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Preparation and Determination of Optical Purity of γ-Lysine Modified Peptide Nucleic Acid Analogues

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Peptide nucleic acids (PNAs) are DNA analogues in which the nucleic acid backbone is replaced by a pseudopeptide backbone and nucleobases are attached to the backbone by methylene carbonyl linkers. γ-Carbon modification of the PNA structure allows monomers, and subsequently oligomers, with improved properties to be obtained. In this study, we report the convenient synthesis of γ-lysine-modified PNA monomers for pyrimidine bases (thymine and cytosine) with high optical purity (> 99.5%) and direct enantiomer separation of γ-lysine-modified PNA analogs, using chiral HPLC to determine the optical purity.

Key words: Peptide nucleic acid, γ-Lysine modified PNA, Optical purity

INTRODUCTION

Peptide nucleic acids (PNAs) are neutral DNA mimics that bind to complementary DNA or RNA sequences with high affinity and sequence specificity (Nilsen et al., 1991). Recently, a number of modifications to the basic PNA monomeric units have been reported in order to improve the binding stability to the complementary nucleic acid and the specificity of complexation (Summerton and Weller, 1997; Koshkin et al., 1998; Obika et al., 1998; Falkieicz et al., 1999; Hickman et al., 2000; Sforza et al., 2003; Noguchi and Asanuma, 2007; Dose and Seitz, 2008; Ishizuka et al., 2008). Among them, the most promising modification is the introduction of the stereogenic center at the γ-carbon atom of the *N*-aminoethylglycine unit (Englund and Appella, 2005; de Koning et al., 2006; Dragulescu-Andrasi et al., 2008; Sahu et al., 2009). Compared to unmodified normal PNAs, the γ-modified PNAs have several advantages such as improved solubility, better permeability to cells, increased stability of PNA-DNA duplexes, and opportunities for further functionalization (Englund and Appella, 2005; de Koning et al., 2006; Dragulescu-Andrasi et al., 2008; Kleiner et al., 2008; Sahu et al., 2009; Totsingan et al., 2009). Therefore, the preparation of chiral PNA monomers with high optical purity for PNA oligomers, which afford high binding affinity to the complementary nucleic acids, has become an important research area. To that end, indirect analytical methods using HPLC, GC and ^{19}F-NMR to determine the optical purity of PNA monomers and/or oligomers have been developed and applied (Szokan et al., 1988; Corradini et al., 1999; Sforza et al., 2003; Sahu et al., 2009). However, all these indirect analytical methods require chiral derivatization or hydrolysis and consequently have the potential complication of racemization during analyte preparation.

In this paper, we report the preparation of enantiopure γ-lysine-modified fluorenylmethoxycarbonyl (Fmoc) PNA monomers and the convenient direct enantiomer separation for Fmoc-protected PNA intermediates and monomers without any derivatization for determination of the optical purity. This is the first report of direct enantiomer separation of γ-lysine-modified Fmoc-protected PNA intermediates and monomers by chiral HPLC.

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MATERIALS AND METHODS

Chemicals and general experimental procedures

Boc: *tert*-butoxycarbonyl; DCC: *N,N*-dicyclohexylcarbodiimide; DhbtOH: 3-hydroxy-1,2,3-benzotriazin-4(*3H*)-one; DIPEA: diisopropylethylamine; DMF: *N,N*-dimethylformamide; DMSO: dimethyl sulfoxide; Fmoc: fluoren-9-yl-methoxycarbonyl; HBTU: *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; THF: tetrahydrofuran. All starting materials, reagents and dry solvents were purchased from the commercial suppliers (Sigma Aldrich and Fluka) and were used without further purification. Flash-column chromatography was performed on Merck Kieselgel 60 (230-400 mesh). Thin-layer chromatography (TLC) was performed on Merck Kieselgel 60 TLC plates. NMR spectra were recorded on a Varian Inova 600 MHz spectrometer. Chemical shifts (δ values) are in ppm relative to DMSO-d_6 (2.50 ppm for proton and 76.9 ppm for carbon). Electrospray mass spectra (ESI-MS) were obtained using LTQ mass spectrometer and nominal mass measurement. Chromatography was performed at room temperature using an HPLC Breeze system consisting of a Waters model 1525 binary pump, a Rheodyne model 7125 injector with a 20 μL loop, and a dual absorbance detector (Waters 2487 detector). Chiralpak IB column (250 mm L × 4.6 mm I.D., 10 μm) was purchased from Daicel Chemical Company. The chromatographic conditions were methanol-ethanol-hexane = 5:15:80 (v/v/v) for Fmoc-L-Lys(Boc)-PNA backbone (**4**) and alcohol-hexane-trifluoroacetic acid = 15-20:80-85:0.1 (v/v/v) for γ-lysine-modified PNA monomers as the mobile phase at 1.0 mL/min of flow rate with UV detection at 254 nm.

Procedure for the preparation (from 2 to 6_T and 6_C)

Fmoc-L-Lys(Boc)-N,O-dimethyl (2)

This compound was prepared according to the previously reported methods (Kleiner et al., 2008; Totsingan et al., 2009).

Fmoc-L-Lys(Boc)-PNA backbone (4)

To a solution of Fmoc-L-Lys(Boc)-N,O-dimethyl (23.0 g, 45.0 mmol) in dry THF (150 mL) was added dropwise 2 M solution of LAH in THF (22.5 mL, 45 mmol) at −78°C. The reaction mixture was stirred at −78°C until HPLC showed no starting amide. The reaction mixture was quenched with H_2O (50 mL) and ethyl acetate (50 mL) mixture with stirring below −10°C. Extracted ethyl acetate mixture was evaporated below room temperature. Glycine ethyl ester HCl (6.28 g, 45.0 mmol) was added to the residue and dissolved in methanol (200 mL). The reaction mixture was cooled to 0°C in an ice bath and $NaBH_3CN$ (2.83 g, 45.0 mmol), CH_3CO_2H (2.57 mL, 45.0 mmol) and DIEPA (7.84 mL, 45.0 mmol) were added to the stirred solution. The reaction was then allowed to stir for 2 h at 0°C. After the solvent was evaporated, the residue was dissolved in ethyl acetate (150 mL) and washed with saturated $KHSO_4$, saturated $NaHCO_3$ and brine. The organic layer was dried over magnesium sulfate, filtered, and evaporated to obtain an oil. The oil was purified by flash chromatography (ethyl acetate-hexane, 1:1). The product was a pale yellow oil. Yield: 16.5 g (2 steps, 68%).

^{1}H-NMR (600 MH, DMSO-d_6): δ 7.83 (d, 2H, Fmoc-*H*), 7.65 (d, 2H, Fmoc-*H*), 7.36 (t, 2H, Fmoc-*H*), 7.27 (t, 2H, Fmoc-*H*), 7.03 (d, 1H, Fmoc-N*H),* 6.71 (t, 1H, Boc-N*H*), 4.15-4.28 (m, 3H, Fmoc-CH_2-O + Fmoc-C*H*), 4.02 (q, 2H, CO_2-CH_2), 3.21-3.42 (m, 3H, N-C*H*-CH_2-N + N-CH_2-CO), 2.83-2.85 (m, 2H, N-CH-CH_2-N), 2.45-2.47 (m, 2H, Boc-NH-CH_2), 1.30-1.52 (m, 6H, Boc-NH-CH_2-CH_2-CH_2-CH_2), 1.43 (s, 9H, *tert*-butyl-CH_3), 1.17 (t, 3H, CO_2-CH_2-CH_3). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 172.82, 156.64, 156.22, 144.62, 141.39, 128.16, 127.61, 125.90, 120.77, 77.91, 65.75, 60.53, 53.31, 51.34, 50.92, 47.49, 32.59, 30.14, 28.94, 28.87, 23.50, 14.78, 14.73. ESI-MS: found 540 (MH^+), calcd 540; for $C_{30}H_{41}N_3O_6$.

Fmoc-L-Lys(Boc)-PNA-thymine-OEt monomer (5_T)

Carboxymethylthymine (0.86 g, 4.68 mmol) was dissolved in dry DMF (20 mL) at 0°C, together with DhbtOH (0.76 g, 4.68 mmol) and DCC (0.97 g, 4.68 mmol). The solution was stirred for 1.5 h at 0°C, then for 30 min at room temperature. The *N,N*-dicyclohexylurea was then filtered and the Fmoc-L-Lys(Boc)-PNA backbone (1.25 g, 2.34 mmol) in DMF (20 mL) was added to the mixture. The solution was stirred for 2 h and the DMF was evaporated. The residue was dissolved in ethyl acetate and washed with saturated $KHSO_4$ (twice) and saturated $NaHCO_3$ (twice). The organic layer was dried over magnesium sulfate and filtered. After the solvent was removed, the residue was purified by solidification with diethyl ether (50 mL) to afford 1.42 g (yield: 86%) of $\mathbf{5_T}$ as a pale yellow solid.

^{1}H-NMR (600 MHz, DMSO-d_6): δ major rotamer 11.25 (s, 1H, imide-N*H*), 7.84 (d, 2H, Fmoc-*H*), 7.63 (m, 2H, Fmoc-*H*), 7.06-7.38 (m, 6H, Fmoc-*H* + thymine-*H* + Fmoc-N*H*), 6.73 (m, 1H, Boc-N*H*), 3.89-4.71 (m, 9H, thymine-CH_2-CO, N-CH_2-CO_2H + Fmoc-CH-CH_2-O + CO_2CH_2 + Fmoc-C*H*-CH_2), 2.84-3.65 (m, 5H, Fmoc-NH-CH-CH_2-N, Fmoc-NH-C*H*-CH_2-N + Boc-NH-CH_2),

2.04-2.15 (m, 2H, Boc-NH-CH_2-CH_2), 1.64 (s, 3H, thymine-CH_3), 1.35 (s, 9H, *tert*-butyl-CH_3), 1.42-1.54 (m, 4H, Boc-NH-CH2-CH_2-CH_2), 1.42 (t, 3H, $CO_2CH_2CH_3$); minor rotamer 1.68 (s, 3H, thymine-CH_3), 1.33 (s, 9H, *tert*-butyl-CH_3). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 169.83, 169.40, 168.48, 168.24, 165.02, 156.74, 156.60, 156.23, 151.60, 144.65, 144.51, 144.45, 142.49, 141.41, 128.33, 127.75, 125.70, 120.72, 108.82, 108.78, 77.97, 65.95, 65.58, 61.11, 52.17, 50.37, 48.84, 48.43, 47.46, 31.87, 29.96, 28.94, 28.88, 23.49, 14.59, 12.56. ESI-MS: found 723 (MNH_4^+); 728 (MNa^+), calcd 723; 728, for $C_{37}H_{47}N_5O_9$.

Fmoc-L-Lys(Boc)-PNA-cytosine(Bhoc)-OEt monomer (5_C)

^{1}H-NMR (600 MHz, DMSO-d_6): δ major rotamer 10.94 (s, 1H, Bhoc-N*H*), 6.70-7.84 (m, 22H, Fmoc-*H* + Bhoc-*H* + cytosine-*H* + Fmoc-N*H* + Boc-N*H*), 3.90-4.84 (m, 10H, Bhoc-C*H* + Fmoc-C*H*-CH_2 + Fmoc-CH-CH_2-O + N-CH_2-CO + CO-CH_2-cytosine + CO_2CH_2), 2.68-3.64 (m, 5H, Fmoc-NH-CH-CH_2-N, Fmoc-NH-C*H*-CH_2-N + Boc-NH-CH_2), 1.31 (s, 9H, *tert*-butyl-CH_3), 1.18 (t, 3H, $CO_2CH_2CH_3$); minor rotamer 10.92 (s, 1H, Bhoc-N*H*), 1.33 (s, 9H, *tert*-butyl-CH_3). ^{13}C-NMR (150 MHz, DMSO-d_6); δ 169.86, 169.40, 168.38, 168.11, 163.70, 156.76, 156.61, 156.24, 155.62, 152.99, 151.41, 144.68, 144.55, 144.51, 141.41, 141.06, 129.26, 128.54, 127.65, 127.15, 127.03, 125.79, 120.74, 94.46, 78.07, 77.97, 65.95, 61.80, 61.12, 50.46, 48.90, 47.46, 46.61, 31.87, 29.95, 28.95, 28.88, 23.51. ESI-MS: found 901 (MH^+); 923 (MNa^+), calcd; 901; 923, for $C_{50}H_{56}N_6O_{10}$.

Fmoc-L-Lys(Boc)-PNA-thymine-OH monomer (6_T)

Fmoc-L-Lys(Boc)-thymine-OEt (1.09 g, 1.55 mmol) was dissolved in dry THF (10 mL) at 0°C. To the stirred solution was added 1 M NaOH solution (4.7 mL, 4.67 mmol) and then stirred for 1 h at room temperature. The reaction mixture was adjusted pH to 9 by adding 1 N HCl solution; Fmoc-OSu (0.63 g, 1.86 mmol) was then added to the solution and stirred for 1 h at room temperature. The reaction mixture was adjusted to pH = 3 with 1 N HCl at 0°C, extracted with ethyl acetate (10 mL), washed with saturated $NaHCO_3$ solution (twice) and dried over magnesium sulfate. The organic layer was filtered, evaporated, and purified by solidification with diethyl ether (50 mL) to afford 0.93 g (yield: 89%) of $\mathbf{6_T}$ as a white solid.

^{1}H-NMR (600 MHz, DMSO-d_6): δ major rotamer 11.25 (s, 1H, imide-N*H*), 7.84 (d, 2H, Fmoc-*H*), 7.63 (t, 2H, Fmoc-*H*), 7.08-7.38 (m, 6H, Fmoc-*H* + thymine-*H* + Fmoc-N*H*), 6.71-6.75 (m, 1H, Boc-N*H*), 3.83-4.70 (m, 7H, thymine-CH_2-CO, N-CH_2-CO_2H + Fmoc-C*H*-CH_2 + Fmoc-CH-CH_2-O), 2.82-3.65 (m, 5H, Fmoc-NH-C*H*-CH_2 + Fmoc-NH-CH-CH_2-N + Boc-NH-CH_2), 1.64 (s, 3H, thymine-CH_3), 1.06-1.43 (m, 6H, BocNH-CH_2-CH_2-CH_2-CH_2), 1.31 (s, 9H, *tert*-butyl-CH_3); minor rotamer 11.23 (s, 1H, imide-N*H*), 1.67 (s, 3H, thymine-CH_3), 1.31 (s, 9H, *tert*-butyl-CH_3). ^{13}C-NMR (150 MHz, DMSO-d_6); δ 171.29, 170.91, 168.42, 168.02, 165.03, 156.75, 156.62, 156.23, 151.62, 144.67, 144.53, 144.44, 142.67, 141.41, 128.23, 127.75, 125.89, 125.78, 120.81, 120.72, 108.78, 77.98, 65.97, 65.58, 52.05, 48.54, 48.37, 47.46, 31.87, 29.95, 28.94, 28.88, 23.51, 15.79, 12.55. ESI-MS: found 695 (MNH_4^+); 700 (MNa^+), calcd 695; 700, for $C_{35}H_{343}N_5O_9$.

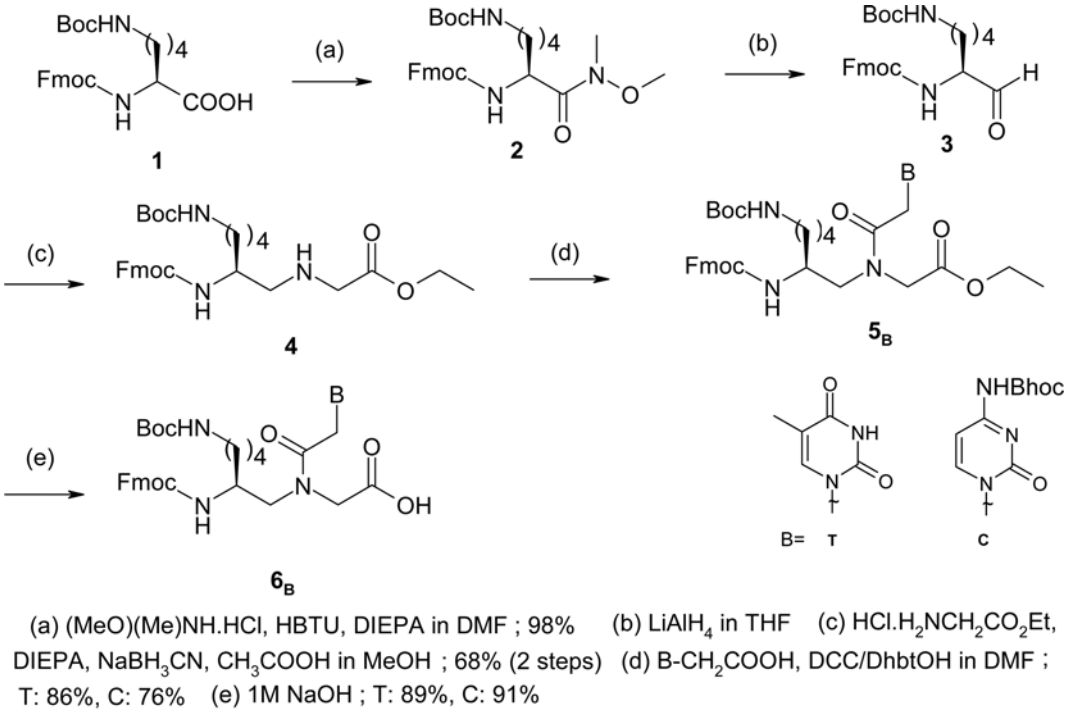
Fmoc-L-Lys(Boc)-PNA-cytosine(Bhoc)-OH monomer (6_C)

^{1}H-NMR (600 MHz, DMSO-d_6): δ major rotamer 6.70-7.87 (m, 22H, Fmoc-*H* + Bhoc-*H* + cytosine-*H* + Fmoc-N*H* + Boc-N*H*), 3.85-4.85 (m, 10H, Bhoc-C*H* + Fmoc-C*H*-CH_2 + Fmoc-CH-CH_2-O + N-CH_2-CO + CO-CH_2-cytosine + CO_2CH_2), 2.82-3.67 (m, 5H, Fmoc-NH-CH-CH_2-N, Fmoc-NH-C*H*-CH_2-N + Boc-NH-CH_2), 1.31 (s, 9H, *tert*-butyl-CH_3); minor rotamer 10.92 (s, 1H, Bhoc-N*H*), 1.33 (s, 9H, *tert*-butyl-CH_3). ^{13}C-NMR (150 MHz, DMSO-d_6); δ 173.45, 171.31, 170.92, 170.37, 168.33, 167.95, 163.70, 163.65, 156.78, 156.64, 156.25, 155.59, 153.05, 151.47, 144.70, 144.56, 144.50, 143.28, 141.41, 141.06, 129.86, 129.26, 129.22, 129.17, 128.54, 128.32, 127.67, 127.15, 127.02, 126.53, 125.82, 120.80, 120.71, 94.48, 78.08, 77.99, 66.00, 65.59, 52.17, 51.55, 50.20, 47.46, 46.61, 32.11, 31.89, 29.97, 28.95, 28.88, 25.90, 23.54, 15.95, 15.79. ESI-MS: found 873 (MH^+); 895 (MNa^+), calcd 873; 895, for $C_{48}H_{52}N_6O_{10}$.

All characterization data of D-(**4**), D-($\mathbf{5_T}$), D-($\mathbf{5_C}$), D-($\mathbf{6_T}$) and D-($\mathbf{6_C}$) matched (**4**), ($\mathbf{5_T}$), ($\mathbf{5_C}$), ($\mathbf{6_T}$) and ($\mathbf{6_C}$), respectively.

RESULTS AND DISCUSSION

The chiral γ-lysine-modified PNA monomers for pyrimidine nucleobase [thymine (T) and cytosine (C)] were prepared as outlined in Scheme 1, by modifying the previously reported methods (Englund and Appella, 2005; de Koning et al., 2006; Dragulescu-Andrasi et al., 2008; Kleiner et al., 2008; Sahu et al., 2009; Totsingan et al., 2009). The commercially available Fmoc-L-lys (Boc)-OH (**1**) was selected as a starting material. The diprotected L-lysine (**1**) was transformed into Fmoc-L-Lys(Boc)-*N*-methoxy-*N*-methyl amide (**2**) with *N*-methoxy-*N*-methylamine hydrochloride using HBTU activation in DMF. The amide (**2**) was then reduced with $LiAlH_4$ in THF at low temperature and the subsequent hydrolysis gave Fmoc-L-Lys(Boc)-H (**3**). The



Scheme 1. Synthesis of γ-L-lysine-modified PNA monomers

crude aldehyde (**3**) was immediately used for reductive amination with ethyl glycine hydrochloride and $NaBH_3CN$ in the presence of acetic acid and DIEPA in methanol to make the γ-L-Lys(Boc)-PNA monomer ester backbone (**4**). After purification of the chiral PNA backbone by column chromatography, the pure backbone was converted into Fmoc-L-Lys(Boc)-PNA-thymine-OEt monomer ($\mathbf{5_T}$) by reaction with thymine acetic acid using the DCC/DhbtOH coupling method in DMF. After hydrolysis with 1 M NaOH in THF (deprotection of Fmoc occurs during hydrolysis, so the subsequent re-protection *in situ* was performed by using Fmoc-OSu), the desired γ-L-Lys(Boc)-PNA-thymine-OH monomer ($\mathbf{6_T}$) was obtained as a white solid by solidification, in which a further purification process was not performed. For the other pyrimidine base, cytosine-derived γ-lysine PNA analogue monomer ($\mathbf{6_C}$) was synthesized by the same procedure described in Scheme 1.

Also, Fmoc-D-Lys(Boc)-PNA-thymine-OH [D-($\mathbf{6_T}$)] and Fmoc-D-Lys(Boc)-PNA-cytosine(Bhoc)-OH [D-($\mathbf{6_C}$)] were synthesized from Fmoc-D-Lys(Boc)-OH [D-(**1**)] as a starting material. Applying the modified direct enantiomer separation method developed in our group (Jin et al., 2006, 2007), we determined the optical purities of Fmoc-L-Lys(Boc)-PNA-thymine-OH ($\mathbf{6_T}$) and Fmoc-L-Lys(Boc)-PNA-cytosine(Bhoc)-OH ($\mathbf{6_C}$) as well as D-($\mathbf{6_T}$) and D-($\mathbf{6_C}$). In the chromatographic conditions of thymine- and cytosine-derived γ-lysine PNA analogs, mobile phases of ethanol-hexane-trifluoroacetic acid = 20:80:0.1 (v/v/v) and methanol-ethanol-hexane-trifluoroacetic acid = 5:10:85:0.1 (v/v/v/v) were used on Chiralpak IB, respectively. Not only ($\mathbf{6_T}$) (L:D = >99.50 :<0.50) and ($\mathbf{6_C}$) (L:D = >99.50:<0.50) but also D-($\mathbf{6_T}$) (L:D = 0.41:99.59) and D-($\mathbf{6_C}$) (L:D = 0.06:99.94) analytes showed extremely high optical purity, which indicates almost no racemization during all PNA monomer synthesis procedures, including the final basic hydrolysis step. This is the first report of direct enantiomer separation of γ-lysine-modified Fmoc-protected PNA intermediates and monomers by chiral HPLC. Other research groups have used indirect analytical methods using HPLC and ^{19}F-NMR to determine the diastereomeric excess after chiral derivatization (Sforza et al., 2003; Sahu et al., 2009). Typical chromatograms demonstrating the optical purity of Fmoc-D and L-Lys(Boc)-PNA-thymine-OH ($\mathbf{6_T}$) are presented in Fig. 1.

Racemization can potentially occur during the reduction step (**2**→**3**) in the preparation of γ-lysine-modified Fmoc-protected PNA monomers, since it is well-known that *N*-electron withdrawing group-substituted α-amino aldehydes are thermally unstable and easy to racemize (Jurczak and Golebiowski, 1989; Gryko et al., 2003; Soto-Cairoli et al., 2008). Therefore, we examined the degree of racemization under various conditions at the reduction step (**2**→**3**), which is an important step for the optical purity of PNA-monomers. Because Fmoc-lysinal (**3**) was not isolated and

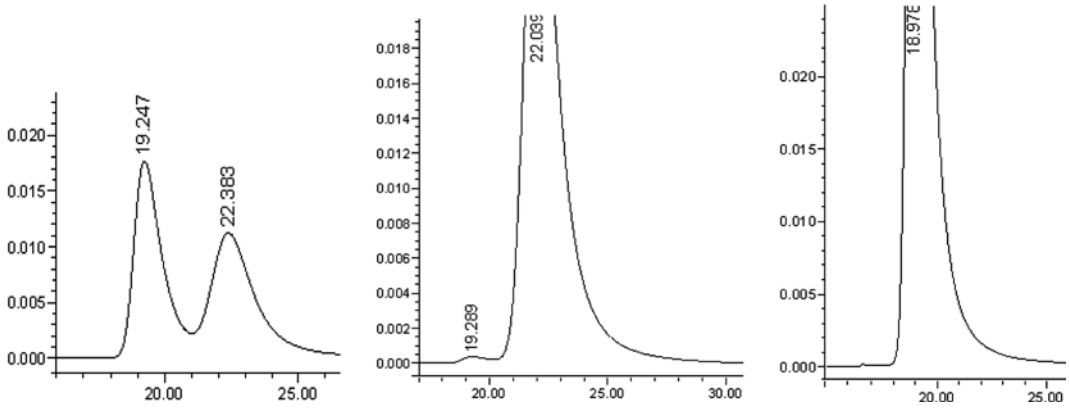


Fig. 1. Determination of optical purity and typical chromatograms of enantiomer separation of racemic Fmoc-Lys(Boc)-PNA-thymine-OH (left), Fmoc-D-Lys(Boc)-PNA-thymine-OH (D-**6**$_T$) (middle, L:D = 0.41:99.59) and Fmoc-L-Lys(Boc)-PNA-thymine-OH (**6**$_T$) (right, L:D = >99.50 : <0.50) on Chiralpak IB; Mobile phase: ethanol-hexane-trifluoroacetic acid = 20:80:0.1 (v/v/v); Flow rate = 1 mL/min; UV 254 nm.

immediately reacted with glycine ethyl ester *in situ*, the degree of racemization in the reduction was examined at the next step of the reductive-aminated intermediate (**4**) by using our direct enantiomer separation method (Jin et al., 2006, 2007). As shown in Table I, we found that the quenching temperature was more important than the complexation temperature. Racemization did not occur at the complexation step below 0°C but occurred at the quenching step above 0°C, due to formation of a stable aluminum-complex with a Weinreb amide. We also observed that the degree of racemization increased very slightly as the quenching temperature increased. However, the yields were dependent on the complexation temperature rather than the quenching temperature. Presumably, the aluminum ion of $LiAlH_4$ could bind to the Weinreb amide (**2**) or Fmoc-carbamate or Boc-carbamate moiety, but at low temperature it might migrate to the Weinreb amide to form a more stable complex with the β-methoxy group of the Weinreb amide without cleavage of protection group (Nahm and Weinreb, 1981). Therefore, Fmoc-deprotection could be minimized at the quenching step and the aldehyde compound (**3**) could be obtained in higher yield at low temperature. Typical chromatograms of determination of the optical purity of Fmoc-L-Lys(Boc)-PNA-backbone (**4**) obtained after reduction of Fmoc-L-Lys(Boc)-*N*,O-dimethyl (**2**) and the subsequent amination of Fmoc-L-Lys(Boc)-H (**3**) are presented in Fig. 2.

In conclusion, we synthesized the enantiopure (> 99.5%) Fmoc-L and D-Lys(Boc)-PNA-thymine-OH and Fmoc-L and D-Lys(Boc)-PNA-cytosine(Bhoc)-OH as γ-lysine-modified PNA pyrimidine base (thymine and cytosine) monomers starting from the commercially

Table I. Determination of optical purity and yield of the reduction of Fmoc-L-Lys(Boc)-N,O-dimethyl (2) under various reaction conditions

Exp. No.	Temperature (°C)		Optical purity* (L:D)	Yield** (%)
	Complexation	Quenching		
1	−78	−10	99.96:0.04	68.0
2	−78	0	99.95:0.05	62.2
3	−10	−10	99.95:0.05	59.4
4	0	−10	99.95:0.05	46.1
5	0	0	99.73:0.27	44.1
6	0	r.t.	99.20:0.80	42.0

*The optical purity of Fmoc-L-Lys(Boc)-PNA backbone (**4**) obtained after reduction of Fmoc-L-Lys(Boc)-*N*,O-dimethyl (**2**) and the subsequent amination of Fmoc-L-Lys(Boc)-H (**3**) was determined on Chiralpak IB [Mobile phase: methanol-ethanol-hexane = 5:15:80 (v/v/v); Flow rate 1 mL/min; UV 254 nm].

Isolation yield obtained after column chromatography of Fmoc-L-Lys(Boc)-PNA backbone (4**).

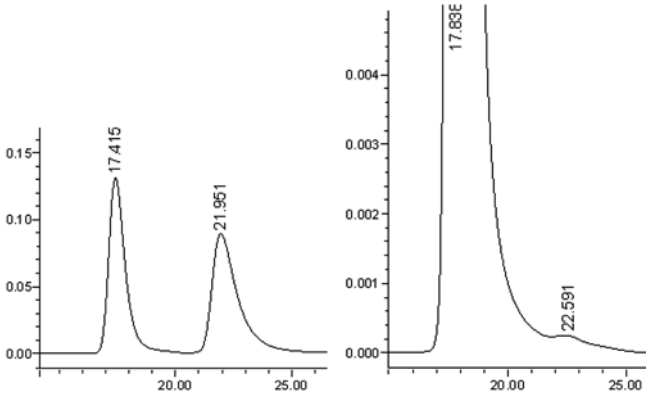


Fig. 2. Determination of optical purity and typical chromatograms of enantiomer separation of racemic Fmoc-Lys(Boc)-PNA backbone (left) and Fmoc-L-Lys(Boc)-PNA backbone (right, L:D = 99.96:0.04) on Chiralpak IB; Mobile phase: methanol-ethanol-hexane = 5:15:80 (v/v/v); Flow rate = 1 mL/min; UV 254 nm.

available Fmoc-L-Lys(Boc)-OH (**1**) and D-(**1**). For determination of their optical purity, the convenient direct enantiomer separation of γ-lysine-modified PNA intermediates and monomers by chiral HPLC was developed and applied.

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