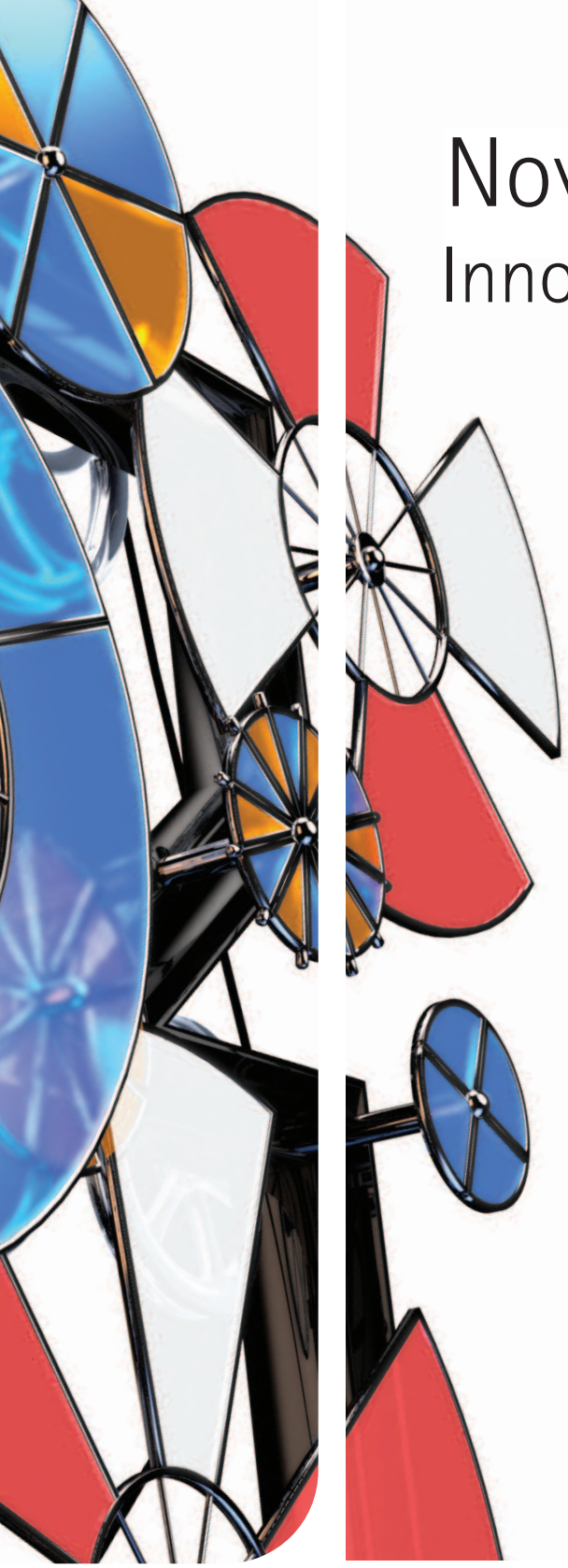

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Synthesis of FBP28WW domains using pseudoproline and isoacyl dipeptides

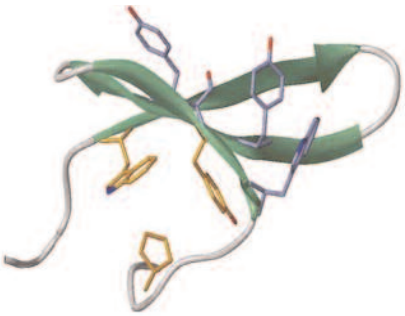

Fig. 1: 3D Structure of FBP28WW domains [1].

WW domains are the smallest three-stranded β-sheet domains known to date. They are named after the two conserved tryptophan residues and serve as non-catalytical domains of signaling proteins. The FBP28WW domain is reported to be stable and monomeric, making it an ideal model system for studying β-sheet stability and folding. The synthesis of such β-sheet forming peptides is notoriously difficult, as they have a high propensity to aggregate on the solid phase during peptide assembly, which leads to incomplete acylation and Fmoc deprotection reactions with the consequent negative effects on the overall product yield and purity.

In this innovation, we present the results from the group of Dr. Beyermann in the synthesis of the Asn^{15} analog of FBP28WW. Unsurprisingly, initial attempts to prepare N(15)-FBP28WW using standard Fmoc amino acid building blocks failed. Fortunately, they were able to prepare this difficult peptide easily using either isoacyl or pseudoproline dipeptides in comparable purities and yields. This work is reproduced here by kind permission of Dr. Beyermann and has been reported elsewhere [2].

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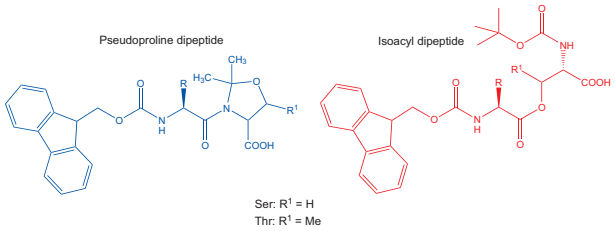
Pseudoproline and isoacyl dipeptides



Pseudoproline [3] and isoacyl dipeptides [4] are powerful tools for expediting the synthesis of difficult peptides. Both pseudoproline and isoacyl dipeptides consist of dipeptides containing C-terminal Ser or Thr residues. In the case of a pseudoproline dipeptide, the Ser or Thr has been reversibly protected as a TFA-cleavable oxazolidine, whereas in the case of isoacyl dipeptides the two amino acids are linked by an ester bond formed with the β-hydroxyl of the Ser or Thr residue. When coupled into a peptide chain in place of Aaa-Ser or Aaa-Thr, these derivatives temporarily introduce a kink into the peptide chain that helps prevent the formation of those secondary structures responsible for aggregation (Figure 2). 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. Novabiochem® Innovations

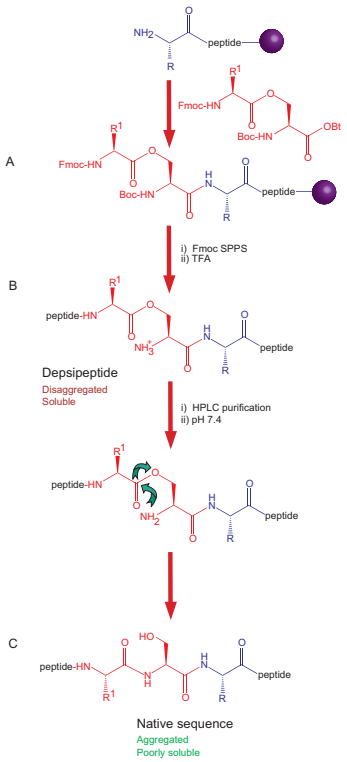


Fig. 2: Use of isoacyl dipeptides in Fmoc SPPS.

1/07 [5] contain guidelines for selecting the optimum locations of pseudoproline dipeptides. The same principles can be applied to isoacyl dipeptides.

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 in 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 [6 - 11]. 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 2).

Synthesis of FBP28WW

Peptide synthesis was carried out manually in fritted plastic syringes as summarized in Table 1. Full synthetic details are given in reference [2].

Assembly of N(15)-FBP28WW was carried out three times. In the first attempt, standard amino acid building blocks were used. Analysis of the product by HPLC indicated the peptide to be highly heterogeneous (Figure 4). The second synthesis was carried out in an identical manner except those residues highlighted in blue in Figure 3 were introduced using pseudoproline dipeptides. For the dipeptide Fmoc-Lys(Boc)-Thr($\Psi^{Me,Me}$pro)-OH, it was found necessary to use HATU activation. This preparation gave a crude product of excellent quality (Figure 5), which after purification was isolated in 25% yield. In the final synthesis, isoacyl dipeptides were incorporated at the sites highlighted in red. These were generated by on-resin *O*-acylation of Boc-Ser or Boc-Thr with the appropriate Fmoc-amino acid, as the corresponding pre-formed isoacyl dipeptides were not yet available from Novabiochem. For this particulary sequence, it was found necessary to use *N*-Bsmoc protection for residues Ala^{4}, Tyr^{11} and Gly^{16} in order to prevent diketopiperazine formation. Cleavage of this material from the resin gave a product in excellent quality containing 4 depsipeptide bonds (Figure 6). Dissolution of this material in 0.1 M $NaHCO_3$ led to *O*- to *N*-migration and generation of native N(15)-FBP28WW.

Table1: Conditions used for SPPS of N(15)-FBP28WW.

	Conditions
Resin	NovaSyn TGR resin
Coupling	Fmoc-Aaa-OH/HBTU/DIPEA (6:6:12), 2 x 30 min
Deblock	20% Piperidine in DMF (1 min. 10 min)
Cleavage	TFA/water/TIS/phenol (85:5:5:5) for 3 h

Fig. 3: Primary sequence of N(15)-FBP28WW. Sites of pseudoproline substitution are marked in blue. Sites of isoacyl dipeptide substitution are marked in red.

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

H-Gly-Ala-Thr-Ala^4-Val-Ser-Glu-Trp-Thr-Glu-Tyr^{11}-Lys-Thr-Ala-Asn-Gly^{16}-Lys-Thr-Tyr-Tyr-Tyr-Asn-Asn-Arg-Thr-Leu-Glu-Ser-Thr- Trp-Glu-Lys-Pro-Gln-Glu-Leu-Lys-NH_2

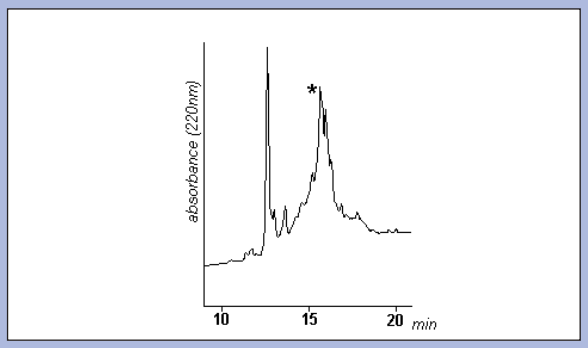


Fig. 4: HPLC profile of N(15)-FBP28WW prepared using standard amino acid building blocks.

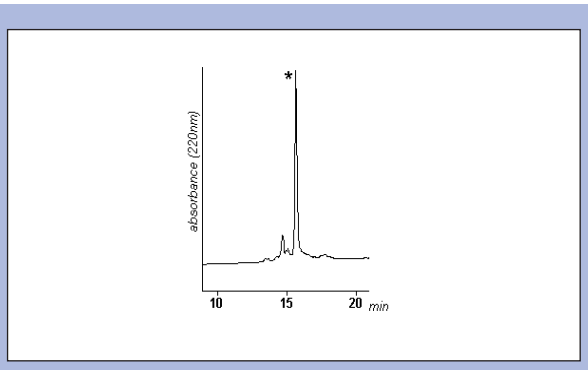


Fig. 5: HPLC profile of crude N(15)-FBP28WW prepared using three pseudoproline dipeptides.

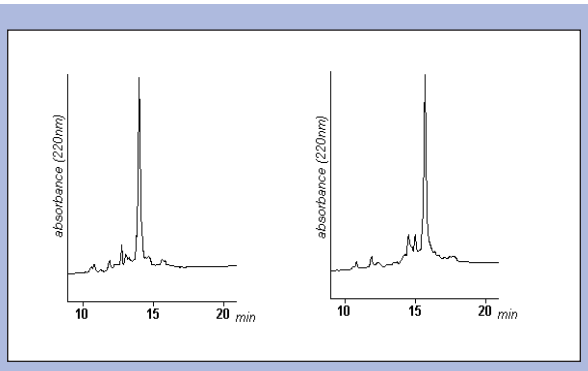


Fig. 6: HPLC profile of crude a) N(15)-FBP28WW depsipeptide and b) N(15)-FBP28WW made using the isoacyl approach.

Conclusions

Peudoproline and isoacyl dipeptides are extremely effective tools for improving yields and purities of difficult and aggregated sequences. For routine applications, pseudoproline dipeptides appear to be the reagents of choice since sequence diketopiperazine formation can occur during Fmoc removal of the second amino acid following introduction of the isoacyl dipeptide unit. Nevertheless, the opportunity provided by the isoacyl dipeptide method to purify insoluble aggregated sequences as a depsipeptide, prior to conversion to the native form, will make this approach an extremely valuable strategy for the synthesis of insoluble and amyloidogenic sequences.

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Ordering information

05-20-0022	Boc-Ser(Fmoc-Asn(Trt)-OH		1 g
NEW		€	5 g
05-20-0023	Boc-Ser(Fmoc-Gln(Trt))-OH	€	1 g
NEW		€	5 g
05-20-0019	Boc-Thr(Fmoc-Ile)-OH	€	1 g
NEW		€	5 g
05-20-0018	Boc-Thr(Fmoc-Val)-OH	€	1 g
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05-20-0015	Boc-Ser(Fmoc-Ala)-OH	€	1 g
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05-20-0010	Boc-Ser(Fmoc-Phe)-OH	€	1 g
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05-20-0013	Boc-Ser(Fmoc-Ser(tBu))-OH	€	1 g
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05-20-0014	Boc-Ser(Fmoc-Thr(tBu))-OH	€	1 g
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05-20-1000	Fmoc-Ala-Ser($\Psi^{Me,Me}$pro)-OH	€	1 g
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