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# Peculiarities of Solid-Phase Synthesis of Negatively Charged Chiral Polyamides as Nucleic Acid Mimics

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**Abstract**—Homopyrimidine polyamide nucleic acid mimics based on L-glutamic acid were prepared by solid-phase synthesis. The effect of the conditions of condensation and cleavage reactions with the use of polymeric support on the yield of the target sequences was studied. Possible ways of formation of by-products were revealed by mass spectrometry.

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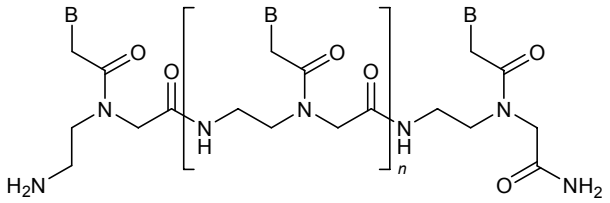
The synthesis and study of various nucleic acid analogs and mimics has become a central line in biotechnology due to their potential use as diagnostic and therapeutic agents in medicine and tools for molecular biology [1]. Among various nucleic acid mimics, the most common are peptide nucleic acids (PNA) [2]. They are structural analogs of natural nucleic acids in which the ribose phosphate backbone is replaced by a polyamide chain consisting of *N*-(2-aminoethyl)-glycine units. The structures of aminoethylglycine PNA (**A**) and polyamide nucleic acid mimics based on L-glutamic acid (**B**) are shown below.

Molecules like **A** and **B** can be readily modified via introduction of various functional substituents, in particular side chains of optically active amino acids [3]. The presence of chiral centers and/or charged fragments [4, 5] in a monomer unit largely determines physicochemical and biological properties of oligomer sequences, in particular preorganization of single-chain sequences, selectivity of complementary recognition of target molecules, solubility in physiological media, and permeability through biological membranes [6].

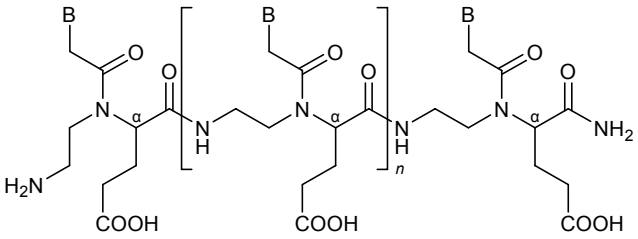
We have proposed structures of such negatively charged PNAs whose physicochemical properties approach those of natural nucleic acids to a greater extent, whereas their stability and hybridization properties remain similar to aminoethylglycine PNAs. The proposed modification implies introduction of a negatively charged group as carboxyethyl residues of L-glutamic acid into each monomer unit. We previously described a scheme of synthesis of a PNA monomer unit containing a carboxyethyl group in the α-position [7, 8].

There are almost no published data on the relations between the structure of peptide nucleic acids based on dicarboxylic amino acids and their hybridization properties. Exceptions are a few studies where the negative charge was introduced either singly into the terminal unit or in the middle with alternation through two achiral monomer units [3].

Most known syntheses of various PNA oligomers are based on the solid-phase strategy (Boc and Fmoc protocols) utilizing up-to-date condensing agents [9, 10]. However, the existing methods are not general,



**A**



**B**

B is a nucleobase.

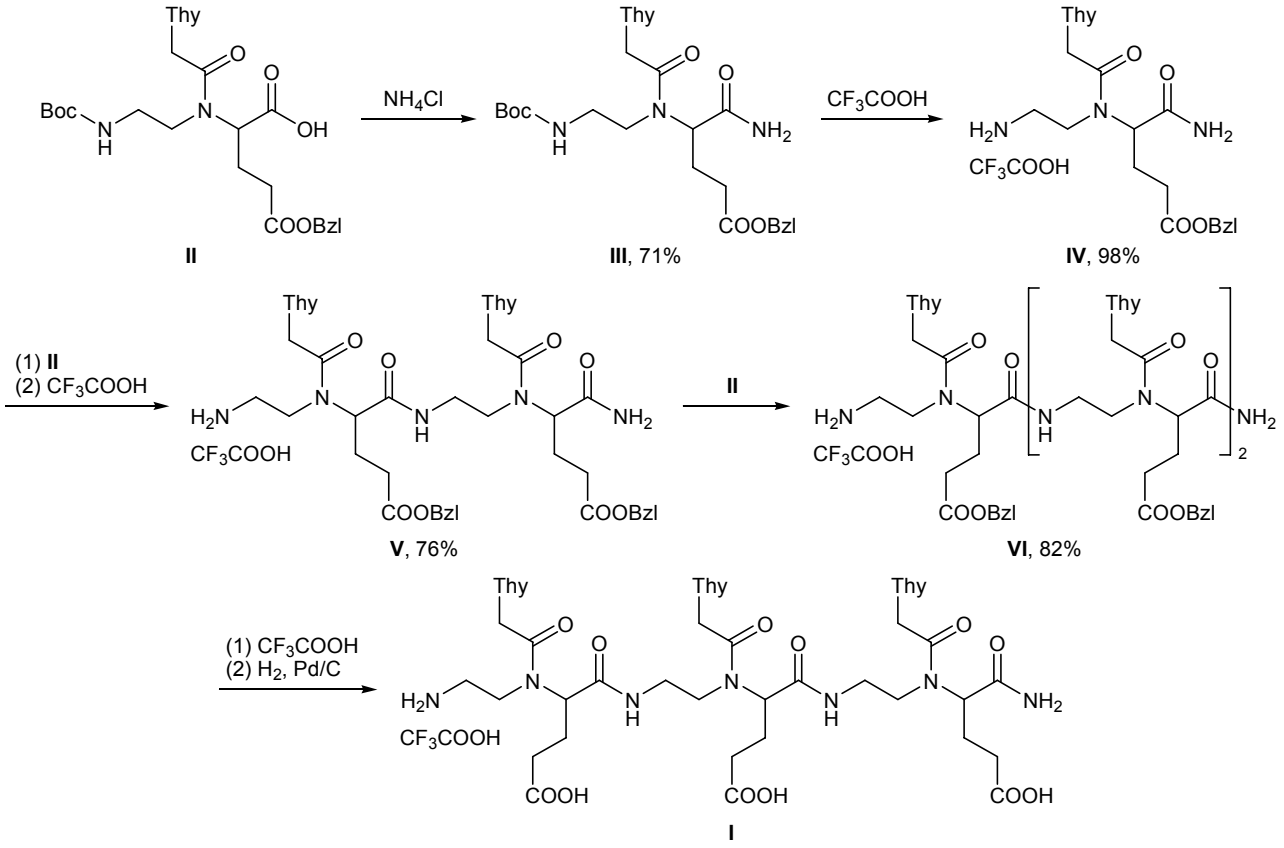
and the conditions of syntheses have not been standardized. Therefore, development of efficient procedures for the synthesis PNA oligomers, including modified ones, remains a quite important problem since the formation of various by-products could strongly reduce the yield of the target sequences. In this article we report the results of our experiments directed toward preparing short PNA oligomers containing carboxyethyl groups in the α-positions and optimizing the main steps in their solid-phase synthesis.

In the first step of our study we selected as model oligomer thymine-containing tripeptide α-$Thy_3$ (**I**) having a carboxyethyl group in the α-position of each amino acid unit [11]. The synthesis of **I** was carried out both in solution and on a solid support under analogous conditions for the main chain augmentation (activating agent, base, and reactants and radical scavengers for elimination of Boc protecting groups). The synthesis in solution followed Scheme 1. Amide **III** was prepared by mixing thymine-containing monomer **II** with ammonium chloride using (benzotriazol-1-yloxy)tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate(V) as condensing agent. The subsequent standard deprotection of the amino group by the action of 50% trifluoroacetic acid (TFA) in methylene chloride gave trifluoroacetate **IV** in quantitative yield, and compound **IV** was brought into condensation with 3 equiv of monomer **II** using 3 equiv of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate(V) (reaction time 30 min). However, we failed to achieve complete conversion of **IV** under these conditions. Repeated Boc-deprotection and subsequent condensation with **II** under analogous conditions afforded Boc/Bzl-protected tripeptide **VI**. Its structure, as well as the structure of all intermediate products, was confirmed by $^1$H NMR. Compounds **III**, **V**, and **VI** were purified by column chromatography.

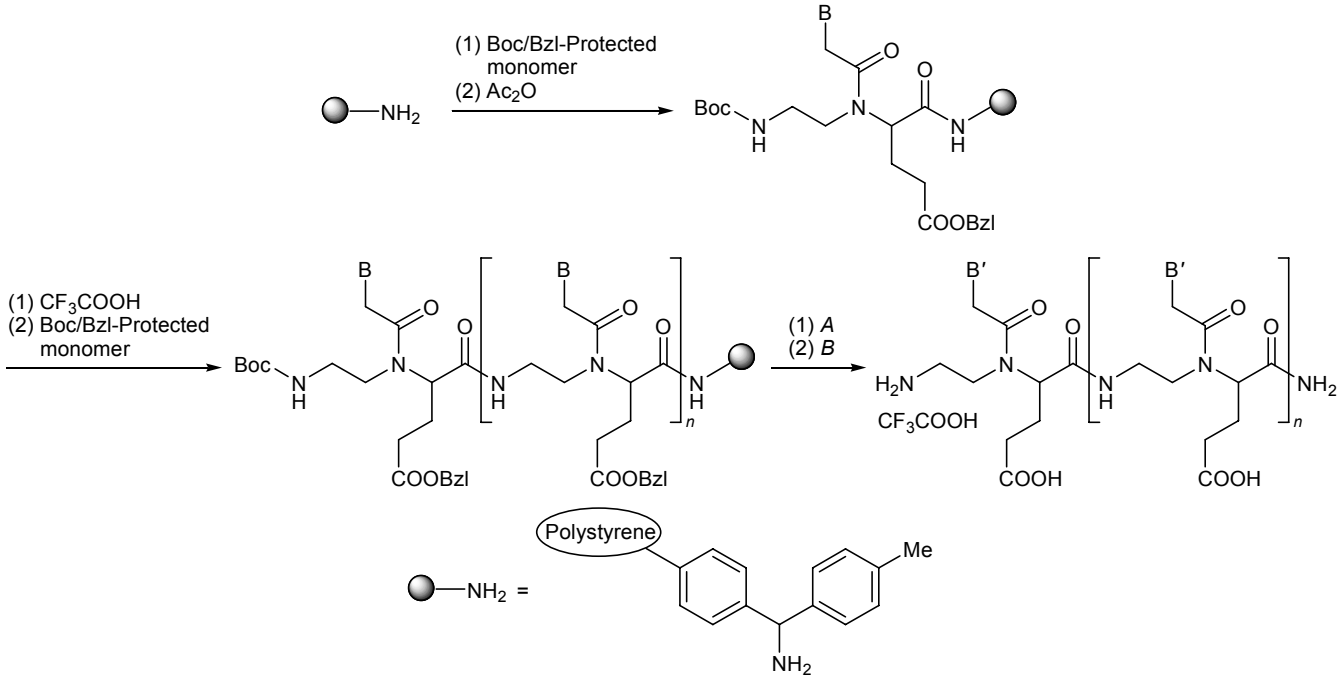
Trimer **VI** was deprotected in two steps. In the first step, the *N*-Boc protecting group was removed by the action of TFA in methylene chloride, and the ester groups were debenzylated by catalytic hydrogenation over 10% Pd/C. The target unprotected tripeptide **I** was recrystallized from anhydrous diethyl ether, and its structure was confirmed by the LC/MS data (*m*/*z* 1031.3 $[M]^+$).

For comparison, solid-phase synthesis of trimer **I** was performed according to a modified Boc-protocol [11] with the use of cross-linked (divinylbenzene,

**Scheme 1.**



**Scheme 2.**




B = Thy, Cyt$^{Cbz}$, Ade$^{Cbz}$, Gua$^{Cbz,Bzl}$; B′ = Thy, Cbz, Ade, Gua; **I**, α-carboxyethyl-Thy$_3$, $n$ = 2; **VII**, α-carboxyethyl-Thy$_6$, $n$ = 5; **VIII**, α-carboxyethyl-Cyt$_4$Thy$_6$, $n$ = 9; **IX**, α-carboxyethyl-ThyAdeCyt, $n$ = 2; **X**, α-carboxyethyl-ThyAdeGuaThyAdeCyt, $n$ = 5; Thy = thymine, Cyt = cytosine, Ade = adenine, Gua = guanine, Cbz = benzyloxycarbonyl, Bzl = benzyl.

1–2%) polystyrene containing 4-[amino(4-methylphenyl)methyl]phenyl groups (Scheme 2). It is known that polymer support load with 0.1–0.2 mmol of $NH_2$ groups per gram is the most efficient in solid-phase PNA syntheses [9]. Therefore, monomer **II** was immobilized on the resin using threefold excess of the latter. Unreacted amino groups in the polymer were capped by treatment with a 1 : 1 mixture of acetic anhydride with DMF (2 h). As a result, the amount of immobilized monomer was 0.15 mmol of $NH_2$ groups per gram of the resin (according to the quantitative Kaiser ninhydrin test) [12].

As condensing agent we used *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate(V), and the reaction was carried out in anhydrous DMF (reaction time 2 h) in the presence of ethyl(diisopropyl)amine as a base. To raise the yield, 3 equiv of **II** and condensing agent were taken. The progress of the condensation process and deprotection was monitored by the quantitative Kaiser test. Excess monomer and condensing agent were removed by thorough washing of the polymer support with DMF.

The Boc protection was removed by treatment with a mixture of TFA with *m*-cresol (95 : 5 by volume). It was found that the concentration of free amino groups (i.e., growing oligomer chains) decreased by 10–15% even at the stage of addition of the second monomer unit, which considerably impaired the yield of the target product. This may be caused by several factors, e.g., by cleavage of growing oligomer chains from the polymer support and side reactions leading to chain termination. One of the most probable side processes is transfer of *N*-acyl groups, which blocks terminal primary amino groups.

This process is typical under basic conditions when amino groups are not protonated [13]. With a view to minimize it, the subsequent condensation steps were carried out without preliminary neutralization, by adding tertiary amine directly to the condensation reaction mixture (*in situ* neutralization) [10]. However, this procedure only slightly improved the efficiency of the synthesis, and we believe that the problem associated with reduction of the amount of terminal amino groups requires special study.

Another important problem in the synthesis of PNA oligomers is the choice of optimal procedure for deprotection of the target oligopeptides and their detachment from the polymer support. Numerous procedures for this purpose are available in modern peptide chemistry, but specific features of the protecting groups and

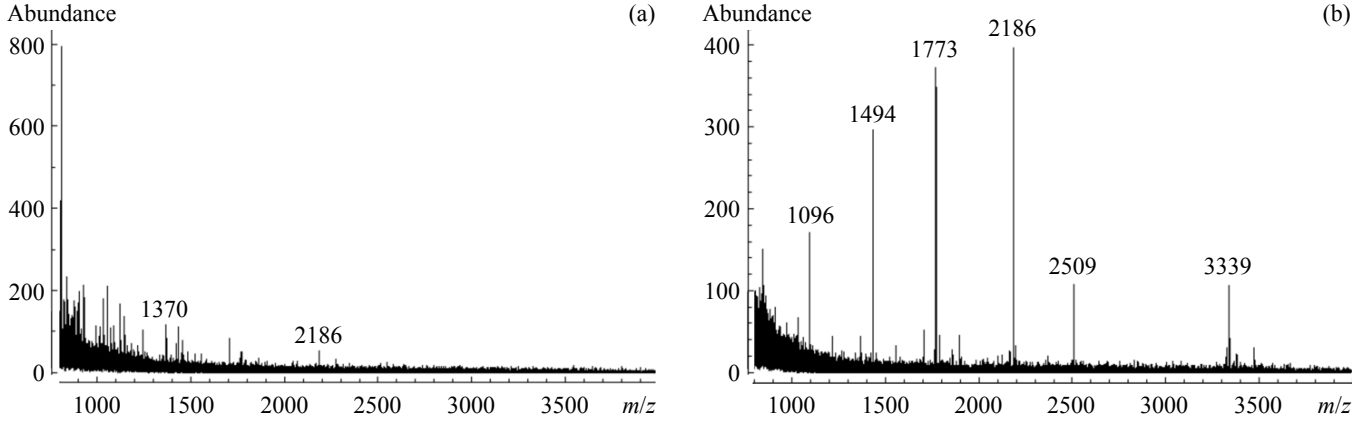
polymer support (cross-linked polystyrene modified by benzhydrylamine fragments) used in the present work require fairly strong acids. For instance, the deprotection/cleavage of trimer **I** was carried our by standard procedure including initial elimination of all protecting groups (*A*: TFA–*m*-cresol–DMSO–$CF_3SO_3H$, 11:2:6:1 by volume) and subsequent cleavage of the oligomer from the resin (*B*: $CF_3SO_3H$–TFA–*m*-cresol, 1:8:1 by volume). The substrate was kept in the above systems for 15 and 20 min, respectively, at different temperatures. The resulting oligomer was crystallized from anhydrous diethyl ether and analyzed by LC/MS. When the reaction was carried out at room temperature, the molecular ion of the major product had an *m*/*z* value of 1014.0 (no target tripeptide **I**), while the reaction at 0°C gave the product with *m*/*z* 1032.0 which coincided with the calculated value for compound **I**.

Thus we have shown the possibility for synthesizing homothymine PNA sequence based on L-glutamic acid both in solution and on a solid support.

Likewise, the above conditions were applied to the solid-phase synthesis of homothymine PNA hexamer α-$Thy_6$ (**VII**) containing a carboxyethyl group in the α-position of each monomer unit. According to the reversed phase (RP) HPLC data, the concentration of hexamer **VII** exceeded 80%, indicating a high efficiency of the cleavage procedure.

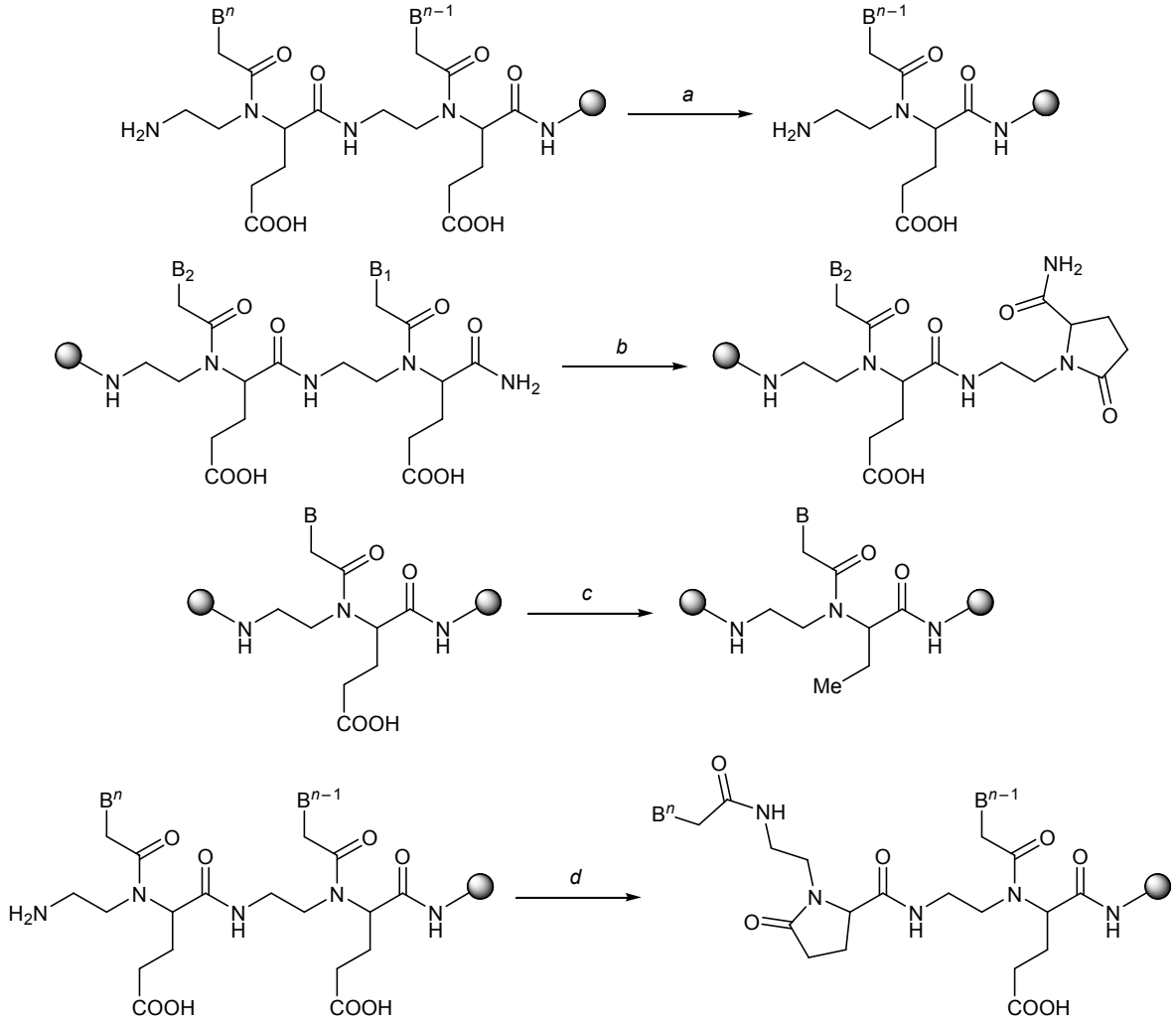
Next, we focused on the synthesis of homopyrimidine decamer α-$Cyt_4Thy_6$ (**VIII**) having carboxyethyl residues in each monomer unit. For this purpose, previously obtained immobilized hexapeptide **VII** was subjected to four cycles of coupling with Cbz (benzyloxycarbonyl)-protected cytosine-containing monomer [8]. The condensation process was monitored by the qualitative Kaiser test [12]. The deprotection and cleavage were accomplished as described above using systems *A* (15 min) and *B* (20 min), respectively. The product was isolated and purified by RP HPLC and analyzed by MALDI TOF mass spectrometry. However, the mass spectrum contained no molecular ion peak corresponding to target sequence **VIII**. Presumably, it decomposed at some step of the solid-phase synthesis, most probably during the cleavage procedure because of the presence of strong acids.

In order to verify this assumption, the reaction mixtures isolated after treatment with systems *A* and *B* were analyzed by mass spectrometry before RP HPLC purification (see figure). The reaction mixture obtained after treatment with system *A* contained almost no oligomeric products, but a large number of molecular ion peaks with *m*/*z* values lower than 1250 were observed. These peaks correspond to fragments with a molecular weight lower than that of the tetramer. In the mass spectrum of the reaction mixture isolated after treatment with system *B*, we observed both molecular ion peak with *m*/*z* 3339 (oligomer **VIII**) and those resulting from possible decomposition products. Scheme 3 illustrates probable side reactions occurring at the cleavage stage. These reactions include mainly cleavage of amide bonds both between monomer units in the chain (*a*) and between the chain and pyrimidinylacetyl fragments, followed by cyclization (*b*), decarboxylation of side carboxyethyl groups (*c*), and transfer of pyrimidinylacetyl group to the primary amino group, followed by ring closure (*d*). The presence of a carboxyethyl group in the α-position of the growing chain is likely to favor the acyl group transfer process.




MALDI TOF mass spectra of the reaction mixtures obtained after treatment of the resin loaded with oligomer **VII** with the systems (a) TFA–*m*-cresol–DMSO–$CF_3SO_3H$ (*A*) and (b) $CF_3SO_3H$–TFA–*m*-cresol (*B*).

**Scheme 3.**



B is a nucleobase.

We then proceeded with the synthesis of PNA trimer α-ThyAdeCyt (**IX**) comprising two pyrimidine (thymine, cytosine) and one purine nucleobase (adenine). Trimer **IX** was obtained by cleavage from the resin with the system TFA–*m*-cresol–$CF_3SO_3H$–DMSO (8 : 1 : 1 : 1 by volume, *C*) at 0°C (30 min). However, the product was isolated in a poor yield.

Presumably, both decapeptide **VIII** and tripeptide **IX** undergo decomposition concurrently with the oligomer cleavage from the resin; therefore, search for optimal reaction conditions is necessary, including variation of the reagent concentration and/or composition. In the synthesis of α-ThyAdeGuaThyAdeCyt hexamer (**X**), apart from the standard system [10], we used a mixture containing triethylsilane. After treatment with system *C*, we failed to isolate hexamer **X**, whereas it was isolated when the resin was treated with $CF_3SO_3H$–TFA–triethylsilane (1 : 3 : 0.1 by volume, *D*). Oligomers **IX** and **X** were isolated by RP HPLC, and their structure was confirmed by the MALDI mass spectra.

In summary, we have synthesized a series of L-glutamic acid-based peptide nucleic acids containing both pyrimidine and purine bases. Their structure was confirmed by MALDI mass spectrometry. However, the coupling/cleavage conditions for aminoethylglycine PNAs turned out not to be optimal as applied to anionic PNAs based on L-glutamic acid. The final step of the solid-phase synthesis is accompanied by various side processes. Variation of the cleavage conditions, namely the use of a triethylsilane-containing system, allowed us to considerably improve the Boc protocol for the manual solid-phase synthesis of anionic PNAs. Nevertheless, further studies in this

field are desirable in order to elucidate in detail the effects of the main synthesis parameters (temperature, reaction time, reactant ratio, post-cleavage neutralization) on the synthesis efficiency.

## EXPERIMENTAL

Liquid chromatography/mass spectrometry was performed on a Thermo Scientific instrument with an LCQ Fleet MS detector (quadrupole ion trap) using a 100×4.6-mm Hypersil Gold C18 column (5 μm); the eluent was prepared from a 10 mM aqueous solution of $NH_4OAc$ and a 90:10 mixture of acetonitrile with 10 mM aqueous $NH_4OAc$ taken at a ratio of 25:75; flow rate 0.6 mL/min; temperature 25°C.

Semipreparative reversed-phase HPLC was implemented on a Bruker instrument equipped with a Beckman 163 variable wavelength UV detector (λ 254 nm) using the following systems: (*1*) gradient elution with 0.1 M ammonium acetate (A)–methanol (B), 2.5 to 27.5% of the latter, 25 min, 4.6×250-mm Ultrasphere ODS C18 column (5 μm) or (*2*) gradient elution, 3 to 28% of methanol in buffer A, 25 min, Diasfer-110 C18 column. In addition, a Knauer instrument equipped with a Knauer Variable Wavelength Monitor (λ 254 nm) was used; (*3*) 4×150-mm Luna C18 (2) column (5 μm); gradient elution, 0 to 30% of methanol in A, 30 min.

Thin-layer chromatography was performed on DC-Alufolien Kieselgel 60 $F_{254}$ plates (Merck) using ethyl acetate–methanol (9:1, A) as eluent, as well as on a reversed-phase sorbent using acetonitrile–methanol–trifluoroacetic acid (2:8:0.1, B) as eluent. Spots were detected by treatment with a 0.5% solution of ninhydrin in ethanol, followed by heating, and under UV light.

The MALDI TOF mass spectra were recorded on Bruker UltraFlex and Bruker MicroFlex instruments equipped with UV lasers (Nd, λ 354 nm, and $N_2$, λ 337 nm, respectively) using a standard Bruker MSP polished steel target; the matrix was a solution of 2,5-dihydroxybenzoic acid in aqueous acetonitrile (1:1) or a saturated solution of 3-hydroxypyridine-2-carboxylic acid in the same solvent. The $^1$H NMR spectra were obtained at 25°C on a Bruker DPX-300 spectrometer (300 MHz) from solutions in DMSO-$d_6$ using tetramethylsilane as internal reference. The melting points were measured on a Boetius melting point apparatus. The elemental compositions were determined on a Flash 1112 CHNS analyzer; the results were in a satisfactory agreement with the calculated values.

Solvents were distilled off from solutions on a rotary evaporator under reduced pressure (20 mm). The products were dried in a high vacuum (oil pump, 0.5 mm). Monomers for solid-phase syntheses were prepared according to the procedures described previously [7, 8].

**Benzyl (2*S*)-5-amino-4-{*N*-[2-(*tert*-butoxycarbonylamino)ethyl]-2-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]-1-oxoethylamino}-5-oxopentanoate (III).** A solution of 70.0 mg (0.13 mmol) of thymine monomer **II** in 2.5 mL of DMF was cooled to 0°C, 67.0 mg (0.13 mmol) of (benzotriazol-1-yloxy)-tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate was added under vigorous stirring, the mixture was stirred for 5–10 min, 6.9 mg (0.13 mmol) of ammonium chloride and 56.0 μL (0.32 mmol) of ethyl(diisopropyl)amine were added, and the mixture was stirred for 2 h at room temperature. The solvent was removed, the residue was dissolved in 4 mL of ethyl acetate, and 2 mL of water was added. The aqueous phase was separated and extracted with ethyl acetate (3×2 mL). The extracts were combined with the organic phase, washed with a saturated solution of $NaHCO_3$ (2 mL), 0.1 M solution of $KHSO_4$ (2 mL), and brine (2 mL), and dried over $Na_2SO_4$. The solvent was removed, and the residue was subjected to column chromatography (gradient elution with ethyl acetate–methanol, 0 to 10% of the latter). The product was dissolved in 5 mL of anhydrous diethyl ether, two drops of ethyl acetate was added, and the mixture was stirred first on cooling and then for 5 h at room temperature. The white solid was filtered off and dried under reduced pressure (oil pump). Yield 49.5 mg (71%), $R_f$ 0.61 (A), mp 183°C. $^1$H NMR spectrum, δ, ppm: 1.40 s (9H, *t*-Bu), 1.78 s (3H, $CH_3$, thymine), 1.89 m and 2.21 m (1H each, β-$CH_2$, glutamic acid), 2.38 m (2H, γ-$CH_2$, glutamic acid), 3.09 m (1H) and 3.30 m (3H) ( HNC**H**$_2$C**H**$_2$N), 4.31 m (1H, α-CH, glutamic acid), 4.61 s [2H, NC(O)$CH_2$], 5.10 s (2H, C**H**$_2$Ph), 7.15 m (1H, N**H**-Boc), 7.32 s (1H, CH, thymine), 7.39 m (5H, Ph), 7.41 s and 7.58 s (1H each, $CONH_2$), 11.30 s (1H, NH, thymine). Found, %: C 57.14; H 6.51; N 12.76. *m*/*z* 546.0 $[M]^+$. $C_{26}H_{35}N_5O_8$. Calculated, %: C 57.24; H 6.47; N 12.84. *M* 545.3.

**Trifluoroacetate IV.** Amide **III**, 21.6 mg (39.6 μmol), was dissolved in 1.5 mL of methylene chloride, 1 mL of trifluoroacetic acid was added, and

the mixture was stirred for 20 min at room temperature. The solvent was removed, the residue was dissolved/evaporated with toluene (3×2 mL) and with diethyl ether (3×2 mL) and ground with anhydrous diethyl ether, and the solid was filtered off and washed with anhydrous diethyl ether; the resulting white crystals were dried in a high vacuum and used in the next step without structure determination. Yield 19.3 mg (87%).

**Trifluoroacetate V.** Monomer **II**, 49.0 mg (89.6 μmol), was dissolved in 1 mL of DMF, 31.2 μL (0.18 mmol) of ethyl(diisopropyl)amine was added, and a solution of 26.0 mg (80.6 μmol) of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate in 0.8 mL of DMF was then added. The mixture was stirred for 2 min and added to a mixture of 25.0 mg (44.8 μmol) of trifluoroacetate **IV** and 11.7 μL (0.07 mmol) of ethyl(diisopropyl)amine in 0.5 mL of DMF. The mixture was stirred for 30 min at room temperature, the solvent was removed, and the residue was treated with 5 mL of water and 5 mL of ethyl acetate. The aqueous layer was separated and extracted with ethyl acetate (2×5 mL), the extracts were combined with the organic phase, washed with a saturated solution of $NaHCO_3$ (2 mL), 0.1 M solution of $KHSO_4$ (2 mL), and brine (2 mL), and dried over $Na_2SO_4$, the solvent was removed, and the product was isolated by column chromatography (gradient elution with ethyl acetate–methanol, 0 to 20% of the latter). The solvent was removed, and the product was dried in a high vacuum. Yield of Boc/Bzl-protected amide 33.2 mg (76%), $R_f$ 0.45 (A), mp 201°C. $^1$H NMR spectrum, δ, ppm: 1.35 s (9H, *t*-Bu), 1.72 s (6H, $CH_3$, thymine), 1.89 m (1H) and 2.20 m (3H) (β-$CH_2$, glutamic acid), 2.40 m (4H, γ-$CH_2$, glutamic acid), 3.11 m and 3.30 m (4H each, HNC**H**$_2$C**H**$_2$N), 4.42 m (2H, α-CH, glutamic acid), 4.65 s [4H, $NCH_2C(O)N$], 5.10 s (4H, C**H**$_2$Ph), 7.19 m (1H, N**H**-Boc), 7.33 s (2H, CH, thymine), 7.35 m (10H, Ph), 7.41 s and 7.58 s (1H each, $CONH_2$), 11.25 s (2H, NH, thymine). Found, %: C 57.91; H 6.15; N 12.89. *m*/*z* 974.2 $[M]^+$. $C_{47}H_{59}N_9O_{14}$. Calculated, %: C 57.96; H 6.11; N 12.94. *M* 973.4.

The Boc-protection was removed as described above for compound **IV** using 11.2 mg (11.5 μmol) of the Boc/Bzl-protected amide and 0.6 mL of TFA in 1 mL of methylene chloride. Yield 11.3 mg (99%).

**Trifluoroacetate I.** The third monomer unit was attached as described above for compound **V** using 27.3 mg (50.0 μmol) of Boc-protected amide in 0.5 mL of DMF, 17.4 μL (0.10 mmol) of ethyl(diisopropyl)amine, and 15.3 mg (47.5 μmol) of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate in 0.6 mL of DMF. Yield of **VI** 14.4 mg (82%), $R_f$ 0.67 (A), mp 209°C. $^1$H NMR spectrum, δ, ppm: 1.41 s (9H, *t*-Bu), 1.70 s (9H, $CH_3$, thymine), 1.90 m (2H) and 2.24 m (4H) (β-$CH_2$, glutamic acid), 2.35 m (6H, γ-$CH_2$, glutamic acid), 3.10 m and 3.29 m (6H each, HNC**H**$_2$C**H**$_2$N), 4.37 m (3H, α-CH, glutamic acid), 4.64 s [6H, $NCH_2(C=O)$], 5.15 s (6H, C**H**$_2$Ph), 7.23 m (1H, N**H**-Boc), 7.35 s (3H, CH. thymine), 7.39 m (15H, Ph), 7.41 s and 7.58 s (1H each, $CONH_2$), 11.22 s (3H, NH, thymine). Found, %: C 58.18; H 6.00; N 12.94. *m*/*z* 1402.5 $[M]^+$. $C_{68}H_{83}N_{13}O_{20}$. Calculated, %: C 58.24; H 5.97; N 12.98. *M* 1401.6.

The Boc-protection was removed as described above using 30.0 mg (21.4 μmol) of the Boc/Bzl-protected amide and 1.6 mL of TFA in 2 mL of methylene chloride. Yield of the Bzl-protected amide trifluoroacetate 29.9 mg (99%). A 15-mg (10.5-μmol) portion of this product was dissolved in 0.42 mL of methanol–acetic acid–water (6 : 1 : 1 by volume), 4.5 mg of 10% Pd/C was added, and hydrogen was passed through the mixture over a period of 1.5 h under vigorous stirring at room temperature. The catalyst was filtered off and washed with 0.2 mL of water, the filtrate was evaporated at 40°C under reduced pressure (oil pump), the residue was ground with anhydrous diethyl ether, and the white crystalline solid was filtered off, washed with anhydrous diethyl ether, dried in a high vacuum, and analyzed by LC/MS. Yield 15.7 mg, $R_f$ 0.87 (B). Found: *m*/*z* 1032.0 $[M + H]^+$. $C_{42}H_{57}N_{13}O_{18}$. Calculated: *M* + H 1031.4.

**Solid-phase synthesis of PNA oligomers.** The polymer support was prepared as follows. Cross-linked polystyrene resin modified with 4-[amino(4-methylphenyl)methyl]phenyl groups was kept for 30 min in methylene chloride and washed in a separatory funnel with methylene chloride (20 mL per gram of the resin) until the phase boundary was clearly seen, the solvent was removed by filtration, and the resin was dried under reduced pressure (oil pump). It was then washed on a filter in succession with a 5% solution of ethyl(diisopropyl)amine in methylene chloride (2×20 mL per gram of the resin) and methylene chloride (2×20 mL per gram of the resin), the solvent was removed, and the resin was dried under reduced pressure (oil pump) and analyzed for amino groups by the quantitative Kaiser test (0.6 mmol $NH_2$ per gram of the resin).

The first monomer unit was immobilized on the resin by adding to a solution of monomer (0.2 mmol per gram of the resin) in DMF ethyl(diisopropyl)amine (2 equiv with respect to the monomer) and a 0.085 M solution of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (1 equiv with respect to the monomer) in DMF until a final monomer concentration of 0.05 M. The mixture was kept for 5 min at room temperature and added to the resin, and the resulting mixture was stirred for 2 h by passing a stream of argon. The resin was filtered off and washed with DMF (4 mL, 2×2 min) and DMF–$CH_2Cl_2$ (1:1 by volume; 4 mL, 2×2 min). The resin load was determined by the quantitative Kaiser test. Unreacted amino groups on the polymer support were capped by treatment with 2 mL of $Ac_2O$–DMF (1:1 by volume) and 2 vol % of ethyl(diisopropyl)amine. The mixture was stirred for 2 h in a stream of argon and filtered, and the resin was washed with DMF (4 mL, 2×2 min) and DMF–$CH_2Cl_2$ (1:1 by volume; 4 mL, 2×2 min). If necessary, the procedure was repeated until negative qualitative Kaiser test.

**General procedure for the solid-phase synthesis of PNA oligomers I and VII–X.** The procedure included the following steps:

(1) Removal of the Boc-protecting group from the terminal amino group; 2 mL of TFA–*m*-cresol (95:5 by volume), 1×10 min and 1×20 min;

(2) Washing with DMF–$CH_2Cl_2$ (1:1 by volume; 4 mL, 2×2 min); washing with DMF (4 mL, 2×2 min);

(3) Condensation in combination with *in situ* neutralization; a 0.085 M solution of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (1 equiv with respect to the monomer) in DMF was added to a solution of monomer (3 equiv with respect to the free amino groups in the resin) and ethyl(diisopropyl)amine (2 equiv with respect to the monomer) in DMF until a final monomer concentration of 0.05 M. The mixture was kept for 5 min at room temperature, and the condensation time was 2 h;

(4) Washing with DMF (4 mL, 2×2 min) and DMF–$CH_2Cl_2$ (1:1 by volume; 4 mL, 2×2 min);

(5) Qualitative Kaiser test. If the test was positive, the condensation step was repeated; otherwise, the next cycle was launched to obtain oligomer containing a required number of monomer units.

All syntheses followed the general procedure; only reagent systems and deprotection/cleavage conditions varied in some cases.

**Thymine trimer α-carboxyethyl-$Thy_3$ (I).** The synthesis was performed with 300 mg of the resin loaded with 0.15 mmol of the monomer per gram of the resin. After the third condensation cycle, 76.5 mg of the resin was cooled to 0°C, and system *A* (10 μL per milligram of the resin) cooled to 0°C was added. The mixture was kept for 1 h at room temperature and filtered, the resin was washed with TFA (3×2 mL) and cooled to 0°C, system *B* (5 μL per milligram of the resin) cooled to 0°C was added, and the mixture was kept for 1 h at room temperature. The filtrates were collected, the resin was washed with TFA (3×2 mL), the combined filtrates were evaporated, the residue was dried under reduced pressure (oil pump), and 3 mL of anhydrous diethyl ether was added. Water was added dropwise to the precipitate until complete dissolution, and extraction was carried out. The organic phase was washed with water (100 μL), and the aqueous phase was purified by RP HPLC (system *1*) without preliminary lyophilization. Yield 12 mg. Found: *m*/*z* 1032.0 $[M + H]^+$. $C_{42}H_{57}N_{13}O_{18}$. Calculated: *M* 1031.4.

**Thymine hexamer α-carboxyethyl-$Thy_6$ (VII).** The synthesis was performed with 150 mg of the resin (0.15 mmol/g). The qualitative Kaiser tests at the second and fourth condensation cycles were positive; therefore, repeated condensations were undertaken. After the sixth cycle, the oligomer was deprotected and removed from 10 mg of the resin by successive treatment with systems *A* and *B*. The conditions and isolation procedure were the same as above, but the reaction times were shortened to 15 and 20 min, respectively. The aqueous phase was purified by RP HPLC (system *2*) without preliminary lyophilization. Found: *m*/*z* 2046.3 $[M + H]^+$. $C_{84}H_{111}N_{25}O_{36}$. Calculated: *M* 2045.8.

**Decamer α-carboxyethyl-$Cyt_4Thy_6$ (VIII).** The synthesis was continued with PNA sequence **VII** which was subjected to four condensation cycles. The condensation at the eighth cycle was repeated (positive qualitative Kaiser test). After the tenth cycle, the decamer was deprotected and cleaved from 10 mg of the resin by successive treatment with systems *A* and *B*. The conditions and isolation procedure were the same as in the synthesis of **VII**. The aqueous phase was purified by RP HPLC (system *2*) without preliminary lyophilization. Found: *m*/*z* 3339.0 $[M + H]^+$. $C_{136}H_{179}N_{45}O_{56}$. Calculated: *M* 3338.3.

**Trimer α-carboxyethyl-ThyAdeCyt (IX).** The synthesis was performed with 100 mg of the resin (0.31 mmol/g). After the third condensation cycle,

10 mg of the resin was dried under reduced pressure (oil pump) and cooled to 0°C, system *B* (10 μL/mg) cooled to 0°C was added, and the mixture was kept for 35 min at 0°C. It was then treated as in previous experiments. The aqueous phase was purified by RP HPLC (system *3*) without preliminary lyophilization. Found; *m*/*z* 1024.0 $[M + H]^+$. $C_{41}H_{45}N_{17}O_{15}$. Calculated: *M* 1025.4.

**Hexamer α-carboxyethyl–ThyAdeGuaThyAdeCyt (X).** The synthesis was continued with PNA sequence **IX** which was subjected to three condensation cycles. After the sixth cycle, 10 mg of the resin was dried under reduced pressure (oil pump) and cooled to 0°C, system *D* (10 μL/mg) cooled to 0°C was added, and the mixture was kept for 40 min at 0°C and treated as described above. The aqueous phase was purified by RP HPLC (system *3*) without preliminary lyophilization. Found: *m*/*z* 2076.0 $[M + H]^+$. $C_{83}H_{107}N_{35}O_{30}$. Calculated: 2073.8.

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