ARTICLE

OBC
www.rsc.org/obc

Synthesis of potent β-secretase inhibitors containing a hydroxyethylamine dipeptide isostere and their structure–activity relationship studies

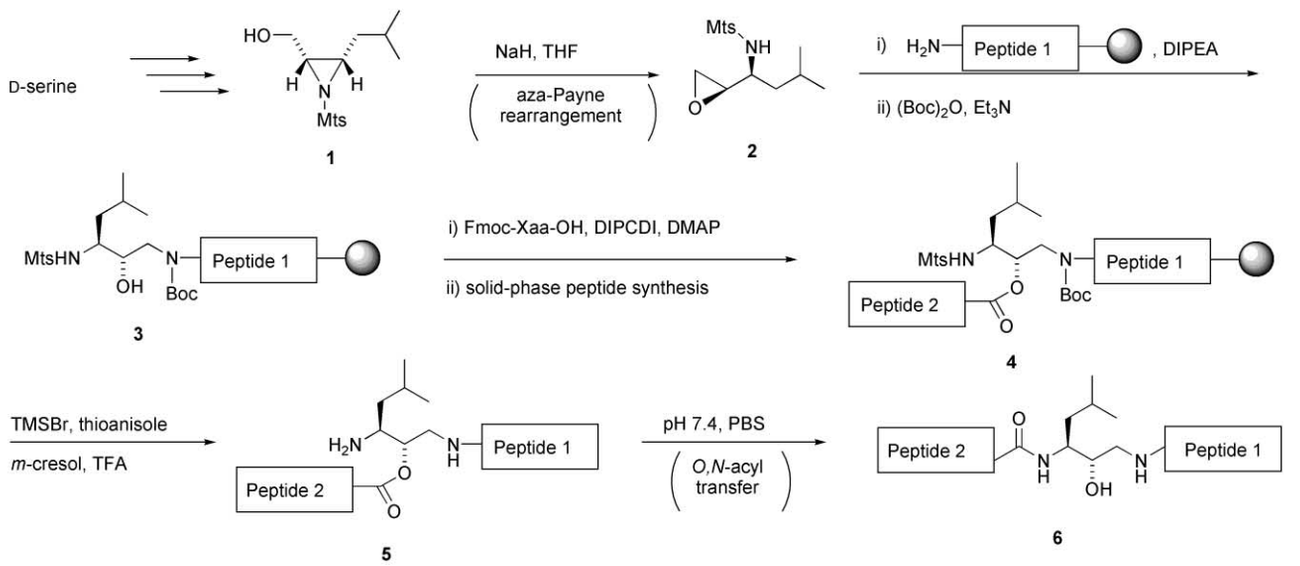
Hirokazu Tamamura,* Terukazu Kato, Akira Otaka and Nobutaka Fujii *
Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan. E-mail: tamamura@pharm.kyoto-u.ac.jp; nfujii@pharm.kyoto-u.ac.jp; Fax: +81-75-753-4570; Tel: +81-75-753-4551

Received 6th May 2003, Accepted 29th May 2003
First published as an Advance Article on the web 10th June 2003

Several β-secretase inhibitors were designed based on hydroxyethylamine dipeptide isostere (HDI) structures and were synthesized by a methodology using the aza-Payne rearragement and *O*,*N*-acyl transfer reactions to study their structure–activity relationships. Among these pseudopeptides, effective compounds were developed as the first β-secretase inhibitors containing the HDI transition state mimic with potent enzyme inhibitory activity (IC_{50} < 100 nM).

Introduction

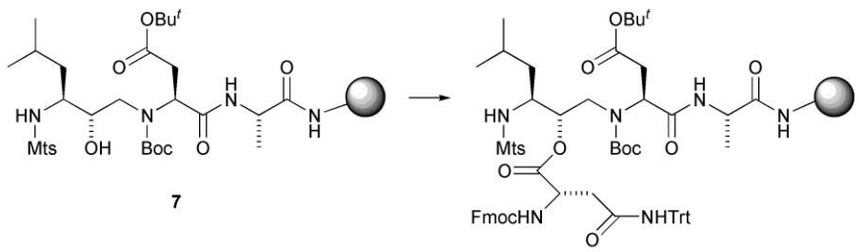
The accumulation of β-amyloid peptide (Aβ) in the brain is thought to be a primary cause for the progression of Alzheimer's disease (AD).[1] Since Aβ is generated from the cleavage of β-amyloid precursor protein (APP) by proteolytic enzymes, β- and γ-secretases,[2] these two secretases represent potential therapeutic targets. The identification of β-secretase[3] prompted us to develop effective inhibitors against this enzyme. β-Secretase belongs to an aspartyl protease family, similar to HIV protease. The efficacy of hydroxyethylamine dipeptide isosteres (HDIs) as the enzyme–substrate transition state mimic structure at the position P_1–$P_{1'}$ of aspartyl protease inhibitors has been well documented.[4] To date, β-secretase inhibitors, which contain statine- and hydroxyethylene-type dipeptide isosteres at the position P_1–$P_{1'}$, have been reported by Tung *et al.*[5] and Ghosh *et al.*,[6] respectively. Thus, we have been engaged in investigating whether pseudopeptides containing the HDI structure at the position P_1–$P_{1'}$ show β-secretase inhibitory activity. Recently, we reported useful methodology utilizing the aza-Payne rearrangement[7] and *O*,*N*-intramolecular acyl transfer[8] reactions for the solid-phase synthesis of HDI-containing peptidomimetics (Scheme 1).[9] An *N*-2,4,6,-trimethylphenylsulfonyl (Mts)-protected (and activated) aziridine **1**, which is prepared from D-serine, is converted to an epoxy amine **2** by the aza-Payne rearrangement reaction using NaH in THF. Reaction of **2** with the resin-bound protected Peptide 1 in Pr^iOH–DIPEA followed by $(Boc)_2O$ treatment yields an HDI-containing peptidyl resin **3**, *via* the regioselective S_N2 ring-opening reaction at position 3 of **2**. Esterification of the secondary hydroxy group of **3** with an N^{α}-Fmoc-protected amino acid affords an *O*-acylated compound followed by Fmoc-based solid-phase peptide synthesis. Treatment of the resin-bound protected *O*-acyl peptide **4** with 1 M TMSBr–thioanisole/TFA yields the deprotected compound **5**. Subsequent *O*,*N*-intramolecular acyl transfer reaction by incubation in phosphate buffer at pH 7.4 affords the corresponding *N*-acyl compound **6**, which is the desired HDI-containing peptidomimetic. Herein, refinement of this methodology in terms of chemical yields was attempted and its applicability to the synthesis of relatively large peptidomimetics was examined in detail. Furthermore, several substrate-based β-secretase inhibitors that contain the HDI transition state mimic were designed and synthesized utilizing the above methodology. The structure–activity relationships (SAR) of the synthetic inhibitors are also discussed.



Scheme 1 An *N*-terminal NH of Peptide 1 and a *C*-terminal CO of Peptide 2 are intentionally shown for easy understanding.

DOI: 10.1039/b304842j

Table 1 Examination of esterification of the secondary hydroxy group



7 **8**

Entry	Reagents[a]	Solvents	Time/h	Yield (%)[b]
1	DIPCDI (5 equiv.), DMAP (0.3 equiv.)	CH_2Cl_2, DMF[c]	15	64[d]
2	MSNT (5 equiv.), NMI (20 equiv.)	CH_2Cl_2	4	69[e]
3	MSNT (15 equiv.), NMI (60 equiv.)	CH_2Cl_2	4	96
4	MSNT (30 equiv.), NMI (60 equiv.)	CH_2Cl_2	4	quant.
5	DIPCDI (15 equiv.), DMAP (0.3 equiv.)	CH_2Cl_2, DMF[c]	4	97
6	DIPCDI (15 equiv.), DMAP (0.3 equiv.)	DMF	4	0
7	DIPCDI (15 equiv.), DMAP (0.3 equiv.)	CH_2Cl_2	4	quant.[f]
8	DIPCDI (15 equiv.), DMAP (0.3 equiv.)	CH_2Cl_2	8	quant.
9	DIPCDI (15 equiv.), DMAP (0.1 equiv.)	CH_2Cl_2	4	90[g]
10	DIPCDI (15 equiv.), DMAP (0.2 equiv.)	CH_2Cl_2	4	quant.[h]

[a] Fmoc–Asn(Trt)–OH (equimoler with DIPCDI or MSNT) was used. [b] Yields were calculated based on HPLC peak areas of crude deprotected-cleaved peptides having Fmoc and Mts groups after 95% TFA treatment of products. These contain the yields of an epimer of **8**. [c] CH_2Cl_2 : DMF = 9 : 1 (v/v). [d] Epimerization detected by HPLC analysis, 11%. [e] Epimerization 32%. [f] Epimerization 17%. [g] Epimerization 11%. [h] Epimerization 15%.

Results and discussion

Examination of the esterification conditions

The overall chemical yields of HDI-containing peptidomimetics composed of 4–7 amino acid residues were relatively low in the previous study (6–15%).[9] In order to improve the esterification step of the secondary hydroxy group of **3**, which is thought to have low reactivity, conversion of a model pseudopeptide resin **7** into **8** was examined with several coupling conditions (see Table 1, A Rink-Amide-CLEAR resin[10] was used as a solid support.). Reaction of **7** with Fmoc–Asn(Trt)–OH (5 equiv.), *N*,*N*′-diisopropylcarbodiimide (DIPCDI) (5 equiv.) and DMAP (0.3 equiv.) in CH_2Cl_2–DMF (9 : 1 (v/v)) at room temp. for 15 h, under the conditions in the previous paper,[9] did not afford a sufficient yield of **8** (64%) accompanied with 11% epimerization (entry 1). Reaction using 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT) (5 equiv.) and *N*-methylimidazole (NMI) (20 equiv.) in CH_2Cl_2[11] at room temp. for 4 h caused relatively high (32%) epimerization in spite of a similar yield (entry 2), while use of MSNT (15–30 equiv.) and NMI (60 equiv.) significantly improved chemical yields. Several reaction conditions using DIPCDI (15 equiv.) and DMAP (0.1–0.3 equiv.) in CH_2Cl_2 and/or DMF (entry 5–10) were tested to investigate whether CH_2Cl_2 is preferred to DMF as a solvent, providing that the esterification is completely performed with DIPCDI (15 equiv.) and DMAP (0.2–0.3 equiv.) in CH_2Cl_2 for 4 h, but accompanied with 15–17 % epimerization.

Examination as to whether the diketopiperazine formation occurs

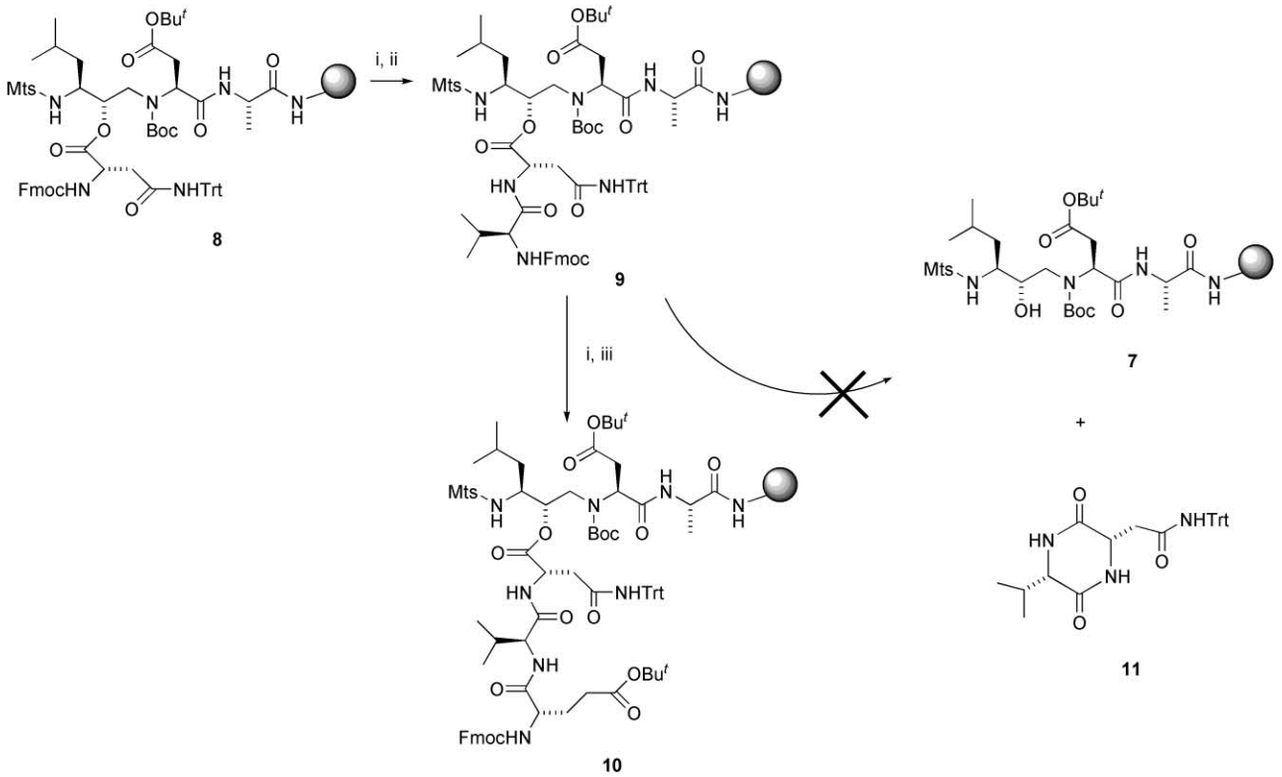
Next, we examined whether this methodology can be applied to the synthesis of longer peptidomimetics, which is likely to involve diketopiperazine formation in elongation of the peptide chain from the secondary hydroxy group. A model peptide resin **9**, derived from **8** by coupling with Fmoc–Val–OH, was treated by 20% (v/v) piperidine–DMF followed by coupling with Fmoc–Glu(OBu^t)–OH (Scheme 2). However, HPLC analysis of the deprotected-cleaved sample having Fmoc and Mts groups revealed that regeneration of **7** by formation of diketopiperazine **11** did not occur practically and that the chain elongation from the secondary hydroxy group was successfully achieved by the usual solid-phase peptide synthesis since the above reactions yielded only the desired product **10**.

Design and synthesis

Since it has been reported that the $P_{1'}$–$P_{3'}$ or $P_{1'}$–$P_{4'}$ residues in the *C*-terminal part of the compounds are essential for the intrinsic β-secretase inhibitory activity,[5a] the length of the *C*-terminal part of the target compounds was fixed at $P_{1'}$–$P_{4'}$. Compounds with various lengths of peptide chains in the *N*-terminal section were prepared to examine their biological effect. Based on the preferred residues for the β-secretase cleavage of the substrate at each position P_4–$P_{4'}$,[12] the compound sequences were determined (Table 2). In TK-4–9, the amino acid residues of positions P_5–P_{10} were derived from the sequence of APP. In TK-10, 11 and 12, the second-preferred residues were adopted at the positions P_2, P_3 and P_4, respectively. These compounds containing the HDI unit were synthesized by the refined methodology (Scheme 1) using the esterification condition of entry 10 in Table 1. The unfavourable dropout of dipeptide from the secondary hydroxy group by the diketopiperazine formation was not observed in the synthesis of TK-3–12. The synthetic yields were improved compared with the previous study: The overall yields of 6–10 residue peptides (TK-1–5 and TK-10–12) were 17–25% except for TK-10. It is reasonable that the elongation of the peptide chain caused a marked decrease in yields (TK-8, 9).

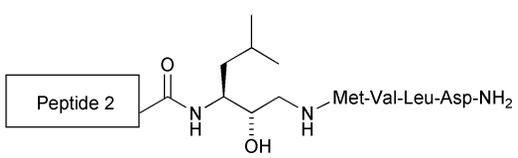
Biological evaluation

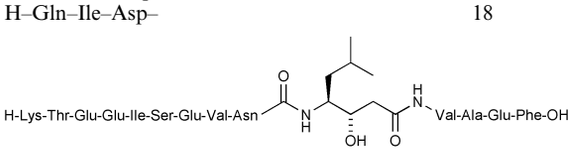
β-Secretase inhibitory activity of the synthetic compounds was evaluated by the homogeneous time-resolving fluorescent (HTRF) assay[13] (Table 2). The shortest compound, TK-1, which contains only the P_2–$P_{4'}$ residues, did not show detectable inhibitory activity, whereas the addition of the P_3-residue resulted in potency enhancement (TK-2, IC_{50} = 0.24 μM), suggesting that P_3–Ile is a critical residue. TK-2–9 showed significant inhibitory activity (IC_{50} < 1.2 μM). The potency of TK-3 was 4-fold stronger than that of a statine-based inhibitor reported by Tung *et al.*,[5a] while the molecular size of TK-3 (MW 962) is much smaller than that of the statine-based inhibitor (MW 1652). It is noted that the P_5–P_{10} residues can be removed without reduction in potency, and that the adoption



Scheme 2 *Reagents*: i, 20% (v/v) piperidine–DMF; ii, Fmoc–Val–OH, DIPCDI, *N*-hydroxybenzotriazole (HOBt); iii, Fmoc–Glu(OBu^t)–OH, DIPCDI, HOBt.

Table 2 Structures of synthetic pseudopeptides, their overall yields and inhibitory activity against β-secretase



Compound	Peptide 2	Yield (%)	IC_{50}/μM [a]
TK-1	H–Asp–	25	>100
TK-2	H–Ile–Asp–	20	0.24
TK-3	H–Glu–Ile–Asp–	17	0.082
TK-4	H–Ser–Glu–Ile–Asp–	18	1.2
TK-5	H–Ile–Ser–Glu–Ile–Asp–	24	0.68
TK-6	H–Glu–Ile–Ser–Glu–Ile–Asp–	11	0.40
TK-7	H–Glu–Glu–Ile–Ser–Glu–Ile–Asp–	13	0.22
TK-8	H–Thr–Glu–Glu–Ile–Ser–Glu–Ile–Asp–	9.0	0.63
TK-9	H–Lys–Thr–Glu–Glu–Ile–Ser–Glu–Ile–Asp–	6.9	0.84
TK-10	H–Glu–Ile–Asn–	4.8	0.38
TK-11	H–Glu–Val–Asp–	23	0.21
TK-12	H–Gln–Ile–Asp–	18	0.24
Statine-based inhibitor [b]			0.38

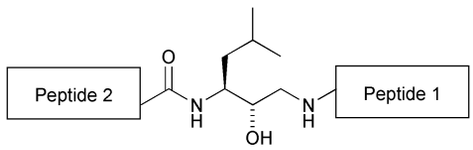
Statine-based inhibitor

[a] IC_{50} values are based on inhibitory activity against β-secretase. [b] Peptide Institute, Inc.[5a]

of the HDI unit as the enzyme–substrate transition state mimic structure led to the development of β-secretase inhibitors, as in the case of statine- and hydroxyethylene-type dipeptide isosteres. The replacement of the P_2, P_3 or P_4 residue by the corresponding second-preferred residue caused significant loss of potency (TK-10–12), suggesting that the enzyme has high preference at positions P_2, P_3 and P_4 for Asp, Ile and Glu, respectively.

Next, TK-13–21 were prepared in a similar way for Ala scan studies through the sequence of TK-3 and SAR studies by deletion from the *C*-terminus (Table 3). The Ala scan revealed that the order of importance of each subsite residue for β-secretase inhibitory activity is the following: P_3–Ile > $P_{2'}$–Val > P_4–Glu > $P_{3'}$–Leu > P_2–Asp > $P_{1'}$–Met > $P_{4'}$–Asp. The Ala-substitution for P_3–Ile (TK-14) caused a marked decrease in potency compared with the Val-substitution (TK-11), whereas

Table 3 Structures of synthetic pseudopeptides with Ala-substitution and *C*-terminal truncation of TK-3, their overall yields and inhibitory activity against β-secretase



Compound	Peptide 2	Peptide 1	Yield (%)	IC_{50}/μM
TK-3	H–Glu–Ile–Asp–	–Met–Val–Leu–Asp–NH_2		0.049
TK-13	H–*Ala*–Ile–Asp–	–Met–Val–Leu–Asp–NH_2	20	0.42
TK-14	H–Glu–*Ala*–Asp–	–Met–Val–Leu–Asp–NH_2	12	1.2
TK-15	H–Glu–Ile–*Ala*–	–Met–Val–Leu–Asp–NH_2	9.0	0.090
TK-16	H–Glu–Ile–Asp–	–*Ala*–Val–Leu–Asp–NH_2	19	0.078
TK-17	H–Glu–Ile–Asp–	–Met–*Ala*–Leu–Asp–NH_2	16	0.47
TK-18	H–Glu–Ile–Asp–	–Met–Val–*Ala*–Asp–NH_2	21	0.16
TK-19	H–Glu–Ile–Asp–	–Met–Val–Leu–*Ala*–NH_2	16	0.047
TK-20	H–Glu–Ile–Asp–	–Met–Val–Leu–NH_2	20	0.087
TK-21	H–Glu–Ile–Asp–	–Met–Val–NH_2	17	0.71
Statine-based inhibitor				0.26

the Ala- and Asn-substitutions for P_2–Asp (TK-15 and TK-10, respectively) resulted in slight loss of potency. The Ala-substitutions at the P_2 and $P_{1'}$ sites (TK-15 and TK-16, respectively) did not cause significant decrease in β-secretase inhibitory activity in spite of the proximity of the HDI central core (the center of the enzyme recognition site). The Ala-replacement and the removal of $P_{4'}$–Asp (TK-19 and TK-20, respectively) revealed the dispensability of this residue, whereas the truncation of the $P_{3'}$–$P_{4'}$ residues (TK-21) disclosed the importance of $P_{3'}$–Leu.

In conclusion, the synthetic procedure of HDI-containing pseudopeptides, previously reported by us,[9] was refined, and the procedure was proven to be applicable to the synthesis of relatively large peptidomimetics. Furthermore, several β-secretase inhibitors were synthesized by the above procedure to find useful leads, such as TK-3 and TK-19, which have potent enzyme inhibitory activity (IC_{50} < 100 nM). These compounds are the first β-secretase inhibitors containing the HDI structure. Of note, the HDI unit can be also adopted as the transition state mimic structures for β-secretase inhibitors, in addition of statine- and hydroxyethylamine-type isosters. The present results in the SAR study will give rise to the rational design and synthesis of a new type of β-secretase inhibitors.

Experimental

General

HPLC solvents were H_2O and CH_3CN, both containing 0.1% (v/v) TFA. For analytical HPLC, a Cosmosil 5C18-AR column (4.6 × 250 mm, Nacalai Tesque Inc., Kyoto, Japan) was eluted with a linear gradient of CH_3CN at a flow rate of 1 mL min^{-1} on a Waters™ 717 plus autosampler (Nihon Millipore, Ltd., Tokyo, Japan) equipped with a Hitachi D-2500 chromato-integrator (Tokyo, Japan). Preparative HPLC was performed on a Waters Delta Prep 4000 equipped with a Cosmosil 5C18-AR column (20 × 250 mm, Nacalai Tesque Inc.) using a linear gradient of CH_3CN at a flow rate of 15 mL min^{-1}. Ion-spray (IS)-mass spectra was obtained with a Sciex API*III*E triple quadrupole mass spectrometer (Toronto, Canada). Optical rotation of a compound in aqueous solution was measured with a Horiba high-sensitive polarimeter SEPA-200 (Kyoto, Japan). Fmoc-protected amino acids were purchased from Watanabe Chemical Industries, Ltd. (Hiroshima, Japan) or Calbiochem-Novabiochem Japan, Ltd. (Tokyo, Japan). A Rink-Amide-CLEAR resin was purchased from Peptide Institute, Inc. (Osaka, Japan). The statine-based inhibitor reported by Tung *et al.*[5a] was purchased from Peptide Institute, Inc. All the other chemicals were purchased from either Nacalai Tesque Inc. or Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Resin-bound compound 8 and its deprotected pseudopeptides (examination of esterification of the secondary hydroxy group, entry 10 in Table 1)

A model pseudopeptide resin **7** (20 mg, 8.0 μmol), which was previously prepared,[9] was treated with Fmoc–Asn(Trt)–OH (72 mg, 120 μmol), DIPCDI (19 μL, 120 μmol) and DMAP (200 μg, 1.6 μmol) in CH_2Cl_2 (1 mL) at room temperature for 4 h. After washing the resin with $CHCl_3$ (10 mL × 5) and drying *in vacuo*, the resin **8** was treated with aqueous 95% TFA (1 mL) at room temp. for 2 h, followed by removal of the resin with filtration. The filtrate was concentrated under reduced pressure, and ice-cold dry diethyl ether (5 mL) was added to the residue. The resulting powder was collected by centrifugation, washed with diethyl ether (5 mL × 3), and then dissolved in 1 M AcOH–MeOH (1 : 1 (v/v)) (2 mL). 10 μL of the solution was analyzed by analytical HPLC with a linear gradient of CH_3CN (30–70%, 30 min) and with an isocratic mode of CH_3CN (46%). HPLC peaks of deprotected compounds (Fmoc and Mts groups were retained) derived from **7** and **8** were identified by the IS-MS analysis. Their amounts were quantified from the corresponding peak areas.

Resin-bound compound 10 and its deprotected pseudopeptide (examination as to whether the diketopiperazine formation occurs)

A model pseudopeptide resin **9** (140 mg, 50 μmol) was normally treated with 20% (v/v) piperidine–DMF followed by standard coupling of Fmoc–Glu(OBu′)–OH (3 equiv.) with DIPCDI (3 equiv.)–HOBt (3 equiv.). The obtained resin **10** was treated with aqueous 95% TFA (5 mL) to afford the corresponding deprotected-cleaved compound, which was analyzed by analytical HPLC with a linear gradient of CH_3CN (30–60%, 30 min) in a similar way as described in the Resin-bound compound **8** and its deprotected psedopeptides. An HPLC peak of deprotected compound of **10** (Fmoc and Mts groups was retained) was identified by the IS-MS analysis. An HPLC peak corresponding to deprotected compound of **7** was not detected.

TK-5 (representative compound of TK-1–21)

The protected TK-5 resin was manually constructed according to Scheme 1 (compound **1** to **4**) using the esterification condition of entry 10 in Table 1 on a Rink-Amide-CLEAR resin (0.40 mmol g^{-1}, 0.2 mmol scale). The obtained TK-5 resin (N^{α}-Fmoc-deprotected, 9.2 μmol) was treated with 1 M

Table 4 Characterization data of the synthetic compounds (TK-1–21)

Compound	$[a]_D$	$c(H_2O)$	Temp./°C	Formula	IS-MS (M + H)$^+$ Found	Calcd
TK-1	18.2	0.055	30	$C_{31}H_{56}N_7O_{10}S$	721.0	720.4
TK-2	19.6	0.051	30	$C_{37}H_{67}N_8O_{11}S$	834.0	833.5
TK-3	40.8	0.049	30	$C_{42}H_{74}N_9O_{14}S$	963.0	962.5
TK-4	−38.5	0.052	31	$C_{45}H_{79}N_{10}O_{16}S$	1050.0	1049.6
TK-5	0.00	0.058	31	$C_{51}H_{90}N_{11}O_{17}S$	1163.5	1162.6
TK-6	−22.2	0.045	31	$C_{56}H_{97}N_{12}O_{20}S$	1292.0	1291.7
TK-7	−218	0.055	31	$C_{61}H_{104}N_{13}O_{23}S$	1421.5	1420.7
TK-8	200	0.045	31	$C_{65}H_{111}N_{14}O_{25}S$	1522.5	1521.8
TK-9	0.00	0.068	31	$C_{72}H_{124}N_{15}O_{26}S$	1652.0	1648.9
TK-10	47.6	0.021	31	$C_{42}H_{75}N_{10}O_{13}S$	962.0	961.5
TK-11	−69.0	0.058	31	$C_{41}H_{72}N_9O_{14}S$	949.5	948.5
TK-12	−40.0	0.050	31	$C_{42}H_{75}N_{10}O_{13}S$	962.0	961.5
TK-13	−164	0.110	23	$C_{40}H_{72}N_9O_{12}S$	904.5	904.5
TK-14	−35.7	0.112	24	$C_{39}H_{68}N_9O_{14}S$	921.0	920.5
TK-15	−58.3	0.103	25	$C_{41}H_{74}N_9O_{12}S$	920.0	918.5
TK-16	−55.0	0.109	25	$C_{40}H_{70}N_9O_{14}$	903.5	902.5
TK-17	−18.5	0.108	25	$C_{40}H_{70}N_9O_{14}S$	936.0	934.5
TK-18	53.1	0.113	25	$C_{39}H_{68}N_9O_{14}S$	921.5	920.5
TK-19	9.52	0.105	25	$C_{41}H_{74}N_9O_{12}S$	919.0	918.5
TK-20	29.1	0.103	26	$C_{38}H_{69}N_8O_{11}S$	849.0	847.5
TK-21	−63.6	0.110	26	$C_{32}H_{58}N_7O_{10}S$	735.5	734.4

TMSBr—thioanisole–TFA (2 mL) in the presence of *m*-cresol (80 μL) at 0 °C for 15 h, followed by removal of the resin with filtration. The filtrate was concentrated under reduced pressure, and ice-cold dry diethyl ether (10 mL) was added to the residue. The resulting powder was collected by centrifugation, washed with diethyl ether (10 mL × 3), and then dissolved in phosphate buffer (pH 7.4, 1 mL) at 0 °C to perform the *O*,*N*-acyl transfer reaction. After 30 min incubation, the crude product solution was purified by preparative HPLC and lyophilized to give a white powder of compound TK-5: yield 3.1 mg (overall 24% based on the Rink-Amide-CLEAR resin). The side product derived from epimerization in the esterification was eliminated by preparative HPLC at the final step.

Characterisation data of all the synthetic pseudopeptides (TK-1–21) are shown in Table 4.

β-Secretase inhibition assay

An aqueous 300 mM AcONa, pH 4.5 assay buffer was used. To the mixture of human recombinant β-secretase (residues 1–460, R & D Systems Inc., Minneapolis, MN, USA) (44 ng 10 μL^{-1}) and various concentrations of test compounds (100 nmol, 10 μL) was added a modified β-secretase substrate based on the Swedish APP mutant sequence[3a] (SEVNLDAEFRKRR–NH_2) (10 μL), and the mixture was incubated at 23 °C for 1 h followed by quenching with 1.2 M K_2HPO_4 (pH 10, 10 μL). β-Secretase inhibitory activity was determined by the HTRF method.[13] Details of this assay will be described elsewhere.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan and a Health and Labour Sciences Research Grant on Health Sciences focusing on Drug Innovation.

References

1 D. J. Selkoe, *Nature*, 1999, **399A**, 23.
2 S. Sinha and I. Lieberburg, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 11049.
3 (*a*) R. Vassar, B. D. Bennett, S. Babu-Khan, S. Kahn, E. A. Mendiaz, P. Denis, D. B. Teplow, S. Ross, P. Amarante, R. Loeloff, Y. Luo, S. Fisher, J. Fuller, S. Edenson, J. Lile, M. A. Jarosinski, A. L. Biere, E. Curran, T. Burgess, J.-C. Louis, F. Collins, J. Treanor, G. Rogers and M. Citron, *Science*, 1999, **286**, 753; (*b*) R. Yan, M. J. Bienkowski, M. E. Shuck, H. Miao, M. C. Tory, A. M. Pauley, J. R. Brashler, N. C. Stratman, W. R. Mathews, A. E. Buhl, D. B. Carter, A. G. Tomasselli, L. A. Parodi, R. L. Heinrikson and M. E. Gurney, *Nature*, 1999, **402**, 533; (*c*) S. Shinha, J. P. Anderson, R. Barbour, G. S. Basi, R. Caccavello, D. Davis, M. Doan, H. F. Dovey, N. Frigon, J. Hong, K. Jacobson-Croak, N. Jewett, P. Keim, J. Knops, I. Lieberburg, M. Power, H. Tan, G. Tatsuno, J. Tung, D. Schenk, P. Seubert, S. M. Suomensaari, S. Wang, D. Walker, J. Zhao, L. McConlogue and V. John, *Nature*, 1999, **402**, 537; (*d*) I. Hussain, D. Powell, D. R. Howlett, D. G. Tew, T. D. Meek, C. Chapman, I. S. Gloger, K. E. Murphy, C. D. Southan, D. M. Ryan, T. S. Smith, D. L. Simmons, F. S. Walsh, C. Dingwall and G. Christie, *Mol. Cell. Neurosci.*, 1999, **14**, 419; (*e*) X. Lin, G. Koelsch, S. Wu, D. Downs, A. Dashti and J. Tang, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 1456.
4 (*a*) D. H. Rich, J. Green, M. V. Toth, G. R. Marshall and S. B. H. Kent, *J. Med. Chem.*, 1990, **33**, 1285; (*b*) N. A. Roberts, J. A. Martin, D. Kinchington, A. V. Broadhurst, J. C. Craig, I. B. Duncan, S. A. Galpin, B. K. Handa, J. Kay, A. Krohn, R. W. Lambert, J. H. Merrett, J. S. Mills, K. E. B. Parkes, S. Redshaw, A. J. Ritchie, D. L. Taylor, G. J. Thomas and P. J. Machin, *Science*, 1990, **248**, 358; (*c*) K. E. B. Parkes, D. J. Bushnell, P. H. Crackett, S. J. Dunsdon, A. C. Freeman, M. P. Gunn, R. A. Hopkins, R. W. Lambert, J. A. Martin, J. H. Merrett, S. Redshaw, W. C. Spurden and G. J. Thomas, *J. Org. Chem.*, 1994, **59**, 3656; (*d*) J. P. Vacca, B. D. Dorsey, W. A. Schleif, R. B. Levin, S. L. McDaniel, P. L. Darke, J. Zugay, J. C. Quintero, O. M. Blahy, E. Roth, V. V. Sardana, A. J. Schlabach, P. I. Graham, J. H. Condra, L. Gotlib, M. K. Holloway, J. Lin, I.-W. Chen, K. Vastag, D. Ostovic, P. S. Anderson, E. A. Emini and J. R. Huff, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 4096; (*e*) D. J. Kempf, K. C. Marsh, J. F. Denissen, E. McDonald, S. Vasavanonda, C. A. Flentge, B. E. Green, L. Fino, C. H. Park, X.-P. Kong, N. E. Wideburg, A. Saldivar, L. Ruiz, W. M. Kati, H. L. Sham, T. Robins, K. D. Stewart, A. Hsu, J. J. Plattner, J. M. Leonard and D. W. Norbeck, *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 2484; (*f*) E. E. Kim, C. T. Baker, M. D. Dwyer, M. A. Murko, B. G. Rao, R. D. Tung and M. R. Navia, *J. Am. Chem. Soc.*, 1995, **117**, 1181; (*g*) S. W. Kaldor, V. J. Kalish, J. F. Davis II, B. V. Shetty, J. E. Fritz, K. Appelt, J. A. Burgess, K. M. Campanale, N. Y. Chirgadze, D. K. Clawson, B. A. Dressman, S. D. Hatch, D. A. Khalil, M. B. Kosa, P. P. Lubbehusen, M. A. Muesing, A. K. Patick, S. H. Reich, K. S. Su and J. H. Tatlock, *J. Med. Chem.*, 1997, **40**, 3979; (*h*) A. K. Ghosh, J. F. Kincaid, W. Cho, D. E. Walters, K. Krishnan, K. A. Hussain, Y. Koo, H. Cho, C. Rudall, L. Holland and J. Buthod, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 687; (*i*) H. Tamamura, Y. Koh, S. Ueda, Y. Sasaki, T. Yamasaki, M. Aoki, K. Maeda, Y. Watai, H. Arikuni, A. Otaka, H. Mitsuya and N. Fujii, *J. Med. Chem.*, 2003, **46**, 1764.
5 (*a*) J. S. Tung, D. L. Davis, J. P. Anderson, D. E. Walker, S. Mamo, N. Jewett, R. K. Hom, S. Sinha, E. D. Thorsett and V. John, *J. Med. Chem.*, 2002, **45**, 259; (*b*) R. K. Hom, L. Y. Fang, S. Mamo,

J. S. Tung, A. C. Guinn, D. E. Walker, D. L. Davis, A. F. Gailunas, E. D. Thorsett, S. Sinha, J. E. Knops, N. E. Jewett, J. P. Anderson and V. John, *J. Med. Chem.*, in press.
6 (*a*) A. K. Ghosh, D. Shin, D. Downs, G. Koelsch, X. Lin, J. Ermolieff and J. Tang, *J. Am. Chem. Soc.*, 2000, **122**, 3522; (*b*) L. Hong, G. Koelsch, X. Lin, S. Wu, S. Terzyan, A. K. Ghosh, X. C. Zhang and J. Tang, *Science*, 2000, **290**, 150; (*c*) A. K. Ghosh, G. Bilcer, C. Harwood, R. Kawahama, D. Shin, K. A. Hussain, L. Hong, J. A. Loy, C. Nguyen, G. Koelsch, J. Ermolieff and J. Tang, *J. Med. Chem.*, 2001, **44**, 2865; (*d*) L. Hong, R. T. Turner III, G. Koelsch, D. Shin, A. K. Ghosh and J. Tang, *Biochemistry*, 2002, **41**, 10963.
7 T. Ibuka, K. Nakai, H. Habashita, Y. Hotta, A. Otaka, H. Tamamura, N. Fujii, N. Mimura, Y. Miwa, T. Taga, Y. Chounan and Y. Yamamoto, *J. Org. Chem.*, 1995, **60**, 2044.
8 (*a*) T. Wakamiya, Y. Tarumi and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1974, **47**, 2686; (*b*) J. M. Stewart, in *The Peptides*, eds. E. Gross and J. Meienhofer, Academic Press, New York, 1981, vol. 3, pp. 170–201; (*c*) T. R. Hurley, C. E. Colson, G. Hicks and M. J. Ryan, *J. Med. Chem.*, 1993, **36**, 1496; (*d*) T. Kimura, J. Ohtake, S. Nakata, H. Enomoto, H. Moriwaki, K. Akaji and Y. Kiso, in *The Peptide Chemistry 1994*, ed. M. Ohno, Protein Research Foundation, Osaka, 1995, pp. 157–160.
9 H. Tamamura, T. Hori, A. Otaka and N. Fujii, *J. Chem. Soc., Perkin Trans. 1*, 2002, 577.
10 M. Kempe and G. Barany, *J. Am. Chem. Soc.*, 1996, **118**, 7083.
11 J. Nielsen and L. O. Lyngso, *Tetrahedron Lett.*, 1996, **37**, 8439.
12 R. T. Turner III, G. Koelsch, L. Hong, P. Castanheira, A. Ghosh and J. Tang, *Biochemistry*, 2001, **40**, 10001.
13 (*a*) G. Mathis, *Clin. Chem.*, 1993, **39**, 1953; (*b*) M. Preaudat, J. Ouled-diaf, B. Alpha-Bazin, G. Mathis, T. Mitsugi, Y. Aono, K. Takahashi and H. Takemoto, *J. Biomol. Screen.*, 2002, **7**, 267.