4.36–4.39 (m, 1, glu α CH), 2.33 (t, 2, glu γ CH_2, J = 7.37 Hz), 1.85–2.16 (m, 2, glu β CH_2); FAB/MS, 473 (M + 1). Anal. ($C_{21}H_{21}N_6O_5Cl·CF_3COOH·H_2O$) C, H, N. The presence of CF_3COOH of solvation was confirmed by ^{19}F NMR.

6-[(*p*-Carboxyanilino)methyl]-5-chloro-2,4-diaminoquinazoline, *tert*-Butyl Ester, 13. 5-Chloro-6-cyano-2,4-diaminoquinazoline (6.5 g, 0.029 mol) was dissolved in 70% AcOH (300 mL). After the reaction mixture was warmed until complete dissolution occurred, *tert*-butyl *p*-aminobenzoate (6.6 g, 0.034 mol) and 2 g of Raney Ni slurry (50% catalyst by volume) were added. The reaction mixture was shaken at room temperature in a low-pressure Parr hydrogenator until H_2 uptake ceased. After 3 g of charcoal was added, the reaction mixture was filtered through a Celite bed. Evaporation of the solvent under reduced pressure gave the crude product, which was dissolved in $CHCl_3$ and applied to a silica gel column. The product was isolated by gradient elution ($CHCl_3$–MeOH, 99:1, to $CHCl_3$–MeOH, 92:8). Pooled TLC-homogeneous fractions were evaporated to obtain 5.3 g (45.7%) of bright-yellow powder: mp 236–237 °C; TLC ($CHCl_3$–MeOH, 9:1); HPLC; NMR (90 MHz, Me_2SO-d_6) δ 7.55 (d, 2, 2′, 6′, J_o = 9 Hz), 7.37 (d, 1, H_8, J_o = 8 Hz + br s, 4, NH_2), 7.08 (d, 1, H_7, J_o = 8.0 Hz), 6.51 (d, 2, 3′, 5′, J_o = 9 Hz), 6.05 (br s, 1, CH_2NH), 4.30 (br s, 2, CH_2N), 1.42 (s, 9, $C(CH_3)_3$). Anal. ($C_{20}H_{22}N_5O_2Cl$) C, H, N, Cl.

6-[(*p*-Carboxyanilino)methyl]-5-chloro-2,4-diaminoquinazoline, 14. Compound **13** (2 g, 5 mmol) was dissolved in CF_3COOH (13 mL) and $CHCl_3$ (27 mL). After the reaction mixture was stirred at room temperature for 3 h, 200 mL of cold Et_2O was added to it. The precipitate was filtered and washed with cold Et_2O. The crude product was dissolved in 500 mL of H_2O, and the resulting suspension was basified to pH 12 with 30% NH_4OH. The solution was slowly brought to pH 6 with concentrated HCl and then 2 N HCl. The precipitate was separated by filtration and washed with H_2O and then Et_2O. After drying in high vacuum at 100 °C, 1.6 g (93%) of yellow crystalline powder was obtained: mp 228–235 °C (lit.[12] mp 232–235 °C); TLC ($CHCl_3$–MeOH, 9:1); HPLC; NMR (400 MHz, Me_2SO-d_6) δ 7.65 (d, 2, 2′, 6′, J_o = 8.71 Hz), 7.43 (d, 1, H_8, J_o = 8.69 Hz + br s, 2, NH_2), 7.13 (d, 1, H_7, J_o = 8.69 Hz), 7.02 (t, 1, CH_2NH, J = 5.69 Hz), 6.57 (d, 2, 3′, 5′, J_o = 8.71 Hz), 6.19 (br s, 2, NH_2), 4.36 (d, 2, CH_2N, J = 5.54 Hz). Anal. ($C_{16}H_{14}N_5O_2Cl·1.5H_2O$) C, H, N.

Di-*tert*-butyl 5-Chloro-5,8-dideazaaminopterin, 15. To a solution of **14**·1.5H_2O (0.25 g, 0.67 mmol) in dry DMF (20 mL) were added Et_3N (0.205 g, 2 mmol) in 2 mL of DMF and diethyl phosphorocyanidate (0.33 g, 2 mmol) in 2 mL of DMF. After 30 min of heating at 65 °C, the reaction mixture was cooled to room temperature and a mixture of L-glutamic acid di-*tert*-butyl ester hydrochloride (Sigma Chemical Co.) (0.204 g, 0.69 mmol) and Et_3N (0.070 g, 0.69 mmol) in dry DMF (10 mL) was added. A second portion of Et_3N (0.068 g, 0.67 mmol) was added, and stirring at 65 °C was continued for 18 h under N_2. The reaction mixture was evaporated under reduced pressure, 30 mL of H_2O was added to the residue, and the H_2O phase was extracted with EtOAc. The organic layer was washed with H_2O and saturated solution of NaCl, dried over $MgSO_4$, and evaporated. The crude product was applied to a silica gel column. Very mobile impurities were eluted from the column with $CHCl_3$, and the product **15** was eluted with $CHCl_3$–MeOH (9:1). Evaporation of pooled TLC-homogeneous fractions gave an orange solid, which was recrystallized from $CHCl_3$–*n*-hexane, 1:4, to yield 0.297 g (63%): melting point poorly defined; TLC ($CHCl_3$–MeOH, 4:1); HPLC; NMR (400 MHz, Me_2SO-d_6) δ 8.13 (d, 1, CON*H*, J = 7.46 Hz), 7.64 (d, 2, 2′, 6′, J_o = 8.47 Hz), 7.57 (d, 1, H_8, J_o = 8.50 Hz), 7.26 (d, 1, H_7, J_o = 8.50 Hz + br s, 2, NH_2), 6.91 (br s, 1, CH_2NH), 6.56 (d, 2, 3′, 5′, J_o = 8.47 Hz + br s, 2, NH_2), 4.39 (br s, 2, CH_2N), 4.26–4.28 (m, 1, glu α CH), 2.28 (t, 2, glu γ CH_2, J = 7.35 Hz), 1.87–1.98 (m, 2, glu β CH_2), 1.38 (s, 9, $C(CH_3)_3$), 1.36 (s, 9, $C(CH_3)_3$). Anal. ($C_{29}H_{37}N_6O_5Cl·4.5H_2O·0.22Et_3N$) C, H; N: calcd, 12.66; found, 11.64. The presence of Et_3N was indicated by ^1H NMR.

5-Chloro-5,8-dideazaaminopterin, 2b. Compound **15** (0.160 g, 0.22 mmol) was dissolved in CF_3COOH (7 mL). After the solution was stirred at room temperature under N_2 for 1 h, it was evaporated under reduced pressure. Next, 15 mL of Et_2O was added to the dry residue, and the precipitate was separated by filtration and washed with 10 mL of Et_2O and 10 mL of Me_2CO. The crude product was dissolved in 15 mL of H_2O, and the resulting suspension was basified to pH 10–11 with 1 N NaOH. Traces of insoluble material were removed by filtration. The solution was brought to pH 3.5 with 1 N HCl. After 15 min of stirring at room temperature, the precipitate was filtered, washed with 3 × 10 mL of cold H_2O, and dried under vacuum, at 100 °C, yielding 0.074 g (66%) of crystalline powder: mp 195–200 °C (lit.[11] mp 220–222 °C); TLC (1-propanol–H_2O–HOAc, 3:1:1); HPLC; NMR (400 MHz, Me_2SO-d_6) δ 8.09 (d, 1, CON*H*, J = 7.49 Hz), 7.64 (d, 2, 2′, 6′, J_o = 8.60 Hz), 7.51 (br s, 2, NH_2), 7.44 (d, 1, H_8, J_o = 8.67 Hz), 7.14 (d, 1, H_7, J_o = 8.67 Hz), 6.83 (t, 1, CH_2NH, J = 5.61 Hz), 6.56 (d, 2, 3′, 5′, J_o = 8.60 Hz), 6.29 (br s, 2, NH_2), 4.37 (d, 2, CH_2N, J = 5.61 Hz), 4.28–4.34 (m, 1, glu α CH), 2.30 (t, 2, glu γ CH_2, J = 7.33 Hz), 1.84–2.05 (m, 2, glu β CH_2). Anal. ($C_{21}H_{21}N_6O_5Cl·1.2H_2O$) C, H, N.

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Lysosomotropic Agents. 7.[1] Broad-Spectrum Antifungal Activity of Lysosomotropic Detergents

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Lysosomotropic detergents, which kill mammalian cells by disrupting lysosomal membranes, have now been found to be antifungals also. All strains in our assay are susceptible. The mode of action is as yet undetermined, but intracellular vacuoles may be the primary targets.

We report on a new class of broad-spectrum antifungals. Lysosomotropic detergents (LD's)[2] are amines of intermediate pK (5.5–7.6) that bear long alkyl chains. As free bases they are lipophilic and nonsurface-active, but when protonated they become powerful detergents. Thus they do not harm the outer membranes of cells, which reside in a neutral medium where LD's are hardly protonated. In the acidic milieu of lysosomes, however, LD's are largely

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(1) Paper 6: Firestone, R. A.; Pisano, J. M.; Falck, J. R.; McPhail, M. M.; Krieger, M. *J. Med. Chem.* **1984**, *27*, 1037.
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Table I. Disk Diffusion Assay of *N*-Dodecylimidazole (DDI) and *N*-Dodecylmorpholine (DDM)[a]

filamentous fungi	strain	zone, mm DDI	DDM
Alternaria solani	MF 3550	20	8H
Aspergillus flavus	MF 383	16	7V
Aspergillus fumigatus	MF 4839	22F	20F
Aspergillus niger YED	MF 442	21F	19F
Aspergillus niger	MF 11	24	20F
Botyris allii	MF 3587	24F	8V
Cephalosporium sp.	MF 4641	13	12S
Ceratocystis ulmi	MF 4042	31F	17S
Cercospora beticola	MF 4608	22F	8H
Cochliobolus miyabeanus	MF 4626	25F	23F
Fusarium oxysporum	MF 4014	18	15F
Penicillium sp.	MF 5014	22F	17S
Penicillium sp.	MF 5020	21F	15F
Penicillium sp.	MF 5016	25F	18F
Phoma sp.	MF 4332	23F	13S
Rhizomucor miehei	MF 4784	15	9H
Scopulariopsis communis	MF 3769	23F	13S
Trichoderma lignorum	MF 3560	24F	13F
Trichoderma sp.	MF 4064	18	16F
Ustilago zeae	MF 1996	28F	14S
Verticillium serrae	MF 3794	20	13F

yeasts	strain	zone, mm DDI	DDM
Brettanomyces bruxellensis	MY 315	16F	7V
Candida albicans YED	MY 992	17F	9S
Candida albicans YNB	MY 992	9S	0
Candida albicans	MY 1028	18F	14S
Candida albicans	MY 1029	15F	15S
Candida albicans	MY 1055	16F	15S
Candida albicans	MY 1099	18F	16H
Candida albicans YNB/GLU	MY 1099	9F	7V
Candida guilliermondii	MY 1019	23F	8H
Candida pseudotropicalis	MY 1100	14F	19S
Candida rugosa	MY 1022	30F	12S
Candida tropicalis	MY 1012	25F	
Cryptococcus albidus	MY 1070	23F	9V
Cryptococcus laurentii	MY 1074	16S	7V
Cryptococcus laurentii	MY 1073	17S	0
Cryptococcus laurentii	MY 1077	22F	9H
Kluyveromyces fragilis	MY 1113	13F	15S
Saccharomyces cerevisiae YED	MY 34	15	8S
Saccharomyces cerevisiae	MY 410	9F	0
Torulopsis glabrata	MY 1062	11	7V
Torulospora hansenii	MY 321	20S	0

[a] Samples of drug in methanol, 250 μg/mL, were applied to filter paper disks (25 μL/6.2 mm disk), dried at room temperature, and then placed on the surface of seeded agar plates and rewet with 25 μL of 10% aqueous Me_2SO. Inhibition zones were measured following overnight incubation. Culture media were unbuffered and acidic (pH ca. 4.5–5.6). Except where noted, media for filamentous fungi were potato dextrose agar and for yeasts, sabouraud dextrose agar. At 500 and 1000 μg/mL all zones were >0. Abbreviations: F, fuzzy edge; S, slightly hazy; H, hazy; V, very hazy; YNB, yeast nitrogen base (contains 76 mM ammonium sulfate as the sole nitrogen source); YED, yeast extract glucose; GLU, glucose.

protonated and thereby trapped, so that their concentration soon grows many times greater than that outside the cell or in the cytoplasm. At some point, the concentration inside the lysosomes becomes sufficient to break up the lysosomal membrane, releasing its contents into the cytoplasm, which is attacked by the lysosomal enzymes, leading eventually to cell death.

The evidence for this picture is strong.[3] The correlation between alkyl chain length of LD's vs. cytotoxic power on the one hand, with chain length of common detergents vs. detergent power on the other, is excellent. Short-chain

(3) Miller, D. K.; Griffiths, E.; Lenard, J.; Firestone, R. A. *J. Cell. Biol.* **1983**, *97*, 1841.

Table II. Dose Response of Lysosomotropic Detergents and Ketoconazole vs. Fungi[a]

concn, μg/mL	mean zone diameters, mm ketoconazole F	ketoconazole Y	DDI F	DDI Y	DDM Y	DDM Y
1000	22.8	27.2	NT	NT	21.9	19.0
500	20.0	24.8	NT	NT	17.1	12.8
250	17.8	23.3	21.7	16.8	14.2	11.4
125	14.1	20.6	20.5	15.8	10.7	10.2
62.5	NT	NT	18.9	14.4	8.7	8.6

[a] The protocol was the same as in Table I and included the full panel of test organisms, 21 yeasts and 21 filamentous fungi. Zones of 0 mm were excluded from the mean zone diameters. NT, not tested, Y, yeasts; F, filamentous fungi.

Table III. Antifungal Activity of Lysosomotropic Detergents and Amphotericin B (Agar Dilution Assay)[a]

fungus	strain	MIC, μg/mL DDI	DDM	AMB
Cryptococcus neoformans	MY 1051	4	16	0.25
Cryptococcus neoformans	MY 1146	4	32	0.5
Candida albicans	MY 1058	32	32	0.5
Candida albicans	MY 1055	32	32	0.5
Candida albicans	MY 0992	32	64	1.0
Candida albicans	MY 1013	16	32	0.5
Candida albicans	MY 1029	32	32	0.5
Candida parapsilosis	MY 1009	32	64	4.0
Candida parapsilosis	MY 1010	16	16	2.0
Candida tropicalis	MY 1011	32	32	1.0
Candida tropicalis	MY 1012	8	16	>128
Candida pseudotropicalis	MY 1040	16	32	1.0
Candida krusei	MY 1020	32	32	1.0
Candida rugosa	MY 1022	32	32	0.5
Candida guilliermondii	MY 1019	16	32	0.5
Candida stellatoidea	MY 1017	16	32	0.5
Torulopsis glabrata	MY 1059	16	64	1.0
Saccharomyces cerevisiae	MY 1027	8	32	0.5
Aspergillus fumigatus	MF 4839	16	64	1.0
Aspergillus flavus	MF 0383	16	64	1.0
Penicillium italicum	MF 2819	4	32	1.0

[a] Standardized agar dilution assay.[9] Kimmig agar supplemented with 5% glycerol. DDI and DDM were solubilized in 10% Me_2SO; desoxycholate stabilized AMB (Fungizone, Squibb) was diluted and titrated in sterile distilled water. Each drug was diluted twofold with sterile distilled water to yield final concentrations in agar from 128 to 0.06 μg/mL.

LD's are inactive. Cells without lysosomes are spared, and the susceptibility of cells to LD's is in proportion to their lysosomal content.[4] Cells whose lysosomal pH has been raised above normal are more resistant to LD's,[3] as are cells whose lysosomal enzyme content has been reduced below normal. Inhibitors of certain lysosomal hydrolases protect cells against LD's.[5] When cells are treated with LD's, this sequence of events can be observed under the microscope: cells with (1) normal, (2) bloated, (3) burst lysosomes. Only after this do the cells die.[3]

Fungi possess vacuoles that are not nearly as well described as mammalian cells' lysosomes but which might be targets for LD's. The first experiments[5] showed that *Saccharomyces cerevisiae* was indeed highly susceptible to *N*-dodecylimidazole (DDI; pK = 6.6), which disrupted the vacuoles and prevented growth at ≥5 μg/mL. The

(4) Thus, bone marrow stem cells are resistant but not immune to LD's. Mughal, T. I.; Robinson, W. A.; Drebing, C. J.; Firestone, R. A.; Entringer, M. A. *Exp. Hematol. (N.Y.)* **1983**, *11*, 118 (Suppl 14). Douay, L. Gorin, N. C.; Gerota, I.; Najman, A.; Duhamel, G. *Exp. Hematol. (N.Y.)* **1982**, *10*, 113.

(5) Personal communication from Prof. J. Lenard, Rutgers Medical School.

dose-response was sigmoidal, as is generally the case with LD's. Thus *S. cerevisiae* is more sensitive to DDI than any mammalian cell tested to date. Lowering the pH of the medium (which reduces lysosomotropism) conferred some protection, as did added ammonium ion (which raises lysosomal pH)[6-8] as the sulfate. These observations are all similar to those with mammalian cells.[7,8]

These results prompted us to put LD's through our antifungal screens. The results are given in Tables I–III.

It is clear that DDI and *N*-dodecylmorpholine (DDM; pK = 7.6) are broad-spectrum antifungals since all organisms and strains are susceptible, even those resistant to other antifungals. DDI is more active than DDM as has been observed invariably with mammalian cells.[2-4] In activity, DDI surpasses ketoconazole and is somewhat less active than amphotericin B (AMB) except for AMB-resistant *Candida tropicalis* strain MY 1012. Since LD's kill cells not by chemical but rather by cooperative physical action, very low MIC's are not expected, and their potency is good considering their low toxicity. Furthermore, the slightly acidic pH of the medium in some experiments tends to reduce the apparent activity of LD's by inhibiting their entry into acidic intracellular compartments[7,8] (v.s.). At pH 7 their potency would be greater.

Whether LD's act on fungi by the same mechanism previously reported for mammalian cells is still uncertain. Supporting the idea is our observation that adding ammonium ion (76 mM) to the culture medium (v.s.) reduces the cytotoxicity of LD's to fungi (see Table I). In any event, LD's constitute a new class of broad-spectrum antifungal compounds whose mode of action differs from that of all previously known classes. They should prove to be useful tools in research, and possibly in therapy.

Experimental Section

Lysosomotropic detergents were synthesized according to ref 2. Limits of error: disk diffusion assay, Table I, ±1 mm.; agar dilution assay, Table III, ±1 stage of dilution (each stage is twice the previous one).

Registry No. DDI, 4303-67-7; DDM, 1541-81-7; ketoconazole, 65277-42-1.

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Preparation and Antileukemic Screening of Some New 6′-Deoxyhexopyranosyladenine Nucleosides

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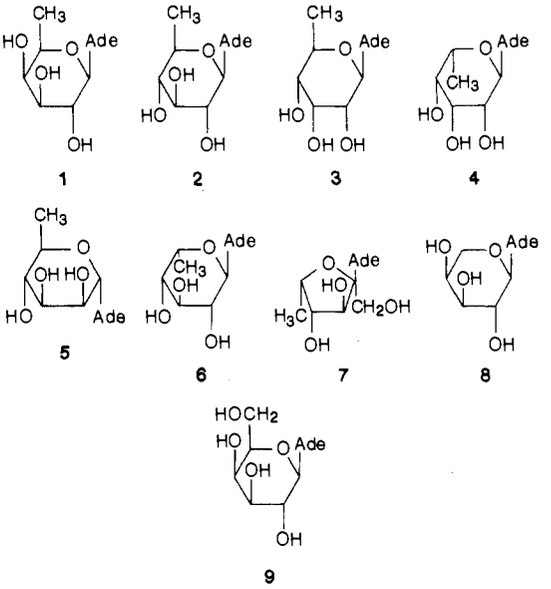
9-β-D-Fucopyranosyladenine (1) has weak antileukemic activity against L1210 cells grown in culture. Several new 6′-deoxyhexopyranosyladenine nucleosides were synthesized by standard procedures and assayed for activity. The new nucleosides were 9-(6-deoxy-β-D-glucopyranosyl)adenine (2), 9-(6-deoxy-β-D-allopyranosyl)adenine (3), 9-(6-deoxy-α-L-talopyranosyl)adenine (4), 9-α-D-rhamnopyranosyladenine (5), and 9-(6-deoxy-α-L-idopyranosyl)adenine (6). In addition, 9-(6-deoxy-α-L-sorbofuranosyl)adenine (7) was isolated from the same preparation as 6. None of the new nucleosides 2–7 had activity against L1210 cells in culture. A number of other known nucleosides related in structure to 1 were also tested for activity. One of these, 9-α-L-arabinopyranosyladenine, had activity, but was significantly weaker than 1.

A number of years ago, 9-β-D-fucopyranosyladenine [9-(6-deoxy-β-D-galactopyranosyl)adenine] (1, Chart I) was synthesized[1] as one of a series of starting materials for the preparation of acyclic nucleosides as inhibitors of adenosine deaminase.[2] No effort was made at the time to investigate the potential for biological activity because relatively few simple aldopyranosyl nucleosides derived from the common bases exhibited activity. Recently, during routine screening of nucleoside analogues, it was discovered that 1 inhibited the growth of leukemia L1210 cells in culture. At 10^{-4} M, this inhibition was about 75% at 48 h. The concentration that caused a 50% inhibition of growth after 48 h was 6×10^{-5} M. Because the sample of 1 was more than 15 years old, its physical properties were carefully checked to make certain that it had remained unchanged over this period of time. To make absolutely certain, the nucleoside was synthesized again starting from D-fucose, and the biological results were confirmed. Although the biological activity of 1 is low compared to other inhibitors of L1210 cells, it is unusual that a nucleoside with this structure would have activity, particularly when the poor record of activity of other hexopyranose nucleosides is considered.[3] Therefore, we set out to prepare a

Chart I



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