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Synthesis of Tryptophan-dehydrobutyrine Diketopiperazines and Analogues

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Abstract

Condensation of N^1-methyl-*cyclo*-Trp-Gly and aldehydes in basic media was studied to confirm the structure of the natural product TDD (*N*-methyl-Tryptophan Dehydrobutyrine Diketopiperazine, **1**) and to prepare analogues with potential activity as GST (Glutathione-*S*-Transferase) inhibitors. This strategy was successful for 1,4-diacetyl-*cyclo*-Trp-Gly but it did not work for N^4-acetyl, N^1-methyl-*cyclo*-Trp-Gly derivatives. Pyrolytic cyclization of *N*-Boc-L-Thr-*N*-methyl-L-Trp methyl ester gave the *Z*-isomer of *N*-methyltryptophan dehydrobutyrine diketopiperazine, which was previously supposed to be the natural product. However, by comparison of melting points and pectroscopic data with those of **1**, we conclude that the proposed structure for TDD must be corrected.

Keywords: Piperazinediones, TDD, Glutathione-*S*-Transferase, Biologically active compounds.

1. Introduction

Many tumour cells become resistant to chemotherapeutic agents by lowering their intracellular active concentrations through multiple mechanisms, such as overexpression of certain membrane proteins that affect the transport of antitumour drugs [1-5], or certain enzymes that are involved in their metabolism. Among these enzymes, one of the most important is the glutathione-*S*-transferase (GST) family [6-9], which uses the abundant intracellular tripeptide glutathione to neutralize electrophilic toxins. It has been suggested that the use of selective GST inhibitors would provide tumour-directed potentiation of conventional cancer chemotherapeutic agents, because many cancers show different distributions of GST isozymes (at least eight different types of human isozymes have been identified) [10].

We became interested in the natural compound tryptophan-dehydrobutyrine diketopiperazine (TDD, **1**), which had been described without a clear assignment of the

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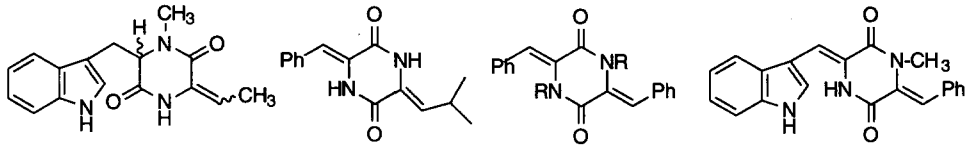
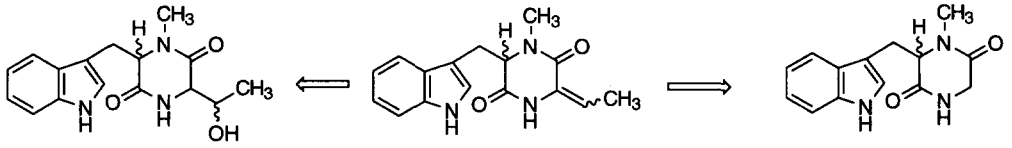
configuration of the C-2 stereocenter and the double bond [11], in connection with our current project on the synthesis of MDR (multi drug resistance) reversal agents [12-16]. Taking into account the activity of **1** as a GST-inhibitor [17], we planned the synthesis of compound **1** and their analogues. Related compounds, such as albonoursin (**2**), have shown antitumour activity. Others, such as the piperafizines A and B (**3**) or compound **4**, are resistance-reversal agents [18-20].



1 (TDD) **2** **3**, R=H, CH_3 **4**

The retrosynthetic analysis of **1**, either by condensation of N^1-methyl-*cyclo*-Trp-Gly (**5**) with acetaldehyde or by dehydration of N^1-methyl-*cyclo*-Trp-Thr (**6**, Scheme 1), seemed straightforward.

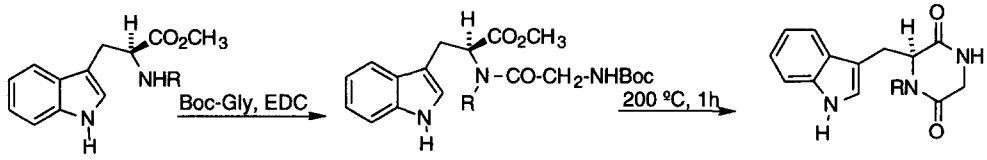


6 **1** **5**

Scheme 1

2. Results and Discussion

Studies about the first route started from L- or D-tryptophan methyl esters (*e.g.* compound **7**), which were transformed into their enantiomerically pure *N*α-methyl derivatives (*S*-**8** and *R*-**8**) [21]. Condensation of *S*-**7** or *S*-**8** with Boc-Gly, using EDC as the coupling reagent [22], gave the corresponding dipeptides **9** and **10** which, after pyrolysis, yielded the cyclodipeptides **11** and **12** in near quantitative yield (Scheme 2).



7, R = H
8, R = Me

9, R = H (88%)
10, R = Me (90%)

11, R = H, (98%)
12, R = Me (90%)

Scheme 2

^{13}C NMR chemical shifts of the carbonyl groups in these compounds clearly reflect the effect of steric interactions between the piperazinedione *C,N*-substituents. Thus, δ values

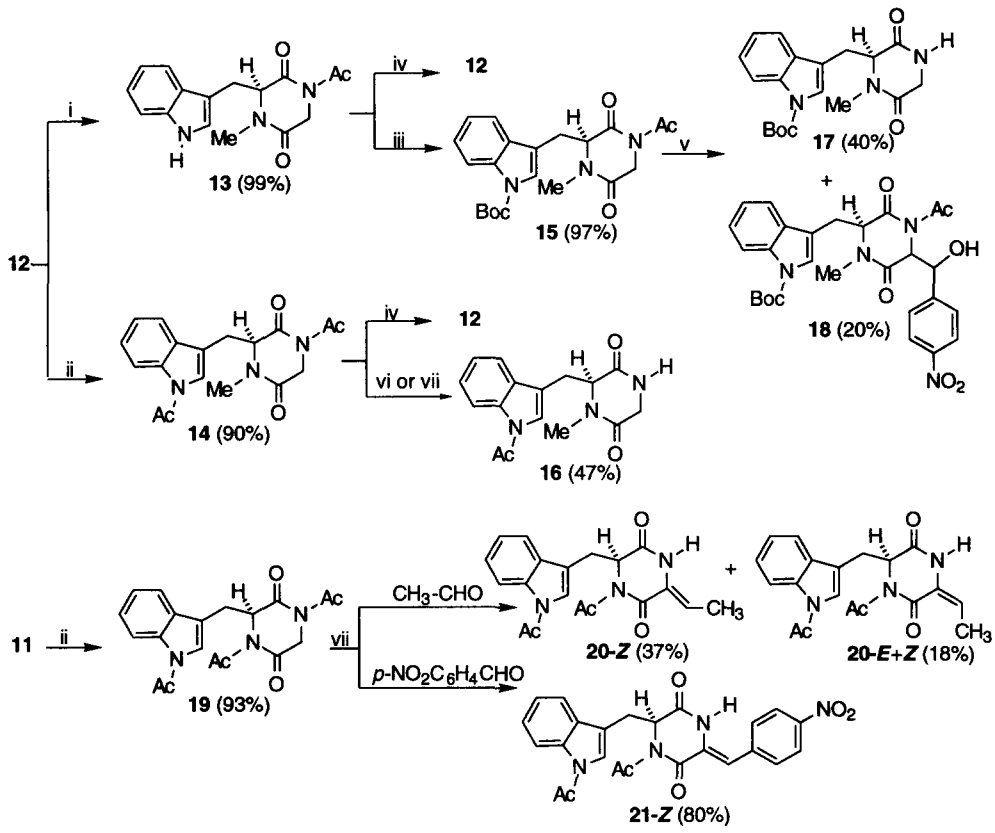
increase from **11** to **12** (164.9 and 166.6, 167.5 and 169.76, respectively). Infrared spectra of compound **11** showed a single carbonyl band (1679 cm^{-1}) while two absorptions (1690 and 1648 cm^{-1}) were observed for **12**.

The most general method to obtain alkylidene derivatives of piperazine-2,5-diones is the aldol condensation between compounds activated by *N*-acetylation and the corresponding aldehyde [23-26]. The *N*-acetyl group enhances the acidity of the heterocyclic methylene protons, and anchimerically assits the aldol reaction through an intramolecular *N-O* shift displacement to give *O*-acetyl aldol intermediates. The final condensation occurs by acetic acid elimination, with loss of the vicinal *N*-acetyl group. The stereochemistry of the condensation favours the *Z*-isomers, which are the thermodynamic products, especially with aromatic aldehydes [27]. Potassium *t*-butoxide, a poor nucleophilic base, has been widely used for this purpose [23-29]. Use of alumina-supported potassium fluoride in aprotic solvents [28,29], with or without ultrasonic irradiation [30,31], is also possible. Neither of these conditions cause epimerization of stereogenic centers.

In spite of these precedents, the reactions of the mono- and diacetyl derivatives of **12** (compounds **13** and **14**, Scheme 3) with different aldehydes (R = Me, *p*-$MeOC_6H_4$, *p*-$NO_2C_6H_4$), using a variety of bases and conditions, were unsuccessful, and the only reaction products were the deacetyl derivatives (**12** or **16**). However, in the reaction of **15** [32], with *t*-BuOK (1*N* DMF solution) and *p*-nitrobenzaldehyde, a 20% yield of the aldol **18** could be obtained as a single diastereomer, besides the deacetyl derivative **17**.

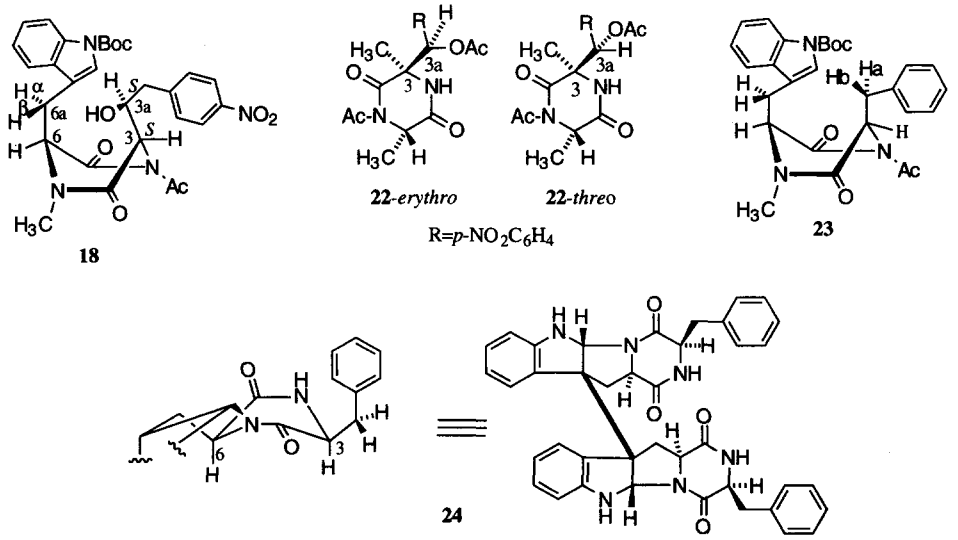
It seems clear that the steric constraints imposed by the N^1-methyl and the C^6-indolylmethyl substituents in **13** and **14** prevent the normal reaction course, as well as the *N,O*-acetyl migration in the case of compound **15**. In fact, compound **19**, which is the triacetyl derivative of **11**, gave the condensation products **20** and **21** shown in Scheme 3.

According to literature precedents the *Z*-isomers are diastereoselectively favoured [16], and the configurations were easily assigned by comparing the δ values of the vinylic protons, which are deshielded by the vicinal carbonyl group in the *Z*-isomers. The stereochemistry of **18** is proposed according to literature data. It has been reported that in the formation of *O*-acetyl intermediates (*e.g.* compound **22** [33]), the new stereogenic center at C-3 is thermodynamically controlled to give the 3,6-*syn* isomers, while the C-3a center is kinetically controlled, generally giving an *erythro/threo* mixture (18:1 in the case of **22**). NMR Data of compound **18** support a 3,6-*syn* configuration and are parallel to those of related compounds (*e.g.* **23** [34] and **24** [35]). Furthermore, the H-3 and H-6 protons are coupled in **24** (^5J = 1.2 Hz), but not in **23** nor in **18**, showing that in the two latter compounds both protons are pseudoequatorial. In compounds **18** and **23**, the H-3a proton is very shielded by the indolyl group, which must be "folded" over the piperazine ring. The vicinal coupling constant $^3J_{3,3a}$ = 16.9 Hz in compound **18**, shows the antiperiplanar conformation of protons H-3 and H-3a, while the coupling constans $^3J_{6,6a\alpha}$ = 4.2 and $^3J_{6,6a\beta}$ = 3.9 Hz, show a *gauche* conformation around the C-6-C-6a bond. Configuration of the C-3a center is tentatively proposed as *S* (*erythro*-form).



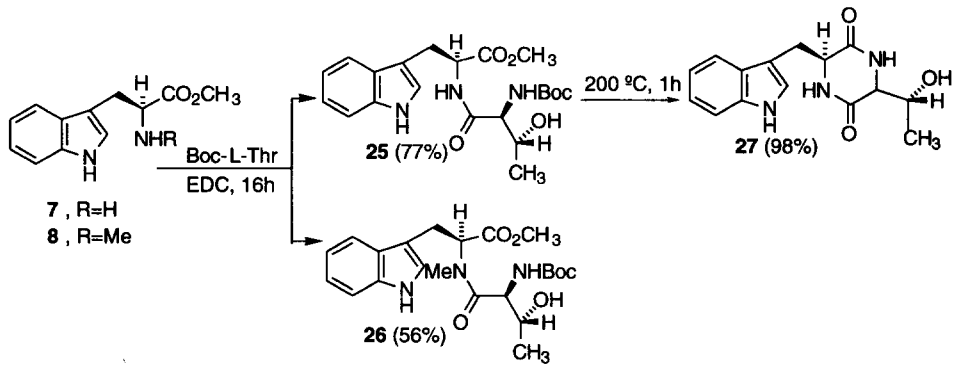
i: Ac_2O, 140 °C, 45 min. ii: Ac_2O, 140 ºC, 6h. iii: Boc_2O, DMAP/CH_3CN. iv: *t*-BuOK/DMF, 0 °C, RCHO, rt, 6 *t*-BuOK/DMF, 0 °C, RCHO, rt,100h or 130 ºC, 23h. vi: Et_3N, 0 °C, RCHO, rt, 16h, 130 °C, 6h. vii: KF/Al_2O_3/DMF, RCH 16h.

Scheme 3



On the other hand, the *Z*-isomer of **20**, shows the vinylic H-3a proton shifted 1 ppm at lower field respect to the same proton in the *E*-isomer, while the H-3b methyl protons are shifted 0.82 ppm at higher field. Finally, the *Z*-configuration of compound **21** was determined by NOE experiments on its *N*-4 acetyl derivative: irradiation on *N*-4 acetyl protons at 2.33 ppm produced enhancements of the H-3c and H-3d benzene signals instead of the H-3a signal.

We concluded that condensation of N^1-methyl-*cyclo*-Trp-Gly derivatives with aldehydes was prevented by the steric constraints imposed by the *N*-methyl group, and therefore we studied the alternative procedure, *i.e.*, the dehydration of *cyclo*-L-Trp-L-Thr (**27**, Scheme 4) and N^1-methyl-*cyclo*-L-Trp- L-Thr (**31**, Scheme 5).



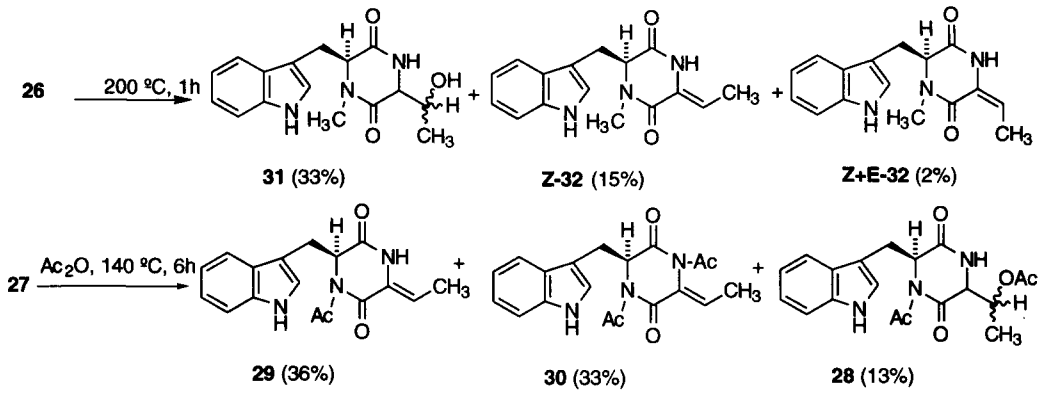
Scheme 4

Amino esters **7** and **8** were converted to the Boc-protected dipeptides **25** and **26**. Pyrolysis of **25** gave quantitatively the *cyclo*-dipeptide **27** (Scheme 4) which, by treatment with acetic anhydride, yielded three acetyl derivatives that were easily separated by flash chromatography (Scheme 5). Probably, the *N,O*-diacetyl derivative **28**, losses acetic acid to give **29**, while an unisolated *N,N,O*-triacetylderivative, also by loss of acetic acid, produces **30**. NOE experiments confirmed the proposed structures. Thus, to determine the *Z* or *E*-configuration of **29**, as well as the position of the *N*-acetyl group, we irradiated at the resonance frequency of the doublet at 0.90 ppm, corresponding to the H-3b methyl protons, observing enhancement of the signals corresponding to H-4 (N-H) and H-3a (vinylic) protons.

After irradiation at the acetyl protons frequency (δ = 2.51 ppm), the signals corresponding to H-2' and H-6 also suffered an small enhancement.

On the contrary, the pyrolysis of **26** gave a mixture from which the expected product **31** was isolated as an inseparable mixture of isomers, together with the dehydration compound ***Z*-32** and traces of the mixture ***Z*+*E*-32** (Scheme 5).

Since all the synthetic process is stereocontrolled, the sterogenic center at C-6 of **32** is *S* (from L-tryptophan). On the other hand, ^{1}H NMR data of the mixture of ***Z*+*E*-32** showed that the vinylic (H-3a) and H-3b protons, that could be clearly differenciated in both isomers, are affected by the diamagnetic anisotropy of the C-2 carbonyl group. In the *Z*-isomer, H-3a



Scheme 5

is deshielded at about 1 ppm with respect to the same proton in the *E*-isomer (δ = 5.41 and 4.50 ppm, respectively). Since the chemical shift values of these protons in **Z-32** and in **1** are identical, the *Z*-configuration of the double bond in TDD seems to be unequivocally established. However, slight differences were observed in other ^{1}H- and ^{13}C-NMR chemical shifts (see Experimental). More significant were the discrepancies found between the melting points of both compounds which were: 121-123 °C (after recrystallisation of **1** in acetone-cyclohexane) [11] and 191-2 °C (the same solvent) or 191-192 °C (methanol) in **Z-32**. Regarding mass spectra, the fragment ion at *m/e* 154, which is one of the two base peaks in **Z-32**, is not mentioned in the description of **1**.

We concluded that **1** must have the *N*-methyl group at the piperazinedione nitrogen adjacent to the ethylidene, instead of the indolylmethyl, substituent. In fact, the proposed structure [11] was mainly based on the identification of α-aminobutyric acid by thin-layer chromatography, after hydrolysis of **1**.

Finally, compound **Z-32** was assayed following Habig's procedure [36] as an inhibitor of human GST-π, which is mostly related to tumour resistances (Sigma G-8642), but it was inactive.

3. Experimental

Infrared spectra were recorded on a Perkin-Elmer Paragon 1000 spectrophotometer. NMR spectra were obtained on a Bruker AC-250 (250 MHz for ^{1}H, 63 MHz for ^{13}C) spectrometer. Elemental analyses of new compounds were determined by the Servicio de Microanálisis, Universidad Complutense, on a Perkin-Elmer 2400 CHN microanalyser. Melting points were measured on a Reichert 273 hot stage microscope, and are uncorrected. Reactions were monitored by thin layer chromatography, on aluminium plates coated with silica gel with fluorescent indicator (Scharlau Cf 530). Separations by flash chromatography were performed on silica gel (SDS 60 ACC, 230-400 mesh and Scharlau Ge 048). All reagents were of commercial quality (Aldrich, Fluka, Merck, SDS, Probus) and were purified

following standard procedures. The expression "petroleum ether" refers to the fraction boiling at 40-60 °C.

3.1. Synthesis of dipeptides. General procedure.

Starting from a magnetically stirred solution of the suitable amino ester (1.0 mmol) in anhydrous dioxane or anhydrous dichloromethane was added EDC (1.0 mmol) . The reaction mixture was kept under argon and protected from light at room temperature during 15 h. After evaporation of the organic solvent, the residue was dissolved in ethyl acetate and washed successively with HCl, $HNaCO_3$ and water to pH 7. The organic layer was dried over anhydrous sodium sulphate and concentrated *in vacuo*, affording the corresponding compounds as solids, which were used without further purification (**9** and **25**) or after flash chromatography (**10** and **26**). NMR data given below correspond to the main rotamer:

Data for **9**. Yield 88%. IR ν_{max} (KBr): 3414, 3331 (NH), 1743, 1670 (C=O) cm-1. ^{1}H-NMR (250 MHz,$CDCl_3$) δ: 8.12 (br s, 1H, NHi); 7.48 (d, 1H, *J* = 8.0 Hz, H-4'); 7.33 (d, 1H, *J* = 7.6 Hz, H-7'); 7.12 (m, 2H, H-5', H-6'); 6.97 (d, 1H, *J* = 2.2 Hz, H-2'); 6.49 (d, 1H, *J* = 7.7 Hz, NH); 4.91 (m, 1H, H-2); 3.73 (d, 2H, *J* = 5.4 Hz, H-3); 3.66 (s, 3H, OCH_3); 3.30 (d, 2H, *J* = 5.2 Hz, H-2"); 1.40 (s, 9H, H-6") ppm. ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 174.0 and 171.2 (C1 and C1"); 157.5 (C4"); 137.9 (C7'a); 128.8 (C3'a); 125.5 (C2'); 122.7 (C5'); 120.2 (C4'); 119.7 (C6'); 113.2 (C7'); 110.9 (C3'); 79.8 (C5"); 54.8 (C2); 53.6 (OCH_3); 44.7 (C2"); 29.9 (C6"); 28.9 (C3) ppm.

Data for **10.** Yield 90%, after chromatography eluting with 7:3 dichloromethane-ethyl acetate. IR ν_{max} ($CHBr_3$): 3335 (NH), 1707, 1652 (C=O) cm-1. ^{1}H-NMR (250 MHz,$CDCl_3$) δ: 8.37 (br s, 1H, NHi); 7.55 (d, 1H, *J* = 7.5 Hz, H-4'); 7.31 (d, 1H, *J* = 7.7 Hz, H-7'); 7.12 (m, 2H, H-5', H-6'); 6.94 (s, 1H, H-2'); 5.46 (s, 1H, NH); 5.26 (m, 1H, H-2); 3.93 (m, 2H, H-3); 3.73 (s, 3H, OCH_3); 3.43 (dd, 1H, *J* = 15.4 and 5.4 Hz, H-2"); 3.24 (dd, 1H, *J* = 15.4 and 10.3 Hz, H-2"); 2.71 (s, 3H, NCH_3); 1.41 (s, 9H, H-6") ppm. ^{13}C-NMR (250 MHz, $CDCl_3$) δ: 171.1 and 169.0 (C1 and C1"); 155.7 (C4"); 136.1 (C7'a); 127.1 (C3'a); 122.3 (C2'); 122.2 (C5'); 122.1 (C4'); 119.5 (C6'); 118.1 (C7'); 111.2 (C3'); 79.6(C5"); 58.1 (C2); 52.3 (OCH_3); 42.4 (C2"); 31.6 (C3); 28.2 (C6"); 24.3 (NCH_3) ppm.

Data for **25.** Yield 77%. IR ν_{max} (KBr): 3405 (OH); 3344 (NH), 1738, 1705, 1660 (C=O) cm-1. ^{1}H-NMR (250 MHz,$CDCl_3$) δ: 8.25 (br s, 1H, NHi); 7.50 (d, 1H, *J* = 7.6 Hz, H-4'); 7.32 (d, 1H, *J* = 7.5 Hz, H-7'); 7.12 (m, 2H, H-5', H-6'); 6.99 (d, 1H, *J* = 2.1 Hz, H-2'); 5.39 (d, 1H, *J* = 8.1 Hz, OH); 4.86 (dd, 1H, *J* = 13.2 and 5.6 Hz, H-2); 4.24 (m, 1H, H-2"a); 4.04 (m, 1H, H-2"); 3.66 (s, 3H, OCH_3); 3.29 (d, 2H, *J* = 5.6 Hz, H-3); 2.97 (br s, 1H, NH); 1.43 (s, 9H, H-6"); 1.11 (d, 3H, *J* = 6.43 Hz, H-2"b) ppm. ^{13}C-NMR (63 MHz, d_6-DMSO) δ: 171.7 and 169.6(C1 and C1"); 155.5 (C4"); 136.1 (C7'a); 127.4 (C3'a); 122.9 (C2'); 122.1 (C5'); 119.5 (C4'); 118.2 (C6'); 111.3 (C7'); 109.3 (C3'); 80.3 (C5"); 70.2 (C2"a); 57.3(C2"); 52.9 (C2); 52.3 (OCH_3); 28.1 (C6"); 227.5 (C3); 15.7 (C2"b) ppm.

Data for **26.** Yield 56%, after chromatography eluting with 6:4 ethyl acetate-hexane.^{1}H-NMR (250 MHz,$CDCl_3$) δ: 8.23 (br s, 1H, NHi); 7.56 (m, 1H, H-4'); 7.33 (d, 1H, *J* = 7.6 Hz, H-7'); 7.14 (m, 2H, H-5', H-6'); 6.98 (br s, 1H, H-2'); 5.41 (m, 1H, OH); 4.34 (m, 1H, H-2);

4.22 (br s, 1H, H-2"a); 4.05 (m, 1H, H-2"); 3.76 (s, 3H, OCH_3); 3.23 (m, 2H, H-3); 3.03 (s, 1H, NH); 2.95 (s, 3H, NCH_3); 1.37 (s, 9H, H-6"); 1.11 (d, 3H, J = 6.4 Hz, H-2"b) ppm. ^{13}C-NMR (63 MHz, d_6-DMSO) δ: 172.7 and 170.5(C1 and C1"); 150.2 (C4"); 136.0 (C7'a); 128.4 (C3'a); 122.4 (C2'); 122.0 (C5'); 119.4 (C4'); 118.2 (C6'); 111.3 (C7'); 109.3 (C3'); 80.2 (C5"); 67.2 (C2"a); 60.2 (C2"); 52.3 (C2); 52.1 (OCH_3); 31.6 (NCH_3); 28.1 (C6"); 22.0 (C3); 16.2 (C2"b) ppm.

3.2. Cyclization of dipeptides. General procedure

Piperazine-2,5-diones **11**, **12** and **27** were obtained by pyrolysis at 200 °C during 1 h of the suitable dipeptides **9**, **10** and **25**, followed by column chromatography and/or recrystallization. Compound **31** was obtained from **26** as an inseparable mixture of isomers, and compound **32**-*E*, which was not isolated from the *Z*-isomer, is described in terms of significant NMR data.

Data for **11.** Yield 98%. M.p. >230 °C (acetone). IR ν_{max} (KBr): 3406, 3184 (NH), 1679 (C=O) cm-^{1}. ^{1}H-NMR (250 MHz,d_6-DMSO) δ: 10.9 (br s, 1H, NHi); 8.11 (s, 1H, NH); 7.77 (s, 1H, NH); 7.53 (d, 1H, J = 7.8 Hz, H-4'); 7.32 (d, 1H, J = 7.9 Hz, H-7'); 6.98 (m, 3H, H-2', H-5', H-6'); 4,00 (m, 1H, H-3); 3.23 (dd, 1H, J = 14.5 and 4.5 Hz, H-3a); 3.23 (d, 1H, J = 17.1 Hz, H-6); 3.00 (dd, 1H, J = 14.5 and 4.5 Hz, H-3a); 2.76 (d, 1H, J = 17.1 Hz, H-6) ppm. ^{13}C-NMR (63 MHz, d_6-DMSO) δ: 169.7 and 167.5 (C2 and C5); 137.7 (C7'a); 129.3 (C3'a); 126.4 (C2'); 122.7 (C5'); 120.5 (C4'); 120.2 (C6'); 112.9 (C7'); 110.1 (C3'); 81.0 (C3); 57.2 (C6); 31.0 (C3a) ppm. Found: C, 64.07; H, 5.19; N, 17.16. $C_{13}H_{13}N_3O_2$ requires C, 64.17; H, 5.39; N, 17.28.

Data for **12.** Yield 90%, after chromatography eluting with 3:1 dichloromethane-petroleum ether. M.p. 84 °C (ethyl acetate). IR ν_{max} (KBr): 3263 (NH), 1690, 1648 (C=O) cm-^{1}. ^{1}H-NMR (250 MHz,$CDCl_3$) δ: 8.28 (br s, 1H, NHi); 7.62 (d, 1H, J = 7.8 Hz, H-4'); 7.32 (d, 1H, J = 7.8 Hz, H-7'); 7.13 (m, 2H, H-5', H-6'); 6.93 (d, 1H, J = 2.3 Hz, H-2'); 5.38 (s, 1H, NH); 4.15 (m, 1H, H-6); 3.57 (dd, 1H, J = 14.9 and 3.0 Hz, H-6a); 3.28 (d, 1H, J = 16.9 Hz, H-3); 3.28 (dd, 1H, J = 14.9 and 4.6 Hz, H-6a); 3.06 (s, 3H, NCH_3); 2.33 (d, 1H, J = 16.9 Hz, H-3) ppm. ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 168.6 and 164.9 (C2 and C5); 136.1 (C7'a); 127.2 (C3'a); 124.1 (C2'); 122.5 (C5'); 120.0 (C4'); 118.8 (C6'); 111.2 (C7'); 108.4 (C3'); 62.9 (C6); 44.3 (C3); 32.5 (C6a); 26.8 (NCH_3) ppm. Found: C, 65.44; H, 5.83; N, 16.37. $C_{14}H_{15}N_3O_2$ requires C, 65.34; H, 5.88; N, 16.34.

Data for **27.** Yield 98%, after chromatography eluting with dichloromethane. M.p. 239-241 °C (ethyl acetate/hexane). IR ν_{max} (KBr): 3413 (OH), 3191 (NH), 1741, 1670 (C=O) cm-^{1}. ^{1}H-NMR (250 MHz, d_6-DMSO) δ: 10.90 (br s, 1H, NHi); 8.37 (s, 1H, NH); 8.09 (s, 1H, NH); 7.51 (d, 1H, J = 7.7 Hz, H-4'); 7.33 (d, 1H, J = 7.7 Hz, H-7'); 7.15 (d, 1H, J = 2.2 Hz, H-2'); 6.98 (m, 2H, H-5', H-6'); 4.87 (m, 1H, OH); 4.01 (m, 1H, H-6); 3.86 (m, 1H, H-3); 3.33 (m, 1H, H-3a); 3.22 (dd, 1H, J = 14.5 and 4.1 Hz, H-6a); 3.07 (dd, 1H, J = 14.5 and 7.6 Hz, H-6a); 1.06 (d, 3H, J = 6.6 Hz, H-3b) ppm. ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 171.1 and 169.4 (C2 and C5); 138.0 (C7'a); 129.2 (C3'a); 124.8 (C2'); 122.6 (C5'); 120.1 (C4' and C6'); 113.1

(C7'); 110.9 (C3'); 72.5 (C3a); 60.0 (C6); 57.2 (C3); 32.7 (C6a); 17.9 (C3b) ppm. Found: C, 62.15; H, 5.66; N, 14.48. $C_{15}H_{17}N_3O_3$ requires C, 62.69; H, 5.97; N, 14.63.

Data for **31.** Yield 33%. IR ν_{max} (KBr): 3412 (OH); 3187 (NH), 1741, 1680 (C=O) cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 8.44 (br s,1H, NHi); 7.58 (d, 1H, *J* = 7.7 Hz, H-4'); 7.30 (d, 1H, *J* = 7.9 Hz, H-7'); 7.13 (m, 2H, H-5', H-6'); 6.86 (d, 1H, *J* = 2.2 Hz, H-2'); 6.05 (s, 1H, NH); 4.15 (m, 1H, H-6); 3.70 (br s, 1H, OH); 3.60 (m, 1H, H-3); 3.52 (dd, 1H, *J* = 14.9 and 3.8 Hz, H-6a); 3.23 (dd, 1H, *J* = 14.9 and 4.5 Hz, H-6a); 3.08 (m, 1H, H-3a); 3.02 (s, 3H, NCH_3); 0.93 (d, 3H, *J* = 6.2 Hz, H-3b) ppm.

Data for **32-*Z***. (compared to those of **TDD**). Yield 15%, after chromatography eluting with 1:1 dichloromethane-ethyl acetate. M.p. 192-194 °C (methanol); 191-192 °C (acetone-cyclohexane); lit: 121-123 °C (acetone-cyclohexane) [11]. IR ν_{max} (KBr) : 3300, 3230 (NH); 1700, 1645 (C=O) cm^{-1}; lit: 3300, 3230, 3100, 1700, 1645, 1600, 1400, 1140, 1110, 740 cm^{-1} [11]. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 8.10 (br s, 1H, NHi); 7.58 (d, 1H, *J* = 7.8 Hz, H-4'); 7.26 (d, 1H, *J* = 10.0 Hz, H-7'); 7.08 (m, 2H, H-5', H-6'); 6.80 (d, 1H, *J* = 2.4 Hz, H-2'); 6.74 (s, 1H, NH); 5.41 (q, 1H, *J* = 7.4 Hz, H-3a); 4.24 (m, 1H, H-6); 3.53 (dd, 1H, *J* = 14.6 and 2.8 Hz, H-6a); 3.23 (dd, 1H, *J* = 14.6 and 4.7 Hz, H-6a); 3.06 (s, 3H, NCH_3); 0.84 (d, 3H, *J* = 7.4 Hz, H-3b) ppm.; lit ($CDCl_3$) [4] δ: 8.19 (br s, 1H, NHi); 7.28* (d, 1H, *J* = 7 Hz, H-4'); 7.62* (d, 1H, *J* = 7 Hz, H-7'); 7.09 (qn, *J* = 7 Hz, 2H, H-5', H-6'); 6.77 (s, 1H, H-2'); 9.05 (bs, 1H, NH); 5.41 (q, 1H, *J* = 6 Hz, H-3a); 4.25 (bt, 1H, *J* = 3 Hz, H-6); 3.56 (dd, 1H, *J* = 15.5 Hz, H-6a); 3.26 (dd, 1H, *J* = 15.3 Hz, H-6a); 3.04 (s, 3H, NCH_3); 1.00 (d, 3H, *J* = 6 Hz, H-3b) ppm. ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 166.1 (C5); 159.9 (C2); 136.1 (C7'a); 127.4 (C3'a); 126.1 (C3); 124.5 (C2'); 122.2 (C5'); 119.7 (C4'); 118.6 (C6'); 111.7 (C3a); 110.7 (C7'); 107.9 (C3'); 63.1 (C6); 32.7 (NCH_3); 27.5 (C6a); 9.5 (C3b) ppm. Lit ($CDCl_3$) [11] δ: 166.9 (C5); 159.5 (C2); 136.4 (C7'a); 127.5 (C3'a); 126.4 (C3); 124.7 (C2'); 122.0 (C5'); 119.4 (C6'); 118.4 (C4'); 113.0 (C3a); 111.0 (C7'); 107.6 (C3'); 63.3 (C6); 32.8 (NCH_3); 27.7 (C6a); 9.9 (C3b) ppm. Found: C, 64.81; H, 6.30; N, 13.69. $C_{16}H_{17}N_3O_2 \cdot CH_3OH$ requires C, 64.76; H, 6.66; N, 13.33. $[\alpha]_D^{21}$ = +13 (c = 0.03, EtOH); lit: $[\alpha]_D^{24.5}$ = +10 (c = 1.1, EtOH). MS: 284 (MH^+; $C_{16}H_{18}N_3O_2^+$; 1%); 283 (M^+, $C_{16}H_{17}N_3O_2^+$; 5%); 154 (M^+-$C_9H_8N^+$; 100%); 130 ($C_9H_8N^+$; 100%); 103.30 ($C_7H_5N^+$; 8%); 77 ($C_6H_5^+$; 12%). Lit [11]: 130, 103, 77.

Data for **32-*E***. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 4.51 (c, 1H, *J* = 7.4 Hz, H-3a); 1.66 (d, 3H, *J* = 7.4 Hz, H-3b) ppm. (Extracted from the mixture *Z*+*E*-**32**).

3.3. Acetyl derivatives of **11** *and* **12.** *Synthesis of* **13**, **14** *and* **19.**

A magnetically stirred solution of **12** (1mmol) in excess of acetic anhydride was heated at 140 °C for 45 min. Evaporation *in vacuo* gave a solid residue that was recrystallized to give **13**. Compounds **14** and **19** were obtained after 6h in the same reaction conditions from **12** and **11**, respectively.

Data for **13.** Yield 99%. M.p. 185 °C (acetone). IR ν_{max} (Br_3CH): 3331 (NH), 1706, 1664 (C=O) cm-^{1}. ^{1}H-NMR (250 MHz,$CDCl_3$) δ: 8.58 (br s, 1H, NHi); 7.60 (d, 1H, *J* = 7.4 Hz, H-4'); 7.33 (d, 1H, *J* = 8.0 Hz, H-7'); 7.12 (m, 2H, H-5', H-6'); 6.92 (d, 1H, *J* = 2.3 Hz, H-2');

4.30 (dd, 1H, J=4.5 and 3.8 Hz, H-3); 4.11 (d, 1H, J=18.1 Hz, H-6); 3.56 (dd, 1H, *J* = 14.9 and 3.8 Hz, H-3a); 3.29 (dd, 1H, J=14.9 and 4.5 Hz, H-3a) 3.04 (s, 3H, NCH_3); 2.43 (s, 3H, $COCH_3$).ppm. ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 171.3 (C1a); 168.9 and 164.7 (C2 and C5); 136.2 (C7'a); 126.7 (C3'a); 124.3 (C2'); 122.9 (C5'); 120.1 (C4'); 118.0 (C6'); 111.8 (C7'); 107.7 (C3'); 64.9 (C3); 45.4 (C6); 32.2 (C4a); 27.8 (C1b); 27.4 (C3a) ppm. Found: C, 63.96; H, 5.64; N, 13.86. $C_{16}H_{17}N_3O_3$ requires C, 64.21; H, 5.68; N, 14.04.

Data for **14.** Yield 90%, after chromatography eluting with 9:1 ethyl acetate-dichloromethane. IR ν_{max} (Br_3CH): 3007 (NH), 1723, 1709, 1681, 1668 (C=O) cm^{-1}. ^{1}H-NMR (250 MHz,$CDCl_3$) δ: 8.41 (d, 1H, *J* = 8.2 Hz, H-4'); 7.33 (d, 1H, *J* = 7.9 Hz, H-7'); 7.27 (m, 2H, H-5', H-6'); 7.15 (s, 1H, H-2'); 4.35 (m, 1H, H-3); 4.35 (d, 1H, *J* = 18.2 Hz, H-6): 3.43 (dd, 1H, *J* = 14.7 and 4.6 Hz, H-3a): 3.28 (dd, 1H, *J* = 14.7 and 4.9 Hz, H-3a); 3.09 (s, 3H, NCH_3); 2.83 (dd, 1H, *J* = 18.2 Hz, H-6): 2.60 (s, 3H, H-1'b); 2.46 (s, 3H, H-1b) ppm. ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 171.2 and 168.4 (C1a and C1'a); 168.3 and 164.4 (C2 and C5); 135.7 (C7'a); 129.3 (C3'a); 126.3 (C2'); 124.8 (C5'); 124.1 (C4'); 118.3 (C6'); 117.0 (C7'); 115.0 (C3'); 64.3 (C3); 45.6 (C6); 32.6 (C4a); 27.7 (C3a); 27.3 and 24.2 (C1b and C1'b) ppm. This product was used without recristalization for the next reactions.

Data for **19.** Yield 93%. M.p. 174-176 °C (ethyl acetate). IR ν_{max} (Br_3CH): 3007 (NH); 1732, 1715, 1701, 1692, 1600 (C=O) cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 8.38 (d, 1H, *J* = 8.1 Hz, H-4'); 7.42 (d, 1H, *J* = 7.8 Hz, H-7'); 7.30 (m, 2H, H-5', H-6'); 7.16 (s, 1H, H-2'); 5.45 (dd, 1H, *J* = 6.4 and 4.2 Hz, H-3); 4.59 (d, 1H, *J* = 19.2 Hz, H-6); 3.47 (dd, 1H, *J* = 14.8 and 4.2 Hz, H-3a); 3.27 (dd, 1H, *J* = 14.8 and 6.4 Hz, H-3a); 2.95 (d, 1H, *J* = 19.2 Hz, H-6); 2.56 (s, 6H, N1-$COCH_3$ and N4-$COCH_3$); 2.47 (s, 3H, N1'-$COCH_3$) ppm. ^{13}C-NMR (63 MHz; $CDCl_3$) δ: 171.4 (C1'a); 171.1 (C2); 168.4 (C1a); 167.9 (C4a); 166.3 (C5); 135.7 (C7'a); 129.4 (C3'a); 126.3 (C2'); 125.1, (C5'); 124.2 (C4'); 118.4 (C6'); 117.0 (C3'); 115.4 (C3'C7'); 58.2 (C3); 46.6 (C6); 28.6 (C3a); 27.2 (C1b); 27.1 (C4b); 24.2 (C1'b) ppm. Found: C, 61.41; H, 5.10; N, 11.22. $C_{19}H_{19}N_3O_5$ requires C, 61.77; H, 5.14; N, 11.38. $[\alpha]_D^{21}$ = +3.6 (c = 0.3, Cl_3CH).

3.4. Synthesis of compound **15.**

It was prepared from **13** following literature references [32] and was used without recrystallization. Yield 97%. IR ν_{max} (Br_3CH): 1734, 1670 (C=O) cm^{-1}. ^{1}H-NMR (250 MHz,$CDCl_3$) δ: 8.11 (d, 1H, *J* = 7.3 Hz, H-4'); 7.44 (d, 1H, *J* = 8.0 Hz, H-7'); 7.39 (d, 1H, *J* = 2.4 Hz, H-2'); 7.26 (m, 2H, H-5' and H-6'); 4.39 (d, 1H, J=18.0 Hz, H-6); 4.33 (dd, 1H, *J* = 4.3 and 4.7 Hz, H-3): 3.41 (dd, 1H, *J* = 14.6 and 4.7 Hz, H-3a); 3.27 (dd, 1H, *J* = 14.6 and 4.3 Hz, H-3a); 2.99 (s, 3H, NCH_3); 2.97 (d, 1H, *J* = 18.0 Hz, H-6); 2.46 (s, 3H, H-1b); 1.66 (s, 9H, H-1'c) ppm. ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 171.2 (C1a); 168.4 (C2); 163.8 (C5); 150.0 (C1'a); 135.3 (C7'a); 129.3 (C3'a); 125.2 (C2'); 122.9 (C5'); 118.2 (C4' and C6'); 115.6 (C7'); 113.1 (C3'); 84.3 (C1'b); 64.5 (C3); 45.5 (C6); 32.5 (C4a); 28.1 (C1'c); 27.7 (C1b); 27.2 (C3a) ppm.

Data for **16.** (This product was obtained in condensation experiments with compound **14**). Yield 47%, after chromatography eluting with 1:5 petroleum ether-ethyl acetate. M.p. 96 °C

(ethyl acetate). IR ν_{max} (Br_3CH): 3293 (NH); 1696, 1652, 1648 (C=O) cm-1. ^{1}H-NMR (250 MHz,$CDCl_3$) δ: 8.39 (d, 1H, *J* = 8.1 Hz, H-7'); 7.52 (d, 1H, *J* = 7.6 Hz, H-4'); 7.27 (m, 2H, H-5' and H-6'); 6.89 (s, 1H, H-2'); 6.42 (br s, 1H, NH); 4.17 (m, 1H, H-6); 3.49 (dd, 1H, *J* = 14.7 and 3.4 Hz, H-6a); 3.20 (dd, 1H, *J* = 14.7 and 4.5 Hz, H-6a); 3.49 (d, 1H, *J* = 17.3 Hz, H-3); 3.05 (s, 3H, H-1a); 2.80 (d, 1H, *J* = 17.3 Hz, H-3); 2.57 (s, 3H, H_1'b) ppm. ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 168.6 (C1'a); 168.3 and 164.6 (C2 and C5); 152.0 (C7'a); 135.7 (C3'a); 129.8 (C2'); 125.9 (C5'); 124.8 (C4'); 124.1 (C6'); 118.9 (C7'); 116.7 (C3'); 62.5 (C6); 46.6 (C3); 32.8 (C1a); 26.9 (C6a); 24.2 (C1'b) ppm. Found: C, 64.12; H, 5.68; N, 14.03. $C_{16}H_{17}N_3O_3$ requires C, 64.19; H, 5.73; N, 14.04.

3.5. Condensation of piperazinediones with aldehydes

Method A: To a magnetically stirred solution of **14** (0.18 mmol) in anhydrous DMF (2 ml) under argon, cooled at 0 °C, *t*-BuOK (1N DMF solution, 0.18 mml) was added. This solution was added to the corresponding aldehyde (0.18 mmol) and the mixture was kept at room temperature for 6 h under argon. The reaction mixture was acidified to pH 5 with acetic acid, poured into water (3 ml) and extracted with CH_2Cl_2 (3 x 10 ml). The organic layers were dried with anhydrous Na_2SO_4 and the solvent was evaporated to dryness. Purification of the residue by column chromathography followed by recrystalization, gave compound **12**.

Method B: To a magnetically stirred solution of **15** (1 mmol) in anhydrous DMF (2 ml) under argon, cooled at 0 °C, *t*-BuOK (1N DMF solution, 0.18 mml) was added. This solution was added to the corresponding aldehyde (1 mmol) and the mixture was kept at room temperature for 100 h or at 130 °C for 23 h under argon. After working-up as described in method A, compounds **17** and **18** were obtained.

Method C: To a magnetically stirred solution of **14** (1 mmol) in anhydrous DMF (2 ml) under argon, cooled at 0 °C, Et_3N (1 mml) was added. This solution was added to the corresponding aldehyde (1 mmol) and the mixture was stirred at room temperature for 16 h under argon and was then heated at 130 °C for 6 h. Compound **16** was obtained.

Method D: To a magnetically stirred solution of **14** or **19** (1 mmol) and the corresponding aldehyde (1 mmol) in anhydrous DMF (15 ml), KF/Al_2O_3 (400 mg) were added. The mixture was stirred at room temperature for 16 h and then DMF (15 ml) was added. The suspension was filtered over celite and washed with DMF (3 x 2 ml). The solvent was concentrated *in vacuo* obtaining compound **16** from **14** and compounds **20-*Z***, **20-*E*** and **21-*Z*** from **19**.

Data for **17.** Yield 40%, after chromatography eluting with 9:1 ethyl acetate-hexanane.^{1}H-NMR (250 MHz,$CDCl_3$) δ: 8.09 (d, 1H, *J* = 8.1 Hz, H-7'); 7.54 (d, 1H, *J* = 7.8 Hz, H-4'); 7.39 (s, 1H, H-2'); 7.27 (m, 2H, H-5' and H-6'); 6.24 (br s, 1H, NH); 4.18 (dd, 1H, *J* = 4.1 and 4.7 Hz, H-6); 3.55 (dd, 1H, *J* = 17.2 and 3.6 Hz, H-3); 3.42 (dd, 1H, *J* = 14.8 and 4.1 Hz, H-6a); 3.24 (dd, 1H, *J* = 14.8 and 4.7 Hz, H-6a); 3.03 (s, 3H, H-1a); 2.95 (d, 1H, *J* = 17.2 Hz, H-3): 1.65 (s, 9H, H_1'c) ppm. ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 168.5 and 164.0 (C2 and C5); 151.4 (C1'a); 129,7 (C7'a); 125.3 (C3'a); 124.9 (C2'); 122.9 (C5'); 118.9 (C4'); 115.2 (C6');

113.6 (C7' and C3'); 84.2 (C1'b); 62.5 (C6); 44.5 (C3); 32.8 (C1a); 28.1 (C6a); 27.1 (C1'c) ppm.

Data for **18.** Yield 20%, after chromatography eluting with 9:1 ethyl acetate-hexane. M.p. 199-201 °C (benzene/hexane). IR ν_{max} (Br_3CH): 3447 (OH); 3113 (NH); 1756, 1734, 1675, 1652 (C=O) cm^{-1}.^{1}H-NMR (250 MHz, $CDCl_3$) δ: 8.09 (d, 1H, *J* = 8.1 Hz, H-4'); 7.92 (d, 1H, *J* = 8.8 Hz, H-3d); 7.79 (s, 1H, H-2'); 7.56 (d, 1H, *J* = 7.4 Hz, H-7'); 7.34 (m, 2H, H-5', H-6'); 6.68 (d, 2H, *J* = 8.8 Hz, H-3c); 4.40 (dd, 1H, *J* = 3.9 and 4.2 Hz, H-6); 3.54 (dd, 1H, *J* = 14.9 and 4.2 Hz, H-6a); 3.51 (d, 1H, *J* = 16.9 Hz, H-3); 3.21 (dd, 1H, *J* = 14.9 and 3.9 Hz, H-6a); 3.12 (s, 3H, H-1a); 2.54 (d, 1H, *J* = 16.9 Hz, H-3a); 2.16 (s, 3H, H-4b); 1.62 (s, 9H, H-1'c) ppm. ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 185.6 (C4a); 168.2 (C5); 165.6 (C2); 163.3 (C1'a); 147.9 (C3e); 140.4 (C3b); 135.0 (C7'a); 129.9 (C3'a); 126.5 (C3c); 125.1 (C2'); 124.9 (C5'); 123.8 (C3d); 123.3 (C4'); 119.0 (C6'); 115.4 (C7'); 113.2 (C3'); 84.6 (C1'b); 77.2 (C3a) 75.1 (C3); 62.8 (C6); 44.4 (C6a); 32.4 (C1a); 28.1 (C1'c); 20.7 (C4b) ppm. MS: 550 (M^+ $C_{28}H_{30}N_4O_8^+$; 0.007%); 492 (M^+ - *t*-BuH; $C_{24}H_{20}N_4O_8^+$; 0.03%); 404 (M^+- *t*-BuH - AcH - CO_2; $C_{21}H_{16}N_4O_5^+$; 0.08%); 150 ($C_7H_4NO_3^+$; 13%); 130 ($C_9H_8N^+$; 100%); 57 ($C_4H_9^+$; 61%).

Data for **20.** Yield 40%, after chromatography eluting with ethyl ether. M.p. 199-201 °C (chloroform/hexane) IR ν_{max} (Br_3CH): 3192 (NH); 1697, 1685, 1647, 1606 (C=O) cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 8.32 (d, 1H, *J* = 8.2 Hz, H-4'); 8.01 (br s, 1H, NH); 7.45 (d, 1H, *J* = 7.8 Hz, H-7'); 7.24 (m, 2H, H-5', H-6'); 6.96 (s, 1H, H-2'); 5.65 (c, 1H-*Z*, *J* = 15.1 and 7.5 Hz, H-3a); 4.76 (c, 1H-*E*, *J* = 15.1 and 7.5 Hz, H-3a)5.27 (dd, 1H, *J* = 4.7 and 3.1 Hz, H-6); 3.44 (dd, 1H, *J* = 14.7 and 3.1 Hz, H-6a); 3.20 (dd, 1H, *J* = 14.7 and 5.4 Hz, H-6a); 2.56 and 2.52 (2s, 6H, H-1b, H-1'b); 0.98 (d, 3H-*Z*, *J* = 7.6 Hz, H-3b); 1.63 (d, 3H-*E*, *J* = 7.6 Hz, H-3b) ppm. ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 172.5 (C1'a); 168.4 (C1a); 166.7 (C5); 161.1 (C2);135.6 (C7'a); 130.0 (C3'a); 126.5 (C3); 126.4 (C2'); 125.6 (C5'); 123.5 (C4'); 118.7 (C6'); 118.0 (C3a); 116.3 (C3'); 115.3 (C7'); 56.6 (C6); 28.6 (C6a); 26.9 and 23.8 (C1'b and C1b); 10.6 (C3b) ppm. Found: C, 64.42; H, 5.15; N, 11.61. $C_{19}H_{18}N_3O_4$ requires C, 64.77; H, 5.15; N, 11.93. $[\alpha]_D^{21}$ = -3.0 (c = 0.02, Cl_3CH).

Data for **21.** Yield 80%, after chromatography eluting with dichloromethane. M.p. 221-223 °C (ethanol). IR ν_{max} (Br_3CH): 3520 (NH), 1702, 1689, 1647 and 1631 (C=O), 1561 (NO_2) cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 8.18 (d, 1H, *J* = 3.4 Hz, H-4'); 7.96 (d, 2H, *J* = 8.6 Hz, H-3d); 7.55 (m, 1H, H-7'); 7.39 (m, 2H, H-5', H-6'); 7.27 (s, 1H, NH); 7.02 (s, 1H, H-2'); 6.48 (s, 1H, H-3a); 6.28 (d, 2H, *J* = 8.6 Hz, H-3c); 5.38 (dd, 1H, *J* = 4.7 and 2.8 Hz, H-6); 3.59 (dd, 1H, *J* = 2.8 and 14.8 Hz, H-6a); 3.29 (dd, 1H, *J* = 4.7 and 14.7 Hz, H-6a); 2.65 and 2.52 (2s, 6H, H-1b, H-1'b) ppm. ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 172.2 (C1'a); 168.2 (C1a); 165.3 (C5); 160.9 (C2); 147.1 (C3e); 138.1 (C3b); 135.6 (C7'a); 129.8 (C3'a); 128.7 (C3d); 127.2 (C2'); 126.7 (C3'); 126.0 (C5'); 124.0 (C3c); 123.9 (C4'); 118.9 (C6'); 116.8 (C7'); 115.0 (C3a); 114.7 (C3); 56.8 (C6); 28.6 (C6a); 26.9 (C1'b); 23.7 (C1b) ppm. Found. C, 62.64; H, 4.00; N, 12.11. $C_{24}H_{19}N_4O_6$ requires C, 62.74; H, 4.17; N, 12.19. $[\alpha]_D^{21}$ = -1.6 (c = 0.06, Cl_3CH).

3.6. Dehydration of piperazinadione **27.**

Products **28** (0.13 mmol), **29** (0.36 mmol) and **30** (0.33 mmol) were obtained by heating the piperazinadione **27** (1 mmol) and acetic anhydride at 140 °C for 6 h, and were separated by column chromatography using dichloromethane/ethyl acetate as eluents.

Data for **28.** Yield 13%. M.p. 94-96 °C (chloroform/hexane). IR ν_{max} (Br_3CH): 3325 (NH), 1742, 1684 (C=O) cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 8.23 (br s, 1H, NHi); 7.59 (d, 1H, *J* = 7.7 Hz, H-4'); 7.40 (d, 1H, *J* = 8.1 Hz, H-7'); 7.16 (m, 2H, H-5', H-6'); 7.08 (d, 1H, *J* = 2.0 Hz, H-2'); 6.05 (s, 1H, NH); 5.28 (m, 2H, H-3, H-6); 4.43 (m, 1H, H-3a); 3.75 (dd, 1H, *J* = 14.2 and 2.9 Hz, H-6a) 3.14 (dd, 1H, *J* = 14.2 and 10.8 Hz, H-6a); 2.56 (s, 3H, H-3b"); 2.09 (s, 3H, H-1b); 1.29 (d, 3H, *J* = 6.1 Hz, H-3b) ppm. ^{13}C-NMR (63 MHz; $CDCl_3$) δ: 171.1 and 169.8 (C1a and C3a"); 169.7 and 165.5 (C2 and C5); 136.7 (C7'a); 126.5 (C3'a); 123.6 (C2'); 122.9 (C5'); 120.3 (C4'); 118.4 (C6'); 111.8 (C7'); 109.5 (C3'); 72.2 (C3); 58.5 (C3a); 58.0 (C6); 31.7 (C6a); 26.2 (C3b"); 21.4 (C1b); 17.9 (C3b) ppm. Found: C, 61.18; H, 5.32; N, 10.98. $C_{19}H_{21}N_3O_5$ requires C, 61.43; H, 5.70; N, 11.32.

Data for **29.** Yield 36%. M.p. 187-189 °C (chloroform/hexane). IR ν_{max} (Br_3CH): 3369 (NH), 1683, 1650 (C=O) cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 8.32 (br s, 1H, NHi); 8.07 (s, 1H, NH); 7.57 (d, 1H, *J* = 7.5 Hz, H-4'); 7.22 (d, 1H, *J* = 6.1 Hz, H-7'); 7.14 (m, 2H, H-5', H-6'); 6.72 (d, 1H, *J* = 2.4 Hz, H-2'); 5.50 (q, 1H, *J* = 7.5 Hz, H-3a); 5.25 (dd, 1H, *J* = 5.3 and 2.7 Hz, H-6); 3.58 (dd, 1H, *J* = 14.7 and 2.7 Hz, H-6a); 3.25 (dd, 1H, *J* = 14.7 and 5.3 Hz, H-6a); 2.51 (s, 3H, H-1b); 0.87 (d, 3H, *J* = 7.0 Hz, H-3b) ppm. ^{13}C-NMR (63 MHz; $CDCl_3$) δ: 172.5 (C1a); 167.3 and 160.9 (C2 and C5); 136.1 (C7'a); 127.5 (C3); 126.4 (C3'a); 125.6 (C2'); 122.3 (C5'); 119.7 (C4'); 118.7 (C6'); 116.9 (C3a); 110.7 (C7'); 108.5 (C3'); 57.2 (C6); 28.7 (C6a); 26.9 (C1b); 10.2 (C3b) ppm. Found: C, 65.26; H, 5.20; N, 13.19. $C_{17}H_{17}N_3O_3$ requires C, 65.57; H, 5.51; N, 13.50.

Data for **30.** Yield 33%. M.p. 130-132 °C (ethyl acetate/hexane). IR ν_{max} (Br_3CH): 3370 (NH), 1721, 1695, 1640 (C=O) cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 8.15 (br s,1H, NHi); 7.44 (d, 1H, *J* = 7.3 Hz, H-4'); 7.28 (d, 1H, *J* = 8.0 Hz, H-7'); 7.10 (m, 2H, H-5', H-6'); 6.79 (d, 1H, *J* = 2.4 Hz, H-2'); 6.08 (q, 1H, *J* = 7.4 Hz, C-3a); 5.52 (dd, 1H, *J* = 5.4 and 4.3 Hz, H-6); 3.60 (dd, 1H, *J* = 15.4 and 4.3 Hz, H-6a); 3.26 (dd, 1H, *J* = 15.4 and 5.4 Hz, H-6a); 2.55 (s, 3H, H-4b); 2.48 (s, 3H, H-1b); 0.64 (d, 3H, *J* = 7.4 Hz, H-3b) ppm. ^{13}C-NMR (63 MHz; $CDCl_3$) δ: 171.9 (C4a); 169.7 (C1a); 167.5 (C5); 163.3 (C2); 136.1 (C7'a); 135.0 (C3); 127.1 (C3'a); 125.5 (C2'); 124.7 (C5'); 122.6 (C4'); 119.9 (C6'); 118.5 (C3a); 111.0 (C7'); 108.9 (C3'); 59.1 (C6); 28.3 (C6a); 26.6 (C4b and C1b); 14.3 (C3b) ppm. Found: C, 64.21; H, 5.40; N, 11.55. $C_{19}H_{19}N_3O_4$ requires C, 64.53; H, 5.38; N, 11.89.

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