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Pentopyranosyl Oligonucleotide Systems. Part 11: Systems with Shortened Backbones: (D)-β-Ribopyranosyl-(4′→3′)- and (L)-α-Lyxopyranosyl-(4′→3′)-oligonucleotides†

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This paper is dedicated to Peter Dervan who, through his pioneering work, has taught organic chemists to look at DNA as 'an organic molecule'

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Abstract—The (L)-α-lyxopyranosyl-(4′→3′)-oligonucleotide system—a member of a pentopyranosyl oligonucleotide family containing a shortened backbone—is capable of cooperative base-pairing and of cross-pairing with DNA and RNA. In contrast, corresponding (D)-β-ribopyransoyl-(4′→3′)-oligonucleotides do not show base-pairing under similar conditions. We conclude that oligonucleotide systems can violate the 'six-bonds-per-backbone-unit' rule by having five bonds instead, if their vicinally bound phosphodiester bridges can assume an antiperiplanar conformation. An additional structural feature that seems relevant to the cross-pairing capability of the (L)-α-lyxopyranosyl-(4′→3′)-oligonucleotide system is its (small) backbone/basepair axes inclination. An inclination which is similar to that in B-DNA seems to be a prerequisite for an oligonucleotide system's capability to cross-pair with DNA.

Introduction

Central to the strategy of our studies toward a chemical etiology of nucleic acid structure[1] are the chemical criteria by which we decide which of the formally conceivable alternative nucleic acid structures should, with priority, be selected for experimental study. The criteria are of two kinds: they are generational and functional in nature, they refer to the type of chemistry to be considered for the generation of a candidate structure under potentially natural conditions, and they relate to the question whether such a structure will likely prove to be an informational base-pairing system, a property considered to be a requisite for any type of oligomer that is supposed to have the functional potential of a genetic system. For nucleic acid alternatives recruited from the close structural neighborhood of RNA, the first criterion demands a candidate structure to be derivable from a $(CH_2O)_n$ sugar ($n=6$, 5, 4) by the same type of potentially natural chemistry that allows the structure type of RNA to be derived from ribose. Applying the second criterion, on the other hand, amounts to predicting a candidate system's capacity of base-pairing. Whereas this can, in principle, be attempted at various levels of theory-assisted molecular modeling, we have developed, and systematically applied, a qualitative conformational analysis of oligonucleotide single strand units as a simple and, in favorable cases, remarkably reliable tool for assessing a given oligonucleotide system's chance of being a base-pairing system.[2,3] Those favorable cases are oligonucleotide backbones containing their sugar units in the pyranose form.

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†For part 10, see ref 18. The paper is also communication No. 33 in the series 'Chemistry of α-aminonitriles'. For No. 32 of this latter series, see ref 18, and for No. 30, see ref 17. The paper that appeared in ref 34 counts as communication No. 31 in this series. For a survey of the numbering within this series, see footnote 1 in ref 17.

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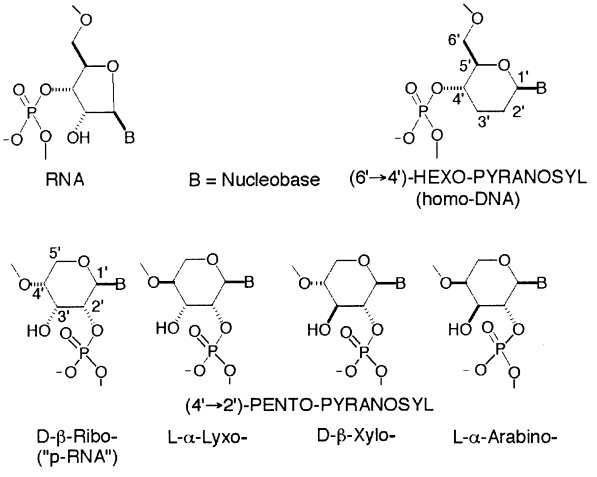
In the task of defining the constitution of candidate systems in the first place, we adhered to an empirical rule concerning the constitutional length of an oligonucleotide system's repetitive backbone unit. That rule[4] requires the number of covalent bonds per repeating backbone unit to be six (number of atomic centers, six) emulating the backbone of the natural nucleic acids. This constitutional rule has been crucial for our choice of the chemical structures of nucleic acid alternatives in both the hexopyranosyl-(6′→4′)- and the pentopyranosyl-(4′→2′)-oligonucleotide series (Scheme 1).[3,5] The finding of strong (orthogonal!) base-pairing in these oligonucleotide families[3,6–8] did greatly strengthen the belief in the relevance of the rule for predicting the base-pairing potential of new oligonucleotide systems. In addition, massive support came from the quarters of anti-sense oriented oligonucleotide chemistry, where a large number of base-pairing systems that cross-pair with RNA and DNA align to the rule with almost no exception.[9] So far, exceptions from the 'six-bonds-per-backbone-unit' rule comprise, at least in phosphodiester-based oligonucleotide systems, lengthened but not shortened oligonucleotide backbones.[10‡] The best known (and etiologically important) case is the (5′→2′)- isomer of RNA, in which the phosphodiester bridge links the sugar units between the 5′- and the 2′-positions, corresponding to seven (instead of six) bonds per backbone unit. The system shows both Watson–Crick self pairing and cross-pairing with RNA, though distinctly weaker than the natural (5′→3′)-isomer.[11] Recent investigation from two laboratories have demonstrated (5′→2′)-DNA-oligonucleotides to behave correspondingly.[12]

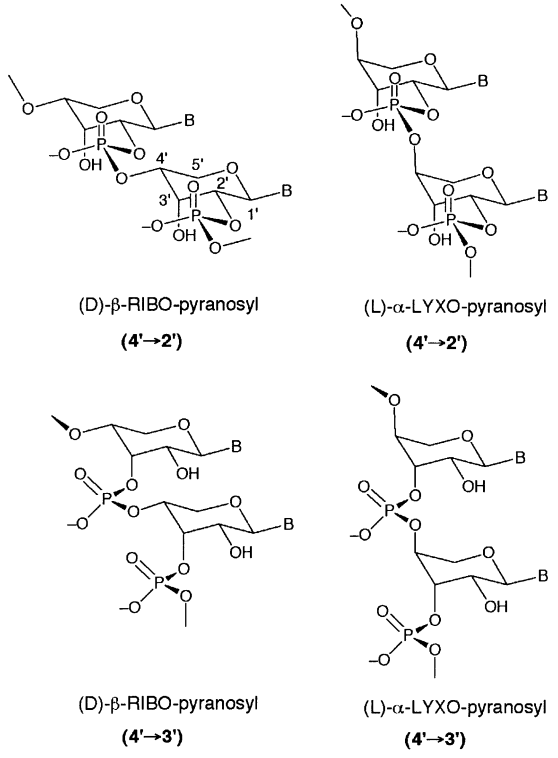
As we reported in a preliminary communication,[13] an example of a phosphodiester-based base-pairing system with a shortened oligonucleotide backbone was encountered in our systematic studies on the properties of pentopyranosyl oligonucleotides, where it was found that



Scheme 1. Constitution and configuration of the repeating units of RNA, homo-DNA, and the family of the four (4′→2′)-pentopyranosyl oligonucleotides.

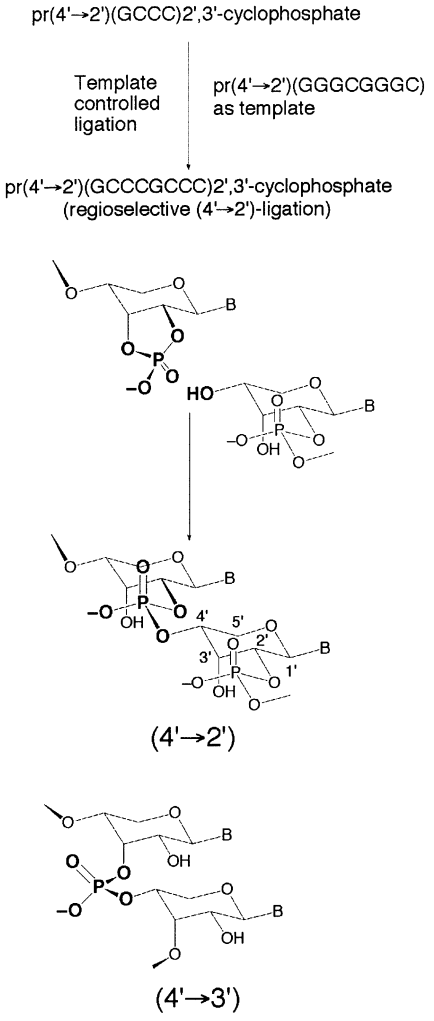
‡Note added in proof: After the present paper was submitted, it came to our attention (Herdewijn, P. *Agnew. Chem.* **2001**, *113*, 2309) that an example of such a system had in fact been described by Koga, M.; Abe, K.; Ozaki, S.; Schneller, S. W. *Nucl. Acids. Symp.* **1994**, *31*, 65.

(L)-α-lyxopyranosyl-(4′→3′)-oligonucleotides (Scheme 2) not only undergo base-pairing by themselves, but also have the capability of (weakly) cross-pairing with DNA and RNA. The access to that discovery had been by no means straightforward. It came about the following way. Template-controlled ligation of (D)-β-ribopyranosyl-(4′→2′)-tetramer cyclophosphates[14] (Scheme 3) had been predicted, as well as observed, to proceed with (4′→2′) regioselectivity. In the course of that work, it had become desirable to strengthen the experimental evidence on this matter by synthesizing, at least for one example, the alternatively possible (4′→3′)-ligation product and determine its chromatographic behavior in order to be able to decide whether the (4′→2′) ligation product can be actually chromatographically distinguished from its (4′→3′) regiosiomer.[15,16] This, in fact, turned out to be the case.[15] However, the corresponding octamer with a (4′→3′) connection at the central ligation site, when mixed with the template strand, surprisingly showed about the same T_m value (74 °C, *c* 3 μM, 0.15 M NaCl) as the duplex derived from the (4′→2′) ligation product ($T_m = 76$ °C). Therefore, it was decided to confirm experimentally what the 'six-bonds-per-backbone-unit' rule was supposed to predict in this series, namely, that β-ribopyranosyl-(4′→3′)-oligonucleotides (Scheme 2) should be devoid of the capacity of base-pairing. To this end, the two (D)-β-ribopyranosyl-(4′→3′)-octamers pr(4′→3′)(A_8) and pr(4′→3′)(T_8) containing exclusively (4′→3′)-phosphodiester links were synthesized. They, in fact, did not undergo duplex formation under conditions where their pr(4′→2′) analogues show distinct base pairing ($T_m = 40$ °C, *c* 10 μM, 0.15 M NaCl).[13] Much later, when our investigations on



Scheme 2. (4′→2′)-(D)-β-ribo- and (4′→2′)-(L)-α-lyxo-pyranosyl oligonucleotides and their corresponding (4′→3′)-pentopyranosyl counterparts in their idealized pairing conformations.

pyranosyl–RNA were extended to the isomeric members of the pentopyranosyl-(4′→2′)-oligonucleotides family and when it was found, to our surprise, that (L)-α-lyxopyranosyl-(4′→2′)-oligonucleotides represent a tendentially even stronger base-pairing system than pyranosyl–RNA,[7] the question of whether the isomeric (4′→3′)-oligonucleotide system with the shortened backbone would be capable of base-pairing came up again, this time, however with a new perspective: In the lyxopyranosyl series, a (4′→3′)-oligonucleotide would have its phosphodiester bridges attached to the neighboring pyranosyl chairs in *diaxial* (instead of axial-equatorial) conformation (Scheme 2). This would imply a conformational fixation of the phosphodiester bridge, and enforce a maximal spatial separation for the two vicinally bound phosphate centers from each other, and so, perhaps, allow for a violation of the 'six-bond-per-backbone-rule'. On the basis of this analysis, work on the synthesis of lyxopyranosyl-(4′→3′)-oligonucleotides was pursued and it was found that in this series the rule is indeed violated.[13] In the present paper, we describe the experimental details of that investigation and also complement the previously published preliminary communication.[13]

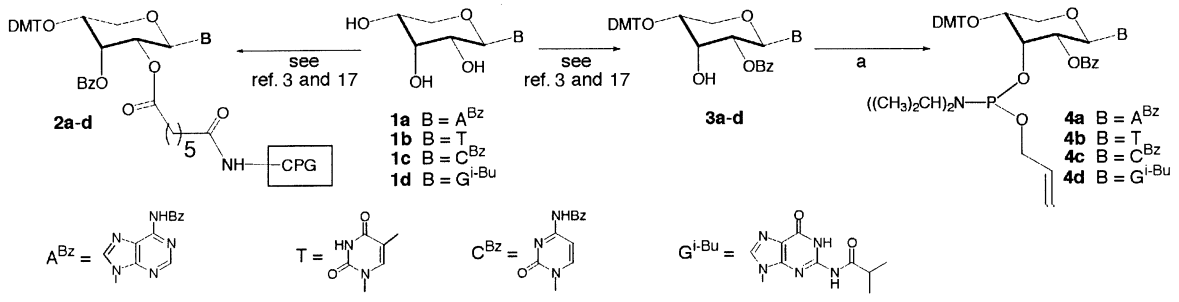


Scheme 3. Template-controlled ligation of (4′→2′)-(D)-β-ribopyranosyl tetramer cyclophosphates leading to regioselective (4′→2′ as against their 4′→3′)-ligation product (from ref 15).

Preparation of Nucleoside Building Blocks

The building blocks required for the synthesis of (4′→3′)-oligonucleotides in the (D)-β-ribopyranosyl- and (L)-α-lyxopyranosyl series were prepared from intermediates which we had previously used in the synthesis of (4′→2′)-oligonucleotides in the (D)-β-ribopyranosyl[3,17] and the (L)-α-lyxopyranosyl series.[7,18] Schemes 4 and 5 summarize the adaptations and extensions of these procedures for the preparation of compounds **4a**–**d** (Scheme 4) and **8a**–**d** (Scheme 5) required for the synthesis of (4′→3′)-oligonucleotide-isomers in the two series. In both the controlled pore glass (CPG) supported nucleosides **2a**–**d** and **9a**–**d** were the same as used previously in the synthesis of the corresponding (4′→2′)-oligonucleotides.

Since in the (4′→3′)-ribopyranosyl series (Scheme 4), the synthesis of oligomers was to be carried out using the allylphosphoramidite chemistry[19] similar to the synthesis of the ribopyranosyl-(4′→2′)-oligomers,[3,17] the *axial* 3′-hydroxyl groups of the intermediates **3a**–**d** were phosphitylated by treatment with (allyloxy)(diisopropylamino)-chlorophosphine[20] in the presence of Hüning's base. The reaction proceeded, not surprisingly, slower than the corresponding phosphitylation of the *equatorial* 2′-hydroxy group in the synthesis of (4′→2′) oligomers, and an excess of the phosphine reagent was needed in order to drive the reaction to completion. Avoiding overly long reaction times was essential, because of the danger that



Scheme 4. Preparation of the A, T, G and C building blocks in the (4′→3′)-(D)-β-ribopyranosyl series. **3a**(**3b**, **3c**, **3d**)→**4a** (**4b**, **4c**, **4d**): (a) (allyloxy)(diisopropylamino)chlorophosphine, diisopropyl ethyl amine, CH_2Cl_2, rt, 94% (78%, 63%, 63%). DMT = 4,4′-dimethoxy trityl. CPG = long-chain-alkylamino-controlled pore glass.

(2′→3′) benzoate migration might interfere during the reaction (for that migration see refs 3 and 17).

The preparation of the building blocks **8a–d** (Scheme 5) required for studying the (L)-α-lyxopyranosyl-(4′→3′)-series made use of components **6a–d** which had served as intermediates in the synthesis of (L)-α-lyxopyranosyl-(4′→2′)-oligonucleotides.[7,18] Removal of the acyl protection of the axial 3′-hydroxyl group by alkaline hydrolysis followed by regioselective benzoylation of the equatorial 2′-hydroxyl group lead to the monobenzoylated intermediates **7a–d**[22] which, in turn, were phosphitylated with (2-cyanoethoxy)(diisopropylamino) chlorophosphine in dichloromethane in the presence of 2,4,6-collidine and *N*-methyl imidazole[23] to give the required phosphitylated derivatives **8a–d** in good yields.

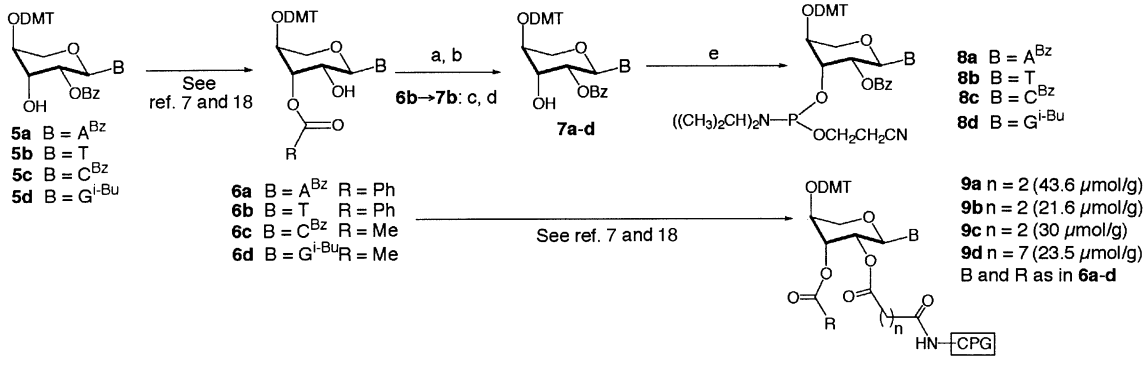
Synthesis, Purification and Characterization of Oligonucleotides

The protocols for the automated synthesis of (4′→3′)-linked oligonucleotides in the ribopyranosyl- and lyxopyranosyl- series as well as their deprotection, purification and characterization, basically followed the procedures developed for the preparation of ribopyranosyl-(4′→2′)-[3,17] and lyxopyranosyl-(4′→2′)-[7,18] oligonucleotides respectively. The syntheses were carried out on 10 μmol scale on automated DNA synthesizers (*Pharmacia's* Gene Assembler Plus, and *Perseptive's* Expedite), coupling, deprotection, and purification procedures adapted where necessary (for details, see Experimental). Individual coupling yields in the (4′→3′)-ribopyranosyl series were, on the average, a mere 88%, in sharp contrast to the yields obtained in the respective (4′→2′)-oligonucleotide series (≥96%). As illustrated for the sequences pr(4′→3′)-(A_4T_4) and pr(4′→3′)-(T_4A_4) in the Experimental, the lower-than-usual coupling yields led to rather complex HPLC traces of the crude oligonucleotide material, rendering purification rather tedious, and yields of isolated and purified oligonucleotide sequences (>95% pure according to HPLC and authenticated by mass spectroscopy) correspondingly low. Average coupling yields in the lyxopyranosyl-(4′→3′) series were not markedly lower than in the synthesis of the corresponding (4′→2′)-oligomers. Two different phosphitylation reagents were used in the two series (allyloxy in the ribopyranosyl- and 2-cyanoethoxy in the lyxopyranosyl series), the deprotection protocols of the syntheses differing correspondingly. Scheme 6 summarizes the deprotection, purification and isolation protocols followed in the two series and Table 1 gives an overall survey of the sequences synthesized, together with relevant analytical information.

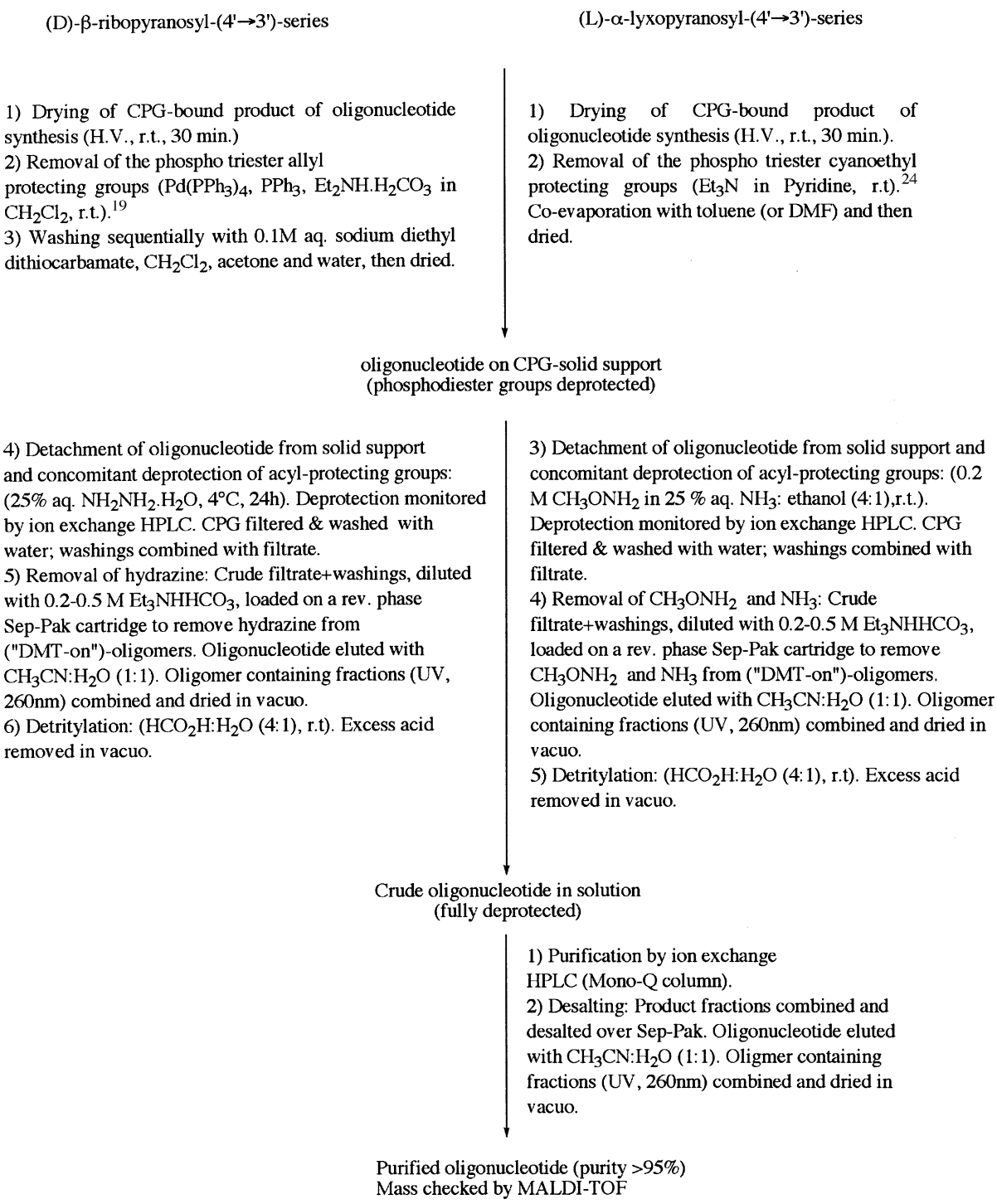
Base-Pairing Properties

The base pairing properties of (D)-β-ribopyranosyl-(4′→3′)- and (L)-α-lyxopyranosyl-(4′→3′)-oligonucleotides were characterized by methods routinely used in oligonucleotide chemistry[25] and used previously in the homo-DNA[6] and p-RNA[3] series, namely, (a) temperature-dependent UV spectroscopy for determination of T_m values, (b) concentration-dependent T_m measurements by UV spectroscopy for determination of thermodynamic data of duplex formation, (c) molar-ratio-dependent UV spectroscopy for determination of complexation stoichiometry and (d) temperature-dependent CD spectroscopy. Measurements were normally made in 10 mM aqueous NaH_2PO_4 buffer containing 0.1 mM Na_2EDTA, 150 mM NaCl at pH 7.0, with a total oligonucleotide concentration of approximately 10 μM, unless otherwise stated. Table 2 gives the T_m values and thermodynamic data of the sequences prepared in this study.

The (D)-β-ribopyranosyl-(4′→3′) octamer strands pr(4′→3′)(A_8) and pr(4′→3′)(T_8) do not form a duplex, as probed by the above UV and CD spectroscopy methods, neither do the formally self-complementary sequences pr(4′→3′)(A_4T_4) and pr(4′→3′)(T_4A_4) as Figure 1a demonstrates. The absence of duplex formation in the ribopyranosyl-(4′→3′) series [under conditions where corresponding ribopyranosyl-(4′→2′)-strands show T_m values in a range up to 45 °C] demonstrates that shortening the backbone unit in pyranosyl–RNA from the (4′→2′) to the (4′→3′) junction leads to loss or marked weakening of the base-pairing capacity. Since, however, in our ligation studies in the pyranosyl–



Scheme 5. Preparation of the A, T, G and C building blocks in the (4′→3′)-(L)-α-lyxopyranosyl series. **6a**(**6c**, **6d**)→**7a** (**7c**, **7d**): (a) aq NaOH in THF, H_2O (2:1), 0 °C; (b) benzoyl chloride, pyridine, 0 °C, 90% (87%, 95%). **6b**→**7b**: (c) K_2CO_3 in MeOH, 0 °C; (d) benzoyl chloride, pyridine, 0 °C, 91%. **7a**(**7b**, **7c**, **7d**)→**8a** (**8b**, **8c**, **8d**): (e) (2-cyanoethoxy)(diisopropylamino)chlorophosphine, 2,4,6-collidine, 0.5 equiv *N*-methylimidazole, CH_2Cl_2, rt, 73% (82%, 70%, 72%). DMT = 4,4′-dimethoxy trityl; CPG = long-chain-alkylamino-controlled pore glass.



Scheme 6. The deprotection, purification and isolation protocols followed in the (4′→3′)-(D)-β-ribo- and (4′→3′)-(L)-α-lyxo-pyranosyl oligonucleotide series.

RNA series[15] we had observed of between a pr(4′→2′) octamer containing a single (4′→3′) connection at the central ligation site with the corresponding (D)-β-ribo-pyranosyl (4′→2′) template strand [the observation that had induced us to study the pr(4′→3′) systems to begin with], it seemed advisable also to check the capability of an all-(4′→3′)-strand to cross-pair with a complementary all-(4′→2′)-strand. Weak duplex formation between pr(4′→3′)(A_8) and pr(4′→2′)(T_8) was in fact observed [$T_m \approx 10\,°C$, *c* 10 μM, 1 M NaCl, as compared to the corresponding (4′→2′)A_8•(4′→2′)T_8 duplex, $T_m \approx 45.5\,°C$, Nos 12 and 1 in Table 2]. In contrast, the reverse combination of pr(4′→3′)(T_8) with pr(4′→2′)(A_8) does not show duplex formation. That pr-(4′→3′) strands have a weak affinity to complementary pr-(4′→2′) strands is also indicated by the T_m value of 23 °C (*c* 5 μM, 150 mM NaCl, No. 14 in Table 2) of the complex formed from pr-(4′→3′)(C_8) and pr-(4′→2′)(G_8).[26]

The data presented in Table 2 concerning the lyxopyranosyl-(4′→3′) series demonstrate that pl-(4′→3′)-oligonucleotides are a fairly well functioning base-pairing system (Fig. 1b and d).[27] Yet, base-pairing strength, as judged by T_m values, is consistently lower than that of corresponding oligonucleotides in the lyxopyranosyl-(4′→2′)-series. Another difference seems to be a marked sequence-dependence of pairing strength; the range of sequences studied is, however, too limited[28] for a reliable analysis of this effect to be feasible. All our observations indicate that duplex formation with antiparallel strand orientation is preferred. The sequence pl-(4′→3′)(TTAAAATA) and its antiparallel complement pl-(4′→3′)(TATTTTAA) form a duplex clearly melting at 12.6 °C (*c* 10 μM, 1 M NaCl, No. 9 in Table 2, Fig. 2a), whereas the T_m value of the corresponding combination with its parallel complement pl-(4′→3′)(AATTTTAT) is below the range of observation (No. 10 in Table 2). The very existence of some of the duplexes listed in Table 2 (Nos 3, 11, 26–31) supports the preference for antiparallel strand orientation. Stoichiometry of duplex formation, tested in three cases, was found to be 1:1 by UV mixing curve determination (Fig. 2b).[27] As judged from pairing tests with the homobasic (A_8) and (T_8) octamers, pl-(4′→3′)-oligonucleotides do not crosspair with either pl-(4′→2′)- nor pr-(4′→2′)-partners.

Table 2 contains also the information we have collected about the capability of lyxopyranosyl-(4′→3′)-oligonucleotides to cross-pair with antiparallel-complementary DNA and RNA sequences. Cross-pairing strength is moderate and somewhat capricious in the sense that it can strongly depend on which of the two complementary base-sequences belong to which of the two participating pairing systems. This is most clearly exemplified by the behavior of the homobasic sequences pl-(4′→3′)(A_n) and pl-(4′→3′)(T_n) ($n = 8$, 12) towards the corresponding complementary DNA and RNA sequences (Fig. 1c). Whereas the combination of pl-(4′→3′)(A_8) with d(T_8) forms a duplex with a T_m value of 42 °C (No. 17), as well as a triplex pl(4′→3′)(A_8)•2d(T_8) (see reproduction of mixing curve in ref 13), no cross-pairing is detected in the inverse combinations pl-(4′→3′)(T_8) with d(A_8) and r(A_8), respectively (Nos 18 and 22). T_m differences of approx 40 and 60 °C are observed with the corresponding dodecamer sequences (Nos 19 and 20) (for a discussion of this kind of phenomenon, see ref 8). Intersystem cross-pairing among sequences in which all four canonical nucleobases are irregularly mixed does not show this kind of backbone dependence (Nos 26–31), however. With regard to the pairing mode, our observations are compatible with the presumption that the self- as well as the cross-pairing of lyxopyranosyl-(4′→3′)-oligonucleotides proceeds by the Watson–Crick mode; alternative modes in duplexes with homobasic sequences are not excluded, however.

Discussion

Although the study of the two members of the pentopyranosyl oligonucleotide family with shortened phos-

Table 1. HPLC and MS data of (D)-β-ribopyranosyl-(4′→3′) and (L)-α-lyxopyranosyl-(4′→3′)-oligonucleotides

Oligonucleotides all (4′→3′)	Deprotection[a] method	Amount made (OD260) (yield after purification)	Analytical HPLC MonoQ-Ion Exchange[b] gradient, t_r (min)	MALDI-TOF MS[c]	
				$[M+H]^+$ (obsd)	$[M+H]^+$ (calcd)
Ribopyranosyl					
pr(A_8)	A	22.0 (2%)[d]	0–100% in 30 min, t_r 15.5	2571	2572
pr(T_8)	A	30.0 (3%)[d]	0–100% in 30 min, t_r 25.5	2500	2500
pr(A_4T_4)	A	4.0 (4%)[d]	0–100% in 30 min, t_r 18.5	2537	2536
pr(T_4A_4)	A	4.0 (4%)[d]	0–100% in 30 min, t_r 18.7	2537	2536
pr(C_8)	A	21.0 (2%)[d]	0–100% in 30 min, t_r 16.1	2376	2379
Lyxopyranosyl					
pl(A_8)	B	23.2 (12%)	0–50% in 30 min, t_r 17.4	2572	2572
pl(T_8)	B	24.0 (15%)	20–80% in 30 min, t_r 26.1	2502	2500
pl(A_{12})	C	12.9 (5%)	0–100% in 30 min, t_r 17.9	3888	3889
pl(T_{12})	C	24.6 (28%)	0–100% in 30 min, t_r 27.4	3780	3780
pl(A_4T_4)	B	18.4 (24%)	0–100% in 30 min, t_r 20.2	2542	2536
pl(T_4A_4)	B	22.1 (14%)	0–100% in 30 min, t_r 20.4	2542	2536
pl($AT)_4$	B	13.8 (18%)	0–100% in 30 min, t_r 20.6	2539	2536
pl($TA)_4$	B	9.6 (6%)	0–100% in 30 min, t_r 20.8	2540	2536
pl(TATTTAA)	B	35.3 (24%)	0–100% in 30 min, t_r 21.4	2528	2527
pl(TTAAATA)	B	39.4 (24%)	0–100% in 30 min, t_r 18.6	2546	2545
pl($CG)_3$	B	11.8 (35%)	0–100% in 30 min, t_r 21.8	1890	1889
pl($GC)_3$	B	7.8 (18%)	0–100% in 30 min, t_r 21.9	1889	1889
pl(ATTCAGCG)	B	5.1 (7%)	0–100% in 30 min, t_r 21.7	2539	2538
pl(CGCTGAAT)	B	4.0 (6%)	0–100% in 30 min, t_r 22.1	2538	2538

[a]Method A: 25% aq NH_2NH_2, 4 °C; ca. 20 h; method B: CH_3ONH_2•HCl in concd aq NH_3 and EtOH (3:1) at 4 °C for approx 6.5 h (ca. 70 h for G,C containing sequences); method C: 40% aq CH_3NH_2 in concd aq NH_3 (1:1) at rt for 6.5 h (ca. 70 h for G,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 1 M NaCl with a flow of 1 mL/min; followed by desalting on Sep-Pak 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 assisted laser-desorption ionisation time-of-flight mass spectroscopy; matrix: 2,4,6-trihydroxyacetophenone and ammonium citrate buffer.

[d]OD yields were measured at 270 nm.

Table 2. T_m values and thermodynamic data

Experiment no.	Duplex	T_m°C (≈10 μM, 1.0 M NaCl				pl(4′→3′)(0.15 M NaCl)		
		pr 4′→2′	pr 4′→3′	pl 4′→2′	pl 4′→3′	ΔG 25 °C	ΔH	TΔS 25 °C
Intra-system pairing								
1.	A_8+T_8	45.5	<0	51.0	41.2	−10.0[b]	−42.1[b]	−32.1[b]
2.	$A_{12}+T_{12}$	67.6		74.0	67.0	−14.0[b]	−51.8[b]	−37.8[b]
3.	T_4A_4	40[a]	<0	49.6	17.5			
4.	A_4T_4	27[a]	<0	41.1	<5			
5.	$(TA)_4$	40[a]		43.2	30.1	−6.3	−33.9	−27.6
6.	$(AT)_4$	38[a]		44.7	26.6	−6.1	−32.5	−26.4
7.	$(CG)_3$	65[a]			45.1	−9.5	−42.5	−33.0
8.	$(GC)_3$	62[a]			35.0	−8.0	−34.3	−26.3
9.	−TTAAAATA + AATTTTAT—	45.9		46.3	12.6			
10.	−TTAAAATA + –AATTTTAT				—			
11.	−ATTCAGCG + TAAGTCGC−	67.6		61.9	39.0	−8.4	−24.9	−16.5
Inter-system cross-pairing								
12.	4′,3′-pr(A_8) + 4′,2′-pr(T_8)				10			
13.	4′,3′-pr(T_8) + 4′,2′-pr(A_8)				—			
14.	4′,3′-pr(C_8) + 4′,2′-pr(G_8)				23.5			
15.	4′,3′-pr(A_8) + d(T_8)				—			
16.	d(A_8) + 4′,3′-pr(T_8)				—			
17.	4′,3′-pl(A_8) + d(T_8)				42			
18.	d(A_8) + 4′,3′-pl(T_8)				—			
19.	4′,3′-pl(A_{12}) + d(T_{12})				63.4			
20.	d(A_{12}) + 4′,3′-pl(T_{12})				17.2			
21.	4′,3′-pl(A_8) + r(U_8)				21			
22.	r(A_8) + 4′,3′-pl(T_8)				—			
23.	4′,3′-pl(A_{12}) + r(T_{12})				62.1			
24.	r(A_{12}) + 4′,3′-pl(T_{12})				<5			
25.	4′,3′-pl(TTAAAATA) + d(AATTTTAT)				—			
26.	d(TTAAAATA) + 4′,3′-pl(AATTTTAT)				28.6	−7.9	−52.6	−44.7
27.	4′,3′-pl(ATTCAGCG) + d(CGCTGAAT)				26.4			
28.	d(ATTCAGCG) + d(CGCTGAAT)				36.3	−9.2	−55.1	−45.9
29.	4′,3′-pl(ATTCAGCG) + r(CGCTGAAT)				42.0			
30.	r(ATTCAGCG) + 4′,3′-pl(CGCTGAAT)				36.5			
31.	r(ATTCAGCG) + r(CGCTGAAT)				52.0	−11.2	−52.8	−41.5

Measurements were made in 10 mM NaH_2PO_4 buffer, 0.1 mM EDTA, 150 mM NaCl, pH 7.0:
[a]In 0.15 M NaCl.
[b]In 1.0 M NaCl. pr = ribopyranosyl, pl = lyxopyranosyl, r = ribofurnaosyl, d = 2′-deoxyribofuranosyl.

phodiester backbones remains rather limited and the observations on these systems incomplete, the study, nevertheless, taught us two significant lessons. The first concerns the range of flexibility of the 'six-bonds-per-backbone-unit' rule toward backbone shortening. The difference in pairing behavior of the two diastereomeric pentopyranosyl-(4′→3′)-oligonucleotide systems clearly indicates that it is most probably the fixed antiperiplanarity in the arrangement of the two vicinal phosphodiester groups at the pyranosyl chair[29] that is the essential conformational feature responsible for the lyxopyranosyl-(4′→3′)-system to be a base-pairing system. The requirement for an antiperiplanar conformation of vicinal phosphodiester groups in a five-bonds-per-backbone-unit can be expected to be a valuable criterion in the design of new oligonucleotide systems possessing base-pairing capability (for one explicit example, see below). It also raises the interesting question whether base-pairing capability would be even compatible with aliphatic backbones containing phosphodiester groups that would assume an antiperiplanar conformation as a result of a tendency to minimize electrostatic repulsion (Scheme 7). At any rate, the findings about the lyxopyranosyl-(4′→3′)-oligonucleotide system widens the constitutional range for the design of new oligonucleotide base-pairing systems.

The second lesson refers to the perhaps most remarkable property of the lyxopyranosyl-(4′→3′)-system, namely its capability to undergo cross-pairing with the natural nucleic acids. After all, none of the four members of the family of pentopyranosyl-(4′→2′)-oligonucleotides[7]—although all of them showing strong Watson–Crick base-pairing by themselves and among themselves- has been observed to possess the capability of cross-pairing with RNA or DNA.

General constitutional criteria that correlate the constitution (including configuration) of an oligonucleotide backbone with a system's capability of cross-pairing with the natural nucleic acids would be valuable tools for oligonucleotide chemistry, but they are notoriously hard to define. It seems to us that the lyxopyranosyl-(4′→3′)-system, besides extending the 'six-bonds-per-backbone-unit' rule, points to one such constitutional criterion, namely, the oligonucleotide system's backbone/basepair axes inclination.[30,31] Among oligonucleotide families, there are at present three groups of base-pairing systems known which are capable of efficiently communicating by base-pairing among themselves, but differ in their 'language' in the sense that they are unable to communicate by cross-pairing with each other. In other words, the base-pairing of each of these

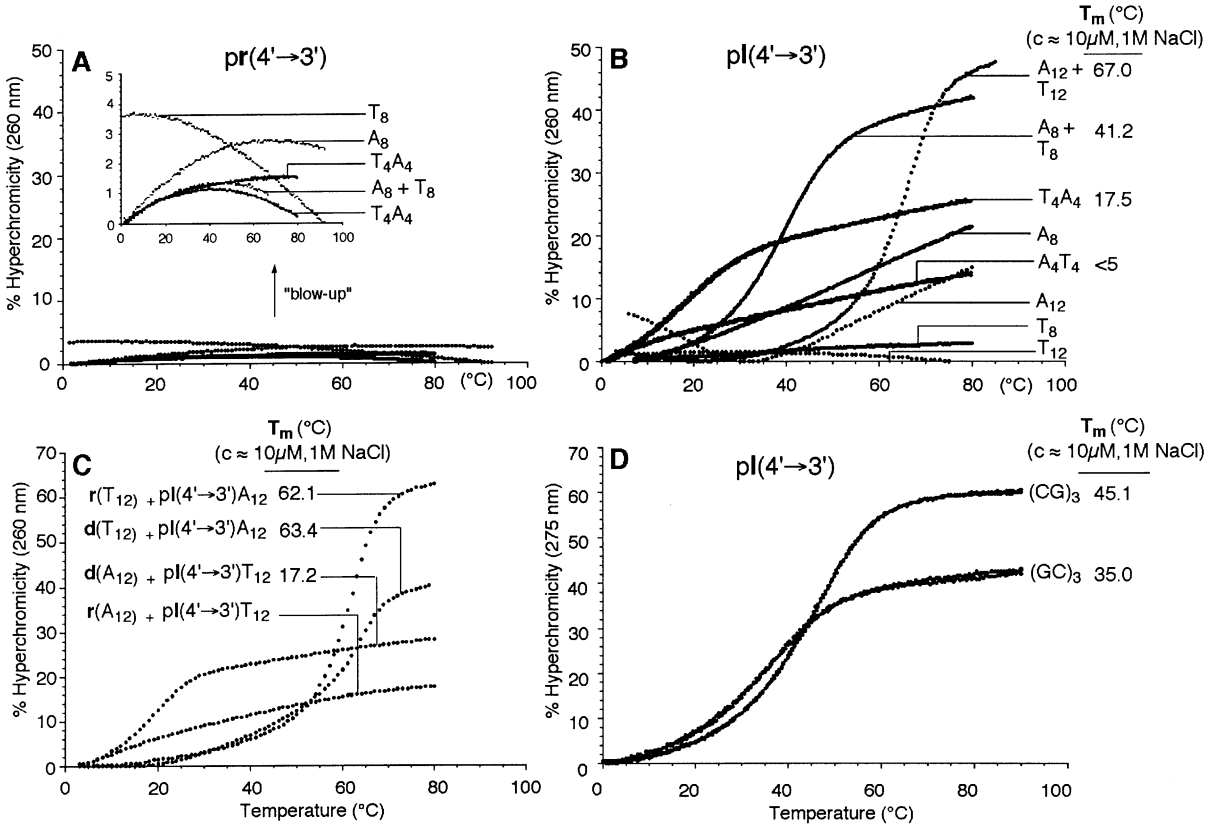


Figure 1. UV–T_m curves documenting the pairing behavior in (A) the (4′→3′)-(D)-β-ribopyranosyl oligonucleotide series; curves of the non-self-complementary single strands are also shown, (B, D) (4′→3′)-(L)-α-lyxoyranosyl oligonucleotide series; curves of the non-self-complementary single strands are also shown, and (C) the intersystem cross-pairing of (4′→3′)-(L)-α-lyxoyranosyl oligonucleotides with RNA and DNA. All measurements were made with total oligonucleotide concentration ≈10 μM in 10 mM aq NaH_2PO_4 buffer containing 0.1 mM Na_2EDTA, 150 mM NaCl at pH 7.0.

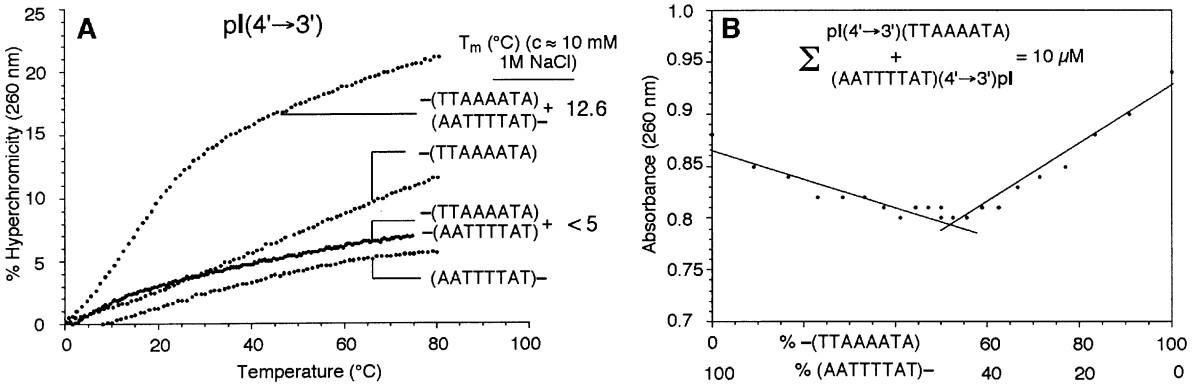


Figure 2. (A) UV–T_m curves documenting the preference of the anti-parallel pairing behavior in the (4′→3′)-(L)-α-lyxoyranosyl oligonucleotide series: pI-(4′→3′)-(TTAAAATA) with its antiparallel complement pI-(4′→3′)(TATTTTAA) strand and its parallel complement pI-(4′→3′)(AATTTTAT) strand. For conditions see caption of Figure 1. (B) Molar-ratio-dependent UV spectroscopy–mixing curve indicating the 1:1 complexation stoichiometry between the sequence pI-(4′→3′)(TATTTTAA) and its antiparallel complement pI-(4′→3′)(TTAAAATA) (260 nm, 0 °C).

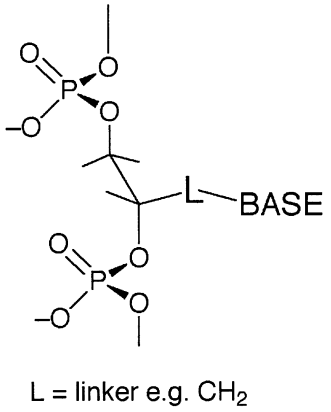
groups is orthogonal to that of the other two (Fig. 3). These three groups are, first, the 'DNA–RNA group' to which belong those systems which are able to cross-pair in the Watson–Crick mode with the natural nucleic acids, second, the 'homo-DNA group' of which the 2′,3′-dideoxy-glucopyranosyl-(6′→4′)-oligonucleotide system ('homo-DNA')[6] is the prototype of the family of potential hexopyranosyl-(6′→4′)-oligonucleotide pairing systems and, third, the 'pyranosyl–RNA group' in which the pyranosyl isomer of RNA is the most representative member of the family of the four diastereomeric pentopyranosyl-(4′→2′)-oligonucleotide systems, all of which are stronger pairing systems than RNA itself.[7] We had implied that the orthogonality of the base-pairing in homo-DNA versus that of the pentopyranosyl-(4′→2′)-systems is the consequence of the opposite sense of the backbone/basepair axes inclination;[30] Figure 4, showing idealized pairing conformations, qualitatively

illuminates the meaning of this statement, and, at the same time, indicates that a shift from a pentopyranosyl-(4′→2′)- to a pentopyranosyl-(4′→3′)-system can be expected to result in a pronounced reduction of that inclination.

In fact, model considerations of (idealized) pl-(4′→2′)- and pl-(4′→3′)-duplexes show that the strong inclination[30–32] of pl-(4′→2′) duplexes largely disappears in shifting to corresponding (4′→3′) duplexes. This shift is accompanied by a change in the constellational nature of base-stacking: What in lyxopyranosyl-(4′→2′) duplexes (in their idealized conformation) is essentially pure *inter*strand-stacking, becomes *intra*-strand stacking in (4′→3′) duplexes. The two alternative stacking modes correlate directly with the degree of (positive or negative) backbone/basepair axes inclination. Systems with only small (or no) inclination are expected to display *intra*strand base-stacking. The prototype of this type of system is B-DNA and, in fact, an analysis of inclinations in known B-DNA X-ray structures[33] confirms that B-DNA-backbones on the average show essentially no backbone/basepair axes inclination.

Such qualitative reasoning encourages us to postulate that one of the criteria which must be fulfilled by an oligonucleotide system (or any other type of base-pairing system) in order to possess the capability of cross-pairing with the natural nucleic acids is that they have access to a pairing conformation that has no, or only a small, backbone/basepair axes inclination. This postulate certainly deserves in future work a more quantitative definition as well as a systematic testing of oligonucleotide and other pairing systems of known structure and known to cross-pair with DNA and RNA.[32]

Finally, our studies on the lyxopyranosyl-(4′→3′)-oligonucleotide system led us to conclude—and this may perhaps be the most consequential lesson we received from it—that we should question our originally held view that tetrofuranosyl-(3′→2′)-oligonucleotides are irrelevant to a search for potentially natural nucleic acid alternatives from the structural neighborhood of RNA.



Scheme 7. A hypothetical aliphatic oligonucleotide backbone system in which the vicinal phosphodiester groups might—as a result of a tendency to minimize electrostatic repulsion—assume an anti-periplanar conformation.

It had been taken for granted that no candidates have to be considered that contain a tetrose sugar as a backbone unit since no oligonucleotide system can be constructed from a tetrose that would satisfy the 'six-bonds-per-backbone-unit' rule. The pairing properties of the lyxopyranosyl-(4′→3′) system as against those of the ribopyranosyl-(4′→3′) isomer led us to extend our screening for potentially natural nucleic acid alternatives to the threofuranosyl-(3′→2′) series, counting on the possibility that their 2′,3′-*trans*-phosphodiester groups at the threofuranose rings might conformationally emulate the diaxial 3′,4′-*trans*-phosphodiester groups of the lyxopyranosyl-(4′→3′)-oligonucleotide system (Scheme 2). As described in a recently published report[34] this conjecture turned out to be fruitful in that it led us to investigate the pairing properties of the threo-(3′→2′)-oligonucleotide system which are, so we think, of considerable interest in the context of nucleic acid etiology.

Experimental

General

Solvents for extraction: technical grade, distilled. Solvents for reaction: reagent grade. Reagents: unless otherwise noted, from Acros, Fluka or Aldrich, highest quality

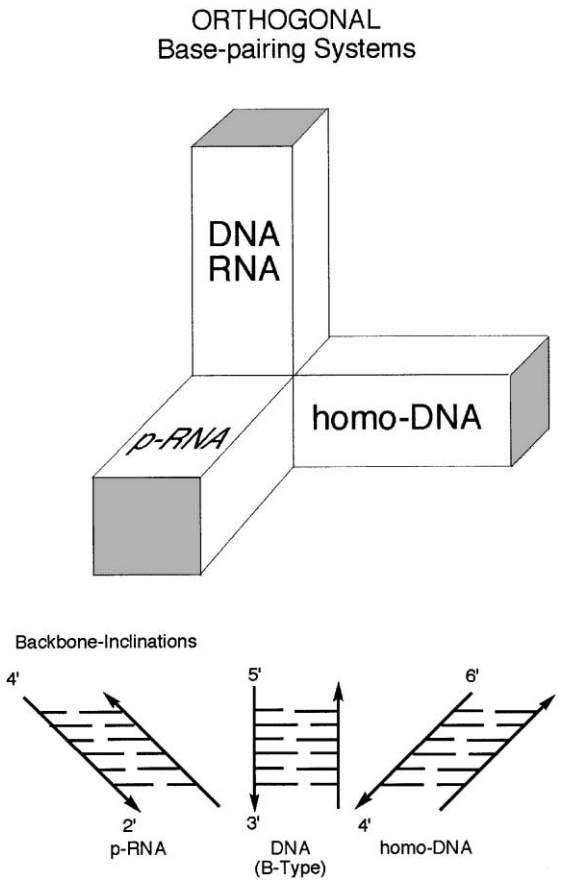


Figure 3. The three groups of base-pairing systems which exhibit strong intrasystem base-pairing but do not cross communicate with each other; they represent orthogonal base-pairing systems. This orthogonality is proposed to be related to the different backbone/base-pair-axes inclinations of these systems.

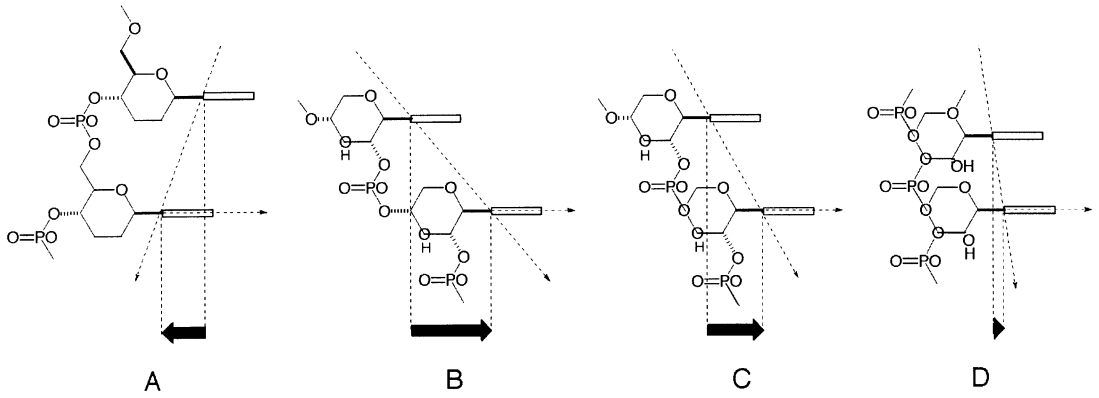


Figure 4. Qualitative vertical projections of formulas of the pairing conformations (idealized, nucleosidic torsion angles −120°) of the four oligonucleotide systems: (A) homo-DNA (g/t phosphodiester conformation); (B) pyranosyl—RNA; (C) (4′→2′)-(L)-α-lyxopyranosyl-, and D: (4′→3′)-(L)-α-lyxopyranosyl-oligonucleotides. Projections allow a rough estimate of the sense and the degree of the four systems' backbone/base-pair-axes inclination.[32] Note that sense and degree of the inclination depends sensitively on the nucleosidic torsion angle.

available. (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). The columns were filled with a suspension of silica gel in MeOH. The MeOH was then replaced by flushing the column with the appropriate solvent mixture. In case of acid sensitive compounds, the silica gel was pre-treated with MeOH containing about 0.5% Et_3N. Melting points (uncorrected) were measured with MEL-TEMP II (Laboratory Devices Inc., USA). NMR: ^1H: δ values in ppm (TMS as internal standard), *J* (Hz), assignments of ^1H resonances were in some cases based on 2-D experiments (^1H–^1H-COSY); ^{13}C: δ values in ppm (TMS as internal standard), *J* (Hz); assignments and multiplicities were based on 2-D experiments (^1H–^{13}C-COSY); ^{31}P: δ values in ppm (85% H_3PO_4 as external standard). FAB^+-MS (matrix-sol): *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) 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 analogue 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, 1 M NaCl, pH = 10.5. UV spectroscopy was measured on a Cary 1 C spectrophotometer (Varian) and a Perkin–Elmer Lambda 2. Melting temperature (T_m) measurements 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. CD spectrum was measured on (A) JASCO J-710 or (B) AVIV 61 DS circular dichroism spectropolarimeter. Concentrations of oligonucleotide solutions were calculated from the UV absorbance of the solutions at 260 nm (pH 7) at ca. 80 °C using the following molar extinction coefficients: εpr(A) = pl(A) = 15,000, εpr(T) = pl(T) = 10,000, εpr(C) = 8400, εpr(G) = 11,900. Abbreviations: CPG: 'controlled pore glass', DMAP: 4-dimethylaminopyridine, DMT: 4,4′-dimethoxytrityl, LCAA-CPG: long-chain aminoalkyl-CPG (500 Å).

Procedure

(Allyloxy)dichlorophosphine. To a mechanically stirred solution of 88 mL (137 g, 1 mol) of PCl_3 in 250 mL of dry ether, 81 mL (79 g, 1 mol) of dry pyridine was slowly added under argon. The slightly turbid solution was chilled with dry ice in acetone. Over a period of 6 h, a soln 61 mL of allyl alcohol in 100 mL of dry diethyl ether was added. On the addition of the alcohol a white precipitate formed and eventually, the mixture became thick and vigorous stirring was needed. On completion of addition, the mixture was allowed to warm up to rt and stirring continued for 16 h. Finally, the mixture was diluted with 100 mL dry diethyl ether and filtered under nitrogen. The precipitate was carefully washed with 3×100 mL portions of dry diethyl ether. The washings were combined with the filtrate and the bulk of the ether was removed by distillation under atmospheric pressure. The residue was subject to fractional distillation under reduced pressure (1 torr). A first fraction with a boiling point of 28–30 °C was collected to give 74 g (47%) of the desired product. A second fraction with a boiling point of 48–50 °C, was collected to give 15 g (8%) of what is considered to be the bis(allyloxy)-chlorophosphine.

Data of (allyloxy)dichlorophosphine. ^{1}H NMR (600 MHz, $CDCl_3$): 4.72 (ddt, J = 8.4, 5.7, 1.4, 2H, OCH_2); 5.31 (dq, J = 10.3, 1.2, 1H, H_2C=); 5.39 (dq, J = 18.5, 1.4, 1H, H_2C=); 5.95 (ddt, J = 16.1, 10.6, 6.5, 1H, HC=).

(Allyloxy)(diisopropylamino)chlorophosphine. To a vigorously stirred solution of 74 g (0.47 mol) of (allyloxy)-dichlorophosphine in 400 mL of dry diethyl ether a soln of 130 mL (94.3 g, 0.93 mol) of diisopropylamine in 100 mL of dry diethyl ether was added within 3 h at −78 °C under nitrogen. After complete addition of the amine, stirring of the thick suspension was continued for 16 h at rt and was diluted with 200 mL of dry ether. The white precipitate was filtered and washed with 3×100 mL portions of dry diethyl ether under nitrogen. The filtrate and washings were combined and the diethyl ether was removed on the rotavapor under nitrogen atmosphere. The residue was carefully distilled under reduced pressure (nitrogen atmosphere, 50 mtorr) and a fraction with a boiling point of 55–60 °C (50 mtorr) was collected to give 69.3 g (66%) of the desired product. The purity of the material was 94% (^{1}H NMR), the impurity being (allyloxy)(diisopropylamino)-H-phosphonate.

Data of allyloxy(diisopropylamino)chlorophosphine. ^{1}H NMR (600 MHz, $CDCl_3$): 1.25–1.29 (m, 12H, CH_3); 3.79–3.85 (*m*, 2H, H-CN); 4.38 (brs, 2H, OCH_2); 5.22 (*d*, J = 10.5, 1H, H_2C=); 5.34 (d, J = 16.9, 1H, H_2C=); 5.98 (ddt, J = 16.4, 10.7, 5.4, 1H, HC=). ^{13}C NMR (150 MHz, $CDCl_3$): 24.2, 23.5 (q, CH_3); 46.2 (d, J_{CP} = 12.9, CH–N); 66.6 (t, J_{CP} = 18.2, CH_2–O); 118.3 (t, CH_2=); 134.3 (d, J_{CP} = 7.9, CHC=). ^{31}P NMR (242 MHz, $CDCl_3$): 183.0.

***N*^{6}-Benzoyl-1-[3′-*O*-((allyloxy)(diisopropylamino)phosphino)-2′-*O*-benzoyl-4′-*O*-((dimethoxytriphenyl)methyl)-β-D-ribopyranosyl-(1′)]adenine (4a).** A solution of 2.52 g (2.9 mmol) of **3a** in 9 mL CH_2Cl_2 was cooled to 0 °C under argon. 1.5 mL (8.7 mmol) Diisopropylethylamine was added, immediately followed by 0.95 mL (4.6 mmol) (allyloxy)(diisoproylamino)phosphine. The reaction mixture was allowed to warm to rt and stirred at rt for 1 h. The reaction was mixture was cooled to 4 °C, 0.95 mL (4.6 mmol) (allyloxy)(diisoproylamino)chlorophosphine, 0.79 mL (4.6 mmol) of diisopropylethylamine were added and stirred at rt After 2 h, 0.36 mL (1.7 mmol) of (allyloxy)(diisoproylamino)phosphine was added (without base). The mixture was stirred for another 1.5 h and 1 mL of methanol was added. After dilution with CH_2Cl_2 and washing with satd aq $NaHCO_3$ soln, the organic phase was dried over Na_2SO_4 and evaporated. Column chromatography over silica gel (acetone/hexanes 2:8 with 1% triethylamine) yielded 2.88 g (94%) of **4a** as a white foam.

Data of 4a. TLC: (hexane/ethyl acetate 3:2) R_f 0.58. ^{1}H NMR (600 MHz, $CDCl_3$): 1.18, 1.23, 1.29 (3d, 12 H, J = 6.8, CH_3(isoprop)); 2.72 (dd, $J_{4'5'}$ = 4.9, J_{gem} = 10.9, 0.6H, H-C(5′)); 2.95 (dd, $J_{4',5'}$ = 4.9, J_{gem} = 10.9, 0.4 H, H-C(5′)); 3.62–3.70 (*m*, 0.4 H, H_2CO(allyl)); 3.82–4.00 (*m*, 10.4 H, CH_3O(DMT), CH(isoprop), H_2CO(allyl), H-C(4′), H-C(5′)); 4.09–4.13 (*m*, 0.6 H, CH_2O(allyl)); 4.19–4.23 (*m*, 0.6 H, CH_2O(allyl)); 4.49 (*d*, $J_{3',P}$ = 10.8, 0.4 H, H-C(3′)); 4.72 (*d*, $J_{3',P}$ = 10.8, 0.6 H, H-C(3′)); 4.85 (*d*, J = 10.3, 0.4 H, H_2C=(allyl)); 4.91 (dd, J = 1.4, 17.2, 0.4 H, H_2C=(allyl)); 5.07 (d, J = 10.2, 0.6 H, H_2C=(allyl)); 5.19 (dd, J = 2.4, 9.5, 0.4 H, H-C(2′)); 5.25 (dd, J = 1.5, 17.1, 0.6 H, H_2C=(allyl)); 5.34 (*d*, $J_{1',2'}$ = 9.7, 0.6 H, H-C(2′)); 5.43 (m, J = 5.3, 10.6, 17.1, 0.4 H, HC-(allyl)); 5.92 (m, J = 5.3, 10.5, 17.1, 0.6 H, HC-(allyl)); 6.26, 6.27 (*d*, $J_{1',2'}$ = 9.5, H-C(1′)); 6.87–8.59 (m, 28 H, H-C(ar)); 8.11, 8.13 (m, H-C(2)), 8.59, 8.64 (s, H-C(8)). ^{13}C NMR (150 MHz, $CDCl_3$): 24.77, 24.81, 25.25, 25.31, 25.4, 25.5 (6q, CH_3(isoprop)); 43.1, 43.2, 43.2 (3d, CH(isoprop)); 55.5 (q, CH_3O(DMT)); 64.1 (t, CH_2O(allyl)); 64.4 (dt, J_{CP} = 19.3, CH_2O(allyl)); 65.8, 66.0 (2t, C(5′)); 69.0, 69.3, 70.8, 71.7, 72.15, 72.23, 72.28 (7d, C(2′), C(3′), C(4′)); 78.8, 79.0 (2d, C(1′)); 87.6, 87.9 (2*s*, CAr); 113.66, 113.71, 113.74, 113.76 (4d, C(ar)); 115.7, 116.4 (2t, H_2C=(allyl)); 127.4, 127.5, 128.3, 128.4, 128.6, 128.77, 128.82, 129.0, 129.2, 129.8, 130.2, 130.5, 130.88, 130.91, 133.3, 133.5, 133.8, 135.75, 135.78, 136.02, 136.07 (21d, C(ar), CH(allyl)); 127.61, 127.65 (2*s*, C(5)); 129.2, 134.3, 136.5, 136.61, 136.64, 136.9, 145.7, 145.9, 159.2, 159.3 (10*s*, C(ar)); 143.2, 143.5 (2d, C(8)); 152.1, 152.1, 153.7, 153.8 (4s, C(4), C(6)); 152.9 (d, C(2)); 165.1, 165.5, 172.61, 172.63 (4*s*, CO); ^{31}P NMR (242 MHz, $CDCl_3$): 150.0, 151.6, HR-FAB-MS (pos, NBA/CsI): 1201.3295 (100, $[M+Cs]^+$).

1-[3′-*O*-((Allyloxy)(diisopropylamino)phosphino)-2′-*O*-benzoyl-4′-*O*-((dimethoxy-triphenyl)methyl)-β-D-ribopyranosyl-(1′)]-thymine (4b). A solution of 1.99 g (3.0 mmol) of **3b** in 9 mL CH_2Cl_2 was cooled to 0 °C under argon. 1.57 mL (9.0 mmol) diisopropylethylamine was added, immediately followed by 0.95 mL (4.6 mmol) (allyloxy)(diisoproylamino)chlorophosphine. The reaction was treated with extra equivalents of the phosphitylating reagent and base as described for the preparation of **4a**. Column chromatography over silica gel (acetone/hexanes 2:8 with 1% triethylamine) yielded 2.0 g (78%) of **4b** as a white foam.

Data of 4b. TLC: (hexane/ethyl acetate 1:1): R_f 0.71. ^{1}H NMR (600 MHz, $CDCl_3$): 1.16, 1.22, 1.29 (3d, J = 6.8, CH_3(isoprop)); 1.841 (*s*, 2.1H, CH_3–C(5)); 1.84 (*s*, 0.9H, CH_3–C(5)); 2.62 (dd, $J_{4',5'}$ = 3.9, J_{gem} = 10.0, 0.7H, H-C(5′)); 2.83–2.88 (m, 0.3 H, H-C(5′)); 3.61–3.66 (m, 0.3H, CH_2–O(allyl)); 3.75–3.95 (m, 10.3H, CH_2–O(allyl), CH_3–O(DMT) HC(isoprop), H-C(4′), H-C(5′)); 4.06–4.11 (m, 0.7H, CH_2–O(allyl)), 4.16–4.21 (m, 0.7H, CH_2–O(allyl)); 4.41 (d, $J_{3',P}$ = 11.1, 0.3H, H-C(3′)); 4.63 (d, $J_{3',P}$ = 10.6, 0.7H, H–C(3′)); 4.67 (dd, J = 2.2, 9.5, 0.3H, CH_2=(allyl)); 4.82–4.85 (m, 1H, H-C(2′)); 4.90 (dq, J = 1.6, 17.2, 0.3H, CH_2=(allyl)); 5.05 (dd, J = 0.9, 10.4, 0.7H, CH_2=(allyl)); 5.23 (dq, J = 1.6, 17.1, 0.7H, CH_2=(allyl)); 5.44 (m, J = 5.3, 10.4, 17.1, 0.3H, HC=(allyl)); 5.90 (*m*, J = 5.3, 10.4, 17.1, 0.7H, HC=(allyl)); 6.21 (d, $J_{1',2'}$ = 9.5, 1H, H–C(1′)); 6.86–6.88 (m, 4 H, H–C(ar)); 7.02 (d, J = 1.1, 1H, H–C(6)); 7.22–7.64 (m, 12H, H–C(ar)); 7.97–8.02 (dd, J = 1.2, 8.2, 2H, H–C(ar)). ^{13}C NMR (150 MHz, $CDCl_3$): 12.6 (q, CH_3–C(5)); 24.7, 25.2 (2q, CH_3(isoprop)); 43.1, 43.2 (2d,

CH(isoprop)); 55.5 (q, CH_3O(DMT)); 63.9, 64.3 (2dt, $J_{CP}=19.3$, CH_2O(allyl)); 65.5, 65.6 (2t, C(5′)); 69.2, 69.4, 70.7, 71.0, 71.5, 72.4 (6d, C(2′), C(3′), C(4′)); 78.1, 78.2 (2d, C(1′)); 87.5, 87.8 (2s, CAr); 111.6, 111.7 (2s, C(5)); 113.6, 113.7 (2d, C(ar)); 115.6, 116.3 (2t, CH_2=(allyl)); 127.4, 127.5, 128.3, 128.6, 128.8, 130.4, 130.7, 130.9, 133.5, 134.0, 135.3, 135.6, 135.9, 136.0, 136.1, 136.2 (16d, C(ar), CH(allyl), C(6)); 129.4, 130.0, 136.5, 136.7, 136.9, 145.7, 145.9 (7s, C(ar)); 150.7, 150.8 (2s, C(2)); 159.2, 159.3 (2s, C(ar)); 163.9 (s, C(4)); 165.6, 165.9 (2s, CO)); ^{31}P NMR (242 MHz, $CDCl_3$): 149.7, 151.6. HR-FAB-MS (pos, NBA/CsI): 984.2641 (100, $[M+Cs]^+$).

N^4-Benzoyl-1-[3′-*O*-((allyloxy)(diisopropylamino)phosphino)-2′-*O*-benzoyl-4′-*O*-((dimethoxy-triphenyl)methyl)-β-D-ribopyranosyl-(1′)]cytosine (4c). To a stirred solution of 540 mg (0.72 mmol) of **3c** in 10 mL of dry CH_2Cl_2, 0.31 mL (1.8 mmol) of diisopropyl ethylamine and 0.23 mL (1.08 mmol) of (allyloxy)(diisoproylamino)-chlorophosphine were added. After 3 h stirring at rt, an additional 0.06 mL (0.36 mmol) of diisopropyl-ethylamine and 0.07 mL (0.36 mmol) of (allyloxy)(diisoproylamino)phosphine were added. After 5 h, the reaction was quenched by the addition of 1 mL of methanol. The solvent was evaporated and the residue was purified by chromatography on silica gel column (hexane/ethyl acetate 5:1 to 2:1). The product fractions were combined, evaporated and the residue was further purified by precipitation from 150 mL of ice cold pentane. This yielded 426 mg (63%) of **4c** as a 1:2 mixture of diastereoisomers.

Data of 4c. TLC (hexane/ethyl acetate 1:1): R_f 0.58. ^{1}H NMR (400 MHz, $CDCl_3$): 1.13 (d, $J=6.8$, 0.9H, H_3C(isoprop)); 1.18, 1.27, 1.43 (3d, $J=6.7$, 11.1H, CH_3(isoprop)); 2.64 (dd, $J_{gem}=4.9$, 10.3, 0.7H, H-C(5′)); 2.83 (dd, $J_{4',5'}=4.9$, $J_{gem}=10.3$, 0.3 H, H-C(5′)); 3.76–3.88 (m, 10 H, CH_3O(DMT), H–C(isoprop), H-C(4′), H-C(5′)); 4.06–4.17 (m, 2H, H_2C–O(allyl)); 4.46 (d(br), $J=10.0$, 0.3H, H–C(3′)); 4.62–4.68 (m, 1 H, H-C(3′), H_2C=(allyl)); 4.82 (m, 1H, H–C(2′)); 4.90 (dd, $J=1.7$, 17.2, 0.3H, H_2C=(allyl)); 5.03 (d(br), $J=10.3$, 0.7H, H_2C=(allyl)); 5.18 (dd, $J=1.6$, 17.1, 0.7H, H_2C=(allyl)); 5.43 (m, $J=5.4$, 10.3, 17.1, 0.3H, HC=(allyl)); 5.82 (m, $J=5.4$, 10.6, 17.1, 0.7H, HC=(allyl)); 6.40–6.46 (m, 1H, H-(C1′)); 6.83 (d, $J=9.0$, 4H, H-C(ar)); 7.19–8.42 (m, 21H, H–C(ar), H–C(5), H–C(6), NH). ^{13}C NMR (100 MHz, $CDCl_3$): 24.47, 24.53, 24.59, 24.95, 25.04, 25.09 (6q, CH_3(isoprop)); 42.8 (dd, $J_{CP}=12.6$, CH(isoprop)); 43.02 (dd, $J_{CP}=12.8$, CH(isoprop)); 55.22 (q, CH_3O); 63.8 (dt, $J_{CP}=19.3$, CH_2–O(allyl)); 65.3, 65.5 (2t, C(5′)); 68.9, 69.2, 70.5, 72.05, 72.13 (5d, C(2′), C(3′), C(4′)); 80.0 (d, C(1′)); 87.2 (d, C(5)); 87.5 (s, CAr); 113.21, 113.26, 113.28, 126.9, 127.0, 127.8, 128.1, 128.26, 128.32, 128.36, 129.0, 129.62, 130.1, 130.34, 130.36, 130.43, 130.46 (17*d*, C(ar)); 115.11, 115.76 (2t, CH_2=(allyl)); 132.9, 133.1, 133.4, 135.8, 136.1, 136.25, 136.28, 136.5, 145.5 (8s, C(ar)); 135.6 (d, CH=(allyl)); 145.3 (d, C(6)); 158.7 (s, C(2)); 165.1, 165.5 (2*s*, C(4), CO). ^{31}P NMR (162 MHz, $CDCl_3$): 138.1; 140.2; FAB-MS (pos, NBA): 1882 (17, $[2M+1]^+$), 941 (40, $[M+1]^+$), 726 (35), 637 (71), 303 (100), 188 (10), 105 (29).

9-[3′-*O*-((Allyloxy)(diisopropylamino)phosphino)-2′-*O*-benzoyl-4′-*O*-((dimethoxy-triphenyl)methyl)-β-D-ribopyranosyl-(1′)]-N^2-isobutyrylguanine (4d). To a stirred solution of 613 mg (0.77 mmol) of **3d** in 10 mL dry CH_2Cl_2 0.25 mL (1.16 mmol) of (allyloxy)(diisopropylamino)-chlorophosphine and 0.34 mL (1.93 mmol) of diisopropylethylamine were added. The reaction was treated with extra equivalents of the phosphitylating reagent and base as described for the preparation of **4c**. The solvent was evaporated, and the residue was purified by chromatography over silica gel (hexane/ethyl acetate 5:1 to 1:1). The product fractions were combined, evaporated and the residue was twice precipitated from 150 mL of ice cold pentane to yield 492 mg (63%) of **4d** as a 1:2 mixture of diastereoisomers.

Data of 4d. TLC (hexane/ethyl acetate 3:2): R_f 0.67. ^{1}H NMR (400 MHz, $CDCl_3$): 1.15, 1.20, 1.21, 1.23, 1.28 (5d, $J=6.8$, 12H, CH_3(isoprop)); 2.62 (dd, $J=4.7$, 10.7, 0.7 H, H-C(5′)); 2.86 (dd, $J_{gem}=4.8$, 10.5, 0.3 H, H–C(5′)); 3.35–3.50 (m, 1H, H–C(isobut)); 3.62–3.70 (m, 0.3H, H_2C–O(allyl)); 3.75–3.98 (m, 10.3H, CH_3–O(DMT), CH_2–O(allyl), CH(isoprop), H-C(4′), H-C(5′)); 4.05–4.12 (m, 0.7 H, CH_2–O(allyl)); 4.16–4.22 (m, 0.7H, CH_2–O(allyl)); 4.54, 4.74 (d, $J_{3',P}=10.7$, 0.7 H, H-C(3′)); 4.83 (d, $J_{1',2'}=10.4$, 0.3 H, H-C(2′)); 4.90 (dd, $J=1.6$, 17.2, 0.3H, H_2C=(allyl)); 4.96–4.99 (m, 2H, H_2C–O(allyl)); 5.05 (*d*, $J_{1',2'}=10.3$, 0.7 H, H–C(2′)); 5.19–5.49 (m, 4 H, H_2C=(allyl), HC=(allyl)), 5.89 (m, $J=5.4$, 10.5, 17.1, 0.7H, HC=(allyl)); 5.99–6.12 (m, 2H, HC=(allyl), H-C(1′)); 6.83–7.82 (m, 25H, H–C(ar), H–C(8)). ^{13}C NMR (100 MHz, $CDCl_3$): 19.11, 19.16, 19.22, 19.42 (4q, CH_3(isobut)); 24.39, 24.45, 24.48, 24.54, 25.00, 25.09, 25.12, 25.2 (8q, CH_3(isoprop)); 34.4 (d, CH(isobut)); 42.8, 42.9 (2dd, $J_{CP}=12.8$, CH(isoprop)); 55.2 (q, CH_3O(DMT)); 63.7 (dt, $J_{CP}=22.1$, CH_2O(allyl)); 64.1 (dt, $J_{CP}=19.8$, CH_2O(allyl)); 65.1, 65.2 (2t, C(5′)); 67.8 (t, CH_2O(allyl)); 68.9, 69.1, 70.5, 70.6, 71.3, 71.8, 71.9 (7d, C(2′), C(3′), C(4′)); 78.7, 78.9 (2d, C(1′)); 87.3, 87.5 (2*s*, CAr); 113.23, 113.27, 133.31 (3d, C(ar)); 115.3, 115.9 (2t, CH_2=(allyl)); 117.9, 118.0 (2*s*, C(5)); 118.6 (t, CH_2=(allyl)); 126.9, 127.0, 127.86, 127.93, 128.1, 128.3, 129.7, 129.9, 130.0, 130.37, 130.40, 132.0, 132.9, 133.3, 135.3, 135.3, 135.5, 135.6 (18d, C(ar), CH–(allyl)); 128.9, 129.5, 136.0, 136.15, 136.15, 136.4, 145.2, 145.5, 158.7, 158.8 (10s, C(ar)); 139.8, 139.9 (2d, C(8)); 151.99, 152.03, 152.9, 153.0 (4s, C(2), C(4)); 160.51, 160.54 (2s, C(6)); 164.6, 164.9, 177.3 (3s, CO); ^{31}P NMR (162 MHz, $CDCl_3$): 138.5, 139.9. FAB-MS (pos, NBA): 1974 (15, $[2M+1]^+$), 987 (48, $[M+1]^+$), 683 (30), 303 (100), 105 (85).

N^6-Benzoyl-9-[2′-*O*-benzoyl-4′-*O*-((4″,4‴-dimethoxytriphenyl)methyl)-α-L-lyxopyranosyl-(1′)]adenine (7a). To a stirred soln of 3.21 g (4.13 mmol) of **6a** in 196 mL THF/H_2O (2:1) at 0 °C was added dropwise 6.42 mL of 15% aq NaOH soln. After stirring for 2 h, 40 mL of an 1 M Et_3NHHCO_3 soln was added and THF was removed by distillation at rt. The soln was diluted with CH_2Cl_2 and the resulting mixture was washed with satd

aq $NaHCO_3$ soln. The org phase was dried (Na_2SO_4) and evaporated. The residue was subjected to column chromatography on silica gel (petroleum ether/acetone 2:1) to afford an amorphous colorless solid. A soln of this compound in 50 mL of dry CH_2Cl_2 and 1.7 mL pyridine was cooled to 0 °C. After dropwise addition of 0.96 mL (8.28 mmol) benzoyl chloride over a period of 30 min, 50 mL of CH_2Cl_2 and 20 mL of satd aq $NaHCO_2$ soln were added. The org phase was washed with 20 mL of satd aq $NaHCO_3$ soln, 20 mL of H_2O, dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography on silica gel (petroleum ether/acetone 2:1) and the combined product fractions were dried (Na_2SO_4) and evaporated. The colorless amorphous product was dried for 12 h in vacuo (~0.1 torr) to furnish 2.60 g (81.1%) of **7a**.

Data of 7a. TLC (Et_2O, 100%): R_f 0.52. ^{1}H NMR (600 MHz, $CDCl_3$): 3.45 (d, J_{gem} = 12.2, H–C(5′)); 3.79 (m, 6H, OC*H*$_3$); 3.88–3.90 (m, 2H, H–C(3′), H–C(4′), overlapping signals); 4.09 (d, J_{gem} = 12.2, H–C(5′)); 6.06 (dd, $J_{2',3'}$ = 2.4, $J_{1',2'}$ = 9.6, H–C(2′)); 6.44 (d, $J_{1',2'}$ = 9.6, H–C(1′)); 6.87–7.98 (m, 23 arom H); 8.37, 8.67 (2s, H–C(2), H–C(8)); 9.14 (s, NH). ^{13}C NMR (150.9 MHz, $CDCl_3$): 55.6 (q, OCH_3); 67.3 (t, C(5′)); 69.2, 71.4 (2d, C(3′), C(4′)); 71.6 (d, C(2′)); 78.5 (d, C(1′)); 88.0 (*s*, C(Ph)$_3$); 113.8 (d, arom C); 122.8 (s, C(5)); 127.6, 128.2, 128.5, 128.7, 129.2, 129.8, 130.7, 133.2, 133.9 (9d, arom C); 134.0, 136.4, 136.5 (3s, arom C); 141.5 (d, C(8)); 145.5, 149.9, 152.1 (3s, arom C); 153.1 (d, C(2)); 159.2 (s, arom C); 164.8, 165.0 (2s, CO). HR-FAB-MS (pos, NBA/CsI): 1042 (21, $[M+H+2Cs]^+$), 910.1822 (100, $[M+H+Cs]^+$). Anal. calcd for $C_{45}H_{39}N_5O_8$: C 69.49, H 5.05, N 9.00, found: C 69.24, H 5.34, N 9.10.

1-[2′-*O*-Benzoyl-4′-*O*-((4″,4‴-dimethoxytriphenyl)-methyl)-α-L-lyxopyranosyl-(1′)]thymine (7b). To a stirred soln of 5.92 g (8.91 mmol) of **6b** in 400 mL dry MeOH was added 400 mg of K_2CO_3 and kept for 4 h at 4 °C. After the addition of 50 mL of 1 M Et_3NHHCO_3 soln, the mixture was evaporated and the residue dissolved in CH_2Cl_2. The org phase was washed with satd aq $NaHCO_3$ soln, dried (Na_2SO_4) and evaporated. The remaining violet solid was dried overnight in vacuo (~0.2 torr). After dissolving this solid in 27 mL of CH_2Cl_2 and cooling to 0 °C, 3.8 mL (47.1 mmol) of dry pyridine was added. To the stirred soln 1.32 mL (11.4 mmol) of benzoyl chloride was added dropwise over a period of 20 min. The soln was washed with satd aq $NaHCO_3$ soln and extracted with CH_2Cl_2. The org phase was dried (Na_2SO_4) and evaporated. Purification of the residue by column chromatography on silica gel (petroleum ether/acetone 2:1) afforded 4.35 g (73.5%) **7b** as an amorphous colorless solid..

Data of 7b. TLC (petroleum ether/acetone 2:1): R_f 0.40. ^{1}H NMR (600 MHz, $CDCl_3$): 1.97 (s, 3H, C*H*$_3$–C(5)); 3.29 (d, J_{gem} = 12.1, H–C(5′)); 3.79 (2s, 6H, OC*H*$_3$); 3.85 (m, H-C(4′)); 3.89 (m, H–C(3′)); 3.95 (d, J_{gem} = 12.1, H–C(5′)); 5.59 (dd, $J_{2',3'}$ = 2.6, $J_{1',2'}$ = 9.6, H–C(2′)); 6.21 (d, $J_{1',2'}$ = 9.6, H–C(1′)); 6.87–7.94 (m, 18 arom H, H–C(6)); 9.36 (s, br, NH). ^{13}C NMR (150.9 MHz, $CDCl_3$): 13.2 (q, *C*H$_3$–C(5)); 55.6 (q, OCH_3); 67.0 (t, C(5′)); 69.3 (d, C(3′)); 70.9 (d, C(2′)); 71.3 (d, C(4′)); 78.1 (d, C(1′)); 87.9 (s, C(Ph)$_3$); 112.1 (s, C(5)); 113.8 (d, arom C); 127.6 (s, arom C); 128.5, 128.9, 129.2, 130.2, 130.67, 130.71, 133.9, 135.9 (8d, arom C); 136.4, 136.5 (2s, arom C); 145.6, 151.2 (2s); 159.2 (s, arom C); 164.1, 165.6 (2s, CO). HR-FAB-MS (pos, NBA/CsI): 797.1498 (15.5 $[M+H^+Cs]^+$). Anal. calcd for $C_{38}H_{36}N_2O_9$: C 68.66, H 5.46, N 4.21, found: C 68.45, H 5.48, N 4.74.

N^4-Benzoy1-[2′-*O*-benzoyl-4′-*O*-((4″,4‴-dimethoxytriphenyl)methyl)-α-L-lyxopyranosyl-(1′)]cytosine (7c). To a soln of 500 mg (0.72 mmol) of **6c** in 30 mL 1,4-dioxane/water (2:1) cooled to 0 °C, was added dropwise, 1.5 mL of 15% aq NaOH soln. The soln was allowed to stir for 2 h, at 0 °C, until the reaction was complete (TLC). The soln was diluted with CH_2Cl_2, at 0 °C, and extracted first with 50 mL of 1 M aq NaH_2PO_4 (pH 6.5), and then with satd aq $NaHCO_3$ soln. The organic layer was dried ($MgSO_4$), filtered and evaporated under vacuum. The residue was dissolved in 10 mL of dry CH_2Cl_2, cooled to 0 °C, and 291 μL (3.6 mmol) of pyridine was added. To the stirred soln, 162 μL (1.4 mmol) benzoylchloride dissolved in 5 mL dry CH_2Cl_2 was added dropwise. The soln was allowed to stir for an additional hour at 0 °C. It was diluted with CH_2Cl_2 and extracted with satd aq $NaHCO_3$ soln. The aqueous layer was extracted twice with CH_2Cl_2, the combined organic phases dried ($MgSO_4$), filtered, and was evaporated under vacuum. The residue was purified by flash chromatography on a silica gel (acetone/hexane 1:2 to 2:3) to afford 516 mg (95%) of **7c**.

Data of 7c. TLC (acetone/hexane 1:1): 0.48. ^{1}H NMR (200 MHz, $CDCl_3$): 3.37 (d, br, J_{gem} = 12.4, H–C(5′)); 3.82 (s, 6H, OCH_3); 3.82–3.90 (m, 2H, H–C(3′), H–C(4′)); 3.99 (d, br, J_{gem} = 12.4, H–C(5′)); 5.62 (dd, $J_{2',3'}$ = 2.4, $J_{1',2'}$ = 9.8, H–C(2′)); 6.47 (d, $J_{1',2'}$ = 9.8, H–C(1′)); 7.25–8.12 (m, 23 arom–H, H–C(5), H–C(6)). FAB-MS (pos, NBA): 303 (74, $[DMTr]^+$); 755 (100, $[M+H]^+$); 1508 (23, $[2M+H]^+$).

9-[2′-*O*-Benzoyl-4′-*O*-((4″,4‴-dimethoxytriphenyl)methyl)-α-L-lyxopyranosyl-(1′)]-N^2-isobutyrylguanine (7d). To a soln of 300 mg (0.43 mmol) **6d** in 30 mL 1,4-dioxane/water (2:1) cooled to 0 °C, was added dropwise, 1 mL of 15% aq NaOH soln and stirred at 0 °C for 3 h. The soln was diluted with CH_2Cl_2 and extracted with 30 mL 1 M aq NaH_2PO_4 soln (pH 6.5) followed by satd aq $NaHCO_3$ soln. The organic phase was dried ($MgSO_4$) and evaporated under vacuum to dryness. The resulting syrup was purified by flash chromatography on silica gel (acetone/hexane 3:2) to afford 263 mg of the debenzoylated intermediate. This was dissolved in 7.5 mL dry CH_2Cl_2, cooled to 0 °C under an argon atmosphere and added dropwise a soln of 92 μL (0.8 mmol) benzoylchloride dissolved in 3 mL CH_2Cl_2. The soln was allowed to stir for 1 h at 0 °C before it was diluted with a mixture of CH_2Cl_2 and satd aq $NaHCO_3$ soln. The aqueous phase was extracted twice with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$) and evaporated under vacuum. The resulting residue was purified by chromatography on a silica gel column (acetone/hexane 1:2 to 3:2) to give 280 mg (87%) of **7d**.

Data of 7d. TLC (acetone/hexane 2:1): 0.62. ^{1}H NMR (300 MHz, $CDCl_3$): 1.14, 1.16 (d, $J=6.9$, $(CH_3)_2CH$)); 2.63 (m, 1H, $(CH_3)_2CH$); 3.29 (d, br, $J_{gem}=12.3$, H–C(5′)); 3.75 (*s*, 6H, OCH_3); 3.81 (d, br, $J_{3',4'}=3.9$, H–C(4′)); 3.89 (d, br, $J_{gem}=12.3$, H-C(5′)); 4.02 (dd, $J_{2',3'}=2.1$, $J_{3',4'}=3.9$, H–C(3′)); 5.96 (d, $J_{1',2'}=9.6$, H–C(1′)); 6.12 (dd, $J_{3',4'}=2.1$, $J_{1',2'}=9.6$, H–C(2′)); 6.84–7.74 (m, 21H, arom); 7.98 (s, H–C(8)); 8.10 (d, $J=8.1$, 1H, arom); 8.58 (d, $J=3.6$, 1H, arom); 9.11, 11.97 (2s, br, 2H, H–N). FAB-MS (pos, NBA): 303 (100, $[DMTr]^+$); 760 (31, $[M+H]^+$); 1520 (7, $[2M+H]^+$).

N^6-Benzoyl-9-[2′-*O*-benzoyl-3′-*O*-((2-cyanoethoxy)(diisopropylamino)phosphino)-4′-*O*-((4″,4‴-dimethoxytriphenyl)methyl)-α-L-lyxopyranosyl-(1′)]adenine (8a). To a soln of 787 mg (1.0 mmol) **7a** in 7 mL of dry CH_2Cl_2, 0.56 mL (4.3 mmol) 2,4,6-collidine, 40 μL (0.5 mmol) *N*-methylimidazole were added followed by 564 μL (2.2 mmol) of 97% (2-cyanoethoxy)(diisopropyl amino)-chlorophosphine and stirred for 1 h at rt The soln was diluted with 5 mL of AcOEt and washed with satd aq $NaHCO_3$ soln. The org phase was dried ($MgSO_4$) and concentrated. The residual oil was purified by column chromatography on silica gel (petroleum ether/AcOEt 1:1) to give 727 mg (73%, mixture of diastereomers ≈3:2 by ^{1}H NMR) of **8a** as a slightly yellowish foam.

Data of 8a. TLC (petroleum ether/AcOEt 1:1): R_f 0.32. ^{1}H NMR (600 MHz, $CDCl_3$): 1.02, 1.09 (2d, $J=6.8$, $(CH_3)_2CH$(a)); 1.12, 1.18 (2d, $J=6.8$, $(CH_3)_2CH$(b)); 2.10–2.31 (m, 2H, $-OCH_2CH_2-CN$); 2.87 (d, $J_{gem}=12.4$, H–C(5′b)); 3.25 (d, $J_{gem}=12.1$, H–C(5′a)); 3.45 (m, H–C(4′a)); 3.56 (2m, $(CH_3)_2CH$; 1H–CH_2O, overlapping signals); 3.68 (m, 1H–CH_2–O); 3.78–3.87 (m, H–C(5′b); 6H, $-OCH_3$(a,b), overlapping signals); 3.97 (d, $J_{gem}=12.1$, H–C(5′a)); 4.02 (d, $J_{3',4'}=4.0$, H–C(4′b)); 4.43 (m, $J_{3',P}=10.9$, H–C(3′a)); 4.65 (m, $J_{3',P}=9.0$, H–C(3′b)); 6.03 (dd, $J_{2',3'}=2.5$, $J_{1',2'}=9.6$, H–C(2′a)); 6.15 (dd, $J_{2',3'}=2.5$, $J_{1',2'}=9.6$, H–C(2′b)); 6.25 (d, $J_{1',2'}=9.6$, H–C(1′b)); 6.34 (d, $J_{1',2'}=9.6$, H–C(1′a)); 6.89–8.0 (m, 23 arom H); 8.37, 8.40, 8.82, 8.87 (4s, H–C(2a,b), H–C(8a,b)); 9.35 (*s*, NH(a,b)). ^{13}C NMR (150.9 MHz, $CDCl_3$): 19.8, 19.9 (t,–CH_2–CN); 24.7, 24.9 (q, CH_3(a)); 24.95, 24.97 (q, CH_3(b)); 43.6 (d, $CH_{i\text{-}pr}$); 57.9 (d, C(4′b)); 59.0 (t, O–CH_2); 67.0 (t, C(5′b)); 67.2 (t, C(5′a)); 70.2 (d, C(3′a)); 70.3, 70.94, 70.99 (3d, C(2′b), C(4′a), C(4′b)); 71.8 (d, C(2′a)); 72.0 (d, C(3′b)); 78.7 (d, C(1′b)); 78.9 (d, C(1′a)); 113.9 (s, arom C); 122.9 (s, CN); 127.6, 128.25, 128.27, 128.56, 128.66, 128.67, 128.8, 128.9, 129.2, 129.3, 129.5, 130.0, 130.2, 130.7, 130.81, 130.84, 131.0, 133.12, 133.16, 133.92, 133.98, 134.1, 134.20, 136.3, 136.33, 136.37, 136.6, 145.5, 145.6, 149.9, 150.0, 152.5, 152.5, 159.3, 159.4 (arom C, C(4a,b), C(5a,b), C(6a,b)); 141.54, 141.63 (2d, C(8a,b)); 153.5 (2d, C(2a,b), overlapping signals); 165.1 (2s, CO, overlapping signals); 165.3, 165.6 (2s, CO). ^{31}P NMR (242.9 MHz, $CDCl_3$): 151.54, 152.35 (2s, (a,b)). FAB-MS (pos, NBA/CsI): 1110.2967 (100.0, $[M+Cs]^+$), 1242 (9.8, $[M+2Cs]^+$). Anal. calcd for $C_{54}H_{56}N_7O_9P$: C 67.42, H 5.87, N 10.19, found: C 66.88, H 6.18, N 9.74.

1-[2′-*O*-Benzoyl-3′-*O*-((2-cyanoethoxy)(diisopropylamino)phosphino)-4′-*O*-((4″,4‴-dimethoxytriphenyl)methyl)-α-L-lyxopyranosyl-(1′)]thymine (8b). To a soln of 620 mg (0.93 mmol) of **7b** in 7 mL of dry CH_2Cl_2, 1.0 mL (7.6 mmol) 2,4,6-collidine and 40 μL (0.5 mmol) *N*-methylimidazole were added followed by 520 μL (2.0 mmol) of 97% (2-cyanoethoxy)(diisopropylamino)chlorophosphine and stirred for 1 h at rt. The soln was diluted with 5 mL AcOEt and washed with satd aq $NaHCO_3$ soln. The org phase was dried ($MgSO_4$) and concentrated. Purification of the resulting oil by column chromatography on silica gel (petroleum ether/AcOEt 3:2) afforded 663 mg (82%, mixture of diastereomers ~1:1 by ^{1}H NMR) of **8b** as a colorless foam.

Data of 8b. TLC (petroleum ether/AcOEt 1:1): R_f 0.48. ^{1}H NMR (600 MHz, $CDCl_3$): 0.95, 1.07 (2d, $J=6.8$, $(CH_3)_2CH$(a)); 1.00, 1.13 (2d, $J=6.8$, $(CH_3)_2CH$(b)); 2.01 (*s*, CH_3–C(5a,b)); 2.33 (t, 2H, $J=6.6$, $-OCH_2CH_2-$CN); 2.78 (d, $J_{gem}=12.4$, H–C(5′b)); 3.07 (d, $J_{gem}=12.2$, H–C(5′a)); 3.42–3.77 (m, H–C(4′a), 2H–OCH_2CH_2CN, H–C(5′b), $(CH_3)_2CH$, overlapping signals); 3.82 (*s*, 6H, OCH_3); 3.86 (d, $J_{gem}=12.2$, H–C(5′a)); 3.94 (m, H–C(4′b)); 4.39 (dt, $J_{2',3'}=2.4$, $J_{3',P}=11.1$, H–C(3′a)); 4.55 (dt, $J_{2',3'}=2.4$, $J_{3',P}=9.3$, H–C(3′b)); 5.55 (dd, $J_{2',3'}=2.4$, $J_{1',2'}=9.6$, H–C(2′a)); 5.64 (dd, $J_{2',3'}=2.4$, $J_{1',2'}=9.62$, H–C(2′b)); 6.16 (d, $J_{1'2'}=9.6$, H–C(1′b)); 6.21 (d, $J_{1',2'}=9.6$, H–C(1′a)); 6.88–7.57 (18 arom H, H–C(6)); 7.97, 8.00 (2d, arom. H); 8.73, 9.22 (2s, br, NH(a,b)). ^{13}C NMR (150.9 MHz, $CDCl_3$): 13.13 (q, CH_3–C(5a,b)); 20.05, 20.09 (2t, $-OCH_2CH_2CN$(a,b)); 24.65, 24.78 (2q, $(CH_3)_2CH$(a,b)); 43.30, 43.38, 43.52, 43.60 (4d, $(CH_3)_2CH$(a,b)); 55.58 (q, OCH_3); 59.08, 59.20 (2t, $-OCH_2CH_2CN$)); 66.91 (t, C(5′b)); 67.01 (t, C(5′a)); 69.82 (d, C(3′a)); 69.92 (d, C(2′b)); 70.85 (d, C(2′a)); 70.95 (2d, C(4′a,b), overlapping signals); 72.14 (d, C(3′b)); 78.10 (d, C(1′b)); 78.20 (d, C(1′a)); 87.84 (*s*, $C(Ph)_3$); 112.21 (*s*, C(5)); 113.82, 113.89 (2d, C(ar)); 118.10 (*s*, CN); 127.63, 128.53, 128.66, 128.86 (4d, arom C); 129.63 (*s*, arom C); 130.11, 130.39 (2d, arom C); 130.67 (s, arom C); 130.75, 130.82 (2d, arom C); 130.87 (s, arom C); 134.01, 135.53, 135.84 (3d, arom C); 136.18, 136.29, 145.61 (3s, arom C); 151.32 (*s*, C(2)); 163.89, 164.05 (2s, CO); 165.76 (s, CO (Bz)). ^{31}P NMR (242.9 MHz, $CDCl_3$): 150.20, 152.51 (2s, (a,b)). HR-FAB-MS (pos, NBA/CsI): 997.2518 (100 $[M+Cs]^+$), 1129 (9, $[M+2Cs]^+$). Anal. calcd for $C_{47}H_{53}N_4O_{10}P$: C 65.27, H 6.18, N 6.48, found: C 65.28, H 5.99, N 6.39.

N^4-benzoyl-1-[2′-*O*-benzyl-3′-*O*-((2-cyanoethoxy)(diisopropylamino)phosphino)-4′-*O*-((4″,4‴-dimethoxytriphenyl)methyl)-α-L-lyxopyranosyl-(1′)]cytosine (8c). A solution of 252 mg (334 μmol) **7c**, 14 μL (0.17 mmol) *N*-methylimidazole and 177 μL (1.4 mmol) 2,4,6-collidine in 3 mL dry CH_2Cl_2 was stirred under argon at rt. To this soln, 203 μL (0.9 mmol) of (2-cyanoethoxy)(diisopropylamino)chlorophosphine was added dropwise and the soln was stirred over night at rt. The soln was concentrated in vacuum and purified by chromatography on a silicagel column (acetone/hexane 1:2 to 3:2) to yield 242 mg (72%) of **8c** as a 4:5 mixture of diastereomers.

Data of 8c. TLC (acetone/hexane 1:1): R_f 0.8. ^{1}H NMR(300 MHz, $CDCl_3$): 0.9–1.14 (m, 12H, $(CH_3)C$); 2.30–2.37 (m, 2H, H–$CN(CH_3)_2$); 2.78 (d, $J_{gem}=12.0$, H–C(5′b)); 3.03 (d, br, $J_{gem}=12.0$, H–C(5′a)); 3.39–3.61 (m, 4H, $NC(CH_2)_2O$); 3.73–3.96 (m, H–C(4′a,b)); 3.80–3.81 (s, 6H, OCH_3); 4.44–4.62 (m, H–C(3′a,b)); 5.55, 5.63 (dd, $J_{2',3'}=2.4$, $J_{1',2'}=9.3$, H–C(2′a,b)); 6.41, 6.48 (d, $J_{1',2'}=9.3$, H–C(1′a,b)); 6.86–8.04 (m, 26H, arom, H–C(5), H–C(6)); 11.35 (s, br, 1H, H–N). ^{31}P NMR (300 MHz, $CDCl_3$): 152.03, 151.14. FAB-MS (pos, NBA): 303 (100, $[DMTr]^+$); 954 (3, $[M+H]^+$); 1909 (4, $[2M+H]^+$).

9-[2′-*O*-Benzoyl-3′-*O*-((2-cyanoethoxy)(diisopropylamino)phosphino)-4′-*O*-((4″,4‴-dimethoxytriphenyl)methyl)-α-L-lyxopyranosyl-(1′)]-N^2-isobutyrylguanine (8d). A solution of 120 mg (158 μmol) **7d**, 8 μL (0.1 mmol) *N*-methylimidazole and 93 μL (0.7 mmol) 2,4,6-collidine in 1 mL dry CH_2Cl_2 was stirred under argon at rt. To this soln, 90 μL (0.4 mmol) of (2-cyanoethoxy) (diisopropylamino)chlorophosphine was added dropwise and the soln stirred overnight at rt. The soln was concentrated in vacuum and purified by chromatography on a silicagel column (AcOEt/hexane 2:3 to 1:0) affording 110 mg (70%) of **8d** as a mixture of diastereomers.

Data of 8d. TLC (AcOEt/hexane 2:1): R_f 0.21. ^{1}H NMR (300 MHz, $CDCl_3$): 0.99–1.21 (m, 6H, $(CH_3)_2CH$); 1.25 (d, $J=6.9$, 6H, $(CH_3)_2CHN$); 1.27 (d, $J=6.6$, 6H, $(CH_3)_2CHN$); 2.31–2.75 (m, 6H, $NC(CH_2)_2O$, H–$CN(CH_3)_2$); 2.92–2.99 (2d, $J_{gem}=12.3$, H–C(5′a,b)); 3.30–3.70 (m, 3H, H–C(4′a,b), H–C(5′a,b), H–$C(CH_3)_2$); 3.79 (s, 6H, OCH_3); 4.70, 4.76 (m, H–C(3′a,b)); 5.81–5.92 (m, H–C(2′b), H–C(1′a)); 6.02 (d, $J_{1',2'}=9.6$, H–C(1′b)); 6.36 (dd, $J_{2',3'}=2.1$, $J_{1',2'}=9.6$, H–C(2′a)); 6.82–7.95 (m, 18H, arom); 8.01, 8.04 (s, 1H, H–C(8a,b)); 9.20 (s, br, 1H, H–N). ^{31}P NMR (121 MHz, $CDCl_3$): 150.4, 151.3. FAB-MS (pos, NBA): 303 (100, $[DMTr]^+$); 960 (13, $[M+H]^+$); 1920 (52, $2M+H]^+$).

Automated solid-phase synthesis on the gene synthesizer

General. The phosphoramidite solutions and the activator solutions were dried over 3 or 4 Å molecular sieves (8–12 mesh, freshly activated by heating at ca. 300 °C under HV overnight) overnight, in a dessicator (containing KOH), at rt prior to use.

pr(4′→3′)-oligonucelotides. p-RNA-Oligonucleotide syntheses were performed in a 10 μmol scale on either a Gene Assembler Plus™ (Pharmacia) or on an Perseptive Expedite 8909 Nucleic Acid Synthesis System (PerSeptive Biosystems). On the Pharmacia synthesizer, the protocol included the following steps: Detritylation for 4.5 min with 6% dichloroacetic acid in dichloroethane, Coupling for 60 min with a 10% (w/v) solution of phosphoramidite in acetonitrile (0.1 M, 500 μL per coupling; $n\times40$ mg of phosphoramidite dissolved in $n\times500$ μL of dry acetonitrile, n = number of couplings). Activation by adding a mixture of 0.15 M 4-nitrophenyl-tetrazole and 0.35 M tetrazole in acetonitrile (600 μL per coupling). Capping for 1.5 min with 0.45 M dimethylaminopyridine in acetonitrile and a mixture of collidine/acetic anhydride/acetonitrile 3:2:5. Oxidation for 2 min with 0.01 M iodine in acetonitrile/sym-collidine/water 100:9.2:46. On the Expedite 8909 synthesizer: Coupling was performed by using a 0.05–0.075 M solution of the 3′-phosphoramidite (4–6 equiv with respect to nucleotide bound to CPG) and a solution of 0.15 M 4-nitrophenyl tetrazole and 0.35 M tetrazole. As the machine does not allow cyclic pumping of the amidite/tetrazole mixture over the CPG-support, the coupling was divided into four steps. In each step the reaction mixture, which contained about 1–1.5 equiv of phosphoramidite with respect to nucleotide bound to the CPG support, was allowed to stand on the support for 900 s. Then it was slowly (within 300 s) washed out by pressing tetrazole solution through the support. Detritylation, capping and oxidation procedures were analogous to the Pharmacia protocol. All oligonucleotides were synthesized in the 'Trityl-on' mode.

pl(4′→3′)-Oligonucelotides. Oligonucleotide synthesis were carried out on a 1 μM scale. The Expedite 8909 synthesizer column was filled with the CPG solid support loaded with the appropriate nucleobase. The substrates and reagents required were prepared as follows. Phosphoroamidites: The amount of phosphoramidite solution was determined as follows: For adenine, thymine or the four-base containing sequences: $(n+1)\times22$ mg of phosphoramidite dissolved in $(n+1)\times312$ μL of dry acetonitrile. For guanine or cytosine containing sequences: $(n+2)\times18$ mg of phosphoramidite dissolved in $(n+2)\times230$ μL of dry acetonitrile (n = number of couplings). The phosphoramidite solution (ca. 0.08 M) was dried over 3 or 4 Å molecular sieves (8–12 mesh, freshly activated by heating at ca. 300 °C under HV overnight) overnight at rt prior to use. The excess of phoshporamidites, depending on the sequence synthesized, ranged from 160 to 323 equiv Activator solution: 5-Ethylthio-1H-tetrazole in dry acetonitrile (0.25 M for adenine or thymine containing sequences and 0.35 M for guanine or cytosine containing sequences). Capping A: A solution of 3.0 g of 4-(dimethylamino)-pyridine in 50 mL of dry acetonitrile and filtered to remove any undissolved solid particles. Capping B. A solution of 10 mL of acetic acid anhydride and 15 mL of 2,4,6-collidine in 25 mL of dry acetonitrile. Oxidizing solution: A solution of 220 mg of iodine and 4.6 mL of 2,4,6-collidine in 23 mL of water and 50 mL of acetonitrile and filtered to remove any undissolved residue. Detritylation reagent: A solution of 6% dichloroacetic acid in 1,2-dichloroethane. The synthesis of oligonucleotides using 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 about 15–16.7 min and (2) the detritylation was accomplished by 6% dichloroacetic acid in 1,2-dichloroethane over a 3 min period. All oligonucleotides were synthesized in the 'Trityl-on' mode.

Post-automation procedures

pr(4′→3′)-Oligonucleotides. The post-automation work up procedures were identical with those developed for the 4′,2′-p-RNA.[3]

Removal of the phosphotriester-allyl protecting group. The CPG solid support, containing the oligonucleotide ('trityl-on') from the synthesizer, was dried in HV for 30 min at rt, and treated with 90 mg (78.0 μmol) of $Pd(PPh_3)_4$, 90 mg (344 μmol) of PPh_3, 90 mg (666 μmol) of $Et_2NH{\cdot}H_2CO_3$ in 5 mL of CH_2Cl_2 at rt, following the procedure of Noyori et al.[21] The CPG was filtered, washed sequentially with 0.1 M aq sodium diethyl dithiocarbamate, CH_2Cl_2, acetone and water, then dried.

Removal of oligonucleotide from solid support and deprotection of acyl-protecting groups. The dried CPG was treated with 25% aq NH_2NH_2 hydrate in water (prepared from 1 mL NH_2NH_2 hydrate and 4 mL water) at 4 °C for 24 h. Deprotection was monitored by ion exchange HPLC. After complete deprotection, the CPG was filtered and washed sequentially with water. The washings were combined with filtrate and were desalted (refer to desalting of oligonucelotides) over a Waters Sepak-C18 cartridge [eluted with 10–15 mL acetonitrile/water (1:1)] to afford the salt free, crude oligonucleotides ('Trityl-on') in solution.

Detritylation. The crude oligonucleotide solution obtained by above was concentrated in vacuo to dryness, the residue treated with about 10 mL of 80% aq formic acid (a red color appears within seconds indicating detritylation) at rt for 15–30 min and concentrated in vacuo to dryness. The residue was dissolved in about 2 mL of water, filtered (Nalgene syringe filter, 0.2 μM) and taken to the next step of HPLC purification (Fig. 5).

pl(4′→3′)-oligonucleotides. The oligonucleotides with the 4′,3′-connection were less stable to the regular deprotection conditions (25% aq hydrazine hydrate). Therefore a modified approach was necessary to minimize strand scission during the cleavage of the oligo from the CPG solid support and the removal of the protecting groups on the base and sugar moieties.

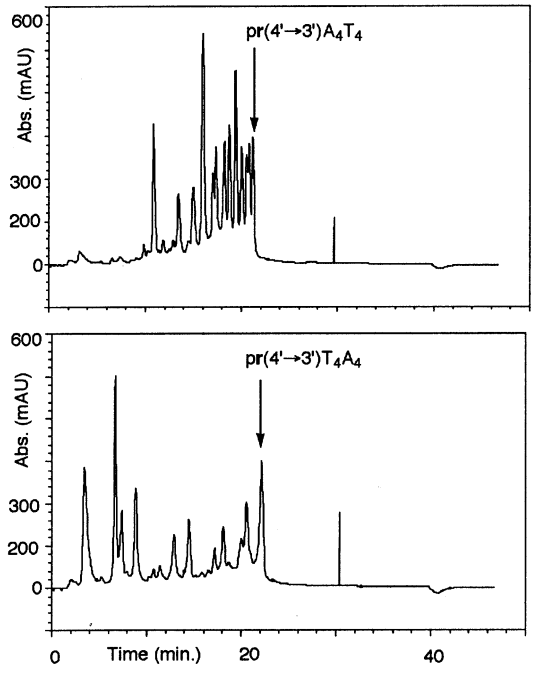


Figure 5. HPLC trace of the crude, fully deprotected, (D)-β-ribopyranosyl(4′→3′)-oligonucleotides, pr(4′→3′)(A_4T_4) and pr(4′→3′)(T_4A_4). For conditions of chromatography, see Table 1.

Removal of phosphotriester-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 at rt, transferred to a pear shaped 10 mL flask and treated with 2.4 mL of pyridine/triethylamine (5:1) for 6.5 h at rt. Evaporation of pyridine and triethylamine in vacuo followed by coevaporation with toluene (or DMF) to dryness—avoiding temperatures over 35 °C—resulted in dry CPG solid support material.

Detachment of oligonucleotide from CPG solid support with concomitant deprotection of sugar and nucleobase protecting groups. One of the following two procedures were used depending on the sequence of the oligonucleotides (Table 1 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 a mixture of 40% aq CH_3NH_2 in concd aq NH_3 (1:1) and shaken at rt for 6.5 h (ca. 70 h for G,C containing sequences). The suspension was co-evaporated with water, carefully (with the temperature always less than 35 °C) to remove the volatiles CH_3NH_2 and NH_3, and filtered. The filtrate was diluted with about 5–10 mL of 0.5 M aq $Et_3NH_2CO_3$ buffer and desalted (refer to desalting of oligonucleotides) over a Waters Sepak-C18 cartridge [eluted with 10–15 mL acetonitrile/water (1:1)] to afford the salt free, crude oligonucleotides ('Trityl-on') in solution.

Method B. To the flask containing the dry CPG solid support was added 2 mL of a solution of $CH_3ONH_2{\cdot}HCl$ dissolved in concd aq NH_3 and EtOH (3:1) and shaken at rt for approx. 6.5 h (ca. 70 h for G,C containing sequences). The suspension was filtered, the filtrate diluted with about 5–10 mL of 0.5 M aq $Et_3NH_2CO_3$ buffer and desalted (refer to desalting of oligonucleotides) over a Waters Sepak-C18 cartridge [eluted with 10–15 mL acetonitrile/water (1:1)] to afford the salt free, crude oligonucleotides ('Trityl-on') in solution.

All of the above deprotections were monitored by anion exchange HPLC for optimum deprotection time.

Detritylation of 'Trityl-on' oligonucleotides. This was performed as described for pr(4′→3′)-oligonucleotides.

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 analogue integrator or (B) Pharmacia Äkta purifier (900) controlled by UNICORN. The oligonucleotides were eluted from the column using a linear gradient of 1 M NaCl in 10 mM aq Na_2HPO_4, pH = 10.5 over a period of 20–30 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, 1 M NaCl, pH = 10.5. Exact gradient conditions are given in Table 1. The pure product containing fractions were collected in Eppendorf vials containing 20 μL of 1M aq acetic acid (to neutralize the high pH), combined and desalted to remove excess salt. In the cases where the first HPLC purification gave still impure oligonucleotides, the combined product fractions were desalted and re-purified by HPLC.

Desalting of oligonucleotides

For desalting, a Waters Sepak-C18 cartridge was equilibrated with 10 mL of acetonitrile/water (1:1) followed by 10 mL of water and finally 10 mL of 0.2 M aq $Et_3NH_2CO_3$. The oligonucleotide solution was diluted with 10 mL of 0.1–0.2 M aq $Et_3NH_2CO_3$ solution and loaded onto the Waters Sepak-C18 cartridge. The cartridge was eluted with 10 mL of 0.2 M (or 0.5 M) $Et_3NH_2CO_3$, followed by 15–30 mL of acetonitrile/water (1:1). The eluted acetonitrile/water fractions containing the oligonucleotide (monitored by UV at 260 nm) were combined and concentrated in vacuo to dryness. The residue was co-evaporated three times with 10 mL water to remove excess buffer. The residue was dissolved in the desired amount of water to give a salt-free stock solution of the oligonucleotide and stored at −20 °C.

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27. For further figures (T_m curves, CD and mixing curves) documenting the pairing behavior of the (4′→3′)-lyxopyranosyl system, see ref 13.
28. One of the limiting factors was difficulties encountered in the preparation of pure G-containing pl-(4′→3′)-oligomers.
29. This reasoning assumes that both the (4′→2′)- and the (4′→3′)-lyxopyranosyl systems have the same type of chair conformation with the equatorial positioning of the nucleobase. For the former, the presence of the conformation is strongly indicated by the system's cross-pairing with all other members of pentopyranosyl-(4′→2′)-family, two of which [ribo-[31] and arabino-pyranosyl (B. Jaun and O. Ebert, ETH, personal communication)] have shown to have this conformation by NMR spectroscopy. For the lyxopyranosyl-(4′→3′)-system, the assignment rests on the reasoning that the inverted chair would force the two (charged) vicinal phosphodiester groups into sterically and electrostatically unfavorable contact in the di-equatorial (syn-clinal) conformation.
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