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Note

Nucleosides having acyclic 2-deoxy-1-thio-D-*arabino*-hexitol and 2-deoxy-1-thio-D-*erythro*-pentitol chains*

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The work described in this report establishes experimental conditions whereby nucleoside analogs having the sugar chain acyclic may be prepared by linking such 2-deoxyalditols as 2-deoxy-D-*erythro*-pentitol and 2-deoxy-D-*arabino*-hexitol with the nucleic acid base analogs 6-chloropurine and 5-fluorouracil. 2′-Deoxynucleosides of various 6-substituted purines have demonstrated antitumor activity[1], and 2′-deoxy-5-fluorouridine is a potent inhibitor of thymidylate synthetase[2]. In relation to synthetic work from this laboratory concerning nucleosides having the sugar chain acyclic, it was of interest to establish procedures for generating such structures from 2-deoxy-aldoses, both for direct study of the resultant, acyclic-sugar 2′-deoxynucleosides and also with a view to possible further modifications, including cyclization of the sugar chain.

In previous studies[3,4] related to the present one, a general procedure developed for coupling a sugar in its open-chain form to a nucleic acid base has involved the conversion of an acylated aldose dialkyl dithioacetal by the action of bromine into a 1-bromo derivative, and subsequent condensation of this reactive α-halo thioether with a suitably activated purine or pyrimidine, to give a 1′-epimeric mixture of acyclic-sugar nucleoside derivatives in which one of the 1′-epimers usually preponderates, presumably because of stereochemical induction by the proximal, chiral center.

In view of the biological importance of 2′-deoxynucleosides, and the high incidence of compounds having antineoplastic activity among analogs of this class, an extension of the synthesis of acyclic-sugar nucleosides to encompass those having a 2-deoxy sugar component was conducted. It was surmised that the acyclic-sugar chain might, according to stereochemical factors, adopt a nonextended conformation (either favored, or energetically readily accessible) that could be isosteric with natural 2′-deoxynucleotides at certain, key structural points; the possibilities for further chemical transformations of these products were also of interest.

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RESULTS AND DISCUSSION

As work previously reported has shown[5], efforts to replace a single ethylthio group by bromine in 3,4,5,6-tetra-*O*-acetyl-2-deoxy-D-*arabino*-hexose diethyl dithioacetal[6] (**1**) by controlled bromination invariably led to removal of both ethylthio substituents. It is presumed that the absence of an (electron-withdrawing) acyloxy group at C-2 so alters the electronic environment at C-1 that the type of halogen replacement possible in the normal aldose derivatives is precluded. It was, therefore, necessary to devise alternative conditions for coupling the 2-deoxy sugar derivative to a nucleic acid base.

Painter and Kurihara[7] have shown, in studies on acyclic-sugar derivatives having a -CH(OAc)SEt group as the chain terminus containing C-1, that replacement reactions of the acyloxy group do not involve neighboring (C-2) acyloxy-group participation, but that such replacements do proceed $\sim 10^3$ times as fast for the 2-deoxy analogs. In view of this report, and the fact that Pedersen and Fletcher[8] were able to obtain a deoxynucleoside directly by condensation of a 5-*O*-acyl-2-deoxypentose diisobutyl dithioacetal with a mercury salt of a purine, it was considered that a protected dithioacetal of a 2-deoxyaldose might react directly with a suitable, activated-base derivative to afford an acyclic-sugar nucleoside.

Direct condensation of equimolar amounts of the dithioacetal **1** with 6-chloro-9-(chloromercuri)purine[9] (**2**) took place during 4 h in boiling toluene, in the presence of cadmium carbonate ($\sim$0.7 molar equiv.) and Celite, to give a mixture of two, coupled products separable by column chromatography on silica gel. The major, slower-migrating component, isolated as a syrup in 62% yield, was the anticipated product (**3**) of coupling of the sugar through C-1 to N-9 of the base, as demonstrated from its elemental analysis ($C_{21}H_{27}ClN_4O_8S$) and u.v. spectrum (λ_{max}^{MeOH} 266 nm). The n.m.r. spectrum indicated that the product was a mixture of C-1′ epimers (two sets of H-2, H-8 signals, and complex, overlapped patterns for protons on the sugar chain), and no separation of these epimers could be achieved by conventional t.l.c., or column chromatography.

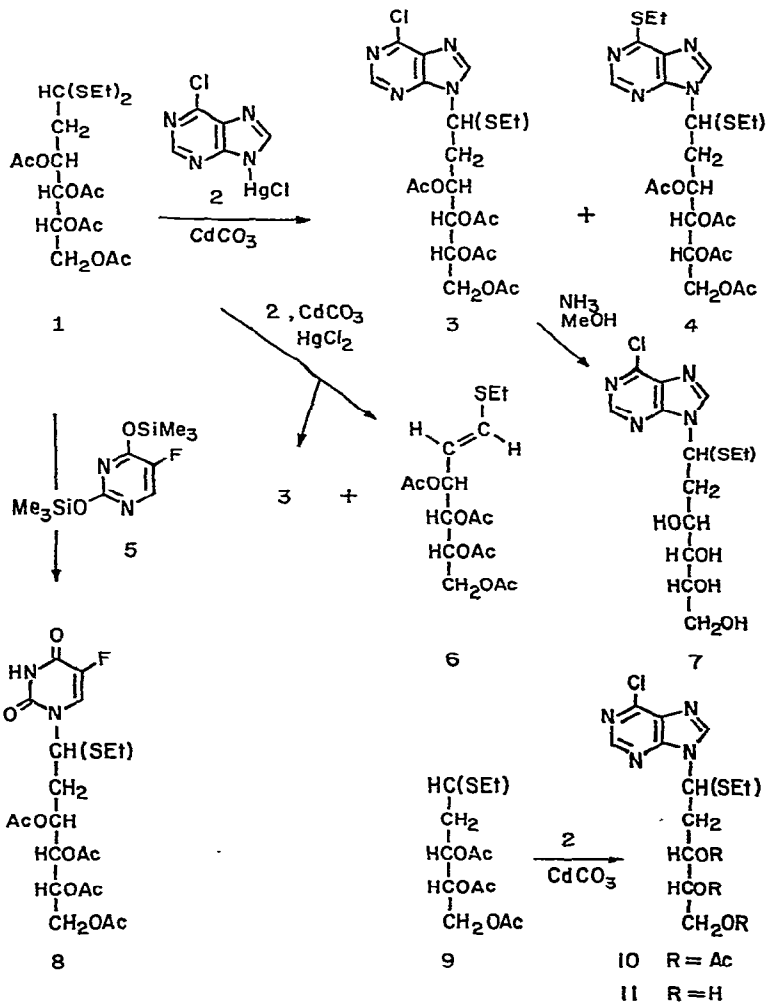
The minor, faster-migrating product was isolated in 8% yield as a syrup that, from its analysis and u.v. spectrum, was identified as a coupled product (**4**) in which the 6-chloro-substituent had been displaced by an ethylthio group. As a total mass-balance was not made for the reaction, mechanistic accounting for formation of **4** is scarcely justifiable, but it is noteworthy that increasing the amount of cadmium carbonate used to $\sim$1.4 molar equiv. caused the yield of **3** to decrease to practically zero.

Incorporation of mercuric chloride (1 molar equiv.) into the mixture gave a two-component product-mixture from which the nucleoside derivative **3** was again isolated, but in only 32% yield, and it was accompanied by a second, faster-migrating compound that was the major product of the reaction; it was obtained crystalline in 54% yield and was formulated, on the basis of its elemental composition ($C_{16}H_{24}O_8S$), and n.m.r. and mass spectra (see Experimental section), as (*E*)-

3,4,5,6-tetra-*O*-acetyl-2-deoxy-1-*S*-ethyl-1-thio-D-*arabino*-hex-1-enitol (**6**). Evidently, the incorporation of mercuric chloride into the reaction mixture leads to a competing elimination of ethanethiol that is favored over the nucleoside coupling-reaction, and no significant increase in the proportion of coupling-product was achieved by use of a 2:1 ratio of purine derivative to dithioacetal.

The enol thioether **6** may be of value in synthesis. A direct procedure was developed for its preparation by the action of mercuric chloride on **1** in hot toluene.

The syrupy, protected nucleoside **3** was routinely deacetylated with methanolic ammonia to give the analytically pure, but syrupy, tetrol **7**, presumably as a mixture of 1′-epimers corresponding to the starting tetraacetate **3**. The coupling reaction evidently has very low stereoselectivity, so that a significantly unequal distribution of 1′-epimers (commonly observed in reactions leading to the 2′-oxygenated counterparts) is not achieved. The low specific rotations observed for **3** and **4** reflect this



distribution; the pure 1′-epimers would be expected[3,4] to show specific rotations of large magnitude.

The same coupling-reaction was conducted in boiling toluene between equimolar amounts of 3,4,5-tri-*O*-acetyl-2-deoxy-D-*erythro*-pentose diethyl dithioacetal[10] (**9**) and 6-chloro-9-(chloromercuri)purine[9] in the presence of an excess of cadmium carbonate. The amorphous, 9-substituted product **10** (λ_{max}^{MeOH} 264 nm) was obtained analytically pure in 60% yield. From the n.m.r. signal-intensities for H-2 and H-8, this product was judged to be an ~3:2 mixture of 1′-epimers. The yield of **10** was severely decreased (to ~27%) when mercuric chloride was incorporated into the reaction mixture, and the favored product was a fast-migrating compound, presumably a C_5 analog of **6**. *O*-Deacetylation of **10** gave the syrupy triol **11**; again, conventional chromatography failed to resolve the 1′-epimers.

Conditions were also established for the corresponding coupling-reaction in the pyrimidine series; 5-fluorouracil was used as the base and compound **1** as the sugar addend. The reaction was conducted in boiling toluene, and a slight molar excess (with respect to **1**) of mercuric chloride was incorporated, together with an excess of 5-fluoro-2,4-bis(trimethylsilyloxy)uracil[11] (**5**) and cadmium carbonate. Processing of the product with aqueous methanol afforded 54% of the coupled product **8** as a syrup that gave an acceptable elemental analysis; its u.v. absorption (270 nm) was close to that of related analogs[5]. The ^{1}H-n.m.r. spectrum clearly showed that **8** was an ~1:1 C-1′-epimeric mixture, in particular by reason of the two proximal doublets for H-6 of the epimers.

In view of the major, separational problems that would be involved, the nucleoside analogs were not resolved into their pure 1′-epimers. The purine derivatives tested (see Experimental) did not display significant antitumor activity.

EXPERIMENTAL

General methods. — These were as described in a previous report[3].

(*1R,1S*)-*3,4,5,6-Tetra-O-acetyl-1-(6-chloropurin-9-yl)-2-deoxy-1-S-ethyl-1-thio*-D-arabino-*hexitol* (**3**). — *Method A.* To an azeotropically dried mixture of 6-chloro-9-(chloromercuri)purine[9] (**2**; 2.0 g, 5.2 mmol), cadmium carbonate (0.6 g, 3.4 mmol), Celite (0.5 g), and toluene (150 mL) was added compound **1** (2.0 g, 5.2 mmol) dissolved in toluene (20 mL), and the stirred mixture was boiled for 4 h under reflux. The hot mixture was filtered, the filtrate successively washed with 30% aqueous potassium iodide (twice), and water (twice), dried (sodium sulfate), and evaporated to give a thick syrup. The syrup was dissolved in the minimal volume of benzene, and applied to a column (65 × 5.0 cm) of silica gel. Elution was initially performed with benzene alone, and then the polarity was gradually increased by incorporating ethyl acetate, until two u.v.-positive components (R_F 0.6 and 0.5, 2:1 benzene–ethyl acetate) were eluted. The fractions containing both products were combined, and concentrated, and the concentrate was applied to a column (205 × 2.0 cm) of silica gel. Careful elution permitted separation of the two compounds.

The slower-moving, syrupy material was found to be the desired nucleoside **3**; yield 1.5 g (62%), $[\alpha]_D^{25}$ +8° (*c* 1.0, chloroform); R_F 0.72 (1:1 benzene–ethyl acetate); λ_{max}^{MeOH} 266 nm (log ε 3.6); ν_{max}^{film} 1770 (C=O of acetate), 1610, 1560, 1450 (purine), 1380, and 1050 cm^{-1} (C-O-C); n.m.r. (C_6D_6): δ 8.70, 8.63, 8.40, 8.34 (4 s, H-2,8 of purine, 2 isomers), 6.2–5.0 (m, H-1',3',4',5'), 4.2 (m, H-6'a,6'b), 2.6–2.1 (m, H-2'a,2'b, SCH_2CH_3), 1.84 s, 1.82, 1.78, 1.74, 1.65 (4 s, 4 OAc), and 0.97 t, 0.93 (t, SCH_2CH_3); *m/e* 530 (M^+), 469 (M^+ — SEt), 377 (M^+ — base), 226 (base-C=S^+Et), and 154 (base H^+).

Anal. Calc. for $C_{21}H_{27}ClN_4O_8S$ (530.98): C, 47.50; H, 5.09; Cl, 6.69; N, 10.55; S, 6.03. Found: C, 47.43; H, 4.84; Cl, 6.51; N, 10.28; S, 6.03.

The peak heights of the signals at δ 8.70 and 8.63 were equal, and more intense than those at δ 8.40 and 8.30, indicating that the 1'-epimers were present in ~4:3 ratio.

The faster-migrating component (R_F 0.6, 2:1 benzene–ethyl acetate) from the foregoing experiment was isolated as a syrup, and identified as (1*R*,1*S*)-3,4,5,6-tetra-*O*-acetyl-2-deoxy-1-*S*-ethyl-1-(6-ethylthiopurin-9-yl)-1-thio-D-*arabino*-hexitol (**4**); yield 0.43 g (8%), $[\alpha]_D^{25}$ +10° (*c* 1.0, chloroform); λ_{max}^{MeOH} 283 nm (log ε 4.2); ν_{max}^{film} 1740 (C=O of acetate), 1560, 1470 (purine), 1360, 1210, and 1040 cm^{-1} (C-O-C).

Anal. Calc. for $C_{23}H_{32}N_4O_8S_2$ (556.65): C, 49.64; H, 5.75; N, 10.07; S, 11.51. Found: C, 49.62; H, 5.62; N, 10.28; S, 11.69.

The n.m.r. spectrum of **4** resembled that of **3**, except for the appearance of a triplet at δ 1.42 and a quartet at δ 3.40 ($CDCl_3$), indicative of an SEt group attached to an electron-withdrawing function.

The yield of compound **4** was decreased to <1% when the proportion of cadmium carbonate used in the reaction was doubled.

Method B. To an azeotropically dried mixture of 6-chloro-9-(chloromercuri)-purine[9] (**2**; 1.0 g, 2.57 mmol), cadmium carbonate (1.0 g, 5.8 mmol), mercuric chloride (0.62 g, 2.28 mmol), Celite (0.3 g), and toluene (85 mL) was added compound **1** (0.95 g, 2.17 mmol) dissolved in toluene (20 mL), and the stirred mixture was boiled for 3 h under reflux. The hot mixture was then filtered, and processed as described in Method *A*. Elution with 9:1 benzene–ethyl acetate from a column (205 × 2.0 cm) of silica gel removed a side-product having a high R_F value. Continued elution, with 1:1 benzene–ethyl acetate, yielded amorphous **3** (0.39 g, 32%). This product was identical by t.l.c. and mass spectrum with the material prepared by Method *A*.

The major side-product in the preparation of **3** by Method *B* was isolated crystalline after column chromatography with 9:1 benzene–ethyl acetate, and was identified as (*E*)-3,4,5,6-tetra-*O*-acetyl-2-deoxy-1-*S*-ethyl-1-thio-D-*arabino*-hex-1-enitol (**6**); yield 1.4 g (54%), m.p. 71–72°, $[\alpha]_D^{25}$ 0° (*c* 0.9, chloroform); ν_{max}^{KBr} 3040, 970 (*trans* -HC=CH-), 1740 (C=O of acetate), 1370, 1220, and 1050 cm^{-1} (C-O-C); n.m.r. ($CDCl_3$): δ 6.46 (d, $J_{1,2}$ 15.6 Hz, H-1), 5.14–5.66 (m, H-2,3,4,5), 4.30 (q, $J_{5,6}$ 3.2 Hz, H-6), 4.16 (q, $J_{5,6'}$ 5.0, $J_{6,6'}$ 13.5 Hz, H-6'), 2.70 (q, SCH_2CH_3),

2.12 s, 2.03 s, 1.99 (s, OAc), and 1.26 (t, SCH_2CH_3); *m/e* 376 (M^+), 315 ($M^+ - SEt$), 274 ($M^+ - Ac_2O$), 231 (C-5/C-6); X-ray powder diffraction data: 8.88 m, 6.83 s (1), 6.38 m, 6.25 m, 4.84 s (2), 4.73 s (3), 4.29 w, 3.69 s, 3.54 m, and 1.95 m.

Anal. Calc. for $C_{16}H_{24}O_8S$ (376.42): C, 51.07; H, 6.38; S, 8.51. Found: C, 51.03; H, 6.39; S, 8.75.

Direct preparation of (E)-*3,4,5,6-tetra*-O-*acetyl-2-deoxy-1*-S-*ethyl-1-thio*-D-arabino-*hex-1-enitol* (**6**). — Mercuric chloride (0.5 g, 1.84 mmol), cadmium carbonate (0.5 g, 2.9 mmol), and Celite (0.2 g) in boiling toluene (70 mL) were stirred under reflux, and a solution of the dithioacetal[6] **1** (0.5 g, 1.14 mmol) in toluene (10 mL) was added. After 3 h, the mixture was filtered, and the filtrate washed successively with 30% aqueous potassium iodide and water, dried (sodium sulfate), and evaporated, and the resultant, thin syrup dissolved in ethanol. Crystals of **6** were formed after 1 week at 0°; yield 0.29 g (68%), identical with **6** from the foregoing reaction.

Compound **6** reacted much more readily with bromine in carbon tetrachloride than did the parent dithioacetal **1**.

(*1*R,*1*S)-*1-(6-Chloropurin-9-yl)-2-deoxy-1*-S-*ethyl-1-thio*-D-arabino-*hexitol* (**7**). — The syrupy, acetylated **3** (0.9 g, 1.69 mmol) was dissolved in methanol (35 mL), and dry, gaseous ammonia was bubbled through the solution for 30 min at 0°. The solution was then kept overnight at 0°, evaporated at ~25 torr, and the residual syrup kept under vacuum for 24 h in an oil bath at 50°. The brownish, syrupy residue was taken up in a small volume of methanol, and applied to a column (205 × 2.0 cm) of silica gel. Gradient elution, beginning with chloroform and ending with 2:1 chloroform–methanol, afforded the desired nucleoside **7** as a syrup; yield 0.41 g (67%), $[\alpha]_D^{25}$ +7° (*c* 1.0, methanol); λ_{max}^{MeOH} 265 nm (log ε 3.7); ν_{max}^{film} 3390 (CH), 1610, 1490, 1450 (purine), 1390, 1230, and 1050 cm^{-1} (C-O-C).

Anal. Calc. for $C_{13}H_{19}ClN_4O_4S$ (362.84): C, 43.03; H, 5.24; Cl, 9.79; N, 15.45; S, 8.83. Found: C, 43.40; H, 5.63; Cl, 9.48; N, 15.88; S, 8.51.

(*1*R,*1*S)-*3,4,5-Tri*-O-*acetyl-1-(6-chloropurin-9-yl)-2-deoxy-1*-S-*ethyl-1-thio*-D-erythro-*pentitol* (**10**). — *Method A.* To an azeotropically dried mixture of 6-chloro-9-(chloromercuri)purine[9] (**2**; 2.1 g, 5.39 mmol), cadmium carbonate (2.0 g, 11.62 mmol), Celite (0.6 g), and toluene (90 mL) was added **9** (1.9 g, 5.2 mmol) in toluene (20 mL), and the stirred mixture was boiled for 3 h under reflux. The general procedure for isolation of **10** was the same as for **3** (Method *A*). The yield of amorphous **10** was 1.5 g (60%), $[\alpha]_D^{25}$ +6° (*c* 0.75, chloroform); λ_{max}^{MeOH} 264 nm (log ε 3.9); ν_{max}^{film} 1770 (C=O of acetate), 1620, 1560, 1450 (purine), 1385, and 1050 cm^{-1} (C-O-C); n.m.r. ($CDCl_3$): δ 8.73, 8.70, 8.45, 8.47 (4 s, H-2,8, 3:2 mixture of epimers), 5.8 (m, H-1′), 5.1 (m, H-3′,4′), 4.1 (m, H-5,5′), 2.4 (m, H-2,2′, SCH_2CH_3), 2.00 s, 1.99 s, 1.97 s, 1.95 s, 1.94 s, 1.88 (s, OAc), and 1.2 (m, SCH_2CH_3).

Anal. Calc. for $C_{18}H_{23}ClN_4O_6S$ (458.9): C, 47.11; H, 5.02; Cl, 7.74; N, 12.21; S, 6.98. Found: C, 47.11; H, 5.44; Cl, 8.07; N, 12.07; S, 7.18.

Method B. The same procedure as that used for the preparation of **3** (Method *B*) was used. Column chromatography with 9:1 benzene–ethyl acetate removed the side-product of high R_F value. Continued elution, with 1:1 benzene–ethyl acetate,

yielded compound **6** (0.92 g, 27%), identical by t.l.c. and mass spectrum with the material prepared by Method *A*.

(1R,1S)-1-(6-Chloropurin-9-yl)-2-deoxy-1-S-ethyl-1-thio-D-erythro-*pentitol* (**11**). — Compound **10** (0.48 g, 1.05 mmol) was treated with ammonia, as in the preparation of **7**, to afford **11** as a chromatographically homogeneous syrup; yield 0.22 g (63%), $[\alpha]_D^{25}$ +6° (*c* 1.0, methanol); λ_{max}^{MeOH} 264 nm (log ε 3.6); ν_{max}^{film} 3390 (OH), 1610, 1490, and 1450 (purine), 1390, 1350, 1235, and 1060 cm^{-1} (C-O-C); *m/e* (c.i., NH_3): 350 ($M \cdot NH_4^+$), 172 (base · NH_4^+), and 155 (base · H^+).

Anal. Calc. for $C_{12}H_{17}ClN_4O_3S$ (332.813): S, 8.37. Found: S, 8.37.

(1R,1S)-3,4,5,6-Tetra-O-*acetyl-2-deoxy-1-S-ethyl-1-(5-fluorouracil-1-yl)-1-thio*-D-arabino-*hexitol* (**8**). — To an azeotropically dried mixture of mercuric chloride (0.7 g, 2.58 mmol), cadmium carbonate (1.5 g, 8.72 mmol), and toluene (70 mL) were added 5-fluoro-2,4-bis(trimethylsilyloxy)uracil[11] (**5**; 2.2 g, 8.59 mmol) and the dithioacetal **1** (1.0 g, 2.28 mmol), and the stirred mixture was boiled for 3 h under reflux. The hot mixture was filtered, and the filtrate successively washed with 30% aqueous potassium iodide and water, and evaporated; the residue was taken up in 4:1 methanol–water, stirred for 30 min, the suspension filtered, and the filtrate evaporated. The residue was taken up in a small volume of benzene, and applied to a column (205 × 2.0 cm) of silica gel. Elution with 9:1 benzene–ethyl acetate removed a side-product (presumed to be **6**, from its R_F value), and further elution, with 1:1 benzene–ethyl acetate, led to elution of the desired product; yield 0.64 g (54%), $[\alpha]_D^{25}$ +9° (*c* 0.8, chloroform); λ_{max}^{MeOH} 270 nm (log ε 3.95); ν_{max}^{film} 1770 (C=O of acetate), 1490, 1475, 1450 (uracil), 1390, 1235, and 1040 cm^{-1} (C-O-C); n.m.r. ($CDCl_3$): δ 7.70, 7.64 (2 d, $J_{6,F}$ 6 Hz, 2 H-6; 1:1 epimeric mixture), 5.8 m, 5.2 (m, H-1',3',4',5'), 4.2 (m, H-5,5'), 1.9 (m, H-2,2'), 2.50 (q, SCH_2CH_3), ~2.1 [group (of s), OAc], and 1.22 (t, SCH_2CH_3).

Anal. Calc. for $C_{20}H_{27}FN_2O_{10}S$ (506.51): C, 47.42; H, 5.33; N, 5.53; S, 6.32. Found: C, 47.69; H, 5.67; N, 5.73; S, 6.34.

Biological testing. — Compounds **3** (NSC 245306) and **7** (NSC 245851) were essentially inactive (T/C 105 at 200 mg/kg, and 108 at 100 mg/kg, respectively) in the NCI, *in vivo*, murine L-1210 leukemia screen (data from Dr. Harry B. Wood, Jr., and the NCI Division of Cancer Treatment). In cell-culture assays with L-1210 leukemia cells, courtesy of Dr. Alexander Block of the Roswell Park Memorial Institute, Buffalo, NY, compound **3** had ID_{50} 50μM, and **7** had ID_{50} >100μM.

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