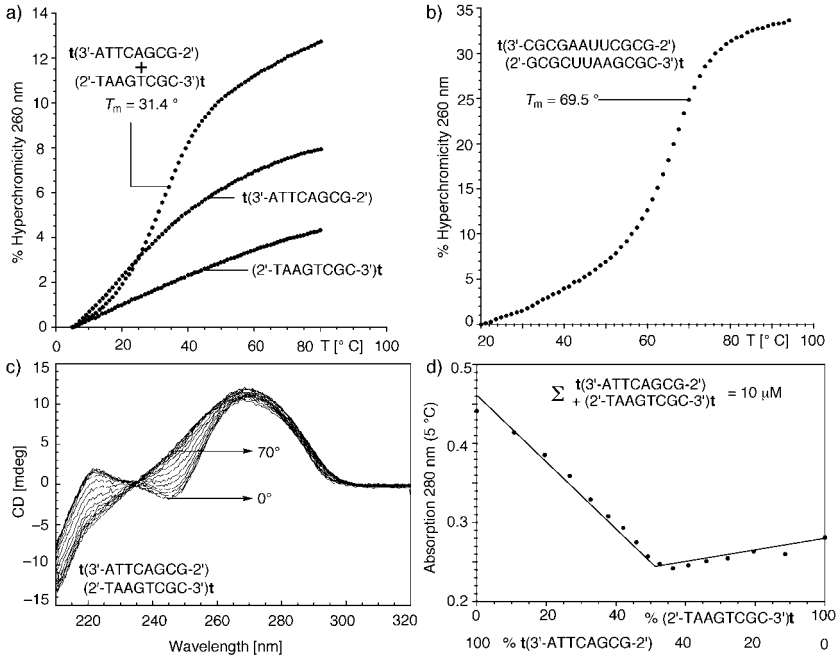


Fig. 5. *UV- and CD-spectroscopic data documenting the pairing behavior of TNA sequences containing A, T, U, C, and G: a*) UV-spectroscopic T_m curves of the duplex formed by **t**(3′-ATTCAGCG-2′) with its antiparallel complement **t**(3′-CGCTGAAT-2′) and T_m curves of the individual strands. *b*) UV-Spectroscopic T_m curves of the duplex formed by the self-complementary sequence **t**(3′-CGCGAAUUCGCG-2′). *c*) Temperature-dependent CD curves of the duplex formed by **t**(3′-ATTCAGCG-2′) with its antiparallel complement **t**(3′-CGCTGAAT-2′); temperature range: $0° \rightarrow 70°$. *d*) Molar-ratio dependance of UV absorption (280 nm, at $5.0 \pm 0.3°$) ('mixing curve') indicating 1:1 duplex stoichiometry for the pairing between **t**(3′-ATTCAGCG-2′) and its antiparallel complementary strand **t**(3′-CGCTGAAT-2′). Total oligonucleotide concentrations in all measurements were *ca.* 10 μM unless otherwise indicated. For conditions of measurement, see caption of *Fig. 4*.

enhance the stability of cross-pairing of TNA with RNA and DNA (*Table 6*). All these observations are consistent with, and are strongly pointing to, *Watson-Crick* as the base-pairing mode assignment for both the intra- and intersystem pairing of TNA[21]).

Interestingly, TNA sequences were also found to cross-pair weakly with complementary sequences of the ribofuranosyl-(5′ → 2′)-oligonucleotide and α-L-lyxopyranosyl-(4′ → 3′)-oligonucleotide series (*Table 5*, *Entries 11–14*)[22]).

---

[21]) The structural fit as well as the *Watson-Crick* pairing mode in TNA's cross-pairings with oligonucleotides of the natural series has recently been demonstrated by *C. J. Wilds*, *Z. Wawrzak*, and *M. Egli* (see a forthcoming publication) by an X-ray analysis of the DNA/TNA duplex $[d,t(CGCGAATTCGCG)]_2$ in which the first T-nucleotide unit in each strand is replaced by a corresponding TNA unit. Furthermore, a comprehensive NMR structure anlysis of the TNA duplex derived from the self-complementary strands t(CGAATTCG) has been carried out by *Jaun* and *Ebert* (ETH, Zürich) and will be described in a forthcoming publication.

[22]) Not unexpectedly, no cross-pairing was observed between TNA and pyranosyl-RNA, in as far the combinations $\mathbf{t}(A_{16}) + \mathbf{pr}(T_{12})$, $\mathbf{t}(T_{16}) + \mathbf{pr}(A_{12})$, and $\mathbf{t}(D_{12}) + \mathbf{t}(T_{12})$ are concerned.

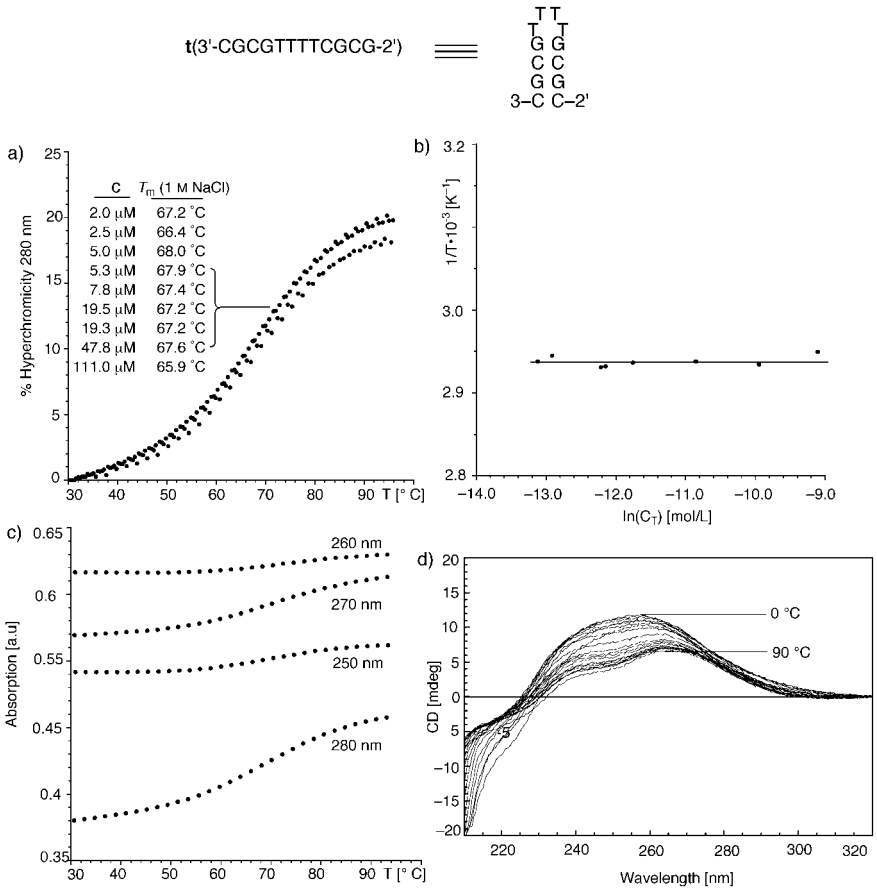


Fig. 6. *UV- and CD-spectroscopic data documenting the formation and the pairing behavior of the hairpin sequence* ***t****(3′-CGCGTTTTCGCG-2′). a*) T_m Curves at different oligonucleotide concentrations. *b*) T_m Invariance with changing concentration. *c*) Temperature dependence of UV absorption at various wavelengths ($c = 5.0$ μM) showing the highest hyperchromicity at 280 nm. *d*) Temperature dependent CD curves ($c =$ 2.5 μM); temperature range: $0° \rightarrow 90°$. For conditions of measurement, see caption of *Fig. 4*.

An intriguing aspect of TNA's cross-pairing behavior is the difference in pairing strengths exhibited by homo-oligomeric sequences *vs.* hetero-oligomeric sequences: whereas the hetero-oligomeric sequences give rise to duplexes that are of comparable stability independent of the type of backbone they inhabit, the stability of the homo-oligomeric duplexes varies widely and depends on to which backbone the all-purine and to which the all-pyrimidine sequence belongs (*Fig. 8, a*, *Table 5*, *Entries 1–14*). For example, the case of cross-pairing between A_{16} and T_{16}: while the combination of $t(A_{16}) + r(T_{16})$ gives rise to a duplex melting at 76°, the duplex of the reverse combination $r(A_{16}) + t(T_{16})$ melts at 28° ($c \approx 10$ μM, 1M NaCl). The same trend is true for TNA cross-pairings with DNA, (5′→2′)-RNA and 3′,4′-lyxopyranosyl-NA. This behavior in intersystem cross-pairing is by no means specific for TNA; it can be encountered in literature with respect to various pairing systems [36]; in our own work,

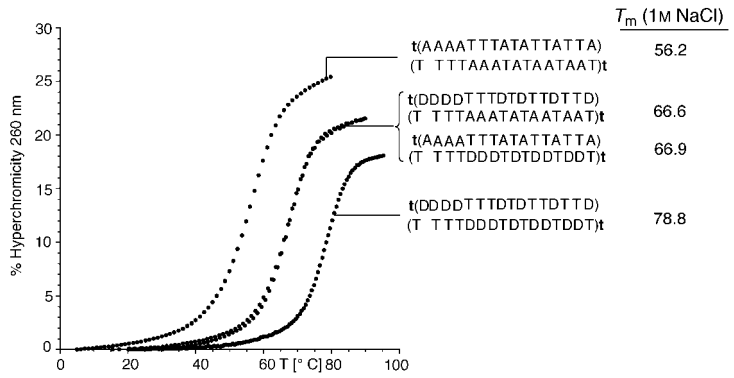


Fig. 7. *UV-Spectroscopic* T_m *curves showing the effect of* $A \rightarrow D$ *replacement on the* T_m *values of the duplex formed from the base sequence* ***t****(3′-AAAATTTATATTATTA-2′) and its antiparallel complement* ***t****(3′-TAATAATATAAATTTT-2′)*. For conditions of measurement, see caption of *Fig. 4*.

it has been extensively seen before in cross-pairing within the family of pentopyranosyl-$(4' \rightarrow 2')$-oligonucleotides [37][23]).

The data in *Tables 5* and *6* contain all presently available comparisons between TNA, and RNA and DNA duplexes with regard to their thermal and thermodynamic stabilities. As a general trend valid for duplexes consisting of hetero-oligomeric strands, the available data indicate that TNA duplexes containing base sequences above a critical length are comparable to corresponding RNA complexes with regard to their thermal stability (as given by T_m values), but tend to be less stable than the RNA complexes with respect to their thermodynamic stability.

Fig. 9 provides a comparison among various base-pairing systems studied in our laboratory referring to the stability of oligonucleotides under conditions of phosphodiester hydrolysis. Under the conditions tested (1.0M NaCl, 0.25M $MgCl_2$, 0.1M *Hepes*, pH = 8, 35°, monitoring with ion-exchange HPLC), r(U_8) decomposes rapidly with a half-life of *ca.* 12 h, while t(T_8) and d(T_8) remain completely unchanged, over a period of at least 8 days. For the TNA sequence HPLC monitoring over a period of months did not reveal any decomposition. t(T_8) and t(A_8) were found to be equally stable over months in the presence of 3.5M KCl or 3.5M LiCl in lieu of the $MgCl_2$/NaCl combination under otherwise identical, conditions. The known importance of the presence of a vicinal *cis*-OH group in such systems for the ease of phosphodiester hydrolysis is well illustrated by the observed intermediate stabilities of the pentopyranosyl-$(4' \rightarrow 2')$-oligonucleotide family.

---

[23]) We consider the origin of this general phenomenon to be primarily related to the fact that, in a duplex consisting of homo-oligomeric base sequences, constellational mismatches between backbone units are bound to accumulate along the duplex strands, when the base sequences are homo-oligomeric, but not necessarily so when they are hetero-oligomeric. Duplex strands may adapt to constellational mismatching of backbone units primarily through changing nucleosidic torsion angles [37], a process that is dependent on whether the nucleobase is a purine or a pyrimidine.

Table 5. T_m *Values and Thermodynamic Data of Inter- and Intrasystem Duplex Formation*

Entry	Oligonucleotide pairing system	T_m [°][a]) TNA duplexes	ΔG (25°)	ΔH	$T\Delta S$ (25°)
		1M NaCl, $c \approx 10$ μM	1M NaCl[a])[b])		
1	**t**(A_{12}) + **r**(T_{12})	10.0	– 6.7	– 28.1	– 21.4
2	**t**(T_{12}) + **r**(A_{12})	< 0*			
3	**t**(A_{16}) + **r**(T_{16})	76.3	– 22.1	– 101.0	– 78.9
4	**t**(T_{16}) + **r**(A_{16})	28.1	– 8.3	– 60.5	– 52.5
5	**t**(A_{16}) + **d**(T_{16})	67.5	– 18.6	– 87.5	– 68.9
6	**t**(T_{16}) + **d**(A_{16})	31.8	– 8.9	– 51.6	– 42.7
7	**r**(A_{16}) + **r**(T_{16})	62.0	– 20.1	– 112.4	– 92.3
8	**d**(T_{16}) + **d**(A_{16})	55.3	– 16.2	– 92.8	– 76.6
9	**r**(A_{16}) + **d**(T_{16})	47.4	– 14.3	– 93.5	– 79.2
10	**d**(T_{16}) + **r**(A_{16})	59.3	– 19.5	– 114.2	– 94.7
11	**t**(A_{16}) + 5′,2′-**r**(T_{16})	< 5			
12	**t**(T_{16}) + 5′,2′-**r**(A_{16})	26.0	– 7.9	– 66.1	– 58.3
13	**t**(A_{16}) + 3′,4′-**pl**(T_{12})	26.8			
14	**t**(T_{16}) + 3′,4′-**pl**(A_{12})	63.5			
15	**t**($T_4A_3TATA_2TA_2T$) + **r**($AT_2AT_2ATAT_3A_4$)	57.3	– 18.3	– 109.8	– 91.5
16	**r**($T_4A_3TATA_2TA_2T$) + **t**($AT_2AT_2ATAT_3A_4$)	50.0			
17	**t**($T_4A_3TATA_2TA_2T$ + **d**($AT_2AT_2ATAT_3A_4$)	42.5			
18	**d**($T_4A_3TATA_2TA_2T$) + **t**($AT_2AT_2ATAT_3A_4$)	35.6			
19	**r**($T_4A_3TATA_2TA_2T$) + **d**($AT_2AT_2ATAT_3A_4$)	40.0			
20	**d**($T_4A_3TATA_2TA_2T$) + **r**($AT_2AT_2ATAT_3A_4$)	41.1			
21	**r**($T_4A_3TATA_2TA_2T$) + **r**($AT_2AT_2ATAT_3A_4$)	57.0	– 21.8	– 145.9	– 124.1
22	**d**($T_4A_3TATA_2TA_2T$) + **d**($AT_2AT_2ATAT_3A_4$)	43.3	– 13.5	– 99.0	– 85.5
23	**t**(ATTCAGCG) + **r**(CGCTGAAT)	40.6			
24	**r**(ATTCAGCG) + **t**(CGCTGAAT)	39.0			
25	**t**(ATTCAGCG) + **d**(CGCTGAAT)	26.3			
26	**d**(ATTCAGCG) + **t**(CGCTGAAT)	25.4			
27	**r**(ATTCAGCG) + **d**(CGCTGAAT)	34.5			
28	**d**(ATTCAGCG) + **r**(CGCTGAAT)	35.6			
29	**r**(ATTCAGCG) + **r**(CGCTGAAT)	52.0			
30	**d**(ATTCAGCG) + **d**(CGCTGAAT)	36.7			
31	**t**,**r**(**r**(A_8)**t**(T_8))	< 5			
32	**t**,**r**(**t**(A_8)**r**(T_8))	61.3	– 15.9	– 85.7	– 69.8
33	**t**,**r**((**rAtT**)$_8$)	50.1	– 14.0	– 96.0	– 82.0
34	**t**,**r**((**tArT**)$_8$)	25.8	– 7.2	– 70.5	– 63.3

[a]) Measurements were made in 0.01M NaH_2PO_4, 0.1 mM Na_2EDTA buffer, pH 7.0 unless otherwise indicated. Error of T_m determination estimated ± 0.5°. Values with asterics (*) were measured in 0.01M *Tris*·HCl buffer, pH 7.0. [b]) Thermodynamic data from plots of T_m^{-1} *vs.* ln c; experimental error estimated in ΔH values ± 5%. **t**: α-L-Threofuranosyl; **r**: β-D-ribofuranosyl; **d**: 2′-deoxy-β-D-ribofuranosyl; p**l**: α-L-lyxofuranosyl

3. Discussion. – Among the nucleic acid alternatives that have been investigated thus far in our laboratory as part of studies toward a chemical etiology of nucleic acid structure, it is the most recently studied member, TNA, that seems to attract the most attention among interested chemists and biologists. Whilst the base-pairing properties of homo-DNA, surprising as they were at the time, had made clear for the first time that the capacity of *Watson-Crick* base pairing is not a property exclusive to the *Watson-Crick* double-helix-type structure, and whereas the chemistry of pyranosyl-RNA had highlighted the remarkable possibility that the very same building blocks that make up

Table 6. T_m *Values and Thermodynamic Data of Duplexes from Inter- and Intrasystem Cross-Pairings*[a])

T_m [°] ΔG (25°) ΔH $T\Delta S$ [kcal/mol]	TNA X = D Y = T	TNA A T	RNA A U	DNA A T
TNA X = D Y = T	**79** – 24.6 – 107.3 – 82.7	**67** – 20.3 – 98.9 – 78.6	**74** – 25.8 – 125.2 – 99.4	**56** – 20.1 – 83.6 – 63.5
TNA A T	**67** – 17.8 – 79.0 – 61.2	**56** – 14.5 – 73.7 – 59.2	**57** – 17.3 – 93.9 – 76.6	**47** – 13.3 – 77.3 – 64.0
RNA A U	**65** – 21.6 – 120.4 – 98.8	**52** – 15.8 – 95.2 – 79.4	**59** – 20.0 – 131.9 – 111.9	**44** – 12.3 – 73.9 – 61.6
DNA A T	**49** – 15.0 – 91.6 – 76.7	**41** – 11.0 – 57.0 – 46.0	**43** – 13.7 – 98.0 – 84.3	**48** – 16.8 – 129.2 – 112.4

(or 5′) 3′-XXXXYYYXYXYYXYYX-2′ (or 3′)
(or 3′) 2′-YYYYXXXYXYXXYXXY-3′ (or 5′)

[a]) Conditions: $c \approx 5 + 5$ µM , 1.0M NaCl, 10 mM NaH_2PO_4, 0.1 mM Na_2(EDTA), pH 7.0; The color of the symbols relates to the oligonucleotide sequences of the same color in the formulae of the duplex given at the bottom of the table. T_m Values in the shaded diagonal refer to intrasystem pairing, the others to intersystem cross-pairing.

today's RNA could have given rise to an isomeric *Watson-Crick* system that, with respect to both base-pairing strength and pairing-mode selectivity, by far excels – on the chemical level – over the natural isomer, TNA's edge in 'popularity' is simply due to its capability to communicate by informational base pairing with RNA and DNA. In this regard, TNA's behavior is in sharp contrast to that of the previously studied model system homo-DNA and the nucleic acid alternatives of the pentopyranosyl series. Those systems may be equally, or even more, articulate than TNA in their base-pairing capability, but they 'speak base-pairing languages' that are different from that 'spoken' by RNA and DNA [10].

We have previously considered the 'backbone inclination' of base-pairing systems containing different backbones to be a (if not the) major structural parameter that determines whether such systems are capable of communicating with each other by cross-pairing or not [10][35]. Intersystem cross-pairing requires the capability of partner strands to conformationally adjust to a common inclination of their backbone axes relative to a common base-pair axis. Homo-DNA and pyranosyl-RNA both have a high backbone inclination, but of opposite orientation, whereas, in the *B*-form of DNA, the axis of the backbone and that of the base pairs are essentially orthogonal to each

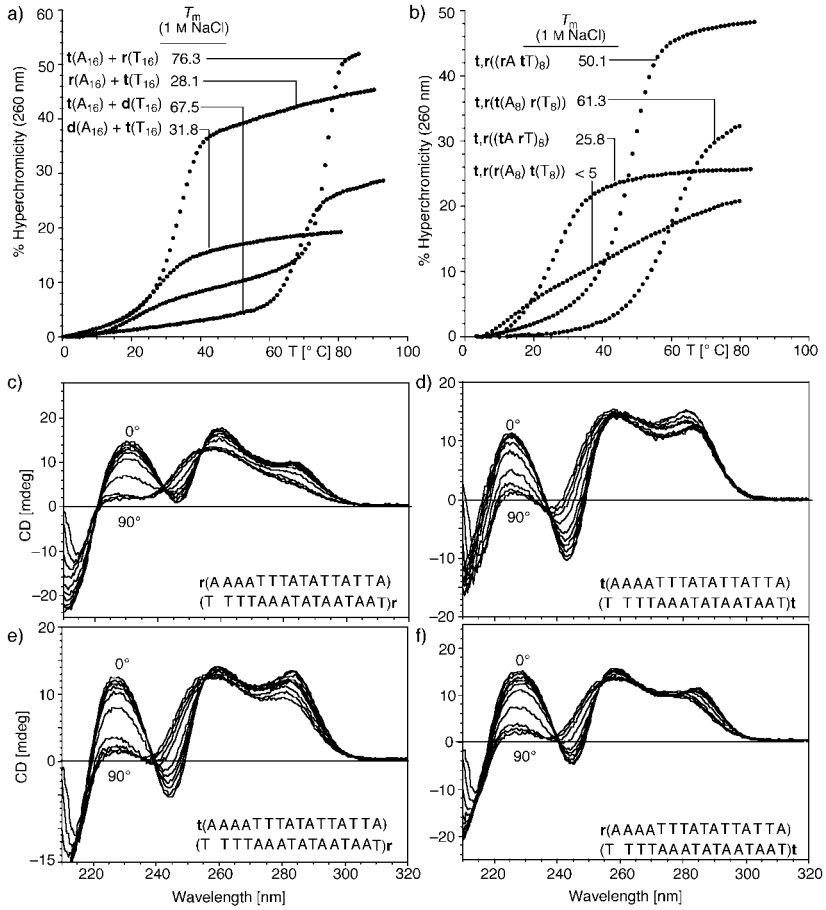


Fig. 8. *UV- and CD-spectroscopic data documenting the inter-system cross-pairing between TNA sequences with corresponding antiparallel complementary RNA and DNA sequences. a*) UV-Spectroscopic T_m curves of indicated duplexes. *b*) UV-spectroscopic T_m curves of duplexes formed by indicated self-complementary RNA-TNA-chimera sequences ($c \approx 6$ μM). *c*)–*e*) Temperature-dependent CD curves of the inter- and intrasystem duplexes formed by AAAATTTATATTATTA strands with its antiparallel complement TAATAATA-TAAATTTT strands between and within the RNA and TNA series; temperature range: $0° \rightarrow 90°$. Total oligonucleotide concentrations in all measurements were *ca.* 10 μM unless otherwise indicated. For conditions of measurement, see caption of *Fig. 4*.

other. A qualitative assessment with the help of molecular models suggest that the backbone inclination of TNA is DNA-like[24]). A more quantitative assessment of all

[24]) A qualitative but experimentally easily accessible indicator for the degree and orientation of a system's backbone inclination are the relative duplex stabilities of self-complementary mirror sequences. In the homo-DNA series, block sequences of the type (purine)$_n$-(pyrimidine)$_n$ give rise to more stable duplexes than (pyrimidine)$_n$-(purine)$_n$ sequences, whereas the opposite is true for pyranosyl-RNA and all other members of the pentopyranosyl-(4′ → 2′)-family [35]. In the TNA series, duplexes derived from such mirror sequences have approximately similar stabilities (see *Table 4*).

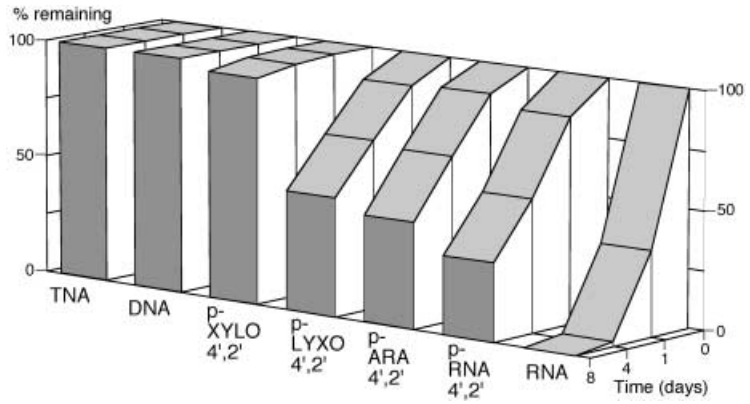


Fig. 9. *Comparison of the hydrolytic stability of the phosphodiester group of T_8 strands in the TNA, DNA, RNA, and the pentopyranosyl-NAs series under the conditions: 1.0*M *NaCl, 0.25*M *$MgCl_2$, 0.1*M *HEPES buffer, pH 8.0, 35°, monitored by ion-exchange HPLC* (including data determined by *T. Müller* [38]). In the case of RNA, the pyrimidine base is U.

these relationships based on a workable structural definition of the backbone inclination[25]) is clearly desirable.

Representing a structure type of obviously lesser complexity than that of the natural nucleic acids, TNA has been referred to in the literature [39][40] as a possible candidate for the ancestry of RNA; such considerations are based on an evolutionary scenario in which an RNA ancestor would have acted as a template for the formation of RNA base-sequences in template-controlled oligomerizations, thereby facilitating RNA's emergence in biological evolution[26]). When, in a context such as this, a system's complexity is to be assessed in comparison to another system, *e.g.*, TNA *vs.* RNA, it becomes necessary to differentiate between molecular complexity referring to the constitutional and spatial parameters of the structure of a molecule, and the complexity referring to the parameters of the formation of that structure within given boundary conditions [42] ('constitutional' *vs.* 'generational' complexity). High constitutional complexity of molecules can go along with a generational complexity that can be low; examples abound, *e.g.*, the structure types of sugars, porphyrins, or fullerenes. Any assessment of generational complexity obviously makes sense only with strict reference to boundary conditions[27]). To consider TNA a 'simpler' nucleic acid than RNA [39] makes sense in as far as its sugar unit appears derivable from one single C_2 building

---

[25]) See a (hopefully) forthcoming publication with *M. Egli* and *P. Lubini*.

[26]) We have previously [41] argued that describing such a process as 'transfer of genetic information' from an RNA ancestor to RNA can be misleading in as far as this expression would make sense only if the 'phenetic information' contained in the chemical phenotype of the ancestor would be transferred concomitantly. Such cannot be assumed to be the case.

[27]) This requirement specifically refers to situations where simple-looking structures are in fact complex from a generational point of view. Examples of this kind, specifically relevant in the present context, are the phosphodiester based nucleic acid systems derived from glycerol that had been proposed as possible RNA ancestors [43]. These systems may look simple, but they are complex with regard to their formation under the constraints of prebiotic conditions.

block ($C_2 + C_2 \rightarrow C_4$), whereas the corresponding derivation of RNA's sugar unit requires the involvement of two of them ($C_2 + C_1 \rightarrow C_3 + C_2 \rightarrow C_5$). These two structure derivations involve the same reaction type (aldolization) and, therefore, refer (in principle) to the same boundary conditions. Considerations of oligonucleotide systems that might have been ancestors of RNA must imply the presumption that the generational complexity of such ancestors is lower than that of RNA with respect to boundary conditions assumed to be prebiotic. For the special case of comparing TNA with RNA, such may be said to be true for the sugar part of the two structures, but less so with respect to those structural features that are mainly responsible for the serious doubts concerning the possibility of an abiological origin of RNA [44]. These features are the nucleosidic junction between sugar and nucleobase, and the phosphodiester linkage between the repeating oligomers unit. With respect to the generational constraints concerning these two structural features, TNA is not less complex than RNA.

Could TNA-related informational oligomer systems exist that might share TNA's simplicity with regard to the $C_2 + C_2 \rightarrow C_4$ pathway, yet would be devoid of the complexities to be associated with the phosphodiester and nucleoside parts of the TNA structure? Considerations based on chemical criteria led us to analyze more broadly the potentially natural chemistry that the $C_2 + C_2 \rightarrow C_4$ pathway of building-block formation may encompass; this chemistry may offer itself as a lead in a systematic search for generational simplicity in TNA-related systems. The library of relationships existing among C_2 and C_4 building blocks at the oxidation level of (the threose precursor) glycolaldehyde, when nitrogen at the oxidation level of ammonia is (formally) allowed to participate in $C_2 + C_2 \rightarrow C_4$ aldolization chemistry (*Scheme 6*), indicates the general direction of our inquiry. From such a perspective, TNA represents the limiting case of the fully oxygenous variant within a library of TNA-related base-pairing systems derived from C_4-backbone units ranging from a fully oxygenous to a fully nitrogenous member.

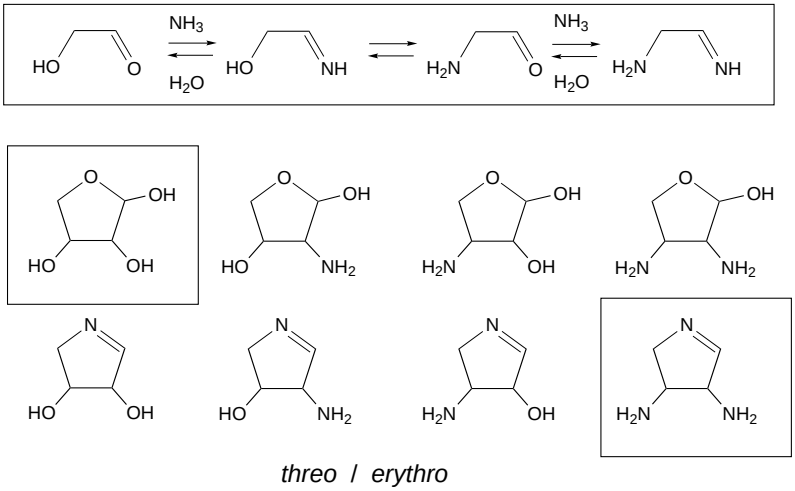
As reported in a short communication [7], two isomeric phospho-monoamidate analogs of TNA, whose structures have been inspired by the reasoning illustrated in *Scheme 6*, have recently been synthesized: both nitrogenous variants are efficient base-pairing systems, and both have the capability to cross-pair with RNA (*Scheme 7*). Furthermore, formation of phosphoramidate linkages by template-controlled ligation was shown in the TNA series to be much more efficient than a formation of corresponding phosphodiester linkages [1][28]). Finally, new aspects of the notorious nucleosidation problem were recognized, in as far it could be shown that cyclic iminium ions derived from 4-deoxy-4-aminothreose derivatives undergo efficient *C*-nucleosidation with appropriate purines and pyrimidines[29]). These are the types of findings that encourage further exploration of nitrogenous TNA variants in the search for generational simplicity among TNA-related informational oligomers.

---

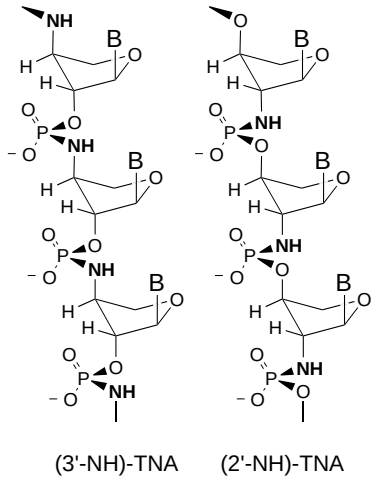
[28]) The higher efficiency of phosphate-to-phosphoroamidate ligations as compared to phosphate-to-phosphodiester ligation in the natural series has been demonstrated before [45].

[29]) Forthcoming publications by *Z. Wang*, *H. K. Huynh*, *B. Han*, *V. Rajwanshi*, *R. Krishnamurthy*, and *A. Eschenmoser*.

Scheme 6. *Survey of Constitutional $C_2 + C_2 \rightarrow C_4$-Relationships on the Oxidation Level of Glycolaldehyde with Participation of Ammonia*



Scheme 7. *The Two Nitrogenous TNA Analogs That Thus Far Have Been Shown to Undergo Intersystem Cross-Pairing with TNA, RNA, and DNA* [7]



The work was supported by the *Skaggs Foundation* (*TSRI*) and *Novartis AG*, Basel. *K. S.*, *P. S.*, *X. W.*, and *S. G.* have been *Skaggs* Postdoctoral Fellows. *G. D.* thanks the *Skaggs Research Foundation* and la *Universidad Nacional Autónoma de México* for support. *S. G.* thanks *NASA/NSCORT, K. S.* and *P. S.* thank the *Deutsche Forschungsgemeinschaft* for postdoctoral fellowships. We thank Prof. *Stefan Pitsch* for advice and help in the synthesis of oligonucleotides.

Experimental Part

General. Solvents for extraction: ACS grade. Solvents for reaction: reagent grade. Reagents: unless otherwise noted, from *Acros*, *Fluka,* or *Aldrich*, highest quality available. Chloro(2-cyanoethoxy)(diisopropylamino)phosphine (97%) was purchased from *Chem-Impex Inc.*, Wood Dale, IL, USA. TLC: Silica gel *60* F_{254} aluminum plates (*Whatman*, Type *Al Sil G/UV*, 250-μm layer); visualization by UV absorption and/or *A*) by dipping in a soln. anisaldehyde /H_2SO_4/AcOH/EtOH 5:5:1:18 or *B*) cerium(IV) sulfate (3 mM)/ammonium molybdate (250 mM) in aq. H_2SO_4 (10%), followed by heating. Flash column chromatography (CC) was performed on silica gel *60* (0.40–0.63 mm, 230–440 mesh, *EM Science*) at low pressure (max. 2 bar). In case of acid-sensitive compounds, the silica gel was pretreated with solvents containing *ca.* 0.5% Et_3N. Melting points (uncorrected) were measured with *MEL-TEMP II* (*Laboratory Devices Inc.*, USA). NMR: ^{1}H: δ values in ppm (TMS as internal standard); *J* [Hz], assignments of ^{1}H resonances were in some cases based on 2D experiments (^{1}H,^{1}H-COSY); ^{13}C: δ values in ppm (TMS as internal standard); *J* [Hz]; assignments and multiplicities were based on 2D experiments (^{1}H,^{13}C-COSY); ^{31}P: δ values in ppm (85% H_3PO_4 as external standard). FAB + -MS (matrix-soln.): *m/z* (intensity in %); performed in the positive-ion mode, with 3-nitrobenzyl alcohol (3-NBA) as matrix, on a *VG ZAB-VSE* double-focusing high-resolution (HR) mass spectrometer equipped with a cesium ion gun. Matrix-assisted laser-desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was performed on a *Voyager-Elite* mass spectrometer (*Perseptive Biosystems*) with delayed extraction with THAP or DHB as the matrix with ammonium citrate added to the sample. Elemental analysis was performed using *Perkin-Elmer PE2400 CHN* analyzer. Oligonucleotides were synthesized on an *Expedite 8909 Nucleic Acid Synthesis* system (*Perseptive Biosystems*) or on a *Pharmacia Gene Synthesizer Plus.* HPLC: Ion-exchange (IE) HPLC was performed on *A*) *Pharmacia GP-250 Gradient Programmer* equipped with two *Pharmacia P-500* pumps, *ABI-Kratos Spectraflow 757* UV/VIS detector and a *Hewlett Packard HP 3396A* analog integrator or *B*) *Pharmacia Äkta Purifier (900)* controlled by *UNICORN* system. Columns: *Mono Q HR 5/5* (*Pharmacia*) or *SAX 1000-8* (*Macherey & Nagel*); Buffer *A:* 10 mM Na_2HPO_4 in H_2O, pH 11.5; Buffer *B:* 10 mM Na_2HPO_4 in H_2O, 1M NaCl, pH 11.5. UV: on a *Cary 1 C* spectrophotometer (*Varian*) or a *Perkin-Elmer Lambda 2*. Melting point (T_m) measurements of oligonucleotides were determined with *Cary 1 Bio* spectrophotometer (*Varian*). CD Spectrum was measured on an *AVIV 61 DS* CD spectropolarimeter. All measurements were made with the 'phosphate buffer', 10 mM aq. NaH_2PO_4 buffer containing 0.1 mM Na_2EDTA, 150 mM (or 1M) NaCl at pH 7.0, with a total oligonucleotide concentration of *ca.* 10 μM, unless indicated otherwise, and the samples were thoroughly degassed, either by heating or by vacuum and ultrasonication. Concentrations of oligonucleotide solns. were calculated from the UV absorbance of the solns. at 260 nm (pH 7) at *ca.* 80° with the following molar extinction coefficients: ε (t(A)) = 15000, ε (t(D)) = 10000, ε (t(C)) = 7600, ε (t(G)) = 11700, ε (t(T)) = 10000, ε (t(U)) = 10000. *Abbreviations:* Ag-Tf = silver trifluoromethanesulfonate, BOP: (benzotriazol-1-yl)oxyltris(dimethylamino)phosphonium hexafluorophosphate; BSA = bis(trimethylsilyl)acetamide, CPG = 'controlled pore glass', DHB = 2,5-dihydroxybenzoic acid, DIBAL-H = diisobutylaluminium hydride, DMAP: 4-(dimethylamino)pyridine, DMT: 4,4′-dimethoxytrityl, LCAA-CPG: long-chain aminoalkyl-CPG (500 Å); TMS-Tf = trimethylsilyl trifluoromethanesulfonate, THAP = 2,4,6-trihydroxyacetophenone, TsOH = toluene-4-sulfonic acid, UF H_2O = ultra-filtered H_2O.

1. Experiments Referring to *Scheme 2.* – *Calcium-*L*-threonate Monohydrate* (**2**) [14][15]. To a soln. of 264 g (1.5 mol) of L-ascorbic acid dissolved in 2.1 l of ultrapure H_2O, 300 g (3 mol) of $CaCO_3$ was slowly added with stirring, and the slurry was cooled to 15°. To this mixture, 600 ml of 30% aq. H_2O_2 soln. was added, at 12–18°, over a period of *ca.* 6 h, and the mixture was stirred at r.t. for 18 h. The mixture, under constant stirring, was treated with 60 g of charcoal and heated to 70°, until no more peroxide was detected (*Merckoquant 1001-1* peroxide test strips). The hot suspension was filtered, and the solid washed with 2 × 50 ml of UF H_2O. The washings were combined with the filtrate and concentrated to *ca.* 2 l under reduced pressure. The resulting soln. was stirred, and 600 ml of MeOH was slowly added over a period of 5 h. After stirring for 5 h, another 600 ml of MeOH was slowly added. The solids were isolated by filtration, washed with 2 × 100 ml of MeOH, and dried under reduced pressure to constant weight. The combined filtrates were concentrated, MeOH was slowly added, and the resulting precipitate was isolated by filtration. The procedure was repeated, and the first two crops gave 246 g (65%) of **2** as a colorless powder. M.p. > 300°. ^{1}H-NMR (300 MHz, D_2O): 3.61 (*dd*, *J* = 12.7, 8.0, H–C(4)); 3.67 (*dd*, *J* = 12.7, 5.7, H–C(4)); 3.97 (*ddd*, *J* = 8.3, 5.7, 2.7, H–C(3)); 4.04 (*dd*, *J* = 2.7, 1.3, H–C(2)).

*2,3-Di-*O*-benzoyl-*L*-threonolactone* (**3**) [16]. To a soln. of 47 g (143 mmol) of **2** in 1 l of warm H_2O, 300 ml of *Dowex 50WX4-50* resin was added, and the mixture was stirred for 30 min at 70°. The resin was removed by filtration and washed with H_2O. The washings were combined with the filtrate and evaporated to dryness under reduced pressure. The residue was dried at 50° to constant weight, suspended in MeCN, and co-evaporated with

MeCN (2×). The residue was suspended in 500 ml of dry MeCN, and 1 g (5 mmol) of TSOH was added. The mixture was refluxed for 1 h, cooled, filtered, and the solvent was removed under reduced pressure. The residue (containing the presumed L-*threonolactone* [12]) was dissolved in 200 ml of pyridine, cooled to 0°, and 50 g (358 mmol) of BzCl was added dropwise. The mixture was allowed to warm to r.t., stirred for 10 h, and diluted with 400 ml of CH_2Cl_2. The resulting mixture was washed sequentially with 3 × 250 ml of ice-cold aq. 1M HCl soln., 200 ml of H_2O, 200 ml of sat. aq. $NaHCO_3$ soln., and 200 ml of sat. aq. NaCl soln. The org. phase was dried ($MgSO_4$) and evaporated to dryness. The residue was crystallized from hexane/AcOEt 6:1 to yield 52 g (60%) of **3**. Colorless needles. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.37 (*m*, H–C(4)); 5.02 (*m*, H–C(4)); 5.80 (*m*, H–C(2); H–C(3)); 7.43–8.12 (*m*, 10 arom. H).

2,3-Di-O-*benzoyl*-L-*threofuranose* (**4**). A soln. of 40 g (120 mmol) of **3** in 500 ml of THF was cooled to −78°. Under Ar, 150 ml of a 1.5M (225 mmol) of DIBAL-H soln. in toluene was added dropwise. The mixture was stirred at −78°, and, as soon as the starting material was completely consumed (TLC, 4–10 h), 20 ml of MeOH was added over a period of 5 min to quench the reaction. The cooling bath was removed, the mixture was diluted with AcOEt, 400 ml of a sat. aq. sodium potassium tartrate soln. was added, and the mixture was stirred vigorously for 3 h. The org. phase was separated, washed with sat. aq. NaCl soln., and dried ($MgSO_4$). Evaporation of the solvent *in vacuo*, followed by CC (silica gel; hexane/AcOEt 2:1), gave a colorless oil (21 g (53%)) consisting of the two diastereoisomers **4a**,**b**[30]).

Data of **4a**,**b**: TLC (hexane/AcOEt 2:1): R_f 0.38. ^{1}H-NMR (600 MHz, $CDCl_3$): 3.97 (*dd*, *J* = 7.5, 3.1, H–C(4) of isomer **b**); 4.28 (*dd*, *J* = 10.4, 4.1, H–C(4) of isomer **a**); 4.60 (*dd*, *J* = 10.4, 6.1, H–C(4) of isomer **a**); 4.63 (*dd*, *J* = 7.9, 4.4, H–C(4) of isomer **b**); 5.48 (*dd*, *J* = 4.3, 4.3, H–C(2) of isomer **b**); 5.55 (*m*, H–C(2), H–C(3) of isomer **a**); 5.61 (*s*, H–C(1) of isomer **a**); 5.78 (*ddd*, *J* = 4.3, 4.3, 3.3, H–C(3) of isomer **b**); 5.89 (*d*, *J* = 4.4, H–C(1) of isomer **b**); 7.43–7.46 (*m*, 8 arom. H); 7.56–7.57 (*m*, 4 arom. H); 8.05–8.28 (*m*, 8 arom. H). ^{13}C-NMR (150 MHz, $CDCl_3$): 69.2 (*q*, C(4) of isomer **b**); 71.7 (*q*, C(4) of isomer **a**); 76.6 (2*d*, C(3) of both isomers); 77.9 (*d*, C(2) of isomer **a**); 81.7 (*d*, C(2) of isomer **b**); 95.3 (*d*, C(1) of isomer **a**); 100.8 (*d*, C(1) of isomer **b**); 128.4 (*d*, arom. C); 129.7 (*d*, arom. C); 129.8 (2*d*, arom. C); 133.4 (*d*, arom. C); 133.5 (*d,* arom. C); 165.5, 165.8, 165.9, 166.1(4*s*). MALDI/TOF-MS (pos., DHB): 351 (100, $[M + Na]^+$).

1-O-*Acetyl-2,3-di*-O-*benzoyl*-L-*threofuranose* (**5**). To a soln. of 19.7 g (60 mmol) of **4** in 200 ml of CH_2Cl_2/pyridine 1:1, 6.8 ml of (62 mmol) of Ac_2O and 50 mg (0.4 mmol) of DMAP were added, and the mixture was stirred at r.t. for 18 h. The mixture was then poured into a stirred, ice-cold mixture of sat. aq. $NaHCO_3$/CH_2Cl_2 and stirred for 1 h. The org. layer was separated, washed sequentially with sat. aq. $NaHCO_3$ soln., H_2O, and sat. aq. NaCl soln., dried ($MgSO_4$), and filtered. The solvent was removed under reduced pressure, and the residue was purified by CC (silica gel; hexane/AcOEt 2:1) to afford 21 g (93%) of a mixture of the two diastereoisomers **5a**,**b** as colorless oil.

Data of **5a**,**b**: TLC (hexane/AcOEt 2:1): R_f 0.32. ^{1}H-NMR (600 MHz, $CDCl_3$): 2.03 (*s*, MeCO); 2.16 (*s*, MeCO); 4.06 (*dd*, *J* = 10.5, 3.5, H–C(4) of isomer **b**); 4.22 (*dd*, *J* = 10.5, 3.7, H–C(4) of isomer **a**); 4.62 (*dd*, *J* = 10.5, 6.4, H–C(4) of isomer **b**); 4.68 (*dd*, *J* = 10.5, 6.1, H–C(4) of isomer **a**); 5.58 (*m*, H–C(3) of isomer **b**); 5.66 (*s*, H–C(2) of isomer **b**); 5.71 (*dd*, *J* = 4.7, 4.7, H–C(2) of isomer **a**); 5.76 (*ddd*, *J* = 6.3, 4.7, 3.7, H–C(3) of isomer **a**); 6.39 (*s*, H–C(1) of isomer **b**); 6.66 (*d*, *J* = 4.6, H–C(1) of isomer **a**); 7.23–7.50 (*m*, 4 H, arom. H); 7.59–7.62 (*m*, 2 H, arom. H); 8.05–8.11 (*m*, 4 H, arom. H). ^{13}C-NMR (150 MHz, $CDCl_3$): 20.8–21.0 (2*q*, *Me*CO); 70.8 (*s*, C(4) of isomer **b**); 73.2 (*s*, C(4) of isomer **a**); 75.9 (2*t*, C(3) of both isomers); 76.6 (*t*, C(2) of isomer **a**); 80.3 (*t*, C(2) of isomer **b**); 94.1 (*t*, C(1) of isomer **b**); 99.2 (*t*, C(1) of isomer **a**); 128.4 (*d*); 128.5 (*d*); 129.7 (*d*); 129.7 (*d*); 129.8 (*d*); 133.5 (*d*); 133.7 (*d*), 166.1, 165.0, 165.5, 165.3, 169.3 (2*s*, Me*C*O). MALDI/TOF-MS (pos., DHB): 393 (100, $[M + Na]^+$).

1,2,3-Tri-O-*benzoyl*-L-*threofuranose* (**6**) [18]. To a soln. of 5 g (15 mmol) of **4** in 50 ml of CH_2Cl_2/pyridine 1:1; 2 ml (17 mmol) of BzCl was added at 0°. The cooling bath was removed, and the mixture was stirred at r.t. for 18 h. The mixture was cooled to 0°, and 20 ml of 0.1M aq. HCl soln. was added. The org. layer was separated, washed with sat. aq. $NaHCO_3$ soln., H_2O, and sat. NaCl soln., dried ($MgSO_4$), and filtered. The solvent was removed under reduced pressure, and the residue was recrystallized from EtOH to afford a single isomer. The ethanolic mother liquor was filtered over silica gel (washed with hexane/AcOEt 2:1 to give the other isomer, for a combined yield of 4.57 g (70%).

Data of α-Isomer: ^{1}H-NMR (300 MHz, $CDCl_3$): 4.13 (*dd*, *J* = 11.6, 3.0, H–C(4)); 4.71 (*dd*, *J* = 11.8, 6.0, H–C(4)); 5.86 (*m*, H–C(2), H–C(3)); 6.88 (*d*, *J* = 4.3, H–C(1)); 7.35–7.75 (*m*, 9 H, arom. H); 7.98–8.22 (*m*, 6 arom. H).

---

[30]) The two diastereoisomers can be obtained as crystals [16].

Data of β-Isomer: ^{1}H-NMR (300 MHz, $CDCl_3$): 4.33 (*dd*, $J = 11.7, 3.7$, H–C(4)); 4.75 (*dd*, $J = 11.7, 6.7$, H–C(4)); 5.64 (*dd*, $J = 6.0, 3.6$, H–C(3)); 5.82 (*s*, H–C(2)); 6.65 (*s*, H–C(1)); 7.38–7.52 (*m*, 6 arom. H); 7.56–7.66 (*m*, 3 arom. H); 8.07–8.14 (*m*, 6 arom. H). MALDI/TOF-MS (pos., DHB): 455 (100, $[M + Na]^+$).

L-*Threose* (**1**). To a soln. of NaOMe (generated from 0.1 g (4.3 mmol) of Na in 45 ml of MeOH (dried and freshly distilled over Na)) cooled to 4°, was added 3.0 g (9.0 mmol) of **4**. The resulting mixture was stirred at 4° for 4 h. The light yellow soln. was neutralized by the addition of *Amberlite IR-120* (H^+) ion-exchange resin (the resin was previously stored in 80% aq. EtOH, filtered, and washed with plenty of H_2O and dried under high vacuum) at 4°. The ion-exchange resin was removed by filtration and washed with MeOH. The washings were combined with the filtrate and evaporated to dryness. The light yellow oil residue was dissolved in 35 ml of UF H_2O and was washed with 4×50 ml of CH_2Cl_2. The aq. phase was evaporated to dryness under reduced pressure to give 1.0 g of light yellow oil. The ^{1}H-NMR spectrum (CD_3OD) of this material in comparison to the spectrum of a commercially available L-threose sample (*Sigma*) indicated the product to be 90–95% pure, which would correspond to *ca.* 90% yield of L-threose (foreign signals in the benzoate region). Since the preparation of TNA-mononucleotides did not require free L-threose as the starting material, the procedure was not optimized.

2. Experiments Referring to *Scheme 3*. – N^6-*Benzoyl-9-(2′,3′-di*-O-*benzoyl-α*-L-*threofuranosyl)adenine* (**7a**). A suspension of 9.65 g (22.3 mmol) of **6** and 6 g (25.1 mmol) of dry N^6-benzoyladenine in 35 ml of abs. MeCN was treated with 11 ml (44.5 mmol) of BSA and heated to 65°. Stirring and heating was continued until a clear soln. was formed (*ca.* 30 min) and 8 ml (36 mmol) of $SnCl_4$ was added in 3 portions over a period of 2 min. After 1 h (monitored by TLC), the mixture was cooled to r.t. and poured into an ice-cold stirred mixture of 300 ml of sat. aq. $NaHCO_3$ soln./AcOEt 1:1. The org. layer was separated, the aq. layer was extracted with AcOEt, and the combined org. layers were washed with sat. aq. NaCl soln. and dried ($MgSO_4$). Evaporation of the solvent and CC (CH_2Cl_2/AcOEt 5:2) gave a crude product, which was recrystallized from EtOH to give 8.95 g (73%) of **7a** as colorless crystals. M.p. 98°. TLC (CH_2Cl_2/AcOEt 1:1): R_f 0.19. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.58 (*dd*, $J = 10.9, 2.2$, H–C(4′)); 4.68 (*dd*, $J = 10.9, 4.7$, H–C(4′)); 5.77 (*ddd*, $J = 6.5, 4.4, 1.9$, H–C(3′)); 6.15 (*dd*, $J = 1.9, 0.5$, H–C(2′)); 6.24 (*d*, $J = 0.5$, H–C(1′)); 6.70–6.85 (*m*, 9 arom. H); 6.94 (*m* , 2 arom. H); 8.05 (*m* , 4 arom. H); 8.17, 8.40 (*s*, H–C(2), H–C(8)); 8.52 (*s*, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 72.8 (*t*, C(4′)); 75.6 (*d*, C(3′)); 79.2 (*d*, C(2′)); 88.1 (*d*, C(1′)); 122.5; 127.1; 127.8; 127.9; 128.0; 128.8; 129.2; 132.0; 133.1; 133.2; 141.3; 148.9; 150.8; 152.1; 163.9; 164.2; 164.6. FAB-MS (pos., 3-NBA): 550 (34, $[M + H^+]$); 311 (58); 105 (100). Anal. calc. for $C_{30}H_{25}N_5O_6$ (549.54): C 65.57, H 4.22, N 12.74, found: C 65.35, H 4.29, N 12.53.

1-(2′,3′-Di-O-*benzoyl-α*-L-*threofuranosyl)thymine* (**7b**). A suspension of 4.4 g (12 mmol) of **5** and 1.5 g (12 mmol) of dry thymine in 35 ml of abs. MeCN was heated to 60° (oil bath) and treated with 5.8 ml (24 mmol) BSA. Stirring and heating was continued until a clear soln. was formed (*ca.* 1 h) and 6.4 ml (36 mmol) of TMS-Tf was added. After total consumption of the starting material (TLC), the mixture was cooled to r.t. and poured into an ice-cold stirred mixture of 120 ml of sat. aq. $NaHCO_3$ soln. and 150 ml of AcOEt. The org. layer was separated, washed with sat. aq. NaCl soln., and dried ($MgSO_4$). Evaporation of the solvent and CC (CH_2Cl_2/AcOEt 7:1) gave crude product, which was crystallized from hexane/AcOEt 4:1 to yield 3.2 g (64%) of **7b**. Colorless needles. M.p. 160–162°. TLC (hexane/AcOEt 1:1): R_f 0.50. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.89 (*s*, Me); 4.48 (*m*, 2 H, H–(C4′)); 5.60 (br. *s*, H–(C3′)); 5.68 (br. *s*, H–C(2′)); 6.22 (*m*, H–C(1′)); 7.27–8.09 (3*m*, 11 arom. H, H–C(6)); 8.39 (br. *s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.6 (Me); 73.5 (*t*, C(4′)); 76.2 (*d*, C(3′)); 80.2 (*d*, C(2′)); 89.8 (*d*, C(1′)); 111.0 (*s*, C(5)); 128.6 (2*d*, arom. C); 128.7 (2*d*, arom. C); 129.7 (2*d*, arom. C); 130.0 (2*d*, arom. C); 133.9–134.0 (2*d*, arom. C); 135.3 (*d*, C(6)); 150.2, 163.9, 164.8, 165.1. FAB-MS (POS., 3-NBA): 437 (46, $[M + H]^+$), 311 (87), 105 (100).

1-(2′,3′-O-*Dibenzoyl-α*- L-*threofuranosyl)uracil* (**7c**). A soln. of 3.45 g (8 mmol) **6** in 40 ml of abs. MeCN was treated with 4.3 ml (17.6 mmol) of BSA and 894 mg (8 mmol) of uracil and then heated to 60° for 1 h. Then, 4.3 ml (24 mmol) of TMS-Tf was added, and heating was continued for another 2 h (monitored by TLC). The mixture was cooled to r.t., diluted with 200 ml of AcOEt, and poured into 400 ml of sat. aq. $NaHCO_3$ soln. with stirring. The org. layer was separated, washed with H_2O, sat. aq. $NaHCO_3$, and sat. aq. NaCl soln. When concentrated under reduced pressure, some product precipitated. It was isolated by filtration and washed with a mixture of hexane/AcOEt/CH_2Cl_2 4:1:1. The combined filtrates were evaporated to dryness and subjected to CC (hexane/AcOEt/CH_2Cl_2 3:1:1); product fractions were combined and evaporated. Crystallization of this residue from MeOH/EtOH 1:1 gave a total of 2.74 g (81%) of **7c**. Colorless crystals. M.p. 173°. TLC (CH_2Cl_2/AcOEt 1:1): R_f 0.53. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.49 (*m*, 2 H–C(4′)); 5.60 (*m*, H–C(3′)); 5.73 (*m*, H–C(2′)); 5.77 (*d*, $J = 8.1$, 1 H–C(5)); 6.16 (*d*, $J = 2.1$, H–C(1′)); 7.55 (*d*, $J = 8.1$, 1 H–C(6)); 7.45–7.65 (*m*, 6 arom. H); 7.97 (*m*, 2 arom. H); 8.07 (*m*, 2 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 73.7; 75.9; 79.8; 90.0; 102.2; 128.3; 128.5; 129.5; 129.8; 133.7; 133.8; 139.2; 148.8; 162.9; 164.5; 164.9. FAB-MS (POS., 3-NBA): 423 (10,

$[M+H]^+$), 311 (52), 105 (100). Anal. calc. for $C_{22}H_{18}N_2O_7$(422.39): C 62.56, H 4.30, N 6.63 found: C 62.73, H 4.50, N 6.52.

*N⁴-Benzoyl-1-(2′,3′-di-*O*-benzoyl-α-*L*-threofuranosyl)cytosine* (**7d**). A soln. of 12.6 g (34.2 mmol) **5** in 100 ml of abs. MeCN was treated with 20.9 ml (85.5 mmol) of BSA and 7.68 g (35.9 mmol) N^4-benzoylcytosine, and heated to 70° for 1 h. 18.5 ml (102.6 mmol) of TMS-Tf was added, and heating was continued for another 60 min (monitored by TLC; use of TMS-Tf as the *Lewis* acid activator was found to be advantageous over $SnCl_4$, due to intractable Sn salts precipitation during workup). The mixture was cooled to r.t., diluted with 300 ml of AcOEt, poured into 400 ml of sat. aq. $NaHCO_3$ soln., and stirred. Stirring was continued for 15 min, and the precipitated product was isolated by filtration. The residue was washed with cold AcOEt, and the combined org. layers were washed with sat. aq. NaCl soln. and then dried ($MgSO_4$). The soln. was concentrated under reduced pressure, which gave some more precipitate. The crude product was crystallized from MeOH to give 16.5 g (94%) of **7d**. Colorless crystals. M.p. 234–236° (dec.). TLC (CH_2Cl_2/AcOEt 1:1): R_f 0.60. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.57 (*dd*, *J* = 11.4, 1.0, H–C(4′)); 4.61 (*dd*, *J* = 11.4, 3.9, H–C(4′)); 5.61 (*dd*, *J* = 3.6, 1.0, H–C(3′)); 5.84 (*s*, H–C(2′)); 6.30 (*s*, H–C(1′)); 7.40–7.65 (*m*, 6 arom. H); 7.87 (*m*, 2 H, arom. H); 7.92 (*d*, *J* = 7.5, H–(C5)); 8.03 (*d*, *J* = 7.5, H–(C6)); 8.11 (*m*, 2 arom. H); 8.73 (br. *s*, NH). ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.65 (*dd*, *J* = 10.9, 4.4, H–C(4′)); 4.71 (*d*, *J* = 11.0, H–C(4′)); 5.65 (*m*, H–C(3′)); 5.81 (*s*, H–C(2′)); 6.16 (*s*, H–C(1′)); 7.41–7.75 (*m*, 9 arom. H); 7.85 (*d*, *J* = 7.5, H–(C5)); 8.07 (*m*, 4 arom. H); 8.37 (*d*, *J* = 7.5, H–(C6)). ^{13}C-NMR (75 MHz, (D_6)DMSO): 74.1; 75.3; 79.2; 91.1; 96.3; 110.0; 118.0; 128.3; 128.4; 128.5; 128.6; 128.8; 129.4; 129.6; 132.6; 133.3; 133.8; 133.9; 145.0; 154.5; 163.8; 164.3; 167.6. FAB-MS (POS., 3-NBA): 1051 (12, $[2M+2H]^+$), 526 (100, $[M+H]^+$), 307 (66), 289 (36), 200 (33). Anal. calc. for $C_{29}H_{23}N_3O_7$ (511.53): C 66.28, H 4.41, N 8.00; found: C 66.32, H 4.50, N 8.01.

N²-Acetyl-O⁶-diphenylcarbamoyl-9-(2′,3′-di-O-benzoyl-α-L-threofuranosyl)guanine (**7e**). A suspension of 2.3 g (5.9 mmol) of N^2-acetyl-O^6-(diphenylcarbamoyl)guanine [21] in 55 ml of $(CH_2Cl)_2$ was treated with 2.9 ml (12 mmol) of BSA and heated to 70° for 1 h. The solvent was evaporated and replaced with 25 ml of dry toluene and 2.1 ml (12 mmol) of TMS-Tf, and a soln. of 2 g (5.4 mmol) of **5** in 15 ml of dry toluene was added. The mixture was heated to 70° for 2.5 h, cooled to r.t. and diluted with AcOEt. Workup and CC (CH_2Cl_2/AcOEt 1:1 containing 1% Et_3N) and drying (first co-evaporation with CH_2Cl_2/toluene, then in high vacuum) gave 2.42 g (64%) **7e**. Colorless foam. TLC (CH_2Cl_2/AcOEt 1:1): R_f 0.81. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.46 (*s*, Me); 4.52–4.67 (*dd*, *J* = 4.5, 1.9, 2 H–C(4′)); 5.74 (*m*, H–C(3′)); 6.19 (br. *s*, H–C(2′)); 6.32 (*d*, *J* = 1.6, H–C(1′)); 7.22–8.01 (*m*, 20 arom. H); 8.21 (*s*, H–C(8)). ^{13}C-NMR (75 MHz, $CDCl_3$): 25.0 (*q*, Me); 52.2 (*q*, MeO); 73.7 (*t*, C(4′)); 76.1 (*d*, C(3′)); 79.6 (*d*, C(2′)); 89.1 (*d*, C(1′)); 127.0 (2*d*); 128.6 (2*d*); 128.7 (2*d*); 129.1 (2*d*); 129.9 (2*d*); 133.8 (*d*); 134.0 (*d*); 141.6 (*d*, C(8)); 141.6; 150.1; 152.3; 154.1; 156.3; 164.7; 165.2; 171.0. FAB-MS (POS., 3-NBA): 699.8 (100, $[M+H]^+$), 196.2 (28), 104.9 (25).

*N²,N⁶-Dibenzoyl-9-(2′,3′-di-*O*-benzoyl-α-L-threofuranosyl)-2,6-diaminopurine* (**7f**). A suspension of 5.74 g (16.5 mmol) of N^2,N^6-dibenzoyl-2,6-diaminopurine [22] (dried in high vacuum at 60°) in 55 ml of dry MeCN was heated under Ar with stirring at 65°, and 8.75 g (10.6 ml, 43.09 mmol) of BSA was added, and the heating was continued for 15 min, until a clear soln. was formed. A soln. of 5.17 g (13.9 mmol) of **5** in 36 ml of dry MeCN was added (*via* syringe) to the soln. of the nucleobase. Additional 10 ml of MeCN was used to rinse the flask. Then, 4.02 g (3.5 ml, 18.07 mmol) of TMS-Tf was added, and the heating with stirring was continued for 3 h. The mixture was then cooled to r.t., transferred to a separatory funnel, diluted with 350 ml of AcOEt, and sat. 450 ml of sat. aq. $NaHCO_3$ soln. was added. Turbidity appeared in the org. phase. The layers were separated and the org. layer was filtered. The solid was filtered and identified as N^2,N^6-dibenzoyl-2,6-diaminopurine (by TLC and ^{1}H-NMR). The filtrate was washed with sat. aq. $NaHCO_3$ soln., sat. aq. NaCl soln., dried (Na_2SO_4), and conc. *in vacuo*. The residue was purified by CC (hexane/AcOEt 3:2 to 1:3, column: ∅5 × 30 cm) and dried in high vacuum to give 5.51 g (59.1%) of **7f**. Yellow solid. M.p. 129–131°. TLC (hexane/AcOEt 1:1): R_f: 0.21. ^{1}H-NMR (500 MHz, (D_6)DMSO): 4.63 (*dd*, *J* = 10.0, 6.5, H_a–C(4)); 4.81 (*dd*, *J* = 10.0, 5.0, H_b–C(4)); 5.89 (*ddd*, *J* = 6.5, 5.0, 3.0, H–C(3′)); 6.48 (*d*, *J* = 3.0, H–C(1′)); 6.51 (*dd*, *J* = 3.0, 3.0, H–C(2′)); 7.40 (*dd*, *J* = 8.0, 1.0, 2 arom. H); 7.46 (*dd*, *J* = 8.0, 1.0, 2 arom. H); 7.56 (*m*, 6 arom. H); 7.65 (*ddd*, *J* = 7.5, 7.5, 1.0, 1 arom. H); 7.69 (*ddd*, *J* = 7.5, 7.5, 1.0, 1 arom. H); 7.90 (*dd*, *J* = 8.0, 1.0, 2 arom. H); 7.92 (*dd*, *J* = 8.0, 1.0, 2 arom. H); 8.05 (*dd*, *J* = 7.5, 1.0, 2 arom. H); 8.09 (*dd*, *J* = 7.5, 1.0, 2 arom. H); 8.60 (*s*, H–C(8)); 10.98 (*br. s*, HN–C(2)); 11.25 (*br. s*, HN–C(6)). ^{13}C-NMR (125 MHz, (D_6)DMSO): 71.57 (*t*, C(4′)); 76.20 (*d*, C(3′)); 79.72 (*d*, C(2′)); 87.84 (*d*, C(1′); 123.67 (*s*, C(5)); 128.07 (*d*, arom. C); 128.45 (*m*, arom. C); 128.54 (*d*, arom. C); 128.81 (*d*, arom. C); 129.39 (*d*, arom. C); 129.64 (*d*, arom. C); 131.87 (*d*, arom. C); 132.45 (*d*, arom. C); 133.20 (*d*, arom. C); 133.51 (*d*, arom. C); 133.96 (*s*, arom. C); 134.15 (*d*, arom. C); 143.21 (*d*, C(8)); 151.02 (*s*, C(6)); 152.41 (*s*, C(2)); 152.75 (*s*, C(4)); 164.93 (*s*, CO); 165.03 (*s*, CO); 165.43 (*s*, CO); 165.68 (*s*, CO). ESI-MS (pos.): 691 (95, $[M+Na]^+$), 669 (100, $[M+H]^+$); ESI-MS (neg.): 667 (100, $[M-H]^-$).

N^6-*Benzoyl-9-(α-*L*-threofuranosyl)adenine* (**8a**). A suspension of 8.5 g (15.5 mmol) of **7a** in 300 ml of THF/MeOH/H_2O 5:4:1 was cooled to 0°, and 18.8 ml (37.5 mmol) of 2N aq. NaOH was added. After 20 min, the soln. was neutralized with 2N aq. HCl. The mixture was concentrated under reduced pressure, until the desired product started to precipitate. The flask was cooled to 10°, and the solid was isolated by filtration. The filtrate was concentrated, and a second precipitate was isolated. The filtrate from this was evaporated to dryness. The residue was dissolved in MeOH and co-evaporated twice with toluene and CC (CH_2Cl_2/EtOH 7:3) resulted in some more product. Crystallization of the combined products from EtOH gave 4.51 g (85%) of **8a**. Colorless crystals. M.p. >195° (dec.). TLC (CH_2Cl_2/AcOEt 1:1): R_f 0.03. ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.16 (*m*, H–C(3′), 2 H–C(4′)); 4.44 (*ddd*, *J* = 6.0, 3.9, 1.8, H–C(2′)); 5.61 (*d*, *J* = 3.0, OH); 5.99 (*d*, *J* = 4.5, OH); 6.06 (*d*, *J* = 1.8, H–C(1′)); 7.56 (*m*, 2 arom. H); 7.65 (*m*, 1 arom. H); 8.05 (*m*, 2 arom. H); 8.55, 8.77 (2*s*, H–C(2), H–C(8)); 11.22 (br. *s*, NH). ^{13}C-NMR (75 MHz, (D_6)DMSO): 75.0 (*t*, C(4′)); 75.1 (*d*, C(3′)); 80.0 (*d*, C(2′)); 90.0 (*d*, C(1′)); 125.5, 128.4 (4*d*); 132.3, 133.3, 143.0, 150.1 (*d* + *s*), 151.5, 151.8 (2*d*, C(2)), C(8)); 165.6 (*s*, arom. C). FAB-MS (pos., 3-NBA): 683 (6, $[2M+H]^+$), 342 (100, $[M+H]^+$), 240 (78), 105 (14). Anal. calc. for $C_{16}H_{15}N_5O_4$ (341.33): C 56.30, H 4.43, N 20.52; found: C 56.46, H 4.45, N 20.68.

*1-(α-*L*-Threofuranosyl)thymine* (**8b**). A suspension of 3.2 g (7.6 mmol) of **7b** in 150 ml of sat. NH_3 in MeOH was stirred at r.t. for 18 h. A clear soln. was formed; NH_3 and MeOH were evaporated, and the resulting conc. soln. was co-evaporated with H_2O (3×). The residue was dissolved in H_2O and washed with CH_2Cl_2. Evaporation of the solvent gave 1.42 g (77%) of **8b** as white powder, which was sufficiently pure for further reaction. For anal. purposes a sample was crystallized from MeOH. Colorless needles. M.p. >300°. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.74 (*s*, Me); 3.38 (br. *s*, 2 OH); 4.00 (*m*, 4 H–C(2′), H–C(3′), 2 H–C(4′)); 5.65 (*m*, H–C(1′)); 7.58 (*m*, H–C(6)); 11.20 (br. *s*, NH). ^{13}C-NMR (75 MHz, (D_6)DMSO): 12.5 (*q*, Me); 75.2 (*d*, C(3′)); 75.3 (*t*, C(4′)); 80.3 (*d*, C(2′)); 91.7 (*d*, C(1′)); 108.5, 137.5 (*d*, C(6)); 150.9, 164.4. FAB-MS (pos., 3-NBA): 229 (39, $[M+H]^+$).

*1-(α-*L*-Threofuranosyl)uracil* (**8c**). A soln. of 5.1 g (12.1 mmol) of **7c** was dissolved in 70 ml of MeOH/H_2O/NEt_3 5:1:1 and heated to reflux until no more starting material was detected (TLC, 2–3 h). The mixture was evaporated to dryness, co-evaporated with MeOH/toluene (3×), and dried to afford 2.55 g (98%) of **8c.** Yellow powder. TLC (CH_2Cl_2/AcOEt 1:1): R_f 0.53. ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.01 (*m*, H–C(2′), H–C(3′), 2 H–C(4′)); 5.40 (br. *s*, OH); 5.57 (*d*, *J* = 7.5, (H–C(6)); 5.64 (*d*, *J* = 0.9, H–C(1′)); 5.75 (*m*, OH); 7.68 (*d*, *J* = 8.1, (H–C(5)); 11.27 (*s*, NH). ^{13}C-NMR (75 MHz, (D_6)DMSO): 74.7; 75.5; 79.9; 91.7; 100.4; 141.3; 150.4; 163.3. FAB-MS (pos., 3-NBA): 429 (22, $[2M+H]^+$); 237 (53); 215 (100, $[M+H]^+$).

N^4-*Benzoyl-1-(α-*L*-threofuranosyl)cytosine* (**8d**). A suspension of 12.5 g (24.4 mmol) of **7d** in 400 ml of THF/MeOH/H_2O 5:4:1 was cooled to 0°, and 40 ml (80 mmol) of 2N aq. NaOH was added. After 30 min of stirring, the mixture was neutralized with 2N HCl. The soln. was concentrated to *ca.* 1/10 of its volume. The precipitated product was isolated by filtration. The filtrate was evaporated, the residue was suspended in MeOH, co-evaporated with toluene, and CC (CH_2Cl_2/MeOH 8:2) gave some more product. The combined product fractions were crystallized from MeOH to afford 7.1 g (96%) of **8d**. Colorless crystals. M.p. 216°. TLC (CH_2Cl_2/MeOH 25:1): R_f 0.02. ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.03 (*dd*, *J* = 1.2, H–C(3′)); 4.08 (*dd*, *J* = 4.2, H–C(4′)); 4.19 (*m*, H–C(2′), H–C(4′)); 5.22 (*d*, *J* = 2.4, OH); 5.67 (*s*, H–C(1′)); 5.83 (*d*, *J* = 4.2, OH); 7.31 (*d*, *J* = 7.2, (H–C(5)); 7.51 (*m*, 2 arom. H); 7.63 (*m*, 1 arom. H); 8.00 (*m*, 2 arom. H); 8.10 (*s*, *J* = 7.8, (H–C(6)); 11.16 (br. *s*, NH). ^{13}C-NMR (75 MHz, (D_6)DMSO): 74.8 (*t*, C(4′)); 76.6 (*d*); 79.6 (*d*); 93.5 (*d*, C(1′)); 94.9, 128.3 (2*d*); 133.2 (*d*); 132.6 (*d*); 145.8; 154.5; 162.9; 167.2. FAB-MS (pos., 3-NBA): 635 (9, $[2M+H]^+$, 318 (100, $[M+H]^+$), 216 (68), 200 (86), 105 (32). Anal. calc. for $C_{15}H_{15}N_3O_5$ (303.31): C 56.78, H 4.76, N 13.21 found: C 56.69, H 4.85, N 13.06.

N^2-*Acetyl*-O^6-*(diphenylcarbamoyl)-9-(α-*L*-threofuranosyl)guanine* (**8e**). A soln. of 2.4 g (3.5 mmol) of **7e** in 200 ml of THF/MeOH/H_2O 5:4:1 was cooled to 0° and treated with 14 ml (28 mmol) of 2N aq. NaOH. After 15 min stirring, 1.6 g (30 mmol) of NH_4Cl was added. Stirring was continued for 30 min, and the solvent was evaporated. The residue was dissolved in CH_2Cl_2/MeOH 5:1 and filtered over silica gel. The filtrate was concentrated until the product started to precipitate. The soln. was cooled, and the solid was isolated by filtration. Drying to constant weight resulted in 978 mg (58%) of **8e**. White powder. TLC (CH_2Cl_2/MeOH 5:1): R_f 0.68. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.20 (*s*, Me); 4.16–4.10 (*m*, H–C(3′), 2 H–C(4′)); 4.43 (*dd*, *J* = 4.3, 2.1, H–C(2′)); 5.52 (*d*, *J* = 3.4, HO–C(3′)); 5.88 (*d*, *J* = 4.3, HO–C(2′)); 5.92 (*d*, *J* = 1.9, H–C(1′)); 7.29–7.50 (*m*, 10 arom. H); 8.53 (*s*, H–C(8)); 10.73 (*s*, NH). ^{13}C-NMR (125 MHz, (D_6)DMSO): 24.5 (*q*, Me); 75.0 (*t*, C(4′)); 75.1 (*d*, C(3′)); 80.1 (*d*, C(2′)); 90.1 (*d*, C(1′)); 120.0, 127.0 (2*d*); 127.3 (2*d*); 129.0 (*d*); 129.4 (4*d*); 141.6, 144.2 (*d*, C(8)); 150.1, 152.2, 154.3, 155.0, 168.9. FAB-MS (pos., 3-NBA): 491.3 (100, $[M+H]^+$).

N^2,N^6-*Dibenzoyl-9-(α-*L*-threofuranosyl)-2,6-diaminopurine* (**8f**). A soln. of 1.18 g (1.77 mmol) of **7f** in 98 ml of THF/MeOH/H_2O 5:4:1 was cooled to 0° under Ar with stirring, and 6.87 ml (13.77 mmol) of 2N aq.

NaOH was slowly added during 10 min. After 5 min (total reaction time of 15 min), the reaction was quenched by addition of 776 mg (14.65 mmol) of solid NH_4Cl, and the stirring was continued for 30 min at 0°. Solvents were removed *in vacuo*, and the residue was co-evaporated with MeOH (2×). This residue was adsorbed on 2 g of silica gel, purified by CC (CH_2Cl_2/MeOH 100:1 to 40:1, ∅ 3 × 19 cm), and dried in high vacuum to afford 703 mg (86.6%) of **8f**. White solid. M.p. 153–155°. TLC (CH_2Cl_2/MeOH 20:1): R_f 0.26. ^{1}H-NMR (500 MHz, (D_6)DMSO): 4.11 (*dd*, *J* = 9.0, 3.0, H–C(4′)); 4.15 (*dd*, *J* = 9.0, 4.0, H–C(4′)); 4.18 (*ddd*, *J* = 3.0, 4.0, 4.0, H–C(3′)), 4.45 (*dd*, *J* = 4.0, 2.0, H–C(2′)); 5.99 (*d*, *J* = 2.0, H–C(1′)); 7.51 (*dd*, *J* = 8.0, 8.0, 2 arom. H); 7.54 (*dd*, *J* = 8.0, 8.0, 2 arom. H); 7.59 (*ddd*, *J* = 8.0, 8.0, 1.0, 1 arom. H); 7.64 (*ddd*, *J* = 8.0, 8.0, 1.0, 1 arom. H); 7.99 (*dd*, *J* = 8.0, 1.0, 2 arom. H); 8.06 (*dd*, *J* = 8.0, 1.0, 2 arom. H); 8.51 (*s*, H–C(8)); 11.00 (*s*, HN–C(2)); 11.18 (*s*, HN–C(6)). ^{13}C-NMR (125 MHz, (D_6)DMSO): 74.90 (*t*, C(4′)); 75.18 (*d*, C(3′)); 80.25 (*d*, C(2′); 89.69 (*d*, C(1′); 123.37 (*s*, C(5)); 128.05 (*d*, arom. C); 128.42 (*d*, arom. C); 131.98 (*d*, arom. C); 132.27 (*d*, arom. C); 133.19 (*s*, arom. C); 134.12 (*s*, arom. C); 142.98 (*d*, C(8)); 150.65 (*s*, C(6)); 152.22 (*s*, C(4)); 152.91 (*s*, C(2)); 165.52 (*s*, CO); 165.62 (*s*, CO). ESI-MS (pos.): 483 (78.2, $[M+Na]^+$), 461 (7.3, $[M+H]^+$), 359 (100); ESI-MS (neg.): 459 (100, $[M-H]^-$).

N^6-*Benzoyl-9-{3′*-O-*[(4″,4′-dimethoxytriphenyl)methyl]-α*-L-*threofuranosyl}adenine* (**9a**), N^6-*Benzoyl-9-{2′*-O-*[(4″,4″-dimethoxytriphenyl)methyl]-α*-L-*threofuranosyl}adenine* (**9aa**), *and* N^6-*Benzoyl-9-{2′,3′*-O-*bis[(4″,4″-dimethoxytriphenyl)methyl]-α*-L-*threofuranosyl}adenine* (**9aaa**). To a soln. of dry 1 g (2.9 mmol) of **8a** in 36 ml of abs. CH_2Cl_2/DMF 1:1, cooled to 10–15°, 1.29 g (3.8 mol) of DMT-Cl, 1.7 ml (14.5 mmol) of 2,6-lutidine, and 870 mg (3.4 mmol) of Ag-Tf were added. After 5 h, 130 mg (0.4 mmol) DMT-Cl and 87 mg (0.34 mmol) Ag-Tf were added, and the mixture was stirred overnight at r.t. After quenching with 2.5 ml of MeOH/H_2O 1:4 and diluting with 150 ml of CH_2Cl_2, the mixture was filtered, the residue was extracted with CH_2Cl_2, and the combined filtrates were washed twice with ice-cold 0.1M aq. HCl soln., with H_2O (2×) and, once with H_2O and sat. aq. NaCl soln. Drying (Na_2SO_4) and evaporation of the solvent gave a residue, which was subjected to CC (column deactivated with CH_2Cl_2/NEt_3 97:3, gradient: CH_2Cl_2/NEt_3 99:1 to CH_2Cl_2/MeOH/NEt_3 98:1:1) to give 1.24 g (66%) of **9a**, 50 mg (3%) of **9aa** as colorless foams, and 830 mg (31%) of **9aaa** as yellow foam.

Data of **9a**: TLC (CH_2Cl_2/MeOH 16:1): R_f 0.24. ^{1}H-NMR (300 MHz, $CDCl_3$): 3.77 (2*s*, 2 MeO); 3.80 (*m*, 2 H–C(4′)); 3.86 (br. *s*, HO–C(2′)); 4.21 (*m*, H–C(2′)); 4.33 (*m*, H–C(3′)); 5.92 (*d*, *J* = 2.2, H–C(1′)); 6.79 (*m*, 4 H, $(MeO)_2$*Tr*); 7.35–7.64 (*m*, 12 arom. H, $(MeO)_2$*Tr*); 8.04 (*m*, 2 arom. H); 8.30 (*s*, arom. H ((H–C(2)); 8.79 (*s*, arom. H ((H–C(8)); 9.13 (br. *s*, NH). ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.45 (*dd*, *J* = 9.3, 5.4, H–C(4′)); 3.59 (*dd*, *J* = 9.3, 3.6, H–C(4′)); 3.73 (2*s*, 2 MeO); 4.17 (*ddd*, *J* = 5.1, 5.1, 3.3, H–C(3′)); 4.50 (*ddd*, *J* = 5.7, 4.8, 2.7, H–C(2′)); 5.91 (*d*, *J* = 2.7, H–C(1′)); 5.92 (*d*, *J* = 5.7, HO–C(2′)); 6.87 (*m*, 4 H, $(MeO)_2$*Tr*); 7.16–7.36 (*m*, 9 H, $(MeO)_2$*Tr*); 7.54–7.70 (*m*, 3 arom. H); 8.07 (*m*, 2 arom. H); 8.55 (*s*, H–C(2)); 8.74 (*s*, H–C(8)); 11.11 (br. s, NH). ^{13}C-NMR (150 MHz, $CDCl_3$): 55.1 (*q*, MeO); 74.7 (*t*, C(4′)); 78.0 (*d*, C(2′)); 79.7 (*d*, C(3′)); 87.6 (*s*, Ar_3C); 91.3 (*d*, C(1′)); 113.3 (*d*); 122.7 (*d*); 125.2 (*d*); 127.9 (*d*); 128.0 (*d*); 128.1 (*d*); 128.7 (*d*); 129.7 (*d*); 129.8 (*d*); 132.7 (*d*); 133.4 (*d*); 135.6 (*d*); 141.6 (*d*); 149.3 (*d*); 150.7, 152.2 (*d*); 158.6, 164.8. FAB-MS (pos., 3-NBA): 644 (98, $[M+H]^+$), 303 (100), 240 (64). Anal. calc. for $C_{37}H_{33}N_5O_6$ (643.70): C 69.04, H 5.17, N 10.88, found: C 68.71, H 6.81, N 11.04.

Data of **9aa**: TLC (CH_2Cl_2/MeOH 16:1): R_f 0.51. ^{1}H-NMR (300 MHz, $CDCl_3$): 3.77, 3.78 (2*s*, 2 MeO); 4.10 (*dd*, *J* = 9.9, 1.0, H–C(4′)); 4.26 (*dd*, *J* = 9.9, 3.9, H–C(4′)); 4.37 (*dddd*, *J* = 10.3, 3.9, 1.2, 1.0, H–C(3′)); 4.76 (*m*, H–(C(2′)); 5.47 (*d*, *J* = 1.2, H–(C(1′)); 6.17 (*d*, *J* = 10.3, HO–C(3′)); 6.74 (*m*, 4 H, $(MeO)_2$*Tr*); 7.20–7.42 (*m*, 9 H, $(MeO)_2$*Tr*); 7.37 (*s*, (H–C(2)); 7.51–7.65 (*m*, 3 arom. H); 8.01 (*m*, 2 arom. H); 8.72 (*s*, (H–C(8)); 9.03 (br. *s*, NH).

Data of **9aaa**: R_f *ca.* 0.7 (CH_2Cl_2/MeOH 16:1).

1-{3′-O-*[(4″,4‴-Dimethoxytriphenyl)methyl]-α*-L-*threofuranosyl}thymine* (**9b**) *and 1-{2′*-O-*[(4″,4‴-Dimethoxytriphenyl)methyl]-α*-L-*threofuranosyl}thymine* (**9bb**). A soln. of dry 82 mg (0.36 mmol) **8b** in 2.5 ml of abs. CH_2Cl_2 and 2.5 ml of abs. DMF was treated with 146 mg (0.43 mmol) DMT-Cl, 0.21 ml (1.8 mmol) 2,6-lutidine, and 102 mg (0.4 mmol) Ag-Tf. The mixture was stirred overnight at r.t., quenched with 0.5 ml of MeOH, diluted with 200 ml of CH_2Cl_2, and filtered. The residue was extracted with CH_2Cl_2, and the combined filtrates were washed sequentially with ice-cold 0.1M aq. HCl soln. (2×), H_2O (2×), sat. aq. $NaHCO_3$, and sat. aq. NaCl soln. Drying (Na_2SO_4) and evaporation of the solvent gave a residue, which was subjected to CC (column deactivated with CH_2Cl_2/Et_3N 97:3, loading with pure CH_2Cl_2, gradient: CH_2Cl_2/Et_3N 99:1 to CH_2Cl_2/MeOH/NEt_3 98:1:1) and yielded 48 mg (25%) of **9b** (3′-*O*-isomer) and 45 mg (24%) of **9bb** (2′-*O*-isomer) as colorless foams.

Data of **9b**: TLC (CH_2Cl_2/MeOH 25:1): R_f 0.27. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.82 (*s*, Me); 3.31–3.92 (*ddd*, *J* = 10.0, 3.7, 1.9, 2 H–C(4′)); 3.70 (*s*, MeO); 3.73 (*s*, MeO); 4.15 (br. *s*, H–C(2′)); 4.22 (br. *d*, *J* ≈ 3.4, H–C(3′)); 4.96 (br. *s*, OH); 5.70 (*s*, H–C(1′)); 6.78–7.45 (*m*, 14 arom. H, H–C(6)); 10.52 (br. *s*, NH). ^{1}H-NMR

(300 MHz, (D_6)DMSO): 1.68 (*s*, Me); 3.57 (*m*, H–C(2′), 2 H–C(4′)); 3.73 (2*s*, MeO); 3.99 (*m*, H–C(3′)); 5.47 (*d*, J = 1.2, H–C(1′)); 5.58 (*d*, J = 4.1, HO–C(2′)); 6.86–7.51 (*m*, 14 arom. H, H–C(6)); 11.27 (*s*, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 12.6 (*q*, Me); 52.2 (2*s*, MeO); 75.5 (*t*, C(4′)); 77.6 (*d*, C(3′)); 80.4 (*d*, C(2′)); 88.0 (*s*, Ar_3C), 93.4 (*d*, C(1′)); 109.6, 113.5 (*d*); 127.7 (*d*); 128.1 (*d*); 129.8 (*d*); 135.7, 135.8, 136.8 (*d*); 144.7; 150.8; 158.8; 158.8; 164.8. MS (FAB, 3-NOBA): 531 (32, $[M+H]^+$), 530 (31), 303 (100), 242 (49), 102 (57).

Data of **9bb**: TLC (CH_2Cl_2/MeOH 25:1): R_f 0.48. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.79 (*s*, Me); 3.74 (*s*, MeO); 3.75 (*s*, MeO, H–C(3′)); 3.95–4.00 (*m*, 2 H–C(4′)); 4.38 (br. *d*, $J \approx 1.6$, H–C(2′)); 5.54 (*d*, J = 2.2, H–C(1′)); 6.77–7.46 (*m*, 14 H, arom. H, H–C(6)); 9.23 (br. *s*, NH). ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.66 (*s*, Me); 3.35 (br. *d*, J = 2.5, H–C(3′)); 3.71 (*s*, MeO); 3.72 (*s*, MeO); 3.83 (br. *s*, 2 H–C(4′)); 3.94 (*d*, J = 1.6, H–C(2′)); 5.12 (*d*, J = 2.8, HO–C(3′)); 5.92 (*d*, J = 1.9, H–C(1′)); 6.81–7.40 (*m*, 14 H, $(MeO)_2Tr$, H–C(6)); 11.33 (*s*, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 12.0 (*q*, Me); 55.1 (2*q*, MeO); 74.5 (*t*, C(4′)); 76.6 (*d*, C(3′)); 83.0 (*d*, C(2′)); 88.0 (s, Ar_3C); 95.0 (*d*, C(1′)); 110.7, 111.0, 113.3 (2*d*); 127.2 (*d*); 128.1 (4*d*); 129.0 (*d*); 130.3 (2*d*); 135.8, 139.1 (*d*); 144.8; 150.4; 159.0; 164.1. FAB-MS (pos., 3-NBA): 530 (1, M^+); 303 (77); 242 (100).

*1-{3′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-threofuranosyl}uracil* (**9c**) *and 1-{2′-*O*-[(4,4′-dimethoxytriphenyl)methyl]-α-*L*-threofuranosyl}uracil* (**9cc**). To a soln. of 1.5 g (7 mmol) of dry **8c** in 6 ml of abs. CH_2Cl_2 and 14 ml of abs. DMF, cooled to 15°, 3.08 g (9.1 mol) of DMT-Cl, 4.1 ml (35 mmol) of 2,6-lutidine and 1.43 g (8.4 mmol) of Ag-Tf were added successively in four portions over a period of 4 min After 5 h of stirring, 150 mg (0.44 mmol) of DMT-Cl and 70 mg (0.3 mmol) of Ag-Tf were added, and the mixture was stirred overnight at r.t. The mixture was quenched with 2 ml of MeOH and 0.5 ml of H_2O, diluted with 200 ml of CH_2Cl_2, and filtered. The residue was extracted with CH_2Cl_2, and the combined filtrates were washed with ice-cold 0.1M aq. HCl soln. (2×); H_2O (2×); sat. aq. $NaHCO_3$, and sat. aq. NaCl soln. Drying (Na_2SO_4) and evaporation of the solvent gave a residue, which was subjected to CC (column deactivated with CH_2Cl_2/Et_3N 98:2, loading with CH_2Cl_2, gradient: CH_2Cl_2/Et_3N 99:1 to CH_2Cl_2/MeOH 99:1) to yield in 0.78 g (22%) of **9c** (3′-*O*-DMT derivative) and 0.63 g (17%) of **9cc** (2′-*O*-DMT derivative) as colorless foams.

Data of **9c**: TLC (CH_2Cl_2/MeOH 16:1): R_f 0.16. ^{1}H-NMR (300 MHz, $CDCl_3$): 3.42 (*d*, J = 11.0, H–C(4′)); 3.72 (*s*, MeO); 3.74 (*s*, MeO); 3.90 (*dd*, J = 11.0, 6.2, H–C(4′)); 4.06 (*s*, H–C(2′)); 4.22 (*d*, J = 3.1, H–C(3′)); 5.64 (*s*, H–C(1′)); 5.73 (*d*, J = 8.1, H–C(5)); 6.83–6.80 (*m*, 4 H, $(MeO)_2Tr$); 7.17–7.37 (*m*, 9 H, $(MeO)_2Tr$); 7.65 (*d*, J = 8.1, H–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 55.1 (*q*, MeO); 55.2 (*q*, MeO); 75.8 (*t*, C(4′)); 77.9 (*d*, C(3′)); 80.2 (*d*, C(2′)); 88.2 (*s*, Ar_3C); 93.8 (*d*, C(1′)); 101.2, 113.4 (*d*); 127.1 (*d*); 127.8 (*d*); 128.1 (*d*); 128.2 (*d*); 129.8 (*d*); 135.6, 135.7, 140.8 (*d*); 144.5 (*d*); 151.1, 158.8, 163.3. FAB-MS (pos., 3-NBA): 516 (1, $[M+H]^+$), 303 (46); 242 (100). Anal. calc. for $C_{29}H_{28}N_2O_7$ (516.54): C 67.43, H 5.46, N 5.42 found: C 68.34, H 6.11, N 4.82.

Data of **9cc**: TLC (CH_2Cl_2/MeOH 16:1): R_f 0.18. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.23 (*s*, H–C(3′)); 3.73 (2*s*, 2 MeO); 3.85 (*m*, 2 H–C(4′)); 3.92 (*s*, H–C(2′)); 5.16 (*d*, J = 2.5, HO–C(3′)); 5.58 (*d*, J = 8.1, H–C(5)); 5.93 (*s*, H–C(1′)); 6.85–6.89 (*m*, 4 H, $(MeO)_2Tr$); 7.22–7.93 (*m*, 9 H, $(MeO)_2Tr$); 7.56 (*d*, J = 8.1, H–C(6)); 11.34 (*s*, NH). ^{1}H-NMR (300 MHz, $CDCl_3$): 3.56 (*m*, H–C(3′)); 3.78 (2*s*, 2 MeO); 3.95 (*d*, J = 10.2, H–C(4′)); 4.02 (*dd*, J = 3.8, 10.2, H–C(4′)); 4.25 (*m*, H–C(2′)); 5.57 (*d*, J = 8.1, H–C(5)); 5.79 (*d*, J = 1.8, H–C(1′)); 6.80–6.86 (*m*, 4 H, $(MeO)_2Tr$); 7.18 (*d*, J = 8.4, H–C(6)); 7.46–7.21 (*m*, 9 H, $(MeO)_2Tr$). ^{13}C-NMR (75 MHz, $CDCl_3$): 55.2 (2*q*, MeO); 74.7 (*t*, C(4′)); 76.1 (*d*, C(3′)); 83.4 (*d*, C(2′)); 88.2 (*s*, Ar_3C); 94.2 (*d*, C(1′)); 102.3 (*d*, C(5)); 113.4 (*d*); 127.3 (*d*); 128.0 (*d*); 128.1 (*d*); 130.3 (*d*); 135.6, 142.8 (*d*); 144.6 (*d*); 150.2; 158.9; 163.4. FAB-MS (pos., 3-NBA): 516 (1, $[M+H]^+$), 303 (46), 242 (100).

*N^4-Benzoyl-1-{3′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-threofuranosyl}cytosine* (**9d**) *and N^4-Benzoyl-1-{2′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-threofuranosyl}cytosine* (**9dd**). To a soln. of 0.5 g (1.6 mmol) dry **8d** in 3 ml of abs. CH_2Cl_2 and 10 ml of abs. DMF, 0.71 g (2.1 mol) of DMT-Cl, 0.94 ml (8 mmol) of 2,6-lutidine and 480 mg (1.9 mmol, in 2 portions) of Ag-Tf were added. After 5 h of stirring at r.t., 50 mg (0.15 mmol) of DMT-Cl and 48 mg (0.2 mmol) of Ag-Tf were added, and the mixture was stirred overnight at r.t. The reaction was quenched with 2 ml of MeOH, 0.5 ml of H_2O, and diluted with 50 ml of CH_2Cl_2. The mixture was filtered, the residue was extracted with CH_2Cl_2 and the combined filtrates were washed with ice-cold 0.1M aq. HCl soln. (2×), H_2O (2×), sat. aq. $NaHCO_3$, and sat. aq. NaCl soln. Drying (Na_2SO_4) and evaporation of the solvent gave a residue, which was subjected to CC (column deactivated with CH_2Cl_2/Et_3N 97:3, gradient: CH_2Cl_2/Et_3N 99:1 to CH_2Cl_2/MeOH/Et_3N 98:1:1) to give 0.44 g (45%) of **9d** (3′-*O*-DMT derivative) and 0.14 g (15%) of **9dd** (2′-*O*-DMT derivative) as colorless foams.

Data of **9d**: TLC (CH_2Cl_2/MeOH 16:1): R_f 0.12. ^{1}H-NMR (300 MHz, $CDCl_3$): 3.38 (*dd*, J = 9.0, 2.4, H–C(4′)); 3.61 (*dd*, J = 9.0, 3.3, H–C(4′)); 3.77 (2*s*, 2 MeO); 3.93 (br. *s*, OH); 4.26 (*m*, H–C(2′), H–C(3′)); 5.64 (*s*, H–C(1′)); 6.81 (*m*, 4 H, $(MeO)_2Tr$); 7.16–7.38 (*m*, 9 H, $(MeO)_2Tr$); 7.51–7.64 (*m*, 4 arom. H); 7.92 (*d*, 1 arom. H); 7.92, 8.05 (2*d*, J = 7.5, H–C(5), H–C(6)); 8.68 (br. *s*, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 55.1; 75.0; 77.8; 80.9; 87.8; 94.4; 96.1; 113.3; 113.4; 125.3; 127.0; 127.6; 127.9; 128.0; 128.2; 129.0; 129.9; 133.0; 133.1; 135.7;

135.9; 144.6; 144.8; 155.8; 158.7; 162.4. FAB-MS (pos., 3-NBA): 620 (15, $[M+H]^+$), 303 (100), 200 (28), 105 (32). Anal. calc. for $C_{36}H_{33}N_3O_7$ (605.68): C 69.78, H 5.37, N 6.78 found: C 69.58, H 5.51, N 6.51.

Data of **9dd**: ^{1}H-NMR (300 MHz, $CDCl_3$): 3.94 (*m*, H–C(3′)); 4.06 (br. *d*, $J \approx 9.7$, H–C(4′)); 4.12 (*dd*, $J =$ 9.7, 3.4, H–C(4′)); 4.64 (br. *s*, H–C(2′)); 5.43 (br. *s*, H–C(1′)); 6.75–6.82 (*m*, 4 H, $(MeO)_2Tr$); 7.08–7.65 (*m*, 14 arom. H); 7.91 (*m*, H–C(6), H–C(5)). ^{13}C-NMR (75 MHz, $CDCl_3$): 55.0; 75.6; 76.3; 82.5; 88.0; 96.6; 97.2; 113.1; 113.2; 127.0; 127.7; 127.9; 128.0; 128.8; 130.2; 130.3; 133.0; 135.6; 135.7; 144.9; 147.8; 158.7; 162.5.

N^2-*Acetyl*-O^6-*(diphenylcarbamoyl)-9-{3′*-O-*[(4″,4‴*-*dimethoxytriphenyl)methyl]*-α-L-*threofuranosyl}guanine* (**9e**) *and* N^2-*Acetyl*-O^6-*(diphenylcarbamoyl)-9-{2′*-O-*[(4″,4‴*-*dimethoxytriphenyl)methyl]*-α-L-*threofuranosyl}guanine* (**9ee**). To a soln. of 2.45 g (5 mmol) of **8e** and 2.3 ml (20 mmol) of 2,6-lutidine in 75 ml of DMF (dried with molecular sieves (4 Å)) was added 3.38 g (10 mmol) of DMT-Cl. The clear soln. was stirred at r.t. for 24 h. Then, an additional 850 mg (2.5 mmol) of DMT-Cl was added, and the mixture was stirred for an additional 6 h. The reaction was quenched with 20 ml of MeOH, diluted with 60 ml of CH_2Cl_2 and washed with sat. aq. $NaHCO_3$. Drying ($MgSO_4$) and evaporation gave a residue, which was subjected to CC (column deactivated with CH_2Cl_2/Et_3N 97:3, gradient: CH_2Cl_2/Et_3N 99:1 to CH_2Cl_2/MeOH/NEt_3 98:1:1) to give 1.38 g (35%) **9e** (3′-*O*-DMT derivative) and 0.81 g (21%) of **9ee** (2′-*O*-DMT derivative) as yellow foams.

Data of **9e**: TLC (CH_2Cl_2/MeOH 25:1): R_f 0.38. ^{1}H-NMR (500 MHz, (D_6)DMSO): 2.22 (*s*, Me); 3.46 (br. *dd*, $J = 9.5, 5.2$, H–C(4′)); 3.60 (br. *dd*, $J = 9.5, 3.3$, H–C(4′)); 3.69 (*s*, MeO); 3.70 (*s*, MeO); 4.15 (br. *dd*, $J = 5.2$, 3.3, H–C(3′)); 4.45 (br. *dd*, $J = 4.6$, 2.7, H–C(2′)); 4.78 (*d*, $J = 4.6$, HO–C(2′)); 5.80 (*d*, $J = 2.6$, H–C(1′)); 6.80–7.50 (*m*, 23 arom. H); 8.50 (*s*, H–C(8)). ^{1}H-NMR (300 MHz, $CDCl_3$): 2.16 (*s*, Me); 3.67 (*m*, H–C(3′)); 3.75 (*s*, MeO); 3.76 (*s*, MeO); 4.35 (*m*, 2 H–C(4′)); 5.09 (*m*, H–C(2′)); 5.68 (br. *s*, H–C(1′)); 6.76–7.46 (*m*, 23 arom. H); 8.01 (*s*, H–C(8)). ^{13}C-NMR (150 MHz, $CDCl_3$): 24.9 (*q*, Me); 55.2 (*q*, MeO); 73.5 (*t*, C(4′)); 77.6 (*d*, C(3′)); 81.1 (*d*, C(2′)); 87.3 (*s*, Ar_3C); 91.4 (*d*, C(1′)); 113.3 (2*d*); 121.5, 126.9 (*d*); 127.7 (*d*); 128.0 (2*d*); 129.2 (2*d*); 130.0 (2*d*); 135.8, 136.0, 141.6, 142.2 (*d*, C(8)); 144.7; 150.4; 151.3; 154.0; 155.8; 158.6; 158.7. FAB-MS (pos., 3-NBA): 793.1 (24, $[M+H]^+$), 600.1 (65), 303.1 (100).

Data of **9ee**: TLC (CH_2Cl_2/MeOH 25:1): R_f 0.29. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.25 (*s*, Me); 3.66 (*s*, MeO); 3.67 (*s*, MeO); 4.09 (*m*, 2 H–C(4′)); 4.32 (*m*, H–C(3′)); 4.81 (br. *s*, H–C(2′)); 5.28 (br. *s*, H–C(1′)); 6.60–7.36 (*m*, 23 arom. H); 7.38 (*s*, H–C(8)). FAB-MS (pos., 3-NBA): 793.0 (12, $[M+H]^+$).

N^2,N^6-*Dibenzoyl-9-{3′*-O-*[(4″,4‴*-*dimethoxytriphenyl)methyl]*-α-L-*threofuranosyl}-2,6-diaminopurine* (**9f**), N^2,N^6-*Dibenzoyl-9-{2′*-O-*[(4″,4‴*-*dimethoxytriphenyl)methyl]*-α-L-*threofuranosyl}-2,6-diaminopurine* (**9ff**), *and* N^2,N^6-*Dibenzoyl-9-{2′,3′*-O-*bis[(4″,4‴*-*dimethoxytriphenyl)methyl]*-α-L-*threofuranosyl}-2,6-diaminopurine* (**9fff**). In a dried one-necked round-bottom 250-ml flask was placed 3.01 g (6.54 mmol) of **8f** and dissolved in 55 ml of CH_2Cl_2/DMF (1:1, previously dried over molecular sieves) at r.t. under Ar and a water bath. To this soln., 3.03 g (8.94 mmol) of DMT-Cl was added with stirring, followed by the addition of 4.5 ml (38.7 mmol) of 2,6-lutidine. Molecular sieves (*ca.* 3 g) were added, and then 2.57 g (10.0 mmol) of Ag-Tf was added over a period of 10 min (the reaction is exothermic) with stirring at r.t. The mixture turned dark red. After 4 h, 399 mg (1.18 mmol) DMT-Cl and 287 mg (1.12 mmol) of Ag-Tf were added, and the reaction was kept, with stirring, in the water bath for additional 20 h. The reaction was quenched by addition of 2 ml of MeOH, diluted with 250 ml of CH_2Cl_2, and filtered. The yellow soln. was cooled (0–4°) and washed with 250 ml of cold 0.1M aq. HCl (2×), with sat. $NaHCO_3$, brine, and dried (Na_2SO_4). The org. phase was concentrated, and residue was purified by CC (column washed with CH_2Cl_2/Et_3N (93:3) to deactivate the silica gel, elution with a mixture of hexane/benzene/CH_2Cl_2/AcOEt/Et_3N/MeOH 10:10:2.5:2.5:0.2:0.1 to 10:10:2.5:2.5:0.2:0.4 and finally with CH_2Cl_2/MeOH 35:1) to afford, apart from pure compound **9f**, fractions containing mixtures of **9f** with **9ff** and **9fff**. These mixtures were re-purified by CC under the conditions mentioned above. The combined purified fractions from the above two CC afforded, after elimination of the solvents and drying in the high vacuum, 400 mg (5.7%) of the bis-tritylated derivative **9fff,** 2.04 g (40.1%) of the 3′-*O*-monotritylated derivative **9f**, 973 mg (19.5%) of the 2′-*O*-monotritylated derivative **9ff**, 890 mg (17.8%) of the mixture **9f**/**9ff**, and 230 mg (7.6%) of the recovered diol **8f**.

Data of **9f**: Amorphous solid. M.p. 157–160°. TLC (benzene/AcOEt/CH_2Cl_2/MeOH 8:1:1:0.2): R_f 0.40. ^{1}H-NMR (500 MHz, $CDCl_3$): 3.14 (*dd*, $J = 9.0, 5.0$, H–C(4′)); 3.37 (*dd*, $J = 9.0, 5.0$, H–C(4′); 3.74 (*s*, 2 MeO), 4.40 (*ddd*, $J = 5.0, 5.0, 5.0$, H–C(3′)); 4.73 (*dd*, $J = 5.0, 4.0$, H–C(2′)); 5.83 (*d*, $J = 4.0$, H–C(1′); 5.80, 5.81 (2*d*, $J = 9.0$, 2 arom. H *ortho* to the MeO); 7.15–7.70 (*m*, 15 arom. H); 7.96, 8.01 (2*dd*, $J = 7.5, 1.5$, 4 arom. H); 8.07 (*s*, H–C(8)); 9.44 (*s*, HN–C(6)); 9.84 (*s*, HN–C(2)). ^{13}C-NMR (125 MHz, $CDCl_3$): 72.80 (*t*, C(4′)); 77.45 (*d*, C(3′)); 81.42 (*d*, C(2′)); 87.18 (*s*, Ar_3C); 90.91 (*d*, C(1′)); 113.32 (*d*, 4 arom. C); 119.56 (*s*, C(5)); 127.71, 127.79 (2*d*, 4 arom. C); 127.95, 128.11, 128.60, 128.81 (4*d*, 8 arom. C); 129.93 (*d*, arom. C); 130.09 (*d*, 2 arom. C); 130.15 (*d*, 2 arom. C); 132.30, 132.88 (2*d*, 2 arom. C); 133.37, 134.07, 135.92, 136.24 (4*s*, arom. C); 140.13 (*d*, C(8)); 144.93 (*s*, arom. C); 149.40 (*s*, C(6)); 151.62 (*s*, C(4)); 152.42 (*s*, C(2)); 158.70 (*s*, 2 arom. C); 164.88 (*s*,

OCN–C(2)); 165.20 (*s*, OCN–C(6)). ESI-MS (pos.): 785 (100, $[M+Na]^+$), 763 (16, $[M+H]^+$). ESI-MS (neg.): 797 (4.1 $[M+Cl]^-$), 761 $[M-H]^-$).

Data of **9fff**: Amorphous solid. M.p. 126–129°. TLC (benzene/AcOEt/CH_2Cl_2/MeOH 8:1:1:0.2): R_f 0.35. ^{1}H-NMR (500 MHz, $CDCl_3$): 3.70 (*s*, MeO); 3.71 (*s*, MeO); 4.21 (*dd*, *J* = 7.0, 5.0, H–C(4′)); 4.29 (*dd*, *J* = 7.0, 5.0, H–C(4′)); 4.64 (*dddd*, *J* = 9.0, 5.0, 5.0, 1.5, H–C(3′)); 5.32 (*d*, *J* = 1.5, H–C(1′)); 5.34 (*dd*, J = 1.5, 1.5, H–C(2′); 5.68 (*d*, *J* = 9.0, HO–C(3′)); 6.68, 6.71 (2*d*, *J* = 9.0, 4 arom. H *ortho* to the MeO); 7.00–7.60 (*m*, 16 arom. H); 7.95, 8.00 (2*d*, *J* = 8.0, 1.5, 4 H); 9.32 (*s*, HN–C(6)); 9.79 (*s*, HN–C(2)). ^{13}C-NMR (125 MHz, $CDCl_3$): 55.13 (2*s*, Me); 74.80 (*t*, C(4′)); 78.17 (*d*, C(3′)); 82.72 (*d*, C(2′)); 87.94 (*s*, Ar_3C); 92.39 (*d*, C(1′)); 113.17, 113.25 (2*d*, 4 arom. C); 127.71, 127.79, 127.92, 128.14, 128.54, 128.90 (6*d*, 12 arom. C); 130.08, 130.19 (2*d*, 4 arom. C); 132.34, 132.98 (2*d*, 2 arom. C); 133.41, 133.93, 135.79. 135.99 (4*s*, 4 arom. C); 143.23 (*s*, C(8)); 144.90 (*s*, arom. C); 149.50 (*s*, C(6)); 152.14 (*s*, C(4)); 152.30 (*s*, C(2)); 158.63, 158.67 (2*s*, 2 arom. C); 164.74 (*s*, OCN–C(6)); 164.81 (*s*, OCN–C(2)). ESI-MS (pos.): 785 (100, $[M+Na]^+$), 763 (57, $[M+H]^+$). ESI-MS (neg.): 761 (100, $[M-H]^-$).

Data of **9ffff**: Amorphous solid. M.p. 144–146°. TLC (benzene/AcOEt/CH_2Cl_2/MeOH 8:1:1:0.2): R_f 0.65. ^{1}H-NMR (500 MHz, $CDCl_3$): 3.51 (br. *d*, *J* = 9.0, H–C(4′)); 3.71 (br. *d*, *J* = 9.0, H–C(4′); 3.64, 3.67, 3.75 (3*s*, 4 MeO); 4.40 (br. *s*, H–C(3′)); 5.10 (br. *s*, H–C(2′)); 5.60 (br. *s*, H–C(1′)); 6.58–6.76, 7.11–7.34 (*m*, 26 arom. H-DMT); 7.45 (*dd*, *J* = 7.5, 7.5, 2 arom. H); 7.48 (*dd*, *J* = 7.5, 7.5, 2 arom. H); 7.53 (*dd*, *J* = 7.5, 7.5, 1 arom. H); 7.56 (*dd*, *J* = 7.5, 7.5, 1 arom. H); 7.90 (br. *d*, *J* = 7.5, 2 arom. H); 8.04 (*s*, H–C(8)); 8.06 (br. *d*, *J* = 7.5, 2 arom. H); 8.86 (*s*, HN–C(6)); 9.54 (*s*, HN–C(2)). ^{13}C-NMR (125 MHz, $CDCl_3$): 55.05, 55.10 (2*q*, MeO); 73.53 (*t*, C(4′)); 78.99 (*d*, C(3′)); 82.42 (*d*, C(2′)); 87.97, 88.06 (2*s*, Ar_3C); 88.33 (*d*, C(1′); 113.12, 113.19, 113.24, (br. *m*, 8 arom. C); 120.07 (*s*, C(5)); 127.07, 126.93 (2*d*, arom. C); 127.45 (*d*, 2 arom. C); 127.87 (br. *d*, 4 arom. C); 127.98, 128.66 (2*d*, 4 arom. C); 130.31 (br. *m*, 8 arom. C); 133.53, 134.32, 135.36, 135.48, 135.61, 135.71 (6*s*, 6 arom. C); 141.57 (*d*, C(8)); 144.74, 144.98 (2*s*, 2 arom. C); 149.70 (*s*, C(6)); 152.08 (*s*, C(4)); 153.18 (*s*, C(2)); 158.64 (*m*, 4 arom. C), 164.34 (s, OCN–C(2)); 164.64 (s, OCN–C(6)). ESI-MS (pos.): 1065 (100, $[M+H]^+$). ESI-MS (neg.): 1063 (100, $[M-H]^-$).

3. Experiments Referring to *Scheme 4*. – *2,2′-Anhydro-1-(α-*L*-threofuranosyl)thymine* (**10b**). The diol **8b** was dried in the high vacuum for 24 h at 60° (*Büchi* gun). In a dried one-necked 100-ml round-bottom flask were placed 1.2 g (5.3 mmol) of **8b**, 2.27 g (10.6 mmol) of diphenyl carbonate, and 134 mg (1.6 mmol) of $NaHCO_3$, dry 4.8 ml of HMPA was added, and the mixture was heated at 150° for 1 h under Ar (TLC control of the reaction was performed by adding 0.5 ml of H_2O and 0.5 ml of $CHCl_3$ to one drop of the mixture, loading the aq. layer to the TLC, and placing the plate in the high vacuum for 15 min (before developing the TLC) to eliminate HMPA). After 1 h, the mixture turned dark brown. The mixture was cooled, poured into 150 ml of H_2O, and washed with 3 × 120 ml of $CHCl_3$ to eliminate HMPA. The aq. layer was filtered through *Celite*, and the combined org. layers were re-extracted with H_2O. The combined aq. phases were concentrated to dryness (H_2O bath at 35°). TLC of this residue showed several spots (presumably due to the presence of mixed carbonates). This residue was suspended with 60 ml of MeOH and heated at 50° for 30 min with stirring (to solvolyze the mixed carbonates). After this time, TLC showed the presence of two main spots. The residue was concentrated to dryness, adsorbed in 1 g of silica gel, and subjected to CC (silica gel, ∅ 3 × 7 cm, with 10 g of silica in AcOEt, elution with AcOEt, followed by AcOEt/MeOH 9:1 to 8:2) to afford 32 mg (4.7%) of thymine as the less polar product (confirmed by MS, ^{1}H- and ^{13}C-NMR, and by direct comparison with an authentic sample; TLC (AcOEt/MeOH 9:1): R_f 0.60) and 740 mg (65.5%) of **10b**. A small amount of **10b** was crystallized from MeOH for anal. purposes. M.p. 208°. TLC (AcOEt/MeOH, 9:1): R_f 0.2. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.79 (*s*, Me–C(5)); 3.24 (*dd*, J_{gem} = 8.8, H–C(4′)); 4.0 (*dd*, *J*(3′,4′) = 6.7, J_{gem} = 8.8, H–C(4′)); 4.31 (*m*, *J*(2′,3′) = 5.25, *J*(3′,4′) = 6.7. *J*(3′,OH) = 6.5, H–C(3′)); 5.17 (*dd*, *J*(1′2′) = (*J*2′,3′) = 5.25, H–C(2′)); 5.71 (*d*, *J*(3′,OH) = 6.5, HO–C(3′)); 6.19 (*d*, *J*(1′,2′) = 5.25, H–C(1′)); 7.71 (*s*, H–C(6)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 14.4 (*q*, *Me*–C(5)); 68.6 (*t*, C(4′)); 70.7 (*d*, C(3′)); 81.4 (*d*, C(2′)); 90.8 (*d*, C(1′)); 117.7 (*s*, C(5)); 132.9 (*d*, C(6)); 161.2 (*s*, C(2)); 172.5 (*s*, CO). ESI-MS (pos.): 443 (100, $[2M+Na]^+$).

Evaluation of the Stability of the Anhydro Compound **10b** *under the Reaction Conditions of its Formation*. In a dried one-necked 5-ml round-bottom flask were placed 5.6 mg (0.027 mmol) of **10b**, 11.4 mg (0.053 mmol) of diphenyl carbonate, and 1.5 mg (0.017 mmol) of $NaHCO_3$ in 0.1 ml of dried HMPA, and the mixture was stirred at 150° for 3 h. The reaction was monitored by TLC (AcOEt/MeOH 9:1) every hour as described above. There appeared a less polar substance identified as thymine (by TLC and co-spotting with an authentic sample); its concentration increased with time, and the spot of the starting material decreased in intensity concomitantly. The soln. turned dark brown (almost black) after 3 h. Threose presumably decomposed under the reaction conditions.

*2,2′-Anhydro-1-(α-*L*-threofuranosyl)uracil* (**10c**). The reaction was performed as described for **10b** with 1.0 g (4.7 mmol) of **8c**, 2.1 g (9.8 mmol) diphenyl carbonate, 4 ml of HMPA, and 0.08 g (0.9 mmol) of $NaHCO_3$.

Workup as described for **10b** afforded 0.84 g (92%) of **10c**. TLC (AcOEt/MeOH, 9:1): R_f 0.2 ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.27 (*dd*, J_{gem} = 9.4, H–C(4′)); 4.01 (*dd*, J(3′,4′ = 6.6, J_{gem} = 9.4, H–C(4′)); 4.33 (*m*, H–C(3′)); 5.19 (*dd*, J(1′2′) = J(2′,3′) = 5.2, H–C(2′)); 5.78 (*d*, J(3′,OH) = 6.0, HO–C(3′)); 5.88 (*d*, J(5,6) = 7.5, H–C(5); 6.20 (*d*, J(1′,2′) = 5.2, H–C(1′)); 7.80 (*d*, J(5,6) = 7.5, H–C(6)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 68.7 (*t*, C(4′)); 70.6 (*d*, C(3′)); 81.7 (*d*, C(2′)); 90.5 (*d*, C(1′)); 109.6 (*d*, C(5)); 137.7 (*d*, C(6)); 161.6 (*s*, C(2)); 172.1 (*s*, CO). ESI-MS (pos.): 219 (100, $[M + Na]^+$).

*1-(2′-*O*-Benzoyl-α-*L*-threofuranosyl)thymine* (**11b**). In a dried three-necked 100-ml round-bottom flask equipped with a condenser, septum, and stopper was placed 1.4 g (6.7 mmol) of **10b** and dried in the high vacuum overnight. Under Ar, 3.88 g (26.7 mmol) of BzONa and 26.8 ml of anh. HMPA were added with stirring. A suspension was formed, and this was heated to 150° (oil bath). After 20 min, the flask was lifted from the bath, cooled to r.t., and 0.96 g (7.7 mmol) of BzOH was added. The heating continued at 150° for 2 h with stirring. After this time, the mixture was cooled, diluted with 300 ml of H_2O, and extracted with 3 × 150 ml of AcOEt. HMPA remained in the aq. phase. The org. phases were pooled, washed with brine, dried ($MgSO_4$), and evaporated to dryness, affording an oily residue, which still contained HMPA. This residue was dissolved with AcOEt, and the org. soln. was washed with H_2O (to remove HMPA) and concentrated to dryness. The residue was adsorbed on *ca*. 3 g of silica gel and purified by CC (silica gel suspended in hexane/AcOEt 80:20, eluted with hexane/AcOEt 75:25 to 40:60) to afford 300 mg of benzoic acid, 140 mg (5%) of **7b**, 1.11 g (54.7%) of **11b**, and 37 mg (6%) of the 3′-*O*-benzoyl isomer **11bb**.

Data of **11b**: M.p. 68–72°. TLC (hexane/AcOEt, 1:1): R_f 0.3. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.80 (*s*, Me–C(5)); 4.11 (*dd*, J(3′,4′) = 4.0, J_{gem} = 9.5, H–C(4′)); 4.16 (*dd*, J_{gem} = 9.5, H–C(4′)); 4.38 (br. *s*, H–C(3′)); 5.27 (br. *s*, H–C(2′)); 5.91 (*d*, J(3′,OH) = 3.2, HO–C(3′)); 5.96 (*d*, J(1′,2′) = 2.1, H–C(1′)); 7.6 (*m*, arom. H); 7.66 (*d*, J = 1.0, H–C(6)); 7.71 (*m*, arom. H); 8.02 (*m*, 2 arom. H); 11.46 (*s*, NH). ^{13}C-NMR (150 MHz, (D_6)DMSO): 13.2 (*q*, *Me*–C(5)); 73.4 (*d*, C(3′)); 75.5 (*t*, C(4′)); 82.8 (*d*, C(2′)); 89.6 (*d*, C(1′)); 126.2 (*s*, C(5)); 129.7 (2*d*, arom. C); 130.4 (2*d*, arom. C); 134.7 (*s*, arom. C); 137.5 (*d*, C(6)); 151.2 (*s*, arom. C); 164.7–165.5 (2*s*, CO). ESI-MS (pos.): 355 (100, $[M + Na]^+$), 687 (80, $[2M + Na]^+$).

Data of **11bb**: M.p. 164–165°. TLC (hexane/AcOEt 2:3): R_f 0.31. ^{1}H-NMR (500 MHz, (D_6)DMSO): 1.71 (*d*, J = 1.0, H_3C–C(5)); 4.34 (*ddd*, J = 4.0, 1.0, 1.0, H–C(2′)); 4.36 (*dd*, J = 11.0, 4.0, H–C(4′)); 4.49 (*dd*, J = 11.0, 1.0, H–C(4′)); 5.23 (ddd, J = 4.0, 1.0, 1.0, H–C(3′)); 5.71 (d, J = 1.0, H–C(1′)); 7.48 (dd, J = 8.5, 8.5, arom. H); 7.56 (*q*, J = 1.0, H–C(6)); 7.65 (*dddd*, J = 8.5, 8.5, 1.5, 1.5, arom. H); 7.87 (*dd*, J = 8.5, 1.0, arom. H). ^{13}C-NMR (125 MHz, $CDCl_3$): 12.08 (*q*, *Me*–C(5)); 73.02 (*t*, C(4′)); 77.62 (*d*, C(2′)); 77.94 (*d*, C(3′)); 91.68 (*d*, C(3′)); 108.38 (*s*, C(5)); 128.66 (2*d*, arom. C); 128.97 (*s*, arom. C); 129.30 (2*d*, arom. C); 133.72 (*d*, C(5″)); 136.02 (*d*, C(6)); 150.38 (*s*, C(2)); 163.90 (*s*, C(4)); 164.79 (*s*, CO). ESI-MS (pos.): 355 (100 $[M + Na]^+$), 333 (57.7 $[M + H]^+$), 207 (56.3), 201 (29.6), ESI-MS (neg.): 367 (14.1, $[M + Cl]^-$), 331 (100 $[M - H]^-$), 311 (40.8), 203 (56.3), 183 (60.6), 127 (43.7).

*1-(2′-*O*-Benzoyl-α-*L*-threofuranosyl)uracil* (**11c**). The reaction was performed as described for **11b** with 0.8 g (4.0 mmol) of **10c**, 2.35 g (16.3 mmol) of BzONa, 16 ml of anh. HMPA, and 0.6 g (4.9 mmol) of BzOH. Workup as described for **11b** gave 1.0 g (77%) of **11c**. M.p. 162–64°. TLC (hexane/AcOEt, 1:1): R_f 0.3. ^{1}H-NMR (600 MHz, (D_6)DMSO): 4.12 (*dd*, J(3′,4′) = 3.7, J_{gem} = 9.7, H–C(4′)); 4.17 (*dd*, J_{gem} = 9.7, H–C(4′)); 4.36 (br. *s*, H–C(3′)); 5.27 (br. *s*, H–C(2′)); 5.68 (*d*, J(5, 6) = 7.8, H–C(5)); 5.93 (*d*, J(3′,OH) = 2.7, HO–C(3′)); 5.95 (*s*, H–C(1′)); 7.56 (*m*, 2 arom. H); 7.70 (*m*, arom. H); 7.77 (*d*, J(5, 6) = 7.8, H–C(6)); 8.01 (*m*, 2 arom. H); 11.38 (*s*, NH). ^{13}C-NMR (150 MHz, (D_6)DMSO): 73.2 (*d*, C(3′)); 76.0 (*t*, C(4′)); 82.7 (*d*, C(2′)); 89.9 (*d*, C(1′)); 102.3 (*d*, C(5)); 129.5 (*d*); 129.6 (*d*); 129.7 (*d*); 134.5 (*d*); 141.9 (*d*, C(6)); 151.2 (*s*, CO); 164.1, 165.5 (2*s*, CO). ESI-MS (pos.): 341 (100, $[M + Na]^+$).

*1-{2′-*O*-Benzoyl-3′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-threofuranosyl}thymine* (**12b**). To a soln. of 1.61 g (4.9 mmol) of **11b** in 18 ml of CH_2Cl_2 and 3.8 ml (28.9 mmol) of *sym*-collidine, 2.4 g (7.1 mmol) of DMT-Cl were added at r.t. under Ar with stirring. The reaction was maintained for 16 h under Ar, and, then, additional 240 mg (0.15 eq.) of DMT-Cl was added. After 4 h, another portion of 240 mg (0.15 equiv.) of DMT-Cl was added. The reaction was quenched with 3 ml of MeOH (the soln. turns yellow); diluted with 100 ml of CH_2Cl_2, and the org. phase was washed with sat. aq. $NaHCO_3$ and brine. The org. phase was dried (Na_2SO_4) and concentrated to dryness to yield *ca*. 3.3 g of residue, which was subject to CC (silica gel, (∅5 × 20 cm) suspended in hexane/AcOEt 4:1 containing 5% of Et_3N; eluted with hexane/AcOEt 70:30 to 40:60, containing 5% of Et_3N) to afford 2.51 g (81.8 %) of **12b**. M.p. 75–77°. TLC (hexane/AcOEt 3:2): R_f 0.26. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.68 (*s*, Me–C(5)); 3.62 (*dd*, J_{gem} = 9.9, H–C(4′)); 3.64 (2*s*, MeO); 3.90 (*dd*, J(3′,4′) = 1.9, J_{gem} = 9.9, H–C(4′)); 4.47 (*m*, H–C(3′)); 4.75 (br. *s*, H–C(2′)); 5.75 (*d*, J(1′,2′) = 2.8, H–C(1′)); 6.8 (*m*, 4 arom. H); 7.1–7.3 (*m*, 7 arom. H); 7.46 (*d*, 2 arom. H); 7.5 (*m*, 2 arom. H); 7.60 (*d*, J = 0.8, H–C(6)); 7.68 (*t*, arom. H); 7.93 (*d*, 2 arom. H); 11.44 (*s*, NH). ^{13}C-NMR (150 MHz, (D_6)DMSO): 12.8 (*q*, *Me*–C(5)); 55.8 (2*q*, MeO); 73.5 (*t*,

C(4′)); 76.6 (*d*, C(3′)); 81.4 (*d*, C(2′)); 88.1 (*s*, Ar_3C); 89.2 (*d*, C(1′)); 110.6; 114.3; 127.8; 128.2; 128.9; 129.3; 129.6; 130.4; 130.6; 130.7; 134.7; 135.9; 136.0; 137.3 (*d*, C(6)); 145.6, 150.9, 159.2 (*s*, CO); 164.6 (*s*, CO); 165.3 (*s*, CO). ESI-MS (pos.): 657 (100, $[M+Na]^+$).

*1-{2′-*O*-Benzoyl-3′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-threofuranosyl}uracil* (**12c**). The reaction was performed as described for **12b** with 0.5 g (1.57 mmol) of dry **11c**, 7 ml of anh. CH_2Cl_2, 0.8 g (2.35 mmol) of DMT-Cl and 1.25 ml (9.5 mmol) of 2,4,6-collidine. Workup and purification was performed as described for **12b** to give 0.94 g (96%) **12c**. M.p. 93–95°. TLC (hexane/AcOEt, 1:1): R_f 0.6. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.46 (*dd*, $J_{gem}=9.8$, $J(3',4')=5.3$, H–C(4′)); 3.66, 3.67 (2*s*, MeO); 3.70 (*dd*, $J(3',4')=3.3$, $J_{gem}=9.8$, H–C(4′)); 4.47 (*m*, H–C(3′)); 5.20 (*m*, H–C(2′)); 5.64 (*d*, $J(5',6')=8.2$, H–C(5)); 5.75 (*d*, $J(1',2')=5.9$, H–C(1′)); 6.80–6.85 (*m*, 4 arom. H); 7.24–7.38 (*m*, 7 arom. H); 7.42 (*m*, 2 arom. H); 7.52 (*m*, 2 arom. H); 7.74 (*d*, $J(5,6)=8.2$, H–C(6)); 7.94 (*m*, 2 arom. H); 11.33 (*s*, NH). ^{13}C-NMR (150 MHz, (D_6)DMSO): 55.7 (2*q*, MeO); 73.5 (*t*, C(4′)); 76.5 (*d*, C(3′)); 81.2 (*d*, C(2′)); 88.0 (*s*, Ar_3C); 89.5 (*d*, C(1′)); 102.7 (*d*, C(5)); 114.2, 114.3, 127.8, 128.4, 128.9, 129.3, 129.6, 130.3, 130.5, 130.6, 130.7, 134.8, 136.1, 136.2 (arom. C); 141.9 (*d*, C(6)); 145.6, 151.0 (arom. C); 159.3, 164.0, 165.4 (3*s*, CO). ESI-MS (pos.): 643 (100, $[M+Na]^+$); 659 (30, $[M+K]^+$).

*1-{3′-*O*-[(4″,4‴-Dimethoxytriphenyl)methyl]-α-*L*-threofuranosyl}thymine* (**9b**). A suspension of 3.3 g (5.2 mmol) of **12b** in 350 ml of 2M NH_3 in MeOH was stirred 24 h at r.t. and monitored (TLC). The solvent was evaporated to dryness, the residue was co-evaporated with H_2O (3×), and was subjected to CC (silica gel, column deactivated with 5% Et_3N in hexane; eluted with hexane/AcOEt 2:1) to give 2.75 g (99%) of **9b**. White powder. The ^{1}H- and ^{13}C-NMR-spectral properties were identical to those of **9b** obtained by the direct-tritylation route.

*1-{3′-*O*-[(4″,4‴-Dimethoxytriphenyl)methyl]-α-*L*-threofuranosyl}uracil* (**9c**). Reaction was performed as described for **9b** with 0.9 g (1.5 mmol) **12c** in 100 ml of 2M NH_3 in MeOH. Workup and purification as described for **9b** gave 0.75 g (99%) of **9c**. White powder. The ^{1}H- and ^{13}C-NMR-spectral properties were identical to those of **9c** obtained by the direct-tritylation route.

4. Experiments Referring to *Scheme 5*. – N^6-*Benzoyl-9-{2′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-3′-*O*-[(4,4′-dimethoxytriphenyl)methyl]-α-*L*-threofuranosyl}adenine* (**13a**). A soln. of 2 g (3.1 mmol) of **9a**, 3 ml (17.2 mmol) of EtN(i-Pr)$_2$, and 0.73 ml (3.2 mmol) of chloro(2-cyanoethoxy)(diisopropylamino)phosphine in 20 ml of CH_2Cl_2 was stirred for 18 h at r.t. Quenching with 4 drops of MeOH, evaporation of the solvent, co-evaporation with toluene, and filtration over silica gel (deactivated with CH_2Cl_2/Et_3N 98:2, pure CH_2Cl_2 for elution) gave a crude product, which was subjected to CC (silica gel, deactivation with CH_2Cl_2/Et_2N 97:3, elution with CH_2Cl_2/hexane/Et_3N 80:19:1) to give 1.67 g (64%) of **13a** as a mixture of diastereoisomers. TLC (CH_2Cl_2/MeOH 50:1): R_f 0.34, 0.24. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.11, 1.16, 1.19, 1.21, 1.25 (8*d*, $J=6.8$, 24 H, Me_2CH of both isomers); 2.37–2.44 (*m*, 2 H, OCH_2CH_2CN); 2.44–2.62 (*m*, 2 H, OCH_2CH_2CN); 3.44 (*m*, H–C(4′)); 3.50–3.80 (*m*, 8 H, H–C(4′), OCH_2CH_2CN of both isomers, Me_2CH of both isomers); 3.76 (*s*, 2 MeO of both isomers); 3.77 (*s*, 2 MeO of both isomers); 3.87 (*m*, CH_2O, H–C(4′) of both isomers); 4.27, 4.37 (*m*, H–C(3′) of both isomers); 4.50, 4.95 (*m*, H–C(2′) of both isomers); 6.16, 6.23 (*s*, H–C(1′) of both isomers); 6.77–6.81 (*m*, 8 H, $(MeO)_2Tr$ of both isomers); 7.08–7.24 (*m*, 18 H, $(MeO)_2Tr$ of both isomers); 7.52–7.63 (*m*, 6 arom. H); 8.05 (*m*, 4 arom. H); 8.35, 8.39, 8.81 (*s*, 4 H–C(2), H–C(8) of both isomers); 9.07 (br. *s*, 2 NH of both isomers). ^{13}C-NMR (150 MHz, $CDCl_3$): 20.4 (*t*, $J(C,P)=7.6$, CH_2CN, of one isomer); 20.5 (*t*, $J(C,P)=7.4$, CH_2CN, of one isomer); 24.8, 24.9, 25.0, 25.1(8*q*, Me of both isomers); 43.8, 43.9 (*d*, Me_2CH of both isomers); 55.6 (4*q*, MeO of both isomers); 58.6 (*t*, $J(C,P)=26.8$, CH_2O, of one isomer); 58.7 (*t*, $J(C,P)=24.9$, CH_2O, of one isomer); 75.4, 75.5 (*t*, C(4′) of both isomers); 78.2 (*d*, C(3′) of one isomer); 78.5 (*d*, $J(C,P)=6.7$, C(3′) of one isomer); 81.6 (*d*, $J(C,P)=29.4$, C(2′) of one isomer); 81.7 (*d*, $J(C,P)=33.0$, C(2′) of one isomer); 88.4, 88.5 (*s*, Ar_3C of both isomers); 90.6 (2*d*, C(1′) of both isomers); 113.8 (4*d*); 113.9 (2*d*); 114.0 (*d*); 117.8, 117.9 (*s*, CN of both isomers); 123.6 (*d*); 123.7 (*d*); 125.7 (*d*); 127.5 (*d*); 128.2 (*d*); 128.3 (2*d*); 128.5 (2*d*); 128.6 (*d*); 129.3 (*d*); 129.4 (*d*); 130.2 (2*d*); 130.4 (*d*); 133.1 (*d*); 134.2 (*d*); 135.8 (*d*); 136.0 (*d*); 136.1 (*d*); 138.2 (*d*); 142.1 (*d*); 142.4 (*d*); 144.8 (2*d*); 149.7 (*d*); 149.8 (*d*); 151.7 (*d*); 151.8 (*d*); 152.7 (*d*); 152.9 (*d*); 159.1 (*d*); 159.2 (*d*); 165.1 (2*s*). ^{31}P-NMR (121 MHz, $CDCl_3$): 151.7, 152.7. FAB-MS (pos., 3-NBA): 844 (82, $[M+H]^+$), 743 (80, $[M-N(i\text{-}Pr)_2]^+$), 304 (100). Anal. calc. for $C_{46}H_{50}N_7O_7P$ (843.92): C 65.47, H 5.97, N 11.62, P 3.67 found: C 65.47, H 6.10, N 11.73, P 3.49.

*1-{2′-*O*-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-3′-*O*-[(4,4′-dimethoxytriphenyl)methyl]-α-*L*-threofuranosyl}thymine* (**13b**). A soln. of 842 mg (1.6 mmol) of **9b**, 1.1 ml (6.4 mmol) of EtN(i-Pr)$_2$ and 413 mg (1.8 mmol) of chloro(2-cyanoethoxy)(diisopropylamino)phosphine in 10 ml of CH_2Cl_2 was stirred 18 h at r.t. Evaporation and CC (hexane/AcOEt/Et_3N 80:18:2 to 50:48:2) gave 809 mg (70%) of **13b** as a mixture of diastereoisomers. TLC (CH_2Cl_2/MeOH 25:2): R_f 0.22. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.99, 1.18 (*m*, 4 Me_2CH);

1.77, 1.79 (2 br. *s*, Me); 2.21, 2.41 (*m*, C*H*$_2$CN); 3.44, 3.62 (*m*, 3 H, Me$_2$C*H*), POC*H*$_2$); 3.70, 3.95 (*m*, H–C(4′) of both isomers); 3.77 (*s*, MeO of both isomers); 4.11 (*m*, H–C(3′) of one isomer); 4.18 (br. *d*, *J* ≈ 8.1, H–C(2′) of one isomer); 4.23 (br. *d*, *J* = 3.1, H–C(3′) of one isomer); 4.47 (br. *d*, *J* ≈ 8.7, H–C(2′) of one isomer); 5.76, 5.80 (2 br. *s*, H–C(1′) of both isomers); 6.79–7.43 (*m*, 14 H, arom. H, H–C(6)). ^{13}C-NMR (75 MHz, CDCl$_3$): 11.2, 12.6 (*q*, *Me*–C(5) of both isomers); 19.8, 20.2 (*t*, *C*H$_2$CN of both isomers); 24.2, 24.5 (8*q*, *Me*$_2$CH); 43.4, 43.5 (*d*, Me$_2$*C*H of both isomers); 55.2 (*q*, MeO of both isomers); 57.9, 58.4 (*t*, *C*H$_2$O of both isomers); 74.9, 75.1 (*t*, C(4′) of both isomers); 77.2, 77.7 (*d*, C(3′) of both isomers); 82.0, 82.2 (*d*, C(2′) of both isomers); 88.2, 88.3 (*s*, Ar$_3$*C* of both isomers); 91.4 (*d*, C(1′)); 91.6 (*d*, C(1′)); 109.1; 109.3, 113.5 (*d*); 113.6 (3*d*); 117.4, 118.1, 127.1 (*d*); 127.6 (*d*); 127.7 (*d*); 128.1 (*d*); 128.2 (*d*); 129.7 (2*d*); 129.9 (*d*); 135.4, 135.5, 135.7, 136.9 (2*d*); 144.5; 150.7; 158.2; 158.8; 164.2; 164.3. ^{31}P-NMR (121 MHz, CDCl$_3$): 151.8, 152.6. FAB-MS (pos., 3-NBA): 731.2 (14, [*M* + H]$^+$), 660.2 (31), 630.1 (28), 303.0 (100).

*1-{2′-*O*-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-3′-*O*-[(4,4′-dimethoxytriphenyl)methyl]-α-*L*-threofuranosyl}uracil* (**13c**). A soln. of 1.3 g (2.5 mmol) of **9c**, 2.5 ml (14.2 mmol) of EtN(i-Pr)$_2$, and 0.62 ml (2.77 mmol) of chloro(2-cyanoethoxy)(diisopropylamino)phosphine in 13 ml of CH$_2$Cl$_2$ was stirred 18 h at r.t. Workup as described for **13a** gave as a colorless foam 1.33 g (74%) of **13c** as a mixture of diastereoisomers. TLC (CH$_2$Cl$_2$/MeOH 1:1): R_f 0.29, 0.23. ^{1}H-NMR (600 MHz, CDCl$_3$): 1.01–1.21 (*d*, *J* = 6.7, 8 Me); 2.27–2.70 (3*m*, C*H*$_2$CN of both isomers); 3.16, 3.35 (*d*, *J* = 10.0, H–C(4′) of one isomer); 3.58 (*m*, 4 H, (Me)$_2$C*H*, C*H*$_2$O); 3.78 (*m*, 8 H, MeO, H–C(4′) of one isomer); 3.93 (*m*, C*H*$_2$O); 4.14 (*d*, *J* = 3.2, H–C(3′) of one isomer); 4.25 (*d*, *J* = 9.0, H–C(2′) of one isomer); 4.26 (*d*, *J* = 3.4, H–C(3′) of one isomer); 4.29 (*d*, *J* = 8.0, H–C(2′) of one isomer); 5.69 (*dd*, *J* = 14.6, 8.2, H–C(5) of both isomers); 5.80, 5.83 (2*s*, H–C(1′) of both isomers); 6.86 (*m*, 8 H, (MeO)$_2$*Tr*); 7.16–7.37 (*m*, 18 H, (MeO)$_2$*Tr*); 7.63, 7.75 (*d*, *J* = 8.2, H–C(6) of both isomers). ^{13}C-NMR (150 MHz, CDCl$_3$): 19.7 (*q*, Me); 20.2 (*t*, *C*H$_2$CN); 20.7 (*t*, *C*H$_2$CN); 24.8–25.0 (3*q*, Me); 44.0–43.8 (4d, (Me)$_2$*C*H); 55.7 (2*q*, MeO of both isomers); 58.4, 59.0 (2*t*, CH$_2$O of both isomers); 75.7, 75.9 (2*t*, C(4′) of both isomers); 77.9 (2*d*, C(3′) of both isomers); 82.5, 82.6 (2*d*, C(2′) of both isomers); 88.8 (2*s*, Ar$_3$*C* of both isomers); 92.1, 92.3 (2*d*, C(1′) of both isomers); 101.3, 101.5 (2*d*, C(5) of both isomers); 113.9, 114.0, 117.9–118.6 (2*s*, CN); 128.1, 128.3, 128.5, 128.6, 130.2, 130.4, 135.8, 135.9, 136.1, 141.4–141.5 (2*d*, C(6) of both isomers); 144.8; 144.9; 150.7; 151.3; 159.2; 159.3; 164.4; 164.5. ^{31}P-NMR (121 MHz, CDCl$_3$): 151.7, 152.7. FAB-MS (pos., 3-NBA): 717 (5 [*M* + H]$^+$), 303 (100), 201 (21), 149 (27), 123 (20).

*4-Benzoyl-1-{2′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-3′-*O*-[(4,4′-dimethoxytriphenyl)methyl]-α-*L*-threofuranosyl}cytosine* (**13d**). A soln. of 1 g (1.7 mmol) of **9d**, 1.67 ml (9.6 mmol) of EtN(i-Pr)$_2$, and 446 µl (1.7 mmol) of chloro(2-cyanoethoxy)(diisopropylamino)phosphine in 8 ml of CH$_2$Cl$_2$ was stirred 18 h at r.t. Quenching with 2 drops of MeOH, evaporation of the solvent, co-evaporation with toluene and filtration over silica gel (deactivation and elution with hexane/AcOEt/Et$_3$N 59:39:2) gave a syrup, which was dissolved in 4 ml of CH$_2$Cl$_2$ and then precipitated in 400 ml of hexane. Filtration gave 922 mg (69%) of **13d**. Colorless foam. TLC (CH$_2$Cl$_2$/MeOH 50:1): R_f 0.30. ^{1}H-NMR (600 MHz, CDCl$_3$): 1.00, 1.01, 1.15, 1.16, 1.18, 1.22, 1.24, 1.89 (8*s*, Me); 2.33–2.45 (*m*, CH$_2$CN); 2.71–2.85 (*m*, CH$_2$CN); 3.19 (br. *d*, *J* = 10.0, H–C(4′) of one isomer); 3.29 (*d*, *J* = 9.9, H–C(4′) of one isomer); 3.50–3.60 (*m*, 4 Me$_2$C*H*); 3.65–3.75 (*m*, CH$_2$O); 3.76, 3.77 (2*s*, 2 MeO of both isomers); 3.86–3.90 (*m*, 3 H, H–C(4′) of both isomers, CH$_2$O); 4.06 (*m*, 1 H, C*H*$_2$O); 4.13 (*d*, *J* = 3.4, H–C(3′) of one isomer); 4.26 (*d*, *J* = 3.6, H–C(3′) of one isomer); 4.55 (*d*, *J* = 8.3, H–C(2′) of one isomer); 4.72 (*d*, *J* = 9.2, H–C(2′) of one isomer); 5.87, 5.89 (2*s*, H–C(1′) of both isomers); 6.80–6.83 (*m*, 8 arom. H); 7.17–7.28 (*m*, 14 arom. H); 7.55–7.64 (*m*, 8 arom. H); 7.93–8.03 (*m*, 6 arom. H); 8.70 (br. *s*, 2 H, NH of both isomers). ^{13}C-NMR (75 MHz, CDCl$_3$): 19.7, 20.2 (2*t*, *C*H$_2$CN of both isomers); 24.3–24.6 (8*q*, Me); 43.0 (*d*, Me$_2$*C*H); 43.2 (*d*, Me$_2$*C*H); 55.1 (2*q*, MeO); 57.5, 58.1 (*t*, *C*H$_2$O of both isomers); 75.0, 75.5 (*d*, C(3′) of both isomers); 76.7, 76.8 (*t*, C(4′) of both isomers); 82.0, 82.6 (*d*, C(2′) of both isomers); 88.0–88.2 (*s*, Ar$_3$*C* of both isomers); 92.9 (*d*, C(1′)); 113.2 (*d*); 113.3 (*d*); 113.4 (*d*); 117.3 (*s*, CN); 117.4 (*s*, CN); 127.0 (*d*); 127.1 (*d*); 127.6 (*d*); 127.9 (*d*); 128.1 (2*d*); 128.2 (*d*); 128.9 (*d*); 130.2 (*d*); 130.3 (*d*); 130.5 (2*d*); 133.0 (*d*); 133.1 (*d*); 135.4 (*d*); 135.7 (*d*); 135.8 (2*d*); 144.9 (2*d*); 158.7, 158.7, 161.9, 162.3. ^{31}P-NMR (121 MHz, CDCl$_3$): 150.1, 151.8. FAB-MS (POS., 3-NBA): 1640 (19, [2*M*]$^+$); 820 (57, [*M* + H]$^+$); 719 (65); 303 (100).

N^2*-Acetyl-*O^6*-diphenylcarbamoyl-9-{2′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-3′-*O*-[(4,4′-dimethoxytriphenyl)methyl]-α-*L*-threofuranosyl}guanine* (**13e**). A soln. of 158 mg (0.2 mmol) of **9e**, 215 µl (2 mmol) of EtN(i-Pr)$_2$, and 118 mg (0.5 mmol) of chloro(2-cyanoethoxy)(diisopropylamino)phosphine in 5 ml of CH$_2$Cl$_2$ was stirred for 90 min at r.t. Evaporation and CC (CH$_2$Cl$_2$/Et$_3$N 98:2 to CH$_2$Cl$_2$/acetone/Et$_3$N 90:10:0) gave 133 mg (67%) of **13e** as mixture of diastereoisomers. TLC (CH$_2$Cl$_2$/MeOH 10:1): R_f 0.87, 0.79. ^{1}H-NMR (300 MHz, CDCl$_3$): 1.08–1.18 (*m*, 4 *Me*$_2$CH); 2.18–2.48 (*m*, CH$_2$CN); 2.52, 2.61 (2*s*, Me of both isomers); 3.51–3.68 (*m*, 5 H, H–C(4′) of both isomers, Me$_2$C*H*, POCH$_2$); 3.70, 3.72, 3.73, 3.74 (4*s*, MeO of both isomers); 4.33, 4.45 (2*m*, H–C3′) of both isomers); 4.74 (*d*, *J* = 8.4, H–C(2′) of one isomer); 4.82 (*d*, *J* = 8.1,

H–C(2′) of one isomer); 6.00, 6.07 (2 br. *s*, H–C(1′) of both isomers); 6.76–7.47 (*m*, 23 arom. H); 8.03, 8.18 (br. *s*, NH) of both isomers); 8.27, 8.29 (*s*, H–C(8) of both isomers). ^{13}C-NMR (75 MHz, $CDCl_3$): 19.7–20.0 (2*t*, *C*H_2CN of both isomers); 24.4–25.3 (10*q*, Me of both isomers); 43.3 (2*d*, Me_2*C*H of both isomers); 43.4 (2*d*, Me_2*C*H of both isomers); 55.1 (2*q*, MeO of both isomers); 57.8, 58.0 (2*t*, CH_2O of both isomers); 74.3, 75.1 (2*t*, C(4′) of both isomers); 77.6, 77.7 (2*d*, C(3′) of both isomers); 81.1, 81.3 (2*d*, C(2′) of both isomers); 87.9, 88.0 (2*s*, Ar_3*C* of both isomers); 89.7, 89.8, 90.3, 90.4 (2*d*, C(1′)); 113.4, 117.2 (*d*); 117.5 (*d*); 117.6, 121.3, 127.0 (2*d*); 127.8 (*d*); 127.9 (*d*); 128.0 (2*d*); 129.1 (2*d*); 129.2 (*d*); 129.6 (*d*); 129.7 (*d*); 129.8 (2*d*); 129.9 (*d*); 135.3; 135.4; 135.5; 135.6; 141.7; 142.7; 143.0; 144.2; 144.3; 150.3; 151.9; 154.1; 154.4; 155.9; 158.7; 158.8; 170.5; 171.3. ^{31}P-NMR (121 MHz, $CDCl_3$): 151.48, 151.89. FAB-MS (pos., 3-NBA): 993.1 (100, $[M+H]^+$); 303.1 (32).

N^2, N^6-*Dibenzoyl-9-{2′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-3′-*O*-[(4,4′-dimethoxytriphenyl)methyl]-α-*L*- threofuranosyl}-2,6-diaminopurine* (**13f**). To a soln. of 518 mg (0.68 mmol, 1 equiv.) of **9f** and 360 µl of *Hünig*'s base in 4 ml of dry CH_2Cl_2, stirred under Ar at room temp., 880 µl (3.9 mmol) of chloro(2-cyanoethoxy)(diisopropylamino)phosphine was added dropwise. Workup and isolation as described for **13e** afforded 0.62 g (94%) of **13f** as a mixture of two diastereoisomers. ^{1}H-NMR (600 MHz, $CDCl_3$): 1.08, 1.16, 1.28, 1.29 (4*d*, $J=7.0$, *Me*$_2$CH of both isomers); 2.63, 2.77 (2*m*, CH_2CN of both isomers); 3.56 (*m*, Me_2C*H* of both isomers); 3.61 (*m*, CH_2CH_2CN of one isomer); 3.65–3.82 (*m*, H–C(4′) of both isomers); 3.89 (*m*, CH_2CH_2CN of one isomer); 4.14, 4.21 (2*m*, CH_2CH_2CN of both isomers); 4.30 (br. *s*, H–C(3′) of both isomers); 4.71, 4.73 (2*s*, H–C(2′) of both isomers); 6.26 (*s*, H–C(1′) of both isomers); 6.81 (*d*, $J=8.0$, arom. H *ortho* to the MeO); 7.15–7.70 (*m*, arom. H of both isomers); 8.00, 8.06 (2*d*, $J=7.5$, arom. H of both isomers); 8.35 (s, H–C(8) of both isomers); 9.14, 9.25 (2*s*, NH of both isomers). ^{31}P-NMR (121 MHz, $CDCl_3$): 151.77, 153.13. ESI-MS (pos.): 985 (100, $[M+Na]^+$), 963 (65, $[M+H]^+$). ESI-MS (neg.): 961 (60, $[M-H]^-$), 761 (100).

Preparation of the Starter Units for the Automated Synthesis on Solid Support (**14a–14f**). To a soln. of the (0.1 mmol) trityl compound **9a–9f** in 3 ml of abs. CH_2Cl_2, 18 mg (0.15 mmol) of DMAP and 9.5 mg (0.1 mmol) of succinic anhydride were added. The mixture was stirred at r.t. until no more starting material was detected (TLC, *ca.* 3.5 h). The soln. was diluted with 20 ml of CH_2Cl_2 and poured into a 7.5% aq. citric acid. The org. layer was separated, washed with a sat. aq. NaCl soln., and dried ($MgSO_4$). Evaporation of the solvent gave yellow foam. The dried foam was treated with a soln. of 83 mg (0.19 mmol) of BOP [27] and 31 mg (0.37 mmol) of 1-methyl-1*H*-imidazole in 3 ml of MeCN. 500 mg of LCAA-CPG was added, and the mixture was gently shaken at r.t. for 18 h. The solid material was isolated by filtration, washed with MeCN, then Et_2O, and dried *in vacuo*. After checking the loading capacity (normally between 20 and 35 µmol/g), the material was treated with a soln. of 200 mg of DMAP and 4 ml of Ac_2O in 16 ml of pyridine for 1 h at r.t. (shaken gently). The solid material was isolated by filtration, washed with MeCN, Et_2O, and then dried *in vacuo*. The loading capacity of the nucleoside on the CPG solid support was determined (at $\varepsilon_{498}(DMT^+)=70{,}000$) to be, on an average, 29–34 µmol/g for **14a**, 20–25 µmol/g for **14b**, 21–27 µmol/g for **14c**, 24–31 µmol/g for **14d**, 26–31 µmol/g for **14e**, and 48 µmol/g for **14f** (by the method published in [4]).

5. Automated Solid-Phase Synthesis and Purification of Oligonucleotides. –Oligonucleotide syntheses were carried out on a 1-µM scale on an *Expedite 8909 Nucleic Acid Synthesis* system (*PE Biosystems*) or on a *Pharmacia Gene Synthesizer Plus*. The DNA/RNA synthesizer column was filled with 30–35 mg of CPG-solid support loaded with the appropriate nucleobase. The substrates and reagents required were prepared as follows.

5.1. *Pre-Automation Procedures. For* Expedite 8909 Nucleic Acid Synthesis *System:* 5.1.1. *Phosphoroamidites.* The amount of phosphoramidite soln. was determined as follows: $(n+4)\times(17-22)$ mg of phosphoramidite dissolved in dry MeCN to make a 0.1M soln. The phosphoramidite soln. was dried (4-Å molecular sieves, 8–12 mesh, freshly activated by heating at *ca.* 300° under high vacuum overnight) overnight at r.t. prior to use; 12 pulses of phosphoramidite soln. per coupling (0.22 ml).

5.1.2. *Activator Soln.* 5-Ethylthio-1*H*-tetrazole, 0.35M soln. (1 g in 22 ml of MeCN) or 5-(benzylthio)-1*H*-tetrazole, 0.35M soln. (1.35 g in 20 ml of MeCN); dried over freshly, activated 3-Å or 4-Å molecular sieves; 45 pulses per coupling (0.72 ml).

5.1.3. *Capping A:* Ac_2O/2,6-lutidine/THF (1:1:8); 125 pulses per coupling (2 ml).

5.1.4. *Capping B:* 1-methyl-1*H*-imidazole in THF (16% (*v*/*v*)); 125 pulses per coupling (2 ml).

5.1.5. *Oxidizing Soln.:* I_2/H_2O/Pyridine/THF (2.5 g:2 ml:20 ml:75.5 ml); 180 pulses per coupling (2.9 ml).

5.1.6. *Detritylation Reagent:* 6% $Cl_2CHCOOH$ in $ClCH_2CH_2Cl$; 500 pulses per coupling (8 ml). The synthesis of oligonucleotides on the *Expedite 8909 Nucleic Acid Synthesis* system was accomplished with the following modifications to the protocol provided by the manufacturer for the DNA synthesis: *1*) the detritylation step was conducted with 500 pulses over a period of 400 s. *2*) The phosphoramidite + activator

coupling step was conducted with 12 pulses over a period of 1600 s. Some oligonucleotides were synthesized in the 'Trityl-on' mode, while the majority was synthesized in the 'Trityl-off' mode.

For Pharmacia Gene Synthesizer Plus *System:* It was essentially the same as for the *Expedite 8909 Nucleic Acid Synthesis* system, except the capping solns. *A* and *B* were combined into one bottle:

Capping: 10 ml of Ac_2O, 10 ml of 2,6-lutidine, 10 ml of 1-methyl-1*H*-imidazole in 160 ml of THF.

5.2. *Post-Automation Procedures.* 5.2.1. *Removal of β-Cyanoethyl Protecting Group and Removal of Nucleobase Protecting Groups with Concomitant Detachment from CPG-Solid Support.* After the automated synthesis was completed, the CPG-solid support containing the oligonucleotide was dried *in vacuo* for 30 min, and the removal of the *β*-cyanoethyl protecting group was effected by treatment with Et_3N/dry pyridine 5:1. The CPG-solid support containing the oligonucleotide was dried *in vacuo* for 30 min, placed in *Eppendorf* tubes (1.5 ml), and concomitant deprotection of nucleobase protecting groups and detachment of the oligonucleotides from the CPG-solid support was effected by one of the following methods (*Table 2* lists the specific deprotection method for the specific sequence).

Method A: with 30% NH_3 in H_2O at 50° for 4–12 h.

Method B: with 33% $MeNH_2$ soln. in EtOH/40% $MeNH_2$ soln. in H_2O (1:1) for 4 h, at r.t.

Method C: with a 2M (*Z*)-pyridine-2-carbaldeyde oxime soln. (in dioxane/H_2O 7:1, r.t., 3.5 min), and then with 24% aq. soln. of NH_2NH_2 hydrate for 18 h at 4°. After deprotection, the suspension was diluted in *ca.* 0.5M aq. $Et_3NH \cdot HCO_3$ buffer soln. and loaded over a *Waters Sepak-C18* cartridge (*cf. Sect. 7*) to afford the salt and hydrazine free, crude 'Trityl-on' oligonucleotides in soln.

Method D: with 24% aq. soln. NH_2NH_2 hydrate for 18 h at 4°°. After deprotection, the suspension was diluted in *ca.* 0.5M aq. $Et_3NH \cdot HCO_3$ buffer soln. and loaded over a *Waters Sepak-C18* cartridge (*cf. Sect. 7*) to afford the hydrazine free, crude 'Trityl-on' oligonucleotides in soln.

Method E: with 33% $MeNH_2$ soln. in EtOH/41% $MeNH_2$ soln. in H_2O (1:1) for 18 h, at 50°.

Subsequently, it was found that it was not necessary to have an initial separate deprotection step to remove the *β*-cyanoethyl protecting group. The following single step treatment with $MeNH_2$ yielded satisfactory results: after the automated synthesis was completed, the CPG-solid support containing the oligonucleotide was dried *in vacuo* for 30 min, placed in *Eppendorf* tubes (1.5 ml) and treated with a mixture of 1 ml of $MeNH_2$ soln. in 8M EtOH and 12M H_2O (1:1) for 2 h at r.t. For sequences containing 2,6-diaminopurine (D), it was necessary to heat at 50° (*Method E*) to remove the Bz protecting groups on the nucleobase.

All of the above deprotections were monitored by anion-exchange HPLC for optimum deprotection time. The CPG-solid support containing *Eppendorf* vial was centrifuged. After centrifugation, the supernatant was removed and the CPG-solid support was washed with 3×0.5 ml H_2O. The combined washings and supernatant was lyophilized to dryness with a *SpeedVac* evaporator. The residue was dissolved in 1 ml of H_2O for analysis and purification.

Oligonucleotides synthesized in the 'Trityl-off' mode were directly taken to the next step of purification by IE HPLC.

5.2.3. *Detritylation of 'Trityl-on' Oligonucleotides.* The aq. soln. containing the deprotected crude-oligonucleotides (synthesized in the 'Trityl-on' mode) were concentrated *in vacuo*, the crude-residue was treated with *ca.* 10 ml of 80% aq. HCOOH (a red color appears within seconds, indicating detritylation) at r.t. for 15 min and concentrated *in vacuo* to dryness. The residue was dissolved in *ca.* 2 ml of H_2O, filtered (*Nalgene* syringe filter, 0.2 μ) and taken to the next step, ion-exchange HPLC purification.

6. HPLC Purification of Oligonucleotides. – Oligonucleotides were purified by ion-exchange HPLC. Anion-exchange HPLC was performed either on *A*) *Pharmacia GP-250 Gradient Programmer* equipped with two *Pharmacia P-500* pumps, *ABI-Kratos Spectraflow 757* UV/VIS detector, and a *Hewlett Packard HP 3396A* analog integrator or *B*) *Pharmacia Äkta Purifier (900)* controlled by *UNICORN* system. Columns: *Mono Q HR 5/5* (*Pharmacia*) or *SAX 1000-8* (*Macherey & Nagel*); buffer *A:* 10 mM Na_2HPO_4 in H_2O, pH 10.5; buffer *B:* 10 mM Na_2HPO_4 in H_2O, 1M NaCl, pH 10.5. Product fractions were collected either in 0.1M aq. AcOH soln. or in 1M aq. $Et_3NH \cdot HCO_3$ buffer soln.

7. Desalting of Oligonucleotides [10]. – The product fractions from HPLC purification were combined and diluted with 1M aq. $Et_3NH \cdot HCO_3$ buffer to twice the volume, and applied to a previously conditioned reverse phase *Sep-Pak* cartridge (*C18 Waters*). Successive elution with 0.1–0.5M aq. $Et_3NH \cdot HCO_3$ buffer soln., MeCN/H_2O 1:1 and lyophillization of the MeCN/H_2O fractions containing the product (monitored by UV at 260 nm) afforded the salt-free oligonucleotide. The residue was dissolved in 1 ml of H_2O and stored at −20° as a stock soln.

REFERENCES

[1] X. Wu, G. Delgado, R. Krishnamurthy, A. Eschenmoser, *Org. Lett*. **2002**, *4*, 1283 – 1286.
[2] A. Eschenmoser, *Science*, **1999**, *284*, 2118 – 2124.
[3] a) A. Eschenmoser, M. Dobler, *Helv. Chim. Acta* **1992**, 75, 218 – 259; b) M. Böhringer, H.-J. Roth, J. Hunziker, M. Göbel, R. Krishnan, A. Giger, B. Schweizer, J. Schreiber, C. Leumann, A. Eschenmoser, *Helv. Chim. Acta* **1992**, *75*, 1416 – 1477; c) J. Hunziker, H.-J. Roth, M. Böhringer, A. Giger, U. Diedrichsen, M. Göbel, R. Krishnan, B. Jaun, C. Leumann, A. Eschenmoser, *Helv. Chim. Acta* **1993**, *76*, 259 – 352; d) G. Otting, M. Billeter, K. Wütrich, H. J. Roth, C. Leumann, A. Eschenmoser, *Helv. Chim. Acta* **1993**, *76*, 2701 – 2756; e) K. Groebke, J. Hunziker, W. Fraser, L. Peng, U. Diedrichsen, K. Zimmermann, A. Holzner, C. Leumann, A. Eschenmoser, *Helv. Chim. Acta* **1998**, *81*, 375 – 474.
[4] a) S. Pitsch, S. Wendeborn, B. Jaun, A. Eschenmoser, *Helv. Chim. Acta* **1993**, *76*, 2161 – 2183; b) S. Pitsch, R. Krishnamurthy, M. Bolli, S. Wendeborn, A. Holzner, M. Minton, C. Lesuéur, I. Schlönvogt, B. Jaun, A. Eschenmoser, *Helv. Chim. Acta* **1995**, *78*, 1621 – 1635; c) I. Schlönvogt, S. Pitsch, C. Lesueur, A. Eschenmoser, R. M. Wolf, B. Jaun, *Helv. Chim. Acta* **1996**, *79*, 2316 – 2345; d) M. Bolli, R. Micura, S. Pitsch, A. Eschenmoser, *Helv. Chim. Acta* **1997**, *80*, 1901 – 1951; e) S. Pitsch, S. Wendeborn, R. Krishnamurthy, A. Holzner, M. Minton, M. Bolli, R. Micura, C. Miculka, N. Windhab, M. Stanek, B. Jaun, A. Eschenmoser, *Helv. Chim. Acta*, in preparation.
[5] M. Beier, F. Reck, T. Wagner, R. Krishnamurthy, A. Eschenmoser, *Science* **1999**, *283*, 699 – 703.
[6] K.-U. Schöning, P. Scholz, S. Guntha, X. Wu, R. Krishnamurthy, A. Eschenmoser, *Science* **2000**, *290*, 1347 – 51.
[7] X. Wu, S. Guntha, M. Ferencic, R. Krishnamurthy, A. Eschenmoser, *Org. Lett*. **2002**, *4*, 1279 – 1282.
[8] A. Eschenmoser, *Verh. Ges. Deutsch. Naturf. Aerzte* 116. Vers. Berlin **1990**; 135 – 172; A. Eschenmoser, *Nachr. Chem. Techn. Lab*. **1991**, *39*, 795 – 806.
[9] F. Reck, H. Wippo, R. Kudick, M. Bolli, G. Ceulemans, R. Krishnamurthy, A. Eschenmoser, *Org. Lett*. **1999**, *1*, 1531 – 1534.
[10] H. Wippo, F. Reck, R. Kudick, M. Ramaseshan, M. Bolli, G. Ceulemans, R. Krishnamurthy, A. Eschenmoser, *Bioorg. Med. Chem.* **2001**, *9*, 2411 – 2428.
[11] D. Müller, S. Pitsch, A. Kittaka, E. Wagner, C. E. Wintner, A. Eschenmoser, *Helv. Chim. Acta* **1990**, *73*, 1410 – 1468.
[12] M. Steiger, T. Reichstein, *Helv. Chim. Acta* **1936**, *19*, 1016 – 19; K. Gätzi, T. Reichstein, *Helv. Chim. Acta* **1938**, *21*, 195; A. S. Perlin in 'Methods in Carbohydrate Chemistry', Vol. 1, Eds. R. L. Whistler and M. L. Wolfram, Academic Press: New York, 1962, p. 68, and ref. cit. therein.
[13] S. Kamiya, T. Nakabayashi, *Bitamin*, **1957**, *13*, 246 – 249; *Chem. Abstr*. **1960**, *54*, 7797; J. M. Perel, P. G. Dayton, *J. Org. Chem.* **1960**, *25*, 2044 – 2045, and ref. cit. therein; M. G. Lopez, M. S. Feather, *J. Carbohydrate Chem*. **1992**, *11*, 799 – 806.
[14] H. S. Isbell, H. L. Frush, *Carbohydr. Res.* **1979**, *72*, 301 – 304.
[15] C. C. Wei, S. D. Bernardo, J. P. Tengi, J. Borgese, M. Weigele, *J. Org. Chem*. **1985**, *50*, 3462 – 3467.
[16] F. Micheel, W. Peschke, *Chem. Ber*. **1942**, *75B*, 1603 – 1607; S. Hashimoto, Y. Saito, H. Seki, T. Kamiya, *Tetrahedron Lett*. **1970**, *16*, 1359 – 1362.
[17] G. Nakaminami, H. Edo, M. Nakgawa, *Bull. Chem. Soc. Jpn*. **1973**, *46*, 266 – 269.
[18] A. S. Perlin, C. Brice, *Can. J. Chem*. **1956**, *34*, 541 – 553; A. Maradufu, D. M. Mackie, A. S. Perlin, *Can. J. Chem*. **1972**, *50*, 2617 – 2621.
[19] H. Vorbrüggen, B. Bennua, *Chem. Ber.* **1981**, *114*, 1279 – 1286; see also, *e.g.*, [3b] and [3e].
[20] D. H. Murray, J. Prokop, *J. Pharm. Sci*. **1967**, *56*, 865 – 870.
[21] M. J. Robins, R. Zou, Z. Guo, S. F. Wnuk, *J. Org. Chem*., **1996**, *61*, 9207; R. Zou, M. J. Robins, *Can. J. Chem*., **1987**, *65*, 1436.
[22] H. J. Thomas, J. A. Johnson, W. E. Fitzgibbon, S. J. Clayton, B. R. Baker, in 'Synthetic Procedures in Nucleic Acid Chemistry', Eds. W. W. Zorbach, R. S. Tipson, Interscience Publishers, Interscience, N.Y., 1968, Vol. 1, p. 249.
[23] S. Parel, C. J. Leumann, *Helv. Chim. Acta* **2000**, *83*, 2514 – 2526.
[24] G. Delgado, R. Krishnamurthy, in preparation.
[25] J. P. H. Verheyden, D. Wagner, J. G. Moffat, *J. Org. Chem*. **1971**, *36*, 250 – 254.
[26] A. V. Rama Rao, M. K. Gurjar, S. V. S. Lalitha, *Chem. Commun*. **1994**, 1255 – 1256.
[27] R. Pon, S. Yu, Y. S. Sanghvi, *Bioconjugate Chem*. **1999**, *10*, 1051 – 1057.
[28] F. Reck, H. Wippo, R. Kudick, R. Krishnamurthy, A. Eschenmoser, *Helv. Chim. Acta* **2001**, *84*, 1778 – 1804.

[29] T. Wagner, H. K. Huynh, R. Krishnamurthy, A. Eschenmoser, *Helv. Chim. Acta* **2002**, *85*, 399 – 416.
[30] A. K. Sood, S. A. Narang, *Nucl. Acids Res.* **1977**, *4*, 2757 – 2765.
[31] T. Kamimura, M. Tsuchiya, K.-I. Urakami, K. Kooura, M. Sekine, K. Shinozaki, K.-I. Miura, T. Hata, *J. Am. Chem. Soc*. **1984**, *106*, 4552.
[32] I. Tinoco Jr., K. Sauer, J. C. Wang, in 'Physical Chemistry: Principles and Applications in Biological Sciences', Eds. D. Young, D. Cavanaugh, Prentice Hall, Upper Saddle River, NJ, 1995, pp. 559 – 89.
[33] C. R. Cantor, P. R. Schimmel, in 'Biophysical Chemistry'; Freeman: San Francisco, CA, 1980; Part III (The Behavior of Biological Macromolecules); pp. 1135 – 1139.
[34] L. A. Marky, K. J. Breslauer, *Biopolymers* **1987**, *26*, 1601 – 1620.
[35] R. Micura, R. Kudick, S. Pitsch, A. Eschenmoser, *Angew. Chem., Int. Ed*. **1999**, *38*, 680 – 683.
[36] N. Sugimoto, S. Nakano, M. Katoh, A. Matsumura, H. Nakamuta, T. Ohmichi, M. Yoneyama, M. Sasaki. *Biochemistry* **1995**, *34*, 11211 – 11216; E. A. Lesnik, S. M. Freier, *Biochemistry*, **1995**, *34*, 10807; M. J. Dahma, C. J. Wilds, A. Noronha, I. Brukner, G. Borkow, D. Arion, M. A. Parniak. *J. Am. Chem. Soc*. **1998**, *120*, 12976 – 12977; A. Eggar, C. Leumann, *Synlett*, **1999**, 913.
[37] O. Jungmann, H. Wippo. M. Stanek, H. K. Huynh, R. Krishnamurthy, A. Eschenmoser, *Org. Lett*. **1999**, *1*, 1527 – 1530.
[38] T. Müller, Postdoctoral report, TSRI, Dec. 1996 – Mar. 1998.
[39] L. E. Orgel, *Science*, **2000**, *290*, 1306 – 1307.
[40] P. Herdewijn, *Angew. Chem*., *Int. Ed*. **2001**, *40*, 2249 – 2251.
[41] A. Eschenmoser, *Orig. Life Evol. Biosphere* **1997**, *27*, 535 – 553.
[42] A. Eschenmoser, *Angew. Chem., Int. Ed.* **1988**, *27*, 5.
[43] G. Joyce, L Orgel, S. Miller, A. Schwartz, *Proc. Natl. Acad. Sci. U.S.A*. **1987**, *84*, 4398 – 4402.
[44] G. Joyce and L. Orgel in 'RNA World', Cold Spring Harbor Monograph Series, Cold Spring Harbor, New York, 1999, 2nd Edition (Prospects for Understanding the Origin of the RNA World), pp. 49 – 77.
[45] R. Lohrman, L. E. Orgel, *Nature* **1976**, *261*, 342; W. S. Zielinsky, L. E. Orgel, *Nature* **1987**, *327*, 346; G. v. Kiedrowski, B. Wlotzka, J. Hebning, M. Matzen. S. Jordan, *Angew. Chem., Int. Ed.* **1991**, *30*, 423.

Received July 15, 2002