The product was purified by flash chromatography[21] on silica gel using 40% hexane in EtOAc. The appropriate fractions were combined, evaporated, and recrystallized from EtOAc–hexane: yield 0.23 g (27%); mp 142–143.5 °C; TLC (toluene–EtOH, 4:1, v/v); UV (0.1 N HCl + 20% EtOH) λ_{max} 258.5 nm (ϵ 8800); UV (0.1 N NaOH + 20% EtOH) λ_{max} 255.5 nm (ϵ 5500); NMR (Me_2SO-d_6) δ 11.87 (br s, 1 H, NH), 8.44 (d, 1 H, C-6), 5.18 (s, 2 H, NCH_2O), 4.66 (t, 1 H, OH), 3.53 (s, 4 H, CH_2CH_2). Anal. ($C_8H_9F_3N_2O_4$) C, H, F, N.

5-Fluoro-1-[(2-hydroxyethoxy)methyl]uracil (22). A solution of 1.690 g (6.86 mmol) of 18, 150 mL of a 10% solution of dimethylamine in EtOH, and 15 mL of water was heated on the steam bath for 3 h. The volatiles were evaporated to give a solid that was triturated with Et_2O. The residue was recrystallized from EtOH: yield 1.59 g; mp 144–156 °C. Several recrystallizations from EtOH gave the analytical sample: yield 0.45 g (32%); mp 155–157 °C; TLC (toluene–EtOH, 3:1, v/v); UV (0.1 N HCl + 20% EtOH) λ_{max} 266 nm (ϵ 7700), UV (0.1 N NaOH + 20% EtOH) λ_{max} 266 nm (ϵ 5600); NMR (Me_2SO-d_6) 11.82 (br s, 1 H, NH), 8.11 (d, 1 H, J = 6.6 Hz, C-6), 5.06 (s, 2 H, NCH_2O), 4.64 (br s, 1 H, OH), 3.52 (s, 4 H, CH_2CH_2). Anal. ($C_7H_9FN_2O_4$) C, H, F, N.

1-[(2-Hydroxyethoxy)methyl]-5-iodouracil (23). A solution of 0.500 g (1.41 mmol) of 19, 0.170 g (3.15 mmol) of sodium methoxide, and 20 mL of dry MeOH was heated on a steam bath with protection from moisture for 2 h. The solution was cooled and neutralized with Bio-Rad AG 50W-X4 cationic resin. The resin was removed by filtration and washed with MeOH. The combined filtrate and wash was decolorized with Norit and concentrated to 10 mL. The resultant crystals were collected and dried: yield 0.32 g (72%); mp 171–173 °C. Recrystallization from MeOH gave the analytical sample: yield 0.260 g (59%); mp 172–174 °C; TLC (toluene–EtOH, 3:1, v/v); UV (0.1 N HCl + 20% EtOH) λ_{max} 285 nm (ϵ 6900); UV (0.1 N NaOH + 20% EtOH) λ_{max} 278 nm (ϵ 4800); NMR (Me_2SO-d_6) δ 11.67 (br s, 1 H, NH), 8.24 (s, 1 H, C-6), 5.09 (s, 2 H, NCH_2O), 4.65 (br t, 1 H, OH), 3.51 (s, 4 H, CH_2CH_2). Anal. ($C_7H_9IN_2O_4$) C, H, I, N.

1-[(2-Hydroxyethoxy)methyl]-5-bromouracil (24). To a solution of 2.0 g (10.7 mmol) of 14 in 200 mL of H_2O was added 200 mL of Br_2-saturated H_2O over 10 min when the color persisted. The solution was flash evaporated to a residue that was chromatographed on silica gel by elution with $CHCl_3$–MeOH mixtures. The fractions containing product were evaporated, dissolved in MeOH, and diluted with EtOAc to give 24: yield 1.08 g (38%); mp 147–148 °C (MeOH-EtOAc); UV (EtOH) λ_{max} 276 nm (ϵ 8400); NMR (Me_2SO-d_6) δ 11.8 (br s, 1 H, NH), 8.30 (s, 1 H, C-6), 4.2 (br s, 1 H, OH), 3.55 (s, 4 H, CH_2CH_2). Anal. ($C_7H_9N_2O_4Br$) C, H, N.

Acknowledgment. The authors are indebted to Dr. D. J. Bauer and his group for the antiviral testing, to Ms. N. K. Cohn for the Detroit 98 and mouse L cells cytotoxicity tests, and to Dr. J. A. Fyfe and P. M. Keller for the HSV-1 thymidine kinase results. We thank Lila Beauchamp for providing 2-(benzoyloxy)ethoxymethyl chloride and Dr. B. S. Hurlbert and his staff for the elemental analyses and NMR spectra.

(21) W. C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, 43, 2923 (1978).

Inhibitors of Phenylethanolamine *N*-Methyltransferase and Epinephrine Biosynthesis. 3. Bis[tetrahydroisoquinoline]s[1,2]

R. M. DeMarinis,* W. M. Bryan, L. M. Hillegass, D. McDermott, and R. G. Pendleton

Research and Development Division, Smith Kline & French Laboratories, Philadelphia, Pennsylvania 19101.
Received August 25, 1980

7,8-Dichloro-1,2,3,4-tetrahydroisoquinoline (SK&F 64139) is a potent inhibitor of phenylethanolamine *N*-methyltransferase (IC_{50} = 10 μM) that may have therapeutic utility in man. A series of related compounds in which two 7,8-dichloro-1,2,3,4-tetrahydroisoquinoline molecules have been bridged from nitrogen to nitrogen by an unbranched alkyl chain have been prepared and have demonstrated potent inhibitory properties (0.08 to 2 μM). In contrast, simple substitution on the nitrogen of 7,8-dichloro-1,2,3,4-tetrahydroisoquinoline with a variety of substituents gives compounds with greatly diminished inhibitory potencies (IC_{50} = 2 to >100 μM) relative to SK&F 64139. Kinetic studies with a C_6 analogue have shown that it is competitive with respect to phenylethanolamine and uncompetitive with respect to *S*-adenosylmethionine. The increased potency of some of the bis analogues relative to that seen with the tetrahydroisoquinolines having larger alkyl groups on nitrogen suggests that several of the bis compounds show supplemental or cooperative binding to the enzyme, presumably as a result of the second tetrahydroisoquinoline moiety.

Phenylethanolamine *N*-methyltransferase (PNMT) is the enzyme which catalyzes the final step in epinephrine biosynthesis. It is mainly localized in the adrenal medulla, although it is also found in much lower concentration in other organs.[3] Physiologically, PNMT catalyzes the transfer of a methyl group from *S*-adenosylmethionine (SAM) to norepinephrine (NE) to yield epinephrine and *S*-adenosylhomocysteine (Scheme I). Under resting conditions, the levels of circulating epinephrine are quite low and it is not a significant regulatory hormone.[4,5] However, when the organism is subjected to stress, large quantities of epinephrine are released into the blood stream and this initiates physiological changes, which are associated with the "fight or flight syndrome", which prepare the body to cope with the stressor situation. It is possible that under certain circumstances the incresed metabolic and or physiological activities induced by the efflux of epinephrine from the adrenal medulla may be detrimental to people who are predisposed to disease states such as anxiety or ischemic heart disease.[6] Thus, an agent that can selectively inhibit the final step in epinephrine biosynthesis could be of potential therapeutic utility in the treatment of these disorders.

(1) This paper has been presented in part. See "Abstracts of Papers", 180th National Meeting of the American Chemical Society, Las Vegas, NV, Aug 24–29, 1980, American Chemical Society, Washington, DC, 1980, Abstr MEDI 71.
(2) For Part 2, see B. Blank, A. Krog, G. Weiner, and R. Pendleton, *J. Med. Chem.*, 23, 837 (1980).
(3) J. Axelrod, *J. Biol. Chem.*, 237, 1657 (1962).
(4) S. Udenfriend, J. R. Cooper, C. T. Clark, and J. E. Baer, *Science*, 117, 663 (153).
(5) R. J. Wurtman, *Recent Adv. Biol. Psychiatry*, 9, 350, (1966).
(6) W. Raab, *Am. J. Cardiol.*, 9, 576 (1962).

0022-2623/81/1824-0756$01.25/0 © 1981 American Chemical Society

Scheme I

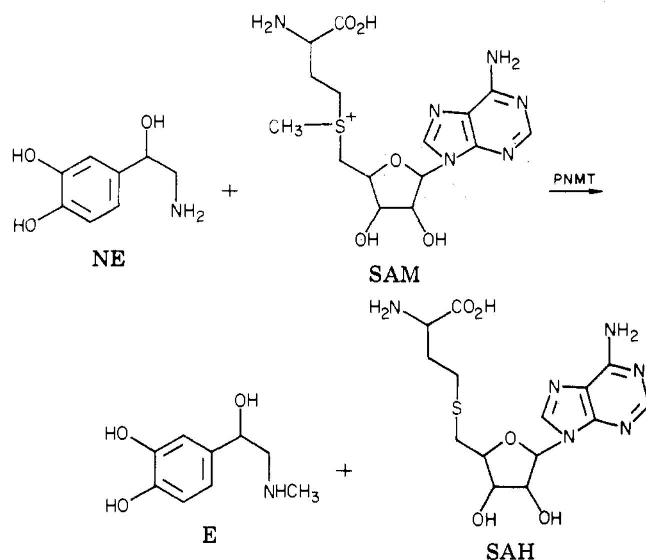


Table I. Bis[tetrahydroisoquinoline]s

n	formula[a]	mp, °C dec
2	$C_{20}H_{20}Cl_4N_2 \cdot 2HCl$	260-265
3	$C_{21}H_{22}Cl_4N_2 \cdot 2NCl$	248-252
4	$C_{22}H_{24}Cl_4N_2 \cdot 2HCl$	269-275
5	$C_{23}H_{26}Cl_4N_2 \cdot 2HCl$	279-285
6	$C_{24}H_{28}Cl_4N_2 \cdot 2HCl$	308-311
7	$C_{25}H_{30}Cl_4N_2 \cdot 2HCl$	280-285
8	$C_{26}H_{32}Cl_4N_2 \cdot 2HCl$	263-268

[a] All compounds for which formulas are indicated analyzed as hydrates for C, H, and N within 0.4% of calculated values.

Investigations at Smith Kline & French have led to the identification of several classes of compounds which are potent, selective inhibitors of PNMT.[7] From among these, 7,8-dichlorotetrahydroisoquinoline (SK&F 64139) emerged as one of the more potent inhibitors both in vitro and in vivo and is being studied for its possible therapeutic utility in man.[8]

Results

We have discovered that a series of bis[7,8-dichlorotetrahydroisoquinoline]s which are connected together by an unbranced chain of from two to eight carbon atoms are potent inhibitors of PNMT. None of these are as potent as SK&F 64139 itself, but all are much more active than would be predicted from previous studies on N-alkylated aralkylamines[9,10] or from the activities seen with a series of N-alkylated tetrahydroisoquinolines. Kinetic studies on a representative C_6 analogue have shown that it is competitive with respect to phenylethanolamine and uncompetitive with respect to SAM. The increase in activity observed when a second tetrahydroisoquinoline is attached via an alkyl chain to SK&F 64139 over that seen upon alkylation of SK&F 64139 with a variety of nonspecific alkylating agents may indicate a second cooperative or supplementary binding as a result of the second tetrahydroisoquinoline.

Table II. Comparison of PNMT-Inhibitory Potency of Bis[tetrahydroisoquinoline]s and N-Substituted Tetrahydroisoquinolines

n	IC_{50}, μM	R	IC_{50}, μM
2	0.08	H	0.01
3	0.3	CH_2CH_3	2.0
4	0.1	$CH_2CH_2NH_2$	2.0
5	10.0[a]	$CH_2CH_2CH_3$	5.0
6	1.0	CH_2Ph	7.0
7	2.0	$(CH_2)_6Ph$	50
8	2.0	$(CH_2)_5CH_2OH$	100
		$(CH_2)_5CH_2Br$	100

[a] Extremely insoluble under assay conditions.

Chemistry. The bis[tetrahydroisoquinoline]s described here were prepared by several general procedures from the previously described 7,8-dichlorotetrahydroisoquinoline (**1**).[11] The analogues having 2, 4, or 5 carbons were obtained by acylation of **1** with the appropriate ω-haloacyl halide, followed by alkylation with a second mole of **1** and reduction of the amide with either diborane or lithium aluminum hydride. Those congeners with 3, 6, 7, or 8 methylenes were synthesized via displacement by **1** on the appropriate dihalides. Compounds were converted to their dihydrochloride salt hydrates and tested as such for PNMT inhibitory activity.

Discussion

The PNMT inhibitory protencies of a series of bis[7,8-dichlorotetrahydroisoquinoline]s are shown in Table II in comparison with a series of N-alkylated 7,8-dichlorotetrahydroisoquinolines. The data obtained from the N-alkylated 7,8-dichlorotetrahydroisoquinolines in this table demonstrate that as the size of the substituent on nitrogen increases, the potency of the compound decreases significantly. Those compounds which have the largest groups on the nitrogen, such as $(CH_2)_6Br$, $(CH_2)_6OH$, or $(CH_2)_6Ph$, are very weak inhibitors of PNMT (IC_{50} = 50 to >100 μM). Other investigators have also shown that N-alkylation of other inhibitors of PNMT significantly decreased their potency.[3,9,10] This effect of substituent size on inhibitory potency presumably acts in a similar fashion in the bis series, but here it is counterbalanced by an opposing effect. As the chain is lengthened to six carbons or greater an increased potency is observed over what would be expected for a simple alkyl substituent. The C_6 dimer is from 50 to 100 times more potent than the phenyl, bromo, or hydroxy compound, which have six methylenes. These structures differ only in the terminal substituent, and the increase in potency must be due to the second tetrahydroisoquinoline moiety. The fact that enhanced potency is seen only when a second tetrahydroisoquinoline is attached but not when hydroxy, bromo, or phenyl is present argues for a second specific interaction rather than

(7) W. E. Bondinell, F. W. Chapin, G. R. Girard, C. Kaiser, A. J. Krog, A. M. Pavloff, M. S. Schwartz, J. S. Silvestri, P. D. Vaidya, B. L. Lam, G. R. Wellman, and R. G. Pendleton, *J. Med. Chem.*, **23**, 506 (1980).
(8) J. W. Dubb, R. M. Stote, F. Alexander, A. P. Intoccia, M. Geczy, and R. G. Pendleton, *Clin. Pharmacol. Ther.*, **25**, 837 (1979).
(9) R. W. Fuller, J. Mills, and M. M. Marsh, *J. Med. Chem.*, 14, 322 (1971).
(10) R. W. Fuller, B. B. Molloy, W. A. Day, B. W. Roush, and M. M. Marsh, *J. Med. Chem.*, **16**, 101 (1973).
(11) R. G. Pendleton, C. Kaiser, and G. Gessner, *J. Pharmacol. Exp. Ther.*, **197**(3), 623 (1976).

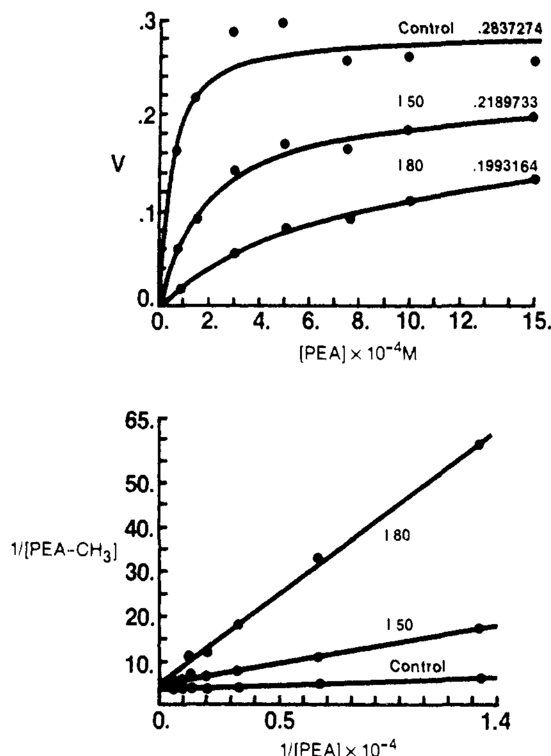


Figure 1. Phenylethanolamine kinetics.

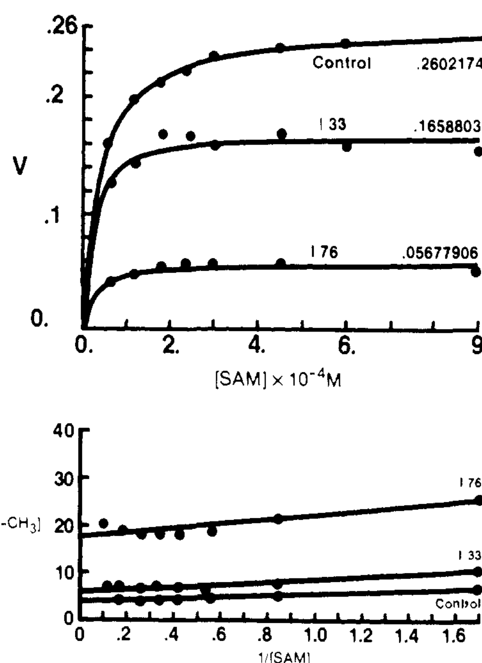


Figure 2. *S*-Adenosylmethionine kinetics.

for a nonspecific lipophilic or steric effect.

The substrate-velocity curves and the Lineweaver–Burk plots on the C_6 dimer indicate that the inhibition produced by this compound at two different concentrations could be overcome by increasing the concentration of the substrate phenylethanolamine (PEA), which shows that it is competitive with respect to PEA (Figure 1). When the variable substrate is SAM, the inhibition is not overcome by increasing the amount of SAM present. In this case, the parallel lines obtained in the Lineweaver-Burk plot of these data show that this inhibitor is uncompetitive with respect to SAM (Figure 2).

We have interpreted the SAR and kinetics of inhibition of this series of compounds to mean that we are looking at an enzyme–inhibitor interaction in which one tetrahydroisoquinoline moiety is binding to an active site of the enzyme and the second tetrahydroisoquinoline is binding in a supplementary fashion to a second site on the enzyme. This series of inhibitors react competitively with PEA and, thus, bind in a reciprocal fashion to the enzyme, most likely at the active site. Their uncompetitive mode of inhibition relative to SAM means that they act with a form of the enzyme with which SAM does not react and that one of the tetrahydroisoquinoline portions is not reacting with a SAM binding site. The significantly increased potency of the C_6 bis compound relative to a series of C_6 alkylated analogues indicates a probable second site of interaction with the enzyme.

Experimental Section

Melting points were determined in open capillary tubes using a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Elemental analyses were performed by the Analytical and Physical Chemistry Department of Smith Kline & French Laboratories. Where analyses are reported by symbols of elements, results were within 0.4% of calculated values. Mass spectra were obtained on a Hitachi Perkin-Elmer RMU-6E spectrometer using Me_4Si or DSS as internal standard. Satisfactory IR and NMR spectral data were obtained for all reported compounds.

2,2′-Ethylenebis[7,8-dichloro-1,2,3,4-tetrahydroisoquinoline] Dihydrochloride Hydrate. A mixture of 2.80 g (70 mmol) of magnesium oxide and 9.76 g (41 mmol) of 7,8-dichloro-1,2,3,4-tetrahydroisoquinoline hydrochloride in 100 mL of CH_2Cl_2 was stirred in an ice bath while 4.75 g (42 mmol) of chloracetyl chloride in 25 mL of CH_2Cl_2 was added over 30 min. The mixture was allowed to warm to room temperature and stirred overnight. The suspended solids were removed by filtration and the filtrate was concentrated to a yellow oil, which was dissolved in about 30 mL of EtOH and placed in the freezer. The resulting crystals were removed by filtration to give solid chloracetamide, mp 110–112 °C. A mixture of 6.95 g (25 mmol) of chloroacetamide, 5.9 g (25 mmol) of 7,8-dichloro-1,2,3,4-tetrahydroisoquinoline, and 5.30 g (50 mmol) of sodium carbonate in 100 mL of absolute EtOH was refluxed for 4 h. The mixture was cooled to 0 °C and filtered. The solid was washed with water and dried to give 10.4 g of cream-colored amide, mp (hydrochloride) 165–175 °C dec. A suspension of 4.44 g (10 mmol) of this amide in 300 mL of dry THF was stirred under argon with 1.0 g of $LiAlH_4$ for 1 h. The mixture was treated with 10 mL of EtOAc, followed by 25 mL of 10% sodium hydroxide. The mixture was evaporated to a residue, treated with 100 mL of water, and extracted with six 50-mL portions of EtOAc. The combined extracts were dried and evaporated to a solid, which was triturated with 10 mL of EtOH. The residue was suspended in 20 mL of EtOH and treated with 10 mL of ethereal HCl to yield a clear solution, which after standing in the cold deposited crystals. The precipitate was removed by filtration, washed with Et_2O, and dried to give white crystals, mp 260–265 °C dec. By a similar procedure, the C_4 and C_5 analogues were prepared.

2,2′-Hexylenebis[7,8-dichloro-1,2,3,4-tetrahydroisoquinoline] Dihydrochloride Hydrate. A suspension of 2.38 g (10 mmol) of 7,8-dichloro-1,2,3,4-tetrahydroisoquinoline and 3.18 g (30 mmol) of sodium carbonate in 50 mL of absolute ethanol was refluxed for 5 min. To this suspension was added 1.22 g (5 mmol) of 1,6-dibromohexane, and the mixture was refluxed overnight. The reaction was cooled and, after standing for 36 h, was filtered of solid precipitate. The resulting solid was suspended in 100 mL of EtOAc and treated with 1 g of $MgSO_4$. The mixture was filtered and the filtrate treated with 10 mL of ethereal HCl. The solution deposited crystals, which were removed by filtration, washed with Et_2O, and dried, mp 308–311 °C dec. By a similar method, the C_3, C_7, and C_8 analogues were prepared.

In Vitro PNMT Inhibition Assay. This test was performed as previously described.[7] Rabbit adrenal PNMT was solubilized in potassium phosphate buffer, and the reaction was conducted in a total volume of 300 μL, constituted as follows: PNMT, 42 μg; potassium phosphate buffer (pH 7.9), 10 μmol; DL-phenylethanolamine, 90 nmol (3×10^{-4} M); [*Me*-^{14}C]SAM (ca. 200 000 dpm), 9 nmol (3×10^{-5} M). The reaction was run for 15 min at

37 °C and was terminated by the addition of 0.5 mL of 0.5 M borate buffer, pH 10. The ^{14}C-labeled product was extracted into 6 mL of toluene–isoamyl alcohol (97:3). One milliliter of the organic layer was added to 10 mL of liquid scintillation cocktail, counted in a Nuclear-Chicago scintillation counter for 10 min, and quantitated in terms of the nanomoles of *N*-(methylphenyl)ethanolamine produced. Percent inhibition was determined by comparison of the quantity of *N*-(methylphenyl)ethanolamine formed in the presence of various concentrations of test compound with controls. Concentrations causing 50% inhibition of PNMT (IC_{50}) were derived graphically from at least four such measurments, at least one of which produced less than 50% inhibition.

Acknowledgment. We thank John Zarembo and his staff in the Analytical and Physical Chemistry Department of Smith Kline & French Laboratories for the elemental analyses. We are also grateful to Dr. Carl Perchonock for the preparation of several reference compounds and for helpful discussions.

Synthesis and Antiviral Properties of (*Z*)-5-(2-Bromovinyl)-2′-deoxyuridine

A. Stanley Jones,* S. George Rahim, Richard T. Walker,

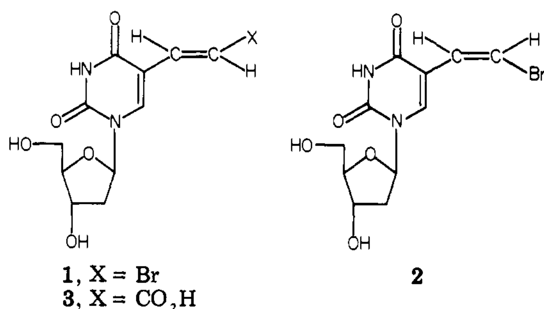
Chemistry Department, University of Birmingham, Birmingham B15 2TT, United Kingdom

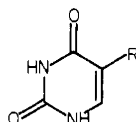
and Erik De Clercq

Rega Institute for Medical Research, University of Leuven, Belgium. *Received December 17, 1980*

(*Z*)-5-(2-Bromovinyl)uracil was obtained by photoisomerization of the *E* isomer. Similarly, (*E*)-5-(2-bromovinyl)-2′-deoxyuridine gave the required *Z* isomer. (*Z*)-5-(2-Bromovinyl)-2′-deoxyuridine is much less active against herpes simplex virus type 1 (HSV-1) and somewhat less active against herpes simplex virus type 2 than is the *E* isomer. Both isomers show similar activity against vaccinia virus. Therefore, the highly potent and selective activity of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine against HSV-1 is due to its *E* configuration.

(*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (**1**) is a potent and





4, R = C≡CBr
5, R = (*Z*)-CH=CHBr
6, R = $CH=CH_2$
7, R = C≡CH
8, R = (*E*)-CH=CHBr

selective inhibitory agent for herpes simplex virus type 1 (HSV-1).[1] The corresponding chloro and iodo compounds are only slightly less active, and (*E*)-5-(propen-1-yl)-2′-deoxyuridine and (*E*)-5-[3,3,3-(trifluoromethyl)propen-1-yl]-2′-deoxyuridine also show high activity.[2] These compounds have attached to the ethylenic double bond, a bulky group which is oriented trans with respect to the nucleoside residue. There have been no reports of the synthesis or antiviral activity of compounds in which these groups are in the cis orientation and so it seemed important to synthesize (*Z*)-5-(2-bromovinyl)-2′-deoxyuridine (**2**) and to determine its antiviral activity.

Compound **1** has been obtained from (*E*)-5-(2-carboxyvinyl)-2′-deoxyuridine (**3**) by treatment with *N*-bromosuccinimide in aqueous potassium acetate.[3] Originally, we found none of the corresponding *Z* isomer; a surprising result in view of the fact that in similar systems either the *Z* isomer is formed exclusively (i.e., in relatively nonpolar solvents) or a mixture of *Z* and *E* isomers is produced.[4–7] Upon completion of the present work it became apparent that in our system, however, about 8% of the *Z* isomer is formed.

To synthesize the required *Z* isomer (**2**), the first approach was to hydrogenate selectively 5-(bromoethynyl)uracil (**4**) by the use of a quinoline-poisoned Lindlar palladium catalyst to obtain (*Z*)-5-(2-bromovinyl)uracil (**5**), which could then be converted into **2** by standard procedures. However, the major product was 5-vinyluracil (**6**). Since traces of 5-ethynyluracil (**7**) were detected as an intermediate in the reaction, it appeared that hydrogenolysis of the C–Br bond preceded the hydrogenation of the triple bond. Attempts to alter this situation by changing the solvent and the catalyst were unsuccessful.

(1) E. De Clercq, J. Descamps, P. De Somer, P. J. Barr, A. S. Jones, and R. T. Walker, *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 2947 (1979); E. De Clercq, J. Descamps, G. Verhelst, A. S. Jones, and R. T. Walker in "Current Chemotherapy and Infectious Diseases", American Society for Microbiology, Washington, DC, 1980, p 1372.
(2) E. De Clercq, J. Descamps, P. J. Barr, A. S. Jones, P. Serafinowski, R. T. Walker, G. F. Huang, P. F. Torrence, C. L. Schmidt, M. P. Mertes, T. Kulikowski, and D. Shugar, in "Antimetabolites in Biochemistry, Biology and Medicine", J. Skoda and P. Langen, Eds., Pergamon Press, Elmsford, NY, 1979, p 275.
(3) A. S. Jones, G. Verhelst, and R. T. Walker, *Tetrahedron Lett.*, 4415 (1979).
(4) E. Grovenstein and D. E. Lee, *J. Am. Chem. Soc.*, **75**, 2639 (1953).
(5) S. J. Crestol and W. P. Norris, *J. Am. Chem. Soc.*, **75**, 632 and 2645 (1953).
(6) W. R. Vaughan, W. F. Cartwright, and B. Heizi, *J. Am. Chem. Soc.*, **94**, 4978 (1972).
(7) D. Vegh, J. Kovac, and M. Dandarova, *Tetrahedron Lett.*, **21**, 969 (1980).
(8) P. J. Barr, A. S. Jones, G. Verhelst, and R. T. Walker, *J. Chem. Soc., Perkin Trans. 1*, in press.

0022-2623/81/1824-0759$01.25/0 © 1981 American Chemical Society