Pentopyranosyl Oligonucleotide Systems

Communication No. 12[1])

The β-D-Xylopyranosyl-(4′ → 2′)-oligonucleotide System

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β-D-Xylopyranosyl-(4′ → 2′)-oligonucleotides containing adenine and thymine as nucleobases were synthesized as a part of a systematic study of the pairing properties of pentopyranosyl oligonucleotides. Contrary to earlier expectations based on qualitative conformational criteria, β-D-xylopyranosyl-(4′ → 2′)-oligonucleotides show *Watson-Crick* pairing comparable in strength to that shown by pyranosyl-RNA.

1. Introduction. – In a preceding communication [3], we described the synthesis and base-pairing properties of α-L-lyxopyranosyl-(4′ → 2′)-oligonucleotides as part of a series of reports to be published in this journal, presenting the experimental data that have accumulated in our studies on the chemistry of the pentopyranosyl-oligonucleotides (*Scheme 1*). The present paper deals with the β-D-xylopyranosyl-(4′ → 2′)-oligonucleotide system and complements the information we have given on this system in two preliminary communications [4][5] (see also [6]).

2. Results. – 2.1. *Synthesis.* 2.1.1. *Preparation of β-D-Xylopyranosyl Nucleoside Building Blocks Containing Adenine (A) and Thymine (T)*[4]). The building blocks required for the synthesis of the β-D-xylopyranosyl-(4′ → 2′)-oligonucleotides were the protected nucleoside phosphoramidite derivatives **7a**,**b**, in which the 2′-position bears the phosphoroamidite group, the 3′- and 4′-OH groups the acetate and the 4′,4″-dimethoxytrityl substituents, respectively, while the amino group of the nucleobase A bears a Bz group (*Scheme 2*). The synthesis of these nucleosides started from tetrabenzoyl-xylopyranose (anomeric mixture **1**), which was prepared according to the procedure of *Fletcher* and *Hudson* [7]. Nucleosidation of **1** with the bases N^6-benzoyladenine (catalyst: $SnCl_4$) and thymine (TMS-triflate) was carried under the *Vorbrüggen-Hilbert-Johnson* conditions [8] and afforded the corresponding xylopyr-

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[1]) Communication No. 11: [1]. The paper is also communication No. 34 in the series '*Chemistry of α-aminonitriles*'. For No. 33, see [1], a summary of the numbering of papers in this series will be given in [2].

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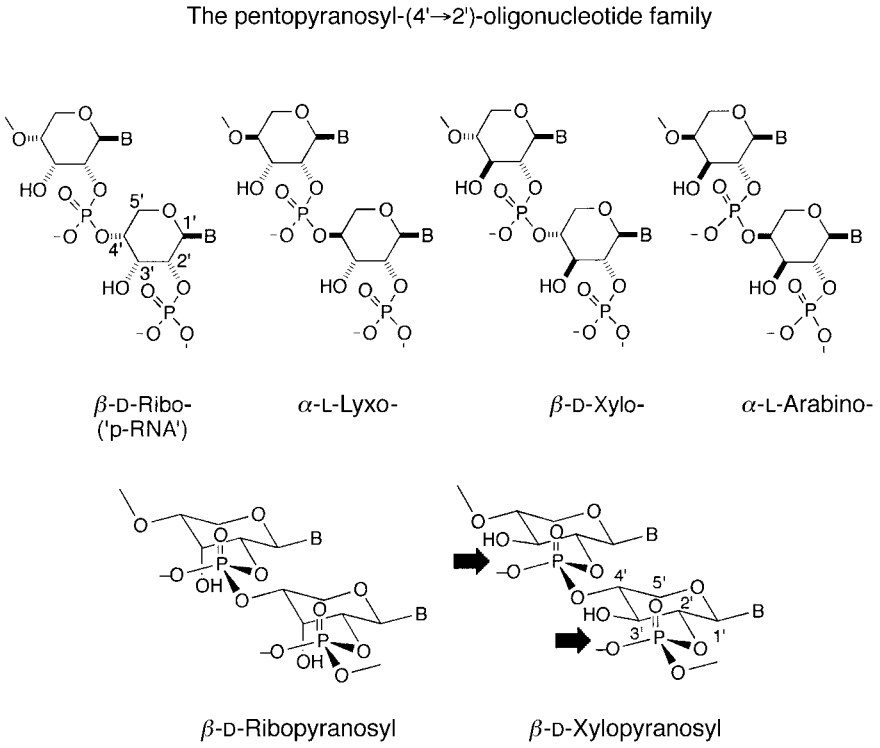
[4]) Xylopyranosyl nucleosides have been prepared before in a different context [9].

Scheme 1. *Constitution and Configuration of the Repeating Units of the Four Diastereoisomeric Pentopyranosyl-(4′ → 2′)-oligonucleotide Systems and Idealized Pairing Conformation of β-D-Ribo-(4′ → 2′)- and β-D-Xylo-(4′ → 2′)-pyranosyl Oligonucleotides.* The arrows (➡) point to sites of severe steric interaction expected to cause a major deviation from the idealized pairing conformation.



anosyl-nucleoside derivatives **2a**[5]) and **2b** in up to 78 and 91% yield, respectively (*Scheme 2*). Base-catalyzed deprotection of **2a** and **2b** gave the trihydroxy-nucleosides **3a** and **3b** in 91 and 88% yield, respectively.

Both nucleosides clearly have the β-configuration, since the ^{1}H-NMR coupling constants between the H–C(1′) and H–C(2′) show the large values (*ca.* 9 Hz, see *Table 1*) characteristic for two vicinal protons in diaxial conformation. For the nucleoside **3b** of the thymine series, an X-ray structure analysis confirmed its constitution, configuration, and preferred conformation (*Fig. 1*)[6]).

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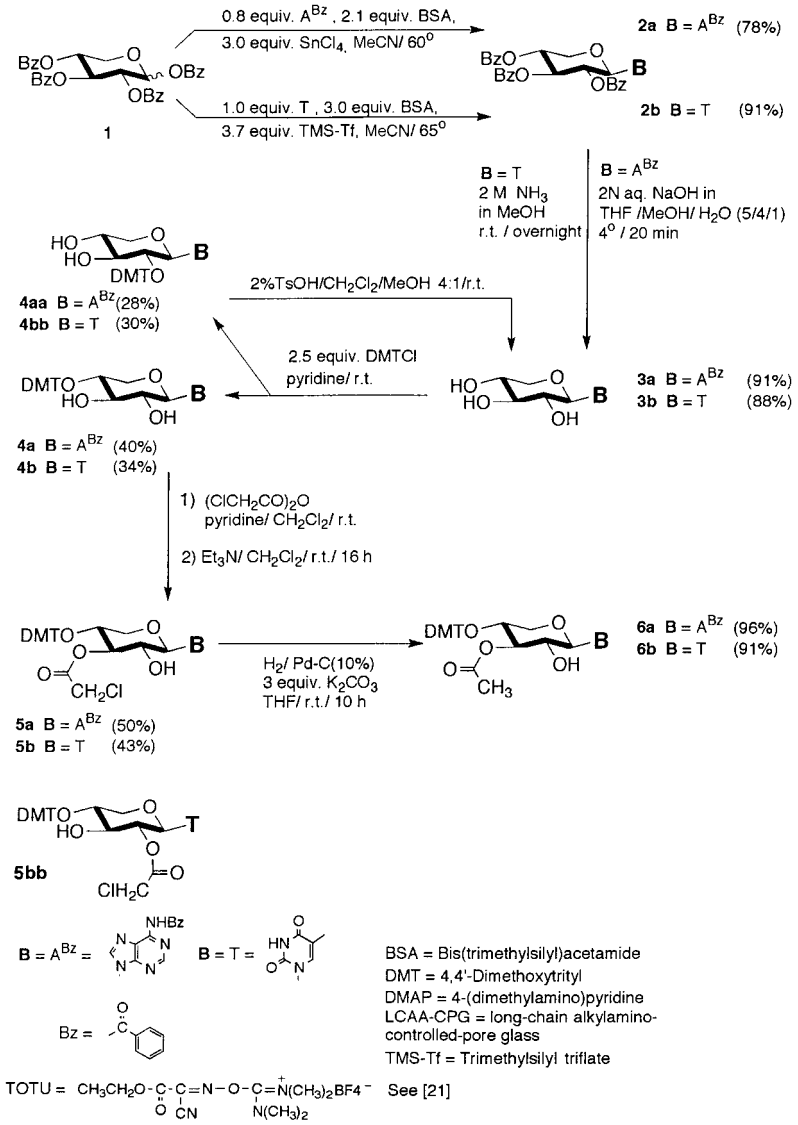
[5]) In one of the nucleosidation experiments with N^6-benzoyladenine, a side product was isolated, which, according to mass spectrum, was isomeric to, but, according to the NMR spectrum, different from, the desired N^9-nucleoside. The constitution of the product (whether it was the N^9-α-isomer or the N^7-regioisomer) was not ascertained further (see *Exper. Part*).

[6]) The X-ray analysis was carried out 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. CCDC 164910. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 union Road, Cambridge CB 12 1EZ UK (fax: +44 (1233) 336033; e-mail: deposit@ccdc.cam.ac.uk).

Scheme 2. *Preparation of A and T Nucleoside Building Blocks for the β-D-Xylopyranosyl Series*



In the further course of the synthesis, the differentiation between the three equatorial OH groups of **3a**,**b** presented, not unexpectedly, the major problem. Attempts to selectively protect OH groups in **3a**,**b** with Bz or Ac under various conditions met with failure. Therefore, it was decided to introduce the trityl group as the first step in the protection strategy, in the hope that such a bulky reagent would be best able to differentiate between the three OH groups. However, the poor solubility of the triols **3a** and **3b** in nonpolar solvents suitable for tritylation (*e.g.*, CH_2Cl_2) complicated the task. While attempts with various solvents (*e.g.*, DMF) and reagents

Table 1. *H–C(1′), H–C(2′) Coupling Constant* (*J*) *Values for Selected Compounds Depicted in* Scheme 2

	J [Hz] ((D$_6$)DMSO)	
Compound	**a**	**b**
	(A)	(T)
2	9.0	9.3
3	9.2	9.3
4	9.0	9.0
5	9.5	–
6	9.0	9.0

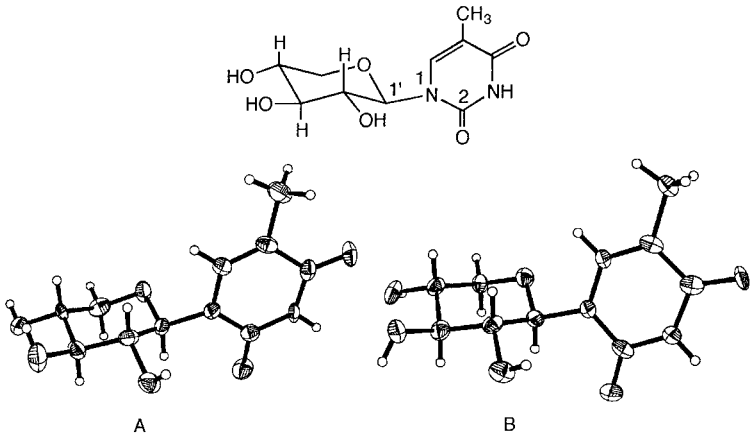


Fig. 1. *Results of an X-ray structure analysis of 2-(β-*D*-xylopyranosyl)thymine* (**3b**). The unit cell shows two types of molecules that differ in their conformations. Nucleosidic torsion angles O–C(1)–N(1)–C(2): –138.0° and –105.8° (see *Footnote 6*).

(*e.g.*, DMT-BF_4) were unsuccessful, pyridine as the solvent and dimethoxytrityl chloride as the reagent gave moderate yields of the desired 4′-*O*-tritylated products **4a** (40%) and **4b** (34%). Also isolated in these experiments, apart from unreacted starting triols (*ca.* 10%), were the 2′-tritylated diol derivatives (28–30%, determined by ^{1}H-NMR) and a compound tentatively assigned by its mass spectrum to be a bis-tritylated product. The tritylated side products were combined and recycled into the synthesis by acid hydrolysis (2% TsOH in CH_2Cl_2/MeOH 4:1, room temperature) to the starting triols **3a**,**b**.

While exploring the course of various acylation reactions in the thymine series, the use of the reactive acetylating agent $(ClCH_2CO)_2O$ led to a surprising as well as useful observation. Chloroacetylation of the tritylated derivative **4b** in CH_2Cl_2/pyridine/0°/1 h gave a mixture of the 3′-chloroacetate **5b** together with its 2′-*O*-chloroacetylated isomer **5bb** in a ratio of *ca.* 3:1 besides minor amounts of the 2′,3′-*O*-bis(chloroacetylated) derivative[7]). The assignment of the constitutions of **5b** and **5bb** was based on ^{1}H-NMR

[7]) The 2′-chloroacetate **5bb** and the 2′,3′-bis(chloroacetyl) derivatives formed in the thymine series as side products in the chloroacetylation step could be recycled by converting them back to the starting trityl derivative **4b**.

spectroscopy chemical-shift values and decoupling experiments (see *Fig. 2* and *Table 2*), as well as by chemical conversion of **5b** to **6b** (see below). When this mixture **5b**/**5bb** was kept in CH_2Cl_2 containing Et_3N (overnight, room temperature) the 3′-*O*-chloroacetyl derivative **5b** was found to have become the major component up to a ratio **5b**/**5bb** 9:1. This change in ratio pointed to the occurrence of a migration of the $ClCH_2CO$ group from the 2′-*O*- to the 3′-*O*-position, a transformation we had observed before in the p-RNA series and has become the central step in the preparation of ribopyranosyl-(4′→2′)-oligonucleotides [6]. It was established that the migration reaches an equilibrium ratio of 9:1 (starting from 0.07M **5bb**, 0.4M TSOH, 0.6M 1-methylimidazole, CH_2Cl_2, *ca.* 10 h, room temperature) in favor of the 3′-chloroacylated derivative as determined by ^{1}H-NMR (*Fig. 2*). When either the 2′-*O*-chloracetyl derivative **5bb** or the 3′-*O*-chloracetyl derivative **5b** was reacted with allyloxycarbonyl chloride in CH_2Cl_2/1-methylimidazole at room temperature, only one product was isolated (88% yield), namely the 2′-(allyloxycarbonyl)-3′-(chloroacetyl)-4′-(dimethoxytrityl)xylopyranosylthymine (see *Exper. Part*). This result demonstrates that the (2′-*O*→3′-*O*) $ClCH_2CO$ migration is taking place faster than the allyloxycarbonylation of either of the two OH groups[8]).

While the migration of the $ClCH_2CO$ group reaches the equilibrium in *ca.* 10 h at room temperature (CH_2Cl_2, Et_3N), the Ac group does not migrate at all under these conditions. For the Ac group, one has to boil the derivative in CH_2Cl_2 in the presence of Et_3N to observe any migration. The assignments of constitutions in the acetate series, again, are based on ^{1}H-NMR chemical-shift values (*Table 2*) and ^{1}H,^{1}H-decoupling experiments.

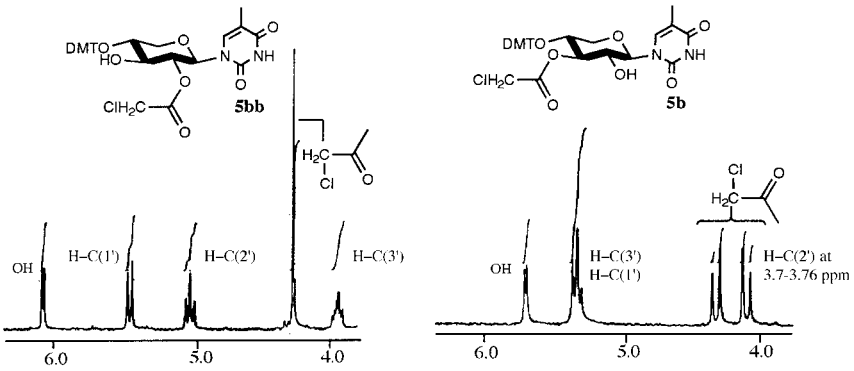


Fig. 2. *^{1}H-NMR Spectra of the 2′-chloroacetyl derivative* **5bb** *and the 3′-chloroacetyl derivative* **5b** *in (D_6)DMSO at room temperature.* The positions of the $ClCH_2COO$ groups are assigned on the basis of the chemical shift of H–C(2′), which is expected to be at lower field in the 2′-*O*-chloroacetyl derivative, identification of H–C(2′) by decoupling from H–C(1′), and further corroborated by decoupling of the OH group from H–C(3′).

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[8]) Surprising to us was not so much that the migration proceeds from the 2′- to the 3′-position (see the corresponding regioselective Bz-migration step in the synthesis of ribopyranosyl nucleosides [6]), but that the presumed intramolecular migration occurs at all, given that the configurational relationship between the two OH groups in the xylopyranosyl is *trans* (and not *cis* as the in the ribo series). As we plan to discuss in more detail in [1] (see also [6]) for the ribopyranosyl case, the origin of the driving force for the 2′→3′ migration in the xylopyranosyl series is probably of partially steric (position of the bulky nucleobase) and partially stereoelectronic (*gauche* effect) origins.

Table 2. *Chemical Shifts* (δ) *and Coupling-Constant* (J) *Values for Selected Protons of the 2′-*O*-Ac and 3′-*O*-Ac Derivatives of Thymine Nucleoside*

DMTO, R^1O, T, OR^2	δ [ppm] (multipl., J [Hz])		
	H–C(1′)	H–C(2′)	H–C(3′)
R^1 = OH, R^2 = Ac	5.59 (d, J = 9.4)	4.82 (t, J = 9.4)	4.05 (t, J = 9.0)
R^1 = Ac, R^2 = OH	5.32 (d, J = 9.0)	3.65–3.74 (m)	5.25 (t, J = 9.2)
R^1 = OH, R^2 = $ClCH_2CO$	5.49 (d, J = 9.3)	5.05 (t, J = 9.3)	3.98 (m)
R^1 = $ClCH_2CO$, R^2 = OH	5.33–5.39 (m)	3.7–3.76 (m)	5.33–5.39 (m)
R^1 = $ClCH_2CO$, R^2 = CH_2=$CHCH_2CO$	5.72 (d, J = 9.5)	5.62 (t, J = 9.4)	4.71 (t, J = 9.5)
R^1 = OH, R^2 = CH_2=$CHCH_2CO$	5.45 (d, J = 9.4)	4.8 (t, J = 9.4)	3.95 (t, J = 9.2)

It was decided[9]) to use the $ClCH_2CO$ instead of the Ac group for the preparation of the 3′-*O*-Ac-protected nucleoside derivatives **6a**,**b**, since exploratory tests showed, and experiments in preparative scales confirmed, that 3′-*O*-chloroacetyl derivatives **5a**,**b** can be converted by catalytic hydrogenation to the corresponding 3′-*O*-Ac derivatives **6a**,**b** in essentially quantitative yield. By means of this chloroacetylation/migration method as a one-pot procedure, 50% of **5a** and 43% of **5b** were obtained. Catalytic reduction of **5a** and **5b** afforded the 3′-*O*-Ac derivatives **6a** and **6b** in 96 and 91% yields, respectively.

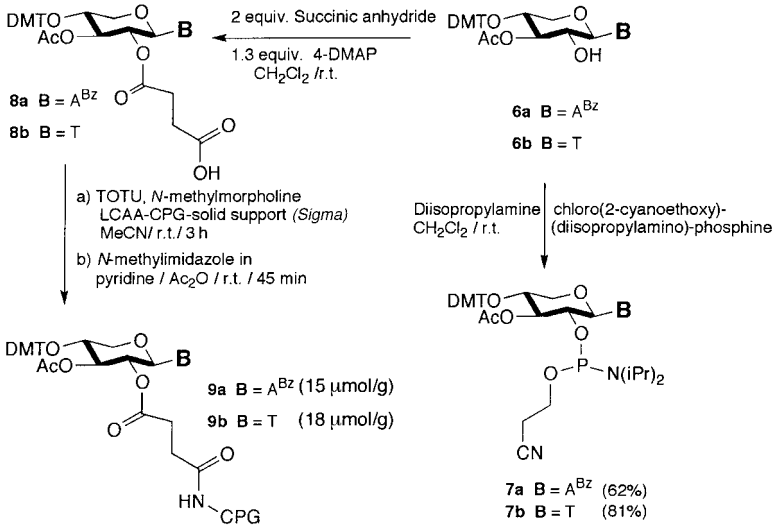
The 3′-*O*-derivatives **6a** and **6b** were the starting materials for the preparation of the building blocks required for the oligonucleotide synthesis on the gene synthesizer. Phosphoroamidites **7a** and **7b** were prepared with $CNCH_2CH_2$ as the protecting group on the phosphoroamidate (*Scheme 3*). Solid-support ('long-chain alkylamine-CPG', *Sigma*) derivatives **9a** and **9b**, were obtained by standard methodology, with succinic anhydride as the linker, as outlined in *Scheme 3* (*cf.*, *e.g.*, [3]).

2.1.2. *Synthesis, Deprotection and Purification of Oligonucleotides.* The preparation of β-D-xylopyranosyl-(4′ → 2′)-oligonucleotides was carried out on a 1-μM scale, as recently described in detail for α-D-lyxopyranosyl-(4′ → 2′)-oligonucleotides series [3], on an automated DNA synthesizer (*Perseptive*'s Expedite) with *ca.* 0.1M solution of phosphoroamidites **7a** and **7b** in MeCN and 35–40 mg of the required solid-support derivatives **9a** and **9b**, respectively. The syntheses were preformed in the 'trityl-on' mode. The deblocking, coupling, capping, and oxidizing protocols in the automated oligonucleotide synthesis had to be adapted to the special problems posed by the xylose system (see *Exper. Part*). For example, while applying the usual conditions [3] in the oxidation step, oligomer synthesis more or less failed; a four-fold extension of the oxidation time (followed by thorough washing) was found to be necessary to have acceptable yields of the desired isomer. We were able to achieve by such modifications coupling efficiencies ranging from 90 to > 95% (trityl assay).

The workup of oligomer syntheses involved treating the dried CPG solid support with dry pyridine/Et_3N 4:1 in order to initiate the removal of the $CNCH_2CH_2$ protecting group, converting the (labile) phosphotriester linkages to phosphodiester linkages, thus stabilizing the oligonucleotide linkage against the strongly basic conditions of the further deprotection steps [10]. Attempts to achieve detachment and deprotection by treatment with aqueous hydrazine hydrate at 4°, a method found

[9]) Decided by *T.W.* (*A.E.*)

Scheme 3. *Preparation of the Phosphoroamidite and CPG Derivatives for the Automated Synthesis of β-D-Xylopyranosyl-(4′→2′)-oligonucleotides* (for abbreviations, see *Scheme 2*)



to be useful in the p-RNA series [6], led to extensive strand scission. Eventually, it was treatment with either a mixture of 40% aq. $MeNH_2$, and 30% aq. NH_3, or a solution of 0.2M $MeONH_2 \cdot HCl$ in conc. aq. NH_3/EtOH 3:1 at 4° that proved to be successful for detaching the oligonucleotides from the CPG solid support and concomitantly removing the 3′-*O*-Ac and the Bz groups at the sugar OH and the adenine amino group, respectively. Desalting with 0.5M $Et_3NH_2CO_3$ over a *Waters Sepak-C_{18}* cartridge removed the excess base[10]). The resulting crude oligonucleotides were detritylated with 80% aqueous HCO_2H at room temperature and subsequently purified by ion-exchange HPLC (target purity at least 95%). Purified oligonucleotides were desalted and stored at −20°. Molecular weights and purities of the oligomers were checked by matrix-assisted laser-desorption inonization time-of-flight (MALDI-TOF) mass spectrometry, concentrations of stock solutions were determined by UV-spectroscopically (at a temperature of *ca.* 80°, wavelength of 260 nm, with values of ε (px(A)) = 15000, ε (px(T)) = 10000). *Table 3* lists the oligonucleotides synthesized, purified, and analyzed by MALDI-TOF MS.

2.2. *Pairing Studies.* The pairing properties of β-D-xylopyranosyl-(4′→2′)-oligonucleotides ('px-oligos') were characterized by temperature-dependent UV spectroscopy (T_m measurements [11]), concentration-dependent T_m measurements (for determination of thermodynamic data, see [12]), molar-ratio-dependent UV spectroscopy (for checking stoichiometry [13]), 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 about

[10]) This desalting procedure to remove the excess base is successful only with sequences that have their final trityl group on. With detritytlated sequences, the efficiency of separation was much less due to the tendency of these oligonucleotides to 'flow-through' with the initial washings.

Table 3. *HPLC and MS Data of β-D-Xylopyranosyl-(4′→2′)-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])	
				$[M+H]^+$ (obs.)	$[M+H]^+$ (calc.)
p**x**(A_8)	*A*	7.0 (6%)	10–50% in 30 min/17.5	2501	2498
p**x**(T_8)	*A*	13.0 (13%)	20–80% in 30 min/21.2	2573	2570
p**x**(A_{12})	*A*	10.1 (7%)	0–100% in 30 min/15.6	3886	3889
p**x**(T_{12})	*B*	9.6 (8%)	0–100% in 30 min/22.6	3782	3780
p**x**(A_4T_4)	*B*	14 (8%)	10–70% in 30 min/21.5	2535	2534
p**x**(T_4A_4)	*A*	25 (16%)	10–70% in 30 min/19.2	2534	2534
p**x**$(AT)_4$	*A*	13 (7%)	15–60% in 30 min/21.2	2557	2534
p**x**$(TA)_4$	*A*	13 (9%)	15–60% in 30 min/19.7	2536	2534
p**x**(TATTTTAA)	*B*	23.5 (22%)	–	2529	2527
p**x**(TTAAAATA)	*B*	12.1 (14%)	0–100% in 30 min/17.4	2547	2545

[a]) *Method A:* 0.2M $MeONH_2 \cdot HCl$ in 25%. aq. NH_3 and EtOH (3:1) at r.t. for *ca.* 6 h. *Method B:* 40% aq. $MeNH_2$ in conc. aq. NH_3 (1:1) at r.t. for 6 h. [b]) 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 *Sep-Pak* cartridges. [c]) Matrix-assisted laser-desorption ionization time-of-flight mass spectrometry; matrix: 2,4,6-trihydroxyacetophenone and ammonium citrate buffer.

10 μM, unless otherwise stated. Our observations are summarized in *Table 4* and illustrated in *Figs. 3* and *4*, which complement figures published in the preliminary communications [4][5].

Though the number of base sequences studied is relatively small, a sufficiently informative picture about the pairing behavior emerges. Base pairing in the β-D-xylopyranosyl series is comparable to that in the ribopyranosyl series (*Table 4*) although the general trend seems to be towards somewhat weaker pairing strength (*Table 4*, *Entries 3*, *6–10*, and *13*). As is the case with the other members of the pentopyranosyl oligonucleotide family [4], strand orientation in px duplexes is antiparallel (*Table 4*, *Entries 11–13*, and *Fig. 4*), and the pairing mode is assigned to be *Watson-Crick*. The latter conclusion is based on the observation that px duplexes undergo efficient intersystem cross-pairing with antiparallel-complementary strands from all other members of the pentopyranosyl oligonucleotide family (for a summary of our observations on intersystem cross-pairing within this series, see *Table 1* in [5]). Operation of *Watson-Crick* pairing mode has been shown by *Jaun* and co-workers for duplexes of the (self-complementary) base sequence $(CGAATTCG)_2$ in both the ribo- and arabinopyranosyl series [14][15].

The duplex formed by the self-complementary block sequence p**x**(A_4T_4) melts at a considerably lower temperature ($T_m = 16.3$; $c = 10$ μM, 150 mM NaCl) than its 'mirror sequence' p**x**(T_4A_4) ($T_m = 40.3$). The same, though to a lesser degree, holds for the two alternating sequences p**x**$(AT)_4$ and p**x**$(TA)_4$ (*Entries 9* and *10* in *Table 4*). This sort of sequence dependence of T_m is common to all four pentopyranosyl systems and has been postulated to be a consequence of the pronounced base-pair-axis inclination, which is characteristic for these systems and which is responsible for the fact that base-stacking is essentially *inter*strand stacking [6][15][16][17]. The T_m difference between block sequences, such as (A_4T_4) and (T_4A_4), is more pronounced in the px and pr series

Table 4. T_m *Values and Thermodynamic Data*

Entry	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]) p**x**(4′→2′) duplexes 1M NaCl ($c \approx 10$ μM)	T_m [°C][a]) p**x**(4′→2′) duplexes 150 mM NaCl ($c \approx 10$ μM)	For comparison: T_m's of p**r**(4′→2′) duplexes 150 mM NaCl ($c \approx 10$ μM)[a])	150 mM NaCl[a])[b]) p**x**(4′→2′) duplexes			Ref.
						ΔG (25°C)	ΔH [kcal/mol]	$T\Delta S$ (25°C)	
1	p**x**(A_8)								[4]
2	p**x**(T_8)								[4]
3	p**x**(A_8) + p**x**(T_8)		47.3*	35.4	40*	– 8.2*	– 39.3*	– 31.1*	[4]
4	p**x**(A_{12})	7.3							[5]
5	p**x**(T_{12})								[5]
6	p**x**(A_{12}) + p**x**(T_{12})		73.2	63.0	60.8	– 17.0	– 82.9	– 65.9	[5]
7	p**x**(A_4T_4)			16.3*	27*	– 6.1*	– 27.4*	– 21.3*	[4]
8	p**x**(T_4A_4)			40.3*	40*	– 8.7*	– 40.1*	– 31.4*	[4]
9	p**x**(AT)$_4$			28.6*	38*	– 6.2*	– 33.9*	– 26.7*	[4]
10	p**x**(TA)$_4$			33.8*	40*	– 7.6*	– 28.7*	– 21.1*	[4]
11	p**x**(TATTTTAA)	23							[5]
12	p**x**(TTAAAATA)	10							[5]
13	p**x**(TATTTTAA) + p**x**(TTAAAATA)		44.4	33.3	38.8	– 8.7	– 39.9	– 30.4	[5]

[a]) Measurements were made in 0.01M NaH_2PO_4, 0.1 mM Na_2(EDTA) buffer, pH 7.0 unless otherwise indicated. Error of T_m determination estimated ±0.5°. Values with asterics (*) were measured in 0.01M *Tris*·HCl buffer, pH 7.0.

[b]) Thermodynamic data from plots of T_m^{-1} *vs.* ln c; experimental error estimated in ΔH values ±5%.

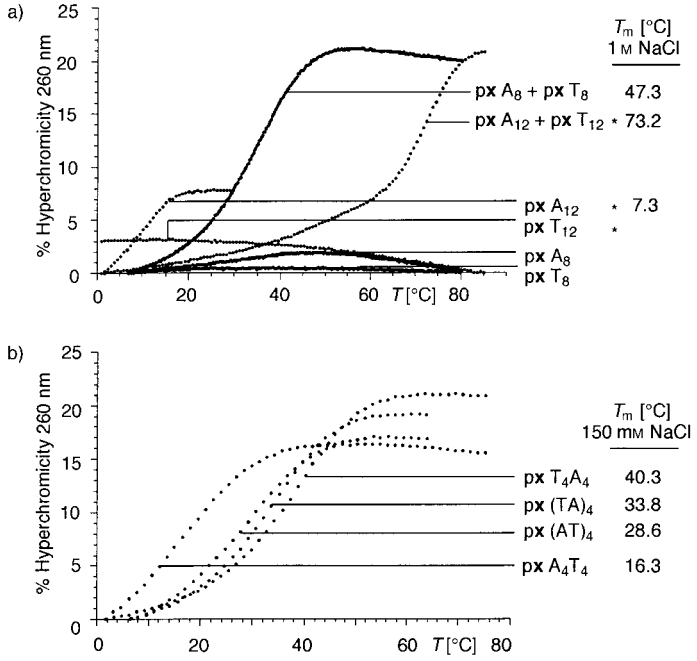


Fig. 3. *UV-*T_m *Data documenting the pairing behavior of A,T-containing (4′ → 2′)-β-*D*-xylopyranosyl sequences. a*) UV-Spectroscopic T_m melting curves (heating) for the individual homobasic sequences and for duplexes formed between px(A_8), px(T_8), and px(A_{12}), px(T_{12}) oligonucleotides. *b*) UV-Spectroscopic T_m curves (heating) of the duplexes formed by self-complementary A,T-containing sequences. T_m Measurements were made in 10 mM aq. *Tris* · HCl buffer, 1M NaCl at pH 7.0, except those marked with an asterisks* (phosphate buffer). Total oligonucleotide concentrations in all measurements were *ca.* 10 μM. All T_m curves were reversible (no hysteresis). T_m Values are calculated from the maxima of the first derivative curve by means of the kaleidagraph software program.

($\Delta T_m = 24°$ and $\Delta T_m = 13°$, resp.) than in the pl- ($\Delta T_m = 8.8°$) and pa series ($\Delta T_m = 8.2°$) [1][4][6][16]. This is consistent with the view that pentopyranosyl systems containing their phosphodiester group in 4′,2′-diequatorial position (px and pr) can be expected to have a more pronounced backbone/basepair-axis inclination than the systems in which this group in the 4′-position is axial (pl and pa) [1][17]. Interstrand stacking must be more important in the former system than in the latter. Interestingly enough, the conformational difference is reflected in differences in the corresponding CD spectra. While the CDs of the px and pr systems (4′-equatorial) are strikingly similar, they are different from those of the pl and pa systems (4′-axial) (see *Fig. 2* in [4]).

3. Discussion. – The finding that duplexes derived from β-D-xylopyranosyl-(4′ → 2′)-oligonucleotide strands display thermal stabilities comparable to those of corresponding β-D-ribopyranosyl duplexes came as a surprise to us. In an early attempt to qualitatively predict the relative strength of base pairing among the members of the pentopyranosyl oligonucleotide family [6], we had ventured to conjecture that, since the (idealized) pairing conformation of xylopyranosyl-(4′ → 2′)-oligonucleotides will be

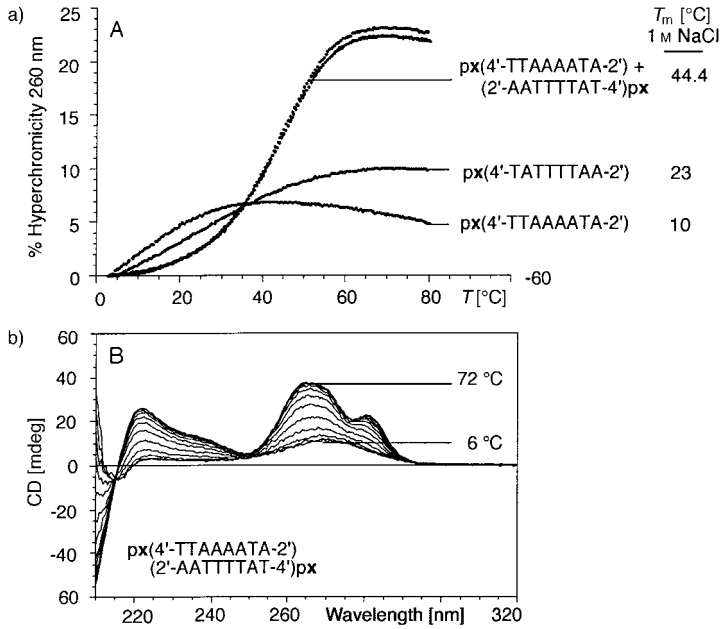


Fig. 4. *UV- and CD-spectroscopic data documenting the pairing behavior of px(4′-TTAAAATA-2′) with its antiparallel complement px(4′-TATTTTAA-2′).* *a*) UV-Spectroscopic T_m curves of the duplex formation and self-pairing of the individual strands. *b*) Temperature-dependent CD curves of the duplex; temperature range: 6° → 75°. Measurements were made in 10 mM aq. NaH_2PO_2 containing 0.1 mM Na_2(EDTA), 1M NaCl at pH 7.0. Total oligonucleotide concentration is *ca.* 10 μM.

sterically strongly hindered (see *Scheme 1* and *Fig. 5*), such strands are expected to pair either not at all or at least more weakly than their p-RNA isomers [6]. In retrospect, it seems worthwhile to analyze possible reasons for this discrepancy between prediction and observation. For this purpose, *Fig. 5* resumes our earlier reasoning with regard to relative pairing strengths in the xylo- and ribopyranosyl series: for each system the three (idealized) staggered phosphodiester conformations arising through rotation around the C(2′)–O bond are depicted. Such formulas represent repetitive conformations and, therefore, possible pairing conformations of corresponding oligomer strands. Whereas in the ribopyranosyl system one of these conformations (**a** in *Fig. 5*) has 'minimal strain'[11]) and was therefore, postulated to represent the pairing-conformation of pyranosyl-RNA (which later turned out to be correct [14]), the corresponding conformation **d** in the xylopyranosyl series is clearly highly strained (see bold arrow). This feature was deemed to prevent population of this specific conformation in a xylopyranosyl oligomer and, therefore, impede or prevent duplex formation. In retrospect, the finding that xylopyranosyl-(4′ → 2′)-oligonucleotides do, in fact, undergo base pairing and, furthermore, do efficiently cross-pair with pyranosyl-RNA [5],

[11]) The term 'minimal strain' in this context is taken to mean that a conformation, while being part of the ensemble of idealized conformations (see Scheme 1 in [6]), does not suffer the type of severe '*Newman* strain' as indicated by ➡ *in Scheme 1* and *Fig. 5* (for the term '*Newman* strain' see *G. Quinkert* [18]).

reminds us of an aspect that we had overlooked in that earlier conformational reasoning, namely, strain in a pairing-conformation will impede duplex formation if, and only if, there are nonrepetitive nonpairing conformations accessible to the system that are more stable than the (repetitive) pairing conformation. To decide whether this might be the case in the xylopyranosyl system has been, and still is, beyond the potential of a qualitative conformational analysis based on idealized conformations.

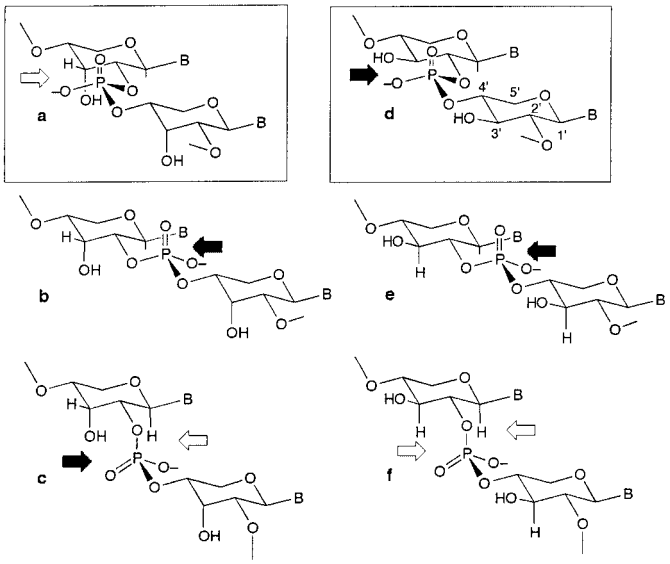


Fig. 5. *Qualitative steric-strain analysis of idealized, repetitive conformations of the repetitive unit of β-*D*-ribopyranosyl-* (left) *and β-*D*-xylopyranosyl-* (4′ → 2′)-oligonucleotides. (right). ⇨ Denotes weak, ➡ strong steric repulsion (*Newman*-strain [18])

The work was supported by the *Skaggs Foundation* (*TSRI*). *T. W.* thanks the *Deutsche Forschungsgemeinschaft* for fellowship support. We thank Dr. *A. Shyvaniuk* for the ORTEP drawing of **2b** (*Fig. 1*).

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-cyanoethoxy)(diisopropylamino)phosphine (97%) was purchased from *Chem-Impex Inc.*, Wood Dale, IL, USA. TLC: silica gel *60* F_{254} aluminum plates (*Whatman*, Type *Al Sil G/UV*, 250 μm layer); visualization by UV absorption and/or *A*) by dipping in a soln. 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 μm, 230–440 mesh, *EM Science*) at low pressure (max. 2 bar). In case of acid-sensitive compounds, the silica gel was pretreated with solvents containing *ca*. 0.5% Et_3N. NMR: ^1H: δ values in ppm (TMS as internal standard), *J* [Hz], assignments of ^1H resonances were in some cases based on 2D experiments (^1H,^1H-COSY); ^{13}C: δ values in ppm (TMS as internal standard), *J* [Hz]; assignments and multiplicities were based on 2D experiments (^1H,^{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 cesium-ion gun. Matrix-assisted laser-desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was performed on a *Voyager-Elite* mass spectrometer (*Perseptive Biosystems*) with delayed extraction with THAP as the matrix with ammonium citrate added to the sample. Elemental analysis were performed using *Perkin-Elmer PE2400 CHN* analyzer. Oligonucleotides were

synthesized on an *Expedite 8909 Nucleic Acid Synthesis* system (*Perseptive Biosystems*). HPLC: Anion exchange (IA)-HPLC was performed on *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 Spectroscopy was measured on a *Cary 1 C* spectrophotometer (*Varian*). Melting-point (T_m) measurements of oligonucleotides were determined with *Cary 1 Bio* spectrophotometer (*Varian*). All measurements were made with the 'phosphate buffer', 10 mM aq. NaH_2PO_4 buffer containing 0.1 mM Na_2(EDTA), 150 mM (or 1M) NaCl at pH 7.0, with a total oligonucleotide concentration of *ca.* 10 μM. Concentrations of oligonucleotide solns. were calculated from the UV absorbance of the solns. at 260 nm (pH 7) with the following molar extinction coefficients: $\varepsilon(px(A)) = 15000$, $\varepsilon(px(T)) = 10000$. CD Spectrum was measured *AVIV 61 DS* CD spectropolarimeter. Abbreviations: BSA: *N,O*-Bis(trimethylsilyl)acetamide, CPG: 'controlled-pore glass', DMAP: 4-(dimethylamino)pyridine, DMF: dimethylformamide, DMT: 4,4′-dimethoxytrityl, LCAA-CPG: long-chain aminoalkyl-CPG (500 Å), TMS-Tf: trimethylsilyl trifluoromethanesulfonate, TOTU: *O*-{[(2-cyanoethoxycarbonyl)methyliden]amino}-1,1,3,3-tetramethyluronium tetrafluoroborate.

1. Experiments Referring to *Scheme 2*. – N^6-*Benzoyl-9-(2′,3′,4′-tri*-O-*benzoyl-β*-D-*xylopyranosyl)adenine* (**2a**). A suspension of 34 g (60 mmol) of **1** [7] and 11.3 g (50 mmol) of N^6-benzoyladenine in 300 ml of dry MeCN was warmed to 60° (oil bath). Addition of 30.5 ml (125 mmol) of BSA resulted in a clear soln. After 30 min, 21.1 ml (180 mmol) of $SnCl_4$ was added dropwise (→ exothermic reaction), and stirring was continued for another 60 min. The mixture 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 and then washed successively with 2 × 100 ml Na_2CO_3, H_2O, and brine. The org. phase was dried ($MgSO_4$) and evaporated. The resulting oil was purified by CC on silica gel (hexane/AcOEt 1:2). The product fractions were combined, evaporated, and dried under high vacuum (*ca.* 0.5 Torr, r.t.) to furnish 26.6 g (78%) of a colorless amorphous solid **2a**. TLC (toluene/EtOAc 1:2): R_f 0.21. ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.25 (*t*, J_{gem} = 10.8, H_{ax}–C(5′)); 4.41 (*dd*, *J* = 10.8, 5.0, H_{eq}–C(5′)); 5.61–5.70 (*m*, H–C(4′)); 6.28 (*t*, *J* = 9.2, 9.0, H–C(3′)); 6.42 (*t*, *J*(1′,2′) = 9.0, *J*(2′,3′) = 9.0, H–C(2′)); 6.59 (*d*, *J* = 9.0, H–C(1′)); 7.31–7.89 (*m*, 20 H of Bz); 8.75 (*s*, H–C(8)); 8.89 (*s*, H–C(2)). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 65.15 (*t*, C(5′)); 69.90, 71.91, 73.87, 81.05 (4*d*, C(1′), C(2′), C(3′), C(4′)); 125.74, 128.6 (2*s*), 129.46, 129.66, 129.73, 129.77, 129.95, 130.14, 133.39, 134.09, 134.72, 134.81, 143.87, 151.4 (*d* + *s*, arom. C); 165.24, 165.86, 166.13, 166.49 (4*s*). FAB-MS (pos., NBA): 706 (100, $[M + Na]^+$), 684 (82, $[M + H]^+$), 445 (23).

In one of the runs of the nucleosidation experiment with N^6-benzoyladenine, a side-product (possibly the epimeric nucleoside) was isolated showing the following data: ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.25 (*t*, J_{gem} = 10.9, H_{ax}–C(5′)); 4.41 (*dd*, *J* = 10.9, 5.6, H_{eq}–C(5′)); 5.61–5.70 (*m*, H–C(4′)); 6.23 (*t*, *J* = 8.7, H–C(3′)); 6.3–6.4 (*m*, H–C(2′), H–C(1′)); 7.31–7.89 (*m*, 20 H of Bz); 8.15 (*s*, H–C(8)); 8.58 (*s*, H–C(2)). HR-FAB-MS (pos., NBA): 706.1886 (15, $[M + Na]^+$); 684 (7, $[M + H]^+$); 602 (87); 580 (100).

1-(2′,3′,5′-Tri-O-*benzoyl-β*-D-*xylopyranosyl)thymine* (**2b**). A suspension of 4.58 g (8.1 mmol) of **1** and 1.02 g (8.1 mmol) of dry thymine in 100 ml of dry MeCN was warmed to 65° (oil bath), followed by the addition of 6.1 ml (25.0 mmol) of BSA. After stirring for 60 min, 4.5 ml (30 mmol) of TMS-Tf was added by syringe to the clear soln., and the mixture was kept for 2 h at 65°. The mixture 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 successively with sat. aq. $NaHCO_3$, H_2O, and brine, then dried (Na_2SO_4). Concentration to dryness gave 4.22 g (91%) of the desired product **2b**. This material was used in the next step without further purification. TLC (AcOEt/hexane 1:1): R_f 0.54. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.82 (*s*, Me); 4.15 (*t*, J_{gem} = 11.0, H_{ax}–C(5′)); 4.35 (*dd*, *J* = 11.5, H_{eq}–C(5′)); 5.70–5.62 (*m*, H–C(4′)); 5.96 (*t*, *J* = 9.3, H–C(2′)); 6.16 (*t*, *J* = 9.3, H–C(3′)); 6.28 (*d*, *J*(1′,2′) = 9.0, H–C(1′)); 7.37–8.06 (*m*, 15 H of Bz, H–C(6)). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 12.76 (*q*, Me); 64.83 (*t*, C(5′)); 69.74, 71.33, 73.81, 80.85, (4*d*, C(1′), C(2′), C(3′), C(4′)); 110.95 (*s*, C(5)); 129.05, 129.51, 129.71, 129.91, 130.09, 134.64, 134.72, 134.84, 137.47 (*d*, C(6)); 149.31 (*d* + *s*, arom. C); 151.40, 164.43, 165.65, 165.87, 166.08 (5 C=O). FAB-MS (pos., NBA): 593 (48, $[M + Na]^+$); 571 (17, $[M + H]^+$), 445 (100).

N^6-*Benzoyl-9-(β*-D-*xylopyranosyl)adenine* (**3a**). To a soln. of 17.0 g (24.9 mmol) of **2a** in 300 ml of a mixture of THF/MeOH/H_2O 5:4:1 was added dropwise 150 ml of aq. NaOH (2N) at 4°. The soln. was stirred for 20 min (checked by TLC). The pH of the resulting soln. was adjusted to 6–7 (pH-paper) by dropwise addition of conc. aq. HCl. Subsequently, the solvent was removed under vacuum (*ca.* 20 Torr, r.t.), and the resulting residue was washed with H_2O, Et_2O, and dried under high vacuum for overnight gave 8.37 g (91%) of **3a** as a white powder. TLC (CH_2Cl_2/MeOH 8:2): R_f 0.17. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.30 (*m*, H–C(2′), H–C(3′)); 3.51 (br. *m*, H–C(4′)); 3.89 (*dd*, *J* = 10.9, 5.0, H_{ax}–C(5′)); 4.1 (br. *m*, H_{eq}–C(5′)); 5.21 (br. *s*, OH–C(4′)); 5.35 (br. *s*, OH–C(3′)); 5.49 (br. *s*, OH–C(2′)); 5.51 (*d*, *J* = 9.2, H–C(1′)); 7.52–7.67 (*m*, 3 H of Bz); 8.67, 8.75 (2*s*, H–C(2), H–C(8)). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 69.25 (*t*, C(5′)); 69.98, 71.18, 78.04,

84.62 (4*d*, C(1′), C(2′), C(3′), C(4′)); 126.37, 129.36, 129.38, 133.37, 134.22, 144.34, 151.17, 152.65 (*d* + *s*, arom. C); 166.58 (C=O). FAB-MS (pos., NBA): 394 (43, $[M+Na]^+$), 372 (100, $[M+H]^+$). Anal. calc. for $C_{17}H_{17}N_5O_5 \cdot H_2O$: C 52.43, H 4.93, N 18.0; found: C 52.23, H 4.81, N 18.43.

*1-(β-*D*-Xylopyranosyl)thymine* (**3b**). A soln. of 27.5 g (48.2 mmol) of **2b** in 600 ml of 2.0M NH_3 in MeOH was stirred overnight at r.t. The precipitate was filtered and washed with cold MeOH and acetone. The filtrate was concentrated to 1/3 of its volume, and the resulting precipitate was combined with the first batch to afford 10.9 g (88%) of **3b**. TLC (CH_2Cl_2:MeOH 10:1): R_f 0.20. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.77 (*s*, Me); 3.1–3.5 (*m*, H–C(3′), H–C(4′), H_{ax}–C(5′), H_{eq}–C(5′)); 3.75 (*dd*, *J*(1′,2′) = 9.3, *J*(2′,3′) = 5.7, H–C(2′)); 5.0–5.4 (br. *s*, OH–C(2′), OH–C(3′), OH–C(4′)); 5.2 (*d*, *J*(1′,2′) = 9.3, H–C(1′)); 7.56 (*s*, H–C(6)). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 12.60 (*q*, Me); 69.09 (*t*, C(5′)); 69.82, 71.20, 73.84, 83.91 (4*d*, C(1′), C(2′), C(3′), C(4′)); 110.30 (*d*, C(5)); 137.70 (*s*, C(2)); 151.91 (*d*, C(6)); 164.68 (*s*, C(4)). FAB-MS (pos., NBA): 281 (100, $[M+Na]^+$), 259 (30, $[M+H]^+$). Anal. calc. for $C_{10}H_{14}N_2O_6$: C 46.51, H 5.46, N 10.85; found: C 46.60, H 5.68, N 10.83.

*N⁶-Benzoyl-9-{4′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-β-*D*-xylopyranosyl}adenine* (**4a**). To a suspension of 10.1 g (27.3 mmol) of **3a** and molecular sieves (4 Å) in 200 ml of dry pyridine was added 23.1 g (68.2 mmol) of DMT-Cl at r.t. The mixture was shaken gently for overnight. After filtration, the soln. was diluted with AcOEt, extracted with H_2O, dried (Na_2SO_4), evaporated, and the residue was co-evaporated with toluene. The residue was purified by CC (silica gel; petroleum ether/AcOEt 1:2 to 0:1) to give 7.3 g (40%) of **4a** and 5.14 g (28%) of the 2′-*O*-DMT derivative **4aa**.

Data of **4a**: TLC (CH_2Cl_2/MeOH 10:1): R_f 0.58. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.19 (*dd*, *J* = 10.9, 4.6, H_{eq}–C(5′)); 2.93 (br. *t*, *J* = 10.9, H_{ax}–C(5′)); 3.82–3.59 (*m*, H–C(3′), H–C(4′), 2 MeO); 4.13–4.05 (*m*, H–C(2′)); 5.41 (*d*, *J*(1′,2′) = 9.0, H–C(1′)); 5.58 (*d*, *J* = 5.5, OH–C(2′)); 5.89 (*d*, *J* = 5.0, OH–C(3′)); 6.87–8.04 (*m*, 13 H of $(MeO)_2$Tr, 5 H of Bz); 8.59, 8.70 (2*s*, H–C(2), H–C(8)). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 55.78 (2*q*, MeO); 67.76 (*t*, C(5′)); 71.79, 72.83, 76.94, 84.14 (4*d*, C(1′), C(2′), C(3′), C(4′)); 86.46 (*s*, Ar_3C); 113.62, 113.98 (2*d*); 126.33, 127.45, 128.56, 129.01, 129.10, 129.35, 129.81, 131.10, 134.20, 137.20, 138.06, 147.11, 151.15, 153.45, 159.13, 159.18 (*d* + *s*, arom. C); 166.50 (C=O). FAB-MS (pos., NBA): 696 (100, $[M+Na]^+$), 674 (95, $[M+H]^+$).

*1-{4′-*O*-[(4″,4‴-Dimethoxytriphenyl)methyl]-β-*D*-xylopyranosyl}thymine* (**4b**). To a suspension of 2.3 g (8.9 mmol) of **3b** and molecular sieves (4 Å) in 50 ml of dry pyridine were added 7.5 g (22.3 mmol) of DMT-Cl at r.t. The mixture was shaken gently overnight. After filtration, the soln. was diluted with AcOEt, extracted with H_2O, dried (Na_2SO_4), evaporated, and the residue was co-evaporated with toluene. The residue was purified by CC (silica gel; petroleum ether/AcOEt 1:2 to 0:1) to give 1.7 g (34%) of **4b** and 1.5 g (30%) of 2′-*O*-DMT derivative **4bb**.

Data of **4b**: TLC (CH_2Cl_2/MeOH 9:1): R_f 0.58. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.70 (*s*, Me); 2.06–2.11 (*dd*, *J* = 10.2, 4.5, H_{ax}–C(5′)); 2.77–2.70 (*t*, *J* = 10.2, H_{eq}–C(5′)); 3.47–3.73 (*m*, 2 MeO, H–C(2′), H–C(3′), H–C(4′)); 5.14 (*d*, *J*(1′,2′) = 9, H–C(1′)); 5.41, 5.76 (2*d*, *J* = 5.0, OH–C(3′), OH–C(2′)); 6.87 (*d*, 4 arom. H); 7.17–7.62 (*m*, 9 H of $(MeO)_2$Tr, H–C(6)). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 12.50 (*q*, Me); 55.78 (2*q*, MeO); 67.69 (*t*, C(5′)); 71.23, 72.69, 76.91, 83.58 (4*d*, C(1′), C(2′), C(3′), C(4′)); 86.37 (*s*, Ar_3C); 110.20 (*s*, C(5)); 113.928, 127.46, 128.51, 129.03, 131.08, 131.32, 137.34 (*d*, C(6)); 137.69, 138.09, 147.08, 151.86 (*s*, C(2)); 159.11, 159.16, 164.62 (C(4)). FAB-MS (pos., NBA): 583 (100, $[M+Na]^+$), 560 (55, $[M+H]^+$).

*Data of the 2′-*O*-DMT Derivative* **4bb**: ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.75 (*s*, Me); 2.92 (apparent *t*, *J* = 9.9, H–C(5′)); 3.18 (apparent *t*, *J* = 7.8, H–C(5′)); 3.6–3.75 (*m*, 2 MeO, H–C(3′)); 3.75–3.85 (*m*, H–C(2′), H–C(4′)); 4.41 (*d*, *J* = 4.3, OH–C(3′)); 4.97 (*d*, *J* = 5.4, OH–C(4′)); 5.05 (*d*, *J*(1′,2′) = 9.1, H–C(1′)); 6.77 (*d*, 4 arom. H); 7.17–7.62 (*m*, 9 H of $(MeO)_2$Tr, H–C(6)).

*N⁶-Benzoyl-9-{3′-*O*-(chloroacetyl)-4′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-β-*D*-xylopyranosyl}adenine* (**5a**). To a soln. of 8.56 g (12.7 mmol) of **4a** in 100 ml of CH_2Cl_2/pyridine 4:1 was added 3.26 g (19.1 mmol) of $(ClCH_2CO)_2O$ at 0°. The soln. was stirred for 1 h, diluted with 200 ml of CH_2Cl_2, and washed twice with 100 ml of sat. aq. $NaHCO_3$ soln. The org. phase was dried (Na_2SO_4), filtered, evaporated, and co-evaporated with toluene. The residue was dissolved in 80 ml of CH_2Cl_2 and 12.35 ml (89.0 mmol) of Et_3N, and the stirring was continued overnight at r.t. The soln. was concentrated, and the residue was purified by CC (silica gel; toluene/AcOEt 1:2) to give 4.3 g (50%) of **5a**. TLC (toluene/EtOAc 1:2): R_f 0.42. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.95 (br. *d*, *J* = 6.4, H_{eq}–C(5′)); 3.75–3.52 (*m*, H–C(4′), H_{ax}–C(5′), 2 MeO); 4.18 (*d*, J_{gem} = 8.0, 1 H, $ClCH_2CO$); 4.38–4.33 (*m*, H–C(2′)); 4.41 (*d*, J_{gem} = 8.0, 1 H, $ClCH_2CO$); 5.51 (br. *t*, *J* = 8.9, H–C(3′)); 5.68 (*d*, *J*(1′,2′) = 9.5, C(1′)); 5.88 (*d*, *J* = 5.5, OH–C(2′)); 6.91–8.03 (*m*, 13 H of $(MeO)_2$Tr, 5 H of Bz); 8.632/8.72 (2*s*, H–C(2), H–C(8)). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 42.16 (*t*, $ClCH_2$); 55.82 (2*q*, OMe); 67.40 (*t*, C(5′)); 69.45, 70.87, 79.08, 83.56 (4*d*, C(1′), C(2′), C(3′), C(4′)); 86.83 (*s*, Ar_3C); 114.13, 126.31, 127.75, 128.42, 128.71, 129.36, 130.85, 130.89, 134.15, 136.74, 136.96, 144.26, 146.81, 151.27, 152.74, 153.43, 159.26, 159.30 (*d* + *s*,

arom. C); 166.50 (*s*); 167.90, 166.52 (2*s*, 2 C=O). FAB-MS (pos., NBA): 772/774 (85/55, $[M+Na]^+$), 750/752 (100/35, $[M+H]^+$). Anal. calc. for $C_{40}H_{36}ClN_5O_8$: C 64.04, H 4.84, N 9.34; found: C 63.74, H 4.82, N 9.35.

*1-{3′-*O*-(Chloroacetyl)-4′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-β-*D*-xylopyranosyl}thymine* (**5b**). A soln. of 2.4 g (4.28 mmol) of **4b** in 50 ml of CH_2Cl_2/pyridine 4:1 were added 1.10 g (6.42 mmol) of $(ClCH_2CO)_2O$ at 0°. The soln. was stirred for 1 h, diluted with 100 ml of CH_2Cl_2, and washed twice with 50 ml of sat. aq. $NaHCO_3$ soln. The org. phase was dried (Na_2SO_4), filtered, evaporated, and co-evaporated with toluene. To bring about the (2′ → 3′)-$ClCH_2CO$ migration, the residue was dissolved in 30 ml of CH_2Cl_2 and 4.16 ml (30.0 mmol) Et_3N, and the stirring was continued overnight at r.t. The soln. was concentrated, and the residue was purified by CC (silica gel; toluene/AcOEt 1:2) to give 1.19 g (43%) of **5b**. TLC (toluene/AcOEt 1:2): R_f 0.54. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.69 (*s*, Me); 2.83–2.78 (*dd*, *J* = 11.1, 4.7, H_{eq}–C(5′)); 3.30–3.38 (*m*, H_{ax}–C(5′)); 3.45–3.49 (*m*, H–C(4′)); 3.70–3.76 (*m*, H–C(2′), 2 MeO); 4.17 (*d*, *J* = 15.5, 1 H, $ClCH_2CO$)); 4.38 (*d*, *J* = 15.5, 1 H, $ClCH_2CO$)); 5.33–5.39 (*m*, H–C(1′), H–C(3′)); 5.73 (*d*, *J* = 5.0, OH–C(2′)); 6.88–7.55 (*m*, 14 H of $(MeO)_2$Tr, H–C(6)). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 12.45 (*q*, Me); 42.09 (*t*, $ClCH_2$); 55.81 (2*q*, MeO); 67.32 (*t*, C(5′)); 68.88, 70.71, 79.15, 83.40 (4*d*, C(1′), C(2′), C(3′), C(4′)); 86.69 (*s*, Ar_3C); 110.40 (*s*, C(5)), 114.10, 127.71, 128.45, 128.67, 129.10, 129.79, 130.83, 130.89, 136.75, 137.02 (C(6)); 137.59, 146.79, 151.78 (C(2)); 159.23, 159.26, 164.58 (C(4)); 167.78 (*d* + *s*, aromat. C). FAB-MS (pos., NBA): 659/661 (90/23, $[M+Na]^+$), 636/638 (100/42, $[M+H]^+$).

*1-{2′-*O*-(Chloroacetyl)-4′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-β-*D*-xylopyranosyl}thymine* (**5bb**) was isolated, as the minor component (apart from the major product **5b**), from exploratory experiments performed in the thymine series with **4b** in CH_2Cl_2/pyridine 4:1 and $(ClCH_2CO)_2O$ at 0°, using the workup described above for the isolation of **5b**.

Data of **5bb**. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.69 (*s*, Me); 2.09 (*dd*, *J* = 11.3, 4.5, H_{eq}–C(5′)); 2.91 (*t*, *J* = 10.8, H_{ax}–C(5′)); 3.69 (*m*, H–C(4′), 2 MeO); 3.98 (*m*, H–C(3′)); 4.31 (*s*, $ClCH_2CO$)); 5.05 (*t*, *J* = 9.3, H–C(2′)); 5.49 (*d*, *J* = 9.3, H–C(1′)); 6.12 (*d*, *J* = 5.0, H–C(3′)); 6.88–7.70 (*m*, 14 H of $(MeO)_2$Tr, H–C(6)).

N^6-*Benzoyl-9-{3′-*O*-acetyl-4′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-β-*D*-xylopyranosyl}adenine* (**6a**). To a soln. of 1.03 g (1.38 mmol) of **5a** in 50 ml of THF were added successively 5.73 mg (4.15 mmol) of K_2CO_3 and 200 mg of Pd/C (10% wt.). The mixture was stirred under H_2 (balloon) for 10 h. The reaction vessel was purged with N_2, the mixture was filtered through *Celite*, and the filtrate was evaporated to give 950 mg (96%) of **6a** as a powder. TLC (toluene/AcOEt 1:2): R_f 0.36. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.04 (*s*, MeCO); 2.82 (*dd*, *J* = 11.1, 4.1, H_{eq}–C(5′)); 3.74–3.36 (*m*, H–C(4′), H_{ax}–C(5′), 2 MeO); 4.32 (*t*, *J* = 9.0, H–C(2′)); 5.41 (*t*, *J* = 9.0, 1 H, H–C(3′)); 5.60 (*d*, *J*(1′,2′) = 9.0, C(1′)); 5.75–5.78 (br. *s*, OH–C(2′)); 6.86–8.03 (*m*, 13 H of $(MeO)_2$Tr, 5 H of Bz); 8.54, 8.64 (2*s*, H–C(2), H–C(8)). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 21.83 (*q*, *Me*CO); 55.78, 55.82 (2*q*, 2 MeO); 67.52 (*t*, C(5′)); 69.48, 71.04, 77.12, 83.76 (4*d*, C(1′), C(2′), C(3′), C(4′)); 86.76 (*s*, Ar_3C); 114.11, 126.31, 127.73, 128.48, 128.55, 128.69, 129.16, 129.38, 130.81,130.93, 136.81, 137.20, 138.11, 146.84, 152.73, 152.76, 153.12, 159.28 (*d* + *s*, arom. C); 166.93, 170.82 (2*s*, 2 C=O). FAB-MS (pos., NBA): 738 (100, $[M+Na]^+$), 716 (55, $[M+H]^+$).

*1-{3′-*O*-Acetyl-4′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-β-*D*-xylopyranosyl}thymine* (**6b**). To a soln. of 2.97 g (4.67 mmol) of **5b** in 100 ml of THF were added successively 1.94 g (14.0 mmol) of K_2CO_3 and 0.5 g of Pd/C (10% wt.). The mixture was stirred under H_2 for 10 h. The reaction vessel was purged with N_2, the mixture was filtered through *Celite*, and the filtrate was evaporated to give 2.55 g (91%) of **6b** as an powder. TLC (toluene/AcOEt 1:1): R_f 0.36. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.70 (*s*, Me); 2.03 (*s*, MeCO); 2.62–2.68 (*dd*, *J* = 10.9, H_{eq}–C(5′)); 3.18–3.25 (*t*, *J* = 10.9,H_{ax}–C(5′)); 3.49–3.52 (*m*, H–C(4′)); 3.65–3.74 (*m*, 2 MeO, H–C(2′)); 5.22–5.27 (*t*, *J* = 9.2, H–C(3′)); 5.32 (*d*, *J*(1′,2′) = 9.0, H–C(1′)); 5.62 (*d*, *J* = 5.0, HO–C(2′)); 6.86–7.54 (*m*, 13 H of $(MeO)_2$Tr, H–C(6)). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 12.47 (*q*, Me); 21.75 (*q*, *Me*CO); 55.80 (*q*, 2 MeO); 67.45 (*t*, C(5′)); 68.93, 70.85, 77.13, 83.21 (4*d*, C(1′), C(2′), C(3′), C(4′)); 86.64 (*s*, Ar_3C); 110.34, 114.08, 126.20, 127.69, 128.50, 128.65, 129.10, 129.79, 130.78, 130.92, 136.83, 137.25 (C(6)); 137.61, 138.25, 146.81, 151.79, 159.22, 159.23, 159.25, 164.58 (C(4)); 170.70 (*d* + *s*, arom. C). FAB-MS (pos., NBA): 625 (100, $[M+Na]^+$), 602 (41, M^+).

2. Experiments Referring to the Chloroacetyl Migration. – *Reaction of 1-{3′-*O*-(Chloroacetyl)-4′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-β-*D*-xylopyranosyl}thymine* (**5b**) *with (Allyloxy)carbonyl Chloride.* To a soln. of 272 mg (0.42 mmol) of **5b** in 4 ml of CH_2Cl_2 were added 270 µl (3.17 mmol) of 1-methylimidazole and 250 µl (2.52 mmol) of (allyloxy)carbonyl chloride at 0°. The soln. was stirred for 24 h at r.t. After 24 h, a soln. of 130 µl (1.3 mmol) of (allyloxy)carbonyl chloride and 130 µl (1.58 mmol) of 1-methylimidazole in 3 ml of CH_2Cl_2 were added, and the mixture was stirred for another 24 h. The mixture was diluted with 25 ml of CH_2Cl_2 and 25 ml of H_2O. The org. phase was dried (Na_2SO_4), filtered, concentrated *in vacuo*, and the residue was dried

under high vacuum to give 240 mg (88%) of crude *1-{2′-O-[(Allyloxy)carbonyl]-3′-O-(chloroacetyl)-4′-O-[(4″,4‴-dimethoxytriphenyl)methyl]-β-*D*-xylopyranosyl}thymine*. TLC (toluene/AcOEt 1:1): R_f 0.67. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.79 (*s*, Me); 3.3–3.37 (*dd*, *J* = 11.8, 5.7, H–C(5′)); 3.30–3.38 (*t*, *J* = 11.8, H–C(5′)); 3.6–3.7 (*m*, H–C(4′)); 3.8 (*m*, 2 MeO, $ClCH_2CO$); 4.5 (*d*, *J* = 5.7, $=CCH_2CO$)); 4.72 (*t*, *J* = 9.5, H–C(2′)); 5.2–5.3 (*m*, $=CH_2$); 5.62 (*t*, *J* = 9.3, H–C(3′)); 5.72 (*d*, *J* = 9.5, H–C(1′)); 5.8 (*m*, =CH); 6.8–7.5 (*m*, 13 H of $(MeO)_2$Tr, H–C(6)).

Similar reaction of 230 mg (0.35 mmol) of *1-{2′-O-(Chloroacetyl)- 4′-O-[(4″,4‴-dimethoxytriphenyl)methyl]-β-*D*-xylopyranosyl}thymine* (**5bb**) with 250 µl (3.12 mmol) of 1-methylimidazole and 250 µl (2.52 mmol) of (allyloxy)carbonyl chloride at 0°, after identical workup and isolation, gave 80 mg (31%) of *1-{2′-O-[(Allyloxy)carbonyl]-3′-O-(chloroacetyl)-4′-O-[(4″,4‴-dimethoxytriphenyl)methyl]-β-*D*-xylopyranosyl}thymine*. Assignment of constitution was based on chemical-shift values (*Table 2*) and ^{1}H-NMR decoupling experiments. Chemical proof for the constitution was obtained by the selective hydrolysis of the $ClCH_2CO$ group with hydrazine thiocarbonate [19] to afford the *1-{2′-O-[(Allyloxy)carbonyl]-4′-O-[(4″,4‴-dimethoxytriphenyl)-methyl]-β-*D*-xylopyranosyl}thymine*. The constitutional assignment of this compound was based on chemical-shift values (*Table 2*) and ^{1}H-NMR decoupling experiments that proved that the OH group is on the C(3′)-position.

3. Experiments Referring to *Scheme 3*. – *N^6-Benzoyl-9-{3′-O-acetyl-2′-O-[(2-cyanoethoxy)(diisopropylamino)phosphino]-4′-O-[(4″,4‴-dimethoxytriphenyl)methyl]-β-*D*-xylopyranosyl}adenine* (**7a**). To a soln. of 950 mg (1.33 mmol) of **6a** in 3 ml of dry CH_2Cl_2 were added successively 920 µl (5.32 mmol) of $EtN(i\text{-}Pr)_2$ and 815 µl (3.99 mmol) of chloro(2-cyanoethoxy)(diisopropylamino)phosphine at r.t. The soln. was stirred for 3 h, diluted with 10 ml of AcOEt, and washed with sat. aq. $NaHCO_3$ soln. The org. phase was dried (Na_2SO_4), filtered, and evaporated. Purification by CC (silica gel; toluene/AcOEt 4:1 to 1:2) gave 750 mg (62%) of **7a** as a mixture of diastereoisomers. TLC (toluene/AcOEt 2:1): R_f 0.31/0.36. ^{1}H-NMR (600 MHz, $CDCl_3$): 0.70, 0.87, 0.91, 0.94 (*d*, *J* = 6.7, 4 (Me_2CH); 2.35–2.55 (*m*, OCH_2CH_2CN); 2.87 (*m*, H_{eq}–C(5′)); 3.2–3.7 (*m*, 6 H, H–C(4′) of minor isomer, H_{ax}–C(5′), (Me_2CH, OCH_2CH_2CN)); 3.80 (*s*, 2 MeO); 3.85 (*m*, H–C(4′) of major isomer); 4.19 (*m*, H–C(2′) of minor isomer); 4.32 (*m*, H–C(2′) of major isomer); 5.59 (overlapping signals, H–C(1′), H–C(3′) of major isomer); 5.61 (overlapping *t*, *J* = 9.2, H–C(3′)); 5.71 (*d*, *J* = 9.0, H–C(1′) of minor isomer); 6.84–8.01 (*m*, 13 H of $(MeO)_2$Tr, 5 H of Bz); 8.08, 8.13, 8.08 (*s*, H–C(2), H–C(8)); 9.1 (br. *s*, NH). ^{13}C-NMR (600 MHz, $CDCl_3$): 20.72, 20.76 (2*t*, OCH_2CH_2CN of both isomers); 24.20, 24.8 (2*q*, Me_2CH of both isomers); 43.3, 43.4 (2*d*, Me_2CH of both isomers); 55 (*q*, MeO of both isomers); 58.1, 58.3 (2*t*, OCH_2CH_2CN of both isomers); 67.2 (*t*, C(5′) of both isomers); 68.8, 68.9 (2*d*, C(2′), C(4′) of both isomers); 70.4, 70.5 (*d*, C(3′) of both isomers); 83.9 (*d*, C(1′) of both isomers); 87.2 (*s*, Ar_3C); 113.6 (*d*, arom. C); 118.3 (*s*, CN), 123.1 (*s*, arom. C); 127.4, 128.2, 128.3, 128.5, 128.6, 129.1, 129.2, 130.1, 130.6, 130.7 (arom. C); 133.0, 133.1 (2*d*, arom. C); 134.0, 134.2 (arom. C); 136.5, 136.7 (2*s*, arom. C); 141.9 (*d*, C(2) or C(8)); 145.9, 149.9 (2*s*); 152.4, 153.3 (*d*, C(2) or C(8)); 159.1, 159.2, 164.8, 165.1 (4*s*, CO of both isomers). ^{31}P-NMR (242.9 MHz, $CDCl_3$): 150.35/150.32 (both isomers). FAB-MS (pos., NBA): 1180 (30, $[M-H+2\,Cs]^+$), 1048 (100, $[M+Cs]^+$).

*1-{3′-O-Acetyl-2′-O-[(2-cyanoethoxy)(diisopropylamino)phosphino]-4′-O-[(4″,4‴-dimethoxytriphenyl)-methyl]-β-*D*-xylopyranosyl}thymine* (**7b**). To a soln. of 400 mg (0.66 mmol) of **6b** in 3 ml of dry CH_2Cl_2 were added successively 343 µl (1.98 mmol) of $EtN(i\text{-}Pr)_2$ and 375 µl (1.65 mmol) of chloro(2-cyanoethoxy)(diisopropylamino)phosphine at r.t. The soln. was stirred for 3 h, diluted with 10 ml of AcOEt, and washed with sat. aq. $NaHCO_3$ soln., org. phase was dried (Na_2SO_4), filtered, and evaporated. Purification by CC (silica gel, toluene/AcOEt 4:1 to 1:1) gave 430 mg (81%) of **7b** as a mixture of diastereoisomers. TLC (toluene/AcOEt 2:1): R_f 0.46/0.53. ^{1}H-NMR (600 MHz, $CDCl_3$): 0.98–0.70 (*m*, 2 Me_3CH); 1.93/1.91 (2*s*, Me); 2.04, 2.04 (2*s*, MeCO); 2.4–2.6 (*m*, OCH_2CH_2CN); 3.1 (overlapping *d*, *J* = 11.5, 5.3, H_{eq}–C(5′) of both isomers); 3.3 (2*t*, *J* = 11.5, H_{ax}–C(5′) of both isomers); 3.35–3.75 (*m*, OCH_2CH_2CN, Me_2CH, H–C(2′), H–C(4′) of both isomers); 3.8 (*s*, 2 MeO); 5.51, 5.55 (2*t*, *J* = 9.2, H–C(3′) of both isomers); 5.60 (overlapping *d*, *J* = 7.0, H–C(1′) of both isomers); 6.84–7.45 (*m*, 13 H of $(MeO)_2$Tr, H–C(6)). ^{13}C-NMR (150.9 MHz, $CDCl_3$): 12.7, 12.8 (2*q*, *Me*–CH, both isomers); 14.6; 20.5, 20.6 (2*t*, OCH_2CH_2CN of both isomers); 24.5, 24.6, 24.7, 24.9 (4*q*, Me_2CH of both isomers); 43.2, 43.4 (2*t*, OCH_2CH_2CN of both isomers); 55.6 (*q*, MeO); 57.7, 58.0 (2*d*, Me_2CH of both isomers); 60.7; 68.5, 67.3 (2*d*, C(5′) of both isomers); 69.4, 69.6 (2*d*, C(4′) of both isomers); 72.3, 72.6 (2*d*, C(3′) of both isomers); 87.1 (*s*, Ar_3C); 111.7 (*s*, C(5) of both isomers); 113.4, 113.6, 113.8 (3*d*, arom. C); 127.4 (*d*, arom. C); 128.2, 128.4, 128.5 (3*d*, arom. C); 128.8, 129.4 (2*d*, arom. C); 129.6, 129.8 (2*s*, arom. C); 130.6, 130 (2*d*, arom. C); 136.5, 136.6, 136.7 (arom. C, C(6)); 146.1, 146.1 (2*s*, arom. C); 151.2, 151.3 (2*s*, C(2) of both isomers); 159.1, 159.33 (2*s*, CO of both isomers); 164.3, 164.4 (2*s*, C(4) of both isomers); 170.3, 170.4 (2*s*, CO of both isomers). ^{31}P-NMR (242.9 MHz, $CDCl_3$): 150.35, 150.24 (both isomers). FAB-MS (pos., NBA): 1067 (10, $[M-H+2\,Cs]^+$), 935 (100, $[M+Cs]^+$).

N^6-*Benzoyl-9-{3′*-O-*acetyl-4′*-O-*[(4″,4‴-dimethoxytriphenyl)methyl]-2′*-O-*succinoyl-β*-D-*xylopyranosyl}-adenine* (**8a**). To a soln. of 96 mg (0.134 mmol) of **6a** in 3 ml of dry CH_2Cl_2 were added successively 21 mg (0.174 mmol) of DMAP and 26 mg (0.26 mmol) of succinic anhydride at r.t. The mixture was stirred for 2 h, diluted with 20 ml of CH_2Cl_2, and washed with 15 ml of 10% aq. citric acid. The org. layer was dried (Na_2SO_4), filtrated, and evaporated. Purification by CC (silica gel; toluene/AcOEt/MeOH 2:1:0 to 5:4:1) gave 66 mg (60%) of **8a**. TLC (toluene/AcOEt 1:2): R_f 0.29. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.92 (*s*, MeCO); 2.37–2.22 (*m*, CH_2CH_2); 3.27 (*dd*, J = 11.1, H_{eq}–C(5′)); 3.48 (*t*, J = 11.1, H_{ax}–C(5′)); 3.84 (overlapping *m*, 2 MeO, H–C(4′)); 5.41 (*t*, J = 9.5, H–C(2′)); 5.63 (*t*, J = 9.5, H–C(3′)); 5.80 (*d*, J(1′,2′) = 9.5, H–C(1′)); 6.83–7.94 (*m*, $(MeO)_2$Tr, 5 H of Bz); 8.09, 8.61 (2*s*, H–C(2), H–C(8)). FAB-MS (pos., NBA): 838 (75, $[M+Na]^+$), 816 (100, $[M+H]^+$).

1-{3′-O-*Acetyl-4′*-O-*[(4″,4‴-dimethoxytriphenyl)methyl]-2′*-O-*succinoyl-β*-D-*xylopyranosyl}thymine* (**8b**). To a soln. of 100 mg (0.166 mmol) of **6b** in 3 ml of dry CH_2Cl_2 were added successively 26 mg (0.216 mmol) of DMAP and 33 mg (0.33 mmol) of succinic anhydride at r.t. The mixture was stirred for 2 h, diluted with 20 ml of CH_2Cl_2, and washed with 15 ml of 10% aq. citric acid. The org. layer was dried (Na_2SO_4), filtered, and evaporated. Purification by CC (silica gel; toluene/AcOEt/MeOH 2:1:0 to 5:4:1) gave 70 mg (60%) of **8b**. TLC (toluene/AcOEt 1:2): R_f 0.24. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.83 (*s*, Me); 1.92 (*s*, MeCO); 1.83–2.56 (*m*, CH_2CH_2); 3.18–3.22 (*m*, H_{eq}–C(5′)); 3.34–3.41 (*m*, H_{ax}–C(5′)); 3.60–3.69 (*m*, H–C(4′)); 3.79 (*s*, 2 MeO); 4.94 (*t*, J = 9.1, H–C(2′)); 5.59 (*t*, J = 9.1, H–C(3′)); 5.72 (*d*, J = 9.5, H–C(1′)); 6.82–7.43 (*m*, 13 H of $(MeO)_2$Tr, H–C(6)). FAB-MS (pos., NBA): 967 (25, $[M-H+2Cs]^+$), 816 (100, $[M+Cs]^+$), 795 (43), 663 (45).

Preparation of Nucleoside-Derivatized Controlled Pore Glass (CPG) (**9a** and **9b**). To a soln. of 20 mg (24 μmol) of **8a** (**8b**) in 7 ml of dry MeCN, 45 μl 1-methylmorpholine, 14 mg (42 μmol) of TOTU, and 350 mg of LCAA-CPG (the CPG was previously washed with 50 ml of CH_2Cl_2, CH_2Cl_2/$ClCH_2CO_2H$ 1:1, CH_2Cl_2, CH_2Cl_2/Et_3N 1:1, CH_2Cl_2, and dried for 2 h *in vacuo*) were successively added. The suspension was gently shaken for 1–1.5 h at r.t. Filtration followed by washing with DMF, MeOH, acetone, and Et_2O afforded the nucleoside-derivatized solid support after drying *in vacuo* (*ca.* 0.2 Torr). A suspension of the nucleoside-derivatized solid support in 10 ml of dry pyridine and 50 mg (0.41 mmol) of DMAP was treated with 1 ml (10.6 mmol) of Ac_2O for 45 min. Filtration, followed by washing with DMF, MeOH, acetone, and Et_2O, afforded capped **9a** (**9b**) after drying *in vacuo* (*ca.* 0.2 Torr) for 3 h. The loading capacity (at 498 nm) was determined to be 15 μmol/g for **9a** and 18 μmol/g for **9b** (by the method published in [20]).

4. Automated Solid-Phase Synthesis on a *Perseptive 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:

4.1. *Pre-Automation Procedures.* 4.1.1. *Phosphoroamidites.* The amount of phosphoramidite soln. was determined as follows: $(n+1) \times 22$ mg of phosphoramidite dissolved in $(n+1) \times 312$ μl of dry MeCN. The phosphoramidite solution (*ca.* 0.1M) was dried over 3-Å molecular sieves (8–12 mesh, freshly activated by heating at *ca.* 300° under high vacuum overnight) overnight at r.t. prior to use. An excess of *ca.* 160 equiv. of phosphoroamidites were used.

4.1.2. *Activator Solution*. Initially, a 0.5M soln. of pyridinium hydrochloride in MeCN was used. Subsequently, it was replaced with the better performing 5-(ethylthio)-1*H*-tetrazole in dry MeCN (0.25M), which was dried over freshly activated 3-Å or 4-Å molecular sieves.

4.1.3. *Capping A*. The standard capping reagent supplied by *Perseptive* was used (part # GEN0898210; 1-methylimidazole in pyridine/THF). Subsequently, it was replaced with a soln. of 3.0 g of DMAP in 50 ml of dry MeCN and filtered to remove any undissolved solid particles.

4.1.4. *Capping B.* The standard capping reagent supplied by *Perseptive* was used (part # GEN089810; Ac_2O in THF). Subsequently, it was replaced with a soln. of 10 ml of Ac_2O and 15 ml of 2,4,6-collidine in 25 ml of dry MeCN.

4.1.5. *Oxidizing Soln.* The standard oxidizing reagent supplied by *Perseptive* was used (part #GEN089850; I_2 in pyridine/THF). Subsequently, it was replaced with a soln. of 220 mg of I_2, 4.6 ml of 2,4,6-collidine in 23 ml of H_2O, and 50 ml of MeCN, and filtered to remove any undissolved residue.

4.1.6. *Detritylation Reagent:* A soln. of 6% Cl_2CHCO_2H 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.* 25 min. *2*) the duration of the oxidation step was increased four-fold (120 pulses), followed by extensive washing with MeCN (90 pulses), and *3*) the detritylation was accomplished by 6% Cl_2CHCO_2H in $ClCH_2CH_2Cl$ over a 3-min period. All oligonucleotides were synthesized in the Trityl-on' mode.

4.2. *Post-Automation Procedures.* 4.2.1. *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 ml of pyridine/Et_3N 5:1 for 3 h at r.t. Evaporation of pyridine and Et_3N *in vacuo*, followed by co-evaporation with DMF, avoiding temp. over 35°, resulted in dry CPG solid support.

4.2.2. *Removal of Sugar and Nucleobase Protecting Groups.* One of the following two procedures was used depending on the sequence of the oligonucleotides (*Table 3* lists the specific deprotection method for the specific sequence):

Method A. To the flask containing the dry CPG solid support was added 2.4 ml of a soln. of 0.2M $MeONH_2 \cdot HCl$ in 25% aq. NH_3/EtOH 3:1, and, the mixture was shaken at r.t. for *ca.* 6 h. After deprotection, the suspension was diluted with *ca.* 5–10 ml of 0.5M aq. $Et_3NH_2CO_3$ buffer and desalted [1][3] over a *Waters Sepak-C_{18}* cartridge (eluted with 10–15 ml MeCN/H_2O 1:1) to afford the salt-free, crude oligonucleotides ('Trityl-on') in soln.

Method B. To the flask containing the dry CPG solid support was added 2.4 ml of a mixture of 40% aq. $MeNH_2$/conc. aq. NH_3 1:1 and shaken at r.t. for 6 h. The suspension was diluted with *ca.* 5–10 ml of 0.5M aq. $Et_3NH_2CO_3$ buffer, loaded over a *Waters Sepak-C_{18}* cartridge [1][3], and eluted with 10–15 ml 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 [1][3] for optimum deprotection time.

4.2.3. *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. formic acid (a red color appears indicating detritylation) at r.t. for 15 min and concentrated *in vacuo* to dryness. The residue was dissolved in *ca.* 2 ml of H_2O, filtered (*Nalgene* syringe filter, 0.2 μM), and taken to the next step, HPLC purification [1][3].

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