SYNTHESIS OF ω-*TERT*-BUTYL ESTERS OF ASPARTIC ACID AND GLUTAMIC ACID VIA B,B-DIFLUOROBOROXAZOLIDONES

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B,B-Difluoroboroxazolidones (DFBONs) were synthesized for the first time from salts of amino acid and $BF_3{\cdot}Et_2O$, and their properties were examined. DFBONs were used in selective preparation of Glu(OBu^t) and Asp(OBu^t) in good yields under catalysis with $BF_3{\cdot}Et_2O$ and H_3PO_4. Amberlite XAD-2 resin was successfully employed to purify the above amino acid derivatives.

KEY WORDS synthetic procedure; B,B-difluoroboroxazolidone; γ-*tert*-butylglutamate; β-*tert*-butylaspartate

The 9-fluorenylmethoxycarbonyl (Fmoc) protective group is increasingly applied in solid-phase peptide synthesis since its introduction by Carpino *et al.*[1] In the Fmoc strategy for synthesis of glutamic acid or aspartic acid containing peptides, the ω-carboxyl groups of Asp and Glu should be protected in the form of their *tert*-butyl (Bu^t) esters. *tert*-Butyl ester is bulky enough to resist some side reactions, easily removed under acidic conditions, and stable under the basic conditions used in Fmoc methodology. Therefore, many efforts have been made for development of facile preparation of Asp(OBu^t) and Glu(OBu^t). However, no significant improvement in this area has been made.[2] The methods used in preparation of these compounds are still labor-intensive and not economical. For example, the anhydride method,[3] which is commonly used in synthesis of Asp(OBu^t), needs multiple steps and gives a total yield of less than 30% (calculated from Z-Asp-OH). For the synthesis of Glu(OBu^t), although 3-benzyloxycarbonyl-5-oxo-4-oxazolidinone propionic acid[4] is chosen as an intermediate in order to avoid separating the α- and γ-esters of Glu, the yield is fairly low (16% calculated from Z-Glu-OH).

To explore a facile preparative method of Asp(OBu^t) and Glu(OBu^t), we targeted compounds with the following properties: (1) precursors are free amino acids; (2) the α-amino and α-carboxyl groups of amino acids are protected simultaneously; and (3) such compound should be easily deprotected to give free amino acids. With such properties, cupric (II) complexes of amino acid are popularly employed in peptide synthesis, for example, the side chain protections of lysine and ornithine[5] and tyrosine.[6] However, their applicability is limited due to low solubility in organic solvents. 2,2-Bis(trifluoromethyl)-1,3-oxazolidin-5-ones[7] which were prepared from α-amino acids and hexafluoroacetone (HFA), were also successfully used as intermediates to prepare some naturally occurring amino acids from aspartic acid. Boroxazolidinone (BON) is another group of compounds that we are interested in, and its synthesis and properties have been reported.[8] However, these two methods are not suitable for preparation of Asp(OBu^t) or Glu(OBu^t) derivatives because of the instability of the intermediates under acidic or alcoholic conditions.

To design a new BON compound that would less likely be affected by acids, we naturally thought of replacing the alkyl substituents of the boron atom with electron-withdrawing ones. With the increased acidity of the boron atom, the polarity of the N→B bond should be increased at the same time, and then the BONs should be expected to be hydrolyzed easily in aqueous solution and meanwhile more resistant to acids. As early 1978, a group of researchers from Denmark[8d] prepared N-substituted B,B-difluoroboroxazolidones (DFBONs) by refluxing N-benzyl-substituted amino acids, $BF_3{\cdot}Et_2O$ and Et_3N in THF. The N-substituted DFBONs thus obtained reacted with diazomethane to give β-methyl ester as the only product, and the ^{19}F-NMR spectra of N-substituted DFBONs showed two multiplets that derived from F atoms which were not equivalent, being situated on either side of the formed ring. These valuable results indicated that DFBONs also strongly tend to form 5-membered heterocycles. But we failed to obtain unsubstituted DFBONs from free α-amino acids following the method described in their report.

Seeking a method for preparation of unsubstituted DFBONs, we have prepared a variety of organic and

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inorganic salts from Asp or Glu by reacting the same equivalent amount of base and amino acid. Salts thus obtained were dried in high vacuo (heated if necessary) to give the anhydrous salts, then $BF_3 \cdot Et_2O$ was reacted

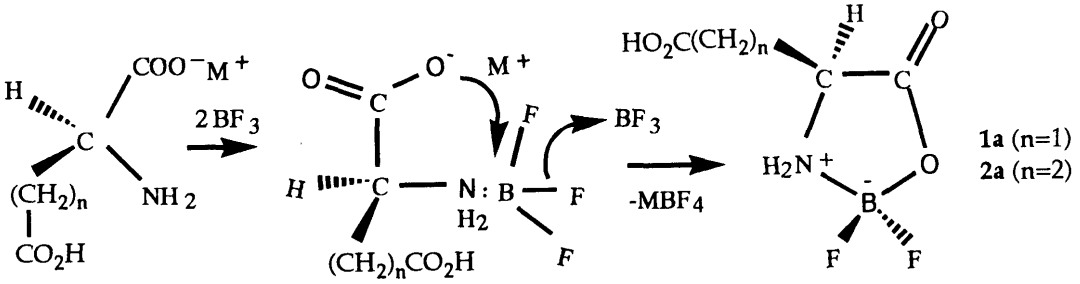

Chart 1

with the salts in THF. To our surprise, only when more than 2 eq of $BF_3 \cdot Et_2O$ was added to the salts (excessive BF_3 could be employed as a catalyst for next reaction), could the formation of DFBONs go to completeness. The ^{19}F-NMR of DFBONs (**1a** in Chart 1), showed two multiplets derived from two fluorine atoms with δ= -69.25 ppm and -70.18 ppm using TFA as an external standard, indicating formation of the ring. When **1a** was treated with diazomethane, the β-methyl ester was the only product (compared with the authentic sample). The results obtained above clearly demonstrate the exclusive formation of more stable 5-membered DFBONs rings in which both the α-amino and α-carboxyl groups are incorporated, even though competing function is present in the substrate molecules. Therefore we assumed the reaction mechanism simply, as shown in Chart 1. The amino group of amino acid formed a complex with BF_3 first (it is also possible to form a complex with carboxylate anion), then an F anion was substituted by attack of α-carboxylate anion to give BF_4^- in tandem with one molecule of BF_3.

Normally, DFBONs are gelatinous solids when precipitated from solvents, easily dissolved in THF, DMF, alcohol, and dioxane and slightly dissolved in ether or dichloromethane. Unlike N-benzyl-substituted DFBON compounds which are stable in aqueous solution, unsubstituted DFBONs are stable for several weeks in anhydrous solutions, while they are sensitive to moisture and become hygroscopic slowly in atmosphere when separated from the solvent, indicating that DFBONs might be suitable intermediates for our purpose. Thus the DFBON derivatives **1a** and **1b** were employed in preparation of Asp(OBu^t) and Glu(OBu^t), respectively[9]; **1a** or **1b** was reacted with isobutylene under the catalyst of BF_3 and H_3PO_4.[9] TLC analysis (sample was taken out during reaction) showed that only the desired compound was produced in each case. After hydrolysis in aqueous sodium bicarbonate (deprotection of α-amino and α-carboxyl groups could be completed in 30 min) and drying, the desired products were dissolved in hot methanol and were used directly to react with Fmoc-OSu.[10] Pure Fmoc-Glu(OBu^t)-OH and Fmoc-Asp(OBu^t)-OH can be obtained in good accordance to the reports in the literature.[11]

Table 1. Properties of Asp and Glu Derivatives

	Asp(OBu^t)	Glu(OBu^t)	FmocAsp(OBu^t)	FmocGlu(OBu^t)
m.p. °C Found Reported	185(dec) 178-178.5[3b]	180-180.5 174-176[4];186-187[3a]	146-147 147-148[11]	79-81 76-77[11]
$[\alpha]_D^{25}$ (c=1.0) Found Reported	in water +10.0 + 8.3[3a]	in water +10.5 +10.1[3a]	in DMF -23.0 -23.2[11]	in EtOAc +0.9 +0.8[11]
Elemental analysis (Cald.) Empirical formula	C 49.73(49.82) H 7.95(8.05) N 7.22(7.26) $C_8H_{15}NO_4 \cdot 1/5H_2O$	C 52.04(52.03) H 8.50(8.49) N 6.80(6.74) $C_9H_{17}NO_4 \cdot 1/4H_2O$	C 67.12(67.16) H 6.12(6.23) N 3.40(3.34) $C_{23}H_{25}NO_6$	C 66.43(66.34) H 6.33(6.49) N 3.15(3.22) $C_{24}H_{27}NO_6 \cdot 1/2H_2O$
Yield %	73.3	69.1	38.9	37.0

Because both the α-amino and α-carboxyl groups of Asp(OBu^t) and Glu(OBu^t) were unblocked, and inorganic salts which formed from BF_3 and DFBON derivatives were soluble in MeOH, it was difficult to remove inorganic salts completely from the desired products. The problem can be circumvented by using Amberlite XAD-2 resin. At pH 6 (the pH that is about the isoeletric point of amino acids), the desired compounds can be easily separated from the salts by the resin which is eluted with water and then aqueous methanol. Thus, pure products were obtained in good yields (Table 1).

In summary, we have prepared DFBONs in quantitative yield using popular laboratory reagents. DFBON compounds, in which the α-amino and α-carboxyl groups of amino acid are simultaneously protected with high selectivity, are much easily deprotected under mild conditions, so that Asp(OBu^t) and Glu(OBu^t) could be obtained in pure form and satisfactory yield without detectable racemization. Compared with the previous methods, this novel procedure has proved to be more convenient and inexpensive, and is expected to become a routine method for the protection of aspartic acid and glutamic acid in Fmoc chemistry. Experiments using DFBON compounds to protect the side chain function of other amino acids are under way.

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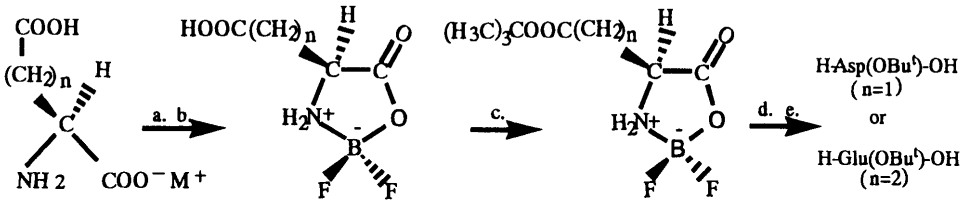
RFERENCES AND NOTES

1) Carpino L. A., Han G.Y., *J. Am .Chem. Soc.*, **92**, 5748-5749 (1970).
2) a) Roeske R. W., *Chem. Ind.* (London), **1959**, 1121-1122. b) Schwyzer R., Dietrich H., *Helv. Chem. Acta*, **44**, 2003-2006 (1961). c) Lajoie G., Crivici A., Adamson G., *Synthesis*, **1990**, 571-572.
3) a) Schröder E., Klieger E., *Liebigs Ann. Chem.*, 673, 208-220 (1964). b) Kemp, D. S., Fotouhi N., Boyd J. G., Carey R. I., Ashton C., Hoare J., *Int. J. Peptide Protein Res.*, **31**, 359-372 (1988).
4) Itoh M., *Chem. Pharm. Bull.*, **17**, 1679-1686 (1969).
5) a) Roeske R., Stewart F. H. C., Stedman R. J., duVigneud V., *J. Am. Chem. Soc.*, **78**, 5883-1587 (1956). b) Erlanger B. F., Sachs H., Brand E., *ibid.*, **76**, 1806-1810 (1954).
6) Wünsch E., Fries G., Zwick A., *Chem. Ber.*, **91**, 542-547 (1958).
7) Golubev A., Sewald N., Burger K., *Tetrahedron Lett.*, **36**, 2037-2040 (1995).
8) a) Lang K., Nuetzel K., Schubert F., *Ger. Pat.*, **1962**, 1130445. b) Tung S. H., Chang K. M., Tah S. L., Lin C. C., Chang S. L., *Ke Xue Tong Bao*, **17**, 414-418 (1966). c) Köster R., Rothgery E., *Liebigs Ann. Chem.*,**1974**, 112-119. d) Halstrøm J., Nebelin E., Pedersen E. J., *J. Chem. Res.(S)*, **1970**, 80-81. e) Rettig S. J., Trotter J., *Can. J. Chem.*, **55**, 958-965 (1977). f) Nefkens G. H. L., Zwanenburg B., *Tetrahedron*, **39**, 2995-2998 (1983). g) Albericio F., Nicolás J., Rizo J., Ruiz-Gayo M., Petroso E., Giralt E. *Synthesis*, **1990**, 119-122.
9) a) Beyermann H. C., Heiszwolf G. J., *J. Chem. Soc.*, 1963, 755-756. b) Smithwich, Edward, Lee Jr., C.A. **79** 32301w, Ger. Offen., 2253924 (1973). c) Synthetic procedure for Asp(OBu^t) and Glu(OBu^t):



a. 4.4 ml BF_3·Et_2O (per 10 mmol of amino acid mono sodium salt in THF), r.t., overnight. b. 50 °C, 1 h.
c. dioxane, liquid isobutylene (15-20 ml), anhydrous H_3PO_4 (0.2 ml), r.t., 3-5 h. d. 1*N* $NaHCO_3$.
e. Amberlite XAD-2 (φ2.8 × 35 cm), Y. 68.0-85.1%. (all yields were calculated from α-amino acid)

Chart 2

10) Lapatsanis L., Milas G., Froussios K., Kolovos M., *Synthesis*, **1983**, 671-673.
11) Chang C. D., Waki M., Ahmad M., Meienhofer J., Lundell E., Haug J. D., *Int. J. Peptide Protein Res.*, **15**, 59-66 (1980).

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