afforded a solid (345 mg, 95%) whose analytical data were identical with that of **13**.

Ornithine Decarboxylase Preparation. Rat liver ODC prepared from the livers of rats that had been injected with thioacetamide (150 mg/kg of body weight) 18 h before sacrifice was purified about 10-fold by acid treatment at pH 4.6 as described by Ono et al.[23] The specific activity of the preparation was 0.2 nmol of CO_2 min^{-1} (mg of protein)$^{-1}$.

Assay of Time-Dependent Inhibition of Ornithine Decarboxylase. Assay and measurement of the kinetic constants of the inhibition were performed as described previously.[3a] Kinetic constants were calculated by the method of Kitz and Wilson[16] by using a least-squares fit of the data points with a Hewlett-Packard 9820 calculator.

Acknowledgment. We are indebted to K. Jund, A. Czermak, and M. C. Chanal for valuable technical assistance. We thank also Dr. J. Wagner and E. Wolf for the measurement of the pK_a values of the putrescine and ornithine derivatives.

(23) M. Ono, H. Inoue, F. Suzuki, and Y. Takeda, *Biochim. Biophys. Acta*, **284**, 285 (1972).

Registry No. **1a**, 82006-42-6; **1b**, 62031-25-8; **2a**, 82031-21-8; **2b**, 86921-74-6; **3a**, 82006-43-7; **3b**, 86921-75-7; **4a**, 82006-44-8; **4b**, 82006-77-7; **5**, 82006-78-8; **6**, 86921-71-3; **7**, 86921-79-1; **7**·2HCl, 82006-79-9; **8**, 82006-45-9; **9a**, 82006-48-2; **10**, 82134-42-7; **10**·HCl, 82006-86-8; (*Z*)-**12a**, 82006-49-3; (*E*)-**12a**, 82006-50-6; (*E*)-**12b**, 82006-62-0; (*Z*)-**12b**, 82006-61-9; **13**, 82006-54-0; **14**, 82006-58-4; **14**·2HCl, 82006-56-2; **15**, 86921-72-4; **16**, 86921-73-5; **17**, 82006-84-6; **17**·HCl, 82006-71-1; *tert*-butyl ethyl malonate, 32864-38-3; allyl bromide, 106-95-6; di-*tert*-butyl malonate, 541-16-2; chlorodifluoromethane, 75-45-6; *N*-bromosuccinimide, 2439-85-2; potassium phthalimide, 1074-82-4; (*E*)-2-(difluoromethyl)-2-(ethoxycarbonyl)-5-phthalimido-3-pentenoyl chloride, 82006-46-0; ethyl (*E*)-2-(difluoromethyl)-2-(azidocarbonyl)-5-phthalimido-3-pentenoate, 82006-47-1; propenylmagnesium bromide, 14092-04-7; fluoroacetonitrile, 503-20-8; 2-(fluoromethyl)-2-phthalimido-3-pentenenitrile, 86921-76-8; phthaloyl dichloride, 88-95-9; (*E*)-2-(fluoromethyl)-2,5-diphathalimido-3-pentenenitrile, 86921-77-9; (*Z*)-1-fluoro-2-phthalimido-3-pentene, 82006-52-8; (*E*)-1-fluoro-2-phthalimido-3-pentene, 82006-53-9; *N*-carbethoxyphthalimide, 22509-74-6; (*E*)-1-fluoro-2,5-diphthalimido-3-pentene, 82006-55-1; 1-bromo-3-ethoxy-1-propene, 86921-78-0; (*E*)-1-fluoro-2-phthalimido-5-ethoxy-3-pentene, 82006-66-4; (*Z*)-1-fluoro-2-phthalimido-5-ethoxy-3-pentene, 82006-65-3; ornithine decarboxylase, 9024-60-6.

Synthesis and Biological Evaluation of Sparsomycin Analogues

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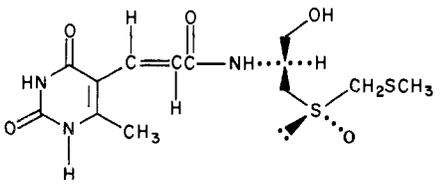
Three series of sparsomycin analogues were prepared and examined for their ability to inhibit DNA or protein synthesis in bone marrow, P388 lymphocytic leukemia, and P815 mastocytoma cells. The compounds of series I and II, distinguished by the inclusion or exclusion of a hydroxymethyl functional group, were designed to elucidate the effect on activity of replacing the oxodithioacetal side chain of sparsomycin with 4-substituted benzyl groups. The series III analogues, which excluded the hydroxymethyl group and replaced the oxodithioacetal moiety of sparsomycin with a benzyl amide group, were designed to investigate the potential interaction of an amide oxygen in contrast to the sulfoxide oxygen of sparsomycin. Overall, the bromobenzyl-substituted analogues imparted the greatest inhibitory activity in the protein synthesis assay, while the methoxybenzyl-substituted analogues displayed the least. The methylbenzyl and the unsubstituted benzyl compounds were intermediate in inhibitory potential. The activity in the protein synthesis assay may correspond to the lipophilic and electronic characteristics of the substituents on the benzyl moiety of the analogues. All of the compounds were inactive in the DNA synthesis assay.

In 1962, Owen, Dietz, and Camiener reported the isolation of a novel antibiotic with antitumor, antifungal, and antibacterial activity from the culture filtrate of *Streptomyces sparsogenes*.[1] The structure of the crystalline antibiotic, named sparsomycin, remained elusive until 1970, when Wiley and MacKellar reported results of spectroscopic and degradation studies that elucidated the structure (**1**).[2,3] The molecule featured a trans olefin bond,



1 (sparsomycin)

a chiral carbon atom with an *S* configuration, and a chiral sulfur atom with a *R* configuration.[4] Due to the presence of the synthetically complex oxodithioacetal side chain, the first total synthesis of sparsomycin was not reported until 1979.[5,6] Since its discovery, the compound has attracted attention not only for its unique structural characteristics and challenging synthesis, but also for the antitumor, antibacterial, antifungal, and antiviral properties attributed to sparsomycin's ability to inhibit protein synthesis at the ribosomal level.[1,7-12] The most promising biological activity of sparsomycin was its antitumor ac-

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(4) Ottenheijm, H. C. J.; Liskamp, R. M. J.; Helquist, P.; Lauher, J. W.; Shekhani, M. S. *J. Am. Chem. Soc.* **1981**, *103*, 1720.
(5) (a) Ottenheijm, H. C. J.; Liskamp, R. M. J.; Tijhuis, M. W. *Tetrahedron Lett.* **1979**, 387. (b) Ottenheijm, H. C. J.; Liskamp, R. M. J.; vanNispen, S. P. J. M.; Boots, H. A.; Tijhuis, M. W. *J. Org. Chem.* **1981**, *46*, 3273.
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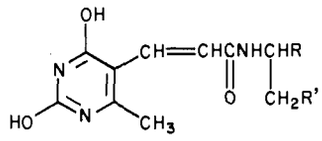
0022-2623/83/1826-1556$01.50/0

Table I. Structure and Properties of Pyrimidinylpropenamides



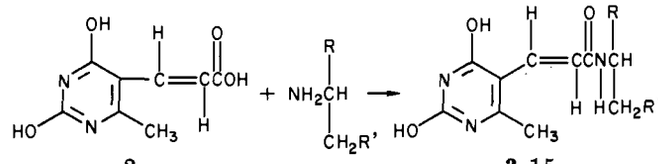
compd	R	R'	X	reaction times, h	yield, %	mp, °C	formula	anal.
				Series I				
3	CH_2OH	C_6H_4-X	H	6	99	191-191.5[a]	$C_{17}H_{19}N_3O_4 \cdot H_2O$	C, H, N
4	CH_2OH	C_6H_4-X	4-CH_3	72	62	187-187.5[a]	$C_{18}H_{21}N_3O_4 \cdot H_2O$	C, H, N
5	CH_2OH	C_6H_4-X	4-OCH_3	72	73	179-181[a]	$C_{18}H_{21}N_3O_5 \cdot {}^1/_2H_2O$	C, H, N
6	CH_2OH	C_6H_4-X	4-Br	24	57	188-189.5[a]	$C_{17}H_{18}BrN_3O_4 \cdot 1{}^1/_4H_2O$	C, H, N
				Series II				
7	H	C_6H_4-X	H	24	59	218 dec[b]	$C_{16}H_{17}N_3O_3 \cdot H_2O$	C, H, N
8	H	C_6H_4-X	4-CH_3	24	83	214.5-215.5[b]	$C_{17}H_{19}N_3O_3 \cdot H_2O$	C, H, N
9	H	C_6H_4-X	4-OCH_3	24	75	200.5-201.5[b]	$C_{17}H_{19}N_3O_4 \cdot H_2O$	C, H, N
10	H	C_6H_4-X	4-Br	24	92	198-199.5[b]	$C_{16}H_{16}BrN_3O_3 \cdot H_2O$	C, H, N
				Series III				
11	H	$CONHCH_2C_6H_4$-X	H	24	72	232.5-233.5[b]	$C_{18}H_{20}N_4O_4 \cdot H_2O$	C, H, N
12	H	$CONHCH_2C_6H_4$-X	4-CH_3	24	60	262-265[c]	$C_{19}H_{22}N_4O_4$	C, H, N
13	H	$CONHCH_2C_6H_4$-X	4-OCH_3	24	71	187-188[b]	$C_{19}H_{22}N_4O_5 \cdot H_2O$	C, H, N
14	H	$CONHCH_2C_6H_4$-X	4-Br	24	56	191-192[b]	$C_{18}H_{19}BrN_4O_4 \cdot H_2O$	C, H, N
15	H	$CONHCH_2C_6H_3$-X	3,4-Cl_2	24	61	262-264[c]	$C_{18}H_{18}Cl_2N_4O_4$	C, H, N

[a] Recrystallized from ethanol. [b] Recrystallized from water. [c] Washed in boiling water.

tivity, which advanced sparsomycin through the research stages to phase I clinical trials. Its progress was impeded, however, by the development of ocular toxicity in the cancer patients.[13,14]

In an effort to eliminate the toxic properties of sparsomycin while maintaining the antitumor activity, analogues of sparsomycin were prepared. Previous investigators examined analogues that incorporated modifications of the uracil ring, the unique oxodithioacetal moiety, and the stereochemical configuration of the chiral centers.[15-18] Vince and Lee reported there was an apparent requirement for the *S* configuration at the asymmetric carbon atom.[16] Overall, however, the small number of sparsomycin analogues prepared and evaluated limited the definitive statements concerning the functional groups required for antitumor activity. In order to expand and clarify the structure–activity relationships, three series of new sparsomycin analogues were prepared (Table I). The compounds of series I and II were designed to elucidate the effect on activity of replacing the oxodithioacetal side chain with hydrophobic groups. In order to more clearly define the interaction, substitutions on the phenyl ring were made that varied the electronic and hydrophobic characteristics of the phenyl side chain. In order to further analyze the requirements for activity, the two series were designed to include (Series I) or exclude (Series II) the hydroxymethyl functional group found in sparsomycin. The compounds of series III were proposed to investigate the potential interactions of an amide oxygen in contrast to the sulfoxide oxygen of sparsomycin by replacing the oxodithioacetal moiety of sparsomycin with an *N*-benzyl-substituted carboxamide. Analogous to series I and II, a hydrophobic phenyl ring with diverse substituents was included to elaborate the structure–activity relationships of sparsomycin. The compounds were not designed to analyze the stereochemical requirements for activity, and the compounds containing chiral centers (series I) were synthesized as the *RS* mixture.

Scheme I



2 16-28 3-15

	R	R'	X
16	CH_2OH	C_6H_4-X	H
17	CH_2OH	C_6H_4-X	4-CH_3
18	CH_2OH	C_6H_4-X	4-OCH_3
19	CH_2OH	C_6H_4-X	4-Br
20	H	C_6H_4-X	H
21	H	C_6H_4-X	4-CH_3
22	H	C_6H_4-X	4-OCH_3
23	H	C_6H_4-X	4-Br
24	H	$CONHCH_2C_6H_4$-X	H
25	H	$CONHCH_2C_6H_4$-X	4-CH_3
26	H	$CONHCH_2C_6H_4$-X	4-OCH_3
27	H	$CONHCH_2C_6H_4$-X	4-Br
28	H	$CONHCH_2C_6H_3$-X	3,4-Cl_2

Chemistry. The structure of each sparsomycin analogue was viewed as consisting of two fragments that could be coupled in the final step of a convergent synthesis. The preparation of one fragment, acid **2**, was well documented in the literature.[2,3,5,19,20] In the final step of the synthesis of each novel compound, acid **2** was coupled with the second fragment, the amine fragment, via formation of an amide bond. *N*-(Ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EEDQ) was the reagent chosen to effect the coupling (Scheme I).

(13) Goldberg, I. H. *Cancer Chemother. Rep.* **1974**, *58*, 479.
(14) Close, H. P.; McFarlane, J. R. *Cancer Chemother. Rep.* **1964**, *43*, 29.
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(16) Lee, C. K.; Vince, R. *J. Med. Chem.* **1978**, *21*, 176.
(17) Lin, C. C. L.; Dubois, R. J. *J. Med. Chem.* **1977**, *20*, 337.
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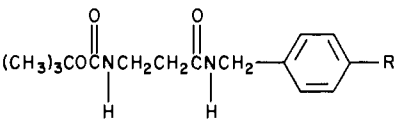
(20) Ottenheijm, H. C. J.; Liskamp, R. M. J. *Tetrahedron Lett.* **1978**, 2437.

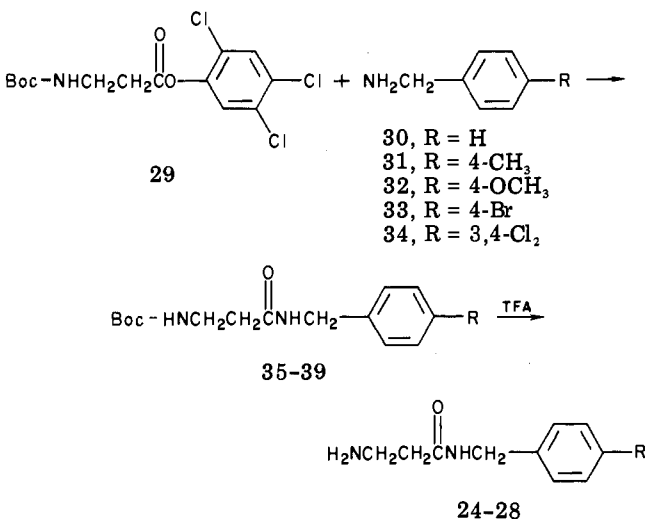
Table II. Properties of *N*-(Substituted-benzyl)-3-[(*tert*-butyloxycarbonyl)amino]propanamides



compd	R	yield, %	mp, °C	formula	anal.
35	H	80	116-118[a]	$C_{15}H_{22}N_2O_3$	C, H, N
36	CH_3	86	124-125.5[a]	$C_{16}H_{24}N_2O_3$	C, H, N
37	OCH_3	67	104-105[a]	$C_{16}H_{24}N_2O_4$	C, H, N
38	Br	57	122.5-124.5[a]	$C_{15}H_{21}BrN_2O_3$	C, H, N
39	3,4-Cl_2	69	124-128[a]	$C_{15}H_{20}Cl_2N_2O_3$	C, H, N

[a] Recrystallized from ethyl acetate/petroleum ether (bp 60-90 °C).

Scheme II



In the series I analogues, **3–6**, the amine fragments of the analogues were derived from substituted phenylalanine compounds. For compound **4**, the methyl-substituted phenylalanine was reduced with lithium aluminum hydride to give the desired alcohol, **17**. Similarly, the methoxy-substituted phenylalanine derivative, **43**, was reduced with lithium aluminum hydride to give **42**, which after hydrolysis of the amide with hydrochloric acid gave the alcohol, **18**. For the bromo-substituted analogue, **6**, 4-bromophenylalanine was reduced with borane in tetrahydrofuran to afford the amino alcohol, **19**.

The compounds of series II, **7–10**, were synthesized by coupling the commercially available phenethylamines with the acid **2** by using EEDQ (Scheme I). Coupling phenethylamine (**20**), 4-methylphenethylamine (**21**), 4-methoxyphenethylamine (**22**), and 4-bromophenethylamine (**23**) with the acid, **2**, gave compounds **7–10**.

The amine fragments for series III analogues **11–15** were derived from substituted β-alanine compounds. The synthesis began by making the *tert*-butyloxycarbonyl (Boc) protected active ester of β-alanine (**29**) by using the procedures of Bentley et al.[21] and Pless and Boissonnas.[22] The active ester **29** was reacted with the appropriately substituted benzylamine, **30–34**, to give the *N*-(substituted-benzyl)-3-[(*tert*-butoxycarbonyl)amino]propanamides **35–39** (Scheme II, Table II). Deprotection of the Boc-substituted amine by treatment with trifluoroacetic acid afforded the amines **24–28**. These amines were coupled with acid **2** as in Scheme I to afford the final products **11–15**.

(21) Bentley, P. H.; Gregory, H.; Laird, A. H.; Morley, J. S. *J. Chem. Soc.* **1964**;, 6130.
(22) Pless, J.; Boissonnas, R. A. *Helv. Chim. Acta* **1963**, *46*, 1609.

Table III. Summary of the Results for the Protein Synthesis Assay

	% inhibition		
compd[b]	P388[a]	P815[a]	BMC[a]
3	6	52[c]	34[c]
4	4	65[c]	40[c]
5	0	0	0
6	44[c]	94[c]	64[c]
7	1	2	34[c]
8	0	11	35[c]
9	0	0	44[c]
10	27[c]	89[c]	42[c]
11[d]			
12[d]			
13	1	0	11
14	23	69[c]	29[c]
15[d]			
puromycin	64[c]	98[c]	61[c]
sparsomycin	48		29

[a] The numbers represent the percent inhibition of [^{75}Se]selenomethionine incorporation in the protein synthesis assay for six replicate wells of 6×10^5 P388 lymphocytic leukemia cells or bone marrow cells or four replicate wells of 6×10^5 P815 mastocytoma cells based upon control incorporation of 13 651 ± 381, 8203 ± 219, and 23 647 ± 920 cpm, respectively. Statistical analysis on the individual counts per minute was done and the standard error was <10%. [b] All compounds were tested at 10^{-3} M, except 5, which was tested at 10^{-4} M in 1% ethanol. Appropriate controls with or without ethanol were used to calculate the percent inhibition. [c] Significant at $p < 0.05$ as compared to controls. [d] These compounds were insoluble at the required concentrations in water or ethanol, which precluded testing.

Results and Discussion

In the early pharmacological evaluation of sparsomycin, experiments were performed to measure the effect of sparsomycin on DNA, RNA, and protein synthesis in *Escherichia coli* cells.[11-13] The initial studies and subsequent more detailed investigations indicated that although sparsomycin inhibited DNA and RNA synthesis at a detectable level, sparsomycin interacted primarily by inhibiting protein synthesis at the ribosomal level. The target compounds reported in this paper (**3–15**) were experimentally examined to determine their effects upon protein synthesis and DNA synthesis in P388 lymphocytic leukemia cells, P815 mastocytoma cells, and bone marrow cells. The results are summarized in Tables III and IV.

In the protein and DNA synthesis assays, the incorporation of the pulse labels [^{75}Se]selenomethionine and ^{125}IUdR, respectively, were quantitated. These values were used as an indirect measurement of protein and DNA synthesis.

Analytical inspection of the data summarized in Table III reveals 3 of the 14 compounds, **6**, **10**, and **14**, were significantly active inhibitors of [^{75}Se]selenomethionine

Table IV. Summary of the Results for the DNA Synthesis Assay

compd[c]	% inhibition P388 cells[a]	% inhibition bone marrow cells[b]
3	5	0
4	0	0
6	34	15
7	9	0
8	0	0
9	2	0
10	17	0
cytarabine (*ara*-C)	99[d]	99[d]

[a] The numbers represent the percent inhibition of ^{125}IUdR incorporation for six replicate wells containing 6×10^5 P388 cells based upon a control incorporation of 21 592 ± 1196 cpm. [b] The numbers represent the percent inhibition of ^{125}IUdR incorporation for six replicate wells containing 6×10^5 bone marrow cells based upon a control incorporation of 20 874 ± 662 cpm. [c] All compounds were tested at 10^{-3} M. [d] $p < 0.05$ as compared to controls. Statistical analysis of the individual counts per minute was done, and the standard error was <10%.

incorporation in P388 lymphoma cells. Closer examination of the data in an attempt to establish a structure–activity relationship, disclosed compounds **5**, **9**, and **13** were usually the least active analogues. Compounds **3**, **4**, **7**, and **8** were moderately active in most of the experiments. The results indicate that the removal of the hydroxymethyl functional group as seen in sparsomycin has a varying effect on activity. In the P815 cells, removal of the hydroxymethyl group decreased the ability of the target compounds to inhibit [^{75}Se]selenomethionine incorporation. In support of this, the series I compounds **3** and **4** were active inhibitors, whereas the series II analogues **7** and **8** were inactive. In the P388 cells and the bone marrow cells, inclusion or exclusion of the hydroxymethyl functional group only effected the bromophenyl- and methoxyphenyl-substituted analogues' ability to inhibit [^{75}Se]selenomethionine incorporation. In the case of the bromophenyl analogues, inclusion of the hydroxymethyl group resulted in an increased inhibition of [^{75}Se]selenomethionine incorporation in the P388 and bone marrow cells (**6** vs. **10**). Conversely, the addition of the hydroxymethyl group to the methoxyphenyl-substituted compound **9** eliminated the inhibitory activity in the bone marrow cells as shown by the inactivity of compound **5**.

The series III analogue **14** was significantly active in the protein synthesis assay but was not as active as **6** or **10**, which suggests that the removal of the hydroxymethyl functional group and substitution of the oxodithioacetal side chain of sparsomycin with a substituted benzyl amide moiety are not beneficial for activity.

Focusing the analysis upon the effect of substitution on the phenyl ring, the bromophenyl-substituted analogues imparted the greatest inhibitory activity. Furthermore, the methoxyphenyl-substituted analogues were inactive in most of the studies. The methylphenyl analogues and unsubstituted phenyl analogues fell in between the bromophenyl- and methoxyphenyl-substituted compounds. The activity may correspond to the lipophilic and electronic characteristics of the substituents on the phenyl moiety of the analogues. It appears that the bromo substituent of high lipophilicity and electron-withdrawing character is optimal for inhibitory activity and, conversely, the methoxyphenyl substituent of hydrophilic and electron-donating character is least desirable. The only exceptions to this pattern were the series II compounds **7–10**, which all had approximately the same activity in the protein synthesis assay using bone marrow cells.

Since 3 of the 14 compounds (**6**, **10**, and **14**) inhibited protein synthesis, as reflected by the incorporation of [^{75}Se]selenomethionine, at levels approaching the control drug puromycin, studies were conducted to detect any effect the compounds may have on DNA synthesis. The data in Table IV summarizes the incorporation of ^{125}IUdR into P388 cells and bone marrow cells including cytarabine as the positive control inhibitor. Within the 2-h time frame of the assay, the compounds had little or no effect on DNA synthesis as measured by the incorporation of ^{125}IUdR into cells.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are reported as uncorrected values. Infrared spectra were obtained from a Beckman AccuLab 8 spectrophotometer using samples of the compounds as a solution in the indicated solvent or neat. The strong, sharp band of polystyrene film at 1601.4 cm^{-1} was used as a reference marker for all infrared spectra. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a Perkin-Elmer Model R-24 spectrometer. Chemical shifts of the compounds in the indicated solvent are denoted in units of parts per million (δ) relative to tetramethylsilane as an internal standard. When trifluoroacetic acid was used as the solvent, the TFA proton at δ 11.3 was used as the reference. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA, and are within 0.4% of theoretical values. All compounds have been characterized by elemental analysis, melting point, and NMR and IR spectral analysis.

Preparation of Pyrimidinylpropenamides. The pyrimidinylpropenamides listed in Table I were prepared by a modification of the procedure described by Lin and Dubois.[17] If the pyrimidinylpropenamide did not crystallize from the reaction mixture, the solvent was removed under reduced pressure, and the residue was triturated with ether to induce crystallization. The preparation of **3** may serve as an example.

(*E*)-(±)-*N*-(1-Hydroxy-3-phenyl-2-propyl)-3-(2,4-dihydroxy-6-methyl-5-pyrimidinyl)-2-propenamide (3). A mixture of **2** and *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (1.56 g, 6 mmol) in dry dimethylformamide (35 mL) was magnetically stirred at 35 °C until solution was effected. A solution of **16** (0.76 g, 5 mmol) in dimethylformamide (5 mL) was added. The solution was stirred at 35 °C for 6 h. The precipitate was collected by filtration to afford 1.63 g (99%) of **3** as a white solid. Recrystallization from ethanol gave **3**: mp 191–191.5 °C; IR (Nujol) 3350 (broad, NH), 3100 (broad, OH), 1710 (strong, C=O), 1650 (strong C=C) cm^{-1}; NMR (Me_2SO-d_6) δ 6.7–7–7.5 (m, 7, 5 Ar H, trans CH=CH), 4.7 (s, 4, NH, 3 OH, exchangeable), 2.5–3.5 (m, 5, CH_2CHCH_2), 2.3 (s, 3, CH_3).

2-Amino-3-(4-methylphenyl)propanol (17). The procedure of Lee and Vince[16] was used. A mixture of lithium aluminum hydride (1.0 g, 26 mmol) and dry tetrahydrofuran (75 mL) was heated at reflux for 2 h under nitrogen. The mixture was cooled in an ice bath, and **40** (2.0 g, 11 mmol) was added in small portions. The mixture was heated at reflux for 12 h under nitrogen. The excess lithium aluminum hydride was decomposed by adding in succession water (2 mL), aqueous sodium hydroxide (10%, 20 mL), and water (5 mL). The mixture was filtered and the precipitate was washed with tetrahydrofuran. The filtrate was concentrated under reduced pressure to leave a yellow oil, which was dissolved in chloroform (50 mL). The chloroform layer was washed with aqueous sodium hydroxide (5%, 25 mL), water (25 mL), and saturated aqueous sodium chloride solution (25 mL). The chloroform extract was dried over anhydrous sodium sulfate and filtered, and the filtrate was evaporated under reduced pressure to give 1.23 g (67%) of **17** as a pale yellow oil. The hydrochloride salt was prepared by the procedure of Matuszak and Matuszak[23] to give a white solid, which was recrystallized from ethanol/ether: mp 173–175 °C; IR (Nujol) 3350 (strong, OH, NH) cm^{-1}; NMR (D_2O) δ 7.35 (s, 4, Ar H), 3.8 (m, 3, OCH_2CH), 3.0 (d, 2, CH_2Ar),

(23) Matuszak, C. A.; Matuszak, A. J. *J. Chem. Educ.* **1967**, *44*, 108.

2.4 (s, 3, CH_3). Anal. ($C_{10}H_{15}NO \cdot HCl$) C, H, N.

2-Amino-3-(4-methoxyphenyl)propanol Hydrochloride (18·HCl). The procedure of Berlinguet[24] was used for the preparation of 18·HCl (58%), mp 190–194 °C (lit.[24] mp 195–197 °C), from **42**.

2-Amino-3-(4-bromophenyl)propanol (19). The procedure of Anhoury et al.[25] was followed. To a solution of 4-bromophenylalanine (1.0 g, 4 mmol) in dry THF (25 mL), under an atmosphere of N_2, was added dropwise, with stirring, 1.0 M borane–tetrahydrofuran solution (5.7 mL). The solution was stirred at room temperature for 15 h, after which additional 1.0 M borane–tetrahydrofuran solution (5.7 mL) was added. The reaction was stirred for another 15 h. The excess borane was destroyed by adding ethanol (about 60 mL) until evolution of hydrogen gas ceased. The solution was acidified with 3 N hydrochloric acid to pH 1. After 2-propanol (10 mL) was added to the flask, the water was azeotropically removed by distillation under reduced pressure to leave a white solid, which was recrystallized from ethanol/ether to give 0.88 g (82%) of 19·HCl. A solution of 19·HCl (0.87 g, 3 mmol) in water (minimum amount) was treated with a 50% solution of sodium hydroxide to a pH of 12–14. After 2-propanol (10 mL) was added, the water was azeotropically removed by distillation under reduced pressure. The residue was dissolved in ether (50 mL), washed with water (25 mL) and saturated sodium chloride solution (20 mL), and dried over anhydrous sodium sulfate, and the ether was removed under reduced pressure. The resulting yellow oil was crystallized by drying under reduced pressure, to give 0.56 g (75%) of **19** as a pale yellow solid, mp 85–87 °C (lit.[26] mp 93–94 °C).

Preparation of *N*-(Substituted-benzyl)-3-[(*tert*-butyloxycarbonyl)amino]propanamides. The compounds listed in Table II were prepared by treatment of the *tert*-butyloxycarbonyl-protected active ester of β-alanine with substituted benzylamines. The preparation of **35** may serve as an example. See Table II for physical properties.

***N*-Benzyl-3-[(*tert*-butyloxycarbonyl)amino]propanamide (35).** A solution of benzylamine (0.65 g, 6 mmol) in methylene chloride (10 mL) was added to a flask containing 2,4,5-trichlorophenyl 3-[(*tert*-butyloxycarbonyl)amino]propanoate[21,22] (2.0 g, 5.4 mmol). The solution was stirred for 4 h at room temperature. The methylene chloride solution was washed with 1 N potassium bisulfate (10 mL), sodium hydroxide solution (5%, 10 mL), and saturated aqueous sodium chloride solution (10 mL). The methylene chloride portion was dried over anhydrous sodium sulfate and filtered, and the filtrate was evaporated under reduced pressure to give **42** as a white solid. Recrystallization from ethyl acetate/petroleum ether (bp 60–90 °C) afforded 1.2 g (80%) of **35**: mp 116–118 °C; IR (Nujol) 3300 (strong, NH), 1690 (strong, carbamate C=O), 1650 (strong, amide C=O) cm^{-1} NMR (Me_2SO-d_6) δ 8.2 (s, 1, carbamate NH), 7.25 (s, 5, Ar H), 6.7 (s, 1, NHC=O), 4.25 (d, 2, CH_2Ar), 3.2 (m, 2, CCH_2N), 2.3 (t, 2, CH_2C=O), 1.3 (s, 9, 3 CH_3).

General Procedure for the Removal of the *tert*-Butyloxycarbonyl Protecting Group. The appropriate protected amine, **35–39** (3 mmol), was dissolved in trifluoroacetic acid (4 mL). The solution was stirred at room temperature for 30 min. The trifluoroacetic acid was removed under reduced pressure, and the residue was dissolved in water (minimum amount). The solution was adjusted to pH 14 with sodium hydroxide solution (5%) and extracted with ethyl acetate (3 × 15 mL). The organic phase was washed with saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and filtered, and the filtrate was concentrated under reduced pressure to give the deprotected 3-aminopropanamide, **24–28**. The crude 3-aminopropanamides were coupled with acid **2** as described for **3** to afford the pyrimidinylpropenamides, **11–15**. See Table I for physical properties.

2-Amino-3-(4-tolyl)propionic Acid (40). The method of Albertson and Archer[27] was followed for the preparation of **40** (98%), mp 207–209 °C (lit.[28] mp 211–218 °C), from **41**.

Diethyl 2-Acetamido-2-(4-methylbenzyl)malonate (41). The procedure of Albertson and Archer[27] was followed to give 8.2 g (51%) of **41** as a white solid (recrystallized from ethanol), mp 111–112 °C. Anal. ($C_{17}H_{23}NO_5$) C, H, N.

2-Acetamido-3-(4-methoxyphenyl)propanol (42). The method of Berlinguet[24] was used for the preparation of **42** (89%), mp 98–104 °C (lit.[24] mp 110–111 °C), from **43**.

Ethyl 2-Acetamido-3-(4-methoxyphenyl)propanoate (43). The method of Berlinguet[24] was used for preparation of **43** (96%), mp 65–65.5 °C (lit.[24] mp 72–73 °C), from **44**.

2-Acetamido-2-carbethoxy-3-(4-methoxyphenyl)propanoic Acid (44). The procedure of Berlinguet[24] was followed for the preparation of **44** (63%), mp 139–140 °C (lit.[24] mp 132–133 °C), from **45**.

Diethyl 2-Acetamido-2-(4-methoxybenzyl)malonate (45). The method of Yamamoto et al.[29] was used for the preparation of **45** (85%), mp 93–95 °C (lit.[29] mp 96–98 °C), from **46**.

4-Methoxybenzyl Bromide (46). The procedures of Birch et al.[30] and Fujii et al.[31] were used. Ice-cooled hydrobromic acid (48%, 15 mL) was added to ice-cooled 4-methoxybenzyl alcohol (8.6 g, 62 mmol), and the mixture was vigorously stirred for 30 min to separate a colorless heavy oil. The oil was extracted with benzene and washed successively with water, saturated aqueous sodium bicarbonate, and saturated sodium chloride. The combined benzene extracts were dried over anhydrous sodium sulfate and filtered, and the filtrate was evaporated under reduced pressure to give an oil, which was distilled in vacuo to afford 7.3 g (58%) of **46** as a colorless liquid, bp 88 °C (1 mm) [lit.[32] bp 131 °C (19 mm)].

Experimental Animals. Male CD-1 and DBA/2 mice were obtained from Charles River Breeding Laboratories, Wilmington, MA. The mice were maintained on Purina Laboratory Chow (Ralson Purina, Inc., Richmond, IN) and tap water ad libitum in temperature-controlled quarters with a 12-h light/dark cycle.

Protein Synthesis Inhibition Assay. (1) Bone Marrow Cells. Bone marrow cells were obtained from the femurs of male CD-1 mice and diluted with Spinner's medium (GIBCO, Grand Island, NY) to a concentration of 3×10^6 cells/mL. Aliquots of 200 μL of the cell suspension were added to each well of a microtiter plate (Microtest II, Falcon Plastics, Oxnard, CA). In addition to the cells, 0.2 μCi L-[^{75}Se]selenomethionine (Amersham, Arlington Heights, IL) in 20 μL of Spinner's medium was added to each well. The compounds to be tested were added to the microtiter plate in 20-μL aliquots of 10^{-2} M aqueous solutions (except compound 4, which was added at 10^{-3} M in 10% ethanol). As a negative control for protein synthesis, 20 μL of 10^{-2} M puromycin or 10^{-2} M sparsomycin was added to six wells of bone marrow cells. The final microtiter plate contained six wells of bone marrow cells (BMC) and [^{75}Se]selenomethionine, six wells of each test compound and BMC and [^{75}Se]selenomethionine, six wells of puromycin or sparsomycin and BMC and [^{75}Se]selenomethionine, and six wells of [^{75}Se]selenomethionine (background).

The cells were incubated with the drugs in the microtiter plate on a rocker platform for 2 h at 37 °C, 5% CO_2/95% air, and 95% humidity. After incubation, the cells were harvested on a Titertek cell harvester and radioassayed on a gamma counter (Model 7000 or 300, Beckman Instruments, Fullerton, CA).

(2) P388 Cells. The P388 tumor line was maintained in DBA/2 male mice by serial intraperitoneal passage of 10^5 cells every 7 to 10 days. The tumor cells were harvested by peritoneal lavage, spun at 300*g* for 10 min. The supernatant was decanted, and the cells were reconstituted at a concentration of 3×10^6 cells/mL in Spinner's medium containing 10% heat-inactivated fetal calf serum. The assay described for bone marrow cells was repeated by using these P388 cells.

(3) P815 Cells. P815 cells, courtesy of Dr. Paal Klykken, Department of Pharmacology, MCV-VCU, were maintained in

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culture flasks in complete Dulbecco's modified Eagle's minimum essential medium (CDMEM, GIBCO, Grand Island, NY). The assay described for bone marrow cells was repeated with P815 and CDMEM instead of Spinner's mdeium.

DNA Synthesis Inhibition Assay. (1) **Bone Marrow Cells.** The assay described for protein synthesis inhibition in bone marrow cells was followed by using Alpha Modification of Eagle's minimum essential medium (αMEM, GIBCO, Grand Island, NY) instead of Spinner's medium and 0.1 μCi ^{125}IUdR–2-deoxyuridine (^{125}IUdR, New England Nuclear, Boston, MA) in 20 μL of 2×10^{-5} M 5-fluorodeoxyuridine (FUdR, Sigma Chemical Co., St. Louis, MO) instead of 0.2 μCi of L-[^{75}Se]selenomethionine. As a negative control for DNA synthesis, 20 μL of 10^{-2} M Cytarabine [*ara*-C, NSC-63878, 4-amino-1-β-D-arabinofuranosyl-2(1*H*)-pyrimidinone) was added to the bone marrow cells instead of puromycin or sparsomycin.

(2) **P388 Cells.** The DNA synthesis inhibition assay described for bone marrow cells immediately preceding this was followed using P388 cells harvested as described previously.

Statistical Analysis. The level of confidence for all experiments was set at 95%. A one-way analysis of variance (ANOVA) with a Dunnett's *t* test[33] was used to compare a control to more than one experimental group.

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Registry No. **2**, 28277-67-0; **3**, 86886-21-7; **4**, 86886-22-8; **5**, 86886-23-9; **6**, 86886-24-0; **7**, 86886-25-1; **8**, 86886-26-2; **9**, 86886-27-3; **10**, 86886-28-4; **11**, 86886-29-5; **12**, 86886-30-8; **13**, 86886-31-9; **14**, 86886-32-0; **15**, 86900-71-2; **16**, 1795-98-8; **17**, 35373-66-1; **18**·HCl, 86941-02-8; **19**, 86886-33-1; **20**, 64-04-0; **21**, 3261-62-9; **22**, 55-81-2; **23**, 73918-56-6; **24**, 64018-20-8; **25**, 86886-34-2; **26**, 86886-35-3; **27**, 86886-36-4; **28**, 86886-37-5; **29**, 3303-86-4; **30**, 100-46-9; **31**, 104-84-7; **32**, 2393-23-9; **33**, 3959-07-7; **34**, 102-49-8; **35**, 71273-81-9; **36**, 86886-38-6; **37**, 86886-39-7; **38**, 86886-40-0; **39**, 86886-41-1; **40**, 4599-47-7; **41**, 82291-79-0; **42**, 86886-42-2; **43**, 70529-51-0; **44**, 86886-43-3; **45**, 53612-87-6; **46**, 2746-25-0; 4-bromophenylalanine, 14091-15-7; 4-methoxybenzyl alcohol, 105-13-5.

β_1-Selective Adrenoceptor Antagonists. 1. Synthesis and β-Adrenergic Blocking Activity of a Series of Binary (Aryloxy)propanolamines

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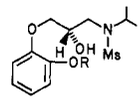
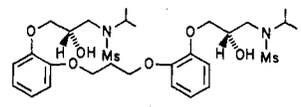
A series of binary (aryloxy)propanolamines has been prepared and examined in vitro and in vivo for β-adrenoreceptor blocking activity. These symmetrical compounds consist of two (*S*)-(phenyloxy)propanolamine pharmacophores coupled through alkylenedioxy or poly(oxyethylenedioxy) linking units of varying lengths. Examples of such binary compounds linked through the 2,2′, 3,3′, and 4,4′ positions in the aromatic rings of the pharmacophores have been prepared. In vitro and in vivo test data indicate that the 2,2′ compounds tend to be selective β_2-adrenergic blocking agents, the 4,4′ binaries tend to be selective β_1-blocking agents, and those compounds with 3,3′ linkages exhibit intermediate selectivities. One of the 4,4′-linked binary compounds, **4s**, exhibited potent, cardioselective β-blockade in vivo, which was of short duration and was accompanied by a prolonged tachycardia.

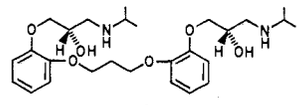
Some time ago, we embarked on a program for the preparation of antihypertensive agents that were designed to act at both the α- and β-adrenoreceptors. Our studies focused on the linking of a β-adrenoreceptor blocking component with various α-blocking moieties via a polymethylene bridge.[2] As part of this work, a reaction was performed in which the β-blocking component **1a** was



1a, R = H
2, R = $(CH_2)_3Br$



3a



4a

alkylated with 1,3-dibromopropane to form **2**; which was then coupled with the α-blocking moiety, phenylpiperazine.[3] A significant side product in the conversion of **1a** to **2** was isolated and identified, not unexpectedly, as the binary compound **3a**. Reductive demesylation of **3a** afforded the corresponding binary (aryloxy)propanolamine **4a**. Interesting results obtained from the initial in vitro screening of **4a** prompted us to develop a series of such binary β-adrenoreceptor blocking agents. These compounds varied mainly in the length and composition of the linking unit, as well as in its position of attachment on the aromatic nuclei of the (aryloxy)propanolamine subunits.

Chemistry. Since the binary (aryloxy)propanolamines have two centers of asymmetry, it was recognized that synthons bearing the oxypropanolamine side chain should be enantiomerically pure prior to their incorporation into the binary structure in order to avoid the problems associated with diastereomeric mixtures. Furthermore, to

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