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Peptide Nucleic Acid Synthesis by Novel Amide Formation

Hyunil Lee,* Jae Hoon Jeon, Jong Chan Lim, Hoon Choi, Yeohong Yoon, and Sung Kee Kim*

PANAGENE INC., 100, Sinsung-dong, Yuseong-gu, Daejeon 305-345, Korea
hileec@panagene.com

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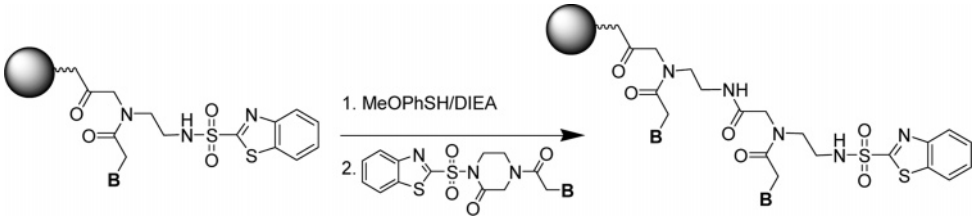
ABSTRACT



Synthesis of self-activated peptide nucleic acid (PNA) monomers and an efficient method for PNA synthesis using a benzothiazole-2-sulfonyl (Bts) group as an amine-protecting group as well as an acid-activating group are reported. Couplings were complete within 120 min, and the deprotection was performed in 10 min. This Bts strategy provides a high purity PNA oligomer and is appropriate for large-scale synthesis. The results of the 15-mer PNA oligomer are described.

Peptide nucleic acid (PNA) is a nucleic acid analogue which was first reported by Nielsen et al. in 1991[1] and has received great attention due to many favorable properties including chemical and thermal stability, resistance to nucleases and proteases, stronger and faster binding affinity to the complementary nucleic acid,[2] hybridization under low salt concentration,[3] and higher specificity and sensitivity to a single mismatch. Specially, PNA has attracted major attention at the interface of chemistry and biology because of its interesting chemical, physical, and biological properties and its potential to act as an active component for diagnostic, molecular biological, and pharmaceutical applications.

Generally, PNA oligomers are synthesized using the well-established solid-phase peptide synthesis protocol.[4] There have been significant improvements in the amide formation techniques as well as in protecting group strategies in the last several years in the field of peptide chemistry. Many kinds of coupling reagents and new protecting groups have been developed to minimize racemization and/or to improve the reactivity.[5]

As is the case in the peptide synthesis, two protection group strategies have been used for the preparation of PNA oligomers: Boc/Cbz and Fmoc/Bhoc.[6,7] However, these methods have serious drawbacks due to harsh reaction conditions and side reactions during either monomer synthesis and/or PNA oligomer synthesis.

Herein we report a new type of cyclic PNA monomer and a new efficient method of PNA oligomer synthesis

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using benzothiazole-2-sulfonyl (Bts) as an amine-protecting group.

Bts has been reported as an amine-protecting group of amino acid, and the stability of sulfonamide and the mild deprotection conditions are attractive properties for a protecting group.[8] Due to the strong electron-withdrawing effect of the sulfonyl group, the acyl group of acylsulfonamide is easily attacked by nucleophiles after alkylation which was applied in the safety-catch strategy[9] or the synthesis of a peptide thioester[10] for native chemical ligation of the peptide. Using these characteristics of Bts, we designed self-activated cyclic PNA monomers.

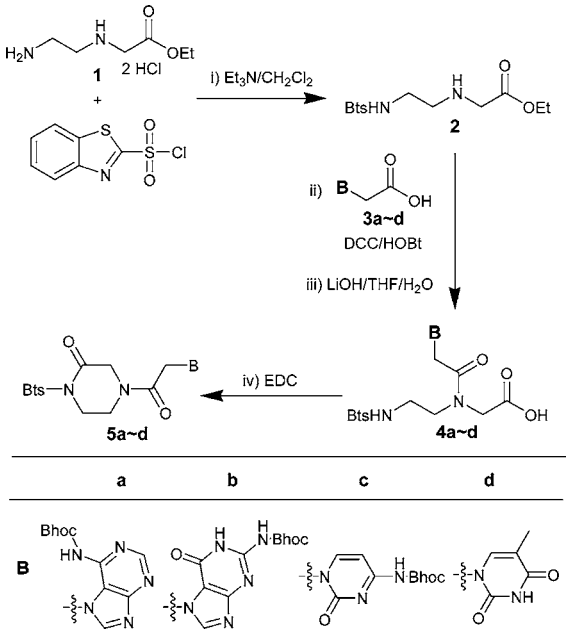
The preparation of cyclic Bts PNA monomers is shown in Scheme 1. The ethyl *N*-[(benzothiazole-2-sulfonylamino)-ethyl]glycinate (**2**) was prepared from the reaction of ethyl *N*-(2-aminoethyl)glycinate (**1**)[11] with benzothiazole-2-sulfonyl chloride[8] in 75% yield. The exocyclic amines of the nucleobase were protected with Bhoc due to its stability toward nucleophiles and ease of deprotection. The corresponding nucleobase acetic acids (**3a**−**d**) were prepared according to the reported procedures[7] and were coupled to **2** under standard coupling conditions (DCC, HOBt), followed by hydrolysis to give the corresponding acids (**4a**−**d**) in 68−82% yield. The acids (**4a**−**d**) were readily cyclized in the presence of EDC to give PNA monomers (**5a**−**d**) in excellent yields.

In these PNA monomers (**5a**−**d**), the Bts group plays an important role not only as a protecting group of the amine of the PNA backbone but also as an activating group for the coupling reaction.

Bts activates the carbonyl of piperazinone to be easily attacked by nucleophiles, such as the primary amine of PNA, and is removed under various thiols in the presence of organic base after a coupling reaction. In spite of activated structure, these monomers are sufficiently stable to be stored in DMF solution for more than a week.

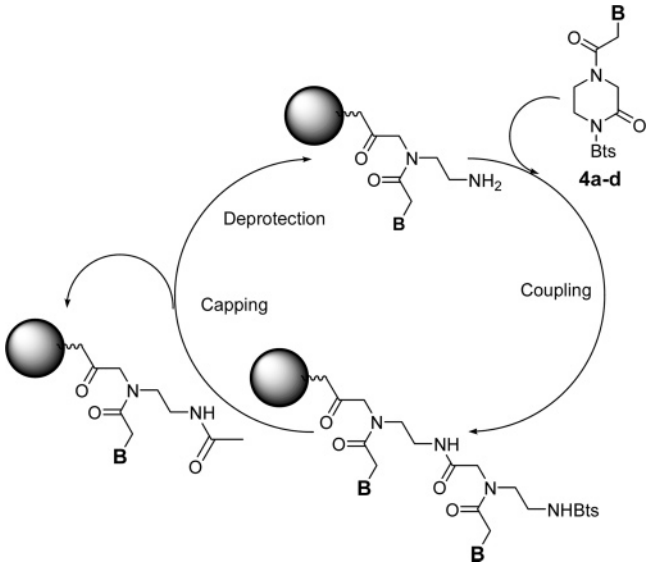
The oligomerization protocols are composed of three steps: deprotection, coupling, and capping steps as shown in Scheme 2.

Scheme 1. Synthesis of Cyclic Bts PNA Monomers (ref 12)[a]



[a] Reagents and conditions: (i) **1** (1.0 equiv), BtsCl (1.0 equiv), triethylamine (4.0 equiv)/CH_2Cl_2, 2 h, 92%; (ii) **2** (1.0 equiv), DCC (1.2 equiv)/HOBt (1.2 equiv); (iii) LiOH (1.2 equiv), THF/water (1/1), 1 h, 72% for **4a**, 68% for **4b**, 80% for **4c**, 72% for **4d** (ii−iii) overall yield); (iv) **4** (1.0, equiv), EDCI (1.2 equiv)/DMF, 6 h, 92% for **5a**, 90% for **5b**, 95% for **5c**, 93% for **5d**.

Scheme 2. Solid-Phase Synthesis of PNA Oligomer by Cyclic Bts Monomer (ref 12)



PNA oligomerization was carried out by the solid-phase synthesis on a CLEAR amino resin (Peptides International Inc., Louisville, KY) loaded with PAL linker (Advanced ChemTech, Louisville, KY) which affords the cleavage of PNA under acidic condition.

At the coupling step, the primary amine of the solid support attacks the carbonyl of piperazinone at the PNA monomer activated by Bts to give the resulting sulfonamide.

The coupling is complete within 120 min over 99%. The trace of unreacted amine of PNA is capped by Ac_2O/lutidine so as not to participate in the next coupling reaction. During the capping step, some parts of sulfonamide may be

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(12) Following abbreviations are used: DCC = dicyclohexylcarbodiimide; EDCI = 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; HOBt = 1-hydroxybenzotriazole; DIEA = *N*,*N*-diisopropylethylamine; Bts = benzothiazole-2-sulfonyl; Bhoc = benzhydryloxycarbonyl.

Table 1. Outlines of the Bts PNA Synthesis Cycles[12]

steps	reaction condition
coupling	0.3 M PNA monomer (20 equiv) and 0.2 M DIEA solution in DMF (120 min, 40 °C)
capping	(1) 5% Ac_2O and 6% lutidine solution in DMF (3 min, rt); (2) 10% piperidine in DMF (3 min, rt)
deprotection	0.8 M 4-methoxybenzenethiol and 0.4 M DIEA in DMF (10 min, 40 °C)

acetylated but can be completely deacetylated by simple treatment of 10% piperidine solution in DMF within a few minutes. The Bts group is easily removed using 1 M 4-methoxybenzenethiol and 1 M DIEA in DMF. This new oligomerization strategy does not require a preactivation step or anhydrous reaction conditions. In addition, the excessively used monomers can be recovered by a simple purification procedure of concentration and filtration since no coupling reagent is involved.

With excellent yield at each of the steps, we carried out the solid-phase synthesis of a 15-mer PNA with Bts PNA monomers. The reaction conditions are summarized in Table 1. After the final coupling step, Bts-bound PNA was cleaved from the resin by treatment with 25% *m*-cresol in TFA for 1.5 h. HPLC and matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) MS results in Figure 1 clearly show the excellent purity of synthesized oligomer (87%; area% in HPLC). The estimated average coupling yield per base is over 99%.

In conclusion, we have developed a novel strategy for the synthesis of PNA oligomers. This process provides excellent purity of PNA oligomers and is scalable since the process is simple and requires neither anhydrous reaction conditions nor the use of coupling reagents.

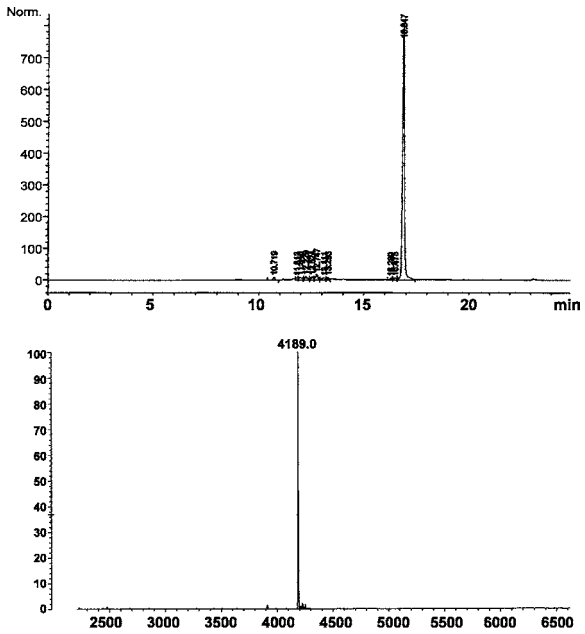

Figure 1. HPLC and MALDI-TOF analysis of crude 15-mer PNA (Bts-**ctcagcacatctaca**).

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Supporting Information Available: Experimental procedures and analytical data for the preparation of PNA monomers and their intermediates, general procedures for PNA synthesis (deprotection, coupling, and capping), and HPLC and MALDI-TOF data of the final 15-mer PNA protected with Bts. This material is available free of charge via the Internet at http://pubs.acs.org.

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