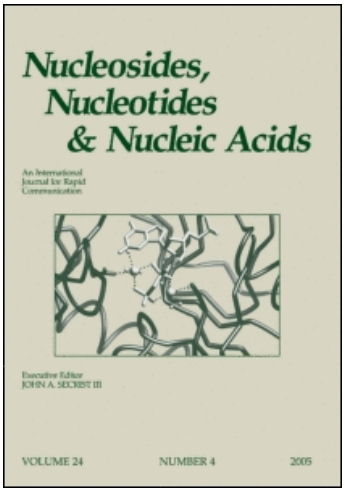

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Heras, Federico G. De las and Camarasa, María-José(1982) 'Synthesis of Alkylating 1-Glycosyl-5-substituted 1,2,4-Triazoles[1]', Nucleosides, Nucleotides and Nucleic Acids, 1: 1, 45 — 56
To link to this Article: DOI: 10.1080/07328318208079401
URL: http://dx.doi.org/10.1080/07328318208079401

NUCLEOSIDES & NUCLEOTIDES, 1(1), 45-56 (1982)

SYNTHESIS OF ALKYLATING 1-GLYCOSYL-5-SUBSTITUTED 1,2,4-TRIAZOLES[1]

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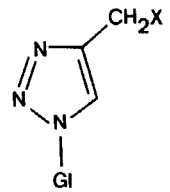
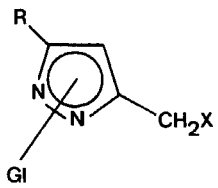
Abstract. A series of alkylating derivatives of 5-substituted 1-glycosyl-1,2,4-triazole having cytostatic activity has been prepared. The compounds synthesized include the 5-hydroxymethyl, 5-halomethyl, 5-(1-aziridino) methyl, and 5-bis (2-chloroethyl) aminomethyl derivatives.

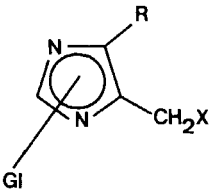
In previous papers in this series the synthesis, cytostatic activity and mechanism of action of halomethyl 1,2,3-triazole 1[2,3,4] pyrazole 2[5], and imidazole 3[6] alkylating N-glycosides was reported. The significant "in vivo" cytostatic activities of 4-bromo- and 4-iodomethyl-1-peracetyl glycosyl-1,2,3-triazoles 1 against Ehrlich carcinoma ascites tumor [2,3] and P-388 Lymphocytic leukemia[2] prompted us to extend our studies to the biologically important[7,8] 1,2,4-triazole series. In this paper we describe the synthesis of a series of triacetyl-β-D-ribofuranosyl and tetraacetyl-β-D-glucopyranosyl derivatives of 1,2,4-triazole substituted at the 5-position with halomethyl, nitrogen mustard and aziridine alkylating groups.



1, X = Halogen
Gl = Glycosyl



2, X = Halogen
Gl = Glycosyl



3, X = Halogen
Gl = Glycosyl

The synthetic route started with the preparation of per-acetyl-β-D-glycosyl-1,2,4-triazoles 4[8]. Fusion of 1,2,3,5--tetra-O-acetyl-β-D-ribofuranose with 1,2,4-triazole at 160ºC for 45 min in the presence of p-toluensulfonic acid as catalyst gave crystalline 4a in 81% yield. 1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-1,2,4-triazole (4c) was synthesized in 81% yield by refluxing a nitromethane solution of 1,2,4--triazole and α-acetobromoglucose in the presence of $(CN)_2Hg$[9]. Attempts to obtain 4c by the fusion procedure using different acids as catalysts and heating at different temperatures, or by the silylation procedure [10] afforded much lower yields of nucleosidic material.

The glycosylation position was assigned, in both cases, as N-1 based on the different chemical shifts of the two triazole protons H-3 and H-5 (TABLE 1). The singlet appearing at lower field was assigned in each case to H-5[11]. This assignation was confirmed by the differences in chemical shifts of H-3 and H-5 in $(CD_3)_2SO$ and $CDCl_3$, since the chemical shift of the proton adjacent to the substituted nitrogen (H-5) in 1,2,4-triazoles is more sensitive to solvent changes than is that of H-3[12]. Thus, the $\Delta\delta = \delta_{(CD_3)_2SO} - \delta_{CDCl_3}$ values for the lower field proton, H-5, were 0.5ppm for both 4a and 4c. The $\Delta\delta$ values for the higher field proton, H-3 were 0.15 ppm for 4a and 0.08 ppm for 4c.

The β anomeric configuration of 4c was determined from the coupling constant $J_{1',2'}$ = 9Hz. The anomeric configuration

TABLE 1. Chemical shift (δ) and coupling constants (Hz) of glucosyl and ribosyl-1,2,4-triazoles 4-10.

Compd.	Solvent	H-1'	H-3	H-5	$CH_2X(5)$	$J_{1',2'}$
4a	DMSO	6.39	8.27	8.88		4
4a	$CDCl_3$	6.09	8.12	8.38		
4b	DMSO	5.91	8.19	8.93		4
4c	DMSO	6.20	8.18	8.96		9
4c	$CDCl_3$	5.72	8.10	8.46		
5a	DMSO	6.43	8.13		4.72	4
5a	$CDCl_3$	6.35	7.97		4.89	
5c	DMSO	6.33	8.07		4.79	8
5c	$CDCl_3$	5.90	7.94		4.93	
6a	$CDCl_3$	6.10	7.93		4.58	3
6c	$CDCl_3$	5.78	7.90		4.60	8
7c	$CDCl_3$	5.79	7.87		4.50	9
8c	$CDCl_3$	5.80	7.90		4.77	8
9c *	$CDCl_3$	6.37	7.87		4.18	9
10c **	$CDCl_3$	6.30	7.85		3.63	9

*δ 2.98 (t,4,CH_2N), 3.60 (t,4,CH_2Cl)

**δ 1.36 , 1.85 (2m, 4H, aziridine)

of 4a could not be determined from the pmr spectrum, since $J_{1',2'}$ = 3Hz could correspond to both α and β anomers[13]. So, compound 4a was deacetylated by treatment with methanolic ammonia.Comparison of the physical properties of the resulting deprotected 1,2,4-triazole riboside 4b with those of the known 1- (β-D-ribofuranosyl)-1,2,4-triazole[11] indicated that both compounds were identical.

5-Hydroxymethyl-1,2,4-triazole riboside 5a and glucoside 5c were obtained in 22 and 61% yield respectively by treatment of 4a and 4c with aqueous formaldehyde in dioxane at 105º and 115ºC, respectively, in a sealed tube. Hydroxymethylation

of the ribosyl-1,2,4-triazole 4a gave also several partially deacetylated 5-hydroxymethyl-1,2,4-triazole ribosides. No 3-hydroxymethyl derivatives were detected.

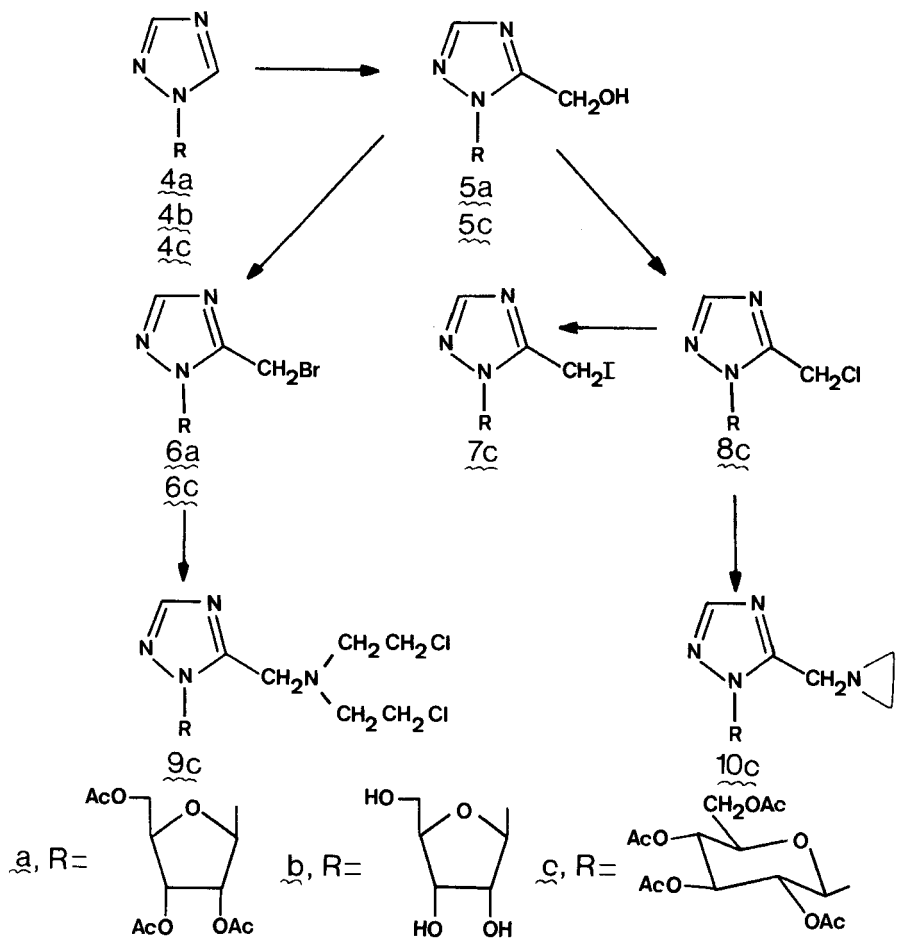
The 5-substitution of the hydroxymethyl group was determined from the pmr spectra, which, in the aromatic protons zone only showed the higher field singlet corresponding to H-3. The small chemical shift differences of this singlet in $(CD_3)_2SO$ and $CDCl_3$ $\Delta\delta$ =0.16 for 5a and $\Delta\delta$= 0.13 for 5c was also in agreement with its assignation to H-3, according to the above mentioned criterion[12].

Reaction of 5a and 5c with triphenylphosphine and bromine gave the 5-bromomethyl analogues 6a, as an unstable syrup, and 6c as a solid. This, and the already observed lower stability of triacetylribofuranosyl-halomethyl-1,2,3-triazole in relation to the corresponding tetraacetylglucopyranosyl analogues, reccomended to only continue this study with the also cytostatic glucopyranosyl series[14]. Reaction of 5-hydroxymethyl-1,2,3-triazole 5c with triphenylphosphite and CCl_4 afforded 5-chloromethyl derivative 8c in 79% yield. Transhalogenation reaction of 8c with sodium iodide gave iodomethyl derivative 7c in 83% yield. Unprotected alkylating halomethyl-1,2,3-triazole nucleosides are unstable[3], while the corresponding acetylated derivatives are usually stable and show significant cytostatic activities[2,3]. Thus, no attempts have been made to deacetylate the alkylating 1,2,4-triazole nucleosides of the present paper.

Nitrogen mustard and aziridine groups have been used as the alkylating residue in certain anticancer drugs[15]. They have been also incorporated into antitumor nucleosides. 5-[N,N-bis-(2-chloroethyl) aminomethyl] uridine is active against leukemia[16] and 6-(1-aziridinyl)-9-(β-D-ribofuranosyl)-purine shows activity against adenocarcinoma 755[17]. In order to compare their cytostatic activities with those of the halomethyl-1,2,4-triazole glycosides synthesized above, having a halomethyl azole as a new benzylic type alkylating group,

we prepared other alkylating 1,2,4-triazole glycosides, having some of the classical alkylating groups such as nitrogen mustard and aziridine. Thus, 5-[N,N-bis-(2-chloroethyl) aminomethyl]-1,2,4-triazole 9c was prepared in quantitative yield by treatment of 5-bromomethyl-1,2,4-triazole 6c with bis (2-chloroethyl)amine, freshly generated by reaction of the corresponding hydrochloride with aqueous KOH. The 5-aziridinomethyl derivative 10c was prepared in 50% yield by reaction of the 5-chloromethyl-1,2,4-triazole 8c with aziridine.

The cytostatic activity against HeLa cells of all the alkylating derivatives 6-10 reported in this paper has been evaluated[2,18]. The bromomethyl and iodomethyl-1,2,4-triazole



derivatives 6c and 7c showed significant ID_{50} = 2.5 μg/ml values. However, cytostatic activities of chloromethyl, N,N-bis- (2-chloroethyl)-aminomethyl and aziridinomethyl derivatives 6c, 9c and 10c respectively, were not significant (ID_{50} = 25 ug/ml).

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Proton nuclear magnetic resonance spectra were recorded with a varian EM-390 or a Perkin-Elmer R-12 spectrometer using Me_4Si as internal standard. Analytical thin-layer chromatography was performed on aluminum sheets coated with a 0.2 mm layer of silica gel 60 F_{254} (Merck). Preparative layer chromatography was performed on 20 x 20 cm glass plates coated with a 2 mm layer of silica gel PF_{254} (Merck). Compounds were detected with a UV light (254 nm) or by spraying the plate with a ethanol-sulfuric acid (3:7) mixture and heating. Column chromatography was performed on glass columns filled with silica gel 60 70-230 mesh (Merck).

1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole (4a). A mixture of 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (10.2g,0.03 mol), 1,2,4-triazole (5.6 g, 0.08 mol) and a catalytic amount of p-toluenesulfonic acid was heated at 160ºC for 45 min. at reduced pressure. After cooling, the reaction mixture was dissolved in boiling ethanol, treated with charcoal and filtered. On cooling 4a crystallized (8.4g,81%), m.p. 110-111ºC.

Anal. Calcd. for $C_{13}H_{17}N_3O_7$: C,47.70; H,5.23; N, 12.83. Found: C,47.45; H,5.42; N,13.20.

1-(β-D-Ribofuranosyl)-1,2,4-triazole (4b). Compound 4a (1 g, 0.003 mol) was treated with a saturated solution of methanolic ammonia. After standing at room temperature for

48 h. the solution was evaporated to dryness and the residue crystallized from EtOAc to give 4b (0.54 g, 88%) m.p. 144-145. (lit[11] m.p. 143-145ºC).

1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-1,2,4-triazole. (4c). To a mixture of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (25 g, 0.061 mol), mercuric cyanide (16.2 g, 0.063 mol) and 4 Å molecular sieves (1 g), a solution of 1,2,4-triazole (9 g, 0.132 mol) in dry nitromethane (652 mL) was added. The resulting mixture was refluxed in the absence of humidity for 2.5 h. Then it was filtered, while still hot, in order to remove the insoluble residue which was washed with more hot nitromethane. The filtrate was evaporated to dryness at reduced pressure and the residue obtained was treated with chloroform and filtered to separate the solid formed. The chloroform extract was washed with 30% aqueous potassium iodide, water, and then dried over anhydrous sodium sulfate. On evaporation, a white solid (23 g) was obtained which after crystallization from EtOAc-hexane gave 4c (19.70 g, 81%)m.p.145-146 ºC.

Anal. Calcd. for $C_{16}H_{21}N_3O_9$: C, 48.11; H, 5.30; N, 10.52
Found: C, 48.30; H, 5.47; N, 10.71.

5-Hydroxymethyl-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole (5a). A solution of 4a (5 g, 0.015 mol) in dioxane (10 mL) and 35% aqueous formaldehyde (15 mL) was heated in a sealed tube at 105ºC for 16 h. On cooling, the mixture was evaporated to dryness and the residue purified by column chromatography, using EtOAc as eluant, to give 5a (1.17 g, 22%) as a colorless syrup.

Anal. Calcd. for $C_{14}H_{19}N_3O_8$: C, 47.05; H, 5.35; N, 11.76
Found: C, 46.79; H, 5.78; N, 11.35.

5-Hydroxymethyl-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-1,2,4-triazole (5c). A solution of 4c (19.64g, 0.049 mol) in dioxane (50 mL) and 35% aqueous formaldehyde (133 mL) was heated in sealed tube at 115ºC for 16.5h. On cooling, the

mixture was evaporated to dryness. The residue was suspended in EtOAc (25 mL) and filtered through silica gel 60 70-230 mesh (Merck), which was washed with more EtOAc. Filtrate and washing were evaporated to dryness and the residue was purified by column chromatography using EtOAc as eluant, to give 5c (12.88 g, 61%) as a colorless syrup which on seeding crystallized from EtOAc-Petroleum ether. Seed crystals were obtained from pure syrupy 5c, which spontaneously crystallized after standing at room temperature for one year m.p. 136ºC.

Anal. Calcd. for $C_{17}H_{23}N_3O_{10}$: C, 47.55; H, 5.39; N, 9.78. Found: C, 47.22; H, 5,73; N, 9.42.

5-Bromomethyl-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)--1,2,4-triazole (6a). To a solution of 5a (2.17 g, 0.0066 mol) in anhydrous 1,2-dimethoxyethane (50 mL), tryphenylphosphite (3mL, 0.0099 mol) was added. The mixture was stirred at 0ºC for 30 min. Bromine (0.49 mL, 0.0099 mol) was added and the resulting mixture was stirred at room temperature for 1 h. Then,the solution was evaporated to dryness and the residue was purified by preparative tlc, using EtOAc - hexane (3:1) as solvent, to give 0.60 g of a syrup, the nmr spectrum of which showed that it was a mixture of 6a (major product) and some minor components. This syrup was not chromatographically homogeneous and was purified repeatedly by the same system. Analytical tlc of the syrup after every chromatography showed the same by-products that have been eliminated in the prevíous preparative tlc. m/e 422 (M^++1,1%), 420 (M^+-1, 1%).

5-Bromomethyl-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-1,2,4-triazole (6c). To a solution of 5c (2 g, 0.0046 mol) in anhydrous 1,2,-dimethoxyethane (30mL), triphenylphosphite (2.15 g, 0.0069 mol) was added. The mixture was stirred at 0ºC in the absence of humidity for 30 min. Bromine (0.96 g, 0.006 mol) was added and the resulting mixture was stirred at

room temperature for 1 h. Then, the solution was concentrated in vacuo and the residue was purified by preparative tlc, using EtOAc - $CHCl_3$ (1:1) as eluant, to give pure 6c (1.2 g, 52%), m.p. 137-138ºC (EtOAc-Hexane).

Anal. Calcd. for $C_{17}H_{22}BrN_3O_9$: C, 41,46: H, 4.50; Br, 16.24; N, 8.53. Found: C, 41,42; H, 4,49; Br 16.08; N, 8.91.

5-Iodomethyl-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl) -1,2,4-triazole (7c). A mixture of 8c (0.151 g, 0.34 m mol) anhydrous acetone (4mL) and sodium iodide (0.0757 g, 0.5 m mol) was heated to reflux for 5 min. The solid precipitated (NaCl) was filtered and the filtrate evaporated to dryness. The residue was dissolved in EtOAc and treated with an aqueous solution of sodium thiosulfate to decoloration. Then, the organic phase was washed twice with water, dried over sodium sulfate and evaporated to dryness to give 7c (0.152 g, 83%) as a light yellow solid m.p. 136-137ºC (EtOAc-hexane).

Anal. Calcd. for $C_{17}H_{22}IN_3O_9$: C, 37.86; H, 4.11; I, 23.52; N, 7.79. Found: C, 38.17; H, 3.99; I, 23.27; N, 7.82.

5-Chloromethyl-1- (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl-1,2,4-triazoles (8c). To a solution of 5c (0.85 g, 0.0021 mol) in anhydrous acetonitrile (27 mL) triphenylphosphine (1.07 g, 0.0039 mol) and CCl_4 (20mL) were added. The mixture was stirred at room temperature for 30 min and then evaporated to dryness. The residue was stirred for 30 min with cold anhydrous ether (50 mL) and the solid which precipitated was eliminated by filtration. The filtrate was evaporated to dryness and the residue was purified by preparative tlc , using chloroform as eluant, to give 8c (0.69 g, 78.5 %) m.p. 156-7ºC (EtOAc-Hexane).

Anal. Calcd. for $C_{17}H_{22}ClN_3O_9$: C, 45.60; H, 4.95; Cl, 7.89; N, 9.38. Found: C, 45.99; H, 4.95; Cl, 7.86; N, 9,67.

5-[Bis(2-chloroethyl) aminomethyl] -1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-1,2,4-triazole (9c). A solution of 6c (0. 56 g, 0.0011 mol) in dry acetone (10 mL) was treated with bis (2-chloroethyl) amine (0.5 g, 0.0036 mol) freshly liberated. This compound was obtained as a liquid by treatment of bis-(2-chloroethyl) amine hydrochloride with a cold aqueous KOH solution, extration of the mixture with ether, drying and evaporation of the organic phase. The acetone solution of 6c and bis-(2-chloroethyl) amine was allowed to stand at room temperature for 48 h. Then, the solvent was removed and the residue was purified by preparative tlc, using chloroform as solvent to give pure 9c (0.67 g, 97%) as a syrup.

Anal. Calcd. for $C_{21}H_{30}Cl_2N_4O_9$: C, 45.59; H,5.46; Cl, 12.78; N, 10.12. Found: C, 45.60; H, 5.61; Cl,12.93; N, 9.79.

Compound 9c was further characterized as the corresponding hydrochloride which crystallized from EtOAc, m.p. 203-204ºC.

Anal. Calcd for $C_{12}H_{30}Cl_2N_4O_9 \cdot HCl$. C, 42.77; H, 5.30; Cl, 17.99; N, 9.50. Found: C, 43.02; H, 5.26; Cl, 17,63; N, 9.50.

5-[(1-Aziridino)methyl] -1-(2,3,4,6-tetra-O-acetyl-β--D-glucopyranosyl)-1,2,4-triazole (10c). To a solution of 8c (0.6 g, 0.0013 mol) in dry acetone (10 mL) aziridine (0.1 mL, 0.0019 mol) was added. The mixture was stirred at room temperature for 72 h. The solvent was evaporated and the residue was purified by preparative tlc, using EtOAc-Hexane (3:1) as eluant to yield 10c (0.33 g, 50%) as a light yellow syrup which crystallized on standing m.p. 144-146ºC.

Anal. Calcd. for $C_{19}H_{26}N_4O_9$: C, 50.21; H, 5.76; N, 12.33. Found: C, 49.88, H, 6.00; N, 12.05.

ACKNOWLEDGEMENT

The authors thank Mrs. Emilia Bayo for technical assistance.

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