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Use of Alloc-amino Acids in Solid-Phase Peptide Synthesis. Tandem Deprotection-Coupling Reactions Using Neutral Conditions.[1]

Nathalie Thieriet,[a,b] Jordi Alsina,[a] Ernest Giralt,[a] François Guibé,[b,*] and Fernando Albericio[a,*]

[a]Departament de Química Orgànica, Universitat de Barcelona, Martí i Franquès, 1-11, E-08028 Barcelona, Spain;
[b]Institut de Chimie Moléculaire d'Orsay, Laboratoire des Réactions Organiques Sélectives, URA 1497, Bât. 420, Université Paris-Sud, 91405 Orsay, France.

***Abstract*:** The use of Alloc group in SPPS for the N^{α} protection of amino acids is an alternative to the Boc and Fmoc protecting groups. The smooth removal of Alloc group in neutral conditions with catalytic amounts of $Pd(PPh_3)_4$ in the presence of $PhSiH_3$ as a scavenger for the allyl system permits orthogonality with the most common protecting groups. Furthermore, a tandem deprotection-coupling reaction allows the suppression of DKP formation in cases where this side reaction is troublesome.
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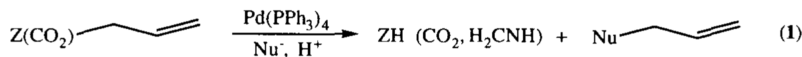
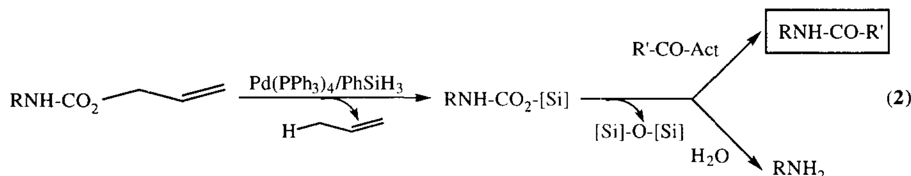
The solid-phase peptide synthesis (SPPS) methodology developed by R.B. Merrifield[2] allows the rapid and efficient synthesis of a large number of peptides.[3] The fluorenylmethoxycarbonyl (Fmoc)/*t*-butyl (*t*-Bu)-based groups for the protection of the N^{α}-amino function and the side-chains, respectively, has become a more popular technique than the first historical approach based on the use of *t*-butoxycarbonyl (Boc) and benzyl (Bzl) protecting groups.[3,4] The mild conditions of Fmoc/*t*Bu procedures and the avoidance of harsh acids such as HF allow the preparation of sequences containing acid-labile constituents such as glyco-, nucleo-, sulfo-, and hindered amino acids. Even the relative mild conditions used in the Fmoc/*t*-Bu strategy precludes very often the preparation of extremely labile peptides. Thererefore, there is an increasing need for the development and use of protecting groups that can be removed in neutral conditions. An attractive alternative to the *t*-Bu and fluorenylmethyl (Fm) based groups are those derived from the allylic system[5]: allyloxycarbonyl (Alloc) for amines, alcohols, indoles (Ind); allyl (All) for carboxylic acids,[6] phenols, imidazoles (Im); and allyloxycarbonylaminomethyl (Allocam) for thiols.[7] Furthermore, these allyl-based protecting groups are completely orthogonal with the *t*-Bu and Fm based groups and have been used in conjunction for the solid-phase preparation of cyclic, branched, and protected peptides.[8] The deprotection step involves a palladium-catalyzed transfer of the allyl unit to various nucleophiles/scavengers in the presence of a proton source (Eq. 1).



Z = R-NH, R-O, R-CO$_2$, Ar-O, R-Im, R-Ind, R-SCH$_2$-NH

While there is a consensus that $Pd(PPh_3)_4$ is usually the best catalyst, special care has to be taken for the choice of the nucleophile. Thus, the use of a nucleophilic secondary amine such as morpholine as the allyl acceptor leads to deprotection of Fmoc group.[8d] Similarly, the choice of Me_2NSiMe_3—$CF_3CO_2SiMe_3$[9] is not compatible with *t*-Bu-based protecting groups.[10] Recently, phenyltrihydrosilane ($PhSiH_3$) has been reported as a new neutral allyl group scavenger.[11] Allyl carbamates, carbonates, and esters are cleaved in solution in the

presence of $Pd(PPh_3)_4$ (0.02 equiv) and $PhSiH_3$ (1.2-2 equiv). The silyl derivatives are almost instantaneously hydrolysed by adventitious water upon simple exposure of the reaction mixture to ambient atmosphere (Eq. 2). Furthermore, when the removal of allyl carbamates is carried out in the presence of acetic anhydride, di-*tert*-butyl dicarbonate, or active species of protected amino acids [pentafluorophenyl esters, fluorides, or *N*-carboxyanhydrides (NCA)], a rapid and virtually quantitative acylation reaction is observed (Eq. 2).[11,12] This report discusses the extension of these techniques to the SPPS methodology, particularly the tandem deprotection-coupling reaction for the suppression of diketopiperazine (DKP) formation in the Fmoc/*t*-Bu approach.



Optimization of the tandem reaction in SPPS. Initially, the conditions incorporated in solution for the tandem deprotection-coupling reaction were validated on solid-phase. Alloc-Ala-Gly-Val-O-AB-Ile-MBHA[13] was chosen as a model and the protocol shown in the Figure 1 was used. Following treatment of the model peptide with a given deprotection reagent, four posssible resin-bound species can be obtained: Alloc-Ala-Gly-Val from no further elaboration, Fmoc-Leu-Ala-Gly-Val from the tandem reaction, H-Ala-Gly-Val from the incomplete coupling, and All-Ala-Gly-Val from palladium catalyzed decarboxylation rearrangement[9] of the Alloc-Ala residue. These possibilities were distinguished by a sequence involving coupling of Fmoc-Phe-OH, prolonged treatment with $Pd(PPh_3)_4/PhSiH_3$, coupling with Fmoc-Asp(*t*Bu)-OH, and evaluation by amino acid analysis (AAA) of the peptides obtained after cleavage from the resin.

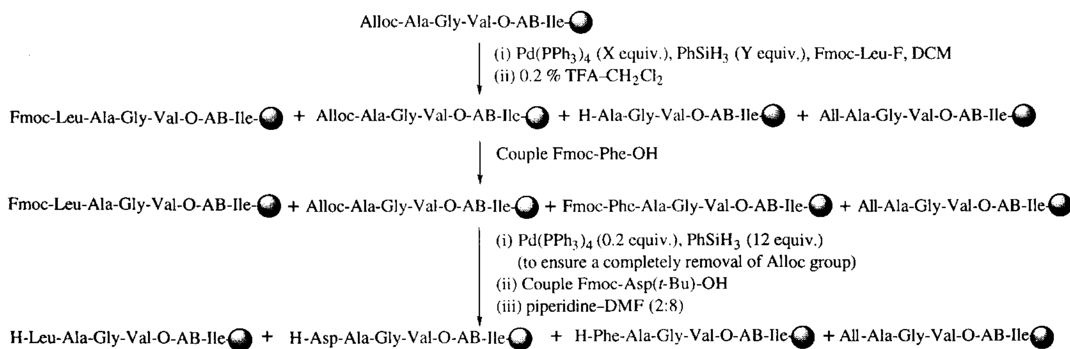


Figure 1

Results from AAA indicated ratios of Leu/Ala, Gly, Val of 0.98 and Phe/Ala/Gly, Val of 0.02, with not detectable amount of Asp, in all cases using different amounts of $Pd(PPh_3)_4$—$PhSiH_3$ (0.10:6, 0.15:9, 0.20:12, 1:12 equiv.). This data suggests that Alloc group removal was quantitative in all cases and that 2% of the tripeptide was not acylated with Fmoc-Leu-F. Since the ratio of products was independent of both the Pd catalyst and nucleophile, 0.10 equiv. of $Pd(PPh_3)_4$ and 6 equiv. of $PhSiH_3$ was used for subsequent experimentation.

Suppresion of DKP formation. This intramolecular aminolysis has been established to be either acid or base catalyzed.[14] A method to minimize this side-reaction incorporates acid labile protecting groups for the

N-amino function of the second residue (Boc and Trt for Boc/Bzl and Fmoc/t-Bu strategies, respectively) followed by removal of the protecting group and, without previous neutralization, coupling of the third residue with phosphonium salts (PyBOP or, preferably, PyAOP) in the presence of a base.[15] An alternative strategy involves the use of Alloc group for the protection of the second residue in conjunction with the tandem deprotection-coupling reactions. Formation of the new amide bond leading to the tripeptide resin under neutral conditions should be more facile than the DKP formation. Based upon previous experience with DKP formation, the dipeptide D-Val-Pro,[14a,d,e,15] was chosen as a model to test this hypothesis. Following construction of Alloc-D-Val-Pro-AB-Ile-MBHA by a standard coupling protocol, the tandem deprotection-coupling reaction was carried out with $Pd(PPh_3)_4$ (0.10 equiv), $PhSiH_3$ (6 equiv), and Fmoc-Leu-F (5 equiv) in CH_2Cl_2. Analysis of the HPLC and AAA data of both the peptide resin prior to cleavage and the corresponding peptide following release from the support confirmed that DKP did not occur.[17] Similar experiments were carried out substituting Fmoc-Leu-F for Fmoc-Leu-NCA and for an equimolar mixture of Fmoc-Leu-OH, PyAOP, and DIEA. In the former case, DKP formation was 2% whereas in the latter, DKP was not detected *via* AAA but the coupling of the third residue occurred in only 37% yield.

SPPS using Alloc-amino acids. The sequence of Leu-enkephalin was synthesized on HO-AB-Ile-MBHA and Fmoc-PAL-Ile-PEG-PS resins to afford C-terminal peptide acid and amide, respectively. Several conditions for removal of Alloc groups were examined. In order to minimize the formation of allylamines, optimal conditions involved addition of $PhSiH_3$ (24 equiv.) in CH_2Cl_2 to the resin, followed by $Pd(PPh_3)_4$ (0.10 equiv.) in CH_2Cl_2. Leu-enkephalin peptides were obtained with a yield superior to 90% and with only minor impurities <2% corresponding to the corresponding allylpeptides.

Experimental Procedures

Materials. $Pd(PPh_3)_4$ was prepared as previously described and maintained under Ar atmosphere.[16] $PhSiH_3$ was obtained from Fluka and used without further purification. CH_2Cl_2 was filtered through aluminium oxide, and stored over CaH_2 and under Ar. Alloc-amino acids were liberated before use from their dicyclohexylamonium salts in the presence of H_2SO_4. Syntheses were performed manually in a 5 mL polypropylene syringe fitted with a polyethylene disc.

Alloc removal. In a typical experiment, to a peptide resin (200 mg) washed with CH_2Cl_2 (5x30 sec) under Ar was added a solution of $PhSiH_3$ (24 equiv.) in CH_2Cl_2 (1 mL) and the resin was manually stirred. A solution of $Pd(PPh_3)_4$ (0.10 equiv.) in CH_2Cl_2 (3 mL) was added, Ar was passed through the resin and the reaction was mechanically stirred for 10 min. The peptide resin was washed with CH_2Cl_2 (8x30 sec) and the process was repeated once.

Tandem deprotection-coupling. The reaction was carried out as described above except that the corresponding active species of the protected amino acids were added at the same time that $Pd(PPh_3)_4$ and $PhSiH_3$, and the process was not repeated.

Coupling of protected amino acids and cleavage of peptides from the resin. These reactions were performed as described in earlier publications from our laboratories.[6d,e,8f,15b]

Conclusions

The use of the Alloc group in SPPS for the N^{α} protection of amino acids is an alternative to the Boc and Fmoc protecting groups. The smooth removal in neutral conditions of Alloc group with catalytic amounts of $Pd(PPh_3P)_4$ (0.10 equiv) in the presence of $PhSiH_3$ as a scavenger for the allyl system permits orthogonality

with the most common protecting groups. The tandem deprotection-coupling reaction allows the suppression of DKP in cases where this side reaction may occur. The neutral conditions used are compatible with a broad range of acid labile protecting groups and handles, such as trityl and HMPB, respectively.

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References and Notes

1. Abbreviations used in this article: AAA, amino acid analysis; AB, 3-(4-hydroxymethylphenoxy) propionic acid; Act, activating groups; All, allyl; Alloc, allyloxycarbonyl; Allocam, allyloxycarbonylaminomethyl; Boc, *t*-butoxycarbonyl; Bzl, benzyl; *t*-Bu, *t*-butyl; DIEA, *N,N*-diisopropylethylamine; DKP, diketopiperazine; Fm, 9-fluorenylmethyl; Fmoc, 9-fluorenylmethoxycarbonyl; HMPB, 4-(4-hydroxymethyl-3-methoxyphenoxy)-butyric acid; HPLC, high performance liquid chromatography; HOAc, acetic acid; Ind, indole; Im, imidazole; NCA, *N*-carboxyanhydride; PAL, 5-(4-aminomethyl-3,5-dimethoxyphenoxy)valeric acid; PEG-PS, polyethylene glycol-polystyrene graft; PyAOP, 7-azabenzotriazol-1-yl-oxytris(pyrrolidino)phosphonium hexafluorophosphate; PyBOP, benzotriazol-1-yl-oxytris(pyrrolidino)phosphonium hexafluorophosphate; SPPS, solid-phase peptide synthesis; TFA, trifluoroacetic acid; Trt, triphenylmethyl (trityl). Amino acid denotes L-configuration unless otherwise noted.
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13. Fmoc-Ile-OH was attached directly to a *p*-methylbenzhydrylamine resin by a standard coupling protocol and served as an internal standard for estimating the stability of the peptide-resin linkage. 3-(4-Hydroxymethylphenoxy) propionic acid was coupled to the Ile by the same coupling method. Fmoc-Val-OH was incorporated using DIPCDI in the presence of DMAP (0.3 fold excess relative to the amino function), and Fmoc-Gly-OH and Alloc-Ala-OH using a standard coupling protocol. A standard protocol in Fmoc/^{t}Bu solid-phase peptide synthesis was as follows: Swelling of the resin by washing with DMF (3x1 min), removal of the Fmoc group with piperidine–DMF (2:8) (2x1min, 1x10 min), washing with DMF (5x1min). Fmoc-AA-OH and HOAt (3 fold excess relative to the amino function in both cases) were added as a solution in DMF followed by DIPCDI (3 equiv.). The mixture was left 60 min and then filtered, and washed with DMF (5x1 min).
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17. These results compare favorably with the previous reported method (see ref. 15b), because the removal of Alloc group does not involved the presence of acids, and therefore there is not risk of premature cleavage of the peptide from the resin.

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