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Engineering D-amino acid containing novel protease inhibitors using catalytic site architecture

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Abstract—The mechanism of proteolysis by serine proteases is a reasonably well-understood process. Typically, a histidine residue acting as a general base deprotonates the catalytic serine residue and the hydrolytic water molecule. We disclose here, the use of an unnatural D-amino acid as a strategic residue in P1 position, designed de novo based on the architecture of the protease catalytic site to impede the catalytic histidine residue at the stage of acyl-enzyme intermediate. Several probe molecules containing D-homoserine or its derivatives at P1 position are evaluated. Compounds **1**, **6**, and **8**–**10** produced up to 57% loss of activity against chymotrypsin. More potent and specific inhibitors could be designed with structure optimization as this strategy is completely general and can be used to design inhibitors against any serine or cysteine protease.

1. Introduction

Proteases are ubiquitous in nature and are involved in key cellular processes.[1] Based on their catalytic mechanism, they are primarily classified into five different classes: (i) serine proteases, (ii) cysteine proteases, (iii) metalloproteases, (iv) aspartic proteases, and (v) threonine proteases. With the exception of metalloproteases, all other enzymes possess a conserved nucleophilic residue in the catalytic site and a basic residue to activate this nucleophilic residue in the catalytic site. For instance, a serine residue serves as the catalytic nucleophilic residue in serine proteases and either a histidine or a lysine residue in some cases functions as a basic residue activating the serine residue. Serine and cysteine proteases share the general principle of the mechanism of proteolytic hydrolysis, and incidentally, more than 50% of the proteases in the human genome belong to serine proteases and cysteine proteases.[1,2]

Several inhibitors have been developed that take advantage of the underlying mechanism of proteolysis by these proteases.[3] For example, in serine proteases such as chymotrypsin, the catalytic serine residue is activated by the His residue and reacts with the peptide substrate to form a covalent species known as the acyl-enzyme intermediate, as illustrated by species **II** (Fig. 1). During the formation of acyl-enzyme intermediate, the C-terminal portion after the scissile bond is released. A hydrolytic water molecule is then activated by the catalytic His residue to regenerate the catalytic Ser residue (Species III, Fig. 1) and release the amino terminal of the peptide substrate.[4] Based on the catalytic mechanism, the molecular architecture of the catalytic residues, and the substrate binding site, we envisioned that D-amino acids, specifically D-homoserine at P1 position in the peptide substrate could, interfere with the function of the catalytic histidine residue during proteolysis.

We modeled the acyl-enzyme intermediate for compound **1** in the active site of chymotrypsin and human

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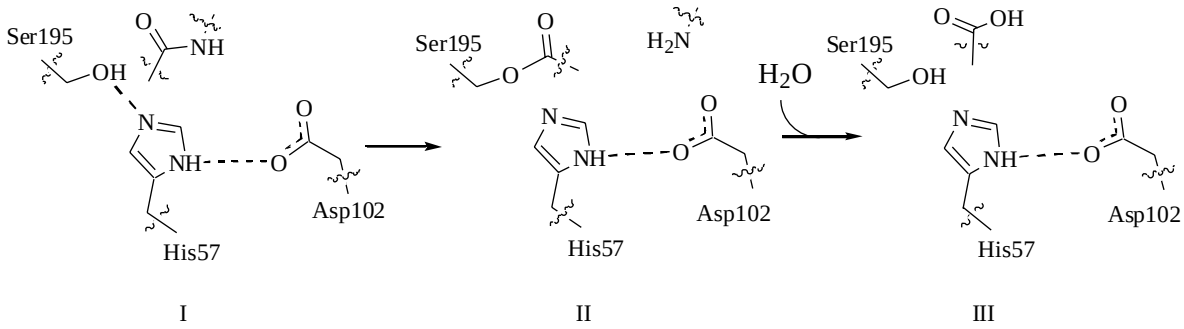


Figure 1. Schematic of the mechanism of proteolysis by chymotrypsin, a serine protease.

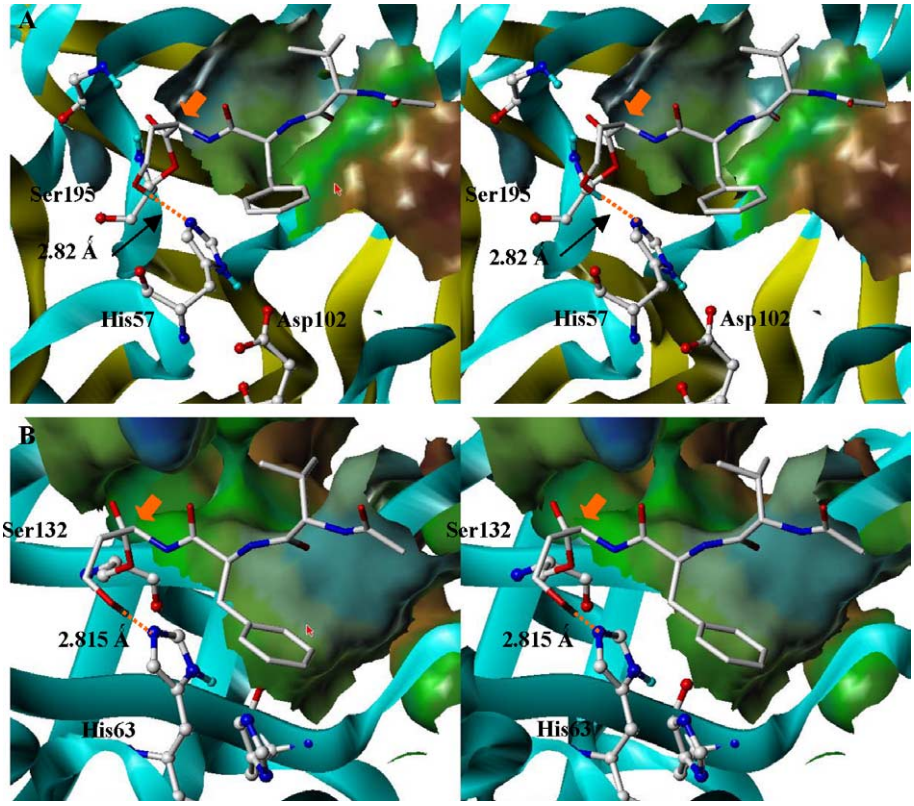


Figure 2. Computer models of the protease acyl-enzyme complex with compound **1**. Stereo view of the energy-minimized models of the acyl-enzyme intermediates of chymotrypsin (A) and HCMV protease (B) bound by compound **1**. The backbone of the enzyme is shown in a ribbon representation and a partial active site is rendered as a Connolly surface. Compound **1** is shown in a capped-stick representation with color-coded atoms (C, gray; N, blue; O, red), and key enzyme active site residues are shown in ball-and-stick representation. The hydrogen bond between the hydroxyl moiety on the inhibitor and N_ε of the catalytic His residue is shown by broken line in orange and the hydrogen bonding distance is indicated by an arrow.

cytomegalovirus protease (HCMV) to investigate the possibility of any interactions of D-amino acids with the catalytic residues (Fig. 2). In the catalytic site of chymotrypsin (Fig. 2A), the hydroxyethyl side chain of D-homoserine is suitably positioned to coordinate with the catalytic His57 residue via hydrogen bonding (distance of 2.8 Å). Specifically, the hydroxyl moiety in D-homoserine is well positioned to interfere with the catalytic His57 residue by interacting with it via (i) a hydrogen bond diminishing its ability to activate the catalytic Ser195 residue and/or (ii) excluding the hydrolytic water molecule to hydrolyze the acyl-enzyme intermediate species. Such close interaction of the hydroxy ethyl side chain of D-homoserine with the His63 residue via a hydrogen bond (2.8 Å) in the active site of HCMV protease was also observed (Fig. 2B). Amino acid at P2 position, Phe in compound **1** is in fact a preferred residue for HCMV protease.[5] To test the design hypothesis, we designed a library of compounds carrying D-serine (**3**), D-homoserine (**1**), D-hydroxynorvaline (**4**), and L-homoserine (**2**). Compounds **3**, **1**, and **4** are homologs with side chains in *R*-configuration and compound **2** has the hydroxyethyl moiety in the *S*-configuration. A library of quaternary amino acids **7**–**19** carrying a hydroxyethyl moiety in *R*-configuration and a side chain in pro-*S*-configuration were also synthesized. The side chains in the pro-*S*-configuration are designed to occupy S1 pocket in the protease binding site. Amino acids in

the P2 and P3 positions were chosen according to the preferences for substrates for chymotrypsin and/or HCMV protease. Compounds **5** and **6** carry a benzothiazole moiety at the scissile amide bond which is anticipated to increase the stability of the acyl-enzyme intermediate complex.[5]

Here, the de novo design of a novel class of inhibitors with a D-homoserine residue is highlighted, taking advantage of the positioning of various catalytic residues in the binding site of proteases, specifically serine proteases. The strategy is applicable to any serine or cysteine protease and the inhibitors could be optimized for drug discovery against proteases.

1.1. Synthesis

Syntheses of compounds **1**–**19** were carried out as described in Figure 3. Homoserine derivatives **1** and **2** (D- and L-isomers, respectively) were obtained as shown in Figure 3A. Coupling of the dipeptide **20** with *R*-(+)-α-amino-γ-butyrolactone hydrochloride using EDAC/HOBt conditions yielded compound **21** (Fig. 3A).[6–8] It should be noted here that racemization occurred at the C_α center in *R*-(+)-α-amino-γ-butyrolactone during the amino acid coupling reaction yielding 20% of the minor diastereomer. Even at a lower temperature, racemization was observed. Similar racemization was observed with *S*-(−)-α-amino-γ-butyrolactone producing the corresponding *R*-(+)-isomer. Treatment of the lactone **21** with benzyl amine under basic conditions followed by silylation of the hydroxyl group produced compounds **22a** and **22b** as a mixture of diastereomers. *R*-(+) and *S*-(−)-isomers (**22a** and **22b**, respectively) were separated and were treated separately with trifluoroacetic acid to remove the Boc moiety. Further acylation followed by removal of TBDPSi group with TBAF afforded D- and L-homoserine derivatives **1** and **2** in good yields.

Compound **3**, which is a (*R*)-D-serine derivative was synthesized as described in Figure 3A. Dipeptide **20** was coupled to *R*-(+)-NH_2-Ser-(*O*-TBDMSi)-OMe to yield the corresponding tripeptide. Hydrolysis of the methyl ester using LiOH hydrolytic conditions followed by condensation with benzyl amine yielded compound **23** (Fig. 3A).[9] Deprotection of the Boc-protecting group, followed by acylation and deprotection of the TBDMSi group, afforded the D-serine derivative **3** in good yield. D-Hydroxynorvaline analog **4** was synthesized according to Figure 3A. Compound **24** was coupled to the dipeptide **20** using EDAC/HOBt followed by the deprotection of methyl ester and coupling to benzyl amine gave the tripeptide **25** (Fig. 3A).[10,11] Standard protection and deprotection protocols yielded the target compound **4**.

Compounds **5** and **6** were synthesized according to Figure 3B. Silylation of the hydroxyl group in compound **26** with TBDPSiCl yielded compound **27** (Fig. 3B).[12] Compound **27** was then converted into the corresponding Weinreb amide using CDI coupling conditions[5] followed by treatment with benzothiazole and *n*-BuLi afforded compound **28**, which was isolated as an inseparable mixture of diastereomers (3:2 ratio). Deprotection of the Boc moiety in compound **28** followed by coupling to the dipeptide **20** yielded compound **29**. Further removal of the silyl and Boc-protecting groups gave the target tripeptide **5**. Compound **5** was isolated as a mixture of inseparable diastereomeric mixture (3:2 ratio). In a separate reaction, oxidation of **29** using Dess–Martin periodinate followed by the removal of the silyl and Boc groups gave compound **6** in good yield as an inseparable mixture of diastereomers (Fig. 3B).[12,13]

Strategy for the synthesis of tripeptide derivatives with quaternary amino acids at P1 position is shown in Figure 3C, starting from D-methionine.[14] The oxazolidinone derivative **30** was isolated as an inseparable mixture of diastereomers (*cis*:*trans* 86:14). Alkylation of compound **30** using sodium hexadimethylsilazane and the corresponding alkyl halide yielded the alkyl derivatives **31a** and **31b** (Fig. 3C). These alkylated products were isolated as an inseparable mixture of diastereomers and were used as such without further separation.

S-Alkylation of **31a** and **31b** using trimethyloxonium tetrafluoroborate, followed by refluxing in aqueous sodium bicarbonate solution, yielded the corresponding homoserine derivatives **32a** and **32b**, respectively.[15,16] Reduction of the olefinic bonds in compounds **32a** and **32b**, followed by lactonization under acidic conditions, gave the lactones **33a** and **33b**, respectively.[15] At this stage, relative enantiomeric ratios in **33a** and **33b** were determined by using ^{1}H NMR with the chiral shift reagent, (*R*)-(−)-2,2,2-trifluoro-1-(9-anthryl)-ethanol.[17] Compounds **33a** and **33b** were then coupled to various amino acids followed by hydrolysis of the lactone which gave the corresponding hydroxy carboxylate derivatives.[18] Protection of the hydroxyl moiety as a silyl ether and further coupling to benzyl amine gave the dipeptide derivatives **34a**–**34d**. Removal of the carbamate protecting groups on **34a**–**34d** and coupling of appropriate amino acids at P3 using standard procedures gave the tripeptides **35a**–**35m**. Deprotection of the silyl moiety followed by the removal of the carbamate moiety, as appropriate, yielded the target compounds **7**–**19** in excellent yields. During the deprotection of TBDPSi moiety using TBAF for the synthesis of compounds **9**, **10**, **13**, **14**, **18**, and **19**, benzyl ester deprotection was also observed.[19] Target compounds **7**–**19** were isolated as inseparable mixtures of diastereomers.

1.2. Enzyme inhibition studies

Stock solutions for chymotrypsin and its substrate were prepared in Tris buffer, pH 7.8 (100 mM Tris/HCl, 10 mM $CaCl_2$) for UV-based assays, and in TES, pH 8.0 (50 mM TES, 10 mM $CaCl_2$, and 1% DMSO) for fluorescence-based assays. Stock solutions of the inhibitors were prepared in methanol for UV-based assays and in DMSO for fluorescence-based assays. Time-dependent loss of activity of chymotrypsin was studied by first incubating the enzyme and inhibitor at 25 °C and the enzyme activity was determined at various time points by adding the substrate. A time course curve was

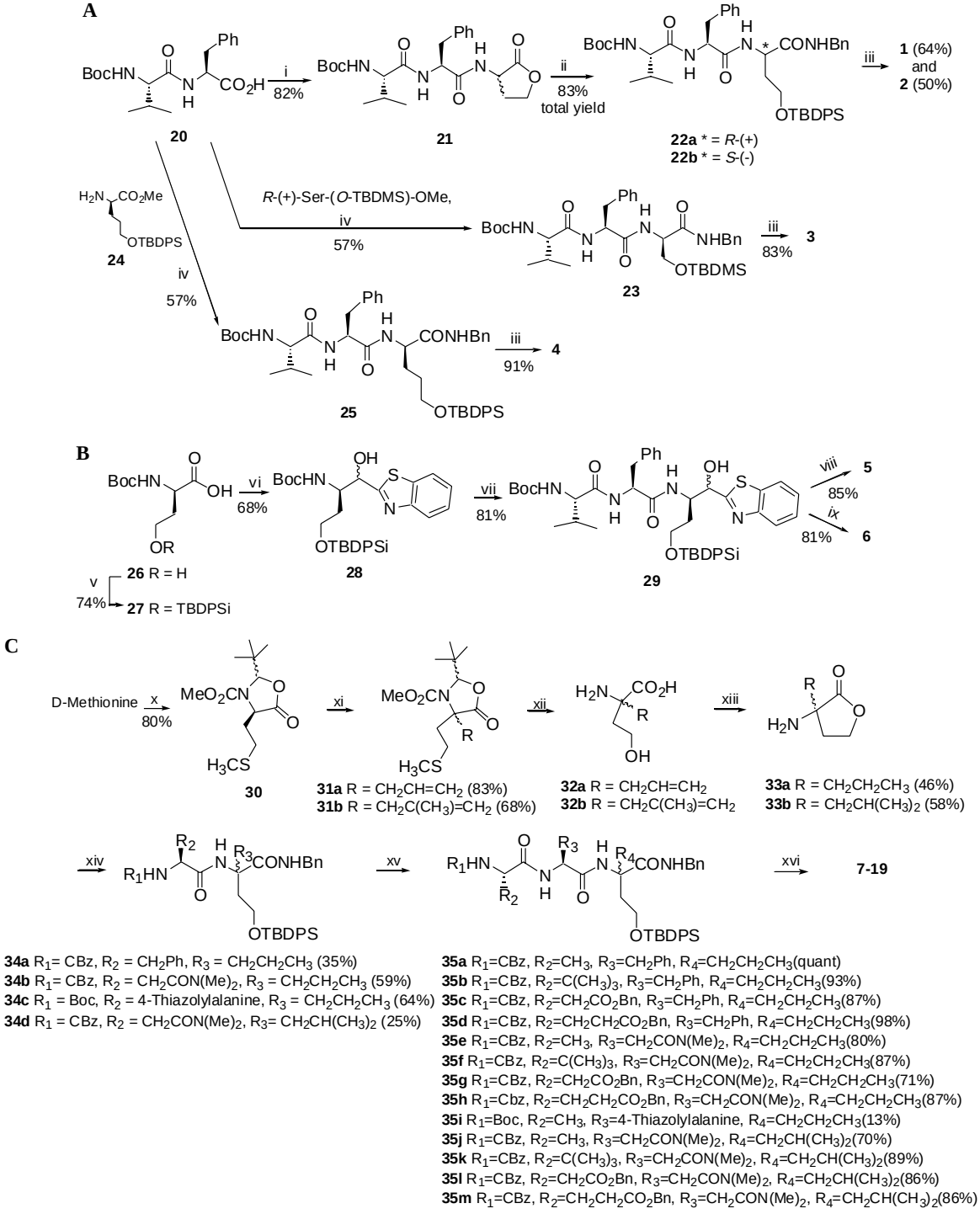


Figure 3. Synthesis of inhibitor molecules, **1**–**19**. Reagents and conditions: (i) *R*-(+)-α-NH_2-γ-butyrolactone HCl, EDAC, HOBt, Et_3N, DMF; (ii) a—$BnNH_2$, Et_3N, DMAP, THF/DMF (5:1); b—TBDPSiCl, imidazole, CH_2Cl_2; (iii) a—20% TFA in CH_2Cl_2, 0 °C; b—Ac_2O, Et_3N, CH_2Cl_2/DMF (10:1); c—TBAF, THF; (iv) a—EDAC, HOBt, Et_3N, CH_2Cl_2; b—LiOH, THF/H_2O (7:1), 0 °C; c—$BnNH_2$, EDAC, HOBt, Et_3N, THF/DMF (5:1); (v) TBDPSiCl, imidazole, CH_2Cl_2; (vi) a—*O*,*N*-dimethylhydroxylamine, CDI, Et_3N, CH_2Cl_2; b—$LiAlH_4$, THF, 0 °C; c—benzothiazole, *n*-BuLi, THF, −78 °C; (vii) a—20% TFA in CH_2Cl_2, 0 °C; b—compound **20**, EDAC, HOBt, CH_2Cl_2:DMF; (viii) a—TBAF, THF; b—20% TFA in CH_2Cl_2, 0 °C; (ix) a—Dess–Martin periodinane, CH_2Cl_2; b—TBAF, THF; c—20% TFA in CH_2Cl_2, 0 °C; (x) a—1 N NaOH; b—pyvalaldehye, pentane, Dean–Stark; c—methyl chloroformate, CH_2Cl_2; (xi) NaHMDS, RX, THF, −78 °C; (xii) a—$(CH_3)_3OBF_4$, CH_2Cl_2; b—$NaHCO_3$, H_2O, reflux, 16 h; (xiii) a—Pd–C/H_2, MeOH; b—4 N HCl, reflux; (xiv) a—RNH–CH(R′)–OH, BOP, DIPEA, CH_2Cl_2; b—LiOH, THF/H_2O, 0 °C; c—TBDPSiCl, imidazole, CH_2Cl_2; d—$BnNH_2$, EDAC, HOBt, Et_3N, CH_2Cl_2; (xv) a—Pd–C/H_2, MeOH or 20% TFA, CH_2Cl_2; b—RNH–CH(R′)–CO_2H, EDAC, HOBt, CH_2Cl_2; (xvi) a—TBAF, THF; b—Pd–C/H_2, MeOH or 20% TFA, CH_2Cl_2.

obtained for 5 min to record the enzyme activity. The incubation time periods for the enzyme and inhibitor were 0, 1, 2, 4, 6, 8, and 16 h. Control experiments typically contained the enzyme but no inhibitor. All experiments were conducted in triplicate. For chymotrypsin assays using 96-well plates, fluorescence detection was

used. Four different concentrations for each inhibitor 50, 150, 250, or 400 μM were used and the concentration of chymotrypsin in each reaction well was 0.4 μM. Enzyme–inhibitor reactions were incubated for 0, 1, 2, 4, and 8 h at 25 °C and the enzyme activity was measured using the substrate Glutaryl-Phe-AMC (λ_{ex} = 370 nm and λ_{em} = 446 nm). Enzyme reaction was monitored for 10 min and each reaction mixture (total volume 200 μL) contained 0.4 μM chymotrypsin, 400 μM substrate, inhibitor (either 50, 150, 250, or 400 μM), and buffer (50 mM TES, 10 mM $CaCl_2$, and 1% DMSO, pH 8.0). Each reaction well and the controls contained a final concentration of 6% DMSO.

HCMV protease assays were conducted in the time-course mode of the fluorometer and were monitored using the excitation wavelength at λ_{ex} = 360 nm and the emission wavelength at λ_{em} = 440 nm for 5000 s. Solution of the HCMV protease substrate, AcHN-Tbg-Tbg-Asn(Me)$_2$-Ala-AMC, was prepared in the incubation buffer, Tris–HCl (50 mM, pH 8.0), Na_2SO_4 (500 mM), NaCl (50 mM), EDTA (0.1 mM), tris-(2-carboxyethyl)-phosphine hydrochloride (1 mM), 3% DMSO (v/v), and 0.05% casein. Inhibitor stock solutions were prepared in DMSO. Stock solution of HCMV protease (20 mM NaOAc), EDTA (0.1 mM), DTT (1 mM), and NaCl (50 mM) was diluted with the incubation buffer. Time-dependent loss of HCMV protease activity was measured similar to that described for chymotrypsin assay above.

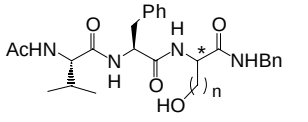
1.3. Computer modeling

Acyl-enzyme models of compound **1** in the active site of chymotrypsin and HCMV protease were built using the X-ray crystal structures of chymotrypsin and HCMV protease (PDB codes: 1AB9 and 1JQ7, respectively). Inhibitors were docked into the active sites appropriately by positioning the side chains into the subsites on the protease. Acyl-enzyme model was built by a covalent link to the catalytic Ser residue in the protease and the complex was energy-minimized using the Sander module in the Amber suite of programs (UCSF).[20]

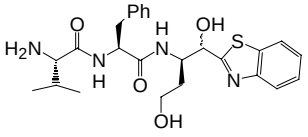
2. Results

Compounds **1**–**6** were synthesized by coupling appropriate amino acids using standard amino acid coupling chemistry (Fig. 3A). Racemization was observed at the Cα atom in D-homoserine during the preparation of compounds **1** and **2**. However, the diastereomeric mixture could be separated yielding pure enantiomers. For compounds **5** and **6**, a slightly different strategy was used: First, benzothiazole moiety was coupled to D-homoserine via reduction/alkylation, followed by appropriate amino acid coupling (Fig. 3B). Compounds **7**–**19** were synthesized starting from D-methionine (Fig. 3C). During alkylation to prepare quaternary amino acid derivatives (**31a** and **31b**), slight racemization occurred and a mixture of diastereomers were isolated. Upon opening the oxazolidinone derivative and lactonization to obtain **33a** and **33b**, enantiomeric ratio in favor of *R*-enantiomer was estimated to be 92:8 based on the NMR data, also supported by the literature.[15,16] This enantiomeric mixture could not be separated further and the final compounds **7**–**19** were isolated and used as a mixture of diastereomers, with the desired diastereomer having (*R*) configuration for P1 amino acid in excess ratio in comparison to the minor undesired diastereomer in (*S*) configuration. Since the proof of principle of the functional role of D-homoserine (*R*-configuration) would be established unequivocally (vide infra) by compounds **1**–**5** in favor of compound **1** with (*R*)-D-configuration, minor diastereomer in (*S*) configuration in compounds **7**–**19** is tolerable.

Compounds **1**–**5** were first evaluated in time-dependent inhibition assays using HCMV protease and chymotrypsin, to evaluate the hypothesis that (*R*)-D-homoserine



1 n = 2, * = *R*
2 n = 2, * = *S*
3 n = 1, * = *R*
4 n = 3, * = *R*



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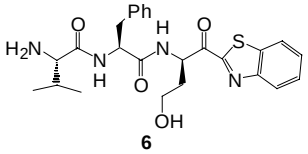

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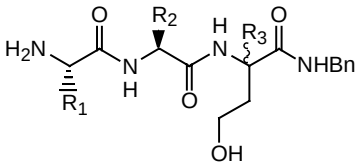


Compd	R_1	R_2	R_3
7	CH_3	CH_2Ph	*n*-C_3H_7
8	$C(CH_3)_3$	CH_2Ph	*n*-C_3H_7
9	CH_2CO_2H	CH_2Ph	*n*-C_3H_7
10	$(CH_2)_2CO_2H$	CH_2Ph	*n*-C_3H_7
11	CH_3	$CH_2CON(Me)_2$	*n*-C_3H_7
12	$C(CH_3)_3$	$CH_2CON(Me)_2$	*n*-C_3H_7
13	CH_2CO_2H	$CH_2CON(Me)_2$	*n*-C_3H_7
14	$(CH_2)_2CO_2H$	$CH_2CON(Me)_2$	*n*-C_3H_7
15	CH_3	4-Thiazolylalanine	*n*-C_3H_7
16	CH_3	$CH_2CON(Me)_2$	$CH_2CH(CH_3)_2$
17	$C(CH_3)_3$	$CH_2CON(Me)_2$	$CH_2CH(CH_3)_2$
18	CH_2CO_2H	$CH_2CON(Me)_2$	$CH_2CH(CH_3)_2$
19	$(CH_2)_2CO_2H$	$CH_2CON(Me)_2$	$CH_2CH(CH_3)_2$

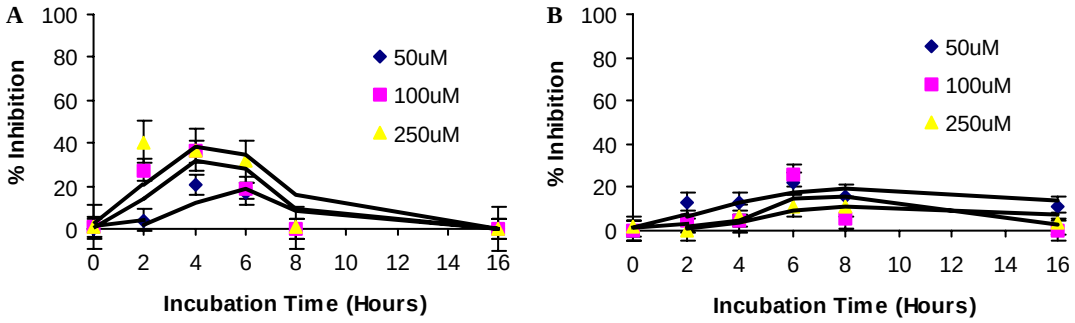


Figure 4. Time-dependent loss of enzyme activity of HCMV protease using the compounds **1** and **2** (A and B, respectively). % Inhibition of the enzyme activity is plotted against time after incubation of the enzyme with the inhibitor (**1** or **2**).

impedes the proteolytic activity of proteases. Figure 4 shows the profiles of compounds **1** and **2** against HCMV protease. Compounds **6**–**19** were then tested for their inhibitory activities at a concentration of 250 μM for up to 8 h incubation with chymotrypsin and HCMV protease in a 96-well plate reader to quickly identify potential inhibitors (Fig. 5A and B). Compounds with most promising inhibitory profile (**6**, **8**, **9**, and **10**) against chymotrypsin were then selected and kinetic analyses were performed (Fig. 6). Inactivation constants K_I and k_{inact} (concentration of inhibitor required to achieve half-maximal enzyme inactivation, and maximal inactivation rate, respectively) were calculated for each of the four compounds using Kitz–Wilson method for mechanism-based enzyme inactivation.[21] Figure 6A shows the events occurring in this type of enzyme inhibition (vide infra). While computing the half-lives and estimating the rate constants, only linear slopes during the initial 2 h were considered after the incubation of the inhibitors with the enzyme. Enzyme kinetics were used to understand the profile of these inhibitors keeping in mind that the acyl-enzyme intermediate is undergoing a slow-turn-over. The goal here is to prove that the design hypothesis and the inhibitors could function as mechanism-based inhibitors rather than to study the enzymatic hydrolysis of these compounds by proteases.

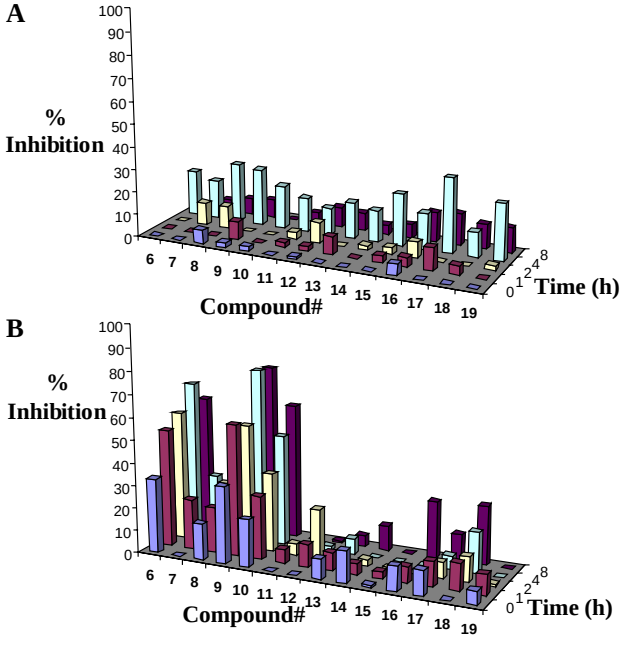
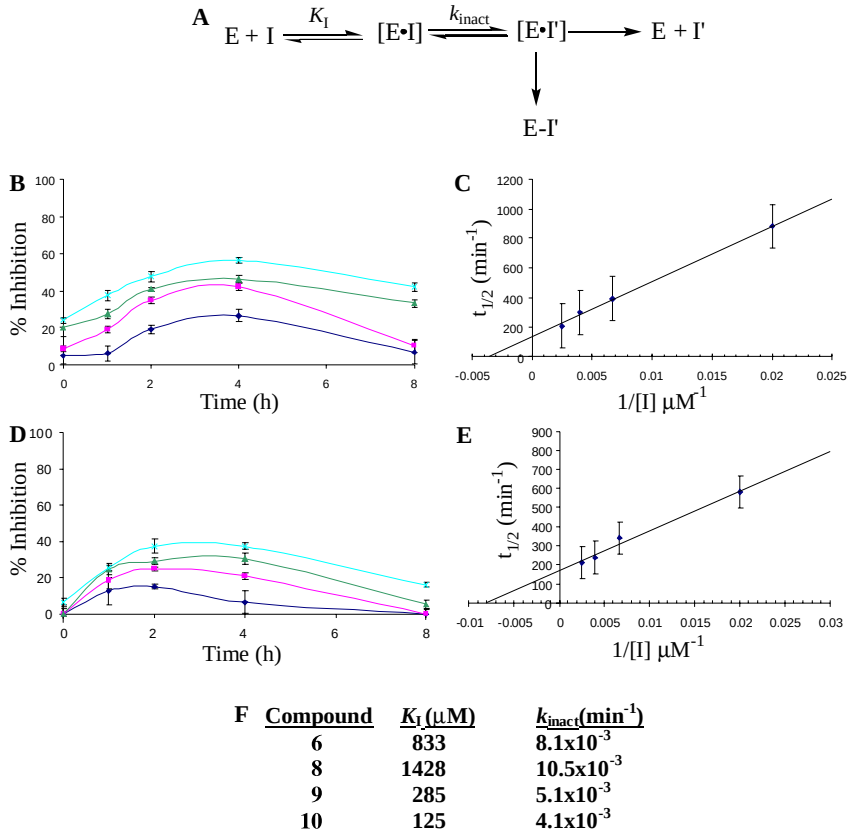


Figure 5. Semi-throughput assays for compounds **6**–**19** against HCMV protease and chymotrypsin. (A) HCMV Protease inhibition studies with compounds **6**–**19** at 250 μM conc at various time intervals up to 8 h. (B) Chymotrypsin inhibition studies with compounds **6**–**19** at 250 μM conc at various time intervals up to 8 h.

3. Discussion

Proteases command attention in biology due to their importance in the biological processes as well as their therapeutic potential. Due to this reason, they are perhaps the most well-studied and somewhat better understood enzymes in nature. Details of the catalytic mechanism and reliable three-dimensional structural information are available widely for proteases. Novel strategies to modulate protease activities thus will find a wide-spectrum of uses in biology and medicine. Here, we made use of the three-dimensional structural information of the catalytic site and the underlying mechanism in serine proteases to design novel D-amino acid containing inhibitors **1**–**19**. We show that these inhibitors impede the catalytic process in proteases.

To test the design principles, initially, we tested compounds **1**–**5** in a time-dependent enzyme inhibition assay against chymotrypsin and HCMV protease. Compound **1** at a concentration of 250 μM did not inhibit HCMV protease at $t = 0$ in a time-dependent assay. Lack of inhibition at $t = 0$ at various concentrations of compound **1** ruled out the possibility of competitive inhibition. After 4 h of incubation with compound **1**, chymotrypsin lost 31% of its activity in comparison to the control without the inhibitor (Fig. 4A). Most of the enzyme activity, however, was recovered after 16 h of incubation, implying that compound **1** was slowly turned over by HCMV protease (Fig. 4A). Compound **1** at the same concentration of 250 μM inhibited 43% of chymotrypsin activity after 8 h of incubation and the complete enzyme activity was recovered after 16 h (Figure S-1, Supplementary data). Inhibition of the enzyme activity of either chymotrypsin or HCMV protease increased at higher concentrations of the inhibitor **1** ranging from 50 to 250 μM, implying a



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Compound	K_I (μM)	k_{inact}(min^{-1})
6	833	8.1x10^{-3}
8	1428	10.5x10^{-3}
9	285	5.1x10^{-3}
10	125	4.1x10^{-3}

Figure 6. A schematic of the kinetic profile for mechanism-based inhibitors (A). Time-dependent enzyme activity profiles for chymotrypsin when incubated with 50 μM (blue), 150 μM (magenta), 250 μM (green), and 400 μM (cyan) of the inhibitors **9** and **10** (B and D, respectively). Kitz–Wilson plots for compounds **9** and **10** are shown in C and E, respectively, with the concentration for half-maximal inhibition (K_I) and rates of inactivation (k_{inact}) indicated for each compound. Standard error is shown in the error bars.

concentration-dependent as well as a time-dependent pattern, which is one of the key characteristics of mechanism-based inhibition. The dissociation constant, K_i, for compound **1** against HCMV protease, is 817 μM (Figure S-3, Supplementary data). Compound **2** did not show a concentration-dependent inhibition of HCMV protease activity. Compound **2** at 100 μM concentration inhibited ca. 20% enzyme activity after 6 h of incubation (Fig. 4B). Compound **2** at three concentrations of 50, 100, and 250 μM against chymotrypsin showed a similar inhibition pattern for up to 16 h of incubation, inhibiting around 20% of enzyme activity. From these observations, it appears that the L-homoserine analog **2**, unlike compound **1**, does not inhibit the activities of HCMV protease and chymotrypsin in a time-dependent manner.

These profiles of inhibitions of compound **1** versus **2**, that is, a D-homoserine analog versus an L-homoserine analog, and the recovery of enzymatic activities after several hours of incubation suggested that compound **1** is inactivating the protease in a time-dependent fashion, however, the inhibitor is going through a very slow turnover process. Compounds **3**–**5** did not show any significant inhibition of protease activities up to 1 mM concentration (data not shown). Compound **3** with a shorter P1 side chain, hydroxymethyl moiety, and compound **4** with a longer P1 side chain of hydroxypropyl moiety than that in compound **1** in D-configuration did not exhibit significant inhibitions against both proteases. There was about 20% loss of enzyme activity in the assays against HCMV protease and was not time-dependent. No further kinetic characterization was performed using these compounds.

These results strengthened the hypothesis generated through computer modeling that hydroxyethyl side chain in compound **1**, perhaps via interactions with the catalytic His residue in the protease and/or via excluding the hydrolytic water, impedes the deacylation process and thus proteolysis (Fig. 2). Although enzyme inhibition studies suggested that such inactivation of the protease is in fact happening, due to the lack of a side chain in the L-configuration in compound **1**, interactions with the S1 pocket of the protease are poor. Absence of interactions with the S1 pocket (highlighted by an arrow in orange, Fig. 2) may have multiple (and undesirable) effects on the enzyme–inhibitor interactions such as poor affinity of the inhibitor. It is also conceivable that lack of interactions at the important S1 subsite between the ligand and the enzyme may result in the covalent inhibitor moiety undergoing dynamic conformational changes outside the binding pocket. Such conformational flexibility could result in either (i) the loss of critical hydrogen bond interactions between the D-homoserine side chain and the catalytic His57 leading to

a turnover event, or (ii) the hydroxyl group of the side chain itself being activated by the catalytic His57 leading to an intramolecular turnover event. These possibilities might explain the recovery of activity of chymotrypsin after 4 h of incubation with the inhibitor and only a partial time-dependent loss of activity for up to 6 h in the case of HCMV protease (Fig. 4). Based on this reasoning, a new generation of compounds **7**–**19** carrying side chains in pro-(*S*)-configuration on the D-homoserine (quaternary amino acids) for enhanced interactions at the S1 subsite were designed and synthesized. The benzothiazole moiety in compound **6** is proposed to function as an electron-withdrawing group and stabilize the tetrahedral acyl-enzyme intermediate. Thus, we replaced the benzyl amine moiety with benzothiazole to enhance the stability of the acyl-enzyme intermediate and was anticipated to provide a better inhibition profile than its benzyl amine counterpart, **1**. Compound **5** is a reduced form of compound **6** and we evaluated its enzyme inhibition potential, although we did not anticipate a covalent species formation.

Compounds **6**–**19** were evaluated for their inhibitory potential against chymotrypsin and HCMV protease in a semi-throughput assay using 96-well plates. Enzyme activities were determined after 0, 1, 2, 4, and 8 h of incubation with each inhibitor at a concentration of 250 μM (Fig. 5). Compounds **6**–**19** did not show significant inhibition of HCMV protease in the semi-throughput assay (Fig. 5A). However, compounds **6** and **8**–**10** showed significant inhibition of chymotrypsin which was concentration- and time-dependent (Fig. 5B).

Based on these results, kinetic analyses for the inhibition of chymotrypsin by compounds **6** and **8**–**10** were undertaken. Based on the proposed mechanism of inactivation, inhibitor [I] is anticipated to bind to the active site of the protease and form a non-covalent complex [E·I] (Fig. 6A). Then the catalytic Ser residue is activated by the His residue and a covalent, acyl-enzyme species will be formed [E·I′]. Such species could have two different fates. This could be turned over by the protease, thus releasing the modified inhibitor species [I′] and the enzyme joins the catalytic process. Alternately, [E·I′] could become an irreversible complex E–I′ and inhibit the protease, which may involve a conformational change(s) in the inhibitor and/or protease.

In separate enzyme kinetic assays using compounds **6** and **8**–**10**, chymotrypsin exhibited a progressive loss of enzyme activity for up to 4 h after incubation, as anticipated according to the mechanism of their inhibition (Fig. 6). Maximum inhibition was observed due to compound **9** (57%) at 400 μM concentration, and after 8 h, significant inhibition (>42%) was still retained (Fig. 6B). Compounds **6**, **8**, and **10** showed a slightly lower degree of inhibition ranging from 36% to 42% also at 400 μM inhibitor concentration, and most of the enzyme activity was recovered after 8 h of incubation.

Additionally, it may also be possible that the hydroxyl moiety on D-homoserine may be assisting the cleavage of the covalent acyl-enzyme moiety slowly turning over the acyl-enzyme intermediate. However, an appropriate side chain in the pro-(*S*) configuration might eliminate such a turnover process as has been demonstrated already using the examples of compound **1** (complete recovery of activity after 8 h) versus compound **9** (over 42% enzyme activity is inhibited even after 8 h).

Compound **9** at 400 μM concentration, however, maintained its ability to inhibit chymotrypsin even after 8 h. Dissociation constants were derived for each compound (for compounds **9** and **10** refer to Figures 6C and E) based on the method developed by Kitz and Wilson.[21] Compounds **9** and **10** exhibited the lowest K_I values reflecting reasonably good affinities against chymotrypsin (285 and 125 μM, respectively), while K_I for the inhibitors **6** and **8** were 833 μM and 1.4 mM, respectively (Fig. 6F). Rates of inactivation for compounds **9** and **10** are slower, almost half, in comparison to those of the other two inhibitors, **6** and **8** (Fig. 6F). Although we anticipated that compound **6** with a benzothiazole moiety might have a better inhibition profile in comparison to compound **1**, inhibition assays indicated no significant difference between compounds **1** and **6**. Half-maximal concentrations for inhibition (K_I) and the rates of inactivation taken together suggest that compounds **9** and **10** are behaving as slow, irreversible inactivators of chymotrypsin, however, a complete irreversible inhibition will need further structural modifications. The highest and the longest lasting inhibition by compound **9** (Fig. 6) could possibly be attributed to the efficient formation of covalent complex within the enzyme active site.

While the kinetics support the mechanism of inhibition by the designed inhibitors, optimization of the side chains on these inhibitors using traditional medicinal chemistry tools is perhaps necessary to achieve complete inhibition of the activity of proteases. Thus, optimization of the side chains may enhance the binding affinities of the inhibitors leading to higher potency. Imparting rigidity to the inhibitor, to decrease the entropic penalty on the inhibitor during binding and after the acyl-enzyme complex formation, might also enhance the stability of the acyl-enzyme inhibitor leading to a complete irreversible loss of enzyme activity. These results confirm D-homoserine-based inhibitors do indeed impede the catalytic activity of proteases significantly, although there is a slow turnover of the inhibited complex (formation of E + I′). These principles form a basis for further side-chain structural modifications at P1 position.

4. Conclusion

Here, we reveal the design of D-homoserine and its quaternary amino acid analogs at P1 position as designer moieties to impede proteolysis, based on the architecture of the catalytic residues in serine proteases. Since cysteine proteases function in a similar fashion to serine proteases, these design principles will be equally applicable. With an appropriate 'fine-tuning' of the side chains on the inhibitors, specific and potent inhibitors of proteases can be designed and this opens doors toward the design of novel drugs against proteases. In fact, catalytic

sites of most of the serine and cysteine proteases share a similar architecture, thus by keeping the D-amino acid structure constant and varying other side chains, specific protease inhibitors could be designed. These design principles can be applied to any protease that shares the mechanistic similarities.

5. Experimental

5.1. General information

5.1.1. Synthesis. All anhydrous reactions were performed under argon atmosphere. All reagents were obtained from commercial sources and were used as received. Chromatographic purification was performed using silica gel 60 Å (70–230 mesh). ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, on a Bruker spectrometer. Chemical shifts were reported in δ parts per million using tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained at the Mass spectral Facility in the Department of Chemistry, University of Toronto.

5.1.2. Enzyme inhibition studies. UV–Vis spectra were recorded on a Shimadzu UV-5401PC spectrophotometer equipped with a temperature controller and a 12-cell holder unit. Fluorescence measurements were recorded on a Shimadzu RF-5301PC spectrofluorometer equipped with a temperature controller and a 4-cell holder unit. A Molecular Devices 96-well plate reader was used to perform semi-throughput assays. Chymotrypsin, its substrates (*N*-succ-Ala-Ala-Pro-Phe-*p*-nitroanilide and glutaryl-Phe-AMC), and HCMV protease substrate (Ac-*t*-Bu-Gly-*t*-Bu-Gly-Asn(Me)$_2$-Ala-AMC) were obtained from commercial sources. HCMV protease (wild type) was prepared as reported earlier.[22]

5.1.3. {(1*S*)-1-[(1*S*)-1-(2-oxo-tetrahydro-furan-3-ylcarbamoyl)-2-phenyl-ethyl carbamoyl]-2-methyl-propyl}-carbamic acid *tert*-butyl ester (21). A solution of compound **20** (0.4 g, 1.09 mmol) in anhydrous DMF (2 mL) was treated with HOBt (0.14 g, 1.09 mmol), EDAC (0.28 g, 1.48 mmol), Et_3N (0.26 mL, 1.86 mmol), and *R*-(+)-α-amino-γ-butyrolactone hydrochloride (0.13 g, 0.98 mmol) at 0 °C and stirred for 1 h at the same temperature. Solvent was evaporated under reduced pressure and the crude was dissolved in ethyl acetate, washed with 10% aqueous citric acid, 10% $NaHCO_3$ solution, brine, and dried (Na_2SO_4). Organic layer was concentrated under reduced pressure to obtain compound **21** (0.42 g, 82%) as an inseparable mixture of diastereomers in 4:1 ratio. ^{1}H NMR ($CDCl_3$) δ 0.68–0.90 (m, 6H), 1.38, 1.39 (2s, 9H), 1.86–2.15 (m, 1H), 2.30–2.45 (m, 1H), 2.57–2.75 (m, 1H), 3.06–3.25 (m, 2H), 3.80 (t, 1H, J = 5.4 Hz), 4.19–4.30 (m, 2H), 4.33–4.50 (m, 1H), 4.72–4.91 (m, 2H), 6.18–6.36 (m, 1H), 7.06–7.34 (m, 6H).

5.1.4. ((1*S*)-1-{(1*S*)-1-[(1*R*) or (1*S*)-1-Benzylcarbamoyl-3-(*tert*-butyl-diphenyl-silanyloxy)-propyl carbamoyl]-2-phenyl-ethylcarbamoyl}-2-methyl-propyl)-carbamic acid *tert*-butyl ester (22a and 22b). A solution of compound **21** (0.4 g, 0.89 mmol) in anhydrous THF/DMF (3 mL, 5:1) was treated with Et_3N (0.15 mL, 1.07 mmol), $BnNH_2$ (0.11 mL, 0.98 mmol), and DMAP (catalytic quantity), and was stirred for 24 h at rt. Solvent was evaporated under reduced pressure and the crude was purified by silica gel column chromatography ($CHCl_3$/MeOH, 98:2) to obtain the corresponding benzyl amide derivative (0.44 g, 90%) as an inseparable mixture of diastereomers in 4:1 ratio. ^{1}H NMR (DMSO-d_6) δ 0.70–0.87 (m, 6H), 1.34, 1.37 (2s, 9H), 1.80–2.07 (m, 3H), 2.98–3.11 (m, 2H), 3.55–3.69 (m, 2H), 4.29–4.98 (m, 6H), 6.62, 6.74 (2d, 1H, J = 6.0, 6.0 Hz), 7.00 (br s, 1H), 7.14–7.42 (m, 10H).

A solution of the above benzyl amide derivative (0.43 g, 0.77 mmol) in anhydrous CH_2Cl_2/DMF (5 mL, 10:1) was treated with imidazole (0.63 g, 0.93 mmol) followed by TBDPSiCl (0.2 mL, 0.77 mmol) at 0 °C and stirred for 1 h at rt. The solvent was evaporated under reduced pressure and the crude was dissolved in ethyl acetate, washed with water, brine, and dried (Na_2SO_4). The organic layer was concentrated under reduced pressure and the crude was purified by silica gel column chromatography ($CHCl_3$/MeOH, 99:1) to obtain compounds **22a** and **22b** (0.4 g, and 0.1 g, respectively, total yield 83%) as a separable mixture of diastereomers in 4:1 ratio. Compound **22a**: solid, mp 201–203 °C, ^{1}H NMR ($CDCl_3$) δ 0.71 (d, 3H, J = 6.3 Hz), 0.78 (d, 3H, J = 6.3 Hz), 1.04 (s, 9H), 1.36 (s, 9H), 1.86–2.00 (m, 2H), 2.16–2.24 (m, 1H), 2.89 (dd, 1H, J = 8.1, 13.6 Hz), 3.13 (dd, 1H, J = 5.4, 13.6 Hz), 3.59 (br t, 1H), 3.69–3.78 (m, 2H), 4.28 (dd, 1H, J = 6.0, 14.8 Hz), 4.40 (dd, 1H, J = 8.7, 9.0 Hz), 4.56–4.68 (m, 2H), 4.89 (d, 1H, J = 6.9 Hz), 6.12 (d, 1H, J = 6.3 Hz), 6.83 (br s, 1H), 7.01 (d, 1H, J = 7.2 Hz), 7.07–7.45 (m, 16H), 7.61–7.64 (m, 4H); Compound **22b**: syrup, ^{1}H NMR ($CDCl_3$) δ 0.76 (d, 3H, J = 6.9 Hz), 0.84 (d, 3H, J = 6.9 Hz), 1.02 (s, 9H), 1.42 (s, 9H), 1.68–1.78 (m, 1H), 1.84–1.94 (m, 1H), 1.98–2.08 (m, 1H), 2.88–3.03 (m, 2H), 3.60–3.67 (m, 1H), 3.74–3.81 (m, 2H), 4.13–4.20 (m, 1H), 4.40 (d, 2H, J = 5.6 Hz), 4.59–4.65 (m, 1H), 4.80 (br d, 1H), 6.34 (d, 1H, J = 5.7 Hz), 6.97 (dd, 1H, J = 3.0, 6.9 Hz), 7.08 (d, 1H, J = 7.8 Hz), 7.18–7.43 (m, 16H), 7.54–7.60 (m, 4H).

5.1.5. (2*S*)-2-Acetylamino-*N*-[(1*S*)-1-{(1*R*)-1-benzylcarbamoyl-3-hydroxypropyl carbamoyl}-2-phenyl-ethyl]-3-methyl butyramide (1). Compound **22a** (0.275 g, 0.34 mmol) was treated with 20% TFA in CH_2Cl_2 (4 mL) at 0 °C and stirred for 4 h at same temperature. The reaction mixture was then treated with excess solid $NaHCO_3$ and the solid was filtered. The filtrate was concentrated under reduced pressure to obtain the crude amine product. A solution of above crude amine in CH_2Cl_2/DMF (3 mL, 10:1) was treated with Et_3N (0.04 mL, 0.34 mmol), DMAP (catalytic quantity), and Ac_2O (0.03 mL, 0.34 mmol) at 0 °C. The reaction mixture was brought to rt and stirred for 1 h. Solvent was evaporated under reduced pressure, the crude was dissolved in ethyl acetate and washed with saturated $NaHCO_3$ solution, water, brine, and dried (Na_2SO_4). Solvent was evaporated under reduced pressure and the crude was purified by silica gel column chromatography

($CHCl_3$/MeOH, 98:2) to obtain the acetyl derivative (0.18 g) as a syrup. ^{1}H NMR ($CDCl_3$) δ 0.68 (d, 3H, J = 6.6 Hz), 0.81 (d, 3H, J = 6.9 Hz), 1.04 (s, 9H), 1.73 (s, 3H), 1.82–1.97 (m, 2H), 2.15–2.20 (m, 1H), 2.86 (dd, 1H, J = 8.7, 14.1 Hz), 3.18 (dd, 1H, J = 5.7, 14.1 Hz), 3.70–3.78 (m, 3H), 4.28 (dd, 1H, J = 5.7, 14.7 Hz), 4.41 (dd, 1H, J = 6.3, 14.7 Hz), 5.83 (d, 1H, J = 6.9 Hz), 6.21 (d, 1H, J = 7.5 Hz), 6.88 (t, 1H, J = 5.7 Hz), 7.09–7.44 (m, 17H), 7.61–7.66 (m, 4H).

A solution of above compound (0.17 g, 0.23 mmol) in dry THF (3 mL) was treated with TBAF (0.23 mL, 0.23 mmol, 1 M solution in THF) and stirred for 2 h at rt. Solvent was evaporated under reduced pressure and the crude was purified by column chromatography ($CHCl_3$/MeOH, 96:4) to obtain compound **1** (0.1 g, 64% over three steps) as a solid: mp 250–252 °C; ^{1}H NMR (DMSO-d_6) δ 0.50 (d, 3H, J = 6.6 Hz), 0.61 (d, 3H, J = 6.6 Hz), 1.64–1.92 (m, 6H), 2.73 (dd, 2H, J = 11.1, 13.6 Hz), 3.10 (dd, 2H, J = 3.6, 13.6 Hz), 3.29–3.31 (m, 2H), 3.39–3.45 (m, 2H), 3.98 (t, 1H, J = 7.5 Hz), 4.26–4.38 (m, 2H), 4.48–4.53 (m, 1H), 7.15–7.33 (m, 10H), 7.90 (d, 1H, J = 7.8 Hz), 8.04 (d, 1H, J = 8.1 Hz), 8.09 (t, 1H, J = 5.7 Hz), 8.39 (d, 1H, J = 8.1 Hz); MS (EI) *m*/*z* (%) 496 (11), 452 (10), 363 (13), 289 (57), 120 (100), 91 (24), 72 (37); HRMS calcd for $C_{27}H_{36}N_4O_5$: 496.266597, observed: 496.268571.

5.1.6. (2*S*)-2-Acetylamino-*N*-[(1*S*)-1-{(1*S*)-1-benzylcarbamoyl-3-hydroxy propyl carbamoyl}-2-phenyl ethyl]-3-methyl butyramide (2). Compound **22b** (0.095 g, 0.11 mmol) was treated with 20% TFA in CH_2Cl_2 (3 mL) at 0 °C and was purified as described for compound **1** to obtain the corresponding amine derivative.

A solution of above crude amine in CH_2Cl_2/DMF (2 mL, 10:1) was treated with Et_3N (0.01 mL, 0.11 mmol), DMAP (catalytic), and Ac_2O (0.01 mL, 0.11 mmol) at 0 °C, and was purified as described for compound **1** to obtain the acetyl derivative (56 mg) as a syrup; ^{1}H NMR ($CDCl_3$) δ 0.80 (d, 3H, J = 2.7 Hz), 0.82 (d, 3H, J = 2.7 Hz), 1.03 (s, 9H), 1.67–1.89 (m, 2H), 1.93 (s, 3H), 1.96–2.03 (m, 1H), 2.91 (dd, 1H, J = 6.9, 13.1 Hz), 3.00 (dd, 1H, J = 8.1, 13.1 Hz), 3.60–3.66 (m, 1H), 3.73–3.81 (m, 1H), 4.13–4.24 (m, 2H), 4.39 (d, 2H, J = 5.7 Hz), 4.62–4.68 (m, 1H), 5.83 (d, 1H, J = 8.7 Hz), 6.60 (d, 1H, J = 5.4 Hz), 6.98–7.05 (m, 2H), 7.17–7.73 (m, 20H).

A solution of the acetyl derivative (0.047 g, 0.06 mmol) in dry THF (2 mL) was treated with TBAF (0.06 mL, 0.06 mmol, 1 M solution in THF) and was purified as described for compound **1** to obtain compound **2** (24 mg, 50% over three steps) as a solid. Mp 247–249 °C; ^{1}H NMR (DMSO-d_6) δ 0.73, (d, 6H, J = 6.3 Hz), 1.17–1.32 (m, 1H), 1.57–1.65 (m, 1H), 1.78–1.87 (m, 4H), 2.83 (dd, 1H, J = 8.7, 13.5 Hz), 3.01 (dd, 1H, J = 6.0, 13.5 Hz), 3.14–3.31 (m, 3H), 4.03 (t, 1H, J = 7.8 Hz), 4.23–4.28 (m, 2H), 4.46–4.50 (m, 1H), 7.17–7.32 (m, 10H), 7.80 (d, 1H, J = 8.1 Hz), 8.08 (d, 1H, J = 8.1 Hz), 8.17 (d, 1H, J = 7.2 Hz), 8.27 (t, 1H, J = 6.0 Hz); MS (EI) *m*/*z* (%) 496 (8), 452 (7), 362 (10), 289 (47), 120 (100), 91 (28), 72 (40), HRMS calcd for $C_{27}H_{36}N_4O_5$: 496.267232, observed: 496.268571.

5.1.7. ((1*S*)-1-{(1*S*)-1-[(1*R*)-1-Benzylcarbamoyl-2-(*tert*-butyldimethylsilyloxy)-ethylcarbamoyl]-2-phenyl-ethylcarbamoyl}-2-methyl propyl)-carbamic acid *tert*-butyl ester (23). A solution of compound **20** (0.15 g, 0.41 mmol) in anhydrous CH_2Cl_2 (3 mL) was treated with HOBt (0.05 g, 0.41 mmol), EDAC (0.11 g, 0.61 mmol), Et_3N (0.05 mL, 0.41 mmol), and H_2N-D-Ser-(*O*-TBDMSi)-OMe (0.095 g, 0.41 mmol) at 0 °C, and was purified as described for compound **21** to obtain the tripeptide derivative (0.14 g, 59%) as an oil. ^{1}H NMR ($CDCl_3$) δ 0.10 (s, 6H), 0.68 (s, 9H), 0.81 (d, 6H, J = 6.3 Hz), 1.34 (s, 9H), 1.94–2.04 (m, 1H), 2.95–2.99 (m, 2H), 3.44–3.47 (m, 1H), 3.58 (s, 3H), 3.83–3.86 (m, 2H), 4.43 (dd, 1H, J = 1.5, 7.8 Hz), 4.61 (dd, 1H, J = 1.0, 7.5 Hz), 4.85 (d, 1H, J = 6.0 Hz), 6.35 (d, 1H, J = 7.8 Hz), 6.49 (d, 1H, J = 5.4 Hz), 7.08–7.18 (m, 5H).

A solution of above compound (0.135 g, 0.23 mmol) in THF/H_2O (2 mL, 7:1) was treated with LiOH (0.005 g, 0.23 mmol) at 0 °C and stirred for 3 h at same temperature. Solvent was evaporated under reduced pressure and the crude was purified by silica gel column chromatography ($CHCl_3$/MeOH/AcOH, 97:2:1) to obtain the corresponding carboxylate derivative (0.12 g, 91%) as a syrup; ^{1}H NMR ($CDCl_3$) δ 0.02–0.03 (m, 6H), 0.82–0.89 (m, 15H), 1.42 (s, 9H), 1.94–2.06 (m, 1H), 2.95 (d, 1H, J = 7.2, 7.5 Hz), 3.67–3.71 (m, 1H), 3.98–4.02 (m, 2H), 4.53–4.55 (m, 1H), 4.93 (q, 1H), 5.18 (d, 1H, J = 9.0 Hz), 6.84 (d, 1H, J = 6.0 Hz), 7.10 (d, 1H, J = 6.0 Hz), 7.14–7.25 (m, 6H).

A solution of the above carboxylate (0.12 g, 0.21 mmol) in anhydrous CH_2Cl_2 (2 mL) was treated with HOBt (0.028 g, 0.21 mmol), EDAC (0.06 g, 0.31 mmol), Et_3N (0.029 mL, 0.21 mmol), and $BnNH_2$ (0.02 mL, 0.21 mmol), and was purified as described for compound **21** to obtain compound **23** (0.08 g, 57%) as a syrup; ^{1}H NMR ($CDCl_3$) δ 0.01 (s, 6H), 0.77–0.87 (m, 15H), 1.43 (s, 9H), 1.93–1.95 (m, 1H), 3.04 (d, 1H, J = 6.9 Hz), 3.24 (t, 1H, J = 9.6 Hz), 3.80–3.90 (m, 1H), 3.99 (d, 2H, J = 9.6 Hz), 4.36 (d, 2H, J = 4.8 Hz), 4.41 (d, 2H, J = 5.7 Hz), 4.85 (d, 1H, J = 7.2 Hz), 6.37 (d, 1H, J = 7.2 Hz), 6.67 (br s, 1H), 7.16–7.26 (m, 10H).

5.1.8. (2*S*)-2-Acetylamino-*N*-[(1*S*)-1-{(1*R*)-1-benzylcarbamoyl-2-hydroxyethyl carbamoyl}-2-phenyl-ethyl]-3-methyl butyramide (3). Compound **23** (0.2 g, 0.30 mmol) was treated with 20% TFA in CH_2Cl_2 (5 mL) at 0 °C and was purified as described for compound **1** to obtain the corresponding amine derivative. A solution of the crude amine in CH_2Cl_2 (3 mL) was treated with Et_3N (0.08 mL, 0.61 mmol), DMAP (catalytic), and Ac_2O (0.02 mL, 0.30 mmol) at 0 °C and was purified as described for compound **1** to obtain the corresponding acetyl derivative (0.08 g, 41% over two steps) as a syrup; ^{1}H NMR ($CDCl_3$) δ 0.01–0.02 (m, 6H), 0.87–0.89 (m, 15H), 1.73–1.85 (m, 2H), 1.96 (s, 3H), 3.03 (d, 1H, J = 7.5 Hz), 3.21 (dd, 1H, J = 6.3 Hz), 3.98 (dd, 1H, J = 3.3 Hz), 4.14 (dd, 1H, J = 1.5, 6.9 Hz),

4.36–4.44 (m, 2H), 5.80 (d, 1H, J = 6.3 Hz), 6.42 (d, 1H, J = 7.5 Hz), 6.60 (d, 2H, J = 6.3 Hz), 7.14–7.30 (m, 10H). A solution of the above compound (0.06 g, 0.10 mmol) in anhydrous THF (3 mL) was treated with TBAF (0.1 mL, 0.10 mmol, 1 M solution in THF) and was purified as described for compound **1** to obtain compound **3** (0.025 g, 83%) as a solid. Mp 234–236 °C; ^{1}H NMR (DMSO-d_6) δ 0.74 (d, 6H, J = 6.3 Hz), 0.90 (t, 1H, J = 6.9 Hz), 1.23–1.31 (m, 1H), 1.79–1.87 (m, 1H), 1.93 (s, 3H), 2.87 (d, 1H, J = 9.0 Hz), 3.03 (d, 1H, J = 9.0 Hz), 3.51–3.59 (m, 1H), 4.01 (t, 1H, J = 7.2 Hz), 4.20–4.30 (m, 2H), 4.44–4.56 (m, 1H), 4.91 (br s, 1H), 7.23–7.26 (m, 10H), 7.85 (d, 1H, J = 8.1 Hz), 8.01 (d, 1H, J = 8.1 Hz), 8.15 (d, 1H, J = 6.6 Hz), 8.27 (br s, 1H); MS (ESI) m/z (%) 521 (M+K, 23), 505 (M+Na, 100), 483 (MH^+, 22).

5.1.9. {(1*S*)-1-{(1*S*)-1-[(1*R*)-1-Benzylcarbamoyl-4-(*tert*-butyl diphenyl silyloxy)-butyl carbamoyl]-2-phenyl ethylcarbamoyl}-2-methyl propyl}-carbamic acid *tert*-butyl ester (25). A solution of compound **20** (0.23 g, 0.65 mmol) in anhydrous CH_2Cl_2 (3 mL) was treated with HOBt (0.13 g, 0.97 mmol), EDAC (0.18 g, 0.97 mmol), and compound **24** (0.25 g, 0.65 mmol) at 0 °C, and the same procedure as described for compound **21** was followed to obtain the corresponding tripeptide derivative (0.3 g, 65%) as a syrup; ^{1}H NMR ($CDCl_3$) δ 0.78 (d, 3H, J = 6.9 Hz), 0.90 (d, 3H, J = 6.9 Hz), 1.05 (s, 9H), 1.41 (s, 9H), 1.43–1.45 (m, 2H), 1.65–1.98 (m, 2H), 2.04–2.20 (m, 1H), 3.06–3.13 (m, 2H), 3.69 (s, 3H), 3.70–3.71 (m, 1H), 3.84 (t, 1H, J = 5.7, 12.0 Hz), 4.47 (dd, 1H, J = 1.2, 5.1 Hz), 4.69 (dd, 1H, J = 1.5, 7.2 Hz), 4.84 (d, 1H, J = 5.4 Hz), 6.45 (d, 1H, J = 7.2 Hz), 6.56 (d, 1H, J = 7.2 Hz), 7.17–7.27 (m, 6H), 7.35–7.41 (m, 5H), 7.62–7.64 (m, 4H). A solution of above compound (0.3 g, 0.41 mmol) in THF/H_2O (5 mL, 7:1) was treated with LiOH (0.01 g, 0.41 mmol) at 0 °C and was purified as described for compound **23** to obtain the corresponding carboxylate derivative (0.25 g, 85%) as a syrup; ^{1}H NMR ($CDCl_3$) δ 0.81 (d, 3H, J = 6.4 Hz), 0.87 (d, 3H, J = 6.4 Hz), 1.03 (s, 9H), 1.39 (s, 9H), 1.42–1.56 (m, 2H), 1.60–1.98 (m, 2H), 2.01–2.04 (m, 1H), 3.04 (d, 2H, J = 6.6 Hz), 3.57 (t, 2H, J = 5.4, 11.1 Hz), 3.92–3.94 (m, 1H), 4.45 (dd, 1H, J = 1.2, 5.4 Hz), 4.90 (d, 1H, J = 4.5 Hz), 5.04 (d, 1H, J = 6.9 Hz), 6.83 (d, 1H, J = 3.5 Hz), 7.13–7.18 (m, 6H), 7.37–7.39 (m, 5H), 7.61–7.63 (m, 4H). A solution of the carboxylate derivative (0.25 g, 0.35 mmol) in anhydrous CH_2Cl_2/DMF (3 mL) was treated with HOBt (0.07 g, 0.53 mmol), EDAC (0.1 g, 0.53 mmol), and $BnNH_2$ (0.05 mL, 0.53 mmol), and a similar procedure that was followed for compound **21** was used to obtain compound **25** (0.16 g, 57%) as a solid. Mp 126–128 °C; ^{1}H NMR ($CDCl_3$) δ 0.76 (d, 3H, J = 6.3 Hz), 0.84 (d, 3H, J = 6.3 Hz), 1.03 (s, 9H), 1.32–1.37 (m, 11H), 1.56–1.68 (m, 2H), 1.90–1.94 (m, 1H), 3.02–3.04 (m, 2H), 3.57 (br s, 2H), 3.85 (m, 1H), 4.30–4.60 (m, 4H), 4.80–4.83 (m, 1H), 5.01–5.05 (m, 1H), 6.40–6.42 (m, 1H), 6.60–6.62 (m, 1H), 6.91–6.95 (m, 1H), 7.14–7.25 (m, 11H), 7.35–7.37 (m, 5H), 7.60–7.62 (m, 4H).

5.1.10. (2*R*)-2-[(2*S*)-2-{(2*S*)-2-Acetylamino-3-methyl-butyrylamino}-3-phenyl-propionylamino]-5-hydroxy pentanoic acid benzylamide (4). Compound **25** (0.11 g, 0.13 mmol) was treated with 20% TFA in CH_2Cl_2 (3 mL) at 0 °C and a procedure similar to that for compound **1** was followed to obtain the corresponding crude amine derivative. A solution of the crude amine in CH_2Cl_2/DMF (3 mL, 10:1) was treated with Et_3N (0.02 mL, 0.26 mmol), DMAP (catalytic), and Ac_2O (0.02 mL, 0.26 mmol) at 0 °C, and a protocol similar to that for compound **1** was followed to obtain the acetyl derivative (0.07 g, 60% over two steps) as a solid. Mp 228–230 °C; ^{1}H NMR ($CDCl_3$) δ 0.69 (d, 3H, J = 6.9 Hz), 0.80 (d, 3H, J = 6.9 Hz), 1.03 (s, 9H), 1.40–1.44 (m, 2H), 1.55–1.60 (m, 2H), 1.85–1.87 (m, 4H), 3.01–3.04 (m, 2H), 3.55–3.57 (m, 2H), 4.26–4.28 (m, 1H), 4.34 (t, 2H, J = 5.4, 8.4 Hz), 4.45–4.50 (m, 1H), 4.84–4.90 (m, 1H), 5.94 (br s, 1H), 6.40 (br s, 1H), 6.60 (br s, 1H), 6.91 (br s, 1H), 7.12–7.22 (m, 11H), 7.31–7.39 (m, 5H), 7.59–7.61 (m, 4H).

A solution of the above acetyl derivative (0.07 g, 0.09 mmol) in anhydrous THF (3 mL) was treated with TBAF (0.09 mL, 0.09 mmol, 1 M solution in THF) and following a procedure similar to that for compound **1** yielded compound **4** (0.044 g, 91%) as a solid. Mp 258–260 °C; ^{1}H NMR (DMSO-d_6) δ 0.73 (d, 3H, J = 6.9 Hz), 0.75 (d, 3H, J = 6.9 Hz), 1.18–1.60 (m, 4H), 1.72–1.80 (m, 1H), 1.82 (s, 3H), 2.84–3.00 (m, 2H), 3.22–3.30 (m, 2H), 4.03–4.58 (m, 4H), 7.17–7.32 (m, 10H), 7.77 (d, 1H, J = 8.1 Hz), 8.03 (d, 1H, J = 7.8 Hz), 8.15 (d, 1H, J = 6.9 Hz), 8.31 (d, 1H, J = 5.4 Hz); MS (ESI) m/z (%) 549 (M+K, 7), 533 (M+Na, 44), 511 (MH^+, 61), 370 (11), 186 (100).

5.1.11. (2*R*)-2-*tert*-Butoxycarbamoyl-4-(*tert*-butyldiphenylsilyloxy)-butyric acid (27). A solution of compound **26** (1.7 g, 7.76 mmol) in anhydrous CH_2Cl_2 (15 mL) was treated with imidazole (1.58 g, 23.28 mmol) and TBDPSiCl (2.22 mL, 8.53 mmol) at 0 °C and a procedure similar to that for compound **22a** was followed to obtain compound **27** (2.62 g, 74%) as a syrup. ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 9H), 1.44 (s, 9H), 1.94–2.18 (m, 2H), 3.70–3.85 (m, 2H), 4.40–4.50 (m, 1H), 5.91 (d, 1H, J = 6.9 Hz), 7.35–7.46 (m, 6H), 7.64–7.67 (m, 4H), 10.72 (br s, 1H).

5.1.12. [(1*R*)-1-(1-Benzothiazol-2-yl-hydroxy methyl)-3-(*tert*-butyldiphenyl silyloxy)-propyl]-carbamic acid *tert*-butyl ester (28). A solution of compound **27** (2.62 g, 5.73 mmol) in anhydrous CH_2Cl_2 (30 mL) was treated with *N*,*N*′-carbonyldiimidazole (1.2 g, 7.45 mmol) at 0 °C. After stirring for 30 min, Et_3N (1.03 mL, 7.45 mmol) and *N*,*O*-dimethylhydroxylamine (0.72 g, 7.45 mmol) were added, and a procedure similar to that for compound **21** was used to obtain the Weinreb amide (2.49 g, 87%) as a syrup. ^{1}H NMR ($CDCl_3$) δ 1.06 (s, 9H), 1.43 (s, 9H), 1.68–1.78 (m, 1H), 1.98–2.10 (m, 1H), 3.19 (s, 3H), 3.65–3.83 (m, 5H), 4.80–4.90 (m, 1H), 5.49 (d, 1H, J = 8.1 Hz), 7.35–7.44 (m, 6H), 7.66–7.72 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 19.27, 26.92, 28.52, 32.31, 34.74, 48.93, 60.79, 61.69, 79.46, 127.82, 129.79, 133.47, 133.63, 135.69, 135.77, 155.79, 173.22. A solu-

tion of the above compound (0.225 g, 0.45 mmol) in anhydrous THF (3 mL) was treated with $LiAlH_4$ (0.034 g, 0.90 mmol) at 0 °C and stirred for 2 h at same temperature. The reaction was quenched with 10% aqueous citric acid and stirred for an additional 30 min at rt. Compound was extracted into ether, washed with brine and dried (Na_2SO_4). Ether layer was evaporated under reduced pressure to obtain the corresponding crude aldehyde (0.2 g) as a syrup. ^1H NMR ($CDCl_3$) δ 1.05 (s, 9H), 1.44 (s, 9H), 1.94–2.18 (m, 2H), 3.70–3.85 (m, 2H), 4.40–4.50 (m, 1H), 5.91 (d, 1H, J = 6.9 Hz), 7.35–7.46 (m, 6H), 7.64–7.67 (m, 4H), 10.72 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 19.21, 26.94, 27.71, 28.49, 29.87, 31.70, 58.87, 60.58, 80.23, 128.00, 130.06, 133.05, 135.72, 155.84, 200.22. A solution of benzothiazole (0.14 mL, 1.36 mmol) in anhydrous THF (2 mL) was treated with *n*-BuLi (0.77 mL, 1.24 mmol) at −78 °C. Above aldehyde (0.2 g, 0.45 mmol) in anhydrous THF (1 mL) was added after stirring for 30 min and stirred for an additional 3 h at the same temperature. Reaction mixture was quenched with sat NH_4Cl solution, warmed to rt, and the aqueous layer was extracted using ethyl acetate. The combined ethyl acetate layer was washed with brine and dried (Na_2SO_4). Ethyl acetate layer was evaporated under reduced pressure and the crude was purified by silica gel column chromatography (EtOAc/hexanes, 1:9) to obtain compound **28** (0.175 g, 68% over two steps) as an inseparable mixture of diastereomers in 1.5:1 ratio: Foam, ^1H NMR ($CDCl_3$) δ 1.01, 1.07 (2s, 9H), 1.25–1.42 (m, 9H), 1.83–2.07 (m, 2H), 3.72–3.85 (m, 2H), 4.25–4.39 (m, 1H), 5.19–5.33 (m, 1H), 5.56–5.69 (m, 1H), 7.29–7.48 (m, 10H), 7.57–7.68 (m, 5H), 7.87–7.91 (m, 1H), 7.97 (d, 1H, J = 7.8 Hz); ^{13}C NMR ($CDCl_3$) δ 19.24, 19.29, 26.99, 27.05, 28.42, 28.49, 31.96, 33.41, 54.79, 61.45, 75.13, 80.21, 80.56, 121.93, 121.97, 122.23, 123.09, 123.15, 124.27, 125.01, 126.07, 127.92, 127.99, 128.02, 129.94, 130.04, 130.07, 133.09, 133.16, 133.29, 135.26, 135.33, 135.70, 135.72, 135.77, 153.29, 153.47, 157.17.

5.1.13. {(1*S*)-1-{(1*S*)-1-[(1*R*)-1-(Benzothiazol-2-yl-hydroxy methyl)- 3-(*tert*-butyl diphenyl silyloxy)-propylcarbamoyl]-2-phenyl-ethylcarbamoyl}-2-methyl-propyl}- carbamic acid *tert*-butyl ester (29). Compound **28** (0.165 g, 0.28 mmol) was treated with 20% TFA in CH_2Cl_2 (5 mL) at 0 °C and a procedure similar to that for compound **1** was followed to obtain the corresponding amine derivative as an inseparable mixture of diastereomers in 1.5:1 ratio. Then, a solution of compound **20** (0.11 g, 0.31 mmol) in dry CH_2Cl_2/DMF (3 mL, 5:1) was treated with HOBt (0.077 g, 0.57 mmol) and EDAC (0.065 g, 0.34 mmol) at 0 °C. After stirring for 30 min, Et_3N (0.039 mL, 0.28 mmol) and the above diastereomeric mixture were added and a protocol similar to that described for compound **21** was followed to obtain compound **29** (0.19 g, 81%) as an inseparable mixture of diastereomers (1.5:1 ratio): syrup, ^1H NMR ($CDCl_3$) δ 0.67–0.83 (m, 6H), 0.95–1.06 (m, 9H), 1.36–1.39 (m, 9H), 1.69 (br s, 1H), 1.82–2.04 (m, 2H), 2.83–3.11 (m, 2H), 3.50–4.08 (m, 3H), 4.50–4.69 (m, 2H), 5.07–5.28 (m, 2H), 5.67, 5.80 (2d, 1H, J = 4.8 Hz), 6.81 (t, 1H, J = 10.5 Hz), 6.92–7.64 (m, 17H), 7.82–7.96 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 17.49, 17.76, 19.19, 19.22, 19.31, 23.31, 26.93, 26.99, 28.38, 28.42, 28.46, 30.90, 31.17, 31.38, 31.54, 33.16, 33.42, 36.58, 38.21, 39.21, 52.76, 53.44, 54.66, 54.82, 60.29, 60.61, 60.74, 61.30, 73.32, 74.05, 77.43, 79.99, 80.33, 121.80, 121.85, 121.94, 122.92, 123.03, 123.11, 124.87, 124.93, 125.93, 126.02, 126.81, 126.96, 127.66, 127.71, 127.83, 127.92, 128.44, 128.63, 128.68, 129.18, 129.27, 129.31, 129.69, 129.86, 129.92, 133.18, 133.26, 133.33, 133.57, 134.92, 134.96, 135.01, 135.06, 135.61, 135.65, 135.69, 136.25, 136.48, 136.60, 153.16, 153.28, 153.34, 153.58, 156.02, 162.73, 170.88, 171.20, 171.32, 171.41, 171.54, 171.80, 171.94, 174.23, 174.30, 174.57, 175.34.

5.1.14. (2*S*)-2-Amino-*N*-{(1*S*)-1-[(1*R*)-1-(benzothiazol-2-yl-hydroxymethyl)-3-hydroxypropyl carbamoyl]-2-phenylethyl}-3-methyl butyramide (5). A solution of compound **29** (0.175 g, 0.21 mmol) in anhydrous THF (3 mL) was treated with TBAF (0.21 mL, 0.21 mmol, and 1 M solution in THF) and a procedure similar to that described for compound **1** was followed to obtain the corresponding alcohol derivative (0.105 g, 85%) as an inseparable mixture of diastereomers (1.5:1 ratio): syrup; ^1H NMR ($CDCl_3$) δ 0.64–0.94 (m, 6H), 1.25–1.40 (m, 9H), 1.52–1.61 (m, 1H), 1.64–1.74 (m, 1H), 1.86–2.05 (m, 2H), 2.58–2.70 (m, 1H), 2.79–3.14 (m, 2H), 3.43–3.73 (m, 2H), 3.83–3.96 (m, 1H), 4.61–4.94 (m, 2H), 5.09–5.43 (m, 2H), 6.87–6.98 (m, 2H), 7.04–7.26 (m, 3H), 7.31–7.45 (m, 3H), 7.53–7.95 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 14.01, 17.60, 17.78, 17.97, 19.21, 19.40, 20.65, 28.46, 28.50, 30.84, 31.01, 31.23, 33.61, 34.21, 38.47, 38.84, 52.39, 52.64, 53.08, 54.80, 58.44, 58.77, 60.15, 60.64, 73.30, 73.46, 73.71, 77.42, 80.21, 80.33, 80.65, 121.98, 123.00, 125.08, 126.17, 126.90, 127.09, 128.47, 128.53, 128.72, 128.76, 129.20, 129.37, 129.49, 134.84, 134.89, 136.15, 136.33, 136.42, 136.56, 153.02, 153.12, 153.29, 153.40, 156.20, 156.35, 171.78, 171.91, 172.11, 172.20, 172.30, 174.75, 174.89. The above compound (0.1 g, 0.17 mmol) was then treated with 20% TFA in CH_2Cl_2 (5 mL) at 0 °C and a procedure similar to that described for compound **1** was used to obtain compound **5** (0.08 g, 98%) as an inseparable mixture of diastereomers (1.5:1 ratio); foam; ^1H NMR (CD_3OD/D_2O) δ 0.61–1.06 (m, 6H), 1.60–1.72 (m, 1H), 1.81–2.24 (m, 2H), 2.80–3.43 (m, 3H), 3.55–3.73 (m, 2H), 4.45–4.57 (m, 1H), 4.65–4.73 (m, 1H), 5.11–5.21 (m, 1H), 7.05–7.32 (m, 5H), 7.41–7.56 (m, 2H), 7.94–8.00 (m, 2H); MS (ESI) *m*/*z* (%) 487 (38), 485 (M^+, 100); HRMS (ESI) calculated for $C_{25}H_{33}N_4O_4S$, calculated: 485.2217, observed: 485.2237.

5.1.15. (2*S*)-2-Amino-*N*-{(1*S*)-1-[(1*R*)-1-(benzothiazol-2-yl-carbonyl)-3-hydroxypropylcarbamoyl]-2- phenylethyl}-3-methyl butyramide (6). A solution of compound **29** (0.5 g, 0.60 mmol) in anhydrous CH_2Cl_2 (5 mL) was treated with Dess–Martin periodinate (0.25 g, 0.60 mmol) and stirred for 2 h in dark. The reaction mixture was diluted with ethyl acetate, 10% aq $Na_2S_2O_3$, and satd $NaHCO_3$ solution (1:1). After stirring for 15 min, organic layer was washed with brine and dried (Na_2SO_4). Organic layer was concentrated under reduced pressure and the crude was purified by silica gel column chromatography ($CHCl_3$/MeOH, 98:2) to ob-

tain the oxidized product (0.42 g, 85%) as a foam. ^{1}H NMR ($CDCl_3$) δ 0.68–0.91 (m, 6H), 0.98–1.07 (m, 9H), 1.33–1.44 (m, 9H), 2.04–2.42 (m, 3H), 2.88–3.11 (m, 2H), 3.45–3.93 (m, 3H), 4.66–4.75 (m, 1H), 4.86–4.98 (m, 1H), 5.75–5.91 (m, 1H), 6.44–6.52 (m, 1H), 6.79–6.90 (m, 1H), 7.04–7.64 (m, 17H), 7.94–8.01 (m, 1H), 8.14–8.17 (m, 1H). A solution of the above-oxidized compound (0.02 g, 0.02 mmol) in anhydrous THF (3 mL) was treated with TBAF (0.024 mL, 0.02 mmol, 1 M solution in THF) and a procedure similar to that described for compound **1** was followed to obtain the alcohol derivative (0.17 g, 60%) as a syrup, ^{1}H NMR ($CDCl_3$) δ 0.70–0.91 (m, 6H), 1.35–1.43 (m, 9H), 1.78–2.17 (m, 2H), 2.24–2.53 (m, 1H), 2.86–3.15 (m, 2H), 3.35–3.70 (m, 1H), 3.88–4.00 (m, 1H), 4.12–4.27 (m, 1H), 4.60–4.92 (m, 1H), 5.00–5.28 (m, 1H), 5.42–5.89 (m, 1H), 6.81–7.60 (m, 9H), 7.78–8.20 (m, 2H). This alcohol derivative (0.165 g, 0.28 mmol) was treated with 20% TFA in CH_2Cl_2 (5 mL) at 0 °C, and a procedure similar to that described for compound **1** was followed to obtain compound **6** (0.11 g, 81%) as a foam, ^{1}H NMR (CD_3OD) δ 0.49–0.84 (m, 6H), 1.73–2.35 (m, 3H), 2.69–3.20 (m, 3H), 3.51–3.69 (m, 1H), 4.12–4.39 (m, 2H), 4.42–4.76 (m, 2H), 7.04–7.25 (m, 5H), 7.35–7.60 (m, 2H), 7.89–8.20 (m, 2H); MS (ESI) *m/z* (%) 485 (32), 483 (M^+, 100), 465 (56), 219 (18); HRMS (ESI): Empirical formula $C_{25}H_{31}N_4O_4S$, calculated: 483.2060, observed: 483.2077.

5.1.16. (4*R*)-2-*tert*-Butyl-4-(2-methylsulfanyl-ethyl)-5-oxo-oxazolidine-3-carbonylic acid methyl ester (30). D-Methionine (20 g, 134.22 mmol) was treated with 1 N sodium hydroxide solution (134 mL) and stirred for 1 h at room temperature. Solvent was evaporated under reduced pressure and co-evaporated with toluene (×2) to obtain the crude sodium salt. A stirred suspension of above crude product in pentane (300 mL) was treated with pivalaldehyde (17.7 mL, 161.07 mmol) and the resulting suspension was refluxed for overnight (16 h) using Dean–Stark apparatus. The reaction mixture was brought to rt and the solvent was evaporated to obtain the crude imine. A suspension of this crude imine in anhydrous CH_2Cl_2 (200 mL) was treated with methyl chloroformate (12.4 mL, 161.07 mmol) at 0 °C. The reaction mixture was brought to rt and stirred for 48 h. The reaction mixture was dissolved in CH_2Cl_2 and washed with water, satd $NaHCO_3$ solution, brine, and dried (Na_2SO_4). Organic layer was concentrated under reduced pressure and the crude was purified by column chromatography (EtOAc/hexanes, 1:9) to obtain compound **30** (29.5 g, 80%) as an inseparable mixture of diastereomers (86:14 ratio). Syrup, ^{1}H NMR ($CDCl_3$) δ 0.96, 0.98 (2s, 9H), 2.24–2.27 (m, 5H), 2.70–2.88 (m, 2H), 3.75, 3.78 (2s, 3H), 4.51 (t, 1H, J = 6.9, 7.2 Hz), 5.56, 5.62 (2s, 1H); ^{13}C NMR ($CDCl_3$) δ 15.15, 24.89, 30.61, 32.38, 37.01, 53.44, 55.86, 96.44, 156.63, 172.46.

5.1.17. 4-Allyl-2-*tert*-butyl-4-(2-methylsulfanyl-ethyl)-5-oxo-oxazolidine-3-carbonylic acid methyl ester (31a). A solution of compound **30** (10 g, 36.36 mmol) in anhydrous THF (100 mL) was treated with NaHMDS (54 mL, 54.5 mmol) at −78 °C. After 30 min of stirring at the same temperature, allyl iodide (3.6 mL, 40 mmol) was added to the reaction mixture and the stirring was continued for another 2 h. Reaction mixture was quenched with satd NH_4Cl solution and was brought to rt. Reaction mixture was then extracted with ethyl acetate, washed with brine, and dried (Na_2SO_4). Solvent was evaporated under reduced pressure and the crude was purified by silica gel column chromatography (EtOAc/hexanes, 1:9) to obtain compound **31a** as an inseparable mixture of diastereomers (9.42 g, 83%): ^{1}H NMR ($CDCl_3$) δ 0.97–1.06 (m, 9H), 2.10–2.42 (m, 5H), 2.48–2.65 (m, 2H), 2.81–2.91 (m, 2H), 3.15 (br s, 1H), 3.70, 3.73 (2s, 3H), 5.11–5.19 (m, 2H), 5.44–5.57 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 15.63, 25.60, 28.94, 37.69, 38.15, 52.77, 66.27, 95.52, 121.76, 130.42, 174.02.

5.1.18. 2-*tert*-Butyl-4-(2-methyl-allyl)-4-(2-methylsulfanyl-ethyl)-5-oxo-oxazolidine-3-carbonylic acid methyl ester (31b). A protocol similar to that for compounds **31a** was used, and compound **31b** was isolated as an inseparable mixture of diastereomers (yield: 68%), syrup, ^{1}H NMR ($CDCl_3$) δ 0.98 (s, 9H), 1.69 (s, 3H), 2.11 (s, 3H), 215–2.27 (m, 1H), 2.34–2.49 (m, 2H), 2.59 dt, 1H, J = 4.8, 12.4 Hz), 2.86 (dt, 1H, J = 4.8, 12.4 Hz), 3.17 (br s, 1H), 3.72 (s, 3H), 4.70 (br s, 1H), 4.93 (t, 1H, J = 1.5 Hz), 5.47 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 15.56, 23.14, 25.77, 28.85, 38.28, 38.63, 52.55, 65.44, 95.47, 117.30, 139.27, 174.18; MS (EI) *m/z* (%) 329 (M^+, 17), 274 (18), 168 (19), 61 (100).

5.1.19. 3-Amino-3-propyldihydrofuran-2-one (33a). A solution of compound **31a** (9.3 g, 29.52 mmol) in anhydrous CH_2Cl_2 (100 mL) was treated with trimethyloxonium tetrafluoroborate (5.3 g, 35.42 mmol) at rt and stirred for 12 h. Solvent was evaporated under reduced pressure to obtain the crude salt as an inseparable mixture of enantiomers (92:8 ratio): ^{1}H NMR ($CDCl_3$) δ 0.95–1.01 (m, 9H), 2.30–2.40 (m, 1H), 2.46–2.51 (m, 2H), 2.97 (s, 3H), 2.98 (s, 3H), 3.20–3.32 (m, 1H), 3.60–3.75 (m, 5H), 5.13–5.21 (m, 2H), 5.38–5.50 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 24.87, 25.34, 31.75, 38.12, 39.09, 53.31, 65.32, 96.08, 122.59, 129.59, 155.46, 173.53. Above crude salt was treated with 2N sodium hydroxide solution (320 mL) and refluxed overnight (16 h). Water was evaporated under reduced pressure and the crude residue was extracted using hot ethanol. Organic layer was then concentrated under reduced pressure to obtain compound **32a** as an inseparable mixture of enantiomers (92:8 ratio): ^{1}H NMR (D_2O) δ 1.88–1.97 (m, 1H), 2.03–2.14 (m, 1H), 2.29–2.41 (m, 1H), 2.60–2.67 (m, 1H), 3.68–3.77 (m, 2H), 5.25–5.29 (m, 2H), 5.73–5.87 (m, 1H); ^{13}C NMR (D_2O) δ 16.81, 27.48, 39.34, 43.11, 57.42, 58.33, 61.73, 119.97, 132.21, 180.08.

A solution of compound **32a** (6.17 g, 38.80 mmol) in anhydrous MeOH (70 mL) was treated with Pd–C (1.0 g) and stirred overnight (14 h) at rt. Reaction mixture was filtered through Celite®s and was washed with methanol. Combined organic layers were concentrated under reduced pressure to obtain propyl derivative as an inseparable mixture of enantiomers (92:8 ratio); foam; ^{1}H NMR (CD_3OD/D_2O) δ 0.89–0.96 (m, 3H), 1.19–1.57 (m, 3H), 1.71–1.88 (m, 2H), 1.92–2.00

(m, 1H), 3.64–3.77 (m, 2H). Above crude compound (6 g, 37.26 mmol) was treated with 2.5 M hydrochloric acid (100 mL) and refluxed overnight (16 h). Solvent was evaporated under reduced pressure, crude was dissolved in chloroform, and Et_3N (10 mL) was added drop-wise and stirred for 15 min. Organic layer was washed with water, brine and dried (Na_2SO_4), concentrated under reduced pressure and the crude was purified by silica gel column chromatography ($CHCl_3$/MeOH, 95:5) to obtain compound **33a** (2.4 g, 46% over four steps) as an inseparable mixture of enantiomers (92:8 ratio): oil, ^{1}H NMR ($CDCl_3$) δ 0.94–0.98 (m, 3H), 130–1.69 (m, 6H), 2.08–2.18 (m, 1H), 2.26–2.34 (m, 1H), 4.18–4.26 (m, 1H), 4.30–4.37 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.26, 16.84, 35.36, 39.86, 57.60, 64.68, 180.90.

5.1.20. 3-Amino-3-isobutyl-dihydrofuran-2-one (33b). A solution of compound **31b** (8.9 g, 27.01 mmol) in anhydrous CH_2Cl_2 (100 mL) was treated with trimethyloxonium tetrafluoroborate (4.8 g, 32.46 mmol) at rt and stirred for 12 h. Solvent was evaporated under reduced pressure to obtain the crude salt as an inseparable mixture of enantiomers (84:16 ratio). ^{1}H NMR ($CDCl_3$) δ 0.99–1.27 (m, 9H), 1.69 (s, 3H), 2.31–2.60 (m, 3H), 2.97 (s, 3H), 2.98 (s, 3H), 3.24–3.40 (m, 1H), 3.59–3.76 (m, 5H), 4.74 (s, 1H), 4.96 (s, 1H), 5.50 (s, 1H), 5.96 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 23.15, 24.85, 25.52, 32.64, 38.31, 39.07, 53.21, 64.77, 96.13, 118.12, 138.57, 155.28, 173.80. Above crude salt was refluxed in 2 N sodium hydroxide solution (300 mL) overnight. Water was evaporated under reduced pressure and the crude residue was extracted using hot ethanol. Organic layer was concentrated under reduced pressure to obtain compound **32b** as an inseparable mixture of enantiomers (84:16 ratio); ^{1}H NMR (D_2O) δ 1.76 (s, 3H), 1.95–1.98 (m, 1H), 2.03–2.10 (m, 1H), 2.42 (d, 1H, J = 13.8 Hz), 2.72 (d, 1H, J = 13.8 Hz), 3.67–3.76 (m, 2H), 4.89 (br s, 1H), 5.05 (br s, 1H).

A solution of compound **32b** (3.1 g, 17.91 mmol) in anhydrous MeOH (30 mL) was treated with Pd–C (0.5 g) and a procedure similar to that for compound **33a** was used to obtain the isobutyl derivative as an inseparable mixture of enantiomers (84:16 ratio); foam, ^{1}H NMR (CD_3OD) δ 0.89–1.02 (m, 6H), 1.50–1.60 (m, 1H), 1.74–2.00 (m, 4H), 3.68–3.76 (m, 2H); ^{13}C NMR (CD_3OD) δ 23.62, 25.02, 25.25, 28.93, 41.64, 60.18, 64.25, 170.54, 179.67.

Above compound (4.5 g, 25.71 mmol) was treated with 2.5 M hydrochloric acid (50 mL) and a protocol similar to that for compound **33a** was followed to obtain compound **33b** (2.3 g, 58% over four steps) as an inseparable mixture of enantiomers (84:16 ratio): foam, ^{1}H NMR ($CDCl_3$) δ 0.97 (d, 3H, J = 6.6 Hz), 1.00 (d, 3H, J = 6.6 Hz), 1.51 (dd, 1H, J = 7.2, 14.4 Hz), 1.67 (dd, 2H, J = 5.1, 14.4 Hz), 1.81–1.93 (m, 1H), 2.07–2.17 (m, 1H), 2.30–2.39 (m, 1H), 4.20–4.28 (m, 1H), 4.30–4.38 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 23.92, 24.43, 24.85, 35.90, 45.93, 57.73, 64.85, 181.22.

5.1.21. {(1*S*)-1-{1-Benzylcarbomoyl-1-[2-(*tert*-butyl diphenyl silyloxy)-ethyl]-butylcarbamoyl}-2-phenylethyl}-carbamic acid benzyl ester (34a). A solution of CBzNH-L-Phe-OH (1.67 g, 5.58 mmol) was treated with BOP (2.96 g, 6.70 mmol) at 0 °C. After stirring for 15 min, DIPEA (0.97 mL, 5.58 mmol) and compound **33a** (0.8 g, 5.58 mmol) were added, and a procedure described for compound **21** was used to obtain the dipeptide derivative (1.8 g) as an inseparable mixture of diastereomers (92:8 ratio): foam, ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, J = 6.9 Hz), 1.10–1.29 (m, 2H), 1.54–1.73 (m, 2H), 2.23–2.30 (m, 1H), 2.47–2.60 (m, 1H), 3.02 (d, 2H, J = 6.6 Hz), 4.13 (q, 1H), 4.35–4.50 (m, 2H), 4.97–5.07 (m, 2H), 5.81 (d, 1H, J = 8.1 Hz), 6.67 (br s, 1H), 7.14–7.34 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 14.02, 16.61, 32.83, 37.90, 38.47, 55.90, 58.91, 65.51, 66.98, 127.02, 127.95, 128.18, 128.54, 128.66, 129.41, 129.60, 136.23, 136.48, 156.14, 170.97, 176.44. A solution of above compound (1.8 g, 4.24 mmol) in THF/H_2O (25 mL, 4:1) was treated with LiOH (0.1 g, 4.24 mmol) at 0 °C, and following a similar procedure as that for compound **23**, the crude hydroxy carboxylate (1.8 g) was obtained.

A solution of the above crude hydroxyl carboxylate (1.8 g, 4.07 mmol) in anhydrous CH_2Cl_2 (25 mL) was treated with imidazole (1.38 g, 20.36 mmol) followed by TBDPSiCl (1.16 mL, 4.47 mmol) at 0 °C and following a procedure similar to that for compound **22a**, silylated product (1.66 g) was obtained. A solution of the silylated compound (1.66 g, 2.44 mmol) in dry CH_2Cl_2 (25 mL) was treated with HOBt (0.32 g, 2.44 mmol) and EDAC (0.56 g, 2.92 mmol) at 0 °C. After stirring for 30 min, Et_3N (0.34 mL, 2.44 mmol) and $BnNH_2$ (0.53 mL, 4.88 mmol) were added at the same temperature, and the protocol similar to that for compound **21** was used to obtain compound **34a** (1.5 g, 35% from **33a**) as an inseparable mixture of diastereomers (92:8 ratio): foam, ^{1}H NMR ($CDCl_3$) δ 0.77–0.89 (m, 4H), 0.95–1.12 (m, 10H), 1.91–2.25 (m, 4H), 2.84 (dd, 1H, J = 8.1, 13.8 Hz), 2.94 (dd, 1H, J = 6.9, 13.8 Hz), 3.66–3.86 (m, 2H), 4.09 (q, 1H), 4.39 (d, 2H, J = 5.7 Hz), 4.66 (d, 1H, J = 12.3 Hz), 4.86 (d, 1H, J = 12.3 Hz), 5.22–5.25 (m, 1H), 6.95–7.06 (m, 2H), 7.17–7.44 (m, 20H), 7.56–7.64 (m, 4H), 7.80 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.29, 16.64, 19.33, 27.20, 37.03, 37.83, 38.04, 43.59, 57.50, 61.62, 64.59, 67.04, 127.14, 127.49, 127.92, 128.00, 128.16, 128.23, 128.54, 128.81, 129.16, 130.08, 132.90, 133.30, 135.62, 135.86, 136.15, 138.87, 156.43, 170.80, 172.97.

5.1.22. {(1*S*)–1-{1-Benzylcarbomoyl-1-[2-(*tert*-butyldiphenylsilyloxy)-ethyl]-butylcarbamoyl}-2-dimethylcarbamoyl ethyl}-carbamic acid benzyl ester (34b). A solution of CBzNH-L-Asp(NMe)$_2$-OH (1.0 g, 3.40 mmol) was treated with BOP (1.8 g, 4.08 mmol) at 0 °C. After 15 min, DIPEA (0.59 mL, 3.40 mmol) and compound **33a** (0.48 g, 3.40 mmol) were added at the same temperature and following a procedure similar to that described for compound **21** to obtain the dipeptide derivative (1.29 g, 90%) as an inseparable mixture of diastereomers (92:8 ratio): foam, ^{1}H NMR ($CDCl_3$) δ 0.90–0.96 (m, 3H), 1.35–1.46 (m, 2H), 1.71 (t, 2H,

J = 9.0 Hz), 2.22–2.29 (m, 1H), 2.58–2.72 (m, 2H), 2.92–3.07 (m, 7H), 4.18 (q, 1H), 4.44 (dt, 1H, J = 3.0, 9.4 Hz), 4.64 (dt, 1H, J = 3.0, 7.8 Hz), 5.10 (s, 2H), 6.35 (d, 1H, J = 7.2 Hz), 7.28–7.35 (m, 5H), 7.88 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.06, 16.54, 32.35, 35.45, 36.19, 37.25, 38.14, 50.63, 58.71, 65.23, 66.88, 77.42, 127.99, 128.12, 128.48, 136.30, 155.85, 170.87, 171.06, 176.59. A solution of the dipeptide derivative (1.29 g, 3.07 mmol) in THF/H_2O (20 mL, 4:1) was treated with LiOH (0.073 g, 3.07 mmol) at 0 °C, and following a procedure similar to that for compound **23** to obtain the crude hydroxy carboxylate (1.22 g). This compound (1.22 g, 2.79 mmol) in anhydrous CH_2Cl_2 (20 mL) was treated with imidazole (0.95 g, 13.95 mmol) followed by TBDPSiCl (0.79 mL, 3.07 mmol) at 0 °C and a protocol similar to that described for compound **22a** was used to obtain the silylated compound (1.63 g). A solution of the above-silylated compound (1.63 g, 2.41 mmol) in anhydrous CH_2Cl_2 (25 mL) was treated with HOBt (0.32 g, 2.41 mmol) and EDAC (0.55 g, 2.89 mmol) at 0 °C. After stirring for 30 min, $BnNH_2$ (0.52 mL, 4.82 mmol) and Et_3N (0.33 mL, 2.41 mmol) were added at the same temperature and a procedure similar to that described for compound **21** was used to obtain compound **34b** (1.55 g, 59% from **33a**) as an inseparable mixture of diastereomers (92:8 ratio): foam; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H, J = 7.2 Hz), 1.03–1.27 (m, 11H), 1.89–2.27 (m, 4H), 2.49 (dd, 1H, J = 4.5, 16.5 Hz), 2.57 (s, 3H), 2.82 (s, 3H), 3.13 (dd, 1H, J = 3.0, 16.5 Hz), 3.69–3.81 (m, 2H), 4.33–4.51 (m, 3H), 4.75 (d, 1H, J = 12.3 Hz), 5.03 (d, 1H, J = 12.3 Hz), 5.86 (d, 1H, J = 8.7 Hz), 7.19–7.48 (m, 18H), 7.59–7.66 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 14.33, 16.78, 19.39, 27.16, 34.96, 35.84, 37.05, 38.61, 43.42, 52.19, 60.96, 63.36, 67.20, 126.92, 127.47, 127.86, 127.97, 128.34, 128.40, 128.60, 129.85, 129.90, 133.66, 133.76, 135.58, 135.64, 136.09, 139.14, 156.43, 170.65, 173.03.

5.1.23. {(1*S*)-1-{1-Benzylcarbomoyl-1-[2-(*tert*-butyldiphenylsilyloxy)-ethyl]-butylcarbamoyl}-2-thiazol-4-yl-ethyl}-carbamic acid benzyl ester (34c). A solution of BocNH-L-4-Thiazolylalanine-OH (0.85 g, 3.12 mmol) was treated with BOP (1.65 g, 3.75 mmol) at 0 °C. After 15 min, DIPEA (0.54 mL, 3.12 mmol) and compound **33a** (0.44 g, 3.12 mmol) were added at the same temperature, and a procedure similar to that for compound **21** was used to obtain the dipeptide derivative (1.2 g, 97%) as an inseparable mixture of diastereomers (92:8 ratio): foam, ^{1}H NMR ($CDCl_3$) δ 0.87–0.93 (m, 3H), 1.17–1.45 (m, 10H), 1.60–1.78 (m, 2H), 2.30–2.44 (m, 1H), 2.54–2.66 (m, 2H), 3.22 (dd, 1H, J = 6.0, 14.7 Hz), 3.33 (dd, 1H, J = 5.7, 14.7 Hz), 4.21 (q, 1H), 4.42–4.52 (m, 2H), 6.16 (d, 1H, J = 7.8 Hz), 7.18 (s, 1H), 7.52 (s, 1H), 8.78 (d, 1H, J = 1.5 Hz); ^{13}C NMR ($CDCl_3$) δ 13.98, 16.55, 28.22, 33.01, 33.26, 36.79, 37.64, 53.67, 58.68, 58.74, 65.45, 77.44, 79.91, 116.07, 152.87, 155.55, 171.10, 176.46.

A solution of the dipeptide derivative (1.2 g, 3.02 mmol) in THF/H_2O (25 mL, 4:1) was treated with LiOH (0.07 g, 3.02 mmol) at 0 °C and following a procedure similar to that for compound **23** yielded the crude hydroxyl carboxylate. A solution of the crude hydroxy carboxylate in dry CH_2Cl_2 (25 mL) was treated with imidazole (1.02 g, 15.11 mmol) and TBDPSiCl (0.86 mL, 3.32 mmol) at 0 °C. Using a similar protocol that was used for compound **22a**, silylated product (1.61 g, 82% over two steps) was obtained as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR ($CDCl_3$) δ 0.82–0.91 (m, 12H), 1.20–1.40 (m, 12H), 1.75–1.82 (m, 1H), 2.33–2.46 (m, 2H), 2.70–2.90 (m, 1H), 3.41 (d, 1H, J = 11.4 Hz), 3.59 (t, 2H, J = 6.7 Hz), 4.50–4.68 (m, 1H), 5.46–5.50 (m, 1H), 6.96 (s, 1H), 7.18–7.37 (m, 7H), 7.57–7.61 (m, 4H), 8.22 (s, 1H), 8.79 (s, 1H). A solution of the above-silylated carboxylate (1.6 g, 2.45 mmol) in anhydrous CH_2Cl_2 (25 mL) was then treated with HOBt (0.33 g, 2.45 mmol) and EDAC (0.56 g, 2.94 mmol) at 0 °C. After stirring for 30 min, Et_3N (0.34 mL, 2.45 mmol) and $BnNH_2$ (0.53 mL, 4.90 mmol) were added at same temperature, and following a protocol similar to that for compound **21**, compound **34c** (1.45 g, 64% from **31a**) was obtained as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR ($CDCl_3$) δ 0.78 (t, 3H, J = 7.2 Hz), 0.89–1.13 (m, 11H), 1.34 (s, 9H), 1.90–2.40 (m, 4H), 3.05 (dd, 1H, J = 5.4, 14.7 Hz), 3.8 (dd, 1H, J = 6.3, 14.7 Hz), 3.60–3.76 (m, 2H), 4.24–4.44 (m, 3H), 6.10 (br s, 1H), 6.86 (s, 1H), 7.15–7.39 (m, 12H), 7.61–7.65 (m, 4H), 7.73 (br s, 1H), 8.51 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.09, 16.46, 19.05, 26.88, 28.13, 32.44, 37.61, 43.41, 55.63, 60.73, 63.16, 80.01, 115.65, 126.88, 127.41, 127.71, 128.31, 129.70, 133.18, 133.25, 135.44, 138.67, 152.55, 153.03, 155.80, 170.34, 172.74.

5.1.24. {((1*S*)-1-{1-Benzylcarbomoyl-1-[2-(*tert*-butyl diphenylsilyloxy)-ethyl]-3-methyl-butylcarbamoyl}-2-dimethylcarbamoyl-ethyl}-carbamic acid benzyl ester (34d). A solution of CBzNH-L-Asp(NMe)$_2$-OH (3.7 g, 12.58 mmol) was treated with BOP (6.1 g, 13.84 mmol) at 0 °C. After stirring for 15 min, DIPEA (4.3 mL, 25.17 mmol) and compound **33b** (1.6 g, 12.58 mmol) were added at the same temperature. The reaction was completed using a procedure similar to that described for compound **21** to obtain the dipeptide derivative (2.8 g) as an inseparable mixture of diastereomers (84:16 ratio); foam, ^{1}H NMR ($CDCl_3$) δ 0.88–0.99 (m, 6H), 1.67–1.69 (m, 2H), 1.80–1.91 (m, 1H), 2.28–2.36 (m, 1H), 2.54–2.74 (m, 2H), 2.89–3.11 (m, 7H), 4.16–4.24 (m, 1H), 4.40–4.52 (m, 1H), 4.60–4.68 (m, 1H), 5.02–5.20 (m, 2H), 6.27 (d, 1H, J = 6.9 Hz), 7.27–7.36 (m, 5H), 7.81 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 23.79, 24.33, 24.35, 32.75, 35.58, 36.22, 37.39, 44.19, 50.82, 58.74, 65.33, 67.11, 77.42, 128.18, 128.31, 128.66, 136.38, 155.99, 170.87, 171.19, 176.84. A solution of the above dipeptide (2.8 g, 6.46 mmol) in THF/H_2O (30 mL, 4:1) was treated with LiOH (0.15 g, 6.46 mmol) at 0 °C and a procedure similar to that for compound **23** was used to obtain the crude hydroxy carboxylate.

A solution of the above crude carboxylate in anhydrous CH_2Cl_2 (30 mL) was treated with imidazole (2.2 g, 32.33 mmol) and TBDPSiCl (1.68 mL, 6.46 mmol) at 0 °C and a protocol similar to that for compound **22a** was used to obtain the silylated

compound (2.68 g) as an inseparable mixture of diastereomers (84:16 ratio): foam, ^{1}H NMR ($CDCl_3$) δ 0.74–1.01 (m, 15H), 1.58–1.76 (m, 2H), 2.32–2.66 (m, 4H), 2.77–2.96 (m, 7H), 3.50–3.58 (m, 2H), 4.62–4.69 (m, 1H), 5.06–5.11 (m, 2H), 6.23 (br d, 1H), 7.25–7.37 (m, 11H), 7.59–7.63 (m, 4H), 8.04 (br s, 1H). A solution of the above-silylated compound (0.1 g, 0.14 mmol) in anhydrous CH_2Cl_2 (3 mL) was treated with HOBt (0.02 g, 0.14 mmol) and EDAC (0.03 g, 0.17 mmol) at 0 °C. After stirring for 30 min, Et_3N (0.02 mL, 0.14 mmol) and $BnNH_2$ (0.017 mL, 0.15 mmol) were added at the same temperature and a protocol similar to that described for compound **21** was used to obtain compound **34d** (0.085 g, 25% from **33b**) as an inseparable mixture of diastereomers (84:16 ratio): foam, ^{1}H NMR ($CDCl_3$) δ 0.83 (d, 3H, J = 6.6 Hz), 0.87 (d, 3H, J = 6.6 Hz), 1.00, 1.03 (2, 9H), 1.58–1.67 (m, 1H), 1.97–2.04 (m, 1H), 2.08–2.17 (m, 2H), 2.52–2.34 (m, 1H), 2.48 (dd, 1H, J = 5.4, 16.5 Hz), 2.58 (s, 3H), 2.79, 2.81 (2s, 3H), 3.11 (dd, 1H, J = 3.3, 16.5 Hz), 3.66–3.83 (m, 2H), 4.31 (dd, 1H, J = 5.7, 15.1 Hz), 4.41–4.48 (m, 2H), 4.80 (d, 1H, J = 12.0 Hz), 5.04 (d, 1H, J = 12.0 Hz), 5.93 (d, 1H, J = 8.4 Hz), 7.17–7.43 (m, 16H), 7.59–7.66 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 19.40, 23.66, 24.02, 24.91, 27.18, 35.00, 35.63, 37.07, 39.18, 42.52, 43.62, 52.40, 60.87, 63.21, 67.22, 127.01, 127.65, 127.87, 127.96, 128.33, 128.43, 128.62, 129.85, 129.90, 133.70, 133.84, 135.63, 136.19, 138.97, 156.40, 170.58, 170.75, 172.97.

5.1.25. [(1*S*)-1-((1*S*)-1-{1-Benzylcarbamoyl-1-[2-(*tert*-butyl diphenyl silyloxy)-ethyl]-butylcarbamoyl}-2-phenyl-ethyl carbamoyl)-ethyl]-carbamic acid benzyl ester (35a). A solution of compound **34a** (1.5 g, 1.95 mmol) in anhydrous MeOH (30 mL) was treated with Pd–C (0.4 g) and a protocol similar to that for compound **33a** was used to obtain the amine derivative (1.24 g, quantitative, inseparable mixture of diastereomers): foam, ^{1}H NMR ($CDCl_3$–CD_3OD) δ 0.73 (t, 3H, J = 6.6 Hz), 0.93–1.21 (m, 11H), 1.83 (t, 2H, J = 7.8 Hz), 1.97–2.04 (m, 1H), 2.09–2.18 (m, 1H), 3.00 (d, 2H, J = 7.8 Hz), 3.51–3.62 (m, 2H), 4.08–4.43 (m, 3H), 7.11–7.45 (m, 17H), 7.58–7.63 (m, 4H), 7.79 (t, 1H, J = 5.7 Hz). A solution of CBzHN-L-Ala-OH (0.1 g, 0.44 mmol) in anhydrous CH_2Cl_2 (5 mL) was treated with HOBt (0.06 g, 0.44 mmol) and EDAC (0.1 g, 0.53 mmol) at 0 °C. After stirring for 30 min, Et_3N (0.06 mL, 0.44 mmol) and the above amine (0.28 g, 0.44 mmol) were added at the same temperature and a protocol similar to that described for compound **21** was used to obtain compound **35a** (0.36 g, 95%, inseparable mixture of diastereomers): foam, ^{1}H NMR ($CDCl_3$) δ 0.76–0.90 (m, 4H), 0.99–1.42 (m, 12H), 1.91–2.20 (m, 4H), 2.81–2.98 (m, 2H), 3.64–3.78 (m, 2H), 3.81–3.84 (m, 1H), 4.08–4.12 (m, 1H), 4.20–4.45 (m, 3H), 5.02–5.08 (m, 2H), 5.18 (d, 1H, J = 7.8 Hz), 6.62 (br s, 1H), 6.92–6.95 (m, 1H), 7.13–7.41 (m, 21H),7.47 (s, 1H), 7.58–7.67 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 14.28, 16.65, 18.36, 19.35, 27.04, 27.21, 37.06, 37.55, 37.96, 43.69, 50.16, 56.29, 61.52, 64.46, 67.10, 77.42, 127.15, 127.61, 127.94, 128.01, 128.17, 128.27, 128.34, 128.55, 128.66, 128.82, 129.13, 129.25, 129.93, 130.10, 132.91, 133.36, 135.64, 135.67, 135.82, 136.28, 138.78, 155.96, 170.29, 172.86, 172.97.

5.1.26. [(1*S*)-1-((1*S*)-1-{1-Benzylcarbamoyl-1-[2-(*tert*-butyldiphenylsilyloxy)-ethyl]-butylcarbamoyl}-2-phenyl-ethylcarbamoyl)-2-methyl-propyl]-carbamic acid benzyl ester (35b). Isolated as an inseparable mixture of diastereomers (92:8 ratio); yield: 93%; foam; ^{1}H NMR ($CDCl_3$) δ 0.74–0.80 (m, 4H), 0.88 (s, 9H), 0.97–1.05 (m, 10H), 1.87–2.21 (m, 4H), 2.81–2.97 (m, 2H), 3.59–3.69 (m, 1H), 3.73–3.81 (m, 1H), 3.94 (d, 1H, J = 9.3 Hz), 4.15–4.25 (m, 2H), 4.47(dd, 1H, J = 6.6, 15.1 Hz), 5.00–5.10 (m, 2H), 5.42 (d, 1H, J = 9.3 Hz), 6.47 (d, 1H, J = 3.9 Hz), 6.90–6.93 (m, 2H), 7.10–7.46 (m, 21H), 7.57–7.62 (m, 4H), 7.80 (t, 1H, J = 5.4 Hz); ^{13}C NMR ($CDCl_3$) δ 14.23, 16.58, 19.35, 26.45, 27.02, 27.20, 34.72, 36.95, 38.04, 43.66, 56.64, 61.68, 62.49, 64.61, 67.14, 77.42, 127.06, 127.23, 127.56, 127.65, 127.89, 128.00, 128.10, 128.26, 128.47, 128.62, 128.86, 129.08, 130.08, 130.11, 132.84, 133.34, 135.58, 135.62, 136.36, 138.70, 156.42, 170.14, 171.02, 172.94.

5.1.27. (3*S*)-*N*-[(1*S*)-1-{1-Benzylcarbamoyl-1-[2-(*tert*-butyl diphenyl silyloxy)-ethyl]-butyl carbamoyl}-2-phenyl-ethyl]-3-benzyloxycarbonylamino-succinamic acid benzyl ester (35c). Isolated as an inseparable mixture of diastereomers (92:8 ratio); yield: 87%, foam, ^{1}H NMR ($CDCl_3$) δ 0.77–0.85 (m, 3H), 0.98–1.12 (m, 10H), 1.86–2.42 (m, 5H), 2.55–2.70 (m, 1H), 2.77–3.10 (m, 2H), 3.66–3.71 (m, 2H), 3.81–3.88 (m, 1H), 4.09–4.41 (m, 3H), 4.52–4.63 (m, 1H), 4.96–5.10 (m, 4H), 5.56, 5.65 (2d, 1H, J = 7.8 Hz), 6.91–7.44 (m, 27H), 7.52 (s, 1H), 7.56–7.66 (m, 5H), 7.79 (t, 1H, J = 5.7 Hz); ^{13}C NMR ($CDCl_3$) δ 14.34, 16.61, 19.23, 19.36, 26.99, 27.05, 27.19, 27.26, 35.79, 36.83, 37.47, 38.03, 43.54, 50.49, 56.83, 61.76, 64.84, 66.92, 67.01, 67.33, 67.44, 127.03, 127.11, 127.18, 127.52, 127.70, 127.84, 127.89, 127.97, 128.01, 128.05, 128.17, 128.26, 128.35, 128.44, 128.53, 128.63, 128.69, 128.83, 129.13, 129.91, 130.13, 130.16, 132.79, 133.31, 135.36, 135.64, 135.66, 135.77, 136.10, 138.89, 155.91, 170.32, 171.00, 171.04, 171.13, 172.05, 173.00.

5.1.28. (4*S*)-4-((1*S*)-1-{1-Benzylcarbamoyl-1-[2-(*tert*-butyl diphenyl silyloxy)-ethyl]-butyl carbamoyl}-2-phenyl-ethyl carbamoyl)-4-benzyloxy carbonylamino-butyric acid benzyl ester (35d). Isolated as an inseparable mixture of diastereomers (92:8 ratio); yield: 98%, foam, ^{1}H NMR ($CDCl_3$) δ 0.76–0.85 (m, 3H), 1.02–1.07 (m, 9H), 1.57–1.78 (m, 2H), 1.87–2.12 (m, 4H), 2.19–2.44 (m, 5H), 2.77–2.95 (m, 2H), 3.63–3.70 (m, 1H), 3.81–3.87 (m, 1H), 4.12–4.18 (m, 2H), 4.29 (dd, 1H, J = 5.4, 14.8 Hz), 4.48 (dd, 1H, J = 6.3, 14.8 Hz), 5.05 (d, 3H, J = 12.3 Hz), 5.32 (d, 1H, J = 7.8 Hz), 6.90–6.92 (m, 2H), 7.11–7.43 (m, 20H), 7.52 (s, 1H), 7.60–7.65 (m, 4H), 7.83 (t, 1H, J = 5.4 Hz); ^{13}C NMR ($CDCl_3$) δ 14.29, 16.56, 19.34, 27.01, 27.22, 28.16, 30.31, 36.86, 37.53, 38.07, 43.57, 53.41, 56.75, 61.72, 64.79, 66.58, 66.69, 67.08, 77.42, 127.01, 127.13, 127.54, 127.64, 127.91, 128.01, 128.11, 128.28, 128.34, 128.40, 128.47, 128.61, 128.66, 128.77, 129.08, 129.88,

130.09, 130.12, 132.78, 133.27, 135.59, 135.63, 135.67, 135.70, 136.27, 138.85, 156.07, 170.41, 171.84, 172.97, 173.51.

5.1.29. [(1*S*)-1-((1*S*)-1-{1-Benzylcarbamoyl-1-[2-(*tert*-butyl diphenyl silyloxy)-ethyl]-butyl carbamoyl}-2-dimethylcarbamoyl-ethylcarbamoyl)-ethyl]-carbamic acid benzyl ester (35e). A solution of compound **34b** (1.5 g, 1.96 mmol) in anhydrous MeOH (30 mL) was treated with Pd–C (0.5 g) and a protocol similar to that for compound **33a** was used to obtain the amine (1.23 g, quantitative) as an inseparable mixture of diastereomers (92:8 ratio); foam; ^{1}H NMR ($CDCl_3$) δ 0.76 (t, 3H, J = 6.9 Hz), 1.00 (s, 9H), 1.12–1.26 (m, 1H), 1.84–2.23 (m, 4H), 2.59–2.89 (m, 8H), 3.12 (dd, 1H, J = 5.1, 16.9 Hz), 3.58–3.72 (m, 2H), 4.21–4.34 (m, 2H), 4.42 (dd, 1H, J = 6.3, 14.8 Hz), 7.11–7.41 (m, 11H), 7.59–7.62 (m, 4H), 7.68 (t, 1H, J = 5.7 Hz), 8.30 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.32, 16.75, 19.21, 27.01, 34.78, 35.42,35.71, 37.20, 37.68, 43.47, 51.10, 60.26, 63.00, 126.93, 127.74, 127.85, 127.87, 128.43, 129.82, 133.46, 133.56, 135.61, 135.63, 139.20, 168.48, 169.68, 172.94.

A solution of CBzHN-L-Ala-OH (0.1 g, 0.44 mmol) in dry CH_2Cl_2 (5 mL) was treated with HOBt (0.06 g, 0.44 mmol) and EDAC (0.1 g, 0.53 mmol) at 0 °C. After stirring for 30 min, Et_3N (0.06 mL, 0.44 mmol) and the above amine (0.28 g, 0.44 mmol) were added at the same temperature. A protocol similar to that used for compound **21** was followed to obtain compound **35e** (0.3 g, 80%) as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR ($CDCl_3$) δ 0.81 (t, 3H, J = 7.2 Hz), 1.01–1.05 (m, 10H), 1.21–1.39 (m, 4H), 1.88–2.07 (m, 2H), 2.26 (br s, 2H), 2.40–2.60 (m, 4H), 2.75–2.83 (m, 3H), 3.06 (br d, 1H, J = 15.3 Hz), 3.65–3.80 (m, 2H), 3.94–3.98 (m, 1H), 4.27–4.43 (m, 2H), 4.63–4.67 (m, 1H), 5.04, 5.08 (2s, 2H), 5.52 (d, 1H, J = 6.0 Hz), 7.15–7.40 (m, 16H), 7.44 (d, 1H, J = 8.1 Hz), 7.60–7.63 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 14.11, 14.33, 16.59, 18.53, 19.22, 26.53, 26.82, 27.09, 27.36, 34.65, 35.27, 36.17, 37.38, 37.71, 38.03, 43.32, 49.31, 50.22, 50.86, 51.07, 51.32, 60.28, 62.50, 67.08, 126.99, 127.43, 127.69, 128.19, 128.47, 128.64, 128.94, 129.36, 130.16, 133.56, 133.71, 135.23, 135.83, 136.19, 139.05, 156.23, 169.99, 170.76, 172.53, 173.05.

5.1.30. [(1*S*)-1-((1*S*)-1-{1-Benzylcarbamoyl-1-[2-(*tert*-butyl diphenyl silyloxy)-ethyl]-butyl carbamoyl}-2-dimethylcarbamoyl-ethylcarbamoyl)-2-methyl-propyl]-carbamic acid benzyl ester (35f). Isolated as an inseparable mixture of diastereomers (92:8 ratio); yield: 87%, foam, ^{1}H NMR ($CDCl_3$) δ 0.80 (t, 3H, J = 6.9 Hz), 0.93–1.26 (m, 20H), 1.82–2.00 (m, 2H), 2.20–2.30 (m, 2H), 2.45–2.52 (m, 1H), 2.58 (s, 3H), 2.73 (s, 3H), 2.90 (d, 1H, J = 13.8 Hz), 3.65–3.80 (m, 2H), 3.86 (d, 1H, J = 8.4 Hz), 4.31 (dd, 1H, J = 5.4, 15.1 Hz), 4.40 (dd, 1H, J = 5.7, 15.1 Hz), 4.62–4.68 (m, 1H), 5.01–5.06 (m, 2H), 5.52 (d, 1H, J = 8.4 Hz), 7.13–7.41 (m, 18H), 7.61–7.74 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 14.08, 14.31, 16.60, 19.14, 26.49, 26.77, 27.04, 27.32, 34.38, 34.77, 35.23, 36.79, 37.21, 43.29, 50.33, 50.58, 60.26, 62.69, 63.11, 63.38, 67.06, 127.12, 127.49, 127.57, 127.80, 128.01, 128.28, 128.50,128.80, 129.46, 130.02, 133.50, 133.62, 135.31, 135.79, 136.22, 138.97, 156.43, 169.93, 170.47, 70.63, 172.74.

5.1.31. (3*S*)-*N*-((1*S*)-1-{1-Benzylcarbamoyl-1-[2-(*tert*-butyl diphenyl silanyloxy)-ethyl]-butyl carbamoyl}-2-dimethylcarbamoyl-ethyl)-3-benzyloxycarbonylamino succinamic acid benzyl ester (35g). Isolated as an inseparable mixture of diastereomers (92:8 ratio); yield: 71%, foam, ^{1}H NMR ($CDCl_3$) δ 0.80–0.87 (m, 3H), 1.00–1.19 (m, 4H), 1.84–1.96 (m, 1H), 2.10–2.51 (m, 8H), 2.69–3.18 (m, 5H), 3.62–3.84 (m, 2H), 4.24–4.50 (m, 3H), 4.62–4.78 (m, 1H), 4.98–5.08 (m, 4H), 5.93 (d, 1H, J = 7.8 Hz), 7.13–7.36 (m, 21H), 7.63 (d, 4H, J = 6.3 Hz); ^{13}C NMR ($CDCl_3$) δ 14.18, 16.45, 19.08, 25.85, 26.77, 26.92, 35.15, 35.99, 37.27, 37.86, 43.07, 48.05, 49.23, 50.19, 50.79, 60.08, 62.04, 62.29, 66.82, 67.12, 126.82, 127.12, 127.58, 127.95, 128.13, 128.62, 129.06, 130.08, 133.59, 134.86, 135.12, 135.73, 135.98, 139.08, 155.91, 169.86, 170.28, 170.54, 171.11, 172.91.

5.1.32. (4*S*)-4-((1*S*)-1-{1-Benzylcarbamoyl-1-[2-(*tert*-butyl diphenyl silyloxy)-ethyl]-butyl carbamoyl}-2-dimethylcarbamoyl-ethyl carbamoyl)-4-benzyloxycarbonylamino-butyric acid benzyl ester (35h). Isolated as an inseparable mixture of diastereomers (92:8 ratio); yield: 87%, foam, ^{1}H NMR ($CDCl_3$) δ 0.80 (t, 3H, J = 6.9 Hz), 1.01–1.26 (m, 10H), 1.86–2.14 (m, 4H), 2.27–2.58 (m, 8H), 2.72 (s, 3H), 3.02 (br d, 1H, J = 14.4 Hz), 3.60–3.78 (m, 3H), 3.96–4.04 (m, 1H), 4.28 (dd, 1H, J = 5.4, 15.4 Hz), 4.41 (dd, 1H, J = 6.0, 15.4 Hz), 4.65 (br s, 1H), 4.99–5.08 (m, 4H), 5.93 (d, 1H, J = 6.0 Hz), 7.13–7.38 (m, 21H), 7.52 (d, 1H, J = 8.1 Hz), 7.63 (d, 4H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$) δ 14.09, 14.27, 16.51, 19.11, 26.45, 26.73, 26.87, 26.98, 30.20, 34.43, 35.19, 36.48, 37.25, 37.64, 43.15, 49.34, 50.23, 50.87, 54.77, 55.00, 60.21, 62.48, 66.55, 67.01, 126.80, 127.25, 127.66, 128.00, 128.06, 128.58, 128.97, 129.19, 130.10, 133.51, 133.65, 134.86, 135.18, 135.56, 136.07, 139.06, 156.37, 169.89, 170.50, 171.20, 172.98, 173.13.

5.1.33. [(1*S*)-1-((1*S*)-1-{1-Benzylcarbamoyl-1-[2-(*tert*-butyl diphenyl silyloxy)-ethyl]-butyl carbamoyl}-2-thiazol-4-yl-ethylcarbamoyl)-ethyl]-carbamic acid *tert*-butyl ester (35i). Compound **34c** (1.4 g, 1.88 mmol) was treated with 20% TFA in CH_2Cl_2 (30 mL) at 0 °C and a protocol similar to that for compound **1** was used to obtain the amine derivative (0.31 g, 26%) as an inseparable mixture of diastereomers (92:8 ratio); syrup, ^{1}H NMR ($CDCl_3$) δ 0.80 (t, 3H, J = 7.2 Hz), 0.92–1.26 (m, 11H), 1.37 (t, 2H, J = 9.6 Hz), 1.86–2.13 (m, 2H), 2.28–2.38 (m, 1H), 3.02 (q, 1H), 3.09–3.21 (m, 2H), 3.58–3.67 (m, 2H), 3.74 (t, 1H, J = 5.4 Hz), 4.24 (dd, 1H, J = 5.4, 14.8 Hz), 4.33–4.40 (m, 1H), 4.45 (dd, 1H, J = 6.0, 14.8 Hz), 6.88 (d, 1H, J = 1.5 Hz), 7.16–7.42 (m, 10H), 7.58–7.74 (m, 6H), 8.14 (s, 1H), 8.33 (d, 1H, J = 3.0 Hz); ^{13}C NMR ($CDCl_3$) δ 8.47, 14.18, 16.75, 19.10, 19.13, 26.68, 26.94, 28.26, 28.35, 34.52, 37.04, 37.93, 43.62, 45.69, 54.48, 60.55, 62.87, 77.42, 116.10, 127.19, 127.61, 17.70, 127.79, 127.82, 128.52, 129.40, 129.83, 133.28, 133.45, 134.93, 135.53, 135.54, 135.94, 138.80, 152.63, 153.26, 171.99, 173.14.

A solution of BocHN-L-Ala-OH (0.05 g, 0.26 mmol) in anhydrous CH_2Cl_2 (5 mL) was treated with HOBt (0.035 g, 0.26 mmol) and EDAC (0.06 g, 0.31 mmol) at 0 °C. After stirring for 30 min, Et_3N (0.036 mL, 0.26 mmol) and the above amine (0.17 g, 0.26 mmol) were added to the reaction mixture at the same temperature, and a protocol similar to that for compound **21** was used to obtain compound **35i** (0.1 g, 13% from **34c**, inseparable mixture of diastereomers) as a syrup. ^{1}H NMR ($CDCl_3$) δ 0.77 (t, 3H, J = 7.5 Hz), 0.96–1.12 (m, 10H), 1.19–1.44 (m, 13H), 1.80–1.94 (m, 2H), 2.22–2.34 (m, 2H), 3.05 (dd, 1H, J = 5.4, 14.7 Hz), 3.25 (dd, 1H, J = 6.0, 14.7 Hz), 3.41–3.72 (m, 2H), 4.04 (t, 1H, J = 6.9 Hz), 4.36–4.41 (m, 3H), 5.04 (d, 1H, J = 5.4 Hz), 6.84 (s, 1H), 7.17–7.41 (m, 13H), 7.60–7.85 (m, 5H), 8.57 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.23, 16.72, 18.45, 19.24, 26.62, 26.74, 27.03, 28.45, 31.89, 36.86, 37.73, 43.64, 50.86, 54.63, 60.75, 63.30, 70.71, 77.43, 80.22, 116.15, 127.08, 127.71, 127.88, 128.49, 129.54, 129.87, 133.53, 134.98, 135.64, 135.70, 138.92, 152.54, 153.27, 155.72, 169.77, 173.00, 173.45.

5.1.34. [(1*S*)-1-((1*S*)-1-{1-Benzylcarbamoyl-1-(2-*tert*-butyl diphenyl silyloxy-ethyl)-3-methyl-butyl carbamoyl}-2-dimentylcarbamoyl-ethylcarbamoyl)-ethyl]-carbamic acid benzyl ester (35j). A solution of compound **34d** (0.08 g, 0.10 mmol) in anhydrous MeOH (3 mL) was treated with Pd–C (0.05 g) and a procedure similar to that for compound **33a** was followed to obtain the amine derivative (0.048 g, 73%) as an inseparable mixture of diastereomers (84:16 ratio); foam, ^{1}H NMR ($CDCl_3$) δ 0.72–0.90 (m, 6H), 0.95–1.03 (m, 9H), 1.25 (br s, 2H), 1.43–1.51 (m, 1H), 1.84 (dd, 1H, J = 6.0, 14.5 Hz), 1.99 (dd, 1H, J = 5.7, 15.6 Hz), 2.13–2.20 (m, 1H), 2.27–2.34 (m, 1H), 2.63, 2.65, 2.72, 2.73 (4s, 6H), 2.87 (dd, 1H, J = 5.4, 17.2 Hz), 3.09 (d, 1H, J = 4.5, 17.2 Hz), 3.54–3.68 (m, 2H), 4.24 (dd, 1H, J = 5.1, 14.5 Hz), 4.35–4.42 (m, 2H), 7.13–7.43 (m, 11H), 7.57–7.61 (m, 4H), 8.27 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.30, 19.31, 23.96, 24.07, 24.54, 27.08, 29.87, 34.06, 35.62, 37.26, 38.43, 41.69, 43.81, 50.95, 60.26, 63.00, 127.19, 127.94, 127.98, 128.10, 128.58, 129.95, 133.45, 133.68, 135.69, 138.94, 167.20, 169.41, 172.71. A solution of CBzHN-L-Ala-OH (0.1 g, 0.44 mmol) in anhydrous CH_2Cl_2 (5 mL) was treated with HOBt (0.06 g, 0.44 mmol) and EDAC (0.1 g, 0.53 mmol) at 0 °C. After stirring for 30 min, Et_3N (0.06 mL, 0.44 mmol) and the above amine (0.28 g, 0.44 mmol) were added to the reaction mixture at the same temperature and a procedure similar to that for compound **21** was used to obtain compound **35j** (0.37 g, 70% from **34d**) as an inseparable mixture of diastereomers (84:16 ratio); foam, ^{1}H NMR ($CDCl_3$) δ 0.76–0.88 (m, 6H), 1.00–1.05 (m, 9H), 1.26–1.41 (m, 3H), 1.51–1.60 (m, 1H), 1.90–2.10 (m, 2H), 2.24–2.34 (m, 2H), 2.42 (dd, 1H, J = 5.1, 16.5 Hz), 2.57, 2.59 (2s, 3H), 2.79 (s, 3H), 3.08 (d, 1H, J = 15.6 Hz), 3.65–3.78 (m, 2H), 3.96–4.06 (m, 1H), 4.24 (dd, 1H, J = 5.4, 15.3 Hz), 4.41 (d, 1H, J = 6.3, 15.3 Hz), 4.64–4.72 (m, 1H), 5.03–5.12 (m, 2H), 5.42 (d, 1H, J = 6.0 Hz), 7.10 (t, 1H, J = 5.1 Hz), 7.16–7.44 (m, 16H), 7.59–7.65 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 18.63, 19.28, 23.55, 23.89, 24.71, 27.02, 34.65, 35.01, 37.09, 38.75, 41.68, 43.56, 50.10, 51.20, 60.34, 62.29, 67.14, 126.97, 127.56, 127.82, 127.84, 128.16, 128.27, 128.37, 128.63, 129.79, 129.83, 133.58, 133.81, 135.57, 135.62, 136.25, 138.89, 156.12, 169.94, 170.88, 172.35, 173.07; MS (ESI) m/z (%) 872 (M+Na, 28), 851 (MH^+, 59), 850 (M^+, 100), 687 (37), 594 (30).

5.1.35. [(1*S*)-1-((1*S*)-1-{1-Benzylcarbamoyl-1-(2-*tert*-butyl diphenyl silyloxy-ethyl)-3-methyl-butyl carbamoyl}-2-dimentylcarbamoyl-ethyl carbamoyl)-2-methyl-propyl]-carbamic acid benzyl ester (35k). Isolated as an inseparable mixture of diastereomers (84:16 ratio); yield 89%, foam; ^{1}H NMR ($CDCl_3$) δ 0.78 (d, 3H, J = 6.3 Hz), 0.83 (d, 3H, J = 6.3 Hz), 0.90–1.04 (m, 18H), 1.50–1.59 (m, 1H), 1.87–2.01 (m, 2H), 2.26–2.53 (m, 3H), 2.57 (s, 3H), 2.70 (s, 3H), 2.88 (br d, 1H, J = 16.2 Hz), 3.64–3.73 (m, 2H), 3.90 (d, 1H, J = 7.8 Hz), 4.33 (d, 2H, J = 5.1 Hz), 4.60–4.70 (m, 1H), 5.07 (s, 2H), 5.61 (d, 1H, J = 7.8 Hz), 7.16–7.38 (m, 18H), 7.62 (d, 4H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 19.07, 23.58, 23.73, 24.43, 26.57, 26.84, 34.30, 34.89, 35.10, 36.84, 38.36, 42.29, 43.38, 50.36, 60.16, 62.36, 63.20, 66.93, 126.79, 127.42, 127.66, 127.94, 128.08, 128.18, 128.45, 129.65, 133.40, 133.61, 135.44, 136.18, 138.71, 156.38, 169.74, 170.35, 170.47, 172.66.

5.1.36. (3*S*)-*N*-[(1*S*)-1-{1-Benzylcarbamoyl-1-(2-*tert*-butyl diphenyl silyloxy-ethyl)-3-methyl butyl carbamoyl}-2-dimethylcarbamoyl-ethyl carbamoyl]-3-benzyloxycarbonyl amino succinamic acid benzyl ester (35l). Isolated as an inseparable mixture of diastereomers (84:16 ratio); yield 86%, foam, ^{1}H NMR ($CDCl_3$) δ 0.78–0.90 (m, 6H), 0.98–1.05 (m, 9H), 1.48–1.59 (m, 1H), 1.91–2.15 (m, 2H), 2.30–2.60 (m, 7H), 2.72 (s, 3H), 2.76–2.90 (m, 2H), 3.01–3.20 (m, 1H), 3.64–3.78 (m, 2H), 4.15–4.26 (m, 1H), 4.38–4.51 (m, 2H), 4.65–4.73 (m, 1H), 5.00–5.12 (m, 3H), 5.80–5.84 (m, 1H), 7.13–7.39 (m, 21H), 7.58–7.63 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 19.11, 19.17, 23.38, 2.47, 23.72, 23.78, 24.75, 24.88, 26.92, 34.74, 34.86, 36.12, 36.88, 38.77, 38.89, 40.71, 43.20, 43.31, 49.77, 50.08, 51.63, 60.07, 60.17, 61.92, 62.20, 66.96, 67.10, 67.24, 67.48, 126.60, 126.73, 127.21, 127.33, 127.70, 127.72, 128.08, 128.17, 128.19, 128.26, 128.34, 128.41, 128.50, 128.55, 128.59, 128.63, 129.61, 129.66, 133.57, 133.71, 134.99, 135.17, 135.50, 135.52, 135.81, 136.00, 139.01, 139.17, 155.94, 169.88, 170.14, 170.18, 170.64, 170.84, 171.26, 171.77, 172.91, 172.95.

5.1.37. (4*S*)-*N*-[(1*S*)-1-{1-Benzylcarbamoyl-1-(2-*tert*-butyldiphenylsilyloxy-ethyl)-3-methyl butylcarbamoyl}-2-(*N*,*N*-dimethylcarbamoyl)-ethylcarbamoyl]-4-benzyloxy-carbonylamino-butyric acid benzyl ester (35m). Isolated as an inseparable mixture of diastereomers (84:16 ratio); yield 86%, foam, ^{1}H NMR ($CDCl_3$) δ 0.79–0.90 (m, 6H), 0.97–1.05 (m, 9H), 1.52–1.64 (m, 2H), 1.85–2.11(m, 4H), 2.24–2.61 (m, 7H), 2.74, 2.80 (2s, 3H), 2.96–3.10 (m, 1H), 3.64–3.79 (m, 2H), 3.98–4.08 (m, 1H), 4.22 (dd, 1H, J = 5.1, 15.3 Hz), 4.36–4.46 (m, 1H), 4.60–4.72 (m, 1H), 5.00–5.13 (m, 4H), 5.87 (d, 1H, J = 5.4 Hz), 7.14–7.39 (m, 22H), 7.49 (d, 1H, J = 8.4 Hz), 7.60–7.67 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 19.18, 23.47, 23.57, 23.76, 24.05, 24.71, 24.92, 26.92, 27.08, 30.32, 34.71, 34.88, 36.80, 36.91, 38.67, 41.16, 43.38, 50.10, 55.01, 60.27, 62.32, 62.95, 66.64, 67.09, 126.65, 126.76, 127.39, 127.43, 127.74, 128.04, 128.17, 128.21, 128.32, 128.52,

128.58, 129.71, 133.54, 133.68, 133.75, 135.48, 135.52, 135.60, 136.14, 138.97, 156.43, 169.87, 170.60, 171.04, 172.98, 173.23, 173.37.

5.1.38. 2-[(2*S*)-2-((2*S*)-2-Amino-propionylamino)-3-phenyl-propionylamino]-2-(2-hydroxyethyl)-pentanoic acid benzylamide (7). A solution of compound **35a** (0.35 g, 0.41 mmol) in anhydrous THF (5 mL) was treated with TBAF (0.45 mL, 0.45 mmol, 1.0 M solution in THF) and following a protocol similar to that for compound **1**, alcohol derivative(0.23 g, 92%) was obtained as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR ($CDCl_3$) δ 0.81 (t, 3H, J = 6.9 Hz), 1.05–1.19 (m, 4H), 1.82–2.18 (m, 4H), 2.94–3.13 (m, 2H), 3.51 (br s, 3H), 4.05–4.15 (m, 1H), 4.28–4.42 (m, 3H), 4.91–5.04 (m, 2H), 5.62 (d, 1H, J = 6.3 Hz), 7.09–7.34 (m, 15H), 7.55 (br s, 1H), 7.77 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.26, 16.76, 18.29, 36.78, 37.19, 37.30, 43.63, 50.52, 56.13, 58.84, 63.95, 67.17, 77.43, 127.05, 127.18, 127.47, 128.10, 128.37, 128.53, 128.64, 128.71, 129.26, 136.07, 136.70, 138.57, 156.33, 170.29, 173.41, 173.53. A solution of the above compound (0.21 g, 0.34 mmol) in anhydrous MeOH (5 mL) was treated with Pd–C (0.05 g) and a protocol similar to that for compound **33a** was used to obtain compound **7** (0.14 g, 82% from **35a**) as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR (DMSO-d_6/D_2O) δ 0.76 (t, 3H, J = 7.2 Hz), 1.00–1.08 (m, 2H), 1.35 (d, 3H, J = 6.9 Hz), 1.70–1.78 (m, 1H), 1.90–1.99 (m, 1H), 2.18–2.29 (m, 1H), 2.35–2.45 (m, 1H), 2.74–2.90 (m, 1H), 2.98–3.23 (m, 3H), 3.75 (q, 1H), 4.21–4.37 (m, 2H), 4.45 (dd, 1H, J = 4.8, 9.6 Hz), 7.18–7.33 (m, 10H), 7.67, 7.70 (2s, 1H), 8.48 (t, 1H, J = 6.0 Hz); MS (ESI) *m*/*z* (%) 491 (M+23, 21), 470 (MH^+, 20), 469 (M^+, 63), 451 (100), 362 (95), 291 (42); HRMS (ESI): Empirical formula: $C_{26}H_{37}N_4O_4$, exact mass calculated: 469.2809, observed: 469.2793.

5.1.39. 2-[(2*S*)-2-((2*S*)-2-Amino-3-methyl-butyrylamino)-3-phenyl-propionylamino]-2-(2-hydroxyethyl)-pentanoic acid benzylamide (8). A solution of compound **35b** (0.3 g, 0.34 mmol) in anhydrous THF (5 mL) was treated with TBAF (0.37 mL, 0.37 mmol, 1.0 M solution in THF) and a procedure similar to that for compound **1** was followed to obtain the alcohol derivative (0.19 g, 88%) as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR ($CDCl_3$) δ 0.79 (t, 3H, J = 6.9 Hz), 0.88 (s, 9H), 1.05–1.20 (m, 1H), 1.81–1.97 (m, 2H), 2.04–2.20 (m, 2H), 2.94–3.11 (m, 2H), 3.30 (br s, 1H), 3.42 (br s, 2H), 4.01 (d, 1H, J = 8.7 Hz), 4.28–4.47 (m, 3H), 4.93 (d, 1H, J = 12.0 Hz), 5.06 (d, 1H, J = 12.0 Hz), 5.66 (d, 1H, J = 9.0 Hz), 7.10–7.35 (m, 15H), 7.42 (t, 1H, J = 5.7 Hz), 7.55 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.21, 16.80, 26.50, 34.69, 37.38, 37.57, 37.94, 43.79, 56.62, 58.90, 62.76, 64.07, 67.22, 127.23, 127.30, 127.58, 128.15, 128.35, 128.61, 128.66, 128.85, 129.27, 136.17, 136.29, 138.46, 156.68, 170.35, 171.24, 173.17.

A solution of the above compound (0.18 g, 0.27 mmol) in anhydrous MeOH (5 mL) was treated with Pd–C (0.05 g) and a method similar to that for compound **33a** was used to obtain compound **8** (0.12 g, 75% from **35b**) as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR (DMSO-d_6/D_2O) δ 0.62–0.80 (m, 4H), 0.92–1.08 (m, 10H), 1.65–1.74 (m, 1H), 1.85–1.95 (m, 1H), 2.05–2.14 (m, 1H), 2.21–2.30 (m, 1H), 2.86 (dd, 1H, J = 8.1, 13.9 Hz), 2.96–3.09 (m, 3H), 3.51 (s, 1H), 4.19 (dd, 1H, J = 5.7, 15.3 Hz), 4.30 (dd, 1H, J = 6.0, 15.3 Hz), 4.55 (t, 1H, J = 7.5 Hz), 7.18–7.32 (m, 10H), 7.66 (s, 1H), 8.26 (t, 1H, J = 5.7 Hz); MS (ESI) *m*/*z* (%) 512 (MH^+, 46), 511 (M^+, 100), 493 (61), 404 (91), 291 (62); HRMS (ESI): Empirical formula $C_{29}H_{43}N_4O_4$, exact mass calculated: 511.3278, observed: 511.3285.

5.1.40. (3*S*)-3-Amino-*N*-{(1*S*)-1-[1-benzylcarbamoyl-1-(2-hydroxyethyl)-butylcarbamoyl]-2-phenyl-ethyl}-succinamic acid (9). A solution of compound **35c** (0.34 g, 0.34 mmol) in anhydrous THF (5 mL) was treated with TBAF (0.76 mL, 0.76 mmol, 1.0 M solution in THF) and a protocol similar to that for compound **1** was used to obtain the alcohol derivative (0.2 g, 89%) as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR (CD_3OD/D_2O) δ 0.79–0.89 (m, 3H), 0.99–1.15 (m, 1H), 1.35–1.42 (m, 1H), 1.60–1.71 (m, 1H), 1.86–2.03 (m, 2H), 2.16–2.28 (m, 1H), 2.59–2.72 (m, 2H), 2.86–2.93 (m, 1H), 3.04–3.12 (m, H), 3.38–3.50 (m, 2H), 4.21–4.50 (m, 4H), 5.03 (s, 2H), 7.14–7.32 (m, 15H).

A solution of the above compound (0.19 g, 0.29 mmol) in anhydrous MeOH (5 mL) was treated with Pd–C (0.05 g) and a procedure similar to that for compound **33a** was used to obtain compound **9** (0.125 g, 74% from **35c**) as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR (DMSO-d_6/D_2O) δ 0.77 (t, 3H, J = 7.2 Hz), 0.86–0.95 (m, 1H), 1.04–1.08 (m, 1H), 1.23–1.35 (m, 1H), 1.50–1.62 (m, 1H), 1.77–2.03 (m, 3H), 2.15–2.29 (m, 2H), 2.59–2.82 (m, 2H), 3.04–3.40 (m, 3H), 4.27–4.36 (m, 3H), 7.21–7.32 (m, 10H); MS (ESI) *m*/*z* (%) 514 (MH^+, 35), 513 (M^+, 100), 406 (35), 242 (37); HRMS (ESI): Empirical formula $C_{27}H_{37}N_4O_6$, exact mass calculated: 513.2707, observed: 513.2683.

5.1.41. (4*S*)-4-Amino-4-{(1*S*)-1-[1-benzylcarbamoyl-1-(2-hydroxyethyl)-butylcarbamoyl]-2-phenyl-ethylcarbamoyl}-butyric acid (10). A solution of compound **35d** (0.375 g, 0.37 mmol) in anhydrous THF (5 mL) was treated with TBAF (0.83 mL, 0.83 mmol, 1.0 M solution in THF) and a procedure similar to that for compound **1** was used to obtain the alcohol derivative (0.18 g, 72%) as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR (DMSO-d_6/D_2O) δ 0.73–0.90 (m, 4H), 1.00–1.10 (m, 1H), 1.62–1.92 (m, 4H), 2.01–2.13 (m, 3H), 2.21–2.31 (m, 1H), 2.74 (dd, 1H, J = 9.9, 13.8 Hz), 3.02 (dd, 1H, J = 5.4, 13.8 Hz), 3.13–3.28 (m, 2H), 3.86 (q, 1H), 4.20–4.36 (m, 3H), 5.02 (s, 2H), 7.19–7.35 (m, 15H).

A solution of the above compound (0.17 g, 0.25 mmol) in anhydrous MeOH (5 mL) was treated with Pd–C (0.05 g) and a method similar to that for compound **33a** was used to obtain compound **10** (0.125 g, 67% from **35d**) as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR (DMSO-d_6/D_2O) δ 0.77 (t, 3H,

J = 6.9 Hz), 0.84–0.90 (m, 1H), 1.00–1.10 (m, 1H), 1.74–1.96 (m, 5H), 2.01–2.08 (m, 1H), 2.17–2.29 (m, 2H), 2.72–2.79 (m, 1H), 3.03–3.08 (m, 1H), 3.15–3.38 (m, 3H), 4.21–4.38 (m, 3H), 7.21–7.33 (m, 5H); MS (ESI) *m/z* (%) 528 (MH^+, 38), 527 (M^+, 100), 420 (18), 291 (20); HRMS (ESI): Empirical formula $C_{28}H_{39}N_4O_6$, exact mass calculated: 527.2864, observed: 527.2844.

5.1.42. (2*S*)-2-[(2*S*)-2-Aminopropionyl]-N^1-[1-benzyl carbamoyl-1-(2-hydroxyethyl)-butyl]-N^4,N^4-dimethyl-succinamide (11). A solution of compound **35e** (0.29 g, 0.34 mmol) in dry THF (10 mL) was treated with TBAF (0.34 mL, 0.34 mmol, 1.0 M solution in THF) and a protocol similar to that for compound **1** was used to obtain the alcohol derivative (0.185 g, 89%) as an inseparable mixture of diastereomers (92:8 ratio); syrup, ^{1}H NMR ($CDCl_3$) δ 0.84–0.89 (m, 3H), 1.04–1.37 (m, 6H), 1.79–1.80 (m, 2H), 2.02–2.11 (m, 1H), 2.41–2.46 (m, 4H), 2.79 (s, 3H), 3.24 (d, 1H, J = 15.9 Hz), 3.65 (br s, 2H), 3.86 (br s, 1H), 4.22 (t, 1H, J = 7.2 Hz), 4.33 (dd, 1H, J = 5.4, 15.4 Hz), 4.52 (dd, 1H, J = 6.9, 15.4 Hz), 4.73–4.76 (m, 1H), 5.05 (s, 2H), 5.83 (d, 1H, J = 6.9 Hz), 7.12–7.34 (m, 10H), 7.61 (d, 1H, J = 8.4 Hz), 7.87 (t, 1H, J = 5.4 Hz), 8.30 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.14, 14.38, 16.74, 17.77, 18.11, 22.66, 31.59, 34.07, 34.47, 35.07, 35.30, 36.61, 37.23, 43.13, 49.04, 49.64, 50.01, 50.61, 50.84, 51.21, 59.20, 64.66, 67.24, 126.38, 126.66, 126.98, 127.36, 127.57, 127.89, 128.21, 128.56, 128.96, 136.01, 139.14, 156.69, 170.51, 171.27, 172.54, 173.81.

A solution of the above compound (0.18 g, 0.30 mmol) in anhydrous MeOH (5 mL) was treated with Pd–C (0.05 g) and a protocol similar to that for compound **33a** was used to obtain compound **11** (0.12 g, 80% from 35e) as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR (DMSO-d_6/D_2O) δ 0.77 (t, 3H, J = 6.6 Hz), 0.86–0.95 (m, 1H), 1.14–1.20 (m, 1H), 1.36 (d, 3H, J = 6.9 Hz), 1.70–1.82 (m, 1H), 1.96–2.11 (m, 2H), 2.26–2.36 (m, 1H), 2.48–2.53 (m, 5H), 2.92 (s, 3H), 3.19–3.37 (m, 2H), 3.85 (q, 1H), 4.23–4.35 (m, 2H), 4.62 (t, 1H, J = 6.0 Hz), 7.23–7.32 (m, 5H), 7.81 (s, 1H), 8.47 (t, 1H, J = 5.1 Hz); MS (EI) *m/z* (%) 465 (MH^+, 22), 464 (M^+, 74, 446 (49), 214 (49), 116 (100), 72 (61); HRMS (EI): Empirical formula $C_{23}H_{38}N_5O_5$, exact mass calculated: 464.287295, observed: 464.286179.

5.1.43. (2*S*)–2-[(2*S*)-2-Amino-3-methylbutyryl]-N^1-[1-benzylcarbamoyl-1-(2-hydroxyethyl)-butyl]-N^4,N^4-dimethyl-succinamide (12). A solution of compound **35f** (0.35 g, 0.39 mmol) in anhydrous THF (10 mL) was treated with TBAF (0.39 mL, 0.39 mmol, 1.0 M solution in THF), and a procedure similar to that for compound **1** was followed to obtain the alcohol derivative (0.23 g, 90%) as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR ($CDCl_3$) δ 0.88–1.08 (m, 10H), 1.11–1.26 (m, 4H), 1.85–1.97 (m, 2H), 2.12–2.17 (m, 1H), 2.27–2.36 (m, 1H), 2.61 (s, 3H), 2.72–2.77 (m, 1H), 2.82 (s, 3H), 3.00 (dd, 1H, J = 5.4, 16.8 Hz), 3.60 (br s, 2H), 3.75 (br s, 1H), 4.00 (d, 1H, J = 8.7 Hz), 4.42 (m, 2H), 4.60–4.70 (m, 1H), 5.04 (q, 2H), 5.54 (d, 1H, J = 8.7 Hz), 7.15–7.31 (m, 10H), 7.73 (d, 1H, J = 7.2 Hz), 7.83 (br s, 1H), 7.90 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.09, 14.35, 16.73, 22.03, 26.11, 26.39, 26.68, 26.98, 31.56, 34.54, 35.41, 36.70, 37.30, 43.39, 50.10, 50.91, 51.57, 58.55, 58.94, 62.59, 62.82, 63.74, 64.08, 67.18, 126.49, 127.10, 127.66, 127.89, 128.17, 128.40, 128.60, 128.95, 136.06, 138.98, 156.85, 170.02, 170.80, 171.22, 173.55.

A solution of the above compound (0.22 g, 0.34 mmol) in anhydrous MeOH (5 mL) was treated with Pd–C (0.05 g) and a procedure similar to that for compound **33a** was used to obtain compound **12** (0.17 g, 88% from **35f**) as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR (DMSO-d_6/D_2O) δ 0.76 (t, 3H, J = 6.6 Hz), 0.86–1.06 (m, 10H), 1.10–1.26 (m, 1H), 1.64–1.78 (m, 1H), 1.90–2.14 (m, 2H), 2.19–2.29 (m, 1H), 2.58–2.80 (m, 5H), 2.91 (s, 3H), 3.17–3.37 (m, 2H), 3.53 (s, 1H), 4.23 (dd, 1H, J = 5.4, 15.3 Hz), 4.34 (dd, 1H, J = 6.0, 15.3 Hz), 4.65 (dd, 1H, J = 5.1, 7.9 Hz), 7.23–7.31 (m, 5H), 7.87 (s, 1H), 8.34 (t, 1H, J = 5.7 Hz); MS (EI) *m/z* (%) 507 (MH^+, 31), 506 (M^+, 88), 488 (50), 116 (98), 115 (69), 86 (100), 72 (55); HRMS (EI): Empirical formula $C_{26}H_{44}N_5O_5$, exact mass calculated: 506.334245, observed: 506.332986.

5.1.44. (3*S*)-3-Amino-*N*-{(1*S*)-1-[1-benzylcarbamoyl-1-(2-hydroxyethyl)-butylcarbamoyl]-2- dimethylcarbamoyl-ethyl}-succinamic acid (13). A solution of compound **35g** (0.33 g, 0.34 mmol) in anhydrous THF (10 mL) was treated with TBAF (0.68 mL, 0.68 mmol, 1.0 M solution in THF) and a procedure similar to that for compound **1** was used to obtain the alcohol derivative (0.167 g, 77%) as an inseparable mixture of diastereomers (92:8 ratio): foam, ^{1}H NMR (DMSO-d_6/D_2O) δ 0.78 (br t, 3H), 0.86–1.24 (m, 2H), 1.77–1.99 (m, 4H), 2.10–2.15 (m, 1H), 2.46–2.86 (m, 9H), 3.31–3.40 (m, 2H), 4.26–4.32 (m, 3H), 4.52 (t, 1H, J = 4.8 Hz), 5.01 (s, 2H), 7.21–7.35 (m, 10H).

A solution of the above compound (0.16 g, 0.24 mmol) in anhydrous MeOH (5 mL) was treated with Pd–C (0.05 g) and was purified as described for compound **33a** to obtain compound **13** (0.12 g, 73% from **35g**) as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR (DMSO-d_6/D_2O) δ 0.80 (t, 3H, J = 6.9 Hz), 0.93–1.24 (m, 2H), 1.78–2.11 (m, 4H), 2.36–2.69 (m, 6H), 2.74–2.88 (m, 4H), 3.30–3.44 (m, 2H), 3.53–3.59 (m, 1H), 4.22–4.33 (m, 2H), 4.52 (t, 1H, J = 4.8 Hz), 7.21–7.31 (m, 5H); MS (ESI) *m/z* (%) 509 (MH^+, 34), 508 (M^+, 100), 490 (34), 401 (31); HRMS (ESI): Empirical formula $C_{24}H_{38}N_5O_7$, exact mass calculated: 508.2765, observed: 508.2751.

5.1.45. (4*S*)-4-Amino-4-{(1*S*)-1-[1-benzylcarbamoyl-1-(2-hydroxyethyl)-butyl carbamoyl]-2-dimethylcarbamoyl-ethyl carbamoyl} butyric acid (14). A solution of compound **35h** (0.4 g, 0.40 mmol) in anhydrous THF (10 mL) was treated with TBAF (0.81 mL, 0.81 mmol, 1.0 M solution in THF) and a protocol similar to that for compound **1** was used to obtain the alcohol derivative (0.16 g, 60%) as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR (DMSO-d_6/D_2O) δ

0.77 (t, 3H, J = 7.2 Hz), 0.80–0.98 (m, 1H), 1.10–1.23 (m, 1H), 1.72–2.00 (m, 5H), 2.10–2.24 (m, 3H), 2.65–2.75 (m, 5H), 2.88 (s, 3H), 3.24–3.41 (m, 2H), 3.92 (dd, 1H, J = 3.9, 9.0 Hz), 4.22–4.34 (m, 2H), 4.51 (t, 1H, J = 6.0 Hz), 4.97–5.07 (m, 2H), 7.22–7.40 (m, 10H), 7.99 (s, 1H), 8.38 (t, 1H, J = 6.0 Hz).

A solution of the above compound (0.155 g, 0.23 mmol) in anhydrous MeOH (5 mL) was treated with Pd–C (0.05 g) and a protocol similar to that for compound **33a** was followed to obtain compound **14** (0.11 g, 55% from 35h) as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR (DMSO-d_6/D_2O) δ 0.79 (t, 3H, J = 7.5 Hz), 0.88–1.04 (m, 1H), 1.09–1.23 (m, 1H), 1.81–2.00 (m, 4H), 2.07–2.22 (m, 1H), 2.29 (t, 2H, J = 7.5 Hz), 2.63–2.84 (m, 5H), 2.88 (s, 3H), 3.20–3.46 (m, 4H), 4.23–4.29 (m, 2H), 4.52 (t, 1H, J = 5.4 Hz), 7.20–7.32 (m, 5H); MS (ESI) m/z (%) 523 (MH^+, 33), 522 (M^+, 100), 513 (23), 301 (27), 241 (23); HRMS (ESI): Empirical formula $C_{25}H_{40}N_5O_7$, exact mass calculated: 522.2922, observed: 522.2926.

5.1.46. 2-[(2*S*)-2-((2*S*)-2-Amino-propionyl amino)-3-thiazol-4-yl-propionyl amino]-2-(2-hydroxyethyl)-pentanoic acid benzylamide (15). A solution of compound **35i** (0.1 g, 0.12 mmol) was treated with 40% TFA in CH_2Cl_2 (5.0 mL) at 0 °C and a procedure similar to that for compound **1** was used to obtain compound **15** (0.04 g, 73%) as an inseparable mixture of diastereomers (92:8 ratio); foam, ^{1}H NMR (CD_3OD/D_2O) δ 0.83 (t, 3H, J = 7.2 Hz), 0.90–1.24 (m, 2H), 1.31 (d, 3H, J = 6.9 Hz), 1.84–1.94 (m, 1H), 2.00–2.12 (m, 2H), 2.31–2.41 (m, 1H), 3.22 (dd, 1H, J = 7.5, 15.0 Hz), 3.42–3.52 (m, 3H), 3.58–3.72 (m, 1H), 4.41–4.46 (m, 2H), 4.66 (t, 1H, J = 6.6 Hz), 7.21–7.34 (m, 6H), 8.85 (d, 1H, J = 2.1 Hz); MS (ESI) m/z (%) 498 (M+Na, 18), 477 (MH^+, 34), 476 (M^+, 100), 458 (42), 369 (15); HRMS (ESI): Empirical formula $C_{23}H_{34}N_5O_4S$, exact mass calculated: 476.2311, observed: 476.2326.

5.1.47. (2*S*)-2-[(2*S*)-2-Aminopropionyl]-*N*^{1}-[1-benzyl carbamoyl-1-(2-hydroxyethyl)-3-methyl- butyl]-*N*^{4},*N*^{4}-dimethyl succinamide (16). A solution of compound **35j** (0.75 g, 0.88 mmol) in anhydrous THF (5 mL) was treated with TBAF (0.8 mL, 0.88 mmol, 1.0 M solution in THF) and a procedure similar to that for compound **1** was used to obtain the alcohol derivative (0.465 g, 86%) as an inseparable mixture of diastereomers (84:16 ratio); foam, ^{1}H NMR ($CDCl_3$) δ 0.86 (d, 3H, J = 6.6 Hz), 0.90 (d, 3H, J = 6.6 Hz), 1.36 (d, 3H, J = 7.2Hz), 1.61–1.72 (m, 1H), 1.89 (dd, 2H, J = 4.8, 14.5 Hz), 2.10–2.19 (m, 1H), 2.41–2.44 (m, 4H), 2.76 (s, 3H), 3.20 (br d, 1H, J = 15.4 Hz), 3.63 (br s, 2H), 3.86 (br s, 1H), 4.20–4.32 (m, 2H), 4.52 (dd, 1H, J = 6.9, 15.4 Hz), 4.68–4.74 (m, 1H), 5.01–5.09 (m, 2H), 6.04 (d, 1H, J = 7.5 Hz), 7.11–7.34 (m, 10H), 7.62 (d, 1H, J = 8.7 Hz), 7.87 (t, 1H, J = 5.7 Hz), 8.30 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.59, 17.79, 20.12, 23.34, 23.73, 25.00, 34.62, 34.76, 36.78, 37.67, 41.41, 43.12, 49.92, 50.90, 58.95, 64.48, 66.99, 126.55, 126.92, 127.75, 128.07, 128.43, 135.99, 138.92, 156.57, 170.54, 171.07, 172.41, 173.65.

A solution of the above compound (0.45 g, 0.73 mmol) in anhydrous MeOH (10 mL) was treated with Pd–C (0.1 g) and a procedure similar to that described for compound **33a** was followed to obtain compound **16** (0.33 g, 81% from **35j**) as an inseparable mixture of diastereomers (84:16 ratio); foam, ^{1}H NMR (DMSO-d_6/D_2O) δ 0.68 (d, 3H, J = 6.6 Hz), 0.77 (d, 3H, J = 6.6 Hz), 1.23–1.49 (m, 4H), 1.71 (dd, 1H, J = 6.6, 14.2 Hz), 1.87–1.96 (m, 1H), 2.16 (dd, 1H, J = 5.4, 14.2 Hz), 2.37–2.40 (m, 1H), 2.52–2.75 (m, 5H), 2.80 (s, 3H), 3.03–3.16 (m, 1H), 3.23–3.31 (m, 1H), 3.85 (q, 1H), 4.20–4.36 (m, 2H), 4.64 (t, 1H, J = 6.0 Hz), 7.21–7.33 (m, 5H), 7.75 (s, 1H), 8.28 (s, 1H), 8.55 (t, 1H, J = 5.7 Hz); MS (ESI) m/z (%) 500 (M+23, 9), 479 (MH^+, 43), 478 (M^+, 100), 460 (92), 371 (29); HRMS (ESI): Empirical formula $C_{24}H_{40}N_5O_5$, exact mass calculated: 478.3014, observed: 478.3023.

5.1.48. (2*S*)-2-[(2*S*)-2-Amino-3-methylbutyryl]-*N*^{1}-[1-benzylcarbamoyl-1-(2-hydroxyethyl)-3-methyl- butyl]-*N*^{4},*N*^{4}-dimethyl-succinamide (17). A solution of compound **35k** (0.6 g, 0.67 mmol) in anhydrous THF (10 mL) was treated with TBAF (0.67 mL, 0.67 mmol, 1.0 M solution in THF) and a procedure similar to that for **1** was used to obtain the alcohol derivative (0.41 g, 95%) as an inseparable mixture of diastereomers (84:16 ratio); foam, ^{1}H NMR ($CDCl_3$) δ 0.83 (d, 3H, J = 6.6 Hz), 0.89 (d, 3H, J = 6.6 Hz), 0.95 (s, 9H), 1.62–1.68 (m, 1H), 1.92–1.99 (m, 2H), 2.21–2.28 (m, 2H), 2.62 (s, 3H), 2.69–2.81 (m, 4H), 2.98 (dd, 1H, J = 5.1, 16.8 Hz), 3.57 (br s, 2H), 3.66 (br s, 1H), 4.04 (d, 1H, J = 9.3 Hz), 4.41 (d, 2H, J = 5.1 Hz), 4.60–4.70 (m, 1H), 5.03 (q, 2H), 5.61 (d, 1H, J = 9.0 Hz), 7.12–7.35 (m, 10H), 7.76–7.84 (m, 2H), 7.91 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 23.43, 23.81, 24.78, 26.49, 34.53, 35.02, 36.94, 37.87, 42.35, 43.44, 50.98, 58.80, 62.58, 63.93, 67.01, 77.43, 126.76, 127.50, 127.92, 128.17, 128.46, 136.07, 138.78, 156.71, 169.93, 170.64, 171.09, 173.36.

A solution of the above compound (0.4 g, 0.61 mmol) in anhydrous MeOH (10 mL) was treated with Pd–C (0.1 g) and a procedure similar to that for compound **33a** was used to obtain compound **17** (0.3 g, 89% from **35k**) as an inseparable mixture diastereomers (84:16 ratio); foam, ^{1}H NMR (DMSO-d_6/D_2O) δ 0.69 (d, 3H, J = 6.3 Hz), 0.79 (d, 3H, J = 6.6 Hz), 0.92 (s, 9H), 1.46–1.58 (m, 1H), 1.73–1.80 (m, 1H), 1.88–2.10 (m, 2H), 2.23–2.36 (m, 1H), 2.68–2.80 (m, 5H), 2.92 (s, 3H), 3.19–3.34 (m, 3H), 4.28 (br s, 2H), 4.59 (t, 1H, J = 6.3 Hz), 7.20–7.33 (m, 5H), 7.85 (br s, 1H), 8.43 (t, 1H); MS (ESI) m/z (%) 521 (MH^+, 42), 520 (M^+, 100), 502 (39), 413 (14); HRMS (ESI): Empirical formula $C_{27}H_{46}N_5O_5$, exact mass calculated: 520.3493, observed: 520.3485.

5.1.49. (3*S*)-3-Amino-*N*-{(1*S*)-1-[1-benzylcarbamoyl-1-(2-hydroxyethyl))-3-methyl-butylcarbamoyl]-2-dimethyl-carbamoyl-ethyl}-succinamic acid (18). A solution of compound **35l** (0.38 g, 0.38 mmol) in anhydrous THF (10 mL) was treated with TBAF (0.38 mL, 0.38 mmol, 1.0 M solution in THF) and a protocol similar to that

for compound **1** was followed to obtain the corresponding alcohol derivative (0.21 g, 83%) as an inseparable mixture of diastereomers (84:16 ratio); foam, ^{1}H NMR (DMSO-d_6/D_2O) δ 0.69–0.96 (m, 6H), 1.42–1.60 (m, 1H), 1.82–2.00 (m, 3H), 2.19–2.31 (m, 1H), 2.40–2.77 (m, 7H), 2.84, 2.86 (2s, 3H), 3.22–3.42 (m, 2H), 4.10–4.20 (m, 1H), 4.27 (br s, 2H), 4.53 (t, 1H, J = 5.7 Hz), 5.00 (s, 2H), 7.20–7.39 (m, 10H), 8.00, 8.07 (2s, 1H), 8.33 (t, 1H, J = 6.3 Hz).

A solution of the above compound (0.2 g, 0.30 mmol) in anhydrous MeOH (5 mL) was treated with Pd–C (0.075 g) and a procedure similar to that for compound **33a** was used to obtain compound **18** (0.146 g, 76% from **35l**) as an inseparable mixture of diastereomers (84:16 ratio); foam, ^{1}H NMR (DMSO-d_6/D_2O) δ 0.70 (d, 3H, J = 6.9 Hz), 0.77 (d, 3H, J = 6.6 Hz), 1.40–1.54 (m, 1H), 1.80–1.94 (m, 3H), 2.15–2.26 (m, 1H), 2.33–2.53 (m, 2H), 2.63–2.80 (m, 5H), 2.86 (s, 3H), 3.17–3.51 (m, 3H), 4.24 (s, 2H), 4.50–4.54 (m, 1H), 7.16–7.28 (m, 5H); MS (ESI) m/z (%) 523 (MH^+, 23), 522 (M^+, 100), 504 (23), 280 (51); HRMS (ESI): Empirical formula $C_{25}H_{40}N_5O_7$, exact mass calculated: 522.2922, observed: 522.2935.

5.1.50. (4*S*)-4-Amino-4-{(1*S*)-1-[1-benzylcarbamoyl-1-(2-hydroxyethyl)-3-methyl-butylcarbamoyl]-2-dimethylcarbamoyl-ethylcarbamoyl} butyric acid (19). A solution of compound **35m** (0.32 g, 0.32 mmol) in anhydrous THF (5 mL) was treated with TBAF (0.32 mL, 0.32 mmol, 1.0 M solution in THF) and a procedure similar to that for compound **1** to obtain the alcohol derivative (0.15 g, 70%) as an inseparable mixture of diastereomers (84:16 ratio); foam, ^{1}H NMR (DMSO-d_6/D_2O) δ 0.64–0.91 (m, 6H), 1.38–1.46 (m, 1H), 1.73–1.97 (m, 6H), 2.11–2.29 (m, 2H), 2.63–2.72 (m, 5H), 2.84 (s, 3H), 3.14–3.34 (m, 2H), 3.78–3.86 (m, 1H), 4.24 (d, 2H, J = 5.7 Hz), 4.49 (t, 1H, J = 5.7 Hz), 4.97 (s, 2H), 7.16–7.35 (m, 10H), 7.90 (s, 1H), 8.09 (d, 1H, J = 7.8 Hz), 8.40 (t, 1H, J = 5.7 Hz).

A solution of the above compound (0.14 g, 0.20 mmol) in anhydrous MeOH (5 mL) was treated with Pd–C (0.05 g) and a procedure similar to that for compound **33a** was used to obtain compound **19** (0.1 g, 63% from **35m**) as an inseparable mixture of diastereomers (84:16 ratio); foam, ^{1}H NMR (DMSO-d_6–D_2O) δ 0.68 (d, 3H, J = 6.6 Hz), 0.76 (d, 3H, J = 6.6 Hz), 1.41–1.50 (m, 1H), 1.79–1.96 (m, 5H), 2.18–2.30 (m, 3H), 2.63–2.69 (m, 5H), 2.86 (s, 3H), 3.14–3.31 (m, 3H), 4.25 (s, 2H), 4.50 (t, 1H, J = 5.7 Hz), 7.17–7.29 (m, 5H); MS (ESI) m/z (%) 537 (MH^+, 35), 536 (M^+, 100), 421 (18), 287 (40); HRMS (ESI): Empirical formula $C_{26}H_{42}N_5O_7$, exact mass calculated: 536.3078, observed: 536.3053.

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Supplementary data

Inhibition studies of compounds **1** and **2** against chymotrypsin. Dixon plot on the inhibition studies of compound **1** against HCMV protease. Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmc.2005.08.019.

References and notes

1. Puente, X. S.; Sanchez, L. M.; Gutierrez-Fernandez, A.; Velasco, G.; Lopez-Otin, C. *Biochem. Soc. Trans.* **2005**, *33*, 331–334.
2. Barrett, A. J. *Curr. Opin. Drug Discov. Devel.* **2004**, *7*, 334–341.
3. Docherty, A. J. P.; Crabbe, T.; O'Connell, J. P.; Groom, C. R. *Biochem. Soc. Sym.* **2003**, *70*, 147–161.
4. Silverman, R. B. *The Organic Chemistry of Enzyme-Catalyzed Reactions*; Academic Press: New York, 2000, pp 39–60.
5. Ogilvie, W.; Bailey, M.; Poupart, M.-A.; Abraham, A.; Bhavsar, A.; Bonneau, P.; Bordeleau, J.; Bousquet, Y.; Chabot, C.; Duceppe, J.-S.; Fazal, G.; Goulet, S.; Grand-Maitre, C.; Guse, I.; Halmos, T.; Lavallee, P.; Leach, M.; Malenfant, E.; O'Meara, J.; Plante, R.; Plouffe, C.; Poirier, M.; Soucy, F.; Yoakim, C.; Deziel, R. *J. Med. Chem.* **1997**, *40*, 4113–4135.
6. Najera, C.; Abellan, T.; Sansano, J. M. *Eur. J. Org. Chem.* **2000**, 2809–2820.
7. Graham, S. L.; Desolms, S. J.; Giuliani, E. A.; Kohl, N. E.; Mosser, S. D.; Oliff, A. I.; Pompliano, D. L.; Rands, E.; Breslin, M. J.; Deana, A. A.; Garsky, V. M.; Scholz, T. H.; Gibbs, J. B.; Smith, R. L. *J. Med. Chem.* **1994**, *37*, 725–732.
8. Corey, E. J.; Narasaka, K.; Shibasaki, M. *J. Am. Chem. Soc.* **1976**, *98*, 6417–6418.
9. Novacheck, K. A.; Meyers, A. I. *Tetrahedron Lett.* **1996**, *37*, 1743–1746.
10. Cordova, A.; Reed, N. N.; Ashley, J. A.; Janda, K. D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3119–3122.
11. Kolasa, T.; Miller, M. J. *J. Org. Chem.* **1999**, *55*, 1711–1721.
12. Ozinskas, A. J.; Rosenthal, G. A. *J. Org. Chem.* **1986**, *51*, 5047–5050.
13. Robinson, R. P.; Donahue, K. M. *J. Org. Chem.* **1992**, *57*, 7309–7314.
14. Scheidt, K. A.; Roush, W. R.; McKerrow, J. H.; Selzer, P. M.; Hansell, E.; Rosenthal, P. J. *Bioorg. Med. Chem.* **1998**, *6*, 2477–2494.
15. Guzzo, P. R.; Trova, M. P.; Inghardt, T.; Linschaten, M. *Tetrahedron Lett.* **2002**, *43*, 41–43.
16. Boyle, P. H.; Davis, A. P.; Dempsey, K. J.; Hosken, G. D. *Tetrahedron: Asymmetry* **1995**, *6*, 2819–2828.
17. Pirkle, W. H.; Sikkenga, D. L. *J. Org. Chem.* **1997**, *42*, 1370–1374.
18. Baldwin, J. E.; Adlington, R. M.; Godfrey, C. R. A.; Patel, V. K. *Tetrahedron* **1993**, *49*, 7837–7856, We also investigated various amino acid coupling conditions for this reaction and BOP reagent provided the best yields.

19. Ueki, M.; Aoki, H.; Katoh, T. *Tetrahedron Lett.* **1993**, *34*, 2783–2786.
20. Case, D. A.; Pearlman, D. A.; Caldwell, J. W.; Cheatham, T. E., III., Wang, J.; Ross, W. S.; Simmerling, C. L.; Darden, T. A.; Merz, K. M.; Stanton, R. V. others. AMBER 7. University of California, San Francisco, CA, 2002.
21. Kitz, R.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3245–3249.
22. Batra, R.; Khayat, R.; Tong, L. *Nat. Struct. Biol.* **2001**, *8*, 809–816.