Synthesis of Peptide Nucleic Acid Dimer Containing Modified Cytosine

Yeong Deok Lee,[†] Kyung Sun Yoon,[‡] and Keun Ho Chun[†,*]

[†]*Department of Chemistry, Soongsil University, Seoul 06978, Republic of Korea.*
**E-mail: kchun@ssu.ac.kr*
[‡]*Department of Information Communication, Materials, and Chemistry Convergence Technology, Soongsil University, Seoul 06978, Republic of Korea*
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Peptide nucleic acid (PNA) has been widely used in the development of antisense drugs and gene diagnosis sensors since it was released by Neilson *et al.* in 1991.[1] Although PNA has excellent binding ability with natural DNA or RNA, it has been pointed out that it is difficult to be synthesized in a large scale, and has low cell membrane permeability. To overcome these problems, new synthetic methodologies and development of various derivatives have been proposed. Most of these derivatives were designed to increase complementary binding capacity and cell permeability by introducing a new functional group into the peptide backbone or modifying DNA bases.[2] Recently Israel researchers have found that a PNA dimer forms a specific quaternary structure by self-replication of bases at a specific pH and exhibits a unique fluorescence property, which can be used as an organic light-emitting diode (OLED) device. This result is significant in that PNA has a new application for an electronic material in addition to the biological field.[3]

To further investigate the applicability of PNA as an electronic material, we tried to make a PNA dimer with a modified cytosine base (C*, Figure 1). The C* was designed to have strong complementary bonds by G-clamp formation.[4] Therefore, we aimed to investigate the effect of modified cytosine on the self-replication pattern and fluorescence properties. For this purpose, we needed to make C*G and GC* PNA dimers. Modified base C* could be synthesized through the method described in the literature.[5]

Typical PNA oligomer synthesis is performed by solid phase peptide synthesis using fluorenylmethyloxycarbonyl (fmoc) resin. However, the synthesis of PNA dimer using PNA monomers protected by fmoc group in the N-terminal amine is very likely to occur acyl migration, a fatal side reaction, when the fmoc protecting group is removed during peptide coupling or final deprotection of fmoc group (Figure 2(a)).[6] In addition, unlike natural C, it was found the PNA monomer containing C* showed significantly reduced reactivity during peptide coupling step with G-monomer. Therefore, in our synthesis, monomethoxy trityl (MMTr) was chosen for the N-terminal amine protection of the PNA monomer because MMTr could be selectively removed at the weak acidity.

MMTr protection is not often used for PNA oligomer synthesis, but acyl migration is rarely observed in the deprotection step, and also it can be selectively removed in the presence of the Boc protecting groups, which were already existing in C*, G bases. Starting from ethylene diamine, compound **6** was synthesized in three steps. C* or G base could be coupled with **6** by EDC coupling, and hydrolysis of ester group gave C* or G monomer, respectively (**9a**, **9b**) in which N-terminal was protected with MMTr (Scheme 1).

However, the direct coupling between the PNA monomer **9a** or **9b** and other N-deprotected PNA monomers also showed severe acyl migration as a side reaction (Figure 2 (b)). To prevent such acyl migration, we first prepared a PNA unit **11**, which had no DNA base attached and tried to couple

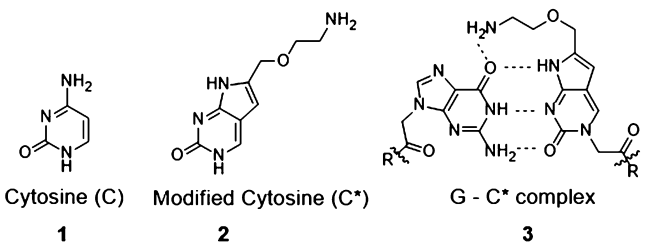


Figure 1. Modified cytosine–guanine complex.

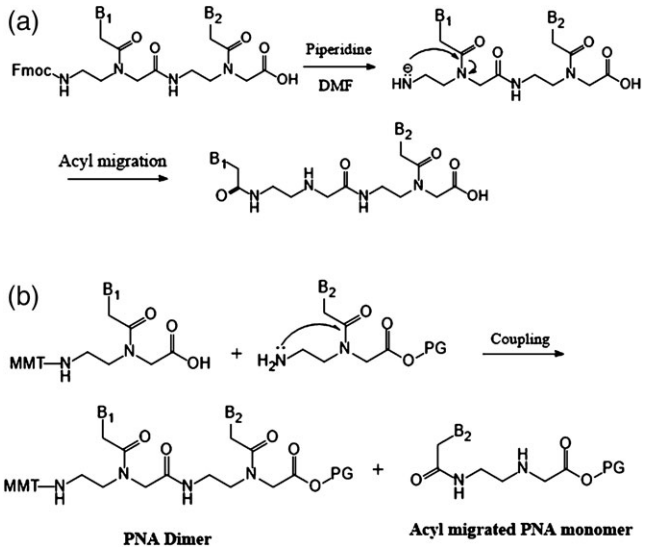
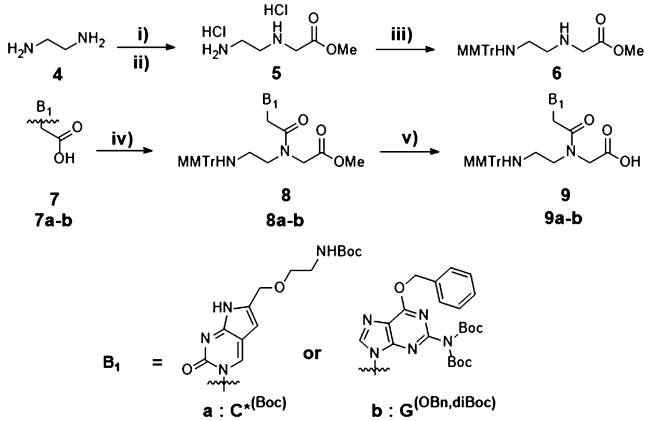
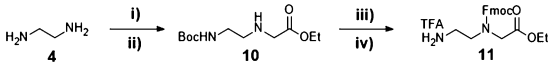


Figure 2. Acyl migration of PNA.



Scheme 1. Synthesis of C* and G PNA monomer. Reagent and conditions: (i) chloroacetic acid, 22 h, 96%; (ii) HCl, MeOH, reflux 3 h, 98%; (iii) TEA, MmtCl, CH_2Cl_2 65%; (iv) **7a** or **7b**, EDC, HOBt, CH_2Cl_2, **6**, 22 h, 91%; (v) **8a** or **8b**, 1 M LiOH, 1,4-dioxane, 6 h, 95%.

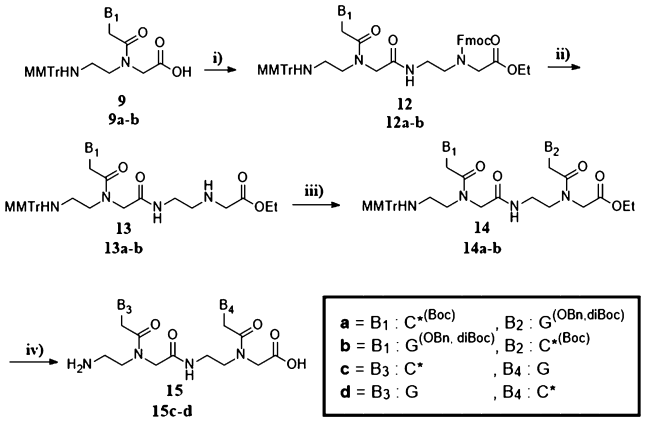


Scheme 2. Synthesis of PNA unit. Reagent and conditions: (i) $(Boc)_2O$, CH_2Cl_2, 22 h, 82%; (ii) ethyl bromoacetate, TEA, CH_3CN, 2 h, 55%; (iii) FmocCl, TEA, CH_2Cl_2, 30 min, 82%; (iv) TFA/CH_2Cl_2, 1:1, 4 h, 99%.

11 with PNA monomer **9a** or **9b** (Scheme 2). Although coupling of **11** with PNA monomer **9a** or **9b** can avoid acyl migration, the protection group of *sec*-amine of **11** should be carefully chosen. Without *sec*-amine protection, during the coupling reaction with PNA monomer, side products are generated because *sec*- and *pri*-amine are competing.[7]

In the literature, as a protecting group for *sec*-amine, a relatively rare and expensive protection group such as Alloc or Troc group is generally used.[8] However, in our synthesis, the fmoc group could be used as the *sec*-amine protecting group of **10**. The fmoc protection of *sec*-amine in **10** was successful, and coupling between **11** and PNA monomer **9a** or **9b** showed the best results in the reaction with isobutyl chloroformate (Scheme 33).[9] After the fmoc group of **12** was removed, C* or G base was introduced by EDC coupling to yield **14**. The ethyl ester of the C-terminal and the MMTr of the N-terminal were sequentially removed, and finally C*G and GC* PNA dimers were effectively synthesized.

In conclusion, the synthesis of PNA dimer containing modified cytosine required a different synthetic strategy than the conventional method in order to minimize the acyl migration and improve the yield of coupling process between PNA monomers. The N-terminal protection using MMTr was successful. Adding the second base later after attaching the PNA unit **11** first led to a significant



Scheme 3. Synthesis of C*G and GC* PNA dimers. Reagent and conditions: (i) **9a–b**, NMM, $ClCO_2iBu$, CH_2Cl_2, **11**, 60 min, 91%; (ii) 20% piperidine, CH_2Cl_2, 60 min, 55%; (iii) $C^{*(Boc)}$ acid or $G^{(OBn,\ diBoc)}$ acid, EDC, HOBt, CH_2Cl_2, **13a–b**, 2 h, 94%; (iv) **14a–b**, 1 M LiOH, 1,4-dioxane, TFA/CH_2Cl_2, 92%.

improvement in the yield, and finally the desired C*G and GC* PNA dimers could be synthesized effectively.

Supporting Information. Additional supporting information is available in the online version of this article.

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