SYNTHESIS AND ANTICANDIDAL ACTIVITIES OF OPTIMIZED ANALOGS OF ANTIBIOTIC Sch 37137

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Peptide analogues of Sch 37137 the antifungal antibiotic have been synthesized and evaluated *in vitro* against *Candida* sp. Di- and tripeptides containing methionine, leucine, norvaline, lysine, glutamic acid and N^3-(*trans*-epoxysuccinamoyl)-L-2,3-diaminopropanoic acid, (EADP) were obtained. Peptides containing (D)-, and (L)-*trans*-epoxysuccinamic acid were also prepared. All of the analogues examined displayed in general higher anticandidal activity than a mixture of diastereomers of Sch 37137.

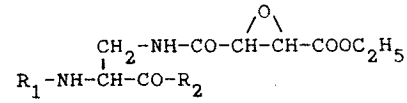
Fungal infections caused mainly by *Candida albicans* have increased rapidly in the last decades especially in immunocompromised patients such as patients with AIDS, organ transplant recipients, cancer patients undergoing chemotherapy and can cause secondary infections that are life threatening[1~4]. Unfortunately, antifungal drugs currently released for clinical use have not offered substantive improvements over amphotericin B, still the drug of choice for most fungal diseases[5]. Therefore, there is still a need for new, safer and more potent antifungal agents.

Fungal cell wall biosynthesis can provide important targets for antifungal agents[6]. Glucosamine-6-phosphate synthase, an enzyme which enables the biosynthetic formation of glucosamine containing macromolecules of the fungal cell wall, has been proposed by us as a target for the design of antifungal agents[7]. Our efforts towards rational design of antifungal agents based on this approach have been focused on glucosamine-6-phosphate synthase inhibitors, N^3-(4-methoxyfumaroyl)- and N^3-iodoacetyl-L-2,3-diaminopropanoic acids and their peptide conjugates with antifungal activity[8~10]. Recently Schering group isolated[11] and synthesized[12] antibiotic Sch 37137, *i.e.* N^2-L-alanyl-N^3-(D-*trans*-epoxysuccinamoyl)-L-2,3-diaminopropanoic acid. Structural similarity of this antibiotic to antibiotic A 19009[13~15] (which differs from Sch 37137 by containing a double bond instead of an epoxide ring) and to N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP) derivatives[9], both being the glutamine analogs, suggests its mechanism of antifungal action by inhibition of glucosamine-6-phosphate synthase. Our previous report on Sch 37137[16], showed that its diastereoisomeric mixture exhibited a high degree of activity against *Candida* species. This prompted us to synthesize a series of Sch 37137 analogues in order to improve their *in vitro* anticandidal activities and to gain information on their structure activity relationships. This report describes the synthesis and *in vitro* anticandidal evaluation of a series of peptide analogs of Sch 37137.

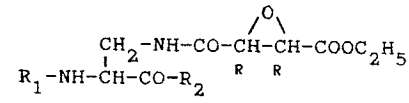
Chemistry

Classical reactions used in peptide chemistry were employed for the preparation of a series of analogs of Sch 37137, including synthesis of a diastereomeric mixtures as well as both enantiomers. The 2*R*,3*R*

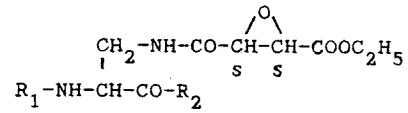
Chart 1.



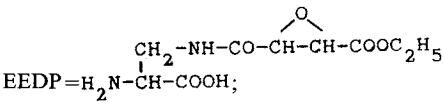
1	R_1=H;	R_2=OH
2	R_1=Boc-Nva;	R_2=OH
3	R_1=Boc-Met;	R_2=OH
4	R_1=Boc-Leu;	R_2=OH
5	R_1=Nva;	R_2=OH
6	R_1=Boc-Nva-Nva;	R_2=OH
7	R_1=Boc-Lys(Boc)-Nva;	R_2=OH
8	R_1=Boc-Glu(OBzl)-Nva;	R_2=OH
9	R_1=Boc-Glu-Nva;	R_2=OH
10	R_1=Boc;	R_2=Nva
11	R_1=H;	R_2=Nva
12	R_1=Boc-Nva;	R_2=Nva

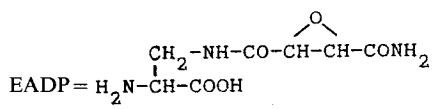


13	R_1=H;	R_2=OH
14	R_1=Boc-Nva;	R_2=OH
15	R_1=Nva;	R_2=OH
16	R_1=Boc-Lys(Boc)-Nva;	R_2=OH



17	R_1=H;	R_2=OH
18	R_1=Boc-Nva;	R_2=H





Boc =*tert*-Butoxycarbonyl

configuration of the epoxide moiety of natural Sch 37137 has been established independently by other authors[12]. Most of the syntheses were performed with the use of the racemic *trans*-epoxysuccinic acid. Our method of synthesis was based in general on the procedure reported earlier for the preparation of Sch 37137 derivatives[16]. Compounds obtained during the synthesis are summarized in Chart 1. Treatment of N^3-(DL-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic acid (EEDP) **1** with *N*-hydroxy-succinimide esters[17] of Boc-Nva, Boc-Met and Boc-Leu afforded the protected dipeptides **2**, **3** and **4**, respectively, in good yields. Dipeptide Boc-Nva-EEDP **2** after deptotection of the Boc group was coupled again using the active ester strategy with Boc-Nva, Boc-Lys(Boc) and Boc-Glu(OBzl)[18] to give the protected tripeptides **6**, **7** and **8**. Boc-EEDP-OSu[16] was reacted with norvaline to give dipeptide **10** which was reacted either with ammonia first followed by deblocking with trifluoroacetic acid to obtain the deprotected dipeptide **10a** or deblocked with trifluoroacetic acid first and reacted with active ester of Boc-Nva to furnish the protected tripeptide Boc-Nva-EEDP-Nva **12**. Racemic *trans*-epoxysuccinic acid was resolved into (D)- and (L)-isomers with (D)- or (L)-arginine by diastereomeric salt formation[19]. (D)- and (L)-*trans*-epoxysuccinic acids were converted into their monomethyl esters according to the procedure described earlier[19] and activated with DCC and HOSu to give the active esters which were allowed to react with N^2-(*tert*-butoxycarbonyl)-L-2,3-diaminopropanoic acid (Boc-A_2pr)[20], deblocked and coupled with protected amino acids using the earlier reported methodology to obtain the peptides **14**, **16** and **18**. The protected peptides after treatment with ammonia followed by acidolysis with trifluoroacetic acid gave the Sch 37137 analogs, **2a**~**18a** (Table 1) for antifungal testing.

Results and Discussion

The *in vitro* activity of Sch 37137 analogs was examined against selected *Candida* sp. by the broth

Table 1. Analytical data of deprotected peptides.

No.	Compound	Yield[a] (%)	$[\alpha]_{578}^{25}$ (*c* 1, MeOH)	MP (°C)	Formula analyses
2a	Nva-EADP	75	+ 9.8	—	$C_{12}H_{20}N_4O_6CF_3COOH$ Calcd: C 38.88, H 5.32, N 12.95 Found: C 38.44, H 5.04, N 12.67
3a	Met-EADP	68	+ 6.2	—	$C_{12}H_{20}N_4O_6SCF_3COOH$ Calcd: C 36.36, H 4.57, N 12.11 Found: C 36.22, H 4.55, N 11.95
4a	Leu-EADP	79	+ 5.8	—	$C_{13}H_{22}N_4O_6CF_3COOH$ Calcd: C 40.54, H 5.21, N 12.60 Found: C 40.34, H 5.06, N 12.42
6a	Nva-Nva-EADP	72	+ 4.0	—	$C_{17}H_{29}N_5O_7CF_3COOH$ Calcd: C 41.77, H 5.84, N 13.53 Found: C 41.72, H 5.66, N 13.63
7a	Lys-Nva-EADP	66	− 4.8	—	$C_{18}H_{32}N_6O_72CF_3COOH$ Calcd: C 42.92, H 5.56, N 13.65 Found: C 42.77, H 5.32, N 13.76
9a	Glu-Nva-EADP	81	− 1.2	—	$C_{17}H_{27}N_5O_9CF_3COOH$ Calcd: C 40.78, H 5.04, N 12.51 Found: C 40.69, H 4.89, N 12.56
10a	EADP-Nva	70	− 8.0	—	$C_{12}H_{20}N_4O_6CF_3COOH$ Calcd: C 38.88, H 5.32, N 12.95 Found: C 38.66, H 5.25, N 12.98
12a	Nva-EADP-Nva	77	+ 8.2	—	$C_{17}H_{29}N_5O_7CF_3COOH$ Calcd: C 41.77, H 5.84, N 13.53 Found: C 41.52, H 5.82, N 13.31
14a	Nva-EADP[b]	77	−20.2	120～122	$C_{12}H_{20}N_4O_6CF_3COOH$ Calcd: C 38.88, H 5.32, N 12.95 Found: C 38.68, H 5.29, N 13.05
16a	Lys-Nva-EADP[b]	68	− 9.8	—	$C_{18}H_{32}N_6O_72CF_3COOH$ Calcd: C 42.92, H 5.56, N 13.65 Found: C 42.84, H 5.41, N 13.66
18a	Nva-EADP[c]	80	+25.8	138～140	$C_{12}H_{20}N_4O_6CF_3COOH$ Calcd: C 38.88, H 5.32, N 12.95 Found: C 38.77, H 5.19, N 12.79

In most cases, trifluoroacetate salts of peptides form hygroscopic, amorphous powders without reproducible melting points.

[a] Yield of ammonolysis and deprotection.

[b] D-Configuration of the epoxy acid.

[c] L-Configuration of the epoxy acid.

dilution method in YNB medium[21] (agar dilution method was also applied for comparison purposes). As shown in Table 2 most of the synthesized peptides appeared to be more active than a racemic mixture of Sch 37137 previously prepared in our laboratory[16]. However, among the peptides obtained, the activity changed depending on the amino acid incorporated into the peptide chain. Dipeptide with norvaline residue in the *N*-terminal position was shown to be the most active peptide tested. Also lengthening of a peptide chain altered the anticandidal activity. In general, tripeptides were somewhat less potent than dipeptides. Chirality of the *trans*-epoxysuccinic acid was another factor influencing on the activity. Thus, dipeptide having D-*trans*-epoxysuccinamic acid (2*R*,3*R* isomer) showed higher activity than compound with the L- acid (approximately 20 times higher) suggesting that the D chirality is essential for antifungal activity. Accordingly, the tripeptide Lys-Nva-EADP with the (2*R*,3*R*) configuration of the epoxy acid exhibited higher activity than corresponding peptide with a racemic epoxy acid. Interestingly, peptide **11**

Table 2. *In vitro* activity of Sch 37137 analogues.

Compound	MIC[a] (μg/ml)					
	Candida albicans ATCC 26278 YNB medium		*Candida krusei*	*Candida glabrata*	*Candida famata* 1940	*Candida albicans* 2043
	Broth	Agar				
Ala-EADP[b]	1.2	2.0	NT	1.5	NT	1.0
Nva-EADP	0.25	0.75	0.35	0.75	0.50	0.25
Met-EADP	0.35	0.75	1.0	1.25	0.5	0.5
Leu-EADP	0.3	0.75	0.5	0.75	0.5	0.25
Nva-Nva-EADP	0.75	2.0	1.2	1.0	1.0	0.75
Lys-Nva-EADP	0.50	1.0	1.5	0.75	1.0	0.50
Glu-Nva-EADP	0.75	1.5	1.2	1.5	0.75	0.50
EADP-Nva	0.5	1.5	1.0	1.25	0.75	0.75
Nva-EADP-Nva	0.75	2.0	1.5	1.5	1.0	0.50
Nva-EADP[c]	0.15	0.50	0.35	0.5	0.35	0.10
Lys-Nva-EADP[c]	0.20	0.50	0.75	0.50	0.50	0.25
Nva-EADP[d]	7.5	15.0	10.0	12.5	10.0	6.50
EEDP-Nva	12.5	25.0	>15.0	>15.0	>15.0	>15.0
Lys-Nva-FMDP	0.75	1.5	1.0	1.5	0.75	0.7

Peptides were tested as trifluoroacetate salts.

[a] Yeast nitrogen base (Difco) - YNB medium containing 200 μg/ml of sodium glutamate, 10^6 cfu/ml, 30°C, 24 hours; agar method (YNB medium with 2% agar).

[b] Diasteromeric mixture of Sch 37137.

[c] D-Configuration of the epoxy acid.

[d] L-Configuration of the epoxy acid.

Table 3. MIC, transport and intracellular cleavage rates of selected EADP-peptides in *Candida albicans* ATCC 26278.

Compound	MIC (μg/ml)	Transport rate (nmol/minute /mg protein)	Intracellular cleavage rate (nmol/minute /mg protein)
Nva-EADP	0.25	2.0	20.4
Leu-EADP	0.3	2.3	27.0
Lys-Nva-EADP[a]	0.2	4.2	37.5
Nva-Nva-EADP	0.75	1.4	16.3
Nva-EADP-Nva	0.75	2.4	4.9

[a] D-Configuration of the epoxy acid.

Table 4. Change of antifungal activity of EADP-peptides against *Candida albicans* ATCC 26278 in serum protein solution[a] expressed as % of standard activity.

Compound	5% HSA[b]	HS[b]
Leu-EADP	100	25
Nva-EADP	100	10
Lys-Nva-EADP	85	20
Nva-Nva-EADP	75	25
Nva-EADP-Nva	60	30

[a] Growth was performed in the 5% solution of serum albumin in YNB modified medium or 1:1 mixture of YNB modified medium and serum, but peptides were previously preincubated in the presence of serum proteins for 2 hours at 37°C before inoculation.

[b] HSA—Human serum albumin, HS—Human serum.

(EEDP-Nva) containing an ethyl ester instead of an amide group in the *trans*-epoxy acid moiety showed weak anticandidal activity. The most active peptide containing N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP), *i.e.* Lys-Nva-FMDP[9] was also used for comparative testing. This compound however was less active than the Sch 37137 analogues obtained in this study.

The uptake of various peptides with the racemic epoxide moiety (Table 3) into fungal cells showed approximately the same range of velocities. Moreover the rate of intracellular cleavage by peptidases ranged from 4.9 (for Nva-EADP-Nva) to a high value of 37.5 nmol/minute/mg protein (for Lys-Nva-EADP) and showed good correlation with antifungal activity. We have also shown that the peptides were partially

inactivated by human serum components, thus affecting the effectiveness of the Sch 37137 and its analogs against fungal cells. The same phenomenon was observed earlier by us for N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid peptides[22]. Incubation of the peptides (Table 4) with 5% human serum albumin and especially in complete human resulted in the significant loss of their antifungal efficacy. It suggested the interaction of serum proteins with the peptides by an unknown mechanism which needs further investigation.

In conclusion, a series of Sch 37137 analogs have been synthesized and evaluated for *in vitro* anticandidal activity. These peptides are taken up into cells *via* peptide permeases ("portage" transport[23]), followed by enzymatic hydrolysis inside the cell and intracellular generation of the enzyme inhibitor. Anticandidal activity is influenced by the length of the peptide chain, the kind of amino acid incorporated into the peptide and the chirality of the *trans*-epoxysuccinic acid.

Experimental

^{1}H NMR spectra were recorded at 60 MHz on a Varian 360 instrument with Me_4Si as internal reference. Optical rotations were measured in a Polamat A (Carl Zeiss Jena) polarimeter. Melting points were determined in an open capillary tubes and are uncorrected. TLC was carried out using Kieselgel 60 (Merck) plates. The analytical results obtained for C, H and N were within $\pm 0.4\%$ of the theoretical values.

The *N*-hydroxysuccinimide esters of *N*-(*tert*-butoxycarbonyl)amino acids (Nva, Lys, Met, Leu and Glu(OBzl)) were prepared by the previously described method[17]. Monoethyl L- and D-*trans*-epoxysuccinate were prepared according to the published procedure[19].

Representative synthetic route to a Sch 37137 analogue.

N^2-(*tert*-Butoxycarbonyl-L-norvalyl)-N^3-(DL-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic Acid **2** (Boc-Nva-EEDP)

To a cooled solution of N^3-(DL-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic acid trifluoroacetate salt[16] **1** (1.44 g, 4 mmol) and $NaHCO_3$ (0.69 g, 8 mmol) in a mixture of water-methanol (1:1 v/v) (10 ml) was added Boc-Nva-OSu (1.25 g, 4 mmol) in MeOH (10 ml) with stirring. The reaction mixture was stirred for 1 hour in an ice-bath then at room temperature overnight. After the usual work-up and evaporation of the solvents compound **2** (1.69 g, 90% yield) was obtained. MP 69~70°C, $[\alpha]_{578}^{25}$ −14.2° (*c* 1, MeOH), ^{1}H NMR (DMSO-d_6) δ 0.9 (3H, m), 1.1 (3H, t, J=7 Hz), 1.2 (9H, s), 1.3~1.5 (4H, m), 3.5~3.7 (4H, m), 3.8 (1H, m), 4.1 (1H, m), 4.3 (2H, q, J=7 Hz), 6.6~6.8 (2H, m), 7.2 (1H, m).

Anal Calcd for $C_{19}H_{31}N_3O_9$: C 51.22, H 7.01, N 9.43.
Found: C 51.38, H 7.27, N 9.21.

The following compounds were also prepared.

N^2-(*tert*-Butoxycarbonyl-L-methionyl)-N^3-(DL-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic Acid **3**

1.46 g (77% yield). MP 81~83°C, $[\alpha]_{578}^{25}$ −12.8° (*c* 1, MeOH), ^{1}H NMR (DMSO-d_6) δ 1.1 (3H, t, J=7 Hz), 1.1~1.3 (2H, m), 1.4 (9H, s), 2.1 (3H, s), 2.2~2.4 (2H, m), 2.6 (2H, m), 3.5~3.7 (4H, m), 3.8 (1H, m), 4.2 (1H, m), 6.7 (2H, m), 7.2 (1H, m).

Anal Calcd for $C_{19}H_{31}N_3O_9S$: C 47.78, H 6.54, N 8.79.
Found: C 47.44, H 6.62, N 8.59.

N^2-(*tert*-Butoxycarbonyl-L-leucyl)-N^3-(DL-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic Acid **4**

1.5 g (82% yield). MP 79~80°C, $[\alpha]_{578}^{25}$ −26.2° (*c* 1, MeOH), ^{1}H NMR (DMSO-d_6) δ 0.9~1.4 (21H, br m), 1.5 (2H, m), 3.4~3.7 (4H, m), 3.8 (1H, m), 4.1 (1H, m), 4.3 (2H, q, J=7 Hz), 6.6~6.8 (2H, m),

7.2 (1H, m).

Anal Calcd for $C_{20}H_{33}N_3O_9$: C 52.26, H 7.23, N 9.15.
Found: C 52.04, H 7.26, N 9.22.

N^2-L-Norvalyl-N^3-(DL-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic Acid Trifluoroacetate Salt **5**

Compound **2** (1.33 g, 3 mmol) was dissolved in cold trifluoroacetic acid and kept at room temperature for 2 hours. The solvent was removed *in vacuo*, the residue triturated with dry ethyl ether and dried over KOH to give 1.3 g (95% yield) of **5** as an amorphous hygroscopic trifluoroacetic acid salt. $[\alpha]_{578}^{25}$ −5.2° (*c* 1, MeOH), ^{1}H NMR (D_2O) δ 1.0 (3H, m), 1.2 (3H, t, J=7 Hz), 1.3~1.5 (4H, m), 3.5~3.6 (2H, m), 3.7 (2H, m), 3.8 (1H, m), 4.0 (2H, q, J=7 Hz), 4.1 (1H, m).

Anal Calcd for $C_{14}H_{23}N_3O_7CF_3COOH$: C 41.83, H 5.26, N 9.14.
Found: C 41.66, H 5.14, N 9.02.

N^2-[(*tert*-Butoxycarbonyl-L-norvalyl)-L-norvalyl]-N^3-(DL-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic Acid **6**

This compound was prepared from Boc-Nva-OSu and compound **5** according to the procedure described for the preparation of **2**. 0.47 g (83% yield). MP 87~90°C, $[\alpha]_{578}^{25}$ −38.0° (*c* 1, MeOH).

Anal Calcd for $C_{24}H_{40}N_4O_{10}$: C 52.92, H 7.40, N 10.28.
Found: C 52.71, H 7.22, N 10.06.

N^2-[N^α,N^ε-Bis(*tert*-butoxycarbonyl-L-lysyl)-L-norvalyl]-N^3-(DL-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic Acid **7**

According to the methodology described for **2**, peptide **7** was prepared, 0.54 g (80%). MP 62~65°C, $[\alpha]_{578}^{25}$ −25.8° (*c* 1, MeOH).

Anal Calcd for $C_{30}H_{51}N_5O_{12}$: C 53.47, H 7.63, N 10.39.
Found: C 53.21, H 7.56, N 10.42.

N^2-[(*tert*-Butoxycarbonyl-γ-*O*-benzyl-L-glutamyl)-L-norvalyl]-N^3-(DL-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic Acid **8**

Tripeptide **8** was obtained from Boc-Glu(OBzl)OSu and peptide **5** according to the method described for **2**. 0.59 g (83%). MP 68~70°C, $[\alpha]_{578}^{25}$ −24.0° (*c* 1, MeOH).

Anal Calcd for $C_{31}H_{44}N_4O_{12}$: C 55.92, H 6.67, N 8.42.
Found: C 55.66, H 6.63, N 8.54.

N^2-[(*tert*-Butoxycarbonyl-L-glutamyl)-L-norvalyl]-N^3-(DL-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic Acid **9**

Peptide **8** 0.58 g (0.9 mmol) was dissolved in 20 ml MeOH and hydrogenolyzed in the presence of 0.05 g of 10% palladium catalyst on carbon. Then the catalyst was filtered off, the filtrate was concentrated and the residue crystallized from methanol-ethyl ether-hexane mixture to give 0.48 g (95%) of **9**. MP 92~94°C, $[\alpha]_{578}^{25}$ −32.0° (*c* 1, MeOH).

Anal Calcd for $C_{24}H_{38}N_4O_{12}$: C 50.16, H 6.66, N 9.75.
Found: C 50.08, H 6.68, N 9.44.

(N^2-(*tert*-Butoxycarbonyl)-N^3-[(DL-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoyl])-L-norvaline **10**

From N^2-(*tert*-butoxycarbonyl)-N^3-(DL-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic acid succinimido ester[16] and L-norvaline by the procedure used to prepare **2**, compound **10** was obtained. 1.12 g (75%). MP 72~74°C, $[\alpha]_{578}^{25}$ −14.0° (*c* 1, MeOH).

Anal Calcd for $C_{19}H_{31}N_3O_9$: C 51.22, H 7.01, N 9.43.
Found: C 51.37, H 6.82, N 9.66.

(N^2-(*tert*-Butoxycarbonyl-L-norvalyl)-N^3-[(DL-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoyl])-L-norvaline **12**

12 was prepared similarly to **2** from freshly deprotected peptide **11** (after trifluoroacetic acid treatment of **10**) and Boc-Nva-OSu. 0.41 g (71%). MP 103~107°C, $[\alpha]_{578}^{25}$ −30.2° (*c* 1, MeOH).

Anal Calcd for $C_{24}H_{40}N_4O_{10}$: C 52.92, H 7.40, N 10.28.
Found: C 52.77, H 7.34, N 10.16.

N^3-(D-*trans*-4-Ethoxyepoxysuccinyl)-L-2,3-diaminopropanolic Acid Trifluoroacetate Salt **13**

N-Succinimido ethyl D-*trans*-epoxysuccinate [MP 96~98°C, $[\alpha]_{578}^{25}$ −70.4° (*c* 1, THF), ^{1}H NMR (DMSO-d_6); δ 1.3 (3H, t, J=7 Hz), 2.9 (4H, s), 3.85 (1H, d, J=2 Hz), 3.95 (1H, d, J=2 Hz), 4.35 (2H, q, J=7 Hz). *Anal* Calcd for $C_{10}H_{11}NO_2$: C 46.69, H 4.31, N 5.44. Found: C 46.58, H 4.40, N 5.51.] was prepared according to the procedure described for the preparation of the racemic compound[16]. This was reacted with N^2-(*tert*-butoxycarbonyl)-L-2,3-diaminopropanoic acid, following the procedure published for the preparation of N^3-(DL-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic acid[16], yielding 0.31 g (96%) of compound **13**. MP 164~166°C, $[\alpha]_{578}^{25}$ −71.2° (*c* 1, H_2O).

Anal Calcd for $C_9H_{14}N_2O_6CF_3COOH$: C 36.67, H 4.19, N 7.77.
Found: C 36.30, H 4.05, N 7.42.

N^2-(*tert*-Butoxycarbonyl-L-norvalyl)-N^3-(D-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic Acid **14**

14 was prepared from Boc-Nva-OSu and **13** as described for the preparation of **2** in 85% yield. MP 76~78°C, $[\alpha]_{578}^{25}$ −49.8° (*c* 1, MeOH).

Anal Calcd for $C_{19}H_{31}N_3O_9$: C 51.22, H 7.01, N 9.43.
Found: C 51.12, H 7.12, N 9.38.

N^2-L-Norvalyl-N^3-(D-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic Acid Trifluoroacetate Salt **15**

From 1.1 g (2.5 mmol) of peptide **14** after trifluoroacetic acid treatment 0.93 g (90%) of **15** was obtained. MP 102~106°C, $[\alpha]_{578}^{25}$ −26.2° (*c* 1, MeOH).

Anal Calcd for $C_{14}H_{23}N_3O_7CF_3COOH$: C 41.83, H 5.26, N 9.14.
Found: C 41.51, H 5.12, N 9.03.

N^2-[N^α,N^ε-Bis(*tert*-butoxycarbonyl-L-lysyl)-L-norvalyl]-N^3-(D-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic Acid **16**

This compound was prepared from Boc-Lys(Boc)-OSu and **15** by the method used for the preparation of the racemic **7**, 1.16 g (82%). MP 79~81°C, $[\alpha]_{578}^{25}$ −47.2° (*c* 1, MeOH).

Anal Calcd for $C_{30}H_{51}N_5O_{12}$: C 53.47, H 7.63, N 10.39.
Found: C 53.34, H 7.44, N 10.44.

N^3-(L-*trans*-4-Ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic Acid Trifluoroacetate Salt **17**

17 was obtained analogously to the preparation of **13** using *N*-succinimido ethyl L-*trans*-epoxysuccinate [MP 97.5~98.5°C, $[\alpha]_{578}^{25}$ +68.8° (*c* 1, THF) ^{1}H NMR (DMSO-d_6) δ 1.25 (3H, t, J=7 Hz), 2.9 (4H, s), 3.8 (1H, d, J=2 Hz), 3.9 (1H, d, J=2 Hz), 4.35 (2H, q, J=7 Hz). *Anal* Calcd for $C_{10}H_{11}NO_7$: C 46.69, H 4.31, N 5.44. Found: C 46.54, H 4.33, N 5.45.] and N^2-(*tert*-butoxycarbonyl)-L-2,3-diaminopropanoic acid, following the procedure used for the preparation of N^3-(DL-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic acid[16]. 0.35 g (95%). MP 150~152°C and $[\alpha]_{578}^{25}$ +29.8° (*c* 1, H_2O).

Anal Calcd for $C_9H_{14}N_2O_6CF_3COOH$: C 36.67, H 4.19, N 7.77.
Found: C 36.41, H 3.98, N 7.54.

N^2-(*tert*-Butoxycarbonyl-L-norvalyl)-N^3-(L-*trans*-4-ethoxyepoxysuccinyl)-L-2,3-diaminopropanoic Acid **18**

18 was prepared similarly to **14** from Boc-Nva-OSu and **17** with 78% yield. $[\alpha]_{578}^{25}$ +30.2° (*c*, MeOH).

Anal Calcd for $C_{19}H_{31}N_3O_9$: C 51.22, H 7.01, N 9.43.
Found: C 51.01, H 7.21, N 9.48.

General Procedure for Ammonolysis and Deprotection of Peptides

The appropriate peptide **2**, **3**, **4**, **6**, **7**, **9**, **10**, **12**, **14**, **16** and **18** (1 mmol) was stirred in a cold ammonia solution (29%), 20 ml for 2 hours, then ammonia was evaporated, the crude residue dissolved in 5 ml of water and passed through a short column of Amberlite CG 50 (H^+), washed with water, evaporated and dried over KOH, then treated with 5 ml cold trifluoroacetic acid for 2 hours. The solvent was removed under reduced pressure leaving the deprotected peptides **2a**, **3a**, **4a**, **6a**, **7a**, **9a**, **10a**, **12a**, **14a**, **16a** and **18a**. Yields and analytical data are collected in Table 1.

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