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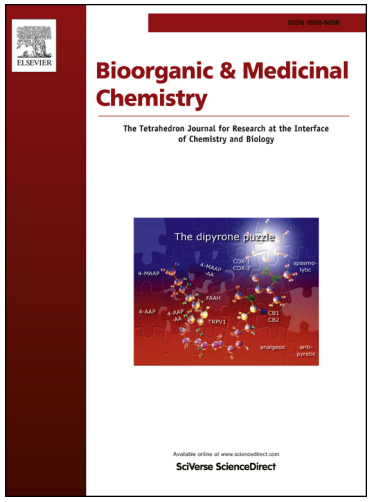

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Evaluation of transition-state mimics in a superior BACE1 cleavage sequence as peptide-mimetic BACE1 inhibitors

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KEY WORDS

BACE 1, inhibitor, transition state mimic, hydroxyethylamine, hydroxymethylcarbonyl

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Abstract

A superior substrate sequence for BACE1 containing transition-state mimics at the scissile site was evaluated as a protease inhibitor. Hydroxymethylcarbonyl (HMC) and hydroxyethylamine (HEA) isosteres were selected as the transition state mimics, and incorporated into the scissile site of the superior sequence covering the P_4 to P_1' sites (Glu-Ile-Thi-Thi*Nva; *denotes the cleavage site). Isosteres having different absolute configurations of the hydroxyl group were synthesized separately, and the effect of the configuration was evaluated. Configuration of the hydroxyl group of each isostere showed a marked effect on the inhibitory activity; *anti*-configuration to the scissile site substituent had potent inhibitory activity in an HMC-type inhibitor, whereas *anti*-configuration of HEA-type inhibitors showed no inhibitory activity. Structural evaluations based on X-ray crystallographic analyses of recombinant BACE1 in complex with each inhibitor provided insights into the protein-ligand interactions, especially at the prime sites.

1. Introduction

Alzheimer's disease (AD) is the most common cause of senile dementia and is thought to be caused by the increased production and accumulation of amyloid peptide (Aβ) in the brain[1]. Most Aβs consist of 40 or 42 amino acid residues and derive from a type I transmembrane protein APP (amyloid precursor protein) by cleavage with two aspartic proteases, β- and γ-secretase. β-Secretase, also called BACE1 (β-site APP cleaving enzyme 1), cleaves the luminal domain of APP at the N-terminus of Aβ, which is followed by γ-secretase cleavage at the C-terminus of Aβ located in the membrane. Thus, an inhibitor of BACE1 would prevent Aβ production at the initial processing step, and could be a promising anti-AD drug[2-8]. Inhibition of BACE1 is expected to be clinically feasible, since BACE1 null mice proved viable with few phenotypical abnormalities.

BACE1 is a transmembrane protease active at a mildly acidic pH of about 5.5, which suggests that it would act in endosomes. The extracellular catalytic domain of mature BACE1 takes a flap open conformation, whereas the flap is closed when the substrate or transition-state mimic inhibitor is bound[9]. The flap-open conformation is energetically stable and destabilization of this conformation is required for substrate/inhibitor binding. This destabilization is compensated to some extent by interactions with the substrate/inhibitor with the flap region covering Tyr71 to Lys75 of BACE1.

In our previous paper[10], we reported an efficient procedure for the preparation of a large amount of recombinant BACE1 (rBACE1) corresponding to positions 47-454 of an extracellular domain. The purified 45-kDa rBACE1 had kinetic parameters of K_m=5.5 μM and k_{cat}=1719 s^{-1}. Using substrate peptide libraries to be digested with rBACE1, it was found that a unique peptide containing unnatural amino acids, Ile-Ser-Glu-Ile-Thi-Thi*Nva-Ala-Glu-Phe-Arg-His-NH_2 (*denotes the cleavage site) could be cleaved 10 times faster than a Swedish mutant substrate. Taking advantage of rBACE1 and the superior cleavage sequence, we incorporated transition-state mimics at the cleavage site of this superior sequence, and evaluated the inhibitory activity for rBACE1. Two types of the most widely used isosters, hydroxymethylcarbony (HMC)[11] and hydroxyethylamine (HEA)[12], were selected as the transition-state mimics. Inhibitory activities of synthesized inhibitors varied significantly depending on the stereo-structures of transition-state mimics. To evaluate the interactions of these inhibitors with rBACE1, structure-based analyses of rBACE1 in complex with each inhibitor were conducted by X-ray crystal analyses.

2. Results and Discussions

2.1. Chemistry

Structures of synthesized HMC- and HEA-type inhibitors are summarized in Figure 1. In both type inhibitors, each isostere with a specific conformation of the key hydroxyl group was separately prepared and incorporated in the superior substrate sequence covering the P_4 to P_1' sites, (*R*)- or (*S*)-**1** and (*R*)- or (*S*)-**2**. In addition, several analogs containing different P_1 site substituents were also synthesized to evaluate additional effects of side chain structures at the scissile site.

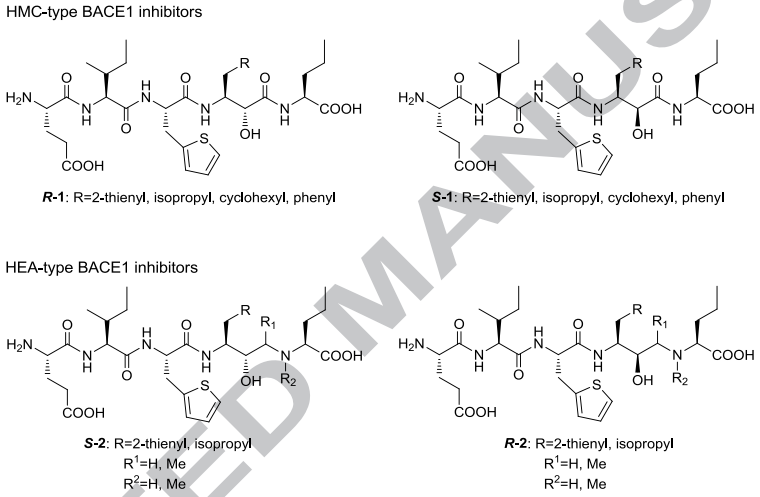
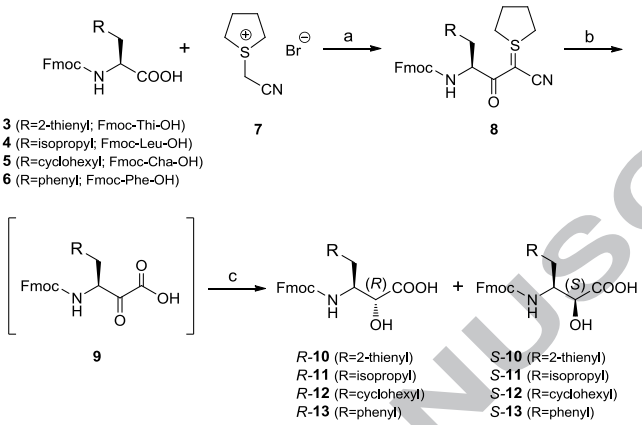


Figure 1

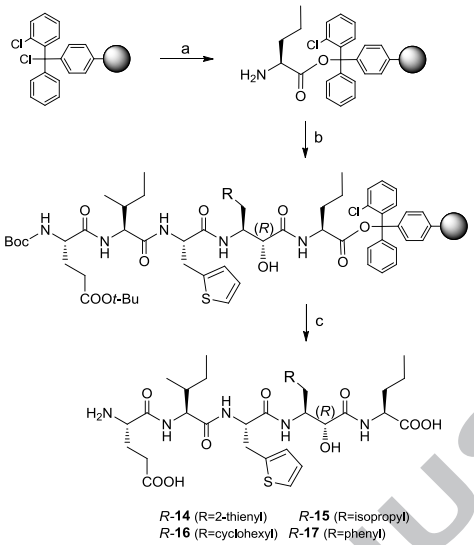
In HMC-type inhibitors, (*R*)- and (*S*)-hydroxyl inhibitors with linear or cyclic substituents at the P_1 site were synthesized, respectively. Thus, four different amino acids, Thi (R=2-thienyl), Leu (R=isopropyl), Cha (R=cyclohexyl), and Phe (R=phenyl), were selected as the starting amino acids. Key intermediate, α-hydroxyl carboxylic acid, was synthesized according to the route shown in Scheme 1. Starting from commercially available Fmoc amino acids, a sulfur ylide derivative **8** was prepared by HATU-mediated coupling[13] with sulfonium salt **7**. Oxidation of **8** with Oxone® [14] yielded **9** which was then reduced with $NaBH_4$ to give the desired α-hydroxyl carboxylic acid as a diastereomer mixture. Each diastereomer was easily separated by preparative HPLC to give (*R*)- or (*S*)-hydroxyl derivatives **10** to **13** (Figure S1). Absolute configuration of the purified diasteromer was determined by ^{1}H NMR using a chiral anisotropic reagent, phenylglycine methyl ester (PGME), as reported by Kusumi *et al*[15]. Each purified diastereomer was coupled with (*S*)- and (*R*)-phenyl glycine methyl ether and ^{1}H NMR was recorded. Signals of α-proton and P_1 site side chain protons

were shifted by the effects of a benzene ring of (*S*)- or (*R*)-PGME. The positive or negative shifts ($\Delta\delta=\delta_S-\delta_R$) were clearly observed as expected from the literature to determine each absolute configuration (Figure S2).



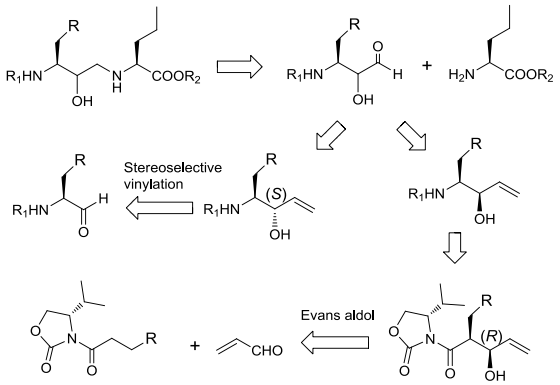
Scheme 1

Using the (*R*)-hydroxymethyl carboxylic acid thus obtained, HMC-type inhibitors were synthesized by conventional Fmoc-based solid phase peptide synthesis (Scheme 2). Fmoc-Nva-OH was coupled with 2-chlorotrityl chloride resin and the Fmoc group was removed by 20% piperidine. Fmoc-protected (*R*)-hydroxyl carboxylic acid *R*-**10**, **11**, **12**, or **13** was then condensed by an EDCI/HOBt-mediated reaction. Fmoc-Thi-OH and Fmoc-Ile-OH were then condensed successively by the combination of piperidine-mediated Fmoc-deprotection and DIC/HOBt-mediated coupling. N-terminal Boc-Glu(O*t*-Bu)-OH was similarly incorporated and the resulting resin was treated with TFA/H_2O/thioanisole. The crude product was purified by preparative HPLC to give *R*-**14**, **15**, **16**, or **17**, each having a single major peak on analytical HPLC (Figure S3). An HMC-type inhibitor having (*S*)-hydroxyl configuration containing 2-thienyl *S*-**14**, isopropyl *S*-**15**, cyclohexyl *S*-**16**, or phenyl *S*-**17** group at the P_1 site was similarly synthesized and purified.



Scheme 2

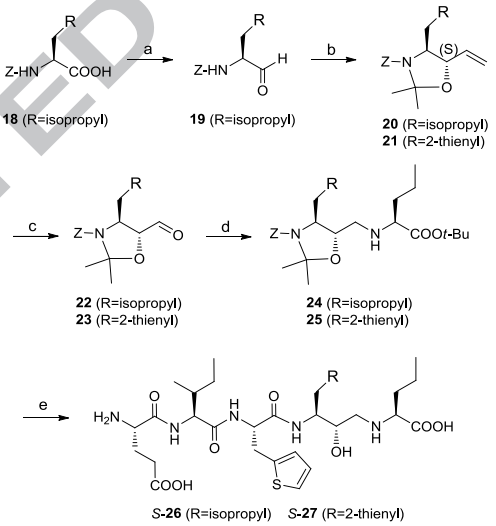
In the synthesis of HEA-type inhibitors, the isostere hydroxyl group was introduced stereo-selectively, since the above PGME method could not be applied to determine the hydroxyl configuration in the HEA scaffold. The HEA backbone was constructed by reductiveamination of α-hydroxyl β-amino aldehyde with a norvaline derivative (Scheme 3). The α-hydroxyl group in the α-hydroxyl β-amino aldehyde was stereo-selectively prepared according to the published procedure. The (*S*)-hydroxyl group of an olefinic 1, 2-amino alcohol derivative was constructed by stereo-selective vinylation[16] of amino aldehydes, and the Evans[17] aldol reaction was employed to construct the (*R*)-hydroxyl group.



Scheme 3

An HEA inhibitor containing an (*S*)-hydroxyl group was synthesized according to the route shown in Scheme 4. A Z-protected amino aldehyde derivative **19** was prepared

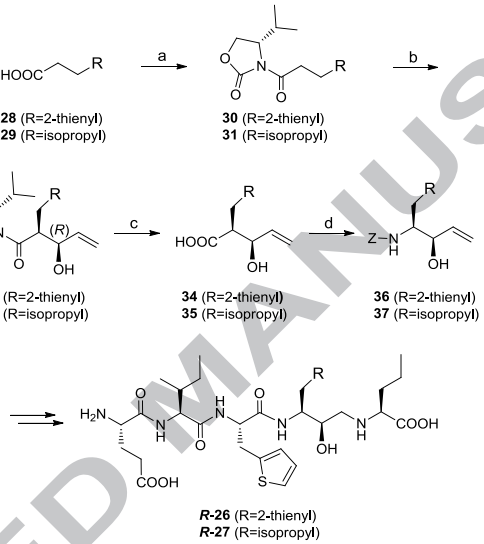
from Z-protected amino acid **18** through the corresponding Weinreb-amide. The aldehyde was reacted with 2-trimethylsilylethyliden triphenylphosphorane according to the published procedure[16] followed by protection as an acetonide to give **20** as a single diastereomer through Cram chelation control. In preparation of the corresponding 2-thienyl derivative **21**, however, the selectivity and isolation yield were insufficient to yield diastereomixtures containing the desired product as a major one. Thus, a simple Grignard reaction using vinylmagnesium chloride was employed for the synthesisis of **21**. Undesired diastereomers could be epimerized to the desired ones after conversion to **23** by treatment with K_2CO_3.[18] Dihydroxylation of the olefin bond of **20** or **21** and $NaIO_4$ mediated oxidation yielded aldehyde **22** or **23**. Reductive amination in the presence of Lewis acid followed by catalytic hydrogenation gave the desired HEA isostere **24** or **25**. Fmoc-Thi-OH, Fmoc-Ile-OH, and N-terminal Boc-Glu(O*t*-Bu)-OH were then condensed successively by the combination of EDCI-mediated coupling and Et_2NH-mediated Fmoc-deprotection. The product was finally treated with TFA/H_2O/thioanisole, and the crude product was purified by preparative HPLC to give a homogeneous product **26** or **27** (Figure S3).



Scheme 4

An HEA inhibitor containing a (*R*)-hydroxyl group was synthesized using the Evans aldol reaction as shown in Scheme 5. Evans asymmetric auxiliary, (*S*)-4-isopropyl-2-oxazolidinone, was introduced to 3-substituted carboxylic acid **28** or **29** through acid anhydride to yield **30** or **31**[19]. 2-Propenal was reacted with Z-enolate of **30** or **31** to yield the desired (*R*)-hydroxyl allyl alcohol derivative **32** or **33** as expected. Removal of the Evans asymmetric auxiliary and subsequent hydrolysis yielded

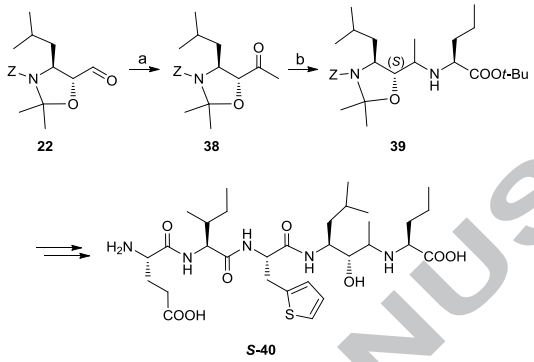
carboxylic acid **34** or **35**. The key intermediate, olefinic 1, 2-amino alcohol **36** or **37** was obtained by Curtius rearrangement followed by protection with the Z group of the resulting amino group. A (*R*)-hydroxyl HEA isostere intermediate was prepared by acetonide formation and following reductive amination with a norvaline derivative as above. Peptide chain elongation to the isoster intermediate and deprotection gave the desired (*R*)-HEA inhibitor ***R*-26** or ***R*-27** without difficulty.



Scheme 5

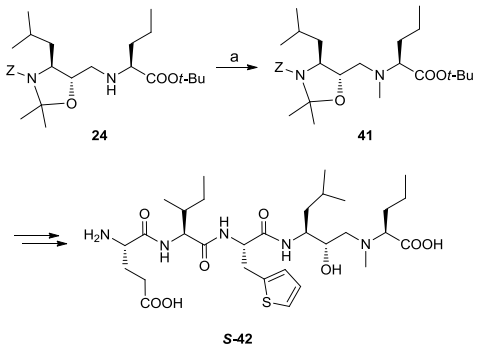
In the synthesis of a substituted HEA-type inhibitor having methyl group at the α-position of the isostere hydroxyl group, coupling with H-Nva-O*t*-Bu with an intermediate ketone by reductiveamination was used (Scheme 6). Previously prepared α-(*S*)-hydroxyl aldehyde **22** was first reacted with MeMgCl followed by oxidation to yield methyl ketone **38**. Imination of **38** with H-Nva-O*t*-Bu proceeded in the presence of 5 equivalent of Ti(O*i*-Pr)$_4$ and following reduction with $NaBH_3CN$ to give the desired product **39** with 74% yield. No imination reaction was observed in the absence of the Lewis acid, and excess or less to 5 equivalent of the Lewis acid lowered the yield. The product was an inseparable diastereomixtures of 3:1. Using this mixture, a substituted HEA-type inhibitor ***S*-40** was synthesized by successive coupling of the corresponding amino acid derivatives and following deprotection as above. The two diastereomers of inhibitor ***S*-40** derived from the substituted methyl group could be separated on HPLC (Figure S4) at this stage, and each product was used for evaluation of the inhibitory activity without determining the absolute configuration. Starting from α-(*R*)-hydroxyl aldehyde, an epimer of **22**, another substituted HEA-type inhibitor ***R*-40** having the

opposite configuration of isostere hydroxyl group was similarly prepared. Diastereomers of the synhtesized inhibitor having an α-(*R*)-hydroxyl group could not be separated on HPLC (Figure S4), and used for the evaluation of the inhibitory activity as a diasteromixture.



Scheme 6

An *N*-Methylated HEA-type inhibitor was synthesized according to the route shown in Scheme 7. Reductiveaminated intermediate synthesized above was methylated by reaction with paraformaldehyde and $NaBH_3CN$ to yield *N*-methylated derivative **41** without difficulties. The product was then used as the starting compound for the *N*-methylated HEA-type inhibitor ***S*-42**. The desired product was purified by preparative HPLC as above. Another *N*-methylated inhibitor ***R*-42** containing an opposite hydroxyl configuration was similarly synthesized.



Scheme 7

2.2. BACE1 inhibitory activity

BACE1 inhibitory activities of the synthesized inhibitors were evaluated using rBACE1[10] with the kinetic parameters of K_m=5.5 μM and k_{cat}=1719 sec^{-1}. The protease

was incubated with an eicosa peptide amide (IKTEEISEVNL*DAEFRHDSG-NH_2; * shows the site of cleavage by BACE1) corresponding to the initial cleavage site of Swedish (SW) mutant APP in the presence of each inhibitor. The mixture was subjected to an analytical HPLC and IC_{50} value calculated from a decrease in the substrate at various inhibitor concentrations was used to evaluate the inhibitory activity (Table 1). A typical sigmoidal curve used to estimate the IC_{50} value is shown in Figure S5.

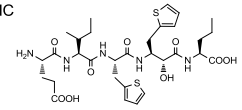
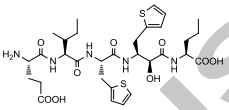
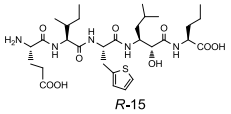
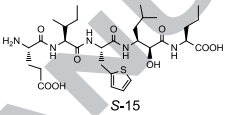
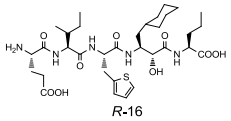
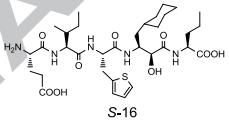
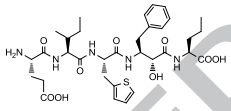
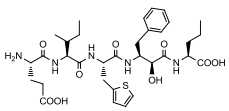
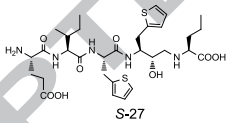
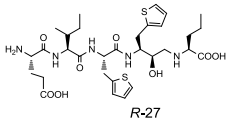
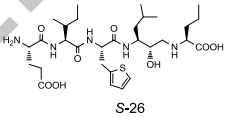
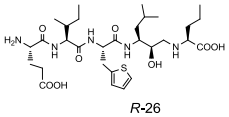
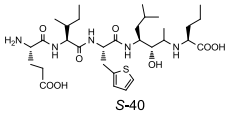
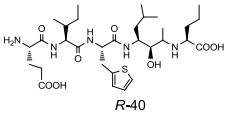
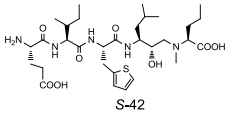
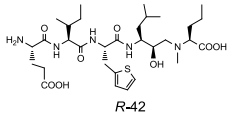
	anti	IC_{50}	*syn*	IC_{50}
HMC	 *R*-14	26 μM	 *S*-14	870 μM
	 *R*-15	56 μM	 *S*-15	> 1mM
	 *R*-16	64 μM	 *S*-16	480 μM
	 *R*-17	34 μM	 *S*-17	870 μM
HEA	 *S*-27	> 2 mM	 *R*-27	9.4 μM
	 *S*-26	> 1 mM	 *R*-26	72 μM
	 *S*-40	> 1 mM	 *R*-40	No inhibition
	 *S*-42	> 1 mM	 *R*-42	680 μM

Table 1

In HMC-type inhibitors, (*R*)-isomer, an *anti*-configuration of the isostere hydroxyl group to the scissile site side-chain substituent, showed inhibitory activity (***R*-14**, ***R*-15**, ***R*-16**, and ***R*-17**) whereas the (*S*)-isomer showed no (***S*-15**; IC_{50}>1 mM) or weak

inhibitory activity (***S*-14**, ***S*-16**, and ***S*-17**). As the P_1 site structure, the aromatic structure (***R*-14** and ***R*-17**) was better than aliphatic structure (***R*-15** and ***R*-16**), and an inhibitor containing the superior sequence including P_1 2-thienyl (***R*-14**) was the most potent inhibitor as expected (IC_{50}=26 μM). (*S*)-isomers having ring structure at the P_1 site (***S*-14**, ***S*-16**, and ***S*-17**) showed weak, but distinct, inhibitory activities (IC_{50}=480~870 μM), which suggest that the P_1 site structure may bring some conformational change taking the (*S*)-hydroxyl group to an accessible distance from the active site Asp residue of BACE1.

In contrast, (*R*)-isomer of HEA-type inhibitors, a *syn*-configuration of the isostere hydroxyl group to the scissile site side-chain substituent, showed inhibitory activity (***R*-27** and ***R*-26**), and no inhibitory activity (IC_{50}>1 mM) was observed for the (*S*)-isomer (***S*-27** and ***S*-26**). An inhibitor containing the superior sequence including P_1 2-thienyl showed 8 times higher inhibitory activity (***R*-27**, IC_{50}=9.4 μM) than the P_1 Leu-type inhibitor (***R*-26**, IC_{50}=72 μM). HEA-type inhibitor ***R*-27** containing the superior sequence had higher activity than the corresponding HMC-type inhibitor **14**, which suggests that the HEA isostere is more suitable than the HMC isostere to integrate into the superior substrate sequence. Introduction of a methyl group at the α-position of the isostere hydroxyl group abolished the inhibitory activity (***R*-** and ***S*-40**), which was restored to some extent by the introduction at the β-position of the isostere hydroxyl group (***R*-42**, IC_{50}=680 μM). The result suggests that interactions of HEA-type inhibitors with BACE1 may be significantly influenced by the prime-site substituent.

2.3. Evaluation of the interactions

The results obtained above strongly suggest that the necessary conformation of the hydroxyl group for potent inhibition depends on the isoster backbone, as well as the prime-site substituent. To clarify the differences by evaluating each interaction with BACE1, the structure of rBACE1 in complex with the inhibitor was revealed by X-ray crystallography. Thus, co-crystals of inhibitor ***R*-14**, ***R*-27**, and ***R*-26** with rBACE1 were prepared and analyzed. Structures of rBACE1 in complex with inhibitors ***R*-14**, ***R*-27**, and ***R*-26** were refined to a resolutions of 2.80 Å, 3.20 Å, and 2.80 Å, respectively (PDB code 4TRY, 4TRZ, and 4TRW). The data obtained were summarized in Table 2.

PDB ID	4TRY complexed with ***R*-14**	4TRZ complexed with ***R*-27**	4TRW complexed with ***R*-26**
Space group	$P12_11$	$P12_11$	$P12_11$
Unit cell parameters			
Length a	82.33	81.87	82.28
Length b	102.62	102.42	103.66
Length c	101.29	101.59	102.00
Angle alpha	90	90	90
Angle beta	103.53	103.49	102.73
Angle gamma	90	90	90
Resolution	2.75	3.25	2.85
Observations			
Unique observations	42426	27835	38857
Redundancy	4.1	3.8	4.1
Completeness	99.7	99.2	99.1
Mean I/sigma(I)	2.31(at 2.77Å)	2.18(at 3.25Å)	2.29(at 2.86Å)
R merge	0.091	0.173	0.10
Refinement			
Resolution range	49.2-2.75	49.1-3.25	49.7-2.85
Rcryst	0.179	0.263	0.177
Rfree	0.252	0.295	0.238
RMSZ from ideal			
Bond length	0.62	0.90	0.61
Bond angle	0.80	0.75	0.80

Table 2

The X-ray crystallography revealed that the overall structures were similarly folded on these inhibitors (Figure 2-a). Each inhibitor was located in the active site cleft taking a linear form. The P_1 and P_3 site substituents directed to rBACE1, whereas the P_2 and P_4 site substituents directed toward outside (Figure S6). The isostere hydroxyl group and its α-positon carbonyl group of the HMC isostere ***R*-14** (IC_{50}=26 μM), and the β-position nitrogen of HEA ***R*-27** (IC_{50}=9.4 μM) and ***R*-26** (IC_{50}=72 μM) were in a hydrogen bond distance from the active center Asp32 and/or Asp228 of rBACE1 (Figure 2-b and S6). The results indicate that the superior substrate sequence contributes to underlying interactions of rBACE1 with the isostere functional groups. Based on these X-ray crystallographic analyses, the interactions of less potent (*S*)-hydroxyl HMC-type inhibitors, containing an opposite configuration hydroxyl group, were estimated using a package for molecular structure analyses, MOE (Molecular Operating Environment). The simulation gave two stable conformation of ***S*-14** (IC_{50}=870 μM); one of which could interact with Asp32 and 228 like the potent inhibitor ***R*-14** (IC_{50}=26 μM), but the other could only interact with Asp32 (Figure S7-a). The predicted low

contribution of the hydroxyl group could explain the lower inhibitory activity of (*S*)-HMC-type inhibitors. The same MOE simulation was also conducted with an (*S*)-HEA-type inhibitor ***S*-26** which showed no inhibitory activity. No predicted stable structure for ***S*-26** could interact with both active center Asp32 and 228 at their hydroxyl or β-site nitrogen atoms like as the active HEA-type inhibitor ***R*-26** (IC_{50}=72 μM) (Figure S7-b). These simulation studies support the essential role of the hydroxyl group of the HEA isosteres incorporated into the superior sequence.

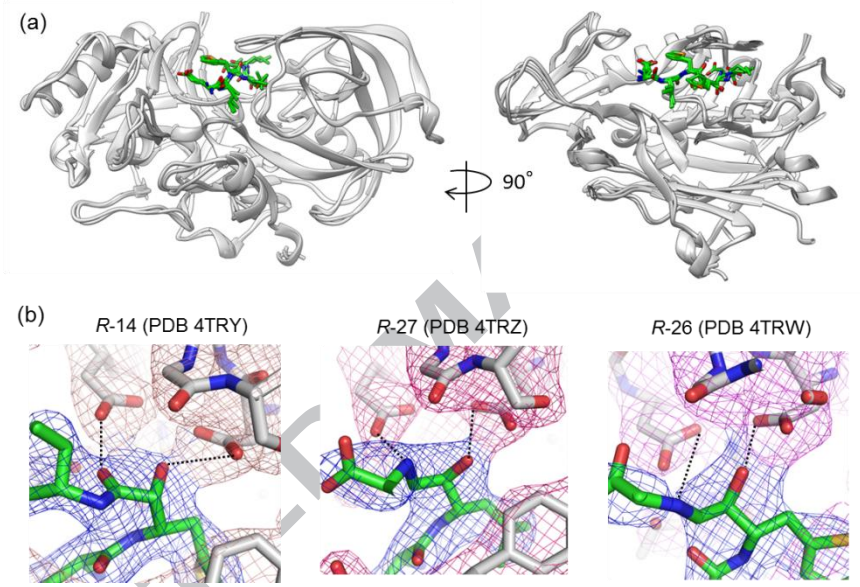


Figure 2

Among the substituents directed toward outside of rBACE1, side-chain carboxylic acid and α-carbonyl of P_4 site Glu in both HMC- and HEA-type inhibitors form hydrogen bonds with side-chain amide of Asn233 and Gln73 of rBACE1, respectively (Figure 3-a). Thus, the P_4 site Glu residue in the superior sequence functions as a kind of hydrogen bond mediator to locate the inhibitor in the flap-closed structure of BACE1. These interactions make it possible to effectively locate the neighboring inside-directed P_3 site substituents in a hydrophobic S_3 pocket formed by Ile110 and a turn structure formed by Ser10 to Gln12 of rBACE1 (Figure 3-b).

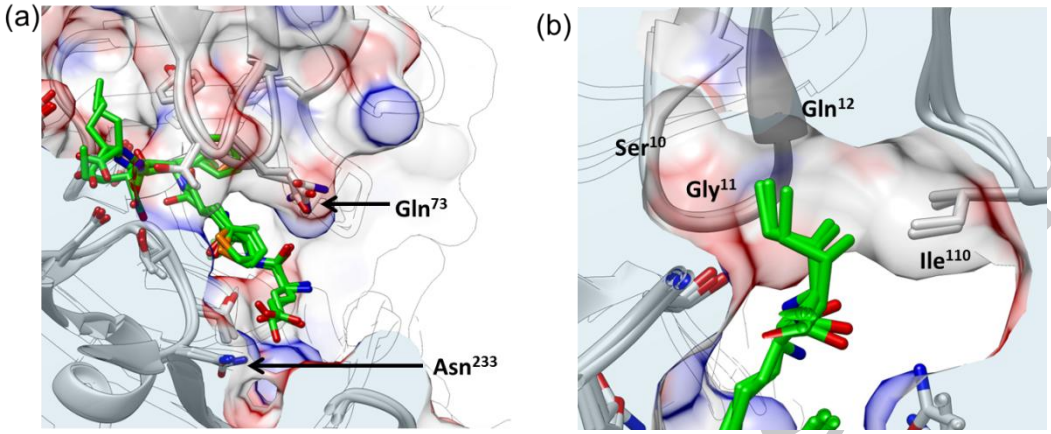


Figure 3

The P_1 site substituents are also directed toward a hydrophobic S_1 pocket formed by Tyr71, Phe108, and Ile118. Although the conformations are nearly the same in ***R*-14, *R*-27, and *R*-26** (Figure 4-a), subtle differences are observed between aliphatic isopropyl and aromatic 2-thienyl substituents. The planer 2-thienyl group of the HEA-type inhibitor ***R*-27** (IC_{50}=9.4 μM) was slightly twisted compared with inhibitors containing the aliphatic isoproyl substituent ***R*-26** (IC_{50}=72 μM). This slight change was transferred to the conformations of neighboring sites, which were forced to add several interactions of inhibitor ***R*-27** with BACE1. Thus, distances between the prime-site carboxyl of Nva and side-cahin hydroxyl group of Thr231 (3.024 Å) and that between the nonprime-site P_2-Thi sulfur atom and side-chain hydroxyl group of Thr72 at the flap region (3.896 Å), as well as the *N*-terminus carboxyl of ***R*-27** and side-cahin amide of Asn233 (2.959 Å) fell into the range of the hydrogen-bond distance (Figure 4-b). These slight changes, as well as the hydrophobic interactions at P_1 and P_3 sites make the HEA-type inhibitor ***R*-27** a suitable isostere to keep rBACE1 in the flap-closed conformation[20]. The important contribution at the prime site interactions was also supported by the results that introduction of a methyl group at the prime site abolished the inhibitory activity (compound ***R*-26** vs. ***R*-40**) probably due to the interception of interactions at these sites, and the activity was recovered to some extent by shifting the substitution position by one-carbon far from the essential hydroxyl group (compound ***R*-40** vs. ***R*-42**).

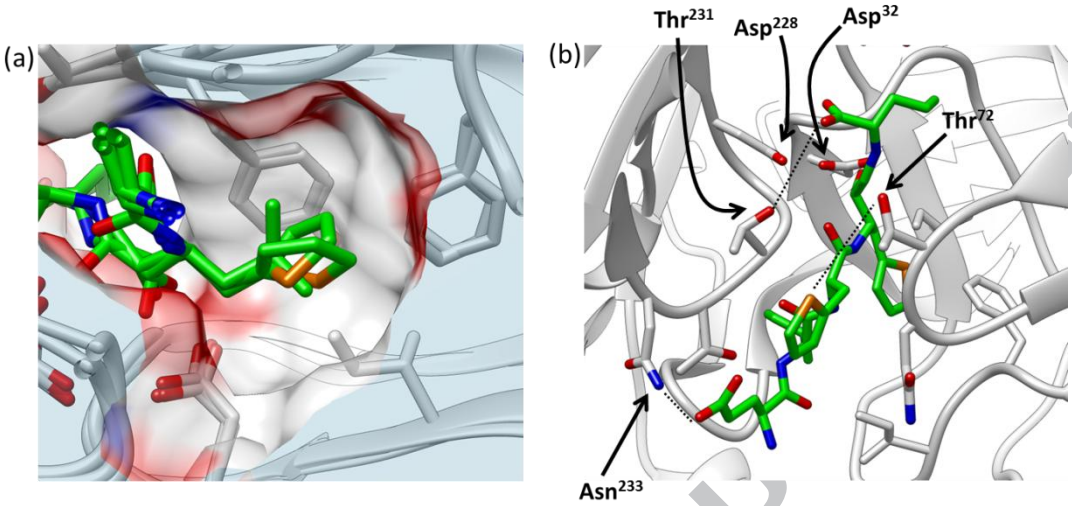


Figure 4

3. Conclusion

Incorporation of transition state mimics for an aspartic protease such as hydroxymethylcarbonyl (HMC)- or hydroxyethylamine (HEA)-isostere into a superior substrate sequence for BACE1 was found to be effective as an initial step for the development of a BACE1 inhibitor. Opposite absolute configurations at the essential hydroxyl group in these isosteres are necessary to bring the isosters in hydrogen bond distances of active center Asp32 and Asp228 of rBACE1. Incorporation of the HEA isostere gave a more potent inhibitor than the incorporation of the HMC isostere. X-ray crystallographic analyses revealed that both inhibitors had similar modes of interaction with rBACE1 at non-prime sites, especially at P_4 and P_3 sites. By P_4 site interactions, both inhibitors are forced to locate at the active center cleft of rBACE1, taking a flap-closed conformation. Hydrophobic side-chains at P_3 and P_1 sites in HMC- and HEA-type inhibitors interacted similarly with the corresponding S_3 and S_1 pockets. Subtle changes in interactions at the P_1 site between the aliphatic isopropyl and aromatic substituents makes the inhibitor ***R*-27** take a twisted conformation at the prime site, resulting in improved interactions with rBACE1. In addition, introduction of a methyl group at the α-position of the HEA-type inhibitors completely abolished the inhibitory activity. Taken together, it was strongly suggested that the inhibitory activity of the HEA-type inhibitors is significantly affected by interactions at the prime site. Replacement of the Nva residues with small aromatic substituents to enhance the interactions at the prime site is now underway.

4. Experimental Section

4.1. General

Melting point was determined with a Yanaco apparatus and was uncorrected. Analytical TLC was performed on silica gel (Silica gel 70 F_{254} TLC Plate-Wako). Column chromatography was carried out on Wakogel® C-200E (particle size, 75-150 μm) or Wakogel® C-300E (particle size, 45-75 μm). ^{1}H NMR spectra (400 or 300 MHz, tetramethylsilane at δ = 0.00 ppm, CH_3COCH_3 δ = 2.04 ppm, or CH_3OH δ = 3.35 ppm as an internal standard) and ^{13}C NMR spectra (100 or 75 MHz, $CDCl_3$ at δ = 77.0 ppm, CD_3COCD_3 δ = 29.3 ppm, or CD_3OD δ = 49.3 ppm as an internal standard) were measured with an Agilent UNITY INOVA 400 NB or Bruker AV-300 FT-NMR spectrometers. The coupling constants were given in Hz. Mass spectra was obtained on JEOL JMS-SX 102A (FAB) or JEOL JMS-GCmate II (EI and CI) mass spectrometers. Optical rotations were determined with a HORIBA SEPA-300 polarimeter.

Preparative HPLC was performed using a COSMOSIL 5C18-ARII column (20 × 250 mm) with a linear gradient of CH_3CN in 0.1% aqueous TFA at a flow rate of 5.0 mL/min on a HITACHI ELITE LaChrom system (OD, 220 nm). For analytical HPLC, a COSMOSIL 5C18-ARII column (4.6 × 150 mm) was employed with a linear gradient of CH_3CN in 0.1% aqueous TFA at a flow rate of 0.9 mL/min on a HITACHI ELITE LaChrom system (OD, 230 nm).

4.1.1. (3*S*)-3-([{(9H-Fluoren-9-yl)methoxy}carbonyl]amino)-2-hydroxy-4-(thiophen-2-yl) butanoic acid *R*- and *S*-10

To a solution of bromide salt **7** (344 mg, 1.65 mmol) was added HATU (531 mg, 1.40 mmol), DIPEA (0.66 mL, 3.8 mmol), and Fmoc-Thi-OH (500 mg, 1.27 mmol) in DMF (4 mL) at 0 °C. After stirring for 14 h at room temperature, the mixture was poured into saturated aqueous NH_4Cl at 0 °C and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was roughly purified by silica gel column chromatography (Ether/Acetone = 2:1). This compound was immediately used for the next step without further purification. The resultant residue was dissolved in DMF/H_2O (1:1, 10 mL). Oxone® (2.13 g, 3.46 mmol) was added to the mixture. After stirring for 30 min at room temperature, the reaction was quenched with 1 mol/L HCl, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. This compound was immediately used for the next step without purification. The residue was dissolved in MeOH (5 mL) and $NaBH_4$ (378 mg, 10.0

mmol) was added at 0 °C. The mixture was stirred for 1.5 h at room temperature. The reaction was quenched with 1 mol/L HCl, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by HPLC to give ***R*-10** (91 mg, 23%) and ***S*-10** (79 mg, 20%).

***R*-10**: $[\alpha]^{28}_D$ – 62 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, CD_3COCD_3): δ = 7.81 (d, *J* = 7.6 Hz, 2H), 7.64-7.61 (m, 2H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.33-7.26 (m, 3H), 6.97-6.92 (m, 2H), 6.44 (d, *J* = 9.2 Hz, 1H), 4.37-4.31 (m, 1H), 4.27 (d, *J* = 2.4 Hz, 2H), 4.24-4.15 (m, 2H), 3.27 (dd, *J* = 14.6, 7.4 Hz, 1H), 3.13 (dd, *J* = 14.6, 7.6 Hz, 1H), 2.02-1.99 (m, 2H); ^{13}C NMR (100 MHz, CD_3COCD_3): δ = 173.6, 156.2, 144.54, 144.49, 141.5, 140.8, 128.0, 127.44, 127.40, 127.2, 126.6, 125.7, 124.5, 120.2, 70.8, 66.6, 55.8, 47.4, 32.1; HRMS (FAB) Calcd. For $C_{23}H_{22}NO_5S$ $[M+H]^+$: 424.1219. Found: 424.1212.

***S*-10**: $[\alpha]^{28}_D$ –13 (*c* 1.0 , MeOH); ^{1}H NMR (400 MHz, CD_3COCD_3): δ = 7.80 (d, *J* = 7.6 Hz, 2H), 7.62-7.61 (m, 2H), 7.35 (t, *J* = 7.4 Hz, 2H), 7.26 (t, *J* = 7.4 Hz, 2H), 7.19 (d, *J* = 4.4 Hz, 1H), 6.90-6.85 (m, 2H), 6.64 (brd, *J* = 9.2 Hz, 1H), 4.33 (d, *J* = 4.0 Hz, 1H), 4.26 (dd, *J* = 8.4, 5.6 Hz, 2H), 4.18-4.11 (m, 2H), 3.16 (dd, *J* = 15.2, 10.4 Hz, 1H), 3.04 (dd, *J* = 15.0, 3.4 Hz, 1H), 2.03-1.99 (m, 2H); ^{13}C NMR (100 MHz, CD_3COCD_3): δ = 173.6, 156.3, 144.51, 144.50, 141.9, 141.1, 127.9, 127.4, 127.0, 126.4, 125.7, 125.6, 124.3, 120.2, 72.8, 66.5, 56.1, 56.0, 47.4; HRMS (FAB) Calcd. For $C_{23}H_{22}NO_5S$ $[M+H]^+$: 424.1219. Found: 424.1214.

4.1.2. (3*S*)-3-([{(9H-Fluoren-9-yl)methoxy}carbonyl]amino)-2-hydroxy-5-methylhexanoic acid *R*- and *S*-11

Title compounds were similarly prepared starting from Fmoc-Leu-OH as above.

***R*-11**: $[\alpha]^{28}_D$ –24 (*c* 0.10, MeOH); ^{1}H NMR (400 MHz, CD_3COCD_3): δ = 7.84 (d, *J* = 7.6 Hz, 2H), 7.70-7.65 (m, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.32-7.29 (m, 2H), 4.28 (d, *J* = 6.8 Hz, 2H), 4.20-4.17 (m, 3H), 2.06 (dd, *J* = 6.8, 4.4 Hz, 1H), 1.69-1.60 (m, 2H), 1.47-1.31 (m, 2H), 0.94 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.0, 156.9, 143.8, 143.4, 141.3, 141.2, 127.7, 127.0, 125.03, 124.99, 119.9, 72.0, 67.2, 51.6, 47.0, 41.1, 24.6, 23.0, 21.9; HRMS (FAB) Calcd. For $C_{22}H_{25}NNaO_5$ $[M+Na]^+$: 406.1630. Found: 406.1636.

***S*-11**: $[\alpha]^{28}_D$ –14 (*c* 0.10, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ = 7.81 (d, *J* = 7.2 Hz, 2H), 7.70-7.69 (m, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.35-7.31 (m, 2H), 4.43 (dd, *J* = 10.8, 7.2 Hz, 1H), 4.35 (dd, *J* = 10.8, 6.8 Hz, 1H), 4.25-4.22 (m, 2H), 4.11-4.08 (m, 1H), 1.66-1.61 (m, 2H), 1.17-1.11 (m, 1H), 0.96 (d, *J* = 6.4 Hz, 3H), 0.92 (d, *J* = 6.4 Hz,

3H); ^{13}C NMR (100 MHz, CD_3OD): δ = 176.0, 158.8, 145.7, 145.5, 142.9, 129.0, 128.42, 128.40, 126.5, 121.2, 74.7, 68.0, 53.5, 48.8, 39.0, 26.0, 24.4, 22.0; HRMS (FAB) Calcd. For $C_{22}H_{25}NNaO_5$ $[M+Na]^+$: 406.1630. Found: 406.1636.

4.1.3.

(3*S*)-3-([{(9H-Fluoren-9-yl)methoxy}carbonyl]amino)-4-cyclohexyl-2-hydroxybutanoic acid *R*- and *S*-12

Title compounds were similarly prepared starting from Fmoc-Cha-OH as above.

***R*-12**: $[\alpha]^{28}_D$ –26.6 (*c* 1.01, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.71 (d, *J* = 7.6 Hz, 2H), 7.61 (m, 1H), 7.50 (d, *J* = 6.8 Hz, 2H), 7.35 (t, *J* = 7.4 Hz, 2H), 7.28-7.25 (m, 2H), 6.47 (brs, 1H), 5.23 (d, *J* = 9.2 Hz, 1H), 4.33 (d, *J* = 6.0 Hz, 2H), 4.23-4.14 (m, 4H), 1.79 (d, *J* = 12.0 Hz, 1H), 1.65-1.45 (m, 5H), 1.26-1.13 (m, 4H), 0.96-0.83 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.3, 156.9, 143.5, 141.25, 141.20, 127.7, 127.0, 125.1, 125.0, 119.9, 72.1, 67.3, 51.0, 47.0, 39.6, 34.1, 33.6, 32.6, 26.4, 26.2, 26.0; HRMS (FAB) Calcd. For $C_{25}H_{29}NNaO_5$ $[M+Na]^+$: 446.1943. Found: 446.1940.

S-12: $[\alpha]^{28}_D$ –9.88 (*c* 1.03, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.75 (d, *J* = 7.6 Hz, 2H), 7.55 (d, *J* = 6.4 Hz, 2H), 7.39 (t, *J* = 7.2 Hz, 2H), 7.29 (t, *J* = 7.2 Hz, 2H), 5.12 (m, 1H), 4.41 (m, 2H), 4.32 (brs, 1H), 4.20-4.19 (m, 1H), 4.10 (m, 1H), 1.78-1.75 (d, *J* = 12.0 Hz, 1H), 1.64-1.55 (m, 5H), 1.25-1.13 (m, 5H), 0.93-0.77 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.6, 157.3, 143.6, 141.3, 127.8, 127.1, 127.0, 125.0, 124.9, 120.0, 74.1, 67.1, 51.8, 47.1, 36.7, 34,0, 32.1, 26.4, 26.2, 26.0; HRMS (FAB) Calcd. For $C_{25}H_{29}NNaO_5$ $[M+Na]^+$: 446.1943. Found: 446.1946.

4.1.4.

(3*S*)-3-([{(9H-Fluoren-9-yl)methoxy}carbonyl]amino)-2-hydroxy-4-phenylbutanoic acid *R*-and *S*-13

Title compounds were similarly prepared starting from Fmoc-Phe-OH as above.

***R*-13**: $[\alpha]^{28}_D$ –51.3 (*c* 0.785, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3COCD_3): δ = 7.80 (d, *J* = 7.6 Hz, 2H), 7.61 (t, *J* = 7.0 Hz, 2H), 7.37-7.14 (m, 9H), 6.38 (brd, *J* = 9.2 Hz, 1H), 4.31-4.29 (m, 1H), 4.21-4.10 (m, 4H), 2.99 (dd, *J* = 13.2, 7.6 Hz, 1H), 2.89 (dd, *J* = 13.2, 7.6 Hz, 1H), 2.03-1.99 (m, 1H); ^{13}C NMR (100 MHz, CD_3COCD_3): δ = 174.0, 156.1, 144.53, 144.47, 141.5, 138.9, 129.7, 128.7, 127.9, 127.42, 127.38, 126.6, 125.7, 120.2, 70.8, 66.6, 55.8, 47.4, 38.1; HRMS (FAB) Calcd. For $C_{25}H_{24}NO_5$ $[M+H]^+$: 418.1654. Found: 418.1649.

***S*-13**: $[\alpha]^{28}_{D}$ −168 (*c* 0.565, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3COCD_3): δ = 7.79 (d, *J* = 7.6 Hz, 2H), 7.57 (d, *J* = 7.6 Hz, 2H), 7.35 (t, *J* = 7.4 Hz, 2H), 7.29-7.19 (m, 6H), 7.13-7.10 (m, 1H), 6.58 (brd, *J* = 8.8 Hz, 1H), 4.34-4.29 (m, 2H), 4.17-4.08 (m, 3H), 2.92-2.81 (m, 2H), 2.03-2.00 (m, 1H); ^{13}C NMR (100 MHz, CD_3COCD_3): δ = 173.6, 156.2, 144.53, 144.47, 141.5, 139.2, 129.6, 127.9, 127.37, 127.35, 126.5, 125.7, 125.6, 120.2, 73.1, 66.5, 56.0, 47.4, 35.3; HRMS (FAB) Calcd. For $C_{25}H_{24}NO_5$ $[M+H]^+$: 418.1654. Found: 418.1651.

4.1.5. Glu-Ile-Thi-(*R*)HMC(2-thienyl)-Nva ***R*-14**

After agitating 2-chlorotrityl resin (500 mg) in DMF for 20 min, Fmoc-Nva-OH (407 mg, 1.20 mmol) and DIPEA (0.21 mL, 1.20 mmol) was added with DMF. The mixture was agitated for 2 h. After the resin was washed with DMF, MeOH and DIPEA (0.41 mL, 2.40 mmol) in DMF was added to the resin. After agitating for 30 min, the resin was washed with DMF, $CHCl_3$, and DMF to give protected Nva on resin (575 mg). After washed with DMF, piperidine (20%) in DMF was added to the resin. After agitating for 20 min, the deprotected resin was washed with DMF. After agitating in DMF, $CHCl_3$, and DMF for 20 min, ***R*-10** (34.9 mg, 0.0827 mmol), HOBt•H_2O (25.3 mg, 0.165 mmol), EDCI•HCl (31.7 mg, 0.165 mmol), and DIPEA (28 μL, 0.165 mmol) in DMF were added to this resin (114 mg, 0.0532 mmol). The mixture was agitated for 18 h. After the resin was washed with DMF, piperidine (20%) in DMF was added to the resin. After agitating for 20 min, the deprotected resin was washed with DMF. To this resin were added Fmoc-AA-OH (0.0827 mmol), HOBt•H_2O (25.2 mg, 0.165 mmol), DIC (26 μL, 0.165 mmol), and DIPEA (28 μL, 0.099 mmol) in DMF. The mixture was agitated for 2 h. After the resin was washed with DMF, piperidine (20%) in DMF was added to the resin. After agitating for 20 min, the deprotected resin was washed with DMF. Completion of each coupling reaction was ascertained using the Kaiser ninhydrin test. Finally, coupling of Boc-Glu(O*t*-Bu)-OH (25.1 mg, 0.0827 mmol) was carried out with HOBt•H_2O (25.2 mg, 0.165 mmol), DIC (26 μL, 0.165 mmol), and DIPEA (28 μL, 0.165 mmol) in DMF. The mixture was agitated for 2 h, and the resin was washed with DMF. The resin was dried under reduced pressure. TFA/H_2O/thioanisole (95:2.5:2.5) was added to the resin and the mixture was agitated for 2 h. The mixture was filtered, and solvents of the filtrate were removed under reduced pressure. Ether was added to the residue to form the precipitate. The precipitate was washed with ether and purified by preparative HPLC to yield ***R*-14** (28.0 mg, 76%). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.24 (dd, *J* = 5.0, 1.4 Hz, 1H), 7.20 (dd, *J* = 5.0, 1.4 Hz, 1H), 6.97-6.90 (m, 4H), 4.72 (dd, *J* = 9.6, 4.4 Hz, 1H), 4.50 (dd, *J* = 7.5, 2.1 Hz, 1H), 4.42 (d, *J* = 7.8, 5.0 Hz, 1H),

4.33 (d, *J* = 7.6 Hz, 1H), 4.13 (d, *J* = 2.4 Hz, 1H), 3.97 (d, *J* = 6.4 Hz, 1H), 3.31 (d, *J* = 4.0 Hz, 1H), 3.21 (dd, *J* = 14.8, 7.6 Hz, 1H), 3.12 (dd, *J* = 15.2, 9.6 Hz, 1H), 3.05 (dd, *J* = 14.6, 7.4 Hz, 1H), 2.50-2.40 (m, 2H), 2.10 (d, *J* = 3.2 Hz, 1H), 2.06 (d, *J* = 8.4 Hz, 1H), 1.91-1.71 (m, 3H), 1.58-1.52 (m, 1H), 1.45-1.37 (m, 2H), 1.20-1.13 (m, 1H), 0.99-0.91 (m, 9H); HRMS (FAB) Calcd. For $C_{31}H_{46}N_5O_9S_2$ $[M+H]^+$: 696.2737. Found: 696.2740.

4.1.6. Glu-Ile-Thi-(*R*)HMC(isopropyl)-Nva *R*-15

^{1}H NMR (400 MHz, CD_3OD): δ = 7.76 (brd, *J* = 9.2 Hz, 1H), 7.20 (dd, *J* = 5.0, 1.0 Hz, 1H), 6.95 (m, 1H), 6.92 (dd, *J* = 5.2, 3.6 Hz, 1H), 4.69 (dd, *J* = 9.6, 4.4 Hz, 1H), 4.43-4.40 (m, 2H), 4.35 (d, *J* = 7.6 Hz, 1H), 4.06 (d, *J* = 2.4 Hz, 1H), 3.97 (t, *J* = 6.4 Hz, 1H), 3.31 (d, *J* = 4.4 Hz, 1H), 3.13 (dd, *J* = 15.0, 9.6 Hz, 1H), 2.45-2.44 (m, 2H), 2.08 (q, *J* = 7.1 Hz, 2H), 1.96-1.74 (m, 3H), 1.63-1.53 (m, 3H), 1.46-1.39 (m, 3H), 1.21-1.14 (m, 1H), 1.00-0.91 (m, 15H); HRMS (FAB) Calcd. For $C_{30}H_{50}N_5O_9S$ $[M+H]^+$: 656.3329. Found: 656.3326.

4.1.7. Glu-Ile-Thi-(*R*)HMC(cyclohexyl)-Nva *R*-16

^{1}H NMR (400 MHz, CD_3OD): δ = 7.20 (dd, *J* = 5.0, 1.4 Hz, 1H), 6.95-6.94 (m, 1H), 6.91 (dd, *J* = 5.0, 3.4 Hz, 1H), 4.71 (dd, *J* = 9.6, 4.4 Hz, 1H), 4.44-4.40 (m, 2H), 4.36 (d, *J* = 8.0 Hz, 1H), 4.05 (d, *J* = 2.4 Hz, 1H), 3.99 (t, *J* = 6.4 Hz, 1H), 3.30 (d, *J* = 4.4 Hz, 1H), 3.12 (dd, *J* = 15.2, 10.0 Hz, 1H), 2.44 (dd, *J* = 13.4, 7.8 Hz, 2H), 2.10 (dd, *J* = 14.4, 7.6 Hz, 2H), 1.92-1.71 (m, 9H), 1.60-1.38 (m, 6H), 1.33-1.14 (m, 5H), 1.02-0.92 (m, 9H); HRMS (FAB) Calcd. For $C_{33}H_{54}N_5O_9S$ $[M+H]^+$: 696.3642. Found: 696.3637.

4.1.8. Glu-Ile-Thi-(*R*)HMC(phenyl)-Nva *R*-17

^{1}H NMR (400 MHz, CD_3OD): δ = 7.32-7.28 (m, 4H), 7.23-7.21 (m, 1H), 7.19 (dd, *J* = 4.6, 1.8 Hz, 1H), 6.91-6.89 (m, 2H), 4.72 (dd, *J* = 9.6, 4.4 Hz, 1H), 4.51 (td, *J* = 7.6, 1.9 Hz, 1H), 4.41-4.37 (m, 1H), 4.34 (d, *J* = 8.0 Hz, 1H), 4.05 (d, *J* = 2.0 Hz, 1H), 4.01-3.98 (m, 1H), 3.30 (dd, *J* = 15.0, 4.2 Hz, 1H), 3.10 (dd, *J* = 15.2, 9.6 Hz, 1H), 2.96 (dd, *J* = 13.4, 7.8 Hz, 1H), 2.86 (dd, *J* = 13.4, 7.4 Hz, 1H), 2.47-2.42 (m, 2H), 2.09-2.06 (m, 2H), 1.90-1.70 (m, 3H), 1.56-1.51 (m, 1H), 1.50-1.33 (m, 2H), 1.17-1.11 (m, 1H), 0.96-0.86 (m, 9H); HRMS (FAB) Calcd. For $C_{33}H_{48}N_5O_9S$ $[M+H]^+$: 690.3173. Found: 690.3176.

4.1.9. Glu-Ile-Thi-(*S*)HMC(2-thienyl)-Nva *S*-14

^{1}H NMR (400 MHz, CD_3OD): δ = 7.24 (dd, *J* = 5.0, 1.4 Hz, 1H), 7.20 (dd, *J* = 5.0,

1.4 Hz, 1H), 6.97-6.90 (m, 4H), 4.72 (dd, *J* = 9.6, 4.4 Hz, 1H), 4.50 (dd, *J* = 7.5, 2.1 Hz, 1H), 4.42 (d, *J* = 7.8, 5.0 Hz, 1H), 4.33 (d, *J* = 7.6 Hz, 1H), 4.13 (d, *J* = 2.4 Hz, 1H), 3.97 (d, *J* = 6.4 Hz, 1H), 3.34 (d, *J* = 1.6 Hz, 1H), 3.21 (dd, *J* = 14.8, 7.6 Hz, 1H), 3.12 (dd, *J* = 15.2, 9.6 Hz, 1H), 3.05 (dd, *J* = 14.6, 7.4 Hz, 1H), 2.50-2.40 (m, 2H), 2.10 (d, *J* = 7.2 Hz, 1H), 2.06 (d, *J* = 8.4 Hz, 1H), 1.93-1.71 (m, 3H), 1.58-1.52 (m, 1H), 1.46-1.33 (m, 2H), 1.21-1.13 (m, 1H), 0.99-0.91 (m, 9H); HRMS (FAB) Calcd. For $C_{31}H_{46}N_5O_9S_2$ $[M+H]^+$: 696.2737. Found: 696.2732.

4.1.10. Glu-Ile-Thi-(*S*)HMC(isopropyl)-Nva *S*-15

^{1}H NMR (300 MHz, CD_3OD): δ = 7.99 (brd, *J* = 8.7 Hz, 1H), 7.20 (dd, *J* = 4.8, 1.8 Hz, 1H), 6.91-6.89 (m, 2H), 4.64 (dd, *J* = 8.7, 5.7 Hz, 1H), 4.43 (dd, *J* = 8.3, 5.0 Hz, 1H), 4.35-4.30 (m, 2H), 4.05 (d, *J* = 2.4 Hz, 1H), 3.94 (t, *J* = 6.3 Hz, 1H), 3.15 (dd, *J* = 14.9, 8.6 Hz, 1H), 2.46-2.41 (m, 2H), 2.06 (q, *J* = 7.0 Hz, 2H), 1.89-1.71 (m, 3H), 1.64-1.49 (m, 3H), 1.46-1.31 (m, 2H), 1.21-1.11 (m, 1H), 1.07-1.00 (m, 1H), 0.97-0.86 (m, 12H), 0.82 (d, *J* = 6.6 Hz, 3H); HRMS (FAB) Calcd. For $C_{30}H_{50}N_5O_9S$ $[M+H]^+$: 656.3329. Found: 656.3333.

4.1.11. Glu-Ile-Thi-(*S*)HMC(cyclohexyl)-Nva *S*-16

^{1}H NMR (400 MHz, CD_3OD): δ = 7.24 (dd, *J* = 5.0, 1.4 Hz, 1H), 6.97-6.97 (m, 1H), 6.95 (dd, *J* = 5.0, 3.4 Hz, 1H), 4.69 (dd, *J* = 8.8, 5.6 Hz, 1H), 4.47 (dd, *J* = 8.2, 5.0 Hz, 1H), 4.39-4.37 (m, 1H), 4.35 (d, *J* = 8.0 Hz, 1H), 4.09 (d, *J* = 3.2 Hz, 1H), 3.98 (t, *J* = 6.2 Hz, 1H), 3.37 (dd, *J* = 7.6, 6.0 Hz, 1H), 3.21 (dd, *J* = 14.8, 8.4 Hz, 1H), 2.47 (td, *J* = 7.6, 2.7 Hz, 2H), 2.11 (dd, *J* = 14.0, 7.6 Hz, 2H), 1.89-1.77 (m, 4H), 1.67-1.56 (m, 6H), 1.47-1.41 (m, 2H), 1.33-1.16 (m, 8H), 1.01-0.93 (m, 9H); HRMS (FAB) Calcd. For $C_{33}H_{54}N_5O_9S$ $[M+H]^+$: 696.3642. Found: 696.3635.

4.1.12. Glu-Ile-Thi-(*S*)HMC(phenyl)-Nva *S*-17

^{1}H NMR (400 MHz, CD_3OD): δ = 7.28-7.21 (m, 4H), 7.18-7.15 (m, 1H), 6.94-6.92 (m, 2H), 4.61 (dd, *J* = 8.8, 5.2 Hz, 1H), 4.52-4.46 (m, 2H), 4.24 (d, *J* = 8.0 Hz, 1H), 4.19 (d, *J* = 3.2 Hz, 1H), 3.97 (t, *J* = 6.4 Hz, 1H), 3.27 (dd, *J* = 13.6, 8.4 Hz, 1H), 3.13 (dd, *J* = 15.0, 9.0 Hz, 1H), 2.81 (d, *J* = 7.2 Hz, 1H), 2.45 (td, *J* = 7.6, 3.2 Hz, 1H), 2.09 (q, *J* = 6.9 Hz, 2H), 1.92-1.64 (m, 4H), 1.51-1.42 (m, 2H), 1.37-1.33 (m 3H), 1.14-1.11 (m, 1H), 0.99 (t, *J* = 7.0 Hz, 3H), 0.90 (t, *J* = 7.2 Hz, 3H), 0.76 (d, *J* = 6.8 Hz, 3H); HRMS (FAB) Calcd. For $C_{33}H_{48}N_5O_9S$ $[M+H]^+$: 690.3173. Found: 690.3173.

4.1.13. (4*S*,5*S*)-Benzyl 4-isobutyl-2,2-dimethyl-5-vinyloxazolidine-3-carboxylate 20.

To a suspension of $LiAlH_4$ (36 mg, 0.96 mmol) in THF (mL) was added Z-Ile-N(OMe)Me (380 mg, 0.96 mmol) in THF (5 mL) at 0 ^oC, stirred for 15 min at same temperature. The reaction was quenched with saturated aqueous H_2O, filtered, and concentrated. The residue was roughly purified by silica gel column chromatography (hexane/AcOEt = 4:1) to give Z-Leu-al **19**, which was immediately used for the next step without further purification. To a solution of methyltriphenylphosphonium bromide (686 mg, 1.92 mmol) in THF (5 mL) was added *n*-BuLi (1.3 mL, 2.1 mmol, 1.6 mol/L in hexane) at 0 ^oC. The mixture was stirred for 1 h at room temperature, and then methyltrimethylsillyl iodide (286 μL, 1.92 mmol) was added at 0 ^oC. The resultant mixture was stirred for 1 h at room temperature. *n*-BuLi (1.3 mL, 2.1 mmol, 1.6 mol/L in hexane) was added to the mixture at −78 ^oC. The reaction mixture was stirred for 1 h at room temperature, and then Z-Leu-al **19** in ether (3 mL) was added. The resultant mixture was stirred for 1 h at room temperature. The reaction was quenched with saturated aqueous NH_4Cl, and the whole was extracted with ether. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was dissolved in THF (3 mL) and TBAF (0.96 mL, 0.96 mmol, 1.0 mol/L in THF) was added at 0 ^oC and stirred for 1 h at room temperature. The reaction was quenched with saturated aqueous NH_4Cl, and the whole was extracted with ether. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was roughly purified by silica gel column chromatography (hexane/EtOAc = 3:1). The resultant residue was dissolved in acetone (8 mL) and 2,2-dimethoxypropane (2.5 mL) and BF_3•Et_2O (25 μL, 0.20 mmol) was added. The mixture was stirred for 5 h at room temperature. The reaction was quenched with Et_3N at 0 ^oC, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 6:1) to give **20** as a single diastereomer (663 mg, 18%, 3 steps). $[\alpha]^{25}_D$ +15 (*c* 0.49, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.37-7.31 (m, 5H), 5.95 (ddd, *J* = 17.2, 10.1, 7.1 Hz, 1H), 5.32 (brd, *J* = 17.1 Hz, 1H), 5.21 (d, *J* = 10.2 Hz, 1H), 5.18 (d, *J* = 12.0 Hz, 1H), 5.09 (brd, *J* = 12.3 Hz, 1H), 4.30 (dd, *J* = 7.2, 3.6 Hz, 1H), 3.84 (brs, 1H), 1.65-1.47 (m, 9H), 0.97-0.80 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 152.2, 137.8, 136.4, 128.5, 128.10, 128.05, 117.6, 94.6, 82.0, 66.8, 60.3, 43.4, 27.4, 25.4, 23.8, 21.3; HRMS (EI) Calcd. For $C_{19}H_{27}NO_3$ $[M]^+$: 317.1991. Found: 317.1987.

4.1.14. Z-Thi-N(OMe)Me

To a solution of *N*,*O*-dimethylhydroxyamine hydrochloride (0.68 g, 7.0 mmol) was added Z-Thi-OH (1.8 g, 5.8 mmol), BOP (2.8 g, 6.4 mmol), and NMM (1.3 g, 12 mmol) in CH_2Cl_2 (12 mL) at 0 ^oC. The mixture was stirred for 48 h at room temperature. The

reaction was quenched with water and the whole was extracted with EtOAc. The organic layer was washed with 1 mol/L HCl, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 1:1) to give Z-Thi-N(OMe)Me (2.0 g, 97%). $[\alpha]^{29}_D$ +17.6 (*c* 1.18, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37-7.29 (m, 5H), 7.15 (dd, *J* = 5.2, 0.8 Hz, 1H), 6.91 (dd, *J* = 5.2, 3.6 Hz, 1H), 6.81 (d, *J* = 3.2 Hz, 1H), 5.59 (d, *J* = 8.8 Hz, 1H), 5.12 (d, *J* = 12.4 Hz, 1H), 5.07 (d, *J* = 12.4 Hz, 1H), 4.99-4.97 (m, 1H), 3.72 (s, 3H), 3.30 (dd, *J* = 15.0, 5.4 Hz, 1H), 3.22-3.16 (m, 1H), 3.20 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.1, 155.7, 137.9, 136.3, 128.5, 128.1, 128.0, 126.9, 126.6, 124.6, 66.8, 61.6, 52.2, 32.6, 32.2; HRMS (EI) Calcd. For $C_{17}H_{20}N_2O_4S$ $[M]^+$: 348.1144. Found: 348.1140.

4.1.15. Benzyl {(2S,3S)-3-hydroxy-1-(thiophen-2-yl)pent-4-en-2-yl}carbamate 21

To a solution of Z-Thi-N(OMe)Me (0.63 g, 1.8 mmol) in CH_2Cl_2 (10 mL) was added DIBALH (3.6 mL, 3.6 mmol, 1.0 mol/L in hexane) at –78 °C, stirred for 15 min at the same temperature under an argon gas atomosphere. The reaction was quenched with MeOH and the temperature was gradually raise upto room temperature and then filtered through silica gel and celite pad and concentrated. The residue was roughly purified by silica gel column chromatography (hexane/EtOAc = 3:1). The resulting Z-Thi-al was immediately used for the next step without further purification. To a solution of vinylmagnesium chloride (12 mL, 18 mmol, 1.45 mol/L in THF) was added Z-Thi-al in THF (3 mL) under an argon gas atmosphere at 0 °C. The mixture was stirred for 12 h at the same temperature. The reaction was quenched with saturated aqueous NH_4Cl, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was roughly purified by silica gel column chromatography (hexane/EtOAc = 3:1). To a solution of the residue and 2,2-dimethoxypropane (0.54 mL, 4.4 mmol) in acetone (4 mL) was added BF_3•Et_2O (1 drop from syringe). The mixture was stirred for 4 h at room temperature. The reaction was quenched with Et_3N and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 10:1) to give **21** (0.22 g, 33%, 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$, 2:1 diasteromer mixture, major isomer): δ = 7.39-7.30 (m, 5H), 7.15 (d, *J* = 4.8 Hz, 1H), 6.92 (dd, *J* = 4.4, 3.6 Hz, 1H), 6.73 (brs, 1H), 5.86-5.76 (m, 1H), 5.25-5.10 (m, 2H), 5.20 (brs, 2H), 4.40 (t, *J* = 6.2 Hz, 1H), 3.88 (td, *J* = 6.8, 2.0 Hz, 1H), 3.16-3.10 (m, 2H), 1.68 (s, 1.5H), 1.65 (brs, 1.5H), 1.60 (s, 1.5H), 1.54 (brs, 1.5H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.2, 140.9, 136.3, 131.8, 128.5, 128.2, 128.1, 126.8, 126.1, 124.5, 118.9, 93.5, 79.8, 77.7, 67.0, 62.2, 31.7, 31.1, 27.1, 23.7; HRMS (EI)

Calcd. For $C_{20}H_{23}NO_3S$ $[M]^+$: 357.1399. Found: 357.1401.

4.1.16. (4*S*,5*R*)-Benzyl 5-formyl-2,2-dimethyl-4-(thiophen-2-ylmethyl)oxazolidine-3-carboxylate 23

To a suspension of $K_2OsO_2(OH)_4$ (4.4 mg, 0.012 mmol), $K_3Fe(CN)_6$ (0.60 g, 1.8 mmol) K_2CO_3 (0.25 g, 1.8 mmol) and $MeSO_2NH_2$ (57 g, 0.60 mmol) in *t*-BuOH/H_2O (1:1, 3 mL) was added **21** (0.22 g, 6.0 mmol) in *t*-BuOH/H_2O (1:1, 3 mL) at 0 ^oC. After stirring for 20 h at the same temperature, the reaction was quenched with aqueous Na_2SO_3, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was roughly purified by silica gel column chromatography (hexane/EtOAc = 3:1). To the product in Et_2O/H_2O (1:1, 3 mL) was added $NaIO_4$ (0.25 g, 1.2 mmol) at 0 ^oC. After stirring for 20 min, the reaction was diluted with water, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was roughly purified by silica gel column chromatography ($CHCl_3$/MeOH = 20:1). The product **23** was immediately used without further purification. ^1H NMR (400 MHz, $CDCl_3$, 2:1 diasteromer mixture): δ = 9.72 (m, 0.67H), 9.47 (m, 0.33H), 7.39-7.32 (m, 5H), 7.17 (dd, *J* = 5.2, 1.2 Hz, 1H), 6.94-6.92 (m, 0.67H), 6.90-6.88 (m, 0.33H), 6.77 (brs, 0.67H), 6.70 (brs, 0.33H), 5.23-5.10 (m, 2H), 4.94 (d, *J* = 12.0 Hz, 0.125H), 4.71 (m, 0.25H), 4.62 (m, 0.125H), 4.55 (d, *J* = 5.6 Hz, 0.25H), 4.48 (td, *J* = 9.2, 2.9 Hz, 0.5H), 4.31 (brs, 0.5H), 4.14 (m, 0.25H), 3.41-3.05 (m, 2H), 1.71-1.55 (m, 6H).

4.1.17. Z-(*S*)HEA(2-thienyl)-Nva-Ot-Bu 25

Compound **23** in MeOH (3 mL) was added K_2CO_3 (80 mg, 0.58 mmol). After stirring for 2 h, the mixture was filtered and washed bith brine, dried over Na_2SO_4, filtered, and concentrated. The residue was roughly purified by silica gel column chromatography ($CHCl_3$/MeOH = 100:1). This compound was immediately used for the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$): δ = 9.72 (m, 1H), 7.39-7.34 (m, 5H), 7.18-7.13 (m, 1H), 6.94-6.89 (m, 1H), 6.78-6.74 (m, 1H), 5.20-5.07 (m, 2H), 4.73-4.72 (m, 0.25H), 4.50-4.47 (m, 0.5H), 4.31 (brs, 0.5H), 4.13-4.11 (m, 0.25H), 4.04-4.02 (m, 0.5H), 3.49-3.07 (m, 2H), 1.57 (brs, 6H).

To a mixture of the resultant residue and HCl•H_2N-Nva-O*t*-Bu (96 mg, 0.46 mmol) was added Ti(O*i*-Pr)$_4$ (0.68 mL, 2.3 mmol). After stirring for 3 h, the mixture was diluted with EtOH (2 mL) and then, $NaBH_3CN$ (58 mg, 0.92 mmol) was added at 0 ^oC. The resulting mixture was stirred for 5 min at room temperature. The reaction was quenched with H_2O and the whole was extracted with EtOAc. The organic layer was

washed with 1 mol/L HCl, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 6:1) to give **25** (83 mg, 27%, 4 steps from **21**). $[\alpha]^{28}_D$ –3.8 (*c* 0.98, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42-7.31 (m, 5H), 7.14 (dd, *J* = 5.2, 1.2 Hz, 1H), 6.91 (dd, *J* = 5.0, 3.4 Hz, 1H), 6.81-6.75 (m, 1H), 5.20-5.13 (m, 2H), 4.11 (quint., *J* = 4.3 Hz, 1H), 3.90 (brs, 1H), 3.36-3.09 (m, 2H), 3.00 (brt, *J* = 6.6 Hz, 1H), 2.71 (dd, *J* = 11.8, 8.6 Hz, 1H), 2.32 (dd, *J* = 11.6, 4.4 Hz, 1H), 1.72 (brs, 3H), 1.65 (brs, 3H), 1.54-1.46 (m, 2H), 1.44 (s, 9H), 1.38-1.26 (m, 3H), 0.89 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 174.6, 152.2, 136.3, 128.6, 128.13, 128.08, 126.9, 126.4, 124.4, 95.2, 81.9, 79.3, 66.9, 62.1, 61.3, 51.4, 35.7, 33.3, 28.1, 27.5, 26.6, 19.0, 13.9; HRMS (EI) Calcd. For $C_{28}H_{40}N_2O_5S$ $[M]^+$: 516.2658. Found: 516.2664.

4.1.18. Z-(*S*)HEA(isopropyl)-Nva-O*t*-Bu 24

To a suspension of $K_2OsO_2(OH)_4$ (3.1 mg, 8.36 μmol), $K_3Fe(CN)_6$ (2.06 g, 6.27 mmol) K_2CO_3 (867 mg, 6.27 mmol) and $MeSO_2NH_2$ (199 mg, 2.09 mmol) in *t*-BuOH/H_2O (1:1, 5 mL) was added **20** (663 mg, 2.09 mmol) in *t*-BuOH/H_2O (1:1, 5 mL) at 0 °C. After stirring for 15 h at the same temperature, the reaction was quenched with aqueous Na_2SO_3, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was roughly purified by silica gel column chromatography (hexane/EtOAc = 3:1) to give the corresponding diol. To a solution of the product diol in Et_2O/H_2O (1:1, 3 mL) was added $NaIO_4$ (0.25 g, 1.2 mmol) at 0 °C. After being stirred for 1 h, the reaction was diluted with water, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was roughly purified by silica gel column chromatography (hexane/EtOAc = 3:1) to give **22**. The product was immediately used for the next step without further purification. To a mixture of the residue and HCl•H_2N-Nva-O*t*-Bu (438 mg, 2.09 mmol) was added Ti(O*i*-Pr)$_4$ (3.1 mL, 10.5 mmol). The mixture was diluted with EtOH (5 mL) and then, $NaBH_3CN$ (263 mg, 4.18 mmol) was added at 0 °C. The resulting mixture was stirred for 10 min at room temperature. The reaction was quenched with H_2O, and the whole was extracted with EtOAc. The organic layer was washed with saturated aqueous NH_4Cl, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 3:1) to give **24** (798 mg, 80%). $[\alpha]^{22}_D$ +5.3 (*c* 0.79, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 9:1 amide rotamer, major isomer): δ = 7.36-7.31 (m, 5H), 5.17 (d, *J* = 12.3 Hz, 1H), 5.07 (brd, *J* = 12.3 Hz, 1H), 3.98 (m, 1H), 3.80 (m, 1H), 3.07 (t, *J* = 6.6 Hz, 1H), 2.80 (dd, *J*

= 11.4, 8.7 Hz, 1H), 2.44 (dd, *J* = 11.4, 5.6 Hz, 1H), 1.76 (brs, 3H), 1.65-1.50 (m, 8H), 1.45 (s, 9H), 1.41-1.25 (m, 3H), 0.96-0.86 (m, 6H), 0.90 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 174.7, 152.2, 136.4, 128.4, 128.0, 94.6, 80.9, 66.7, 62.2, 59.1, 52.3, 43.4, 35.9, 29.7, 29.1, 28.1, 27.6, 25.6, 23.8, 21.1, 19.0, 13.9; HRMS (EI) Calcd. For $C_{27}H_{44}N_2O_5$ $[M]^+$: 476.3250. Found: 476.3244.

4.1.19. Glu-Ile-Thi-(*S*)HEA(2-thienyl)-Nva *S*-27

The mixture of **25** (83 mg, 0.16 mmol) and Pd-black (83 mg) in MeOH/Et_3N (3:1, 1 mL) was stirred under hydrogen atmosphere. After stirring for 48 h, the reaction mixture was filtered and concentrated to afford deprotected product. To a solution of the deprotected product in DMF was added Fmoc-Thi-OH (83 mg, 0.21 mmol), HOBt•H_2O (37 mg, 0.24 mmol), EDCI•HCl (46 mg, 0.24 mmol), and NMM (88 μL, 0.80 mmol). After being stirred for 4 h, the reaction mixture was quenched with water and the whole was extracted with ether. The organic layer was washed with saturated aqueous NH_4Cl, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtered, and concentrated. The residue was dissolved in Et_2NH/MeCN (1:1, 1 mL). After stirring for 1 h, solvents were removed under reduced pressure, and the residue was dissolved in DMF. To the solution were added Fmoc-Ile-OH (100 mg, 0.29 mmol), HOBt•H_2O (37 mg, 0.24 mmol), EDCI•HCl (46 mg, 0.24 mmol), and NMM (88 μL, 0.80 mmol). After stirring for 4 h, the reaction mixture was diluted with water and the whole was extracted with ether. The organic layer was washed with brine and dried over Na_2SO_4, filtered, and concentrated. The residue was dissolved in Et_2NH/MeCN (1:1, 1 mL). After being stirred for 1 h, solvents were removed under reduced pressure. The residue was dissolved in DMF and Boc-Glu(O*t*-Bu)-OH (88 mg, 0.29 mmol), HOBt•H_2O (37 mg, 0.24 mmol), EDCI•HCl (46 mg, 0.24 mmol), and NMM (88 μL, 0.80 mmol) were added. After stirring for 4 h, the reaction mixture was diluted with water and the whole was extracted with ether. The organic layer was washed with brine and dried over Na_2SO_4, filtered, and concentrated. Finally, TFA/H_2O/TIS (95:2.5:2.5, 6 mL) was added to the resin and the mixture was agitated for 3 h. The mixture was concentrated. Ether was added to the residue to form the precipitate. The precipitate was washed with ether and purified by preparative HPLC to yield **27** (11 mg, 10%, 7 steps). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.24 (dd, *J* = 4.6, 1.8 Hz, 1H), 7.21 (dd, *J* = 7.0, 3.8 Hz, 1H), 6.93-6.90 (m, 4H), 4.63 (dd, *J* = 8.4, 6.4 Hz, 1H), 4.49 (d, *J* = 5.6 Hz, 0.5H), 4.36 (d, *J* = 5.6 Hz, 0.5H), 4.14 (d, *J* = 7.2 Hz, 1H), 4.07-4.04 (m, 1H), 4.00-3.97 (m, 2H), 3.76 (brt, *J* = 6.0 Hz, 1H), 3.19-3.13 (m, 2H), 3.06 (dd, *J* = 14.8, 8.0 Hz, 1H), 2.88 (dd, *J* = 12.4, 4.0 Hz, 1H), 2.77 (dd, *J* = 12.2, 9.6 Hz, 1H), 2.58-2.51 (m, 2H), 2.20-2.15 (m, 2H), 1.89-1.84 (m, 2H), 1.78-1.76 (m,

1H), 1.52-1.35 (m, 3H), 1.16-1.09 (m, 1H), 0.97 (t, *J* = 7.2 Hz, 3H), 0.96-0.81 (m, 6H); HRMS (FAB) Calcd. For $C_{31}H_{48}N_5O_8S_2$ $[M+H]^+$: 682.2944. Found: 682.2951.

4.1.20. Glu-Ile-Thi-(*S*)HEA(isopropyl)-Nva *S*-26

Compound ***S*-26** was similarly synthesized using **24** (113 mg, 0.237 mmol) as above. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.94 (d, *J* = 9.9 Hz, 1H), 7.28 (dd, *J* = 4.1, 2.6 Hz, 1H), 6.98-6.95 (m, 2H), 4.54 (t, *J* = 7.7 Hz, 1H), 4.27 (d, *J* = 7.8 Hz, 1H), 4.07 (t, *J* = 6.3 Hz, 1H), 3.90 (brd, *J* = 9.9 Hz, 2H), 3.79 (d, *J* = 5.4 Hz, 1H), 3.75 (d, *J* = 3.9 Hz, 1H), 3.26 (dd, *J* = 9.8, 8.0 Hz, 1H), 3.12 (dd, *J* = 12.6, 3.3 Hz, 1H), 2.97 (dd, *J* = 12.3, 9.6 Hz, 1H), 2.54 (t, *J* = 7.7 Hz, 2H), 2.20-2.08 (m, 2H), 1.96-1.88 (m, 3H), 1.61-1.44 (m, 4H), 1.28-1.18 (m, 3H), 1.03 (t, *J* = 7.4 Hz, 3H), 1.02-0.94 (m, 6H), 0.88 (d, *J* = 6.0 Hz, 3H), 0.83 (d, *J* = 6.0 Hz, 3H); HRMS (FAB) Calcd. For $C_{30}H_{52}N_5O_8S$ $[M+H]^+$: 642.3537. Found: 642.3541.

4.1.21. (*S*)-4-Isopropyl-3-{3-(thiophen-2-yl)propanoyl}oxazolidin-2-one 30

To 2-thiophene-propionic acid **28** (5.23 g, 33.5 mmol) in THF (60 mL) were added Et_3N (5.6 mL, 40 mmol) and isobutylchloroformate (5.0 mL, 36.8 mmol) at 0 °C. After stirring for 30 min, the mixture was added to the lithio-(4*S*)-4-isopropyl-2-oxazolidinone, prepared by dropwise addition of *n*-BuLi (14.2 mL, 36.8 mmol, 2.6 mol/L in hexane) to (4*S*)-4-isopropyl-2-oxazolidinone (4.76g, 36.8 mmol) in THF (60 mL) at −78 °C, and the mixture was stirred for 1 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and the whole was extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 5:1) to yield **30** (3.68 g, 97%). $[\alpha]^{28}_D$ +78 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.12 (dd, *J* = 5.2, 1.2 Hz, 1H), 6.91 (dd, *J* = 5.2, 3.2 Hz, 1H), 6.86-6.85 (m, 1H), 4.44 (td, *J* = 8.1, 3.6 Hz, 1H), 4.26 (dd, *J* = 9.2, 8.0 Hz, 1H), 4.21 (dd, *J* = 9.2, 3.2 Hz, 1H), 3.41-3.25 (m, 2H), 3.24-3.19 (m, 2H), 2.41-2.33 (m, 1H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.85 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.7, 153.9, 143.0, 126.7, 124.8, 123.4, 63.4, 58.3, 37.2, 28.2, 24.3, 17.8, 14.5; HRMS (EI) Calcd. For $C_{13}H_{17}NO_3S$ $[M]^+$: 267.0929. Found: 267.0933.

4.1.22. (*S*)-3-{(2*S*,3*R*)-3-Hydroxy-2-(thiophen-2-ylmethyl)pent-4-enoyl}-4-isopropyloxazolidin-2-one 32

To a solution of **30** (8.68 g, 32.5 mmol) in CH_2Cl_2 (60 mL) were added *n*-Bu_2BOTf

(5.70 g, 20.8 mmol) and DIEA (5.80 mL, 32.5 mmol) at 0 °C. After the mixture had been stirred for 40 min, acrolein (0.87 mL, 13 mmol) in CH_2Cl_2 (10 mL) was added. After stirring for 2 h, the temperature was gradually raised up to room temperature. The reaction was quenched with saturated aqueous NH_4Cl and 30% H_2O_2. The mixture was extracted with EtOAc and the whole was washed with saturated aqueous $NaHCO_3$, water, and brine. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 4:1) to yield **32** (6.63 g, 63%). $[\alpha]^{28}_D$ +9.1 (*c* 0.77, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.09 (dd, *J* = 5.2, 1.2 Hz, 1H), 6.86 (dd, *J* = 5.0, 3.4 Hz, 1H), 6.84-6.83 (m, 1H), 5.93 (ddd, *J* = 17.0, 10.6, 6.4 Hz, 1H), 5.35 (td, *J* = 17.2, 1.5 Hz, 1H), 5.25 (td, *J* = 10.4, 1.4 Hz, 1H), 4.67 (td, *J* = 11.1, 4.6 Hz, 1H), 4.45 (m, 1H), 4.43 (td, *J* = 8.4, 3.5 Hz, 1H), 4.21 (t, *J* = 8.8 Hz, 1H), 4.12 (dd, *J* = 9.2, 3.2 Hz, 1H), 3.34 (dd, *J* = 14.4, 11.0 Hz, 1H), 3.12 (dd, *J* = 14.8, 4.2 Hz, 1H), 2.44 (m, 1H), 2.17-2.10 (m, 1H), 0.80 (d, *J* = 6.8 Hz, 3H), 0.50 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.3, 154.1, 140.9, 136.9, 126.7, 125.7, 123.8, 117.1, 73.6, 62.9, 58.5, 49.9, 28.0, 27.9, 17.8, 13.9; HRMS (EI) Calcd. For $C_{16}H_{21}NO_4S$ $[M]^+$: 323.1191. Found: 323.1189.

4.1.23.

(*S*)-3-{(2*S*,3*R*)-3-Hydroxy-2-isobutylpent-4-enoyl}-4-isopropyloxazolidin-2-one 33

Compound **33** was similarly prepared as above starting from 2-isopropyl-propionic acid **29.** $[\alpha]^{27}_D$ +63 (*c* 1.3, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.88 (ddd, *J* = 17.1, 10.5, 6.0 Hz, 1H), 5.28 (td, *J* = 17.2, 1.4 Hz, 1H), 5.20 (td, *J* = 10.5, 1.4 Hz, 1H), 4.50 (td, *J* = 8.1, 3.5 Hz, 1H), 4.39-4.30 (m, 2H), 4.25 (d, *J* = 8.1 Hz, 1H), 4.20 (dd, *J* = 9.3, 3.3 Hz, 1H), 2.39-2.33 (m, 2H), 1.80 (ddd, *J* = 13.3, 10.3, 4.7 Hz, 1H), 1.53-1.45 (m, 1H), 1.36 (ddd, *J* = 13.0, 9.1, 3.7 Hz, 1H), 0.92 (d, *J* = 6.9 Hz, 3H), 0.914 (d, *J* = 6.6 Hz, 3H), 0.910 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.9, 154.3, 137.2, 116.7, 74.4, 63.0, 58.7, 45.8, 37.0, 28.3, 26.3, 23.4, 21.9, 18.0, 14.5; HRMS (EI) Calcd. For $C_{15}H_{25}NO_4$ $[M]^+$: 283.1784. Found: 283.1788.

4.1.24. (2*S*,3*R*)-Methyl 3-hydroxy-2-(thiophen-2-ylmethyl)pent-4-enoate

To a solution of the **32** (6.63 g, 20.5 mmol) in MeOH (40 mL) was added NaOMe (3.32 g, 61.5 mmol) at −15 °C and stirred for 20 min. The reaction was quenched with saturated aqueous NH_4Cl. The mixture was extracted with EtOAc and the whole was washed with 1 mol/L HCl, saturated aqueous $NaHCO_3$, water, and brine. The combined organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 3:1) to afford title

compound (3.34 g, 72%). $[\alpha]^{28}_{D}$ –28 (*c* 0.71, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.13 (dd, *J* = 5.2, 1.2 Hz, 1H), 6.90 (dd, *J* = 5.2, 3.6 Hz, 1H), 6.80 (dd, *J* = 3.4, 1.0 Hz, 1H), 5.90 (ddd, *J* = 16.9, 10.7, 6.1 Hz, 1H), 5.36 (td, *J* = 17.1, 1.4 Hz, 1H), 5.25 (td, *J* = 10.4, 1.4 Hz, 1H), 4.39 (brt, *J* = 5.4 Hz, 1H), 3.65 (s, 3H), 3.26 (dd, *J* = 15.0, 9.8 Hz, 1H), 3.14 (dd, *J* = 15.0, 5.0 Hz, 1H), 2.91 (td, *J* = 9.9, 5.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 141.3, 137.1, 126.8, 125.5, 123.9, 117.1, 72.8, 53.4, 51.9, 27.6; HRMS (EI) Calcd. For $C_{11}H_{14}O_3S$ $[M]^+$: 226.0664. Found: 226.0667.

4.1.25. (2*S*,3*R*)-Methyl 3-hydroxy-2-isobutylpent-4-enoate

$[\alpha]^{28}_{D}$ –6.6 (*c* 0.71, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.85 (ddd, *J* = 17.1, 10.5, 6.0 Hz, 1H), 5.30 (td, *J* = 17.1, 1.5 Hz, 1H), 5.19 (td, *J* = 10.4, 1.4 Hz, 1H), 4.30-4.29 (m, 1H), 3.70 (s, 3H), 2.65 (ddd, *J* = 10.4, 5.0, 4.1 Hz, 1H), 2.55 (brd, *J* = 3.9 Hz, 1H), 1.67 (ddd, *J* = 13.2, 10.5, 5.0 Hz, 1H), 1.58-1.50 (m, 1H), 1.37 (ddd, *J* = 13.2, 9.0, 2.1 Hz, 1H), 0.91 (d, *J* = 6.6 Hz, 3H), 0.89 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.3, 137.5, 116.5, 73.5, 51.7, 49.1, 36.2, 26.2, 23.2, 21.7; HRMS (CI) Calcd. For $C_{10}H_{19}O_3$ $[M+H]^+$: 187.1334. Found: 187.1326.

4.1.26. (2*S*,3*R*)-3-Hydroxy-2-(thiophen-2-ylmethyl)pent-4-enoic acid 34

Methyl ester of **34** obtained above (3.34 g, 14.8 mmol) was dissolved in THF/H_2O (1:1, 15 mL) and 1 mol/L NaOH (15 mL) was added. After stirring for 2 h, the reaction was quenched with 1 mol/L HCl. The mixture was extracted with EtOAc, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 2:1) to yield **34** (3.04 g, 97%). $[\alpha]^{28}_{D}$ –30 (*c* 0.93, $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.14 (dd, *J* = 5.0, 1.0 Hz, 1H), 6.91 (dd, *J* = 5.0, 3.4 Hz, 1H), 6.83-6.82 (m, 1H), 5.91 (ddd, *J* = 17.0, 10.6, 6.2 Hz, 1H), 5.37 (d, *J* = 16.8 Hz, 1H), 5.28 (d, *J* = 10.4 Hz, 1H), 4.43 (brt, *J* = 5.4 Hz, 1H), 3.27 (dd, *J* = 15.0, 9.0 Hz, 1H), 3.09 (dd, *J* = 15.0, 5.2 Hz, 1H), 2.95 (dd, *J* = 9.6, 4.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.3, 141.0, 136.4, 126.9, 125.7, 124.0, 117.8, 72.7, 53.0, 27.2; HRMS (EI) Calcd. For $C_{10}H_{12}O_3S$ $[M]^+$: 212.0507. Found: 212.0504.

4.1.27. (2*S*,3*R*)-3-Hydroxy-2-isobutylpent-4-enoic acid 35

$[\alpha]_D^{28}$ +81 ($CHCl_3$, *c* 1.5); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.00 (brs, 1H), 5.88 (ddd, *J* = 17.0, 10.7, 6.2 Hz, 1H), 5.32 (td, *J* = 17.2, 1.4 Hz, 1H), 5.22 (td, *J* = 10.5, 1.4 Hz, 1H), 4.36 (dd, *J* = 6.0, 5.1 Hz, 1H), 2.69-2.63 (m, 1H), 1.73-1.56 (m, 2H), 1.40-1.30 (m, 1H), 0.93 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.0, 136.9, 117.0, 73.5, 48.9, 35.8, 26.1, 23.2, 21.6; HRMS (EI) Calcd. For $C_9H_{16}O_3$

$[M]^+$: 172.1099. Found: 172.1100.

4.1.28. Benzyl {(2*S*,3*R*)-3-hydroxy-1-(thiophen-2-yl)pent-4-en-2-yl}carbamate 36

To a solution of **34** (3.04 g, 14.3 mmol) in toluene (30 mL) was added DPPA (4.0 mL, 18.6 mmol) and Et_3N (2.6 mL, 18.6 mmol). After stirring for 2 h, 4 mol/L KOH/EtOH (1:1, 8 mL) was added. The mixture was stirred for 2 h under reflux. After cooled to room temperature, the reaction mixture was acidified with 6 mol/L HCl and the whole was extracted with ether. The aqueous layer was bacified with saturated aqueous $NaHCO_3$ and was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue and $NaHCO_3$ (1.44 g, 17.2 mmol) were dissolved in acetone/H_2O (2:1, 30 mL) and then Z-OSu (4.28 g, 17.2 mmol) were added at 0 ^oC. The resultant mixture was stirred for 19 h at room temperature. The reaction was quenched with 1 mol/L HCl and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 3:1) to yield **36** (3.03 g, 67%). M.p. 93-96 ^oC; $[\alpha]^{28}_D$ –29 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.37-7.30 (m, 5H), 7.15 (dd, *J* = 5.2, 1.2 Hz, 1H), 6.92 (dd, *J* = 5.2, 3.2 Hz, 1H), 6.83 (d, *J* = 2.8 Hz, 1H), 5.92 (ddd, *J* = 17.1, 10.9, 5.9 Hz, 1H), 5.36 (d, *J* = 17.6 Hz, 1H), 5.28 (td, *J* = 10.6, 1.3 Hz, 1H), 5.07 (s, 2H), 4.93 (m, 1H), 4.27 (brs, 1H), 4.01 (m, 1H), 3.12-3.04 (m, 2H), 2.47 (brs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.5, 139.8, 136.6, 136.3, 128.4, 128.1, 127.9, 126.9, 126.1, 124.2, 117.3, 74.1, 66.8, 56.5, 29.8; HRMS (EI) Calcd. For $C_{17}H_{19}NO_3S$ $[M]^+$: 317.1086. Found: 317.1084.

4.1.29. Benzyl {(3*R*,4*S*)-3-hydroxy-6-methylhept-1-en-4-yl}carbamate 37

$[\alpha]^{25}_D$ –25 (*c* 1.3, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.36-7.29 (m, 5H), 5.83 (ddd, *J* = 17.2, 10.7, 5.5 Hz, 1H), 5.32 (d, *J* = 17.1 Hz, 1H), 5.22 (td, *J* = 10.5, 1.5 Hz, 1H), 5.10 (s, 2H), 4.84 (brd, *J* = 8.4 Hz, 1H), 4.22 (brs, 1H), 3.89-3.86 (m, 1H), 2.71 (m, 1H), 1.73-1.53 (m, 1H), 1.34-1.24 (m, 2H), 0.92 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.0, 136.6, 136.3, 128.5, 128.1, 128.0, 116.7, 75.5, 66.9, 53.8, 38.8, 24.6, 23.4, 21.6; HRMS (EI) Calcd. For $C_{16}H_{23}NO_3$ $[M]^+$: 277.1678. Found: 277.1681.

4.1.30. Z-(*R*)HEA(2-thienyl)-Nva-O*t*-Bu

$[\alpha]^{28}_D$ –24 (*c* 0.99, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.35-7.31 (m, 5H), 7.10 (d, *J* = 4.8 Hz, 1H), 6.87 (dd, *J* = 5.1, 3.6 Hz, 1H), 6.75 (d, *J* = 3.3 Hz, 1H), 5.11 (d, *J* = 12.3 Hz, 1H), 4.78 (d, *J* = 12.3 Hz, 1H), 4.24-4.16 (m, 2H), 3.21-3.16 (m, 1H), 3.11 (t, *J*

= 6.6 Hz, 1H), 2.96 (dd, *J* = 14.4, 5.3 Hz, 1H), 2.73 (t, *J* = 5.4 Hz, 1H), 1.63 (s, 3H), 1.57 (s, 3H), 1.54-1.46 (m, 3H), 1.43 (s, 9H), 1.38-1.20 (m, 3H), 0.90 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.4, 152.1, 140.7, 136.3, 128.4, 128.1, 126.7, 125.8, 123.9, 93.6, 80.9, 66.7, 62.0, 60.0, 46.5, 35.3, 30.5, 28.1, 27.0, 23.6, 21.8, 18.9, 13.9; HRMS (EI) Calcd. For $C_{28}H_{40}N_2O_5S$ $[M]^+$: 516.2658. Found: 516.2664.

4.1.31. Z-(*R*)HEA(isopropyl)-Nva-O*t*-Bu

$[\alpha]^{25}_D$ −13 (*c* 0.96, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 2:1 amide rotamer, major isomer): δ 7.37-7.31 (m, 5H), 5.15 (d, *J* = 7.2 Hz, 1H), 5.09 (d, *J* = 6.9 Hz, 1H), 4.10-4.06 (m, 1H), 3.17 (t, *J* = 6.8 Hz, 1H), 2.76 (dd, *J* = 11.7, 4.8 Hz, 1H), 2.66 (dd, *J* = 12.0, 7.8 Hz, 1H), 1.59 (s, 3H), 1.57 (s, 3H), 1.46 (s, 9H), 1.42-1.20 (m, 6H), 0.92 (t, *J* = 7.4 Hz, 3H), 0.76 (d, *J* = 6.3 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.5, 152.3, 136.4, 128.6, 128.4, 128.1, 119.2, 93.3, 80.9, 66.7, 62.1, 56.8, 39.6, 35.4, 28.1, 27.2, 24.8, 23.7, 23.1, 22.9, 22.0, 19.0, 13.9; HRMS (EI) Calcd. For $C_{27}H_{44}N_2O_5$ $[M]^+$: 476.3250. Found: 476.3255.

4.1.32. Glu-Ile-Thi-(*R*)HEA(2-thienyl)-Nva *R*-27

^{1}H NMR (400 MHz, CD_3OD): δ 7.28 (dd, *J* = 4.8, 1.6 Hz, 1H), 7.23 (t, *J* = 3.2 Hz, 1H), 6.99-6.94 (m, 4H), 4.65 (t, *J* = 3.4 Hz, 1H), 4.26 (d, *J* = 8.0 Hz, 1H), 4.12-3.95 (m, 2H), 3.85-3.77 (m, 1H), 3.64-3.55 (m, 1H), 3.44 (d, *J* = 15.2 Hz, 1H), 3.30 (dd, *J* = 14.4, 6.8 Hz, 1H), 3.21 (dd, *J* = 14.8, 8.0 Hz, 1H), 3.04 (dd, *J* = 15.4, 9.8 Hz, 1H), 3.01-2.97 (m, 1H), 2.65 (dd, *J* = 12.8, 7.6 Hz, 1H), 2.50 (t, *J* = 3.6 Hz, 2H), 2.12 (dd, *J* = 14.4, 7.2 Hz, 2H), 1.90 (q, *J* = 7.3 Hz, 2H), 1.81-1.80 (m, 1H), 1.58-1.49 (m, 3H), 1.21-1.14 (m, 1H), 1.04 (t, *J* = 7.2 Hz, 3H), 0.92 (t, *J* = 7.2 Hz, 3H), 0.86 (t, *J* = 6.8 Hz, 3H); HRMS (FAB) Calcd. For $C_{31}H_{48}N_5O_8S_2$ $[M+H]^+$: 682.2944. Found: 682.2938.

4.1.33. Glu-Ile-Thi-(*R*)HEA(isopropyl)-Nva *R*-26

^{1}H NMR (300 MHz, CD_3OD): δ 7.30 (dd, *J* = 3.8, 2.6 Hz, 1H), 7.01-6.99 (m, 2H), 4.70 (t, *J* = 7.5 Hz, 1H), 4.31 (d, *J* = 7.5 Hz, 1H), 4.00 (t, *J* = 6.0 Hz, 1H), 3.81 (t, *J* = 8.4 Hz, 1H), 3.59-3.56 (m, 1H), 3.38 (d, *J* = 4.2 Hz, 1H), 3.23 (dd, *J* = 14.9, 7.7 Hz, 1H), 2.87 (brd, *J* = 10.5 Hz, 1H), 2.50-2.48 (m, 3H), 2.13-2.08 (m, 2H), 1.89-1.85 (m, 3H), 1.67-1.42 (m, 6H), 1.26-1.16 (m, 2H), 1.04 (t, *J* = 7.4 Hz, 3H), 0.96 (d, *J* = 6.0 Hz, 3H), 0.95 (d, *J* = 6.0 Hz, 3H), 0.93 (t, *J* = 7.2 Hz, 3H), 0.88 (d, *J* = 6.0 Hz, 3H); HRMS (EI) Calcd. For $C_{30}H_{52}N_5O_8S$ $[M+H]^+$: 642.3537. Found: 642.3541.

4.1.34. (4*S*,5*R*)-Benzyl

5-(1-hydroxyethyl)-4-isobutyl-2,2-dimethyloxazolidine-3-carboxylate

To a soution of aldehyde **22** (1.49 g, 4.93 mmol) in THF (15 mL) was added methylmagnesium bromide (12 mL, 12 mmol, 1.0 mol/L in THF) at 0 ^{o}C under an argon atomosphere and the mixture was stirred for 1.5 h. The reaction was quenched with saturated aqueous NH_4Cl and the whole was extracted with ether. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 5:1) to yield the title compound (984 mg, 49%). ^{1}H NMR (300 MHz, $CDCl_3$, 4:1 diastereomer mixture, major isomer): δ 7.36-7.31 (m, 5H), 5.20-5.01 (m, 2H), 4.12-4.08 (m, 0.5H), 3.97-3.93 (m, 0.5H); 3.90-3.84 (m, 1H), 3.76 (dd, *J* = 8.4, 4.5 Hz, 0.5H); 3.69 (dd, *J* = 8.7, 4.5 Hz, 0.5H); 1.79-1.37 (m, 7H), 1.31 (d, *J* = 6.0 Hz, 3H); 1.28-1.15 (m, 2H), 0.80 (d, *J* = 6.6 Hz, 3H), 0.77 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 152.4, 136.3, 128.6, 128.4, 128.3, 93.3, 81.4, 66.6, 65.2, 56.7, 39.6, 27.2, 24.2, 23.7, 22.9, 22.8, 22.3; HRMS (EI) Calcd. For $C_{19}H_{29}NO_4$ $[M]^+$: 335.2097. Found: 335.2100.

4.1.35. (4*S*,5*S*)-Benzyl 5-(1-hydroxyethyl)-4-isobutyl-2,2-dimethyloxazolidine-3-carboxylate

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37-7.30 (m, 5H), 5.19 (d, *J* = 12.0 Hz, 1H), 5.07 (brd, *J* = 12.0 Hz, 1H), 4.13-4.09 (m, 1H), 3.79-3.70 (m, 1H); 3.61-3.58 (m, 1H), 1.66-1.40 (m, 7H), 1.45-1.22 (m, 2H), 1.27 (d, *J* = 6.3 Hz, 3H); 0.97-0.89 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 152.2, 136.3, 128.4, 128.1, 128.0, 94.5, 85.2, 68.4, 66.8, 58.0, 43.6, 27.7, 25.3, 23.8, 21.1; HRMS (EI) Calcd. For $C_{19}H_{29}NO_4$ $[M]^+$: 335.2097. Found: 335.2102.

4.1.36. (4*S*,5*R*)-Benzyl 5-acetyl-4-isobutyl-2,2-dimethyloxazolidine-3-carboxylate 38

To a suspension of above alcohol obtained in **4.1.34.** (984 mg, 2.93 mmol) and celite® (1.17 g) in CH_2Cl_2 (10 mL) was added PCC (1.90 g, 8.79 mmol). After stirring for 6 h, the mixture filtered through silica gel pad. The fitrate was concentrated and subjected to silica gel column chromatography (hexane/EtOAc = 3 :1) to yield ketone **38** (721 mg, 74%). $[\alpha]^{25}_D$ -24 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 2:1 amide rotater, major isomer): δ 7.37-7.32 (m, 5H), 5.13 (d, *J* = 11.7 Hz, 1H), 5.06 (brd, *J* = 12.3 Hz, 1H), 4.48 (m, 2H), 2.26 (s, 3H), 1.69 (s, 3H), 1.59 (s, 3H), 1.31-1.22 (m, 3H), 0.75 (d, *J* = 6.0 Hz, 3H), 0.71 (d, *J* = 5.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 205.8, 152.1, 136.0, 128.6, 128.5, 128.2, 94.4, 82.0, 66.9, 56.9, 40.4, 28.4, 27.3, 24.5, 23.7, 22.7; HRMS (EI) Calcd. For $C_{19}H_{27}NO_4$ $[M]^+$: 333.1940. Found: 333.1936.

4.1.37. (4*S*,5*S*)-Benzyl 5-acetyl-4-isobutyl-2,2-dimethyloxazolidine-3-carboxylate

$[\alpha]^{26}_{D}$ +6.4 (*c* 0.71, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37-7.31 (m, 5H), 5.18 (d, *J* = 12.3 Hz, 1H), 5.05 (brd, *J* = 11.7 Hz, 1H), 4.42 (m, 1H), 4.12 (m, 1H), 2.29 (s, 3H), 1.60-1.46 (m, 8H), 1.36-1.19 (m, 1H), 0.97-0.87 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 208.2, 151.9, 136.2, 128.5, 128.1, 95.6, 85.3, 66.9, 55.7, 43.4, 27.4, 26.2, 25.6, 23.7, 21.1; HRMS (EI) Calcd. For $C_{19}H_{27}NO_4$ $[M]^+$: 333.1940. Found: 333.1937.

4.1.38. Z-Me-(*S*)HEA(isopropyl)-Nva-O*t*-Bu 39

To a mixture of **38** (721 mg, 2.17 mmol) and HCl•H_2N-Nva-O*t*-Bu (455 mg, 2.17 mmol) was added Ti(O*i*-Pr)$_4$ (3.22 mL, 10.9 mmol) After stirring for 1 h, the reaction mixture was diluted with EtOH (5 mL) and then $NaBH_3CN$ (273 mg, 4.34 mmol) was added. The resultant mixture was stirred for 10 min. The reaction was quenched with water and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 3:1) to yield **39** (782 mg, 74%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35-7.31 (m, 5H), 5.19 (d, *J* = 12.0 Hz, 1H), 5.04 (d, *J* = 12.3 Hz, 1H), 4.08 (m, 0.25H), 3.97 (d, *J* = 6.9 Hz, 0.25H), 3.82 (d, *J* = 10.2 Hz, 0.5H), 3.54 (d, *J* = 9.6 Hz, 1H), 3.24 (brt, *J* = 6.8 Hz, 1H), 2.72-2.66 (m, 1H), 2.20 (m, 1H), 1.68-1.52 (m, 10H), 1.44 (s, 9H), 1.38-1.19 (m, 3H), 0.95-0.85 (m, 9H), 0.91 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.9, 151.9, 136.4, 128.4, 128.2, 128.0, 94.7, 86.2, 80.8, 66.7, 57.9, 57.7, 52.4, 43.9, 36.5, 29.1, 28.2, 28.0, 25.3, 24.0, 20.9, 19.1, 16.2, 13.9; HRMS (EI) Calcd. For $C_{28}H_{46}N_2O_5$ $[M]^+$: 490.3407. Found: 490.3412.

4.1.39. Z-Me-(*R*)HEA(isopropyl)-Nva-O*t*-Bu

^{1}H NMR (300 MHz, $CDCl_3$, 2:1 diastereomer mixture, major isomer): δ 7.37-7.33 (m, 5H), 5.15 (d, *J* = 11.7 Hz, 1H), 5.00 (d, *J* = 12.0 Hz, 1H), 4.11-4.05 (m, 1H), 3.63 (dd, *J* = 9.0, 4.2 Hz, 1H), 3.22 (t, *J* = 6.6 Hz, 1H), 2.87-2.76 (m, 1H), 1.78-1.50 (m, 1H), 1.59 (s, 3H), 1.57 (s, 3H), 1.52-1.32 (m, 6H), 1.45 (s, 9H), 1.26-1.23 (m, 1H), 1.13-1.11 (m, 3H), 0.94-0.88 (m, 3H), 0.83 (d, *J* = 6.6 Hz, 3H), 0.78 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.0, 152.8, 136.3, 128.7, 128.5, 128.4, 93.2, 81.4, 80.9, 66.7, 57.6, 56.1, 49.3, 38.6, 36.5, 28.1, 27.4, 24.0, 23.7, 21.9, 21.8, 19.1, 16.8, 13.9; HRMS (EI) Calcd. For $C_{28}H_{46}N_2O_5$ $[M]^+$: 490.3407. Found: 490.3412.

4.1.40. Glu-Ile-Me(*S*)HEA(isopropyl)-Nva *S*-40

***S*-40a:** ^{1}H NMR (300 MHz, CD_3OD): δ = 7.31 (d, *J* = 4.5 Hz, 1H), 7.01-6.96 (m,

2H), 4.69-4.65 (m, 1H), 4.33 (d, *J* = 7.5 Hz, 1H), 4.10-4.05 (m, 1H), 3.01(t, *J* = 6.3 Hz, 1H), 3.68-3.62 (m, 1H), 3.51-3.48 (m, 1H), 3.19 (d, *J* = 6.9 Hz, 1H), 2.54-2.49 (m, 3H), 2.23-2.05 (m, 2H), 1.90-1.88 (m, 3H), 1.68-1.42 (m, 5H), 1.35-1.14 (m, 3H), 1.17 (d, *J* = 7.2 Hz, 3H), 1.04 (t, *J* = 6.9 Hz, 3H), 0.98-0.94 (m, 12H); HRMS (FAB) Calcd. For $C_{31}H_{54}N_5O_8S$ $[M+H]^+$: 656.3693. Found: 656.3697.

***S*-40b:** ^{1}H NMR (300 MHz, CD_3OD): δ = 7.28 (dd, *J* = 5.0, 1.4 Hz, 1H), 7.00-6.96 (m, 2H), 4.79-4.74 (m, 1H), 4.33 (d, *J* = 7.5 Hz, 1H), 3.99 (t, *J* = 6.3 Hz, 2H), 3.61-3.58 (m, 2H), 3.44-3.38 (m, 1H), 3.24 (dd, *J* = 15.2, 8.0 Hz, 1H), 2.85 (t, *J* = 6.6 Hz, 1H), 2.52-2.47 (m, 2H), 2.16-2.08 (m, 2H), 1.90-1.85 (m, 3H), 1.69-1.56 (m, 5H), 1.26 (d, *J* = 6.6 Hz, 3H), 1.17 (m, 2H), 1.05 (t, *J* = 7.4 Hz, 3H), 0.96 (d, *J* = 6.9 Hz, 9H), 0.91 (d, *J* = 6.3 Hz, 3H); HRMS (FAB) Calcd. For $C_{31}H_{54}N_5O_8S$ $[M+H]^+$: 656.3693. Found: 656.3690.

4.1.41. Glu-Ile-Me(*R*)HEA(isopropyl)-Nva *R*-40

^{1}H NMR (300 MHz, CD_3OD): δ 7.32 (dd, *J* = 5.1, 1.2 Hz, 1H), 7.01-6.97 (m, 2H), 4.60 (t, *J* = 7.6 Hz, 1H), 4.25 (d, *J* = 7.2 Hz, 1H), 3.99 (t, *J* = 6.0 Hz, 1H), 3.87-3.84 (m, 1H), 3.68-3.65 (m, 1H), 3.51-3.48 (m, 1H), 3.40-3.39 (m, 1H), 3.20 (dd, *J* = 14.6, 7.7 Hz, 1H), 2.85-2.83 (m, 1H), 2.51 (t, *J* = 6.9 Hz, 2H), 2.11-2.08 (m, 2H), 1.85 (m, 3H), 1.68-1.45 (m, 5H), 1.38-1.26 (m, 2H), 1.23-1.13 (m, 3H), 1.02 (t, *J* = 7.4 Hz, 3H), 0.97-0.91 (m, 12H); HRMS (FAB) Calcd. For $C_{31}H_{54}N_5O_8S$ $[M+H]^+$: 656.3693. Found: 656.3701.

4.1.42. Z-(*S*)HEA(isopropyl)-N-Me-Nva-O*t*-Bu 41

To a solution of **24** (5.2 mg, 0.011 mmol) in EtOH (1 mL) was added *p*-formaldehyde (50 mg), $NaBH_3CN$ (26 mg, 0.42 mmol), and AcOH (0.024 mL, 0.42 mmol) at 0 ^{o}C. The mixture was stirred for 24 h at room temperature. The reaction was quenched with saturated aqueous $NaHCO_3$ and the whole was extracted with ether. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 6:1) to yield **41** (2.0 mg, 36%). $[\alpha]_D^{30}$ –294 ($CHCl_3$, *c* 0.196); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36-7.31 (m, 5H), 5.17 (d, *J* = 12.0 Hz, 1H), 5.08 (brd, *J* = 11.7 Hz, 1H), 3.97-3.95 (m, 1H), 3.83 (m, 1H), 3.10 (m, 1H), 2.78 (dd, *J* = 13.1, 5.9 Hz, 1H), 2.61 (brdd, *J* = 12.9, 7.8 Hz, 1H), 2.33 (s, 3H), 1.63-1.59 (m, 4H), 1.54-1.50 (m, 5H), 1.45 (s, 9H), 1.41-1.23 (m, 3H), 1.25 (dd, *J* = 6.3, 3.0 Hz, 1H), 0.98-0.87 (m, 9H); ^{13}C NMR (75 MHz,$CDCl_3$): δ 172.1, 152.3, 136.5, 128.4, 128.1, 128.0, 94.5, 80.8, 79.1, 67.9, 66.7, 59.7, 43.6, 37.4, 31.9, 29.7, 28.3, 28.0, 27.7, 25.6, 23.8, 21.3, 19.5, 13.9; HRMS (EI) Calcd. For

$C_{28}H_{46}N_2O_5$ $[M]^+$: 490.3407. Found: 490.3403.

4.1.43. Z-(*R*)HEA(isopropyl)-N-Me-Nva-O*t*-Bu

$[\alpha]_D^{28}$ –14 ($CHCl_3$, *c* 0.90); ^{1}H NMR (300 MHz, $CDCl_3$, 2:1 amide rotamer, major isomer): δ 7.36-7.31 (m, 5H), 5.13 (d, *J* = 11.4 Hz, 1H), 5.04 (brd, *J* = 12.3 Hz, 1H), 4.17-4.07 (m, 1H), 3.94-3.88 (m, 1H), 3.19 (t, *J* = 7.5 Hz, 1H), 2.80-2.66 (m, 2H), 2.40 (s, 3H), 1.72-1.49 (m, 7H), 1.46 (s, 9H), 1.42-1.23 (m, 5H), 1.25 (dd, *J* = 6.2, 2.3 Hz, 1H), 0.98-0.89 (m, 6H), 0.77 (dd, *J* = 6.3, 3.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.1, 152.4, 136.4, 128.7, 128.4, 93.2, 80.7, 66.7, 66.6, 57.2, 52.6, 39.6, 39.3, 32.1, 32.0, 28.3, 27.3, 24.7, 23.7, 22.9, 22.8, 19.6, 14.0; HRMS (EI) Calcd. For $C_{28}H_{46}N_2O_5$ $[M]^+$: 490.3407. Found: 490.3412.

4.1.44. Glu-Ile-Thi-(*S*)HEA(isopropyl)-N-Me-Nva *S*-42

^{1}H NMR (300 MHz, CD_3OD): δ 7.29-7.27 (m, 1H), 6.97-6.96 (m, 2H), 4.53-4.47 (m, 1H), 4.30 (d, *J* = 7.8 Hz, 1H), 4.04 (t, *J* = 6.5 Hz, 2H), 3.95-3.89 (m, 1H), 3.83 (dd, *J* = 8.6, 4.4 Hz, 1H), 3.29 (dd, *J* = 11.7, 3.9 Hz, 1H), 3.24-3.19 (m, 2H), 2.98 (s, 3H), 2.55-2.50 (m, 2H), 2.18-2.11 (m, 2H), 1.96-1.82 (m, 3H), 1.74-1.41 (m, 4H), 1.33-1.19 (m, 4H), 1.06 (t, *J* = 7.2 Hz, 3H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.98 (d, *J* = 5.7 Hz, 3H), 0.88 (d, *J* = 6.3 Hz, 3H), 0.84 (d, *J* = 6.0 Hz, 3H); HRMS (FAB) Calcd. For $C_{31}H_{54}N_5O_8S$ $[M+H]^+$: 656.3693. Found: 656.3690.

4.1.45. Glu-Ile-Thi-(*R*)HEA(isopropyl)-N-Me-Nva *R*-42

^{1}H NMR (300 MHz, CD_3OD): δ 7.31 (dd, *J* = 4.4, 2.0 Hz, 1H), 7.01-6.83 (m, 2H), 4.66 (t, *J* = 7.4 Hz, 1H), 4.32 (d, *J* = 7.5 Hz, 1H), 4.00 (t, *J* = 6.3 Hz, 1H), 3.85-3.81 (m, 2H), 3.79-3.73 (m, 1H), 3.21 (dd, *J* = 15.0, 7.8 Hz, 1H), 3.04-2.93 (m, 2H), 2.90 (s, 3H), 2.50 (m, 2H), 2.13 (dd, *J* = 13.8, 7.5 Hz, 2H), 1.92-1.82 (m, 3H), 1.67-1.47 (m, 6H), 1.35-1.15 (m, 2H), 1.06 (t, *J* = 7.2 Hz, 3H), 0.96 (d, *J* = 6.9 Hz, 9H), 0.88 (d, *J* = 6.3 Hz, 3H); HRMS (FAB) Calcd. For $C_{31}H_{53}N_5NaO_8S$ $[M+Na]^+$: 678.3513. Found: 678.3507.

4.2. Estimation of IC_{50} values

Peptide substrate [H-Ile-Lys-Thr-Glu-Glu-Ile-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-NH_2][10] (105 μM) in a reaction solution (25 μL of 40 mM AcONa buffer pH 4.5) was incubated with the rBACE 1 (100 nM) at 37 °C for 80 min in the presence of various inhibitor concentrations. The cleavage reaction was monitored by analytical HPLC [Cosmosil 5C18 column (4.6 x 150 mm), a linear gradient of CH_3CN (10-25 %) in an

aq.0.1% TFA over 30 min], and the cleavage rates were calculated from the reduction in the substrate peak area. Each IC_{50} value was obtained from the sigmoidal dose-response curve (see Figure S5 for a typical sigmoidal curve). Each experiment was repeated 3 times and the results were averaged.

4.3. X-ray crystallography

Purified rBACE1 in a final buffer composition of 20mM Tris-HCl pH8.0, 150mM NaCl, and 2mM DTT was concentrated to 8 mg/mL. Co-crystals of rBACE1 in complexes with inhibitors were grown at 20^{o}C using a sitting-drop vapor diffusion method. Co-crystals of rBACE1 in complex with ***R*-14** were grown by mixing an equal volume of rBACE1-inhibitor complex (final inhibitor concentration of 1.5mM) and a precipitant solution containing 100 mM sodium citrate pH5.0, 200 mM ammonium sulfate, and 22% PEG10000. Co-crystals of rBACE1 in complex with **R-27** were obtained using a precipitant solution containing 100 mM sodium citrate pH5.0, 200 mM ammonium sulfate, and 23% PEG10000. Co-crystals of rBACE1 in complex with ***R*-26** were obtained using a precipitant solution containing 100 mM sodium citrate pH5.0, 200 mM ammonium sulfate, and 14% PEG10000. The co-crystals were transferred into a cryobuffer containing 100 mM Sodium citrate pH5.0, 200 mM ammonium sulfate, 14% PEG10000, 1.5% DMSO, and 12.5% ethylene glycol, and then flash-frozen in liquid nitrogen.

X-ray diffraction data of rBACE1 in complexes with inhibitors ***R*-14**, ***R*-27** and ***R*-26** were collected at the SPring-8, beamline BL44XU with a Rayonix MX300HE CCD detector at the wavelength of 0.900Å. The structures of rBACE1 in complex with inhibitors were determined by molecular replacement using the Molrep[21] program with a previously reported structure (PDB code 2QP8[22]) as the search model. Rigid body refinement and subsequent restrained refinement protocols were performed with the program Refmac 5[23] of the CCP package[24]. The Coot program[25] was used for manual model rebuilding. Water molecules were added using Coot only after the refinement of protein structures had converged. Ligands generated on the PRODRG[26] web site or JLigand[27] software were directly built into the corresponding difference electron density, and the model was then subjected to an additional round of refinement. The figures of structural representation in this paper were generated on Pymol[28] or ucsf chimera[29] software.

5. PDB ID codes

4TRY, 4TRZ, and 4TRW

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Supplementary data

Supplementary data (the PGME data for HMC-type inhibitors, the HPLC data for HMC-/HEA-type inhibitors, a typical sigmoidal curve used to obtain the IC_{50} value, schemes of interactions between potent HMC-/HEA-type inhibitors with rBACE1 obtained by X-ray crystallographic analyses, schemes of interactions between less potent HMC- and HEA-type inhibitors with rBACE1 obtained by MOE simulations, and NMR data of synthesized compounds) associated with this article can be found in the online version, at http://

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Figure Legends

Figure 1 Structures of HMC- and HEA-type inhibitors

Figure 2
(i) Superimposed structures of ***R*-14**, ***R*-27** and ***R*-26** complexed with BACE1. The inhibitors are shown as a stick model. (ii) Interactions of ***R*-14** (PDB code 4TRY), ***R*-27** (PDB code 4TRZ), and ***R*-26** (PDB code 4TRW) at the active center of BACE1. Red and blue meshes represent observed electron densities corresponding to the BACE1 and inhibitors, respectively.

Figure 3
Interaction of inhibitors with BACE1 around P_4 (left panel) and P_3 (right panel). Side chain structures of residues interacting with the inhibitors are shown on the superimposed structure.

Figure 4
(i) Ineraction at the P_1 site. The isobutyl or thiophenyl groups occupied the S_1 pocket.
(ii) Interaction of ***R*-27** with BACE1. The P_1 side chain is directed deeper into the protein, and the flap region is in the opposite direction.

Scheme 1 Syntheses of α-hydroxyl carboxylic acids
(a) HATU/DIPEA (b) Oxone® DMF/H_2O (1:1) (c) $NaBH_4$/MeOH

Scheme 2 Syntheses of HMC-type inhibitors ***R*-14** to ***R*-17**
(a) (i) Fmoc-Nva-OH/DIPEA, (ii) 20% piperidine (b) (i) ***R*-10**, **11**, **12**, or **13**/EDCI/HOBt, (ii) 20% piperidine, (iii) Fmoc-Thi-OH/DIC/HOBt, (iv) 20% piperidine, (v) Fmoc-IIe-OH/DIC/HOBt, (vi) 20% piperidine, (vii) Boc-Glu(O*t*-Bu)-OH/DIC/HOBt (c) TFA/H_2O/thioanisole

Scheme 3 Construction of the HEA backbone

Scheme 4 Syntheses of HEA-type inhibitors **26** and **27**
(a) (i) CH_3NHOCH_3/BOP (ii) $LiAlH_4$ (b) (i) for **20**; $CH_3PPh_3 \cdot Br$/*n*-BuLi then $ICH_2Si(CH_3)_3$/*n*-BuLi then TBAF, for **21**; CH_2=CHMgCl (ii) 2,2-dimethyloxypropane/$BF_3 \cdot Et_2O$ (c) (i) $K_2OsO_2(OH)_4/K_3Fe(CN)_6/K_2CO_3$; for 21 (ii)

$NaIO_4$ (d) H-Nva-O*t*-Bu/Ti(O*i*-Pr)$_4$ then $NaBH_3CN$ (e) (i) Pd-C/H_2 for **24**, Pd black/H_2 for **25** (ii) Fmoc-Thi-OH/EDCI/HOBt/NMM (iii) Et_2NH (iv) Fmoc-Ile-OH/EDCl/HOBt/NMM (v) Et_2NH (vi) Boc-Glu(O*t*-Bu)-OH/EDCl/HOBt/NMM (vii) TFA/H_2O/TIS

Scheme 5 Syntheses of HEA-type inhibitors ***R*-26** and ***R*-27**
(a) (i) IBCF/Et_3N (ii) (*S*)-4-isopropyl-2-oxazolidinone/*n*-BuLi (b) 2-propenal/DIPEA/*n*-Bu_2BOTf (c) (i) NaOMe/MeOH (ii) 1N NaOH (d) (i) DPPA then 4N KOH (ii) Z-OSu/DIPEA

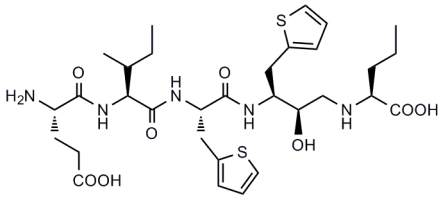
Scheme 6 Syntheses of HEA-type inhibitor ***S*-40**
(a) (i) CH_3MgBr (ii) PCC (b) H-Nva-O*t*-Bu/Ti(O*i*-Pr)$_4$ then $NaBH_3CN$

Scheme 7 Synthesis of HEA-type inhibitor ***S*-42**
(a) $(CH_2O)_n$/AcOH/$NaBH_3CN$

Table 1 Inhibitory Activities of the HMC- and HEA-type inhibitors

Table 2 Data Collection and Refinement Statistics for the BACE1 in Complexes with Compounds ***R*-14**, ***R*-27** and ***R*-26**

Graphical abstract



R-27