integrated One-Way ANOVA data-analysis program. Those values achieving a $p < 0.05$ vs the control were deemed significant.

In Vivo Cardiac Arrhythmias.[34] Adult male mongrel dogs weighing 11–20 kg were anesthetized (sodium pentobarbital, 30 mg/kg iv) and a thoracotomy was performed under aspetic conditions at the left fourth interspace. A two-stage occlusion (90 min) of the left anterior descending coronary artery (LAD) and subsequent reperfusion through a critical stenosis produced a myocardial infarction. The animals were then closely monitored during recovery from anesthesia and returned to their kennel for complete surgical recovery.

The animals where then studied 3–9 days after the myocardial infarction. On the day of study, the animals were reanesthetized with pentobarbital and placed on a respirator, and a thoracotomy was performed to insert electrodes for cardiac stimulation and recording of cardiac conduction. Arterial blood gases were monitored during the experiment and respiration adjusted to maintain physiologic conditions. Isolation of the right carotid artery allowed for placement of a quadripolar HIS-bundle catheter. The heart was exposed and suspended in a pericardial cradle. An electromagnetic flow probe (Carolina Medical Electronics, Inc.) measured coronary artery blood flow through the LAD to establish patency of the vessel.

A quadripolar electrode was sewn onto the left atrial appendage to control the heart rate by atrial pacing and to record an atrial electrogram. A plunge bipolar electrode was sewn on the anterior ventricular wall at the junction of the left and right ventricle, i.e., interventricular septum, for introducing premature ventricular stimuli (4-ms duration; 2 × diastolic threshold square-wave pulses).

A preset control module (W-P Instruments, Inc., Model 842) triggered the ventricular stimulus from the R wave of the lead II EKG or from the normal zone electrogram. A Grass S8 stimulator and SIU-478 stimulus isolation unit were used for pacing during PES. The lead II EKG, arterial blood pressure, composite electrograms, atrial electrogram, and HIS-bundle electrogram were displayed on a multichannel oscillographic recorder (Model VR-12, Electronics for Medicine) and recorded on photographic paper.

Programmed electrical stimulation involved the introduction of one (S_2), two (S_2S_3), and three ($S_2S_3S_4$) premature ventricular stimuli to the right outflow tract during normal sinus rhythm or atrial pacing (180–220 bpm, 4-ms duration, 2 × threshold). In this manner, the effective refractory period (ERP) of normal myocardium was obtained and ventricular arrhythmias were induced. In this study, nonsustained tachycardia (NSVT) involved the production of at least three spontaneous ventricular beats in response to premature ventricular stimuli. Ventricular tachycardia lasting for at least 30 s was considered sustained (VT) and usually required an intervention to convert the animal to normal sinus rhythm. Animals that failed to display at least 2 "runs" of ventricular tachycardia at baseline were considered noninducible (NI).

Baseline electrophysiologic values (at a paced rate of 180–220 bpm), atrial and ventricular ERPs, and reproducible inducibility were determined as control data. Then, the dogs received cumulative intravenous doses of drug. Compounds **2** and **25** were tested as the (*E*)-2-butenedionate (2:1) salts which were dissolved in glass-distilled water (pH 6.7 and 6.1, respectively). Compound **20** (sotalol hydrochloride) was dissolved in saline and neutralized with NaOH (pH 6.7–7.0). Control experiments showed that these vehicles had no electrophysiologic activity (data not shown).

Statistical Analysis. A general linear models procedure was used to determine a two-way analysis of variance for data comparison. In this study, significance is defined as $p < 0.05$. All data are expressed as mean ± standard error of the mean.

Acknowledgment. We are indebted to Dr. D. J. Duchamp and his associates for physical and analytical data, to Dr. M. W. McMillan, Dr. M. A. Lyster, Dr. K. W. Fields, D. J. Houser, B. G. Conway, and R. L. Johnson for the preparation of chemical intermediates, and to D. M. Squires for technical assistance.

Registry No. 1, 100632-57-3; 2, 100632-81-3; **2**·$^1/_2$fumarate, 130350-52-6; 3, 100632-58-4; 4, 100632-59-5; **5**·HCl, 130350-53-7; 6, 3984-34-7; 7, 130350-54-8; 8, 130350-55-9; **8**·$^3/_4$fumarate, 130350-56-0; 9, 100633-01-0; 10, 6328-00-3; 11, 130350-57-1; 12, 130350-58-2; 13, 130377-67-2; 14, 100632-78-8; 15, 5317-89-5; 17, 100632-63-1; 18, 5577-42-4; **19**·HCl, 130350-59-3; 20, 959-24-0; 21, 68379-03-3; 22, 130350-60-6; **23**·$^1/_2$fumarate, 130350-62-8; 24, 100632-86-8; **25**·$^1/_2$fumarate, 130377-68-3; 26, 100632-84-6; 27, 130350-63-9; 28, 100632-82-4; **29**·$^1/_2$fumarate, 130350-65-1; 30, 130350-66-2; 31, 130350-67-3; 32, 130350-68-4; 33, 130350-69-5; 34, 130350-70-8; 35, 100632-83-5; 39, 130350-71-9; 40, 130350-72-0; isobutyl chloroformate, 543-27-1; 4-methylpiperidine, 626-58-4; heptamethyleneimine, 1121-92-2; 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide hydrochloride, 7084-11-9; potassium sodium tartrate, 304-59-6; ethylheptylamine, 66793-76-8; methanesulfonyl chloride, 124-63-0; methanesulfonanilide, 1197-22-4; succinic anhydride, 108-30-5.

Boron-Containing Thiouracil Derivatives for Neutron-Capture Therapy of Melanoma

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Boron-containing derivatives of 2-thiouracil and 2,4-dithiouracil and the corresponding 6-propyl compounds, containing a dihydroxyboryl group in the 5-position, have been prepared. These compounds accumulate in B16 melanoma in mice in concentrations up to 30 μg of boron per gram tissue. The uptake persists. The toxicity of both 2-thiouracil derivatives is low. These compounds are therefore good candidates for boron neutron-capture therapy of malignant melanoma.

Melanomas are tumors that derive from melanin-forming cells. They therefore are different in their metabolism from almost all other cells of the body due to their greatly enhanced synthesis of melanin. This metabolic difference can be used to selectively deliver substances to the tumor cells.

Cyclic thioureas, and especially 2-thiouracils, are known as specific melanoma seekers.[1,2] They are bound covalently to the newly formed melanin polymer via the sulfur. Their accumulation in melanomas therefore is both selective and persistent.

Neutron-capture therapy utilizes the property of the boron-10 nucleus to capture thermal (i.e. slow) neutrons and then undergo nuclear disintegration. The nuclear fragments (a ^{4}He and a ^{7}Li nucleus) are able to selectively

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(1) Dencker, L.; Larsson, B.; Olander, K.; Ullberg, S.; Yokota, M. *Br. J. Cancer* **1979**, *39*, 449.
(2) Fairchild, R. G.; Packer, S.; Greenberg, D.; Som, P.; Brill, A. B.; Fand, I.; McNally, W. P. *Cancer Res.* **1982**, *42*, 5126.

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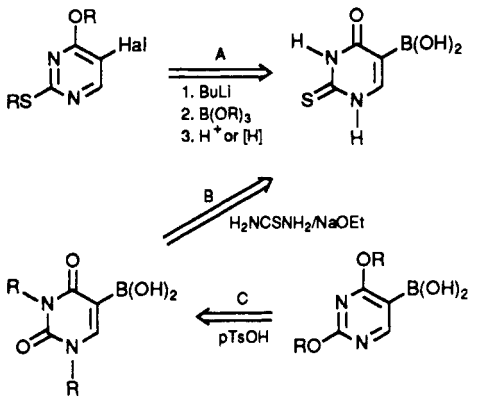
Scheme I



and effectively kill those cells in which the capture reaction takes place.[3] In order to be utilized successfully in cancer therapy, it is necessary that boron accumulates selectively in tumor cells. Boron-containing thiouracils have been proposed for neutron-capture therapy.[2] *nido*-Carborate containing thioureas have recently been described by us.[4]

We wish to report here the synthesis of 5-(dihydroxyboryl)-substituted 2-thio- and 2,4-dithiouracils. These substances show a very selective accumulation in mouse melanomas. The compounds prepared are of low toxicity.

Chemistry

The synthesis of 2-thiouracils that contain a dihydroxyboryl group in the 5-position can be achieved via two different paths (Scheme I). One possibility would be to introduce the dihydroxyboryl group into a 2-thiouracil derivative (Scheme I, path A). Alternatively, the 2-thio function can be added to a uracil derivative after the attachment of the dihydroxyboryl group (path B). In both cases, the dihydroxyboryl compound would be obtained from a reaction of the corresponding 5-halo compound with butyl lithium[5] and subsequent reaction with butyl borate.[6,7]

The chemistry following path A would be possible when the amide functions have been suitably protected. Schinazi and Prusoff[7] had used a trimethylsilyl group to introduce a dihydroxyboryl substituent into 5'-bromo-2-deoxyuridine. For path B, the base-catalyzed exchange of a 1,3-dialkylurea group of a uracil derivative with thiourea could be feasible.[8]

We were only successful with path A, and also this reaction did not proceed completely without problems. Thus, in our hands, the reaction analogous to that of Schinazi and Prusoff,[7] using *O,S*-bis(trimethylsilyl)-protected 5-iodo-2-thiouracil [Scheme I, path A, R = $(CH_3)_3Si$] did not result in the desired product, but instead led in 63% yield to 2-(*n*-butylthio)-5-(trimethylsilyl)uracil.[9] Similar rearrangements with trimethylsilyl-protected heterocycles have been observed by Bailey and Taylor[10] and Bassindale and Walton.[11]

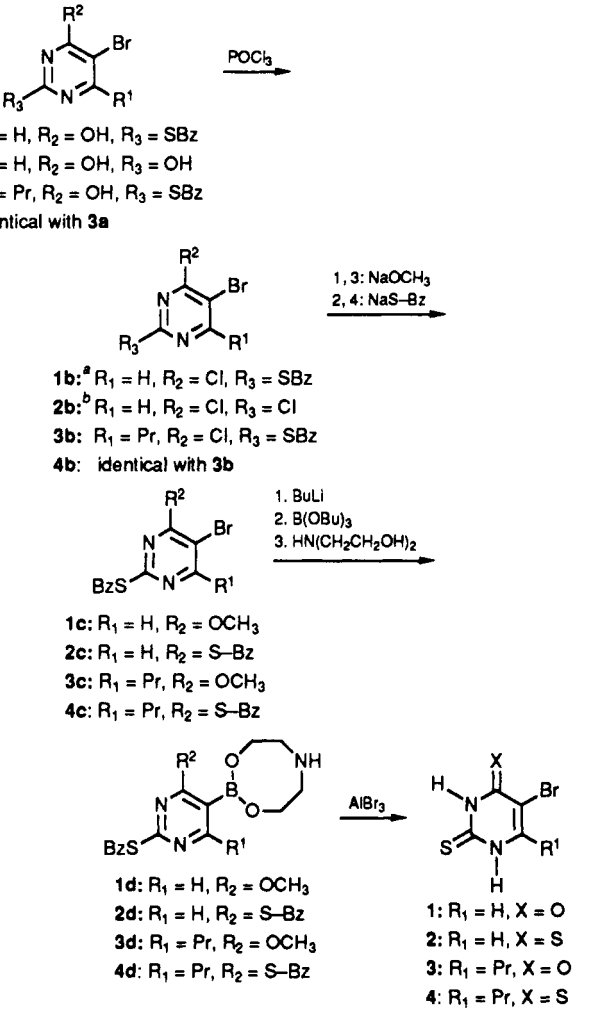
S- and *O*-alkyl groups could be successfully employed as protecting groups for the introduction of the dihydroxyboryl group. The general route to the substances is shown in Scheme II. For sulfur, the benzyl group was chosen, and for oxygen, the methyl group was chosen. Deprotection of *O*-benzyl dihydroxyboryl derivatives of uracil had been achieved previously by catalytic hydrogenation.[6,7] In the case of the sulfur-containing derivatives, equimolar amounts of the catalyst would have to be used. We found that the cleavage of the *S*-benzyl group could be effected by $AlBr_3$ in toluene[12] without concomitant loss of the dihydroxyboryl group. We found that under these conditions also the *O*-methyl group was removed.

The synthesis (Scheme I) thus started with the S-benzylated thiouracils (for **1** and **3**). The 4-carbonyl group was converted to the chlorides in **1b** and **3b**, and subsequent reaction with sodium methylate yielded **1c** and **3c**.

For the corresponding compounds **2** and **4**, the thiobenzyl group was introduced by analogy with the step from **1b** to **1c**.

The boronation was found to be critically dependent on the temperature of the reaction. Only between −100 and

Scheme II



[a] Reference 19. [b] Reference 20.

(3) Gabel, D.; Fairchild, R. G.; Larsson, B.; Börner, H. *Radiat. Res.* **1984**, *98*, 307.
(4) Ketz, H.; Tjarks, W.; Gabel, D. *Tetrahedron Lett.* **1990**, *31*, 4003.
(5) Langley, B. W. *J. Am. Chem. Soc.* **1956**, *78*, 2136.
(6) Liao, T. K.; Prodrebarac, E. G.; Cheng, C. C. *J. Am. Chem. Soc.* **1964**, *86*, 1869.
(7) Schinazi, R. F.; Prusoff, W. H. *J. Org. Chem.* **1985**, *50*, 841.
(8) Hirota, K., Watanabe, K. A., Fox, J. J. *J. Org. Chem.* **1978**, *43*, 1193.
(9) Because of the unsuccessful synthetic attempts, the resulting unwanted compounds and their precursors were not submitted for elemental analysis.
(10) Bailey, F. P.; Taylor, R. J. *J. Chem. Soc. (B)* **1971**, 1446.
(11) Bassindale, A. R.; Walton, D. R. M. *J. Organomet. Chem.* **1970**, *25*, 389.
(12) Harnish, D. P.; Tarbell, D. S. *J. Am. Chem. Soc.* **1948**, *70*, 4123. Lancilotti, A. E.; Ziegler, J. B.; Shabica, A. C. *J. Am. Chem. Soc.* **1954**, *76*, 3666.

Table I

no.	amount admin, μg/g body wt (μg boron/g body wt)	time after admin, h	boron concn, μg/g tumor	boron concn, μg/g blood	ratio tumor/blood[d]
1[a]	300 (15.6)	4	32 ± 8.3	8.0 ± 7.1	4
		13	15.3 ± 9.0	0.5 ± 0.4	31
2[b]	200 (9.5)	4	30, 28	4, 4	7
3[c]	250 (14.0)	3.5	1.7	<0.1	>15

[a] Mean ± SD of four animals. [b] Two animals. [c] One animal. [d] Calculated from the mean values.

-85 °C could satisfactory yields be obtained.

The use of diethanolamine (originally described by Schinazi and Prusoff[7]) greatly facilitated the isolation of the S- and O-protected dihydroxyboryl derivatives, as they could be precipitated from ethyl ether.

The final purification of the compounds after removal of the protecting groups was achieved with charcoal. This was necessary because of the presence of minor amounts of impurities. This procedure reduced the yields considerably, compared to that of the raw product.

Mass spectrometric analysis of the compounds could only be achieved with fast atom bombardment ionization and a non-hydroxyl-containing solvent.

The introduction of sulfur into 1,3-dialkyluracil derivatives via a base-catalyzed exchange of the 1,3-dialkylurea with thiourea[8] (Scheme I, path B) proved unsuccessful. Thus, the reaction of 1,3-dimethyl-5-(dihydroxyboryl)uracil (prepared from the 5-bromo compound as described below) with thiourea to the corresponding thiouracil led to 2-thiouracil in 74% yield.

1,3-Dialkyluracils can be obtained via acid-catalyzed rearrangement of 2,4-dialkyluracils. We attempted the rearrangement of 2,4-dibenzyl-5-(dihydroxyboryl)uracil[7] to 1,3-dibenzyl-5-(dihydroxyboryl)uracil with catalytic amounts of *p*-toluenesulfonic acid according to the method of Kato et al.[13]

The only compounds that could be isolated (as judged by ^{1}H NMR) appeared to be 3-benzyluracil (13% yield) and 1,5-dibenzyluracil (16% yield). No boron-containing compound besides boric acid was present, as judged by neutron-capture radiography[14] of the thin-layer chromatograms. Rearrangement of the benzyl group does not appear to be possible, probably due to steric hindrance, under conditions that had been used successfully by others[13] for methyl, ethyl, allyl, and 2-butenyl groups. Attempts to synthesize the desired 1,3-alkyl compound by acid-catalyzed rearrangements of one of the latter groups were not carried out, as hydrolysis of the dihydroxyboryl group during the rearrangement and/or exchange reactions appeared inevitable.

The final products were stable ($t_{1/2}$ > 8 h) in water and in the buffers used for injection. This is in contrast to the reported instability of the dihydroxyboryl group in other heterocyclic compounds.[15,16]

Biodistribution

Tumor accumulation of the compounds was studied in B16 melanomas in female C57/bl mice. As can be seen from the results in Table I, selective accumulation in melanoma could be found. Preliminary experiments indicated that oral administration also led to a high degree of tumor accumulation. The concentrations found in the tumor are sufficient for a successful neutron-capture therapy.[17] The selectivity of tumor uptake for all compounds is high. 5-(Dihydroxyboryl)-2,4-dithiouracil (2) and its 6-propyl analogue 4 showed, however, a greatly reduced absolute level of uptake. In addition, the two latter compounds appeared to be more toxic; of 4, only around 50 μg of compound per gram of body weight could be administered before adverse reactions (lethargy, cramps) were observed. Therefore, no detailed biodistribution study was attempted with this compound.

Both 2-thiouracil derivatives were well-tolerated. This behavior was expected from the known tolerance to thiourea derivatives used in long-term treatment of hyperthyroidism. A detailed distribution study of these compounds in different human and murine tumor models is in progress (D. Gabel, W. Tjarks, B. Allen, J. A. Coderre, to be published).

It should be noted that boric acid, a possible degradation product of the compounds used here, distributes differently in the same models with little if any accumulation in tumor over blood and with a rather short half-life in the body (R. G. Fairchild, unpublished observations).

In addition to thiouracils, *p*-(dihydroxyboryl)phenylalanine has been discussed as a tumor seeker for melanomas.[18] This compound shows a transient but large accumulation of boron in the tumor, but does not allow accumulation for similarly long periods as the thiouracils. It has been proposed[18] that this compound is taken up through the transport system for tyrosine, but not metabolized further. In contrast, the thioureas are incorporated covalently into the melanin. For the treatment of melanoma by neutron-capture therapy it is therefore possible to use both classes of compounds in combination and thereby accumulate boron by two independent mechanisms.

Experimental Section

Proton and carbon-13 NMR spectra were recorded on a Bruker WH 360. Chemical shifts were reported in ppm downfield from an internal tetramethylsilane standard. IR spectra were recorded on an FTIR Nicolet DX. Melting points were determined on a Büchi 512 apparatus and are reported uncorrected. Mass spectra were recorded with Varian MAT instruments CH7A or 8222. Ionization was through electron impact (EI) or fast atom bombardment with Xe (FAB). Elemental analyses were performed by Mikroanalytisches Labor Beller, Göttingen, FRG. Substances were identified as indicated by ^{1}H and ^{13}C NMR spectroscopy, by mass spectroscopy, and by elemental analysis. IR spectra were in accordance with the proposed structures. Petroleum ether had a boiling range of 58–62 °C.

Diethanolamine Derivative of 2-(Benzylthio)-5-(dihydroxyboryl)-4-methoxypyrimidine (1d). 2-(Benzylthio)-5-bromouracil[19] (1a) was reacted[20] with $POCl_3$ for 2 h to give 4-

(13) Kato, T.; Oda, N.; Ito, I. *Chem. Pharm. Bull.* **1977**, *25*, 215.
(14) Gabel, D.; Holstein, H.; Larsson, B.; Gille, L.; Ericson, G.; Sacker, D.; Som, P.; Fairchild, R. G. *Cancer Res.* **1987**, *47*, 5451.
(15) Yamamoto, Y.; Seko, T.; Nemoto, H. *J. Org. Chem.* **1989**, *54*, 4734.
(16) Yamamoto, Y.; Seko, T.; FengGoang, R.; Nemoto, H. *Tetrahedron Lett.* **1989**, *30*, 7191.
(17) Fairchild, R. G.; Bond, V. P. *Int. J. Radiat. Oncol. Biol. Phys.* **1985**, *11*, 831.
(18) Coderre, J. A.; Glass, J. D.; Fairchild, R. G.; Roy, U.; Cohen, S.; Fand, I. *Cancer Res.* **1987**, *47*, 6377.

chloropyrimidine **1b**: yield 59%; purification by Kugelrohr distillation, bp 170 °C (0.03 mm); mp 56–57 °C; MS (EI) 314 (M^+); ^1H NMR ($CDCl_3$) 4.31 (s, 2 H, SCH_2), 7.12–7.55 (m, 5 H, ArH) 8.60 (d, 1 H, C6H). Anal. ($C_{11}H_8N_2SClBr$) C, H, N, S.

To a suspension of 3.25 g (60 mmol) of sodium methylate in 100 mL of dry toluene was added 17 g (54 mmol) of **1b** in 50 mL of toluene under cooling and stirring such that the temperature did not exceed 25 °C. Stirring was continued overnight. The residue was filtered off, and the toluene was evaporated. The resulting 2-(benzylthio)-5-bromo-4-methoxypyrimidine (**1c**) was purified by Kugelrohr distillation: yield 88%; bp 170 °C (0.03 mm); mp 48–49 °C. MS (EI) 310 (M^+); ^1H NMR ($CDCl_3$) 4.00 (s, 3 H, OCH_3), 4.38 (s, 2 H, SCH_2) 7.19–7.53 (m, 5 H, ArH), 8.40 (s, 1 H, C6H). Anal. ($C_{12}H_{11}N_2SOBr$) C, H, N, S.

1c (5.0 g, 16 mmol) was dissolved in 150 mL of dry THF and cooled to −100 °C under a nitrogen atmosphere. Over the course of 10 min, 11 mL (17.5 mmol) of a 1.6 M solution of *n*-butyllithium in hexane, cooled to −80 °C, was injected through a septum. After stirring for an additional 10 min, 5 mL (18.5 mmol) of tributyl borate (95% ^{10}B-enriched or natural isotope abundance), cooled to −80 °C, was injected. The reaction mixture was allowed to warm to room temperature over a period of 1.5 h and concentrated under reduced pressure. The residue was dissolved in diethyl ether and washed with aqueous 0.01 M HCl. The ether phase was dried with magnesium sulfate and concentrated under reduced pressure to 40 mL. A saturated solution of diethanolamine in diethyl ether was added, and the mixture was left overnight in a refrigerator. The crystals formed were filtered off and dissolved in a small amount of ethanol, and petroleum ether was added until the solution became turbid. It was left in the refrigerator; the precipitate was filtered off and dried at 100 °C: yield 63%; white crystals; mp 185–186 °C; ^1H NMR ($CDCl_3$) δ 2.69–2.80 (m, 2 H, NCH_2), 3.27–3.40 (m, 2 H, NCH_2), 3.75–3.85 (m, 2 H, OCH_2), 3.88 (s, 3 H, OCH_3), 3.9–4.0 (m, 2 H, OCH_2), 4.39 (s, 2 H, SCH_2), 5.65 (s, br, 1 H, NH), 7.18–7.47 (m, 5 H, ArH), 8.33 (m, 1 H, C6H); MS (EI) 345 (M^+, ^{11}B), 232 ([M − $C_4H_8NO_2{}^{11}B$ + H]$^+$), 199 ([232 − HS]$^+$). Anal. ($C_{16}H_{20}N_3O_3SB$) (95% ^{10}B, 5% ^{11}B) C, H, N, S. B.

2-(Benzylthio)-5-bromo-4-methoxy-6-propylpyrimidine (3c) was prepared from 2-(benzylthio)-6-propyluracil[21] by analogy with the 6*H*-uracil[19] via 2-(benzylthio)-5-bromo-6-propyluracil (**3a**) [yield 54%; white crystals; mp 137 °C; MS (EI) 338 (M^+); ^1H NMR (DMSO-d_6) 0.7–0.98 (tt, 3 H, CCH_3), 1.39–1.91 (qt, 2 H, CH_2), 2.53–2.74 (qt, 2 H, $C6CH_2$), 4.38 (s, 2 H, SCH_2, 7.10–7.58 (m, 5 H, ArH). Anal. ($C_{14}H_{15}N_2OSBr$) C, H, N, S] to give 2-(benzylthio)-5-bromo-4-chloro-6-propyluracil (**3b**) (according to the method of Hilbert and Jansen,[20] with a reduced reaction time of 2 h) [yield 94%; colorless high-boiling liquid, purified by column chromatography on silica gel in dichloromethane/petroleum ether 7:3; MS (EI) 356 (M^+); ^1H NMR ($CDCl_3$) 0.86–1.10 (tt, 3 H, CCH_3, 1.44–2.05 (qt, 2 H, CH_2), 2.73–3.00 (qt, 2 H, $C6CH_2$), 4.39 (s, 2 H, SCH_2) 7.14–7.55 (m, 5 H, ArH). Anal. ($C_{14}H_{15}N_2OSClBr$) C, H, N, S].

3b was reacted to 2-(benzylthio)-5-bromo-4-methoxy-6-propylpyrimidine (**3c**) in a manner analogous to that for **1c** [yield 69%; purification by Kugelrohr distillation; bp 173 °C (0.02 mm); MS (EI) 352 (M^+); ^1H NMR ($CDCl_3$) δ 0.84–1.09 (tt, 3 H, CCH_3), 1.41–2.02 (q, 2 H, CH_2), 2.67–2.9 (q, 2 H, C_6CH_2), 4.02 (s, 3 H, OCH_3), 4.41 (s, 3 H, SCH_2), 7.17–7.57 (m, 5 H, ArH). Anal. ($C_{15}H_{17}N_2OSBr$) C, H, N, S.

2,4-Bis(benzylthio)-5-bromopyrimidine (2c) was prepared from 5-bromo-2,4-dichloropyrimidine (**2b**)[20] and a 2-fold excess of sodium benzylthiolate[22] in toluene as described above for **1c**. After filtration the residual liquid was evaporated and purified by Kugelrohr distillation: yield 66%; white crystals; bp 200 °C (0.03 mm); mp 66–67 °C; MS (EI) 402 (M^+); ^1H NMR ($CDCl_3$) δ 4.28 (s, 4 H, 2 SCH_2), 7.12–7.43 (m, 10 H, ArH), 8.19 (s, 1 H, C6H). Anal. ($C_{18}H_{15}N_2S_2Br$) C, H, N, S.

2,4-Bis(benzylthio)-5-bromo-6-propylpyrimidine (4c) was prepared from **3b**: **3b** was reacted with equimolar amounts of sodium benzylthiolate in toluene, as described for **2c** to give 2,4-bis(benzylthio)-5-bromo-6-propyluracil: yield 79%; liquid; bp 230 °C (0.05 mm); purification by column chromatography in dichloromethane/petroleum ether 8:2; MS (EI) 444 (M^+); ^1H NMR ($CDCl_3$) 0.8–1.03 (tt, 3 H, CH_3), 1.37–1.80 (qt, 2 H, CH_2), 2.57–2.80 (qt, 2 H, $C6CH_2$), 4.22, (s, 2 H, $C4SCH_2$), 4.27 (s, 2 H, $C2SCH_2$), 6.95–7.40 (m, 10 H, ArH). Anal. ($C_{21}H_{21}N_2S_2Br$) C, H, N, S.

Diethanolamine Derivatives of 2,4-Bis(benzylthio)-5-(dihydroxyboryl)pyrimidine (2d), 2-(Benzylthio)-5-(dihydroxyboryl)-4-methoxy-6-propylpyrimidine (3d), and 2,4-Bis(benzylthio)-5-(dihydroxyboryl)-6-propylpyrimidine (4d). These compounds were prepared by analogy with the method of **1d**.

2d: yield 56%; white crystals; mp 157–158 °C; MS (EI) 437 (M^+, ^{10}B); ^1H NMR ($CDCl_3$) δ 2.69–2.77 (m, 2 H, NCH_2), 3.34–3.42 (m, 2 H, NCH_2), 3.86–4.00 (m, 4 H, OCH_2), 4.34 (s, $C4SCH_2$), 4.40 (s, 2 H, $C2SCH_2$), 5.25–5.32 (t, br, 1 H, NH), 7.19–7.41 (m, 10 H, ArH), 8.33 (s, 1 H, C6H). Anal. ($C_{16}H_{20}N_3O_2S_2B$) (18.83% ^{10}B, 81.17% ^{11}B) C, H, N, S, B.

3d: yield 69%; white crystals; mp 178 °C; MS (FAB$^-$, HMPT) 385 (M^+); ^1H NMR ($CDCl_3$) δ 0.96–1.00 (tt, 3 H, CH_3), 1.69–1.81 (qt, 2 H, CH_2), 2.83–2.90 (m, 2 H, NCH_2), 2.91–2.96 (q, 2 H, $C6CH_2$), 3.33–3.43 (m, 2 H, NCH_2), 3.80–3.87 (m, 2 H, OCH_2), 3.89 (s, 3 H, OCH_3), 4.04–4.11 (m, 2 H, OCH_2), 4.41 (s, 2 H, SCH_2), 6.13 (s, br, 1 H, NH), 7.18–7.45 (m, 5 H ArH). Anal. ($C_{19}H_{26}N_3O_3SB$) (95% ^{10}B, 5% ^{11}B) H, N, S, B; C: calcd, 59.04; found, 58.30.

4b: yield 49%; white crystals; mp 155 °C; MS (FAB$^+$, DMPU) 479 (M + H$^+$, ^{10}B); ^1H NMR ($CDCl_3$) δ 0.88–0.94 (tt, 3 H, CH_3), 1.54–1.65 (q, 2 H, CH_2), 2.77–2.84 (q, 2 H, $C6CH_2$), 2.86–2.94 (m, 2 H, NCH_2), 3.18–3.29 (m, 2 H, NCH_2), 3.79–3.93 (m, 4 H, $(OCH_2)_2$), 4.19 (s, 2 H, $C4SCH_2$), 4.36 (s, 2 H, $C2SCH_2$), 6.96–7.02 (t, br, 1 H, NH), 7.18–7.41 (m, 10 H, ArH). Anal. ($C_{25}H_{30}N_3O_2S_2B$) (95% ^{10}B, 5% ^{11}B) C, N, S, B; H: calcd, 6.32; found, 6.81.

5-(Dihydroxyboryl)-2-thiouracil (1). To a solution of 15.5 g (58 mmol) of $AlBr_3$ in dry toluene (100 mL) was added 5.0 g (14.5 mmol) of **1d**. The reaction mixture was stirred at 50–60 °C for 4 h and cooled. Ice water was added; the raw product was filtered, dissolved in 75 mL of an aqueous 1 M NaOH solution, and extracted with diethyl ether. The aqueous solution was acidified with concentrated hydrochloric acid to pH = 2, and the precipitate was filtered off. This residue was dissolved in refluxing ethanol (100 mL), and charcoal was added until the solution was decolorized. After hot filtration, the eluate was concentrated under reduced pressure until turbid. Water was added and the product (48% yield) was filtered off and dried at 50 °C: white crystals; mp >250 °C; IR (KBr) 1660 (s, C=0), 1560 (s, NH), 1415 (m, ^{10}B–C, isotope-dependent), 1380 (s, ^{10}B–O, isotope-dependent), 1185 (s, C=S); ^1H NMR (DMSO-d_6) δ 3.4 (s, 0.5 H, H_2O), 7.67 (d, 1 H, C6H), 8.08 (s, br, 2 H, $B(OH)_2$), 12.60 (d, 1 H, N1H), 12.74 (d, 1 H, N_3H); H–D exchange δ 3.70 (H_2O), 7.69 (s, C6H); ^{13}C NMR (DMSO-d_6, ^1H-decoupled) δ 104.7 (C5), 148.3 (C6), 167.4 (C4), 176.2 (C2); DEPT CH, CH_3, 148.3 (C6); MS (FAB[−], HMPT) 171 [(M − H)$^-$, ^{11}B], 153 [(M − H_2O − H)$^-$], 127 [(M − H^{11} − BO_2 − H)$^-$]. Anal. ($C_4H_5N_2O_3SB$) (95% ^{10}B, 5% ^{11}B) C, H, N, S, B.

5-(Dihydroxyboryl)-2,4-dithiouracil (2), 5-(Dihydroxyboryl)-6-propyl-2-thiouracil (3), and 5-(Dihydroxyboryl)-2,4-dithio-6-propyluracil (4). These compounds were prepared by analogy with **1**.

2: yield 40%; yellow crystals; mp >250 °C; ^1H NMR (DMSO-d_6) δ 3.4 (0.3 H, H_2O), 7.59 (s, 1 H, C6H), 8.55 (s, br, 2 H, $B(OH)_2$), 13.25 (s, br, 1 H, N1H), 13.81 (s, br, 1 H, N3H); MS (FAB[−], HMPT) 187 [(M − H)$^-$, ^{11}B], 169 [(M − H_2O − H)$^-$], 143 [(M − $H^{11}BO_2$ − H)$^-$]. Anal. ($C_4H_5N_2O_2S_2B$) (18.83% ^{10}B, 81.17% ^{11}B) C, H, N, S, B.

3: yield 53%; white crystals; mp 193 °C; ^1H NMR (DMSO-d_6) δ 0.84–0.90 (t, 3 H, CH_3), 1.49–1.60 (qt, 2 H, CH_2), 2.66–2.72 (t, 2 H, $C6CH_2$), 8.60 (s, 2 H, $B(OH)_2$), 12.41 (s, br, 1 H, N1H), 12.65 (s, br, 1 H, N3H); MS (FAB[−], DMPU) 212 [(M − H)$^-$, ^{10}B], 194 [(M − H_2O − H)$^-$], 169 [(M − $H^{10}BO_2$ − H)$^-$]. Anal. ($C_7H_{11}N_2O_3SB$) (95% ^{10}B, 5% ^{11}B) C, H, N, S, B.

4: yield 19%; yellow crystals; mp 185 °C; ^1H NMR (DMSO-d_6) δ 0.83–0.90 (t, 2 H, CH_3), 1.50–1.63 (q, 2 H, CH_2), 2.22–2.29 (t,

(19) Barrett, H. W.; Goodman, I.; Dittmer, K. *J. Am. Chem. Soc.* **1948**, *57*, 1753.
(20) Hilbert, G. E.; Jansen, E. F. *J. Am. Chem. Soc.* **1934**, *56*, 134.
(21) Bucher, K. *Pharm. Acta Helv.* **1951**, *26*, 145.
(22) Equimolar amounts of NaH (60% in mineral oil) and benzyl mercaptan were stirred in toluene at 50 °C for 0.75 h, until the hydrogen evolution had ceased.

2 H, C6CH$_2$), 3.4 (s, 2.5 H, H$_2$O), 8.20 (s, br, 2 H, B(OH)$_2$), 12.73 (s, br, 1 H, N1H), 13.40 (s, br, 1 H, N3H); MS (FAB[-], DMPU) 228 [(M - H)$^-$, ^{10}B], 210 [(M - H$_2$O - H)$^-$], 185 [(M - H^{10}BO$_2$ - H)$^-$]. Anal. ($C_7H_{11}N_2O_2S_2B$) (95% ^{10}B, 5% ^{11}B) C, H, N, S, B.

Attempted Reaction of *O,S*-Bis(trimethylsilyl)-5-iodo-2-thiouracil with Butyllithium and Tributyl Borate. 5-Iodo-2-thiouracil (2.45 g, 10 mmol) was refluxed with 0.3 mL (2.4 mmol) of chlorotrimethylsilane and 26 mL of hexamethyldisilazane according to ref 23, and the resulting *O,S*-bis(trimethylsilyl)-5-iodo-2-thiouracil (yield 51%) was purified by Kugelrohr distillation (bp 135 °C, 0.01 mm). Its reaction with butyl lithium and tributyl borate according to the preparation of 1 led to 2-(*n*-butylthio)-5-(trimethylsilyl)uracil: yield 63%; white crystals; mp 117 °C; purification by column chromatography in CHCl$_3$/MeOH 9:1; MS (EI) 256 (M$^+$); ^{1}H NMR (CDCl$_3$) 0.28 (s, 9 H, Si(CH$_3$)$_3$), 0.94-3.17 (m, 9 H, *n*-butyl), 7.84 (s, 1 H, C6H), 12.85 (s, 1 H, N3H); ^{13}C NMR (CDCl$_3$) (-4)-0.5 (q, SiC$_3$), 11.5-33 (m, *n*-butyl), 119.7 (d, $^2J_{C,H}$ = 16.2 Hz, C5), 159.3 (d, $^1J_{C,H}$ = 178 Hz, C6), 163.25-163.58 (d, $^3J_{C,H}$ = 14 Hz + t, $^3J_{C,H}$ = 5.1 Hz, C2), 167.55 (d, $^3J_{C,H}$ = 9.2 Hz, C4).

Attempted Reaction of 5-(Dihydroxyboryl)-1,3-dimethyluracil with Thiourea. 5-Bromo-1,3-dimethyluracil[12] was converted to the 5-dihydroxyboryl derivative as described above (**1d**), increasing the time span between the addition of butyllithium and tributyl borate to 45 min [yield 27%; white crystals; mp 167 °C; MS (EI) 183 (M$^+$); ^{1}H NMR (DMSO-d_6) 3.19 (s, 3 H, N1-CH$_3$), 3.37 (s, 3 H, N3CH$_3$), 8.00 (s, 1 H, C6H), 8.13 (s, 2 H, B(OH)$_2$)].

Reaction of this compound with a 5-fold excess of thiourea and sodium ethylate in ethanol led to 2-thiouracil (yield 74%).

Attempted Rearrangement of 2,4-Bis(benzyloxy)-5-(dihydroxyboryl)pyrimidine. 2,4-Bis(benzyloxy)-5-bromopyrimidine[7] (2 g, 5.4 mmol) was heated with 0.2 g of water-free *p*-toluenesulfonic acid to 165 °C for 3 min. The residue was dissolved in 20 mL of H$_2$O, adjusted to pH = 12, and extracted with ether. Following acidification to pH = 2, two compounds could be extracted with ether. These were purified by column chromatography in CHCl$_3$/MeOH 9:1 and shown to be 1,5-dibenzyluracil [yield 16%; white crystals; mp 132 °C; ^{1}H NMR (CDCl$_3$) 3.49 (s, 2 H, C5CH$_2$), 4.78 (s, 2 H, N1CH$_2$), 7.2 (m, 11 H, C6H + ArH), 9.92 (s, br, 1 H, N3-H)] and 3-benzyluracil [yield 8%, not purified to homogeneity; ^{1}H NMR (CDCl$_3$) 4.98 (s, 2 H, N3CH$_2$), 5.55 (d, 1 H, C5-H), 7.35 (s, 5 H, ArH), 7.62 (d, 1 H, C6-H)].

Animal Distribution Studies. The tumor model used was B16 melanoma (obtained through Dr. J. A. Coderre, Brookhaven National Laboratory) in C57/bl mice. Animals were obtained from Jackson Laboratories, Bar Harbor, ME, or Zentrallaboratorium für Versuchstierzucht, Hannover, FRG. The B16 tumor was propagated in cell culture and implanted into mice by injecting subcutaneously 10^6 cells. The weight of the mice was around 20 g. The tumor size at the time of the experiment was between 0.1 and 0.5 g.

Solutions of 1 and 2 (0.6 and 0.5 mg/mL, respectively) were prepared in 0.1 M Tris base and adjusted to pH = 8. Solutions of 3 and 4 (0.4 and 0.2 mg/mL) were prepared by dissolving the appropriate amount in 0.1 M NaOH and diluting with 10 volumes of water. The pH of this solution was around 9. Of these solutions, 0.5 mL were injected intraperitoneally.

Boron distribution was evaluated with quantitative neutron-capture radiography,[13] with the Brookhaven Medical Research Reactor as neutron source. Frieze-dried sections of 50 μm thickness, obtained in a cryomicrotome, were placed in close contact with a solid-state track detector (Kodak-Pathé LR115, Type 1) and exposed to 10^{12} n cm^{-2}. The exposed film was etched with 10% NaOH for 50-60 min. The tracks that developed were evaluated with an image analyzer and compared to suitable standards.

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(23) Vorbrüggen, H.; Strehlke, P. *Chem. Ber.* **1973**, *106*, 3039.

Substituted Dihydrobenzopyran and Dihydrobenzofuran Thiazolidine-2,4-diones as Hypoglycemic Agents

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A series of dihydrobenzofuran and dihydrobenzopyran thiazolidine-2,4-diones (compounds **3-26**) was synthesized from the corresponding aryl aldehydes **1** in two steps. These compounds represent conformationally restricted analogues of the novel hypoglycemic ciglitazone. The series was evaluated by hypoglycemic effects in vitro by measuring stimulation of 2-deoxyglucose uptake in L6 myocytes and stimulation of expression of the glucose transporter protein in 3T3-L1 adipocytes. In vivo hypoglycemic effects were evaluated in the genetically obese ob/ob mouse, and structure-activity relationships are discussed. On the basis of this in vivo potency, we have selected the 2(*R*)-benzylbenzopyran derivative to be further studied in a clinical setting.

Diabetes mellitus is a complex, chronic, progressive disease which eventually can adversely affect function of the kidneys, eyes, and nervous and vascular systems. Of the estimated 5.8 million individuals diagnosed with diabetes mellitus in the United States,[1,2] approximately 90% are characterized[3] as non-insulin-dependent (NIDDM, Type II). Most of these NIDDM patients exhibit hyperglycemia, peripheral insulin resistance, and obesity. Besides diet and exercise, current drug therapy for the treatment of diabetes mellitus is aimed at improving glycemic control.[4] The most commonly employed oral hypoglycemics are the sulfonylureas (SU),[5,6] whose mecha-

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(1) Herman, W. H.; Sinnock, P.; Brenner, E.; Brimberry, J. L.; Langford, D.; Nakashima, A.; Sepe, S. J.; Teutsch, S. M.; Mazze, R. S. *Diabetes Care* **1984**, *7*, 367.
(2) National Diabetes Data Group In *Diabetes in America, 1985*; National Institutes of Health: Bethesda, MD. This survey also estimates that 4-5 million diabetics are undiagnosed.
(3) National Diabetes Data Group, *Diabetes* **1979**, *28*, 1039.
(4) *The Physicians Guide To Type II Diabetes (NIDDM): Diagnosis and Treatment*; H. Rifkin, Ed.; American Diabetes Association: Alexandria, VA, 1984; p 67.
(5) Sarges, R. *Prog. Med. Chem.* **1981**, *18*, 191.

0022-2623/91/1834-0319$02.50/0