Pergamon

Tetrahedron Letters, Vol. 35, No. 29, pp. 5173-5176, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01031-5

A Convenient Synthesis Of Chiral Peptide Nucleic Acid (PNA) Monomers

Larisa Kosynkina, Wei Wang and T. Chyau Liang*

Department of Biochemistry and Molecular Biology, University of Texas Medical School,
P.O. Box 20708, Houston, TX 77225

Abstract: Chiral peptide nucleic acid monomers containing amino acid side chains can be easily prepared from the BOC-protected amino acids.

Oligonucleotides (both DNA and RNA) have been extensively used in diagnostic and antisense applications. However, the susceptibility of DNA and RNA to enzymatic degradation makes them undesirable for *in vivo* application. Therefore, there has been an intense interest in the search for analogs of oligo DNA or RNA. Several modifications have been reported to give oligonucleotides with improved properties. These modifications include phosphate methyl ester,[1] phosphorothioate,[2] phosphonate[3] and other modifications replacing the phosphate linkers entirely.[4]

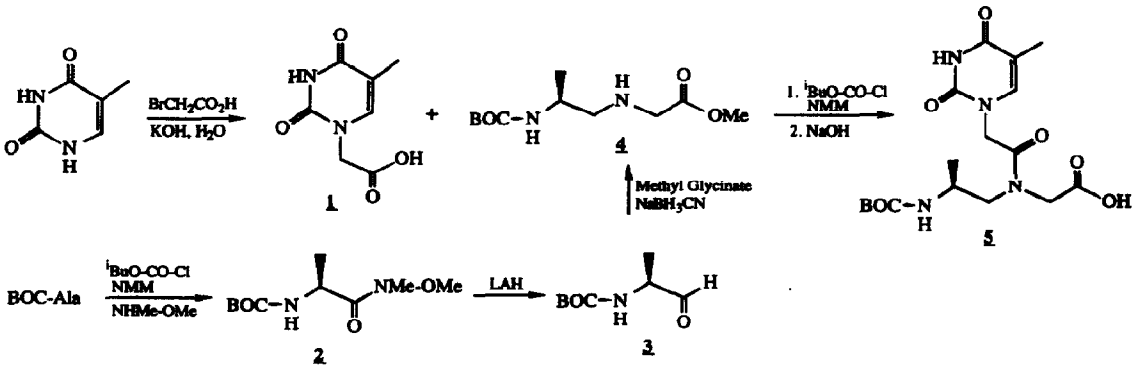
Peptide nucleic acids (PNA) are analogs of oligonucleotides with peptide bonds in the place of the sugar and phosphate backbone.[5,6] PNA have been shown to have several unique properties: (i) They can hybridize to the complementary DNA or RNA with significantly higher affinities than their oligonucleotide counterparts. (ii) They can discriminate against mismatches as effectively as (or even better than) regular oligonucleotides. (iii) They are resistant to DNase or RNase degradation. (iv) They can be prepared on large scales with relatively low cost. These advantages together make PNA the reagent of choice for many applications including antisense and *in vitro* diagnostics. For this reason, PNA has attracted much attention since they were reported by Nielsen et al..[5,6]

The tighter binding of PNA to DNA and RNA is attributable to two factors: (i) the lack of negative charge on PNA; hence, no unfavorable charge interactions between PNA-DNA or PNA-RNA duplexes, and (ii) rigidity of the peptide backbone, which arises from the sp^2 hybridization as well as the presence of tertiary amide linkages. In fact, due to the presence of tertiary amide linkage, the monomers of PNA can be detected by NMR spectroscopy as mixtures of *cis* and *trans* isomers in solution, indicating slow conversion between these isomers.

In order to probe the contribution of backbone rigidity to the PNA-DNA or PNA-RNA stability and to explore ways to enhance this rigidity, it is desirable to develop procedures for preparing PNA with modifications in the backbone. However, these modifications should be carried out in a stereospecific manner. Otherwise, one will end up with 2^n isomers for a PNA molecule with n monomers in length. Fortunately, one can incorporate residues into the backbone of PNA in a stereospecific manner by using naturally occurring amino acids. By using natural amino acids, one can also take advantages of the vast amount of information in the literature regarding the physical and chemical properties of various amino acids. For example, tryptophan containing peptides are found to form hydrogen bonds with guanine base of a nucleic acid or to intercalate into DNA.[7] In addition, incorporation of lysine or arginine side chains may confer favorable charge interactions to enhance the stability of PNA-DNA or PNA-RNA duplexes.

Towards that aim, we have developed a new synthesis of PNA monomers which allows for incorporation of various amino acid side chains into PNA in a stereo specific manner (Scheme 1). The key intermediate, **1**, which was prepared in two steps in the original synthesis reported by Nielsen et al.,[5] can be prepared in one step with good yield starting from thymine and bromoacetic acid. The other key intermediate, **4**, can be prepared by reductive amination of glycine methyl ester with a BOC-amino acid aldehyde, which in turn can be prepared by lithium aluminum hydride reduction of the corresponding BOC-amino acid N,O-dimethyl hydroxylamide. The peptide nucleic acid monomer, **5**, is then prepared by coupling **1** with **4** via mixed anhydride, followed by alkaline hydrolysis of the methyl ester. The overall yield for **5** is 49.6% based on thymine. These procedures offer a convenient synthesis of the PNA monomer with amino acid side chains in a stereospecific manner, since both lithium aluminum hydride reduction and reductive amination do not destroy the original chirality of a BOC-amino acid.

These procedures can be used to prepare the uridine-containing monomer without modification. For the synthesis of monomers containing adenosine, guanidine, and cytosine, one needs to protect the exocyclic amino groups on these bases with CBZ groups either before the bases are reacted with bromoacetic acid (cytosine) or after the base is reacted with bromoacetic acid (adenine & guanidine).[10] With these procedures, various PNA monomers with desired properties can be easily prepared. This should greatly expand the range of application involving PNA.



Scheme 1. Procedures for the synthesis of a chiral PNA monomer

Experimental Procedures:

The following procedures and Scheme 1 use BOC-L-alanine as an example. These procedures are suitable for most properly protected BOC-amino acids with the exception of Asp and Glu, whose side chain protected esters will be reduced by LAH. For these two amino acids, the corresponding BOC-protected aldehydes can be prepared via the corresponding BOC-protected alcohols according to the procedures of Chapman.[8] For protected arginine aldehyde, one can use the procedures as outlined in Scheme 1. Alternatively, one can prepare BOC-Arg(CBZ)-H according to the procedures of Bajusz et al..[9]

Thymine acetic acid (1): Thymine (3.78 g, 30 mmol) was dissolved in a solution of potassium hydroxide (6.45g, 115 mmol) in 20 ml of water. While this solution was warmed in a 40 °C water bath, a solution of bromoacetic acid (6.25 g, 45 mmol) in 10 ml of water was added over 30 minutes. The reaction was stirred for another 30 minutes at this temperature. It was allowed to cool to room temperature and the pH was adjusted to 5.5 with conc. HCl. The solution was then cooled in a refrigerator for 2 hours. Any precipitate (unreacted

thymine) formed was removed by filtration. The solution was then adjusted to pH 2 with conc. HCl and put in a freezer for 2 hours. The white precipitates were collected by filtration and dried in a vacuum oven at 40 °C for 6 hours. The yield was 4.4 g (24 mmol; 80% of theoretical yield based on thymine). TLC: R_f = 0.2 (product) and 0.45 (thymine) in EtOAc/MeOH/AcOH (75:20:5). ^{1}H-NMR (D_2O): δ 7.0 (s, 1H), 4.4 (s, 2H), and 2.0 (s, 3H).

BOC-L-Alanyl-N-methoxy-N-metylamide (2): BOC-L-alanine (1.89 g, 10 mmol) and N-methylmorpholine (1.1 ml, 10 mmol) were dissolved in 60 ml dry THF. While the solution was stirred at -20 °C, isobutyl chloroformate (1.3 ml, 10 mmol) was added in one portion. After 2 minutes, triethylamine (1.53 ml, 11 mmol) was added, followed by a solution of N,O-dimethylhydroxylamine hydrochloride (0.97 g, 10 mmol) in 20 ml of DMF, which had been chilled to the same temperature. After stirring for 30 minutes at -20 °C, the reaction mixture was allowed to warm to room temperature. White precipitates were removed by filtration. The filtrate was diluted with 70-80 ml of water and THF was removed in vacuo. The solution was then cooled in freezer for 3 hours. White precipitates were collected by filtration and briefly washed with deionized water. After drying in a vacuum oven at 40 °C for 3 hours, the product weighed 1.7 g (73% of theoretical yield based on BOC-L-alanine). TLC: R_f = 0.56 in $CHCl_3$/EtOAc/AcOH (100:99:1). ^{1}H-NMR ($CDCl_3$) δ 5.7 (br.s, 1H), 4.6 (m, 1H), 3.8 (s, 3H), 3.2 (s, 3H), 1.4 (s, 9H) and 1.2 (d, 3H).

BOC-L-alaninal (3): A suspension of $LiAlH_4$ (152 mg, 4 mmol) in 10 ml dry THF was stirred under nitrogen in a flask sealed with a rubber septum. A solution of **2** (1.62 g, 7 mmol) in 30-40 ml of THF was injected with a syringe. After 30 minutes, a solution of $NaHSO_4$ (2.4 g, 20 mmol) was added and the reaction mixture was extracted with EtOAc (3x25 ml). The combined organic extracts were washed with 5% sodium bicarbonate and saturated sodium chloride. The organic layer was then dried ($MgSO_4$) and evaporated on a rotavap to give a white solid, which was dried over night in a vacuum oven (40 °C) to give 1.15 g of the desired product (95% of theoretical). TLC: R_f = 0.7 in $CHCl_3$/EtOAc/AcOH (100:99:1). ^{1}H-NMR ($CDCl_3$) d 9.7 (s, 1H), 5.2 (s, 1H), 4.3 (m, 1H), 1.5 (s, 9H) and 1.4 (d, 3H).

Methyl N-(2-BOC-aminopropyl)glycinate trifluoroacetate salt (4): A solution of **3** (1.38 g, 8 mmol), glycine methyl ester HCl salt (5.0 g, 40 mmol) and $NaBH_3CN$ (0.5 g, 8 mmol) in 50 ml of dry methanol was stirred at room temperature for 3 hrs. At this time, the reaction was complete and methanol was removed in vacuo. The residue was dissolved in water, adjusted to pH 3.0 with 1N HCl and purified on a low pressure C-18 column (LoBar LiChroPrep, 3.0x31cm, from E. Merck) to give 2.44 g of pure product after lyophilization (84% of theoretical yield based on **3**). TLC: R_f = 0.5 in n-butanol/acetic acid/water (8:2:2, v/v). ^{1}H-NMR ($CDCl_3$): δ 5.4 (d, 1H), 4.05 (m, 1H), 3.9 (dd, 2H), 3.8 (s, 3H), 3.2 (d, 2H), 1.4 (s, 9H) and 1.2 (d, 3H).

N-(2-BOC-aminopropyl),N-(Thyminylacetyl)glycinate (5): Thymine acetic acid, **1**, (0.73 g, 4 mmol) and N-methylmorpholine (0.44 ml, 4 mmol) were dissolved in 10 ml of DMF. While the solution was stirred at -20 °C, isobutyl chloroformate (0.52 ml, 4 mmol) was added in one portion. After 2 minutes, triethylamine (0.56 ml, 4 mmol) was added, followed by a solution of **4** (1.24 g, 3.44 mmol) in 3 ml of DMF, which had been chilled to the same temperature. After stirring for 30 minutes at -20 °C, the reaction mixture was allowed to warm to room temperature. White precipitates were removed by filtration. The filtrate was dried in vacuo to give a yellow oil, which was dissolved in 20 ml of 1N NaOH. The mixture was stirred at room temperature for 1 hour. This was then acidified to pH 2.5 with 1N HCl and purified on a low pressure reverse phase C-18 column (LoBar, LiChroPrep, 3.0x31 cm from E. Merck) to give 0.85 g of white powder after lyophilization (62% of theoretical yield based on **4**). TLC: R_f = 0.34 for the product in EtOAc/MeOH/AcOH (75:20:5). ^{1}H-NMR (cis + trans isomers; D_2O) δ 7.38 (s, 1H), 4.78 (m, 2H), 4.4-4.0 (m, 3H), 3.8 (m, 1H), 3.5 (m, 2H),

1.9 (s, 3H), 1.4 (s, 9H), and 1.2 & 1.1 (d, 3H); FAB-MS: m/e = 399.2 (MH^+); $[\alpha]_{546}^{20}$ = -19.5 + 0.5 (c=2.0 in methanol).

Acknowledgment: This work was supported by a grant from SynPep Corporation (Dublin, CA).

REFERENCES:

1. For Review see: Uhlman, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 544-584.
2. Stein, C.; Mori, K.; Loke, S.; Subasinghe, C.; Shinozuka, K.; Cohen, J.; Neckers, L. *Gene*, **1988**, *72*, 333-341.
3. For review see: Miller P. S. *Bio/Technology*, **1991**, *9*, 358-362.
4. Matteucci, M. *Tetrahedron Lett.* **1990**, *17*, 2385-2388.
5. Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O., *Science*, **1991**, *254*, 1497-1500.
6. Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H., *J. Amer. Chem. Soc.* **1992**, *114*, 1895-1897.
7. Rajeswari, M. R.; Bose, H. S.; Kukreti, S.; Gupta, A.; Chauhan, V. S.; Roy, K. B. *Biochemistry* **1992**, 31, 6237-6241.
8. Chapman, K. T. *Bioorg. & Med. Chem. Lett.* **1992**, *2(6)*, 613-618.
9. Bajusz, S.; Szell, E.; Bagdy, D.; Barabas, E.; Horvath, G.; Dioszegi, M.; Fittler, Z.; Szabo, G.; Juhasz, A.; Tomori, E.; Azilagyi, G. *J. Med. Chem.* **1990**, *33*, 1729-1735.
10. Egholm, M.; Behrens, C.; Christensen, L.; Berg, R. H.; Nielsen, P. E.; Buchardt, O. *J. Chem. Soc., Chem. Commun.*, **1993**, 800-801.

(*Received in USA* 14 *April* 1994; *revised* 23 *May* 1994; *accepted* 25 *May* 1994)