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Synthesis of anionic peptide nucleic acid oligomers including γ-carboxyethyl thymine monomers

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Synthesis of a modified thymine monomer based on L-Glu, solid phase synthesis of anionic peptide nucleic acid (PNA) oligomers containing the modified monomer and optimization of the deblocking procedure and cleavage conditions of the PNA oligomers from resin are accomplished.

Peptide nucleic acids (PNAs)[1] are used in wide range of biomedical technologies.[2] The original aminoethylglycine (aeg) PNAs (Figure 1, **B**) are achiral non-charged molecules, which are not difficult to produce.[3] The development of this trend produced the numerous aeg-PNA modifications including chiral acyclic α- and γ-modifications (Figure 1, **C**).[4] Such γ-PNA oligomers have a number of advantages, in particular, L-amino acid derivatives are preorganizated into a right-handed helix, and more effective and specific to complementary nucleic acids targets.[5] However, nowadays broad use of PNAs and their modifications has the number of limitations, namely, relatively low solubility of the aeg-PNAs and another non-charged chiral PNAs,[6] obstacles with delivery of potential therapeutics PNA oligomers to cellular targets,[7] as well efficiency and selectivity of molecular recognition of complementary targets.[4] Introducing γ-monomers based on dicarboxyl acids into PNA may solve the problems of PNA solubility, since the presence of a carboxyl function makes modified PNA somewhat analogues to nucleic acids (Figure 1, **A**). Negative charge enables to form stable complex with cationic transfectants such as cell-penetrating peptides[8] and lipid-like substances.[9] *S*-Chiral center at γ-position effects the required preorganization of PNA oligomer and, hence, more directed interaction with targets.[10]

Recently, the attention to negatively charged PNAs appeared again[11–14] 10 years later publications devoted to phosphono-PNA.[15,16] The reported[11] Fmoc-protocol for the synthesis of oligomers including the thymine monomers based on L-Asp provided good complexing of negatively charged PNAs at medium and high concentrations of salts with nucleic acids targets, especially RNA. Therefore, here we describe the synthesis of PNA oligomers containing γ-carboxyethyl thymine monomers based on L-Glu following Boc-protocol, which is more convenient in terms of monomer synthesis.

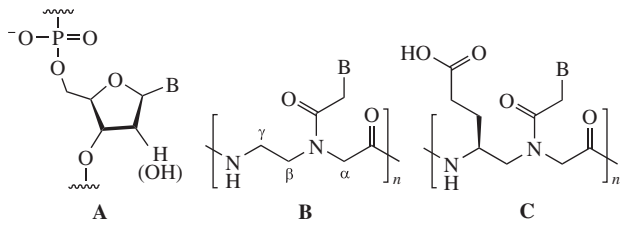
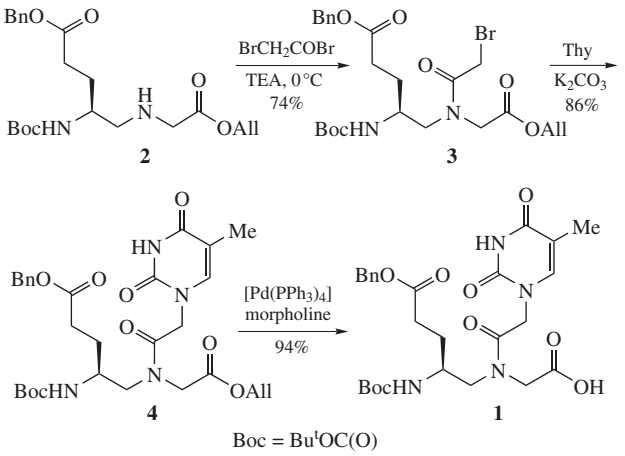


Figure 1 Structures of DNA (RNA) **A**, aeg-PNA **B** and γ-carboxyethyl-PNA **C**.

The synthesis of aeg-PNA monomers was performed by known methods.[17] The preparation of γ-modified thymine monomer **1** using condensation of thymin-1-ylacetic acid with secondary amine **2** was described earlier.[18] However, high tendency of the latter to form side cyclic β-lactam[19] makes us to use the alternative strategy[20–23] of alkylation of thymine with bromoacetyl derivative **3** (Scheme 1).† The latter was obtained from secondary amine **2**[19] by the reaction with bromoacetyl bromide in the presence of TEA. The analysis of ^{13}C NMR spectrum of fully protected monomer **4** showed that alkylation of thymine occurs regioselectively at N^1-position: the chemical shift of C^5 atom of alkylated thymine was 109.5 ppm and equal to that obtained by another synthetic approach[18] (in case of N^3-alkylation, chemical shift of C^5 atom is 107.9 ppm[24]). The target monomer **1** was produced by cleavage of allyl protecting group under catalysis with [Pd(PPh$_3$)$_4$] in morpholine as scavenger.[25] The overall yield was 85% that is higher than that in case of conventional synthetic route (65%).[18]

Tetramer ACA$^{\gamma}$T (Figure S1, Online Supplementary Materials) was synthesized according to standard protocol[26] with some distinctions: capping of amino groups was carried out with Ac_2O instead of Rappoport's reagent,[27] 3 monomer equiv. instead of 5 equiv. were used at one condensation stage, and the resin was washed with DMF–DCM system instead of pyridine. Earlier we showed[28,29] for α-PNA synthesis that when standard deblocking



Scheme 1

† For synthetic procedures, see Online Supplementary Materials.

protocol is employed for cleavage of aegPNA ('low-high TfOH'[30]), the isomeric α-PNAs oligomers underwent degradation to amide bonds, so it is preferably to use the deblocking cocktail including scavengers based on trialkylsilanes for their cleavage from the polymer support.

The cleavage of tetramer ACA$^{\gamma}$T was carried out using two deblocking protocols: (I) the standard 'low-high TfOH' treatment of resin with solution of 'low TfOH'–TFA/*m*-cresol/DMS/TfOH (11 : 2 : 6 : 1, by volume) for 15 min at 0 °C and solution 'high TfOH'–TFA/*m*-cresol/TfOH (8 : 1 : 1, by volume) for 20 min at 0 °C with preliminary cooling of resin to –30 °C and (II) mixture TFA/TfOH/$Pr^{i}_{3}SiH$ (3 : 1 : 0.1, by volume) for 45 min at 0 °C with preliminary cooling of resin to –30 °C.[29] RP HPLC of produced reaction mixture are presented in Figure 2. In case of employing protocol II with $Pr^{i}_{3}SiH$, the formation of side products was not observed. The yield of oligomer was 31% after purification by preparative RP HPLC. The initial loading of MBHA resin was 0.14 μmol g^{-1}. It was 0.114 μmol g^{-1} after four condensation stages.[31] Therefore, the average yield of condensation cycle was 95% before the cleavage of oligomer from resin, which is similar to that for aeg-PNAs[26] (97%). Thus, the major losses occur on the final deblocking step and cleavage from the resin, though the average coupling yield is 75% in this case. It is important to note that earlier synthesis of α- and γ-carboxyethyl thymine decamers gave very low overall yields (0.2% and 0.7%, respectively; the results will be published elsewhere).

The hexamer CA$^{\gamma}$TCA$^{\gamma}$T (Figure S3, Online Supplementary Materials) including two γ-modified thymine monomers **1** was synthesized like tetramer ACA$^{\gamma}$T.† The cleavage of oligomer was carried out with deblocking protocol II, its yield was 27% (an average yield per oligomerization round is 81%) after purification by RP HPLC.[32] The structure of oligomers ACA$^{\gamma}$T and CA$^{\gamma}$TCA$^{\gamma}$T was confirmed by MALDI-TOF MS data (calc. for tetramer $[M+H]^+$ *m*/*z* 1156.5, found 1157.6; calc. for hexamer $[M+H]^+$ *m*/*z* 1745.7, found 1746.5).

In conclusion, we prepared γ-carboxyethyl thymine PNA monomer by regioselective alkylation of thymine. Two new carboxyethyl PNAs oligomers were obtained by solid phase synthesis. Application of new deblocking protocol with $Pr^{i}_{3}SiH$ and temperature control for the oligomer cleavage from the resin provides effective oligomer synthesis.

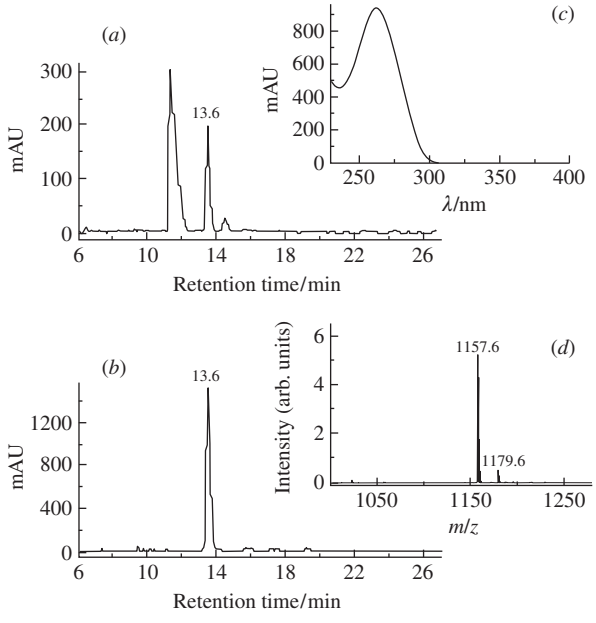


Figure 2 Profiles of RP HPLC of reaction mixture produced when cleaved tetramer ACA$^{\gamma}$T from polymer support using deblocking protocols (*a*) I and (*b*) II, (*c*) UV and (*d*) MALDI-TOF mass spectra of oligomer ACA$^{\gamma}$T.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2015.01.017.

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