

238–240 °C; (IR) (KBr) 1660, 1625, 1500, 1450, 1425 cm^{-1} ; ^1H NMR ($\text{Me}_2\text{SO}-d_6$) δ 4.65 (s, 2 H), 7.18–7.66 (m, 8 H), 7.78 (s, 1 H), 8.07 (s, br, NH), 8.23 (d, $J = 8.0$ Hz, 1 H), 8.41 (s, 1 H), 11.56 (s, indole 1 H); MS (CI, CH_4), 274 ($M + 1$). Anal. ($\text{C}_{18}\text{H}_{15}\text{N}_3\text{HCl} \cdot 1/3\text{EtOH}$) C, H, N.

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Registry No. 2, 114819-74-8; 2 (free base), 91985-82-9; 3, 114819-73-7; 3 (free base), 91985-81-8; 4, 114819-75-9; 4 (free base), 91985-83-0; 5, 91985-80-7; 6, 114819-76-0; 6 (free base), 114819-77-1; 7, 114819-79-3; 8, 114819-72-6; 8 (free base), 73834-77-2; 8 (dihydrochloride), 114819-78-2; 9, 91985-78-3; 13, 114819-81-7; 13 (free base), 114819-80-6; 18, 106613-33-6; benzaldehyde, 100-52-7.

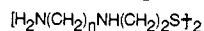
Structure-Activity Relationships among Benextramine-Related Tetraamine Disulfides. Chain Length Effect on α -Adrenoreceptor Blocking Activity¹

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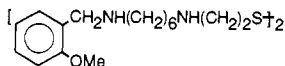
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Several N' -substituted N,N'' -(dithiodi-2,1-ethanediyl)bis(1, ω -alkanediamines) were prepared and evaluated for their blocking activity on α -adrenoreceptors in the isolated rat vas deferens and human blood platelets. The results were compared with those obtained for benextramine (N,N'' -(dithiodi-2,1-ethanediyl)bis[N' -(2-methoxyphenyl)methyl]-1,6-hexanediamine], 10). Bendotramine (N,N'' -(dithiodi-2,1-ethanediyl)bis[N' -(2-methoxyphenyl)methyl]-1,12-dodecanediamine], 16) proved to be as active as 10 on α_1 -adrenoreceptors, showing that optimum activity is associated with two carbon chain lengths separating inner from outer nitrogens of tetraamine disulfides. On the other hand, 16 had no activity up to 20 μM at α_2 -adrenoreceptors. The optimum activity at this receptor subtype was associated with a six to eight carbon chain (10–12). Furthermore, 10 proved to be more selective toward α_2 -adrenoreceptors whereas 16 was a selective α_1 -antagonist. The tetraamine disulfides were shown also to be potent inhibitors of human platelet aggregation induced by ADP or epinephrine. The potency increased with the carbon chain length. However, the results on platelets did not parallel those found in the rat vas deferens, indicating that differences exist between the α -adrenoreceptor subtypes investigated. In conclusion, 10 may be a useful tool in characterizing α_2 -adrenoreceptors whereas 16 might help in investigating α_1 -adrenoreceptors.

The development of polymethylene tetraamines, whose main feature is a cystamine moiety carrying aminoalkyl substituents on the nitrogens, as α -antagonists started from the observation that the linear tetraamine disulfide 1 ($n = 5$), originally developed as a radioprotective agent,² showed a relatively weak but apparently selective and irreversible α -blocking activity. Extensive structure-activity relationships were carried out with the aim of improving the potency of 1 and elucidating the active-site topography in the region of the target thiol.^{3,4} These studies led to the discovery of compounds such as benextramine (10),^{4a} pyrextamine,^{4g} or 4 ($n = 8$)^{4a} displaying an α_1 -blocking activity about 2 orders of magnitude higher than that of the parent compound 1.



1-8: $n = 5-12$



10 (Benextramine)

It was shown that α_1 -adrenoreceptor inhibition by tetraamine disulfides is the result of covalent bond formation between a receptor target thiol and the disulfide bridge of the antagonist through a disulfide-thiol interchange reaction.^{3,4a} It was also found that optimum activity is associated with two different carbon chain lengths separating the inner from the outer nitrogens and depends on the type of substituents on the terminal nitrogens. Thus, optimum α_1 -blocking activity in the series with benzyl-type substituents on the terminal nitrogens is associated with a six-carbon chain, as in 10, on rat vas deferens^{4a,g} and rabbit aorta^{4d} and left atrium⁵ whereas in the unsubstituted series it is associated with an eight-carbon chain, as in 4, on rat vas deferens^{4a} and a seven-carbon chain, as in 3, on rabbit aorta.^{4d}

The prototype of tetraamine disulfides 10 has been fairly well investigated in both functional studies and binding experiments.^{4a,g,6-27} Compound 10 irreversibly blocked

- (1) Some aspects of this work were presented at the French-Italian Joint Meeting on Medicinal Chemistry, September 22–26, 1987, Pisa, Italy; Abstract L10, p 18.
- (2) Démarée, G. E.; Brockenton, R. E.; Heiffer, M. H.; Rothie, W. E. *J. Pharm. Sci.* 1971, 60, 1743.
- (3) Lippert, B.; Belleau, B. *Frontiers in Catecholamine Research*; Usdin, E., Snyder, S. H., Eds.; Pergamon: London, 1973; p 369.
- (4) (a) Melchiorre, C.; Young, M. S.; Benfey, B. G.; Belleau, B. *J. Med. Chem.* 1978, 21, 1126. (b) Ueda, Y.; Melchiorre, C.; Lippert, B.; Belleau, B.; Chona, S.; Triggle, D. J. *Farmaco Ed. Sci.* 1978, 33, 479. (c) Melchiorre, C.; Giardinà, D.; Brasili, L.; Belleau, B. *Farmaco Ed. Sci.* 1978, 33, 999. (d) Melchiorre, C.; Giannella, M.; Brasili, L.; Benfey, B. G.; Belleau, B. *Eur. J. Med. Chem.* 1981, 16, 111. (e) Brasili, L.; Giannella, M.; Melchiorre, C.; Belleau, B.; Benfey, B. G. *Eur. J. Med. Chem.* 1981, 16, 115. (f) Giardinà, D.; Brasili, L.; Melchiorre, C.; Belleau, B.; Benfey, B. G. *Eur. J. Med. Chem.* 1981, 16, 569. (g) Angeli, P.; Brasili, L.; Brancia, E.; Giardinà, D.; Quaglia, W.; Melchiorre, C. *J. Med. Chem.* 1985, 28, 1643.

- (5) Benfey, B. G.; Belleau, B.; Brasili, L.; Giannella, M.; Melchiorre, C. *Can. J. Physiol. Pharmacol.* 1980, 58, 1477.
- (6) Brasili, L.; Angeli, P.; Brancia, E.; Melchiorre, C. *Eur. J. Pharmacol.* 1984, 103, 181.
- (7) Furchgott, R. F. *Vascular Neuroeffector Mechanisms*; Bevan, J. A., Godfraind, T., Maxwell, R. A., Vanhoutte, P. M., Eds.; Raven: New York, 1980; p 245.
- (8) Benfey, B. G.; Yong, M. S.; Belleau, B.; Melchiorre, C. *Can. J. Physiol. Pharmacol.* 1979, 57, 510.
- (9) Reader, T. A.; Brière, R.; Grondin, L. *Neurochem. Res.* 1986, 11, 9.
- (10) Redaer, T. A.; Brière, R.; Grondin, L. *J. Neural. Transm.* 1987, 68, 79.
- (11) Brasili, L.; Cassinelli, A.; Angeli, P.; Melchiorre, C. *Life Sci.* 1986, 38, 1633.
- (12) Mottram, D. R.; Thakar, Y. *Br. J. Pharmacol.* 1983, 80, 562P.
- (13) Benfey, B. G.; Yong, M. S.; Belleau, B.; Melchiorre, C. *Can. J. Physiol. Pharmacol.* 1979, 57, 41.

Table I. α -Adrenoreceptor Blocking Activity in the Isolated Rat Vas Deferens and Inhibition of Human Blood Platelet Aggregation^a
[ArCH₂NHYNR(CH₂)₂S]₂

no.	antagonist ^b Y	rat vas deferens			human blood platelet	
		α_1 IC ₅₀ , μ M, against norepinephrine	α_2 IC ₅₀ , μ M, against clonidine	α_2/α_1 selectivity ratio ^c	IC ₅₀ , μ M, against epinephrine	IC ₅₀ , μ M, against ADP
9	(CH ₂) ₅	55.80 \pm 4.1	25.80 \pm 7.10	2.16	46.0 \pm 3.6	87.0 \pm 8.8
10	(CH ₂) ₆	7.55 \pm 0.68	0.82 \pm 0.086	9.20	31.3 \pm 2.5	97.0 \pm 12.9
11	(CH ₂) ₇	55.20 \pm 9.1	1.04 \pm 0.21	53.08	20.6 \pm 2.6	41.3 \pm 0.9
12	(CH ₂) ₈	98.50 \pm 16.5	1.22 \pm 0.12	80.74	5.4 \pm 0.2	22.7 \pm 1.8
13	(CH ₂) ₉	68.00 \pm 9.8	inactive ^e	<3.4	2.3 \pm 0.5	6.0 \pm 0.1
14	(CH ₂) ₁₀	62.80 \pm 11.3	inactive ^e	<3.14	2.2 \pm 0.4	2.8 \pm 0.3
15	(CH ₂) ₁₁	51.30 \pm 10.0	inactive ^e	<2.57	1.7 \pm 0.1	2.1 \pm 0.4
16	(CH ₂) ₁₂	8.23 \pm 1.47	inactive ^e	<0.41	1.7 \pm 0.1	2.1 \pm 0.1
17	(CH ₂) ₁₂	7.30 \pm 5.4	inactive ^e	<0.37		
18	(CH ₂) ₁₂	46.90 \pm 0.9	inactive ^e	<2.35		
21	(CH ₂) ₂ CONH(CH ₂) ₈	inactive ^d				
22	(CH ₂) ₅ CONH(CH ₂) ₆	inactive ^d				
23	(CH ₂) ₃ NH(CH ₂) ₈	inactive ^d				
24	(CH ₂) ₆ NH(CH ₂) ₆	inactive ^d				
adenosine					1.8 \pm 0.3	2.5 \pm 0.4

^a IC₅₀ values are expressed as the mean \pm standard error of at least four independent observations. ^b 9-16, 21-24: Ar = 2-MeOC₆H₄, R = H. 17: Ar = 2-pyrrolyl, R = H. 18: Ar = 2-MeOC₆H₄, R = Me. ^c The α_2/α_1 selectivity ratio is a measure of the selectivity and is the antilog of the difference between the $-\log$ IC₅₀ values at α_2 - and α_1 -adrenoreceptors. ^d Inactive up to a concentration of 20 μ M (see the Experimental Section). ^e Inactive up to a concentration of 20 μ M (see the Experimental Section).

Table II. Physical Characteristics of Polyamine Disulfides

no.	X	R	Y	[XNH(CH ₂) _n N(CH ₂) ₂ S] ₂		mp, ^a $^{\circ}$ C	purifn solvent ^b	formula ^c
				7. 13-18	19-24			
7	H	H		11		289-291	A	C ₂₆ H ₅₈ N ₄ S ₂ ·4HCl
13	2-MeOC ₆ H ₄ CH ₂	H		9		166-168	A/B	C ₃₈ H ₉₆ N ₄ O ₂ S ₂ ·4HCl·H ₂ O
14	2-MeOC ₆ H ₄ CH ₂	H		10		195-197	C	C ₄₀ H ₇₀ N ₄ O ₂ S ₂ ·4HCl·H ₂ O
15	2-MeOC ₆ H ₄ CH ₂	H		11		198-200 ^d	A-B	C ₄₂ H ₇₄ N ₄ O ₂ S ₂ ·4HCl·H ₂ O
16	2-MeOC ₆ H ₄ CH ₂	H		12		192-194	A-B	C ₄₄ H ₇₈ N ₄ O ₂ S ₂ ·4HCl·2H ₂ O
17	pyrrol-2-ylmethyl	H		12		indef ^e	D	C ₃₈ H ₇₂ N ₆ S ₂ ·4H ₂ C ₂ O ₄ ·H ₂ O
18	2-MeOC ₆ H ₄ CH ₂	Me		12		149-151 ^f	A-B	C ₄₆ H ₈₂ N ₄ O ₂ S ₂ ·4H ₂ C ₂ O ₄
19	H		CO	8	2	225-227	A-D	C ₂₆ H ₅₆ N ₆ O ₂ S ₂ ·4HBr
20	H		CO	6	5	192-197	C	C ₂₈ H ₆₀ N ₆ O ₂ S ₂ ·4HBr
21	2-MeOC ₆ H ₄ CH ₂		CO	8	2	191-192	A-D	C ₄₂ H ₇₂ N ₆ O ₄ S ₂ ·4H ₂ C ₂ O ₄ ·H ₂ O
22	2-MeOC ₆ H ₄ CH ₂		CO	6	5	186-188	A-D	C ₄₄ H ₇₆ N ₆ O ₄ S ₂ ·4H ₂ C ₂ O ₄ ·1.5H ₂ O
23	2-MeOC ₆ H ₄ CH ₂		CH ₂	8	2	237-239	A	C ₄₂ H ₇₆ N ₆ O ₂ S ₂ ·6HCl·1.5H ₂ O
24	2-MeOC ₆ H ₄ CH ₂		CH ₂	6	5	235-237	C	C ₄₄ H ₈₀ N ₆ O ₂ S ₂ ·6HCl·3H ₂ O

^a All compounds were crystallized at least three times and decolorized with charcoal when necessary. Their purity was uniformly checked by TLC (silica). IR and NMR spectra of all compounds were in agreement with the expected structures. The heating rate was 1 $^{\circ}$ C/min for melting point determinations. ^b A, methanol; B, 2-propanol; C, absolute ethanol; D, water. ^c Analyses for C, H and N were within \pm 0.4% of the theoretical value required. ^d Decomposition began at 180 $^{\circ}$ C. ^e Compound 17 has indefinite melting point. Decomposition began at 110 $^{\circ}$ C. ^f It crystallizes also with two water molecules: mp, 128-131 $^{\circ}$ C.

both α_1 - and α_2 -adrenoreceptors.^{4,11} However, nothing is known about the structural requirements for optimum

occupancy of α_2 -adrenoreceptors by tetraamine disulfides. To this end we investigated the effect of the chain length separating the inner from the outer nitrogens of tetraamine disulfides on the α_2 -blocking activity of isolated rat vas deferens and human blood platelets. Since at α_1 -adrenoreceptors only tetraamine disulfides 9-12 having five to eight methylenes between the nitrogens were investigated,^{4a} we extended that study to compounds 13-16 in order to achieve a better understanding of the chain length effect on α_1 -blocking activity. Since 16 ($n = 12$) turned out to be as active as 10 ($n = 6$), some derivatives were designed with the aim of correlating the binding sites of 16 with those of 10 or alternatively 4. Thus, hexamine disulfides 23 and 24 were investigated to verify whether 16 may bind with two additional receptor anionic sites situated at an

- (14) Benfey, B. G.; Brasili, L.; Melchiorre, C.; Belleau, B. *Can. J. Physiol. Pharmacol.* 1980, 58, 984.
 (15) Belleau, B.; Benfey, B. G.; Benfey, T. J.; Melchiorre, C. *Br. J. Pharmacol.* 1982, 75, 573.
 (16) Belleau, B.; Benfey, B. G.; Melchiorre, C. *Br. J. Pharmacol.* 1982, 75, 617.
 (17) Belleau, B.; Benfey, B. G.; Melchiorre, C.; Mountambault, M. *Br. J. Pharmacol.* 1982, 76, 253.
 (18) Melchiorre, C.; Gallucci, P. *Farmaco Ed. Sci.* 1983, 38, 950.
 (19) Brazenor, R. M.; Angus, J. A. *J. Pharmacol. Exp. Ther.* 1981, 218, 530.
 (20) Coates, J.; Weetman, D. F. *Br. J. Pharmacol.* 1983, 80, 343.
 (21) Kenakin, T. P. *J. Pharmacol. Exp. Ther.* 1981, 216, 210.
 (22) Lew, M. J.; Angus, J. A. *Eur. J. Pharmacol.* 1984, 98, 27.
 (23) Plotek, Y.; Atlas, D. *Eur. J. Biochem.* 1983, 133, 539.
 (24) Plotek, Y.; Atlas, D. *Highlights in Receptor Chemistry*; Melchiorre, C., Giannella, M., Eds.; Elsevier Science: Amsterdam, 1984; p 97.
 (25) Vohra, M. M.; Chaudhry, A. *Can. J. Physiol. Pharmacol.* 1984, 62, 1147.

- (26) Timmermans, P. B. M. W. M.; Thoolen, M. J. M. C.; Mathy, M. J.; Wilffert, B.; de Jonge, A.; van Zwieten, P. A. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1985, 329, 404.
 (27) Mohammed, T.; Berlan, M.; Lafontan, M. *Mol. Pharmacol.* 1987, 31, 89.

appropriate distance from the binding sites for 10 or 4. In fact, 23 and 24 have a chain length separating the inner from the outer nitrogens that is similar to that in 16 while bearing additional nitrogens inserted in the carbon chain at the same position of the outer nitrogens of 10 and 4, respectively. N-methylation of the inner nitrogens of 10 and 4 resulted in a different effect on activity, suggesting that 10 and 4 can hardly interact with the same set of anionic sites.^{4a,28,29} Thus, a similar structural modification was performed on the inner nitrogens of 16 yielding the *N,N'*-dimethyl analogue 18 to gain information on its binding sites. To the same end, the effect of terminal nitrogen substitution of 16 affording 17 was investigated.

Chemistry. Benextramine analogues were synthesized by standard procedures and their structure is shown in Table II.

The previously unreported tetraamine disulfide 7 (*n* = 11) was obtained through the reaction of thiazolidine and *N*-(11-bromoundecyl)phthalimide,³⁰ affording *N*-[11-(1,3-thiazolidin-3-yl)undecyl]phthalimide. Oxidative ring opening and dimerization by treatment with iodine followed by removal of the phthalimido protecting group by hydrazine treatment gave tetraamine disulfide 7. The substituents on the terminal nitrogens were easily introduced by condensation of 1-8^{4a} with the appropriate aromatic aldehyde and subsequent reduction of the intermediate Schiff bases affording 9-17. Substituted tetraamine disulfides 13-17 were previously unreported.

3-(12-Aminododecyl)-1,3-thiazolidine was obtained by the procedure already reported for other analogues³¹ and was converted to *N,N'*-(dithiodi-2,1-ethanediy)bis[*N'*-(benzyloxycarbonyl)-1,12-dodecanediamine] by reaction with benzyloxycarbonyl chloride followed by oxidative ring opening and dimerization with iodine. Methylation of the inner nitrogens was carried out by the Eschwiler-Clarke method³² followed by removal of the benzyloxycarbonyl protecting group to give *N,N'*-(dithiodi-2,1-ethanediy)-*N,N'*-dimethylbis(1,12-dodecanediamine), which was transformed into 18 as described for 9-17.

3-(6-Aminoethyl)- and 3-(8-aminoethyl)-1,3-thiazolidines³¹ were *N*-acylated with *N*-(benzyloxycarbonyl)-6-aminohexanoic acid or *N*-(benzyloxycarbonyl)-3-aminopropionic acid. Oxidative ring opening and dimerization with iodine followed by acid hydrolysis of the benzyloxycarbonyl protecting group gave tetraamine diamide disulfides 22 and 21. Terminal substitution was carried out as already described for 9-17. Finally, reduction of tetraamine diamide disulfides 21 and 22 followed by oxidation gave hexamine disulfides 23 and 24.

Pharmacology. The biological profile of hexamine and tetraamine disulfides at α -adrenoreceptors was assessed on isolated rat vas deferens and human blood platelets (Table I and Figure 1).

α_1 -Adrenoreceptor blocking activity was assessed by antagonism of (-)-norepinephrine-induced contractions of the epididymal portion of the vas deferens. α_2 -Adrenoreceptor blocking activity was determined by antagonism of the clonidine-induced depression of the twitch responses of the field-stimulated prostatic portion of vas deferens.

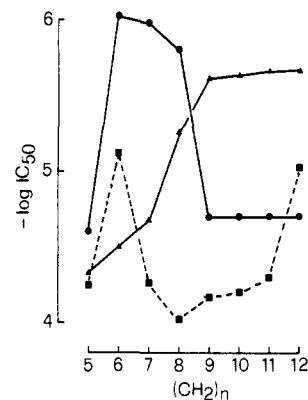


Figure 1. Comparative potencies of the tetraamine disulfides 10-16 at rat vas deferens α_1 - (■) and α_2 -adrenoreceptors (●), and human blood platelet α -adrenoreceptors (▲) vs length of the carbon chain [(CH₂)_n] separating inner from outer nitrogens. The results are expressed as $-\log IC_{50}$ values calculated from Table I. Note that the values for 13-16 at α_2 -adrenoreceptors were calculated assuming IC_{50} values of 20 μ M (see table I).

In order to allow comparison of the results, we used the same techniques and statistical evaluation of the bioassays as for other tetraamine disulfides.^{4a,g} Thus, the noncompetitive (irreversible) α_1 - or α_2 -antagonism was determined at three different concentrations after a 30-min incubation followed by 30 min of washing. The decrease in maximum response was expressed as a percentage of the control value.

α -Adrenoreceptors of human platelets are often said to be similar to α_2 -adrenoreceptors, but there are differences.^{17,33} Thus, tetraamine disulfides 9-16 were investigated on human platelet aggregation to verify whether there is a correlation with the potency displayed at rat vas deferens. To this end the ability of 9-16 to inhibit epinephrine-induced as well as ADP-induced platelet aggregation, in comparison with that of adenosine, was measured in vitro in human platelet-rich plasma.

The potency for each compound is expressed as IC_{50} values, the concentrations that produce 50% inhibition of the agonist maximal response.

Results and Discussion

The biological results obtained in the present investigation are assembled in Table I and are graphically shown in Figure 1. It is evident that benextramine analogues 9-18 have a significant and irreversible α_1 -blocking activity whereas compounds 21-24 have no activity up to a concentration of 20 μ M. It was previously found that with terminal 2-methoxybenzyl substituents optimum activity is strongly associated with a six-carbon chain as in 10.^{4a,28,29} Present results show that a 12-carbon chain is as effective as a six-carbon one. In fact, bendotramine (16) was as active as benextramine (10) (Figure 1). It was advanced that substituted and unsubstituted tetraamine disulfides, exemplified by 10 and 4, respectively, bind with two distinct sets of sites disposed like a cross while sharing a common target thiol.^{4c,28,29,34} This hypothesis was based on the observation that N-methylation of the common cystamine segments of 10 and 4 had a significant effect on the activity (decrease) of only one (10) of the two prototypes and that the unsymmetrical tetraamine disulfide which results from the combination of half of 10 with half

(28) Melchiorre, C.; Belleau, B. *Adrenoceptors and Catecholamine Action*, Kunos, G., Ed.; Wiley: New York, 1981; Part A, p 181.

(29) Melchiorre, C. *Trends Pharmacol. Sci.* 1981, 2, 209.

(30) Elderfield, C. R.; Gensler, J. W.; Bembry, H. T.; Brody, F.; Widerhold, L.; Newman, B. *J. Am. Chem. Soc.* 1946, 68, 1568.

(31) Melchiorre, C.; Giardinà, D.; Angeli, P. *J. Heterocycl. Chem.* 1980, 17, 1215.

(32) (a) Eschwiler, W. *Ber Dtsch.-Chem. Ges.* 1905, 38, 880. (b) Clarke, H. T.; Gillespie, H. B.; Weisshaus, S. *Z. J. Am. Chem. Soc.* 1933, 55, 4571.

(33) Bylund, D. B. *Pharmacol. Biochem. Behav.* 1985, 22, 835.

(34) Melchiorre, C.; Benfey, B. G.; Brasili, L.; Bolger, G.; Belleau, B. *Recent Advances in Receptor Chemistry*; Gualtieri, F., Giannella, M., Melchiorre, C., Eds.; Elsevier/North-Holland: Amsterdam, 1979; p 207.

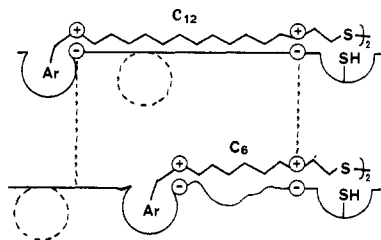


Figure 2. Hypothetical conformational change in the rat vas deferens α_1 -adrenoreceptor induced by terminal *N*-(2-methoxybenzyl)benzyl (Ar) substitution and chain length separating the nitrogens of tetraamine disulfides bendotramine (16) and benextramine (10).

of 4 retained the same activity as the prototypes.^{4c} Furthermore, on the basis of protection experiments and substituent effects we have advanced the idea that the terminal nitrogens of 10 may interact with the anionic site recognized by the neurotransmitter while the 2-methoxybenzyl substituent would bind to an accessory area.^{28,29} Present results raise the question of whether the receptor binding sites recognized by the tetraamine disulfide 16 are identical with those for 10 and 4, nonidentical but connected, or unconnected.

As preliminary remarks, we note that 4 can be excluded because of the observation that *N*-methylation of the inner nitrogens of 16, giving 18, had the same effect on activity (decrease) (Table I) as in 10 (in contrast to 4),^{4c} suggesting that the inner nitrogens of 16 and those of 4 can hardly interact with the same set of sites. Furthermore, the finding that the anionic sites for the terminal nitrogens of 4 do not accept benzyl-type substituents⁴ eliminates also the possibility of a common binding site for the terminal nitrogens of 16 and 4.

We will limit our discussion to the first two possibilities because our data is not sufficient to confirm or exclude the third one. The simplest explanation of the results obtained with 10 and 16 would be provided either of the following possibilities: (a) the two antagonists bind with a single set of sites that adapts conformationally at either one of the two structures or (b) the terminal nitrogens of 16 bind with two additional receptor anionic sites whereas the cystamine moiety interacts with the same sites for 10 (inner nitrogens). The latter hypothesis was ruled out by the observation that the hexamine disulfide 24, bearing two additional cationic nitrogens at the same distance as in 16 (outer nitrogens), had no activity at a concentration (20 μM) (Table I) at which both 10 and 16 inhibit completely rat vas deferens α_1 -adrenoreceptors (not shown). In favor of possibility a, we observe that the substitution of 2-methoxybenzyl groups of 16 for pyrrol-2-ylmethyl groups, giving 17, resulted in a slight increase of activity as in 10. Taken together, the parallel effects observed by substituting the inner and outer nitrogens of 10 and 16 point to a common set of sites (hypothesis a). That the binding sites be identical requires the distance separating the receptor anionic sites, when 16 is the ligand, must be shortened by six bond lengths. This could be accomplished by a receptor conformational change induced by the different chain length of 10 (Figure 2).

Table I and Figure 1 clearly show that the structural requirements for optimum α -adrenoreceptor occupancy by tetraamine disulfides are different at the two receptor subtypes. Compounds 9–12 have an irreversible α_2 -blocking activity that is significantly higher than that observed at the α_1 -adrenoreceptor. In contrast, tetraamine disulfides 13–18 have no activity up to 20 μM concentration. Thus, optimum activity at rat vas deferens α_2 -adrenoreceptors is now associated with a six to eight carbon

chain length separating inner and outer nitrogens. The finding that 16 has no α_2 -blocking activity up to 20 μM is of interest as this is a concentration that completely inhibits α_1 -adrenoreceptors. This clearly indicates that 16 is a selective and irreversible α_1 -adrenoreceptor antagonist whereas 10 is more selective toward α_2 -adrenoreceptors having IC_{50} values of 7.55 and 0.82 μM for α_1 - and α_2 -adrenoreceptors, respectively. The higher selectivity of 10 for α_2 -adrenoreceptors has also been confirmed by binding experiments.^{9,10} However, tetraamine disulfides 11 and 12 bearing a seven- and an eight-carbon chain, respectively, proved to be the most selective toward α_2 -adrenoreceptors with an α_2/α_1 selectivity ratio of about 2 orders of magnitude while being as active as 10 (Table I). The lack of activity of 16 at α_2 -adrenoreceptors may be relevant for delineating differences between active-site topographies in the region of the target thiol of α_1 - and α_2 -adrenoreceptors. If 16 interacted with α_1 -adrenoreceptors according to the hypothesis outlined in Figure 2, its inactivity toward α_2 -adrenoreceptors would indicate that this receptor carries four complementary anionic sites around a target thiol for the binding of 10 but is unable to accommodate 16 by way of a conformational change.

Tetraamine disulfides 9–16 were effective inhibitors of human platelet aggregation induced by ADP and epinephrine (Table I). The results clearly indicate that there is a strict correlation between inhibitory activity and carbon chain length. As to the ADP-induced platelet aggregation, compounds 9 ($n = 5$, $\text{IC}_{50} = 87 \mu\text{M}$) and 10 ($n = 6$, $\text{IC}_{50} = 95 \mu\text{M}$) are about 45 times less effective than 16 ($n = 12$, $\text{IC}_{50} = 2.1 \mu\text{M}$), the inhibitory activity of compounds 14–16 being approximately the same. Also in the case of epinephrine-induced aggregation the inhibitory potency correlates with carbon chain length. Thus, compound 9 ($\text{IC}_{50} = 46 \mu\text{M}$) is about 30 times less active than 15 and 16 ($\text{IC}_{50} = 1.7 \mu\text{M}$). It is worth noting that benextramine analogues gave a completely different pattern at platelet α_2 -adrenoreceptors when compared to that observed at rat vas deferens α_1 - and α_2 -adrenoreceptors.

In a previous study it was observed that the biological profile of 10 differs from that of phentolamine, which has a much higher α -adrenoreceptor blocking potency in platelets. In fact, it has been shown that¹⁷ phentolamine causes 51% inhibition of the epinephrine effect in platelets in a concentration of 100 nM, whereas it only slightly affects the ADP-induced aggregation. In the present study we conclude that the analogues of 10 antagonize aggregation induced by ADP in concentrations slightly higher than those that inhibit aggregation induced by epinephrine. It is also seen that the IC_{50} values for compounds 14–16 are similar to those for adenosine both in ADP- and in epinephrine-induced aggregation.

In conclusion, the present investigation has confirmed and extended the view that tetraamine disulfides are effective antagonists of rat vas deferens α -adrenoreceptors and human platelet aggregation induced by epinephrine. However, the structural requirements for optimum occupancy were shown to be different at the receptor subtypes investigated.

Experimental Section

Chemistry. Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and NMR spectra were recorded on Perkin-Elmer 297 and Varian EM-390 instruments, respectively. Although the IR and NMR spectral data are not included (because of the lack of unusual features), they were obtained for all compounds reported and were consistent with the assigned structures. The microanalyses were performed by the Microanalytical Laboratory of our department, and the elemental compositions of the compounds agreed to within $\pm 0.4\%$

of the calculated value. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.063–0.200 mm, Merck). The term "dried" refers to the use of anhydrous sodium sulfate.

***N*-[11-(1,3-Thiazolidin-3-yl)undecyl]phthalimide.** A solution of *N*-(11-bromoundecyl)phthalimide³⁰ (3.8 g, 10.0 mmol) and an excess of thiazolidine (3.0 g) in absolute EtOH (40 mL) was heated under reflux for 40 h under nitrogen. The solvent was removed under reduced pressure, and the residue was taken up in 1 N NaOH (25 mL) and extracted with chloroform (3 × 50 mL). Extracts were dried and then evaporated to give an oil, which was purified by column chromatography by eluting with cyclohexane–chloroform–ethyl acetate (7:2:1), to give 1.25 g of desired compound, which was used in the next step without further purification.

***N,N'*-(Dithiodi-2,1-ethanediyl)bis(1,11-undecanediamine) Tetrahydrochloride (7).** A solution of *N*-[11-(1,3-thiazolidin-3-yl)undecyl]phthalimide (1.25 g, 3.22 mmol) in chloroform was treated dropwise with a solution of 0.1 N iodine in 2.5% potassium iodide (32.2 mL) with stirring. The reaction mixture was treated with 2 N NaOH (25 mL) and extracted with chloroform (3 × 50 mL). Removal of dried solvents gave an oil, which was dissolved in absolute EtOH (25 mL) and treated with hydrazine monohydrate (0.17 g). The solution was heated under reflux for 24 h. Removal of solvent gave a residue, which was taken up in 6 N HCl (10 mL) and heated at 50 °C for 15 min. The reaction mixture was diluted with warm water (200 mL), and the precipitate was filtered off and washed with water. The solution was washed with chloroform (30 mL) and then made basic with 6 N NaOH (10 mL) and extracted with chloroform (4 × 30 mL). Extracts were dried and then evaporated to give 0.63 g of 7, which was purified by crystallization of the tetrahydrochloride salt (Table II).

***N,N'*-(Dithiodi-2,1-ethanediyl)bis[*N'*-(2-methoxyphenyl and pyrrol-2-yl)methyl]-1,ω-alkyldiamine] Tetrahydrochlorides (13–16) and Tetraoxalate (17).** These compounds were synthesized from the unsubstituted tetraamine disulfide⁴⁸ (5–8) and the appropriate aldehyde (2-methoxybenzaldehyde or pyrrole-2-carboxaldehyde) following the procedure described for benextramine (10) and analogues.^{48a} Compounds 13–16 were purified by crystallization of the tetrahydrochloride salts whereas 17 was characterized as tetraoxalate salt (Table II). Yields were in the range of 55–70%.

3-(12-Aminododecyl)-1,3-thiazolidine Dihydrochloride. This compound was synthesized in 82% yield from *N*-(12-aminododecyl)cysteamine dihydrochloride⁴⁸ via the procedure described for 3-(6-aminoethyl)-1,3-thiazolidine.³¹ mp 135–139 °C (from 2-PrOH). Anal. (C₁₅H₃₆Cl₂N₂S) C, H, N.

***N,N'*-(Dithiodi-2,1-ethanediyl)bis[*N'*-(benzyloxycarbonyl)-1,12-dodecanediamine].** Benzyl chloroformate (1.25 g, 7.34 mmol) was added dropwise to a stirred and cooled solution of 3-(12-aminododecyl)-1,3-thiazolidine as the free base (2.0 g, 7.34 mmol) and Et₃N (0.75 g, 7.41 mmol) in chloroform (100 mL). After the mixture stood for 1 h, the solvent was removed under reduced pressure to give a residue, which was taken up in EtOAc–cyclohexane (1:1). The precipitate was removed by filtration. Removal of solvents gave an oil, which was purified by column chromatography by eluting with ethyl acetate–cyclohexane (14:86), to give 1.1 g (37%) of a white solid (mp 59–60 °C). This solid (2.71 mmol) was dissolved in chloroform (100 mL) and treated with a solution of 0.1 N iodine in 2.5% KI (27.1 mL) with stirring. After 1 h, the reaction mixture was made basic with 2 N NaOH (25 mL) and was extracted with chloroform (3 × 50 mL). Extracts were dried and then evaporated to give 0.9 g (84%) of desired compound (mp 80–82 °C), which was used in the next step without further purification.

***N,N'*-(Dithiodi-2,1-ethanediyl)-*N,N'*-dimethylbis(1,12-dodecanediamine).** *N,N'*-(Dithiodi-2,1-ethanediyl)bis[*N'*-(benzyloxycarbonyl)-1,12-dodecanediamine] (0.9 g, 1.14 mmol) was treated with HCOOH (5 mL) and 40% HCHO (5 mL) for 8 h at reflux temperature, and, after cooling, the mixture was made basic with 40% NaOH (20 mL) and was extracted with chloroform (3 × 50 mL). Extracts were dried and then evaporated to give a residue, which was dissolved in AcOH (4 mL) and treated with HBr gas for 10 min with stirring. After 1 h at room temperature, ether was added to the solution, which was kept overnight at 0

°C. The precipitate was filtered and then dissolved in water; the resulting solution was made basic with 2 N NaOH (5 mL), saturated with NaCl, and extracted with chloroform (3 × 30 mL). Extracts were dried and then evaporated to give 0.29 g (50%) of desired compound as an oil, which was used without further purification in the next step.

***N,N'*-(Dithiodi-2,1-ethanediyl)-*N,N'*-dimethylbis[*N'*-(2-methoxyphenyl)methyl]-1,12-dodecanediamine] Tetraoxalate (18), *N,N'*-(Dithiodi-2,1-ethanediyl)bis[*N'*-(3-[(2-methoxyphenyl)methyl]amino)propanoyl]-1,8-octanediamine] and *N,N'*-(Dithiodi-2,1-ethanediyl)bis[*N'*-(6-[(2-methoxyphenyl)methyl]amino)hexanoyl]-1,6-hexanediamine] Tetraoxalates (21 and 22).** Compounds 18, 21, and 22 were synthesized from *N,N'*-(dithiodi-2,1-ethanediyl)-*N,N'*-dimethylbis(1,12-dodecanediamine), 19 and 20, respectively, and 2-methoxybenzaldehyde following the procedure described for 10.⁴⁸ Crude 18 was purified as the free base by column chromatography by eluting with concentrated ammonia–MeOH (2:98). It was converted into its oxalate salt by treating an alcoholic solution with 4 equiv of oxalic acid dihydrate. After cooling, the solid was collected, washed with ether, and recrystallized to give pure 18 in 60% yield (Table II).

Crude 21 and 22 were purified in 75% yield by crystallization of the tetraoxalate salts (Table II).

***N,N'*-(Dithiodi-2,1-ethanediyl)bis[*N'*-(3-amino)propanoyl]-1,8-octanediamine] and *N,N'*-(Dithiodi-2,1-ethanediyl)bis[*N'*-(6-amino)hexanoyl]-1,6-hexanediamine] Tetrahydrobromides (19 and 20).** Ethyl chlorocarbonate (2.51 g, 23.0 mmol) was added dropwise to a stirred and cooled (ice) solution of *N*-(benzyloxycarbonyl)-3-aminopropionic acid (5.15 g, 23.0 mmol) and Et₃N (2.34 g, 23.0 mmol) in chloroform (300 mL), followed after 30 min by the addition of a solution of 3-(8-aminooctyl)-1,3-thiazolidine³¹ (5.0 g, 23.0 mmol) in chloroform (30 mL). The resulting reaction mixture was stirred overnight at room temperature and then was washed with 2 N HCl, 2 N NaOH, and finally with water. Extracts were dried and then evaporated to give a white solid, which was washed with ethyl acetate and then ether to yield 6.0 g (62%) of a solid (mp 91–92 °C) that was used in the next step without further purification. A solution of 0.1 N iodine in 2.5% KI (142 mL) was added dropwise with stirring to a solution of the compound obtained above (6.0 g, 14.2 mmol) in chloroform (180 mL). After 1 h, the reaction mixture was made basic with 2 N NaOH and extracted with chloroform (3 × 50 mL). Extracts were dried and then evaporated to give 4.2 g of a yellow solid (mp 115–119 °C), which was dissolved in AcOH (15 mL) and treated with HBr gas for 10 min with cooling (ice). After 1 h at room temperature, ether was added to the solution, which was kept overnight at 0 °C. The precipitate was collected and purified by crystallization to give 3.18 g of 19 as the tetrahydrobromide salt.

Compound 20 was similarly obtained starting from 3-(6-aminoethyl)-1,3-thiazolidine³¹ and *N*-(benzyloxycarbonyl)-6-aminohexanoic acid (Table II).

***N,N'*-(Dithiodi-2,1-ethanediyl)bis[*N'*-(3-[(2-methoxyphenyl)methyl]amino)propyl]-1,8-octanediamine] and *N,N'*-(Dithiodi-2,1-ethanediyl)bis[*N'*-(6-[(2-methoxyphenyl)methyl]amino)hexyl]-1,6-hexanediamine] (23 and 24).** A solution of 10 M BH₃CH₃SCH₃ (0.1 mL) in dry diglyme (5 mL) was added dropwise at room temperature to a solution of 22 (0.43 g, 0.52 mmol) in dry diglyme (30 mL) with stirring under a stream of dry nitrogen with exclusion of moisture. When the addition was completed, the reaction mixture was heated at 120 °C for 8 h. After the mixture was cooled at 0 °C, excess borane was destroyed by cautious dropwise addition of 20 mL of MeOH. The resulting mixture was left at room temperature for 5 h with stirring, treated with HCl gas for 10 min, and then was heated at 120 °C for 4 h. Removal of the solvent under reduced pressure gave a residue, which was crystallized from MeOH, yielding 0.27 g (51%) of the corresponding thiol of 24. This solid was dissolved in water (1 mL), the pH was adjusted to 8–9 by the addition of 1 N NaOH, and the solution treated with potassium ferricyanide (0.47 g) in water (1 mL). After being allowed to stand 30 min, the reaction mixture was made basic with NaOH pellets (0.5 g) while being cooled in ice and then was saturated with NaCl and extracted with chloroform (3 × 25 mL). Extracts were washed (saturated NaCl), dried, and then evaporated to give 0.1 g (47%)

of 24 (free base) as an oil, which was dissolved in absolute EtOH, and the solution was treated with HCl gas. Removal of the solvent gave 24 as the hexahydrochloride salt.

Compound 23 was similarly obtained in a global yield of 28% starting from 21 (Table II).

Pharmacology. Rat Vas Deferens. Male albino rats (175–200 g) were killed by a sharp blow on the head and both vasa deferentia were isolated, freed from adhering connective tissue, and transversely bisected. Prostatic (12 mm in length) and epididymal portions (14 mm in length) were prepared and mounted individually in baths of 20-mL working volume containing physiological salt solution of the following composition (PSS) (mM): NaCl, 118.4; KCl, 4.7; CaCl₂, 2.52; MgSO₄·7H₂O, 1.2; KH₂PO₄, 1.2; NaHCO₃, 25.0; glucose, 11.1. The MgSO₄·7H₂O concentration was reduced to 0.6 mM when twitch response to field stimulation was studied. The medium was maintained at 37 °C and gassed with 95% O₂–5% CO₂. The loading tension used to assess α_1 - or α_2 -blocking activities was 0.5 g, respectively, and the contractions were recorded by means of force transducers connected to a two-channel Gemini 7070 polygraph.

Field stimulation of the tissue was carried out by means of two platinum electrodes, placed near the top and bottom of the vas deferens, at 0.1 Hz with square pulses of 3-ms duration at a voltage of 10–15 V. The stimulation voltage was fixed throughout the experiments.

Propranolol hydrochloride (1 μ M) and cocaine hydrochloride (10 μ M) were present in the PSS throughout the experiments outlined below to block β -adrenoreceptors and neuronal uptake mechanisms, respectively.

Postsynaptic α_1 -adrenoreceptor blocking activity was determined on the epididymal portion of the vas deferens. The tissues were allowed to equilibrate for at least 1 h before the addition of any drug. Norepinephrine dose–response curves were obtained cumulatively, the first one being discarded and the second one taken as a control. After incubation with the antagonist for 30 min followed by 30-min washing, a third dose–response curve was obtained. Responses were expressed as a percentage of the maximal response obtained in the control curve. Parallel experiments, in which tissues did not receive any antagonist, were run in order to correct for time-dependent changes in agonist sensitivity.³⁵ It was generally verified that the third dose–response curve was identical with the second because the change in dose–ratio is less than 2, which usually represents a minimal correction.

α_2 -Adrenoreceptor blocking activity was assessed on the prostatic portion of the vas deferens with clonidine as the agonist. The procedure was substantially the same as described for unstimulated vasa, with the only exception being that two dose–response curves to clonidine were constructed and the first one was taken as a control.

Each antagonist was tested at three different concentrations and each concentration was investigated at least four times. Compounds 21–24, however, showed no α_1 -adrenoreceptor blocking activity up to a concentration of 20 μ M. At higher concentrations they gave only a slight displacement to the right of norepinephrine dose–response curve without affecting the maximum. Similarly compounds 13–18 showed no α_2 -adrenoreceptor blocking activity up to 20 μ M. They could not be studied at higher concentrations because they markedly depressed the twitch of the field stimulated vas deferens in a dose-dependent fashion. The twitch was abolished at 100 μ M (results not shown). However, it was found that it was probably not an α_2 -agonistic effect since the depression was not reversed by the addition of a selective antagonist-like idazoxan.

The α -blockade for each compound is expressed as IC₅₀ values, which were estimated from graphical plots of percent inhibition

vs log molar concentration and are defined as the concentrations that produce 50% inhibition of the maximal response to the agonist.

Human Blood Platelets. Blood was obtained by venipuncture in the forearms of apparently healthy humans and collected in polyethylene tubes containing ¹/₁₀ final volume of 3.8% trisodium citrate. Platelet-rich plasma (PRP) was prepared by centrifugation of the blood for 10 min at 200g and platelet-poor plasma (PPP) was obtained by centrifugation for 20 min at 1500g. PRP was adjusted to 350 000 platelets/mL by adding PPP. Platelet aggregation was measured photometrically³⁶ with a BIO/DATA Platelet aggregation profiler (Model PAP-3) and calculated as the percentage decrease in optical density of the PRP, 100% being that of PPP.

The potential inhibitors were dissolved in 10% dimethyl sulfoxide, which was present in a final concentration of 0.5% or less in all test samples and controls, and had no effect at this concentration on platelet aggregation. ADP and (–)-epinephrine were used as aggregating agents. In a typical experiment, 940 μ L of PRP was pipetted into each 1-mL siliconized glass cuvette containing 20 μ L of the test compound or control. The cuvette was allowed to incubate at 37 °C for 5 min, after which 10 μ L of an aqueous solution of ADP (final concentration 2–6 μ M) or epinephrine (final concentration 4–6 μ M) was added to induce platelet aggregation.

Inhibition of platelet aggregation by a test compound was calculated by dividing the maximum deflection in the optical density curve by that observed in the control, then multiplying by 100. Each antagonist was tested at three different concentrations and each concentration was investigated at least four times. IC₅₀ values for each inhibitor were estimated from graphical plots of % inhibition vs log molar concentration and were defined as the concentrations that produce 50% inhibition of the aggregatory response to ADP or epinephrine. Data were expressed as the mean \pm standard error.

Statistical Evaluation. Student's *t* test was used to assess the significance of the experimental results.

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Registry No. 5, 75575-23-4; 6, 77799-84-9; 7, 115483-35-7; 8, 82414-72-0; 9, 68535-78-4; 10, 69790-18-7; 11, 75575-15-4; 12, 82414-71-9; 13, 115483-57-3; 13·4HCl, 115483-39-1; 14, 115483-58-4; 14·4HCl, 115483-40-4; 15, 115483-59-5; 15·4HCl, 115483-41-5; 16, 115483-60-8; 16·4HCl, 115483-42-6; 17, 115483-43-7; 17·4H₂C₂O₄, 115483-44-8; 18, 115483-45-9; 18·4H₂C₂O₄, 115483-46-0; 19, 115483-61-9; 19·4HBr, 115483-47-1; 20, 115483-62-0; 20·4HBr, 115483-48-2; 21, 115483-49-3; 21·4H₂C₂O₄, 115483-50-6; 22, 115483-63-1; 22·4H₂C₂O₄, 115512-51-1; 23, 115483-64-2; 23·6HCl, 115483-51-7; 24, 115483-65-3; 24·6HCl, 115483-52-8; *N*-(11-bromoundecyl)phthalimide, 115483-33-5; thiazolidine, 504-78-9; *N*-[11-(1,3-thiazolidin-3-yl)undecyl]phthalimide, 115483-34-6; 2-methoxybenzaldehyde, 135-02-4; pyrrole-2-carboxaldehyde, 1003-29-8; 3-(12-aminododecyl)-1,3-thiazolidine dihydrochloride, 115483-36-8; 3-(12-aminododecyl)-1,3-thiazolidine, 115483-37-9; 3-[12-[(benzyloxycarbonyl)amino]dodecyl]-1,3-thiazolidine, 115483-38-0; *N,N'*-(dithio-2,1-ethanediyl)bis[*N'*-(benzyloxycarbonyl)-1,12-dodecanediamine], 115483-53-9; *N,N'*-(dithio-2,1-ethanediyl)-*N,N'*-dimethylbis(1,12-dodecanediamine), 115483-54-0; *N*-(benzyloxycarbonyl)-3-aminopropionic acid, 2304-94-1; 3-(8-aminoctyl)-1,3-thiazolidine, 115483-55-1; *N*-[8-[[3-[(benzyloxycarbonyl)amino]-1-oxopropyl]amino]octyl]-1,3-thiazolidine, 115483-56-2; 3-(6-aminoethyl)-1,3-thiazolidine, 88346-63-8; *N*-(benzyloxycarbonyl)-6-aminohexanoic acid, 1947-00-8; 2-[[[6-[(2-methoxybenzyl)amino]hexyl]amino]hexyl]amino]ethanethiol, 115512-52-2.

(35) Furchgott, R. F. *Catecholamines*; Blaschko, H., Muscholl, E., Eds.; Springer Verlag: Berlin, 1972; p 283.

(36) Michal, F.; Born, G. V. R. *Nature (London)* 1971, 231, 220.