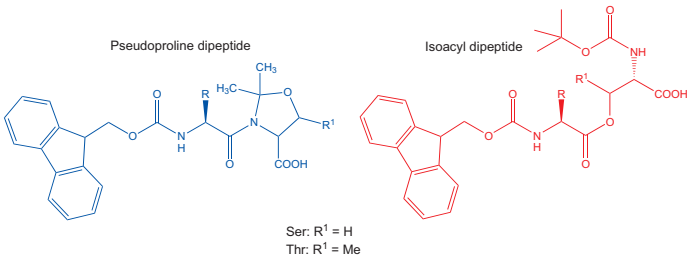
Novabiochem®

innovations 4/07

Comparison of pseudoproline and isoacyl dipeptides in the synthesis of difficult sequences



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 offer the same benefits as pseudoproline dipeptides, but with the added advantage that the depsipeptides obtained directly from the TFA cleavage reaction are often markedly more soluble than the native target sequence. This property allows insoluble aggregated sequences such as β-amyloid to be purified in the soluble isoacyl form prior to conversion to the insoluble native form (Figure 1).

In this innovation, we compare the application of pseudoproline dipeptides and isoacyl dipeptides in the synthesis of a number of challenging peptides selected from our in-house research or the literature with respect to their ease of use, yield, and purity of the final products.

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How isoacyl dipeptides work

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 in which the amide bond between Aaa and Ser or Thr is replaced by an ester linkage (Figure 1A). 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 - 8]. In contrast to pseudoproline dipeptides, the product cleaved when using isoacyl dipeptides is the depsipeptide and not the native peptide sequence (Figure 1B). 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 - 8]. Once the depsipeptide form is purified, it can be easily converted to the native form by adjusting the pH to 7.4 (Method 1) 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 1C).

Coupling of isoacyl dipeptides

Activation of isoacyl dipeptides with base-mediated coupling methods such as PyBOP®/DIPEA or HBTU/DIPEA has be shown to cause β-elimination of the Fmoc-amino acid from the serine or threonine side chain. 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 (Method 2) appears to eliminate this problem (Figure 2) [9].

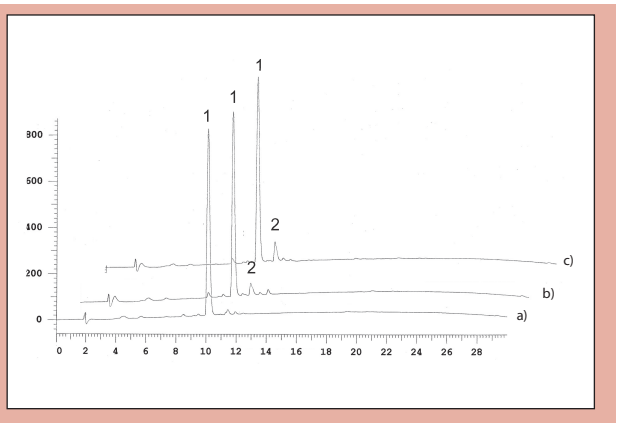

Fig. 2: HPLC profiles of crude H-Tyr-Phe-Ser-Leu-OH prepared on H-Leu-Wang resin using Boc-Ser(Fmoc-Phe)-OH under different coupling conditions: a) Boc-Ser(Fmoc-Phe)-OH/DIPCDI/HOBt (1:1:1); b) Boc-Ser(Fmoc-Phe)-OH/PyBOP®/NMM (1:1:1); c) Boc-Ser(Fmoc-Phe)-OH/HBTU/NMM (1:1:1). Peak1: desired product; peak 2: H-Tyr-Phe-Leu-OH.

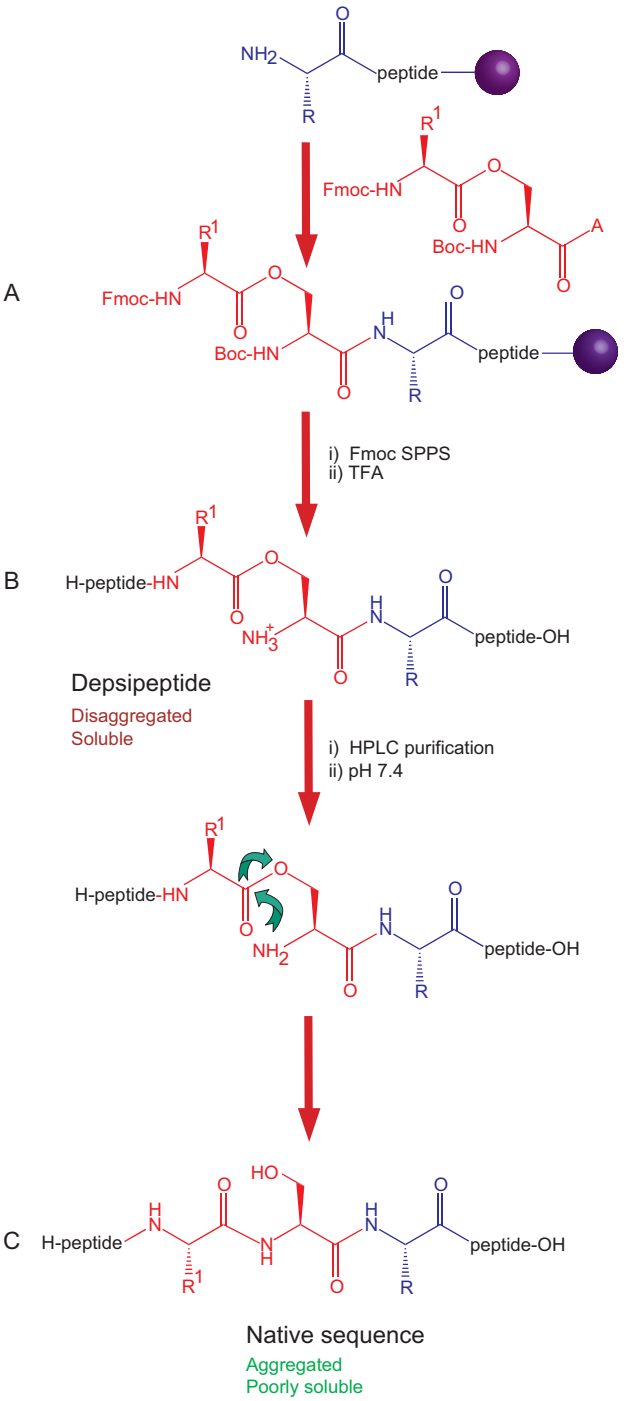

Fig.1: Use of isoacyl dipeptides in Fmoc SPPS.

Method 1: O to N migration

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

Method 2: Coupling of isoacyl dipeptides

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

[a]relative to resin loading

Comparison of pseudoproline and isoacyl dipeptides

Peptides **1**, **2** and **3** (Table 1) were prepared as shown in Table 2 with standard amino acid building blocks and with pseudoproline and isoacyl dipeptides substitutions at the positions marked in Table 1. Isoacyl dipeptides were all coupled using the optimized conditions previously described. Peptide **1** was selected because we have extensive experience in the synthesis of this difficult sequence under a wide range of conditions. Peptides **2** & **3** were identified by Sampson, *et al.* [10] as difficult peptides that could only be made satisfactorily using pseudoprolines.

Table 1: Peptide sequences prepared in this study. Locations where pseudoproline or isoacyl dipeptides were used are indicated in red.

	Sequence
1	H-Val-Thr-Arg-Tyr-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-OH
2	H-Met-Glu-Asp-Ser-Thr-Tyr-Lys-Ala-Ser-Lys-Gly-Cys-NH_2
3	H-Pro-Lys-Tyr-Leu-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr-Gly-Met-Arg-Asn-Val-Pro-Glu-Lys-Gln-Thr-Thr-OH

The products were identified and characterized by LC-ESMS. In every case, the use of either pseudoproline or isoacyl dipeptides resulted in remarkable enhancements in product purities compared to the use of standard Fmoc-amino acid derivatives (Figures 3 - 5). Interestingly, all of the products obtained using either pseudoproline dipeptides or isoacyl dipeptides appeared to be of almost identical purity and composition.

*Table 2: General reaction conditions used to prepare peptides **1** - **3**.*

	Conditions
Resin	Peptide **1**: Fmoc-Gln(Trt)-NovaSyn® TGA Peptide **2**: Sieber amide resin Peptide **3**: Fmoc-Thr(tBu)-NovaSyn® TGA
Instrument	PTi Symphony Peptide Synthesizer
Coupling	Fmoc-Aaa-OH/PyBOP®/NMM (1:1:2), 30 min Fmoc-Aaa-Thr/Ser($\Psi^{Me,Me}$pro)-OH/PyBOP®/NMM (1:1:2), 60 min Boc-Ser/Thr(Fmoc-Aaa)-OH/DIPCDI/HOBt (1:1:1), 60 min
Deblock	20% Piperidine in DMF (2 x 3 min)
Cleavage	Peptide **1**: TFA/water/TIS (95:2.5:2.5) for 3 h Peptides **2** & **3**: TFA/water/TIS/EDT (92.5:2.5:2.5:2.5) for 3 h

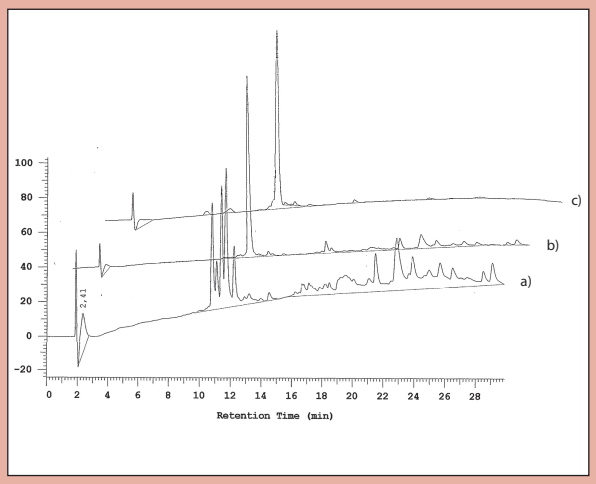

*Fig. 3: HPLC profiles of crude peptide **1** prepared a) with standard Fmoc-amino acid derivatives; b) pseudoproline dipeptide; c) isoacyl dipeptide.*

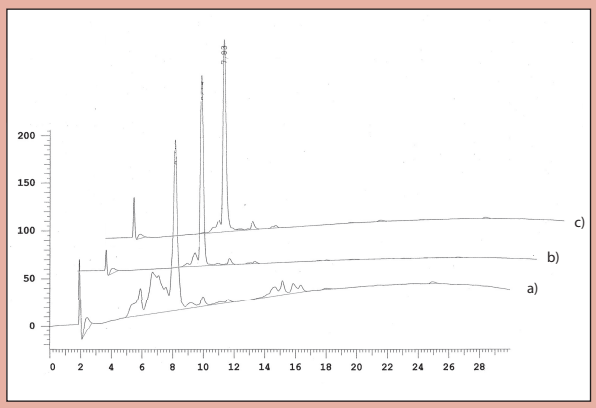

*Fig. 4: HPLC profiles of crude peptide **2** prepared a) with standard Fmoc-amino acid derivatives; b) pseudoproline dipeptide; c) isoacyl dipeptide.*

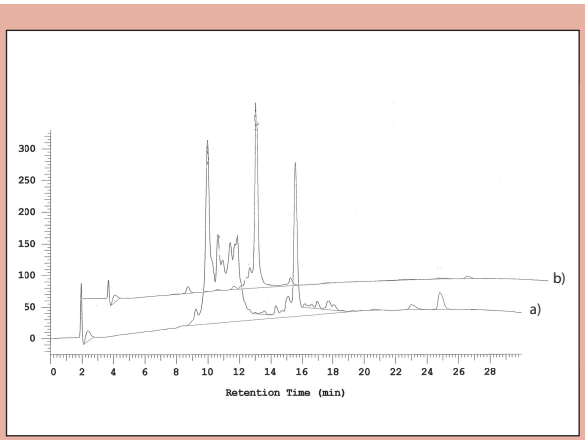

*Fig. 5: HPLC profiles of crude peptide **3** prepared a) with standard Fmoc-amino acid derivatives; b) isoacyl dipeptide.*

Ordering information

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

Other products used in this Innovation

05-20-1000	Fmoc-Ala-Ser($\Psi^{Me,Me}$pro)-OH	1 g
		5 g
05-20-1005	Fmoc-Ala-Thr($\Psi^{Me,Me}$pro)-OH	1 g
		5 g
05-20-1121	Fmoc-Phe-Ser($\Psi^{Me,Me}$pro)-OH	1 g
		5 g
01-64-0059	Sieber Amide resin	1 g
		5 g
		25 g
04-12-2674	Fmoc-Gln(Trt)-NovaSyn® TGA	1 g
		5 g
04-12-2668	Fmoc-Thr(tBu)-NovaSyn® TGA	1 g
		5 g

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