Structural Modification of Fas C-Terminal Tripeptide and Its Effects on the Inhibitory Activity of Fas/FAP-1 Binding

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Received November 3, 1998

We report the structural requirements of the C-terminal tripeptide derivative of Fas (Ac-Ser-Leu-Val-OH, **1**) for the inhibitory activity of Fas/FAP-1 binding. The presence of a carboxyl group and a L-Val residue at the C-terminus is essential for the inhibitory activity, and the hydroxyl group of Ser plays an important role as the donor of a hydrogen bond. The introduction of hydrophobic groups to the N-terminal region of **1**, especially the phenylaminocarbonyl group (**41**), showed a remarkable increase in potency. Further improvement was observed by the attachment of the Glu residue to the *meta*-position of the phenyl ring of **41** (**51**). The ester derivative of **41** (**56**) had the ability to induce apoptosis which was dependent on the concentration of anti-Fas antibody in the colon cancer cell line, DLD-1, which expresses both Fas and FAP-1 and is resistant to Fas-induced apoptosis. We are now investigating whether FAP-1 is a main target of **56** and whether the inhibition of Fas/FAP-1 binding by **56** retrieves the apoptotic signal.

Introduction

Fas (APO-1/CD95) is a cell surface receptor, which is a member of the tumor necrosis factor receptor (TNFR) superfamily, including p55-TNFR, the p75-nerve growth factor receptor (NGFR), and CD40.[1] Binding of the anti-Fas antibody or ligand for Fas induces apoptosis of cells expressing Fas.[2−4] Genetic mutations of Fas or its ligand have been related to lymphoproliferative and autoimmune diseases in mice.[5,6] Furthermore, changes in the Fas expression level have been associated with the induction of apoptosis of unnecessary cells such as human immunodeficiency virus (HIV)-infected T-cells.[7] Recently, Fas is reported to interact with several signal-transducing molecules, such as Fas-associated phosphatase-1 (FAP-1),[8] FADD,[9−11] and RIP[12] which were identified using the yeast two-hybrid system and biochemical method. FAP-1 binds to the negative regulatory region (C-terminal 15 amino acids) of Fas with its third PDZ (GLGF) domain and suppresses Fas-mediated apoptosis. It is reported that FAP-1 is involved in the acquisition of resistance to anti-Fas antibody-mediated apoptosis in human pancreatic adenocarcinoma cells[13] and AIDS-associated Kaposi's sacroma cells.[14]

We think that an inhibitor of Fas/FAP-1 binding will be a good candidate as an anticancer agent. We have already reported that the C-terminal tripeptide (Ac-SLV-OH, **1**) of Fas was necessary and sufficient both for binding to the third PDZ domain of FAP-1 and for inhibiting Fas/FAP-1 binding in vitro.[8,16] Furthermore, the inhibitory activity of Fas/FAP-1 binding was studied using the scanned tripeptides to determine the essential amino acids for inhibition. Replacement of the first position from the C-terminus[15] with other amino acids except for Ile or Leu considerably reduced the potency. Additionally, the potency was reduced by replacement of the third position with other amino acids except for Thr. However, replacement of the second position with other amino acids retained the potency with the exception of Gly. On the basis of these results, we previously proposed the consensus motif of tS/T-X-V/L/I.[16] In this report, we detail our continuing efforts to identify the structural requirements for inhibition against Fas/FAP-1 binding and to develop more potent inhibitors.

Table 1. Fas/FAP-1 Binding Inhibitory Potency of Tripeptides Replaced at Each Residue with D- or *N*-Methylated Amino Acids at a Concentration of 1 mM

Compound		% binding inhibition[a]
1	Ac-Ser-Leu-Val-OH	100.7
2	Ac-Ser-Leu-**D-Val**-OH	-6.8
3	Ac-Ser-**D-Leu**-Val-OH	1.2
4	Ac-**D-Ser**-Leu-Val-OH	59.2
5	Ac-Ser-**NMe-Leu**-Val-OH	33.4
6	Ac-**NMe-Ser**-Leu-Val-OH	20.6

[a] Inhibitory activity was calculated by the methods described under In Vitro Binding Assay (see Experimental Section).

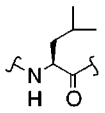
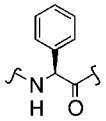
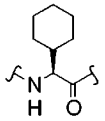
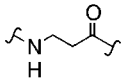
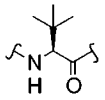
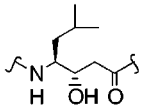
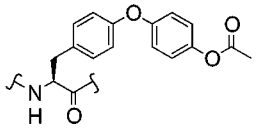
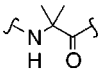
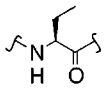
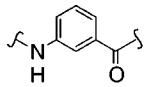
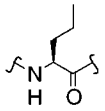
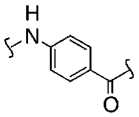
Results and Discussion

To determine the structural requirements for the inhibitory activity of Fas/FAP-1 binding, our initial studies focused on the replacement of each amino acid of the C-terminal tripeptide derivative of Fas (Ac-Ser-Leu-Val-OH, **1**) with several unusual amino acids. These peptides were synthesized by the ordinary solid-phase method.

First of all, we replaced the amino acid residues of **1** with D- and *N*-methylated analogues, and the results of the in vitro inhibition assay of Fas/FAP-1 binding are shown in Table 1. Each substitution of the first and second residues with corresponding D-amino acids displayed a detrimental effect on the inhibitory potency (compounds **2** and **3**), suggesting that the L-stereochem-

10.1021/jm980617f CCC: $18.00
Published on Web 08/05/1999

Table 2. Fas/FAP-1 Binding Inhibitory Potency of Tripeptides Replaced at the 2nd Position Leu with Unusual Amino Acids at a Concentration of 30 μM

Ac-Ser-**X**-Val-OH				Ac-Ser-**X**-Val-OH			
Compound	X		% binding inhibition[a]	Compound	X		% binding inhibition[a]
1	Leu		31.1	**12**	Phg		18.5
7	Chg		34.7	**13**	β-Ala		13.6
8	tBuGly		54.6	**14**	Sta		4.9
9	Thy(OAc)		46.7	**15**	Aib		-4.0[b]
10	Abu		21.1	**16**	3-Abz		9.5[b]
11	NorVal		6.0	**17**	4-Abz		16.5[b]

[a] See Table 1. [b] Concentration is 1 mM.

istry at both positions is essential for the activity. However, the D-configuration at the third position (compound **4**) did not reduce the potency as much as those in the first and second positions, indicating that both configurations at the third position were probably acceptable in the case of the tripeptide derivatives. On the other hand, *N*-methylated analogues (compounds **5** and **6**) significantly reduced the potency, which indicates that the amide nitrogens play an important role in interacting with FAP-1 to possess the inhibitory activity.

Previously, we described that the consensus motif of tS/T-X-V/L/I is essential for inhibition of Fas/FAP-1 binding; therefore, we next investigated the effect of replacement of the most tolerable second position with unusual amino acids. At a concentration of 1 mM for each peptide, the inhibitory potency was retained in most of the cases. Further investigation at a lower concentration (30 μM) of each peptide was carried out, and the results are shown in Table 2. The potency was retained by replacement with the hindered residues Chg (**7**), tBuGly (**8**), and Thy(OAc) (**9**), but it was reduced by replacement with the less hindered residues Abu (**10**), NorVal (**11**), and Phg (**12**). On the other hand, replacement with β-Ala (**13**) or statine (**14**) reduced the potency. These results suggest the existence of a large hydrophobic pocket around the side chain of the second position. Furthermore, replacement of Leu with the α,α-disubstituted residue Aib (**15**) or the conformationally restricted residue aminobenzoic acid (**16** and **17**) caused a significant loss in potency. This result suggests that these rigid analogues cannot have the active conformation of the initial tripeptide **1**.

The inhibitory potency was also studied by replacement of the first Val position. As mentioned above, Val, Ile, or Leu is essential to retain the potency at the first position, so the unusual amino acids having a hydrophobic side chain were tried as replacements. However, as shown in Table 3, all trials to improve the activity by replacing Val with Abu (**18**), NorVal (**19**), tBuGly (**20**), tBuAla (**21**), or Chg (**22**) ended in failure. This result suggests that the isopropyl group is very suitable for fitting into the hydrophobic pocket of FAP-1.

Final replacement with unusual amino acids was carried out at the third position. These results are shown in Table 4. As previously described, the hydroxyl group at the third residue is essential for the inhibitory activity. Therefore, the unusual amino acids having a hydroxyl group were used for replacement. All attempts by replacement of Ser with Hse (**23**), cisHyp (**24**), transHyp (**25**), or statine (**26**) failed to improve the potency, suggesting that the spatial position of the hydroxyl group is important to maintain the inhibitory activity.

Next, we proved the importance of the hydroxyl group at the third position of Ser by modifying it. Any modification, such as acetylation (**28**), benzoylation (**30**), and benzylation (**31**), resulted in considerable losses of potency, suggesting that this hydroxyl group plays an important role as a donor of a hydrogen bond (Table 5).

Furthermore, it was confirmed by the following several modifications that the presence of a carboxyl group at the C-terminus was essential for the inhibitory activity. Conversion of the carboxylic group to the corresponding methyl ester (**33**) had a harmful effect

Table 3. Fas/FAP-1 Binding Inhibitory Potency of Tripeptides Replaced at the 1st Position Val with Unusual Amino Acids at a Concentration of 50 μM

Ac-Ser-Leu-**X**-OH

Compound	X	% binding inhibition[a]
1	Val	44.3
18	Abu	18.7
19	NorVal	16.6
20	tBuGly	7.2
21	tBuAla	10.1
22	Chg	7.7

[a] See Table 1.

Table 4. Fas/FAP-1 Binding Inhibitory Potency of Tripeptides Replaced at the 3rd Position Ser with Unusual Amino Acids at a Concentration of 50 μM

Ac-**X**-Leu-Val-OH

Compound	X	% binding inhibition[a]
1	Ser	47.5
23	HSer	13.3
24	cisHyp	9.6
25	transHyp	2.4
26	Sta	9.9

[a] See Table 1.

Table 5. Fas/FAP-1 Binding Inhibitory Potency of Tripeptides Modified in the Hydroxyl Group at the 3rd Position Ser at a Concentration of 100 μM

Ac-Ser(O**R**)-Leu-Val-OH

Compound	R	% binding inhibition[a]
1	H	87.6
28	Ac	47.9
30	Bz	36.2
31	Bn	10.4

[a] See Table 1.

on the potency, indicating that negative charge is necessary at the C-terminus (Table 6). However, conversion into a tetrazole derivative (**38**), which is regarded as an isoster of a carboxylic acid and was prepared as shown in Scheme 1, showed a remarkable loss in potency. This suggested that the spatial volume of the negative charge is probably a crucial factor for the inhibitor to fit into the FAP-1 pocket.

To improve the inhibitory potency, we finally investigated the introduction of more hydrophobic groups to the N-terminal region of Ac-SLV-OH (**1**), instead of the acetyl group (Table 7). Several acyl groups were introduced by coupling the free amino group of the growing peptide on the resin with appropriate acids: as a result, benzoyl (**39**) and cyclohexylcarbonyl (**40**) groups increased the potency. Further improvements were observed when modified with appropriate isocyanates, and the introduction of the phenylaminocarbonyl group (**41**) gave a remarkable increase in activity. Compound **41** was more potent than the derivative possessing 6-amino acids of the Fas C-terminus (**43**). These hydrophobic groups probably fit the hydrophobic region which is occupied by the side chains of Glu and Ile at the sixth and fifth positions of the Fas C-terminal peptides.

We also examined the effect of the introduction of the Glu residue at the phenyl ring of **41**, because our preliminary data showed that the presence of Glu at the sixth position improved the inhibitory potency. Three derivatives (**47**, **51**, and **55**) were prepared as shown in Scheme 2, and the introduction of Glu at the *meta*-position is most efficient for the activity (Table 8). *Ortho*-substitution has almost no influence on the activity, while *para*-substitution slightly improved it.

Next, we studied whether these peptide derivatives had the ability to induce apoptosis in the colon cancer cell line, DLD-1, which expresses both Fas and FAP-1 and is resistant to Fas-induced apoptosis. We prepared an ester derivative of **41** to improve its permeability into cells. As shown in Figure 1, DLD-1 cells survived when they are incubated with only the anti-Fas antibody. On the other hand, Fas-induced apoptosis, which was dependent on the concentration of the anti-Fas antibody, was observed during the existence of the tripeptide ester

Scheme 1

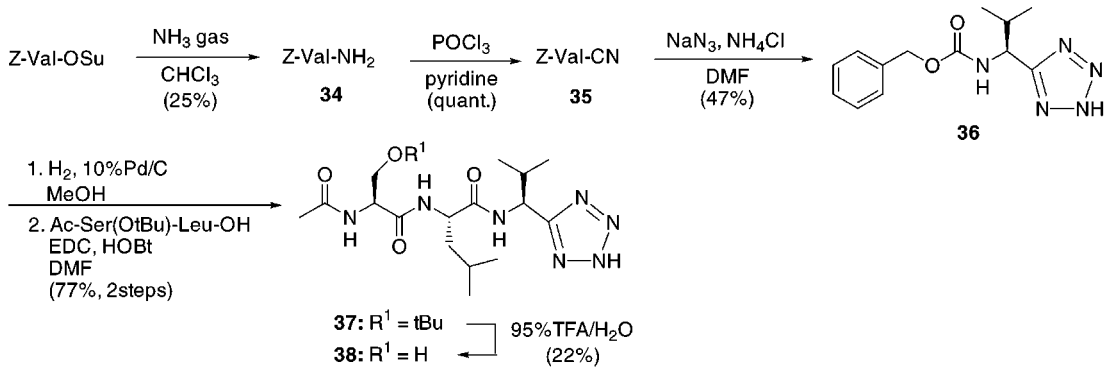


Table 6. Fas/FAP-1 Binding Inhibitory Potency of Tripeptides Modified in the C-Terminal Region at a Concentration of 1 mM

Ac-Ser-Leu-Val-**X**

Compound	X	% binding inhibition[a]
1	OH	100.7
33	OMe	-0.5
38	Tet	14.4

[a] See Table 1.

Table 7. Fas/FAP-1 Binding Inhibitory Potency of Tripeptides Modified in the N-Terminal Region at a Concentration of 10 μM

X-Ser-Leu-Val-OH

Compound	X	% binding inhibition[a]
1	Ac	-1.3
39	Bz	28.0
40	Cyh-CO	16.0
41	Ph-NHCO	51.5
42	Cyh-NHCO	29.1
43	Ac-Glu-Ile-Gln	29.6

[a] See Table 1.

Ph-NHCO-SLV-OEt (**56**). In a previous report,[16] we confirmed that microinjection of **1** induced apoptosis in DLD-1 cells and the methyl ester **33** did not show any Fas/FAP-1 binding inhibition in vitro; therefore, **56** was probably hydrolyzed and converted into the active form of **41** in the DLD-1 cells.

These obtained structure−activity relationships are almost in good agreement with the following information which was obtained from the X-ray structure of the third PDZ domain of the synaptic protein PSD-95 in complex with its peptide ligand.[17] (1) The C-terminal carboxyl group of the peptide makes tight hydrogen bonds with amide nitrogens in the carboxylate-binding loop of the PDZ domain. (2) The side chain of the C-terminal Val dips deeply into a prominent cavity on the surface of the PDZ domain and makes tight contacts with it. (3) The side chain of the second residue is away from the interactive surface. (4) The hydroxyl oxygen of the third residue Thr makes hydrogen bonds with the N-3 nitrogen of His-372 of PSD-95. (5) Only the C-terminal four residues of the peptide are clearly defined in the crystal structure of the PDZ−peptide complex.

The first and second results agree with our results that the presence of a carboxyl group and a Val residue at the C-terminus is essential for the inhibitory activity. The third one is in agreement with our result that the side chain of the second position is tolerable for the inhibitory potency. The fourth result supports our study that the hydroxyl group at the third position of Ser plays an important role as a donor of a hydrogen bond. Finally, the fifth result suggests that there is a possibility for further improvement in the inhibitory activity and specificity by introducing an appropriate group to the N-terminal region for interaction with the PDZ domain. Indeed, the introduction of hydrophobic groups into the N-terminal region of the tripeptide showed a significant increase in the inhibitory activity against Fas/FAP-1 binding.

As already mentioned, the active conformation of the C-terminal tripeptide of Fas binds to the third PDZ domain of FAP-1 in a way similar to that observed in the case of PSD-95 and its binding peptide. So it would be effective to introduce several new groups into the N-terminal region of the peptide for developing a specific inhibitor for Fas/FAP-1 binding. Furthermore, it is necessary to restrict the conformation of the inhibitor to fit the PDZ cavity of FAP-1 and increase the inhibitory potency. We are now investigating these subjects in detail.

On the other hand, many PDZ domain-containing proteins were recently found to bind target proteins through interaction with the consensus C-terminal sequence motif (tS/T-X-V).[18,19] Therefore, **56** may bind to several PDZ domain-containing proteins related to the apoptotic signal transduction through Fas in addi-

Scheme 2

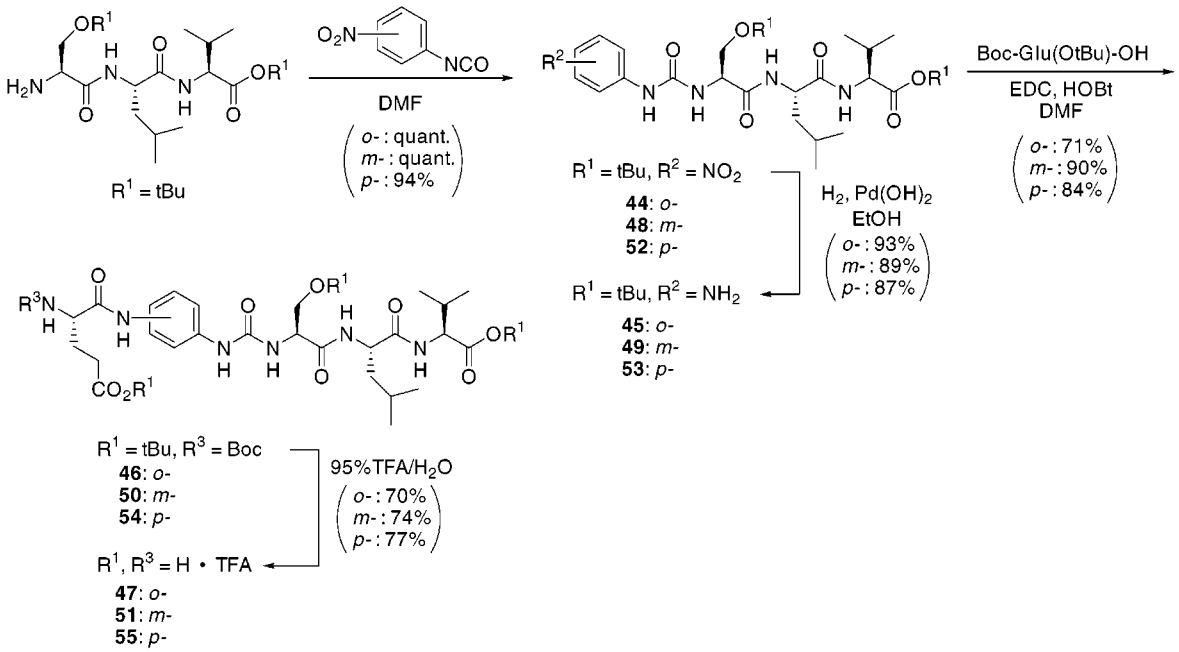
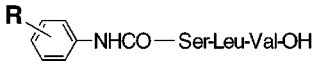


Table 8. Fas/FAP-1 Binding Inhibitory Potency of Peptides Introduced by a Glu Residue in the N-Terminal Region at a Concentration of 10 μM



Compound	R	% binding inhibition[a]
41	H	51.5
47	*o*-Glu	47.8
51	*m*-Glu	76.8
55	*p*-Glu	66.6

[a] See Table 1.

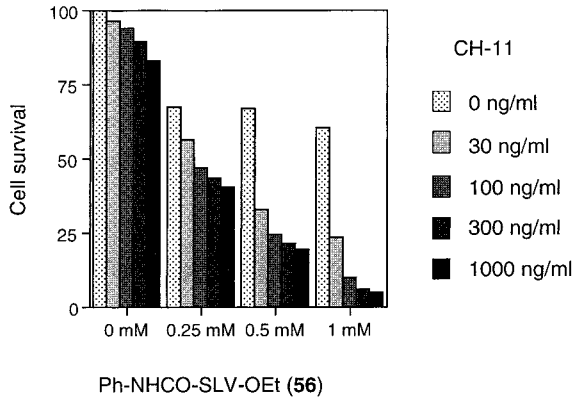


Figure 1. Fas-induced apoptosis that was dependent on the concentration of anti-Fas antibody (CH-11) during the existence of the tripeptide ester PhNHCO-SLV-OEt (**56**).

tion to FAP-1, and so further work needs to investigate whether FAP-1 is a main target of **56** and whether the inhibition of Fas/FAP-1 binding by **56** retrieves the apoptotic signal.

Experimental Section

Peptide Synthesis. Peptides were synthesized by the solid-phase method using an Advanced ChemTech (ACT) 357

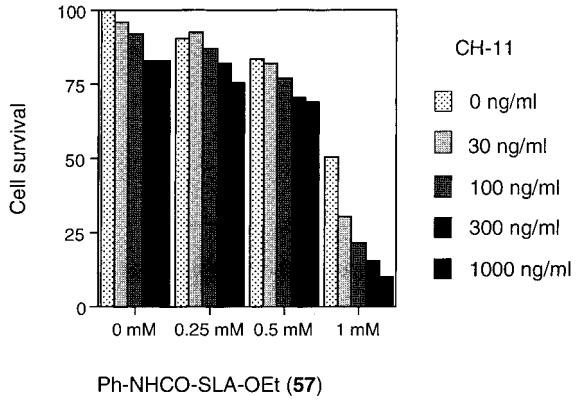


Figure 2. Fas-induced apoptosis that was dependent on the concentration of anti-Fas antibody (CH-11) during the existence of the tripeptide ester PhNHCO-SLV-OEt (**57**).

peptide synthesizer on a 0.045−0.2-mmol scale except for compounds **27**−**38** and **44**−**57**. *N*-Fmoc protection was used for all amino acids during the solid-phase synthesis. Peptides with a C-terminal carboxylate were synthesized by employing Wang resin (ACT or Watanabe Chemical Industries), and a peptide with a C-terminal amide was synthesized using Rink amide resin (ACT). Each amino acid was sequentially coupled to the peptide chain from the C- to N-terminus employing the *N*-hydroxybenzotriazole (HOBt) active ester coupling via diisopropylcarbodiimide (DIC) and subsequent 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) as the coupling agents. Removal of the *N*-Fmoc protective group was effected with 20% piperidine in DMF. After the last amino acid was coupled, the growing peptide on the resin was capped by acetic anhydride, appropriate acids, or isocyanates. Peptides were cleaved from the resin with simultaneous deprotection using TFA at room temperature for 2−3 h in the presence of 5% water or 2.5% water + 2.5% triisopropylsilane as the scavenger. The resin was filtered and washed twice with TFA. The filtrate was evaporated in vacuo, and the residue was triturated with ether. The crude peptide was collected by centrifuging, washed twice with ether, and dried in vacuo.

Purification and Characterization. The crude peptides were purified by preparative reverse-phase HPLC (Nomura Chemical Develosil ODS-HG-5, 20 × 250 mm) using various

Table 9. HPLC Analysis Data[a]

		0.1% TFA-0.1% TFA/CH_3CN			0.1% NH_4HCO_3-CH_3CN		
		gradient[b]	purity (%)	tR (min)	gradient[c]	purity (%)	tR (min)
1	Ac-SLV-OH	2	100.00	15.68	1	100.00	15.49
2	Ac-SL-(D)V-OH	2	99.21	15.49	1	98.39	15.46
3	Ac-S-(D)LV-OH	2	99.45	15.43	1	99.04	15.48
4	Ac-(D)SLV-OH	2	100.00	15.34	1	100.00	15.50
5	Ac-S-(NMe)LV-OH	2	99.35	17.04	1	99.28	16.53
6	Ac-(NMe)SLV-OH	2	98.38	16.85	1	98.26	16.58
7	Ac-S-Chg-V-OH	2	98.68	18.18	1	99.27	18.09
8	Ac-S-tBuGly-V-OH	2	99.04	14.37	1	99.14	13.97
9	Ac-S-Thy(OAc)-V-OH	2	99.16	19.71	1	98.86	19.31
10	Ac-S-Abu-V-OH	2	98.60	10.05	1	100.00	9.21
11	Ac-S-NorVal-V-OH	2	100.00	12.90	1	98.52	11.24
12	Ac-S-Phg-V-OH	2	99.41	14.98	1	99.29	12.99
13	Ac-S-βAla-V-OH	2	98.37	8.45	1	98.63	8.02
14	Ac-S-Sta-V-OH	2	100.00	15.17	1	98.67	14.39
15	Ac-S-Aib-V-OH	2	98.59	10.08	1	98.69	9.47
16	Ac-S-3-Abz-V-OH	2	99.17	17.19	1	99.57	14.87
17	Ac-S-4-Abz-V-OH	2	98.80	16.81	1	99.44	14.86
18	Ac-SL-Abu-OH	2	98.68	13.33	1	99.16	11.40
19	Ac-SL-NorVal-OH	2	98.93	16.36	1	100.00	14.05
20	Ac-SL-tBuGly-OH	2	100.00	17.95	1	100.00	17.51
21	Ac-SL-tBuAla-OH	2	100.00	21.28	1	100.00	19.14
22	Ac-SL-Chg-OH	2	98.19	21.76	1	100.00	20.07
23	Ac-Hse-LV-OH	2	100.00	15.44	1	98.54	14.31
24	Ac-cisHyp-LV-OH	2	100.00	15.89	1	100.00	14.36
25	Ac-transHyp-LV-OH	2	100.00	15.34	1	98.57	14.21
26	Ac-Sta-LV-OH	2	98.84	23.04	1	100.00	23.57
28	Ac-S(OAc)LV-OH	2	98.54	18.65	1	98.56	16.82
30	Ac-S(OBz)LV-OH	3	99.59	10.23	2	99.68	17.94
31	Ac-S(OBn)LV-OH	3	100.00	11,43	2	99.26	19.05
33	Ac-SLV-OMe	2	100.00	19.96	2	98.04	19.74
38	Ph-NHCO-SLV-Tet	3	98.95	9.14	2	98.18	17.42
39	Bz-SLV-OH	2	98.86	23.21	1	99.26	20.71
40	Cyh-CO-SLV-OH	2	98.23	25.70	1	98.67	23.05
41	Ph-NHCO-SLV-OH	3	99.44	8.81	2	99.42	17.96
42	Cyh-NHCO-SLV-OH	3	98.40	9.76	2	98.63	19.43
43	Ac-EIQSLV-OH	2	98.93	20.52	1	98.30	16.86
47	*o*-E-Ph-NHCO-SLV-OH	2	98.98	18.33	1	99.74	16.90
51	*m*-E-Ph-NHCO-SLV-OH	2	99.88	17.63	1	100.00	15.57
55	*p*-E-Ph-NHCO-SLV-OH	2	99.72	16.11	1	99.73	13.55
56	Ph-NHCO-SLV-OEt	3	99.75	15.14	1	98.43	15.35
57	Ph-NHCO-SLA-OEt	3	98.43	10.50	3	98.94	10.91

[a] HPLC on a column of Develosil ODS-HG-5 (4.6 × 15 mm). [b] 2: B, 5% → 53% in 32 min. 3: B, 30% → 73% in 32 min. A: 0.1% TFA. B: 0.1% TFA in CH_3CN. [c] 1: B, 0% → 32% in 32 min. 2: B, 5% → 53% in 32 min. 3: B, 30% → 73% in 32 min. A: 0.1% NH_4HCO_3. B: CH_3CN.

linear gradients of water containing 0.1% TFA and acetonitrile containing 0.1% TFA at a flow rate of 10 mL/min for elution. The fractions containing the product were pooled and lyophilized. The peptides were characterized by NMR and MS. The purity was further checked by analytical reverse-phase HPLC (Nomura Chemical Develosil ODS-HG-5, 4.6 × 150 mm) using various linear gradients of water containing 0.1% TFA and acetonitrile containing 0.1% TFA and also 0.1% aqueous ammonium hydrocarbonate−acetonitrile solvent systems at a flow rate of 1 mL/min with UV detection at 220 nm. All compounds were found to be homogeneous in both HPLC systems (Table 9). Total yield of peptides were different from one another; for example, compound **41** was obtained in 15% yield after HPLC purification (from Fmoc-Val-Wang resin).

Ac-Ser-Leu-Val-OH (1): FAB-MS ($M^+ + H$) 360; ^{1}H NMR (500 MHz, CD_3OD) δ 4.48 (dd, J = 10.4, 4.9 Hz, 1H), 4.44 (t, J = 6.1 Hz, 1H), 4.29 (d, J = 6.1 Hz, 1H), 3.76 (m, 2H), 2.15 (m, 1H), 1.99 (s, 3H), 1.73−1.55 (m, 3H), 0.95 (m, 12H).

Ac-Ser-Leu-D-Val-OH (2): FD-MS ($M^+ + H$) 360; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (br, 1H), 7.61 (br, 1H), 4.67 (m, 2H), 4.37 (m, 1H), 3.84 (br, 1H), 3.76 (m, 1H), 3.61 (m, 1H), 2.18 (m, 1H), 2.04 (s, 3H), 1.72−1.56 (m, 3H), 1.00−0.90 (m, 12H)

Ac-Ser-D-Leu-Val-OH (3): FD-MS ($M^+ + H$) 360; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$) δ 4.52 (dd, J = 8.8, 5.9 Hz, 1H), 4.47 (m, 1H), 4.36 (d, J = 5.1 Hz, 1H), 4.00 (dd, J = 11.5, 3.7 Hz, 1H), 3.66 (dd, J = 11.5, 4.6 Hz, 1H), 2.22 (m, 1H), 2.06 (s, 3H), 1.73−1.54 (m, 3H), 0.99−0.91 (m, 12H).

Ac-D-Ser-Leu-Val-OH (4): FAB-MS ($M^+ + H$) 360; ^{1}H NMR (500 MHz, CD_3OD) δ 4.47 (dd, J = 9.2, 5.5 Hz, 1H), 4.40 (t, J = 5.5 Hz, 1H), 4.29 (d, J = 5.5 Hz, 1H), 3.76 (m, 2H), 2.16 (m, 1H), 2.00 (s, 3H), 1.68 (m, 2H), 1.62 (m, 2H), 0.95 (m, 12H).

Ac-Ser-NMe-Leu-Val-OH (5): FAB-MS ($M^+ + Na$) 396; ^{1}H NMR (500 MHz, CD_3OD) δ 5.25 (dd, J = 9.8, 5.5 Hz, 1H), 4.97 (m, 1H), 4.28 (m, 1H), 3.75 (d, J = 7.3 Hz, 2H), 3.11 (s, 3H), 2.15 (m, 1H), 1.97 (s, 3H), 1.77−1.66 (m, 2H), 1.53 (m, 1H), 0.96−0.89 (m, 12H).

Ac-NMe-Ser-Leu-Val-OH (6): FAB-MS ($M^+ + H$) 374; ^{1}H NMR (500 MHz, CD_3OD) δ 4.60 (m, 1H), 4.45 (m, 1H), 4.27 (m, 1H), 3.97 (m, 1H), 3.88 (m, 1H), 2.65 (s, 3H), 2.18 (m, 1H), 2.15 (s, 3H), 1.78−1.60 (m, 3H), 0.95 (m, 12H).

Ac-Ser-Chg-Val-OH (7): FAB-MS ($M^+ + H$) 386; ^{1}H NMR (500 MHz, CD_3OD) δ 4.48 (m, 1H), 4.31 (d, J = 7.3 Hz, 1H), 4.28 (d, J = 5.5 Hz, 1H), 3.74 (m, 2H), 2.15 (m, 1H), 2.00 (s, 3H), 1.81−1.65 (m, 6H), 1.26−1.07 (m, 5H), 0.96 (d, J = 6.7 Hz, 6H).

Ac-Ser-tBuGly-Val-OH (8): FAB-MS ($M^+ + H$) 360; ^{1}H NMR (500 MHz, CD_3OD) δ 4.48 (t, J = 5.5 Hz, 1H), 4.37 (m, 1H), 4.29 (m, 1H), 3.75 (d, J = 5.5 Hz, 2H), 2.14 (m, 1H), 2.01 (s, 3H), 1.02 (s, 9H), 0.96 (d, J = 6.7 Hz, 6H).

Ac-Ser-Thy(OAc)-Val-OH (9): FAB-MS ($M^+ + H$) 544; ^{1}H NMR (500 MHz, CD_3OD) δ 7.24 (d, J = 8.6 Hz, 2H), 7.06 (d, J = 9.2 Hz, 2H), 6.97 (d, J = 9.2 Hz, 2H), 6.89 (d, J = 8.6 Hz, 2H), 4.70 (m, 1H), 4.40 (m, 1H), 4.29 (m, 1H), 3.70 (m, 2H), 3.17 (dd, J = 14.0, 4.9 Hz, 1H), 2.93 (dd, J = 14.0, 8.5 Hz, 1H), 2.26 (s, 3H), 2.15 (m, 1H), 1.96 (s, 3H), 0.96 (m, 6H).

Ac-Ser-Abu-Val-OH (10): FAB-MS ($M^+ + H$) 322; ^{1}H NMR (500 MHz, CD_3OD) δ 4.45 (m, 1H), 4.35 (m, 1H), 4.30 (m, 1H), 3.78 (dd, J = 11.0, 5.5 Hz, 1H), 3.73 (dd, J = 11.0, 6.1 Hz, 1H), 2.16 (m, 1H), 2.00 (s, 3H), 1.89 (m, 1H), 1.68 (m, 1H), 0.97 (m, 9H).

Ac-Ser-NorVal-Val-OH (11): FAB-MS ($M^+ + H$) 346; ^{1}H NMR (500 MHz, CD_3OD) δ 4.44 (m, 2H), 4.29 (m, 1H), 3.77 (dd, J = 11.0, 5.5 Hz, 1H), 3.73 (dd, J = 11.0, 6.1 Hz, 1H), 2.16 (m, 1H), 2.00 (s, 3H), 1.82 (m, 1H), 1.64 (m, 1H), 1.41 (m, 2H), 0.95 (m, 9H).

Ac-Ser-Phg-Val-OH (12): FAB-MS ($M^+ + H$) 380; ^{1}H NMR (500 MHz, CD_3OD) δ 7.45 (d, J = 7.3 Hz, 2H), 7.33 (m, 3H), 5.60 (s, 1H), 4.50 (m, 1H), 4.33 (m, 1H), 3.78 (m, 2H), 2.17 (m, 1H), 2.00 (s, 3H), 0.98 (d, J = 6.7 Hz, 6H).

Ac-Ser-β-Ala-Val-OH (13): FAB-MS ($M^+ + H$) 318; ^{1}H NMR (500 MHz, CD_3OD) δ 4.36 (dd, J = 5.5, 4.9 Hz, 1H), 4.32 (d, J = 6.1 Hz, 1H), 3.78 (dd, J = 11.0, 5.5 Hz, 1H), 3.73 (dd, J = 11.0, 4.9 Hz, 1H), 3.50 (m, 1H), 3.45 (m, 1H), 2.49 (m, 2H), 2.18 (m, 2H), 2.02 (s, 3H), 0.98 (m, 6H).

Ac-Ser-Sta-Val-OH (14): FAB-MS ($M^+ + H$) 404; ^{1}H NMR (500 MHz, CD_3OD) δ 4.40 (t, J = 5.5 Hz, 1H), 4.31 (d, J = 5.5 Hz, 1H), 4.02−3.94 (m, 2H), 3.79 (d, J = 5.5 Hz, 2H), 2.38 (m, 2H), 2.18 (m, 1H), 2.02, (s, 3H), 1.67−1.55 (m, 2H), 1.32 (m, 1H), 0.98 (d, J = 7.3 Hz, 6H), 0.93 (d, J = 6.1 Hz, 3H), 0.90 (d, J = 6.7 Hz, 3H).

Ac-Ser-Aib-Val-OH (15): FAB-MS ($M^+ + H$) 322; ^{1}H NMR (500 MHz, CD_3OD) δ 4.36 (m, 1H), 4.28 (d, J = 5.5 Hz, 1H), 3.75 (m, 2H), 2.14 (m, 1H), 1.99 (s, 3H), 1.49 (d, J = 6.1 Hz, 6H), 0.95 (d, J = 6.7 Hz, 3H), 0.91 (d, J = 6.7 Hz, 3H).

Ac-Ser-3-Abz-Val-OH (16): FAB-MS ($M^+ + H$) 366; ^{1}H NMR (500 MHz, CD_3OD) δ 7.97 (s, 1H), 7.72 (m, 1H), 7.75 (d, J = 7.9 Hz, 1H), 7.41 (t, J = 7.9 Hz, 1H), 4.50 (m, 2H), 3.83 (m, 2H), 2.28 (m, 1H), 2.14 (s, 3H), 1.05 (d, J = 6.7 Hz, 6H).

Ac-Ser-4-Abz-Val-OH (17): FAB-MS ($M^+ + H$) 366; ^{1}H NMR (500 MHz, CD_3OD) δ 7.82 (d, J = 8.5 Hz, 2H), 7.67 (d, J = 8.5 Hz, 2H), 4.49 (m, 2H), 3.87 (m, 1H), 3.83 (m, 1H), 2.27 (m, 1H), 2.14 (s, 3H), 1.04 (d, J = 6.7 Hz, 6H).

Ac-Ser-Leu-Abu-OH (18): FAB-MS ($M^+ + H$) 346; ^{1}H NMR (500 MHz, CD_3OD) δ 4.48 (dd, J = 10.4, 4.9 Hz, 1H), 4.43 (t, J = 6.1 Hz, 1H), 4.26 (dd, J = 8.5, 4.9 Hz, 1H), 3.78 (dd, J = 10.4, 6.1 Hz, 1H), 3.73 (dd, J = 10.4, 6.1 Hz, 1H), 2.00 (s, 3H), 1.91−1.84 (m, 1H), 1.77−1.56 (m, 4H), 0.99−0.92 (m, 9H).

Ac-Ser-Leu-NorVal-OH (19): FAB-MS ($M^+ + H$) 360; ^{1}H NMR (500 MHz, CD_3OD) δ 4.48 (dd, J = 10.4, 4.9 Hz, 1H), 4.43 (t, J = 6.1 Hz, 1H), 4.33 (dd, J = 9.2, 4.9 Hz, 1H), 3.78 (dd, J = 11.0, 5.5 Hz, 1H), 3.73 (dd, J = 11.0, 6.1 Hz, 1H), 2.00 (S, 3H), 1.84−1.56 (m, 5H), 1,45−1.36 (m, 2H), 0.97−0.92 (m, 9H).

Ac-Ser-Leu-tBuGly-OH (20): FAB-MS ($M^+ + H$) 374; ^{1}H NMR (500 MHz, CD_3OD) δ 4.50 (m, 1H), 4.45 (t, J = 6.1 Hz, 1H), 4.29 (m, 1H), 3.75 (m, 2H), 2.00 (s, 3H), 1.75−1.57 (m, 3H), 1.01 (s, 9H), 0.96 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 6.1 Hz, 3H).

Ac-Ser-Leu-tBuAla-OH (21): FAB-MS ($M^+ + H$) 388; ^{1}H NMR (500 MHz, CD_3OD) δ 4.48−4.42 (m, 3H), 3.74 (m, 2H), 2.00 (s, 3H), 1.80−1.54 (m, 5H), 0.95 (s, 9H), 0.94 (m, 6H).

Ac-Ser-Leu-Chg-OH (22): FAB-MS ($M^+ + H$) 400; ^{1}H NMR (500 MHz, CD_3OD) δ 4.49 (dd, J = 10.4, 4.9 Hz, 1H), 4.44 (t, J = 6.1 Hz, 1H), 4.28 (d, J = 6.1 Hz, 1H), 3.75 (m, 2H), 2.00 (s, 3H), 1.82−1.56 (m, 9H), 1.30−1.09 (m, 5H), 0.96 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 6.1 Hz, 3H).

Ac-Hse-Leu-Val-OH (23): FAB-MS ($M^+ + H$) 374; ^{1}H NMR (500 MHz, CD_3OD) δ 4.46 (m, 2H), 4.30 (d, J = 5.5 Hz, 1H), 3.62 (m, 2H), 2.17 (m, 1H), 2.00 (m, 1H), 1.97 (s, 3H), 1.72 (m, 1H), 1.60 (m, 2H), 0.96 (d, J = 6.7 Hz, 9H), 0.92 (d, J = 6.7 Hz, 3H).

Ac-cisHyp-Leu-Val-OH (24): FAB-MS ($M^+ + H$) 386; ^{1}H NMR (500 MHz, CD_3OD) δ 4.45 (m, 2H), 4.37 (m, 1H), 4.28 (d, J = 6.1 Hz, 1H), 3.75 (dd, J = 11.0, 4.9 Hz, 1H), 3.57 (m, 1H), 2.38 (m, 1H), 2.17 (m, 1H), 2.08 (s, 3H), 2.00 (m, 1H), 1.77 (m, 1H), 1.70−1.59 (m, 2H), 0.98−0.91 (m, 12H).

Ac-transHyp-Leu-Val-OH (25): FAB-MS ($M^+ + H$) 386; ^{1}H NMR (500 MHz, CD_3OD) δ 4.58−4.43 (m, 3H), 4.30 (d, J = 5.5 Hz, 1H), 3.75 (dd, J = 11.0, 4.3 Hz, 1H), 3.51 (m, 1H), 2.38−2.00 (m, 3H), 2.07 (s, 3H), 1.78 (m, 1H), 1.62 (m, 2H), 0.99−0.93 (m, 12H).

Ac-Sta-Leu-Val-OH (26): FAB-MS ($M^+ + H$) 430; ^{1}H NMR (500 MHz, CD_3OD) δ 4.46 (dd, J = 9.2, 6.1 Hz, 1H), 4.30 (d, J = 6.1 Hz, 1H), 3.97 (m, 2H), 2.32 (m, 2H), 2.17 (m, 1H), 2.03 (s, 3H), 1.72 (m, 1H), 1.62−1.49 (m, 4H), 1.33 (m, 1H), 0.97−0.90 (m, 18H).

Ac-Ser(OAc)-Leu-Val-OtBu (27). To a solution of Ac-Ser-Leu-Val-OtBu (50 mg, 0.12 mmol) in CH_2Cl_2 (1 mL) were added Ac_2O (15 mg, 0.15 mmol) in CH_2Cl_2 (0.5 mL) and Et_3N (0.03 mL, 0.22 mmol). The mixture was stirred for 6 h. Additional Ac_2O (7.5 mg, 0.074 mmol) in CH_2Cl_2 (1 mL), Et_3N (0.03 mL, 0.22 mmol), and CH_2Cl_2 (1 mL) were added. The mixture was stirred for an additional 16 h and partitioned between water and ethyl acetate. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was adsorbed on a plate of silica gel, and the plate was developed with 10% methanol/chloroform to afford 35 mg (64%) of **27**: FD-MS ($M^+ + H$) 458; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76 (br, 1H), 6.66 (br, 1H), 6.60 (br, 1H), 4.75 (m, 1H), 4.53 (m, 1H), 4.41 (dd, J = 9.2, 4.9 Hz, 1H), 4.31 (dd, J = 11.0, 5.5 Hz, 1H), 4.18 (dd, J = 11.0, 6.1 Hz, 1H), 2.15 (m, 1H), 2.07 (s, 3H), 2.05 (s, 3H), 1.70−1.53 (m, 3H), 1.47 (s, 9H), 0.94 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 6.7 Hz, 3H), 0.88 (d, J = 6.7 Hz, 3H).

Ac-Ser(OAc)-Leu-Val-OH (28). A solution of **27** (33 mg, 0.072 mmol) in trifluoroacetic acid (TFA; 1 mL) and water (0.025 mL) was stirred for 1 h and concentrated. The residue was dissolved in ethyl acetate, and the solution was washed with water and brine. The organic layer was dried (Na_2SO_4)

and concentrated. The residue was washed with Et_2O to completely remove TFA and dried under vacuum to afford 19 mg (66%) of **28**: FAB-MS ($M^+ + H$) 402; ^1H NMR (500 MHz, $CDCl_3$) δ 7.58 (br, 1H), 7.11 (br, 1H), 6.95 (br, 1H), 4.74 (m, 1H), 4.50 (m, 1H), 4.30 (dd, J = 11.0, 5.5 Hz, 1H), 4.25 (dd, J = 11.0, 5.5 Hz, 1H), 2.20 (m, 1H), 2.05 (s, 3H), 2.04 (s, 3H), 1.66−1.55 (m, 3H), 0.94 (m, 12H).

Ac-Ser(OBz)-Leu-Val-OtBu (29). To a solution of Ac-Ser-Leu-Val-OtBu (50 mg, 0.12 mmol) in THF (6 mL) were added benzoyl chloride (0.017 mL, 0.15 mmol) and Et_3N (0.03 mL, 0.22 mmol). The mixture was then stirred for 18 h. Additional benzoyl chloride (0.009 mL, 0.078 mmol) and Et_3N (0.02 mL, 0.14 mmol) were added, and the mixture was stirred for an additional 3 h. To the mixture was added 4-(dimethylamino)-pyridine (7 mg, 0.06 mmol). The mixture was stirred for 1.5 h and partitioned between water and ethyl acetate. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was adsorbed on a plate of silica gel, and the plate was developed with 10% methanol/chloroform to afford 36 mg (60%) of **29**: FD-MS ($M^+ + H$) 520; ^1H NMR (500 MHz, $CDCl_3$) δ 8.05 (d, J = 7.3 Hz, 2H), 7.57 (t, J = 7.3 Hz, 1H), 7.44 (t, J = 7.3 Hz, 2H), 6.83 (d, J = 7.9 Hz, 1H), 6.65 (d, J = 7.3 Hz, 1H), 6.56 (d, J = 8.5 Hz, 1H), 4.87 (m, 1H), 4.63 (dd, J = 11.6, 6.1 Hz, 1H), 4.51 (m, 1H), 4.50 (dd, J = 11.6, 5.5 Hz, 1H), 4.35 (dd, J = 8.5, 4.3 Hz, 1H), 2.07 (m, 1H), 2.05 (s, 3H), 1.72−1.55 (m, 3H), 1.46 (s, 9H), 0.90 (d, J = 6.1 Hz, 6H), 0.83 (d, J = 6.1 Hz, 3H), 0.81 (d, J = 6.7 Hz, 3H).

Ac-Ser(OBz)-Leu-Val-OH (30). The titled compound was prepared in 70% yield according to the procedure as described for **28** using **29** instead of **27**: FAB-MS ($M^+ + H$) 464; ^1H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, J = 7.9 Hz, 2H), 7.75 (br, 1H), 7.57 (t, J = 7.3 Hz, 1H), 7.44 (t, J = 7.3 Hz, 1H), 7.13 (br, 1H), 4.87 (t, J = 5.5 Hz, 1H), 4.54 (t, J = 4.9 Hz, 1H), 4.50 (m, 2H), 4.37 (d, J = 4.9 Hz, 1H), 2.12 (m, 1H), 2.04 (s, 3H), 1.66−1.53 (m, 3H), 0.90 (d, J = 6.7 Hz, 3H), 0.89 (d, J = 6.7 Hz, 3H), 0.85 (d, J = 6.7 Hz, 6H).

Ac-Ser(OBn)-Leu-Val-OH (31). A solution of Ac-Ser(OBn)-Leu-Val-OtBu (100 mg, 0.198 mmol) in TFA (2 mL) and water (0.05 mL) was stirred for 2.5 h and concentrated. The residue was washed with Et_2O and dried under vacuum to afford 83 mg (93%) of **31**: FD-MS (M^+) 449; ^1H NMR (500 MHz, CD_3OD) δ 7.35−7.25 (m, 5H), 4.59−4.50 (overlapped, 4H), 4.27 (m, 1H), 3.74 (dd, J = 9.8, 5.5 Hz, 1H), 3.70 (dd, J = 9.8, 5.5 Hz, 1H), 2.13 (m, 1H), 2.00 (s, 3H), 1.68 (m, 1H), 1.59 (m, 2H), 0.93−0.90 (m, 12H).

H-Ser(OtBu)-Leu-Val-OMe (32). A solution of Fmoc-Ser-(OtBu)-Leu-Val-OMe (200 mg, 0.33 mmol) in 20% piperidine/DMF (1 mL) was stirred for 25 min. After water was added, the mixture was extracted with ethyl acetate. The extract was washed with brine (3×), dried (Na_2SO_4), and concentrated. The residue was chromatographed (silica gel, hexane/ethyl acetate = 9/1 → $CHCl_3$/MeOH = 100/1 → 100/2 → 100/5) to afford 128 mg (quantitative) of **32**: FAB-MS ($M^+ + H$) 388; ^1H NMR (90 MHz, $CDCl_3$) δ 6.64 (m, 1H), 4.50 (m, 2H), 3.73 (s, 3H), 3.55 (m, 3H), 2.04 (m, 1H), 1.69−1.54 (m, 3H), 1.18 (s, 9H), 0.93 (m, 12H).

Ac-Ser-Leu-Val-OMe (33). A solution of **32** (135 mg, 0.35 mmol), Ac_2O (0.04 mL, 0.42 mmol), and Et_3N (0.06 mL, 0.43 mmol) in CH_2Cl_2 (2 mL) was stirred for 15 h at room temperature under argon. The mixture was partitioned between $CHCl_3$ and water. The organic layer was dried (Na_2SO_4) and concentrated. The residue was dissolved in TFA (2 mL) and water (0.1 mL). The solution was stirred for 35 min, diluted with ice-cooled water, and extracted with ethyl acetate. The extract was washed with saturated aqueous $NaHCO_3$ and brine. Concentrating under vacuum gave a syrup, which was washed with Et_2O to afford 31 mg (24%) of **33**: FAB-MS ($M^+ + H$) 374; ^1H NMR (500 MHz, $CDCl_3$) δ 6.99 (br, 1H), 6.84 (br, 1H), 6.59 (br, 1H), 4.56 (m, 2H), 4.46 (m, 1H), 4.02 (m, 1H), 3.74 (s, 3H), 3.61 (dd, J = 11.0, 7.3 Hz, 1H), 2.15 (m, 1H), 2.04 (s, 3H), 1.75−1.57 (m, 3H), 0.92 (m, 12H).

Z-Val-NH_2 (34). Ammonia gas was bubbled into a solution of Z-Val-OSu (1.0 g, 2.87 mmol) in $CHCl_3$ (10 mL) at 0 °C for 15 min with stirring. The mixture was stirred for 1 h at room temperature and concentrated. The residue was washed with Et_2O and partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4) and concentrated to afford 181 mg (25%) of **34**: FD-MS (M^+) 250; ^1H NMR (500 MHz, $CDCl_3$) δ 7.40−7.30 (m, 5H), 5.78 (br, 1H), 5.39 (br, 1H), 5.30 (br, 1H), 5.12 (s, 2H), 4.03 (t, J = 7.3 Hz, 1H), 2.16 (m, 1H), 1.00 (d, J = 6.7 Hz, 3H), 0.95 (d, J = 6.7 Hz, 3H).

Z-Val-CN (35). To a suspension of **34** (170 mg, 0.68 mmol) in pyridine (3 mL) was added $POCl_3$ (0.175 mL, 1.88 mmol) at −15 °C. The mixture was stirred for 1.5 h at the same temperature. After water was added, the solution was extracted with ethyl acetate. The extract was washed with water, 1 N HCl, saturated aqueous $NaHCO_3$, and brine and was dried (Na_2SO_4) and concentrated to afford **35** (158 mg, quantitative) which was used without further purification: FD-MS (M^+) 232; ^1H NMR (500 MHz, $CDCl_3$) δ 7.40−7.30 (m, 5H), 5.14 (s, 2H), 4.51 (dd, J = 7.3, 7.3 Hz, 1H), 2.04 (m, 1H), 1.09 (d, J = 6.7 Hz, 3H), 1.07 (d, J = 7.3 Hz, 3H).

Z-Val-Tet (36). A solution of **35** (140 mg, 0.60 mmol), NaN_3 (41 mg, 0.63 mmol), and NH_4Cl (36 mg, 0.67 mmol) in DMF (2 mL) was stirred for 6 h at 105 °C. Additional NaN_3 (20 mg, 0.30 mmol) and NH_4Cl (20 mg, 0.37 mmol) were added, and the mixture was stirred for 16 h at 105 °C. Additional NaN_3 (41 mg, 0.63 mmol) and NH_4Cl (36 mg, 0.67 mmol) were added, and the mixture was stirred for 10 h at 120 °C. After ice chips and 1 N HCl were added, the solution was extracted with ethyl acetate. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was washed with Et_2O/hexane to afford **36** (78 mg, 47%) which was used without further purification: FD-MS (M^+) 275; ^1H NMR (500 MHz, $CDCl_3$) δ 13.68 (br, 1H), 7.40−7.30 (m, 5H), 5.60 (d, J = 7.9 Hz, 1H), 5.14 (d, J = 12.2 Hz, 1H), 5.11 (d, J = 12.2 Hz, 1H), 4.78 (br, 1H), 2.48 (m, 1H), 1.07 (d, J = 6.1 Hz, 3H), 0.92 (d, J = 7.3 Hz, 3H).

Ac-Ser(OtBu)-Leu-Val-Tet (37). Palladium on carbon (10%, 20 mg) was added to a solution of **36** (200 mg, 0.72 mmol) in MeOH (15 mL). A balloon containing hydrogen gas was attached to the flask, and the mixture was stirred for 2 h. The mixture was filtered through Celite, and the filtrate was concentrated. The residue was dissolved in DMF (15 mL). To the solution were added Ac-Ser(OtBu)-Leu-OH (230 mg, 0.72 mmol), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC; 150 mg, 0.78 mmol), and HOBt (110 mg, 0.81 mmol), and the mixture was stirred for 8 h. After water was added, the solution was extracted with ethyl acetate. The extract was washed with brine (3×), dried (Na_2SO_4), and concentrated. The residue was washed with Et_2O to afford **37** (243 mg, 77%) which was used without further purification: FD-MS ($M^+ + H$) 440; ^1H NMR (500 MHz, DMSO-d_6) δ 16.18 (br, 1H), 8.33 (d, J = 8.5 Hz, 1H), 7.95 (d, J = 7.9 Hz, 1H), 7.85 (d, J = 7.9 Hz, 1H), 4.93 (t, J = 7.9 Hz, 1H), 4.40 (m, 1H), 4.29 (m, 1H), 3.45 (dd, J = 9.2, 5.5 Hz, 1H), 3.40 (dd, J = 9.2, 5.5 Hz, 1H), 2.20 (m, 1H), 1.89 (s, 3H), 1.60−1.36 (m, 3H), 1.09 (s, 9H), 0.91 (d, J = 6.7 Hz, 3H), 0.83 (d, J = 6.1 Hz, 3H), 0.81 (d, J = 6.1 Hz, 3H), 0.75 (d, J = 6.7 Hz, 3H).

Ac-Ser-Leu-Val-Tet (38). A solution of **37** (100 mg, 0.228 mmol) in TFA (1.90 mL) and water (0.1 mL) was stirred for 1.5 h. After the solvent was removed under vacuum, the residue was subjected to preparative reverse-phase HPLC (Nomura Chemical Develosil ODS-HG-5, 20 × 250 mm) using a linear gradient of (A) water containing 0.1% TFA and (B) acetonitrile containing 0.1% TFA (5−70% B, in 40 min) at a flow rate of 10 mL/min. Fractions containing the major peak were pooled and lyophilized to yield 19 mg (22%) of **38**: FD-MS ($M^+ + H$) 384; ^1H NMR (500 MHz, DMSO-d_6) δ 16.15 (br, 1H), 8.32 (d, J = 8.6 Hz, 1H), 8.02 (d, J = 7.9 Hz, 1H), 7.98 (d, J = 7.9 Hz, 1H), 4.92 (t, J = 7.9 Hz, 1H), 4.30 (m, 2H), 3.53 (m, 2H), 2.20 (m, 1H), 1.86 (s, 3H), 1.57 (m, 1H), 1.46 (m, 2H), 0.91 (d, J = 6.7 Hz, 3H), 0.85 (d, J = 6.7 Hz, 3H), 0.81 (d, J = 6.7 Hz, 3H), 0.75 (d, J = 6.7 Hz, 3H).

Bz-Ser-Leu-Val-OH (39): FAB-MS ($M^+ + H$) 422; ^1H NMR (500 MHz, CD_3OD) δ 7.87 (d, J = 7.3 Hz, 2H), 7.53 (m, 1H), 7.46 (m, 2H), 4.68 (t, J = 6.1 Hz, 1H), 4.52 (dd, J = 9.8, 4.9

Hz, 1H), 4.29 (d, J = 5.5 Hz, 1H), 3.90 (m, 2H), 2.16 (m, 1H), 1.75 (m, 1H), 1.65 (m, 2H), 0.95 (m, 12H).

Cyh-CO-Ser-Leu-Val-OH (40): FAB-MS ($M^+ + H$) 428; ^{1}H NMR (500 MHz, CD_3OD) δ 4.44 (m, 2H), 4.29 (d, J = 5.5 Hz, 1H), 3.73 (m, 2H), 2.24 (m, 1H), 2.15 (m, 1H), 1.78 (m, 4H), 1.66 (m, 4H), 1.41 (m, 2H), 1.28 (m, 3H), 0.94 (m, 12H).

Ph-NHCO-Ser-Leu-Val-OH (41): FAB-MS ($M^+ + H$) 437; ^{1}H NMR (500 MHz, CD_3OD) δ 7.35 (d, J = 8.5 Hz, 2H), 7.24 (m, 2H), 6.97 (m, 1H), 4.50 (dd, J = 9.8, 4.9 Hz, 1H), 4.40 (dd, J = 6.1, 4.9 Hz, 1H), 4.29 (d, J = 5.5 Hz, 1H), 3.86 (dd, J = 10.4, 4.9 Hz, 1H), 3.74 (dd, J = 10.4, 6.1 Hz, 1H), 2.17 (m, 1H), 1.77−1.60 (m, 3H), 0.95 (m, 12H).

Cyh-NHCO-Ser-Leu-Val-OH (42): FAB-MS ($M^+ + H$) 443; ^{1}H NMR (500 MHz, CD_3OD) δ 4.48 (m, 1H), 4.29 (m, 2H), 3.78 (dd, J = 10.4, 5.5 Hz, 1H), 3.66 (m, 1H), 3.47 (m, 1H), 2.16 (m, 1H), 1.85 (m, 2H), 1.71 (m, 3H), 1.60 (m, 2H), 1.34 (m, 3H), 1.17 (m, 3H), 0.95 (m, 12H).

Ac-Glu-Ile-Gln-Ser-Leu-Val-OH (43): FAB-MS ($M^+ +$ Na) 753; ^{1}H NMR (500 MHz, CD_3OD) δ 4.48 (m, 1H), 4.40 (m, 2H), 4.29 (m, 2H), 4.13 (d, J = 7.3 Hz, 1H), 3.86 (dd, J = 11.0, 5.5 Hz, 1H), 3.78 (dd, J = 11.0, 5.5 Hz, 1H), 2.41−2.33 (m, 4H), 2.18−2.09 (m, 3H), 1.99 (s, 3H), 1.92−1.86 (m, 2H), 1.72 (m, 1H), 1.66 (m, 3H), 1.56 (m, 1H), 1.22 (m, 1H), 0.97−0.90 (m, 18H).

***o*-NO_2-Ph-NHCO-Ser(OtBu)-Leu-Val-OtBu (44).** To a solution of H-Ser(OtBu)-Leu-Val-OtBu (500 mg, 1.16 mmol) in DMF (3 mL) was added 2-nitrophenyl isocyanate (210 mg, 1.28 mmol). The mixture was stirred for 10 min at room temperature and cooled to 0 °C. The mixture was partitioned between water and ethyl acetate. The organic layer was washed with water and brine and dried ($MgSO_4$). The solvent was removed under vacuum to afford **44** (691 mg, 100%) which was used without further purification: FD-MS (M^+) 593; ^{1}H NMR (500 MHz, CD_3OD) δ 8.19 (d, J = 8.5 Hz, 1H), 8.02 (d, J = 6.7 Hz, 1H), 7.63 (t, J = 7.3 Hz, 1H), 7.16 (t, J = 7.3 Hz, 1H), 4.47 (t, J = 7.3 Hz, 1H), 4.29 (t, J = 4.9 Hz, 1H), 3.99 (d, J = 6.7 Hz, 1H), 3.56 (dd, J = 9.2, 5.5 Hz, 1H), 3.45 (m, 1H), 2.00 (m, 1H), 1.63 (m, 1H), 1.48 (t, J = 7.3 Hz, 2H), 1.39 (s, 9H), 1.12 (s, 9H), 0.89 (d, J = 6.7 Hz, 3H), 0.86 (d, J = 6.7 Hz, 3H), 0.85 (d, J = 6.7 Hz, 6H).

***o*-NH_2-Ph-NHCO-Ser(OtBu)-Leu-Val-OtBu (45).** Palladium hydroxide on carbon (18%, 120 mg) was added to a solution of **44** (667 mg, 1.14 mmol) in EtOH (50 mL). A balloon containing hydrogen was attached to the flask, and the mixture was stirred for 16 h. The mixture was filtered through Celite, and the filtrate was concentrated under vacuum. The residue was chromatographed (silica gel, 25:1 $CHCl_3$:MeOH). Fractions containing the product were pooled and concentrated to afford 600 mg (93%) of **45**: FD-MS (M^+) 563; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.99 (d, J = 7.9 Hz, 1H), 7.96 (s, 1H), 7.85 (d, J = 6.7 Hz, 1H), 7.29 (d, J = 6.7 Hz, 1H), 6.79 (t, J = 7.9 Hz, 1H), 6.68 (d, J = 6.7 Hz, 1H), 6.51 (t, J = 7.9 Hz, 1H), 6.41 (d, J = 7.9 Hz, 1H), 4.74 (s, 2H), 4.51 (q, J = 7.9 Hz, 1H), 4.24 (m, 1H), 3.99 (dd, J = 7.9, 6.1 Hz, 1H), 3.60 (dd, J = 9.2, 4.3 Hz, 1H), 3.42 (dd, J = 9.2, 4.9 Hz, 1H), 2.00 (m, 1H), 1.63 (m, 1H), 1.47 (t, J = 7.3 Hz, 2H), 1.39 (s, 9H), 1.10 (s, 9H), 0.87 (m, 12H).

***o*-(Boc-Glu(OtBu)-NH)-Ph-NHCO-Ser(OtBu)-Leu-Val-OtBu (46).** To a solution of **45** (200 mg, 0.355 mmol) in DMF (2 mL) were added Boc-Glu(OtBu)-OH (130 mg, 0.426 mmol), EDC (82 mg, 0.426 mmol), and HOBt (66 mg, 0.426 mmol). The mixture was stirred for 20 h and partitioned between water and ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated. The residue was chromatographed (silica gel, 30:1 $CHCl_3$:MeOH) to afford **46** (215 mg, 71%): FD-MS (M^+) 848; ^{1}H NMR (500 MHz, CD_3-OD) δ 7.55 (d, J = 7.9 Hz, 1H), 7.42 (d, J = 7.3 Hz, 1H), 7.12 (m, 1H), 7.04 (m, 1H), 4.52 (m, 1H), 4.30 (m, 1H), 4.08 (m, 1H), 3.98 (d, J = 6.1 Hz, 1H), 3.62 (m, 1H), 3.44 (m, 1H), 2.28 (m, 2H), 1.99 (m, 2H), 1.81 (m, 1H), 1.63 (m, 1H), 1.48 (m, 2H), 1.40 (s, 18H), 1.39 (s, 9H), 1.10 (s, 9H), 0.87 (m, 12H).

***o*-(Glu-NH)-Ph-NHCO-Ser-Leu-Val-OH (47).** A solution of **46** (100 mg, 0.118 mmol) in 19:1 TFA:water (2 mL) was stirred for 2.5 h and concentrated. To the residue was added toluene (5 mL), and the suspension was concentrated. The residue was dissolved in a 50/50 mixture of 50% AcOH and DMSO and subjected to preparative reverse-phase HPLC (Nomura Chemical Develosil ODS-HG-5, 20 × 250 mm) using a linear gradient of (A) water containing 0.1% TFA and (B) acetonitrile containing 0.1% TFA (5−60% B, in 35 min) at a flow rate of 10 mL/min. Fractions containing the major peak were pooled and lyophilized to yield 48 mg (70%) of **47**: FAB-MS ($M^+ + H$) 581; ^{1}H NMR (500 MHz, CD_3OD) δ 7.60 (m, 1H), 7.38 (m, 1H), 7.22 (m, 2H), 4.49 (m, 1H), 4.38 (m, 1H), 4.27 (d, J = 5.5 Hz, 1H), 4.18 (m, 1H), 3.88 (m, 1H), 3.79 (m, 1H), 2.57 (m, 2H), 2.25 (m, 2H), 2.13 (m, 1H), 1.75−1.60 (m, 3H), 0.93 (m, 12H).

***m*-NO_2-Ph-NHCO-Ser(OtBu)-Leu-Val-OtBu (48).** The titled compound was prepared in quantitative yield according to the procedure as described for **44** using 3-nitrophenyl isocyanate instead of 2-nitrophenyl isocyanate: FD-MS (M^+) 593; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.42 (s, 1H), 8.49 (s, 1H), 7.93 (d, J = 8.6 Hz, 1H), 7.89 (d, J = 8.6 Hz, 1H), 7.73 (d, J = 7.9 Hz, 1H), 7.56 (d, J = 7.9 Hz, 1H), 7.48 (t, J = 7.9 Hz, 1H), 6.47 (m, 1H), 4.48 (m, 1H), 4.28 (m, 1H), 3.97 (m, 1H), 3.59 (m, 1H), 3.43 (m, 1H), 1.99 (m, 1H), 1.60 (m, 1H), 1.45 (m, 2H), 1.37 (s, 9H), 1.09 (s, 9H), 0.84 (m, 12H).

***m*-NH_2-Ph-NHCO-Ser(OtBu)-Leu-Val-OtBu (49).** The titled compound was prepared in 89% yield according to the procedure as described for **45** using **48** instead of **44**: FD-MS (M^+) 563; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.52 (s, 1H), 7.91 (d, J = 8.5 Hz, 1H), 7.79 (d, J = 7.9 Hz, 1H), 6.79 (t, J = 7.9 Hz, 1H), 6.65 (s, 1H), 6.49 (d, J = 7.3 Hz, 1H), 6.26 (d, J = 7.3 Hz, 1H), 6.09 (d, J = 7.9 Hz, 1H), 4.84 (s, 2H), 4.47 (m, 1H), 4.24 (m, 1H), 3.98 (m, 1H), 3.57 (m, 1H), 3.36 (dd, J = 9.2, 4.9 Hz, 1H), 1.99 (m, 1H), 1.59 (m, 1H), 1.44 (m, 2H), 1.38 (s, 9H), 1.09 (s, 9H), 0.86 (m, 12H).

***m*-(Boc-Glu(OtBu)-NH)-Ph-NHCO-Ser(OtBu)-Leu-Val-OtBu (50).** The titled compound was prepared in 90% yield according to the procedure as described for **46** using **49** instead of **45**: FD-MS (M^+) 848; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.88 (s, 1H), 8.93 (s, 1H), 7.98 (d, J = 7.9 Hz, 1H), 7.90 (d, J = 9.1 Hz, 1H), 7.70 (s, 1H), 7.13 (m, 3H), 7.03 (m, 1H), 6.37 (d, J = 7.9 Hz, 1H), 4.50 (m, 1H), 4.29 (m, 1H), 4.00 (m, 1H), 3.98 (m, 1H), 3.60 (m, 1H), 3.42 (m, 1H), 2.25 (m, 2H), 2.01 (m, 1H), 1.88 (m, 1H), 1.79 (m, 1H), 1.62 (m, 1H), 1.47 (m, 2H), 1.38 (s, 27H), 1.10 (s, 9H), 0.87 (m, 12H).

***m*-(Glu-NH)-Ph-NHCO-Ser-Leu-Val-OH (51).** The titled compound was prepared in 74% yield according to the procedure as described for **47** using **50** instead of **46**: FAB-MS ($M^+ + H$) 581; ^{1}H NMR (500 MHz, CD_3OD) δ 7.78 (s, 1H), 7.23 (m, 2H), 7.08 (d, J = 8.0 Hz, 1H), 4.52 (m, 1H), 4.39 (m, 1H), 4.28 (m, 1H), 4.02 (m, 1H), 3.86 (m, 1H), 3.71 (m, 1H), 2.52 (m, 2H), 2.18 (m, 3H), 1.65 (m, 3H), 0.94 (m, 12H).

***p*-NO_2-Ph-NHCO-Ser(OtBu)-Leu-Val-OtBu (52).** The titled compound was prepared in 94% yield according to the procedure as described for **44** using 4-nitrophenyl isocyanate instead of 2-nitrophenyl isocyanate: FD-MS (M^+) 593; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.73 (br, 1H), 8.23 (d, J = 9.2 Hz, 2H), 8.04 (m, 2H), 7.70 (d, J = 9.2 Hz, 2H), 6.69 (m, 1H), 4.60 (m, 1H), 4.41 (m, 1H), 4.09 (m, 1H), 3.71 (dd, J = 9.2, 3.7 Hz, 1H), 3.54 (dd, J = 9.2, 4.3 Hz, 1H), 2.11 (m, 1H), 1.71 (m, 1H), 1.56 (t, J = 7.3 Hz, 2H), 1.49 (s, 9H), 1.20 (s, 9H), 0.97 (m, 12H).

***p*-NH_2-Ph-NHCO-Ser(OtBu)-Leu-Val-OtBu (53).** The titled compound was prepared in 87% yield according to the procedure as described for **45** using **52** instead of **44**: FD-MS (M^+) 563; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H), 7.96 (d, J = 7.9 Hz, 1H), 7.79 (d, J = 8.6 Hz, 1H), 7.01 (d, J = 8.5 Hz, 2H), 6.46 (d, J = 8.5 Hz, 2H), 6.12 (d, J = 7.3 Hz, 1H), 4.65 (s, 2H), 4.49 (t, J = 7.9 Hz, 1H), 4.22 (m, 1H), 3.99 (dd, J = 8.5, 6.7 Hz, 1H), 3.58 (dd, J = 9.2, 4.3 Hz, 1H), 3.38 (dd, J = 9.2, 4.9 Hz, 1H), 2.02 (m, 1H), 1.62 (m, 1H), 1.46 (t, J = 7.3 Hz, 2H), 1.39 (s, 9H), 1.10 (s, 9H), 0.87 (m, 12H).

***p*-(Boc-Glu(OtBu)-NH)-Ph-NHCO-Ser(OtBu)-Leu-Val-OtBu (54).** The titled compound was prepared in 84% yield according to the procedure as described for **46** using **53** instead of **45**: FD-MS ($M^+ + H$) 849; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.73 (s, 1H), 8.80 (s, 1H), 7.90 (d, J = 7.9 Hz, 1H), 7.84 (d, J

= 8.5 Hz, 1H), 7.41 (d, J = 9.2 Hz, 2H), 7.28 (d, J = 8.5 Hz, 2H), 6.92 (br, 1H), 6.30 (br, 1H), 4.46 (m, 1H), 4.24 (m, 1H), 4.02 (m, 1H), 3.97 (m, 1H), 3.57 (m, 1H), 3.39 (m, 1H), 2.21 (m, 2H), 1.99 (m, 1H), 1.85 (m, 1H), 1.76 (m, 1H), 1.60 (m, 1H), 1.45 (m, 2H), 1.37 (s, 27H), 1.08 (s, 9H), 0.85 (m, 12H).

***p*-(Glu-NH)-Ph-NHCO-Ser-Leu-Val-OH (55).** The titled compound was prepared in 77% yield according to the procedure as described for **47** using **54** instead of **46**: FAB-MS (M^+ + H) 581; ^{1}H NMR (500 MHz, CD_3OD) δ 7.48 (d, J = 8.5 Hz, 2H), 7.36 (d, J = 8.5 Hz, 2H), 4.52 (dd, J = 9.8, 4.9 Hz, 1H), 4.38 (t, J = 5.5 Hz, 1H), 4.28 (d, J = 6.1 Hz, 1H), 4.01 (t, J = 6.1 Hz, 1H), 3.86 (dd, J = 10.4, 5.5 Hz, 1H), 3.73 (dd, J = 10.4, 6.1 Hz, 1H), 2.53 (t, J = 7.3 Hz, 2H), 2.20 (m, 3H), 1.80−1.55 (m, 3H), 0.95 (m, 12H).

Ph-NHCO-Ser-Leu-Val-OEt (56). A solution of **41** (192 mg, 0.442 mmol) in 10% HCl/EtOH (1.5 mL) was stirred for 12 h and concentrated. The residue was adsorbed on a plate of silica gel, and the plate was developed with 10% methanol/chloroform to afford 41 mg (20%) of **56**: FD-MS (M^+) 464; ^{1}H NMR (500 MHz, $CDCl_3/CD_3OD$) δ 7.37−7.26 (m, 5H), 4.44 (m, 2H), 4.39 (m, 1H), 4.18 (m, 2H), 3.90 (dd, J = 11.0, 4.3 Hz, 1H), 3.65 (dd, J = 11.0, 7.3 Hz, 1H), 2.15 (m, 1H), 1.73−1.56 (m, 3H), 1.29 (t, J = 3.7 Hz, 3H), 0.92 (m, 12H).

Ph-NHCO-Ser-Leu-Ala-OEt (57). A solution of Leu-Ala (2.0 g, 9.1 mmol) in saturated HCl/EtOH (50 mL) was stirred for 4 h at room temperature. Concentration and subsequent separation between EtOAc and saturated aqueous $NaHCO_3$ gave an organic layer, which was washed with brine, dried over Na_2SO_4, and concentrated to afford Leu-Ala-OEt (0.51 g, 2.22 mmol) which was used without further purification. A solution of Leu-Ala-OEt (0.50 g, 2.17 mmol), Fmoc-Ser(OtBu)-OH (0.83 g, 2.22 mmol), and EDC (0.45 g, 2.35 mmol) in DMF (10 mL) was stirred at room temperature for 3.5 h. After water was added, the solution was extracted with ethyl acetate. The extract was washed with brine (3×), dried over Na_2SO_4, and concentrated. Obtained residue was dissolved in 20% piperidine/DMF (10 mL) and stirred for 50 min. The solution was partitioned between ethyl acetate and water. The organic layer was washed with brine (3×), dried over Na_2SO_4, and concentrated. The crude products were washed with MeOH, and the soluble part was subjected to a silica gel column (hexane → hexane/ethyl acetate = 9/1 → $CHCl_3$/MeOH = 100/1 → 100/5) to afford 588 mg (5.8%, 3 steps) of Ser(OtBu)-Leu-Ala-OEt: FD-MS (M^+) 373; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, J = 8.6 Hz, 1H), 6.74 (d, J = 6.7 Hz, 1H), 4.51 (m, 1H), 4.43 (m, 1H), 4.19 (qd, J = 7.3, 1.2 Hz, 2H), 3.56 (m, 2H), 3.50 (m, 1H), 1.78 (s, 2H), 1.69 (m, 2H), 1.58 (m, 1H), 1.39 (d, J = 6.7 Hz, 3H), 1.28 (t, J = 7.3 Hz, 3H), 1.18 (s, 9H), 0.95 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 6.1 Hz, 3H).

A solution of Ser(OtBu)-Leu-Ala-OEt (400 mg, 1.07 mmol) and phenyl isocyanate (150 μM, 1.38 mmol) in DMF (5 mL) was stirred for 80 min at room temperature, and then it was diluted with water and extracted with ethyl acetate. The insoluble product was removed by filtration, and the filtrate was washed with brine (3×), dried over Na_2SO_4, and concentrated to give a solid residue. The solid residue was suspended in ethyl acetate and separated into soluble and insoluble parts. Insoluble products were combined into afford 396 mg of Ph-NHCO-Ser(OtBu)-Leu-Ala-OEt. A solution of Ph-NHCO-Ser-(OtBu)-Leu-Ala-OEt (390 mg) in TFA (5 mL) and water (0.25 mL) was stirred for 1 h at room temperature and concentrated. The oily residue were partitioned between water and ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and then concentrated. Crystalline solid was washed with Et_2O/hexane to afford 318 mg (68%, 2 steps) of **57**: FD-MS (M^+) 464; ^{1}H NMR (500 MHz, $CDCl_3/CD_3OD$) δ 7.37−7.26 (m, 5H), 4.44 (m, 2H), 4.39 (m, 1H), 4.18 (m, 2H), 3.90 (dd, J = 11.0, 4.3 Hz, 1H), 3.65 (dd, J = 11.0, 7.3 Hz, 1H), 2.15 (m, 1H), 1.73−1.56 (m, 3H), 1.29 (t, J = 3.7 Hz, 3H), 0.92 (m, 12H).

In Vitro Binding Assay. HFAP-10 cDNA[8] subcloned into the Bluescript vector pSK-II (Stratagene) was in vitro-translated from an internal methionine codon in the presence of [^{35}S]-L-methionine using a coupled in vitro transcription/translation system (Promega, TNT lysate) and T7 RNA polymerase. The C-terminus regions of Fas (191−335) and FasΔ15 (191−320, Fas lacks FAP-1 binding region) were subcloned into pGEX-4T-1 (Amersham Pharmacia Biotech). GST-fusion protein was purified with Glutathione-Sepharose (Amersham Pharmacia Biotech) according to instructions of the manufacturer. The resulting ^{35}S-labeled protein was incubated with GST-Fas fusion proteins that had been immobilized on GST-Sepharose 4B affinity beads (Amersham Pharmacia Biotech) with a synthetic peptide in a buffer containing 150 mM NaCl, 50 mM Tris (pH 8.0), 5 mM dithiothreitol, 2 mM EDTA, 1.1% Nonidet P-40, 1 mM phenylmethanesulfonyl fluoride, 50 μg/mL leupeptin, 1 mM benzamidine, and 7 μg/mL pepstatin for 16 h at 4 °C. After four vigorous washings in the same buffer, associated proteins were recovered with the Glutathione-Sepharose beads by centrifugation, and radioactivity was monitored by liquid scintillation counter. Specific binding was calculated by subtraction of the binding to FasΔ15 from the binding to Fas. The binding ratio (%) was calculated as follows: [specific binding with peptides]/[specific binding without peptides] × 100 (%). The "percent binding inhibition" was calculated by subtraction of binding ratio (%) from 100 (%). Thus, 100% inhibition is defined as no binding between Fas and FAP-1.

Cytotoxicity Assay. After preincubation for 24 h, the human colon cancer cell line, DLD-1 (2×10^4), was incubated 20 h at 37 °C with a graded concentration of Fas antibody CH-11 (Medical & Biological Laboratories Co., Ltd., Aichi, Japan) and synthetic peptide in 96-well plates. Each well was washed twice by phosphate-buffered saline, and surviving cells were determined using the MTT assay (Chemicon International Inc., El Segundo, CA).

Abbreviations: FAP-1, Fas-associated phosphatase-1; Ac, acetyl; Chg, cyclohexyl-L-glycine; tBu, *tert*-butyl; tBuGly, *tert*-butyl-L-glycine; Thy(OAc), 4-acetoxyphenyl-L-tyrosine; Abu, 2-aminobutyric acid; NorVal, norvaline (2-aminovaleric acid); Phg, phenyl-L-glycine; Sta, statine; Aib, aminoisobutyric acid; Abz, aminobenzoic acid; tBuAla, *tert*-butyl-L-alanine; Hse, homoserine; Hyp, 4-hydroxy-L-proline; Bz, benzoyl; Bn, benzyl; Z, benzyloxycarbonyl; Su, succinimide; Tet, tetrazole; Cyh, cyclohexyl; Ph, phenyl; Boc, *tert*-butoxycarbonyl; Fmoc, 9-fluorenylmethoxycarbonyl; HOBt, *N*-hydroxybenzotriazole; DIC, diisopropylcarbodiimide; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride; TFA, trifluoroacetic acid; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; GST, glutathione *S*-transferase; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate.

Acknowledgment. We thank the staff in our laboratory for the MS analyses. We also thank Ms. Yoshiko Yanagisawa for the HPLC analyses. We are grateful to Dr. Hiroshi Iijima for helpful discussions.

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JM980617F