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The fate of the tryptophan stereocenter in the synthesis of 7,10,16,16a-tetrahydro-11*H*-quinazolino[2′,3′:3,4]-pyrazino[1,2-*b*]β-carboline-5,8-diones

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Abstract

Condensation reactions of anthranilic acid with iminoethers **14**–**17** derived from tetracycles **9**–**13** to give the title hexacyclic compounds reflect a preferred *trans*-relationship for H(10)–H(16a) protons in C(7)-unsubstituted products and a *cis*-relatioship for H(7)–H(16a) protons in C(10)-unsubstituted analogs. This synthetic strategy is limited by the steric hindrance of the substituent at C(10). Theoretical calculations are in agreement with the experimental results. The regioselectivity in favor of the linear tetracyclic compound **11** with respect to **12** has also been confirmed. © 2000 Published by Elsevier Science Ltd.

1. Introduction

We are currently studying the synthesis of hexacyclic compounds related to the fungal metabolite *N*-acetylardeemin[1] such as **C** and **D**. Given the activity of *N*-acetylardeemin as a reversor of Pgp-mediated multidrug resistance (MDR), compounds **C** and **D** could be potentially useful in combination with antitumor drugs to maintain the therapeutic intracellular concentrations when the 170 kD membrane glycoprotein Pgp is overexpressed.[2] In fact, some of the analogs so far studied have shown an interesting reversal activity of Pgp-mediated resistance.[3]

The synthesis of hexacycles **C** and **D** starts with the transformation of tryptophan methyl esters to the tricyclic pyrroloindoles and β-carbolines, respectively, followed by *N*-acylation and condensation with amino acids to the corresponding tetracyclic piperazine-2,5-diones **A** and **B**. Although direct condensation of the C(1)–N(2) amide function of these compounds with

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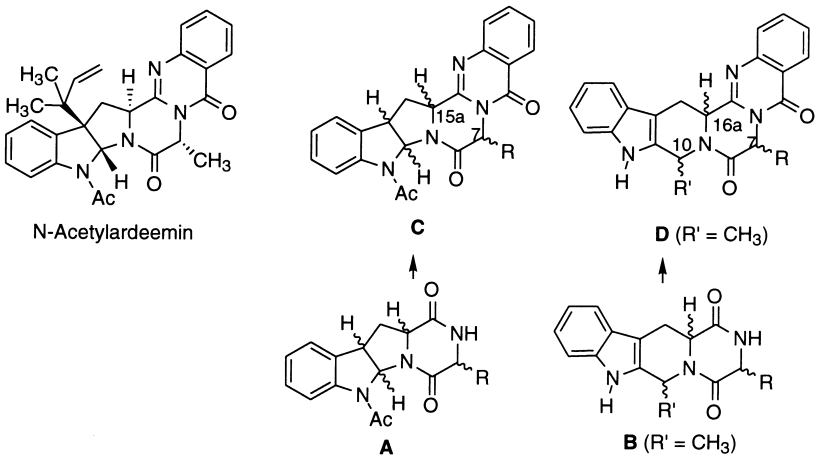
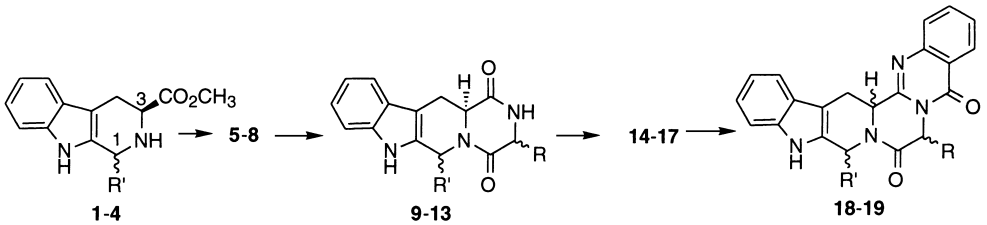
anthranilic acid and thionyl chloride[4] failed, iminoethers were efficient derivatives to activate the reaction substrate. Alternatively, **C** and **D** have been obtained by *N*(2)-acylation with *o*-azidobenzoyl chloride and KHMDS, followed by intramolecular aza-Wittig reaction.[5,6] Epimerization of the 'tryptophan' stereocenter [C(15a) in **C** and C(16a) in **D**] in the last step of the first mentioned strategy or at the *N*-acylation in the second, was observed in some instances. The lability of this proton, which is due to its α-position to an imino group, has also been described in condensations of anthranilic acid with iminoethers derived from proline-containing diketopiperazines to form fused pyrrolo–pyrazino–quinazoline compounds.[7]



The results with the pyrazino–pyrrolo–indoles **A** precursors of compounds **C** so far studied indicated a lower stability for the isomers with the H(7) and H(15a) protons in a *cis*-relationship.[5] However, the results with pyrazino-β-carbolines precursors of **D**[8] were more intriguing, since epimerization of the C(16a)-stereocenter seemed to take place independently to the *cis*- or *trans*-relationship between H(7) and H(16a)-protons.[6]

In order to explore the effect of the C(10)-methyl substituent we study here the fate of the tryptophan stereocenter in the reaction sequence to C(10)-unsubstituted analogs **18** (Scheme 1).



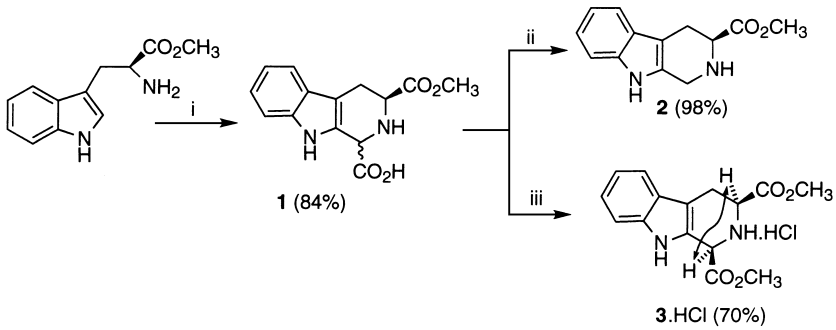
Compound	R'	H(1) - H(3)relationship in **1-4**
1	COOH	*cis/trans*
2	H	—
3	$COOCH_3$	*cis*
4	C_6H_{11}	*trans*

Scheme 1.

On the other hand, since most of compounds **D** so far studied derived from a tetrahydro-β-carboline with a 1,3-*cis*-stereochemistry,[8] we investigate the chemical behavior of tetracycles **13** derived from a β-carboline with a 1,3-t*rans*-relationship **4**. Finally, we also study the regioselectivity of the cyclization in dipeptide anhydrides derived from the tetrahydro-β-carboline **3**, to give linear **11** or angular-fused tetracycles **12**.

2. Results and discussion

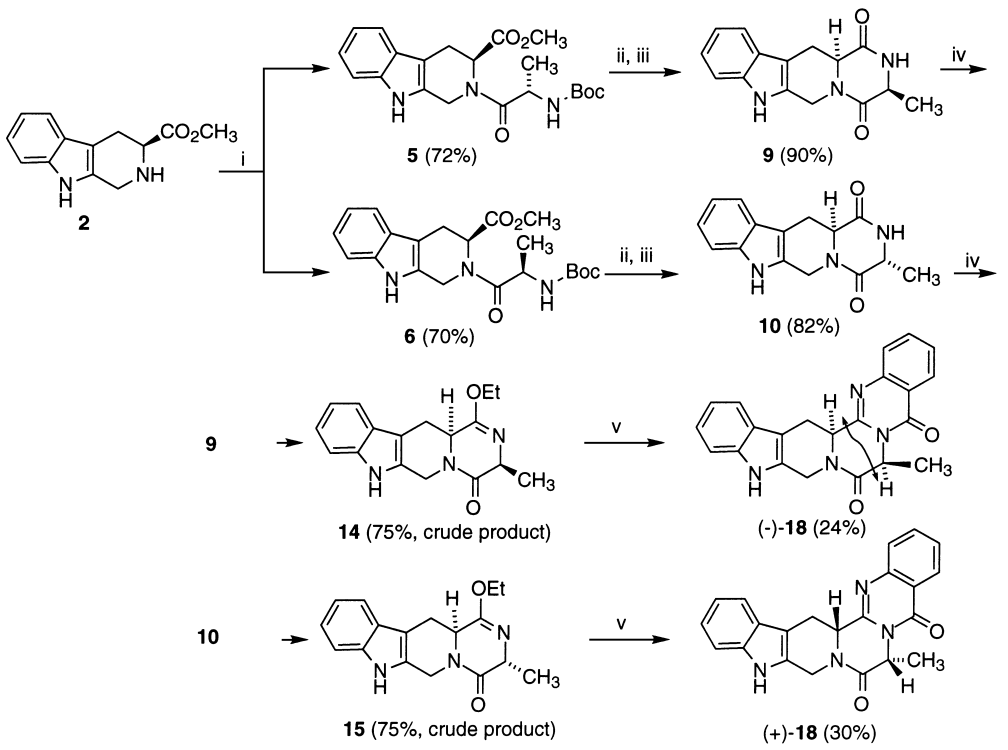
A modification of the Pictet–Spengler reaction between L-tryptophan methyl ester and glyoxylic acid gave 1[9] (Scheme 2). Compounds **2** or **3** resulted from subsequent decarboxylation of **1** in refluxing xylene or esterification in dry methanol–thionyl chloride. The expected H(1)–H(3) *cis*-relationship of **1** was confirmed by NOE experiments in compound **3**·HCl.



Scheme 2. Reagents and conditions: (i) HOC-CO_2H, EtOAc, rt, 16 h; (ii) xylene, 200°C, 2 h; (iii) dry MeOH, $SOCl_2$, rt, 48 h, Et_2O

Treatment of **2** with *N*-Boc-L- or D-alanine using EDC [1-ethyl-3-[3′-(dimethylamino)-propyl]carbodiimide] as the coupling reagent gave compounds **5** or **6** which, after *N*-deprotection by acid, afforded **9** or **10**, respectively (Scheme 3). Unlike 6-methyl analogs **B**, which showed clearly distinguished by ^{1}H NMR both *s*-*E* and *s*-*Z* rotamers at room temperature,[8] peptides **5** and **6** showed a single NH signal because of their side chain free rotation. The crude iminoethers **14** and **15** derived from **9** and **10** were condensed with anthranilic acid to give enantiomers (−)-**18** and (+)-**18**, respectively, as single reaction products. To check the possible existence of the minor diastereomers in the crude reaction mixtures, the $CHCl_3$ extracts were submitted to a fast column-chromatography. The excess of anthranilic acid was first eliminated with hexane, a mixture of the condensated products **18** and the starting iminoethers was later eluted by using chloroform–ethyl acetate as solvents. The piperazinediones, formed by partial hydrolysis of iminoethers, were finally recovered by eluting with methanol. The weight of the two latter fractions roughly corresponded to that of the reagents. The yields of these cyclizations are in the range of those previously observed with other substrates.[6]

The retention of the tryptophan stereocenter configuration in the condensation of **14** was confirmed by NOE experiments, while its epimerization in the condensation of **15** is in accordance to the physicochemical properties of the products, which are identical except for the specific rotation values, which are opposite.

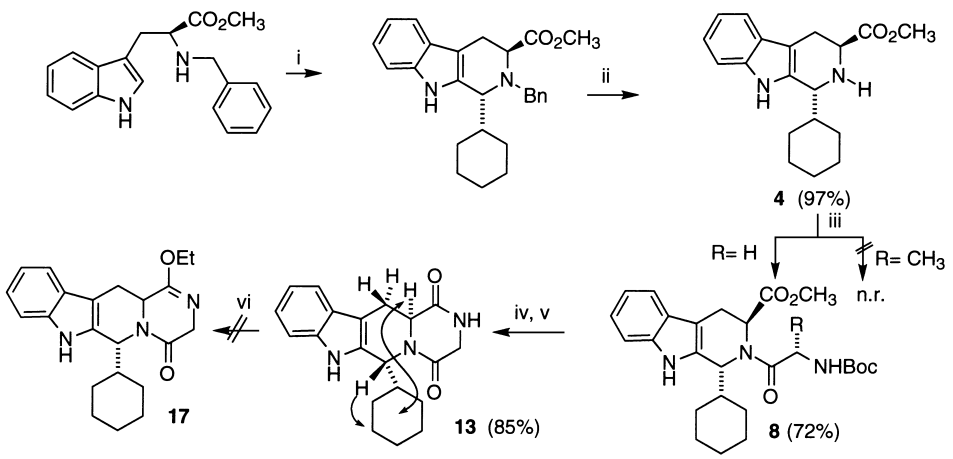


Scheme 3. Reagents and conditions: (i) Boc-L- or D-Ala, EDC/CH_2Cl_2, 16 h, rt; (ii) F_3CCO_2H/CH_2Cl_2, rt, 4 h; (iii) 10% NH_4OH; (iv) $Et_3O^+F_4B^-/CH_2Cl_2$, Na_2CO_3, rt, 16 h;(v) anthranilic acid, 130°C, 2 h

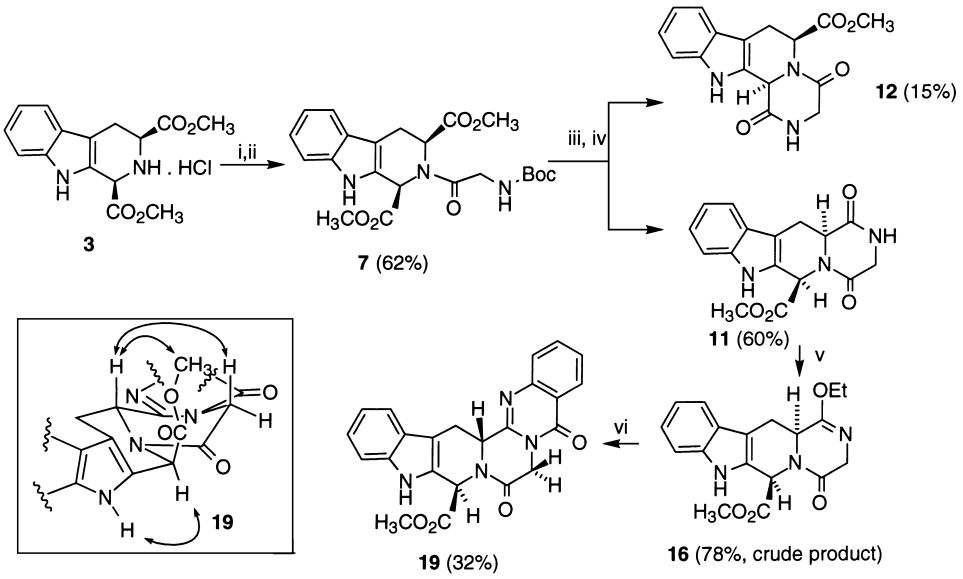
These results clearly show that the H(7)–H(16a) *cis*-relationship is thermodynamically preferred, which is in agreement with its calculated lower heat of formation [54.0 Kcal/mol for (+)-**18** and 55.6 Kcal/mol for its 16a-epimer][10] and with the results found in 1,2,4-trisubstituted pyrazino[2,1-*b*]quinazoline-3,6-diones.[11]

The tetrahydro-β-carboline **4**, with the 1,3-stereocenters in a *trans*-relationship, was obtained quantitatively by reductive deprotection of its previously described *N*-benzyl derivative, which is the product of a Pictet–Spengler reaction between methyl *N*-benzyltryptophanate and cyclohexanecarbaldehyde.[12] Steric interactions did not permit the subsequent *N*-acylation of **4** with Boc-protected L-Ala, but the reaction worked with Boc-Gly to give **8**, which after deprotection cyclized to **13**. Its stereochemistry was confirmed by NOE experiments, but the insolubility of this tetracyclic piperazinedione precluded its activation to the required iminoether **17** and its acylation with *o*-azidobenzoyl chloride (Scheme 4).

As in the case of β-carboline **4**, compound **3** could only be acylated with the less hindered *N*-Boc-α-amino acid, to give **7** in moderate yield (Scheme 4). The subsequent deprotection regioselectively gave the cyclized product **11**, being the angular compound **12** much less favored. Conversion of **11** into the iminoether **16**, followed by condensation with anthranilic acid, gave hexacycle **19** isolated as the only reaction product using the methodology described for compounds **18** (Scheme 5).



Scheme 4. Reagents and conditions: (i) $C_6H_{11}CHO$/benzene, reflux, 18 h; (ii) H_2/C-Pd, MeOH, rt, 1.5 h; (iii) Boc-L-Ala or Boc-Gly, EDC, CH_2Cl_2, rt, 16 h; (iv) F_3CCO_2H, CH_2Cl_2, rt, 4 h; (v) 10% NH_4OH; (vi) $Et_3O^+F_4B^-$/CH_2Cl_2, Na_2CO_3, rt, 16 h



Scheme 5. Reagents and conditions: (i) K_2CO_3, Et_2O; (ii) Boc-Gly, EDC/CH_2Cl_2, 16 h, rt; (iii) F_3CCO_2H/CH_2Cl_2, rt, 4 h; (iv) 10% NH_4OH; (v) $Et_3O^+F_4B^-$/CH_2Cl_2, Na_2CO_3, rt, 16 h; (vi) anthranilic acid, 130°C, 2 h

According to NOE experiments, the H(10) and H(16a) protons in **19** are in a *trans*-relationship, since after irradiation of the H(16a) proton weak enhancements of the singlet corresponding to the OCH_3 group and of the doublet at a higher field [H(7)ax proton] were observed. This result was also in agreement with the calculated heats of formation for both possible diastereoisomers [18.1 Kcal/mol for the *trans*-isomer and 19.3 Kcal/mol for the *cis*-isomer].[10]

Although the *trans* stereochemistry could arise from epimerization of any of the two stereocenters, we conclude that epimerization occurs at the C(16a) stereocenter following ^{1}H NMR spectroscopic evidence. Thus, if we compare the chemical shift values (ppm) of the H(1) protons in **2** (3.88) with that of the H(1) proton in **3** (5.78); the H(1) protons in **5** (4.5 and 5.8 for *axial* and *equatorial*, respectively), with the H(1) proton in **7** (6.07); the H(6) protons in **9** (4.27 and 5.57 for axial and *equatorial*, respectively), with the H(6) proton in **11** (6.28); the H(6) protons in **14** (4.14 and 5.54 for *axial* and *equatorial*, respectively), with the H(6) proton in **16** (6.34); and the H(10) protons in **18** (4.26 and 5.80 for *axial* and *equatorial*, respectively), with the H(10) proton in **19** (6.54); it can be seen that a $\Delta\delta \approx 2$ ppm is maintained for the proton α to the methoxycarbonyl group in all derivatives in respect of the corresponding protons in the unsubstituted analogs. Epimerization of the C(10) stereocenter would imply a much greater chemical shift for this proton in **19**, since it would suffer the anisotropic effect of the C(8) carbonyl group. Furthermore, the observed NOE between H-16a and H-7_{ax} (H-7_{eq} is deshielded by the anisotropic effect of the neighboring C(5) carbonyl[11]) also confirms the epimerization at C(16a).

From these results we conclude that in C(10)-unsubstituted hexacycles the *cis*-relationship between H(7) and H(16a)-protons is preferred, while a *trans*-relationship between H(10) and H(16a)-protons is favored in the C(7)-unsubstituted compounds.

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. Reactions were monitored by thin layer chromatography, on aluminum plates coated with silica gel with a fluorescent indicator (Macherey–Nagel Alugram Sil G/UV_{254}). 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 a Perkin–Elmer Paragon 1000 FT-IR spectrophotometer, 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 or 300 MHz for ^{1}H, 63 MHz for ^{13}C), with $CDCl_3$ or DMSO-d_6 as solvents (Servicio de Espectroscopía, Universidad Complutense). When necessary, assignments were aided by DEPT, COSY and ^{13}C–^{1}H correlation experiments. Exchangeable assignments are marked with the symbol (*). 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. (1*R,*3*S*)-3-Methoxycarbonyl-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid* **1**

To a stirrred solution of methyl thryptophanate (4 g, 18.35 mmol) in 20 ml of ethyl acetate were added 1.687 g (18.35 mmol) of glyoxalic acid, and the mixture was maintained at room temperature over 16 h. The precipitate formed was filtered and washed with ethyl acetate to give 4.02 g (84%) of **1**.

Data for **1**: mp: 143–144°C. IR (KBr): 3390 (NH), 2694 (NH), 1634 (COOH) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 10.68 (br s, 1H, H-9), 7.40 (m, 2H, H-5 and H-8), 6.98 (m, 2H, H-6 and H-7), 4.80 (m, 1H, H-1), 4.27 (m, 1H, H-3), 3.77 (s, 3H, OCH_3), 3.02 (m, 2H, H-4*ax* and *eq*) ppm. ^{13}C NMR (DMSO-d_6) δ: 174.1 ($COOCH_3$), 166.8 ($COOH$), 135.9 (C-8a), 129.2 (C-9a), 125.9 (C-4b), 120.8 (C-7), 118.1 (C-6), 117.3 (C-5), 111.7 (C-8), 104.5 (C-4a), 62.5 (C-1), 59.7 (C-3), 55.4 ($COOCH_3$), 18.5 (C-4) ppm. Anal. calcd for $C_{14}H_{14}N_2O_4$: C, 61.31; H, 5.14; N, 10.21. Found: C, 61.58; H, 5.19; N, 10.05.

3.2. Methyl (3S)-1,2,3,4-tetrahydro-β-carboline-3-carboxylate **2**

A solution of 2.2 g (8.02 mmol) of **1** in 20 ml of xilene, was heated at 200°C over 2 h. The organic layer was evaporated to give 1.8 g (98%) of **2**.

Data for **2**: mp: 99–100°C. IR (KBr): 3310 (NH), 2800 (NH), 1728 ($COOCH_3$) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 8.18 (s, 1H, H-9), 7.1–7.6 (m, 4H, H-5 to 8), 3.88 (m, 2H, H-1), 3.74 (m, 1H, H-3), 3.48 (s, 3H, OCH_3), 3.0–3.2 (m, 2H, H-4*ax* and *eq*) ppm. ^{13}C NMR (Cl3CD) δ: 175.9 ($COOCH_3$), 136.0 (C-8a), 132.2 (C-9a), 127.0 (C-4b), 121.8 (C-7), 119.6 (C-6), 117.9 (C-5), 110.9 (C-8), 107.5 (C-4a), 56.0 (C-3), 52.3 ($COOCH_3$), 42.2 (C-1), 25.5 (C-4) ppm. Anal. calcd for $C_{13}H_{14}N_2O_2$: C, 67.79; H, 6.13; N, 12.17. Found: C, 68.08; H, 6.29; N, 11.95.

3.3. Dimethyl (1R,3S)-1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylate·HCl **3**

To a cooled (0°C), stirred solution of **1** (1 g, 3.64 mmol) in dry methanol was dropwise added thionyl chloride 0.62 ml (8.5 mmol) over 30 min and the stirring was continued at room temperature up to 48 h. To the reaction mixture was added 3 ml of ethyl eter and the precipitated crystals were filtered to give 0.82 g (70%) of **3**·HCl.

Data for **3**: mp: 120–121°C. IR (KBr): 3638 (NH), 1741 and 1627 (2 $COOCH_3$) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 11.11 (s, 1H, H-9), 7.48 (m, 2H, H-5 and H-8), 7.15 (t, 1H, H-6, $J=7$ Hz), 7.04 (t, 1H, H-7, $J=7$ Hz), 5.78 (s, 1H, H-1), 4.74 (dd, 1H, H-3, $J=5$ and 11 Hz), 3.94 and 3.85 (2s, 6H, (C-1)-$COOCH_3$ and (C-3)-$COOCH_3$), 3.28 (dd, 1H, H-4*eq*, $J=5$ and 16 Hz), 3.15 (m, 1H, H-4*ax*) ppm.^{13}C NMR (DMSO-d_6) δ: 170.3 and 167.8 (2$COOMe$)*, 143.2 (C-8a), 138.6 (C-9a), 127.1 (C-4b), 124.5 (C-7), 121.3 (C-6), 120.0 (C-5), 113.8 (C-8), 108.1 (C-4a), 82.5 (C-1), 56.3 (C-3), 55.6 and 55.0 (OCH_3)*, 23.5 (C-4) ppm. Anal. calcd for $C_{15}H_{17}N_2O_4Cl$: C, 55.48; H, 5.38; N, 8.63. Found: C, 55.75; H, 5.41; N, 8.55.

3.4. N-Acylation of tetrahydro-β-carbolines. General Method. Synthesis of compounds **5–8**

N-Boc-glycine, L- or D-alanine (5.32 mmol) and EDC (4.04 mmol) were added to the corresponding tetrahydro-β-carboline (2.7 mmol). The reaction was kept in the dark with an anhydrous calcium chloride tube and was magnetically stirred for 24 h. After concentration to dryness at low pressure, the residue was extracted with a mixture of chloroform (15 ml) and 1N hydrochloric acid (16 ml). The separated organic phase was washed with 1N sodium bicarbonate (15 ml), dried and concentrated. Purification of the residue by column chromatography with dichloromethane as solvent, gave compounds **5–8**.

*3.4.1. Methyl (3*S,*2′*S*)-1,2,3,4-tetrahydro-2-*N′-tert-*butoxycarbonylalanyl-β-carboline-3-carboxylate* ***5***

Starting from **2** (0.62 g, 2.7 mmol) dissolved in dichloromethane (1 ml) Boc-L-alanine (1 g, 5.32 ml) and EDC (0.776 g, 4.04 mmol) a yield of 0.77 g (72%) of **5** was obtained.

Data for **5**: mp: 267–268°C. IR (KBr): 3326 (NH), 1742 ($COOCH_3$), 1699 (CONH), 1651 (OCONH) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 8.94 (s, 1H, H-9), 7.48 (d, 1H, H-8, $J=7$ Hz), 7.37 (d, 1H, H-5, $J=7$ Hz), 7.12 (m, 2H, H-6 and 7), 5.85 (d, 1H, N*H*, $J=5.5$ Hz), 5.18 (d, 1H, $J=16.9$ Hz, H-1*eq*), 4.92 (m, 2H, H-3 and H-2′), 4.5 (d, 1H, H-1*ax*, $J=16.8$ Hz), 3.52 (s, 3H, OCH_3), 3.38 (m, 1H, H-4*eq*), 2.82 (m, 1H, H-4*ax*) 1.45 (m, 12H, $C(CH_3)_3$ and (C-2′)-CH_3) ppm. ^{13}C NMR ($CDCl_3$) δ: 173.9 and 169.5 (*C*OOMe and C-1′)*, 154.8 (NH-*C*OO), 134.5 (C-8a), 129.6 (C-9a), 126.4 (C-4b), 122.1 (C-6), 119.7 (C-7), 118.8 (C-5), 111.6 (C-8), 105.1 (C-4a), 82.8 ($C(CH_3)_3$), 52.5 (OCH_3), 51.0 (C-2′), 48.0 (C-3), 40.3 (C-1), 28.2 ($C(CH_3)_3$), 22.6 (C-4), 15.5 (C-2′-CH_3) ppm. Anal. calcd for $C_{21}H_{27}N_3O_5$: C, 62.83; H, 6.78; N, 10.47. Found: C, 62.90; H, 6.82; N, 10.26.

*3.4.2. Methyl (3*S,*2′*R*)-1,2,3,4-tetrahydro- 2-*N′-tert-*butoxycarbonylalanyl-β-carboline-3-carboxylate* ***6***

Starting from **2** (1 g, 4.30 mmol) dissolved in dichloromethane (1 ml) Boc-D-alanine (1.61 g, 8.58 ml) and EDC (1.258 g, 6.5 mmol) a yield of 1.21 g (70%) of **6** was obtained.

Data for **6**: mp: 258–259°C. IR (KBr): 3340 (NH), 1757 ($COOCH_3$), 1667 (CONH), 1647 (OCONH) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 9.33 (9.12) (s, 1H, H-9), 7.46 (m, 1H, H-6), 7.30 (d, 1H, H-5, $J=7.5$ Hz), 7.10 (m, 2H, H-6 and 7), 5.78 (m, 1H, N*H*), 5.30 (d, 1H, H-1, $J=16.9$ Hz), 5.20 (m, 1H, H-3), 4.45 (d, 1H, H-1, $J=16.9$ Hz), 4.73 (m, 1H, (C-2′)-C*H*), 3.54 (s, 3H, OCH_3), 3.44 (m, 1H, H-3*eq*), 3.02 (m, 1H, H-3*ax*), 1.46 (s, 9H, $C(CH_3)_3$), 1.35 (d, 3H, (C-2′)-CH_3, $J=6.7$ Hz) ppm. ^{13}C NMR ($CDCl_3$) δ: 174.0 and 171.4 (COOMe and C-1′)*, 155.4 (NH-COO), 136.7 (C-8a), 129.4 (C-9a), 126.6 (C-4b), 122.1 (C-6), 119.5 (C-7), 118.2 (C-5), 111.2 (C-8), 106.2 (C-4a), 78.0 ($C(CH_3)_3$), 53.0 (OCH_3), 51.4 (C-2′), 47.2 (C-3), 41.5 (C-1), 28.5 ($C(CH_3)_3$), 23.0 (C-4) 16.0 (C-2′-CH_3) ppm. Anal. calcd for $C_{21}H_{27}N_3O_5$: C, 62.83; H, 6.78; N, 10.47. Found: C, 62.97; H, 6.86; N, 10.39.

*3.4.3. Dimethyl (1*R,*3*S*)-1,2,3,4-tetrahydro-2-N′-*tert-*butoxycarbonylglycinyl-β-carboline-1, 3-dicarboxylate* ***7***

From a solution of compound **3** (0.32 g, 0.98 mmol), liberated and extracted from **3**·HCl by treatment with aqueous K_2CO_3 in Et_2O, in dichloromethane (1 ml), Boc-glycine (0.34 g, 1.94 mmol) and EDC (0.281 g, 1.47 mmol) a yield of 0.27 g (62%) of **7** was obtained.

Data for **7**: mp: 204–206°C. IR (KBr): 3320 (NH), 1750 ($COOCH_3$), 1690 (CONH), 1647 (OCONH) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 8.62 (s, 1H, H-9), 7.43 (d, 1H, H-8, $J=7.5$ Hz), 7.36 (d, 1H, H-5, $J=7.5$ Hz), 7.11 (m, 2H, H-6 and 7), 6.07 (s, 1H, H-1), 5.99 (m, 1H, H-3), 5.57 (m, 1H, (C-2′)-N*H*), 4.62 (m, 2H, (C-2′)-CH_2), 3.70 and 3.62 (2s, 6H, (C-3)-COOCH_3 and (C-1)-COOCH_3)*, 3.20 (m, 2H, H-4), 1.41 (s, 9H, $C(CH_3)_3$) ppm. ^{13}C NMR ($CDCl_3$) δ: 173.1 and 172.2 (C-3)-*C*OOCH_3 and (C-1)-*C*OOCH_3)*, 169.7 (C-1′), 156.1 (NH-*C*OO), 136.6 (C-8a), 131.2 (C-9a), 126.10 (C-4b), 122.8 (C-6), 119.9 (C-7), 118.6 (C-5), 111.4 (C-8), 106.4 (C-4a), 84.3 ($C(CH_3)_3$), 53.2 y 52.4 (C-1 and C-3)*, 53.1 and 52.7 ((C-1)-COOCH_3 and (C-3)-COOCH_3)*, 45.3 (C-2′), 28.4 ($C(CH_3)_3$), 23.0 (C-4) ppm. Anal. calcd for $C_{22}H_{27}N_3O_7$: C, 59.32; H, 6.11; N, 9.43. Found: C, 59.96; H, 6.45; N, 9.21.

*3.4.4. Methyl (1*R,*3*S*)-1-cyclohexyl-1,2,3,4-tetrahydro-2-*N′-tert-*butoxycarbonylglycinyl-β-carboline-3-carboxylate* ***8***

Starting from **4** (0.5 g, 1.6 mmol) dissolved in dichloromethane (1 ml) Boc-glycine (0.554 g, 3.16 mmol) and EDC (0.460 g, 2.4 mmol) a yield of 0.54 g (72%) of **8** was obtained.

Data for **8**: mp: 267-268°C. IR (KBr): 3326 (NH), 1742 ($COOCH_3$), 1699 (CONH), 1651 (OCONH) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 8.13 (s, 1H, H-9), 7.47 (d, 1H, H-8, $J=7.4$ Hz), 7.3 (d, 1H, H-5, $J=7.4$ Hz), 7.12 (m, 2H, H-6 and 7), 5.46 (m, 1H, H-3), 5.19 (s, 1H, (C-2′)-N*H*), 4.25 (d, 1H, H-1, $J=5$ Hz), 4.01 (m, 1H, H-4*eq*), 3.76 (s, 3H, OCH_3), 3.24 (m, 3H, H-4*ax* and (C-2′)-CH_2), 1.38 (s, 9H, $C(CH_3)_3$), 1–2 (m, 11H, C_6H_{11}) ppm. ^{13}C NMR ($CDCl_3$) δ: 172.1 (C-3)-$COOCH_3$, 170.6 (C-1′), 156.0 (NH-*C*OO), 136.2 (C-8a), 132.8 (C-9a), 126.3 (C-4b), 122.1 (C-6), 119.8 (C-7), 118.3 (C-5), 111.3 (C-8), 108.5 (C-4a), 80.0 ($C(CH_3)_3$), 55.6 (C-3), 52.3 ((C-3)-COOCH_3), 42.8 (C-1), 42.5 (C-2′), 30.4 and 30.0 (C-1″, C-2″ and C-6″)*, 29.7 ($C(CH_3)_3$), 26.6, 26.4, y 26.4 (C3″-5″)*, 22.4 (C-4) ppm. Anal. calcd for $C_{26}H_{35}N_3O_5$: C, 66.50; H, 7.51; N, 8.95. Found: C, 66.90; H, 7.87; N, 8.44.

3.5. General Method. Synthesis of compounds ***9–13***

A solution (2.49 mmol) of the corresponding *N*-aminoacyl derivative in a 1/2 mixture of trifluoroacetic acid/dichloromethane, was magnetically stirred for 4h at rt. After neutralization with 10% ammonium hydroxide, the dichloromethane extracts were dried under anhydrous sodium sulfate, filtered and evaporated, to give a residue that, after column chromatography on silica gel (chloroform), gave the corresponding piperazinedione.

*3.5.1. (3*S,*12a*S*)-3-Methyl-2,3,6,7,12,12a-hexahydropyrazino[1,2-*b*]β-carboline-1,4-dione* ***9***

Starting from **5** (1 g, 2.49 mmol) dissolved in a 1/2 mixture of trifluoroacetic acid/dichloromethane (4 ml) a yield of 0.6 g (90%) of **9** was obtained.

Data for **9**: mp: 205–206°C. IR (KBr): 3388 (NH), 1649 and 1625 (CONH) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 8.37 (s, 1H, H-9), 7.47 (d, 1H, H-8, $J=7.4$ Hz), 7.32 (d, 1H, H-11, $J=7.4$ Hz), 7.12 (m, 2H, H-9 and 10), 6.42 (s, 1H, H-2), 5.57 (m, 1H, H-6*eq*), 4.27 (m, 3H, H-3, H-12a, and H-6*ax*), 3.5 (dd, 1H, H-12*eq*, $J=2.5$ and 15 Hz), 2.94 (m, 1H, H-12*ax*), 1.54 (d, 3H, (C-3)-CH_3, $J=7$ Hz) ppm. ^{13}C NMR ($CDCl_3$) δ: 167.1 (C-4), 165.8 (C-1), 136.0 (C-11a), 128.4 and 126.3 (C-7a and C-11b)*, 122.3 (C-10), 120.0 (C-9), 118.0 (C-8), 110.8 (C-11), 106.9 (C-6a), 54.5 (C-12a), 51.4 (C-3), 40.2 (C-6), 27.4 (C-12), 22.7 ((C-3)-CH_3) ppm. Anal. calcd for $C_{15}H_{15}N_3O_2$: C, 66.90; H, 5.61; N, 15.60. Found: C, 66.97; H, 5.72; N, 15.41.

*3.5.2. (3*R,*12a*S*)-3-Methyl-2,3,6,7,12,12a-hexahydropyrazino[1,2-*b*]β-carboline-1,4-dione* ***10***

Starting from **6** (0.5 g, 1.24 mmol) dissolved in a 1/2 mixture of trifluoroacetic acid/dichloromethane (1 ml) a yield of 0.274 g (82%) of **10** was obtained.

Data for **10**: mp: 217–218°C. IR (KBr): 3326 (NH), 1657 and 1620 (CONH) cm^{-1}. H NMR ($CDCl_3$) δ: 8.72 (s, 1H, H-9), 7.5 (d, 1H, H-8, $J=7.5$ Hz), 7.44 (d, 1H, H-11, $J=7.4$ Hz), 7.12 (m, 2H, H-9 and H-10), 6.59 (s, 1H, H-2), 5.59 (d, 1H, H-6*eq*, $J=16.5$ Hz), 4.14 (m, 3H, H-3, H-12a, and H-6*ax*), 3.42 (dd, 1H, H-12*eq*, $J=3.2$ and 14.2 Hz), 2.91 (m, 1H, H-12*ax*), 1.57 (d, 3H, (C-3)-CH_3, $J=7$ Hz) ppm. ^{13}C-NMR ($CDCl_3$) δ: 167.6 (C-4), 166.0 (C-1), 136.0 (C-11a), 128.6 and 126.3 (C-7a and C-11b)*, 122.1 (C-10), 119.8 (C-9), 117.9 (C-8), 111.0 (C-11), 106.8 (C-6a), 57.0 (C-12a), 50.5 (C-3), 40.4 (C-6), 26.8 (C-12), 20.1 ((C-3)-CH_3) ppm. Anal. calcd for $C_{15}H_{15}N_3O_2$: C, 66.90; H, 5.61; N, 15.60. Found: C, 67.02; H, 5.87; N, 15.97.

*3.5.3. Methyl (6*R,*12a*S*)-1,4-dioxo-2,3,6,7,12,12a-hexahydropyrazino[1,2-*a*]β-carboline-6-carboxylate* ***11***

Starting from **7** (1 g, 2.24 mmol) dissolved in a 1/2 mixture of trifluoroacetic acid/dichloromethane (2 ml) a yield of 0.42 g (60%) of **11** was obtained.

Data for **11**: mp: 210–211°C. IR (KBr): 3252 (NH), 1731 ($COOCH_3$) 1687 and 1658 (CONH) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 8.49 (s, 1H, H-9), 7.49 (d, 1H, $J=7.7$ Hz, H-8), 7.37 (d, 1H, $J=7.7$ Hz, H-11), 7.31 (s, 1H, H-2), 7.15 (m, 2H, H-9 and H-10), 6, 28 (s, 1H, H-6), 4.51 (dd, 1H, $J=3.9$ and 11.68 Hz, H-12a), 4.2 (m, 2H, H-3), 3.81 (s, 3H, OCH_3), 3.52 (dd, 1H, $J=4.1$ and 15.8 Hz, H-12*eq*), 2.95 (m, 1H, H-12*ax*). ^{13}C NMR ($CDCl_3$) δ: 168.3 ($COOCH_3$), 167.5 (C-4), 163.4 (C-1), 136.43 (C-11a), 126.1 (C-7a), 124.7 (C-11b), 123.2 (C-10), 120.3 (C-9), 118.7 (C-8), 11.4 (C-11), 108.6 (C-6a), 54.8 (C-12a), 53.4 ($COOCH_3$), 44.8 (C-3), 26.7 (C-12). Anal. calcd for $C_{16}H_{15}N_3O_4$: C, 61.34; H, 4.83; N, 13.41. Found: C, 61.47; H, 4.99; N, 13.17.

*3.5.4. Methyl (6*S,*12b*R*)-1,4-dioxo-2,3,6,7,12,12b-hexahydropyrazino[2,1-*a*]β-carboline-6-carboxylate* ***12***

Starting from **7** (1 g, 2.24 mmol) dissolved in a 1/2 mixture of trifluoroacetic acid/dichloromethane (2 ml) a yield of 0.105 g (15%) of **12** was obtained.

Data for **12**: mp: 104–105°C. IR (KBr): 3344 (NH), 1739 ($COOCH_3$), 1683 and 1662 (CONH) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 9.24 (s, 1H, H-12), 7.49 (d, 1H, H-11, $J=7.9$ Hz), 7.36 (d, 1H, H-8, $J=7.9$ Hz), 7.16 (m, 2H, H-9 and H-10), 6.49 (s, 1H, H-2), 5.92 (d, 1H, H-6, $J=6.3$ Hz), 5.77 (s, 1H, H-12b), 4.11 (m, 2H, H-3), 3.65 (s, 3H, OCH_3), 3.45 (d, 1H, H-7*eq*, $J=16$ Hz), 3.16 (dd, 1H, H-7*ax*, $J=4.7$ and 14.0 Hz) ppm. ^{13}C NMR ($CDCl_3$) δ: 170.4 ($COOCH_3$), 166.0 and 163.8 (C-1 and C-4), 136.5 (C-7b), 126.1 (C-7a), 125.7 and 123.2 (C-11a and C-7a)*, 122.1 (C-9), 119.6 (C-10), 118.3 (C-11), 111.4 (C-8), 107.1 (C-12a), 52.8 ($COOCH_3$), 52.3 (C-6), 50.9 (C-12b), 45.0 (C-3), 22.2 (C-7) ppm. Anal. calcd for $C_{16}H_{15}N_3O_4$: C, 61.34; H, 4.83; N, 13.41. Found: C, 61.48; H, 5.07; N, 13.27.

*3.5.5. (6*R,*12a*S*)-6-Cyclohexyl-2,3,6,7,12,12a-hexahydropyrazino[1,2-*b*]β-carboline-1,4-dione* ***13***

Starting from **8** (1 g, 2.2 mmol) dissolved in a 1/2 mixture of trifluoroacetic acid/dichloromethane (2 ml) a yield of 0.632 g (85%) of **13** was obtained.

Data for **13**: mp: 334–335°C. IR (KBr): 3284 (NH), 1697 and 1644 (CONH) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 10.88 (s, 1H, H-9), 8.30 (s, 1H, H-2), 7.42 (d, 1H, H-8, $J=7.6$ Hz), 7.32 (d, 1H, H-11, $J=7.9$ Hz), 7.02 (m, 2H, H-9 and H-10), 4.37 (d, 1H, H-6, $J=8.4$ Hz), 4.30 (dd, 1H, H-12a, $J=4.5$ and 11.7 Hz), 4.06 (d, 1H, H-3*eq*, $J=17.7$ Hz), 3.85 (dd, 1H, H-3*ax*, $J=2.9$ and 17.7 Hz), 3.14 (dd, 1H, H-12*eq*, $J=4.6$ and 15.4 Hz), 2.88 (dd, 1H, H-12*ax*, $J=15.4$ and 11.7 Hz), 1-2 (m, 11H, C_6H_{11}) ppm. ^{13}C NMR (DMSO-d_6) δ: 168.7 (C-4), 165.3 (C-1), 137.7 (C-11a), 134.5 (C-7a), 127.8 (C-11b), 123.0 (C-10), 121.0 (C-9), 119.6 (C-8), 112.9 (C-11), 107.5 (C-6a), 55.0 (C-12a), 45.7 (C-3), 42.3 (C-6), 31.8 and 31.2 (C-1′, C-2′, and C-6′)*, 28.0, 27.5, and 27.3 (C-12, C-3′, C-4′, C-5′)* ppm. Anal. calcd for $C_{15}H_{15}N_3O_2$: C, 71.19; H, 6.87; N, 12.45. Found: C, 71.42; H, 6.97; N, 12.27.

3.6. Activation of piperazinediones. General Method. Synthesis of iminoethers ***14–16***

To a solution of the starting piperazinedione (0.34 mmol) and triethyloxonium tetrafluoroborate (3 equiv.) in dry dichloromethane (10 ml) was added anhydrous sodiun carbonate (5 equiv.). The suspension was stirred at room temperature under a stream of argon for 16 h,

and was then poured onto ice water. The combined organic layers were dried over sodium sulphate and evaporated under reduced pressure. The residue was used in the next reaction without further purification.

Data for **14**: ^{1}H NMR ($CDCl_3$) δ: 8.66 (s, 1H, H-7), 7.41 (d, 1H, H-8, $J=7$ Hz), 7.3 (d, 1H, H-11, $J=7$ Hz), 7.10 (m, 2H, H-9 and 10), 5.54 (d, 1H, H-6*eq*, $J=16.5$ Hz), 4.14 (m, 5H, H-3, H-12a, H-6*ax* and OCH_2CH_3), 3.07 (dd, 1H, H-12*eq*, $J=6.7$ and 19.5 Hz), 2.75 (m, 1H, H-12*ax*), 1.47 (d, 3H, (C-3)-CH_3, $J=7.2$ Hz), 1.35 (t, 3H, OCH_2CH3, $J=7$ Hz) ppm.

Data for **15**: ^{1}H NMR ($CDCl_3$) δ: 8.74 (s, 1H, H-7), 7.44 (d, 1H, H-8, $J=7.5$ Hz), 7.29 (d, 1H, H-11, $J=7.5$ Hz), 7.10 (m, 2H, H-9 and H-10), 5.5 (d, 1H, H-6*eq*, $J=16.5$ Hz), 4.2 (m, 5H, H-3, H-12a, H-6*ax*, and OCH_2CH_3), 3.28 (dd, 1H, H-12*eq*, $J=4.0$ and 14.8 Hz), 2.78 (m, 1H, H-12*ax*), 1.02 (d, 3H, (C-3)-CH_3, $J=7$ Hz), 1.02 (t, 3H, OCH_2CH3, $J=7$ Hz) ppm.

Data for **16**: ^{1}H NMR ($CDCl_3$) δ: 8,69 (s, 1H, H-7), 7.5 (d, 1H, H-8, $J=7.7$ Hz), 7.34 (d, 1H, H-11, $J=7.7$ Hz), 7.15 (m, 2H, H-9 and H-10), 6, 34 (s, 1H, H-6), 4.47 (m, 1H, H-12a), 4.25 (m, 2H, H-3), 4.17 (q, 2H, OCH_2CH_3, $J=7$ Hz), 3.78 (s, 3H, OCH_3), 3.38 (dd, 1H, H-12*eq*, $J=4.0$ and 15.5 Hz), 2.81 (dd, 1H, H-12*ax*, $J=15.5$ and 12.0 Hz), 1.35 (t, 3H, OCH_2CH_3, $J=7$ Hz) ppm.

*3.7. Synthesis of 7,10,16,16a-tetrahydroquinazolino[2′,3′:3,4]pyrazino[1,2-*b*]β-carboline-5,8-diones. General method. Synthesis of (+)-**18**, (−)-**18**, and **19***

A mixture of the corresponding iminoether (0.88 mmol) and anthranilic acid (3 equiv.) was melted at 130°C under stream of argon for 2 h. The melt was cooled and triturated with 20% aqueous ammonium hydroxide (2 ml), and the mixture was extracted with chloroform. The combined organic layers were dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was chromatographed on silica gel as previously described, and the final purification was performed by a second column chromatography eluting with dichloromethane.

Starting from **14** (0.1 g, 0.33 mmol) and anthranilic acid (0.138 g, 1.01 mmol) a yield of 0.03 g (24%) of (−)-**18** was obtained.

Data for (−)-**18**: mp: 235–236°C. IR (KBr): 3297 (NH), 1660 and 1628 (CONH) and 1622 (C=N) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.35 (s, 1H, H-11), 8.29 (d, 1H, H-4, $J=7$ Hz), 7.79 (t, 1H, H-2, $J=7$ Hz), 7.7 (d, 2H, H-1, $J=7$ Hz), 7.54 (m, 2H, H-3 and 15), 7.37 (d, 1H, H-12, $J=7.5$ Hz), 7.20 (m, 2H, H-13 and 14), 5.8 (d, 1H, H-10*eq*, $J=16.2$ Hz), 5.47 (q, 1H, H-7, $J=7$ Hz), 5.0 (dd, 1H, H-16a, $J=11.8$ and 4.2 Hz), 4.26 (d, 1H, H-10*ax*, $J=16.3$ Hz), 3.67 (dd, 1H, H-16*eq*, $J=4.9$ and 11.8 Hz), 3.18 (m, 1H, H-16*ax*), 1.64 (d, 3H, (C-7)-CH_3, $J=7$ Hz) ppm. ^{13}C NMR ($CDCl_3$) δ: 166.4 y 160.8 (C-8 and C-$_5$)*, 150.1 (C-16b), 147.5 and 136.3 (C-17a and C-4a)*, 135.0 (C-4), 132.5 (C-15a), 129.1 (C-15b), 127.3 (C-1), 127.1 (C-2), 127.0 (C-3), 126.6 (C-11a), 122.6 (C-14), 120.2 (C-13), 118.11 (C-12), 111.3 (C-15), 107.3 (C-15b), 58.3 (C-16a), 52.0 (C-7), 38.8 (C-10), 29.8 (C-16), 20.3 ((C-10)-CH_3) ppm. Anal. calcd for $C_{22}H_{18}N_4O_2$: C, 71.34; H, 4.90; N, 15.13. Found: C, 71.86; H, 5.01; N, 15.02. $[\alpha]_D^{25}$ −353 (*c* 0.07, $CHCl_3$).

Starting from **15** (0.15 g, 0.5 mmol) and anthranilic acid (0.207 g, 1.5 mmol) a yield of 0.056 g (30%) of (+)-**18** was obtained.

Data for (+)-**18**, with the exception of the specific rotation value, were identical to those of compound (−)-**18**. $[\alpha]_D^{25}$ +350 (*c* 0.07, Cl_3CH).

Starting from **16** (0.3 g, 0.88 mmol) and anthranilic acid (0.361 g, 2.64 mmol), a yield of 0.116 g (32%) of **19** was obtained.

Data for **19**: mp: 253–254°C. IR (KBr): 3347 (NH), 1735 ($COOCH_3$), 1685 and 1662 (CONH) and 1598 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 8.43 (s, 1H, H-11), 8.30 (d, 1H, H-4, $J=8$ Hz), 7.77 (m, 2H, H-2 and H-1), 7.52 (m, 2H, H-3 and H-15), 7.39 (d, 1H, H-12, $J=8$ Hz), 7.21 (m, 2H, H-13 and H-14), 6.54 (s, 1H, H-10), 5.20 (dd, 1H, H-16a, $J=4$ and 12 Hz), 4.52 and 5.10 (2d, 2H, H-7*eq* and H-7*ax*, $J=19.5$ Hz)*, 3.83 (s, 3H, OCH_3), 3.64 (dd, 1H, H-16*eq*, $J=4$ and 15.5 Hz), 3.07 (m, 1H, H-16*ax*) ppm. ^{13}C NMR ($CDCl_3$) δ: 168.2 ($COOCH_3$), 163.2 and 160.7 (C-8 and C-5)*, 149.6 (C-16b), 147.4 and 132.1 (C-17a and C-4a)*, 135.1 (C-4), 132.1 (C-15a), 125.1 (C-15b), 127.5 (C-1), 127.3 (C-2), 126.9 (C-3), 126.0 (C-11a), 123.3 (C-14), 120.4 (C-13), 118.7 (C-12), 111.4 (C-15), 108.9 (C-15b), 56.4 (C-10), 53.6 ($COOCH_3$), 52.2 (C-16a), 44.8 (C-7), 29.1 (C-16) ppm. Anal. calcd for $C_{23}H_{18}N_4O_4$: C, 66.66; H, 4.38; N, 13.52. Found: C, 66.88; H, 4.57; N, 13.11. $[\alpha]_D^{25}$ +50 (*c* 0.1, CH_3COCH_3).

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