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Communications

An Efficient Preparation of Cyclo(Leu-Enkephalin) *via* the 4-(Methylthio)phenyl Ester Method[1]

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Although the number of literatures describing synthesis of cyclic peptide has steadily grown, no decisive synthetic protocol has been established yet[2]. The popularity of the azide method[3] is in part due to the convenience of preparing the peptide precursors for cyclization. However, it is generally approved that the separative steps of the activation and cyclization provide a better control of the cyclization reaction and often give better yields. Furthermore, the impurities of the peptide precursors have harmful effects on the cyclization reaction in the azide procedure.

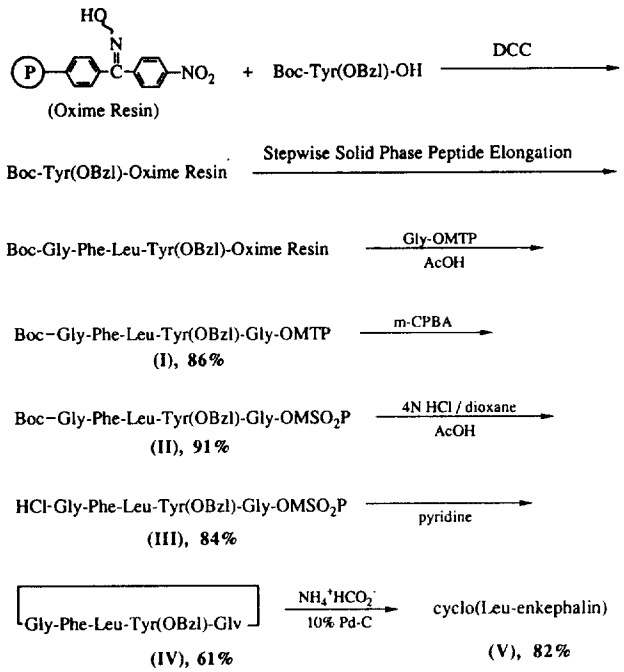
The active ester procedure[5] has the advantages that the activation and cyclization steps can be cleanly separated. However, the preparation of the linear peptide precursor usually requires tedious reaction steps. In addition, efficient methods of obtaining the peptide acid[6] for the next C-terminal activation are hardly available. Therefore, the establishment of an efficient method for the preparation of cyclic peptide precursors is essential for the successful synthesis of cyclic peptides. An alternative method is possible if peptide 4-(methylthio)phenyl[MTP][7] ester is utilized as the cyclic peptide precursor. The C-terminal MTP peptide can easily be converted to the corresponding 4-(methylsulfonyl)phenyl[MSO_2P][8] ester, which has been previously utilized in the active ester method. Futhermore, as described in our recent report[1], the peptide fragment can be prepared efficiently by using various nitro-aromatic oxime resins and C-terminal amino acid MTP esters. It was also reported that such effectiveness was due to the strong charge-transfer interaction[9] between the MTP group and the nitro-aromatic group in the oxime resins.

If the linear peptide precursor is prepared by the solid phase method using nitro-aromatic oxime resins and C-terminal amino acid MTP esters, the activation and cyclization steps can be simplified by simple conversion of the peptide MTP ester to the corresponding MSO_2P ester without deblocking the carboxyl protecting group from the linear peptide. These ideas led us to design an efficient method for the preparation of cyclo(Leu-enkephalin)[10], [cyclo(Tyr-Gly-Gly-Phe-Leu)], using nitrobenzophenone oxime resin and amino acid MTP esters. According to this strategy, the linear precursor, Boc-Gly-Phe-Leu-Tyr(OBzl)-Gly-OMTP, was prepared by the solid phase method, and activation and deprotection of the peptide and then cyclization of the resulting peptide, Gly-Phe-Leu-Tyr(OBzl)-Gly-$OMSO_2P$, were done in the solution phase. We now herein wish to report on a preliminary result of the preparation of cyclo(Leu-enkephalin) *via* hybrid strategy of solid and solution phases.

As a linear precursor of cyclo(Leu-Enkephalin), HCl·Gly-Phe-Leu-Tyr(OBzl)-Gly-$OMSO_2P$ was prepared as follows. The original amino acid sequence in Tyr-Gly-Gly-Phe-Leu was arranged so that both N- and C-terminal meet Gly residues as in the method of Izumiya[11]. This rearrangement was supposed to be ideal for both the maximal cyclomonomerization which suppresses side products from intermolecular reactions and the minimal racemization. The cyclomonomerization of peptide is often difficult and is still regarded as an unsettled problem in peptide chemistry, due to the competition of cyclodimerization or polycondensation.

4-Nitrobenzophenone oxime resin[12] and the amino acid MTP esters were prepared in the reported manner[1,12]. Boc-Tyr(OBzl)-OH was anchored onto the oxime resin by DCC to give Boc-Tyr(OBzl)-oxime resin (sub. level, 0.30 mmol/g resin). The desired peptide chain length was attained by conventional chain elongation with symmetric anhydrides of the corresponding protected amino acid derivatives(Boc-Leu, Boc-Phe, and Boc-Gly), yielding Boc-Gly-Phe-Leu-Tyr(OBzl)-nitrobenzophenone oxime resin.

Peptide fragment for the synthesis of cyclo(Leu-enkephalin) derivative, Boc-Gly-Phe-Leu-Tyr(OBzl)-Gly-OMTP (I), was prepared in 86% yield from the tetrapeptide-oxime resin and Gly-OMTP (3 eq) in methylene chloride with acetic acid as a catalyst (2 eq). The peptide bond formation between



Scheme 1.

Boc-Gly-Phe-Leu-Tyr(OBzl)-nitrobenzophenone oxime resin and Gly-OMTP was finished within 10 min due to the charge-transfer interaction. I was treated with m-CPBA to give Boc-Gly-Phe-Leu-Tyr(OBzl)-Gly-$OMSO_2P$ (II) in 91% yield, and then with 4 N HCl/AcOH-dioxane to give HCl·Gly-Phe-Leu-Tyr(OBzl)-Gly-$OMSO_2P$ (III) in 84% yield. III (95 mg, 0.07 mmol) was added in a portionwise manner to a large volume of pyridine (400 m*l*) for 40 min, giving a final concentration of 3×10^{-4} M. The reaction mixture was stirred for 3 days at 70℃.

The reaction mixture was concentrated to an oily residue and dissolved in 95% aq. MeOH (170 m*l*). The product mixture was purified on Amberlite IRC-400 and Dowex 1×8 ion exchange resin. From successive fractional crystallization of the crude product from EtOAc-ether, two pure products were isolated; one (cyclomonomer, 43 mg, 61%, mp. 209-210 ℃) in major and the other (mp. 146-147℃, assumed as a cyclodimer) in trace amounts. The major fraction was treated with ammonium formate (7 eq.) in DMF/MeOH/AcOH for a catalytic transfer hydrogenolysis[13] yielding a cyclo(Leu-enkephalin) (30 mg, 82%, mp.>300℃)[14].

The overall yield of cyclo(Leu-enkephalin) from the Boc-Tyr(OBzl)-4-nitrobenzophenone oxime resin was 33%. The molecular weight was determined to be 600 by ebulliometry, which corresponds to the cyclomonomer with 7 or 8 moles of combined water. The physical data including NMR, mp, and other methods of analysis were identical with those reported[10].

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References and Notes

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14. The result of the reactions and the physical data for the peptide products are as follows. I: yield, 86%; mp. 178-180℃; $[\alpha]_D^{20}$ −1.88 (c=1, HOAc); Anal. (%) Calcd. for $C_{47}H_{57}N_5O_9S$ (868.07): C, 65.03; H, 6.62; N, 8.07. Found: C, 64.63; H, 7.08; N, 8.49. II: yield, 91%; mp. 173-174℃; $[\alpha]_D^{20}$ −3.11 (c=1, HOAc); Anal. (%) Calcd. for $C_{47}H_{57}N_5O_{11}S$ (900.07), C, 62.71; H, 6.27; N, 8.71. Found: C, 62.60; H, 6.39; N, 8.40. III: yield, 84%; mp. 142-143℃; $[\alpha]_D^{20}$ −2.63 (c=1, HOAc); Anal. (%) Calcd. for $C_{42}H_{50}N_5O_9SCl$ (836.41): C, 60.31; H, 6.03; N, 8.37. Found: C, 59.99; H, 6.10; N, 8.29. IV: yield, 61%; mp. 209-210℃; Anal. (%) Calcd. for $C_{35}H_{41}N_5O_6\cdot3H_2O$ (681.80): C, 61.66; H, 6.95; N, 10.27. Found: C, 62.01; H, 6.77; N, 9.96. V: yield, 82%; mp. >300℃ (lit. value, *ca.* 350℃, ref. 10); Anal. (%) Calcd. for $C_{28}H_{35}N_5O_6\cdot5.5H_2O$ (636.71): C, 52.82; H, 7.28; N, 11.00. Found: C, 52.60; H, 7.39; N, 10.81.