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PLURIPOTENTIAL AMINO ACIDS I. (L)-p-DIHYDROXYBORYLPHENYLALANINE (L-Bph) AS A PRECURSOR OF L-Phe AND L-Tyr CONTAINING PEPTIDES; SPECIFIC TRITIATION OF L-Phe-CONTAINING PEPTIDES AT A FINAL STEP IN SYNTHESIS

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SUMMARY: p-Dihydroxyborylphenylalanine has been resolved and incorporated into simple di and tripeptides; treatment of these with hydrogen peroxide or diammino silver salts results respectively in formation of tyrosine and phenylalanine peptides.

Earlier, we have reported[1] the application of a phenylboronic acid derivative as a protecting group with "handle"[2] properties. The p-dihydroxyborylbenzyloxycarbonyl amino group (Dobz) is a urethane-type protective group that is closely related to the familiar carbobenzoxy group. The presence of the boronic acid function allows three unusual transformations. First, Dobz-bearing derivatives can be reversibly extracted into aqueous solutions of chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) as boronic acid-diol complexes. By this means, a separation from non-Dobz containing substances is easily achieved. Second, the boronic acid function undergoes catalytic protiodeboronation in high yield, forming carbobenzoxy derivatives by treatment with aqueous solutions of the silver diammine cation. Third, the Dobz group can be removed selectively by a reaction sequence that is initiated by replacement of boron by hydroxyl, using hydrogen peroxide under controlled conditions.

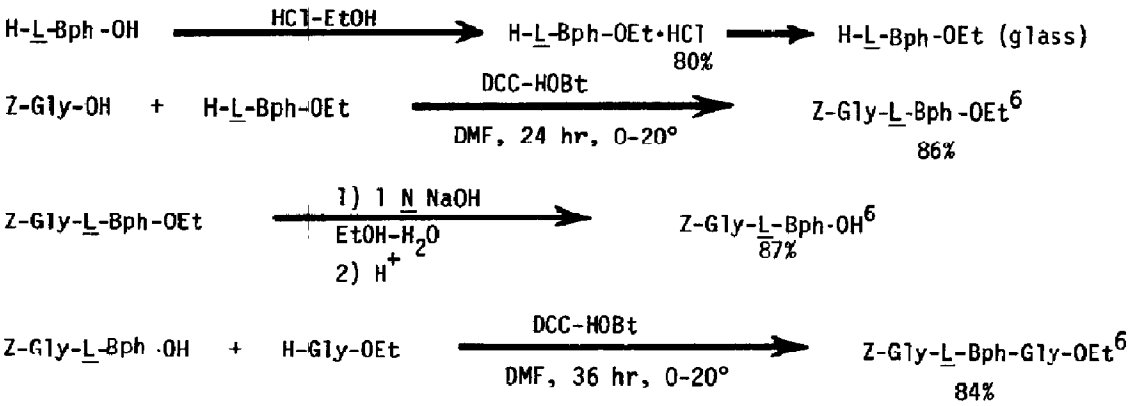
It appeared to us that an affinity site within a peptide chain that offered the option of conversion to either phenylalanine or tyrosine has considerable promise for increasing the ease of synthesis of peptides bearing these amino acids. Accordingly, we examined peptides containing p-dihydroxyborylphenylalanine (Bph) residues.

Earlier reports of the synthesis of this amino acid described it as the racemate, obtained by hydrolysis and decarboxylation of the product of alkylation of the sodium salt of diethyl acetamidomalonate with p-dihydroxyborylbenzyl bromide.[3] Resolution of this substance was achieved by conversion to the ethyl ester (Fischer esterification, 80%), followed by α-chymotrypsin-catalyzed hydrolysis.[4] The L-enantiomer of the amino acid was obtained in 95%

yield as a insoluble powder, $[\alpha]_D^{23°}$ -8.2° (0.7, 0.1 N HCl), L-Bph.

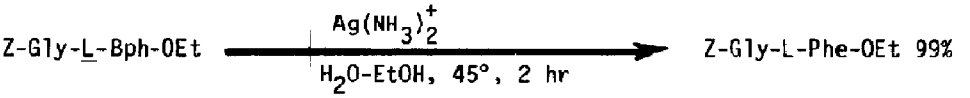
Following the reaction sequences of Scheme I, L-Bph was converted to the dipeptide derivatives, Z-Gly-L-Bph-OEt and Z-Gly-L-Bph-OH, and the tripeptide derivative, Z-Gly-L-Bph-Gly-OEt. As indicated in the scheme, the widely used DCC-HOBt coupling reagent was used to form amide bonds,[5] and the preparations of this scheme can be taken to demonstrate the compatibility of the boronic acid group of the Bph residue with some of the commonly used reagents of peptide synthesis.

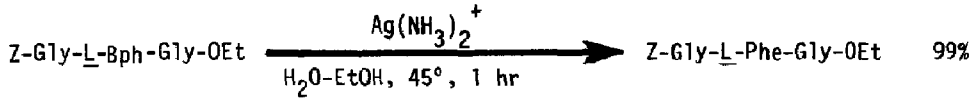
SCHEME I: SYNTHESIS OF DI AND TRIPEPTIDES CONTAINING Bhe RESIDUES



During these manipulations, it became apparent that air oxidation is more problematic with peptides containing the Bph residue than with peptides bearing the Dobz group. The result of oxidation by air or by peroxides is the formation of traces of the corresponding peptide in which the Bph residue is replaced by L-tyrosine. To prevent these slow oxidation processes, all extractions of Bph-containing peptides were carried out under nitrogen, using solvents that had been equilibrated with sodium bisulfite. Tyrosine-containing by-products could be separated from Bph products by chromatography on silica gel or by extraction with aqueous chromotropic acid, buffered at pH7 and containing sodium bisulfite. Acidification to pH 3 allows recovery of the Bph derivative by repeated extraction with ethyl acetate.

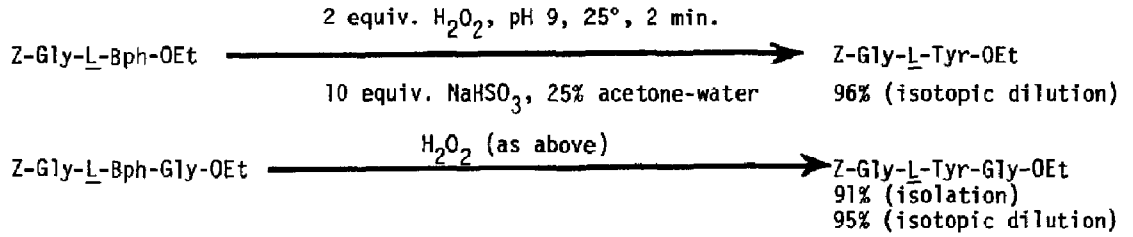
The peptides Z-Gly-L-Bph-OEt and Z-Gly-L-Bph-Gly-OEt were subjected to protiodeboronation by treatment with an equivalent of silver diammine nitrate in water at 45°, pH 9, 1-2 hr. Extraction, evaporation, removal of boric acid by azeotropic distillation of trimethyl borate with methanol, and chromatography (silica gel) gave the expected phenylalanine derivatives in essentially quantitative yield, identical in all respects with authentic samples.





When these reactions are carried out in tritiated water, phenylalanine-containing peptides are obtained that bear C-T bonds. This procedure offers a simple alternative to sequential manipulations involving radiolabeled phenylalanine as starting material. Unfavorable kinetic and equilibrium isotope effects might be anticipated for the tritiodeboronation reaction, and in fact, we observed only 5.4% of the theoretical level of tritium incorporation for the formation of Z-Gly-L-Phe-Gly-OEt from the Bph tripeptide. In view of the availability of tritiated water of high specific activity, this feature of the reaction does not appear to decrease its preparative value.

The hydrogen peroxide-induced conversion of Bph to Tyr peptides was observed to proceed satisfactorily in aqueous solvent mixtures that contain acetone. As indicated in the following examples, under the proper conditions the reaction proceeds in excellent yield.



The possibility of replacing the dihydroxyboryl group under mild conditions with other functionalities, such as halogens[7] or heavy metals[8] further enhances the potential of this versatile amino acid.

ACKNOWLEDGEMENT

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