cedure C. A mixture of aniline (138 μL, 1.5 mmol) and compound **4** (170 mg, 0.42 mmol) was heated at 83 °C for 48 h. The reaction mixture was cooled and chromatographed directly on silica gel (hexane–ethyl acetate, 2:1 v/v) to give the analytical products (R_f 0.22).

Preparation of *N*-[5-(2-Fluorophenyl)-3-(1*H*-indol-3-ylmethyl)-3*H*-1,4-benzodiazepin-2-yl]glycine (12). Procedure D. A solution of 220 mg (0.44 mmol) of 1,1-dimethylethyl [5-(2-fluorophenyl)-3-(1*H*-indol-3-ylmethyl)-3*H*-1,4-benzodiazepin-2-yl]acetate (prepared according to procedure A) in 50 mL of dry ethyl acetate was cooled to 0 °C and saturated with hydrogen chloride gas. The reaction flask was capped, and the reaction mixture was warmed to room temperature over 5 h. Solvent and excess reagent were removed under reduced pressure to give 200 mg of an orange-brown powder. PTLC (chloroform–methanol–acetic acid, 84:15:1.5 v/v) on silica gel afforded the analytical sample.

Acknowledgment. We acknowledge with pleasure the assistance of Dr. S. M. Pitzenberger and J. Murphy (NMR), Dr. H. Ramjit and J. Smith (MS), C. F. Homnick (HPLC), and J. P. Moreau (elemental analysis). We are grateful to Drs. P. S. Anderson and D. F. Veber for their support and encouragement during the course of this study.

Synthesis and Antiviral Properties of (*E*)-5-(2-Bromovinyl)-2′-deoxycytidine-Related Compounds

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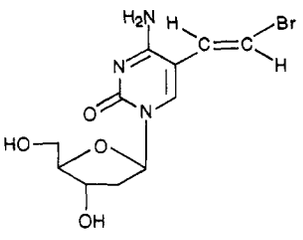
Treatment of 3′,5′-di-*O*-acetyl-(*E*)-5-(2-bromovinyl)-2′-deoxyuridine (**2**) with *p*-chlorophenyl phosphorodichloridate and 1,2,4-triazole gave 1-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-(*E*)-5-(2-bromovinyl)-4-(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (**3**). Reaction of **3** with ammonia gave (*E*)-5-(2-bromovinyl)-2′-deoxycytidine (**1**), the overall yield from **2** being 60%. A similar 4-(1,2,4-triazol-1-yl) derivative (**4**) was obtained from 3′,5′-di-*O*-acetylthymidine by the use of phosphoryl chloride as the condensing agent. Treatment of thymidine with trimethylsilyl chloride and then with phosphoryl chloride and 1,2,4-triazole gave upon workup 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-methyl-4(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (**5**). (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVDU) when similarly treated gave the corresponding (*E*)-5-(2-bromovinyl) compound **7**. A minor product formed in both cases was a 4-(1,2,4-triazol-1-yl) derivative in which the nucleoside 5′-hydroxyl group had been replaced by chlorine (**6** and **8**). Whereas compounds **4–6** and **8** did not exhibit a selective antiviral effect, compounds **1–3** and **7** proved almost as active as the reference compound BVDU. In particular, compound **7**, the 4-triazolyl derivative of BVDU, would seem worth pursuing for its potential as an inhibitor of herpes simplex virus type 1 and varicella-zoster virus.

(*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVDU) is a potent antiviral agent against herpes simplex virus type 1 (HSV-1) and varicella-zoster virus (VZV) in cell culture and animals, and the compound has been shown to be effective in the clinic.[1] (*E*)-5-(2-Bromovinyl)-2′-deoxycytidine (BVDC, **1**) has been shown to be almost as active as BVDU in cell culture. Its toxicity to cells is even less than that of BVDU, so its chemotherapeutic index is similar.[2] The clinical use of BVDC has not been reported. The synthesis of BVDC was first carried out by us by a route that was similar to that used for BVDU, namely by the formation of (*E*)-5-(2-carboxyvinyl)-2′-deoxycytidine (via the 5-chloromercuri and 5-chloropalladium derivatives of 2′-deoxycytidine) and reaction of this with *N*-bromosuccinimide.[3] Subsequent attempts to repeat these reactions have met with difficulties, and therefore an alternative synthesis has been developed.



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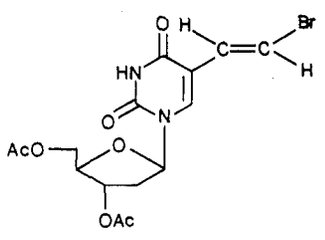

2

Chemistry

3′,5′-Di-*O*-acetyl-(*E*)-5-(2-bromovinyl)-2′-deoxyuridine (**2**)[4] was treated with *p*-chlorophenyl phosphorodichloridate and 1,2,4-triazole according to the procedure described by Sung[5] to give 1-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-(*E*)-5-(2-bromovinyl)-4-(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (**3**) as a crystalline solid in 25% yield. This product was characterized by its UV and NMR spectra and elemental analysis. The NMR spectrum showed sharp singlets at δ 9.39 and 8.46, which were assigned to the H-5 and H-3 protons of the triazolyl ring. There was a noticeable downfield shift of the 2-vinylic and pyrimidine H-6 resonances compared to those of **2**. The λ_{max} of 340 nm was also consistent with the presence of the triazolyl ring. Treatment of **3** with ammonia gave (*E*)-5-(2-bromovinyl)-2′-deoxycytidine (BVDC, **1**), which was characterized by its UV and NMR spectra and elemental analysis. The low yield of **3** was due to loss upon workup. It was found that direct conversion of **2** to **1** without isolation of **3** gave an overall yield of 60%.

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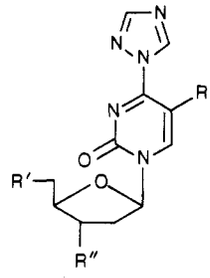
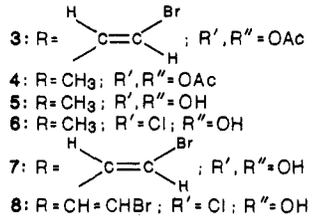
Table I. Antiviral Activity in Primary Rabbit Kidney Cell Cultures

compd	minimum cytotoxic concn[b]	minimum antiviral concentration,[a] μg/mL							
		HSV-1			HSV-2			vaccinia virus	vesicular stomatitis virus
		KOS	F	McIntyre	G	196	Lyons		
1	>400	0.1	0.2	0.2	20	>400	>400	>400	>400
2	≥300	0.1	0.1	0.2	20	100	40	40	>300
3	≥200	0.1	0.2	0.07	20	20	20	20	>200
4	≥40	>40	>40	>40	>40	>40	>40	>40	>40
5	≥40	>40	>40	20	20	20	20	>40	>40
6	≥10	>10	>10	>10	>10	>10	>10	>10	>10
7	≥200	0.07	0.07	0.1	20	>200	70	7	>200
8	≥100	70	>100	>100	>100	>100	>100	>100	>100
BVDU	≥400	0.02	0.02	0.02	2	30	7	10	>400

[a] Concentration required to reduce virus-induced cytopathogenicity by 50%. [b] Concentration required to cause a microscopically detectable alteration of normal cell morphology.

The BVDC gave similar biological results to those already reported.[2] It was found that, like **1** and **2**, compound **3** also had a marked activity against HSV-1, so an improved method for its synthesis was developed. The procedure, which was based on that used by Matsuda et al.,[6] was first tried with 3′,5′-di-*O*-acetylthymidine. This was added to a mixture of phosphoryl chloride and pyridine, and after a short interval, 1,2,4-triazole was added and allowed to react. From this reaction, 1-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-5-methyl-4-(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (**4**) was obtained in 80% yield and characterized in the usual way. Similarly, compound **3** was obtained from **2** in 70% yield.





3: R = ; R′,R″ = OAc
4: R = CH_3; R′,R″ = OAc
5: R = CH_3; R′,R″ = OH
6: R = CH_3; R′ = Cl; R″ = OH
7: R = ; R′,R″ = OH
8: R = CH=CHBr; R′ = Cl; R″ = OH

The biological results for **3** indicated that it would be of interest to obtain the deacetylated compound. This could not be obtained by the direct deacetylation of **3**, so the formation of the triazolyl compound was carried out with the 3′,5′-di-*O*-trimethylsilyl derivative of the nucleoside. In order to evaluate the procedure, it was first applied to thymidine. This was trimethylsilylated and then treated with phosphoryl chloride followed by 1,2,4-triazole to give upon workup 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-methyl-4-(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (**5**) in 37% yield. A second product (less polar than **5** in TLC) was also obtained (in 10% yield). This had very similar UV and NMR spectra to that of **5** except that it only had one exchangeable hydroxyl proton, which appeared as a doublet at δ 5.5. The FAB mass spectrum showed that the sugar portion of the molecule appeared at *m/z* 135/137 with a pattern typical of a chlorine substituent. From the spectral data and the elemental analysis, it was concluded that the compound was 1-(5-chloro-2,5-dideoxy-β-D-*erythro*-pentofuranosyl)-5-methyl-4-(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (**6**). Treatment of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine in a similar way gave (*E*)-5-(2-bromovinyl)-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (**7**) in 30% yield. A minor product was also formed in this reaction. Its ^1H NMR spectrum indicated that it was a mixture of two compounds in the ratio of 2:1 (based on the integration of the two pyrimidine H-6 resonances at δ 8.71 and 8.37). There were two pairs of vinylic H resonances, δ 7.62 and 6.86, J = 16 Hz, and δ 7.32 and 6.80, J = 9 Hz. These results and the FAB mass spectrum and elemental analysis showed that the product was a mixture of the *E* and *Z* isomers of 5-(2-bromovinyl)-1-(5-chloro-2,5-dideoxy-β-D-*erythro*-pentofuranosyl)-4-(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (**8**). The chloro compounds **6** and **8** probably arose from the use of an excess of trimethylsilyl chloride by a mechanism described by Jung and Lyster.[7] The formation of **8** as an *E*/*Z* mixture was probably an artifact because it is known that the tranformation from *E* to *Z* occurs under the influence of light.[8]

Biological Evaluation

As shown in Table I, compound **7** exhibited a marked activity against HSV-1 (strains KOS, F, and McIntyre), lesser activity against HSV-2 (strains G and Lyons) and vaccinia virus, and no activity against vesicular stomatitis virus. Compound **7** was also evaluated against a number of herpes viruses of veterinary importance, i.e. suid herpes virus type 1 (SHV-1), bovid herpes virus type 1 (BHV-1), equid herpes virus type 1 (EHV-1), and herpes virus platyrrhinae (HVP).[9] The minimum antiviral concentration of **7** against SHV-1, BHV-1, EHV-1, and HVP in primary rabbit kidney cell cultures was 0.2, 0.7, >100, and 0.4 μg/mL, respectively. The corresponding values for BVDU were 0.07, 0.2, 150, and 0.02 μg/mL, respectively (see also ref 9). Hence, the antiviral activity spectrum of **7** is remarkably similar to that of BVDU.

The 3′,5′-di-*O*-acetyl derivative of **7**, namely **3**, showed an antiviral activity comparable to that of **7**, as did compounds **1** and **2**. The thymidine analogues **4**–**6** had no appreciable antiviral effect. The antiviral activity noted with **1** and **2** is similar to that reported previously.[2,4] The inactivity of **8** is not surprising in view of a similar lack

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of antiviral activity noted with the 5′-chloro derivative of BVDU.[10]

The salient feature of the present investigations was the marked anti-HSV-1 activity of **3** and **7**. The antiviral activity of **7** has also been confirmed in virus yield reduction experiments where the compound was found to reduce the 24-h yield of HSV-1 (KOS) in primary rabbit kidney cells by log 6.5, 4.5, and 3.5 if added to the cells at a concentration of 100, 10, or 1 μg/mL, respectively (data not shown). Compound **7** was also found effective against VZV (strains YS and Oka) in human embryonic lung cells at a concentration of 0.02 μg/mL; compound **7** was only cytotoxic, i.e. inhibited the growth of the host cells, at a concentration of 100 μg/mL, thus achieving a therapeutic index of 5000 against VZV (T. Sakuma and E. De Clercq, unpublished data). Compound **7** therefore yields considerable promise as a selective inhibitor of HSV-1 and VZV and should be further pursued for its chemotherapeutic potentials in this regard.

Experimental Section

NMR spectra were recorded on Varian XL100 (100 MHz), Brucker 240 MHz, JEOL FX 90Q (90 MHz), and JEOL GX 270 (270 MHz) instruments with Me_2SO-d_6 as solvent. UV spectra were measured on a Perkin-Elmer 552 spectrophotometer, and mass spectra were measured on a Kratos MS80 mass spectrometer. Column chromatography was carried out on silica gel, Keiselgel 60 type 7734 (0.063–0.200 mm, 70–230 mesh ASTM) (E. Merck A.G., Darmstadt, West Germany). All experiments were carried out under scrupulously dry conditions unless otherwise indicated. Evaporations were carried out under reduced pressure.

1-(3,5-Di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-(*E*)-5-(2-bromovinyl)-4-(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (3). To a suspension of 3′,5′-di-*O*-acetyl-(*E*)-5-(2-bromovinyl)-2′-deoxyuridine (**2**; 3.0 g, 9.0 mmol)[4] in pyridine at 20 °C was added, with stirring, *p*-chlorophenyl phosphorodichloridate (2.5 g, 10 mmol). After a few minutes, 1,2,4-triazole (1.0 g, 14 mmol) was added, and the stirring was continued at ~20 °C for 6 h. Then methanol (20 mL) was added, and the mixture was evaporated to dryness to give a red gum. This was purified by column chromatography with chloroform as the eluant. The appropriate fractions were evaporated to dryness to give a white solid, which was crystallized from propan-2-ol to give the product as colorless needles (1.05 g, 25% yield): UV λ_{max} 242 nm (ε 19900), 339 nm (ε 6200), λ_{min} 298 nm (ε 2100) (ethanol); ^{1}H NMR δ 1.10 (6 H, s, $COCH_3$), 2.60 (2 H, m, H-2), 4.38 (3 H, m, H-4, H-5), 5.27 (1 H, m, H-3), 6.18 (1 H, t, H-1), 6.95 (1 H, d, vinylic H, J = 15 Hz), 7.55 (1 H, d, vinylic H, J = 15 Hz), 8.40 (1 H, s, uracil H-6), 8.46 (1 H, s, triazole H-3), 9.39 (1 H, s, triazole H-5). Anal. Calcd for $C_{17}H_{18}BrN_5O_6$: C, 43.6; H, 3.9; N, 15.0. Found: C, 43.4; H, 3.9; N, 14.8.

(*E*)-5-(2-Bromovinyl)-2′-deoxycytidine (1). Compound **2** (3.04 g, 9 mmol) was stirred for 12 h at ~20 °C in pyridine (50 mL) with *p*-chlorophenyl phosphorodichloridate (2 mL, 12 mmol) and 1,2,4-triazole (1.70 g, 24 mmol). To the mixture were added aqueous ammonia (sp gr 0.88, 5 mL) and methanol (10 mL). After being stirred for 12 h at ~20 °C, the mixture was evaporated to dryness by coevaporation with toluene. The residue was purified by column chromatography with chloroform–methanol (4:1) as the eluent. Appropriate fractions were evaporated to dryness to give the product (2.0 g, 60% yield), which was crystallized from water–acetone: UV λ_{max} 252 nm (ε 17800), 295 nm (ε 7000), λ_{min} 232 nm (ε 1400), 280 nm (ε 6600) (H_2O, pH 7); ^{1}H NMR δ 2.22 (2 H, m, H-2′), 3.60 (2 H, m, H-5′), 3.80 (1 H, m, H-4′), 4.22 (1 H, m, H-3′), 5.10 (1 H, t, OH-5′), 5.18 (1 H, d, OH-3′), 6.12 (1 H, t, H-1′), 6.72 (1 H, d, vinylic H, J = 15 Hz), 7.08 (1 H, d, vinylic H, J = 15 Hz), 7.25 (2 H, s, NH_2), 8.12 (1 H, s, H-6). Anal. Calcd for $C_{11}H_{14}BrN_3O_4$: C, 39.8; H, 4.2; N, 12.6. Found: C, 39.7; H, 4.2; N, 12.5.

1-(3,5-Di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-5-methyl-4-(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (4). 3′,5′-Di-*O*-acetylthymidine (1.7 g, 4.3 mmol) was dissolved in pyridine (20 mL), freshly distilled phosphoryl chloride (1.5 g, 10 mmol) and 1,2,4-triazole (2.0 g, 30 mmol) were added, and the reaction mixture was stirred at ~20 °C for 48 h. Dichloromethane (100 mL) was then added, and the solution was washed with water (2 × 50 mL). The organic layer was dried and evaporated to dryness, and the residue was crystallized from propan-2-ol to give the product (1.6 g, 80% yield): UV λ_{max} 249 nm (ε 11000), 325 nm (ε 8000), λ_{min} 233 nm (ε 7120), 283 nm (ε 1720); ^{1}H NMR δ 2.10 (6 H, s, $COCH_3$), 2.37 (3 H, s, CH_3), 2.50 (2 H, m, H-2), 4.40 (3 H, m, H-4, H-5), 5.30 (1 H, m, H-3), 6.20 (1 H, t, H-1), 8.30 (1 H, s, uracil H-6), 8.40 (1 H, s, triazole H-3), 9.35 (1 H, s, triazole H-5). Anal. Calcd for $C_{16}H_{19}N_5O_6$: C, 50.9; H, 5.1; N, 18.6. Found: C, 50.9; H, 4.8; N, 18.6.

Synthesis of 4-(1,2,4-Triazol-1-yl) Nucleosides (5–8). The nucleoside (ca. 2 g) was treated with trimethylsilyl chloride (2.2 equiv) in pyridine (50 mL). After the mixture was stirred at ~20 °C for 1 h, freshly distilled phosphoryl chloride (1.5 equiv) was added. After the mixture stirred for a further 5–10 h, 1,2,4-triazole (2.5 equiv) was added. The rection mixture was stirred for a further 18 h at ~20°C and then evaporated to dryness. The residue was suspended in acetone–water (1:1, 50 mL) and stirred at ~20 °C for 5 h. Evaporation of the suspension to dryness gave a brown solid, which was fractionated by column chromatography by elution with a gradient of chloroform → chloroform–ethanol (5:1).

From thymidine (1.8 g, 7.4 mmol), the first fraction eluted was a minor product, which was crystallized from propanol (240 mg, 10% yield) and shown to be **1-(5-chloro-2,5-dideoxy-β-D-*erythro*-pentofuranosyl)-5-methyl-4-(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (6)**: UV λ_{max} 248 nm (ε 10400), 325 nm (ε 8290), λ_{min} 283 nm (ε 1650) (ethanol); ^{1}H NMR δ 2.20 (2 H, m, H-2), 2.30 (3 H, s, CH_3), 3.20 (1 H, m, H-5), 3.90 (1 H, m, H-4), 4.32 (1 H, m, H-3), 5.50 (1 H, d, OH-3′), 6.18 (1 H, t, H-1), 8.25 (1 H, s, uracil H-6), 8.36 (1 H, s, triazole H-3), 9.32 (1 H, s, triazole H-5); FAB mass spectrum, m/z 312 [(M + H)$^+$, 15], 178 [(Base + H)$^+$, 100], 135 [(sugar)$^+$, 5]. Anal. Calcd for $C_{12}H_{14}ClN_5O_3$: C, 46.2; H, 4.5; N, 22.5. Found: C, 46.2; H, 4.2; N, 22.2.

The second product eluted was crystallized from propanol to give **1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-methyl-4-(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (5)**: (800 mg, 37% yield); UV λ_{max} 248 nm (ε 10600), 326 nm (ε 8650), λ_{min} 282 nm (ε 1470) (ethanol); ^{1}H NMR δ 2.20 (2 H, m, H-2), 2.30 (3 H, s, CH_3), 3.20 (1 H, m, H-5), 3.90 (1 H, m, H-4), 4.32 (1 H, m, H-3), 5.50 (2 H, m, OH-3, OH-5), 6.18 (1 H, t, H-1), 8.25 (1 H, s, uracil H-6), 8.36 (1 H, s, triazole H-3), 9.32 (1 H, s, triazole H-5). Anal. Calcd for $C_{12}H_{15}N_5O_4$: C, 49.1; H, 5.2; N, 23.9. Found: C, 49.4; H, 5.0; N, 24.0.

From (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (2.0 g, 4.8 mmol) the first fraction eluted was a minor product, which was obtained as a syrup (200 mg, 10% yield) and which was a mixture of **(*E*)-** and **(*Z*)-5-(2-bromovinyl)-1-(5-chloro-2,5-dideoxy-β-D-*erythro*-pentofuranosyl)-4-(1,2,4-triazol-1-yl)pyrimidin-2-(1*H*)-one (8)**: UV λ_{max} 243 nm (ε 20000), 336 nm (ε 5280), λ_{min} 298 nm (ε 2220) (ethanol); ^{1}H NMR δ 2.35 (2 H, m, H-2), 3.90 (2 H, m, H-5), 4.14 (1 H, m, H-4), 4.30 (1 H, m, H-3), 5.66 (1 H, m, OH-3), 6.20 (1 H, m, H-1), 6.90 ($^1/_3$ H, d, vinylic H, J = 9 Hz), 6.86 ($^2/_3$ H, d, vinylic H, J = 16 Hz), 7.33 ($^1/_3$ H, d, vinylic H, J = 9 Hz), 7.62 ($^2/_3$ H, d, vinylic H, J = 16 Hz), 8.37 ($^2/_3$ H, s, uracil H-6), 8.46 (1 H, s, triazole H-3), 8.71 ($^1/_3$ H, s, uracil H-6), 9.35 (1 H, s, triazole H-5); FAB mass spectrum, m/z 404 [[M(^{81}Br + ^{35}Cl and ^{79}Br + ^{37}Cl) + H]$^+$, 18], 406 [[M(^{81}Br + ^{37}Cl) + H]$^+$, 4], 402 [[M(^{79}Br + ^{35}Cl) + H]$^+$, 12]. Anal. Calcd for $C_{13}H_{13}BrClN_5O_3$: C, 38.8; H, 3.25; N, 17.4. Found: C, 38.6; H, 3.1; N, 17.1.

The second product eluted was crystallized from acetone to give **(*E*)-5-(2-bromovinyl)-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-(1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (7)** (630 mg, 34% yield); UV λ_{max} 243 nm (ε 18600), 340 nm (ε 5200), λ_{min} 298 nm (ε 1600) (ethanol); ^{1}H NMR δ 2.32 (2 H, m, H-2), 3.70 (2 H, m, H-5), 3.90 (1 H, m, H-4), 4.30 (1 H, m, H-3), 5.40 (2 H, m, OH-3, OH-5), 6.12 (1 H, t, H-1), 6.80 (1 H, d, vinylic H, J = 15 Hz), 7.61 (1 H, d, vinylic H, J = 15 Hz), 8.40 (1 H, s, uracil H-6), 9.00 (1 H, s, triazole H-3), 9.35 (1 H, s, triazole H-5); FAB mass spectrum, m/z 384 [(M + H)$^+$, 15], 268 [(Base + H)$^+$, 100], 117 [(sugar)$^+$, 30]. Anal. Calcd for $C_{13}H_{14}BrN_5O_4$: C, 40.6; H, 3.7;

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N, 18.2. Found: C, 40.3; H, 3.6; N, 18.1.

Antiviral Evaluation. The compounds were assayed for antiviral activity in primary rabbit kidney cells by using a viral cytopathogenicity inhibition method as described previously.[11]

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Registry No. 1, 74131-09-2; 2, 91593-18-9; 3, 111160-27-1; 4, 80991-41-9; 5, 109389-25-5; 6, 111160-28-2; 7, 111160-31-7; (*E*)-8, 111160-29-3; (*Z*)-8, 111160-30-6; Ac-dThd-Ac, 6979-97-1; H-dThd-H, 50-89-5; 1,2,4-triazole, 288-88-0; (*E*)-5-(2-bromovinyl)-2′-deoxyuridine, 69304-47-8.

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N^6-(Arylalkyl)adenosines. Identification of N^6-(9-Fluorenylmethyl)adenosine as a Highly Potent Agonist for the Adenosine A_2 Receptor

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Several N^6-(arylalkyl)adenosines related to N^6-benzyladenosine were synthesized, and their A_1 and A_2 adenosine receptor binding affinities were determined. The annulated derivative N^6-(1-naphthylmethyl)adenosine resulted in a very potent A_2 agonist (A_1 K_i = 24 nM, A_2 K_i = 9.1 nM), whereas N^6-(9-anthracenylmethyl)adenosine was virtually inactive (A_1 K_i = 9000 nM, A_2 K_i = 29 000 nM). Interestingly, the structurally similar N^6-(9-fluorenylmethyl)adenosine was the most potent A_2 agonist reported to date, with a K_i of 4.9 nM in A_2 binding and 5.1 nM in A_1 binding. The homologues N^6-9-fluorenyladenosine and N^6-[2-(9-fluorenyl)ethyl]adenosine showed little or no activity at either adenosine receptor. Effects of these agents on heart rate and coronary flow in the isolated rat heart paralleled their A_1 and A_2 binding affinities, respectively. These data suggest that for high affinity at the A_2 receptor a planar hydrophobic function at a certain distance and angle from the N^6 nitrogen is required.

Adenosine and adenosine receptor agonists have been shown to produce a variety of pharmacological effects, including vasodilation, negative inotropy, hypotension, inhibition of platelet aggregation, anticonvulsant activity, inhibition of locomotor activity, inhibition of neurotransmitter release, and antilipolytic activity.[1] These actions are due to the activation of membrane-bound adenosine receptors, which are divided into two major subtypes: A_1 receptors, which inhibit adenylate cyclase, and A_2 receptors, which stimulate adenylate cyclase.[2,3] The two receptors have similar but distinguishable structure–activity relationships.[4] Receptor binding assays have been developed for the A_1 receptor and, more recently, the A_2 receptor.[5]

A significant number of agonists have been evaluated for potency in A_1 binding and A_1-mediated pharmacodynamic responses, revealing in part the structural requirements for high A_1 affinity and selectivity.[6,7] Numerous potent, A_1-selective agonists are known.[6-8] As yet, only a few potent and/or selective A_2 receptor agonists have been identified. 2-(Phenylamino)adenosine (CV-1808) (Figure 1) has been reported to be a selective coronary vasodilator,[9] and the A_2 selectivity of this compound has been confirmed in receptor binding.[5] NECA (1-(6-amino-9*H*-purin-9-yl)-1-deoxy-*N*-ethyl-β-D-ribofuranuronamide) possesses high potency in A_2 receptor binding[5] and as a coronary vasodilator,[10] but also has high affinity for the A_1 receptor.[5] N^6-Modified adenosines with high potency or selectivity for the A_2 receptor have not yet been reported. However, N^6-benzyladenosine and N^6-[(*R*)-1-methyl-2-phenylethyl]adenosine (*R*-PIA) have been found to possess appreciable affinities at the A_2 receptor in rat striatal membranes, with K_i values of 280 nM and 120 nM, respectively.[5] Although *R*-PIA is highly A_1 selective, N^6-benzyladenosine has almost equal affinity in A_1 and A_2 binding.[5] In light of the above findings, we have evaluated the A_2 affinities of other N^6-(arylalkyl)adenosines, including several novel agents with bi- or tricyclic aryl moieties. Of particular interest is N^6-(9-fluorenylmethyl)adenosine (1) (Figure 2), a highly potent adenosine A_2 receptor agonist with a K_i value of 4.9 nM in [^{3}H]NECA binding.

Table I. Physical–Chemical Properties of Novel N^6-Substituted Adenosines

compd	mp,[a] °C	(formula) anal.
1	139–142	($C_{24}H_{23}N_5O_4$) C, H, N
4	129–136	($C_{25}H_{23}N_5O_4$) C, H, N
5	210–212	($C_{23}H_{21}N_5O_4$) C, H, N
6	120–123	($C_{25}H_{25}N_5O_4$) C, H, N
7	104–106	($C_{24}H_{23}N_5O_5$) C, H, N
8	89–95	($C_{26}H_{27}N_5O_4$) C, H, N

[a] Melting points are uncorrected.

Chemistry. Adenosine analogues were synthesized at Warner-Lambert/Parke-Davis according to standard chemical procedures[11,12] in which 6-chloropurine riboside

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