measurement of plasma renin activity (PRA) were collected before and after administration of the compounds as indicated. The blood samples were taken by direct puncture of the saphenous vein.

Acknowledgment. We wish to extend our thanks to Dr. Volker Eiermann and Helmut Müller for the measurement and interpretation of NMR and mass spectra. For their skillful experimental work, we would like to thank Ralf Emmerich, Christine Heiner, Dieter Koethe, Dieter Kux, Rolf Löffler, Gabriele Mahr, Barbara Rothenstein, and Ludwig Weigand. We also thank Marion Gerbig for preparing and typing the manuscript.

Antitumor Properties of 2(1*H*)-Pyrimidinone Riboside (Zebularine) and Its Fluorinated Analogues

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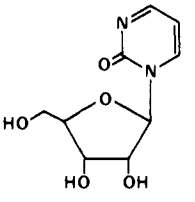
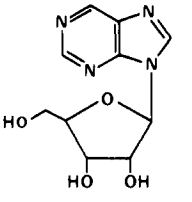
2(1*H*)-Pyrimidinone riboside (zebularine, **1b**) and its 5-fluoro (**6b**) and 2′-*ara*-fluoro (**7b**) analogues have been synthesized and evaluated in vivo as antitumor agents. Zebularine provides increase in life span (ILS) values of ca. 70% against intraperitoneal (ip) murine B16 melanoma and 50% against P388 leukemia. This compound is active when administered either ip or orally against ip or subcutaneously implanted L1210 leukemia, producing ILS values of about 100% at an optimum dose of 400 mg/kg. **1b** is also active (60% ILS) against *ara*-C-resistant L1210. The analogous unsubstituted purine riboside nebularine (**2**) has modest activity against P388 leukemia (60% ILS). While 2′-*ara*-fluorozebularine (**7b**) is only marginally active (40% ILS) at high doses against L1210 leukemia, 5-fluoro analogue **6b** is more active than zebularine and is ca. 100 times more potent. Although the activity of **6b** is about the same as that of **1b** against P388 leukemia, greater potency also is realized in this model. Zebularine is a strong inhibitor of cytidine deaminase, but in contrast to tetrahydrouridine, **1b** is acid-stable. In an attempt to use this property to advantage in oral administration, **1b** and *ara*-C have been orally coadministered to mice with ip L1210 leukemia. When zebularine is given in divided doses, up to a 2-fold increase in activity is realized, relative to treatment with the same dose of *ara*-C alone.

Earlier biochemical investigations with 1-β-D-ribofuranosyl-1,2-dihydropyrimidin-2-one (**1b**, zebularine, 2-(1*H*)-pyrimidinone riboside), established this compound as an inhibitor of cytidine deaminase (CDA).[1-4] The transition-state hypothesis[1] that lead to our initial work on **1b** as a CDA inhibitor has recently been examined in detail.[5] Although **1b**, with a 2 μM K_i, is about 10 times less potent than tetrahydrouridine (THU) as an inhibitor of mouse kidney CDA, it is still a good inhibitor of this enzyme. The stability of **1b** relative to THU in an acid-induced furanose to pyranose deactivation reaction[6] makes it a potentially useful adjuvant for oral combination studies with drugs which are substrates for CDA.

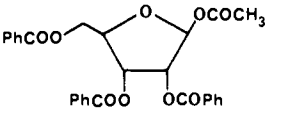
Zebularine (**1b**) was synthesized and evaluated as a bacteriostat in the early 1970s.[7,8] Our observation[1] that **1b** was cytotoxic to cultured L1210 leukemia cells, as well as active in vivo against P388 leukemia, prompted us to examine further the antitumor properties of this compound and two of its analogues. For comparison, data are included for nebularine (**2**), the purine counterpart of **1b**.



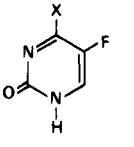
1b



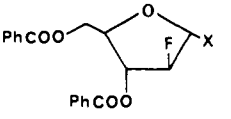
2



3



4a. X = OH
b. X = SH
c. X = H



5a, X = OCOPh
b, X = Br

Chemistry

Zebularine (**1b**) was prepared by deblocking its tri-*O*-benzoyl derivative **1a** which had been synthesized by the method of Niedballa and Vorbruggen[9] utilizing the sugar intermediate **3** (Scheme I). 5-Fluorozebularine (**6b**) has been prepared by a number of methods, including the direct fluorination of **1a**.[10] Our route to **6b** utilized 5-fluoro-2(1*H*)-pyrimidinone (**4c**), which was prepared by the treatment of 5-fluorouracil (**4a**) with P_2S_5 followed by reduction with Raney nickel.[11] The tin chloride catalyzed

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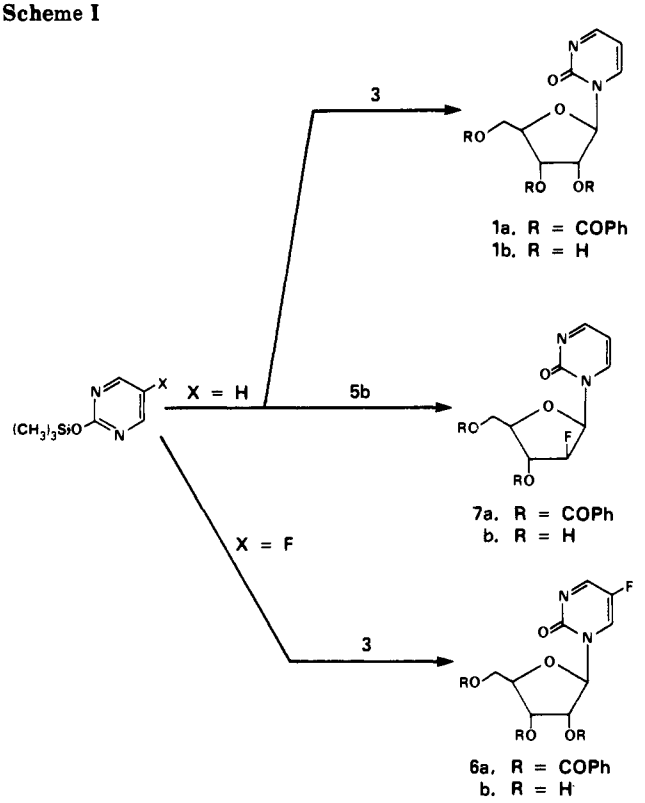
Scheme I



condensation[9] between **3** and silylated **4c** gave **6a**, which was deblocked with methanolic ammonia to give **6b** (Scheme I).

Table I. Antitumor Activity of Zebularine (1b)[a]

tumor[b]	dose, mg/kg per injection	weight change difference,[c] g	% ILS[d]
ip B16	800	0.0	38
	400	−0.1	80
ip B16	400	−0.2	66
	200	−0.5	36
ip P388	400	−2.3	52
	200	−2.0	34
ip P388	600	−1.3	45
	400	−1.2	37
	200	−0.7	25
ip L1210	800	−2.5	93
	400	−2.1	75
ip L1210	800	−2.8	89
	400	−0.4	81
ip L1210[e]	1600	−2.3	112
	800	−0.6	77
sc L1210	800	−2.4	110
	400	−1.1	75
sc L1210[e]	800	−0.8	81
	400	−0.8	39
ip L1210/*ara*-C[f]	800	−3.4	60
	400	−1.8	51
	200	−1.6	40

[a] Intraperitoneal drug treatment unless otherwise noted. QD 1–9 treatment schedule. Physiologic saline vehicle used for compound administration and for vehicle-treated, tumored controls. [b] B16 melanoma, L1210 leukemia, P388 leukemia, ip = intraperitoneal, sc = subcutaneous. [c] Difference in average body weight change between tumored, drug-treated mice and vehicle-treated controls. [d] Percent increase in life span of tumored, drug-treated mice relative to tumored, vehicle-treated controls. [e] Oral treatment. [f] *ara*-C-resistant L1210 leukemia.

Table II. Antitumor Activity of **2**, **6b**, and **7b**[a]

compd	tumor	dose, mg/kg per injection	weight change difference, g	% ILS
2	P388[b]	6.25	−1.0	40
		3.13	0.0	60
	P388[b]	6.25	1.0	34
		3.13	−1.0	52
6b	L1210	2.5	−1.0	137
		1.25	0.0	75
		0.68	0.0	62
6b	L1210	2.5	−2.0	150
		1.67	−1.0	118
		1.11	0.0	75
6b	P388	2.5	−2.0	54
		1.67	−2.0	45
6b	P388	3.75	−2.0	60
		2.5	−2.0	60
		1.67	−2.0	50
		1.11	−1.0	50
7b	L1210	800	1.0	43
		400	0.0	37
		200	1.0	31

[a] Intraperitoneal tumor implantation. See Table I for other definitions. [b] Three mice/dose. All other experiments use six mice/dose.

Fluoro sugar **5**, required for the synthesis of 2′-fluoro-2′-deoxy analogue **7b**, was prepared by the procedure of Tann et al.[12] Conversion to the α-bromo derivative with HBr/HOAc and condensation with silylated pyrimidin-2(1*H*)-one (Scheme I) gave a mixture of anomers. Separation of β-anomer **7a**, followed by deblocking, gave 2′-deoxy-2′-fluoro-*ara*-zebularine (**7b**).

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In Vivo Single Agent Studies

Our earlier observation that zebularine (**1b**) had modest activity against P388 leukemia[1] prompted further study in additional tumor models. Good, albeit not spectacular, activity is observed against B16 melanoma, L1210 leukemia, and P388 leukemia (Table I). The relatively high doses required are also indicative of the low toxicity of **1b**.

On a daily times nine (QD 1–9) treatment schedule, zebularine produced at least a 66% increase in life span (ILS) in B16 melanoma tumored mice at a dose of 400 mg/kg (Table I). Toxicity, as indicated by the difference in weight between drug-treated and untreated control mice, was minimal, even at twice the optimum dose. Repeat P388 leukemia data are consistent with our original report[1] of activity at the 50% ILS level.

More detailed studies were carried out using the L1210 murine leukemia model. Employing a standard NCI testing protocol[13] [intraperitoneal tumor implantation/intraperitoneal drug treatment (ip/ip)], ILS values of ca. 90% are observed at 400–800 mg/kg (Table I). Using oral treatment, good activity (81–112% ILS) is obtained with either ip or subcutaneous (sc) L1210 tumor implantation. As is often the case, the optimum dose for oral treatment is somewhat higher than for ip treatment because of drug bioavailability considerations. Although subcutaneously implanted L1210 leukemia is normally more difficult to treat than the ip implanted tumor, a 110% ILS is seen in the sc/ip system (Table I). Activity was also observed against L1210 leukemia which had been made resistant to *ara*-C. Zebularine (**1b**) is inactive against the following tumors in vivo: murine CD8F1 mammary adenocarcinoma; colon 38 carcinoma; Lewis lung carcinoma; M5076 sarcoma; and three human tumor xenografts, CX-1 adenocarcinoma, LX-1 lung, and the MX-1 mammary carcinoma.

Earlier studies with 5-fluorozebularine (**6b**) showed that it was the most potent inhibitor of cytidine deaminase among a number of zebularine analogues, with a K_i value equivalent to that of THU.[1] This in vitro potency and activity has been shown to carry over to in vivo antitumor experiments (Table II). While the activity of **6b** against murine P388 leukemia was about the same as that of zebularine (50–60% ILS), **6b** was 100 times more potent than **1b** under the same experimental conditions. Against L1210 leukemia, **6b** was significantly more active than **1b** (140% ILS vs 90%) with the same 100-fold potency increase noted in the P388 tumor model.

(2′-Deoxyarabinofuranosyl)pyrimidine nucleosides with 2′-fluoro substitution have demonstrated excellent preclinical antiviral properties.[14] In order to evaluate a pyrimidin-2(1*H*)-one with fluorine substitution in the sugar rather than in the base, compound **7b** was synthesized (Scheme I) and tested against L1210 leukemia (Table II). Statistically significant antitumor activity was observed (40% ILS), but the doses required were so high that testing was not extended to other tumor models.

Nebularine (**2**), a well-known natural product[15] and the purine counterpart of zebularine (**1b**), had previously been reported to have in vitro cytotoxicity to sarcoma 180 cells.[16]

Table III. Oral Combination Studies with *ara*-C and Zebularine against L1210 Leukemia[a]

oral dose,[b] mg/kg		weight change	%
ara-C	zebularine	difference, g	ILS
500		−0.5	44
	500[c]	−0.9	27
500	500[d]	−0.7	74
500	500[e]	−2.5	80
500	100[e]	−1.0	68
500	10[e]	−2.2	64

[a] See Table I definitions. [b] Intraperitoneal tumor implantation. Treatment on day 1 only, 24 h after tumor implantation. [c] Two 250 mg/kg doses on day 1 spaced 30 min apart. [d] One dose administered immediately before *ara*-C treatment. [e] One-half total dose 30 min prior to *ara*-C administration, one-half dose immediately after.

This compound was found to possess moderate activity against P388 leukemia in vivo and to be much more potent than **1b** (Table II).

In Vivo Combination Studies

Acidic conditions have been shown to inactivate THU as a cytidine deaminase inhibitor by converting this nucleoside from a ribofuranose to a ribopyranose.[6] In contrast, zebularine (**1b**) is an acid-stable inhibitor of cytidine deaminase,[6] making it a candidate for oral combination experiments with antitumor drugs which are inactivated by this enzyme. Studies were performed to determine whether **1b** would enhance the oral activity of arabinofuranosylcytosine (*ara*-C) in a mouse tumor model. Although the mouse is an imperfect model of the human situation since it has a somewhat higher stomach pH (2.5)[17] and its liver cytidine deaminase levels are lower,[18] it is possible that the combination of *ara*-C and zebularine could produce a greater increase in life span (ILS) than either drug used alone.

A nonoptimum treatment schedule for *ara*-C (one dose on the day after tumor implantation) was chosen so that any enhancement by **1b** would be obvious. *ara*-C alone produced a 44% ILS against ip L1210 leukemia at an oral dose of 500 mg/kg (Table III). A similar single dose of **1b** alone produced a marginal response (27% ILS). When two 250 mg/kg divided oral doses of zebularine were administered in combination with oral *ara*-C, ILS values increased to 74–80% without significant toxicity. A zebularine dose response can be seen. Oral THU had been shown earlier to be inactive against L1210 leukemia.[19] Our results with oral THU (10 mg/kg) plus 500 mg/kg *ara*-C were similar to those of Neil et al.[19] (ILS of 68 and 43%, respectively), although in our tests increasing the THU in this combination to 50 mg/kg resulted in toxicity. While not definitive, these experiments suggest that orally administered zebularine may increase the response of L1210 leukemia to *ara*-C without significantly increasing toxicity.

Experimental Section

Compound **3** was purchased from Pfanstiehl Laboratories Inc. **5a** was synthesized by Ash-Stevens Inc. (Detroit, MI) under contract to the Pharmaceutical Resources Branch, NCI. Nebularine (**2**) was provided by the Drug Synthesis and Chemistry

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Branch, NCI. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Proton and ^{13}C NMR spectra were obtained on Varian XL-200 and Bruker 250 MHz instruments. Proton chemical shifts are expressed as δ values with reference to Me_4Si. For ^{13}C NMR, the peak positions were determined by reference to dioxane (δ 67.3). Specific rotations were measured in a 4-dm cell with a Perkin-Elmer Model 241 polarimeter. Normal-phase column chromatography was run on silica gel (J. T. Baker 60-200 mesh), and analytical TLC was performed on Analtech Uniplates silica gel GF with the solvents indicated for the individual experiments. Elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN) and Atlantic Microlabs (Norcross, GA).

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one (1a). This compound was prepared on a small scale by the general method of Niedballa and Vorbruggen[9] to give a white powder which was recrystallized from ethanol, mp 152–154 °C (lit.[9] mp 155–158 °C). This synthesis was scaled-up by Ash-Stevens Inc. (Detroit, MI), giving >2 kg (62%) of analytically pure **1a**, mp 165–167 °C. Anal. ($C_{30}H_{24}N_2O_8$) C, H, N.

1-β-D-Ribofuranosyl-1,2-dihydropyrimidin-2-one (1b, 2-(1*H*)-Pyrimidinone Riboside, Zebularine). This compound was prepared on a 1-g scale from **1a** by removal of the benzoyl protecting groups with sodium methoxide (procedure of Pischel[20]). By the same procedure, the compound was prepared from **1a** by Ash-Stevens on a 500-g scale, giving 57% of analytically pure, white material: mp 158–159 °C (lit. mp 150–155 °C,[7] 156.5–157 °C[21]); $[\alpha]_D^{25}$ +160.9° (*c* 0.76, MeOH); ^1H NMR (D_2O) δ 8.44 (dd, J = 2.6 and 4.3 Hz, 1 H, H4), 8.38 (dd, J = 2.6 and 6.8 Hz, 1 H, H6), 6.59 (dd, J = 4.3 and 6.7 Hz, 1 H, H5), 5.72 (d, J = 1.8 Hz, 1 H, H1′), 4.14 (dd, J = 1.8 and 4.4 Hz, 1 H, H2′), 3.94–4.09 (m, 2 H, H3′ and H4′), 3.85 (dd, J = 2.2 and 13.0 Hz, 1 H, H5′), 3.68 (dd, J = 3.7 and 13.0 Hz, 1 H, H5″); ^{13}C NMR (D_2O, 1 drop dioxane) δ 166.61, 156.56, 145.23, 106.33, 92.00, 83.61, 74.51, 68.21, 59.91. Anal. ($C_9H_{12}N_2O_5$) C, H, N.

4-Thio-5-fluorouracil (4b). A mixture of 5-fluorouracil (**4a**, 5.50 g, 42 mmol) P_2S_5 (9.0 g, 40.5 mmol) in 120 mL of *p*-dioxane was heated under reflux with vigorous mechanical stirring for 3 h to provide a brown solution. The solution was filtered and, upon cooling, crystals appeared which were filtered to provide 2.30 g of yellow product. A second crop (3.31 g) was obtained by concentrating the filtrate to give a total yield of 5.61 g (91%), mp 273–274 °C (lit.[22] mp 275 °C).

5-Fluoro-2(1*H*)-pyrimidinone (4c). A solution of **4b** (0.900 g, 6.16 mmol) in 90 mL of 5:4 H_2O–concentrated NH_4OH mixture was heated under reflux with vigorous mechanical stirring. Raney nickel (4.0 g) was added to the mixture. After 1 h the reaction was essentially complete. The hot solution was filtered through Celite and the Celite cake washed with hot water. The washings were combined and evaporated to dryness in vacuo to provide 420 mg (60%) of a yellow solid which was recrystallized from ethyl acetate: mp 168–170 °C (lit.[11] mp 170–171 °C); NMR (DMSO-d_6) δ 8.38 (s).

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-5-fluoropyrimidin-2(1*H*)-one (6a). The general procedure of Holy[10] was used. 5-Fluoro-2-pyrimidinone (**4c**, 0.340 mg, 3 mmol) was dried thoroughly in vacuo. Dry CH_3CN (15 mL) and 4 mL (15.5 mmol) of bis(trimethylsilyl)trifluoroacetamide (BSTFA) were added, and the mixture was stirred at room temperature for 3 h. The excess reagents were evaporated in vacuo to give a yellowish syrup. This syrup was dissolved in 40 mL of dry 1,2-dichloroethane, and acetyl 2,3,5-tri-*O*-benzoylribofuranoside (**3**, 2.00 g, 3.96 mmol) was added followed by a solution of 0.35 mL (1.9 mmol) of freshly distilled $SnCl_4$ in 10 mL of dry 1,2-dichloroethane. The mixture was stirred at room temperature for 18 h under dry conditions. The reaction mixture was shaken with saturated $NaHCO_3$ solution and the milky organic extract filtered through Celite. The filtrate was dried with $MgSO_4$ and evaporated in vacuo to provide a foamy syrup. The syrup was chromatographed on prep TLC plates (Analtech Silica Gel GF, 2000 μm, ca. 300 mg per plate) using 97:3 CH_2Cl_2–MeOH as the elution solvent. The nucleoside (R_f 0.65) was isolated as a dry foam (440 mg, 26%) and used as such in the next reaction.

1-β-D-Ribofuranosyl-5-fluoropyrimidin-2(1*H*)-one (6b). Compound **6a** (200 mg) was treated with methanol saturated with ammonia in a pressure bottle at 8 °C overnight. The solution was evaporated to dryness and the residue separated on a prep TLC plate (Analtech silica gel GF, 2000 μm) using 9:1 CH_2Cl_2–MeOH as the eluting solvent. The free nucleoside (R_f 0.43) was isolated as yellowish crystals by trituration with ether (50 mg, 57%); mp 73–75 °C; λ_{max} (MeOH) 325 nm (lit.[10] λ_{max} 325 nm); $[\alpha]_D^{25}$ −12.5° (*c* 0.72, MeOH); ^1H NMR (D_2O) δ 8.73 (m, 2 H, H4 and H6), 5.87 (s, 1 H, H1′), 3.6–4.4 (m, sugar); ^{13}C NMR (D_2O) δ 157.54 (d, $^2J_{C,F}$ = 23.5 Hz, C6), 154.93, 147.01 (d, $^1J_{C,F}$ = 238.9 Hz, C5), 131.87 (d, $^2J_{C,F}$ = 34.8 Hz, C4), 92.17, 83.69, 74.53, 67.90, 59.60.

1-(2′-Deoxy-2′-fluoro-3′,5′-dibenzoyl-β-D-arabinofuranosyl)pyrimidin-2(1*H*)-one (7a). Compound **5a** (0.5 g, 1.08 mmol) was dissolved in CH_2Cl_2 (10 mL) and treated with 30% HBr/acetic acid solution (0.8 mL). After stirring overnight at room temperature, argon was bubbled through the solution for 10 min. The solvents were evaporated in vacuo. The resulting red-brown syrup was taken up in CH_2Cl_2 and the organic layer was washed first with water, then saturated sodium bicarbonate solution, and brine, dried (Na_2SO_4), and concentrated to give bromo sugar **5b** as a syrup (465 mg, 93%). Meanwhile, 2(1*H*)-pyrimidinone (170 mg, 1.77 mmol) was suspended in dry acetonitrile (10 mL), treated with bis(trimethylsilyl)trifluoroacetamide (1 mL), and stirred at room temperature until the solution was homogeneous (1 h). The solution was concentrated in vacuo to ca. 0.5 mL. The flask was flushed with argon and a solution of the bromo sugar in dichloroethane (10 mL) was added by syringe. The homogeneous solution was refluxed for 18 h. After evaporation of the solvents, the crude residue was immediately purified by flash chromatography (silica). The desired β-nucleoside **7a** eluted with 50% ethyl acetate/petroleum ether as part of an α–β-mixture. Recrystallization from ether gave 307 mg (65%) of a 1:5 α–β-mixture as white flakes: mp 166–168 °C; ^1H NMR ($CDCl_3$) δ 8.63 (dd, J = 2.8 and 4.1 Hz, 1 H, H4), 8.01–8.15 (m, 5 H, H6 and aromatic), 7.4–7.7 (m, 6 H, aromatic), 6.37 (dd, J = 4.1 and 6.8 Hz, 1 H, H5), 6.34 (dd, J = 2.7 and 20.8 Hz, 1 H, H1′), 5.63 (dd, J = 2.5 and 16.4 Hz, 1 H, H3′), 5.59 (dd, J = 2.7 and 49.3 Hz, 1 H, H2′), 4.80 (br d, 2 H, H5′, H5″), 4.61 (br q, 1 H, H4′); ^{13}C NMR ($CDCl_3$) δ 166.65, 154.95, 144.27, 144.23, 134.13, 133.40, 129.95, 129.69, 128.69, 128.50, 103.98, 91.7 (d, $^1J_{C,F}$ = 191.4 Hz, C2′), 87.02 (d, $^2J_{C,F}$ = 17.1 Hz, C3′), 82.35, 63.24. Anal. ($C_{23}H_{19}FN_2O_6$) C, H, N.

1-(2′-Deoxy-2′-fluoro-β-D-arabinofuranosyl)pyrimidin-2(1*H*)-one (7b). Compound **7a** (200 mg, 0.46 mmol) was placed in a pressure bottle with a saturated solution of NH_3/MeOH at 0 °C. After stirring for 1.5 h, argon was bubbled through the solution and the solvent was evaporated. The resulting syrup was dissolved in water and was washed with CH_2Cl_2 (3×). The aqueous layer was concentrated and freeze dried to a white powder. This was purified by flash chromatography on silica (5% MeOH/CH_2Cl_2 eluant), affording 54 mg of the diol **7b** (55%). A small sample was recrystallized from water: mp 86–88 °C, $[\alpha]_D^{25}$ +196.3° (*c* 1.18, MeOH); ^1H NMR (D_2O) δ 8.52 (dd, J = 2.6 and 4.4 Hz, 1 H, H4), 8.27 (dd, J = 2.6 and 6.7 Hz, 1 H, H6), 6.63 (dd, J = 4.5 and 6.7 Hz, 1 H, H5), 6.15 (dd, J = 3.7 and 17.1 Hz, H1′), 5.19 (ddd, J = 2.3, 3.7 and 50.9 Hz, 1 H, H2′), 4.29 (ddd, J = 2.3, 4.2, and 17.4 Hz, 1 H, H3′), 4.05 (m, 1 H, H4′), 3.71 (center of AB set of dd of ABX, 2 H, H5′, H5″); ^{13}C NMR (D_2O) δ 167.07, 156.17, 146.12, 106.20, 94.47 (d, $^1J_{C,F}$ = 190.8 Hz, C2′), 86.48 (d, $^2J_{C,F}$ = 16.7 Hz, C3′), 84.83, 73.51 (d, $^3J_{C,F}$ = 25.6 Hz, C4′), 60.52. Anal. ($C_9H_{11}FN_2O_4 \cdot H_2O$) C, H, N.

Antitumor Evaluation. Antitumor testing was conducted under contract by Southern Research Institute (Birmingham, AL) and Hazleton Laboratories (Vienna, VA) using standard, published NCI protocols.[13] Briefly, tumors were implanted on day 0 using BDF_1 mice for B16 melanoma (0.5 mL of 1:10 tumor brei) and CDF_1 mice for L1210 (10^5 cells) and P388 (10^6 cells) leukemias.

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A QD 1-9 treatment schedule was used. Tumored, untreated control mice died between days 8-15 (B16), 8-11 (L1210), and 9-13 (P388).

Acknowledgment. We thank Drs. Karl Flora and B. Rao Vishnuvajjala, Pharmaceutical Resources Branch, NCI, and Dr. Ven Narayanan and Mrs. Nancita Lomax, Drug Synthesis and Chemistry Branch, NCI, for their cooperation in scheduling the large-scale synthesis of several compounds under their contracts with the Ash-Stevens Corporation (M. S. Khan and R. L. Kalamas) (Detroit, MI) and SISA Corporation (J. Quick) (Cambridge, MA).

Synthesis, Characterization and Myocardial Uptake of Cationic Bis(arene)technetium(I) Complexes

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Received February 26, 1991

A series of bis(arene)technetium(I) complexes has been synthesized from $^{99m}TcO_4^-$ in order to study their organ distribution. Syntheses using either ultrasound/Al/$AlCl_3$ or Zn/HCl gave products relatively free from transalkylation. The identity of the complexes was verified by comparison to the ^{99}Tc complexes. Equivalence of the ^{99}Tc and ^{99m}Tc complexes was demonstrated by HPLC techniques. Biodistribution studies in rats reveal substantial myocardial uptake for many members of the series, especially those containing benzene rings substituted with about four to six carbon atoms. The myocardial uptake is related to the lipophilicity of the complexes as measured by octanol/buffer partition ratios (OBPR). Optimal ranges of lipophilicity for maximal myocardial uptake occur for OBPR from 2 to 9. Rat and human plasma binding of the complexes increases with lipophilicity after a threshold value is exceeded.

Introduction

Since the demonstration that cationic complexes of technetium can show substantial uptake in myocardial tissue,[2] several classes of complexes have been discovered to have such properties. Among the more notable cationic classes are the hexakisisonitriles,[3,4] the hexakis(trimethylphosphite) and related complexes,[5,6] and the 1,2-bis(dimethylphosphino)ethane series.[2,7] For these classes there usually exists a compound which accumulates in the myocardium at a much higher level than closely related complexes. Drawbacks of these classes include high plasma-binding values, especially for the complexes of ligands containing phosphorus, and the lack of a correlation between myocardial uptake in animals and humans. Moreover, the nature of these classes is such that when the ligand is changed slightly, this change is multiplied over the six identical ligands and results in gross changes in physical properties. Thus, solution of the clinical problems through structure–distribution studies is hindered due to the inability to vary subtly the properties of the complexes.

Neutral BATO complexes of technetium also show myocardial uptake.[8,9] In these complexes the substituent on the capping boron atom of the BATO complexes can be varied to give complexes with finely tuned biodistributions. Until recently, a series of cationic complexes with such versatility has not been available.

$Tc(arene)_2^+$ complexes have been known for years.[10-12] The benzene and hexamethylbenzene complexes are stable in aqueous solution as cations. Since only two arene rings are coordinated to the metal, the class is much more amenable to subtle variations in structure than other cationic systems since changing one substituent on the arene ring introduces only two changes in the complex.

This work reports in detail the synthesis, characterization, biodistribution in rats, and plasma binding for a wide range of $Tc(arene)_2^+$ complexes with subtle structural variations. Significant progress has been made in the synthesis of isomerically pure $Tc(arene)_2^+$ complexes in order to gain access to this class of complexes. The structure–distribution relationships reveal that heart up-

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0022-2623/91/1834-3284$02.50/0