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The synthesis of 6-methyl-7-( $\beta$ -D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (8-methyl-2-azinosine (**2**)) and 6-methyl-7-( $\beta$ -D-glucopyranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**5**) by diazotization of 5-amino-1-( $\beta$ -D-ribofuranosyl)-2-methylimidazole-4-carboxamide (**1**) and diazotization of 5-amino-1-(2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl)-2-methylimidazole-4-carboxamide (**3**), followed by deacetylation of the resulting compound **4**, is described. The preparation of 6-methyl-5-( $\beta$ -D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**10**) and 6-methyl-5-( $\beta$ -D-glucopyranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**11**) by glycosylation of 6-methylimidazo[4,5-*d*]-*v*-triazin-4-one (8-methyl-2-azahypoxanthine, (**7**)) is also described. Structural assignments were made on basis of analytical and  $^1\text{H}$ -nmr and uv spectral data.

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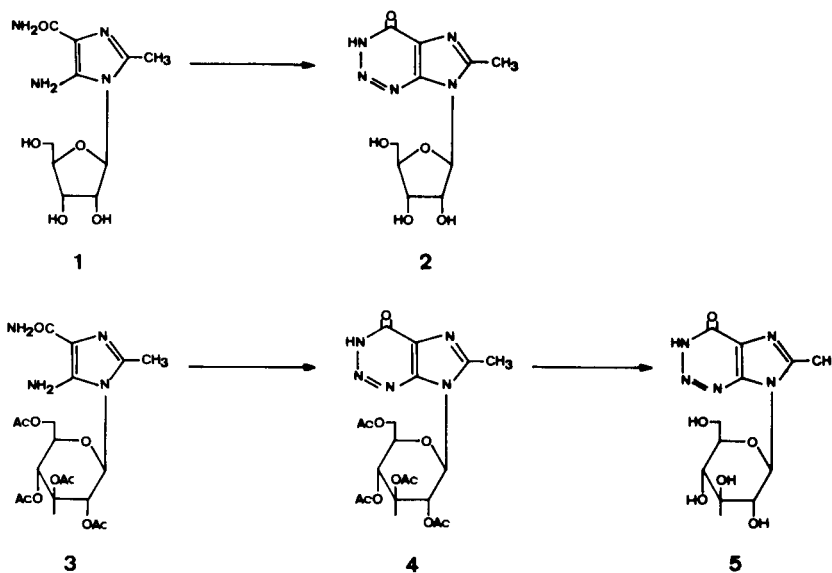
Replacement of the C-2 atom of adenosine and inosine by a nitrogen atom has led to the corresponding 2-azapurine nucleosides, namely 4-amino-7-( $\beta$ -D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazine (2-azaadenosine) and 7-( $\beta$ -D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (2-azinosine) which show interesting biological properties. 2-Azaadenosine has anticancer activity while 2-azinosine and its 2',3',5'-tri-*O*-acetyl derivative show antiviral activity [1, 2a]. On the other hand, 2-azinosine has been proposed to be a potential antimetabolite which can be viewed as a "masked" 5-amino-1-( $\beta$ -D-ribofuranosyl)imidazole-4-carboxamide (AICA riboside) [3]. Based on these facts, we have considered of interest the preparation of new 2-azinosine analogs as potential chemotherapeutic agents.

This paper describes the synthesis of 6-methyl-7-( $\beta$ -D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (8-methyl-2-azinosine) and its glucosyl analog from the correspon-

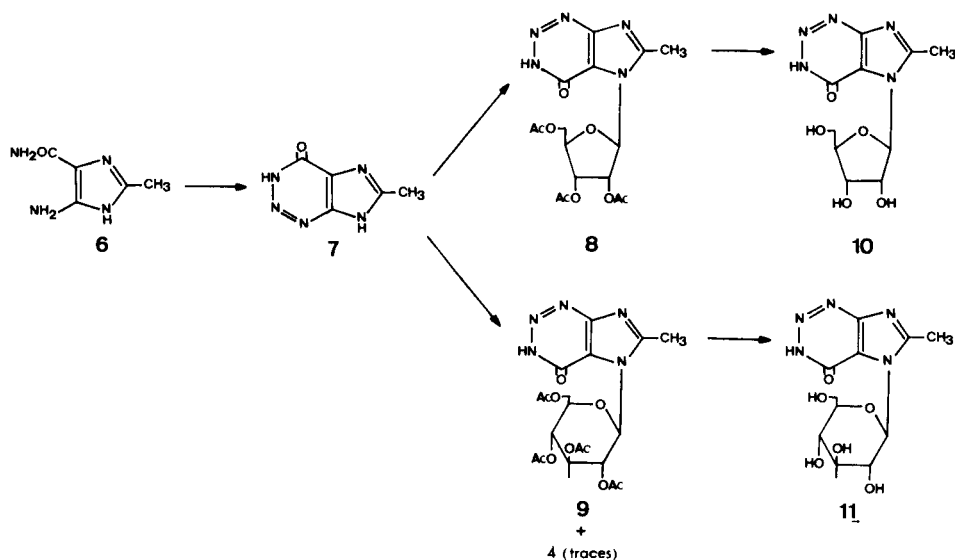
ding 5-amino-1-glycosyl-2-methylimidazole-4-carboxamide. It also reports the preparation of the 5-glycosyl substituted isomers by direct glycosylation of 6-methylimidazo[4,5-*d*]-*v*-triazin-4-one (8-methyl-2-azahypoxanthine).

The procedure described in the literature for the synthesis of 2-azinosine and its isomer, 5-( $\beta$ -D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one, consists on the cyclization *via* diazotization of AICA riboside and *iso*-AICA riboside respectively [2]. The optimum conditions for this reaction were found to be treatment of the *o*-aminocarboxamide nucleoside of its per-*O*-acetyl derivative in 6*N* hydrochloric acid at  $-25$  or  $-20^\circ$  with sodium nitrite [2]. In our case, following this procedure and using the same reaction conditions, 5-amino-1-( $\beta$ -D-ribofuranosyl)-2-methylimidazole-4-carboxamide (**1**) [4] led to 6-methyl-7-( $\beta$ -D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (8-methyl-2-azinosine, (**2**)) in 52% yield. A similar reaction at  $-10^\circ$ ,

SCHEME I



SCHEME II



using 5-amino-1-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-2-methylimidazole-4-carboxamide (**3**) [4], furnished 6-methyl-7-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**4**) in 70% yield which was deacetylated with methanolic ammonia to give 6-methyl-7-(β-*D*-glucopyranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**5**) (Scheme I). It should be noted that no reaction occurred when the 2',3'-*O*-isopropylidene derivative of the ribofuranosylimidazole **1** was diazotized at  $-30^{\circ}$  under the above conditions. Diazotization of this derivative at  $-10^{\circ}$  led to the acidic hydrolysis of the ribosyl linkage along with the formation of 6-methylimidazo[4,5-*d*]-*v*-triazin-4-one (**7**) as free base. Compound **7** was also obtained by direct diazotization of 4(5)-amino-2-methylimidazole-5(4)-carboxamide (**6**). Compound **6** was previously described [5] by reaction of α-amino-α-cyanoacetamide with ethyl acet-

imide hydrochloride followed by treatment of the resulting linear intermediate with ethanolic ammonia. In our case, this compound was directly obtained by reaction of α-amino-α-cyanoacetamide [6] with benzyl thioacetimidate hydrochloride [7].

Structural assignments of these compounds were made on the basis of elemental analysis and spectroscopic data. Thus, comparison of the uv spectra of **2**, **4** and **7** (two absorption maxima) with those of the imidazoles **1**, **3** and **6** used as starting materials (one absorption maximum) and with those of 7-methylimidazo[4,5-*d*]-*v*-triazin-4-one, 2-azanosine and 2-azahypoxanthine [2a,8] revealed that ring annulation occurred. The  $^1\text{H}$ -nmr spectra of the nucleosides **2**, **4** and **5** confirmed the assigned structures (Table 1).

Table 1

$^1\text{H}$  NMR Data of 5- and 7-Glycosylimidazo[4,5-*d*]-*v*-triazin-4-ones at 90 MHz With TMS as Internal Standard

Compound	Solvent	H-1'	Chemical Shifts (δ)		J <sub>1',2'</sub> (Hz)
			CH <sub>3</sub> -6	NH-3	
<b>2</b>	DMSO + D <sub>2</sub> O	5.93	2.60	—	7
<b>4</b>	CDCl <sub>3</sub>	6.05	2.80	13.70	9
<b>4</b>	DMSO	6.40	2.66	15.30	9
<b>5</b>	DMSO + D <sub>2</sub> O	5.56	2.60	—	9
<b>8</b>	CDCl <sub>3</sub>	6.12	2.75	13.70	7
<b>8</b>	DMSO	6.30	2.66	15.33	5
<b>9</b>	CDCl <sub>3</sub>	6.38	2.75	13.70	9.5
<b>10</b>	DMSO + D <sub>2</sub> O	6.03	2.70	—	6
<b>11</b>	DMSO + D <sub>2</sub> O	5.93	[a]	—	9

[a] Included with DMSO.

The second method used for the synthesis of nucleosides of 8-methyl-2-azahypoxanthine consisted on the direct glycosylation of **7** with per-*O*-acetylated glycosyl halides in dimethylformamide (Scheme II). As in the case of alkylation of 2-azahypoxanthine with 1-benzoyloxy-2-chloromethoxyethane, this procedure gave low yields of products, the only or the major one being the 5-substituted imidazo[4,5-*d*]-*v*-triazin-4-one isomer [9]. Thus, ribosylation of **7** with 2,3,5-tri-*O*-acetyl-*D*-ribofuranosyl chloride afforded 6-methyl-5-(2,3,5-tri-*O*-acetyl- $\beta$ -*D*-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**8**) in 10% yield. When the reaction was achieved with 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -*D*-glucopyranosyl bromide, 6-methyl-5-(2,3,4,6-tetra-*O*-acetyl- $\beta$ -*D*-glucopyranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**9**) was obtained in 10% yield along with traces of the isomeric compound **4**. Deacetylation of **8** and **9** with methanolic ammonia at room temperature gave 6-methyl-5-( $\beta$ -*D*-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one and 6-methyl-5-( $\beta$ -*D*-glucopyranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**10** and **11**) respectively.

The glycosylation site of compounds **8**, **9**, **10** and **11** was determined from their <sup>1</sup>H-nmr and uv spectra. Thus, the appearance of an NH signal at very low field ( $\delta$  DMSO = 15.33 and  $\delta$  (deuteriochloroform) = 13.70) in the <sup>1</sup>H-nmr spectra of **8** and **9**, which seems to be a characteristic feature of the NH-3 of imidazo[4,5-*d*]-*v*-triazin-4-ones [2b], and a  $\delta$  value for the methyl protons of these compounds comparable with that of the 7-glycosyl substituted derivative **4**, suggested that the glycosylation took place on the imidazole ring. This similarity in the chemical shift of the methyl group was also observed when deprotected nucleosides **10** and **11** were compared with the deprotected 7-glycosyl substituted derivatives **2** and **5**. If glycosylation had occurred on the triazine ring, the methyl protons of the 6-methylimidazo[4,5-*d*]-*v*-triazine system would have appeared at higher field than those of 5- or 7-glycosyl derivatives as a consequence of the deshielding effect of the glycosyl moiety, which at 5- or 7-position is adjacent to the methyl group [10]. Therefore, compounds **8**, **9**, **10** and **11** were initially assigned as 5-glycosylimidazo[4,5-*d*]-*v*-triazin-4-ones. This assignment was clearly supported by comparing the uv spectra of these compounds with those of 5-methylimidazo[4,5-*d*]-*v*-triazin-4-one and 5-( $\beta$ -*D*-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one [2a]. As in the case of isomeric 5- and 7-( $\beta$ -*D*-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-ones [2a], a great similarity was observed in mp, chromatographic mobilities and <sup>1</sup>H-nmr spectra of each isomeric pair **2**, **10**; **4**, **9** and **5**, **11**. However, comparison of the <sup>1</sup>H nmr of each pair revealed that the signal for the anomeric proton of the 5-glycosyl substituted compounds **9**, **10** and **11** appeared at lower field than that corresponding to the 7-glycosyl substituted isomers **4**, **2** and **5** respectively (Table 1). This downfield shift of the anomeric proton is consistent with the presence of an anisotropic carbonyl

group adjacent to the site of glycosylation in compounds **9**, **10** and **11** [11]. Finally, the anomeric configuration was clearly ascertained as  $\beta$  for glucopyranosyl derivatives **9** and **11** on the basis of their corresponding  $J_{1',2'}$  values. In the case of ribofuranosyl derivatives **8** and **10**, the value of the coupling constant did not allow an unequivocal assignment ( $J_{1',2'} > 2$  Hz). However, they were assigned as  $\beta$ , since the chemical shift for the anomeric proton of the deacetylated riboside **10** ( $\delta$  DMSO = 6.03 ppm) is very close to that of 5-( $\beta$ -*D*-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one [2a] ( $\delta$  DMSO = 6.25 ppm) and a downfield shift for this proton of **10** would be expected in the case of an  $\alpha$  configuration.

## EXPERIMENTAL

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. The <sup>1</sup>H-nmr spectra were recorded at 90 MHz on a Varian EM-390 spectrometer, with TMS as internal standard. The uv spectra were taken with a Perkin-Elmer 402 spectrophotometer. Analytical tlc was performed on aluminium sheets coated with a 0.2 mm layer of silica gel 60 F<sub>254</sub> (Merck) and preparative tlc on 20 × 20 cm glass plates coated with a 2 mm layer of silica gel PF<sub>254</sub> (Merck). Silica gel PF<sub>254</sub> (Merck) was also used for column chromatography. The compounds were detected with uv light of 254 nm or by spraying with sulfuric acid in ethanol 30%.

6-Methyl-7-( $\beta$ -*D*-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (8-Methyl-2-azinosine, (**2**)).

To a cold solution (−25°) of 0.62 g (2 mmoles) of **1** [4] in 18 ml of 6*N* hydrochloric acid was added dropwise a solution of 0.40 g (6 mmoles) of sodium nitrite in 2 ml of water. After the addition was complete, the reaction mixture was stirred for 1 hour, keeping the temperature between −25 and −30°. The reaction solution was diluted with 18 ml of cold ethanol (−30°) and the pH was adjusted to 7 with concentrated ammonium hydroxide. The reaction mixture was evaporated *in vacuo* and the residue was treated with methanol. The solid formed was removed by filtration and the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column with chloroform-methanol (4:1) to give 0.30 g (52%) of **2** with mp 200° dec (from methanol); uv (water):  $\lambda$  max 245 nm ( $\epsilon$  5,200), 293 (5,800).

Anal. Calcd. for C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>5</sub>·½CH<sub>3</sub>OH: C, 42.14; H, 5.02; N, 23.41. Found: C, 41.95; H, 5.29; N, 23.26.

6-Methyl-7-(2,3,4,6-tetra-*O*-acetyl- $\beta$ -*D*-glucopyranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**4**).

To a solution of 0.94 g (2 mmoles) of **3** [4] in 10 ml of 6*N* hydrochloric acid at −10° was added dropwise a solution of 0.40 g (6 mmoles) of sodium nitrite at −10°. After the addition was complete, the reaction mixture was stirred for 1 hour and then neutralized with concentrated ammonium hydroxide. Up to this point the temperature was kept between −10 and −15°. The product was extracted with chloroform (3 × 20 ml) and the organic extracts were dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed by preparative tlc using acetone-chloroform (1:1) to give 0.67 g (70%) of **4** as a white foam; uv (water);  $\lambda$  max 245 nm ( $\epsilon$  1000), 292 (1000).

Anal. Calcd. for C<sub>19</sub>H<sub>23</sub>N<sub>5</sub>O<sub>10</sub>: C, 47.40; H, 4.78; N, 14.55. Found: C, 47.52; H, 4.59; N, 14.19.

6-Methyl-7-( $\beta$ -*D*-glucopyranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**5**).

A solution of 0.24 g (0.5 mmoles) of **4** in 40 ml of saturated methanolic ammonia was allowed to stand at room temperature overnight. The solution was evaporated to dryness to yield 0.15 g (100%) of **5**, recrystallized from methanol-ether, mp 200° dec; uv (water):  $\lambda$  max 245 nm ( $\epsilon$  400), 293 (500).

Anal. Calcd. for C<sub>11</sub>H<sub>15</sub>N<sub>5</sub>O<sub>6</sub>·CH<sub>3</sub>OH: C, 41.73; H, 5.50; N, 20.28. Found: C, 41.71; H, 5.56; N, 19.94.

4(5)-Amino-2-methylimidazole-5(4)-carboxamide Hydrochloride (**6**).

A solution of 9.90 g (100 mmoles) of  $\alpha$ -amino- $\alpha$ -cyanoacetamide [**6**] and 20.15 g (100 mmoles) of benzyl thioacetimidate hydrochloride [**7**] in a mixture of 120 ml of dry acetonitrile and 40 ml of dry methanol was refluxed for 6 hours, and then was allowed to stand at room temperature overnight. The precipitate formed was filtered and washed with chloroform to give 9.80 g (60%) of **6** with mp 243° dec (from methanol) mp lit [5] 242-243°; uv (water):  $\lambda$  max 270 nm ( $\epsilon$  7270).

6-Methylimidazo[4,5-*d*]-*v*-triazin-4-one (**8**-Methyl-2-azahypoxanthine, (**7**)).

A stirred solution of 3.79 g (55 mmoles) of sodium nitrite in 100 ml of water was kept between -5 and 0° while a solution of **6** in 60 ml of 1*N* hydrochloric acid was added dropwise. A crystalline precipitate began to form after adding a small portion of the aminoimidazole solution. After an additional 2 hours stirring, the precipitate was removed by filtration, washed with water and dried *in vacuo* over phosphorus pentoxide to give 6.70 g (90%) of **7** with mp 230° explosive dec (from water); uv (water):  $\lambda$  max 250 nm ( $\epsilon$  1550), 285 (1100).

*Anal.* Calcd. for  $C_5H_5N_5O$ : C, 39.79; H, 3.31; N, 46.35. Found: C, 39.82; H, 3.07; N, 45.95.

6-Methyl-5-(2,3,5-tri-*O*-acetyl- $\beta$ -D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**8**).

To a solution of 1.51 g (10 mmoles) of **7** and 2.75 ml (20 mmoles) of triethylamine in 30 ml of dry *N,N*-dimethylformamide was added 4.41 g (15 mmoles) of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride. The reaction mixture was stirred for 15 hours and the solvent was removed under reduced pressure to have an oil which was treated with 200 ml of ethyl acetate and 100 ml of water. The organic phase was washed with water, dried over sodium sulfate and evaporated to dryness. The residue was chromatographed on a silica gel column with chloroform-acetone (3:1) to give 0.40 g (10%) of **8**, recrystallized from ethyl acetate-hexane as a white solid, mp 145-146°; uv (water):  $\lambda$  max 255 nm ( $\epsilon$  750), 280 (sh) (550).

*Anal.* Calcd. for  $C_{16}H_{19}N_5O_8 \cdot AcOEt$ : C, 48.29; H, 5.43; N, 14.09. Found: C, 48.32; H, 5.53; N, 14.49.

6-Methyl-5-(2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**9**).

To a solution of 1.51 g (10 mmoles) of **7** and 2.75 ml (20 mmoles) of triethylamine in 30 ml of dry *N,N*-dimethylformamide was added 6.15 g (15 mmoles) of 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-glucopyranosyl bromide. The reaction mixture was stirred for 15 hours and the solvent was removed under reduced pressure to leave an oil which was treated with 200 ml of ethyl acetate and 100 ml of water. The organic layer was washed with water, dried over sodium sulfate and evaporated to dryness. The residue was chromatographed on a silica gel column with chloroform-acetone (3:1) to give 0.45 g (10%) of **9** as a foam; uv (ethanol):  $\lambda$  max 253 nm ( $\epsilon$  1400) 288 (1300).

*Anal.* Calcd. for  $C_{19}H_{23}N_5O_{10}$ : C, 47.40; H, 4.78; N, 14.55. Found: C, 47.61; H, 4.46; N, 14.20.

6-Methyl-5-( $\beta$ -D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**10**).

A solution of 0.20 g (0.5 mmoles) of **8** in 40 ml of saturated methanolic ammonia was allowed to stand at room temperature overnight. The solution was evaporated to dryness to give 0.14 g (100%) of **10**, recrystallized from methanol, mp 190° dec; uv (water)  $\lambda$  max 255 nm ( $\epsilon$  1800), 280 (1350).

*Anal.* Calcd. for  $C_{10}H_{13}N_5O_5 \cdot \frac{1}{2}CH_3OH$ : C, 42.14; H, 5.02; N, 23.41. Found: C, 41.88; H, 5.21; N, 23.31.

6-Methyl-5-( $\beta$ -D-glucopyranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**11**).

A solution of 0.24 g (0.5 mmoles) of **9** in 40 ml of saturated methanolic ammonia was allowed to stand at room temperature overnight. The solution was evaporated to dryness to yield 0.15 g (100%) of **11**, recrystallized from methanol-ether, mp 190° dec; uv (water):  $\lambda$  max 253 nm ( $\epsilon$  313), 294 (300).

*Anal.* Calcd. for  $C_{11}H_{15}N_5O_6 \cdot CH_3OH$ : C, 41.73; H, 5.50; N, 20.28. Found: C, 41.59; H, 5.62; N, 20.03.

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