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NEW PROTECTIVE GROUPS FOR PEPTIDE SYNTHESIS--I THE BIC GROUP

BASE AND SOLVENT LABILITY OF THE 5-BENZISOXAZOLYLMETHYLENEOXYCARBONYLAMINO FUNCTION

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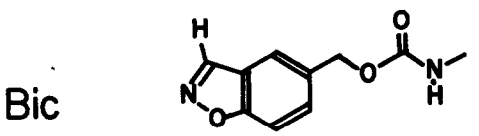
Despite continuing research,[1] the groups available for protection of amino and carboxyl functions of peptides fail to meet the most stringent demands of synthesis. Particularly needed are protective groups which can survive the routine operations of synthesis, purification, and cleavage of conventional groupings, yet which can be removed selectively under the mildest of conditions.

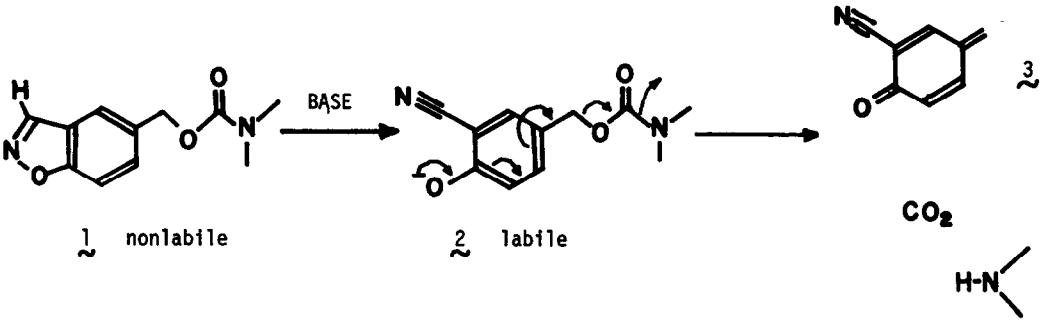
We report preparation and properties of the 5-benzisoxazolylmethyleneoxycarbonyl (Bic) grouping, 1, a new urethane-type protective group for amine functions. The Bic group withstands most manipulations of conventional peptide synthesis, including treatment with trifluoroacetic acid. It is cleaved by hydrogenolysis or by treatment with hydrogen bromide in acetic acid. It is also cleaved in a novel two-step sequence, consisting of treatment with an aprotic base in a dipolar aprotic solvent, followed by solvolysis in water at pH 7.



1

The ease of acid-catalyzed cleavage of protective groups of the carbobenzoxy class has long been recognized to vary markedly with ring substituent.[2] Rates of base-catalyzed isomerization of benzisoxazoles have been found to be highly solvent dependent and are slowed by a protic medium.[3] We sought to exploit these facts in designing the Bic group. In its conjugating capacity, the

5-benzisoxazolyl function was anticipated to be mildly deactivating, relative to phenyl. On the other hand, at pH values above its pK_a of 7, the isomeric cyanophenol moiety was anticipated to be strongly electron donating. Thus it was hoped that 1 would be nonlabile under normal reaction conditions, but that it could be isomerized to the labile 2 by treatment with aprotic bases in dipolar aprotic solvents.



1 nonlabile 2 labile 3

The key intermediate in the synthesis of Bic functions, 5-hydroxymethylbenzisoxazole, is prepared in three steps by chloromethylation,[4] hydrolysis, and reaction with hydroxylammonium O-sulfonate[5,6]. The corresponding chloroformate is obtained as an oil by treatment of the hydroxymethyl derivative with phosgene; synthesis of Bic amino acids is conveniently carried out under standard conditions in an aqueous bicarbonate buffer.[7]

Experiment confirmed all features of the predicted behavior of 1 and 2 . In an nmr experiment, the Bic group was found to be more stable than the carbobenzoxy group to anhydrous trifluoroacetic acid. Isomerization of 1 to 2 was conveniently effected by 30 min contact with 2 equiv. of triethylamine in acetonitrile or DMF,25°. Reaction of 2 derived from BicValOH with trifluoroacetic acid for 90 min released Val in 95 % yield.[8]

Solvolysis of species 2, is also possible under alkaline conditions. Thus, 4-benzoyloxymethyl-2-cyanophenol in aqueous Tris buffer, pH 7.5, 40°, for 2 hr released benzoate ion in 98% yield. Development of an efficient trapping agent for the quinonemethide, 3, was a necessary preliminary to the application of this solvolysis procedure to the cleavage of urethanes, 2, for it could be shown that 3 selectively reacts in aqueous medium with the liberated amine. Sulfite ion proved to be convenient. When BicValOH is treated with triethylamine in acetonitrile for 30 min, followed by aqueous-ethanolic sodium sulfite buffer, pH 7, 40°, 3 hr, Val is released in 92 % yield.

As well as being removable under generally applicable conditions, a new protective group must be compatible with the routine operations of isolation and synthesis. Of particular concern for the Bic group are operations which involve bases.

It was anticipated that the features of equilibria involving weak acids and bases in dipolar aprotic solvents would govern the conversion of 1 to 2.[9] Thus, salts of amines and

carboxylic acids, which are largely undissociated in aprotic media, are expected to be poor catalysts. When BicAlaOH, 0.2 M in acetonitrile, is treated with 0.5 equiv. of triethylamine, 25°, the half time for isomerization to 2 is found to be ca. 55 hr. In the presence of 0.9 equiv. or of 1.2 equiv. of amine, half times of 2 and 0.3 hr are observed. In these three experiments, the principal bases are assigned, following Kolthoff[9], as $R\text{-}CO_2^-\cdots HO_2C\text{-}R$ $Et_3\overset{+}{N}H$, $R\text{-}CO_2^-\cdots\overset{+}{H}NEt_3$, and Et_3N, respectively. The half time for the isomerization to 2 in water, pH 9, 30°, is ca. 1 day, and roughly 3% conversion occurs in 1 hr. No decomposition of BicAlaOH could be observed after 12 hr, 25°, in aqueous phosphate buffer, pH 7. With all Bic derivatives we have studied, the isomerization to 2 could be easily followed by disappearance of the pmr singlet at 8.8 δ and the shift of methylene resonance from 5.3 δ to 4.8 δ.

The following transformations involving bases have been found to be compatible with the Bic grouping. Bic amino acids and peptide acids can be extracted into and recovered from aqueous sodium bicarbonate. They can be converted to dicyclohexylamine salts,[10] which are stable to storage. The normal mixed anhydride coupling procedure, which prior to addition of ethyl chlorocarbonate involves salt formation with a tertiary amine, can be employed with Bic amino acids or peptides. As with the normal mixed anhydride procedure, an excess of amine base must be avoided. Coupling reactions in dipolar aprotic solvents such as DMF can be carried out successfully using the DCC-HBT procedure. The Bic group is not stable to the presence of excess tertiary amine bases in dipolar aprotic solvents such as DMF, acetone, acetonitrile, or DMSO. Nonhydrogen bonded oxyanions are unusually effective catalysts for isomerization in these solvents, and operations such as recrystallization which may require prolonged contact with these solvents are best carried out in the presence of a trace of acetic acid. Though the point can only be established by further experimentation, we tentatively conclude that the base lability of the Bic group, though necessitating some attention to experimental design, need pose no more of an experimental problem than the corresponding acid lability of the Bpoc group.

As a test of the Bic group in practical synthesis, we have prepared BicArg(NO_2)-Val-Tyr-Ile-His-Pro-AlaONb by a route previously employed for the synthesis of the corresponding Cbz-blocked heptapeptide and modeled after earlier angiotensin syntheses.[11] (Yields for the Cbz-based synthesis are reported in parentheses.) Reaction of BicArg(NO_2)OH with triethylamine in DMF, then ethyl chlorocarbonate, -10°, followed in 4 min by H-Val-TyrN_2H_2Boc gave after workup, 50-80 % (60-70%) of BicArg(NO_2)-Val-TyrN_2H_2Boc.[12] Reaction with 90% Tfa, 30 min, 25°, then with $NaNO_2$ -HCl in DMF-water, -10°, followed by ethyl acetate extraction, bicarbonate wash, and concentration gave the azide, which was coupled in DMF at 0° with H-Ile-His-Pro-AlaONb·2HBr and tributylamine (2 equ.) to yield BicArg(NO_2)-Val-Tyr-Ile-His-Pro-AlaONb,[12] 60-70% (65-75%). Treatment of the blocked heptapeptide with triethylamine, 3 equiv., in DMF for 30 min., 25°, followed by solvolysis in an aqueous ethanolic sodium sulfite buffer, pH 7, 40°, 3 hr, basification, and extraction allowed isolation of H-Arg(NO_2)-Val-Tyr-Ile-His-Pro-AlaONb in 91 % yield.

In summary, the Bic group can be viewed as complementary to the Bpoc, the Boc, and the t-butyl ester functions, since it is inert to the acidic conditions used to cleave these functions. It is cleanly removed by the hydrogenolysis or HBr/HOAc conditons used to cleave Cbz functions. All of these groupings are inert to the mildly basic conditions which are optimal for Bic group

removal. The integrity of the Bic group can be monitored using the 8.8 δ pmr resonance of the isoxazole proton. More definitive tests of the utility of the Bic function are in progress and will be reported subsequently.

ACKNOWLEDGEMENTS

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6. Chloromethylsalicylaldehyde[4] was hydrolyzed as a stirred suspension (1g/25ml water), 25°, for 70 hr. This solution was used directly in the procedure of ref. 5; 5-hydroxymethylbenzisoxazole, 82 %, was obtained, m.p.52-55°, anal. sample m.p.,54-55°.[12]

7. A solution of the hydroxymethylbenzisoxazole in CH_2Cl_2 is treated with a 12-fold excess of phosgene. After 6 hr, the solvent was removed, readded and evaporated for four cycles. The chloroformate was stored in CH_2Cl_2 solution and recovered by evaporation before use. In a typical acylation, HAlaOH in water at pH 8.5-9, 0°, was treated with chlorocarbonate in acetonitrile for 1 hr. Extraction, acidification, extraction, and evaporation yielded 6.6 g solid, recryst. from EtOAc-cyclohexane to give 5.6 g (63%), m.p. 137-138.5°.[12]

8. Yield assessed by isotopic dilution.

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12. Satisfactory elemental analysis has been obtained for this substance.