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Synthesis and Antitumor Activity of Novel Dolastatin 10 Analogs

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Dolastatin 10 (1) is a potent antineoplastic pentapeptide. Novel dolastatin 10 analogs each modified at one of the constituent amino acid derivatives, were synthesized and their antitumor activity was evaluated against P388 leukemia in mice. The structural requirements for antitumor activity are discussed. Some of the analogs, 31c, 35c, 38b, and 50c showed excellent activity *in vivo*. Highly active 50c, which lacks the thiazole group of 1, was selected for further development as an antitumor agent.

Key words dolastatin 10 analog; antitumor agent; P388 leukemia; β-phenethylamide analog

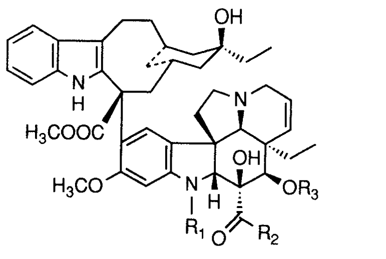
Dolastatin 10 (**1**), a pentapeptide isolated from the marine mollusk *Dolabella auricularia*, has potent antineoplastic and antimitotic activity.[1] This peptide consists of five subunits, namely, dolavaline (Dov),[2] valine (Val), dolaisoleuine (Dil), dolaproine (Dap), and dolaphenine (Doe) (Fig. 1). The stereochemistry of **1** was determined by Pettit *et al.*[3a] through total synthesis. Stereoselective synthesis of the individual subunits and total synthesis of **1** have subsequently been achieved by several groups.[4-6] However, thus far there have been no reports on dolastatin 10 analogs except for two chiral-modification studies by Pettit and co-workers.[7,8] They reported that one chiral isomer, with reversal of configuration at the side chain of the Dil unit, showed 10-fold more potent cytostatic activity against murine P388 leukemia cells,[7] and that two chiral isomers, with reversal of configuration at the side chain of either the Dil or the Doe unit, were similar to **1** in their cytotoxicity to murine L1210 leukemia cells,[8] but they gave no *in vivo* data.

Compound **1** has been reported to inhibit microtubule assembly by binding to tubulin near the vinca alkaloid site.[9] Compound **1**, vinca alkaloids, and maytansine (Fig. 1) have a similar mode of action.[10] Some of these compounds are in clinical use as anticancer drugs. Thus, **1** and its analogs appear to be promising candidates as anticancer drugs.

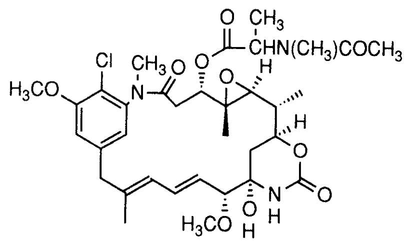
Our efforts have therefore been directed towards the development of dolastatin 10 analogs which have more potent antitumor activity than the parent compound (**1**). In order to elucidate the structural requirements for antitumor activity, we examined the effects of deletion of subunits and the modification of a side chain or a functional group at one of each subunit of **1**.

Chemistry

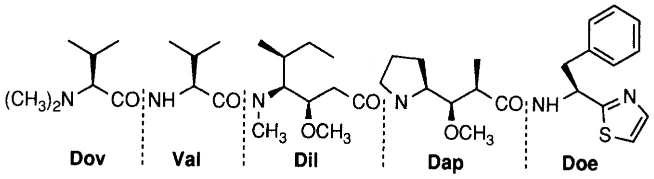
Synthesis of Amino-Acid Derivatives The syntheses of the Dil-related compounds are illustrated in Chart 1. β-Hydroxyesters **3a**—**e** with 3*R*-configuration were prepared from amino acids **2a**—**e** according to the reported procedure.[4a,c] β-Hydroxyesters **3a**—**e** were converted



vinblastine : R_1=CH_3, R_2=OCH_3, R_3=$COCH_3$
vincristine : R_1=CHO, R_2=OCH_3, R_3=$COCH_3$
vindecine : R_1=CH_3, R_2=NH_2, R_3=H



maytansine



1: dolastatin 10

Fig. 1. Antimitotic Agents Which Bind to the Vinca Alkaloid Site of Tubulin

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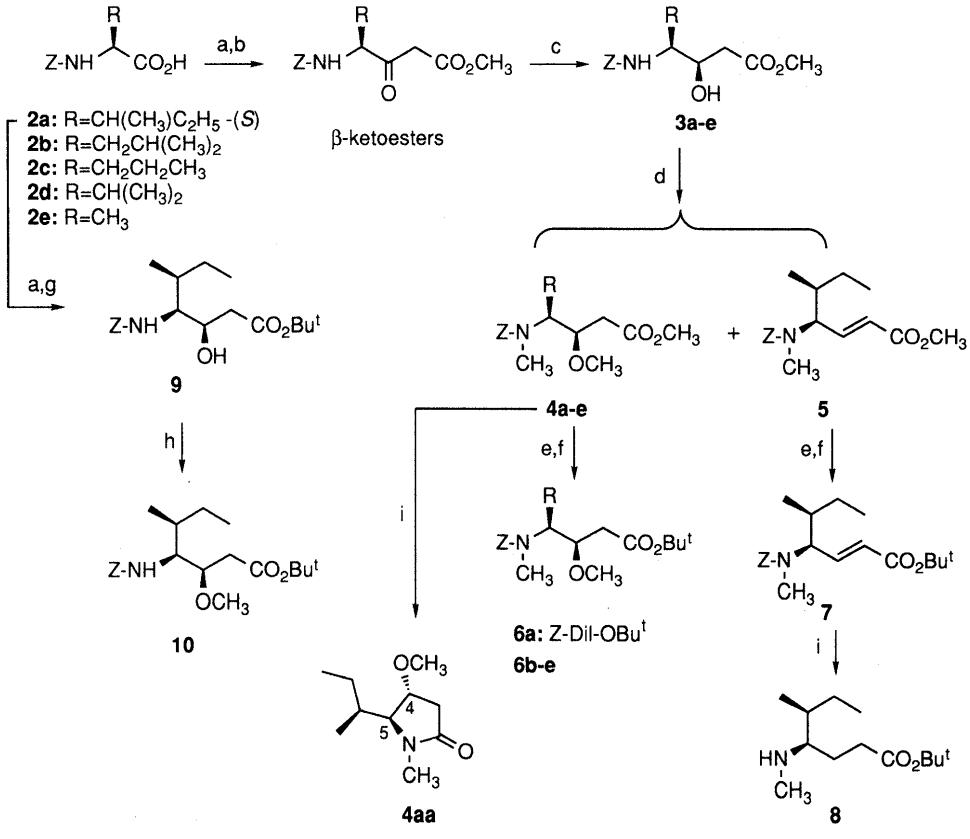
into β-methoxyesters **4a**—**e** by treatment with MeI and Ag_2O in *N*,*N*-dimethylformamide (DMF). The dehydrated compound **5** was produced simultaneously with **4a** in this procedure. First, we attempted to remove the Z group of **4a** by hydrogenolysis prior to the next reaction. However, **4a** was easily converted into the stereochemically known γ-lactam **4aa**, which was identified from its ^{1}H-NMR spectrum.[3b)] Thus, in order to avoid the cyclization, the methyl esters **4a**—**e** and **5** were converted into the corresponding *tert*-butyl esters **6a**—**e** and **7**, respectively, by treatment with aqueous NaOH followed by acid-catalyzed esterification with isobutene. Catalytic hydrogenation of the unsaturated compound **7** gave the 3-demethoxy-Dil derivative **8**.

Compound **9**, prepared according to the literature method,[5)] was treated with CH_2N_2–$BF_3 \cdot OEt_2$ to give the *N*-demethyl-Dil derivative **10**.

The syntheses of the Dap-related compounds are illustrated in Chart 2. Aldol reaction of Boc-L-prolinal with $LiCH(CH_3)CO_2Bzl$ followed by purification by column chromatography on silica gel gave β-hydroxyesters **11** and **12**, which were converted into β-hydroxy acids **13** and **14**, respectively, by catalytic hydrogenolysis. They were proved to be identical to ones prepared according to the method of Shioiri and co-workers[4c)] in terms of ^{1}H-NMR spectra, specific rotations, and retention times on HPLC. Treatment of **11** and **12** with CH_2N_2–$BF_3 \cdot OEt_2$ afforded Boc-Dap-OBzl (**15**) and its (2*S*)-epimer **16**, respectively, in 30—40% yields. Compounds **15** and **16** could be alternatively prepared from **11** and **12** by treatment with MeI and NaH in dry DMF, respectively, in improved yields (80—94%). This reaction proceeded without C-2 epimerization, since **15** and **16** were each homogeneous on HPLC (see Experimental).

Aldol reaction of Boc-L-prolinal with $LiCH_2CO_2Bzl$ according to the reported procedure[11)] gave **18**, which was treated with TFA followed by neutralization with aqueous K_2CO_3 to give the stereochemically known hydroxy pyrrolizidinone **18a**,[11)] and this compound was identified from the ^{1}H-NMR spectrum and specific rotation. The β-hydroxyester **18** was *O*-methylated by treatment with MeI and NaH in dry DMF to give the 2-demethyl-Dap derivative **19**. Compounds **11** and **18** were converted into the 3-demethoxy-Dap derivative **17** and the 2-demethyl-3-demethoxy-Dap derivative **20**, respectively, by treatment with 1,1′-thiocarbonyldiimidazole, followed by reaction with *n*-Bu_3SnH and 2,2′-azobis(isobutyronitrile).

Synthesis of Dolastatin 10 and Its Analogs Compound **1** and most of its analogs were synthesized by a similar method to that of Pettit *et al.*[3a)] as illustrated in Charts 3—7. DEPC, DCC, and BOP were used as coupling reagents. Catalytic hydrogenolysis, HCl in dioxane, and

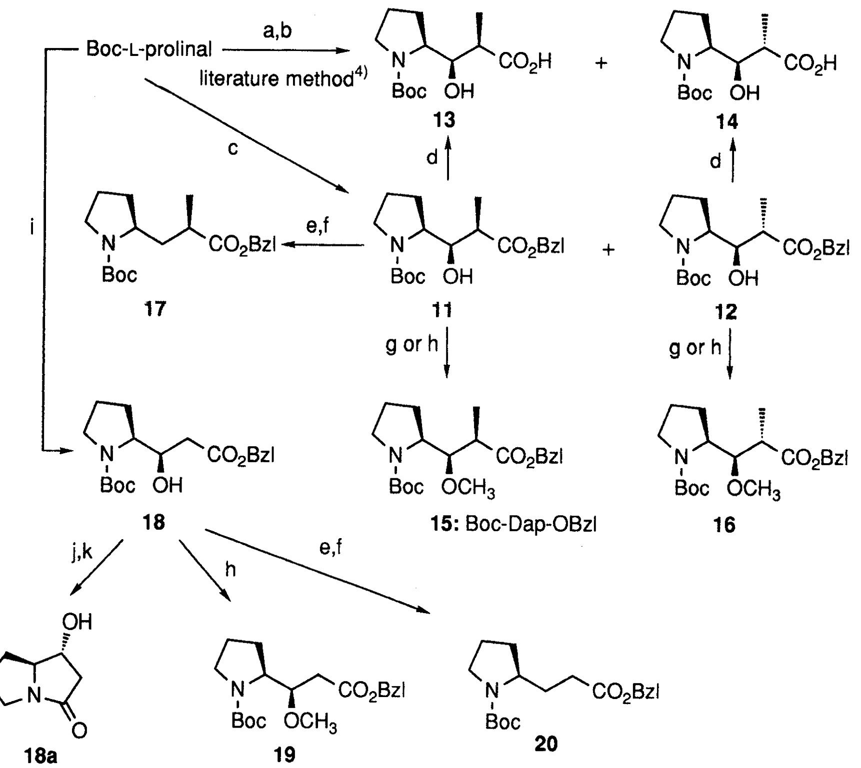


(a) CDI, THF; (b) $CH_2(CO_2CH_3)CO_2K$, $MgCl_2$, THF; (c) $NaBH_4$, MeOH; (d) CH_3I, Ag_2O, DMF;

(e) aqueous NaOH, dioxane; (f) isobutene, H^+; (g) $LiCH_2CO_2Bu^t$, THF; (h) CH_2N_2, $BF_3 \cdot OEt_2$, CH_2Cl_2;

(i) H_2, Pd-C, *tert*-BuOH/H_2O

Chart 1



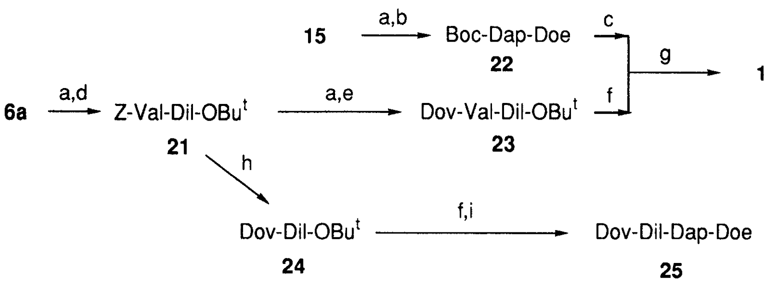
(a) (4*R*,5*S*)-4-methyl-5-phenyl-3-propionyl-2-oxazolidinone, *n*-Bu_2BOTf, Et_3N, CH_2Cl_2; (b) LiOH, H_2O_2, MeOH;

(c) $LiCH(CH_3)CO_2Bzl$, THF; (d) H_2, Pd-C, *tert*-BuOH/H_2O; (e) 1,1'-thiocarbonyldiimidazole, toluene;

(f) *n*-Bu_3SnH, 2,2'-azobis(isobutyronitrile), toluene; (g) CH_2N_2, BF_3•OEt_2, CH_2Cl_2; (h) CH_3I, NaH, DMF;

(i) $LiCH_2CO_2Bzl$, THF; (j) TFA; (k) aqueous K_2CO_3, EtOH

Chart 2



(a) H_2, Pd-C, *tert*-BuOH/H_2O; (b) H-Doe•TFA, BOP, *iso*-Pr_2NEt, CH_3CN; (c) HCl in dioxane;

(d) Z-Val-OH, DCC, CH_2Cl_2; (e) Dov, DEPC, Et_3N, DMF; (f) TFA, CH_2Cl_2; (g) DEPC, Et_3N, DMF;

(h) H_2, Pd-C, CH_2O, MeOH; (i) H-Dap-Doe•HCl (from **22**), DEPC, Et_3N, DMF

Chart 3

TFA were generally used to remove the Z, Boc, and *tert*-butyl ester groups, respectively.

DCC was used for the coupling of the Val unit and the Dil unit (*e.g.*, **6a**) to give the Val–Dil unit (*e.g.*, **21**)[3a] in good yield. It is noteworthy that the DCC method was very efficient in this coupling reaction of the *N*-methylamino ester (from **6a**), because the pivaloyl anhydride method or bromo tris(dimethylamino)phosphonium hexafluorophosphate method was used in this step previously.[3a,4c] After deprotection of the Val–Dil unit, coupling with the Dov unit provided the Dov–Val–Dil unit (*e.g.*, **23**).[3a] On the other hand, coupling reaction of the Dap unit (*e.g.*, **15**) with the Doe unit using BOP and iso-Pr_2NEt in CH_3CN provided the Dap–Doe unit (*e.g.*, **22**).[3a] After deprotection of the Dov–Val–Dil unit (*e.g.*, **23**) and the Dap–Doe unit (*e.g.*, **22**), coupling of the two components using DEPC and Et_3N in DMF provided the target peptide (*e.g.*, **1**).

Successive deprotection and reductive dimethylation of the Z-dipeptide ester **21** with formaldehyde in methanol afforded the *N,N*-dimethylamino derivative **24**. After deprotection of **22** and **24**, coupling of the two components using DEPC and Et_3N in DMF provided des-Val[2]-dolastatin 10 (**25**).

Chart 4 shows the synthesis of **28a** and **28b** in which the Dov unit (position 1) was replaced by Val or *N*-methylvaline. Coupling of H-Val–Dil–OBu[t] (from **21**) with Z-Val–OH or Z-*N*-methylvaline using DEPC and Et_3N in DMF gave **26a** or **26b**, respectively. Deblocking the *tert*-butyl ester group from **26a** and **26b** followed by coupling with H-Dap–Doe·HCl (from **22**) yielded **27a** and **27b**, respectively. Treatment of compounds **27a** and **27b** with TFA in the presence of thioanisole and *m*-cresol followed by aqueous HCl treatment, gave the corresponding hydrochlorides **28a** and **28b**, respectively.

As illustrated in Chart 4, coupling reaction of **29a—d** and H-Val–Dil–OBu[t] (from **21**) provided tripeptides **30a—d**. After deprotection of **30a—d**, coupling with H-Dap–Doe·HCl (from **22**) provided analogs **31a—d** modified at position 1.

Similarly, analogs **35a—c** modified at position 2 (the Val unit), **38b—e** and **42a—d** modified at position 3 (the Dil unit), and **44a—e** modified at position 4 (the Dap unit) were prepared as illustrated in Charts 5, 6, and 7, respectively, through the same sequence as used in the synthesis of **1** (Chart 3).

Chart 8 shows the synthesis of analogs **49** and **50a—d** modified at position 5 (the Doe unit). The thiazole amino compound **46** was prepared by dehydrogenation of the thiazolidine **45** using battery-grade MnO_2 according to the literature method.[3a)] The tetrapeptide **47**, prepared from the tripeptide **23** and Boc–Dap–OBzl (**15**), was deprotected and coupled with the thiazole amino compound **46** or aralkylamines **48a—d** to give analogs **49** and

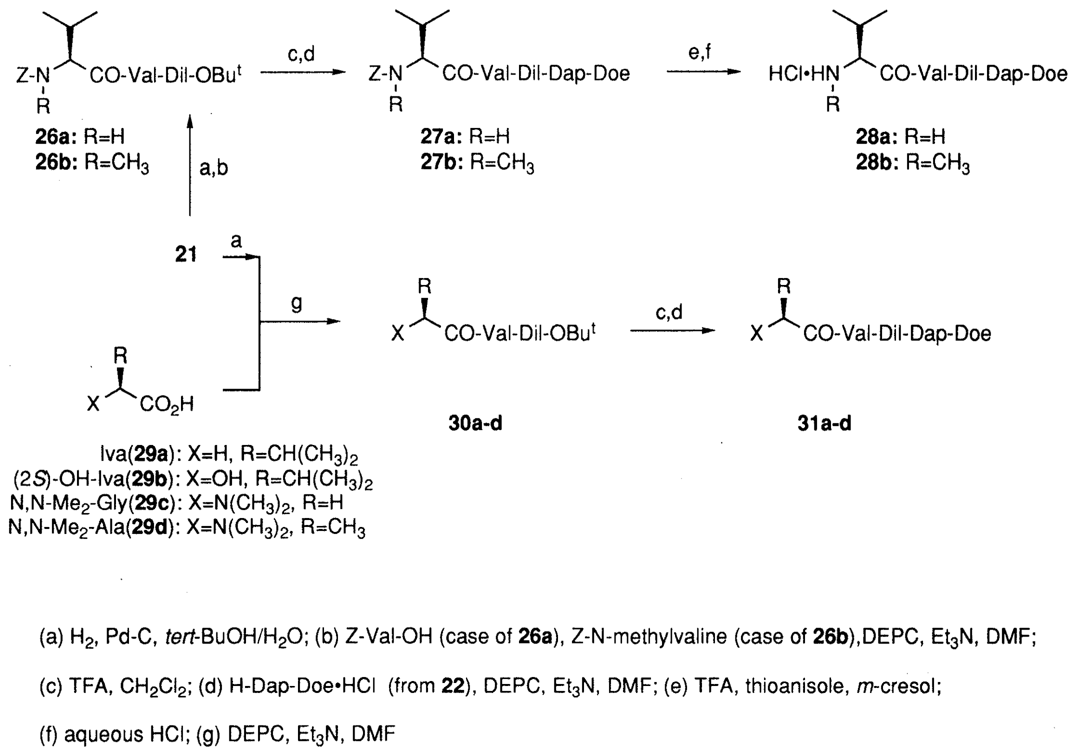


Chart 4. Synthesis of Analogs Modified at the Dov Unit

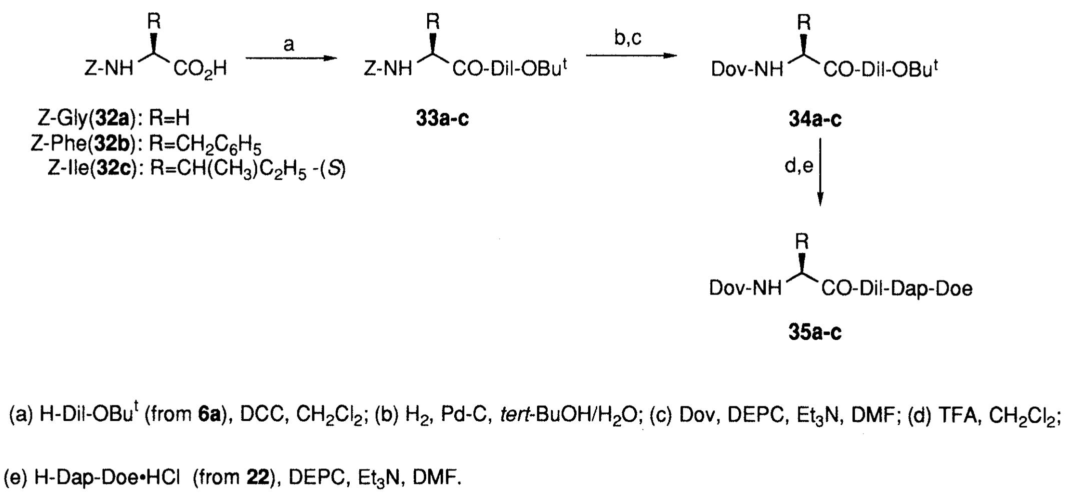
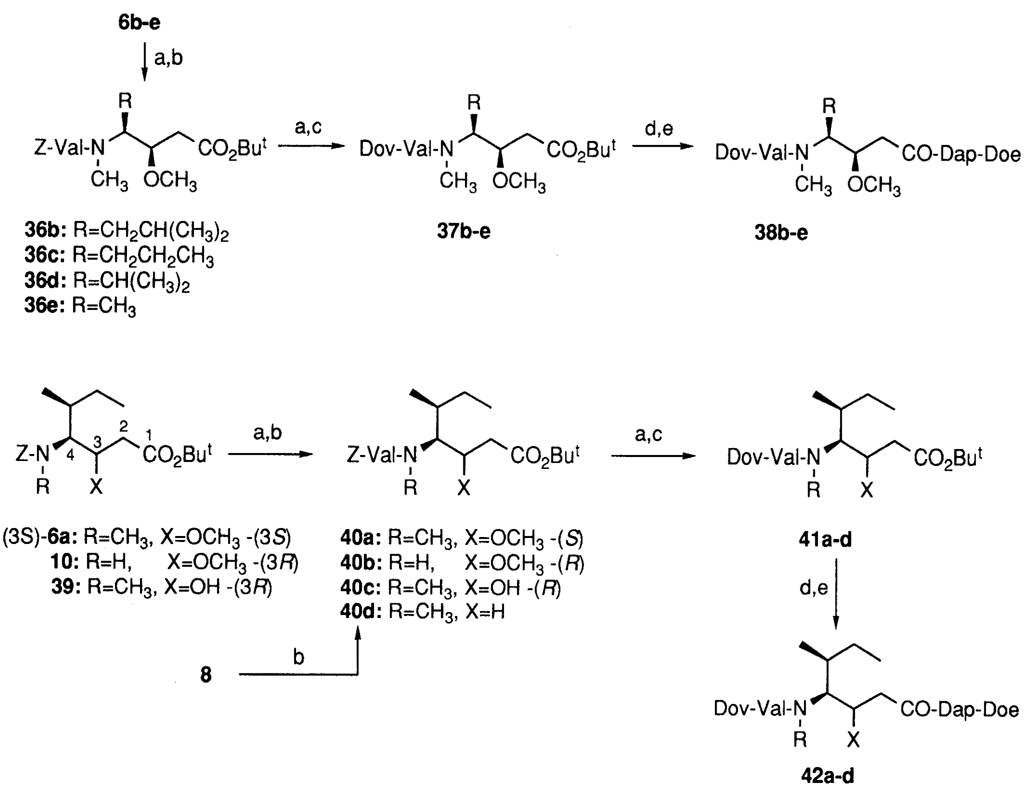


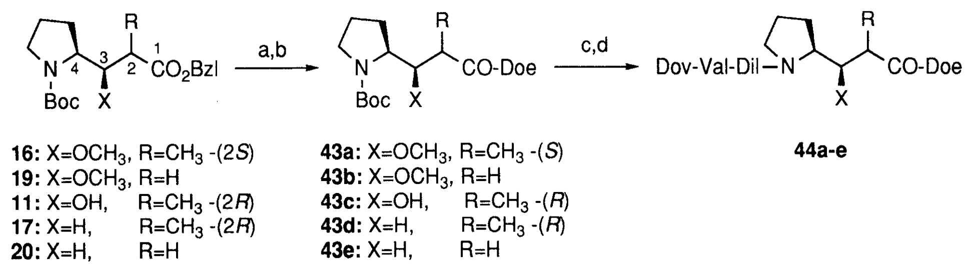
Chart 5. Synthesis of Analogs Modified at the Val Unit



(a) H_2, Pd-C, *tert*-BuOH/H_2O; (b) Z-Val-OH, DCC, CH_2Cl_2; (c) Dov, DEPC, Et_3N, DMF; (d) TFA, CH_2Cl_2;

(e) H-Dap-Doe•HCl (from **22**), DEPC, Et_3N, DMF

Chart 6. Synthesis of Analogs Modified at the Dil Unit



(a) H_2, Pd-C, *tert*-BuOH/H_2O; (b) H-Doe•TFA, BOP, *iso*-Pr_2NEt, CH_3CN; (c) HCl in dioxane;

(d) Dov-Val-Dil-OH (from **23**), DEPC, Et_3N, DMF.

Chart 7. Synthesis of Analogs Modified at the Dap Unit

50a—d, respectively.

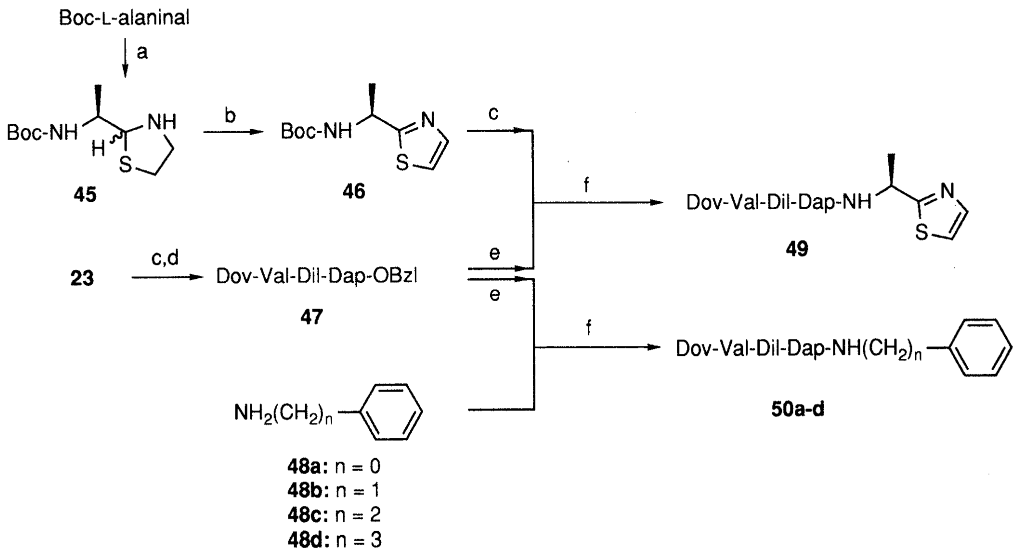
The physical properties of these analogs are shown in Tables 1—5.

Biological Results and Discussion

Antitumor activity was tested against P388 leukemia in mice according to the standard protocols of the United States National Cancer Institute.[12] Maximal increases in life span (ILS_{max}, %) and optimal doses of all dolastatin 10 analogs are summarized in Table 6. Any compound with an ILS_{max} of less than 30% was regarded as inactive. We will discuss the structural requirements for antitumor activity on the basis of these ILS_{max} values.

Boc–Dap–Doe (**22**) and Dov–Val–Dil–OBu^t (**23**) are synthetic intermediates of **1**. Compound **22** consists of two amino acids from the C-terminus of **1**, and **23** consists of three amino acids from the N-terminus. Dov–Dil–Dap–Doe (**25**) is the Val^2-deleted analog. These three analogs had no antitumor activity. Thus, shortening the peptide led to loss of activity. These findings suggest that the full five-subunit length corresponding to that of **1** is essential for antitumor activity. Subsequently, further studies on modification of individual subunits were carried out.

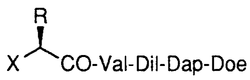
First, we discuss the activity of the analogs (**28a**, **b**, and **31a—d**) modified at position 1 (the Dov unit). Replacement of the *N*,*N*-dimethylamino group by an amino group (**28a**) resulted in loss of activity. The *N*-monomethylamino analog **28b**, on the other hand, retained



(a) $HSCH_2CH_2NH_2$, benzene; (b) MnO_2, dioxane; (c) TFA, CH_2Cl_2; (d) H-Dap-OBzl•HCl (from **15**), DEPC, Et_3N, DMF; (e) H_2, Pd-C, *tert*-BuOH/H_2O; (f) DEPC, Et_3N, DMF

Chart 8. Synthesis of Analogs Modified at the Doe Unit

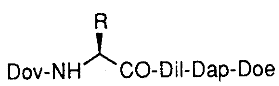
Table 1. Physical Properties of Analogs Modified at Position 1



No.	X	R	Purification method[a)]	HPLC %[b)]	HPLC t_R[c)]	HPLC Purity (%)	mp (°C[d)])	$[\alpha]_D$ (°)[e)]	Formula	Analysis (%)[f)] Calcd (Found) C	H	N
28a	$NH_2 \cdot HCl$	$CH(CH_3)_2$	IV	35	5.5	93	112—116	−69.3	$C_{40}H_{64}N_6O_6S \cdot 0.75HCl$	61.25 (61.14	8.32 8.04	10.71 10.35)
28b	$NHCH_3 \cdot HCl$	$CH(CH_3)_2$	IV	35	6.1	99	100—103	−71.8	$C_{41}H_{66}N_6O_6S \cdot HCl$	60.98 (60.98	8.36 8.51	10.41 10.17)
31a	H	$CH(CH_3)_2$	I	50	7.3	>99	92—95	−85.0	$C_{40}H_{63}N_5O_6S$	64.75 (64.61	8.56 8.67	9.44 9.41)
31b	OH	$CH(CH_3)_2$	I	50	7.4	>99	99—102	−82.8	$C_{40}H_{63}N_5O_7S \cdot 0.8H_2O$	62.20 (62.52	8.43 8.40	9.07 8.67)
31c	$N(CH_3)_2$	H	II	38	3.6	94	89—92	−79.3	$C_{39}H_{62}N_6O_6S \cdot H_2O$	61.55 (61.55	8.48 8.38	11.04 10.65)
31d	$N(CH_3)_2$	CH_3	I	35	4.9	>99	81—88	−80.6	$C_{40}H_{64}N_6O_6S \cdot H_2O$	61.99 (61.82	8.58 8.61	10.84 10.61)

a) See Experimental. *b)* Solvent system: isocratic, percentage of CH_3CN in 0.1% aqueous TFA. Flow rate: 1.0 ml/min (column conditions in Experimental). *c)* Retention time (min). *d)* All analogs were obtained as amorphous powders. *e)* Specific rotation in MeOH (c=0.3—1.3)at 23—28°C. *f)* Analyses for C, H, and N were within ±0.4% of the theoretical values.

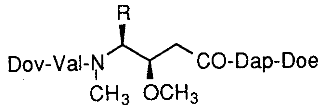
Table 2. Physical Properties of Analogs Modified at Position 2



No.	R	Purification method[a)]	HPLC %[b)]	HPLC t_R[c)]	HPLC Purity (%)	mp (°C[d)])	$[\alpha]_D$ (°)[e)]	Formula	Analysis (%)[f)] Calcd (Found) C	H	N
35a	H	II	35	5.5	>99	86—89	−61.6	$C_{39}H_{62}N_6O_6S \cdot 0.2H_2O$	62.74 (62.48	8.42 8.48	11.26 11.14)
35b	$CH_2C_6H_5$	II	40	5.4	>99	91—94	−67.8	$C_{46}H_{68}N_6O_6S \cdot 0.5H_2O$	65.61 (65.54	8.26 8.21	9.98 9.88)
35c	$CH(CH_3)C_2H_5$-(*S*)	I	38	6.2	>99	90—96	−70.4	$C_{43}H_{70}N_6O_6S \cdot 2.0MeOH$[g)]	62.61 (62.30	9.11 8.79	9.74 9.63)

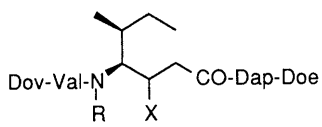
a—f) See footnotes *a—f)* in Table 1. *g)* HR-MS *m/z*: Calcd for $C_{43}H_{71}N_6O_6S$ (MH^+): 799.5153. Found: 799.5174.

Table 3. Physical Properties of Analogs Modified at Position 3



No.	R	Purification method[a)]	HPLC %[b)]	HPLC t_R[c)]	HPLC Purity (%)	mp (°C[d)])	$[\alpha]_D$ (°)[e)]	Formula	Analysis (%)[f)] Calcd (Found) C	H	N
38b	$CH_2CH_2(CH_3)_2$	II	40	5.5	>99	93—95	−89.0	$C_{42}H_{68}N_6O_6S \cdot 0.5H_2O$	63.53 (63.35	8.76 8.73	10.58 10.30)
38c	$CH_2CH_2CH_3$	II	35	5.8	99	92—94	−82.9	$C_{41}H_{66}N_6O_6S \cdot 2.0MeOH$[g)]	61.84 (62.16	8.93 8.56	10.06 9.75)
38d	$CH(CH_3)_2$	IV	35	5.1	99	94—97	−79.4	$C_{41}H_{66}N_6O_6S \cdot 0.4H_2O$	63.27 (62.90	8.65 8.89	10.80 10.66)
38e	CH_3	II	35	4.1	98	86—89	−79.1	$C_{39}H_{62}N_6O_6S \cdot 0.6H_2O$	62.14 (62.34	8.45 8.59	11.15 10.76)

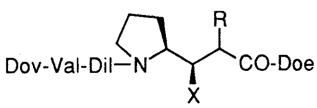
Table 3. (continued)



No.	R	X (config.)	Purification method[a)]	HPLC %[b)]	HPLC t_R[c)]	HPLC Purity (%)	mp (°C[d)])	$[\alpha]_D$ (°)[e)]	Formula	Analysis (%)[f)] Calcd (Found) C	H	N
42a[10)]	CH_3	OCH_3-(*S*)	II	35	9.0	>99	85—88	−90.2	$C_{42}H_{68}N_6O_6S$	64.25 (64.24	8.73 8.91	10.70 10.76)
42b	H	OCH_3-(*R*)	II	35	5.2	99	>97 (gradual)	−70.9	$C_{41}H_{66}N_6O_6S \cdot 0.5MeOH$[h)]	63.33 (63.23	8.71 8.86	10.68 10.29)
42c	CH_3	OH-(*R*)	II	35	5.1	91	96—99	−78.6	$C_{41}H_{66}N_6O_6S \cdot 0.6H_2O$	62.98 (63.13	8.66 8.78	10.75 10.37)
42d	CH_3	H	I	35	6.7	99	82—85	−84.7	$C_{41}H_{66}N_6O_5S \cdot 0.5H_2O$	64.45 (64.42	8.84 8.87	11.00 10.60)

a—f) See footnotes *a—f*) in Table 1. *g*) HR-MS *m/z*: Calcd for $C_{41}H_{67}N_6O_6S$ (MH^+): 771.4840. Found: 771.4855. *h*) HR-MS *m/z*: Calcd for $C_{41}H_{67}N_6O_6S$ (MH^+): 771.4840. Found: 771.4859.

Table 4. Physical Properties of Analogs Modified at Position 4



No.	X	R (config.)	Purification method[a)]	HPLC %[b)]	HPLC t_R[c)]	HPLC Purity (%)	mp (°C[d)])	$[\alpha]_D$ (°)[e)]	Formula	Analysis (%)[f)] Calcd (Found) C	H	N
44a[10)]	OCH_3	CH_3-(*S*)	II	35	7.6	>99	96—97	−84.3	$C_{42}H_{68}N_6O_6S \cdot 1.1MeOH$	63.10 (62.72	8.90 8.99	10.24 9.86)
44b	OCH_3	H	III	35	6.1	93	80—83	−76.1	$C_{41}H_{66}N_6O_6S$	63.87 (63.57	8.63 8.87	10.90 10.75)
44c	OH	CH_3-(*R*)	I	35	4.0	>99	106—109	−77.2	$C_{41}H_{66}N_6O_6S \cdot 0.5H_2O$	63.13 (62.85	8.66 8.75	10.77 10.38)
44d	H	CH_3-(*R*)	II	35	7.2	97	85—88	−62.1	$C_{41}H_{66}N_6O_5S$	65.22 (65.13	8.81 9.09	11.13 11.01)
44e	H	H	II	35	5.6	96	76—79	−60.0	$C_{40}H_{64}N_6O_5S$	64.83 (64.92	8.71 8.97	11.34 10.95)

a—f) See footnotes *a—f*) in Table 1.

Table 5. Physical Properties of Analogs Modified at Position 5

Dov–Val–Dil–Dap–NH–R

No.	R	Purification method[a)]	HPLC %[b)]	HPLC t_R[c)]	HPLC Purity (%)	mp (°C[d)])	$[\alpha]_D$ (°)[e)]	Formula	Analysis (%)[f)] Calcd (Found) C	H	N
49	$CH(CH_3)$-2-thiazole-(*S*)	IV	28	6.8	97	86—89	−84.9	$C_{36}H_{64}N_6O_6S$	60.99 (60.66	9.10 9.31	11.85 11.68)
50a	C_6H_5	II	38	5.8	96	96—97	−52.8	$C_{37}H_{63}N_5O_6$ ·1.5MeOH[g)]	64.05 (64.41	9.63 9.44	9.70 9.24)
50b	$CH_2C_6H_5$	III	38	4.6	95	>116 (gradual)	−35.8	$C_{38}H_{65}N_5O_6$ ·0.6MeOH[h)]	65.56 (65.85	9.61 9.69	9.90 9.40)
50c	$CH_2CH_2C_6H_5$	II	35	7.0	>99	75—78	−38.0	$C_{39}H_{67}N_5O_6$	66.73 (66.76	9.62 9.72	9.98 9.97)
50d	$CH_2CH_2CH_2C_6H_5$	III	38	8.0	96	74—78	−47.1	$C_{40}H_{69}N_5O_6$ ·0.4MeOH[i)]	66.58 (66.92	9.76 10.08	9.61 9.25)

a—f) See footnotes *a—f)* in Table 1. *g)* N analysis was unsatisfactory. HR-MS *m/z*: Calcd for $C_{37}H_{64}N_5O_6$ (MH^+): 674.4854. Found: 674.4867. *h)* N analysis was unsatisfactory. HR-MS *m/z*: Calcd for $C_{38}H_{66}N_5O_6$ (MH^+): 688.5009. Found: 688.5021. *i)* HR-MS *m/z*: Calcd for $C_{40}H_{70}N_5O_6$ (MH^+): 716.5323. Found: 716.5336.

Table 6. Antitumor Activity of Dolastatin 10 Analogs

Dov–Val–Dil–Dap–Doe
position 1 2 3 4 5

No.	Modified position	ILS_{max}[a)]	Optimal dose (mg/kg per inj.)	No.	Modified position	ILS_{max}[a)]	Optimal dose (mg/kg per inj.)
1		50	0.05	**42a**	3	53	0.5
22		7	1.0	**42b**	3	49	0.1
23		7	0.5	**42c**	3	32	0.5
25		17	1.0	**42d**	3	16	0.5
28a	1	24	1.0	**44a**	4	29	0.5
28b	1	57	0.05	**44b**	4	55	0.5
31a	1	18	0.5	**44c**	4	64	0.1
31b	1	22	0.5	**44d**	4	61	1.0
31c	1	94	0.5	**44e**	4	0	0.5
31d	1	18	1.0	**49**	5	45	1.0
35a	2	16	0.1	**50a**	5	13	0.5
35b	2	41	0.1	**50b**	5	16	0.5
35c	2	80	0.5	**50c**	5	83	0.5
38b	3	74	2.0	**50d**	5	11	0.1
38c	3	60	0.5				
38d	3	49	0.02	5FU[b)]		60—85	120
38e	3	16	0.5	VCR[c)]		100	2.0

a) Maximal increase in life span (see Experimental). *b)* 5-Fluorouracil. *c)* Vincristine.

activity comparable to that of **1**. Removal of the *N*,*N*-dimethylamino group (**31a**) led to loss of activity. Compound **31b**, which has a hydroxy group instead of the *N*,*N*-dimethylamino group, also showed no activity. These findings suggest that either a secondary or a tertiary amino group at position 1 is necessary. As for the modification of the side chain at position 1 (an isopropyl group in **1**), **31c** and **31d** were tested. Compound **31c** (no side chain) showed high activity, but **31d** (having a methyl group) was inactive. The side chain at this position may play an important role, but there seems to be no correlation between the size of the side chain and antitumor activity.

Second, we tested the analogs (**35a—c**) modified at position 2 (the Val unit). The glycine analog **35a** was inactive, the phenylalanine analog **35b** was slightly less active than **1**, and the isoleucine analog **35c** displayed more potent activity. These findings suggest that a bulky side chain here is favorable for activity.

Third, among the side-chain modified analogs (**38b—e**) at position 3 (the Dil unit), **38b** (R = isobutyl) and **38c** (R = *n*-propyl) were more potent than **1** (R = *sec*-butyl), and **38d** (R = isopropyl) was equipotent to **1**. Compound **38e** (R = methyl), on the other hand, was inactive. It seems that the side chain here must be bulkier than a methyl group.

The (3*S*)-methoxy-Dil analog **42a**, a chiral isomer of **1**, was equipotent to **1**, while the (3*R*)-hydroxy-Dil analog **42c**, the *O*-demethylated derivative of **1**, showed weak activity, and the 3-demethoxy-Dil analog **42d** was inactive. These findings suggest that activity does not depend on the configuration of the 3-methoxy group in the Dil unit, but this moiety is necessary for activity. Additionally, the *N*-demethyl-Dil analog **42b** had activity comparable to that of **1**, showing that this methyl group has no significant

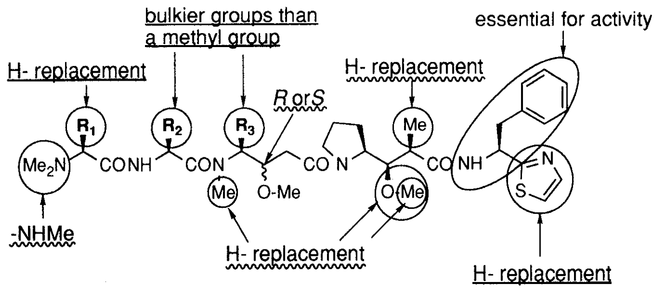


Fig. 2. Structural Requirements of Dolastatin 10

Some modifications (underlined with a straight line) resulted in more potent activity than that of dolastatin 10 (**1**), while other modifications (underlined with a wavy line) yielded activity equivalent to that of **1**.

effect on the activity.

Fourth, we tested the analogs modified at position 4 (the Dap unit). The (2*S*)-methyl-Dap analog **44a** displayed no activity, while the activity of the 2-demethyl-Dap analog **44b** was equal to that of **1**. Both the (3*R*)-hydroxy-Dap analog **44c** and the 3-demethoxy-Dap analog **44d** were more active than **1**. The 2-demethyl-3-demethoxy-Dap analog **44e** had no activity. Deletion of either the 2-methyl group or the 3-methoxy group from the Dap unit did not affect activity, but deletion of both resulted in loss of activity.

Finally, modification at position 5 (the Doe unit) by replacement of the side chain with a methyl group yielded analog **49**, which was slightly less potent than **1**. The β-phenethylamide analog **50c**, which had no thiazole ring but had the same length of methylene chain between N and phenyl, showed high activity, while the anilide analog **50a**, the benzylamide analog **50b**, and the γ-phenylpropylamide analog **50d** were inactive. These findings suggest that the β-phenethylamide moiety in the Doe unit is essential for activity.

Conclusions

The structural requirements of **1** for antitumor activity were evaluated *in vivo*. The key structural variations are summarized in Fig. 2. Replacement of the *N*,*N*-dimethylamino group with an *N*-monomethylamino group resulted in analog **28b** which was equipotent to **1**. Compounds **31c** (R_1=H), **35c** (R_2=CH(CH_3)C_2H_5), and **38b** (R_3=CH_2CH(CH_3)$_2$) showed more potent activity than **1**. The *N*-methyl group in the Dil unit and the 2-methyl group in the Dap unit could be replaced with H without loss of activity (**42b** and **44b**). Similarly, the methoxy group in the Dap unit could be replaced with H or a hydroxy group without loss of activity (**44c** and **44d**). The inversion of configuration of the methoxy group in the Dil unit caused no difference in efficacy (**1** and **42a**). It is especially noteworthy that deletion of the thiazole group in the Doe unit (**50c**) resulted in higher antitumor activity than that of the parent compound **1**. In the absence of the thiazole group, the β-phenethylamide moiety could not be replaced by other aralkylamide moieties (**50a, 50b**, and **50d**) without loss of activity.

The fact that compound **50c** was superior to **1** encouraged us to assess further modifications at the Doe unit. The synthesis and evaluation of analogs with a variety of modifications of the Doe unit will be reported elsewhere.

Experimental

General Melting points were measured with a Mettler FP800HT melting point apparatus and are uncorrected. ^{1}H-NMR spectra were measured on a 90-MHz Hitachi R-90H instrument or a 500-MHz JEOL TNM FX-500 instrument with tetramethylsilane as an internal standard. Electron-impact mass spectra (EI-MS) were recorded on a Shimadzu GCMS-QP1000 spectrometer. High-resolution mass spectra (HR-MS) and secondary ion mass spectra (SI-MS) were measured on a Hitachi M-2500 mass spectrometer. Specific rotations were determined on a JASCO DIP-370 digital polarimeter. Elemental analysis was performed using a Hitachi 026 CHN analyzer. HPLC was carried out using a Tosoh CCPE high-pressure liquid chromatograph. Thin-layer chromatography (TLC) was done on Merck Silica gel 60F-254 plates, and preparative TLC on Whatman Silica gel 60A plates. Column chromatography was performed using Wakogel C-200 (75—150 μm) for gravity columns and Wakogel FC-40 (20—40 μm) for flash columns.

DMF, DMSO, and CH_2Cl_2 were dried over 4A molecular sieves. Dry tetrahydrofuran (THF) was obtained by distillation from sodium benzophenone ketyl immediately prior to use. Reagent-grade CH_3CN, MeOH, and EtOH were dried over 3A molecular sieves.

Materials All chemicals used are commercially available and were used without further purification. (2*S*)-Hydroxyisovaleric acid (**29b**) was derived from Val by the method of Johnson.[13] *N*,*N*-Dimethylvaline (Dov), *N*,*N*-dimethylglycine (**29c**), and *N*,*N*-dimethylalanine (**29d**) were prepared by reductive dimethylation of Val, Gly, and Ala, respectively, according to the procedure reported by Bowman and Stroud.[14] *O*-Demethyl-Dil derivative (**39**)[3b] and (*S*)-dolaphenine·TFA (H-Doe·TFA)[3a] were prepared according to the method of Pettit *et al.*

Purification of the Target Peptide Method I: Crude peptide was purified by preparative reversed-phase HPLC using a YMC-packed column D-ODS-5 S-5 (2×25 cm, 5 μm particle size), eluting isocratically with a mixture of aqueous TFA and CH_3CN. The main fraction was pooled and concentrated *in vacuo*, and the residue was dissolved in CH_2Cl_2. The CH_2Cl_2 phase was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was then purified by column chromatography on Sephadex LH-20 with hexane–CH_2Cl_2–MeOH (4 : 15 : 5) as the eluent, giving the desired peptide as an amorphous powder.

Method II: The crude peptide was purified by flash chromatography on silica gel or preparative TLC using CH_2Cl_2–MeOH (50—10 : 1). The following column chromatography on Sephadex LH-20 was carried out using the above eluent to give the desired peptide as an amorphous powder.

Method III: Flash chromatography on silica gel or preparative TLC.

Method IV: Column chromatography on Sephadex LH-20.

Homogeneity and Characterization of the Target Peptide Peptides were assessed for homogeneity by analytical reversed-phase HPLC and TLC. Analytical HPLC was carried out on a GL Sciences Inertsil ODS-2 packed column (0.46×15 cm, 5 μm particle size) with a mixture of aqueous TFA and CH_3CN (see footnote *b*) in Table 1). All peptides were homogeneous on TLC. They were characterized by EI-MS, ^{1}H-NMR spectroscopy, optical rotation, and elemental analysis or HR-MS (Tables 1—5).

Methyl (3*R*,4*S*)-4-(*N*-Benzyloxycarbonylamino)-3-hydroxypentanoate (3e) a) CDI (3.89 g, 24 mmol) was added to a solution of **2e** (4.46 g, 20 mmol) in THF (60 ml) in one portion, and the mixture was stirred at room temperature for 5.5 h. A mixture of $CH_2(CO_2CH_3)CO_2K$ (6.90 g, 44 mmol) and anhydrous $MgCl_2$[15] (3.00 g, 32 mmol) in THF (70 ml) was stirred at 55—60 °C for 6 h, and then cooled in an ice bath, followed by the addition of the above imidazolide solution in one portion. The reaction mixture was stirred at room temperature for 1 d. The solvent was evaporated and the oily residue was partitioned between EtOAc and ice-cold 2 N HCl. The organic phase was washed successively with 2 N HCl, water, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using EtOAc–hexane (1 : 1) as the eluent to give the β-ketoester [methyl (4*S*)-4-(*N*-benzyloxycarbonylamino)-3-oxopentanoate] (4.78 g, 86% yield) as a colorless oil, $[\alpha]_D^{26}$ −17.7° (c=1.0, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, d, J=7.1 Hz), 3.55 (2H, s), 3.72 (3H, s), 4.45 (1H, quintet, J=7.1 Hz), 5.11 (2H, s), 5.25—5.55 (1H, m), 7.34 (5H, s). EI-MS m/z: 279 (M^+). b) The above β-ketoester was reduced with $NaBH_4$ according to the method of Shioiri and co-workers[4c] to give **3e** in 85% yield as colorless crystals (recrystallized from isopropyl ether), mp 78 °C, $[\alpha]_D^{28}$ −4.4° (c=1.0, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.15 (3H, d, J=6.8 Hz), 2.35—2.55 (2H, m), 3.15—3.3 (1H, m), 3.70 (3H, s), 3.7—3.85 (1H, m), 3.9—4.15 (1H, m), 4.9—5.1 (1H, br s), 5.09 (2H, s), 7.34 (5H, s). *Anal.* Calcd for $C_{14}H_{19}NO_5$: C, 59.77; H, 6.81; N, 4.98. Found: C, 59.83; H, 6.92; N, 5.07.

Compounds **3a—d** were derived from **2a—d**, respectively, as described above.

3a: Colorless crystals (86% yield), mp 77 °C (isopropyl ether), $[\alpha]_D^{27}$ −6.9° (c=1.0, MeOH). *Anal.* Calcd for $C_{17}H_{25}NO_5$: C, 63.14; H, 7.79; N, 4.33. Found: C, 63.12; H, 7.78; N, 4.35.

(3*S*)-**3a**: This compound was obtained as a by-product in the preparation of **3a** starting with **2a**, in 9% yield as a colorless oil, $[\alpha]_D^{30}$ −32.9° (c=1.0, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.8—1.1 (6H, m), 1.1—1.3 (1H, m), 1.4—1.8 (2H, m), 2.4—2.6 (2H, m), 3.1—3.45 (2H, m), 3.70 (3H, s), 4.28 (1H, m), 5.11 (2H, s), 5.1—5.3 (1H, m) 7.34 (5H, s). EI-MS m/z: 323 (M^+). *Anal.* Calcd for $C_{17}H_{25}NO_5$: C, 63.14; H, 7.79; N, 4.33. Found: C, 62.94; H, 7.83; N, 4.53.

3b: Colorless crystals (82% yield), mp 100 °C (isopropyl ether), $[\alpha]_D^{26}$ −21.5° (c=1.0, MeOH). *Anal.* Calcd for $C_{17}H_{25}NO_5$: C, 63.14; H, 7.79; N, 4.33. Found: C, 63.05; H, 7.76; N, 4.62.

3c: Colorless crystals (88% yield), mp 126 °C (isopropyl ether), $[\alpha]_D^{27}$ −13.6° (c=1.0, MeOH). *Anal.* Calcd for $C_{16}H_{23}NO_5$: C, 62.12; H, 7.49; N, 4.53. Found: C, 61.99; H, 7.51; N, 4.75.

3d: Colorless crystals (80% yield), mp 81 °C (isopropyl ether), $[\alpha]_D^{28}$ +9.6° (c=1.0, MeOH). *Anal.* Calcd for $C_{16}H_{23}NO_5$: C, 62.12; H, 7.49; N, 4.53. Found: C, 62.16; H, 7.51; N, 4.70.

(4*R*,5*S*,1′*S*)-*N*-Methyl-5-(1′-methylpropyl)-4-methoxypyrrolidin-2-one (4aa)[3b] This compound was obtained by hydrogenation in quantitative yield as a colorless oil, starting with **4a**. ^{1}H-NMR ($CDCl_3$) δ: 0.71 (3H, d, J=6.8 Hz), 1.00 (3H, t, J=7.6 Hz), 1.2—1.54 (2H, m), 1.78 (1H, m), 2.39 (1H, br d, J=17.0 Hz), 2.55 (1H, dd, J=17.0, 6.1 Hz), 2.81 (3H, s), 3.29 (3H, s), 3.45 (1H, dd, J=3.5, 1.7 Hz), 3.68 (1H, ddd, J=6.1, 3.1, 1.7 Hz). EI-MS m/z: 185 (M^+), 128, 96, 71, 55.

***tert*-Butyl (3*R*,4*S*)-4-(*N*-Benzyloxycarbonyl-*N*-methylamino)-3-methoxypentanoate (6e)** a) Ag_2O (40 g, 172 mmol) and MeI (50 ml, 0.8 mol) were added to a solution of the β-hydroxyester **3e** (9.78 g, 34.8 mmol) in DMF (100 ml). The mixture was stirred at 35 °C for 5 h, filtered and washed with DMF. The filtrate and washings were combined and concentrated *in vacuo*. The residue was extracted with EtOAc. The organic phase was washed with 5% aqueous $Na_2S_2O_3$ and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was then purified by column chromatography on silica gel using benzene–EtOAc (5 : 1) as the eluent to give **4e** (7.63 g, 71% yield) as a pale yellow oil, $[\alpha]_D^{25}$ −39.8° (c=1.0, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.21 (3H, d, J=6.8 Hz), 2.47 (2H, d, J=6.2 Hz), 2.80 (3H, s), 3.38 (3H, s), 3.64 (3H, s), 5.13 (2H, s), 7.34 (5H, s). b) A 1 N aqueous NaOH solution (23.5 ml, 23.5 mmol) was added to an ice-cooled solution of the β-methoxyester **4e** (6.6 g, 21.4 mmol) in dioxane (100 ml). The mixture was stirred at room temperature for 3 h, and the resulting solution was acidified to pH 4 by the addition of 20% aqueous citric acid. The mixture was then concentrated *in vacuo* and extracted with EtOAc. The organic phase was washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated *in vacuo* to give the β-methoxy acid. A solution of this acid and concentrated H_2SO_4 (0.8 ml) in CH_2Cl_2 (60 ml) was treated with liquid isobutene (25 ml) and the mixture was shaken for 48—96 h in a pressure bottle, then poured into a mixture of saturated aqueous $NaHCO_3$ and EtOAc. The organic phase was washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel using benzene–EtOAc (10 : 1) as the eluent to give **6e** (6.31 g, 84% yield) as a colorless oil, $[\alpha]_D^{27}$ −33.0° (c=1.0, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.21 (3H, d, J=6.8 Hz), 1.44 (9H, s), 2.38 (2H, d, J=6.2 Hz), 2.82 (3H, s), 3.38 (3H, s), 3.5—3.85 (1H, m), 3.85—4.4 (1H, m), 5.13 (2H, s), 7.34 (5H, s). EI-MS m/z: 319 ($M-MeOH$)$^+$. SI-MS m/z: 352 (MH^+). HR-MS m/z: Calcd for $C_{19}H_{30}NO_5$ (MH^+): 352.2133. Found: 352.2142.

Compounds **6a**,[3b] (3*S*)-**6a**,[3b] and **6b—d** were prepared from **3a**, (3*S*)-**3a**, and **3b—d**, respectively, as described above.

6b: Colorless oil (76% yield), $[\alpha]_D^{26}$ −28.9° (c=1.0, MeOH). EI-MS m/z: 337 ($M-C_4H_9+H$)$^+$, 320. SI-MS m/z: 394 (MH^+). HR-MS m/z: Calcd for $C_{22}H_{36}NO_5$ (MH^+): 394.2592. Found: 394.2607.

6c: Colorless oil (84% yield), $[\alpha]_D^{27}$ −42.0° (c=1.0, MeOH). EI-MS m/z: 347 ($M-MeOH$)$^+$, 323, 306. SI-MS m/z: 380 (MH^+). HR-MS m/z: Calcd for $C_{21}H_{34}NO_5$ (MH^+): 380.2436. Found: 380.2434.

6d: Colorless oil (75% yield), $[\alpha]_D^{27}$ −17.8° (c=1.0, MeOH). EI-MS m/z: 379 (M^+), 347, 323. SI-MS m/z: 380 (MH^+). HR-MS m/z: Calcd for $C_{21}H_{34}NO_5$ (MH^+): 380.2436. Found: 380.2436.

Compound **7** was similarly prepared from **3a** *via* **5**. Compound **5** was obtained simultaneously with **4a**, starting with **3a**. Compound **8** was obtained from **7** by hydrogenation using 5% Pd–C, and used in the next reaction without further purification.

7: Colorless oil (79% yield), $[\alpha]_D^{26}$ −28.5° (c=1.0, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.7—1.0 (6H, m), 1.1—1.2 (2H, m), 1.48 (9H, s), 1.7—1.82 (1H, m), 2.80 (3H, s), 4.2—4.6 (1H, br s), 5.14 (2H, s), 5.81 (1H, d, J=15.6 Hz), 6.80 (1H, dd, J=15.6, 7.3 Hz), 7.34 (5H, s). EI-MS m/z: 361 (M^+). SI-MS m/z: 362 (MH^+). HR-MS m/z: Calcd for $C_{21}H_{32}NO_4$ (MH^+): 362.2330. Found: 362.2350.

8: Colorless oil (quantative yield), $[\alpha]_D^{27}$ 17.3° (c=1.0, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.79—0.97 (6H, m), 1.06—1.32 (2H, m), 1.45 (9H, s), 1.5—1.8 (3H, m), 2.13—2.46 (3H, m), 2.38 (3H, s). EI-MS m/z: 214 ($M-CH_3$)$^+$.

***tert*-Butyl (3*R*,4*S*,5*S*)-4-(*N*-Benzyloxycarbonylamino)-3-methoxy-5-methylheptanoate (10)** The β-hydroxyester **9**[5] (183 mg, 0.5 mmol) was *O*-methylated with ethereal CH_2N_2 (large excess) and $BF_3 \cdot OEt_2$ (70 µl, 0.55 mmol) to give **10** (38 mg, 20% yield) as a pale yellow oil, $[\alpha]_D^{25}$ −11.3° (c=1.0, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$) δ: 0.75—1.1 (8H, m), 1.44 (9H, s), 1.5—1.7 (1H, m), 2.40 (2H, d, J=5.7 Hz), 3.36 (3H, s), 3.55—3.9 (2H, m), 4.45—4.75 (1H, m), 5.10 (2H, s), 7.33 (5H, s). *Anal.* Calcd for $C_{21}H_{33}NO_5$: C, 66.46; H, 8.77; N, 3.69. Found: C, 66.68; H, 9.02; N, 3.90.

Benzyl (2*R*,3*R*,2′*S*)-3-(*N*-*tert*-Butoxycarbonyl-2′-pyrrolidinyl)-3-hydroxy-2-methylpropanoate (11) and Its 2*S* Epimer (12) A 1.6 M solution of lithium diisopropylamide (LDA) (100 ml) in THF was cooled to −78 °C, and dry benzyl propionate (26.3 g, 0.16 mol) was added to it under an N_2 atmosphere. A solution of Boc-L-prolinal (23.9 g, 0.12 mol) in THF (100 ml) was added, and the reaction mixture was stirred for 30 min. A saturated aqueous solution of NH_4Cl was added, and the solvent was removed. The residue was extracted with EtOAc, and the organic phase was washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel using ether–petroleum ether (1 : 2) as an eluent to give oily **11** (7.63 g, 18% yield) and **12** (4.81 g, 11% yield). Other diastereomers were not obtained because of the low yields. **11**: $[\alpha]_D^{27}$ −28.4° (c=0.82, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.3 (3H, d, J=7.0 Hz), 1.45 (9H, s), 1.6—2.05 (4H, m), 2.61 (1H, quintet, J=7.0 Hz), 3.0—3.6 (2H, m), 3.7—3.9 (1H, br s), 4.06 (1H, dd, J=5.1, 4.8 Hz), 5.13 (2H, s), 7.34 (5H, s). EI-MS m/z: 345 ($M-H_2O$)$^+$. SI-MS m/z: 364 (MH^+). HR-MS m/z: Calcd for $C_{20}H_{30}NO_5$ (MH^+): 364.2123. Found: 364.2110. **12**: $[\alpha]_D^{25}$ −70.2° (c=0.31, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, d, J=7.3 Hz), 1.46 (9H, s), 1.6—2.1 (4H, m), 2.59 (1H, quintet, J=7.3 Hz), 3.1—3.6 (2H, m), 3.8—4.15 (1H, m), 3.8—4.15 (2H, m), 5.15 (2H, s), 7.35 (5H, s). EI-MS m/z: 363 (M^+), 345. SI-MS m/z: 364 (MH^+). HR-MS m/z: Calcd for $C_{20}H_{30}NO_5$ (MH^+): 364.2123. Found: 364.2131.

(2*R*,3*R*,2′*S*)-3-(*N*-*tert*-Butoxycarbonyl-2′-pyrrolidinyl)-3-hydroxy-2-methylpropanoic Acid (13)[4c] and Its 2*S* Epimer (14)[4c] The β-hydroxy acids **13** and **14** were obtained by hydrogenation in quantitative yields, starting with **11** and **12**, respectively. **13**: A white solid, mp 86—89 °C (lit.,[4c] 86 °C), $[\alpha]_D^{26}$ −56.8° (c=0.52, MeOH) [lit.,[4c] $[\alpha]_D^{22}$ −54.5° (c=1.0, MeOH)]. ^{1}H-NMR (DMSO-d_6) δ: 1.09 (3H, d, J=6.8 Hz), 1.39 (9H, s), 1.6—2.0 (4H, m), 2.25 (1H, m), 3.1—3.4 (2H, m), 3.67 (1H, m), 3.83 (1H, dd, J=7.5, 4.2 Hz), 4.7—5.1 (1H, br), 11.7—12.3 (1H, br). EI-MS m/z: 273 (M^+). HPLC, t_R: 4.1 min (33% of CH_3CN in 0.1% aqueous TFA). **14**: Colorless crystals, mp 151—152 °C (ether–hexane) (lit.,[4c] 153—154 °C), $[\alpha]_D^{26}$ −98.8° (c=0.51, MeOH) [lit.,[4c] $[\alpha]_D^{22}$ −94.7° (c=1.0, MeOH)]. ^{1}H-NMR (DMSO-d_6) δ: 1.02 (3H, d, J=7.0 Hz), 1.41 (9H, s), 1.5—1.95 (4H, m), 2.23 (1H, m), 3.1—3.35 (2H, m), 3.76 (1H, m), 3.96 (1H, m), 4.6—5.0 (1H, br), 11.6—12.0 (1H, br). EI-MS m/z: 273 (M^+). HPLC, t_R: 5.5 min (33% of CH_3CN in 0.1% aqueous TFA).

Benzyl (2*R*,3*R*,2′*S*)-3-(*N*-*tert*-Butoxycarbonyl-2′-pyrrolidinyl)-3-methoxy-2-methylpropanoate (15) NaH (60% oil suspension, 3.7 g, 93.6 mmol) was added to an ice-cooled solution of the β-hydroxyester **11** (17 g, 46.8 mmol) and MeI (14.6 ml, 234 mmol) in DMF (100 ml). Stirring was continued at 0 °C for 0.5 h, and then ice-cooled 5% aqueous $KHSO_4$ was added to the mixture. The solution was extracted with benzene–EtOAc (1 : 4). The organic phase was washed with 5% aqueous

$Na_2S_2O_3$ and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using benzene–EtOAc (10 : 1) as the eluent to give **15** (16.6 g, 94% yield) as a colorless oil, $[\alpha]_D^{24}$ −23.1° (c=0.32, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, d, J=7.0 Hz), 1.45 (9H, s) 1.6—2.05 (4H, m), 2.56 (1H, quintet, J=7.0 Hz), 3.1—3.5 (2H, m), 3.38 (3H, s), 3.6—3.95 (2H, br s), 5.05 (1H, d, J=12.0 Hz), 5.19 (1H, d, J=12.0 Hz), 7.34 (5H, s). EI-MS *m/z*: 345 $(M-MeOH)^+$. SI-MS *m/z*: 378 (MH^+). HR-MS *m/z*: Calcd for $C_{21}H_{32}NO_5$ (MH^+): 378.2279. Found: 378.2288. HPLC, t_R: 9.4 min (65% of CH_3CN in 0.1% aqueous TFA).

Compound **16** was prepared from **12**, as described above.

16: Colorless oil (80% yield), $[\alpha]_D^{24}$ −88.5° (c=0.38, MeOH). EI-MS *m/z*: 345 $(M-MeOH)^+$. SI-MS *m/z*: 378 (MH^+). HR-MS *m/z*: Calcd for $C_{21}H_{32}NO_5$ (MH^+): 378.2279. Found: 378.2279. HPLC, t_R: 8.4 min (65% of CH_3CN in 0.1% aqueous TFA).

Compounds **15** and **16** could be alternatively prepared from **11** and **12** by *O*-methylation with CH_2N_2 and $BF_3 \cdot OEt_2$ in 41% and 34% yields, respectively.

Benzyl (3*R*,2′*S*)-3-(*N*-*tert*-Butoxycarbonyl-2′-pyrrolidinyl)-3-hydroxypropanoate (18) This compound was prepared according to the literature method[11] using lithiobenzyl acetate in place of lithioethyl acetate, starting with Boc-L-prolinal, in 32% yield as an oil, $[\alpha]_D^{23}$ −23.7° (c=1.3, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 1.6—2.0 (4H, m), 2.47 (2H, d, J=6.8 Hz), 3.1—3.6 (2H, br s), 3.7—4.0 (1H, m), 4.1—4.3 (1H, m), 5.15 (2H, s), 7.34 (5H, s). EI-MS *m/z*: 331 $(M-H_2O)^+$. SI-MS *m/z*: 350 (MH^+). HR-MS *m/z*: Calcd for $C_{19}H_{28}NO_5$ (MH^+): 350.1966. Found: 350.1974.

(1*R*,8*S*)-Hexahydro-1-hydroxy-3*H*-pyrrolizin-3-one (18a)[11] The above compound **18** was treated with TFA to give a crystalline amine salt, mp 117—120 °C (EtOH–ether, 63% yield), $[\alpha]_D^{25}$ −13.3° (c=1.0, EtOH). This salt was then neutralized with aqueous K_2CO_3 to produce **18a** as colorless crystals, mp 81—83 °C (EtOAc–hexane, 82% yield), $[\alpha]_D^{25}$ −92.2° (c=0.36, $CHCl_3$). [lit.,[11] mp 84—86 °C, $[\alpha]_D^{22}$ −91.5° (c=1.0, $CHCl_3$)]. ^{1}H-NMR ($CDCl_3$) δ: 1.25—1.64 (1H, m), 1.86—2.32 (3H, m), 2.70—2.79 (2H, m), 2.9—3.2 (2H, m), 3.43—3.87 (2H, m), 4.1—4.4 (1H, m). EI-MS *m/z*: 141 (M^+).

Benzyl (3*R*,2′*S*)-3-(*N*-*tert*-Butoxycarbonyl-2′-pyrrolidinyl)-3-methoxypropanoate (19) This compound was prepared in a similar manner to that used for the synthesis of **15**, starting with **18**, in 65% yield as a colorless oil, $[\alpha]_D^{25}$ −58.7° (c=0.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 1.6—2.0 (4H, m), 2.47 (2H, d, J=7.5 Hz), 3.1—3.5 (2H, br s), 3.34 (3H, s), 3.6—3.8 (1H, m), 4.1—4.25 (1H, m), 5.14 (2H, s), 7.34 (5H, m). EI-MS *m/z*: 331 $(M-MeOH)^+$. SI-MS *m/z*: 364 (MH^+). HR-MS *m/z*: Calcd for $C_{20}H_{30}NO_5$ (MH^+): 364.2123. Found: 364.2127.

Benzyl (2*R*,2′*S*)-3-(*N*-*tert*-Butoxycarbonyl-2′-pyrrolidinyl)-2-methylpropanoate (17) 1,1′-Thiocarbonyldiimidazole (124 mg, 0.69 mmol) was added to a solution of the β-hydroxyester **11** (210 mg, 0.58 mmol) in dioxane (2 ml), and the mixture was stirred at room temperature for 24 h. The solvent was then removed, and the residue was purified by preparative TLC using hexane–EtOAc (2 : 3) as a developing solvent to give the thioimidazolide (161 mg, 59% yield) as a yellowish oil. A mixture of the thioimidazolide (56 mg, 0.12 mmol), *n*-Bu_3SnH (52 mg, 0.18 mmol), and 2,2′-azobis(isobutyronitrile) (1 mg, 0.006 mmol) in toluene (0.5 ml) was heated to 110 °C for 4 h under an N_2 atmosphere. The solvent was then removed, and the residue was purified by preparative TLC using hexane–EtOAc (2 : 1) as a developing solvent to give **17** (55.7 mg, 81% yield from the thioimidazolide) as a colorless oil, $[\alpha]_D^{26}$ −25.9° (c=0.58, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, d, J=7.0 Hz), 1.44 (9H, s), 1.6—1.95 (4H, m), 1.95—2.3 (2H, m), 2.35—2.75 (1H, m), 3.15—3.5 (2H, m), 3.75—3.95 (1H, m), 5.11 (2H, s), 7.34 (5H, s). EI-MS *m/z*: 347 (M^+). SI-MS *m/z*: 348 (MH^+). HR-MS *m/z*: Calcd for $C_{20}H_{30}NO_4$ (MH^+): 348.2174. Found: 348.2178.

Compound **20** was similarly prepared from **18**.

20: 22% yield (calculated from **18**) as a colorless oil, $[\alpha]_D^{25}$ −36.6° (c=1.3, MeOH). EI-MS *m/z*: 333 (M^+). SI-MS *m/z*: 334 (MH^+). HR-MS *m/z*: Calcd for $C_{19}H_{28}NO_4$ (MH^+): 334.2017 Found: 334.1995.

General Procedure for the Synthesis of the Val–Dil Unit Synthesis of **36e**: The catalyst, 5% Pd on activated carbon (0.1 g), was added to a solution of **6e** (0.7 g, 2.0 mmol) in *tert*-BuOH/H_2O (9 : 1) (20 ml). The mixture was stirred under an atmosphere of H_2 for 2 h, then filtered, and the filtrate was concentrated. Z-Val–OH (0.56 g, 2.23 mmol) and the residue were dissolved in CH_3CN (10 ml) and cooled to 0 °C. DCC (0.43 g, 2.1 mmol) was added, and the whole was stirred at 0 °C for 3 h, and then at room temperature for 16 h. After the usual work-up, purification by column chromatography on silica gel using benzene–EtOAc (5 : 1) as the eluent gave **36e** (0.67 g, 74% yield) as a colorless oil, $[\alpha]_D^{28}$ −31.4° (c=1.0, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, d, J=6.8 Hz), 1.00 (3H, d, J=6.8 Hz), 1.17 (3H, d, J=6.8 Hz), 1.45 (9H, s), 1.8—2.1 (1H, m), 2.25—2.45 (2H, m), 3.0 (3H, s), 3.37 (3H, s), 3.68 (1H, dd, J=12.1, 6.2 Hz), 4.35—4.75 (2H, m), 5.09 (2H, s), 5.56 (1H, br s), 7.33 (5H, s). EI-MS *m/z*: 395 $(M-C_4H_9+2H)^+$, 377. SI-MS *m/z*: 451 (MH^+). HR-MS *m/z*: Calcd for $C_{24}H_{39}N_2O_6$ (MH^+): 451.2807. Found: 451.2803.

Compounds **33a—c** and **36b—d** were similarly prepared from **32a—c** and **6b—d**. Compounds **40a**[7] and **40b—d** were also prepared from (3*S*)-**6a**,[3b] **10**, **39**, and **8**, respectively.

33a: Colorless oil (59% yield), $[\alpha]_D^{27}$ −11.0° (c=1.0, MeOH). EI-MS *m/z*: 418 $(M-MeOH)^+$, 394, 377. SI-MS *m/z*: 451 (MH^+). HR-MS *m/z*: Calcd for $C_{24}H_{39}N_2O_6$ (MH^+): 451.2807. Found: 451.2793.

33b: Colorless oil (59% yield), $[\alpha]_D^{26}$ −4.8° (c=1.0, MeOH). EI-MS *m/z*: 467 $(M-OC_4H_9)^+$. SI-MS *m/z*: 541 (MH^+). HR-MS *m/z*: Calcd for $C_{31}H_{45}N_2O_6$ (MH^+): 541.3276. Found: 541.3274.

33c: Colorless oil (63% yield), $[\alpha]_D^{24}$ −26.5° (c=1.0, MeOH). EI-MS *m/z*: 433 $(M-OC_4H_9)^+$. SI-MS *m/z*: 507 (MH^+). HR-MS *m/z*: Calcd for $C_{28}H_{47}N_2O_6$ (MH^+): 507.3432. Found: 507.3457.

36b: Colorless oil (85% yield), $[\alpha]_D^{26}$ −41.1° (c=1.0, MeOH). EI-MS *m/z*: 437 $(M-C_4H_9+2H)^+$, 419. SI-MS *m/z*: 493 (MH^+). HR-MS *m/z*: Calcd for $C_{27}H_{45}N_2O_6$ (MH^+): 493.3276. Found: 493.3250.

36c: Colorless oil (80% yield), $[\alpha]_D^{27}$ −46.2° (c=1.0, MeOH). EI-MS *m/z*: 478 (M^+), 446. SI-MS *m/z*: 479 (MH^+). HR-MS *m/z*: Calcd for $C_{26}H_{43}N_2O_6$ (MH^+): 479.3119. Found: 479.3140.

36d: Colorless oil (74% yield), $[\alpha]_D^{27}$ −32.9° (c=1.0, MeOH). EI-MS *m/z*: 423 $(M-C_4H_9+2H)^+$, 405. SI-MS *m/z*: 479 (MH^+). HR-MS *m/z*: Calcd for $C_{26}H_{43}N_2O_6$ (MH^+): 479.3119. Found: 479.3114.

40b: Colorless crystals (65% yield), mp 121—122 °C (EtOAc–hexane), $[\alpha]_D^{26}$ −3.9° (c=1.1, MeOH). EI-MS *m/z*: 446 $(M-MeOH)^+$, 423, 405. *Anal.* Calcd for $C_{26}H_{42}N_2O_6$: C, 65.25; H, 8.84; N, 5.85. Found: C, 65.35; H, 9.04; N, 5.96.

40c: Colorless oil (16% yield, The main product in this reaction was the acylurea derived from Z-Val–OH.), $[\alpha]_D^{25}$ −4.0° (c=1.2, $CHCl_3$). EI-MS *m/z*: 478 (M^+), 423. SI-MS *m/z*: 479 (MH^+). HR-MS *m/z*: Calcd for $C_{26}H_{43}N_2O_6$ (MH^+): 479.3119. Found: 479.3101.

40d: Colorless oil (78% yield), $[\alpha]_D^{25}$ −31.6° (c=1.0, MeOH). EI-MS *m/z*: 462 (M^+). SI-MS *m/z*: 463 (MH^+). HR-MS *m/z*: Calcd for $C_{26}H_{43}N_2O_5$ (MH^+): 463.3170. Found: 463.3148.

General Procedure for the Synthesis of the Dov–Val–Dil Unit Synthesis of **37e**: The dipeptide **36e** (0.65 g, 1.44 mmol) was deprotected by hydrogenolysis as described for **36e**. The deprotected product was dissolved together with Dov–OH (0.25 g, 1.72 mmol) in DMF (6 ml) and the solution was cooled to 0 °C. DEPC (0.29 g, 1.78 mmol) was added to the solution, followed by the addition of Et_3N (0.17 g, 1.68 mmol) in DMF (1 ml). The reaction mixture was stirred at 0 °C for 4 h, and at room temperature for 16 h, then diluted with benzene–EtOAc (1 : 4), washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using hexane–EtOAc (1 : 1) as the eluent to give **37e** (0.46 g, 72% yield) as a white solid (readily soluble in hexane), mp 75—78 °C, $[\alpha]_D^{27}$ −56.5° (c=1.0, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.8—1.1 (12H, m), 1.17 (3H, d, J=7.0 Hz), 1.45 (9H, s), 1.8—2.2 (2H, m), 2.27 (6H, s), 2.3—2.6 (3H, m), 3.05 (3H, s), 3.38 (3H, s), 3.55—3.85 (1H, m), 4.35—4.65 (1H, m), 4.65—4.95 (1H, m), 6.88 (1H, br s). EI-MS *m/z*: 443 (M^+), 440. *Anal.* Calcd for $C_{23}H_{45}N_3O_5$: C, 62.27; H, 10.22; N, 9.47. Found: C, 62.25; H, 10.48; N, 9.39.

Compounds **26a**, **26b**, **30a—d**, **34a—c**, **37b—d**, **41a**,[7] and **41b—d** were similarly prepared from Z-Val–OH, Z-*N*-methylvaline, **29a—d**, **33a—c**, **36b—d**, **40a**,[7] and **40b—d**, respectively.

26a: White solid (91% yield, readily soluble in hexane), mp 49—51 °C, $[\alpha]_D^{26}$ −47.7° (c=1.1, MeOH). *Anal.* Calcd for $C_{32}H_{53}N_3O_7$: C, 64.95; H, 9.03; N, 7.10. Found: C, 65.04; H, 9.22; N, 7.08.

26b: Colorless oil (94% yield), $[\alpha]_D^{29}$ −82.2° (c=1.0, MeOH). SI-MS *m/z*: 606 (MH^+). HR-MS *m/z*: Calcd for $C_{33}H_{56}N_3O_7$ (MH^+): 606.4117. Found: 606.4127.

30a: White solid (89% yield, readily soluble in hexane), mp 65—67 °C, $[\alpha]_D^{25}$ −47.3° (c=1.0, MeOH). EI-MS *m/z*: 385 $(M-C_4H_9)^+$, 369. *Anal.* Calcd for $C_{24}H_{46}N_2O_5$: C, 65.12; H, 10.48; N, 6.33. Found: C, 65.30; H, 10.85; N, 6.41.

30b: Colorless crystals (94% yield), mp 75—78 °C (hexane), $[\alpha]_D^{27}$

$-51.5°$ ($c=1.0$, MeOH). EI-MS m/z: 459 (MH^+), 403, 385. *Anal.* Calcd for $C_{24}H_{46}N_2O_6$: C, 62.85; H, 10.11; N, 6.11. Found: C, 63.08; H, 10.41; N, 6.15.

30c: Colorless oil (73% yield), $[\alpha]_D^{24}$ $-25.7°$ ($c=0.3$, $CHCl_3$). EI-MS m/z: 443 (M^+), 386. SI-MS m/z: 444 (MH^+). HR-MS m/z: Calcd for $C_{23}H_{46}N_3O_5$ (MH^+): 444.3436. Found: 444.3451.

30d: Colorless oil (62% yield), $[\alpha]_D^{26}$ $-42.9°$ ($c=1.0$, MeOH). EI-MS m/z: 457 (M^+), 414. SI-MS m/z: 458 (MH^+). HR-MS m/z: Calcd for $C_{24}H_{48}N_3O_5$ (MH^+): 458.3593. Found: 448.3588.

34a: Colorless oil (90% yield), $[\alpha]_D^{26}$ $-14.5°$ ($c=1.0$, MeOH). EI-MS m/z: 443 (M^+), 400. SI-MS m/z: 444 (MH^+). HR-MS m/z: Calcd for $C_{23}H_{46}N_3O_5$ (MH^+): 444.3436. Found: 444.3456.

34b: Colorless oil (86% yield), $[\alpha]_D^{27}$ $-18.0°$ ($c=1.0$, MeOH). EI-MS m/z: 533 (M^+), 490. SI-MS m/z: 534 (MH^+). HR-MS m/z: Calcd for $C_{30}H_{52}N_3O_5$ (MH^+): 534.3906. Found: 534.3915.

34c: White solid (73% yield, readily soluble in hexane), mp 101—104 °C, $[\alpha]_D^{27}$ $-45.7°$ ($c=1.0$, MeOH). *Anal.* Calcd for $C_{27}H_{53}N_3O_5$: C, 64.89; H, 10.69; N, 8.41. Found: C, 64.89; H, 10.97; N, 8.32.

37b: White solid (79% yield, readily soluble in hexane), mp 62—64 °C, $[\alpha]_D^{26}$ $-57.8°$ ($c=1.0$, MeOH). EI-MS m/z: 485 (M^+), 442. *Anal.* Calcd for $C_{26}H_{51}N_3O_5$: C, 64.29; H, 10.58; N, 8.65. Found: C, 64.19; H, 10.75; N, 8.61.

37c: White solid (70% yield, readily soluble in hexane), mp 70—72 °C, $[\alpha]_D^{28}$ $-62.8°$ ($c=1.0$, MeOH). EI-MS m/z: 471 (M^+), 428. *Anal.* Calcd for $C_{25}H_{49}N_3O_5$: C, 63.66; H, 10.47; N, 8.91. Found: C, 63.77; H, 10.66; N, 8.82.

37d: Colorless crystals (78% yield), mp 120—122 °C (ether–hexane), $[\alpha]_D^{27}$ $-51.0°$ ($c=1.0$, MeOH). EI-MS m/z: 428 ($M-CH(CH_3)_2)^+$. *Anal.* Calcd for $C_{25}H_{49}N_3O_5$: C, 63.66; H, 10.47; N, 8.91. Found: C, 63.58; H, 10.73; N, 8.80.

41b: Colorless crystals (70% yield), mp 146 °C (hexane), $[\alpha]_D^{26}$ $-22.5°$ ($c=0.53$, MeOH). EI-MS m/z: 471 (M^+), 428. *Anal.* Calcd for $C_{25}H_{49}N_3O_5$: C, 63.66; H, 10.47; N, 8.91. Found: C, 63.58; H, 10.83; N, 8.74.

41c: Colorless crystals (80% yield), mp 130—133 °C (ether–hexane), $[\alpha]_D^{26}$ $-45.1°$ ($c=0.76$, MeOH). EI-MS m/z: 471 (M^+), 428. *Anal.* Calcd for $C_{25}H_{49}N_3O_5$: C, 63.66; H, 10.47; N, 8.91. Found: C, 63.62; H, 10.79; N, 8.69.

41d: White solid (65% yield, readily soluble in hexane), mp 76—78 °C, $[\alpha]_D^{27}$ $-42.9°$ ($c=1.0$, MeOH). EI-MS m/z: 455 (M^+), 412. *Anal.* Calcd for $C_{25}H_{49}N_3O_4$: C, 65.90; H, 10.84; N, 9.22. Found: C, 65.68; H, 11.19; N, 9.07.

General Procedure for the Synthesis of the Dap–Doe Unit Synthesis of **43b**: The 2-demethyl-Dap derivative **19** (220 mg, 0.604 mmol) was deprotected by hydrogenolysis as described for **36e**. The deprotected product was dissolved together with Doe·TFA[3a)] (192 mg, 0.604 mmol) and BOP (267 mg, 0.604 mmol) in CH_3CN (3 ml), and iso-Pr_2NEt (195 mg, 1.51 mmol) was added to this solution at 0 °C. The mixture was stirred at room temperature for 4 h. After evaporation, the residue was dissolved in CH_2Cl_2. The CH_2Cl_2 phase was washed with 10% aqueous NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was then purified by column chromatography on silica gel using CH_2Cl_2–MeOH (50 : 1) as the eluent to give **43b** (262 mg, 94% yield) as colorless crystals, mp 130—132 °C (ether–hexane), $[\alpha]_D^{27}$ $-86.4°$ ($c=0.43$, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s), 1.7—2.1 (4H, m), 2.29 (2H, d, $J=$ 6.2 Hz), 3.27 (3H, s), 3.2—3.45 (4H, m), 3.6—4.1 (2H, br s), 2.63 (1H, m), 7.1—7.3 (6H, m), 7.24 (1H, d, $J=3.3$ Hz), 7.40 (1H, d, $J=3.3$ Hz). EI-MS m/z: 427 ($M-MeOH)^+$. *Anal.* Calcd for $C_{24}H_{33}N_3O_4S$: C, 62.72; H, 7.24; N, 9.14. Found: C, 62.55; H, 7.37; N, 8.79.

Compounds **43a**[3c)] and **43c—e** were similarly prepared from **16**, **11**, **17**, and **20**, respectively.

43c: Colorless crystals (80% yield), mp 69—72 °C (ether–hexane), $[\alpha]_D^{25}$ $-79.1°$ ($c=0.37$, MeOH). EI-MS m/z: 459 (M^+), 441. *Anal.* Calcd for $C_{24}H_{33}N_3O_4S$: C, 62.72; H, 7.24; N, 9.14. Found: C, 62.36; H, 7.37; N, 8.83.

43d: Pale yellow oil (92% yield), $[\alpha]_D^{27}$ $-57.5°$ ($c=0.60$, MeOH). EI-MS m/z: 443 (M^+), 370. SI-MS m/z: 444 (MH^+). HR-MS m/z: Calcd for $C_{24}H_{34}N_3O_3S$ (MH^+): 444.2319. Found: 444.2316.

43e: Pale yellow oil (95% yield), $[\alpha]_D^{26}$ $-64.3°$ ($c=1.5$, MeOH). EI-MS m/z: 429 (M^+). SI-MS m/z: 430 (MH^+). HR-MS m/z: Calcd for $C_{23}H_{32}N_3O_3S$ (MH^+): 430.2163. Found: 430.2144.

General Procedure A for the Synthesis of Analogs Synthesis of **38e**: a) A mixture of Boc–Dap–Doe (**22**)[3c)] (18 mg, 0.038 mmol) and 4 N HCl in dioxane (0.2 ml) was stirred at room temperature for 1 h. After evaporation, the residue was dried under a vacuum to yield the amine component. A mixture of the tripeptide **37e** (16.8 mg, 0.038 mmol) and 50% TFA in CH_2Cl_2 (0.2 ml) was stirred at room temperature for 1 h. After evaporation, the residue was dried under a vacuum to yield the carboxylic acid component.

b) The carboxylic acid and amine components described above were dissolved in DMF (0.5 ml) and the solution was cooled to 0 °C. DEPC (8.4 mg, 0.049 mmol) was then added, followed by the addition of Et_3N (27 μl, 0.114 mmol). The whole was stirred at 0 °C for 2 h, and at room temperature for 16 h, then diluted with benzene–EtOAc (1 : 4), and the whole was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by method II to give **38e** (19 mg, 68% yield) as a white amorphous powder, mp 86—89 °C, $[\alpha]_D^{25}$ $-79.1°$ ($c=0.67$, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.14 (3H, d, $J=7.0$ Hz), 1.18 (3H, d, $J=6.6$ Hz), 0.8—1.4 (12H, m), 1.5—1.9 (4H, m), 1.9—2.2 (2H, m), 2.52 (6H, br s), 2.3—2.7 (3H, m), 3.02 (3H, s), 3.33 (6H, s), 3.2—3.5 (4H, m), 3.7—4.0 (2H, m), 4.0—4.2 (1H, m), 4.70 (1H, m), 4.8—5.0 (1H, m), 5.56 (1H, m), 7.07 (1H, m), 7.1—7.3 (6H, m), 7.73 (1H, d, $J=3.3$ Hz). EI-MS m/z: 742 (M^+), 699. *Anal.* Calcd for $C_{39}H_{62}N_6O_6S\cdot0.6H_2O$: C, 62.14; H, 8.45; N, 11.15. Found: C, 62.34; H, 8.59; N, 10.76.

Analogs **31a—d**, **35a—c**, **38b—d**, **42a**,[7)] **42b—d**, and **44a—e** were similarly prepared from **30a—d**, **34a—c**, **37b—d**, **41a**,[7)] **41b—d** and **43a—e**, respectively. The physical properties of these analogs are shown in Tables 1—4.

Dov-Dil-OBu^t (24) Formaldehyde (37% solution, 2 ml) and 5% Pd on activated carbon (0.5 g) was added to a solution of **21**[3a)] (0.81 g, 1.65 mmol) in MeOH (30 ml). The mixture was stirred under an atmosphere of H_2 for 1 d, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using $CHCl_3$–MeOH (20 : 1) to give **24** (0.5 g, 78% yield) as colorless crystals. An analytical sample was recrystallized from hexane, mp 72—74 °C. $[\alpha]_D^{28}$ $-2.4°$ ($c=1.0$, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.8—1.15 (14H, m), 1.45 (9H, s), 1.6—1.82 (1H, m), 2.0—2.2 (1H, m), 2.3—2.6 (2H, m), 2.43 (6H, s), 2.91 (3H, s), 3.36 (3H, s), 3.8—4.2 (2H, m), 4.73 (1H, dd, $J=9.9$ and 5.5 Hz). EI-MS m/z: 386 (M^+), 371, 343. *Anal.* Calcd for $C_{21}H_{42}N_2O_4$: C, 65.24; H, 10.95; N, 7.25. Found: C, 65.21; H, 11.18; N, 7.00.

Dov-Dil-Dap-Doe (25) This compound was obtained by general procedure A, starting with **22**[3c)] and **24**, in 61% yield as a white amorphous powder, mp 86—89 °C, $[\alpha]_D^{24}$ $-65.1°$ ($c=0.95$, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.7—1.3 (17H, m), 1.6—2.0 (5H, m), 2.3—2.8 (4H, m), 2.54 (6H, s), 2.95 (3H, s), 3.2—3.7 (4H, m), 3.32 (3H, s), 3.34 (3H, s), 3.8—4.0 (2H, m), 4.0—4.25 (2H, m), 4.6—5.0 (1H, m), 5.4—5.8 (1H, m), 6.2—6.4 (1H, m), 7.21 (6H, s), 7.73 (1H, d, $J=3.3$ Hz). EI-MS m/z: 685 (M^+), 642, 594. SI-MS m/z: 686 (MH^+). HR-MS m/z: Calcd for $C_{37}H_{60}N_5O_5S$ (MH^+): 686.4312. Found: 686.4318. *Anal.* Calcd for $C_{37}H_{59}N_5O_5S\cdot1.7H_2O$: C, 62.02; H, 8.78; N, 9.77. Found: C, 62.27; H, 8.46; N, 9.18.

H-Val-Val-Dil-Dap-Doe·HCl (28a) Z-Val–Val–Dil–Dap–Doe (**27a**) was prepared according to general procedure A, starting with **26a**, in 85% yield as a white solid. The Z-peptide **27a** (48 mg, 0.054 mmol) was treated with TFA (1.5 ml) in the presence of thioanisole (0.15 ml) and *m*-cresol (0.15 ml) at room temperature for 8 h. Volatiles were removed *in vacuo*. The residue was dissolved in Et_2O, followed by the addition of 1 N aqueous HCl, and the aqueous phase was washed with Et_2O and concentrated *in vacuo*. The residue was then purified by method IV to give **28a** (31 mg, 71% yield) as a white amorphous powder, mp 111—116 °C, $[\alpha]_D^{24}$ $-69.3°$ ($c=0.38$, MeOH). ^{1}H-NMR ($CDCl_3$, selected data) δ: 3.03 (3H, s), 3.32 (6H, s), 7.21 (5H, s), 7.74 (1H, d, $J=2.9$ Hz). *Anal.* Calcd for $C_{40}H_{64}N_6O_6S\cdot0.75HCl$: C, 61.25; H, 8.32; N, 10.71. Found: C, 61.64; H, 8.04; N, 10.35.

***N*-Me-Val-Val-Dil-Dap-Doe·HCl (28b)** Z-*N*-Me–Val–Val–Dil–Dap–Doe (**27b**) was prepared according to general procedure A, starting with **26b**, in 69% yield as a white solid. The Z-peptide **28b** was prepared as described for the synthesis for **28a**, starting with **26b**, in 71% yield as a white amorphous powder, mp 100—103 °C, $[\alpha]_D^{24}$ $-71.8°$ ($c=0.33$, MeOH). ^{1}H-NMR ($CDCl_3$, selected data) δ: 2.46 (3H, s), 3.03 (3H, s), 3.33 (6H, s), 7.21 (5H, s), 7.73 (1H, d, $J=3.3$ Hz). *Anal.* Calcd for $C_{41}H_{66}N_6O_6S\cdot HCl$: C, 60.98; H, 8.36; N, 10.41. Found: C, 60.98; H, 8.51; N, 10.17.

(2*RS*,1'*S*)-2-(1'-*N*-*tert*-Butoxycarbonylaminoethyl)thiazolidine (45) This compound was prepared according to the method of Shioiri *et al.*,[4d,e)] starting with Boc-L-alaninal, in 43% yield as colorless crystals, mp 90—91 °C. EI-MS m/z: 232 (M^+). *Anal.* Calcd for $C_{10}H_{20}N_2O_2S$: C, 51.7; H, 8.68; N, 12.06. Found: C, 51.93; H, 8.88; N, 11.82.

(S)-2-(1′-N-tert-Butoxycarbonylaminoethyl)thiazole (46) This compound was obtained according to the literature method,[3a)] starting with **45**, in 10% yield as a pale yellow oil, $[\alpha]_D^{22}$ −36.0° (c=1.3, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s), 1.56 (3H, d, J=7.2 Hz), 4.9—5.2 (2H, m), 7.26 (1H, d, J=3.3 Hz), 7.67 (1H, d, J=3.3 Hz). EI-MS m/z: 228 (M^+). SI-MS m/z: 229 (MH^+). HR-MS m/z: Calcd for $C_{10}H_{17}N_2O_2S$ (MH^+): 229.1010. Found: 229.1035.

Dov-Val-Dil-Dap-OBzl (47) This compound was obtained by general procedure A, starting with **15** and **23**,[3a)] in 85% yield as a foam, $[\alpha]_D^{26}$ −44.0° (c=0.80, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.82 (3H, m), 0.90—1.05 (16H, m), 1.28 (3H, d, J=7.0 Hz), 1.3—1.42 (1H, m), 1.6—1.9 (3H, m), 1.95—2.15 (4H, m), 2.26 (6H, s), 2.4—2.5 (3H, m), 2.62 (1H, quintet, J=7.0 Hz), 3.02 (3H, s), 3.29 (3H, s), 3.33 (3H, s), 3.38—3.5 (3H, m), 4.01 (1H, m), 4.08—4.18 (2H, m), 4.80 (1H, dd, J=8.8, 6.4 Hz), 5.08—5.2 (2H, m), 6.9 (1H, br s), 7.2—7.4 (5H, m). EI-MS m/z: 688 (M^+), 645. *Anal.* Calcd for $C_{38}H_{64}N_4O_7 \cdot 0.4H_2O$: C, 65.56; H, 9.38; N, 8.05. Found: C, 65.30; H, 9.46; N, 7.70.

General Procedure B for the Synthesis of Analogs Synthesis of **50c**: The tetrapeptide **47** (69 mg, 0.1 mmol) was deprotected by hydrogenolysis as described for **36e**. The deprotected product was coupled with β-phenethylamine (13 mg, 0.11 mmol) using DEPC (19 mg, 0.11 mmol) and Et_3N (15 μl, 0.11 mmol) in DMF (0.3 ml). After the usual work-up, the crude product was purified by method II to give **50c** (56 mg, 80% yield) as a white amorphous powder, mp 75—78 °C, $[\alpha]_D^{25}$ −38.0° (c=0.57, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.80 (3H, t, J=7.3 Hz), 0.85—1.1 (16H, m), 1.19 (3H, d, J=7.0 Hz), 1.2—1.3 (1H, m), 1.3—1.4 (1H, m), 1.6—1.8 (3H, m), 1.9—1.96 (1H, m), 1.96—2.05 (2H, m), 2.05—2.1 (2H, m), 2.22 (6H, s), 2.3—2.4 (2H, m), 2.42 (1H, d, J=7.0 Hz), 2.81 (2H, t, J=7.0 Hz), 3.00 (3H, s), 3.29 (3H, s), 3.33 (3H, s), 3.4—3.5 (4H, m), 3.82 (1H, dd, J=8.1, 2.0 Hz), 4.0—4.07 (1H, m), 4.07—4.15 (1H, m), 4.77 (1H, dd, J=9.0, 6.4 Hz), 6.47 (1H, br s), 6.86 (1H, d, J=9.0 Hz), 7.1—7.3 (5H, m). EI-MS m/z: 701 (M^+), 658. *Anal.* Calcd for $C_{39}H_{67}N_5O_6$: C, 66.73; H, 9.62; N, 9.98. Found: C, 66.76; H, 9.79; N, 9.97.

Analogs **49**, **50a**, **50b**, and **50d** were similarly prepared from **46**, **48a**, **48b**, and **48d**, respectively. The physical properties of these analogs are shown in Tables 5.

***In Vivo* Antileukemic Evaluation** Murine P388 leukemia cells were obtained from the Cancer Chemother. Jpn. Fnd. Cancer Res. and maintained by weekly intraperitoneal (i.p.) transplantation into DBA/2 female mice (Charles River Japan, Inc.). Evaluation of the antitumor activity of the analogs was performed in accordance with the standard protocols of the United States National Cancer Institute.[12)] In brief, CDF_1 female mice were injected i.p. with 10^6 P388 cells on day zero, and solutions of compounds in 0.1% DMSO/0.1% Tween 80/physiological saline were administered i.p. on day 1 and day 5. The treated group consisted of six animals, and the control group of twelve animals. The medium survival times of the treated (T) and control (C) groups were determined, and the increase in life span (ILS) was calculated by using the following equation: $\%ILS=(T/C-1)\times 100$.

References and Notes

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2) All amino acids are in the L configuration unless otherwise noted. Other abbreviations used are as follows: Dov = dolavaline, Dil = dolaisoleuine, Dap = dolaproine, Doe = dolaphenine, Iva = isovaleric acid, TFA = trifluoroacetic acid, DEPC = diethyl phosphorocyanidate, DCC = 1,3-dicyclohexylcarbodiimide, BOP = benzotriazolyloxytris(dimethylamino)phosphonium hexafluorophosphate, CDI = 1,1′-carbonyldiimidazole, DMF = dimethylformamide, DMSO = dimethyl sulfoxide, Z = benzyloxycarbonyl, Boc = *tert*-butyloxycarbonyl, Bzl = benzyl, Bu^t = *tert*-butyl, ILS = increase in life span.
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