

γ^3 PNA: Peptide Analogue of Glycol Nucleic Acid[†]

Taedong Ok, Joohee Lee, and Hee-Seung Lee*

Molecular-Level Interface Research Center, Department of Chemistry, KAIST, Daejeon 305-701

*E-mail: hee-seung_lee@kaist.ac.kr

Received February 7, 2011, Accepted February 9, 2011

Key Words : Peptide nucleic acids, GNA

In the research of the chemical etiology of nucleic acid structure, the studies from Eschenmoser group¹ and Nielsen group² have demonstrated that the Watson-Crick base pairing can be supported by various backbone derivatives. Inspired by the groundbreaking results, other researchers have devoted their attention to finding artificial nucleic acids that can form duplexes, in which both synthetic accessibility and new characteristics for potential therapeutic (or diagnostic) use are desirable aims.³ In this context, GNA (glycol nucleic acid, Figure 1A) is a promising artificial nucleic acid because it is structurally very simple with a high atom economy and forms a duplex by Watson-Crick base pairing.⁴

Despite of its acyclic and negatively charged phosphate backbone, GNA shows exceptional duplex stability compared to that of DNA.⁵ However, the direct use of GNA for biological applications would be hampered by drawbacks such as chemical instability, promiscuous serum binding, and poor cellular uptake. In order to make GNA more practically applicable, we designed a peptide version of GNA (named γ^3 PNA, Figure 1C), in which the phosphate backbone of GNA was replaced by neutral amides, expecting that γ^3 PNA would have both binding characteristics of GNA and *aeg*PNA (Figure 1B).

γ^3 PNA is a chiral peptide nucleic acid composed of γ^3 X monomers (X = A, T, G and C), which are γ^3 -amino acids attached with the corresponding nucleic acid bases. Previous study from our group has demonstrated that incorporation of a few γ^3 T into the *aeg*PNA backbone (a chimeric PNA) can induce a preorganized helical propensity, by which the parent *aeg*PNA was endowed with an improved binding preference to a target RNA.⁶ This initial result has encouraged us to investigate the possibility of self-duplex formation of γ^3 PNA. Herein we report the synthetic methods for the preparation of monomers with other nucleobases (A, C, and G) as well as the thermal stability of γ^3 PNA duplex.

Given the protocol for the synthesis of γ^3 T, we were able to synthesize other monomers (γ^3 A, γ^3 C, and γ^3 G) with high enantiomeric purities. In contrast to the case in γ^3 T, they have nucleophilic exocyclic amino groups on the nucleobases that should be protected for the solid-phase peptide synthesis by Fmoc chemistry. After screening of acid-labile protection groups (such as Bhoc, Boc, and Mmt), we found that the best

chemical yield were obtained with Mmt for γ^3 C and γ^3 G, Boc for γ^3 A, respectively. In addition, the order of reactions at the initial steps turned out to be important for efficient synthesis. For example, in case of γ^3 C, the protection of the amino groups should be prior to the substitution reaction, whereas the other route worked better for the purine monomers (γ^3 A and γ^3 G).

The detailed synthetic procedures are summarized in Scheme 1. The common intermediate bromide **1** was easily prepared from (*S*)-epichlorohydrin (>99%ee) in three steps (34% overall yield).^{6,7} For the synthesis of γ^3 A, the bromide **1** was reacted with unprotected adenine base in the presence of sodium hydride in DMF for 48 hr at room temperature. After completion of the substitution reaction, the crude adduct was treated with excess amount of Boc anhydride, which unexpectedly resulted in bis-Boc protected product **3** in 61% overall yield. We fortunately found that only one Boc-group could be cleaved during the following ester hydrolysis to give the desired mono-Boc acid **4** in 86% yield. Subsequent reduction of azide followed by Fmoc protection provided γ^3 A in 44% overall yield (Scheme 1a).

For γ^3 G, the bromide **1** was treated with unprotected 2-amino-6-chloropurine in the presence of K₂CO₃ to get **6** in 83% yield. Mmt protection of **6** provided N²-Mmt-protected **7** in 90% yield. The transformation of chloride to carbonyl group and ester hydrolysis occurred simultaneously under the reflux condition with 10% aqueous NaOH, which gave **8** in 90% yield. The azide reduction of **8** followed by Fmoc protection furnished γ^3 G in 60% yield (Scheme 1b). In the case of γ^3 C, cytosine was reacted with Mmt-Cl to prepare N⁴-Mmt-cytosine, which was reacted with **1** to give **10** in good yield (90%). Further sequential manipulation gave the desired

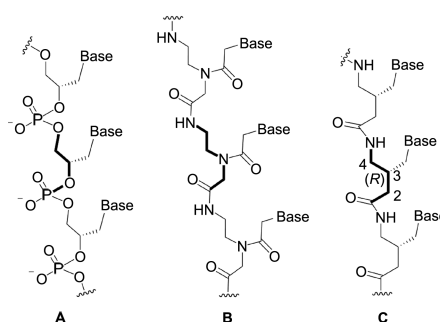
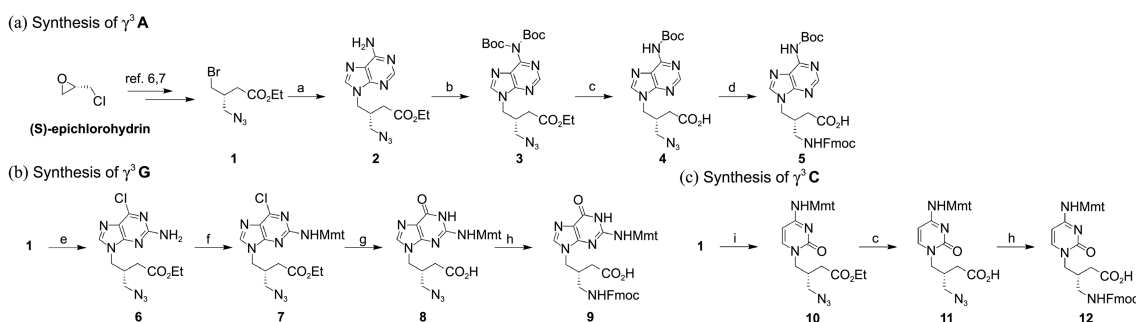


Figure 1. Structural relationship among GNA (A), *aeg*PNA (B), and γ^3 PNA(C); Base = T, A, C, or G.

[†]This paper is dedicated to Professor Eun Lee on the occasion of his honourable retirement.



Scheme 1. (a) Adenine, NaH, DMF (b) Boc₂O, DMAP, THF (2 step from **1** 61%) (c) LiOH, THF:MeOH:H₂O (6:3:1) (86% from **3**, 100% from **10**) (d) i) Me₃P, H₂O, THF, 70 °C ii) Fmoc-OSu, Na₂CO₃, H₂O/CH₃CN (44%) (e) 2-Amino-6-chloropurine, K₂CO₃, 18-Crown-6 ether, DMF (83%) (f) MmtCl, Et₃N, pyridine (90%) (g) 10% NaOH(aq), reflux (90%) (h) i) Me₃P, H₂O, THF, 70 °C ii) Fmoc-OSu, Na₂CO₃, H₂O/Acetone (60% from **8**, 40% from **11**) (i) N⁴-Mmt-cytosine, NaH, DMSO (90%).

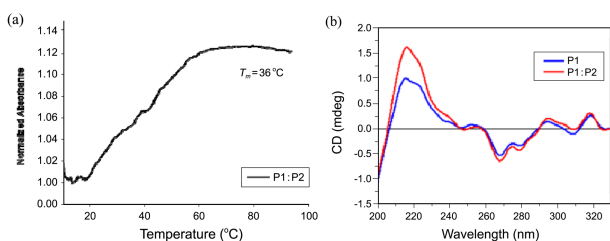


Figure 2. (a) Temperature-dependent UV melting curve. (b) Circular dichroism (CD); **P1**: H-Lys-GTAGTCACT-NH₂, **P2**: H-Lys-AGTGATCTAC-NH₂; The data were measured for the duplex (**P1:P2**, 2 μM) in a sodium phosphate buffer (10 mM, pH 7.0) containing 100 mM NaCl and 0.1 mM EDTA.

γ^3 C in 40% overall yield (Scheme 1c).

With all four monomers in hand, two strands of γ^3 PNA (**P1** and **P2**) with complementary base sequence were synthesized. To improve water solubility, single L-Lys residue was attached at the N-termini of the oligomers. The peptide coupling reaction was carried out manually on Nova TG Sieber resin, in which a solution of DIC and HOBt in DMF was used as coupling reagent and 20% piperidine in DMF was used for the deprotection of Fmoc group. The resulting γ^3 PNAs were cleaved from the resin with TFA. The crude oligomers were purified by reverse-phase HPLC, and confirmed by MALDI-TOF.⁸

To see if the γ^3 PNAs can form a stable duplex, we performed temperature-dependent UV-melting experiment at 260 nm to measure the T_m value of a 1:1 mixture of **P1** and **P2**. To our delight, we observed a typical sigmoid curve for the mixture (Figure 2a), supporting that γ^3 PNA forms a duplex through Watson-Crick base pairing. The thermal stability of the γ^3 PNA duplex **P1:P2** ($T_m = 36$ °C, measured) exceeds the stability of the analogous DNA duplex ($T_m = 29$ °C, calculated⁹) by 7 °C. To get an insight into the solution conformation of γ^3 PNA, circular dichroism (CD) study was carried out. The γ^3 PNA strand **P1** showed a significant Cotton effect (with a max. at 220 nm and a min. at around 270 nm), suggesting a helical preorganization of the backbone in the single strand (Figure 2b). The CD signal for the duplex **P1:P2** is similar to that of single strand **P1**, but showed higher intensity especially at 220 nm. The CD signal enhancement can be attributed to the duplex formation of the γ^3 PNAs. Although γ^3 PNA is structurally similar to GNA, it is still possible that the struc-

ture of γ^3 PNA duplex can be different from that of GNA duplex because the amide backbone is conformationally more rigid than the phosphodiester backbone.

In summary, inspired by the structure of GNA, we have designed a new artificial nucleic acid composed of neutral amide backbone with structural simplicity and chirality. We were able to gain access to all four types of γ^3 PNA monomers in a simple synthetic route. Preliminary studies showed that γ^3 PNA seems to form a stable 1:1 duplex with Watson-Crick base pairing. Further systematic studies are in progress on the thermal stability of γ^3 PNA in various sequences and length as well as the duplex conformation.

Acknowledgment. This work was supported by Korea Research Foundation Grant funded by Korea Government (MOEHRD, Basic Research Promotion Fund, KRF-2005-205-C00043) and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0001318).

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- Exact mass for **P1**: 2425.1413, found: 2425.2336; Exact mass for **P2**: 2425.1413, found: 2425.8586.
- Calculation using 2 μM of 5'-GTAGTCACT-3' in 100 mM salt concentration gave $T_m = 29$ °C. The calculation was done using the source code provided by <http://www.biophp.org>.