1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-ethyluracil. A Highly Selective Antiherpes Simplex Agent

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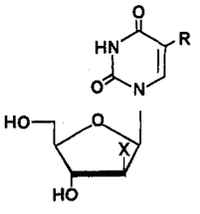
Received March 31, 1986

The protected nucleoside 1-(2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)-5-ethyluracil (**10**) was prepared by condensation of 3,5-dibenzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide (**9**) with 2,4-bis-*O*-(trimethylsilyl)-5-ethyluracil (**8**). The ratio in this coupling reaction has been raised to 17:1 in favor of the desired β-anomer. Deprotection by aminolysis gave 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-ethyluracil (FEAU, 1) in 67% isolated yield from the bromo sugar **9**. In vitro data show that FEAU has activity against herpes simplex virus types 1 and 2 comparable to that of 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-methyluracil (FMAU, **2**), 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil (FIAU, **3**), and acyclovir (ACV, **12**). The cellular toxicity of FEAU was found to be much lower than that of the other nucleoside analogues. Biochemical experiments indicate that FEAU has similar affinity toward thymidine kinases encoded by HSV 1 and 2 and a much lower affinity for cellular thymidine kinase than thymidine. The in vivo antiviral effects of FEAU, FMAU, FIAU, and ACV were evaluated against herpes infection in a systemic mouse encephalitis model and a cutaneous guinea pig model. While FEAU showed activity comparable to that of ACV in the systemic infection model, it was superior in the cutaneous herpes infection model.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-ethyluracil (FEAU, 1) is a member of a class of fluoroarabinofuranosyl 5-substituted pyrimidine nucleosides discovered and pioneered by Fox and his co-workers.[1,2] Compounds of this class have been extensively studied for their potential antiherpes activity. For activity, these compounds require



1	R = Et	X = F
2	R = Me	X = F
3	R = I	X = F
4	R = Me	X = H
5	R = Et	X = H
6	R = I	X = H

the presence of the virus-specified thymidine kinase.[3] These nucleosides are monophosphorylated on the 5′-hydroxyl group[4] by this kinase and subsequently converted to the 5′-di-[5] and then the 5′-triphosphates by cellular kinases. The triphosphate derivative inhibits virus DNA polymerase and thereby inhibits virus replication.[6] Inasmuch as these compounds are poor substrates for cellular kinases, the initial phosphorylation by the virus-specified thymidine kinase in infected cells is responsible for some selectivity. Although a synthesis[2] of FEAU (**1**) and in vitro[2,3] and some in vivo[7] data have been previously reported by Fox and his co-workers, no biochemical[8] or extensive in vivo biological results are published.

Among other 5-substituted derivatives, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-methyluracil (FMAU, **2**) and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil (FIAU, **3**) are particularly interesting as analogues of thymidine (**4**) that are active against both herpes simplex virus types 1 and 2 (HSV 1 and 2). For recognition as a thymidine analogue, the size of the 5-substituent is very important.[1b,7,9] If the substituent is larger than ethyl, then the activity against HSV 2 decreases significantly,[2c] because neither the cellular nor the virus-specified thymidine kinases efficiently convert the 5′-monophosphate to the 5′-diphosphate required for further activation. FMAU, however, shows very high potency in vivo against HSV 1 and 2,[9] and it shows good in vitro activity against varicella zoster[10,11] and cytomegalovirus.[12] Unfortunately, FMAU is relatively toxic to uninfected cells. FIAU, although less toxic to cells than FMAU, still showed cardiotoxicity as well as myelosuppression and bone marrow depletion at high doses.[13]

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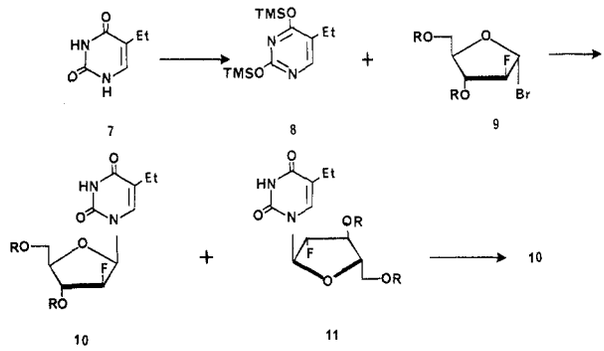
Scheme I[a]



[a] R = COPh.

An additional effect of the 5-substituent in these compounds is on the pK_a of the N^3-H of the nucleoside. FEAU would be expected to have a pK_a similar to that of 5-ethyldeoxyuridine (EDU, **5**), which is 9.8[14] as compared to 9.6 for thymidine. 5-Iododeoxyuridine (IUDR, **6**), the thymidine analogue in which the 5-methyl substituent has been replaced by iodine, has a pK_a of 8.25.[14] IUDR is mutagenic and can only be used topically. The difference between the pK_a of the N^3-H between IUDR and thymidine, which can potentially lead to base mispairing during DNA synthesis,[14] may be responsible for the mutagenicity of IUDR. Since EDU shows no demonstrable mutagenicity,[15] FEAU, like EDU, would also be expected to be less mutagenic. The presence of the fluorine may also reduce the potential of mutagenicity. Marquardt et al. have already shown that FMAU is not mutagenic.[16]

In addition to the possible reduction in mutagenicity, the 2′-fluoro substituent also confers two other main advantages over the 2′-deoxy analogues. First, the 2′-fluoro compounds are much less readily metabolized by pyrimidine nucleoside phosphorylase,[17] which cleaves the sugar from the base. Furthermore, those 2′-fluoroarabino derivatives that have been prepared and studied have shown greater selectivity compared to their 2′-deoxy counterparts.[18]

On the basis of the above arguments, we expected that FEAU would be a stable (nucleoside phosphorylase resistant), nonmutagenic, potent, and selective agent against herpes simplex virus. We describe a short, direct, and efficient synthesis of FEAU. We also provide biochemical and biological data that demonstrate that FEAU is a highly selective antiherpes agent.

Chemistry

5-Ethyluracil (**7**) was prepared according to the method of Kaul et al.[19] Simply heating **7** in hexamethyldisilazane (HMDS) at reflux with a minimal amount of DMF gave the bis-silylated base **8** (Scheme I). Although **8** was isolated by distillation in the earlier experiments, it can be used directly without purification once the excess HMDS has been removed. The silylated compound was coupled with the bromo sugar **9**.[20] Heating the reactants **8** and **9** at reflux in acetonitrile for 2 h gave the products **10** and **11** in 63% yield in a 3:2 ratio in favor of the desired β-anomer **10**. In the related 2′-chloro series, Ritzmann et al. had shown that these condensation reactions are very solvent dependent.[21,22] Using the optimum conditions described by Ritzmann, namely, heating **8** and **9** to reflux in dichloromethane, we were able to raise the ratio to 6:1 in favor of the desired β-anomer **10**. We later found that this ratio could be further improved to 17:1 if the reaction was carried out at reflux in chloroform.[23] The isolated yield of **10** in the chloroform reaction was 76%.

Although the two anomers co-migrate on TLC, the ratio can easily be obtained by either analytical HPLC[24] or ^1H NMR. Typically, the anomeric proton in the β-anomer appears downfield of the proton in the corresponding α-anomer.[2] In this case the H-1′ proton in the predominant β-anomer appeared as a double doublet at δ 6.32, approximately 0.2 ppm downfield of the H-1′ proton for the α-anomer.[2] The method of choice for separation of the two anomers was to recrystallize from absolute ethanol; the desired β-anomer crystallizes from the solution first.

On a small scale, simply stirring the protected compound **10** in a saturated solution of ammonia in methanol at room temperature overnight cleanly removed both benzoate protecting groups to give FEAU in 90% yield. On a large scale, longer reaction times are required to fully deprotect the 5′-monobenzoate intermediate, and after a reaction time of 3 days, the yield for this deprotection step was 93%.[25] The overall yield for pure recrystallized FEAU

Table I. Comparative Antiviral Efficacies of FMAU, FEAU, FIAU, and ACV against Herpes Simplex Virus[a]

compound	ID_{50},[b] μg/mL HSV 1 (BW^S)	ID_{50},[b] μg/mL HSV 2 (MS)
FEAU	0.99	2.7
FIAU	0.96	0.64
FMAU	1.3	0.13
ACV	0.7	1.1

[a] Antiviral test was performed in Vero cell cultures. [b] The 50% inhibitory dose.

Table II. Cellular Toxicity of FMAU, FIAU, FEAU, and ACV on Vero Cells[a]

compound	$TCID_{50}$,[b] μg/mL at 72 h[c]	$TCID_{50}$,[b] μg/mL at 120 h[c]
FIAU	43	11
FMAU	14	8
FEAU	>200	>200
ACV	28	30

[a] Cellular toxicity was assayed on Vero cells. [b] The 50% tissue culture inhibitory dose. [c] Postexposure.

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(25) The HPLC using 50% CH_3CN, 50% H_2O, UV detection at 254 nm, and a flow rate of 2 mL/min gave retention times of 0.94 min for β-FEAU and 3.89 min for dibenzoyl-FEAU.

Table III. The Kinetic Constants of Nucleoside Analogues for Viral and Cellular Thymidine Kinases

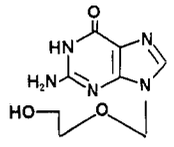
thymidine kinase	K_m, μM Thd	K_i, μM FEAU	FIAU	FMAU	ACV
Vero	0.81	3154.7	53	60	>4920[a]
HSV 1	0.29	0.09	0.14	0.18	45
HSV 2	0.66	0.44	0.95	1.40	180

[a] At saturating concentration of ACV, no inhibition could be detected.

from the bromo sugar **10** was 67% for two steps. This simple, direct method has been used to prepare multigram quantities of FEAU (**1**).

Biological Results and Discussion

The antiviral effects of FEAU, FMAU, FIAU, and acyclovir (ACV, **12**) were compared for selective inhibition of herpes virus replication in Vero cells. The data in Table I demonstrate the in vitro antiviral potency of each compound as expressed by the 50% inhibitory concentration (ID_{50}) against HSV 1 (strain BW^S) and HSV 2 (strain MS). All of these compounds exhibited similar antiherpetic activity against HSV 1 and HSV 2.



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Rapidly growing Vero cells were used to conduct a cell viability assay to ascertain the cellular toxicity of FEAU, FMAU, FIAU, and ACV. The results (Table II) show the 50% tissue culture inhibitory dose ($TCID_{50}$) after 72- or 120-h exposure to these nucleosides. While the $TCID_{50}$ for FMAU, FIAU, and ACV were all below 50 μg/mL at 72 or 120 h, the value for FEAU was greater than 200 μg/mL for both time periods. These data clearly show the lack of cellular toxicity of FEAU compared to the other compounds in this assay system.[7]

To better understand the lack of cellular toxicity of FEAU (**1**) as compared to the other test compounds, biochemical experiments were conducted. Since the selectivity of these nucleosides depends, in part, on their being good substrates for the virus-specified thymidine kinase but not the host thymidine kinase, the affinity (K_i) for these two enzymes was measured (Table III). The K_i of the fluoroarabinosyl nucleosides FEAU, FMAU, and FIAU for virus-specified kinases was slightly higher than for thymidine. FEAU, however, has a very low affinity for Vero cell thymidine kinases when compared to thymidine (3154 and 0.81 μM, respectively). Note also the considerable difference between FEAU and FMAU and FIAU. These results indicate that in uninfected cells FEAU would be phosphorylated at a very low rate in the presence of thymidine. This explains, at least in part, the impressive selectivity that FEAU demonstrated compared to the other nucleosides in the cellular cytotoxicity assay.

The in vivo antiviral efficiency of FEAU was compared with that of FIAU and ACV by using the herpes encephalitis mode. The results (Table IV) showed that the systemic treatment with all three test compounds decreased the mortality and increased the mean survival time of mice previously inoculated with HSV 2. Whereas ACV and FEAU significantly increased the number of survivors at doses of 50 and 100 mg/kg per day, respectively, FIAU showed significant activity at 25 mg/kg per day in this animal model. These data independently confirm other recently published data[7,26] and present further comparative results for other nucleoside analogues.

The effects of topical treatment with 5% preparations of FEAU, FMAU, and ACV on HSV 1 induced cutaneous lesions in guinea pigs are summarized (Table V). There were significant differences in the mean peak lesion score between untreated control or placebo control and FIAU-, FMAU-, and FEAU-treated animals, FMAU being the most effective. In contrast, no significant differences were observed between the ACV- and control-treated areas. The time to healing in guinea pigs treated with FMAU, FIAU, and FEAU was significantly reduced when compared to placebo-treated animals. The potency of FEAU, FMAU, and FIAU was also evaluated in terms of the lesion formation and healing time by using the area under the lesion score–day curve. Topical treatment with these compounds showed significant effects when compared to the ACV-treated and placebo-treated animals.

These results clearly show that FEAU is a highly selective compound with good activity against herpes simplex virus. While FEAU showed activity comparable to that of ACV in the systemic infection model, it was superior in the cutaneous herpes infection model.

Experimental Section

Melting points were determined on an Electrothermal capillary apparatus and are uncorrected. TLC was performed on silica gel 60 F-254 plates purchased from E. Merck Co., and column chromatography was performed on flash silica gel (40-μm particle size, Baker). Elemental analyses were performed by the Analytical Department, Bristol-Myers, Syracuse. ^1H and ^{13}C NMR spectra were recorded on an AM360 Bruker NMR spectrometer using tetramethylsilane as an internal standard; chemical shifts are reported in parts per million. Analytical HPLC was performed on an IBM C_{18} reverse-phase column (4.5 × 150 mm).

2,4-Bis-*O*-(trimethylsilyl)-5-ethyluracil (8). A solution of 5-ethyluracil (**7**)[19] (2.4 g, 16.9 mM) in hexamethyldisilazane (14 mL) and dimethylformamide (1.2 mL) was heated at reflux for 12 h under an inert atmosphere. The dark brown reaction mixture was allowed to cool and then filtered under nitrogen. The excess hexamethyldisilazane was removed at reduced pressure; the residual dark brown oil was then distilled on a Kugelrohr apparatus to give a colorless oil (2.7 g, 56%), which was used directly in the subsequent experiment: bp 70–80 °C (0.1 mm).

1-(2-Deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)-5-ethyluracil (10). Procedure A. A solution of **8** (1.3 g, 4.5 mm) in CH_3CN (5 mL) was added to a solution of the bromo sugar **9**[20] (2 g, 4.78 mM) in CH_3CN (5 mL), and the reaction mixture was heated at reflux for 2 h. The reaction was quenched with ice water, and then CH_2Cl_2 was added (50 mL). The CH_2Cl_2 solution was washed with water (2 × 25 mL) and dried ($MgSO_4$). The organic solution was filtered and then concentrated to leave 1.3 g (63%) of a pale yellow oil. This oil was a mixture of the two anomers **10** and **11**, as seen by analytical HPLC and ^1H NMR. Crystallization from ethanol gave the desired β-anomer **10** as a white crystalline powder (0.8 g, 38%): mp 149–153 °C; ^1H NMR ($CDCl_3$) 8.15–7.4 (m, 10 H, Ar H), 7.22 (s, 1 H, H-6), 6.35 (dd, 1 H, H-1′), 5.62 (dd, 1 H, H-3′), 5.31 (dd, 1 H, H-2′, J_{H-F} = 52 Hz), 4.8 (m, 2 H, 2 H-5′), 4.64 (br m, 1 H, H-4′), 2.2 (q, 2 H, CH_2), 0.95 (t, 3 H, CH_3). Anal. ($C_{25}H_{23}FN_2O_7$) C, H, N.

1-(2-Deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)-5-ethyluracil (10). Procedure B. Hexamethyldisilazane (96.5 mL) and ammonium sulfate (4.83 g, 36 mM) were added to a suspension of 5-ethyluracil (**7**) (57.2 g, 405 mM) in CH_3CN (1.45 L). The reaction mixture was then heated to reflux for 3 h and then allowed to cool. The solution was then stirred carefully under reduced pressure (aspirator) to remove excess NH_3 and then evaporated to leave an oily residue. Chloroform (200 mL) was added to the residue and the solution concentrated again; this procedure was repeated once more. The crude product was dissolved in $CHCl_3$ (300 mL) and transferred to a 3-L single-neck

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Table IV. Effects of FEAU, FIAU, and ACV Treatment on HSV 2 Induced Mortality in Mice

compound	dose,[a] mg/kg per day	survivors/total (%)	P value[b]	mean survival time, days	P value[c]
placebo		0/12		7	
FEAU	200	6/12 (50)	0.014	19	<0.001
	100	5/10 (50)	0.010	18	<0.001
	50	3/10 (30)	0.078	12	<0.001
	25	0/10 (0)	NS	9	0.001
	12.5	2/10 (20)	0.195	8	0.06
FIAU	200	6/10 (60)	0.003	21	<0.001
	100	3/10 (30)	0.078	13	<0.001
	50	5/10 (50)	0.010	18	<0.001
	25	5/10 (50)	0.010	18	<0.001
	12.5	2/10 (20)	0.195	10	0.010
ACV	100	3/8 (38)	0.049	13	<0.001
	50	4/10 (40)	0.029	10	<0.001
	25	3/9 (34)	0.063	10	0.001
	12.5	2/10 (20)	0.195	8	0.016

[a] The dose was administered ip, b.i.d. for 5 consecutive days starting 1 h postinfection. [b] Probability value of statistical significance, determined by the two-tailed Fisher exact test. [c] Probability value of statistical significance, determined by the Gehan–Wilcoxon test.

Table V. Effects of FEAU, FIAU, FMAU, and ACV Treatment on Lesion Score, Duration, and Healing Time in HSV 1 Cutaneous Infection of Guinea Pigs

group	no. of areas	mean peak lesion score	maximum score, days	time to start healing, days	mean area under lesion score–day curve
control	6	2.67	2.83	6.67	18.1
placebo control	3	3.0	3.67	5.67	16.7
5% ACV	4	2.5	4.0	6.25	15.2
5% FEAU	4	1.5	3.0	6.50	10.0[a,b]
5% FIAU	3	1.0	3.0	4.67	9.6[a,b]
5% FMAU	3	0.75	2.67	5.33	2.7[a,b]

[a] $P < 0.05$ compared to placebo-treated animals. [b] $P < 0.05$ compared to ACV-treated animals.

flask. To this was added a solution of freshly prepared bromo sugar **9** (145 g, 343 mM) in $CHCl_3$ (700 mL). Additional $CHCl_3$ (700 mL) was added, and the reaction mixture was heated to reflux with stirring for 20 h. The reaction mixture was allowed to cool and then washed with water (2 × 2 L). The organic phase was dried (Na_2SO_4) and then concentrated to give 153 g (94%) of the coupled products as a mixture of the two anomers. The crude product was dissolved in hot ethanol (1.5 L) and the solution allowed to cool at room temperature. After 64 h, the material was filtered to give the desired product **10** as a white crystalline solid (126 g, 76%): mp 149–152 °C.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-ethyluracil (1). Dibenzoate **10** (172 g, 356 mM) was added portionwise over 5 min to a cold (0–5 °C) solution of NH_3 (300 mL) in MeOH (1.72 L). The reaction vessel was stoppered and the slurry stirred at room temperature for 65 h. Analytical HPLC[25] showed the reaction to be complete. The reaction mixture was concentrated at reduced pressure to leave 189 g of an oily residue, which crystallized on standing. The residue was dissolved in boiling CH_3CN (700 mL) and left to crystallize. FEAU was isolated as a white crystalline solid (90.8 g, 93%): mp 168–170 °C; ^1H NMR (Me_2SO) 7.55 (s, 1 H, H-6), 6.1 (dd, 1 H, H-1′), 5.1 (m, 1 H, H-2′, J_{H-F} = 51 Hz), 4.1 (m, H, H-3′), 3.7 (m, 1 H, H-4′), 3.6 (m, 2 H, H-5′), 2.2 (q, 2 H, CH_2), 1.0 (t, 3 H, CH_3); ^{13}C NMR (Me_2SO) 165.19 (C, C_2), 150.04 (C, C_4), 135.02 (CH, C_6), 114.37 (1 C, C_5), 96.69, 94.58 (d, CH, C_2', J_{C-F} = 196 Hz), 83.01, 82.94 (d, CH, C_4'), 82.20, 82.01 (d, CH, C_3'), 72.40, 72.14 (d, CH, C_1'), 59.51 (s, CH_2, C_5'), 19.62 (s, CH_2, ethyl), 12.90 (s, CH_3, ethyl). Anal. ($C_{11}H_{15}FN_2O_5$) C, H, N.

Biochemical Data. Thymidine kinases from LMTK$^-$ cells infected with HSV 1 (KOS strain) or HSV 2 (MS strain) were purified.[27,28] Enzymes were changed to the ATP·Mg^{2+} form before being used in kinetic studies by passage through a Sephadex G-25-40 column (Sigma Chemical Co., St. Louis, MO), equilibrated with 1 mM ATP·Mg^{2+}, 10 mM Tris, pH 7.5, 10% glycerol, and were eluted with the same buffer. Thymidine kinase from Vero cells was purified as described above. The assay for enzyme activities and analysis of kinetic data were done as described previously.[29]

Antiviral Activity. a. In Vitro Antiviral Evaluation. The compounds were evaluated for antiviral activity in vitro by the dye-uptake method.[30] The 50% inhibitory dose (ID_{50}) for each drug was determined by linear regression analysis.

b. In Vitro Cellular Cytotoxicity. The cellular toxicity assay was determined in rapidly growing Vero cells. The toxic effect of the tested antiviral compounds was determined by a reduction in the number of viable cells in culture. Twenty-four-well plates (Costar #3424, Cambridge, MA) were seeded with 5 × 10^3 cells/well. On the following day, various concentrations of the test compounds were added to triplicate cultures. The plates were then incubated at 37 °C in 5% CO_2. At 72 and 120 h post-treatment, cells were trypsinized and viable cells were counted with a hemacytometer by using the trypan blue exclusion method. The percent viable cell reductions in treated cultures were determined by using the formula

$$100 - [(\text{no. of viable cells in treated culture} - \text{no. of viable cells in untreated culture})/(\text{no. of viable cells in mock treated culture} - \text{no. of viable cells in untreated culture})] \times 100$$

The 50% tissue culture inhibitory dose ($TCID_{50}$) for each drug was then determined by the Reed–Muench method.[31]

c. In Vivo Herpes Encephalitis Infection in Mice. Female Swiss mice weighing 16–18 g (Charles River, Wilmington, MA) were anesthetized (Ketaset, Bristol-Myers Co., Syracuse, NY) and inoculated intracerebrally (ic) with 50 μL (10 LD_{50}) of HSV 2, G strain. Mice were then randomized into groups of 8–12 mice each. Intraperitoneal (ip) treatment with the drug was initiated 1 h postinfection and continued twice daily for 5 consecutive days.

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Infected placebo control mice were injected with the vehicle [0.2% (carboxymethyl)cellulose with 0.2% Tween 80 in water (CMC/T)]. Mice were observed, for 21 days, for mortality. Statistical analysis and the probablity (P) value for the percent survival and the mean survival time were determined by Fisher's exact and Gehan–Wilcoxon tests, respectively.

d. Cutaneous HSV Infection in Guinea Pigs. We used the method described by Alenius and Oberg[32] with slight modification. Briefly, guinea pigs weighing 250–300 g (Charles River, Wilmington, MA) were anesthetized (Ketaset, Bristol-Myers Co., Syracuse, NY) and shaved and depilated on the back. The depilated areas were divided into six squares, and 20 μL of HSV 1, HL-34 strain with a titer of 6.5×10^7 PFU/mL was applied with a multiple puncture apparatus (Downs Surgical, Inc., Wilmington, MA). Treatment was initiated 3 h postinfection and continued twice daily for 5 consecutive days. Four areas on each animal were treated topically with preparations of either 5% FEAU, FMAU, or FIAU (prepared fresh daily in CMC/T in polyethylene glycol (PEG)) or with 5% acyclovir ointment (Zovirax, Burroughs Wellcome Co., Research Triangle, NC). One area on each animal was treated with PEG (placebo control) and one area was not treated (untreated control). Fifty microliters of the compound solution was applied each treatment and spread over the infected site. Each drug was tested in three to five animals, with one area/animal for each compound.

The scoring system used was similar to that described by Alenius and Oberg:[32] 1 = erythema and one or two small vesicles; 2 = erythema and numerous small vesicles; 3 = numerous large vesicles, if in close juxtaposition, coalesced; III = vesicles dried, large crusts; II = 50% of the crusts fallen off; I = 10% of the crusts remains; 0 = complete healing. Scoring was done blindly every day for 14 days. Statistical analysis and the mean area under the lesion score–day curve were determined by using a two-sample t test with a pooled error term. Comparisons were based on a logarithmic transformation of the areas. A P value less than 5% was considered significant.

Acknowledgment. We express our appreciation to Dan J. Speelman and Randy R. Brutkiewiez for their technical assistance in obtaining the biological data. We thank also the Bristol-Myers Analytical Department.

Registry No. 1, 83546-42-3; 7, 4212-49-1; 8, 31167-05-2; 9, 97614-44-3; 10, 95740-18-4; 11, 106835-91-0.

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Structure–Activity Relationships in the 2-Arylcarbapenem Series: Synthesis of 1-Methyl-2-arylcarbapenems

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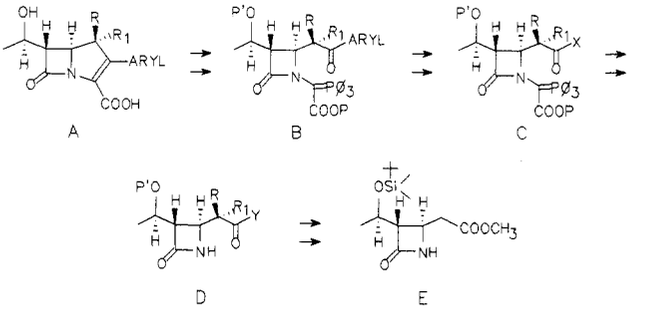
Received January 10, 1986

The labile *tert*-butyldimethylsilyl esters of the azetidinones **6–8b** served as the crucial synthons in the preparation of the potentially useful ylide pyridyl thio esters **18–20**. These intermediates were utilized to synthesize a host of title carbapenems **25–30d**, **32**, and **49–53**. The antimicrobial properties and DHP-I susceptibility of these carbapenems were studied with reference to thienamycin.

The discovery of thienamycin[1] and related members of the carbapenem family[2] ushered in a new era in antibiotics research. Excitement over the unprecedented potency and breadth of spectrum of these natural products was soon tempered by the recognition that many of the agents that showed the most promising antibacterial properties also suffered from both chemical instability and susceptibility to a mammalian dehydropeptidase, DHP-I.[3a,b] Previous work[3b] from these laboratories has shown that the chemical stability of thienamycin could be significantly enhanced by conversion to the N-formimidoyl derivative, imipenem.

Scheme I



However, this modification has little impact on metabolic stability, imipenem being approximately 90% as susceptible to DHP-I-mediated hydrolysis as thienamycin. Subsequent total synthesis studies[4,5] resulted in the discovery of two subclasses of carbapenems that combine chemical and metabolic stability with enhanced antibacterial potency. The properties of these structural variants, i.e., 1-substituted carbapenems and 2-arylcarbapenems, have been the subjects of previous reports from these laboratories.[4,5] Herein we detail the syntheses and structure–activity relationships for 1-methyl-2-arylcarbapenems, a new hybrid class of compounds that com-

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