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Synthesis and Stereochemistry of Indolactam-V, An Active Fragment of Teleocidins. Structural Requirements for Tumor-Promoting Activity.

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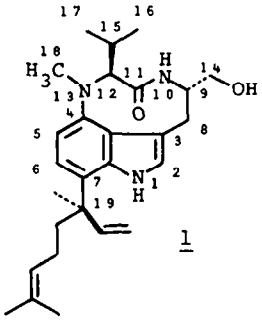
Abstract: (-)-Indolactam-V, which is an active fragment of the potent tumor promoters teleocidins and has also been isolated as a Streptoverticillium metabolite, has been synthesized starting from 4-nitrogramine. The absolute stereochemistry of (-)-indolactam-V has been determined to be (9S, 12S), which suggests that teleocidins and related compounds are biosynthesized from L-amino acids. Three unnatural diastereoisomers were also synthesized.

The NMR spectra of (-)- and (±)-indolactam-V and its derivatives showed that they exist in two conformational states in solution. The structures of the two conformers were deduced from the chemical shifts, coupling constants and nuclear Overhauser effects to be SOFA and TWIST form which are characterized by trans and cis amide bonds, respectively. The calculated ratio of the two conformers was consistent with the observed ratio.

Tumor promoters stimulate tumor formation in animals treated with a suboptimal amount of chemical carcinogens.[1] Until recently, relatively little was known about tumor promoters and their mechanisms of action. Within the past several years, studies mainly on 12-O-tetradecanoyl-phorbol-13-acetate (TPA) have demonstrated a very wide range of biological effects: the most important effects are neoplastic transformation in normal cells, enhancement of expression of transformation markers in cells treated with chemical carcinogens, and modulation of differentiation of normal and neoplastic cells. These specific activities make potent tumor promoters valuable for studies on molecular biology and cancer biology.[2] Searches for naturally occurring tumor promoters, other than TPA and phorbol esters, have been carried out, and one fruitful result has been the discovery of the activity of dihydroteleocidin B by Fujiki and coworkers:[3] this compound shows activity for skin tumor promotion that is equivalent to or higher than that of TPA, and it binds to the putative TPA receptor on cell membranes[4] and exerts many biological effects which are similar to those of TPA.[5] Teleocidin B was first isolated from mycelia of Streptomyces mediocidicus as a highly irritant substance by Takashima and Sakai,[6] and the structure was determined by X-ray crystallography in 1966.[7,8] On the other hand, lyngbyatoxin A (1), which is structurally related to teleocidin B, was isolated from the irritative blue-green alga, Lyngbya majuscula, by Moore et al.[9] Recently, a teleocidin fraction from S. mediocidicus was separated into six components, i.e., two teleocidin A isomers (one of them corresponds to lyngbyatoxin A (1 and 2)),[10] and four teleocidin B isomers (B-1 (3), B-2 (4), B-3 (5), B-4 (6)) in the order of elution on reversed-phase HPLC).[11] These four isomers are diastereomeric at C-19 and C-22 (this, in addition to the introduction of the eleventh



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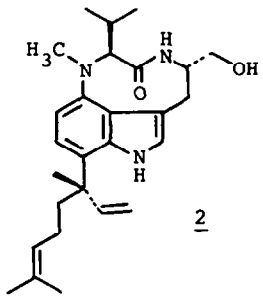

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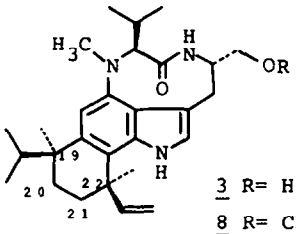

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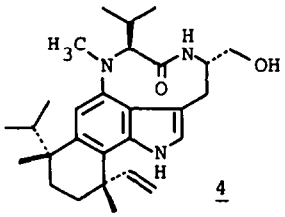

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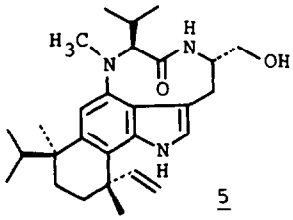

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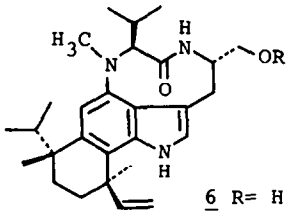

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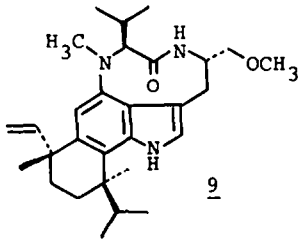

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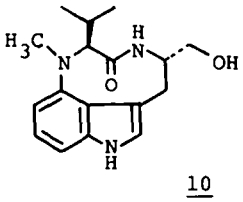

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methyl group, is of interest from a biogenetic stand- point). Teleocidin B-4 is identical with the reported teleocidin B.[12] The structures of the other three isomers were also unambiguously determined.[11] These six isomers were proved to be equally potent as skin tumor promoters.[13] As related compounds, olivoretins A (7), B (8) and C (9), and des-O-methylolivoretin C were isolated from Streptoverticillium olivoreticuli[14] and from S. blastmyceticum.[15]

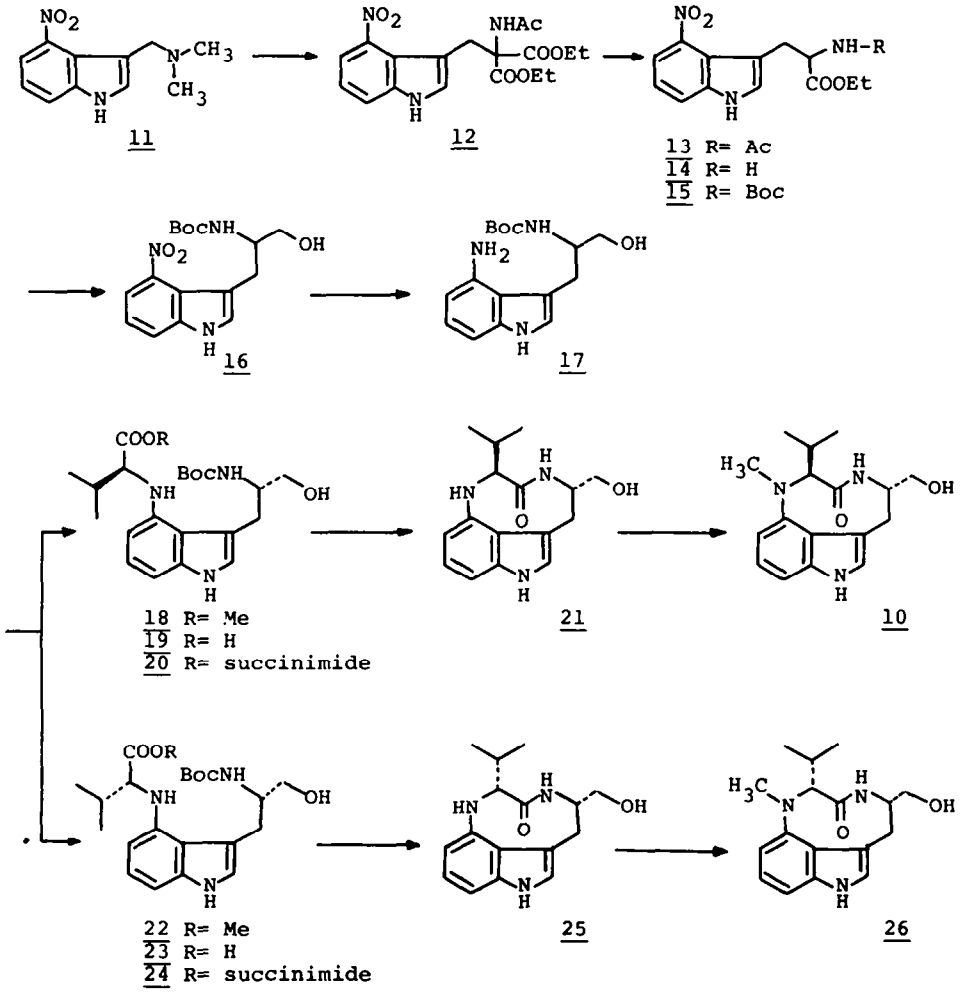
The activity of these teleocidins seems not to be dependent on a specific structure of the terpenoid moiety. All of them have the same modified dipeptide structure, which consists of an indole moiety and a nine-membered lactam ring. Since this unique ring system seems to be indispensable for the biological activity, we planned to synthesized a possible active structural fragment of teleocidins, which we named (-)-indolactam-V ((-)-10),[16] as well as enantiomer ((+)-indolactam-V((+)-10) and diastereoisomers ((+)-epi-indolactam-V, (-)-epi-indolactam-V). This work has established the absolute stereochemistry of teleocidins. Conformational analysis of indolactam-V is also described. Studies on the biological activity of the synthetic compounds revealed that (-)-indolactam-V is an active fragment of the tumor promoters and that the inversion of even one of the chiral centers at C-9 or C-12 abolishes the biological activity. (-)-Indolactam-V((-)-10), together with teleocidin B-4, has been isolated from S. blastmyceticum as an active compound which induces Epstein-Barr virus early antigen.[17]

Synthesis of (-)- and (+)-indolactam-V and (+)- and (-)-epi-indolactam-V.

The terpenoid moieties of the six teleocidins are structurally and chirally different from each other. In particular, these are marked differences of the C_{10}

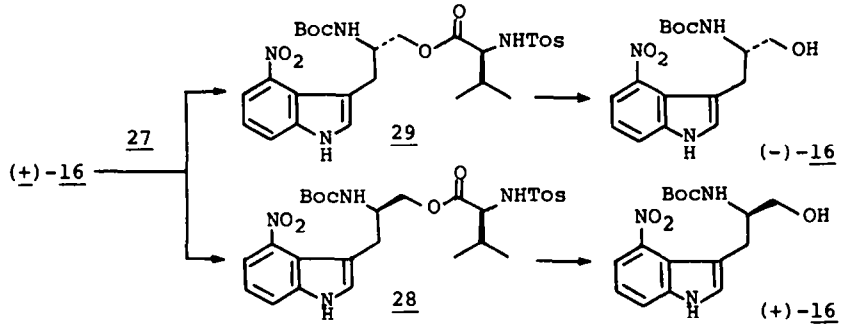
and C_{11} terpenoid side structures of teleocidin B and lyngbyatoxin A. Therefore, these moieties seem to have a rather non-specific role in relation to the putative receptor. That is, it is plausible that a compound lacking the terpenoid moieties, i.e., the common fragment of teleocidins, indolactam-V, might still exhibit the tumor-promoting activity. In addition to testing this hypothesis, we wished to determine the absolute configuration of teleocidins, since it has not been established. The determination of the absolute stereochemistry of teleocidins is essential for a full understanding of the structure-activity relationships of these tumor promoters. Another interesting question is whether or not the enantiomer or the epimers are active. Therefore, we needed all four diastereoisomers in order to establish the structural requirements of teleocidins for activity. This might best be achieved by synthesizing racemic compounds followed by optical resolution, rather than by stereospecific asymmetric synthesis. There are two potential problems in the synthesis: the introduction of the nitrogen group at the 4-position of indole and the formation of the 9-membered lactam ring. Fortunately, 4-nitrogramine is a useful starting material: this route also enabled the synthesis of 6,7-substituted compounds. The lactam ring formation was successfully accomplished by using the active succinimide ester. Synthesis of (±)-indolactam-V was attempted first.

Scheme 1.



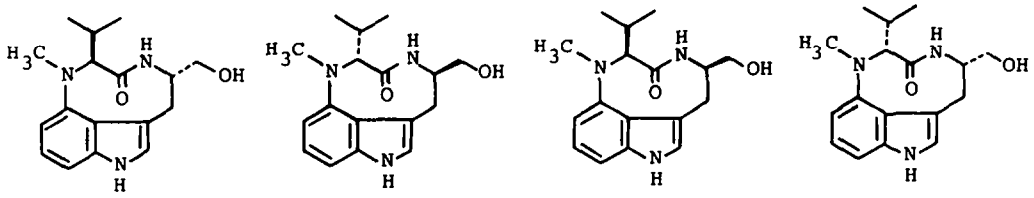
The starting material, 4-nitrogramine (11),[18] was converted to DL-4-nitrotryptophan ethyl ester (14) by a general method for tryptophan synthesis (Scheme 1).[19] The amino group of 14 was protected with a Boc group to give 15 in 95% yield. Reduction of 15 with lithium borohydride gave N-Boc-4-nitrotryptophanol (16) in quantitative yield. The nitro group was catalytically reduced over Pd-charcoal in ethyl acetate to give 17 in 87% yield. Treatment of 17 with methyl 2-oxoisovalerate, and followed by reduction with sodium cyanoborohydride in THF gave the amino esters in 51% yield. The two diastereomeric isomers (18, 22; 3:2) were separated by column chromatography on silica gel. Then 18 was hydrolyzed with 2 N KOH aq. in methanol, and treated with N-hydroxysuccinimide-DCC in acetonitrile to give the activated ester 20 (57%). Deprotection of the Boc group of 20 employing CF_3COOH gave an amino ester, which was treated with weak aqueous alkali ($NaHCO_3$ aq./ethyl acetate) to give the lactam 21 (64%). N-Methylation of 21 employing methyl iodide gave (±)-indolactam-V (10) in 57% yield. The ^{1}H-NMR spectrum of 10 corresponds well to that of teleocidins A and B in the chemical shifts: all the signals in the NMR spectrum of 10 were split in a 2:1 ratio in CD_3OD as in the cases of teleocidins, and all the chemical shifts and coupling constants could be assigned in accordance with those of teleocidins and lyngbyatoxins. The details of the conformational conversion will be described later. The diastereomeric ester (22) prepared from 17 was converted into (±)-epi-indolactam-V (26) in a manner similar to that used for the preparation of (±)-indolactam-V. The ^{1}H-NMR spectrum of 26 was greatly different from that of 17 and the signals were not split, which indicated that the stereochemistry of 26 is different from that of teleocidins, and 26 exists in a conformation different from that of 10. These NMR results clearly show that 10 is the racemate having the same stereochemistry as the natural teleocidins, and 26 is the racemate of the epimeric stereochemistry.

For the purpose of determination of the absolute configuration and investigation of structure-activity relationships involving the two chiral centers on the nine-membered lactam ring moiety, we synthesized optically active 10 and 26. Optical resolution was carried out on N-Boc-4-nitrotryptophanol (16): 16 was treated with (+)-N-tosylvaline chloride (27), prepared from L-valine, to give a mixture of diastereomeric esters (28 and 29), which were separated by column chromatography on silica gel. Hydrolysis of 28 and 29 afforded (-)-16 and (+)-16, respectively. The recovery of the asymmetric isomer was about 85% in each case.



The absolute configuration of (-)-16 was determined by chemical transformation. Optically active (-)-16 was benzoylated (PhCOCl, pyridine), deprotected removal of the Boc group with CF_3COOH, benzoylated ((1) PhCOCl, pyridine, (2) NaH, followed by PhCOCl) to give 4-nitro-N,N,O-tribenzoyltryptophanol ((-)-30), which was catalytically reduced, diazotized ($NaNO_2$, conc. HCl), and then reductively dediazoniated (H_3PO_2) to give (-)-N,N,O-tribenzoyltryptophanol ((-)-31) in 34% overall yield. This product, (-)-31, was identical with S-31 prepared from L-tryptophan ethyl ester. Thus, (-)-16 was converted into (-)-indolactam-V ((-)-10), which has S

stereochemistry at position 12 as well as S stereochemistry at position 9, since 10 unambiguously corresponds to teleocidins, whose relative stereochemistry is certain. Further, (-)-16 was also converted into (+)-epi-indolactam-V ((+)-26) by



(-)-10 (+)-10 (-)-26 (+)-26

the same procedure as described for the preparation of racemic indolactam-V. On the other hand, (+)-16 was converted into (+)-indolactam-V ((+)-10) and (-)-epi-indolactam-V ((-)-26) by similar procedures.

Circular dichroism curves of (-)-10 and (+)-26 are antipodal to those of (+)-10 and (-)-26, respectively, and the CD curve of (-)-10 are approximates well to that of teleocidin B-4.(Figure 1) Thus, the chiral centers on the nine-membered ring of teleocidins must have (9S, 12S) configuration. This conclusion is supported by the observation that only (-)-10 has teleocidin-type activity, and by the co-existence of (-)-10 in the culture medium with teleocidins.[17]

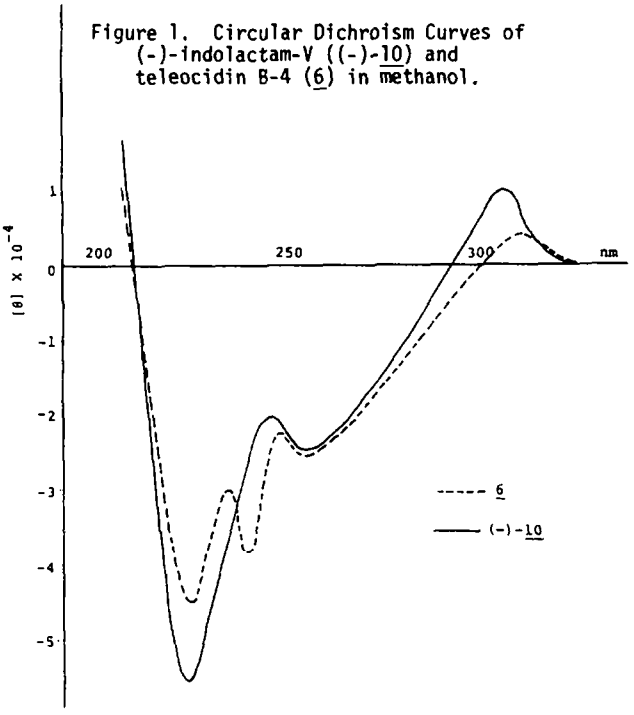


Figure 1. Circular Dichroism Curves of (-)-indolactam-V ((-)-10) and teleocidin B-4 (6) in methanol.

Conformational analysis of indolactam-V and teleocidin derivatives

During the synthesis of indolactam-V, we found that the ^{1}H-NMR spectrum of (±)-10 suggested the existence of two stable conformational states in solution.[20]

Figure 2. 400 Mhz ^{1}H-NMR spectrum of (±)-indolactam-V ((±)-10) in CD_3OD at 23°C. Asterisks mark peaks from conformer A.

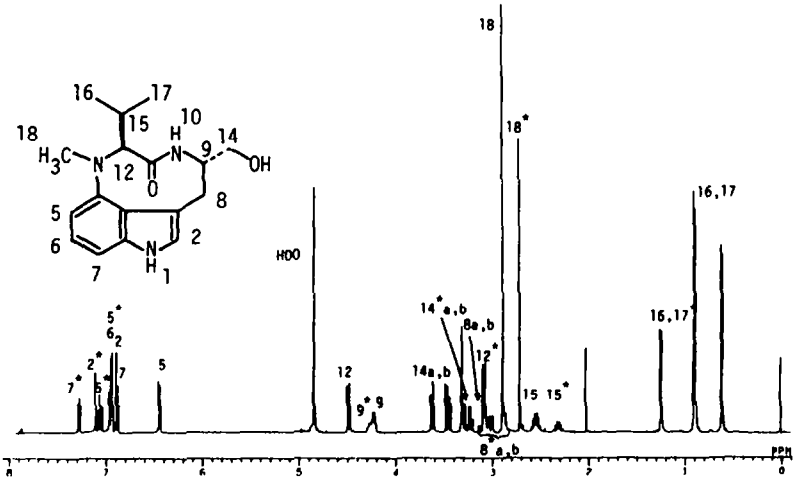
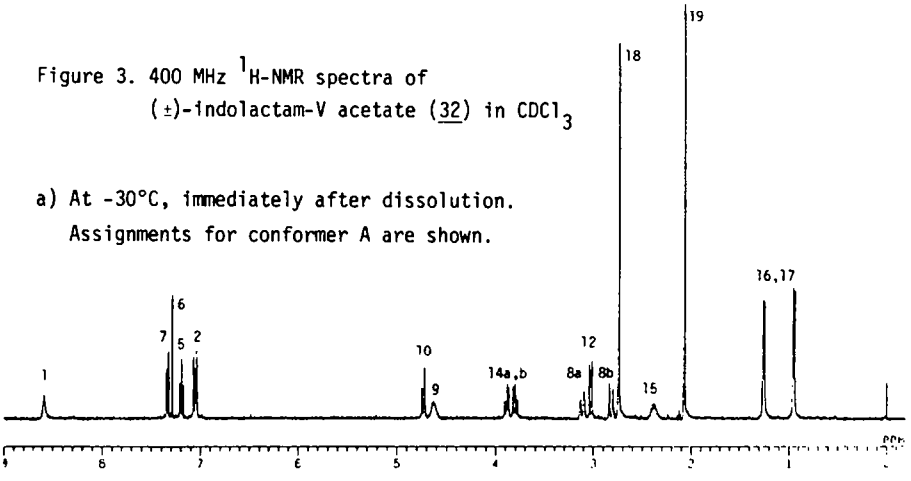


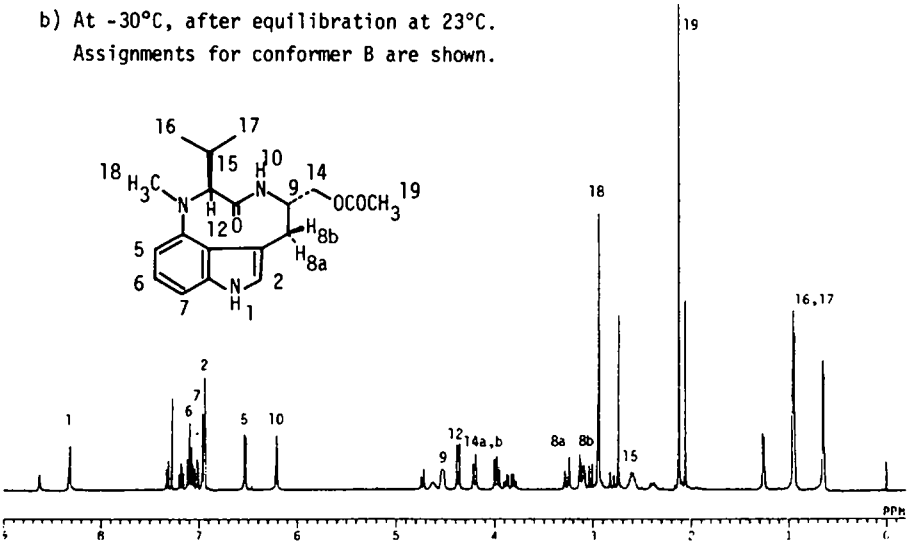
Figure 2 shows the spectrum of (±)-10 in CD_3OD at 23°C. It can be interpreted in terms of two sets of components A and B in a ratio of 1:2. The presence of extra signals in the NMR spectrum of lyngbyatoxin A (1) was reported, though the possible coexistence of an isomer could not be ruled out.[9] We reexamined the NMR spectra of teleocidins. All the teleocidin derivatives also showed NMR spectra consisting of two sets of components A and B, though in a somewhat different ratio. The existence of two stable conformational states of these tumor promoters in solution was confirmed on the basis of a thermodynamic analysis and NMR study of indolactam-V acetate ((±)-32), which has the same biological activity as (±)-10, and the quite similar chemical shifts for the two sets of signals in the NMR spectra of (±)-10 and teleocidins. The ^{1}H-NMR spectra of 32 showed duplicate signals corresponding to ratios of components A and B of 1:2.6 in $CDCl_3$ and 3:2 in CD_3OD when the solutions were prepared at 23°C. If the barrier to the conformational conversion is sufficiently high, it should be possible to dissolve crystalline 32 at low temperature and to observe the ^{1}H-NMR spectrum of the single isomer before it isomerizes. When the ^{1}H-NMR spectrum of 32 was recorded at -30°C by dissolving the crystalline 32 (crystallized from ethanol) in $CDCl_3$ previously cooled to -40°C (Figure 3a), only component A was detectable. When the solution was warmed to 23 °C and the spectrum was again recorded at -30°C (Figure 3b) an equilibrated spectrum was obtained, where the ratio of components A and B was 1:2.8.

Figure 3. 400 MHz ^{1}H-NMR spectra of (±)-indolactam-V acetate (32) in $CDCl_3$

a) At -30°C, immediately after dissolution. Assignments for conformer A are shown.



b) At -30°C, after equilibration at 23°C. Assignments for conformer B are shown.



When the crystals recovered from the equilibrated solution were redissolved in precooled $CDCl_3$ and the NMR spectrum was again recorded at -30°C, it showed only the signals of component A. This result clearly eliminates the possibility that the synthetic indolactams and teleocidins are mixtures of two compounds.

The thermodynamic parameters of the conversion between the two conformers were determined by NMR measurements of the conversion rates from conformer A to B. The rates were slow enough to be determined by the usual kinetic method of following the time-course at three temperature points, i.e. -20°C, -10°C and 0°C. Crystalline 32 was dissolved in $CDCl_3$ at -40°C and spectra were recorded at suitable intervals at each temperature by using a PG-200 autostacking system. The rates of the reversible reaction $A \underset{k'}{\overset{k}{\rightleftharpoons}} B$ were calculated by using the following equation for a first order approach to equilibrium.

$\ln C_0-C_e/C-C_e = (k+k')t$

where C_0 = 100% = the initial percentage of conformer A, C_e = per-centage of conformer A in the equilibrated solution, C = percentage of conformer A at each time, k = rate constant for conversion of conformer A to B, k' = rate constant for conversion of conformer B to A, and t = time. The rate constants (k+k') were calculated from the slopes of the plots of $\ln C_0-C_e/C-C_e$ vs. time, and (k+k') values were converted to rate constants k and k' by using the following equation: $kC_e = k'(C_0-C_e)$. Thus, the rate constants of conversion of A to B were 1.13×10^{-4} sec^{-1} at -20°C, 5.99×10^{-4} sec^{-1} at -10°C and 2.80×10^{-3} sec^{-1} at 0°C. The equilibrium constant between A and B was 2.77 at -10°C. This corresponds to a free-energy difference of 0.53 kcal/mol. From the rate constant at -10°C, the free energy of acti-vation $\Delta G^{\#}$ is 19.2 kcal/mol at -10°C. The Table 1 also shows the values of activation enthalpy $\Delta H^{\#}$ and the activation entropy $\Delta S^{\#}$.

Table 1.

Conformational Conversion Parameters for Conformer A to B in $CDCl_3$ Solution

temp. °C	rate constant s^{-1}	$\Delta G^{\#}$ kcal/mol	$\Delta H^{\#}$ kcal/mol	$\Delta S^{\#}$ cal/K·s^{-1}
-20	1.13×10^{-4}	19.3		
-10	5.99×10^{-4}	19.2	21.6±0.1	+9.4±0.4
0	2.86×10^{-3}	19.1		

The structures of the two conformers were deduced from the ^1H-NMR spectra of 10 and 32. It is noteworthy that some corresponding signals of the two conformers differ significantly in chemical shifts (Table 2,3). In conformer A, protons H-10 and H-12 are subject to high field shielding by the aromatic ring current anisotropy, and the low field shielding of the aromatic protons H-5 and H-7 is considered to be due to the fixation of the molecule in a conformation which decreases the resonance between the lone pair electrons on the nitrogen atom and the aromatic electrons, while the large coupling constant between H-9 and H-10 indicates that the dihedral angle between these protons is near to 180°. On the other hand, in conformer B, protons H-16 and H-17 are subject to high field shielding by the aromatic current anisotropy and the chemical shifts of aromatic protons H-5 and H-7 indicate the fixation of the molecule in a conformation which increases the resonance between the lone pair electrons on the nitrogen atom and the aromatic electrons.

Table 2.

^{1}H-NMR Chemical Shifts of (±)-Indolactam-V ((±)-10) and (±)-Epi-indolactam-V ((±)-26) in CD_3OD Solution at 23 °C.

proton	(±)-10		(±)-26
	conformer A	conformer B	
H_2	7.11 (s)	6.94 (s)	6.90 (s)
H_5	6.95 (dd,J=8.0,1.1)	6.44 (dd,J=8.0,1,1)	6.66 (ABX)
H_6	7.05 (t,J=8.0)	6.95 (t,J=8.0)	6.92 (ABX)
H_7	7.28 (dd,J=8.0,1.1)	6.88 (dd,J=8.0,1.1)	6.91 (ABX)
H_8	2.87 (dd,J=15.0,1.8)	3.05 (dd,J=15.1,3.7)	2.94 (dd,J=15.1,2.2)
	3.02 (dd,J=15.0,4.1)	3.11 (dd,J=15.1,2.0)	3.10 (dd,J=15.1,3.7)
H_9	4.26 (m)	4.23 (m)	3.80 (m)
H_{12}	3.08 (d,J=12.0)	4.48 (d,J=10.5)	4.03 (d,J=10.5)
H_{14}	3.22 (dd,J=10.9,6.5)	3.45 (dd,J=11.3,9.0)	3.72 (dd,J=11.5,8.1)
	3.30 (dd,J=10.9,7.9)	3.62 (dd,J=11.3,4.1)	3.77 (dd,J=11.5,5.8)
H_{15}	2.31 (dsept,J=12.0,6.9)	2.55 (dsept,J=10.5,6.9)	2.57 (dsept,J=10.5,6.9)
$H_{16,17}$	0.90 (d,J=6.9)	0.61 (d,J=6.9)	0.69 (d,J=6.9)
	1.24 (d,J=6.9)	0.89 (d,J=6.9	0.75 (d,J=6.9)
H_{18}	2.77 (s)	2.88 (s)	3.08 (s)

Table 3.

^{1}H-NMR Chemical Shifts of (±)-Indolactam-V acetate ((±)-32) and (±)-Epi-indolactam-V acetate ((±)-26 acetate) in $CDCl_3$ Solution at 23°C.

proton	(±)-32		(±)-26 acetate
	conformer A	conformer B	
H_1	8.29 (s)	8.02 (s)	8.10 (s)
H_2	6.99 (s)	6.90 (s)	6.96 (s)
H_5	7.05 (d,J=8.1)	6.52 (d,J=8.1)	6.78 (dd,J=8.1,1.8)
H_6	7.17 (t,J=8.1)	7.07 (t,J=8.1)	7.05 (t,J=8.1)
H_7	7.28 (d,J=8.1)	6.91 (d,J=8.1)	6.84 (dd,J=8.1,1.8)
H_8	2.78 (d,J=16.2)	3.09 (d,J=16.2,3.6)	2.90 (dd,J=15.8,3.7)
	3.12 (dd,J=16.2,3.9)	3.24 (d,J=16.2)	3.15 (dd,J=15.8,3.7)
H_9	4.60 (m)	4.50 (m)	4.07 (m)
H_{10}	4.73 (d,J=11.5)	6.08 (s)	6.10 (d,J=7.5)
H_{12}	2.98 (d,J=10.7)	4.32 (d,J=9.8)	3.88 (d,J=10.8)
H_{14}	3.83 (ABX)	3.99 (dd,J=11.6,8.1)	4.29 (ABX)
	3.85 (ABX)	4.20 (dd,J=11.6,3.3)	4.35 (ABX)
H_{15}	2.39 (dsept,J=10.7,6.7)	2.61 (dsept,J=9.8,6.7)	2.64 (dsept,J=10.8,6.8)
$H_{16,17}$	0.94 (d,J=6.7)	0.64 (d,J=6.7)	0.74 (d,J=6.8)
	1.24 (d,J=6.7)	0.93 (d,J=6.7)	0.78 (d,J=6.8)
H_{18}	2.75 (s)	2.92 (s)	3.12 (s)
H_{19}	2.02 (s)	2.09 (s)	2.14 (s)

On the basis of these observations and exhaustive examination of molecular models, conformer A and conformer B are presumed to be in SOFA conformation and in TWIST conformation, respectively. The SOFA conformation is characterized by the trans conformation of the amide C-N bond, and the TWIST conformation is characterized by the cis conformation of the amide C-N bond. The two conformations are supported by the nuclear Overhauser difference spectra of 10 and 32 in $CDCl_3$. In conformer A (SOFA), saturation of the H-10 proton (NH) results in characteristic enhancement of the H-12 signal. In conformer B (TWIST), saturation of the H-12 produces NOE at the H-8a signal. These molecular model structure were refined by

empirical force field calculation using Allinger's MM2 program.[21] When the calculation was done using torsional barriers of 20 kcal/mol and 9.5 kcal/mol for the amide C-N bond and the N-13 - C-4 bond, respectively, the free energy difference (0.48 kcal/mol)[22] between the conformers is in agreement with the value derived from the kinetics data. The optimized structures for 10 are illustrated in Figure 4.

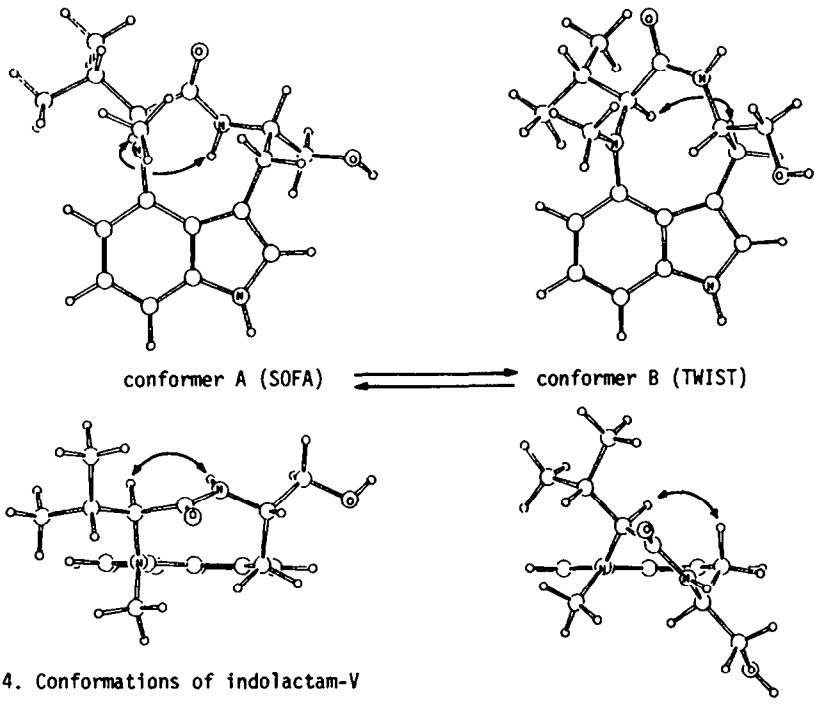


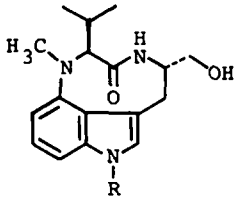
Figure 4. Conformations of indolactam-V
The arrows indicate typical NOE enhancements.
Top: views from the face of the indole ring.
Bottom: views from the side of the indole ring.

The resulting SOFA conformation is very similar to the crystalline conformation of olivoretin B (8)[14] and the resulting TWIST conformation is very similar to the crystalline conformation of teleocidin B-4 (6).[11] Low-temperature NMR measurements of 6 and 8 were taken. As expected from the above results, the spectrum of 8 in $CDCl_3$ at -30 °C, obtained immediately after dissolution of crystalline 8 in $CDCl_3$ precooled to -40°C, showed only signals assigned to the SOFA conformation. When the solution was warmed to 23 °C, then a spectrum of an equilibrated solution (SOFA:TWIST = 1:4) was obtained. On the other hand, when the spectrum of 6 was taken in $CDCl_3$ at -30°C immediately after dissolution of crystalline 6, only signals assigned to the TWIST conformation were detected. The solution was warmed to 23°C, and an equilibrated spectrum (SOFA:TWIST = 1:7) was obtained. Thus, the conversion of the two conformational states in solution seems to occur generally in teleocidin-type tumor promoters.

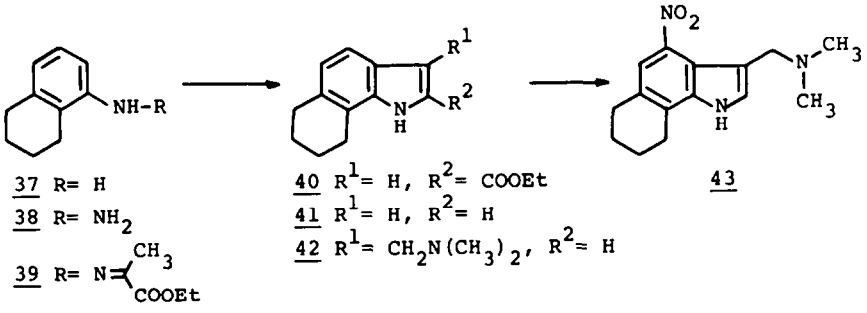
Synthesis of indolactam-V derivatives

Indolactam-V and its derivatives are especially well suited for studies of the structure-activity relationships of teleocidin type tumor promoters. In view of the potential importance of hydrophilic and hydrophobic substituents in relation to the biological activity of teleocidins, the folowing thre points were of interest: i) effect of the free 1-imino group ii) effect of the free 14-hydroxyl group iii) effect of the terpenoid hydrocarbon moiety at the C-6 and C-7 positions on the indole nucleus. In order to examine the first point, 1-substitutedindolactam-Vs were prepared. N-Methyl ((±)-33), N-prenyl ((±)-34) and N-geranylindolactam-V ((±)-35) were prepared from indolactam-V acetate ((±)-32) by treatment with the

appropriate alkyl halide in the presence of NaH, followed by hydrolysis. Concerning the second point, the 14-O-methyl derivative was obtained as a by-product in the preparation of N-methylindolactam-V. In order to investigate the third point, an indolactam derivative having an alkyl moiety at the C-6, C-7 positions, (-)-indolactam-V-tetramethylene ((-)-36), was synthesized as follows. 5,6,7,8-Tetrahydro-1-naphthylamine (37) was diazotized, then reduced with $TiCl_3$ to give the hydrazine (38) in 44% yield.[23] Treatment with ethyl pyruvate yielded the hydrazone (39), which was cyclized with sulfuric acid, hydrolyzed and decarboxylated to give 6,7,8,9-tetrahydrobenzo[g]indole (40) in 20% yield. This product (40) was converted to the gramine derivative (41), and nitration afforded 3-dimethylamino-4-nitro-6,7,8,9-tetrahydrobenzo[g]indole (42) in 35% yield. Compound 42, which corresponds to 4-nitrogramine (11), was converted into the 4-nitrotryptophanol derivative (43). Optical resolution was carried out at this stage, and (-)-43 was converted to (-)-36 by a procedure similar to that used for the preparation of (-)-10.



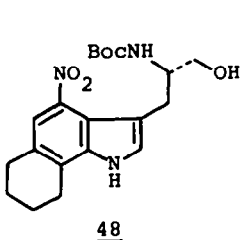
33 R= CH_3
34 R= prenyl
35 R= geranyl



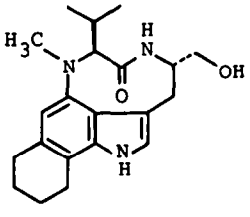
37 R= H
38 R= NH_2
39 R= $N{=}C(CH_3)COOEt$

40 R^1= H, R^2= COOEt
41 R^1= H, R^2= H
42 R^1= $CH_2N(CH_3)_2$, R^2= H

43



48



(-)-36

Discussion

Indolactam-V ((-)-10), its enantiomer ((+)-10) and the two C-9 and C-12 diastereomeric isomers ((+)-26, (-)-26) were synthesized in order to investigate the structure-activity relationships, and to determine the absolute configurations of teleocidins, which were not known. (-)-Indolactam-V ((-)-10) is the teleocidin skeleton without the terpenoid side chain, and it exists in essentialy the same two conformational states in solution as teleocidins. The absolute configuration of (-)-indolactam-V ((-)-10) and teleocidins was determined as (9S,12S). This suggests that telocidins and (-)-indolactam-V are biosynthesized from L-Trp and L-Val. Recently, a possible biosynthetic intermediate, N-methyl-L-valyl-L-tryptophanol was isolated from mycelia of *S. mediocidicus* and *S. olivoreticuli*. (-)-Indolactam-V ((-)-10) is a very plausible biosynthetic intermediate of teleocidins, and this hypothesis is strongly supported by the isolation of this compound from *S. blastmy-*

ceticum.[17] (-)-Indolactam-V was found to exhibit several of the known biological effects of tumor promoters.[24] This means that (-)-indolactam-V is an active fragment: the hydrophobic moiety (the terpene moiety) of teleocidins is not necessarily required. This conclusion is in contrast to the case of TPA, whose basic skeleton without the tetradecanoate and acetate side chains (phorbol) shows no activity. (+)-Indolactam-V ((+)-10) and the two isomers of epi-indolactam-V ((+)-26, (-)-26) showed no biological effects.[24]

Indolactam-V and teleocidin derivatives exist in two stable conformational states in solution. From the ratio of the two conformers in solution (CD_3OD or $CDCl_3$), the free-energy difference between the two conformers was calculated to be 0-1.5 kcal/mol in all teleocidin derivatives. The two conformers, SOFA form and TWIST form, are characterized by the trans amide bond and cis amide bond, respectively. It is noteworthy that in the SOFA conformer optimized by MM2, the dihedral angle between the trans amide C=O bond and the N-H bond is approximately 173°, i.e., not completely planar. On the other hand, in the TWIST conformer, the dihedral angle between the cis amide C=O bond and the N-H bond is very close to 0°. Our minimized energy calculations for indolactam-V ((±)-10) gave values of 45.4 kcal/mol and 44.9 kcal/mol for the SOFA form and the TWIST form, respectively: the difference between the two energy values corresponds well to the observed energy diference (0.53 kcal).

The free energy of activation $\Delta G^{\#}$ (19.2 kcal/mol at -10°C) in the conversion of the SOFA form to the TWIST form can be accounted for by the $\Delta G^{\#}$ (about 20 kcal/mol)[25] in the rotation of the amide bond from cis to trans. The free energy of activation indicates that rotation of the amide bond is restricted but possible. From the conversion parameters, $\Delta H^{\#}$, and $\Delta S^{\#}$, the 50 % equilibration times are calculated to be 14 min at -10°C, and 6 sec at +20°C. Thus, isolation of the two conformers in solution is not possible.[26] The results of conformational analysis of indolactam-Vs in solution suggest that the conformational state, i.e., TWIST form and/or SOFA form, may play an important role in the appearance of activity. In order to resolve this problem, we are proceeding with the synthesis of conformationally fixed indolactam derivatives.

Structural modification of indolactam-V affects the biological activity.[24,27,28] The importance of the 14-hydroxyl group is proved by the loss of the activity in the O-methylated compound.[27] 1-Methyl- ((±)-33) and 1-prenylindolactam-V ((±)-34) showed activity slightly weaker than that of (±)-10, but N-geranylindolactam-V ((±)-35) was found to exhibit stronger activity than (±)-10.[28] The presence of the hydrophobic group may cause an enhancement of the activity that is greater than the decreasing effect due to blocking of the 1-NH group.[28] The difference of activity between (-)-indolactam-V and teleocidins may be interpreted in terms of the absence or presence of the terpenoid side structure. In accordance with this, (-)-indolactam-V-tetramethylene ((-)-36), which has a hydrophobic group of intermediate size, was 4 times more effective than (-)-10,[26] although its effect was ten-fold weaker than that of teleocidin.

The present syntheses of teleocidin-type tumor promoters pave the way for detailed analysis of the structure-activity relationships of tumor promoters, the molecular design of new tumor promoters and antagonists, and receptor mapping of the putative receptor: these goals all require a correct structural understanding. Since the indole tumor promoters as well as phorbol tumor promoters are undoubtedly most important epigenetic chemical modulaters of cell prolification and cell differentiation, further chemical researches to establish the basis of their actions should be helpful for understanding and treating cancer.

Experimental Section

General Remarks. Melting points were obtained on a Yanagimoto micro hot stage and are uncorrected. Spectra were recorded with the following instruments: ^{1}H-NMR spectra, JEOL JMN-FX-100 (100 MHz) and JEOL JMN-FX-400 (400 MHz); mass spectra, JEOL JMS-DX-300; IR spectra, JASCO DS-402G. NMR spectra were recorded with $(CH_3)_4Si$ as an internal standard and the chemical shifts are given as δ values from TMS. The IR data are presented in cm^{-1}. Optical rotations were determined with JASCO DIP-181 polarimeter. All the elemental analyses agreed with the calculated values to within ±0.3%. Column chromatography was performed on silica gel (Merck 7734 or 9385 (flash chromatography)).

Diester (12) Ethyl acetamidomalonate (23.8 g, 0.109 mol) was added to a solution of 2.5 g (0.109 mol) of sodium in 150 ml of dry ethanol, then 20 g (0.109 mol) of 4-nitrogramine (11) was added. The mixture was chilled with ice-water and 22.6 g (0.179 mol) of dimethyl sulfate was added dropwise with stirring. Stirring was continued at room temperature for 4 h, then the mixture was poured into water. The resulting suspension was chilled in ice, and the precipitated solid was collected and dissolved with CH_2Cl_2. This solution was washed with 1 N HCl and water, dried over $MgSO_4$ and concentrated in vacuo to give the crude diester. Crystallization from $CH_3COOC_2H_5$ afforded the diester (12)as bright yellow cubes (27.2 g, 76%); mp 189-191°C. IR (KBr) 1665 (s), 1740 (s); ^{1}H-NMR ($CDCl_3$) 1.23 (t, 6H, J=7 Hz, $-CH_2-CH_3$), 1.80 (s, 3H, $NHCOCH_3$), 3.96 (s, 2H, $Ar-CH_2-$), 4.20 (q, 4H, J=7 Hz, $-O-CH_2-CH_3$), 6.55 (bs, 1H, NH-CO), 7.14 (t, 1H, J=8 Hz, 6-CH), 7.37 (d, 1H, J=3 Hz, 2-CH), 7.55 (d, 1H, J=8 Hz, 7-CH), 7.82 (d, 1H, J=8 Hz, 5-CH), 9.15 (bs, 1H, 1-NH); Anal. ($C_{18}H_{21}N_3O_7$): C,H,N.

N-Acetyl-4-nitrotryptophan ethyl ester (13) The diester (12) (27.2 g, 0.07 mol) was added a solution of 13 g (0.325 mol) of sodium hydroxide in 120 ml of water. The mixture was refluxed for 70 min, then cooled in ice, and acidified with conc. HCl. The precipitate was collected, washed with saturated NaCl aq., and dried at 80°C in vacuo over P_2O_5. The crude diacid was heated with 10 ml of water at 100°C for 2 h. After cooling with ice, the resultant precipitate was collected and dried at 80°C in vacuo over P_2O_5 for 8 h. The crude monoacid was added to a solution of 20 ml of thionyl chloride in 90 ml of dry ethanol (prepared at -10°C) with stirring at -10°C. The mixture was stirred for 1 h at room temperature and 3 h at 60°C, then concentrated in vacuo to remove most of the ethanol. The residue was dissolved in $CH_3COOC_2H_5$ and washed with water, saturated Na_2CO_3, and water. The solution was dried over $MgSO_4$ and concentrated. The residue was dissolved in ethanol and treated with active carbon. After filtration, the solvent was evaporated off to leave crude 13. Crystallization from $CH_3COOC_2H_5$ afforded N-acetyl-4-nitrotryptophan ethyl ester as yellow needles (15.1 g, 68%) ; mp 162-164°C; IR (KBr) 1650 (s), 1730 (s); ^{1}H-NMR ($CDCl_3$-DMSO-d_6) 1.19 (t, 3H, J=7 Hz, $-CH_2-CH_3$), 1.93 (s, 3H, $NHCOCH_3$), 3.3-3.5 (m, 2H, $Ar-CH_2$), 4.12 (q, 2H, J=7 Hz, $O-CH_2$), 4.76 (m, 1H, -CH), 6.88 (bs, 1H, -NH-CO), 7.16 (t, 1H, J=8 Hz, 6-cH), 7.25 (d, 1H, J=2 Hz, 2-CH), 7.68 (dd, 1H, J=8, 1 Hz, 7-CH), 7.84 (dd, 1H, J=8, 1 Hz, 5-CH), 12.6 (bs, 1H, 1-NH); Anal. ($C_{15}H_{17}N_3O_5$) C,H,N.

4-Nitrotryptophan ethyl ester (14) Compound 13 (15.0 g, 0.047 mol) was added to a saturated solution of HCl in 300 ml of ethanol. The reaction mixture was refluxed for 48 h, then cooled with ice-water, and 70 ml of ether was added. The suspension was chilled and the resultant precipitate was collected and dried; 13.1 g (88.7%) of the HCl salt of 14 was obtained. The HCl salt was dissolved in a minimum amount of water, neutralized with $NaHCO_3$, and extracted with $CH_3COOC_2H_5$. The solvent was removed at below 40°C in vacuo to give 4-nitrotryptophan ethyl ester (11.1 g); mp 96-98°C, IR (KBr) 1735 (s); ^{1}H-NMR ($CDCl_3$) 1.20 (t, 3H, J=7 Hz, $-CH_2-CH_3$), 3.08 (dd, 1H, J=6. 8 Hz, Ar-CH), 3.45 (dd, 1H, J=6, 14 Hz, Ar-CH), 3.72 (dd, 1H, J=6, 8 Hz, CH), 4.14 (q, 2H, J=7 Hz, $-O-CH_2-$), 7.13 (t, 1H, J=8 Hz, 6-CH), 7.23 (d, 1H, J=2 Hz, 2-CH), 7.52 (dd, 1H, J=8, 1 Hz, 7-CH), 7.84 (dd, 1H, J=8, 1 Hz, 5-CH), 9.15 (bs, 1H, 1-NH); Anal. ($C_{13}H_{15}N_3O_4$) C,H,N.

N-Boc-4-nitrotryptophan ethyl ester (15) Compound 14 (11g, 0.04 mol) was dissolved in 50 ml of dioxane and 50 ml of water, and then 8.34 g of $NaHCO_3$ and 11.3 g (0.08 mol) of tert-butoxycarbonyl azide were added. The solution was heated at 40-45°C for 40 h with stirring. Removal of most of the solvent in vacuo gave crude 15 as a yellow solid, which was dissolved in $CH_3COOC_2H_5$ and washed with water, 0.5 N $NaHCO_3$ aq., 0.5 N citric acid aq., and water. The solution was dried over $MgSO_4$ and concentrated to give N-Boc-4-nitrotryptophan ethyl ester (15)(13.5 g, 90%). mp 193-194°C (from $CH_3COOC_2H_5$); IR (KBr) 1670 (s), 1720 (s); ^{1}H-NMR ($CDCl_3$-DMSO-d_6) 1.19 (t, 3H, J=7 Hz, $-CH_2-CH_3$), 1.34 (s, 9H, $-(CH_3)_3$), 3.40 (m, 2H, $Ar-CH_2$), 4.40 (m, 1H, CH), 4.12 (q, 2H, J=7 Hz, $-CH_2-CH_3$), 5.40 (bs, 1H, -NH-CO), 7.16 (t, 1H, J=7 Hz, 6-CH), 7.32 (d, 1H, J=2 Hz, 2-CH), 7.69 (d, 1H, J=7 Hz, 7-CH), 7.84 (d, 1H, J=7 Hz, 5-CH), 11.0 (bs, 1H, 1-NH); Anal. ($C_{18}H_{23}N_3O_6$), C,H,N.

N-Boc-4-nitrotryptophanol (16) $LiBH_4$ (7.4 g, 0.34 mol) was added portionwise to a solution of 13.2 g (0.035 mol) of 15 in 20 ml of THF at 0°C with stirring, and the mixture was stirred at

room temperature for 3 h, then poured into water, and extracted with $CH_3COOC_2H_5$. The extract was washed with water, dried over $MgSO_4$ and concentrated. Crystallization of the residue from ethanol-n-hexane gave 11.5 g of N-Boc-4-nitrotryptophanol (16)(98%). mp 153-154°C; IR (KBr) 1960 (s); ^{1}H-NMR ($CDCl_3$) 1.30 (s, 9H, $-C(CH_3)_3$), 2.9-3.2 (m, 2H, Ar-CH_2-), 3.5 (m, 2H, $-CH_2$-OH), 4.35 (m, 1H, -CH), 5.66 (bs, 1H, -NH-COO-), 7.15 (t, 1H, J=8 Hz, 6-CH), 7.37 (d, 1H, J=2 Hz, 2-CH), 7.69 (d, 1H, J=8 Hz, 7-CH), 7.77 (d, 1H, J=8 Hz, 5-CH), 11.30 (bs, 1H, 1-NH); Anal. ($C_{16}H_{21}N_3O_5$) C,H,N.

N-Boc-4-aminotryptophanol (17) A mixture of 10.1 g (0.03 mol) of 16 and 8 g of 10% Pd-charcoal in 350 ml of $CH_3COOC_2H_5$ containing 1% water was vigorously stirred under 1 atm of H_2 at room temperature for 2 h, then filtered. The filtrate was concentrated in vacuo and separated by column chromatography on silica gel (CH_2Cl-$CH_3COOC_2H_5$ 1:1) to give N-Boc-4-nitrotryptophanol (17) as a viscous liquid (8.16 g, 89%). ^{1}H-NMR($CDCl_3$) 1.48 (s, 9H, $-C(CH_3)_3$), 3.0-3.2 (m, 2H, Ar-CH_2-), 3.50 (m, 2H, $-CH_2$-OH), 4.80 (m, 1H, -CH), 5.40 (bd, 1H, -NH-COO-), 6.40 (dd, 1H, J=8, 2 Hz, 5-CH), 6.8-7.0 (m, 3H, 2-CH, 6-CH, 7-CH), 8.19 (s, 1H, 1-NH).

Methyl 2-oxoisovalerate L-Valine (50 g, 0.427 mol) was added portionwise to 120 g (0.571 mol) of trifluoroacetic anhydride at 0°C. The mixture was heated on oil bath under reflux for 30 min at 80°C, and for 30 min at 120-130°C, then excess trifluoroacetic anhydride and trifluoroacetic acid were evaporated off at the same temperature. The residue was partitioned between ether and water, and the organic layer was washed with 5% $NaHCO_3$ aq., dried ($MgSO_4$), and concentrated. Distillation of the crude product gave 58.3 g (70%) of the oxazolone; bp. 56-57°C (11 mm). The oxazolone was added to a solution of 13.2 g of NaOH in 150 ml of water, and the mixture was stirred at room temperature for 15 h. The aqueous solution was washed with ether, and acidified with 5% HCl aq. and extracted with ether. The ethereal layer was washed with water and dried over $MgSO_4$. Removal of the solvent and distillation of the residue afforded 21.7 g (63%) of 2-oxoisovaleric acid; bp 70-72°C (14 mm); fp 31°C; ^{1}H-NMR ($CDCl_3$) 1.20 (d, 6H, J=7 Hz, $CH(CH_3)_2$), 3.39 (sept, 1H, J=7 Hz, $-CH(CH_3)_2$), 8.51 (s, 1H, COOH) A solution of 21g (0.181 mol) of 2-oxoisovaleric acid in 100 ml of ether was treated dropwise with diazomethane ether solution at 0°C until the yellow color of diazomethane no longer disappeared. The solution was washed with 5% $NaHCO_3$ aq. and concentrated. Distillation of the crude product gave 19.7 g (84%) of methyl 2-oxoisovalerate; bp 66-67°C (20 mm); ^{1}H-NMR ($CDCl_3$) 1.20 (d, 6H, J=7 Hz, $-CH(CH_3)_2$), 3.31 (sept, 1H, J=7 Hz, $-CH(CH_3)_2$), 3.90 (s, 3H, $COOCH_3$).

Diastereomeric esters (18 and 22) A mixture of 7.93 g (24.2 mmol) of 17 and 10.4 g (78 mmol) of methyl 2-oxoisovalerate in 100 ml of chloroform was heated to reflux for 18 h under an Ar atmosphere. After removal of the solvent and excess methyl 2-oxoisovalerate, the resulting residue was dissolved in 200 ml of THF. Then 3.1 g (49 mmol) of $NaBH_3CN$ was added portionwise with stirring at room temperature. The reaction mixture was stirred at room temperature for 8 h, poured into ice-water, acidified with 0.5 M citric acid aq. solution, and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. Separation by column chromatography on silica gel using CH_2Cl_2-$CH_3COOC_2H_5$ (8:3) as the eluent gave two isomers. The less polar isomer was the ester 18 (3.81 g, 35%) and the more polar isomer was 22 (2.50 g, 23%). 18; mp 166-169°C (from CH_2Cl_2-n-hexane); ^{1}H-NMR ($CDCl_3$) 1.04 (d, 3H, J=7 Hz, $-CH(CH_3)_2$), 1.15 (d, 3H, J=7 Hz, $-CH(CH_3)_2$), 1.04 (s, 9H, $-C(CH_3)_3$), 2.10 (m, 1H, $-CH(CH_3)_2$), 3.0-3.2 (m, 2H, Ar-CH_2), 3.6-3.8 (m, 2H, $-CH_2$-OH), 3.75 (s, 3H, $COOCH_3$), 3.9-4.1 (m, 2H, 2 X aliphatic CH) 5.30 (bs, 1H, -NH-COO-), 6.17 (d, 1H, J=8 Hz, 5-CH), 6.76 (d, 1H, J=8 Hz, 7-CH), 6.93 (d, 1H, J= 2Hz, 2-CH), 6.96 (t, 1H, J=8 Hz, 6-CH), 8.11 (s, 1H, 1-NH). Anal. ($C_{22}H_{33}N_3O_5$) C,H,N. 19; mp 181-184°C (from CH_2Cl_2-n-hexane) ^{1}H-NMR($CDCl_3$) 1.05 (d, 3H, J=7 Hz, $-CH(CH_3)_2$), 1.13 (d, 3H, J=7 Hz, $-CH(CH_3)_2$), 1.45 (s, 9H, $-C(CH_3)_3$), 2.10 (sept, 1H, J=7 Hz, $-CH(CH_3)_2$), 3.08 (d, 1H, J=8, 15 Hz, Ar-CH_2), 3.28 (dd, 1H, J=5, 15 Hz, Ar-CH_2), 3.60 (m, 2H, $-CH_2$-OH), 3.9-4.1 (m, 2H, 2 X aliphatic CH), 5.08 (d, 1H, -NH-COO-), 6.20 (d, 1H, J=8 Hz, 5-CH), 6.78 (d, 1H, J=8 Hz, 7-CH), 6.87 (d, 1H, J=2 Hz, 2-CH), 6.99 (t, 1H, J=8 Hz, 6-CH), 8.12 (s, 1H, 1-NH). Anal. ($C_{22}H_{33}N_3O_5$) C,H,N.

Activated ester (20) A mixture of a solution of 3.35 g (8 mmol) of 18 in 200 ml of methanol and 40 ml (80 mmol) of 2 N KOH aq. solution was kept at room temperature for 24 h. Methanol was evaporated off in vacuo and the residue was diluted with 50 ml of ice-water. The aqueous solution was acidified with 0.5 M citric acid aq. solution at 0°C, and extracted with $CH_3COOC_2H_5$. The extract was dried over $NaSO_4$ and concentrated to give the crude acid (19). The acid and 1.84 g (16 mmol) of N-hydroxysuccinimide were dissolved in 50 ml of CH_3CN, and then a solution of 2.47 g (12 mmol) of dicyclohexylcarbodiimide in 15 ml of CH_3CN was added at 0°C with stirring. Stirring was continued for 1 h at room temperature, then the solvent was removed in vacuo and the residue was dissolved in $CH_3COOC_2H_5$. A precipitate (dicyclohexylurea) was removed by filtration, and the filtrate was concentrated. The residue was dissolved in CH_2Cl_2 and the solution was washed with water, dried ($NaSO_4$) and concentrated. The residue was chromatographed

on silica gel using CH_2Cl_2-$CH_3COOC_2H_5$ (1:1) as the eluent to give an the activated ester (20) (2.28 g, 57%). ^{1}H-NMR ($CDCl_3$) 1.23 (d, 6H, J=7 Hz, -CH(CH_3)$_2$), 1.45 (s, 9H, -C(CH_3)$_3$), 2.40 (m, 1H, -CH(CH_3)$_2$), 2.79 (s, 4H, -CO-CH_2-CH_2-CO-), 3.1-3.3 (m, 2H, Ar-CH_2-), 3.60 (m, 2H, -CH_2-OH), 3.80 (m, 1H, -CH), 4.31 (d, 1H, J=7 Hz, -CH), 5.22 (bs, 1H, -NH-COO-), 6.37 (d, 1H, J=8 Hz, 5-CH), 6.83 (d, 1H, J=8 Hz, 7-CH), 6.93 (d, 1H, J=2Hz, 2-CH), 7.06 (t, 1H, J=8 Hz, 6-CH), 8.08 (bs, 1H, 1-NH).

Lactam (21) Trifluoroacetic acid (50 ml) was added to a solution of 2.01 g (4 mmol) of the activated ester (20) in 50 ml of CH_2Cl_2 at 0°C with stirring. The mixture was stirred for 1 h, at 0°C under an Ar atmosphere, then the trifluoroacetic acid was removed *in vacuo* at below 30°C. The residue was dissolved in $CH_3COOC_2H_5$ (400 ml), then 10 ml of saturated $NaHCO_3$ aq. solution was added and the mixture was refluxed for 1 h with vigorous stirring. The organic layer was separated and the aqueous layer was extracted with $CH_3COOC_2H_5$. The combined $CH_3COOC_2H_5$ extract was dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography on silica gel using $CH_3COOC_2H_5$ as the eluent to afford 770 mg (67%) of the lactam (21). mp 240-242°C (from methanol); ^{1}H-NMR (CD_3OD) Two conformers existed. Signals due to the major conformer were assigned as follows. 1.04 (d, 3H, J=6.5 Hz, -CH(CH_3)$_2$), 1.24 (d, 3H, J=6.5 Hz, -CH(CH_3)$_2$), 2.28 (d sept, 1H, J=6.5, 9.5 Hz, -CH(CH_3)$_2$), 2.99 (d, 1H, J=9.5,15 Hz, Ar-CH_2-), 3.14 (dd, 1H, J=6.5, 15 Hz, Ar-CH_2-), 3.57 (d, 1H, J=9.5 Hz, Ar-NH-CH-), 3.62 (dd, 1H, J=7, 11 Hz, -CH_2-OH), 3.70 (dd, 1H, J=5,10 Hz, -CH_2-OH), 5.11 (bs, 1H, -CH-CH_2-OH), 6.66 (dd, 1H, J=7.5, 1.0 Hz, 5-CH), 6.94 (t, 1H, J=7.5 Hz, 6-CH), 6.95 (s, 1H, 2-CH), 7.02 (dd, 1H, J=7.5, 1.0 Hz, 7-CH); MS 287(M^+); Anal. ($C_{16}H_{21}N_3O_2$) C,H,N.

Indolactam-V ((±)-10) A mixture of 574 mg (2 mmol) of 21, 840 mg (10 mmol) of $NaHCO_3$ and 50 ml of CH_3I in 50 ml of methanol was heated to reflux for 60 h, under an Ar atmosphere. The solvent was removed *in vacuo* and the residual solid was partitioned between $CH_3COOC_2H_5$ and water. The organic layer was dried and concentrated to give crude 10. Purification by column chromatography on silica gel using $CH_3COOC_2H_5$ gave (±)-indolactam-V ((±)-10). (373 mg, 62%); mp 261-264°C (from methanol); IR (KBr) 1640 (s); ^{1}H-NMR (CD_3OD) signals are given in the text. MS 301 (M^+); Anal. ($C_{17}H_{23}N_3O_2$) C,H,N.

Activated ester (24) The procedure was the same as that used for the preparation of 20,employing 2.1 g (5 mmol) of 22, 150 ml of methanol, and 25 ml (50 mmol) of 2 N KOH aq. solution. The acid (23) was isolated and converted to the activated ester (24), using 1.15 g (10 mmol) of N-hydroxysuccinimide, 30 ml of CH_3CN, and 1.55 g (7.5 mmol) of dicyclohexylcarbodiimide, to give 1.76 g (70%) of the activated ester (24); ^{1}H-NMR ($CDCl_3$) 1.23 (d, 6H, J=7 Hz, -CH(CH_3)$_2$), 1.40 (s, 9H, -C(CH_3)$_3$), 2.50 (m, 1H, -CH(CH_3)$_2$), 2.76 (s, 4H, -CO-CH_2-CH_2-CO-), 3.00 (dd, 1H, J=15, 8 Hz, Ar-CH_2-), 3.30 (dd, 1H, J=15, 8 Hz, Ar-CH_2-), 3.5 (m, 2H, -CH_2-OH), 3.89 (m, 1H, -CH-), 4.28 (m, 1H, -CH-), 5.00 (bs, 1H, -NH-COO-), 6.37 (d, 1H, J=8 Hz, 5-CH), 6.80 (d, 1H, J=8 Hz, 7-CH), 6.84 (d, 1H,J=2 Hz, 2-CH), 7.05 (t, 1H, J=8 Hz, 6-CH), 8.04 (bs, 1H, 1-NH).

Lactam (25) The procedure was the same as that used for the preparation of 21, employing 1.75 g (3.49 mmol) of 24, 45 ml of CH_2Cl_2, and 45 ml of trifluoroacetic acid. After work-up, a residue was treated with 350 ml of $CH_3COOC_2H_5$ and 90 ml of saturated $NaHCO_3$ aq. solution at reflux temperature to give 748 mg (75%) of the lactam (25): mp 138-140°C (methanol); ^{1}H-NMR (CD_3OD) 0.95 (d, 3H, J=6.5 Hz, -CH(CH_3)$_2$), 1.13 (d, 3H, J=6.5 Hz,-CH(CH_3)$_2$), 2.17 (dsept, 1H,J=6.5, 10 Hz, -CH(CH_3)$_2$), 3.0-3.2 (m, 2H, Ar-CH_2-), 3.70 (m, 2H, -CH_2-OH), 3.89 (m, 1H, -CH-), 4.60 (m, 1H, -CH-), 6.54 (dd, 1H, J=8, 1 Hz, 5-CH), 6.85-6.95 (m, 3H, 2-CH,6-CH,7-CH),; MS 287(M^+) Anal. ($C_{16}H_{21}N_3O_2$) C,H,N.

Epi-indolactam-V (26) The procedure was the same as that used for the preparation of 10 employing 574 mg (2 mmol) of 25, 840 mg (10 mmol) of $NaHCO_3$, 50 ml of CH_3I, and 50 ml of methanol for 40 h at refluxing temperature. The yield of epi-indolactam-V was 476 mg (79%): mp 214-216°C (chloroform); ^{1}H-NMR (CD_3OD) signals are described in the text. MS 301 (M^+); Anal. ($C_{17}H_{23}N_3O_2$) C,H,N.

(S)-(+)-N-Tosylvaline chloride (27) The procedure was similar to that used for the synthesis of (±)-N-tosylvaline chloride.[29] (S)-N-Tosylvaline (5.42g, 20 mmol) and PCl_5 (6.26 g, 30 mmol) were suspended in 40 ml of dry ether and shaken until all the organic material had dissolved, then for a further 30 min. Excess PCl_5 was removed by fitration, 200 ml of n-hexane was added, and the solution was set aside at 0°C for 8 h. The resultant crystalline acid chloride was collected, washed with n-hexane and dried to give 4.34 g (75%) of (S)-N-tosylvaline chloride (27): mp 64-65°C; $[\alpha]^{27}_D$ +53.5°(c 4.5, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) 0.90 (d, 3H, J=7 Hz, -CH(CH_3)$_2$), 1.06 (d, 3H, J=7 Hz, -CH(CH_3)$_2$), 2.32 (m, 1H, -CH(CH_3)$_2$), 2.42 (s, 3H, Ar-CH_3), 4.06 (d, 1H, J=4, 10 Hz, -NH-CH-CO-), 5.18 (d, 1H, J=10 Hz, -NH-SO_2-), 7.30 (d, 2H, J=8 Hz, Ar-3,5-CH), 7.72 (d, 2H, J=8 Hz, Ar-2,6-CH).

(S)-(-)-N-Boc-4-nitrotryptophanol ((-)-16) and (R)-(+)-N-Boc-4-nitrotryptophanol ((+)-16)

A mixture of 3.20 g (9.55 mmol) of (±)-16 and 4.15 g (14.3 mmol) of 27 in 30 ml of pyridine was allowed to react for 18 h at room temperature, then the solvent was removed in vacuo and the residue was dissolved in $CH_3COOC_2H_5$. The solution was washed with water and dried over $MgSO_4$. Evaporation of the solvent gave a yellow crystalline solid. The crude product was dissolved in a minimum amount of acetone and chromatographed on silica gel using CH_2Cl_2-acetone (10:1) as the eluent. Two diastereomeric isomers were separated. The less polar isomer was 28 (2.32 g) and the more polar isomer was 29 (2.21 g). Then 2.16 g (3.67 mmol) of 29 was dissolved in 350 ml of methanol, and 50 ml of 2 N KOH aq. solution was added. The mixture was stirred at room temperature for 6 days, and concentrated in vacuo. The solution was diluted with 100 ml of water and extracted with $CH_3COOC_2H_5$ to give a crystalline solid. Purification of the product by column chromatography on silica gel using CH_2Cl_2-$CH_3COOC_2H_5$ (1:1) gave 1.08 g of (-)-16 as yellow cubes: mp 164-166°C (dec.)(from chloroform); $[\alpha]^{25}_D$ -246.0°(c, 1.0, $CHCl_3$). The other product (28) was converted into (+)-16 by the same procedure as used for the preparation of (-)-16 employing 2.25 g of 28, 70 ml of methanol and 10 ml of 2 N KOH aq. solution: 1.26 g of (+)-16 was obtained. mp 164-166°C (dec.)(from chloroform); $[\alpha]^{25}_D$ +248.2°(c, 1.0, $CHCl_3$).

Determination of absolute configuration of (-)-16 A mixture of 35 mg (0.104 mmol) of (-)-16 and 45 mg (0.32 mmol) of benzoyl chloride in 0.2 ml of pyridine was allowed to stand at room temperature for 15 h, then the solvent was removed in vacuo, and the residue was dissolved in CH_2Cl_2, washed with water and concentrated. Purification by column chromatography on silica gel gave the O-benzoylated product (40 mg, 85%). Deprotection of the Boc group was carried out by treatment with 1 ml of CH_2Cl_2 and 1 ml of trifluoroacetic acid to give O-benzoyl-4-nitrotryptophanol in quantitative yield. This product was dissolved in 0.2 ml of pyridine, 40 mg (0.285 mmol) of benzoyl chloride was added, and the mixture was allowed to react at room temperature for 15 h to give 31 mg (77%) of N,O-dibenzoyl-4-nitrotryptophanol. The compound was treated with 5.6 mg (0.14 mmol) of NaH (60% in oil) in 1 ml of THF at 0°C for 30 min, followed by treatment with 20 mg (0.14 mmol) of benzoyl chloride at room temperature for 10 h, to give 36 mg (94%) of N,N,O-tribenzoyl-4-nitrotryptophanol (30). This product (30) was catalytically reduced using 40 mg of 10% Pd-charcoal in 35 ml of ethanol under 1 atm of H_2 for 1 h to give the 4-amino derivative (28 mg, 82%). The amide (28 mg, 0.054 mmol) was suspended in 1 ml of conc. HCl, and 5.6 mg (0.08 mmol) of $NaNO_2$ was added at 0°C. The solution was stirred for 1 h at 0°C. A large excess of H_3PO_2 was added, and the mixture was stirred for 1 h, diluted with 10 ml of water and extracted with $CH_3COOC_2H_5$. The extract was concentrated to give a residue. Purification of the crude product by column chromatography on silica gel using CH_2Cl_2-n-hexane-$CH_3COOC_2H_5$ (8:2:1) gave 12 mg of N,N,O-tribenzoyltryptophanol (31)(44%). $[\alpha]^{20}_D$ -6.9°(c, 0.5, $CHCl_3$). The product was identified by comparison of its IR spectrum with that of an authentic sample. The authentic sample of 31 was prepared from L-valine in a total yield of 45%. $[\alpha]^{20}_D$ -6.7°(c, 0.5, $CHCl_3$).

(-)-Indolactam-V ((-)-10) and (+)-epi-indolactam-V ((+)-26) Optically active (-)-16 (1.195g, 3.57 mol) was converted into (-)-10 and (+)-26 by the same procedures as used for racemic 10 and 26. Yields and physical data of synthetic precursors and products were as follows. From (-)-16 to (-)-17: 86%, (-)-17, colorless viscous liquid, $[\alpha]^{20}_D$ -12.6°(c, 1.0, $CHCl_3$). From (-)-17 to (-)-18 (28%) and (+)-22 (23%): (-)-18, colorless leaflets ($CHCl_3$-n-hexane) mp 186-189°C, $[\alpha]^{20}_D$ -70.8° (c, 0.96, $CHCl_3$); (+)-22, amorphous powder, $[\alpha]^{20}_D$ +16.8°(c, 1.02, $CHCl_3$). From (-)-18 to the lactam ((-)-21): 31%, (-)-21, amorphous powder, $[\alpha]^{20}_D$ -76.0°(c, 0.72, methanol). From (-)-21 to (-)-10: 58%, amorphous powder, $[\alpha]^{20}_D$ -134.5°(c, 0.70, methanol). From -22 to the lactam ((+)-25): 50%, (+)-25, amorphous powder, $[\alpha]^{20}_D$ +29.7°(c, 0.85, methanol). From (+)-25 to (+)-26: 72%, (+)-26, colorless needles ($CHCl_3$), mp 134-136°C, $[\alpha]^{20}_D$ +86.5° (c, 0.83, methanol).

(+)-Indolactam-V ((+)-10) and (-)-epi-indolactam-V ((-)-26) Optically active (+)-16 (1.253g, 3.74 mmol) was converted into (+)-10 and (-)-26 by the same procedures as used for racemic 10 and 26. Yields and physical data of synthetic precursors and products were as follows. From (+)-16 to (+)-17: 83%, (+)-17, colorless viscous liquid, $[\alpha]^{20}_D$ +11.4°(c, 1.0, $CHCl_3$). From (+)-17 to (+)-18 (32%) and (-)-22 (28%): (+)-18, colorless leaflets ($CHCl_3$-n-hexane) mp 187-190°C, $[\alpha]^{20}_D$ +72.6°(c, 0.9, $CHCl_3$); (-)-22, amorphous powder, $[\alpha]^{20}_D$ -17.3°(c,1.04,$CHCl_3$). From (+)-18 to the lactam ((+)-21): 30%, (+)-21, amorphous powder, $[\alpha]^{20}_D$ +77.5° (c, 0.75, methanol). From (+)-21 to (+)-10: 59%, (+)-10, amorphous powder, $[\alpha]^{20}_D$ +136.5°(c, 0.56, methanol). From (-)-22 to the lactam ((-)-25): 48%, (-)-25, amorphous powder, $[\alpha]^{20}_D$ -30.4°(c, 0.87, methanol). From (-)-25 to (-)-26: 74%, (-)-26, colorless needles ($CHCl_3$), mp 134-136°C, $[\alpha]^{20}_D$ -84.9° (c, 0.75, methanol).

(±)-Indolactam-V acetate (32) A mixture of 301 mg (1 mmol) of indolactam-V (10) and 500 mg (4.9 mmol) of acetic anhydride in 2 ml of pyridine was allowed to react at room temperature for 18 h. After removal of the solvent in vacuo, the residue was dissolved in 50 ml of CH_2Cl_2 and the

solution was washed with water, dried and concentrated. Purification by column chromatography on silica gel using CH_2Cl_2-$CH_3COOC_2H_5$ (8:1) gave 298 mg of indolactam-V acetate (32) (87%). 32: colorless needles, mp 187-188°C, (ethanol-n-hexane); ^{1}H-NMR ($CDCl_3$) Conformer A:conformer B = 1:2.6. The spectral data are presented in Table 3; ^{1}H-NMR (CD_3OD) conformer A:conformer B = 3:2. conformer A: 0.91 (d, 3H, J=6.8 Hz, -CH($C\underline{H}_3$)$_2$), 1.24 (d, H, J=6.8 Hz, -CH($C\underline{H}_3$)$_2$), 2.02 (s, 3H, 19-$C\underline{H}_3$), 2.31 (dsept, 1H, J=11.3, 6.8 Hz, -$C\underline{H}$(CH_3)$_2$), 2.70 (s, 3H, 18-$C\underline{H}_3$), 2.71 (dd, 1H, J=14.5, 1.6 Hz, 8-$C\underline{H}_2$), 3.08 (dd, 1H, J=14.5, 4.6 Hz, 8-$C\underline{H}_2$), 3.10 (d, 1H, J=1.3 Hz, 12-C$\underline{H}$), 3.76 (dd, 1H, J=11.6, 7.8 Hz, 14-$C\underline{H}_2$), 3.90 (d, 1H, J=11.6, 6.4 Hz, 14-$C\underline{H}_2$), 4.50 (m, 1H, 9-C$\underline{H}$), 6.90 (dd, 1H, J=8.1, 1.0 Hz, 5-C$\underline{H}$), 7.07 (s, 1H, 2-C$\underline{H}$), 7.08 (t, 1H, J=8.1 Hz,6-C$\underline{H}$), 7.28 (dd, 1H, J=8.1 Hz, 7-C$\underline{H}$). conformer B: 0.62 (d, 3H, J=6.8 Hz, -CH($C\underline{H}_3$)$_2$), 0.90 (d, 3H, J=6.8 Hz, -CH($C\underline{H}_3$)$_2$), 2.05 (s, 3H, 19-$C\underline{H}_3$), 2.54 (dsept, 1H, J=12.5, 6.8 Hz, -$C\underline{H}$(CH_3)$_2$), 2.89 (s, 3H, 18-$C\underline{H}_3$), 3.15 (d, 1H, J=17.4 Hz, 8-$C\underline{H}_2$), 3.21 (dd, 1H, J=17.4, 3.8 Hz, 8-$C\underline{H}_2$), 4.09 (dd, 1H, J=11.6, 11.0 Hz, 14-$C\underline{H}_2$), 4.13 (dd, 1H, J=11.6, 4.4 Hz, 14-$C\underline{H}_2$), 4.43 (m, 1H, 9-C$\underline{H}$), 4.44 (d, 1H, J=12.5 Hz, 12-C$\underline{H}$), 6.47 (d, 1H, J=8.1 Hz, 5-C$\underline{H}$), 6.94 (dd, 1H, J=8.1, 1.0 Hz, 7-C$\underline{H}$), 6.96 (t, 1H, J=8.1 Hz, 6-C$\underline{H}$), 6.96 (s, 1H, 2-C$\underline{H}$); MS 343 (M^+) ; Anal. ($C_{19}H_{25}N_3O_3$) C,H,N.

Kinetic study of the conformational conversion of 32 The measurements for determination of the rate constants were carried out by using a JEOL JMN-FX-100 spectrometer and JEOL NM-5471 variable-temperature controller which employs a copper-constantan thermocouple situated 2-3 cm below the sample tube in the probe. Each sample was prepared as follows. 4 mg of 32 was dissolved in 0.4 ml of $CDCl_3$ at -40°C, and the spectrum was recorded at suitable intervals at each temperature using the JEOL PG-200 autostacking system. The conditions were at -20°C, interval of 484.0 sec (accumulation 6 sec X 12 times); at -10°C, interval of 123.5 sec (accumulation 6 sec X 12 times); at 0°C, interval of 51.4 sec (accumulation 6 sec X 8 times). Each spectrum was recorded and the ratio of conformer A and conformer B was calculated by measuring the peak height ratio of the -OCOCH_3 methyl proton resonance at each time.

1-Methylindolactam-V (33) A 15 mg (0.375 mol) portion of NaH (60% in oil) was washed with n-hexane and suspended in 1 ml of THF, and 86 mg (0.25 mol) of 32 was added to the suspension at 0°C. After vigorous stirring for 30 min at 0 C, 1 ml of CH_3I was added and the whole was stirred for 18 h at room temperature. Then, 1 ml of 2 N KOH aq. solution was added. The reaction mixture was stired for 3 h, and partitioned between CH_2Cl_2 and water. The organic layer was dried over $MgSO_4$ and concentrated. Purification of the residue by column chromatography on silica gel using CH_2Cl_2-$CH_3COOC_2H_5$ (2:1) as the eluent to gave 33 mg (53%) of 1-methylindolactam-V (33): mp 235°C (methanol); ^{1}H-NMR ($CDCl_3$) Conformer A: conformer B ratio was 1:5. conformer A: 0.94 (d, 3H, J=6.8 Hz, -CH($C\underline{H}_3$)$_2$), 1.25 (d, 3H, J=6.8 Hz, -CH($C\underline{H}_3$)$_2$), 2.40 (dsept, 1H, J=10.8, 6.8 Hz, -$C\underline{H}$(CH_3)$_2$), 2.74 (s, 3H, 18-$C\underline{H}_3$), 2.80 (dd, 1H, J=14.5, 1.7 Hz, 8-$C\underline{H}_2$), 2.98 (d, 1H, J=10.8 Hz, 12-C$\underline{H}$), 3.08 (dd, 1H, J=14.5, 4.8 Hz, 8-$C\underline{H}_2$), 3.43-3.45 (m, 2H, 14-$C\underline{H}_2$), 3.77 (s, 3H, 1-$C\underline{H}_3$), 4.46 (m, 1H, 9-C$\underline{H}$), 6.90 (s, 1H, 2-C$\underline{H}$), 7.06 (m, 1H, 5-C$\underline{H}$), 7.20 (m, 1H, 6-C$\underline{H}$), 7.20 (m, 1H, 7-C$\underline{H}$). conformer B: 0.62 (d, 3H, J=6.8 Hz, -CH($C\underline{H}_3$)$_2$), 0.92 (d, 3H, J=6.8 Hz, -CH($C\underline{H}_3$)$_2$),2.60 (dsept, 1H, J=10.2, 6.8 Hz, -$C\underline{H}$(CH_3)$_2$), 2.92 (s, 3H, 18-$C\underline{H}_3$), 3.01 (dd, 1H, J=17.1, 3.4 Hz, 8-$C\underline{H}_2$), 3.18 (d, 1H, J=17.1 Hz, 8-$C\underline{H}_2$), 3.56 (dd, 1H,J=14.1, 8.8 Hz,14-C$\underline{H}$), 3.69 (s, 3H, 1-$C\underline{H}_3$), 3.74 (dd, 1H, J=11.4, 3.7 Hz, 14-$C\underline{H}_2$), 4.30 (m, 1H, 9-C$\underline{H}$), 4.41 (d, 1H, J=10.2 Hz, 12-C$\underline{H}$), 6.51 (d, 1H, J=8Hz, 5-C$\underline{H}$), 6.76 (s, 1H, 2-C$\underline{H}$), 6.84 (d, 1H, J=8 Hz, 7-C$\underline{H}$), 7.10 (t, 1H, J=8 Hz, 6-C$\underline{H}$), 7.11 (s, 1H, 10-N$\underline{H}$). MS 315 (M^+); Anal. ($C_{18}H_{25}N_3O_2$) C,H,N.

1-Prenylindolactam-V (34) The procedure was the same as that used for 33, employing 56 mg (0.375 mmol) of 3,3-dimethylallyl bromide instead of CH_3I: 38 mg (41%) of 1-prenylindolactam-V was obtained. mp 235-236.5°C (ethanol); ^{1}H-NMR ($CDCl_3$) Conformer A: conformer B ratio was 1:5. conformer A: 0.94 (d, 3H, J=6.5 Hz, -CH($C\underline{H}_3$)$_2$), 1.25 (d, 3H, J=6.5 Hz, -CH($C\underline{H}_3$)$_2$), 1.77 (s, 6H, -$C\underline{H}_3$), 2.40 (dsept, 1H, J=10.5, 6.5 Hz, -$C\underline{H}$(CH_3)$_2$), 2.74 (s, 3H, 18-$C\underline{H}_3$), 2.80 (d, 1H, J=14.6 Hz, 8-$C\underline{H}_2$), 2.99 (d, 1H, J=10.5 Hz, 12-C$\underline{H}$), 3.08 (dd, 1H, J=14.6, 4.6 Hz, 8-$C\underline{H}_2$), 3.41-3.46 (m, 2H, 14-$C\underline{H}_2$), 4.45 (m, 1H, 9-C$\underline{H}$), 4.60 (d, 2H, J=6.8 Hz, 1-N-$C\underline{H}_2$), 5.10 (m, 1H, olefinic C$\underline{H}$), 6.90 (s, 1H, 2-C$\underline{H}$), 7.07 (d, 1H, J=8 Hz, 5-C$\underline{H}$), 7.19 (t, 1H, J=8 Hz, 6-C$\underline{H}$), 7.21 (d, 1H, J=8 Hz, 7-C$\underline{H}$). conformer B: 0.64 (d, 3H, J=6.5 Hz, -CH($C\underline{H}_3$)$_2$), 0.91 (d, 3H, J=6.5 Hz, -CH($C\underline{H}_3$)$_2$), 1.77 (s, 6H, -$C\underline{H}_3$), 2.60 (dsept, 1H, J=10.1, 6.5 Hz, -$C\underline{H}$(CH_3)$_2$), 2.91 (s, 3H, 18-$C\underline{H}_3$), 3.00 (dd, 1H, J=17.2, 3.5 Hz, 8-$C\underline{H}_2$), 3.18 (d, 1H, J=17.2 Hz, 8-$C\underline{H}_2$), 3.55 (dd, 1H, J=11.4, 8.7 Hz, 14-C$\underline{H}$), 3.75 (dd, 1H, J=11.4, 3.7 Hz, 14-$C\underline{H}_2$), 4.29 (m, 1H, 9-C$\underline{H}$), 4.40 (d, 1H, J=10.1 Hz, 12-C$\underline{H}$), 4.55 (d, 2H, J=6.8 Hz, 1-N-$C\underline{H}_2$-), 5.18 (m, 1H, olefinic $\underline{H}$), 6.50 (d, 1H, J=8 Hz, 5-C$\underline{H}$), 6.76 (s, 1H, 2-C$\underline{H}$), 6.86 (d, 1H, J=8 Hz, 7-C$\underline{H}$), 7.09 (t, 1H, J=8 Hz, 6-C$\underline{H}$), 7.12 (bs, 1H, 10-N$\underline{H}$); MS 369 (M^+); Anal. ($C_{22}H_{31}N_3O_2$) C,H,N.

1-Geranylindolactam-V (35) The procedure was the same as that used for 33, employing 82 mg (0.378 mmol) of geranyl bromide instead of CH_3I: 51 mg (47%) of 1-geranylindolactam-V (35) was

obtained. mp 160-162°C (CH_2Cl_2-n-hexane); ^{1}H-NMR ($CDCl_3$) Conformer A: conformer B ratio was 1:3. conformer A: 0.94 (d, 3H, J=6.5 Hz, -CH($C\underline{H}_3$)$_2$), 1.24 (d, 3H, J=6.5 Hz, -CH($C\underline{H}_3$)$_2$), 1.59 (s, 3H, -$C\underline{H}_3$), 1.67 (s, 3H, -$C\underline{H}_3$), 1.80 (s, 3H, -$C\underline{H}_3$), 2.05-2.15 (m, 4H, -$C\underline{H}_2$-$C\underline{H}_2$-), 2.40 (dsept, 1H, J=10.7, 6.5 Hz, -$C\underline{H}$(CH_3)$_2$), 2.74 (s, 3H, 18-$C\underline{H}_3$), 2.79 (d, 1H, J=14.9 Hz, 8-$C\underline{H}_2$), 2.98 (d, 1H, J=10.7 Hz, 12-$C\underline{H}$), 3.09 (dd, 1H, J=14.9, 4.8 Hz, 8-$C\underline{H}_2$), 3.41 (dd, 1H, J=11.3, 6.9 Hz, 14-$C\underline{H}_2$), 3.48 (dd, 1H, J=11.3, 6.0 Hz, 14-$C\underline{H}_2$), 4.45 (m, 1H, 9-$C\underline{H}$), 4.68 (d, 2H, J=6.3 Hz, 1-N-$C\underline{H}_2$), 4.74 (d, 1H, J=11.9 Hz, 10-N$\underline{H}$), 5.08 (m, 1H, olefinic $C\underline{H}$), 5.33 (dt, 1H, J=6.3, 0.5 Hz, olefinic $C\underline{H}$), 6.91 (s, 1H, 2-$C\underline{H}$), 7.06 (d, 1H, J=8 Hz, 5-$C\underline{H}$), 7.18 (t, 1H, J=8 Hz, 6-$C\underline{H}$), 7.21 (d, 1H, J=8 Hz, 7-$C\underline{H}$). conformer B: 0.63 (d, 3H, J=6.5 Hz, -CH($C\underline{H}_3$)$_2$), 0.92 (d, 3H, J=6.5 Hz, -CH($C\underline{H}_3$)$_2$), 1.59 (s, 3H, -$C\underline{H}_3$), 1.67 (s, 3H, -$C\underline{H}_3$), 1.80 (s, 3H, -$C\underline{H}_3$), 2.05-2.15 (m, 4H, -$C\underline{H}_2$-$C\underline{H}_2$-), 2.60 (dsept, 1H, J=10.1, 6.5 Hz, -$C\underline{H}$(CH_3)$_2$), 2.92 (s, 3H, 18-$C\underline{H}_3$), 3.01 (dd, 1H, J=17.9, 3.8 Hz, 8-$C\underline{H}_2$), 3.17 (d, 1H, J=17.9 Hz, 8-$C\underline{H}_2$), 3.55 (dd, 1H, J=11.6, 8.9 Hz, 14-$C\underline{H}$), 3.74 (dd, 1H, J=11.6, 3.6 Hz, 14-$C\underline{H}_2$), 4.30 (m, 1H, 9-$C\underline{H}$), 4.41 (d, 1H, J=10.1 Hz, 12-$C\underline{H}$), 4.59 (d, 2H, J=6.6 Hz, 1-N-$C\underline{H}_2$-), 5.08 (m, 1H, olefinic $\underline{H}$), 5.37 (dt, 1H, J=6.6, 0.5 Hz, olefinic $C\underline{H}$), 6.51 (d, 1H, J=8 Hz, 5-$C\underline{H}$), 6.77 (s, 1H, 2-$C\underline{H}$), 6.85 (d, 1H, J=8 Hz, 7-$C\underline{H}$), 7.03(bs, 1H, 10-N$\underline{H}$), 7.07 (t, 1H, J=8 Hz, 6-$C\underline{H}$). MS 437 (M^+); Anal. ($C_{27}H_{39}N_3O_2$) C,H,N.

6,7,8,9-Tetrahydrobenzo[g]indole-2-carboxylic acid ethyl ester (40) A solution of 47.6 g (0.24 mol) of the hydrazine hydrochloride (38·HCl)[23] in 500 ml of water was treated with 24.4 g (0.25 mol) of CH_3COOK. Then 41.8g (0.36 mol) of ethyl pyruvate was added at 40°C with stirring, and the suspension was stirred for 1 h at 40°C. The precipitate was collected, and dissolved in CH_2Cl_2. This solution was washed with water, dried over Na_2SO_4, and concentrated to give 61.1 g (98%) of the hydrazone (39). An material was used directly in the next step of the synthesis. The analytical sample was obtained as colorless plates by crystallization from n-hexane. mp 114-116 °C, ^{1}H-NMR ($CDCl_3$); 1.39 (t, 3H, J=7 Hz, -$OCH_2C\underline{H}_3$), 1.82 (m, 4H, -$CH_2C\underline{H}_2C\underline{H}_2CH_2$-), 2.11 (s, 1H, -$C\underline{H}_3$), 2.53 (bt, 1H, J=7 Hz, Ar-$C\underline{H}_2$-), 2.77 (bt, 1H, J=7 Hz, Ar-$C\underline{H}_2$-), 4.31 (q, 2H, J=7 Hz, -O-$C\underline{H}_2CH_3$), 6.71 (d, 1H, J=8 Hz, 2-$C\underline{H}$), 7.09 (t, 1H, J=8 Hz, 3-$C\underline{H}$), 7.43 (d, 1H, J=8 Hz, 4-$C\underline{H}$), 7.48 (bs, 1H, Ar-N$\underline{H}$-); Anal. ($C_{15}H_{20}N_2O_2$) C,H,N. The hydrazone (39)(60 g, 0.23 mol) was refluxed with 10 ml of sulfuric acid and 400 ml of ethanol for 2.5 h under an Ar atmosphere. The mixture was poured into ice-water, and the whole was extracted with CH_2Cl_2, washed with water and 5% $NaHCO_3$ aq. solution, and dried over $MgSO_4$. The solution was concentrated and the residue was purified by column chromatography on silica gel using CH_2Cl_2-n-hexane (3:2) to give 25.2 g (45%) of 6,7,8,9-tetrahydrobenzo[g]indole-2-carboxylic acid ethyl ester (40): colorless plates (CH_2Cl_2-n-hexane), mp 157-159°C; ^{1}H-NMR ($CDCl_3$); 1.40 (t, 3H, J=7 Hz, -$OCH_2C\underline{H}_3$), 1.90 (m, 4H, 7,8-$C\underline{H}_2$), 2.87 (m, 4H, 6,9-$C\underline{H}_2$), 4.40 (q, 2H, J=7 Hz, -O-$C\underline{H}_2CH_3$), 6.88 (d, 1H, J=8 Hz, 5-$C\underline{H}$), 7.16 (d, 1H, J=2Hz, 3-$C\underline{H}$), 7.40 (d, 1H, J=8 Hz, 4-$C\underline{H}$), 8.72 (bs, 1H, 1-N$\underline{H}$); Anal. ($C_{15}H_{17}NO_2$) C,H,N.

6,7,8,9-Tetrahydrobenzo[g]indole (41) A mixture of 25 g (0.103 mol) of 40 and 8.8 g (0.157 mol) of KOH in 250 ml of ethanol and 15 ml of water was heated to reflux for 1 h. After removal of the solvent, the residue was dissolved in 40 ml of water, acidified with 5% HCl aq. and extracted with $CH_3COOC_2H_5$. The solution was dried over $MgSO_4$ and concentrated to give 6,7,8,9-tetrahydrobenzo[g]indole-2-carboxylic acid. Copper powder (8.4 g) was added to a solution of the acid in 160 ml of quinoline, and the suspension was heated at 220-230°C for 2 h. The mixture was poured into conc. HCl and ice, and extracted with benzene. The organic layer was washed with 5% HCl aq., 5% $NaHCO_3$ aq., and water, and dried over $MgSO_4$. Concentration and purification of the residue by column chromatography on silica gel using CH_2Cl_2-n-hexane (1:2) gave 7.64 g of 6,7,8,9-tetrahydrobenzo[g]indole (41)(43%): colorless needles (n-hexane); mp 88-89°C; ^{1}H-NMR ($CDCl_3$); 1.90 (m, 4H, 7,8-$C\underline{H}_2$), 2.86 (m, 4H, 5,9-$C\underline{H}_2$), 6.48 (m, 1H, 3-$C\underline{H}$), 6.84 (d, 1H, J=8 Hz, 5-$C\underline{H}$), 7.11 (m, 1H, 2-$C\underline{H}$), 7.38 (d, 1H, J=8 Hz, 4-$C\underline{H}$), 7.93 (bs, 1H, 1-N$\underline{H}$); Anal. ($C_{12}H_{13}N$) C,H,N.

3-Dimethylaminomethyl-6,7,8,9-tetrahydrobenzo[g]indole (42) A mixture of 7.49 g (43.8 mmol) of 41, 50% aqueous dimethylamine (7.95 g, 88 mmol), formalin (6.72 g, 83 mmol) and acetic acid (12.5 g, 208 mmol) was heated at 80°C for 10 min. The mixture was poured into 15% KOH aq. with ice cooling and stirring, and the whole was extracted with CH_2Cl_2, and dried over Na_2SO_4. Evaporation of the solvent gave 42 quantitatively. This material was used directly in the next step of the synthesis. An analytical sample was obtained as colorless leaflets by crystallization from CH_2Cl_2-n-hexane: mp 124-125°C; ^{1}H-NMR ($CDCl_3$); 1.91 (m, 4H, 7,8-$C\underline{H}_2$), 2.27 (s, 6H, -N($C\underline{H}_3$)$_2$), 2.85 (m, 4H, 5,9-$C\underline{H}$), 3.60 (s, 2H, Ar-$C\underline{H}_2$-N), 6.85 (d, 1H, J=8 Hz, 5-$C\underline{H}$), 7.03 (d, 1H, J=2.2 Hz, 2-$C\underline{H}$), 7.43 (d, 1H, J=8 Hz, 4-$C\underline{H}$), 7.94 (bs, 1H, 1-N$\underline{H}$); Anal. ($C_{15}H_{20}N_2$) C,H,N.

3-Dimethylaminomethyl-4-nitro-6,7,8,9-tetrahydrobenzo[g]indole (43) A solution of 13.2 ml of nitric acid in 44 ml of acetic acid was added to a solution of 9.4 g (41.2 mmol) of 42 in 8 ml of acetic acid at 0°C with stirring. Stirring was continued for 15 min at 0°C, then the solution was poured into ice-water, made strongly basic with 20% KOH aq., and extracted with CH_2Cl_2. The

organic layer was extracted with 20% HCl aq., and the aqueous layer was neutralized with solid $NaHCO_3$ then extracted with CH_2Cl_2. This extract was dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel using CH_2Cl_2-$CH_3COOC_2H_5$ (1:2) saturated with ammonia as the eluent to give 4.5 g (40%) of 43: yellow needles (CH_2Cl_2-n-hexane); mp 126-129°C; ^{1}H-NMR ($CDCl_3$); 1.90 (m, 4H, 7,8-C$\underline{H}_2$), 2.26 (s, 6H, -N(C$\underline{H}_3$)$_2$), 2.86 (m, 4H, 5,9-C$\underline{H}_2$), 3.63 (s, 2H, Ar-C$\underline{H}_2$-N), 7.18 (bs, 1H, 2-C$\underline{H}$), 7.54 (s, 1H, 5-C$\underline{H}$), 8.42 (bs, 1H, 1-N$\underline{H}$); Anal. ($C_{15}H_{19}N_3O_2$) C,H,N.

Diester (44) The procedure was similar to that used for the synthesis of 12, employing 4.47 g (16.4 mmol) of 43, 4.26 g (19.6 mmol) of diethyl acetamidomalonate, 0.452 g (19.6 mmol) of sodium and 30 ml of dry ethanol. After work-up, the reaction mixture was chromatographed on silica gel using CH_2Cl_2-$CH_3COOC_2H_5$ (2:1) as the eluent to give 5.25 g (72%) of the diester (44): yellow cubes ($CH_3COOC_2H_5$); mp 196-198°C; ^{1}H-NMR ($CDCl_3$); 1.25 (t, 6H, J=7 Hz, -OCH$_2$C$_3$), 1.84 (s, 3H, -NHCOC$\underline{H}_3$), 1.85 (m, 4H, 7,8-C$\underline{H}_2$), 2.64 (m, 2H, 6-C$\underline{H}_2$), 2.86 (m, 2H, 9-C$\underline{H}_2$), 3.97 (s, 2H, Ar-C$\underline{H}_2$), 4.23 (q, 4H, J=7 Hz, -OC$\underline{H}_2$CH$_3$), 6.53 (s, 1H, -N$\underline{H}$CO-), 7.16 (s, 1H, 5-C$\underline{H}$), 7.33 (d, 1H, J=2 Hz, 2-C$\underline{H}$), 8.92 (bs, 1H, 1-N$\underline{H}$); Anal. ($C_{22}H_{27}N_3O_7$) C,H,N.

N-Acetyl ester (45) The procedure was similar to that used for the preparation of 13: 5.2 g (1.7 mmol) of 44 was converted into the N-acetylester (45)(2.75 g, 63%): yellow needles ($CH_3COOC_2H_5$); mp 205-207°C; ^{1}H-NMR ($CDCl_3$-DMSO-d_6); 1.18 (t, 3H, J=7 Hz, -OCH$_2$C$\underline{H}_3$), 1.90 (m, 4H, 7,8-C$\underline{H}_2$), 1.92 (s, 3H, -NHCOC$\underline{H}_3$), 2.90 (m, 4H, 6,9-C$\underline{H}_2$), 3.23 (dd, 1H, J=15, 9 Hz, Ar-C$\underline{H}_2$), 3.43 (dd, J=15, 6 Hz, Ar-C$\underline{H}_2$), 4.10 (q, 2H, J=7 Hz, -O-C$\underline{H}_2$CH$_3$), 4.67 (m, 1H, -C$\underline{H}$), 7.24 (d, 1H, J=2 Hz, 2-C$\underline{H}$), 7.30 (bs, 1H, -N$\underline{H}$CO-), 7.62 (s, 1H, 5-C$\underline{H}$), 10.8 (bs, 1H, 1-N); Anal. ($C_{19}H_{23}N_3O_5$) C,H,N.

Amino ester (46) The N-acetyl ester (45) (2.7g, 7.24 mmol) was converted to 1.94 g of the amino ester (46) (81%) by a method similar to that used for the preparation of 14. 46: yellow needles (CH_2Cl_2-n-hexane); mp 143-145°C; ^{1}H-NMR (CDC_3); 1.28 (t, 3H, J=7 Hz, -OCH$_2$C$\underline{H}_3$), 1.80 (m, 4H, 7,8-C$\underline{H}_2$), 2.55 and 2.80 (m, 4H, 6,9-C$\underline{H}_2$), 3.00 (dd, 1H, J=16, 9 Hz, Ar-C$\underline{H}_2$), 3.55 (dd, 1H, J=16, 5 Hz, Ar-C$\underline{H}_2$), 3.72 (m, 1H, -C$\underline{H}$), 4.20 (q, 2H,J=7 Hz, -O-C$\underline{H}_2$CH$_3$), 7.08 (d, 1H, J=2 Hz, 2-C$\underline{H}$), 7.64 (s, 1H, 5-C$\underline{H}$), 9.20 (bs, 1H, 1-N$\underline{H}$); Anal. $C_{17}H_{21}N_3O_4$) C,H,N.

N-Boc-ester (47) The amino ester (46) (1.94 g, 5.86 mmol) was converted into 2.25 g (89%) of the N-Boc-ester (47) by a method similar to that used for the preparation of 15. 47: yellow powder ($CH_3COOC_2H_5$); mp 235-237°C; ^{1}H-NMR ($CDCl_3$-DMSO-d_6); 1.18 (t, 3H, J=7 Hz, -O-CH$_2$C$\underline{H}_3$), 1.36 (s, 9H, -C(C$\underline{H}_3$)$_3$), 1.90 (m, 4H, 7,8-C$\underline{H}_2$), 2.92 (m, 4H, 6,9-C$\underline{H}_2$), 3.2-3.5 (m, 2H, Ar-C$\underline{H}_2$), 4.08 (q, 2H, J=7 Hz, -O-C$\underline{H}_2$CH$_3$), 4.30 (m, 1H, -C$\underline{H}$), 6.16 (d, 1H, J=8 Hz, -N$\underline{H}$COO-), 7.24 (d, 1H, J=3 Hz, 2-C$\underline{H}$), 7.60 (s, 1H, 5-$\underline{H}$), 11.10 (bs, 1H, 1-N$\underline{H}$); Anal. ($C_{22}H_{29}N_3O_6$) C,H,N.

N-Boc-alcohol (48) The above product (7) (2.0 g, 4.64 mmol) was converted into 1.70 g (94%) of the N-Boc-alcohol (48) by a method similar to that used for the preparation of 16. 48: yellow powder ($CH_3COOC_2H_5$); mp 231°C(dec.); ^{1}H-NMR ($CDCl_3$-DMSO-d_6); 1.35 (s, 9H, -C(C$\underline{H}_3$)$_3$), 1.91 (m, 4H, 7,8-C$\underline{H}_2$), 2.75 (m, 4H, 6,9-C$\underline{H}_2$), 2.8-3.2 (m, 2H, Ar-C$\underline{H}_2$), 3.65 (m, 2H, -C$\underline{H}_2$-OH), 3.70 (m, 1H, -C$\underline{H}$), 5.18 (d, 1H, J=8 Hz, -N$\underline{H}$-COO-), 7.2 (d, 1H, 2-C$\underline{H}$), 7.62 (s, 1H, 5-C$\underline{H}$), 11.2 (bs, 1H, 1-N$\underline{H}$); Anal. ($C_{20}H_{27}N_3O_5$) C,H,N.

(-)-N-Boc-alcohol ((-)-48) The procedure was similar to that used for the preparation of (-)-16. After esterification of 630 mg (0.617 mmol) of (±)-48 with (+)-N-tosylvaline chloride, the crude product was separated by column chromatography on silica gel using $CHCl_3$-acetone (10:1) as the eluent to give a less polar isomer (415 mg) and a more polar isomer (420 mg). The polar isomer (400 mg) was hydrolyzed with 2 N KOH aq. in 200 ml of methanol. Purification by silica gel column chromatography using $CHCl_3$-acetone (8:3) as the eluent gave 233 mg of (-)-48: yellow cubes (acetone); mp 206-207°C, $[\alpha]^{20}_D$ -70.2° (c, 0.25, $CHCl_3$). The less polar isomer was converted to (+)-48: yellow cubes (acetone); mp 205-207°C; $[\alpha]^{20}_D$ +69.4°(c, 0.24, $CHCl_3$).

(-)-N-Boc-amino alcohol ((-)-49) The above product ((-)-48) (225 mg, 0.578 mmol) was converted into 185 mg (89%) of the (-)-N-Boc-aminoalcohol ((-)-49) by a method similar to that used for the preparation of 17. (-)-49: colorless leaflets (CH_2Cl_2-n-hexane); mp 160-162°C, $[\alpha]^{20}_D$ -7.0°(c, 1.02, $CHCl_3$); ^{1}H-NMR ($CDCl_3$); 1.48 (s, 9H, -C(C$\underline{H}_3$)$_3$), 1.88 (m, 4H, 7,8-C$\underline{H}_2$), 2.76 (m, 4H, 6,9-C$\underline{H}_2$), 3.04 (dd, 1H, J=15, 5 Hz, Ar-C$\underline{H}_2$), 3.23 (dd, 1H, J=15, 5 Hz, Ar-C$\underline{H}_2$), 3.40 (dd, 1H, J=15, 4 Hz, -C$\underline{H}_2$-OH), 3.58 (dd, 1H, J=15, 3Hz, -C$\underline{H}_2$-OH), 4.10 (m, 1H, -C$\underline{H}$), 5.36 (bd, 1H, J=8 Hz, -N$\underline{H}$-COO-), 6.20 (s, 1H, 5-C$\underline{H}$), 6.93 (d, 1H, J=2 Hz, 2-C$\underline{H}$), 8.00 (bs, 1H, 1-N$\underline{H}$); Anal. ($C_{20}H_{29}N_3O_3$) C,H,N.

Ester ((-)-50) The procedure was similar to that used for the preparation of 18. After condensation of (-)-49 (150 mg, 0.418 mol) with methyl 2-oxoisovalerate and reduction with $NaBH_3CN$, the crude product was separated by column chromatography on silica gel using CH_2Cl_2-$CH_3COOC_2H_5$ (8:3) as the eluent to give a less polar isomer ((-)-50, 53 mg, 27%) and a more polar isomer (epimer of (-)-50, 43 mg, 22%). (-)-50: amorphous gum; $[\alpha]^{20}_D$ -81.3°(c, 0.88, $CHCl_3$); ^{1}H-NMR ($CDCl_3$); 1.03 (d, 3H, J=7 Hz, -CH(C$\underline{H}_3$)$_2$), 1.14 (d, 3H, J=7 Hz, -CH(C$\underline{H}_3$)$_2$), 1.46 (s, 9H, -C(C$\underline{H}_3$)$_3$),

1.85 (m, 4H, 7,8-C<u>H</u>$_2$), 2.10 (m, 1H, -C<u>H</u>(CH$_3$)$_2$), 2.70 (m, 4H, 6,9-C<u>H</u>$_2$), 3.0-3.2 (m, 2H, Ar-C<u>H</u>$_2$), 3.55-3.65 (m, 2H, -C<u>H</u>$_2$-OH), 3.9-4.1 (m, 2H, -C<u>H</u> X 2), 3.75 (s, 3H, -COOC<u>H</u>$_3$), 5.35 (bd, 1H, J=8 Hz, -N<u>H</u>-COO-), 5.95 (s, 1H, 5-C<u>H</u>), 6.90 (d, 1H, J=2 Hz, 2-C<u>H</u>), 7.99 (bs, 1H, 1-N<u>H</u>); MS 473 (M^+).

Lactam ((-)-52) A solution of 35 mg (0.074 mmol) of (-)-50 in 1 ml of methanol was treated with 0.5 ml (1 mmol) of 2 N KOH aq., and the mixture was allowed to react at room temperature for 10 h. The solvent was removed in vacuo and the residue was dissolved in 3 ml of ice water at below 0°C. The aqueous solution was acidified with 0.5 M aqueous citric acid solution at 0°C, and extracted with $CH_3COOC_2H_5$ at 0°C. The extract was dried over Na_2SO_4 and concentrated in vacuo at below 0°C to give the crude acid. The acid and 26 mg (0.226 mmol) of N-hydroxysuccinimide were dissolved in 0.5 ml of CH_3CN, and then a solution of 31 mg (0.15 mol) of dicyclohexylcarbodiimide in 0.2 ml of CH_3CN at 0°C was added with stirring. Stirring was continued for 1 h at room temperature, then the solvent was removed in vacuo and the residue was dissolved in $CH_3COOC_2H_5$. The precipitate (dicycohexylurea) was removed by filtration, and the filtrate was concentrated. The residue was dissolved in CH_2Cl_2 and chromatographed on silica gel using $CH_3COOC_2H_5$-n-hexane (2:1) as the eluent to give 22 mg (53%) of the activated ester ((-)-51). A solution of 22 mg of (-)-51 in 0.5 ml of CH_2Cl_2 was treated with 0.5 ml of trifluoroacetic acid at 0°C. The mixture was stirred for 1 h at 0°C under an Ar atmosphere, then the acid was removed in vacuo at below 30 °C. The residue was dissolved in 20 ml of $CH_3COOC_2H_5$, then 1 ml of saturated aq. $NaHCO_3$ solution was added and the mixture was refluxed for 1 h with vigorous stirring. The organic layer was separated and the aqueous layer was extracted with $CH_3COOC_2H_5$. The combined $CH_3COOC_2H_5$ solution was dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography on silica gel using $CH_3COOC_2H_5$ as the eluent to give 10 mg (74%) of the lactam ((-)-52): colorless powder ($CH_3COOC_2H_5$); mp 262-265°C; $[\alpha]^{20}_D$ -91.5° (c, 0.20, CH_3OH).

(-)-Indolactam-V-tetramethylene ((-)-36) A mixture of 9 mg (0.0264 mmol) of 51, 20 mg (0.24 mmol) of $NaHCO_3$, 4 ml of CH_3I and 4 ml of methanol was heated under reflux for 24 h. The solvent was removed in vacuo and the residual solid was partitioned between $CH_3COOC_2H_5$ and water. The organic layer was dried and concentrated to give crude (-)-36. Purification by column chromatography on silica gel using $CH_3COOC_2H_5$ gave 7.1 mg (76%) of (-)-36: mp 158-160°C (CH_2Cl_2-n-hexane); $[\alpha]^{20}_D$ -138.6° (c, 0.71, CH_3OH); ^{1}H-NMR (CD_3OD) conformer A: conformer B ratio was 1:1 in this solution; conformer A: 0.92 (d, 3H, J=7 Hz, -CH(C<u>H</u>$_3$)$_2$), 1.24 (d, 3H, J=7 Hz, -CH(C<u>H</u>$_3$)$_2$), 1.88 (m, 4H, 20,21-C<u>H</u>$_2$), 2.30 (m, 1H, -C<u>H</u>(CH$_3$)$_2$), 2.69 (s, 3H, 18-C<u>H</u>$_3$), 2.76 (m, 4H, 19,22-C<u>H</u>$_2$), 2.8-3.1 (m, 2H, 8-C<u>H</u>$_2$), 3.04 (d, 1H, J=11 Hz, 12-C<u>H</u>), 3.2-3.3 (m, 2H, 14-C<u>H</u>$_2$), 4.20 (m, 1H, 9-C<u>H</u>), 6.86 (s, 1H, 5-C<u>H</u>), 7.01 (s, 1H, 2-C<u>H</u>), conformer B: 0.64 (d, 3H, J=7 Hz, -CH(C<u>H</u>$_3$)$_2$), 0.90 (d, 3H, J=7 Hz, -CH(C<u>H</u>$_3$)$_2$), 1.88 (m, 4H, 20,21-C<u>H</u>$_2$), 2.5 (m, 1H, -C<u>H</u>(CH$_3$)$_2$), 2.76 (m, 4H, 19,22-C<u>H</u>$_2$), 2.86 (s, 3H, 18-C<u>H</u>$_3$), 2.8-3.2 (m, 2H, 8-C<u>H</u>), 3.46 (dd, 1H, J=11, 1 Hz, 14-C<u>H</u>$_2$), 3.62 (dd, 1H, J=11, 5 Hz, 14-C<u>H</u>$_2$), 4.20 (m, 1H, 9-C<u>H</u>), 4.43 (d, 1H, J=10 Hz, 12-C<u>H</u>), 6.16 (s, 1H, 5-C<u>H</u>), 6.69 (s, 1H, 2-C<u>H</u>); MS m/e 355.2247, calcd. for $C_{21}H_{29}N_3O_2$ 355.2266.

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