Pergamon

*Tetrahedron Letters*, Vol. 38, No. 48, pp. 8363-8366, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)10269-6

# Dinucleotide-Analogous Tetrapeptides. Specific Triplex Formation with Complementary Polynucleotides

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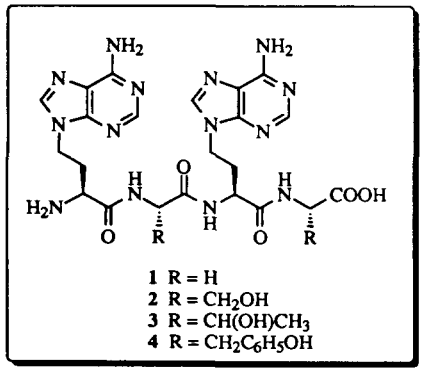
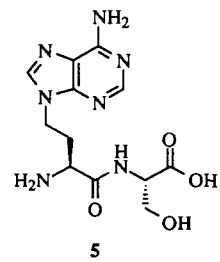
**Abstract:** Several tetrapeptides (**1** – **4**) having two adenyl moieties at the side chains interacted complementary base-specifically with poly (dT) or poly (U). The hypochromic effects and CD spectra of the complexes suggested the formation of triplexes, which are more stable than those between natural ApA and the polynucleotides.


The double-stranded helical structure of DNA is maintained in part by the stacking of complementary base pairs linked to the ribose-phosphodiester backbones.[1] Recent investigations targeting antigene or antisense strategies for modifying replication and transcription have shown that the ribose-phosphodiester linkage can be replaced with various modifications.[2] For example, the extensive work on peptide nucleic acids (PNAs) by Nielsen *et al.* showed that oligonucleotide analogs with pseudopeptide backbones can form duplexes with natural DNAs or by self-hybridization, or form triplexes composed of PNA-PNA-DNA (or -PNA).[3] Several attempts to use real peptides as alternative oligonucleotide linkages have been reported, but none of them has resulted in the successful specific recognition of DNA.[4-6] Here, we describe adenylyladenosine analogs with a real peptide backbone, which form more stable triplexes with the complementary polynucleotides than those of natural dinucleotide–polynucleotides.



**1** R = H
**2** R = $CH_2OH$
**3** R = $CH(OH)CH_3$
**4** R = $CH_2C_6H_5OH$

Fig. 1



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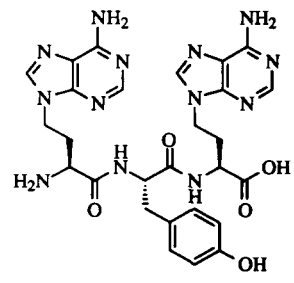
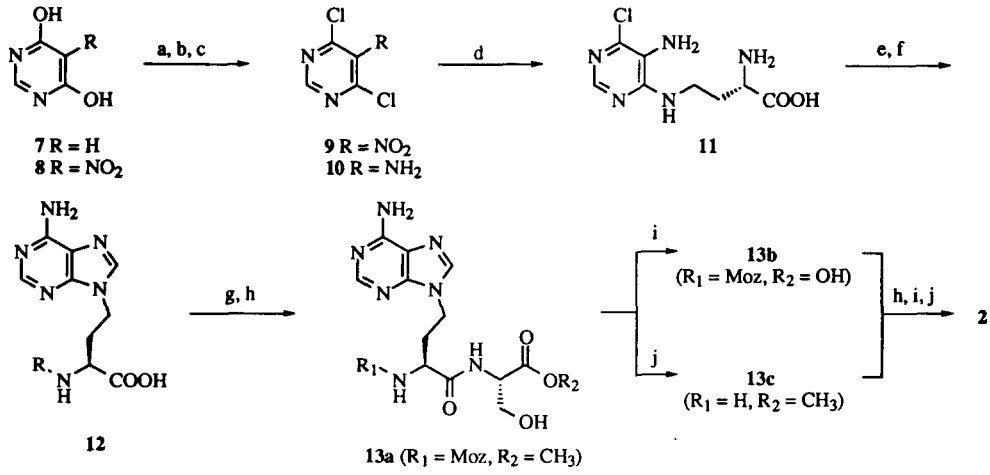


**6**

Fig. 2 Synthetic Route to the Tetrapeptide 2




(a) $HNO_3$ / AcOH (b) $POCl_3$ / $PhN(CH_3)_2$ (c) $SnCl_2$ / HCl / EtOH (d) *L*-2,4-diaminobutyric acid / TEA / DMF-$H_2O$ (e) $HC(OEt)_3$ / HCl (f) $NH_4OH$ (sealed) (g) Moz-on / TEA / dioxane-$H_2O$ (h) L-serine methyl ester / WSC / HOBt / TEA / DMF (i) NaOH / MeOH (j) TFA

On the basis of our previous related studies[7,8] and the structures of PNAs,[3] we designed and synthesized four tetrapeptides (**1** – **4**), consisting of two γ-adenyl-α-aminobutyric acid and two natural amino acid moieties linked alternately, and two related compounds (**5** and **6**, Fig. 1). These compounds were designed such as to have base-base and/or base-backbone distances similar to those of natural DNAs. Synthesis of **2** is illustrated in Fig. 2. 4,6-Dihydroxypyrimidine (**7**) was nitrated (72 %), and then the hydroxyl groups were converted to chloro groups to afford **9** (58 %). After reduction of the nitro group (61 %), **10** was reacted with *L*-2,4-diaminobutyric acid in DMF–triethylamine (TEA)–$H_2O$ at 57 °C for 2 days to give **11** in 46 % yield. This was reacted with ethyl orthoformate to construct the purine ring (80 %), and then with aqueous ammonia at 100 °C in a sealed tube to give an *L*-amino acid having an adenyl group at the side chain (**12**, 93 %).[9] **12** was used to construct tetrapeptides through the conventional peptide synthesis using the WSC–HOBt method, and compounds **1** – **4** thus obtained were purified by HPLC (ODS, $CH_3CN$ / $H_2O$ / 10 mM triethylammonium acetate buffer, pH 7.0). Their structures were confirmed by $^1H$ NMR and FAB MS.[10]

The interactions of the tetrapeptides (**1**–**4**) with polynucleotides were examined in terms of the hypochromic effects of the complexes in UV spectra. All the compounds examined showed large hypochromicity with both poly (dT) and poly (U) in 10 mM Tris / 10 mM $MgCl_2$ buffer (pH 7.4) at 0 °C (Table 1). The UV-mixing curves for interaction of each tetrapeptide and polynucleotide showed the maximum hypochromicity at the 1 : 2 molar ratio of tetrapeptide to polynucleotide. The extent of hypochromicity was similar for each compound, being approximately 40 % and 30 % for the interactions with poly (dT) and poly (U), respectively. Compound **2** did not show any hypochromicity when mixed with poly (dA) or poly (C) under the same conditions, which indicates that the interaction is complementary base-specific. In order to elucidate the structural properties and stability of the tetrapeptide–polynucleotide complexes, we examined the interactions of a natural-type dinucleotide, adenylyladenosine (ApA), with the polynucleotides. ApA exhibited similar hypochromicity (28 %) with the maximum at the 1 : 2 ratio of ApA to poly (U), but no hypochromicity was seen with poly (dT) at 0 °C.[11] The melting temperature (*Tm*) was measured to assess the stability of the complexes. The 1 : 2 complexes of the tetrapeptides with poly (dT) or poly (U) possess *Tm* values of around 20 °C, which is higher than that of ApA–poly (U) complex (Table 1). Among them, the *Tm* values of

Table 1 Interactions of Tetrapeptides (**1** – **4**) with Polynucleotides

| compound | poly (dT) | | | poly (U) | | |
|---|---|---|---|---|---|---|
| | hypochromicity [a] (%) | stoichiometry [b] (ratio) | *Tm* [c] (°C) | hypochromicity [a] (%) | stoichiometry [b] (ratio) | *Tm* [c] (°C) |
| **1** | 43 | 1 : 2 | 16 | 30 | 1 : 2 | 18 |
| **2** | 42 | 1 : 2 | 21 | 27 | 1 : 2 | 21 |
| **3** | 42 | 1 : 2 | 21 | 29 | 1 : 2 | 19 |
| **4** | 41 | 1 : 2 | 20 | 32 | 1 : 2 | 19 |
| ApA | 0 | | < 10 | 28 | 1 : 2 | 13 |

[a] The hypochromicity was calculated at 266 nm for poly (dT) and 259 nm for poly (U). No hypochromicity was oberved at 60 °C.

[b] The molar ratio of the tetrapeptide to polynucleotide which gave the maximum hypochromicity.

[c] The melting temperature (*Tm*) was measured for the complexes of 1 : 2 molar ratio of the tetrapeptide to polynucleotide at the above wavelengths.

compound **1** having glycine units with poly (dT) and with poly (U) are reproducibly lower than those of the other compounds. Thus, the tetrapeptides **1** – **4** interact base-specifically with poly (dT) and poly (U) to form 1 : 2 complexes, in the same manner as ApA, but more potently. Compound **5**, corresponding to a monomeric compound, did not interact with polynucleotides. On the other hand, compound **6**, a derivative of **4** with a tripeptide backbone, exhibited similar interactions with poly (dT) or poly (U), the *Tm* values being 20 °C or 23 °C, respectively. This result showed that the C-terminal natural-type amino acid unit is not necessary for the interaction.

CD studies afforded structural information on the tetrapeptides and their complexes with polynucleotides. In contrast to the large ellipticity observed for ApA (curve C in Fig. 2a), tetrapeptide **2** showed a weak CD spectrum (curve A), like monomeric **5** (curve B) or adenylic acid (curve D). This means that there is no significant base-stacking in the structure of **2** as well as the other tetrapeptides (not shown). On the other hand, the CD spectra of the 1 : 2 complexes of the tetrapeptides with poly (dT) resemble those of poly (dA) and poly (dT) (Fig. 2b). All of the spectra examined have large negative ellipticity at around 250 nm to similar extents. The shapes of the CD spectra at the longer wavelength region differ from each other, and tetrapeptides **2** and **4** have negative ellipticity at around 260 – 300 nm. This is considered to reflect the

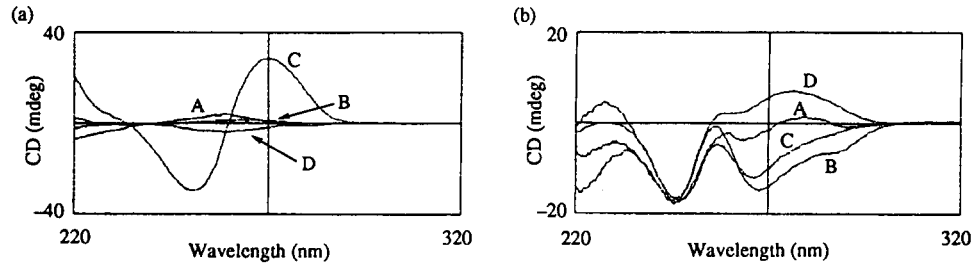



Fig. 3 (a) CD spectra of (A) **2**, (B) **5**, (C) adenyladenosine (ApA) and (D) adenosine in 10 mM Tris / 10 mM $MgCl_2$ (pH 7.4) at 0 °C. (b) CD spectra of the 1 : 2 complexes of the tetrapeptides with poly (dT) in the same buffer at 0 °C. The adenine derivative is (A) **1**, (B) **2**, (C) **4**, and (D) poly (dA).

conformation of the peptide bond, since compound **1** having achiral glycine units shows a weak band in this region.[12]

In conclusion, we have observed the stable complex formation between dinucleotide analogs with a real peptide backbone and complementary natural polynucleotides. The structures of the complexes are expected to resemble the triple helix composed of natural polynucleotides, as deduced from (1) the stoichiometry of the UV-mixing curves (1 : 2 ratio), (2) the similar extents of hypochromicity, reflecting the stacking of base pairs, and (3) the similarity in CD spectra. The ability to form the triplex or duplex depends on the electronic or conformational properties of the backbones and their lengths.[13] Therefore, it is interesting that even small molecules with a dinucleotide unit show good ability to form triplex structures. This may reflect the lack of the negative charge repulsion which exists between natural oligonucleotides, and also the relatively large stacking stabilization in the tetrapeptide–polynucleotide complexes due to the lack of base-stacking in the tetrapeptides themselves. The tetrapeptides (**2** – **4**) with different substituents at the α position of the natural amino acid moiety showed similar potencies, so further modification at this position may be feasible to increase the stability of the complexes or to modulate the DNA recognition ability. The structures of the complexes and their utility in antisense / antigene strategies are under detailed study.

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9 No detectable racemization occurred during the synthesis of **9**.

10 FAB MS data: Compound **1**, $[C_{22}H_{28}N_{14}O_5+H]^+$ 569; **2**, $[C_{24}H_{32}N_{14}O_7+H]^+$ 629; **3**, $[C_{26}H_{36}N_{14}O_7+H]^+$ 659, **4**, $[C_{36}H_{40}N_{14}O_7+H]^+$ 781,

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12 The fact that compound **1** with the lowest *Tm* values showed weak ellipticity at around 260 – 300 nm may be significant, since the ellipticity of **2**–poly (dT) complex in this region strongly decreased in 10 mM NaCl buffer (*Tm* 14 °C) compared to 10 mM $MgCl_2$ buffer (*Tm* 21 °C).

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(*Received in Japan* 29 *August* 1997; *revised* 22 *September* 1997; *accepted* 25 *September* 1997)