147. Why Pentose- and Not Hexose-Nucleic Acids?

Part VII[1])

Pyranosyl-RNA ('p-RNA')

Preliminary Communication

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In memoriam Jakob Schreiber

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Qualitative conformational analysis of the entirety of conceivable hexo- and pento*pyranosyl* oligonucleotide systems derived from the diastereoisomeric aldohexoses $(CH_2O)_6$ and aldopentoses $(CH_2O)_5$ predicts the existence of a variety of pairing systems which have not been experimentally investigated so far. In particular, the analysis foresees the existence of a ribo*pyranosyl* isomer of RNA ('p-RNA'), containing the phosphodiester linkage between the positions C(4') and C(2') of neighboring ribopyranosyl units. Double strands of p-RNA oligonucleotides are expected to have a linear structure and to show purine-pyrimidine and purine-purine (*Watson-Crick*) pairing comparable in strength to that observed in homo-DNA. Experimentally, synthetic β-D-ribopyranosyl (4'→2')-oligonucleotides derived from adenine and uracil confirm this prognosis: *adenine-uracil pairing in p-RNA duplexes is stronger than in the corresponding RNA duplexes.* Importantly, adenine in p-Ribo(A_8) does not show (reverse-*Hoogsteen*) self-pairing, in sharp contrast to its behavior in the homo-DNA series. The sheer existence of strong and selective pairing in a system that is *constitutionally isomeric* to RNA and can be predicted to have a linear structure has implications for the problem of RNA's origin. In this context, a comprehensive experimental study of the pairing properties of p-RNA, of its potential for constitutional assembly, self-replication, and intra-duplex isomerization to RNA seems mandatory.

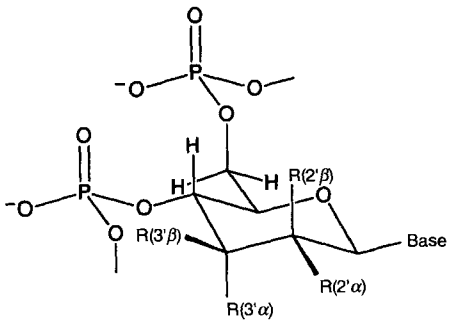
Our experimental studies on pyranosyl oligonucleotides initiated and conducted by the question '*Why Pentose- and not Hexose-Nucleic Acids?*' are meant to be steps towards a chemical etiology[3]) of the natural nucleic acid's structure [1] [14] [15]. So far, we have reported on the pairing properties of β-D-2',3'-dideoxyglucopyranosyl (6'→4')-oligonucleotides ('homo-DNA') [1–4]. Although that work – within the concept of investigation defined by the question in the title – represents only a comprehensive model study, it

[1]) For Parts I–IV of this series, *cf.* [1–4], respectively. Part V ('Purine-Purine Pairing in the Homo-DNA Series: Guanine, Isoguanine, 2,6-Diaminopurine, and Xanthin' [5], *cf.* the theses of *J. Hunziker* [6], *L. Peng* [7], *K. Groebke* [8]) and Part VI ('Synthesis and Pairing Properties of (4'→6')-Oligonucleotides Containing β-D-Allopyranose, β-D-Altropyranose, and β-D-Glucopyranose' [9], *cf.* the theses of *R. W. Fischer* [10], *A. Helg* [11], *U. Diederichsen* [12], *A. Giger* [13]) are in preparation and will be submitted to this journal. The present paper is also Communication No. 11 in the series 'Chemistry of α-Aminonitriles'.

[2]) Postdoctorate ETH-Zürich, since fall 1992.

[3]) Etiology = 'science of causes or origins' ('*Webster*'s New Twentieth Century Dictionary of the English Language Unabridged', 2nd edn., 1983).

has uncovered divergences in structure properties and pairing behavior between homo-DNA and DNA oligonucleotides that – above all – reveal the basic importance of the sugar ring in natural DNA (and RNA) being five-membered. These studies have recently been extended to allopyranosyl, altropyranosyl, and glucopyranosyl (6′→4′)-oligo-



Oligonucleotides	R(2'α)	R(2'β)	R(3'α)	R(3'α)	Reference
2',3'-dideoxy-β-D-glucopyranosyl ('homo-DNA')	H	H	H	H	[1–5]
β-D-allopyranosyl	OH	H	OH	H	[9–11]
β-D-altropyranosyl	H	OH	OH	H	[8][9]
2'-deoxy-β-D-allopyranosyl	H	H	OH	H	[9]
3'-deoxy-β-D-allopyranosyl	OH	H	H	H	[9]
β-D-glucopyranosyl	OH	H	H	OH	[9][12]

Fig. 1. *Hexopyranosyl-oligonucleotides investigated so far*

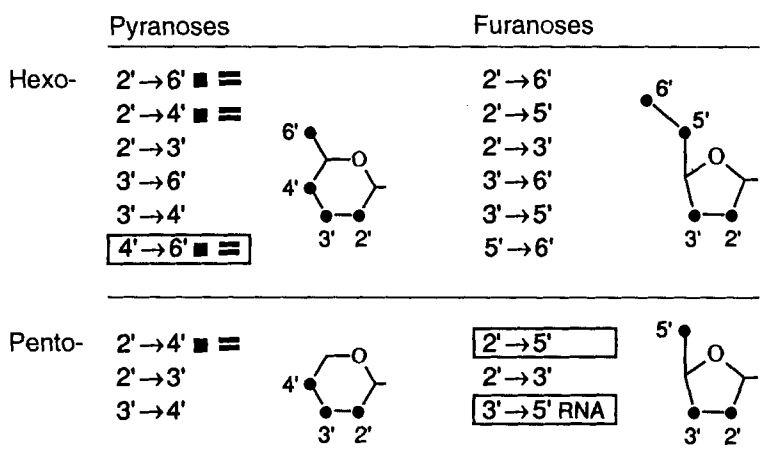


Fig. 2. *Survey on the constitution of conceivable oligonucleotide systems*

nucleotides [10] [11] [8] [12] [13] [15] (*Fig. 1*), all of them hexopyranosyl systems, containing sugar building blocks for which, in contrast to the dideoxy-sugar of homo-DNA, both retrosynthetic considerations and chemical experience would not exclude that they may have occurred naturally before nucleic acids. The pairing properties of these systems are found to be in part similar to, but in significant respects drastically different from the pairing behavior of homo-DNA [15]. The observations and interpretations available so far coherently point to the existence of a whole series of intrinsic steric handicaps hampering base pairing in fully hydroxylated hexopyranosyl oligonucleotides, in sharp contrast to the corresponding 2′,3′-dideoxy derivatives (homo-DNA oligonucleotides) in which base pairing has been found to be uniformly stronger than the pairing in corresponding DNA oligonucleotides. These studies on fully hydroxylated hexopyranosyl (6′→4′)-oligonucleotides are still being extended, and the results will be reported in detail in *Part VI* [9][1]) of this series.

A comprehensive experimental involvement in the problems of a chemical etiology of the structure of the natural nucleic acids[4]) would require a systematic extension of the study onto hexopyranosyl (as well as to hexofuranosyl) oligonucleotide systems which have their phosphodiester link between positions *other* than the (6′→4′)-link of the structures investigated so far (*Fig. 2*). In the course of systematic screening of the potential pairing propensities of the *hexo*pyranosyl systems by a qualitative conformational analysis of the type described for homo-DNA [1] [3], we were led to extend the analysis also to *pento*pyranosyl oligonucleotides systems derived from the four natural aldopentoses[5]). The analysis of both the hexo- and pentopyranosyl cases predicts the existence of a variety of pairing systems which have not been experimentally investigated so far and, above all and most interestingly, foresees the existence of a ribopyranosyl isomer of RNA, which contains the phosphodiester linkages between positions C(4′) and C(2′) of the neighboring ribopyranosyl nucleotide units [15] (*Fig. 3*). *Scheme 1* summarizes the outcome of the analysis of the single-strand oligonucleotide backbone of this system (for the selection criteria used in the analysis, see [1] [3]).

Among a total of $2 \cdot 3^4$ idealized conformations of the mononucleotide backbone unit, there exists – allowing for phosphodiester conformation of both the *gauche/gauche*- and the *gauche/trans*-type [1] [3] – an ensemble of nine least-strained conformers among which *only one is conformationally repetitive and, therefore, prone to base pairing* in

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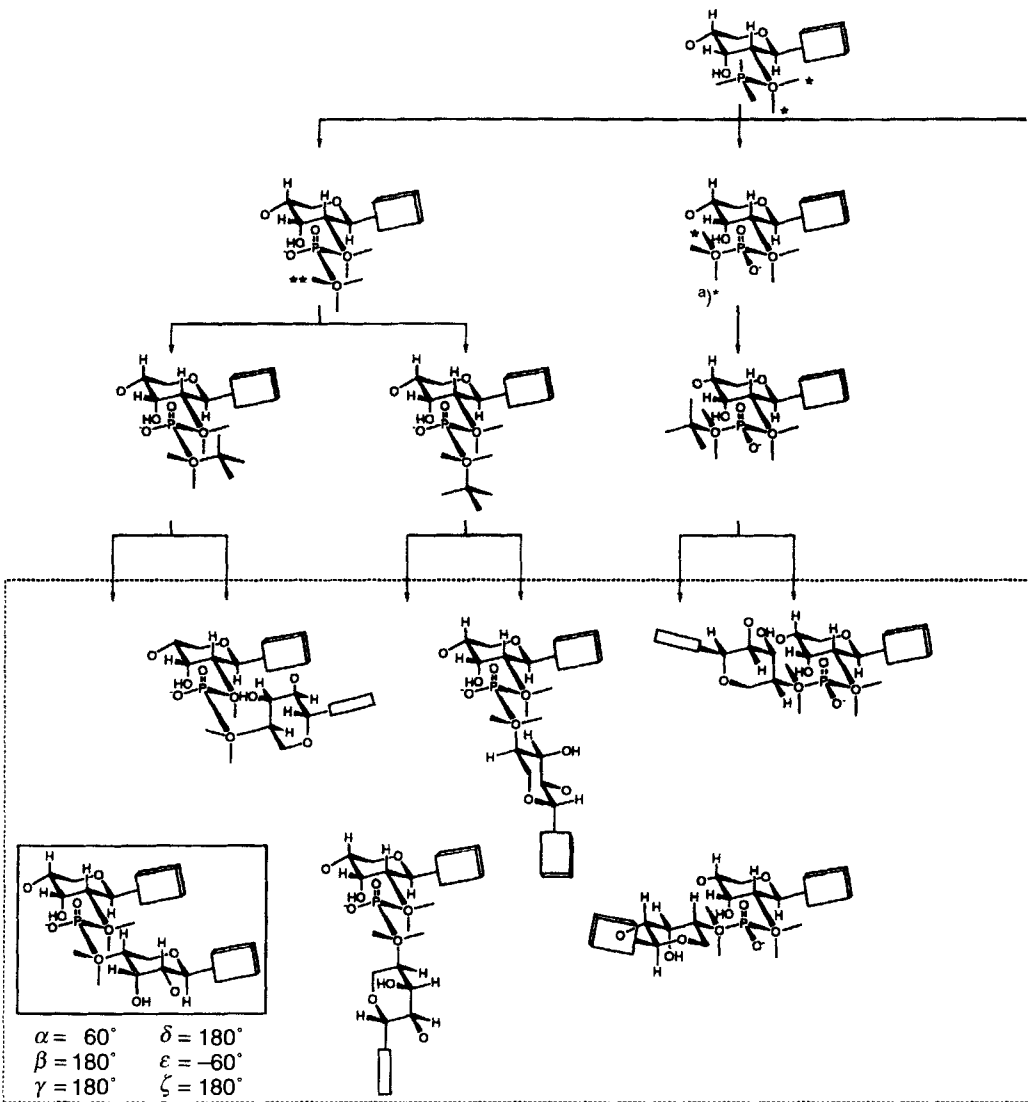
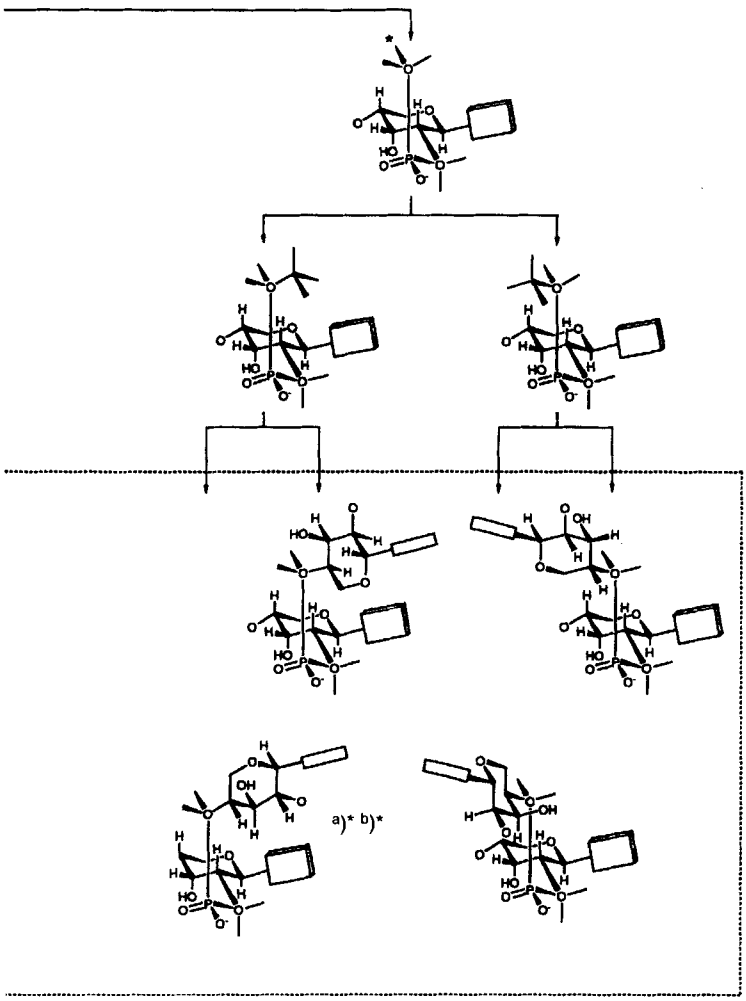
[4]) An early discussion of etiological nature concerning the sugar-backbone structure of nucleic acids had been brought up by *Usher*'s [16a] question why RNA contains (5′→3′)- and not (5′→2′)-phosphodiester links [16] [17]. Experiments of the type performed in the homo-DNA series referring to that question were carried out only very recently [18–20]. Relevant in this respect are also *Sequin*'s [21] early proposal that oligo(2′-deoxy-α-D-ribofuranosyl) nucleotides should be capable of forming a double helix by base pairing and the recent experimental confirmation [22] of the proposal, as well as the experimental studies on the pairing properties of oligo(2′-deoxy-β-D-xylofuranosyl) and of oligo(2′-deoxy-β-D-lyxofuranosyl) nucleotides by *Seela* and coworkers [23] [24].

[5]) This extension was actually inspired by the constitutional and conformational analysis of hexopyranosyl oligonucleotide systems, the constitution of which would be (formally) derivable *via* the aldolization pathway to oligonucleotide backbones starting from bis(glycolaldehyde) phosphodiester and 1 equiv. of glycolaldehyde phosphate; replacement of the latter by 1 equiv. of formaldehyde leads to pentopyranosyl (4′→2′)-oligonucleotides (see the three relevant Schemes in [15]). We shall discuss these relationships in more detail in the context of a forthcoming paper on the formation of glycolaldehyde phosphate and bis(glycolaldehyde) phosphodiester from oxiranecarbonitrile and phosphate anion [25] [26]).

Scheme 1. *Qualitative Conformational Analysis of the*



oligomers with a mononucleotide as the conformationally repetitive unit. Phosphodiester linkages in this pairing conformation are of the *gauche/trans*-type. The single strand of an oligomer in this conformation (*Fig. 4*) is linear and, since its backbone does not contain a bond pointing *quasi*-orthogonally to the average plane defined by strand and base-pair axes in their idealized *Watson-Crick* orientation (see Fig. 31 in [3]), duplexes derived from it are expected also to be linear (*Fig. 5*), provided that their base pairs have

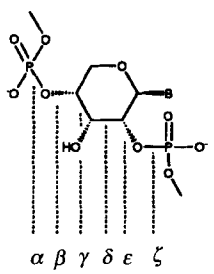


* option forbidden for steric reasons (1,5-repulsion)

** option forbidden for stereo-electronic reasons (*t,t* - phosphodiester group)

a)* option forbidden for steric reasons (1,7-repulsion)

b)* conformer forbidden by long-range steric hindrance between sugar residue and base of preceding unit



the *Watson-Crick* constitution[6]). Strand and *Watson-Crick* base-pair axes are strongly inclined towards each other; the deviation from orthogonality (inclination angle *ca.* 30°) is greater than in homo-DNA (*ca.* 60° in the *g*/*g*-type and *ca.* 45° in the *g*/*t*-type

[6]) The importance of this restriction has been recognized as a consequence of the (at first, surprising) observation that there is no adenine self-pairing in p-Ribo(A_8), see below.

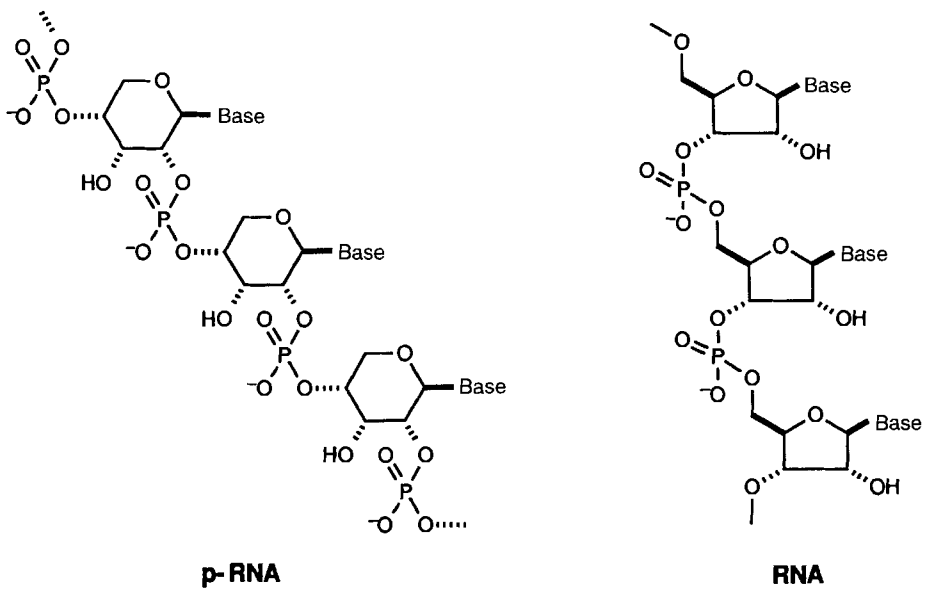


Fig. 3. *Constitution and configuration of p-RNA in comparison to RNA*

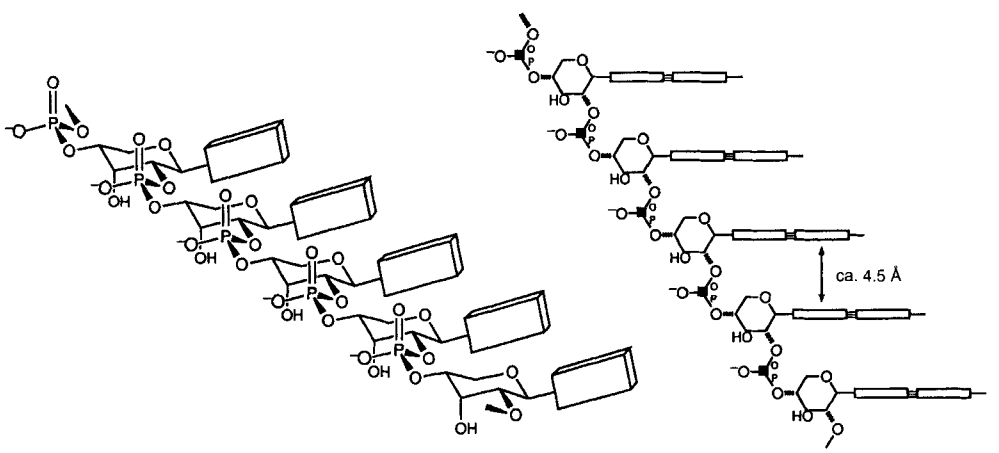


Fig. 4. *The unique (idealized) conformation of the p-RNA backbone expected to be the pairing conformation in p-RNA duplexes*

conformation [4]). Idealized p-RNA duplex models, therefore, show a smaller orthogonal base-pair distance (*ca.* 4.5 Å) than corresponding homo-DNA models and, furthermore, they show a positioning of neighboring base pairs relative to each other which is favorable for *inter*strand (as against *intra*strand) stacking; both model properties can be expected to reflect themselves in enhanced duplex stability. In view of the large inclination mentioned above, p-RNA duplexes are further expected to show an accordingly

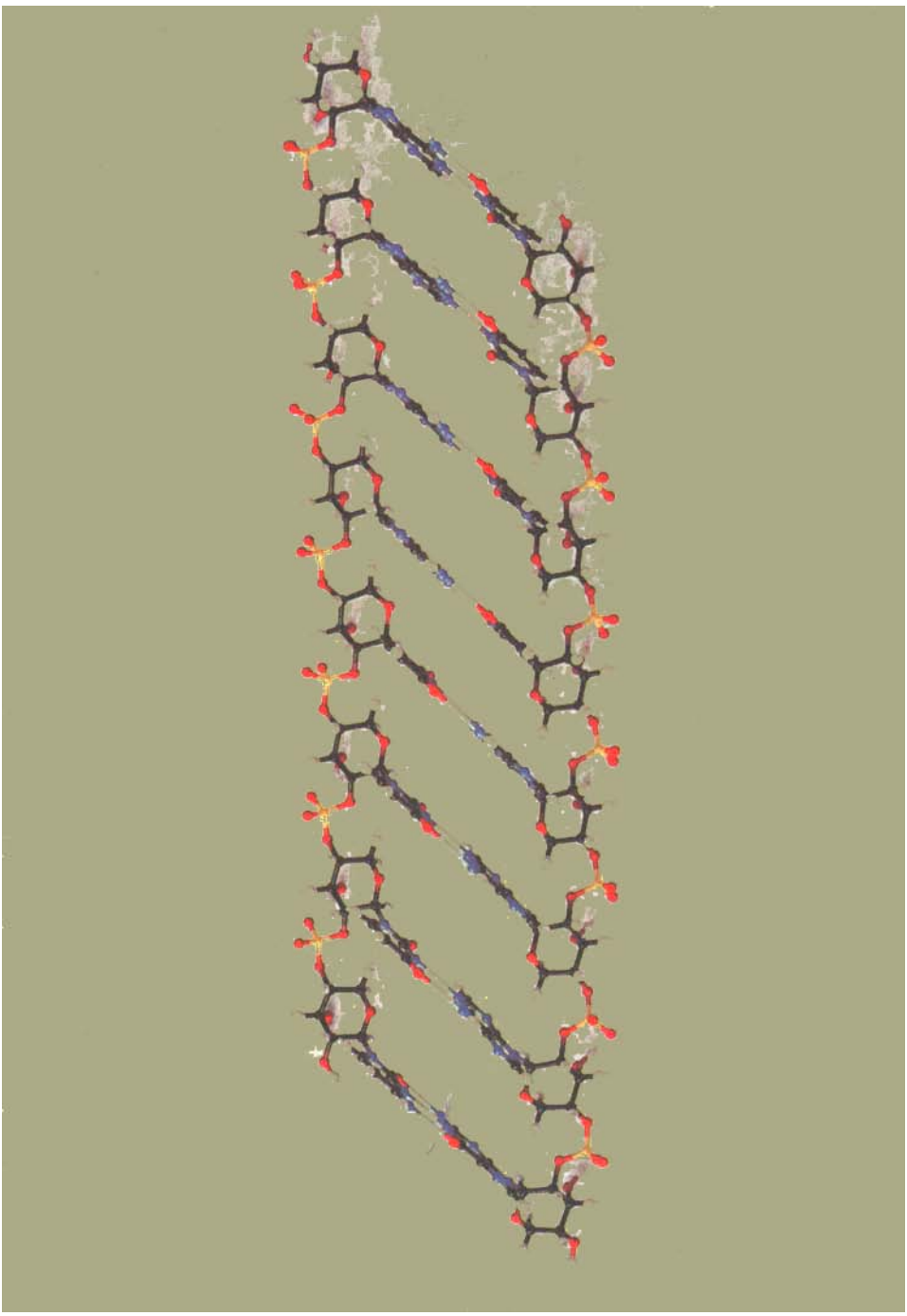
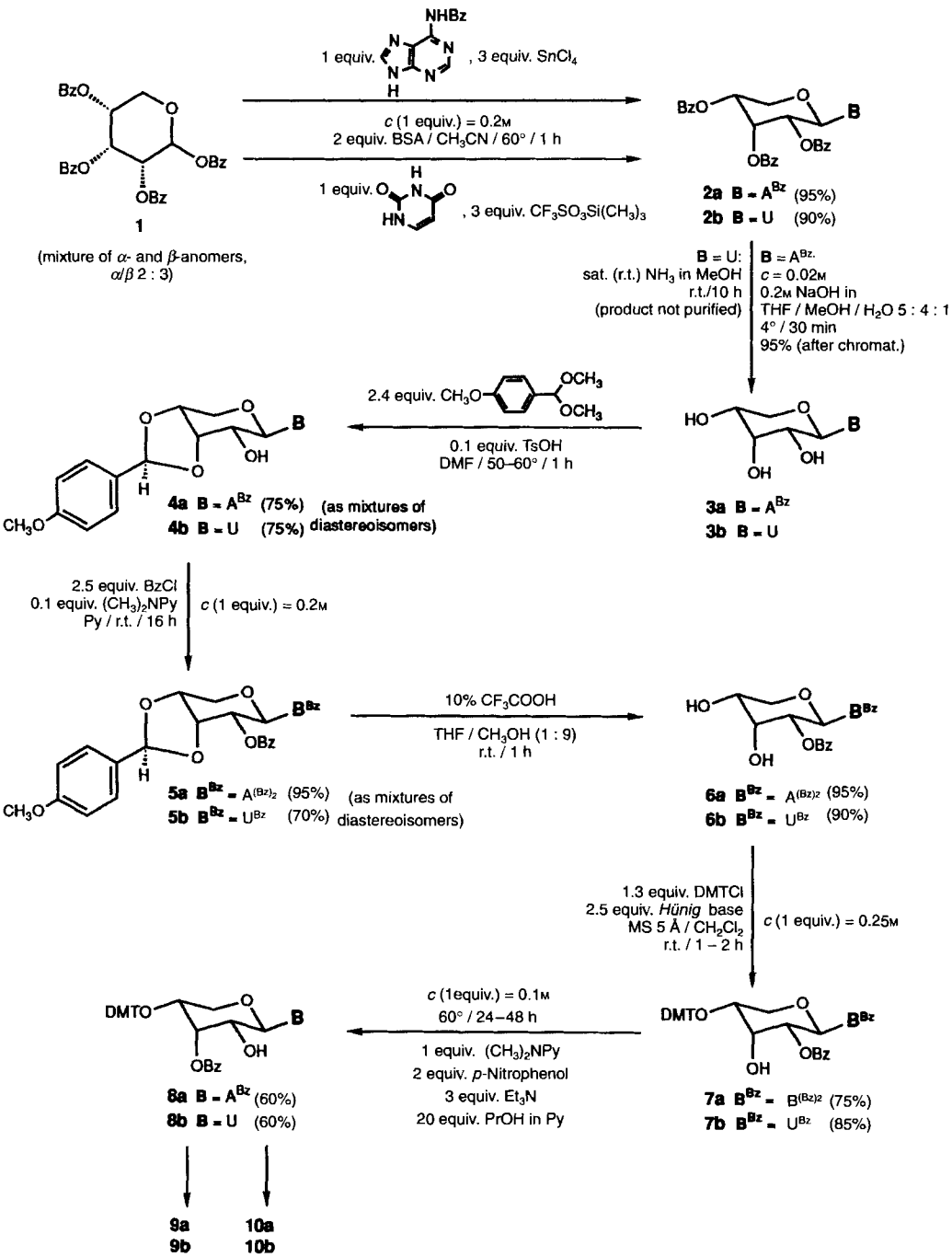


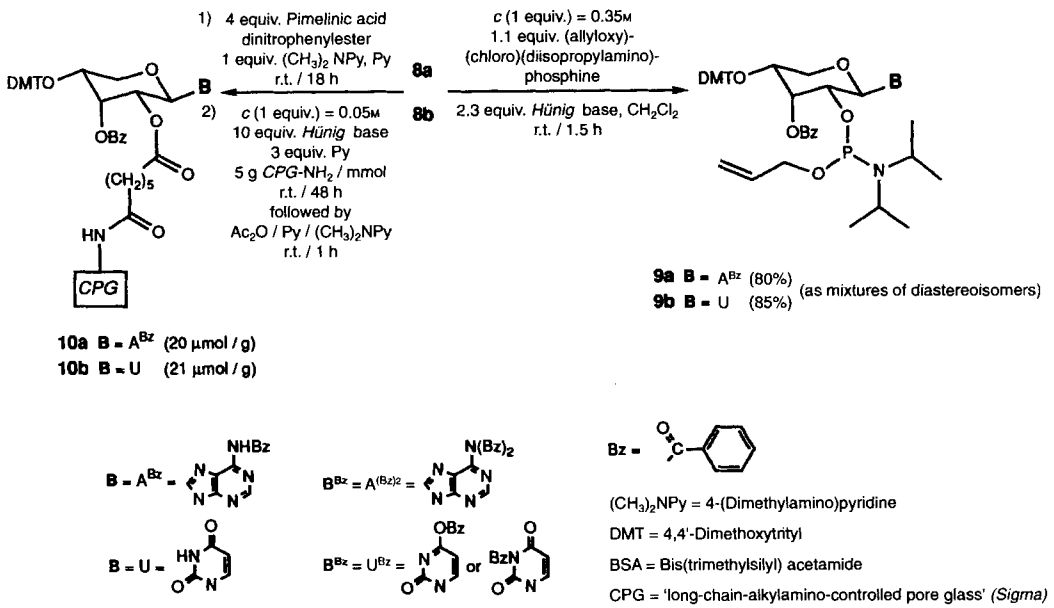
Photo: Dr. Dorothee Felix

Fig. 5. Maruzen *model of the p-Ribo(A_4–U_4) duplex*

Scheme 2. *Preparation of Nucleoside Derivatives for the Automated Synthesis of p-Ribo(A,U)-oligonucleotides*



Scheme 2 (cont.)



strong and unambiguous preference for antiparallel (as against parallel) strand orientation. Given the overall similarity of the linear single-strand models of p-RNA and of homo-DNA and extrapolating the (known) pairing behavior of homo-DNA to that of p-RNA, the latter can be (and has been [15]) expected to represent a strong and selective, autonomous pairing system. Here, we report the synthesis of adenine- and uracil-containing β-D-ribopyranosyl (4′→2′)-oligonucleotides and the first observations on their pairing properties.

The *preparation* of the four *starting* materials **9a**, **9b**, and **10a**, **10b** required for the automated synthesis of adenine- and uracil-containing ribopyranosyl (4′→2′)-oligonucleotide sequences is summarized in *Scheme 2*. *Vorbrüggen-Hilbert-Johnson* nucleosidation [2] of an α/β-anomer mixture of tetrabenzoyl-ribopyranose (**1**) [27] with *N*-benzoyladenine (catalyst: $SnCl_4$) or uracil (trimethylsilyl triflate) produced the corresponding pyranosyl-nucleoside derivatives **2a** and **2b**, respectively, each in over 90% yield[7]). Acid-catalyzed ketalization of nucleosides **3a** and **3b** with *p*-methoxybenzaldehyde proceeds with regioselection in favor of the 3′,4′-isomers by a factor of *ca.* 5:1 (^{1}H-NMR), and with diastereoselection of *exo*-ketals **4a** and **4b** by a factor of *ca.* 3:1 (^{1}H-NMR). In both series, the mixture of diastereoisomeric 3′,4′-ketals is separated from the 2′,3′-regioisomer mixture by chromatography, perbenzoylated to **5a** and **5b** as the mixture of diastereoisomers, and eventually deketalized to the regio- and diastereoisomerically pure (tri- and di)-benzoyl derivatives **6a**/**6b**, of which the ^{1}H-NMR chemical shifts of the signals corre-

[7]) Derivatives of all five β-D-ribopyranosyl nucleosides containing natural nucleic-acid bases were prepared previously by other authors in different contexts [28].

sponding to H–C(3′) and H–C(4′) establish the position of the *O*-benzoyl group at C(2′) (*Table 1*). Regioselective dimethoxytritylation of the 4′-OH group in these derivatives leading to **7a**/**7b** was found to be straightforward.

The crucial step in our current preparation of building blocks for the synthesis of ribopyranosyl oligonucleotides is the benzoyl-migration step **7a(b)** → **8a(b)**. The occurence of such a migration under deprotonating condition was (unexpectedly) observed in attempts to introduce a protecting group at the axial OH group of **7a** by alkylation, an observation that led to the choice of benzoyl as the protection group instead[8]). The rather complex cocktail of components (see *Scheme 2*) for carrying out the migration step on preparative scale is the result of a careful optimization of the reaction conditions: PrOH serves the purpose of accepting the benzoyl groups from the benzoylated uracil ring of **7b** and the dibenzoylated adenine amino group of **7a**, avoiding, thereby, transfer of these benzoyl equivalents to the OH groups at C(2′); *p*-nitrophenol – by reasons which have not been determined yet – is found to be beneficial for the rate and the product yield of the process. Under the reaction conditions used, the equilibrium between the 2′β-(benzoyloxy)-3′α-hydroxy- and the 2′β-hydroxy-3′α-(benzoyloxy) form of the 4′-(dimethoxytritylated)-nucleoside derivatives is observed to lie on the side of the latter by a factor of at least *ca.* 1:10[9]). The constitutional assignment of educts **7a**/**7b** and products **8a**/**8b** of the migration step is based on the ^{1}H-NMR spectral data listed in *Table 1*. Compound **7a** and the *N*,*N*-dibenzoylated analogue of **8a** were shown to give the same tetrabenzoyl derivative, when *O*-benzoylated with benzoyl chloride in pyridine in the presence of 4-(dimethylamino)pyridine; benzoylation of the **7a** proceeded much more slowly than that of the **8a** analogue.

Table 1. *Chemical Shifts* (δ [ppm]) *of Non-exchangeable Sugar Protons of Synthetic Intermediates*

	H–C(1′)	H–C(2′)	H–C(3′)	H–C(4′)	H_β–C(5′)	H_α–C(5′)	J(1′,2′) [Hz]
2a ($CDCl_3$)	6.50	6.01	6.41	5.70	4.33–4.46		9.5
3a (CD_3OD)	5.92	4.37	4.26	3.9	3.80	3.9	9.3
6a ($CDCl_3$)	6.30	5.55	4.56	4.10	3.83–4.00		9.4
7a ($CDCl_3$)	6.26	5.35	3.74	4.05	3.38	4.02	9.6
8a ($CDCl_3$)	5.81	4.40	5.92	4.05	2.79	3.8	9.2

The *preparation of oligonucleotides* in *ca.* 1-μM scale was carried out as described in *Part II* of this series [2] (see also [29]) for homo-DNA oligonucleotides using a DNA synthesizer (*Pharmacia Gene-Assembler Plus*), solid support derivatives derived from **10a**/**10b**, and coupling intermediates **9a**/**9b**, as well as adopting the following changes in reaction conditions: coupling during 15 min (instead of 6 min) using a 0.5M solution of 1*H*-tetrazole/5-(4-nitrophenyl)-1*H*-tetrazole (0.35:0.15), detritylation during 5 min (instead of 1 min) using a 6% (instead of 3%) dichloroacetic-acid solution (average coupling yields > 99%, by detritylation assay).

[8]) We had already successfully used benzoyl as protecting group for the two axial OH groups in the synthesis of altropyranosyl oligonucleotides [8] [9].

[9]) The position of this equilibrium is remarkable and presumably determined mainly by steric factors; on the other hand, an acyloxy group that violates the *gauche*-effect (*e.g.*, the 2′β-benzoyloxy group) is expected to be more electrophilic than a acyloxy group situated in accord with the *gauche*-effect (*e.g.*, the 3′α-benzoyloxy group).

Before detachment from the solid support, the p-RNA-oligonucleotide chains were first deprotected at the phosphotriester linkages by removing the allyl groups under the conditions described by *Noyori* and coworkers [30] for DNA-oligonucleotides. Attempts to deprotect the 3′α-benzoyloxy groups of p-ribo-oligonucleotides with concomitant detachment under conditions successfully used in the altropyranosyl series [8] [9] (conc. NH_3 in H_2O/EtOH 3:1, room temperature, 40 h) led only to partial debenzoylation and also to substantial strand scission[10]). In the case of p-Ribo(A_8) and other homo-adenine sequences, detachment and complete deprotection without discernible strand scission was achieved by hydrazinolysis under mild conditions (yield of HPLC-purified octamer *ca.* 30%). Not unexpectedly, this method fails with p-RNA sequences containing uracil residues due to the sensitivity of the pyrimidine ring towards hydrazine [31]; however, a (not fully satisfactory, but nevertheless acceptable) solution to this deprotection problem was found in using *O*-methylhydroxylamine in aqueous ethanolic ammonia solution under carefully controlled conditions (*Table 2*)[11]).

Deprotected p-RNA-oligonucleotides were purified by HPLC (same systems as described in [2]) exceeding 95% purities (*Fig. 8*), and their composition controlled by

Table 2. *HPLC and MS Data of p-Ribo-oligonucleotides*

Oligonucleotide	Deprotection[a])	Analytical HPLC		MALDI-TOF MS[d])	
		Reverse phase[b]) t_R [min]	Ion exchange[c]) t_R [min]	$[M-H]^-$ (obs.)	$[M-H]^-$ (calc.)
p-Ribo(A_3)	*A*	17.3		923.3	924.7
p-Ribo(A_8)	*A*	21.0		2574.6	2570.7
p-Ribo(U_8)	*B*	15.7		2386.1	2386.4
p-Ribo((U-A)$_4$)	*B*	18.4	16.0	2481.4	2478.6
p-Ribo((A-U)$_4$)	*B*	18.2	15.4	2480.4	2478.6
p-Ribo(A_4-U_4)	*B*	18.6	15.5	2479.3	2478.6
p-Ribo(U_4-A_4)	*B*	19.0			

a) *Method A: ca.* 10% NH_2NH_2 in H_2O/4°/*ca.* 20 h; *Method B:* 0.2M $CH_3ONH_2 \cdot HCl$ in NH_3 conc. (aq.)/EtOH 3:1/r.t./2 × 20 h[11]). All oligonucleotides were purified by chromatography on *Spherisorb* 300 Å, *C-18*, 10 μm, 250 × 9.0 mm; elution with 0.1M NEt_3/0.1M HOAc (pH 7.0), and a linear gradient of CH_3CN, flow 3 ml/min; followed by desalting on *Sepak*® cartridges [2].

b) *Aquapore RP-300*, 7 μm, 220 × 4.6 mm; elution with 0.1M NEt_3/0.1M AcOH (pH 7.0), and a linear gradient of CH_3CN (0–32% within 30 min), flow 1 ml/min; peak purity (260 nm) 99–100% in each case.

c) *Nucleogen-DEAE 60-7*, 125 × 4.0 mm; elution with 20 mM K_2HPO_4/KH_2PO_4 (pH 6.0), 20% CH_3CN and a linear gradient of KCl (0.1–1.0M within 30 min); peak purity (260 nm) 95–99%.

d) Matrix-Assisted Laser-Desorption Ionization Time-Of-Flight Mass Spectrometry; matrix: 2,4,6-trihydroxyacetophenone, see [33][12]). Samples were measured in the form of their triethylammonium salts.

[10]) Presumably *via* (2′→3′)- and/or (4′→3′)-cyclic phosphate intermediates.

[11]) Strand scission under these conditions is much less pronounced, but still not completely absent. The compromise was adopted to interrupt the procedure well before (20 h) the deprotection is completed (HPLC, $t_{1/2}$ of p-Ribo((U-A)$_4$) under these conditions *ca.* 40 h), to separate the desired product by HPLC and to subject the partially deprotected fractions to the reaction conditions again (overall yield of chromatographically pure (HPLC) p-ribo(A,U)-oligonucleotides *ca.* 20–25%). The p-RNA-phosphodiester backbones are more stable in alkaline solutions with respect to strand scission than the RNA backbone ($t_{1/2}$ of p-Ribo(U_2) *ca.* 28 min, for r(U_2) *ca.* 7 min [32] in 50 mM phosphate buffer, pH 12.1, 60°).

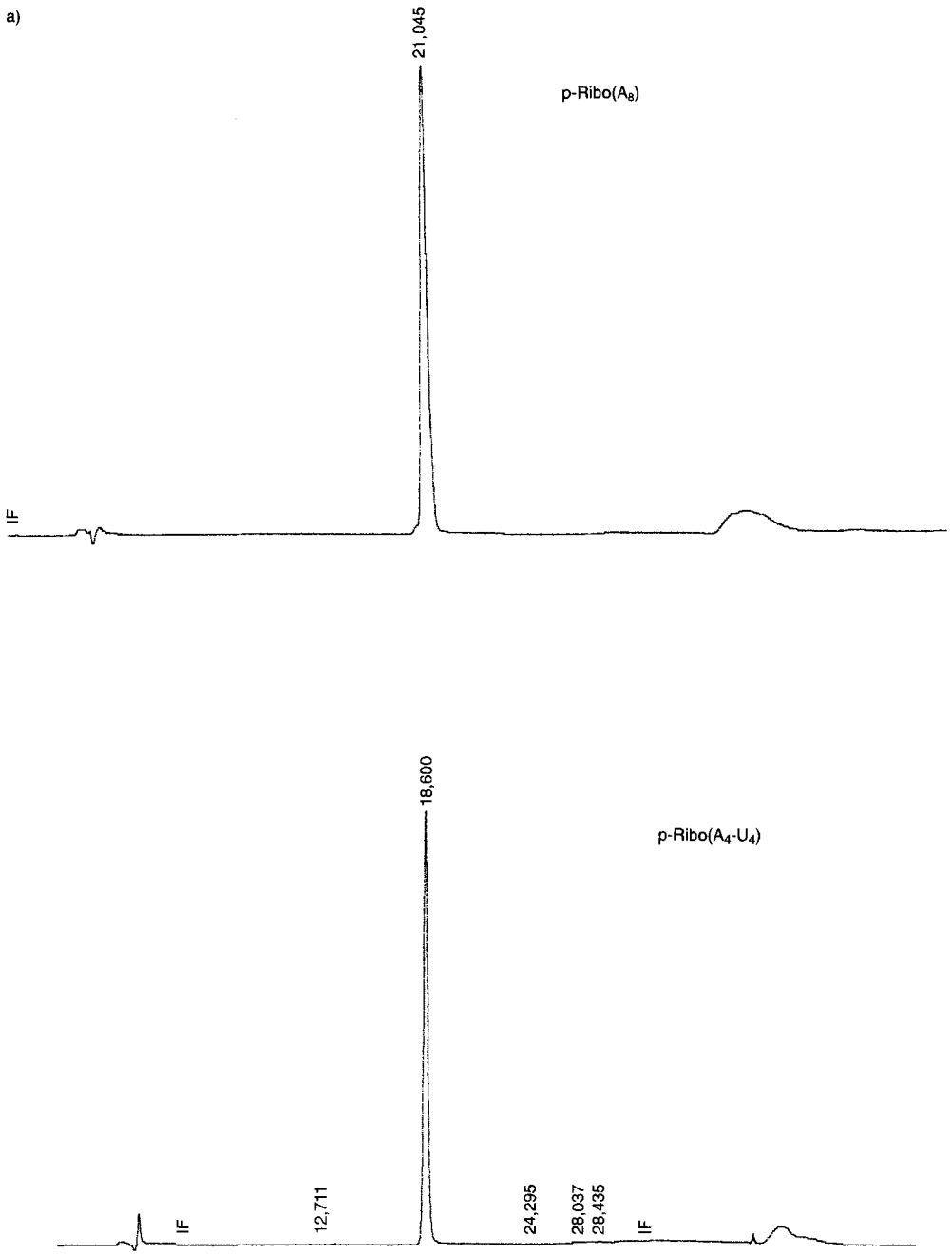
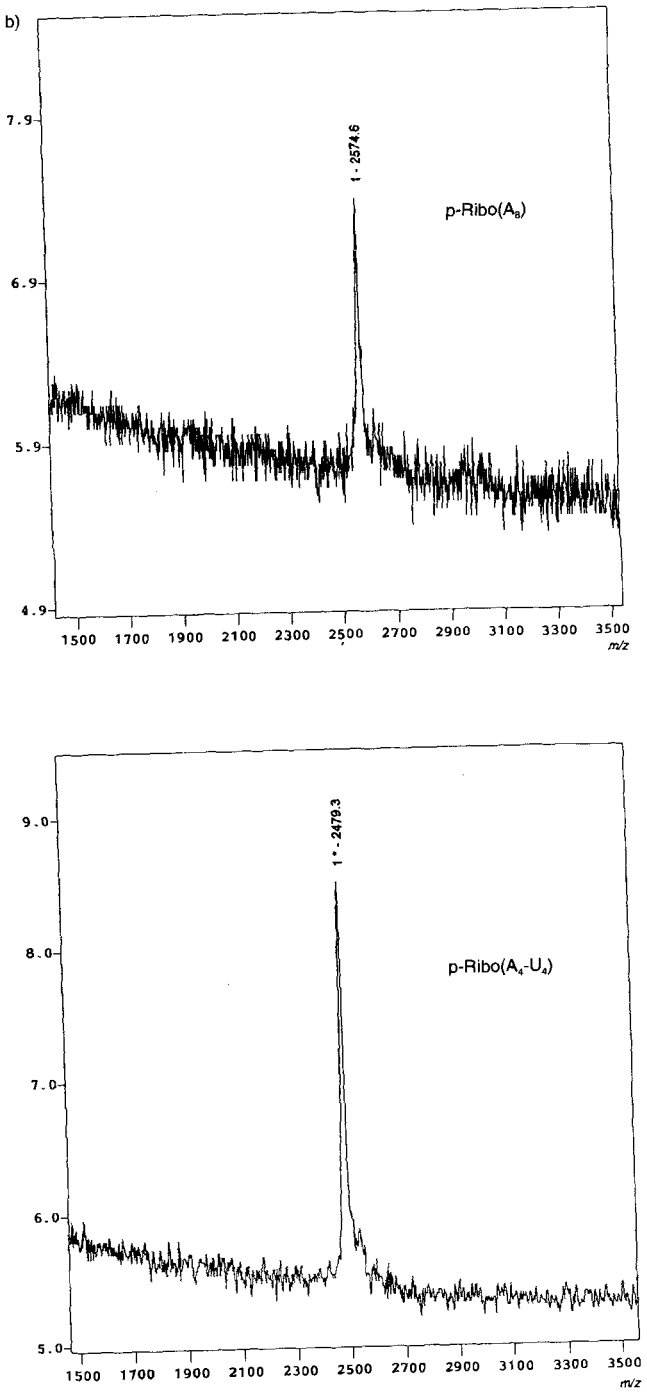


Fig. 6. *Data of HPLC and MALDI-TOF mass spectrometry for*



p-Ribo(A_8) and p-Ribo(A_4–U_4). For details, see *Table 2.*

matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) mass spectrometry[12]) (*Fig. 6* and *Table 2*).

For checking the synthetic procedure by spectroscopic product characterization, p-Ribo (A_3) was prepared in 100-μmol scale (*ca.* 15 mg) and its structure verified by ^{1}H-, ^{31}P-, and ^{13}C-NMR spectroscopy (*Table 3*) [34]. The spin systems corresponding to the three ribosyl units were identified from a TOCSY spectrum, and all individual sugar protons could be assigned unequivocally by double quantum-filtered COSY (*Table 3, a*). The coupling constants observed for all three sugar units (*Table 3, b*) correspond to the pattern expected for a ribopyranosyl ring in a chair conformation. Strong cross-peaks between H–C(4′$_2$), H–C(2′$_1$), and P_1 as well as between H–C(4′$_3$), H–C(2′$_2$), and P_2 in the ^{31}P,^{1}H-COSY spectrum demonstrate the (4′→2′)-mode of both phosphate linkages. The adenine H–C(8) protons could be assigned to their corresponding nucleoside units from their ROESY cross-peaks with p-ribosyl H–C(2′) and H–C(1′) protons. These cross-peaks also indicate the population of both *anti*- and *syn*-conformers with respect to the nucleosidic bonds in all three nucleoside units.

Methods and conditions for the *characterization of the pairing behavior* of p-RNA oligonucleotides were the same as those used in the homo-DNA study [3], namely, temperature-, concentration-, and molar-ratio-dependent UV spectroscopy as well as temperature-dependent CD spectroscopy in concentrations of the range of 5→100 μmol in 0.15M NaCl, 0.01M *Tris* hydrochloride-buffer, pH 7.0. The thermodynamic-data determination for the self-complementary octamer p-Ribo(A_4–U_4) was also carried out in a solution containing 1.00M NaCl for the sake of direct comparison with published data [35] for the corresponding RNA octamer r(A_4–U_4).

The expectation that p-RNA would show pairing properties similar to those of homo-DNA is – as evidenced by the documentation given in *Fig. 7* and *8* – confirmed, with one drastic, at first surprising, but then highly welcome exception: *p-Ribo(A_8) does not pair with itself* (*Fig. 7*[13])) in sharp contrast to the homo-DNA series, where ddGlc(A_8) forms a reverse-*Hoogsteen* duplex with a melting temperature of 63° under similar conditions [3]. This divergence (and the reason for the change of our attitude towards it) will be discussed below.

The two single strands (*Fig. 7*), p-Ribo(A_8) and p-Ribo(U_8), form a complex on mixing which we consider to be the *Watson-Crick*-paired duplex according to the following observations: the perfectly reversible UV melting curves (*Fig. 7, a*), whose dependence from concentration (*Fig. 7, b* and *c*) demonstrate intermolecularity of the pairing interaction, the dependence of UV absorption (260 nm) at 5° from the molar ratio (*Fig. 7, d*) which establishes 1:1 stoichiometry, the temperature-dependent CD spectra (*Fig. 7, e* and *f*) as well as the behavior of the single strands and their 1:1 mixture in gel electrophoresis (*Fig. 7, g*). The octamer sequences p-Ribo(A_4–U_4), p-Ribo(U_4–A_4), p-Ribo((A–U)$_4$), and p-Ribo((A–U)$_4$) are *self*-complementary with respect to antiparallel pairing; all four form cleanly and reversibly melting complexes by self-pairing as shown in *Fig. 8*. The thermo-

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[12]) We acknowledge the help of Dr. *U. Pieles* and Dr. *H. Moser* (*Ciba AG*, Basel) in the determination of these spectra in their laboratory; for the methodology and accuracy of the method in the field of oligonucleotides, see [33].

[13]) Absence of adenine self-pairing was also observed in (preliminary) UV experiments with samples of p-Ribo(A_5) and p-Ribo(A_7).

Table 3. a) *Chemical Shifts* (δ [ppm]) *of Non-exchangeable Protons and of P-Atoms of p-Ribo(A_3)* (A_1 = nucleoside unit at the 4′-end, A_3 = nucleoside unit at the 2′-end)

	H–C(1′)[a]	H–C(2′)[a]	H–C(3′)[a]	H–C(4′)[a]	H_β–C(5′)[a]	H_α–C(5′)[a]	H–C(8)[b]	H–C(2)	Phosphate group	$\delta(^{31}P)$[c]
A_1	5.88	4.80	4.55	4.19	3.9	3.9	8.32			
								8.11,	P_1	−1.22
A_2	5.72	4.52	4.46	3.79	3.28	3.50	8.32	8.14,		
								8.15	P_2	−1.61
A_3	5.55	4.03	4.10	3.55	3.33	3.6	8.22			

b) *Coupling Constants* [Hz] *of Selected Protons of p-Ribo(A_3)*

	J(1′,2′)[d]	J(2′,3′)[d]	J(3′,4′)[d]	J(4′,5′β)[d]	J(4′,5′α)[d]	J(5′β,5′α)[d]	$J(P_1,2')$[e]	$J(P_1,4')$[e]	$J(P_2,2')$[e]	$J(P_2,4')$[e]
A_1	9.4	2.6	2.6	6.9	9.6	[g]	8.05			
A_2	9.3	2.8	2.8	5.4	9[f]	11.0		8.05	8.05	
A_3	9.5	2.8	2.8	3.8	[g]	10.0				8.05

c) ^{13}*C-Chemical Shifts* (δ [ppm])[h] *and P,C Coupling Constants*[i] *of p-Ribo(A_3)*

	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C(2)	C(4)	C(5)	C(6)	C(8)
A_1	81.9	75.3	72.5	68.3	67.5					
	(9.2 Hz)	(4.8 Hz)						121.1, 121.2,		
						155,5,		121.4, 151.5,		143.1,
A_2	81.1	74.9	71.4	71.9	65.8	155.6,		151.6, 151.7,		143.1,
	(9.3 Hz)	(5.2 Hz)	(4.8 Hz)	(5.6 Hz)	(3.3 Hz)	155.6		158.2, 158.2,		143.5
								158.3		
A_3	82.3	70.4	72.2	72.3	65.6					
			(4.4 Hz)	(5.2 Hz)	(4.6 Hz)					

[a]) Assignment based on TOCSY and COSY.
[b]) Assignment based on ROESY.
[c]) Assignment based on H,P-COSY.
[d]) Determined from the 1D spectrum.
[e]) Determined from the fully coupled ^{31}P spectrum.
[f]) Estimated from DQF-COSY crosspeaks.
[g]) Proton resonances nearly isochronous.
[h]) Assignment based on ^{13}C,^{1}H correlation (HMQC).
[i]) ^nJ(P,C) determined from the ^{1}H-broad-band-decoupled ^{13}C-NMR spectrum.

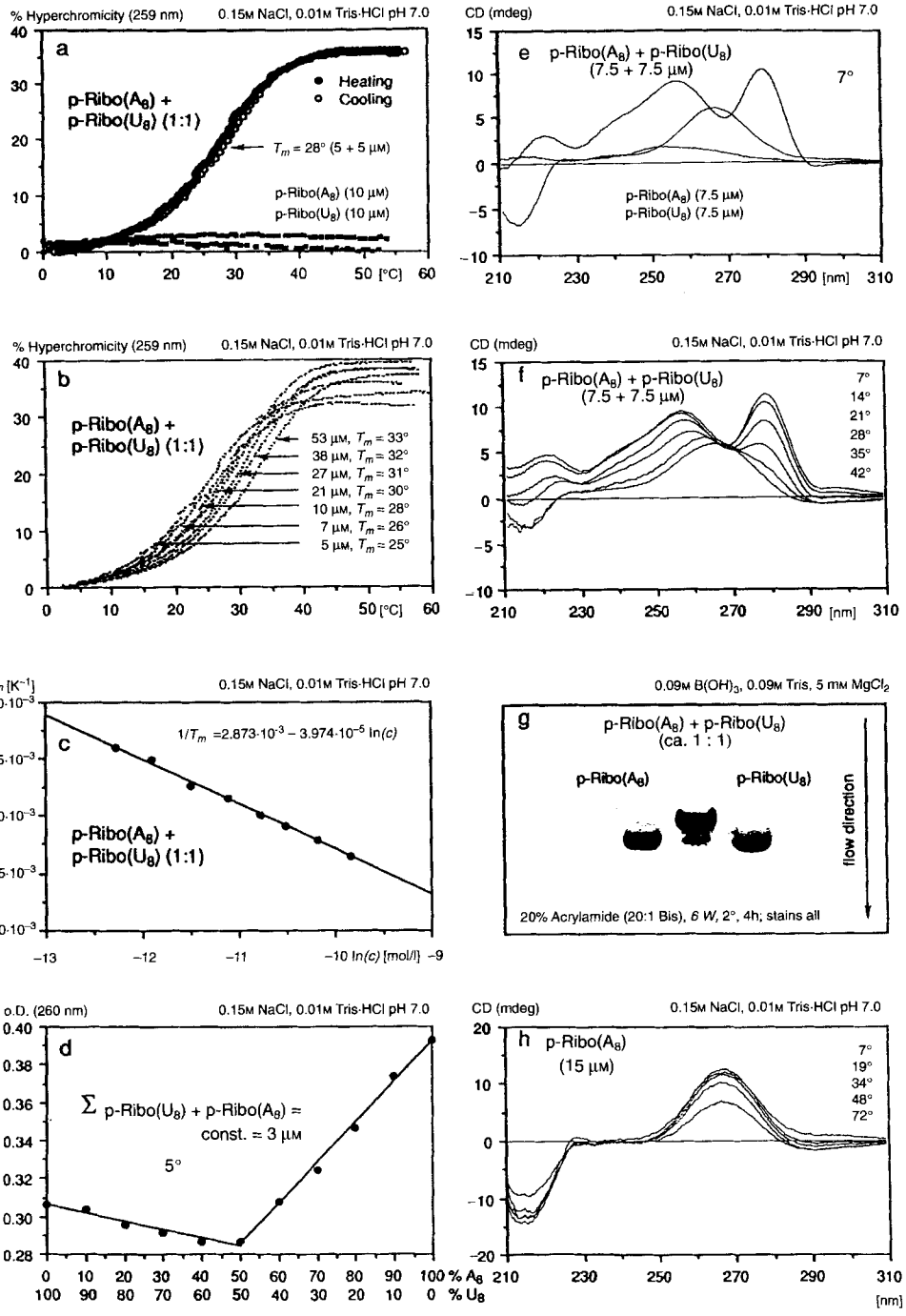

Fig. 7. *Experimental data of the single strands and the 1:1 mixture p-Ribo(A_8)/p-Ribo(U_8)*

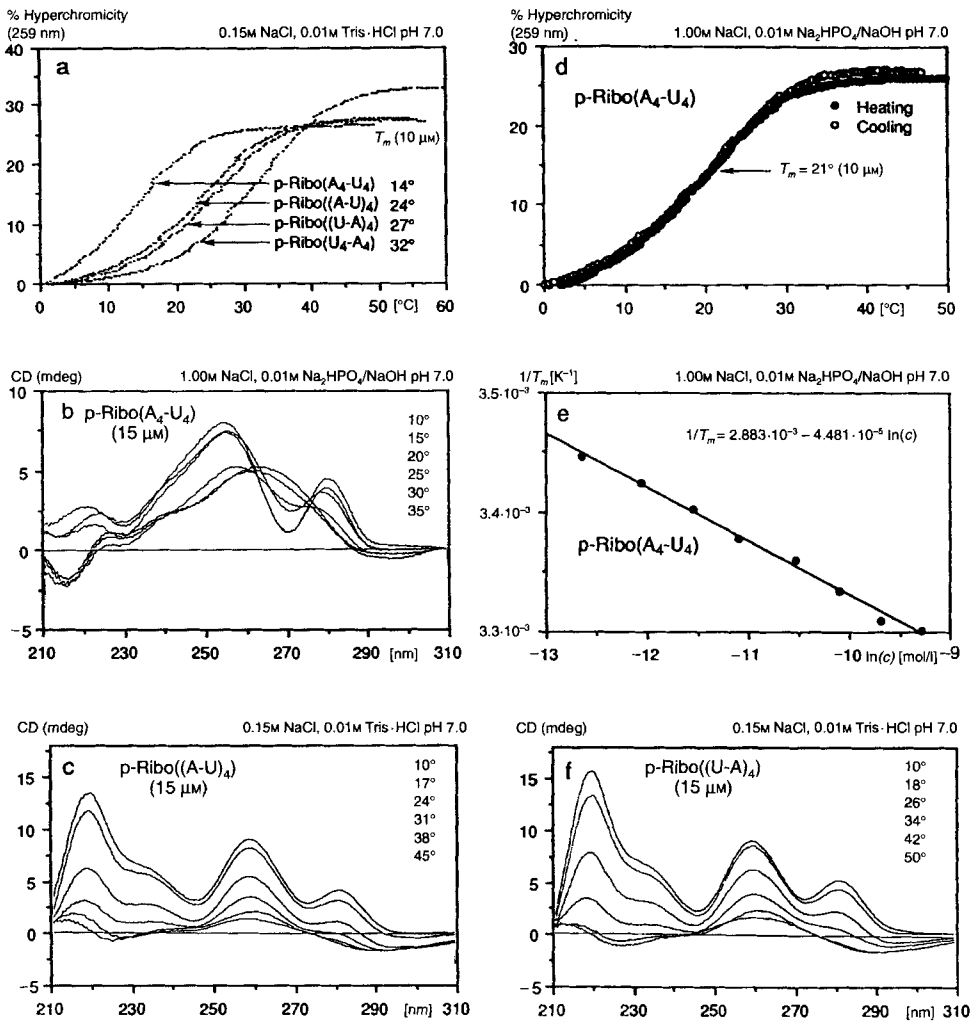

Fig. 8. *Experimental data of p-Ribo(A,U)-oligonucleotides with self-complementary base sequences*

dynamic data of all (compositionally isomeric) complexes (determined by the method described by *Breslauer* and coworkers [36] using the dependence of T_m values from oligonucleotide concentrations; see *Table 4*) support the assumption that all these complexes are duplexes. Above all, the comparison of the data for p-Ribo(A_4–U_4) with those published for the RNA duplex of r(A_4–U_4) [35] demonstrates that adenine-uracil pairing in the p-RNA series is much stronger than in the natural RNA series[14]). Thermodynami-

[14]) Interestingly, the ΔG (and correspondingly the T_m) value shown by the octamer containing the block sequence A-A-A-A-U-U-U-U is somewhat lower than the values of the other octamer duplexes. Whether this specific deviation is connected with competitive hairpin formation needs further investigation.

Table 4. *Thermodynamic Data of Duplex Formation of p-Ribo(A,U)-oligonucleotides*

Base sequence	c (NaCl)	T_m [°C]		Thermodynamic data of duplex formation[d])		
		10 μM	100 μM	ΔG°_{298} [kcal/mol]	$T\Delta S^\circ_{298}$ [kcal/mol]	ΔH° [kcal/mol]
p-Ribo(A_8) + p-Ribo(U_8) (1:1)	0.15M[a])	28	35[c])	−8.0	−42.1	−50.1
p-Ribo((U-A)$_4$)	0.15M[a])	27	35	−7.2	−41.2	−48.4
p-Ribo((A-U)$_4$)	0.15M[a])	24	33	−6.6	−44.5	−51.1
p-Ribo(U_4-A_4)	0.15M[a])	32	40	−8.1	−46.8	−54.9
p-Ribo(A_4-U_4)	0.15M[a])	14	23	−5.2	−37.3	−42.5
p-Ribo(A_4-U_4)	**1.00M[b])**	**21**	**30**	**−6.2**	**−38.1**	**−44.3**
Ribo(A_4-U_4)[e])	**1.00M[b])**	**5[c])**	**12**	**−3.3**	**−47.7**	**−51.0**

[a]) 0.15M NaCl, 0.01M Tris-HCl, pH 7.0.
[b]) 1.00M NaCl, 0.01M Na_2HPO_4-NaOH, 10^{-4}M EDTA, pH 7.0.
[c]) Extrapolated from thermodynamic data.
[d]) Determined from concentration dependence of melting temperatures according to [36].
[e]) Data taken from [35].

cally, the reason for this is the same as for the analogous phenomena encountered in the homo-DNA *vs.* DNA series [3]: the higher stability of the (presumably linear) p-RNA duplex as compared to the corresponding (helical) RNA duplex presents itself again as being not the consequence of a more favorable pairing enthalpy, but of an entropy change opposing duplex formation from the single strand less strongly than in the RNA case[15]).

The property of p-RNA that deserves special comment is the important difference in self-pairing behavior of the homopurine oligomers p-Ribo(A_8) and its homo-DNA analogue ddGlc(A_8).

Model considerations with p-RNA single strands in their idealized linear conformation make it evident that duplexes with antiparallel strand orientation can be expected to be linear as well, if, and only if, the constitutional type of base pairing corresponds to the C_2 symmetry of the strand arrangement; this is the case with the *Watson-Crick*[16]), but not the reverse-*Hoogsteen*[17]) pairing (see *Fig. 9*). The anti*parallel* positioning of two linear strands of an (antiparallel) *Watson-Crick*-paired duplex changes into a *clinal* positioning of these strands, when the partners of a *Watson-Crick* pair are brought into the reverse-*Hoogsteen*[17]) arrangement by rotation around an axis perpendicular to the base-pair plane; such strand deparallelization is the consequence of the axis of that rotation lying perpendicular to the C_2 axis of the *Watson-Crick*-paired double strand on the one hand, and of the inclination between strand and base-pair axes on the other. Base pairing between the resulting clinally positioned linear strands requires (left-handed) helical deformation of both strands. In the light of this analysis, absence of adenine-adenine self-pairing in p-RNA as opposed to its presence in homo-DNA has to be related to a

[15]) For conformational pre-organization due to the rigidity of the pyranose chair as a possible reason for this phenomenon, see the discussions in [1] [3] [4].
[16]) *Sensu stricto* would this be valid only for *Watson-Crick* pairs between isomorphous pairing partners, *e.g.*, guanine and isoguanine [6–8] [14] [15] or adenine-iminoadenine [3].
[17]) Reverse-*Hoogsteen* is the only pairing mode available to antiparallel adenine self-pairing, if *anti*-conformation at the nucleosidic bonds is maintained throughout.

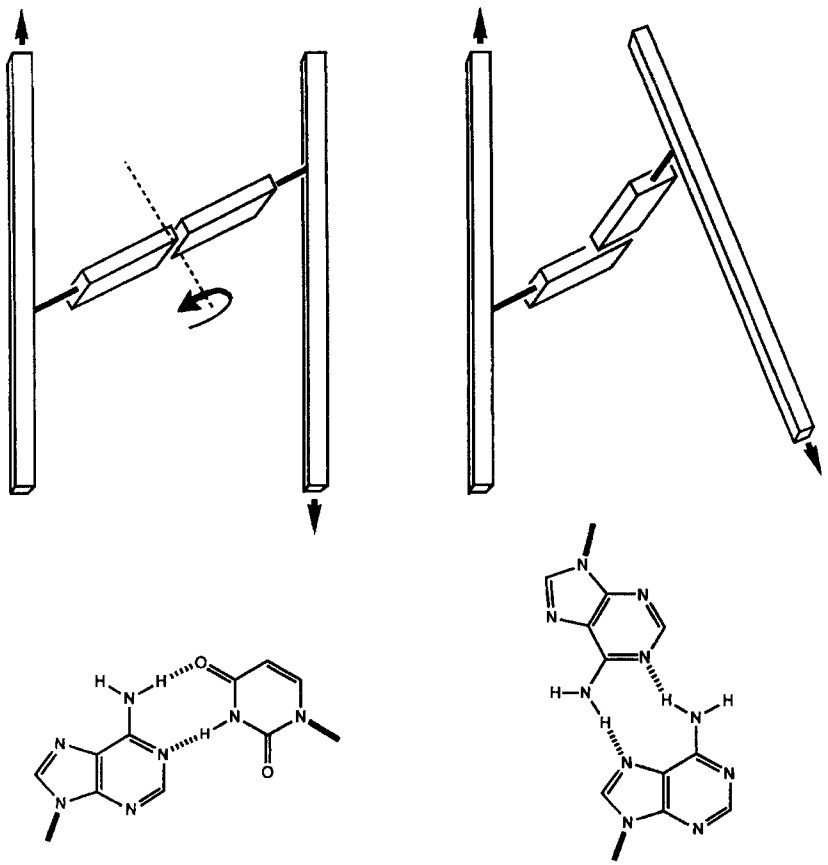

Fig. 9. *Topological contrast in strand arrangement for (antiparallel)* Watson-Crick vs. *reverse*-Hoogsteen *pairing*

resistance of p-RNA strands to undergo such a deformation as a consequence of the constitutionally and conformationally implemented rigidity of the p-RNA backbone, as well as of the inclination of its strand to base-pair axes; both properties are expected to be much more pronounced in p-RNA than they are in homo-DNA[18]). Furthermore, homo-DNA strands in their *gauche/gauche* phosphodiester conformation contain – in contrast to the p-RNA strand – one 'axial' bond per repetitive unit [1] [3]; this conformation feature *per se* induces a *right*-handed helical deformation for *Watson-Crick* pairing [3] (see discussion in *Part III* [3]) and, therefore, tendentially compensates for the *left*-handed helicalization referred to above.

The close relationship between the idealized *structural models* of homo-DNA and p-RNA single strands in conjunction with the so far *observed pairing behavior* of the two

[18]) The p-RNA backbone contains *two* (homo-DNA: only one) rigid endocyclic C–C bonds per repetitive unit and, furthermore, *two* (homo-DNA: only one) constrained secondary phosphodiester carbon junctions.

pairing systems allow us to make the following predictions concerning the properties of p-RNA: guanine will form strong *Watson-Crick* pairs with cytosine, but also with isoguanine, and a comparably strong isomorphous *Watson-Crick* pair is expected to form between 2,6-diaminopurine and xanthine[19]). The topological interpretation delineated above concerning the phenomenon of non-self-pairing of adenine in p-RNA points to the possibility that not only adenine, but also guanine will not pair with itself, neither in the *Hoogsteen*, nor in the reverse-*Hoogsteen* mode, and this in contrast not only to homo-DNA, but also to the natural series. 2,6-Diaminopurine and isoguanine – as derivatives of adenine – show self-pairing in homo-DNA[19]); again, they are expected not to do so in p-RNA. The occurrence of strong purine-pyrimidine and purine-purine pairing in the *Watson-Crick* mode combined with an absence of purine-purine pairing in both the *Hoogsteen* and reverse-*Hoogsteen* mode would render p-RNA the most selective of the pairing systems encountered so far[20]). This selectivity should specifically refer also to strand orientation, because preference for antiparallel *Watson-Crick* pairing should increase with increasing inclination between strand and base-pair axes [3].

Within the family of four diastereoisomeric pentopyranosyl oligonucleotide single strands, the *ribo*pyranosyl member is expected to occupy a unique position in that it is the only one for which conformational analysis on the level of idealized conformations predicts *Watson-Crick*-type pairing to be unhampered and to give rise to a strictly linear double-strand structure (*Fig. 10*). A *xylo*pyranosyl single strand could formally adopt the analogous (idealized) pairing conformation as the *ribo*pyranosyl isomer; however, prohibitive steric hindrance between the equatorial (instead of axial) 3′-OH group and a phosphodiester O-atom should hamper population of this conformation and, therefore, hamper pairing. *Xylo*pyranosyl oligonucleotides are, therefore, expected to pair either not at all, or much more weakly than their p-RNA isomers[21]). As illustrated by the juxtaposition of the four vertical projections of (idealized) linear backbone conformations of the pentopyranosyl series (*Fig. 10*), both the *lyxo*pyranosyl and the *arabino*pyranosyl oligonucleotide systems are expected to pair. However, duplex formation in the *lyxo*pyranosyl series is expected to be handicapped by larger orthogonal base-pair distances (*gauche*/*trans* phosphodiester groups, less pronounced backbone inclination), whereas the *arabino*pyranosyl system (*gauche*/*gauche*-phosphodiester groups) will be handicapped by a repulsive interaction between the bases and the axial 2′-phospho-

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[19]) There is strong *Watson-Crick* pairing between guanine and isoguanine as well as between 2,6-diaminopurine and xanthine in homo-DNA; isoguanine and 2,6-diaminopurine also pair strongly with themselves (presumably in the reverse-*Hoogsteen* mode, as adenine does) [3] [5–8]. For a short preliminary summary of purine-purine pairing in the homo-DNA series, see [14] [15]. We have recently shown that there is, interestingly enough, no guanine-isoguanine *Watson-Crick* pairing in DNA (unpublished experiments by *A. Holzner*, *G. Delgado*, and *J. Hunziker*).

[20]) The significance of an absence (or a weakening relative to RNA) of purine-purine self-pairing as a consequence of structural factors such as those discussed above in connection with adenine is evidenced by the studies of *Orgel* [37] on template-assisted oligonucleotide synthesis in the RNA series. According to *Orgel*, it is mainly the self-pairing of guanine that seriously hampers non-enzymatic replication of RNA-type oligonucleotide sequences (such processes can nevertheless occur in special cases, see [38] [39]).

[21]) The type of steric hindrance revealed by the idealized model of the linear xylopyranosyl backbone conformation is also present in the two linear conformations (*gauche*/*gauche*- and *gauche*/*trans*-phosphodiester groups) of the glucopyranosyl (6′→4′)-oligonucleotide backbone. Experimentally, neither adenine-adenine nor adenine-uracil pairing could be observed in the glucopyranosyl series [9] [12].

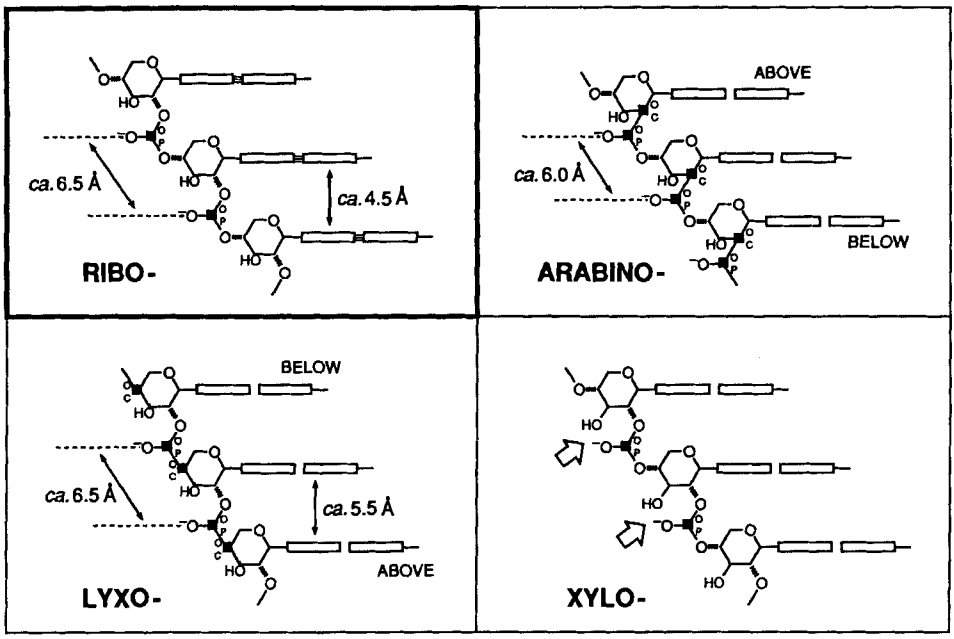

Fig. 10. *Evaluation of relative pairing propensities for the four pentopyranosyl systems by qualitative conformational analysis*

diester O-atoms[22]). Due to the axial positioning of one backbone bond together with short orthogonal base-pair distances (*gauche*/*gauche*-phosphodiesters!), *arabino*pyranosyl duplexes will require a backbone deformation into a helical shape (left-handed; see discussion of pairing topologies in [3]). As a matter of fact, on the level of the qualitative analysis of idealized backbone conformations, the ribopyranosyl system is unique among the four pentopyranosyl systems in that it is expected, first, to show the strongest base pairing of all four; second, to have the highest strand-to-base-pair axis inclination (and, therefore, strand-orientation selectivity), and finally, to be the only system among the four which can adopt a strictly linear double-strand structure without any steric or topic constraint.

Apart from the intrinsic chemical interest that an efficient oligonucleotidal pairing system consitutionally isomeric to RNA deserves, there are three aspects of potentially *biological* relevance which strongly encourage a comprehensive investigation of the chemical properties of p-RNA: first, a linear, strongly paired double-stranded p-RNA would be expected to possess a higher potential for constitutional (especially mineral-assisted) self-assembly than RNA would appear to have; second, an *absence* of purine-purine self-pairing of reverse-*Hoogsteen* and *Hoogsteen* type in p-RNA would facilitate

[22]) Interactions of this type are expected to induce repetitive propeller twists in antiparallel base pairing and, therefore, to hamper duplex formation. Experimentally, altropyranosyl (6′→4′)-oligonucleotides (axial 2′-OH group) show a very weak adenine-uracil pairing [9].

non-enzymic template-directed oligonucleotide syntheses on purine templates, a process critically hampered in the RNA series as shown by the pioneering work of *Orgel* [37], and, third, reaction paths can be envisaged along which a p-RNA double strand could undergo a proton- or *Lewis*-acid-catalyzed p-RNA→RNA transition by intramolecular phosphodiester transesterification and nucleoside isomerization under retention of the constitutional information stored in the base sequence. It will depend from the outcome of such investigations, whether the letter 'p' in 'p-RNA' will adopt another connotation.

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