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Degradation-promoters of cellular inhibitor of apoptosis protein 1 based on bestatin and actinonin

Shinichi Sato,[a] Masashi Tetsuhashi,[a] Keiko Sekine,[a,b] Hiroyuki Miyachi,[a] Mikihiko Naito,[a] Yuichi Hashimoto[a] and Hiroshi Aoyama[a,*]

[a]*Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan*
[b]*Pharmaceutical Research Laboratories, Research & Development Group, Nippon Kayaku Co., Ltd, 3-31-12, Shimo, Kita-ku, Tokyo 115-8588, Japan*

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Abstract—A series of hybrid compounds of bestatin (**1**) and actinonin (**3**), which promote degradation of cellular inhibitor of apoptosis protein 1 (cIAP1), were designed and synthesized. Structure–activity relationship studies indicated that absolute configuration, hydrophobicity at the α-position of the internal amide carbonyl group, and the presence of a small substituent at the α-position of the ester group are important factors for the expression of potent cIAP1 degradation-promoting activity. HAB-5A (**30b**) showed the most potent activity (IC_{50} = 0.53 μM) among the compounds prepared.

1. Introduction

Programmed cell death, often called apoptosis, is required for normal embryonic development, growth, differentiation, and homeostasis of multicellular organisms.[1–4] Apoptosis can be triggered by distinct extracellular and intracellular stimuli, and it can involve the activation of a unique class of cysteine proteases known as caspases.[5–8] The function of caspases is regulated by another set of proteins called inhibitor of apoptosis proteins (IAPs).[9–11]

IAPs are members of a group of intracellular survival proteins, first identified in baculoviruses, whose function is to keep the host cells alive while the virus continues to replicate.[12,13] IAPs have two distinctive motifs, that is, one to three copies of baculovirus IAP repeat (BIR) domains, which are zinc-binding domains of about 80 amino acid residues, and a second zinc-binding motif known as really interesting new gene (RING) domain, which exhibits E3-ubiquitin-ligase activity.[14–16] Several human IAPs have been shown to bind directly to, and to inhibit, caspases via their BIR domains.[11,17] So far, at least eight kinds of human IAPs, neuronal apoptosis inhibitory protein (NAIP),[18] cellular inhibitor of apoptosis protein 1 (cIAP1),[19] cellular inhibitor of apoptosis protein 2 (cIAP2),[19] x-linked inhibitor of apoptosis protein (XIAP),[20] Livin,[21] apollon,[22] ILP-2,[23] and survivin,[24] have been identified.

cIAP1 was first found as a binding factor to TNF receptor associated factor-1 (TRAF-1) and TNF receptor associated factor-2 (TRAF-2) in the signaling pathway mediated by tumor necrosis factor 2 (TNFR2).[19] In this pathway, cIAP1 directly inhibits the activity of caspase-3, caspase-7, and caspase-9.[25,26] cIAP1 is highly expressed in various organs, such as kidney, small intestine, and lung, and one of the factors causing treatment resistance in cancer patients has been suggested to be the apoptosis-inhibiting activity of cIAP1 in these organs. Thus, inhibition of cIAP1 function is regarded as a potential therapeutic target.

Generally, inhibition of apoptosis by XIAP, a potent anti-apoptosis mediator in mammals, is overcome by Smac, an apoptosis stimulator, which binds to XIAP. Stimulation depends on the relative concentrations of Smac and XIAP, as a high concentration of XIAP can protect cells against expression of Smac.[27–30] However, cIAPs inhibit the inhibitory effect of Smac on XIAP.[31,32]

Keywords: Bestatin; Actinonin; cIAP1; Structural development; Inhibitor.
* Corresponding author. Tel.: +81 03 5841 7848; fax: +81 03 5841 8495; e-mail: aoyama@iam.u-tokyo.ac.jp

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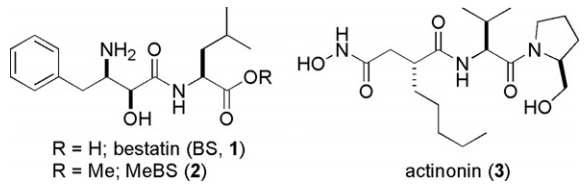


Figure 1. Structures of bestatin (**1**), MeBS (**2**), and actinonin (**3**).

Thus, cIAPs have become one of the molecular targets for drugs to treat cancer. Recently, some Smac mimics were reported to possess potent inhibitory activity not only against XIAP-mediated inhibition of caspase activity, but also against cIAPs-mediated inhibition of Smac activity.[33–37]

In addition, it was reported that Smac and Smac mimics enhance auto-ubiquitination of cIAP1 and cIAP2,[38,39] and this might provide the basis for a novel anti-tumor therapy. However, some problems, such as low membrane permeability and insufficient in vivo stability of these compounds, still remain.

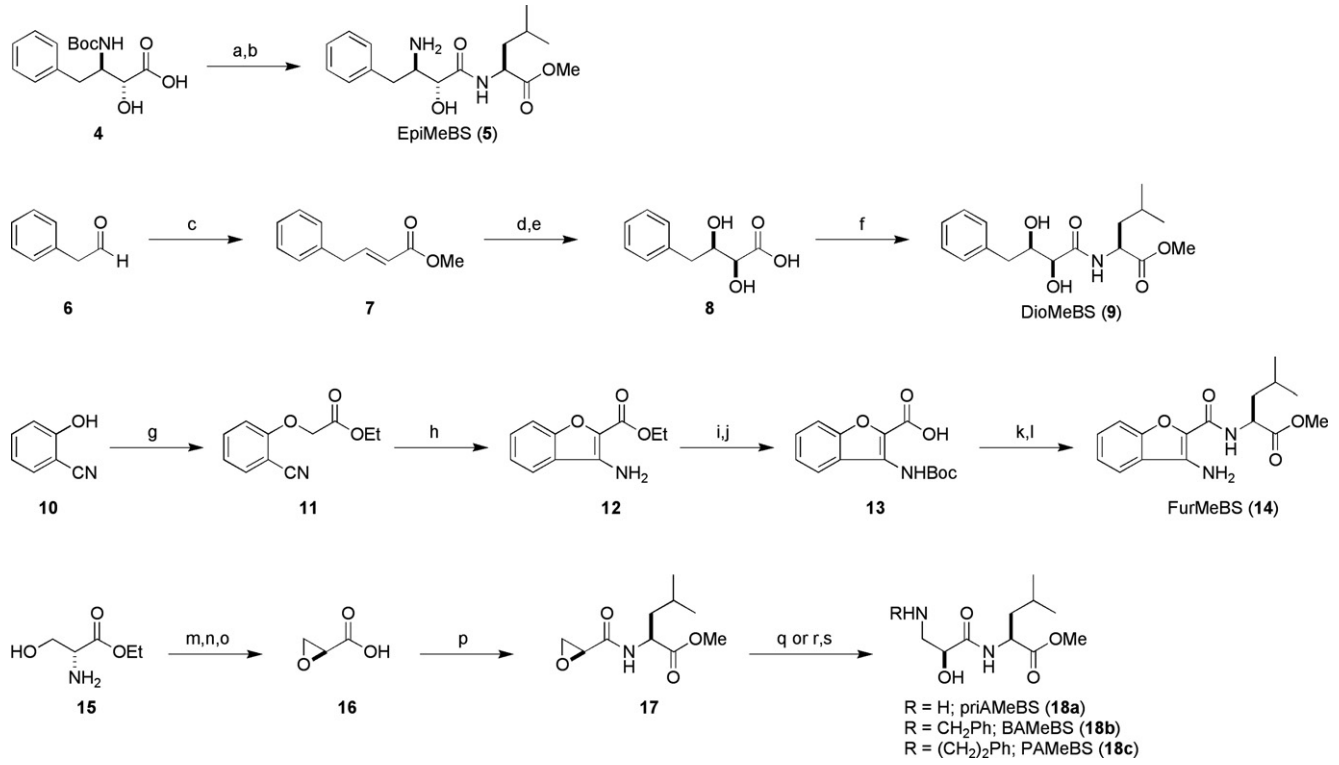
Bestatin (**1**, Fig. 1), *N*-[(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine, was isolated from *Streptomyces olivoreticulithe* in 1976,[40] and shown to be a potent competitive inhibitor of aminopeptidase B and leucine aminopeptidase.[41] Bestatin (**1**) also possesses immunomodulatory effects through stimulation of humoral and cell-mediated immune responses and inhibition of aminopeptidases.[42] We recently found that bestatin ester derivatives, such as MeBS (**2**), possess potent cIAP1 degradation-promoting activity based on auto-ubiquitination followed by proteasome-dependent digestion.[43] We also found that actinonin (**3**, Fig. 1),[44] an antibiotic and an inhibitor of leucine aminopeptidase and aminopeptidase N,[45] possesses similar cIAP1 degradation-promoting activity.

Based on these previous studies, bestatin (**1**) and actinonin (**3**) were expected to be suitable lead compounds for the development of compounds with more potent cIAP1 degradation-promoting activity. Here, we examined the structure–activity relationships of bestatin esters and hybrid compounds based on bestatin (**1**) and actinonin (**3**) structures.

2. Results and discussion

2.1. Synthesis

Bestatin methyl ester analogs **5**, **9**, **14**, and **18a**–**c** were synthesized as shown in Scheme 1. Briefly, condensation of (2*R*,3*R*)-3-[*N*-(benzyloxycarbonyl)amino]-2-hydroxy-4-phenylbutanoic acid (**4**) and L-leucine methyl ester, followed by deprotection of the Boc group with TFA gave EpiMeBS (**5**). Wittig reaction of phenylacetaldehyde (**6**) and methyl (triphenylphosphoranylidene)acetate, fol-



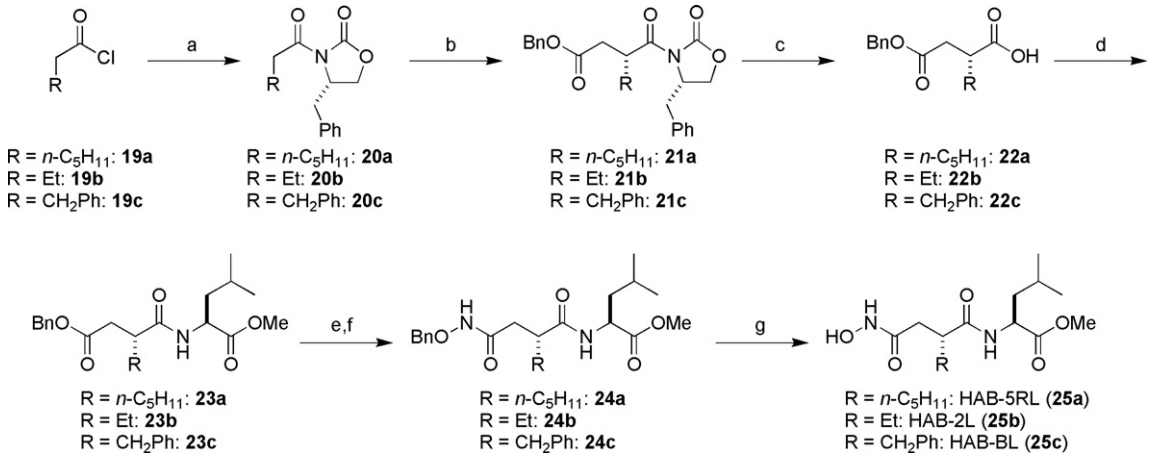
Scheme 1. Reagents and conditions: (a) L-leucine methyl ester hydrochloride, EDCI, HOBt, CH_2Cl_2, rt; (b) TFA, CH_2Cl_2, rt; (c) $Ph_3P{=}CHCOOCH_3$, toluene, reflux; (d) K_2OsO_4, AD-mix-β, *tert*-butanol–H_2O, 0 °C–rt; (e) TFA, H_2O, 60 °C; (f) L-leucine methyl ester hydrochloride, EDCI, HOBt, CH_2Cl_2, rt; (g) $BrCH_2COOEt$, K_2CO_3, acetone, rt; (h) NaH, DMF, 0 °C; (i) $(Boc)_2O$, Et_3N, DMAP, CH_2Cl_2, rt; (j) 3.73 N NaOH aq, MeOH–H_2O, rt; (k) L-leucine methyl ester hydrochloride, EDCI, HOBt, CH_2Cl_2, rt; (l) TFA, CH_2Cl_2, rt; (m) $NaNO_2$, 6 N HCl, 0 °C; (n) KOH, EtOH, 0 °C; (o) 2 N HCl, rt; (p) L-leucine methyl ester hydrochloride, EDCI, HOBt, CH_2Cl_2, rt; (q) RNH_2, CH_2Cl_2, 70 °C; (r) NaN_3, NH_4Cl, THF–H_2O, rt; (s) H_2, Pd/C, MeOH, rt.

lowed by dihydroxylation with osmium reagent, hydrolysis, and condensation with leucine methyl ester gave DioMeBS (**9**). Application of Williamson ether synthesis using 2-cyanophenol (**10**) and ethyl bromoacetate, followed by cyclization reaction, afforded the benzofuran intermediate **12**. Subsequent protection of the amino group with Boc group, hydrolysis of the ethyl ester, condensation with leucine methyl ester, and deprotection of the amino group afforded FurMeBS (**14**). Intermediate **17** for the synthesis of compounds **18a**–**c** was prepared by the treatment of D-serine (**15**) with sodium nitrate, potassium hydroxide, and aqueous HCl, followed by condensation of the product with leucine methyl ester. The intermediate **17** was treated with sodium azide, benzylamine, or phenethylamine to afford priAMeBS (**18a**), BAMeBS (**18b**), or PAMeBS (**18c**), respectively. In the case of the preparation of **18a**, hydrogenation under a hydrogen atmosphere using Pd/C catalyst was needed.

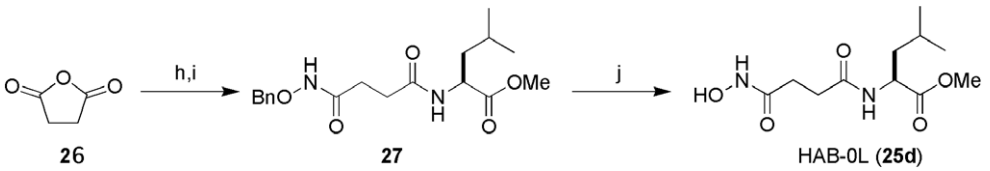
Hybrid compounds of bestatin and actinonin, **25a**–**d** and **30a**–**e**, were prepared from the corresponding acid chlorides based on Evans's asymmetric alkylation method (Schemes 2–4). As shown in Scheme 2, (*S*)-*N*-*n*-alkanoyl-4-benzyloxazolidinone **20a**–**c**, prepared from *n*-alkanoyl chloride **19a**–**c** and 4-(*S*)-benzyloxazolidinone, was treated with benzyl 2-bromoacetate, followed by the removal of the chiral auxiliary to afford the chiral benzyl ester intermediate **22a**–**c**. The product was condensed with leucine methyl ester, then hydrogenated with hydrogen gas over Pd/C, and condensed with benzyloxyamine to afford the benzylated hydroxamic acid **24a**–**c**. Finally, hydrogenation of **24a**–**c** with hydrogen gas in the presence of Pd/C afforded the (2*S*,5*R*)-configured product, HAB-5RL [(2*S*,5*R*)-**25a**], HAB-2RL [(2*S*,5*R*)-**25b**], and HAB-BRL [(2*S*,5*R*)-**25c**], respectively. The configurational isomer of (2*S*,5*R*)-**25a**, HAB-5SL [(2*S*,5*S*)-**25a**], was prepared by the similar method using an enantiomer of **20a** as a starting material.

The dealkylated derivative of **25** at the α-position of the internal amide carbonyl group (HAB-0L, **25d**) was prepared from succinic anhydride (**26**) in three steps (Scheme 3). Derivatives modified at the α-position of ester carbonyl group of (2*S*,5*R*)-**25a** were similarly prepared from intermediate **22a** using methyl ester derivatives of glycine (HAB-5G: **30a**), L-alanine (HAB-5A: **30b**), L-valine (HAB-5V: **30c**), L-*tert*-leucine (HAB-5TL: **30d**), and L-phenylalanine (HAB-5F: **30e**), instead of L-leucine methyl ester (Scheme 4).

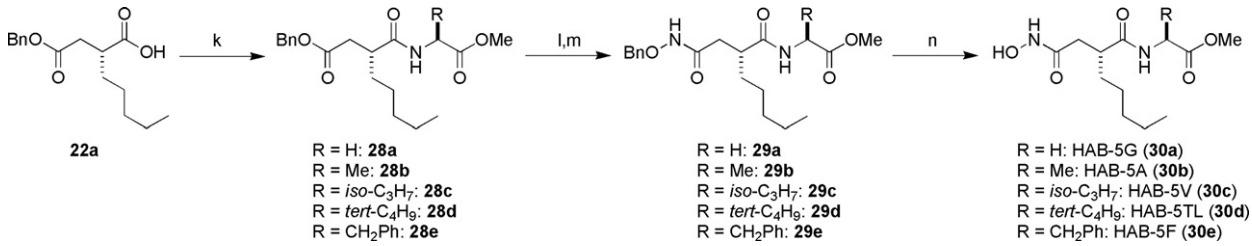
HAB-A0L (**36a**), bearing an amino group at the α-position of the internal amide carbonyl group of **25d**, and its *N*-butylated derivative [HAB-A4L (**36b**)] and *N*,*N*-dibutylated derivative [HAB-A44L (**36c**)] were prepared from D-aspartic acid (**31**) (Scheme 5). As shown in the scheme, benzyl *N*-Boc-D-aspartate (**32**), prepared from D-aspartic acid (**31**) by the general protective method in two steps, was condensed with leucine methyl ester. The product was hydrogenated with hydrogen gas over Pd/C and then condensed with *tert*-butyldimethylsilyloxyamine to afford the *O*-silylated hydroxamic acid **35a**. The product was treated with trifluoroacetic acid, followed by deprotection of the *tert*-butyldimethylsilyl group using trifluoroacetic acid, to give HAB-A0L



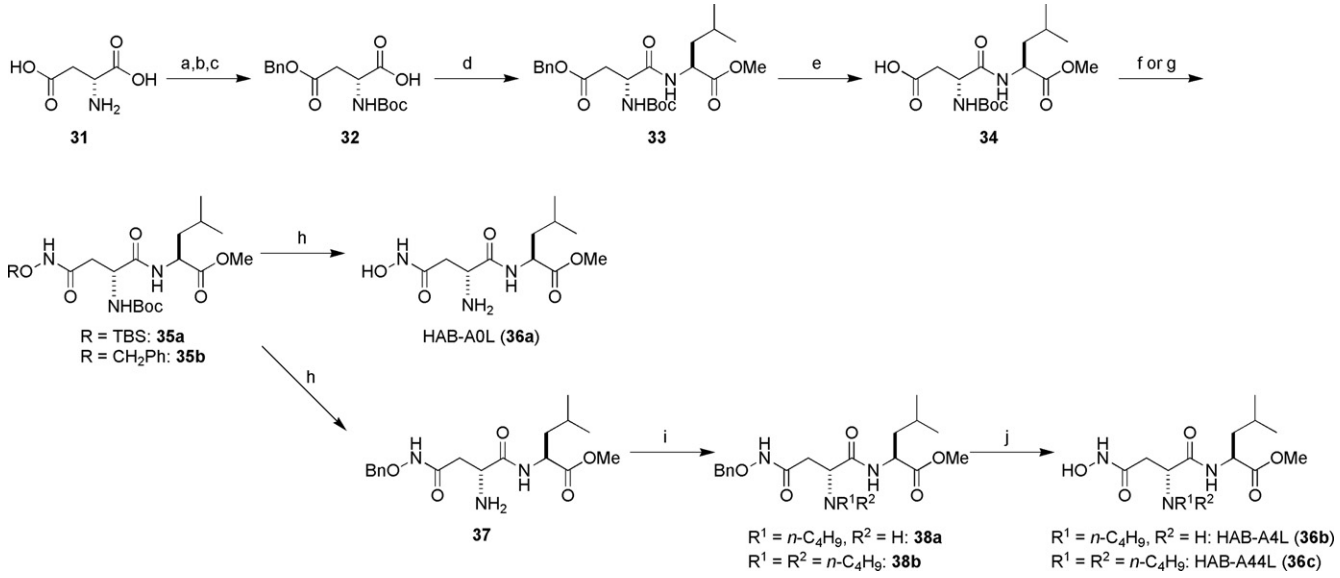
Scheme 2. Reagents and conditions: (a) (*S*)-*N*-*n*-alkanoyl-4-benzyloxazolidinone, *n*-BuLi, THF, −78 °C–rt; (b) $BrCH_2COOBn$, LiHMDS, THF, −78 to 0 °C; (c) 30% H_2O_2 aq, LiOH, THF–H_2O, 0 °C–rt; (d) L-leucine methyl ester hydrochloride, EDCI, HOBt, DIPEA, CH_2Cl_2, rt; (e) H_2, Pd/C, EtOAc, rt; (f) $BnONH_2HCl$, EDCI, DIPEA, CH_2Cl_2, rt; (g) H_2, Pd/C, MeOH, rt.



Scheme 3. Reagents and conditions: (h) L-leucine methyl ester hydrochloride, DMAP, Et_3N, CH_2Cl_2, rt; (i) $BnONH_2HCl$, EDCI, DIPEA, CH_2Cl_2, rt; (j) H_2, Pd/C, MeOH, rt.



Scheme 4. Reagents and conditions: (k) L-amino acid methyl ester hydrochloride, EDCI, HOBt, DIPEA, CH_2Cl_2, rt; (l) H_2, Pd/C, EtOAc, rt; (m) $BnONH_2HCl$, EDCI, DIPEA, CH_2Cl_2, rt; (n) H_2, Pd/C, MeOH, rt.



Scheme 5. Reagents and conditions: (a) BnOH, *p*-$TsOHH_2O$, benzene, reflux; (b) 20% NaOH aq, acetone–H_2O, 13 °C; (c) $(Boc)_2O$, Et_3N, acetone–H_2O, rt; (d) L-leucine methyl ester hydrochloride, EDCI, HOBt, DIPEA, CH_2Cl_2, rt; (e) H_2, Pd/C, EtOAc, rt; (f) $TBSONH_2$, EDCI CH_2Cl_2, rt; (g) $BnONH_2HCl$, EDCI, DIPEA, CH_2Cl_2, rt; (h) TFA, CH_2Cl_2, 0 °C; (i) *n*-butylaldehyde (1 equiv or 2 equiv), $NaBH(OAc)_3$, AcOH, THF, rt; (j) H_2, Pd/C, MeOH, rt.

(**36a**). On the other hand, condensation with benzyloxyamine instead of *tert*-butyldimethylsilyloxyamine at step 'g' in Scheme 5 afforded the *O*-benzylated hydroxamic acid **35b**. After deprotection of the Boc group with trifluoroacetic acid, the resulting amino derivative **37** was treated with 1 equiv or 2 equiv of *n*-butylaldehyde for reductive amide alkylation using sodium triacetoxyborohydride, and then hydrogenated over Pd/C to give HAB-A4L (**36b**) and HAB-A44L (**36c**).

2.2. Biological evaluation

In our previous studies, various esters derived from bestatin (**1**) were found to enhance CH11 (anti-Fas antibody which has been established to act as an Fas agonist)[46]-induced apoptosis more efficiently than bestatin (**1**).[43] In particular, the methyl ester derivative (MeBS, **2**) exhibited potent enhancing activity on CH11-induced apoptosis of HT1080 cells (Fig. 2). On the basis of this information, our preliminary efforts for the structural optimization of MeBS (**2**) analogs were focused on altering the β-amino-alcohol moiety, that is, compounds **5**, **9**, **14**, **18a**–**c**, and (2*S*,5*R*)-**25a** (Fig. 3). The cIAP1 degradation-promoting activity of these analogs was evaluated by using human fibrosarcoma HT-1080 cells stably expressing FLAG-tagged cIAP1, and treated with the prepared compounds,

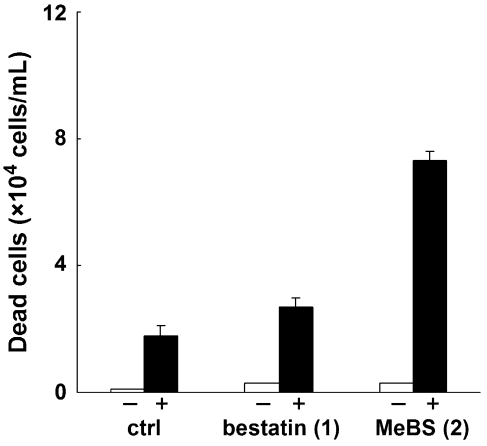


Figure 2. HT1080 cells were treated with 30 μM bestatin (**1**) or MeBS (**2**) in the presence (+) or absence (−) of 100 ng/mL CH11[46] for 24 h.

and the results are summarized in Figure 3 and Table 1. The diastereomer of MeBS (EpiMeBS: **5**), the diol compound (DioMeBS: **9**), the benzofuran-type compound (FurMeBS: **14**), and compounds debenzylated at the 3-position (priAMeBS: **18a**; BAMeBS: **18b**; PAMeBS: **18c**) possessed no or very weak cIAP1 degradation-promoting activity, even though they have an ester moiety,

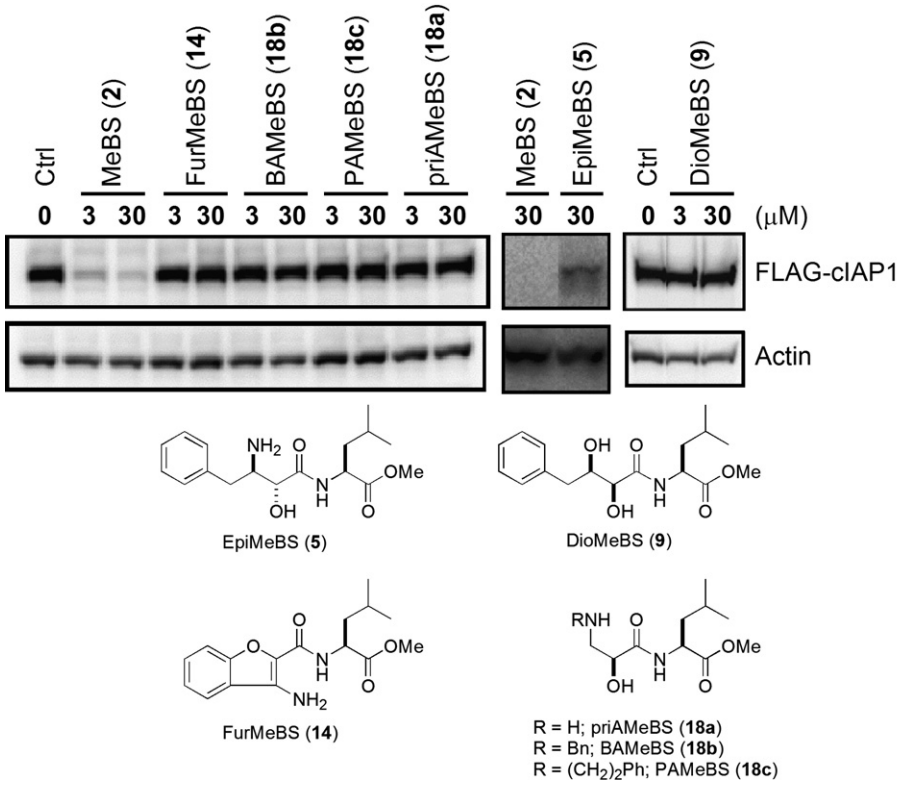
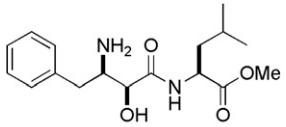
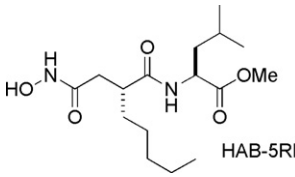


Figure 3. The effects of MeBS (**2**) derivatives on cIAP1 degradation.

except for **5**, which exhibited moderate activity (Fig. 3). In contrast, the hybrid compound [HAB-5RL: (2*S*,5*R*)-**25a**] composed of the L-leucine part of MeBS (**2**) and the hydroxamic acid part of actinonin (**3**) exhibited potent cIAP1 degradation-promoting activity with an IC_{50} value of 1.06 μM, which is comparable with that of MeBS (IC_{50} = 0.69 μM) (Table 1). This result suggested that a putative zinc-binding structure, such as a β-aminoalcohol moiety and/or a hydroxamic acid moiety, as well as an ester moiety, is essential for potent cIAP1 degradation-promoting activity. In addition, the putative zinc-binding structure seemed to be critically recognized by the target molecule(s), because the stereoisomer of MeBS (EpiMeBS: **5**) showed lower activity than MeBS (**2**) (Fig. 3). The down-regulation of cIAP1 caused by MeBS (**2**) and some of the prepared derivatives can plausibly be attributed to the enhancement of cIAP1 degradation in the cells, because incubation of the cells with the compounds for only 3 h was sufficient for manifestation of the degradation-promoting activity. We suggest that these compounds promote degradation, analogously with Yang's and Eugene's conclusion that Smac and Smac mimics enhanced auto-ubiquitination of cIAP1.[38,39]

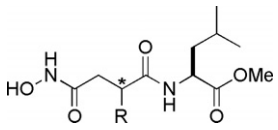
Table 1. cIAP1 production-inhibiting activity of MeBS (**2**) and HAB-5RL [(2*S*,5*R*)-**25a**]

Compound	IC_{50}[a] (μM)
 MeBS (**2**)	0.69
 HAB-5RL [(2*S*,5*R*)-**25a**]	1.06

[a] IC_{50} is the concentration that decreases cIAP1 production to 50%.

On the basis of the initial screening results mentioned above, further structural modification of HAB-5RL [(2*S*,5*R*)-**25a**] was conducted. First, we evaluated the effects on the cIAP1 degradation-promoting activity of various substituents at the α-position of the internal amide carbonyl group (Table 2). The stereoisomer [HAB-5SL: (2*S*,5*S*)-**25a**] showed lower activity (IC_{50} = 12.1 μM, Table 2, entry 2) than (2*S*,5*R*)-**25a** (IC_{50} = 1.06 μM, Table 2, entry 1). The non-substituted compound [HAB-0L: **25d**], and the compound with an ethyl [HAB-2L: (2*S*,5*R*)-**25b**] or a benzyl [HAB-BL: (2*S*,5*R*)-**25c**] group at the corresponding position also exhibited lower activity (IC_{50} values of 19.9–45.2 μM, Table 2, entries 3–5) than (2*S*,5*R*)-**25a** (Table 2, entry 1). The activity decreased in the order of: R = *n*-C_5H_{11} [HAB-5RL: (2*S*,5*R*)-**25a**] > R = H (HAB-0L: **25d**) > R = C_2H_5 (HAB-2L: **25b**) > R = CH_2Ph (HAB-BL: **25c**). These results indicated that derivatives with the *R* configuration at the α-position of the internal amide carbonyl group are more effective than the corresponding derivatives with the *S* configuration. Additionally, the expression of potent cIAP1 degradation-promoting activity seems to require a long/flexible alkyl chain, such as an *n*-pentyl group, at the α-position of the

Table 2. cIAP1 production-inhibiting activity of compounds **25a–d**



Entry	Compound	Config.	R	IC_{50}[a] (μM)
1	HAB-5RL [(2*S*,5*R*)-**25a**]	*R*	*n*-C_5H_{11}	1.06
2	HAB-5SL [(2*S*,5*S*)-**25a**]	*S*	*n*-C_5H_{11}	12.1
3	HAB-2L (**25b**)	*R*	C_2H_5	24.1
4	HAB-BL (**25c**)	*R*	CH_2Ph	45.2
5	HAB-0L (**25d**)		H	19.9

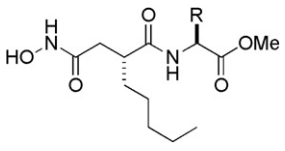
[a] IC_{50} is the concentration that decreases cIAP1 production to 50%.

internal amide carbonyl group. Introduction of spherical/rigid groups, such as a benzyl group (HAB-BL: **25c**), decreased the activity.

Next, we examined the effect on cIAP1 degradation-promoting activity of substituents at the α-position of the ester carbonyl group (Table 3). The compound with a *tert*-butyl group (HAB-5TL: **30d**) was almost inactive (Table 4, entry 4). In contrast, the unsubstituted compound (HAB-5G: **30a**), and the compound with a methyl (HAB-5A: **30b**), an isopropyl (HAB-5V: **30c**), or a benzyl (HAB-5F: **30e**) group exhibited potent cIAP1 degradation-promoting activity (Table 4, entries 1–3, and 5). HAB-5A (**30b**) showed the most potent activity (IC_{50} = 0.53 μM) among the prepared compounds, including the lead compound (2*S*,5*R*)-**25a**. As shown in Figure 4, HAB-5A (**30b**) showed cIAP1 degradation-promoting activity with the potency comparable to that of MeBS (**2**) as far as that measured by Western blot analysis (Fig. 4, panel a). The degradation-promoting activity elicited by HAB-5A (**30b**) seems to be based on auto-ubiquitination followed by proteasome-dependent digestion, as that elicited by MeBS (**2**) does, because addition of proteasome inhibitor MG132[47] caused accumulation of cIAP1 and its ubiquitinated form (Fig. 4, panel b). As expected, HAB-5A (**30b**) showed more potent enhancing activity than that of MeBS (**2**) on apoptosis induced by actinomycin D, cisplatin, etoposide, or camptothecin (Fig. 4, panels c and d).

The activity of the derivatives of **30** decreased in the order of: R = CH_3 (HAB-5A: **30b**) > R = CH_2Ph (HAB-5F: **30e**) > R = *i*-C_3H_7 (HAB-5V: **30c**) > R = H (HAB-5G: **30a**) ≫ R = *t*-C_4H_9 (HAB-5TL: **30d**). The potent activity (IC_{50} values of 0.53–1.25 μM) of HAB-5A (**30b**), HAB-5V (**30c**), and HAB-5F (**30e**), in contrast to HAB-5TL (**30d**) and HAB-5G (**30a**) (IC_{50} values of >20 μM and 1.54 μM, respectively), implies that the presence of a hydrogen atom (small substituent) on the β-carbon at the α-position of ester carbonyl group is necessary for potent cIAP1 degradation-promoting activity.

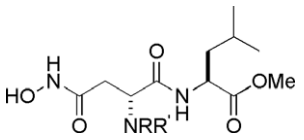
Table 3. cIAP1 production-inhibiting activity of compounds **30a–e**



Entry	Compound	R	IC_{50}[a] (μM)
1	HAB-5G (**30a**)	H	1.54
2	HAB-5A (**30b**)	CH_3	0.53
3	HAB-5V (**30c**)	*i*-C_3H_7	1.25
4	HAB-5TL (**30d**)	*t*-C_4H_9	>20
5	HAB-5F (**30e**)	CH_2Ph	0.84

[a] IC_{50} is the concentration that decreases cIAP1 production to 50%.

Table 4. cIAP1 production-inhibiting activity of compounds **36a–c**



Entry	Compound	R	R^1	IC_{50}[a] (μM)
1	HAB-A0L (**36a**)	H	H	N.A.
2	HAB-A4L (**36b**)	H	*n*-C_4H_9	4.69
3	HAB-A44L (**38c**)	*n*-C_4H_9	*n*-C_4H_9	9.20

[a] IC_{50} is the concentration that decreases cIAP1 production to 50%.

As regards the alkyl chain at the α-position of the internal amide carbonyl group, we introduced a hetero atom(s), and examined the effect on cIAP1 degradation-promoting activity (Table 4). Although the non-substituted amino compound (HAB-A0L: **36a**) was inactive (Table 4, entry 1), the compound with a mono-*n*-butylamino group (HAB-A4L: **36b**), which can be regarded as an aza-analog of HAB-5RL [(2*S*,5*R*)-**25a**] (Table 2), exhibited moderate cIAP1 degradation-promoting activity (IC_{50} = 4.89 μM, Table 4, entry 2), though the activity was lower than that of HAB-5RL [(2*S*,5*R*)-**25a**]. Further introduction of an *n*-butyl group, that is, the compound with a di-*n*-butylamino group (HAB-A44L: **36c**), caused a decrease of the activity (IC_{50} = 9.20 μM, Table 4, entry 2). These results suggest that the introduction of hetero atom(s) at the α-position of the internal amide carbonyl group is permissible, but decreases the activity.

3. Conclusions

We have developed a number of potent cIAP1 degradation-promoting compounds. Among the compounds prepared, HAB-5A (**30b**) exhibited the most potent activity for promotion of cIAP1 degradation, with an IC_{50} value of nanomolar order (IC_{50} = 0.53 μM). Further structure–activity relationship studies based on HAB-5A (**30b**) and a search for compounds targeting other IAPs are in progress.

4. Experimental

4.1. Cell culture

Human fibrosarcoma HT-1080 cells stably expressing FLAG-tagged cIAP1 were cultured in RPMI 1640 con-

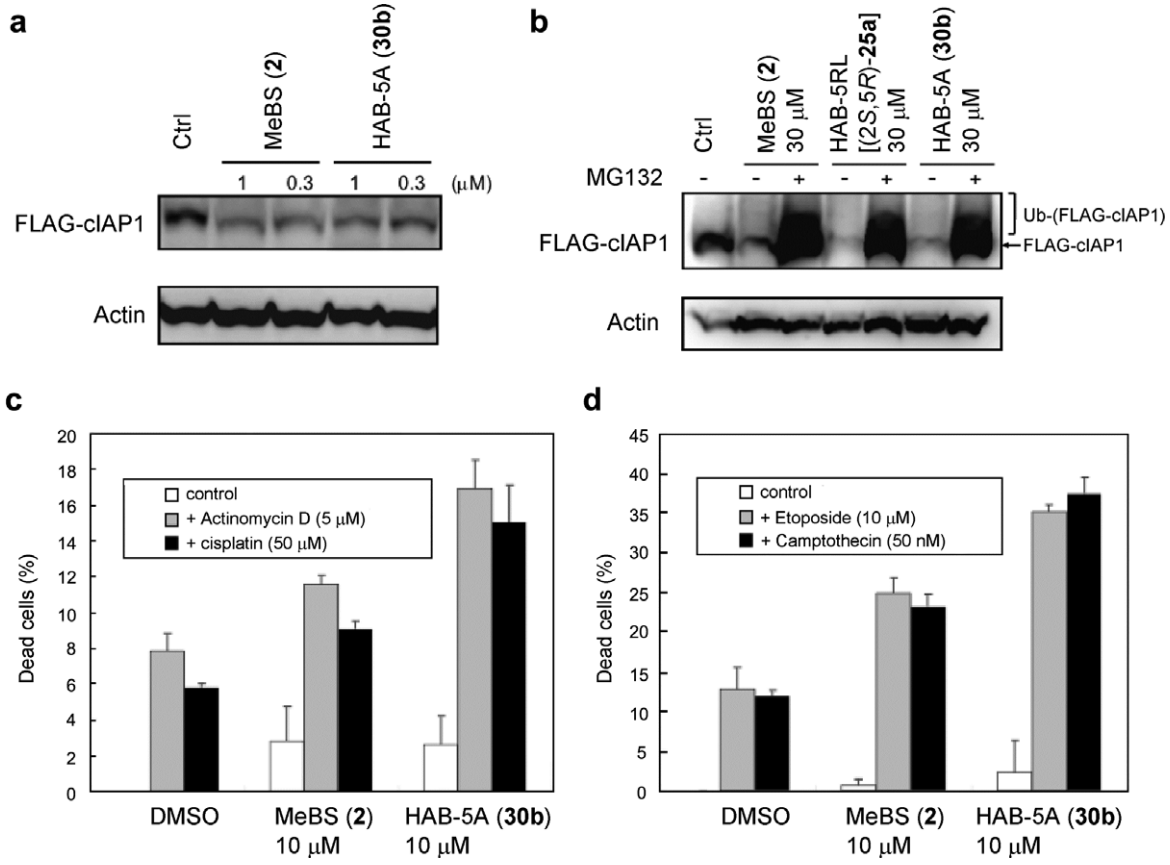


Figure 4. The effects of HAB-5A (**30b**) on cIAP1 degradation and apoptosis. (a) Western blot analysis of FLAG-tagged cIAP1 extracted from HT1080 cells stably expressing FLAG-tagged cIAP1. (b) Inhibition of cIAP-1 degradation-promoting activity of HAB-5A (**30b**)/MeBS (**2**) by proteasome inhibitor, MG132.[47] (c) Effects of HAB-5A (**30b**) and MeBS (**2**) on actinomycin- and cisplatin-induced cell apoptosis. (d) Effects of HAB-5A (**30b**) and MeBS (**2**) on etoposide- and camptothecin-induced cell apoptosis.

taining 10% heat-inactivated fetal bovine serum (FBS) and antibiotic–antimycotic mixture (Nacalai) at 37 °C in a humidified atmosphere of 5% CO_2 in air.

4.2. cIAP1 production inhibition

HT1080 cells stably expressing FLAG-tagged cIAP1 (5×10^5) in six-well plates were treated with serial dilutions of compound for 3 h. The cells were harvested and lysed with lysis buffer (1% SDS, 10% glycerol, 0.1 M Tris, pH 7.4). Cell lysates containing equal amounts of protein were separated by 4–20% gradient polyacrylamide gel electrophoresis, transferred to PVDF membranes, and Western-blotted using appropriate antibodies (anti-FLAG and anti-action antibodies). Protein bands were detected using Enhanced Chemiluminescense detection kits and the quantity of protein was determined with image analysis software (MetaMorph).

4.3. Chemistry

4.3.1. General. ^{1}H NMR (500 MHz) spectra were recorded on a JEOL JNM-α500 spectrometer. Mass spectra were obtained on JEOL JMA-HX 110 spectrometer with *m*-nitrobenzyl alcohol. Flash column chromatography was performed on silica gel 60 Kanto Kagaku (40–100 μm). Optical purity of optically active compounds prepared were confirmed to be more than 97% ee at the lowest, by HPLC equipped with chiral column [CHIRALPAK AD-H: eluted with a mixture of hexane and isopropanol (1:2) containing 0.1% v/v TFA].

4.3.1.1. *N*-[(2*R*,3*R*)-3-Amino-2-hydroxy-4-phenylbutanoyl]-L-leucine methyl ester (EpiMeBS: 5). To a solution of compound **4**[48] (50.0 mg, 0.109 mmol) in dry CH_2Cl_2 (4 mL) were added L-leucine methyl ester hydrochloride (21.9 mg, 0.120 mmol), HOBt (16.3 mg, 0.120 mmol), and EDC (25.2 mg, 0.131 mmol). The reaction mixture was stirred at rt for 2 h and quenched with water. The organic layer was collected, dried over $MgSO_4$, and concentrated under reduced pressure. The intermediate was obtained after column chromatography (AcOEt/Hex = 1:1) (28.5 mg, 62%). To a solution of the intermediate (28.5 mg, 0.068 mmol) in dry CH_2Cl_2 (4 mL) was added TFA (0.4 mL). The reaction mixture was stirred at rt for 60 min and concentrated under reduced pressure. Pure **5** was obtained by means of PLC (CH_2Cl_2/MeOH = 7:1) (17.4 mg, 80%). ^{1}H NMR (500 MHz, CD_3OD) δ: 7.88 (d, J = 8.1 Hz, 1H), 7.74 (d, J = 7.7 Hz, 1H), 7.75–7.24 (m, 5H), 4.49 (m, 1H), 4.38 (d, J = 3.0 Hz, 1H), 3.77 (m, 1H), 3.70 (s, 3H), 3.10 (dd, J = 14.5, 4.3 Hz, 1H), 2.88 (dd, J = 14.5, 10.7 Hz, 1H), 1.75–1.67 (m, 3H), 0.96 (d, J = 6.0 Hz, 3H), 0.93 (d, J = 6.0 Hz, 3H); HRMS (FAB) calcd for $C_{17}H_{27}N_2O_4$ 323.1971; found: 323.1949 $(M+H)^+$; $[\alpha]_D^{22}$ −1.71 (*c* 0.70 in MeOH).

4.3.1.2. (*E*)-Methyl 4-phenylbut-2-enoate (7). To a solution of phenyl acetaldehyde (500 mg, 4.2 mmol) in toluene (50 mL) was added (triphenylphosphoranylidene)acetic acid methyl ester (1.4 g, 4.2 mmol). The reaction mixture was stirred at reflux for 3 h. Pure **7** was obtained by means of column chromatography (AcOEt/Hex = 1:2) (667 mg, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.28–7.12 (m, 5H), 7.06 (dt, J = 15.4, 6.8 Hz, 1H), 5.79 (d, J = 15.4 Hz, 1H), 3.67 (s, 3H), 3.47 (d, J = 6.8 Hz, 2H).

4.3.1.3. Methyl (2*S*,3*R*)-2,3-dihydroxy-4-phenylbutanoate (8). To a solution of **7** (667 mg, 3.8 mmol) in ^{t}BuOH/H_2O (20 mL:20 mL) were added AD-mix-β (5.3 g), $CH_3SO_2NH_2$ (360 mg, 3.8 mmol), and K_2SOsO_4 (210 mg, 0.57 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 50 min and then stirred at rt for 3 h. The reaction mixture was quenched with $NaSO_3$ (5.3 g) and water, and the product was extracted with AcOEt. The extract was dried over $MgSO_4$. Pure **8** was obtained by means of column chromatography (AcOEt/Hex = 1:2) (583 mg, 73%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.34–7.25 (m, 5H), 4.17 (tdd, J = 7.3, 8.1, 1.7 Hz, 1H), 4.08 (dd, J = 5.1, 1.7 Hz, 1H), 3.80 (s, 3H), 3.05 (d, J = 5.1 Hz, 1H), 2.96 (dd, J = 13.7, 7.3 Hz, 1H), 2.95 (dd, J = 13.7, 7.3 Hz, 1H), 2.00 (d, J = 8.1 Hz, 1H); MS (FAB) 211 $(M+H)^+$; $[\alpha]_D^{24}$ +38.55 (c 1.01 in $CHCl_3$).

4.3.1.4. *N*-[(2*S*,3*R*)-2,3-Dihydroxy-4-phenylbutanoyl]-L-leucine methyl ester (DioMeBS: 9). Compound **8** (50.0 mg, 0.24 mmol) was dissolved in a mixture of H_2O (10 mL) and TFA (6 mL). The reaction mixture was stirred at 60 °C for 12 h. The pure intermediate {the corresponding carboxylic acid, $[\alpha]_D^{24}$ +28.20 (c 0.58 in MeOH)}[49,50] was obtained by chromatography eluted with AcOEt (30.0 mg, 64%). To a solution of the intermediate (28.0 mg, 0.14 mmol) in dry THF (15 mL) were added L-leucine methyl ester hydrochloride (28.6 mg, 0.16 mmol), HOBt (27.0 mg, 0.20 mmol), EDCI (41.0 mg, 0.21 mmol), and DIPEA (29.0 μL, 0.17 mmol) under an argon atmosphere. The reaction mixture was stirred at rt for 90 min and quenched with NH_4Cl aq. The organic layer was collected, dried over $MgSO_4$, and concentrated under reduced pressure. Pure **9** was obtained by means of column chromatography (AcOEt/Hex = 1:2) (46.1 mg, 100%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.79 (m, 1H), 7.45 (m, 1H), 7.33–7.23 (m, 3H), 7.07 (d, J = 8.1 Hz, 1H), 4.66 (m, 1H), 4.26 (m, 1H), 4.15 (d, J = 3.4 Hz, 1H), 3.75 (s, 3H), 2.99 (dd, J = 13.7, 4.3 Hz, 1H), 2.77 (dd, J = 13.7, 9.8 Hz, 1H), 1.72–1.58 (m, 3H), 0.94 (d, J = 6.0 Hz, 6H); HRMS (FAB) calcd for $C_{17}H_{26}NO_5$ 324.1811; found: 324.1851 $(M+H)^+$; $[\alpha]_D^{24}$ −29.89 (c 0.51 in MeOH).

4.3.1.5. Ethyl 2-(2-cyanophenoxy)acetate (11).[51] 2-Cyanophenol (**10**) (1.00 g, 8.40 mmol), ethyl 2-bromoacetate (1.8 g, 12.0 mmol) and K_2CO_3 (1.5 g, 10.9 mmol) were dissolved in acetone (100 mL). The reaction mixture was stirred at rt for 64 h. K_2CO_3 was removed by filtration, and pure **11** was obtained by concentration of the filtrate (1.59 g, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.61–6.84 (m, 4H), 4.77 (s, 2H), 4.25 (q, J = 7.3 Hz, 2H), 1.29 (t, J = 7.3 Hz, 3H); MS (FAB) 206 $(M+H)^+$.

4.3.1.6. Ethyl 3-aminobenzofuran-2-carboxylate (12).[51] To a solution of **11** (500 mg, 2.44 mmol) in DMF was added NaH (64.5 mg, 2.68 mmol) at 0 °C, and the mixture was stirred for 1 h and quenched with water. Pure **12** was obtained by extraction with Et_2O and column chromatography (AcOEt/Hex = 3:7) (307 mg, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.58–7.25 (m, 4H), 4.46 (q, J = 6.8 Hz, 2H), 1.45 (t, J = 6.8 Hz, 3H).

4.3.1.7. 3-(*N*-*tert*-Butoxycarbonyl)aminobenzofuran-2-carboxylic acid (13). To a solution of **12** (157 mg, 0.77 mmol) in CH_2Cl_2 (20 mL) were added Boc_2O (250 mg, 1.15 mmol), DMAP (93.4 mg, 0.77 mmol), and NEt_3 (2 mL). The mixture was stirred for 2.5 h at rt and quenched with 1 N HCl. The organic layer was collected, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was dissolved in MeOH/H_2O (4 mL:2 mL), and 15% v/v NaOH aq (1 mL) was added. Stirring was continued for 1.5 h at rt, then CH_2Cl_2 was added. The aqueous layer was acidified with 1 N HCl. Pure **13** was obtained by extraction with AcOEt (187 mg, two steps, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.60–7.26 (m, 4H), 1.40 (s, 9H).

4.3.1.8. *N*-(3-Aminobenzofuran-2-carbonyl)-L-leucine methyl ester (FurMeBS: 14). To a solution of **13** (10.0 mg, 0.036 mmol) in dry CH_2Cl_2 (1 mL) were added HOBt (6.8 mg, 0.050 mmol), EDCI (10.3 mg, 0.037 mmol), and L-leucine methyl ester hydrochloride (7.2 mg, 0.040 mmol). The reaction mixture was stirred at rt for 1 h and quenched with water. The organic layer was collected, dried over $MgSO_4$, and concentrated under reduced pressure. The intermediate was dissolved in dry CH_2Cl_2 (2 mL), TFA (0.2 mL) was added, and the mixture was stirred for 1 h. Pure **14** was obtained by PLC (AcOEt/Hex = 1:2) (56 mg, two steps, 56%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.54–7.23 (m, 4H), 6.50 (d, J = 8.1 Hz, 1H), 4.84 (m, 1H), 3.78 (s, 3H), 1.79–1.68 (m, 3H), 1.00 (d, J = 4.3 Hz, 3H), 0.99 (d, J = 4.3 Hz, 3H); HRMS (FAB) calcd for $C_{16}H_{21}N_2O_4$ 305.1501; found: 305.1530 $(M+H)^+$.

4.3.1.9. (*S*)-Oxirane-2-carboxylic acid (16).[52] D-Serine (2.0 g, 19.0 mmol) was dissolved in 6 N HCl at 0 °C, $NaNO_2$ was added, and the mixture was stirred at 0 °C for 6 h, then extracted with Et_2O, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was dissolved in EtOH (5 mL), KOH (1.26 g, 22.5 mmol) was added, and the mixture was stirred at 0 °C for 3 h then at rt for 13 h. The precipitate was removed by filtration and recrystallized (AcOEt/MeOH). The crystalline potassium salt was dissolved in water (20 mL), then 2 N HCl was added, and the mixture was stirred for 30 min at rt. Pure **16** was obtained by extraction with AcOEt and concentration (403 mg, three steps, 24%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.49 (t, J = 3.4 Hz, 1H), 3.03–3.00 (m, 2H).

4.3.1.10. *N*-[(*S*)-Oxirane-2-carbonyl]-L-leucine methyl ester (17). To a solution of **16** (403 mg, 4.58 mmol) in CH_2Cl_2 (30 mL) were added HOBt (862 mg, 6.38 mmol), EDCI (1.32 g, 6.89 mmol), and L-leucine methyl ester hydrochloride (862 mg, 4.74 mmol). The reaction mixture

was stirred at rt for 30 min and quenched with water. The organic layer was collected, dried over $MgSO_4$, and concentrated under reduced pressure. Pure **17** was obtained by column chromatography (AcOEt/Hex = 1:3) (617 mg, 63%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.48 (d, J = 8.1 Hz, 1H), 4.46 (m, 1H), 3.74 (s, 3H), 3.46 (dd, J = 4.7, 2.6 Hz, 1H), 3.00 (dd, J = 5.6, 4.7 Hz, 1H), 2.75 (dd, J = 5.6, 2.6 Hz, 1H), 1.64 (m, 1H), 1.57–1.49 (m, 2H), 0.91 (d, J = 6.4 Hz, 3H), 0.90 (d, J = 6.4 Hz, 3H); MS (FAB) 216 $(M+H)^+$.

4.3.1.11. *N*-[(*S*)-3-Amino-2-hydroxypropanoyl]-L-leucine methyl ester (priAMeBS: 18a). To a solution of NaN_3 (29.0 mg, 0.45 mmol) and NH_4Cl (6.8 mg, 0.44 mmol) in H_2O (2 mL) was added a solution of **17** (47.8 mg, 0.22 mmol) in THF (4 mL), and the mixture was stirred for 24 h at rt. The intermediate azide was obtained by column chromatography (AcOEt/Hex = 1:1) (34.0 mg, 59%). This intermediate (34.0 mg, 0.13 mmol) was dissolved in MeOH (15 mL), then 10% Pd–C (3.4 mg) was added, and the mixture was stirred under H_2 gas for 1 h. Pure **18a** was obtained by filtration of Pd–C (27.2 mg, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.49 (d, J = 8.1 Hz, 1H), 4.61 (m, 1H), 4.02 (m, 1H), 3.73 (s, 3H), 3.07 (dd, J = 12.8, 5.1 Hz, 1H), 2.98 (dd, J = 12.8, 5.1 Hz, 1H), 1.70–1.56 (m, 3H), 0.94–0.92 (m, 6H); HRMS (FAB) calcd for $C_{18}H_{29}N_2O_4$ 233.1501; found: 233.1507 $(M+H)^+$.

4.3.1.12. *N*-[(*S*)-2-Hydroxy-3-phenethylaminopropanoyl]-L-leucine methyl ester (PAMeBS: 18c). *General procedure.* To a solution of **17** (50.0 mg, 0.23 mmol) in CH_2Cl_2 (50 mL) was added dropwise 2-phenylethylamine (280 mg, 2.31 mmol) over 30 min. The reaction mixture was stirred for 1 h at rt, quenched with water, and extracted with AcOEt. The extract was concentrated under reduced pressure. Pure **18c** was obtained by means of column chromatography (CH_2Cl_2/MeOH = 20:1, containing 1% NEt_3) (7.0 mg, 36%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.73 (d, J = 8.1 Hz, 1H), 7.32–7.18 (m, 5H), 4.59 (m, 1H), 3.98 (dd, J = 6.8, 6.0 Hz, 1H), 3.73 (s, 3H), 3.00 (dd, J = 12.4, 6.0 Hz, 1H), 2.99–2.88 (m, 3H), 2.81–2.77 (m, 3H), 0.93 (d, J = 6.4 Hz, 3H), 0.92 (d, J = 6.4 Hz, 3H); HRMS (FAB) calcd for $C_{18}H_{29}N_2O_4$ 337.2127; found: 337.2111 $(M+H)^+$.

4.3.1.13. *N*-[(*S*)-3-Benzylamino-2-hydroxypropanoyl]-L-leucine methyl ester (BAMeBS: 18b). This compound was prepared from **17** according to the general procedure (9%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.75 (d, J = 8.6 Hz, 1H), 7.35–7.28 (m, 5H), 4.60 (m, 1H), 4.08 (dd, J = 6.0, 6.4 Hz, 1H), 3.83 (s, 2H), 3.73 (s, 3H), 3.05–2.96 (m, 2H), 1.67–1.58 (m, 3H), 0.92 (d, J = 6.4 Hz, 3H), 0.91 (d, J = 6.4 Hz, 3H); HRMS (FAB) calcd for $C_{17}H_{27}N_2O_4$ 323.1971; found: 323.2006 $(M+H)^+$.

4.3.1.14. (*S*)-4-Benzyl-3-heptanoyloxazolidin-2-one (20a). *General procedure.*[53] (*S*)-Benzyl-2-oxazolinone (10 g, 56.5 mmol) was dissolved in THF (70 mL) under an argon atmosphere, *n*-BuLi (1.6 M in hexane) (35.3 mL, 56.5 mmol) was added, and the mixture was stirred for 1 h at −78 °C. To the mixture was added **19a** (8.0 mL, 51.7 mmol), and the whole was stirred for 2 h at rt, quenched with sat. NH_4Cl aq and satd $NaHCO_3$ aq. Extraction with AcOEt and concentration under reduced pressure afforded the product. Pure **20a** was obtained by column chromatography (AcOEt/Hex = 1:6) (14.25 g, 95%). ^{1}H NMR (500 MHz,$CDCl_3$) δ: 7.35–7.20 (m, 5H), 4.67 (m, 1H), 4.17 (m, 2H), 3.30 (dd, J = 13.3, 3.4 Hz, 1H), 3.00–2.86 (m, 2H), 2.77 (dd, J = 13.3, 9.8 Hz, 1H), 1.69 (m, 2H), 1.40–1.31 (m, 6H), 0.90 (t, J = 6.9 Hz, 3H); MS (FAB) 290 $(M+H)^+$.

4.3.1.15. (*S*)-4-Benzyl-3-butyryloxazolidin-2-one (20b). This compound was prepared from **19b** according to the general procedure (91%). ^{1}H NMR (500 MHz,$CDCl_3$) δ: 7.39–7.25 (m, 5H), 4.72 (m, 1H), 4.25–4.19 (m, 2H), 3.35 (dd, J = 13.7, 3.4 Hz, 1H), 3.04–2.89 (m, 2H), 2.81 (dd, J = 13.7, 10.7 Hz, 1H), 1.78 (qdd, J = 7.3, 7.3, 2.6 Hz, 2H), 1.05 (t, J = 7.3 Hz, 3H); MS (FAB) 248 $(M+H)^+$.

4.3.1.16. Benzyl 4-[(*S*)-4-benzyl-2-oxooxazolidin-3-yl]-3-pentyl-4-oxobutanoate (21a). *General procedure.* To a solution of **20a** (3.5 g, 12.1 mmol) in THF (70 mL) was added LiHMDS (1.0 M in THF) (14.6 mL, 14.6 mmol) at −78 °C, and the mixture was stirred for 20 min at 0 °C. To this solution was added dropwise benzyl bromoacetate (3.59 g, 15.7 mmol) in THF (50 mL) over 90 min at −78 °C. The reaction mixture was stirred for 30 min at 0 °C and for 17 h at rt, then quenched with sat. NH_4Cl aq and satd $NaHCO_3$ aq. Extraction with AcOEt and concentration under reduced pressure afforded the product. Pure **21a** was obtained by means of column chromatography (AcOEt/Hex = 1:5) (4.35 g, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.27–7.13 (m, 10H), 5.02 (s, 2H), 4.54 (m, 1H), 4.19–4.02 (m, 2H), 3.16 (dd, J = 13.3, 3.4 Hz, 1H), 2.89 (dd, J = 16.7, 10.7 Hz, 1H), 2.53 (dd, J = 16.7, 3.9 Hz, 1H), 2.40 (dd, J = 13.3, 9.8 Hz, 1H), 1.38 (m, 1H), 1.27–1.18 (m, 6H), 0.79 (t, J = 6.9 Hz, 3H).

4.3.1.17. Benzyl 4-[(*S*)-4-benzyl-2-oxooxazolidin-3-yl]-3-benzyl-4-oxobutanoate (21c). This compound was prepared from **19c** according to the general procedure (two steps, 44%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.37–7.20 (m, 15H), 5.06 (s, 2H), 4.57 (m, 1H), 4.48 (m, 1H), 4.03 (dd, J = 8.6, 2.1 Hz, 1H), 3.91 (dd, J = 8.6, 8.1 Hz, 1H), 3.19 (dd, J = 13.3, 3.0 Hz, 1H), 3.05–2.97 (m, 2H), 2.65 (dd, J = 13.3, 9.4 Hz, 1H), 2.53–2.44 (m, 2H); MS (FAB) 458 $(M+H)^+$.

4.3.1.18. (*R*)-3-Benzyloxycarbonyl-2-pentylpropanoic acid (22a). *General procedure.* To a solution of **21a** (4.35 g, 9.95 mmol) in THF/H_2O (72 mL:18 mL) were added 30% H_2O_2 aq (6.0 mL) and a solution of $LiOH{\cdot}H_2O$ (600 mg) in H_2O (24 mL). The mixture was stirred for 2 h at 0 °C and 2 h at rt, then acidified with 2 N HCl. Extraction with AcOEt and concentration under reduced pressure afforded the product. Pure **22a** was obtained by means of column chromatography (AcOEt/Hex = 1:5) (1.8 g, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.38–7.30 (m, 5H), 5.13 (d, J = 4.3 Hz, 2H), 2.90 (m, 1H), 2.77 (dd, J = 16.7, 9.4 Hz, 1H), 2.51 (dd, J = 16.7, 5.1 Hz, 1H), 1.68 (m, 1H), 1.55 (m, 1H), 1.35–1.26 (m, 6H), 0.87 (t, J = 6.8 Hz, 3H).

4.3.1.19. (*R*)-3-Benzyloxycarbonyl-2-benzylpropanoic acid (22c). This compound was prepared from **21c** according to the general procedure (89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.36–7.15 (m, 10H), 5.09 (s, 2H), 3.22–3.12 (m, 2H), 2.79 (dd, J = 11.3, 8.6 Hz, 1H), 2.70 (dd, J = 17.1, 9.0 Hz, 1H), 2.46 (dd, J = 17.1, 4.7 Hz, 1H); MS (FAB) 299 $(M+H)^+$.

4.3.1.20. *N*-[(*R*)-3-Benzyloxycarbonyl-2-pentylpropanoyl]-L-leucine methyl ester (23a). *General procedure.* To a solution of compound **22a** (273 mg, 0.98 mmol) in dry CH_2Cl_2 (20 mL) were added L-leucine methyl ester hydrochloride (214 mg, 1.18 mmol), HOBt (147 mg, 1.09 mmol), EDCI (245 mg, 1.28 mmol), and DIPEA (186 μL, 1.09 mmol) under an argon atmosphere. The reaction mixture was stirred at rt for 2 h and quenched with 0.5 N HCl. The organic layer was collected, dried over $MgSO_4$, and concentrated under reduced pressure. Pure **23a** was obtained by means of column chromatography (AcOEt/Hex = 1:3) (339 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.45–7.30 (m, 5H), 6.08 (d, J = 8.1 Hz, 1H), 5.12 (d, J = 12.4 Hz, 1H), 5.08 (d, J = 12.4 Hz, 1H), 4.62 (m, 1H), 3.74 (s, 3H), 2.79 (dd, J = 16.7, 9.4 Hz, 1H), 2.64 (m, 1H), 2.45 (dd, J = 16.7, 5.1 Hz, 1H), 1.66–1.51 (m, 3H), 1.38–1.36 (m, 2H), 1.33–1.26 (m, 6H), 0.97–0.90 (m, 6H), 0.87 (t, J = 6.8 Hz, 3H); MS (FAB) 406 $(M+H)^+$.

4.3.1.21. *N*-[(*R*)-3-Benzyloxycarbonyl-2-ethylpropanoyl]-L-leucine methyl ester (23b). This compound was prepared from **20b** according to the general procedure (three steps, 32%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.38–7.30 (m, 5H), 6.00 (d, J = 8.1 Hz, 1H), 5.11 (d, J = 12.0 Hz, 1H), 5.08 (d, J = 12.0 Hz, 1H), 4.62 (m, 1H), 3.72 (s, 3H), 2.80 (dd, J = 16.7, 9.4 Hz, 1H), 2.58 (m, 1H), 2.46 (dd, J = 16.7, 4.3 Hz, 1H), 1.72–1.58 (m, 3H), 1.55–1.46 (m, 2H), 0.96–0.90 (m, 9H); MS (FAB) 364 $(M+H)^+$.

4.3.1.22. *N*-[(*R*)-3-Benzyloxycarbonyl-2-benzylpropanoyl]-L-leucine methyl ester (23c). This compound was prepared from **22c** according to the general procedure (57%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.36–7.16 (m, 10H), 5.88 (d, J = 8.6 Hz, 1H), 5.07 (s, 2H), 4.55 (m, 1H), 3.67 (s, 3H), 2.99 (dd, J = 13.3, 7.5 Hz, 1H), 2.91 (m, 1H), 2.82 (dd, J = 17.1, 9.4 Hz, 1H), 2.72 (dd, J = 13.3, 7.5 Hz, 1H), 2.46 (dd, J = 17.1, 4.0 Hz, 1H), 1.59–1.52 (m, 2H), 1.45 (m, 1H), 0.89–0.88 (m, 6H); MS (FAB) 426 $(M+H)^+$.

4.3.1.23. *N*-[(*R*)-3-*O*-Benzyloxycarbamoyl-2-pentylpropanoyl]-L-leucine methyl ester (24a). *General procedure.* To a solution of **23a** (339 mg, 0.84 mmol) in AcOEt (25 mL) was added 10% Pd–C (30 mg), and the mixture was stirred under H_2 gas for 1 h. The intermediate was obtained by removal of the Pd–C by filtration, and dissolved in CH_2Cl_2 (20 mL). To a solution of the intermediate were added *O*-benzylhydroxylamine hydrochloride (152 mg, 0.95 mmol), HOBt (147 mg, 1.09 mmol), EDCI (183 mg, 0.95 mmol), and DIPEA (113 μL, 0.87 mmol) under an argon atmosphere. The reaction mixture was stirred for 2 h at rt and quenched with 0.5 N HCl. The organic layer was collected, dried over $MgSO_4$, and concentrated under reduced pressure. Pure **24a** was obtained by means of column chromatography (AcOEt/Hex = 1:3 to 1:1) (245 mg, two steps, 70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.39–7.35 (m, 5H), 6.07 (s, 1H), 4.87 (s, 2H), 4.58 (m, 1H), 3.72 (s, 3H), 2.69 (m, 1H), 2.37 (m, 1H), 2.21 (m, 1H), 1.66–1.55 (m, 4H), 1.42 (m, 1H), 1.32–1.26 (m, 6H), 0.94 (d, J = 6.0 Hz, 3H), 0.93 (d, J = 6.0 Hz, 3H), 0.87 (t, J = 6.8 Hz, 3H).

4.3.1.24. *N*-[(*R*)-3-*O*-Benzyloxycarbamoyl-2-ethylpropanoyl]-L-leucine methyl ester (24b). This compound was prepared from **23b** according to the general procedure (two steps 13%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.47–7.35(m, 5H), 6.58 (d, J = 8.1 Hz, 1H), 4.88 (s, 2H), 4.59 (m, 1H), 3.72 (s, 3H), 2.63 (m, 1H), 2.38 (m, 1H), 2.20 (m, 1H), 1.67–1.49 (m, 5H), 0.96–0.91 (m, 9H); MS (FAB) 379 $(M+H)^+$.

4.3.1.25. *N*-[(*R*)-3-*O*-Benzyloxycarbamoyl-2-benzylpropanoyl]-L-leucine methyl ester (24c). This compound was prepared from **23c** according to the general procedure (two steps 82%). ^{1}H NMR (DMSO-d_6) δ: 10.98 (s, 1H), 8.30 (d, J = 7.7 Hz, 1H), 7.36–7.16 (m, 10H), 4.70 (s, 2H), 4.25 (m, 1H), 3.56 (s, 3H), 3.03 (dd, J = 7.3, 6.8 Hz, 1H), 2.96 (dd, J = 13.7, 7.3 Hz, 1H), 2.51 (m, 1H), 2.20 (dd, J = 15.0, 8.1 Hz, 1H), 1.90 (dd, J = 15.0, 6.0 Hz, 1H), 1.62–1.50 (m, 2H), 1.45 (m, 1H), 0.85 (d, J = 6.4 Hz, 3H), 0.79 (d, J = 6.4 Hz, 3H); MS (FAB) 441 $(M+H)^+$.

4.3.1.26. *N*-[(*R*)-3-Hydroxycarbamoyl-2-pentylpropanoyl]-L-leucine methyl ester (HAB-5RL: 25a). *General procedure.* To a solution of **24a** (210 mg, 0.84 mmol) in MeOH (20 mL) was added 10% Pd–C (20 mg), and the mixture was stirred under H_2 gas for 40 min. Pure **25a** was obtained by removal of Pd–C by filtration (160 mg, 97%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.36 (s, 1H), 8.67 (s, 1H), 8.23 (d, J = 7.7 Hz, 1H), 4.23 (m, 1H), 3.32 (s, 3H), 2.62 (m, 1H), 2.09 (m, 1H), 1.98(m, 1H), 1.67–1.51 (m, 2H), 1.48–1.36 (m, 2H), 1.27–1.12 (m, 7H), 0.88–0.79 (m, 9H); HRMS (FAB) calcd for $C_{16}H_{30}N_2NaO_5$ 353.2052; found: 353.2040 $(M+Na)^+$.

4.3.1.27. *N*-[(*R*)-3-Hydroxycarbamoyl-2-ethylpropanoyl]-L-leucine methyl ester (HAB-2L: 25b). This compound was prepared from **24b** according to the general procedure (87%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.35 (s, 1H), 8.66 (s, 1H), 8.21 (d, J = 8.1 Hz, 1H), 4.23 (m, 1H), 3.59 (s, 1H), 2.59 (m, 1H), 2.12 (dd, J = 14.5, 6.4 Hz, 1H), 1.98 (dd, J = 14.5, 7.7 Hz, 1H), 1.65–1.35 (m, 5H), 0.87 (d, J = 6.4 Hz, 3H), 0.87 (d, J = 6.4 Hz, 3H), 0.83–0.81 (m, 6H); HRMS (FAB) calcd for $C_{13}H_{25}N_2O_5$ 289.1763; found: 289.1768 $(M+H)^+$.

4.3.1.28. *N*-[(*R*)-3-Hydroxycarbamoyl-2-benzylpropanyl]-L-leucine methyl ester (HAB-BL: 25c). This compound was prepared from **24c** according to the general procedure (97%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.33 (s, 1H), 8.93 (s, 1H), 8.26 (d, J = 7.7 Hz, 1H), 7.35–7.15 (m, 5H), 4.21 (m, 1H), 3.54 (s, 3H), 3.00 (dd, J = 7.3, 6.8 Hz, 1H), 2.86 (dd, J = 13.7, 7.3 Hz,

1H), 2.52 (m, 1H), 2.19 (dd, J = 15.0, 7.7 Hz, 1H), 1.90 (dd, J = 15.0, 6.8 Hz, 1H), 1.59–1.49 (m, 2H), 1.44 (m, 1H), 0.86 (d, J = 6.4 Hz, 3H), 0.80 (d, J = 6.4 Hz, 3H); HRMS (FAB) calcd for $C_{18}H_{27}N_2O_5$ 351.1920; found: 351.1870 $(M+H)^+$.

4.3.1.29. *N*-[*N*-Benzyloxysuccinamyl]-L-leucine methyl ester (27). To a solution of **26** (1.0 g, 10.0 mmol) in CH_2Cl_2 (200 mL) were added L-leucine methyl ester hydrochloride (1.50 g, 8.26 mmol), DMAP (1.02 g, 8.36 mmol), and NEt_3 (1.15 mL). The mixture was stirred for 17 h at rt, then the reaction was quenched with 10% $NaHCO_3$ aq. The aqueous layer was acidified with 2 N HCl and extracted with AcOEt. Concentration under reduced pressure afforded the pure intermediate (1.68 g, 83%). To a solution of intermediate (320 mg, 1.31 mmol) were added *O*-benzylhydroxylamine hydrochloride (250 mg, 1.57 mmol), EDCI (300 mg, 1.47 mmol), and DIPEA (222 μL, 1.31 mmol) under an argon atmosphere. The reaction mixture was stirred for 1 h at rt and quenched with 0.5 N HCl. The organic layer was collected, dried over $MgSO_4$, and concentrated under reduced pressure. Pure **27** was obtained by means of column chromatography (CH_2Cl_2/MeOH = 15:1) and recrystallization (AcOEt/hexane) at −78 °C (281 mg, 67%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.0 (s, 1H), 8.22 (d, J = 7.7 Hz, 1H), 7.38–7.35 (m, 5H), 4.75 (s, 2H), 4.26 (m, 1H), 3.62 (s, 3H), 2.44–2.33 (m, 3H), 2.18 (m, 1H), 1.61(m, 1H), 1.51 (m, 1H), 1.46 (m, 1H), 0.88 (d, J = 6.4 Hz, 3H), 0.82 (d, J = 6.4 Hz, 3H).

4.3.1.30. *N*-[*N*-Hydroxysuccinamiyl]-L-leucine methyl ester (HAB-0L: 25d). To a solution of **27** (81.0 mg, 0.23 mmol) in MeOH (14 mL) was added 10% Pd–C (7.0 mg), and the mixture was stirred under H_2 gas for 40 min. Pure **25d** was obtained by the removal of Pd–C by filtration (59.0 mg, 98%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.36 (s, 1H), 8.68 (s, 1H), 8.24 (d, J = 6.8 Hz, 1H), 4.25 (m, 1H), 3.60 (s, 3H), 2.38–2.31 (m, 3H), 1.63 (m, 1H), 1.60 (m, 1H), 1.54–1.42 (m, 2H), 0.87 (d, J = 6.4 Hz, 3H), 0.82 (d, J = 6.4 Hz, 3H); HRMS (FAB) calcd for $C_{11}H_{21}N_2O_5$ 261.1450; found: 261.1458 $(M+H)^+$.

4.3.1.31. *N*-[(*R*)-3-Benzyloxycarbonyl-2-pentylpropanoyl]glycine methyl ester (28a). This compound was prepared from **22a** according to the general procedure (76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.37–7.31 (m, 5H), 6.13 (m, 1H), 5.13 (d, J = 12.4 Hz, 1H), 5.08 (d, J = 12.4 Hz, 1H), 4.07 (dd, J = 18.4, 5.6 Hz, 1H), 3.92 (dd, J = 18.4, 4.7 Hz, 1H), 3.74 (s, 3H), 2.79 (dd, J = 16.7, 9.4 Hz, 1H), 2.64 (m, 1H), 2.47 (dd, J = 16.7, 4.3 Hz, 1H), 1.66 (m, 1H), 1.41 (m, 1H), 1.32–1.23 (m, 6H), 0.86 (t, J = 6.8 Hz, 3H); MS (FAB) 350 $(M+H)^+$.

4.3.1.32. *N*-[(*R*)-3-Benzyloxycarbonyl-2-pentylpropanoyl]-L-alanine methyl ester (28b). This compound was prepared from **22a** according to the general procedure (96%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.37–7.31 (m, 5H), 6.11 (d, J = 7.3 Hz, 1H), 5.12 (d, J = 12.4 Hz, 1H), 5.08 (d, J = 12.4 Hz, 1H), 4.57 (quant, J = 7.3 Hz, 1H), 3.74 (s, 3H), 2.79 (dd, J = 16.7, 9.4 Hz, 1H), 2.59 (m, 1H), 2.45 (dd, J = 1.67, 4.7 Hz, 1H), 1.64 (m, 1H), 1.40 (m, 1H), 1.32 (d, J = 7.3 Hz, 3H), 1.33–1.25 (m, 6H), 0.86 (t, J = 6.8 Hz, 3H); MS (FAB) 364 $(M+H)^+$.

4.3.1.33. *N*-[(*R*)-3-Benzyloxycarbonyl-2-pentylpropanoyl]-L-valine methyl ester (28c). This compound was prepared from **22a** according to the general procedure (92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.36–7.30 (m, 5H), 6.12 (d, J = 8.6 Hz, 1H), 5.10 (s, 2H), 4.55 (m, 1H), 3.73 (s, 3H), 2.80 (dd, J = 16.7, 9.4 Hz, 1H), 2.64 (m, 1H), 2.46 (dd, J = 16.7, 3.4 Hz, 1H), 2.14 (m, 1H), 1.65 (m, 1H), 1.40 (m, 1H), 1.31–1.24 (m, 6H), 0.92–0.84 (m, 9H); MS (FAB) 392 $(M+H)^+$.

4.3.1.34. *N*-[(*R*)-3-Benzyloxycarbonyl-2-pentylpropanoyl]-L-*tert*-leucine methyl ester (28d). This compound was prepared from **22a** according to the general procedure (100%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.37–7.31 (m, 5H), 6.19 (d, J = 16.7, 9.4 Hz, 1H), 5.10 (s, 2H), 4.45 (d, J = 9.4 Hz, 1H), 3.71(s, 3H), 2.80 (dd, J = 16.7, 9.4 Hz, 1H), 2.64 (m, 1H), 2.46 (dd, J = 16.7, 3.9 Hz, 1H), 1.64 (m, 1H), 1.39 (m, 1H), 1.29–1.22 (m, 6H), 0.95 (s, 9H), 0.86(t, J = 6.8 Hz, 3H); MS (FAB) 406 $(M+H)^+$.

4.3.1.35. *N*-[(*R*)-3-Benzyloxycarbonyl-2-pentylpropanyl]-L-phenylalanine methyl ester (28e). This compound was prepared from **22a** according to the general procedure (100%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.37–7.13 (m, 10H), 6.09 (d, J = 7.7 Hz, 1H), 5.11 (d, J = 12.4 Hz, 1H), 5.06 (d, J = 12.4 Hz, 1H), 4.89 (dt, J = 7.7, 6.0 Hz, 1H), 3.70 (s, 3H), 3.09 (dd, J = 14.1, 6.0 Hz, 1H), 3.05 (dd, J = 14.1, 6.0 Hz, 1H), 2.43 (dd, J = 16.7, 9.0 Hz, 1H), 2.58 (m, 1H), 2.43 (dd, J = 16.7, 4.7 Hz, 1H), 1.59 (m, 1H), 1.38 (m, 1H), 1.27–1.22 (m, 6H), 0.84 (t, J = 6.8 Hz, 3H); MS (FAB) 440 $(M+H)^+$.

4.3.1.36. *N*-[(*R*)-3-*O*-Benzyloxycarbamoyl-2-pentylpropanoyl]glycine methyl ester (29a). This compound was prepared from **28a** according to the general procedure (two steps 74%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.00 (s, 1H), 8.36 (t, J = 6.0 Hz, 1H), 7.37–7.34 (m, 5H), 4.75 (s, 2H), 3.83 (dd, J = 17.1, 6.0 Hz, 1H), 3.73 (dd, J = 17.1, 6.0 Hz, 1H), 3.59 (s, 3H), 2.66 (m, 1H), 2.15 (dd, J = 14.5, 6.8 Hz, 1H), 1.98 (dd, J = 14.5, 8.1 Hz, 1H), 1.38 (m, 1H), 1.26–1.16 (m, 7H), 0.84 (t, J = 6.8 Hz, 3H); MS (FAB) 365 $(M+H)^+$, 242 $(M-BnONH)^+$.

4.3.1.37. *N*-[(*R*)-3-*O*-Benzyloxycarbamoyl-2-pentylpropanoyl]-L-alanine methyl ester (29b). This compound was prepared from **28b** according to the general procedure (two steps 68%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.99 (s, 1H), 8.31 (d, J = 6.8 Hz, 1H), 7.37–7.33 (m, 5H), 4.74 (s, 2H), 4.21 (qd, J = 7.3, 6.8 Hz, 1H), 3.58 (s, 3H), 2.64 (m, 1H), 2.14 (dd, J = 14.5, 7.3 Hz, 1H), 1.98 (dd, J = 14.5, 7.3 Hz, 1H), 1.38 (m, 1H), 1.28–1.16 (m, 6H), 1.24 (d, J = 7.3 Hz, 3H), 0.85 (t, J = 6.8 Hz, 3H); MS (FAB) 379 $(M+H)^+$, 256 $(M-BnONH)^+$.

4.3.1.38. *N*-[(*R*)-3-*O*-Benzyloxycarbamoyl-2-pentylpropanoyl]-L-valine methyl ester (29c). This compound was prepared from **28c** according to the general proce-

dure (two steps, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.37–7.36 (m, 5H), 6.24 (s, 1H), 4.88 (s, 2H), 4.51 (m, 1H), 3.73 (s, 3H), 2.75 (m, 1H), 2.40 (m, 1H), 2.23–2.16 (m, 2H), 1.60 (m, 1H), 1.41(m, 1H), 1.29–1.24 (m, 6H), 0.93 (dd, J = 12.4, 6.8 Hz, 6H), 0.87 (t, J = 6.8 Hz, 3H); MS (FAB) 407 $(M+H)^+$, 284 $(M-BnONH)^+$.

4.3.1.39. *N*-[(*R*)-3-*O*-Benzyloxycarbamoyl-2-pentylpropanoyl]-L-*tert*-leucine methyl ester (29d). This compound was prepared from **28d** according to the general procedure (two steps, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.38–7.36 (m, 5H), 6.32 (s, 1H), 4.87 (s, 2H), 4.41 (d, J = 7.7 Hz, 1H), 3.72 (s, 3H), 2.75 (m, 1H), 2.39 (m, 1H), 2.22 (m, 1H), 1.59 (m, 1H), 1.40 (m, 1H), 1.29–1.23 (m, 6H), 0.98 (s, 9H), 0.87 (t, J = 6.8 Hz, 3H); MS (FAB) 421 $(M+H)^+$, 298 $(M-BnONH)^+$.

4.3.1.40. *N*-[(*R*)-3-*O*-Benzyloxycarbamoyl-2-pentylpropanoyl]-L-phenylalanine methyl ester (29e). This compound was prepared from **28e** according to the general procedure (two steps, 60%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.38–7.13 (m, 10H), 6.17 (s, 1H), 4.87 (s, 2H), 4.83 (m, 1H), 3.70 (s, 3H), 3.09 (d, J = 6.0 Hz, 2H), 2.64 (m, 1H), 2.32 (m, 1H), 2.15 (m, 1H), 1.56 (m, 1H), 1.38 (m, 1H), 1.26–1.21 (m, 6H), 0.85 (t, J = 6.8 Hz, 3H); MS (FAB) 455 $(M+H)^+$, 332 $(M-BnONH)^+$.

4.3.1.41. *N*-[(*R*)-3-Hydroxycarbamoyl-2-pentylpropanoyl]glycine methyl ester (HAB-5G: 30a). This compound was prepared from **29a** according to the general procedure (100%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.37 (s, 1H), 8.69 (s, 1H), 8.41 (m, 1H), 3.84 (dd, J = 17.1, 6.4 Hz, 1H), 3.71 (dd, J = 17.1, 5.6 Hz, 1H), 3.60 (s, 3H), 2.64 (m, 1H), 2.14 (dd, J = 14.5, 6.4 Hz, 1H), 1.98 (dd, J = 14.5, 8.1 Hz, 1H), 1.40 (m, 1H), 1.27–1.16 (m, 7H), 0.84 (t, J = 6.8 Hz, 3H); HRMS (FAB) calcd for $C_{12}H_{23}N_2O_5$ 275.1607; found: 275.1588 $(M+H)^+$.

4.3.1.42. *N*-[(*R*)-3-Hydroxycarbamoyl-2-pentylpropanoyl]-L-alanine methyl ester (HAB-5A: 30b). This compound was prepared from **29b** according to the general procedure (99%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.35 (s, 1H), 8.70 (s, 1H), 8.29 (d, J = 6.7 Hz, 1H), 4.20 (td, J = 7.3, 6.7 Hz, 1H), 3.58 (s, 3H), 2.63 (m, 1H), 2.12 (dd, J = 14.6, 6.7 Hz, 1H), 1.96 (dd, J = 14.6, 7.9 Hz, 1H), 1.39–1.21 (m, 8H), 1.24 (d, J = 7.3 Hz, 3H), 0.84 (t, J = 6.7 Hz, 3H); HRMS (FAB) calcd for $C_{13}H_{25}N_2O_5$ 289.1763; found: 289.1758 $(M+H)^+$.

4.3.1.43. *N*-[(*R*)-3-Hydroxycarbamoyl-2-pentylpropanoyl]-L-valine methyl ester (HAB-5V: 30c). This compound was prepared from **29c** according to the general procedure (100%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.34 (s, 1H), 8.67 (s, 1H), 8.15 (d, J = 7.7 Hz, 1H), 4.09 (dd, J = 7.7, 6.4 Hz, 1H), 3.58 (s, 3H), 2.75 (m, 1H), 2.13 (dd, J = 14.5, 6.8 Hz, 1H), 2.03–1.96 (m, 2H), 1.37 (m, 1H), 1.29–1.14 (m, 7H), 0.88 (d, J = 6.8 Hz, 3H), 0.85 (d, J = 6.8 Hz, 3H), 0.84 (t, J = 6.8 Hz, 3H); HRMS (FAB) calcd for $C_{15}H_{29}N_2O_5$ 317.2076; found: 317.2073 $(M+H)^+$.

4.3.1.44. *N*-[(*R*)-3-Hydroxycarbamoyl-2-pentylpropanoyl]-L-*tert*-leucine methyl ester (HAB-5TL: 30d). This compound was prepared from **29d** according to the general procedure (97%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.35 (s, 1H), 8.67 (s, 1H), 8.03 (d, J = 8.1 Hz, 1H), 4.10 (d, J = 8.1 Hz, 1H), 3.58 (s, 3H), 2.83 (m, 1H), 2.12 (dd, J = 14.5, 6.8 Hz, 1H), 1.99 (dd, J = 14.5, 7.7 Hz, 1H), 1.36 (m, 1H), 1.29–1.12 (m, 7H), 0.93 (s, 9H), 0.83 (t, J = 6.8 Hz, 3H); HRMS (FAB) calcd for $C_{16}H_{31}N_2O_5$ 331.2233; found: 331.2247 $(M+H)^+$.

4.3.1.45. *N*-[(*R*)-3-Hydroxycarbamoyl-2-pentylpropanoyl]-L-phenylalanine methyl ester (HAB-5F: 30e). This compound was prepared from **29e** according to the general procedure (93%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.35 (s, 1H), 8.68 (s, 1H), 8.42 (d, J = 7.3 Hz, 1H), 7.27–7.17 (m, 5H), 4.43 (ddd, J = 9.0, 7.3, 6.0 Hz, 1H), 3.54 (s, 3H), 3.00 (dd, J = 13.7, 5.6 Hz, 1H), 2.90 (dd, J = 13.7, 9.4 Hz, 1H), 2.62 (m, 1H), 2.48 (m, 1H), 1.91 (m, 1H), 1.33 (m, 1H), 1.22–1.13 (m, 7H), 0.83 (t, J = 6.8 Hz, 3H); HRMS (FAB) calcd for $C_{19}H_{29}N_2O_5$ 365.2076; found: 365.2044 $(M+H)^+$.

4.3.1.46. (*R*)-2-*tert*-Butoxycarbonylaminosuccinic acid 4-benzyl ester (32). To a solution of **15** (6.66 g, 50 mmol) in benzene (250 mL) were added *p*-toluenesulfonic acid monohydrate (10.5 g, 55 mmol) and benzyl alcohol (35 mL, 339 mmol). The mixture was stirred at reflux with continuous removal of generated H_2O for 3.5 h. Benzene was removed under reduced pressure and the residue was dissolved in acetone:H_2O (62 mL:47 mL). To this solution was added 20% NaOH aq (15 mL), and the mixture was stirred at 13 °C for 30 min. Then NEt_3 (10 mL) and Boc_2O (16 g, 73 mmol) were added, and the whole was stirred for 2 h at rt. Acetone was removed under reduced pressure, and the aqueous layer was acidified with 2 N HCl. Pure **32** was obtained by extraction with AcOEt and concentrated under reduced pressure (7.73 g, three steps, 48%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.37–7.26 (m, 5H), 5.55 (d, J = 8.1 Hz, 1H), 5.16 (d, J = 12.4 Hz, 1H), 5.12 (d, J = 12.4 Hz, 1H), 4.63 (m, 1H), 3.07 (dd, J = 17.1, 3.8 Hz, 1H), 2.89 (dd, J = 17.1, 5.1 Hz, 1H), 1.45 (s, 9H).

4.3.1.47. *N*-[(*R*)-3-Benzyloxycarbonyl-2-*tert*-butoxycarbonylaminopropanoyl]-L-leucine methyl ester (33). To a solution of compound **32** (6.60 g, 20.4 mmol) in dry CH_2Cl_2 (100 mL) were added L-leucine methyl ester hydrochloride (4.08 g, 22.5 mmol), HOBt (3.04 g, 22.5 mmol), and EDCI (4.70 g, 34.5 mmol), under an argon atmosphere. The reaction mixture was stirred at rt for 2 h and quenched with 0.5 N HCl. The organic layer was collected, dried over $MgSO_4$, and concentrated under reduced pressure. The intermediate was obtained by means of column chromatography (AcOEt/Hex = 1:4 to 1:1) (4.76 g, 52%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.36–7.32 (m, 5H), 6.86 (d, J = 6.8 Hz, 1H), 5.67 (d, J = 7.3 Hz, 1H), 5.16 (d, J = 12.0 Hz, 1H), 5.11 (d, J = 12.0 Hz, 1H), 4.59–4.55 (m, 2H), 3.71 (s, 3H), 3.01 (dd, J = 17.1, 3.8 Hz, 1H), 2.74 (dd, J = 17.1, 6.0 Hz, 1H), 1.66–1.52 (m, 3H), 1.46 (s, 9H), 0.91 (d, J = 6.0Hz, 6H); MS (FAB) 451 $(M+H)^+$.

4.3.1.48. *N*-[(*R*)-3-*O*-Benzyloxycarbamoyl-2-*tert*-butoxycarbonylaminopropanoyl]-L-leucine methyl ester (35b). *General procedure.* To a solution of **33** (1.2 g, 2.66 mmol) in AcOEt (50 mL) was added 10% Pd–C (120 mg). The mixture was stirred under H_2 gas for 1 h. The intermediate was obtained by the removal of Pd–C by filtration, and dissolved in CH_2Cl_2 (30 mL). To this solution were added *O*-benzylhydroxylamine hydrochloride (467 mg, 2.93 mmol), HOBt (395 mg, 2.92 mmol), EDCI (612 mg, 3.19 mmol), and DIPEA (226 μL, 1.33 mmol) under an argon atmosphere. The reaction mixture was stirred for 2 h at rt and quenched with 0.5 N HCl. The organic layer was collected, dried over $MgSO_4$, and concentrated under reduced pressure. Pure **35b** was obtained by column chromatography (AcOEt/Hex = 2:3 to 3:2) (758 mg, two steps, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.41–7.35 (m, 5H), 4.89 (s, 2H), 4.52 (m, 1H), 4.45 (m, 1H), 3.71 (s, 3H), 2.67 (m, 1H), 2.46 (m, 1H), 1.67–1.56 (m, 3H), 1.49 (s, 9H), 0.94–0.90 (m, 6H).

4.3.1.49. *N*-[(*R*)-3-*O*-*tert*-Butyldimethylsilyloxycarbamoyl-2-*tert*-butoxycarbonylaminopropanoyl]-L-leucine methyl ester (35a). This compound was prepared from **33** according to the general procedure (two steps 29%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.51 (m, 1H), 4.43 (m, 1H), 3.72 (s, 3H), 2.72 (m, 1H), 2.47 (m, 1H), 1.68–1.55 (m, 3H), 1.45 (s, 9H), 0.95 (s, 9H), 0.17 (s, 6H); MS (FAB) 490 $(M+H)^+$.

4.3.1.50. *N*-[(*R*)-3-Hydroxycarbamoyl-2-aminopropanoyl]-L-leucine methyl ester (HAB-A0L: 36a). 35a (50 mg, 0.102 mmol) was dissolved in TFA/CH_2Cl_2 (2 mL:2 mL) and the solution was stirred for 5 h at 0 °C. The TFA was removed under reduced pressure, and pure **36a** was obtained by recrystallization (AcOEt/Hex) (38.7 mg, 100%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.63 (s, 1H), 8.91 (m, 2H), 4.26 (m, 1H), 3.63 (m, 1H), 3.62 (s, 3H), 2.86–2.67 (m, 2H), 2.52–2.42 (m, 2H), 1.65–1.47 (m, 5H), 1.32–1.27 (m, 2H), 0.89 (d, J = 6.4 Hz, 3H), 0.87 (t, J = 7.7 Hz, 3H), 0.83 (d, J = 6.4 Hz, 3H); HRMS (FAB) calcd for $C_{11}H_{22}N_3O_5$ 276.1559; found: 276.1561 $(M+H)^+$.

4.3.1.51. *N*-[(*R*)-3-*O*-Benzyloxycarbamoyl-2-butylaminopropanyl]-L-leucine methyl ester (38a). 35b (62 mg, 0.133 mmol) was dissolved in TFA/CH_2Cl_2 (0.5 mL: 5 mL) and the solution was stirred for 1 h at rt. The TFA was removed under reduced pressure, and the residue was dissolved in THF (5 mL). To this solution were added *n*-butylaldehyde (13.2 μL, 0.146 mmol), $NaBH(OAc)_3$ (42.4 mg, 0.200 mmol), and AcOH (8 μL), and the mixture was stirred for 30 min at rt. Pure **38a** was obtained by means of column chromatography (AcOEt/Hex = 1:1 to AcOEt/Net_3 = 50:1) (39.1 mg, two steps, 70%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.37 (s, 1H), 7.38–7.36 (m, 5H), 4.78 (d, 2H), 4.29 (m, 1H), 4.15 (m, 1H), 3.71 (s, 3H), 2.84 (m, 2H), 2.59 (d, J = 5.6 Hz, 1H), 2.49 (m, 1H), 1.68–1.48 (m, 5H), 1.31 (dd, J = 15.0, 7.3 Hz, 2H), 0.88 (t, J = 7.7 Hz, 3H), 0.87(d, J = 6.4 Hz, 3H), 0.81 (d, J = 6.4 Hz, 3H); MS (FAB) 422 $(M+H)^+$.

4.3.1.52. *N*-[(*R*)-3-*O*-Benzyloxycarbamoyl-2-dibutylaminopropanoyl]-L-leucine methyl ester (38b). Compound **35b** (100 mg, 0.215 mmol) was dissolved in TFA/CH_2Cl_2 (0.5 mL:5 mL) and the solution was stirred for 1 h at rt. The TFA was removed under reduced pressure, and the residue was dissolved in THF (5 mL). To this solution were added *n*-butylaldehyde (48.5 μL, 0.538 mmol), $NaBH(OAc)_3$ (137 mg, 0.646 mmol), and AcOH (26 μL), and the mixture was stirred for 30 min at rt. Pure **38b** was obtained by means of column chromatography (AcOEt/Hex = 1:1 to AcOEt/Net_3 = 50:1) (49 mg, two steps 48%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.0 (s, 1H), 7.88 (d, J = 8.1 Hz, 1H), 7.39–7.33 (m, 5H), 4.75 (s, 2H), 4.31 (td, J = 8.6, 4.7 Hz, 1H), 3.79 (dd, J = 7.7, 5.6 Hz, 1H), 3.60 (s, 3H), 2.35 (t, J = 6.8 Hz, 4H), 2.30 (dd, J = 14.5, 7.7 Hz, 1H), 2.15 (dd, J = 14.5, 5.6 Hz, 1H), 1.59–1.50 (m, 3H), 1.41–1.36 (m, 4H), 1.29–1.23 (m, 4H), 0.88–0.83 (m, 12H); MS (FAB) 478 $(M+H)^+$.

4.3.1.53. *N*-[(*R*)-3-Hydroxycarbamoyl-2-butylaminopropanoyl]-L-leucine methyl ester (HAB-A4L: 36b). *General procedure.* To a solution of **38a** (23.7 mg, 0.056 mmol) in MeOH (4 mL) was added 10% Pd–C (2.4 mg) and the mixture was stirred under H_2 gas for 40 min. Pure **36b** was obtained by the removal of Pd–C by filtration (18.6 mg, 100%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.63 (s, 1H), 8.91 (m, 2H), 4.26 (m, 1H), 3.63 (m, 1H), 3.62 (s, 3H), 2.86–2.67 (m, 2H), 2.52–2.42 (m, 2H), 1.65–1.47 (m, 5H), 1.32–1.27 (m, 2H), 0.89 (d, J = 6.4 Hz, 3H), 0.87 (t, J = 7.7 Hz, 3H), 0.83 (d, J = 6.4 Hz, 3H); HRMS (FAB) calcd for $C_{15}H_{30}N_3O_5$ 332.2185; found: 332.2207 $(M+H)^+$.

4.3.1.54. *N*-[(*R*)-3-Hydroxycarbamoyl-2-dibutylaminopropanyl]-L-leucine methyl ester (HAB-A4L: 36c). This compound was prepared from **38b** according to the general procedure (99%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.34 (s, 1H), 8.65 (s, 1H), 7.86 (d, J = 8.6 Hz, 1H), 4.30 (m, 1H), 3.78 (m, 1H), 3.60 (s, 3H), 2.36 (t, J = 6.8 Hz, 4H), 2.31 (dd, J = 15.0, 7.3 Hz, 1H), 2.13 (dd, J = 15.0, 5.6 Hz, 1H), 1.58–1.50 (m, 3H), 1.42–1.36 (m, 4H), 1.29–1.22 (m, 4H), 0.88–0.83 (m, 12H); HRMS (FAB) calcd for $C_{19}H_{38}N_3O_5$ 388.2811; found: 388.2791 $(M+H)^+$.

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