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Synthesis of Two New Chiral Blocks for the Construction of Peptide Nucleic Acid (PNA)

Li Gang ZHANG, Ji Mei MIN, Li He ZHANG*

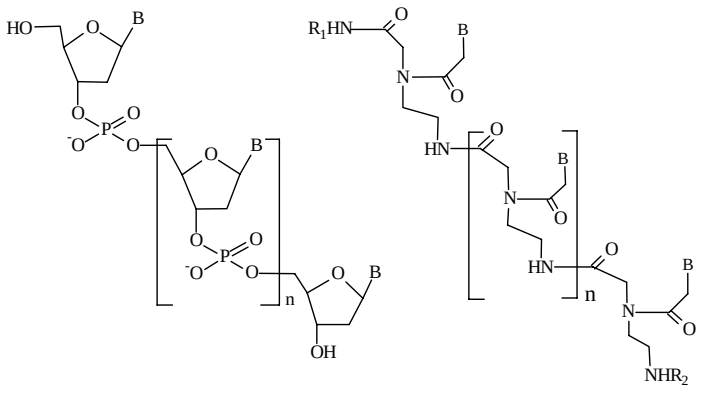
National Laboratory of Natural and Biomimetic Drugs, Beijing Medical University, Beijing 100083

Abstract: Protected (L) and (D)-lysine were used respectively as starting materials to synthesize two new types of chiral blocks for the construction of PNA. Nucleobase was linked to α-NH_2 of lysine *via* -CH_2C (O)- spacer in type I, and -C (O)- was used in type II. The corresponding oligomers were constructed in solution.

Keywords: Peptide nucleic acid (PNA); lysine; synthesis.

A new type of DNA mimic termed PNA (Peptide Nucleic Acid), was first prepared by Nielsen *et al*[1] in the early 1990s. It is an oligonucleotide analogue in which the entire deoxyribose phosphate backbone has been replaced by a chemically completely different, but structurally homomorphous achiral, uncharged peptide backbone, composed of N-(2-aminoethyl) glycine units. The nucleobases were attached to the glycine nitrogens *via* methylene carbonyl linkers (**Figure 1**). Subsequent studies have demonstrated that it can hybridize sequence-specially to complementary DNA and RNA with surprisingly high affinity, and also show very high biological stability. Ever since then, a lot of modified PNA molecules have been synthesized by various groups, including extention of the original PNA backbone at different sites, use of some other linkers for the nucleobases to

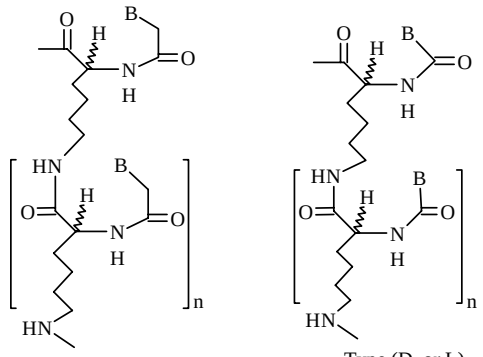
Figure 1.



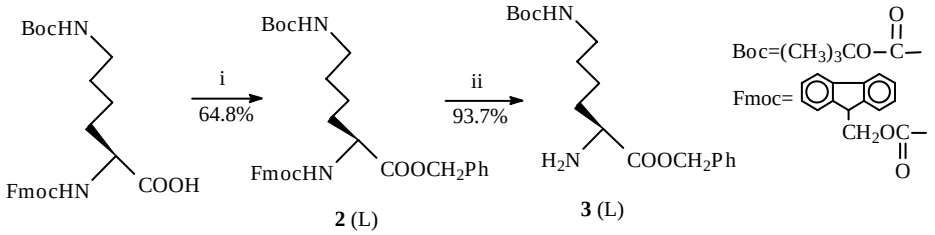
attach to the main chain, as well as introduction of chirality by replacing glycine in the original backbone with various natural and unnatural amino acids[2]. In order to understand the relationship between activities and structures, it is necessary to synthesize more PNAs of different types.

In most cases, PNAs were constructed with a lysine residue at the C-terminal of the oligomers[1, 2], which was designed to improve the solubility of the whole molecule or avoid self-aggregation. Since lysine is a natural amino acid and commercially available, we envisaged to synthesize two new types of chiral PNA molecules with lysine as the main chain unit. In type I, nucleobase was linked to α-NH_2 group of lysine by methylene carbonyl group and in type II, carbonyl was used as a linker (II) (**Figure 2**). The two PNA backbones were designed to differ from each other only in the bond numbers between the nucleobases and the main chain, so that we may have an understanding of the relationship between activities and the length of the linkers in both kinds of molecules. Although only the D-lysine series were supposed to mimic natural oligonucleotides as referring to configuration, the more readily available and inexpensive L-lysine series were also investigated. We report the construction of the corresponding blocks of both kinds herein.

Figure 2.



Scheme 1.



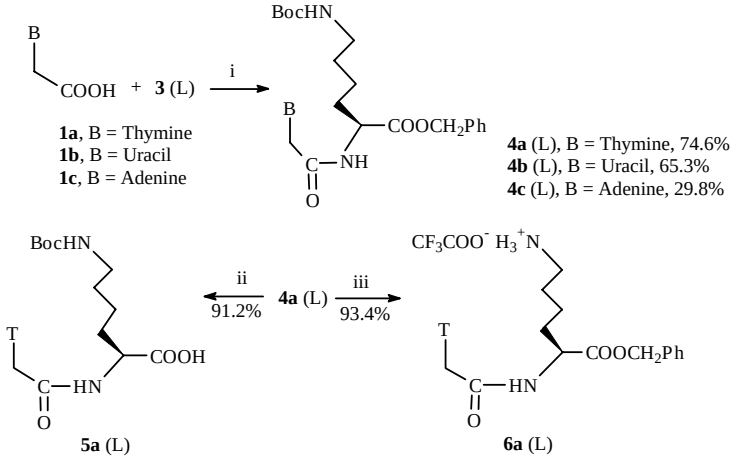
2 (L) **3** (L)

i. BnBr/TEA, DMF, r. t., 5 hr ii. Et_2NH/DMF, r. t., 5 min

Starting from Fmoc-L-Lys (Boc)-OH, the common intermediate of L-configuration of type I and type II PNA backbones, compound **3** (L), was prepared in two steps. The carboxylic acid was first converted to the corresponding benzyl ester with benzyl bromide and triethyl amine, and then Fmoc can be selectively removed by 50% Et_2NH/DMF (**Scheme 1**). The enantiomeric compound **3** (D) was prepared using exactly the same procedure with Fmoc-D-Lys (Boc)-OH as starting material.

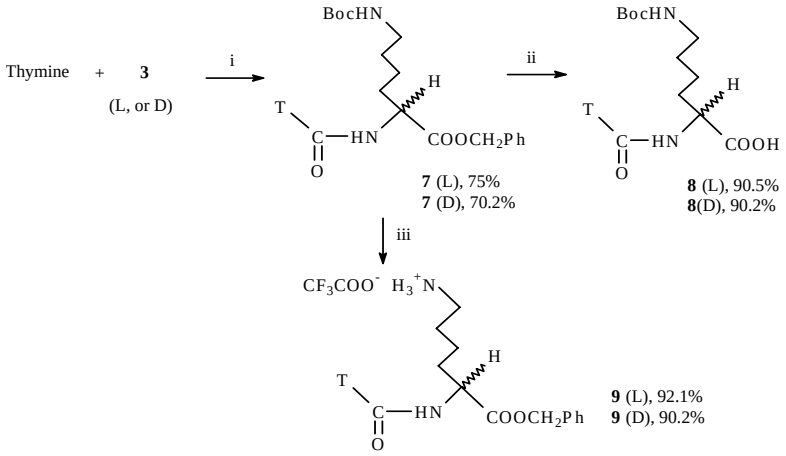
To build the PNA block of type I, the compound with free α-NH_2 **3** (L) as an example, **Scheme 2** was condensed with the base (1-yl) acetic acid[4] **1** under standard conditions. DCC and HOBT were used as coupling agents. The corresponding PNA blocks (**4**a-c)were obtained. In an aim to build the corresponding PNA oligomer, compound **5**a (L)[3] and **6**a (L) were synthesized for solid phase synthesis or synthesis in solution. Also, the D-isomers were prepared following similar routes by using compound **3** (D).

Scheme 2.



i. DCC, HOBT/DMF, 6 hrs ii. H_2, 5% Pd/C, ethanol iii. 33% TFA/DCM, r. t. , 5 min

Scheme 3



i. triphosgene, DCM ii. H_2, 5% Pd/C, ethanol iii. 33% TFA/DCM, r. t., 5 min

To synthesize the block of type II, we first tried ethyl chloroformate as a linker, but failed to get the desired product in the resulting mixtures. Phosgene is a reagent requently used to deliver a carbonyl group, but it is not so easy to handle. Thus, we finally found CO $(OCCl_3)_2$ (triphosgene), which was used as a solid substitute for phosgene in many reactions[5]. The pair of enantiomeric PNA blockss of type II were synthesized respectively by the corresponding compound **3** (L-, or D-), triphosgene, and nucleobases in one pot reactions (**Scheme 3**). Thymine derivatives of **7** (L)[6] and **7** (D) were obtained. Deprotected blocks **8** (L, D) and **9** (L, D) were used to prepare for the oligomerization reactions in solution.

A decamer has been synthesized from **5**a (L) and **6**a (L) in solution in reasonable yield.

Acknowledgments

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References

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2. G. Haaima, A. Lohse, O. Buchardt, *et al Angew. Chem. Int. Ed. Engl.*, **1996**, *35* (17), 1939.
3. Data of compound **5**a (L). ^{1}H-NMR (DMSO-d_6) δ (ppm): 1. 75-1. 18 (18H, m, $CH_3\times3$, $CH_2\times3$, 5-CH_3); 2. 89 (2H, m, ε-CH_2); 4. 17 (1H, m, α-CH); 4. 34 (2H, s, -CH_2); 6. 79 (1H, t, J=6Hz, ε-NH); 7. 42 (1H, s, 6-H); 8. 44 (1H, d, J=7. 5Hz, α-NH); 11. 27 (1H, s, N^3-H). ^{13}C-NMR (DMSO-d_6) δ (ppm): 11. 94 (5-CH_3); 22. 66 (γ-C); 28. 30 ($CH_3\times3$); 29. 13 (β-C); 31. 00 (δ-C); 49. 01 (T-CH_2); 52. 04 (α-C); 77. 39 (-C $(CH_3)_3$); 107. 83 (5-C); 142. 42 (6-C); 150. 86 (2-C=O); 155. 55 (-t-Bu-C=O); 164. 32 (4-C=O); 166. 86 (α-NH-C=O); 173. 38 (-COOH). $[\alpha]^{26}_D$-4. 58 (c 0. 24, DMSO). FAB-MS: 413 (M+1), 435 (M+23). Anal. Calcd for $C_{18}H_{28}N_4O_7$: C, 52. 42; H, 6. 84; N, 13. 58; Found: C, 52. 39; H, 7. 00; N, 13. 28.
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6. Data of compound **7** (L). ^{1}H-NMR (DMSO-d_6) δ (ppm): 1. 18-1. 85 (18H, m, t-Bu, $3\times CH_2$, 5-CH_3); 2. 88 (2H, m, ε-CH_2); 4. 48 (1H, m, α-CH); 5. 19 (2H, d, -CH_2); 6. 79 (1H, t, J=6Hz, ε-NH); 7. 33-7. 39 (5H, m, aromatic H); 8. 09 (1H, s, 6- H); 9. 68 (1H, d, J=7. 5Hz, α-NH); 11. 90 (1H, s, N^3-H). ^{13}C-NMR (ppm): 12. 11 (5-CH_3); 22. 08 (γ-C); 28. 25 ($3\times CH_3$); 29. 06 (β-C); 30. 79 (δ-C); 53. 67 (α-C); 66. 45 (-CH_2Ph); 77. 37 (Boc-C); 111. 79 (C-5); 127. 95, 128. 21, 128. 49, 133. 54 (-Ph-); 135. 71 (C-6); 150. 02 (2-C=O); 151. 73 (-ε-NH-C=O); 155. 59 (Boc -C=O); 163. 45 (4-C=O); 170. 96 (-COOCH_2Ph). $[\alpha]^{26}_D$ -1. 02 (c 0. 39, $CHCl_3$) Anal. Calcd for $C_{24}H_{32}N_4O_7$: C, 59. 01; H, 6. 60; N, 11. 47. Found: C, 58. 75; H, 6. 60; N, 11. 09.

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