137517-48-7; (±)-**9c**, 137539-89-0; (±)-**9d**·maleate, 137517-50-1; (±)-**9** (R = H), 137517-51-2; (±)-**10**, 137517-52-3; (±)-**11a**, 137517-53-4; (±)-**11b**, 137517-54-5; (±)-**11c**, 137517-55-6; (±)-**11d**, 137517-56-7; (±)-**12**, 137540-34-2; (±)-**13**, 137517-57-8; (±)-**13** (6-methylene derivative), 137540-35-3; (±)-**14**, 137517-58-9; (±)-**15**, 137517-59-0; (±)-**15**·maleate, 137517-60-3; (±)-**15** (des-cyclopropylmethyl derivative), 137517-61-4; (±)-**16**, 137517-62-5; (±)-**17**, 137517-63-6; (±)-**18**, 137517-64-7; (±)-**18** (6-methylene derivative), 137517-65-8; (±)-**19**, 137517-66-9; (±)-**19** (des-cyclopropylmethyl derivative), 137517-67-0; (±)-**20**, 137517-68-1; (±)-**21**, 137517-69-2; (±)-**21** (*N*-desmethyl derivative), 137517-70-5; (±)-**22a**, 137517-71-6; (±)-**22b**, 137517-72-7; (±)-**22b** methyl ether·HCl, 137517-73-8; (±)-**22c**, 137517-74-9; (±)-**22d**, 137517-75-0; (±)-**23a**, 137517-76-1; (±)-**23a**·HCl, 137517-77-2; (±)-**23b**, 137517-78-3; (±)-**24a**, 137517-79-4; (±)-**24b**, 137517-80-7; (±)-**25**, 137517-81-8; (±)-**26**, 137517-82-9; (±)-**27a**, 137517-83-0; (±)-**27a** ketone, 137517-84-1; (±)-**27b**, 137517-85-2; (±)-**28a**, 137517-86-3; (±)-**28a** methyl ether, 137517-87-4; (±)-**28b**, 137517-88-5; (±)-**29a**, 137517-89-6; (±)-**29b**, 137517-90-9; (±)-**30a**, 137517-91-0; (±)-**30b**, 137517-92-1; $BrCH_2C(=CH_2)CH_2CH_2Br$, 82359-61-3; (*Z*)-$ClCH_2CH=CHCH_2Cl$, 1476-11-5; $BrCH_2$-c-C_3H_5, 7051-34-5; ClCO-c-C_4H_7, 5006-22-4; (±)-2,3,4,4a,5,8-hexahydro-4a-(3-methoxyphenyl)-2-methylisoquinoline, 137517-93-2; ethenyl (±)-*trans*-3,4,4a,5,8,8a-hexahydro-4a-(3-methoxyphenyl)-2(1*H*)-isoquinolinecarboxylate, 137517-94-3.

Supplementary Material Available: NMR data of selected target compounds (2 pages). Ordering information is given on any current masthead page.

Di- and Triester Prodrugs of the Varicella-Zoster Antiviral Agent 6-Methoxypurine Arabinoside

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6-Methoxypurine arabinoside (9-β-D-arabinofuranosyl-6-methoxy-9*H*-purine, 1) has potent and selective activity against varicella-zoster virus in vitro. An unfavorable metabolic profile observed with oral dosing in the rat led to the preparation of a variety of 2′,3′,5′-triesters (**2a–n**) and several 2′,3′-, 2′,5′-, and 3′,5′-diesters of this arabinoside (**3a–n**, **4a–f**, and **5a–j**, respectively). The compounds were evaluated as prodrugs by measuring the urinary levels of 1 in rat urine after oral dosing. With the exception of triacetate **2a**, the triesters failed to significantly enhance bioavailability. Administration of compound **2a** resulted in a 3-fold increase in systemic availability of 1, possibly because of its increased water solubility (1.6 times more soluble than 1) and only slightly increased relative log *P* value (1.93 vs 0.50 for 1). The longer chain aliphatic triesters and aromatic triesters had lower water solubilities and increased lipophilic partitioning. These factors might account for the lower systemic bioavailability of these compounds. In contrast, the diesters, especially the aliphatic diesters, showed significantly improved systemic availability. This might be a consequence of the higher aqueous solubilities and enhanced partition coefficients seen with these compounds. 2′,3′-Diacetate **3a** showed the best combination of high systemic availability and water solubility of all the prodrugs of 1.

The potent activity of 6-methoxypurine arabinoside (9-β-D-arabinofuranosyl-6-methoxy-9*H*-purine, 1) as an anti-varicella-zoster agent in vitro and the molecular basis for its selectivity have been described.[1] However, in vivo and enzyme studies indicated that adenosine deaminase is responsible for catabolism of the compound to hypoxanthine arabinoside[2] and accounts for the pharmacokinetic limitations of the compound following oral dosing.[3] A similar limitation for adenine arabinoside (*ara*-A) has been observed, leading to the preparation of a wide variety of prodrugs designed to increase resistance to adenosine deaminase, increase water solubility, and modify lipophilicity.[4-9] These studies indicated that modification of the 2′- or 5′-hydroxyls of adenine arabinoside, but not the 3′-hydroxyl, increases resistance of adenine arabinoside to enzymatic deamination.[4-6] A limited series of 2′,3′- and 3′,5′-di-*O*-acyl derivatives of *ara*-A were examined, with the 2′,3′-diacetate showing enhanced water solubility. Good in vitro and in vivo antiviral activity toward herpes simplex type 2 was also noted.[5] Haskell examined the antiviral activity of a series of triesters of *ara*-A, concluding that in vitro activity was consistent with the ease of aqueous or enzymatic hydrolysis to the parent nucleoside.[7] The tri-*O*-formyl derivative possessed the best antiviral activity, although limited aqueous solubility was found. With the goal of achieving improvements in the bioavailability and solubility of 1, we prepared and evaluated a series of di- and triester prodrugs of 6-methoxypurine arabinoside.

Chemistry

The triesters of 6-methoxypurine arabinoside (1) were synthesized by adding the appropriate acid anhydride to a solution of 1 in acetonitrile and pyridine (Scheme I). As the aliphatic chain became more hindered, or in the case of the aromatic esters, it sometimes became necessary to heat the solution to reflux to drive the reaction to completion.

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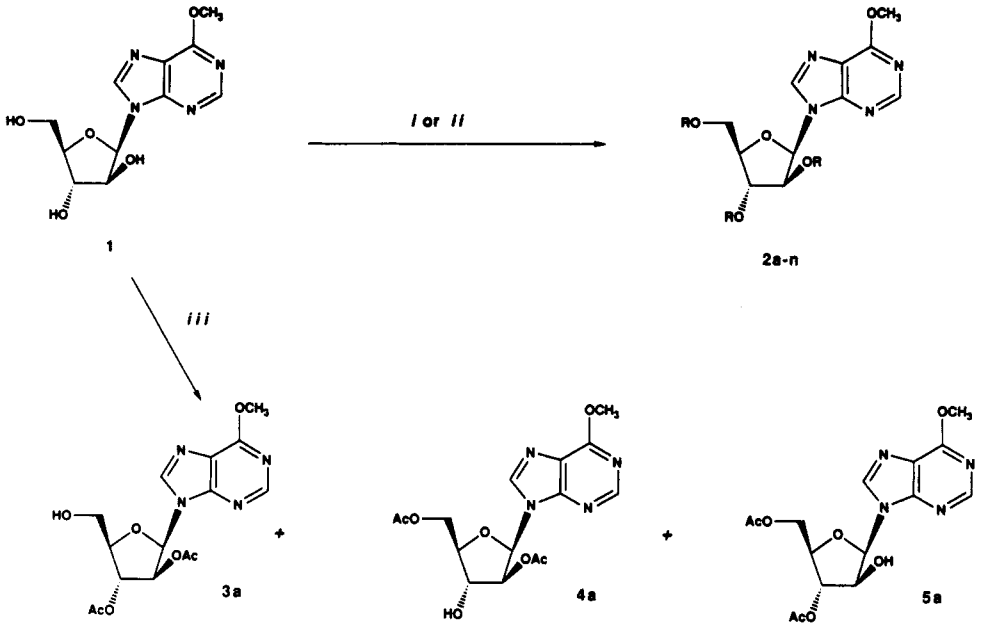
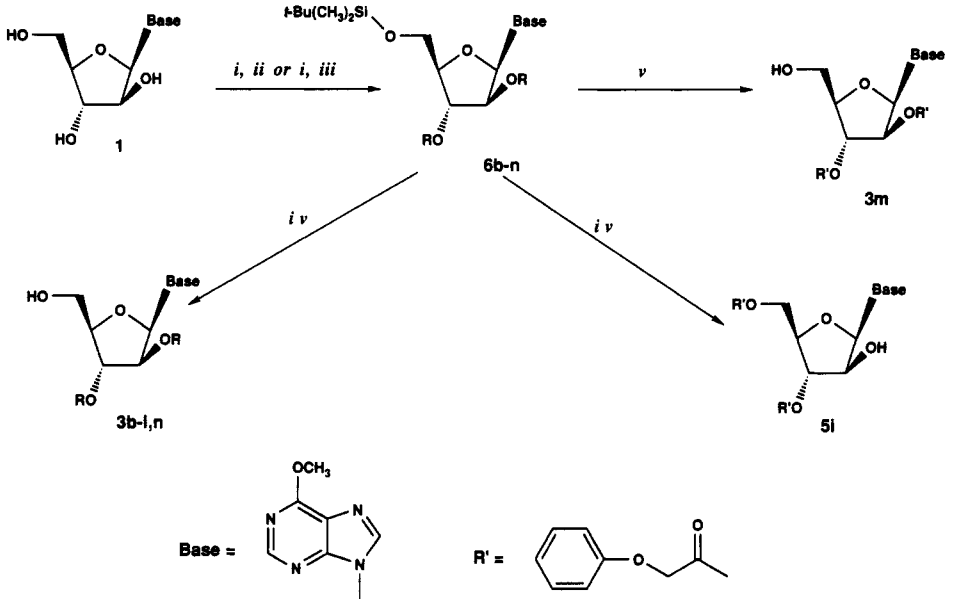
Scheme I.[a] Synthetic Scheme for Triester and Diacetyl Ester Prodrugs of 6-Methoxypurine Arabinoside



[a] (i) Acid anhydride, CH_3CN, pyridine; (ii) acid chloride (RCl), CH_3CN, pyridine; (iii) $(CH_3CO)_2O$, CH_3CN, pyridine.

Scheme II.[a] Synthetic Scheme for 2′,3′-Diester Prodrugs of 6-Methoxypurine Arabinoside



[a] (i) *tert*-Butyldimethylsilyl chloride, CH_3CN, pyridine; (ii) acid chloride (RCl), CH_3CN, pyridine; (iii) acid anhydride, CH_3CN, pyridine; (iv) tetrabutylammonium fluoride (3.3 equiv), THF, H_2O; (v) 80% acetic acid, 50 °C.

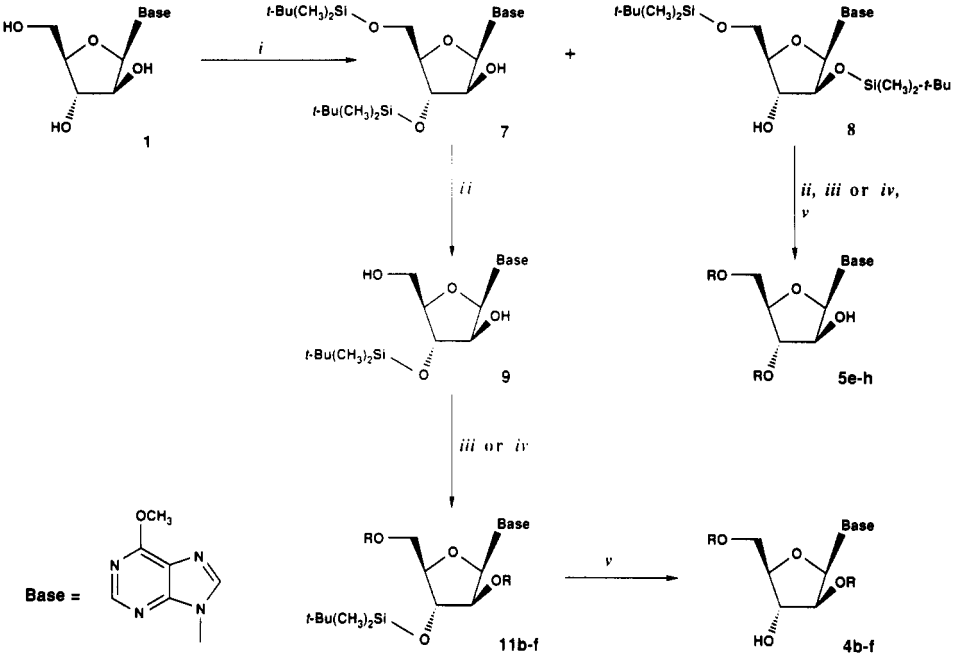
Three of the triesters, **2c**, **2j**, and **2m**, were prepared using acid chlorides as alternatives to the anhydrides (Scheme I). If pyridine was used as both the base and the solvent and the reaction was performed at room temperature, the desired product was isolated in low yields. When triethylamine was used as the base, only extensive decomposition of the starting material was seen. This was attributed to N-1 or N-7 acylation followed by ring opening.[7]

The diesters of **1** were prepared by a variety of methods. The 2′,3′- and 2′,5′-diacetates (**3a** and **4a**) were obtained by preparative HPLC of a 3:5:1 mixture of the 2′,3′-, 2′,5′, and 3′,5′-diacetates. Since this method was both time-consuming and produced low yields, it was used only for these two compounds.

The 2′,3′-diesters were preferentially synthesized by protecting the 5′-position with *tert*-butyldimethylsilyl chloride and then acylating with the appropriate acid chloride or anhydride; this was followed by deprotection with tetrabutylammonium fluoride (Scheme II). The deprotection procedure had to be modified for the phenoxyacetate ester (**6m**). If tetrabutylammonium fluoride was used, 3′,5′-diester **5i** was produced exclusively by migration of the 2′-acyl group. When the deprotection of **6m** was performed in 80% acetic acid at 50 °C for 3 h, only the expected 2′,3′-diester **3m** was produced.

2′,5′-Diesters **4b–f** were synthesized by preparation of the disilylated intermediate **7**. Treatment of **7** for 6 h at 50 °C in 80% acetic acid led to selective removal of the 5′-silyl group (Scheme III). The monosilylated intermediate **9** was acylated to yield the 2′,5′-diacylated intermediates **11b–f**, which, in turn, were deprotected using tetrabutylammonium fluoride to give the desired 2′,5′-diesters.

Scheme III.[a] Synthetic Scheme for 2′,5′- and 3′,5′-Diester Prodrugs of 6-Methoxypurine Arabinoside



[a] (i) *tert*-Butyldimethylsilyl chloride, DMF, imidazole; (ii) 80% acetic acid; (iii) acid anhydride, *N,N*-(dimethylamino)pyridine, pyridine, CH_3CN; (iv) acid chloride, *N,N*-(dimethylamino)pyridine, pyridine, CH_3CN; (v) tetrabutylammonium fluoride, THF, H_2O.

Table I. ^1H NMR Chemical Shift Assignments for 6-Methoxypurine Arabinoside and the Valerate Ester Prodrugs

no.	H-2	H-8	H-1′	H-2′, H-3′	H-4′, H-5′	OCH_3	2′-R	3′-R	5′-R
1	8.54	8.45	6.36	4.24, 4.13	3.84–3.62	4.10	5.66 (OH)	5.58 (OH)	5.12 (OH)
2′-valerate	8.52	8.50	6.53	5.34, 4.44	3.89–3.83, 3.73–3.65	4.07	2.05–1.93 (CH_2CO), 1.87–1.72 (CH_2CO), 0.99–0.82 (CH_2)$_2$, 0.57 (CH_3)	5.82 (OH)	5.07 (OH)
3′-valerate	8.53	8.45	6.36	4.35, 5.25	3.99–3.95, 3.70–3.66	4.09	5.98 (OH)	2.39 (CH_2CO), 1.58–1.50 (CH_2), 1.40–1.23 (CH_2), 0.88 (CH_3)	5.16 (OH)
5′-valerate	8.53	8.33	6.38	4.25–4.14	4.06–3.89 (4′), 4.39, 4.30 (5′)	4.09	5.78 (OH)	5.73 (OH)	2.29 (CH_2CO), 1.55–1.16 (CH_2)$_2$, 0.86 (CH_3)
2d	8.56	8.45	6.63	5.77, 5.66	4.38–4.29	4.08	2.08–1.87 (CH_2CO), 1.00–0.79 (CH_2)$_2$, 0.62–0.55 (CH_3)	2.42–2.27 (CH_2CO), 1.56–1.20 (CH_2)$_2$, 1.00–0.79 (CH_3)	2.42–2.27 (CH_2CO), 1.56–1.20 (CH_2)$_2$, 1.00–0.79 (CH_3)
3d	8.56	8.54	6.60	5.64–5.60	4.12–4.07 (4′), 3.73–3.65 (5′)	4.07	2.05–1.93 (CH_2CO), 1.87–1.72 (CH_2CO), 0.99–0.82 (CH_2)$_2$, 0.59 (CH_3)	2.36 (CH_2CO), 1.65–1.44 (CH_2), 1.35–1.20 (CH_2), 0.99–0.82 (CH_3)	5.19 (OH)
4b	8.54	8.42	6.58	5.37–5.30 (2′), 4.63–4.60 (3′)	4.06–4.04 (4′), 4.38–4.30 (5′)	4.08	2.07–1.85 (CH_2CO), 0.94–0.72 (CH_2)$_2$, 0.56 (CH_3)	6.00 (OH)	2.31 (CH_2CO), 1.52–1.23 (CH_2)$_2$, 0.83 (CH_3)
5d	8.54	8.37	6.39	4.49–4.13 (2′, 4′, 5′) 5.29–5.15 (3′)		4.09	6.14 (OH)	2.40 (CH_2CO), 1.62–1.16 (CH_2)$_2$, 0.88 (CH_3)	2.30 (CH_2CO), 1.62–1.16 (CH_2)$_2$, 0.82 (CH_3)

3′,5′-Diesters **5a–d** were prepared by first acylating the 3′-position of 1 using an enzyme (lipase) and an activated ester, such as 2,2,2-trichloroethyl propionate (Scheme IV). After these compounds were isolated in pure form, the 5′-positions were acylated using Lipozyme IM 20 with the same activated ester. These were very clean, high-yielding reactions unless a sterically hindered aliphatic (e.g., isobutyrate or pivalate) or aromatic ester was used, in which cases the reaction failed completely. The remaining 3′,5′-diesters (**5e–h**) were prepared from 8 in a manner analogous to that of the 2′,5′-diesters.

All compounds were characterized on the basis of ^1H NMR, IR, UV, and mass spectra and elemental analyses. A characteristic downfield shift of the sugar protons due to the deshielding effects of the acyl substituents was observed in the ^1H NMR spectra of both the di- and triesters (Table IV and Appendix 3, supplementary material); this is analogous to the effect seen with prodrugs of adenine arabinoside.[6] Darzynkiewicz et al. found that the chemical shifts of the individual methyl groups of the three monomethyl ethers of *ara*-A each exhibit a characteristic value. These values do not change significantly with the presence of additional methyl ethers. This allowed the identification of the three diethers and the triether derivative from the ^1H NMR spectra.[10] We have now found that this observation can be extended to the aliphatic and aromatic di- and triesters of 1 by comparison of their ^1H NMR spectra to those of the respective monoesters (Table I). The distinctive ^1H NMR spectra exhibited by the 2′-monoesters, specifically, the diastereotopic shift of the two α-protons and the characteristic upfield shift of all

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Table II. Reaction and Purification Conditions and Physical and Spectral Properties of Triester Prodrugs of 6-Methoxypurine Arabinoside

no.	ester	% yield	reaction time (h)	purification[a] method	mp, °C	formula[b]	UV λ_{max}, nm (ϵ)[c]	IR, cm^{-1}
2a	CH_3CO	71	0.17	A	viscous oil	$C_{17}H_{20}N_4O_8$	246.5 (10700)	1748.2, 1600.2
2b	CH_3CH_2CO	84	22.0	B	viscous oil	$C_{20}H_{26}N_4O_8$	246.5 (11600)	1751.7, 1599.4
2c	$CH_3(CH_2)_2CO$	35	3.0	B	viscous oil	$C_{23}H_{32}N_4O_8$	246.8 (11100)	1747.0, 1599.4
2d	$CH_3(CH_2)_3CO$	60	48.0	B, C	viscous oil	$C_{26}H_{38}N_4O_8$	246.3 (11300)	1746.4, 1599.3
2e	$(CH_3)_2CHCO$	72	25.0	C	viscous oil	$C_{23}H_{32}N_4O_8$	246.5 (10700)	1742.3, 1598.4
2f	$(CH_3)_3CCO$	67	72.0[d]	C, D	135–136	$C_{26}H_{38}N_4O_8$	246.2 (11000)	1732.8, 1598.1
2g	C_6H_5CO	ND[e]	72.0	B, D	151–152	$C_{32}H_{26}N_4O_8$	232.5 (42000)	1733.6, 1719.3, 1599.3
2h	$4\text{-}CH_3OC_6H_4CO$	19	24.0	B, D	98–102	$C_{35}H_{32}N_4O_{11}$	259.5 (60600)	1718.3, 1605.4
2i	$4\text{-}CH_3C_6H_4CO$	54	144.0	B, E	140–142	$C_{35}H_{32}N_4O_8$	242.4 (51300)	1718.3, 1598.1
2j	$4\text{-}ClC_6H_4CO$	26	1.0	A, E	126–128	$C_{32}H_{23}N_4O_8Cl_3$	243.5 (62000)	1730.4, 1719.4, 1596.6
2k	$4\text{-}CH_3C_6H_4SO_2$	22	120.0	B, F	168–170	$C_{32}H_{32}N_4O_{11}S_3$	226.0 (55800)	1599.5, 1371.9, 1353.6, 1191.8, 1177.7
2l	CH_3SO_2	94	24.0	B	80–85 dec	$C_{14}H_{20}N_4O_{11}S_3$[f]	245.4 (12200)	1601.3, 1351.8, 1177.6
2m	$C_6H_5OCH_2CO$	26	24.0	B, C	64–66	$C_{35}H_{32}N_4O_{11}$	250.9 (11000)	1768.4, 1599.8
2n	$C_6H_5CH_2CO$	68	2.33	B, C	viscous oil	$C_{35}H_{32}N_4O_8$[g]	247.2 (11700)	1747.4, 1599.3

[a] Solvent used with silica gel flash column and/or special purification steps taken were as follows: (A) isocratic elution with $CHCl_3$, (B) gradient of $CHCl_3$ to $CHCl_3$/acetone, (C) isocratic elution with $CHCl_3$/heptane, (D) recrystallized from MeOH/H_2O, (E) recrystallized from EtOAc/heptane, (F) recrystallized from acetone/heptane. [b] Satisfactory elemental analyses (±0.4%) were obtained for all compounds. Solvates were confirmed by ^1H NMR. [c] UV spectra were recorded in 95% ethanol. [d] Solution was held at ambient temperature for 24.0 h, then refluxed for an additional 48.0 h. [e] ND: not determined. This product was isolated as an impurity in a reaction preparing the 2′-benzoate. [f] (·0.20C_3H_6O·0.15$CHCl_3$·0.30H_2O). [g] (·0.75H_2O).

Table III. Reaction and Purification Conditions and Physical and Spectral Properties of Diester Prodrugs of 6-Methoxypurine Arabinoside

no.	ester	% yield[a]	synthetic method[b]	purification method[c]	mp, °C	formula[d]	UV λ_{max}, nm (ϵ)[e]	IR, cm^{-1}
3a	CH_3CO	15	C	A, B	138–139	$C_{15}H_{18}N_4O_7$	247.6 (10800)[f]	1741.0, 1604.1
3b	CH_3CH_2CO	5	D	A, B, C, D	viscous oil	$C_{17}H_{22}N_4O_7$·0.15C_6H_{14}	249.0 (10200)[f]	1750.1, 1601.2
3c	$CH_3(CH_2)_2CO$	25	D	A, E	78–80	$C_{19}H_{26}N_4O_7$·0.10C_6H_{14}	248.3 (10700)[f]	1741.2, 1601.2
3d	$CH_3(CH_2)_3CO$	20	E	A	viscous oil	$C_{21}H_{30}N_4O_7$·0.30H_2O	247.1 (11300)[f]	1748.6, 1601.0
3e	$CH_3(CH_2)_4CO$	19	D	E, C	viscous oil	$C_{23}H_{34}N_4O_7$·0.50$CHCl_3$	247.9 (9300)[f]	1749.9, 1600.6
3f	$CH_3(CH_2)_5CO$	37	D	E	viscous oil	$C_{25}H_{38}N_4O_7$	247.0 (9870)[f]	1749.2, 1601.3
3g	$CH_3(CH_2)_6CO$	24	D	E	viscous oil	$C_{27}H_{42}N_4O_7$	246.7 (12600)[f]	1749.9, 1601.4
3h	$(CH_3)_2CHCO$	13	D	A, E	129–131	$C_{19}H_{26}N_4O_7$	249.7 (9800)[f]	1741.1, 1600.4
3i	$(CH_3)_3CCO$	62	D	E	121–123	$C_{21}H_{30}N_4O_7$	247.9 (9600)[f]	1727.7, 1600.9
3j	C_6H_5CO	32	D	E, F	151–153	$C_{25}H_{22}N_4O_7$	233.6 (33400)	1728.4, 1598.8
3k	$CH_3OC_6H_4CO$	44	D	E, F	168–169	$C_{27}H_{26}N_4O_9$	259.8 (38400)	1707.7, 1603.7
3l	$CH_3C_6H_4CO$	40	D	E, F	169–170	$C_{27}H_{26}N_4O_7$	242.6 (44500)	1733.5, 1721.1, 1603.1
3m	$C_6H_5OCH_2CO$	65	D[g]	E	viscous oil	$C_{27}H_{26}N_4O_9$[h]	249.0 (10600)	1768.3, 1774.7, 1602.2
3n	$C_6H_5CH_2CO$	9	E	A, G	viscous oil	$C_{27}H_{26}N_4O_7$[i]	247.7 (11300)	1747.5, 1602.0
4a	CH_3CO	10	C	A, H, I	169–171	$C_{15}H_{18}N_4O_7$	246.7 (12300)	1747.8, 1603.4
4b	$CH_3(CH_2)_3CO$	73	F	N	viscous oil	$C_{21}H_{30}N_4O_7$	247.9 (9800)[f]	1742.4, 1601.1
4c	$(CH_3)_2CHCO$	77	F	J	viscous oil	$C_{19}H_{26}N_4O_7$	247.7 (9750)[f]	1738.0, 1603.2
4d	$(CH_3)_3CCO$	69	F	J	brittle foam	$C_{21}H_{30}N_4O_7$[j]	247.8 (11900)[f]	1734.4, 1601.7
4e	C_6H_5CO	91	F	N	77–79	$C_{25}H_{22}N_4O_7$[k]	234.2 (28100)[f]	1719.3, 1601.3
4f	$4\text{-}CH_3OC_6H_4CO$	90	F	N, F	167–168	$C_{27}H_{26}N_4O_9$	254.6 (31900)[f]	1710.3, 1606.5
5a	CH_3CO	67	J	C	viscous oil	$C_{15}H_{18}N_4O_7$·0.15$CHCl_3$	247.2 (11800)	1748.9, 1604.1
5b	CH_3CH_2CO	65	J	A, K	113–115	$C_{17}H_{22}N_4O_7$[l]	247.4 (11500)	1748.2, 1605.1
5c	$CH_3(CH_2)_2CO$	73	J	A	109–111	$C_{19}H_{26}N_4O_7$·0.09C_3H_6O	247.3 (10000)	1740.9, 1603.6
5d	$CH_3(CH_2)_3CO$	92	J	A	viscous oil	$C_{21}H_{30}N_4O_7$[m]	247.1 (12100)	1744.8, 1603.4
5e	$(CH_3)_2CHCO$	93	H	A	viscous oil	$C_{19}H_{26}N_4O_7$·0.25$CHCl_3$	247.0 (10200)	1740.6, 1603.0
5f	$(CH_3)_3CCO$	76	H	C	viscous oil	$C_{21}H_{30}N_4O_7$[n]	247.9 (10100)[f]	1739.5, 1604.9
5g	C_6H_5CO	44	H	L	161–163	$C_{25}H_{22}N_4O_7$·0.20$C_4H_8O_2$	234.6 (22000)[f]	1723.5, 1602.2
5h	$4\text{-}CH_3OC_6H_4CO$	34	I	C	68–70	$C_{27}H_{26}N_4O_9$·0.25$CHCl_3$	259.8 (21000)[f]	1717.3, 1605.3
5i	$C_6H_5OCH_2CO$	45	D	E, M	viscous oil	$C_{27}H_{26}N_4O_9$[o]	250.3 (13200)	1764.5, 1602.3
5j	$C_6H_5CH_2CO$	25	E[q]	E, C	viscous oil	$C_{27}H_{26}N_4O_7$[p]	247.2 (13400)	1746.0, 1603.4

[a] Yields for the 2′,3′-diesters were overall yields from 1. Yields for the 2′,5′- and 3′,5′-diesters were calculated from the acylation step to final compound. [b] Synthetic methods are described in the Experimental Section of this paper. [c] Solvent used and purification techniques were as follows: (A) silica gel flash column with a $CHCl_3$ to $CHCl_3$/acetone gradient, (B) silica gel preparative plate with a $CHCl_3$/acetone eluant, (C) chromatotron rotor (silica, 4 mm) with a $CHCl_3$/acetone gradient, (D) acetonitrile/hexane extraction, (E) silica gel glash column with a heptane/EtOAc gradient, (F) recrystallization from EtOAc/heptane, (G) silica gel preparative plate with a $CHCl_3$/EtOAc gradient, (H) MPLC tandem silica columns (1.5 × 25.0 cm and 1.5 × 100.0 cm) using an isocratic EtOAc/acetone eluant, (I) recrystallized from acetone/heptane, (J) chromatotron rotor (silica, 4 mm) and a $CHCl_3$ eluant, (K) recrystallized from $CHCl_3$/heptane, (L) chromatotron rotor (silica, 2 mm) eluted with hexane/EtOAc, (M) silica gel preparative plate with a heptane/EtOAc eluant, (N) MPLC tandem silica columns with $CHCl_3$/acetone eluant. [d] Satisfactory elemental analyses (±0.4%) were obtained for all compounds. Solvates were confirmed by ^1H NMR. [e] UV spectra were recorded in 95% ethanol unless noted otherwise. [f] UV spectrum recorded in pH 7.00 phosphate buffer. [g] See the Experimental Section for specific deprotection method. [h] ·0.10H_2O·0.10C_3H_6O. [i] ·0.30H_2O·0.20C_3H_6O. [j] ·0.05$CHCl_3$·0.05C_3H_6O. [k] ·0.05$CHCl_3$·0.25 C_3H_6O. [l] ·0.30$CHCl_3$·0.10H_2O. [m] ·0.05$CHCl_3$·0.05H_2O·0.05C_3H_6O. [n] ·0.10$CHCl_3$·0.30C_3H_6O. [o] ·0.25$CHCl_3$·0.15H_2O. [p] 0.15$CHCl_3$·0.35H_2O·0.05C_3H_6O. [q] Intermediate **6n** was treated with 10.0 equiv of tetrabutylammonium fluoride to generate the rearranged **5j**.

substituent protons of the 2′-ester relative to the 3′- and 5′-esters,[11,12] were also observed in these derivatives. The ^1H NMR also indicated that the purine ring was intact, since the chemical shifts of the protons at H-2, H-8, and

Table IV. Solubilities, Stabilities, Estimated Partition Coefficients, and Urinary Recoveries of 6-Methoxypurine Arabinoside and Di- and Triester Prodrugs

no.	R	solubility,[a] mM	stability,[b] $T_{1/2}$, days	estimated log P	% urinary recovery[c]
1		49.8	*d*	0.50	3.8 (oral) 25.9 (ip)
2a	CH_3CO	81.1	15.2	1.93	8.3
2b	CH_3CH_2CO	3.55	127	2.75	0.8
2c	$CH_3(CH_2)_2CO$	0.102	ND[e]	4.07	0.7
2d	$CH_3(CH_2)_3CO$	0.021	ND	>4.50	2.2
2e	$(CH_3)_2CHCO$	0.141	ND	3.96	2.1
2f	$(CH_3)_3CCO$	0.014	ND	>4.50	5.4
2g	C_6H_5CO	0.008	ND	>4.50	0.9
2h	4-$CH_3OC_6H_4CO$	<0.005	ND	>4.50	4.1
2i	4-$CH_3C_6H_4CO$	<0.005	ND	>4.50	5.6
2j	4-ClC_6H_4CO	<0.005	ND	>4.50	4.5
2k	4-$CH_3C_6H_4SO_2$	0.005	ND	>4.50	3.0
2l	CH_3SO_2	3.22	>70	0.58	4.4
2m	$C_6H_5OCH_2CO$	0.022	ND	>4.50	4.6
2n	$C_6H_5CH_2CO$	<0.005	ND	>4.50	4.1
3a	CH_3CO	172	19.1	1.33	19.2
3b	CH_3CH_2CO	23.5	5.25	2.07	8.7
3c	$CH_3(CH_2)_2CO$	9.60	13.1	2.90	4.8
3d	$CH_3(CH_2)_3CO$	0.694	7.02	3.83	9.1
3e	$CH_3(CH_2)_4CO$	0.140	ND	>4.40	4.8
3f	$CH_3(CH_2)_5CO$	0.030	ND	>4.40	10.5
3g	$CH_3(CH_2)_6CO$	0.036	ND	>4.40	12.6
3h	$(CH_3)_2CHCO$	3.30	28.9	2.82	2.3
3i	$(CH_3)_3CCO$	0.640	72.7	3.61	1.6
3j	C_6H_5CO	0.013	ND	3.71	0.1
3k	4-$CH_3OC_6H_4CO$	0.005	ND	4.03	0.2
3l	4-$CH_3C_6H_4CO$	0.005	ND	>4.40	0.2
3m	$C_6H_5OCH_2CO$	0.067	ND	3.40	7.6
3n	$C_6H_5CH_2CO$	0.204	ND	3.48	2.7
4a	CH_3CO	7.10	22.5	1.40	10.6
4b	$CH_3(CH_2)_3CO$	1.30	51.6	3.58	15.3
4c	$(CH_3)_2CHCO$	9.11	53.6	2.70	4.2
4d	$(CH_3)_3CCO$	1.35	178	3.40	15.7
4e	C_6H_5CO	<0.005	ND	3.39	5.8
4f	4-$CH_3OC_6H_4CO$	0.006	ND	3.65	0.2
5a	CH_3CO	12.3	30.6	1.75	16.3
5b	CH_3CH_2CO	2.39	30.2	2.70	11.0
5c	$CH_3(CH_2)_2CO$	0.422	ND	3.67	11.8
5d	$CH_3(CH_2)_3CO$	0.078	ND	>4.40	12.8
5e	$(CH_3)_2CHCO$	0.428	19.5	3.63	17.7
5f	$(CH_3)_3CCO$	0.032	ND	>4.40	1.2
5g	C_6H_5CO	0.033	ND	>4.40	4.3
5h	4-$CH_3OC_6H_4CO$	0.045	ND	>4.40	0.2
5i	$C_6H_5OCH_2CO$	0.058	ND	4.06	3.8
5j	$C_6H_5CH_2CO$	<0.005	ND	4.37	1.5

[a] At 37 °C in phosphate-buffered saline. [b] At 37 °C in 50 mM potassium phosphate buffer (pH 7.00). [c] Urinary recoveries were reported as percent of total dose recovered in the urine as compound 1 following oral administration. None of the respective esters were detected in the urine. [d] No decomposition was seen after 60 days of incubation. [e] Compound's solubility in pH 7.00 buffer was so poor that its half-life could not be determined.

the exocyclic methoxy group were extremely stable.

The infrared spectra (Tables II and III) confirmed the presence of an intact purine ring (~1600 cm^{-1}) and an ester carbonyl (1730–1768 cm^{-1}). Only on a few occasions were multiple ester carbonyl bands present (**2g,j,k,3k,n**). The presence of sulfonate esters (**2k** and **2l**) was indicated by absorbances in the ranges of 1352–1372 and 1177–1191 cm^{-1}.

Both the EI and CI mass spectra (supplementary material) of the di- and triesters followed the characteristic fragmentation patterns described by McCloskey.[13] The molecular ion (M + H) was seen in each case as well as two abundant and characteristic ions at m/z 179 and 151. The fragment at m/z 179 was thought to contain the heterocyclic base, C-1′, and the sugar ether oxygen. The ion at m/z 179 was thought to eliminate CO to provide the second ion at m/z 151. With the aliphatic esters, fragments corresponding to the fully acylated sugar (M – base) as well as the various stages of deacylation of those sugars and the corresponding acyl groups were routinely observed.

Biological Evaluation and Discussion

The bioavailability of each prodrug was evaluated using 48-h urinary recoveries of intact 1 following oral administration.[14,15] The results are summarized in Table IV. Of

(11) Chamberlain, S. D.; Moorman, A. R.; Jones, L. A.; de Miranda, P.; Reynolds, D. J.; Koszalka, G. W.; Krenitsky, T. A. Unpublished results.

(12) Moorman, A. R.; Chamberlain, S. D.; Jones, L. A.; de Miranda, P.; Reynolds, D. J.; Peoples, M. E.; Krenitsky, T. A. Unpublished results.

(13) McCloskey, J. A. In *Basic Principles in Nucleic Acid Chemistry*; Ts'o, P. O. P., Ed.; Academic Press: New York, 1974; Vol. 1, p 209–309.

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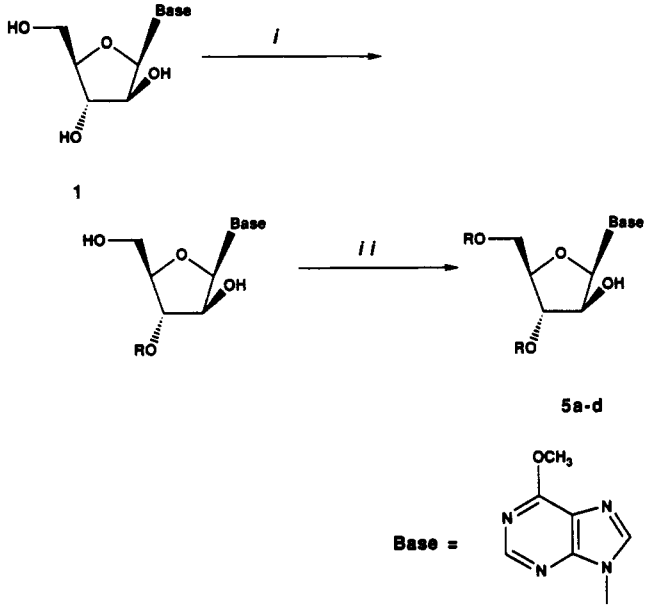
(15) Krasny, H. C.; de Miranda, P.; Blum, M. R.; Elion, G. B. *J. Pharmacol. Exp. Ther.* **1981**, *216*, 281.

Scheme IV.[a] Enzymatic Synthesis of 3′,5′-Diester Prodrugs of 6-Methoxypurine Arabinoside



[a] (i) Lipase, 2,2,2-trichloroethyl ester, acetonitrile; (ii) Lipozyme IM-20, 2,2,2-trichloroethyl ester.

the fully acylated compounds, the triacetate was two times more water soluble than 1 and showed a 2-fold improvement in bioavailability, but this series appeared to have an optimal lipophilicity. Although a great percentage of the triesters led to improved bioavailability of 1, the improvement was modest, and the water solubilities, with the exception of that for the triacetate, were very low. It was not determined whether these compounds were being sequestered in the fat tissues or were simply not well absorbed, but no intact prodrug was seen in the urine.

The diesters were divided into two distinct groups—the aliphatic diesters, which increased the bioavailability of 1, and the aromatic diesters, which did not. The reasons for this dichotomy are not understood at this time.

2′,3′-Diacetate **3a** showed a 5-fold increase in bioavailability and a 3-fold increase in water solubility, giving it the best combination of these properties of any prodrug described in this paper. 2′,5′-Diacetate **4a** and 3′,5′-diacetate **5a** displayed a 3- and 4-fold increase in bioavailability, respectively. Both had lower water solubilities compared to that of 1.

The enhanced water solubility, important to the anticipated development of an intravenous formulation, and improved bioavailability seen with 2′,3′-diacetate **3a** make it worthy of further study. The full therapeutic value of this and related[11,12] compounds awaits further study.

Experimental Section

Materials. 6-Methoxypurine arabinoside (1) was prepared as previously described.[1] Toluenesulfonyl chloride was freshly recrystallized from CCl_4 immediately prior to use. Phenylacetic anhydride was purchased from Parish Chemical Company (Orem, UT). Lipase (EC 3.1.1.3, type II, porcine pancreas) was purchased from Sigma Chemical Co. (St. Louis, MO). Lipozyme IM 20 (EC 3.1.1.3, immobilized *Mucor miehei* lipase) was purchased from Novo Laboratories (Danbury, CT). All other chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI); these were of the highest purity available and were used without further purification. Silica gel products were purchased from EM Science (Cherry Hill, NJ). Flash chromatography was carried out using Kieselgel 60, 230–400 mesh. Analytical TLC was performed on Kieselgel 60 F_{254} (0.2-mm thickness) plastic-backed sheets. Preparative-layer chromatography was performed on Kieselgel 60 F_{254} (2.0-mm thickness) glass-backed plates. The 0.22-μm nylon filters used for filter sterilization were purchased from Millipore (Bedford, MA), and the 0.2-μm nylon filters used for HPLC sample preparation were purchased from Alltech Associates (Avondale, PA). HPLC was carried out on a Perkin-Elmer System 4 liquid chromatograph equipped with a Perkin-Elmer LC-95 variable-wavelength detector, ISS-100 autoinjector, and LCI-100 integrator, unless otherwise indicated. The columns used were a Zorbax ODS, 4.6 × 250 mm, 5-μm column (Wilmington, DE) and an Alltech/Applied Sciences C-18, 4.6 × 250 mm, 10-μm column (State College, Pa).

General Methods. Melting points were determined on a Thomas Hoover UniMelt apparatus and are uncorrected. Ultraviolet spectra were recorded using a Perkin-Elmer Lambda 7 spectrometer equipped with a thermoelectric cell holder set to either 25.0 or 37.0 °C, as indicated by a digital readout from a thermistor in each cell holder. ^1H NMR spectra were recorded on a Varian XL-200 (200.058 MHz) or a Varian XL-300 (299.945 MHz) spectrometer and are reported relative to tetramethylsilane. Assignments of H-8 and H-2 were not unequivocal and may be reversed. Mass spectra were recorded by Oneida Research Services (Whitesboro, NY). A Mattson Cygnus 100 Fourier transform spectrometer interfaced to a Pixel 80 microcomputer was used in recording infrared spectra. Elemental analyses were performed by Atlantic Microlabs (Atlanta, GA).

General Synthesis of 2′,3′,5′-Triesters of 6-Methoxypurine Arabinoside (2a,b,d–i,l,n). Method A. 6-Methoxypurine arabinoside (1, 1.00 g, 3.54 mmol) was suspended in anhydrous acetonitrile (70 mL), and pyridine (20 mL) was added to effect complete solution. The appropriate acid anhydride (14.0 mmol) was added and the mixture was stirred at room temperature under argon for the time specified in Table I. The reaction was quenched by adding an excess of ethanol (20 mL) and evaporating to dryness, followed by coevaporation with additional ethanol (2 × 100 mL). The residue was then purified by flash chromatography on silica gel (25 g/mmol) with the solvent system indicated in Table I.

General Synthesis of 2′,3′,5′-Triesters of 6-Methoxypurine Arabinoside (2c,j,k,m). Method B. 6-Methoxypurine arabinoside (1, 1.00 g, 3.54 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (0.02 g, 0.19 mmol) were dissolved in pyridine (20 mL). With the solution at room temperature, the appropriate acid chloride (19.3 mmol) was added and the solution was stirred under argon for the time specified in Table I. At that point, the reaction was quenched with an excess of ethanol (20 mL) and purified in the same manner as reactions using an anhydride.

9-(2,3-Di-*O*-acetyl-β-D-arabinofuranosyl)-6-methoxy-9*H*-purine (3a) and 9-(3,5-Di-*O*-acetyl-β-D-arabinofuranosyl)-6-methoxy-9*H*-purine (4a). Method C. Compound 1 (3.00 g, 10.3 mmol) was dissolved in a mixture of acetonitrile (50 mL) and pyridine (50 mL). Acetic anhydride (21.2 mmol) in acetonitrile (20 mL) was added over 3.0 h at room temperature under argon. The reaction was quenched with ethanol (50 mL) after 7 h. After coevaporation with ethanol (2 × 100 mL), the residue was purified by flash chromatography on silica gel (25.0 g/mmol) with the solvent system indicated in Table II. Fractions containing a mixture of the three diesters were further purified by preparative HPLC (Zorbax ODS, 21.2 mm × 25 cm; isocratic eluant of 65% acetonitrile and 35% water at a flow rate of 8.0 mL/min).

General Synthesis of 2′,3′-Diesters of 6-Methoxypurine Arabinoside (3a–c,e–l). Method D. Compound 1 (1.00 g, 3.54 mmol) was suspended in a mixture of acetonitrile (20 mL) and pyridine (20 mL), the solution was chilled to 5 °C, and *tert*-butyldimethylsilyl chloride (1.00 g, 1.2 mmol) was added. The reaction was allowed to stir overnight under argon and warm to ambient temperature. The mixture was again cooled to 5 °C and the appropriate acid chloride was added. The reaction was allowed to stir for 0.5–16 h and was then quenched by adding methanol or ethanol (3–10 mL). After removal of half of the solvent, the residue was partitioned between hexane or chloroform (150 mL) and water (3 × 50 mL). The combined aqueous phase was back-extracted, and the combined organic phases were dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (50 g of silica gel/g of product, chloroform/heptane eluant) to afford the acylated intermediates **6a–c** and **6e–m**.

Intermediates **6a–c,e–l** (1.5 mmol) were dissolved in tetrahydrofuran (40 mL) and chilled to 5 °C. Water (1.0 mL) and

tetrabutylammonium fluoride in tetrahydrofuran (1.0 M, 5.0 mL) were added, and the solution was stirred for 1 h at ice-bath temperature. The reaction was quenched by diluting with chloroform (40 mL) and passing through a filter pad of silica gel (10 g) using an eluant of chloroform/acetone (1:1, 500 mL). The solvent was removed under reduced pressure and the residue was purified by the method indicated in Table II. Fractions containing the product were combined to afford the desired compound.

6-Methoxy-9-[2,3-*O*-bis(phenoxyacetyl)-β-D-arabinofuranosyl]-9*H*-purine (3m). Intermediate **6m** (1.4 mmol) was deprotected by suspension in 80% acetic acid (100 mL) and heating to 50 °C for 3 h. Ethanol (100 mL) was added, and the volatiles were removed on a rotary evaporator. The residue was purified as indicated in Table II.

General Synthesis of 2′,3′-Diesters of 6-Methoxypurine Arabinoside (3d,n). Method E. This synthesis was identical to method D except that the appropriate acid anhydride was added in place of the acid chloride. Acylated intermediates **6d** and **6n** were produced by this method.

9-[3,5-*O*-Bis(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]-6-methoxy-9*H*-purine (7) and 9-[2,5-*O*-Bis(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]-6-methoxy-9*H*-purine (8). Compound 1 (2.82 g, 10.0 mmol) was dissolved in anhydrous dimethylformamide (50 mL). Imidazole (3.40 g, 50 mmol) and *tert*-butyldimethylsilyl chloride (3.77 g, 25 mmol) were added. The mixture was stirred at ambient temperature under argon for 48 h, evaporated to dryness, and partitioned between chloroform (100 mL) and water (40 mL). The aqueous portion was extracted with additional chloroform (2 × 50 mL), and the combined organic layers were dried ($MgSO_4$). After filtration and evaporation, the residue was purified by column chromatography on silica gel (250 g, 15.0 × 5.0 cm) with an eluant of 0.5% ethanol in chloroform/heptane (3:1). The first compound eluted was 9-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]-6-methoxy-9*H*-purine. The second compound eluted was 3,5-di-*O*-(*tert*-butyldimethylsilyl)-6-methoxypurine arabinoside (1.47 g, 29% yield, 7). The last compound eluted was 2,5-di-*O*-(*tert*-butyldimethylsilyl)-6-methoxypurine arabinoside (0.99 g, 19% yield, 8).

9-[3-*O*-(*tert*-Butyldimethylsilyl)-β-D-arabinofuranosyl]-6-methoxy-9*H*-purine (9). Compound 7 (8.68 g, 17.0 mmol) was suspended in 80% acetic acid (170 mL) at 50 °C for 6 h to remove the 5′-*tert*-butyldimethylsilyl group. The reaction was cooled to ambient temperature, evaporated to dryness, and recrystallized from ethyl acetate. This gave compound **9** in 68% yield.

9-[2-*O*-(*tert*-Butyldimethylsilyl)-β-D-arabinofuranosyl]-6-methoxy-9*H*-purine (10). Compound 8 (0.51 g, 1.0 mmol) was suspended in 80% acetic acid (10 mL) at 80 °C for 1 h to remove the 5′-*tert*-butyldimethylsilyl group. The reaction was cooled to ambient temperature, evaporated to dryness, and purified by flash chromatography on silica gel (25.0 g) using chloroform/acetone (6:1). This gave compound **10** in 51% yield.

General Synthesis of 2′,5′-Diesters of 6-Methoxypurine Arabinoside (4b–e). Method F. Compound **9** (0.60 g, 1.50 mmol) was dissolved in acetonitrile (20 mL) and pyridine (0.26 g, 3.3 mmol). 4-(*N*,*N*-Dimethylamino)pyridine (0.012 g, 0.1 mmol) and the appropriate acid anhydride (4.50 mmol) were added, and the reaction was allowed to stir at ambient temperature under argon for 0.5–16 h. At this point, the reaction was quenched by adding ethanol (10 mL), and the solution was evaporated to dryness and then coevaporated with additional ethanol (2 × 5 mL). The residue was purified by flash chromatography on silica gel.

The appropriate 2′,5′-bisacylated-3′-(*tert*-butyldimethylsilyl)-6-methoxypurine arabinoside (**11b–e**, 1.15 mmol) was dissolved in tetrahydrofuran (23 mL) and water (1.2 mL) was added. This solution was treated with tetrabutylammonium fluoride in tetrahydrofuran (1.0 M, 1.25 mL), stirred for 1 h at ambient temperature, and then quenched by diluting with chloroform (20 mL) and applied directly to a silica gel pad (20 g). The pad was washed with 1:1 chloroform/acetone (200–500 mL) and the volatiles were removed. The residue was purified as indicated in Table II.

6-Methoxy-9-[2,5-*O*-bis(4-methoxybenzoyl)-β-D-arabinofuranosyl]-9*H*-purine (4f). Method G. Compound **9** (0.50 g, 1.26 mmol) was dissolved in acetonitrile (17 mL) and triethylamine (4.2 mL). 4-Methoxybenzoyl chloride (0.645 g, 3.78 mmol) was added and the reaction was stirred at 5 °C under argon for 3 h. At this point, the reaction was quenched by adding 2-propanol (10 mL), and the solution evaporated to dryness and then coevaporated with ethanol (2 × 5 mL). The residue was purified by flash chromatography on silica gel.

9-[3-*O*-(*tert*-Butyldimethylsilyl)-2,5-*O*-bis(4-methoxybenzoyl)-β-D-arabinofuranosyl]-6-methoxy-9*H*-purine (**11f**, 0.386 g, 0.58 mmol) was dissolved in THF (11.6 mL), to which was added 0.58 mL of water. The reaction was then treated with a 1 M solution of tetrabutylammonium fluoride (0.67 mL), stirred for 3 h at 5 °C, and then warmed to room temperature, more tetrabutylammonium fluoride (1.0 mL) was added, and the mixture was stirred for 2.5 h at room temperature. This was then diluted with chloroform (20 mL) and applied directly to a flash column of silica gel (20 g) in chloroform. The product was eluted with chloroform (200 mL) followed by 1:1 chloroform/acetone (200 mL). The product was further purified as described in Table II.

General Synthesis of 3′,5′-Diesters of 6-Methoxypurine Arabinoside (5e–g). Method H. Compound 10 was converted to the desired 3′,5′-diesters in a manner identical to method F.

6-Methoxy-9-[3,5-*O*-bis(4-methoxybenzoyl)-β-D-arabinofuranosyl]-9*H*-purine (5h). Method I. This synthesis was identical to method H except that 4-methoxybenzoyl chloride was added in place of the corresponding acid anhydride. Acylated intermediate **12h** was produced by this method.

General Synthesis of 3′,5′-Diesters of 6-Methoxypurine Arabinoside (5a–d). Method J. Compound 1 (1.00 g, 3.50 mmol) was dissolved in a mixture of acetonitrile (200 mL) and the appropriate 2,2,2-trichloroethyl ester (35.0 mmol), which was prepared by the addition of the desired acid chloride to 2,2,2-trichloroethanol in pyridine. Lipase (11 g) was added and the reaction was stirred at 40 °C for 8–12 h under argon. At this point, the reaction was filtered and the filtrate was concentrated to an oil. This crude material was purified by flash chromatography to yield the 3′-monoester as an uncharacterized intermediate.

The 3′-monoester (1.2 mmol) was dissolved in the appropriate 2,2,2-trichloroethyl ester (5–10 mL) and Lipozyme IM 20 (1.34 g) added. The reaction was heated to 60 °C, with stirring, for about 1 h and then filtered to remove the enzyme. The filtrate was concentrated and this crude material was purified by the method indicated in Table II to yield the desired 3′,5′-diester.

Determination of Solubilities. A sufficient amount of each of the esters to prepare a saturated solution was mixed with 1.0 mL of pH 7.2 phosphate-buffered saline (8.0 g of sodium chloride, 0.15 g of disodium hydrogen phosphate, and 0.03 g of potassium dihydrogen phosphate in water to make 1.0 L) and agitated at 37 °C for 2 h. The samples were then filtered through 0.2-μm nylon filters and the ultraviolet spectrum was recorded. The absorbance at the λ_{max} was used to determine the concentration of the saturated solution.

Determination of Stabilities. Solutions of each of the esters (10–20 μM, 50 mL) were prepared in 50 mM potassium phosphate buffer (pH 7.00), filter sterilized into ca. 2-mL aliquots, and incubated at 37 °C. At various times, samples were withdrawn and analyzed directly by HPLC on a Zorbax ODS column. The sample was eluted at 2.0 mL/min with a linear gradient from 10% CH_3CN in water to 50% CH_3CN over 10 min, followed by a linear gradient to 100% CH_3CN over 5 min. A variable-wavelength detector set at 254 nm was used to monitor the eluate. Original conditions were regenerated with a 5-min linear gradient followed by a 15-min reequilibration phase. The rate of hydrolysis of 6-methoxypurine arabinoside (1) was determined. No decomposition of compound 1 was observed under these conditions over a period of 9 weeks.

Estimation of Octanol–Water Partition Coefficients. Octanol–water partition coefficients (log *P*) for the esters were estimated by correlation with their chromatographic capacity factors (k') according to the method of Haky and Young, using an Alltech RP-18, 4.6 × 250 mm, 10-μm column.[16] The mobile phase used was 55% methanol, 45% aqueous ammonium phosphate buffer (0.05 M, pH 7.0) at a flow rate of 2.0 mL/min.

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Samples were dissolved in methanol (1 mg/mL) for injection. Results are the average of three separate determinations.

Prodrug Disposition Studies. Two Long Evans male rats were dosed by gavage through an intragastric tube with 10 mg/kg of 6-methoxypurine arabinoside (1), or the molar equivalent of a prodrug, dissolved or uniformly suspended in water containing 0.1% Tween 80. The animals were then housed together in a metabolic cage for 48 h and given food and water ad libitum. Urine was collected for 0-24 and 24-48 h following administration of the test compound. Samples of the pooled urine were then filtered through 0.22-μm nylon filters. A WISP automatic injector was used to inject 100 μL of the filtered urine sample onto an Alltech/Applied Sciences C-18 column. The sample was eluted with a 45-min linear gradient of 0-20% CH_3CN in 25 mM phosphoric acid previously adjusted to pH 7.2 with ammonium hydroxide, followed by a 10-min purge of 80% CH_3CN in distilled water. Two variable-wavelength detectors set at 252 and 259 nm were used to monitor the eluate. Original conditions were regenerated with a 4-min linear gradient followed by a 15-min reequilibration phase. The standard error of these determinations was found to be less than 15% of the reported value as determined by multiple experiments (n = 3-7) with representative compounds.

Acknowledgment. We thank Mr. Aris Ragouzeos for assistance in obtaining the ^1H NMR spectra. Large quantities of compound 1 were prepared by Dr. B. Sickles of the Burroughs Wellcome Co. Chemical Development Labs. The assistance of Mr. Allen Jones and Ms. Laura Mansberg in proofreading this manuscript is gratefully acknowledged.

Registry No. 1, 91969-06-1; **2a**, 121032-46-0; **2b**, 137057-42-2; **2c**, 137057-43-3; **2d**, 137057-44-4; **2e**, 137057-45-5; **2f**, 137057-46-6; **2g**, 137057-47-7; **2h**, 137057-48-8; **2i**, 137057-49-9; **2j**, 137057-50-2; **2k**, 137057-51-3; **2l**, 137057-52-4; **2m**, 137057-53-5; **2n**, 137057-54-6; **3a**, 137057-55-7; **3b**, 137057-56-8; **3c**, 137091-64-6; **3d**, 137057-57-9; **3e**, 137057-58-0; **3f**, 137057-59-1; **3g**, 137057-60-4; **3h**, 137057-61-5; **3i**, 137057-62-6; **3j**, 137057-63-7; **3k**, 137057-64-8; **3l**, 137057-65-9; **3m**, 137057-66-0; **3n**, 137057-67-1; **4a**, 137057-68-2; **4b**, 137057-69-3; **4c**, 137057-70-6; **4d**, 137057-71-7; **4e**, 137057-72-8; **4f**, 137057-73-9; **5a**, 137057-74-0; **5b**, 137057-75-1; **5c**, 137091-65-7; **5d**, 137057-76-2; **5e**, 137057-77-3; **5f**, 137057-78-4; **5g**, 137057-79-5; **5h**, 137057-80-8; **5i**, 137057-81-9; **5j**, 137057-82-0; **6b**, 137057-83-1; **6c**, 137057-84-2; **6d**, 137057-85-3; **6e**, 137091-66-8; **6f**, 137057-86-4; **6g**, 137057-87-5; **6h**, 137057-88-6; **6i**, 137057-89-7; **6j**, 137057-90-0; **6k**, 137057-91-1; **6l**, 137057-92-2; **6m**, 137057-93-3; **6n**, 137057-94-4; **7**, 137057-95-5; **8**, 137057-96-6; **9**, 137057-97-7; **10**, 137058-03-8; **11b**, 137057-98-8; **11c**, 137057-99-9; **11d**, 137058-00-5; **11e**, 137058-01-6; **11f**, 137091-51-1; **12h**, 137058-02-7.

Supplementary Material Available: Analytical and spectral (^1H NMR assignments, multiplicities, and coupling constants, and mass spectral fragments) data for compounds **2a-n**, **3a-n**, **4a-f**, **5a-j**, **6b-n**, **7-10**, **11b-f**, **12e-h** (37 pages). Ordering information is given on any current masthead page.

Total Synthesis of Uracil Analogues of Sinefungin

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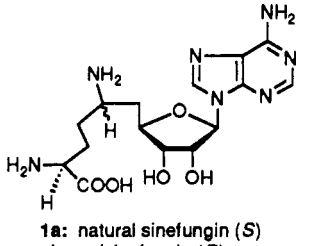
Analogues of sinefungin derivatives **18a** and **18b** have been prepared from uridine and L-aspartic acid. The key step in the synthesis was the coupling of the radical derived from **14** with the unsaturated amide **13**. The latter was produced from the known *N*-hydroxy-2-thiopyridone ester of L-aspartic acid **12** with the olefin **11**. Thus, the essential carbon skeleton was constructed by way of two radical coupling reactions. These analogues as well as **1a** and **1b** synthesized previously were tested for their antileishmanial effect in vivo and for their inhibitory activity of protein carboxymethylase (protein methylase II). The replacement of the adenine moiety by uracil or dihydrouracil considerably decreases the antiparasitic activity and the affinity for protein methylase II. The synthetic (*S*)-sinefungin was as active as the natural one. Interestingly, the C-6′ epimer **1b** was 50% less active in vitro than the natural sinefungin, but both had identical affinities for the target enzyme.

Natural sinefungin (**1a**) was isolated in 1973 from the cultures of *Streptomyces grisoleus*.[1] Its structure (**1a**) is composed of an adenosine unit to which has been attached at the C-5′ position an ornithine residue. The C-6′ chiral center has the *S* configuration, as has been confirmed by the recent synthesis of Rapoport[2] and in agreement with our own synthesis.[3]



1a: natural sinefungin (*S*)
b: episinefungin (*R*)

Natural sinefungin is a powerful antifungal agent particularly efficient against *Candida albicans*.[4] It possesses also a strong antiparasitic activity, especially against various species of *Leishmania*[5] and *Trypanosoma*.[6] However, sinefungin has serious side effects (nephrotoxicity in the dog and toxicity for bone marrow cells). In an effort to improve the therapeutic index of sinefungin, Secrist and co-workers have recently reported the synthesis of a series of analogues.[7] We ourselves disclosed a new

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0022-2623/92/1835-0063$03.00/0