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Reexamination of some reactions of 3-(D-galactosylidenehydrazino)-1,2,4-triazino[5,6-*b*]indole

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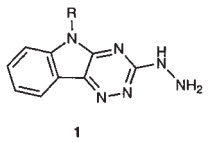
Incorrect structures described in the literature for the products of reactions of the title compound **6** were reexamined and corrected. Thus, the product of acetylation of **6** with acetic anhydride in the presence of pyridine was found to be the mono-*N*-penta-*O*-acetyl derivative **10** and not the previously described di-*N*-penta-*O*-acetyl derivative **7** [11]. Assignment of structure **10** was based on ^{1}H NMR data as well as its ability to undergo oxidative cyclization with Br_2/AcOH to give **12**. The previously assigned structure **7** would be incapable of undergoing such cyclization. The linear structure **12** rather than the angular regioisomer **3c** was assigned on the basis of its UV absorption pattern and ^{1}H NMR NOE spectra. Attempted preparation of **7** by increasing the duration of the reaction gave only compound **10**. A di-*N*-acetyl-penta-*O*-acetyl derivative, however, was obtained with acetyl bromide in the presence of pyridine to which structure **8** rather than structure **7** or **9** was assigned on the basis of ^{1}H NMR NOE studies. Acetylation of the triazolo-triazino-indole **11** gave a product identical to **12**; structure **15** previously assigned [11] to this product is, therefore, in error. Finally, the angular annelated structure **3e** previously ascribed [23] to the oxidative cyclization product of the 5-methylhydrazone congener of **6 (13)** is now rectified to the linear annelated structure **14**; the latter was found to be identical to the product obtained by *N*-methylation of the unequivocally linear 1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*]indole **11**. Compound **8** was found to exist in the preponderantly populated sickle (bent) conformation **18** in contrast to compounds **10** and **12** which were found to adopt the extended planar zigzag conformations **19** and **20** respectively.

1. Introduction

During our investigations on heterocyclizations of 3-hydrazino-1,2,4-triazino[5,6-*b*]indoles **(1a, b)** with one-carbon cyclizing reagents to condensed 1,2,4-triazolo-1,2,4-triazino[5,6-*b*]indoles [1–4], we came across some divergent structural assignments of the cyclization products. Whereas the linear annelated 1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*]indole structure **2** was assigned to the cyclization products by some investigators [5–13], the angular annelated 1,2,4-triazolo[3,4-*c*]1,2,4-triazino[5,6-*b*]indole structure **3** was adopted by others [14–23]. Non of the angular annelated structure assignments was based on coherent proof [14–23]. In some cases, results reported by the same research group have even fluctuated between the linear [11–13] and the angular structures [22, 23] without offering plausible rationales. Divergent results were also reported [22, 23] to the products of cyclization of **1** with related one-carbon cyclizing reagents known to afford one and the same product. Thus, cyclocondensation of **1** with acetic acid was reported to produce the linear annelated 3-methyl-1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*]indole **(2)** in contrast to the dehydrogenative cyclization of the hydrazone derived from **1** and acetaldehyde which was reported to yield the angular annelated 1-methyl-1,2,4-triazolo[3,4-*c*]1,2,4-triazino[5,6-*b*]indole **(3)** [22, 23]. We have been able to refute the angular structure **(3)** in favor of the linear structure **(2)** on the basis of chemical, UV-spectral, computational, and X-ray crystallographic evidences [1–4, 24]. In addition, we established that cyclization of **1** with acetic



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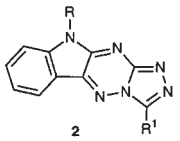

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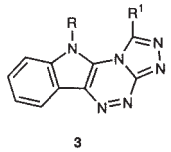

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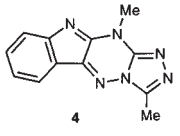

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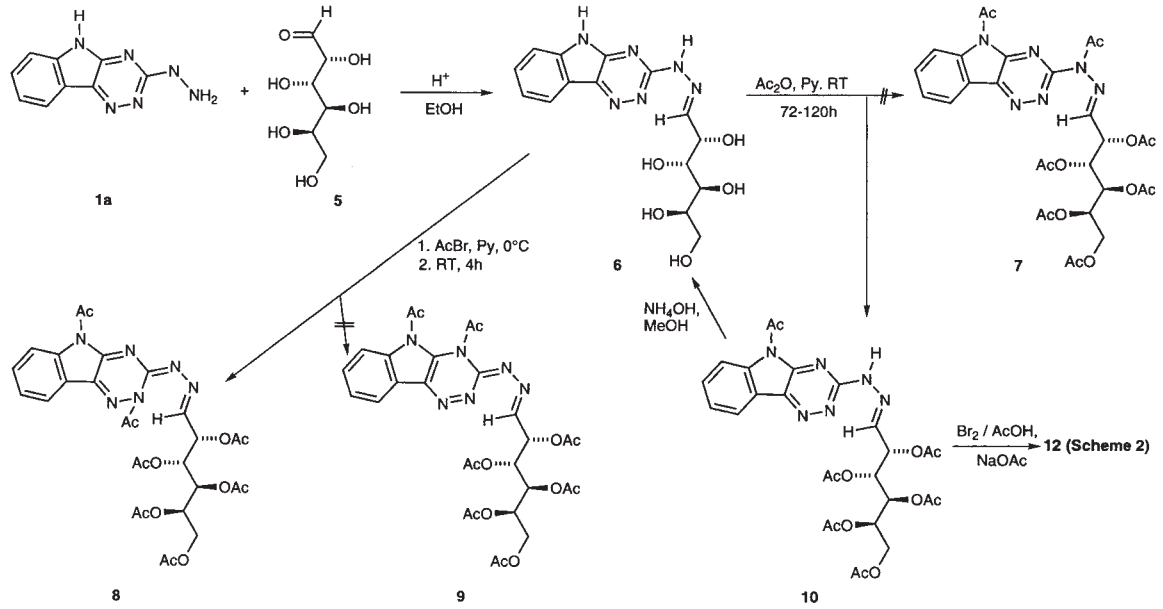
a, R = H
b, R = Me
c, R = H; R^1 = (1*S*, 2*S*, 3*S*, 4*R*)-1,2,3,4,5-pentaacetoxylpent-1-yl
d, R = Ac; R^1 = Me
e, R = Me; R^1 = (1*S*, 2*S*, 3*S*, 4*R*)-1,2,3,4,5-pentaacetoxylpent-1-yl
f, R = R^1 = Me

Scheme 1

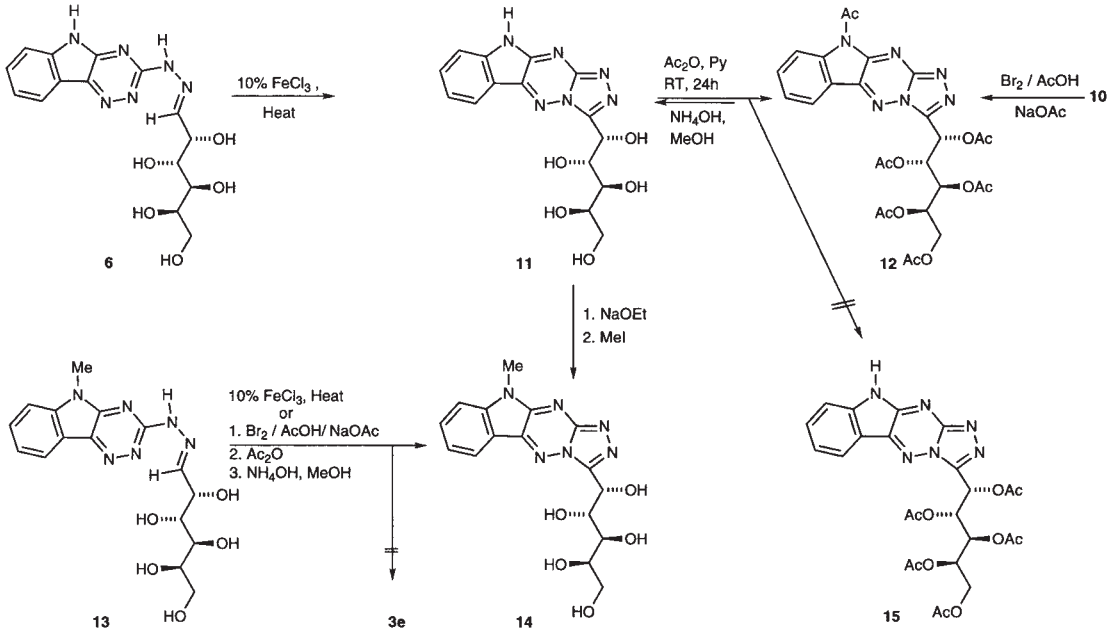


acid or cyclization of its acetaldehyde hydrazone gave one and the same linear product **2** [24]. In the present article we report on the rectification of some further erroneous results described in the literature concerning the reactions of 3-(D-galactosylidene-hydrazino)-1,2,4-triazino[5,6-*b*]indole **(6)**; the hydrazone derived from **1a** and D-galactose **(5)**. 3-Hydrazino-1,2,4-triazino[5,6-*b*]indoles **(1)** and hydrazones derived therefrom as well as 1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*] indoles possess multifarious biological activities including: antibacterial [1–3, 18, 25–29], antiviral [6, 30–34], hypotensive [15, 34, 35], blood-platelet aggregation inhibition [15, 35], antitumor [14], and thromboxane synthetase inhibition [35] activities. The logic of undertaking the task of rectifying inaccurate results is attributed to the importance of the structure-biological activity relationship in pinpointing the mode of action of active agents in order to modifying prototypes and achieve the best profile of activity. Evidently, erroneous structure assignments would jeopardize this systematic process.

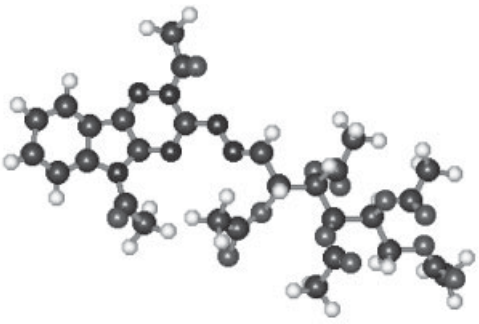
2. Investigations, results and discussion

3-(D-galactosylidenehydrazino)-1,2,4-triazino[5,6-*b*]indole **(6)** was prepared by El Ashry et al. [11] by condensation of 3-hydrazino-1,2,4-triazino[5,6-*b*]indole **(1a)** and D-galactose **(5)** (Scheme 1). Treatment of **6** with acetic anhydride in the presence of pyridine for 72 h at ambient temperature was reported [11] to effect *N*-acetylation of the pyrrole ring nitrogen and the hydrazone moiety as well as *O*-acetylation of the galactosylidene residue to give 5-acet-

Scheme 2



yl-3-[1-acetyl-1-(2,3,4,5,6-penta-*O*-acetyl-D-galactosylidene)]hydrazino-1,2,4-triazino[5,6-*b*]indole **(7)**. This result contradicted our findings on the protective acetylation of sugar heterylhydrazones using the same aforementioned conditions; these acetylations led only to *O*-acetylation of the hydroxyl groups of the sugar residue without *N*-acetylation of the hydrazone residue [2, 3, 36, 37]. Attempting to reproduce El Ashry's results, we subjected **6** to acetylation at the same conditions [11] to obtain a single crystalline product that melted at 218 °C [Ref. 11, m.p. for compound **7**: 206–207 °C] and showed IR absorptions due to NH (3443), *O*Ac (1751), and *N*Ac groups (1701 cm^{-1}) [Ref 11: *O*Ac (1750) and *N*Ac (1700 cm^{-1})]. ^1H NMR of the product revealed six 3H singlet signals at δ 3.05 (*N*Ac), 2.53, 2.14, 2.11, 2.02 and 2.01 ppm (5 *O*Ac) [Ref. 11 δ : 3.00 (*N*Ac), 2.49 (*N*Ac), 2.10, 2.07, and 2.00 ppm (unspecified number of *O*Ac groups)]. It analyzed for $C_{27}H_{30}N_6O_{11}$ and is, accordingly, assigned the structure of 5-acetyl-3-(2,3,4,5,6-penta-*O*-acetyl-D-galactosylidene)hydrazino-1,2,4-triazino[5,6-*b*]indole **(10)** rather than the previously described [11] di-*N*-acetyl-penta-*O*-acetylhydrazone structure **7** (Scheme 1). In support of structure **10** is its successful oxidative heterocyclization with Br_2/AcOH in the presence of NaOAc to 10-acetyl-3-[(1*S*, 2*S*, 3*S*, 4*R*)-1,2,3,4,5-pentaacetoxypent-1-yl]-1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*]indole **(12)** (Schemes 1 and 2). Structure **7** would be incapable of undergoing a similar cyclization due to the abscence of an imino hydrogen. Assignment of the linear structure **(12)** rather than the corresponding angular structure **3c** was based on the identity of the UV absorption pattern of the product with that of the unequivocally linear 10-acetyl-3-methyl-1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*]indole **(2d)**; both showed absorptions at 317, 270–269, and 263–262 (sh) nm [24]. It is worth mentioning that structural isomers such as anthracene and phenanthrene [38] and 6,7-diphenyl-1,2,4-triazolo[4,3-*b*] and [5,1-*c*]1,2,4-triazines [39, 40] have been distinguished depending on their UV absorption patterns. In addition, the nuclear Overhauser enhancement (NOE) difference spectrum of the obtained cyclization product revealed that selective irradiation of the pyrrole ring *N*Ac protons (δ 3.47 ppm) did not enhance any of the pentaacetoxypentyl chain protons as would be expected from the linear structure **12**. Efforts to obtain the heptaacetate derivative **7** by increasing the duration of acetylation to 120 h were unsuccessful; the hexaacetyl derivative **10** was the only product obtained. Applying more forcing conditions of heating **6** with acetic anhydride at reflux for 4 h led to a tarry residue containing intractable products. However, when hydrazone **6** was treated with acetyl bromide in the presence of pyridine for 4 h, a heptaacetyl derivative was obtained (Scheme 1). It showed IR absorptions at 1756 (*O*Ac), 1725 (*N*Ac), and 1700 cm^{-1} (*N*Ac); ^1H NMR of seven 3 H singlet signals at δ 2.96 and 2.64 (2 *N*Ac) and 2.25, 2.11, 2.08; 2.02 and 1.75 ppm (5 *O*Ac). The product analyzed for $C_{29}H_{32}N_6O_{12}$ and its MS revealed the molecular ion [M^+] at m/z 656.2. These data agree with structure **7**, **8** or **9** (Scheme 1) for the heptaacetyl derivative. The NOE difference spectra of the heptaacetyl derivative obtained by selective irradiation of either of the two *N*Ac group signals at δ 2.96 and 2.64 ppm revealed that neither caused enhancement of the other. Irradiation of either of the two *N*Ac groups, however, caused enhancement of one of the five *O*Ac groups of the penta-*O*-acetyl-D-galactosylidene residue. Thus, irradiation of the *N*Ac signal at δ 2.96 ppm resulted in 17% positive enhancement of the *O*Ac at δ 1.63 ppm. Saturation of the



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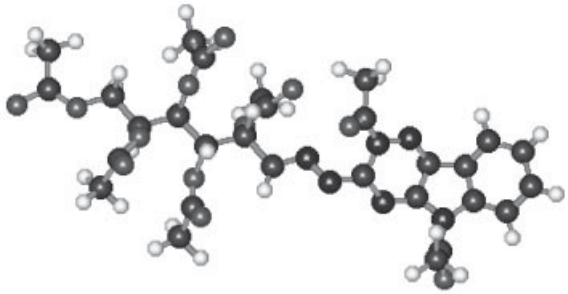

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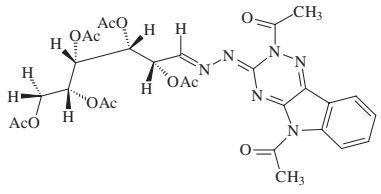
*N*Ac group at δ 2.64 ppm caused 26% positive enhancement of the same *O*Ac (δ 1.63 ppm). These results are in agreement with the 2,5-diacetyl-3-(2,3,4,5,6-penta-*O*-acetyl-D-galactosylidene)hydrazino-1,2,4-triazino[5,6-*b*]indole structure **(8)** rather than with the alternative isomeric structures **7** or **9**. Molecular models and computer optimized geometries of compound **8** indicated that the penta-*O*-acetyl-D-galactosylidene C2-*O*Ac is in proximity to the pyrrole ring *N*Ac in rotamer **16** and to the triazine ring *N*2-Ac in rotamer **17**. The models and optimized geometries also indicated that the triazine ring *N*2-Ac recline nearer to the alditolylidene C2-*O*Ac in rotamer **17** than the pyrrole *N*Ac to the alditolylidene C2-*O*Ac in rotamer **16**. It is relevant, therefore, to assign the ^1H NMR *N*Ac signal at δ 2.64 ppm to the triazine ring *N*2-Ac which effected the larger enhancement (26%) and the *N*Ac signal at δ 2.96 ppm to the pyrrole ring *N*Ac which effected the smaller enhancement (17%) to the alditolylidene C2-*O*Ac.



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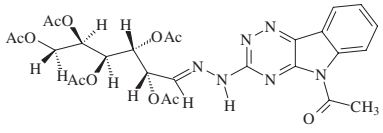

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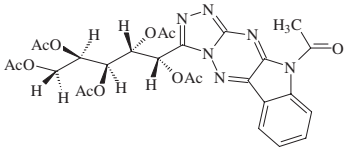

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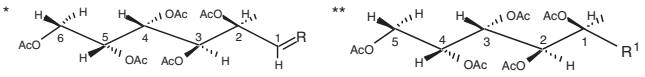
Table: Comparison of the found coupling constants (J Hz) of the polyacetoxyalky chain vicinal CH protons of 8, 10 and 12 with those expected [43–47] from their ideal extended planar zigzag conformations.

compound	J value								
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{4,5'}$	$J_{5,5'}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
Expected for the extended planar zigzag conformation of **8** and **10***	<4	<4	>7	<4	–	–	<4	>7	>10
Found for **8**	5.8	5.2	5.5	3.5	–	–	5.6	6.3	11.2
Found for **10**	3.9	2.7	10.8	3.5	–	–	7.0	9.8	12.5
Expected for the extended planar zigzag conformation of **12****	<4	>7	<4	<4	>7	>10	–	–	–
Found for **12**	2.5	9.8	2.5	4.9	7.3	11.6	–	–	–



The *N*-acetylation of 3-ethylidenehydrazino-1,2,4-triazino[5,6-*b*]indole as a simpler relevant hydrazone model has been studied from a quantum chemical point of view using Austin Model 1 [41]. Reactivity of the reactants, intermediates, transition states, and products as well as heats of formation have been taken into consideration [42]. The study decisively predicted that the first acetylation step is that involving the pyrrole ring nitrogen rather than any of the three nitrogens of the amidine system consisting of: the hydrazone residue N2 and the triazine ring N2 and N4. The study, however, failed to predict the preference of formation of any of the three possible heptaacetyl derivatives **7**, **8**, or **9** [42].

Acetylation of compound **11** at ambient temperature with Ac_2O/pyridine for an overnight has been claimed [11] to effect *O*-acetylation of the polyhydroxylalkyl chain but not *N*-acetylation of the pyrrole ring nitrogen to give **15** (Scheme 2). *N*- and *O*-Acetylation of **11** to give **12** was reported to take place only after a much longer time (3 days) [11]. Acetylation of the sugar chain OH groups prior to the NH of the pyrrole ring appeared unreasonable. Accordingly, we carried out the acetylation of **11** for 24 h using the same reported conditions [11] and obtained compound **12** which was found to be indistinguishable from that prepared by oxidative cyclization of **10** (Schemes 1 and 2). Deacetylation of **12** with NH_4OH/MeOH gave **11**.

Dehydrogenative cyclizations of the D-galactose hydrazones **6** and **13** derived from the unsubstituted hydrazine **1a** and its 5-methyl congener **1b** respectively with 10% $FeCl_3$/EtOH were reported to afford products of divergent structural isomerism [11, 23] (Scheme 2). Whereas the cyclic product from hydrazone **6** was assigned the linear annelated product **11** [11], the cyclic product from hydrazone **13** was assigned the corresponding angular annelated isomer **3e** [23]. In terms of our previous results [1–4, 24] this divergency seemed inexplicable and needs reexamination. We subjected the 5-methylhydrazone **13** [2, 23] to cyclization with 10% $FeCl_3$/EtOH according to El Ashry et al. [23] (Scheme 2) and obtained a product identical to the linearly annelated 3-[(1*S*, 2*S*, 3*S*, 4*R*)-1,2,3,4,5-pentaacetoxypent-1-yl]-10-methyl-1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*]indole **(14)** prepared by: (a) one-pot oxidative cyclization-acetylation of the methylhydrazone **13** with Br_2/AcOH in the presence of NaOAc, followed by treatment with Ac_2O and de-*O*-acetylation of the obtaind product with NH_4OH/MeOH [2] and (b) *N*-methylation of the unequivocally linear **11**. Compound **14** prepared by the three routes possessed the same R_f value on TLC, melted with decomposition at 257 °C [Ref. 23: the product erroneously ascribed the angular structure **3e**, m.p. 238–240 °C without decomposition], and showed UV absorptions at 320–318, 269, and 264 (sh) nm. The latter UV absorption pattern is identical to that of the unequivocally linear 3,10-dimethyl-1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*]indole (**2f**) [2] and the inevitably linear 3,11-dimethyl-1,2,4-triazolo[4,3-*b*]1,2,4-triazino[5,6-*b*]indole (**4**) [24]. This result confirmed the linear annelated structure **14** and disagrees with the angular annelated structure **3e** assigned [23] for the product cyclization of hydrazone **13**.

The coupling constants (J values) of the polyacetoxyalkyl chain vicinal CH protons of compounds **8**, **10**, and **12** were found amenable for analysis as a tool to provide their in-solution most preponderantly populated conformations at ambient temperature. Table 1 includes the measured J values for these protons compared with the values expected [43–47] for vicinal CH–CH in gauche (dihedral angle 60, J: < 4 Hz) and antiparallel orientation (dihedral angle 180°, J: > 7 Hz). Compound **8** showed slight deviations of H1-H2, H2-H3, and H4-H5 from the gauche orientations and a divergence of H3-H4 from the antiparallel orientation to be expected for the ideal extended planar zizag conformation. The deviation of the H3-H4 is explained to be due to rotation around the C3-C4 bond of the polyacetoxyalkyl chain to bring H3-H4 in a gauche orientation and, accordingly, relive the adverse steric interactions of the poly-acetoxyalkyl chain C2-*O*Ac with the pyrrole ring *N*Ac and the triazine ring *N*2-Ac groups. Accordingly, compound **8** exists in the most abundantly populated sickle (bend) conformation **18**. The coupling constants pattern of compound **10** is: H1-H2, H2-H3, and H4-H5 possess values typical of gauche orientations while H3-H4 possesses an antiparallel orientation. These orientations are in agreement with the extended planar zigzag conformation **19**. The measured values of $J_{5,6}$ and $J_{5,6'}$ (Table) indicated a moderate rotation around the C5-C6 bond in order alleviate the interaction between C5-*O*Ac and C6-*O*Ac groups. Finally, the coupling constants pattern of compound **12** (Table) shows that H1-H2, H3-H4, and H4-H5 are in gauche orientations whereas H2-H3 are in an antiparallel orientation. These data are in agreement with the extended planar zigzag conformation **20**.

3. Experimental

Melting points were determined on MEL-TEMP II melting point apparatus in open glass capillaries and are uncorrected. The UV spectrum were recorded on a Shimadzu UV-160A UV/Vis spectrophotometer. The IR spectra were recorded for potassium bromide discs on a Pye-Unicam SP1025 spectrophotometer. ^{1}H NMR spectra were carried out at ambient temperature (~25 °C) with a Varian EM-390, with a Brucker AC-250, or with a Jeol JNM-ECA 500 spectrometers using tetramethylsilane (TMS) as an internal standard. MS was recorded on a Hewlett-Packard 5995 GC-MS system. Molecular modeling and optimized geometries were obtained using Hyperchem release 6.03 for Windows Molecular Modeling System, Copyright 2000, Hypercube, Inc. Homogeneity of the products and follow up of the reactions were checked by ascending TLC on plates precoated with silica gel G (E. Merck; layer thickness 0.25 mm), used without pretreatment. All ratios of the used solvent systems were volume to volume (V/V); the distance of the solvent travel was 5 cm and the spots were visualized by exposure to iodine vapour for a few minutes. Elemental microanalyses were performed at the Microanalytical Unit, Cairo University, Cairo, Egypt. The prepared compounds gave satisfactory elemental analyses to +/− 0.3% .

3.1. 3-(D-Galactosylidenehydrazino)-1,2,4-triazino[5,6-b]indole (6)

3.1.1. Method A: Direct condensation of hydrazine **1a** and D-galactose (5)

A suspension of hydrazine **1a** (5 mmol) in EtOH (50 ml) was treated with a solution of D-galactose (**5**, 5 mmol) in H_2O (5 ml) containing 2 drops of acetic acid and the mixture was heated under reflux with stirring for 2 h. The mixture was left to attain room temperature and the product was filtered, washed with H_2O, dried, and crystallized from DMF to give **6** as pale yellow crystals (75%), m.p.: 221–223 °C (decomp), Lit. [11], m.p.: 195–197 °C; TLC in 1 : 1 $CHCl_3$/MeOH, R_f: 0.57; IR: 3355 (broad, OH + NH), and 1614 cm^{-1} (C=N).
$C_{15}H_{18}N_6O_5$(362)

3.1.2. Method B: Deacetylation of compound **10**

A mixture of **10** (2 mmol) in MeOH (50 ml) was treated with 20% aqueous NH_4OH solution (15 ml) and kept at room temperature for 24 h. Evaporation of the solvent gave a residue which crystallized from DMF to give **6** (62%), m.p. and mixed m.p.: 221–223 °C (decomp), TLC and IR data are identical to those of **6** prepared by method A.

3.2. 5-Acetyl-3-(2,3,4,5,6-penta-O-acetyl-D-galactosylidene)hydrazino-1,2,4-triazino[5,6-b]indol (10)

3.2.1. Method A: Acetylation of hydrazone **6** for 72 h

A mixture of **6** (3 mmol), pyridine (25 ml) and acetic anhydride (50 ml) was stirred at room temperature for 72 h. The reaction mixture was poured onto crusted ice and the product was filtered, washed with H_2O, dried, and crystallized from a $CHCl_3$/EtOH mixture (1 : 1) to give **10** as colorless reedles (70%), m.p. 218 °C; TLC in 19 : 1 $CHCl_3$/MeOH, R_f: 0.61; IR: 3443 (NH), 1751 (*O*Ac), 1701 (*N*Ac) and 1618 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 8.74 (d, 1 H, triazino-indole H6, $>J_{6,7}$ 8.7 Hz), 8.52 (d, 1 H, triazino-indole H9, $J_{9,8}$ 7.5 Hz),7.82 (dd, 1H, triazino-indole H7, $J_{7,8}$ 7.5 Hz), 7.63 (dd, 1 H, triazino-indole H8), 6.52 (d, 1H, CH=N, $J_{1,2}$ 3.9 Hz), 5.57 (dd, 1 H, penta-*O*-acetyl-D-galactosylidene H2, $J_{2,3}$ 2.7 Hz), 5.41 (dd, 2 H, penta-*O*-acetyl-D-galactosylidene H3 + H4, $J_{3,4}$ 10.8 Hz, $J_{4,5}$ 3.5 Hz), 5.35- 5.30 (m, 1 H, penta-*O*-acetyl-D-galactosylidene H5) 4.24, (dd, 1 H, penta-*O*-acetyl-D-galactosylidene H6, $J_{5,6}$ 7.0 Hz), 3.87 (dd, 1 H, penta-*O*-acetyl-D-galactosylidene H6′, $J_{5,6'}$ 9.8 Hz, $J_{6,6'}$ 12.5 Hz), 3.05 (s, 3 H, *N*Ac), 2.53, 2.14, 2.11, 2.02 and 2.01 ppm (5 s, 3 H each, 5 *O*Ac).
$C_{27}H_{30}N_6O_{11}$ (614)

3.2.2. Method B: Acetylation of hydrazone **6** for 120 h

A mixture of **6** (2.8 mmol), pyridine (5 ml) and acetic anhydride (50 ml) was stirred for 120 h. The product was filtered, washed with H_2O, dried and crystallized from a $CHCl_3$/EtOH mixture (1 : 1) to give **10** (70%); m.p. and mixed m.p.: 218 °C.

3.3. Attempted preparation of 7

A mixture of **6** (3 mmol) and acetic anhydride (50 ml) was heated under reflux for 4 h. The mixture was evaporated to give a dark syrupy residue. TLC of this residue showed that it contained a large number of products.

3.4. 2,5-Diacetyl-3-(2,3,4,5,6-penta-O-acetyl-D-galactosylidene)hydrazino-1,2,4-triazino[5,6-b]indole (8)

A mixture of **6** (3 mmol) and pyridine (7 ml) was cooled to 0 °C and gradually treated with acetyl bromide (10 ml) while striring. The mixture was kept at room temperature for 4 h, poured onto an ice-water mixture, and the product which separated was filtered, washed with H_2O, dried. It crystallized from EtOH to give **8** as colorless needles (72%), m.p.: 212 °C, TLC in 19 : 1 $CHCl_3$/MeOH, R_f: 0.60; IR: 1756 (*O*Ac), 1725 (*N*Ac), 1700 (*N*Ac), and 1644 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 8.73 (d, 1 H, triazino-indole H6, $J_{6,7}$ 8.6 Hz), 8.44 (d, 1 H, triazino-indole H9, $J_{9,8}$ 7.5 Hz), 7.82 (dd, 1 H, triazino-indole H7, $J_{7,8}$ 8.0 Hz), 7.62 (dd, 1 H, triazino-indole H8), 6.42 (d, 1 H, CH=N, $J_{1,2}$ 5.8 Hz), 5.69 (t, 1 H, penta-*O*-acetyl-D-galactosylidene H2, $J_{2,3}$ 5.2 Hz), 5.10 (t, 1 H, penta-*O*-acetyl-D-galactosylidene H3, $J_{3,4}$ 5.5 Hz), 4.97–4.95 (m, 1 H, penta-*O*-acetyl-D-galactosylidene H4), 3.89 (dd, 1 H, penta-*O*-acetyl-D-galactosylidene H6, $J_{6,5}$ 5.6 Hz), 3.80 (dd, 1 H, penta-*O*-acetyl-D-galactosylidene H5, $J_{5,4}$ 3.5 Hz), 3.71 (dd, 1 H, penta-*O*-acetyl-D-galactosylidene H6′, $J_{6,5'}$ 6.3 Hz, $J_{6,6'}$ 11.2 Hz), 2.96 (pyrrole ring *N*-Ac), 2.64 (triazine ring *N*2-Ac) 2.25, 2.11, 2.08, 2.02 (4 s, 3 H each, penta-*O*-acetyl-D-galactosylidene 4 *O*Ac), and 1.63 ppm (s, 3 H, penta-*O*-acetyl-D-galactosylidene C2-*O*Ac); MS: m/z 656.2 (M^+, 3.5%).
$C_{29}H_{32}N_6O_{12}$ (656)

3.5. 3-[(1S, 2S, 3S, 4R)-1,2,3,4,5-Pentaacetoxypent-1-yl]-10H-1,2,4-triazolo[4,3-b]1,2,4-triazino[5,6-b]indole (11)

3.5.1. Method A: Dehydrogenative cyclization of **6** with $FeCl_3$/EtOH

A suspension of **6** (3 mmol) in EtOH (100 ml) was gradually treated with 10% ethanolic $FeCl_3$ solution (10 ml) and heated at reflux for 30 min. The solvent was removed and the obtained residue was washed with H_2O (3 × 10 ml) and crystallized from DMF to give **11** as yellow crystals (58%), m.p.: 275–278 °C (decomp), Lit. [11]; m.p.: 273–275 °C; TLC in 1 : 1 $CHCl_3$/MeOH, R_f: 0.55; IR: 3389 (OH), 3211 (NH), and 1616 cm^{-1} (C=N); λ_{max}^{EtOH} nm (log ε): 320 (4.79), 2.70 (5.35) and 262 (sh).
$C_{15}H_{16}N_6O_5$ (360)

3.5.2. Method B: Deacetylation of compound **12**

A solution of **12** (7.6 mmol) in MeOH (50 ml) was treated with 20% aqueous NH_4OH solution (15 ml) and kept at room temperature for 24 h. Evaporation of the solvent gave a residue which crystallized from DMF to give **11** (62%); m.p. and mixed m.p. with **11** prepared by method A: 275–278 °C. TLC and IR data are identical to those of **11** prepared by method A.

3.6. 10-Acetyl-3-[(1S, 2S, 3S, 4R)-1,2,3,4,5-pentaacetoxypent-1-yl]-1,2,4-triazolo[4,3-b]1,2,4-triazino[5,6-b]indole (12)

3.6.1. Method A: Acetylation of compound **11**

A mixture of **11** (3 mmol), pyridine (5 ml) and acetic anhydride (25 ml) was stirred at room temperature for 24 h and then poured onto crushed ice. The obtained product was filtered, washed with H_2O, dried and crystallized from EtOH to give **12** as yellow needles (80%), m.p.: 227–230 °C, Lit. [11], m.p.: 220–221 °C; TLC in 19 : 1 $CHCl_3$/MeOH, R_f: 0.64; IR: 1748 (*O*Ac), 1723 (*N*Ac), and 1605 cm^{-1} (C=N) λ_{max}^{EtOH} nm (log ε): 317 (5.05), 270 (5.74) and 262 (sh); ^{1}H NMR ($CDCl_3$): δ 8.71 (d, 1 H, triazolo-triazino-indole H6, $J_{6,7}$ 8.6 Hz), 7.27 (d, 1 H, triazolo-triazino-indole H9, $J_{9,8}$ 7.1 Hz), 7.81 (t, 1 H, triazolo-triazino-indole H7, $J_{7,8}$ 8.0 Hz), 7.57 (t, 1 H, triazolo-triazino-indole H8), 6.16 (d, 1 H, pentyl H1, $J_{1,2}$ 2.5 Hz), 5.75 (dd, 1 H, pentyl H2, $J_{2,3}$ 9.8 Hz), 5.65 (dd, 1 H, pentyl H3, $J_{3,4}$ 2.5 Hz), 5.46–5.44 (m, 1 H, pentyl H4), 4.33 (dd, 1 H, pentyl H5, $J_{5,4}$ 4.9 Hz), 3.98 (dd, 1 H, pentyl, H5′, $J_{5',4}$ 7.3 Hz, $J_{5,5'}$ 11.6 Hz), 3.47 (s, 3 H, *N*Ac), 2.25 (s, 6 H, 2 *O*Ac), 2.05 (s, 3 H, *O*Ac), and 1.97 ppm (s, 6 H, 2 *O*Ac).
$C_{27}H_{28}N_6O_{11}$ (612)

3.6.2. Method B: Oxidative cyclization of compound **10** with Br_2/AcOH

A solution of bromine (2 mmol) in glacial acetic acid (5 ml) was gradually added at ambient temperature to a stirred mixture of **10** (7 mmol) and anhydrous sodium acetate (6 mmol) in glacial acetic acid (20 ml). Stirring was continued for three additional hours in the dark and the mixture was poured onto crushed ice and the separated product was filtered, washed with H_2O and dried. It crystallized from EtOH to give **12** (78%), m.p. and mixed m.p. with **12** prepared by method A: 227–230 °C. R_f, IR, UV, and ^{1}H NMR are identical to those of **12** prepared by method A.

3.7. 3-[(1S, 2S, 3S, 4R)-1,2,3,4,5-Pentahydroxypent-1-yl]-10-methyl-1,2,4-triazolo[4,3-b]1,2,4-triazino[5,6-b]indole(14)

3.7.1. Method A: Methylation of compound **11**

Compound **11** (3 mmol) was treated with a solution of sodium (3 mmol) in absolute EtOH (25 ml) and the mixture was stirred at room temperature for 30 min. The product which separated was filtered and washed with ether to give the sodium salt of **11** (2.5 mmol). A mixture of the sodium salt (2.4 mmol) and methyl iodide (2.5 mmol) in a closed tube was heated at 100 °C for 20 min and then allowed to attain ambient temperature and then cooled in a mixture of dry ice and acetone. The tube was opened and the obtained mass was crystallized from H_2O/EtOH to give **14**, m.p.: 257 °C; TLC in 1 : 1 $CHCl_3$/MeOH, R_f: 0.59; IR: 3364 (OH) and 1610 cm^{-1} (C=N); λ_{max}^{EtOH} nm (log ε): 320 (3.75), 269.8 (4.24) and 264 (sh); ^{1}H NMR [$(CD_3)_2SO$]: δ 7.95 (d, 1 H, ArH), 7.50–7.15 (m, 3 H, 3 ArH), 5.35 (m, 1 H, pentyl H), 4.65 (d, 1 H, exchangeable, OH), 4.40 (t, 1 H, pentyl H), 4.20 (m, 2 H, pentyl H), and 3.65 ppm (s, 3 H, NCH_3), the rest of the pentyl chain H and OH signals appeared as a large signal centered at δ 3.35 due to association with the DMSO-d_6.
$C_{16}H_{18}N_6O_5$ (374)

3.7.2. Method B: Dehydrogenative cyclization of hydrazon **13** with $FeCl_3$/EtOH

A suspension of hydrazone **13** (4 mmol) in EtOH (50 ml) was treated with 10% ethanolic $FeCl_3$ solution (20 ml) and heated at reflux for 30 min. The product which separated after attaining room temperature was filtered and crystallized from a H_2O-MeOH-mixture to give **14** (60%) identical to **14** prepared according to method A.

3.7.3. Method C: One-pot oxidative cyclization-acetylation of hydrazone **13**

Cyclization of **13** with Br_2/AcOH-NaOAc followed by acetylation with Ac_2O and de-*O*-acetylation with NH_4OH/MeOH was performed as previously described [2].

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