Articles

Modifications on the Heterocyclic Base of Acyclovir: Syntheses and Antiviral Properties

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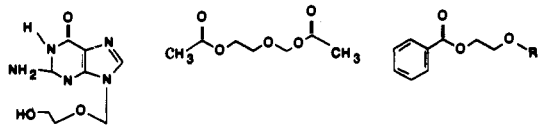
A group of compounds was prepared in which variations of the ring portion of the acyclovir (ACV) structure were made. These modifications included monocyclic (isocytosine, triazole, imidazole), bicyclic (8-azapurine, pyrrolo[2,3-*d*]pyrimidine, pyrazolo[3,4-*d*]pyrimidine) and tricyclic (linear benzoguanine) congeners. The derivatives were evaluated against herpes simplex virus type 1 (HSV-1) by the plaque-inhibition and plaque-reduction methods with only the 8-azapurine analogue **28** showing some activity. In a test measuring the ability of these compounds to inhibit the HSV-1 thymidine kinase, **28** and the tricyclic derivative **38** exhibited competition with ACV for binding to the enzyme. The inability of the group to exert significant antiherpetic action is attributed to their lack of phosphorylation to the requisite triphosphate stage.

For a number of years we have been engaged in these laboratories in a research program on acyclic nucleosides, i.e., heterocyclic nitrogen bases that are attached by a glycosidic linkage to acyclic chains, structurally designed to resemble the cyclic carbohydrate portion of naturally occurring nucleosides.[1,2] The potent antiviral drug acyclovir [9-[(2-hydroxyethoxy)methyl]-9*H*-guanine, **1**, Zovirax], which has activity against the viruses of the herpes family,[1,2] evolved from these studies.

To determine the effect that modifications on the heterocyclic base of acyclovir may have on its antiviral properties, we have prepared a group of analogues, all of which retain the (2-hydroxyethoxy)methyl acyclic chain and possess some of the features of the guanine molecule. Presented in this paper are the syntheses and testing results of these compounds.

Chemistry. In general, the preparation of acyclic nucleosides in this series utilized one of three synthetic approaches, in each of which the appropriate base was first converted to a more reactive species. In the fusion method, a polyacetylated base or other intermediate was combined with the acyclic chain 2-(acetoxymethoxy)ethyl acetate (**2**)[3] and, in the presence of a catalyst, heated at a high temperature under vacuum until a liquid melt was obtained. This procedure is exemplified by method A in the Experimental Section. An alternate synthesis consisted of the reaction of a silylated base with 2-(chloromethoxy)ethyl benzoate (**3**). The latter was prepared by chloromethylation of 2-hydroxyethyl benzoate (**4**).[4] A typical example is illustrated by method B. The third synthetic pathway was the direct alkylation with **3** of the anion of the appropriate heterocyclic base, generated in situ by a strongly basic reagent, which also acted as an acid acceptor. Three compounds were prepared in this manner, described as method C.

In most experiments, two or more isomeric components were formed, which were separated by column chromatography. The protected hydroxy terminus in the acyclic chain of the desired intermediates was then unblocked by suitable means to yield the target compounds. Structural assignments were made by NMR and MS determinations and also, in some cases, by comparison of the UV spectra with that of known ribofuranosyl analogues.



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Monocyclic Derivatives. The imidazole derivative 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (**5**), which as the 5′-phosphate (AICAR) is an intermediate in the de novo biosynthesis of purine nucleic acids, has a broad spectrum of biological properties. Synthesis of the corresponding acyclic analogue **6a** was achieved in a modest yield by treatment of 5-aminoimidazole-4-carboxamide **7** with **3** and triethylamine in refluxing acetonitrile, followed by hydrolysis of the terminal ester group in the acyclic chain of the separated isomer **6b** with aqueous $MeNH_2$. Isolation of the final product was facilitated by conversion to its hydrochloride salt. Two other groups have reported the preparation of this compound.[5,6]

A structure closely related to AICA ribonucleoside is the well-known broad-spectrum antiviral agent ribavirin or Virazole (**9**).[7] To prepare the acyclic derivative, the method of Witkowski et al. was used,[7] i.e., fusion of 1,2,4-

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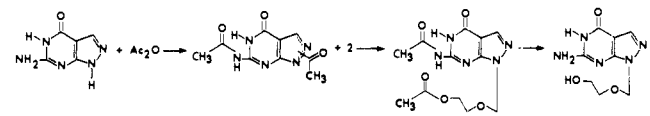
triazole-3-carbonitrile (**10**), with **2**, using bis(*p*-nitrophenyl) phosphate as the catalyst. This reaction, on purification, gave three products: **11–13**. ^{1}H NMR and IR spectral examinations identified **11** and **12** as the substituted 3- and 5-cyano isomers, respectively. Treatment of **11** with concentrated ammonium hydroxide at 100 °C produced a compound that, on the basis of comparison of its UV and NMR spectra with that of ribavirin, was identified as the desired 1-alkylated isomer **14**. Intermediate **13**, which was determined to contain a carboxamide rather than a cyano group by IR analysis, yielded on ammonolysis the 3-substituted isomer **15**. The latter had comparable UV properties to the corresponding ribonucleoside and was identical (^{1}H NMR, melting point) to the product obtained from similar treatment of **12**. The lower shift value for the NCH_2O group in the ^{1}H NMR spectrum of **15** compared with that of **14** (see Experimental Section) is due to the effect of the neighboring carboxamide group and supports the position of alkylation by the acyclic chain.

Synthesis of the isocytosine acyclic derivative **20** was initially attempted by reaction of the silylated base with **3**, as in method B, but only starting material was isolated from the reaction mixture. The compound was ultimately made by condensation of the isocytosine anion, generated in situ with sodium hydride in DMF, with 1 equiv of **3**, but extensive purification was required to resolve the complex reaction mixture. Separation by repeated column chromatography gave the two expected isomeric components **17** and **19** as well as a third product, **18**. The latter was identified by ^{1}H NMR as an amidine derivative, 2-[[2-[[(dimethylamino)methylene]amino]-1,4-dihydro-4-oxo-1-pyrimidinyl]methoxy]ethyl benzoate, produced by reaction of **17** with the solvent DMF. Both **17** and **18** yielded identical products, **20**, on deblocking with aqueous $MeNH_2$. The other isomer was not reacted further. The synthesis of this isocytosine derivative **20** has been reported by other investigators.[8]

Bicyclic Derivatives. Three bicyclic analogues of acyclovir were prepared in which the modifications were restricted to the imidazole portion of the molecule.

The pyrazolo[3,4-*d*]pyrimidine congener **24** was synthesized by a fusion procedure with the polyacetylated base **22**, which afforded a satisfactory yield (58%) of the desired intermediate **23**, without the necessity for column purification. Deprotection of the compound gave the acyclic analogue **24** for an overall yield of 33%. The position of the acyclic chain was determined principally by ^{13}C NMR analysis, as a consequence of previous studies of ribofuranosyl derivatives (unpublished) that have established that, in N-2-substituted analogues, the chemical shift value for the C-3 atom in the pyrazole moiety is approximately 125 ppm whereas those of N-1 congeners were further downfield at about 132 ppm, a value comparing nicely with the 135 ppm assignment found in the spectrum of **23**. Examination of the mother liquors of **23** by TLC (in 10% MeOH–CH_2Cl_2 on silica gel) indicated that another component was present in this fraction, and on unblocking with aqueous $MeNH_2$, a small amount of solid (44 mg) was obtained by acetone trituration of the evaporated solution. Proton NMR spectrum of this material showed two sets of peaks for the amino group as well as the pyrazole hydrogen but only a single peak for the methylene group attached to the ring nitrogen, which had the same shift value as that of **24**. On the basis of this evidence, it was concluded that the second component was the starting base **21** and not the other possible N-2-alkylated product.



21 22 23 24

Alkylation of the trissilylated base of 8-azaguanine **25** with several potential reaction sites produced only two isomers **26** and **27**, which were separated by differential solubilities in boiling MeOH. The more soluble component, identified by ^{13}C NMR as the 3-substituted isomer **26**, on hydrolysis with $MeNH_2$ solution yielded the 8-aza analogue of acyclovir **28**. The 1-substituted congener **27** had little solubility in MeOH and was recrystallized from hot Me_2SO to give analytically pure material.

The substitution positions of the acyclic chain in the two isomers were ascertained by comparison of the shift values in their respective ^{13}C NMR spectrum for the the carbons in the bridgehead positions, C-8 and C-9 (analogous to C-5 and C-4 of a purine ring). In the spectrum of **27**, the assignment of 113.13 for C-8 is upfield from that of **26** (124.16) as would be expected due to the effect of the adjacent N-1 substituent and the tautomeric amidic carbonyl group at position 7. Conversely, in **26**, the value for C-9 is lower (upfield, 151.8 vs. 161.29 for **27**) due to the effect of the acyclic chain on the adjacent triazolic N-3. Confirmatory evidence for the structure of **26** was obtained from comparison of the UV spectrum of the hydrolyzed product **28** with that of the corresponding N-3-substituted ribofuranosyl congener.[9]

Preparation of the 7-deaza congener of acyclovir **30** involved the most complex chemistry of the series. Initially, it was attempted by using a method based on that of Townsend and co-workers,[10] employing the trissilylated derivative of 2-amino-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (**29**) with **3** and the combined catalysts mercuric oxide and mercuric bromide. However, this reaction after workup and unblocking produced less than 1% of **30**. Alternately, direct substitution of the anion of the 2-acetylated derivative **31**[10] generated by lithium diisopropylamide (LDA) resulted in a rather complex mixture of products due to competing alkylation of the pyrimidine ring of the dianionic molecule. The monosubstituted analogue **32** was the sole product obtained from the reaction of **31** and 1 equiv each of LDA and **3**. If two equivalents of the lithium salt were used, three compounds, **32–34**, were produced in varying ratios, depending on the reaction time.

Both **33** and the bisalkylated product **34** yielded 7-deazaacyclovir (**30**) by deprotection with aqueous $MeNH_2$, but the use of this reagent on **32** cleaved the acyclic chain to regenerate the parent base **29**. An alternate approach to **30** has been demonstrated by Seela and colleagues,[11] which commences with the 4-methoxy analogue of **29**.

Tricyclic Derivative. The synthesis of the only tricyclic derivative in this group was modeled on the work of Keyser and Leonard concerning the conformation and biological properties of "stretched" nucleosides, so called because their synthesis results in extension of the heterocyclic base as if by insertion of a benzene ring in the

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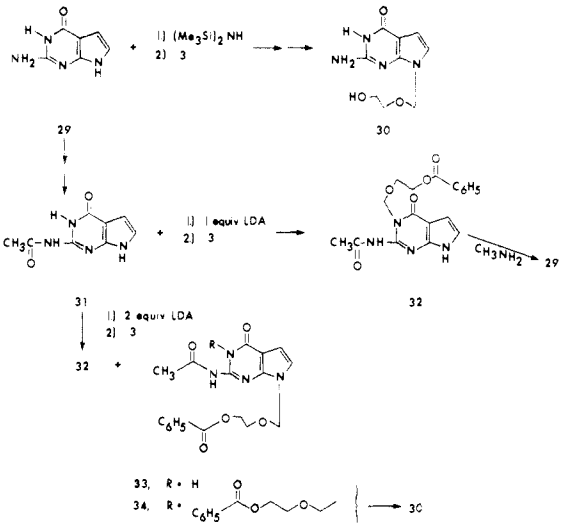
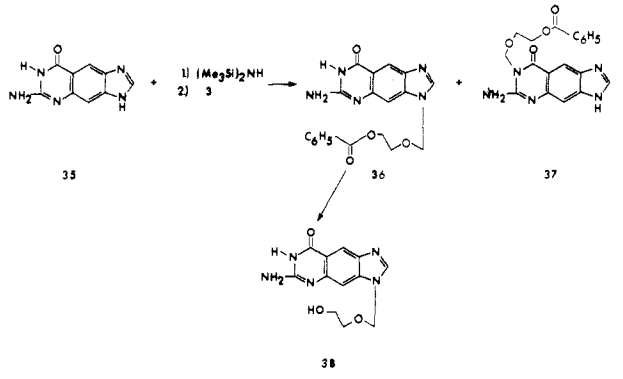


center of the heterocycle moiety.[12] Using this terminology, one could consider the acyclic congener of linear benzoguanine, 6-amino-3,7-dihydro-8*H*-imidazo[4,5-*g*]-quinazolin-8-one (**35**) (or "stretched" guanine), as "stretched" acyclovir. This novel compound was obtained through the reaction of the trissilylated base derivative of **35** with **3**, which gave the usual isomeric mixture, **36** and **37**. The desired component was separated and unblocked to give the product **38** in which the position of the acyclic chain was ascertained through ^{1}H NMR–NOE experiments. This study showed increased sensitivity of the 2-



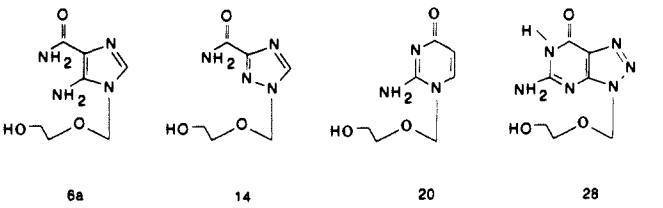
and 4-protons relative to that of the 9-position on irradiation of the 5.7 ppm frequency (NCH_2O group), a consequence of the nuclear Overhauser effect of the acyclic methylene group in position 3. The full preparation is described as method B in the Experimental Section.

Biological Results and Discussion

Table I summarizes the results of screening this group of compounds against herpes simplex virus type 1 (HSV-1) by the plaque-inhibition and plaque-reduction methods.[13] Also included are the testing data from their evaluation as substrates or inhibitors of the HSV-1 thymidine kinase (TK).

Only one compound, the 8-azaguanine derivative **28**, showed some inhibition of HSV-1, and it was more than 1000-fold less active than acyclovir. The remaining compounds had no inhibitory effect on the viral plaques at the concentrations tested.

The first assay with HSV-1 TK shown in Table I is a measurement of the ability of the test substrate (300 μM) to compete with 150 μM of [^{14}C]ACV for the binding site(s)



on the viral enzyme.[14] In this test, only **28** and **38** exhibited any appreciable competition with the parent structure **1**. In the second assay measuring phosphorylation rates, the former two had very low substrate velocities relative to thymidine, the natural substance of the enzyme, which is arbitrarily set at 100%.

The mechanism of action of acyclovir has been well documented.[1,2] It is phosphorylated by the herpes-induced enzyme (TK) to the monophosphate derivative and ultimately to the triphosphate form by cellular kinases.[15,16] The latter nucleotide is an inhibitor of the viral DNA polymerase and also acts as a chain terminator. While it is possible, of course, for compounds to inhibit HSV by other mechanisms, one would expect the derivatives in this series to operate by a similar mode of action as the parent acyclic nucleoside. The data in Table I show that most of the compounds listed lack appreciable phosphorylation by the viral enzyme due to low substrate activity and hence fail to undergo the critical first step of the inhibitory process by acyclovir, i.e., conversion to the monophosphate species. In the cases of those analogues cited as having been made by others (**20** and **6a**),[8] this lack of antiherpetic activity has been confirmed. No biological data has been reported on **30**.[11]

None of the derivatives in this group exhibited any significant activity in other viral screens against measles, influenza, vaccinia, respiratory syncytial, Semliki, Sindbis, Bunyamwera, Mengo, rhino 1B, and adenoviruses. Testing in antibacterial, antiprotozoal, and antitumor assays showed negative results for the group.

Experimental Section

Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded at ambient temperature on either a Varian XL-100 spectrometer, a Hitachi Perkin-Elmer R-24A spectrometer, or a Varian FT-80A spectrometer in $CDCl_3$ or Me_2SO-d_6 with Me_4Si as the internal standard. Carbon nuclear magnetic resonance spectra (^{13}C NMR) were obtained with a Varian CFT-20 spectrometer in Me_2SO-d_6 with reference to internal Me_4Si. Ultraviolet spectra were recorded on a Norelco Unicorn SP-820 spectrometer and the infrared spectra on a Beckman 1R 8 infrared spectrometer. Mass spectra were obtained with a Varian MAT 731 instrument using either EI or FD techniques. Melting points were obtained with a Thomas-Hoover apparatus or a Kofler hot stage and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA, or by the Analytical Services Section of the Wellcome Research Laboratories, and the results are within 0.4% of the theoretical values. Thin-layer chromatography studies were done with Analtech 250-μM silica gel (GDF) or alumina G plates. Silica gel 60 (230–400 mesh) for column chromatography was purchased from Brinkmann Instruments and W200 neutral alumina from ICN. HPLC studies were performed with a Waters Model 600A delivery solvent system using an ultraviolet detector on a Waters C18 μBondapak column at a flow rate of 2 mL/min. The starting material 8-azaguanine was obtained from Aldrich Chemical Co. and 5-amino-4-imidazolecarboxamide hydrochloride and isocytosine were from Sigma Chemical Co. Solvents were dried over 3A molecular sieves for a minimum of 24 h.

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Table I. Testing Results on HSV Type 1

	HSV-1 sensitivity		HSV-1 TK assay[a]	
no.	b	IC_{50},[c] μM	inhibn	rel substrate velocity, %
1 (ACV)	+	0.1	69[d]	36[d]
20	-	ND[e]	0, 7[d]	<3
6a	-	ND	1	ND
14	-	>200	0	ND
28	+	129	27, 23[d]	9, 5,[d] 9[d]
30	-	ND	6[d]	3[d]
24	-	ND	0	ND
38	-	ND	24	<3

[a] Measure of the ability of 300 μM test substrate to compete for binding site(s) on the HSV thymidine kinase with 150 μM [^{14}C]H-ACV. [b] Detection of antiviral activity by the plaque-inhibition test in vero cells, measuring the zone of inhibition of plaque formation with disks containing 50 μg of test compound. (+) 11–50-mm zone of inhibition. [c] Plaque-reduction assay determination of IC_{50}, the concentration required to inhibit the growth of viral plaques by 50% of controls. [d] Mac strain, other values are for the H29 strain. [e] Not determined.

Table II. UV Spectral Characteristics[a]

	λ_{max} (ε)		
no.	pH 1.0	pH 7.0	pH 13.0
6a	245 (8820)	267 (11 470)	267 (11 840)
	268 (9900)		
14		207 (8570)	
20	255 (7770)	221 (15 210)	221 (15 210)
		255 sh (5770)	252 sh (5590)
24	252 (15 860)	252.5 (16 550)	264 (12 300)
28	254 (16 960)	254 (16 960)	278 (14 130)
	269 sh (11 740)	269 sh (11 740)	
30	258.5 (13 200)	258 (13 700)	261 (11 950)
		276 sh (8250)	
38	230 sh (33 100)	238 (38 950)	238 sh (29 400)
	236 (36 050)	256.5 (22 150)	247.5 (35 300)
	243 (36 750)	276 (5150)	253 sh (33 800)
	260 sh (5600)	321 (5150)	277 sh (4250)
	317 (5300)	330 sh (4550)	286 (5450)
	325 sh (4700)		295 sh (3400)
			339 (5850)

[a] Compounds were dissolved in water and aliquots diluted 10-fold with 0.1 N aqueous HCl, H_2O, or 0.1 N aqueous NaOH solutions.

2-(Chloromethoxy)ethyl Benzoate (3). Dry HCl gas was passed through a mixture of 41.5 g (0.25 mol) of 2-hydroxyethyl benzoate (**4**)[4] in 100 mL of dry $C_2H_4Cl_2$ and 22.5 g (0.25 mol) of paraformaldehyde at 0 °C with magnetic stirring for 3 h. The solution was dried over $CaCl_2$ for 24 h, filtered, and evaporated in vacuo. The residual oil was distilled to give 49.6 g (93%) of **3**, bp 126–129 °C (0.5 mm).

Method A. 6-Amino-1,5-dihydro-1-[(2-hydroxyethoxy)-methyl]-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (24). A mixture of 1.23 g (7.9 mmol) of **21**[17] in 100 mL of Ac_2O was refluxed with stirring for 18 h. The resultant amber solution was evaporated in vacuo and the residual solid was triturated with acetone to yield, after drying 3 h at 100 °C (3.5 mm), 1.34 g (72%) of **22** as a tan powder. The ^{1}H NMR spectrum (Me_2SO-d_6) indicated that the solid was a mixture of the two diacetylated isomers in a 1:1 ratio: δ 11.9 (br s, 4 H, NHCO), 8.97 (s, 1 H, C^3-H), 8.18 (s, 2 H, C^3-H), 2.67, 2.65 (d, J = 2 Hz, 6 H, CH_3CON), 2.3, 2.15 (d, J = 2 Hz, 6 H, CH_3CON). Anal. ($C_9H_9N_5O_3$) C, H, N (N = 0.47 off).

A mixture of 0.6 g (2.55 mmol) of **22**, 1.7 g (9.65 mmol) of **2**, and 0.01 g (0.05 mmol) of *p*-toluenesulfonic acid monohydrate was heated, with stirring, under water aspirator pressure at reflux (oil bath temperature 140 °C) until a clear solution was obtained (20 min). The amber liquid was cooled to room temperature, causing a crystalline precipitate to form. The crystals were filtered with the aid of benzene, washed with Et_2O, and dried to give 0.46 g (58%) of a tan powder, **23**. An additional crop was obtained from the mother liquors by dilution of the filtrate with hexane. A solution of 20 mL of 40% aqueous $MeNH_2$ and 0.44 g (1.42 mmol) of **23** was heated for 20 min at 100 °C, the solution evaporated in vacuo, and the residue triturated with acetone. Recrystallization from hot EtOH gave 0.175 g (55%) of the title compound **24**: mp 250–253 °C; ^{1}H NMR (Me_2SO-d_6) δ 10.59 (s, 1 H, NHCO), 7.82 (s, 1 H, C^3-H), 6.72 (s, 2 H, NH_2), 5.41 (s, 2 H, NCH_2O), 4.57 (br s, 1 H, OH), 3.49 (s, 4 H, OCH_2CH_2O), 3.37 (s, 1 H, H_2O); ^{13}C NMR (Me_2SO-d_6) δ 157.85 (C^4), 155.81 (C^8), 155.09 (C^6), 135.23 (C^3), 99.73 (C^9), 74.99 (NCH_2O), 70.53 (OCH_2), 59.94 (CH_2OH); HPLC (20% MeOH–H_2O), single peak, retention time 3.4 min. Anal. ($C_8H_{11}N_5O_3 \cdot {}^1/_4H_2O$) C, H, N.

Method B. 6-Amino-3,7-dihydro-3-[(2-hydroxyethoxy)-methyl]-8*H*-imidazo[4,5-*g*]quinazolin-8-one (38). A mixture of 0.41 g (2.0 mmol) of **35**,[12] 0.18 g (1.36 mmol) of $(NH_4)_2SO_4$, and 15 mL of hexamethyldisilazane (HMDS) was refluxed under N_2 with stirring for 18 h. The solution was evaporated in vacuo to a dark oil, dissolved in 10 mL of dry toluene, and combined with 0.7 mL (5 mmol) of Et_3N and 0.45 g (2.1 mmol) of **3**. The reaction solution was refluxed under N_2 for 4 h and then evaporated to dryness. The residue was stirred with 1:1 (v/v) EtOH–H_2O, a negligible amount of solid was filtered off, and the filtrate was evaporated in vacuo. The residue was dissolved in 25 mL of H_2O and 5 mL of Et_3N and reevaporated. The residual oil was purified by column chromatography on 115 g silica gel eluting initially with 5% MeOH in CH_2Cl_2, progressing by 1% differentials to 14% MeOH–CH_2Cl_2. Fractions were monitored by TLC (silica gel) in 11% MeOH–CH_2Cl_2 (developed 4 times). The material obtained from the 11% through the initial 14% eluates contained two spots on TLC, but the later (14%) fractions showed only the lower R_f spot. The latter, on evaporation and recrystallization from H_2O, gave 0.117 g (17%) of **36**: mp 246–250 °C; MS, m/e 379 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 8.52 (s, 1 H, C^2-H), 8.19 (s, 1 H, C^9-H), 7.44 (m, 8 H, C^4-H, NH_2, phenyl), 5.76 (s, 2 H, NCH_2O), 4.34 (m, 2 H, OCH_2C), 3.57 (m, 2 H, CH_2CO). A solution of 0.112 g (0.3 mmol) of **36** and 30 mL of 40% aqueous $MeNH_2$ was heated at 100 °C for 5 min and then evaporated in vacuo. The residue was triturated with EtOAc and recrystallized from H_2O to yield 0.047 g (56%) of **38**: mp 243–249 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 8.40 (s, 1 H, C^2-H), 8.18 (s, 1 H, C^9-H), 7.38 (s, 1 H, C^4-H), 6.3 (br s, 2 H, NH_2), 5.67 (s, 2 H, NCH_2O), 3.44 (br s, 4 H, OCH_2CH_2O), OH masked by H_2O peak. Anal. ($C_{12}H_{13}N_5O_3 \cdot H_2O$) C, H, N.

Method C. 2-Amino-3,7-dihydro-7-[(2-hydroxyethoxy)-methyl]-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (30). One Equivalent of LDA. To a stirred solution of 8.38 mmol of LDA (generated in situ from *n*-butyllithium and diisopropylamine) in 40 mL of dry Me_2SO was added 1.54 g (7.99 mmol) of **31**[10] in 20 mL of Me_2SO at room temperature. After 5 min, 1.71 g (7.99 mmol) of **3** in 20 mL of Me_2SO was added and the reaction mixture was stirred under N_2 at room temperature for 10 min. The solution was poured into 800 mL of 5% aqueous HCl at 0 °C and the aqueous phase was extracted 3 times with 200 mL of CH_2Cl_2. The organic extracts were dried (Na_2SO_4) and filtered, and the filtrate was evaporated in vacuo. The residue was recrystallized once with Et_2O and once with EtOH to yield 223 mg (7.5%) of **32** as tan crystals: mp 195–197 °C; ^{1}H NMR (Me_2SO-d_6) δ 12.08 (br s, 1 H, NHCO), 11.9 (br s, 1 H, NHCO), 7.61 (m, 5 H, phenyl H's), 7.06 (d, J = 3.4 Hz, 1 H, C^6-H), 6.48 (d, J = 3.3 Hz, 1 H, C^5-H), 5.25 (s, 2 H, NCH_2O), 4.38 (m, 2 H, CH_2OCO), 3.89 (m, 2 H, CH_2ON), 2.12 (s, 3 H, CH_3CO). Anal. ($C_{18}H_{18}N_4O_5$) C, H, N.

Two Equivalents of LDA. To a stirred solution of 43.7 mmol of LDA in 50 mL of Me_2SO under N_2 was added, at room temperature, a solution of 4.0 g (20.8 mmol) of **31** in 50 mL of Me_2SO. A precipitate formed immediately. After stirring of the mixture for 5 min, a solution of 4.47 g (20.8 mmol) of **3** in 50 mL of Me_2SO was added to the suspension, giving a pale brown transparent solution. After 5 min of additional stirring, the reaction solution was poured into 3 L of 5% aqueous HCl at 0 °C and the mixture was extracted 3 times with 350 mL of CH_2Cl_2. The combined organic extracts were washed with H_2O, dried (Na_2SO_4), filtered, and evaporated in vacuo. The residual oil was triturated once with 5:1 (v/v) Et_2O–hexane and once with 200 mL of Et_2O and recrystallized from EtOH–hexane to yield 1.05 g of an off-white crystalline solid. TLC examination (cellulose in 1:6:3 *n*-PrOH,

(17) Hitchings, G. H.; Falco, E. A. U.S. Patent 3 474 098, 1969.

5% aqueous $(NH_4)_2SO_4$, 1:1 NH_4OH in H_2O) showed three spots, one corresponding to **32**, R_f 0.896. Trituration of the material with 10% EtOAc–Et_2O gave 330 mg (4%) of a solid homogeneous by TLC, R_f 0.79. Recrystallization of 80 mg from EtOAc–hexane yielded 51 mg of **33**: mp 184–186 °C; ^1H NMR (Me_2SO-d_6) δ 10.0 (br s, 2 H, 2 NHCO), 7.64 (m, 5 H, phenyl H's), 7.18 (d, J = 3.5 Hz, 1 H, C^6-H), 6.47 (d, J = 3.5 Hz, 1 H, C^5-H), 5.48 (s, 2 H, NCH_2O), 4.34 (m, 2 H, CH_2OCO), 3.78 (m, 2 H, CH_2ON), 2.14 (t, 3 H, CH_3CO). Anal. ($C_{18}H_{18}N_4O_5$) C, H, N.

The filtrate from the trituration of **33** on evaporation and trituration with toluene gave 220 mg of a solid, which was shown to be a mixture of **32** and **33** by TLC. The toluene extracts produced on evaporation in vacuo 0.5 g of a solid, which consisted largely of the highest R_f material of the original three component mixture, identified as **34** on the basis of its proton NMR spectrum: ($CDCl_3$) δ 8.48 (br s, $^1/_2$ H, HNCO), 7.69 (m, 14 H, phenyl H's), 6.91 (d, J = 3.54 Hz, 1 H, C^6-H), 6.65 (d, J = 3.51, 1 H, C^5-H), 5.65 (s, 2 H, pyrrolic NCH_2O), 5.46 (s, 2 H, pyrimidinic NCH_2O), 4.42 (m, 4 H, 2 CH_2CO), 3.97 (m, 2 H, CCH_2O pyrimidine chain), 3.77 (m, 2 H, CCH_2O, pyrrole chain), 2.37 (s, 3 H, CH_3CO).

Deprotection Studies. A 220-mg (0.6 mmol) sample of **33** was stirred in 22 mL of 40% aqueous $MeNH_2$ for 1.5 h. After evaporation in vacuo, the solid was triturated with Et_2O and azeotropically dried with MeOH and acetone. Recrystallization from acetone gave 0.086 g (64%) of the title compound **30**: mp 217.5–219.5 °C (lit.[11] mp 220 °C); ^1H NMR (Me_2SO-d_6) δ 10.26 (br s, 1 H, NHCO), 6.8 (d, J = 4 Hz, 1 H, C^6-H), 6.25 (d, J = 4 Hz, 3 H, NH_2, C^5-H), 5.30 (s, 2 H, NCH_2O), 4.5 (br s, 1 H, OH), 3.41 (s, 4 H, OCH_2CH_2O). Anal. ($C_9H_{12}N_4O_3$) C, H, N.

Similar treatment of **34**, at room temperature for 3 days, yielded a product identical with **30** by TLC and ^1H NMR, whereas **32** under the same conditions for 4 h gave the starting material **29**, identified by TLC (silica gel in 16% MeOH–CH_2Cl_2) and ^1H NMR.

5-Amino-1-[(2-hydroxyethoxy)methyl]imidazole-4-carboxamide Hydrochloride (6a). The title compound was prepared according to method C, using 3.6 g (22 mmol) of **7**, 9.8 mL (70 mmol) of dry Et_3N, and 10.38 g (48.4 mmol) of **3** in 210 mL of dry CH_3CN. After refluxing for 6 h, the reaction mixture was evaporated and purified by flash column chromatography on silica gel using 20% MeOH in CH_2Cl_2 to elute, successively, the desired 3- and 1-substituted isomers 2-[(4-amino-5-carbamoyl-1*H*-imidazol-1-yl)methoxy]ethyl benzoate (**8b**; 0.65 g) and 2-[(5-amino-4-carbamoyl-1*H*-imidazol-1-yl)methoxy]ethyl benzoate (**6b**; 1.74 g). The former compound was contaminated with an unknown higher R_f component and an intermediate fraction containing both isomers was also obtained (1.74 g). A portion of the final pure fraction of **6b** was recrystallized twice from benzene to an analytically pure, pale violet sample, mp 135–137 °C, on drying at 100 °C (3.5 mm) for 18 h. Treatment of 600 mg of **6b** with 40% aqueous $MeNH_2$ at 100 °C for $^1/_2$ h yielded on evaporation a yellow oil, which was purified by column chromatography with 10% MeOH in CH_2Cl_2. The resultant pure oil (0.197 g, 52%) was dissolved in EtOH and chilled (0 °C) and the pH adjusted to 1.0 with ethereal HCl. The yellow precipitate was quickly filtered and dried at room temperature (1.6 mm, 18 h) to give 176 mg of a rose powder **6a** as the hydrochloride salt: mp 155–157 °C; HPLC (5% MeOH–H_2O) single peak, retention time 5.2 min; M/S, m/e 200 (M^+); ^1H NMR (Me_2SO-d_6) δ 8.73 (s, 1 H, C^2-H), 7.5 (br s, 5 H, NH_2CO, NH_3), 5.5 (s, 2 H, NCH_2O), 3.53 (s, 4 H, OCH_2CH_2O). Anal. ($C_7H_{12}N_4O_3$-HCl-$^1/_4H_2O$) C, H, N.

1-[(2-Hydroxyethoxy)methyl]-1*H*-1,2,4-triazole-3-carboxamide (14). As described in the text, this compound was synthesized by method A, utilizing 4.7 g (0.05 mol) of 1,2,4-triazole-3-carbonitrile (**10**) with 8.8 g (0.05 mol) of **2** and 0.05 g (0.147 mmol) of bis(*p*-nitrophenyl) phosphate at 153 °C for 24 min. Purification was achieved by column chromatography on 200 g of silica gel. After initial elution with benzene to remove the acyclic chain byproducts, the column was eluted with increasing concentrations of Et_2O in benzene (2, 5, 7, 10, 50, and 100%). The 7 and 10% eluates yielded on evaporation 0.816 g (5.9%) of 2-[(5-cyano-1*H*-1,2,4-triazol-1-yl)methoxy]ethyl acetate (**12**) as determined by comparison of the ^1H NMR shift values for the triazole C^2-H (8.16 ppm) with those of the corresponding ribofuranosyl analogue in ref 7 (8.05 ppm). Subsequent fractions obtained with the 10% elution gave 7.16 g (68%) of the 3-cyano compound 2-[(3-cyano-1*H*-1,2,4-triazol-1-yl)methoxy]ethyl acetate (**11**): ^1H NMR ($CDCl_3$) δ 8.4 (s, 1 H, C^5-H), 5.63 (s, 2 H, NCH_2O), 4.05 (m, 4 H, OCH_2CH_2O), 2.05 (s, 3 H, CH_3CO). The last fraction from this eluate yielded more of **11** and an unknown product (0.82 g), which was the sole constituent of the 50 and 100% eluates. The latter on evaporation produced 0.97 g of a white solid whose IR spectrum (Nujol) showed no nitrile group and had CO (1720 cm^{-1}), $CONH_2$ (1620 cm^{-1}), and NH_2 (3350, 3180 cm^{-1}) groups. ^1H NMR ($CDCl_3$) δ 7.85 (s, 1 H, C^2-H), 6.95 (d, J = 54 Hz, 2 H, $CONH_2$), 6.0 (s, 2 H, NCH_2O), 4.05 (m, 4 H, OCH_2CH_2O), 2.05 (s, 3 H, CH_3CO). On the basis of these data and the results of the subsequent basic hydrolysis of the compound, the structure of this component was assigned as the carboxamide derivative 2-[(5-carbamoyl-1*H*-1,2,4-triazol-1-yl)methoxy]ethyl acetate (**13**). Both **12** and **13** yielded the identical compound, 2-[(2-hydroxyethoxy)methyl]-2*H*-1,2,4-triazole-3-carboxamide (**15**), on stirring with concentrated NH_4OH (100 °C, 1 h): mp 114–115 °C; ^1H NMR (Me_2SO-d_6) δ 8.11 (d, J = 23 Hz, 2 H, NH_2), 8.14 (s, 1 H, C^2-H), 5.91 (s, 2 H, NCH_2O), 4.64 (t, J = 5 Hz, 1 H, OH) 3.5 (m, 4 H, OCH_2CH_2O). Anal. ($C_6H_{10}N_4O_3$) C, H, N. Compound **11** on similar treatment gave 2.29 g (55%) of the title compound **14**: mp 152–154 °C; ^1H NMR (Me_2SO-d_6) δ 8.75 (s, 1 H, C^5-H), 7.65 (d, J = 12 Hz, 2 H, NH_2), 5.6 (s, 2 H, NCH_2O), 3.55 (s, 9 H, OCH_2CH_2O, OH, H_2O). Anal. ($C_6H_{10}N_4O_3$) C, H, N.

2-Amino-1-[(2-hydroxyethoxy)methyl]-4(1*H*)-pyrimidinone (20). A mixture of 4.44 g (40 mmol) of isocytosine (**16**) in 440 mL of dry DMF was heated on a steam bath until the solid dissolved. The solution was cooled to room temperature and 2.0 g (48 mmol) of a 57% NaH mineral oil dispersion was added with stirring. After 30 min, the mixture was chilled to 10 °C and 8.58 g (40 mmol) of **3** was added. The suspension was stirred for 18 h at ambient temperature and then poured into 400 mL of ice and H_2O and extracted with Et_2O (3 × 500 mL). The aqueous phase was chilled and the pH adjusted to 7.0 with concentrated HCl. After evaporation in vacuo, the resultant oil was purified by column chromatography on 300 g of silica gel. Elution with successively 6, 8, and 10% MeOH in $CHCl_3$ gave on evaporation several fractions, most of which still showed contamination with isocytosine by TLC (alumina in 5% MeOH–$CHCl_3$) and contained the isomeric components. One homogeneous fraction was obtained from the 8% eluate, which on evaporation and recrystallization from acetone gave 0.5 g of lustrous white crystals of 2-[[2-[[(dimethylamino)methylene]amino]-1,4-dihydro-4-oxo-1-pyrimidinyl]methoxy]ethyl benzoate (**18**): mp 168 °C; ^1H NMR ($CDCl_3$) δ 11.5 (s, 1 H, CONH), 7.73 (m, 6 H, phenyl H's and C^6-H), 5.97 (d, J = 7 Hz, 1 H, C^5-H), 5.47 (s, 2 H, NCH_2O), 4.18 (m, 4 H, OCH_2CH_2O), 3.13 (s, 3 H, NCH_3), 3.08 (s, 3 H, NCH_3). Anal. ($C_{17}H_{20}N_4O_4$) C, H, N. This fraction on treatment with aqueous $MeNH_2$ yielded a compound identical with **20** (^1H NMR, TLC).

The solids from evaporation of the 10% eluate were extracted in a Soxhlet apparatus with CH_3CN and the evaporated extracts (2.6 g) chromatographed on alumina with 10% MeOH in $CHCl_3$ to yield 1.94 g (17%) of 2-[(2-amino-1,4-dihydro-4-oxo-1-pyrimidinyl)methoxy]ethyl benzoate (**17**). Hydrolysis of 0.5 g of **17** with 40% aqueous $MeNH_2$ gave after recrystallization from EtOH 0.23 g (72%) of the title compound **20**: mp 200–204 °C; MS, m/e 185 (M^+); ^1H NMR (Me_2SO-d_6) δ 7.42 (d, J = 7.5 Hz, 1 H, C^6-H), 6.83, (br s, 2 H, NH_2), 5.52 (d, J = 7.6 Hz, 1 H, C^5-H), 5.1 (s, 2 H, NCH_2O), 4.76 (br s, 1 H, OH), 3.5 (s, 4 H, OCH_2CH_2O). The position of the acyclic chain was established as N-1 by comparison of the UV spectrum to that of 2′,3′-*O*-isopropylideneisocytidine.[18] Anal. ($C_7H_{11}N_3O_3$) C, H, N. Later fractions from the column of the acetone extracts gave mixtures of isomers, which were not used.

5-Amino-3,6-dihydro-3-[(2-hydroxyethoxy)methyl]-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (28). After preparation of the trissilylated derivative of 8-azaguanine as in method B, the evaporated solution was dissolved in 30 mL of dry CH_3CN and 2.83 g (13.15 mmol) of **3** in 3 mL of CH_3CN was added. The reaction solution was stirred at ambient temperature for 6 days. After removal of the solvent by flash evaporation, the residual oil was heated on a steam bath with 75 mL of EtOH for 15 min,

(18) Brown, D. M.; Todd, A. R.; Varadarajan, S. *J. Chem. Soc.* **1957**, 868.

cooled, and filtered. The precipitate, which appeared to be a mixture of two isomeric components in a 2:1 ratio by ^1H NMR, was extracted in a Soxhlet apparatus with boiling MeOH, and the extracts were concentrated and cooled to give successively two crops of 0.39 and 0.29 g. The first crop was recrystallized from MeOH to yield 0.32 g of 2-[(5-amino-6,7-dihydro-7-oxo-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)methoxy]ethyl benzoate (**26**), mp 236–239 °C. Anal. ($C_{14}H_{14}N_6O_4$) C, H, N. This was identical with the second unrecrystallized crop by ^1H NMR except the former still contained 8% of the other isomeric component. ^{13}C NMR (Me_2SO-d_6) δ 165.46 (C=O), 155.81 (C^5), 155.54 (C^7), 151.8 (C^9), 133.24 (phenyl C^4), 129.39 (phenyl C^1), 128.94 and 128.56 (phenyl C-2, -3, -5 and -6), 124.16 (C^8), 74.05 (NCH_2O), 67.02 (CCH_2O), 63.38 (CH_2OC=O). The MeOH insolubles from the Soxhlet extraction step were recrystallized from 20 mL of hot Me_2SO to yield 0.457 g of a white solid, mp 263–267 °C. The ^{13}C NMR spectrum identified this as the N-1-substituted isomer 2-[(5-amino-6,7-dihydro-7-oxo-1*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-1-yl)methoxy]ethyl benzoate (**27**) and was identical with the minor (8%) component in the isomer described above. ^{13}C NMR (Me_2SO-d_6) δ 165.5 (C=O), 161.29 (C^9), 153.89 (C^5), 153.48 (C^7), 133.26 (phenyl C^4), 129.39 (phenyl C^1), 128.98 and 128.62 (phenyl C-2, -3, -5, and -6) 113.3 (C^8), 77.7 (NCH_2O), 66.96 (OCH_2C), 63.38 (CH_2OC=O). Anal. ($C_{14}H_{14}N_6O_4$) C, H, N. A second crop of **27** (0.245 g) was obtained from the mother liquor by dilution with EtOH. The overall yield of the two isomers was 32%.

A solution of 0.235 g (0.71 mmol) of **26** in 6 mL of 40% aqueous $MeNH_2$ was heated on a steam bath for 30 min. The solution was evaporated, and the residue was partitioned between Et_2O and H_2O. The aqueous layer was evaporated in vacuo and recrystallized from EtOH to give the title compound **28**, 0.128 g (80%), mp 239–241 °C. The ^1H NMR showed only a single component (Me_2SO-d_6) δ 7.00 (br s, 2 H, NH_2), 5.64 (s, 2 H, NCH_2O), 4.65 (br s, 1 H, OH), 3.53 (m, 4 H, OCH_2CH_2O). Anal. ($C_7H_{10}N_6O_3$) C, H, N.

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Potential Radiosensitizing Agents. 7. 4(5)-Iodo-5(4)-nitroimidazole Derivatives[1]

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A series of 4(5)-iodo-5(4)-nitro-1-substituted-imidazoles has been synthesized and tested for their ability to selectively radiosensitize hypoxic Chinese hamster cells (V-79) to the lethal effect of radiation. The reaction of 4(5)-iodo-5(4)-nitroimidazole with 1,2-epoxy-3-methoxypropane and ethyl α-chloroacetate produced two isomeric products in each case, which were identified by their NMR spectra. The ethyl esters were further reacted with 3-picolylamine to produce corresponding amides. The 5-iodo-4-nitroimidazole-1-*N*-(3-picolyl)acetamide on further reaction with *m*-chloroperbenzoic acid produced the corresponding *N*-oxide. These compounds were generally more toxic to V-79 cells than the 2-nitroimidazole derivatives and were found to be more effective radiosensitizers in vitro. The 5-iodo-4-nitroimidazole derivatives were more efficient as sensitizers than the 4-iodo-5-nitroimidazole derivatives, and the sensitizing efficiency of this class of agents was found to have significant correlation with their partition coefficients.

In continuation of our efforts to develop potent and effective radiosensitizers to selectively sensitize the relatively resistant hypoxic tumor cells toward ionizing radiation, we have synthesized a series of 4(5)-iodo-5(4)-nitroimidazole derivatives.[1] It has been demonstrated that the radiosensitizing efficiency of a sensitizer is directly related to its electron affinity.[2] However, 5-chloro, 5-bromo, and 5-sulfonamido analogues of 1-methyl-4-nitroimidazole have been reported recently to sensitize the hypoxic cells at concentrations 50–100 times lower than misonidazole.[3-6] Since our preliminary communication of iodonitroimidazoles,[1] Stratford et al.[7] have reported the radiosensitizing properties of two isomeric compounds of 4(5)-iodo-5(4)-nitroimidazoles. These agents have been termed as anomalous radiosensitizing compounds since under in vitro conditions they sensitize the hypoxic cells much more effectively than that predicted from their one-electron reduction potential. Corresponding 4-substituted analogues of 1-methyl-5-nitroimidazole were found to be comparatively less active as sensitizers. Rapid mix experiments have demonstrated that the dissociation of the ortho-substituted leaving group to produce the radical anion was not responsible for the observed radiosensiti-

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