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Design and synthesis of a novel water-soluble Aβ1-42 isopeptide: an efficient strategy for the preparation of Alzheimer's disease-related peptide, Aβ1-42, via *O–N* intramolecular acyl migration reaction

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Abstract—A novel water-soluble isopeptide of Alzheimer's disease-related peptide Aβ1-42, '26-*O*-acyl isoAβ1-42', which could efficiently convert to intact Aβ1-42 under physiological conditions via *O–N* intramolecular acyl migration, was synthesized providing a new system useful for investigation of biological function of Aβ1-42.

Amyloid β peptides (Aβ) are the main proteinaceous component of the amyloid plaques found in the brains of Alzheimer's disease (AD) patients.[1] Neuritic plaques, pathognomonic features of AD, contain abundant fibrils formed from Aβ, which have been found to be neurotoxic in vivo and in vitro.[2] The predominant forms of Aβ consist of mainly 40- and 42-residue peptides (designated Aβ1-40 and Aβ1-42, respectively), which are proteolytically produced from amyloid precursor protein (APP) by enzymatic reactions.[3] Since Aβ1-42 is suggested to play a more critical role in amyloid formation and the pathogenesis of AD than Aβ1-40, many studies using synthetic Aβ1-42 have been carried out to clarify the involvement of Aβ1-42 in AD.[4]

However, Aβ1-42 is a highly hydrophobic peptide and forms aggregates in various media. This aggregation is attributed to its intermolecular hydrophobic interaction and hydrogen bond formation among peptide chains, leading to the formation of extended β-sheet structures.[5] Hence, the highly agglutinative potency of Aβ1-42 results in the synthetic difficulty of this peptide,[5] so-called 'difficult sequence'-containing peptide.[6] Due to the low solubility and broad elution of Aβ1-42 under acidic or neutral conditions, the conventional HPLC purification of synthesized Aβ1-42 in the aqueous TFA–acetonitrile system is too laborious to remove impurities accumulated during the solid-phase peptide synthesis (SPPS). Furthermore, the biological experiments using Aβ1-42 are problematic because of the large extent of aggregation in a standard storage solution such as dimethylsulfoxide (DMSO).[7] Therefore, the 'in situ' system that can prepare intact Aβ1-42 in solubilized form under physiological conditions would be a powerful tool in understanding the inherent pathological function of Aβ1-42. To create such system, (1) a novel propeptide possessing a high solubility during HPLC purification and long-term storage as a solution and (2) a capability of intact Aβ1-42 production under physiological conditions are required.

O–N intramolecular acyl migration reaction is a well-known reaction observed in Ser/Thr-containing peptides.[8] In our previous study regarding the synthesis of difficult sequence-containing peptides, we disclosed a novel and efficient method based on this migration reaction of synthesized '*O*-acyl isopeptides'. This method remarkably improved the synthetic yields of difficult sequence-containing pentapeptides.[9] The result also indicated that the branched ester structure in *O*-acyl isopeptides could suppress the unfavorable nature seen in the difficult sequence-containing peptides. Namely, the insertion of the ester bond into peptide chain can

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probably disrupt the secondary structure formed by the inherent peptide chain, leading to the improvement of coupling and deblocking efficacy during SPPS. In addition, *O*-acyl isopeptides with the newly formed amino group can possess reasonable H_2O- and MeOH-solubility required in HPLC purification by the formation of salt. Furthermore, from recent research of water-soluble prodrugs[10] and *O*-acyl isopeptides[9] based on *O–N* intramolecular acyl migration, it is established that the purified *O*-acyl isoform can completely be converted to the original *N*-acyl form in a short time with no side reaction at pH 7.4.

Based on this background, we conceived the idea that the *O–N* intramolecular acyl migration of *O*-acyl isopeptides could be applied to the synthesis of Aβ1-42 via a novel water-soluble isopeptide of Aβ1-42, that is, '26-*O*-acyl isoAβ1-42 (26-AIAβ42, **2**)'. This idea would overcome the problems in the synthesis and storage of Aβ1-42 (Fig. 1). Although there are two Ser residues in Aβ1-42 at the positions 8 and 26 with the capability of *O–N* intramolecular acyl migration, we selected the Ser^{26} for the *O*-acylation, since the adjacent Gly^{25} does not epimerize during ester bond formation (Fig. 1).

As depicted in Scheme 1, Fmoc-Ala-O-chlorotrityl resin (0.465 mmol/g, **3**) was employed and Fmoc-protected amino acids were sequentially coupled using the DIPCDI–HOBt method (2 h) after removal of each Fmoc group with 20% piperidine–DMF (20 min) to give peptide resin **4**. After Boc-Ser-OH was introduced to **4**, the obtained **5** was coupled with Fmoc-Gly-OH at the β-hydroxy group of Ser using the DIPCDI–DMAP method in CH_2Cl_2 to obtain ester **6**. 26-AIAβ1-42-resin (**7**) was obtained through the coupling of additional amino acid residues by the conventional manner. Finally, 26-AIAβ1-42 (**2**) was obtained as a major product by the treatment of TFA–*m*-cresol–thioanisole–H_2O (92.5:2.5:2.5:2.5) for 90 min followed by NH_3I–dimethylsulfide for 60 min in TFA–H_2O (2:1).

In HPLC analysis of crude products (Fig. 2A), Aβ1–25 (DAEFRHDSGYEVHHQKLVFFAEDVG) was not observed as a by-product, although a very low rate (1.6%, HPLC yield) of Aβ26-42 (SNKGAIIGLMVGGVVIA) was detected. This indicates that (1) the esterification of the β-hydroxy group of Ser was successfully completed on the solid support and (2) the formed ester bond between Gly and Ser was stable in both piperidine and

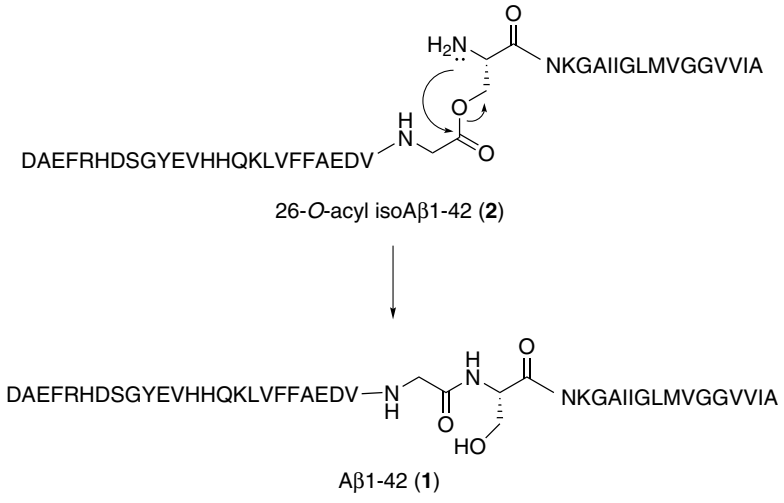
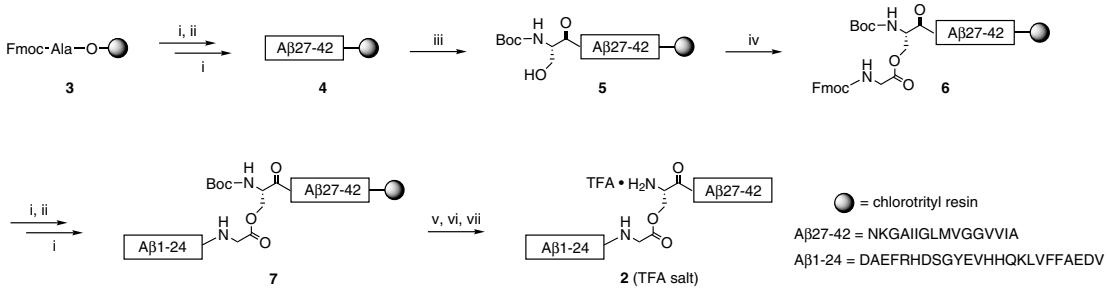


Figure 1. The production of Aβ1-42 (**1**) via the *O–N* intramolecular acyl migration reaction of 26-*O*-acyl isoAβ1-42 (**2**).



Scheme 1. Reagents and conditions: (i) 20% piperidine/DMF, 20 min; (ii) Fmoc-AA-OH (2.5 equiv), DIPCDI (1,3-diisopropylcarbodiimide, 2.5 equiv), HOBt (2.5 equiv), DMF, 2 h; (iii) Boc-Ser-OH (2.5 equiv), DIPCDI (2.5 equiv), HOBt (2.5 equiv), DMF, 2 h; (iv) Fmoc-Gly-OH (3.0 equiv), DIPCDI (3.0 equiv), DMAP (0.2 equiv), CH_2Cl_2, 16 h × 2; (v) TFA–*m*-cresol–thioanisole–H_2O (92.5:2.5:2.5:2.5), 90 min; (vi) NH_3I (20 equiv), dimethylsulfide (20 equiv), TFA–H_2O (2:1), 60 min, 0 °C; (vii) preparative HPLC (a linear gradient of CH_3CN in 0.1% aqueous TFA).

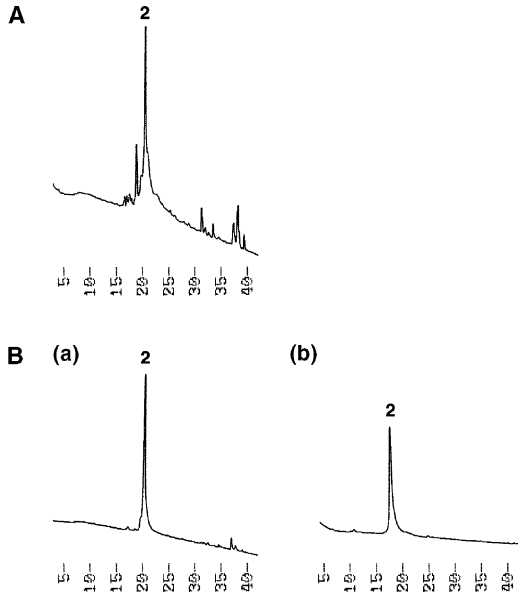


Figure 2. HPLC profiles of (A) crude and (B) purified 26-AIAβ1-42 (**2**). Analytical HPLC was performed using a C18 reverse phase column (4.6×150 mm; YMC Pack ODS AM302) with binary solvent system: a linear gradient of CH_3CN (0–100% CH_3CN, 40 min for A and B-a, 25–55% CH_3CN, 60 min for B-b) in 0.1% aqueous TFA at a flow rate of 0.9 mL min^{-1} (temperature: 40 °C), detected at 230 nm.

TFA. The crude *O*-acyl isopeptide **2** was dissolved in hexafluoroisopropanol, applied to preparative HPLC, and eluted using 0.1% aqueous TFA–CH_3CN. Since **2** was eluted as a narrow single peak, we could easily purified by preparative scale HPLC to give pure **2** (Fig. 2B) as TFA salt with the total isolated yield of 33.6%, calculated from the original loading of chlorotrityl resin.[11] This yield was higher than that obtained in the synthesis of **1** by a standard Fmoc-based SPPS (7.2%). Since **1** was eluted as a broad peak in preparative scale HPLC purification, it was laborious to isolate **1** from impurities as reported.[5] In addition, in the synthesis of **2**, no conversion to **1** was observed.

The water-solubility of **2** (TFA salt) was 15 mg mL^{-1}, which was 100-fold higher than that of Aβ1-42 (**1**, 0.14 mg mL^{-1}). Interestingly, the fact that a slight modification of peptide chain by the insertion of one ester bond could drastically increase the solubility of the insoluble original peptide with 42-residues suggests that *O*-acyl isopeptide totally break the secondary structures responsible for the insolubility of the original peptide. As we demonstrated that *O*-acyl isopeptide could suppress the unfavorable nature of difficult sequence-containing pentapeptides in the previous study,[9] the present result in the synthesis of **2** indicates that this method is a powerful strategy for increasing the solubility even in large peptides.

As shown in Figure 3, purified **2** was completely converted to Aβ1-42 (**1**) at room temperature in phosphate buffered saline (PBS, pH 7.4) with no side reaction. This migration was rapid with a half-life of 2.6 min, while TFA salt of **2** was stable at 4 °C for at least 30 days as either a solid state or a DMSO solution. Moreover, a slower migration was observed at pH 4.9 (PBS) with a

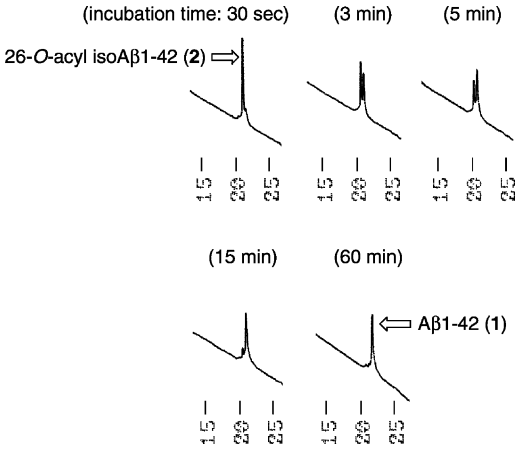


Figure 3. HPLC profiles of the conversion of 26-AIAβ1-42 (**2**) to Aβ1-42 (**1**) via *O–N* intramolecular acyl migration in PBS (pH 7.4, 25 °C). Analytical HPLC was performed using a C18 reverse phase column (4.6×150 mm; YMC Pack ODS AM302) with binary solvent system: a linear gradient of CH_3CN (0–100% CH_3CN for 40 min) in 0.1% aqueous TFA at a flow rate of 0.9 mL min^{-1} (temperature: 40 °C), detected at 230 nm.

half-life of 3 h, and no migration at pH 3.5 (acetate buffer) after incubation for 3 h. This rapid migration under physiological conditions enables to produce intact monomer Aβ1-42 in situ to investigate inherent biological function of Aβ1-42 in AD. The conversion of **2** (TFA salt) in water for 48 h at room temperature followed by lyophilization yielded Aβ1-42 (**1**) quantitatively as TFA salt with the purity of >95%.[12]

In conclusion, we synthesized a novel water-soluble isopeptide of Aβ1-42, (26-AIAβ42, **2**), which is a 26-*O*-acyl isopeptide of Aβ1-42. This *O*-acyl isopeptide has a higher water-solubility than that of Aβ1-42 (**1**), and can migrate to intact Aβ1-42 (**1**) under various conditions while it is stable under storage conditions. This suggests that the synthesis of Aβ1-42 via 26-AIAβ42 could overcome the solubility problem and give a novel tool for the biological evaluation system, in which 26-AIAβ42 can be stored in a solubilized state before the use and rapidly produce the intact Aβ1-42 in situ during biological experiments.

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11. Spectral data for **2**: MALDI-MS (TOF): M_{calc}: 4514.04; $M + H_{found}$: 4515.26; HPLC analysis at 230 nm: purity was >96%.
12. Spectral data for **1**: MALDI-MS (TOF): M_{calc}: 4514.04; $M + H_{found}$: 4515.48; HPLC analysis at 230 nm: purity was >95%; the retention time on HPLC (0–100% CH_3CN for 40 min, 230 nm) of synthesized **1** was identical to that of commercially available Aβ1-42 (Peptide Institute, Inc., Osaka, Japan).