Synthesis and Evaluation of a Series of 2′-*O*-Acyl Derivatives of 9-β-D-Arabinofuranosyladenine as Antiherpes Agents

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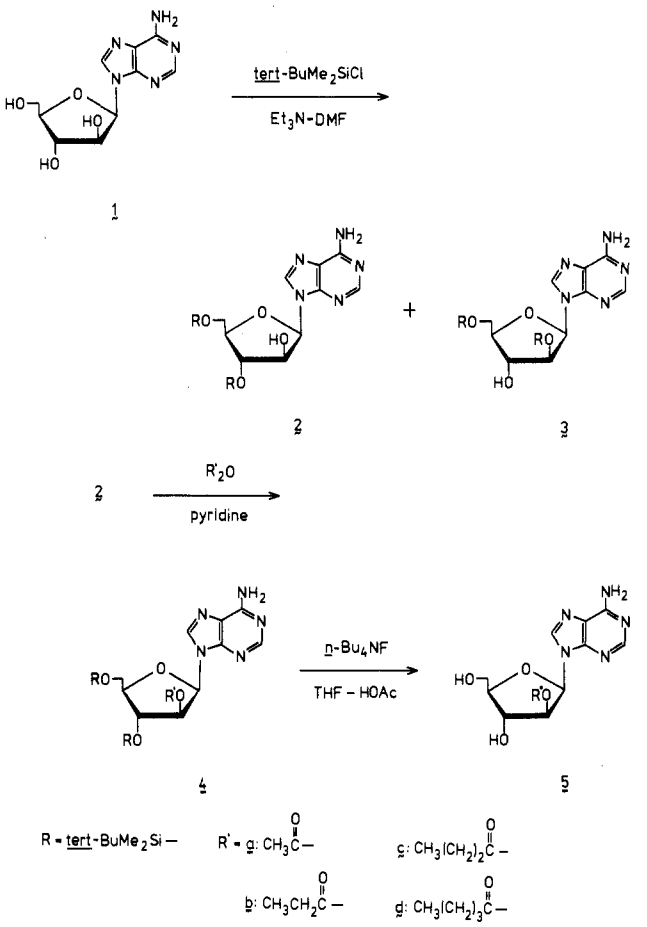
A series of four 9-(2-*O*-acyl-β-D-arabinofuranosyl)adenines (**5a–d**) was synthesized by acylation of 9-[3,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (**2**), followed by removal of the *tert*-butyldimethylsilyl groups under conditions (HOAc, tetra-*n*-butylammonium fluoride) that prevented acyl migration. The four 2′-*O*-acyl derivatives **5a–d** showed activity in vitro against herpes type 1 viruses [virus ratings = 1.5–2.6; MIC_{50} = 26–72 μg/mL (8.48–21.3 × 10^{-5} M)]. The 2′-*O*-acetyl (**5a**) and 2′-*O*-valeryl (**5d**) derivatives were evaluated in a guinea pig model for genital herpes (herpes type 2); only **5a** showed potent activity when given 6 or 24 h postinfection.

Prodrugs of 9-β-D-arabinofuranosyladenine (**1**, *ara*-A, Vira-A) have been synthesized[1–3] in an effort to improve the efficacy of the parent drug, which, despite its demonstrated usefulness in combating severe herpesvirus infections of the central nervous system and the eye,[4] suffers some limitations. These include (1) a low solubility (ca. 0.4 mg/mL at 25 °C); (2) a reluctance to transport across biological membranes, as evidenced by a negative log *P* ([octanol]/[water]), and (3) a rapid deamination to the inactive 9-β-D-arabinofuranosylhypoxanthine by the enzyme adenosine deaminase (ADase), which singly or together account for the drug's failure as a general topical agent against cutaneous herpesvirus infections and make systemic administration of the drug dificult to manage. 5′-*O*-Acyl derivatives of **1**, which have been synthesized with both straight and branched chains of C_1–C_8 units, inclusive, offer various ranges of solubility and lipophilicity, as well as resistance to ADase.[1] Several of these (C_1–C_4 and C_8 straight-chain examples) have been found topically active in a guinea pig intravaginal herpes type 2 model.[5] Diesters of *ara*-A that show activity in the guinea pig model are limited to a 2′,3′-diester, 2′,3′-di-*O*-acetyl-*ara*-A, a highly water-soluble (33 mg/mL at 23 °C), yet lipophilic [log *P* = +0.2 (pentanol–water)] species, that has emerged from the in vivo studies as the decidedly superior prodrug.[2,5] Curiously, the latter compound was also found to be resistant to ADase, owing to its being esterified at the 2′-position, a fact that was ferreted out of studies that examined both 2′- and 3′-*O*-acyl derivatives of **1** and found ADase resistance only in the former.[2] These compounds, the 2′-*O*-acyl-*ara*-A derivatives, are thus of interest in their own right as possible antiviral prodrugs. Heretofore these were available only via a tedious enzyme-mediated procedure.[2] Herein is described a straightforward chemical synthesis of the 2′-*O*-acyl derivatives of *ara*-A, as well as a study of their physicochemical properties and antiviral activities, both in vitro and in vivo.

Results and Discussion

Chemistry. The strategy to prepare the 2′-*O*-acyl derivatives of **1** requires the protection of both the 3′- and 5′-OH groups, acylation at the 2′-OH, and then deprotection without affecting the labile ester function. Our choice of protecting group was the *tert*-butyldimethylsilyl (*t*-$BuMe_2Si$) group, which had functioned well in the past on **1** for the preparation of *N*-benzoyl-9-(2,3-di-*O*-benzoyl-β-D-arabinofuranosyl)adenine[6] and both 2′,3′- and 3′,5′-di-*O*-acyl derivatives of **1**.[2] Reaction of **1** with 2.5 equiv of *tert*-butylchlorodimethylsilane under the above-referenced conditions (i.e., with *N*,*N*-dimethylformamide as solvent and imidazole as base)[6] gave a complex, nondiscriminating mixture of di- and triprotected nucleosides. It was found that the selectivity of the reaction, however,

Scheme I



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(1) Baker, D. C.; Haskell, T. H.; Putt, S. R. *J. Med. Chem.* **1978**, *21*, 1218, and references 12–14 cited therein.
(2) Baker, D. C.; Haskell, T. H.; Putt, S. R.; Sloan, B. J. *Ibid.* **1979**, *22*, 273.
(3) Haskell, T. H.; Hanessian, S. U.S. Patent 3 651 045, 1972.
(4) Pavan-Langston, D.; Buchanan, R. A.; Alford, C. A., Eds. "Adenine Arabinoside, an Antiviral Agent"; Raven Press: New York, 1975.
(5) Shannon, W. M.; Arnett, G.; Baker, D. C., Kumar, S. D.; Higuchi, W. I. *Antimicrob. Agents Chemother.* **1983**, *24*, 706.
(6) Baker, D. C.; Haskell, T. H.; Putt, S. R. In "Nucleic Acid Chemistry"; Townsend, L. B.; Tipson, R. S., Eds.; Wiley: New York, 1978; Part 2, p 505.

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Table I. Physical Data for 9-(2-O-Acyl-β-D-arabinofuranosyl)adenines (5a–d)

compd[a]	yield,[b] %	mp, °C	elution solvent ($CHCl_3$-MeOH)	$[\alpha]^{25}_D$, deg (c 1, EtOH)	UV λ_{max} (MeOH), nm ($\epsilon \times 10^{-3}$)
5a	88	195–197	90:10	−33.4	258 (14.7)
5b	85	207–210	90:10	−42.4	257 (16.0)
5c	78	209–210	80:20	−38.2	259 (14.5)
5d	86	164–166	94:6	−39.9	259 (14.8)

[a] Each compound analyzed for C, H, and N within ±0.3% of the theoretical values. [b] Yields are based on recrystallized, analytically pure samples.

was particularly affected by the base employed, and studies[7] showed that of nine organic bases investigated, a triethylamine–*N*,*N*-dimethylformamide combination directly gave a 76% yield of the desired 3′,5′-*O*-*tert*-butyldimethylsilyl derivative **2**,[8] along with only minor amounts of the 2′,5′-bis-*O*-(*tert*-butyldimethylsilyl) derivative **3** (4%), as well as 9-[5-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (11%).[6]

Acylation of **2** with the appropriate acid anhydride then gave the intermediates **4a–d** in high yield. These were easily purified by column chromatography, and the glassy products were used directly in the deprotecting reactions. Deprotection of **4a–d** proceeded smoothly and in high yield (78–88%) by the addition of excess tetra-*n*-butylammonium fluoride to a solution of **4a–d** that contained 1.25 equiv of acetic acid per *t*-$BuMe_2Si$ group. Acetic acid has been found effective[2] in preventing 2′ → 5′ *O*-acyl migration, a process that presumably involves an oxy anion formed from Si–O bond cleavage. The crude 2′-*O*-acyl derivatives so produced were purified by column chromatography and recrystallized to give analytically pure samples. (See Experimental Section and Table I for details.) The structures were confirmed by ^1H NMR spectroscopy wherein H-2′ was typically shifted downfield by ca. 1 ppm from that observed for the H-2′ of **1** due to the deshielding effects of the 2′-*O*-acyl function. (See Table VI, supplementary material, for ^1H NMR spectral data. See paragraph at the end of paper concerning Supplementary material.)

Solubility and Partition Coefficient Determinations. Aqueous solubilities for **5a–d** were determined by equilibrating the crystalline compounds with distilled water at 37 °C and pH 7.3 over a period of up to 13.5 h. The prodrug concentrations were determined by HPLC (see Experimental Section for details), and the values obtained are listed in Table II. As expected, based on the findings with other monoesters of **1**,[1] the 2′-*O*-acyl derivatives **5a–d** showed dramatic increases in solubility over that for **1**. In fact, the 2′-*O*-acetyl-*ara*-A (**5a**) is the most soluble ester yet studied. Its solubility of 93.6 mg/mL (0.30 M) at 37 °C represents a ~170-fold increase over the solubility of the parent *ara*-A hydrate [solubility ~0.5 mg/mL (1.75×10^{-3} M) at 37 °C]. While the others show less dramatic solubilities (Table II), all represent considerable improvement in solubility over the parent **1**.

Partition coefficients were determined by equilibrating dilute aqueous solutions of **5a–d** with 1-octanol at 37 °C. (See Experimental Section for details and Table III for data.) Compounds **5a–c** showed, in contrast to their 5′-*O*-acyl counterparts,[1] a negative value for log *P*, while **5d** gave log *P* = +0.41. A plot of log *P* vs. chain length gave a linear plot, with a *y* intercept = −1.77 and a slope of 0.436 (correlation = 0.9998). This behavior is in line with the expected ~3-fold increase in *P* per additional methylene group, a phenomenon that has been observed in other systems.[9] It is interesting that, while solubility decreases

Table II. Solubility Data for Compounds 5a–d in Aqueous Buffer at pH 7.3 and 37 °C[a]

sample[b]	amt, mg	hour	concn, mg/mL	mean concn, mg/mL
5a	150	4.0	93.6	93.6
		8.0	93.6	
		13.5	c	
5b[d]	20	4.0	11.1	11.7 (±1.13)
		8.0	11.0	
		13.5	13.0	
5c	10	4.0	3.38	3.41 (±0.118)
		8.0	3.54	
		13.5	3.31	
5d	20	4.0	9.28	10.4 (±0.941)
		8.0	10.8	
		13.5	11.0	

[a] During the solubility experiments, prodrug decomposition invariably resulted and was corrected for. The amount of decomposition at 13.5 h was as follows: for 5a, 11%; for 5b, 12%; for 5c, 7.8%; for 5d, 7.9%. This pattern fits quite well that expected for chemical stability, based on findings for the 5′-monoesters of *ara*-A (see ref 1). [b] Compounds were shown to be pure by HPLC at the outset of the experiment. [c] Buffer capacity exceeded due to prodrug decomposition. All other samples showed an insignificant lowering of ~0.12 pH unit, maximum. [d] An unknown peak that increased with time was noted by HPLC.

Table III. Octanol-Water Partition Coefficient Data for Compounds 5a–d at 37 °C

compd	hour	O/W[a] *P*	mean *P*	log of mean *P*
5a	1.0	0.149	0.130 (±0.012)	−0.886
	3.0	0.134		
	4.5	0.118		
	10.5	0.127		
	24.5	0.122		
5b	1.0	0.336	0.335 (±0.015)	−0.475
	3.0	0.359		
	4.5	0.318		
	10.5	0.335		
	24.5	0.329		
5c	1.0		0.952 (±0.035)	−0.0214
	3.0	0.911		
	4.5	0.969		
	10.5			
	24.5	0.975		
5d	1.0	2.5	2.6 (±0.31)	+0.41
	3.0	2.5		
	4.5	2.2		
	10.5	3.0		
	24.5	2.8		

[a] Octanol/water distribution of the drug, as determined by HPLC analysis. These results were verified by a duplicate run.

(7) Crews, R. P.; Kumar, S. D.; Baker, D. C., unpublished work.
(8) Compound **2** has been reported to be prepared on a small scale by using *t*-$BuClMe_2Si$/THF/$AgNO_3$. We found no advantage to this procedure. See: Ogilvie, K. K.; Hakimelahi, G. H.; Proba, Z. A.; McGee, D. P. C. *Tetrahedron Lett.* **1982**, *23*, 1997.
(9) Flynn, G. L.; Yalkowsky, S. H. *J. Pharm. Sci.* **1972**, *61*, 838.

Table IV. In Vitro Antiviral Data for Compounds 5a-d[a]

compd	MIC_{50}[b] μg/mL	MIC_{50}[b] M × 10^5	VR[c]
5a	26.2	8.48	2.3
5b	29.3	9.06	1.9
5c	71.9	21.3	1.5
5d	56.8	16.2	2.6
1 (control)	9.5	3.45[d]	2.0

[a] Testing procedure (see ref 10): 0.1 mL of test compound (320 to 0.32 μg/mL) and 0.1 mL of virus suspension [32 CC ID_{50} units (i.e., cell culture infectious dose, 50%) per well] were added to triplicate wells of Microtest II plates containing Vero cell monolayers. The plates were sealed and incubated at 37 °C for 3 days, at which time they were examined microscopically for cytopathogenic effects. Test medium: Eagle's essential medium plus 2% fetal bovine serum. [b] MIC_{50} (minimum inhibitory concentration, 50%) is the minimum concentration required to inhibit virus-induced cytopathogenic effects by 50%. [c] Virus rating (VR) is a weighted measurement of in vitro antiviral activity based on the inhibition of virus-induced cytopathogenic effects and on the cytotoxicity exhibited by the drug (see ref 1); VR > 1.0, active; VR = 0.5-0.9, marginal; VR < 0.5, inactive. [d] Calculated as $1 \cdot H_2O$.

drastically with increasing ester chain length of **5a–c** (Table II), **5d** shows an abrupt increase in solubility, perhaps attributable to a less densely packed crystal lattice from that of its analogues. This difference in crystal packing is also reflected in the lowered melting point of **5d** over the melting points of its analogues. (See Table I.)

Biological Evaluation. Compounds **5a–d** were evaluated in vitro against herpes simplex virus type 1 (strain 377).[10] As the data reveal (see Table IV), all compounds were active, showing a virus rating (VR)[11] between 1.5 and 2.6 (VR = 2.0 for 1 in a side by side comparison test), with MIC_{50} values ranging from 26.2 μg/mL (8.48×10^{-5} M) for **5a** to 71.9 μg/mL (21.3×10^{-5} M) for **5c**, which are considerably higher than that for 1 [MIC_{50} = 9.5 μg/mL (3.45×10^{-5} M)]. Such in vitro results have been typical of other C_1–C_9 straight-chain prodrugs of 1.[1,2]

Compounds **5a** and **5d**, which represent the extremes in the series under study insofar as solubility and lipophilicity are concerned, were selected for in vivo evaluation in a guinea pig model[12] for herpes type 2 infections. The drugs were administered intravaginally both 6 and 24 h after initial virus infection at dosages of 0.10 mL of 10%, w/v, of drug in a polyethylene glycol/propylene glycol/stearyl alcohol cream[13] twice daily for 7 days. Appropriate untreated and placebo-treated control animals were used, and the antiviral effects were evaluated by a lesion-score

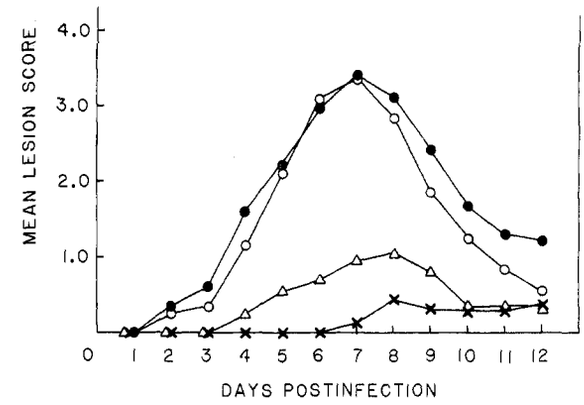

Figure 1. Effect of 9-(2-*O*-acetyl-β-D-arabinofuranosyl)adenine (**5a**) on herpes type 2 infection in the guinea pig: (●) HSV-infected, untreated control; (○) HSV-infected placebo-treated control; (×) HSV + *ara*-A 2′-acetate (**5a**), 6 h postinfection; (△) HSV + *ara*-A 2′-acetate (**5a**), 24 h postinfection.

rating (0 = no lesions; 4 = maximum lesion severity). The 2′-*O*-valeryl derivative **5d** was inactive when applied beginning either 6 h or 24 h postinfection, as evidenced by mean lesion scores at or near those for the control groups. The 2′-*O*-acetyl derivative **5a**, on the other hand, showed significant reduction of lesions for both the 6- and 24-h postinfection applications. (See Figure 1.) While the mean lesion scores peaked for the control animals at about day 7 (peak mean lesion score ~3.4), only a slight evidence of any lesions was noted in the group treated beginning 6 h postinfection. The group treated beginning 24 h postinfection, which presented a far more severe challenge, registered a mean lesion score of 1.0 on day 8, with that value falling to ~0.35 by day 10. Such results indicate that **5a** offers a definite therapeutic effect even when administered beginning 24 h after initial virus inoculation of the animals. Only the 2′,3′-di-*O*-acetyl derivative[2,5] has shown comparable activity among *ara*-A prodrugs studied to date.[14]

Conclusions

The 2′-*O*-acyl-derivatives of *ara*-A are a soluble, albeit a relatively nonlipophilic, group of drugs, of which the 2′-*O*-acetyl derivative **5a** is very active in the in vivo model against herpes type 2 virus infections. The reason for this enhanced activity in **5a** alone is not clear. The following facts, however, are known. (1) The more soluble compounds among the members of each series studied have shown the greatest (and comparable) in vivo activity: (a) 5′-*O*-butyryl-*ara*-A (solubility ~16.1 mg/mL),[1,5] (b) 2′,3′-di-*O*-acetyl-*ara*-A (solubility ~33 mg/mL),[2,5] and (c) 2′-*O*-acetyl-*ara*-A (solubility 93.6 mg/mL). (2) A large and positive log *P* ([octanol]/[water]) is not required for high activity in the guinea pig model. In a recent study[15,16] it

(10) Shannon, W. M.; Arnett, G.; Schabel, F. M., Jr.; North, T. W.; Cohen, S. S. *Antimicrob. Agents Chemother.* **1980**, *18*, 598.
(11) Determined by the method of Ehrlich et al., as modified by Sidwell et al.: Ehrlich, J.; Sloan, B. J.; Miller, F. A.; Machamer, H. E. *Ann. N.Y. Acad. Sci.* **1965**, *130*, 5. Sidwell, R. W.; Arnett, G.; Dixon, G. J.; Schabel, F. M., Jr. *Proc. Soc. Exp. Biol. Med.* **1969**, *131*, 1223.
(12) The protocol is essentially that of Kern et al., as modified by Shannon et al.: Kern, E. R.; Glasgow, L. A.; Overall, J. C., Jr.; Reno, J. M.; Boezi, J. A. *Antimicrob. Agents Chemother.* **1978**, *14*, 817. Shannon, W. M.; Arnett, G.; Drennen, D. J. *Ibid.* **1982**, *21*, 513.
(13) The formulation consisted of polyethylene glycol 4000/propylene glycol/stearyl alcohol, 20:90:20 (by weight). The polyethylene glycol 4000 and stearyl alcohol were gently warmed on a hot water bath, and the propylene glycol was added with stirring to give a smooth cream. The drug was then blended in.
(14) It is interesting to note that both *ara*-A and its water-soluble, nonlipophilic prodrug, *ara*-AMP, show activity against herpes simplex virus type 2 when administered 3–6 h postinfection. (See ref 5. Compare: Richards, J. T.; Kern, E. R.; Overall, J. C., Jr.; Glasgow, L. A. "Abstracts", Interscience Conference on Antimicrobial Agents and Chemotherapy, 17th, New York, 1977; American Society for Microbiology: Washington, DC, 1978; Abstr 128. Kern, E. R.; Overall, J. C., Jr.; Glasgow, L. A. *Antiviral Res.* **1982**, *2*, 27.
(15) Hsu, C.-C. Ph.D. Dissertation, University of Michigan, Ann Arbor, MI, 1980.
(16) Higuchi, W. I.; Kusai, A.; Fox, J. L.; Gordon, N. A.; Ho, N. F. H.; Hsu, C.-C.; Park, J. Y.; Baker, D. C.; Shannon, W. M. In "Controlled Release Delivery Systems"; Roseman, T. J.; Mansdorf, S. Z., Eds.; Marcel Dekker: New York, 1983; Chapter 3.

was shown that even though the partition coefficients for the 5′-O-acyl compounds increase with increasing chain length as do those for the 2′-O-acyl compounds **5a–d** (Table III), the mouse vaginal membrane permeability coefficients for the 5′-O-acyl derivatives increase only slightly with increasing chain length. If this relative insensitivity of the permeation coefficient also holds for the 2′-O-acyl derivatives **5a–d** in the guinea pig vaginal membrane, then solubility would indeed be expected to be the primary factor in determining transport across biological membranes, thus offering a plausible explanation for the present observed in vivo results. Finally, (3) each prodrug is resistant to ADase, at least while the ester group(s) is attached.[1,2] It is likely that in vivo efficacy arises from a subtile combination of all three factors: solubility, lipophilicity, and ADase resistance.

Transport alone would not, however, be expected to be the only important factor for in vivo efficacy. Propensity for the given ester to hydrolyze to the parent drug at or near the site of action, yet remain resistant to ADase in the prodrug form, most certainly contributes to in vivo efficacy. Also, the possible importance of systemic effects, which may arise from a combination of high prodrug membrane transport rates and slow to moderate release of the active drug, remains an unanswered question. All of these factors are under study with both the 5′-O-acyl and 2′-O-acyl derivatives (**5a–d**) of *ara*-A.

Finally, most interesting is the relationship between 2′,3′-di-O-acetyl-*ara*-A and 2′-O-acetyl-*ara*-A (**5a**), as the former has been demonstrated to enzymically hydrolyze to the latter, both in vitro[2] and in vivo.[17] The implication is that the 2′-O-acetyl derivative **5a** might well serve as an ADase-resistant, intermediary depot form of the diester or serve as *the* active drug on its own. This possibility will be the subject of future investigations.

Experimental Section

All solutions were evaporated at aspirator vacuum at ~40 °C unless otherwise specified. Melting points (mp) were determined with a Thomas-Hoover Unimelt capillary tube device equipped with a Cole-Parmer Model 8520-50 Digi-Sense thermocouple thermometer with a 8520-55 probe; as such melting points are considered corrected. IR spectra were determined with a Perkin-Elmer 710B instrument; UV measurements were made with a Cary-14 spectrophotometer; 200-MHz ^{1}H NMR spectra were determined with a Nicolet NT-200 instrument on ~0.1% solutions in the indicated solvent with tetramethylsilane as an internal reference (δ of Me_4Si 0.0; an asterisk indicates that the proton was observed to exchange with deuterium oxide). Opical rotations were measured in a 1-dm cell on a Perkin-Elmer Model 241 spectropolarimeter in the indicated solvent. Chromatography products were E. Merck's silica gel 60 (TLC: catalog no. 5539; column chromatography: catalog no. 7734, 20–60 μm). Solvent systems used were chloroform–methanol mixtures in the following ratios: A, 97:3; B, 96:4; C, 90:10. HPLC was carried out on a Waters Associates unit equipped with a M-45 pump, automatic sampler and 254-nm detection (0.45 × 30 cm, 10 μm, C-18 column; 10:90 acetonitrile–water). Reagents were reagent grade and were used as supplied. *N,N*-Dimethylformamide was distilled in vacuo from calcium hydride and stored over 4A molecular sieves; pyridine was distilled from calcium hydride and satored over 4A molecular sieves. microanalyses were carried out by Atlantic Microlab, Inc., Atlanta, GA. Analyses followed by the elemental symbol indicates that results were ±0.3% of the theoretical values.

9-[3,5-Bis-O-(*tert*-Butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (2) and 9-[2,5-Bis-O-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (3). To a stirred suspension of 5.32 g (20 mmol) of dry 9-β-D-arabinofuranosyladenine (1) in 100 mL of dry *N,N*-dimethylformamide was added 13.90 mL (10.12 g, 5 equiv) of triethylamine, followed by 7.54 g (2.5 equiv) of *tert*-butylchlorodimethylsilane (Petrarch). The mixture was stirred unde- nitrogen at room temperature for 36 h, at the end of which time the solvent was removed at ~60 °C in vacuo. The syrupy residue was dissolved in ethyl acetate and then washed with water, and the aqueous layer was extracted with 2 × 50 mL of ethyl acetate. The combined, dried (magnesium sulfate) organic layers were evaporated to give a clear syrup, which showed by TLC two components (solvent A). Silica gel column chromatography (solvent B) gave 7.57 g (76.4%) of **2** and 0.40 g (4.0%) of **3**, along with 0.83 g (10.8%) of 9-[5-O-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine:[6] total yield 91.2%.

Physical and spectroscopic data for **2**: mp 176–178 °C; R_f 0.28 (solvent A); $[\alpha]^{21}_D$ −11.0° (*c* 1, 1:1 THF–$CHCl_3$); UV (MeOH) max 259 nm (ε 15 500); ^{1}H NMR ($CDCl_3$) δ 0.14 (s, 12 H, 2 $SiMe_2$), 0.93 (s, 18 H, 2 *t*-Bu), 3.80–4.16 (m, 4 H, H-3′, H-4′, H-5′), 4.36 (s, 1 H, H-2′), 4.9–5.1 (br, s, 1 H, OH*), 5.82 (s, 2 H, NH_2*), 6.33 (d, 1 H, $J_{1',2'}$ = 2.4 Hz, H-1′) 8.28 and 8.33 (2 s, 1 H, 1 H, H-2, H-8). Anal. Calcd for $C_{22}H_{41}N_5O_4Si_2$: C, 53.30; H, 8.33; N, 14.13. Found: C, 53.22; H, 8.37; N, 14.11.

Physical and spectroscopic data for **3**: mp 191–192 °C; R_f 0.24 (solvent A); $[\alpha]^{21}_D$ −7.68° (*c* 1, 1:1 THF–$CHCl_3$); UV (MeOH) max 259 nm (ε 15 400); ^{1}H NMR ($CDCl_3$) δ 0.07 (s, 6 H, $SiMe_2$), 0.10 (s, 6 H, $SiMe_2$), 0.68 (s, 9, *t*-Bu), 0.92 (s, 9 H, *t*-Bu), 3.89–4.03 (m, 3 H, H-4′), 6.51 (d, 1 H, $J_{1',2'}$ = 3.5 Hz, H-1′), 8.1 and 8.33 (2 s s, 1 H, 1 H, H-2, H-8). Anal. Calcd for $C_{22}H_{41}N_5O_4Si_2$: C, 53.30; H, 8.33; N, 14.13. Found: C, 53.15; H, 8.35; N, 14.12.

9-[2-O-Acyl-3,5-bis-O-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenines (4a–d). To a stirred suspension of 4.95 g (0.01 mol) of 9-[3,5-bis-O-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (**2**) in 50 mL of dry pyridine cooled to 0 °C was added 0.015 mol of the appropriate acid anhydride, and the mixture was stirred under a nitrogen atmosphere at 0–5 °C for 48 h, at the end of which time the reaction was terminated by the addition of a few milliliters of water. The solvent was evaporated, and the residue was dissolved in ethyl acetate, washed with aqueous sodium bicarbonate and then with water, dried (magnesium sulfate), and evaporated to give a syrup. Silica gel column chromatography of the crude products with solvent B as eluent gave pure **4a–d** as glassy solids. Yields were as follows: **4a**, 90%; **4b**, 88%; **4c**, 92%; **4d**, 95%. All were homogeneous by TLC and ^{1}H NMR spectroscopy. These products, characterized by ^{1}H NMR spectroscopy (see Table V, supplementary material, for ^{1}H NMR data), were directly used in the following preparation.

9-(2-O-Acyl-β-D-arabinofuranosyl)adenines (5a–d). To a stirred solution of 75 mmol of the appropriate 9-[2-O-acyl-3,5-bis-O-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine in 100 mL of dry tetrahydrofuran (THF) was added 10.7 mL (11.2 g, 187 mmol) of glacial acetic acid, followed by 151 g (0.6 mol) of tetra-*n*-butylammonium fluoride,[18] and the reaction was monitored by TLC (solvent C). After TLC indicated complete removal of the protecting groups, the solution was passed through a bed of ~200 g of silica gel, and the product was eluted with THF to give, after column chromatography with the indicated solvent (Table I), the 2′-O-acyl derivatives **5a–d**. Crystallization afforded analytically pure samples. For yields and physical data, see Table I. For ^{1}H NMR data, see Table VI, supplementary material.

Determination of Partition Coefficients for Compounds 5a–b. One milligram of each drug, **5a–d**, was dispersed in a mixture of 0.5 mL of octanol (Fisher) and 0.5 mL of doubly distilled water in 1.5-mL micro test tubes that were shaken intermittently on a vortex shaker (Eppendorf, Model 5432 mixer) and maintained at 37 °C (Eppendorf Model 5320 thermostat). At sampling intervals, the tubes were centrifuged, and a 50-μL sample was removed from the octanol layer, diluted with 0.95 mL of methanol, and stored at −20 °C for analysis. The remaining octanol layer was removed, and a 50-μL portion of the water layer was sampled and diluted with 0.95 mL of methanol as above; the

(17) Glazko, A. J.; Schneider, H. G., unpublished results.

(18) The reagent is best prepared by neutralizing at 0 °C a ~10% aqueous solution of tetra-*n*-butylammonium hyroxide by the dropwise addition of 48% hydrofluoric acid (final pH 4.5 by pH paper), followed by lyophilization to dryness to give a powdery product that is stable for a few days when stored fully dried at −20 °C.

remaining portion was pipetted into a new tube, and the process was repeated, with samples being taken at the intervals shown in Table III.

The samples so obtained were analyzed by HPLC using 10-μL injections, with peak areas or peak heights being used to obtain the ratios.

Determination of the Solubilities of Compounds 5a–d. The indicated amount (Table II) for each drug, **5a–d**, was mixed with 1 mL of phosphate-buffered saline (9.195 g of sodium dihydrogen phosphate hydrate, 5.20 g of sodium chloride in 1 L water, adjusted to pH 7.3) and agitated at 37 °C (Eppendorf Model 5432 mixer and Model 5320 thermostat). At the specified times, the samples were centrifuged at 14000 ± 500 rpm for 5 min, and 25-μL samples were taken, diluted with 0.975 mL of HPLC mobile phase, and assayed by HPLC against known standard concentrations of the drugs. The results are tabulated in Table II.

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Registry No. 1, 5536-17-4; 2, 82870-42-6; 3, 82845-91-8; 4a, 87970-03-4; 4b, 87970-04-5; 4c, 87970-05-6; 4d, 87984-85-8; 5a, 65174-95-0; 5b, 65174-99-4; 5c, 87970-06-7; 5d, 87970-07-8; *tert*-butylchlorodimethylsilane, 18162-48-6; 9-[5-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine, 87970-08-9.

Supplementary Material Available: Complete ^{1}H NMR data for compounds **4a–d** (Table V) and **5a–d** (Table VI) (2 pages). Ordering information is given on any current masthead page.

Adenosine Deaminase Inhibitors. Synthesis of Deaza Analogues of *erythro*-9-(2-Hydroxy-3-nonyl)adenine

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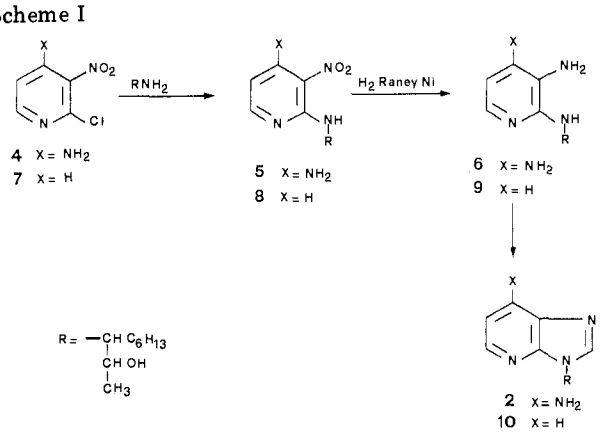
Structural analogues of *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA), in which the adenine moiety of the molecule was modified, were prepared in order to investigate the structural requirement of EHNA as an inhibitor of adenosine deaminase (ADA). Thus, 1- and 3-deaza-EHNA and their 6-deamino analogues were synthesized and evaluated as inhibitors of ADA from calf intestine. Inhibition studies indicated that isosteric substitution of pyrimidine nitrogens by carbons could be tolerated at the enzymatic binding site. In fact, 3-deaza-EHNA was found to have an inhibitory activity comparable to EHNA itself, and 1-deaza-EHNA, though less potent, is a good inhibitor. The 6-amino group gives an important contribution to the enzymatic binding if the N^1 nitrogen is also present, conferring on the compound the characteristic of a semitight inhibitor.

The ubiquitous enzyme adenosine demainase (ADA) catalyzes the hydrolytic N^6-deamination of adenine nucleoside analogues of chemotherapeutic interest to inactive or considerably less active hypoxanthine derivatives.[1] It was demonstrated that ADA inhibitors are able to enhance the cytotoxic activity of a variety of adenosine analgoues, such adenosine arabinoside (*ara*-A), 8-azaadenosine, and formycin.[2] Among these ADA inhibitors, pentostatin[3,4] and coformycin,[5] two naturally occurring nucleoside antibiotics, are extremely potent (K_i = 10^{-11} to 10^{-12} M).[6,7] Interest has been generated in tight-binding inhibitors of ADA because, in addition to potentiating the effect on nucleoside-type antitumor or antiviral agents, they may induce cellular deficiency of ADA and allow one to study of the effect of genetic deficiency of the enzyme and to clarify its function in severe combined immunodeficiency syndrome.[8] Among the synthetic compounds, the most active inhibitor is the *erythro*-9-(2-hydroxy-3-nonyl)-adenine (EHNA, 1),[9] which has been classified as a semitight-binding inhibitor with a K_i = 1.6 × 10^{-9} M.[7] As a result of the much faster reactivation of inhibited ADA observed in the case of inhibition by EHNA than that by pentostatin and coformycin, EHNA is advocated as a possible inhibitor of choice for use in viral chemotherapy.[10]

The tight binding of EHNA and of a previous series of 9-hydroxyalkylpurines might occur in an auxiliary site near that which normally binds the ribose moiety of adenosine. In the course of their research on nonnucleosidic adenine

Scheme I



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