Facile Solid-Phase Synthesis of Peptide-7-*p*-Nitroanilide (*p*NA) Analog Containing Conjugates Using a Novel Wang or Rink Amide Resin

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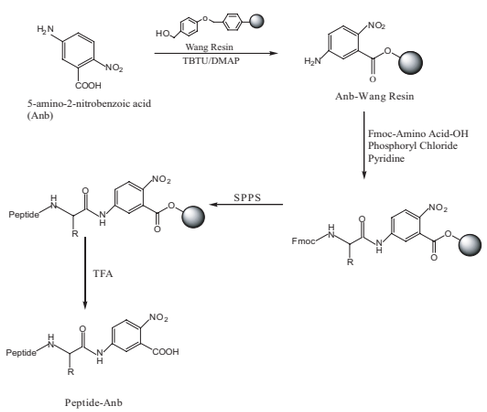
Introduction

Proteases play a key role in literally all biological processes, and are of great interest especially to the pharmaceutical industry. Colorimetric based Peptide-*p*-Nitroanilide conjugates (peptide-*p*NAs), with absorbance at approximately 408 nm, have historically been and are still widely used substrates for the study of protease activity. The preparation of peptide-*p*NA however, presents several technical challenges. Firstly, the amino group of *p*NA has a low nucleophilic property due to the electron-withdrawing effect of the nitro group. Secondly, poor solubility of a *p*-nitroanilide intermediate and lastly, coupling in solution phase by DCC, azide or active ester, techniques commonly used are not effective.



Scheme 1. Solid-phase synthesis of peptide-Anbs using Anb-Wang resin.

Based on a paper by Hojo et. al., in which they described the introduction of Anb^{5,2} to a p-methylbenzhydrylamine (MB) resin, here we report the development of two novel supports for facile solid phase syntheses, namely, Wang-resin and Rink Amide–resin conjugated with a *p*NA analog, 5-amino-2-nitrobenzoic acid (Anb^{5,2}). In scheme 1, 5-amino-2-nitrobenzoic acid (Anb^{5,2}) is loaded to Wang resin. The Anb-Resin is then coupled to a fluorenylmethoxycarbonyl (Fmoc) containing-amino acid using phosphoryl chloride in pyridine. Peptide synthesis can subsequently proceed using standard Fmoc-chemistry, followed by cleavage with trifluoroacetic acid to obtain Anb-peptide.

Materials and Methods

- 5-amino-2nitrobenzoic acid (Anb^{5,2}) was used as a *p*NA analog
- Attachment of Anb^{5,2} to Wang resin was achieved using TBTU/DMAP. Loading was determined to be 1.0 mmol/g
- The first amino acid was coupled using $POCl_3$ / pyridine at different temperatures
- Peptides were synthesized using standard solid-phase Fmoc chemistry
- A peptide substrate of Caspase-8, Ac-IETD-Anb was selected as the model compound and was compared with an authentic sample, Ac-IETD-*p*NA

Results

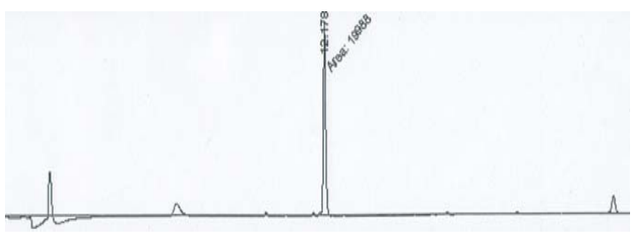


Figure 1. HPLC spectrum of crude Ac-IETD-Anb.

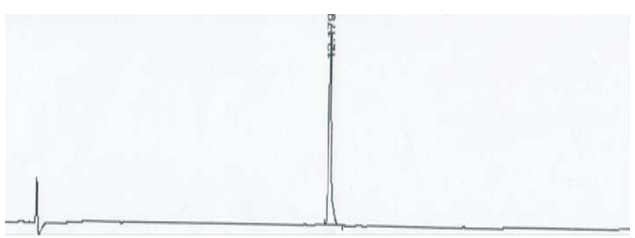


Figure 2. HPLC spectrum of purified Ac-IETD-Anb.

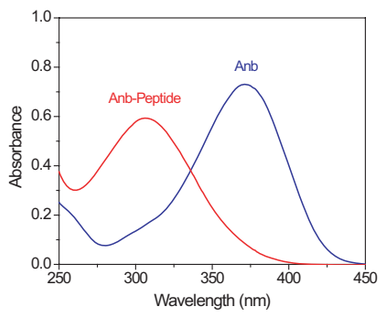


Figure 3. UV-Vis spectrum of Ac-IETD-Anb and Anb.

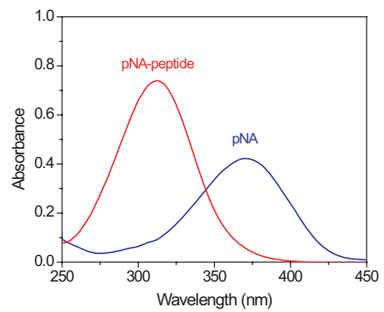


Figure 4. UV-Vis spectra of Ac-IETD-*p*NA and *p*NA

Figures 1 and 2 show that Ac-IETD-Anb was obtained in good crude purity and yields. The spectra of Ac-IETD-Anb is similar to Ac-IETD-*p*NA before and after cleavage by enzymes (Figures 3 and 4).

Conclusions

We have successfully synthesized *p*NA analog peptides (Anb-peptides) using Wang and Rink Amide resins for facile solid-phase synthesis. The approach is straightforward and versatile. Technical difficulties in making *p*NA-peptides can be circumvented by the use of these *p*NA analog containing resins. Moreover, enzyme cleavage of Anb-peptides is similar to that of *p*NA-peptides. The availability of Anb-resins will greatly facilitate the synthesis of peptide-*p*NA-like chromogenic substrate for protease analysis.

Reference

Hojo, K. et al. *Chem. Pharm. Bull. U.S.A.* **48**, 1740 (2000).