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A Useful Method for the Preparation of Fully Protected Peptide Acids and Esters

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Abstract: Resin bound peptides can be cleaved from the Kaiser oxime resin using various oxygen nucleophiles (H_2O, CH_3OH, $PhCH_2OH$) with DBU. Fully protected peptide acids and esters are obtained rapidly and with good yields using this new method of cleavage.

The synthesis of larger peptides and proteins is often achieved by the condensation of segments in solution or in the solid phase.[2] In this regard, the 4-nitrobenzophenone oxime resin (Kaiser resin)[3-5] is widely used as a solid support for the production of fully protected peptide segments[6] under mild cleavage conditions. However no simple and direct procedure for obtaining peptide esters or acids using this resin has, to our knowledge, been reported until now. Kaiser[7] described a two-step procedure for preparing peptide acids. His method involves a nucleophilic attack on the oxime ester bond using N-hydroxypiperidine to obtain the ester which is then treated with Zn/AcOH to give the acid. Lansbury[8] also described a procedure for obtaining peptide acids involving the use of tetra-n-butylammonium amino acid salts as cleavage reagents. In this article, we describe a convenient method of preparing fully protected peptide acids and esters (Scheme 1). Our method involves the use of 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) as a transacylation catalyst. This catalyst was used by Seebach[9] in transesterification reactions and to cleave peptides of PAM and Wang resins.

Scheme 1

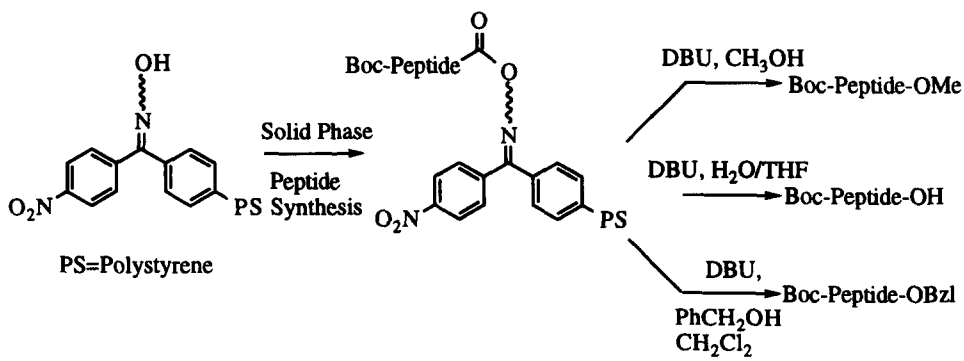


Table 1. Cleavage of Boc-Peptide-Oxime Resin using DBU and Different Nucleophiles

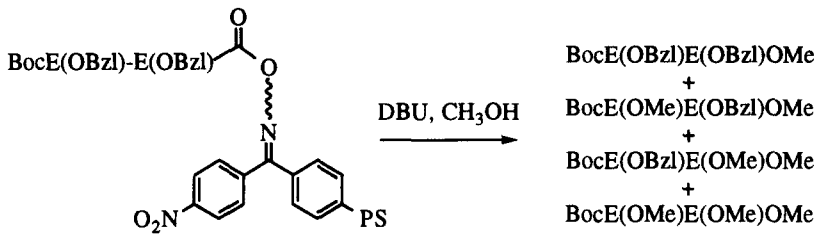
resin-bound peptide[a]	cleavage condition	protected peptide	isolated yield
BocL-CE-L_3- -CE-L~Resin	2 eq DBU, 5% H_2O/THF(4h)	BocL-CE-L_3- -CE-LOH **1**	95%
BocE(OBzl)- -E(OBzl)~Resin	1,2 eq DBU, 4% $PhCH_2OH/CH_2Cl_2$(2h)	BocE(OBzl)- -E(OBzl)OBzl **2**	66%
BocA-E(TPP)-A_3- -E(TPP)-A~Resin	3,5 eq DBU, CH_3OH(2h)	BocA-E(TPP)-A_3- -E(TPP)-AOMe **3**	40%
BocA-E(TPP)-A_6- -E(TPP)-A~Resin	3,5 eqDBU, CH_3OH(2h)	BocA-E(TPP)-A_6- -E(TPP)-AOMe **4**	48%

(a) L=L-leucine; CE=(21-crown-7) L-phenylalanine; E=L-glutamic acid; A=L-alanine; TPP=5-p-aminophenyl-10,15,20-triphenylporphyrine.

Several peptides were synthesized on the oxime resin and were cleaved using various nucleophiles in the presence of DBU. The results show that the cleavage conditions used gave good yields of peptide acids and esters. In all cases, complete cleavage was observed as demonstrated by negative ninhydrin test[10] on the remaining resins after treatment with 25 % TFA/CH_2Cl_2. An example is the excellent yield obtained (95%) of the heptapeptide acid **1** as shown in table 1. The procedure involved treating the Boc-L-CE-L_3-CE-L~Resin with 2 eq of DBU and a solution of 5% H_2O in THF. Following 4 hours of mechanical shaking, the resin was washed several times with chloroform, methylene chloride and methanol and the filtrates were combined and evaporated to dryness. The crude product was dissolved in methylene chloride and the solution washed twice with 1 N HCl and water. The organic phase was dried with $MgSO_4$ and evaporated to give an oily residue. The pure peptide acid **1** was obtained as a white solid by trituration with ether. All the peptides were characterized by FAB Mass Spectrometry and by High Resolution ^1H NMR.

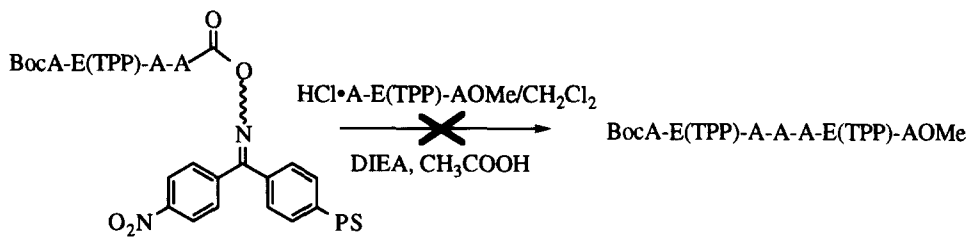
The isolated yields for the other peptides using similar cleavage conditions, although not optimized, were inferior to those observed with peptide **1** mainly due to problems of purification. It is important to mention that cleavage with DBU-CH_3OH was tried initially and that a mixture of products was obtained with a small amount of BocE(OBzl) -E(OBzl)OMe. After isolation of these secondary products, it was possible to identify products of transesterification in the side chains (Scheme 2). This transesterification problem was resolved by using $PhCH_2OH$ as the nucleophile. The cleavage conditions used for the peptide **2** were slightly different than those used for the peptide **1** as shown in table 1. In addition, the procedure used to purify peptide **2** was more difficult as isolation of this peptide required fractionation by flash chromatography of the crude product followed by HPLC on a reverse phase C-18 column (RP-HPLC).

Scheme 2



The pure isolated peptide methyl esters **3** and **4** were obtained in not optimized yields of 40 and 48% respectively by using DBU as the catalyst and methanol as the nucleophile. These cleavages were shown to be particularly efficient and useful as compared to the frequently used method , tried in our laboratory, of synthesizing a monoporphyrin peptide on the oxime resin followed by cleavage with a monoporphyrin segment containing a free amino group (scheme 3). In our case, this strategy turned out to be totally inefficient in spite of several attempts which was probably due to steric hindrance of the nucleophile. The cleavage procedure used to obtain peptides **3** and **4** was similar to that described for the two other peptides except that the crude products obtained were purified by size exclusion chromatography (CH_3OH : $CHCl_3$, 1 : 9) using a LH-20 gel (Pharmacia Fine Chemicals).

Scheme 3



It is useful to point out that no racemization was observed by NMR and by reverse-phase HPLC in the cleavage reactions described in this work. Based on our results, we have demonstrated that utilization of DBU as a transesterification catalyst with the oxime resin results in the formation of protected peptide acids and esters in very good yields and without notable racemization. The method is simple and rapid and the results obtained in the synthesis of peptides **3** and **4** show that in this case, the method of cleavage is more efficient than the aminolysis method of cleavage using a peptide segment with a free amino function (scheme 3) and is applicable to complex peptides.

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