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# NEW PROTECTIVE GROUPS FOR PEPTIDE SYNTHESIS--II THE DOBZ GROUP

## BORON-DERIVED AFFINITY PROTECTION WITH THE p-DIHYDROXYBORYLBENZYLOXYCARBONYLAMINO FUNCTION

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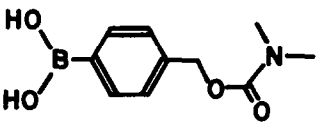
Protecting groups which permit modification of solubility properties of peptide derivatives or which can act as "handles",[1] allowing isolation by selective affinity, offer great potential for facilitating the manipulations of peptide chemistry.[1,2] We report preparation and properties of p-dihydroxyborylbenzyloxycarbonyl (Dobz) amino acid derivatives. As a boronic acid derivative, the Dobz group has very versatile solubilizing and affinity properties.

Dobz 


Transformations or manipulations of Dobz derivatives offer the unusual feature that the reactive hydroxyl functions of the boronic acid ($pK_a \sim 9$) can be masked by rapid, reversible reaction with diols such as catechol, alkyldiethanolamine, or naphthalene-1,8-diol derivatives. The Dobz group is unexceptional in other respects. Thus it is introduced as an amine protecting group by a Schotten-Baumann reaction[3] in water, using Dobz chloride, protected as its catechol complex.[4]

In the absence of complexing species, boronic acids are in reversible equilibrium with their cyclic trimers and water; N-Dobz amino acids [7] are partially converted to boronic anhydrides by solution in ethyl acetate or acetonitrile and removal of water by azeotropic evaporation. Addition of water regenerates the boronic acid. No other special reactivity has been found to attend the Dobz group or its anhydrides under the normal reaction conditions of peptide chemistry.

A free Dobz group is compatible with peptide coupling by the p-nitrophenyl ester, acyl azide, or DCC-HBT[8] coupling procedures. The latter two appear to be the coupling methods of choice.

The Dobz group must be protected (e.g. as the methyldiethanolamine complex) when a Dobz-bearing peptide acid is coupled by the mixed anhydride procedure.

Saponification of N-Dobz amino acid esters with 2 N sodium hydroxide, followed by acid generates Dobz amino acids in yields of 80 %. Cleavage of the Dobz group can be effected under conditions standard for removal of the carbobenzoxy function (HBr/HOAc or $H_2$/Pd). The Dobz group is somewhat more resistant than the latter to anhydrous trifluoroacetic acid. [9]

Addition of a Dobz-bearing peptide in dioxane-water to a water solution of the sodium salt of chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) at pH 7 results in immediate formation of a water soluble complex which is decomposed to its constituents at pH 1-2 or in the presence of a large excess of boric acid. By this means, Dobz-bearing peptides may be selectively extracted from impurities which do not bear boronic acid residues and recovered from the extraction. For example, the lipophilic tripeptide, $^{\alpha}$Dobz$^{\varepsilon}$ZLys-Ala-ValOEt, is solubilized and recovered by this procedure in 98 % yield. The sole instance in which we have been unsuccessful in achieving solubilization is the pentapeptide, BpocAsn-Phe-Phe-Try-Lys($^{\varepsilon}$Dobz)OtBu, which represents an extreme of lipophilicity. More work is needed to define the usual number of amino acid residues which can be solubilized by a single Dobz-chromotropic acid complex.

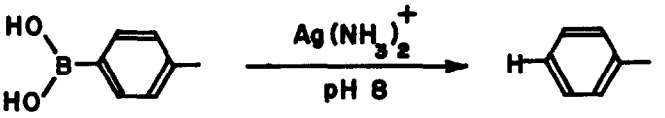
A new protective group must be established as showing inertness to normal reaction conditions as well as trouble-free removal. To establish the first point, we have compared results for two syntheses of a heptapeptide analog of angiotensin, a conventional synthesis involving Z($NO_2$)Arg and an analogous synthesis involving Dobz($NO_2$)Arg. For each, intermediates were obtained in comparable purity. Coupling of Dobz($NO_2$)ArgOH with H-Val-Tyr$N_2H_2$Boc in DMF with DCC-HBT yielded Dobz($NO_2$)Arg-Val-Tyr$N_2H_2$Boc [10] in 82% yield (60-70% for a mixed anhydride coupling in the conventional synthesis). Reaction with Tfa removed the Boc group selectively, and diazotization at -10° in water-DMF, 0.2 M in HCl, followed by basification, extraction into ethyl acetate, drying, concentration, and addition of a DMF solution of H-Ile-His-Pro-AlaONb at 0° yielded after 24 hr, 80 % (75 % by conventional route ) of Dobz($NO_2$)Arg-Val-Tyr-Ile-His-Pro-AlaONb as a white solid. [10] Purification of this substance and of the tripeptide Boc hydrazide by the chromotropic acid method proceeded satisfactorily.

Although only preliminary results are available, it seems appropriate to outline further potential of this function for affinity separations and solubilization. DobzAlaOH is solubilized in carbon tetrachloride through the addition of the lipophilic complexing agent, N-octadecyldiethanolamine, and the free amino acid, ($^{\varepsilon}$Dobz)Lys, can be solubilized in acetonitrile and in dichloromethane by the addition of equivalents of the ditetrabutylammonium salt of chromotropic acid and of pyridine. Thus it may be possible to adjust the solubility properties of Dobz-bearing peptides through use of appropriate complexing agents.

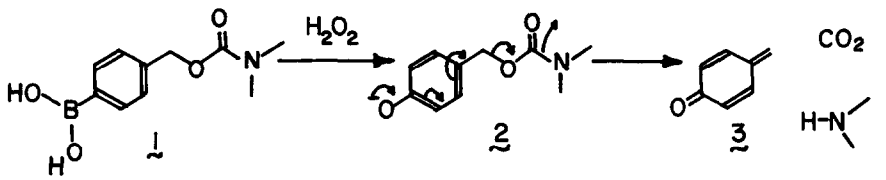
A natural experiment suggested by the demonstration by several groups of nucleotide separations on boron-bearing resins or solid supports [11] is the selective binding of Dobz peptides to solid supports bearing suitable diol functions. Although preliminary experiments appear promising, we have yet to find the optimal system. Loading levels, solvent sensitivity, and reproducibility are problems which remain to be solved.

Two remarkable reactions which achieve deboronation of Dobz derivatives greatly extend the versatility of this function. The first is conversion of the Dobz group to a carbobenzoxy group, a transformation which provides a simple means of characterization, if the Cbz derivative is known.

It has long been recognized[12] that reaction of arylboronic acids with metal ions results in boron loss. We have observed that reaction of Dobz peptides with water is catalyzed by the silver diammine cation, and that high yields of the corresponding carbobenzoxypeptides are thereby formed. The reaction proceeds in water-ethanol at 25° at pH 7.5-8, and an approximate half time




of 16 minutes is observed for the conversion of 0.5 M phenylboronic acid to benzene (catalyst concentration, 1 M.) In a representative reaction, $^{\alpha}$Dobz$^{\varepsilon}$ZLys-GlyOEt, 0.2 M in ethanol, was treated with an equivalent of aqueous silver diammine nitrate, prepared by titrating silver nitrate solution with sufficient ammonia to dissolve all precipitate (pH 7.7). After 24 hr at 25°, the mixture was extracted with ethyl acetate, from which 90% of $^{\alpha}$Z$^{\varepsilon}$ZLys-GlyOEt was obtained, m.p. 89-92°, rep. 92-93°.[13] Application of this procedure to Dobz($NO_2$)Arg-Val-Tyr$N_2H_2$Boc gave after dry column chromatography on silica gel, 71 % of the corresponding Z tripeptide derivative, m.p. 144-148°, identical with an authentic sample.




A second deboronation reaction of interest involves the well-known[14] peroxide-induced conversion of arylboronic acids to phenols. By this means, cleavage of the Dobz group can be achieved under exceptionally mild conditions. The reaction sequence poses two problems; hydrogen peroxide is capable of reacting with sensitive amino acids such as methionine and tryptophane, and the quinonemethide formed as an intermediate is capable of reacting selectively with peptide nucleophiles, such as the liberated amino grouping. Both problems appear to be resolved by the following experimental procedure: An aqueous solution of Dobz derivative, 25°, pH 9.5 in borate buffer, containing five equiv. of sulfite anion, is treated with 1.1 equiv. of hydrogen peroxide. After ca. 5 min, the pH is reduced and the product is isolated. By this means, DobzAlaOH was converted to Ala, 93 % in 1 min, 97% in 40 min,[15] and $^{\alpha}$Dobz$^{\varepsilon}$ZLysOH was converted to $^{\varepsilon}$ZLys in an isolated yield of 90 %.

The success of the method depends on the following factors. The reaction of boronic acids with peroxide is pH dependent and very rapid at pH 9.5.[14] Any residual peroxide is consumed by excess sulfite, with which it reacts with half times of seconds. Sulfite also acts as a good scavenger for quinonemethide. In control experiments, oxidation of tolueneboronic acid was

carried out in the presence of ZGly-MetOH and of ZGly-TryOH, under the above conditions. No oxidation of either amino acid derivative could be detected by pmr. Kinetics indicated that the methionine peptide is oxidized ca 400 times slower than the boronic acid, tryptophane $10^5$times.

Evaluation of the worth of this highly promising functionality of course must await its success in practical synthesis, and we will report subsequently on further synthetic applications.

ACKNOWLEDGEMENT

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