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Sterically Controlled Regiospecific Cyclization of Aldose-5-ethyl-1,2,4-triazino[5,6-*b*]indol-3-ylhydrazones to Linearly Annelated 3-Polyhydroxyalkyl-10-ethyl-1,2,4-triazolo-[4′,3′:2,3]-1,2,4-triazino[5,6-*b*]indoles

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Summary. Condensation of aldoses with 5-ethyl-3-hydrazino-1,2,4-triazino[5,6-*b*]indole gave the corresponding aldose-5-ethyl-1,2,4-triazino[5,6-*b*]indol-3-ylhydrazones which were acetylated to their poly-O-acetyl derivatives. The latter underwent sterically controlled regiospecific oxidative cyclization with bromine in acetic acid and sodium acetate to sterically favourable linearly annelated 3-polyacetoxyalkyl-10-ethyl-1,2,4-triazole[4′,3′:2,3]-1,2,4-triazino[5,6-*b*]indoles rather than to their sterically unfavourable angularly annelated regioisomers. The regiospecific outcome of this heterocyclization is discussed in terms of electronic as well as steric factors, and the assigned structures have been corroborated on the basis of chemical as well as spectroscopic evidence. De-O-acetylation of the acetoxyindoles with ammonium hydroxide in methanol gave the title compounds. Representative members of the prepared compounds were tested for antimicrobial activity.

Keywords. Sugar hydrazones; Heterocyclization; Acyclic C-nucleosides; Antimicrobial activity.

Introduction

Heterocycles carrying carbon-carbon linked alditolyl chains constitute an important class of acyclic C-nucleosides because of their various biological activities and medicinal applications [1, 2]. A few naturally occurring members of this class of compounds, pyridindolol [3, 4] and the two antibiotics CV-1 [5] and gualamycin [6–8], have been isolated from different species of *Streptomyces*. The majority of these compounds, however, have been prepared synthetically [1, 2]. Valuable biological activities are known to be associated with the 1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-*b*]indole skeleton. Thus, 3-hydrazino-1,2,4-triazino[5,6-*b*]indoles (*e.g.* **2**) show antihypertensive [9,10], antiviral [9], blood-platelet aggregation inhibitory [10, 11], analgesic [12], and antibacterial activities [13]. Hydrazones derived from **2**

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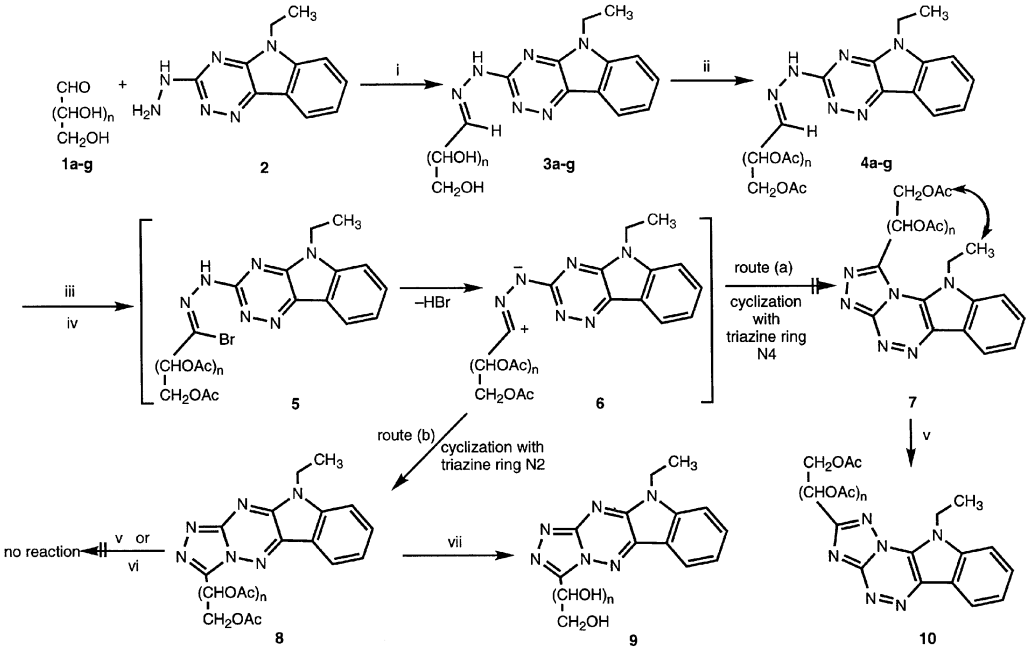
have been found to possess antiumor activity against P388 lymphocytic leukemia in mice [14] and antibacterial activity [15, 16]. In addition, 1,2,4-triazolo[3′,4′:3,4]- and -[4′,3′:2,3]-1,2,4-triazino[5,6-*b*]indoles (*e.g.* **7** and **8**) exhibit antiviral [17–20] and antibacterial properties [21]. In continuation of our studies on the synthesis of biologically active acyclic C-nucleosides [22–26], we here report the synthesis of the title compounds. In principle, the glycosyl and alditolyl residues of C-nucleosides and their acyclic analogues render them more hydrophilic compared to the parent heterocyclic bases. Accordingly, the former compounds are usually expected to possess much more potent activities as a result of overcoming the blood-brain and/or the blood-cerebrospinal fluid barrier [27] and hence ensure better pervasion into biological systems [28].

Results and Discussion

Reaction of aldopentoses or aldohexoes like *D*-arabinose (**1a**), *L*-arabinose (**1b**), *D*-ribose (**1c**), *D*-xylose (**1d**), *D*-galactose (**1e**), *D*-glucose (**1f**), and *D*-mannose (**1g**) with 5-ethyl-3-hydrazino-1,2-4-triazino[5,6-*b*]indole (**2**) [9] gave the corresponding yellow to orange aldose-5-ethyl-1,2,4-triazino[5,6-*b*]indol-3-ylhydrazones **3a**–**g** (Scheme 1). These hydrazones showed OH, NH, and C=N IR adsorptions as well as ^{1}H NMR hydrazono (=N–NH, exchangeable with D_2O), azomethine (–CH=N–), benzo group and ethyl group proton signals. In most cases, the alditolyl group protons were associated with the solvent absorption (*DMSO*-d_6) forming a



Scheme 1. Reagents and conditions: i, H_2O/EtOH, 100°C, 15 min, RT, 24 h; ii, Ac_2O/pyridine, RT, 24 h; iii, Br_2/AcOH; iv, NaOAc, RT, 2 h ; v, AcOH, reflux, 2 h; vi, fusion; vii, NH_4OH/MeOH, RT, 24 h $n = 3$: **a** = *D*-arabino-, **b** = *L*-arabino-, **c** = *D*-ribo-, **d** = *D*-xylo-; $n = 4$: **e** = *D*-galacto-, **f** = *D*-gluco-, **g** = *D*-manno-

broad signal at $\delta = 3.70\text{-}3.35$ ppm. The mass spectrum of **3b** showed an $M^+ + 1$ peak at $m/z = 361$.

Acetylation of **3a**–**g** with acetic anhydride in the presence of pyridine at room temperature gave the corresponding poly-O-acetyl derivatives **4a**–**g**, showing the expected OAc and C=N IR absorptions as well as ^{1}H NMR signals of four or five O-acetyl groups.

Oxidative cyclization of the hydrazone acetates **4a**–**g** with bromine in acetic acid in the presence of anhydrous sodium acetate or one-pot oxidative cyclization/acetylation of hydrazones **3a**–**g** with the same reagent followed by treatment with acetic anhydride afforded a single crystalline product in each case that lacked the IR absorption of the NH group and the ^{1}H NMR signals of the azomethine (CH=N) and hydrazone (=N–NH–) protons characteristic for the parent hydrazones **3a**–**g** and their acetates **4a**–**g**. These spectroscopic data together with the elemental analyses of the cyclization products were in agreement both with the angularly annelated 1-polyacetoxyalkyl-10-ethyl-1,2,4-triazolo[3′,4′:3,4]-1,2,4-triazino[5,6-*b*]indole (**7**) and the linearly annelated 3-polyacetoxyalkyl-10-ethyl-1,2,4-triazole[4′,3′:2,3]-1,2,4-triazino[5,6-*b*]indole (**8a**–**g**) structure types (Scheme 1). Formation of **7** or **8** presumably took place *via* hydrazonoyl bromide (**5**) and nitrilimine (**6**) intermediates [29] as a result of nucleophilic attack of the triazine ring N4 (route a) or N2 (route b) on the nitrilimine carbon.

Previous results on heterocyclization of 5-hydrazino-1,2,4-triazino[5,6-*b*]indoles with one-carbon cyclizing reagents indicate that the choice of routes (a [10, 30–34] or b [17, 18, 35–41]) depends on electronic factors that enhance the nucleophilicity of N4 or N2 of the triazine ring [42–52]. Surprisingly, steric factors that may dominate electronic factors in determining the regiospecificity (or regioselectivity) of this cyclization have been disregarded. Examination of molecular models and computer optimized geometries predicted that N5-unsubstituted 3-hydrazino-1,2,4-triazino[5,6-*b*]indoles may cyclize with one-carbon cyclizing reagents to give linearly annelated 3-substituted 1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-*b*]indoles and/or the regioisomeric angularly annelated 1-substituted 1,2,4-triazole[3′,4′:3,4]-1,2,4-triazino[5,6-*b*]indoles; both structures are free of unfavourable steric interactions. In fact, results reported in the literature did assign products of this cyclization either angular [10,30–32] or linear [38–41] structures only on the basis of electronic factors. In no case, however, the formation of a mixture of both regioisomers has been documented. In contrast, molecular models and computer optimized geometries predicted that cyclization of N5-substituted 3-hydrazino-1,2,4-triazino[5,6-*b*]indoles (*e.g.* **2**) with one-carbon cyclizing reagents [53] would be sterically controlled to preferably produce the linearly annelated 3,10-disubstituted regioisomer (*e.g.* **8**) rather than the angularly annelated 1,10-disubstitutesd regioisomer (*e.g.* **7**). The angular structure (*e.g.* **7a**) suffers crowding of the C1 and N10 substituents (Fig. 1).

Entry 3 shows the adverse steric interaction between the ethyl group and the alditolyl chain even when the methyl portion of the ethyl group is directed away from the alditolyl chain. Entry 4 shows, on the other hand, that the linear structure (*e.g.* **8a**) is free of such adverse steric interactions. This argument is taken to favor the assignment of the linearly annelated structure (**8**) to the cyclization products which also concords with electronic factors favoring cyclization at the more nucleophilic

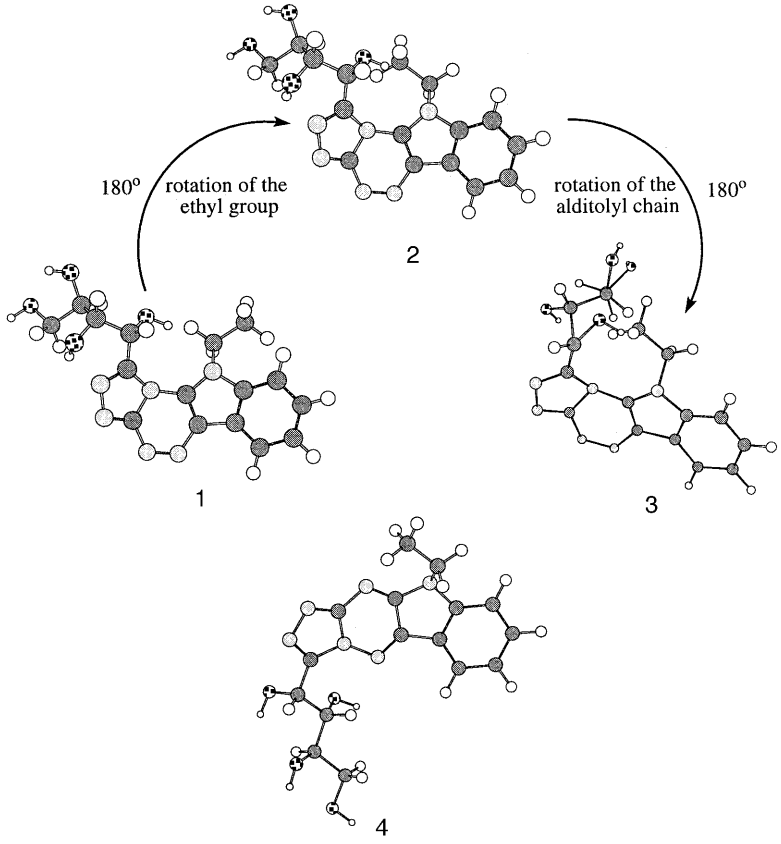


Fig. 1. Computer optimized geometries of three rotomers of angularly annelated **7a** (1–3) and its linearly annelated regioisomer **8a** (4)

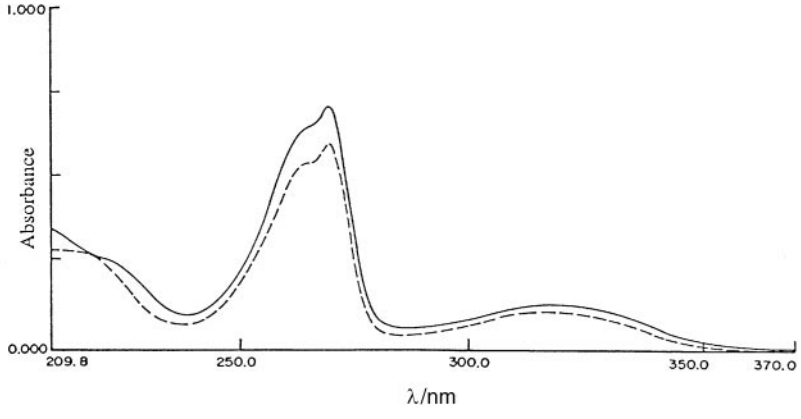
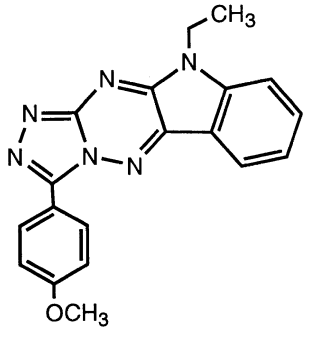


Fig. 2. UV spectra of **8c** (-, 1.71×10^{-6} *M*) and the unequivocally prepared compound **14** (——, 3.75×10^{-7} *M*) in methanol

N2 of the 1,2,4-triazine ring rather than at the less nucleophilic N4 [42–49]. Evidently, both electronic and steric factors operate synergistically towards the regiospecific production of the linear isomer (**8**). Experimental evidence in favor of the linear structure **8** are:

(a) Attempted acid or thermally induced *Dimroth* rearrangement by heating compounds **8** in acetic acid or by fusion above their melting points gave the unchanged starting compounds. These results are in harmony with the 1,2,4-triazino[4,3-*b*]-1,2,4-triazine type of fusion (*e.g.* **8**) known to be incapable of undergoing *Dimroth* rearrangement and contrasts the facile rearrangement of the 1,2,4-triazino[3,4-*c*]-1,2,4-triazine type of fusion (*e.g.* **7**) to the 1,2,4-triazole[5,1-*c*]-1,2,4-triazine type (*e.g.* **10**) [44–46] (Scheme 1).

(b) The UV spectra of the cyclization products **8a–g** were found to be identical with the spectrum of the linearly annelated 10-ethyl-3-methyl-1,2,4-triazolo-[4′,3′:2,3]-1,2,4-triazino[5,6-*b*]indole (**14**) independently prepared by cyclocondensation of 3,4-diamino-5-methyl-1,2,4-triazole hydrochloride (**11**) [47] and 1-ethylisatin (**12**) [54] (Scheme 2). An unequivocal synthesis of the 3,10-dimethyl congener **15** has been previously accomplished by cyclocondensation of **11** and **13** [35]; the assignment of the linear structure **15** was based on the preference of nucleophilic attack of the more reactive C4-NH_2 of **11** at the more reactive C3-carbonyl carbon of **13** [47]. Compounds **8a–g** and **14** showed two absorption maixma at λ 321-318 and 270-268 nm and a shoulder at λ 265-264 nm (Fig. 2). X-Ray crystal analysis [47] would be the tool of choice to differentiate between structures **7** and **8**. Unfortunately, attempts to develop suitable crystals were unsuccessful. Fortunately, however, crystals of 10-ethyl-3-(4-methoxyphenyl)-1,2-4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-*b*]indole (**16**), prepared by a similar



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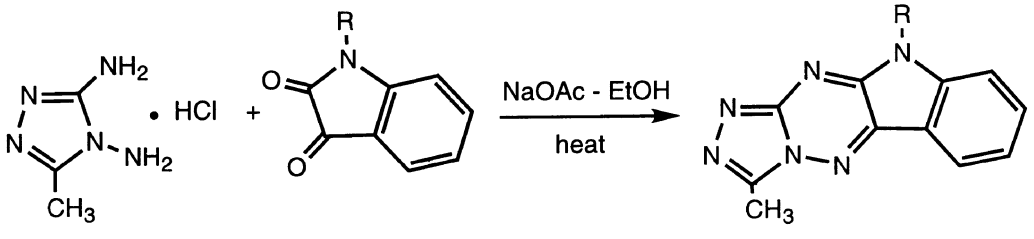

11 **12** (*R* = Et) **13** (*R* = Me) **14** (*R* = Et) **15** (*R* = Me)

Scheme 2

Table 1. Antibacterial and antifungal activities of selected compounds; inhibition zones of less than 10 mm in diameter are considered to indicate weak activity

	Inhibition zones (mm)					
	Conc. (mg/ml)	*Escherichia coli*	*Bacillus subtilis*	*Staphylococcus aureus*	*Candida albicans*	*Aspergiltus niger*
3c	0.24	9	10	10	–	8
3f	0.24	8	9	–	8	8
9c	0.24	8	8	–	8	8
9f	0.24	7	7	–	8	9
Ampicillin	0.24	21	21	17	–	–
Streptomycin	0.24	30	35	22	–	–
DMF	solvent	6.5	6.5	–	–	9

oxidative cyclization of the corresponding hydrazone [55] were found amenable for X-ray analysis and shown to possess the linear and not the angular skeleton [56]. This X-ray analysis result may reasonably be extended to confirm the assigned linear structure **8**. MS of **8b** showed its molecular ion peak at $m/z = 526$; the base peak appeared at $m/z = 267$ and corresponds to the protonated formyl heterocyclic moiety (B+30). The latter fragment is known to be diagnostic of C-nucleoside and acyclic C-nucleoside structres [57].

De-O-acetylation of **8a**–**g** with ammonium hydroxide in methanol gave the acyclic C-nucleosides **9a**–**g** (Scheme 1) that revealed two UV spectroscopic maxima at λ 321-319 and 270-267 nm and a shoulder at λ 265 nm. These maxima are also identical to those of the unequivocally prepared reference compound **14**. Compounds **9a**–**g** showed OH and C=N IR absorptions and ^{1}H NMR benzo group and ethyl group proton signals; in most cases, the alditolyl group protons were associated with the solvent absorption (*DMSO*-d_6) to give a broad signal at 3.65-3.50 ppm. The MS of **9d** revealed the molecular ion peak at $m/z = 358$.

Compounds **3c**, **3f**, **9c**, and **9f** showed weak to negative antibacterial activity *in vitro* against the gram-negative bacterium *Escherichia coli* and the two gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* as well as weak antifungal activity against *Candida albicans* and *Aspergillus niger* using the agar diffusion method [58] (Table 1).

Experimental

Melting points were determined on a MEL-TEMP II melting point apparatus in open glass capillaries and are uncorrected. The UV spectra were recorded on a Perkin-Elmer Lambda 4B UV/Vis spectrophotometer. The IR spectra were obtained from potassium bromide discs on a Pye-Unicam SP1025 spectrophotometer. ^{1}H NMR spectra were measured at ambient temperature (25°C) with Varian EM-390 or or Bruker AC-250 spectrometers using *TMS* as an internal standard. Mass spectra were run at 70 eV on an analytical system consisting of a DuPont 21–419 mass spectrometer interfaced with a DuPont 492-094 data acquisition station or on a Hewlett-Packard 5995 GC-MS system. Structure geometries were optimized using the molecular mechanics program Chem 3D Plus. Homogeneity of the products and completeness of the reactions were checked by ascending

thin-layer chromatography on plates precoated with silica gel G (E. Merck; layer thickness 0.25 mm) and used without pretreatment. All ratios of the used solvent systems were v/v; the distance start-front 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 obtained results agreed satisfactorily with the calculated values.

Aldose-5-ethyl-1,2,4-triazino[5,6-b]indol-3-ylhydrazones (**3a–g**); *generate procedure*

A solution of **2** (0.005 mol) in ethanol (30 cm^3) was added to the appropriate sugar (**1a–g**, 0.005 mol) in water (2 cm^3), heated on a boiling water bath for 15 min, kept at room temperature for 24 h, and the crystalline product which separated was filtered, washed with ethanol, and recrystallized from water-ethanol.

D-Arabinose-5-ethyl-1,2,4-triazino[5,6-b]indol-3-ylhydrazone (**3a**; $C_{16}H_{20}H_6O_4$)

Yield: 1.41 g (88%); pale yellow crystals; m.p.: 168°C; TLC ($CHCl_3/CH_3OH = 1:1$): $R = 0.67$; IR: $\nu = 3371$ (OH+NH), 1612 (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6) $\delta = 11.30$ (s, 1H,=N-NH, exchangeable), 8.20 (d, 1H, arom), 7.85-7.25 (m, 4H, -CH=N+3 arom-H), 5.20 (m, 1H, OH, exchangeable), 4.95 (d, 1H, OH, exchangeable), 4.60 (m, 2H, 2 OH, exchangeable, 4.40 (m, 4H, N-C$H_2$$CH_3$+2 tetrahydroxybutyl=H), 1.30 (t, 3H, N-CH_2CH_3) ppm.

L-Arabinose-5-ethyl-1,2,4-triazino[5,6-b]indol-3-ylhydrazone (**3b**; $C_{16}H_{20}N_6O_4$)

Yield: 1.34 g (84%); pale yellow crystals; m.p.: 179°C; TLC ($CHCl_3/CH_2OH = 1:1$): $R_f = 0.79$; IR:$\nu = 3241$ (OH+NH), 1614 (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 11.25$ (s, 1H, =N-NH, exchangeable), 8.05 (d, 1H, arom), 7.70-7.10 (m, 4H, -CH=N+3 arom-H), 4.95 (m, 1H, OH, exchangeable), 4.55 (m, 3H, OH, exchangeable), (m, 2H, 2 OH, exchangeable +tetrahydroxybutyl-H), 4.55 (m, 4H, N-C$H_2$$CH_3$+2 tetrahydroxybutyl-H), 1.35 (t, 3H, N-CH_2CH_3) ppm; MS: *m/z* (%) = 361 (M^++1, 0.6), 269 (0.6), 239 (10.8), 213 (100), 198 (1.3), 184 (6.5), 157 (80.5), 156 (39.4).

D-Ribose-5-ethyl-1,2,4-triazino[5,6-b]indol-3-ylhydrazone (**3e**: $C_{16}H_{20}N_6N_4$)

Yield: 1.12 g (70%); pale yellow crystals; m.p.: 166 C; TLC ($CHCl_3/CH_3OH = 1.1$): $R_f = 0.74$; IR: $\nu = 3316$ (OH+NH), 1585 (C=N) cm^{-1} ^1H NMR (*DMSO*-d_6): $\delta = 11.30$ (br s, 1H, =N-NH, exchangeable), 8.10 (d, 1H, arom), 7.75-7.15 (m, 4H, -CH=N- +3 arom-H), 4.35 (m, 3H, N-C$H_2$$CH_3$+tetrahydroxybutyl-H), 1.35 (t, 3H, N-CH_2CH_3) ppm.

D-Xylose-5-ethyl-1,2,4-triazino[5,6-b]indol-3-ylhydrazone (**3d**; $C_{16}H_{20}N_6O_4$)

Yield: 1.20 g (75%); pale yellow crystals; m.p.: 162°C; TLC ($CHCl_3/CH_3OH = 1:1$): $R_f = 0.66$; IR: $\nu = 3197$ (OH+NH), (C=N) 1593 cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 11.50$ (br s, 1H, =N-NH, exchangeable), 8.25 (d, 1H, arom), 7.85-7.30 (m, 4H, -CH=N-+3 arom-H), 5.35 (m, 1H, OH, exchangeable) 4.85-4.20 (m, 7H, N-C$H_2$$CH_3$+2 OH, exchangeable +3 tetrahydroxybutyl-H), 1.50 (t, 3H, N-CH_2CH_3) ppm.

D-Galactose-5-ethyl-1,2,4-triazino[5,6-b]indol-3-ylhydrazone (**3e**; $C_{17}H_{22}N_6O_5$)

Yield: 1.45 g (85%); orange crystals; m.p.: 168°C; TLC ($CHCl_3/CH_3OH = 1:1$): $R_f = 0.69$; IR: $\nu = 3397$ (OH+NH), 1609 (C=N) cm^{-1} ^1H NMR (*DMSO*-d_6): $\delta = 11.25$ (s, 1H, =N-NH, exchangeable), 8.15 (d, 1H, arom), 7.80-7.20 (m, 4H, -CH=N- +3 arom-H), 4.90 (d, 1H, OH, exchangeable) 4.75-4.05 (m, 7H, N-C$H_2$$CH_3$+OH, exchangeable +4 pentahydroxypentyl-H), 1.35 (t, 3H, N-CH_2CH_3) ppm.

D-Glucose-5-ethyl-1,2,4-triazino[5,6-b]indol-3-ylhydrazone (**3f**; $C_{17}H_{22}N_6O_5$)

Yield: 1.36 g (80%); pale yellow crystals; m.p.: 187°C; TLC ($CHCl_3/CH_3OH = 1:1$): $R_f = 0.62$; IR: $\nu = 3341$ (OH+NH), (C=N) 1614 cm^{-1}; 1H NMR (*DMSO*-d_6) $\delta = 11.25$ (s, 1H, =N-NH, exchangeable), 8.05 (d, 1H, arom), 7.65-7.10 (m, 4H, -CH=N- +3 arom-H), 5.75, 5.15 (2m, 1H each, 2 OH, exchangeable) 4.80 (m, 2H, 2 OH, exchangeable) 4.25 (m, 7H, N-C*H*$_2$CH$_3$+5 pentahydroxypentyl-H), 1.35 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

D-Mannose-5-ethyl-1,2,4-triazino[5,6,b]indol-3-ylhydrazone (**3g**; $C_{17}H_{22}H_6O_5$)

Yield: 1.33 g (78%); pale yellow crystals; m.p.: 177°C; TLC ($CHCl_3/CH_3OH = 1:1$): $R_f = 0.62$; IR:$\nu = 3365$ (OH+NH), (C=H) 1586 cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 11.30$ (s, 1H, =N-NH, exchangeable), 8.25 (d, 1H, arom), 7.85-7.25 (m, 4H, -CH=N- +3 arom-H), 5.75 (d, 1H, OH, exchangeable), 4.40 (m, 7H, N-C*H*$_2$CH$_3$+5 pentahydroxypentyl-H), 1.35 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

Aldose-poly-O-acetyl-5-ethyl-1,2,4-triazino[5,6-b]indol-3-ylhydrazones (**4a–g**); *general procedure*

A solution of the respective aldose-5-ethyl-1,2,4-triazino[5,6-*b*]indol-3-ylhydrazone (**3a–g**), 0.004 mol) in pyridine (3 cm^3) was treated with Ac_2O (15 cm^3) for 24 h at ambient temperature. The reaction mixture was poured onto ice and water and extracted with $CHCl_3$ (3×20 cm^3), and the $CHCl_3$ extract was washed with 10% $NaHSO_4$ (2×20 cm^3) and water and dried (Na_2SO_4). The solvent was evaporated, and the obtained redisue was crystallized from methanol.

2,3,4,5-Tetra-O-acetyl-D-arabinose-5-ethyl-1,2,4-triazino[5,6-b]indol-3-ylhydrazone (**4a**; $C_{24}H_{28}N_6O_8$)

Yield: 1.45 g (66%); yellow crystals; m.p.: 114°C; TLC ($CHCl_3/CH_3OH = 9:1$): $R_f = 0.69$; IR: $\nu = 3449$ (NH), 1746 (OAc), 1581 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): $\delta = 8.40$ (d, 1H, arom), 7.80-7.30 (m, 4H, -CH=N- +3 arom-H), 6.30 (d, 1H, tetraacetoxybutyl H-1), 5.85 (dd, 1H, tetraacetoxybutyl H-2), 5.30-4.85 (m, 2H, tetraacetoxybutyl H-3+H-4), 4.60-4.00 (m, 3H, N-C*H*$_2$CH$_3$+tetraacetoxybutyl H-4′), 2.40, 2.16, 2.10, 2.00 (4s, 3H each, 4 OAc), 1.40 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

2,3,4,5-Tetra-O-acetyl-L-arabinose-5-ethyl-1,2,4-triazino[5,6-b]indol-3-ylhydrazone (**4a**; $C_{24}H_{28}N_6O_8$)

Yield: 0.93 g (62%); yellow crystals; m.p.: 108°C; TLC ($CHCl_3/CH_3OH = 9:1$): $R_f = 0.65$; IR: $\nu = 3467$ (NH), 1747 (OAc), 1581 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): $\delta = 8.35$ (d, 1H, arom), 7.80-7.25 (m, 4H-CH=N- +3 arom-H), 6.25 (d, 1H, tetraacetoxybutyl H-1), 5.75 (dd, 1H, tetraacetoxybutyl H-2), 5.35-4.80 (m, 1H, tetraacetoxybutyl H-3), 4.55-3.65 (m, 4H, N-C*H*$_2$CH$_3$+tetraacetoxybutyl H-4+H-4′), 1.95 (s, 12H, 4 OAc) 1.45 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

2,3,4,5-Tetra-O-acetyl-D-ribose-5-ethyl-1,2,4-triazino[5,6-b]indol-3-ylhydrazone (**4a**; $C_{24}H_{28}N_6O_8$)

Yield: 0.90 g (60%); yellow crystals; m.p.: 100°C; TLC ($CHCl_3/CH_3OH = 9:1$): $R_f = 0.63$; IR: $\nu = 3468$ (NH), 1748 (OAc), 1581 (C=N) cm^{-1}, ^{1}H NMR ($CDCl_3$): $\delta = 8.40$ (d, 1H, arom), 7.80-7.25 (m, 4H-CH=N- + 3 arom-H), 6.40 (d, 1H, tetraacetoxybutyl H-1), 5.55 (dd, 1H, tetraacetoxybutyl H-2), 5.35-4.93 (m, 1H, tetraacetoxybutyl H-3), 4.60-4.00 (m, 4H, N-C*H*$_2$CH$_3$+tetraacetoxybutyl H-4+H-4′), 2.05 (s, 3H, OAc) 2.00 (s, 2 OAc), 1.95 (s, 3H, OAc), 1.40 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

2,3,4,5-Tetra-O-acetyl-D-xylose-5-ethyl-1,2,4-triazino[5,6-b]indol-3-ylhydrazone (**4a**; $C_{24}H_{28}N_6O_8$)

Yield: 0.95 g (63%); yellow crystals; m.p.: 96°C; TLC ($CHCl_3$//$CH_3OH = 9{:}1$): $R_f = 0.69$; IR: $\nu = 3450$ (NH), 1747 (OAc), 1581 (C=N) cm^{-1}, ^1H NMR ($CDCl_3$): $\delta = 8.40$ (d, 1H, arom), 7.80-7.30 (m, 4H-CH=N- +3 arom-H), 5.95 (d, 1H, tetraacetoxybutyl H-1), 5.65-5.00 (m, 2H, tetraacetoxybutyl H-2+H-3), 5.68-3.95 (m, 4H, N-C*H*$_2$CH$_3$ tetraacetoxybutyl H-4+H-4′), 2.10 (s, 12H, 4 OAc), 1.50 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

2,3,4,5,6-Penta-O-acetyl-D-galactose-5-ethyl-1,2,4-triazino[5,6-b]indol-3-ylhydrazone (**4e**; $C_{27}H_{32}N_6O_{10}$)

Yield: 1.38 g (60%); yellow crystals; m.p.: 93°C; TLC ($CHCl_3/CH_3OH = 9{:}1$): $R_f = 0.71$; IR: $\nu = 3466$ (NH), 1748 (OAc), 1582 (C=N) cm^{-1}, ^1H NMR ($CDCl_3$): $\delta = 8.35$ (d, 1H, arom), 7.75-7.20 (m, 4H-CH=N- +3 arom-H), 5.05 (d, 1H, pentaacetoxypentyl H-1), 5.55 (dd, 1H, -pentaacetoxypentyl H-2), 5.45-4.70 (m, 2H, pentaacetoxypentyl H-3+H-4), 4.30 (q, 2H, N-C*H*$_2$CH$_3$), 3.70-3.40, 3.20-3.00 (2m, 1H each, pentaacetoxypentyl H-5+H-5′), 2.5 (s, 3H, OAc), 2.33, 2.05, 1.95, 1.90, 1.85 (5s, 3H each, 5 OAc) 1.40 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

2,3,4,5,6-Penta-O-acetyl-D-glucose-5-ethyl-1,2,4-triazino[5,6-b]indol-3-ylhydrazone (**4f**; $C_{27}H_{32}N_6O_{10}$)

Yield: 1.24 g (54%); yellow crystals; m.p.: 95°C; TLC ($CHCl_3/CH_3PH = 9{:}1$): $R_f = 0.76$; IR: $\nu = 3456$ (NH), 1748 (OAc), 1582 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 8.40$ (d, 1H, arom), 7.85-7.25 (m, 4H-CH=N- +3 arom-H), 6.15 (d, 1H, pentaacetoxypentyl H-1), 5.55-4.90 (m, 2H, pentaacetoxypentyl H-2+H-3), 4.55-3.90 (m, 4H, N-C*H*$_2$CH$_3$ pentaacetoxypentyl H-4+H-5) 3.90-3.35 (m, 1H, pentaacetoxypentyl H-5′), 2.40 (s, 3H, OAc), 2.05, 1.95, (2s, 6H each, 4 OAc) 1.45 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

2,3,4,5,6-Penta-O-acetyl-D-mannose 5-ethyl-1,2,4-triazino[5,6-b]indol-3-yl-hydrazone (**4g**; $C_{27}H_{32}N_6O_{10}$)

Yield: 1.27 g (55%); yellow crystals; m.p.: 112°C; TLC ($CHCl_3/CH_3OH = 9{:}1$): $R_f = 0.63$; IR: $\nu = 3456$ (NH), 1748 (OAc), 1581 (C–N) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 8.35$ (d, 1H, arom), 7.75-7.25 (m, 4H-CH=N- +3 arom-H), 6.10 (d, 1H, pentaacetoxypentyl H-1), 5.60 (dd, 1H, pentaacetoxypentyl H-2), 5.15-4.75 (m, 1H, pentaacetoxypentyl H-3), 4.45-3.95 (m, 4H, N-C*H*$_2$CH$_3$ pentaacetoxypentyl H-4+H-5), 3.95-3.10 (m, 1H pentaacetoxypentyl H-5′), 2.50, 2.15 (s, 3H each, 2 OAc), 2.00, (s, 6H, 2 OAc), 1.90 (s, 3H, OAc), 1.45 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

3-Polyacetoxyalkyl-10-ethyl-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indoles (**8a–g**); *general procedure*

Method A. A solution of 0.002 mol Br_2 in 5 cm^3 glacial acetic acid was gradually added at ambient temperature to a stirred mixture of the appropriate hydrazone acetate (**4a–g**, 0.002 mol) and anhydrous sodium acetate (0.006 mol) in 15 cm^3 glacial acetic acid. Stirring was continued for 2 h, and the mixture was evaporated under reduced pressure. The $CHCl_3$ soluble portion of the residue was extracted, evaporated, and the product was crystallized from methanol.

Method B. A solution of 0.003 mol Br_2 in 5 cm^3 glacial acetic was added dropwise at ambient temperature to a stirred mixture of the respective hydrazone (**3a–g**, 0.003 mol) and anhydrous sodium acetate (0.009 mol) in 15 cm^3 glacial acetic acid. The reaction mixture was stirred for 2 h and then treated with 15 cm^3 acetic anhydride. The reaction mixture was kept at ambient temperature for

24 h and evaporated to dryness. The $CHCl_3$ soluble portion of the obtained residue was evaporated, and the product was crystallized from methanol.

3-(D-Arabino-1,2,3,4-tetraacetoxybut-1-yl)-10-ethyl-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**8a**; $C_{24}H_{26}N_6O_8$)

Yield: 0.65 g (65%) (*A*), 0.60 g (60%) (*B*); yellow crystals; m.p.: 102°C; TLC ($CHCl_3/CH_3OH = 9:1$): $R_f = 0.69$; UV (MeOH); λ_{max} (logε) = 320 (4.28), 269 (4.96), 265 (sh) nm; IR: $\nu = 1746$ (OAc), 1602 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 8.15$ (d, 1H, arom), 7.65 (t, 1H, arom-H), 7.30 (m, 2H, 2 arom-H), 6.65 (d, 1H, tetraacetoxybutyl H-1), 5.75 (dd, 1H, tetraacetoxybutyl H-2) 5.40 (m, 1H, tetraacetoxybuty H-3) 4.25 (m, 4H, N-C*H*$_2$CH$_3$+tetraacetoxybutyl H-4+H-4′), 2.15, 2.10 (2s, 3H each, 2 OAc), 2.05 (s, 6H, 2 OAc), 1.45 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

3-(L-Arabino-1,2,3,4-tetraacetoxybut-1-yl)-10-ethyl-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**8b**; $C_{24}H_{26}N_6O_8$)

Yield: 0.62 g (62%) (*A*) 0.58 g (58%) (*B*); yellow crystals; m.p.: 118°C; TLC ($CHCl_3/CH_3OH = 9:1$): $R_f = 0.66$; UV (MeOH); λ_{max} (logε) = 319 (4.26, 270 (4.93), 265 (sh) nm; IR: $\nu = 1748$ (OAc), 1602 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 8.15$ (d, 1H, arom), 7.65 (t, 1H, arom-H), 7.30 (m, 2H, 2 arom-H), 6.65 (d, 1H, tetraacetoxybutyl H-1), 5.75 (dd, 1H, tetraacetoxybutyl H-2) 5.45 (m, 1H, tetraactoxybutyl H-3) 4.25 (m, 4H, N-C*H*$_2$CH$_3$+tetraacetoxybutyl H-4+H-4′), 2.15, 2.10 (2s, 3H each, 2OAc), 2.00 (s, 6H, 2 OAc), 1.45 (t, 3H, N-CH$_2$C*H*$_3$) ppm; MS: *m/z* (%) = 526 (M^+, 2.4), 484 (3.7), 424 (5.5), 407 (7.2), 347 (2.4), 309 (5.8), 267 (100), 237 (5.6).

10-Ethyl-3-(D-ribo-1,2,3,4-tetraacetoxybut-1-yl)-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**8c**; $C_{24}H_{26}N_6O_8$)

Yield: 0.60 g (60%) (*A*) 0.55 g (55%) (*B*); yellow crystals; m.p.: 120°C; TLC ($CHCl_3/CH_3OH = 9:1$): $R_f = 0.56$; UV (MeOH). λ_{max} (logε) = 319 (4.89), 270 (5.61), 264 (sh) nm; IR: $\nu = 1757$ (OAc), 1587 (C=N) cm^{-1}, ^1H NMR ($CDCl_3$): $\delta = 8.40$ (d, 1H, arom), 7.90-7.20 (m, 3H, 3 arom-H), 6.10 (d, 1H, tetraacetoxybutyl H-1), 5.85-4.90 (m, 2H, tetraacetoxybutyl H-2+H-3) 4.65-3.90 (m, 3H, N-C*H*$_2$CH$_3$+tetraacetoxybutyl H-4) 3.90-3.40 (m, 1H, tetraacetoxybutyl H-4′) 2.10 (s, 12H, 4 OAc), 1.45 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

10-Ethyl-3-(D-xylo-1,2,3,4-tetraacetoxybut-1-yl)-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**8d**; $C_{24}H_{26}N_6O_8$)

Yield: 0.52 g (52%) (*A*) 0.50 g (50%) (*B*); yellow crystals; m.p.: 95°C; TLC ($CHCl_3/CH_3OH = 9:1$): $R_f = 0.64$; UV (MeOH): λ_{max} (logε) = 320 (4.25), 270 (4.95), 265 (sh) nm; IR: $\nu = 1747$ (OAc), 1602 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 8.10$ (d, 1H, arom), 7.65 (t, 2H, 2 arom-H), 7.30 (d, 1H, arom-H), 6.60 (d, 1H, tetraacetoxybutyl H-1), 6.05 (dd, 1H, tetraacetoxybutyl H-2), 5.20 (m, 1H, tetraacetoxybutyl H-3), 4.35 (q, 2H, N-C*H*$_2$CH$_3$), 4.10 (m, 2H, tetraacetoxybutyl H-4+H-4′), 2.10, 2.00 (2s, 6H each, 4OAc), 1.45 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

10-Ethyl-3-(D-galacto-1,2,3,4,5-pentaacetoxypent-1-yl)-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**8e**; $C_{27}H_{30}N_6O_{10}$)

Yield: 0.62 g (62%) (*A*) 0.51 g (51%) (*B*); yellow crystals; m.p.: 115°C; TLC ($CHCl_3/CH_3OH = 9:1$): $R_f = 0.75$; UV (MeOH): λ_{max} (log ε) = 320 (4.55), 270 (5.25), 264 (sh) nm; IR: $\nu = 1750$ (OAc), 1604 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 8.30$ (d, 1H, arom), 7.75 (t, 1H, arom-H), 7.40 (m, 2H, 2

arom-H), 6.60 (d, 1H, pentaacetoxypentyl H-1), 5.70 (m, 2H, pentaacetoxypentyl H-2+H-3), 5.40 (m, 1H, pentaacetoxypentyl H-4), 3.35 (q, 2H, N-C*H*$_2$CH$_3$), 3.95 (m, 2H, pentaacetoxypentyl H-5+H-5′), 2.25 (s, 6H, 2OAc), 2.15 (s, 3H, OAc), 2.05 (s, 6H, 2 OAc), 1.50 (t, 3H, N-CH$_2$C*H*$_3$O ppm.

10-Ethyl-3-(D-gluco-1,2,3,4,5-pentaacetoxypent-1-yl)-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**8f**; $C_{27}H_{30}N_6O_{10}$)

Yield: 0.58 g (58%) (*A*) 0.50 g (50%) (*B*); yellow crystals; m.p.: 95°C; TLC ($CHCl_3/CH_3OH = 9:1$): $R_f = 0.59$; UV (MeOH): λ_{max} (logε) = 319 (4.29), 268 (4.95), 265 (sh) nm; IR: $\nu = 1745$ (OAc), 1605 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): $\delta = 8.30$-7.20 (m, 4H, 4 arom H), 6.55 (d 1H, pentaacetoxypentyl H-1), 6.15 (m 1H, pentaacetoxypentyl H-2), 5.20 (m, 2H, pentaacetoxypentyl H-3+H-4), 4.20 (m, 4H, N-C*H*$_2$CH$_3$+pentaacetoxypentyl H-5+H-5′), 2.10 (s, 12H, 4OAc), 1.90 (s, 3H, OAc), 1.50 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

10-Ethyl-3-(D-manno-1,2,3,4,5-pentaacetoxypent-1-yl)-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**8g**; $C_{27}H_{30}N_6O_{10}$)

Yield: 0.60 g (60%) (*A*) 0.52 g (52%) (*B*); yellow crystals; m.p.: 162°C; TLC ($CHCl_3/CH_3OH = 9:1$): $R_f = 0.60$; UV (MeOH): λ_{max} (logε) = 321 (4.33), 269 (5.02), 264 (sh)nm; IR: $\nu = 1739$ (OAc), 1600 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$); $\delta = 8.40$-7.10 (m, 4H, 4 arom-H), 6.45 (d, 1H, pentaacetoxypentyl H-1), 5.68-5.00 (m, 2H, pentaacetoxypentyl H-2+H-3), 4.65-3.40 (m, 5H, N-C*H*$_2$CH$_3$+pentaacetoxypentyl H-4+H-5+H-5′), 2.15 (s, 15H, 5OAc), 1.45 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

3-Polyhydroxyalkyl-10-ethyl-1,2,4,triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indoles (**9a**–**g**); *general procedure*

A solution of the appropriate acetate (**8a**–**g**), 0.002 mol) in 50 cm^3 methanol was treated with 10 cm^3 of 20% aqueous ammonia solution and kept at ambient temperature for 24 h. Evaporation of the solvents under reduced pressure gave a residue which crystallized from a water-methanol mixture.

3-(D-Arabino-1,2,3,4-tetrahydroxybut-1-yl)-10-ethyl-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**9a**; $C_{16}H_{18}N_6O_4$)

Yield: 1.55 g (55%); yellow crystals; m.p.: 294°C; TLC ($CHCl_3/CH_3OH = 1:1$): $R_f = 0.69$; UV (MeOH): λ_{max} (logε) = 320 (4.04), 270 (4.71), 265 (sh) nm; IR: $\nu = 3427$ (OH), 1604 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d$_6$): $\delta = 8.05$ (d, 1H, arom), 7.60 (m, 2H, 2 arom-H), 7.25 (t, 1H, arom-H), 5.65 (m, 1H, OH exchangeable), 5.35 (d, 1H, tetraacetoxybutyl-H), 4.65 (m, 2H, 2OH, exchangeable), 4.30 (m, 5H, N-C*H*$_2$CH$_3$+3 tetraacetoxybutyl-H), 1.35 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

3-(L-Arabino-1,2,3,4-tetrahydroxybut-1-yl)-10-ethyl-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**9b**; $C_{16}H_{18}N_6O_4$)

Yield: 1.50 g (50%); yellow crystals; m.p.: 228°C; TLC ($CHCl_3/CH_3OH = 1:1$): $R_f = 0.55$; UV (MeOH) λ_{max} (logε) = 319 (4.45), 267 (5.11), 265 (sh) nm; IR: $\nu = 3348$ (OH), (C=N) 1604 cm^{-1}; ^{1}H NMR (*DMSO*-d$_6$) $\delta = 8.00$ (d, 1H, arom), 7.55 (m, 2H, 2 arom-H), 7.20 (t, 1H, arom-H), 5.40 (m, 2H, OH, exchangeable +tetraacetoxybutyl-H), 4.95-4.40 (d, 3H, 3 OH, exchangeable), 4.35-3.90 (m, 6H, N-C*H*$_2$CH$_3$+4 tetraacetoxybutyl-H), 1.60 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

10-Ethyl-3-(D-ribo-1,2,3,4-tetrahydroxybut-1-yl)-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**9c**; $C_{16}H_{18}N_6O_4$)

Yield: 1.49 g (49%); yellow crystals; m.p.: 218°C; TLC ($CHCl_3/CH_3OH = 1:1$): $R_f = 0.73$; UV (MeOH); λ_{max} (logε) = 320 (4.38), 270 (5.05), 265 (sh) nm; IR: $\nu = 3396$ (OH), (C=N) 1600 cm^{-1}

^{1}H NMR (*DMSO*-d_6): $\delta = 7.95$ (d, 1H, arom), 7.70-7.05 (m, 3H, 3 arom-H), 5.30 (d, 1H, tetraacetoxybutyl-H), 4.25 (1, 2H, N-C*H*$_2$CH$_3$), 1.60 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

10-Ethyl-3-(D-xylo-1,2,3,4-tetrahydroxybut-1-yl)-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**9d**; $C_{16}H_{18}N_6O_4$)

Yield: 1.51 g (51%); yellow crystals; m.p.: 242°C; TLC ($CHCl_3$/CH_3OH = 1:1): $R_f = 0.72$; UV (MeOH): λ_{max} (logε) = 319 (5.84), 270 (6.63), 265 (sh) nm; IR: $\nu = 3344$ (OH), (C=N) 1604 cm^{-1}; ^{1}H NMR (*DMSO*-d_6): (d, 1H, arom), 7.55 (m, 2H, 2 arom-H), 7.30 (t, 1H, arom-H), 5.60, 5.35, 4.65 (3m, 1H each, 3 tetraacetoxybutyl-H), 4.35 (m, 4H, N-C*H*$_2$CH$_3$+2 tetraacetoxybutyl-H), 1.65 (t, 3H, N-CH$_2$C*H*$_3$) ppm; MS: *m*/*z* (%) = 358 (M^+, 3.9), 340 (1.9), 327 (3.2), 267 (100), 239 (76.7).

10-Ethyl-3-(D-galacto-1,2,3,4,5-pentahydroxypent-1-yl)-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**9e**; $C_{17}H_{20}N_6O_5$)

Yield: 1.63 g (63%); yellow crystals; m.p.: 158°C; TLC ($CHCl_3$/CH_3OH = 1:1): $R_f = 0.54$: UV (MeOH): λ_{max} (logε) = 319 (4.24), 270 (4.93), 265 (sh) nm; IR: $\nu = 3237$ (OH), (C=N) 1600 cm^{-1}; ^{1}H NMR (*DMSO*-d_6) $\delta = 8.05$ (d, 1H, arom), 7.55 (m, 2H, 2 arom-H), 7.30 (m, 1H, arom-H, 5.45 (m, 2H, pentaacetoxypentyl-H) 4.70 (m, 1H, OH, exchangeable), 4.20 (m, 5H, N-C*H*$_2$CH$_3$+3OH, exchangeable), 1.65 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

10-Ethyl-3-(D-gluco-1,2,3,4,5-pentahydroxypent-1-y;)-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**9f**; $C_{17}H_{20}N_6O_5$)

Yield: 1.50 g (50%) yellow crystals; m.p.: 214°C; TLC ($CHCl_3$/CH_3OH = 1:1): $R_f = 0.54$; UV (MeOH) λ_{max} (logε) = 320 (4.11), 270 (4.77), 265 (sh) nm; IR: $\nu = 3312$ (OH), (C=N) 1610 cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 8.05$ (d, 1H, arom), 7.60 (t, 1H, arom-H), 7.30 (t, 2H, 2 arom-H), 5.65 (m, 1H, OH, exchangeable) 5.40 (d, 1H, pentaacetoxypentyl-H)), 4.60 (m, 1H, pentaacetoxypentyl-H), 4.35 (m, 4H, N-C*H*$_2$CH$_3$+2OH, exchangeable), 1.40 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

10-Ethyl-3-(D-manno-1,2,3,4,5-pentahydroxypent-1-yl)-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**9g**; $C_{17}H_{20}N_6O_5$)

Yield: 1.52 g (52%); yellow crystals; m.p.: 262°C; TLC ($CHCl_3$/CH_3OH = 1:1): $R_f = 0.64$; UV (MeOH): λ_{max} (logε) = 312 (6.12), 269 (6.79), 265 (sh) nm; IR: $\nu = 3400$ (OH), (C=N) 1605 cm^{-1}; ^{1}H NMR (*DMSO*-d_6): 7.10 (m, 4H, arom), 4.10 (q, 2H, N-C*H*$_2$CH$_3$), 1.25 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

10-Ethyl-3-methyl-1,2,4-triazolo[4′,3′:2,3]-1,2,4-triazino[5,6-b]indole (**14**; $C_{13}H_{12}N_6$)

A mixture of 3,4-diamino-5-methyl-1,2,4-triazole hydrochloride [47] (**11**, 0.001 mol), 1-ethylisatin [54] (**12**, 0.001 mol), and sodium acetate (0.001 mol) in 25% aqueous ethanol (25 cm^3) was heated at reflux for 1 h. Acetic acid (0.2 cm^3) was added, and heating was continued for 2 h. The product which separated after attaining ambient temperature was filtered and crystallized from ethanol.

Yield: 1.18 g (61%); yellow crystals; m.p.: 343°C; TLC ($CHCl_3$/CH_3OH = 9:1): $R_f = 0.53$; UV (MeOH): λ_{max} (logε) = 318 (5.48), 270 (6.24), 265 (sh) nm; IR: $\nu = 1605$ cm^{-1} (C=N); ^{1}H NMR (*DMSO*-d_6) $\delta = 8.70$ (q, 1H, arom), 7.65 (t, 1H, arom-H), 7.00 (m, 2H, 2 arom-H), 4.25 (q, 2H, N-C*H*$_2$CH$_3$), 2.70 (s, 3H, CH_3), 1.35 (t, 3H, N-CH$_2$C*H*$_3$) ppm.

Attempted acid or thermally induced Dimroth rearrangement of **8**

Solutions of **8** (0.001 mol) in 10 cm^3 acetic acid were refluxed for 2 h and then evaporated under reduced pressure. The obtained residues were crystallized from ethanol. TLC, m.p., mixed m.p., and

UV spectra of the products were identical with those of the corresponding starting compound **8**. Attempted thermal rearrangement of compounds **8** by fusion at a temperature 10°C above the corresponding melting point for 1 h also gave the unchanged compounds.

Antimicrobial screening

Sterile nutrient agar plates (100 cm^3) were separately inoculated with a 24 h broth culture (1 cm^3) of Escherichia *coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger*. Solutions (0.06 cm^3) of the tested compounds (0.24 mg) in *DMF* (1 cm^3) were placed in wells (6 mm diam.) cut in the agar media, and the plates were incubated at 37°C in the case of bacteria and at 25°C in the case of yeast. The diameters of the resulting inhibition zones were measured after 28 h for bacteria and after 96 h for yeast [58].

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