Synthesis of Cyclopentapeptides Using an Organophosphorus Reagent DEPBT

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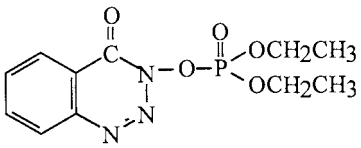
(Received April 11, 2000; CL-000339)

Two cyclopentapeptides cyclo(ProTyrLeuAlaGly)**(1)** and cyclo(AlaTyrLeuAlaGly)**(2)** isolated and identified from a Chinese medicinal herb were chosen as model cyclopeptides to evaluate a novel coupling reagent DEPBT (3-(diethoxy-phosphoryloxy-1,2,3-benzotriazin-4(3H)-one). DEPBT was used for synthesis of cyclic peptides in one-pot procedure. Compared with some other coupling reagents, the cyclization yields were satisfactory.

Many cyclic peptides of natural or synthetic analogs have important bioactivities,[1,2] Therefore, the study on the synthesis of cyclopeptide has become attractive project for peptide chemists. Cyclic peptides are usually prepared by suitably free linear precursors, which are selectively activated and cyclized in a highly dilute solution. However, the cyclization yields are always very low, especially pentapeptides are often not the best starting materials for cyclization. Because the cyclodecapeptides or linear decapeptides as by-products are easily generated even under high dilution. The ring closure is affected by many important factors such as the structure of linear precursor, coupling reagent, concentration of reaction solution, solvent, reaction temperature etc.

Cyclo(ProTyrLeuAlaGly) **(1)** and cyclo(AlaTyrLeuAlaGly) **(2)** were isolated and identified from one kind of Chinese medicinal herbs named *Stellaria yunnanensis Franch(M)*,[3,4] The two cyclopentapeptides were chosen as model cyclopeptides to evaluate the cyclization effect of DEPBT, 3-(diethoxy-phosphoryloxy-1,2,3-benzotriazin-4(3H)-one), which was developed by our group. It is a colorless stable crystalline compound and easy to prepare. It has been successfully used for synthesis of linear peptides by both solution method and solid phase method. DEPBT mediate amide formation with a remarkable resistance to racemization. Moreover, the side chain of Tyr or Ser need not be protected.[5–7]



DEPBT

Protected linear pentapeptides BocProTyrLeuAlaGlyOMe and BocAlaTyrLeuAlaGlyOMe were synthesized using DEPBT as coupling reagent by one-pot method[5] with the overall yields 60% and 52% respectively. Free linear pentapeptides were used for the synthesis of the corresponding cyclic pentapeptides after deprotection and saponification. Because the solubilities of the linear pentapeptides in DCM and THF are poor, cyclizations of the two free linear precursors were carried out in DMF solution (10^{-3} M) by DEPBT. In order to let the reaction finish completely, excessive DEPBT (4eq) was added to the solution. Et_3N was used for keeping pH = 8–9. The reaction time was usually for 4–5 days. After evaporation of the DMF in vacuum, the crude product was obtained and purified by a silica gel column (gradient elution: $CHCl_3/CH_3OH$ = 50:1–10:1) and then TLC plate(**1**: $CHCl_3/CH_3OH$ = 6:1, R_f = 0.50; **2**: $CHCl_3/CH_3OH$ = 5:1, R_f = 0.52) to give pure cyclopentapeptide. The purity of the product was analyzed by HPLC (>95%) (Figure 1). The structures of the two cyclopentapeptides were identified by FAB-MS (**1**: $[M+H]^+$ *m/z*: 502, **2**: $[M+H]^+$ *m/z*: 476), ^1H NMR and amino acid analysis (**1**: Pro 1.0 (1), Tyr 0.89 (1), Leu 1.1 (1), Ala 0.96 (1), Gly 1.0 (1); **2**: Ala 2.0 (2), Tyr 0.9 (1), Leu 1.03 (1), Gly 1.03 (1)). The cyclization with DEPBT proceeded smoothly and only trace cyclodecapeptides (<5%) were formed in the synthesis of the two cyclopentapeptides. Excessive DEPBT was readily removed through a silica gel column.

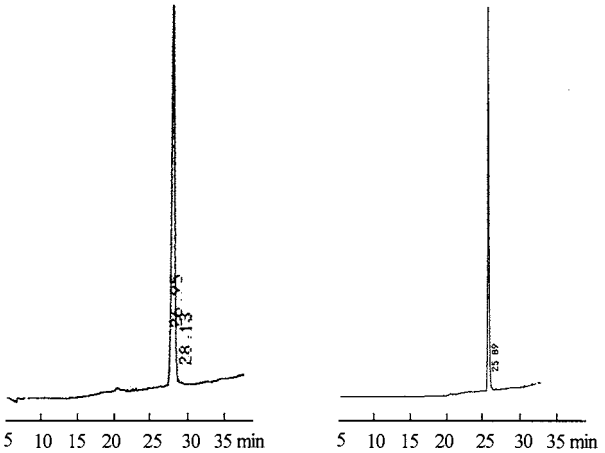


Figure 1. HPLC analyses of the cyclopentapeptides (**1** and **2**) purified by TLC. HPLC column: Nova-C_{18} 3.9 × 150 mm; eluent: linear gradient 20-80% CH_3CN/H_2O (with 0.1% TFA) for 30 min; flow rate: 0.5 ml/min; UV detection at 220 nm.

Other coupling reagents BOP, EDCI, HBTU, DPPA, TBTU and DCC[8] were also used for synthesis of the two cyclopentapeptides under the same reaction conditions as those for DEPBT. The cyclization yields by DEPBT were higher than those of BOP, EDCI or HBTU (Table 1).

Using the strongly active reagents BOP, HBTU or TBTU, only traces of linear precursor were detectable by TLC after a reaction time of 2 h. However, more by-products were observed on TLC during the reaction and the product was not

easy to purify. Coupling reactions with DCC or EDCI proceeded much more slowly than with BOP, HBTU or TBTU, and the cyclization yields were unsatisfactory since even more by-products were formed. DPPA is also an efficient coupling reagent for synthesis of the two cyclopentapeptides. There is no racemization problem in the cyclization reaction since the C-terminal was Gly residue, so there were no remarkable differences among the specific optical rotation values of the products synthesized with those coupling reagents.

Table 1. Yields of cyclopentapeptide **1** and **2**

Cyclopeptide	Coupling reagent	Yield /%	$[\alpha]_D^{15}$	m.p./℃
1	DEPBT	54	-131.0[a]	184-186
	BOP	35	-127.0[a]	183-185
	EDCI	25	-133.0[a]	184-186
	HBTU	40	-128.2[a]	182-184
	DPPA	52	-131.5[a]	183-186
2	DEPBT	52	-104.0[b]	284-286(dec.)
	TBTU	45	-103.8[b]	283-285(dec.)
	DCC	15	-103.4[b]	280-285(dec.)

[a] c 0.50, MeOH. [b] c 0.10, MeOH.

In conclusion, DEPBT is an efficient coupling reagent for cyclization and the results showed that the formation of the corresponding cyclodecapeptides as by-product was not serious during the cyclization reaction. DCC and EDCI gave low yields. BOP, TBTU and HBTU gave moderate yields. Like DEPBT, DPPA gave high cyclization yield, but DPPA is oil, which is not so convenient to use as other solid coupling reagents. Our study indicated that DEPBT is a useful coupling reagent for synthesis of both linear and cyclic bioactive peptides.

The authors would like to thank the National Natural Science Foundation of China for financial support (No. 29772001).

References and Notes:

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8 Notes: BOP, benzotriazoyloxy-tris-(dimethylamino) phosphonium hexafluorophosphate; DCC, *N, N'*-dicyclohexylcarbodiimide; DPPA, diphenylphosphoryl azide; EDCI, 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; TBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate.