Part One

Nucleic Acids
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Searching for Nucleic Acid Alternatives

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“Back of the envelope” methods have their place in experimental chemical research; they are effective mediators in the generation of research ideas; for instance, for the design of molecular structures. Their qualitative character is part of their strength, rather than a drawback for the role they have to play. Qualitative conformational analysis of oligonucleotide and other oligomer systems on the level of idealized conformations is one such method; it has played a helpful role in our work on the chemical etiology of nucleic acid structure. This article, while giving a short overview of that work, shows how.

Chemists understand by comparing, not ab initio. To perceive and to create opportunities for drawing conclusions on the basis of comparisons is the organic chemist’s way of interpreting and exploring the world at the molecular level. Comparing the properties of molecules of unknown constitution with those of already known constitution was the basic strategy in classical chemistry of structure determination by chemical methods, and the same still largely holds for more recent
structure determinations in chemical laboratories by physical methods; it is only with regard to X-ray structure analysis that the statement ceases to hold in so far as chemistry is concerned. Model studies, an approach characteristically chosen by chemists when confronted with structural or transformational complexity, serve the purpose of creating opportunities to compare the behaviors of complex systems with those of simpler ones. Enzymic reactions and enzyme models are examples.

To reach an understanding of structures and structural transformations through comparing is not what chemists alone are aiming at; it is also true for biologists, dealing both with their own spatial and temporal resolution and with the conceptual resolution of their objects. Yet, biology is also a historic science: ever since the time of Charles Darwin, biologists – besides and beyond studying structure and function – have pondered the origin of their objects. Chemists do not have to; pursuing the question of origin with regard to the constituents of the periodic table is the job of cosmologists. However, with the progressive breakdown of borders between biology and (a very large part of) contemporary chemistry, the search for origins is bound to leap over from biology to chemistry; the quest for comprehending the evolution of living organisms will have its extension in the quest for an understanding of the origin of biomolecular structures. I do not mean the understanding of the origin of biomolecules in terms of their biosynthesis, but rather the origin of these biosyntheses themselves and, in the long term, of biogenesis, at the molecular level.

To systematically compare selected chemical properties of structural nucleic acid analogs with corresponding properties of the natural system is part of a project pursued in my research group(s) since 1986; the aim is to explore the potential of organic chemistry to arrive at an understanding of how and why Nature came to choose the specific structure type of the nucleic acids we know today as the molecular basis of genetic function. The specific property to be compared in this work is a given nucleic acid alternative’s capacity for informational Watson–Crick nucleobase-pairing, the overall criterion for the selection for study of a given system being a structure’s potential for constitutional self-assembly compared with that envisaged for the structure of the natural system itself [1].

There is another motive for pursuing in organic and medicinal chemistry laboratories the search for nucleic acid analogs: the worldwide hunt (also since the late 1980s) for nucleic acid substitutes that might be useful in medicinal antisense technology [2]. This kind of search is not subjected to the above-mentioned selection criterion that rather
strictly narrows the choice of oligomer systems for study by demanding them to be potentially natural systems. Nevertheless, the antisense-technology-driven search for nucleic acid substitutes is not just very successful (chemically) in pursuing its own goals, some of its results importantly also complement etiology-oriented research on nucleic acid alternatives by corroborating and extending one of the major conclusions to be drawn from the latter, namely that, in contrast to what was believed before, the capability of informational Watson–Crick base-pairing is by no means limited to the structure type of the Watson–Crick double helix. Quite the contrary, it is widespread among oligomers containing backbones that may be quite different from one of the natural nucleic acids. Nielsen’s peptide nucleic acid (PNA) exemplifies perhaps most instructively the degree of structural backbone variation Watson–Crick base-pairing is found to be compatible with. This wide spread of biology’s genetic type of recognition process notwithstanding, an oligomer system’s capability to show informational base-pairing demands rather stringent prerequisites to be fulfilled regarding the backbone structure and the three-dimensional relationship between backbone units and recognition elements (nucleobases). There is not just the question “base-pairing, yes or no?”; informational oligomer systems may “speak different base-pairing languages,” in the sense that they can be capable of undergoing intrasystem base-pairing with themselves, while being unable to communicate with each other by intersystem cross-pairing. To deduce from empirical and theoretical sources the structural constraints that control an oligomer system’s capability of base-pairing in a given language would be important for eventually being able, on a qualitative level, to estimate in advance, if not to predict, whether or not a given type of oligomer structure could act as an informational oligomer. This challenge is clearly addressed to researchers in both the etiology-oriented and antisense-technology-driven branches of nucleic acid chemistry mentioned above.

From its very beginning, our own work was assisted by a set of such empirical qualitative criteria, a set that evolved with time and experience. In retrospect, it seems quite instructive to look at the balance between success and failure experienced so far in applying these criteria for “predicting” the base-pairing capability for new oligomer systems. I review them in this article by pointing to their origin, summarizing their essentials, commenting on their strengths and weaknesses as well as on their potential to be extended and applied for oligomer systems structurally different from those for which these rules were deduced and developed. I am, of course, not unaware of the fact that in contemporary
chemistry the task of predicting for practical purposes the structure (and sometimes implicitly the properties) of molecules is to be pursued by theory-based and computer-assisted tools; yet this does not mean that efforts to reach such estimates by empirical arguments on a qualitative level have become obsolete. After all, the experimental chemist’s pragmatic reasoning in terms of his classical formulae language coexists quite successfully with the kind of reasoning feasible on the level of quantum chemistry.

Our entry into the research field of nucleic acid chemistry was motivated by our interest in the broader context of an organic chemistry of biogenesis [3], and consisted in an experimental study on the formation and properties of oligo-dipeptamidinium salts (1982–1986, with Heinz Moser and Arthur Steiger [4]). At the outset of this project stood the recognition of the fact that the constitutional periodicity of a negatively charged oligonucleotide chain happens to be identical (six covalent bonds) with that of the chain of a positively charged oligo-dipeptamidinium salt; that is, of a polypeptide chain in which each second backbone amide group is replaced by a cationic amidinium group (Figure 1.1). The driving force behind the project was the question as to whether such an (etiologically not necessarily accidental) identity of constitutional periodicity in the two major types of biopolymer could give rise to a mutual catalysis in the formation of oligonucleotide and oligo-dipeptamidinium strands by oligomerization of their respective mono-

![Figure 1.1 Six-bond periodicity in oligonucleotides and oligo-dipeptamidinium salts (facsimile from Ref. [4])](image-url)
mers, given that oligo-dipeptamidinium salts can be considered to be oligomerization products of (the ammonium form of) dipeptide nitriles and could transform to corresponding oligopeptides under mild hydrolytic conditions. Experiments turned out to negate neither the feasibility of the oligomerization step (preferably catalyzed by thiols such as cysteine [5]), nor the hydrolytic conversion to oligopeptides, yet uncovered strand fission to be a competing process in that hydrolysis and, moreover, the overall stability of oligo-dipeptamidium salts toward such cleavage near neutral pH to be quite low [6].

One special aspect of that early project, namely the remarkable constitutional relationship between an oligo-nucleotide and an oligo-dipeptide chain, was to induce our next move. Only little was needed to realize that the complementarity of charges may be extended to the complementarity of other recognition elements; most appealingly, of course, nucleobases. Such a step called for nucleic acid alternatives composed of homochiral oligo-dipeptamidinium and also oligo-dipeptide chains in which each second $\alpha$-amino acid would be tagged by a nucleobase (Figure 1.2a and b). We studied (with Peter Lohse [6]) the formation and the properties of such nucleobase-tagged oligo-dipeptamidinium salts with partial success, yet eventually succumbed to their relative instability. The literature existing at the time (1986) already contained reports on pioneering experimental efforts to prepare oligopeptides composed of nucleo-base-tagged $\alpha$-amino acids; however, these reports were devoid of considerations according to which tagged oligomer units should be a dipeptide unit [7]. Encouraged by an ETH-built (only slightly “suffering”) mechanical model of a double-helical hybrid duplex between an oligonucleotide chain and a nucleobase-tagged oligo-dipeptide chain, Gerhard Baschang of (former) CIBA-Geigy’s newly formed “antisense group” synthesized in 1986 an octamer of an L-alanyl-glycyl-type oligomer in which each L-alanine was replaced by an L-serine-derived L-(1-thyminyl)-alanine (Figure 1.2b). Disappointingly, no base-pairing with poly-d-adenosine was observed of that (presumably earliest) sample of a constitutionally and stereochemically defined nucleobase-tagged oligo-dipeptide.\(^1\) In the light of this experience, Peter Nielsen’s pragmatically and ingeniously designed and, in turn, dramatically successful “PNA” a few years later amounted to an

\(^1\) Unpublished; however, see Ref. [6]. In retrospect, we realize that the sequence length of an 8-mer (with thymin as nucleobase) is far too short to draw conclusions about an oligomer system’s capability of base-pairing. Had we in the TNA series (see later) investigated the 8-mer only, we would have failed to observe the system’s pairing capacity.
overwhelming discovery [8]. Reassuringly, the periodicity of the nucleobase-tagging of PNA’s backbone nicely corresponded to that of an oligo-dipeptide-type of oligomer structure (Figure 1.2c).

Gradually overlapping with our early steps toward oligo-dipeptide-based informational systems in the 1980s was our search for nucleic acid alternatives that contain phosphodiester backbones derived from hexopyranoses in place of ribofuranose units (“Why pentose and not
hexose nucleic acids?” [9]; Figure 1.3). While our results of an extensive study of the (diastereoselective) aldolization of glycolaldehyde phosphate [10] had initiated these studies, it was mainly the following aspect that eventually led us to rigorously concentrate on this project: an assignment of etiological relevance to a given nucleic acid alternative will be more reliable the closer the structural and generational relationships between the alternative and the natural system are. The structure of an alternative should be derivable from an alternative aldose by the same type of chemistry that allows us to derive the structure of RNA from ribose. What, in addition, made such a study appealing from a purely chemical point of view was the following: oligonucleotide strands composed of hexopyranose sugar units would be amenable to qualitative conformational rationalization, in sharp contrast to the conformationally far more complex pentofuranose-based natural systems, the base-pairing capability of which had, at the time, never been rationalized at the level of the organic chemist’s qualitative way of conformational reasoning.

Figure 1.4 reproduces the results of the kind of conformational analysis that allowed us to “predict” that pyranose analogs of DNA (“homo-DNA”) might have the capability of informational base-pairing. The analysis was based on the following framework of assumptions and stipulations [9, 11]:

1. search for the ensemble of (formally) least strained conformers of an oligomer chain’s constitutionally repeating monomer unit by restricting the rotational continuum around each covalent bond of the backbone to three ideally staggered (i.e. “idealized”) conformations (bidentate oxygen centers containing two electron lone-pairs taken as tetrahedral centers);
2. least strained conformers of a monomer unit are those that have either no or the smallest possible number of, “1,5-repulsions” (i.e. repulsions resulting whenever in a five-center-chain \(a\–b\–c\–d\–e\) the two bonds \(a\–b\) and \(d\–e\) are positioned parallel to each other and, at the same time, neither \(a\) nor \(e\) is a hydrogen atom);
3. phosphodiester group conformations are allowed to be gauche-gauche or gauche-trans, but not trans-trans;2

2For a discussion of X-ray structures of phosphodiester groups, see Figure 9 in Ref. [10]. Whereas the gauche-gauche conformation appears intrinsically favored, the trans-trans conformation apparently has not been not observed. Stereoelectronic reasoning (generalized anomeric effect) led us originally to believe that the gauche-trans conformation should be disallowed; however, the structure of pyranosyl-RNA, for example, taught us to think differently (see below).
Figure 1.3 Three (potentially natural) hexopyranosyl alternatives of RNA studied in our laboratory [19–21]. The model system homo-DNA lacks the hydroxyl groups in positions 2' and 3'.
Figure 1.4  Result of the qualitative conformational analysis of the homo-DNA backbone using idealized conformations [9, 11]. The figure depicts the ensemble of least-strained monomer-unit conformers of which the two pairing conformations are framed (-sc/-sc = -g/-g and ap/-sc = -g/t; sc = synclinal, g = gauche, ap = antiplanar, t = trans)
4. an oligomer system can be expected to be a base-pairing system if the ensemble of (formally) least strained monomer-unit conformers contains at least one conformer that is in a “pairing conformation”;
5. a “pairing conformation” of a monomer unit conformer is a conformation that repeats itself in both neighboring monomer units.

It is to be noted that, in such an analysis, based on idealized conformations, the criteria “conformational repetitivity of a monomer unit” and “pairing conformation” are assessable in such a straightforward way only if the constitutional periodicity of the oligomer backbone corresponds to an even number of bonds, as is the case, for example, in homo-DNA.

The analysis of the homo-DNA system (Figure 1.4) reveals an ensemble of four least-strained conformers, one of them conformationally repetitive, with the gauche–gauche (-gl/-g) conformation of the phosphodiester group [12]. When the latter is allowed to assume the trans–gauche conformation, there are six additional least-strained conformers; again, with one of them (tl/-g) conformationally repetitive. Therefore, a homo-DNA strand in a homo-DNA duplex can assume two types of conformation: the gauche–gauche phosphodiester type with (idealized) torsion angles $\alpha = -60^\circ$, $\beta = 180^\circ$, $\gamma = +60^\circ$, $\delta = +60^\circ$, $\epsilon = 180^\circ$ and $\zeta = -60^\circ$; or the trans–gauche type with $\alpha = 180^\circ$, $\beta = 180^\circ$, $\gamma = 180^\circ$, $\delta = +60^\circ$, $\epsilon = 180^\circ$ and $\zeta = -60^\circ$ (Figure 1.4). These are exactly the two structure types that an NMR-structure analysis of the homo-d(A5T5)2 duplex shows to coexist in aqueous solution [13]. Duplex models, constructed out of homo-DNA monomer units in either the -gl/-g or the tl/-g type of pairing conformation with all torsion angles taken to be idealized and (for the sake of argument) with all bond lengths identical, would have a linear shape. In reality, the intrinsic nonideality of all structural parameters, assisted by the necessity of a duplex to reach optimal nucleobase stacking distances by either helicalization or by adapting axes inclination, is bound to result in a helical twist of the duplex structure, the degree and sense of which, however, the NMR-structure analysis was unable to determine.3

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3A whole series of attempts in various laboratories to translate the X-ray refraction data of the beautifully crystalline and high-melting homo-DNA duplex of the self-complementary octamer sequence dd(CGAATTGC) into a consistent X-ray structure turned out to be unexpectedly difficult. According to a personal communication of Martin Egli (Vanderbilt University), it is only recently that the structure could be solved by making use of the new methodology of Se-labeling of oligonucleotide strands (unpublished; for the method, see Ref. [14]).
Perhaps the most interesting aspect of the outcome of this kind of qualitative conformational analysis of the homo-DNA structure is the following: out of the two possible (idealized) structures of a homo-DNA duplex, the (-g/-g)-structure, the one in which the (formal) nucleobase stacking distance is nearer to the optimal value, turns out to be identical in type with the structure observed by X-ray analyses as the A-type structure of DNA-duplexes [9, 13] and the structure of duplex RNA (Figure 1.5). This indicates that **content and outcome of the conformational analysis of the homo-DNA structure implies a rationalization of**

![Figure 1.5](image-url)  
**Figure 1.5** Pictorial comparison of idealized (-g/-g)-pairing conformations of homo-DNA with experimental data of A- and B-type DNA duplexes [1]
the A-type structure of DNA and, at the same time, of RNA, at the level of qualitative organic stereochemistry (Figure 1.6). Furthermore, the finding that the A-type structure type of duplex DNA corresponds to a homo-DNA structure that is deduced by conformational analysis of a single strand must mean (assuming the latter finding would still hold for a conformational analysis of a DNA single strand) that DNA (and RNA) single strands are preorganized toward duplexation. Finally, the reasoning about homo-DNA and DNA in terms of idealized conformations provides a transparent rationalization of a well-known and biologically important fact, namely that duplex DNA has the option of assuming either the A-type or its characteristic B-type structure, whereas the A-type structure is mandatory for duplex RNA (1,5-repulsion between 2′-hydroxyl and one of the phosphodiester oxygen centers in B-type RNA; see Figure 1.7) and, therefore, that DNA is forced to structurally adapt to RNA (not vice versa) when the two are cross-pairing with each other. Analyzing and depicting the natural nucleic
acids’ duplex structures in terms of idealized conformations within the framework of rules listed above allows the organic chemist to rationalize and appreciate these remarkable facts at a level of stereochemical reasoning that biologists tend to achieve (Figure 1.7).

The synthesis and first steps in our rather comprehensive exploration of the homo-DNA system (pioneered by Christian Leumann, Hans-Jörg Roth, Jürg Hunziker and Markus Boehringer [11, 15, 16]) had in our laboratory the status of a model study that served the purpose of developing experimental methodologies besides determining the influence of
a simple insertion of a methylene group into DNA’s furanose ring might have on the system’s pairing capability. Homo-DNA was not considered to be a potentially natural nucleic acid alternative because the dideoxy-hexose sugar building block is a generationally much more complex sugar in comparison with a nonreduced hexose; it is in this sense that the homo-DNA project represented only a model study.

The base-pairing properties of synthetic homo-DNA strands turned out to be remarkable, to say the least. Strands with complementary nucleobase sequences showed regular Watson–Crick pairing much stronger than in DNA, but did not display any cross-pairing with complementary DNA (or RNA) strands. In homo-DNA, there is strong purine–purine pairing of guanine with isoguanine and of 2,6-diaminopurine and xanthine in the Watson–Crick mode, as well as homo-purine–purine self-pairing of adenine and of guanine in the reverse-Hoogsteen mode. Comparison of thermodynamic parameters of homo-DNA and DNA duplexations clearly revealed homo-DNA’s stronger Watson–Crick pairing to be of entropic rather than enthalpic origin and, therefore, presumably to reflect a higher degree of conformational strand preorganization toward duplex formation in homo-DNA than in DNA; and very plausibly so, considering the difference in flexibility between a pyranose chair and a furanose ring (Figure 1.8).

It was Christian Leumann who first drew the consequences of these observations by initiating and pioneering with his “bicyclo-DNA” project [17] an important strategy in antisense-oriented nucleic acid research (design of oligomer backbones that are conformationally pre-organized toward duplexation with DNA and RNA), a strategy that turned out to be highly successful in his and a number of other laboratories (Figure 1.9) [2d, 18].

Figure 1.10 gives – in terms of the formulae of monomer units depicted in their (potential) pairing conformation – an overview of all the families of nucleic acid alternatives we investigated at ETH and TSRI. All (except homo-DNA) are taken from the close structural neighborhood of RNA and deemed to be potentially natural nucleic acid alternatives, since they relate generationally to their respective building blocks in the same way as RNA does to ribose, purines, pyrimidines, and phosphate. Grey scale formulae denote oligomer systems found experimentally to be Watson–Crick base-pairing systems, identical color indicates systems that are capable of communicating with each other through informational intersystem cross-pairing, and nongrey members denote oligomer systems that are devoid of any significant base-pairing capability in the Watson–Crick mode.
Figure 1.8  Comparison of thermodynamic data of duplexations in the homo-DNA versus the DNA series [11]. The larger negative ΔG values of the homo-DNA duplexations are entropic in origin. Homo-DNA single strands are more strongly pre-organized toward duplexation than RNA single strands, and the latter (see Figure 1.7) more strongly than DNA single strands.

Figure 1.9  Pictorial juxtaposition (in the form of idealized conformations) of homo-DNA with the natural systems and important analogs of the latter (for these analogs, see Ref. [18]).
A remarkable and etiologically significant finding is the discrepancy between the base-pairing potential of the model system homo-DNA and the three members of the family of homo-DNA’s “natural” analogs studied, namely the fully hydroxylated oligonucleotide systems derived from allose, altrose, and glucose [19–21]. The latter show neither efficient, nor consistent Watson–Crick base-pairing. Exploratory base-pairing tests with mono-hydroxylated models of the allose- and altrose-derived oligomers supported the hypothesis that the source of this crucial difference in pairing capability is steric hindrance involving the two additional hydroxyl groups of the fully hydroxylated hexopyranose unit, as well as neighboring nucleobases, precluding the population of the unit’s pairing conformation in oligomers [22]. Clearly, RNA -alternatives derived from hexopyranoses in place of ribofuranose could not have acted as functional competitors of RNA in Nature’s evolution of a genetic system [23].

Apart from homo-DNA, by far the most extensive of our experimental efforts invested in the nucleic acid etiology project were directed to the synthesis (pioneered by Stephan Pitsch and Sebastian Wendeborn [24]) and investigation of the structure and properties of the pyranosyl
isomer of RNA ("p-RNA"), the nucleic acid alternative that is composed of the very same building blocks as natural RNA itself. In contrast to the backbone design for RNA alternatives in the hexopyranosyl series, where the phosphodiester group can link the sugar units in a way completely analogous to that in RNA (4’-6’-link in hexopyranose and 3’-5’-link in ribofuranose), the design of the backbone of a pyranosyl-RNA demanded a distinct deviation from that constitutional pattern in as far as a six-bond periodicity of the pento-pyranose backbone would only be compatible with a (2’-4’)-phosphodiester junction. Applying this criterion and defining the nucleoside to have the β-configuration (in order to have the nucleobase at the ribopyranose chair in an equatorial position) resulted in the p-RNA backbone depicted in Figure 1.11. p-RNA’s pairing conformation, equally depicted in Figure 1.11, emerged in a remarkably unambiguous way from a conformational analysis carried out with the very same criteria used already for homo-DNA: the ensemble of nine least-strained monomer-unit conformers contains only a single member displaying a repetitive conformation and, therefore, embodying the pairing conformation of an p-RNA duplex (Figure 1.12). It was not only this singularity that allowed us to predict with high confidence that p-RNA will turn out to be a base-pairing system, it was above all our experience that the pairing model for homo-DNA, built

Figure 1.11 Constitution and pairing conformation of pyranosyl-RNA
Figure 1.12  Qualitative conformational analysis of pyranosyl-RNA (facsimile from Ref. [24a])
with the same criteria as that of p-RNA had, in fact, found its counter-part in reality and, therefore, there was reasoned hope that the p-RNA model would do so, too. In fact, the agreement between the predicted model and the model that emerged from Bernhard Jaun’s X-ray analysis and Romain Wolf’s molecular mechanics-based modeling of the p-RNA-octamer duplex pr(CGAATTCG)$_2$ was exquisite [25].

While such unambiguous agreement between “theory” and experiment is of course welcomed, it is the encountering of clear-cut disagreement with specific details of a “theory’s” predictions that reveals to us the limits of that “theory” and, therefore, should to be welcomed, too. We encountered such disagreement when we studied the entire family of the four possible diastereomeric pentopyranosyl-oligonucleotide systems (containing equatorial nucleobases) [26], namely (besides the beta-D-ribo-system) the beta-D-xylo- [27], the alpha-L-lyxo- [28], and the alpha-L-arabino- [29] member (Figure 1.13). It was the arabinopyranosyl member that turned out to be the strongest base-pairing system by far of the entire family (Figure 1.14), while we had expected it to be a member that would definitely pair less strongly than p-RNA [24a].

The alpha-L-arabino-pyranosyl system is, in fact, one of the strongest oligonucleotide-type Watson–Crick pairing systems known today. The lesson which this discrepancy between expectation and fact taught us was (at least) twofold. First, in cases where the conformational analysis in terms of idealized conformations leads to an ensemble of monomer-unit conformers in which – according to the standard criteria – all members (the conformationally repetitive one(s) inclusive) are heavily strained, the task of judging on the level of idealized conformations as

Figure 1.13 The four diastereomeric pentopyranosyl-RNA variants (with equatorial nucleobases) studied at ETH and TSRI. Arrows point to severe steric hindrance in (idealized) pairing conformation
to whether a conformationally repetitive conformer is among the least unstable conformers is no longer straightforward; neither is, therefore, any prediction with regard to such a system’s base-pairing capability.

Second, the relative base-pairing strength of a system is intrinsically codetermined by inter-monomer-unit repulsions that are not taken into account by the routine analysis of the monomer unit conformations. However, perhaps the major lesson that our study of the pentopyranosyl-oligonucleotide family as a whole taught us was the one given by the α-L-lyxopyranosyl system [26, 28]; it was the lesson that determined the next project in our work and came about as follows.

One of the most remarkable properties observed for pyranosyl-RNA was its capacity for template-controlled ligations, especially the capability of hemi-self-complementary p-RNA-tetramer-2′,3′-cyclo-phosphates to regio- and chiro-selectively self-template their gradual growth into duplexes of long complementary p-RNA sequences (pioneered by Stephan Pitsch and Martin Bolli [30, 31]). The study of this type of ligation chemistry demanded a careful proof of its regioselectivity, in connection of which iso-p-RNA-oligomers with (3′–4′)- instead of (2′–4′)-phosphodiester bridges were synthesized for comparison purposes (Figure 1.15). Not unexpectedly, such isomeric p-RNA strands

Figure 1.14  Comparison of base-pairing strength of pentopyranosyl-oligonucleotides and RNA (A8/T8-duplexes; in 0.15 M NaCl, 10 μM NaH2PO4, pH 7; see Refs [24, 26–29])
Figure 1.15 (4′→2′)- versus (4′→3′)-phosphodiester bridges in the pentopyranosyl RNA series
lacked the capability of either intrasystem or intersystem (with DNA) base-pairing (five-bond periodicity). However, the analogous modification in the α-L-lyxopyranosyl series, in which a (3′–4′)-phosphodiester bridge is diaxially attached to the pentopyranosyl chairs, produced oligomer strands that, though not completely unexpectedly, revealed themselves as being capable of intrasystem base-pairing and, surprisingly, of intersystem cross-pairing with DNA. It seemed clear that the reason for such a drastic six-bond-rule disdaining behavior must be lying in the unique trans-diaxial arrangement of the phosphodiester bridge [32].

Sugar units consisting of tetrooses (which can form only furanoses) had originally been explicitly neglected as building blocks of nucleic acid alternatives because it is not possible to derive from them tetrofuranosyl-oligonucleotides with a backbone periodicity of six bonds. Our observations with the lyxopyranosyl system, however, made us rethink the matter and etiology-driven reasoning quickly associated the structure of an α-L-threofuranosyl-oligonucleotide strand in which the trans-phosphodiester bridge might be held in quasi-diaxial conformation by electrostatic reasons and, therefore, give rise to a behavior similar to that of an α-L-lyxopyranosyl oligonucleotide strand (Figure 1.16). Why should such a system be of etiological interest? It would be very much so, because the four-carbon sugar threose is a generationally simpler sugar than ribose.

α-L-Threofuranosyl-oligonucleotides (“TNA”, Figure 1.17; pioneered by Uwe Schönig and Peter Scholz [33]) turned out to be a marvelous Watson–Crick base-pairing system of a pairing strength comparable to that of RNA and the capability to cross-pair with equal efficiency with the natural system (Figure 1.18). Bernhard Jaun’s NMR-structure analysis of the duplex t(CGAAATTGC)₂ showed an RNA-like right-handed helix [34] [and Jaun and Ebert (unpublished results)], and Martin Egli’s two X-ray structure analyses of both an A- and B-type DNA duplex of a self-complementary dodecamer sequence containing a single TNA unit demonstrated how the quasi-diaxial phosphodiester bridge adjusts to the DNA double helix, revealing at the same time TNA’s distinct preference for the A-type structure paralleling the observation that TNA cross-pairs more strongly with RNA than with DNA [35].

Among molecular biologists, TNA has become more popular than, for example, the pyranosyl isomer of RNA. This is as a simple consequence of the fact that TNA does not speak a “foreign” language as p-RNA does. In Jack Szostak’s laboratory it was shown that TNA strands in the presence of a “tolerant” DNA-polymerase can template
Figure 1.16  The conceptual leap from observing the properties of the (4′–3′)-lyxo-pyranosyl system to studying the (3′–2′)-threofuranosyl system

Figure 1.17  Juxtaposition of the (idealized) pairing conformations of RNA and TNA
Figure 1.18  \( T_m \)-values and thermodynamic data of self- and cross-pairing in duplex formation within and in between the TNA-, RNA-, and DNA-series [33]

DNA-synthesis and, most remarkably, DNA strands can serve as template for the faithful synthesis of complementary TNA strands (so far up to 50-mer) from \( \alpha \)-l-threofuranosyl-nucleotide-2'-triphosphates [36]. The authors of these findings consider such experiments as first steps in a project that aims at an \textit{in vitro} evolution of TNA (with the help of contemporary enzymes) to the functional level of an artificial genotype possessing phenotypic properties. The model vision behind this work is the role assigned to RNA in a hypothetical world (the “RNA-world”), considered to have preceded our own, in which RNA is thought to have fulfilled both genotypic and phenotypic functions [37].
Can the structural and generational simplicity of an informational oligomer system such as TNA be pushed even further? In one of our papers [32], we had commented on such an extrapolation of the TNA structure to an even simpler system by hypothesizing that acyclic phosphodiester-based oligomers of the type depicted in Figure 1.19 might prove to behave like TNA, since the \textit{trans}-antiperiplanar arrangement of the negatively charged phosphodiester groups – a violation of the gauche effect notwithstanding – might be favored by electrostatic repulsion. We refrained from testing the hypothesis experimentally because we supposed the simplicity of oligomers, such as the one derived from the C3-backbone \((n = 1)\), is deemed to be structural, not generational (its backbone unit corresponds to a reduced sugar), and, therefore, to be a system that lacks the attribute of being potentially natural. Fortunately for nucleic acid chemistry, such a constraint is not what everybody adheres to, and so we know today, thanks to recent work by Eric Meggers [38], that the \((n = 1)\)-oligomer of Figure 1.19 is an impressively efficient base-pairing system, able to cross-pair with the natural nucleic acids.

The available NMR-structure analyses for duplexes of the homo-DNA- [13], pyranosyl-RNA- [25], arabinopyranosyl-RNA- [39], and TNA- [34] [and Jaun and Ebert (unpublished results)] series revealed structural models that fall into three different groups where the degree

\[\text{Figure 1.19} \quad \text{A hypothetical aliphatic oligonucleotide backbone system in which the vicinal phosphodiester groups might (as a result of a tendency to minimize electrostatic repulsion) assume an antiperiplanar conformation (excerpt from Ref. [32])}\]
Figure 1.20 The three groups of base-pairing systems which exhibit strong intra-system base-pairing but do not cross-communicate with each other; they represent orthogonal base-pairing systems. This orthogonality is proposed to be related to the different backbone–base-pair axes’ inclinations of these systems (excerpt from Ref. [32])

and orientation of the inclination between the (averaged) backbone axis and the (averaged) base-pair axis [40] is concerned. The ladder-like, feebly helical models of homo-DNA and the two pentopyranosyl-nucleic acids show a strong inclination of these axes, yet (significantly) in opposite orientation [40], whereas the compactly helical model of TNA resembles the right-handed helices of the natural nucleic acids of which, characteristically, the B-type helix of DNA has essentially no inclination. This difference in degree and orientation of backbone inclination is related to the base-pairing orthogonality of the three groups of nucleic acids (Figure 1.20), giving rise to barriers to conformational adjustments necessary for base-pairing, even though the inclination is (formally) efficiently adjustable through rotation around nucleosidic bonds (Figure 1.21) [32]. The degree of inclination of these axes (in any orientation) correlates with two further structural properties of informational oligomers: one is a system’s propensity to pair in parallel, versus antiparallel, strand orientation [11]; the other is the nature of the base-stacking in duplexes, namely intra- versus inter-strand [25, 39, 40]. The more strongly inclined a system’s backbone is, the more strongly forbidden is base-pairing in parallel strand orientation, and the more pronounced interstrands is the base-stacking.

Have we reached the boundaries of the structural landscape of the carbohydrate-derived phosphodiester-type base-pairing systems by what we know today? The answer is a clear no from a purely chemical point
of view, and a cautious *very probably not* when referring to systems that fall into the category of potentially natural nucleic acid alternatives. What at this stage, however, seems more urgent than to go on and close this remaining gap of knowledge may be to abandon the selection criterion that determined the course of our work so far, namely that the structure of a nucleic acid alternative has to be taken from RNA’s structural neighborhood in the sense that it must be derivable from a \((\text{CH}_2)_n\) aldose sugar by the same type of generational chemistry that allows the structure of RNA to be derived from ribose. There is that other class of fundamental biomolecules, the polypeptides and proteins, whose backbone can also serve as the skeleton of informational oligomers – as recent work on nucleobase-tagged peptide-like oligoamide backbones
has amply shown [6, 41–47, 49]. The polypeptide instead of oligonucleotide type of structure could serve as reference in a search for nucleobase-tagged oligomers, whose repeating units would have to be tagged dipeptide units, as recognized at the very beginning of our work [41]. We would again demand candidate structures to be potentially natural, in the sense that they have to be derivable from their building blocks by the same type of basic chemistry that connects polypeptides with the proteinogenic $\alpha$-amino acids and, moreover, that these nucleobase-tagged building blocks should be derivable from natural $\alpha$-amino acids by potentially primordial reactions.

The last part of this article deals with the question as to what extent the kind of conformational reasoning used in the search for oligonucleotide type nucleic acid alternatives could assist such a search in the field of nucleobase-tagged oligo-dipeptides and related systems and, moreover, could provide a qualitative rationalization of the facts already known in this field.

Figure 1.22 recapitulates the two types of (idealized) pairing conformations of the repeating monomer unit of homo-DNA, transcribed into the corresponding formulae of DNA (and RNA) backbones (see also Figure 1.6), the (-$g$/-$g$)-variant of the two representing the type of conformation characteristically observed for A-type duplexes of DNA and RNA. Important characteristics of this backbone conformation are the two $180^\circ$ torsion angles $\beta$ and $\epsilon$ (bonds in bold). In a further transcription of the conformation into a backbone variant that lacks the cyclic part of the natural systems, the bold bonds of the backbone formula define the positions at which double bonds could be accommodated without changing in any major way the overall shape of the backbone thread (Figure 1.23, formula a).\(^5\) Interestingly, the relative positions of these double bonds correspond to the relative positions of the (planar) amide bonds in oligopeptides. This means that this specific (idealized) type of polypeptide backbone conformation corresponds to the -$g$/-$g$ pairing conformation of the backbone in A-DNA (and RNA),

\(^4\)For studies on $\delta$-peptide analogs of pyranosyl-RNA with Gerhard Quinkert at the University of Frankfurt, see Ref. [49].

\(^5\)A formal transformation of the idealized conformation of a saturated four-center chain containing two vicinal substituents in antiplanar arrangement into the idealized conformation of a $2,3$-unsaturated chain with the two substituents in trans arrangement would in fact require two $60^\circ$ rotations around the resulting sp$^3$/sp$^3$ bonds in order to reach the (idealized) most stable conformations at the allylic positions (allylic hydrogen atoms synplanar with the double-bond axis). Allowing for this implicit conformational change would have to be part of a more detailed analysis of such an adaptation of the pairing conformation of an oligonucleotide to that of an oligopeptide. However, for the sake of pictorial simplicity and clarity, this aspect is not taken into account by Figures 1.22–1.24.
and that nucleobase-tagged oligo-dipeptides, depending on the structural details of the nucleobase-bearing chains alternatingly attached at tetrahedral positions of the backbone (see below), may represent systems that harbor the capacity of base-pairing with themselves and of cross-pairing with DNA and RNA.

As Figure 1.23 (formula b) indicates, there is another way of maintaining the conformational character of the A-DNA-type backbone within an aliphatic backbone thread, namely by putting electronegative atoms at the positions labeled in the figure as black spots. If such a backbone were to follow the organic chemist’s “gauche-effect” in choosing its preferred conformations, the specific positioning of the electronegative centers would support the maintenance of the thread’s specific folding and have an effect on the backbone’s conformational preferences similar to that of the aforementioned double bonds. Taking the NH-group as the electronegative center, the oligomer backbone becomes that of an oligo-ethylenimine, pointing to the possibility that, again depending on the structural details of the attachment of the
nucleobase, such a type of structure might serve as the backbone of oligomer systems that cross-pair with DNA and RNA. A similar prediction could be made for a correspondingly nucleobase-tagged oligo-ethylenoxide backbone.

Finally, there is a third way of maintaining the special conformational relationship of an aliphatic backbone with the $(-g/-g)$-conformer equated to the conformation of the backbones of aliphatic oligomer systems containing double bonds (or equivalents thereof) or electronegative centers (black dots)

Figure 1.23  The idealized conformation of the backbone-core of A-DNA’s $(-g/-g)$-conformer equated to the conformation of the backbones of aliphatic oligomer systems containing double bonds (or equivalents thereof) or electronegative centers

Figure 1.24 gives the generalized formula of nucleobase-tagged oligo-dipeptide oligomers drawn in the two conformations that correspond
Figure 1.24  The two (idealized) backbone conformations of oligo-dipeptide base-pairing systems (a and b), and three experimentally studied systems taken from the literature, depicted in an (idealized) pairing conformation (c–e)
Most, if not all, of these variants do not belong to the landscape of potentially primordial oligomer systems. However, Nielsen’s PNA itself has been considered in this context [46, 48]. Important informational chemistry involving conventional nucleobases and peptidic backbones not based on the oligo-dipeptide (six-bond periodicity) concept exists: see the work of Ulf Diederichsen on nucleobase-tagged alanyl- and homoalanyl-PNAs [44] and that of Philip Garner on α-helical peptide nucleic acids (α-PNAs) [45].

The residues labeled as R can be the residue of any α-amino acid, preferable one that facilitates water solubility. Only a few representatives of the potentially large diversity of such oligo-dipeptide systems are described in the literature [42]; however, data about the self-pairing capabilities of such systems (for example, of oligomers of type b in Figure 1.24) are absent and the reports about their capability to cross-pair with the natural systems are conflicting. DNA- and RNA- cross-pairing capabilities of such systems seem, indeed, to depend on the nature of the nucleobase-bearing side-chain, as Masayuki Fujii’s well-studied and successful oligo-dipeptide system (Figure 1.24d) demonstrates [41]. Of special interest in this context is the oligomer of Figure 1.24e, which corresponds to the type of system pointed to in Figure 1.23c and which shows efficient cross-pairing with DNA and RNA [43], in remarkable contrast to the oligomer of Figure 1.24c, which is reported [43a] not to do so.

In a search for potentially primordial genetic systems within the landscape of nucleobase-tagged oligodipeptides, the main selection criterion for choosing the structure of systems to be studied will have to be a given oligomer system’s generational simplicity relative to that of normal natural α-oligopeptides and their building blocks, the α-amino acids. Thereby, the exploration of the structural diversity of generationally simple heterocycles that could serve as alternative nucleobases and especially of the kind of “nucleosidation step” they would allow for will be challenges to be met.

In recent years, a huge amount of empirical information about the structure and base-pairing properties of a large variety of structurally modified analogs of Nielsen’s PNA has become available [46, 47]. To what extent these data fit into the kind of formal stereochemical exercise

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4 Important informational chemistry involving conventional nucleobases and peptidic backbones not based on the oligo-dipeptide (six-bond periodicity) concept exists: see the work of Ulf Diederichsen on nucleobase-tagged alanyl- and homoalanyl-PNAs [44] and that of Philip Garner on α-helical peptide nucleic acids (α-PNAs) [45].

7 Most, if not all, of these variants do not belong to the landscape of potentially primordial oligomer systems. However, Nielsen’s PNA itself has been considered in this context [46, 48].
discussed here, of connecting (idealized) pairing conformations of peptide-type oligomer systems with the “allowed” (idealized) pairing conformations of homo-DNA, is a question that might deserve a separate analysis.

REFERENCES


12. For a complete pictorial presentation of the sequence of stereochemical arguments leading to this result, see Quinkert, G., Egert, E., Griesinger, C.


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