

Mapping the Landscape of Potentially Primordial Informational Oligomers: Oligodipeptides and Oligodipeptoids Tagged with Triazines as Recognition Elements**

Gopi Kumar Mittapalli, Kondreddi Ravinder Reddy, Hui Xiong, Omar Munoz, Bo Han, Francesco De Riccardis, Ramanarayanan Krishnamurthy,* and Albert Eschenmoser*

One of the challenges that current thinking on the problem of biogenesis poses to contemporary organic chemistry is to map, by chemical synthesis, the landscape of the chemical structures of potentially primordial informational^[2] oligomers. Knowledge of such a landscape in terms of chemical facts will offer a guide on one of the directions along which the search for the chemistry of life's origin has to proceed. The very existence of such a landscape is to be assumed in view of the outcome of the extensive experimental studies toward a chemical etiology of nucleic acid structure^[3] as well as of the comprehensive search in various laboratories for nucleic acid substitutes that are functionally competent with respect to the goals of the medicinal antisense project.^[4]

It has long been conjectured that RNA was not the first genetic system, a notion adumbrating a transient existence of primordial genetic systems that have preceded RNA.^[5,6] In fact, the capability of informational Watson–Crick base pairing is surprisingly widespread among oligomer systems of quite different backbone structures, ranging from non-canonical oligonucleotides and oligonucleosides^[3,7] to oligo-amides^[8] and to oligopeptides.^[9] Furthermore, Watson–Crick pairing is found to mediate a variety of “base-pairing languages” that are orthogonal to each other in the sense that base sequences bound to a given type of oligomer backbone may be devoid of the capability to communicate with corresponding complementary base sequences attached to another type of backbone and yet are capable of efficient

intrasystem Watson–Crick base pairing in their own “language”.^[3,9c–d,10] The primary chemical criterion for deciding whether a given candidate system may belong to the landscape of primordial oligomers refers primarily to a system's generational^[11] properties in reference to boundary conditions of the type considered in prebiotic chemistry.^[12] For our work, we are adopting a pragmatic criterion according to which an oligomer system can qualify as potentially primordial, if it is generationally (not necessarily structurally) at least as simple as, yet preferably, simpler than, the structure types of oligonucleotides, oligopeptides, and oligosaccharides within constraints that refer to the nature of starting materials, reaction types, and reaction conditions deemed to be prebiotically realistic.^[13] This is structurally far broader a selection criterion than that used in our earlier studies,^[3] in as far as candidates may belong to any type of oligomer structure as long they satisfy the premises and deserve to be called informational.^[2] Backbones may be achiral or chiral; backbone chirality as such is not considered a reason for excluding a system from being assigned to the landscape, the underlying presumption being that replication of a chiral informational oligomer would be intrinsically chiroselective^[14] and, therefore, an oligomer system capable of replication would eventually become homochiral through the very process of replicating.^[15]

The majority of the experimental work on nucleic acid alternatives carried out thus far has concentrated on backbone variation, making conservative use of the family of canonical nucleobases as recognition elements. Studies in various laboratories dealing with nucleobase variation^[17] have referred to alternative bases that were chosen for study without reference to etiological constraints.^[18] Yet, there are heterocycle families that can be envisaged to have been capable of acting as alternative nucleobases while, at the same time, are derived from the same type of starting materials from which the canonical nucleobases are supposed to have been formed.^[19] Of special interest is the possibility that such heterocycles may offer ways of becoming attached to backbones by reactions that are different from the notorious nucleosidation process required for nucleosides to be formed from aldoses and nucleobases, a reaction type that, after half a century of prebiotic chemistry, has still not been convincingly demonstrated to be capable of proceeding efficiently under reaction conditions deemed to be potentially primordial.^[5,20]

This and the following Communication^[45] report results of exploratory work on the pairing properties of heterocycles

[*] Dr. G. K. Mittapalli, Dr. K. R. Reddy, Dr. H. Xiong, Dr. O. Munoz, Prof. Dr. F. De Riccardis, Prof. Dr. R. Krishnamurthy, Prof. Dr. A. Eschenmoser
Department of Chemistry and
The Skaggs Institute for Chemical Biology
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-9573
E-mail: rkrishna@scripps.edu
Eschenmoser@org.chem.ethz.ch

Dr. B. Han, Prof. Dr. A. Eschenmoser
Laboratorium für Organische Chemie
Eidgenössische Technische Hochschule
HCI Hönggerberg, 8093 Zürich (Switzerland)
Fax: (+41) 1-632-1043

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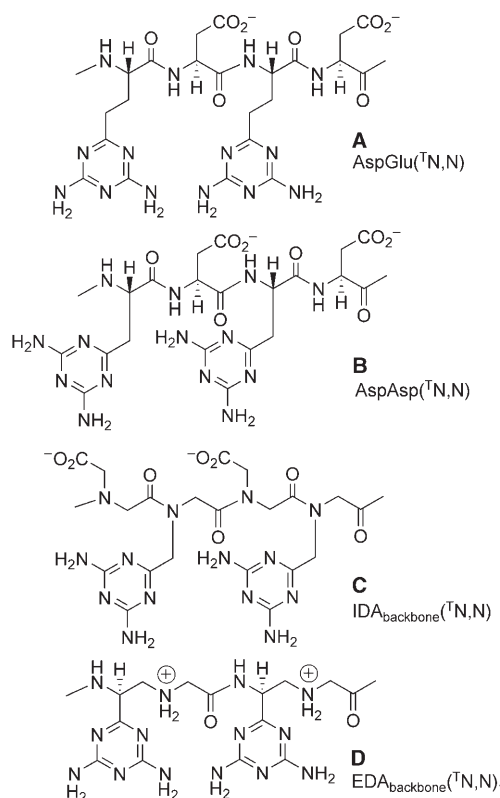
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that are derivatives of 2,4-diamino- and 2,4-dioxotriazines in one of the studies, and derivatives of the family of 2,4-disubstituted 5-aminopyrimidines in the other. Herein we describe oligomer systems that contain backbones of the structure types of oligopeptides, oligodeoxidipeptides, and oligodipeptoids tagged with triazines as recognition elements that are derived from carboxyl functions of α -amino acids, for example, aspartic, glutamic, α,β -diamino propionic, and imino diacetic acids.^[21,22] Scheme 1 depicts the chemical structure of the four types of oligomer systems **A–D** (exemplified as 2,4-diaminotriazine derivatives **A**(^TN,N)–**D**(^TNN)) that this report deals with.

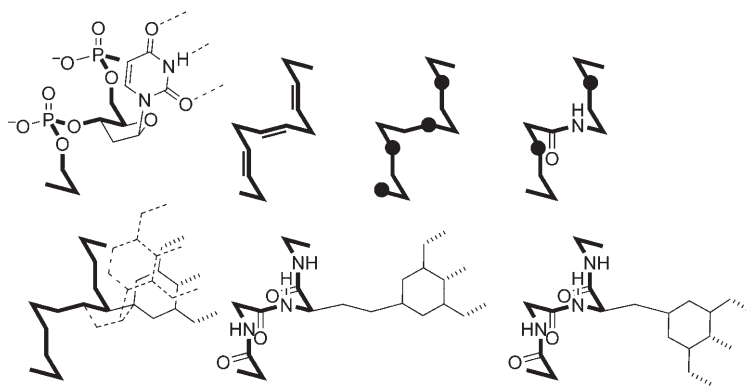
1,3,5-Triazines are a family of heterocycles that have been widely investigated in chemical molecular-recognition studies.^[23] With a few exceptions,^[23e–g] these studies refer to the behavior of triazines as recognition elements in an organic medium and a few investigations dealt with triazines as alternative nucleobases in nucleic acid chemistry.^[24] 1,3,5-Triazines are generationally simple heterocycles in the sense that they can be derived from carboxylic acid functions with activating agents and reaction partners under conditions that are deemed to be compatible with the constraints of prebiotic chemistry.^[21] We became involved in the chemistry of this type of recognition element in the context of our recent work on allopurines.^[25] The choice of oligoamide backbones for testing triazines as recognition elements in oligomers was prompted by the vast amount of information that has become available in the wake of Nielsen's discovery of the pairing properties of peptide nucleic acids (PNAs)^[4b,26] involving a wide variety of variants^[4c,f,8] as well as genuine peptide analogues of nucleic acids.^[9] We were further attracted to this project by an enticingly simple and rough^[3d] way to extrapolate the (idealized) pairing conformations of A- and B-type DNA to potential pairing conformations of oligomer systems that contain noncyclic backbones tagged with noncanonical recognition elements, such as triazines or any other potential nucleobase alternative. Scheme 2 and its caption explain how this was done.

The types of triazine-tagged oligomers **A–C** depicted in Scheme 1 are built of monomer units that are dipeptides, a requirement that corresponds to a basic constitutional complementarity between the structure types of oligonucleotides and oligopeptides^[27] and conspicuously follows from the conformational reasoning referred to above (Scheme 2). In all three systems, the repeating unit contains (in addition to the recognition element) a free carboxyl group, the function of which is to ensure solubility of oligomers in aqueous solution, whereas in oligomers of type **D**, solubility is promoted by the presence of an ammonium group in the backbone's monomer unit. The peptoid oligomers derived from iminodiacetic acid (type **C**) are achiral;^[28] the other oligomers are derived from the corresponding (enantiopure) L- α -amino acids.

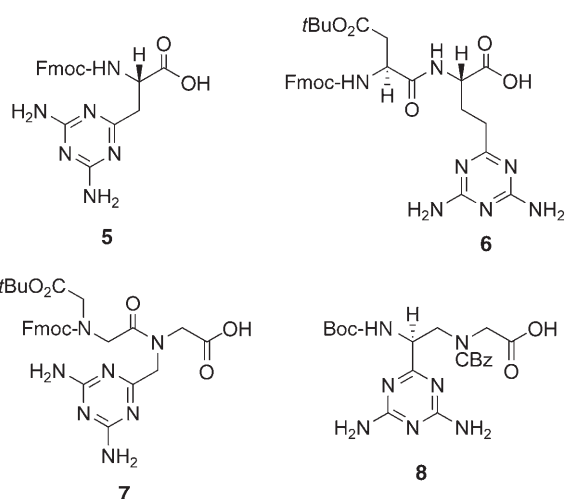
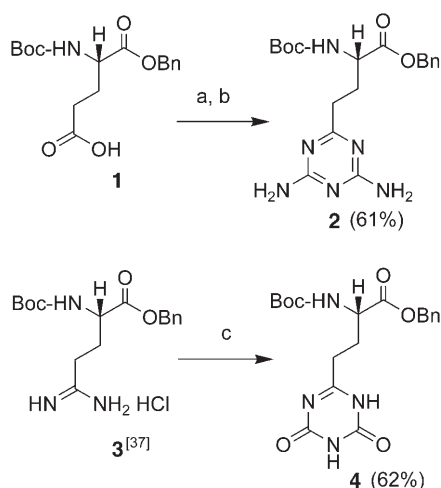
Given that the goal of our studies was primarily the screening of pairing capabilities, the synthesis



Scheme 1. Structures of the four triazine-tagged oligomeric systems dealt with in this report. End groups are either free NH_2 and CO_2H or CH_3CONH and CONH_2 . Asp = aspartyl; Glu = glutamyl; IDA = iminodiacetic acid; EDA = ethylenediamine; (^TN,N) = (2,4-diamino)triazin-6-yl.



Scheme 2. An approximate derivation of an (idealized) pairing conformation of triazine-tagged aliphatic oligomer backbones from the (idealized) (–g/–g)-pairing conformation of A-type DNA (and RNA). The two bonds of the latter conformation with torsion angles of 180° (β and ϵ) correspond to bond positions of an aliphatic backbone thread in which double bonds (or equivalents thereof, for example, amide bonds) can be accommodated. Also compatible with this conformation are electronegative centers in the solid-sphere positions of the (saturated) aliphatic backbone where they satisfy the gauche effect. Alternation of such electronegative centers with, for example, amide bonds, represents a third possibility. The figure also depicts positioning and lengths of the side chain that bears the recognition element such that the pairing axis of the triazine ring approximates that of a pyrimidine ring in the pairing conformation of oligonucleotides. An alternative pairing conformation of an aliphatic backbone can be derived analogously from the (t/–g)-pairing conformation of A-type DNA or the (g/t)-conformation of B-type DNA (for details, see reference [3d]).



Scheme 4. The monomeric (**5**), dipeptidic (**6**), dipeptidic (**7**), and monopeptidic (**8**) building blocks used in the solid-support synthesis of oligomers of type **A–D** (see Scheme 1). CBz = benzyloxycarbonyl, Fmoc = 9-fluorenylmethoxycarbonyl.

of specific members of the oligomer systems followed conventional methodologies of chemical peptide and peptoid synthesis with no consideration for etiological constraints. Scheme 3 exemplifies, in the case of glutamic acid, the tagging procedures, and Scheme 4 depicts the four different types of monomer units **5**, **6**, **7**, and **8** that served as starting materials for the synthesis of the oligomers of type **A–D**, which were prepared by standard automated solid-phase peptide synthesis. Notably, all these manipulations could be conducted without the need for protecting the amino or oxo groups of the triazine moiety.^[31]

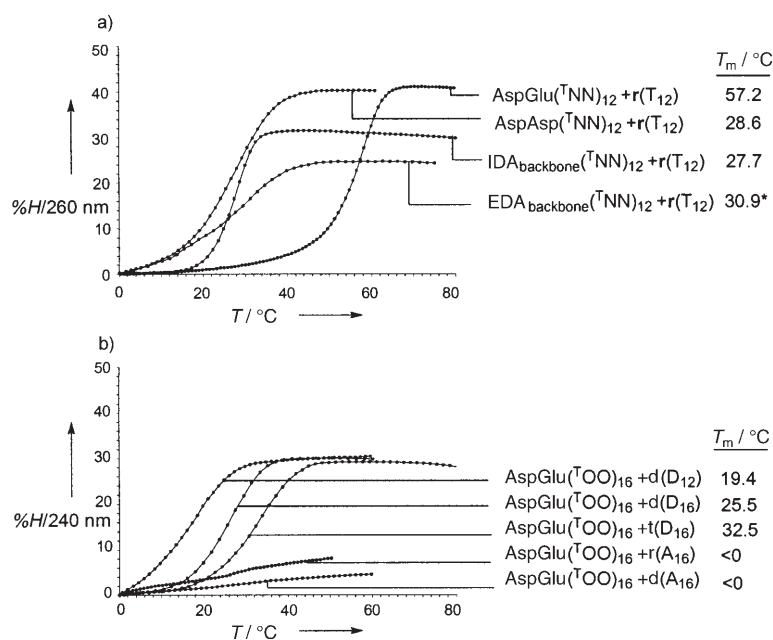


Figure 1. UV spectroscopic *T_m* curves of selected duplexes formed by a) 2,4-diaminotriazine-tagged dodecamer sequences from oligomeric systems **A–D** with RNA (*r*(*T*₁₂)) and b) 2,4-diketotriazine-tagged hexadecamer sequences, AspGlu(^TOO)₁₆, with complementary DNA (*d*), RNA (*r*), and TNA (*t*) sequences. For conditions of measurement refer to the caption for Table 1. No self-pairing was observed for individual partner stands, except in the case of AspGlu(^TNN)₁₆ (see Table 1, entry 11). The asterisk (*) indicates measurement in 0.15 M NaCl.

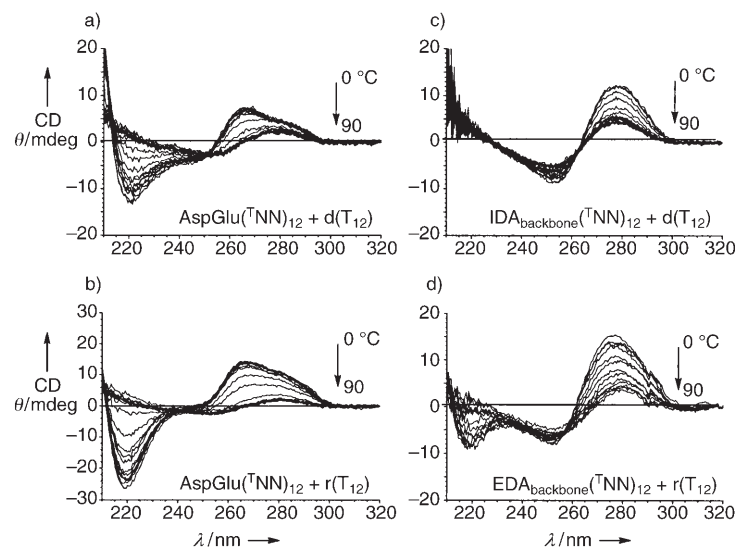


Figure 2. Temperature-dependent CD spectra of duplexes formed by the cross-pairing of 2,4-diaminotriazine-tagged **A–D** dodecamer sequences (see Scheme 1) with the complementary DNA and RNA sequences: a) AspGlu(^TNN)₁₂ + d(*T*₁₂), *T_m* = 50 °C; b) AspGlu(^TNN)₁₂ + r(*T*₁₂), *T_m* = 55 °C; c) IDA_{backbone}(^TNN)₁₂ + d(*T*₁₂), *T_m* = 20 °C; d) EDA_{backbone}(^TNN)₁₂ + r(*T*₁₂), *T_m* = 25 °C. Measurements were made with the respective **A**, **B**, **C**, or **D** (5 μM) and 5 μM complementary DNA/RNA sequence in 1 M NaCl, 10 mM NaH₂PO₄, 0.1 mM Na₂EDTA, pH 7.0; d) in 0.15 M NaCl. Temperature increments in 5 °C steps; *T_m*(CD) at approximately 270 nm.

Most of our exploratory screening of the pairing properties was done with homobase sequences containing the 2,4-

Table 1: T_m data of triazine-tagged oligomeric sequences derived from backbones depicted in Scheme 1.

Entry	Pairing system ^[a]	T_m (UV) (λ [nm]) ^[b]	T_m (CD) (λ [nm]) ^[b]	Comments ^[c]
1	$H_2NOCAspGlu(TNN)_6 + \text{poly-d(T)}$	41.7 (260)		
2	+ poly-r(U)	28.6 (260)		0.15 M NaCl
3	+ d(T) ₁₂	50.0 (260)		0.15 M NaCl
4	+ r(T) ₁₂	35.8 (260)		0.15 M NaCl
		32.2 (260)		0.15 M NaCl
		28.2 (260)		0.15 M NaCl
		42.8 (260)		0.15 M NaCl
		31.1 (260)		0.15 M NaCl
5	$H_2NOCAspGlu(TNN)_{12}$	< 0 (250)		
6	+ poly-d(T)	59.4 (260)		0.15 M NaCl
7	+ poly-r(U)	52.2 (260)		0.15 M NaCl
8	+ d(T) ₁₂	65.0 (260)	50 (270)	0.15 M NaCl
9	+ r(T) ₁₂	51.4 (260)	50 (270)	0.15 M NaCl
10	+ t(T) ₁₂	53.8 (260)		0.15 M NaCl
		49.2 (260)		0.15 M NaCl
		57.2 (260)		0.15 M NaCl
		51.2 (260)		0.15 M NaCl
		35.8 (260)		
11	$AcNHAspGlu(TNN)_{16}$	≈ 21 (250)	22 (222)	self-pairing of (TNN) ₁₆ -mer
12	+ poly-d(T)	34.3 (250)		100 μM
13	+ poly-r(U)	50.8 (260)	49 (260)	Ref. [40]
14	+ d(T) ₁₆	54.0 (260)	51 (260)	Ref. [40]
		50.0 (260)		1:1 duplex by Job plot (UV); Ref. [40]
15	$AcNHAspGlu(TOO)_{12} + d(D)_{12}$	19.4 (240)		Ref. [44]
16	+ poly-r(A)	< 0 (240)		Ref. [44]
17	+ t(D) ₁₂	≈ 14 (240)		Ref. [44]
18	+ $NH_2AspGlu(TNN)_{12}$	≈ 13 (240)		1:1 duplex by Job plot (UV); Ref. [44]
19	+ $H_2NOCAspAsp(TNN)_{12}$	< 10 (240)		pH 5.0
		< 0 (240)		Ref. [44]
20	$AcNHAspGlu(TOO)_{16} + d(A)_{16}$	< 0 (240)		Ref. [44]
21	+ d(D) ₁₆	< 0 (240)	25 (250)	pH 5.0, acetate buffer solution; Ref. [44]
		25.5 (240)		2:1 triplex by Job plot (UV); Ref. [44]
		25.3 (240)		pH 8.0; Ref. [44]
		25.7 (240)		pH 6.0; Ref. [44]
		24.3 (240)		pH 5.5, acetate buffer solution; Ref. [44]
		18.4 (240)		pH 5, acetate buffer solution; Ref. [44]
22	+ poly-r(A)	< 10 (240)		Ref. [44]
23	+ r(A) ₁₆	< 0 (240)		Ref. [44]
24	+ t(D) ₁₆	32.5 (240)		Ref. [44]
		38.6 (240)		50 + 50 μM
25	+ $NH_2AspGlu(TNN)_{16}$	≈ 11 (240)	13 (252)	10 + 10 μM, 1:1 duplex by Job plot (UV); Refs. [40, 44]
26	$AcNHAspGlu[(TNN)(TOO)]_6$	< 0 (240)		Ref. [44]
		< 0 (240)		100 μM
27	$HOOCAspAsp(TNN)_6 + \text{poly-d(T)}$	< 10 (260)		
28	+ poly-r(U)	≈ 10 (260)		
29	$HOOCAspAsp(TNN)_{12} + \text{poly-d(T)}$	26.6 (260)	25 (270)	
30	+ poly-r(U)	33.1 (260)	30 (270)	
31	+ d(T) ₁₂	11.6 (260)		
32	+ r(T) ₁₂	28.6 (260)	30 (270)	
		24.4 (260)		0.15 M NaCl
33	$NH_2IDA_{\text{backbone}}(TNN)_8 + \text{poly-d(T)}$	26.7 (270)		
34	+ poly-r(U)	19.7 (270)		
35	$NH_2IDA_{\text{backbone}}(TNN)_{12} + \text{poly-d(T)}$	35.2 (270)	35 (277)	1:1 duplex by Job plot (CD)
36	+ poly-r(U)	29.1 (270)	25 (265)	
37	+ d(T) ₁₂	22.7 (270)	20 (265)	

Table 1: (Continued)

Entry	Pairing system ^[a]	T_m (UV) (λ [nm]) ^[b]	T_m (CD) (λ [nm]) ^[b]	Comments ^[c]
38	+ r(T) ₁₂	27.7 (270)		
39	+ NH ₂ IDA _{backbone} (^T OO) ₆	< 0 (240)		1:2 ratio
40	NH ₂ IDA _{backbone} (^T OO) ₆ + poly-d(A)	< 0 (240)		Ref. [44]
41	+ poly-r(A)	< 0 (240)		Ref. [44]
42	NH ₂ IDA _{backbone} (^T OO) ₇ + t(D) ₁₂	< 0 (240)		Ref. [44]
43	NH ₂ EDA _{backbone} (^T NN) ₁₂ + poly-d(T)	47.4 (270)	45 (275)	0.15 M NaCl
44	+ poly-r(U)	31.7 (270)	30 (275)	0.15 M NaCl
45	+ d(T) ₁₂	33.1 (270)	30 (275)	0.15 M NaCl
46	+ r(T) ₁₂	30.9 (270)	30 (275)	0.15 M NaCl

[a] Oligodipeptide sequences are written from the COOH terminus (except when stated otherwise) and oligodipeptoid and oligoamide sequences are written from the NH₂ terminus, with every second amino acid residue tagged with the heterocycle; Asp = aspartyl; Glu = glutamyl; IDA = iminodiacetic acid; EDA = ethylenediamine; (^TNN) = (2,4-diamino)triazin-6-yl; (^TOO) = (2,4-dioxo)triazin-6-yl; (^TNO) = (2-amino-4-oxo)triazin-6-yl; A = adenine; D = 2,6-diaminopurine; T = thymine; U = uracil; d = DNA; r = RNA, t = TNA. [b] Measurements were made at the indicated wavelength (nm), the pairing systems (each $\approx$ 5 μ M) in phosphate buffer solution (1 M NaCl, 10 mM NaH₂PO₄, 0.1 mM Na₂EDTA; pH 7.0) except when stated otherwise. T_m values [$^{\circ}$ C] are derived from maxima of the first derivative of the heating curve (Kaleidagraph software). [c] In acetate buffer solution (1 M NaCl, 10 mM NaOAc/HOAc, 0.1 mM EDTA). The end NH₂ group of oligopeptide sequences 11, 15, 20, and 26 are acetylated.

diaminotriazine nucleus. Not unexpected (see Scheme 2), yet nevertheless remarkable was the finding that such homobase sequences in all four oligomer systems show efficient cross-pairing with complementary sequences of RNA and DNA in aqueous solution. This is shown by the T_m curves reproduced in Figure 1 a, the CD curves of Figure 2, and the T_m data summarized in Table 1. Furthermore, the T_m data illustrate that, in apparent agreement with the conformational reasoning about the directionality of base-pairing axes, the glutamic acid tagged homo-oligomers AspGlu(^TNN)_{*n*=6,12} cross-pair with the complementary counterparts of RNA and DNA more strongly than the corresponding aspartic acid tagged homo-oligomers AspAsp(^TNN)_{*n*=6,12}. Additionally, a corresponding strand with no methylene group in the backbone-triazine linker, EDA_{backbone}(^TNN)₁₂, again shows more efficient cross-pairing.^[32] Job plots^[33] (see the Supporting Information) demonstrate the absence of triplex formation for the cross-pairing of AspGlu(^TNN)₁₆ with d(T)₁₆ and of IDA_{backbone}(^TNN)₁₂ with r(T)₁₂. The affinities of AspGlu(^TNN) sequences for RNA and DNA are comparable, with RNA prevailing slightly. The cross-pairing capability extends to (3'→2')- α -L-threofuranosyl oligonucleotides (TNA)^[36] as a partner, yet as the T_m value of the interaction between AspGlu(^TNN)₁₂ and t(T)₁₂ (Table 1, entry 10) indicates, is somewhat weaker than that found in natural systems.

Homobase sequences containing the 2,4-dioxotriazine base complementary to the 2,4-diaminotriazine base were synthesized with the backbones of the oligomer systems **A**, **B**, and **C**. To our surprise and (transient) disappointment,^[34] the 2,4-dioxotriazine nucleus in these oligomers is found to act as a drastically weaker pairing partner to adenine in DNA and RNA than the 2,4-diaminotriazine base acts toward thymine in the natural backbones (Figure 1b). No cross-pairing was discernable from T_m or CD curves of a (1:1) mixtures of the homobase sequence AspGlu(^TOO)₁₆ and either r(A)₁₆ or d(A)₁₆ (Table 1). This behavior is assumed to be caused by the electronic properties of the 2,4-dioxotriazine nucleus rather

than a steric incompatibility of triazine–purine base pairs within hetero-backbone duplexes (as compared with triazine–pyrimidine base pairs). We came to this conclusion as we observed unambiguous (yet still comparably weak) base pairing of both AspGlu(^TOO)–dodeca- and hexadecamers with the corresponding homobase sequences of DNA and TNA containing a 2,6-diaminopurine instead of adenine as the complementary base (Figure 1b).

In a careful spectroscopic study of the 5-aza analogue of uracil, Jonas et al.^[35] could not find any evidence for this compound to exist in aqueous solution in a tautomeric form other than the dilactam form, and for the solid state of another 2,4-dioxo triazine derivative, the lactam form is confirmed by an X-ray study.^[36] The comparison of the pK_a value of 2,4-dioxotriazines with that of 2,4-dioxypyrimidines is of significance: the first dissociation constant of the 6-(Boc-aminomethyl)-2,4-dioxo-1,3,5-triazine^[37] in aqueous solution, as determined by the pH-dependent UV spectrum,^[37] was found to be 6.0, that is, approximately 4 pK units lower than the pK value of uracil.^[38] Remarkably, the observed T_m value of the duplex AspGlu(^TOO)₁₆–d(D)₁₆ appears essentially unchanged at pH values in the range of pH 5.5 to 8 (Table 1, entry 21), whereas its UV spectrum indicates that the dioxotriazine rings in the duplex are deprotonated, at least in part, at pH 8 and protonated at pH 5.^[37,39]

The pronounced imbalance in the pairing behavior of the 2,4-diamino and the 2,4-dioxo member of the 1,3,5-triazine family (see Figure 1 and Table 1) augurs badly for the relevance of triazine heterocycles as a family of recognition elements of potentially primordial informational oligomer systems. Indeed, intrasystem base pairing between the homobase dodecamer and hexadecamer sequences AspGlu(^TNN)_{*n*} and AspGlu(^TOO)_{*n*} (*n* = 12 and 16) under standard conditions were found to be very weak (UV, CD) and comparable in strength with self-pairing of the hexadecamer AspGlu(^TNN)₁₆ (Table 1 and Figure 3).^[40] The (formally self-complementary) dodecamer containing the two bases in

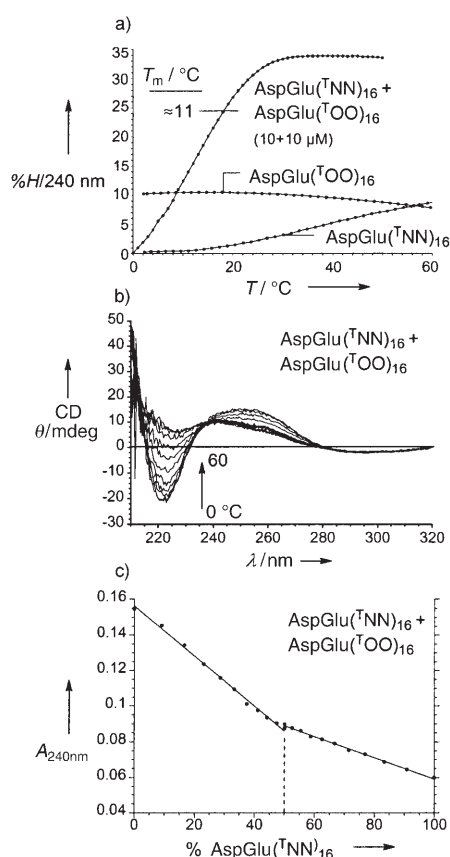


Figure 3. Intrasytem pairing experiments. a) T_m (UV) curves of the duplex formed from cross-pairing of AspGlu(TNN)₁₆ with AspGlu(TOO)₁₆; also shown is the behavior of individual partner strands; b) CD spectrum of the corresponding duplex; c) Job plot^[33] showing the 1:1 ratio of the pairing partners in the duplex formed (at 0°C). CD spectrum of AspGlu(TNN)₁₆ alone shows self-pairing, $T_m = 22^\circ\text{C}$ at 222 nm (see the Supporting Information), whereas AspGlu(TOO)₁₆ alone shows no temperature dependence in the CD spectrum. Measurements were made with AspGlu(TNN)₁₆ and AspGlu(TOO)₁₆ ($c \approx 10 \mu\text{M}$, 1:1) in 1 M NaCl, 10 mM NaH₂PO₄, 0.1 mM Na₂EDTA, pH 7.0. CD temperature increments in 5°C steps.

alternating positions AspGlu[(TNN)(TOO)]₆ fails to show a T_m value through either UV or CD measurements (Table 1, entry 26). Exploratory tests with homo-2,4-dioxotriazine sequences in the oligomer systems AspAsp and IDA_{backbone} had been equally discouraging.^[41]

The unfitness of the pair of 2,4-diamino- and 2,4-dioxotriazine nuclei for the efficient base-pairing in aqueous solution is exacerbated by the observation that oligomer strands containing 2,4-dioxotriazines are found to be appreciably more sensitive toward hydrolysis than corresponding strands that contain 2,4-dioxypyrimidines.^[42] Although UV spectroscopic T_m determinations involving AspGlu(TOO)₁₆ strands within the temperature range of 0–60°C (1°min^{-1}) did not reveal any instability of the substrate, subjecting the dioxo 16mer alone to two heating cycles reaching the temperature of 90°C under otherwise identical conditions led to a partial constitutional change involving the UV-active recognition elements which, on the basis of UV, HPLC, and MS analysis, is to be interpreted as hydrolytic opening of

dioxotriazine rings.^[37] In comparison, the 2,4-diaminotriazine ring is very stable.

The systematic screening of the pairing properties of oligomer systems, which according to the criterion of generational simplicity could be members of a potentially primordial landscape, will perhaps more often than not lead to a negation of such a membership on the basis of functional criteria. Our observations show this to be the case, presumably irrespective of backbone constitution, for oligomers containing 2,4-dioxo derivatives of 1,3,5-triazines as recognition elements. On the other hand, they also show that 2,4-diaminotriazine in dipeptidic oligomers undergoes strong base pairing in aqueous solution with 2,4-dioxypyrimidines bound to the DNA or RNA backbones. We consider this remarkable difference in base-pairing fitness of the 2,4-dioxo derivatives of 1,3,5-triazine compared with 2,4-dioxo derivatives of 1,3-pyrimidine to be relevant to etiological reasoning on nucleic acid structure. The observations described in the following communication^[45] corroborate this notion by providing further insight into the constitutional and physicochemical prerequisites that heterocycles must fulfill to be capable of efficient base-pairing in aqueous solution.

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- [2] In the present context, an oligomer system is considered to be “informational” if it can accommodate constitutional diversity in the positioning of recognition elements such that any specific constitutional pattern of them can be “read” intermolecularly and (in principle) reproduced by self-replication.
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