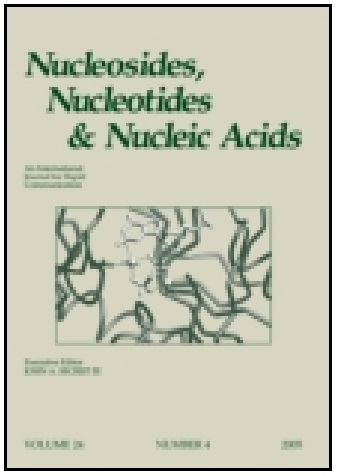

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Synthesis of New Chiral Peptide Nucleic Acid (PNA) Monomers by a Simplified Reductive Animation Method

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SYNTHESIS OF NEW CHIRAL PEPTIDE NUCLEIC ACID (PNA) MONOMERS BY A SIMPLIFIED REDUCTIVE AMINATION METHOD

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ABSTRACT: The aim of this work was the preparation of four new peptide nucleic acid (PNA) monomer backbone by reductive amination of N^{α}-Boc-protected chiral amino aldehydes, derived from Leu, Phe, Tyr(Bzl), and Thr(Bzl), with methyl glycinate. To the crude 2-substituted methyl N-(2-Boc-aminoethyl)glycinates obtained, thymin-1-ylacetic acid was coupled using TBTU procedure in a one-pot reaction. PNA monomers were isolated and characterized.

Peptide nucleic acids (PNA) incorporating nucleic acid bases into a polyamide backbone are relatively novel DNA analogues [1]. They are intensively investigated due to their potential as gene-targeted drugs with antigene and antisense properties and are expected to become one of the most useful molecular tools in molecular biology [2].

In PNA the deoxyribose phosphate backbone of the nucleic acid is replaced by a chiral or achiral pseudopeptide backbone and natural nucleobases are attached to the backbone by methylene carbonyl linkers (Fig. 1). This structure gives increased stability of PNA-containing duplexes with DNA or RNA, and increased resistance of PNA oligomers to nucleases and proteases in comparison with natural nucleic acids [2b,i]. PNA have emerged as useful agents for recognition of single- and double-stranded nucleic acids and as a DNA mimic exhibiting several unique hybridization characteristics [2].

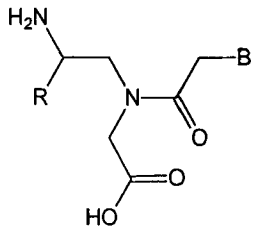


FIG. 1. Unprotected chiral PNA monomer.

PNA oligomers can be obtained by standard peptide chemistry methods. The PNA structure is easy to modify and it is probable that synthesis of altered monomers gives the possibility to obtain oligomers with improved properties, e.g. with better permeability through cellular membranes. Recently, we developed a new method of synthesis of various aminoethyl- or glycine residue-modified PNA monomers [3] with the use of the Mitsunobu reaction [4]. Syntheses of chiral PNA monomer backbones (Fig. 1, B: nucleic acid base) with the use of reductive amination of amino acid-derived amino aldehydes were also proposed: Kosynkina et al. [6] have described a method of the chiralization of the aminoethyl residue of PNA backbone; Brown et al. [7] and Farese et al. [8] used a procedure of the same type to obtain protected N-(2-aminoethyl)glycine-based PNA monomers; alternative chiral modifications of the glycine residue in PNA monomers were also proposed [9,10]. All the procedures cited need two purification steps - after reductive amination and acylation procedures [6-10], or are based on unstable N-protected amino acetaldehyde [7-10]. Following these experiments we developed a simplified and efficient procedure of reductive amination of amino acid-derived amino aldehydes for the synthesis of chiral PNA monomer backbone and have applied it to obtain several PNA monomers with aliphatic, aromatic, and hydroxy amino acid side chains.

RESULTS AND DISCUSSION

According to the scheme (Fig. 2), we have prepared a series of four new chiral PNA monomers based on Leu, Phe, Tyr(Bzl), and Thr(Bzl) aldehydes (PNA-Leu, PNA-Phe, PNA-Tyr(Bzl), and PNA-Thr(Bzl), respectively; Fig. 3). The corresponding N-Boc-

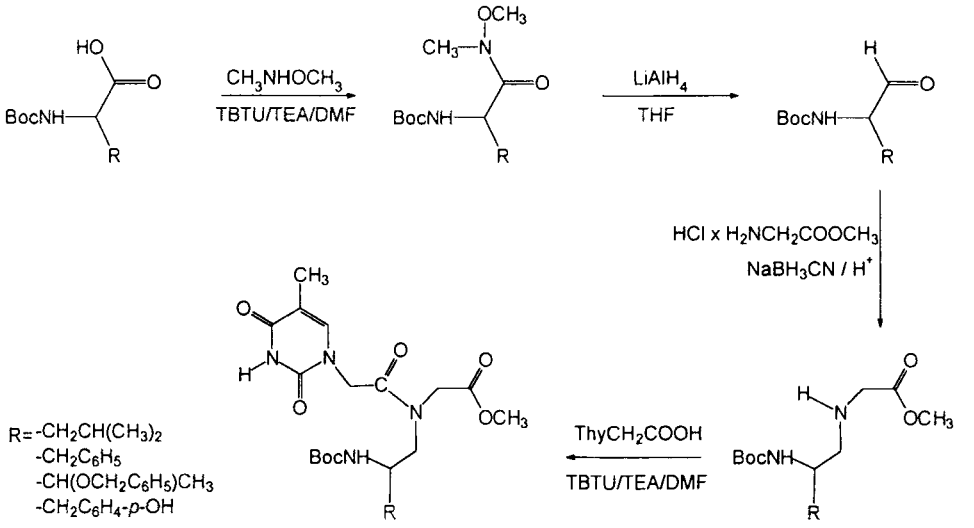


FIG. 2. Scheme of the chiral PNA monomers synthesis.

protected amino acids were transformed into their N,O-dimethylhydroxamate derivatives which were then reduced using $LiAlH_4$. The crude amino aldehydes were subjected to reductive amination with methyl glycinate following more or less the procedure described by Kosynkina et al. [6]. The protected pseudodipeptides obtained after standard work-up were directly acylated with thymin-1-ylacetic acid using TBTU procedure. Our attempts to purify 2-substituted methyl N-(2-Boc-aminoethyl)glycinates by reverse-phase high performance liquid chromatography (RP-HPLC) or by low pressure liquid chromatography gave the products in so low yields that we decided to simplify the procedure described [6] and to acylate crude pseudodipeptide. The details of the procedure are given in the Experimental. The structure of the products was confirmed by analysis of the ^{1}H-NMR spectra and MS analysis. The NMR spectra indicate the presence of two rotamers around the tertiary amide bond. The relative proportions of the rotamers were (in $CDCl_3$): PNA-Leu: 36/64; PNA-Phe: 46/54; PNA-Tyr(Bzl) 43/57, PNA-Thr(Bzl) 41/59 (as calculated from ^{1}H-NMR spectra analysis, room temperature).

The proposed procedures simplified the isolation and purification of the desired products, since the purification was limited to the last step of the synthesis. The applied procedures give chiral products; therefore the method is extremely useful in the synthesis

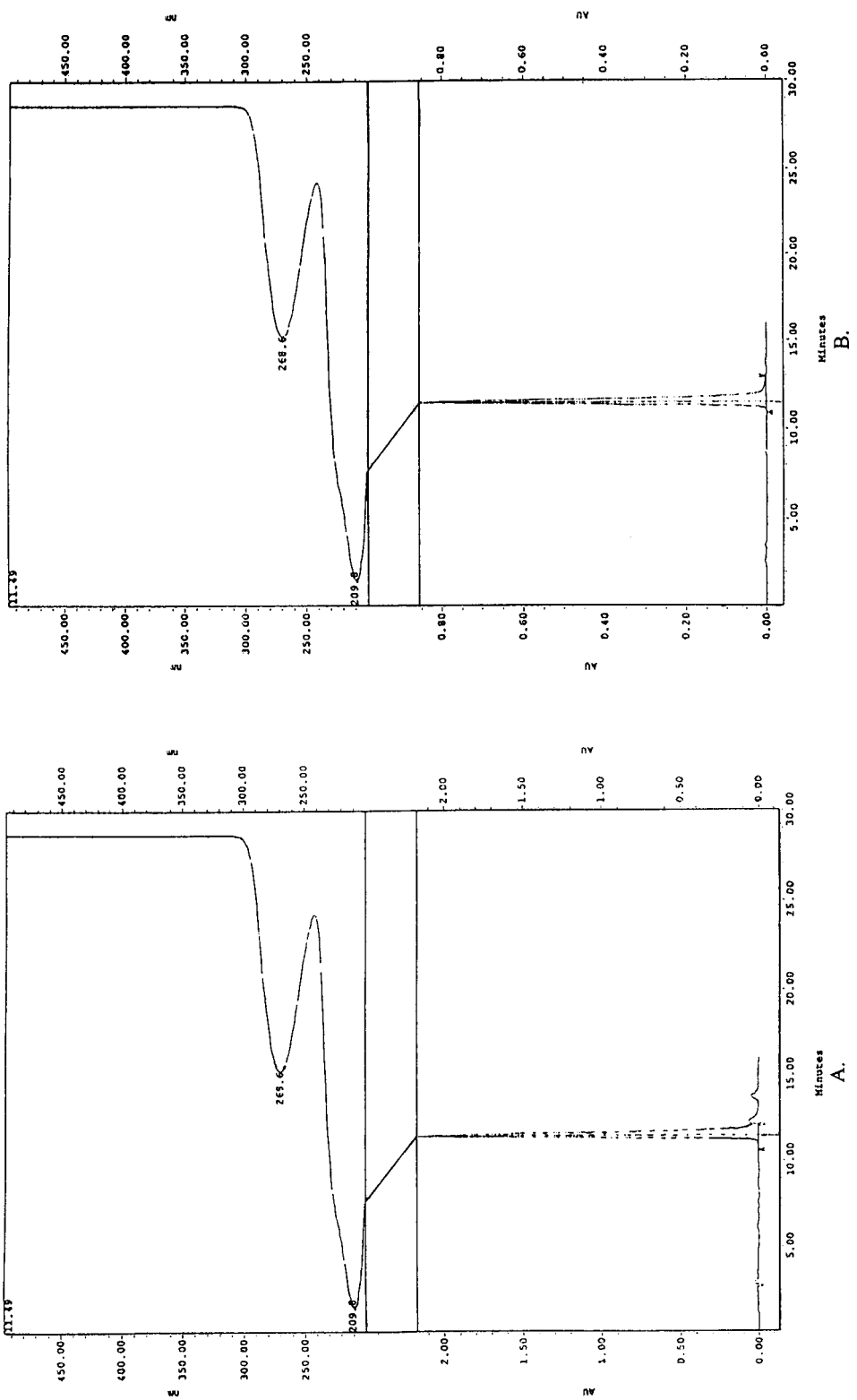


FIG. 3. Sample analytical RP-HPLC chromatograms of pure PNA-Tyr(Bzl) monomer. Flow 1 ml/min, isocratic elution using 50% acetonitrile in water containing 0.1% TFA. A. Eluent monitored at 223 nm. B. Eluent monitored at 254 nm.

of the various chiral PNA monomers which can easily be applied to synthesis of oligomers [11]. The proposed method is of general applicability and allows for various modifications of PNA structure by using different α-amino aldehydes or amino acid esters.

EXPERIMENTAL

General: The 500 MHz ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Varian Unity 500 Plus spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in ppm downfield TMS. For analytical RP-HPLC, a Varian Vista 5500 system and analytical Kromasil 100-5C8 column were used. Thin layer chromatography (TLC) was performed on Merck Kieselgel 60 F_{254} sheets. Optical rotations were obtained at 19-22 °C with a Perkin Elmer 141 polarimeter. Evaporations were performed under diminished pressure at <50 °C.

Thymin-1-ylacetic acid: the title compound was synthesized from thymine and ethyl bromoacetate according to the described procedure [12] with minor modifications.

N^{α}-Boc-L-amino acid N-methoxy-N-methylamides (general procedure): 1.07 g (11 mmol) of N-methoxy-N-methylamine hydrochloride and 1.53 ml (11 mmol) of TEA were dissolved in 10 ml of DMF and the solution was added to N^{α}-Boc-L-amino acid (10 mmol) in 20 ml of DMF and cooled to 0 °C. Then TBTU (3.53 g, 11 mmol) and TEA (2.77 ml, 20 mmol) were added to the vigorously stirred solution. When there was no substrate detectable in the solution (TLC inspection), the solvents were evaporated and the oily residue was dissolved in 100 ml of AcOEt and washed with 0.1 M $KHSO_4$ (1x30 ml), brine (1x30 ml), 5% $NaHCO_3$ (1x30 ml), and brine (2x30 ml). Organic layer was dried with $MgSO_4$. Evaporation of the solvent gave the desired compounds in 80-99% yield.

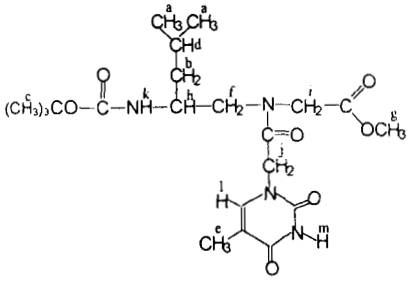
N^{α}-Boc-L-amino aldehydes (general procedure): 10 mmol of N^{α}-Boc-L-amino acid N-methoxy-N-methylamide in 20 ml of THF was cooled to 0°C and $LiAlH_4$ (0.95 g, 25 mmol) was added portionwise over a period 15 min to the stirred solution. Then AcOEt (100 ml) and 10% aqueous citric acid (80 ml) were added, and the mixture was stirred over additional 15 min in 0°C. The aqueous phase was separated and additionally extracted with AcOEt (3x30 ml). The organic phases were combined and washed successively with 5% $NaHCO_3$ (1x50 ml), water (1x50 ml), 1 M HCl (1x50 ml), brine

(1x50 ml), then dried with anhydrous $MgSO_4$. The solution was evaporated and crude aldehyde was immediately used for reductive amination.

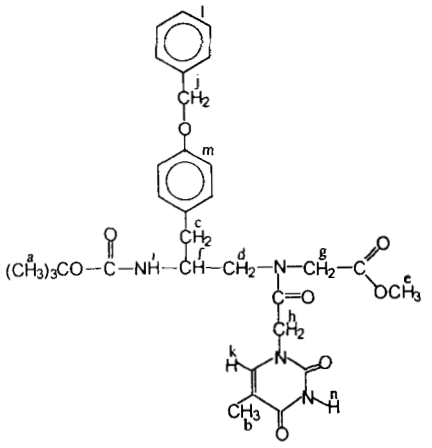
Synthesis of pseudodipeptides by reductive amination of the aldehydes (general procedure): 1.26 g (10 mmol) of methyl glycinate hydrochloride was added to 10 mmol of aldehyde in 25 ml of 3% acetic acid in methanol, followed by a portionwise addition of $NaBH_3CN$ (0.85 g, 13.5 mmol) over a period of 30 min. When there was no unreacted aldehyde (about 2 hours, TLC inspection), 200 ml of AcOEt and 80 ml of 5% $NaHCO_3$ were added. The aqueous layer was separated and additionally extracted with AcOEt (3x100 ml), the organic layers were combined, washed successively with 5% $NaHCO_3$ (1x80 ml) and brine (1x80 ml), and dried with $MgSO_4$. The solvents were evaporated and a crude pseudodipeptide (82-99% yield) was immediately used in the next step of the synthesis.

Acylation of the pseudodipeptide with thymin-1-ylacetic acid (general procedure): thymin-1-ylacetic acid (2.02 g, 11 mmol), TEA (1.53 ml, 11 mmol) and TBTU (3.53 g, 11 mmol) were suspended in 5 ml of dry DMF at 0°C and stirred vigorously over 30 min. Then the mixture was added to a solution of pseudodipeptide (10 mmol) and TEA (1.53 ml, 11 mmol) in DMF (25 ml). pH was kept at 8-9 by TEA addition. After 24 hours the solvent was evaporated and the residue was partitioned between water (50 ml) and AcOEt (150 ml). Organic phase was washed with 0.1 M $KHSO_4$ (2x50 ml), water (6x50 ml), brine (3x50 ml), and dried with $MgSO_4$. The solvent was evaporated to dryness. The crude product were dissolved in 15% acetonitrile in water containing 0.1% TFA and purified on a preparative HPLC Kromasil C_8 column using a linear gradient of 15-50% acetonitrile in water containing 0.1% TFA over 60 min; eluent monitored at 223 nm. Fractions containing only the desired product were pooled and lyophilized. The sample analytical HPLC chromatograms of purified PNA-Tyr(Bzl) monomer obtained are shown in Fig. 3.

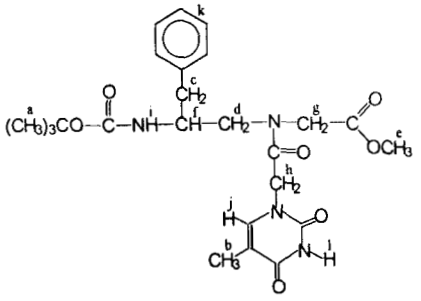
Methyl N-(2-Boc-amino-4-methylpentyl)-N-(thymin-1-ylacetyl)glycinate {PNA-Leu} (Fig. 4): synthesis scale - 10 mmol of N^{α}-Boc-Leu, yield 42.5%, $[\alpha]_{589nm}^{r.t.} = -2.1^{\circ}$ (c = 1, MeOH). FAB MS molecular ion peak analysis: calculated (found): PNA-Leu ($M + H^+$): 455.2 (455.2). ^{1}H-NMR ($CDCl_3$, TMS): δ = 0.88; 0.95 (6H, 2d, Ha); δ = 1.28 (2H, m, Hb); δ = 1.38; 1.45 (9H, 2s, Hc); δ = 1.70 (1H, m, Hd); δ = 1.91; 1.92 (3H, 2s, He); δ = 3.4 (2H, m, Hf); δ = 3.74; 3.81 (3H, 2s, Hg); δ = 3.9; 4.6 (5H, m, Hh, Hi, Hj); δ = 5.06 (1H, m, Hk); δ = 7.05; 7.10 (1H, 2s, Hl); δ = 9.21; 9.22 (1H, 2ss, Hm).



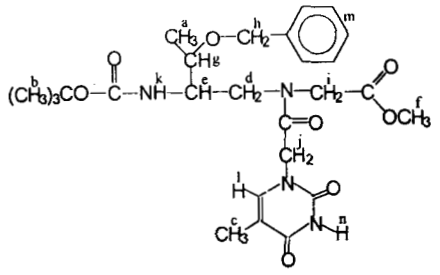
PNA-Leu



PNA-Tyr(Bzl)



PNA-Phe



PNA-Thr(Bzl)

FIG. 4. Chiral PNA monomers obtained.

Methyl N-(2-Boc-amino-3-phenylpropyl)-N-(thymin-1-ylacetyl)glycinate {PNA-Phe} (Fig. 4): synthesis scale - 10 mmol of N^{α}-Boc-Phe, yield 40%, $[\alpha]^{r.t.}_{589nm}$ = +6.7° (c = 1, MeOH). FAB MS molecular ion peak analysis: calculated (found): PNA-Phe (M + H^+): 489.2 (489.3). ^{1}H-NMR ($CDCl_3$, TMS): δ = 1.40; 1.42 (9H, 2s, Ha); δ = 1.95 (3H, s, Hb); δ = 2.90 (2H, m, Hc); δ = 3.52 (2H, m, Hd); δ = 3.75; 3.8 (3H, 2s, He); δ = 3.9 ÷ 4.8 (5H, m, Hf, Hg, Hh); δ = 5.10 (1H, d, Hi); δ = 7.07 (1H, ss, Hj); δ = 7.35 (5H, m, Hk); δ = 8.52; 8.60 (1H, ss, Hl).

Methyl N-[2-Boc-amino-3-(4'-benzyloxyphenyl)propyl]-N-(thymin-1-ylacetyl) glycinate {PNA-Tyr(Bzl)} (Fig. 4): synthesis scale - 10 mmol of N^{α}-Boc-Tyr(Bzl), yield 54%, $[\alpha]^{r.t.}_{589nm}$ = +3.1° (c = 1, MeOH). FAB MS molecular ion peak analysis: calculated (found): PNA-Tyr(Bzl) ($M + H^+$): 595.3 (595.3). ^{1}H-NMR ($CDCl_3$, TMS): δ = 1.37; 1.40 (9H, 2s, Ha); δ = 1.92; 1.93 (3H, 2s, Hb); δ = 2.82 (2H, m, Hc); δ = 3.3 ÷ 4.1 (3H, m, Hd, Hf); δ = 3.73; 3.80 (3H, 2s, He); δ = 4.15 (2H, s, Hg/Hh); δ = 4.42 (2H, d, Hh/Hg); δ = 4.65 (1H, ss, Hi); δ = 5.05 (2H, s, Hj); δ = 6.9 ÷ 7.4 (10H, m, Hk, Hl, Hm); δ = 8.52 (1H, ss, Hn).

Methyl N-(2-Boc-amino-3-benzyloxybutyl)-N-(thymin-1-ylacetyl)glycinate {PNA-Thr(Bzl)} (Fig. 4): synthesis scale - 10 mmol of N^{α}-Boc-Thr(Bzl), yield 48%, $[\alpha]^{r.t.}_{589nm}$ = -6.8° (c = 1, MeOH). FAB MS molecular ion peak analysis: calculated (found): PNA-Thr(Bzl) ($M + H^+$): 533.2 (533.2). ^{1}H-NMR ($CDCl_3$, TMS): δ = 1.21; 1.32 (3H, 2d, Ha); δ = 1.43 (9H, s, Hb); δ = 1.90; 1.94 (3H, 2s, Hc); δ = 3.46 (2H, d, Hd); δ = 3.5 ÷ 4.0 (7H, 2s + m, He, Hf, Hg, Hh); δ = 4.1 ÷ 4.8 (4H, m, Hi, Hj); δ = 5.13 (1H, d, Hk); δ = 6.93; 7.08 (1H, 2s, Hl); δ = 7.33 (5H, m, Hm); δ = 8.52 (1H, ss, Hn).

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