Rapid parallel synthesis of 584 betides, peptides composed largely of beta-amino acids with side-chains not found in natural peptides

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Introduction

Structure activity studies require the use of novel building blocks for exploration of structural and functional features of peptide analogs. Optically pure beta amino acids bring additional flexibility to the peptide chain and can be used for detailed study of the importance of side chain location. We have developed a technique capable of producing up to 768 peptides in a single run [1,2] and we have applied it to the synthesis of an array of 584 betides, peptides composed either solely, or in large proportion, of beta amino acids.

Results and Discussion

The structures of the optically active beta amino acids used in this study are given in Fig. 1. The synthesis of betides was performed in a centrifugal synthesizer (Compas 768.2) using five microtiterplates. Benzhydrylamine resin (3 mg) was distributed into individual wells, and standard DIPCDI/HOBt couplings were performed and monitored by the bromophenol blue method [3]. Fmoc groups were removed by 50% piperidine in DMF. At the end of the synthesis the side-chain protecting groups were removed by a 50:45:5 TFA/DCM/anisole mixture. The resin was washed and dried, and the product was detached from the resin by gaseous HF [1,4,5]. The betides were extracted by acetic acid, lyophilized and analyzed by HPLC and MS. One plate of identical compounds was synthesized on aminomethyl polystyrene with Knorr linker and betides were cleaved by a TFA/H_2O/anisole (92:5:3) mixture. The quality of products from both cleavages is compared in Fig. 2. The two step deprotection/cleavage procedure provided products of superior quality in comparison to the products prepared on TFA cleavable linker. Betides were designed to mimic the structure of recently identified ligands for the mu, kappa, and delta opiate receptors [6] (Tyr-D-Nva-Gly-Nal-NH_2, D-Phe-D-Phe-D-Ile-D-Arg-NH_2, Trp-D-Tyr-Asn-Arg-NH_2) and their screening provided new analogs. The results of the screening will be presented elsewhere.

Acknowledgment

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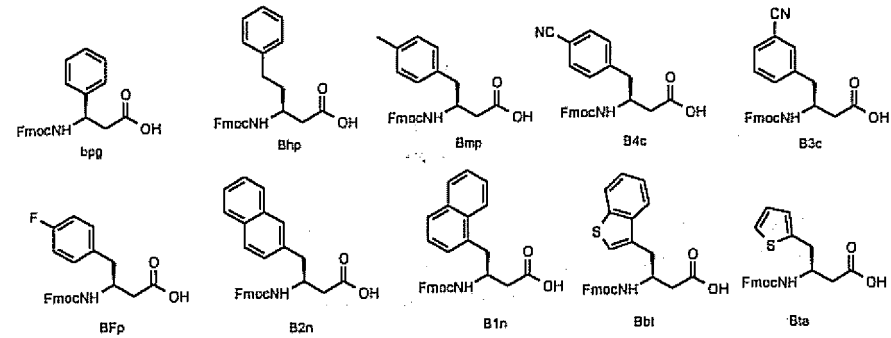

Fig. 1. Structure of beta amino acids used in the synthesis of 584 betides.

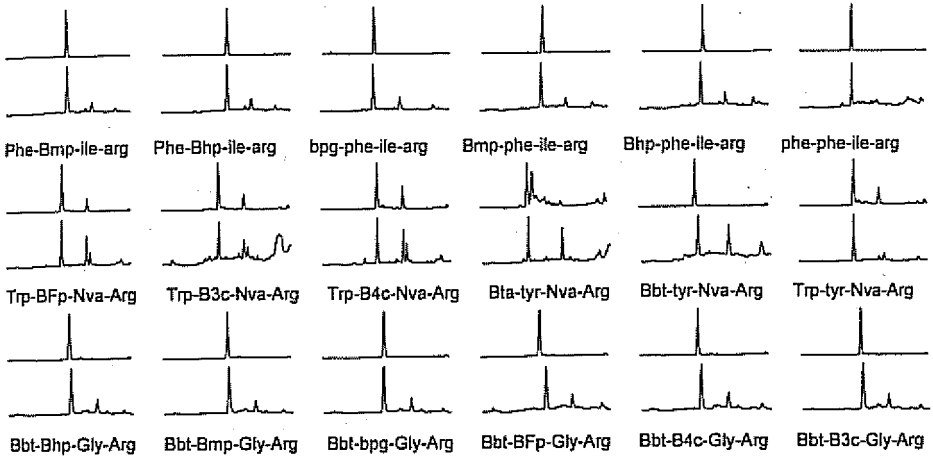

Fig. 2. HPLC traces of "difficult" betides. Upper traces - cleavage by HF from the p-methylbenzhydrylamine resin; Lower traces - cleavage by TFA mixture (Knorr linker).

References

1. Lebl, M., Bioorg. Med. Chem. Lett. 9 (1999) 1305.
2. Lebl, M., elsewhere in this volume.
3. Krchnak, V., Vagner, J., Safar, P., and Lebl, M., Coll. Czech. Chem. Comm. 53 (1988) 2542.
4. Lebl, M. and Krchnak, V., In Epton, R. (Ed.) Innovation And Perspectives In Solid Phase Synthesis and Combinatorial Libraries, Mayflower Scientific, Birmingham, 1999, p. 43.
5. Lebl, M., Pires, J., Poncar, P., and Pokorny, V., J. Comb. Chem., in press.
6. Dooley, C.T., Ny, P., Bidlack, J.M., and Houghten, R.A., J. Biol. Chem. 273 (1998) 18848.