collected. The pure peptide (16 mg) was secured from fractions 7–13. Subsequent fractions yielded 8 mg less pure material. The pure product was homogeneous on TLC, R_f (B) 0.35. On descending paper chromatograms in the freshly prepared solvent system *n*-BuOH–AcOH–H_2O (4:1:5, upper layer), 8-L-methionine-oxytocin moved as a single spot with R_f 0.54. Oxytocin used as reference traveled with R_f 0.61. Amino acid analysis: Asp, 1.08; Glu, 1.05; Pro, 0.96; Gly, 1.01; $^1/_2$-Cys, 1.97; Met, 0.95; Ile, 1.08; Tyr, 0.85. From the recovery, a peptide content of 98% was calculated. This value was confirmed by the absorption at 278 nm of a sample of XII in H_2O. This material was used in the biological assays.

A sample of XII (purified by partition chromatography, cf. above) was subjected to LC on a DuPont 850 liquid chromatograph with a Zorbax ODS 4.6 × 25 mm column and 0.01 N NH_4OAc (pH 4)–CH_3CN system as eluent. The elution was monitored by absorption at 275 nm. A flow rate of 2.5 mL/min was maintained. A solvent gradient of 25–50% CH_3CN in NH_4OAc was used. A single peak was recorded at 39% CH_3CN composition (6 min).

Pharmacological Assays. Rat uterine assays were performed on isolated horns from virgin rats in natural estrus according to the method of Holton,[14a] as modified by Munsick,[14b] with the use of Mg^{2+}-free van Dyke-Hastings solution as bathing fluid. Avian vasodepressor assays were performed on conscious chickens by the method of Coon,[15a] as described in the U.S. Pharmacopeia,[15b] as modified by Munsick et al.[15c] Pressor assays were carried out on anesthetized male rats as described in the U.S. Pharmacopeia.[16] Antidiuretic assays were performed on anesthetized male rats according to the method of Jeffers et al.,[17a] as modified by Sawyer.[17b] The four-point assay design of Schild[25] was used to obtain specific activities as compared to U.S. Pharmacopeia posterior pituitary reference standard.

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Prodrugs of 9-(β-D-Arabinofuranosyl)adenine 2. Synthesis and Evaluation of a Number of 2′,3′- and 3′,5′-Di-*O*-acyl Derivatives[1]

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A number of 2′,3′- and 3′,5′-di-*O*-acyl derivatives of 9-β-D-arabinofuranosyladenine (1) were prepared and evaluated as antivirals. These compounds, designed as prodrugs of 1, offer a range of solubilities and lipophilicities, as well as a resistance to adenosine deaminase, that render some as being attractive as possibly useful antiviral agents. Of particular note is 9-(2,3-di-*O*-acetyl-β-D-arabinofuranosyl)adenine that was found to be effective as a topical agent in a guinea pig model of genital herpes.

In the preceding paper,[2] a series of prodrugs of the potent antiviral agent 9-β-D-arabinofuranosyladenine (VIRA-A,® vidarabine, *ara*-A, 1) was described. These compounds, which are stable, 5′-*O*-acyl derivatives of 1,

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were designed to provide suitable prodrugs that would increase the aqueous solubility over that of the parent drug,[3a] provide greater lipophilicity and, hence, increase the potential for membrane transport, and provide, in addition, resistance to adenosine deaminase, the enzyme responsible for the deamination and deactivation[3b] of **1**. Solubilities were found, indeed, to increase for the short-chain acyl derivatives, giving rise to drug concentrations of 21 to 53 μmol/mL, for the 2- to 5-carbon ester derivatives with an increase in lipophilicity commensurate with increasing chain length. Furthermore, a total resistance to adenosine deaminase was found in vitro, a finding in agreement with the resistances found in general for adenine nucleosides lacking a free 5′-hydroxy group.[4] These 5′-*O*-acyl compounds thus furnish potential candidates for both parenteral and topical prodrugs of **1**.

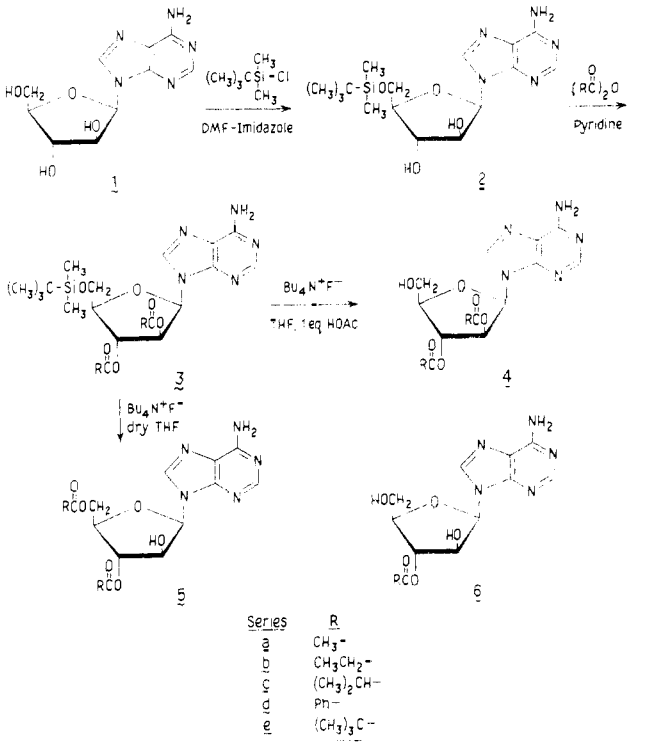
It was deemed important to investigate the effects of protecting the secondary alcoholic groups at the 2′ and 3′ positions to offer an intermediate stage of hydroxyl-group protection between that of afforded by the 5′-*O*-acyl derivatives[2] and that of the fully derivatized 2′,3′,5′-tri-*O*-acyl derivatives.[5] To this end was designed a synthesis for 2′,3′-di-*O*-acyl derivatives of **1**. In the course of the research, there evolved a synthesis of the isomeric 3′,5′-di-*O*-acyl analogues that presented yet another interesting class of prodrugs. As byproducts, both the 2′- and 3′-monoesters were encountered.

Chemical Synthesis. A traditional approach[6] for the synthesis of 2′,3′-di-*O*-acyl nucleosides has been the sequence of: (1) 5′-tritylation,[7] (2) acylation, and (3) detritylation. However, earlier experience with a trityl derivative of **1**,[8] using the highly acid-labile 5′-methoxytrityl group of Khorana,[6] left much to be desired in the way of yields and ease of handling in this sequence. An alternative to the trityl ether, the *tert*-butyldimethylsilyl group, described by Corey[9] and first applied to nucleoside synthesis by Ogilvie,[10a] was found to be an ideal protecting group. Reaction of a suspension of **1** with a slight excess of *tert*-butyldimethylsilyl chloride in *N*,*N*-dimethylformamide with 2 equiv of imidazole[9] furnished the crystalline 9-[5-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (**2**) in >90% preparative yield (see Scheme I). Acylation with the appropriate acid anhydride in cold pyridine gave the 2′,3′-di-*O*-acyl derivatives **3a–c** as thick syrups that were near homogeneous by thin-layer chromatography (TLC) and were used in the deblocking reaction without further purification.

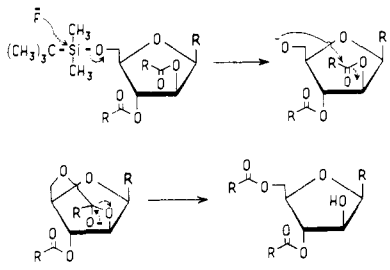
Removal of the 5′-*O*-*tert*-butyldimethylsilyl protecting group with tetrabutylammonium fluoride in tetrahydrofuran gave in most cases the desired 2′,3′-di-*O*-acyl nucleosides **4a–c**; however, for reasons at first unknown, other products, indistinguishable by adsorption-mode TLC or high-pressure liquid chromatography (HPLC) on silica gel but decidedly different in their ^{1}H NMR spectra, were occasionally encountered. LC, operating in the reverse-phase (partition) mode using an octadecylsilyl-derivatized silica gel and an aqueous acetonitrile eluent, showed the presence of two products from the deblocking of the 2′,3′-di-*O*-(2-methylpropionyl) derivative **3c** that were easily resolved by this method. Preparative LC afforded clean separation, and NMR analysis of an isolated sample confirmed the identity of the desired 9-[2,3-di-*O*-(2-methylpropionyl)-β-D-arabinofuranosyl]adenine (**4c**), as well as a second, more lipophilic (i.e., with a longer retention time on reverse-phase LC) component, identified by its ^{1}H NMR spectrum (see Table IV; see paragraph at the end of this paper concerning supplementary material) as the isomeric 9-[3,5-di-*O*-(2-methylpropionyl)-β-D-arabinofuranosyl]adenine (**5c**). The latter compound undoubtedly arose via a 2′ → 5′-*O*-acyl migration. The two were clearly distinguished in the NMR (see Table IV, supplementary material), as **4c** gave a signal at δ 3.72 that integrated for two protons (AB of an ABX) in a region similar to that for the 5′-methylene group of **1**. Two overlapping signals that were shifted downfield (relative to those of **1**) by 1.48 ppm to δ 5.59 confirmed the acylation of the 2′- and 3′-hydroxyl groups. In contrast, the component **5c** showed a downfield shift of ~0.6 ppm for the 5′-methylene group in respect to either **4c** or **1**. A pseudotriplet at δ 5.25 indicated an O-acylated methinyl group, while the signal at δ 4.3 that showed simplification upon addition of deuterium oxide indicated that **5c** was a 3′,5′-diester derivative. These latter assignments were confirmed in a double-resonance experiment whereby the H-1′ doublet (δ 6.26) was irradiated, showing a collapse of the more upfield δ 4.3 methinyl signal, indicating that the 2′-hydroxyl group was nonacylated. That the amino group on the adenine moiety remained free for both **4c** and **5c** was shown by the UV spectra [λ_{max} (MeOH) = 259 nm].[10b]

Careful evaluation of the conditions of the reaction using the 2′,3′-di-*O*-(2-methylpropionyl) derivative **3c** showed that, by maintaining a rigorously dry medium during the deprotecting step with tetrabutylammonium fluoride on an acid-free sample of **3c** (rendered acid free by the exhaustive washing of an organic extract of crude **3c** with aqueous sodium hydrogen carbonate), the 3′,5′-di-*O*-(2-methylpropionyl) derivative **5c** could be obtained as the major product, as revealed by LC analysis. Furthermore, it was found that upon the addition of 1 equiv of acetic acid to the tetrahydrofuran solution of **3c** before the addition of the fluoride reagent the near exclusive product was the nonrearranged 2′,3′-di-*O*-(2-methylpropionyl) derivative **4c**. The findings for the 2-methylpropionyl derivatives **4c** and **5c** were successfully extended to the other derivatives **4a**,**b**,**d** and **5a**,**b** as well. Thus, by maintaining control over the acid content of the mixture during deprotection of **3a–c**, the reaction conditions could be altered to furnish either the 2′,3′- or the 3′,5′-diesters

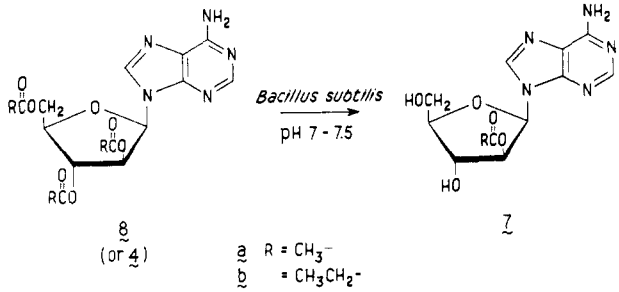
Scheme I



Scheme II



Scheme III



at will, as depicted in Scheme I. The products were generally isolable directly by crystallization.

A plausible mechanism to explain the observed results is offered in Scheme II. It is proposed that there is an initial attack by the fluoride ion upon the silicon atom, with transfer of negative charge onto the neighboring oxygen. The anion is then favored to react intramolecularly with the carbonyl center of the sterically acessible 2′-*O*-acyl group, resulting in a scission of the 2′-O–C linkage, giving rise to a 2′ → 5′-acyl migration. In the presence of a good proton source (e.g., acetic acid), the anion is effectively quenched, thereby preserving the 2′,3′-di-*O*-acyl species. Acetic acid was found by experiment to be superior to alcohols, water, mineral acids,[11] or other lower alkyl carboxylic acids, which were either ineffective in controlling the acyl migration or altogether prevented desilylation. Other workers have noted[11] frustrations in dealing with similar acyl migrations on *tert*-butyldimethylsilylated lipids.

Associated with preparative-scale (>0.10 mol) syntheses of **4a–c**, a low yield of each of the 2′- and 3′-*O*-acyl derivatives **7a,b** and **6a–c**, respectively, was isolated by silica gel chromatography of the mother liquors of the reaction. These products arose apparently either from a lack of complete acylation of **2** or from consequences of the deprotection of **3**.

On account of a particular interest (see Discussion and Results) in obtaining the 2′-*O*-acyl derivatives **7a,b** in reasonable yields, a synthesis was devised whereby the more sterically accessible 3′- and 5′-*O*-acyl groups could be selectively removed from the known tri-*O*-acyl derivatives.[5] In order to avoid the possibility of acyl migrations, an exceedingly mild process using the crude, nonspecific acylase from a cell paste of *Bacillus subtilis*[12] was employed. A stirred suspension of the crude cell paste and **8**, maintained at ca. pH 7.5 and 37 °C, gave a good yield of the 2′-*O*-acyl derivatives **7a,b** (see Scheme III). Under identical conditions, the more soluble 2′,3′-di-*O*-acyl derivatives **4a,b** could be even more efficiently converted to **7a,b**.

The structures of the 2′-*O*-acyl derivatives **7a,b** were confirmed by NMR spectroscopy. For **7a**, a pseudotriplet at δ 5.23, downfield and distinct from the methinyl signal at δ 4.40 whose identity was determined by irradiation of H-1′, together with the signal for the 5′-methylene group that was relatively unchanged from that of **1**, **4a–c**, or **6a–c**, clearly pinpointed the site of acylation at O-2′. A similar NMR study identified the compounds **6a–c** as 3′-*O*-acylated derivatives.

The acylation of **1** with pivalyl chloride in pyridine was carried out prior to the development of either the foregoing procedures or a process that favors 5′-monoacylation.[2] The complex mixtures of mono- and di-*O*-acyl compounds were separated by column chromatography on silica gel and identified by their NMR spectra as the 5′-*O*-pivalyl derivative,[2] the 3′,5′-di-*O*-pivalyl derivative **5e** (see Table I), and the 2′,5′-di-*O*-pivalyl compound (see the Experimental Section).

Discussion and Results

Physical Properties and in Vitro Biological Evaluation. The 3′,5′-di-*O*-acyl derivatives **5a–e**, most closely related structurally to both the known 5′-*O*-acyl[2] and the 2′,3′,5′-tri-*O*-acyl derivatives,[5] were found to possess greater aqueous solubilities than the parent **1** only for the shortest chain examples, **5a,b** (see Table I). As expected, for both **5a** and **5b**, a greater aqueous solubility was achieved in respect to their respective 9-(2,3,5-tri-*O*-acyl) counterparts,[13] while little was sacrificed in lipophilicity, indicating that the 2′-*O*-acyl group contributes little to the overall partition coefficients as measured in a pentanol–water system. With the four- and five-carbon 3′,5′-*O*-acyl compounds **5c** and **5e**, the solubilities fell far below that for **1**, with large increases being noted in the log *P* values.

The antiviral activities, as measured in a plaque-reduction screen against herpes simplex type 1 virus, show that both **5a** and **5b** do possess antiviral activity even in vitro, while the larger, branched-chain examples **5c** and **5e** are inactive (see Table II), presumably indicating that these latter examples are resistant to deacylation, at least in the cell cultures under study. These results are analogous to the findings in the companion study[2] where 9-(5-*O*-pivalyl-β-D-arabinofuranosyl)adenine was essentially inactive in vitro, indicating possibly a true prodrug function for both **5a** and **5b**. As expected, having the 5′-hydroxyl group blocked, these drugs **5a–e** were not deaminated by adenosine deaminase in vitro,[14] a finding that is in accord with known[4] structure–activity relationships established for adenine ribonucleosides.

In comparison with the 3′,5′-di-*O*-acyl compounds **5a–c**, the corresponding 2′,3′-di-*O*-acyl analogues **4a–c** were found to be markedly more water soluble (see Table I), with partition coefficients of about the same magnitude as those for **5a–c**, indicating that the sterically prominent 5′-hydroxyl group figures importantly in determining aqueous solubility. As with their 3′,5′-di-*O*-acyl counterparts **5a,b**, only the short-chain compounds **4a,b** possessed appreciable antiviral activity (see Table II). Again the branched-chain analogue **4c** showed diminished activity in vitro, while the 2′,3′-di-*O*-benzoyl compound **4d** was inactive, even at high concentrations.

Most noteworthy, however, was the fact that the 2′,3′-di-*O*-acyl compounds **4a–d** were found to be completely resistant to deamination by calf mucosal adenosine deaminase in vitro,[14] despite the fact that the 5′-hydroxymethyl group remained free. In an effort to determine precisely what functional group was responsible for this lack of substrate activity, both the 3′-*O*-acyl analogues **6a–c** and 2′-*O*-acyl analogues **7a,b** were examined for their in vitro adenosine deaminase substrate activity. The 3′-*O*-acyl compounds **6a–c** were found to be deaminated[14] at rates of 0.16, 0.48, and 0.60, respectively, that of **1**.[15] In

Table I. Physical and Chemical Data for 2'- and 3'-*O*-Acyl and 2',3'- and 3',5'-Di-*O*-acyl Compounds

compd	R_2'	R_3'	R_5'	yield,[a] %	mp,[b] °C	$[\alpha]^{23}_D$ (*c* 1, MeOH)	λ_{max}(MeOH), nm ($\epsilon \times 10^{-3}$)	analyses, C, H, N	aq solubility, mg/mL (pH 7)	log $P(\frac{[\text{pentanol}]}{[\text{water}]})$
					2',3'-di-*O*-acyl compounds					
4a	CH_3-	CH_3-	H-	63	138–139	-4.1°	259 (15)	$C_{14}H_{17}N_5O_6 \cdot 0.2H_2O$	33	0.20
b	CH_3CH_2-	CH_3CH_2-	H-	51	172–173	-4.1°	259 (15.4)	$C_{16}H_{21}N_5O_6 \cdot 0.25H_2O$	4.5	1.10
c	$(CH_3)_2CH$-	$(CH_3)_2CH$-	H-	34	207–208		259 (15.3)	$C_{18}H_{25}N_5O_6$	0.42	1.74
d	C_6H_5-	C_6H_5-	H-	48	215–216	-58°	259 (15.7) 232 (31.3)	$C_{24}H_{21}N_5O_6$	<0.01	
					3',5'-di-*O*-acyl compounds					
5a	H-	CH_3-	CH_3-	52	167.5–169	+9.0°	258 (15.5)	$C_{14}H_{17}N_5O_6$	4.7	0.72
b	H-	CH_3CH_2-	CH_3CH_2-	61	*c*	+8.1°	259 (15.0)	$C_{16}H_{21}N_5O_6$	2.3	1.54
c	H-	$(CH_3)_2CH$-	$(CH_3)_2CH$-	58	217.5–218.5		259 (15.5)	$C_{18}H_{25}N_5O_6$	0.02	1.66
e	H-	$(CH_3)_3C$-	$(CH_3)_3C$-	32	136–137	-1.2°	259 (15.3)	$C_{20}H_{29}N_5O_6$	<0.01	
					3'-*O*-acyl compounds					
6a	H-	CH_3-	H-	6[d]	195–201 dec		259 (15.0)	$C_{12}H_{15}N_5O_5$	vs[f]	0.72
b	H-	CH_3CH_2-	H-	5[d]	167–169		259 (14.9)	$C_{13}H_{17}N_5O_5 \cdot 0.2H_2O$	vs[f]	0.59
c	H-	$(CH_3)_2CH$-	H-	6[d]	198–198.5		258 (14.0)	$C_{14}H_{19}N_5O_5$	vs[f]	1.06
					2'-*O*-acyl compounds					
7a	CH_3-	H-	H-	53[e]	*c*		259 (15.0)	$C_{12}H_{15}N_5O_5$	vs[f]	-0.18
b	CH_3CH_2-	H-	H-	48[e]	206.5–207.5		259 (14.7)	$C_{13}H_{17}N_5O_5$	vs[f]	0.19

[a] Yields were based on recrystallized, analytically pure products. [b] Uncorrected values (°C) were determined using a Thomas-Hoover Unimelt melting point apparatus. [c] Product failed to crystallize and was isolated by column chromatography as a glassy solid. [d] Yields as byproducts from the preparation of the 2',3'-di-*O*-acyl compounds 4a–c (see Experimental Section). [e] Yields based on the enzymatic deacylation of di- and tri-*O*-acyl derivatives (see Experimental Section). [f] Solubility, vs = very soluble (i.e., >> vidarabine 1, determined qualitatively).

Table II. In Vitro Antiviral Testing Data[a]

compd	compounds: concn, M × 10⁵	compounds: concn, μg/mL	compounds: % plaque reduct	control,[b] % plaque reduct
	2',3'-di-O-acyl compounds			
4a	1.75	6.15	68	100
	0.18	0.62	0	40
b	1.83	6.95	15	90
	0.18	0.70	8	60
c	1.71	6.95	2	90
	0.53	2.17	0	73
d	5.47	26.0	0	100
	3',5'-di-O-acyl compounds			
5a	1.75	6.15	81	87
	0.54	1.90	5	79
b	1.76	6.70	33.8	76
	0.18	0.70	0	23
c	1.75	7.15	0	90
e	1.67	7.26	0	100
	3'-O-acyl compounds			
6a	1.71	5.30	83	87
	0.17	0.53	9	36
b	1.76	5.68	80	87
	0.18	0.57	5	36
c	1.75	5.90	99	90
	0.18	0.59	26	60
	2'-O-acyl compounds			
7a	1.71	5.30	84	90
	0.17	0.53	0	60
b	1.76	5.70	35	88
	0.18	0.57	4	36

[a] Procedure of G. D. Hsiung and J. L. Melnick, *Virology*, 1, 533 (1955), using herpes simplex type 1. [b] A control using *ara*-A was conducted alongside the test sample at the same molar concentration, using an identical virus challenge (125–300 plaque-forming units). All data were confirmed by duplicate assay (±10%).

sharp contrast, the two 2'-O-acyl analogues **7a,b** were found totally resistant to the enzyme, even when enzyme concentrations were increased 10- and 100-fold. LC analysis of the enzyme-reaction mixture revealed the unchanged 2'-O-acyl compounds, a result that rules out a possible 2' → 5'-O-acyl migration that could have possibly accounted for the resistance to adenosine deaminase.

This unexpected lack of substrate activity for adenosine deaminase in the case of the 2'-O-acyl derivatives is of interest. As noted from the method of synthesis using a bacterial enzyme preparation, one might reasonably expect a similar activity by tissue and blood serum acylases in vivo. These results could well imply that either a 2',3'-di- or a 2',3',5'-tri-O-acyl compound could serve as a lipophilic prodrug, and the 2'-O-acyl compound, that is slow to deacylate and resistant to adenosine deaminase, yet active as an antiviral in vitro (see Table II), would be retained as an initial metabolite, providing a slower release form for **1**. This hyphothesis is under further study.

In Vivo Antiviral Studies on 9-(2,3-Di-O-acyl-β-D-arabinofuranosyl)adenine Derivatives 4a,b. A guinea pig model system for intravaginal herpes simplex type 2 (Magill strain) infections was established following a modification of that previously reported.[16] A group of 12 young female albino guinea pigs (125–150 g) was washed intravaginally with 2 mL of saline solution 1 h prior to infection with 0.1 mL of virus stock solution ($10^{2.82}$ plaque-forming units) into the vagina using a 7.6-cm cannula attached to a 1-mL syringe. The test drugs, incorporated into an ointment base in either 3 or 10% concentrations, were instilled directly into the vagina from tubes, filling the entire cavity. Drugs were given twice daily for 4 days, starting 4-h postinfection. Animals were observed daily for clinical signs of infection, i.e., inflammation, vulvovaginal swelling, vesiculations of the labia and/or vulva, vaginal discharge, paralysis, and death.

Results tabulated in Table III for the two drugs tested in vivo show that the 2',3'-di-O-acetyl derivative **4a** had significant therapeutic activity, preventing the development of clinical signs of infection. The 2',3'-di-O-propionyl compound **4b**, on the other hand, was less active, with clinical evidence of infection developing in a number of the animals and paralysis developing in 1 of 12 animals. The activity of **4a** at 3% concentration is essentially equivalent to that of 9-(β-D-arabinofuranosyl)adenine 5'-monophosphate, sodium salt (*ara*-AMP, vidarabine 5'-monophosphate), at a 10% concentration in the same test. *ara*-AMP[17] is a water-soluble, nonlipophilic prodrug that has been demonstrated to be as effective as, or superior to, **1**[18] in this model system.

Summary. The 2',3'- and 3',5'-di-O-acyl compounds (**4a–d** and **5a–c**, respectively) present a variety of solubilities and lipophilities that pose interesting possibilities as prodrugs of **1**. The fact that both of these classes of compounds possess resistance to adenosine deaminase is noteworthy, especially the finding that acylation of the 2' position renders the compound inert to the enzyme. In vitro antiviral activity is significant only for the shorter-chain derivatives. Of the two compounds tested topically in vivo against genital herpes, the 2',3'-di-O-acetyl

Table III. Intravaginal Treatment of Genital Herpes Simplex Virus Type 2 (Magill Strain) Infections in Guinea Pigs[a] With Compounds 4a, 4b, and *ara*-AMP[b]

compd[c]	% concn	norm and/or cured anim group[e,f]	symptoms of infect,[d] (during 16-days postinfect. period): inflammat[g]	vesicles and/or lesions	vaginal discharge	vulvovaginal swelling	paralysis	deaths
4a	3	12/12	3/12	0/12	0/12	0/12	0/12	0/12
4b	3	11/12	5/12	2/12	1/12	3/12	1/12	0/12
ara-AMP[b]	10	12/12	0/12	0/12	0/12	2/12	0/12	0/12
infected controls		5/7	2/7	2/7	1/7	2/7	1/7	0/7
infected controls	placebo	2/7	5/7	5/7	3/7	5/7	1/7	0/7

[a] Animals infected intravaginally with $1 \times 10^{2.82}$ PFU contained in 0.1 mL. [b] *ara*-AMP = 9-(β-D-arabinofuranosyl)adenine 5'-monophosphate, sodium salt. [c] Animals were treated intravaginally (topical) with drug ointments beginning at 4-h postinfection and then twice daily for 4 days. Sufficient drug was administered from 1/8-oz tubes to completely fill the vagina. [d] Animals positive over animals infected. [e] Animals either failed to develop any symptoms and appeared normal or developed one or more symptoms that healed by day 16 postinfection. [f] Data listed as animals cured/animals infected. [g] No evidence of irritation or toxic response was observed in uninfected control animals treated with the various drugs, with the exception of two animals treated with the 2',3'-di-O-propionyl derivative 4b; both animals developed erythema and one animal died 11 days after treatment began.

derivative 1 is definitely therapeutically active. Full determination of the therapeutic value of these compounds awaits further studies.

Experimental Section

General Methods. The general experimental methods, as well as solubility and partition coefficient determinations, have been described in a previous paper.[2] High-pressure liquid chromatography (LC) was carried out in the reverse-phase mode using an instrument of the author's design[19] with an octadecylsilyl-derivatized column (Waters Associates, μ-Bondapak C-18, 4 × 300 mm) with aqueous acetonitrile as the eluting phase and with 254-nm UV detection.

9-[5-*O*-(*tert*-Butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (2). To a stirred suspension of 26.7 g (0.1 mol) of dry 9-(β-D-arabinofuranosyl)adenine (1) in 500 mL of dry *N*,*N*-dimethylformamide was added 16.3 g (0.24 mol) of imidazole, followed by 18.1 g (0.12 mol) of *tert*-butyldimethylchlorosilane. The mixture was stirred with protection from moisture at room temperature for 20 h, at the end of which time the solvent was removed at 50 °C in vacuo. The residue was dissolved in 300 mL of ethyl acetate, the solution was washed with 200 mL of water, and the extract was dried ($MgSO_4$) and evaporated to give a syrup. Crystallization from 240 mL of hot chloroform, to which was added hot hexane to the point of opalescence, followed by slow cooling to room temperature, gave upon filtration of the product and drying at 40 °C/0.1 Torr, 35 g (92%) of pure, crystalline **2**; mp 157–158 °C; UV (MeOH) λ_{max} 259 nm (ε 15 000); $[\alpha]^{23}_D$ +4.1° (c 1, methanol); solubility in water 0.23 mg/mL; R_f 0.52, using tetrahydrofuran as eluent; for NMR data, see Table IV (supplementary material).

General Procedures for the Preparation of 9-(2,3-Di-*O*-acyl-β-D-arabinofuranosyl)adenines (4a–d) and 9-(3,5-Di-*O*-acyl-β-D-arabinofuranosyl)adenines (5a–c). (a) **Acylation of 9-[5-*O*-(*tert*-Butyldimethylsilyl)-β-D-arabinofuranosyl]adenine.** To a stirred suspension of 7.62 g (20 mmol) of 9-[5-*O*-(*tert*-butyldimethylsily)-β-D-arabinofuranosyl]adenine in 100 mL of dry pyridine cooled to 0 °C was added 41–45 mmol of the appropriate acid anhydride, and the mixture was stirred with protection from moisture at 0–5 °C for 20 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 crude **3a–c** was dissolved in ~100 mL of chloroform, washed with 2 × 100 mL of saturated sodium hydrogen carbonate and then with water, dried (magnesium sulfate), and evaporated to give a thick, clear syrup that was near homogeneous by TLC (9:1 chloroform–methanol). The product was rigorously dried in vacuo at ~25 °C and used directly in the following procedures.

(b) **Deprotection of 3a–d to Give 9-(2,3-Di-*O*-acyl-β-D-arabinofuranosyl)adenines (4a–d).** To a stirred solution of the above acylated product **3a–d** in 200 mL of dry THF was added 1.2 mL (1.25 g, 21 mmol) of glacial acetic acid, followed by 10–15.6 g (40–60 mmol) of tetrabutylammonium fluoride,[20] and the reaction was monitored by TLC (9:1 chloroform–methanol). Upon conversion of **3a–d** to the slower migrating **4a–d** (ca. 1–1.5 h), the fluoride was removed from the mixture by filtration through a layer of ~200 g of silica gel, and the products were eluted with tetrahydrofuran. The crude product, obtained upon evaporation of the solvent, was crystallized directly from acetone to yield pure **4a–d**. For physical constants, see Table I. NMR data are listed in Table IV (supplementary material).

(c) **Deprotection of 3a–c and Acyl Migration to Give 9-(3,5-Di-*O*-acyl-β-D-arabinofuranosyl)adenines 5a–c.** The deprotecting reaction on compounds **3a–c** using tetrabutylammonium fluoride was carried out exactly as in b, except that the equivalent of acetic acid was omitted from the mixture. After ca. 1–1.5 h, TLC (9:1 chloroform–methanol) indicated conversion of **3a–c** to **5a–c**. The product, obtained upon workup as in b, was crystallized from acetone to yield pure **5a** and **5c**; product **5b** repeatedly failed to crystallize, and it was isolated as an analytically pure, glassy solid after column chromatography over silica gel (30 g of silica gel/1 g of product) using the TLC solvent as eluent. Physical constants are listed in Table I; for NMR data see Table IV (supplementary material).

Isolation of 9-(3-*O*-Acyl-β-D-arabinofuranosyl)adenines 6a–c and 9-(2-*O*-Acyl-β-D-arabinofuranosyl)adenines 7a–b. The residues from evaporation of the mother liquors from the crystallization of the 2′,3′-di-*O*-acyl compounds **4a–c** (foregoing section b) were subjected to column chromatography (50 g of silica gel/1 g of product) using a linear gradient of pure chloroform, increasing to 80:20 chloroform–methanol, collected in 10-mL fractions. Following an early fraction consisting of a mixture of both 2′,3′- and 3′,5′-di-*O*-acyl compounds, the 3′-*O*-acyl derivative **6a–c** and the 2′-*O*-acyl derivatives **7a,b** were isolated, respectively, in that order. Crystallizations were effected from ethanol; for yields and physical constants see Table I; NMR data are tabulated in Table IV (supplementary material).

9-(2-*O*-Acetyl-β-D-arabinofuranosyl)adenine (7a). To a suspension of 0.5 g (1.27 mmol) of 9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)adenine (**8a**)[5] in 200 mL of 0.1 M, pH 7, phosphate buffer was added 0.5 g of crude cell paste[12] from *Bacillus subtilis*, and the mixture was stirred vigorously at ca. 35 °C for 22 h while maintaining the pH at 7–7.5 by periodic addition of aqueous sodium carbonate. TLC (9:1 chloroform–methanol) revealed near complete conversion of **8a** (R_f 0.40) to **7a** (R_f 0.23) and some **1** (R_f 0.05); LC (80:20 acetonitrile–water) showed a ratio of **8a**/**7a**/**1** of 6:92:2. The mixture was poured into an equal volume of methanol, and the precipitated cellular debris was filtered through Celite and washed with 1:1 water–methanol. The methanol was evaporated, and the aqueous solution was lyophilized to dryness. Column chromatography of the lyophilisate over 30 g of silica gel using a linear gradient of 250 mL of chloroform–250 mL of 30:70 chloroform–methanol gave 208 mg of pure **7a** as a noncrystalline glass; yields and physical constants are recorded in Table I; for NMR data see Table IV (supplementary material). The product was in all respects identical with that isolated as a byproduct in the synthesis of the 2′,3′-di-*O*-acetyl compound **4a** (vide supra).

Enzymatic deacylation of the 2′,3′-di-*O*-acetyl derivative **4a** was conducted in the same manner using 0.5 g (1.42 mmol) of **4a** and 0.5 g of crude cell paste in 50 mL of aqueous buffer at pH 7–7.5 over 9 h to give an identical yield of **7a**.

9-(2-*O*-Propionyl-β-D-arabinofuranosyl)adenine (7b). As in the foregoing procedure, 0.5 g (1.32 mmol) of the 2′,3′-di-*O*-propionyl compound **4b** in 300 mL of pH 7 buffer was enzymatically deacylated using 0.5 g of crude *B. subtilis* cell paste over 18 h; LC (80:20 acetonitrile–water) revealed an 8:88:4 ratio of **8b**/**7b**/**1**, respectively. Isolation as for **7a** gave 244 mg of a glassy solid that crystallized from ethanol to give 205 mg of pure **7b**; yields and physiochemical data are recorded in Table I; for NMR data see Table IV (supplementary material).

Enzymatic deacylation of the 2′,3′,5′-tri-*O*-propionyl derivative **8b** proceeded slowly on a suspension of this water-insoluble compound in buffer, as determined by LC and TLC as above.

Acylation of 9-(β-D-Arabinofuranosyl)adenine (1) with Pivalyl Chloride. To a stirred suspension of 2.67 g (10 mmol) of dry **1** in 100 mL of dry pyridine was added 1.48 mL (1.45 g, 12 mmol) of pivalyl chloride, dropwise at ca. 25 °C. The mixture was stirred for 4 h, at the end of which time a solution had formed. The reaction was terminated by the addition of ice, and the mixture was poured into 100 mL of water and extracted with 3 × 50 mL of chloroform. The organic extract was washed with water, dried (magnesium sulfate), and evaporated to a clear, yellow syrup. Examination by TLC (tetrahydrofuran) revealed three products at R_f values 0.37 (major), 0.66 (minor), and 0.76 (minor).

The crude product was loaded onto a 3 × 60 cm column of silica gel and eluted with a linear gradient consisting of 500 mL of chloroform to 500 mL of 70:30 tetrahydrofuran–chloroform, and finally with tetrahydrofuran, 20-mL fractions being collected. Fractions 68–79 were pooled and evaporated to give 101 mg (2.3%) of 9-(2,5-di-*O*-pivalyl-β-D-arabinofuranonyl)adenine as a glassy, noncrystalline solid: $[\alpha]^{23}_D$ 2.1° (c 1, methanol); UV λ_{max} (MeOH) 259 nm (ε 13 700); NMR ($CDCl_3$) δ 0.86–1.21 (s, s, 9, 9, acyl), 3.99 (m, width 16 Hz, H-4′), 4.40 (m, 2, H-5′,5′a), 4.59 (t, 1, H-3′, $J_{2,3}$ = 4.5 Hz), 5.27 (confirmed by irradiation at H-1′, dd, 1, H-2′), 5.86 (disappears upon exchange with deuterium oxide, br s, 2, NH_2), 6.52 (d, 1, H-1′, $J_{1,2}$ = 5.6 Hz), 8.27, 7.91 (s, s, 1, 1, H-2, H-8). Anal. C, H, N. Fractions 81–100 furnished 517 mg (12%) of 9-(3,5-di-*O*-pivalyl-β-D-arabinofuranosyl)adenine **5e** that was crystallized from acetone; for physical constants see Table I; for NMR data, see Table IV (supplementary material). Fractions 101–130 gave 956 mg (26%) of pure 9-(5-*O*-pivalyl-β-D-arabi-

nofuranosyl)adenine, identical in all respects to the sample previously described.[2]

Supplementary Material Available: Table IV containing ^{1}H NMR spectral data for 2′- and 3′-*O*-acyl and 2′,3′- and 3′,5′-di-*O*-acyl compounds (1 page). Ordering information is given on any current masthead page.

References and Notes

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(15) Interestingly, it is to be pointed out that the in vitro antiviral activities (see Table II) for the 3′-*O*-monoacyl analogues **6a–c** were found to be quite high, even for the branched-chain example **6c** whose di-*O*-acyl counterparts **4c** and **5c** were inactive.
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(20) Tetrabutylammonium fluoride is conveniently prepared by neutralization of 0.1 M tetrabutylammonium hydroxide at 0 °C with concentrated hydrofluoric acid, followed by lyophilization to give a white, flaky solid that may be stored up to a few hours in vacuo.

New Bronchodilators. Synthesis and Bronchodilating Activity of Some 3-(Alkoxymethyl)-α-(N-substituted aminomethyl)-4-hydroxybenzyl Alcohols

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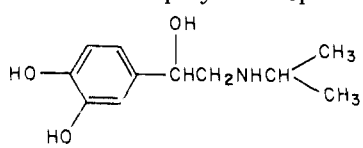
A series of 3-(alkoxymethyl)-α-(N-substituted aminomethyl)-4-hydroxybenzyl alcohols was synthesized as potential bronchodilators. The ability to prevent effects against histamine-induced bronchoconstriction in guinea pigs was studied to determine their bronchodilating activity. Introduction of a methoxymethyl group in place of the *m*-hydroxyl group of β-adrenergic catecholamines afforded compounds especially effective in delaying histamine-induced bronchoconstriction in guinea pigs. Appropriate N-substitution also enhanced the potency of these catecholamine analogues. 4-Hydroxy-3-(methoxymethyl)-α-[*N*-[4-(methoxymethyl)-α-methylphenyl]aminoethyl]benzyl alcohol hemifumarate (**3r**) was the most potent compound in this series.

β-Adrenergic receptors are classified into two types, β_1 and β_2.[1-3] The β_1 receptors mediate stimulation of cardiac muscle, relaxation of intestinal muscle, and glycogenolysis, while β_2 receptors mediate relaxation of bronchial, vascular, and uterine muscle and lipolysis. Isoproterenol (**1**) is a



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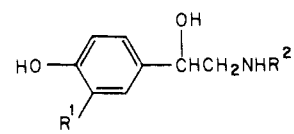
potent β-adrenergic stimulant which is widely used clinically as a bronchodilator in respiratory disorders. However, **1** stimulates both β_1 and β_2 receptors, and clinical application of **1** frequently causes adverse reactions such as palpitation, tachycardia, cardiac necrosis, and tremor. In addition, **1** has disadvantages of being short acting and low in activity by oral application owing to rapid conversion to ineffective metabolites,[4-7] *O*-sulfate and *m*-OMe derivatives. Numerous attempts have been made for structural modification of **1** to separate the bronchodilating effect from these side effects. The compounds **2a–d** in



2a,[8] $R^1 = CH_2OH$; $R^2 = t$-Bu
b,[9] $R^1 = NHSO_2Me$; $R^2 = i$-Pr
c,[10] $R^1 = NHCONH_2$; $R^2 = t$-Bu
d,[11] $R^1 = CH_2SO_2Me$; $R^2 = t$-Bu

which the *m*-phenolic OH was replaced with various substituents were reported[8-11] as improved bronchodilators with bronchial selectivity and oral activity.

In order to investigate the effect of introducing more lipophilic moieties into the meta position for bronchodilating activity, we prepared a series of 3-(alkoxymethyl)-α-(*N*-alkylaminomethyl)-4-hydroxybenzyl alcohols (**3a-i**) in which the meta substituents of **1** and **2a–d** were replaced by alkoxymethyl groups and examined their

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