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SYNTHESIS OF NEW INDOLACTAM ANALOGUES BY MICROBIAL CONVERSION

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Abstract: *Ten indolactam congeners with L-Ala, Abu, γ,δ-Δ-Nva, Nva, Nle, tert-Leu, Leu, Ile, allo-Ile, Phg instead of L-Val in (-)-indolactam-Val, were synthesized from their seco-compounds (N-methyl-L-amino acidyl-L-tryptophanol) by microbial conversion.*

Introduction

Teleocidins are potent tumor promoters[1] produced by actinomycetes.[2] Their peculiar structures of a nine-membered lactam ring and complex monoterpenoid side chains attract much attention in the area of organic chemistry. Especially (-)-indolactam-Val (ILV),[3] which has the fundamental structure of teleocidins and is the minimum unit for tumor-promoting activity,[4] is useful as a lead compound for structure-activity studies[5] and receptor analysis.[6] Hitherto, total syntheses of ILV and its analogues have been intensively investigated.[7] However, more convenient syntheses are desirable from the view point of synthesizing various indolactam congeners for structure-activity studies.

We have demonstrated that ILV was biosynthesized from L-tryptophan, L-valine and L-methionine via *N*-methyl-L-valyl-L-tryptophanol,[8] using *Streptoverticillium blastmyceticum* NA34-17,[9] which has the characteristic feature of producing ILV in quantity. Since *N*-methyl-L-valyl-L-tryptophanol, which was first isolated by Sakai *et al.* from *Streptoverticillium olivoreticulli*,[10] can be chemically synthesized without difficulty, utilization of the microbial cyclization enzyme would be a convenient synthetic method of various indolactam analogues for structure-activity studies. In fact, we have recently shown that (-)-indolactam-Ile (Figure 1) can be efficiently synthesized from *N*-methyl-L-isoleucyl-L-tryptophanol by use of *S. blastmyceticum*.[11] We have successively metabolized twelve seco-compounds and obtained ten indolactam congeners in the optically active form. This paper describes the synthesis of new indolactam analogues by microbial conversion, and discusses the substrate specificity and stereochemistry in the cyclization step.

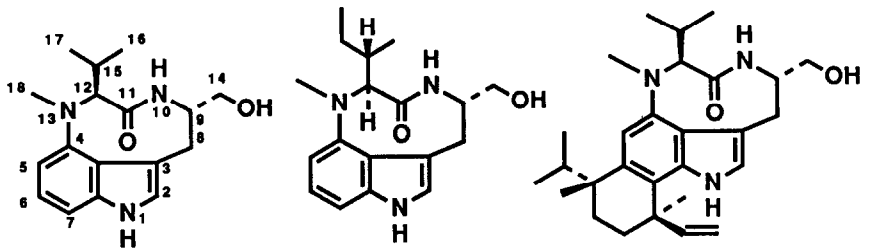


Fig. 1 Structure of naturally occurring teleocidin-related compounds.

Results

Time course study on ILV production

First we closely examined the time course of (-)-indolactam-Val production. As shown in Figure 2, the pH of the culture medium varied with time, and ILV production did not begin until the pH had recovered to 6.6. Since beginning of the ILV production was unsettled, depending on the cultures and their conditions, the optimum time to add the precursors and to harvest the culture broth was guided by pH: a precursor was added to the medium at the pH bottom and the culture broth was harvested 12hr after the pH recovered to 6.6.

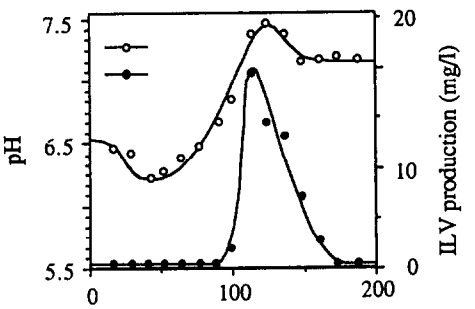


Fig. 2 Time course study on ILV production.

Synthesis of cyclization precursors, N-methyl-L-amino acidyl-L-tryptophanol

Synthesis of cyclization precursors, *N*-methyl-L-X-L-tryptophanols (X=L-Thr(OMe), Ala, Abu, γ,δ-Δ-Nva, Nva, Nle, *tert*-Leu, Leu, Ile, *allo*-Ile, Phg, Phe), was carried out as shown in Figure 3. *tert*-Butoxycarbonyl-*N*-methyl-L-amino acids (Boc-*N*-methyl-L-amino acids) were prepared by the method of Cheung and Benoiton.[12] In brief, each amino acid protected with 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON) was methylated with sodium hydride and iodomethane in tetrahydrofuran. Condensation of each Boc-*N*-methyl-L-amino acid with L-tryptophanol, followed by removal of the Boc-group, gave a corresponding *N*-methyl-L-amino acidyl-L-tryptophanol. To examine the stereochemistry in the cyclization step, four deuterium-labelled stereoisomers of *N*-methyl-valyl-tryptophanol (*N*-CD3-L-valyl-L-tryptophanol, *N*-CD3-L-valyl-D-tryptophanol, *N*-CD3-D-valyl-L-tryptophanol and *N*-CD3-D-valyl-D-tryptophanol) were also synthesized using iodomethane-*d3*.

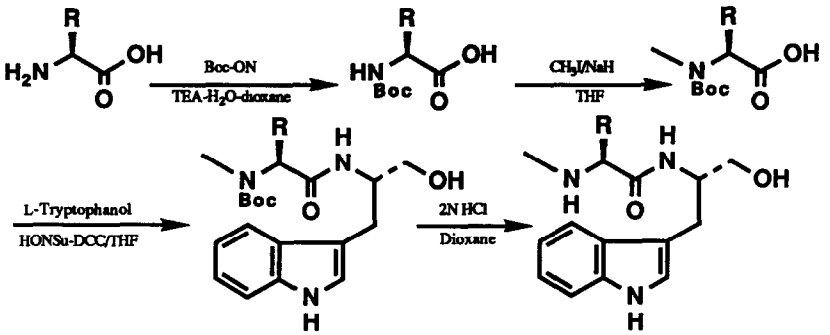


Fig. 3 Synthetic scheme of *N*-methyl-L-amino acidyl-L-tryptophanol.

Microbial conversion of N-methyl-L-amino acidyl-L-tryptophanol into indolactams

Table I shows the incorporation ratio of the four deuterium-labelled stereoisomers of *N*-methyl-valyl-tryptophanol. The results were derived from a simultaneous experiment using the same seed culture. *N*-CD3-L-valyl-L-tryptophanol was efficiently converted into ILV as reported previously[8a], whereas its enantiomer *N*-CD3-D-valyl-D-tryptophanol did not result in cyclization product. On the other hand, *N*-CD3-L-valyl-D-tryptophanol was significantly converted into (-)-epi-indolactam V,[3b] though *N*-CD3-D-valyl-L-tryptophanol was not.

Cyclization yield and the side chain structure of each cyclization precursor are summarized in Table II. The cyclization yield changed drastically in each precursor. *N*-Methyl-L-isoleucyl-L-tryptophanol and *N*-methyl-L-norvalyl-L-tryptophanol were most efficiently converted into the corresponding cyclization products, (-)-indolactam-Ile and (-)-indolactam-Nva, whose yields were even higher than that of the natural ligand, *N*-CD3-L-valyl-L-tryptophanol (Table I). Very little *N*-methyl-L-alanyl-L-tryptophanol with a smaller substituent at position 12 and *N*-methyl-L-norleucyl-L-tryptophanol with a longer substituent at position 12 were converted into (-)-indolactam-Ala and (-)-indolactam-Nle, respectively. Furthermore, *N*-methyl-L-phenylalanyl-L-tryptophanol with the largest substituent at position 12 did not yield a cyclization product. None of the precursors in Table I and II inhibited ILV production.

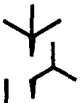
Table I Incorporation of four deuterium-labelled stereoisomers of *N*-CD_3-valyl-tryptophanol into indolactam-Val

compound	indolactam-Val			epi-indolactam-Val		
	Prod.[a]	%D[b]	%I.R.[c]	Prod.	%D	%I.R.
N-CD_3-L-Val-L-Trp-ol	32	15	10	ND[d]		
N-CD_3-L-Val-D-Trp-ol	27	0	0	1.0	100	2.0
N-CD_3-D-Val-L-Trp-ol	28	0	0	ND		
N-CD_3-D-Val-D-Trp-ol	28	0	0	ND		

[a] Production (mg/l). [b] Deuterium content (%). [c] Incorporation ratio (%). [d] Not detected.

Table II

Microbial conversion of *N*-methyl-L-amino acidyl-L-tryptophanol into indolactams

No.	compound	side-chain	cyclization yield
1.	*N*-Me-L-Thr(OMe)-L-Trp-ol		ND [a]
2.	*N*-Me-L-Ala-L-Trp-ol		+
3.	*N*-Me-L-Abu-L-Trp-ol		+ +
4.	*N*-Me-L-γ,δ-Δ-Nva-L-Trp-ol		+ + +
5.	*N*-CD_3-L-Val-L-Trp-ol		+ + +
6.	*N*-Me-L-Nva-L-Trp-ol		+ + + +
7.	*N*-Me-L-Nle-L-Trp-ol		+
8.	*N*-Me-L-*tert*-Leu-L-Trp-ol		+ + +
9.	*N*-Me-L-Leu-L-Trp-ol		+ + +
10.	*N*-Me-L-Ile-L-Trp-ol		+ + + +
11.	*N*-Me-L-*allo*-Ile-L-Trp-ol		+ + +
12.	*N*-Me-L-Phg-L-Trp-ol		+ +
13.	*N*-Me-L-Phe-L-Trp-ol		ND

[a]ND: Not detected, + : <1%, ++ : 1~5%, +++ : 5~15%, ++++ : >15%

Discussion

The above synthesis of new indolactam analogues using microbial conversion has two advantages. The first is that optically pure indolactams with 9*S* and 12*S* configurations, which are necessary for tumor-promoting activity,[5a] are obtainable even if a corresponding precursor contains a small amount of its enantiomer. As shown in Table I, feeding experiments with the four stereoisomers of *N*-methyl-valyl-tryptophanol indicates that L-stereochemistry at position 12 is strictly recognized by the cyclization enzyme. The second is that synthesis of the cyclization precursors can be easily attained. The synthesis of *N*-methyl-L-amino acidyl-L-tryptophanol consists basically of only two steps, methylation and condensation as shown in Figure 3. Since the ten indolactam congeners in Table II were easily synthesized, and since teleocidin-producing micro-organisms are very common,[2a,2c,10,13] this microbial conversion might be a simple and convenient method to prepare various indolactam analogues. Kogan *et al.* have recently tried to cyclize 8-oxo-*N*-methyl-L-valyl-L-tryptophanol by means of regiospecific thallation in an analogy to the biosynthesis of ILV.[7g] However, this attempt was disappointing.

The cyclization yield tends to increase with increasing the number of carbons in the substituents up to 3 (compounds 2~6 in Table II), suggesting that hydrophobicity of the substituents plays an important role in the cyclization yield. However, very little *N*-methyl-L-norleucyl-L-tryptophanol, with a four-carbon straight side chain, was converted to (-)-indolactam-Nle though *N*-methyl-L-*tert*-leucyl-L-tryptophanol, *N*-methyl-L-leucyl-L-tryptophanol, *N*-methyl-L-isoleucyl-L-tryptophanol and *N*-methyl-L-*allo*-isoleucyl-L-tryptophanol were converted efficiently to the corresponding products. Furthermore, *N*-methyl-L-phenylalanyl-L-tryptophanol was not converted at all. These facts show that not only a hydrophobic factor but also a steric factor dominate the conversion efficiency. From the results in Table II, it seems that there is at least a cavity the size of cyclohexane in the cyclization enzyme. Needless to say, other factors such as membrane permeability and metabolism must be involved in the cyclization efficiency. In fact, less than 10% of (-)-indolactam-Ile was subjected to introduction of a terpenoid side chain like teleocidin B-4. (data not shown) Closer examination on the substrate specificity should be, therefore, carried out after the isolation of this cyclization enzyme. Precursors such as *N*-methyl-L-isoleucyl-L-tryptophanol and *N*-methyl-L-norvalyl-L-tryptophanol are especially useful as substrates for isolation of this enzyme because the cyclization products, for example, (-)-indolactam-Ile and (-)-indolactam-Nva, rarely occur naturally and their cyclization efficiency is very high.

We evaluated (manuscript in preparation) the tumor-promoting activity of these new indolactam analogues by two biological tests related to tumor promotion: binding ability to the 12-*O*-tetradecanoylphorbol-13-acetate (TPA) receptor[14] and stimulation of radioactive inorganic phosphate incorporation into the phospholipids of HeLa cells.[15] These two biological activities correlated very well for each derivative. Effects of the substituents at position 12 on the TPA receptor binding were analyzed quantitatively using physicochemical substituent parameters and regression analysis. The results indicated that both the hydrophobicity and the bulkiness of the substituents at position 12 increased the binding ability to the TPA receptor, demonstrating the recent hypothesis[16] that the isopropyl group at position 12 of (-)-indolactam-Val is involved in the hydrophobic interaction on the receptor site.

Acknowledgements

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Experimental

General remarks

Melting points are not corrected. The following spectroscopic and analytical instruments were used: UV, Shimadzu UV-200; ORD, Jasco Model J-5; ^{1}H NMR, JEOL GX400 (400MHz, ref. TMS, 27°C); HPLC, Waters Model 600E with a UV detector; MS, JEOL JMS-DX300 (70eV, 300μA).

HPLC was carried out on YMC packed SH-343 (ODS, 20mm i. d. x 250mm), SH-342 (ODS, 20mm i. d. x 150mm), AQ-323 (ODS, 10mm i. d. x 250mm) and A-023 (silica gel, 10mm i. d. x 250mm) column (Yamamura Chemical Laboratory) and μ-Bondasphere C_{18} (19mm i. d. x 150mm) column (Waters Associates). Wako C-100 gel (silica gel, Wako Pure Chemical Industries) were used for column chromatography. All other chemicals and reagents were purchased from chemical companies and were special grade.

Synthesis of N-methyl-L-amino acidyl-L-tryptophanol

i) Preparation of Boc-L-amino acids: Each amino acid (10mmol) was stirred with triethylamine (TEA) (15mmol) and Boc-ON (11mmol, 6ml dioxane solution) in water (6ml) for 4hr at room temperature. This solution was evaporated to dryness and partitioned between water and EtOAc. The aqueous layer was acidified to pH 3 with citric acid and then extracted three times with EtOAc. The EtOAc extracts, which were dried over P_2O_5, were used for the next step without further purification.

ii) Methylation of Boc-L-amino acids: Each Boc-L-amino acid (4mmol) and CH_3I (32mmol) were dissolved in dry tetrahydrofuran (THF) (25ml) and cooled to 0°C. NaH (12mmol) was added to this solution with vigorous stirring. After stirring at room temperature for 24hr, EtOAc (3ml) was added carefully to the mixture, and this was evaporated to dryness after adding water (5ml). After dissolution in water (100ml), the reaction mixture was washed twice with ether. The aqueous layer was acidified to pH 3 with citric acid, then extracted twice with EtOAc. The EtOAc layer was washed successively with 5% $NaHCO_3$, 5% $Na_2S_2O_3$ and water to give each Boc-*N*-methyl-L-amino acid.

iii) Preparation of L-tryptophanol[17]: L-Tryptophan methyl ester hydrochloride (3g) was added carefully to a dry THF solution containing $LiAlH_4$ (1.4g). The reaction mixture was refluxed for 1hr. To the reaction mixture, wet THF was added carefully to decompose remaining $LiAlH_4$. The mixture was filtered and evaporated to dryness. The resultant oil was extracted three times with EtOAc. The EtOAc extracts were purified by column chromatography on Wako C-100 gel (i-PrOH-CH_2Cl_2, stepwise).

iV) Condensation of Boc-L-amino acid with L-tryptophanol: Each Boc-*N*-methyl-L-amino acid (1.6mmol), L-tryptophanol (1.6mmol) and *N*-hydroxysuccinimide (HONSu) (1.6mmol) were dissolved in dry THF (20ml) and cooled to -20°C. To the solution, 1,3-dicyclohexylcarbodiimide (DCC) (2.4mmol, 4ml THF solution) was added. The solution was stirred at -20°C for 1 hr and at room temperature for 24 hr. The reaction mixture was acidified to pH3 with citric acid and extracted twice with EtOAc. The EtOAc extracts were purified by column chromatography on Wako C-100 gel (acetone-toluene, stepwise), followed by preparative HPLC on μ-Bondasphere C_{18} column with 60~70% MeOH to give each Boc-*N*-methyl-L-amino acidyl-L-tryptophanol.

V) Removal of the Boc-group: Each Boc-*N*-methyl-L-amino acidyl-L-tryptophanol was treated with 2M HCl in dioxane for 50min. The reaction mixture was adjusted to pH10 by $NaHCO_3$, and extracted three times with EtOAc. The EtOAc extracts were purified by preparative HPLC on YMC SH-342 with 17~22% CH_3CN containing 0.1% trifluoroacetic acid (TFA) to give each *N*-methyl-L-amino acidyl-L-tryptophanol as follows.

N-Me-L-Thr(OMe)-L-Trp-ol: 5.0% yield from Boc-L-Thr. Amorphous, $[\alpha]_D^{20}$ -54.6° (*c*=0.52, MeOH). UV λmax (MeOH) nm (ε): 290 (3900), 282 (4600), 276 (4200), 222 (26,000). ^{1}H NMR δ (CD_3OD, 0.06M) ppm: 0.94 (3H, d, *J*=6.4Hz), 2.14 (3H, s), 2.79 (1H, d, *J*=6.7Hz), 2.91 (1H, dd, *J*=14.7, 8.6Hz), 3.05 (1H, ddd, *J*=14.7, 5.8, 0.6Hz), 3.17 (1H, quintet, *J*=6.4Hz), 3.20 (3H, s), 3.59 (2H, d, *J*=5.5Hz), 4.29 (1H, m), 6.99 (1H, ddd, *J*=7.8, 7.0, 0.9Hz), 7.07 (1H, dt, *J*=7.6, 1.2Hz), 7.09 (1H, s), 7.30 (1H, dd, *J*=7.3, 0.9Hz), 7.62 (1H, dd, *J*=7.9, 0.9Hz). HR-EI-MS *m/z*: 319.1882 (M^+, calcd. for $C_{17}H_{25}N_3O_3$, 319.1896).

N-Me-L-Ala-L-Trp-ol: 21.6% yield from Boc-*N*-Me-L-Ala. Amorphous, $[\alpha]_D^{27}$ -35.6° (*c*=0.72, MeOH). UV λmax (MeOH) nm (ε). 290 (4300), 282 (5000), 276 (sh., 4600), 222 (29,600). ^{1}H NMR δ (CD_3OD, 0.06M) ppm: 1.14 (3H, d, *J*=6.7Hz), 2.05 (3H, s), 2.91 (1H, dd, *J*=14.3, 7.9Hz), 3.00 (1H, q, *J*=6.7Hz), 3.05 (1H, dd, *J*=14.3, 7.0Hz), 3.57 (1H, dd, *J*=11.0, 5.5Hz), 3.60 (1H, dd, *J*=11.0, 5.2Hz), 4.25 (1H, m), 6.99 (1H, dt, *J*=7.0, 1.2Hz), 7.06 (1H, dt, *J*=7.0, 1.2Hz), 7.08 (1H, s), 7.30 (1H, dd, *J*=7.3, 0.9Hz), 7.62 (1H, dd, *J*=7.0, 1.2Hz). HR-EI-MS *m/z*: 275.1622 (M^+, calcd. for $C_{15}H_{21}N_3O_2$, 275.1634).

N-Me-L-Abu-L-Trp-ol: 7.3% yield from Boc-L-Abu. Colorless leaflets from CH_3CN, mp 92~93°C, $[\alpha]_D^{22}$ -27.3° (*c*=0.75, MeOH). UV λmax (MeOH) nm (ε): 290 (4600), 282 (5300), 276 (sh., 4800), 222 (30,000). ^{1}H NMR δ (CD_3OD, 0.12M) ppm:

0.82 (3H, t, *J*=7.3Hz), 1.51 (2H, quintet, *J*=7.3Hz), 2.03 (3H, s), 2.81 (1H, t, *J*=6.7Hz), 2.90 (1H, dd, *J*=14.7, 8.5Hz), 3.07 (1H, dd, *J*=14.7, 5.5Hz), 3.59 (2H, m), 4.31 (1H, m), 6.99 (1H, dt, *J*=7.6, 0.9Hz), 7.06 (1H, dt, *J*=8.2, 1.2Hz), 7.08 (1H, s), 7.30 (1H, dd, *J*=7.9, 0.9Hz), 7.63 (1H, dd, *J*=7.9, 0.9Hz). EI-MS *m/z*: 289 (M^+). Anal. Calcd. for $C_{16}H_{23}N_3O_2$: C, 66.41; H, 8.01; N, 14.52. Found: C, 66.44; H, 8.04; N, 14.57.

N-Me-L-γ,δ-Δ-Nva-L-Trp-ol: 12.3% yield from L-γ,δ-Δ-Nva. Colorless needles from CH_3CN, mp 104~106°C, $[\alpha]_D^{21}$ -0.45° (*c*=0.45, MeOH). UV λmax (MeOH) nm (ε): 290 (4500), 281 (5200), 274 (sh., 4900), 222 (29,400). ^1H NMR δ (CD_3OD, 0.05M) ppm: 2.07 (3H, s), 2.16 (1H, m), 2.26 (1H, m), 2.91 (2H, m), 3.07 (1H, dd, *J*=14.7, 6.1Hz), 3.56 (1H, dd, *J*=10.7, 5.2Hz), 3.60 (1H, dd, *J*=10.7, 5.2Hz), 4.27 (1H, m), 4.97~5.04 (2H, m), 5.64 (1H, m), 6.99 (1H, dt, *J*=7.0, 0.9Hz), 7.06 (1H, dt, *J*=7.9, 1.2Hz), 7.08 (1H, s), 7.30 (1H, d, *J*=7.9Hz), 7.62 (1H, d, *J*=7.0Hz). EI-MS m/z: 301 (M^+). Anal. Calcd. for $C_{17}H_{23}N_3O_2 \cdot 1/2CH_3CN$: C, 67.16; H, 7.67; N, 15.23. Found: C, 67.40; H, 7.73; N, 15.08.

N-CD3-L-Val-L-Trp-ol: 5.5% yield from Boc-L-Val. Amorphous, $[\alpha]_D^{25}$ -36.4° (*c*=0.53, MeOH). UV λmax (MeOH) nm (ε): 290 (6100), 282 (7100), 276 (sh., 6400), 222 (39,000). ^1H NMR δ (CD_3OD, 0.07M) ppm: 0.85 (3H, d, *J*=6.7Hz), 0.86 (3H, d, *J*=6.7Hz), 1.77 (1H, m), 2.64 (1H, d, *J*=6.4Hz), 2.90 (1H, dd, *J*=14.7, 8.5Hz), 3.07 (1H, ddd, *J*=14.7, 6.1, 0.6Hz), 3.57 (1H, dd, *J*=11.0, 5.5Hz), 3.61 (1H, dd, *J*=11.0, 5.2Hz), 4.31 (1H, m), 6.99 (1H, ddd, *J*=7.9, 7.0, 0.9Hz), 7.06 (1H, ddd, *J*=8.1, 7.0, 1.2Hz), 7.08 (1H, s), 7.30 (1H, dd, *J*=7.9, 0.9Hz), 7.63 (1H, dd, *J*=7.9, 0.9Hz). HR-EI-MS *m/z*: 306.2155 (M^+, calcd. for $C_{17}H_{22}D_3N_3O_2$, 306.2135).

N-CD3-D-Val-D-Trp-ol: 4.5% yield from Boc-D-Val. Amorphous, $[\alpha]_D^{24}$ +31.5° (*c*=0.55, MeOH). HR-EI-MS *m/z*: 306.2134 (M^+, calcd. for $C_{17}H_{22}D_3N_3O_2$, 306.2135). The UV and ^1H NMR spectra coincided with those of *N*-CD3-L-Val-L-Trp-ol.

N-CD3-D-Val-L-Trp-ol: 4.6% yield from Boc-D-Val. Amorphous, $[\alpha]_D^{25}$ -21.8° (*c*=0.47, MeOH). UV λmax (MeOH) nm (ε): 290 (4700), 281 (5600), 274 (sh., 5300), 222 (31,900). ^1H NMR δ (CD_3OD, 0.07M) ppm: 0.78 (3H, d, *J*=7.0Hz), 0.81 (3H, d, *J*=6.7Hz), 1.76 (1H, m), 2.65 (1H, d, *J*=5.8Hz), 2.91 (1H, dd, *J*=14.7, 7.9Hz), 3.08 (1H, dd, *J*=14.7, 6.4Hz), 3.57 (1H, dd, *J*=11.0, 5.5Hz), 3.61 (1H, dd, *J*=11.0, 5.5Hz), 4.31 (1H, m), 6.99 (1H, ddd, *J*=7.9, 7.0, 0.9Hz), 7.07 (1H, dt, *J*=8.2, 1.2Hz), 7.08 (1H, s), 7.30 (1H, dd, *J*=8.2, 0.9Hz), 7.62 (1H, dd, *J*=7.6, 0.9Hz). HR-EI-MS *m/z*: 306.2164 (M^+, calcd. for $C_{17}H_{22}D_3N_3O_2$, 306.2135).

N-CD3-L-Val-D-Trp-ol: 5.5% yield from Boc-L-Val. Amorphous, $[\alpha]_D^{25}$ +23.9° (*c*=0.48, MeOH). HR-EI-MS *m/z*: 306.2145 (M^+, calcd. for $C_{17}H_{22}D_3N_3O_2$, 306.2135). The ^1H NMR spectra coincided with those of *N*-CD3-D-Val-L-Trp-ol.

N-Me-L-Nva-L-Trp-ol: 7.2% yield from Boc-L-Nva. Colorless leaflets from CH_3CN, mp 119~121°C, $[\alpha]_D^{25}$ -33.2° (*c*=0.70, MeOH). UV λmax (MeOH) nm (ε): 290 (5200), 281 (6000), 275 (sh., 5500), 222 (29,000). ^1H NMR δ (CD_3OD, 0.06M) ppm: 0.85 (3H, t, *J*=7.3Hz), 1.23 (2H, m), 1.42 (2H, m), 2.03 (3H, s), 2.89 (2H, m), 3.07 (1H, dd, *J*=14.7, 6.1Hz), 3.58 (2H, m), 4.30 (1H, m), 6.99 (1H, ddd, *J*=7.9, 7.0, 0.9Hz), 7.06 (1H, ddd, *J*=7.9, 7.0, 0.9Hz), 7.08 (1H, s), 7.30 (1H, dd, *J*=8.0, 0.9Hz), 7.62 (1H, dd, *J*=7.9, 0.9Hz). EI-MS *m/z*: 303 (M^+). Anal. Calcd. for $C_{17}H_{25}N_3O_2$: C, 67.30; H, 8.31; N, 13.85. Found: C, 67.17; H, 8.25; N, 13.87.

N-Me-L-Nle-L-Trp-ol: 35.2% yield from Boc-L-Nle. Colorless needles from CH_3CN, mp 128~129°C, $[\alpha]_D^{24}$ -29.1° (*c*=0.51, MeOH). UV λmax (MeOH) nm (ε): 290 (5000), 282 (5900), 276 (sh., 5400), 222 (28,100). ^1H NMR δ (CD_3OD, 0.06M) ppm: 0.85 (3H, t, *J*=7.3Hz), 1.15~1.28 (4H, m), 1.45 (2H, m), 2.03 (3H, s), 2.86 (1H, t, *J*=7.0Hz), 2.90 (1H, dd, *J*=14.7, 8.6Hz), 3.07 (1H, dd, *J*=14 7, 6.1Hz), 3.58 (1H, dd, *J*=11.0, 5.5Hz), 3.61 (1H, dd, *J*=11.0, 5.2Hz), 4.30 (1H, m), 6.99 (1H, ddd, *J*=7.9, 7.0, 0.9Hz), 7.06 (1H, dt, *J*=7.9, 0.9Hz), 7.08 (1H, s), 7.30 (1H, dd, *J*=7.9, 0.9Hz), 7.62 (1H, dd, *J*=7.0, 0.9Hz). EI-MS *m/z*: 317 (M^+). Anal. Calcd. for $C_{18}H_{27}N_3O_2 \cdot 2/5CH_3CN$: C, 67.64; H, 8.51; N, 14.26. Found: C, 67.39; H, 8.59; N, 13.98.

N-Me-L-Ile-L-Trp-ol: 14.5% yield from Boc-*N*-Me-L-Ile. Amorphous, $[\alpha]_D^{26}$ -38.2° (*c*=0.50, MeOH). UV λmax (MeOH) nm (ε): 290 (5000), 281 (5800), 275 (sh., 5400), 222 (33,700). ^1H NMR δ ($CDCl_3$, 0.06M) ppm: 0.75 (3H, t, *J*=7.3Hz), 0.78 (3H, d, *J*=7.0Hz), 0.92 (1H, m), 1.21 (1H, m), 1.66 (1H, m), 2.27 (3H, s), 2.79 (1H, d, *J*=4.6Hz), 2.99 (1H, dd, *J*=14.7, 7.9Hz), 3.06 (1H, ddd, *J*=14.7, 6.7, 0.6Hz), 3.67 (1H, dd, *J*=11.0, 6.1Hz), 3.77 (1H, dd, *J*=11.0, 3.5Hz), 4.26 (1H, m), 7.06 (1H, d, *J*=2.1Hz), 7.11 (1H, dt, *J*=7.9, 1.2Hz), 7.19 (1H, dt, *J*=8.2, 1.2Hz), 7.35 (1H, dd, *J*=7.9, 0.9Hz), 7.49 (1H, br.d, *J*=7.0Hz), 7.65 (1H, dd, *J*=8.2, 0.9Hz), 8.23 (1H, br.s). HR-EI-MS *m/z*: 317.2101 (M^+, calcd. for $C_{18}H_{27}N_3O_2$, 317.2103).

N-Me-L-allo-Ile-L-Trp-ol: 7.7% yield from Boc-*N*-Me-L-*allo*-Ile. Amorphous, $[\alpha]_D^{26}$ -31.0° (*c*=0.41, MeOH). UV λmax (MeOH) nm (ε): 290 (4600), 281 (5600), 276 (sh., 4900), 222 (31,300). ^1H NMR δ (CD_3OD, 0.06M) ppm: 0.76 (3H, d, *J*=6.7Hz), 0.85 (3H, t, *J*=7.3Hz), 1.10 (1H, m), 1.38 (1H, m), 1.60 (1H, m), 2.10 (3H, s), 2.76 (1H, d, *J*=5.2Hz), 2.92 (1H, dd, *J*=14.6, 8.2Hz), 3.06 (1H, dd, *J*=14.6, 6.4Hz), 3.58 (1H, dd, *J*=10.7, 5.2Hz), 3.61 (1H, dd, *J*=10.7, 5.2Hz), 4.29 (1H, m), 6.99 (1H, ddd, *J*=7.9, 7.0, 1.2Hz), 7.06 (1H, dt, *J*=8.2, 1.2Hz), 7.08 (1H, s), 7.30 (1H, dd, *J*=8.2, 0.9Hz), 7.63 (1H, dd, *J*=7.9, 0.9Hz). HR-EI-MS *m/z*: 317.2115 (M^+, calcd. for $C_{18}H_{27}N_3O_2$, 317.2103).

N-Me-L-tert-Leu-L-Trp-ol: 0.4% yield from L-*tert*-Leu. Amorphous, $[\alpha]_D^{23}$ -24.4° (*c*=0.47, MeOH). UV λmax (MeOH) nm (ε): 290 (3200), 282 (3900), 276 (3500), 222 (22,000). ^{1}H NMR δ (CD_3OD, 0.06M) ppm: 0.90 (9H, s), 1.98 (3H, s), 2.58 (1H, s), 2.89 (1H, dd, *J*=14.7, 8.6Hz), 3.08 (1H, dd, *J*=14.7, 6.1Hz), 3.57 (1H, dd, *J*=10.7, 5.5Hz), 3.62 (1H, dd, *J*=10.7, 5.2Hz), 4.34 (1H, m), 6.98 (1H, dt, *J*=7.0, 1.2Hz), 7.06 (1H, dt, *J*=8.2, 1.2Hz), 7.08 (1H, s), 7.29 (1H, d, *J*=8.2Hz), 7.63 (1H, d, *J*=7.0Hz). HR-EI-MS *m/z*: 317.2111 (M^+, calcd. for $C_{18}H_{27}N_3O_2$, 317.2103).

N-Me-L-Leu-L-Trp-ol: 12.4% yield from Boc-*N*-Me-L-Leu. Colorless leaflets from CH_3CN, mp 82~84°C, $[\alpha]_D^{24}$ -29.2° (*c*=0.88; MeOH). UV λmax (MeOH) nm (ε): 290.5 (4500), 282 (5200), 275 (sh., 4800), 222 (30,200). ^{1}H NMR δ (CD_3OD, 0.09M) ppm: 0.84 (3H, d, *J*=6.4Hz), 0.88 (3H, d, *J*=6.7Hz), 1.23 (1H, m), 1.36 (1H, m), 1.54 (1H, m), 2.04 (3H, s), 2.91 (1H, dd, *J*=14.7, 8.6Hz), 2.93 (1H, t, *J*=7.3Hz), 3.07 (1H, dd, *J*=14.7, 5.5Hz), 3.58 (1H, dd, *J*=11.0, 5.5Hz), 3.61 (1H, dd, *J*=11.0, 5.2Hz), 4.30 (1H, m), 6.99 (1H, dt, *J*=7.0, 0.9Hz), 7.06 (1H, dt, *J*=7.0, 0.9Hz), 7.08 (1H, s), 7.30 (1H, dd, *J*=7.9, 0.9Hz), 7.63 (1H, dd, *J*=7.9, 0.9Hz). HR-EI-MS *m/z*: 317.2117 (M^+, calcd. for $C_{18}H_{27}N_3O_2$, 317.2103).

N-Me-L-Phg-L-Trp-ol: 6.1% yield from *N*-Me-L-Phg. Colorless rods from MeOH, mp 92~94°C, $[\alpha]_D^{21}$ +9.3° (*c*=0.49, MeOH). UV λmax (MeOH) nm (ε): 288 (4800), 280 (5600), 273 (sh., 5200), 220 (36,000). ^{1}H NMR δ (CD_3OD, 0.05M) ppm: 2.18 (3H, s), 2.93 (1H, dd, *J*=14.7, 8.2Hz), 3.07 (1H, dd, *J*=14.7, 6.1Hz), 3.56 (2H, m), 3.99 (1H, s), 4.24 (1H, m), 6.99 (1H, dt, *J*=7.9, 1.2Hz), 7.02 (1H, s), 7.07 (1H, dt, *J*=7.9, 1.2Hz), 7.22 (5H, m), 7.32 (1H, dd, *J*=7.3, 0.9Hz), 7.62 (1H, dd, *J*=7.9, 0.9Hz). EI-MS *m/z*: 337 (M^+). Anal. Calcd. for $C_{20}H_{23}N_3O_2 \cdot 1/2MeOH$: C, 69.66; H, 7.13; N, 11.89. Found: C, 69.51; H, 7.03; N, 12.02.

N-Me-L-Phe-L-Trp-ol: 12.8% yield from Boc-*N*-Me-L-Phe. Colorless leaflets from CH_3CN, mp 113~115°C, $[\alpha]_D^{28}$ -17.6° (*c*=0.88, MeOH). UV λmax (MeOH) nm (ε): 290 (4700), 282 (5400), 275 (sh., 4900), 221 (31,800). ^{1}H NMR δ ($CDCl_3$, 0.15M) ppm: 2.15 (3H, s), 2.51 (1H, dd, *J*=14.0, 9.2Hz), 2.92~3.03 (3H, m), 3.14 (1H, dd, *J*=9.2, 4.6Hz), 3.60 (1H, dd, *J*=11.0, 5.8Hz), 3.68 (1H, dd, *J*=11.0, 3.7Hz), 4.25 (1H, m), 6.95 (1H, d, *J*=2.4Hz), 7.08~7.37 (9H, m), 7.60 (1H, d, *J*=7.9Hz), 8.37 (1H, br.s). EI-MS *m/z*: 351 (M^+). Anal. Calcd. for $C_{21}H_{25}N_3O_2 \cdot 2/3CH_3CN$: C, 70.81; H, 7.18; N, 13.56. Found: C, 70.82; H, 7.18; N, 13.54.

Feeding experiments with cyclization precursors

S. blastmyceticum NA34-17 maintained on Waksman's medium was transferred to a 500ml flask containing 100ml of a medium consisting of 1% glucose, 1% polypeptone (Daigo Eiyo Kagaku), 1% meat extract (Wako Pure Chemical Industries) and 0.5% NaCl (pH 6.6), and the flask was shaken at 28°C for 70hr. Two milliliters of the seed culture thus obtained was transferred to a 500ml flask containing 100ml of medium (2% glucose, 1% polypeptone, 1% meat extract, 0.5% NaCl). The pH of the culture broth was checked at 12hr intervals. A cyclization precursor (5mg) dissolved in dimethylsulfoxide (DMSO) (0.5ml), was sterilized with a membrane filter (0.45μm), then added to the flask at the pH bottom. The culture fermentation broth was harvested 12hr after the pH had recovered to 6.6, and filtered to remove the mycelia. The filtrate was extracted three times with CH_2Cl_2, and the CH_2Cl_2 extracts were purified by eluation through Wako C-100 gel with toluene and increasing volumes of acetone. The 40% acetone-eluate was further purified by preparative HPLC on YMC SH-343 (60% MeOH for (-)-indolactam-Val, Ile, Nva, γ,δ-Δ-Nva, Nle, Leu, *allo*-Ile, and *tert*-Leu; 55% MeOH for (-)-indolactam-Abu and Phg; 0~100% MeOH linear gradient for (-)-indolactam-Ala), followed on YMC A-023 (80% hexane, 10% 2-propanol, 10% $CHCl_3$ for (-)-indolactam-Val, Abu, γ,δ-Δ-Nva, Nle, Leu, Ile, *allo*-Ile and Phg; 75% hexane, 15% 2-propanol, 10% $CHCl_3$ for (-)-indolactam-Ala; 80% hexane, 7% 2-propanol, 13% $CHCl_3$ for (-)-indolactam-Nva and *tert*-Leu) to give the corresponding derivatives as follows.

(-)-Indolactam-Ala: Amorphous. EI-MS *m/z* (%): 273 (M^+ 40), 255 (10), 228 (8), 215 (25), 199 (15), 185 (55), 171 (100). The ^{1}H NMR spectrum was not measured because of its small quantity. The identification of (-)-indolactam-Ala was carried out by co-chromatography with the authentic sample[7d] supplied by Dr. Koichi Shudo of the Faculty of Pharmaceutical Sciences at Tokyo University under the following condition: column, YMC A-311 (ODS); eluent, 40% MeOH; flow rate, 1.0ml/min; t_R=15.5min.

(-)-Indolactam-Abu : 5.0% yield. Amorphous. UV λmax (MeOH) nm (ε): 294 (6900), 282 (6900), 226 (27,400). ^{1}H NMR δ ($CDCl_3$, 0.004M, twist only) ppm: 0.79 (3H, t, *J*=7.3Hz), 1.79 (1H, m), 2.01 (1H, m), 2.83 (3H, s), 3.07 (1H, dd, *J*=17.2, 3.7Hz), 3.13 (1H, br.d, *J*=17.2Hz), 3.57 (1H, dd, *J*=11.4, 8.4Hz), 3.78 (1H, dd, *J*=11.4, 4.0Hz), 4.50 (1H, t, *J*=7.3Hz), 4.61 (1H, m), 6.58 (1H, d, *J*=7.3Hz), 6.81 (1H, br.s), 6.90 (1H, s), 6.97 (1H, d, *J*=8.1Hz), 7.08 (1H, t, *J*=8.1Hz), 8.00 (1H, br.s). EI-MS *m/z* (%): 287 (M^+, 95), 269 (15), 256 (8), 242 (7), 228 (5), 215 (50), 199 (30), 185 (28), 171 (100). HR-EI-MS *m/z*: 287.1642 (M^+ calcd. for $C_{16}H_{21}N_3O_2$, 287.1634).

(-)-Indolactam-γ,δ-Δ-Nva: 7.1% yield. Amorphous. UV λmax (MeOH) nm (ε): 295 (6200), 280 (5900), 226 (24,000). ^{1}H NMR δ (CD_3OD, 0.008M, twist only) ppm: 2.49 (1H, m), 2.69 (1H, m), 2.82 (3H, m), 2.98 (1H, dd, *J*=17.1, 2.8Hz), 3.10 (1H,

dd, *J*=17.1, 4.3Hz), 3.50 (1H, dd, *J*=11.3, 8.6Hz), 3.64 (1H, dd, *J*=11.3, 4.6Hz), 4.62 (2H, m), 4.8~5.0 (2H, m), 5.67 (1H, m), 6.54 (1H, m), 6.93 (1H, s), 6.96 (2H, m). EI-MS *m/z* (%): 299 (M^+, 20), 281 (50), 258 (30), 240 (35), 183 (45), 171 (100). HR-EI-MS *m/z*: 299.1638 (M^+, calcd. for $C_{17}H_{21}N_3O_2$, 299.1634).

(-)-Indolactam-Nva: 16.7% yield. Amorphous, $[\alpha]_D^{19}$ -228° (*c*=0.17, MeOH). UV λmax (MeOH) nm (ε): 296 (6900), 284 (6800), 228 (26,900). ^1H NMR δ ($CDCl_3$, 0.004M, twist only) ppm: 0.75 (3H, t, *J*=7.3Hz), 1.18 (2H, m), 1.75 (1H, m), 1.92 (1H, m), 2.08 (1H, m), 2.83 (3H, s), 3.09 (2H, m), 3.57 (1H, m), 3.78 (1H, m), 4.60 (2H, m), 6.57 (1H, d, *J*=7.3Hz), 6.65 (1H, br.s), 6.90 (1H, d, *J*=0.6Hz), 6.97 (1H, dd, *J*=7.9, 0.6Hz), 7.08 (1H, t, *J*=7.9Hz), 7.99 (1H, br.s). EI-MS *m/z* (%): 301 (M^+, 95), 283 (18), 270 (10), 254 (20), 242 (10), 215 (55), 199 (10), 183 (20), 171 (100). HR-EI-MS *m/z*: 301.1794 (M^+, calcd. for $C_{17}H_{23}N_3O_2$, 301.1790).

(-)-Indolactam-Nle: 0.9% yield. Amorphous. UV λmax (MeOH) nm (ε): 295 (7800), 283 (7800), 226 (30,000). ^1H NMR δ (CD_3OD, 0.003M, twist only) ppm: 0.74 (3H, t, J=7.0Hz), 1.15 (4H, m), 1.79 (1H, m), 1.89 (1H, m), 2.79 (3H, s), 2.98 (1H, dd, *J*=17.2, 3.3Hz), 3.10 (1H, dd, *J*=17.2, 4.0Hz), 3.50 (1H, dd, *J*=11.4, 8.8Hz), 3.64 (1H, dd, *J*=11.4, 4.8Hz), 4.50 (1H, t, *J*=7.0Hz), 4.68 (1H, m), 6.56 (1H, dd, *J*=5.5, 2.9Hz), 6.93~6.97 (3H, m). EI-MS *m/z* (%): 315 (M^+, 60), 297 (55), 286 (10), 254 (50), 240 (20), 227 (20), 215 (45), 199 (25), 183 (47), 171 (100). HR-EI-MS *m/z*: 315.1949 (M^+, calcd. for $C_{18}H_{25}N_3O_2$, 315.1947).

(-)-Indolactam-Ile: 16.8% yield. Amorphous, $[\alpha]_D^{25}$ -72.3° (*c*=0.16, MeOH). UV λmax (MeOH) nm (ε): 301 (6900), 290 (6600), 228 (25,800). ^1H NMR δ ($CDCl_3$, 0.04M, twist:sofa=3.5:1) ppm; for twist conformer: 0.58 (3H, t, *J*=7.0Hz), 0.63 (1H, m), 0.90 (3H, d, *J*=6.6Hz), 1.37 (1H, m), 2.41 (1H, m), 2.91 (3H, s), 3.04 (1H, dd, *J*=17.6, 4.0Hz), 3.05 (1H, m, OH), 3.18 (1H, br.d, *J*=17.6Hz), 3.56 (1H, m), 3.74 (1H, m), 4.28 (1H, m), 4.52 (1H, d, *J*=10.3Hz), 6.49 (1H, d, *J*=7.7Hz), 6.88 (1H, s), 6.89 (1H, d, *J*=7.3Hz), 7.06 (1H, t, *J*=7.7Hz), 7.33 (1H, br.s), 8.02 (1H, br.s); for sofa conformer: 0.90 (3H, d, *J*=6.6Hz), 1.06 (3H, t, *J*=7.2Hz), 1.33 (1H, m), 1.57 (1H, m), 2.07 (1H, m), 2.20 (1H, m), 2.73 (3H, s), 2.83 (1H, dd, *J*=12.6, 1.8Hz), 3.10 (1H, d, *J*=10.6Hz), 3.43 (2H, m), 4.45 (1H, m), 4.74 (1H, br.d, *J*=11.0Hz), 7.16 (1H, t, *J*=7.7Hz), 8.37 (1H, br.s). Other peaks of the sofa conformer had weak intensities and/or overlapped the peaks of the twist conformer. EI-MS *m/z* (%): 315 (M^+, 45), 297 (20), 284 (7), 268 (35), 258 (15), 240 (12), 227 (10), 215 (40), 199 (12), 183 (32), 171 (100). HR-EI-MS *m/z*: 315.1963 (M^+, calcd. for $C_{18}H_{25}N_3O_2$, 315.1947).

(-)-Indolactam-allo-Ile: 10.0% yield. Amorphous, $[\alpha]_D^{24}$ -128° (*c*=0.10, MeOH). UV λmax (MeOH) nm (ε): 298 (6800), 285 (6500), 228 (24,700). ^1H NMR δ ($CDCl_3$, 0.004M, twist:sofa=2.4:1) ppm; for twist conformer: 0.61 (3H, d, *J*=7.0Hz), 0.85 (3H, t, *J*=7.3Hz), 0.8~1.5 (2H, m), 2.42 (1H, m), 2.93 (3H, s), 3.02 (1H, dd, *J*=17.2, 3.7Hz), 3.18 (1H, br.d, *J*=17.2Hz), 3.54 (1H, dd, *J*=11.0, 8.8Hz), 3.75 (1H, dd, *J*=11.0, 4.0Hz), 4.29 (1H, m), 4.50 (1H, d, *J*=10.6Hz) 6.51 (1H, d, *J*=7.7Hz), 6.77 (1H, br.s), 6.89 (1H, s), 6.90 (1H, d, *J*=8.1Hz), 7.07 (1H, t, *J*=8.1Hz), 7.97 (1H, br.s); for sofa conformer: 1.23 (1H, d, *J*=6.6Hz), 2.23 (1H, m), 2.74 (3H, s), 2.83 (1H, dd, *J*=14.7, 1.5Hz), 3.08 (1H, d, *J*=11.0Hz), 3.12 (1H, dd, *J*=14.7, 4.4Hz), 3.45 (2H, m), 4.45 (1H, m), 4.72 (1H, br.d, *J*=10.6Hz), 7.17 (1H, t, *J*=8.1Hz), 7.28 (1H, d, *J*=8.1Hz), 8.26 (1H, br.s). Other peaks of the sofa conformer had weak intensities and/or overlapped the peaks of the twist conformer. EI-MS *m/z* (%): 315 (M^+, 100), 297 (8), 284 (10), 268 (12), 258 (25), 240 (5), 227 (10), 215 (60), 199 (10), 185 (20), 171 (98). HR-EI-MS *m/z*: 315.1953 (M^+, calcd. for $C_{18}H_{25}N_3O_2$, 315.1947).

(-)-Indolactam-tert-Leu: 5.6% yield. Amorphous. UV λmax (MeOH) nm (ε): 297 (7000), 226 (23,600). EI-MS *m/z* (%): 315 (M^+, 35), 297 (25), 282 (38), 258 (100), 240 (25), 230 (20), 215 (18), 200 (22), 183 (17), 171 (90). ^1H NMR of (-)-indolactam-*tert*-Leu coincided with that reported previously.[7d]

(-)-Indolactam-Leu: 5.2% yield. Amorphous, $[\alpha]_D^{25}$ -222° (*c*=0.14, MeOH). UV λmax (MeOH) nm (ε): 298 (7000), 286 (6900), 229 (26,800). EI-MS *m/z* (%): 315 (M^+, 60), 297 (17), 284 (10), 270 (5), 254 (55), 240 (12), 227 (17), 215 (50), 199 (15), 183 (45), 171 (100). ^1H NMR of (-)-indolactam-Leu coincided with that reported previously.[7d]

(-)-Indolactam-Phg: 1.2% yield. Amorphous. UV λmax (MeOH) nm (ε): 288 (6000), 224 (23,500). ^1H NMR δ ($CDCl_3$, 0.003M, twist:sofa=6:1) ppm; for twist conformer: 2.74 (3H, s), 3.05 (1H, dd, *J*=16.1, 5.1Hz), 3.37 (1H, dd, *J*=16.1, 5.9Hz), 3.58 (1H, dd, *J*=11.0, 7.0Hz), 3.64 (1H, dd, *J*=11.0, 4.8Hz), 5.09 (1H, m), 5.27 (1H, s), 6.63 (1H, d, *J*=7.3Hz), 6.99 (1H, t, *J*=7.5Hz), 7.00 (1H, s), 7.08 (1H, d, *J*=8.1Hz), 7.32 (3H, m), 7.42 (2H, m). The peaks of the sofa conformer had week intensities and/or overlapped the peaks of the twist conformer. EI-MS *m/z* (%): 335 (M^+, 95), 317 (55), 304 (15), 292 (17), 274 (10), 259 (10), 248 (45), 233 (15), 224 (15), 218 (10), 198 (25), 183 (80), 171 (70). HR-EI-MS *m/z*: 335.1631 (M^+, calcd. for $C_{20}H_{21}N_3O_2$, 335.1634).

The $[\alpha]_D$ values of (-)-indolactam-Ala, Abu, γ,δ-Δ-Nva, Nle, *tert*-Leu and Phg were not measured because of insufficient quantity.

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