CHEMISTRY OF POTENTIALLY PREBIOLOGICAL NATURAL PRODUCTS

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Abstract. A relationship between what might be called a kinetic version of Le Chatelier's principle and chemical self-organization is considered. Some aspects of the search for a pre-RNA genetic system are discussed. Results of an experimental investigation on the pairing properties of alternative nucleic acid systems – including those of pyranosyl-RNA ('p-RNA'), a constitutional isomer of RNA – are summarized.

1. Remarks on the Problem of Life's Origin*

* To demonstrate *experimentally* in a chemical model how 'life' can have emerged from a natural environment is a major challenge for bioorganic synthetic chemistry. In the author's view the challenge is to demonstrate a constitutional self-assembly of (a family of) autocatalysts which must (a) contain, replicate and mutate combinatorial structural information and (b) link information with autocatalytic function, in order to have the potential to evolve along gradients of increasing efficiency, diversity and control of catalytic function, toward self-sustainment.

Emergence of an evolving system in Nature according to such a model requires a chemical environment contained far from thermodynamic equilibrium by kinetic barriers. As the environment moves toward equilibrium its random exploration of chemical space ('tinkering')** can result in constitutional self-assembly of a type of structure that acts as a catalyst for the environment's overall equilibration *by the very process of its own replication and propagation.* Given the fortuitous convergence of self-assembly of such a family of catalysts and an environment with kinetic barriers and physical conditions in the appropriate relationships to the catalysts' reactivity, self-replication of these catalysts in that environment is a thermodynamic *necessity*; this is not in spite of, but because of the second law of thermodynamics. The replicators' propagation is mandated because any chemical environment that is immobilized by kinetic barriers *must* undergo selective partial equilibration when one of the barriers is lifted due to the presence of an appropriate catalyst. Progressive partial equilibration through propagation of such catalysts would bring

* Sections 1 and 2 of this paper are an amendment to the material discussed in the inaugural lecture 'Chemistry of Potentially Prebiological Natural Products' at the Barcelona conference; the lecture's content is covered by Section 3, where the authentic sequence of slide copies is reproduced. The author wishes to acknowledge the intense and valuable discussions with Professor Claude Wintner (Haverford College, USA) of the ideas presented in Sections 1 and 2 and of their formulation in English.

** Borrowed from the term 'evolutionary tinkering' (Jacob, 1982).

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the replication process to an eventual end; thus, maintainance of a steady, as opposed to a transient, replication process demands a quasi-unlimited reservoir, or else a steady influx, of requisite starting materials. In a stationary state such a process of self-replication of catalysts constitutes a dynamic (free) energy dissipating process and implies, on the molecular level, an organizational structure reminiscent of what, on the macroscopic level, is referred to as a dissipative structure (Prigogine and Nicolis, 1967).

Let us allow ourselves what is normally taboo in physical science, namely, to look at this situation from a teleological* point of view. Encouragement for so doing is drawn from the fact that any reference to a process as being driven by the second law of thermodynamics can be considered to have a connotation that is fundamentally teleological in its nature.

Formally, the phenomenon of catalysis of a chemical environment's thermodynamic equilibration by the self-assembly of self-replicating catalysts may be regarded *as if* the environment were to induce the catalysts' assembly so as to enhance the rate by which it can move toward equilibrium. In other words, it appears *as if* the environment acts according to what might be considered a *kinetic* version of Le Chatelier principle. In one of its formulations the (thermodynamic) Le Chatelier principle states that 'a system *at equilibrium* reacts to an applied stress in such a way as to reduce the effect of that stress'.** A chemical system unfettered by kinetic barriers does so by re-organizing itself in order to regain equilibrium. Analogously, *a chemical environment constrained by kinetic barriers will react to the stress of being kept far from equilibrium in such a way as to seek maximization of its equilibration rate.* The proposition is made that it can do so by chemical self-organization, that is, through the generation of autocatalytic dissipative structures along gradients of increasing rates of the environment's overall (free) energy dissipation. According to this view *circumvention* of kinetic barriers is at the heart of chemical self-organization.*** The proposition provides a sense for what had to be chance and what was necessity in the emergence and propagation of self-replicating autocatalytic systems that might have evolved into a living system.

Since it is known that the (thermodynamic) Le Chatelier principle is also applicable to equilibria involving physical rather than chemical changes, it is tempting to extrapolate and to ask whether such a kinetic principle might apply to the formation of *any* self-organized dissipating structure in *physical* systems far from equilibrium, thus providing the sort of intuitive and qualitative comprehension of why and

* The general term 'teleological' ('end-directed' or 'goal-directed') is meant here in the sense discussed and specified by Ernst Mayr (Mayr, 1974). According to Mayr's proposal, the term 'teleomatic' is to be used when referring to a process which reaches an end state purely as the result of the operation of natural laws (e.g. gravitation or the second law of themodynamics); alternatively, Mayr proposes that the term 'teleonomic' (Pittendrigh, 1958) be used when referring to a process or a behavior which owes goal-directedness to the operation of a program (e.g., a genetic program).

** Formulation according to Gray and Haight (1967).

*** Mechanistically, self-organization is the result of a complex interplay between catalysis and inhibition, positive and negative feedback, as well as boundary conditions (see, e.g., Haken, 1981).

how environments react by self-organization to being kept far from equilibrium, as the thermodynamic principle does for the 'self-organizing' behavior of systems near equilibrium.[‡]

Formed by constitutional self-assembly within an environment, a replicator would be totally dependent on that environment; its status, with respect to the question of whether it is 'living' or not, would not be considered without strict reference to its environment (as should be true, in principle, for any living system). Whether a replicator has the potential to evolve depends on whether differences in structurally stored information can express themselves in functional differences, that is, whether the system as a genotype can code for function (Eigen, 1971; Kuhn, 1972; Eigen and Schuster, 1977, 1978). If so, then the potential to evolve would have to be directed primarily at moving toward a degree of chemical and physical independence of the replicating system from the environment. Self-sustainment is the elongated arm of self-replication. A system could move toward it by gradually taking control, first of function and then of synthesis, of an expanding spectrum of cooperating catalysts, as well as by developing and taking advantage of compartmentalization. The ultimate achievement in such a cascade of takeovers would be the enslavement of an entire class of servant-catalysts with essentially limitless structural and functional versatility, by achieving strict control over their synthesis through a coding machinery. The gradients of increasing efficiency, selectivity, and diversity of catalytic function will be, at the same time, gradients of increasing structural and systemic complexity. Reasons for the increase of the *structural* complexity have their origin in the detailed structural demands of the mechanisms of molecular recognition and catalysis.

Along the path from self-organization to self-sustainment in any model for the origin of life a new quality must gradually emerge: the quality of a 'biological entity'. We recognize this quality in, and – in part – define it by, the evolving system's potential for purposeful (teleonomic, as opposed to teleomatic) behavior, by its capacity to move to higher and more diversified levels of structural and systemic complexity through evolution (Eigen, 1971; Kuhn, 1972). Under no circumstances would we any longer refer to the maximization of the environment's equilibration rate as being the system's 'raison-d'être', and no longer would we say that the

[‡] Prigogine's theorem of 'minimum entropy production' ('least dissipation') (Prigogine, 1945; Glansdorff and Prigogine, 1971) has been referred to as implying an extension of Le Chatelier's principle to dynamic systems *near* equilibrium, being valid within the 'thermodynamic branch' of a system's dynamic states. However, '... far from equilibrium the thermodynamic behavior could be quite different, in fact, even opposite to that indicated by the theorem of minimum S-production' (Prigogine, 1978). Glansdorff and Prigogine's 'evolution criterion' for the formation of dissipative structures far from equilibrium (valid within the 'non-linear branch') assigns a central role to 'excess entropy production,' for which, however, theory does not assign a defined sign and, therefore, does not suggest either a maximization or a minimization principle for the formation of dissipative structures far from equilibrium (Glansdorff and Prigogine, 1971; Nicolis and Prigogine, 1977). A well documented example of a macroscopie dissipative structure, the formation of which is accompanied by an increase of dissipation, is the Bénard convection cell (Prigogine, 1978) (cf. also a discussion by Baranowski, 1989).

environment 'makes use' of the system; now, we would adopt the conventional way of referring to the *system* by stating that it requires and processes energy from the environment in order to live.

2. Concerning the Search for 'the First Genetic System'

The widespread idea that a more primitive genetic system might have preceded RNA is a daring, but important and challenging proposition (Orgel, 1986; Joyce *et al.*, 1987). The first challenge is to demonstrate experimentally whether a proposed type of structure is, in fact, an informational system, e.g., a pairing system, in other words, whether it has the capacity to recognize and read the structural information it would be assumed to have carried and transmitted. Another requirement expected to be fulfilled by such a candidate sytem is that it must be constitutionally elementary and not only be 'simpler looking' than the RNA system. This requirement is to be assessed by chemical reasoning, by judging the system's potential for constitutional self-assembly, taking into account the criteria of prebiotic chemistry.

How 'simple' would such a system have to be? It seems worthwhile to question the validity of the widespread idea that a backbone – let us say of an oligonicleotide system – should be not only constitutionally, but also configurationally as simple as possible. Selectivity in molecular recognition – this is the basis for any 'genetic' system's capacity for making use of its structural information – intrinsically demands a broad variety of molecular shapes. What conveys such variety to molecules is constitutional and configurational complexity. To consider a crucial example: an important part of the pairing selectivity of an oligonucleotide system resides in the distinct preference for (either) an antiparallel (or a parallel) orientation when strands recognize (complex with) each other. Such a preference requires a given strand to be able to present a partner strand with a sufficiently different molecular shape when facing it in antiparallel versus parallel orientation. A prerequisite for such dependence of strand orientation on strand shape is the absence of a corresponding strand symmetry. Such strand asymmetry quite naturally requires a degree of molecular complexity.

The experimentally documented example of high diastereoselectivity in the aldomerization of glycolaldehyde phosphate to *rac*-allose-2,4,6-triphosphate in the aldohexose, and *rac*-ribose-2,4-diphosphate in the aldopentose series (Müller *et al.* 1990) is a case that demonstrates that *there can be an intrinsic preference for a given diastereoisomer in the formation of molecular structures that, in fact, are configurationally complex molecules but, at the same time, have an elementary structure.* The major source of the molecular complexity that might be required for achieving pairing and replication selectivity in a primitive oligonucleotide-type genetic system is contained in, as well as determined by, the sugar building block. It well might be a mistake to think that the structure of such a building block should be devoid of configurational isomerism. One is tempted to paraphrase a remark

attributed to Einstein, by stating that the structure of a proposed first genetic system 'should be as simple as possible, but not simpler'.

The idea of a genetic forerunner system is normally connected with the concept of a genetic takeover (Cairns-Smith, 1982). A number of questions come up in connection with the concept of successive genetic systems. One of these questions has attracted the attention of two advocates of a pre-RNA world (Joyce and Orgel, 1993): 'What selective advantage could a simpler, metabolically competent system derive from the synthesis of oligonucleotides?' It is, in fact, quite difficult to see, as the authors have pointed out, how the formation of the components of a follow-up genetic system by a forerunner system can serve the forerunner's teleonomic interest. However, the difficulty is specific to the formulation of the question within the framework of evolutionary thinking. The question should also be asked with reference to the factors that drive teleomatic self-organization rather than Darwinian selection: Could the presence of a rudimentary, but operating, genetic system improve the probability for the *teleomatic* emergence of a superior genetic system relative to the chance of the superior system's *ab initio* emergence from a virgin environment?

When the question is asked in this manner, it becomes clear that the answer can be affirmative. The character of the answer is hardly dependent on details about the nature of the two genetic systems and on how they might relate to each other. This is so even for the simplest case, where the forerunner system would not be able to achieve anything other than growth (and would, therefore, not really be a genetic system). Such a system would, at least, change the chemical environment; even if it would not contribute anything more than to accumulate a given type of organic material in a given locale, it could greatly influence the chance for the emergence of a new genetic system in such an environment, not to speak of the impact on the environment of a genetic system that advances to the level of producing organic catalysts. The occurrence of symbiosis, also at such a level, is conceivable. In principle, the scenario not only of one takeover but of a whole cascade of genetic takeovers in moving up to the RNA world seems admissable, if (and this is a big if) a correspondingly broad variety of organic structures had been available.

The contrasting answers one may arrive at when Orgel's question is asked from the point of view of evolutionary selection on the one hand and self-organization on the other provide a good illustration of the distinction to be made between the concepts of teleonomic and teleomatic processes. The example has an interesting connection with the ongoing debate concerning the gap between the credos in the camps of the 'geneticists' and the 'metabolists' on the question of life's origin. The geneticists' credo is that life started with the emergence of a genetic system (Eigen, 1971; Kuhn, 1972). The 'metabolists' oppose this view and adhere to the faith that it was the emergence of autocatalytic metabolic cycles that marked life's origin (see, e.g., Kauffman, 1986, 1993; Wächtershäuser, 1990; de Duve, 1991); for them, the emergence of life's genetic system was a later accomplishment of metabolic life's evolution. What has been said in chapter one about self-organization within

a chemical environment leading to a genetic system is, in principle, equally valid for an emergence of autocatalytic metabolic cycles. A teleomatically driven self-organization within a chemical environment could, in principle, take a course toward autocatalytic metabolic cycles which, by definition, would have to have the capacity to grow. Such dissipative systems would modify the chemical environment and, therefore, influence the chances for (and this, now, is the geneticist's way of saying it) a *teleomatic* emergence of a genetic system in the environment. The geneticist's interpretation of such a scenario is dictated by an attitude toward the problem of defining life that is reluctant to assign the quality of life to a dissipative chemical system as long as it does not act as – or under the control of – a replicating entity that embodies combinatorial structural information and evolves by virtue of using it as genetic information that codes for a phenotype.

Chemical theories concerning the origin of life are significant if, and only if, they lead to experiments which extend chemical knowledge. This admittedly restrictive, but pragmatic, imperative might be the experimentalist's lead for rendering the debate between the geneticists and the metabolists fruitful.

3. Toward a Chemical Etiology of the Structure of Nucleic Acids

According to the prevailing view within the 'camp of the geneticists' (see above) a chemical etiology* of life will be primarily concerned with the type of molecular structure that we encounter today in Nature's nucleic acids and, more specifically, in the structure of RNA. Since 1986, extended experimental work has been carried out at the ETH toward such a chemical etiology of the nucleic acids' structure. The concept according to which this investigation continues to proceed is illustrated in Figure 6. The problem of the origin of the nucleic acids' structure – or any other type of biomolecular structure – can be approached experimentally by systematically studying the chemistry of *structural alternatives*, molecular structures which – according to chemical reasoning – could have been, but were not, chosen by Nature to become (or to survive as) biomolecules. The structure of an alternative is derived from a *chemical hypothesis* about the constitutional self-assembly that could have given rise to the biomolecule's origin. The structure is chosen according to two criteria: first, a judgment about whether it has a potential for constitutional self-assembly comparable to that of the biomolecule itself and, second, a judgment about whether its chemical properties might be such that the alternative could, in principle, fulfill the type of biological function accomplished by the actual biomolecule. By chemically synthesizing such an alternative structure and comparing its relevant chemical properties with those of the actual biomolecule, we can expect to learn about the reasons why the latter, and not the alternative, was chosen by Nature to become a biomolecule. Such information will deepen our understanding of the structural basis of the actual biomolecule's functioning and, if we are lucky, we

* Etiology is the 'science of causes and origins', cf. Webster's New Twentieth Century Dictionary of the English Language, Unabridged 2nd edn., 1983.

may come across molecular structures which reveal themselves as candidates for having been intermediates (if there were any) along the evolutionary path toward the biomolecule we know today.

Obviously, the choice of the alternative's structure is crucial. In choosing it, one draws from the wealth of experience about the reactivity and synthesis of organic molecules, as well as from the findings and concepts of prebiotic chemistry (Miller and Orgel, 1974; Oro, 1960; Ferris and Hagan, 1984). In practice not one, but a whole range, of alternative structures may offer themselves. The concept's strength resides in the iterative nature of the experimental approach that it proposes (Figure 7), in the feedback that the outcome of one cycle can have on the choice of the structural alternative for the next.

Historically, the question, 'Why pentose- and not hexose-nucleic acids?* stood at the outset of our experimental studies on alternative nucleic acid structures (Eschenmoser, 1991; Eschenmoser and Dobler, 1992). The question came from the investigation of the aldomerization chemistry of glycolaldehyde phosphate (Müller *et al.*, 1990; Pitsch *et al.*, 1994), where we observed *rac*-ribose-2,4-diphosphate to be the kinetically favored product in the pentose family and – in the absence of formaldehyde – *rac*-allose-2,4,6-triphosphate in the hexose family, the latter forming with ease and selectivity comparable to the former (Figures 4–6.**) In a sequence of investigations hexopyranosyl-(6' → 4')-oligonucleotides derived from 2',3'-dideoxy-D-glucose (the building block of 'homo-DNA'), D-allose, 2'-deoxy- and 3'-deoxy-D-allose, D-altrose and D-glucose were synthesized and their pairing properties compared with those of corresponding DNA-oligonucleotides.*** These studies presented us with a cascade of surprises and, consequently, of insights. Hexopyranosyl-(6' → 4')-oligonucleotides in the model system homo-DNA show Watson-Crick purine-pyrimidine pairing that is uniformly *stronger* than the pairing in the DNA series and, in addition, display unprecedented purine-purine pairing to give duplexes in the reverse-Hoogsteen mode. On the other hand, corresponding oligonucleotides derived from the *natural* hexopyranoses D-allose, D-altrose and D-glucose show pairing that is in some respects similar to, but in others drastically different from, and above all, uniformly much weaker than the pairing in homo-DNA (Figures 9–28). In view of the pairing properties shown by the 2'-deoxy-

* For other 'why-questions' referring to the natural nucleic acids' structure see, e.g., Westheimer, 1987; Usher, 1972, 1977; Dingra and Sarma, 1978; Jin *et al.*, 1993

** Figures 1–3 refer to the background of the work that led to the aldolization studies with glycolaldehyde phosphate (Eschenmoser, 1988; Eschenmoser and Loewenthal, 1992; Ksander *et al.*, 1987; Wagner *et al.*, 1990; Drenkard *et al.*, 1990; Müller *et al.*, 1990; Pitsch *et al.*, 1994; Xiang *et al.*, 1994).

*** This work is discussed and described in detail in: Eschenmoser and Dobler, 1992 (introduction to homo-DNA), Böhringer *et al.*, 1992 (synthesis of homo-DNA oligonucleotides), Hunziker *et al.*, 1993 (pairing properties of homo-DNA oligonucleotides), Otting *et al.*, 1993 (nmr spectroscopy of a homo-DNA duplex), Groebke, 1993 (purine-purine pairing in homo-DNA and altropyranosyl-(6' → 4')-oligonucleotides), Peng, 1993 (purine-purine pairing in homo-DNA), Fischer, 1992 (allopyranosyl oligonucleotides), Helg, 1994 (allopyranosyl oligonucleotides), Giger, 1992 (homo-DNA and allopyranosyl oligonucleotides), Diederichsen, 1993 (glucopyranosyl oligonucleotides). For a summary of the work see Eschenmoser, 1993.

and the 3'-deoxy-allopyranosyl systems (Hammer *et al.*, 1992), it can be argued convincingly that the reason for this divergence is intrastrand steric hindrance in the pairing conformation. The three examined $(CH_2O)_6$ hexopyranose sugars (and, foreseeably, also the five remaining diastereomers) are too bulky to serve as building blocks of efficient pairing systems. The short answer to the question 'Why pentose, and not hexose-nucleic acids?' that emerges from these studies simply seems to be: '*Too many atoms*!'

A comprehensive experimental involvement in the problems of a chemical etiology of the nucleic acids' structure would require a systematic extension of the study into hexo- and pentopyranosyl (as well as hexo- and pentofuranosyl) oligonucleotide systems which have their phosphodiester link between positions *other* than the (6' → 4')- or the (5' → 3')-link of the structures investigated so far (Figure 29). Screening the pyranosyl section of this structure space by qualitative conformational analysis predicts the existence of a variety of experimentally untouched pairing systems and, above all, foresees a *pyranosyl isomer* of RNA ('p-RNA') that contains the phosphodiester linkage between positions C-2' and C-4' of neighboring ribopyranosyl units and is expected to show purine-pyrimidine and purine-purine (Watson-Crick) pairing comparable in strength to that observed in homo-DNA (Figures 30–35). *Experimentally* (Pitsch *et al.*, 1993), not only does Watson-Crick pairing in p-RNA turn out to be *stronger* than in both RNA and DNA (and even stronger than in homo-DNA), but p-RNA also appears to be the most *selective* oligonucleotide pairing system observed so far, including the natural systems (Figures 36–43). This statement is based on the observation that the oligomers p-Ribo (A_n) and p-Ribo (G_n) $(n \leq 10)$* show neither reverse-Hoogsteen, nor Hoogsteen self-pairing, in sharp contrast to homo-DNA and – with respect to guanine – also in contrast to DNA and RNA. These (together with other, so far conjectured) properties (see Figure 43) render this constitutional *isomer* of RNA of great interest in the context of the problem of RNA's origin.

* Since the lecture was given, it has been found that the conjecture mentioned in Figure 43, according to which not only adenine but also guanine should show no self-pairing in p-RNA, is in fact correct (unpublished work by Sebastian Wendeborn, Armin Holzner and Stefan Pitsch, ETH, 1993).

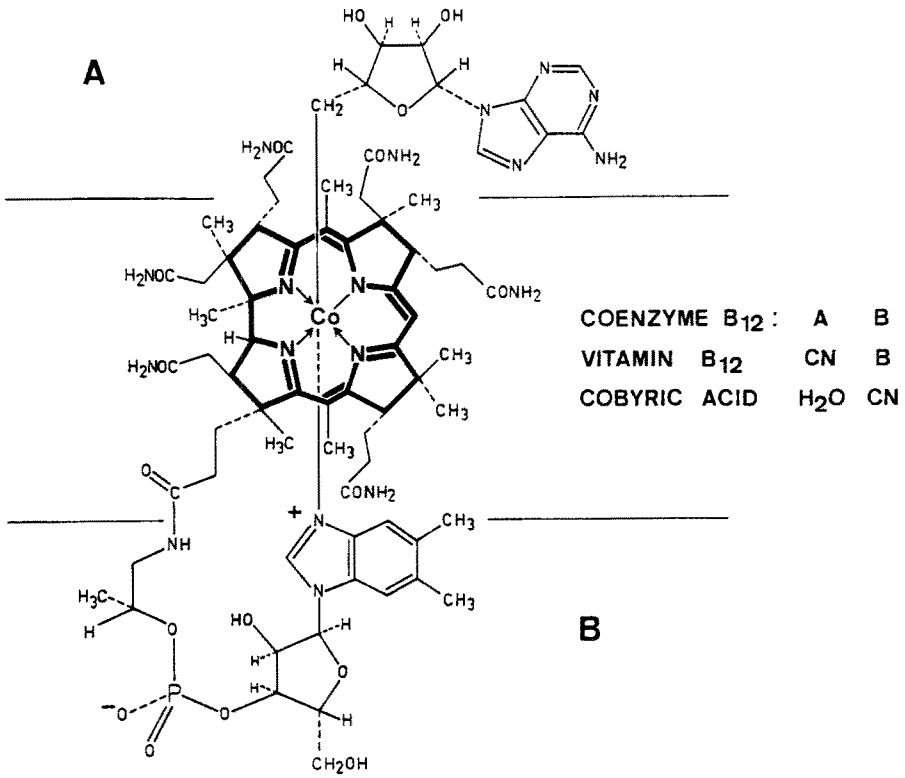

Fig. 1. Our investigations directed toward a chemical etiology of biomolecular structures have their origin in the work on the chemistry of vitamin B_{12} (Eschenmoser, 1988).

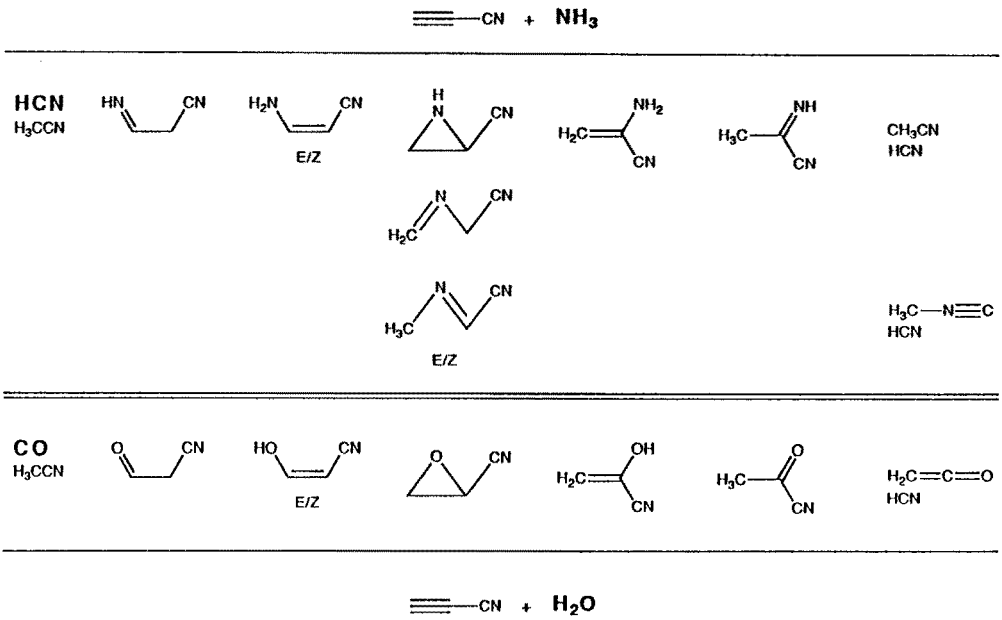

Fig. 2. Chemistry of α-aminonitriles (Ksander *et al.*, 1987; Drenkard *et al.*, 1990; Wagner *et al.*, 1990; Xiang *et al.*, 1994).

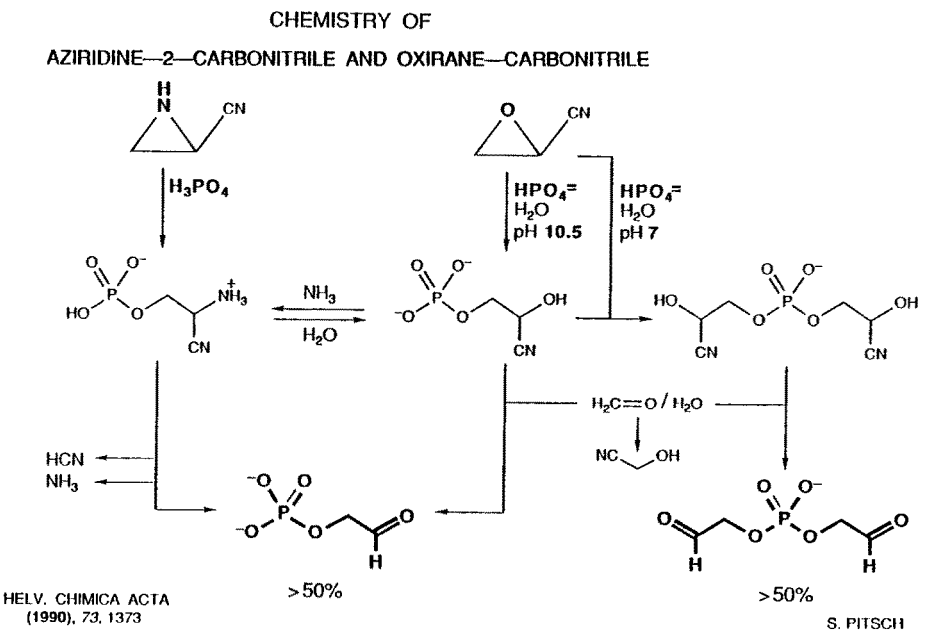

Fig. 3. Chemistry of aziridine-2-carbonitrile and oxirane-carbonitrile (Wagner *et al.*, 1990; Pitsch *et al.*, 1994).

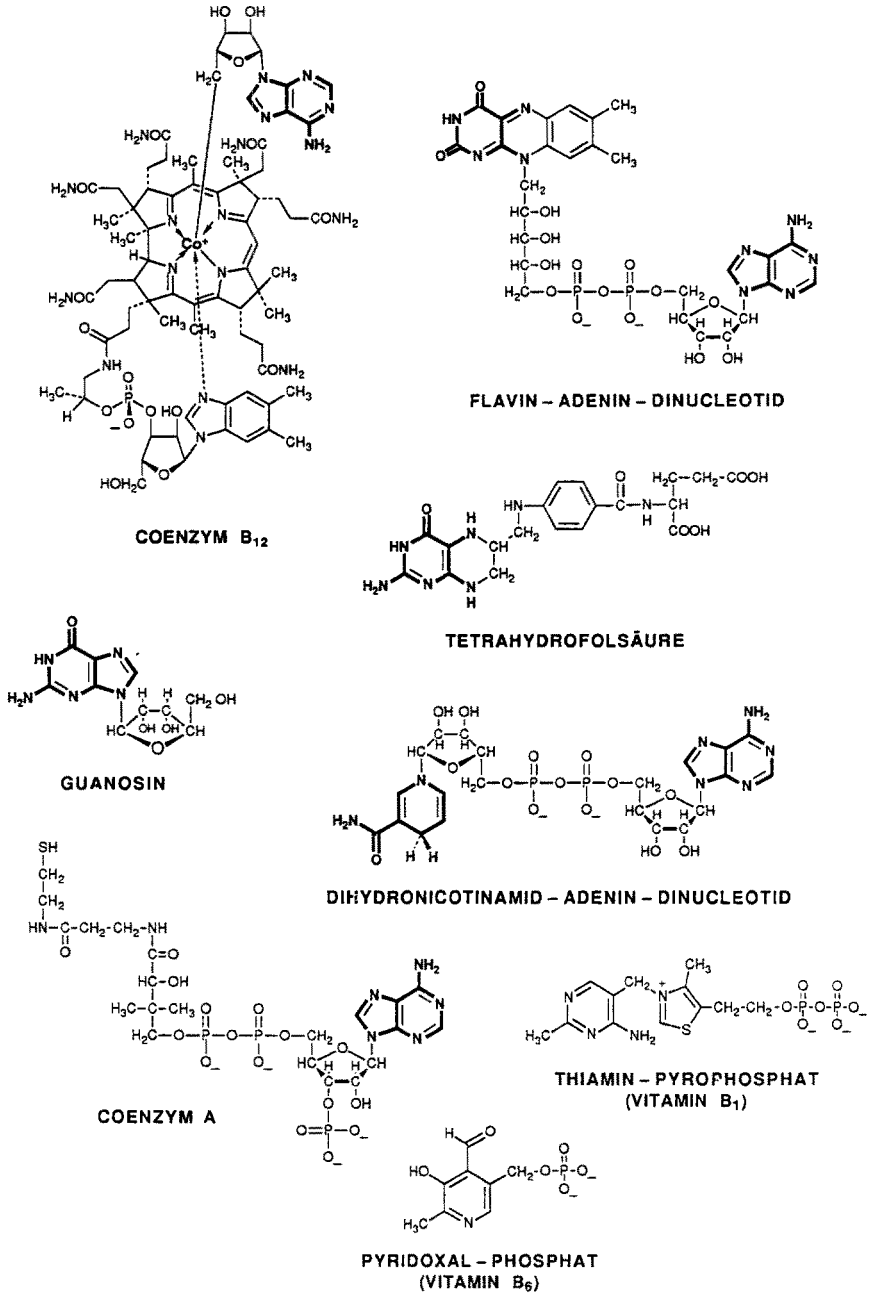

Fig. 4. Coenzyme structures.

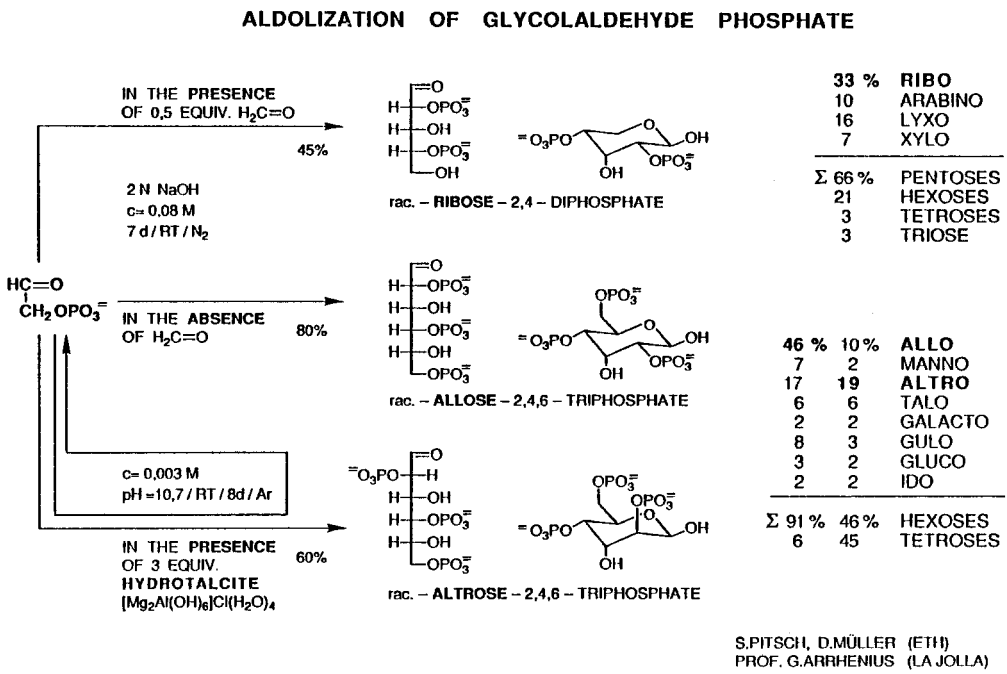

Fig. 5. Aldolization chemistry of glycolaldehyde phosphate (Müller *et al.*, 1990; Pitsch *et al.*, 1994).

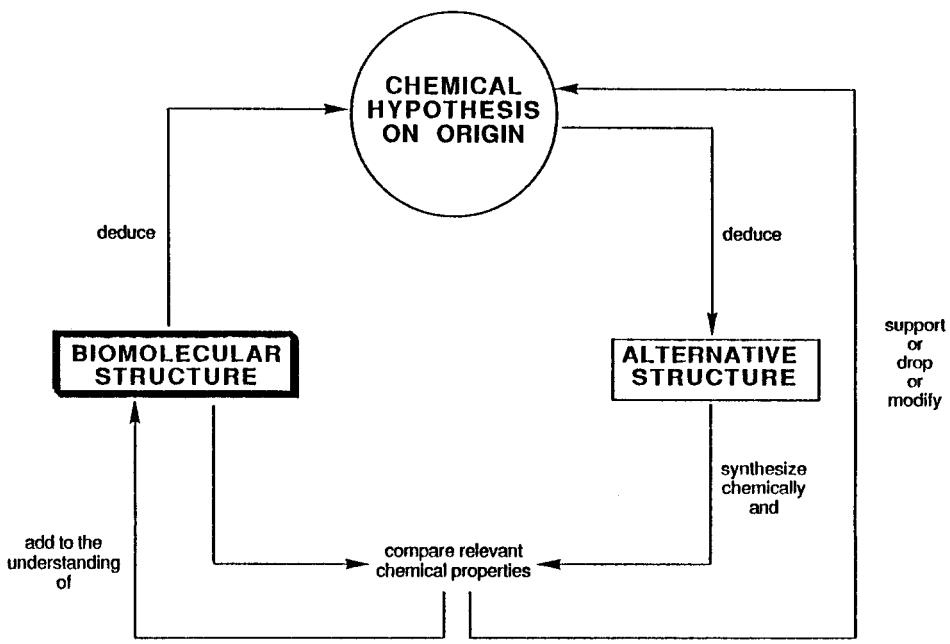

Fig. 6. General concept for experiments directed at a chemical etiology of biomolecular structures.

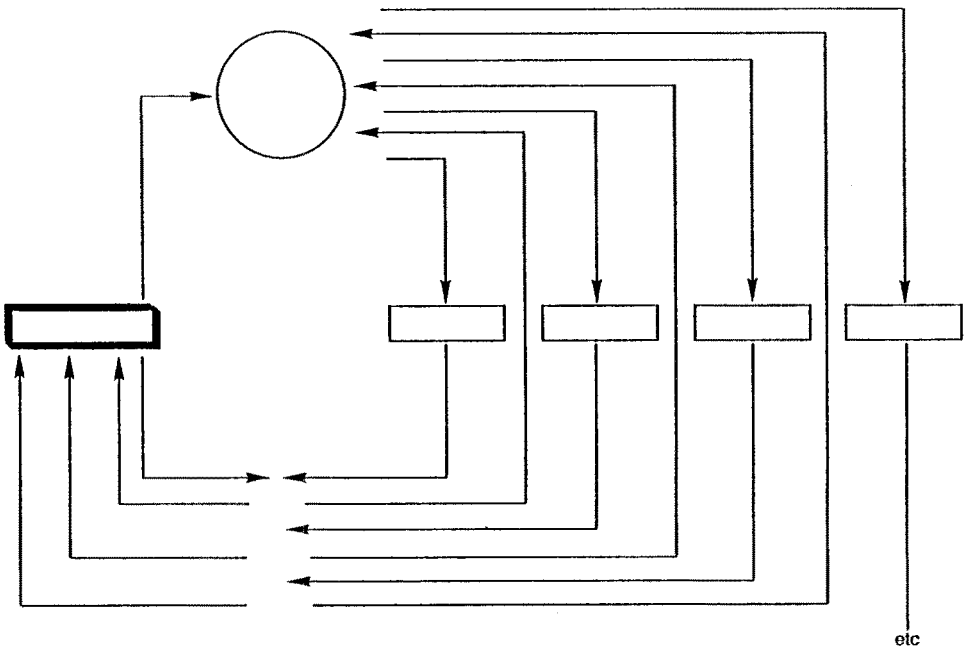

Fig. 7. Iterations within the concept presented in Figure 6.

Why purines and pyrimidines	and not .....
Why a sugar	and not .....
Why a pentose	and not a hexose
Why ribose	and not another pentose
Why ribofuranose	and not ribopyranose
Why phosphate	and not .....

Fig. 8. Cascade of 'why-questions' directed at a chemical etiology of the natural nucleic acids' structure. Such 'why-questions' must specify alternatives, in order to define experiments.

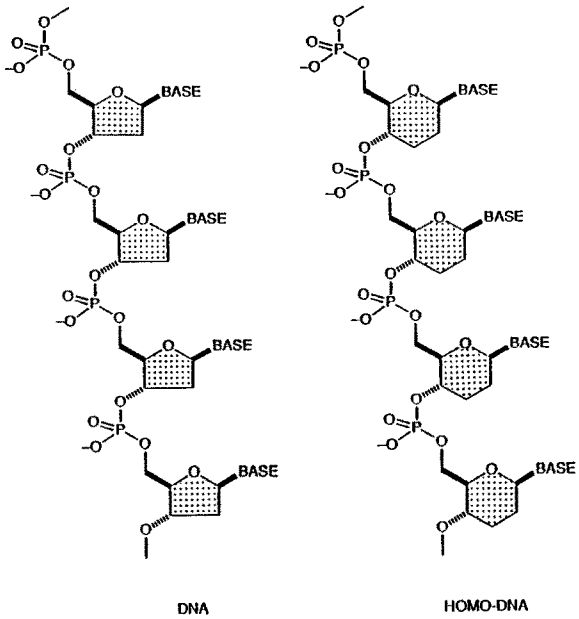

Fig. 9. Constitution and configuration of homo-DNA compared to DNA.

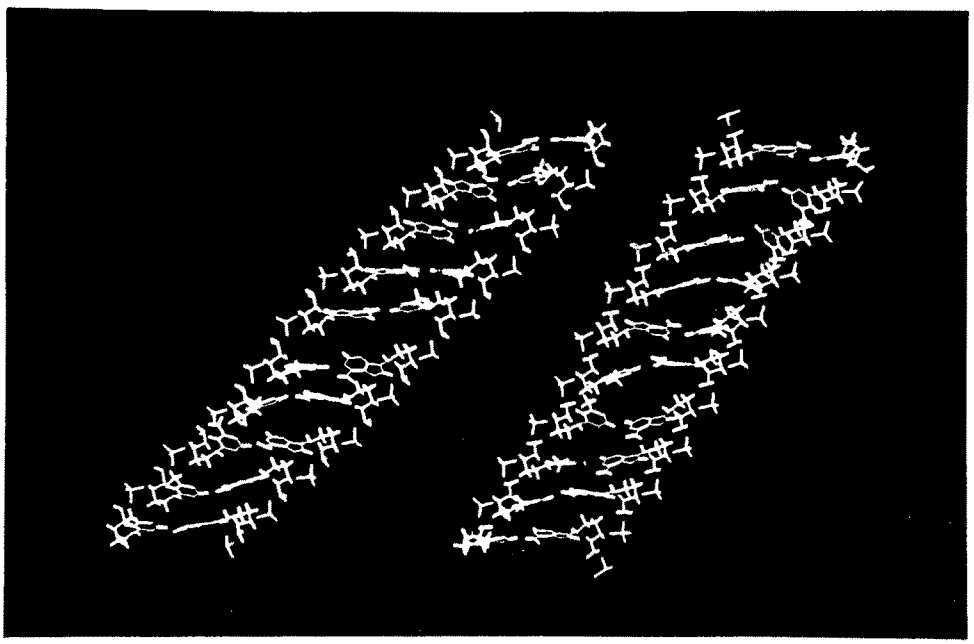

Fig. 10. Structure models of the self-complementary homo-DNA duplex ddGlc(A_5-T_5), according to nmr spectroscopy (Otting *et al.*, 1993).

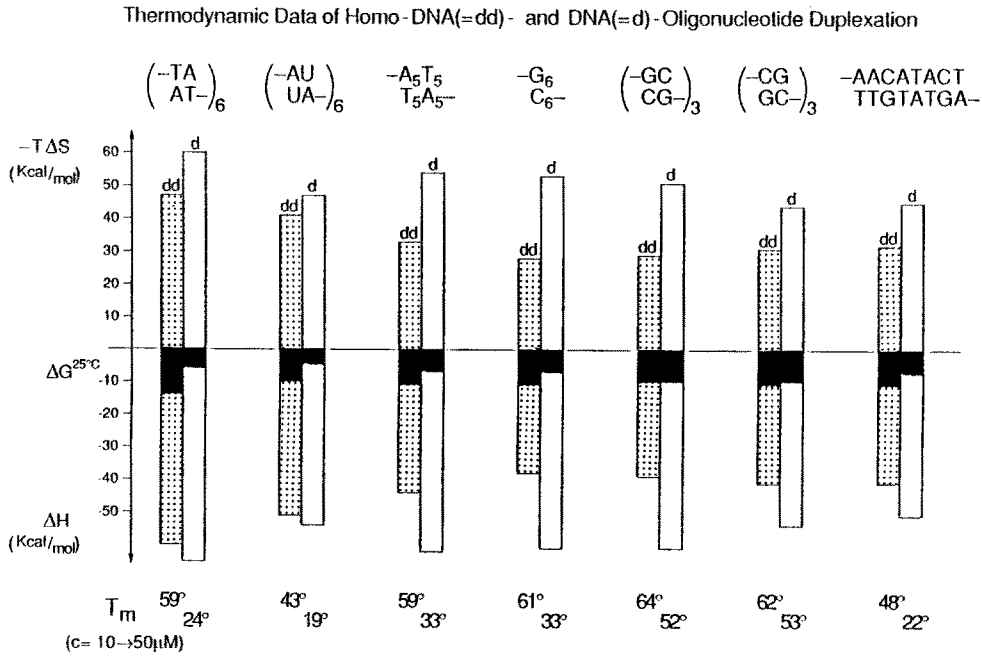

Fig. 11. Homo-DNA duplexes are uniformly more stable than the corresponding DNA duplexes. The stability difference is entropic in origin (Hunziker *et al.*, 1993).

MELTING TEMPERATURES (°C) OF HEXAMER – DUPLEXES

15 - 20 μM Nucleotide, 150 mM NaCl, 10 mM Tris pH 7

dd-BBBBBB
BBBBBB-dd
dd-BBBBBB
BBBBBB-dd

HOMO-DNA

	A	T	G	C
A	47			
T	20	—		
G	<15	—	38	
C	<15	—	58	—

DNA

	A	T	G	C
A	—			
T	< 5	—		
G	—	—	+	
C	—	—	48	—

MARKUS BOEHRINGER, HANS-JÖRG ROTH, JÜRG HUNZIKER, FREDI GIGER, DR. CHRISTIAN LEUMANN

Fig. 12. Melting temperatures (in degrees centigrade), under standard conditions, of representative homo-DNA hexamer duplexes, as compared to corresponding DNA hexamer duplexes (Hunziker *et al.*, 1993).

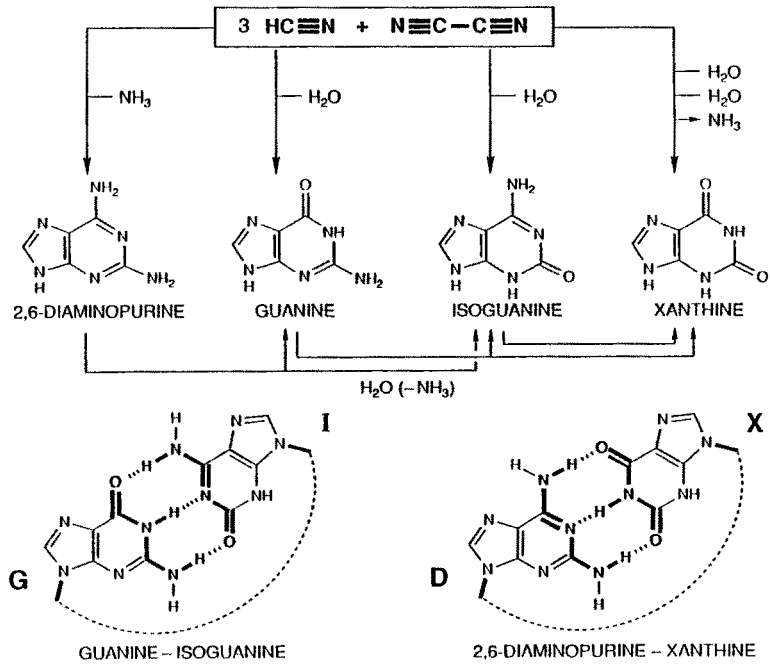

Fig. 13. Constitutional assignment of the strong pairing between guanine and isoguanine, as well as between 2,6-diamino-purine and xanthine, observed in the homo-DNA series (Groebke, 1993; Peng, 1993; Giger, 1993).

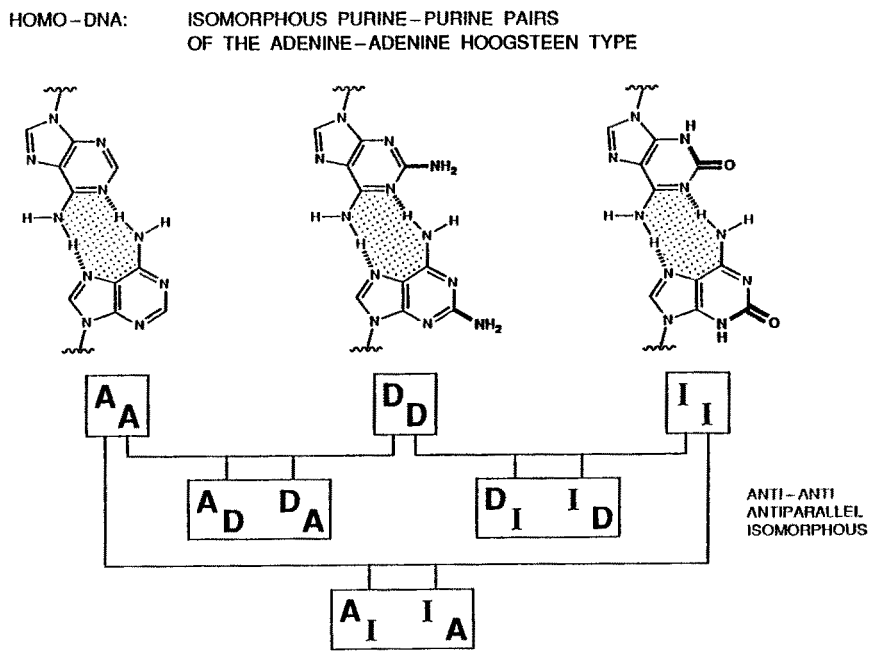

Fig. 14. Constitutional assignment of the purine-purine self-pairing of adenine (and adenine derivatives) observed in the homo-DNA series (Hunziker *et al.*, 1993; Groebke, 1993; Peng, 1993; Giger, 1993).

HOMO – DNA : PURINE – PURINE PAIRING

MELTING TEMPERATURES (°C) OF HEXAMER – DUPLEXES:

15-20μM Nucleotide
150mM NaCl
10mM Tris pH 7

dd-BBBBBB
BBBBBB-dd

dd-BBBBBB
BBBBBB-dd

dd-BBBBBB
BBBBBB-dd

dd-BBBBBB
BBBBBB-dd

		A	H	D	X	I	G
ADENINE	A	47					
HYPOXANTHINE	H	< 7	—				
2,6-DIAMINOPURINE	D	41	< 5	36			
XANTHINE	X	14	—	**63**	—		
ISOGUANINE	I	43	12	39	16	42	
GUANINE	G	<15	<15	<15	18	**61**	38

~40 HOOGSTEEN (BIDENTATE)

~60 WATSON-CRICK (TRIDENTATE)

— NO PAIRING OBSERVED

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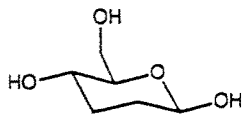
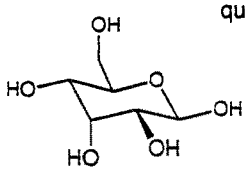
Fig. 15. Relative strength of purine-purine self-pairing in the reverse-Hoogsteen mode (see Figure 14) and purine-purine pairing in the Watson-Crick mode (see Figure 13) in the homo-DNA series (melting temperatures of relevant hexamer duplexes under standard conditions, A = adenine, H = hypoxanthine, D = 2,6-diamino-purine, X = xanthine, I = isoguanine, G = guanine) (Groebke, 1993).



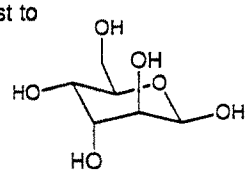
2-3-DIDEOXY-GLUCOSE

is NOT to be considered
a potentially
prebiological sugar

quite in contrast to



or



ALLOSE ALTROSE

Fig. 16. Within the concept of Figure 6, the investigation of the properties of homo-DNA is only a model study.

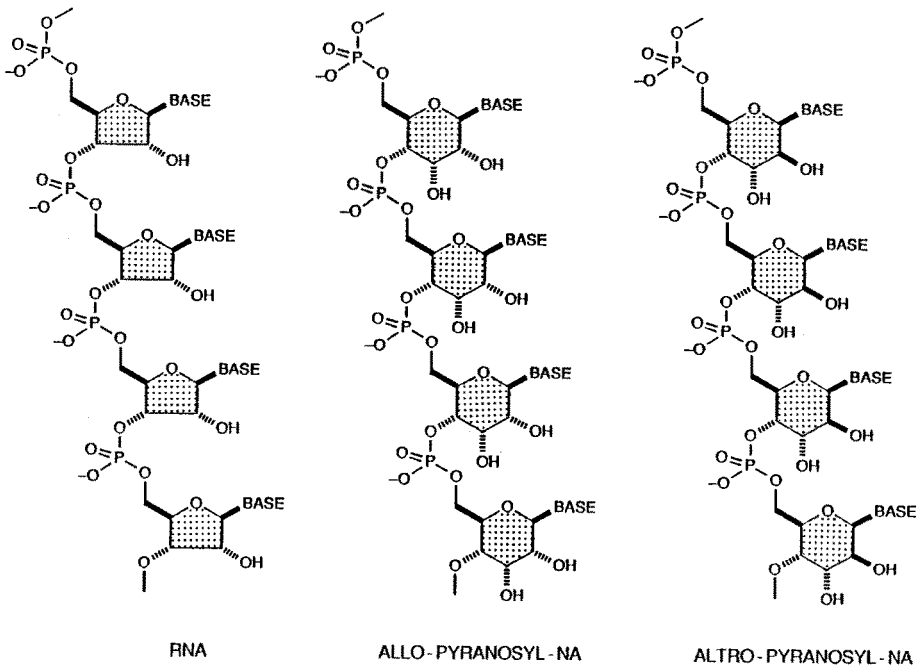
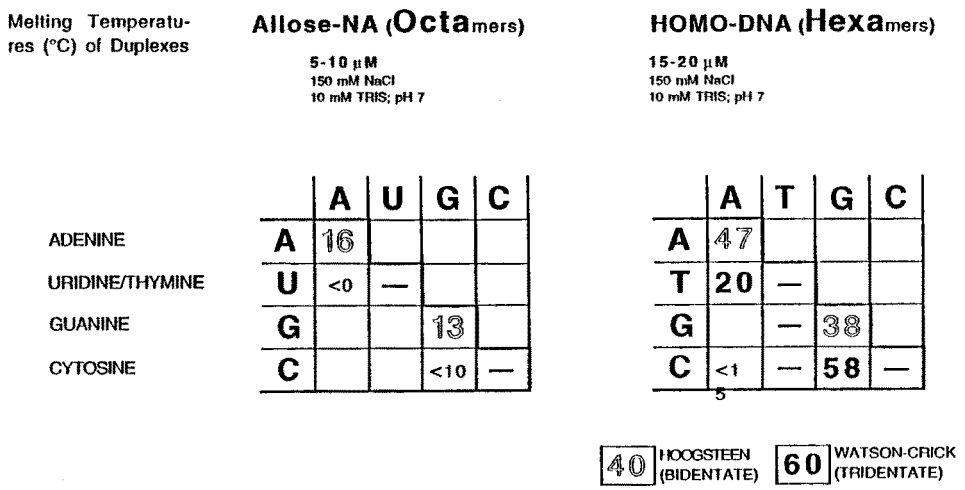


Fig. 17. Constitution and configuration of D-allopyranosyl- and D-altropyranosyl-(6' → 4')-oligonucleotides.



Markus Böhringer, Hans-Jörg Roth, Jürg Hunziker, Andreas Helg, Reto Fischer, Alfred Giger, Dr. William Fraser, Dr.Christian Leumann

Fig. 18. Melting temperatures of *octamer* duplexes in the allopyranosyl series compared to corresponding *hexamer* duplexes of the homo-DNA series. Base pairing in the allopyranosyl series is drastically weaker, compared to both the homo-DNA and DNA series (Fischer, 1992; Helg, 1994).

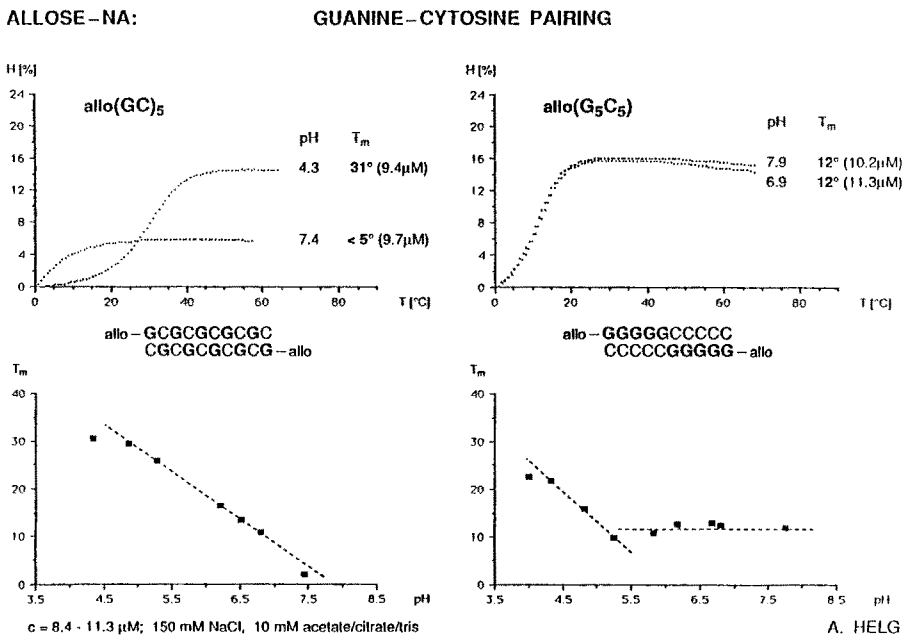


Fig. 19. Guanine-cytosine pairing in the allopyranosyl series is very weak and depends on the base pair sequence as well as pH (Helg, 1994).

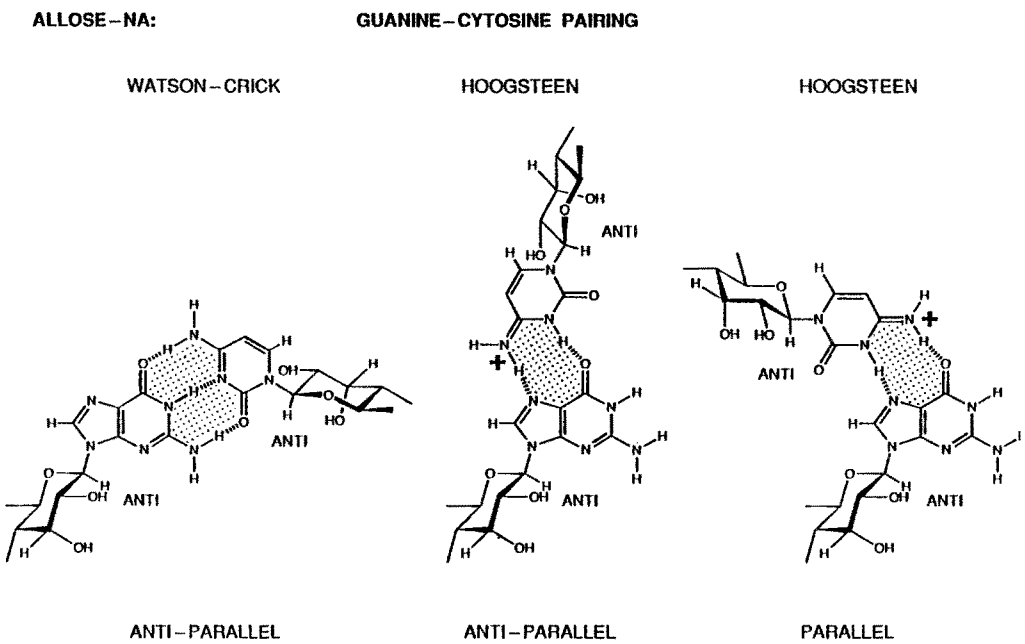


Fig. 20. Most of the guanine-cytosine pairing in the allopyranosyl series very probably is of the reverse-Hoogsteen type (acidified solutions).

		Tm (10 μM Oligomer, 150 mM NaCl, 10 mM buffer)	
		pH 7	pH 4.3
DNA	d - CGCG AATT CGCG GCGC TTAA GCGC - d	58°	44°
HOMO-DNA	dd - ——— - dd	86°	75°
ALLOSE-NA	allo - ———UU——— - allo ———UU———	< 3°	20°

R. FISCHER
C. LEUMANN

Fig. 21. Relative stability of dodecamer duplexes in the DNA, homo-DNA and allopyranosyl series (Fischer, 1992).

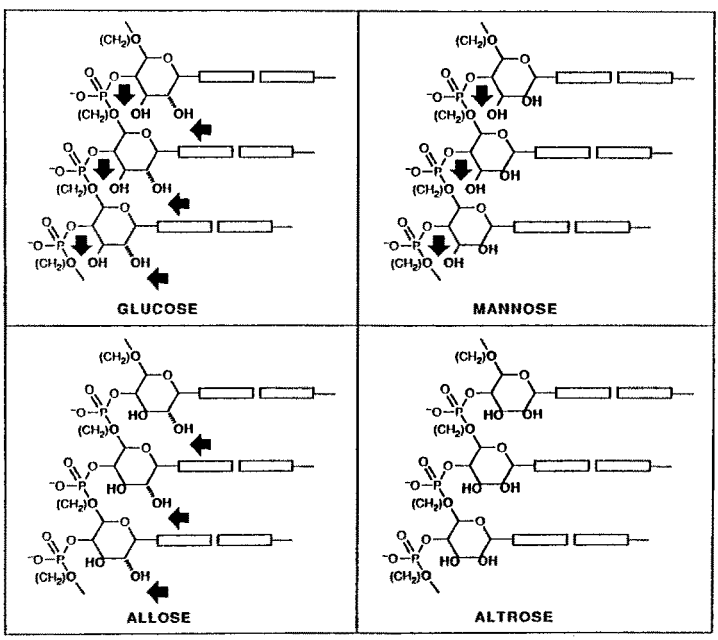


Fig. 22. Intrastrand steric hindrance in the pairing conformation of hexo-pyranosyl-(6' → 4')-oligonucleotide backbones. In an (idealized) allo-pyranosyl duplex model the equatorial hydroxy group at position C-2' runs directly into the neighboring base pair (see arrow).

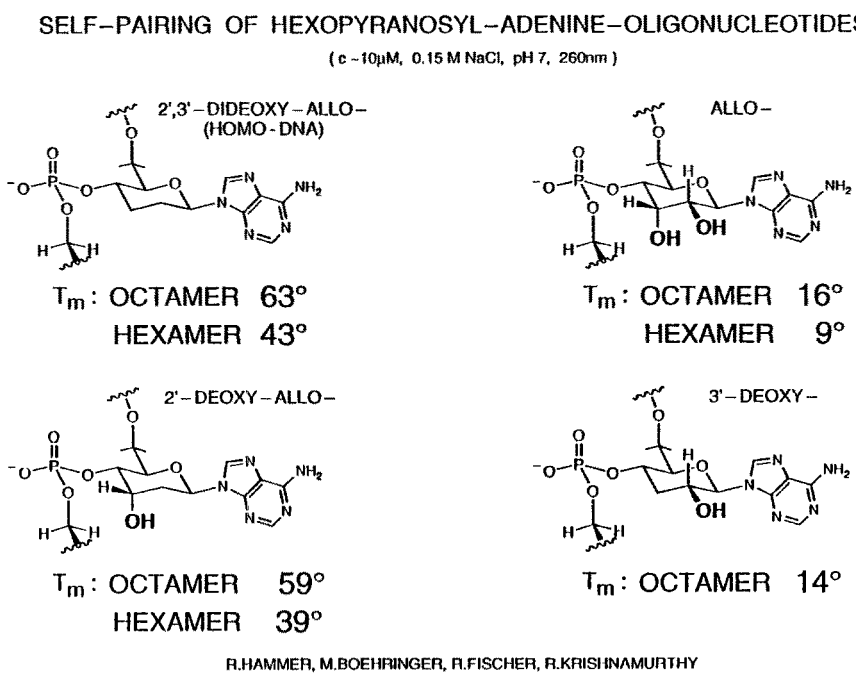


Fig. 23. Experimental evidence supporting the hypothesis that the equatorial 2'-hydroxy group, and not the axial 3'-hydroxy group, is responsible for the destabilization of reverse-Hoogsteen pairing to duplexes in the allopyranosyl series (Hammer, 1992).

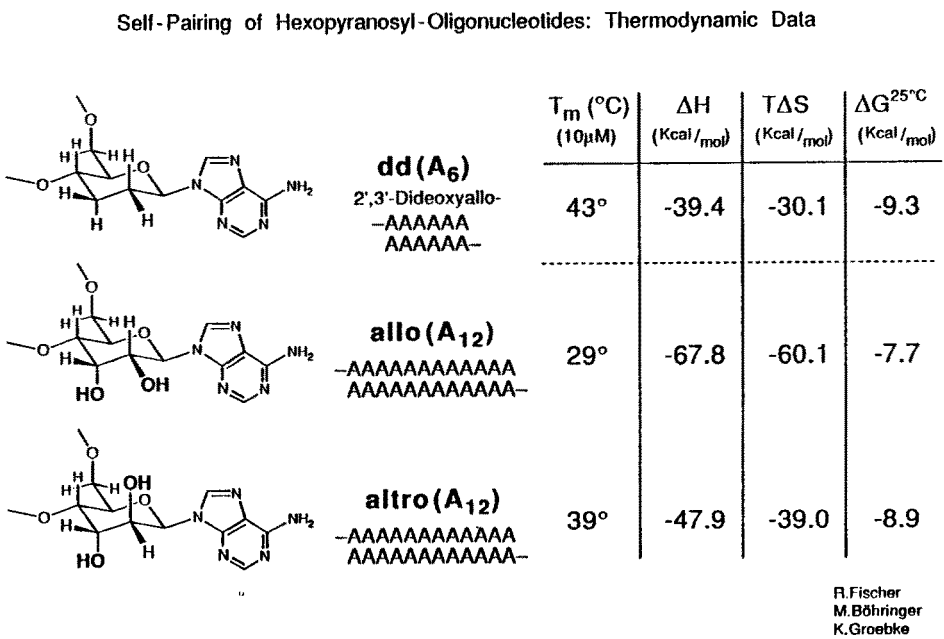


Fig. 24. Adenine-adenine pairing (reverse-Hoogsteen) in the altropyranosyl series is stronger than in the allopyranosyl series, but still drastically weaker than in the homo-DNA series (Groebke, 1993).

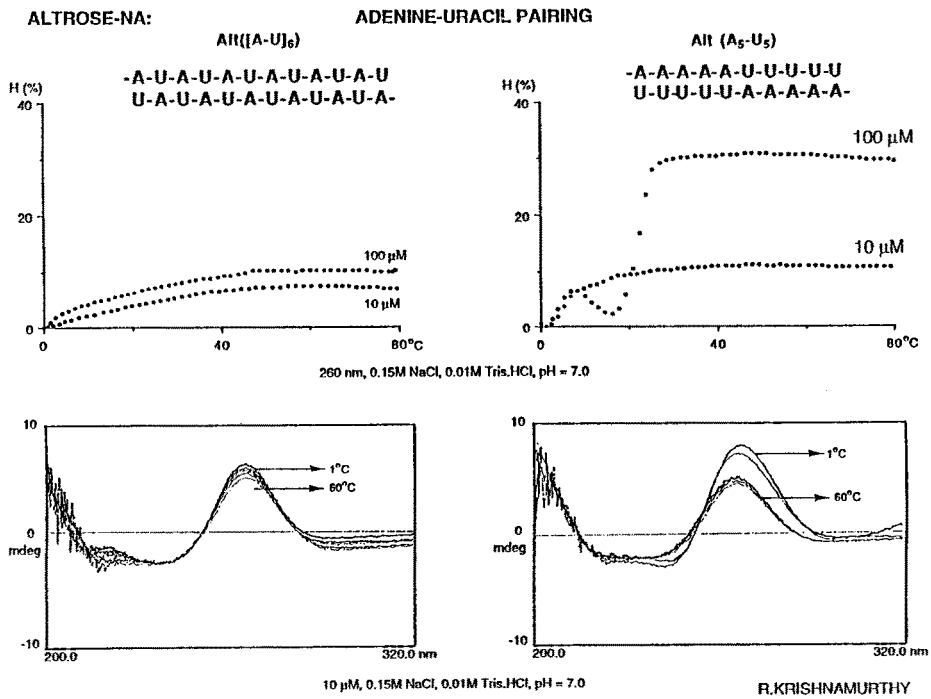


Fig. 25. Adenine-uracil pairing (presumably Watson-Crick) in the altropyranosyl series is very much weaker than in the homo-DNA series (Krishnamurthy, 1993).

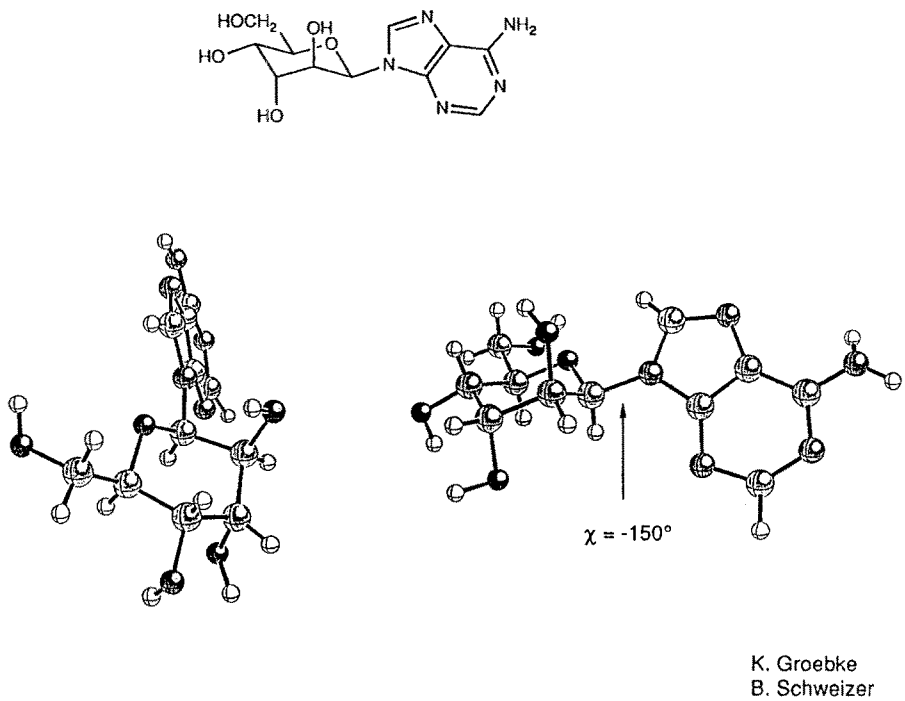


Fig. 26. An x-ray analysis of D,β-(1-altropyranosyl)-adenine demonstrates steric hindrance between the adenine moiety and the axial 2'-hydroxy group (Groebke, 1993).

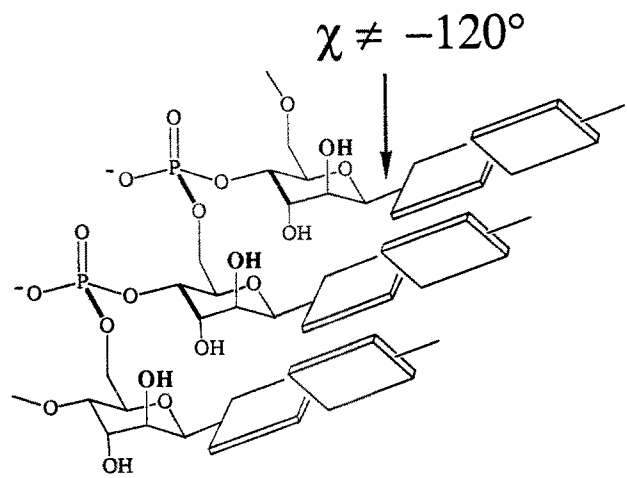


Fig. 27. Propeller twists within the base pairs are the probable reason for the weakness of the pairing in the altropyranosyl series (Groebke, 1993).

Why Pentose and not Hexose Nucleic Acids?

Conclusions (preliminary)
based on experimental observations on Gluco-, Allo- and Altropyranosyl-(6' → 4')-oligonucleotides:

Hexopyranosyl-(6' → 4')-oligonucleotide analogues of RNA derived from $(CH_2O)_6$-hexoses are pairing systems drastically inferior to RNA, if they are pairing system at all. The reason is intrastrand steric hindrance in the pairing conformation.

("too many atoms")

Fig. 28. Preliminary conclusions.

Constitutionally Isomeric Oligonucleotide Backbones
(Phosphodiester junctions between sugar postions)

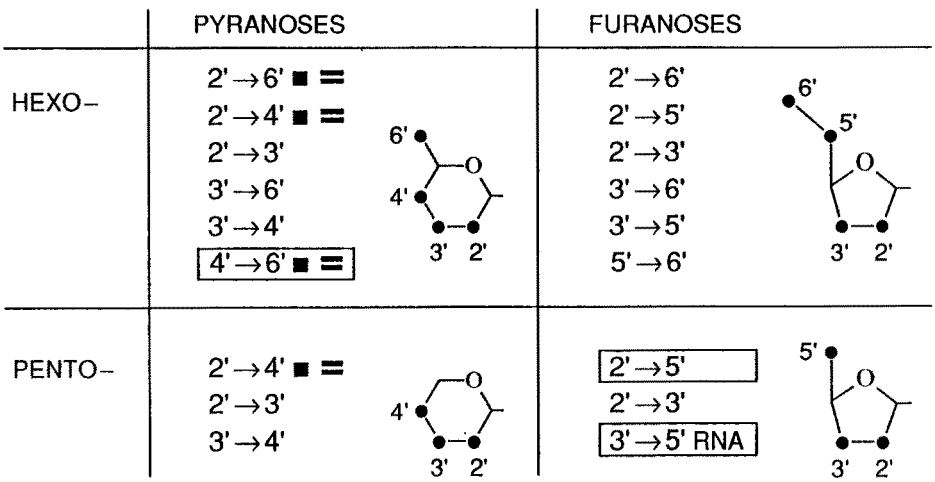


Fig. 29. Survey of the constitutions of (formally) conceivable oligonucleotide systems derived from aldohexoses and aldopentoses.

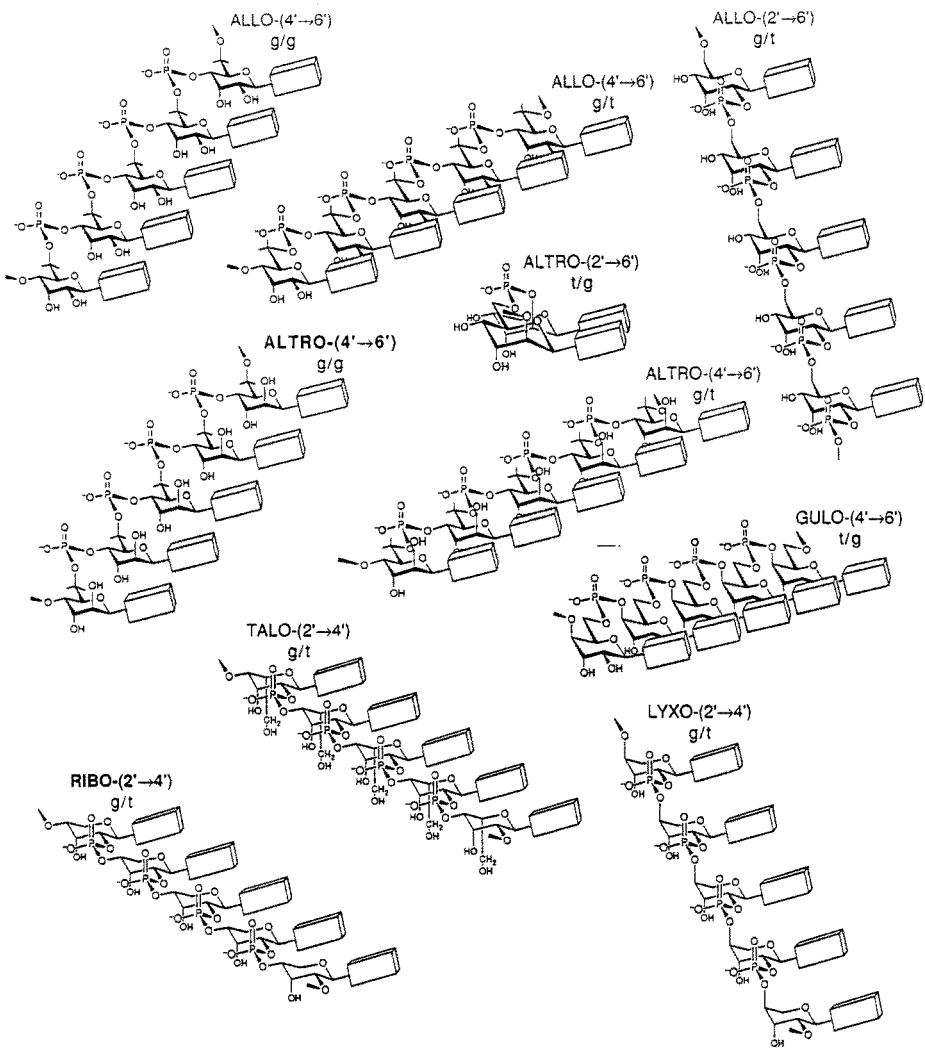


Fig. 30. Qualitative conformational analyses, at the level of idealized conformations, of hexo- and pentopyranosyl oligonucleotide systems predicts a variety of potential pairing systems, some of which, so far, are experimentally untouched.

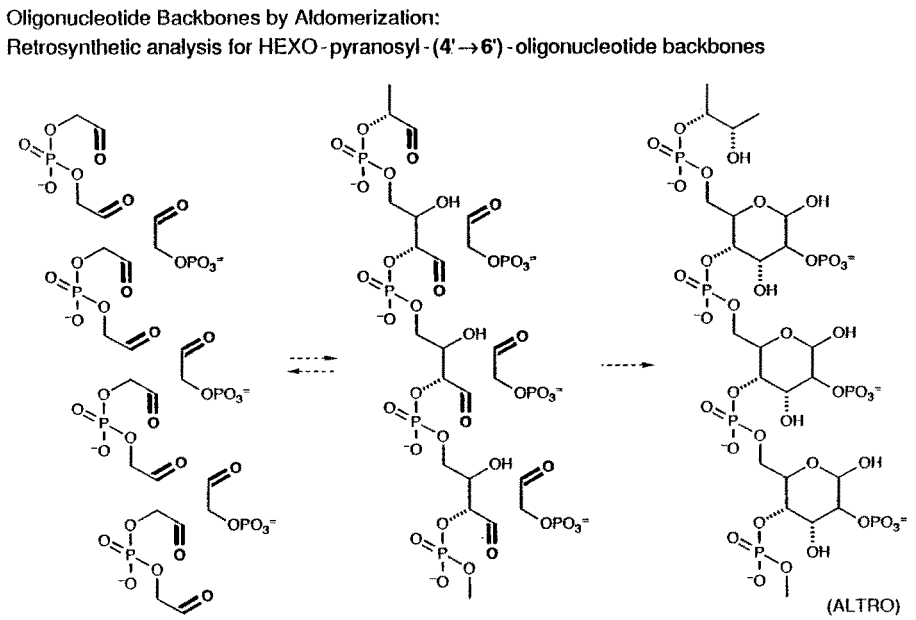


Fig. 31. *Formal* derivation of the constitution of hexopyranosyl-(6' → 4')-oligonucleotides via the aldomerization pathway (for a discussion of the aldomerization pathway see Wagner *et al.*, 1990; Pitsch *et al.*, 1994).

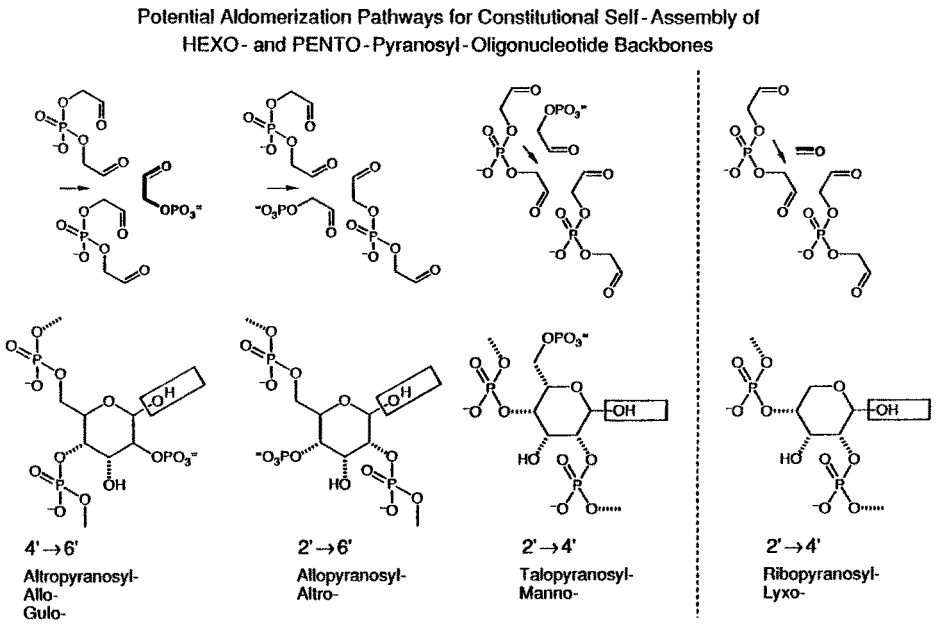


Fig. 32. Not only (6' → 4'), but also (6' → 2')- and (4' → 2')-*hexo*-pyranosyl oligonucleotide systems can be formally derived via the aldomerization pathway. The backbones of all diastereomeric (4' → 2') systems are sterically hindered in their pairing conformation; not so, the corresponding (4' → 2')-*pento*-pyranosyl systems.

Oligonucleotide Backbones by Aldomerization:
Retrosynthetic analysis for PENTO-pyranosyl-(2' → 4')-oligonucleotide backbones

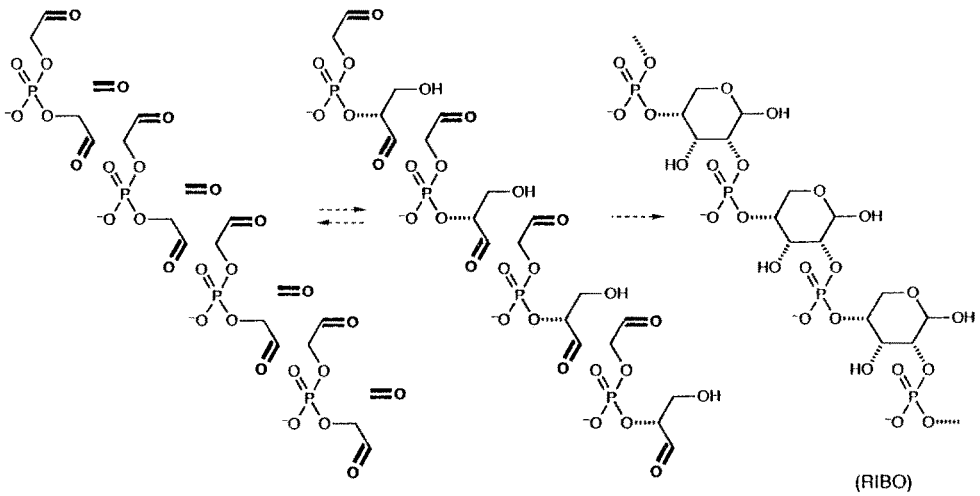


Fig. 33. *Formal* derivation of the constitution of pentopyranosyl-(4' → 2')-oligonucleotides by the aldomerization pathway.

β-RIBO-PYRANOSYL-(4' → 2')-OLIGONUCLEOTIDES
"PYRANOSYL - RNA", an ISOMER of RNA
a target of chemical synthesis and of studies on the constitutional self-assembly of potentially self-replicating systems :

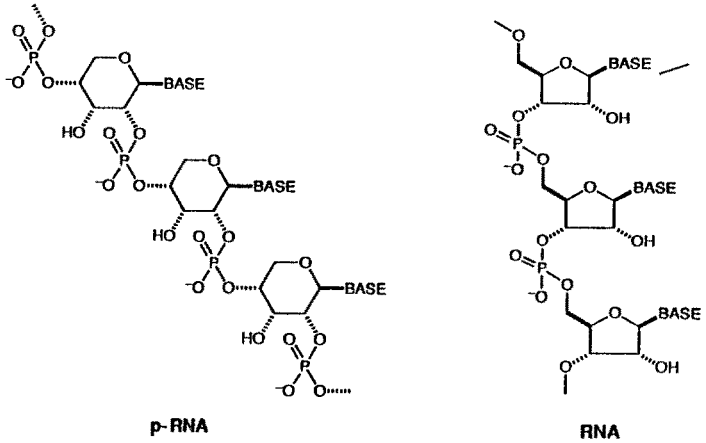


Fig. 34. Constitution of pyranosyl-RNA ('p-RNA').

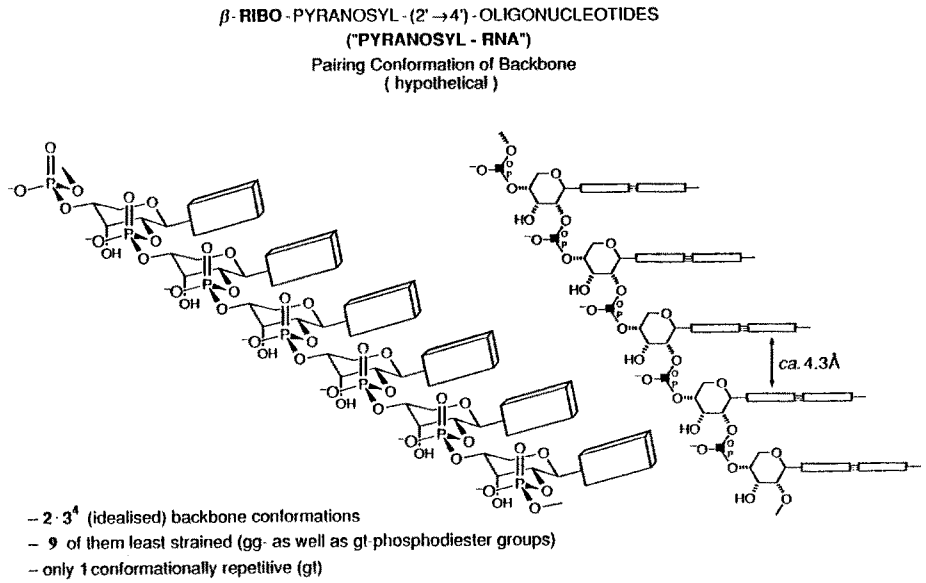


Fig. 35. Qualitative conformational analysis predicts that the backbone of p-RNA has a single pairing conformation. This conformation belongs to the ensemble of least strained backbone conformations. Therefore, it is predicted that p-RNA should form duplexes comparable in stability to homo-DNA duplexes.

p-Ribo (U - U - U - U - U - U - U - U)
(A - A - A - A - A - A - A - A) p-Ribo

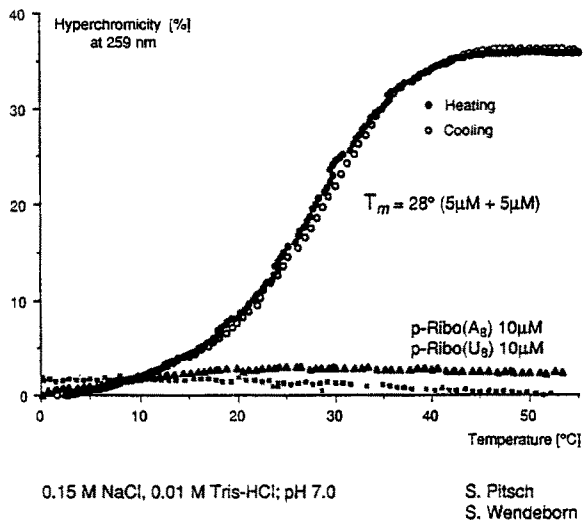


Fig. 36. UV melting curve of a p-RNA duplex. The curves shown by the strands p-Ribo (A_8) and p-Ribo (U_8) before mixing demonstrate that there is no adenine-adenine self-pairing in the p-RNA series, in sharp contrast to homo-DNA. For the synthesis of p-RNA oligonucleotides (see Pitsch *et al.*, 1993).

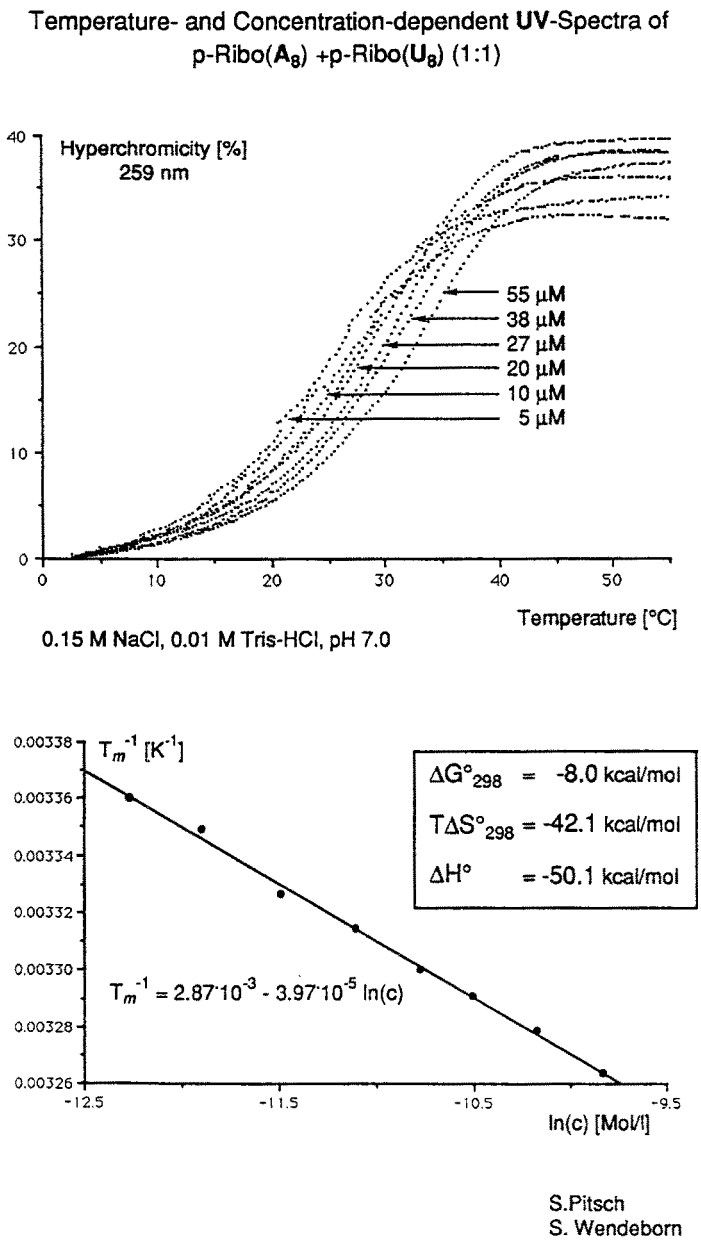


Fig. 37. Determination of the thermodynamic data of the pairing between p-Ribo (A_8) and p-Ribo (U_8).

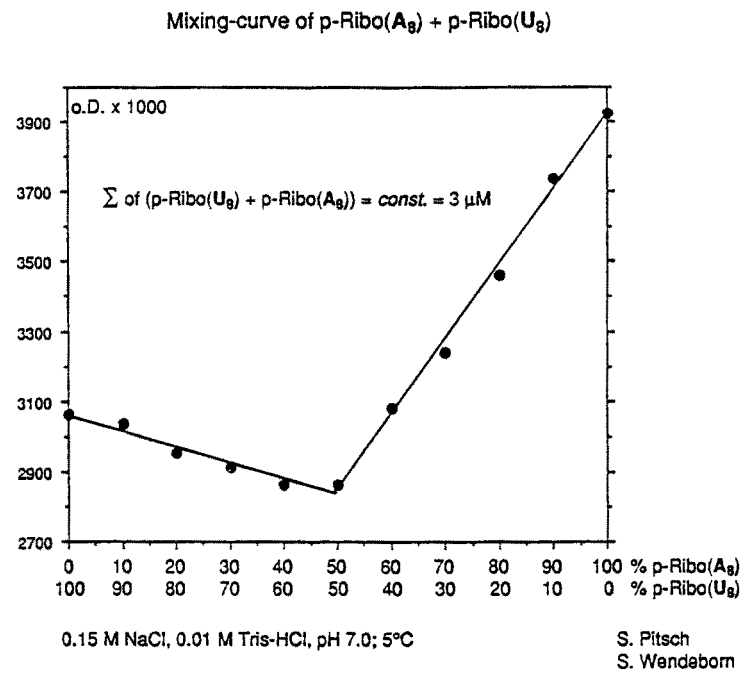


Fig. 38. Determination of the stoichiometry of the pairing between p-Ribo (A_8) and p-Ribo (U_8) (Pitsch *et al.*, 1993).

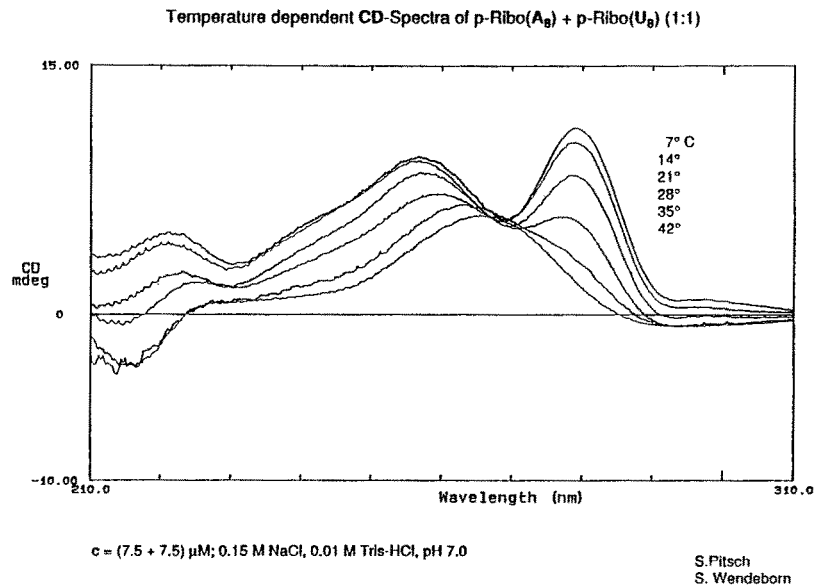


Fig. 39. Temperature-dependent CD spectra of the p-Ribo (A_8)/p-Ribo (U_8) duplex (Pitsch *et al.*, 1993).

p-Ribo (A - A - U - A - U - U - U - A)
(U - U - A - U - A - A - A - U) p-Ribo

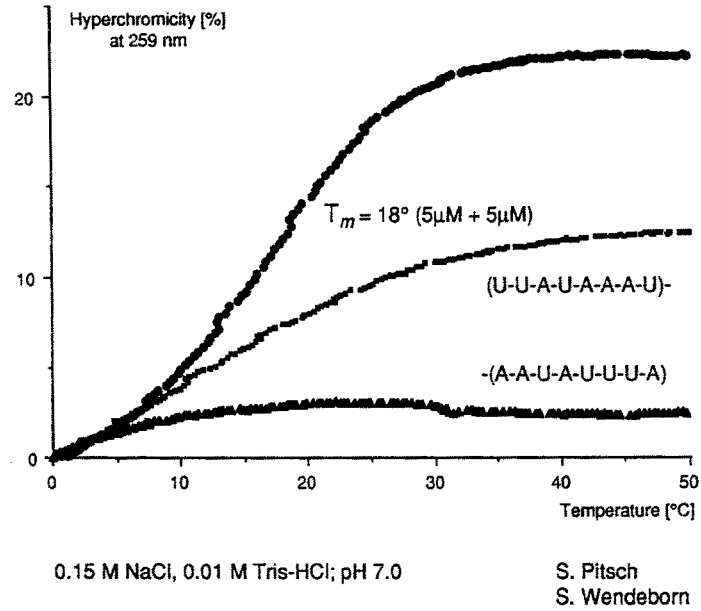


Fig. 40. Testing the strand orientation in p-RNA duplexes.

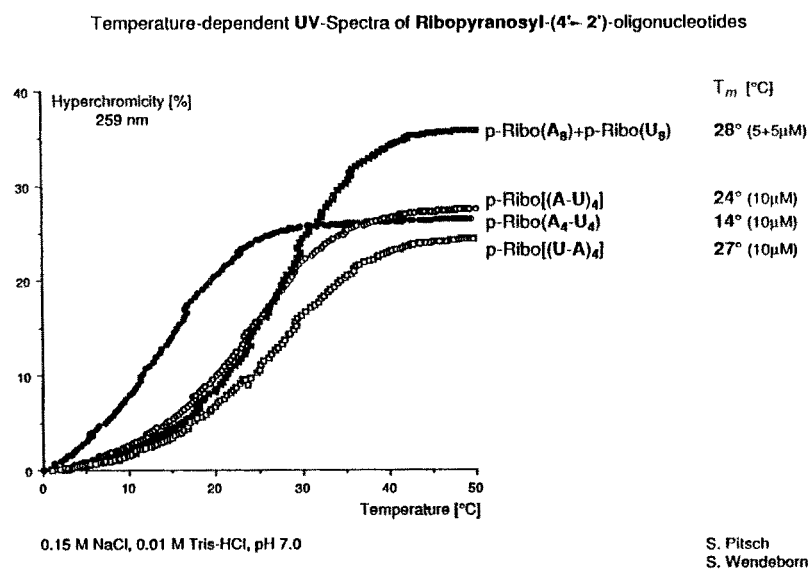


Fig. 41. Comparing the melting temperatures of isomeric (self-complementary) p-RNA duplexes (Pitsch *et al.*, 1993).

Thermodynamic Data of **Ribopyranosyl-(4'→2')-oligonucleotides**

Sequence	Conditions (pH 7.0)	T_m (10 μM) [°C]	T_m (100 μM) [°C]	$\Delta G°_{298}$ [kcal/Mol]	$T\Delta S°_{298}$ [kcal/Mol]	$\Delta H°$ [kcal/Mol]
p-Ribo[(U-A)$_4$]	0.15 M NaCl	27	35	-7.2	-41.2	-48.4
p-Ribo[(A-U)$_4$]	"	24	33	-6.6	-44.5	-51.1
p-Ribo(A$_4$-U$_4$)	"	14	23	-5.2	-37.3	-42.5
p-Ribo(A$_4$-U$_4$)	1.00 M NaCl	21	30	-6.2	-38.1	-44.3
Ribo(A$_4$-U$_4$)[2)]	"	5[1)]	12	-3.3	-47.7	-51.0

[1)] from thermodynamic data by extrapolation

[2)] data taken from: P.N. Borer, B. Dengler, I. Tinocco Jr., *J. Mol. Biol.*, 86, 843 (1974)

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Fig. 42. Comparison of thermodynamic data of p-RNA duplexes. By comparing p-Ribo (A$_4$-U$_4$) with Ribo (A$_4$-U$_4$) it was first shown that p-RNA duplexes are more stable (relative to their single strands) than the corresponding RNA duplexes (Pitsch *et al.*, 1993).

PYRANOSYL-RNA ("p-RNA")

Observations	- Adenine pairs with uracil more strongly than it does in RNA - Adenine does NOT pair with itself (as it does in homo-DNA)
Predictions	- Strong pairing (Watson-Crick) between: G and C (purine-pyrimidine pairing) D and U G and I (purine-purine pairing) D and X - No self-pairing (reverse-Hoogsteen) of G, I, D and X - Strict preference for antiparallel strand orientation - No cross-pairing with RNA - Duplexes have a linear structure
Conjectures	- The potential for constitutional selfassembly of p-RNA is greater than that of RNA - p-RNA can isomerize to RNA intramolecularly within a duplex

Fig. 43. Summary of observations and predictions about the properties of p-RNA oligonucleotides.

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Dr. F. Kreppelt

Post-Retirement Postdoctoral Crew:

Stefan Pitsch
Sebastian Wendeborn
Armin Holzner
Georg Issakides
Rama Krishnamurthy
Guillermo Delgado

Fig. 44. Names of the collaborators who have participated in the project on alternative nucleic acids.

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References

Baranoski. B.: 1989, 'Dissipative Strukturen als ein energetisches Problem', *Nova Acta Leopoldina* NF **60**, Nr. 265, 91.

Böhringer, M., Roth,, H-J., Hunziker, J., Göbel, M., Krishnan, R., Giger, A., Schweizer, B., Schreiber, J., Leumann, C., and Eschenmoser, A.: 1992, 'Why Pentose and Not Hexose Nucleic Acids? Part II. Preparation of Oligonucleotides Containing 2',3'-Dideoxy-β-D-glucopyranosyl Building Bocks', *Helv. Chim. Acta* **75**, 1416.

Cairns-Smith, A. G.: 1982, 'Genetic Takeover and the Mineral Origins of Life', Cambridge University Press, Cambridge.

de Duve, C.: 1991, 'Blueprint for a Cell. The Nature and Origin of Life', Neil Patterson Publishers, Burlington, North Carolina.

Diederichsen, U.: 1993, 'A. Hypoxanthin-Basenpaarungen in Homo-DNA-Oligonucleotiden. B. Zur Frage des Paarungsverhaltens von Glucopyranosyl-Oligonucleotiden', Thesis No. 10122, ETH Zürich.

Dingra, M. M. and Sarma, R. H.: 1978, 'Why do Nucleic Acids Have 3',5'-phosphodiester Bonds?', *Nature* **272**, 798.

Drenkard, S., Ferris, J. P., and Eschenmoser, A.: 1990, 'Chemistry of α-Aminonitriles. Aziridine-2-carbonitrile: Photochemical Formation from 2-Aminopropenenitrile', *Helv. Chim. Acta* **73**, 1373.

Eigen, M.: 1971, 'Selforganization of Organic Matter and the Evolution of Biological Macromolecules', *Naturwissenschaften* **58**, 465.

Eigen, M. and Schuster, P.: 1977, 'The Hypercycle, a Prinicple of Natural Self-Organisation', Part A, *Naturwissenschaften* **64**, 541.

Eigen, M. and Schuster, P.: 1978, 'The Hypercycle, a Principle of Natural Self-Organisation', Part B, *Naturwissenschaften* **65**, 7; Part C, *Naturwissenschaften* **65**, 341.

Eschenmoser, A.: 1988, 'Vitamin B12: Experiments Concerning the Origin of Its Molecular Structure', *Angew. Chem. Int. Ed. Engl.* **27**, 5.

Eschenmoser, A.: 1991, 'Warum Pentose- und nicht Hexose-Nucleinsäuren?', *Nachr. Chem. Tech. Lab.* **39**, 795.

Eschenmoser, A.: 1993, 'Towards a Chemical Etiology of the Natural Nucleic Acids' Structure', in Proceedings of the R. A. Welch Foundation Conference on Chemical Research XXXVII: '40 Years of the DNA Double Helix', A. Welch Foundation, Houston, p. 201.

Eschenmoser, A. and Dobler, M.: 1992, 'Why Pentose- and Not Hexose Nucleic Acids? Part I. Introduction to the Problem, Conformational Analysis of Oligonucleotide Single Strands Containing 2',3'-Dideoxyglucopyranosyl Building Blocks ('Homo-DNA'), and Reflections on the Conformation of A- and B- DNA', *Helv. Chim. Acta* **75**, 218.

Eschenmoser, A. and Loewenthal, E.: 1992, 'Chemistry of Potentially Prebiological Natural Products', *Chem. Soc. Rev.* **21**, 1.

Ferris, J. P., Hagan, W. J., 1984, 'HCN and Chemical Evolution: The Possible Role of Cyano Compounds in Prebiotic Synthesis' Tetrahedron **40**, 1093.

Fischer, R. W.: 1992, 'Allopyranosyl-Nukleinsäure: Synthese, Paarungseigenschaften und Struktur von Adenin-/Uracil-haltigen Oligonukleotiden', Thesis No. 9971, ETH Zürich.

Giger, A.: 1992, 'Untersuchungen über Oligonucleotide mit 2-Deoxy-D-ribose, 2,3-dideoxy-D-glucopyranose und D-allopyranose als Zuckerbausteine', Thesis No. 9975, ETH Zürich.

Glansdorff, P. and Prigogine, I.: 1971, 'Thermodynamic Theory of Structure, Stability and Fluctuations', Wiley-Interscience Publ.

Gray, H. B. and Haight, G. P.: 1967, 'Basic Principles of Chemistry', W. A. Benjamin Inc., New York, p. 365.

Groebke, K.: 1993, 'Über Purin-Purin-Paarungen bei Hexopyranose-Nukleinsäuren', Thesis No. 10149, ETH Zürich.

Haken, H.: 1981, 'Erfolgsgeheimnisse in der Natur. Synergetik: Die Lehre vom Zusammenwirken', Deutsche Verlags-Anstalt GMbH., Stuttgart.

Hammer, R.: 1992, unpublished work, ETH.

Helg, A. G.: 1994, 'Allopyranosyl-Nukleinsäure: Synthese, Paarungseigenschaften und Struktur von Guanin-/Cytosin-enthaltenden Oligonukleotiden', Thesis No. 10464, ETH Zürich.

Hunziker, J., Roth, H.-J., Böhringer, M., Giger, A., Diederichsen, U., Göbel, M., Krishnan, R., Jaun, B., Leumann, C., and Eschenmoser, A.: 1993, 'Why Pentose- and Not Hexose-Nucleic Acids? Part III. Oligo(2',3'-dideoxy-β-D-glucopyranosyl)nucleotides. ('Homo-DNA'): Base-Pairing Properties', *Helv. Chim. Acta* **76**, 259.

Jacob, F.: 1982, 'The Possible and the Actual', Pantheon Books, New York.

Jin, R., Chapman, W. H., Srinivasan, A. R., Olson, W. K., Breslow, R., and Breslauer, K. J.: 1993, 'Comparative spectroscopic calorimetric and computational studies of nucleic acid complexes with 2',5''- versus 2',5'' -phosphodiester linkages', *Proc. Natl. Acad. Sci. USA* **90**, 10568.

Joyce, G. F. and Orgel, L. E.: 1993, 'Origin of the RNA World', in: Gesteland, R. F. and Atkins, J. F. (eds.), 'The RNA World', Cold Spring Harbour Laboratory Press, p. 1.

Joyce, G. F., Schwartz, A. W., Miller, S. L., and Orgel, L. E.: 1987, 'A Case for an Ancestral Genetic System Involving Simple Analogues of the Nucleosides', *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4398.

Kauffman, S. A.: 1986, 'Autocatalytic Sets of Proteins', *J. Theor. Biol.* **119**, 1.

Kauffman, S. A.: 1993, 'The Origins of Order. Self-Organization and Selection in Evolution', Oxford University Press.

Krishnamurthy, R.: 1993, unpublished work, ETH.

Ksander, G., Bold, G., Lattmann, R., Lehmann, C., Früh, T., Xiang, Y.-B., Inomata, K., Buser H.-P., Schreiber, J., Zass, E., and Eschenmoser, A.: 1987, 'Chemistry of α-Aminonitriles I. Introduction and Pathways to Uroporphyrinogen-octanitriles', *Helv. Chim. Acta* **70**, 1115.

Kuhn, H.: 1972, 'Selforganization of Molecular Systems and Evolution of the Genetic Apparatus', *Angew. Chem. Int. Ed. Engl.* **11**, 798.

Mayr, E.: 1974, 'Teleological and Teleonomic, a New Analysis', in Cohen, R. S. and Wartofsky, M. W. (eds.), *Boston Studies in the Philosophy of Science*, Vol. XIV, 'Methodological and Historical Essays in the Natural and Social Sciences', D. Reidel Publ. Co., Dordrecht, Boston, p. 91.

Miller, S. L. and Orgel, L. E.: 1974, 'The Origins of Life on Earth', Prentice-Hall Inc., Englewood Cliffs, N. J.

Müller, D., Pitsch, S., Kittaka, A., Wagner, E., Wintner, C. E., and Eschenmoser, A.: 1990, 'Chemistry of α-Amininitriles. Aldomerisation of Glycolaldehyde Phosphate to rac-Hexose 2,4,6-Triphosphates and (in Presence of Formaldehyde) rac-Pentose 2,4-Diphosphates: rac-Allose 2,4,6-Triphosphate and rac-Ribose 2,4-Diphosphate Are the Main Reaction Products', *Helv. Chim. Acta* **73**, 1410.

Nicolis, G. and Prigogine, I: 1977, 'Self-Organization in Non-Equilibrium Systems: From Dissipative Structures to Order through Fluctuations', Wiley-Interscience Publ.

Orgel, L. E.: 1986, 'RNA Catalysis and the Origin of Life', *J. Theor. Biol.* **123**, 127.

Oro, J., 1960, 'Synthesis of Adenine from Ammonium Cyanide' Biochem. Biophys. Res. Comm **2**, 407.

Otting, G., Billeter, M., Wüthrich, K., Roth, H.-J., Leumann, C., and Eschenmoser, A.: 1993, 'Why Pentose- and Not Hexose-Nucleic Acids? Part IV. 'Homo-DNA': 1H-, 13C-, 31P,- and 15N-NMR-Spectroscopie Investigation of ddGlc(A-A-A-A-A-T-T-T-T-T) in Aqueous Solution', *Helv. Chim. Acta* **76**, 2701.

Peng, L.: 1993, '1. Synthese und Eigenschaften von Oligo-2'-deoxy-1',2'-seco-D-ribonukleotiden. 2. Purin-Purin-gepaarte Oligonukleotide der homo-DNA-Reihe', Thesis No. 10035, ETH Zürich.

Pitsch, S., Wendeborn, S., Jaun, B., and Eschenmoser, A.: 1993, 'Why Pentose- and Not Hexose-Nucleic Acids? Part VII. Pyranosyl-RNA ('p-RNA')', *Helv. Chim. Acta* **76**, 2161.

Pitsch, S., Pombo-Villar, E., and Eschenmoser, A.: 1994, 'Chemistry of α-Aminonitriles, Part 13. On the Formation of Glycolaldehyde Phosphate Esters from Oxirane Carbonitrile – and on a (so far formal) Constitutional Relationship Between bis-Glycolaldehyde Phosphate and Pyranosyl-Oligonucleotide Backbones', *Helv. Chim. Acta* (in press).

Pittendrigh, C. S.: 1958, 'Behaviour and Evolution', in Roe, A. and Simpson, G. G. (eds.), Yale University Press, New Haven.

Prigogine, I.: 1945, 'Modération et transformations irréversibles des systèmes ouverts', *Bull. Cl. Sci. Acad. R. Belg.* **31**, 600.

Prigogine, I.: 1978, 'Time, Structure, and Fluctuations', *Science* **201**, 777.

Prigogine, I. and Nicolis, G.: 1967, 'On Symmetry-Breaking Instabilities in Dissipative Systems', *J. Chem. Phys.* **46**, 3542.

Usher, D. A.: 1972, 'RNA Double Helix and the Evolution of the 3'5'-Linkage', *Nature* **235**, 207.

Usher, D. A.: 1977, 'Early Chemical Evolution of Nucleic Acids: A Theoretical Model', *Science* **196**, 311.

Wächtershäuser, G.: 1990, 'Evolution of the First Metabolic Cycles', *Proc. Natl. Acad. Sci. USA* **87**, 200.

Wagner, E., Xiang, Yi-Bin, Baumann, K., Gück, J., and Eschenmoser, A.: 1990, 'Chemistry of α-Aminonitriles. Aziridine-2-carbonitrile, a Source of Racemic-O^3-Phosphoserinenitrile and Glycolaldehyde-Phosphate', *Helv. Chim. Acta* **73**, 1391.

Westheimer, F. H.: 1987, 'Why Nature Chose Phosphates', *Nature* **235**, 1173.

Xiang, Yi-Bin, Drenkard, S., Baumann, K., Hickey, D., Felix, D., and Eschenmoser, A.: 1994, 'Chemistry of α-Aminonitriles. Part 12. Exploratory Experiments on Thermal Reactions of α-Aminonitriles', *Helv. Chim. Acta* (in press).