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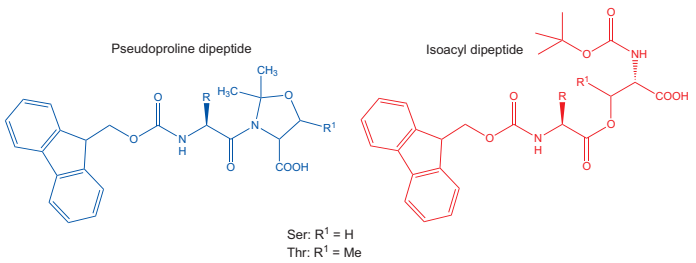
Innovations 2/10

Guide to the use of isoacyl dipeptides in Fmoc SPPS



Since the introduction of the O-acyl isopeptide method or depsipeptide technique almost simultaneously by Kiso [1], Beyermann/Carpino [2] and Mutter [3] in 2004, isoacyl dipeptides [4, 5] have proven to be invaluable tools for the synthesis of difficult and cyclic peptides and for epimerization-free fragment condensation reactions.

Isoacyl dipeptides consist of a Boc-protected serine or threonine derivative in which the β-hydroxyl group is acylated by an Fmoc-amino acid. When coupled into a peptide chain in place of Aaa-Ser or Aaa-Thr, these derivatives temporarily introduce a kink into the peptide chain, in much the same manner as a pseudoproline, that helps prevent the formation of those secondary structures responsible for aggregation. Their use leads to better and more predictable acylation and deprotection kinetics, which results in higher purities and solubilities of crude products, easier HPLC purification and improved yields.

Cleavage of the product from the resin with TFA regenerates the native peptide in the case of a pseudoproline dipeptide containing sequence, whereas with an isoacyl dipeptide a depsipeptide is produced. 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 [1 - 3, 6 - 9]. 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 (Figure 1).

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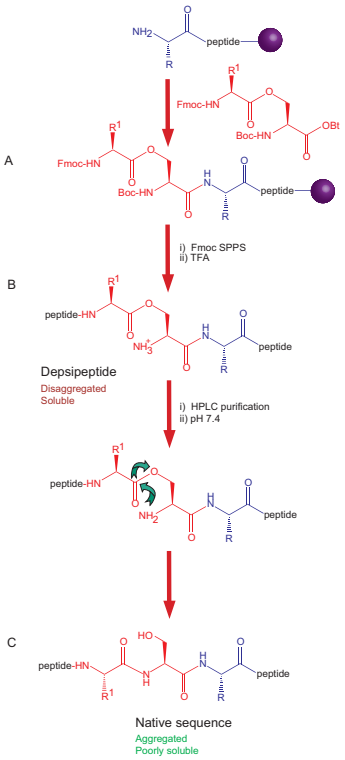

Fig. 1: Use of isoacyl dipeptides in Fmoc SPPS.

Guidelines for introduction of isoacyl dipeptides

- Optimal results are obtained if the isoacyl dipeptides are spaced 5-6 residues apart throughout the sequence.
- The optimum separation between an isoacyl dipeptide and a Pro residue is 5-6 amino acid residues.
- The minimum separation between an isoacyl dipeptide and another isoacyl dipeptide or Pro residue is 2 residues.
- Aim to insert a isoacyl dipeptide before regions of hydrophobic residues.

Coupling

Activation of isoacyl dipeptides with base-mediated coupling methods such as PyBOP®/DIPEA or HBTU/DIPEA has been shown to cause β-elimination of the Fmoc-amino acid from the serine or threonine side chain (Figure 2). This can lead to the formation of peptides omitting serine/threonine or by-products derived from dehydroresidues. Coupling under non-basic conditions using HOBt/DIPCDI in DCM (Method 1) appears to eliminate this problem [10, 11].

Method 1: Coupling of isoacyl dipeptides

1. Dissolve the isoacyl dipeptide (4 eq.[a]) and HOBt (4 eq.[a]) in DCM.
2. Add DIPCDI (4.4 eq.[a]) and agitate for 10 mins.
3. Add solution to peptidyl resin.

[a]relative to resin loading

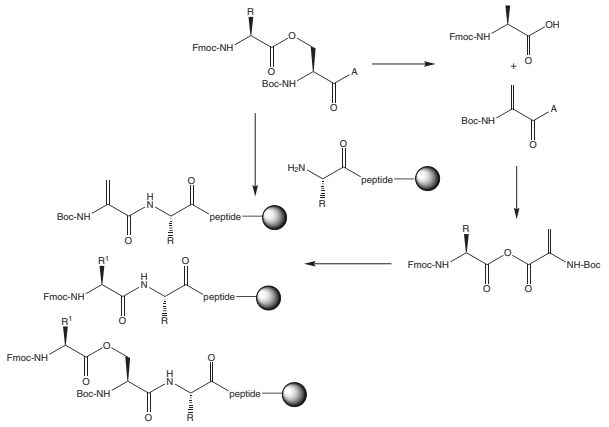

Fig. 2: β-Elimination of isoacyl dipeptides during activation.

DKP formation

Occasionally sequence dependent cleavage of the ester bond has been observed. This presumably arises from diketopiperazine formation during the removal of Fmoc from the residue following introduction of the isoacyl dipeptide, and is particularly problematic when the non-hindered residues are present in the ester-linked dipeptide. Beyermann and colleagues eliminated this problem by employing Bsmoc-protected amino acids for introduction of the residue immediately following the isoacyl dipeptide [12]. More recently, T. Yoshiya, *et al.* have shown that this side reaction can be suppressed by using 1-methylpyrrolidine/ hexamethyl-eneimine/HOBt in NMP/DMSO [13] for Fmoc removal. The use of this mixture should only be necessary for removing the Fmoc from the residue following the isoacyl dipeptide.

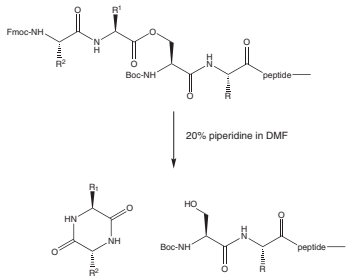

Fig. 3: Diketopiperazine formation during Fmoc removal.

O- to N-Shift

Conversion of the depsipeptide to the native sequence can normally be achieved by dissolving the depsipeptide in pH 7 -8 buffer (Method 2). This reaction is facile and is usually complete in a few minutes. The process can be monitored by RP-HPLC; the native peptide is invariably more strongly retained on the column than the depsipeptide.

Method 2: O to N migration

1. Dissolve depsipeptide in PBS (pH 7.4) or 0.05 M sodium bicarbonate. Monitor reaction by HPLC until complete.
2. Desalt peptide by gel filtration, solid phase extraction or HPLC.

Applications

Difficult peptides

Isoacyl dipeptides and pseudoproline dipeptides have been found to perform equally well in expediting the synthesis of aggregated sequences, as illustrated by the examples in Figures 4 and 5.

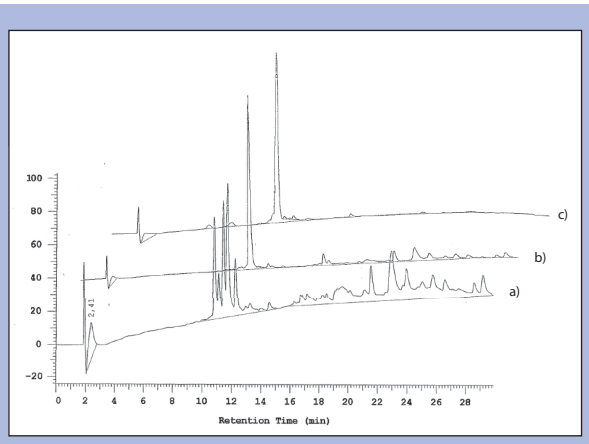

Fig. 4: HPLC profiles of H-Val-Thr-Arg-Tyr-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-OH prepared a) with Fmoc-amino acid derivatives; b) FS pseudoproline dipeptide; c) FS isoacyl dipeptide.

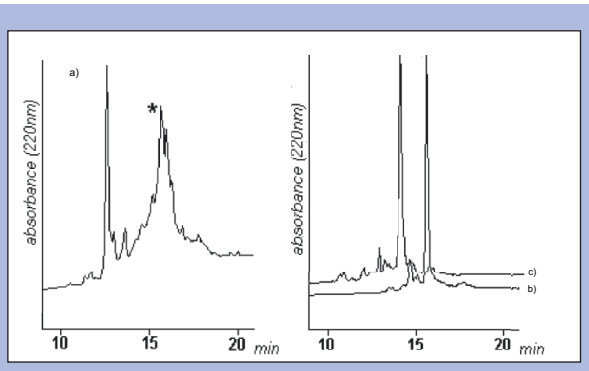

Fig. 5: HPLC profile of N(15)-FBP28WW (H-Gly-Ala-Thr-Ala-Val-Ser-Glu-Trp-Thr-Glu-Tyr-Lys-Thr-Ala-Asn-Gly-Lys-Thr-Tyr-Tyr-Tyr-Asn-Asn-Arg-Thr-Leu-Glu-Ser-Thr- Trp-Glu-Lys-Pro-Gln-Glu-Leu-Lys-NH_2) prepared using a) Fmoc amino acid building blocks, b) pseudoproline dipeptides, and c) isoacyl dipeptides [12].

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 [11, 14]. 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 6). 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. Furthermore, protected depsipeptides are more soluble and couple significantly faster than the corresponding native sequences.

Activation of the depsipeptide fragment is best achieved using DIPCDI/HOBt in DCM, to suppress β-elimination of the ester containing Ser or Thr residue. This approach has recently been used to prepare humanin in excellent purity [13].

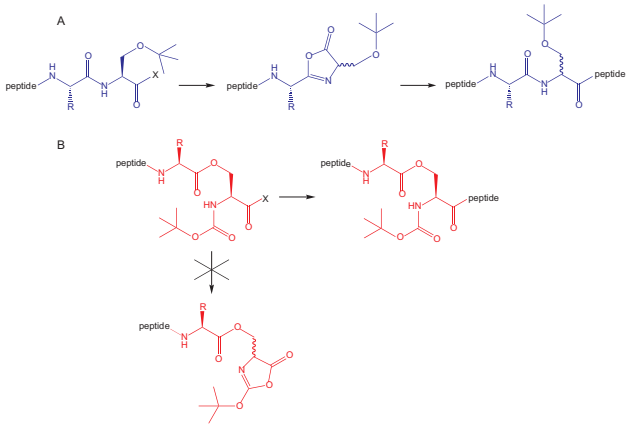

Fig. 6: A: Epimerization during fragment condensation via oxazolone formation; B: Epimerization-free fragment coupling using isoacyl peptide.

Cyclizations

Protected peptides incorporating an isoacyl dipeptide at the C-terminus are excellent intermediates for the preparation of cyclic peptides, as they couple rapidly and undergo carboxyl activation without the risk of epimerization. Cyclic depsipeptides produced in the manner will rearrange to the native sequence upon dissolution in pH 7.4 buffer or piperidine in DMF (Figure 7). This approach afforded all L-amino acid cyclic pentapeptides that are extremely difficult to access by conventional methods [15].

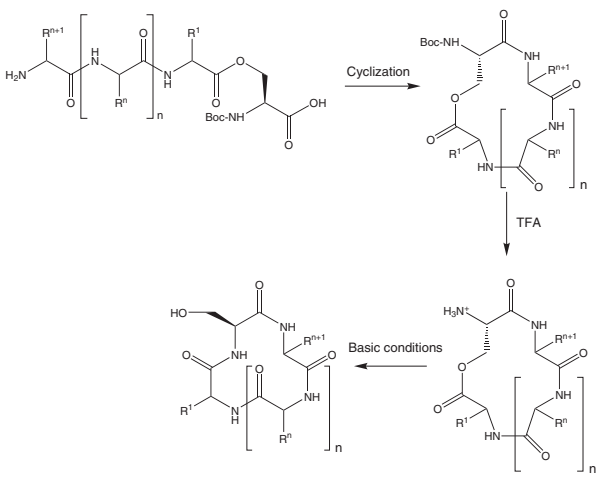

Fig. 7: Synthesis of cyclic peptides via depsipeptides.

Ordering information

852174	Boc-Ser(Fmoc-Ala)-OH	1 g 5 g
852249	Boc-Ser(Fmoc-Arg(Pbf)-OH	1 g 5 g
852257	Boc-Ser(Fmoc-Asn(Trt)-OH	1 g 5 g
852298	Boc-Ser(Fmoc-Asp(OtBu))-OH	1 g 5 g
852256	Boc-Ser(Fmoc-Gln(Trt))-OH	1 g 5 g
852295	Boc-Ser(Fmoc-Glu(OtBu))-OH	1 g 5 g
852168	Boc-Ser(Fmoc-Gly)-OH	1 g 5 g
852250	Boc-Ser(Fmoc-Ile)-OH	1 g 5 g
852262	Boc-Ser(Fmoc-Leu)-OH	1 g 5 g
852293	Boc-Ser(Fmoc-Met)-OH	1 g 5 g
852169	Boc-Ser(Fmoc-Phe)-OH	1 g 5 g
852172	Boc-Ser(Fmoc-Ser(tBu))-OH	1 g 5 g
852173	Boc-Ser(Fmoc-Thr(tBu))-OH	1 g 5 g
852290	Boc-Ser(Fmoc-Val)-OH	1 g 5 g
852170	Boc-Thr(Fmoc-Ala)-OH	1 g 5 g
852294	Boc-Thr(Fmoc-Arg(Pbf))-OH	1 g 5 g
852297	Boc-Thr(Fmoc-Asp(OtBu))-OH	1 g 5 g
852296	Boc-Thr(Fmoc-Glu(OtBu))-OH	1 g 5 g
852171	Boc-Thr(Fmoc-Gly)-OH	1 g 5 g
852252	Boc-Thr(Fmoc-Ile)-OH	1 g 5 g
852263	Boc-Thr(Fmoc-Leu)-OH	1 g 5 g
852292	Boc-Thr(Fmoc-Met)-OH	1 g 5 g
852299	Boc-Thr(Fmoc-Thr)-OH	1 g 5 g
852253	Boc-Thr(Fmoc-Val)-OH	1 g 5 g

References

1. Y. Sohma, et al. (2004) *Chem. Commun*., 124.
2. L. A. Carpino, et al. (2004) *Tetrahedron Lett.*, **45**, 7519.
3. M. Mutter, et al. (2004) *Angew. Chem. Int. Ed.*, **43**, 4172.
4. Y. Sohma, et al. (2006) *Tetrahedron Lett.*, **47**, 3013; T. Yoshiya, et al. (2007) *Org. Biomol. Chem.*, **5**, 1720.
5. I. Coin, et al. (2006) *J. Org. Chem.*, **71**, 6171.
6. Y. Sohma, et al. (2004) *Peptide Science*, **76**, 344.
7. Y. Sohma, et al. (2005) *J. Pept. Sci.*, **11**, 441
8. Y. Sohma, et al. (2006) *ChemBioChem*, **7**, 1549
9. S. Dos Santos, et al. (2005) *J. Am. Chem. Soc.*, **127**, 11888.
10. A. Taniguchi, et al. (2007) *J. Pept. Sci.*, **13**, 868.
11. I. Coin, et al. (2008) *J. Pept. Sci.*, **14**, 299.
12. I. Coin, et al. (2007) *Nature Protocols*, **2**, 3247.
13. T. Yoshiya, et al. (2009) *Org. Biomol. Chem.*, **7**, 2894.
14. T. Yoshiya, et al. (2006) *Tetrahedron Lett.*, **47**, 7905.
15. J. Lécaillon, et al. (2008) *Tetrahedron Lett.*, **49**, 4674.