Novabiochem®

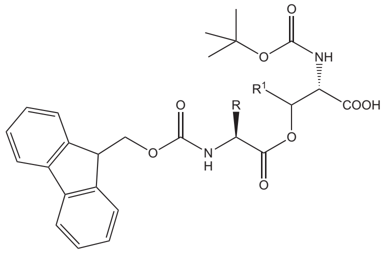
Letters: 2/07

Contents

Product Focus: Derivatives for enhancing peptide synthesis

NEW Structure breaking isoacyl dipeptides

Boc-Ser/Thr(Fmoc-Aaa)-OH



Features & Benefits

- Improved yields and purities of insoluble aggregated peptides
- Purification can be carried out on soluble depsipeptide prior to conversion to native sequence
- Ideal for synthesis of amyloidogenic peptides
- Routine use provides same benefits as pseudoproline dipeptides

Isoacyl dipeptides are remarkable new tools for enhancing synthetic efficiency in Fmoc SPPS that consist of a Boc-protected serine or threonine derivative in which the β-hydroxyl group is acylated by an Fmoc-amino acid [1, 2]. They perform the same role, and are used in exactly the same manner, as pseudoproline dipeptides. Substitution of Aaa-Ser or Aaa-Thr in a peptide sequence with an isoacyl dipeptide results in the formation of a depsipeptide analog of the native sequence

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in which the amide bond between Aaa and Ser or Thr is replaced by an ester linkage. This modification results in a marked change in the conformation of the peptide chain which leads to disruption of aggregation in much the same way as would insertion of a pseudoproline or N-Dmb/Hmb-residue [3 - 5]. However, the real benefits of using isoacyl dipeptides become apparent once the peptide is released from the solid phase. In contrast to pseudoproline dipeptides, the product cleaved when using isoacyl dipeptides is the depsipeptide and not the native peptide sequence (Figure 1). Such depsipeptide analogs of aggregation prone peptides have been found to be more soluble and consequently more easily purified than the highly structured native peptide [3 - 5]. For example, isoacyl β-amyloid has a solubility of 15 mg/ml in water, whereas for the natural peptide it is only 0.14 mg/ml [6]. Once the depsipeptide form is purified, it can be easily converted to the native form by adjusting the pH to 7.4 when spontaneous O- to N-acyl migration occurs, with formation of an amide bond between the Ser or Thr residue and the next amino acid. This pH controlled switching of the peptide conformation can be very advantageous in the study of amyloidogenic peptides, where the initial aggregation state of the peptide to be used in the assay is often unknown. For instance, a non-aggregated depsipeptide can be introduced into a bioassay and the native peptide formed *in situ* under physiological conditions, enabling activity of the native sequence in a non-aggregated state to be determined [7, 8].

A further important application of isoacyl dipeptides is in fragment condensation. By employing an isoacyl dipeptide as the C-terminal dipeptide within the sequence of the carboxyl component, epimerization during fragment coupling can be avoided [9]. This is because the amine group of the Ser/Thr is protected as a urethane and therefore can not easily become involved in oxazolone formation (Figure 2). This approach effectively doubles the number of sites available in a given peptide sequence for epimerization-free fragment condensation from Gly and Pro to now include Ser and Thr.

05-20-0015 ***NEW***	Boc-Ser(Fmoc-Ala)-OH	1 g 5 g
05-20-0009 ***NEW***	Boc-Ser(Fmoc-Gly)-OH	1 g 5 g
05-20-0010 ***NEW***	Boc-Ser(Fmoc-Phe)-OH	1 g 5 g
05-20-0013 ***NEW***	Boc-Ser(Fmoc-Ser(tBu))-OH	1 g 5 g
05-20-0014 ***NEW***	Boc-Ser(Fmoc-Thr(tBu))-OH	1 g 5 g
05-20-0011 ***NEW***	Boc-Thr(Fmoc-Ala)-OH	1 g 5 g
05-20-0012 ***NEW***	Boc-Thr(Fmoc-Gly)-OH	1 g 5 g

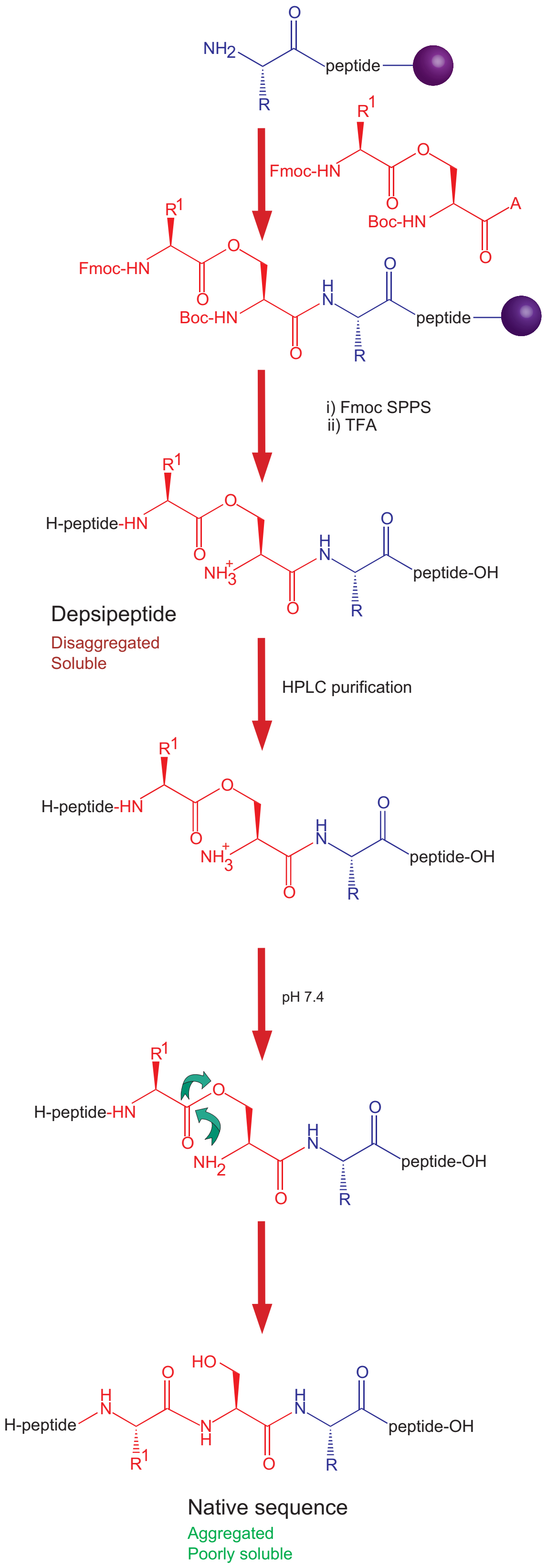
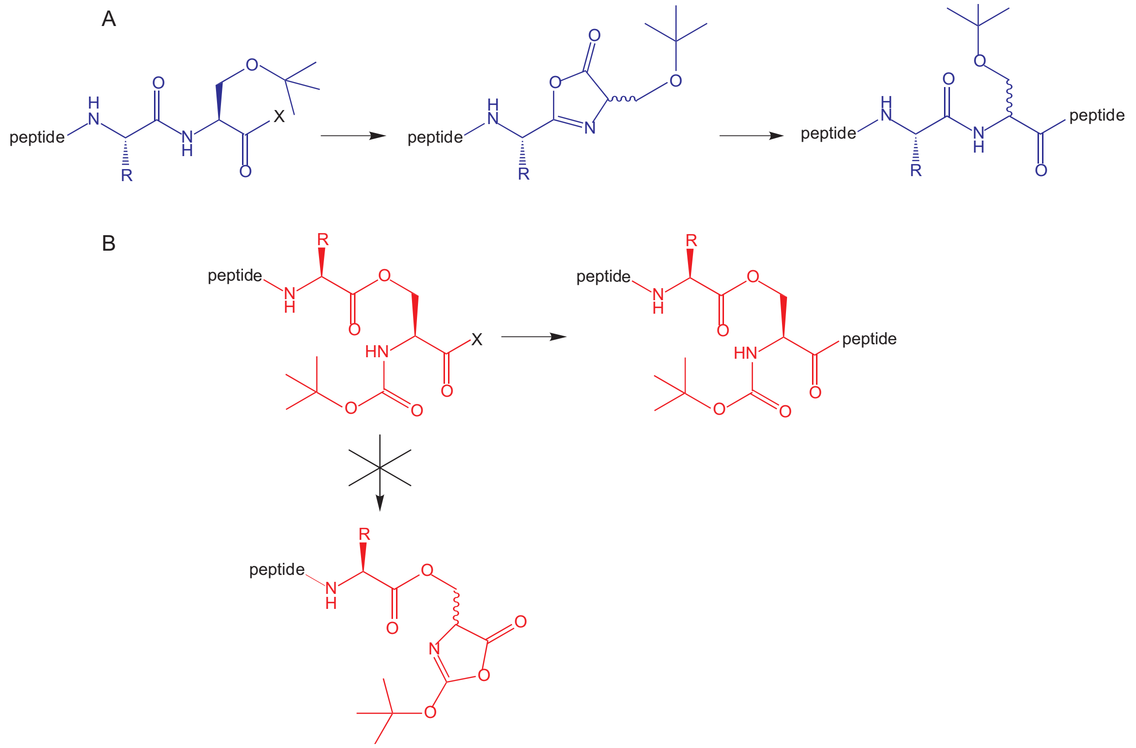


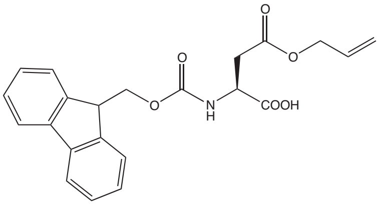
Fig. 1: Principles of peptide synthesis with isoacyl dipeptides, illustrated with Ser. A = leaving group. R and R^1 = amino-acid side chains.

Fig. 2: A. Epimerization via oxazolone formation during conventional fragment condensation. B. Fragment condensation using a C-terminal isoacyl dipeptide without epimerization. X = leaving group. R = amino-acid side chain.

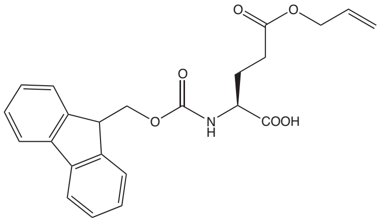


NEW Allyl-protected amino-acid derivatives for Fmoc SPPS

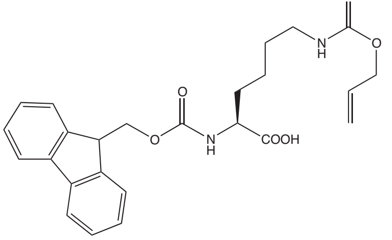
Fmoc-Asp(OAll)-OH



Fmoc-Glu(OAll)-OH



Fmoc-Lys(Alloc)-OH



Features & Benefits

- Allyl-based protection removed by Pd-catalysis
- Allyl-protection is stable to TFA and piperidine
- Ideal reagents for on-resin synthesis of cyclic and side-chain modified peptides

We are pleased to offer Fmoc-Lys(Alloc)-OH, Fmoc-Asp(OAll)-OH, and Fmoc-Glu(OAll)-OH as the latest additions to our range of orthogonally protected amino acids for Fmoc SPPS. Allyl-based protecting groups such as allyl esters (OAll) and allyloxycarbonyl (Alloc) are completely stable to piperidine and TFA but can be easily removed under mild conditions by Pd(0) catalyzed allyl transfer [10]. These properties make amino acids bearing allyl-based side-chain protection ideally suited to the on-resin synthesis of cyclic peptides and side-chain modified peptides, as their side chains can be selectively unmasked on the solid support in the presence of the standard t-butyl protecting groups [11, 12].

A variety of methods has been developed for allyl removal, but perhaps most useful for on-resin cleavage is that of Kates, *et al.* using $Pd(PPh_3)_4/CHCl_3$/HOAc/NMM as described in Method 1 [13]. This procedure can be adapted to be carried out automatically on peptide synthesizers that utilize nitrogen agitation.

Allyl-based protection is orthogonal to both ivDde- and trityl-based chemistry, facilitating the synthesis of multicyclic, and differentially branched peptides, and regioselectively addressable peptide templates *via* multidimensional protection strategies [12, 14]. However, when using allyl-based protection in conjunction with ivDde, it is recommended that a small amount of allyl alcohol is added to the hydrazine reagent used to cleave the ivDde group [15]; the allyl alcohol serves to scavenge any diazine that may be present. It is thought that diazine can cause reduction of the double bond of the allyl group, thereby rendering it intractable to Pd-mediated cleavage.

Method 1: Removal of allyl protecting groups

1. Weigh the peptidyl resin into a test tube and dry at 40°C under high vacuum. Seal the tube with a rubber septum. Flush the vessel with a stream of Ar delivered via a needle inserted through the septum.
2. Weigh $Pd(PPh_3)_4$ (3 eq.) into a dry test tube, add $CHCl_3$-AcOH-N-methylmorpholine (37:2:1) (15 ml/g of resin), dissolve catalyst by bubbling a stream of Ar through the solution, and seal the tube with a rubber septum.
3. Transfer this mixture using an Ar flushed gas-tight syringe to the tube containing the resin. Leave to stand for 2 h with occasional gentle agitation.
4. Transfer the resin to a sintered glass funnel and wash consecutively with 0.5% DIPEA in DMF and sodium diethyldithiocarbamate (0.5% w/w) in DMF to remove the catalyst.

The above procedure can also be carried out on any automated peptide synthesizer which uses N_2 agitation for dissolution and transfer of reagents. The $Pd(PPh_3)_4$ catalyst should be weighed out into an amino acid cartridge and dissolved in $CHCl_3$-AcOH-N-methylmorpholine (37:2:1) with Ar agitation. The cartridge should be sealed and placed in the instrument autoloader as normal. The instrument should then be programmed to transfer the contents of the vial to reaction vessel or column without the addition of further reagents. If the instrument has any spare solvent reservoirs, these can be filled with 0.5% DIPEA in DMF and sodium diethyldithiocarbamate (0.5% w/w) in DMF to allow automated washing of the resin following allyl deprotection.

Care should also be taken when preparing peptides using Asp(OAll), as in contrast to t-butyl, the unhindered allyl group offers little protection against aspartimide formation. With Asp(OAll), not only has this side reaction been observed during Fmoc SPPS, but it has also been seen during coupling [15]. This problem can be eliminated by using a N-Dmb/Hmb-protected amino acid derivative for introduction of the residue before the Asp residue [16].

04-12-1303	Fmoc-Asp(OAll)-OH	5 g
NEW		25 g
04-12-1304	Fmoc-Glu(OAll)-OH	5 g
NEW		25 g
04-12-1305	Fmoc-Lys(Alloc)-OH	5 g
NEW		25 g

References

1. Y. Sohma, et al. (2006) *Tetrahedron Lett.*, **47**, 3013; T. Yoshiya, et al. (2007) *Org. Biomol. Chem.*, **5**, 1720.
2. I. Coin, et al. (2006) *J. Org. Chem.*, **71**, 6171.
3. Y. Sohma, et al. (2004) *Biopolymers*, **76**, 344.
4. M. Mutter, et al. (2004) *Angew. Chem. Int. Ed.*, **43**, 4172.
5. L. A. Carpino, et al. (2004) *Tetrahedron Lett.*, **45**, 7519.
6. Y. Sohma, et al. (2005) *J. Pept. Sci.*, **11**, 441.
7. Y. Sohma & Y. Kiso (2006) *ChemBioChem*, **7**, 1549.
8. S. Dos Santos, et al. (2005) *J. Am. Chem. Soc.*, **127**, 11888.
9. T. Yoshiya, et al. (2006) *Tetrahedron Lett.*, **47**, 7905.
10. A. Loffet & H. X. Zhang (1993) *Int. J. Peptide Protein Res.*, **42**, 346.
11. S. A. Kates, et al. (1993) *Tetrahedron Lett.*, **34**, 1549.
12. G. B. Bloomberg, et al. (1993) *Tetrahedron Lett.*, **34**, 4709.
13. S. A. Kates, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 13th American Peptide Symposium", R. S. Hodges & J. A. Smith (Eds.), ESCOM, Leiden, 1994, pp. 113.
14. B. Rohwedder, et al. (1998) *Tetrahedron Lett.*, **39**, 1175.
15. S. C. Vigil-Cruz & J. V. Aldrich (1999) *Lett. Pept. Sci.*, **6**, 71.
16. J. Offer, et al. (1996) *J. Chem. Soc., Perkin Trans. I*, 175.

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