Pentopyranosyl Oligonucleotide Systems

Communication No. 10[1])

The α-L-Lyxopyranosyl-(4′ → 2′)-oligonucleotide System

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Edgar Heilbronner zum 80. Geburtstag gewidmet, in Erinnerung an die 'guten alten Zeiten'

To determine whether the remarkable chemical properties of the pyranosyl isomer of RNA as an informational *Watson-Crick* base-pairing system are unique to the pentopyranosyl-(4′ → 2′)-oligonucleotide isomer derived from the RNA-building block D-ribose, studies on the entire family of diastereoisomeric pyranosyl-(4′ → 2′)-oligonucleotide systems deriving from D-ribose, L-lyxose, D-xylose, and L-arabinose were carried out. The result of these extended studies is unambiguous: not only pyranosyl-RNA, but all members of the pentopyranosyl-(4′ → 2′)-oligonucleotide family are highly efficient *Watson-Crick* base-pairing systems. Their synthesis and pairing properties will be described in a series of publications in this journal. The present paper describes the α-L-lyxopyranosyl-(4′ → 2′)-system.

1. Introduction. – When, in 1992, our systematic search for potentially natural nucleic acid alternatives recruited from the structural neighborhood of RNA [2][3] switched from *hexo*pyranosyl-(6′ → 4′)- to *pento*pyranosyl-(4′ → 2′)-oligonucleotides [4], it was natural to chose among the pentopyranosyl family's four diastereomers (*Scheme 1*) the member derived from D-ribose with priority. This isomer is built from the same building blocks as RNA itself, it is the pyranosyl isomer of RNA ('p-RNA'). The system proved to have a variety of remarkable properties: first of all, it turned out to be a stronger *Watson-Crick* base-pairing system than RNA itself [5]. It forms duplexes and hairpins in antiparallel strand orientations similar to the natural system [6]. Base sequences of it can be transcribed *via* template-controlled ligations of short ligands, *e.g.*, tetramers, using 2′,3′-cyclophosphates as a mild form of phosphate activation, and such ligations were shown to proceed sequence-, regio-, as well as chiroselectively [7]. The self assembly of duplexes of long p-RNA sequences *via* self-templated chiroselective oligomerization of hemi-self-complementary tetramer-2′,3′-cyclophosphates has also been observed [8]. Significantly, replication of p-RNA sequences was achieved in a

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[1]) For communication No. 9, see [1]. The paper is also communication No. 32 in the series '*Chemistry of α-Aminonitriles*'. For a survey of the numbering of papers in this series, see [1].

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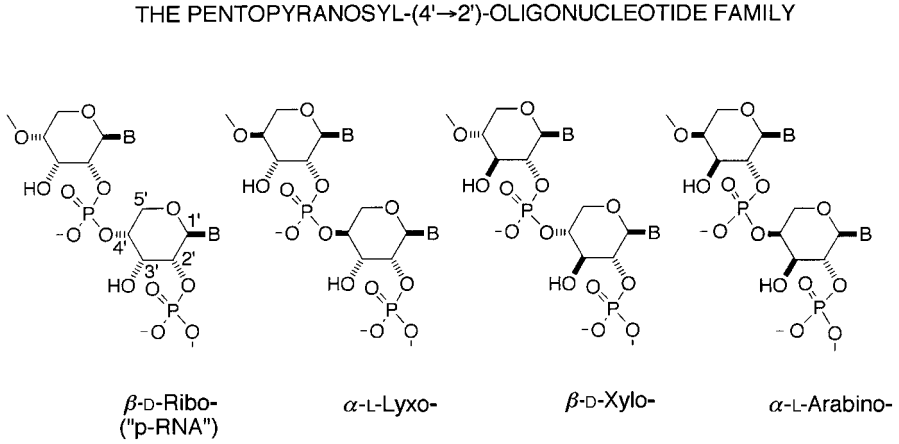
stepwise manner only; attempts to observe autocatalytic replication with turnover have failed [9]. Nevertheless, such failure could not really detract from sustaining the view that the pyranosyl form of RNA could have been, in principle, an evolutionary alternative to the RNA structure since, after all, comprehensive non-enzymic(!) autocatalytic replication with turnover is not known for RNA oligonucleotides either[4]).

Scheme 1. *Constitution and Configuration of the Repeating Units of the Four Diastereoisomeric Pentopyranosyl-(4′ → 2′)-oligonucleotide Systems*



In this situation, it appeared important to establish experimentally whether the remarkable potential of pyranosyl-(4′ → 2′)-oligonculeotides is unique for the ribopyranosyl series, or whether any of the three other diastereoisomers of the pentopyranosyl-(4′ → 2′)-oligonucleotide family might possess comparably interesting properties. Therefore, we extended our studies from p-RNA to all the other members of the pentopyranosyl-(4′ → 2′)-oligonucleotide family and found – admittedly to our surprise – that the potential for strong informational *Watson-Crick* base pairing within this family is by no means a privilege of the ribopyranosyl isomer. Quite the opposite turned out to be true: the remaining three other members, namely, α-L-lyxopyranosyl-(4′ → 2′)-, α-L-arabinopyranosyl-(4′ → 2′)-, and β-D-xylopyranosyl-(4′ → 2′)-oligonucleotides (*Scheme 1*) are all highly efficient *Watson-Crick* base-pairing systems [13]. Among them, the arabinopyranosyl member is the most astonishing; it is, in fact, one of the strongest oligonucleotide-type base-pairing systems known so far. All four pentopyranosyl-(4′ → 2′)-oligonucleotide systems form a compact family of base-pairing systems whose base-pairing is orthogonal to that of RNA in the sense that all of them show

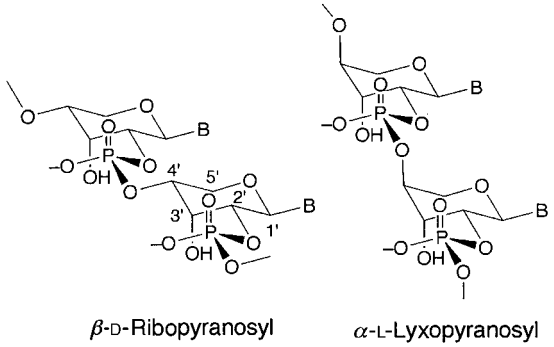
[4]) The failure to demonstrate sequence-replication with turnover in the p-RNA series is due to the problems of product inhibition, competition by hydrolytic deactivation, and slow ligation rates of 2′,3′-cyclophosphates [1]. Clearcut examples of autocatalytic oligomerization by template-controlled ligation have been demonstrated by *von Kiedrowsky et al.* [10] as well as *Zielinski* and *Orgel* [11] for short (modified) 2-deoxyribofuranosyl-oligonucleotide hexamers. So far, there is no evidence that these observations could be generalized and extrapolated to longer DNA or RNA sequences. For a recent review on nonenzymic molecular self-replication, see [12].

promiscuous cross-pairing among each other, yet none of them does cross-pair with natural RNA or DNA [14].

We have reported a summary of the first findings in this project in two preliminary communications [13][14] and promised to publish the full study in a series of papers in this journal. The present paper describes the synthesis and the pairing properties of oligonucleotides of the α-L-lyxopyranosyl-$(4' \rightarrow 2')$ series.

2. Synthesis. – For a significant comparison between the base-pairing properties of β-D-ribopyranosyl-$(4' \rightarrow 2')$-oligonucleotides ('p-RNA') and other members of the pentopyranosyl family, it was essential to use for the latter the right enantiomer. For the member derived from lyxose, the enantiomer in which the local chirality and conformation at the nucleosidic center (nucleobase equatorial) correspond to the β-D-ribopyranosyl series, is the α-L-lyxopyranosyl enantiomer (see *Scheme 2*). Overall, the synthesis of α-L-lyxopyranosyl-$(4' \rightarrow 2')$-oligonucleotides described here follows the pattern developed earlier in the synthesis of oligonucleotides of the p-RNA series [1][5].

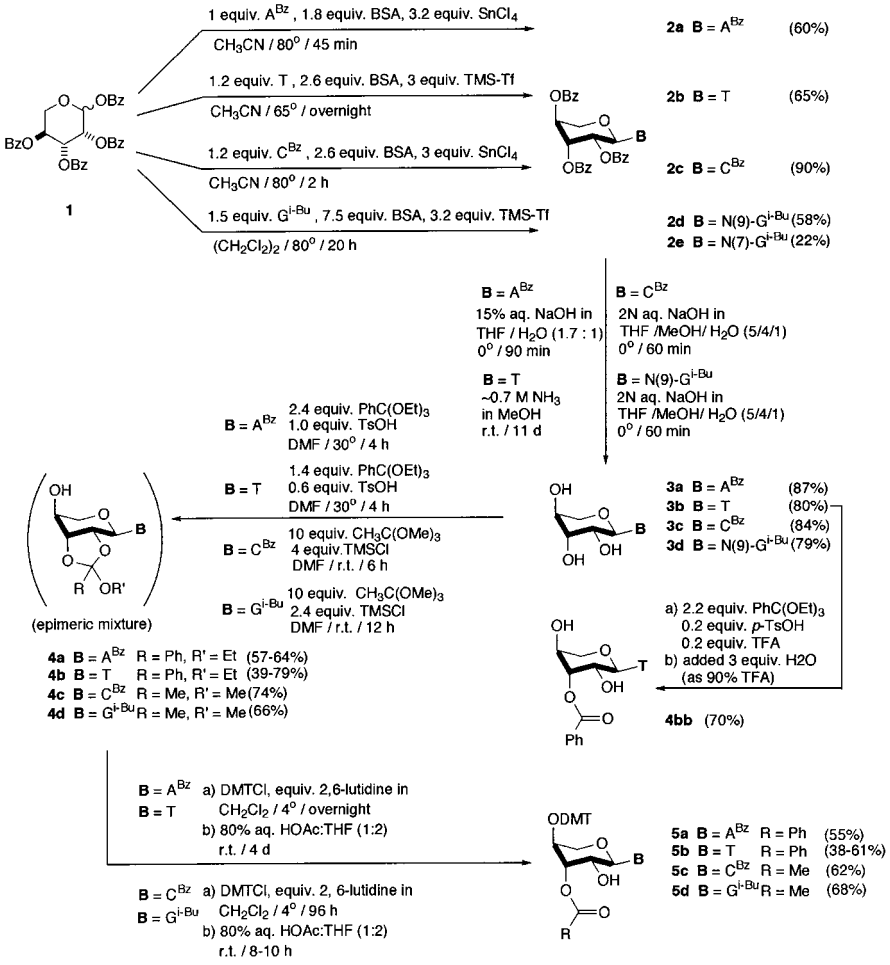
Scheme 2. *Idealized Pairing Conformation of β-D-Ribo-$(4' \rightarrow 2')$- and α-L-Lyxo-$(4' \rightarrow 2')$-pyranosyl-oligonucleotides*



2.1. *Synthesis of α-L-Lyxopyranosyl Nucleoside Building Blocks Containing Adenine (A), Thymine (T), Guanine (G), and Cytosine (C)* (*Scheme 3*). Primary targets in the preparation of the building blocks for the synthesis of α-L-lyxopyranosyl-$(4' \rightarrow 2')$-oligonucleotides were the protected nucleoside derivatives **5a**–**5d** in which the 4′-OH groups bear a 4′,4″-dimethoxytrityl substituent, the 3′-OH groups an acyl function (benzoyl (Bz) or acetyl (Ac)), and the nucleobases A, C, and G the conventional protecting groups Bz and isobutyroyl, respectively. Central intermediates on the way to these compounds were the 2′,3′,4′-trihydroxy derivatives **3a**–**3d** of the nucleobase-protected nucleosides, easily obtained by selective hydrolysis of the corresponding 2′,3′,4′-tribenzoyl derivatives **2a**–**2d**. The latter were synthesized in yields of 80–90% by coupling the α/β-anomeric mixture of tetrabenzoyl-lyxopyranose **1** [15] with the corresponding bases, namely, N^6-benzoyladenine (nucleosidation catalyst: $SnCl_4$), thymine (trimethylsilyl triflate (TMS-Tf)), N^4-benzoylcytosine ($SnCl_4$), and N^2-isobutyrylguanine (trimethylsilyl triflate) under the *Vorbrüggen-*

Hilbert-Johnson conditions [16][5]) (*Scheme 1*). Only the α-nucleosides with equatorial conformation of the nucleobases at the pyranose chairs (see *Schemes 3* and *4*) were formed (^{1}H-NMR). In the reaction with *N*-isobutyrylguanine, chromatographic separation of the N(9)-regioisomer (**2d**, 58%) from the N(7)-regioisomer (**2e**, 22%) was necessary. The N(7)-regioisomer, after isolation, could be equilibrated by refluxing in CH_2Cl_2 in the presence of TMS-Tf, to give further amounts of N(9)-regioisomer so that the overall yield of the latter was 70%. Assignment of N(9) *vs.* N(7) constitution was based on the ^{1}H-NMR spectrum, in which the compound assigned to the N(9)-regioisomer **2d** shows the *doublet* for H–C(1′) at higher field ($\delta = 6.12$ ppm, $J = 7.5$ Hz, in $CDCl_3$) as compared to the N(7)-isomer **2e** ($\delta = 6.83$ ppm, *d*, $J = 9.5$ Hz, in $CDCl_3$)

Scheme 3. *Preparation of the A,T,G, and C Nucleoside Building Blocks for the α-L-Lyxopyranosyl Series*



[5]) The α-L-lyxopyranosyl-nucleosides of adenine, cytosine, and uracil (unprotected) have been previously synthesized by *Fuertes et al.* [17] by another method and in a different context.

[16]. Base-catalyzed selective removal of the *O*-acyl protecting groups in (**2a**–**2d**) afforded the triols (**3a**–**3d**) in 79–87% yields.

Fig. 1 presents the results of an X-ray structure analysis of the 2′, 3′, 4′-tribenzoyloxy-nucleoside **2b** of the thymine series, demonstrating its constitution, configuration, and preferred conformation[6]). That the latter is also the preferred conformation in solution can be deduced from the coupling constants of the H–C(1′) with H–C(2′), the value of which ($J = 9.8$ Hz, in $CDCl_3$) is typical for two vicinal protons in diaxial conformation. *Table 1* lists the values of the corresponding coupling constants of all the intermediates formulated in *Scheme 3*. These values demonstrate that all these nucleosides have the α-configuration of the nucleobase, and that the preferred conformation of them in solution (NMR solvents) is the one depicted in their respective formulas of *Scheme 3*.

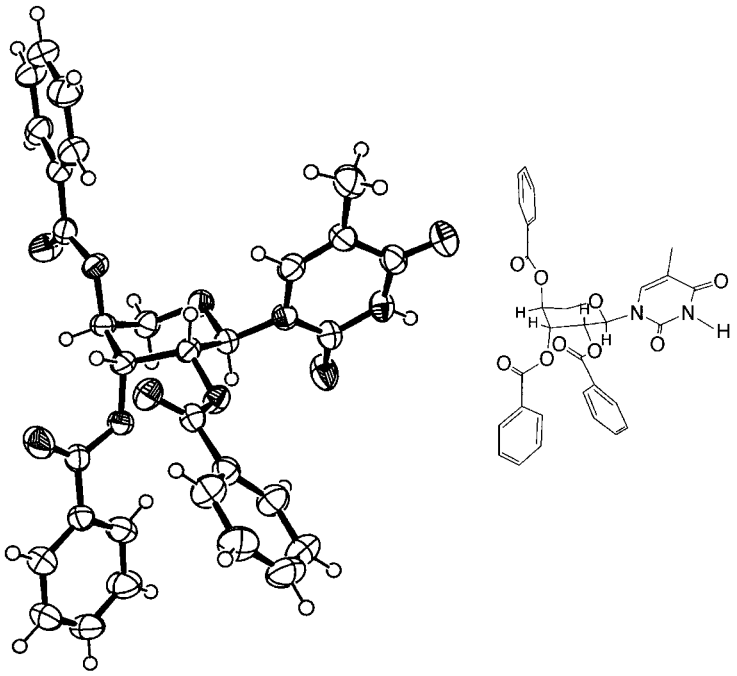


Fig. 1. *X-Ray structure of the 1-(2′,3′,4′-tri*-O-*benzoyl-α*-L-*lyxopyranos-1′-yl)thymine*, **2b**. Nucleosidic torsion angle O–C(1′)–N(1)–C(2): –91.1°[6]).

Regioselective protection of the 3′-OH and 4′-OH functions of **3a**–**3b** with the benzoyl- and the 4′,4″-dimethoxytrityl groups, respectively, was the major problem on the way to the synthesis of lyxopyranosyl-oligonucleotides. Whereas the choice of Bz as the protecting group for the 3′-OH was suggested by our previous experience in the

[6]) The X-ray analysis was carried by *Raj K. Chadha*, TSRI. Crystallographic data (excluding structural factors) for the structure reported in this paper has been deposited with the *Cambridge Crystallographic Data Center* as deposition No. CDCC 160498. 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).

Table 1. *J(H–C(1′), H–C(2′)) Values* [Hz] *for Selected Compounds Depicted in* Scheme 3

Compound	**a** (A)	**b** (T)	**c** (C)	**d** (G)
2	9.6 ($CDCl_3$)	9.8 ($CDCl_3$)	10.2 ((D_6)DMSO)	7.5 $CDCl_3$
3	9.6 ((D_6)DMSO)	9.8 (D_2O)	9.4 ((D_6)DMSO)	9.6 ((D_6)DMSO)
4	7.6	8.1	8.1	
(*exo*/*endo*)	8.3 ((D_6)DMSO)	8.5 ((D_6)DMSO)	8.4 ((D_6)DMSO)	
4b		9.7 ((D_6)DMSO)		
5	9.7 ($CDCl_3$)	9.6 ((D_6)DMSO)	9.4 ($CDCl_3$)	9.0 ($CDCl_3$)

p-RNA series [1][5], the method for its regioselective introduction was not, since the two series differ in the relative configurations of the 3′- and 4′-OH groups. A nearly ideal (and chemically appealing) solution[7]) to the problem was found in the application of a remarkable regioselectivity documented in the literature [18] referring to the acid-catalyzed ring opening of orthoesters implying vicinal *cis*-diol groups at pyranose chairs: mild treatment of such orthoesters with aqueous acid leads regioselectively to corresponding *cis*-diol monoesters with the ester function occupying the axial position.

Reaction of the mono nucleosides **3a** and **3b** with triethyl orthobenzoate and TsOH in DMF under anhydrous conditions at 30° under a vacuum of 20 Torr afforded the orthobenzoates **4a** and **4b** as *endo*/*exo*-mixtures in 64 and 74% yields (isolated). Successive removal of the EtOH formed during the reaction by working under vacuum was crucial for high conversion. It was also essential to perform the reaction at slightly elevated temperature, because when, for example, the reaction with **3a** was done at room temperature, formation of an acyclic diethyl-orthobenzoate derivative (presumably the 4′-isomer) was observed as main product. This acyclic orthoester could be converted to the cyclic orthoester **4a** in the presence of TsOH by increasing the temperature to 30°. Apparently, the 4′-OH group reacts to form first an acyclic orthoester, and, at slightly elevated temperatures, the *trans*-orthoesterification proceeds further to the more stable cyclic orthoester, involving the two *cis*-vicinal OH groups. Tritylation of **4a** and **4b** with dimethoxytrityl chloride and lutidine in CH_2Cl_2 at 0° proceeded in almost quantitative yield (as observed by TLC) to the corresponding 4′-*O*-tritylated orthoesters, which were opened to the 3′-*O*-benzoates **5a** and **5b** by addition of aqueous AcOH to the reaction mixture. It was important to perform the tritylations at low temperature, because, when the reaction was carried out at room temperature or higher, then the conversion of **4a** and **4b** remained incomplete. The trityloxy group is relatively stable under the conditions of the partial orthoester hydrolysis. Compounds **5a** and **5b** are obtained in 55 and 61% yield, respectively, over

7) Initiated and carried through by *F.R.* (*A.E.*)

both steps, tritylation followed by orthoester-hydrolysis being carried out as a convenient one-pot procedure.

In exploratory experiments in the thymine series, the orthoester **4b** (as a mixture of epimers) was originally opened before tritylation to give the crystalline 3′-*O*-benzoate **4bb** regioselectively in 70% yield. No isomeric 2′-*O*-benzoate was isolated[8]). However, tritylation of this derivative proved to be difficult, due to the substrate's low solubility in noncoordinating solvents that are suitable for the tritylation step (*e.g.*, CH_2Cl_2). Orthoesters **4a** and **4b**, on the other hand, are much more soluble in such solvents and, therefore, much more efficiently tritylated.

In the cytosine and guanine series, it was observed that formation of the orthoesters **4c** and **4d** suffered from low yields when triethyl orthobenzoate was used as the reagent. Therefore, the orthobenzoate reagent was replaced by trimethylorthoacetate (in the presence of TMS-Cl) according to observations published by other authors for the ribofuranosyl series [19][20]; it gave the orthoesters **4c** and **4d** in 74 and 66% yields, respectively. Tritylation, carried out as in the adenine and thymine series and followed by acid-catalyzed ring opening, afforded regioselectively the 3′-Ac derivatives **5c** and **5d**. In the chromatographic purification of these compounds on silica gel, caution was demanded to avoid partial ($3' \rightarrow 2'$) migration of the Ac groups during chromatography (see *Exper. Part*).

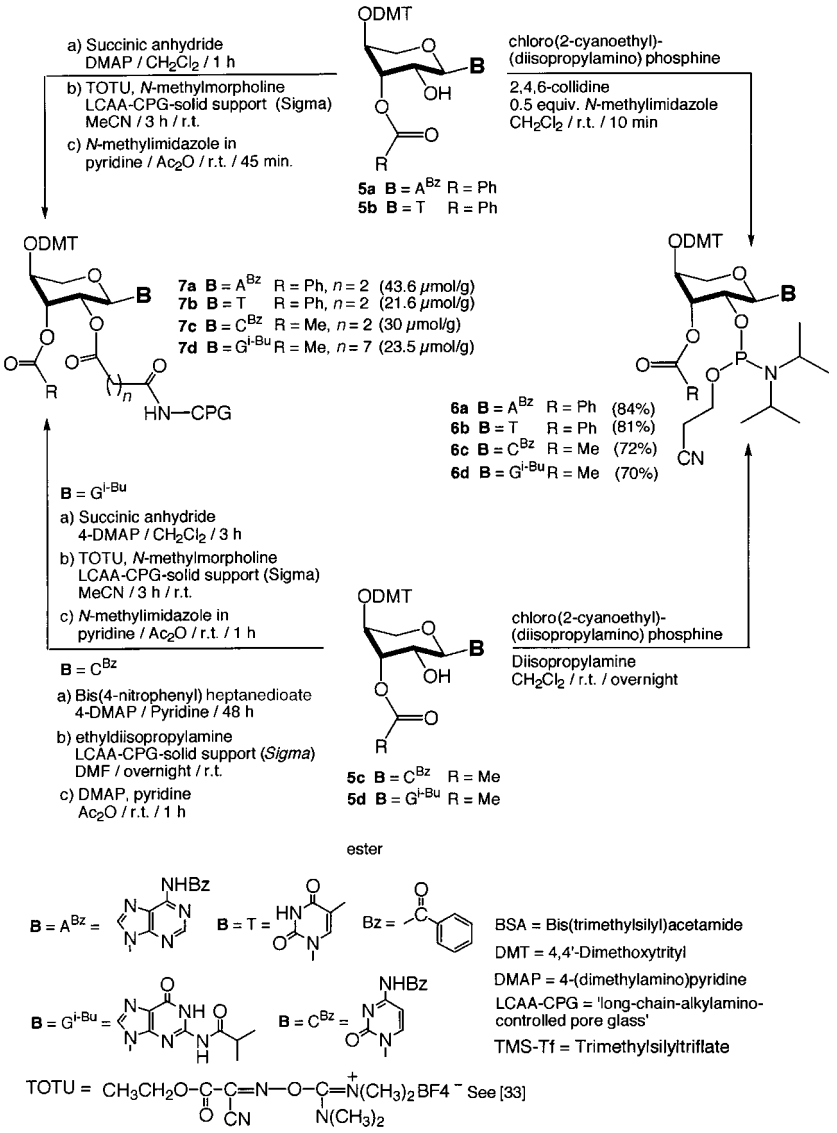
Phosphoroamidites **6a**–**6d** were synthesized with the 2-cyanoethyl moiety as the protecting group. Solid support ('long-chain alkylamine-CPG'; *Sigma*) derivatives **7a**–**7d** required for the automated synthesis of oligonucleotides were synthesized from intermediates **5a**–**5d** through standard methodology outlined in *Scheme 4*.

2.2. *Synthesis and Purification of Oligonucleotides.* The preparation of α-L-lyxopyranosyl-($4' \rightarrow 2'$)-oligonucleotides was carried out on a 1-μM scale as previously done in the homo-DNA [21] and p-RNA series [1][5] on automated DNA synthesizers (*Pharamacia*'s *Gene Assembler Plus* and *Perseptive*'s *Expedite*) with *ca.* 0.08M solution of phosphoroamidites **6a**–**6d** in MeCN and *ca.* 35 mg of solid support derivatives **7a**–**7d**. The syntheses were performed in the 'trityl-on' mode. The following modifications to the protocols used earlier were made: *a*) coupling of phosphoroamidites over a period of 16.7 min with 0.25–0.35M 5-(ethylthio)-1*H*-tetrazole in MeCN, and *b*) detritylation with 6% $Cl_2CHCOOH$ in 1,2-dichloroethane over a 3-min period. Coupling efficiencies ranged from 95 to greater than 99%.

Post-synthesis handling of the dried CPG-solid support included, as the first step, treatment with dry pyridine Et_3N 5:1 to induce the eliminative removal of the 2-cyanoethyl protecting group, thus converting the labile phosphotriester linkages to the stable phosphodiester linkages [22]. This step minimizes the strand scission that can occur in the deprotection steps under basic conditions. These conditions were – depending on the specific oligonucleotide sequence – either treatment with 25% aq. hydrazine hydrate at 4° (*Method A*, see *Table 2*), or with aq. $MeNH_2/NH_3$ at room temperature (*Method B*, *Table 2*), or with aq. $MeONH_2/NH_3$ at 4° (*Method C*, *Table 2*). All these methods led to the detachment of the oligonucleotides from the CPG-solid support with the concomitant deprotection of the acyloxy groups, as well as the acylamino groups of adenine, cytosine, and guanine. A special workup procedure after

[8]) For assignment of the constitution of **4bb**, see *Exper. Part.*

Scheme 4. *Preparation of the Phosphoroamidite and CPG Derivatives for the Automated Synthesis of α-L-Lyxopyranosyl-(4′→2′)-oligonucleotides*



deprotection was crucial, at least when deprotection was carried out by the hydrazine treatment[9]). The product mixture is diluted with 0.5M aq. $Et_3NH_2CO_3$ buffer and loaded over a *Waters Sepak-C18* cartridge, to remove the excess hydrazine. The use of 0.2–0.5M aq. $Et_3NH_2CO_3$ buffer greatly enhances the efficiency of separation when

[9]) Attempts to remove hydrazine by evaporation led to increased strand scission.

compared to the loading of the oligos on the *Sepak* cartridge with H_2O alone. This strategy is successful only with 'trityl-on' sequences[10]).

The crude oligonucleotides were detritylated with 80% aq. HCO_2H at room temperature, subsequently purified by ion-exchange HPLC (target purity 95%), desalted[11]) again, and stored at $-20°$. The concentrations of the purified oligonucleotide stock solutions were determined by UV at 260 nm (at *ca.* 80°), and the purified oligonucleotides checked for the correct mass by matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) mass spectrometry [23]. *Table 2* lists the oligonucleotides synthesized, purified, and controlled by MALDI-TOF MS.

Table 2. *HPLC and MS Data of α-L-Lyxopyranosyl-(4′→2′)- and Chimeric (p***r***-pl)-Oligonucleotides*

Base sequences **all (4′→2′)**	Deprotection[a]) *Method*	OD260 (yield)	Analytical HPLC *MonoQ*-Ion exchange[b]) gradient t_R [min]	MALDI-TOF MS[c])	
				$[M+H]^+$ (found)	$[M+H]^+$ (calc.)
pl(A_8)	*A*	49.0 (25%)	10–50% in 30 min/18.7	2572	2573
pl(T_8)	*A*	20.7 (14%)	20–80% in 30 min/24.8	2500	2500
pl(A_{12})	*A*	26.4 (10%)	10–50% in 30 min/25.5	3890	3889
pl(T_{12})	*B*	13.6 (15%)	0–100% in 30 min/26.3	3780	3780
pl(A_4T_4)	*A*	13.5 (7%)	20–80% in 30 min/18.6	2536	2534
pl(T_4A_4)	*A*	47.4 (28%)	20–80% in 30 min/18.2	2536	2534
pl$(AT)_4$	*A*	9.2 (4%)	20–80% in 30 min/18.3	2536	2536
pl$(TA)_4$	*A*	30.6 (17%)	20–80% in 30 min/18.6	2536	2535
pl(TATTTTAA)	*A*	36.2 (22%)	0–100% in 30 min/21.2	2526	2527
pl(TTAAAATA)	*A*	26.5 (15%)	0–100% in 30 min/18.7	2547	2545
pl(G_6)	*A*	2.1 (3%)	0–100% in 30 min/29.5	2009	2010
pl(C_6)	*A*	26.3 (43%)	0–100% in 30 min/12.4	1770	1770
pl(C_3G_3)	*A*	5.6 (10%)	0–100% in 30 min/22.4	1889	1889
pl(ATTCAGCG)	*C*	10.2 (13%)	0–100% in 30 min/21.5	2538	2538
pl(CGCTGAAT)	*C*	4.2 (5.9%)	0–100% in 30 min/21.0	2538	2538
pl(TATAAAAATAA)	*A*	14.5 (59%)	0–100% in 30 min/20.3	3529	3532
(p**r**-pl)$_4$(T_4A_4)[d])	*C*	2.7 (2%)	0–100% in 30 min/20.7	2536	2536
(p**l**-p**r**)$_4$(T_4A_4)	*A*	1.0 (1%)	0–100% in 30 min/19.7	2538	2536

[a]) *Method A:* 25% aq. NH_2NH_2 inH_2O at 4° for *ca.* 6.5 h; *Method B:* 40% aq. $MeNH_2$ conc. aq. NH_3 1 : 1 at r.t. for 6.5 h; *Method C:* 0.2M $MeONH_2 \cdot HCl$ in 25% aq. NH_3 and EtOH (3 : 1) at r.t. for *ca.* 6 h (66 h for G- and C-containing sequences). All oligonucleotides were purified by ion-exchange chromatography on *Mono Q HR 5/5* column (58 × 6.0 mm, *Pharmacia*); elution with 10 mM Na_2HPO_4 in H_2O and a linear gradient of 1M NaCl with a flow of 1 ml/min; followed by desalting on *Sepak* cartridges.

[b]) *MonoQ HR 5/5* column; elution with 10 mM Na_2HPO_4 in H_2O and a linear gradient of NaCl, pH ≈ 10.5, flow 1 ml/min; peak purity (260 nm) 95–99%.

[c]) Matrix: 2,4,6-trihydroxyacetophenone and ammonium citrate buffer.

[d]) Chimera: p**r**(T)-pl(T)-p**r**(T)-pl(T)-p**r**(A)-pl(A)-p**r**(A)-pl(A); p**r** = β-D-ribopyranosyl; pl = α-L-lyxopyranosyl.

[10]) When conducted with 'trityl-off' sequences, it was noticed that clean separation from hydrazine was not achieved as the oligos had a tendency to 'flow-through' with the initial loading to the *Sepak* cartridge.

[11]) This process gave high reproducibility of salt-free oligonucleotides as evidenced by clean spectra in MALDI-TOF mass spectrometry.

3. Base-Pairing Studies. – The pairing properties of (4′→2′)-α-L-lyxopyranosyl-oligonucleotides were characterized by methods with which we were familiar from our earlier studies in the homo-DNA [21] and p-RNA series [1][5–8]. These methods are temperature-dependent UV spectroscopy (for T_m-measurements [24]), concentration-dependent T_m-measurement by UV spectroscopy (for determination of thermodynamic data [25]), molar-ratio-dependent UV spectroscopy (for checking stoichiometry [26]), and temperature-dependent CD spectroscopy. All measurements were made in 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 otherwise stated.

Table 3. T_m *Values and Thermodynamic Data*

No.	Base sequence **all (4′→2′)**	T_m [°C][a]) Self-pairing of non-complementary single strands 1M NaCl ($c \approx 10$ μM)	T_m [°C][a]) **pl**(4′→2′) duplexes 1M NaCl ($c \approx 10$ μM)	T_m [°C][a]) **pl**(4′→2′) duplexes 150 mM NaCl ($c \approx 10$ μM)	For comparison: T_m's of **pr**(4′→2′) duplexes 150 mM NaCl ($c \approx 10$ μM)[a])	ΔG (25°)	ΔH	$T\Delta S$ (25°)	Ref.
						150 mM NaCl[a])[b]) **pl**(4′→2′) duplexes			
1	**pl**(A_8)	14.8*							
2	**pl**(T_8)	<5*							
3	**pl**(A_8) + **pl**(T_8)		51.0	47.0*	40*	−12.3*	−69.5*	−57.2*	[13]
4	**pl**(A_{12})	19.9 (16.4*)							
5	**pl**(T_{12})	16.2							
6	**pl**(A_{12}) + **pl**(T_{12})		74.3	68.0	60.8	−19.8	−97.1	−77.3	[14]
7	**pl**(A_4T_4)		41.1*	38.2*	27*	−9.4*	−60.7*	−51.3*	[13]
8	**pl**(T_4A_4)		49.6*	47.0*	40*	−11.4*	−67.0*	−53.6*	[13]
9	**pl**$(AT)_4$		44.7*	38.3*	38*	−9.5*	−61.4*	−52.0*	[13]
10	**pl**$(TA)_4$		43.2*	37.9*	40*	−9.4*	−62.9*	−53.5*	[13]
11	**pl**(TATTTTAA)	19.6*							
12	**pl**(TTAAAATA)	23.2*							
13	**pl**(TATTTTAA) + **pl**(TTAAAATA)		46.4*	41.8*	38.8	−10.6	−55.5	−44.9	[14]
14	**pl**(G_6) + **pl**(C_6)		52		61[5c]	−10.4	−33.3	−22.9	
15	**pl**(C_3G_3)		61		68*	−11.2‡	−42.1‡	−30.9‡	
16	**pl**(ATTCAGCG)	<0							
17	**pl**(CGCTGAAT)	9.9							
18	**pl**(ATTCAGCG) + **pl**(CGCTGAAT)		61.9	57.6	61.4	−13.0	−54.9	−41.9	[14]
19	**pl**(TATAAAAATAA) hairpin	36.5**				−1.2†	−31.7†	−30.5†	
20	(p**r**-p**l**)$_4$(T_4A_4)			41.8					
21	(p**l**-p**r**)$_4$(T_4A_4)			43.3					

[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 asterisk (*) were measured in 0.01M Tris-HCl buffer, pH 7.0; **: in 150 mM NaCl. [b]) Thermodynamic data from plots of T_m^{-1} *vs.* ln *c*; experimental error estimated in ΔH values ±5%. ‡: in 1M NaCl. †: Thermodynamic data calculated for hairpin according to [25] (p. 1610).

Table 3 summarizes our observation on the base-pairing capability of α-L-lyxopyranosyl-(4′→2′)-oligonucleotides, and *Fig. 2* and *3* give selected illustrations of it, such that they complement the Figures already reproduced in the preliminary communications [13][14].

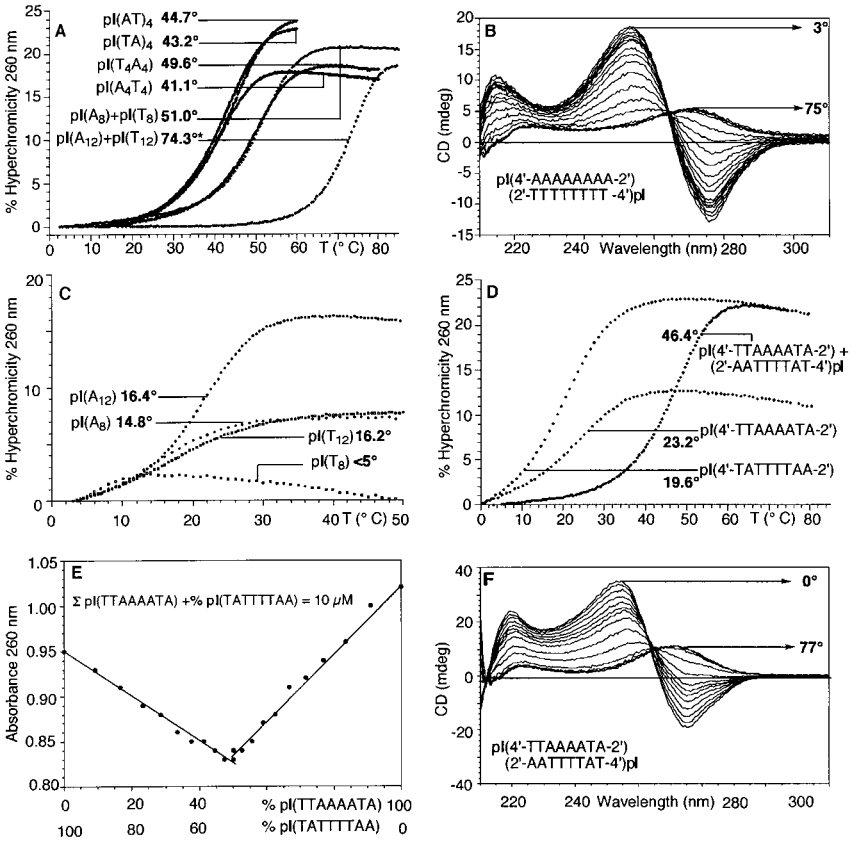


Fig. 2. *UV and CD data documenting the pairing behavior of A,T-containing (4′→2′)-α-L-lyxopyranosyl sequences. A*) UV-Spectroscopic T_m curves (heating) of selected duplexes. *B*) Temperature-dependent CD curves; temperature range: 3°→75°. *C*) Self-pairing of the homobasic strands pI(A_8), pI(A_{12}), pI(T_8), and pI(T_{12}) as demonstrated by UV-spectroscopic T_m curves (heating). *D*) UV-Spectroscopic T_m curves of the duplex formed by pI(4′-TTAAAATA-2′) with its antiparallel complement pI(4′-TATTTTAA-2′) and self-pairing T_m curves of the individual strands. *E*) Molar-ratio-dependance of UV absorption (260 nm, 0.3 ± 0.3° ('mixing curve') indicating 1:1 duplex stoichiometry for the pairing between pI(4′-TTAAAATA-2′) and its antiparallel complementary strand pI(4′-TATTTTAA-2′). *F*) Temperature-dependent CD curves of the duplex between pI(4′-TTAAAATA-2′) and its antiparallel complementary strand pI(4′-TATTTTAA-2′); temperature range: 0°→77°. T_m Measurements were made in 10 mM aq. *Tris*·HCl buffer, 1M NaCl at pH 7.0 except the one marked with an asterisk * (phosphate buffer). All CD and mixing curves were measured in 10 mM aq. NaH_2PO_4 containing 0.1 mM Na_2EDTA, 1M NaCl at pH 7.0. Total oligonucleotide concentrations in all measurements were *ca.* 10 μM. 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.

The tendency of lyxopyranosyl-(4′→2′)-oligonucleotides to undergo somewhat stronger base pairing than p-RNA is most clearly expressed by sequences that contain A and T only, and is also evident in the behavior of homobasic sequences like pI(A_8), pI(A_{12}), or pI(T_{12}) (see *Table 3*), which show weak self-pairing under conditions where the corresponding p-RNA sequences do not [1][5][14]. A series of sequence combinations (No. *7*, *8*, *13*, *15*, and *18* in *Table 3*) demonstrate the preferred strand

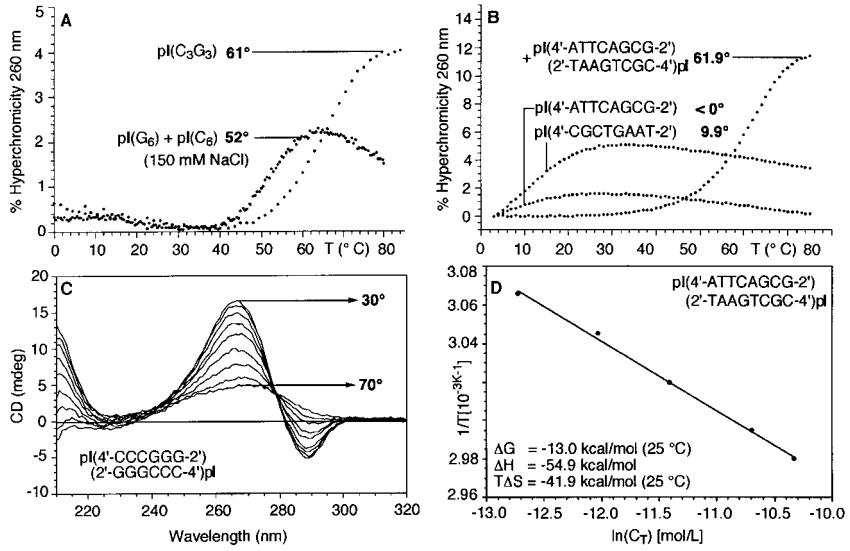


Fig. 3. *UV,* T_m*, CD, and thermodynamic data documenting the pairing behavior of G,C-containing (4′ → 2′)-α-*L*-lyxopyranosyl sequences. A*) UV-Spectroscopic T_m melting curves for self-pairing of pI(C_3G_3) and pairing between pI(G_6) and pI(C_6). *B*) UV-Spectroscopic T_m curves of the duplex formed between pI(4′-ATTCAGCG-2′) with its antiparallel complement pI(4′-CGCTGAAT-2′), and corresponding curves of individual strands. *C*) Temperature-dependent CD curves of self-complementary pI(C_3G_3) strand, temperature range: 30° → 70° (T_m = 55°). For conditions of measurements see caption of *Fig. 2*. T_m Curves fully reversible (no hysteresis). *D*) Thermodynamic data of the formation of the duplex shown, determined from concentration-dependence of T_m values (in 10 mM aq. NaH_2PO_4 containing 0.1 mM Na_2EDTA, 150 mM NaCl at pH 7.0). For method, see [25].

orientation in lyxopyranosyl-(4′ → 2′)-duplexes to be antiparallel, as it has been proven to be the case in the p-RNA series. Remarkable is the high efficiency by which antiparallel complementary base-sequences of the lyxo- and ribopyranosyl-sequences undergo inter-system cross-pairing with each other (*Table 4*). This cross-pairing is, at the same time, strong evidence for the correctness of the postulate that the base pairing in the lyxopyranosyl-(4′ → 2′)-oligonucleotide series proceeds by the *Watson-Crick* mode, as it is the case for the ribopyranosyl series, where it has been proven by a comprehensive NMR-structure analysis of a p-RNA duplex [5b].

The efficient intersystem cross-pairing between the lyxopyranosyl- and the ribopyranosyl-(4′ → 2′)-system makes it very probable that pairing should also occur between chimera strands, where both lyxo- and ribopyranosyl-nucleoside building blocks occur in a given strand, while the two strands still possess antiparallel complementary base-sequences (*Fig. 4*). We have made two such examples and have found that their pairing behavior is as expected (Nos. *20* and *21* in *Table 3*).

The sequence pI(TATAAAAATAA) (No. *19*) has been constructed in order to test the lyxopyranosyl system's capability to form hairpins. It has previously been shown that pyranosyl-RNA does form hairpins with surprising ease (considering the presumed nonflexibility of a pyranosyl ring relative to a furanosyl ring) [6]. Sequence No. *19* does from a hairpin that is remarkably stable (see *Fig. 5*), given that it contains three A-T

Table 4. *T_m Values of Duplexes Formed by Cross-Pairing between α-L-Lyxopyranosyl* (**pl**) *and β-D-Ribopyranosyl* (**pr**)*-oligonucleotides*[a])

		pr	pl	
self-pairing		-	15	I
		(5)	20	II
		15	23	III
		6	-	IV
-	pr	46	31	I
-		68	52	II
22		46	30	III
30		68	57	IV
(5)	pl	43	51	I
16		66	74	II
20		40	46	III
10		55	62	IV

I 4'-AAAAAAAA-2' / 2'-TTTTTTTT-4'

II 4'-AAAAAAAAAAAA-2' / 2'-TTTTTTTTTTTT-4'

III 4'-TTAAAATA-2' / 2'-AATTTTAT-4'

IV 4'-ATTCAGCG-2' / 2'-TAAGTCGC-4'

[a]) Conditions: $c = 5 + 5$ μM, 1.0M NaCl, 10 mM NaH_2PO_4, 0.1 mM Na_2EDTA, pH 7.0. The color of the symbols relates to the oligonucleotide sequences of the same color in the formulas of duplex **I – IV** given at the side of the table. T_m Values in black refer to intersystem cross-pairing and those in the shaded diagonal to intrasystem pairing; T_m values in color refer to self-pairing of corresponding strands.

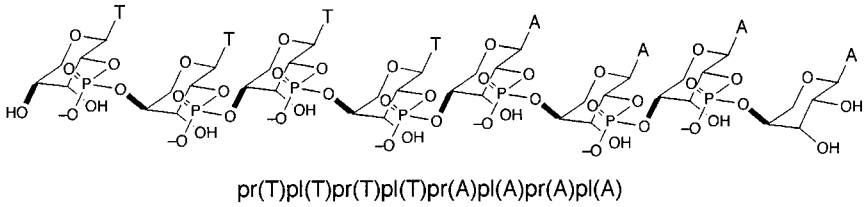


Fig. 4. *Idealized pairing conformation of a chimeric oligonucleotide containing alternating β-D-ribo-(4′ → 2′)-* and *α-L-lyxo-(4′ → 2′)-pyranosyl units* (No. *20* in *Table 3*)

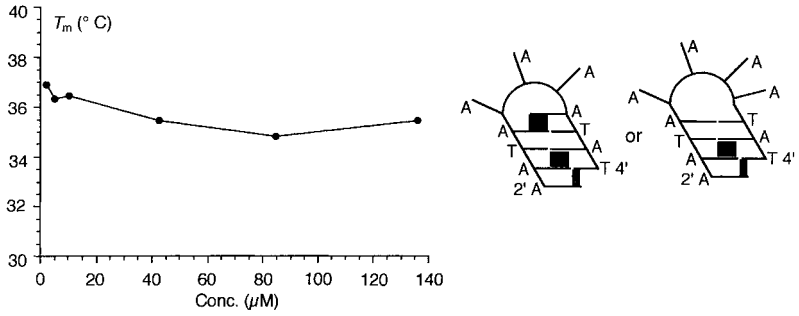


Fig. 5. *Invariance of T_m with variation of oligonucleotide concentration of hairpin-forming sequence pl(4′-TATAAAAATAA-2′)* (10 mM aq. NaH_2PO_4 containing 0.1 mM Na_2EDTA, 150 mM NaCl at pH 7.0). Hairpin formulae indicate two possible 'interstrand' base-stacking patterns.

base pairs only. This seems to be still another example of a characteristic property of pentopyranosyl-(4′ → 2′)-oligonucleotides, namely, that their base-pairing draws much of its strength from interstrand-base-stacking [27][28]. The surprisingly strong self-pairing of the two complementary, but not self-complementary, strands No. *11* and *12* (see also *Fig. 2*,*D* and *Fig. 6*) is quite probably another illustration of this relationship.

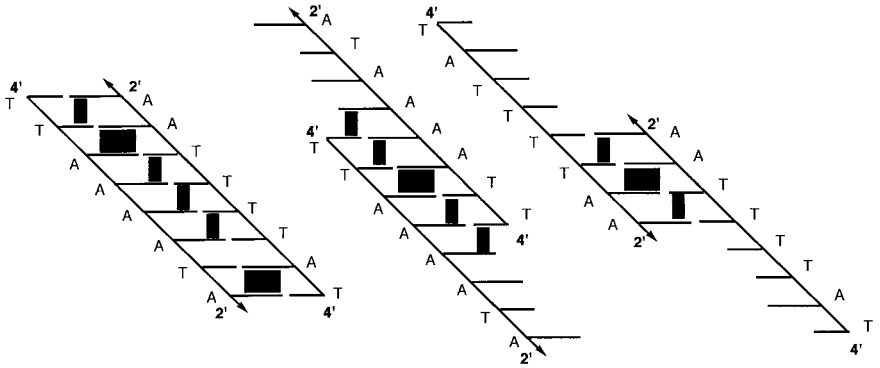


Fig. 6. *Representation of the postulated interstrand base-stacking in the duplex formed by pl(4′-TTAAAATA-2′) with its antiparallel complement pl(4′-TATTTTAA-2′), and in duplexes formed by the self-pairing of the (not self-complementary) individual strands*

4. Discussion. – In an early comparative analysis of the (then) hypothetical base-pairing behavior of pentopyranosyl-(4′ → 2′)-oligonucleotide systems based on considerations of idealized single-strand pairing conformations, we hypothesized that lyxopyranosyl oligonucleotides will show base pairing, but one that should be weaker than that of pyranosyl-RNA [5a]. Experimental findings qualified this conjecture: α-L-lyxopyranosyl-(4′ → 2′)-oligonucleotides do, in fact, show *Watson-Crick* pairing, but it is – at least with respect to adenine-thymine pairing – tendentially stronger than that of corresponding oligonucleotides of the pyranosyl-RNA series. In fact, the investigation of the lyxopyranosyl system was the first in a series of experimental studies that uncovered the limitations of our originally applied criteria for predicting base-pairing properties [5a][29]. In the lyxopyranosyl case, a pairing weaker than p-RNA had been expected on the basis of the argument that the backbone/base-pair axes inclination [5b][27] of the lyxo-system is less than that of the ribo-system (see *Fig. 7*) and, therefore, that the stacking distance between neighboring base pairs – too large already in the (idealized) pairing conformation of p-RNA – will be even larger in the (idealized) lyxopyranosyl system (*ca.* 5.5 Å). Implicit in this reasoning was the assumption that shortening the stacking distance towards the optimal range between 3.5 and 4.0 Å by either increasing the backbone/base-pair-axes inclination, or by helicalization of the duplex strands, would induce strain within the backbone, as a consequence of which pairing strength would decrease. In the meantime, an NMR-structure analysis of a p-RNA duplex has revealed that such duplexes, indeed, have optimal base-stacking distances, as well as a slightly twisted (left-handed) helical shape [5b]. Since then, it has become clear that considerations on idealized pairing conformations deduced by qualitative conformational analysis of repeating oligonu-

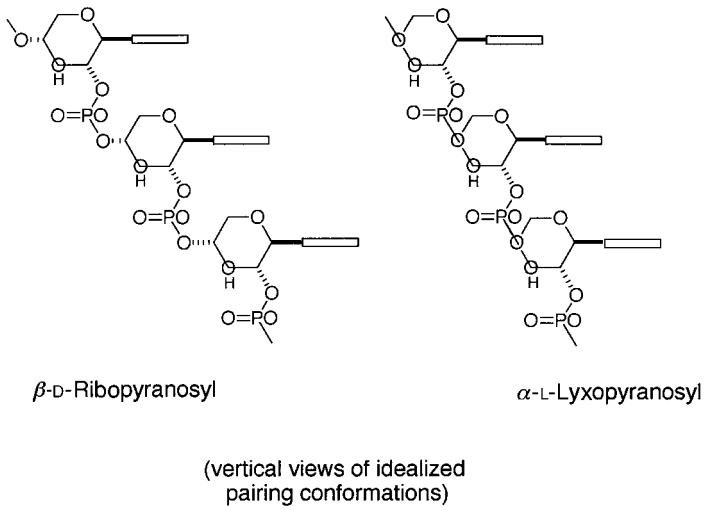


Fig. 7. *Vertical projection formulae of the idealized pairing conformation* (nucleosidic torsion angles $-120°$) *of the ribo- and lyxo-pyranosyl-(4′ → 2′)-oligonucleotide systems, pointing to the difference in the two systems' backbone/base-pair-axes inclination.* See also [5b][27].

cleotide units [5a][29] are useful for making qualitative predictions about the base-pairing capability as such, but are too simplistic for predicting relative base-pairing strengths as a function of configurational and conformational differences in pentopyranosyl-oligonucleotide backbones.

In retrospect, and in light of the presently available knowledge on the structure of pentopyranosyl-(4′ → 2′)-oligonucleotide duplexes [5b][30], we think that is a specific type of steric hindrance occurring in duplexes of pentopyranosyl-(4′ → 2′)-oligonucleotides that acts as a major determinant of the relative base-pairing strengths of diastereoisomeric members in the series. This type of steric hindrance refers to the spatial closeness of the equatorial H-atom at C(5′) of a pyranose chairs to the plane of the neighboring nucleobase situated upstream in the same strand (*Fig. 8*). Provided that this closeness acts indeed as a source of repulsion in p-RNA duplexes (phosphodiester bridges diequatorial), then such repulsion would be expected to be less important in lyxopyranosyl duplexes: phosphodiester bridges that are axially attached to C(4′) of the pyranose chairs will put the critical H-atom at C(5′) farther apart from the plane of its neighboring nucleobase than is the case in corresponding p-RNA duplexes. The difference has recently been substantiated by *Jaun* and *Ebert* [30] – not in the lyxopyranosyl, but the arabinopyranosyl series – by an NMR-structure analysis of the α-L-arabinopyranosyl-(4′ → 2′)-$(CGAATTCG)_2$ duplex, allowing a comparison of the structural environment of the equatorial H-atoms at C(5′) in duplexes with an axial-equatorial *vs.* a diequatorial C(4′)-phosphodiester group. The equatorial H-atoms at C(5′) of pyranosyl-RNA duplexes are found to be distinctly more deshielded in their chemical shifts by upstream-nucleobase neighbors than corresponding H-atoms at C(5′) of arabinopyranosyl-duplexes (*Fig. 9*). We conjecture that a corresponding difference in intrastrand steric repulsion between neighboring

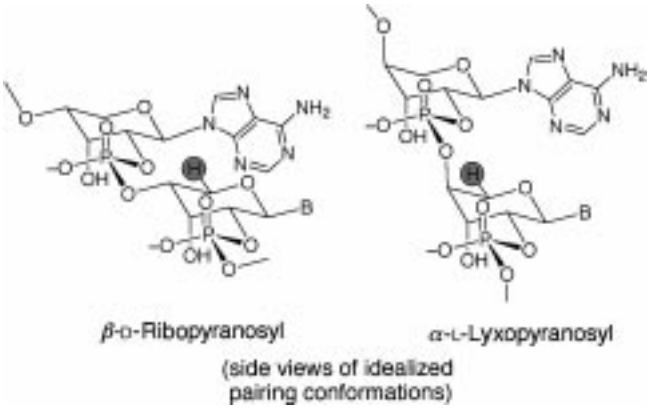


Fig. 8. *Side view of the idealized pairing conformation of the two diastereoisomeric (4′ → 2′)-pentopyranosyl oligonucleotide systems, pointing to the difference in the steric hindrance between the equatorial hydrogen at C(5′) of the pyranose chair and the neighboring (upstream) nucleobase*

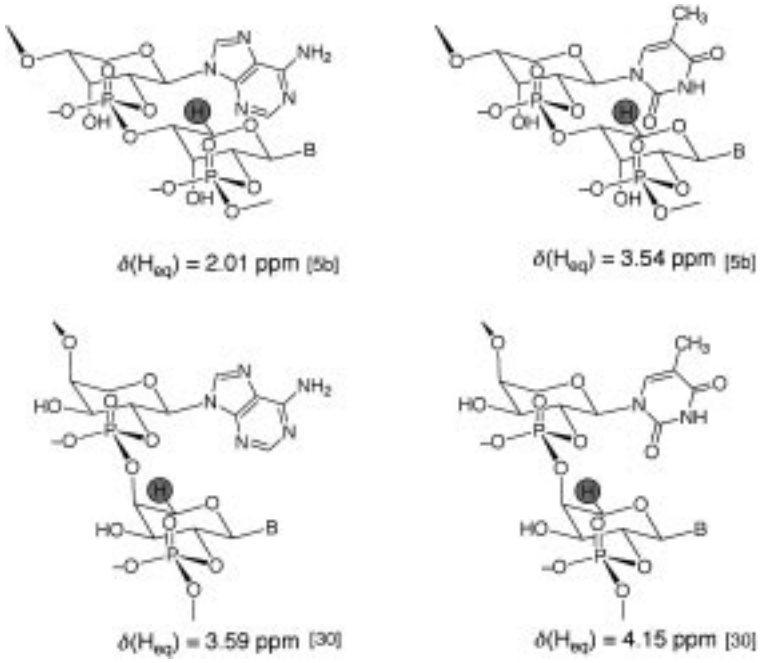


Fig. 9. *^{1}H-NMR Chemical shifts of the equatorial H-atoms at C(5′) in the (4′-CGAATTCG-2′)$_2$ duplexes of the β-*D*-ribopyranosyl-(4′ → 2′)- and the α-*L*-arabinopyranosyl-(4′ → 2′)-series* (data taken from *Jaun* and co-workers [5a][30])

nucleoside units in lyxopyranosyl duplexes may be a significant factor in rendering them tendentially more stable than corresponding p-RNA duplexes.

The properties of α-L-lyxopyranosyl-(4′ → 2′)-oligonucleotides have provided the first of a series of lessons that the systematic investigation of the pentopyranosyl-(4′ →

2′)-oligonucleotide family has given us. A more drastic correction of our earlier predictions on relative base-pairing strength of oligonucleotide systems had come from studying the α-L-arabinopyranosyl-(4′→2′)-system, which we will describe in a forthcoming paper of this series. On the other hand, it was the work in the lyxopyranosyl series that turned out to exert the most incisive influence – *via* a study of the α-L-lyxopyranosyl-(4′→3′)-oligonucleotide system [28][31] – on the further course of our investigation in the field of nucleic acid etiology [32].

The work was supported by the *Skaggs Foundation (TSRI)* and *Novartis AG*, Basel. We thank Professor *Bernhard Jaun* (ETH) for allowing us to include in this paper unpublished data from his laboratory. We thank Dr. *A. Shyvaniuk* for help in the ORTEP drawing of **2b**.

Experimental Part

General. Solvents for extraction: technical grade, distilled. Solvents for reaction: reagent grade. Reagents: unless otherwise noted, from *Acros*, *Fluka*, or *Aldrich*, highest quality available. Chloro(2-cyanoethyoxy)(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. of H_2SO_4/H_2O/EtOH 14:4:1, 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* (40–63 μ, 230–440 mesh, *EM Science*) at low pressure (max. 2 bar). In case of acid-sensitive compounds, the silica gel was pre-treated with appropriate solvents containing *ca*. 0.5% Et_3N. M.p. (uncorrected): *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 on a *VG ZAB-VSE* double focusing high-resolution mass spectrometer equipped with a Cs^+ 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 as the matrix with ammonium citrate added to the sample. Elemental analyses were performed with a *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: Anion exchange (IA)-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*); 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. UV Spectra were measured on a *Cary 1 C* spectrophotometer (*Varian*) and a *Perkin-Elmer Lambda 2*. Melting-points (T_m) of oligonucleotides were determined with *Cary 1 Bio* spectrophotometer (*Varian*) or with *Perkin-Elmer Lambda 2* equipped with *Perkin-Elmer Digital Controller/Temperature Programmer C570*. 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 (pr(A)) = ε (pl(A)) = 15000, ε (pr(T)) = ε (pl(T)) = 10000, ε (pr(C)) = ε (pl(C)) = 8400, ε (pr(G)) = ε (pl(G)) = 11900. CD Spectra were measured on *A*) *JASCO J-710 or B*) *AVIV 61 DS* CD spectropolarimeter. *Abbreviations:* BSA: *N*,*O*-Bis(trimethylsilyl)acetamide; CPG: 'controlled pore glass', DMAP: 4-(dimethylamino)pyridine; DMT: 4,4′-dimethoxytrityl; LCAA-CPG: long-chain aminoalkyl-CPG (500 Å); TMS-Tf: trimethylsilyl trifluoromethanesulfonate; TOTU: *O*-{[(2-cycanoethoxycarbonyl)methyliden]amino}-1,1,3,3-tetramethyluronium tetrafluoroborate.

1. ***Experiments Referring to*** **Scheme 3**. – *1′,2′,3′,4′-Tetra*-O-*benzoyl-α/β*-L-*lyxopyranose* (**1**). To an ice-cold stirred soln. of 78 ml (665.8 mmol) BzCl and 130 ml (1.6 mol) pyridine in 400 ml of dry CH_2Cl_2 were added 20.0 g (131.9 mmol) of (+)-L-lyxose in small portions within 30 min. The mixture was stirred for another 2 h, and a colorless substance precipitated. After addition of 150 ml of H_2O, the mixture was washed successively with 20 ml of 1M aq. HCl, 18 ml of 2-aminoethanol, 50 ml of H_2O, 20 ml of 1M aq. HCl, 20 ml sat. aq. $NaHCO_3$ soln., and 50 ml of H_2O. The org. phase was dried ($MgSO_4$) and evaporated. The residual oil was dissolved in CH_2Cl_2, followed by co-evaporation with toluene. The slightly yellowish foam was dried *in vacuo* (*ca*. 0.5 Torr r.t.) for several hours to afford 68.5 g (91.7%) of product. It was clean by TLC and ^{1}H-NMR, and was used in subsequent reactions without further purification. Crystallization of a small amount from toluene gave analytically pure **1**.

M.p. 136–137°. TLC (petroleum ether/acetone 3:1): R_f 0.55. ^{1}H-NMR (600 MHz, $CDCl_3$): 4.11 (*dd*, $J(4',5'b) < 0.5$, $J_{gem} = 11.6$, $H_\beta - C(5')$); 4.40 (*dd*, $J(4',5'\alpha) = 5.2$, $J_{gem} = 11.6$, $H_\alpha - C(5')$); 5.85 (*m*, H–C(4')); 5.93 (*m*, H–C(2')); 6.11 (*dd*, $J(2',3') = 3.5$, $J(3',4') = 9.2$, H–C(3')); 6.57 (*d*, $J(1',2') = 2.9$, H–C(1')); 7.35–7.70 (*m*, 12 arom. H); 7.92–8.22 (*m*, 8 arom. H). ^{13}C-NMR (150.9 MHz, $CDCl_3$): 62.70 (*t*, C(5')); 67.81 (*d*, C(4')); 69.56 (*d*, C(3')); 69.62 (*d*, C(2')); 91.88 (*d*, C(1')); 128.93, 128.97, 129.09, 129.18 (4*d*, arom. C); 129.41 (*s*, arom. C); 130.23, 130.31, 130.45, 130.57 (4*d*, arom. C); 133.96, 134.04, 134.17, 134.44 (4*s*, arom. C); 164.74, 165.79, 166.04, 166.15 (4*s*, CO). ES-MS (pos.): 589 (24.8, $[M + Na]^+$), 445 (29.7, $[M - PhCOO]^+$), 381 (9.9), 360 (17.5), 304 (52.5), 282 (100.0).

N^6-*Benzoyl-9-(2',3',4'-tri*-O-*benzoyl-α*-L-*lyxopyranosyl)adenine* (**2a**). A suspension of 40 mg (70.6 mmol) of **1** and 17.0 g (71.1 mmol) of 6-benzoyladenine in 375 ml of dry MeCN was heated to 80° (oil bath). Addition of 32 ml (125.6 mmol) of BSA resulted in a clear soln. After 15 min, 26.3 ml (222.5 mmol) of $SnCl_4$ was added dropwise (→ exothermic reaction), the soln. became dark, and stirring was continued for another 30 min. The soln. was cooled to r.t. and poured into a mixture of cold sat. aq. $NaHCO_3$ soln./AcOEt 1:1 (*v*/*v*) with stirring. The aq. phase was extracted with 3×150 ml AcOEt. The org. phase was dried ($MgSO_4$) and evaporated. Purification of the residual oil was accomplished by CC (silica gel; petroleum ether/AcOEt 1:1 to 1:2 and finally 1:3). The product fractions were combined, evaporated, and dried *in vacuo* (*ca.* 0.5 Torr r.t.) to furnish 28.7 g (59.5%) of a colorless amorphous solid, **2a**. TLC (petroleum ether/acetone 2:1): R_f 0.40. ^{1}H-NMR (600 MHz, $CDCl_3$): 4.46 (*d*, $J_{gem} = 13.5$, H–C(5')); 4.55 (*d*, $J_{gem} = 13.5$, H–C(5')); 5.48 (*d*, $J(3',4') = 3.7$, H–C(4')); 6.15 (*m*, H–C(3')); 6.29 (*dd*, $J(2',3') = 3.2$, $J(1',2') = 9.6$, H–C(2')); 6.50 (*d*, $J(1',2') = 9.6$, H–C(1')); 7.20–8.33 (*m*, 20 arom. H); 8.33, 8.83 (2*s*, H–C(2), H–C(8)); 9.12 (*s*, NH). ^{13}C-NMR (150.9 MHz, $CDCl_3$): 66.81 (*t*, C(5')); 68.56 (*d*, C(2')); 68.69 (*d*, C(3')); 69.31 (*d*, C(4')); 79.48 (*d*, C(1')); 122.93 (*s*, arom. C); 128.28 (*d*, arom. C); 128.44 (*s*, arom. C); 128.86 (*d*, arom. C); 129.18 (*s*, arom. C); 129.23, 129.26, 129.33 (3*d*, arom. C); 129.38 (*s*, arom. C); 130.13, 130.39, 130.50, 133.23 (4*d*, arom. C); 134.04 (*s*, arom. C); 134.19, 134.41 (2*d*, arom. C); 134.51 (*s*, arom. C); 141.24, 153.65 (2*d*, C(2), C(8)); 150.16, 152.40 (2*s*, arom. C), 165.10, 165.21, 165.26, 165.65 (4*s*, CO). FAB-MS (pos., NBA/CsI): 948 (27.0, $[M + 2\ Cs]^+$), 816.1040 (100.0, $[M + Cs]^+$), 684 (9.1, $[M + H]^+$).

1-(2',3',4'-Tri-O-*benzoyl-α*-L-*lyxopyranosyl)thymine* (**2b**). A suspension of 15.84 g (28.0 mmol) of **1** and 4.20 g (33.0 mmol) of dry thymine in 170 ml of dry MeCN was heated to 65° (oil bath), followed by the addition of 18.5 ml (72.6 mmol) of BSA. After 40 min, 15.2 ml (83.1 mmol) of TMS-Tf were added by syringe to the clear soln., and was kept overnight at 65°. The soln. was cooled to r.t. and poured into *ca.* 1 l of an ice-cold mixture of sat. aq. $NaHCO_3$ soln./AcOEt 1:1 (*v*/*v*) with stirring. The org. phase was washed with sat. aq. $NaHCO_3$ soln. and dried (Na_2SO_4). Precipitation by addition of *ca.* 100 ml EtOH afforded 10.3 g (64.5%) of an amorphous product. A small amount was crystallized from acetone to give **2b**. M.p. 194°. TLC (petroleum ether/AcOEt 1:1): R_f 0.50. ^{1}H-NMR (600 MHz, $CDCl_3$): 1.94 (*s*, Me–C(5)); 4.39 (*d*, $J_{gem} = 13.4$, H–C(5')); 4.41 (*d*, $J_{gem} = 13.4$, H–C(5')); 5.37 (*m*, H–C(4')); 5.73 (*dd*, $J(2',3') = 3.2$, $J(1',2') = 9.8$, H–C(2')); 6.05 (*m*, H–C(3')); 6.50 (*d*, $J(1',2') = 9.8$, H–C(1')); 7.10–8.15 (*m*, 15 arom. H, H–C(6)); 9.56 (br. *s*, NH). ^{13}C-NMR (150.9 MHz, $CDCl_3$): 13.06 (*q*, *Me*–C(5)); 67.04 (*t*, C(5')); 67.71 (*d*, C(2')); 69.13 (*d*, C(3')); 69.65 (*d*, C(4')); 78.78 (*d*, C(1')); 112.41 (*s*, C(5)); 128.62 (*s*, arom. C); 128.97 (*d*, arom. C); 129.13 (*s*, arom. C); 129.18, 129.23 (2*d*, arom. C); 129.40 (*s*, arom. C); 130.27, 130.33 (2*d*, arom. C); 134.18, 134.32, 134.34 (3*d*, arom. C); 134.92 (*d*, C(6)); 150.87 (*s*, C(2)); 163.78 (*s*, C(4)); 165.21, 165.44, 165.52 (3*s*, CO). FAB-MS (pos., NBA/CsI): 703.0669 (17.6, $[M + Cs]^+$). Anal. calc. for $C_{31}H_{26}N_2O_9$: C 65.26, H 4.59, N 4.91, found: C 65.32, H 4.76, N 5.05.

N^4-*Benzoyl-1-(2',3',4'-tri*-O-*benzoyl-α*-L-*lyxopyranosyl)cytosine* (**2c**). The reaction was performed as described for **2a**. In one of the experiments, 4.19 g (19.2 mmol) of *N*^{4}-benzoylcytosine, 9.1 g (16.1 mmol) of **1**, and 9.7 ml (40 mmol) of BSA in 100 ml of dry MeCN followed by addition of 6.1 ml (52 mmol) of $SnCl_4$. Following the workup described for **2a** and purification by chromatography (silica gel; AcOEt/hexane 1:1 to 2:1) afforded 9.4 g (90%) of **2c**. M.p. 118–120°. TLC (petroleum ether/AcOEt 7:3): R_f 0.45. ^{1}H-NMR (200 MHz, (D_6)DMSO): 4.45 (br. *s*, 2 H–C(5')); 5.39 (br. *d*, $J(3',4') = 3.6$, H–C(4')); 5.74 (*dd*, $J(2',3') = 3.4$, $J(1',2') = 10.2$, H–C(2')); 6.05 (*dd*, $J(2',3') = 3.4$, $J(3',4') = 3.6$, H–C(3')); 6.75 (*d*, $J(1',2') = 10.2$, H–C(1')); 7.25–8.23 (*m*, 20 arom. H, H–C(5), H–C(6)).

N^2-*Isobutyryl-9-(2',3',4'-tri*-O-*benzoyl-α*-L-*lyxopyranosyl)guanine* (**2d**) *and* N^2-*Isobutyryl-7-(2',3',4'-tri*-O-*benzoyl-α*-L-*lyxopyranosyl)guanine* (**2e**). To a suspension of 715 mg (3 mmol) of *N*^{2}-isobutrylguanine monohydrate and 1.13 g (2 mmol) of **1** in 20 ml of dry 1,2-dichloroethane, 3.75 ml (15 mmol) of BSA were added, and the mixture was refluxed for 1 h. During heating, the suspension became a clear soln., 1.5 ml (6.5 mmol) of TMS-triflate were added by a syringe, and the soln. was refluxed for an additional 20 h. The mixture was cooled, solid $NaHCO_3$ was added, and the soln. was stirred for 10 min. To this suspension, a mixture of H_2O and CH_2Cl_2 was added, and the org. phase washed twice with H_2O and dried (Na_2SO_4). The org. phase

was filtered and evaporated *in vacuo* to dryness. The solid residue was dissolved in AcOEt, and hexane was added carefully until the soln. became turbid. The N^7-isomer crystallized from the soln. to give 0.3 g (22%) of **2e**. The mother liquor was decanted and purified by CC (silica gel; CH_2Cl_2/MeOH 100:0 to 95:5 (*v*/*v*)) to afford 0.76 g (58%) of **2d**.

Data of **2d**: TLC (petroleum ether/AcOEt 7:3): R_f 0.16. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.21, 1.24 (*d*, J = 7.9, Me); 2.69 (*m*, J = 7.9, Me_2CH); 4.21 (br. *d*, J_{gem} = 11.2, H–C(5′)); 4.46 (br. *d*, J_{gem} = 11.2, H–C(5′)); 5.54 (*m*, H–C(4′)); 6.12 (*d*, J(1′,2′) = 7.5, H–C(1′)); 6.32 (*dd*, J(2′,3′) = 3.2, J(3′,4′) = 3.4, H–C(3′)); 6.42 (*dd*, J(1′,2′) = 7.5, J(2′,3′) = 3.2, H–C(2′)); 7.31–8.13 (*m*, 15 arom. H, H–C(8)); 9.15, 12.1 (*s*, NH). ^{13}C-NMR (50.3 MHz, $CDCl_3$): 18.93, 19.00 (*q*, Me_2CH); 36.76 (*d*, Me_2CH); 65.41, 68.24, 68.74, 69.28 (C(5′), C(2′), C(3′), C(4′)); 81.40 (*d*, C(1′)); 128.80, 128.93, 129.01, 129.21, 129.28, 130.01, 130.16, 130.20, 134.10, 134.22 (arom. C), 138.23 (C(5)); 147.92 (C(4)); 155.59 (C(6)); 165.40, 165.41, 165.60 (3*s*, CO); 178.48 (*s*, CO^iBu). FAB-MS (pos., NBA): 666 (100, $[M+H]^+$).

Data of **2e**: TLC (petroleum ether/AcOEt 7:3): R_f 0.25. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.14, 1.17 (*d*, J = 6.9, Me); 2.92 (*m*, J = 6.9, Me_2CH); 4.44–4.51 (br. *m*, 2 H–C(5′)); 5.46 (br. *d*, J(3′,4′) = 3.8, H–C(4′)); 6.04 (*m*, H–C(2′)); 6.08 (*m*, H–C(3′)); 6.83 (*d*, J(1′,2′) = 9.5, H–C(1′)); 7.22–8.26 (*m*, 15 arom. H, H–C(8)); 10.23, 12.30 (*s*, NH). ^{13}C-NMR (50.3 MHz, $CDCl_3$): 18.47, 18.66 (*q*, Me_2CH); 35.52 (*d*, Me_2CH); 66.44, 68.38, 68.72, 69.01 (C(5′), C(2′), C(3′), C(4′)); 80.60 (*d*, C(1′)); 111.61, 127.74, 128.06, 128.37, 128.47, 129.30, 129.98, 133.23, 133.45, 133.58 (arom. C), 141.01 (C(5)); 147.77 (C(4)); 152.31 (C(2)); 156.15 (C(6)); 164.22, 164.53, 164.81 (3*s*, CO); 179.48 (*s*, CO^iBu).

Isomerization of **2e** *to a Mixture of* **2d**/**2e**. To a suspension of 2.2 g (3.27 mmol) of **2e** and 389 mg (1.64 mmol) of N^2-isobutylguanine monohydrate in 40 ml of $(CH_2Cl)_2$, 1.25 ml (5 mmol) of BSA was added. Under Ar, the suspension was heated under reflux for *ca*. 1 h (until all material was dissolved). To that refluxing soln., 2.0 ml (8.2 mmol) of TMS-Tf was added by syringe, and the soln. was heated for additional 20 h. The cooled soln. was poured into sat. aq. $NaHCO_3$ soln. and diluted with CH_2Cl_2. The phases were separated, and the org. layer was washed for a second time with sat. aq. $NaHCO_3$ soln. and dried ($MgSO_4$). The soln. was filtered, evaporated under vacuum, and subjected to CC (CH_2Cl_2/hexane 6:4 with 2–10% MeOH) to afford 1.36 g (62%) of **2d** and 0.65 g (30%) of **2e**.

N^6-*Benzoyl-9-(α-*L*-lyxopyranosyl)adenine* (**3a**). A soln. of 28.3 g (41.4 mmol) of **2a** in 425 ml of THF and 250 ml of H_2O was cooled to 0°, and 320 ml of 15% aq. NaOH soln. were added dropwise over a period of 90 min. To keep the soln. homogenous, H_2O was added dropwise. The pH of the resulting soln. was adjusted to 6–7 (checked by *Merck* universal indicator paper) by dropwise addition of conc. aq. HCl. Subsequently, THF was removed under vacuum (*ca*. 20 Torr r.t.), and the pH was adjusted to 2–3 with conc. aq. HCl. The aq. phase was extracted twice with 100 ml of Et_2O, and the pH of the aq. phase was brought to 7 by dropwise addition of 15% aq. NaOH soln. Concentration of the soln. to *ca*. 800 ml and cooling to 4° gave (after 10 days) 13.45 g (87.4%) of **3a** as colorless crystals. M.p. 195°. TLC (petroleum ether/AcOEt 1:1): R_f 0.18. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.68 (*d*, J(3′,OH) = 3.3, H–C(3′)); 3.71 (*d*, J_{gem} = 11.6, H–C(5′)); 3.99 (*m*, H–C(4′)); 4.01 (*d*, J_{gem} = 11.6, H–C(5′)); 4.51 (*dd*, J(2′,3′) = 2.6, J(1′,2′) = 9.6, H–C(2′)); 5.18–5.40 (3 br. *s*, 3 OH); 5.84 (*d*, J(1′,2′) = 9.6, H–C(1′)); 7.54 (*m*, 2 arom. H); 7.64 (*m*, 1 arom. H); 8.06 (*m*, 2 arom. H); 8.68, 8.76 (2*s*, H–C(2), H–C(8)); 11.20 (br. *s*, NH). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 66.8 (*d*, C(2′)); 68.3 (*t*, C(5′)); 70.3 (*d*, C(3′)); 71.9 (*d*, C(4′)); 81.0 (*d*, C(1′)); 126.2 (*s*); 129.3, 129.5, 133.3 (3*d*, arom. C); 134.2 (*s*); 144.2 (*d*, C(2)); 151.0 (*s*); 152.6 (*d*, C(8)); 153.7 (*s*); 166.5 (*s*, CO). FAB-MS (pos., NBA/NaI): 394 (50.3, $[M+Na^+]$), 372.1302 (18.9, $[A^{Bz}+H]^+$), 227 (100.0), 249 (98.0).

*1-(α-*L*-lyxopyranosyl)thymine* (**3b**). A soln. of 38.3 g (67.1 mmol) **2b** in 710 ml *ca*. 0.7M methanolic NH_3 was stirred for 11 days at r.t. NH_3 and MeOH were evaporated, and the resulting soln. was co-evaporated twice with 200 ml of dry MeOH. The residue was subjected to CC (silica gel; $CHCl_3$/MeOH 8:1 to 6:1) to afford 13.9 g (80.2%) of **3b**. M.p. 233°. TLC ($CHCl_3$/MeOH 6:1): R_f 0.17. ^{1}H-NMR (600 MHz, D_2O): 1.79 (*s*, Me); 3.78 (*dd*, J(4′,5′) = 1.04, J_{gem} = 12.5, H–C(5′)); 3.81 (*m*, H–C(3′)); 4.00 (*dd*, J(4′,5′) = 0.95, J_{gem} = 12.5, H–C(5′)); 4.02 (*dd*, J(2′,3′) = 3.3, J(1′,2′) = 9.8, H–C(2′)); 4.09 (*Ψt*, H–C(4′)); 5.66 (*d*, J(1′,2′) = 9.8, H–C(1′)); 7.56 (*s*, H–C(6)). ^{13}C-NMR (150.9 MHz, D_2O): 11.79 (*q*, Me); 66.04 (*d*, C(2′)); 67.80 (*t*, C(5′)); 69.33 (*d*, C(3′)); 70.85 (*d*, C(4′)); 80.69 (*d*, C(1′)); 112.34 (*s*, C(5)); 137.63 (*d*, C(6)); 152.57, 166.58 (2*s*, C(2), C(4)). FAB-MS (pos., NBA/NaI): 539 (8.1, $[2M+Na]^+$), 517 (10.8, $[2M+H]^+$), 439 (4.8), 391 (47.0, $[2M+H-thym]^+$), 286 (72.3), 281 (77.7, $[M+Na]^+$), 259.0936 (100.0, $[M+H]^+$).

N^4-*Benzoyl-1-(α-*L*-lyxopyranosyl)cytosine* (**3c**). A soln. of 9.0 g (13.7 mmol) of **2c** in 550 ml of THF/MeOH/H_2O 5:4:1 (*v*/*v*/*v*) was cooled to 0° (NaCl/ice-bath). To this soln., 82.2 ml of 2M aq. NaOH was added dropwise, and the mixture was stirred for additional 45 min at 0°. The resulting soln. was neutralized with 1M aq. HCl and reduced to a volume of 100 ml. This suspension was heated until a clear soln. was obtained and was

allowed to stand overnight at 4°. The product crystallized as colorless needles. After filtration, the mother liquor was further concentrated and left at 4° to afford a second batch of product. The combined batches of crystals were dried *in vacuo* (*ca.* 0.5 Torr r.t.) to afford 3.3 g (70%) of **3c**. M.p. 153–155°. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.12. ^{1}H-NMR (200 MHz, (D_6)DMSO): 3.91–4.03 (br. *m*, 2 H–C(5′)); 4.12–4.30 (br. *m*, H–C(2′), H–C(3′), H–C(4′)); 5.99 (*d*, *J*(1′,2′) = 9.4, H–C(1′)); 7.50–8.25 (*m*, 5 arom. H, H–C(5), H–C(6)); 10.23 (*s*, NH). FAB-MS (pos., NBA): 695 (15, $[2M+H]^+$), 348 (95, $[M+H]^+$), 216 (100, $[BzCyt]^+$).

N^2-*Isobutyryl-9-(α-*L*-lyxopyranosyl)guanine* (**3d**). To a soln. of 1.6 g (2.4 mmol) of **2d** in 90 ml THF/MeOH 5:4 (*v*/*v*) cooled to 0° in an ice-bath were added 10 ml of 2M aq. NaOH soln., and the mixture was stirred for 50 min. After the reaction was complete (TLC), the cold soln. was neutralized with 1M aq. HCl. The soln. was evaporated to dryness and co-evaporated twice with toluene. The dry residue was dissolved in MeOH, adsorbed on 10 g of silica gel, loaded on the top of a short silica gel column, and eluted from the column (CH_2Cl_2/MeOH 95:5 to 6:1) to afford 705 mg (79%) of **3d**. TLC (CH_2Cl_2/MeOH 4:1): R_f 0.10. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.12 (*d*, *J* = 6.9, Me); 2.77 (*m*, *J* = 6.9, Me_2CH); 3.61 (*m*, H–C(4′)); 3.65 (*d*, J_{gem} = 11.2, H–C(5′)); 3.88–3.93 (*m* + *d*, J_{gem} = 11.2, H–C(3′), H–C(5′)); 4.27 (*m*, H–C(2′)); 5.11 (*d*, *J* = 6.8, HO); 5.17 (*d*, *J* = 5.0, OH); 5.30 (*d*, *J* = 3.6, OH); 5.60 (*d*, *J*(1′,2′) = 9.6, H–C(1′)); 8.15 (*s*, H–C(8)); 11.73, 12.08 (*s*, NH). FAB-MS (pos., NBA): 354 (100, $[M+H]^+$).

N^6-*Benzoyl-9-{2′,3′-*O*-[ethoxy(phenyl)methylidene]-α-*L*-lyxopyranosyl}adenine* (**4a**). To a soln. of 7.63 g (20.6 mmol) of **3a** in 23 ml of dry DMF (previously dried over activated molecular sieves (3 Å)) was added a small amount of molecular sieves (3 Å) for further drying. The soln. was kept for 1 h at r.t. and was transferred by syringe into a dry 100-ml flask under N_2, followed by the addition of a soln. of 3.92 g (20.3 mmol) of TsOH · H_2O (melted and dehydrated *in vacuo* (*ca.* 0.5 Torr) before use) in 20 ml of MeCN and 11.5 ml (49.3 mmol) of dry triethyl orthobenzoate (stored over molecular sieves, 3 Å). The reaction flask was attached to a rotavap equipped with a drying tower (filled with *Drierite*™) and heated under vacuum (*ca.* 20 Torr) at 30° (water bath) without distillation of DMF. After 4 h under vacuum, the reaction was complete (TLC), and the soln. was poured into an ice-cold mixture of sat. aq. $NaHCO_3$ soln./AcOEt 1:1 (*v*/*v*) with stirring. The aq. phase was extracted with 3 × 100 ml of AcOEt, and the combined org. phase was dried (Na_2SO_4) and concentrated to *ca.* 100 ml. Dropwise addition of CH_2Cl_2 initiated precipitation. The precipitate was filtered and dried (*in vacuo*, r.t., 24 h) to afford 3.97 g of amorphous product, **4a**. The mother liquor was concentrated and purified by CC (silica gel; conditioned with MeOH (containing *ca.* 0.5% Et_3N); $CHCl_3$/AcOEt 4:1, then toluene/acetone 1:1), which gave an additional 1.93 g of product, **4a**. Combined yields: 5.9 g (56.8%) of **4a** (mixture of diastereoisomers a and b; (purified by CC). TLC (petroleum ether/acetone 1:1): R_f 0.48. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.07, 1.16 (2*t*, *J* = 7.1, *Me*CH_2O); 3.31–3.63 (3*m*, MeCH_2O, H_2O, overlapping signals); 3.95–4.11 (3*m*, 2 H–C(5′), H–C(4′) of both epimers); 4.29 (*m*, H–C(3′) of epimer b); 4.84 (*dd*, *J*(3′,4′) = 3.0, *J*(2′,3′) = 5.5, H–C(3′) of epimer a); 5.38 (*dd*, *J*(2′,3′) = 5.7, *J*(1′,2′) = 8.3, H–C(2′) of epimer b); 5.48 (*dd*, *J*(2′,3′) = 5.5, *J*(1′,2′) = 7.6, H–C(2′) of epimer a); 5.61 (*d*, *J*(4′,OH) = 4.7, HO–C(4′) of epimer a); 5.64 (*d*, *J*(1′,2′) = 7.6, H–C(1′) of epimer a); 5.68 (*d*, *J*(4′,OH) = 4.7, HO–C(4′) of epimer b); 6.20 (*d*, *J*(1′,2′) = 8.3, H–C(1′) of epimer b); 7.39–8.07 (*m*, 10 arom. H); 8.74 (*s*, H–C(2) of epimer a); 8.81 (*s*, H–C(2) of epimer b, H–C(8) of epimer a, overlapping signals); 8.91 (*s*, H–C(8) of epimer b); 11.28, 11.31 (2 br. *s*, NH of both epimers). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 15.8 (*q*, *Me*CH_2O); 59.5, 60.0 (2*t*, MeCH_2O); 65.1 (2*d*, C(4′) of both epimers, overlapping signals); 69.1, 69.5 (2*t*, C(5′) of both epimers); 72.6, 73.3 (2*d*, C(2′) of both epimers); 78.5, 78.8 (2*d*, C(3′) of both epimers); 81.8, 81.9 (2*d*, C(1′) of both epimers); 121.0, 122.0 (2*s*, arom. C); 126.2, 126.7, 126.8, 129.1, 129.2, 129.4 (6*d*, arom. C); 130.0, 130.1 (2*s*, arom. C); 133.4 (*d*, arom. C); 134.1, 138.0, 139.2 (3*s*, arom. C); 144.0 (2*d*, C(8) of both epimers, overlapping signals); 151.5 (*s*, arom. C); 152.9, 153.2 (2*d*, C(2) of both epimers). FAB-MS (pos., NBA/NaI): 526.1720 (100.0, $[M+Na]^+$), 504 (17.6, $[M+H]^+$), 458 (9.1, $[M-OEt]^+$).

*1-{2′,3′-*O*-[ethoxy(phenyl)methylidene]-α-*L*-lyxopyranosyl}thymine* (**4b**). The reaction was performed as described for **4a** with 19.43 g (75.2 mmol) of **3b** in 50 ml of dry DMF soln. of 9.10 g (47.1 mmol) TsOH · H_2O (melted and dehydrated *in vacuo*, *ca.* 0.5 Torr, before use) in 40 ml of MeCN and 24.5 ml (105 mmol) of dry triethyl orthobenzoate. Following similar workup procedure, the org. phase was dried (Na_2SO_4) and evaporated. The residual oil was purified by CC (silica gel; conditioned with MeOH (containing *ca.* 0.5% Et_3N); petroleum ether/acetone 3:1 to 1:1) afforded 11.55 g (39.4%) of **4b** as a 60:40 mixture of diastereoisomers (purified by CC). TLC (petroleum ether/acetone 1:1): R_f 0.67. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.07, 1.13 (2*t*, *J* = 7.1, *Me*CH_2O of both epimers); 1.81, 1.82 (2*s*, Me–C(5) of both epimers); 3.28–3.37 (2*m*, MeCH_2O of epimer a, H_2O, overlapping signals); 3.55–3.68 (*m*, H–C(5′) of both epimers, MeCH_2O of epimer b, overlapping signals); 3.81 (*d*, J_{gem} = 12.3, H–C(5′) of both epimers); 3.91–3.96 (2*m*, H–C(4′) of both epimers); 4.08 (*dd*, *J*(3′,4′) = 2.3, *J*(2′,3′) = 5.8, H–C(3′) of epimer b); 4.66–4.67 (*m*, H–C(3′) of epimer a); 4.75 (*dd*, *J*(2′,3′) = 6.0, *J*(1′,2′) =

8.5, H–C(2′) of epimer b); 4.87 (*dd*, *J*(2′,3′) = 6.1, *J*(1′,2′) = 8.1, H–C(2′) of epimer a); 5.36 (*d*, *J*(1′,2′) = 8.1, H–C(1′) of epimer a); 5.48 (*d*, *J*(4′, OH) = 5.7, HO–C(4′) of epimer b); 5.57 (*d*, *J* = 5.7, HO–C(4′) of epimer a); 5.91 (*d*, *J*(1′,2′) = 8.5, H–C(1′) of epimer b); 7.39–7.95 (*m*, 5 arom. H, H–C(6) of both epimers); 11.42 (br. *s*, NH of both epimers). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 12.8 (*q*, Me–C(5)); 15.6, 15.8 (2*q*, *Me*CH_2O of both epimers); 59.4, 59.6 (2*t*, MeC*H_2*O of both epimers); 64.8, 64.9 (2*s*, C(4′) of both epimers); 69.4, 69.6 (2*d*, C(5′) of epimer b); 71.9 (*d*, C(2′) of epimer b); 72.7 (*d*, C(2′) of epimer a); 78.0 (*d*, C(3′) of epimer b); 78.6 (*d*, C(3′) of epimer a); 81.7 (*d*, C(1′) of epimer b); 82.1 (*d*, C(1′) of epimer a); 111.0, 111.1, 120.9, 121.8 (4*s*); 126.7, 127.0 (2*d*, arom. C); 129.0, 129.1 (2*d*, arom. C); 129.9, 130.1 (2*s*, arom. C); 137.4, 137.5, 137.6 (arom. C); 139.5 (*s*, arom. C); 151.6, 164.3 (2*s*, CO). FAB-MS (pos., NBA/NaI): 435 (7.1, $[M+2\ Na]^+$), 413.1335 (100.0, $[M+Na]^+$), 345 (45.3, $[M+H-OEt]^+$), 265 (20.6, $[M+H-thym]^+$), 241 (9.5), 223 (23.3), 219 (29.7, $[M+H-thym-OEt]^+$).

*1-(3′-*O*-Benzoyl-α-*L*-lyxopyranosyl)thymine* (**4bb**). To a soln. of 300 mg (1.2 mmol) of **3b** in 1 ml of DMF was added a soln. of 5% TsOH and 3% CF_3COOH acid in 1 ml of MeCN, followed by a soln. of 200 µl (0.9 mmol) of triethyl orthobenzoate in 3 ml of MeCN. The MeCN was removed by evaporation, and the mixture was kept for 2 h under vacuum at 30°, without distillation of the DMF (TLC: $CHCl_3$/MeOH 6:1). Addition of triethyl orthobenzoate (300 µl) and evaporation was repeated twice. Aq. CF_3COOH (90%, 500 µl) was added, and the reaction left overnight upon which the product crystallized. It was filtered and washed with MeCN to afford 296 mg (70%) of **4bb**. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.82 (*s*, Me), 3.76 (*m*, H–C(4′)); 3.79 (br. *d*, 1 H, H–C(5′)); 3.96 (br. *d*, J_{gem} = 12.3, 1 H, H–C(5′)); 4.23 (*m*, H–C(2′)); 5.40 (*t*, *J*(2′,3′) ≈ *J*(3′,4′) ≈ 3.0, H–C(3′)); 5.57 (*d*, *J* = 5.8, OH); 5.65 (*d*, *J* = 5.6, OH); 5.78 (*d*, *J*(1′,2′) = 9.7, H–C(1′)), 8.10–7.50 (*m*, 5 arom. H, H–C(6)). On addition of D_2O to the above NMR sample, the signal assigned to H–C(2′) reduced to a *dd* (*J*(1′,2′) = 9.7, *J*(2′,3′) = 3.3 Hz), while the signal assigned to H–C(3′) remained unchanged. The assignment of H–C(2′) was based on the coupling to H–C(1′) (*J*(1′,2′) = 9.7 Hz).

N^4*-Benzoyl-1-{2′,3′-*O*-[methoxy(methyl)methylidene]-α-*L*-lyxopyranosyl}cytosine* (**4c**). To a soln. of 1.84 g (5.3 mmol) of **3c** and 6.7 ml (53 mmol) of trimethylorthoacetic acid in 15 ml of dry DMF, stirred under Ar at r.t., were added 1.7 ml (21.2 mmol) of TMS-Cl by a syringe. The soln. was stirred for 6 h, quenched with pyridine, and concentrated *in vacuo*. The residue was purified by CC (silica gel; acetone/hexane 1:3 to 3:2) to afford 1.58 g (74%) of **4c** as a 4:1 mixture of diastereoisomers. TLC (CH_2Cl_2/MeOH 12:1): R_f 0.48. ^{1}H-NMR (200 MHz, (D_6)DMSO): 1.43, 1.56 (*s*, Me of both epimers); 3.18–3.30 (*s*, MeO of both epimers); 3.91–4.03 (br. *m*, H–C(5′), H–C(4′) of both epimers); 4.34, 4.42 (*m*, H–C(3′) of both epimers); 4.51, 4.65 (*dd*, *J*(1′,2′) = 8.1, *J*(2′,3′) = 5.7, H–C(2′) of both epimers); 5.48 (*d*, *J*(4′, OH) = 5.4, OH); 5.95, 5.64 (*d*, *J*(1′,2′) = 8.1, 8.4, H–C(1′) of both epimers); 7.38–8.22 (*m*, 5 arom. H, H–C(5), H–C(6)); 11.3 (*s*, NH). FAB-MS (pos., NBA): 426 (19, $[M+Na]^+$), 404 (38, $[M+H]^+$), 372 (93, $[M+H-MeO]^+$), 216 (77, $[BzCyt]^+$), 105 (100, $[PHCO]^+$). A crystallized sample (M.p. 207–209°; from acetone/hexanes) was subjected to elemental analysis. Anal. calc. for $C_{19}H_{21}N_3O_7$: C 56.67, H 5.25, N 10.42, found: C 56.63, H 5.31, N 10.40.

N^2*-Isobutyryl-9-{2′,3′-*O*-[methoxy(methyl)methylidene]-α-*L*-lyxopyranosyl}guanine* (**4d**, impure). To a soln. of 900 mg (2.5 mmol) of **3d** and 3.14 ml (25 mmol) of trimethylorthoacetic acid in 10 ml of dry DMF under Ar were added 760 µl (6 mmol) of TMS-Cl. The soln. was allowed to stir for 16 h and evaporated to afford a thick syrup. The syrup was purified by CC (silica gel; acetone/hexane 2:3 to 2:1) to yield 681 mg (66%) of **4d** as a mixture. ^{1}H-NMR Data tentatively compatible with **4d** as major component. TLC (acetone/hexane 2:1): R_f 0.36. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.12 (*d*, *J* = 6.9, *Me*$_2$CH); 2.08, 2.11 (2*s*, Me of both epimers); 2.79 (*m*, *J* = 6.9, Me_2CH); 3.34–3.37 (*s*, MeO of both epimers); 3.62–4.11 (br. *m*, H–C(5′), H–C(4′) of both epimers); 4.52, 4.55 (*m*, H–C(3′) of both epimers); 5.14–5.18 (*m*, H–C(2′) of both epimers); 5.41 (*d*, *J*(4′, OH) = 5.0, OH); 5.51, 5.61 (*d*, *J*(1′,2′) = 9.8 and/or 7.2 (?), H–C(1′) of both epimers); 8.12, 8.35 (*s*, H–C(8)); 11.7, 12.1 (2*s*, NH). FAB-MS (pos., NBA): 448 (27, $[M+K]^+$), 432 (100, $[M+Na]^+$), 410 (17, $[M+H]^+$).

N^6*-Benzoyl-9-{3′-*O*-benzoyl-4′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-lyxopyranosyl}adenine* (**5a**). A suspension of 7.16 g (14.2 mmol) of **4a** in 250 ml CH_2Cl_2 and 10 ml (85.0 mmol) 2,6-lutidine was cooled to 0°, and 14.3 g (40.1 mmol) of 95% DMT-Cl were added in small portions. Stirring overnight at 4° resulted in complete conversion of **4a** (TLC). To this mixture, 135 ml of 80% aq. AcOH in 270 ml of THF was added, and the mixture was stirred for 4 d at r.t. The mixture was washed with sat. aq. $NaHCO_3$ soln., and the aq. phase was extracted with 3 × 200 ml CH_2Cl_2. Evaporation of the combined org. layers and purification of the residue by CC silica gel (conditioned with MeOH (containing *ca.* 0.5% Et_3N), acetone, petroleum ether/acetone 2:1; petroleum ether/acetone 2:1 to 1:1) afforded, after drying (Na_2SO_4), 6.08 g (54.9%) of **5a**. TLC (petroleum ether/acetone 2:1): R_f 0.25. ^{1}H-NMR (600 MHz, $CDCl_3$): 3.10 (*d*, J_{gem} = 12.5, H–C(5′)); 3.70 (*d*, J_{gem} = 12.5, H–C(5′)); 3.82 (*s*, 2 MeO); 3.94 (*d*, *J*(3′,4′) = 3.6, H–C(4′)); 4.13–4.17 (br. *m*, OH); 4.30 (*dt*, *J*(2′,3′) = 2.7, *J*(1′,2′) = 9.7, H–C(2′)); 5.35 (*Ψs*, H–C(3′)); 6.00 (*d*, *J*(1′,2′) = 9.7, H–C(1′)); 6.86–7.55 (*m*, 23 arom. H); 7.99

(2*s*, H–C(2), H–C(8)); 9.66 (*s*, NH). ^{13}C-NMR (150.9 MHz, $CDCl_3$): 55.7 (*q*, MeO); 67.1 (*t*, C(5′)); 68.0 (*d*, C(2′)); 69.5 (*d*, C(4′)); 73.4 (*d*, C(3′)); 81.2 (*d*, C(1′)); 88.2 (*s*, $(MeOC_6H_4)_2CPh$); 112.4 (*s*, arom. C); 113.8, 113.9, 127.7 (3*d*, arom. C); 128.5, 128.7 (2*d*, arom. C); 129.7 (*d*, arom. C); 130.3 (*d*, arom. C); 130.5, 130.6 (2*d*, C(2), C(8)); 133.8, 135.6 (3*d*, arom. C); 136.1, 136.3, 145.4, 151.9, 159.3, 163.3 (6*s*, arom. C); 163.9, 165.5 (2*s*, CO). FAB-MS (pos., NBA/CsI): 1042 (19.9, $[M+2\,Cs]^+$), 910.1886 (100.0, $[M+Cs]^+$), 778 (5.4, $[M+H]^+$).

*1-{3′-*O*-Benzoyl-4′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-lyxopyranosyl}thymine* (**5b**). The reaction was performed as described for **5a** with 14.76 g (37.8 mmol) of **4b** in 470 ml of dry CH_2Cl_2, 31 ml (26.3 mmol) of 2,6-lutidine, 43.2 g (121.1 mmol) of 95% DMT-Cl. Then, 240 ml 80% aq. AcOH in 470 ml of THF was added, and the mixture was stirred for 5 d at 4° and 2 d at r.t. The soln. was diluted with 30 ml of CH_2Cl_2 and poured into an ice-cold mixture of sat. aq. $NaHCO_3$ soln./AcOEt 1:1 (*v*/*v*). Extraction with 500 ml of AcOEt, drying of the org. phase (Na_2SO_4), and evaporation of the org. solvent afforded a colorless oil, which was subjected to CC (silica gel; conditioned with MeOH (containing *ca.* 0.5% Et_3N); petroleum ether/acetone 2:1 to 1:1) and gave 9.55 g (38.1%) of **5b**. TLC (petroleum ether/acetone 3:2): R_f 0.56. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.89 (*s*, Me–C(5)); 2.90 (*d*, $J_{gem}=12.4$, H–C(5′)); 3.65 (*d*, $J_{gem}=12.4$, H–C(5′)); 3.75 (*s*, 2 MeO); 3.77 (*d*, *J*(3′,4′) = 3.4, H–C(4′)); 4.46 (*m*, H–C(2′)); 5.32 (*m*, H–C(3′)); 5.62 (*d*, *J*(2′, OH) = 6.0, OH); 5.69 (*d*, *J*(1′,2′) = 9.6, H–C(1′)); 6.91–7.96 (*m*, 18 arom. H, H–C(6)); 11.40 (*s*, NH). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 13.2 (*q*, Me–C(5)); 55.9 (*q*, MeO); 65.2 (*d*, C(2′)); 67.0 (*t*, C(5′)); 70.0 (*d*, C(4′)); 73.4 (*d*, C(3′)); 81.4 (*d*, C(1′)); 87.9 (*s*, $(MeOC_6H_4)_2CPh$); 110.6 (*s*, C(5)); 114.2, 114.3, 127.9, 128.6, 128.9, 129.5, 130.3, 130.3, 130.8, 134.3 (10*d*, arom. C); 136.4, 136.5 (2*s*, arom. C); 137.1 (*d*, arom. C); 146.0, 151.8 (2*s*, arom. C); 159.2 (*s*, arom. C); 164.5, 165.1 (3*s*, CO). FAB-MS (pos., NBA/CsI): 929 (12.2, $[M+2\,Cs]^+$), 797.1497 (100.0, $[M+Cs]^+$).

*N^4-Benzoyl-1-{3′-*O*-acetyl-4′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-lyxopyranosyl}cytosine* (**5c**). To a soln. of 310 mg (0.75 mmol) of **4c** in 5 ml of dry CH_2Cl_2 cooled to 0° were added a soln. 560 µl of 2,6-lutidine and 813 mg of DMT-Cl, and the mixture was stirred for 96 h at 4°. The soln. was evaporated, and the residue was purified by CC (silica gel; acetone/hexane 3:1 to 2:1 with 2% Et_3N) to afford 292 mg (55%) of the tritylated intermediate. The tritylated intermediate was dissolved in 60 ml of THF, 30 ml of 80% aq. AcOH was added, and the mixture was stirred at r.t. until the reaction was complete (*ca.* 19 h, TLC). The mixture was diluted with CH_2Cl_2, neutralized with sat. aq. $NaHCO_3$ soln., and the aq. phase was extracted twice with CH_2Cl_2. The combined org. phases were dried ($MgSO_4$), filtered, and evaporated. The residue was purified by CC (silica gel; acetone/hexane 1:3 to 3:2 with 1% pyridine) to give 177 mg (62%) of **5c**. TLC (acetone/hexane 1:1): R_f 0.2. ^{1}H-NMR (600 MHz, $CDCl_3$): 2.09 (*s*, MeOH); 3.05 (*dd*, $J_{gem}=12.6$, *J*(4′,5′) = 4.7, H–C(5′)); 3.59 (*d*, $J_{gem}=12.6$, H–C(5′)); 3.82 (*s*, 2 MeO); 3.83 (*m*, H–C(4′)); 4.26 (*m*, H–C(2′)); 5.13 (br. *s*, H–C(3′)); 5.97 (*d*, *J*(1′,2′) = 9.4, H–C(1′)); 6.86–8.1 (*m*, 18 arom. H, H–C(5), H–C(6)); 9.1 (*s*, NH). FAB-MS (pos., NBA): 692 (29, $[M+H]^+$), 303 (100, $[DMTr]^+$).

*N^2-Isobutyryl-9-{3′-*O*-acetyl-4′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-lyxopyranosyl}guanine* (**5d**). The reaction was performed as described for **5c** with 1.28 g (3.1 mmol) of **4d**, 15 ml of dry CH_2Cl_2, 3.65 ml (31.3 mmol) dry 2,6-lutidine, and 3.19 g (9.4 mmol) of DMT-Cl (5 d at 4°). To this mixture were added 60 ml of THF, 30 ml of 80% aq. AcOH, and the mixture was stirred until the reaction was complete (*ca.* 13 h, TLC). Following the workup as described for **5c** and purification by CC (silica gel, acetone/hexane 1:2 to 2:1 with 1% pyridine) afforded 1.49 g (68%) of **5d**. TLC (acetone/hexane 2:1): R_f 0.24. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.05, 1.15 (*d*, *J* = 6.9, Me_2CH); 2.09 (2*s*, MeO); 2.38 (*m*, Me_2CH); 3.20 (*d*, $J_{gem}=11.6$, H–C(5′)); 3.61 (*d*, $J_{gem}=11.6$, H–C(5′)); 3.77 (br. *m*, H–C(4′)); 3.81 (*s*, 2 MeO); 4.6–5.25 (*m*, H–C(3′), H–C(2′), OH); 5.46 (*d*, *J*(1′,2′) = 9.0, H–C(1′)); 6.85–7.66 (*m*, 13 arom. H); 7.76 (2*s*, H–C(8)); 8.62, 11.89 (br. *s*, NH). FAB-MS (pos., NBA): 2094 (5, $[3M+H]^+$), 1418 (22, $[2M+Na]^+$), 720 (24, $[M+Na]^+$), 698 (45, $[M+H]^+$), 303 (100, $[DMTr]^+$).

2. ***Experiments Referring to*** **Scheme 4.** – *N^6-Benzoyl-9-{3′-*O*-benzoyl-2′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-4′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-lyxopyranosyl}adenine* (**6a**). To a soln. of 440 mg (0.57 mmol) of **5a** in 5 ml of dry CH_2Cl_2, 0.56 ml (4.2 mmol) of 2,4,6-collidine, and 23 µl (0.29 mmol) *N*-methylimidazole, 316 µl (1.2 mmol) of 97% chloro(2-cyanoethoxy)(diisopropylamino)phosphine were added by syringe. The mixture was stirred for 10 min. at r.t., diluted with 5 ml of AcOEt, and washed with sat. aq. $NaHCO_3$ soln. The org. phase was dried ($MgSO_4$), concentrated, and subjected to CC (silica gel; petroleum ether/AcOEt 1:1) to furnish 0.473 g (84.2%, mixture of diastereoisomers *ca.* 2.7:1 by ^{1}H-NMR) of **6a** as a syrupy liquor. TLC (petroleum ether/AcOEt 1:1): R_f 0.33. ^{1}H-NMR (600 MHz, $CDCl_3$): 0.54, 0.91 (2*d*, *J* = 6.8, Me_2CH of epimer b); 0.96 (2*d*, *J* = 5.6, Me_2CH of epimer a), overlapping signals); 2.34 (*m*, CH_2CN of epimer a);

2.49 (*m*, CH_2CN of epimer b); 3.13 (*d*, J_{gem} = 12.7, 1 H–C(5′) of both epimers); 3.21 (2*m*, 1 H; CH_2CH$_2$CN of epimer a), Me$_2$C*H* of both epimers); 3.30, 3.53, 3.68 (3*m*, 2 H; CH_2CH$_2$CN of both epimers); 3.80–3.84 (*m*, 1 H–C(5′) of both epimers, MeO, overlapping signals); 3.98 (*m*, H–C(4′) of both epimers); 5.10, 5.23 (2*m*, H–C(2′) of both epimers); 5.62 (*m*, H–C(3′) of both epimers); 6.05, 6.09 (2*d*, *J*(1′,2′) = 9.3, 9.2, H–C(1′) of both epimers); 6.88–8.09 (*m*, 23 arom. H); 8.38, 8.43, 8.91, 8.94 (4*s*, H–C(2), H–C(8)); 9.15 (br. *s*, NH of both epimers). ^{13}C-NMR (150.9 MHz, $CDCl_3$): 20.30, 20.34 (2*t*, *C*H$_2$CN of both epimers); 24.3, 24.8 (2*q*, *Me*$_2$CH of both epimers); 43.3, 43.4 (2*d*, Me$_2$*C*H of both epimers); 55.7 (*q*, MeO of both epimers); 58.2, 58.3 (2*t*, *C*H$_2$CH$_2$CN); 67.2 (*t*, C(5′) of both epimers); 69.4, 69.6 (2*d*, C(2′), C(4′) of both epimers); 72.2, 72.4 (*d*, C(3′) of both epimers); 81.1 (*d*, C(1′) of both epimers); 88.3 (*s*, (MeOC$_6$H$_4$)$_2$*C*Ph of both epimers); 113.9 (*d*, arom. C); 117.9 (*s*, CN), 123.2 (*s*, arom. C); 127.7, 128.2, 128.3, 128.5, 128.6, 129.0, 129.3, 130.1, 130.6, 130.7 (arom. C); 133.2, 133.8 (2*d*, arom. C); 134.1, 134.2 (arom. C); 136.1, 136.4 (2*s*, arom. C); 142.3 (*d*, C(2) or C(8)); 145.3, 149.9 (2*s*), 152.5, 152.9 (*d*, C(2) or C(8)); 159.3, 159.3, 164.9, 165.0 (4*s*, CO of both epimers). ^{31}P-NMR (242.9 MHz, $CDCl_3$): 150.54, 151.79 (2*s*, P of both epimers). FAB-MS (pos., NBA/CsI): 1242 (18.2, [*M* + 2 Cs]$^+$), 1110.2974 (100.0, [*M* + Cs]$^+$).

*1-{3′-*O*-Benzoyl-2′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-4′-*O*-[(4″,4‴-dimethoxytriphenyl)-methyl]-α-*L*-lyxopyranosyl}thymine* (**6b**). To a soln. of 500 mg (0.75 mmol) of **5b** in 5 ml of dry CH_2Cl_2, 0.75 ml (5.7 mmol) of 2,4,6-collidine, and 30 µl (0.4 mmol) of *N*-methylimidazole, 420 µl (1.6 mmol) of 97% chloro(2-cyanoethoxy)(diisopropylamino)phosphine was added by syringe. The soln. was stirred for 10 min. at r.t., diluted with 5 ml of AcOEt, and washed with sat. aq. $NaHCO_3$ soln. The org. phase was dried ($MgSO_4$), concentrated, and subjected to CC (silica gel; petroleum ether/AcOEt 2:1 to 3:2) to afford 0.523 g (80.6%, mixture of diastereoisomers *ca.* 1:1 by ^{1}H-NMR) of **6b** as a syrup. TLC (petroleum ether/acetone 3:2): R_f 0.56. ^{1}H-NMR (600 MHz, $CDCl_3$): 0.93, 1.04, 1.11, 1.12 (4*d*, *J* = 6.8, Me$_2$CH of both epimers); 2.05, 2.06 (2*s*, Me–C(5) of both epimers); 2.38, 2.51 (2*m*, CH_2CN of both epimers); 2.88, 3.06 (2*d*, J_{gem} = 12.6, H–C(5′) of both epimers); 3.36–3.79 (*m*, CH_2CH$_2$CN, Me$_2$C*H*, 1 H–C(5′) of both epimers, Me$_2$C*H*); 3.82 (*s*, 2 MeO); 3.92 (*m*, H–C(4′) of both epimers); 4.48, 4.56 (2*m*, H–C(2′) of both epimers); 5.57, 5.65 (2*m*, H–C(3′) of both epimers); 6.06 (*m*, H–C(1′) of both epimers); 6.86–8.03 (*m*, 18 arom. H, H–C(6)); 8.33 (br. *s*, NH of both epimers). ^{13}C-NMR (150.9 MHz, $CDCl_3$): 13.1, 13.2 (2*q*, *Me*CH of both epimers); 20.4, 20.6 (2*t*, *C*H$_2$CN of both epimers); 24.6, 24.75, 24.80, 24.9 (4*q*, Me$_2$CH of both epimers); 43.4, 43.5 (2*t*, *C*H$_2$CH$_2$CN of both epimers); 55.7 (*q*, MeO); 58.0, 58.9 (2*d*, Me$_2$*C*H of both epimers); 66.8, 67.3 (2*d*, C(5′) of both epimers); 69.4, 69.6 (2*d*, C(4′) of both epimers); 72.3, 72.6 (2*d*, C(3′) of both epimers); 88.3 (*s*, (MeOC$_6$H$_4$)$_2$*C*Ph of both epimers); 111.7 (*s*, C(5) of both epimers); 113.82, 113.86, 113.90 (3*d*, arom. C); 127.7 (*d*, arom. C); 128.41, 128.47, 128.53 (3*d*, arom. C); 128.9, 129.0 (2*d*, arom. C); 129.80, 129.9 (2*s*, arom. C); 130.08, 130.11 (2*d*, arom. C); 130.5, 130.6, 130.7 (arom. C, C(6)); 145.4, 145.5 (2*s*, arom. C); 150.8, 151.0 (2*s*, C(2) of both epimers); 159.30, 159.33 (2*s*, CO of both epimers); 163.7, 163.8 (2*s*, C(4) of both epimers); 165.0, 165.1 (2*s*, CO of both epimers). ^{31}P-NMR (242.9 MHz, $CDCl_3$): 150.18, 151.16 (2*s*, P of both epimers). FAB-MS (pos., NBA/CsI): 1129 (12.8, [*M* + 2 Cs]$^+$), 997.2587 (100.0, *M* + Cs]$^+$).

N^4*-Benzoyl-1-{3′-*O*-acetyl-2′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-4′-*O*-[(4″,4‴-dimethoxy-triphenyl)methyl]-α-*L*-lyxopyranosyl}cytosine* (**6c**). To a soln. of 1.4 g (1.9 mmol) of **5c** and 856 µl (5 mol) of EtN(i-Pr)$_2$ in 5 ml of dry CH_2Cl_2, stirred under Ar at r.t., were added 643 µl (2.85 mol) of chloro(2-cyanoethoxy)(diisopropylamino)phosphine dropwise by a syringe. The mixture was stirred overnight at r.t., concentrated *in vacuo*, and purified by CC (silica gel; acetone/hexane: 1:2 to 2:1) to afford 1.31 g (72%) of **6c** as a mixture of diastereoisomers. TLC (AcOEt/hexane 2:1): R_f 0.28, 0.31 (both epimers). ^{1}H-NMR (600 MHz, $CDCl_3$): 1.03, 1.09, 1.13, 1.19 (4*d*, *J* = 6.8, *Me*$_2$CH of both epimers); 2.05–2.07 (2*s*, MeO); 2.53–2.78 (2*m*, CH_2CN of both epimers); 2.83, 3.02 (2*d*, J_{gem} = 12.7, H–C(5′) of both epimers); 3.42–3.72 (*m*, CH_2CH$_2$CN, Me$_2$C*H*, H–C(5′) of both epimers); 3.73, 3.88 (2*m*, H–C(4′) of both epimers); 3.82 (*s*, 2 MeO–C$_6$H$_4$); 4.37, 4.47 (2*m*, H–C(2′) of both epimers); 5.36, 5.42 (2*m*, H–C(3′) of both epimers); 6.16 (br. *s*, H–C(1′) of both epimers); 6.88–7.95 (*m*, 18 arom. H, H–C(5), H–C(6)); 8.76 (br. *s*, NH of both epimers). ^{31}P-NMR (242.9 MHz, $CDCl_3$): 151.29, 151.59 (2*s*, P of both epimers). FAB-MS (pos., NBA): 930 (10, [*M* + K]$^+$); 914.3548 (100, [*M* + Na]$^+$); 892 (20, [*M* + H]$^+$).

N^2*-Isobutyryl-9-{3′-*O*-acetyl-2′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-4′-*O*-[(4″,4‴-dimethox-ytriphenyl)methyl]-α-*L*-lyxopyranosyl}guanine* (**6d**). The reaction was performed as described for **6c** with 1.06 g (1.53 mmol) of **5d**, 1.6 ml (4.6 mmol) of EtN(i-Pr)$_2$, 5 ml of dry CH_2Cl_2, 1.04 ml (4.6 mmol) of chloro(2-cyanoethoxy)(diisopropylamino)phosphine (overnight, r.t). The mixture was concentrated *in vacuo* and purified by CC (silica gel; acetone/hexane: 1:2 to 2:1) to afford 985 mg g (70%) of **6d** as a mixture of diastereoisomers. TLC (acetone/hexane 2:1): R_f 0.45, 0.48 (both epimers). ^{1}H-NMR (600 MHz, $CDCl_3$): 0.83, 1.04, 1.08, 1.11 (2*d*, *J* = 7.3, *Me*$_2$CH of both epimers, overlapping signals); 1.17, 1.19 (*d*, *J* = 6.8, *Me*$_2$CH); 2.09 (2*s*,

MeO of both epimers); 2.4–2.6 (*m*, Me_2CH, CH_2CN of both epimers); 3.11 (*m*, 1 H–C(5′) of both epimers); 3.41 (*m*, 2 H, CH_2CH_2CN of epimer a, Me_2CH of both epimers); 3.5–3.6 (*m*, 2 H, CH_2CH_2CN of epimer b, 1 H–C(5′) of both epimers); 3.72–3.82 (*m*, H–C(4′) of both epimers, $2\,MeOC_6H_4$); 5.03 (br. *m*, H–C(2′) of both epimers); 5.39, 5.45 (*m*, H–C(3′) of both epimers); 5.46, 5.57 (2*d*, $J(1',2') = 8.8$, H–C(1′) of both epimers); 6.82–7.59 (*m*, 13 arom. H); 7.79, 7.92 (2*s*, H–C(8) of both epimers); 12.03 (br. *s*, NH of both epimers). ^{13}C-NMR (150.9 MHz, $CDCl_3$): 18.9, 18.7 (2*q*, Me_2CH of both epimers); 20.9, 20.3 (2*t*, CH_2CN of both epimers); 24.1, 24.5 (2*q*, Me_2CH of both epimers); 36.2, 36.3 (2*d*, Me_2CH of both epimers); 42.9, 43.0 (2*d*, Me_2CH of both epimers); 55.2 (*q*, MeO of both epimers); 57.8, 58.0 (2*t*, CH_2CH_2CN); 66.3 (*t*, C(5′) of both epimers); 68.2, 67.6 (2*d*, C(2′) of both epimers); 68.9, 69.2 (C(4′) of both epimers); 71.5, 71.7 (*d*, C(3′) of both epimers); 81.1, 82.1 (*d*, C(1′) of both epimers); 87.7 (*s*, $MeOC_6H_4$); 113.3, 117.7, 121.3, 127.1, 128.0, 130.2, 136.0, 137.9, 138.7, 145.2, 147.1, 147.3, 148.4, 155.5, 157.5, 158.7 (CN, arom. C, C(2), C(4), C(6), C(8)); 168.8, 178.3, 178.6 (3*s*, CO of both epimers). ^{31}P-NMR (242.9 MHz, $CDCl_3$): 151.5 (br. *s*, of both epimers). FAB-MS (pos., NBA): 920 (100, $[M + Na]^+$), 898 (24, $[M + H]^+$).

Preparation of Nucleoside-Derivatized Controlled Pore Glass (CPG) **7a** *and* **7b**. A soln. of 0.18–0.19 mmol of **5a** (**5b**), 0.36–0.39 mmol of succinic anhydride, and 0.24–0.25 mmol of DMAP in 2.4–3.0 ml of dry CH_2Cl_2 was stirred for 1 h at r.t. After dilution with CH_2Cl_2, the soln. was successively washed with 10% aq. citric acid, sat. aq. $NaHCO_3$ soln., and H_2O. The org. phase was dried (Na_2SO_4) and evaporated. The residue was purified by CC (silica gel; petroleum ether/acetone 2:1 to CH_2Cl_2/MeOH 6:1). The product fractions were collected and dried (Na_2SO_4), and concentrated *in vacuo* (*ca*. 0.2 Torr, 40 min., r.t.). The residue was dissolved in 7 ml of dry MeCN, followed by successive addition of 45 µl of *N*-methylmorpholine, 75 mg (0.229 mmol) of TOTU [33], and 1.8 g of LCAA-CPG. The suspension was shaken gently for 3 h at r.t. Filtration, followed by washing with DMF, MeOH, acetone, and Et_2O, afforded **7a** (**7b**) after drying *in vacuo* (*ca*. 0.2 Torr). A suspension of the nucleoside-derivatized solid support **7a** (**7b**) in 27.5 ml of dry pyridine and 2.8 ml of *N*-methylimidazole was treated with 2.8 ml of Ac_2O for 30 min. Filtration, followed by washing with DMF, MeOH, acetone, and Et_2O, afforded capped **7a** (**7b**) after drying *in vacuo* (*ca*. 0.2 Torr) for 3 h. The loading capacity (at 498 nm) was determined to be 43.6 µmol/g for **7a** and 21.6 µmol/g for **7b** [21].

Preparation of Nucleoside-Derivatized Controlled Pore Glass (CPG) **7c**. To a soln. of 120 mg (174 µmol) of **5c** in 2 ml of dry pyridine under Ar were added 490 mg (1.22 mmol) of bis(4-nitrophenyl)heptanedioate, 22 mg (180 µmol) of DMAP and stirred for 48 h at r.t. The mixture was concentrated and co-distilled twice with toluene. The syrup was purified by CC (silica gel; acetone/hexane 1:2 to 2:3). The product fractions were collected and concentrated *in vacuo* (*ca*. 0.2 Torr, 40 min., r.t.). To this residue were added 500 mg of LCAA-CPG (LCAA-CPG was previously washed with 50 ml of CH_2Cl_2, CH_2Cl_2/$ClCH_2COOH$ 1:1, CH_2Cl_2, CH_2Cl_2/Et_3N 1:1, CH_2Cl_2, and dried for 2 h *in vacuo*), dried for 1 h *in vacuo* (*ca*. 0.2 Torr), followed by addition of 7.5 ml of dry DMF, 110 µl (0.6 mmol) of $EtN(i\text{-}Pr)_2$ 16 µl (180 µmol) of dry pyridine and shaken for 3 h. The solid material was washed with 20 ml of DMF, 30 ml of MeOH, 30 ml of acetone, 30 ml of Et_2O, and was dried *in vacuo*. The dry material was suspended in 25 ml of dry pyridine and 2.5 ml of Ac_2O, 22 mg of DMAP and shaken for 1 h. The solid material was placed on a fritted-glass funnel and washed with 20 ml of DMF, 30 ml of MeOH, 30 ml of acetone, and 30 ml of Et_2O, and dried *in vacuo* (*ca*. 0.2 Torr) overnight. The loading capacity (at 498 nm) was determined to be 30 µmol/g [21].

Preparation of Nucleoside-Derivatized Controlled Pore Glass (CPG) **7d**. The reaction was performed as described for **7a** (**7b**) with 151 mg (0.216 mmol) of **5d**, 44 mg (0.43 mmol) of succinic anhydride, 38 mg (0.3 mmol) of DMAP, 3 ml of dry CH_2Cl_2. The residue after workup was treated with 7.5 ml of dry MeCN, 66 µl (0.6 mmol) of 4-methylmorpholine, 97 mg (0.3 mmol) of TOTU, 2 g of LCAA-CPG, and worked up as described for **7a** (**7b**). The capping was performed as described for **7a** (**7b**) with 25 ml of dry pyridine and 2.5 ml of Ac_2O. After workup, drying *in vacuo* (*ca*. 0.2 Torr) overnight afforded capped **7d**. The loading capacity was determined to be 23.5 µmol/g [21].

3. ***Automated Solid-Phase Synthesis on a Perceptive Expedite Gene Synthesizer.*** – Oligonucleotide syntheses were carried out on a 1 µM scale. The DNA/RNA synthesizer column was filled with the CPG-solid support loaded with the appropriate nucleobase. The substrates and reagents required were prepared as follows.

3.1. *Pre-automation Procedures.* 3.1.1. *Phosphoroamidites.* The amount of phosphoramidite soln. was determined as follows: for adenine, thymine, or the four-base containing sequences: $(n+1) \times 22$ mg of phosphoramidite dissolved in $(n+1) \times 312$ µl of dry MeCN. For guanine- or cytosine-containing sequences: $(n+2) \times 18$ mg of phosphoramidite dissolved in $(n+2) \times 230$–260 µl of dry MeCN. The phosphoramidite soln. (*ca*. 0.08M) was dried over 3-Å or 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. The excess of phosphoramidites, depending on the sequence synthesized, ranged from 160–323 equiv.

3.1.2. *Activator Soln.* 5-(Ethylthio)-1*H*-tetrazole in dry MeCN (0.25M for adenine- or thymine-containing sequences, and 0.35M for guanine- or cytosine-containing sequences) was dried over freshly activated 3-Å or 4-Å molecular sieves.

3.1.3. *Capping A*. A soln. of 3.0 g of DMAP in 50 ml of dry MeCN and filtered to remove any undissolved solid particles.

3.1.4. *Capping B.* A soln. of 10 ml of Ac_2O and 15 ml of 2,4,6-collidine in 25 ml of dry MeCN.

3.1.5. *Oxidizing Soln.* A mixture of 220 mg of I_2, 46 ml of 2,4,6-collidine in 23 ml of H_2O, and 50 ml of MeCN, and filtered to remove any undissolved residue.

3.1.6. *Detritylation Reagent.* A soln. of 6% $Cl_2CHCOOH$ in $ClCH_2CH_2Cl$.

The synthesis of oligonucleotides with the *Perseptive Expedite Gene Synthesizer* required the following modifications to the protocol provided by *Perseptive* for the DNA/RNA synthesis: *1*) the duration of the coupling time of phosphoramidite was *ca*. 15–16.7 min and *2*) the detritylation was accomplished by 6% $Cl_2CHCOOH$ in $ClCH_2CH_2Cl$ over a 3-min period. All oligonucleotides were synthesized in the 'Trityl-on' mode.

3.2. *Post-automation Procedures.* 3.2.1. *Removal of β-Cyanoethyl Protecting Group*. After the automated synthesis was completed, the CPG-solid support containing the oligonucleotide ('Trityl-on') was dried *in vacuo* for 30 min, transferred to a pear-shaped 10-ml flask and treated with 2.4 ml of pyridine/Et_3N 5:1 for 6.5 h at r.t. Evaporation of pyridine and Et_3N *in vacuo* followed by co-evaporation with toluene – avoiding temps. over 35° – resulted in dry CPG-solid support.

3.2.2. *Removal of Sugar- and Nucleobase-Protecting Groups.* One of the following three procedures was used depending on the sequence of the oligonucleotides (*Table 2* lists the specific deprotection method for the specific sequence).

Method A. To the flask containing the dry CPG-solid support was added 2 ml of 25% aq. $NH_2NH_2 \cdot H_2O$ (prepared from 1 ml of $NH_2NH_2 \cdot H_2O$ and 4 ml of H_2O) and shaken at 4° (6–7 h for adenine- or thymine-containing sequences, and *ca*. 20 h for guanine- or cytosine-containing sequences). After deprotection, the suspension was diluted with *ca*. 5–10 ml of 0.5M aq. $Et_3NH_2CO_3$ buffer, loaded over a *Waters Sepak-C18* cartridge (*cf. Sect. 5*) and eluted with 10–15 ml MeCN/H_2O 1:1 to afford the hydrazine-free, crude oligonucleotides ('Trityl-on') in soln.[9])[10]).

Method B. To the flask containing the dry CPG-solid support was added 2 ml of a mixture of 40% aq. $MeNH_2$ in conc. aq. NH_3 1:1 and shaken at r.t. for 6.5 h (*ca*. 70 h for G,C-containing sequences). The suspension was co-evaporated carefully with H_2O (with the temp. always less than 35°) to remove the volatiles $MeNH_2$ and NH_3, and filtered. The filtrate was diluted with *ca*. 5–10 ml of 0.5M aq. $Et_3NH_2CO_3$ buffer, loaded over a *Waters Sepak-C18* cartridge (*cf. Sect. 5*) and eluted with 10–15 ml MeCN/H_2O 1:1 to afford the salt free, crude oligonucleotides ('Trityl-on') in soln.

Method C. To the flask containing the dry CPG-solid support was added 2 ml of a soln. of 0.2M $MeONH_2 \cdot$ HCl in 25% aq. NH_3 and EtOH 3:1, and shaken at r.t. for *ca*. 6.5 h (*ca*. 66 h for G,C-containing sequences). After deprotection, the suspension was diluted with *ca*. 5–10 ml 0.5M aq. $Et_3NH_2CO_3$ buffer and desalted (*cf. Sect. 5*) over a *Waters Sepak-C18* cartridge (eluted with 10–15 ml of MeCN/H_2O 1:1) to afford the salt free, crude oligonucleotides ('Trityl-on') in soln.

All of the above deprotections were monitored by anion-exchange HPLC (*cf. Sect. 4*) for optimum deprotection time.

3.2.3. *Detritylation of 'Trityl-on' Oligonucleotides.* The crude oligonucleotide soln. obtained by desalting was concentrated *in vacuo*, the residue was treated with *ca*. 10 ml of 80% aq. HCOOH (a red color appears within seconds indicating detritylation) at r.t. for 15–30 min, and concentrated *in vacuo* to dryness. The residue was dissolved in *ca*. 2 ml of H_2O, filtered (*Nalgene* syringe filter, 0.2 μM), and taken to the next step, HPLC purification.

4. *HPLC Purification of Oligonucleotides.* – The crude oligonucleotides were purified by anion-exchange (IA)-HPLC system, over a *Mono Q HR 5/5* (*Pharmacia*) column, 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*. The oligonucleotides were eluted from the column with a linear gradient of 1M NaCl in 10 mM of aq. Na_2HPO_4, pH 10.5 over a period of 20 to 30 min with the following buffer systems: 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. Exact conditions are given in *Table 2*. The

pure fractions were collected in *Eppendorf* vials containing 20 µl of 1M aq. AcOH (to neutralize the high pH), combined, and desalted to remove excess salt.

5. *Desalting of Oligonucleotides.* – For desalting, a *Waters Sepak-C18* cartridge was equilibrated with 10 ml of MeCN/H_2O 1:1 followed by 10 ml of H_2O and finally 10 ml of 0.2M aq. $Et_3NH_2CO_3$. The oligonucleotide soln. was diluted with 10 ml of 0.1–0.2M aq. $Et_3NH_2CO_3$ soln. and loaded onto the *Waters Sepak-C18* cartridge. The cartridge was eluted with 10 ml of 0.2 or 0.5M $Et_3NH_2CO_3$, followed by 15 to 30 ml of MeCN/H_2O 1:1. The eluted MeCN/H_2O fractions containing the oligonucleotide (monitored by UV at 260 nm) were combined and concentrated *in vacuo*. The residue was co-evaporated three times with 10 ml of H_2O to remove excess buffer. The residue was dissolved in the desired amount of H_2O to give a salt-free soln. of the oligonucleotide.

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