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Note Added in Proof. After the completion of this research and after the preparation of the final draft of this manuscript, a paper was published by Professor J. A. Pople and co-workers (*Int. J. Quantum Chem. Quantum Biol. Symp.* **1989**, *16*, 311-322) on L-alaninediamides. The numerical results of the above paper and our present paper at the 3-21G basis set level are remarkably similar. However, the purposes of the two papers are obviously different and therefore they are complementary to one another.

Registry No. OHC-Gly-NH_2, 4238-57-7; OHC-L-Ala-NH_2, 134453-10-4; OHC-D-Ala-NH_2, 54046-46-7; Ac-Gly-$NHCH_3$, 7606-79-3; Ac-L-Ala-$NHCH_3$, 19701-83-8; Ac-D-Ala-$NHCH_3$, 128657-13-6; Ac-Gly-NH_2, 2620-63-5; Ac-D-Ala-NH_2, 71806-49-0; Ac-L-Ala-NH_2, 15962-47-7.

Communications to the Editor

Molecular Recognition by Circular Oligonucleotides: Increasing the Selectivity of DNA Binding

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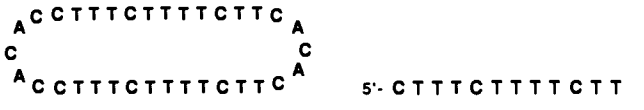
Sequence selectivity in the molecular recognition of DNA and RNA is an essential factor in the formation of polynucleotide secondary structure and in accurate copying of genetic information. In addition, recent interest in the possible use of oligonucleotides and analogues as therapeutic agents[1] has underscored the importance of the specificity of polynucleotide recognition.

We recently found that certain circular oligonucleotides can display strong binding affinities for single-stranded DNA and RNA by complexing the strand on two sides.[2] In this report it is shown that such a circular oligonucleotide can display higher sequence selectivity for its complement than does a standard DNA oligomer.

The circular compounds in this study were designed to bind strongly to complementary single-stranded purine sequences by forming hydrogen bonds from two sides of a circle to the central DNA target. Thus, a triple helical complex is formed,[3a-f] bounded by the two unpaired loop ends of the circle (see Figure 1). One side of a circle is complementary in the Watson-Crick sense (antiparallel), while the other side is complementary in the Hoogsteen sense (parallel).[4] This results in the formation of T-A-T and C+G-C base triads.[5] These pyrimidine-rich circles can thus be used to recognize purine sequences in single-stranded polynucleotides.

To measure the sequence selectivity of the circular ligand **1**, a set of complementary purine substrate oligomers with one variable base (X or Y) was constructed. Binding energies for the circle complexed with these oligomers were measured; the selectivity is defined as the free energy difference between binding of the correct sequence and the mismatched sequences. The selectivity obtained with the circular structure was then directly compared to the selectivity of standard linear oligomer **2**.



1 **2**

DNA oligomers were machine synthesized with standard β-cyanoethyl phosphoramidite chemistry. The circular ligand **1** was prepared from the linear precursor 5′-pTCTTTCCACACCTTTCTTTTCTTCACACTTCTTT and was cyclized by assembly around the template 5′-AAGAAAA-GAAAG, BrCN/imidazole being used to close the final bond.[2,6,7] The circular structure was confirmed by its resistance both to the 3′-exonuclease activity of T4 DNA polymerase and to calf alkaline phosphatase.

Thermal denaturation of the complexes was carried out in the presence of 10 mM $MgCl_2$, 100 mM NaCl, and 10 mM Tris·HCl (pH 7.0), with strand concentrations of 3 μM each. Free energies of association were obtained by fitting the data with a two-state model.[8,9]

Figure 1 shows the probe oligomers **1** and **2** hybridized with the variable-base (X or Y) oligomers. In the first set of four, T in the duplex is matched with X. These duplexes are then compared to the second set, in which opposing T's in the circle are paired with X. Similarly, in the third set, C in the duplex is matched with Y, while in the fourth, opposing C's in the circle are paired with Y.

Table I displays the results of the mismatch experiments. First, experiments 1-4 show the effects of a T-X mismatch on a DNA duplex. As expected, the true match (X = A) gives the most favorable complex ($-\Delta G°_{37}$ = 10.3 kcal/mol); the mismatches (X = G, C, T) result in a loss of 3.2-4.4 kcal/mol in binding energy, in good agreement with published mismatch studies.[10] Experiments 5-8, by comparison, show the effects of a T-X-T mismatch on circle complex strength. Once again, the true match

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(8) Thermal denaturation experiments were carried out in duplicate and the results averaged. Melting was monitored at 260 nm, and the resulting temperature vs absorbance curves showed a single transition from bound to free strands. Free energies of association were obtained by fitting the data with a two-state model.[9] In two cases the association energies were also determined from plots of log C_T vs $1/T_m$; good agreement (±7%) was seen between the two methods.
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Figure 1. Sequences of the complexes in experiments 1-16. Bases X and Y represent A, G, C, and T. Boxes mark the variable base pair or triad.

Table I. Free Energies of Association ($-\Delta G°_{37}$) and Selectivities of Oligomers **1** and **2** Hybridized to Oligomers with One Variable Base (X or Y)[a]

expt no.	variable base	T_m, °C	$-\Delta G°_{37}$, kcal/mol	selectivity, kcal/mol
duplex				
1	X = A	43.8	10.3	-
2	G	33.8	7.1	3.2
3	C	28.3	5.9	4.4
4	T	31.1	6.4	3.9
circle complex				
5	X = A	62.3	16.4	-
6	G	44.2	10.2	6.2
7	C	39.8	8.8	7.6
8	T	40.8	9.1	7.3
duplex				
9	Y = A	26.2	5.1	5.2
10	G	43.8	10.3	-
11	C	22.2	4.5	5.8
12	T	27.0	5.0	5.3
circle complex				
13	Y = A	39.9	9.0	7.4
14	G	62.3	16.4	-
15	C	41.3	9.3	7.1
16	T	39.6	8.9	7.5

[a] Uncertainty in T_m is estimated as ± 0.5 °C and in $\Delta G°$, ±5%.

(X = A) gives the most favorable complex ($-\Delta G°_{37}$ = 16.4 kcal/mol). The mismatches (X = G, T, C), however, result in a considerably larger loss of binding energy (6.2-7.6 kcal/mol) than for the duplex. Thus, the selectivity of circle **1** for its complement in this case is 6.2-7.6 kcal/mol, compared to 3.2-4.4 kcal/mol for oligomer **2**.

Similarly, experiments 9-12 give the effects of a C-Y mismatch on the duplex. The matched base (Y = G) gives a free energy of duplex association of -10.3 kcal/mol. The mismatches (Y = A, T, C) result in a loss of 5.2-5.8 kcal/mol of binding energy, once again in reasonable agreement with published data.[10] By contrast, the effects of a C-Y-C mismatch in the circle complex are greater (experiments 13-16): the match (Y = G) gives a binding energy of -16.4 kcal/mol, and the mismatches (Y = A, T, C) are less stable by 7.1-7.5 kcal/mol.

Thus, in all cases studied, the circular ligand shows greater selectivity for its correctly matched sequence than does the standard linear oligomer. The selectivity advantage ranges from 1.3-2.2 kcal/mol for the C-Y-(C) series to 3.0-3.4 kcal/mol for the T-X-(T) series. These are large differences, considering they arise from a single base change. For example, in the T-X-(T) series, the circular ligand is more selective than the linear reference oligomer by 1-2 orders of magnitude in binding constant at 37 °C.

There are two factors that may explain this high selectivity. First, because the circular ligand forms close contacts with *two sides* of the central complexed strand, it can, in effect, check the sequence twice for correct matching. A mismatch results in unfavorable interactions in both binding domains of the complex. Secondly, protonation of cytosine within a C+G-C triad may also be a factor in increasing selectivity. This protonation is likely to be favored only when there is a correct match, so that guanine can share the added proton; evidence suggests that the pK_a of cytosine within a triplex is 2-3 units higher than that of deoxycytidine monophosphate.[11,12]

We conclude that circular oligomers can have higher selectivity than can be achieved with standard Watson-Crick complementary oligomers and that they can have higher binding affinities as well. These properties are shared with other known macrocyclic hosts. For example, crown ethers and related cyclic ligands display high selectivity and strong binding for specific guests, as do the "crown nucleotides" in this study. We are currently investigating further the unusual binding properties of circular oligonucleotides and their analogues, and we anticipate that these properties may prove useful in the design of more efficient DNA- and RNA-binding molecules.

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Remarkably "Pair"-Selective and Regioselective Carbon-Carbon Bond Forming Reaction of Zirconacyclopentane Derivatives with Grignard Reagents

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We report herein (i) "pair"-selective and regioselective formation of 3-alkyl- or 2-aryl-1-zirconacyclopentanes (**1**) via ethyl-alkene coupling reactions[2] of zirconocene-alkene complexes,[3] (ii) clean and regioselective transfer of the substituted tetramethylene groups of **1** from Zr to Mg to produce **2**, and (iii) formation of **3** as the byproduct, which has been identified as **4**. We further report that

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