amino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (3; 1.238 g, 3 mmol), dimethylformamide dimethyl acetal (1.785 g, 15 mmol), and dimethylformamide (18 mL) was stirred for 48 h at room temperature. The solvent was evaporated in vacuo at room temperature and the syrupy intermediate 15 was dissolved in dioxane (14 mL). The solution was stirred with excess dry ice and then with water (14 mL). The resulting solution was stirred for an additional hour, and the solvent was evaporated to dryness in vacuo. The oily product was crystallized from alcohol to provide 1.2 g (80%) of 13: mp 158–159 °C dec; $[\alpha]^{26}_D$ −74.7° (*c* 1.004, MeOH); ^{1}H NMR (Me_2SO-d_6) δ 9.03, 8.53, and 8.26 (3 s, 3, N=C*H*N, H_3 and H_6), 6.21 (d, 1, $J_{1',2'}$ = 5.5 Hz, HI'), 3.23 [d, 6, $N(CH_3)_2$]. Anal. ($C_{13}H_{18}N_6O_4$) C, H, N.

p-[Bis(2-chloroethyl)amino]benzaldehyde (1-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4-yl)hydrazone (14). 4-Hydrazino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (6; 1.415 g, 5 mmol) was suspended with stirring in absolute *N*,*N*-dimethylformamide (50 mL), and *p*-[*N*,*N*-bis(2-chloroethyl)-amino]benzaldehyde (1.35 g, 5.5 mmol) was added. After the mixture was stirred at room temperature for 1 h, molecular sieves (5 Å, 25 g) were added and stirring was continued overnight. Another fresh quantity (11 g) of molecular sieves was then added and stirring continued for another 60 h (the reaction was monitored by TLC). The reaction mixture was filtered, and the residue was washed with dimethylformamide (4 × 50 mL). The combined filtrate and washings were evaporated in vacuo at <40 °C to provide a gum. Absolute ethanol (200 mL) was added to the gum to precipitate the crude product (1.6 g), mp 195–200 °C dec. For purification, this product was dissolved in dimethylformamide (80 mL), the solution was filtered through Celite, and absolute methanol (≃200 mL) was added to the filtrate to induce a cloud point. The solution was then allowed to stand at room temperature for 18 h. The solid which had separated was collected by filtration and dried over P_2O_5 in a vacuum dessicator at room temperature to afford 2 g (78.5%) of 14: mp 225–226 °C dec; $[\alpha]^{25}_D$ −1.4° (*c* 1.043, DMF); ^{1}H NMR (Me_2SO-d_6) δ 11.93 (s, 1, NH), 8.53, 8.4, 8.23 (3 s, 3, H_3, H_6, and aldehyde C*H*), 7.72 and 6.91 (2 d, 4 aromatic protons), 6.27 (d, 1, $J_{1',2'}$ = 4.5 Hz, HI'). Anal. ($C_{21}H_{25}N_7O_4Cl_2$) C, H, N.

Antitumor Studies. The in vitro cytotoxicity against L1210 was evaluated as described previously.[3e] L1210 cells were grown in static suspension culture using Fischer's medium for leukemic cells of mice, and the growth rate over a 3-day period was determined in the presence of various concentrations of the test compound. The ID_{50} was defined as the concentration required to reduce the growth rate to 50% of the control.

The in vivo antitumor data was furnished by the Division of Cancer Treatment using standard National Cancer Institute protocols for evaluation of compounds against the mouse leukemias L1210 and P388.[23]

Acknowledgment. The authors are indebted to Steven J. Manning for the large-scale syntheses of certain nucleosides and Suzanne Mason, Randy Philpot, C. Butter Pruitt, Norma Griffin, and Kirk Tarlo for technical assistance. This research was supported by Research Contract NIH NCI-CM-77142 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Service, and Grant R01-CA-26032 from the National Cancer Institute, National Institutes of Health.

Supplementary Material Available: Table I listing ultraviolet spectral data for certain pyrazolo[3,4-*d*]pyrimidines (2 pages). Ordering information is given on any current masthead page.

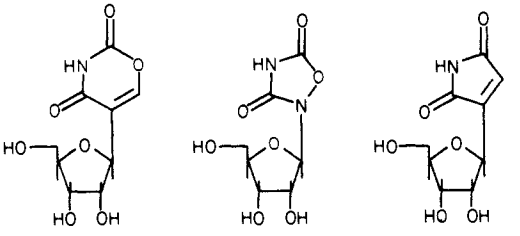
Synthesis and Biological Activity of Certain Derivatives of Oxazinomycin and Related Oxadiazole Nucleosides

Prem C. Srivastava* and Roland K. Robins

Cancer Research Center, Department of Chemistry, Brigham Young University, Provo, Utah 84602. *Received April 30, 1981*

Oxazinomycin was converted into 2',3',5'-tri-*O*-acetyloxazinomycin (2) and 2',3'-*O*-isopropylideneoxazinomycin (3), respectively. Compound 3 was iodinated and reduced to provide 5'-deoxy-2',3'-*O*-isopropylideneoxazinomycin (5) which, after acid hydrolysis, provided 5'-deoxyoxazinomycin (6). Alternatively, the iodination of oxazinomycin followed by catalytic hydrogenation also provided 6. Oxazinomycin was treated with 2-acetoxybenzoyl chloride to provide 3'-*O*-acetyl-2'-chloro-2'-deoxyoxazinomycin (8) which, after reduction with tributyltin hydride, provided 3'-*O*-acetyl-2'-deoxyoxazinomycin (9). Oxazinomycin was also converted into oxazinomycin 5'-phosphate (10) and into O^4,2'-anhydrooxazinomycin (11). 1,2,4-Oxadiazole-3,5-dione (12) was glycosylated to provide 2-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-oxadiazole-3,5-dione (13) which, after deacetylation, provided 2-β-D-ribofuranosyl-1,2,4-oxadiazole-3,5-dione (14). Similarly, 12 provided 2-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-3,5-dione (17); 14 was also converted into the corresponding 2',3'-*O*-isopropylidene derivative 15. Compound 14 showed significant antiviral activity against herpes simplex virus type 1, in vitro.

Various pyrimidine analogue nucleosides, such as cytosine arabinoside (*ara*-C), 3-deazauridine, 5-azacytidine, and 5-azacytosine arabinoside, have shown great promise in cancer chemotherapy.[1] A close examination would reveal that much of the interest in the synthetic nucleosides is due to their varied biological activities, which result from the close structural relationship of these synthetic molecules to the "natural" nucleoside metabolites. Deletion, introduction, or exchange of a hetero atom in aglycon and a modification in the sugar moiety could have interesting effects on the biological activity. Oxazinomycin[2] (minimycin,[3] 1) is a C-nucleoside antibiotic and is structurally



1 14 showdomycin

related to uridine and pseudouridine. However, due to the oxazinedione ring system, oxazinomycin is quite susceptible to hydrolytic cleavage and is consequently less stable

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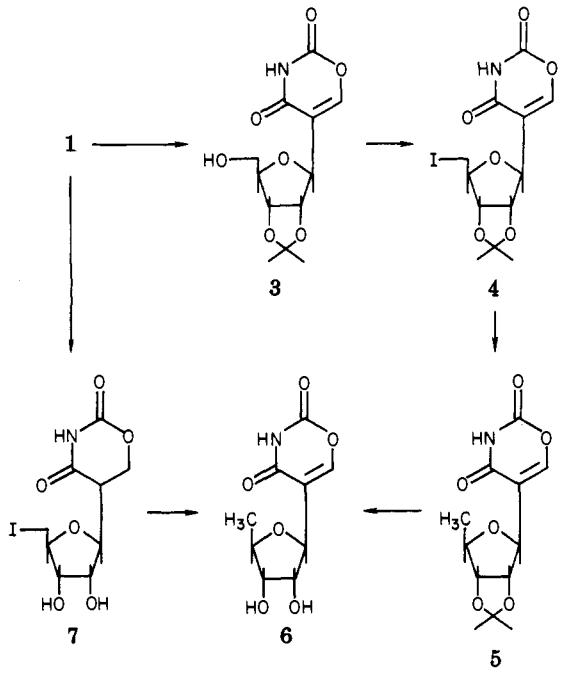
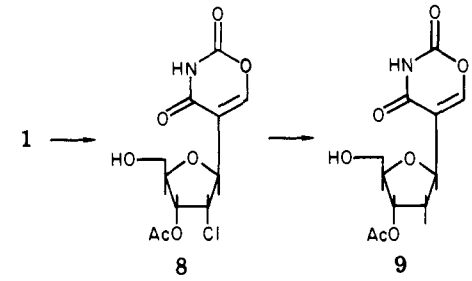
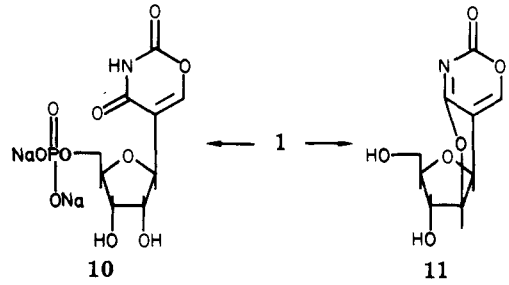
Scheme I



Scheme II



Scheme III



than uridine or pseudouridine. Oxazinomycin is active against Ehrlich ascites carcinoma[2] and several solid tumors in mice.[4] It is possible that the antitumor activity of oxazinomycin might be due to its instability. Similar instability in the case of 5-azacytidine and 5-azacytosine arabinoside is suggested to contribute to the biosynthesis of less stable nucleic acids, leading to disruption of secondary structure, chromosomal breakage, and, consequently, to antitumor activity.[5,6] Recently, some quantity of oxazinomycin (minimycin) has become available,[7] which prompted us to synthesize certain derivatives of oxazinomycin and related oxadiazole nucleosides for biological evaluation.

Chemistry. For the synthesis of oxazinomycin derivatives, the hydroxyl groups of oxazinomycin were protected to provide valuable reaction intermediates with solubility in suitable organic solvents. Treatment of oxazinomycin with acetic anhydride in pyridine provided 2′,3′,5′-tri-*O*-acetyloxazinomycin (**2**) in 92% yield. Reaction of oxazinomycin with acetone, dimethoxypropane, and perchloric acid provided 2′,3′-*O*-isopropylideneoxazinomycin (**3**) in 50% yield. We have earlier described our interest and approach for the synthesis of various 5′-deoxynucleosides as potential medicinal agents.[8] Compound **3** on reacton with methyltriphenoxyphosphonium iodide[9] in dichloromethane provided 5′-deoxy-5′-iodo-2′,3′-*O*-isopropylideneoxazinomycin (**4**) in crystalline form. Compound **4**, after hydrogenation using Pd/C as the catalyst, provided the dehalogenated product, 5′-deoxy-2′,3′-*O*-isopropylideneoxazinomycin (**5**). The hydrolysis of the isopropylidene group of **5** with formic acid provided the desired 5′-deoxyoxazinomycin (**6**). Compound **6** was also synthesized by an alternate route. Oxazinomycin, on direct iodination with methyltriphenoxyphosphonium iodide in dimethylformamide, provided the corresponding 5′-deoxy-5′-iodooxazinomycin (**7**) which, after catalytic hydrogenation, provided 5′-deoxyoxazinomycin (**6**) identical with the sample of **6** obtained from **5** (Scheme I).

The synthesis of 2′-deoxyoxazinomycin was also investigated due to its structural similarity with various naturally occurring pyrimidine 2′-deoxyribonucleosides. A general procedure for the synthesis of 2′-chloro-2′-deoxy-3′-*O*-acetylpyrimidine nucleosides has been described by Reichman et al.[10] Following a similar procedure, oxazinomycin was refluxed with 2-acetoxybenzoyl chloride in acetonitrile to provide 3′-*O*-acetyl-2′-chloro-2′-deoxyoxazinomycin (**8**). The structure of **8** was determined on the basis of elemental analysis and ^{1}H NMR. Reduction of **8** with tributyltin hydride in the presence of 2,2-azobisisobutyronitrile in tetrahydrofuran provided 3′-*O*-acetyl-2′-deoxyoxazinomycin (**9**) in crystalline form (Scheme II). Our attempts to deacetylate **9** at pH $\geq$ 8 were always accompanied by opening of the oxazinedione ring as indicated by IR and ^{1}H NMR of the product, which was not further characterized. The oxazinomycin derivatives exhibited in IR a signal at $\sim$1790 cm^{-1} for O=CO and in ^{1}H NMR a signal at δ $\sim$7.8 for aromatic H_6.

The 5′-phosphates of various biologically active nucleosides are active metabolites. The synthesis of oxazinomycin 5′-phosphate (**10**) was therefore also considered. The phosphorylation of oxazinomycin using phosphoryl chloride and trimethyl phosphate provided nucleotide **10** as the disodium salt after chromatographic purification (Scheme III).

Oxazinomycin, due to its sensitivity to various organic reagents, has mainly eluded our attempts at modification, especially at the 2 position. The only modification involving the aglycon was the synthesis of O^4,2′-anhydrooxazinomycin (**11**) under the mild conditions used for the synthesis of O^2,2′-anhydrouridine.[11] Such anhydronucleosides are potentially useful precursors which provide the corresponding arabinonucleosides on hydrolysis of the

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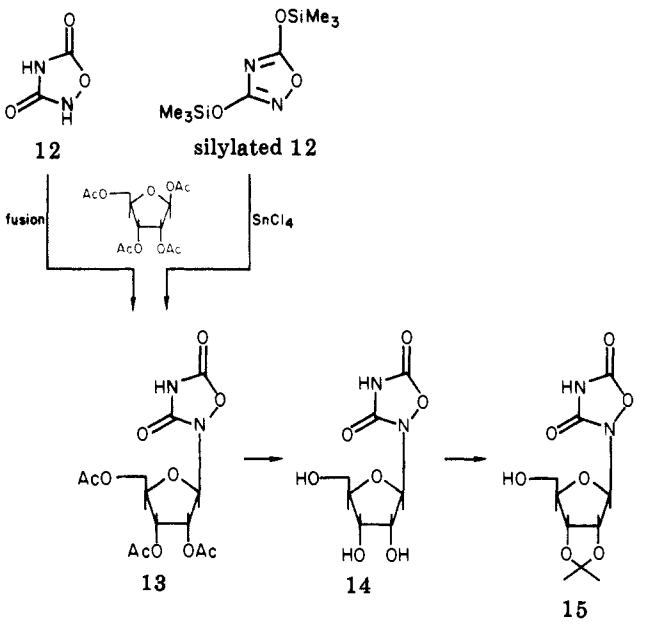
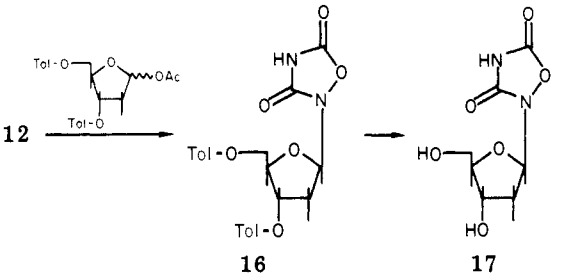
Scheme IV



anhydro bond. O^4,2′-Anhydrooxazinomycin was synthesized in low yield when **1** was reacted with diphenyl carbonate in the presence of sodium bicarbonate in dimethylformamide (Scheme III).

In view of the biological activity of oxazinomycin, we also set out to synthesize a novel nucleoside, 2-β-D-ribofuranosyl-1,2,4-oxadiazole-3,5-dione (**14**, Scheme IV), which has the structural features similar not only to oxazinomycin but also to the antitumor nucleoside antibiotic showdomycin.[12,13] The aglycon 1,2,4-oxadiazole-3,5-dione (**12**) was synthesized by a modification of the literature procedure.[14] Treatment of hydroxylamine with ethoxycarbonyl isocyanate provided N^1-(ethoxycarbonyl)-N^3-hydroxyurea which, in the presence of 1.1 equiv of potassium hydoxide in water at 50 °C, cyclized to provide **12** as the potassium salt. Neutralization of the potassium salt with Dowex 50 (H^+) resin provided **12** as shining white crystals. It was surprising to note that **12** was found highly soluble and acidic (pH 1–2) in water and was also readily soluble in organic solvents like ethyl ether and acetone. The structure of **12** was confirmed by melting point, mass spectrum, ^{1}H NMR, and elemental analysis. Fusion of **12** with tetra-*O*-acetyl-β-D-ribofuranose in the presence of a catalytic amount of bis(*p*-nitrophenyl) phosphate at 125 °C provided 2-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-oxadiazole-3,5-dione (**13**) as the major product. An alternate route for the synthesis of **13** was also investigated. Silylation of **12** with hexamethyldisilazane provided the corresponding bis(trimethylsilyl) derivative of **12**, which was condensed with tetra-*O*-acetyl-β-D-ribofuranose in the presence of catalytic amount of stannic chloride to provide **13** in 70% yield. The fusion procedure was, however, found comparatively more convenient. Compound **13** was deacetylated with NaOMe in MeOH at pH 8.5–9. It is important to mention that first 1 equiv of NaOMe was consumed to neutralize the acidic proton in **13** and slightly more than 1 equiv (~1.1) of NaOMe was needed to adjust the solution to pH 8.5–9. The deacetylated compound,

Scheme V



2-β-D-ribofuranosyl-1,2,4-oxadiazole-3,5-dione (**14**), was formed as a sodium salt which, when neutralized with Dowex 50 (H^+) resin, provided **14** as shining crystals. The structure of **14** was confirmed on the basis of elemental analysis, mass spectrum, and ^{1}H NMR. The β configuration of **14** was confirmed by converting **14** into the corresponding 2′,3′-*O*-isopropylidene derivative **15** (Scheme IV). In the ^{1}H NMR spectrum of **15**, the difference in the chemical shifts of the methyl groups of the isopropylidene moiety was equal to 0.16 ppm, which is in accordance to the β configuration of the ribonucleosides as reported by Imbach.[15] Further proof for the β configuration[16] of **14** was evident from the fact that in the ^{1}H NMR spectrum (Me_2SO-d_6) the anomeric proton appeared as a doublet with a coupling constant ($J_{1'2'}$) of 4.5 Hz, which was diminished to ≤1.5 Hz for the same proton in the corresponding isopropylidene derivative **15**.

The site of ribosylation in aglycon **12** at N-2 was expected from steric considerations. The fact that the ribosyl moiety is, indeed, linked to N-2 in **14** was confirmed by ^{13}C NMR spectroscopy. In the ^{13}C NMR spectrum of **12** the two peaks at 159.3 and 155.0 ppm were assigned to C-3 and C-5, respectively. These assignments were based on the observation that in ^{13}C NMR the signal for a substituted urea carbon (such as C-3) is shifted 6-ppm downfield as compared to that for a carbamate carbon (such as C-5) as reported in the literature.[17] In the ^{13}C NMR spectrum of **14** the signal for C-3, which appeared at 152.7 ppm, was shifted upfield by 6.6 ppm as compared to the signal for C-3 of **12**, whereas the signal at 158 ppm for C-5 of **14** was shifted downfield by 3 ppm as compared to the signal for C-5 of **12**. A relatively large upfield shift for C-3 as compared to a small downfield shift for C-5 suggested that in **14** C-3 and C-5 must be at positions α and β, respectively, relative to the ribosylation site N-2. These data are in accordance with the previous reports[18–20] that nitrogen substitution resulted in a substantial upfield shift for the carbon α to the substituted nitrogen, while a relatively small downfield shift was observed for the β carbon.

The 2′-deoxy analogue of this novel ribonucleoside, **14**, was also synthesized. Fusion of **12** with 1-*O*-acetyl-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranose[21] in the presence of a catalytic amount of bis(*p*-nitrophenyl)

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phosphate provided 2-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-3,4-dione (**16**) as the major product. Removal of the toluoyl blocking groups with NaOMe in MeOH provided, after treatment with Dowex 50 (H^+), the desired 2-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-3,5-dione (**17**) as shining fine needles in 63% yield (Scheme V). The structure of **17** was confirmed on the basis of elemental analysis and ^1H NMR. In ^1H NMR the anomeric proton of **17** appeared as a triplet centered at δ 5.8 with a peak width of ~13.4 Hz and an apparent splitting constant of ~6.7 Hz. These ^1H NMR properties are characteristics of a β configuration of the deoxynucleosides.[16] The site of glycosylation in **17** would be expected at N-2, analogous to that in **14**.

Biological Evaluation. The antitumor activity of oxazinomycin has been reported previously from different laboratories.[2,4] Dr. Alexander Bloch's[22] recent study on the antitumor activity of oxazinomycin indicated that oxazinomycin has an IC_{50} of 3×10^{-7} M for L1210 cells and 5×10^{-7} M for HeLa cells. At a concentration of 3×10^{-6} M, oxazinomycin causes extensive lysis when incubated with L1210 cells for 2 h. The growth inhibitory effect of oxazinomycin on L1210 is extensively reversed by uridine and cytidine but to much lesser extent by the corresponding deoxyribonucleosides, indicating a possible effect on the path leading to the formation of ribonucleotides. Oxazinomycin also showed about 5% inhibition of RNA, DNA, and protein synthesis in L1210 cells at a concentration of 8×10^{-7} M, after 24 h of incubation. Oxazinomycin derivatives **6** and **9–11** and oxadiazole derivatives **12**, **14**, and **17** were inactive when tested in cell culture against leukemia L1210 and P388. They were also inactive against a variety of bacteria and fungi when tested at 200 and 100 mg/mL, respectively, in defined medium using the agar dilution technique.

Compounds **12**, **14**, and **17** were also tested for antiviral activity in vitro (KB cells)[23] against herpes simplex virus (HSV) type 1, HSV type 2, and parainfluenza virus type 3 in concentrations ranging from 1000 to 1 μg/mL. The antiviral activity was determined in terms of virus rating (VR) by observing inhibition of viral cytopathic effect after a 72-h incubation at 37 °C. A VR of ≥0.5 was indicative of moderate to definite antiviral activity. Ribavirin[24] (Virazole) and acyclovir[25,26] were used as control for this study. Compound **14** showed significant antiviral activity (VR = 0.65) against HSV/1 but was less active than either ribavirin (VR = 1.2) or acyclovir (VR = 1.4). Compounds **12** and **17** were inactive.

Experimental Section

The physical properties were determined with the following instruments: melting point, Thomas-Hoover apparatus (uncorrected); IR, Beckman Acculab 2 (KBr); UV, Cary 15 UV spectrophotometer; ^1H NMR, Varian EM-390 (Me_4Si). A fourier transform NMR spectrometer, JEOL-FX-900, was used for ^{13}C NMR. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Where analyses are indicated by only symbols of the elements, the analytical results for those elements were within ±0.4% of the theoretical value. Precoated (silica gel $60F_{254}$) TLC sheets were used to examine the purity of compounds.

2′,3′,5′-Tri-*O*-acetyloxazinomycin (2). Oxazinomycin[7] (6.1 g, 24.8 mmol) was dissolved in anhydrous pyridine (35 mL). To this was added acetic anhydride (15 mL) and the reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated in vacuo and the residue partitioned between ethyl acetate and water. The ethyl acetate portion was dried ($MgSO_4$) and evaporated in vacuo to provide a residue, which was crystallized from 2-propanol to yield 8.5 g (92%) of **2** as white crystals: mp 125–126 °C; ^1H NMR (Me_2SO-d_6) δ 4.73 (d, 1, J = 4 Hz, $C_{1'}$ H), 7.92 (s, 1, C_6 H), 2.04 (s, 9, acetyl protons), and other sugar protons. Anal. ($C_{15}H_{17}NO_{10}$) C, H, N.

2′,3′-*O*-Isopropylideneoxazinomycin (3). Oxazinomycin (2.45 g, 10 mmol) was added to a cold solution of anhydrous acetone (50 mL), dimethoxypropane (16 mL), and 70% perchloric acid (1.6 mL), and the solution was stirred at 0 °C for 15 min. The reaction solution was poured into an ice-cold stirred solution of NH_4OH (10 mL) and water (40 mL). The solvent was evaporated under vacuum, and the residue was dried by repeated coevaporation with ethanol. The dried residue was extracted with $CHCl_3$ (300 mL). The $CHCl_3$ portion was evaporated in vacuo to provide a residue, which was crystallized from ethanol to yield 1.4 g (50%) of crystalline **3**: mp 181–182 °C; ^1H NMR (Me_2SO-d_6) δ 7.90 (s, 1, C_6H), 1.3 and 1.5 (2 s, 3 and 3, isopropylidene methyls), and other protons. Anal. ($C_{12}H_{15}NO_7$) C, H N.

5′-Deoxy-5′-iodo-2′,3′-*O*-isopropylideneoxazinomycin (4). Methyltriphenoxyphosphonium iodide[9] (3.8 g) was added under a N_2 atmosphere to a solution of **3** (1.0 g, 3.5 mmol) in anhydrous DMF (15 mL), and the reaction solution was stirred at room temperature for 30 min. The solvent was evaporated in vacuo, and the residue was taken in ethyl acetate (100 mL) and washed with 10% sodium thiosulfate (100 mL), followed by water (100 mL). The ethyl acetate portion was dried ($MgSO_4$) and evaporated in vacuo and the residue (syrup) was passed through a column of silica gel (150 g) packed in $CHCl_3$. The column was eluted with 25% ethyl acetate in $CHCl_3$ to provide **4**, which was crystallized from $CHCl_3$: yield 1.0 g (72%); mp 189–190 °C. Anal. ($C_{12}H_{14}INO_6$) C, H, N.

5′-Deoxy-2′,3′-*O*-isopropylideneoxazinomycin (5). To a solution of **4** (500 mg, 1.26 mmol) in ethanol (25 mL) was added sodium acetate (110 mg) and 10% Pd/C (110 mg), and the mixture was hydrogenated at 42 psi for 90 min. The catalyst and the insoluble product were filtered and washed with ethanol. The filtrate and the washings were combined and evaporated in vacuo to provide a residue, which was crystallized from chloroform and ligroin to yield 200 mg (59%) of **5**: mp 141–142 °C. Anal. ($C_{12}H_{15}NO_6$) C, H, N.

5′-Deoxyoxazinomycin (6). Method A. Compound **5** (100 mg, 0.37 mmol) was dissolved in 80% formic acid (5 mL) and stored at room temperature for 1 h and then overnight at 0 °C. The solvent was evaporated in vacuo and the residual syrup coevaporated repeatedly with water. The residue, after chromatographic purification on a silica gel column, provided 40 mg (49%) of pure **6** identical (mp, TLC, ^1H NMR) with **6** obtained by method B.

Method B. To a solution of 5′-deoxy-5′-iodooxazinomycin (**7**; 750 mg, 2.1 mmol) in ethanol (25 mL) was added 10% Pd/C (180 mg) and sodium acetate (165 mg), and the mixture was hydrogenated at room temperature at 40 psi for 5 h. The catalyst and the insoluble material were removed by filtration, and the filtrate was evaporated in vacuo. The residue was passed through a column of silica gel (150 g) packed in $CHCl_3$. The pure product was eluted with 25% $CHCl_3$ in ethyl acetate. The fractions containing the product were combined and concentrated in vacuo to 5 mL, which, on keeping, provided 300 mg (62%) of **6**: mp 150 °C (softens at 134–135 °C); ^1H NMR (D_2O) δ 7.76 (s, 1, C_6 H), 1.35 (d, 3, $C_{5'}$ H_3), and other protons. Anal. ($C_9H_{11}NO_6$) C, H, N.

5′-Deoxy-5′-iodooxazinomycin (7). Methyltriphenoxyphosphonium iodide (6.4 g) was added under a N_2 atmosphere to a solution of oxazinomycin (2.4 g, 10 mmol) in DMF (50 mL), and the reaction solution was stirred for 1 h. Sodium thiosulfate (4.26 g) was added, followed by the addition of methanol (1 mL).

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The reaction mixture was stirred for 5 min. The insoluble material was removed by filtration, and the filtrate was concentrated to 10 mL in vacuo. It was passed through a column of silica gel (150 g) packed in $CHCl_3$. Elution with 25% $CHCl_3$ in ethyl acetate provided the product as a syrup, which was crystallized from chloroform to yield 1.2 g (34%) of **7**: mp 150 °C. Anal. ($C_9H_{10}INO_6$) C, H, I, N.

3′-*O*-Acetyl-2′-chloro-2′-deoxyoxazinomycin (8). 2-Acetoxybenzoyl chloride (4.0 g) was added to a suspension of oxazinomycin (1.25 g) in acetonitrile (15 mL). The mixture was refluxed for 5 min and cooled immediately. The solvent was evaporated to dryness, and the residue was dissolved in methanol and left at 0 °C for 15 h. The solvent was evaporated again, and the residue was triturated with chloroform (15 mL). The insoluble material was removed by filtration, and the filtrate (chloroform portion) was evaporated in vacuo to provide a residue, which was passed through a column (3 × 40 cm) of silica gel packed in chloroform. The column was eluted with 25% ethyl acetate in chloroform to provide fractions containing **8**. These fractions were combined, and the solvent was evaporated in vacuo to provide a mobile syrup. Addition of ethyl ether to the syrup provided **8** as light yellow crystals: mp 149–151 °C; ^1H NMR (Me_2SO-d_6) δ 7.98 (s, 1, C_6 H), 4.81 (d, 1, $C_{1'}$ H), 2.31 (s, 3, acetyl). Anal. ($C_{11}H_{12}ClNO_7$) C, H, Cl, N.

3′-*O*-Acetyl-2′-deoxyoxazinomycin (9). A solution of **8** (306 mg, 1 mmol), 2,2′-azobisisobutyronitrile (71 mg) and tributyltin hydride (1.14 g) in anhydrous tetrahydrofuran (15 mL) was heated under gentle reflux with exclusion of moisture for 18 h. The solvent was evaporated in vacuo. The residual syrup after chromatographic purification on a silica gel column (solvent: 40% chloroform in ethyl acetate) provided **9**, which was crystallized from chloroform and ethyl acetate: yield 120 mg (44%); mp 137–138 °C; IR (KBr) 1790, 1710 (>C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.84 (s, 1, C_6 H), 4.8 (pseudo t, 1, $C_{1'}$ H), 2.2 (m, 2, $C_{2'}$ H_2), 2.07 (s, 3, acetyl). Anal. ($C_{11}H_{13}NO_7$) C, H, N.

Disodium Oxazinomycin 5′-Phosphate (10). Oxazinomycin (1.25 g, 5 mmol) was added to a precooled (0 °C) solution of $POCl_3$ (3.0 g, 20 mmol) and trimethyl phosphate (10 mL). The reaction mixture was stirred at 0–4 °C for 4 h under anhydrous conditions. The reaction solution was poured into crushed ice (~15 g) and extracted with ethyl ether (2 × 15 mL). The water portion was adjusted to pH 7 with $NaHCO_3$ and passed through a column of Sephadex (DEAE, A25, HCO_3^- form, 15 mL). A gradient elution (0–0.5 M, triethylammonium bicarbonate) provided oxazinomycin 5′-phosphate in crude form. The crude compound was dissolved in water and passed through a charcoal column (2 × 15 cm). The column was washed with triethylammonium bicarbonate (0.5 M, 800 mL). Elution with ethanol/water/triethylamine (50:50:1) provided a fraction containing oxazinomycin 5′-phosphate. The fraction was evaporated in vacuo below 20 °C, and the residue was dissolved in water (2 mL). The water portion was passed through a column of Dowex 50W-X8, 20–50 mesh, Na^+ form (10 mL). The column was eluted with water (25 mL), which, after lyophilization, provided oxazinomycin 5′-phosphate as the disodium salt: IR (KBr) 1790, 1755 (>C=O) cm^{-1}. Anal. ($C_9H_{10}NO_{10}P \cdot Na_2 \cdot 2H_2O$) C, H, N, P.

O^4-2′-Anhydrooxazinomycin (11). A mixture of oxazinomycin (500 mg, 2.0 mmol), diphenyl carbonate (600 mg), and $NaHCO_3$ (10 mg) in DMF (1 mL) was stirred at room temperature for 5 min and then heated (bath temperature 135–136 °C) with stirring for 7 min. The dark brown solution was cooled in ice and triturated with ethyl ether (50 mL). The supernatant was discarded, and the solid was passed through a column of silica gel (50 g) packed in ethyl acetate. The column was eluted with ethyl acetate, and the fractions (15 mL each) were collected. Fractions 10–14, which contained the product, were combined and evaporated under vacuum. The residue on trituration with ethyl ether gave a crystalline product, which was recrystallized from water to provide **11**: mp 219–220 °C (dec, turns black at 214–215 °C); IR (KBr) 1798 (OC=) cm^{-1}. Anal. ($C_9H_9NO_6$) C, H, N.

1,2,4-Oxadiazole-3,5-dione (12). To a suspension of N^1-(ethoxycarbonyl)-N^3-hydroxyurea[14] (95.6 g, 640 mmol) in H_2O (515.2 mL) was added 3 N KOH (235.7 mL, 710 mmol). The suspension was heated in a water bath (~50 °C) to provide a clear solution. The reaction was complete after ~5 min of additional heating at 50 °C. The progress of the reaction was followed by TLC (solvent: ethyl acetate/1-propanol/water, 4:1:2; top layer) and $FeCl_3$ spray which gave a purple color for the cyclized final product. The solution was immediately cooled in an ice bath and then passed through a column of Dowex 50W-X8, 20–50 mesh, H^+ form (1100 mL). The column was washed with water (~2.5 L), and the fractions with acidic pH were collected and evaporated in vacuo to provide a white solid. The solid was dissolved in ethanol, and the solution evaporated under vacuum. The residue was crystallized from ethyl ether and petroleum ether to provide shining white crystals of **12** in 91% yield: mp 107–108 °C (lit.[14] mp 103–105 °C); IR (KBr) 1810 and 1740 (C=O) cm^{-1}; MS, *m/e* 102 (m^+). Anal. ($C_2H_2N_2O_3$) C, H, N.

2-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-oxadiazole-3,5-dione (13). Method A. Finely powdered **12** (1.02 g, 10 mmol) and tetra-*O*-acetyl-β-D-ribofuranose (3.18 g, 10 mmol) were mixed thoroughly and heated with stirring in a pear-shaped flask at 122–125 °C (bath temperature). The mixture melted to a clear syrup, to which was added a catalytic amount of bis(*p*-nitrophenyl) phosphate (~10 mg), and the vacuum from a water aspirator was applied immediately. The reaction mixture was heated with stirring under vacuum for 45 min to 1 h. The mixture was cooled to room temperature and dissolved in $CHCl_3$ (125 mL). A solution of $NaHCO_3$ (1.68 g) in H_2O (50 mL) was added, and the mixture was stirred for 15 min. The water portion was separated, cooled in ice, and added to a stirred mixture of Dowex 50W-X8, 20–50 mesh, H^+ form (20 mL), in $CHCl_3$ (85 mL). The resin was removed by filtration and washed with chloroform (50 mL) and H_2O (50 mL). The filtrate and washings were combined, and the chloroform layer was separated and dried ($MgSO_4$). Evaporation of chloroform under vacuum provided 2.7 g (75%) of **13** as a syrup which was of sufficient purity for further reaction. An analytical sample was obtained by chromatographic purification: IR (KBr) 1740 (>C=O), 1650 and 1635 (OAc) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.1 (s, 9, 3 × OAc). Anal. ($C_{13}H_{16}N_2O_{10}$) C, H, N.

Method B. A suspension of **12** (430 mg, 4.2 mmol) and a catalytic amount of ammonium sulfate (~10 mg) in hexamethyldisilazane (4.0 mL) was heated under reflux (bath temperature 130 °C) for 4 h. The suspension became clear and the solvent was evaporated in vacuo. The residual solid was dissolved in anhydrous dichloroethane (20 mL). Tetra-*O*-acetyl-β-D-ribofuranose (1.33 g, 4.2 mmol) was added, followed by the addition of stannic chloride (0.48 mL, 1.09 g) at 0 °C. The reaction mixture was stirred at room temperature with exclusion of moisture for 16 h. The clear solution was poured into a stirred saturated solution of $NaHCO_3$ (50 mL). Dichloroethane (50 mL) was added. The aqueous layer containing the product was separated and worked up exactly as described in method A to provide **12** (70%) as a syrup, which had identical TLC mobility, IR, and ^1H NMR when compared with those of **13** obtained by method A.

2-β-D-Ribofuranosyl-1,2,4-oxadiazole-3,5-dione (14). A solution of **13** (foam, 2.8 g, 7.77 mmol) in methanol (25 mL) was adjusted to pH 8.5 with NaOMe and allowed to stand at room temperature for 2.5 h. The progress of the reaction was followed by TLC which indicated complete deacetylation. The solvent was evaporated, and the residue was dissolved in water and passed through a column packed with Dowex 50W-X8, 20–50 mesh, H^+ form (15 mL).

The column was washed with water (45 mL), and the fraction with acidic pH was collected and evaporated at <30 °C in vacuo. The residue was coevaporated with methanol and crystallized from methanol and chloroform to provide 1.3 g (71.5%) of **14** as shining white crystals: mp 174–175 °C dec; IR (KBr) 1808 and 1735 (>C=O) cm^{-1}; MS, *m/e* 234 (M^+); ^1H NMR (Me_2SO-d_6) δ 5.3 (d, 1, $J_{1',2'}$ = 4.5 Hz, $C_{1'}$ H), 4.2 (t, 1, *J* = 4.5, $C_{2'}$ H), 3.85 (m, 2, $C_{3'}$ H, $C_{4'}$ H), 3.4 (t, 2, $C_{5'}$ H_2), and other protons which were exchangeable with D_2O. Anal. ($C_7H_{10}N_2O_7$) C, H, N.

2-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-1,2,4-oxadiazole-3,5-dione (15). Compound **14** (234 mg, 1 mmol) was added to a stirred solution of anhydrous acetone (5 mL), 2,2-dimethoxypropane (1.6 mL), and 70% perchloric acid (0.16 mL). The reaction mixture was stirred for 5 h and evaporated in vacuo. The isopropylidene derivative, **15**, was isolated after chromatographic purification, using a preparative silica gel plate (solvent: Et OAc/1-propanol/H_2O, 4:1:2; top layer). The product was crystallized from $CHCl_3$ to provide 192 mg (70%) of **15**: mp 142–143

°C; ^{1}H NMR (Me_2SO-d_6) δ 5.4 (d, 1, $J_{1',2'} \leq 1.5$ Hz, $C_{1'}$ H), 1.3 and 1.46 (2 s, 3 and 3, isopropylidene methyls, difference in chemical shifts of methyl groups = 0.16 ppm). Anal. ($C_{10}H_{14}N_2O_7$) C, H, N.

2-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-3,5-dione (17). A mixture of **12** (1.02 g, 10 mmol), 1-*O*-acetyl-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranose[21] (4.13 g, 10 mmol), and a catalytic amount of bis(*p*-nitrophenyl) phosphate was finely powdered and heated in a pear-shaped flask at 125 °C (bath temperature). When the mixture melted to a clear syrup, the vacuum from a water aspirator was applied and the heating continued for an additional 30 min. The reaction mixture was cooled to room temperature, and the thick syrup was dissolved in $CHCl_3$ (200 mL) and washed with water saturated with NaCl. The $CHCl_3$ portion was separated, dried ($MgSO_4$), and evaporated in vacuo. The crude residue was passed through a column (3.5 × 36 cm) packed with silica gel in $CHCl_3$. Elution with 25% ethyl acetate in $CHCl_3$ provided the chromatographically pure 2-(deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-3,5-dione (**16**), which was crystallized from benzene and cyclohexane: mp 149–151 °C (softens at 142 °C); ^{1}H NMR ($CDCl_3$) δ 8.5 (br s, 1, NH, exchangeable with D_2O), 7.82 and 7.15 (2 m, 4 and 4, toluene ring protons), 6.02 (t, 1, $C_{1'}$ H), 2.35 (s, 6, methyls), and other sugar protons. Compound **16** was dissolved in methanol (50 mL), adjusted to pH 8.5 with NaOMe, and allowed to stand at room temperature for 48 h. The solent was evaporated in vacuo, and the residue was taken in water (25 mL), which was extracted with $CHCl_3$ (15 mL × 2). The water portion was passed through a column of AG 50W-X8, 20–50 mesh, H^+ form (15 mL). The column was washed with additional water (30 mL). The water fractions were combined, washed with $CHCl_3$ (45 mL), and lyophilized to provide a residue, which was crystallized from acetone and ethyl ether to provide 1.3 g (63%) of **17**: mp 140–141 °C; IR (KBr) 1805 and 1730 (>C=O); ^{1}H NMR (Me_2SO-d_6) δ 5.8 (t, 1, $J_{H_{1'},H_{2'}} = 6.7$ Hz, peak width 13.4 Hz, $C_{1'}$ H), 4.08 and 3.6 (2 m, 1 and 1, $C_{3'}$ H and $C_{4'}$ H), 3.3 (m, 2, $C_{5'}$ H_2), 2.1 (m, 2, $C_{2'}$ H_2). Anal. ($C_7H_{10}N_2O_6$) C, H, N.

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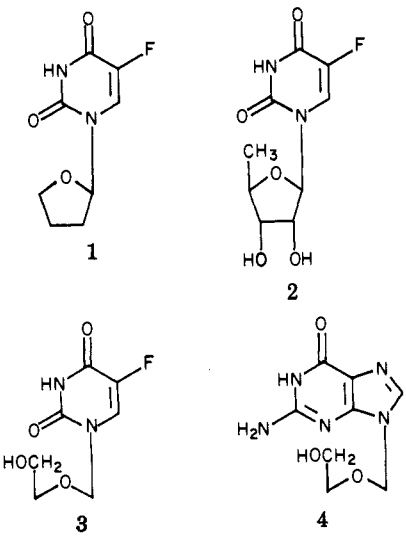
Synthesis and Antitumor Activity of an Acyclonucleoside Derivative of 5-Fluorouracil

Andre Rosowsky,* Sun-Hyuk Kim, and Michael Wick

Sidney Farber Cancer Institute and the Departments of Pharmacology and Dermatology, Harvard Medical School, Boston, Massachusetts 02115. *Received April 28, 1981*

The pyrimidine acyclonucleoside 5-fluoro-1-[(2-hydroxyethoxy)methyl]uracil (**3**) was synthesized as part of a program aimed at the development of new 5-fluorouracil derivatives with fewer side effects and a broader margin of safety. Condensation of 5-fluoro-2,4-bis[(trimethylsilyl)oxy]pyrimidine with 2-acetoxyethyl acetoxymethyl ether (**6**) in the presence of $SnCl_4$ afforded the acetate ester **7**, which on deprotection with NaOMe gave **3** in 50–60% overall yield. The 5-bromo and 5-iodo analogues **10** and **11**, respectively, were obtained similarly. Reaction of 5-fluoro-4-(methylthio)-2-[(trimethylsilyl)oxy]pyrimidine with 2-acetoxyethyl acetoxymethyl ether and $SnCl_4$, followed by ammonolysis, yielded 5-fluoro-1-[(2-hydroxyethoxy)methyl]cytosine (**12**). Deamination of **12** with nitrous acid produced **3**, thereby confirming that alkylation of 5-fluoro-2,4-bis[(trimethylsilyl)oxy]pyrimidine had occurred at N^1. The ID_{50} of **3** against L1210 mouse leukemia cells in culture was 1.7×10^{-5} M, as compared with 1×10^{-6} M for FU. The 5-fluorocytosine analogue **12** was inactive at up to 1×10^{-4} M, and the other halogenated derivatives **10** and **11** had no effect even at 1×10^{-3} M. When **3** was given ip in water to P388 leukemic mice at 400 mg/kg (b.i.d. × 4) or 240 mg/kg (q.d. 1–9), a 75% increase in survival was observed relative to untreated controls, and there was no evidence of any host toxicity.

Novel prodrug derivatives of 5-fluorouracil (FU) possessing a broader spectrum of antitumor activity and fewer toxic side effects than FU have been sought diligently in a number of laboratories. One such derivative which has received attention in recent years is 1-(2-tetrahydrofuranyl)-5-fluorouracil (**1**, Ftorafur).[1] In addition to several clinical studies,[2] the human and animal pharmacology of this compound has been extensively investigated,[3] and several improved methods of chemical synthesis have been developed.[4] Another FU prodrug that has aroused interest more recently is 5-fluoro-5′-deoxyuridine (**2**, 5-DFUR).[5] This compound is reported to be therapeutically superior to both Ftorafur and 5-fluoro-2′-deoxyuridine (FUDR) against several murine tumors, including P388 and L1210 leukemias, Lewis lung carcinoma, and Crocker sarcoma S180.[6,7] Among the reported advantages of the



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