Notes

Improved Synthesis and Antitumor Activity of 2-Bromo-2′-deoxyadenosine

Min-chi Huang,† Thomas L. Avery,† Raymond L. Blakley,*,† John A. Secrist III,‡ and John A. Montgomery‡

Division of Biochemical and Clinical Pharmacology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101, and The Southern Research Institute, Birmingham, Alabama 35225. *Received September 26, 1983*

A more convenient synthetic route to 2-bromo-2′-deoxyadenosine (**5**) is reported, and results indicating significant antitumor activity of **5** against three murine tumors (L1210 leukemia, B16 melanoma, and M5076 ovarian carcinoma) are presented. The antitumor activity is very schedule dependent, being much greater when the drug is given q 3 h (×8) every 3rd or 4th day than when given by single daily administration. Toxicity of **5** for the tumor-bearing host is also very schedule dependent. Thus, on the q 3 h schedule of administration, a greater cumulative dose is tolerated by the host, and the therapeutic effectiveness of **5** is enhanced accordingly.

We previously reported[1] the synthesis of a number of analogues of 2′-deoxyadenosine from analogues of adenine by the use of nucleoside deoxyribosyltransferase (EC 2.4.2.6). Of the nucleosides synthesized, the most cytotoxic for cultures of human lymphoblastic cells (CCRF-CEM) were 2-bromo-2′-deoxyadenosine (**5**; NSC 341936) and 2-chloro-2′-deoxyadenosine. The 2-bromo derivative (**5**) is not a substrate for adenosine deaminase[2] and, thus, is resistant to catabolism. 2-Chloro-2′-deoxyadenosine has previously been shown to have significant antitumor activity.[3] In view of the marked cytotoxicity of the 2-bromo derivative, it was desirable to synthesize a larger quantity for investigation of antitumor activity. The major limitation to the synthesis of larger quantities of **5** was the relative inaccessibility of the base, 2-bromoadenine (**4**).

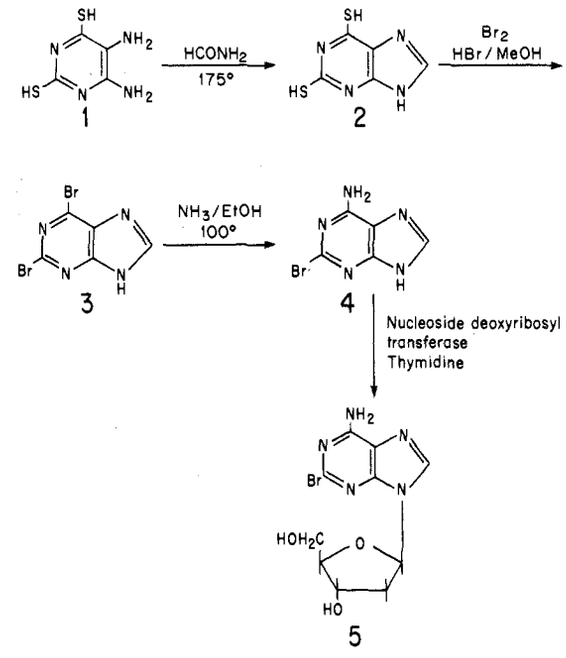
Chemistry. Our initial synthesis of **5** was carried out according to Scheme I. 2,6-Dibromopurine (**3**) was synthesized by the literature procedure[4] from 2,6-dimercaptopurine (**2**), which in turn was formed[5] from 4,5-diamino-2,6-dimercaptopyrimidine (**1**). However, the current unavailability of **4**[6] and the irreproducibility of the **2** to **3** conversion prompted a search for a different synthesis of **4** from guanosine (Scheme II).

Synthesis of **9** from **6** followed the known route,[7,8] and treatment of **9** with ethanolic ammonia at 100 °C in a steel pressure vessel afforded an 81% yield of 2-bromoadenosine (**10**).[9] Hydrolysis of **10** with hydrobromic acid produced **4**, which was converted to **5** enzymatically in the usual manner.[1] The overall yield of **5** from **6** was 29%. A conceivable alternative procedure for the conversion of **10** to **5** is the four-step procedure of Robins and Wilson;[10] however, the synthesis of **5** by this procedure has not been reported, and the method may not be applicable because of possible loss of the 2-bromo group.

Antitumor Activity and Host Toxicity. When **5** was administered once on day 1 (i.e., approximately 24 h after inoculation of animals with L1210 cells) at doses that ranged, by increments of 20 mg, from 30 to 230 mg/kg, **5** did not extend life span beyond that of untreated control mice. On this schedule, no toxic deaths were caused by doses at or below 160 mg/kg. Deaths within 48 h were caused by higher doses as follows: 180 mg/kg, 2/6; 200 mg/kg, 3/6; 230 mg/kg, 12/12.

When **5** was administered in multiple doses, both the therapeutic effect and the host toxicity were schedule dependent. When the drug was administered once daily on days 1, 4 and 7, or each day on days 1 to 7, it produced negligible therapeutic effects (Table I). In agreement with previous observations,[11] however, significant increases in

Scheme I



†St. Jude Children's Research Hospital.
‡Southern Research Institute.

(1) M.-C. Huang, K. Hatfield, A. W. Roetker, J. A. Montgomery, and R. L. Blakley, *Biochem. Pharmacol.*, **30**, 2663 (1981).
(2) L. L. Bennett, Jr., et al., unpublished results.
(3) D. A. Carson, D. B. Wasson, J. Kaye, B. Ullman, D. W. Martin, Jr., R. K. Robins, and J. A. Montgomery, *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 6865 (1980).
(4) A. G. Beaman, J. F. Gerster, and R. K. Robins, *J. Org. Chem.*, **27**, 986 (1962).
(5) A. G. Beaman and R. K. Robins, *J. Am. Chem. Soc.*, **83**, 4038 (1961).
(6) Originally purchased from Pfaltz and Bauer, Inc., Stamford, CT.
(7) M. J. Robins and B. Uznanski, *Can. J. Chem.*, **59**, 2601 (1981).
(8) M. J. Robins and B. Uznanski, *Can. J. Chem.*, **59**, 2608 (1981).
(9) J. A. Montgomery and K. Hewson, *J. Heterocycl. Chem.*, **1**, 213 (1964).
(10) M. J. Robins and J. S. Wilson, *J. Am. Chem. Soc.*, **103**, 932 (1981).
(11) J. A. Montgomery, *Cancer Res.*, **42**, 3911 (1982).

0022-2623/84/1827-0800$01.50/0

Table I. Response of Mice Inoculated with L1210 Leukemia to 2-Bromo-2′-deoxyadenosine (5)

dose,[a] mg/kg	life span,[b] days	ILS, %	LTS[c]
Experiment 1 (Inoculum: 1 × 10⁶ Cells; Drug: qd (× 3), Days 1, 4, 7)			
untreated controls[d]	6.33 ± 0.89		
70	6.50 ± 0.55	3	0/6
90	7.00 ± 0.63	11	0/6
110	8.17 ± 1.83	29	0/6
140	8.00 ± 1.26	26	0/6
180	5.00 ± 3.10	0	0/6
Experiment 1 (Inoculum: 1 × 10⁶ Cells; Drug: qd (× 7), Days 1-7)			
untreated controls	6.33 ± 0.89		
30	7.67 ± 0.82	21	0/6
50	9.00 ± 1.41	42	0/6
70	7.00 ± 1.10	11	0/6
90	4.83 ± 2.48	0	0/6
110	4.17 ± 1.47	0	0/6
Experiment 2 (Inoculum: 1 × 10⁶ Cells; Drug: q 3 h (× 8), Days 1, 4, 7)			
untreated controls	6.33 ± 0.49		
5.6	11.08 ± 0.67	75	0/12
11.3	12.92 ± 0.79	104	0/12
22.5	15.00 ± 1.04	137	0/12
45.0	13.50 ± 5.96	113	0/12
Experiment 3 (Inoculum: 1 × 10⁵ Cells; Drug: q 3 h (× 8), Days 1, 4, 7)			
untreated controls	7.33 ± 0.49		
5.6	11.75 ± 0.97	60	0/12
11.3	14.33 ± 1.15	95	0/12
22.5	23.75 ± 17.05	224	2/12
45.0	37.92 ± 27.33	417	7/12
Experiment 4 (Inoculum: 1 × 10⁵ Cells; Drug: q 3 h (× 8), Days 1, 5, 9)			
untreated controls	7.75 ± 0.62		
5.6	12.36 ± 0.50	59	0/12
17.3	14.00 ± 1.00	81	0/12
22.5	21.42 ± 12.62	176	1/12
45.0	37.73 ± 21.68	387	5/12
Experiment 5 (Inoculum: 1 × 10⁵ Cells; Drug: q 3 h (× 8), Days 1, 4, 7, 10, 13, 16)			
untreated controls	8.81 ± 0.60		
5.6	11.83 ± 1.75	45	0/12
11.3	18.67 ± 13.22	128	1/12
22.5	20.67 ± 4.52	153	0/12
Experiment 6 (Inoculum: 1 × 10⁶ Cells; Drug: q 3 h (× 8), Days 1, 4, 7, 10, 13, 16)			
untreated controls	7.09 ± 0.83		
5.6	10.50 ± 1.38	48	0/12
11.3	13.17 ± 0.94	86	0/12
22.5	16.08 ± 3.32	127	0/12

[a] Dose administered at each injection. [b] Mean plus or minus standard deviation. [c] LTS, long-term survivor, mice surviving 60 days or more after injection with leukemic cells. Life span of these survivors was treated as 60 days in the calculation of ILS. [d] The untreated control groups contained 12 mice in experiments 1, 3, and 4, 18 in experiment 2, and 11 in experiments 5 and 6. Control animals were injected with a comparable volume of 0.154 M NaCl.

life span were obtained when it was administered every 3 h on days 1, 4, and 7 (Table I). The only early, toxicity-related deaths in any of the experiments involving the q 3 h (×8) schedule were those involving mice receiving 45 mg/kg.

It is evident from Table I that the maximum tolerated dose was schedule dependent. The maximum tolerated

Scheme II

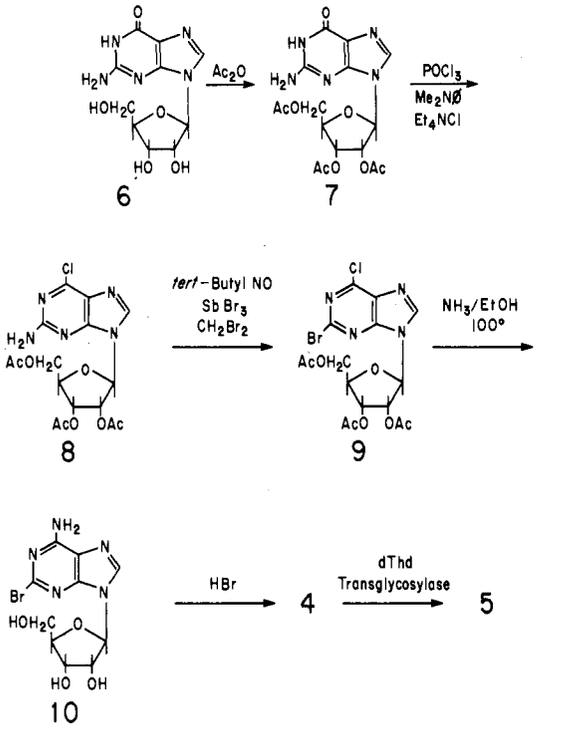


Table II. Treatment with 2-Bromo-9-(2-deoxyribofuranosyl)adenine of Mice Inoculated with M5076 Ovarian Carcinoma or B16 Melanoma[a]

dose,[b] mg/kg	mean life span, days	ILS, %	tumor vol, mm³
Experiment 1 (Tumor: M5076; Schedule: q 3 h (×8), Days 1, 4, 7, 15, 18, 21)			
untreated controls	34.33 ± 4.97		1012 ± 375
22.5	43.33 ± 6.17	26	456 ± 166
Experiment 2 (Tumor: M5076; Schedule: q 3 h (×8), Every 3rd day, days 1-34)			
untreated controls	32.11 ± 3.05		1635 ± 497
5.6	34.44 ± 3.17	7	1152 ± 391
11.3	39.72 ± 2.42	24	784 ± 241
22.5	46.41 ± 5.18[c]	46[c]	298 ± 139[c]
Experiment 4 (Tumor: B16; Schedule: q 3 h (×8), Days 1, 4, 7, 10, 13, 16)			
untreated controls	17.50 ± 4.74		
22.5	19.22 ± 1.70	10	
Experiment 3 (Tumor: B16; Schedule: q 3 h (×8), Days 1, 4, 7, 10)			
untreated controls	13.94 ± 1.21		
11.3	23.11 ± 10.17	66	
22.5	20.89 ± 8.40	50	

[a] Each control and treatment group contained 18 animals, except as indicated. Controls were treated as described in Table I. [b] Dose administered at each injection. [c] Only 11 animals in this treatment group.

cumulative doses were 330 mg/kg on a qd (×3) schedule, 350 mg/kg on a qd (×7) schedule, and 540 mg/kg on a q 3 h (×8) days 1, 4, 7 schedule. Later results (Table II) indicated that doses of 22.5 mg/kg, q 3 h (×8), could be given on every 3rd day untill day 34 without toxic deaths. This corresponds to a cumulative dose of 2160 mg/kg and even more clearly indicates the high tolerance of the host to the drug given in smaller, repeated doses. The therapeutic effect of the drug against L1210 was no greater when given q 3 h (×8) every 3rd day to day 16 than when given

q 3 h (×8) on days 1, 4, and 7 (Table I), and, in fact, the therapeutic effect of the drug was somewhat less on the former schedule, perhaps due to sublethal toxicity to the host or decreased antitumor immunity over the longer period. Nevertheless, the therapeutic and toxicity data together indicate that a rapid removal of the active form of the drug must occur.

Results of the treatment of mice inoculated with M5076 ovarian carcinoma or B16 melanoma are shown in Table II. M5076 has for many years been designated as an ovarian carcinoma, since it arose spontaneously in the ovary of C57B1/6 mouse. More recently it has been suggested that it is a reticulum cell sarcoma that appears to have originated from histiocytic macrophages.[12] Depending on its stage of growth, M5076 exhibits a doubling time of 3–7 days[13] and metastasizes preferentially to the peritoneal viscera, liver, ovaries, spleen, and kidney.[14] When tested for responsiveness to **5**, the optimal dosage and schedule gave significant ILS and 82% inhibition of tumor volume, with proportionally smaller responses to lower doses (Table II). Prolonging the treatment schedule (injections every 3rd day) to the 34th day resulted in considerably better responses than treatment for shorter periods. The response of mice with B16 melanoma was about 66% ILS with a dose of 11.3 mg/kg, q 3 h (×8), days 1, 4, 7, 10, 13, 16, but double this dose gave no further increase in life span.

Experimental Section

Physical properties were determined by the following instruments: Fischer digital melting point analyzer, Model 355; UV spectra, Cary 219 spectrophotometer. Elemental analyses were performed by Midwest Microlab, Indianapolis, IN. Where analyses are indicated by only symbols of the elements, the analytical results for those elements were within 0.4% of the theoretical value. A Parr stirred pressure reactor, Bench Model 4521 (capacity 2000 mL) was used for the ammonolysis reaction. A Beckman-Altex HPLC Model 332 fitted with a 254-nm UV detector and a Waters μBondapak C_{18} semipreparative column (7.8 mm × 30 cm) or analytical column (3.9 mm × 30 cm) was used to purify compounds, monitor the progress of reactions, or check the purity of compounds. Elution was performed at a flow rate of 2 mL/min for the semipreparative column or at 1 mL/min for the analytical column, with an increase from 0 to 24% methanol over 10 min, maintenance of 24% methanol for 15 min, followed by an increase from 24 to 80% methanol over 10 min and maintenance of 80% methanol for 5 min.

2-Bromoadenosine (10). A solution of **9** (15.0 g, 0.306 mol) in 100% ethanol (750 mL) was saturated with anhydrous ammonia at 0 °C and then heated in a stirred pressure reactor at 100 °C. The reaction was complete in 5 h, and the reactor was then allowed to cool overnight. After the reaction solution had been evaporated to dryness in vacuo, the oily residue was dissolved in hot 95% ethanol (60 mL). After cooling, the solution was stored overnight at 4 °C. The crystalline precipitate was filtered, washed twice with chloroform (50 mL), and dried in vacuo to give the first crop of **10**. Concentration of the mother liquor to about 30 mL and treatment as before gave a second crop: total yield 8.6 g (81%). The material was 94–99% pure as determined by analytical HPLC. The major byproduct, which was identified as 2,6-diaminopurine riboside by HPLC, was difficult to remove by recrystallization from 95% ethanol, but after the next step in Scheme II, 2,6-diaminopurine could be easily removed.

Analytically pure (HPLC) **10** was obtained by HPLC on the semipreparative column. Evaporation of homogeneous fractions gave white crystals: mp indefinite; UV λ_{max} (pH 1) 267 nm (ϵ 14200) [lit.:[9] 266 nm (ϵ 14300)]; UV λ_{max} (pH 13) 265 nm (ϵ 15600) [lit.[9] 265 nm (ϵ 14900)].

2-Bromoadenine (4). A solution of pure **10** (0.1 g, 0.288 mmol) in 0.1 N HBr (10 mL) was heated under reflux. A crystalline precipitate gradually appeared during heating. After 2.5 h, the reaction mixture was allowed to cool, neutralized with 1 N NaOH, and stored at 4 °C overnight. The precipitate was filtered, washed with water (10 mL), and dried in vacuo to give 58 mg of **4** (94%). Analytic HPLC showed a single peak. When partially purified **10** (1.8 g, 5.2 mmol) was used for hydrolysis, it gave 1 g of **4** (89%). Analytic HPLC showed that this product was also homogeneous.

Therapeutic Evaluations. BDF_1, DBA/2, and C57B1/6 female mice weighing about 18 g were obtained from the Jackson Laboratories in Bar Harbor, ME. The mice were housed in a central animal facility that provided controlled temperature and humidity, free access to food and water, and defined periods of light. L1210 leukemia was serially propagated by the passage at weekly intervals of 1×10^5 cells into the peritoneal compartments of DBA/2 mice. Both the B16 and the M5076 tumors were propagated by serial passage in C57B1/6 mice. For B16, tumor fragments about 20 mg in weight were implanted subcutaneously in the flank at intervals of 10–14 days, and M5076 was passaged similarly at intervals of 2–3 weeks. Therapeutic evaluations were performed exclusively in BDF mice. L1210 recipients were inoculated intraperitoneally with 1×10^5 or 1×10^6 leukemia cells, while M5076 hosts received subcutaneous implants similar to those used for serial passage, and B16 hosts were inoculated intraperitoneally with 0.5 mL of tumor brei prepared by homogenizing 1 g of tumor with 10 mL of cold balanced salt solution. The mice were housed in groups of six with a maximum intercage variation in weight of 2 g. Drug was prepared immediately before use and was injected at the rate of 0.01 mL/g of mouse weight. During and after treatment, mice were observed for morbidity or death. Death was attributed to tumor on the basis of specific necropsy findings, such as organomegaly, solid tumor masses in the pancreas and mesentery, peritoneal or thoracic fluid containing neoplastic cells, and the absence of manifestation of toxicity. Toxicity was monitored on the basis of generalized morbidity, patterns of weight change, examinations of lungs and abdominal viscera, and patterns of death. When mice without evidence of tumor or organomegaly died earlier than animals treated with lower dosages of drug, prior to the deaths of control mice, or two standard deviations earlier than treatment mates, their deaths were attributed to acute drug toxicity. For studies with L1210 and B16, evaluations of therapeutic effectiveness were based solely on duration of survival. This end point reflects the dose–response relationship for both the tumor and the host. For studies with M5076, drug effects on tumor size as well as on duration of survival were assessed. Mean values and standard deviations were calculated for survival times and for tumor size, and statistical inference was assessed by the "t" test.

Acknowledgment. This work was supported by grants from the American Lebanese Syrian Associated Charities, USPHS CORE Center Grant P30-CA 21765, and Grant P01-CA 34200 both from the National Cancer Institute, National Institutes of Health. We thank R. Finch, H. Pasha, and H. Clariette for assistance in performing the determination of antitumor activity and Vicki Gray for typing the manuscript.

Registry No. **4**, 28128-25-8; **5**, 89178-21-2; **9**, 40896-58-0; **10**, 146-76-9.

(12) J. E. Talmage, M. E. Key, and I. R. Hart, *Cancer Res.*, 41, 1271 (1981).
(13) L. Simpson-Herren, D. P. Griswold, and D. J. Dykes, *Proc. Am. Assoc. Cancer Res.*, **20**, 80 (1979).
(14) I. R. Hart, J. E. Talmadge, and I. J. Fidler, *Cancer Res.*, 41, 1281 (1981).