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SYNTHESIS OF ALKYLATING 1-GLYCOSYL-5-SUBSTITUTED 1,2,4-TRIAZOLE$^1$

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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$^2$, pyrazole$^5$, and imidazole$^6$ 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$^2$ 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-$\beta$-D-ribofuranosyl and tetraacetyl-$\beta$-D-glucopyranosyl derivatives of 1,2,4-triazole substituted at the 5-position with halomethyl, nitrogen mustard and aziridine alkylating groups.

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The synthetic route started with the preparation of per-acetyl-β-D-glycosyl-1,2,4-triazoles 48. Fusion of 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose with 1,2,4-triazole at 160°C 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)₂Hg²⁻. 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-511. This assignment was confirmed by the differences in chemical shifts of H-3 and H-5 in (CD₃)₂SO and CDCl₃, 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-312. Thus, the Δδ = δ(CD₃)₂SO - δCDCl₃ values for the lower field proton, H-5, were 0.5 ppm for both 4a and 4c. The Δδ 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 J₁₁',₂₁' = 9 Hz. The anomeric configuration...
TABLE 1. Chemical shift (δ) and coupling constants (Hz) of glucosyl and ribosyl-1,2,4-triazoles 4-10.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Solvent</th>
<th>H-1'</th>
<th>H-3</th>
<th>H-5</th>
<th>CH₂X(5)</th>
<th>J₁',₂'</th>
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</thead>
<tbody>
<tr>
<td>4a</td>
<td>DMSO</td>
<td>6.39</td>
<td>8.27</td>
<td>8.88</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>4a</td>
<td>CDCl₃</td>
<td>6.09</td>
<td>8.12</td>
<td>8.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>DMSO</td>
<td>5.91</td>
<td>8.19</td>
<td>8.93</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>4c</td>
<td>DMSO</td>
<td>6.20</td>
<td>8.18</td>
<td>8.96</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>4c</td>
<td>CDCl₃</td>
<td>5.72</td>
<td>8.10</td>
<td>8.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5a</td>
<td>DMSO</td>
<td>6.43</td>
<td>8.13</td>
<td></td>
<td>4.72</td>
<td>4</td>
</tr>
<tr>
<td>5a</td>
<td>CDCl₃</td>
<td>6.35</td>
<td>7.97</td>
<td></td>
<td>4.89</td>
<td></td>
</tr>
<tr>
<td>5c</td>
<td>DMSO</td>
<td>6.33</td>
<td>8.07</td>
<td></td>
<td>4.79</td>
<td>8</td>
</tr>
<tr>
<td>5c</td>
<td>CDCl₃</td>
<td>5.90</td>
<td>7.94</td>
<td></td>
<td>4.93</td>
<td></td>
</tr>
<tr>
<td>6a</td>
<td>CDCl₃</td>
<td>6.10</td>
<td>7.93</td>
<td></td>
<td>4.58</td>
<td>3</td>
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<tr>
<td>6c</td>
<td>CDCl₃</td>
<td>5.78</td>
<td>7.90</td>
<td></td>
<td>4.60</td>
<td>8</td>
</tr>
<tr>
<td>7c</td>
<td>CDCl₃</td>
<td>5.79</td>
<td>7.87</td>
<td></td>
<td>4.50</td>
<td>9</td>
</tr>
<tr>
<td>8c</td>
<td>CDCl₃</td>
<td>5.80</td>
<td>7.90</td>
<td></td>
<td>4.77</td>
<td>8</td>
</tr>
<tr>
<td>9c *</td>
<td>CDCl₃</td>
<td>6.37</td>
<td>7.87</td>
<td></td>
<td>4.18</td>
<td>9</td>
</tr>
<tr>
<td>10c **</td>
<td>CDCl₃</td>
<td>6.30</td>
<td>7.85</td>
<td></td>
<td>3.63</td>
<td>9</td>
</tr>
</tbody>
</table>

*δ 2.98 (t, CH₂N), 3.60 (t, CH₂Cl)
**δ 1.36, 1.85 (2m, 4H, aziridine)

of 4a could not be determined from the pmr spectrum, since J₁',₂' = 3Hz could correspond to both α and β anomers. 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 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 105° and 115°C, 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$_3$)$_2$SO 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, recommended 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-(6-D-ribofuranosyl)purine shows activity against adenocarcinoma 75517. 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, \( 5\{N,N\text{-bis-(2-chloroethyl) aminomethyl}\}-1,2,4\text{-triazole} \) 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 $6\text{c}$ and $7\text{c}$ showed significant $ID_{50} = 2.5 \mu g/ml$ values. However, cytostatic activities of chloromethyl, N,N-bis-(2-chloroethyl)-aminomethyl and aziridinomethyl derivatives $6\text{c}, 9\text{c}$ and $10\text{c}$ respectively, were not significant ($ID_{50} = 25 \mu g/ml$).

**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$_4$Si 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-0-acetyl-β-D-ribofuranosyl)-1,2,4-triazole** ($4\text{a}$). A mixture of 1,2,3,5-tetra-0-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 160°C for 45 min. at reduced pressure. After cooling, the reaction mixture was dissolved in boiling ethanol, treated with charcoal and filtered. On cooling $4\text{a}$ crystallized (8.4g, 81%), m.p. 110-111°C.

**Anal. Calcd.** for C$_{13}$H$_{17}$N$_3$O$_7$: 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** ($4\text{b}$). Compound $4\text{a}$ (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\textsuperscript{11} m.p. 143-145\degree C).

1-(2,3,4,6-Tetra-O-acetyl-\beta-D-glucopyranosyl)-1,2,4-triazole. (4c). To a mixture of 2,3,4,6-tetra-O-acetyl-\beta-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 \degree C.

Anal. Calcd. for C\textsubscript{16}H\textsubscript{19}N\textsubscript{3}O\textsubscript{5}: 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-\beta-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 105\degree C 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\textsubscript{14}H\textsubscript{19}N\textsubscript{3}O\textsubscript{5}: C, 47.05; H, 5.35; N, 11.76. Found: C, 46.79; H, 5.78; N, 11.35.

5-Hydroxyethyl-1-(2,3,4,6-tetra-O-acetyl-\beta-D-glucopyranosyl)-1,2,4-triazole (5c). A solution of 4c (19.64 g, 0.049 mol) in dioxane (50 mL) and 35\% aqueous formaldehyde (133 mL) was heated in sealed tube at 115\degree C for 16.5 h. 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 syrup 5c, which spontaneously crystallized after standing at room temperature for one year m.p. 136°C.

Anal. Calcd. for C_{17}H_{23}N_{3}O_{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), triphenylphosphite (3 mL, 0.0099 mol) was added. The mixture was stirred at 0°C 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 previous 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 (30 mL), triphenylphosphite (2.15 g, 0.0069 mol) was added. The mixture was stirred at 0°C 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 - CHCl₃ (1:1) as eluant, to give pure 6c (1.2 g, 52%), m.p. 137-138°C (EtOAc-Hexane).

Anal. Calcd. for C₁₇H₂₂BrN₃O₉: 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. 136-137°C (EtOAc-hexane).

Anal. Calcd. for C₁₇H₂₂I₃N₃O₉: 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 CCl₄ (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. 156-78°C (EtOAc-Hexane).

Anal. Calcd. for C₁₇H₂₂Cl₁₂N₃O₉: 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, extraction 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_{2}N_{4}O_{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. 203-204°C.

Anal. Calcd for C_{12}H_{30}Cl_{2}N_{4}O_{9}. 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. 144-146°C.

Anal. Calcd. for C_{19}H_{26}N_{4}O_{9}: C, 50.21; H, 5.76; N, 12.33. Found: C, 49.88, H, 6.00; N, 12.05.
ACKNOWLEDGEMENT

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REFERENCES

1. Paper no 8 in the series "Alkylating Nucleosides". For previous paper in the series see ref. 2a.


14. See reference 1 and 3


