β-Adrenoceptor Antagonist Activity of Bivalent Ligands. 1. Diamide Analogues of Practolol

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Two series of bivalent ligands (P–X–P) containing the (*R*,*S*)-3-[(4-aminoaryl)oxy]-1-(isopropylamino)propan-2-ol pharmacophore and a connecting α,ω-dicarbonylpoly(methylene) [X = $-OC(CH_2)_nCO-$] or α,ω-*N*,*N*′-bis(carbonylmethylene)polymethylenediamine [X = $-OCCH_2NH(CH_2)_nNHCH_2CO-$] spanner were synthesized and evaluated for β-adrenoceptor antagonist activity in rat heart and lung membrane preparations. The target compounds were obtained as a mixture of stereoisomers in modest yields by using a three to four step sequence beginning with *N*-benzylpractolol. The results from the competitive binding studies indicated that binding affinity increased by a factor of up to 160 by increasing the length of the group spanning the pharmacophore moieties. Modest increases in cardioselectivity were also obtained. The data suggest that further increases in spanner length and lipophilicity and optical resolution may improve the potential of a labeled bivalent β_1-adrenoceptor antagonist to function as a myocardial imaging agent.

As part of our program to develop receptor-site-directed radiopharmaceuticals for the external visualization of the myocardium, we have examined the radioiodinated analogues of the β-adrenoceptor antagonists.[1,2] The data obtained from initial studies indicated that analogues that demonstrate a high in vitro affinity for the receptors possessed neither pharmacologic nor distributional selectivity for the myocardium. On the other hand, compounds such as the practolol analogues that did display pharmacologic and distributional selectivity for the myocardium possessed receptor affinities that were too low to be useful for noninvasive in vivo receptor mapping.[2]

One approach to the problem of how to retain cardioselectivity while simultaneously enhancing receptor affinity was to employ bivalent ligands based on the structure of practolol. Such bivalent ligands would incorporate the 3-(aryloxy)-1-(isopropylamino)propan-2-ol pharmacophore (P) linked by a spanner group (X) through an amino group at the 4′-position of the aryloxy substituent. The bivalent ligand would be expected to show enhanced receptor affinity relative to the monovalent ligand (PX) when the spanner group was long enough to permit simultaneous binding of the pharmacophore to two receptors or one receptor and one accessory binding site.[3] This effect has been recently demonstrated in β-adrenoceptors[4,5] and several other biological systems including binding to opioid receptors,[6-9] DNA intercalation,[10-13] α-adrenoceptors,[14] and peptide hormone receptors.[15-17]

In this paper we report on our studies to evaluate the influence of the length and nature of the spanner on the affinity and cardioselectivity of the bivalent ligand for the β-adrenoceptors in the rat heart and lung which possess relatively high percentages of the two β-adrenoceptor subtypes (β_1 in the heart and β_2 in the lung). As a practical result of the structure–activity relationships derived from this study, radioiodinated derivaties of the compounds that demonstrate the greatest affinity and β_1 selectivity would be prepared and evaluated as potential myocardial imaging agents.

Chemistry

In the design of the bivalent ligands, practolol (1), a selective β_1 adrenoceptor antagonist, was used as the pharmacophore. Although there were several potential sites for inserting a spanner group, the 4′-amino moiety was selected for this study (Figure 1).

The two types of spanner groups that were employed in this investigation were the α,ω-polydicarbonyl(methylene) [$-OC(CH_2)_nCO-$] and α,ω-*N*,*N*′-bis(carbonylmethylene)polymethylenediamine [$-OCCH_2NH(CH_2)_nNHCH_2CO-$] units. The availability of the desired poly(methylene)diacyl dichlorides and polymethylenediamines (n = 2–8) allowed the synthesis of two series of bivalent ligands possessing the desired variation in spanner lengths. The inclusion of the diamine into the spanner unit would permit some differentiation of spanner length and lipophilic effects. In order to evaluate the contribution of the spanner, the monovalent ligand practolol (1) was included in the study.

The first series of bivalent ligands was synthesized as shown in Scheme I. The *N*-benzyl derivative 4 was synthesized and deacetylated via the method described by Crowther et al.[18] Reaction of the 4′-amino free base with

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(1) Hanson, R. N.; Holman, B. L.; Davis, M. A. *J. Med. Chem.* **1978**, *21*, 830.
(2) Hanson, R. N.; Davis, M. A.; Holman, B. L. *J. Nucl. Med.* **1980**, *21*, 436.
(3) Dwyer, J. D.; Bloomfield, V. A. *Biopolymers* **1981**, *20*, 2323.
(4) Pitha, J.; Milecki, J.; Czajkowska, T.; Kusiak, J. W. *J. Med. Chem.* **1983**, *26*, 7.
(5) Kierstead, R. W.; Faraone, A.; Mennona, F.; Mullin, J.; Guthrie, R. W.; Crowley, H.; Simko, B.; Blaber, L. C. *J. Med. Chem.* **1983**, *26*, 1561.
(6) Shimohigashi, Y.; Costa, T.; Matsuura, S.; Chen, H.-C.; Rodbard, D. *Mol. Pharmacol.* **1982**, *21*, 558.
(7) Erez, M.; Takemori, A. E.; Pórtoghese, P. S. *J. Med. Chem.* **1982**, *25*, 847.
(8) Portoghese, P. S.; Ronsisvalle, G.; Larson, D. L.; Yim, C. B.; Sayre, L. M.; Takemori, A. E. *Life. Sci.* **1982**, *31*, 1283.
(9) Portoghese, P. S.; Larson, D. L.; Yim, C. B.; Sayre, L. M.; Ronsisvalle, G.; Lipkowski, A. W.; Takemori, A. E.; Rice, K. C.; Tam, S. W. *J. Med. Chem.* **1985**, *28*, 1140.
(10) Le pecq, J. B.; Le Bret, M.; Barbet, J.; Roques, B. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2915.
(11) Fico, R. M.; Chen, T. K.; Canellakis, E. S. *Science (Washington, D.C.)* **1977**, *198*, 53.
(12) Chen, T. K.; Fico, R.; Canellakis, E. S. *J. Med. Chem.* **1978**, *21*, 868.
(13) Pelaprat, D.; Delbarre, A.; LeGuen, I.; Roques, B. P. *J. Med. Chem.* **1980**, *23*, 1336.
(14) Melchiorre, C.; Yong, M. S.; Benfey, B. G.; Belleau, B. *J. Med. Chem.* **1978**, *21*, 1126.
(15) Shimoyanagi, Y.; Costa, T.; Matsuura, S.; Chen, H. C.; Rodband, P. *Mol. Pharmacol.* **1982**, *21*, 558.
(16) Conn, P. M.; Rogers, D. C.; Stewart, J. M.; Wiedel, J.; Sheffield, T. *Nature (London)* **1982**, *296*, 653.
(17) Fauchere, J.-L.; Rossier, M.; Capponi, A.; Vallotton, M. B. *FEBS Lett.* **1985**, *183*, 283.
(18) Crowther, A. F.; Howe, R.; Smith, L. H. *J. Med. Chem.* **1971**, *14*, 511.

0022-2623/87/1830-0722$01.50/0

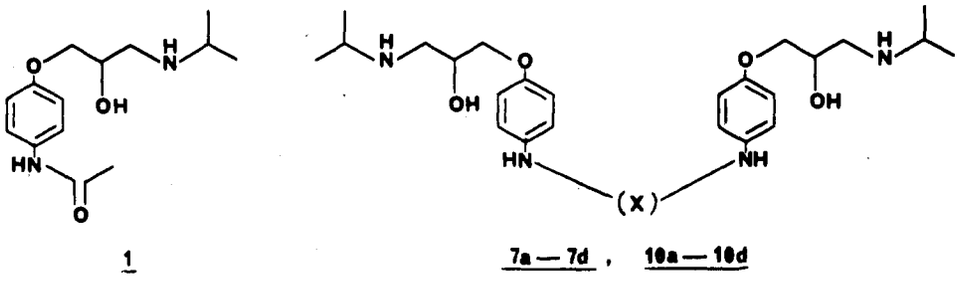


Figure 1. Practolol (1) and general structure proposed for bivalent practolol analogues.

Scheme I

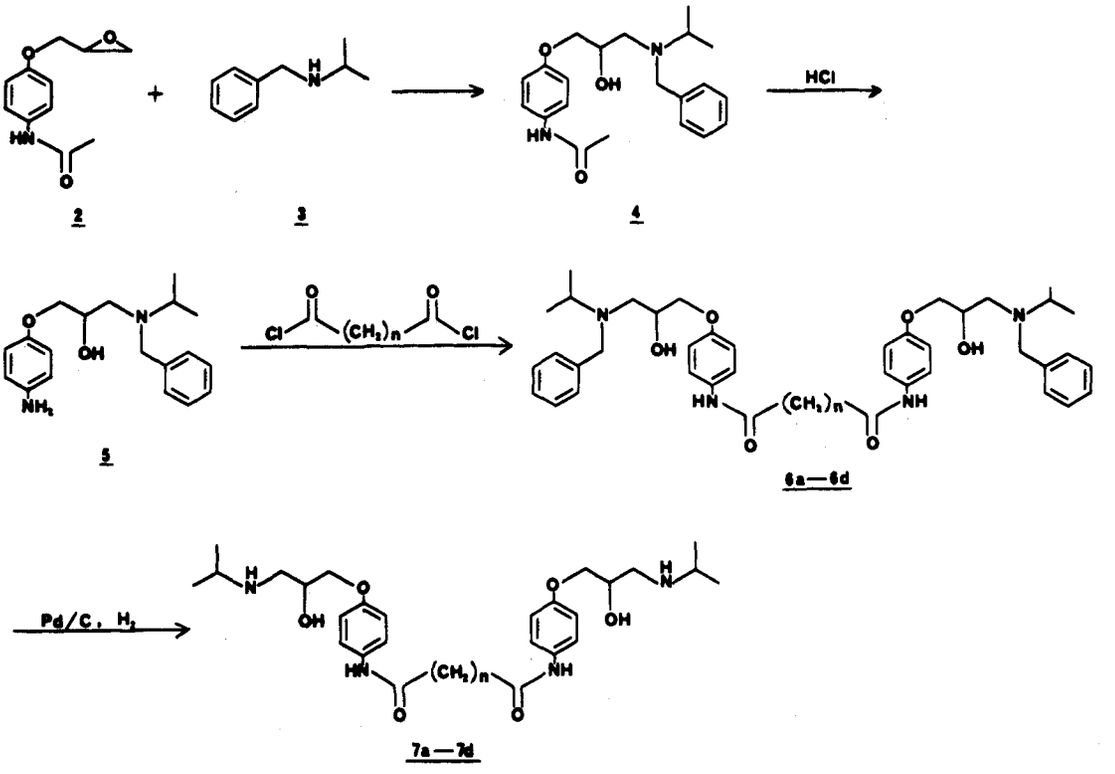


Table I. Yields and Properties of *N,N'*-Dibenzyl Intermediates

compd	*n*	mp, °C	yield,[b] %	recrystn solvent	formula	anal.
6a	2	133–135	18	acetone	$C_{42}H_{54}N_4O_6$	C, H, N
6b	4	141–143	77	EtOH	$C_{44}H_{58}N_4O_6$	C, H, N
6c	6	129–131	60	EtOH	$C_{46}H_{62}N_4O_6$	C, H, N
6d	8	122–125	45	EtOH	$C_{48}H_{66}N_4O_6$	C, H, N
9a	4	163–166	28	EtOH	$C_{46}H_{64}N_4O_6$	C, H, N
9b	6	128–131	8	EtOH	$C_{48}H_{68}N_4O_6$	C, H, N
9c	8	oil	15		$C_{50}H_{72}N_4O_6$	C, H, N
9d	2 + 2[a]	ND[c]	53		$C_{46}H_{62}N_4O_6$	ND

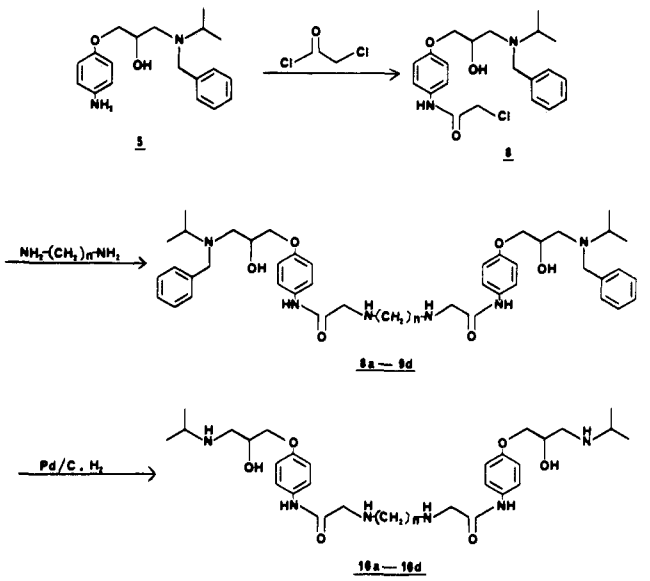
[a] Piperazinyl. [b] Based on **2** or **8** used. [c] ND, not determined.

Table II. Physical Properties of Dimeric β-Antagonists

compd[a]	*n*	mp, °C	yield,[b] %	crystal solvent	λ_{max}, nm	ϵ_{max} ($\times 10^{-4}$)	R_f[d]	formula	anal.
7a	2	203–205	47	MeOH	246	2.25	0.16	$C_{28}H_{42}N_4O_6$	C, N, H
7b	4	188–189	30	EtOH	246	2.44	0.23	$C_{30}H_{46}N_4O_6$	C, N, H
7c	6	172–173	35	EtOH	246	2.61	0.35	$C_{32}H_{50}N_4O_6$	C, N, H
7d	8	167–168	41	EtOAc	246	2.80	0.36	$C_{34}H_{54}N_4O_6$	C, N, H
10a	4	147–148	37	EtOH	246	2.48	0.11	$C_{32}H_{52}N_6O_6$	C, N, H
10b	6	108–112	74	EtOAc	250	2.34	0.17	$C_{34}H_{56}N_6O_6$	C, N, H
10c	8	89–91	36	EtOAc	250	2.55	0.21	$C_{36}H_{60}N_6O_6$	C, N, H
10d	2 + 2[c]	146–147	26	EtOAc	250	2.68	0.27	$C_{32}H_{50}N_6O_6$	C, N, H
practolol (1)		138–139					0.53	$C_{14}H_{22}N_2O_3$	

[a] All the compounds are diastereomeric mixtures of unknown composition. [b] Based on *N,N'*-dibenzyl intermediate used. [c] Piperazinyl. [d] Solvent system C.

Scheme II



the diacyl dichloride in diethyl ether in the presence of triethylamine provided the *N,N'*-dibenzyl bivalent ligands **6a–d** in 29–91% isolated yields (Table I). These intermediates were debenzylated by hydrogenation over palladium-on-carbon to give the desired products **7a–d** in 30–47% yields, after crystallization (Table II).

For the second series, which contained the diamine moiety, the *N*-benzyl-4-amino intermediate **5** was first reacylated with chloroacetyl chloride (Scheme II). The resulting chloroacetanilide derivative was then dissolved in ethanol, and equivalent quantities of sodium iodide and sodium carbonate were added, followed by 0.5 equiv of the α,ω-diamine or piperazine. The reaction mixture was heated at reflux, and the resulting products were separated by column chromatography. The desired *N,N'*-dibenzyl intermediates **9a–d** were isolated in 8–28% yields (Table I). Catalytic debenzylation with hydrogen over palladium-on-carbon gave the final bivalent ligands **10a–d**, in 26–74% yields after crystallization (Table II).

Binding Studies

The dissociation studies were performed according to the method described by Williams et al.[19] using membrane preparations derived from rat hearts and lungs. The total specific binding for both tissues was determined by using (2–6) × 10^{-9} M of [^{3}H]dihydroalprenolol ([^{3}H]DHA) in the presence or absence of 10^{-5} M (±)-propranolol. With use of this method, both the β-adrenoceptor concentration and the percentage of the radioligand bound were higher in the lung membranes (630 fmol/mg of protein) than in the heart membranes (80 fmol/mg of protein). This compares favorably with the previously reported values by Williams et al.[20] and Bieth et al.[21]

The dissociation constants for the bivalent ligands in the heart and lung were obtained by competitive displacement of [^{3}H]DHA in each membrane preparation. Practolol and propranolol were also evaluated in this manner to provide indices of receptor affinity and cardioselectivity. The ability of the unlabeled antagonists to compete with [^{3}H]DHA for the binding sites was determined at various concentrations while constant concentrations of the receptor and the radioligand were maintained. The apparent dissociation constants for the compounds can be calculated from the equation described by Cheng and Prusoff.[22] The IC_{50} values for each compound were determined by plotting the percent [^{3}H]DHA bound vs. the logarithm of the concentration of the compound (10^{-3}–10^{-8} M). The cardioselectivity values of the compounds were calculated from the ratio of the membrane affinities (K_D' of lung/K_D' of heart).

Table III. Apparent Dissociation Constant (K_D') and Cardioselectivity of Bivalent Ligands

compd	*n*	K_D' (heart), 10^{-6} M	K_D' (lung), 10^{-6} M	K_D' (lung)/K_D' (heart)
7a	2	16.3	28.1	1.7
7b	4	9.0	67.5	7.5
7c	6	4.4	3.9	0.9
7d	8	0.1	1.4	14.0
10a	4	32.2	185.6	5.8
10b	6	5.0	28.1	5.6
10c	8	0.5	3.9	7.8
10d	2 + 2[a]	2.1	28.1	13.4
(±)-practolol		6.2	36.5	5.9
(±)-propranolol		0.002	0.004	2.0

[a] Piperazinyl.

The results of these competitive binding studies are shown in Table III. The dissociation constants for the bivalent β-adrenergic antagonists demonstrate consistent trends within each series. Those compounds with the shorter spacer chain have the higher K_D' values in both the heart and lung preparations, e.g., the values for **7a** and **10a** are 1.63 × 10^{-5} and 3.22 × 10^{-5} M in the heart and 5.0 × 10^{-4} and 3.0 × 10^{-4} M in the lungs. Sequential extention of the spacer chain by two carbon units progressively lowers the K_D' values for **7d** and **10c** to 1.0 × 10^{-7} and 5.0 × 10^{-7} M in the heart and 1.4 × 10^{-6} and 3.9 × 10^{-6} M in the lungs. These latter values are substantially lower than that determined for monovalent practolol but still somewhat greater than that obtained for potent, nonselective propranolol. The bivalent ligand **10d**, which contains the piperazinyl moiety as the spacer chain, exhibited the K_D' values similar to those of **10b**.

Discussion

The synthesis of the target compounds was achieved. However, the overall yields in the last two steps, the linking with the spanner moiety and the catalytic debenzylation, provided low to modest yields (8–77% and 26–74%, respectively). The synthetic scheme described in Schemes I and II produces a mixture of enantiomers and diastereomers. No attempts were made to isolate the desired *S,S* isomer and thus all the compounds are diastereomeric mixtures of unknown composition. The K_D' values determined in this study therefore reflected the aggregate values of these isomers and the structure–activity relationships are based upon it. We assume in the interpretation of the data that the pure *S,S* isomer would have a lower K_D' value than that measured in this study and that the overall substituent effects observed with mixtures would also apply to the pure isomer.

Upon examination of our results several substituent effects are apparent. The first is that increasing the length of the spanner group produces a beneficial effect upon receptor binding and the cardioselectivity in each series; the K_D' value drops and the cardioselectivity modestly increase as the length of the spanner group increases.

(19) Williams, L. T.; Jarett, L.; Lefkowitz, R. J. *J. Biol. Chem.* **1976**, *251*, 3096.
(20) Williams, L. T.; Lefkowitz, R. J.; Watanabe, A. M.; Hathaway, D. R.; Besch, H. R., Jr. *J. Biol. Chem.* **1977**, *252*, 2787.
(21) Bieth, N.; Rouot, B.; Schwartz, J.; Velly, J. *Br. J. Pharmacol.* **1980**, *68*, 563.
(22) Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.

Among the practolol and related analogues, the lipophilicity contribution from the substituents on the amino group of the side chain and the *p*-anilino function produces additive effects on β-adrenergic activity but not on the cardioselectivity.[18,23-25] On the other hand, modest increases in the cardioselectivity by increasing the length of spanner chain suggest that bivalent ligands bind to multiple binding sites. There is some evidence, however, that enhanced binding affinity may be a nonreceptor effect since **7d** and **10a**, which possess similar spanner lengths, differ by a factor of greater than 100 in their K_D' values. The major difference here is related to their lipophilicities since **10a** contains two –NH– groups within the spanner moiety. The second major effect, or lack thereof, is that the increase in cardioselectivity is not as great as was anticipated. This may be because the optimal spanner length needed to permit chiral receptor site interaction has not been reached or that the distance between adjacent receptors for both the β_1 and β_2 subtypes has not been reached. The trends for both the K_D' and cardioselectivity effects suggest that further extention of the spanner group may be necessary.

As potential myocardial imaging agents, the bivalent β_1-adrenoceptor antagonists show promise, but not necessarily with the specific examples identified in this study. The best K_D' measure here with **7d** is still at least 1 order of magnitude higher than that suggested by several reviews in the literature (i.e., $K_D = 10^{-8}$–10^{-9} M).[26-28] Also the cardioselectivity would need to be increased substantially to compensate for the higher concentration of β_2 subtype in the lungs that would comprise a significant background. The results suggest that improvements may be achieved by further extention of the spanner group and the resolution of *S,S* stereoisomers. Preliminary study to evaluate the effect of bivalency upon tissue distribution and pharmacokinetics compared to the monovalent practolol derivatives would provide an initial basis for the development of such myocardial imaging agents. This would be accompanied by continued modification of the structure of the parent bivalent ligands with an emphasis on enhanced β_1-adrenoceptor affinity and selectivity.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on either a Perkin-Elmer Model 700 or 599B infrared spectrophotometer. Ultraviolet (UV) spectra were taken on a Beckman grating spectrophotometer. Proton magnetic resonance spectra (^{1}H NMR) were taken on a Varian T-60 NMR spectrometer for 4–8% solutions containing Me_4Si as an internal standard. Chemical shifts are reported on the δ scale with peak multiplicities: d; doublet; m, multiplet; s, singlet. Elemental analyses were performed by Schwartzkopf Microanalytical Laboratory, Inc. and were within ±0.4% of the theoretical values. Thin-layer chromatography was performed with Analtech GF silica gel plates in three solvent systems: solvent A, *n*-BuOH/HOAc/H_2O (4:2:1, v/v/v); solvent B, *n*-BuOH/HOAc/H_2O (8:2:1, v/v/v); solvent C, MeOH/$CHCl_3$/NH_4OH (58%) (10:90:1, v/v/v). Column chromatography was performed on alumina and flash chromatography was carried out on silica gel (230–400 mesh, E. Merck).

4′-(2,3-Epoxypropoxy)acetanilide (2). The oxirane was prepared according to the procedure outlined in the literature.[13] To a stirred solution consisting of 18 g (120 mmol) of 4-acetamidophenol, 6 g (150 mmol) of NaOH, and 150 mL of H_2O was added 14 mL (220 mmol) of epichlorohydrin. The reaction mixture was stirred for 16 h at room temperature and filtered to give 18.1 g of white residue. Recrystallization from MeOH yielded 12.4 g (50%) of the product: mp 119–122 °C (lit.[13] mp 110 °C); TLC R_f 0.55 (solvent A); ^{1}H NMR ($CDCl_3$) δ 2.15 (s, 3 H), 2.7–3.1 (m, 2 H), 3.25–3.6 (m, 1 H), 3.95–4.3 (m, 2 H), 6.8–7.7 (AB, 4 H), 8.0 (s, 1 H).

4′-[3-(Benzylisopropylamino)-2-hydroxypropoxy]acetanilide (4). A solution consisting of 17 g (82.1 mmol) of **2**, 12.6 g (82.1 mmol) of *N*-isopropylbenzylamine, and 150 mL of MeOH was heated at reflux for 5 h. After removal of the solvent the colorless oil was crystallized from diethyl ether to give 26.0 g (89%) of the product: mp 98–100 °C (reported as a pale yellow syrup[15]); TLC R_f 0.74 (solvent B); ^{1}H NMR (Me_2SO-d_6) δ 0.85–1.15 (d, 6 H), 2.1 (s, 3 H), 2.4–2.7 (m, 3 H), 2.7–3.1 (m, 1 H), 3.35 (s, 1 H), 3.65 (s, 2 H), 3.7–4.1 (m, 3 H), 6.1–7.8 (AB, 9 H), 9.8 (s, 1 H).

4′-[3-(Benzylisopropylamino)-2-hydroxypropoxy]aniline (5). Compound **4** (10.7 g, 30 mmol) was deacetylated in 180 mL of a mixture of absolute EtOH and concentrated HCl (2:1, v/v) by heating at reflux for 5 h. The yellowish oil that was obtained after evaporation of the solvent was dissolved in water, basified, and extracted with CH_2Cl_2. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. The solution was filtered and evaporated to dryness to give a brownish oil, which crystallized upon cooling (7.8 g, 82.5%): mp 74–75 °C (lit.[15] bp 198–200 °C (0.15 mm); TLC R_f 0.34 (solvent A); ^{1}H NMR (CCl_4) δ 0.9–1.17 (m, 6 H), 2.5–2.7 (d, 2 H), 2.8–3.1 (m, 1 H), 3.2 (s, 3 H), 3.65 (s, 2 H), 3.7–3.95 (m, 3 H), 6.5–6.7 (AB, 4 H), 7.3 (s, 5 H).

4′-[3-(Benzylisopropylamino)-2-hydroxypropoxy]chloroacetanilide (8). To a solution consisting of 3.14 g (10 mmol) of **5**, 1.5 g (15 mmol) of triethylamine, and 140 mL of diethyl ether was added dropwise 1.7 g (15 mmol) of chloroacetyl chloride. The reaction mixture was stirred at room temperature for 3 h and washed with H_2O. The organic layer was evaporated and the residue was crystallized from MeOH to give 2.73 g (70%) of the product: mp 142–143 °C; TLC R_f 0.81 (solvent C); ^{1}H NMR ($CDCl_3$) δ 0.9–1.25 (m, 6 H), 2.5–2.75 (m, 2 H), 2.8–3.2 (m, 1 H), 3.35 (s, 1 H), 3.55–3.8 (d, 2 H), 3.95 (s, 2 H), 6.75–7.65 (AB, 9 H), 8.2 (s, 1 H); IR (KBr) 1690 (C=O), 3280 cm^{-1} (N–H). Anal. ($C_{21}H_{27}ClN_2O_3$) C, H, N.

General Method for the Preparation of *N,N′*-Bis[4-[2-hydroxy-3-[(1-methylethyl)benzylamino]propoxy]phenyl]-poly(methylene)dicarboxamides 6a–d. To a stirred solution consisting of 2 equiv of the 4-substituted aniline **5** and triethylamine in diethyl ether or methylene chloride was added dropwise 1 equiv of the appropriate diacyl dichloride. The reaction mixture was stirred for 2–3 h at room temperature and washed with 5% Na_2CO_3 solution. The organic layer was separated, washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated to dryness. Column chromatography (Al_2O_3) and crystallization gave the pure compounds. Physical constants are given in Table I.

General Method for the Preparation of 2,2′-(α,ω-Alkanediyldiimino)bis[*N*-[4-[2-hydroxy-3-[(1-methylethyl)-benzylamino]propoxy]phenyl]acetamides] 9a–d. To a solution consisting of 2 equiv of 4-substituted chloroacetanilide **8**, NaI, and Na_2CO_3 in absolute EtOH was added dropwise 1 equiv of the appropriate alkyldiamine. The reaction mixture was heated at reflux for 2 h, filtered, and evaporated to dryness. The residue was dissolved in $CHCl_3$, washed with $Na_2S_2O_3$ solution, H_2O, and brine, and dried over $MgSO_4$. The organic layer was evaporated to dryness and purified by flash chromatography (silica gel). Crystallization from EtOH gave the pure compound. Physical constants are given in Table I.

General Method for the Deprotection of *N,N′*-Dibenzyl Intermediates. Catalytic Hydrogenation. Removal of the *N*-benzyl protective groups of the intermediates **6a–d** and **9a–d**

(23) Clarkson, R. *Antihypertensive Agents*; Engelhardt, Ed.; American Chemical Society: Washington, DC, 1976; pp 1–26.
(24) Smith, L. H. *J. Med. Chem.* **1976**, *19*, 1119.
(25) Erez, M.; Shtacher, G.; Welnstock, M. *J. Med. Chem.* **1978**, *21*, 982.
(26) Gibson, R. E.; Eckelman, W. C.; Rzeszotarski, W. J.; Jian, V.; Mazaitis, A.; Paik, C.; Riba, R. C. In *Principles of Radiopharmacology*; Colombetti, L., Ed.; CRC: Boca Raton, FL, 1979; pp 17–39.
(27) Eckelman, W. C.; Reba, R. C.; Gibson, R. E.; Rzeszotarski, W. J.; Vieras, F.; Mazaitis, J. K.; Francis, B. *J. Nucl. Med.* **1979**, *20*, 350.
(28) Eckelman, W. C.; Gibson, R. E.; Vieras, F.; Rzeszotarski, W. J.; Francis, B.; Reba, R. C. *J. Nucl. Med.* **1980**, *21*, 436.

was carried out in a Parr hydrogenation apparatus using 5% Pd-C. The intermediate was dissolved in 10 mL of MeOH containing 10% HOAc and added to the suspension of 500 mg of the catalyst in 20 mL of MeOH. The reaction mixture was shaken with hydrogen for 16–24 h at room temperature, filtered, and evaporated to dryness. Crystallization gave the pure compounds. Physical constants are given in Table II.

***N,N'*-Bis[4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]decanediamide (7d).** To a stirred solution consisting of 0.63 g (2 mmol) of **5**, 0.2 g (2 mmol) of triethylamine, and 20 mL of CH_2Cl_2 was added dropwise 0.24 g (1 mmol) of sebacoyl chloride. The reaction mixture was stirred for 3 h at room temperature and was washed with 5% Na_2CO_3 solution. The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by column chromatography using Al_2O_3. Crystallization from MeOH gave 0.36 g (45%) of **7d**: mp 122–125 °C; TLC R_f 0.67 (solvent C); ^{1}H NMR ($CDCl_3$) δ 0.9–1.15 (m, 12 H), 1.15–1.4 (m, 8 H), 1.4–2.0 (m, 4 H), 2.0–2.4 (m, 4 H), 2.4–2.7 (m, 4 H), 2.7–3.1 (m, 2 H), 3.6 (s, 4 H), 3.65–3.95 (m, 6 H), 6.55–7.05 (AB, 18 H), 7.9 (s, 2 H); IR (KBr) 1660 (C=O), 3300 cm^{-1} (N–H). Anal. ($C_{48}H_{66}N_4O_6$) C, H, N. The *N,N'*-dibenzyl intermediate **6d** (0.57 g, 0.72 mmol) was dissolved in 10 mL of MeOH containing 10% of HOAc and the mixture was added to the suspension of the catalyst (500 mg) in 20 mL of MeOH. The reaction mixture was shaken with hydrogen for 20 h at room temperature, filtered, and evaporated to dryness. Crystallization from EtOAC gave 0.18 g (41.2%) of the bivalent compound **7d**: mp 167–168 °C; TLC R_f 0.42 (solvent A); ^{1}H NMR (Me_2SO-d_6) 0.9 (d, 12 H), 1.2–1.5 (m, 8 H), 1.5–2.0 (m, 8 H), 2.1–2.5 (m, 4 H), 2.5–3.0 (m, 10 H), 3.8–4.0 (m, 6 H), 6.1–7.7 (m, 8 H), 9.7 (s, 2 H); IR (KBr) 1655 (C=O), 3300 cm^{-1} (N–H). Anal. ($C_{34}H_{54}N_4O_6$) C, H, N.

2,2'-(1,8-Octanediyldiimino)bis[*N*-[4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]acetamide] (10c). To a solution, consisting of 3.9 g (10 mmol) of **8**, 1.5 g (10 mmol) of NaI, 1.06 g (10 mmol) of Na_2CO_3, and 25 mL of EtOH was added dropwise 0.74 g (5 mmol) of 1,8-octanediamine and the mixture was heated at reflux for 2 h. The reaction mixture was filtered and evaporated to give a brownish oil. The oil was dissolved in $CHCl_3$, washed with $Na_2S_2O_3$ solution, H_2O, and brine, and dried over $MgSO_4$. The organic layer was evaporated to dryness and purified by flash chromatography (silica gel) to give 0.65 g (15.3%) of a yellow oil (**9c**): TLC R_f 0.75 (solvent C); ^{1}H NMR ($CDCl_3$) δ 0.96–1.2 (m, 12 H), 1.1–1.7 (m, 12 H), 2.5–3.3 (m, 4 H), 3.4 (s, 4 H), 3.7 (d, 4 H), 3.85–4.10 (m, 6 H), 6.8–7.75 (AB, 18 H), 9.3 (s, 2 H); IR (KBr) 1700 (C=O), 3330 cm^{-1} (N–H). Anal. ($C_{50}H_{72}N_6O_6$) C, H, N. The *N,N'*-dibenzyl intermediate **9c** (1.16 g, 1.26 mmol) was dissolved in 10 mL of MeOH containing 10% of HOAc and the mixture was added to the suspension of the catalyst (500 mg) in 20 mL of MeOH. The reaction mixture was shaken with hydrogen for 20 h at room temperature, filtered, and evaporated to dryness. Crystallization from EtOAc gave 0.33 g (35.6%) of **10c**: mp 89–91 °C; TLC R_f 0.21 (solvent C); ^{1}H NMR ($CDCl_3$) δ 1.10 (d, 12 H), 1.20–1.55 (m, 12 H), 3.3 (s, 4 H), 3.85–4.0 (m, 6 H), 6.7–7.5 (AB, 8 H), 9.1 (s, 2 H); IR (KBr) 1660 (C=O), 3300 cm^{-1} (N–H). Anal. ($C_{36}H_{60}N_6O_6$) C, H, N.

Radioligand Binding Assay. All binding assays were performed in disposable culture tubes (12 × 75 mm) in triplicate according to the method described by Williams et al.[19] using membrane preparations derived from rat hearts and lungs according to the method described by Williams and Lefkowitz.[20] The protein concentration of the final membrane preparation was determined by the Lowry procedure[29] using a standardized bovine serum solution. The membrane protein ranging from 100 to 300 μg was incubated with (2–6) × 10^{-9} M of [^{3}H]DHA in a final volume of 150 μL of the assay buffer (50 mM Tris·HCl, 10 mM $MgCl_2$, pH 7.4 at 37 °C) for 12 min at 37 °C. Incubations were terminated by adding 2 mL of the ice-cold assay buffer followed by rapid vacuum filtration of the suspension through a Whatman GF/C glass fiber filter. The filter was then placed in a scintillation vial and 10 mL of scintillation cocktail (Formula-963, NEN, Boston) was added. The vial was vortexed for 5 s and radioactivity counted in a Packard Tricarb 3325 liquid scintillation spectrometer. In each experiment, "nonspecific" binding was determined by measuring the amount of radioactivity retained on filters when incubations were performed in the presence of 10^{-5} M (±)-propranolol. The "specific" binding was determined by subtracting the "nonspecific" binding from the total counts bound.

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Registry No. (±)-**2**, 106247-87-4; (±)-**4**, 106163-09-1; (±)-**5**, 43203-60-7; (±)-**6a**, 106163-11-5; *meso*-**6a**, 106163-26-2; (±)-**6b**, 106163-12-6; *meso*-**6b**, 106163-27-3; (±)-**6c**, 106163-13-7; *meso*-**6c**, 106163-28-4; (±)-**6d**, 106163-14-8; *meso*-**6d**, 106163-29-5; (±)-**7a**, 106163-18-2; *meso*-**7a**, 106163-30-8; (±)-**7b**, 106163-19-3; *meso*-**7b**, 106163-31-9; (±)-**7c**, 106163-20-6; *meso*-**7c**, 106191-51-9; (±)-**7d**, 106163-21-7; *meso*-**7d**, 106163-32-0; (±)**8**, 106163-10-4; (±)-**9a**, 106163-15-9; *meso*-**9a**, 106163-33-1; (±)-**9b**, 106163-16-0; *meso*-**9b**, 106163-34-2; (±)-**9c**, 106163-17-1; *meso*-**9c**, 106163-35-3; (±)-**9d**, 106191-50-8; *meso*-**9d**, 106191-52-0; (±)-**10a**, 106163-22-8; *meso*-**10a**, 106163-36-4; (±)-**10b**, 106163-23-9; *meso*-**10b**, 106163-37-5; (±)-**10c**, 106163-24-0; *meso*-**10c**, 106163-38-6; (±)-**10d**, 106163-25-1; *meso*-**10d**, 106163-39-7; $ClCO(CH_2)_2COCl$, 543-20-4; $ClCO(CH_2)_4COCl$, 111-50-2; $ClCO(CH_2)_6COCl$, 10027-07-3; $ClCO(CH_2)_8COCl$, 111-19-3; $H_2N(CH_2)_4NH_2$, 110-60-1; $H_2N(CH_2)_6NH_2$, 124-09-4; $H_2N(CH_2)_8NH_2$, 373-44-4; 4-acetamidophenol, 103-90-2; (±)-epiclorohydrin, 13403-37-7; *N*-isopropylbenzylamine, 102-97-6; chloroacetyl chloride, 79-04-9; piperazine, 110-85-0.

(29) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.

Benzimidazole Derivatives with Atypical Antiinflammatory Activity

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A number of substituted 2-[(2,2,2-trifluoroethyl)sulfonyl]-1*H*-benzimidazoles (**4**) have demonstrated antiinflammatory activity that appears to have a mechanism distinct from typical cyclooxygenase inhibiting nonsteroidal antiinflammatory drugs. Several of these compounds inhibit adjuvant-induced arthritis in rats at 25 mg/kg while showing no activity in the carrageenan paw edema model at up to 100 mg/kg. Two compounds, **4a** and **4b**, showed no significant inhibition of cyclooxygenase in vitro at concentrations as high as 5 × 10^{-5} M. All compounds **4** active in adjuvant-induced arthritis were also found to inhibit release of lysosomal enzymes from neutrophils, raising the possibility that their antiinflammatory effect is at least partially mediated by an effect on neutrophil function.

A number of reports have appeared in the literature of antiinflammatory heterocycles bearing (polyfluoroalkyl)-sulfonyl or (polyfluoroalkyl)thio side chains.[1-3] A common feature of these structures (**1–3**) is a sulfonylurea or

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