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NPE-resin, a new approach to the solid-phase synthesis of protected peptides and oligonucleotides

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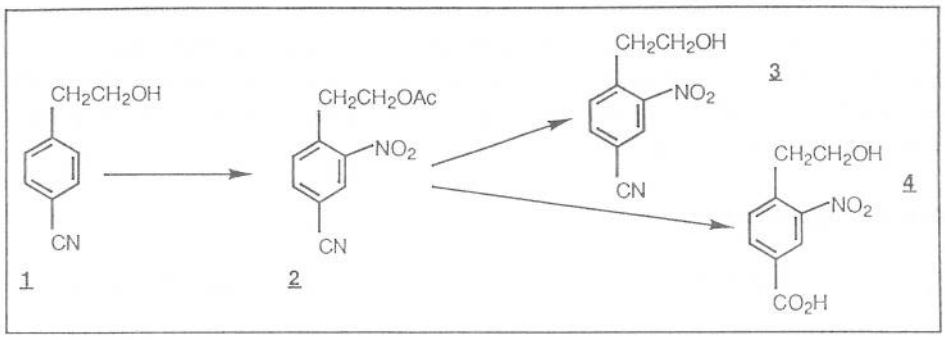
Introduction

Solid-phase methodology has become the most used technique for the preparation of peptides and oligonucleotides. Generally, the nucleoside at the 3'-end of the oligonucleotide and the C-terminal amino acid of the peptide are linked through its 3'-hydroxyl and its carboxyl group to a suitable functionalized support. In oligonucleotide synthesis the most commonly used linkage is the succinate linkage although some alternatives have been proposed [1]. In peptide synthesis, the choice of the linkage between polymeric support and peptide depends on the nature of the peptide and the C-terminal residue and the protection strategy for the α-amino and side chain functions [2]. One of the alternatives to obtain large peptides is the convergent solid-phase approach [3] which involves the preparation and assembly of protected peptides. The preparation of these protected fragments needs a linkage between the peptide and the polymeric support that can be cleaved leaving other protecting groups of the peptide intact.

In the present communication we describe the synthesis of a new polymeric support (NPE-resin) that contains a 2-(2-nitrophenyl)ethyl linkage labile to bases through a ß-elimination process and the use of this new linkage for the synthesis of oligonucleotides and protected peptides.

Results and Discussion

Starting from 2-phenylethanol, 4-(2-hydroxyethyl)-3-nitrobenzonitrile (**3**) was prepared as described in Ref. 4. 4-(2-hydroxyethyl)-3-nitrobenzoic acid (**4**) was prepared by hydrolysis of **3** or directly from 4-(2-acetyloxyethyl)-3-nitrobenzonitrile (**2**) obtained after nitration of 4-(2-hydroxyethyl)benzonitrile (**1**). The active ester was prepared by reaction of **4** and 2,4,5-trichlorophenol in the presence of dicyclohexylcarbodiimide (DCC). The anchoring of 4-(2-hydroxyethyl)-3-nitrobenzoic acid to aminomethyl polymeric supports (polystyrene and controlled pore glass) was achieved by DCC-mediated coupling of **4** or directly with the active ester using DMF as solvent in the presence of 1 equivalent of HOBt. In both cases a negative ninhydrin test was obtained with 2 equivalents of **4** or its active ester after



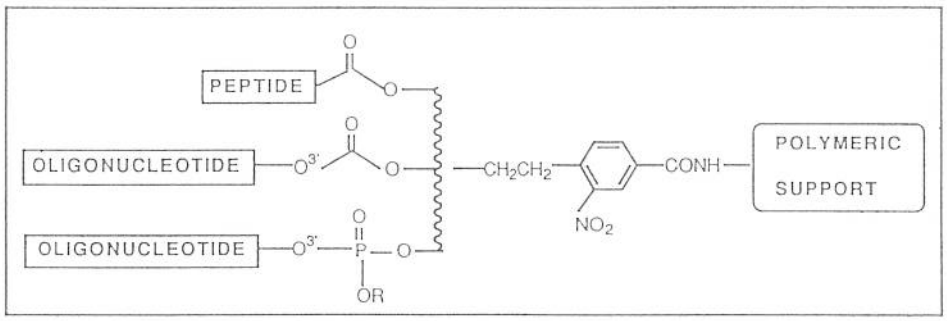
3 h. Attachment of Boc-amino acids to NPE-polystyrene resin was achieved with DCC in the presence of 0.1 equivalent of dimethylaminopyridine.

The following sequences were assembled stepwise using Boc-amino acids and standard protocols:

Boc-Leu-Ala-Gly-Val-OH
Boc-Glu(Bzl)-Ser(Bzl)-Gly-OH
Boc-Lys(Z)-Lys(Z)-Ala-Ala-OH
Boc-Gln-Thr(Bzl)-Thr(Bzl)-Arg(Tos)-Glu(cHex)-Asn-Ile-Met-Lys(ClZ)-OH

At the end of the synthesis, protected peptides were obtained by treatment of peptidyl-resins with a 0.1 M DBU solution in dioxane (2 h, 25°C) or alternatively with 20% piperidine-DMF (2 h, 25°C) followed by Dowex 50 × 8 or Sephadex LH-20 chromatography to remove excess base. Good cleavage yields (> 90%) and high purity of peptides (70–98%) were observed in all cases. Amino acid analyses of purified peptides were in good agreement with the theoretical value and no racemization (< 1%) was detected by the GC-MS method [6].

Furthermore, DMT-nucleosides have been attached to NPE supports through a carbonate or a phosphate linkage. As these linkages are also labile to concentrated ammonia, oligonucleotides and oligonucleotide 3'-phosphates have been prepared on these supports using commercially available 2-cyanoethyl phosphoramidites. When used in conjunction with amidites containing *p*-nitrophenylethyl groups [5]



as base protecting groups, we obtained oligonucleotides using the following protocol for final deprotection: (1) 3-h treatment with 40% triethylamine in pyridine to remove the 2-cyanoethyl phosphate protecting groups, and (2) 3-h treatment with a 0.5 M DBU solution in pyridine. To our knowledge, this is the first time that a method for the preparation of oligonucleotides that completely avoids the use of nucleophiles is described.

Acknowledgements

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