Heterocyclic *N*-Glycosides. VI. The Reaction of Glycosyl Azides with Propiolic Acid and Methyl Propiolate.

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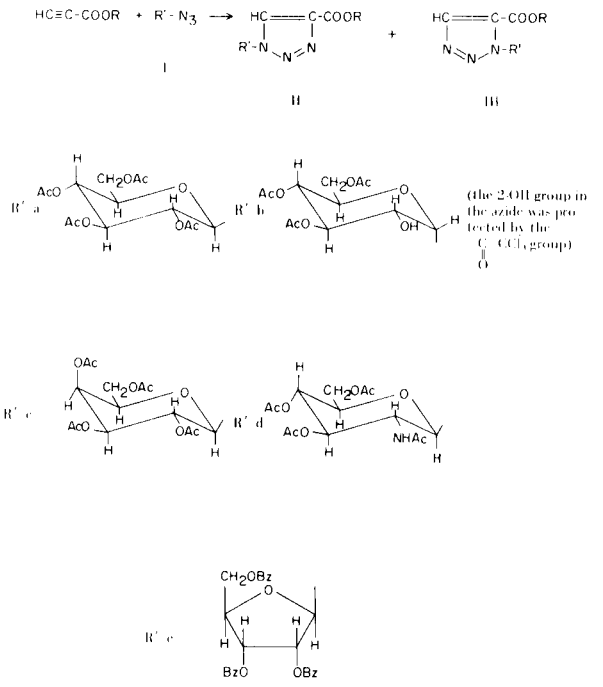
The synthesis of a series of *N*-glycosyl-*v*-triazoles has been accomplished by the 1,3-dipolar cycloaddition of several glycosyl azides to methyl propiolate and propiolic acid. In most of the cases the two isomeric *v*-triazoles were obtained. Structural and anomeric configuration assignments for the *N*-glycosides obtained were made on the basis of NMR data. None of the compounds possessed appreciable biological activity against HeLa cells in culture and mouse Sarcoma 180.

In previous papers in this series (1,2) we reported the application of glycosyl azides to the preparation of heterocyclic *N*-glycosides derived from benzotriazole and 4(5)-phenyl-1,2,3-triazole. As it was shown (2), glycosyl azides added to phenylacetylene to give a mixture of the two possible isomeric *v*-triazoles in almost equal amounts.

In the present investigation glycosyl azides (I) were reacted with methyl propiolate and propiolic acid to yield a pair of isomeric *v*-triazoles (II + III), exceptions being the reaction with 2-*O*-trichloroacetyl-3,4,6-tri-*O*-acetyl-α-D-glycopyranosyl azide and the reactions using propiolic acid which led to only one of the isomers, the corresponding 1,4-substituted *v*-triazoles. Nevertheless, in the other cases when the two isomers were obtained, the 1,4-substituted *v*-triazole (II) was the predominate one.



In order to find a method for recognising the positional isomers of *N*-glycosides the use of NMR spectroscopy appeared to be the most promising. A study of the NMR spectra was made, especially on the chemical shifts of the H-4 protons (1,5-disubstituted derivatives) and H-5 protons (1,4-disubstituted derivatives) as well as on solvent induced shifts. As Elguero, González and Jacquier have shown (3) in a previous study of the chemical shifts of H-4 and H-5 protons in 1-methyl-1,2,3-triazole and H-5 proton in 1-methyl-4-bromo-1,2,3-triazole and also in some other azoles, the proton nearest to the substituted nitrogen atom (H-5) is the most sensible to both solvent induced effects and nature of the substituent on the nitrogen atom.

Table I contains the chemical shifts data obtained in deuteriochloroform and DMSO for H-4 and H-5 protons in the various *N*-glycosyl-*v*-triazoles along with the $\Delta\tau$ values ($\Delta\tau = \tau_{\text{deuteriochloroform}} - \tau_{\text{DMSO}}$).

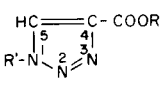
It can be seen that in the cases when both isomers were available and it was possible to record the corresponding NMR spectra in both deuteriochloroform and DMSO, the proton resonance values of H-5 in both solvents were considerably lower than the corresponding H-4. A much higher $\Delta\tau$ value was observed in 1,4-disubstituted compounds than in the other derivatives, showing the existence on those *N*-glycosides of a proton (H-5) (3) near to the nitrogen atom where the sugar moiety is bonded.

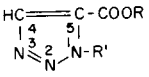
When only one of the isomers was available, the unequivocal structure assignment was made on the basis of the τ value of the corresponding triazolic proton.

The products obtained starting with propiolic acid (only a single isomer in each case), and assigned as the 1,4-disubstituted *v*-triazoles II (R = H, R' = a and R = H,

TABLE I

Chemical Shifts for Triazolic Ring Protons
(10% solutions, τ-values)





		CCl_3D	DMSO	$\Delta\tau$*	CCl_3D	DMSO	$\Delta\tau$*
R = CH_3	R′ = a	1.44	0.77	0.67	1.89	1.54	0.35
R = CH_3	R′ = b	----	1.07	----	----	----	----
R = H	R′ = a	----	0.90	----	----	----	----
R = CH_3	R′ = d	----	0.89	----	1.88	1.59	0.29
R = H	R′ = d	----	1.04	----	----	----	----
R = CH_3	R′ = c	1.51	0.86	0.65	1.83	1.52	0.31
R = CH_3	R′ = e	1.62	0.89	0.73	1.82	1.59	0.23

* $\Delta\tau = \tau_{\text{deuteriochloroform}} - \tau_{\text{DMSO}}$.

R′ = d) on the basis of their NMR spectra, when esterified with diazomethane yielded the compounds II (R = CH_3, R′ = a and R = CH_3, R′ = d) identical to those obtained starting with methyl propiolate.

The configurations at the anomeric carbon atom for the glycopyranosides were established by their NMR spectra. The anomeric protons appeared as doublets and the values of the coupling constants were in the usual range (8-10 Hz) for diaxial (β configuration) coupled protons. In the case of compound II (R = CH_3, R′ = b) the anomeric proton appeared also as a doublet but the value of the coupling constant was 6.1 Hz which is more characteristic for an α configuration. As for the ribofuranosyl derivatives II (R = CH_3, R′ = e) and III (R = CH_3, R′ = e) the anomeric protons also appeared as doublets with a $J_{1',2'}$ values of 2.8 and 1.3 Hz, respectively. Since these values are less than 3.5 Hz (4), the configurations of these *N*-glycosides were assigned as β.

When the carbomethoxy derivatives were treated with methanolic ammonia, the corresponding deacetylated or debenzoylated carboxamido compounds were obtained in good yield.

Attention is called to the fact that in all the cases the 1,5-disubstituted compounds showed a UV absorption maximum at slightly higher wave length than the corresponding 1,4-disubstituted isomers.

All of the *N*-glycosides obtained, including the carboxamido derivatives, were screened in our laboratories for antitumor activity in cell culture tests against HeLa cells at three dose levels (1, 10 and 100 μg/ml.). Only one compound, II (R = H, R′ = d), showed a slight activity. The *N*-glycosides II (R = CH_3, R′ = a; R = CH_3, R′ = c; R = CH_3, R′ = d; R = CH_3, R′ = e; R = H, R′ = a; R = H, R′ = d) were tested for toxicity and then screened against mouse Sarcoma 180 according to the protocols of the Cancer Chemotherapy National Service Center (5). None of these compounds showed a significant activity at doses of 350-400 mg/kg/day.

EXPERIMENTAL

Melting points were taken on a Kofler apparatus, NMR spectra were recorded on a Perkin-Elmer R-10 spectrometer at room temperature with TMS as an internal standard, the ultraviolet spectra with a Perkin-Elmer 350 spectrophotometer and the infrared spectra with a Perkin-Elmer 257 spectrophotometer. Optical rotations were obtained with a Perkin-Elmer 141 polarimeter.

T.l.c. was performed with 0.25 mm chromatoplates of silica gel GF_{254} (Merck) and spots were visualized with UV light of 254 mμ or with sulfuric acid in ethanol, 30%.

General Procedure for the Preparation of the *N*-Glycosides.

A mixture of 0.015 mole of methyl propiolate or propiolic acid and 0.01 mole of the glycosyl azide in 50 ml. of toluene was heated at reflux temperature for 10-12 hours. The reaction was monitored by t.l.c. (ethyl acetate-petroleum ether, 1:1). At the end, the reaction products were separated and purified as specified in each case.

4-Carbomethoxy-1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2,3-triazole II (R = CH_3, R′ = a) and 5-Carbomethoxy-1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2,3-triazole III (R = CH_3, R′ = a).

The solid which separated after cooling the reaction mixture was collected by filtration. T.l.c. of this product (chloroform) showed a single spot, visualized with sulfuric acid in ethanol, 30%. Recrystallization from ethanol gave white needles, m.p. 212°, $[\alpha]_D$ -33.7° (c 1.0, chloroform); U.V. λ max (ethanol), 212 mμ (ϵ, 12,400); NMR (deuteriochloroform, τ), 3.92 doublet (H_1', $J_{1',2'}$ 9.1 Hz). This compound was the *N*-glycoside II (R = CH_3, R′ = a), yield, 57%.

Anal. Calcd. for $C_{18}H_{23}N_3O_{11}$: C, 47.26; H, 5.03; N, 9.18. Found: C, 47.25; H, 4.83; N, 9.07.

The colourless residue obtained by evaporation of the mother liquor was dissolved in a small amount of ethyl acetate and chromatographed on a silica gel column. The column was eluted with mixtures of petroleum ether-ethyl acetate (4:1, 2:1, and 1:1). Two products were separated, first, 0.6 g. of 2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosyl azide. Then 0.8 g. of a product was eluted which was shown to be a mixture of the above *N*-glycoside and a compound visualized with U.V. light. Separation of this mixture was accomplished by thick-layer chromatography after 17 consecutive developments with chloroform (10 plates). From the fluorescent band was obtained 0.37 g. of a solid which was recrystallized from ethyl acetate-petroleum ether to give white needles, m.p. 119°, $[\alpha]_D$ -20.3° (c 1.0, chloroform); U.V. λ max (ethanol), 224 mμ (ε, 7,180); NMR (deuteriochloroform, τ), 3.62 doublet (H_1', $J_{1',2'}$ 9.6 Hz). This compound was III (R = CH_3, R' = a), yield, 8.5%.

Anal. Calcd. for $C_{18}H_{23}N_3O_{11}$: C, 47.26; H, 5.03; N, 9.18. Found: C, 47.36; H, 5.07; N, 8.99.

4-Carbomethoxy-1-(3',4',6'-tri-*O*-acetyl-α-D-glycopyranosyl)-1,2,3-triazole II (R = CH_3, R' = b).

The precipitate, which formed on cooling the reaction mixture at room temperature, was removed by filtration. T.l.c. of this product (visualized with sulfuric acid in ethanol, 30%) showed a single spot. Recrystallization from ethanol gave 24% of II (R = CH_3, R' = b) m.p. 211°, $[\alpha]_D$ +101.6° (c 0.56, chloroform); IR, 3,459 cm^{-1} (OH group); U.V. λ max (ethanol), 213 mμ (ε, 11,050); NMR (dimethylfulfoxide-d_6, τ), 3.59 doublet (H_1', $J_{1',2'}$ 6.1 Hz).

Anal. Calcd. for $C_{16}H_{21}N_3O_{10}$: C, 46.26; H, 5.06; N, 10.12. Found: C, 46.70; H, 4.89; N, 9.84.

4-Carbomethoxy-1-(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl)-1,2,3-triazole II (R = CH_3, R' = c) and 5-Carbomethoxy-1-(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl)-1,2,3-triazole III (R = CH_3, R' = c).

On cooling the reaction mixture, a solid separated. It was filtered off and recrystallized from ethanol to give a white compound m.p. 178°, $[\alpha]_D$ -11° (c 0.6, chloroform); U.V. λ max (ethanol), 211 mμ (ε, 12,960); NMR (deuteriochloroform, τ), 3.98 doublet (H_1', $J_{1',2'}$ 8.7 Hz). This product was II (R = CH_3, R' = c), yield, 69%.

Anal. Calcd. for $C_{18}H_{23}N_3O_{11}$: C, 47.26; H, 5.03; N, 9.18. Found: C, 47.19; H, 5.14; N, 9.07.

The mother liquors were taken to dryness *in vacuo*, the residue (1.12 g.) was dissolved in chloroform and the solution was applied to 8 preparative t.l.c. plates (20 x 20 cm, and 2 mm thickness silica gel, Merck PF_{254}). The plates were developed 2 times in a mixture of chloroform-ethyl acetate (4:1) and 15 times in chloroform resulting in the separation of a compound which was detected by a U.V. lamp (254 mμ). Recrystallization from ethyl acetate-petroleum ether afforded a solid, m.p. 126°, $[\alpha]_D$ -5° (c 0.46, chloroform); U.V. λ max (ethanol), 225 mμ (ε, 8,770); NMR (deuteriochloroform, τ), 3.67 doublet (H_1', $J_{1',2'}$ 8.7 Hz). This product was III (R = CH_3, R' = c), yield, 12.5%.

Anal. Calcd. for $C_{18}H_{23}N_3O_{11}$: C, 47.26; H, 5.03; N, 9.18. Found: C, 47.06; H, 5.08; N, 9.36.

4-Carbomethoxy-1-(2'-acetamido-2'-deoxy-3',4',6'-tri-*O*-acetyl-β-D-glucopyranosyl)-1,2,3-triazole II (R = CH_3, R' = d) and 5-Carbomethoxy-1-(2'-acetamido-2'-deoxy-3',4',6'-tri-*O*-acetyl-β-D-glucopyranosyl)-1,2,3-triazole III (R = CH_3, R' = d).

The solid which separated after cooling the reaction mixture was filtered off and recrystallized from ethanol to give white needles, m.p. 270°, $[\alpha]_D$ -53° (c 0.5, pyridine); U.V. λ max (ethanol), 210 mμ (ε, 12,900); NMR (deuteriochloroform, τ), 3.78 doublet (H_1', $J_{1',2'}$ 9.8 Hz). This compound was II (R = CH_3, R' = d), yield, 58%.

Anal. Calcd. for $C_{18}H_{24}N_4O_{10}$: C, 47.36; H, 5.26; N, 12.28. Found: C, 47.14; H, 5.18; N, 12.03.

Concentration of the filtrate furnished a residue which was purified by thick-layer chromatography. After four consecutive developments with a mixture of ethyl acetate-ethanol (9:1), from the band visualized with a U.V. lamp, a solid was obtained, m.p. 137° (ethyl acetate-petroleum ether), $[\alpha]_D$ +9° (c 0.55, chloroform); U.V. λ max (ethanol), 225 mμ (ε, 8,150); NMR (deuteriochloroform, τ), 3.18 doublet (H_1', $J_{1',2'}$ 9.8 Hz). This product was III (R = CH_3, R' = d), yield, 7%.

Anal. Calcd. for $C_{18}H_{24}N_4O_{10}$: C, 47.36; H, 5.26; N, 12.28. Found: C, 47.64; H, 5.45; N, 12.14.

4-Carbomethoxy-1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,3-triazole II (R = CH_3, R' = e) and 5-Carbomethoxy-1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,3-triazole III (R = CH_3, R' = e).

The insoluble residue which separated on cooling the reaction mixture was filtered and recrystallized from a mixture of ethyl acetate-petroleum ether to yield 1.25 g. of a white solid, m.p. 195°, $[\alpha]_D$ -73.7° (c 0.55, chloroform); U.V. λ max (ethanol), 229 mμ (ε, 49,250); NMR (deuteriochloroform, τ), 3.48 (H_1', $J_{1',2'}$ 2.8 Hz); (dimethylsulfoxide-d_6, τ), 3.20 (H_1', $J_{1',2'}$ 2.4 Hz). This compound was II (R = CH_3, R' = e)

Anal. Calcd. for $C_{30}H_{25}N_3O_9$: C, 63.04; H, 4.37; N, 7.36. Found: C, 62.90; H, 4.36; N, 7.46.

The filtrate was evaporated to dryness *in vacuo* to afford 1.08 g. of a tan solid. Thin layer chromatography using chloroform showed two ultraviolet absorbing species to be present. Thick layer chromatography after seven developments with chloroform was successful in resolving the mixture and the faster moving band furnished 0.28 g. of II (R = CH_3, R' = e). The other band yielded 0.23 g. of a white solid which was recrystallized from ethyl acetate-petroleum ether, m.p. 54.56°, $[\alpha]_D$ -8.5° (c 0.59, chloroform); U.V. λ max (ethanol), 231 mμ (ε, 49,235); NMR (dimethylsulfoxide-d_6, τ), 2.85 doublet (H_1', $J_{1',2'}$ 1.3 Hz). This product was III (R = CH_3, R' = e).

Anal. Calcd. for $C_{30}H_{25}N_3O_9$: C, 63.04; H, 4.37; N, 7.36. Found: C, 62.93; H, 4.14; N, 7.08.

1-(2',3',4',6'-Tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2,3-triazole-4-carboxylic Acid II (R = H, R' = a).

The solid material which formed on cooling the reaction mixture was filtered off and recrystallized from water, m.p. 178°, $[\alpha]_D$ -35° (c 0.5 ethanol); NMR (dimethylsulfoxide-d_6, τ), 3.54 doublet (H_1', $J_{1',2'}$ 9.1 Hz), yield, 39%.

Anal. Calcd. for $C_{17}H_{21}N_3O_{11}$: C, 46.04; H, 4.74; N, 9.48. Found: C, 46.01; H, 4.84; N, 9.50.

The methyl ester, prepared by adding an ethereal solution of diazomethane to an ethyl acetate solution of II (R = H, R' = a), was shown to be identical to the compound II (R = CH_3. R' = a) obtained starting from methyl propiolate by t.l.c. and m.p. comparison.

1-(2'-Acetamido-2'-deoxy-3',4',6'-tri-*O*-acetyl-β-D-glycopyranosyl)-1,2,3-triazole-4-carboxylic Acid II (R = H, R' = d).

The brown residue from the evaporation of the solvent was recrystallized from methanol-ether using active carbon, to give a solid, m.p. 228°, $[\alpha]_D$ -56° (c 0.55, pyridine); NMR (dimethyl-

sulfoxide-d_6, τ), 3.78 doublet (H_1', $J_{1',2'}$ 9.8 Hz), yield, 40%.

Anal. Calcd. for $C_{17}H_{22}N_4O_{10}$: C, 46.15; H, 4.97; N, 12.66. Found: C, 46.40; H, 4.96; N, 12.71.

The methyl ester, prepared as above, was shown to be identical to II (R = CH_3, R' = d) obtained from methyl propiolate.

Deacetylation and Debenzoylation of the above *N*-Glycosides. Preparation of Carboxyamides.

The protected *N*-glycosides (0.01 mole) were dissolved in 60 ml. of dry methanolic ammonia (methanol saturated with ammonia at 0°) and the resulting solution was allowed to stand at room temperature for 24 hours. After removal of the methanol, the compounds were purified as indicated in each case.

4-Carboxamido-1-(β-D-glucopyranosyl)-1,2,3-triazole.

This compound was obtained from II (R = CH_3, R' = a) as a white solid, m.p. 232° (methanol-water, 10:1 and then ether), $[\alpha]_D$ +2° (c 0.5, water), yield, 90%.

Anal. Calcd. for $C_9H_{14}N_4O_6$: C, 39.41; H, 5.10; N, 20.43. Found: C, 39.15; H, 5.15; N, 20.26.

5-Carboxamido-1-(β-D-glucopyranosyl)-1,2,3-triazole.

This compound was prepared from III (R = CH_3, R' = a); m.p. 201-202° (methanol-water, 10:1 and then ether), $[\alpha]_D$ +2° (c 0.77, water), yield, 95%.

Anal. Calcd. for $C_9H_{14}N_4O_6$: C, 39.41; H, 5.10; N, 20.43. Found: C, 39.49; H, 5.19; N, 20.63.

4-Carboxamido-1-(β-D-galactopyranosyl)-1,2,3-triazole.

This compound was prepared by deacetylation of II (R = CH_3, R' = c); m.p. 237° (methanol-water, 10:1 and then ether), $[\alpha]_D$ +31.5° (c 0.54, water), yield, 90%.

Anal. Calcd. for $C_9H_{14}N_4O_6$: C, 39.41; H, 5.10; N, 20.43. Found: C, 39.25; H, 5.22; N, 19.98.

5-Carboxamido-1-(β-D-galactopyranosyl)-1,2,3-triazole.

This compound was obtained from III (R = CH_3, R' = c); m.p. 235-236° (methanol-water, 10:1 and then ether), $[\alpha]_D$ +26° (c 0.43, water), yield, 80%).

Anal. Calcd. for $C_9H_{14}N_4O_6$: C, 39.41; H, 5.10; N, 20.43. Found: C, 39.83; H, 5.14; N, 20.40.

4-Carboxamido-1-(2'-acetamido-2'-deoxy-β-D-glucopyranosyl)-1,2,3-triazole.

This compound was prepared from II (R = CH_3, R' = d); m.p. 250° (methanol-water, 10:1 and then ether), $[\alpha]_D$ -8.5° (c 0.61, water), yield, 95%.

Anal. Calcd. for $C_{11}H_{17}N_5O_6 \cdot \frac{1}{2}CH_3OH$: C, 41.69; H, 5.73; N, 21.14. Found: C, 41.65; H, 5.81; N, 20.96.

4-Carboxamido-1-(β-D-ribofuranosyl)-1,2,3-triazole.

This product was obtained from II (R = CH_3, R' = e); m.p. 204° (methanol-water, 10:1 and then ether), $[\alpha]_D$ -51.5° (c 0.45, water), yield, 70%.

Anal. Calcd. for $C_8H_{12}N_4O_5$: C, 39.32; H, 4.91; N, 22.91. Found: C, 39.34; H, 4.97; N, 23.29.

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