New procedure of the Mitsunobu reaction as the key step in peptide nucleic acid (PNA) monomers synthesis

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ABSTRACT
PNAs are relatively novel DNA analogues, intensively studied due to their potential as gene-targeted drugs with antigenic and antisense properties. In 1996 we elaborated a new method of synthesis of PNA monomer backbones based on the Mitsunobu reaction with N-tosyl-protected (Tos) amino acid esters as acidic components of the reaction. Since the method used for the Tos group removal requires conditions incompatible with various functional groups, here we modified the procedure by replacing the tosyl group with o-nitrobenzenesulfonyl (o-NBS) group. Using the new procedure we obtained protected PNA monomer backbones with various amino acid side chains. The pseudodipeptide secondary amine groups were then deprotected by thiolysis, and after standard work-up acylated with thymine-1-yllactic acid, to give the protected monomers. Since the deprotection of the secondary amine group occurs under mild conditions, the procedure is of general applicability and allows various modifications of PNA structure by using diverse β-amino alcohols and α-amino acid esters.

INTRODUCTION
Peptide nucleic acids (PNA) incorporating nucleic acid bases into polyamide backbone are relatively novel DNA analogues [1]. They mimic oligonucleotides forming Watson-Crick heteroduplexes with complementary DNA or RNA. They have recently been intensively investigated due to their potential as gene-targeted compounds with antigenic and antisense properties [reviewed in 2-4].

In the typical PNA oligomers the phosphodiester backbone of DNA is replaced by N-(aminoethyl)glycine units. The majority of the reported procedures for the synthesis of peptidic part of PNA monomer are based on unstable Boc-aminoacetaldehyde as the key component. The PNA structure is easy to modify and it is probable that synthesis of altered (e.g. chiral [5]) monomers would give the possibility to obtain oligomers with improved properties, e.g. with better permeability through cellular membranes [4].

reaction (the group introduced for amide alkylation by Henry et al. [9] and Edwards et al. [10], and then for sulphonamide alkylation under the Mitsunobu conditions by Papaioannou et al. [11]). Since the removal of Tos group is difficult, modifications of the N-alkylation procedure has recently been devised [12, 13]. Since the method previously used by us requires conditions incompatible with various functional groups, here we present a new, modified and efficient method of synthesis of various peptidic parts of PNA monomers, employing the Mitsunobu reaction (one can see several reviews on the extreme usefulness of the reaction [14-19]).

RESULTS AND DISCUSSION

Methyl o-NBS-glycinate was used as an acidic [12] and N-t-butoxycarbonyl-aminooethanol (Boc-aminooethanol), Boc-phenylalaninol, Boc-phenylalaninol, Boc-phenylalaninol, or Boc-leucinol were used as alcoholic components in the Mitsunobu reaction which is the key step of the syntheses.

Boc-aminooethanol was synthesised from aminooethanol and an excess of (Boc)2O in diethyl ether. Boc-aminooethanol was synthesised by the sodium borohydride reduction of mixed anhydride obtained from Boc-PhCH2OH and isobutyl chloroformate [20].

The Mitsunobu reaction was carried out in THF with the use of triphenylphosphine (TPP) and diethyl azodicarboxylate (DEAD) or disopropyl azodicarboxylate (DIAD) and resulted in fully protected pseudopeptides. The o-NBS group was removed by thiolysis. The thiolysis conditions were optimised using o-NBS-GlyOMe as model compound and various combinations of thiols (2-mercaptopropionic acid, diisopropylethylamine, piperidine, thiolactic acid, 2-thiophenol, and potassium carbonate in dimethylforrnamide [12]) were chosen. The pseudopeptides were isolated from the reaction mixture with C18 cartridges.

Thymin-1-ylacetic acid (ThyAcOH) was synthesized from thymine and ethyl bromoacetate analogously to the procedures described and was subsequently coupled to the peptidic part of PNA monomer using optimised procedure [21]. Then the C-terminal carboxylic group was deprotected using standard saponification. The desired compounds were obtained in high yields after chromatographic work-up. Employing the procedure described, we have synthesized the PNA monomers listed in Fig. 1. NMR and MS data of all obtained compounds were in agreement with expectations.

In summary, we have developed a new and convenient method of PNA monomer synthesis. Since the deprotection of the secondary amine group occurs under mild conditions, the procedure is of general applicability and allows various modifications of the PNA structure. The described procedure can be easily used to the synthesis of PNA monomers containing chiral amino acid residues not only in ,,aminoethyl", but also in the ,,amino acid" part of the PNA backbone [for a review on PNA modifications, see 4], by using various β-amino alcohols and α-amino acid esters.

ACKNOWLEDGEMENTS

This work was supported by the Polish State Committee of Scientific Research (KBN) 3T09A08013 research grant.

REFERENCES