Synthesis and Characterization of Optically Pure Gamma-PNA Backbones by SIBX-Mediated Reductive Amination

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Chiral peptide nucleic acid (PNA) is a derivative of regular PNA by introducing a chiral center to its backbone, and is known to bind more strongly to DNA or RNA than regular PNA. In particular, in the case of a γ-backbone, the *L* isomer stabilizes the PNA/DNA duplex, and the *D*-isomer has the opposite effect. Therefore, the synthesis of an optically pure γ-backbone is very important. Here, we report a novel synthetic strategy for the suppression of epimerization during the synthesis of the γ-PNA backbone. A stabilized form of 2-iodoxybenzoic acid (SIBX) was used as an oxidative reagent in the key intermediate of the N-Boc-amino acetaldehyde synthesis. This paper reports (1) the synthesis and comparison of three different γ-PNA backbones (lysine, alanine, and glutamate) by three different synthetic routes (SIBX, lithium aluminum hydride, and Red-Al) and (2) the determination of chiral purity from their derivative compounds. The enantiomeric excess purity of SIBX-mediated γ-PNA backbones was determined to be more than 99.4%, as ascertained by the high-performance liquid chromatography (HPLC) chromatogram on a standard RP-C18 column. It is comparatively higher than that of the other methods examined in this work.

Keywords: Peptide nucleic acid, γ-Backbone, Optical purity, Stabilized 2-iodoxybenzoic acid, Epimerization

Introduction

Peptide nucleic acids (PNAs) are synthetic DNA analogs in which *N*-(2-aminoethyl) glycine units and nucleobases are linked via a methylene carbonyl group.[1] It has the property of a strong sequence-selective binding to DNA or RNA.[2] Thus, it has been widely used in the fields of chemistry, biology, biotechnology, and medicine.[3–6] In particular, PNAs have been studied a lot as therapeutic agents such as antigene or antisense because of their resistance to the proteolytic and nucleolytic enzymes *in vivo*.[7] However, its application was limited due to a remarkably low cell permeability, low solubility in water, and endosomal escape issues.[8]

To improve these limitations, there has been much research on the modification of the PNA structure.[9–15] One of the most distinct modifications has been the insertion of charged amino acids at the interior of the *N*-(2-aminoethyl) glycine backbone[16–23] (Figure. 1). Nielsen *et al.* reported the first *α*-chiral PNA, in which the glycine unit of the PNA backbone was substituted by alanine.[24] Subsequently, various γ-modified PNA backbones have been reported, including Ala, Ser, Lys, PEG, and Cys-based PNAs. Compared with regular achiral PNA, γ-substituted chiral PNA has advantages in terms of minimal self-aggregation, good solubility, and stronger PNA-DNA duplex formation.[25–28]

In the case of γ-PNA, the *L*-isomer PNA in the chiral center has the property of binding more strongly to complementary DNA or RNA than regular PNA due to the preorganization effect for the α-helical duplex formation. On the other hand, the *D*-isomer PNA destabilizes the PNA/DNA duplex, significantly lowering the melting temperature.[29,30] Therefore, it is particularly important to synthesize chiral PNA monomers with a high optical purity for use in medicinal applications.[31]

Traditionally, reductive amination is the common method to synthesize the gamma chiral backbone, using commercially available Boc or Fmoc-protected amino acids.[32] That is, after synthesizing the corresponding aldehyde derivatives of amino acids, reductive amination between the aldehyde and glycinate ester provides the gamma chiral backbone.

A critical challenge in the chiral PNA backbone synthesis is the epimerization of chiral carbon during amino aldehyde synthesis even under milder conditions.[33,34] Amino aldehyde intermediates have been synthesized either by oxidation of the corresponding alcohols[35,36] or by reduction of the acids and esters.[37,38] A typical method to prepare the optically active amino aldehydes is from *N*-Boc-protected Weinreb amide derivatives by reduction with lithium aluminum hydride (LAH), and previous studies have shown that this harsh treatment is prone to racemization in the preparation of amino aldehyde.[39,40]

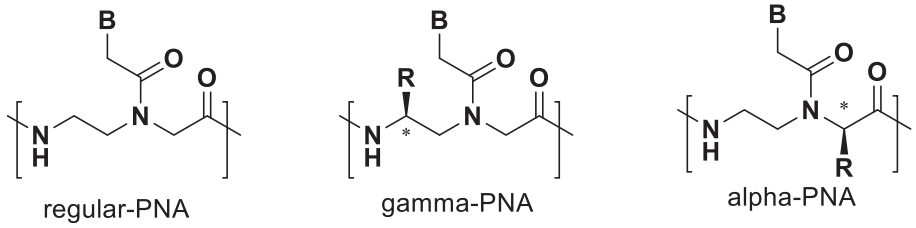


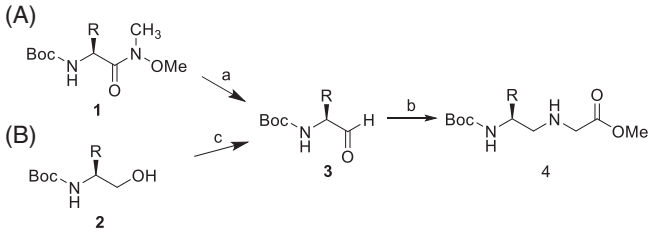
Figure 1. Chemical structures of regular PNA and modified PNAs.

Stabilized 2-iodoxybenzoic acid (SIBX) is a non-explosive formulation of the IBX oxidative reagent that can be used in a variety of solvents, for instance, refluxing with ethyl acetate or tetrahydrofuran to safely oxidize organic alcohol groups into the corresponding aldehydes and ketones.[41,42] The aim of this study was to develop a novel synthetic method to control the epimerization during the *N*-Boc-amino acetaldehyde synthesis. Here, we report that (1) the synthesis of the gamma-lysine, glutamate, and alanine PNA backbones based on SIBX, LAH, and Red-Al mediated the corresponding aldehyde synthesis and (2) an extensive study of these γ-PNA backbones verified their chiral purity using analytical high-performance liquid chromatography (HPLC).

Results and Discussion

The typical method of making the corresponding amino acetaldehyde from protected amino acids is by reducing the Weinreb amide with LAH shown in Scheme 1(A). In this case, epimerization may occur when the α-hydrogen of aldehyde derivative is deprotonated as a hydride reduction condition. In the case of the alanine γ-PNA backbone, the Danith H. Ly group showed that the LAH reduction of the Weinreb amide to aldehyde followed by reductive amination could roughly produce 5% epimerization, which was determined by fluorine NMR after derivatization to Mosher's reagent.[43] An enantiomeric pure product from a small amount of enantiomeric mixture can be purified by the recrystallization method; however, it takes a lot of time and labor such as determining the conditions for recrystallization.

In this study, γ-backbones were synthesized with the traditional LAH reduction method and with Red—Al, a milder reducing agent than LAH, and the optical purities were measured and compared with each other. To confirm the effect of temperature, the γ-backbones were synthesized at two temperatures (−20 °C and 0 °C). In addition, a reagent that converts an alcohol group to an aldehyde group was investigated as an alternative to the hydride reduction reaction, which is vulnerable to epimerization. As a result, a mild oxidation reagent, stabilized IBX reagent, was selected, and it was reported that there is no change in the optical purity when the alcohol derivative of an amino acid is converted to the corresponding aldehyde (Scheme 1(B)).



Scheme 1. Synthetic strategies of γ-chiral backbones, (A) conventional reductive route, (B) SIBX oxidative route; (a) $LiAlH_4$ or Red-Al, (b) reductive amination with $NaHB(OAc)_3$, (c) SIBX, DMSO

The next step is to determine the target molecules to be synthesized. In this study, as shown in Figure 2, the Boc-Lys(*Z*)-aeg-OMe, Boc-Glu(cHx)-aeg-OMe, and Boc-Ala-aeg-OMe γ-backbones were selected as three different targets that have positive, negative, and neutral charges respectively in PNA oligomers.

As a method of confirming the optical purity of the synthesized γ-backbones, the Ly group introduced a chiral auxiliary group containing fluorine atoms into the γ-backbone that generated a diastereomer structure. Thus, the optical purity was measured with F-NMR. In addition, Lee's group proposed a direct method of separating the enantiomers of the γ-backbones through a chiral HPLC column and measured the optical purity without any derivatization process.[44] However, in both cases, expensive reagents and equipment are required, and especially, in the case of chiral columns, it is necessary to optimize the separation conditions.

Here, the (*L*)-Fmoc-amino acid as a chiral auxiliary was attached to the secondary amine of the synthesized γ-backbone to generate the diastereomers, which were directly analyzed by C18-RP HPLC to measure the optical purity of the γ-backbone (Scheme 2).

Both the *L* and *D* form of the γ-backbone references are required to determine how much epimerization took place in the synthesis of the γ-backbone. Thus, each Weinreb amide from *L* and *D* form of the Boc-Lys(*Z*)-OH, Boc-Glu(cHx)-OH, and Boc-Ala-OH, respectively, was prepared, and then, each *L* and *D* form of the γ-backbones was synthesized by the conventional method shown in Scheme 1(A).

The (*L*,*L*)-**5** and (*D*,*L*)-**5** diastereomers were synthesized by the coupling reactions of each of the *L* and *D* form of γ-backbones with various (*L*)-Fmoc-amino acids. Each diastereomer was analyzed with RP-HPLC, and the (*L*)-Fmoc-amino acid with the largest difference in the retention time between the diastereomers was selected. As a result, for

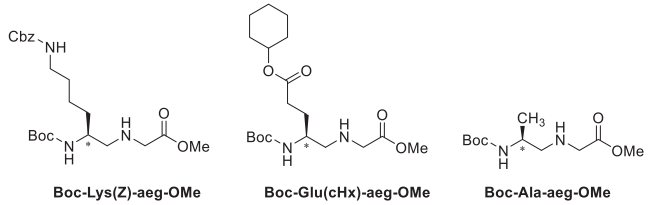
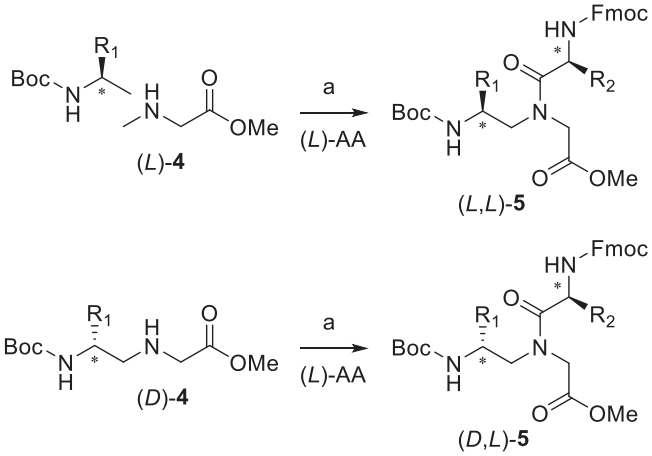


Figure 2. Target structures of the three different side chain modified γ-backbones.

each of the Boc-Lys(*Z*)-aeg-OMe, Boc-Glu(cHx)-aeg-OMe, and Boc-Ala-aeg-OMe, *L* forms of the Fmoc-Leu-OH, Fmoc-Ala-OH, and Fmoc-Phe-OH were optimal as chiral auxiliary, respectively. As shown in Figure 3(a), each diastereomer reference was fully resolved in this analytical condition when each diastereomer was coinjected onto an HPLC column. The (*D*,*L*) diastereomers of the three different γ-backbones were all eluted about 1 min faster than the (*L*,*L*) diastereomers. Using these chiral auxiliary derivatization conditions and HPLC analysis conditions, the degree of epimerization was measured for the various γ-backbone synthetic methods. Representative examples are shown in Figure 3(b), in which the formation of the (*D*,*L*) isomer was almost negligible in the SIBX-mediated oxidative route. By quantification through integration, all three γ-backbones showed less than 0.6% epimerization.

Table 1 shows a summary of the analysis results for the detailed epimerization degree under each synthetic condition. The Boc-Glu(cHx)-aeg-OMe synthesis of the γ-backbone through the LAH reduction route failed because the cyclohexyl ester of the side chain was also reduced by LAH.

From the results, it was observed that an epimerization of 1.4%–9.7% occurred mostly in the case of the γ-backbone synthesized by the hydride reduction pathway. The tendency with temperature seems to improve slightly in the case of the −20 °C reaction condition, but it is negligible. However, in the case of the alanine γ-backbone,



Scheme 2. Synthesis of diastereomers of the γ-backbone using a (*L*)-Fmoc-amino acid as a chiral auxiliary: (a) HATU, DIPEA, DMF

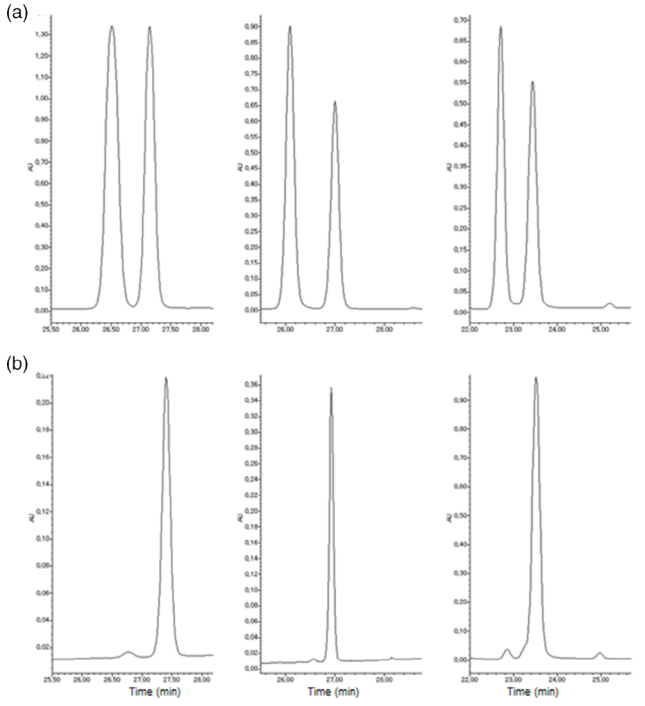


Figure 3. HPLC chromatograms of the diastereomer separation; (a) left, Boc-Lys-(Fmoc-Leu)-aeg-OMe, (*D*,*L*)-26.5 min, (*L*,*L*)-27.1 min; middle, Boc-Glu(Fmoc-Ala)-aeg-OMe, (*D*,*L*)-26.1 min, (*L*,*L*)-27.0 min; right, Boc-Ala(Fmoc-Phe)-aeg-OMe, (*D*,*L*)-22.7 min, (*L*,*L*)-23.4 min. (b) SIBX-mediated γ-backbone; left, Boc-Lys-(Fmoc-Leu)-aeg-OMe; middle, Boc-Glu(Fmoc-Ala)-aeg-OMe; right, Boc-Ala(Fmoc-Phe)-aeg-OMe.

epimerization occurred up to almost 10% under the condition of −20 °C with the Red-Al reduction route. It can be assumed that this was caused by exposure to an unexpected basic environment not only under the reaction conditions but also during the workup and purification in the hydride reduction pathway. The degree of epimerization in the

Table 1. Optical purities of each γ-backbone synthesized in different synthetic conditions.

γ-backbones	Route	Temp. (°C)	*L*-isomer[a]	*D*-isomer[a]
Lys	LAH	0	98.0	2.0
	LAH	−20	98.6	1.4
	Red-Al	0	97.1	2.9
	Red-Al	−20	98.1	1.9
	SIBX	Reflux	99.7	0.3
Glu	Red-Al	0	98.0	2.0
	Red-Al	−20	98.1	1.9
	SIBX	Reflux	99.4	0.6
Ala	LAH	0	96.7	3.3
	LAH	−20	96.2	3.8
	Red-Al	0	96.9	3.1
	Red-Al	−20	90.3	9.7
	SIBX	Reflux	99.4	0.6

[a] Relative % calculated after integration in the HPLC chromatogram.

LAH reduction pathway of the alanine γ-backbone was comparable to that observed for the Ly group.

The most interesting result is that in the case of the γ-backbone synthesized by the SIBX oxidative pathway, the degree of epimerization was significantly reduced for all three γ-backbones. The degree of epimerization for each of the Lys, Glu, and Ala-γ-backbones was reduced by 85%, 70%, and 82% on average compared to the hydride reduction pathway when synthesized by the SIBX oxidative pathway, respectively. This is a novel method of synthesizing optically pure γ-backbones that have not yet been reported as far as we know.

Hydride reagents (LAH, Red-Al, DIBALH, etc.) are particularly flammable, and when used, the reaction must be done at low temperature and in an anhydrous environment. When mass production of target materials is required, special equipment is required for the low temperatures and anhydrous environments, and special attention is required during the quenching step in the workup. On the other hand, the SIBX oxidizing agent is a solid reagent and free from such conditions, and because the reaction conditions are usually reflux conditions, its use is advantageous because the SIBX reagents can be easily removed or regenerated through a filter. Thus, it is particularly suitable for the mass production of optically pure γ-backbones.

Experimental Methods

All reagents used in this experiment were purchased from commercial suppliers and used without further purification unless otherwise noted. ^{1}H NMR and ^{13}C NMR spectra were taken with a Bruker 400 MHz NMR spectrometer; $CDCl_3$ and DMSO-d_6 were used as solvents, and tetramethylsilane was used as an internal standard. Column chromatography was performed with CHROMATOREX GS-40/75 silica gel, and TLC analysis was done with silica gel 60 F-254 precoated aluminum plate. The HPLC instrument was a Waters binary pump and a Waters 2487 detector, and the HPLC analytical column used for the optical purity measurement was INNO C18 (5 μm, 120 Å, 4.6 × 250 mm). The solvent systems used in the HPLC were water and MeCN containing 0.1% TFA, respectively.

Abbreviations: *N*, *N*-dimethylformamide (DMF); dichloro-methane (DCM); tetrahydrofuran (THF); dichloroethane (DCE); *N*,*N*-diisopropylethylamine (DIEA); *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′*N*′-tetramethyluronium hexafluorophosphate (HATU); dimethyl sulfoxide (DMSO); acetic acid (AcOH); *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU); 1,1′-carbonyldiimidazole (CDI); stabilized 2-iodoxybenzoic acid (-SIBX); sodium borohydride ($NaBH_4$); sodium triacetoxyborohydride ($NaBH(AcO)_3$; lithium aluminum hydride (LAH); sodium bis(2-methoxyethoxy) aluminum hydride (Red-Al).

In general, all γ-modified PNA backbones were synthesized from commercially available *N*-(Boc)-protected *L*- and *D*-amino acids, followed by aldehyde synthesis and reductive amination, respectively. The main intermediate amino acetaldehyde was obtained by three different routes mentioned in Scheme 1.

***N*-(Boc)-Protected Amino Acid Weinreb Amide (1).** *N*,*O*-dimethylhydroxylamine hydrochloride, HBTU (1.1 eq.) and DIEA (3 eq.) were added to a stirred solution of *N*-(Boc) protected amino acids in DMF. The resulting mixture was stirred for 30 min. Upon completion of the reaction, as confirmed by the TLC, the solvent was evaporated under reduced pressure. The resulting crude was dissolved in ethyl acetate, washed multiple times with 1 *N* HCl followed by saturated $NaHCO_3$ and brine solution, and dried over anhydrous Na_2SO_4. The concentrated crude mixture was purified by flash chromatography to give the corresponding Weinreb amides as a white color solid (**1**) at a good yield.

***N*-(Boc)-Protected Amino Alcohol (2).** CDI (3 eq.) was portion-wise added to a solution of *N*-(Boc) protected amino acid in anhydrous THF. The solution was stirred for 5 min. Subsequently, the solution of $NaBH_4$ (3 eq.) in water was added dropwise for 10 min (effervescence occurred). After completion of the reaction, a large volume of EtOAc was added and washed multiple times with 1 *N* HCl solution, followed by saturated $NaHCO_3$ solution. The combined organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated using a rotary evaporator. The resulting crude was purified by column chromatography to obtain the target alcohol compound **2**.

***N*-(Boc)-Protected Amino Acetaldehyde (3).** Path 1: First a 2.6 M solution of LAH (1.2 eq.) was added to a chilled solution of *N*-(Boc) Weinreb amide (**1**) in anhydrous THF, and then the solution was stirred for 30 min. The reaction was then quenched with 1 *N* HCl and washed with saturated $NaHCO_3$ followed by saturated brine solution. The combined organic phase was dried over anhydrous Na_2SO_4, and the solvent was concentrated with a rotary evaporator. This crude mixture **3** was used in the next reductive amination reaction without further purification.

Path 2: First, a 3.6 M solution of Red-Al (1.5 eq.) was added to a cold solution of *N*-(Boc) Weinreb amide (**1**) in anhydrous THF, and then the solution was stirred for 30 min. The reaction was then quenched with 1 *N* HCl and washed with saturated $NaHCO_3$ followed by saturated brine solution. The combined organic phase was dried over anhydrous Na_2SO_4, and the solution was concentrated with a rotary evaporator. This crude mixture **3** was used in the next reductive amination reaction without further purification.

Path 3: SIBX (5 eq.) was added to a solution of *N*-(Boc) amino alcohol in EtOAc in a two-neck round bottom flask, and then the reaction mixture was refluxed for 5 min. Next, DMSO (5 eq.) was added to the solution, and the reflux was continued. After completion of the reaction, the reaction mixture was filtered through a celite pad and washed with a saturated $NaHCO_3$ solution. The organic phase was again washed with a saturated brine solution, and the solution was concentrated with a rotary evaporator after drying

over anhydrous Na_2SO_4. The resulting crude compound (**3**) was used in the subsequent reductive amination reaction without any further purification.

***N*-(Boc)-Protected γ-Backbone (4).** Methyl glycinate hydrochloride (1.5 eq.), DIEA (1.5 eq.), acetic acid (1.0 eq.), and 1.5 eq. of $NaBH(OAc)_3$ were added to a solution of aldehyde (**3**) in DCE at 0 °C. Then, the temperature was raised to ambient temperature, and the reaction was stirred for 1 hr. Upon the completion of the reaction, an excess volume of DCM was added, and the organic layer was washed with saturated $NaHCO_3$ and a brine solution. The extracted DCM was dried over anhydrous Na_2SO_4, and the solvent was evaporated with a rotary evaporator. The resulting crude was purified by flash column chromatography to yield a colorless syrup compound (**4**).

Fmoc-Amino Acid Attached γ-Backbones (5). In a 1.5 mL Eppendorf tube, (*L*)-Fmoc-amino acid in DMF was added to Boc-Lys(*Z*)-aeg-OMe (**4**) (1.0 eq.), HATU (1.1 eq.), and DIEA (6.0 eq.). The Fmoc-amino acids used as a chiral auxiliary were Fmoc-Leu-OH, Fmoc-Ala-OH, and Fmoc-Phe-OH for the Lys, Glu, and Ala-γ-backbone, respectively. The solution was left to react for 1 hr, and then the diastereomer products were monitored by HPLC at 260 nm, flowing from 50% MeCN to 95% MeCN for 30 min at a flow rate of 1 mL/min.

The following compounds were prepared, purified, and characterized using the above general procedure, and their spectral data are given below.

Boc-Lys(Z)-Weinreb Amide (1a). Yield; 93.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.19 (m, 5H), 5.30 (d, J = 8.1 Hz, 1H), 5.10 (d, J = 30.2 Hz, 3H), 4.63 (s, 1H), 3.72 (s, 3H), 3.16 (s, 5H), 1.67 (d, J = 6.4 Hz, 1H), 1.59–1.44 (m, 3H), 1.40 (s, 11H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.92, 156.32, 155.54, 136.49, 128.30, 127.92, 127.84, 79.37, 66.31, 61.41, 49.90, 40.56, 32.31, 31.89, 29.06, 28.19, 22.29.

Boc-Lys(Z)-Alcohol (2a). Yield; 81.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.29 (m, 5H), 5.20–5.05 (m, 2H), 4.90 (d, J = 36.2 Hz, 2H), 3.59 (ddd, J = 14.5, 11.5, 4.1 Hz, 3H), 3.27–3.15 (m, 2H), 2.77 (s, 1H), 1.61–1.48 (m, 3H), 1.45 (s, 9H), 1.42–1.27 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.70, 156.51, 152.64, 136.59, 128.52, 128.11, 79.56, 66.68, 65.48, 52.48, 40.41, 30.78, 29.77, 28.40, 22.77.

Boc-Lys(Z)-aeg-OMe γ-Backbone (4a). Yield; obtained through LAH reaction at 0 °C; 75.0%, at −20 °C; 70.0%; Red-Al reaction at 0 °C; 97.3%, at −20 °C; 99.5% and SIBX reaction; 57.1% respectively. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.29 (m, 5H), 5.09 (s, 2H), 4.97 (s, 1H), 4.77 (s, 1H), 3.72 (s, 3H), 3.63 (s, 1H), 3.41 (q, J = 17.4 Hz, 2H), 3.19 (dd, J = 12.6, 6.4 Hz, 2H), 2.71–2.56 (m, 2H), 1.67 (s, 1H), 1.60–1.29 (m, 15H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.92, 156.45, 155.91, 136.66, 128.44, 128.06, 127.99, 79.14, 66.51, 52.94, 51.71, 50.72, 50.09, 40.70, 32.74, 29.56, 28.36, 22.88.

Boc-Glu(cHx)-Weinreb Amide (1b). Yield; 96.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.24 (dd, J = 4.8, 4.0 Hz, 1H), 4.67 (td, J = 8.9, 4.2 Hz, 2H), 3.71 (s, 3H), 3.13 (s, 3H), 2.31 (t, J = 7.5 Hz, 2H), 1.98 (qd, J = 7.7, 4.8 Hz, 1H), 1.83–1.71 (m, 3H), 1.68–1.59 (m, 2H), 1.52–1.41 (m, 1H), 1.35 (s, 9H), 1.28 (dd, J = 15.1, 5.8 Hz, 3H), 1.18 (ddd, J = 13.2, 10.9, 3.2 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.33, 172.00, 155.29, 79.31, 72.47, 61.43, 49.70, 31.88, 31.40, 30.33, 28.12, 27.60, 25.17, 23.53.

Boc-Glu(cHx)-Alcohol (2b). Yield; 82.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.86–4.69 (m, 2H), 3.66–3.50 (m, 3H), 2.48–2.41 (m, 1H), 2.40–2.30 (m, 2H), 1.91–1.79 (m, 3H), 1.79–1.74 (m, 1H), 1.74–1.66 (m, 2H), 1.58 (s, 1H), 1.53 (ddd, J = 9.4, 5.2, 3.3 Hz, 1H), 1.42 (s, 9H), 1.38–1.33 (m, 2H), 1.33–1.28 (m, 1H), 1.28–1.18 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.27, 156.13, 79.50, 72.95, 64.91, 52.49, 31.63, 31.28, 28.39, 26.07, 25.37, 23.78.

Boc-Glu(cHx)-aeg-OMe γ-Backbone (4b). Yield; obtained through Red-Al reaction at 0 °C; 44.5%, at −20 °C; 62.5% and SIBX route 69.3% respectively. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.75 (td, J = 8.9, 4.0 Hz, 2H), 3.73 (s, 3H), 3.66 (d, J = 2.8 Hz, 1H), 3.42 (q, J = 17.4 Hz, 2H), 2.67 (qd, J = 12.2, 5.6 Hz, 2H), 2.37 (t, J = 7.6 Hz, 2H), 1.91–1.79 (m, 3H), 1.78–1.68 (m, 3H), 1.64 (s, 1H), 1.55 (ddd, J = 9.2, 5.1, 3.3 Hz, 1H), 1.47–1.41 (m, 10H), 1.41–1.36 (m, 2H), 1.35–1.30 (m, 1H), 1.30–1.20 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.89, 172.86, 155.78, 79.23, 72.74, 53.06, 51.74, 50.81, 50.27, 31.64, 31.41, 28.38, 28.21, 25.37, 23.76.

Boc-Ala-Weinreb Amide (1c). Yield; 90.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.25 (d, J = 7.8 Hz, 1H), 4.73–4.62 (m, 1H), 3.76 (s, 3H), 3.20 (s, 3H), 1.43 (s, 9H), 1.30 (d, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.62, 155.16, 79.49, 61.61, 46.51, 32.12, 28.36, 18.68.

Boc-Ala-Alcohol (2c). Yield; 85.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.77 (d, J = 6.5 Hz, 1H), 3.73 (d, J = 4.2 Hz, 1H), 3.60 (ddd, J = 10.1, 5.8, 4.0 Hz, 1H), 3.48 (dt, J = 10.9, 5.5 Hz, 1H), 3.01 (s, 1H), 1.42 (s, 9H), 1.12 (d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.34, 79.63, 67.19, 48.52, 28.36, 17.29.

Boc-Ala-aeg-OMe γ-Backbone (4c). Yield; obtained through LAH reaction at 0 °C; 81.4%, at −20 °C; 83.7%; Red-Al reaction at 0 °C; 79.1%, at −20 °C; 71.1% and through SIBX reaction; 66.7%, respectively. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.00 (s, 1H), 3.52 (s, 4H), 3.23 (q, J = 17.4 Hz, 2H), 2.44 (d, J = 5.9 Hz, 2H), 1.62 (s, 1H), 1.24 (s, 9H), 0.95 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.72, 155.46, 78.60, 54.24, 51.46, 50.35, 45.90, 28.20, 18.78.

^{1}H NMR and ^{13}C NMR data of the various intermediates and γ-backbones synthesized in this study are included in the Supporting Information.

Conclusion

Three types of γ-modified PNA backbones were synthesized from the relatively inexpensive *N*-protected *L*-amino acid. *N*-protected amino acetaldehyde derivatives, which are key intermediates of the γ-backbone synthesis, were synthesized through the LAH, Red-Al reduction, and SIBX oxidative routes, and the final γ-backbone was synthesized by the conventional reductive amination between the Boc-amino acetaldehyde and glycinate ester. The chiral purity of each γ-backbone was determined by the diastereomer formation of the corresponding γ-backbones with the suitable (*L*)-Fmoc-protected amino acids as chiral derivatizing reagents. In the SIBX-mediated route, γ-backbones with an optical purity of over 99.4% were obtained from all three types of γ-backbones. Finally, this methodology is robust and can be applied to the preparation of large-scale γ-PNA backbones without any concerns over loss of optical purity.

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Supporting Information. Additional supporting information may be found online in the Supporting Information section at the end of the article.

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