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'Click peptide': a novel '*O*-acyl isopeptide method' for peptide synthesis and chemical biology-oriented synthesis of amyloid β peptide analogues

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Abstract: After over a decade of studies on aspartic protease inhibitors and water-soluble prodrugs, we have been developing a novel method, since 2003, called '*O*-acyl isopeptide method', for the synthesis of peptides containing difficult sequences. With our recent discoveries of '*O*-acyl isodipeptide unit' and the 'racemization-free segment condensation method', this method has further evolved as a general synthetic method for peptides. Moreover, 'Click Peptide', which could be a powerful tool for identifying the pathological functions of amyloid β peptides in Alzheimer's disease, represents a valuable use of the isopeptide method in Chemical Biology-oriented research. Copyright © 2006 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: *O*-acyl isopeptide method; Alzheimer's disease; amyloid β peptide; click peptide; difficult sequence

INTRODUCTION

Since 2003, we have been developing a novel method, called '*O*-acyl isopeptide method', for the synthesis of peptides containing difficult sequences in which a native amide bond at a hydroxyamino acid residue, such as Ser being isomerized to an ester bond, is followed by an *O*–*N* intramolecular acyl migration reaction [1–10]. Recently, the '*O*-acyl isopeptide method' began to be widely utilized by several other groups [11–15]. In chemical biology-oriented research, we developed a novel 'Click Peptide' based on the *O*-acyl isopeptide method to study the inherent biological functions of native peptides or proteins (Figure 1) [3–7,9].

O-ACYL ISOPEPTIDE METHOD

Several years ago, when we tried to synthesize some peptide derivatives, including phenylnorstatine, for the study of aspartic protease inhibitors [16–18], some of the synthesized compounds could not be purified in preparative scale HPLC owing to their extremely low solubility in various solvents (Figure 2(A)). Thus, these peptide derivatives were considered to be the so-called 'difficult sequence'-containing peptides [19,20].

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On the other hand, for over a decade, we studied the pH-dependent '*O*–*N* intramolecular acyl migration [21,22]'-type water-soluble prodrugs of the peptide mimetic HIV-1 protease inhibitors [23,24]. These prodrugs, which are *O*-acyl isoforms of parent drugs possessing α-hydroxy-β-amino acids, had higher water solubility because of a newly formed and ionized amino group. Moreover, migration to the *N*-acyl parent drugs could be transacted with no side reaction under physiological conditions.

As a consequence, in 2003, we considered that the hydrophilic '*O*-acyl isopeptides' derived from the phenylnorstatine-containing peptide derivatives would overcome the solubility problem in HPLC purification (Figure 2(B)). However, we had a surprising discovery in this research, which showed that not only did the '*O*-acyl isopeptide' possess a higher solubility in various media, but also that the coupling and deprotection efficacy during solid-phase peptide synthesis (SPPS) was improved by modifying the nature of the difficult sequence [1,2]; namely, the isomerization of the peptide backbone from the *N*-acyl to *O*-acyl isopeptide structure, i.e. formation of one single ester bond, significantly changed the unfavorable secondary structure of the native peptides. Thus, this finding led to the development of the '*O*-acyl isopeptide method' as a novel synthetic method in the field of Peptide Chemistry.

We also designed an '*O*-acyl isodipeptide unit', e.g. Boc–Ser/Thr(Fmoc–Xaa)–OH. The use of *O*-acyl isodipeptide units, in which the racemization-inducing

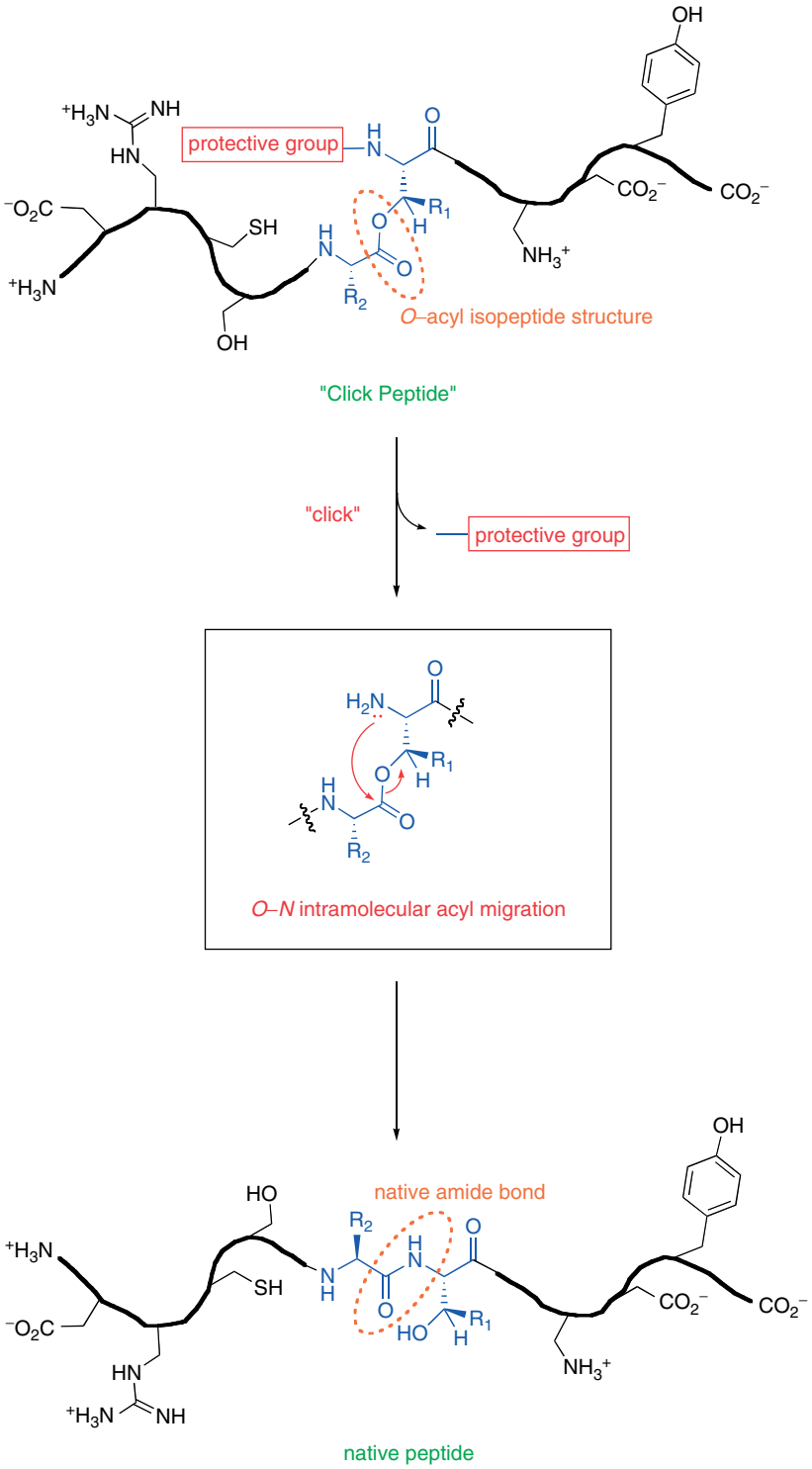

Figure 1 'Click Peptide' based on the '*O*-acyl isopeptide method'.

esterification reaction on the resin could be omitted, allows the application of the '*O*-acyl isopeptide method' to fully automated protocols for the synthesis of long peptides or proteins (Figure 3) [8]. Additionally, very recently, we developed a novel 'racemization-free segment condensation' based on the '*O*-acyl isopeptide method' [10].

The Application of *O*-Acyl Isopeptide Method

Moreover, we have successfully applied the '*O*-acyl isopeptide method' to the chemical biology-oriented synthesis of the Alzheimer's disease (AD)-related amyloid β peptide (Aβ) 1–42 analogues, leading to the development of pH- or photo-triggered 'Click Peptide'

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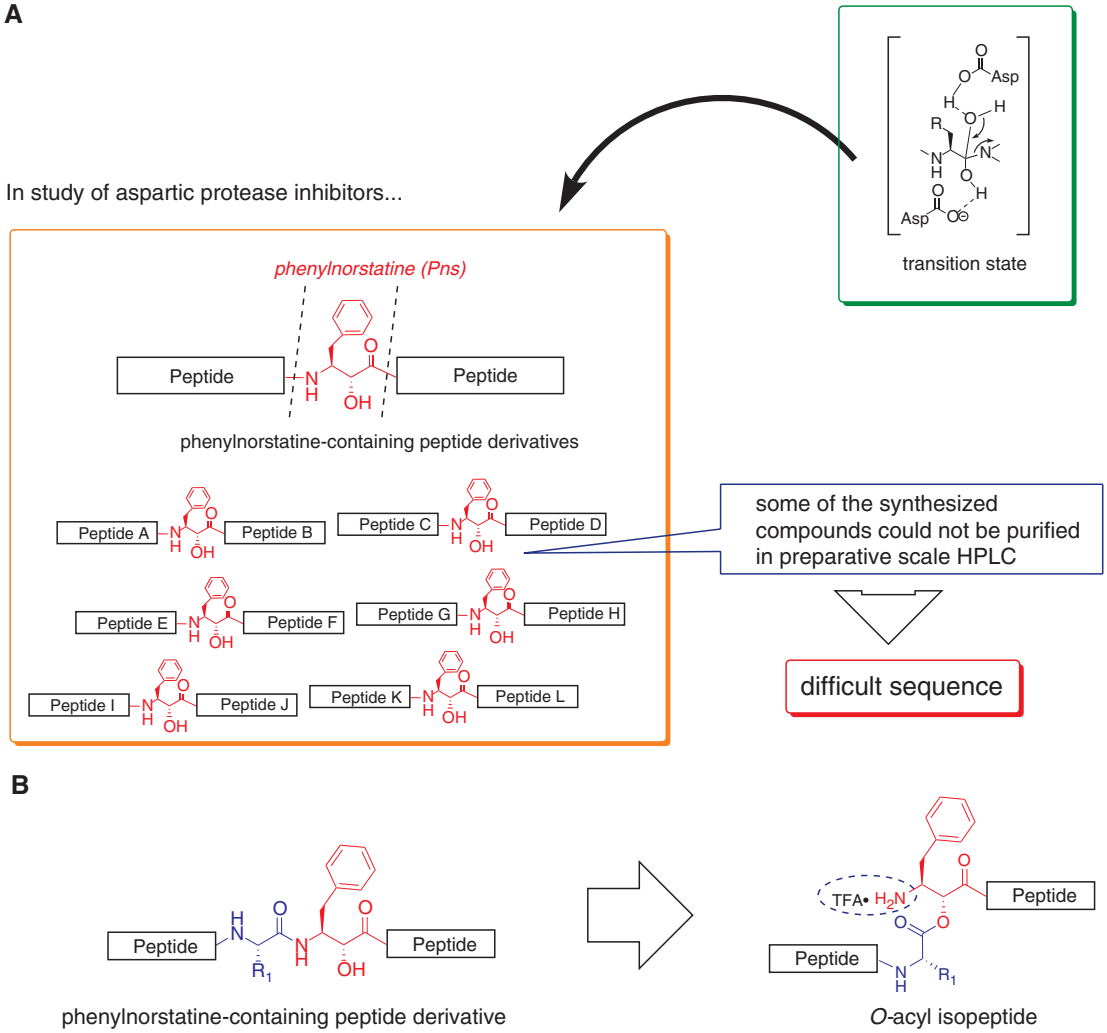

Figure 2 (A) 'Difficult sequence' in our studies of aspartic protease inhibitors, (B) design of *O*-acyl isopeptide derived from phenylnorstatine-containing peptide derivative.

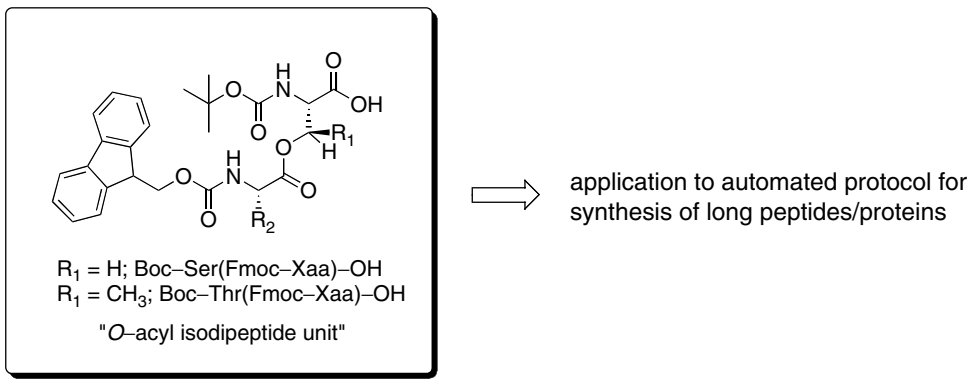

Figure 3 General structure of *O*-acyl isodipeptide unit: application of the '*O*-acyl isopeptide method' to fully automated protocol.

(Figure 1) [3–7,9]. The 'Click Peptide' did not exhibit the self-assembling nature under physiological conditions because of one single ester, and could migrate to the original Aβ1–42 with a quick and easy one-way conversion reaction (so-called 'click') via the *O*–*N* intramolecular acyl migration. A clear understanding of the pathological mechanism of Aβ1–42, a currently unexplained process, would be of great significance in the discovery of novel drug targets against AD [25–28]. Currently, the difficulties in handling Aβ1–42, because

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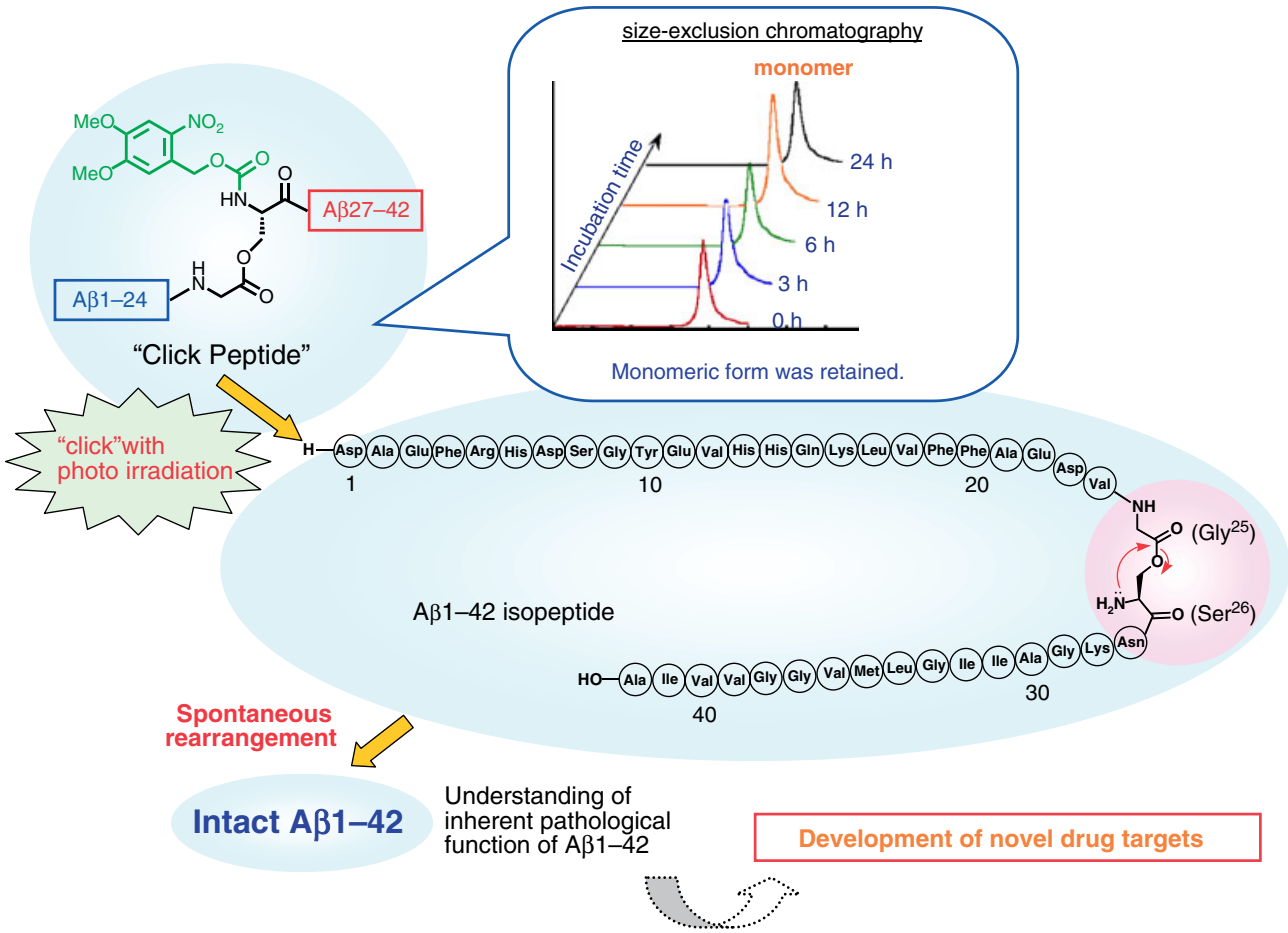

Figure 4 Photo-triggered click peptide. The production of Aβ1–42 by photo-triggered click followed by *O–N* intramolecular acyl migration reaction of Aβ1–42 isopeptide.

of its highly aggregative nature, hamper the progress of Aβ1–42-related AD research [29–32]. The 'Click Peptide' method would open the doors for investigation of the biological functions of Aβ1–42 in AD by inducible activation of Aβ1–42 self-assembly (Figure 4).

Interestingly, shortly after we disclosed the '*O*-acyl isopeptide method' [1,2], several other groups also confirmed the efficacy of this method. Carpino *et al.* synthesized the Jung–Redemann 26-residue peptide efficiently by utilizing the '*O*-acyl isopeptide method', whereas this peptide could not be synthesized by standard SPPS [12]. By carefully evaluating the appropriate protecting group, stability of the ester bond during assembly, and occurrence of side reactions, they concluded that the efficacy of the '*O*-acyl isopeptide method' was comparable to that of the pseudoproline method [15]. Börner *et al.* also synthesized the *O*-acyl isopeptide for efficient preparation of poly(ethylene oxide)–peptide conjugates [14]. Moreover, Mutter *et al.* confirmed by circular dichroism (CD)-based analyses that the secondary structure of *O*-acyl isopeptide structure is significantly different from that of the corresponding *N*-acyl native peptides [11,13], which agrees with our hypothesis. These reports indicate that the '*O*-acyl isopeptide method' is widely advantageous for peptide preparation by disrupting the unfavorable secondary structures of the native peptides.

CONCLUSION

Classical *O–N* intramolecular acyl migration was revived by our group as a powerful key reaction in the field of modern medicinal chemistry in the development of water-soluble prodrugs. After more than a decade of prodrug studies, we recently disclosed the '*O*-acyl isopeptide method' as a novel synthetic method in the field of peptide chemistry and its application to chemical biology-oriented synthesis of Aβ analogues, leading to the development of 'Click Peptide' (Figure 5). We hope that the strategy using the '*O*-acyl isopeptide method', in which a simple isomerization to an *O*-acyl isopeptide remarkably and temporarily changes the physicochemical properties of the native peptide and an *O–N* intramolecular acyl migration triggers the native amide bond formation under physiological conditions,

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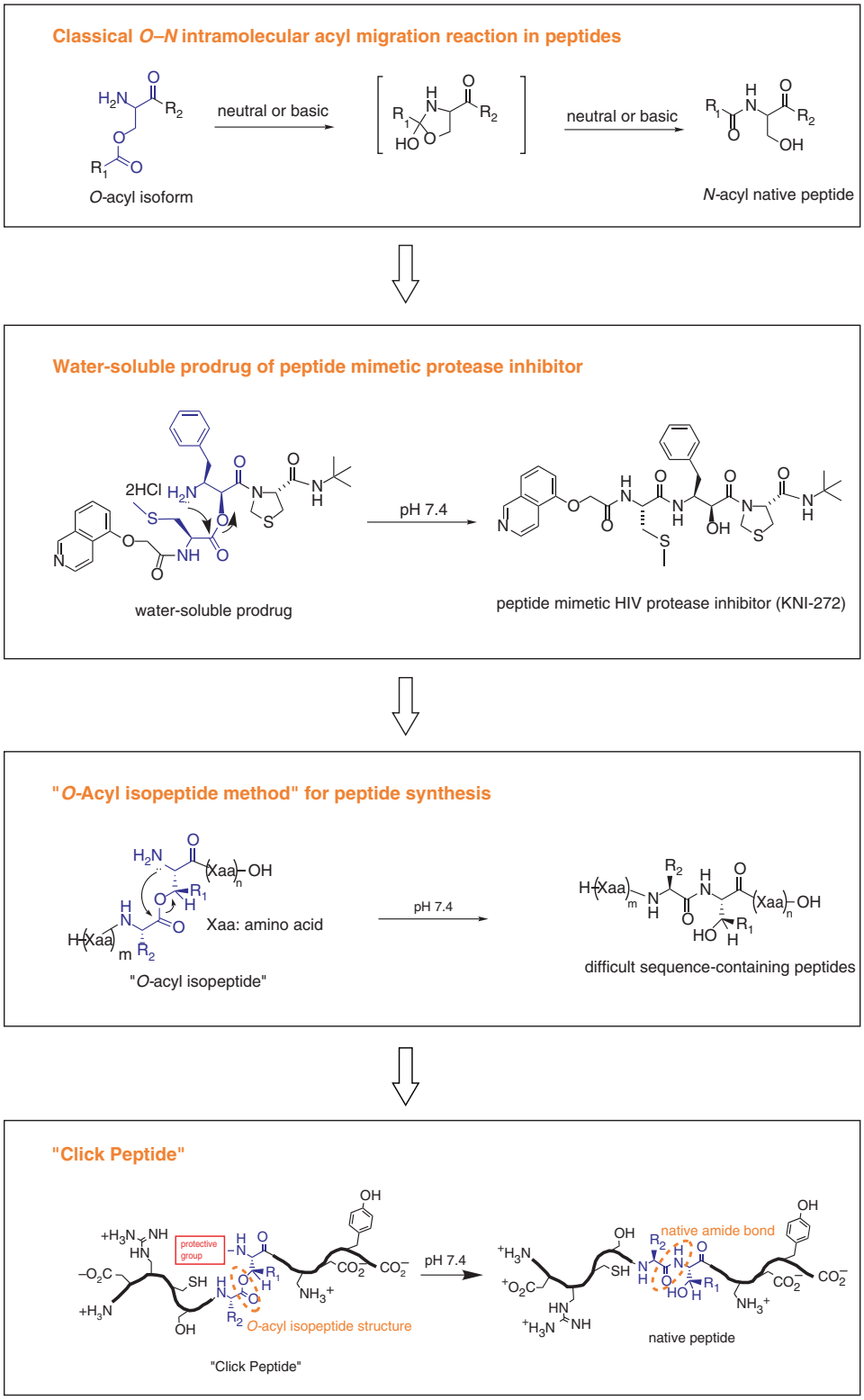

Figure 5 Our workflow on peptide science based on the '*O*–*N* intramolecular acyl migration reaction'.

will further contribute to the study of peptides and proteins. Examples of such studies include the studies of membrane peptides/proteins that are difficult to handle in various conditions because of their high self-assembling characters.

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REFERENCES

1. Sohma Y, Sasaki M, Ziora Z, Takahashi N, Kimura T, Hayashi Y, Kiso Y. A novel approach for the hydrophobic peptides synthesis and purification through *O–N* intramolecular acyl migration reaction. *Peptides, Peptide Revolution: Genomics, Proteomics and Therapeutics*. Kluwer Academic: Netherlands, 2003; 67–68.
2. Sohma Y, Sasaki M, Hayashi Y, Kimura T, Kiso Y. Novel and efficient synthesis of difficult sequence-containing peptides through *O–N* intramolecular acyl migration reaction of *O*-acyl isopeptides. *Chem. Commun.* 2004; 124–125, (First published as an Advanced Article on the web 21st November 2003).
3. Sohma Y, Sasaki M, Hayashi Y, Kimura T, Kiso Y. 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. *Tetrahedron Lett.* 2004; **45**: 5965–5968.
4. Sohma Y, Hayashi Y, Skwarczynski M, Hamada Y, Sasaki M, Kimura T, Kiso Y. O–N intramolecular acyl migration reaction in the development of prodrugs and the synthesis of difficult sequence-containing bioactive peptides. *Biopolymers* 2004; **76**: 344–356.
5. Sohma Y, Hayashi Y, Kimura M, Chiyomori Y, Taniguchi A, Sasaki M, Kimura T, Kiso Y. "*O*-Acyl isopeptide method" for the synthesis of difficult sequence-containing peptides: application in the synthesis of Alzheimer's disease-related amyloid β peptide (Aβ) 1–42. *J. Pept. Sci.* 2005; **11**: 441–451.
6. Sohma Y, Chiyomori Y, Kimura M, Fukao F, Taniguchi A, Hayashi Y, Kimura T, Kiso Y. "*O*-Acyl isopeptide method" for the efficient preparation of amyloid β peptide (Aβ) 1–42 mutants. *Bioorg. Med. Chem.* 2005; **13**: 6167–6174.
7. Taniguchi A, Sohma Y, Kimura M, Okada T, Ikeda K, Hayashi Y, Kimura T, Hirota S, Matsuzaki K, Kiso Y. "Click Peptide" based on the "*O*-acyl isopeptide method": control of Aβ1–42 production from a photo-triggered Aβ1–42 analogue. *J. Am. Chem. Soc.* 2006; **128**: 696–697.
8. Sohma Y, Taniguchi A, Skwarczynski M, Yoshiya T, Fukao F, Kimura T, Hayashi Y, Kiso Y. "*O*-Acyl isopeptide method" for the efficient synthesis of difficult sequence-containing peptides: use of "*O*-acyl isodipeptide unit". *Tetrahedron Lett.* 2006; **47**: 3013–3017.
9. Sohma Y, Kiso Y. "Click Peptide": chemical biology-oriented synthesis of Alzheimer's disease-related amyloid β peptide (Aβ) analogues based on the "*O*-acyl isopeptide method". *Chembiochem* 2006; **7**: 1549–1557.
10. Yoshiya T, Sohma Y, Kimura T, Hayashi Y, Kiso Y. "*O*-Acyl isopeptide method": racemization-free segment condensation in solid phase peptide synthesis". *Tetrahedron Lett.* 2006; **47**: 7905–7909.
11. Mutter M, Chandravarkar A, Boyat C, Lopez J, Santos SD, Mandal B, Mimna R, Murat K, Patiny L, Saucede L, Tuchscherer G. Switch peptides in statu nascendi: induction of conformational transitions relevant to degenerative diseases. *Angew. Chem. Int. Ed. Engl.* 2004; **43**: 4172–4178.
12. Carpino LA, Krause E, Sferdean CD, Schuemann M, Fabian H, Bienert M, Beyermann M. Synthesis of 'difficult' peptide sequences: application of a depsipeptide technique to the Jung-Redemann 10- and 26-mers and the amyloid peptide Aβ(1–42). *Tetrahedron Lett.* 2004; **45**: 7519–7523.
13. Santos SD, Chandravarkar A, Mandal B, Mimna R, Murat K, Saucède L, Tella P, Tuchscherer G, Mutter M. Switch-Peptides: controlling self-assembly of amyloid β-derived peptides in vitro by consecutive triggering of acyl migrations. *J. Am. Chem. Soc.* 2005; **127**: 11888–11889.
14. Hentschel J, Krause E, Börner HG. Switch-Peptides to trigger the peptide guided assembly of poly(ethylene oxide)–peptide conjugates into tape structures. *J. Am. Chem. Soc.* 2006; **128**: 7722–7723.
15. Coin I, Dölling R, Krause E, Bienert M, Beyermann M, Sferdean CD, Carpino LA. Depsipeptide methodology for solid-phase peptide synthesis: circumventing side reactions and development of an automated technique via depsidipeptide units. *J. Org. Chem.* 2006; **71**: 6171–6177.
16. Kiso Y. Design and synthesis of substrate-based peptidomimetic human immunodeficiency virus protease inhibitors containing the hydroxymethylcarbonyl isostere. *Biopolymers* 1996; **40**: 235–244.
17. Kiso A, Hidaka K, Kimura T, Hayashi Y, Nezami A, Freire E, Kiso Y. Search for substrate-based inhibitors fitting the S2′ space of malarial aspartic protease plasmepsin II. *J. Pept. Sci.* 2004; **10**: 641–647.
18. Hamada Y, Igawa N, Ikari H, Ziora Z, Yamani A, Hidaka K, Kimura T, Saito K, Hayashi Y, Ishiura S, Kiso Y. β-Secretase inhibitors: Modification at the P4 position and improvement of inhibitory activity in cultured cell. *Bioorg. Med. Chem. Lett.* 2006; **16**: 4354–4359.
19. Wöhr T, Wahl F, Nefzi A, Rohwedder B, Sato T, Sun X, Mutter M. Pseudo-prolines as a solubilizing, structure-disrupting protection technique in peptide synthesis. *J. Am. Chem. Soc.* 1996; **118**: 9218–9227.
20. For a review, see: Sheppard R. The fluorenylmethoxycarbonyl group in solid phase synthesis. *J. Pept. Sci.* 2003; **9**: 545–552.
21. Stewart JM. Protection of the hydroxyl group in peptide synthesis. In *The Peptides*, vol. 3, Gross E, Meienhofer J (eds). Academic Press: New York, 1981; 169–201.
22. Mouls L, Subra G, Enjalbal C, Martinez J, Aubagnac J-L. O-N-Acyl migration in N-terminal serine-containing peptides: mass spectrometric elucidation and subsequent development of site-directed acylation protocols. *Terahedron Lett.* 2004; **45**: 1173–1178.
23. Kimura T, Ohtake J, Nakata S, Enomoto H, Moriwaki H, Akaji K, Kiso Y. Synthesis of prodrugs of HIV protease inhibitors. In *Peptide Chemistry 1994*, Ohno M (ed.). Protein Research Foundation: Osaka, 1995; 157–160.
24. Hamada Y, Matsumoto H, Yamaguchi S, Kimura T, Hayashi Y, Kiso Y. Water-soluble prodrugs of dipeptide HIV protease inhibitors based on O → N intramolecular acyl migration: design, synthesis and kinetic study. *Bioorg. Med. Chem.* 2004; **12**: 159–170.
25. Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. *Physiol. Rev.* 2001; **81**: 741–766.
26. Geula C, Wu CK, Saroff D, Lorenzo A, Yuan ML, Yankner BA. Aging renders the brain vulnerable to amyloid β-protein neurotoxicity. *Nat. Med.* 1998; **4**: 827–831.
27. Bitan G, Vollers SS, Teplow DB. Elucidation of primary structure elements controlling early amyloid β-protein oligomerization. *J. Biol. Chem.* 2003; **278**: 34882–34889.
28. Catalano SM, Dodson EC, Henze DA, Joyce JG, Krafft GA, Kinney GG. The role of amyloid-beta derived diffusible ligands (ADDLs) in Alzheimer's disease. *Curr. Top. Med. Chem.* 2006; **6**: 597–608.
29. Soto C, Castano EM, Kumar RA, Beavis RC, Frangione B. Fibrillogenesis of synthetic amyloid-β peptides is dependent on their initial secondary structure. *Neurosci. Lett.* 1995; **200**: 105–108.
30. Shen C-L, Murphy RM. Solvent effects on self-assembly of β-amyloid peptide. *Biophys. J.* 1995; **69**: 640–651.
31. Gorman PM, Chakrabartty A. Alzheimer β-amyloid peptides: structures of amyloid fibrils and alternate aggregation products. *Biopolymers* 2001; **60**: 381–394.
32. Stine WB Jr, Dahlgren KN, Krafft GA, Ladu MJ. In vitro characterization of conditions for amyloid-β peptide oligomerization and fibrillogenesis. *J. Biol. Chem.* 2003; **278**: 11612–11622.