PNA Synthesis by Novel Amide Formation

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

Panagene Inc. */ 100, Sinsung-dong, Yuseong-gu, Daejeon, 305-345, Korea /* www.panagene.com

INTRODUCTION

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.

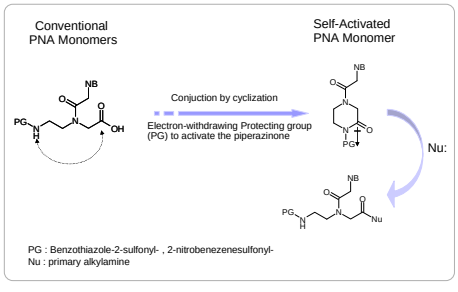
Generally, PNA oligomers are synthesized using the well-established solid-phase peptide synthesis protocol.[4] There have been significant improvements in the amide formation techniques as well as in protecting group strategies in the last several years in the field of peptide chemistry. Many kinds of 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.

Benzothiazole-2-sulfonyl (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 acyl-sulfonamide 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.

Herein we report a new type of cyclic PNA monomer and a new efficient method of PNA oligomer synthesis using Bts as an amine-protecting group.

Novel Self-Activated PNA monomers

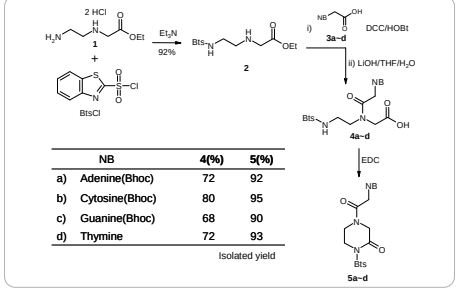
In a conventional PNA synthesis, a carboxylic group of PNA monomer is activated by coupling reagent regardless of amine protecting group. For a efficient PNA oligomerization without coupling reagents, we intended to activate the carboxylic acid by an amine protecting group and it was realized by a cyclization of N-(2-aminoethyl)glycine backbone and a selection of electron withdrawing protecting group. Sulfonyl groups were the promising candidates such as 2-nitrobenzenesulfonyl, benzothiazole-2-sulfonyl and their derivatives, which have been reported as amine protecting groups. The nucleophilicity of sulfonamide and a favorable geometry of cyclization afforded the self-activated PNA monomer.



Synthesis of Monomers

The cyclic Bts monomers are synthesized according to the method described in **Scheme 1**. A Bts protected PNA monomer backbone (**2**) was prepared from the reaction of N-(2-aminoethyl)glycine ethyl ester (**1**) with benzothiazole-2-sulfonyl chloride (BtsCl). Compound **2** was coupled with a nucleobase acetic acid (**3a ~ d**) in the presence of coupling reagent, followed by hydrolysis and cyclization to give self-activated PNA monomers (**5a ~ d**).

Scheme 1. Synthesis of cyclic Bts PNA monomers



NB	4(%)	5(%)
a) Adenine(Bhoc)	72	92
b) Cytosine(Bhoc)	80	95
c) Guanine(Bhoc)	68	90
d) Thymine	72	93

Isolated yield

Synthesis of PNA Oligomer

The oligomerization protocols are composed of three steps: deprotection, coupling, and capping step as shown in **Scheme 2** and the reaction conditions are outlined in **Table 1**. The synthesized PNA oligomers were analyzed with HPLC and confirmed by MALDI-TOF Mass Spectrometry.

Scheme 2. Solid Phase Synthesis of PNA Oligomer by cyclic Bts Monomer

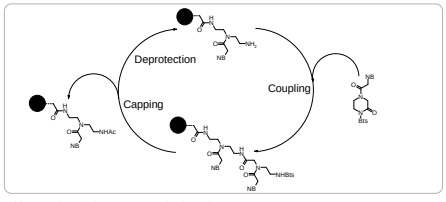


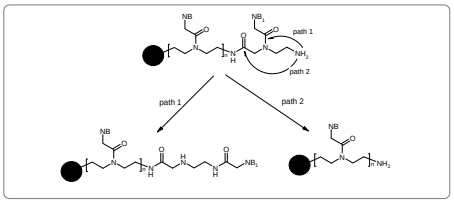
Table 1. Outlines of the Bts PNA Synthesis Cycles

steps	reaction conditions		
	reagents	time (min)	temp
coupling	0.3M PNA monomer (20 eq) and 0.2M DIEA in DMF	120	40 ºC
capping	1) 5% Ac_2O and 6% lutidine solution in DMF	3	rt
	2) 10% piperidine in DMF	3	rt
deprotection	0.8 M 4-methoxybenzenethiol and 0.4 M DIEA in DMF	10	40 ºC

Side Reaction in PNA oligomerization

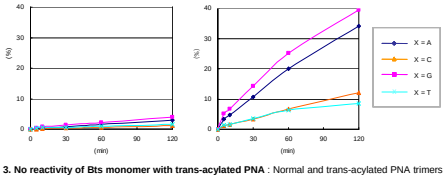
1. Trans-acylation ; Although PNA oligomers are synthesized using the well established solid phase peptide synthesis protocol, trans-acylation of nucleobase acetyl moiety is a common side reaction, because the 2-ethylamino group in PNA is more reactive than that of α-amino acid by the increased basicity and a favorable geometry (**Figure 1**). This side reaction lowers the yield and purity of PNA oligomer, and results in a PNA oligomer with strongly altered hybridization properties as well as difficulties in purification.

Figure 1. Side reactions in PNA synthesis ; path 1 : trans-acylation of the nucleobase acetyl moiety, path 2 : N-terminal detachment of the monomer.



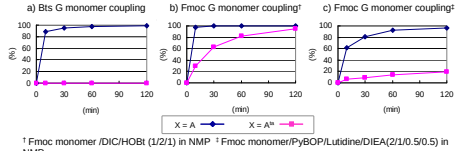
2. Study on the formation of trans-acylated PNA : We compared the formation of trans-acylated PNA under each deprotecting condition of Bts and Fmoc group, respectively. The ratio of trans-acylated PNA was analyzed with HPLC and shown in **Figure 2**. Bts deprotecting conditions showed a 5 to 8 fold lower rate of trans-acylation compared with Fmoc deprotecting conditions.

Figure 2. Trans-acylated PNA (X^{as}) of resin bound PNA trimers under Bts or Fmoc deprotecting condition.



3. No reactivity of Bts monomer with trans-acylated PNA : Normal and trans-acylated PNA trimers were synthesized from the corresponding monomer. Bts or Fmoc monomer showed a good reactivity to a normal PNA(blue diamond in **Figure 3**). Bts Monomer showed no reactivity with trans-acylated PNA but Fmoc monomer showed significant reactivity with that (red square in **Figure 3**).

Figure 3. Reaction of Fmoc or Bts G monomers with normal or tans-acylated PNA.



† Fmoc monomer /DIC/HOBt (1/2/1) in NMP ‡ Fmoc monomer/PyBOP/Lutidine/DIEA(2/1/0.5/0.5) in NMP

HPLC data of PNA oligomer using Bts cyclic monomers

By using Bts PNA monomers, various types of PNA were synthesized and their HPLC profiles are shown in **Figure 4** ~ **Figure 7.**

Figure 4. HPLC profile of Crude PNA oligomer (Bts -CTCAGCACATCTACA)

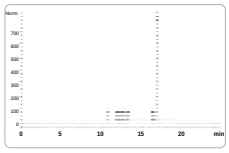


Figure 5. HPLC profile of crude peptide-PNA conjugate (peptide 10mer-O-PNA 10-mer)

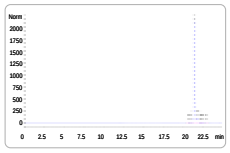


Figure 6. HPLC profile of crude bis PNA (Fmoc-PNA10mer-OOO-PNA 10-mer-k)

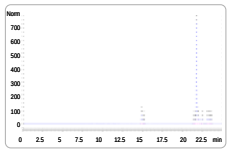
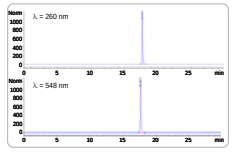


Figure 7. HPLC profile of purified labeled PNA (Cy3-OO-TAACCCTAACCCTAACCC)



CONCLUSION

We have developed a novel strategy for the synthesis of PNA oligomers. The properties of Bts monomers were summarized in **Table 2** compared with the Fmoc monomers. Bts monomers are self-activated ones and good for bulk production. Bts strategy provides excellent purity of PNA oligomers due to mild deprotecting process as well as the high coupling efficiency. And this process is scalable since the process is simple and requires neither anhydrous reaction conditions nor the use of coupling reagents. Since the technology also enables the synthesis of myriad modifications such as peptide-PNA conjugates, bis-PNA, Fluorescently labeled PNA, etc. we believe that their ready availability will enable widespread use of PNA in research, diagnostics and therapeutics.

Table 2. Summary of Bts PNA and Fmoc PNA

Monomers	Bts PNA monomer	Fmoc PNA monomer
Monomer recovery after coupling	70 ~ 80%	-
Large scale synthesis of monomers	Good	-
Solubility of monomers	Good	Moderate
Cost of oligomer synthesis	Low	High
Solvent for oligomer synthesis (Anhydrous requirement)	DMF (Not necessary)	NMP (Necessary)
Coupling reagent	None	required
Trans-acylation during deprotection	+	+++++
Reactivity of monomer with trans-acylated product	Not Detected	high

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