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Acyclovir was synthesized in five steps from 7-formamido-5-methylthiofurazano[3,4-*d*]pyrimidine **2**. Alkylation of **2** with 2-(benzoyloxy)ethoxymethyl chloride, followed by reductive cleavage of the furazan ring gave 9-[2-(benzoyloxy)ethoxy]methyl-2-(methylthio)adenine **5**. Hydrolysis of the 6-amino group of **5**, followed by amination of **7** with ammonia gave 9-(2-hydroxyethoxy)methyl]guanine (**1**, acyclovir).

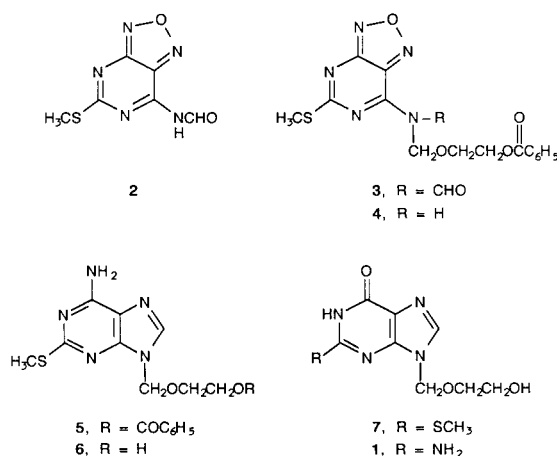
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Subsequent to the initial report [1,2] of the potent antiherpetic activity of acyclovir [9-(2-hydroxyethoxy)methyl]guanine, Zovirax®], several laboratories reported synthetic entries to this new, clinically efficacious antiviral agent. These methods encompass routes that proceed through alkylation of 2,6-dichloropurine [1], 2-chloro-6-iodopurine [3], 2-amino-6-chloropurine [4] or guanine [5]. The 6-amino or 6-chloro moiety of 2-amino-6-substituted purines was hydrolyzed with adenosine deaminase to give acyclovir [4]. Acyclovir was also prepared from 2-amino-9-[(2-hydroxyethoxy)methyl]-9*H*-purine (6-deoxyacyclovir) by oxidation with xanthine oxidase [6].

We were interested in developing an unambiguous synthesis of acyclovir that would be amenable to preparation of 2- and 6-substituted analogs. The furazano[3,4-*d*]pyrimidine route reported by Taylor [7], which leads to unambiguous placement of the purine 9-substituent, appeared to fulfill this requirement. In this approach, the purine 9-substituent is introduced normally by reaction of a primary amine with a 7-aminofurazano[3,4-*d*]pyrimidine. This method was modified by introduction of the eventual 9-substituent through alkylation of 7-formamidofurazano[3,4-*d*]pyrimidine [7] with 2-(benzoyloxy)ethoxymethyl chloride [10] (Scheme I). Alkylation of acylamino pyrimidines for preparation of substituted purines has been previously described [8,9]. We describe the preparation of acyclovir and analogs *via* a synthesis that unambiguously fixes the substitution position of the hydroxyethoxymethyl side chain.

The 7-formamidofurazanopyrimidine **2** was prepared in 86% yield from 7-formamidofurazano[3,4-*d*]pyrimidine by slightly modifying a literature method [7]. Alkylation of **2** with 2-(benzoyloxy)ethoxymethyl chloride [10] in dimethylformamide in the presence of triethylamine gave a dark, oily mixture of desired compound **3** and deformed product **4**. The mixture was reformylated with acetic-formic anhydride to give **3** as a crude oil suitable for further reaction. In one experiment, **3** was isolated as a crystalline solid that exhibited characteristic nmr signals

Scheme I



as singlets at δ 10.05 for the formyl hydrogen and at δ 5.81 for the two methylene hydrogens. Deformylation of **3** to give pure **4** was accomplished in refluxing ethanol. The nmr signal for the methylene in **4** appeared as a doublet at δ 5.28 ($J = 7$ Hz); it collapsed to a singlet with deuterium oxide thus confirming the substitution position in **3** and **4** to be on the exocyclic nitrogen. Reduction of **3** as the crude oil by low-pressure catalytic hydrogenation in acetic acid failed to give desired compound **5**. Only the deformed furazano[3,4-*d*]pyrimidine was isolated. Reductive cleavage of the furazan ring of **3** with zinc dust in acetic acid followed by heating to facilitate cyclization proceeded smoothly to give 2-(methylthio)adenine **5**. The identity of the product as a 2-methylthio-9-substituted adenine was supported by its uv spectrum [11]. The benzoyl group of **5** was cleaved with aqueous methylamine to give **6**. The 2-methylthio group of **6** failed to react in liquid ammonia at 90°. Therefore, the 6-amino group of **6** was transformed with sodium nitrite in acetic acid to give **7**. Subsequent amination with ammonia saturated ethanol at 140° gave **1**. The tlc, uv, nmr, and mass spectral data were the same as that for acyclovir prepared from guanine [5].

EXPERIMENTAL

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian T-60 spectrometer using tetramethylsilane as an internal standard. Ultraviolet absorption spectra were obtained on an Unicam SP 800 spectrophotometer. Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on tlc. Analytical samples gave combustion values for C, H and N that are within 0.4% of theoretical.

7-Formamido-5-(methylthio)furazano[3,4-*d*]pyrimidine (2).

To an ice-bath cooled solution of acetic-formic anhydride [12] formed from 100 ml of acetic anhydride and 40 ml of 97-100% formic acid was added 8.10 g (4.42 mmoles) of 7-aminofurazano[3,4-*d*]pyrimidine [7]. This mixture was stirred in an oil bath at 80° for 2 hours, cooled and spin evaporated *in vacuo* at 50°. The residual orange solid was twice dispersed in ether and evaporated to dryness, then collected and washed with ether, yield, 8.08 g (86%), mp 170-173°, lit [7] mp 173-174°. Recrystallization of a portion from ether-cyclohexane gave the analytical sample of unchanged mp; nmr (DMSO-*d*₆): δ 12.60 (br s, 1H, NH), 9.69 (s, 1H, CHO), 2.60 (s, 3H, CH₃).

Anal. Calcd. for C₆H₈N₄O₂S: C, 34.13; H, 2.39; N, 33.17. Found: C, 34.15; H, 2.35; N, 33.24.

7-[*N*-Formyl-2-(benzoyloxy)ethoxy]methylamino]-5-(methylthio)furazano[3,4-*d*]pyrimidine (3).

To a solution of 6.00 g (28.4 mmoles) of **2** in 50 ml of dimethylformamide cooled on an ice bath and protected from moisture with a calcium chloride drying tube was added 3.00 g (29.7 mmoles) of triethylamine. After five minutes, 8.05 g (37.6 mmoles) of 2-(benzoyloxy)ethoxymethyl chloride in 5 ml of dimethylformamide was added. An additional 1.00 g (9.9 mmoles) of triethylamine was added, the ice bath was removed, and the reaction was stirred at ambient temperature for 15 hours. The reaction mixture was poured over 400 g of crushed ice containing 2 ml of acetic acid. The resultant mixture was extracted with five 50 ml portions of chloroform. The combined extracts were washed with 25 ml of brine and dried with magnesium sulfate. The solvent was removed by spin evaporation *in vacuo* at aspirator pressure and finally at about 1 mm of Hg to remove residual dimethylformamide. The residual dark oil was dissolved in 200 ml of ethyl acetate and filtered through a pad of Superfilitrol # 19. The pad was washed with two 75 ml portions of ethyl acetate. The combined filtrates and wash were spin evaporated *in vacuo* to give an orange, oily mixture of **3** and **4**. The oil was dissolved in acetic-formic anhydride (formed from 100 ml of acetic anhydride and 50 ml of 97-100% formic acid) and heated in an oil bath at 80° for two hours, and at ambient temperature for 15 hours. The reaction was spin evaporated *in vacuo* to give **3** as a crude oil that was used without further purification in the next step.

In an experiment on a 64 mmole scale, crude **3** from alkylation of **2** was dissolved several times in tetrahydrofuran, spin evaporated *in vacuo* and triturated under cyclohexane to give a solid. The cyclohexane was decanted, the solid was dispersed in 60 ml of ether, collected and washed with cyclohexane; yield, 10.27 g (41%), mp 84-85°. Recrystallization of a portion from ether gave the analytical sample, mp 86-88°; nmr (deuteriochloroform): δ 2.65 (s, 3H, CH₃), 4.03 (m, 2H, CH₂OCH₂CH₂), 4.49 (m, 2H, CH₂OCOAr), 5.81 (s, 2H, NCH₂O), 7.36-8.11 (m, 5H, Ar), 10.05 (s, 1H, CHO).

Anal. Calcd. for C₁₆H₁₈N₄O₅S: C, 49.35; H, 3.88; N, 17.99. Found: C, 49.68; H, 3.93; N, 18.02.

7-[[2-(Benzoyloxy)ethoxy]methylamino]-5-(methylthio)furazano[3,4-*d*]pyrimidine (4).

A mixture of 1.00 g (2.57 mmoles) of **3** and 25 ml of ethanol was refluxed with stirring for 83 hours. The resultant solution was cooled and spin evaporated *in vacuo*. The residual oil was triturated with cyclohexane to give a yellow solid, yield, 0.41 g (44%), mp 125-130°. Recrystallization

from cyclohexane-ether gave the analytical sample, mp 135-137°; nmr (deuteriochloroform): δ 2.58 (s, 3H, CH₃), 4.02 (m, 2H, CH₂OCH₂CH₂), 4.52 (m, 2H, CH₂OCOAr), 5.28 (d, 2H, NCH₂O, collapses to singlet with deuterium oxide), 7.66 (m, 6H, Ar and NH).

Anal. Calcd. for C₁₅H₁₅N₄O₄S: C, 49.85; H, 4.18; N, 19.38. Found: C, 50.03; H, 4.16; N, 19.43.

9-[[2-(Benzoyloxy)ethoxy]methyl]-2-(methylthio)adenine (5).

To a stirred solution of **3** as the crude oil in 200 ml of acetic acid that was cooled on a water bath was added 30 g of zinc powder in portions over 20 minutes. The resultant mixture was refluxed with stirring for 1.5 hours. The mixture was cooled and filtered to remove the solids. The solids were washed with acetic acid, and the filtrate and wash was spin evaporated *in vacuo*. The residue was covered with 100 ml of water and turned on a roto evaporator without heating until a solid formed. The aqueous layer was decanted, the solids were digested with 50 ml of ethanol and allowed to cool. The solids were collected, washed with ether and dried, yield, 3.30 g (32%), mp 166-167°. Recrystallization of 0.80 g from ethanol gave the analytical sample, mp 166-168°; uv (0.1*N* hydrochloric acid + 10% ethanol): λ max 270 nm (ε 15000), 300 nm (sh) (ε 7000); (water + 10% ethanol): λ max 276 nm (ε 13600); (0.1*N* sodium hydroxide + 10% ethanol): λ max 276 nm (ε 14900); nmr (DMSO-*d*₆): δ 2.50 (s, 3H, CH₃) 3.94 (m, 2H, CH₂OCH₂CH₂), 4.40 (m, 2H, CH₂OCOAr), 5.60 (s, 2H, NCH₂O), 7.33 (s, 2H, NH₂), 7.43-8.01 (m, 5H, Ar), 8.16 (s, 1H, purine H).

Anal. Calcd. for C₁₆H₁₇N₅O₃S: C, 53.47; H, 4.77; N, 19.49. Found: C, 53.72; H, 4.85; N, 19.56.

9-[[2-(Hydroxyethoxy)methyl]-2-(methylthio)adenine (6).

A mixture of 2.20 g (6.12 mmoles) of **5** and 100 ml of 40% aqueous methylamine was heated on a steam bath with occasional agitation for one hour. The solution was cooled and spin evaporated *in vacuo*. The resultant oil was triturated with five 40 ml portions of hot ether to give a solid, yield, 1.31 g (84%), mp 174-176°. Recrystallization from ethanol gave the analytical sample, mp 175-176°; uv (0.1*N* hydrochloric acid + 10% ethanol): λ max 270 nm (ε 16700), 291 nm (sh) (ε 11900); (water or 0.1*N* sodium hydroxide, + 10% ethanol): λ max 276 nm (ε 15200); nmr (DMSO-*d*₆): δ 2.49 (s, 3H, CH₃), 3.55 (s, 4H, CH₂CH₂), 4.65 (br s, 1H, OH), 5.55 (s, 2H, NCH₂O), 7.31 (s, 2H, NH₂), 8.15 (s, 1H, purine H).

Anal. Calcd. for C₉H₁₃N₅O₂S: C, 42.34; H, 5.13; N, 27.43. Found: C, 42.66; H, 5.10; N, 27.39.

9-[[2-(Hydroxyethoxy)methyl]-2-(methylthio)hypoxanthine (7).

To a stirred solution of 0.50 g (1.96 mmoles) of **6** in 10 ml of acetic acid was added 0.83 g (12.0 mmoles) of sodium nitrite in portions over one hour. The reaction mixture was stirred for 15 hours, the white solids were collected by filtration and washed with 5 ml of acetic acid. The combined filtrate and wash were spin evaporated *in vacuo* to give a solid that was dissolved in 40 ml of water. This solution was applied to an Amberlite XAD-2 column [23 cm x 3 cm] and eluted with 1000 ml of water to remove contaminating salts. The column was eluted with 30% ethanol-water to remove the product. The solution was spin evaporated *in vacuo*, and the residue was recrystallized from ethanol, yield, 0.245 g (48%), mp 190-193°; uv (0.1*N* hydrochloric acid + 10% ethanol): λ max 268 nm (ε 16200); (water + 10% ethanol): λ max 262 nm (ε 15200), 284 nm (sh) (ε 11500); (0.1*N* sodium hydroxide + 10% ethanol): λ max 272 nm (ε 15200); nmr (DMSO-*d*₆): δ 2.58 (s, 3H, CH₃), 3.57 (s, 4H, CH₂CH₂), 4.67 (br s, 1H, OH), 5.56 (s, 2H, NCH₂O), 8.14 (s, 1H, purine H), 12.52 (br s, 1H, NH).

Anal. Calcd. for C₉H₁₂N₄O₃S: C, 42.17; H, 4.72; N, 21.86. Found: C, 42.36; H, 4.72; N, 21.57.

9-[[2-(Hydroxyethoxy)methyl]guanidine (I).

A solution of 0.044 g of **7** in 50 ml of ammonia saturated ethanol was heated in a stainless steel vessel at 140° for 60 hours. The cooled reaction solution was spin evaporated *in vacuo*, and the residual solid was recrystallized from ethanol, yield, 15 mg (39%), mp ~225 dec. The tlc,

uv, nmr, and mass spectral data were the same as that for acyclovir (1) prepared from guanine [5].

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REFERENCES AND NOTES

- [1] H. J. Schaeffer, L. Beauchamp, P. de Miranda, G. B. Elion, D. J. Bauer and P. Collins, *Nature* (London), **272**, 583 (1978).
- [2] G. B. Elion, P. A. Furman, J. A. Fyfe, P. de Miranda, L. Beauchamp and H. J. Schaeffer, *Proc. Nat. Acad. Sci. USA*, **74**, 5716 (1977).
- [3] J. R. Barrio, J. D. Bryant and G. E. Keyser, *J. Med. Chem.*, **23**, 572 (1980).
- [4] M. J. Robins and P. W. Hatfield, *Can. J. Chem.*, **60**, 547 (1982).
- [5] H. J. Schaeffer, U. S. Patent 4,199,574 (1980); *Chem. Abstr.*, **84**, 180300p (1976); This *Chem. Abstr.*, reference refers to German Patent 2,539,963.
- [6] T. A. Krenitsky, W. W. Hall, P. de Miranda, L. M. Beauchamp, H. J. Schaeffer and P. D. Whiteman, *Proc. Nat. Acad. Sci. USA*, **81**, 3209 (1984).
- [7] E. C. Taylor, G. P. Beardsley and Y. Maki, *J. Org. Chem.*, **36**, 3211 (1971).
- [8] J. A. Montgomery and K. Hewson, *J. Org. Chem.*, **26**, 4469 (1961).
- [9] M. D. Fenn and J. H. Lister, *J. Chem. Soc., Perkin Trans. I*, 485 (1975).
- [10] J. L. Kelley, J. E. Kelsey, W. R. Hall, M. P. Krochmal and H. J. Schaeffer, *J. Med. Chem.*, **24**, 753 (1981).
- [11] M. H. Maguire, D. M. Nobbs, R. Einstein and J. C. Middleton, *J. Med. Chem.*, **14**, 415 (1971).
- [12] L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis", Vol 1, John Wiley and Sons, Inc, New York, NY, 1967, p 4.