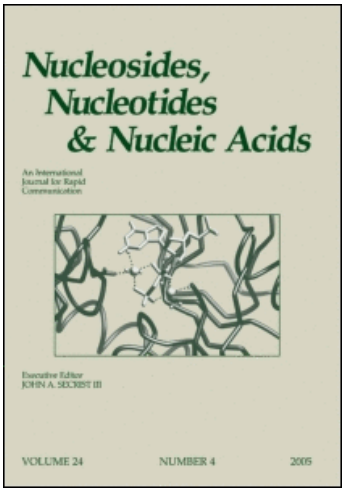

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Synthesis of 2-S-dioxo Isosteres of Purine and Pyrimidine Nucleosides. III. Alkyl And Glycosyl Derivatives of Triazolo-and Thiadiazolothiadiazines.

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SYNTHESIS OF 2-S-DIOXO ISOSTERES OF PURINE AND PYRIMIDINE NUCLEOSIDES. III[1]. ALKYL AND GLYCOSYL DERIVATIVES OF TRIAZOLO- AND THIADIAZOLOTHIADIAZINES.

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Dedicated to Professor M. Lora-Tamayo, on the occasion of his 80th birthday

Abstract. Synthesis of methyl, glucosyl and ribosyl derivatives of 7-amino-2H, 4H-[1, 2, 3]triazolo[4, 5-c][1, 2, 6]thiadiazine 5, 5-dioxide (1a) and 7-amino-4H-[1, 2, 5]thiadiazolo[3, 4-c][1, 2, 6]thiadiazine 5,5-dioxide (2a) is described. The structures of the glycosyl derivatives are discussed on the basis of their PMR- and UV-spectroscopic data.

Continuing with our work on the alkylation and glycosidation of 2-S-dioxo isosteres of purines and pyrimidines[2,3], we now wish to report our results on the preparation of methyl and glycosyl derivatives of triazolo- and thiadiazolothiadiazine 1a[4] and 2a[5], respectively. These glycosidation reactions were carried out so as to prepare analogs of the previously reported 3-(2, 3, 5-tri-O-benzoyl-β-D-ribofuranosyl)-7-aminofurazano[3, 4-c][1, 2, 6]thiadiazine 5,5-dioxide (3)[1] which had shown interesting cytostatic activity against HeLa cells[6].

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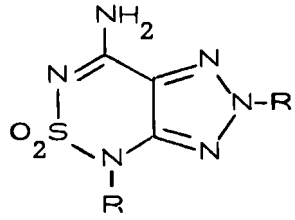
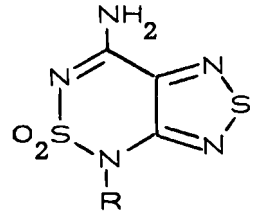
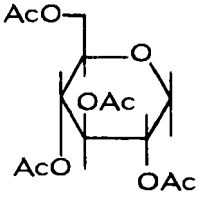




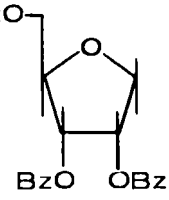
1a, R = H

1b, R = CH_3

1c, R = AcO-



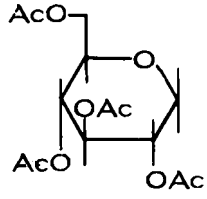
1d, R = BzO-



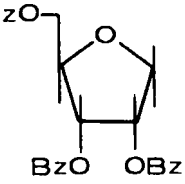
2a, R = H

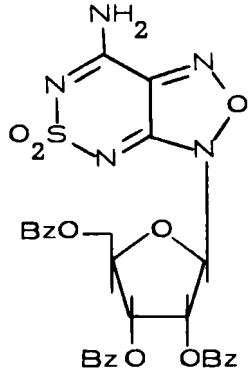
2b, R = CH_3

2c, R = AcO-



2d, R = BzO-





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In order to obtain models for the characterization of the synthesized nucleosides, we have first studied the reaction of 1a and 2a with a methylating agent. Reaction of 1a with dimethyl sulfate in the presence of sodium hydrogen carbonate afforded the dimethyl derivati-

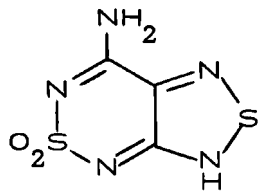
TABLE 1. UV spectroscopic data

Compound	λ_{max}, nm ($\varepsilon \cdot 10^{-3}$)		
1a[4] (neutral form) pH = 1	225 (10.0)	242 (7.5)	292 (6.9)
1b	225 (10.3)	250 (7.7)	293 (7.2)
1c	221 (9.5)	260 (7.0)	285 (6.0)
1d (deblocked)	233 (10.5)	261 (15.3)	290 (11.6)
2a[5] (neutral form) pH = 1	223 (8.5)	--	310 (5.3)
2b	233 (21.7)	281 (9.8)	325 (14.1)
2c	227 (10.5)	282 (5.9)	322 (7.3)
2d (deblocked)	233 (5.2)	278 (4.7)	326 (1.8)
3[1] (deblocked)	217 (6.7)	--	303 (4.3)

ve 1b. The PMR-spectrum of 1b shows two singlets at δ 3.30 and 4.30 ppm, the former corresponding to a methyl group at the N-4 of a fused thiadiazine derivative[7] and the latter to an N-2 methyl group in a 1,2,3-triazole[8]. The structure of 1b was confirmed by single crystal X-ray diffraction techniques[9]. In the same reaction conditions, 2a gave the monomethyl derivative 2b, the PMR spectrum of which shows a singlet at δ 3.40 ppm corresponding to the N-4 methyl group. The position of the methyl group was confirmed by X-ray analysis of a derivative of 2b[10].

The UV spectroscopic data of 1a, 2a and all the newly synthesized compounds are gathered in TABLE 1.

The fact that the UV spectrum of the neutral form (pH = 1) of 2a[5] is different to those of its methyl and glycosyl derivatives 2b, 2c and 2d suggests that this structure is better represented by another



tautomeric form. Besides, its UV spectrum closely resembles that of the deblocked nucleoside 3[1], which shows the same conjugation. This unusual cross-conjugated π-electron system, with the acidic hydrogen localized at a more distant position forming a conjugated mesomeric sulfonamide function, has also been observed in the case of pyrazino [2, 3-c] [1, 2, 6] thiadiazine 2, 2-dioxides which exist in the neutral form as the H-8 tautomers[11].

Due to the low solubility of 1a in organic solvents, the glycosidation procedure chosen was that which uses the silyl derivatives in the presence of Friedel-Crafts catalysts[12]. Silylation was carried out with hexamethyldisilazane and trimethylchlorosilane. In the case of 1a addition of pyridine was necessary in order to achieve complete solution of the starting material. Reaction of the silyl derivative of 1a with 1, 2, 3, 4, 6-penta-O-acetyl-β-D-glucopyranose yielded a diglucoside 1c. The site of glycosidation of 1c as well as the rest of the nucleosides described in this paper, were determined by comparing their UV spectra with those of the model substances previously synthesized. (See TABLE 1).

In the PMR spectrum, the signals of the anomeric protons have been assigned in the same order as the corresponding methyl groups in compound 1b.

Thus, the doublet which appears at lower field corresponds to the anomeric proton of the glucose attached to the triazole ring, whilst the next signal, a distorted doublet belongs to that of the

glucose at the thiadiazine ring. The signals corresponding to H-2' and H-3' of both glucose moieties appear overlapped as well as those corresponding to all H-6'. In order to assign the chemical shifts and coupling constants of every proton, double resonance experiments, an homonuclear J-resolved 2-D spectrum and analysis of seven spin systems were performed. The experimental and calculated spectra from the resulting best values match satisfactorily. The PMR spectroscopic data are given in TABLE 2. The β configuration in both glycosidic bonds was assigned from the values of the coupling constants $J_{1',2'}$ = 9.0 and 7.8 Hz for the glucose attached to the thiadiazine (N^4-glucose) and triazole (N^2-glucose) rings, respectively.

Reaction of the silyl derivative of 1a with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose afforded a diriboside 1d. In the PMR spectrum, the doublet corresponding to the anomeric proton of the ribose attached to the triazole ring appears at lower field than the one linked to the thiadiazine. From the value of the coupling constant of the former $J_{1',2'}$ = 1.7 Hz and taking into account the Karplus equation[13], the β configuration was tentatively assigned to the glycosidic bond. The doublet corresponding to the anomeric proton of the ribose linked to the thiadiazine ring has a coupling constant $J_{1',2'}$ = 5.3 Hz; from this value it was not possible to assign an α or β configuration. Studies of the nuclear Overhauser effect (NOE) were carried out by irradiation at the frequency of H-3'. Irradiation did not affect the intensity of the signals corresponding to H-1' and H-2', and so it was not possible to discard the β configuration. As in the previous case, and in order to assign unequivocally all the chemical shifts and coupling constants, double resonance experiments, homonuclear J-resolved 2-D spectrum (cross sections at different frequencies and J-resolved projection spectrum) and analysis of six spin

TABLE 2. PMR spectral parameters δ (ppm) and J(Hz) of compounds 1c and 1d

Parameter	compound 1c		compound 1d			
	N^4-glucose	N^2-glucose	N^4-ribose calcd.	N^4-ribose J-resolved	N^2-ribose calcd.	N^2-ribose J-resolved
$\delta_{1'}$	5.783	6.624	6.227	6.21	6.697	6.69
$\delta_{2'}$	5.676	5.640	6.405	6.39	6.457	6.46
$\delta_{3'}$	5.593	5.636	6.042	6.03	6.229	6.30
$\delta_{4'}$	4.981	5.076	4.837	4.85	5.043	5.03
$\delta_{5'}$	4.291	4.417	4.764	4.75	4.763	4.75
$\delta_{5''}$	-	-	4.662	4.66	4.681	4.67
$\delta_{6'}$	4.101	4.175	-	-	-	-
$\delta_{6''}$	4.033	4.077	-	-	-	-
$J_{1',2'}$	9.0	7.8	5.3		1.7	
$J_{2',3'}$	9.5	9.5	5.7		5.2	
$J_{3',4'}$	10.4	9.8	5.8		7.2	
$J_{4',5'}$	9.9	9.9	5.0		4.5	
$J_{4',5''}$	-	-	5.7		5.2	
$J_{5',5''}$	-	-	-11.8		-12.0	
$J_{5',6'}$	1.5	5.6	-		-	
$J_{5',6''}$	5.5	5.3	-		-	
$J_{6',6''}$	-12.2	-12.8	-		-	

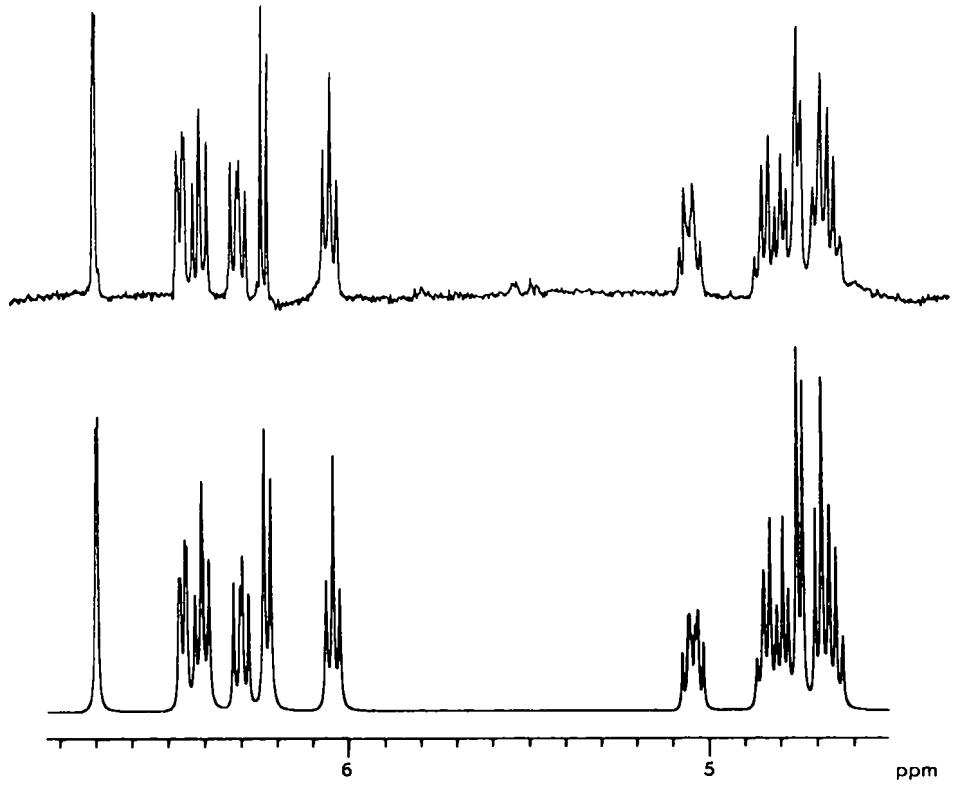


FIG. 1. Experimental and calculated spectra of compound 1d.

systems were performed. The experimental and calculated spectra are depicted in FIGURE 1. The chemical shifts of the calculated and J-resolved spectra and coupling constants are gathered in TABLE 2. In the UV spectrum of 1d the strong absorption of the benzoyl groups masked that corresponding to the nucleoside itself. In order to obtain the UV spectrum of the deblocked nucleoside from 1d, a small amount of 1d was treated with methanolic ammonia and purified by preparative chromatography.

From the reaction of the silyl derivative of 2a and 1, 2, 3, 4, 6-penta-O-acetyl-β-D-glucopyranose, a monoglucoside 2c was obtained. In the PMR spectrum, the signal corresponding to H-1' with a chemical shift (δ = 5.87) similar to that of the anomeric proton of the glucose at

TABLE 3. PMR Spectral parameters δ (ppm) and J(Hz) of compounds 2c and 2d

Parameter (calcd.)	Compound 2c	2d
$\delta_{1'}$	5.870	6.296
$\delta_{2'}$	5.840	6.324
$\delta_{3'}$	5.610	6.099
$\delta_{4'}$	4.994	4.586
$\delta_{5'}$	4.336	4.780
$\delta_{5''}$	-	4.806
$\delta_{6'}$	4.081	-
$\delta_{6''}$	4.080	-
$J_{1',2'}$	8.3	4.9
$J_{2',3'}$	8.6	6.3
$J_{3',4'}$	9.3	6.0
$J_{4',5'}$	10.0	6.4
$J_{4',5''}$	-	7.7
$J_{5',5''}$	-	-11.9
$J_{5',6'}$	3.0	-
$J_{5',6''}$	3.7	-
$J_{6',6''}$	-12.1	-

the thiadiazine ring (5.78) in compound 1c, appears overlapped with the one of H-2'. For this reason, a spectral analysis of the seven spin systems was performed. From the calculated spectrum, all the chemical shifts and coupling constants were unequivocally obtained. The coupling constant values indicate that the glucose has all the protons in a transdiaxial disposition and so the nucleosidic bond configuration is β. The PMR spectroscopic data are given in TABLE 3.

Reaction of the silyl derivative of 2a with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose gave a single monoribosidic product 2d. As in the previous case, in the PMR spectrum the signals corresponding to H-1' and H-2' appear overlapped and very near that corresponding to H-3'. A spectral analysis of the six spin systems established all the chemical shifts and coupling constants. From the value of the coupling constant of the anomeric proton $J_{1',2'} = 4.9$ Hz, it was not possible to assign an α or β configuration to the nucleosidic bond. Deblocking of 2d was achieved in the same way as described for 1d.

The test of the cytostatic activity against HeLa cells of the synthesized compounds are in progress.

EXPERIMENTAL

Melting points were determined with a Kofler apparatus and are uncorrected. 300 MHz-PMR spectra were recorded for solutions in DMSO-d_6 with TMS as internal standard on a Varian XL-300 spectrometer. Analyses of the systems were performed with the iterative programme PANIC 81 on a Bruker Aspect 2000 Computer. The experimental and calculated spectra from the resulting best values match satisfactorily (RMS = 0.08). The data matrix of the J-resolved 2-D spectra were 128 x 1024 with the spectral widths of 40 x 1500 Hz. Infrared spectra were recorded on a Perkin-Elmer 681 spectrometer and ultraviolet spectra on a Perkin-Elmer 402 spectrophotometer. The thin layer chromatography was performed on Merck silicagel plates PF_{254}. The solvents used in the preparation of the glycosides were carefully purified. Acetonitrile was refluxed for two hours over phos-

phorous pentoxide and distilled, the procedure was repeated, and finally, it was stored over 3 Å molecular sieves.

7-Amino-2,4-dimethyl [1,2,3] triazolo [4,5-c] [1,2,6] thiadiazine 5,5-dioxide (1b)

To a stirred solution of 1 g (0.005 mole) of 7-amino-2H,4H-[1,2,3] triazolo [4,5-c] [1,2,6] thiadiazine 5,5-dioxide (1a)[4] in 10 ml of water, sodium hydrogen carbonate was added until pH = 7. The mixture was then treated, dropwise, with 2.04 g (1.5 ml, 0.015 mole) of dimethyl sulfate, and stirred at room temperature for 20 h, keeping the pH at 7 by addition of sodium hydrogen carbonate if necessary. The solid was collected by filtration and crystallized from water-ethanol to give 0.62 g (54 %) of 1b as needles, m.p. 255-256° C. IR (nujol) ν: 3400 and 3320 cm^{-1} (NH_2), 1310 and 1180 cm^{-1} (SO_2). PMR (DMSO-d_6) δ: 8.80 (b.s., 2H, NH_2), 4.30 (s, 3H, $-N^2-CH_3$), 3.30 (s, 3H, $-N^4-CH_3$).

Anal. Calcd. for $C_5H_8N_6O_2S$: C, 27.78; H, 3.73; N, 38.87. Found: C, 27.95; H, 4.00; N, 39.13.

7-Amino-4-methyl [1,2,5] thiadiazolo [3,4-c] [1,2,6] thiadiazine 5,5-dioxide (2b)

Following the procedure described for the preparation of 1b, the reaction of 1 g (0.004 mole) of 7-amino-4H [1,2,5] thiadiazolo [3,4-c] [1,2,6] thiadiazine 5,5-dioxide (2a) with 1.36 g (1 ml, 0.01 mole) of dimethyl sulfate, yielded 0.6 g (56 %) of 2b, m.p. 248° C. IR (nujol) ν: 3420 and 3320 cm^{-1} (NH_2), 1320 and 1140 cm^{-1} (SO_2). PMR (DMSO-d_6) δ: 7.75 (b.s., 2H, NH_2), 3.45 (s, 3H, CH_3).

Anal. Calcd. for $C_4H_5N_5O_2S_2$: C, 21.91; H, 2.30; N, 31.94. Found: C, 22.30; H, 2.12; N, 31.53.

2,4-Bis-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-7-amino [1,2,3] triazolo [4,5-c] [1,2,6] thiadiazine 5,5-dioxide (1c)

To a stirred solution of 2.9 g (0.0075 mole) of 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose in 100 ml of dry acetonitrile, a solution of the silyl derivative of 1a [prepared from 1.50 g (0.008 mole) of 1a by reaction with hexamethyldisilazane (10 ml) and trimethylchlorosilane (1 ml) in the presence of pyridine (30 ml)] in acetonitrile was added. The mixture was treated with 3 ml of boron trifluoride etherate and stirred overnight at room temperature and with exclusion of humidity. The reaction mixture was then evaporated to dryness and the residue dissolved in chloroform and treated with a saturated sodium hydrogen carbonate solution. The organic phase was washed with water, dried over sodium sulfate and evaporated under reduced pressure. Preparative TLC of the residue (1.4 g), using chloroform-ethanol (10:1) as eluent, gave 1 g (31 %) of 1c as a white foam. IR (nujol) ν: 3340 and 3260 cm^{-1} (NH_2), 1750 cm^{-1} (C=O), 1380 and 1185 cm^{-1} (SO_2).

Anal. Calcd. for $C_{31}H_{40}N_6O_{20}S$: C, 43.86; H, 4.71; N, 9.90. Found: C, 43.96; H, 5.08; N, 9.70.

2-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-4-(2,3,5-tri-O-benzoyl-D-ribofuranosyl)-7-amino [1,2,3] triazolo[4,5-c] [1,2,6] thiadiazine 5,5-dioxide (1d)

According to the procedure described for the synthesis of 1c, 3.8 g (0.0075 mole) of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose and the silyl derivative obtained from 1.5 g (0.008 mole) of 1a were used. After work-up, the residue (2.6 g) was chromatographed on silicagel plates and eluted with chloroform-ethanol (20:1) gave 1.4 g (35 %) of 1d as a white foam. IR (nujol) ν: 3430 and 3340

cm^{-1} (NH_2), 1725 cm^{-1} (C=O), 1380 and 1185 cm^{-1} (SO_2).

Anal. Calcd. for $C_{55}H_{44}N_6O_{16}S$: C, 61.33; H, 4.08; N, 7.80 Found: C, 61.20; H, 4.34; N, 7.41.

4-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-7-amino [1,2,5] thiadiazolo [3,4-c] [1,2,6] thiadiazine 5,5-dioxide (2c)

Following the procedure described for the preparation of 1c, 1.56 g (0.004 mole) of 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose and the silyl derivative prepared from 0.82 g (0.004 mole) of 2a, by reaction of 2a with hexamethyldisilazane (10 ml), and trimethylchlorosilane (1 ml), were used. After work-up, the residue was chromatographed on silicagel plates and eluted with chloroform-ethanol (10 : 1). Crystallization from ethanol gave 1.36 g (64 %) of 2c, m.p. 235-236° C. IR (nujol) ν: 3430 and 3340 cm^{-1} (NH_2), 1750 cm^{-1} (C=O), 1380 and 1190 cm^{-1} (SO_2).

Anal. Calcd. for $C_{17}H_{21}N_5O_{11}S_2$: C, 38.13; H, 3.92; N, 13.08. Found: C, 37.87; H, 3.84; N, 12.80.

4-(2,3,5-Tri-O-benzoyl-D-ribofuranosyl)-7-amino [1,2,5] thiadiazolo [3,4-c] [1,2,6] thiadiazine 5,5-dioxide (2d)

According to the procedure described for the synthesis of 1c, 2 g (0.004 mole) of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose, and the silyl derivative prepared from 0.82 g (0.004 mole) of 2a, were used. After work-up, preparative TLC using chloroform-ethanol (20 : 1) as eluent, gave 1.5 g (59 %) of 2d as a foam. IR (nujol) ν: 3410 and 3330 cm^{-1} (NH_2), 1735 cm^{-1} (C=O), 1275 and 1185 cm^{-1} (SO_2).

Anal. Calcd. for $C_{29}H_{23}N_5O_9S_2$: C, 53.62; H, 3.54; N, 10.78. Found: C, 53.98; H, 3.27; N, 10.82.

Acknowledgements

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