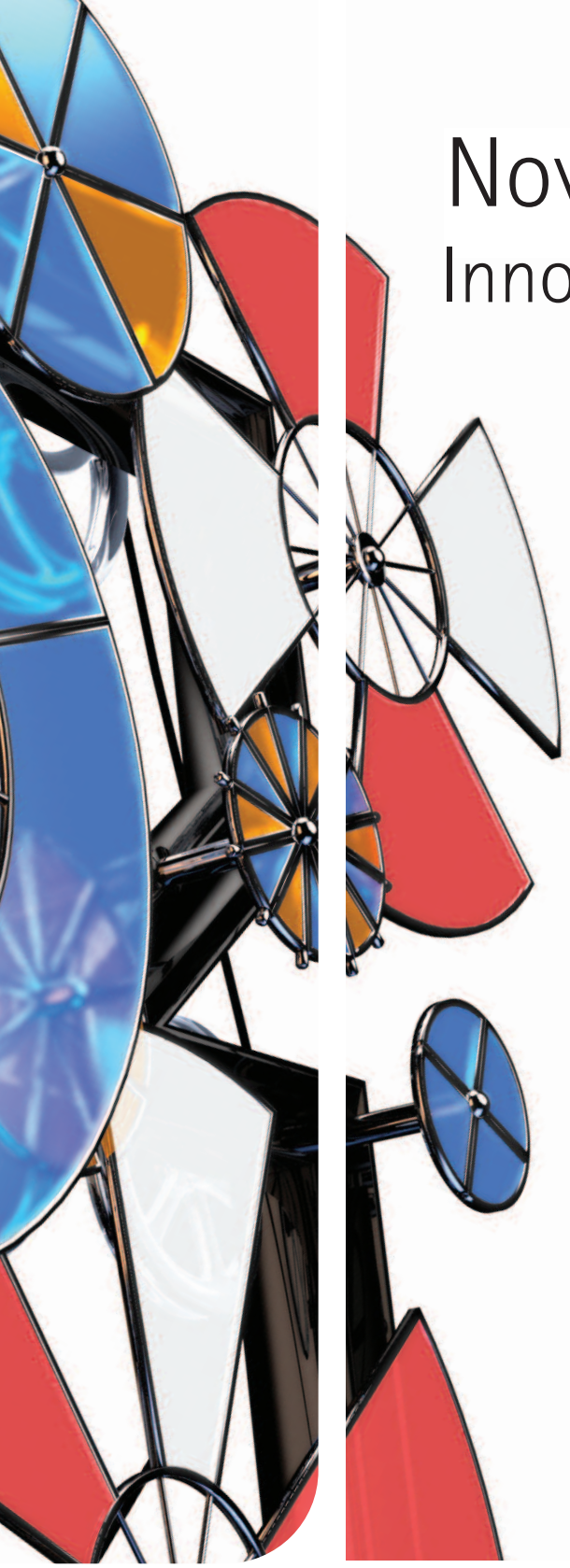

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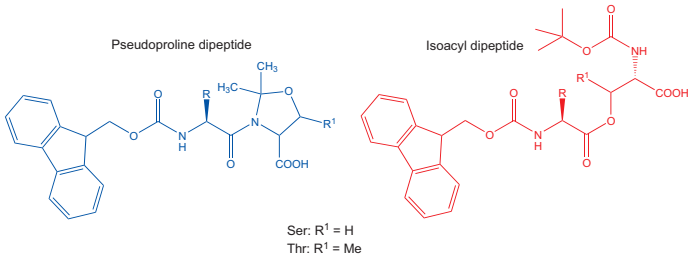
Synthesis of β-amyloid (1-42) using an isoacyl dipeptide



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.

β-Amyloid (1-42) is a notoriously difficult peptide to synthesize. Not only does it undergo serious aggregation on the solid phase during assembly, but it also aggregates in solution, making analysis and purification of the peptide extremely difficult. Kiso [3], Carpino [4], and Mutter [5] have independently described the synthesis of β-amyloid (1-42) using an isoacyl dipeptide at residues Gly-Ser. This measure allowed isoacyl-β-amyloid to be prepared in moderate purity without obvious problems with aggregation during chain assembly. This material exhibited good solubility in aqueous buffer which enabled the product to be purified efficiently by HPLC. The desired product was obtained by allowing the isoacyl peptide to rearrange to the native form in neutral to slightly basic buffer. In this innovation, we demonstrate the efficacy of this approach by the successful preparation of amyloid (1-42).

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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 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 been shown to cause β-elimination of the Fmoc-amino acid from the serine or threonine side chain [9, 10]. 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 (Figure 2) [9, 10].

Method 1: 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 min.
3. Add solution to peptidyl resin.

[a]relative to resin loading

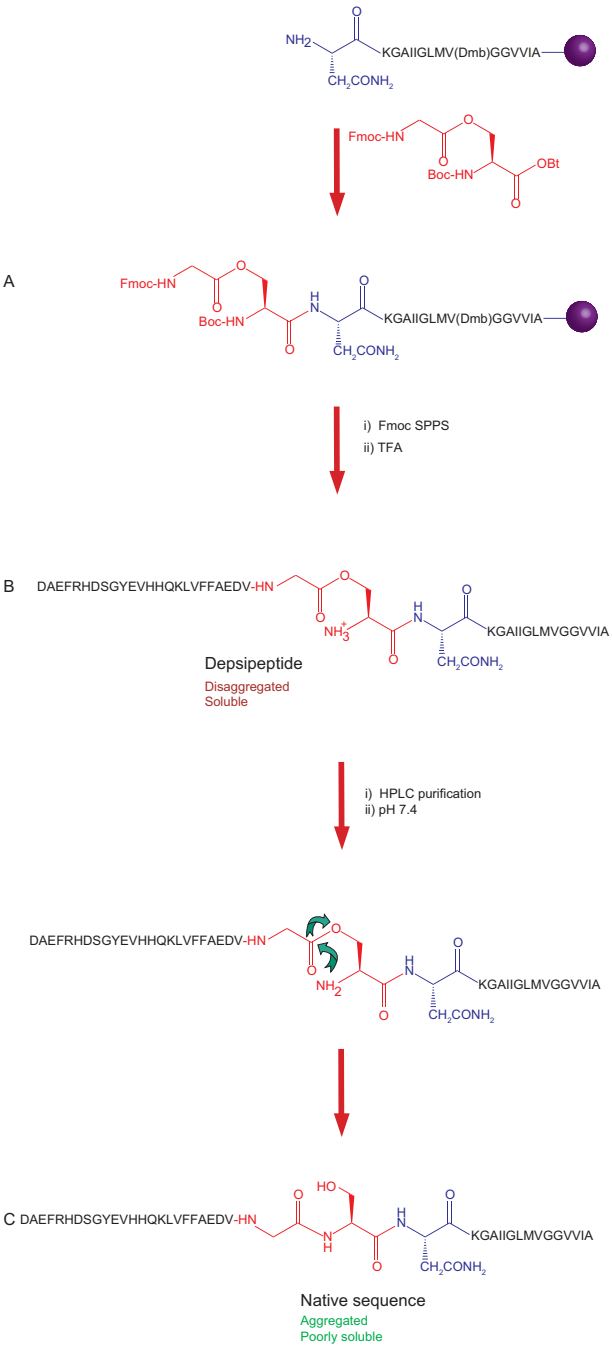

Fig.1: Use of isoacyl dipeptides in Fmoc SPPS.

Peptide assembly of β-amyloid (1-42)

The approach taken for the synthesis of β-amyloid (1-42) is shown in Figure 1. 26-O-Isoacyl-β-amyloid (1-42) was assembled on an ABi 433A peptide synthesizer using modified FastFmoc cycles (Table 1). HCTU was used instead of using HBTU/HOBt as the activator and all Fmoc deprotection reactions were performed 3 times by treatment with 20% piperidine in NMP for 3 minutes. Val-36 and Gly-37 were introduced at the same time using the Dmb dipeptide Fmoc-Val-(Dmb)Gly-OH. This measure was introduced to help effect assembly of the hydrophobic C-terminal region of this peptide. The isoacyl dipeptide Boc-Ser(Fmoc-Gly)-OH was activated using HOBt/DIPCDI in DCM/DMF (3:1) and used to concurrently introduce Gly-25 and Ser-26. The solution of activated derivative was placed in a cartridge and manually transferred to the reaction vessel. A standard 1 hour coupling time was used throughout the synthesis. The peptide was cleaved from the resin and side-chain deprotected by treatment with TFA/water/TIS (95:2.5:2.5) for 3 hours, and the product was isolated by precipitation into cold ether.

Table1: Conditions used for SPPS of 26-O-isoacyl-β-amyloid (1-42).

	Conditions
Resin	Fmoc-Asn(Trt)-Wang resin
Coupling	Fmoc-Aaa-OH/HCTU/DIPEA (10:10:20), 60 min
Deblock	20% Piperidine in NMP (3 x 3 min)
Cleavage	TFA/water/TIS (95:2.5:2.5) for 3 h

The crude peptide was dissolved in formic acid and diluted with water to a 5% formic acid concentration. A sample of this solution was analyzed by HPLC (Figure 2) using a Supelco 300 Å 2.4 mm column. On wider bore columns, the peptide exhibited notable peak broadening, indicating the 26-O-isoacyl peptide retained some potential to aggregate. A sample of this material was purified on the Supelco column and gave the HPLC profile and MS spectrum shown in Figures 3 and 4, respectively. The purified peptide was converted to native β-amlyoid by dissolution in 0.1 M ammonium bicarbonate. The rearrangement appears to be complete after 1 h. The HPLC profile of a co-injection of 26-O-isoacyl-β-amyloid and β-amyloid is shown in Figure 5.

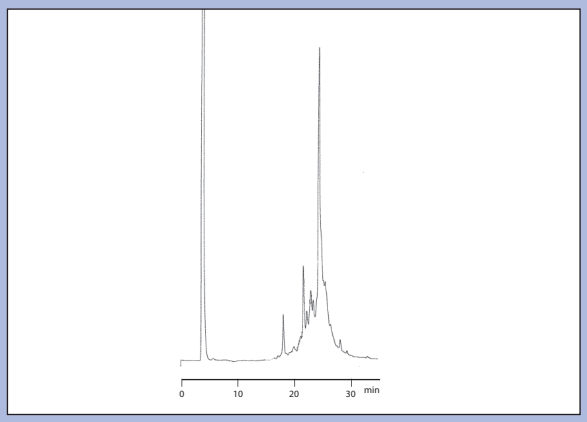

Fig. 2: HPLC profile of crude 26-O-isoacyl-β-amyloid. Column: Supelco 300 Å 2.4 mm. Buffer A: 0.1% TFA aq. Buffer B: MeCN/water/TFA (70:30:0.1). Gradient: 30% B for 5 min then 30 - 100% in 35 min. Flow rate: 0.2 ml/min.

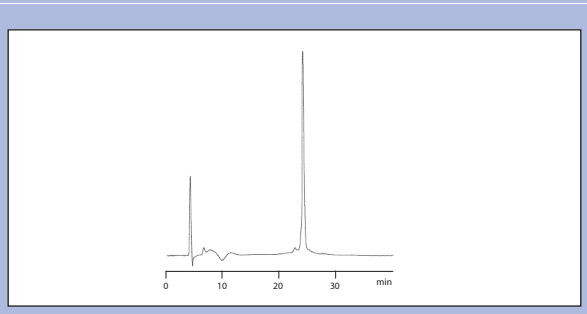

Fig. 3: HPLC profiles of purified 26-O-isoacyl-β-amyloid. HPLC conditions as Figure 2.

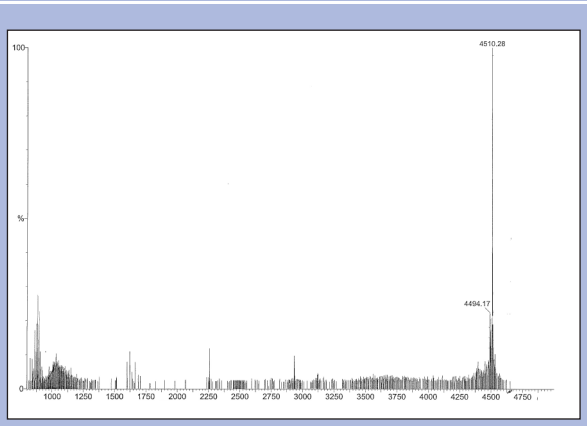

Fig. 4: MALDI-TOF spectrum of purified 26-O-isoacyl-β-amyloid.

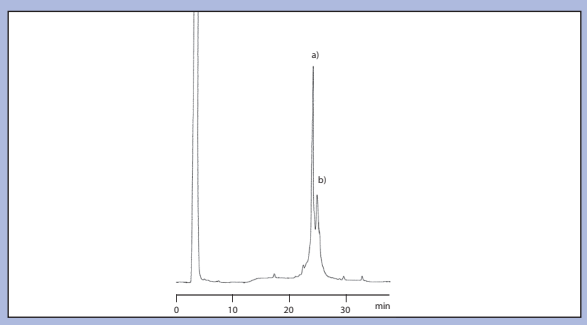

Fig. 5: HPLC profile of a co-injection of a) 26-O-isoacyl-β-amyloid and b) β-amyloid. HPLC conditions as Figure 2.

Ordering information

05-20-0016	Boc-Ser(Fmoc-Arg(Pbf)-OH	1 g
NEW		5 g
05-20-0017	Boc-Ser(Fmoc-Ile)-OH	1 g
NEW		5 g
05-20-0015	Boc-Ser(Fmoc-Ala)-OH	1 g
		5 g
05-20-0009	Boc-Ser(Fmoc-Gly)-OH	1 g
		5 g
05-20-0010	Boc-Ser(Fmoc-Phe)-OH	1 g
		5 g
05-20-0013	Boc-Ser(Fmoc-Ser(tBu))-OH	1 g
		5 g
05-20-0014	Boc-Ser(Fmoc-Thr(tBu))-OH	1 g
		5 g
05-20-0011	Boc-Thr(Fmoc-Ala)-OH	1 g
		5 g
05-20-0012	Boc-Thr(Fmoc-Gly)-OH	1 g
		5 g

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