Nucleosides of 8-Azapurines (*v*-Triazolo[4,5-*d*]pyrimidines)†

John A. Montgomery* and H. Jeanette Thomas

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205. *Received September 7, 1971*

The xylose (**2**), 2′-deoxyribose (**4**), and arabinose (**7**) analogs of 8-azaadenosine have been prepared from the reaction of *N*-nonanoyl-8-azaadenine and the appropriate pentofuranosyl halides. Deamination of the xylose and 2′-deoxyribose compounds gave the corresponding analogs (**3** and **5**) of 8-azainosine, an interesting nucleoside with anticancer activity in experimental animal systems. The cytotoxicity of these nucleosides is discussed.

Our interest in 8-azapurine nucleosides was stimulated by the observation that 8-azainosine (3-β-D-ribofuranosyl-*v*-triazolo[4,5-*d*]pyrimidin-7(6*H*)-one), which is active *in vivo* against both adenocarcinoma 755 and leukemia L1210, is cytotoxic to H.Ep.-2 cells in culture lacking IMP pyrophosphorylase and, therefore, resistant to 8-azahypoxanthine.[1] This finding indicates that the activity of 8-azainosine is due to the intact nucleoside or metabolite thereof and not to cleavage back to 8-azahypoxanthine, and also that this nucleoside is active against cells that have become resistant to 6-mercaptopurine, a fact that could have practical application.

8-Azainosine was first prepared by Davoll[2] by the deamination of 8-azaadenosine, which in turn was prepared from *N*-acetyl-8-azaadenine by the chloromercury coupling procedure. Since, in our hands, this latter procedure was found to be less than satisfactory for the preparation of quantities of material, we developed a synthesis of 8-azaadenosine (7-amino-3-β-D-ribofuranosyl-*v*-triazolo[4,5-*d*]-pyrimidine) from the reaction of *N*-nonanoyl-8-azaadenine (**1**) with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride in refluxing benzene in the presence of Linde Molecular Sieve AW500.[3] We have now applied this general procedure to the preparation of other pentofuranosyl-8-azaadenines. The yield of anomerically pure 9-β-D-xylofuranosyl-8-azaadenine (**2**)‡ from 2,3,5-tri-*O*-acetyl-D-xylofuranosyl chloride based on unrecovered *N*-nonanoyl-8-azaadenine (**1**) was 63%. In the reaction of 3,5-di-(*p*-chlorobenzoyl)-2-deoxy-D-ribofuranosyl chloride with **1**, Et_3N as the acid acceptor§ proved superior to the Molecular Sieve and gave a 44% yield of 9-(2-deoxy-D-ribofuranosyl)-8-azaadenine (**4**),# which was separated into its anomers prior to deacylation by means of silica gel chromatography. The pmr spectrum of one of these nucleosides showed the anomeric proton as a pseudotriplet with a peak width of 13 Hz compatible with the β configuration. The pmr spectrum of the second nucleoside showed the anomeric proton as a pseudotriplet also, instead of the more normal quartet, but with a peak width of 10.5 Hz compatible with the α configuration.[8] After deacylation, however, the signal from the anomeric proton of both nucleosides appeared in their pmr spectra as a pseudotriplet with the same peak width (13 Hz) instead of as a pseudotriplet (peak width 13 Hz) in one case and as a quartet (peak width 10 Hz) in the other, which is typical of β- and α-anomers in the case of 2′-deoxyribonucleosides of purines.[8] Thus it appeared initially that both nucleosides must have the β configuration; and, since the point of attachment of the sugar in both cases was shown to be 9 by their uv spectra, that epimerization of the sugar must have occurred. However, both nucleosides were hydrolyzed in dilute acid and the sugar moiety identified as 2-deoxyribose by its chromatographic travel in four solvent systems and by its conversion to the anilide derivative. The correct identity of the α- and β-anomers was finally confirmed by their conversion to the dimesylate derivatives, which were refluxed in acetonitrile solution. The β-anomer cyclized to the cyclonucleoside, while the α-anomer remained unchanged. The products were identified by their travel on tlc and paper electrophoresis and by their uv and pmr spectra. It must be concluded that the 8-N of 8-azapurines causes a distortion of bond angles of the 2-deoxy-D-ribofuranose ring from those found in the 9-(2-deoxy-α-D-ribofuranosyl)purines so that the empirical rule governing assignment of anomeric configurations does not hold in this case.

The reaction of 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride with **1** followed by treatment with methanolic NaOMe gave 9-(2,3,5-tri-*O*-benzyl-D-arabinofuranosyl)-8-azaadenine (**6**) as a mixture of anomers, in contrast to the reaction of this sugar with *N*-benzoyladenine in which only the β-anomer was formed.[9,10] One anomer, later identified as the β-anomer, crystallized from its MeOH solution. The other anomer (α-**6**) was isolated from the mother liquor as the picrate salt from which it was regenerated by treatment with ion-exchange resin. The total yield was 40% based on unrecovered **1**.

The *O*-benzyl groups were removed from both anomers by hydrogenation using $PdCl_2$ to give 9-β-D-arabinofuranosyl-8-azaadenine (β-**7**) and its α-anomer (α-**7**), although the yields were low owing to concomitant ring reduction. Attempted reduction with Pd/C and with Na-liq NH_3 failed. The α-anomer (α-**7**) was also obtained in 78% yield based on unrecovered **1** by the reaction of 2,3,5-tri-*O*-acetyl-D-arabinofuranosyl chloride with **1** followed by MeO^- treatment. In this case no β-anomer was formed. The assignment of the anomeric configuration of these nucleosides is based on the trans rule,[11] which predicts that the 2,3,5-tri-*O*-acetyl-D-arabinofuranosyl chloride would give the α-(trans) anomer, and on the fact that the signal from the anomeric proton of this nucleoside (α or trans) appears in its pmr spectrum as a

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‡Fusion of *N*-nonanoyl-8-azaadenine with tetra-*O*-acetyl-D-xylofuranose gave an anomeric mixt that was separated by means of paper chromatography.[4]

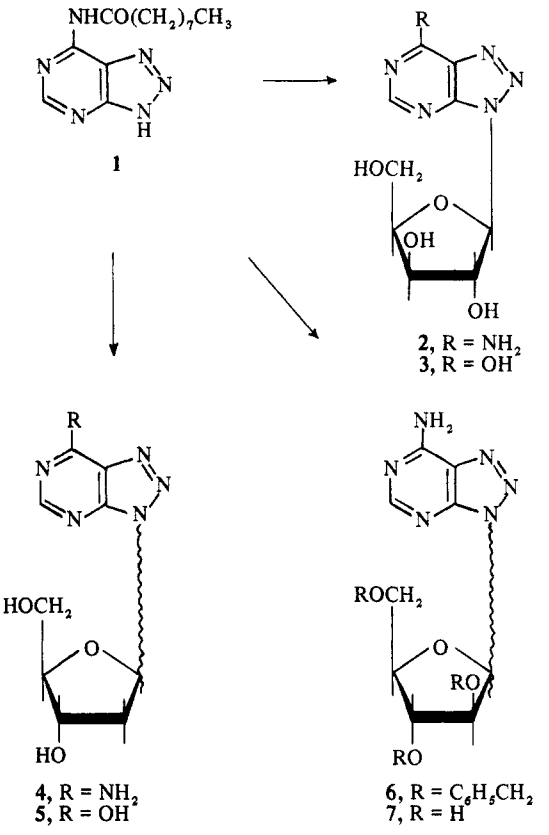
§The use of Et_3N as acid acceptor in the synthesis of 8-azaadenosine was unsuccessful.

#The synthesis of 2′-deoxy-8-azaadenosine and its α-anomer by the chloromercuri procedure in total yield of 2.7% has been reported.[5] These investigators assigned the β configuration to one of their nucleosides because it was a substrate for adenosine deaminase. The other nucleoside was assumed to have the α configuration because it was not a substrate for the enzyme (the position of attachment in both cases was assigned on the basis of the similarity of the uv spectra of these nucleosides to that of 8-azaadenosine, although no data were available on 8-substituted-8-azaadenines). In view of the fact that a compd as different from the normal substrate as 3-β-D-ribofuranosyladenine is a substrate for adenosine kinase[6] and of the well-known low substrate specificity of adenosine deaminase [see Montgomery[7]], we felt that this assignment was open to question and have now confirmed the assignment by chemical means (see Discussion).

Table I. Cytotoxicity of Some 8-Azapurines and Their Nucleosides

Compound	ED_{50}, μmole/l.[a]
8-Azaadenine	20
8-Azahypoxanthine	3.5
8-Azaadenosine	1.0
2′-Deoxy-8-azaadenosine	11
9-(2-Deoxy-α-D-ribofuranosyl)-8-azaadenine	>40[b]
9-β-D-Arabinofuranosyl-8-azaadenine	>40[b]
9-α-D-Arabinofuranosyl-8-azaadenine	2.6
9-β-D-Xylofuranosyl-8-azaadenine	5.0
2′-Deoxy-8-azainosine	*ca.* 80
9-β-D-Xylofuranosyl-8-azahypoxanthine	>75[b]

[a]The concn of compd required to inhibit the growth of treated cells to 50% of that of untreated controls as measured by colony counts (see ref 13). [b]No inhibition at this, the highest level tested.



doublet centered at 6.13 ppm ($J_{1'2'}$ = 6.0 Hz) upfield from that of the β-(cis) anomer (6.43 ppm, $J_{1'2'}$ = 6.0 Hz).[12]

Deamination of **2** to 9-β-D-xylofuranosyl-8-azahypoxanthine (**3**) and of α- and β-**4** to give 2′-deoxy-8-azainosine (β-**5**) and its α-anomer (α-**5**) was accomplished with $NaNO_2$ in AcOH. Treatment of 9-β-D-arabinofuranosyl-8-azaadenine (β-**7**) in this manner caused cleavage of the sugar moiety so that the only product isolated was 8-azahypoxanthine.

Biologic Evaluation. The cytotoxicity of the 8-azapurine nucleosides to human epidermoid carcinoma cells No. 2 in culture[13] is given in Table I. 8-Azainosine and 8-azaadenosine are the most cytotoxic of this group of compounds. The other 8-azaadenine nucleosides are all less toxic, but somewhat surprisingly, the α-anomer (α-**7**) of 9-D-arabinofuranosyl-8-azaadenine is quite active, being 0.4 as toxic as 8-azaadenosine, more than 15 times as toxic as the β-anomer (β-**7**), and 15 times more toxic than α-D-arabinofuranosyladenine. 2′-Deoxy-8-azaadenosine is only 0.125 as toxic as 8-azaadenosine but still about 3 times as toxic as 8-azaadenine and 7 times as toxic as 2′-deoxy-8-azainosine, indicating that its activity is probably due to the intact nucleoside or its phosphate anabolites but not to catabolic products. 9-β-D-Xylofuranosyl-8-azaadenine (**2**) is 0.2 as toxic as 8-azaadenosine, whereas 9-β-D-xylofuranosyl-8-azahypoxanthine is not cytotoxic at the highest level tested.

The correct interpretation of the cytotoxicity data presented above must be based on additional biologic data not yet at hand, because it is known that the azapurine nucleosides, as exemplified by 8-azaadenosine, can be extensively metabolized to compounds known to be cytotoxic.[6,14,15] Recent data tend to support the idea that, although the activity of 8-azaadenosine probably results, at least in cell lines having adenosine kinase,[6] from its phosphorylation to 8-azaadenylic acid,[15] the activity of 8-azainosine may be due to its conversion to 8-azainosinic acid, but not to 8-azaadenylic acid.** No data are yet available on the substrate specificities of the other 8-azapurine nucleosides.

Table II. Preparation of the D-Pentofuranosyl-8-azaadenines

No.	Compd	Pentofuranosyl chloride	Blocking group	Recrystn solvent	Mp, °C	[α]D (Temp, °C; *c*, solvent)	Yield,[a] %	Formula[b]
2	9-β-D-Xylofuranosyl-8-azaadenine	D-Xylose	Acetyl	H_2O	204–206	−85.0 ± 2.6 (23; 0.23, 1 *N* NaOH)	63	$C_9H_{12}N_6O_4$
β-**4**	2′-Deoxy-8-azaadenosine	2-Deoxy-D-ribose	*p*-Chlorobenzoyl	H_2O[c]	200–201	+90.2 ± 1.0 (25; 0.39, H_2O)	18	$C_9H_{12}N_6O_3$
α-**4**	9-(2-Deoxy-α-D-*erythro*-pentofuranosyl)-8-azaadenine			H_2O[c]	195–196	−51.6 ± 1.3 (25; 0.32, H_2O)	9	$C_9H_{12}N_6O_3$
β-**6**	9-(2,3,5-Tri-*O*-benzyl-β-D-arabinofuranosyl)-8-azaadenine	D-Arabinose	Benzyl	MeOH	164–165		22	$C_{30}H_{30}N_6O_4$
α-**6**	9-(2,3,5-Tri-*O*-benzyl-α-D-arabinofuranosyl)-8-azaadenine			MeOH[d]			18	
β-**7**	9-β-D-Arabinofuranosyl-8-azaadenine			MeOH	211–212	−60.0 ± 4.4 (24.5; 0.24, 0.1 *N* NaOH)	31	$C_9H_{12}N_6O_4$
α-**7**	9-α-D-Arabinofuranosyl-8-azaadenine			MeOH	240–241	+83.1 ± 3.5 (25; 0.16, H_2O)	15	$C_9H_{12}N_6O_4$
		D-Arabinose	Acetyl	H_2O	240–241[e]		78	

[a]Based on unrecovered *N*-nonanoyl-8-azaadenine (24–78% recovery). [b]*Anal.* C, H, N. [c]The blocked anomers were separated by pressure column chromatog on Mallinckrodt SilicAr-7 (9:1 C_6H_5–EtOAc) before removal of the acyl groups with NaOMe. [d]Isolated as the picrate from the filtrate of β-**6**. The free base obtained by treatment of a MeOH soln of the picrate with Dowex 1-X8 (CO_3) was a syrup, which was debenzylated without further purification. [e]Identical in all respects with the α-anomer prepared from 2,3,5-tri-*O*-benzyl-D-arabinofuranosyl chloride.

**L. L. Bennett, Jr., personal communication.

Experimental Section

Melting points were detd with a Mel-Temp apparatus and are uncorrected. The optical rotations were detd in the solvents specified with a Rudolph Model 80 polarimeter. The uv spectra of all compds were detd in aq soln with a Cary Model 14 spectrophotometer and were as expected. The ir spectra were detd in pressed KBr discs with Perkin-Elmer Models 221-G, 521, and 621 spectrophotometers and were also normal. The pmr spectra were detd in DMSO-d_6 (TMS) with a Varian A-60A spectrometer; chemical shifts quoted in the case of multiplets are measured from the approximate center. Chromatographic analyses were carried out on tlc plates of silica gel H (Brinkmann). The spots were detected by uv light after spraying the plates with Ultraphor (WT, highly concd). Analytical samples were dried over P_2O_5 (0.07 mm) for 4–7 hr at 78 or 100°.

Preparation of D-Pentofuranosyl-8-azaadenines. A soln of the blocked D-pentofuranosyl chloride in dry C_6H_6 (*ca.* 20 ml/mmole) was added to a flask contg *N*-nonanoyl-8-azaadenine (1 equiv) and Molecular Sieve (1.8–2.5 g/mmole). The mixt was refluxed with stirring for 1 hr, addnl Molecular Sieve added (1.8–2.5 g/mmole), and heating contd for an addnl hr. Filtration of the cooled reaction mixt gave an insol material from which *N*-nonanoyl-8-azaadenine was recovered. A soln of the residue from evapn (*in vacuo*) of the filtrate in dry MeOH contg NaOMe (1.5 equiv) was refluxed for 0.5 hr, chilled, neutralized with AcOH, and evapd to dryness *in vacuo*. A soln of the residue in H_2O was washed with $CHCl_3$, concd, and chilled to give a cryst solid (except in the case of α-**6**, which was isolated as the picrate from the filtrate of the β-anomer). The analytical samples (**2**, α- and β-**4**, and β-**6**) were obtained by recrystn (see Table II). The *O*-benzyl groups of β- and α-**6** were removed by hydrogenolysis at room temp in 1:1 MeOH–2-methoxyethanol at 50 psi for 2 hr using $PdCl_2$ catalyst (0.5 g/mmole).

9-β-D-Xylofuranosyl-8-azahypoxanthine (3-β-D-Xylofuranosyl-*v*-triazolo[4,5-*d*]pyrimidin-7(6*H*)-one, 3). To a soln of 9-β-D-xylofuranosyl-8-azaadenine (815 mg, 3.04 mmoles) in hot H_2O (30 ml) was added $NaNO_2$ (5.06 g, 73.4 mmoles) followed by glacial AcOH (5.06 ml). The reaction soln was stirred at room temp for 16 hr, diluted with H_2O (38 ml), and stirred for an addnl hr. Addn of 1.2 equiv of $Pb(OAC)_2$ followed by 10.3 ml of concd NH_4OH gave a white solid that was collected by filtration. A soln of this solid in 20% aq AcOH was treated with H_2S. The black PbS was removed by filtration and the filtrate evapd to dryness *in vacuo*. The residue crystd from 80% aq EtOH; yield, 334 mg. Addn of another 0.6 equiv of $Pb(OAc)_2$ to the filtrate followed by concd NH_4OH (5 ml) gave addnl material from which a second crop was obtd; yield, 78 mg (total yield 51%). The analytical sample was obtd by recrystn from abs EtOH; mp 174–176°. *Anal.* ($C_9H_{11}N_5O_5$) C, H, N.

2′-Deoxy-8-azainosine [3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-*v*-triazolo[4,5-*d*]pyrimidine-7(6*H*)-one, β-5). Treatment of 2′-deoxy-8-azaadenosine (200 mg, 0.8 mmole) as described above gave the product (β-**5**), which crystd from EtOH; yield, 70 mg (28%). The analytical sample was obtd by recrystn from EtOH; mp 155–156°. *Anal.* ($C_9H_{11}N_5O_4 \cdot 0.1H_2O$) C, H, N.

3-(2-Deoxy-α-D-*erythro*-pentofuranosyl-*v*-triazolo[4,5-*d*]pyrimidin-7(6*H*)-one, α-5). In the same manner, 9-(2-deoxy-α-D-ribofuranosyl)-8-azaadenine (252 mg, 10 mmoles) gave the α-anomer of 2′-deoxy-8-azainosine, which crystd from 90% EtOH; yield 137 mg (54%); mp 143–144°. *Anal.* ($C_9H_{11}N_5O_4$) C, H, N.

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Glutathione. 6. Probable Mechanism of Action of Diazene Antibiotics[1]

Edward M. Kosower* and Tetsuo Miyadera†

Department of Chemistry, State University of New York, Stony Brook, New York 11790. *Received August 6, 1971*

Compounds with the structure AN=NB (and the potential for conversion to that structure, as ANHNHB) are now recognized as a new class of biological control agents, the *diazene antibiotics*. Examples include the antibiotic, hexahydrospinamycin, the glutathione-oxidizing agents, "azoester" and "diamide." Within specific chemical classes of diazene antibiotics, higher antifungal activity can be correlated with higher rate of reaction with glutathione. Reasons are presented for the idea that the antibiotic action of diazenes may involve intracellular oxidation of glutathione to its disulfide, and compounds which could effect this conversion in a catalytic fashion (by cycling through reoxidizable hydrazo compounds) have been designed and synthesized.

Recognition of antibiotic classes serves to accelerate pursuit of effective agents and their mode of action. Three sources provided the stimulus for the classification of compounds with the structure AN=NB as *diazene antibiotics*. First, the antifungal activity of phenylthiosemicarbazide (**1**) derivatives has been known for many years (*cf.* references cited in reference 2). Recently, the diazenes derived by oxidation of **1** have been implicated as the active biological agents (*e.g.*, **2**).[3,‡] Second, reagents introduced by ourselves had clearcut antibiotic activities. These reagents included

$C_6H_5NHNHCSNH_2$ (**1**) $C_6H_5N{=}NCSNH_2$ (**2**)

†On leave from Central Research Laboratories, Sankyo Co., Tokyo, Japan.

‡The care required to demonstrate the intermediacy of diazenes because of hydrolytic instability is not appreciated (*cf.* ref 3b).