Synthesis and Antiviral Activity of Carbocyclic Analogues of 2′-Deoxyribofuranosides of 2-Amino-6-substituted-purines and of 2-Amino-6-substituted-8-azapurines

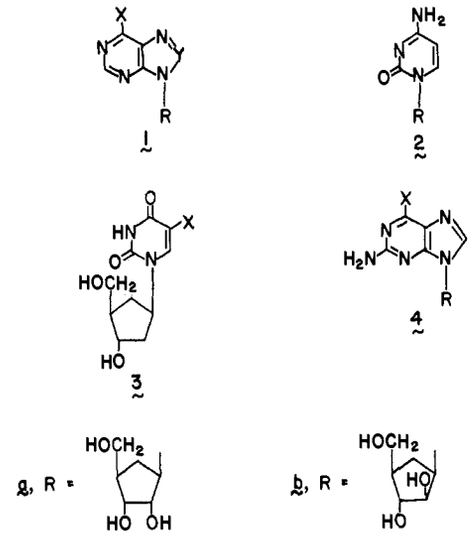
Y. Fulmer Shealy,* C. Allen O'Dell, W. M. Shannon, and Gussie Arnett

Kettering-Meyer Laboratories, Southern Research Institute, 2000 Ninth Avenue, South, Birmingham, Alabama 35255-5305.
Received December 1, 1983

(±)-(1α,2β,4α)-4-[(2,5-Diamino-6-chloro-4-pyrimidinyl)amino]-2-hydroxycyclopentanemethanol (**9**) was synthesized by beginning with 2-amino-4,6-dichloropyrimidine and (±)-(1α,2β,4α)-4-amino-2-hydroxycyclopentanemethanol, preparing the 5-[(4-chlorophenyl)azo] derivative of the resulting pyrimidine, and reducing the azo derivative. The carbocyclic analogue of 2-amino-6-chloropurine 2′-deoxyribofuranoside (**10**) was prepared from **9** and triethyl orthoformate, and the analogous 8-azapurine (**11**) was obtained by diazotizing **9**. From **10** or **11**, the carbocyclic analogues of 2′-deoxyguanosine, 2′-deoxythioguanosine, 2,6-diaminopurine 2′-deoxyribofuranoside, 2′-deoxy-8-azaguanosine, and 2,6-diamino-8-azapurine 2′-deoxyribofuranoside were prepared. All of these 2′-deoxyribofuranoside analogues were active against herpes simplex virus (types 1 and 2) replicating in cells in culture; some demonstrated potent activity.

Nucleoside analogues in which a cyclopentane ring replaces the tetrahydrofuran ring of a pentofuranosyl-substituted purine, pyrimidine, or related heterocycle have been designated carbocyclic analogues of nucleosides.[1] In this report, the trivial name of a carbocyclic analogue will be the trivial name of the corresponding true nucleoside preceded by *C*-. *C*-8-Azaadenosine[2] (**1a**, Y = N; Chart I), *C*-cytidine[3] (**2a**), *C*-1-β-arabinofuranosylcytosine[4] (*C-ara*-C, **2b**), and *C*-thymidine[5] (**3**, X = CH_3) increased survival times of mice bearing transplantable leukemias. Observations of antiviral activity by carbocyclic analogues of nucleosides include the following: several *C*-6-substituted-purine ribofuranosides[6] (**1a**; e.g., *C*-6-(methylthio)purine ribofuranoside,[1] *C*-inosine[1]), *C*-1-β-arabinofuranosyladenine[7] (**1b**, Y = CH), *C*-cytidine[8,9] (**2a**), *C-ara*-C[8,9] (**2b**), *C*-5-substituted-2′-deoxyuridines[10] (**3**; e.g., *C*-thymidine and *C*-IdUrd), *C*-2,6-diaminopurine arabinofuranoside[11] (**4b**, X = NH_2), *C*-2,6-diaminopurine ribofuranoside[12] (**4a**, X = NH_2), and *C*-3-deazaadenosine.[13] Syntheses of carbocyclic analogues of ribofuranosides[12,14] and of arabinofuranosides[11] of 2-amino-6-substituted-purines and of 2-amino-6-substituted-8-azapurines have been described previously. *C*-2,6-Diaminopurine ribofuranoside has potent antiherpetic and antivaccinia activity in vitro.[12] In this report, we describe the synthesis and antiviral evaluation of carbocyclic analogues of 2′-deoxyribofuranosides of 2-amino-6-substituted-purines and of 2-amino-6-substituted-8-azapurines (1,2,3-triazolo[4,5-*d*]pyrimidines).

Chart I



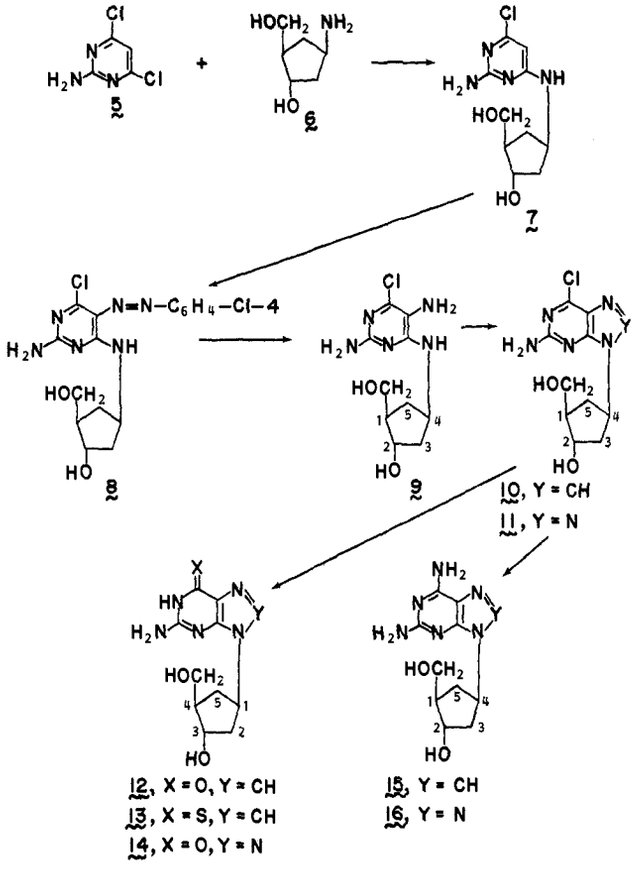
Chemistry. The synthesis of the carbocyclic analogues (**10**–**16**) of 2′-deoxyribofuranosides of 2-amino-6-substituted-purines and of 2-amino-6-substituted-8-azapurines parallels the synthesis of the analogous ribofuranoside analogues.[12,14] Treatment of 2-amino-4,6-dichloropyrimidine (**5**) with (±)-(1α,2β,4α)-4-amino-2-hydroxycyclopentanemethanol (**6**) in refluxing butanol or, for a longer period, in refluxing ethanol and in the presence of triethylamine furnished (cyclopentylamino)pyrimidine **7** (Chart II). The formation of a byproduct during the reaction of **5** and **6** depressed the yields (37–56%) of **7**. The byproduct ($C_{14}H_{17}Cl_2N_7O_2$; M^+, *m/e* 385) contained two pyrimidine nuclei for each cyclopentyl group. Coupling of 4-chlorobenzenediazonium chloride with **7** in aqueous acetic acid buffered with sodium acetate gave the azo derivative **8** in yields of 72–82%. Reduction of **8** with zinc in acetic acid[14] furnished the required 5-aminopyrimidine (**9**).

The 2-amino-6-chloropurine (**10**) was obtained by acid-catalyzed reaction of **9** with triethyl orthoformate (cf. ref 12, 14) followed by treatment of the crude product successively with 50% acetic acid and with 10% methanolic ammonia to liberate derivatized hydroxyl and amino groups. The carbocyclic analogue (**12**) of 2′-deoxyguanosine was isolated after treatment of **10** either with dilute refluxing aqueous base or with refluxing aqueous acid. The carbocyclic analogues (**13**, **15**) of 2′-deoxythioguanosine and of 2,6-diaminopurine 2′-deoxyribofuranoside

(1) Shealy, Y. F.; Clayton, J. D. *J. Am. Chem. Soc.* **1966**, *88*, 3885–3887; **1969**, *91*, 3075–3083.
(2) Shealy, Y. F.; Clayton, J. D. *J. Pharm. Sci.* **1973**, *62*, 858–859.
(3) Shealy, Y. F.; O'Dell, C. A. *J. Heterocycl. Chem.* **1976**, *13*, 1353–1354.
(4) Shealy, Y. F.; O'Dell, C. A. *J. Pharm. Sci.* **1979**, *68*, 668–670.
(5) Shealy, Y. F.; O'Dell, C. A.; Thorpe, M. C. *J. Heterocycl. Chem.* **1981**, *18*, 383–389.
(6) Bennett, L. L., Jr.; Shannon, W. M.; Allan, P. W.; Arnett, G. *Ann. N.Y. Acad. Sci.* **1975**, *255*, 342–358.
(7) Vince, R.; Daluge, S. *J. Med. Chem.* **1977**, *20*, 612–613.
(8) Shealy, Y. F.; O'Dell, C. A. U.S. Patent 4 177 348, Dec 4, 1979.
(9) Shannon, W. M.; Arnett, G.; Westbrook, L.; Shealy, Y. F.; O'Dell, C. A.; Brockman, R. W. *Antimicrob. Agents Chemother.* **1981**, *20*, 769–776.
(10) Shealy, Y. F.; O'Dell, C. A.; Shannon, W. M.; Arnett, G. *J. Med. Chem.* **1983**, *26*, 156–161.
(11) Lee, H.; Vince, R. *J. Pharm. Sci.* **1980**, *69*, 1019–1021.
(12) Shealy, Y. F.; Clayton, J. D.; Arnett, G.; Shannon, W. M. *J. Med. Chem.* **1984**, *27*, 670–674.
(13) Montgomery, J. A.; Clayton, S. J.; Thomas, H. J.; Shannon, W. M.; Arnett, G.; Bodner, A. J.; Kim, I.-K.; Cantoni, G. L.; Chiang, P. K. *J. Med. Chem.* **1982**, *25*, 626–629.
(14) Shealy, Y. F.; Clayton, J. D. *J. Pharm. Sci.* **1973**, *62*, 1432–1434.

0022-2623/84/1827-1416$01.50/0

Chart II[a]



[a] The numbering systems used in the names of compounds 9–16 (Experimental Section) and in the assignments of NMR chemical shifts (Table IV) are shown on the cyclopentyl part of structures 9–16.

were prepared by treating **10**, respectively, with sodium hydrogen sulfide in ethanol or with ammonia at 80 °C.

The carbocyclic analogue (**11**) of 2-amino-6-chloro-8-azapurine 2'-deoxyribofuranoside was prepared by treating pyrimidine **9** with sodium nitrite in aqueous acetic acid. The carbocyclic analogues (**14** and **16**) were readily isolated from reactions of **11**, respectively, with dilute aqueous base or with ammonia at 60 °C.

Biological Evaluation. The 2'-deoxyribofuranoside analogues (**10–16**) were evaluated for antiviral activity in vitro against type 1 (HSV-1) and type 2 (HSV-2) herpes simplex virus. The results are summarized in Table I. The antiviral activity of each compound is expressed as a virus rating (VR), and the potency is given as a minimum inhibitory concentration (MIC_{50}). The VR, determined by the method of Ehrlich et al.,[15] is a weighted measurement of antiviral activity that takes into account both the degree of inhibition of virus-induced cytopathogenic effects and the degree of cytototoxicity produced by the test compound.

All of these 2'-deoxyribofuranoside analogues (**10–16**) showed unequivocal activity against both HSV-1 and HSV-2. Compounds **11–16** were highly active against strain 377 of HSV-1; all were more active (VR, 4-5) and more potent (lower MIC_{50}) than was 9-β-D-arabinofuranosyladenine (*ara*-A). In these tests vs. HSV-1, the carbocyclic analogues of 2'-deoxyguanosine (**12**), 2'-deoxy-8-azaguanosine (**14**), 2,6-diaminopurine 2'-deoxyribofuranoside (**15**), and 2-amino-6-chloro-8-azapurine

(15) Ehrlich, J.; Sloan, B. J.; Miller, F. A.; Machamer, H. E. *Ann. N.Y. Acad. Sci.* **1965**, *130*, 5–16.

Table I. Evaluation of Carbocyclic Analogues of 2'-Deoxyribofuranosides of 2-Amino-6-substituted-purines and 2-Amino-6-substituted-8-azapurines against Herpes Virus in Vitro[a]

compound		type 1, strain 377		type 2, strain MS	
no.	substituent at position 6[b]	VR[c]	MIC_{50},[d] mcg/mL	VR[c]	MIC_{50},[d] mcg/mL
purines					
10	Cl	1.9	8.1	2.0	37
12	O	≥4.6	<0.3	3.7	0.8
13	S	4.9	<3.2	1.7	32
		4.4	1.8		
15	NH_2	4.5	0.6	2.9	2.4
		4.7	0.3		
8-azapurines					
11	Cl	5.0	<1.0	1.9	23
		4.5	1.6		
14	O	4.2	0.6	2.1	6.9
16	NH_2	4.0	<3.2	2.3	10
		4.4	2.0		
ara-A[e]		2.5–3.1	6.4–9.8	1.6–2.3	6.4–30

[a] Antiviral evaluations were performed with HSV-1 and HSV-2 replicating either in secondary cultures of rabbit kidney cells or in Vero cells. Vero cells were the host cells in the tests of **14** and in the tests of **10** and **12** against HSV-2. [b] Purine numbering system for 8-azapurines. [c] A virus rating (VR) equal to or greater than 1.0 indicates definite and significant antiviral activity, a VR of 0.5–0.9 indicates marginal to moderate antiviral activity, and a VR less than 0.5 usually indicates no significant antiviral activity. [d] The MIC_{50} is the concentration of the tested compound required to inhibit virus-induced cytopathogenic effects by 50%. [e] 9-β-D-Arabinofuranosyladenine was tested as a positive control; the range of values of VR and MIC_{50} came from several tests with either rabbit kidney cells or Vero cells as the host cells.

2'-deoxyribofuranoside (**11**) were the most potent compounds, the values of MIC_{50} being less than 1 mcg/mL. The carbocyclic analogue of 2'-deoxyguanosine (**12**) showed excellent activity (VR, 3.7) and high potency (MIC_{50}, 0.8 mcg/mL) against strain MS of HSV-2. Diaminopurine **15** was the next most effective compound (VR, 2.9; MIC_{50}, 2.4 mcg/mL) against HSV-2. The 8-azapurines **11**, **14**, **16** were also quite active against HSV-2. Strain 377 of HSV-1 induces thymidine kinase in host cells; i.e., it is a TK^+ strain. Earlier,[12] the carbocyclic analogue (**4**, X = NH_2) of 2,6-diaminopurine ribofuranoside was evaluated vs. the HF strain, which is a TK^- strain, and was found to be highly active.

Other carbocyclic analogues of nucleosides have been tested against either strain 377 or strain HF of HSV-1. The results of these tests, all of which were performed in these laboratories by the same methods, are summarized in Table II for comparison with the results of tests of **10–16** (Table I). These results indicate that compounds **11–16** are more active against HSV-1 than are any of the previously tested carbocyclic analogues except for *C*-thymidine and the 5-iodo-, 5-bromo-, and 5-(methylamino)-substituted *C*-2'-deoxyuridines. However, *C*-2'-deoxyguanosine (**12**) and *C*-2,6-diaminopurine 2'-deoxyribofuranoside (**15**) are more potent (lower values of MIC_{50}) against HSV-2 than are the *C*-5-substituted-2'-deoxyuridines. In addition, it should be noted that 6-aminopurine nucleosides and nucleotides may serve as substrates for adenosine deaminase and adenylate deaminase, respectively, being converted thereby to 6-oxopurine nucleosides or nucleotides. If enzymatic deamination of 2,6-diaminopurine *C*-ribofuranoside[12] or 2,6-diaminopurine *C*-arabinofuranoside[11] occurs, it would produce the corresponding guanine derivatives, which are not active against HSV-1 (strain HF).[11,12] In contrast, deamination of the highly active 2,6-diaminopurine or 2,6-diamino-8-azapurine *C*-

Table II. Antiherpetic and Antivaccinia Activity of Carbocyclic Analogues of Nucleosides[a]

compound	HSV-1 strain	HSV-1 VR	HSV-1 MIC_{50}, mcg/mL	HSV-2 (strain MS) VR	HSV-2 (strain MS) MIC_{50}, mcg/mL	vaccinia virus[b] VR	vaccinia virus[b] MIC_{50}, mcg/mL	ref
C-6-(methylthio)purine ribofuranoside	HF	2.9	59			2.9	97	6[c]
C-6-methoxypurine ribofuranoside	HF	2.0	7			1.8	21	6
C-6-(hydroxyamino)-purine ribofuranoside	HF	1.6	4.7			1.8	3.3	6
C-6-chloropurine ribofuranoside	HF	0.8	13.8			0.8	13	6
C-inosine	HF	2.1	97			1.9	191	6
C-8-azainosine	HF	1.5	298			1.2	467	6
C-*ara*-A	HF	2.2	9			1.5	9	7
		3.5	2.8			1.7	9	
C-cytidine[d]	HF	0.8	68[e]	3.2[e]	2.4[e]	0.9	57[e]	8
C-*ara*-C	HF	0.6	93[e]					8
	377[e]	1.8	<32					
C-thymidine	377	5.4	0.8	3.2	7.0			10
C-5-iodo-2′-deoxyuridine	377	6.5	0.4	2.9	32			10
		7.9	0.1	3.4	20			
C-5-bromo-2′-deoxyuridine	377	6.2	0.3	1.5	32			10
C-5-(methylamino)-2′-deoxyuridine	377	3.9–4.5	10–25	1.2	229			10
C-5-(butylamino)-2′-deoxyuridine	377	2	290	0.7	1000			10
C-2,6-diaminopurine arabinofuranoside	HF	1.5	205[e]					11
C-2,6-diaminopurine ribofuranoside	HF	4.6	0.3			3.8	2.3	12
C-2,6-diamino-8-azapurine ribofuranoside	HF	1.4	100					12
C-2-amino-6-(methylamino)purine ribofuranoside	HF	1.3	320					12
C-2-amino-6-chloropurine-ribofuranoside	HF	1.7	10	1.2	20			12
C-2-amino-6-chloro-8-azapurine ribofuranoside	HF	0.9	81	0.6				12
C-3-deazaadenosine	377	1.1	238			2.8	1.9	13
2-amino-6-substituted-purine *C*-2′-deoxyribofuranosides		see Table I						
2-amino-6-substituted-8-azapurine *C*-2′-deoxyribofuranosides		see Table I						

[a] See footnotes *c* and *d* of Table I. [b] Lederle Chorioallantoic strain. [c] Values of VR and of MIC_{50} for the first six compounds are averages of two or more determinations. Most of the values of VR for these compounds were taken from ref 6. [d] Highly active in vitro, but not in vivo, against influenza virus (ref 9). [e] Not previously reported.

2′-deoxyribofuranosides (**15** or **16**) would produce compounds (**12** or **14**) that are also highly active against HSV-1 and HSV-2.

Compounds **10–16** were tested for antineoplastic activity against KB cells in culture and against L1210 leukemia in mice (Table III). The 2-amino-6-chloro-8-azapurine (**11**) was cytotoxic (ED_{50}, 10 mcg/mL). At the doses administered (q.d. 1–9), no significant increases in lifespan were observed in the tests against L1210 leukemia. In the latter tests, compounds **11** and **15** were toxic to leukemia-bearing mice at 200 mg/kg per day.

Experimental Section[17]

General Methods. Decomposition and melting temperatures (mp) were determined in capillary tubes heated in a Mel-Temp apparatus. Ultraviolet spectra (UV) were recorded with a Cary Model 17 spectrophotometer and absorption maxima are reported in nanometers; sh = shoulder. Solutions for ultraviolet spectral determinations were prepared by diluting a 5-mL aliquot of a water or ethanol solution to 50 mL with 0.1 N hydrochloric acid, phosphate buffer (pH 7), or 0.1 N sodium hydroxide. Absorption maxima of these solutions are reported as being determined at pH 1, 7, or 13, respectively. Infrared spectra (IR) were recorded with Perkin-Elmer Model 521 or 621 spectrophotometer from samples in pressed potassium bromide disks: vs = very strong, sh = shoulder. Mass spectral data (MS) were taken from low-

Table III. Tests of Componds **10–16** for Antineoplastic Activity

no.	cytotoxicity[b] ED_{50}, mcg/mL	L1210 in vivo[a], q.d. 1–9 dose, mg/kg	wt-change diff,[c] g	T/C[d], %
10	62	200	−2.2	106
		100	−1.0	106
11	10	200	−4.4	78t
		100	−3.4	100
		50	−1.5	105
		25	−1.0	118
12	95	100	−3.7	96
13	>100	200	+0.5	108
		100	−0.2	91
14	>100	200	−3.2	104
		100	−2.4	100
15	>100	200	−5.5	79t
		50	−2.8	104
16	>100	200	−1.1	92
		100	−0.7	96

[a] Tests were performed in accordance with the protocols of the National Cancer Institute.[16] Mice were inoculated intraperitoneally with 10^5 L1210 leukemia cells on day 0. Solutions or suspensions of the compounds were administered intraperitoneally. T = treated mice; C = untreated, leukemic control mice. [b] Cytotoxicity to KB cells (derived from a human epidermoid carcinoma) in culture was determined by the protocol of the National Cancer Institute.[16] [c] The weight-change difference is the average change in weight of the treated mice minus the average change in weight of the control mice, both values being determined on day 5. [d] A dose is considered to be toxic (t) if T/C < 85% or the weight-change difference is greater in magnitude than −4 g.

(16) Geran, R. I.; Greenberg, N. H.; Macdonald, M. M; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 1–103.

(17) In accordance with *Chemical Abstracts* nomenclature, compounds **6–11**, **15**, and **16** are named as cyclopentanemethanols, and substituents on the cyclopentane ring are designated 1α,2β,4α. Because compounds **12–14** have oxo or thioxo substituents on the heterocyclic ring, they are named as cyclopentylpurines or -1,2,3-triazolo[4,5-*d*]pyrimidines; therefore, substituents on the cyclopentane ring are designated 1α,3β,4α.

Table IV. Proton NMR Data[a]

compd	δ in ppm (multiplicity[b], no. of protons, position of protons[c])
9	1.14 (m, 1 H, 5), 1.65 (m, 1 H, 3), 1.83 (m, 2 H, 1 and 5), 2.19 (m, 1 H, 3), 3.39 (m, 2 H, CH_2OH), 3.9 (m, 3 H, 2 + 2OH), 4.48 (s, 1 H, 4), 4.53 (s, 2 H, NH_2), 5.56 (s, 2 H, NH_2); 6.31 (d, 1 H, NH)
10	1.65 (m, 1 H, 5), 2.02 (m, 2 H, 1 and 3), 2.17 (m, 1 H, 3), 2.33 (m, 1 H, 5), 3.47 (m, 2 H, CH_2OH), 4.08 (m, 1 H, 2), 4.67 (m, 1 H, CH_2OH), 4.81 (m, 1 H, sec OH), 4.92, (m, 1 H, 4), 6.88 (s, NH_2), 8.25 (s, purine CH)
12	1.58 (m, 1 H, 5), 1.97 (m, 2 H, 2 and 4), 2.10 (m, 1 H, 2), 2.29 (m, 1 H, 5), 3.47 (m, 2 H, CH_2OH), 4.06 (m, 1 H, 3), 4.63 (t, 1 H, CH_2OH), 4.74 (d, 1 H, secOH) 4.81 (m, 1 H, 1), 6.40 (s, 2 H, NH_2), 7.79 (s, purine CH), 10.53 (s, NH)
13	1.61 (m, 1 H, 5), 1.97 (m, 2 H, 2 and 4), 2.12 (m, 1 H, 2), 2.29 (m, 1 H, 5), 3.47 (m, 2 H, CH_2OH), 4.06 (m, 1 H, 3), 4.63 (t, 1 H, CH_2OH), 4.76 (d, 1 H, *sec* OH), 4.82 (m, 1 H, 1), 6.74 (s, 2 H, NH_2), 8.01 (s, purine CH), 11.85 (s, 1 H, NH)
15	1.63 (m, 1 H, 5), 1.97 (m, 2 H, 1 and 3), 2.13 (m, 1 H, 3), 2.28 (m, 1 H, 5), 3.48 (m, 2 H, CH_2OH), 4.06 (m, 1 H, 2), 4.64 (t, 1 H, CH_2OH), 4.74 (d, 1 H, sec OH), 4.85 (m, 1 H, 4), 5.73 (s, 2 H, NH_2), 6.62 (s, 2 H, NH_2), 7.81 (s, purine CH)
16	1.86 (m, 1 H, 5), 2.02 (m, 2 H, 1 and 3), 2.3 (m, 2 H, 3 and 5), 3.48 (m, 2 H, CH_2OH), 4.13 (m, 1 H, 2), 4.60 (t, 1 H, CH_2OH), 4.78 (d, 1 H, sec OH), 5.08 (m, 1 H, 4), 6.33 (s, 2 H, NH_2), 7.5 (m, 2 H, NH_2)

[a] ^1H NMR spectra were recorded from Me_2SO-d_6 solutions (concentration, 5 mg/mL) at 300.64 MHz (Nicolet 300 NB NMR spectrometer) and with Me_4Si as the internal standard. [b] m = multiplet, s = singlet, d = doublet, t = triplet. [c] The positions on the cyclopentane ring are defined by the numbers on structures 9–16; note that the numbering system for 12 and 13 differs from that of the other compounds in this table.

resolution, electron-impact spectra determined at 70 eV with a Varian/MAT 311A spectrometer unless indicated otherwise. The peaks listed are those arising from the molecular ion (M), those attributable to the loss of certain fragments (M minus a fragment), and some other prominent peaks. Fragments containing the complete purine or pyrimidine moiety may be designated P plus an atom or group. Thin-layer chromatography (TLC) was performed on plates of silica gel, and developed plates were examined by UV light (254 nm).[18]

Reaction of 5 and 6. A. (±)-(1α,2β,4α)-4-[(2-Amino-6-chloro-4-pyrimidinyl)amino]-2-hydroxycyclopentanemethanol (7). A solution of 6.56 g (40 mmol) of 2-amino-4,6-dichloropyrimidine (**5**), 2.60 g (19.8 mmol) of (±)-(1α,2β,4α)-4-amino-2-hydroxycyclopentanemethanol[19,20] (**6**), 4.03 g of triethylamine (40 mmol), and 410 mL of 1-butanol was boiled under reflux for 72 h. Unreacted **5** was recovered in two portions (2.7 and 0.9 g, mp 216–219 °C) as the reaction mixture was being concentrated in vacuo. After the second portion had been separated by filtration, the filtrate was concentrated to a dark syrup, which was concentrated further by evaporating several portions of methanol from it. The residue was mixed thoroughly with 5:1 chloroform–methanol (10 mL), and a pale tan solid that formed was collected by filtration, washed with the solvent mixture, and dried in vacuo at 78 °C: yield, 2.63 g (51%); mp 168–170 °C. This material was used in the subsequent step since the UV and IR spectra showed it to be comparable to the analytical sample. Additional crude **7** was isolated by chromatography on silica gel.

The crude product, after removal of unreacted **5**, of a reaction of **5** and **6** during 10 days in refluxing ethanol was chromatographed on a column of silica gel with chloroform–methanol (9:1) as eluting solvent. Concentration of the product-containing fractions, located by TLC, left a pale yellow syrup that was crystallized from water and dried in vacuo: yield, 46%; mp 171–174 °C, sintering at 168–171 °C (inserted at 70 °C, 3 °C/min); UV max 300 nm (ε 4300), 285 (sh), 274 (8800), 237 (12 500), 214 (18 700) at pH 1; 287 (9900), 238 (11 000), 212 (26 400) at pH 7; 286 (9900), 238 (10 800) at pH 13; MS (direct-probe temperature, 280 °C), *m/e* 258 (M), 241 (M – OH), 227 (M – CH_2OH), 209 (M – CH_2OH – H_2O), 171 (PNH + C_2H_4), 144 (PNH + H); IR (strong and medium-strong bands, 1800–700-cm^{-1} region) 1645, 1575 (vs), 1475, 1365, 1160, 1015, 970, 795. Anal. ($C_{10}H_{15}ClN_4O_2$) C, H, N.

B. Byproduct. 2-Amino-4,6-dichloropyrimidine (31.54 g) was treated with **6** (12.5 g) according to the first procedure described above. After two portions of **5** (10.9 and 3.63 g) had been removed, the filtrate was concentrated to dryness. The residue was mixed with 5:1 chloroform–methanol (50 mL), and additional **5** (2.36 g) was separated by filtration. The filtrate was seeded with **7**, refrigerated, and filtered to collect **7**: weight, 9.71 g (39% yield); mp 168–170 °C. The filtrate was concentrated to dryness, the residue was stirred overnight with 3:1 chloroform–methanol (40 mL), the solid phase (8.61 g) was separated by filtration and stirred for 2 h with 5:1 chloroform–methanol (100 mL) and then with water (50 mL), and the resulting solid (6.3 g) was recrystallized from ethanol–water: weight, 4.76 g; mp 155 °C, resolidified, remelted at 215–218 °C dec; UV max 330 nm (sh), 323 (ε 20 600), 260 (slight sh), 244 (24 600), 237 (sh) at pH 1; 304 (19 100), 260 (sh), 245 (sh, 26 500), 229 (31 500) at pH 7; MS (direct-probe temperature, 300 °C), *m/e* 385 (M for $C_{14}H_{17}Cl_2N_7O_2$), 368 (M – OH), 354 (M – CH_2OH), 327, 295 ($C_8H_6Cl_2N_7$ + C_2H_4), 271 ($C_8H_6Cl_2N_7$ + H); IR (strong and medium-strong abands, 1800–800-cm^{-1} region) 1645, 1610, 1585 (vs), 1555, 1460, 1380, 1330, 1235, 1020, 975, 915, 805. Anal. ($C_{14}H_{17}Cl_2N_7O_2 \cdot H_2O$) C, H, N.

(±)-(1α,2β,4α)-4-[[2-Amino-6-chloro-5-[(4-chlorophenyl)azo]-4-pyrimidinyl]amino]-2-hydroxycyclopentanemethanol (8). A cold solution (at 0–5 °C) of 4-chlorobenzenediazonium chloride (prepared from 421 mg (3.3 mmol) of 4-chloroaniline, 0.92 mL of 12 N hydrochloric acid, 3.2 mL of water, and 254 mg of sodium nitrite (3.68 mmol) in 3 mL of water) was added dropwise during 0.5 h to a solution of 776 mg (3 mmol) of **7**, 6.0 g of sodium acetate trihydrate, 15 mL of acetic acid, and 15 mL of water. The mixture was stirred overnight at room temperature, and a yellow crystalline precipitate was collected by filtration, washed with cold water, and dried in vacuo at 56 °C: yield, 1.01 g (85%); mp 236–240 °C dec. The product was recrystallized from a mixture of dimethylformamide (11 mL) and water (30 mL), washed with cold water, and dried in vacuo at 78 °C: weight, 980 mg (97% recovery); mp 238–240 °C dec (inserted at 100 °C, 3 °C/min); UV max 372 nm (ε 26 400), 280 (8000), 239 (18 200) at pH 1; MS (direct-probe temperature, 50 °C), *m/e* 396 (M), 378 (M – H_2O), 361 (M – Cl), 270 (M – HNC_6H_4Cl); IR (strong and medium-strong bands, 1800–600-cm^{-1} region) 1630, 1565 (vs), 1475, 1460, 1365, 1080, 1045, 825, 780. Anal. ($C_{16}H_{18}Cl_2N_6O$) C, H, N.

(±)-(1α,2β,4α)-4-[(2,5-Diamino-6-chloro-4-pyrimidinyl)amino]-2-hydroxycyclopentanemethanol (9). A vigorously stirred mixture of 4.00 g of **8**, 88 mL of ethanol, 88 mL of water, and 8.8 mL of acetic acid was heated to 70 °C under an atmosphere of nitrogen. Zinc dust (8.2 g) was added in small portions during 40 min, and the mixture was stirred at 70 °C for 1.5 h. The solid phase was separated by filtration, the filtrate (plus washings) was concentrated in vacuo to about one-fifth of the original volume, the solution was extracted with ether to remove

(18) Analytical TLC was performed with plates of silica gel GF. [Precoated thin-layer chromatography plates (fluorescent), 250 μm in thickness, were purchased from Analtech Inc., Blue Hen Industrial Park, Newark, DE 19711.] Preparative TLC was performed with precoated plates of silica gel (Whatman PLK5F, 20 × 20 cm, 1 mm in thickness, Whatman Inc., Clifton, NJ 07014).

(19) Shealy, Y. F.; O'Dell, C. A. *Tetrahedron Lett.* **1969**, 2231–2234.

(20) O'Dell, C. A.; Shealy, Y. F. "Nucleic Acid Chemistry. Improved and New Synthetic Procedures, Methods, and Techniques"; Townsend, L. B., Tipson, R. S., Ed.; Wiley: New York, 1978; Part 1, pp 161–167.

4-chloroaniline, the pH of the aqueous layer was raised to 5.9 with 2 N sodium hydroxide, and the mixture was refrigerated. Crystalline **9** was collected by filtration, washed with cold water, and dried in vacuo at 78 °C: yield, 1.30 g (47%); mp 164–167 °C dec; UV max 298 nm (ε 7800), 237 (15 800), 210 (16 000) at pH 1; 304 (9000), 240 (sh), 225 (sh), 205 (19 400) at pH 7; 304 (9000), 240 (sh), 225 (sh) at pH 13; MS (direct-probe temperature, 20 °C), *m/e* 273 (M), 256 (M – OH), 255 (M – H_2O), 224, 198, 196, 186 (PNH + C_2H_4), 170, 159 (PNH + H). Anal. ($C_{10}H_{16}ClN_5O_2$) C, H, N. A lower melting form (mp 103–106 °C) was also obtained. A mixture of the two forms melted at the higher temperature.

(1α,2β,4α)-(±)-4-(2-Amino-6-chloro-9*H*-purin-9-yl)-2-hydroxycyclopentanemethanol (10). Freshly distilled triethyl orthoformate (3 mL) was added to a solution, chilled to 0 °C, of 400 mg of pyrimidine **9** in 3 mL of dimethylacetamide. Concentrated hydrochloric acid (0.15 mL) was added to the cold solution, and the resulting mixture was stirred in a stoppered flask at room temperature for 24 h. The mixture, containing a solid, was concentrated in vacuo, (oil pump, 30 °C) to a red solid. A solution of the solid in 20 mL of 50% acetic acid was stirred at room temperature for 4 h, volatile components were evaporated in vacuo, and several portions of ethanol were evaporated from the residue. A solution of the partially solid residue in ammonia–methanol (10% NH_3) was stirred at room temperature for 4 h and then concentrated in vacuo to a red gum (460 mg). A methanol solution of the residue was divided into three portions and applied to three preparative TLC plates of silica gel. The product bands were scraped from the plates, combined, and extracted (4 h) in a Soxhlet extractor with ethanol, and the filtered extract was concentrated in vacuo to a solid (375 mg). A solution of the residue in hot water (3 mL) was cooled, filtered to remove a slight amorphous precipitate, and diluted with an equal volume of acetonitrile. The solution was concentrated, and a crystalline precipitate was collected by filtration, washed with acetonitrile–water (1:1), and dried in vacuo at 56 °C for 2 h: weight, 94 mg; mp 136–140 °C. Since a higher melting crystalline form had been obtained earlier from ethanol, this specimen was dissolved in ethanol and the solution was concentrated in vacuo to a white crystalline solid: mp 163–166 °C; TLC, 1 spot (3:1 chloroform–methanol); UV max 314 nm (ε 7000), 242 (5600), 219 (27 300) at pH 1; 307 (7600), 246 (4600), 223 (27 900) at pH 7; 307 (7400), 246 (4500), 223 (27 100) at pH 13; MS (direct-probe temperature, 80 °C), *m/e* 283 (M), 266 (M – OH), 253, 252 (M – CH_2OH), 248 (M – Cl), 236 (M – OH – CH_2OH + H), 196 (P + C_2H_4), 170 (P + 2 H), 169 (P + H). Anal. ($C_{11}H_{14}ClN_5O_2 \cdot 0.25H_2O$) C, H, N.

Concentration to dryness of the acetonitrile–water filtrate from the 94-mg specimen afforded 253 mg of a peach-colored solid; TLC revealed two slight impurities, but this material was entirely suitable for conversion to other 2-amino-6-substituted-purine derivatives, e.g., **12**. Specimens of **10** suitable for conversion to derivatives were obtained without resorting to preparative TLC as follows. The residue from the methanol–ammonia solution was dissolved in water, the hydroxide form of an anion-exchange resin was added until the pH of the stirred solution stabilized at about 11, a small amount of activated charcoal was added, the mixture was stirred and filtered, and the filtrate (plus water washings) was concentrated to dryness; yields 47–50%; TLC, **10** as a major spot plus trace amounts of one or two impurities.

(1α,2β,4α)-(±)-4-(5-Amino-7-chloro-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)-2-hydroxycyclopentanemethanol (11). A solution of 587 mg (8.51 mmol) of sodium nitrite in 13 mL of water was added dropwise to a cold (0 °C) solution of 2.00 g of pyrimidine **9** in 20 mL of water and 6.7 mL of acetic acid. The mixture was stirred for 1 h at 0 °C, and the precipitated product was collected by filtration, washed with cold water, and dried in vacuo at room temperature: weight, 1.30 g; mp 140–148 °C dec. A solution of the product in warm ethanol (32 mL) was treated with activated charcoal, and the warm filtrate (plus washings) was diluted with cyclohexane (80 mL). Compound **11**, which crystallized from the cool solution, was separated by filtration after the mixture had been kept at 5 °C for 4 h, washed with cyclohexane–ethanol (4:1), and dried in vacuo: yield, 1.125 g (54%); mp 152–153 °C dec (inserted at 80 °C, 3 °C/min); TLC, 1 spot (2:1 chloroform–methanol); UV max 316 nm, 245–260 (infl), 226 at pH 1 and in ethanol; 246 and 286 at pH 13; MS[21] (direct-probe temperature, 280 °C), *m/e* 284 (M), 267 (M – OH), 254, 239, 237 (M – OH – CH_2OH + H), 225, 211, 209 (M – 75), 197 (P + C_2H_4), 171 (P + 2H), 170 (P + H), 169 (P). Anal. ($C_{10}H_{13}ClN_6O_2$) C, H, N.

(±)-2-Amino-1,9-dihydro-9-[(1α,3β,4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-6*H*-purin-6-one (12). A solution of 523 mg of the 2-amino-6-chloropurine (**10**) in 25 mL of 0.5 N NaOH was boiled under reflux for 2 h, the pH of the solution was adjusted to 7, and the mixture was concentrated to dryness in vacuo. A water solution of the residue was applied to a column containing 80 mL of Sephadex G-10. The column was eluted with water (0.4 mL/min), and product-containing fractions (located with a UV monitor and by TLC) were combined and concentrated to dryness in vacuo. The residual solid was triturated with ether, collected by filtration, washed with ether, and dried in vacuo; yield, 160 mg (33%). A specimen was recrystallized from water: mp 248–250 °C dec (inserted at 180 °C, 3 °C/min); TLC, 1 spot (40 or 80 mcg applied, butanol–acetic acid–H_2O (5:2:3) or 2-propanol–1 M ammonium acetate (7:3) as developing solvent); UV max 279 nm (ε 8400) and 254 (12 500) at pH 1; 270 (sh, 10 200) and 253 (13 800) at pH 7; 268 (11 800) and 257 (sh, 11 000) at pH 13; MS (direct-probe temperature, 160 °C), *m/e* 265 (M), 248 (M – OH), 235, 234 (M – CH_2OH), 190 (M – 75), 178 (P + C_2H_4), 152 (P + 2H), 151 (P + H); IR 1725 (sh), 1680 (vs), 1625 (vs), 1600 (sh), 1565, 1535, 1480, 1405, 1355, 1160, 1020, 775, 680, other broad or weak bands and shoulders. Anal. ($C_{11}H_{15}N_5O_3 \cdot 1.5H_2O$) C, H, N.

Compound **12** was also isolated by diluting a reaction mixture with water and pouring the filtered solution onto a column of the hydroxide form of an anion-exchange resin (Dowex 1-X8), washing the column with water, and eluting **12** with 5% acetic acid. The glassy solid remaining after the eluate had been concentrated in vacuo was triturated with ethanol, the resulting solid was stirred with water, the mixture was filtered to remove a small amount of insoluble material, the filtrate was lyophilized, and ethanol was evaporated from the residue; yield, 30%. Compound **12** was also obtained by hydrolyzing **10** in refluxing hydrochloric acid (1 N, 3.5 h).

(±)-2-Amino-1,9-dihydro-9-[(1α,3β,4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-6*H*-purine-6-thione (13). Hydrogen sulfide gas was introduced during 1 h into a solution, kept in an ice bath, of 2.05 g of sodium methoxide in 50 mL of anhydrous ethanol. To the resulting solution, under an acetone–dry ice condenser, was added a solution of 615 mg of **10** in 10 mL of anhydrous ethanol. The solution was boiled under reflux for 2 h and then concentrated to dryness in vacuo. A solution of the residue in water (12 mL) was acidified to pH 4.3 with 6 N HCl. A white solid precipitated. The mixture was chilled, and the solid was collected by filtration, washed with water, and dried in vacuo at 56 °C: yield, 442 mg (72%); mp 295–298 °C dec (darkening above 250 °C, capillary inserted at 100 °C, 3 °C/min). Recrystallization of the product from water (35 mL) furnished 399 mg (65% yield, dried in vacuo at 78 °C): mp 295–299 °C dec (inserted at 200 °C, 3 °C/min); TLC, 1 spot (40 or 80 mcg, 3:1 chloroform–methanol as developing solvent); UV max 350 nm (ε 21 600), 263 (7400), 227 (sh), 208 (25 700) at pH 1; 342 (25 400), 264 (7500), 231 (17 400), 208 (21 700) at pH 7; 318 (20 200), 271 (7000), 252 (11 500), 222 (16 300) at pH 13; MS[21] (direct-probe temperature, 400 °C), *m/e* 281 (M), 264 (M – OH), 251, 250 (M – CH_2OH), 234 (M – OH – CH_2OH + H), 214, 194 (P + C_2H_4), 168 (P + 2H), 167 (P + H); IR (strong bands, 1800–600-cm^{-1} region) 1640, 1600 (vs), 1575 (vs), 1555, 1385, 1355, 1175, 1020, 940, 895, 650. Anal. ($C_{11}H_{15}N_5O_2S$) C, H, N.

(±)-5-Amino-3,6-dihydro-3-[(1α,3β,4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (14). A mixture of 65 mg of 11 and 3 mL of 0.25 N NaOH was boiled under reflux for 3.5 h. The solution was treated with a small amount of activated charcoal and filtered, and the filtrate was acidified to pH 3.8 with 6 N HCl to precipitate 14. After the mixture had been chilled, the white crystalline solid was collected by filtration, washed with water, and dried in vacuo at 78 °C: yield, 60 mg (98%); mp 196-199 °C (inserted at 100 °C,

(21) This mass spectrum was determined with a Hitachi-Perkin-Elmer RMU-6D double-focusing mass spectrometer.

3 °C/min); TLC, 1 spot (40 or 80 mcg, 5:2:3 butanol–acetic acid–water or 3:1 chloroform–methanol as developing solvent); UV max 270 nm (sh, ϵ 8200) and 253 (11 800) at pH 1; 270 (8700) and 253 (11 900) at pH 7; 278 (11 600) and 255 (sh) at pH 13; MS (direct-probe temperature, 250 °C), *m/e* 266 (M), 249 (M – OH), 236, 235 (M – CH_2OH), 221, 219 (M – OH – CH_2OH + H), 209, 207, 193, 191, 179 (P + C_2H_4), 165, 163, 153 (P + 2H), 152 (P + H), 151 (P); IR (strong bands, 1800–600-cm^{-1} region) 1700 (vs), 1650 (vs), 1595, 1575 (vs), 1540, 1385, 1295, 1040. Anal. ($C_{10}H_{14}N_6O_3$) C, H, N.

(±)-(1α,2β,4α)-4-(2,6-Diamino-9*H*-purin-9-yl)-2-hydroxycyclopentanemethanol (15). A solution of 500 mg of **10** in 20 mL of liquid ammonia was heated for 18 h at 80 °C in a stainless steel bomb having a glass liner. The bomb was chilled and opened, the ammonia was allowed to evaporate, the residue was dissolved in water, and the hot solution was treated with activated charcoal and filtered. The filtrate, combined with several hot water washes, was concentrated to dryness in vacuo, and the residual solid was recrystallized from water and dried in vacuo at 78 °C: yield, 298 mg (64%); mp 242–246 °C; TLC, 1 spot (40 or 80 mcg, 3:1 chloroform–methanol or 5:2:3 butanol–acetic acid–water as developing solvent); UV max 292 nm (ϵ 9800), 253 (9500), 218 (22 600) at pH 1; 280 (10 500), 255 (8200), 250 (sh), 216 (29 000) at pH 7; 280 (10 500), 255 (ϵ 8200), 250 (sh) at pH 13; MS (direct-probe temperature, 150 °C), *m/e* 264 (M), 247 (M – OH), 234, 233 (M – CH_2OH), 217 (M – OH – CH_2OH + H), 177 (P + C_2H_4), 151 (P + 2H), 150 (P + H); IR (strong bands, 1800–600-cm^{-1} region) 1665, 1630, 1590 (vs), 1475, 1455, 1405, 1030, 785, 635. Anal. ($C_{11}H_{16}N_6O_2$) C, H, N.

(1α,2β,4α)-(±)-4-(5,7-Diamino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)-2-hydroxycyclopentanemethanol (16). A solution of 428 mg of **11** in 25 mL of liquid ammonia was heated for 18 h at 60 °C in a stainless steel bomb containing a glass liner. The bomb was chilled and opened, and ammonia was evaporated with a current of nitrogen. The residual solid was triturated with water (12 mL), collected by filtration, washed with cold water, and dried in vacuo at 56 °C: yield, 359 mg (90%); mp 245–247 °C dec (inserted at 200 °C, 3 °C/min). A second crop of **16** was isolated in the same way from the concentrated filtrate: weight 31 mg (total yield, 98%); mp 244–246 °C dec (inserted at 200 °C, 3 °C/min). A solution of the two portions in boiling water (35 mL) was treated with a small amount of activated charcoal, concentrated to about one-half of the original volume, and refrigerated. White crystalline **16** was separated by filtration, washed with cold water, and dried in vacuo at 56 °C: weight, 350 mg (92% recovery); mp 246–248 °C dec (inserted at 200 °C, 3 °C/min); TLC, 1 spot (40 mcg, 2:1 chloroform–methanol as developing solvent); UV max 285 nm (ϵ 7700), 255 (9800), 214 (26 000) at pH 1; 287 (10 600), 258 (5800), 223 (25 800) at pH 7; 287 (10 700), 258 (5800), 223 (26 000) at pH 13; MS (direct-probe temperature, 220 °C), *m/e* 265 (M), 248 (M – OH), 235, 234 (M – CH_2OH), 218 (M – OH – CH_2OH + H), 206 (M – CH_2OH – N_2), 190 (M – 75), 178 (P + C_2H_4), 152 (P + 2 H), 151 (P + H); IR (strong bands, 1800–600-cm^{-1} region) 1670, 1630, 1600, 1585 (vs), 1480 (vs), 1420, 1020, 785, 670. Anal. ($C_{10}H_{15}N_7O_2$) C, H, N.

Antiviral Evaluations in Vitro. The methods and procedures used to evaluate compounds **10–16** for antiviral activity in vitro have been described previously.[9,22] These compounds were tested for inhibition of cytopathogenic effects produced by strain 377 of HSV-1 or strain MS of HSV-2 replicating in secondary cultures of rabbit kidney cells or Vero cells.

Acknowledgment. We are indebted to Dr. William C. Coburn, Jr., Mary B. Emory, Marion C. Kirk, Christine G. Richards, and Martha C. Thorpe of this Institute for spectroscopic determinations and elemental analyses and to Beverly Washburn for technical assistance in the performance of antiviral evaluations. The chemical and viral aspects of this investigation were supported by Grant RO1-CA25701 from the National Institutes of Health and by contracts with the National Cancer Institue (NO1-CM-43762, NIH-C-73-3712, NIH-71-2021). Testing against leukemia L1210 in vivo was carried out under supervision of Drs. F. M. Schabel, Jr., and W. R. Laster, Jr., and tests for cytotoxicity were performed under the supervision of Dr. L. J. Wilkoff. The tests for antineoplastic activity were supported by contracts with the National Cancer Institute (NO1-CM-43756, NO1-CM-12098).

Registry No. **5**, 56-05-3; **6**, 23722-89-6; **7**, 91296-08-1; **8**, 91296-09-2; **9**, 91296-10-5; **10**, 91296-11-6; **11**, 91311-05-6; **12**, 91296-12-7; **13**, 91296-13-8; **14**, 91296-14-9; **15**, 91296-15-0; **16**, 91296-16-1; 4-chlorobenzenediazonium chloride, 2028-74-2; triethyl orthoformate, 122-51-0.

(22) Shannon, W. M.; Shortnacy, A.; Arnett, G.; Montgomery, J. A. *J. Med. Chem.* **1974**, *17*, 361–363.