# SYNTHESIS OF PEPTIDE SUBSTRATES FOR PORCINE ELASTASE VIA (4-HYDROXY-3-NITRO)BENZYLATED POLYSTYRENE (PHNB)

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## 1. Introduction

Peptide synthesis by means of polymeric active esters was introduced recently as a new approach to the preparation of peptides with a predetermined sequence [1,2]. In this technique amino acids or peptide esters possessing a free α-amino group are coupled with high molecular weight insoluble active esters of N-blocked amino acids and cross-linked hydroxyl polymers. The N- and D-blocked peptides obtained in such a reaction could be separated readily from the insoluble reagent and subsequently elongated by removal of the N-blocking group and by coupling with the insoluble polyfunctional active ester of a desired N-blocked amino acid.

Although the successful preparation of several peptides was reported [1,2] we did not utilize the above approach routinely, as there were certain drawbacks in the physical and chemical properties of the polymeric active esters used [3]. As shown in our previous publication [4], these difficulties were overcome by the use of (4-hydroxyl-3-nitro)benzylated polystyrene (PHNB). In this paper we describe further applications of PHNB active esters in the synthesis of several penta-, hexa- and heptapeptides, designed as substrates for mapping the active site of porcine elastase [5–7].

** *Abbreviations:* PHNB, (4-hydroxy-3-nitro)benzylated polystyrene. Abbreviations for peptides follow IUPAC-IUB rules: Eur. J. Biochem. (1972) 27, 201–207.

## 2. Materials and methods

The active esters of N-blocked amino acids with PHNB were prepared as described previously [4].

Commercially available fluorescent Kieselgel plates (obtained from Riedel-de-Haen, Hannover) were used for thin–layer chromatography. The following solvent systems were used: The organic layer of butanol: acetic acid: water (4:1:4) (I); chloroform:methanol (II).

Peptides were detected either by charring over a flame or under the ultraviolet lamp (when the plates contained a fluorescent indicator). The free peptide substrates were identified by their $R_f$ values in electrophoresis according to Atlas [7]. The electrophoresis was carried out at pH 1.9 on Whatman No. 1 paper for 4 h at 3000 V, and the stains were developed with ninhydrin.

Removal of blocking groups was accomplished by standard methodology; Boc groups were cleaved in 0.5 N HCl hydrogen chloride in dioxane for 20 min. Carbobenzoxy groups were cleaved with 30% hydrogen bromide in acetic acid for 30 min to 60 min [8]. Carbobenzoxy and ONB-groups were simultaneously removed from final products by catalytic hydrogenation. The peptide substrates obtained were identified after enzymatic nydrolysis by porcine elastase [5–7]. Some of the N- and C-blocked peptide intermediates and their hydrogen chloride or hydrogen bromide salts were also identified by elemental analysis and anhydrous titration with sodium methylate [9].

2.1. *Coupling via the PHNB esters*

Dried N-blocked amino acid active ester of PHNB (1.5–2.0 equiv) was reacted at room temperature with one equivalent of the amino acid or the peptide *p*-nitrobenzyl ester in dimethylformamide (Fluka, dried on 5 Å molecular sieve), (the HCl or HBr salts having been neutralized with triethylamine). The amount of solvent used in the coupling procedure was minimal, just sufficient to wet the beads. After several (from 2 to 6) hours when all the amine was reacted as shown by thin-layer chromatography, the polymer was filtered and washed twice with *N,N*-dimethyl-formamide. The combined filtrates were poured slowly into 20 vols of water. The white precipitate was collected and washed with cold water, then dried in high vacuum over $H_2SO_4$ and NaOH. The yields of the collected products were always high (90–98%).

## 3. Results and discussion

By using both PHNB-active ester and classical coupling (via the *N*-hydroxysuccinimide esters of Z-L-Ala, Boc-L-Ala, Z-L-Phe and αBoc, εZ-L-Lys) according to the procedure described above, stepwise synthesis of the predetermined peptides was accomplished (see table 1). Since the polymeric active esters can be separated readily from the reaction mixture large excesses can be used and the resulting peptides are pure and in very high yields. Purity was determined chromatographically and electrophoretically (see Materials and methods) as well as by elemental analysis. The final free peptides which served as substrates for porcine elastase were checked for purity by alkali uptake at total enzymatic hydrolysis.

Peptide synthesis via PHNB active esters is very efficient. The peptides were always obtained in very high yields; coupling procedures were fast and easy removal of the active ester rendered the reaction simple to perform. The usual need for purification by crystallization, acid-base extraction, etc., because of unreacted starting materials was obviated, thus the entire synthetic procedure was relatively short and efficient. An additional advantage of polymeric technique is the capability of reloading the polymer with active ester thus recycling it for additional synthese

## References

[1] Fridkin, M., Patchornik, A. and Katchalski, E. (1968) J. Am. Chem. Soc. 90, 2953.
[2] Fridkin, M., Patchornik, A. and Katchalski, E. (1972) Biochemistry 11, 466.
[3] Patchornik, A., Fridkin, M. and Katchalski, E. (1973) in: The Chemistry of Polypeptides. Essays in Honor of Prof. Zervas, Ch. (Katsoyannis, P. G. ed.) Plenum Press, New York, p. 315.
[4] Kalir, R., Fridkin, M. and Patchornik, A. (1974) Europ. J. Biochem. 42, 151.
[5] Atlas, D. and Berger, A. (1972) Biochemistry 11, 4719.
[6] Atlas, D. and Berger, A. (1973) Biochemistry 12, 2573.
[7] Atlas, D. (1975) J. Mol. Biol. 93, 39.
[8] Ben-Ishai, D. and Berger, A. (1952) J. Org. Chem. 17, 1564.
[9] Patchornik, A., Ehrlich-Rogozinski, S. and Fridkin, M. (1975) in: Peptides, 1974, Proc. 13th Europ. Peptide Symp. (Wolman, Y. ed.) p. 257. Israel University Press, Jerusalem.

Table 1
Peptide substrates prepared via PHNB esters

| Compound | Yield (%) | Optical configuration |
|---|---|---|
| 1. Boc-Ala-ε Z-Lys–Ala-naphtyl ester | 95 | $L_3$ |
| 2. α, ε di Z-Lys–Ala–Ala–Ala-ONB | 93 | $L_4$ |
| 3. Z-Ala–Ala–Ala Leu ε Z Lys Ala ONB | 98,97,98 | $L_6$ |
| 4. Z-Ala–Ala–Ala–Ala ε Z Lys Phe Ala ONB | 97,96 | $L_7$ |
| 5. Z-Ala–Ala–Ala ε Lys Phe D Ala ONB | 96,95 | $L_6D$ |
| 6. Z-Ala–Ala–Ala ε Lys Phe D Ala ONB | 98,95,97 | $L_7D$ |
| 7. Z-Ala–Ala–Ala ε Z Lys Phe D Ala Ala | 99 | $L_6DL$ |
| 8. Z-Ala–Ala–Ala–Ala–Ala ε Z Lys Phe Ala D Ala ONB | 99,99 | $L_8D$ |
| 9. Boc-Ala–Ala–Ala–Ala ε Z Lys Phe Ala–Ala ONB | 98,99,95 | |
| | 94,95,97 | $L_8$ |

The underlined compounds indicate amino acid residues for which the yields are given.