Pergamon

Tetrahedron: *Asymmetry* 9 (1998) 967–981

TETRAHEDRON: *ASYMMETRY*

Stereochemical issues related to the synthesis and reactivity of pyrazino[2′,1′-5,1]pyrrolo[2,3-*b*]indole-1,4-diones

Esmeralda Caballero, Carmen Avendaño * and J. Carlos Menéndez

Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia, Universidad Complutense, 28040 Madrid, Spain

Received 5 January 1998; accepted 12 February 1998

Abstract

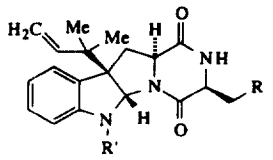
The cyclization of all four diastereoisomers of *cyclo*-(Trp-Ala) to the corresponding 3,5a,6,10b,11,11a-hexahydro-2*H*-pyrazino[2′,1′-5,1]pyrrolo[2,3-*b*]indoles is studied from the stereochemical point of view. Epimerization of the tryptophan stereocenter during the cyclization and during acylation reactions of the N-2 atom of the tetracyclic derivatives is discussed.

1. Introduction

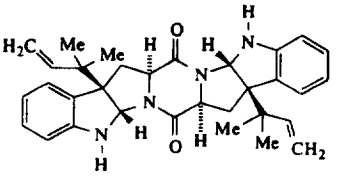
A number of diketopiperazine alkaloids biogenetically derived from amino acids have been isolated from fungi. Among them, 3,5a,6,10b,11,11a-hexahydro-2*H*-pyrazino[2′,1′-5,1]pyrrolo[2,3-*b*]indoles constitute a growing class of natural products, comprising compounds such as the fructigenins A and B,[1,2] amauromine,[3,4] the sporidesmins,[5] *N*-acetylardeemin[6] and ditrytophenaline.[7] Many of them exhibit interesting biological properties; for example, amauromine is a vasodilator acting through calcium antagonism,[3] the sporidesmins, together with other epidithiodiketopiperazines, are interesting as immunomodulators,[8] and *N*-acetylardeemin is one of the most potent known inhibitors of multi-drug resistance (MDR) in tumours.[9]

* Corresponding author. Fax: +34 1 394 1822.

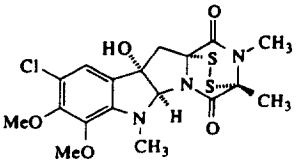
0957-4166/98/$19.00
PII: S0957-4166(98)00068-8



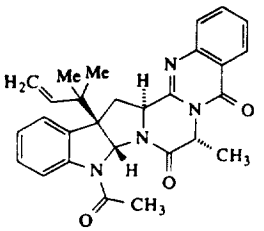
R = C_6H_5 (**fructigenine A**)
R = $CH(CH_3)_2$ (**fructigenine B, or verrucofortine**)



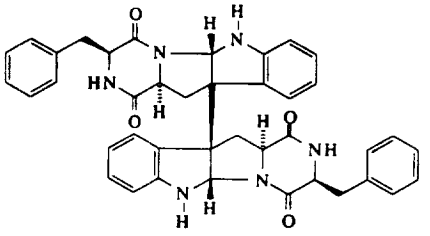
Amauromine



Sporidesmin B

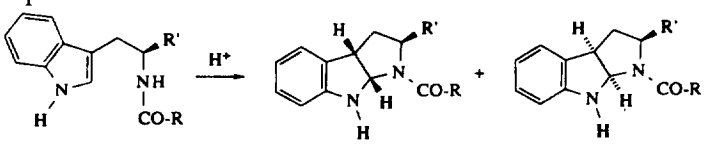


***N*-acetylardeemin**



Ditryptophenaline

The most concise route to pyrrolo[2,3-*b*]indoles is based on the acid-promoted cyclization of tryptamine derivatives.[10,11] For instance, the cyclization of carbamates **I** yields initially the kinetically controlled compounds **II**, which are quickly transformed into their thermodynamically more stable isomers **III** at room temperature.



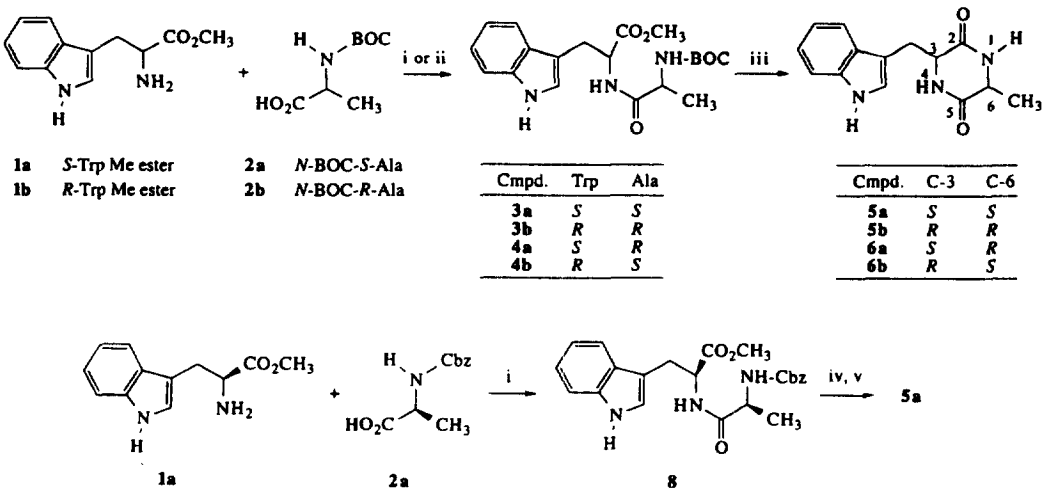
I **II** **III**

2. Results and discussion

An analogous cyclization of 3-(indolylmethyl)-2,5-piperazinediones should provide an efficient synthesis of pyrazino[2′,1′-5,1]pyrrolo[2,3-*b*]indoles, however, this method has received little attention, particularly from the stereochemical point of view.[12] In order to carry out a systematic study of this reaction, all four stereoisomers of *cyclo*-(Trp-Ala) were prepared using the route outlined in Scheme 1. Treatment of tryptophan methyl esters **1**[13] with the suitable Boc-protected alanine derivatives **2** in the presence of DCC or EDC[14] as coupling reagents afforded the dipeptides **3** or **4**, which under pyrolytic conditions underwent one-pot deprotection[15]–cyclization, yielding the diketopiperazines **5** and **6**, respectively. Alternatively, compound **5a** was also prepared by hydrogenolysis of the *N*-Cbz dipeptide **8**, followed again by thermal cyclization.[16]

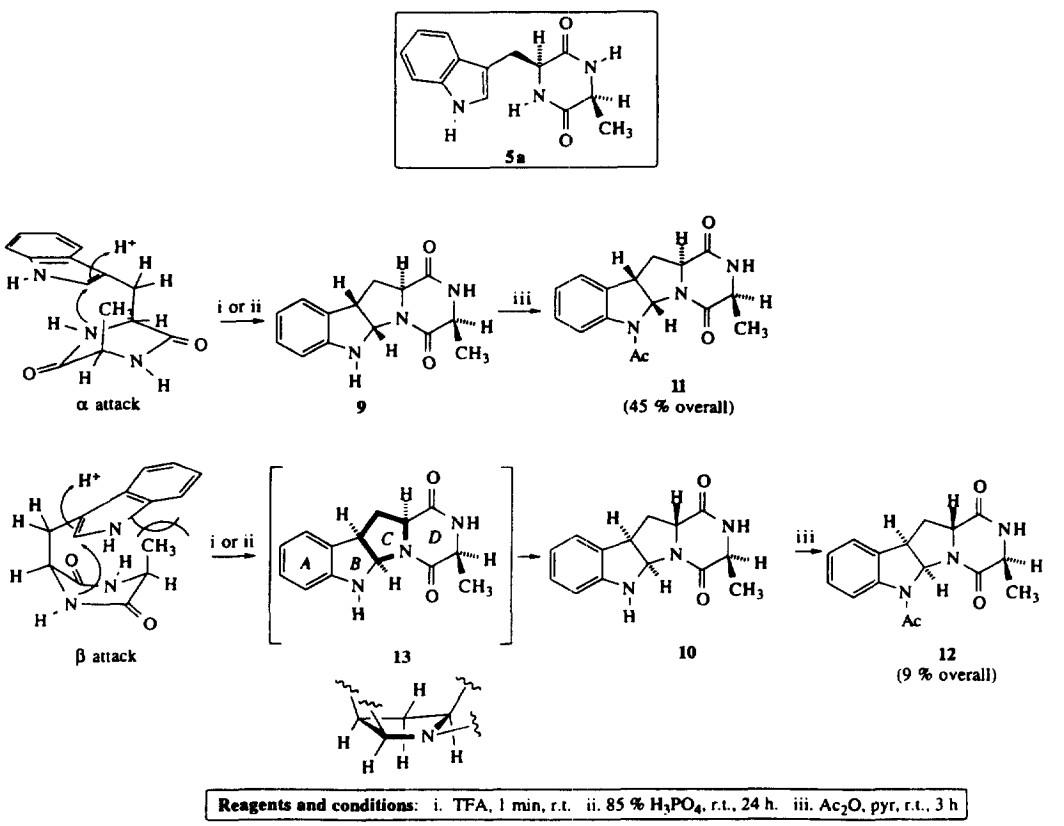
As shown in Scheme 2, *cyclo*-(L-Trp-L-Ala) **5a** was cyclized by brief[17] exposure to neat trifluoroacetic acid to give a mixture of diastereoisomers **9** and **10**, where the hydrogens at the 5a and 10b positions have *cis* and *trans* arrangements, respectively, with respect to H-11a. An identical result was obtained by treatment with 85% phosphoric acid at room temperature for 24 h. The mixture of **9** and **10** was immediately treated with acetic anhydride in pyridine to give the corresponding *N*-acetyl derivatives **11** and **12**, in order to increase their stability by preventing reversion to the starting diketopiperazines.[18]

The fact that the major reaction product arises from attack from the bottom (α) face of the indole ring can be ascribed to more efficient bond formation due to frontal overlap of the p orbitals involved



Reagents and conditions: i. DCC, THF, reflux, 90 min. ii. EDC, CH_2Cl_2, r.t., 24 h. iii. 200 °C, 2-4 h. iv. Pd-C 10%, AcOEt-EtOH, H_2 (42 psi), r.t., 20 h. v. Xylene, reflux, 40 h.

Scheme 1.



Reagents and conditions: i. TFA, 1 min, r.t. ii. 85 % H_3PO_4, r.t., 24 h. iii. Ac_2O, pyr, r.t., 3 h

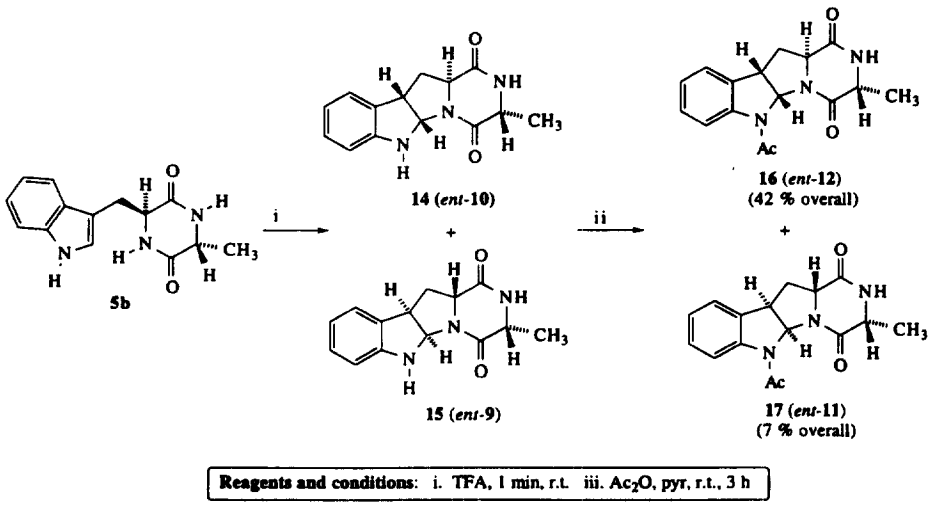
Scheme 2.

in the reaction, which is not possible in the alternative β attack owing to repulsive interactions between the indole ring and the pseudoaxial methyl group of the piperazine ring. Also, compound **9** is probably

more stable than its isomer **10**. Thus, if a solution of **5a** in 85% aqueous phosphoric acid is stirred at room temperature for 72 h, the only reaction product is compound **9** (62% yield). This result can only be explained through an equilibrium between the cyclized and the open forms, and shows that **10** reverts to a diketopiperazine more easily than **9**.

Cyclization from the β face to give compound **10** was accompanied by epimerization of the tryptophan stereogenic center. Since related epimerizations have been observed only in proline-derived diketopiperazines,[19,20] we assume that epimerization takes place after cyclization, and is probably prompted by the considerable steric crowding of the non-isolated derivatives **13**, with an all-*cis* arrangement in the *C* ring in which stereocenters 5a, 10b and 11a have, respectively, *S*, *R* and *S* configurations (Scheme 2). Previous similar cyclizations of the less rigid tryptamine derivatives have never led to a comparable result.[10,11]

Similarly, treatment of *cyclo*-(L-Trp-D-Ala) (**6a**) with trifluoroacetic acid afforded a mixture of compounds **14** and **15**, which was also acetylated to yield **16** and **17** (Scheme 3).



Scheme 3.

The stereochemistry of the cyclizations was established through NOE experiments in the acetylated derivatives **11**, **12**, **16** and **17**. NOE effects with a diagnostic value are summarized in Fig. 1. The enantiomeric relationships between compounds **11–17** and **12–16** were reflected in their specific rotations.

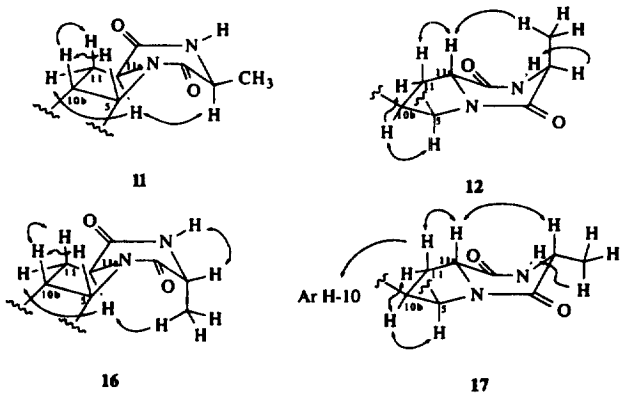
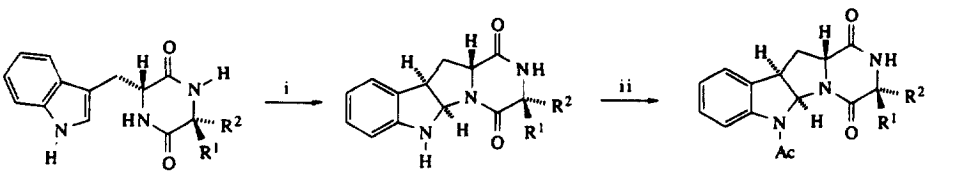


Fig. 1.

Since inversion of the tryptophan stereocenter is not mentioned in the literature precedent to this type of reaction (e.g., the acid-promoted cyclization of *cyclo*-(L-Trp-L-Pro)),[12] structures **12** and **17** required confirmation by an independent method. Therefore, we studied their alternative synthesis starting from derivatives of D-Trp. As shown in Scheme 4, treatment of **5b** and **6b** with 85% phosphoric acid for 72 h gave compounds **12** (41%) and **17** (40%), respectively, as the only isolated products after acetylation of **10** and **15**.



Compound	R^1	R^2
5b	H	CH_3
6b	CH_3	H

Compound	R^1	R^2
10	H	CH_3
15	CH_3	H

Compound	R^1	R^2	Yield, %*
12	H	CH_3	41
17	CH_3	H	40

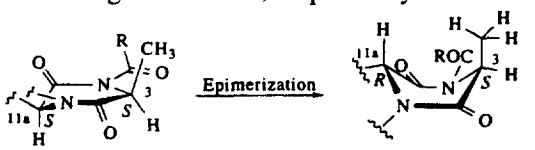
* Isolated, overall yields

Reagents and conditions: i. 85 % H_3PO_4, r.t., 72 h. ii. Ac_2O, pyr, r.t., 3 h

Scheme 4.

Steric effects in other parts of the molecule can also be responsible for epimerization of the C-11a carbon and can override the strain due to an all-*cis* arrangement of hydrogens in the *C* ring. Thus, we have observed that the configuration of the alanine stereogenic center (i.e., C-3) exerts an interesting influence on the reactions of the N-2 nitrogen in the tetracyclic systems. For instance, treatment of compound **11** with refluxing acetic anhydride for 4 h led to equal amounts of the expected derivative **18**, together with compound **19**, formed by epimerization of the 11a atom (Scheme 5). Epimerization takes place after acetylation, as shown by the fact that milder reaction conditions (e.g. Ac_2O, cat. DMAP, r.t., 16 h), under which conversion is not complete, led only to **18** and unreacted **11**, the formation of **19** being observed only for longer reaction times. Similarly, treatment of **11** with Ac_2O in the presence of KHMDS (−78°C to r.t.) afforded **18** and recovered starting material. Benzoylation showed a similar behaviour, as shown in Scheme 5.[21]

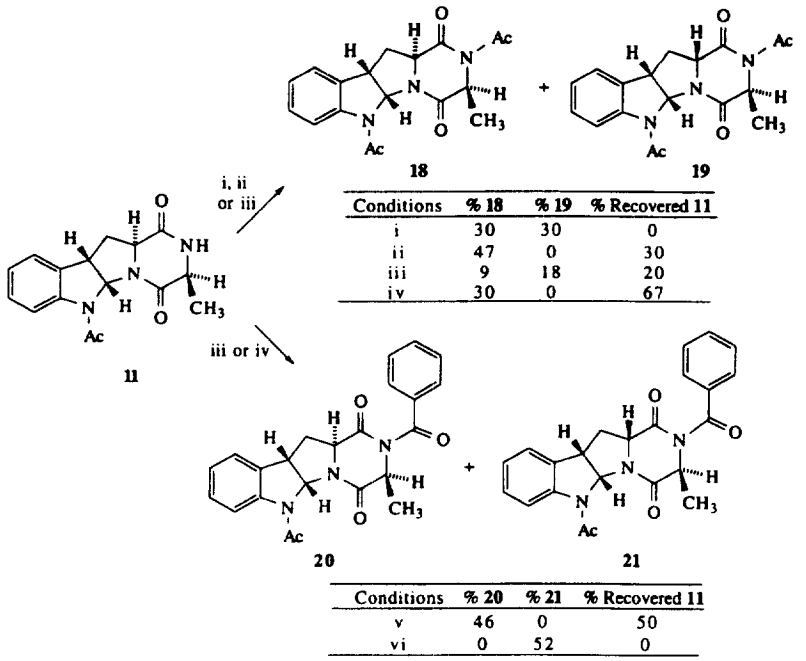
These observations can be explained by the steric compression between the N-2 acyl group, the C-1 oxygen and the C-3 methyl in the most probable conformation for compounds **18** or **20**,[22,23] which is relieved by epimerization of C-11b to give **19** or **21**, respectively.



18 R = $COCH_3$
20 R = COC_6H_5

19 R = $COCH_3$
21 R = COC_6H_5

The mechanism described depends on the steric hindrance associated with the group at C-3, and therefore similar reactions of derivatives with an *R* configuration at C-3 (e.g. compound **16**) should not lead to epimerization. Indeed, treatment of **16** with refluxing acetic anhydride, under the same conditions employed for **11**, led to **22** as the only reaction product in 77% yield, and the reaction between **14** and pivaloyl chloride gave compound **23** in 78% yield as the only tetracyclic product (Scheme 6).[24] Both reaction products lack the interaction mentioned above between the C-3 methyl and the N-2 acyl groups: the NOE effects that substantiate the stereochemical assignments of compounds **18–23** are given in Fig. 2.

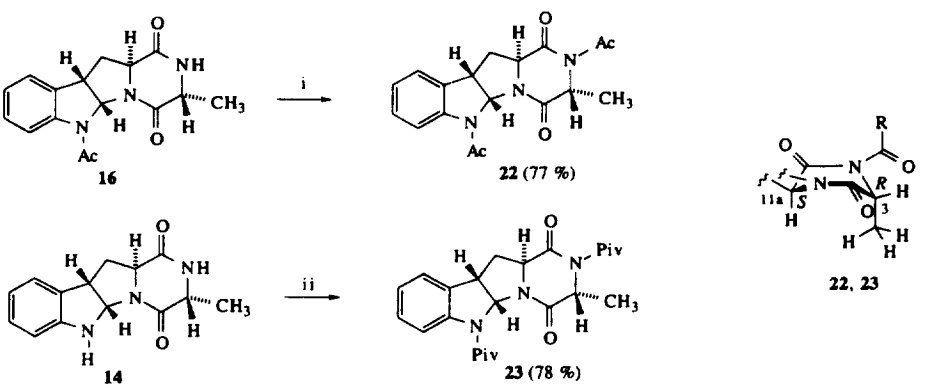


Conditions	% 18	% 19	% Recovered 11
i	30	30	0
ii	47	0	30
iii	9	18	20
iv	30	0	67

Conditions	% 20	% 21	% Recovered 11
v	46	0	50
vi	0	52	0

Reagents and conditions: i. Ac_2O, reflux, 4 h. ii. Ac_2O, DMAP, CH_2Cl_2, r.t., 24 h. iii. Ac_2O, DMAP, CH_2Cl_2, r.t., 96 h. iv. Ac_2O, KHMDS, - 78 °C, 3 h; - 78 °C → r.t., 16 h. v. Benzoyl chloride, KHMDS, - 78 °C, 3 h; - 78 °C → r.t., 21 h . vi. Benzoyl chloride, reflux, 4 h.

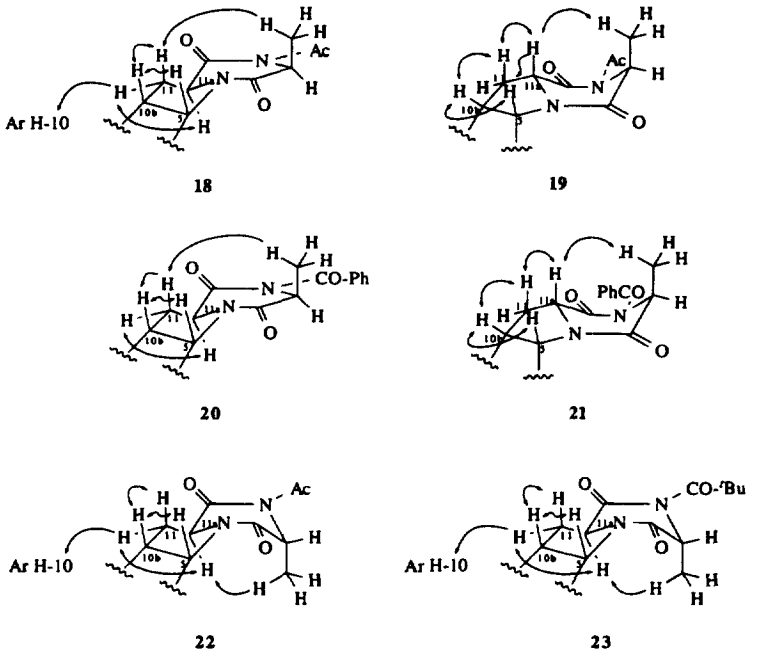
Scheme 5.



Reagents and conditions: i. Ac_2O, reflux, 4 h. ii. Piv-Cl, pyr, r.t., 3 h.

Scheme 6.

In conclusion, we have shown that the stereochemical outcome of the acid-catalyzed cyclization of *cyclo*-(Trp-Ala) derivatives depends mainly on the configuration of the Trp stereocenter, and always leads to compounds where the hydrogen atoms of the *B–C* ring fusion are *trans* with respect to the C-11a carbon. This arrangement involves sometimes an epimerization of the Trp stereocenter, which is believed to occur after cyclization. Acylation reactions of the N-2 atom can also be accompanied by epimerization of the Trp stereocenter when C-3 has the *S* configuration.



18 19 20 21 22 23

Fig. 2.

3. Experimental

All reagents were of commercial quality (Aldrich, Fluka, SDS, Probus) and were used as received. Solvents (SDS, Scharlau) were dried and purified using standard techniques. The expression 'petroleum ether' refers to the fraction boiling at 40–60°C. Reactions were monitored by thin layer chromatography, on aluminium plates coated with silica gel with fluorescent indicator (Macherey–Nagel Alugram Sil G/UV_{254}). Catalytic hydrogenations were carried out using a Parr 3920 shaking reactor. Separations by flash chromatography were performed on silica gel (SDS 60 ACC, 230–400 mesh). Melting points were measured on a Reichert 723 hot stage microsope, and are uncorrected. Infrared spectra were recorded on Buck Scientific 500 and Perkin–Elmer Paragon 1000 FT-IR spectrophotometers, with solid compounds compressed into KBr pellets and liquid compounds placed between two NaCl disks. NMR spectra were obtained on a Bruker AC-250 spectrometer (250 MHz for ^1H, 63 MHz for ^{13}C) spectrometer (Servicio de Espectroscopía, Universidad Complutense), with $CDCl_3$ or DMSO-d_6 as solvents. When necessary, assignments were aided by DEPT, COSY and ^{13}C–^1H correlation experiments. Optical rotations were determined at 25°C on a 1 ml cell, using a Perkin–Elmer 240 polarimeter operating at the emission wavelength of a sodium lamp; concentrations are given in g/100 ml. Elemental analyses were determined by the Servicio de Microanálisis, Universidad Complutense, on a Perkin–Elmer 2400 CHN microanalyzer.

3.1. *Methyl* N-(tert-*butyloxycarbonyl)alanyltryptophanates. General procedures*

Method A: To a solution of (*S*)-tryptophan methyl ester **1a** (2 g, 9.17 mmol) in dry THF (85 ml) was added *N*-Boc-L-alanine **2a** (1.73 g, 9.17 mmol) and DCC (1.89 g, 9.17 mmol). The reaction was refluxed for 90 min. The precipitate of dicyclohexylurea was filtered off and the filtrate was evaporated under

reduced pressure. The residue was chromatographed on silica gel, eluting with ethyl acetate, yielding 2.125 g (60%) of compound **3a**.

Method B: To a solution of the suitable tryptophan methyl ester (2 g, 9.17 mmol) in dry dichloromethane (50 ml) was added the suitable isomer of Boc-alanine (1.73 g, 9.17 mmol) and EDC (1.76 g, 9.17 mmol). The reaction was protected from light and stirred at room temperature for 24 h. The solution was washed with 1 N aqueous HCl (10 ml) and 1 N aqueous $NaHCO_3$ (10 ml). The residue was chromatographed on silica gel, eluting with ethyl acetate.[25] The yields obtained were: compound **3a**, 3.14 g (88%); compound **3b**, 3.26 g (91%); compound **4a**, 3.25 g (91%); compound **4b**, 3.22 g (90%).

Data for **3a**: mp, 121–122°C. IR (KBr): 3320.3 (NH); 1740.1 (CO_2CH_3); 1698.6, 1662.7 (CO–N) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 8.16 (br. s, 1H, NH^i); 7.52 (d, 1H, *J*=7.6 Hz, H-4′); 7.35 (d, 1H, *J*=8.0 Hz, H-7′); 7.15 (m, 2H, H-5′,6′); 7.03 (s, 1H, H-2′); 6.54 (d, 1H, *J*=7.5 Hz, NH); 5.06 (br. s, 1H, NH); 4.91 (m, 1H, Hα-Ala); 4.13 (m, 1H, Hα-Trp); 3.67 (s, 3H, CO_2CH_3); 3.33 (d, 2H, *J*=5.0 Hz, Hβ-Trp); 1.41 (s, 9H, Boc); 1.30 (d, 3H, *J*=6.6 Hz, CH_3-Ala). ^{13}C-NMR ($CDCl_3$) δ: 172.50, 172.21 (CO-Ala, CO_2CH_3); 155.20 ($CO_2C(CH_3)_3$); 136.20 (C-7′a); 127.66 (C-3′a); 123.18 (C-2′); 122.25 (C-4′); 119.66 (C-5′); 118.60 (C-6′); 111.46 (C-7′); 109.73 (C-3′); 81.00 ($CO_2C(CH_3)_3$); 53.04 (Cα-Trp); 52.50 (CO_2CH_3); 50.26 (Cα-Ala); 28.36 ($CO_2C(CH_3)_3$); 27.65 (Cβ-Trp); 18.51 (CH_3-Ala) ppm. $[\alpha]_D^{25}$=+32.4 (0.50, $CHCl_3$). Anal. calcd for $C_{20}H_{27}N_3O_3$: C, 61.68; H, 6.99; N, 10.79. Found: C, 61.30; H, 6.95; N, 10.60.

Data for **3b**: mp, 123–124°C. $[\alpha]_D^{25}$=−32.9 (0.50, $CHCl_3$). Anal. calcd for $C_{20}H_{27}N_3O_3$: C, 61.68; H, 6.99; N, 10.79. Found: C, 61.41; H, 6.94; N, 10.29. Spectral data were identical to those of compound **3a**.

Data for **4a**: mp, 125–126°C. IR (KBr): 3320.4 (NH), 1734.1 (CO_2CH_3); 1699.8, 1669.5 (CO–N) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 8.72 (br. s, 1H, NH^i); 7.50 (d, 1H, *J*=7.6 Hz, H-4′); 7.31 (d, 1H, *J*=7.7 Hz, H-7′); 7.14 (m, 2H, H-5′,6′); 6.96 (d, 1H, *J*=2.3 Hz, H-2′); 5.27 (d, 1H, *J*=7.2 Hz, NH); 4.88 (m, 1H, Hα-Ala); 4.14 (m, 1H, Hα-Trp); 3.59 (s, 3H, CO_2CH_3); 3.28 (d, 2H, *J*=4.8 Hz, Hβ-Trp); 1.41 (s, 9H, Boc); 1.24 (d, 3H, *J*=6.7 Hz, CH_3-Ala). ^{13}C-NMR ($CDCl_3$) δ: 172.37 (CO-Ala, CO_2CH_3); 155.20 ($CO_2C(CH_3)_3$); 136.21 (C-7′a); 127.52 (C-3′a); 123.13 (C-2′); 122.26 (C-4′); 119.67 (C-5′); 118.55 (C-6′); 111.39 (C-7′); 109.74 (C-3′); 81.00 ($CO_2C(CH_3)_3$); 52.60 (Cα-Trp); 52.51 (CO_2CH_3); 50.20 (Cα-Ala); 28.38 ($CO_2C(CH_3)_3$); 27.70 (Cβ-Trp); 18.44 (CH_3-Ala) ppm. $[\alpha]_D^{25}$=+55.6 (0.50, $CHCl_3$). Anal. calcd for $C_{20}H_{27}N_3O_3$: C, 61.68; H, 6.99; N, 10.79. Found: C, 61.22; H, 6.90; N, 10.79.

Data for **4b**: mp, 125–126°C. $[\alpha]_D^{25}$=−55.8 (0.50, $CHCl_3$). Anal. calcd for $C_{20}H_{27}N_3O_3$: C, 61.68; H, 6.99; N, 10.79. Found: C, 62.18; H, 7.14; N, 10.42. Spectral data were identical to those of compound **4a**.

3.2. *(S,S)-Methyl N-(benzyloxycarbonyl)alanyltryptophanate* **8**

To a solution of (*S*)-tryptophan methyl ester **1a** (0.894 g, 4.0 mmol) in dry THF (37 ml) was added (*S*)-Boc-alanine (0.892 g, 4.0 mmol) and DCC (0.825 g, 4.0 mmol). The solution was refluxed for 90 min. The precipitate of dicyclohexylurea was filtered off, and the filtrate was evaporated under reduced pressure. The residue was recrystallized from chloroform–hexane, yielding 1.109 g (64%) of compound **8**. Mp, 120°C. IR (KBr): 3300 (NH); 1725 (CO_2CH_3); 1685, 1640 (CO–N) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 8.07 (br. s, 1H, NH^i); 7.54 (d, 1H, *J*=7.6 Hz, H-4′); 7.48 (m, 6H, H-7′ and CH_2–C_6H_5); 7.13 (m, 2H, H-5′,6′); 6.94 (s, 1H, H-2′); 6.53 (d, 1H, *J*=7.4 Hz, NH); 5.27 (d, 1H, *J*=7.4 Hz, NH); 5.04 (AB system, 2H, CH_2–C_6H_5); 4.90 (m, 1H, Hα-Ala); 4.22 (m, 1H, Hα-Trp); 3.67 (s, 3H, CO_2CH_3); 3.30 (d, 2H, *J*=5.3 Hz, Hβ-Trp); 1.31 (d, 3H, *J*=6.9 Hz, CH_3-Ala). ^{13}C-NMR ($CDCl_3$) δ: 172.19 (CO-Ala, CO_2CH_3); 155.20 ($CO_2CH_2C_6H_5$); 136.16 (C-7′a); 128.67, 128.16 and 128.32 ($CO_2CH_2C_6H_5$); 127.57

(C-3′a); 123.26 (C-2′); 122.27 (C-4′); 119.70 (C-5′); 118.53 (C-6′); 111.47 (C-7′); 109.57 (C-3′); 67.04 ($CO_2CH_2C_6H_5$); 52.99 (Cα-Trp); 52.56 (CO_2CH_3); 50.56 (Cα-Ala); 27.57 (Cβ-Trp); 18.73 (CH_3-Ala) ppm. $[\alpha]_D^{25}$=+44.7 (0.50, $CHCl_3$). Anal. calcd. for $C_{23}H_{25}N_3O_5$: C, 65.25; H, 5.91; N, 9.93. Found: C, 64.92; H, 5.67; N, 9.84.

3.3. 3-Indolylmethyl-2,5-piperazinediones. General procedures

Method A. The suitable Boc-protected dipeptides (8.0–8.5 mmol) were heated at 200°C under a stream of argon for 2–4 h. The off-white to white residues were identified as the pure piperazinediones **5a**, **5b**, **6a**, and **6b**, in quantitative yields.

Method B. To a solution of dipeptide **8** (300 mg, 0.71 mmol) in a 1:1 mixture of ethyl acetate–ethanol (50 ml) was added 10% Pd–C (120 mg). The suspension was hydrogenated at 42 psi for 20 h, filtered through Celite and evaporated. The residue was dissolved in dry xylene and the solution was refluxed for 40 h. Upon cooling to 0°C, a white precipitate was obtained, which was filtered and washed with petroleum ether. Yield, 101 mg (64%) of compound **5a**.

Data for **5a**: mp, 280–281°C (decomp.), lit.,[16] 282–284°C (decomp.). IR (KBr): 3411.1, 3189.8 (NH); 1654.9 (CO–NH). ^{1}H-NMR (d_6-DMSO) δ: 10.90 (br. s, 1H, NH^i); 8.04 (s, 1H, NH); 7.91 (s, 1H, NH); 7.56 (d, 1H, *J*=7.7 Hz, H-4′); 7.30 (d, 1H, *J*=8.0 Hz, H-7′); 6.96 (m, 3H, H-2′,5′,6′); 4.10 (m, 1H, H-3); 3.56 (m, 1H, H-6); 3.24 (dd, 1H, *J*=14.3 and 3.9 Hz, CH_2); 2.99 (dd, 1H, *J*=14.3 and 4.4 Hz, CH_2); 0.39 (d, 3H, *J*=7.0 Hz, C-6–CH_3) ppm. ^{13}C-NMR (d_6-DMSO) δ: 167.66 and 166.68 (2 CO); 135.71 (C-7′a); 127.73 (C-3′a); 124.50 (C-2′); 120.74 (C-4′); 118.92 (C-5′); 118.32 (C-6′); 111.03 (C-7′); 108.41 (C-3′); 55.33 (C-3); 49.71 (C-6); 28.77 (CH_2); 19.49 (C-6–CH_3) ppm. $[\alpha]_D^{25}$=+17.0 (0.05, EtOH). $[\alpha]_D^{25}$=−8.0 (0.50, DMSO), lit.,[26] $[\alpha]_D^{25}$=+18 (0.05, EtOH). Anal. calcd for $C_{14}H_{15}N_3O_2$: C, 65.36; H, 5.88; N, 16.33. Found: C, 65.10; H, 5.71; N, 15.93.

Data for **5b**: mp, 280–281°C (decomp.), lit.,[16] 281–283°C (decomp.). $[\alpha]_D^{25}$=+8.4 (0.50, DMSO), lit.,[16] $[\alpha]_D^{25}$=−10.2 (0.048, EtOH).[27] Anal. calcd for $C_{14}H_{15}N_3O_2$: C, 65.36; H, 5.88; N, 16.33. Found: C, 65.05; H, 6.01; N, 16.08. Spectral data were identical to those of compound **5a**.

Data for **6a**: mp, 265–267°C (decomp.), lit.,[16] 265–267°C (decomp.). IR (KBr): 3359.7, 3193.3 (NH); 1659.8 (CO–NH). ^{1}H-NMR (d_6-DMSO) δ: 10.92 (br. s, 1H, NH^i); 8.07 (s, 1H, NH); 7.93 (s, 1H, NH); 7.54 (d, 1H, *J*=7.7 Hz, H-4′); 7.31 (d, 1H, *J*=8.0 Hz, H-7′); 6.99 (m, 3H, H-2′,5′,6′); 4.03 (m, 1H, H-3); 3.24 (dd, 1H, *J*=14.3 and 4.6 Hz, CH_2); 3.00 (dd, 1H, *J*=14.5 and 4.5 Hz, CH_2); 2.89 (q, 1H, *J*=6.7 Hz, H-6); 1.03 (d, 3H, *J*=6.9 Hz, C-6–CH_3) ppm. ^{13}C-NMR (d_6-DMSO) δ: 168.43 and 168.09 (2 CO); 135.85 (C-7′a); 127.45 (C-3′a); 124.49 (C-2′); 120.84 (C-4′); 118.68 (C-5′); 118.39 (C-6′); 111.12 (C-7′); 108.35 (C-3′); 55.87 (C-3); 48.70 (C-6); 29.08 (CH_2); 18.28 (C-6–CH_3) ppm. $[\alpha]_D^{25}$=+13.6 (0.50, DMSO), lit,[16] $[\alpha]_D^{25}$=+75.6 (0.049, EtOH).[27] Anal. calcd for $C_{14}H_{15}N_3O_2$: C, 65.36; H, 5.88; N, 16.33. Found: C, 65.06; H, 5.76; N, 16.05.

Data for **6b**: mp, 265–267°C (decomp.), lit.,[16] 265–267°C (decomp.). $[\alpha]_D^{25}$=−14.0 (0.50, DMSO), lit,[16] $[\alpha]_D^{25}$=−74.2 (0.051, EtOH).[27] Anal. calcd for $C_{14}H_{15}N_3O_2$: C, 65.36; H, 5.88; N, 16.33. Found: C, 65.08; H, 5.76; N, 16.04. Spectral data were identical to those of compound **6a**.

3.4. 6-Acetylpyrazino[2′,1′-5,1]pyrrolo[2,3-b]indole-1,4-diones. General procedures

Method A. The suitable 3-indolylmethyl-2,5-piperazinedione (0.195 to 1.556 mmol) was added in one portion to trifluoroacetic acid (1 to 8 ml). The suspension was stirred until complete disolution (*ca.* 2 min), and was poured onto a vigorously stirred biphasic system of dichloromethane (7 to 56 ml) and 20% aqueous potassium carbonate (7 to 56 ml), externally cooled with an ice bath. The pH of the aqueous

layer was ajusted to 8 and it was extracted with dichloromethane (20×10 ml). The combined organic layers were dried over anhydrous sodium sulphate and evaporated. The residue was examined by ^{1}H-NMR (data for compounds **9**, **10**, **14** and **15** can be found in Table 1) and was used for the next step without further purification.

A solution of the crude pyrazino[2′,1′-5,1]pyrrolo[2,3-*b*]indole-1,4-dione (**9**, **10**, **14** or **15**) (0.23 to 1.40 mmol) in pyridine (1 to 6 ml) and acetic anhydride (0.25 to 1.50 ml) was stirred at room temperature for 3 h. The solution was diluted with dichloromethane (5 to 10 ml) and filtered to remove the corresponding diketopiperazines, from ring opening of the starting materials. The filtrate was evaporated, and the residue was diluted with dichloromethane (5–10 ml) and washed with water (5–10 ml). The organic layer was dried over anhydrous sodium sulphate and evaporated. The residue was chromatographed on silica gel, eluting with ethyl acetate.

NMR data of compounds **11**, **12**, **16** and **17** are in Tables 1 and 2. Other data follow.

*3.5. (3S,5aR,10bS,11aS)-6-Acetylpyrazino[2′,1′-5,1]pyrrolo[2,3-*b*]indole-1,4-dione,* ***11***

Yield, 45%. Mp, 272–274°C. IR (KBr): 3242.1 (NH), 1676.1 (C=O) cm^{-1}. $[\alpha]_D^{25}$=− 218.0 (0.50, $CHCl_3$). Anal. calcd for $C_{16}H_{17}N_3O_3$: C, 64.20; H, 5.72; N, 14.04. Found: C, 64.04; H, 5.74; N, 13.82.

*3.6. (3S,5aS,10bR,11aR)-6-Acetylpyrazino[2′,1′-5,1]pyrrolo[2,3-*b*]indole-1,4-dione,* ***12***

Yield, 9%. Mp, 294–296°C. IR (KBr): 3264.7 (NH); 1673.9 (C=O) cm^{-1}. $[\alpha]_D^{25}$=+138.9 (0.50, DMSO). Anal. calcd for $C_{16}H_{17}N_3O_3$: C, 64.20; H, 5.72; N, 14.04. Found: C, 63.96; H, 5.92; N, 13.81.

*3.7. (3R,5aR,10bS,11aS)-6-Acetylpyrazino[2′,1′-5,1]pyrrolo[2,3-*b*]indole-1,4-dione,* ***16***

Yield, 42%. Mp, 295–296°C. $[\alpha]_D^{25}$=−140.6 (0.50, DMSO). Anal. calcd for $C_{16}H_{17}N_3O_3$: C, 64.20; H, 5.72; N, 14.04. Found: C, 63.91; H, 5.53; N, 13.82.

*3.8. (3R,5aS,10bR,11aR)-6-Acetylpyrazino[2′,1′-5,1]pyrrolo[2,3-*b*]indole-1,4-dione,* ***17***

Yield, 7%. Mp, 274°C. $[\alpha]_D^{25}$=+216,6 (0.50, $CHCl_3$). Anal. calcd for $C_{16}H_{17}N_3O_3$: C, 64.20; H, 5.72; N, 14.04. Found: C, 64.05; H, 5.48; N, 13.82.

Method B. A solution of 2,5-piperazinediones **5b** or **6b** (0.390 to 1.556 mmol) in 85% aqueous phosphoric acid (4 to 8 ml) was stirred at room temperature for 72 h. After this time, the solution was poured onto a vigorously stirred biphasic system of dichloromethane (10 to 20 ml) and a 20% aqueous solution of potassium carbonate (40 to 80 ml), externally cooled with an ice bath. The pH of the aqueous layer was adjusted to 8 and it was extracted with dichloromethane (20×10 ml) (a precipitate of phosphate salts that normally appeared during the extraction was removed by filtration). The combined organic layers were dried over anhydrous sodium sulphate and evaporated. The residue was found to consist of compounds **10** and **15**, which were essentially pure by ^{1}H-NMR (Table 1). These crude cyclization products were used for the acetylation step, which was performed as indicated under Method A. The overall yields obtained were: starting from **5b**, 41% of **12**; starting from **6b**, 40% of **17**.

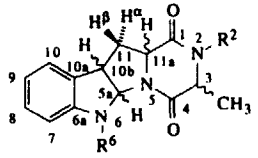


Table 1
^{1}H-NMR data of compounds **9–23**[a]

Cmpd.	H-3	H-5a	H-7	H-8	H-9	H-10	H-10b	H-11	H-11a	R^2	C_3-CH_3	R^6
9	4.05 (m)	5.74 (d, J = 6.8)	6.62 (d, J = 7.5)	7.12 (m)	6.79 (t, J = 7.4)	7.12 (m)	4.05 (m)	2.70 (dd, J = 12.3, 6.4) 2.46 (m)	4.05 (m)	*b*	1.48 (d, J = 6.8)	*b*
10	4.03 (m)	5.81 (d, J = 7.1)	6.62 (d, J = 7.8)	7.12 (m)	6.78 (t, J = 7.4)	7.12 (m)	4.03 (m)	2.73 (dd, J = 12.9, 6.3) 2.41 (m)	4.03 (m)	4.97 (br. s)	1.43 (d, J = 7.2)	6.13 (br. s)
11	4.12 (m)	6.35 (d, J = 6.2)	8.00 (d, J = 7.5)	7.13 (t, J = 7.4)	7.27 (m)		4.12 (m)	2.74 (dd, J = 12.8, 5.8) 2.36 (m)	3.95 (dd, J = 11.7, 5.6)	6.60 (br. s)	1.46 (d, J = 6.8)	2.66 (s, 3H)
12	4.04 (m)	6.47 (d, J = 6.7)	8.05 (d, J = 8.0)	7.13 (t, J = 7.4)	7.26 (m)		4.12 (t, J = 6.8)	2.72 (dd, J = 12.6, 5.4) 2.28 (m)	3.89 (dd, J = 11.8, 5.5)	6.12 (br. s)	1.44 (d, J = 7.1)	2.61 (s, 3H)
14	4.01 (m)	5.80 (d, J = 7.0)	6.61 (d, J = 7.8)	7.10 (m)	6.77 (t, J = 7.3)	7.10 (m)	4.01 (m)	2.71 (dd, J = 12.8, 6.2) 2.40 (m)	4.01 (m)	5.05 (br. s)	1.42 (d, J = 7.2)	*b*
15	4.04 (m)	5.73 (d, J = 6.7)	6.60 (d, J = 7.5)	7.11 (m)	6.78 (t, J = 7.4)	7.11 (m)	4.04 (m)	2.69 (dd, J = 12.3, 6.4) 2.45 (m)	4.04 (m)	5.13 (br. s)	1.47 (d, J = 6.8)	6.45 (br. s)
16	4.04 (m)	6.49 (d, J = 6.7)	8.06 (d, J = 8.1)	7.14 (t, J = 7.4)	7.27 (m)		4.12 (t, J = 6.8)	2.74 (dd, J = 12.6, 5.4) 2.30 (m)	3.91 (dd, J = 11.8, 5.4)	6.51 (br. s)	1.46 (d, J = 7.0)	2.63 (s, 3H)
17	4.11 (m)	6.34 (d, J = 6.2)	7.99 (d, J = 7.5)	7.12 (t, J = 7.4)	7.25 (m)		4.11 (m)	2.72 (dd, J = 12.8, 5.8) 2.34 (m)	3.94 (dd, J = 11.5, 5.6)	6.18 (br. s)	1.45 (d, J = 6.8)	2.65 (s, 3H)
18	5.03 (q, J = 7.0)	6.71 (d, J = 7.6)	8.16 (d, J = 7.9)	7.13 (t, J = 7.4)	7.26 (m)		4.16 (m)	2.60 (dd, J = 12.1, 4.9) 2.24 (m)	4.16 (m)	2.46 (s, 3H)*	1.53 (d, J = 7.0)	2.48 (s, 3H)*
19	4.96 (q, J = 7.4)	6.10 (d, J = 5.7)	7.88 (d, J = 7.7)	7.09 (t, J = 7.0)	7.25 (m)		4.02 (t, J = 6.5)	3.21 (dd, J = 14.8, 1.7) 2.73 (m)	4.39 (dd, J = 10.2, 2.7)	2.57 (s, 3H)	1.48 (d, J = 7.4)	2.21 (s, 3H)
20	4.85 (q, J = 6.9)	6.66 (d, J = 7.2)	8.16 (d, J = 7.5)	7.13 (t, J = 7.4)	7.26 (m)		4.18 (m)	2.60 (m)[c] 2.28 (m)	4.18 (m)	7.50 (m, 5H)	1.64 (d, J = 6.9)	2.59 (s, 3H)
21	4.88 (q, J = 7.1)	6.45 (d, J = 6.3)	8.05 (d, J = 7.9)	7.15 (t, J = 7.4)	7.35 (m)		4.22 (t, J = 6.4)	2.80 (dd, J = 12.8, 5.8) 2.44 (m)	4.08 (dd, J = 11.4, 5.7)	7.53-7.35 (m, 5H)	1.56 (d, J = 7.1)	2.71 (s, 3H)
22	5.02 (q, J = 7.1)	6.30 (d, J = 6.2)	8.01 (d, J = 7.9)	7.15 (t, J = 7.4)	7.28 (m)		4.17 (t, J = 6.5)	2.83 (dd, J = 12.9, 5.9) 2.44 (m)	4.05 (m)	2.52 (s, 3H)	1.35 (d, J = 7.1)	2.66 (s, 3H)
23	4.44 (q, J = 7.1)	6.87 (d, J = 6.0)	7.67 (d, J = 7.2)	7.05 (t, J = 7.4)	7.22 (m)		4.11 (t, J = 7.1)	2.82 (dd, J = 11.8, 5.8) 2.37 (m)	3.95 (dd, J = 11.3, 5.8)	1.45 (s, 9H)	1.37 (d, J = 7.1)	1.27 (s, 9H)

[a] Exchangeable assignments are marked with *. [b]Not detected. [c]Overlapped with the acetyl resonance.

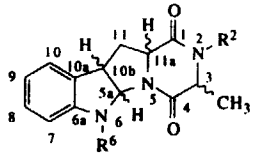


Table 2
^{13}C-NMR data of compounds **13–23**[a]

Cmpd.	C-1*	C-3	C-4*	C-5a	C-6a	C-7	C-8#	C-9	C-10#	C-10a	C-10b	C-11	C-11a	N^2-COR	N^2-COR	C_3-CH_3	N_6-CO-R	N_6-COR
11	169.14	51.30	166.50	77.51	142.94	118.97	125.06	128.88	123.70	130.70	43.93	34.40	58.45	-	-	16.54	170.68	23.89
12	167.32	52.58	166.78	77.37	142.99	117.62	124.48	127.94	124.27	132.05	43.16	34.99	56.87	-	-	20.75	170.36	23.84
16	167.15	52.40	166.63	77.20	142.81	117.46	124.32	127.78	124.10	131.88	42.98	34.79	56.69	-	-	20.57	170.20	23.67
17	169.05	51.31	166.46	77.41	142.95	118.97	125.05	128.88	123.69	130.71	43.94	34.41	58.47	-	-	16.58	170.59	23.87
18	168.02	53.03	167.89	76.48	143.70	117.27	124.90	129.25	124.59	*b*	44.05	38.93	59.15	170.90*	26.50	21.18	170.73*	24.39
19	169.94	55.10	167.56	79.11	141.05	118.77	124.73	128.84	124.57	131.26	44.73	27.89	57.95	168.45	26.99	15.91	171.33	24.08
20	169.06	54.94	167.43	76.89	143.48	117.81	125.03	129.19	124.37	137.12	44.10	37.41	59.06	171.70**	127.47 (1') 128.68 (2',3',5',6') 133.02 (4')	20.04	170.85**	24.35
21	168.98	56.99	166.65	77.14	142.88	119.21	125.21	129.14	123.65	134.61	44.07	35.71	58.70	171.46	130.16 (1') 132.71 (4') 128.50, 128.27 (2',3',5',6')	18.80	170.64	23.91
22	168.75	54.90	166.46	77.67	142.75	119.28	125.17	129.07	123.57	130.36	44.06	34.87	58.79	171.25*	27.54	18.05	170.55*	23.83
23	168.92	53.40	167.20	77.09**	145.47	116.32	128.77	128.77	123.60	134.17	43.52	34.08	58.49	181.49‡	29.05** 77.09**	18.05	185.21‡	27.81** 76.67**

[a]Exchangeable assignments are marked with *, #, ‡ and **. [b]Overlapped with the C-9 signal.

3.9. N-2 Acetylation of 11

Method A. A solution of **11** (82 mg, 0.274 mmol) in acetic anhydride (10 ml) was refluxed in an oil bath at 140°C for 4 h. The solution was evaporated and the residue was chromatographed on silica gel, eluting with ethyl acetate:petroleum ether (1:1), to yield 27 mg (29%) of compound **18** and 28 mg (30%) of compound **19**, as oils.

Method B. A solution of **11** (50 mg, 0,167 mmol), 4-dimethylaminopyridine (2 mg, 0.017 mmol) and acetic anhydride (0.24 μl, 0.25 mmol) in dichloromethane (5 ml) was stirred at room temperature for 24 h. The solution was evaporated and the residue was chromatographed on silica gel, eluting with ethyl acetate, to yield 27 mg (47%) of **18** and 15 mg (30%) of unreacted **11**.

Method C. To a cooled (−78°C) solution of compound **11** (60 mg, 0.200 mmol) in dry THF (10 ml), under an argon atmosphere, was added a 0.5 M solution of potassium hexamethyldisilazide in toluene (0.5 ml, 0.24 mmol). The yellow solution was stirred for 15 min, and acetic anhydride (23 μl, 0.24 mmol) was added. The reaction mixture was stirred at −78°C for 3 h and left to warm to room temperature over 16 h. The solution was poured on an aqueous, saturated solution of ammonium chloride (10 ml), which was extracted with dichloromethane (6×10 ml). The combined organic layers were evaporated under reduced pressure and the residue was chromatographed on silica gel, eluting with 1.5:1 petroleum ether:ethyl acetate. Yield, 40 mg (67%) of unreacted **11** and 20 mg (30%) of compound **18**.

Data for **18**: mp, 172–173°C. IR (KBr): 1710.1 ($COCH_3$); 1659.8 (CO–NH) cm^{-1}. $[\alpha]_D^{25}=-62.3$ (0.175, $CHCl_3$). Anal. calcd for $C_{18}H_{19}N_3O_4$: C, 63.34; H, 5.57; N, 12.32. Found: C, 63.25; H, 5.41; N, 12.55. NMR data are in Tables 1 and 2.

Data for **19** (oil): IR (KBr): 1710.1 ($COCH_3$); 1659.8 (CO–NH) cm^{-1}. $[\alpha]_D^{25}=+65.6$ (0.50, $CHCl_3$). Anal. calcd for $C_{18}H_{19}N_3O_4$: C, 63.34; H, 5.57; N, 12.32. Found: C, 63.14; H, 5.54; N, 12.04. NMR data are in Tables 1 and 2.

3.10. N-2 Benzoylation of 11

Method A: A solution of **11** (60 mg, 0.20 mmol) in dry THF (10 ml), in an argon atmosphere, was cooled to −78°C, treated dropwise with a 0.5 M solution of potassium hexamethyldisilazide in toluene (0.5 ml, 0.24 mmol) and stirred for 15 min. To the yellow solution was added benzoyl chloride (25 μl, 0.22 mmol). The temperature was maintained at −78°C for 2 h, and the reaction was allowed to warm to room temperature over 21 h. After this time, the reaction mixture was evaporated and the residue was chromatographed on silica gel, eluting with ethyl acetate:petroleum ether (1:2). Yield, 37 mg (46%) of compound **20** and 30 mg (50%) of unreacted **11**.

Method B: A solution of **11** (50 mg, 0.195 mmol) in benzoyl chloride (10 ml) was heated at 140°C for 4 h. The solution was washed with a 20% aqueous solution of Na_2CO_3 (10 ml) and evaporated (0.5 torr, 120°C, 3 h). The residue was purified by chromatography on silica gel, eluting with ethyl acetate:petroleum ether (1:1). Yield, 35 mg (52%) of compound **21**.

Data for **20** (oil): IR (KBr): 3379.5 (NH); 1681.9 (CO) cm^{-1}. $[\alpha]_D^{25}=-64.7$ (0.36, $CHCl_3$). Anal. calcd for $C_{23}H_{21}N_3O_4$: C, 68.49; H, 5.21; N, 10.42. Found: C, 68.31; H, 5.50; N, 10.12. NMR data are in Tables 1 and 2.

Data for **21**: mp 238–240°C. IR (KBr): 3349.9 (NH); 1681.8 (CO) cm^{-1}. $[\alpha]_D^{25}=-28.57$ (0.07, $CHCl_3$). Anal. calcd for $C_{23}H_{21}N_3O_4$: C, 68.49; H, 5.21; N, 10.42. Found: C, 68.61; H, 5.27; N, 10.27. NMR data are in Tables 1 and 2.

3.11. Acetylation of 16

A solution of **16** (47 mg, 0.157 mmol) in acetic anhydride (10 ml) was refluxed in an oil bath at 140°C for 4 h. The solution was evaporated and the residue was chromatographed on silica gel, eluting with ethyl acetate:petroleum ether (1:1). Yield, 41 mg (77%) of compound **22**. Mp, 80–82°C. IR (KBr): 3349.6 (NH); 1713.4 ($COCH_3$); 1681.6 (CO–N) cm^{-1}. $[\alpha]_D^{25}=-126.32$ (0.19, $CHCl_3$). Anal. calcd for $C_{18}H_{19}N_3O_4$: C, 63.34; H, 5.57; N, 12.32. Found: C, 63.08; H, 5.23; N, 12.08. NMR data are in Tables 1 and 2.

3.12. Pivaloylation of 14

To a solution of compound **14** (192 mg, 0.747 mmol) in pyridine (2.5 ml) was added pivaloyl chloride (0.79 ml, 6.4 mmol). The reaction was stirred at room temperature for 24 h and filtered to remove the pyridine hydrochloride. The filtrate was evaporated and the residue was dissolved in dichloromethane (10 ml) and washed with water (10 ml). The organic layer was dried over anhydrous sodium sulphate and evaporated. The residue was chromatographed on silica gel, eluting with ethyl acetate:petroleum ether (1:3). Yield, 248 mg (78%) of compound **23** and 38 mg (12%) of **24**,[24] both as oils.

Data for **23**: IR (KBr): 3250.3 (NH); 1684.2 (CO) cm^{-1}. $[\alpha]_D^{25}=-209.17$ (0.36, $CHCl_3$). Anal. calcd for $C_{24}H_{31}N_3O_4$: C, 67.76; H, 7.29; N, 9.88. Found: C, 67.68; H, 7.06; N, 9.61. NMR data are in Tables 1 and 2.

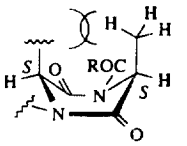
Acknowledgements

We thank CICYT for financial support (grants SAF-94-0517 and SAF-97-0143).

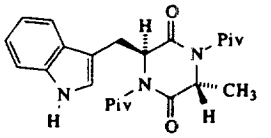
References

1. Arai, K.; Kimura, K.; Mushiroda, T.; Yamamoto, Y. *Chem. Pharm. Bull.* **1988**, *37*, 2937.
2. Fructigenine B has been isolated both from *Penicillium fructigenum* and *Penicillium verrucosum*, and hence the alternative name verrucofortine. See: Hodge, R. P.; Harris, C. M.; Harris, T. M. *J. Nat. Prod.* **1988**, *51*, 66.
3. (a) Takase, S.; Iwami, M.; Ando, T.; Okamoto, M.; Yoshida, K.; Horiai, H.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.* **1984**, *37*, 1320. (b) Takase, S.; Kavai, Y.; Uchida, I.; Tanaka, H.; Akoi, H. *Tetrahedron* **1985**, *41*, 3037. (c) Laws, I.; Mantle, P. G. *Phytochemistry* **1985**, *24*, 1395.
4. A stereoisomer of amauromine has also been isolated from natural sources. See: de Guzmán, F. S.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *J. Nat. Prod.* **1992**, *55*, 931.
5. Isolation and structural determination: Safe, S.; Taylor, A. *J. Chem. Soc., Perkin Trans. 1* **1972**, 472 and references therein. For a commentary on the total synthesis of the sporidesmins, see: Anand, N.; Bindra, J. S.; Ranganathan, S. *Art in Organic Synthesis* (2nd Edition), John Wiley & Sons, 1988, p. 330.
6. Hochlowski, J. E.; Mullaly, M. M.; Spanton, S. G.; Whittern, D. N.; Hill, P.; McAlpine, J. B. *J. Antibiot.* **1993**, *46*, 380.
7. (a) Springer, J. P.; Buchi, G.; Kobbe, B.; Demain, A. L.; Clardy, J. *Tetrahedron Lett.* **1977**, *28*, 2403. (b) Maes, C. M.; Potgieter, M.; Steyn, P. S. *J. Chem. Soc., Perkin Trans. 1* **1986**, 861. For the recent isolation of a related natural product with cytotoxic properties, see: Varoglu, M.; Corbett, T. H.; Valeriote, F. A.; Crews, P. *J. Org. Chem.* **1997**, *62*, 7078.
8. (a) Müllbacher, A.; Waring, P.; Tiwari-Palni, U.; Eichner, R. D. *Mol. Immunol.* **1986**, *23*, 231. (b) Müllbacher, A.; Waring, P.; Braithwaite, A. W.; Eichner, R. D. *Drugs of the Future* **1987**, *12*, 565.
9. Karkowski, J. P.; Jackson, M.; Rasmussen, R. R.; Humphrey, P. E.; Poddig, J. B.; Kohl, W. L.; Scherr, M. H.; Kadam, S.; McAlpine, J. B. *J. Antibiot.* **1993**, *46*, 374.
10. Hino, T.; Nakagawa, M. In *The Alkaloids*, Brossi, A. (ed.), Vol. 34, Pergamon Press, 1988, p. 1.
11. Crich, D.; Lim, L. B. L. *Heterocycles* **1993**, *36*, 1199 and references therein.

12. Taniguchi, M.; Yamamoto, I.; Nakagawa, M.; Hino, M. *Chem. Pharm. Bull.* **1985**, *33*, 4783.
13. Brugger, P. H.; Schreiber, J.; Eschenmoser, A. *Helv. Chim. Acta* **1963**, *46*, 577.
14. Sheehan, J. C.; Ledis, S. L. *J. Am. Chem. Soc.* **1973**, *95*, 875.
15. Elimination of the *N*-Boc protection under thermal conditions: Rawal, V. H.; Jones, R. J.; Cava, M. P. *J. Org. Chem.* **1987**, *52*, 19.
16. Compounds **5** and **6** have been prepared through an alternative route, although no spectral data were given. See: Nakashima, R.; Slater, G. T. *Can. J. Chem.* **1969**, *47*, 2069. Compound **5a** has also been isolated from natural sources (see Ref. 25, for instance).
17. Longer treatment (*ca.* 15 min) leads to reversal of the cyclization by ring opening.
18. Partial reversion to **1** was observed during the acetylation reactions, leading to the isolation of **1a** (28%) and **1b** (24%).
19. Reviews on diketopiperazines: *Natural products containing the diketopiperazine unit*: (a) Johne, S.; Gröger, D. *Pharmazie* **1977**, *32*, 1. (b) Sammes, P. G. *Progress Chem. Nat. Prod.* **1975**, *32*, 51. *Conformational aspects*: Anteunis, M. J. O. *Bull. Soc. Chim. Belg.* **1978**, *87*, 627. *Synthesis and reactivity*: Rajappa, S.; Natekar, M. V. *Adv. Heterocycl. Chem.* **1993**, *57*, 187.
20. Rajappa, S.; Advani, B. G. *Tetrahedron* **1973**, *29*, 1299.
21. The higher degree of epimerization upon heating **11** with benzoyl chloride is probably related to the presence of HCl in the reaction medium, a stronger acid then the AcOH liberated in the reactions with acetic anhydride.
22. The alternative boat conformation is prevented by steric interactions between the pseudoaxial methyl group and the adjacent pentagonal ring.



23. In order to explain the observed NOEs between the C-3 methyl groups and one of the H-11 protons (see Fig. 2), compounds **18** and **20** have been depicted as deformed boats where the methyl group occupies a pseudoaxial position. Such a conformational change partly relieves the steric compression between the N_2-Ac, C_3–Me and C_4=O groups, although not as efficiently as epimerization, and has been previously observed in related compounds.[20]
24. Some reversion (12%) to a diketopiperazine was also observed in this case, leading to compound **24**. ^{1}H-NMR, $CDCl_3$, δ: 8.47 (br. s, 1H, NH); 7.38 (d, 1H, *J*=7.9, H-7′); 7.31 (d, 1H, *J*=8.0, H-4′); 7.19 (t, 1H, *J*=7.1, H-5′); 7.09 (t, 1H, *J*=7.0, H-6′); 6.85 (d, 1H, *J*=2.4, H-2′); 4.80 (m, 1H, H-3); 3.73 (dd, 1H, J=14.8 and 5.0, CH_2); 3.58 (dd, 1H, *J*=14.8 and 2.8, CH_2); 2.87 (q, 1H, *J*=6.8, H-6); 1.33 (s, 9H, ^{t}Bu); 1.26 (s, 9H, ^{t}Bu); 1.09 (d, 3H, *J*=6.9 Hz, C-6–CH_3) ppm.



24

25. Although the crude dipeptides thus obtained are virtually pure, they may contain traces of acid, which can only be completely eliminated by chromatography. Their presence causes epimerization of the tryptophan stereocenter of the diketopiperazines during the pyrolysis step. For example, under these conditions **4b** led to 50% of **6b** and 50% of **5a**.
26. Hamasaki, T.: Nagayama, K.; Hatsuda, Y. *Agr. Biol. Chem.* **1976**, *40*, 2487.
27. There is a remarkable difference between the [α] values of compounds **5** and **6** when measured in ethanol and in DMSO. See, for instance, our polarimetric data for compound **5a**, which are of opposite signs in these two solvents.