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REMOVAL OF BENZHYDRYL-GLYCOLAMIDE (OBg) GROUP WITH TETRABUTYLAMMONIUM FLUORIDE

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Abstract: Tetrabutylammonium fluoride (TBAF) in acetonitrile (MeCN) or in N, N-dimethylformamide (DMF) is an alternative to the potassium carbonate hydrolysis in a mixture of DMF and water for the removal of N-Benzhydryl-glycolamide esters (OBg) in the solution synthesis of peptide.

N-Benzhydryl-glycolamide esters (OBg esters) are a new form of carboxy-protecting group[1] derived from carboxamidomethyl esters (CAM esters).[2, 3] Amino acid-OBg esters have been found to be stable during coupling with BOP, PyBOP and active esters. They are inert and stable in the conditions used for the removal of Z, Boc and Fmoc protecting groups and can be rapidly, selectively and cleanly cleaved in mild alkaline conditions.[1] Furthermore the presence of the large hydrophobic benzhydryl group on the amide makes the OBg esters less water soluble than CAM esters. This additional liposolubility increases yields by reducing the losses associated with the use of CAM esters during the normal workup of reaction mixtures.

Because of these chemical characteristics, the OBg group can act as a temporary three-dimensional[4] orthogonal protection of selected carboxylic acid functions and can be selectively removed at any point during the synthesis.

Interestingly the OBg esters can easily generate C-terminal amides in very mild conditions by reaction with NH_4OH and they also undergo rapid hydrazinolysis in the presence of few equivalents of hydrazine hydrate.[1]

Solution-phase synthesis of linear and cyclic heptapeptides and their corresponding dimeric forms related to the sequence 412-418 of $pp60^{c\text{-}src}$ and its analogs has been achieved by using a combination of *Z*/*tert*-butyl protecting groups together with OBg-protection[5] (Fig. 1). This synthetic route was chosen to permit the preparation of the N^{α}-free and/or C^{α}-free side-chain protected derivatives and of the C^{α}-hydrazides from the totally protected heptapeptides **VI**. These intermediates are key fragments for the synthesis of the linear and cyclic monomeric and dimeric analogs.

Good results were also obtained using the OBg ester as temporary protecting group of the C^{α}-carboxyl of the tyrosine or phenilalanine residues, for the synthesis of the dipeptide 415-416 derivatives **II**.

In the attempts to prepare the C^{α}-free, N^{α}- and side-chain protected heptapeptide analogs by the removal of the OBg ester in the reported conditions[1] (K_2CO_3 2-5 equivalents in a mixture of DMF/water) we have detected extensive α-β shift side reaction involving the aspartic acid 413.

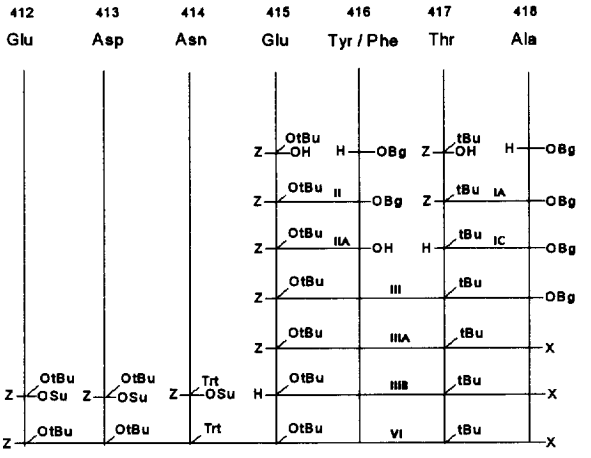

Fig. 1. Scheme of the synthesis of the fragment 412-418 of pp60c-src.
X = -OBg, -OH, NH_2 or -$NHNH_2$

This well documented side-reaction is strictly correlated to the Asp-Asn sequence and is favoured in the mild conditions used by solvents as DMF.[6] On the other band, none of α-β shift reaction at the aspartic acid residue was revealed after treatment of the same intermediates with hydrazine hydrate or ammonia to prepare the corresponding totally protected heptapeptides hydrazides or amides.

For this reason, the synthetic pathway was modified to include the removal of the OBg ester at the tetrapeptide step.

At the same time, alternative OBg deprotection routes using different reagents and conditions were explored.

Fluoride ion has been used in peptide syntheses as a reagent for the removal of protecting groups such as silyl ethers and esters, Fmoc, Ppt, $OpNO_2Bzl$, Teoc, Tce and Pac groups[7] and as a reagent for cleavage of peptide chain from a resin support in solid-phase synthesis.[8] Even if some degree of instability of aspartyl peptide bonds toward this ion has already been noted[9], we have tested its effects on our synthetic intermediates. In this comunication we report the application of tetrabutylammoniun fluoride (TBAF) to the cleavage of the base-labile OBg group.

By treatment with 0.1 M TBAF trihydrate in DMF or in MeCN the OBg esters of protected amino acid or peptides were removed rapidly and in good yields from the carboxyl group. In these experimental conditions Z, Boc and *tert*-butyl groups were fully preserved. No remarkable

difference was observed among the solvents employed and the reaction solvent can be chosen according to the solubility of the substrate.

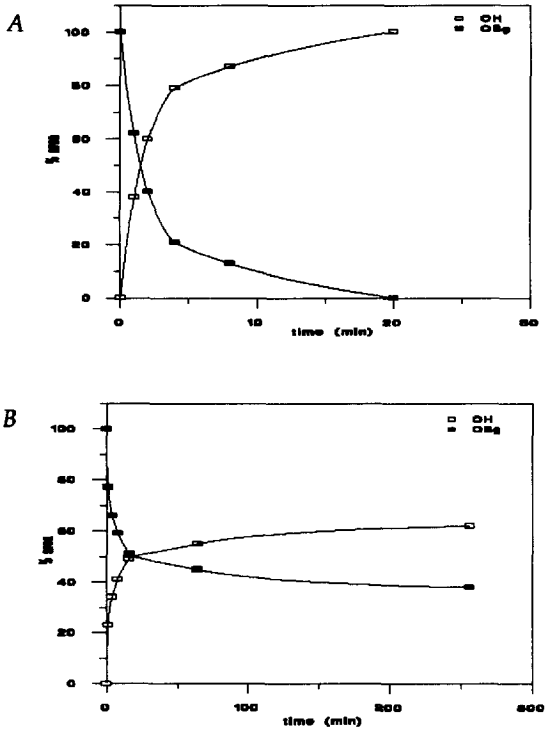

Fig. 2. Effect of TBAF on the removal of OBg ester from the dipeptide Z-Glu(OtBu)-Phe-OBg in MeCN. A - freshly prepared solution; B - three days old solution.

The rate of hydrolysis and the amounts of carboxyl group liberated were followed by HPLC.[10] As shown in Fig. 2 for the Z-Glu(OtBu)-Phe-OBg intermediate, when a fresh solution of the reagent was employed the deprotection of the carboxyl group was achieved in a few minutes (Fig. 2A). On the other hand, when the TBAF solution in DMF was used some days after its preparation, a lower in the recovery of free carboxyl group was obtained (Fig. 2B). Such decrease in the recovery of deprotected carboxyl moiety can probably be attributed to the degradation of TBAF in solution. Similar results were obtained using MeCN as solvent.

The treatment of the heptapeptide sequences in the same conditions lead also to the α-β shift at the Asp 413 peptide bond in an amount comparable with that found during the hydrolysis with K_2CO_3.

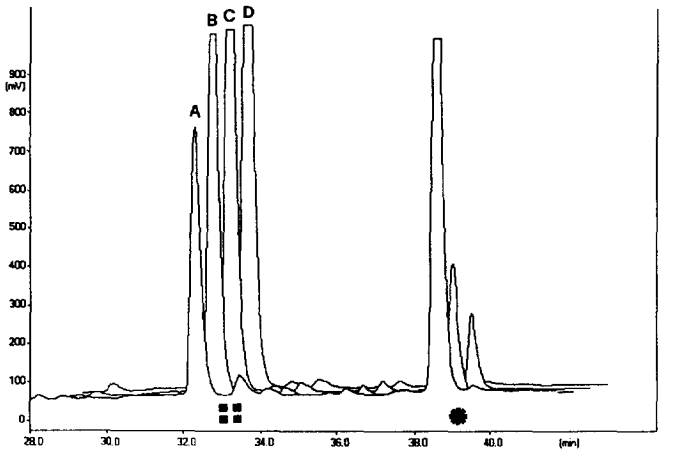

Fig.3. Elution profiles of Z-Glu(OtBu)-Phe-OBg at different times from TBAF addition. A after one minute, B after 3 minutes, C after 8 minutes and D after 20 minutes, with freshly prepared solution. ● OBg ester; ■ free carboxyl group.

Nevertheless, the newly proposed OBg ester removal method in the presence of TBAF permits to avoid the other troubles connected with the use of K_2CO_3 in a mixture of water-organic solvents.

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10. **HPLC elution condition**: eluents: A - 0.05% TFA in water, B - 0.05% TFA in 9:1 MeCN-water; flow rate 1 ml/min, detection at 216 and 280 nm; 3 min at 5% B and then linear gradient from 5% to 100% B in 35 min.

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