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The Allyloxycarbonylaminomethyl Group: a New Allylic Protection for the Thiol Group of Cysteine.

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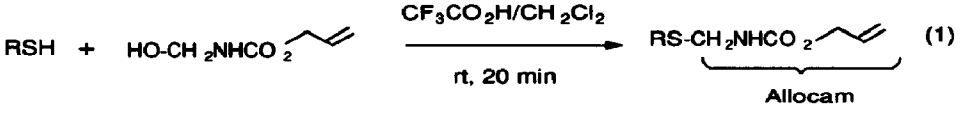
Key words: cysteine protection, allylic protecting group, palladium catalysis, tributyltin hydride.

Abstract: S-Allocam derivatives of thiols in general and of cysteine in particular are selectively and readily deprotected through palladium catalyzed hydrostannolysis. They are perfectly stable in the basic conditions of Fmoc-removal, but only marginally stable in the acidic conditions of t-Bu, Boc removal.

Due to the fact that they are orthogonal to both acid labile (Boc, t-Bu) and base labile (Fmoc) protecting groups, allylic groups which can be easily and selectively removed through π-allyl palladium methodology have emerged, in the recent years, as very promising protecting entitities for peptide chemistry.[1]

Thus the allyl group (All) and the allyloxycarbonyl (Alloc) group have already been used in peptide synthesis for the temporary or permanent protection of carboxylic acid and amine functions,[2] and allylic handles have been devised and used for the synthesis of fully protected peptidic fragments[3]. Many side chain functions of natural amino-acids may be adequately protected by either the All or the Alloc groups, including the carboxylic groups of aspartic and glutamic acids,[4,5] the phenolic group of tyrosine,[2a,4,6] and the nitrogen functions of lysine,[2a,4,7] arginine,[4] or tryptophan[8]. Neither the All nor the Alloc groups however are suitable protections for the thiol function of cysteine[9]. Indeed, allyl thioethers are not cleaved to π−allyl species by zerovalent palladium complexes, due to the poor leaving group ability of the thiolato entity. Alloc derivatives of thiols are cleaved by palladium,[2a] but as most other S-acyl derivatives of cysteine, the S-Alloc derivative of cysteine is not stable enough under basic conditions and intramolecular acylation reactions are observed leading to thiazolidinone formation or/and S to N Alloc group transfer. To circumvent these problems, we have devised a new allylic protecting entity, namely the allyloxycarbonylaminomethyl (Allocam) group for the specific protection of thiols in general and cysteine in particular.

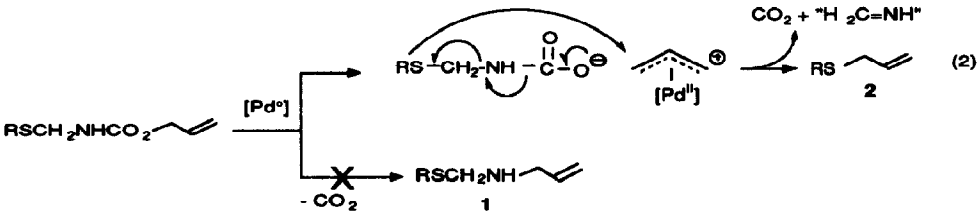
ThioAllocam derivatives are readily prepared (eq.1) by condensation of thiols with allyl N-hydroxymethyl carbamate in acidic medium (CH_2Cl_2/CF_3CO_2H, 1/1, rt, ca 15-20 min). By this method, the Allocam derivatives of benzyl- and 1-naphtylmethylmercaptan were obtained in 70-90% yield after puri-



(1)

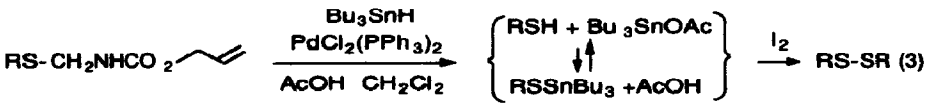
fication. The S-Allocam derivative of cysteine was obtained in more than 95% yield after precipitation as its hydrochloride salt and it was further derivatized into Boc-Cys(Allocam)-OMe, Boc-Cys(Allocam)-NHBzl and Fmoc-Cys(Allocam)-OMe, using conventional procedures[10].

Allyl carbamates in general are known to smoothly rearrange, in the presence of palladium catalyst, into allylamines with loss of CO_2[1a]. We expected that a similar decarboxylative rearrangement would convert thioAllocam derivatives to thioaminals **1** , thus offering a straightforward deprotection procedure since acyclic thioaminals are easy to hydrolyse. In the event, we found that thioAllocam derivatives of thiols do rearrange in the presence of palladium tetrakis(triphenylphosphine) ($3x10^{-2}$mol. equiv. of catalyst, CH_2Cl_2, 35°C, 4 to 6 h) but through a *double* fragmentation process which leads to allyl thioethers **2** with loss of CO_2 *and* methylenimine (eq. 2). Therefore adding a nucleophilic species to the medium appeared to be necessary,



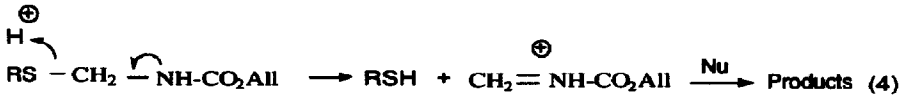
in order to trap the π-allyl entity before rearrangement take place.

N,N-dimethylbarbituric acid[11] or N-trimethylsilylamines[1a] which are efficient nucleophilic allyl group scavengers in the palladium catalyzed deprotection of allyl carbamates in general gave poor results with thioAllocam derivatives; deprotection reactions were incomplete, probably as a result of catalyst poisoning, and furthermore unselective as allyl thioethers were still formed as side products. The ternary system palladium catalyst/ tributyltin hydride/ acetic acid[2a] however led to complete deprotection within a short time (ca 10min., rt) with all the S-Allocam derivatives under study.[12] The presence of acetic acid (ca 4 equiv.) in the medium was found to be essential to prevent any side formation (ca 5-10% without added acetic acid) of allyl thioethers. The hydrostannolytic procedure leads (eq. 3) to a mixture of the thiol, its tributyltin salt and minor amounts of disulfide. For the sake of convenience, the crude reaction mixtures were therefore treated with iodine (ca 1/2 equiv. of I_2 based on starting Allocam derivative, instantaneous) and the deprotected products isolated as their disulfide derivatives.[13] The following yields of purified products[14] were thus obtained: dibenzyl disulfide 78%, di(1-naphtylmethyl) disulfide 73%, bis-Boc-Cystine bis-methyl ester 100%, bis-Boc-Cystine bis-benzylamide 92%, bis-Fmoc-Cystine bis-methyl ester 65%.



Allocam derivatives of thiols are perfectly stable under the basic (piperidine) deprotection conditions of Fmoc derivatives but only marginally stable in the acidic conditions of t-Bu, Boc removal. For instance, the following per-cents decomposition were observed, based on HPLC analysis, for Fmoc-Cys(Allocam)OMe in CH_2Cl_2/CF_3CO_2H 3/1 v/v at 25°C: 3.4% (3 h), 10.3% (20 h).

The first step of the degradation process is very likely to be a proton induced fragmentation reaction (eq.4) leading to the thiol and the N-allyloxycarbonylmethyleniminium cationic species.[15,16] We are currently



investigating the use of other protecting entities, derived from the Allocam group by attachment of an electronegative substituent (EWG) either on the nitrogen (3) or the carbon atom (4) in order to discourage this process.

3 4

Notes and references

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9. For a recent review on the protection of the thiol function in peptide synthesis, see: Cavelier, F.; Daunis, J.; Jacquier, R. *Bull. Soc. Chim. Fr.* **1990**, *127*, 210.
10. All compounds gave satisfactory analytical and spectroscopic data. For instance: Boc-Cys(AllocAM)-OMe : ^{1}H NMR (250 MHz,$CDCl_3$) 5.7 (d, 1H, NH–Boc), 5.45 (t, 1H, NH-Alloc); 5.4-5.2 (two overlapping d, J= 13 and 7 Hz, 2H, Z and E vinylic H); 4.55 (m, 3H, $C^{\alpha}H$ and allylic H); 4.3 (d, J=5 Hz, 2H, SCH_2); 3.7 (s, 3H); 3.1 (ABX system, J_{AB}=12 Hz, J_{AX}= 4.8 Hz, J_{BX}= 6 Hz, 2H, SCH2); 1.4(s, 9H). Anal. Calcd. for $C_{14}H_{24}N_2O_6S$: C,49.96; H, 6.71; N, 7.77. Found : C, 49.93; H, 6.86; N, 7.55.
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12. Experimental procedure: To a lemon-yellow solution of Allocam derivative (2 mmol.), acetic acid (8 mmol.) and $PdCl_2(PPh_3)_2$ (0.08 mmol.) in CH_2Cl_2, tributyltin hydride (4.4 mmol., excess) is dropwise added through a syringe over a period of 2 min. The reaction mixture is further stirred for 20 min at room temperature, at which time an orange-yellow colour has usually developed. In all cases, complete disappearance of Allocam derivative was checked by CCM, IR (in the case of Allocam derivatives of arylmethylthiols) and NMR analysis of the crude reaction mixture. The work-up procedures were carried out as described in note 13.
13. Work-up procedures: Aside from the catalyst, the by-products of the hydrostannolytic procedure are tributyltin acetate and hexabutyldistannane, the latter one arising from the palladium catalyzed decomposition of tributyltin hydride in

excess.[2a] After evaporation of dichloromethane and acetic acid in excess, short column chromatography (silica, cyclohexane/AcOEt as the eluent) allows the elimination of hexabutyldistannane ($R_F \approx 1$) and of the catalyst. The chromatographed product is taken up in CCl_4 and treated dropwise with a CCl_4 solution of iodine until persistence of iodine colour. The oxidation process may be also carried out before chromatographic purification, in which case more iodine is necessary as hexabutyldistannane is also oxidized to Bu_3SnI. In the case of the cystine derivatives which are insoluble in hydrocarbon solvents, the crude reaction mixture from oxidation is concentrated under vacuum, the residue is taken up in 9/1 v/v CH_3CN/H_2O and repeatedly extracted (x 6) with pentane, which allows the complete elimination of all tin by-products (Neumann, W. P. *Synthesis* **1987**, 665). In this way , the methyl ester and benzylamide of Boc-cystine were obtained in pure form.[14] The Fmoc cystine methyl ester was further purified by flash column chromatography (silica, cyclohexane/AcOEt as the eluent). In the case of arylmethyl disulfides which are soluble in hydrocarbon solvents, the reaction mixture from iodine oxidation is concentrated on a Rotovap, taken up in diethyl ether and treated with an excess of a concentrated aqueous solution of KF to convert Bu_3SnI, which tails on chromatography, into Bu_3SnF (Leibner, J. E.; Jacobus J. *J. Org. Chem.* **1979**, *44*, 449). The precipitated Bu_3SnF is then filtered off, the organic phase is concentrated and flash chromatographed on silica.

14. All compounds gave satisfactory analytical and spectroscopic data. For instance: bis-Boc-Cystine bis-methyl ester: ^1H NMR (250 MHz,$CDCl_3$) δ 5.5 (broad, 2H, 2 NH–Boc); 4.6 (broad app. q, J: ca 6-8Hz, 2H, 2 C^{α}-H); 3.74 (s, 6H); 3.2 (d, J=6 Hz, 4H, SCH_2); 1.45 (s, 18H). Anal. Calcd. for $C_{18}H_{32}N_2O_8S$: C, 46.14; H, 6.88; N, 5.98; S, 13.68. Found : C, 46.23; H, 6.90; N, 5.82; S, 13,48.
15. Acyliminium species: Speckamp, W. N.; Hiemstra, H. *Tetrahedron* **1985**, *41*, 4367.
16. The trifluoracetolytic degradation of Allocam derivatives of arylmethylthiols leads to the thioketals $ArCH_2$-S-CH_2-S-CH_2Ar probably through hydrolysis by adventitious water of the N-Allocam methyleniminium cation to formaldehyde followed by thioketalisation.The trifluoroacetolysis of Fmoc-Cys(Allocam)OMe leads to several products whose identity has not yet been determined.

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