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FACILE SOLID PHASE SYNTHESIS OF OCTREOTIDE ANALOGS USING P-CARBOXYBENZALDEHYDE AS A NOVEL LINKER TO ANCHOR FMOC-THREONINOL TO SOLID PHASE RESINS

Ying-Ta Wu[1], Hsing-Pang Hsieh[1], Chi-Yu Wu[1], Hui-Ming Yu[1], Shui-Tein Chen[1*], and Kung-Tsung Wang[1,2]

1. Institute of Biological Chemistry, Academia Sinica, Taipei, 115, Taiwan.
2. Department of Chemistry, National Taiwan University, Taipei, 100, Taiwan.

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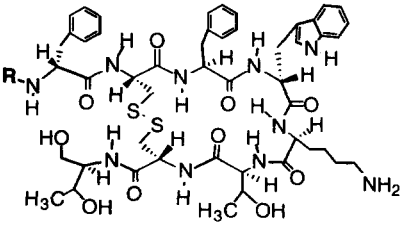
Abstract: A new procedure is described for the synthesis of octreotide and its analogs using p-carboxybenzaldehyde as a linker to anchor Fmoc-threoninol to solid phase resins. Fmoc-threoninol reacted with p-carboxybenzaldehyde to form Fmoc-threoninol p-carboxybenzacetal in 95% yield. The Fmoc-threoninol p-carboxybenzacetal was coupled to an amine-resin and octreotides were successfully synthesized with yields of 74-76%.

Peptides that contain a C-terminal alcohol such as octreotide[1,2] and enkephalin analogs[3,4] have been the focus of recent research because these peptides bind to their receptors with high affinity and display a long half-life *in vivo*. Peptides that contain a C-terminal alcohol, howerer, can not be synthesized by conventional solid-phase peptide synthesis (SPPS) because a free carboxyl group is required for attachment to the resin. Octreotide **1**, a metabolically stable somatostatin analog, inhibits the growth of tumor cells by binding to surface somatostatin receptors[5]. Although many methods have been reported for the synthesis of octreotide,[1,6-8] they are complicated and produce low yields. We describe a new procedure using p-carboxybenzaldehyde as a linker to anchor Fmoc-threoninol to amine-resins for the synthesis of peptide alcohols by SPPS.

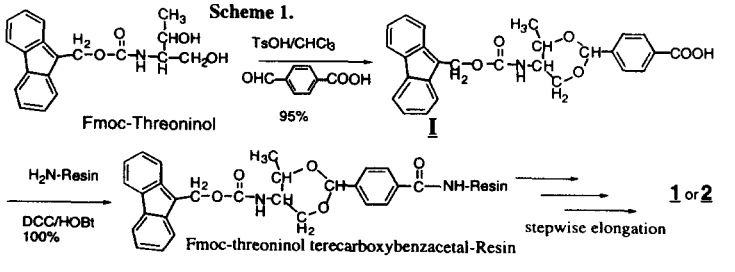


1. R = H,
2. R = Biotin.

The two hydroxyl groups of Fmoc-threoninol reacted with the aldehyde of p-carboxybenzaldehyde to form acetal **I** in 95% yield (Scheme 1). The reaction was catalyzed by p-toluenesulphonic acid in chloroform using a Dean & Stark apparatus. The carboxyl group of **I** can easily be attached to amine-resins by conventional coupling reactions for use as an Fmoc-amino acid derivative in SPPS to synthesize **1** and **2**.

Octreotide **1** and biotin-octreotide **2** were synthesized to study the feasibility of this reaction protocol starting from Fmoc-threoninol p-carboxybenzacetal-resin (0.25 mmol sacle on Rink Amide AM resin, loading 0.56 mmole/g resin). Fmoc-amino acids were activated *in situ* with HOBt/HBTU/DIEA in NMP using FastMoc Chemistry on a synthesizer (ABI/433) with four equivalents of each amino acid derivative. The coupling efficiency was monitored with a built-in conductivity recorder. The acetal was stable during the stepwise elongation of each Fmoc-amino acid as shown by the average coupling yield (>96%). Conjugation of biotin to octreotide was directly performed on the synthesizer

Scheme 1.



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by introducing four equivalents of biotin in the last step of the synthesis process.

Cleavage of the elongated peptide from the resin was carried out by the TFA method (TFA 90%, water 2.5%, thioanisole 5%, EDA 2.5%, and phenol 0.79 w/v). The solution was evaporated under reduced pressure and extracted with aqueous acetic acid (5%) to dissolve the reduced octreotide. Figure 1 shows a chromatogram of the reduced octreotide immediately after extraction. The oxidation to form the disulfide bond was carried out at 4°C after adjusting the pH of the solution to 7.0 with ammonium hydroxide (25%). The progress of the disulfide bond formation was monitored by HPLC (see Figure 2). All the reduced octreotide disappeared and a major peak corresponding to oxidized octreotide appeared after 48 hrs. **1** and **2** were isolated with total yields of 74-76% by preparative HPLC and the structures were further confirmed by Fab mass and amino acid analysis.

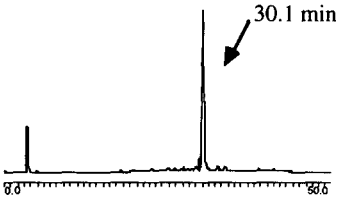

Figure 1. HPLC of reduced octreotide after TFA cleavage and extraction with aqueous acetic acid.

Edwards *et al*[7]. recently isolated octreotide with a total yield of 14% by cleaving the protected peptide with threoninol. Arano *et al.*[8] utilized 2-chlorotrityl chloride resin to load Fmoc-threoninol(Bu^t) as the first amino acid residue by the protocol of Wenschuh *et al.*[9] to obtain octreotide with a total yield of <32%. Schmidt[10] utilized Rink acid resin and carefully controled concentrations of TFA (2.0% at 25°C) to synthesize **1** (yield was not reported). We employed p-carboxybenzaldehyde as a linker to anchor Fmoc-threoninol to the amine-resin. The process takes advantage of SPPS to rapidly prepare **1** and **2** with high yields and purities without the need of further modification. This linker is compatible with the Fmoc peptide synthesis protocol and can be easily applied to large scale preparation of octreotide derivatives. The starting material, Fmoc-threonine, is cheap and easy to prepare via the direct reaction of threonine with Fmoc-OSu, instead of the very expensive Fmoc-Thr(Bu^t)-OH which requires multi-steps to synthesize.

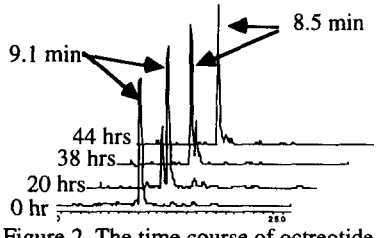

Figure 2. The time course of octreotide oxidation as determined by HPLC analysis. The retention times of reduced and oxidized octreotide were 9.1 min and 8.5 min, respectively.

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References

1. J. Pless, W. Bauer, U. Briner, W. Doepfner, P. Marbach, R. Maurer, T. J. Petcher, J. -C. Reubi, and J. Vonderscher, Scand. J. Gastroenterol., **1986**, 21 suppl. 119, 54.
2. W. Bauer, U. Briner, W. Doepfner, R. Haller, R. Huguenin, P. Marbach, T. J. Petcher, and J. Pless, Life Sci., **1982**, 31, 1133.
3. D. Roemer, H. H. Buescher, R. C. Hill, J. Pless, W. Bauer, F. Cardinaux, A. Closse, D. Hauser, and R. Hugenin , Nature (London), **1977**, 268, 547.
4 D. Roemer and J. Pless, Life Sci., **1979**, 24, 621.
5. G. Weckbecker, F. Raulf, B. Stolz, and C. Bruns, Pharmacol. Ther., **1993**, 60, 245.
6. M. Mergler and R. Nyfeler, In Peptide, ed. C. H. Schneider & A. N. Eberle, **1992**, ESCOM, 1993, p. 177.
7. W. B. Edwards, C. G. Fields, C. J. Anderson, T. S. Pajeau, M. J. Welch, and G. B. Fields, J. Med. Chem., **1994**, 37, 3749.
8. Arano, Y.; Akizawa, H.; Uezono, T.; Akaji, K.; Ono, M.; Funakoshi, S.; Koizumi, M.; Yokoyama, A.; Kiso, Y.; Saji, H. Bioconjugate Chem. **1997**, *8*, 442-446.
9. Wenschuh, H.; Beyermann, M.; Haber, H.; Seydel, J.K.; Krause, E.; Bienert. M. J. Org. Chem. **1995**, *60*, 405-410.
10. M. A. Schmidt, R. R. Wilhelm, and A. Srinivasan, In Abstracts of the Fifteenth American Peptide Symposium, June 14-19, **1997**, Nashville, Tennessee, p. 2-109.