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Cyanuryl-PNA Monomer: Synthesis and Crystal Structure

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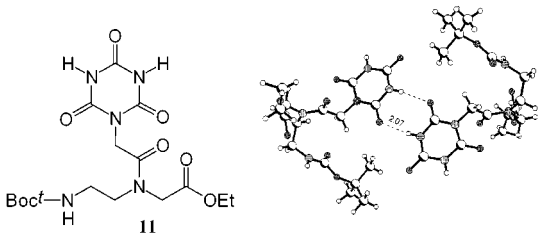
ABSTRACT



The chemical synthesis and crystal structure of the peptide nucleic acid (PNA) monomer 11 having cyanuric acid as the nucleobase is reported. The crystal structure of 11 shows molecular tapes arising from continuous intermolecular dimeric hydrogen bonding, with successive tapes held by single hydrogen bonds in the backbone.

Study of nucleic acid mimics through the design and synthesis of DNA analogues has assumed interest not only from a structural point but also from their applications as new medicinal agents.[1] Among a wide variety of structurally modified nucleic acids synthesized over the past decade, peptide nucleic acids (**1**, PNA) have come to the fore because they recognize the complementary nucleic acids through Watson−Crick base pairing and exhibit strand invasion.[2] Employing nonnatural nucleobase ligands in place of natural nucleobases would help in understanding the recognition process in terms of various factors contributing to the complementation events such as hydrogen bonding and internucleobase stacking.[3] There is increasing interest in modulating and expanding the recognition motifs of standard base pairs as this may have potential applications in diagnostics and nanomaterial chemistry.[2,4] The nonstandard nucleobases employed so far with PNA include 2-aminopurine[5] **2**, 2,6-diaminopurine[6] **3**, φ-isocytosine[7] **4**, E-base[8] **5**, hypoxanthine[9] **6**, 2-thiouracil[10] **7**, and 6-thioguanine[11] **8**, and each offered specific effects on the stability of the derived PNA:DNA hybrids.

A basic requirement for triplex formation is that the central base of the triad must be able to form hydrogen bonds from both sides, and purine bases are ideal for this purpose.[12] Cyanuric acid, **9**, a six-membered cyclic imide with alternate arrangement of hydrogen bond donors and acceptors is potentially well suited for such a purpose. It forms a network

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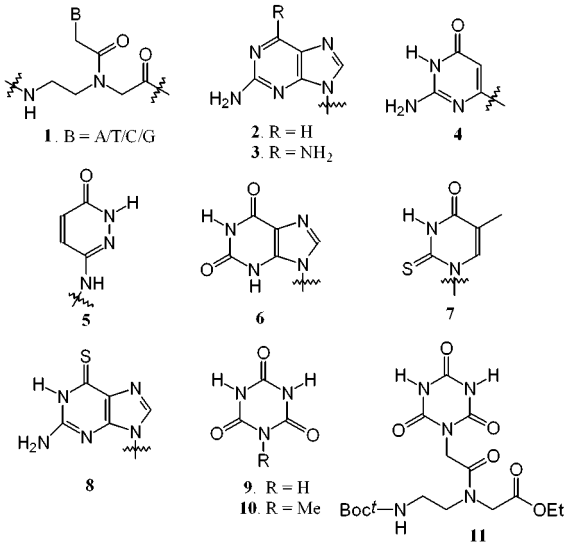
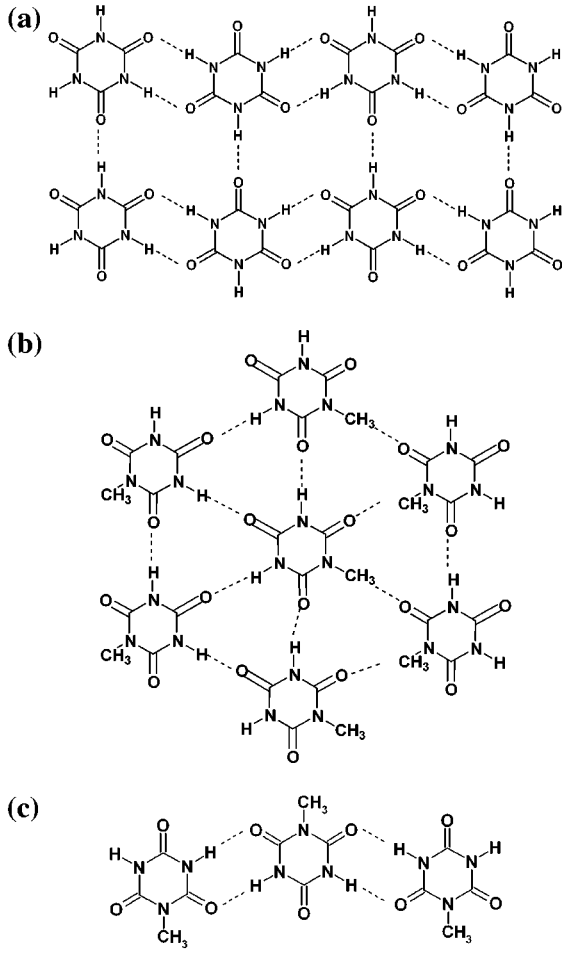


of well-defined robust hydrogen-bonded systems arranged on a molecular tape,[13] with tapes held together by single hydrogen bonds as shown in Scheme 1a. Monosubstitution of cyanuric acid perturbs this hydrogen-bonding network, as shown for *N*-methylcyanuric acid **10** which forms a hexagonal network[13d] (Scheme 1b) instead of the anticipated pattern shown in Scheme 1c. It appears that leaving a potential hydrogen-bonding moiety such as *keto* oxygen unsatisfied in the crystal structure owing to steric factors leads to an improper packing arrangement, which precluded the formation of continuous hydrogen-bonded tapes. Cyanuric acid nucleosides have been shown to originate in radiation-exposed deoxyguanosine samples,[14] and DNA oligomers containing this base have been synthesized.[15]

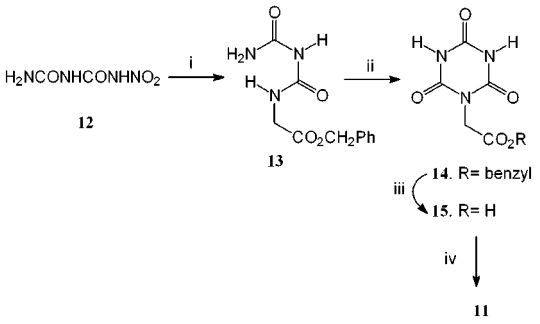
Scheme 1. Hydrogen Bonding in (a) Cyanuric Acid **9**, (b) *N*-Methylcyanuric Acid **10** (Found), (c) **10** (Expected)



The backbone moiety of nucleic acids, in the form of sugar−phosphate linkage in DNA/RNA or peptide in PNA, plays a crucial/indirect role in the formation of dimeric hydrogen bonds.[16] It would therefore be interesting to study the effect of replacing the N-CH_3 group with the putative PNA backbone on its hydrogen-bonding propensity. We report here the chemical synthesis of PNA monomer ethyl *N*-(2-Boc-aminoethyl)-*N*-(cyanuric-1-ylacetyl)glycinate **11** having cyanuric acid as the base and which can be directly used for the solid phase synthesis of PNA oligomers. Its crystal structure interestingly shows a distinct difference from **10** in the pattern of intermolecular hydrogen bonding and crystal packing, with the involvement of the backbone as well.

Selective mono *N*-alkylation of **9** is not an efficient process, unlike that of other nucleobases. Though *N*-carboxymethylcyanuric acid **15** is known to be one of the products in the hydrolysis of *N*-methylenecarboxy melamine,[17] the reported failure to isolate the pure product prompted us to explore an alternative method of its synthesis as the benzylester **14** (Scheme 2). The reaction of nitrobiuret **12**

Scheme 2. Synthesis of Cyanuric Acid PNA Monomer **11**[a]



[a] Reagents: (i) glycine benzyl ester toluene-4-sulfonate, Et_3N, DMF, 80 °C, 6 h; (ii) CDI, pyridine, reflux, 30 min; (iii) KOH, aqueous MeOH, reflux, 45 min; (iv) ethyl *N*-(2-Boc-aminoethyl)-glycinate, HOBT, 0 °C, DCC, DMF.

with the benzyl ester of glycine gave the *N*-benzyloxycarbonylmethyl biuret **13**. The next step involved construction

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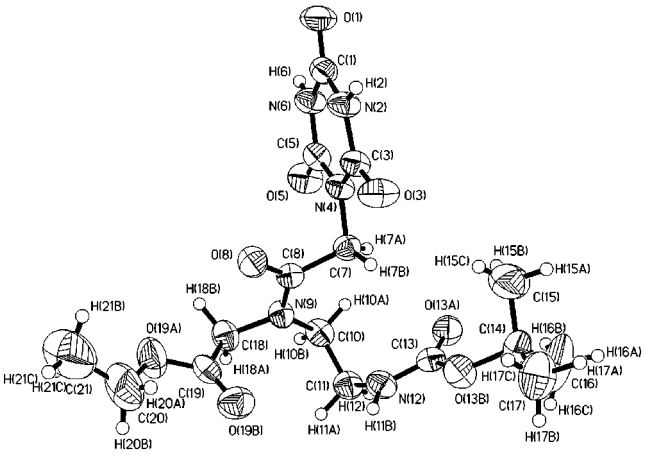


Figure 1. ORTEP drawing of an asymmetric unit in the crystal structure of **11**.

of the heterocyclic ring by carbonyl insertion−cyclization of **13**. It is known that refluxing an equimolar mixture of *N*-monosubstituted biuret and diethyl carbonate with sodium ethoxide leads to *N*-monosubstituted cyanurates.[18] However, this reaction with **13** led to an intractable mixture of products, perhaps due to the presence of the base-sensitive *N*-methylenecarboxy substituent. The procedure was hence modified by use of the more reactive carbonyl diimidazole in the presence of pyridine as base to obtain **14** in good yields. This was saponified to the free acid **15** followed by DCC-mediated coupling with ethyl *N*-(2-Boc-aminoethyl)-glycinate to give the desired product **11**. All products gave satisfactory analytical and spectroscopic data for unambiguous characterization.[19]

Compound **11** crystallizes[20] in an orthorhombic space group, *Pbca*, with one molecule in the asymmetric unit as shown in Figure 1. A packing analysis, shown in Figure 2, reveals that **11** has the anticipated centrosymmetric hydrogen-bonded dimeric structure with an H···O distance of 2.07 Å (Figure 2a). In comparison with the corresponding hydrogen bond distance in the crystal structure of **9** (H···O, 1.78 Å), the strength of the hydrogen bond in **11** is certainly weaker. However, this is not surprising since the presence of the bulky substituent at one of the heterocyclic nitrogens affects the acidity of the remaining protons. It is worth mentioning that the hydrogen bond distance noted in compound **11** is comparable with such distances found in the various molecular complexes of **9**.[13c,d] Furthermore, the adjacent molecules are held together by N−H···O hydrogen bonds formed between the *keto* oxygen of imide and the −NH of the backbone, with an H···O distance of 2.14 Å (Figure 2b). Such an arrangement ultimately leads to the formation of chains of molecules arranged in a helical mode. In the 3-dimensional lattice, the arrangement of the helices viewed down the [010] axis is shown in Figure 2c. Since such an interaction was not feasible in the crystal structure of **10**, it could not form the required dimeric hydrogen bond. It would be interesting to compare the structural features of cyanuryl PNA monomer **11** in solution (^{1}H NMR) and crystals. Earlier structural analyses of PNA monomers and dimers[21] have indicated high barriers of rotation (10−25 kcal M^{-1}) and a low rate of exchange (0.5−2 s^{-1}, 37 °C) around the tertiary amide bond (χ_1), resulting in a *cis*:*trans* ratio of 30:70. In the major *trans* form, the methylene protons next to the nucleobase are toward the 2-aminoethyl unit, while in the minor *cis* isomer, they are on the side of the glycyl unit. The DQF-COSY assignment and observance of diagnostic cross-peak between α-CH_2 of 2-aminoethyl and CH_2 adjacent

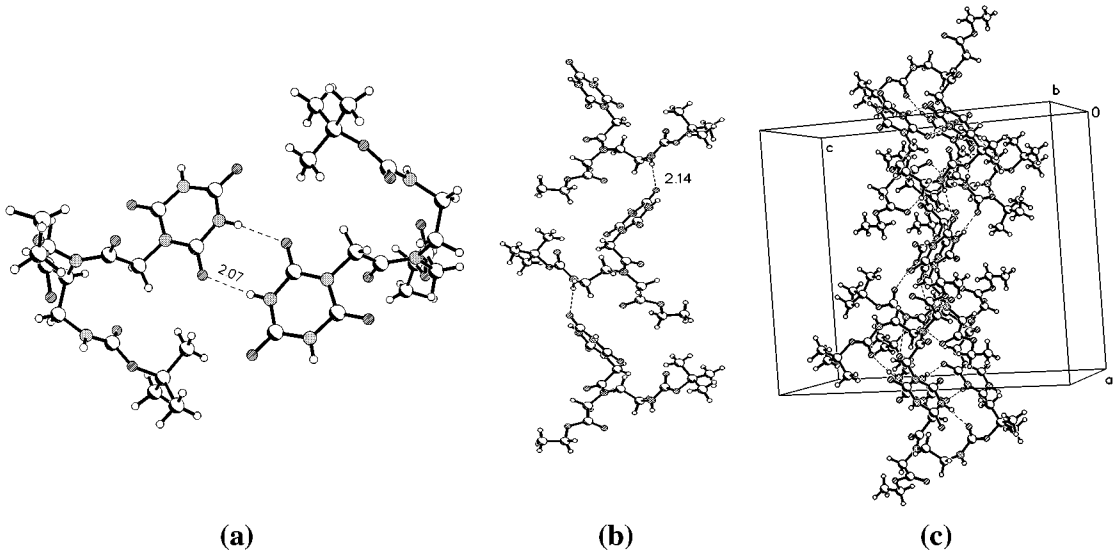
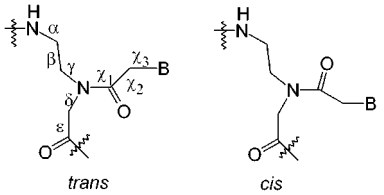


Figure 2. (a) Dimeric hydrogen bond pattern between the cyanuric acid moieties in the crystal structure of **11**. (b) Helical arrangement of adjacent molecules along [100] connected together by a N−H···O hydrogen bond formed between *keto* oxygen of cyanuric acid moiety and −NH of the backbone. (c) Three-dimensional arrangement of helices viewed down [010] in the crystal structure of **11**.

to cyanuryl residue in the NOESY ^{1}H NMR of **11** indicated the major rotamer to be the *trans* isomer,[21] identical to that of the crystal structure.



To our knowledge, the structure of **11** reported here is the first crystal structure of any PNA monomer, with the other crystal structures known being those of a T−T photodimer,[22] a PNA_2:DNA triplex,[23] and a PNA:PNA duplex.[24] Table 1 shows a comparison of some of the torsion angles found in these structures. It is shown that χ_1 in **11** is similar to that in the PNA duplex and triplex and corresponds to the *trans* form, while in the photodimer it is locked in the *cis* form. While the torsion angle χ_2 in the PNA oligomers is close to zero (planar), considerable departure occurred in **11**, perhaps to relieve the mutual repulsion of the amide carbonyl and cyanuryl ring carbonyls. While the torsion angle δ on the glycyl side was similar in both the monomer **11** and the PNA oligomers, the relative sign and magnitude of angle γ on the aminoethylene side suggests opposite conformations. The bulky *tert*-Boc group perhaps forces different values in the torsion angle α for **11** as compared to the oligomers.

Table 1. Torsion Angles[a] in Known PNA Crystal Structures

compd	α	β	γ	δ	χ_1	χ_2
11	−77	−60	−86	118	1	142
PNA dimer[22]	−88	54	−111	−108	−171	166
PNA_2:DNA[23]	−103	73	70	93	1	−175
PNA-PNA[24]						
strand 1	−112	56	73	114	5	−176
strand 2	−118	69	65	106	6	−179

[§] Defined as in ref 24.

In conclusion, we have presented a method for synthesis of cyanuryl-PNA monomer **11** that is useful in the preparation of new PNA analogues. The structural features found in the crystal data of cyanuryl monomer **11** shows conformational similarity to PNA oligomeric structures, around bonds encompassing the tertiary amide group. The results indicate a preferred *trans* orientation of the side chain carrying base in the monomer, identical to that found in the oligomeric PNA complexes. Future efforts are directed toward the synthesis of cyanuric PNA oligomers for studying the consequences on DNA recognition.

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(19) **11** and **14** are present in solution as mixtures of rotamers in ratios of 7:3 and some NMR signals are in multiples on this account. *ma*, major isomer; *mi*, minor isomer. ***N*-Benzyloxycarbonylmethyl cyanuric acid 14**: mp 242−244 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 11.75 (s, 1H), 9.70 (mi), 8.85 (mi), 8.15 (ma), 7.70 (ma), 6.75 (ma) (s, 1 H), 7.35 (s, 5H), 5.15 (m, 2H), 4.45−4.56 (m, 2H); ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ 41.62, 41.87, 42.45, 66.13, 66.25, 66.80, 118.78, 122. 07, 128.05, 128.18, 128.73, 135.78, 136.11, 148.62, 149.75, 155.00, 155.64, 158.45, 159.06, 167.97, 168.83, 170.35. Anal. Calcd for $C_{12}H_{11}N_3O_5$ (277.23): C, 51.98; H, 3.99; N, 15.16. Found: C, 51.63; H, 4.32; N, 15.41. **Ethyl *N*-(2-Boc-aminoethyl)-*N*-(cyanuric-1-ylacetyl)glycinate 11:** mp 178−80 °C; ^{1}H NMR (DMSO-d_6/$CDCl_3$, 500 MHz) δ 11.48 (ma) and 11.13 (mi) (s, 1H), 6.56 (ma) and 6.37 (mi) (br, 1 H), 4.57 (ma) and 4.43 (mi) (s, 2 H), 4.14−4.17 (mi) and 4.05 (ma) (m, 4 H), 3.40 (ma) and 3.33 (mi) (m, 2 H), 3.15 (ma), 3.03 (mi) (m, 2 H), 1.35 (s, 9 H), 1.22 (mi) and 1.17 (ma) (t, 3 H); ^{13}C NMR (DMSO-d_6/$CDCl_3$, 125.75 MHz) δ 167.35, 165.26, 165.17, 154.34, 147.99, 147.00, 59.74. Anal. Calcd for $C_{16}H_{25}N_5O_8$ (415.40): C, 46.26; H, 6.06; N, 16.86. Found: C, 46.49; H, 5.74; N, 16.56.

(20) Crystal data for **11**: ($C_{16}H_{25}N_5O_8$), orthorhombic, space group, *Pbca*, a = 18.779(1), b = 10.669(1), and c = 20.715(1) Å, V = 4150.3(1) Å^3, Z = 8, D_c = 1.330 Mg m^{-3}, λ(Mo Kα) = 0.107 mm^{-1}, $F(000)$ = 1760, λ = 0.71073 Å, $1 < \theta < 24°$ ($-17 \le h \le 20$, $-11 \le k \le 11$, $-23 \le l \le 22$), 15009 total reflections, 2985 independent reflections which were used in the refinement. The structure was solved and refined (Sheldrick, G. M.) to R_1 = 0.046 and wR_2 = 0.133. Hydrogen atoms were obtained from difference Fourier maps. Structure factors available on request from the author.

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