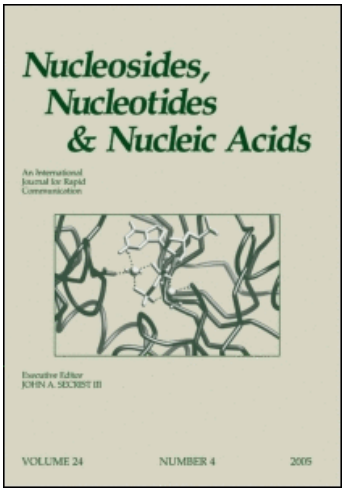

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Synthesis and Cytostatic Activity of Halomethylthiazole C-Nucleosides and Analogues[1]

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SYNTHESIS AND CYTOSTATIC ACTIVITY OF HALOMETHYLTHIAZOLE C-NUCLEOSIDES AND ANALOGUES[1]

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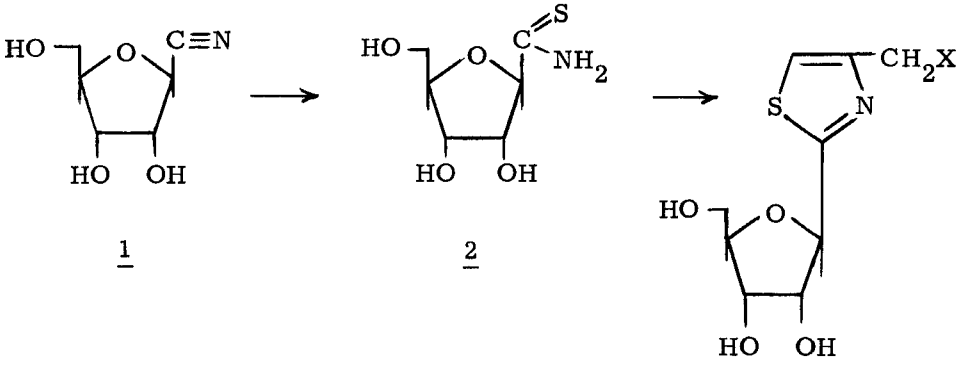
Abstract. The synthesis of 2-(β-D-ribofuranosyl)- and 2-(tetrahydropyran-2-yl)-4-halomethylthiazoles from 2,5-anhydro-D-allonthioamide and tetrahydropyran-2-thiocarboxamide is described. Bromination of 2-(β-D-ribofuranosyl)- and 2-tetrahydropyran-2-yl)-4-methylthiazole with NBS is studied. Cytostatic activity against HeLa cells of all the compounds is reported.

With the aim of exploring the possibilities of benzylic type halides as a new type of cytotoxic alkylating agents, we have reported the synthesis, cytostatic activity and mode of action of a series of N-glycosyl derivatives of several halomethylpentaheterocycles[2]. Studies on structure-activity relationships demonstrated that the presence of the glycosyl moiety is required for cytostatic activity, although the nature of such moiety does not seem to be determinant of activity[2,3,4]. Due to these facts, we considered of interest, in one hand, to prepare C-ribosyl halomethyl heterocycles in order to increase the strenght of the sugar-aglycon bond, and, on the other, to compare their activities with those of the corresponding tetrahydropyranyl derivatives, considering tetrahydropyran as a simple model of sugars.

The present paper describes the synthesis of 2-(β-D-ribofuranosyl)- and 2-(tetrahydropyran-2-yl)-4-halomethylthiazoles. It also describes the study of the bromination of 2-(β-D-ribofuranosyl)- and 2-(tetrahydropyran)-2-yl)-4-methylthiazole with N-bromosuccinimide.

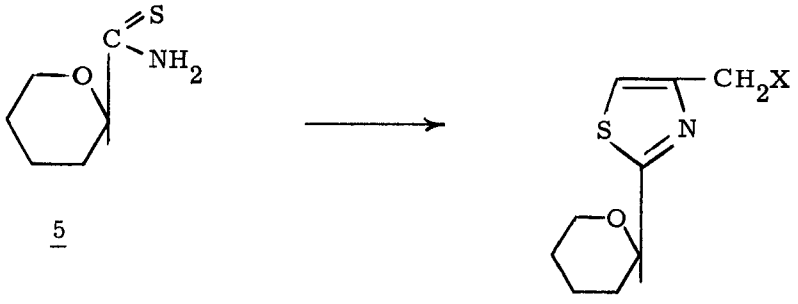
The procedure reported in the literature for the preparation of 2-glycosyl thiazoles involves the condensation of hydroxyl protected glycosylthiocarboxamides with α-halocarbonyl compounds[5,6]. In those cases the use of 2,5-anhydro-3,4,6-tri-O-benzoyl-D-allonthioamide afforded, besides the desired 2-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl) thiazoles, 2-(5-benzoyloxymethylfuran-2-yl) thiazoles resulting from benzoate elimination. In order to avoid such elimination during cyclization reaction, 2,5-anhydro-3,4-O-isopropylidene-D-allonthioamide and 2,5-anhydro-6-O-benzoyl-D-allonthioamide were used in those reactions[5a,6]. In our case, 2,5-anhydro-D-allonthioamide (2) was utilized as the starting material which was readily obtained as the only reaction product in 87% yield when β-D-ribofuranosyl cyanide (1) was treated with hydrogen sulfide in the presence of triethylamine. Reaction of the thioamide 2 with 1,3-dichloroacetone or 1,3-dibromoacetone in ethanol afforded 2-(β-D-ribofuranosyl)-4-chloromethylthiazole (3) or 2-(β-D-ribofuranosyl)-4-bromomethylthiazole (4) in 38 and 25% yield, respectively. It is interesting to note that the use of the hydroxyl deprotected thioamide 2 in the preparation of the halomethylthiazoles 3 and 4 has allowed us to obtain for the first time, deprotected alkylating nucleosides, since all the attempts to remove the sugar hydroxyl protecting groups in the series of N-glycosyl halomethyl heterocycles were unsuccessful[4,7].

Similar condensation reactions of tetrahydropyran-2-thiocarboxamide (5), obtained in 90% yield from 2-cyanotetrahydropyran[8], with 1,3-dichloroacetone or 1,3-dibromoacetone gave 2-(tetrahydropyran-2-yl)-4-chloromethylthiazole (6) or 2-(tetrahydropyran-2-yl)-4-bromomethylthiazole (7) in 55 and 26% yield respectively.



1 2

3 : X = Cl
4 : X = Br



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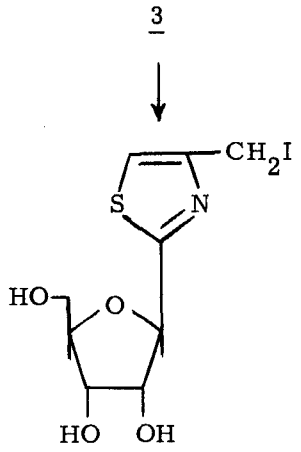
6 : X = Cl
7 : X = Br

Iodomethylthiazole derivatives, namely 2-(β-D-ribofuranosyl)- and 2-(tetrahydropyran-2-yl)-4-iodomethylthiazole (8 and 9) were obtained from the corresponding chloromethyl analogs 3 and 6 by transhalogenation reaction with sodium iodide in acetone.

Pure samples of the ribofuranosyl derivatives 3, 4 and 8 were obtained when recently purified by TLC. However, these compounds, specially the chloro- and the bromomethyl substituted nucleosides 3 and 4, obtained as syrups decomposed on standing.

In order to explore an alternative procedure for the preparation of the bromomethylthiazoles 4 and 7, we attempted a route which had been successfully used in the synthesis of certain N-glycosyl bromo-



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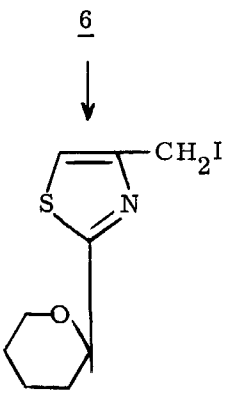

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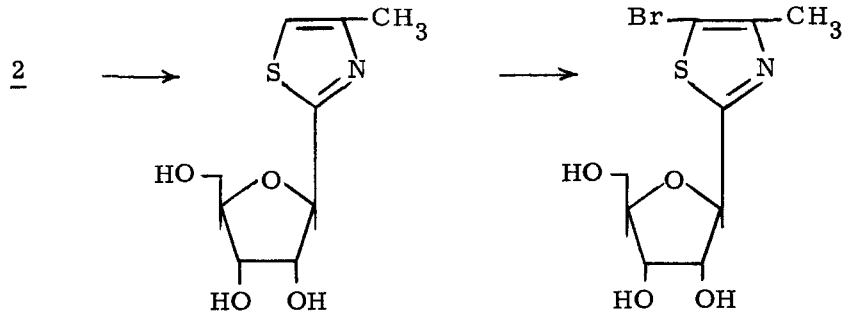
methylpyrazoles [9] and N-glycosyl bromomethylimidazoles [10]. This route consists on the generation of the bromomethyl group on methyl substituted nucleosides, previously formed. With this end, the thioamides 2 and 5 were condensed with chloroacetone to give 2-(β-D-ribofuranosyl)-4-methylthiazole [5a] (10) and 2-(tetrahydropyran-2-yl)-4-methylthiazole (11) in 40 and 44% yield, respectively. It should be noted that compound 10 has been previously prepared by debenzoylation of 2-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-4-methylthiazole, obtained by reaction of 2,5-anhydro-3,4,6-tri-O-benzoyl-D-allonthioamide with chloroacetone. That condensation reaction also led to 2-(5-benzoyloxymethylfuran-2-yl)-4-methylthiazole resulting from elimination of two benzoyloxy groups. As mentioned before, the use of the unprotected thiocarboxamide 2 has allowed us to avoid the easy benzoate elimination during cyclization and to prepare the methylthiazole 10 in one step and in higher yield than that reported [5a]. Reaction of methylthiazoles 10 and 11 with N-bromosuccinimide, irradiating with a 200 wat lamp, provided 2-(β-D-ribofuranosyl)-5-bromo-4-methylthiazole (12) and 2-(tetrahydropyran-2-yl)-5-bromo-4-methylthiazole (13), respectively, as the only reaction products.



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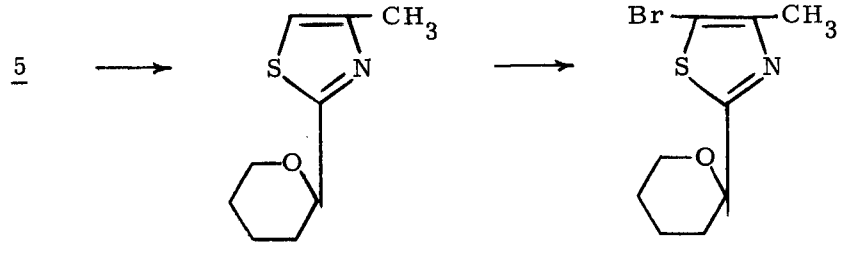

11 13

The absence of the signal corresponding to the H-5 thiazole proton and the presence of the signal corresponding to CH_3-4 in the ^1H NMR spectra of 12 and 13 clearly demonstrated that bromination took place at C-5 of the thiazole ring. (TABLE 1).

All the compounds reported in this paper were evaluated as cytotoxic agents against HeLa cell cultures[11]. Although the cytostatic activity was associated with the presence of the alkylating halomethyl group, only the bromomethyl and iodomethyl derivatives 7 and 9 showed significant activity. The activities of the 2-(β-D-ribofuranosyl) halomethylthiazoles 3, 4 and 8 were not significant (3 and 4: ID_{50} = 10 -100 μg/ml; 8: ID_{50} = 10 μg/ml) and, in all cases, lower than those of the corresponding tetrahydropyranyl derivatives 6, 7 and 9 (ID_{50} = 10, 5 and 4 μg/ml, respectively). The low activity of ribofuranosyl derivatives 3, 4 and 8 may be due to their instability.

TABLE 1. Chemical shift (δ) and coupling constants (Hz) of 2-(β-D-ribofuranosyl)- and 2-(tetrahydropyran-2-yl)-thiazoles.

No	Solvent	H-5	H-1' [a] / H-2' [b]	CH_2X	$J_{1',2'}$ [a] / $J_{2'a,3'a} + J_{2'a,3'e}$ [b]
3	DMSO + D_2O	7.83	5.03	4.90 (X = Cl)	5.5
4	DMSO + D_2O	7.83	4.98	4.78 (X = Br)	4.5
6	DMSO	7.38	4.70	4.83 (X = Cl)	11
7	$CDCl_3$	7.35	4.70	4.62 (X = Br)	11
8	DMSO + D_2O	7.55	4.98	4.55 (X = I)	4
9	$CDCl_3$	7.28	4.70	4.55 (X = I)	12
10	DMSO + D_2O	7.30	4.95	2.38 (X = H)	5
11	DMSO	7.24	4.68	2.36 (X = H)	12
12	DMSO + D_2O	--	4.92	2.35 (X = H)	5
13	DMSO	--	4.65	2.32 (X = H)	11

a) β-D-Ribofuranosyl derivatives

b) Tetrahydropyranyl derivatives.

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Proton nuclear magnetic resonance spectra were recorded at 60 MHz with a Perkin-Elmer R-12 spectrometer using Me_4Si as internal standard. Analytical thin layer chromatograpy was performed on aluminium sheets coated with a 0.02 mm layer of silica gel $60F_{254}$ (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 UV light (254 nm) or by spraying the plate with a ethanol-sulfuric acid (7 : 3) mixture and heat-

ing. Column chromatography was performed on glass columns filled with silica gel 60, 70-230 mesh (Merck).

β-D-Ribofuranosyl cyanide (1). A solution of 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl cyanide[12] (4.67 g, 1.07 mmole) in saturated methanolic ammonia (250 mL) was allowed to stand at room temperature for 24 h. The residue obtained by evaporation of the solvent was chromatographed on a silica gel column. Elution with $CHCl_3$–acetone (9 : 1) and then with acetone furnished 1.23 g (78.5%) of 1 as a syrup which solidified on standing, mp 91-93°C; NMR (DMSO-D_2O): δ 4.46 (d, 1, $J_{1,2}$ = 2.5 Hz, H-1).

Anal. Calcd. for $C_6H_9NO_4$: C, 45.28; H, 5.70; N, 8.80. Found: C, 44.94; H, 5.63; N, 9.20.

2,5-Anhydro-D-allonthioamide (2). Hydrogen sulfide was bubbled into a solution of 1 (1.23 g, 7.73 mmole) in ethanol (50 mL) containing triethylamine (0.90 mL) for 2 h. The solvent was evaporated and the residue was purified by preparative TLC using acetone-$CHCl_3$ (1 : 1). Elution of the major band afforded 1.30 g (87.5%) of 2, mp 116°C (from 2-propanol); NMR (DMSO-D_2O): δ 4.40 (d, 1, $J_{2,3}$ = 2 Hz, H-2).

Anal. Calcd. for $C_6H_{11}NO_4S$: C, 37.30; H, 5.74; N, 7.25; S, 16.56. Found: C, 37.58; H, 6.06; N, 7.29; S, 16.20.

2-(β-D-Ribofuranosyl)-4-chloromethylthiazole (3). A solution of 2 (0.29 g, 1.50 mmole) and 1,3-dichloroacetone (0.189 g, 1.5 mmole) in ethanol (25 mL) was heated under reflux for 8 h. The solvent was removed and the residue was purified by preparative TLC using $CHCl_3$-MeOH (9 : 1). Elution of the major band gave 3 (0.15 g, 38%) as a homogeneous syrup.

Anal. Calcd. for $C_9H_{12}ClNO_4S$: C, 40.70; H, 4.55; N, 5.27; S, 12.05. Found: C, 41.04; H, 4.73; N, 4.89; S, 11.89.

2-(β-D-Ribofuranosyl)-4-bromomethylthiazole (4). A solution of 2 (1.00 g, 4.60 mmole) and 1,3-dibromoacetone (1.10 g, 5.10 mmole) in ethanol (50 mL) was heated under reflux for 1 h. The solvent was evaporated to provide a syrup which was chromatographed by preparative TLC using $CHCl_3$-MeOH (9:1). Elution of the major band gave 4 (0.40 g, 25%) as a homogeneous syrup.

Anal. Calcd. for $C_9H_{12}BrNO_4S$: C, 34.84; H, 3.89; N, 4.51; S, 10.31. Found: C, 34.43; H, 3.56; N, 4.71; S: 9.98.

Tetrahydropyran-2-thiocarboxamide (5). A mixture of 2-cyanotetrahydropyran[8] (12.24 g, 0.13 mmole), triethylamine (3.5 mL) and ethanol (60 mL) was stirred at room temperature for 2 h., while hydrogen sulfide was bubbled into the solution. Evaporation of the solvent left a solid which was recrystallized from EtOH to give 5 (12.8 g, 90%) mp 120°C; NMR ($CDCl_3$): δ 4.20 (dd, 1, $J_{2a,3a}$ = 10 Hz; $J_{2a,3e}$ = 2 Hz, H-2).

Anal. Calcd. for $C_6H_{11}NOS$: C, 49.64; H, 7.63; N, 9.65; S, 22.04. Found: C, 49.83; H, 7.51; N, 10.10; S, 22.22.

2-(Tetrahydropyran-2-yl)-4-chloromethylthiazole (6). A solution of 5 (1.45 g, 10 mmole) and 1,3-dichloroacetone (1.27 g, 10 mmole) in ethanol (25 mL) was refluxed for 1 h. The solvent was evaporated and the residue was purified by preparative TLC using $CHCl_3$. Elution of the major band gave 6 (1.2 g, 55%), mp 91-92°C (from EtOH).

Anal. Calcd. for $C_9H_{12}ClNOS$: C, 49.68; H, 5.55; N, 6.43; S, 14.70. Found: C, 49.81; H, 5.85; N, 6.79; S, 14.99.

2-(Tetrahydropyran-2-yl)-4-bromomethylthiazole (7). 1,3-Dibromoacetone (2.16 g, 10 mmole) was slowly added to a solution of 5 (1.45 g, 10 mmole) in ethanol (25 mL) at room temperature. Then, the solvent was removed and the residue was chromatographed by preparative TLC using $CHCl_3$-hexane (4:1). Elution of the major

band afforded a solid which was recrystallized from cyclohexane to give 7 (0.34 g, 26%), mp 89-91°C.

Anal. Calcd. for $C_9H_{12}BrNOS$: C, 41.22; H, 4.61; N, 5.34; S, 12.20. Found: C, 41.44; H, 4.90; N, 5.49; S, 12.31.

2-(β-D-Ribofuranosyl)-4-iodomethylthiazole (8). A solution of 3 (0.36 g, 1.35 mmole) and NaI (0.30 g, 2 mmole) in dry acetone (50 mL) was heated under reflux until a precipitate of NaCl formed (~ 5 min). After cooling, the NaCl was removed by filtration and washed with dry acetone. The filtrate and washing were evaporated, and the residue chromatographed by preparative TLC using $CHCl_3$-MeOH (9 : 1). Elution of the major band gave 8 (0.30 g, 26%) as a syrup which solidified on standing, mp 83-85°C.

Anal. Calcd. for $C_9H_{12}INO_4S$: C, 30.27; H, 3.38; N, 3.92; S, 8.96. Found: C, 30.66; H, 3.55; N, 4.00; S, 8.77.

2-(Tetrahydropyran-2-yl)-4-iodomethylthiazole (9). A solution of 6 (0.60 g, 2.75 mmole) and NaI (0.82 g, 5.46 mmole) in dry acetone (40 mL) was heated under reflux until a precipitate of NaCl formed (~ 2 min). After cooling, the NaCl was removed by filtration and washed with dry acetone. The filtrate and washing were evaporated. The residue was treated with a saturated solution of sodium thiosulfate and extracted with EtOAc (50 mL). The organic layer was washed with H_2O and dried over Na_2SO_4. Evaporation of the solvent left a yellow syrup which was chromatographed by preparative TLC using $CHCl_3$-hexane (2 : 1). Elution of the major band yielded 9 (0.54 g, 64%), mp 54-55°C (from cyclohexane).

Anal. Calcd. for $C_9H_{12}INOS$: C, 34.95; H, 3.88; N, 4.53; S, 10.35. Found: C, 35.10; H, 4.15; N, 4.80; S, 10.20.

2-(β-D-Ribofuranosyl)-4-methylthiazole (10). A solution of 2 (0.64 g, 3.3 mmole) and chloroacetone (0.30 g, 3.3 mmole) in ethanol (30 mL) was refluxed for 8 h. The solvent was evaporated

and the residue purified by preparative TLC using $CHCl_3$-MeOH (9 : 1). Elution of the major band gave 10 (0.30 g, 40%), mp 122-123° C (from EtOAc-petroleum ether) (lit.[5] mp 122-124°C).

2-(Tetrahydropyran-2-yl)-4-methylthiazole (11). A solution of 5 (2 g, 13.8 mmole) and chloroacetone (1.27 g, 13.8 mmole) in ethanol (50 mL) was refluxed for 5 h. Evaporation of the solvent left a residue which was dissolved in H_2O (10 mL) and neutralized with $NaHCO_3$. This was extracted with EtOAc (25 mL x 2); the organic portion was washed with H_2O and dried over Na_2SO_4. Evaporation of the solvent provided 2 g of a chromatographically homogeneous syrup from which an analytically pure sample could not be obtained. Then, hydrogen chloride was bubbled into a solution of the syrup in ether (60 mL). The precipitated solid was collected by filtration to give pure 11 as its hydrochloride (1.34 g, 44%), mp 108-110°C (from EtOAc).

Anal. Calcd. for $C_9H_{13}NOS \cdot HCl \cdot \frac{1}{2} H_2O$: C, 47.26; H, 6,56; N, 16.12; S, 14.00. Found: C, 47.66; H, 6.77; N, 6.09; S, 14.04.

2-(β-D-Ribofuranosyl)-5-bromo-4-methylthiazole (12). To a solution of 10 (46 mg, 0.20 mmole) in methanol (30 mL) was added N-bromosuccinimide (72 mg, 0.40 mmole) and the mixture was irradiated for 20 min by suspending the flask one centimeter above a 200 watt lamp. Evaporation of the solvent left a residue which was purified by preparative TLC, using $CHCl_3$-acetone (1 : 1) to give 12 (42 mg, 68%), mp 167°C (from acetone).

Anal. Calcd. for $C_9H_{12}BrNO_4S$: C, 34.84; H, 3.89; N, 4.51; S, 10.31. Found: C, 34.89; H, 3.81; N, 4.52; S, 10.68.

2-(Tetrahydropyran-2-yl)-5-bromo-4-methylthiazole (13). To a solution of 11 (1.44 g, 7.8 mmole) in methanol (50 mL) was added N-bromosuccinimide (2.77 g, 15.6 mmole) and the mixture was irradiated for 15 min as it indicated above. Evaporation of the solvent left a residue which was purified by preparative TLC using EtOAc-

hexane (1 : 2) to give 13 (0.76 g, 37%) as a syrup which solidified on standing, mp 37-38°C.

Anal. Calcd. for $C_9H_{12}BrNOS$: C, 41.22; H, 4.61; N, 5.34; S, 12.20. Found: C, 40.91; H, 4.81; N, 5.36; S, 11.80.

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