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IMPROVED SYNTHESIS OF PREFORMED Boc-AMINOACID-BRIDGING GROUPS FOR USE IN SOLID PHASE PEPTIDE SYNTHESIS

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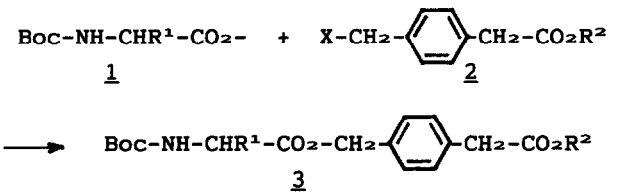
Abstract : Preformed Boc-aminoacid-bridging groups are synthesized by an improved method, using Tmse temporary protection of the starting 4-(halomethyl)phenylacetic acid or bromoacetic acid.

In solid phase peptide synthesis (SPPS), numerous electron-withdrawing bridging groups have to be used in order to increase the acid stability of the peptide ester bond between polymer and peptide[1]. Two of them are of particular interest : 4-(halomethyl) phenylacetic acid[2,3] and bromoacetic acid[4].

In the customary methodology, supports (polystyrene or polyacrylamide) functionalized with amino groups were successively acylated with these bridging molecules and then with Boc-aminoacid salts[2-5].

Whereas the first acylation step is easily monitored as going to completion, analytical control of the second step is difficult to perform, and unreacted halo sites could participate in undesirable side reactions later on during the peptide synthesis[2]. Therefore a second strategy, giving higher loaded resins than the two-step assembly, seemed preferable, but has only been applied to the Pam resin ; the supported amine function was directly acylated with the preformed Boc-aminoacid-bridging group 3 (R^2=H)[6], the reaction being easily monitored using the ninhydrin test. However the key step is the synthesis, in

solution, of the pure preformed building block 3 (R^2=H), the synthesis of which, up until now, was not completely satisfactory.



R^1= CH_3, $CH(CH_3)_2$, CH_2-$CH(CH_3)_2$, CH_2-C_6H_5

In fact several attempts to obtain 3 (R^2=H) have appeared in the literature. In the first, the 4-(halomethyl)phenylacetic acid 2 was unprotected (R^2=H), but results were disappointing[2,3] (34% yield and difficult purification). To avoid side reactions, the carboxyl function has to be protected by a group which must be both stable to the conditions of the benzylester bond formation, and selectively removable without affecting either the N_α-Boc group, or the benzylester link or any aminoacid side-chain protecting group possibly present. Various temporary protecting groups have been investigated: succinimide ester[6] (too reactive), t-butyl ester[7] (impossible to use with a N_α-Boc strategy) and phenacyl ester[6] ; this last affords in fact the best results, although overall yields for preparing preformed Boc-aminoacid bridging group do not exceed 50%[8] ; moreover after the final deprotection step with zinc in acetic acid, it is difficult to completely remove the acetic acid which can later on acylate some of the supported amine functions. Overall yields can be improved (ranging between 55-80% according to the starting aminoacid) by use of the 4-methoxy phenacyl ester[9], cleaved in the last step either by reduction with zinc in acetic acid or better by photolysis[9].

Recently Pane and Heissler[10] described a new way to prepare the phenacyl ester of 3 by esterification of N-Boc aminoacids with 4-(bromomethyl)phenethyl alcohol, followed by a two-step oxidation ; unfortunately the starting alcohol is not commercially available.

We first tried to protect the 4-(halomethyl)phenylacetic acid 2 (R^2=H) as the trimethyl, dimethyl t-butyl, diphenyl t-butyl or triphenyl silyl ester ; although these silyl esters were easily isolated, the next reaction with Boc-aminoacid cesium salts afforded some by-products, separable with difficulty and resulting probably from trans-silylation reactions.

We next prepared the 2-trimethylsilyl ethyl ester 2a (X=Br, R^2=CH_2-CH_2-$SiMe_3$) from 2-trimethylsilyl ethanol (TmseOH) and DCC-DMAP ; compound 2a is easily purified by chromatography on silica gel and isolated nearly

quantitatively ; NMR ($CDCl_3$) δ ppm: 0.00 (s, 9H, $SiMe_3$), 0.99 (t ,J=8.5 Hz, 2H, CH_2-Si), 3.60 (s, 2H, Ar-CH_2-CO), 4.21 (t, J=8.5 Hz, 2H, CO_2CH_2), 4.48 (s, 2H, Br-CH_2), 7.32(q_{AB}, 4H, Ar).

Treatment of Tmse ester 2a with N-Boc aminoacid cesium salts 1 (alanine, valine, leucine or phenylalanine) in DMF at 50°C during 12 h afforded 3a (R^2=CH_2-CH_2-$SiMe_3$) (90% yield). These esters[11] are easily cleaved at 0°C in 85% yield with a small excess of a commercially available 1M solution of tetrabutylammonium fluoride, containing less than 5% of water (if a solution of hydrated fluoride is used, simultaneous partial cleavage of the benzyl ester bond is observed as was recently claimed[12]) ; NMR spectra of 3a in $CDCl_3$ show an AB system (J=13 Hz) at 5.1-5.2 ppm, corresponding to the methylene of CO_2-CH_2Br and with a singulet at 3.6-3.7 ppm corresponding to the methylene of the phenacetic acid moiety.

The same convenient route can be followed for the glycolamido bridge which has been until now used with polyacrylamide supports functionalized with amino groups[4,13]. This bridge allowed peptide chains to be anchored via an ester link almost as stable against acids as Pam resin ; moreover its hydrophilic properties are quite similar to those of the resin and the bound peptide. Finally the ester bond can be cleaved in different conditions, affording peptides with a choice of acid, amide or ester terminal functions[4].

Our first attempts to obtain the preformed Boc-aminoacid-glycolic acid block, using phenacyl bromoacetic ester, gave poor yields (25 to 30%).

$$\underset{\underline{1}}{Boc\text{-}NH\text{-}CHR\text{-}CO_2^-} + \underset{\underline{4}}{Br\text{-}CH_2\text{-}CO_2\text{-}CH_2\text{-}CH_2\text{-}SiMe_3} \longrightarrow \underset{\underline{5}}{Boc\text{-}NH\text{-}CHR\text{-}CO_2\text{-}CH_2\text{-}CO_2\text{-}CH_2\text{-}CH_2\text{-}SiMe_3} \xrightarrow{F^-} \underset{\underline{6}}{Boc\text{-}NH\text{-}CHR\text{-}CO_2\text{-}CH_2\text{-}CO_2H}$$

R=CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, CH_2-C_6H_5

Trimethylsilyl ethyl ester 4 of bromoacetic acid was easily isolated as an oil in 95% yield ; NMR ($CDCl_3$) δ ppm : 0.00 (s,9H, $SiMe_3$), 0.99 (t, 2H, J=8.5 Hz, CH_2-Si), 3.77 (s, 2H, CH_2Br), 4.22 (t, 2H, J=8.5 Hz, CO_2CH_2). Ester 4 reacted with N-Boc aminoacid cesium salts (alanine, valine, leucine or phenylalanine), affording triméthylsilylethyl Boc-aminoacylglycolic ester 5 as oils with yields better than 90%. Compounds 5 were easily purified by chromatography on silica gel. A final treatment at 0°C with a 1M solution of tetrabutylammonium fluoride afforded pure Boc-aminoacyl glycolic acids 6 in 80% yields. NMR spectra of 5 and 6 ($CDCl_3$) showed an AB system (J=16 Hz) corresponding to the glycolic methylene protons at 4.6-4.7 ppm.

By successive couplings of a polyacrylamide resin functionalized with amino groups (0.9 meq per g) with Boc-Val-glycolic acid, Boc-Gly, Boc-Ala and Boc-Leu

according to the symmetric anhydride methodology, a final cleavage with a 1M NaOH solution (iPrOH-H_2O 70/30), afforded Merrifield's peptide in almost quantitative yield.

In conclusion, Boc-aminoacid-bridging group synthesis is a key step in SPPS. The improvement described here, using Tmse ester as temporary protecting group, appears as a very convenient way to easily afford compounds 3 and 6 with high yields (70-80%), easy purification and no production of undesirable by-products; moreover this synthesis can be scaled up. Studies with aminoacids containing functionalized side chains are in progress.

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