Amino Acids and Peptides. XX.[1] Preparation of β-Cyclododecyl Aspartate and Its Application to Synthesis of Fibronectin- and Laminin-Related Peptides

Koichi KAWASAKI,*,[a] Tomohiro MURAKAMI,[a] Kazuyoshi KOSHINO,[a] Machiko NAMIKAWA,[a] Mitsuko MAEDA,[a] Takao HAMA,[a] and Tadanori MAYUMI[b]

Faculty of Pharmaceutical Sciences, Kobe-Gakuin University,[a] Ikawadani-cho, Nishi-ku, Kobe 651–21, Japan and Faculty of Pharmaceutical Sciences, Osaka University,[b] Suita-shi, Osaka 565, Japan.

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β-Cyclododecyl aspartate was prepared and its application to peptide synthesis was examined. Derivatives of β-cyclododecyl aspartate are more likely to crystallize and should be useful for peptide synthesis. The cyclododecyl ester was much stable to bases than the benzyl ester and rather more stable than the cyclohexyl ester. It was removable by HF treatment and trifluoromethanesulfonic acid treatment. β-Cyclododecyl aspartate was used for synthesis of laminin- and fibronectin-related peptides by solid phase and liquid phase methods.

Keywords β-cyclododecyl aspartate; imide formation; aspartylimide; fibronectin-related peptide; laminin-related peptide; metastasis inhibitor

Imide formation at aspartyl bonds[2] is a serious problem during peptide synthesis. To suppress this undesired reaction, various β-esters of Asp (such as cyclopentyl,[3] cyclohexyl(cHx),[4] cycloheptyl,[5] cyclooctyl,[5] menthyl,[6] and adamantyl[7] esters) have been introduced instead of β-benzyl (Bzl) aspartate. These are bulky esters which sterically hinder imide formation. Since cyclododecanol has a bulky alkyl group, we examined cyclododecyl (cDo) ester as a protecting group for the β-carboxyl group of Asp.

β-Cyclododecyl aspartate [H–Asp(OcDo)–OH] was prepared according to the procedure reported by Tam *et al.*[4] for preparation of β-cyclohexyl aspartate as shown in Fig. 1. Boc–Asp–OBzl was esterified with cyclododecanol and dicyclohexylcarbodiimide (DCC) with the aid of 4-dimethylaminopyridine to obtain the diester compound, Boc–Asp(OcDo)–OBzl. The yield of the esterification was 66%. The benzyl group of the diester product was removed by hydrogenation, followed by trifluoroacetic acid treatment to give H–Asp(OcDo)–OH. The intermediates and H–Asp(OcDo)–OH were crystalline compounds and they were easily purified by recrystallization.

The stability of β-cyclododecyl ester to various deblocking agents was examined using Boc–Asp(OcDo)–OH. Boc–Asp(OcDo)–OH was exposed to deblocking agents and results were examined by thin-layer chromatography (TLC), as summarized in Table I. The cyclododecyl ester was removable with HF,[8] 1 M trifluoromethanesulfonic acid (TFMSA)–thioanisole in TFA[9] and 1 M trimethylsilyl trifluoromethanesulfonate (TMSOTf) in TFA,[10] and was stable to TFA, 1 M methanesulfonic acid (MSA)–thioanisole in TFA[9] and 1 M trimethylsilyl bromide (TMSBr)–thioanisole in TFA.[11]

The stability of β-cyclododecyl aspartate to bases was compared with that of β-benzyl aspartate. Boc–Asp(OX)–Y–NH_2 (X = Bzl, cHx, cDo; Y = Val, Gly) peptides were prepared by the mixed anhydride method[12] and treated with equimolar triethylamine (TEA), diisopropylethylamine (DIEA) and *N*-methylmorpholine (NMM) in DMF. The Asp(OBzl)–Gly sequence was reported to be base-sensitive, while the Asp(OBzl)–Val sequence was reported to be relatively stable to base-catalyzed imide formation.[13] Each mixture was examined by TLC. The developed thin layer plate was examined by ninhydrin test after treatment with 6 N HCl and analyzed with a TLC scanner. Prior to the test, spots of imide compound were determined as follows. Excess of TEA was added to a solution of Boc–Asp(OBzl)–Gly–NH_2 and the solution was checked by TLC (silica gel), infrared (IR) spectroscopy and field

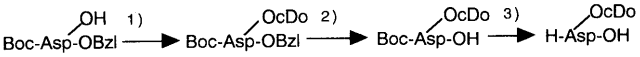

Fig. 1. Synthetic Scheme for H–Asp(OcDo)–OH

1) Cyclododecanol, DCC dimethylaminopyridine. 2) H_2/Pd. 3) TFA.

TABLE I. Stability of β-Cyclododecyl Ester to Acids at 0 °C

Acids	Cyclododecyl ester
HF (10 ml, 60 min)	Removable
1 M TFMSA–thioanisole/TFA (20 eq 60 min)	Removable
1 M TMSOTf–thianisole/TFA (20 eq 60 min)	Removable
TFA (1 ml 60 min)	Stable
1 M MSA–thianisole/TFA (20 eq 60 min)	Stable
1 M TMSBr–thioanisole/TFA (20 eq 60 min)	Stable

Boc–Asp(OcDo)–OH was treated with various acids and the products were examined by TLC.

TABLE II. Stability of Boc–Asp(OR)–Gly–NH_2 to Bases

R	TEA			DIEA			NMM		
	0.5 h	1.0 h	2.0 h	0.5 h	1.0 h	2.0 h	0.5 h	1.0 h	2.0 h
Bzl	73.3	39.5	27.1	64.4	32.8	25.9	95.9	94.4	94.2
cHx	97.0	94.2	92.9	95.9	94.5	92.2	100	100	100
cDo	99.4	98.4	94.7	100	100	100	100	100	100

Numbers in the table indicate unchanged amount (%) of Boc–Asp(OR)–Gly–NH_2. Each peptide was examined in DMF.

We dedicate this paper to Professor Yoshifumi Maki on the occasion of his retirement from Gifu Pharmaceutical University.

TABLE III. Stability of Boc–Asp(OR)–Val–NH_2 to Bases

R	TEA (1 eq)			DIEA (1 eq)			NMM (1 eq)		
	0.5 h	1.0 h	2.0 h	0.5 h	1.0 h	2.0 h	0.5 h	1.0 h	2.0 h
Bzl	97.8	96.6	94.7	99.1	97.3	96.3	100	100	100
cHx	100	100	100	100	100	100	100	100	100
cDo	100	100	100	100	100	100	100	100	100

Numbers indicate unchanged amount (%) of Boc–Asp(OR)–Val–NH_2.

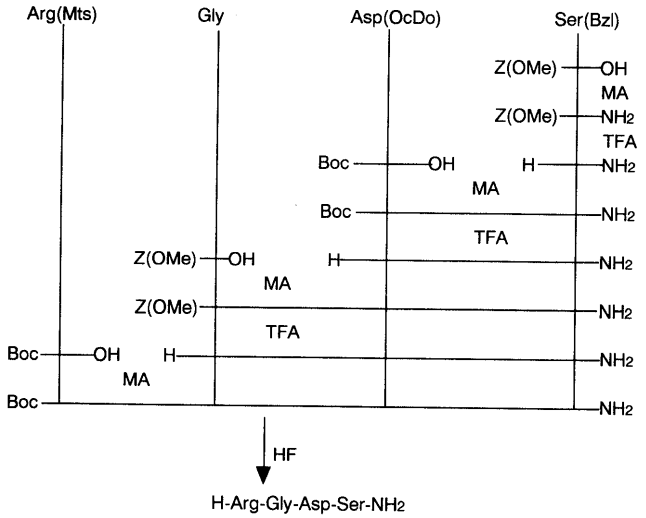

Fig. 2. Synthetic Scheme for H–Arg–Gly–Asp–Ser–NH_2

MA, mixed anhydride method.

desorption mass spectrometry (FD-MS). A new spot (*Rf* 0.33 in the solvent system of $CHCl_3$–MeOH–H_2O) below the spot of Boc–Asp(OBzl)–Gly–NH_2(*Rf* 0.52) on TLC appeared and the spot became increasingly intense. The IR spectrum of the solution indicated a decrease of the ester band. The FD-MS of the product also indicated formation of the imide.

The stability of the aspartylpeptides to various bases is summarized in Tables II and III. The stability of the aspartylpeptides shows that the stability of cyclododecyl ester to bases is much better than that of benzyl ester and somewhat better than that of cyclohexyl ester.

Application of Asp(OcDo) to peptide synthesis was examined by using the solid phase method and the solution method. A fibronectin-related peptide, H–Arg–Gly–Asp–Ser–NH_2 (RGDS), was found to be an inhibitor of experimental metastasis in mice.[14] Since the Asp–Ser sequence was also reported to be base-sensitive,[12] we prepared it using Asp(OcDo). The peptide was synthesized by the solution method as shown in Fig. 2. The yields of the coupling reactions of [Boc–Asp(OcDo)–OH + H–Ser(Bzl)–NH_2] and [Z(OMe)–Gly–OH + H–Asp(OcDo)–Ser(Bzl)–NH_2] were 80% and 78%, respectively. Thus Boc–Asp(OcDo)–OH could react smoothly in spite of the bulky cyclododecyl moiety. The inhibitory effect of the synthetic RGDS on experimental metastasis of B16 melanoma BL6 in mice[15] was examined. The peptide and melanoma cells were mixed and the mixture was injected into the tail vein of mice. Three weeks later, the number of melanoma colonies on the lungs was counted macroscopically. As shown in Fig. 3, RGDS exhibited a dose-dependent inhibitory effect.

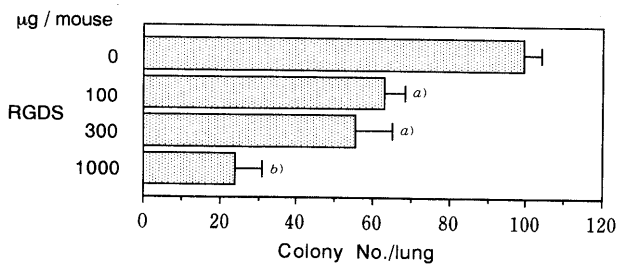

Fig. 3. Inhibitory Effect of RGDS on Formation of Lung Metastasis

B16-BL6 cells (1×10^5) were injected i.v. with various concentrations of RGDS. Lung colonies were examined 3 weeks later. Values are the mean ± S.E. *a*) $p < 0.05$; *b*) $p < 0.01$ compared with untreated control by Student's *t*-test.

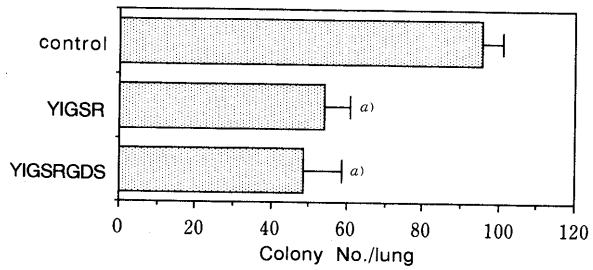

Fig. 4. Inhibitory Effect of YIGSR and YIGSRGDS on Formation of Lung Metastasis

B16-BL6 cells (1×10^5) were injected i.v. with 300 μg/mouse of YIGSR or YIGSRGDS. Lung tumor colonies were examined 3 weeks later. Values are the mean ± S.E. *a*) $p < 0.01$ compared with untreated control by Student's *t*-test.

Next, a laminin- and fibronectin-related octapeptide, H–Tyr–Ile–Gly–Ser–Arg–Gly–Asp–Ser–NH_2 (YIGSRGDS) was prepared by the solid phase method using Boc–Asp(OcDo)–OH. Laminin contains YIGSR sequence and the peptide YIGSR[16] was found to be an inhibitor of experimental metastasis in mice. The above octapeptide, YIGSRGDS, is a hybrid peptide of laminin- and fibronectin-related peptides. The hybrid might bind both RGDS and YIGSR receptors and its inhibitory effect on experimental metastasis is therefore of interest. Boc–Asp(OcDo)–OH and the following amino acid derivatives were used for the synthesis; Boc–Tyr(Bzl)–OH, Boc–Ile–OH, Z(OMe)–Gly–OH, Z(OMe)–Ser(Bzl)–OH, and Boc–Arg(Mts)–OH. These were assembled on *p*-methylbenzhydrylamine resin by an Applied Biosystem 430A peptide synthesizer controlled under the normal program. Since Z(OMe)–Gly–OH was not readily soluble in dichloromethane (DCM), its DMF solution was used when its symmetrical anhydride was prepared. The final deprotection was performed by HF treatment and the product was purified by ion-exchange column chromatography, followed by high performance liquid chromatography (HPLC). The product was shown by HPLC to be identical with YIGSRGDS prepared in the same way using Boc–Asp(OcHx)–OH. The yields of the purified octapeptide based on protected octapeptide-resin were 40% when β-cyclododecyl aspartate was used and 36% when β-cyclohexyl aspartate was used.

The inhibitory effect of the octapeptide on experimental metastasis was examined. As shown in Fig. 4, YIGSRGDS exhibited a remarkable inhibitory effect on the metastasis.

However, the inhibitory level of the octapeptide was same as that of YIGSR and RGDS (Fig. 3) at the dose of 300 μg/mouse. This result suggests that YIGSRGDS might not bind to both fibronectin and laminin receptors at the same time. A suitable spacer between YIGSR and RGDS may be necessary to allow the peptide to bind both receptors.

Derivatives of cyclododecyl aspartate are more likely to crystallize and should be useful for peptide synthesis.

Experimental

Melting points are uncorrected. Solvent systems for ascending TLC on Silica gel G (type 60, E. Merck) are indicated as follows: Rf^1 = BuOH–AcOH–H_2O (4:1:5, upper phase), Rf^2 = BuOH–AcOH–pyridine–H_2O (4:1:1:2), Rf^3 = $CHCl_3$–MeOH–H_2O (8:3:1, lower phase), Rf^4 = AcOEt–benzene (1:1), Rf^5 = $CHCl_3$–MeOH–AcOH (90:8:2), Rf^6 = $CHCl_3$. The stability of aspartyl peptide was examined on TLC with a Shimadzu dual-wavelength TLC scanner. Synthetic peptides were hydrolyzed in 6 N HCl at 110°C for 20 h. Amino acid compositions of acid hydrolysates were determined with a Hitachi 835 amino acid analyzer. Rotations were measured with a JASCO DIP-360 polarimeter.

Boc–Asp(OcDo)–OBzl DCC (1.49 g, 6 mmol) was added to a solution of cyclododecanol (1.11 g, 6 mmol), Boc–Asp–OBzl (1.94 g, 6 mmol) and dimethylaminopyridine (73 mg, 0.6 mmol) in DCM (30 ml) at −10 °C and the mixture was stirred overnight in a cold room. The precipitate was removed by filtration and the solvent was removed *in vacuo*. The residue was dissolved in AcOEt and the solution was washed with 5% citric acid, 5% $NaHCO_3$ and water. The solvent was removed *in vacuo* and the residue was recrystallized from ether/petroleum ether. Yield 1.95 g (66%), mp 121—122 °C, Rf^4 0.78, Rf^6 0.63, $[\alpha]_D^{25}$ −20.6° (c = 1.0, DMF). *Anal.* Calcd for $C_{28}H_{43}NO_6$: C, 68.68; H, 8.85; N, 2.86. Found: C, 68.36; H, 9.13; N, 2.86.

Boc–Asp(OcDo)–OH Boc–Asp(OcDo)–OBzl (1.8 g, 3.67 mmol) was hydrogenated in MeOH (40 ml) with Pd catalyst. The product was recrystallized from AcOEt/petroleum ether. Yield 1.41 g (96%), mp 99—100 °C, Rf^5 0.86, Rf^6 0.47, $[\alpha]_D^{25}$ −15.6° (c = 1.0, DMF). *Anal.* Calcd for $C_{21}H_{37}O_6N$: C, 63.13; H, 9.33; N, 3.51. Found: C, 62.86; H, 9.59; N, 3.56.

H–Asp(OcDo)–OH Boc–Asp(OcDo)–OH (1 g, 2.5 mmol) was treated with 5% anisole/TFA (6 ml) at room temperature for 1 h. Ether was added and the mixture was stored overnight in a cold room. The resulting precipitate was collected by filtration, washed with ether, and dried. Yield 944 mg (92%), mp 186—189 °C, *Anal.* Calcd for $C_{16}H_{29}NO_4 \cdot CF_3COOH$: C, 52.29; H, 7.31; N, 3.38. Found: C, 52.43; H, 7.47; N, 3.40.

Stability of Cyclododecyl Ester to Acidic Conditions Boc–Asp(OcDo)–OH was treated with various acids as described below and the mixtures were examined by TLC.

1) TFA Treatment: Boc–Asp(OcDo)–OH (30 mg) was dissolved in TFA (0.5 ml) and the solution was stirred at 0 °C for 1 h. The mixture was diluted with MeOH (1.5 ml) and was examined by TLC. A single spot (Rf^2 0.62 corresponding to H–Asp(OcDo)–OH) was observed.

2) HF treatment: Boc–Asp(OcDo)–OH (30 mg) was treated with a mixture of HF (9 ml) and anisole (1 ml) at 0 °C for 1 h. HF was removed *in vacuo* and the residue was dried overnight *in vacuo*. The residue was dissolved in water (1.5 ml) and the solution was examined by TLC. A single spot (Rf^2 0.08 corresponding to H–Asp–OH) was observed.

3) Others: Boc–Asp(OcDo)–OH (20 mg, 50 μmol) was treated with 1 mmol of the following acids as a 1 M solution in TFA containg 5% thioanisole: a) 1 M TFMSA–thioanisole/TFA, b) 1 M TMSOTf–thioanisole/TFA, c) 1 M MSA–thioanisole/TFA, d) 1 M TMSBr–thioanisole/TFA. Treatments with a and b gave a single spot (Rf^2 0.08) corresponding to H–Asp–OH on TLC. Treatment with c and d gave a single spot (Rf^2 0.62) corresponding to H–Asp(OcDo)–OH on TLC.

General Procedure for Preparation of Boc–Asp(OR)–X–NH_2 Isobutyl chloroformate (0.32 ml, 2.5 mmol) was added to a solution of Boc–Asp(OR)–OH (2.5 mmol) and NMM (0.28 ml, 2.5 mmol) in DMF (10 ml) at −10 °C and the mixture was stirred for 10 min. H–X–NH_2 (2.5 mmol) was dissolved in DMF (10 ml) and the solution was added to the above mixture. The whole was stirred overnight in a cold room. The solvent was removed *in vacuo* and the residue was extracted with AcOEt, followed by washing with 5% citric acid, 5% $NaHCO_3$ and water. The AcOEt was removed *in vacuo* and the residue was recrystallized from AcOEt/petroleum ether.

Boc–Asp(OcDo)–Gly–NH_2: Yield 71%, mp 100—102 °C, Rf^3 0.52, $[\alpha]_D^{24}$ −3.5° (c = 1.0, DMF). Amino acid ratios in an acid hydrolysate: Asp 1.00, Gly 1.02 (average recovery 82%). *Anal.* Calcd for $C_{23}H_{41}N_3O_6$: C, 60.63; H, 9.07; N, 9.22. Found: C, 60.35; H, 9.13; N, 9.24.

Boc–Asp(OcDo)–Val–NH_2: Yield 75%, mp 97—98 °C, Rf^3 0.67, $[\alpha]_D^{24}$ −2.7° (c = 1.0, DMF). Amino acid ratios in an acid hydrolysate: Asp 1.00, Val 0.95 (average recovery 87%). *Anal.* Calcd for $C_{26}H_{47}N_3O_6$: C,62.74; H, 9.51; N, 8.44. Found: C, 62.73; H, 9.59; N, 8.28.

Boc–Asp(OcHx)–Gly–NH_2: Yield 73%, mp 70—72 °C, Rf^3 0.49, $[\alpha]_D^{24}$ −6.5° (c = 1.0, DMF). Amino acid ratios in an acid hydrolysate: Asp 1.00, Gly 1.04 (average recovery 79%). *Anal.* Calcd for $C_{17}H_{29}O_6N_3$: C, 54.97; H, 7.86; N, 11.31. Found: C, 55.03; H, 7.71; N, 11.13.

Boc–Asp(OcHx)–Val–NH_2: Yield 70%, mp 79—82 °C, Rf^3 0.60, $[\alpha]_D^{24}$ −4.9° (c = 1.0, DMF). Amino acid ratios in an acid hydrolysate: Asp 1.00, Val 1.01 (average recovery 82%). *Anal.* Calcd for $C_{20}H_{35}N_3O_6$: C, 58.09; H, 8.53; N, 10.16. Found: C, 57.99; H, 8.60; N, 9.98.

Boc–Asp(OBzl)–Gly–NH_2: Yield 62%, amorphous powder, Rf^3 0.52. $[\alpha]_D^{24}$ −7.1° (c = 1.0, MeOH). Amino acid ratios in an acid hydrolysate: Asp 1.00, Gly 1.02 (average recovery 86%). *Anal.* Calcd for $C_{18}H_{25}N_3O_6$: C, 56.98; H, 6.64; N, 11.07. Found: C, 56.86; H, 6.83; N, 10.83. During storage, a new spot (Rf^3 0.33) corresponding to the imide compound appeared on TLC. This spot became more intense during storage.

Boc–Asp(OBzl)–Val–NH_2: Yield 84%, mp 188—140 °C, Rf^3 0.61, $[\alpha]_D^{28}$ −24.8° (c = 1.0, MeOH). *Anal.* Calcd for $C_{21}H_{31}N_3O_6$: C, 59.84; H, 7.41; N, 6.73. Found: C, 59.91; H, 7.66; N, 6.74.

Stability of Boc–Asp(OX)–Y–NH_2 to Bases A 1 M solution of a base (TEA, DIEA, NMM) in DMF (40 μl) was added to a 1 M solution of Boc–Asp(OX)–Y–NH_2 (X = cDo, cHx, Bzl. Y = Gly, Val) in DMF (40 μl) and the mixture was stirred at room temperature. An aliquot of the mixture was subjected to TLC and examined by ninhydrin test after treatment with 6 N HCl. Spots were measured with a TLC scanner at 570 nm. The results are shown in Tables II and III.

Imide Formation of Boc–Asp(Bzl)–Gly–NH_2 Boc–Asp(Bzl)–Gly–NH_2 (76 mg, 0.2 mmol) and TEA (56 μl, 0.4 mmol) were dissolved in $CHCl_3$ (3 ml) and the mixture was stirred at room temperature. An aliquot of the mixture was examined by IR spectroscopy and TLC. For measurement of the IR spectrum, an aliquot of the mixture was evaporated down and dried in a desiccator to remove TEA. The residue was dissolved again in $CHCl_3$ and its IR spectrum was measured.

A new spot (Rf^3 0.33) appeared and became more intense with time. The spot (Rf^3 0.52) corresponding to Boc–Asp(OBzl)–Gly–NH_2 became correspondingly weaker. The IR spectrum showed decreased absorbance of the ester band (1695 cm^{-1}). The spot at Rf^3 0.70 almost disappeared after refluxing the mixture. FD-MS also indicated the formation of imide. $[M+1]^+$ 380, corresponding to Boc–Asp(OBzl)–Gly–NH_2, disappeared and $[M+1]^+$ 272, corresponding to the imide compound, became the parent peak.

Imide formation of other Boc–Asp(OX)–Y–NH_2 compounds was also examined in the same manner.

Z(OMe)–Ser(Bzl)–NH_2 This compound was prepared from Z(OMe)–Ser(Bzl)–OH (3.59 g, 10 mmol) and 28% NH_4OH (6 ml) in tetrahydrofuran (THF) by the mixed anhydride method.[12] The solvent was evaporated off and the residue was extracted with AcOEt, followed by washing with 5% citric acid, 5% Na_2CO_3 and H_2O. The AcOEt was evaporated off and the residue was recrystallized from ether. Yield 2.86 g (80%), mp 129—130 °C, Rf^4 0.65, $[\alpha]_D^{24}$ +16.5° (c = 1.0, MeOH). *Anal.* Calcd for $C_{19}H_{22}N_2O_5$: C, 63.67; H, 6.19; N, 7.82. Found: C, 63.49; H, 6.22; N, 7.93.

Boc–Asp(OcDo)–Ser(Bzl)–NH_2 Z(OMe)–Ser(Bzl)–NH_2 (2.33 g, 6.5 mmol) was treated with TFA (3 ml) containing 10% anisole for 45 min and the product was precipitated by addition of ether. The product was washed with ether, dissolved in DMF (20 ml) and reacted with Boc–Asp(OcHx)–OH (2.6 g, 6.5 mmol) by the mixed anhydride method using isobutyl chloroformate (0.84 ml, 6.5 mmol) and NMM (0.72 ml, 6.5 mmol) in THF (15 ml) in the usual manner[12] for 5 h. The solvent was removed and residue was extracted with AcOEt. The extract was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O successively. The AcOEt was removed and the residue was recrystallized from AcOEt/petroleum ether. Yield 2.99 g (80%), mp 139—141 °C, Rf^3 0.89, Rf^5 0.69, $[\alpha]_D^{25}$ −9.2° (c = 1.0, DMF). *Anal.* Calcd for $C_{31}H_{49}N_3O_7$: C,

64.67; H, 8.57; N, 7.29. Found: C, 64.58; H, 9.04; N, 6.99. Amino acid ratios in an acid hydrolysate: Asp 1.00, Ser 0.96 (average recovery 82%).

Z(OMe)–Gly–Asp(OcDo)–Ser(Bzl)–NH_2 Boc–Asp(OcDo)–Ser(Bzl)–NH_2 (1.72 g, 3 mmol) was treated with 5% anisole/TFA (2 ml) for 1 h. The deblocked material was precipitated by addition of ether, washed with ether and dried. The material was coupled with Z(OMe)–Gly–OH (0.72 g, 3 mmol) by the mixed anhydride method in a mixture of THF/DMF for 5 h in the usual manner.[12] The solvent was removed *in vacuo* and the residue was extracted with AcOEt. The AcOEt layer was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O and dried with Na_2SO_4. The solvent was evaporated off and the residue was recrystallized from AcOEt. Yield 1.62 g (78%), mp 142—143 °C, Rf^5 0.65, $[\alpha]_D^{25}$ −11.1° (c=1.0, DMF). *Anal.* Calcd for $C_{37}H_{52}N_4O_9$: C, 63.77; H, 7.52; N, 8.04. Found: C, 63.57; H, 7.42; N, 7.81. Amino acid ratios in an acid hydrolysate: Gly 1.00, Asp 1.01, Ser 0.95 (average recovery 83%).

Boc–Arg(Mts)–Gly–Asp(OcDo)–Ser(Bzl)–NH_2 Z(OMe)–Gly–Asp(OcDo)–Ser(Bzl)–NH_2 (1.39 g, 2 mmol) was treated with 5% anisole/TFA (2 ml) for 45 min. The product was precipitated by addition of ether, washed with ether and reacted with Boc–Arg(Mts)–OH (0.91 g, 2 mmol) by the mixed anhydride method in a mixture of THF and DMF in the usual manner.[12] After the reaction, the solvent was removed *in vacuo* and the residue was extracted with AcOEt. The AcOEt layer was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O, and dried with Na_2SO_4. The solvent was removed and the residue was precipitated from AcOEt/petroleum ether. Yield 1.66 g (85%), mp 104—106 °C, Rf^5 0.45, $[\alpha]_D^{25}$ −9.3° (c=1.0, DMF). *Anal.* Calcd for $C_{48}H_{74}N_8O_{11}S \cdot H_2O$: C, 58.27; H, 7.75; N, 11.33. Found: C, 58.28; H, 7.50; N, 11.27. Amino acid ratios in an acid hydrolysate: Arg 1.00, Gly 1.02, Asp 1.00, Ser 0.98 (average recovery 98%).

H–Arg–Gly–Asp–Ser–NH_2 Boc–Arg(Mts)–Gly–Asp(OcDo)–Ser(Bzl)–NH_2 (1.55 g, 1.6 mmol) was treated with HF (30 ml) containing anisole (3 ml) at 0 °C for 1 h. HF was removed *in vacuo* and the residue was extracted with water, followed by washing with ether. After lyophilization, the material was purified by CM-cellulose column chromatography using 0.01—0.05 M $AcONH_4$ (pH 6.9) as an eluent. The 0.02 M $AcONH_4$ eluate was evaporated and the residue was lyophilized. The material was lyophilized from water containing HCl to convert it into the hydrochloride. Yield 560 mg (69%), hygroscopic powder, Rf^2 0.17, $[\alpha]_D^{25}$ +4.0° (c=1.0, H_2O), FAB-MS m/z: 433 $(M+1)^+$. Amino acid ratios in an acid hydrolysate: Arg 1.00, Gly 1.02, Asp 1.00, Ser 0.98 (average recovery 88%).

H–Tyr–Ile–Gly–Ser–Arg–Gly–Asp–Ser–NH_2 This compound was prepared by the solid phase method using an Applied Biosystems 430A peptide synthesizer. *p*-Methylbenzhydrylamine resin (NH_2 content: 0.64 meq/g, 780 mg) and the following amino acid derivatives were used: Boc–Asp(OcDo)–OH, Boc–Asp(OcHx)–OH, Z(OMe)–Ser(Bzl)–OH, Boc–Arg(Mts)–OH, Z(OMe)–Gly–OH, Boc–Ile–OH, Boc–Tyr(Bzl)–OH. Since Z(OMe)–Gly–OH was not readily soluble in dichloromethane, DMF solution was used for preparation of its symmetrical anhydride.

a) When Boc–Asp(OcDo)–OH was used, 1.5 g of final peptide-resin was obtained, and it was treated with 5% *m*-cresol/HF(20 ml) at 0 °C for 1 h. Purification was performed by CM-cellulose column chromatography using 0.01—0.1 M $AcONH_4$. The desired material was obtained in the 0.02 M $AcONH_4$ eluate. The material was converted to its hydrochloride by lyophilization from HCl-containing water. Yield 184 mg (40%, calculated from the peptide-resin), Rf^2 0.10, $[\alpha]_D^{25}$ −27.5° (c=1.0, H_2O), FAB-MS m/z: 854 $(M+1)^+$. Amino acid ratios in an acid hydrolysate: Tyr 0.92, Ile 0.95, Gly 2.00, Ser 1.99, Arg 1.01, Asp 1.02 (average recovery 76%).

b) When Boc–Asp(OcHx)–OH was used, 165 mg (35%) of the desired material was obtained by the same procedure as described above. Rf^2 0.10, $[\alpha]_D^{25}$ −29.8° (c=1.0, H_2O), FAB-MS m/z: 854 $(M+1)^+$. Amino acid ratios in an acid hydrolysate: Tyr 1.00, Ile 1.03, Gly 2.00, Ser 2.09, Arg 0.97, Asp 1.04 (average recovery 80%). The materials obtained by methods a and b were shown to be identical by HPLC on a YMC AQ-303 column using a mixture of CH_3CN and H_2O as an eluent.

Metastasis Assay The assay was performed as reported.[15]

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References and Notes

1) Standard abbreviations are used for amino acids, protecting groups, and peptides [*Eur. J. Biochem.*, **138**, 9 (1984)]. Other abbreviations include: Mts = mesitylenesulfonyl, DMF = dimethylformamide, TFA = trifluoroacetic acid.
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