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Allyl Based Side-Chain Protection for SPPS

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Abstract

Recent studies have demonstrated the usefulness of an allyl-based linker in the syntheses of labile glycopeptides[1] and of allyloxycarbonyl [Aloc] for exocyclic amino protection in DNA synthesis[2]. T removal of allyl-based protection can be accomplished under mild conditions by Pd(O) catalyse transfer. This concept is potentially applicable tc protection of all trifunctional amino acids in SPP performed by either tBoc or Fmoc methods. To test this possibility a variety of allyl-based side-chain protected amino acids have been synthesized and incorporated into test sequences. With conventional polystyrene supports little deprotection could be accomplished, whereas with PEG-I then quantitative removal, as demonstrated by HPLC and FAB M/S was obtained. This methodology obviates one of the remaining problems of solid-phase synthesis, alkylation of sensitive residues by carbo-cations generated during cleavage and side-chain deblocking can be performed without detachment of the peptide from the support. Specific amide-bonds have been forme between selected side-chain residues using a combination of allyl and tBu protection.

Synthesis and Properties of N-Alpha Fmoc Allyl Side-Chain Protected Amino Acids

Allyl-based side-chain protection is potentially applicable to both tBoc and Fmoc chemistries. may be used in combination with conventional protection for side-chain branching and production of side-chain to side-chain linked cyclic peptides, and offers the advantage that side-chain deprotection may be accomplished by Pd(O) catalysed transfer prior to cleavage.

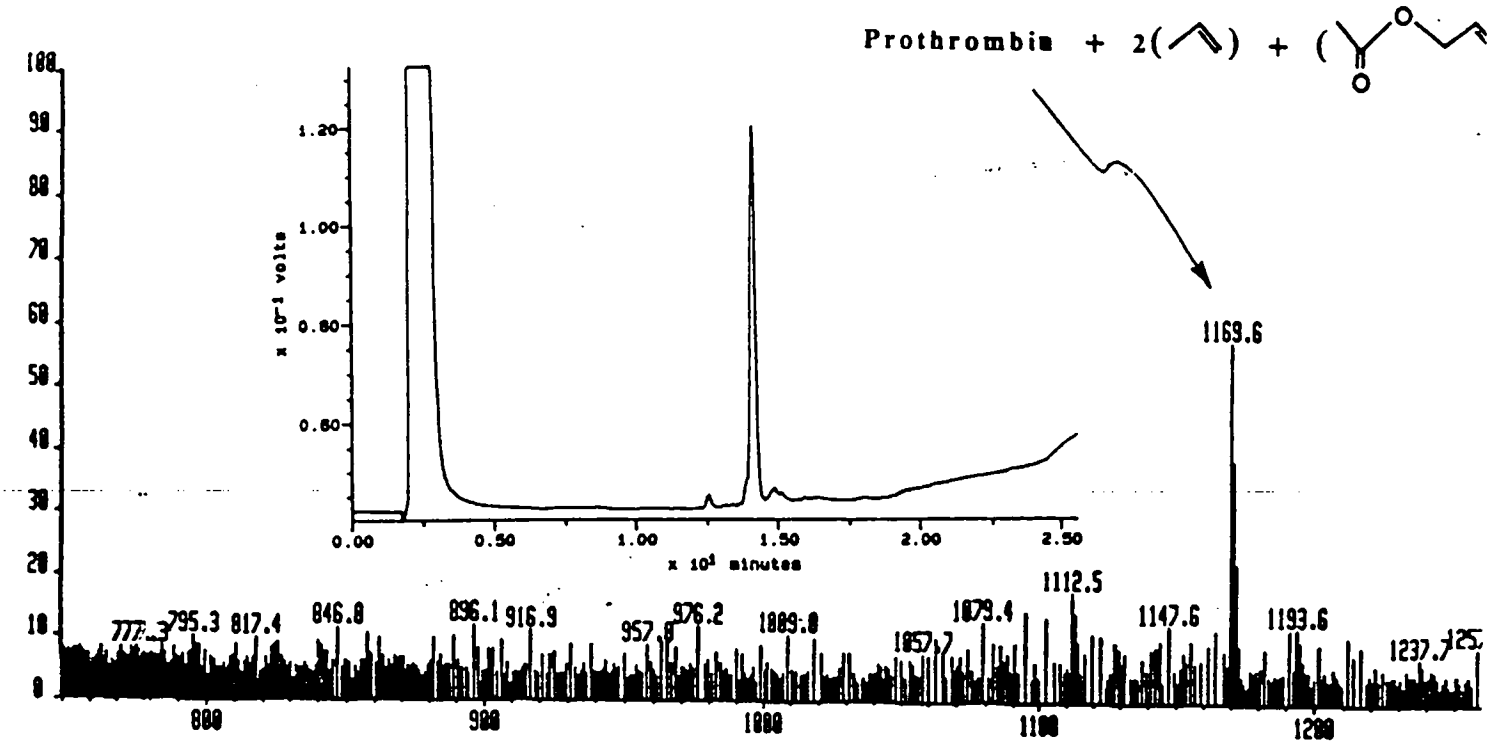
The straightforward preparation of several Fmoc derivatives is shown in Table 1. Note that th Glu allyl derivative cannot be prepared by the method used for Asp. Studies on the amino acid derivatives showed that extended treatment with Fmoc or tBoc removal reagents caused negligible damage to allyl groups.

Table 1: Side-Chain Allyl Protected Fmoc Amino Acids

DERIVATIVE	SYNTHESIS	M.P.(°C)	HPLC	TLC
Fmoc-Asp(OAl)-OH	Asp + allyl alcohol, c.H_2SO_4 –> H-Asp(OAl)-OH, + Fmoc-OSu –> Product	105-108	16.0	0.47(
Fmoc-Glu(OAl)-OH	Glu + Fmoc-OSu –> Fmoc-Glu-OH, + allyl alcohol, c.H_2SO_4 –> Product	66-72	16.5	0.49(
Fmoc-Lys(Aloc)-OH	Lys + Cu + Cl-CO-O-CH_2-CH=CH_2 –> Lys(Aloc)-copper complex, + H_2S, then Fmoc-OSu –> Product	80-85	15.7	0.65(
Fmoc-Orn(Aloc)-OH	Ornithine as Lys	82-85	14.0	0.63(
Fmoc-Cys(Al)-OH	Cys + Fmoc-OSu –> Fmoc-Cys-OH not isolated, + Br-CH_2-CH=CH_2 –> Product	87-91*	18.0	0.52(
Fmoc-Arg(Aloc)$_2$-OH	Arg –> tBoc-Arg-OH –> tBoc-Arg(Aloc)$_2$-OH, + TFA, then Fmoc-OSu –> Product	65-69	14.0	0.75(

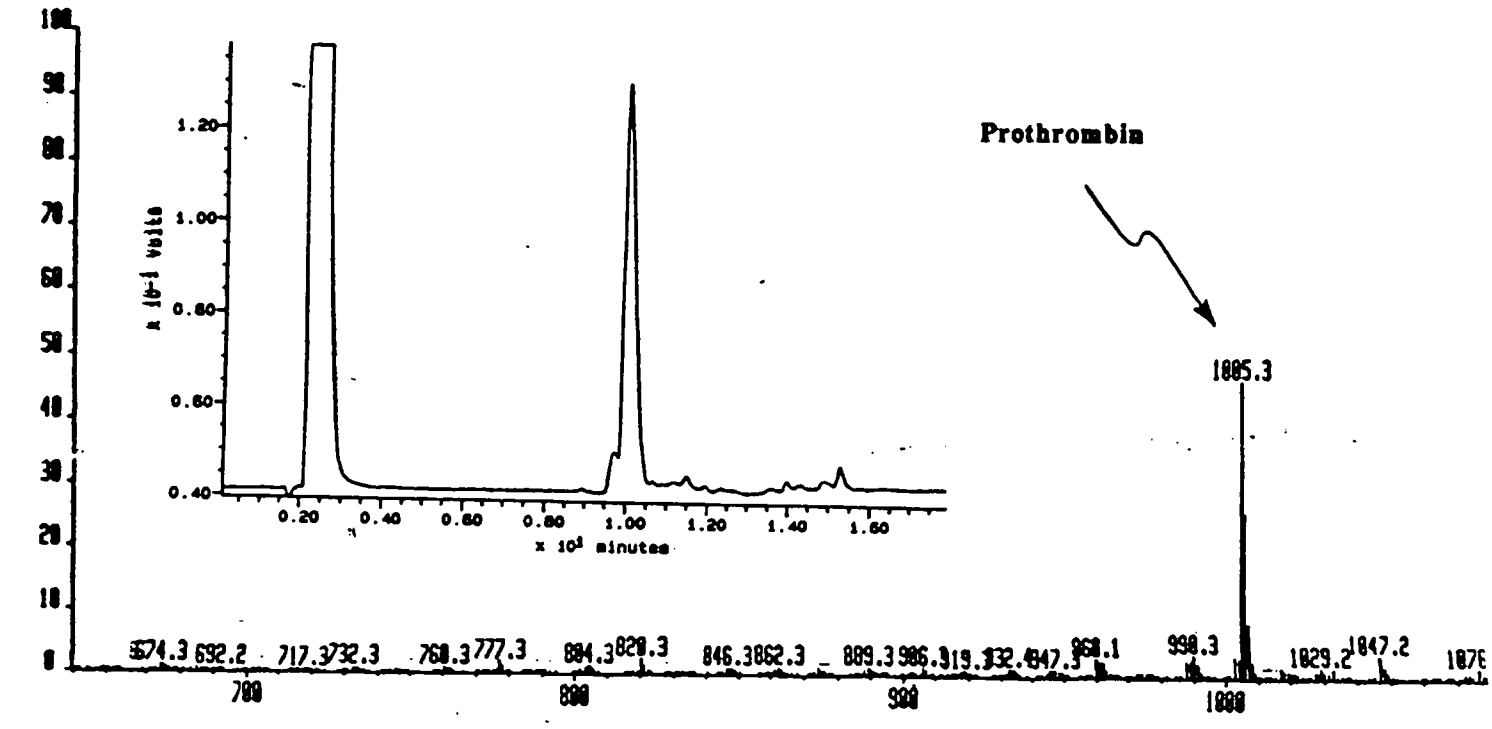
HPLC elution times (minutes) on Waters C-18 column, buffer A 0.1% TFA in water, buffer B 0.1% TFA in CH_3CN, flow 1.7 ml/min., gradient 30% B for 3 minutes, then to 100% B over 20 minutes; TLC on Merck GF254 plates in $CHCl_3$/MeOH/AcOH (90:8:2, a or 77.5:15:7.5 b), *DCHA salt.

Figure 1a.



FAB M/S of *allyl-protected* prothrombin 1-9 ANKGFLEEV amide showing reverse phase HPLC of product

Figure 1b.



FAB M/S of *allyl-deprotected* prothrombin 1-9 ANKGFLEEV amide showing reverse phase HPLC of product

Conditions for Allyl Cleavage

These allyl-protected Fmoc amino acids were found to couple efficiently and were incorporated into the sequence Ile-Ala-X-Gly. Under a variety of conditions Pd(0) treatment of the peptide-polystyrene supports gave incomplete allyl removal. Near quantitative cleavage (FAB M/S, HPLC, TLC) was obtained for all but Cys(Al) when a novel polyethyleneglycolpolystyrene copolymer (PEG-PS)[4] was used, and the N-terminal protecting group was not removed prior to allyl cleavage. Typically 200 mg of peptide resin was placed in a 5 mL polypropylene vial, and treated with dry THF (3 mL), morpholine (200 µL), triphenylphosphine (250 mg), and tetrakistriphenylphosphine palladium(0) complex (50 mg). The vial is sealed, shaken overnight, and the resin washed with THF repeatedly prior to further treatment.

Application to Linear Sequences

The prothrombin sequence 1-9 ANKGFLEEV was prepared as its C-terminal amide on PAL-PEG-PS using Fmoc-Glu(OAl)-OH and Fmoc-Lys(Aloc)-OH. From this synthesis were obtained both allyl-protected and allyl-cleaved peptides, and the free peptide was compared to the same peptide made with tBu-based protection. Analytical data show fully equivalent product purity from both syntheses; the FAB M/S shows complete removal of all allyl protection (Fig 1). In further experiments the phenylalanine in this test sequence was replaced with Trp, Tyr and Met; and the allyl-cleaved peptides were shown to be equivalent to the standard synthesis (data not shown). In an interesting recent example in conventional synthesis of the sequence Ac-Tyr-Lys$_9$-Ala-NH$_2$[5] (with TFA + thiol scavenger cleavage) a significant amount of t-butylated impurity was generated. With the allyl chemistry, although some higher molecular weight materials are present, the FAB M/S shows complete cleavage of the 9 allyloxycarbonyl groups, the product purity being at least as good as in the standard synthesis (cf. Fig. 2a and b).

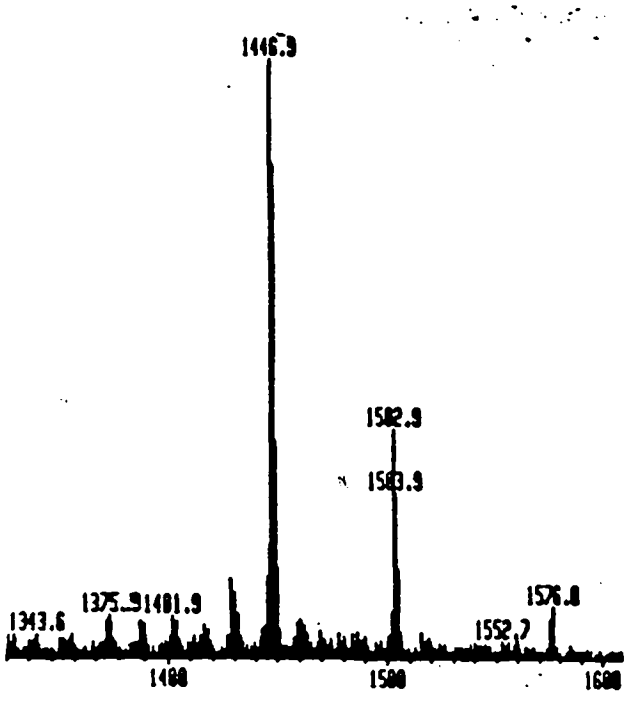


Figure 2a. FAB M/S of conventional synthesis of Ac-Tyr-Lys$_9$-Ala-amide

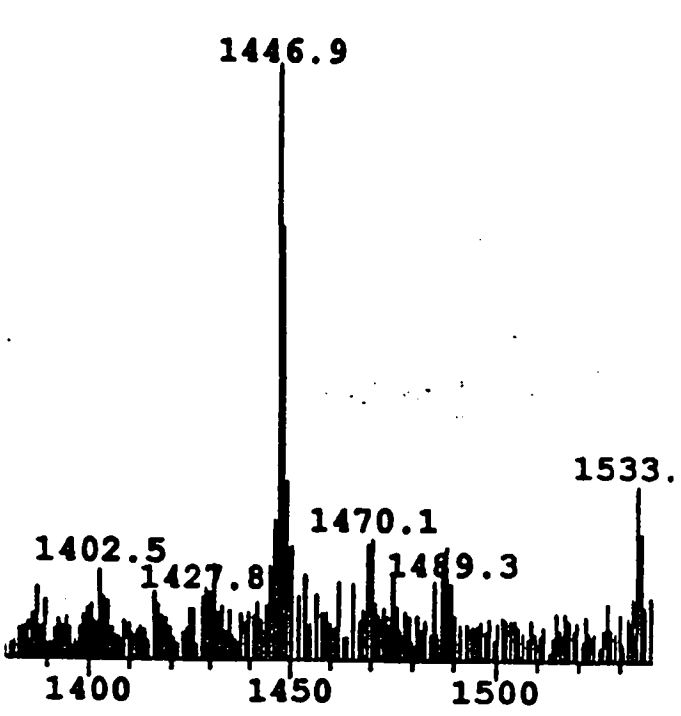


Figure 2b. FAB M/S from allyl-based synthesis of Ac-Tyr-Lys$_9$-Ala-amide

Synthesis of Cyclic Peptides

At present no technique is available for the preparation of side-chain to side-chain linked peptides via the Fmoc synthesis method. The established method, introduced by Felix *et al.* uses fluorenylmethyl-based side-chain protection for bridging residues, benzyl-based groups for other side-chains, and the tBoc synthesis method. We have used the same test sequence derived from human growth hormone 1-29, YADAIFTNSYRKVLGQLSARKLLDIMSR amide incorporating Fmoc-Lys(Aloc)-OH at position 12, and Fmoc-Asp(OAl)-OH at position 3. It should be noted that the assembly of this sequence on PAL-PEG-PS proceeded with high efficiency, as reflected in the HPLC of the uncyclized, fully deprotected peptide (Fig. 3a). The majority of the completed peptide resin was subjected to allyl cleavage, then treated with B reagent to establish the desired inter-side-chain amide bridge. Following conventional cleavage, FAB M/S (Fig. 3b) showed the presence of the desired cyclic material with some higher molecular weight (at present uncharacterised) impurity. Automated Edman sequence analysis (Model 6600 with DITC immobilization protocols) of the cyclic product shows the presence NO PTH-Asp at cycle 3, and a non-natural amino acid at cycle 12, all other residues being determined correctly.

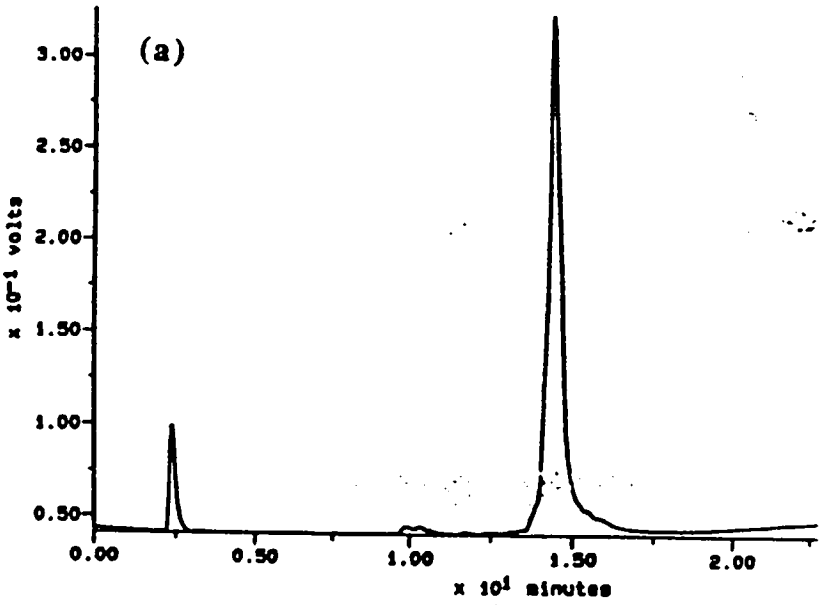
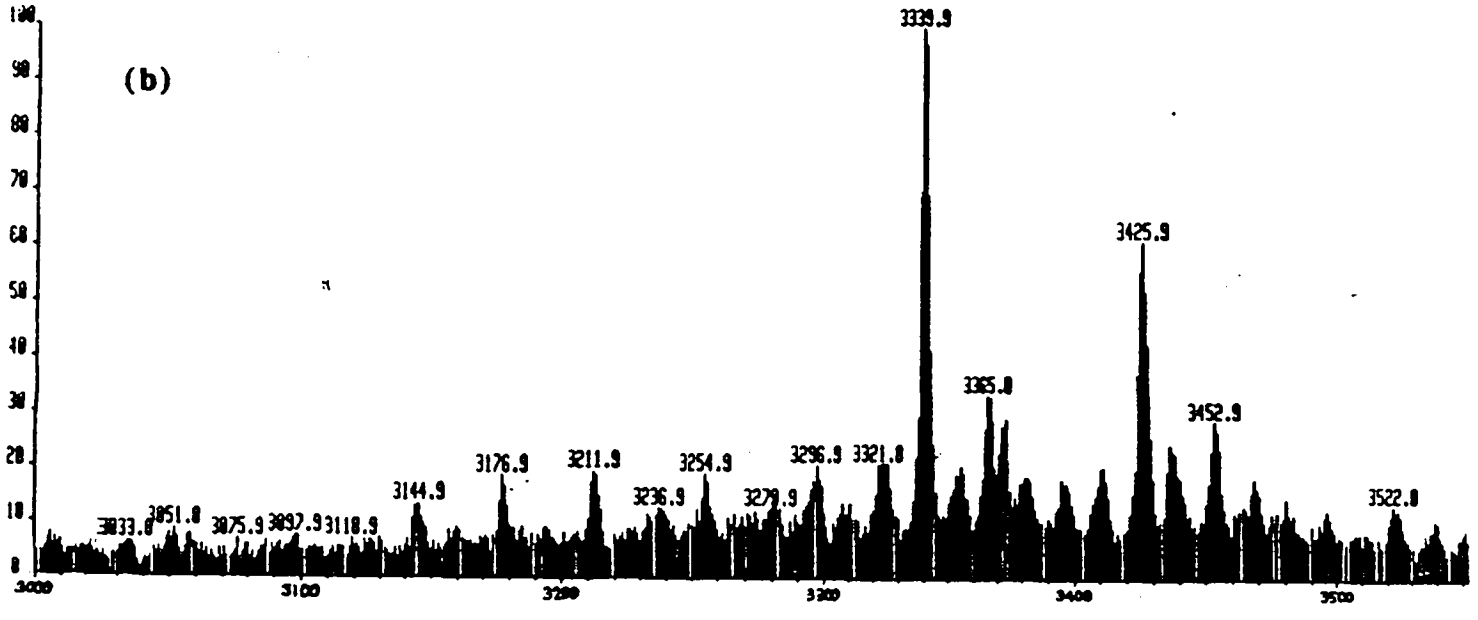


Figure 3.

(a) Reverse phase HPLC analysis of fully deprotected GRF 1-29 amide

(b) FAB M/S of 3, 12 cyclized GRF 1-29 amide



General Applicability

These studies demonstrate the potential of a new methodology which increases the scope of existing solid-phase synthesis techniques; it may also be widely applicable. When used in combination with hyperacid-labile or photo-cleavable handles the need for TFA and noxious scavengers may be entirely eliminated from deprotection and cleavage protocols. We have far been unable to affect removal of the allyl protection from peptides prepared from Fmoc-Cys(Al)-OH. However, other orthogonal protecting groups are available for this amino acid. Although we have been able to synthesize several peptides containing arginine using Fmoc-Arg(Aloc)$_2$-OH, significant quantities of ornithine are formed during piperidine treatment. Studies to overcome these problems, and to extend the idea to the remaining trifunctional amino acids, as well as to apply it to conversion of Orn to Arg, are in progress.

Conclusions

We have shown that allyl protection is particularly useful in Fmoc-mediated syntheses of cyclic peptides. The method is also useful for simple sequences containing any of the amino acids yet prepared. The key to obtaining high cleavage efficiency resides in the use of PEG as a support for synthesis.

Acknowledgments

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