(d, 1 H), 7.96 (d, 1 H), 8.23 (d, 1 H), 8.23 (d, 1 H). Anal. ($C_{20}H_{24}N_2O_8 \cdot H_2O$) C, H, N.

***N*-[2-(Diethylamino)-2-(1-naphthalenyl)ethyl]-4-[(methylsulfonyl)amino]benzamide Hydrochloride (3l).** Reaction of **4l** (10.6 g, 43.7 mmol) with **5** (11.2 g, 48 mmol) as described for **3a** gave 5.9 g (28%) of **3l**: NMR (DMSO-d_6) δ 1.03 (t, 3 H), 1.46 (t, 3 H), 2.86 (m, 2 H), 3.02 (s, 3 H), 3.62 (br, 2 H), 3.96 (m, 1 H), 4.32 (m, 1 H), 5.60 (br, 1 H), 7.13 (d, 2 H), 7.59 (m, 5 H), 8.00 (t, 2 H), 8.24 (d, 1 H), 8.50 (d, 1 H), 8.74 (m, 1 H), 10.15 (s, 1 H), 11.38 (br s, 1 H).

Pharmacology. The experimental protocols describing the intracellular electrophysiological studies in canine Purkinje fibers,[3] intraduodenal activity studies in anesthetized dogs,[3] and the PES efficacy model[3] have been reported previously.

Ouabain-Induced Ventricular Arrhythmias in the Guinea Pig.[9] The guinea pig was anesthetized with 50 mg/kg pentobarbital, iv, and a lead II ECG monitored. The trachea was cannulated and the animal respirated with room air. The right jugular was cannulated for administration of test agent and ouabain. After equilibrating for 5 min, the test agent was administered. Ten minutes later a bolus dose of ouabain (40 μg/kg) was administered followed by a constant fusion of 10 μg/kg/min ouabain. Time to first arrhythmia was measured, beginning at the start of the ouabain infusion.

Acknowledgment. We thank the Berlex Analytical Section for support of this project. We are grateful to Hannah Troy, Mark Caroll, C. Michael Doroshuk, Deborah Moore, and Kenneth Sansone, who carried out the pharmacology experiments. We thank Dr. William Lumma for helpful discussions during the course of the project. For the preparation of the manuscript we thank Christine Juhasz.

Registry No. **3a**, 116855-61-9; **3a** (free base), 116855-32-4; **3b**, 116855-68-6; **3b** (free base), 123507-50-6; **3c**, 116855-69-7; **3c** (free base), 123507-51-7; **3d**, 123507-53-9; **3d** (free base), 123507-52-8; **3e**, 123507-54-0; **3f**, 116855-60-8; **3f** (free base), 116855-31-3; **3g**, 116855-63-1; **3g** (free base), 116855-34-6; **3h**, 116855-62-0; **3h** (free base), 116855-33-5; **3i**, 116855-64-2; **3i** (free base), 116855-40-4; **3j**, 116855-41-5; **3k**, 123507-55-1; **3l**, 116855-66-4; **3l** (free base), 116855-42-6; **4b**, 123507-56-2; **4b**·HCl, 123507-57-3; **4e**, 123507-58-4; **4e** (free base), 123507-59-5; **4g**, 116855-49-3; **4j**, 123507-60-8; **4j** (free base), 116855-55-1; **4l**, 123507-61-9; **4l** (free base), 116855-52-8; **5**, 63421-72-7; **6g**, 116855-48-2; **7j**, 123507-62-0; **8j**, 116855-54-0; **9l**, 123507-63-1; *N*,*N*-diethyl-*N*′-phenyl-1,2-ethanediamine, 1665-59-4; 2-(diethylamino)-*N*-(4-ethylphenyl)acetamide, 56974-52-8; *N*-(4-chlorophenyl)-*N*′,*N*′-diethyl-1,2-ethanediamine, 5427-35-0; *N*,*N*-diethyl-*N*′-(4-methoxyphenyl)-1,2-ethanediamine, 123507-64-2; *N*-(4-aminophenyl)methanesulfonamide, 53250-82-1; (diethylamino)ethyl chloride hydrochloride, 869-24-9; *N*,*N*-diethyl-*N*′-(2,6-dimethylphenyl)-1,2-ethanediamine, 21236-57-7; 2-chloro-*N*-[2,6-bis(1-methylethyl)phenyl]acetamide, 20781-86-6; diethylamine, 109-89-7; *N*,*N*-diethyl-*N*′-(1-naphthalenyl)-1,2-ethanediamine, 5235-86-9; N^2,N^2-diethyl-1-phenyl-1,2-ethanediamine, 31788-87-1; 2-bromo-1-(1-naphthalenyl)ethanone, 13686-51-6; N^1,N^1-diethyl-1-phenyl-1,2-ethanediamine, 31788-97-3; 1-naphthalenecarboxaldehyde, 66-77-3.

1,9-Alkano-Bridged 2,3,4,5-Tetrahydro-1*H*-3-benzazepines with Affinity for the α_2-Adrenoceptor and the 5-HT_{1A} Receptor

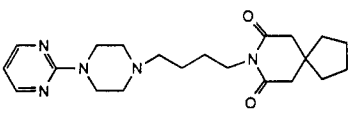
Robin D. Clark,*,† Klaus K. Weinhardt,*,† Jacob Berger,† Lawrence E. Fisher,† Christine M. Brown,‡ Alison C. MacKinnon,‡ Andrew T. Kilpatrick,‡ and Michael Spedding‡

Institute of Organic Chemistry, Syntex Research, Palo Alto, California 94304, and Department of Pharmacology, Syntex Research Center, Edinburgh, Scotland EH14 4AP. *Received December 7, 1988*

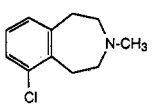
A number of 1,9-alkano-bridged 2,3,4,5-tetrahydro-1*H*-3-benzazepines were prepared and evaluated for 5-HT_{1A} receptor and α_2-adrenoceptor affinity by using radioligand receptor binding techniques. Several compounds displayed 5-HT_{1A} receptor affinity comparable to, or greater than, the known 5-HT_{1A} ligand buspirone. The highest affinity 5-HT_{1A} receptor ligands were *N*-alkyl-, *N*-allyl-5-chloro-, and 5-methoxy-1,2,3,4,8,9,10,10a-octahydronaphth[1,8-*cd*]azapines (**4c**, **4m**, **4n**), which had pK_i values of 7.9–8.1. The *S* enantiomer of **4c** had a higher affinity for the 5-HT_{1A} receptor than the corresponding *R* isomer (pK_i of 8.2 for (***S***)-**4c** vs 7.7 for (***R***)-**4c**). These compounds had a relatively low affinity for the α_2-adrenoceptor (pK_i of 7 or less). On the other hand, the closely related 5-chloro-2-methyl-2,3,4,8,9,9a-hexahydro-1*H*-indeno[1,7-*cd*]azepine (**3b**) had high affinity for both the α_2-adrenoceptor (pK_i = 8.1) and 5-HT_{1A} receptor (pK_i = 7.6). These results indicate that the two receptors may share common recognition sites.

Classification of receptors into subtypes based on binding of selective ligands continues to be an active area of research. For example, reviews on α-adrenoceptor[1-3] and 5-hydroxytryptamine receptor[4,5] classification emphasize the extensive progress which has been made in these areas.

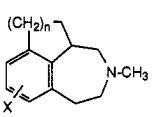
Interest in ligands with high affinity for the 5-HT_{1A} receptor subtype has been stimulated by the finding that one such agent, buspirone (1), is a clinically efficacious anxiolytic.[6a-d] The α_2-adrenoceptor antagonism of the buspirone metabolite 1-(2-pyrimidyl)piperazine[7] prompted us to investigate interrelationships between other α_2-adrenoceptor and 5-HT_{1A} receptor ligands. We determined that the α_2-adrenoceptor antagonist **2** (SKF 86466)[8] had affinity for the 5-HT_{1A} receptor and investigated the effect of structural modification of **2** on this spectrum of receptor affinity. We now wish to report the preparation and α_2-adrenoceptor/5-HT_{1A} receptor affinity of bridged ana-



1 Buspirone



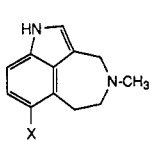
2 SKF 86466



3 n=1

4 n=2

5 n=3



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†Syntex Research, Palo Alto.
‡Syntex Research, Edinburgh.

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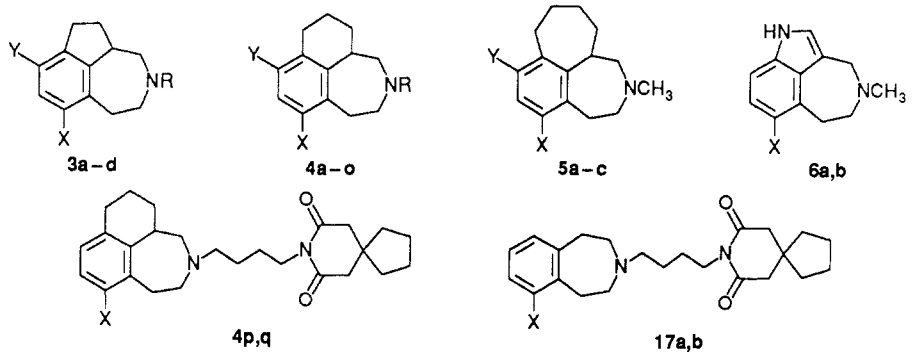
Table I. Physical Properties and Radioligand Binding Data for Bridged Benzazepines



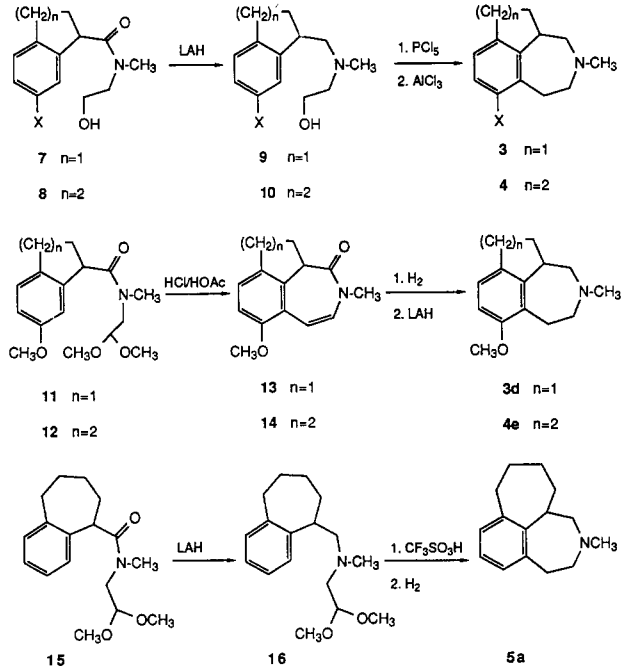
no.	X	Y	R	mp, °C	cryst solvent	formula[a]	radioligand binding data: pK_i[b] [^{3}H]yohimbine (α_2)	[^{3}H]-8-OH-DPAT ($5HT_{1A}$)
3a	H	H	CH_3	262–263	EtOH	$C_{13}H_{17}N$·HBr	6.6 ± 0.1	7.8 ± 0.1
3b	Cl	H	CH_3	279–281	EtOH	$C_{13}H_{16}ClN$·HBr	8.1 ± 0.1[c]	7.6 ± 0.1[c]
3c	H	Cl	CH_3	257–259	EtOH	$C_{13}H_{16}ClN$·HCl	6.8 ± 0.1	7.6 ± 0.1
3d	OCH_3	H	CH_3	250–252	EtOH	$C_{14}H_{19}NO$·HBr	6.9 ± 0.2	7.0 ± 0.2
4a	H	H	H	207–209	EtOH–Et_2O	$C_{13}H_{17}N$·HCl·0.25H_2O	5.9 ± 0.1	6.7 ± 0.1
4b	H	H	CH_3	185–186	EtOH–Et_2O	$C_{14}H_{19}N$·HCl	5.9 ± 0.2	7.3 ± 0.3
(*R*)-4b	H	H	CH_3	180–181	EtOH–Et_2O	$C_{14}H_{19}N$·HCl·0.66H_2O	6.4 ± 0.1	7.2 ± 0.3
(*S*)-4b	H	H	CH_3	177–178	EtOH–Et_2O	$C_{14}H_{19}N$·HCl	5.6 ± 0.1	7.5 ± 0.3
4c	Cl	H	CH_3	233–234	EtOH–Et_2O	$C_{14}H_{18}ClN$·HCl	6.9 ± 0.2	8.0 ± 0.1[d]
(*R*)-4c	Cl	H	CH_3	218–220	EtOH–Et_2O	$C_{14}H_{18}ClN$·HCl	7.0 ± 0.3	7.7 ± 0.1
(*S*)-4c	Cl	H	CH_3	220–221	EtOH–Et_2O	$C_{14}H_{18}ClN$·HCl[e]	6.6 ± 0.1	8.2 ± 0.1
4d	H	Cl	CH_3	224–226	EtOH–Et_2O	$C_{14}H_{18}ClN$·HCl	6.0 ± 0.2	7.1 ± 0.2
(*R*)-4d	H	Cl	CH_3	191–193	EtOH–Et_2O	$C_{14}H_{18}ClN$·HCl	6.4 ± 0.2	6.9 ± 0.1
(*S*)-4d	H	Cl	CH_3	192–193	EtOH–Et_2O	$C_{14}H_{18}ClN$·HCl[f]	5.8 ± 0.2	7.3 ± 0.1
4e	OCH_3	H	CH_3	217–218	EtOH–Et_2O	$C_{15}H_{21}NO$·HCl	6.8 ± 0.1	7.6 ± 0.3
4f	OH	H	CH_3	214–215	EtOH–Et_2O	$C_{14}H_{19}NO$·HBr·0.25H_2O	6.2 ± 0.3	6.5 ± 0.2
4g	$OCH_2CH{=}CH_2$	H	CH_3	201–202	EtOH–Et_2O	$C_{17}H_{23}NO$·HCl	7.5 ± 0.2	6.2 ± 0.1
4h	H	H	CH_2CH_3	199–200	EtOH–Et_2O	$C_{15}H_{21}N$·HCl	6.2 ± 0.1	7.3 ± 0.2
4i	Cl	H	CH_2CH_3	246–248	EtOH–Et_2O	$C_{15}H_{20}ClN$·HCl	6.5 ± 0.1	7.8 ± 0.1
4j	H	H	$(CH_2)_2CH_3$	196–197	EtOH–Et_2O	$C_{16}H_{23}N$·HCl	6.0 ± 0.1	7.3 ± 0.1
4k	Cl	H	$(CH_2)_2CH_3$	204–205	EtOH–Et_2O	$C_{16}H_{22}ClN$·HCl	6.4 ± 0.2	7.5 ± 0.1
4l	H	H	$CH_2CH{=}CH_2$	158–161	EtOH–Et_2O	$C_{16}H_{21}N$·HCl[g]	6.1 ± 0.1	7.7 ± 0.2
4m	Cl	H	$CH_2CH{=}CH_2$	202–204	EtOH–Et_2O	$C_{16}H_{20}ClN$·HCl	6.1 ± 0.3	8.1 ± 0.1
4n	OCH_3	H	$CH_2CH{=}CH_2$	191–192	EtOH–Et_2O	$C_{17}H_{23}NO$·HCl	6.0 ± 0.1	7.9 ± 0.1
4o	H	H	$(CH_2)_2Ph$	204–206	EtOH–Et_2O	$C_{21}H_{25}N$·HCl	6.2 ± 0.1	6.1 ± 0.1
4p	H			170–172	EtOH–Et_2O	$C_{26}H_{36}N_2O_2$·HCl	5.9 ± 0.2	7.4 ± 0.3
4q	Cl			167–168	EtOH–Et_2O	$C_{26}H_{35}ClN_2O_2$·HCl	5.5 ± 0.2	8.1 ± 0.1[d]
5a	H	H		184–186	Me_2CO	$C_{15}H_{21}N$·HCl[h]	5.9 ± 0.1	6.4 ± 0.1
5b	Cl	H		191–193	Me_2CO	$C_{15}H_{20}ClN$·HBr	6.5 ± 0.1	7.1 ± 0.1
5c	H	Cl		211–213	Me_2CO	$C_{15}H_{20}ClN$·HBr[g]	6.2 ± 0.1	5.3[i]
6a	H			225–228	EtOH	$C_{12}H_{14}N_2$·HBr	6.3 ± 0.2	7.0 ± 0.1
6b	Cl			210–214	Me_2CO–EtOH	$C_{12}H_{13}ClN_2$·HCl[j]	6.8 ± 0.1	7.5 ± 0.1
17a	H			188–189	EtOH–Et_2O	$C_{23}H_{32}N_2O_2$·HCl	NT[k]	7.6 ± 0.1
17b	Cl			208–209	EtOH–Et_2O	$C_{23}H_{31}ClN_2O_2$·HCl	NT[k]	8.3 ± 0.2
21				154–156	EtOH–Et_2O	$C_{15}H_{21}NO$·HCl·0.25H_2O	5.9 ± 0.3	5.2 ± 0.1
23				191–192	EtOH–Et_2O	$C_{14}H_{19}NO$·HCl	6.8 ± 0.1	6.7 ± 0.1
25				208–209	EtOH–Et_2O	$C_{14}H_{19}NO$·HCl	NT[k]	5.5[i]
32				263–265[l]	EtOH	$C_{12}H_{14}N_2$·HCl	6.0 ± 0.1	5.9[i]
1 (buspirone)							5.3 ± 0.1	7.9 ± 0.1
2 (SKF 86466)							8.1 ± 0.1	7.0 ± 0.2

[a] Elemental analyses for C, H, N were within 0.4% of the theoretical values for all new compounds unless otherwise noted. [b] Determined in rat cerebral cortical membranes with [^{3}H]yohimbine and [^{3}H]-8-OH-DPAT to label α_2-adrenoceptors and 5-HT_{1A} receptors, respectively. Values are means of three separate determinations (n = 3) ± SEM unless otherwise noted. [c] n = 4. [d] n = 6. [e] C: calcd, 61.77; found, 61.24. [f] C: calcd, 61.77; found, 61.32. [g] Analysis not obtained, satisfactory NMR and mass spectral data. [h] H: calcd, 8.81; found, 9.27. [i] n = 2. [j] N: calcd, 10.89; found, 10.32. [k] Not tested. [l] Base: mp 193–195 °C [lit.[19] mp 195–197 °C (base), 252–257 °C (HCl salt)].

logues of types **3–6** and related structures.[9] Our results indicate that receptor affinity is subtly modified by this bridging, allowing compounds to have affinity for one or both receptors. Several of these bridged compounds that

(1) Timmermans, P. B. M. W. M.; van Zwieten, P. A. *J. Med. Chem.* **1982**, *25*, 1389.
(2) Clark, R. D.; Michel, A. D.; Whiting, R. L. *Progress in Medicinal Chemistry*; Ellis, G. P., West, G. B., Eds.; Elsevier: Amsterdam, 1986; Vol. 23, p 1.
(3) Luttinger, D.; Hlasta, D. J. *Annu. Rep. Med. Chem.* **1987**, *22*, 21.
(4) Middlemiss, D. N.; Hibert, M.; Fozard, J. R. *Annu. Rep. Med. Chem.* **1986**, *21*, 41.
(5) Robertson, D. W.; Fuller, R. W. *Annu. Rep. Med. Chem.* **1988**, *23*, 49.
(6) (a) Bare, T. M.; Resch, J. F.; Patel, J. B. *Annu. Rep. Med. Chem.* **1987**, *22*, 11. (b) Martin, L.; Tegeler, J. J. *Annu. Rep. Med. Chem.* **1988**, *23*, 19. (c) Chopin, P.; Briley, M. *Trends Pharm. Sci.* **1987**, *8*, 383. (d) Gardner, C. R. *Pharm. Biochem. Behav.* **1986**, *24*, 1479.

Scheme I



display high affinity for the 5-HT_{1A} receptor are structurally unrelated to other agents with high affinity for this receptor (e.g. **1**).

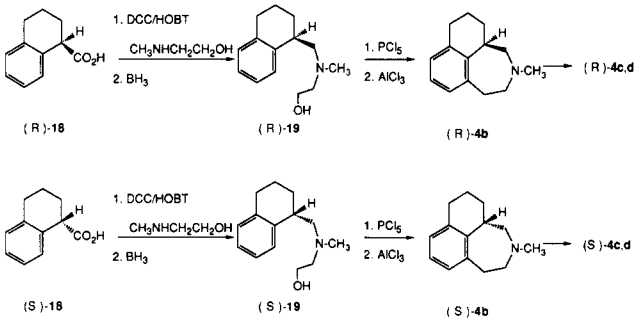
Chemistry

The syntheses of tricyclic benzazepines **3–5** (Table I) are outlined in Scheme I. Conversion of 1-indanones, 1-tetralones, and 1-benzosuberone to the corresponding homologated carboxylic acids was accomplished via the intermediacy of (trimethylsilyl)cyanohydrins.[10] The carboxylic acids were converted to acid chlorides and condensation with 2-(methylamino)ethanol or (methylamino)acetaldehyde dimethyl acetal afforded amides **7** and **8**, and **11**, **12**, and **15**, respectively. Reduction of **7** and **8** with lithium aluminum hydride gave **9** and **10**, respectively, while reduction of **15** furnished **16**.

Reaction of hydroxyethylamines **9** and **10** (X = H) with phosphorus pentachloride followed by aluminum chloride in trichlorobenzene at elevated temperature, essentially the conditions described for the preparation of **2**,[11] afforded **3** (X = H) and **4** (X = H) in yields of 45% and 80%, respectively. Cyclizations of **9** and **10** (X = Cl) gave low yields (<5%) of tricyclic products **3b** and **4c**.

Treatment of amide acetals **11** and **12** with acetic acid–HCl afforded modest yields of unsaturated benzazepinones **13** and **14**, respectively.[12] Sequential catalytic hydrogenation and lithium aluminum hydride reduction gave **3d** and **4e**. Boron tribromide demethylation of **4e** afforded phenol **4f** (Table I), which was alkylated with allyl bromide to give **4g**.

Scheme II



Cyclization of amino acetal **16** was accomplished with trifluoromethanesulfonic acid[13] to afford an unstable unsaturated benzazepine, which was reduced (H_2, Pd–C) to **5a**.

Due to the low yield of chloro derivatives **3b** and **4c** obtained by cyclization of **9** and **10** (X = Cl), chlorination of tricyclic benzazepines **3** (X = H), **4** (X = H), and **5a** was investigated. Treatment of **3** (X = H) with chlorine and $FeCl_3$ in acetonitrile afforded **3b** (Table I) as the major product, which was identical with the product obtained by cyclization of **9** (X = Cl). The structure of minor product **3c** was readily apparent from the NMR spectrum which showed ortho-coupled aromatic protons similar to those of regioisomer **3b**. Chlorinations of **4** (X = H) and **5a** were less selective, furnishing isomers **4c,d** and **5b,c** in approximately equal amounts.

Nitrogen substituents other than methyl (Table I) were introduced in series **4** by von Braun demethylation of **4b,c** and subsequent alkylation of the resultant secondary amines to afford **4h–o**. Alkylation with 8-(4-chlorobutyl)-8-azaspiro[4.5]decane-7,9-dione[14] afforded **4p,q** with the buspirone side chain. Benzazepines **17a,b** (Table I) were prepared by analogous alkylations.

The enantiomers of **4b–d** were prepared from (*R*)- and (*S*)-1,2,3,4-tetrahydro-1-naphthoic acid (**18**)[15] (Scheme II). The resolved acids were coupled with 2-(methylamino)-ethanol in the presence of DCC and the resultant amides were reduced with borane to afford (***R***)-**19** and (***S***)-**19**. Cyclization as previously described gave (***R***)-**4b** and (***S***)-**4b**. Chlorination furnished (***R***)-**4c,d** and (***S***)-**4c,d**, respectively, which were separated by chromatography. The optical purity of (***R***)-**4c** and (***S***)-**4c** was determined to be 98% ee and 93% ee, respectively, by HPLC analysis (Experimental Section).

The positional isomer **21** and the tricyclic tetrahydroisoquinoline analogue **23** were prepared by Bischler–Napieralski cyclization of formamides **20** and **22**, respectively,

(7) We determined a pK_i for 1-(2-pyrimidyl)piperazine of 7.0 for displacement of [^{3}H]idazoxan from rat cerebral cortex. In vivo α_2-adrenoceptor antagonism for this metabolite has been reported: Bianchi, G.; Garattini, S. *Eur. J. Pharmacol.* **1988**, *147*, 343.

(8) DeMarinis, R. M.; Hieble, J. P.; Matthews, W. D. *J. Med. Chem.* **1983**, *26*, 1213.

(9) Ethano-bridged 3-benzazepines with dopaminergic activity have been reported: Weinstock, J.; Oh, H. J.; DeBrosse, C. W.; Eggleston, D. S.; Wise, M.; Flaim, K. E.; Gessner, G. W.; Sawyer, J. L.; Kaiser, C. *J. Med. Chem.* **1987**, *30*, 1303.

(10) Belletire, J. L.; Howard, H.; Donahue, K. *Synth. Commun.*, **1982**, *12*, 763.

(11) Borowski, S. J.; Post, T. A. U.S. Pat. 4,541,954, 1985; *Chem. Abstr.* **1986**, *104*, 88457*d*.

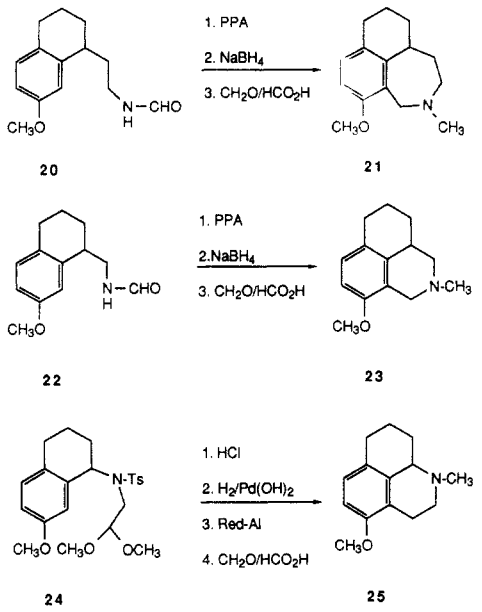
(12) For a related benzazepinone synthesis from cyclization of aldehydoamides, see: (a) Auerbach, J.; Weinreb, S. M. *J. Am. Chem. Soc.* **1972**, *94*, 7172. (b) Lennon, M.; McLean, A.; Proctor, G. R.; Sinclair, I. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 622.

(13) Shah, D.; Lavanchy, P.; Lafferty, J.; DeMarinis, R. *Abstract of Papers* 192nd National Meeting of the American Chemical Society; Anaheim, CA, American Chemical Society: Washington, DC, 1986; Abstract 323.

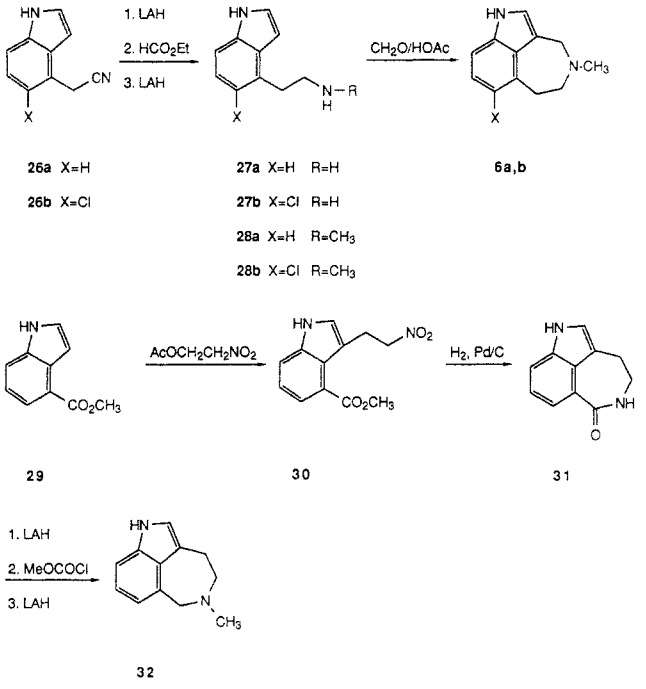
(14) Wu, Y.; Rayburn, J. W.; Allen, L. E.; Ferguson, H. C.; Kissel, J. W. *J. Med. Chem.* **1972**, *15*, 477.

(15) (a) Weidmann, R.; Guette, J. P. *C. R. Acad. Sci., Paris, Ser. C.* **1969**, *268*, 2225; *Chem. Abstr.* **1969**, *71*, 61037*e*. (b) Westman, L. *Ark. Kemi* **1958**, *12*, 161; *Chem. Abstr.* **1958**, *52*, 14574*e*.

Scheme III



Scheme IV



followed by borohydride reduction and N-methylation (Scheme III). Regioisomeric tetrahydroisoquinoline **25** was prepared by a modified Pomeranz–Fritsch cyclization[16] of **24** and subsequent reduction, detosylation, and methylation.

Azepinoindoles **6a,b**[17] were prepared from 4-(cyanomethyl)-1*H*-indole (**26a**) and 5-chloro-4-(cyanomethyl)-1*H*-indole (**26b**)[18] (Scheme IV). Lithium aluminum hydride reduction furnished **27a,b**, which were converted to the *N*-methyl derivatives **28a,b** by reduction (LAH) of the formamides. Pictet–Spengler cyclization of amines **28a,b** gave **6a,b**.

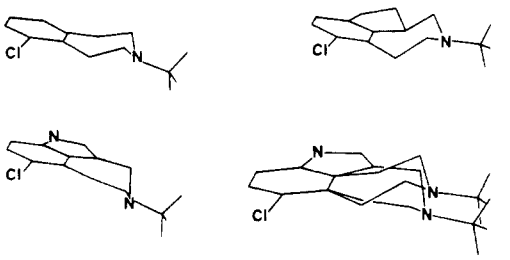


Figure 1. MAXIMIN2 minimum energy structures: (a) upper left, SKF 86466 (**2**), (b) upper right, ethano-bridged benzazepine **3b** (*R* isomer arbitrarily shown), (c) lower left, indole-fused benzazepine **6b**, (d) lower right, FIT least-squares analysis superimposition of **2** and **6b**.

The known azepinoindole **32**[19] was synthesized by a route (Scheme IV) differing in part from the published route. Methyl 1*H*-indole-4-carboxylate (**29**)[20] was condensed with 2-nitroethyl acetate[21] and catalytic hydrogenation of the resulting 3-(2-nitroethyl) analogue **30** furnished lactam **31**. Stepwise conversion of the lactam into **32** was accomplished following the published procedure[19] (reduction of the lactam carbonyl with LAH, acylation of the resulting amine by methyl chloroformate, and LAH reduction of the carbamoyl derivative).

Results and Discussion

The affinities of the bridged benzazepines **3–6** and related compounds for the α_2-adrenoceptor and the 5-HT_{1A} receptor in rat cerebral cortical membranes were determined by radioligand receptor binding with [^{3}H]yohimbine (α_2) and [^{3}H]-8-OH-DPAT (5-HT_{1A}) as selective ligands. The results of these studies are presented in Table I.

α_2-Adrenoceptor Structure–Affinity Relationships. Compound **3b**, the ethano-bridged derivative of **2**, was the only ligand with affinity for the α_2-adrenoceptor comparable to **2**. Bridging with three (**4c**) or four (**5b**) methylenes led to significantly reduced affinity. The enhanced affinity upon chlorine substitution (e.g. **3b** vs **3a**) is consistent with previous results related to analogues of **2** and confirms the importance of a lipophilic interaction provided by chlorine in these benzazepines.[22] The lower affinity of the larger bridged benzazepines **4c** and **5b** may imply a region of limited steric accessibility in the area of the three- and four-carbon bridges. Consistent with this hypothesis is the lower ($^1/_3$) affinity of the 9-allyloxy derivative of **2** for the human platelet α_2-adrenoceptor labeled with [^{3}H]yohimbine.[22]

A rationale for the lack of α_2-adrenoceptor affinity of indoles **6a,b** may relate to conformational changes in the azepine ring of these molecules relative to the high affinity benzazepines **2** and **3b**. To investigate this point, studies were performed with the SYBYL Molecular Modeling System (TRIPOS Associates) with minimization using MAXIMIN2 (Figure 1). The conformation of benzazepine **2** (Figure 1a) appears to be relatively unchanged in the ethano-bridged **3b** (Figure 1b). However, the benzazepine

(16) Birch, A. J.; Jackson, A. H.; Shannon, P. V. R. *J. Chem. Soc., Perkin Trans. 1* 1974, 2185.
(17) Compound **6a** has been claimed in a patent and was prepared by a route differing in part from the one reported herein. Heindl, J.; Schroeder, G. Ger. Offen, DE 3,525,564, 1987; Chem. Abstr. **1987**, *106*, 156272*h*.
(18) Matsumoto, M.; Watanabe, N.; Ishida, Y. *Heterocycles* **1986**, *24*, 3157.
(19) Bowman, R. E.; Evans, D. D.; Guyette, J.; Nagy, H.; Weale, J.; Weyell, D. J.; White, A. C. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1926.
(20) Clark, R. D.; Repke, D. B. *Heterocycles* **1984**, *22*, 195 and references therein.
(21) Flaugh, M. E.; Crowell, T. A.; Clemens, J. A.; Sawyer, B. D. *J. Med. Chem.* **1979**, *22*, 63.
(22) DeMarinis, R. M.; Krog, A. J.; Shah, D. H.; Lafferty, J.; Holden, K. G.; Hieble, J. P.; Matthews, W. D.; Regan, J. W.; Lefkowitz, R. J.; Caron, M. G. *J. Med. Chem.* **1984**, *27*, 918.

conformation is considerably altered in azepinoindole **6b** (Figure 1c). This difference is clearly seen when the structures of **2** and **6b** are superimposed using FIT least-squares analysis (TRIPOS) with overlay of the benzene rings (Figure 1d). The considerable perturbation in the position of the nitrogen atom of **6b** relative to the plane of the benzene ring from the position in **2** offers an attractive explanation for the difference in α_2-adrenoceptor affinity of these two ligands.

5-HT$_{1A}$ Receptor Structure–Affinity Relationships. Both the ethano- and propano-bridged benzazepines of general structures **3** and **4** had significant affinity for the 5-HT$_{1A}$ receptor while extension of the bridge to four carbons (**5**) led to a diminution in affinity. SAR work was centered on series **4**, primarily due to accessibility of intermediates. Within this series, substitution with chlorine ortho to the nitrogen-containing ring (X = Cl, Table I) consistently led to higher affinity relative to either the parent molecule or the other chloro regioisomer (e.g. **4c** vs **4d**). N-Substitution with small alkyl groups or with allyl was tolerated while the large phenethyl group (**4o**) was not.

Among the three enantiomeric pairs which were evaluated (**4b–d**), the *S* isomers had higher affinity than the *R* isomers although the differences were not pronounced (0.3–0.5 log orders), or, in the case of **4b**, statistically significant. This may indicate that the 5-HT$_{1A}$ receptor does not make rigorous stereochemical demands for binding of these structures. An alternative, albeit less likely, explanation could be that the chlorine atom and the carbon bridge in a molecule such as **4c** may interchangably have access to the same lipophilic binding site(s) and allow the enantiomers to bind with the benzazepine rings in the same orientation.[23]

The importance of the position of the nitrogen atom in these tricyclic structures is emphasized by the lack of affinity of the benzylic analogues **21**, **23**, and **25** relative to methoxy-substituted 3-benzazepine congener **4e**.

In light of the relatively high affinity of **4c** for the 5-HT$_{1A}$ receptor, we were interested in evaluating azepinoindoles **6** and **32** as 5-HT$_{1A}$ ligands. However, while the trend to increased affinity upon chlorine substitution was noted (**6b** vs **6a**), the affinities of these indoles were less than **4c**. The lack of affinity of the restricted tryptamine **32** was consistent with the inactivity of the other benzylic analogues (e.g. **21**). Thus, a preliminary hypothesis relating structures **6** and **32** to the endogenous ligand 5-hydroxytryptamine was not borne out.

Based on an elegant computer-modeling approach, a three-dimensional map of the 5-HT$_{1A}$ antagonist recognition site was recently proposed.[24] This model defines the pharmacophore as a basic nitrogen which is 5.6 Å from the center of an aromatic ring and 1.6 Å below (or above) the plane of this ring. (*S*)-2-(Aminomethyl)-1,4-benzodioxan (Figure 2a) was shown to fit this model and addition of the buspirone side chain to the amino group afforded the high affinity (pK_i 9.2) ligand MDL 72832.[24] Comparison of the SYBYL-generated conformation of (***S***)-**4c** (Figure 2b) with this pharmacophore (Figure 2c) indicates that while the basic nitrogen of (***S***)-**4c** is similarly situated below the plane of the aromatic ring, the distance from the center of the ring is ca. 1 Å less. This may account for the lower affinity of (***S***)-**4c** (and **4q**; vide infra) relative to MDL 72832. It is also of interest that this model provides for a region of steric accessibility which would accommodate the three-carbon bridge of (***S***)-**4c**.

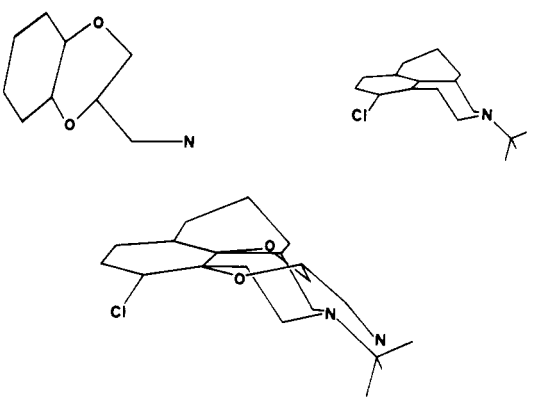


Figure 2. (a) Upper left, putative 5-HT$_{1A}$ antagonist recognition site pharmacophore represented by (*S*)-2-(aminomethyl)-1,4-benzodioxan,[24] (b) upper right, MAXIMIN2 minimum energy structure for (***S***)-**4c**, (c) lower center, FIT least squares analysis superimposition of pharmacophore from Figure 2a[24] and (***S***)-**4c**.

In addition to containing the suitably situated basic nitrogen and aromatic ring which define the 5-HT$_{1A}$ pharmacophore,[24] buspirone and MDL 72832 contain the 8-(4-butyl)-8-azaspiro[4.5]decane-7.9-dione side chain which presumably provides important secondary binding interactions. It was therefore of interest to determine the effect of this side chain on the 5-HT$_{1A}$ receptor affinity of benzazepine **2** and bridged benzazepine **4c**. Virtually no increase in affinity was observed upon replacement of the *N*-methyl group of **4c** (pK_i = 8.0) with the buspirone side chain to give **4q** (pK_i = 8.1). However, the affinity of benzazepine **2** (pK_i = 7.0) was markedly enhanced in the corresponding analogue **17b** (pK_i = 8.3). This indicates that the contribution of this side chain to the affinity of 5-HT$_{1A}$ receptor ligands can be of major importance and can, in certain cases, overcome the limited affinity of the pharmacophore to which it is attached.

Also noteworthy is that whereas buspirone is generally regarded as a partial agonist,[25] the high affinity 5-HT$_{1A}$ ligand **4c** appears to be devoid of agonist properties in certain functional assays in which buspirone is a partial agonist.[26] On the other hand, analogues with the buspirone side chain (e.g. **4q**, **17b**) retain partial agonist properties. These agents, in particular **4c**, may therefore be useful in characterizing 5-HT$_{1A}$ receptor functional responses.

Conclusion

The results presented herein indicate that structural changes in benzazepines **2–6** modify affinity between the α_2-adrenoceptor and the 5-HT$_{1A}$ receptor. In this regard, it can be noted that other α_2-adrenoceptor antagonists such as yohimbine and WY-26703 also have affinity for the 5-HT$_{1A}$ receptor.[27,28] Our structure–affinity relationship and molecular modeling studies on these bridged benzazepines suggest that the structural requirements for

(23) That is, one enantiomer could be "flipped over" in its binding to the receptor relative to the other enantiomer.

(24) Hibert, M. F.; Gittos, M. W.; Middlemiss, D. N.; Mir, A. K.; Fozard, J. R. *J. Med. Chem.* **1988**, *31*, 1087.

(25) Fozard, J. R. *Trends Pharm Sci.* **1987**, *8*, 501.

(26) Spedding, M., unpublished results. Functional pharmacology of **4c** will be described in detail elsewhere.

(27) We have measured pK_i values of 6.5 and 6.9 for WY-26703 and yohimbine, respectively, in displacing [^{3}H]8-OH-DPAT from rat hippocampus homogenate.

(28) A number of α_2-adrenoceptor antagonists have also been shown to be potent antagonists of 5-HT-induced contraction of rat fundic strips although the exact nature of the apparently 5-HT$_1$-like receptor which mediates this contraction has not been established: Clineschmidt, B. V.; Reiss, D. R.; Pettibone, D. J.; Robinson, J. L. *J. Pharmacol. Exp. Ther.* **1985**, *235*, 696.

binding of these compounds to the α_2-adrenoceptor are more rigorous than the requirements for 5-HT_{1A} receptor binding.

The 5-HT_{1A} receptor and α_2-adrenoceptor proteins have recently been shown to display strong sequence homology in the seven membrane-spanning regions.[29] Our findings on mixed α_2-adrenoceptor/5-HT_{1A} receptor ligands, as well as the affinity of other α_2-adrenoceptor antagonists for the 5-HT_{1A} receptor, make it appear likely that the binding sites of these two receptors may also share common features.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. ^1H NMR spectra were recorded with Varian A-60 or Bruker WM 300 instruments and were consistent with the assigned structures. All TLC were done with silica gel GF 2.5 × 10 cm plates (Analtech) and chromatographic separations were performed on silica gel 60 (70–230 mesh) and Merck (230–400 mesh) kieselgel (medium pressure). Microanalyses were performed by the Syntex Analytical Department and where analyses are indicated only by symbols of the elements, results obtained were within ±0.4% (for C, H, N) of the theoretical values unless otherwise noted.

2-Methyl-2,3,4,8,9,9a-hexahydro-1*H*-indeno[1,7-*cd*]azepine (3a). A solution of 19.5 g (120 mmol) of indan-1-carboxylic acid and 20 mL (230 mmol) of oxalyl chloride in 150 mL of CH_2Cl_2 was stirred 1 h at ambient temperature and 1 h at reflux. The mixture was concentrated under reduced pressure and the residue of indan-1-carboxylic acid chloride was dissolved in 40 mL of CH_2Cl_2 and added dropwise to 150 mL of CH_2Cl_2 containing 19 g (250 mmol) of 2-(methylamino)ethanol. After stirring for 16 h, the solvent was removed under vacuum. The residual mixture was stirred rapidly with 400 mL of EtOAc and 300 mL of ether and the insoluble amine hydrochloride was removed by filtration. The filtrate was washed with 100 mL of 5% HCl and brine, dried over K_2CO_3, and filtered. When the filtrate was concentrated to ca. 150 mL there was crystallization of 14.4 g of pure hydroxyethylamine **7** (X = H), mp 96–97 °C. A second crop of 3.1 g (67% total) was obtained upon further concentration to 20 mL. Anal. ($C_{13}H_{17}NO_2$) C, H, N.

A solution of 17.5 g (80 mmol) of **7** (X = H) in 400 mL of THF was added over a period of 90 min to a stirred suspension of 3.1 g (82 mmol) of LAH in 100 mL of THF, allowing a temperature rise to 40–50 °C. After 16 h at room temperature, the mixture was treated with 3 mL of water, 3 mL of 15% NaOH, and an additional 10 mL of water. The mixture was filtered and the filtrate was concentrated and distilled to afford 13.7 g (84%) of amine **9** (X = H), bp 110 °C (0.3 mm). A sample was converted into the HBr salt, mp 115–117 °C (acetone). Anal. ($C_{13}H_{19}NO$·HBr) C, H, N.

A stirred solution of 13.7 g (66.8 mmol) of hydroxyethylamine **9** (X = H) and 5.5 g (26.5 mmol) of PCl_5 in 75 mL of 1,2,4-trichlorobenzene was heated to 120 °C. At this temperature there was added 17.5 g (131 mmol) of $AlCl_3$ and the bath temperature was raised to 200 °C for 3 h. After the temperature was allowed to cool to <100 °C, dilute HCl was carefully added. Nonbasic materials were removed by extraction with EtOAc. The products were extracted into EtOAc after the aqueous solution had been basified (NaOH). Chromatography (0–3% MeOH in CH_2Cl_2 gradient) gave 5.8 g (45%) of tricyclic product **3**, which was converted to the hydrobromide salt in EtOH.

5-Methoxy-2-methyl-2,3,4,8,9,9a-hexahydro-1*H*-indeno-[1,7-*cd*]azepine (3d). A sample of 6 g (37 mmol) of 6-methoxyindan-1-carboxylic acid was converted into its acid chloride as described under the preparation of **3a**. The acid chloride, in 40 mL of CH_2Cl_2, was added dropwise to a stirred mixture of 5.6 g (37 mmol) of (methylamino)acetaldehyde dimethyl acetal, 5 mL (36 mmol) of triethylamine, and 50 mL of CH_2Cl_2. Stirring was continued for 16 h and the solvent was then removed under reduced pressure. The residue was stirred with ether and water, the organic phase was washed with ice-cold, dilute H_2SO_4, water, and saturated $NaHCO_3$, dried over K_2CO_3, filtered, and concentrated to give 8 g (87%) of 2-dimethoxyethyl amide **11** (n = 1) as an oil, IR (film) 1630 cm^{-1}. Anal. ($C_{16}H_{23}NO_4$) H, N; C: calcd, 65.51; found 64.48.

Dimethoxyethyl amide **11** (8 g, 27 mmol) in a mixture of 80 mL of glacial acetic acid and 8 mL of concentrated HCl was stirred at 80 °C for 6 h. The mixture was poured onto ice and extracted with ether. The separated ether extract was stirred and aqueous $NaHCO_3$ was added in portions until the aqueous layer was basic. The ether was removed under vacuum, leaving 1.2 g (19%) of unsaturated lactam **13** (n = 1) as a gum. This material (600 mg) was dissolved in 40 mL of EtOH and hydrogenated over 250 mg of 5% Pt/C for 24 h at atmospheric pressure. After removal of the catalyst by filtration the solvent was evaporated under vacuum to yield 580 mg (96%) of the saturated lactam: mp 141–143 °C; IR (KBr) 1630 cm^{-1}. A solution of 550 mg (2.4 mmol) of this material and 750 mg (20 mmol) of LAH in 100 mL of ether was stirred for 48 h. The reaction was worked up by dropwise addition of 3.5 mL of water. After all solids had turned white, the inorganic matter was removed by filtration. Concentration of the filtrate gave 470 mg of **3d** as an oil. The HBr salt crystallized from EtOH to give 350 mg (47%).

3-Methyl-2,3,4,4a,5,6,7,8-octahydro-1*H*-cyclohepta[*ef*]-benzazepine (5a). Benzocycloheptene-1-carboxylic acid (4.4 g, 23 mmol) was converted into its acid chloride as described for indan-1-carboxylic acid under the preparation of **3a**. This acid chloride was dissolved in 20 mL of CH_2Cl_2 and added to a solution of 4 g (33 mmol) of (methylamino)acetaldehyde dimethyl acetal and 3 mL (22 mmol) of Et_3N in 100 mL of CH_2Cl_2. The solution was stirred for 16 h and washed twice with water, and the organic phase was concentrated under vacuum. The residual oil was purified by chromatography (30–50% EtOAc in hexane) to afford 3.5 g (52%) of **15** as an oil, IR (film) 1660, 1640 cm^{-1}. Anal. ($C_{17}H_{25}NO_3$) C, H, N.

To a stirred slurry of 1 g (26 mmol) of LAH in 100 mL of ether was added a solution of 3.5 g of amide **15** in 20 mL of ether. After 48 h the mixture was worked up as described above for **3d** to afford 3.1 g (90%) of **16** as an oil.

To a stirred, ice-cooled solution of 2.5 g (9 mmol) of **16** in 3 mL of CH_2Cl_2 was added slowly 5 mL of CF_3SO_3H via a syringe. The reaction mixture was stirred in an ice bath for 3 h and at ambient temperature for 16 h. Ice was added to the reaction and after the ice had melted the mixture was diluted with ether and stirred. The layers were separated and K_2CO_3 was added to the aqueous layer. The product was extracted into ether and the ether was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and this solution was passed through a small column of silica to afford 1.3 g of an oil. This material was hydrogenated in 40 mL of EtOH and 0.5 mL of concentrated HCl at atmospheric pressure with 400 mg of 5% Pd/C. After 1 h the catalyst was removed by filtration and the filtrate was concentrated to a small volume. The residue was treated with dilute NH_4OH and the product was extracted into CH_2Cl_2. The CH_2Cl_2 was evaporated and the residue was chromatographed (5% MeOH in CH_2Cl_2) to afford 0.6 g (31% from **16**) of **5a**. The HCl salt was prepared in acetone.

5-Chloro- and 7-Chloro-2-methyl-1,2,3,4,8,9,10,10a-octahydronaphth[1,8-*cd*]azepine (4c and 4d). A mixture of 4.3 g (21 mmol) of **4b** and 5.8 g (21 mmol) of $FeCl_3$·$6H_2O$ in 40 mL of acetonitrile was cooled in an ice bath and a solution of 1.8 g (25 mmol) of chlorine in 20 mL of acetonitrile was added. The mixture was stirred for 15 min, 30 mL of 1 N NaOH and 100 mL of EtOAc were added, and the layers were separated. The EtOAc was dried (Na_2SO_4) and evaporated to 4.2 g of a mixture of **4c,d**. The isomers were separated by medium-pressure chromatography (1% NH_4OH in EtOAc–MeOH 10:1). The less polar isomer was **4c** (oil, 1.7 g, 34%), which was identical by TLC and ^1H NMR analysis with the product obtained in 5% yield by cyclization of **10** (X = Cl, n = 2) under the conditions described for preparation of **3a**. **4c**: ^1H NMR ($CDCl_3$) δ 1.52–1.80 (m, 3 H, H9, H9′, H10), 1.98 (m, 1 H, H10′) 2.03 (m, 1 H, H4), 2.15 (dd, 1 H, J = 9.2, 12 Hz, H1), 2.33 (s, 3 H), 2.66 (m, 2 H, H8, H8′), 2.76 (ddd, 1 H, J = 1.5, 1.5, 12 Hz, H1′), 2.85 (m, 1 H, H3), 3.05 (m, 1 H, H4′), 3.22 (m, 1 H, H10a), 3.54 (m, 1 H, H3′), 6.87 (d, 1 H, J = 8.3 Hz, H7), 7.12 (d, 1 H, J = 8.3 Hz, H6). Proton assignments were made

(29) Fargin, A.; Raymond, J. R.; Lohse, M. J.; Kobilka, B. K.; Caron, M. G.; Lefkowitz, R. J. *Nature* **1988**, *335*, 358.

by decoupling experiments. A small (ca. 0.5 Hz) coupling was evident between H7 and the benzylic H8. The more polar isomer was **4d** (oil, 1.2 g, 24%): ^{1}H NMR ($CDCl_3$) δ 1.56–1.80 (m, 3 H, H9, H9′, H10), 1.95 (m, 1 H, H10′), 2.03 (dd, 1 H, J = 11, 11 Hz, H4), 2.14 (dd, 1 H, J = 9.1, 12 Hz, H1), 2.34 (s, 3 H), 2.64–2.76 (m, 3 H, H8, H8′, H3′), 2.76 (ddd, 1 H, J = 1.5, 1.5, 12 Hz, H1′), 3.02 (m, 1 H, H4′), 3.04–3.22 (m, 2 H, H3, H10a), 6.87 (d, 1 H, J = 8 Hz, H5), 7.12 (d, 1 H, J = 8 Hz, H6). The bases were converted to the HCl salts in ethanol–ether.

5-Chloro- and 7-Chloro-2-methyl-2,3,4,8,9,9a-hexahydro-1*H*-indeno[1,7-*cd*]azepine (3b and 3c). Chlorination of **3a** as described above for **4b** afforded **3b** (68%) and **3c** (5%) after chromatographic purification. The major product was identical by TLC and NMR analysis with the product obtained from **9** (X = Cl, n = 1) in ca. 5% yield.

1,2,3,4,8,9,10,10a-Octahydronaphth[1,8-*cd*]azepine (4a). A solution of 320 mg (3 mmol) of BrCN and 510 mg (2.5 mmol) of **4b** in 15 mL of toluene was stirred at 50 °C for 1 h. The solution was filtered through silica gel and the silica was rinsed with CH_2Cl_2. Evaporation left 440 mg of the N-cyano compound as a white solid: mp 58–60 °C; IR (KBr) 2190 cm^{-1}. This solid was dissolved in a mixture of 2.5 mL of AcOH and 2.5 mL of 6 N HCl. The solution was allowed to stand for 16 h and was then poured into ice-cold, dilute NaOH. Extraction of the product with Et_2O and evaporation of the solvent gave 310 mg (65%) of secondary amine **4a** as an oil that eventually solidified. This product was used in subsequent reactions without purification. The HCl salt was prepared in ethanol–ether.

2-(2-Propen-1-yl)-1,2,3,4,8,9,10,10a-octahydronaphth[1,8-*cd*]azepine (4l). A mixture of 280 mg (1.5 mmol) of secondary amine **4a**, 180 mg (1.5 mmol) of allyl bromide, and 315 mg (2.3 mmol) of K_2CO_3 in 10 mL of EtOH was stirred for 16 h. The solid was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography (5% MeOH in CH_2Cl_2, 0.5% NH_4OH) to afford 120 mg (35%) of **4l** as an oil, which was converted to the HCl salt in ethanol–ether.

2-[4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butyl]-5-chloro-1,2,3,4,8,9,10,10a-octahydronaphth[1,8-*cd*]azepine (4q). Demethylation of **4c** as described for the preparation of **4a** afforded the corresponding secondary amine. A neat mixture of this amine (230 mg, 1 mmol) and 8-(4-chlorobutyl)-8-azaspiro[4.5]decane-7,9-dione (310 mg, 1.2 mmol) was stirred at 150 °C for 30 min. The reaction mixture was applied to a silica gel column and eluted with 3% MeOH–CH_2Cl_2 (1% NH_4OH) to afford 240 mg (54%) of **4q** as an oil. The HCl salt was prepared from ethanol–ether.

(*R*)-2-Methyl-1,2,3,4,8,9,10,10a-octahydronaphth[1,8-*cd*]-azepine ((*R*)-4b). Resolution of 1,2,3,4-tetrahydro-1-naphthoic acid with cinchonine as described by Westman[15b] afforded (*R*)-18. A solution of 7.9 g (38 mmol) of 1,3-dicyclohexylcarbodiimide in 25 mL of CH_2Cl_2 was added to a 0 °C (ice bath) suspension of 5.4 g (40 mmol) of 1-hydroxybenzotriazole and 6.4 g (37 mmol) of (*R*)-18. The resulting mixture was stirred in an ice bath for 45 min. The mixture was filtered and the filtrate was treated with 4 mL (49 mmol) of 2-(methylamino)ethanol at room temperature. After 30 min the suspension was filtered and the filtrate was washed with water, dried (Na_2SO_4), and evaporated. The residue was purified by chromatography (4% MeOH–CH_2Cl_2) to afford 5.9 g (69%) of the amide as an oil. This material was dissolved in 20 mL of THF and 50 mL of 1 M borane in THF was added. The mixture was heated under reflux for 2 h and cooled, and 5 mL of MeOH was carefully added followed by MeOH–HCl (until the mixture was strongly acidic). The mixture was heated under reflux for 3 h. Solvents were removed under vacuum, and the residue was partitioned between water and ether. The aqueous layer was basified with NaOH and extracted with ether. The ether extract was dried and evaporated to 5.1 g (93%) of (*R*)-19. A small sample was converted to the HCl salt (EtOH–ether): mp 153–154 °C; $[\alpha]^{25}_D$ −30° (c 0.25, EtOH). Anal. ($C_{14}H_{21}NO$·HCl) C, H, N.

Cyclization of (*R*)-19 was performed as described for **9** (X = H) to afford (*R*)-**4b** in 73% yield after bulb to bulb distillation of the crude product. The HCl salt was prepared from ethanol–ether, $[\alpha]^{25}_D$ +18° (c 0.7, EtOH).

(*S*)-2-Methyl-1,2,3,4,8,9,10,10a-octahydronaphth[1,8-*cd*]-azepine ((*S*)-4b). This enantiomer was prepared from (*S*)-18[15b] as described above. The intermediate (*S*)-19 had mp 154–155 °C for the HCl salt, $[\alpha]^{25}_D$ +36° (c 0.3, EtOH). **4b**·HCl: $[\alpha]^{25}_D$ −15° (c 1.1, EtOH).

(*R*)-5-Chloro- and (*R*)-7-Chloro-2-methyl-1,2,3,4,8,9,10,10a-octahydronaphth[1,8-*cd*]azepine ((*R*)-4c and (*R*)-4d). Chlorination of (*R*)-**4b** as described for the racemate afforded a ca. 55:45 mixture (TLC analysis) of (*R*)-**4c** and (*R*)-**4d**, which were separated by chromatography as before. (*R*)-**4c**·HCl: $[\alpha]^{25}_D$ −8° (c 0.6, EtOH). (*R*)-**4d**·HCl: $[\alpha]^{25}_D$ +27° (c 0.9, EtOH).

(*S*)-5-Chloro- and (*S*)-7-Chloro-2-methyl-1,2,3,4,8,9,10,10a-octahydronaphth[1,8-*cd*]azepine ((*S*)-4c and (*S*)-4d). These were prepared from (*S*)-**4b** as described above. (*S*)-**4c**·HCl: $[\alpha]^{25}_D$ +10° (c 0.8, EtOH). (*S*)-**4d**·HCl: $[\alpha]^{25}_D$ −27° (c 0.8, EtOH).

Determination of the Enantiomeric Purities of (*R*)-4c and (*S*)-4c. HPLC analysis of the enantiomers was done with a 100 × 4 mm Enantiopac, α_1-acid glycoprotein 10-μm column (LKB). The mobile phase was 2-propanol–phosphate buffer (pH 7) 5:95 v/v and the flow rate was 0.25 mL/min. The retention times for (*S*)-**4c** and (*R*)-**4c** were 37 and 49 min, respectively (resolution factor 1.46, relative retention α 1.37). The detection limit was ca. 1%. Compound (*R*)-**4c** was determined to have an ee of 98% and the ee for (*S*)-**4c** was 93%.

10-Methoxy-2-methyl-1,2,3,4,4a,5,6,7-octahydronaphth[1,8-*cd*]azepine (21). 7-Methoxy-1,2,3,4-tetrahydro-1-naphthaleneacetic acid was prepared as described for the 6,7-dimethoxy congener.[30] Treatment of the acid chloride with ammonia followed by LAH reduction and treatment with ethyl formate at reflux furnished formamide **20**. Compound **20** (5.7 g, 24 mmol) was heated in 20 g of PPA at 120 °C for 6 h. The cooled mixture was dissolved in water and washed with EtOAc. The aqueous layer was basified with NH_4OH and extracted with EtOAc. The extract was dried (Na_2SO_4) and evaporated to a residue which was purified by medium-pressure chromatography (80% EtOAc–hexane) to afford 1.38 g (26%) of 10-methoxy-3,4,4a,5,6,7-hexahydronaphth[1,8-*cd*]azepine. This material was dissolved in 25 mL of EtOH, and 0.5 g of $NaBH_4$ was added. The mixture was concentrated in vacuo and partitioned between EtOAc and water. The EtOAc was dried (Na_2SO_4) and evaporated to a residue, which was treated with 25 mL of formic acid and 20 mL of formalin. The resulting solution was heated under reflux for 30 min, cooled, diluted with water, and washed with ether. The aqueous layer was basified with NH_4OH at 0 °C and extracted with EtOAc. The EtOAc was dried (Na_2SO_4) and evaporated and the crude product was purified by chromatography (7% MeOH–CH_2Cl_2, 1% NH_4OH) to afford 1.2 g (81% for the two steps) of **21** as a foam. The HCl salt was prepared in ethanol–ether.

9-Methoxy-2-methyl-2,3,3a,4,5,6-hexahydro-1*H*-benz[*de*]-isoquinoline (23). 1-(Aminomethyl)-7-methoxytetralin was prepared as described for the corresponding 6,7-dimethoxy analogue.[30] Conversion to **23** (50% overall) was effected as described for the preparation of **21**.

4-Methoxy-1-methyl-2,3,7,8,9,9a-hexahydro-1*H*-benzo[*de*]quinoline (25). Acetal **24** was prepared from 7-methoxy-1-tetralone according to the general literature procedure.[16] A mixture of 3.15 g (7.5 mmol) of **24** in 50 mL of dioxane and 8 mL of 6 N HCl was heated under reflux for 1 h. The cooled mixture was diluted with water and extracted with ether. The ether extract was dried ($MgSO_4$) and evaporated. The crude product was dissolved in 50 mL of ethanol and 20 mL of dioxane and hydrogenated with 0.5 g of 20% $Pd(OH)_2$ at 60 psi for 12 h. Catalyst was removed by filtration and the filtrate was evaporated. Medium-pressure chromatography (CH_2Cl_2) afforded 0.57 g (21%) of 4-methoxy-1-(*p*-tolylsulfonyl)-2,3,7,8,9,9a-hexahydro-1*H*-benzo[*de*]quinoline, mp 164–165 °C. Anal. ($C_{20}H_{23}NO_3S$) C, H, N.

A solution of 0.54 g (1.5 mmol) of the N-tosyl derivative was suspended in 5 mL of toluene and 2 mL of 3.4 M sodium bis(2-methoxyethoxy)aluminum hydride in toluene was added. The resulting mixture was heated under reflux for 12 h and cooled, and 1 mL of 15% NaOH was added. The mixture was partitioned between water and ether, and the organic layer was dried ($MgSO_4$)

(30) Cannon, J. G.; Perez, Z.; Long, J. P.; Ilhan, M. *J. Med. Chem.* **1983**, *26*, 813.

and evaporated. Purification by chromatography (5% MeOH–CH_2Cl_2) afforded 0.15 g (50%) of the secondary amine, which was converted to *N*-methyl derivative **25** as described for compound **21**.

7-Chloro-4-methyl-3,4,5,6-tetrahydro-1*H*-azepino[3,4,5-*cd*]indole (6b). The *N*-tosyl derivative of 5-chloro-4-(cyanomethyl)indole[18] was deprotected with KOH in methanol at 40 °C. A sample of the resulting 5-chloro-4-(cyanomethyl)indole **26b** (1.3 g, 6.8 mmol) and 1.0 g (26 mmol) of LAH in 100 mL of ether and 50 mL of THF was stirred 16 h. The mixture was worked up by dropwise addition of 5 mL of water. The solid was removed by filtration and the filtrate was concentrated to 1.3 g (98%) of 4-(2-aminoethyl)-5-chloroindole (**27b**) as an oil. Reaction of this amine with 20 mL of ethyl formate in 20 mL of THF at reflux for 16 h and subsequent removal of solvents under reduced pressure gave 1.5 g of the 4-(2-formamidoethyl) derivative as an oil, IR (film) 1675 cm^{-1}. A solution of this formamide in 40 mL of THF was added to a stirred slurry of 1 g (26 mmol) of LAH in 100 mL of ether. After allowing the reaction mixture to stir for 16 h, the reaction was worked up as above to afford 1.3 g (93%) of **28b** as an oil, which was used in the next step without purification.

A solution of 420 mg (2 mmol) of **28b**, 1 mL of 37% aqueous formaldehyde, and 0.25 mL of acetic acid in 50 mL of methanol was stirred at room temperature for 24 h. The mixture was concentrated to a small volume, treated with 0.5 N HCl, and washed with ether. The aqueous layer was basified with K_2CO_3 and extracted with ether. Evaporation of solvent afforded a residue, which was purified by chromatography (5% MeOH CH_2Cl_2) to afford 350 mg (68%) of **6b** as an oil. The HCl salt was prepared from acetone–EtOH: ^1H NMR (Me_2SO-d_6) δ 2.85 (s, 3 H), 3.45 (m, 2 H), 3.60–3.80 (m, 2 H), 4.50 (d, 1 H, J = 14.5 Hz), 4.62 (d, 1 H, J = 14.5 Hz), 7.15 (d, 1 H, J = 8.6 Hz), 7.34 (d, 1 H, J = 8.6 Hz), 7.45 (d, 1 H, J = 2.5 Hz), 11.50 (br s, 1 H, exchanges with D_2O), 11.65 (s, 1 H, exchanges with D_2O, indole NH); MS m/e 220 (M^+).

5-Methyl-3,4,5,6-tetrahydro-1*H*-azepino[5,4,3-*cd*]indole (32). A solution of 16.8 g (96 mmol) of methyl 1*H*-indole-4-carboxylate (**29**),[20] 15.8 g (118 mmol) of ethyl nitroacetate, and 60 mg of 4-*tert*-butylcatechol in 120 mL of xylenes was kept at reflux for 6 h. The solvent was removed under reduced pressure and the residual oil was purified by chromatography (25% EtOAc in hexane) to afford 20 g (84%) of the 3-nitroethyl-substituted indole **30**; mp 102–105 °C; IR (KBr) 3340, 1700, 1550 cm^{-1}. Anal. ($C_{12}H_{12}N_2O_4$) C, H, N.

A solution of 8 g (43 mmol) of **30** in 300 mL of MeOH was hydrogenated over 1 g of 5% Pd/C at 50 psi. After 16 h the catalyst was removed by filtration. TLC indicated a major product, R_f 0.4 (5% MeOH in CH_2Cl_2), along with a much more polar product, R_f 0.2 (0.3% $(NH_4)_2CO_3$ in MeOH), presumably uncyclized reduction product. The filtrate was kept at reflux for 2 days and was essentially devoid of the more polar product. The solvent was removed under reduced pressure and the residue was purified by chromatography (1–5% MeOH in CH_2Cl_2 gradient) to afford 3.2 g (48%) of **31**, mp 232–234 °C, (lit.[19] mp 237–239 °C). Stepwise conversion of the lactam into **32** (LAH reduction followed by N-methylation via the methoxycarbonyl derivative) was carried out essentially as previously described.[19]

5-HT_{1A} Radioligand Binding Assay. The radioligand receptor binding studies were performed by a modification of a previously described procedure.[31] Male Sprague–Dawley rats were killed by a blow to the head and the brains were rapidly removed and dissected. The cerebral cortex was homogenized in 25 mL of ice-cold Tris buffer (50 mM Tris HCl, pH 7.7 at 25 °C) and centrifuged at 38000*g* for 10 min. The membrane pellet was repeatedly washed by resuspension and centrifugation. After the third wash the resuspended pellet was incubated at 37 °C for 10 min. Membranes were then collected by centrifugation and the final pellet was resuspended in 50 mM Tris HCl, 5 mM $MgSO_4$, and 0.5 mM EDTA buffer (pH 7.4 at 37 °C).

Aliquots of the final membrane suspension (0.8–1.0 mg of protein) were incubated for 10 min at 37 °C with [^{3}H]-8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) (171 Ci/mmol, pK_i 8.5) (1.5 nM) in the presence or absence of 13 concentrations of the competing drug in a final volume of 2 mL of Tris assay buffer (50 mM Tris HCl, pH 7.4). Nonspecific binding was determined with 10 μM 5-HT or 3 μM buspirone. Bound radioactivity was separated from free by vacuum filtration over Whatman GF/B filters washed with 3 × 5 mL of ice-cold buffer. Radioactivity retained on the filters was determined by liquid-scintillation spectrophotometry.

Competition binding isotherms were analyzed by using iterative curve-fitting techniques,[32] which provided IC_{50} values and Hill coefficients for test compounds. K_i values were determined by the method of Cheng and Prusoff.[33]

α_2-Adrenoceptor Radioligand Binding Assay. The radioligand receptor binding studies were performed by a modification of a previously described procedure.[34] Rat cerebral cortices were homogenized in 20 volumes of Tris buffer (50 mM Tris HCl, 5 mM EDTA, pH 7.4 at 25 °C) with a Polytron PT 10 tissue disruptor. The homogenate was then centrifuged at 38000*g* for 15 min. The pellet obtained was washed three times by resuspension and centrifugation in assay buffer (50 mM Tris HCl, 0.5 mM EDTA, pH 7.4 at 25 °C). The final pellet was resuspended in assay buffer for direct use in binding studies.

Washed rat cerebrocortical membranes (1.0 mg/mL membrane protein) were incubated for 30 min at 25 °C with [^{3}H]yohimbine (89 Ci/mmol, pK_i 7.9) (2.0 nM) in the presence or absence of a range of 13 concentrations of the competing ligands in a total volume of 0.25 mL of Tris assay buffer. Nonspecific binding was defined as the concentration of bound ligand in the presence of 10 μM phentolamine. Following equilibrium (30 min), bound ligand was separated from free by vacuum filtration over Whatman GF/B glass-fiber filters. Radioactivity bound to the glass-fiber filters was determined by liquid-scintillation spectrophotometry. The pK_i values were determined as described for the 5-HT_{1A} receptor assay.

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Registry No. **3a**, 123882-48-4; **3a**·HBr, 123882-49-5; **3b**, 123882-50-8; **3b**·HBr, 123882-51-9; **3c**, 123882-52-0; **3c**·HCl, 123882-53-1; **3d**, 123882-54-2; **3d**·HBr, 123882-55-3; **4a**, 123882-56-4; **4a**·HCl, 123882-57-5; (±)-**4b**, 123882-58-6; (±)-**4b**·HCl, 123882-59-7; (*R*)-**4b**, 123932-19-4; (*R*)-**4b**·HCl, 123932-20-7; (*S*)-**4b**, 123932-21-8; (*S*)-**4b**·HCl, 123932-22-9; (±)-**4c**, 123882-60-0; (±)-**4c**·HCl, 123882-61-1; (*R*)-**4c**, 123932-23-0; (*R*)-**4c**·HCl, 123932-24-1; (*S*)-**4c**, 123932-25-2; (*S*)-**4c**·HCl, 123932-26-3; (±)-**4d**, 123882-62-2; (±)-**4d**·HCl, 123882-63-3; (*R*)-**4d**, 123932-27-4; (*R*)-**4d**·HCl, 123932-28-5; (*S*)-**4d**, 123932-29-6; (*S*)-**4d**·HCl, 123932-30-9; **4e**, 123882-64-4; **4e**·HCl, 123882-65-5; **4f**, 123882-66-6; **4f**·HBr, 123882-67-7; **4g**, 123882-68-8; **4g**·HCl, 123882-69-9; **4h**, 123882-70-2; **4h**·HCl, 123882-71-3; **4i**, 123882-72-4; **4i**·HCl, 123882-73-5; **4j**, 123882-74-6; **4j**·HCl, 123882-75-7; **4k**, 123882-76-8; **4k**·HCl, 123882-77-9; **4l**, 123882-78-0; **4l**·HCl, 123882-79-1; **4m**, 123882-80-4; **4m**·HCl, 123882-81-5; **4n**, 123882-82-6; **4n**·HCl, 123882-83-7; **4o**, 123882-84-8; **4o**·HCl, 123882-85-9; **4p**, 123882-86-0; **4p**·HCl, 123882-87-1; **4q**, 123882-88-2; **4q**·HCl, 123882-89-3; **5a**, 123882-90-6; **5a**·HCl, 123882-91-7; **5b**, 123882-92-8; **5b**·HBr, 123882-93-9; **5c**, 123882-94-0; **5c**·HBr, 123882-95-1; **6a**, 123882-96-2; **6a**·HBr, 123882-97-3; **6b**, 123882-98-4; **6b**·HCl, 123882-99-5; **7** (X = H), 123883-00-1; **7** (X = Cl), 123883-01-2; **8** (X = H), 123883-02-3; **8** (X = Cl), 123883-03-4; **9** (X = H), 123883-04-5; **9** (X = H)·HBr, 123883-05-6; **9** (X = Cl), 123883-06-7; **10** (X = Cl), 123883-07-8; **10** (X = H), 123883-08-9; **11**, 123883-09-0; **12**, 123883-10-3; **13**, 123883-11-4; **14**, 123883-12-5; **15**, 123883-13-6; **16**, 123883-14-7; **17a**, 123883-15-8; **17a**·HCl, 123883-16-9; **17b**,

(31) Gozlan, H.; El Mestikawy, S.; Pichat, L.; Glowinski, J.; Hamon, M. *Nature* **1983**, *305*, 140.
(32) Michel, A. D.; Whiting, R. L. *Br. J. Pharmacol.* **1984**, *83*, 460P.
(33) Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
(34) Cheung, Y. D.; Barnett, D. B.; Nahorski, S. R. *Eur. J. Pharmacol.* **1982**, *84*, 79.

123883-17-0; **17b**·HCl, 123883-18-1; (*R*)-**18**, 23357-47-3; (*S*)-**18**, 85977-52-2; (*R*)-**19**, 123883-19-2; (*R*)-**19**·HCl, 123883-20-5; (*S*)-**19**, 123883-21-6; **20**, 123883-22-7; **21**, 123883-23-8; **21**·HCl, 123883-24-9; **22**, 123883-25-0; **23**, 123883-26-1; **23**·HCl, 123883-27-2; **24**, 123883-28-3; **25**, 123883-29-4; **25**·HCl, 123883-30-7; **26a**, 30933-66-5; **26b**, 123883-31-8; **27a**, 16176-73-1; **27b**, 123883-32-9; **27** (X = Cl, R = CHO), 123883-33-0; **28a**, 94980-84-4; **28b**, 123883-34-1; **29**, 39830-66-5; **30**, 123883-35-2; **31**, 38073-22-2; **32**, 38073-26-6; **32**·HCl, 38073-29-9; $AcO(CH_2)_2NO_2$, 18942-89-7; $MeNH(CH_2)_2OH$, 109-83-1; $MeNHCH_2CH(OMe)_2$, 122-07-6; 8-(4-chlorobutyl)-8-azaspiro[4.5]decane-7,9-dione, 21098-11-3; 2,3-dihydro-1*H*-indene-1-carboxylic acid, 14381-42-1; 6-methoxyindan-1-carboxylic acid, 62956-62-1; 5-methoxy-2-methyl-2,3,4,8,9,9a-hexahydro-1*H*-indeno[1,7-*cd*]azepin-1-one, 123883-36-3; benzocycloheptene-1-carboxylic acid, 14378-56-4; 2-cyano-1,2,3,4,8,9,10,10a-octahydronaphth[1,8-*cd*]azepine, 123883-37-4; 5-chloro-1,2,3,4,8,9,10,10a-octahydronaphth[1,8-*cd*]azepine, 123883-38-5; *N*-(2-hydroxyethyl)-*N*-methyl-1-tetralincarboxamide, 123883-39-6; 7-methoxy-1,2,3,4-tetrahydro-1-naphthaleneacetic acid, 27559-29-1; 10-methoxy-3,4,4a,5,6,7-hexahydronaphth[1,8-*cd*]azepine, 123883-40-9; 7-methoxy-1,2,3,4-tetrahydronaphthalene-1-methanamine, 57314-45-1; 4-methoxy-1-(*p*-tolylsulfonyl)-2,3,7,8,9,9a-hexahydro-1*H*-benzo[*de*]quinoline, 123883-41-0; 4-methoxy-2,3,7,8,9,9a-hexahydro-1*H*-benzo[*de*]quinoline, 123883-42-1; *N*-tosyl-5-chloro-4-(cyanomethyl)indole, 99696-42-1; 1-tetralincarboxylic acid, 1914-65-4; 7-chloro-1-tetralincarboxylic acid, 91193-16-7; 7-methoxy-1-tetralincarboxylic acid, 85858-95-3; 6-chloro-1-indancarboxylic acid, 52651-15-7; 2,3,4,5-tetrahydro-1*H*-3-benzazepine, 4424-20-8; 6-chloro-2,3,4,5-tetrahydro-1*H*-3-benzazepine, 26232-35-9.

Total Synthesis of the Four Stereoisomers of Dihexadecanoyl Phosphatidylinositol and the Substrate Stereospecificity of Human Erythrocyte Membrane Phosphatidylinositol 4-Kinase

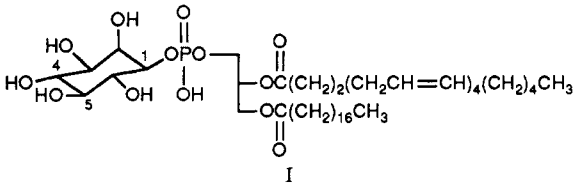
Rodney C. Young,*,† C. Peter Downes,‡ Drake S. Eggleston,§ Martin Jones,† Colin H. Macphee,‡ Kishore K. Rana,† and John G. Ward†

Smith Kline & French Research Limited, The Frythe, Welwyn, Hertfordshire, England AL6 9AR, and Smith Kline & French Laboratories, P.O. Box 1539, King of Prussia, Pennsylvania 19406-0939. *Received May 24, 1989*

A new and convenient method for the preparation of the four stereoisomers of dihexadecanoyl phosphatidylinositol has been developed. An enantiomeric pair of acid-labile, pentaprotected *myo*-inositol building blocks was synthesized in high yield and coupled with chiral phenyl dihexadecanoylglyceryl phosphates to give the fully protected phosphatidylinositols. These were subsequently deprotected by hydrogenolysis and self-hydrolysis in aqueous ethanol to give the desired pure products. Comparison of these compounds as potential substrates for a partially purified phosphatidylinositol 4-kinase (EC 2.7.1.67) derived from human erythrocyte membranes revealed that the chirality of the inositol ring is crucial for efficient phosphorylation, whereas the chirality of the glycerol moiety is relatively unimportant. Moreover, the similarity in phosphorylation rates of the naturally occurring mammalian phospholipid, I, and its synthetic stereochemical counterpart, compound **10a**, suggests that the enzyme is relatively tolerant to changes in fatty acid composition.

The identification of D-*myo*-inositol 1,4,5-tris(phosphate)[1] and diacylglycerol[2] as intracellular second messengers has led to wide interest in enzymes that regulate their synthesis and metabolism. Their precursor is the minor membrane phospholipid phosphatidylinositol 4,5-bis(phosphate) (PIP_2), which is cleaved by a receptor-coupled phospholipase C upon stimulation by a range of neurotransmitters, hormones, and growth factors.[3]

In order to maintain the supply of PIP_2, rapid sequential phosphorylation of the more abundant phospholipid phosphatidylinositol (PI, I) by specific kinases occurs.



I

Initially, the 4-hydroxyl group of PI is phosphorylated by a PI 4-kinase to give phosphatidylinositol 4-phosphate (PIP), followed by a PIP 5-kinase-catalyzed phosphorylation of the 5-hydroxyl group.[3] PI is also a substrate for phospholipases A[4] and C[5] and a 3-kinase.[6] The relative importance of these different enzymes in modulating the production of second messengers is thus far unclear, and more detailed information about their specificity and kinetics requires supplies of pure, chemically and stereochemically well-defined PI analogues. Although various methods have been reported for the synthesis of chiral PI analogues[7] following the pioneering work of Shvets et al.[8] in 1970, many stages, including the resolution of the inositol building block, have been hampered by low yields and lack of reproducibility. Here, we describe an unambiguous route for the preparation of the four stereoisomers of the dihexadecanoyl analogue of I in good yield and high chemical purity, starting from readily available materials, and their use as probes to study the stereochemical requirements for binding to a partially purified PI 4-kinase derived from human erythrocyte membranes.

Chemistry

The observation that 2,3(1):5,6(4)-di-*O*-isopropylidene-D-*myo*-inositol could be regioselectively silylated at the

† Department of Medicinal Chemistry, SK&F England.
‡ Department of Cellular Pharmacology, SK&F England.
§ Research and Development Division, SK&F U.S.A.

(1) Berridge, M. J.; Irvine, R. F. *Nature (London)* **1984**, *312*, 315.
(2) Nishizuka, Y. *Nature (London)* **1984**, *308*, 693.
(3) Downes, C. P.; Michell, R. H. In *Molecular Aspects of Cellular Regulation*; Cohen, P., Houslay, M. D., Eds.; Molecular Mechanisms of Transmembrane Signaling, Vol. 4 Elsevier: Amsterdam, 1985; p 3.
(4) Rittenhouse, S. E. *Cell Calcium* **1982**, *3*, 311.
(5) Dawson, R. M. C.; Clarke, R. *Biochem. J.* **1972**, *127*, 113.
(6) Whitman, M.; Downes, C. P.; Keeler, M.; Keller, T.; Cantley, L. *Nature (London)* **1988**, *332*, 644.
(7) Gigg, R. *Chem. Phys. Lipids* **1980**, *26*, 287.
(8) Zhelvakova, E. G.; Klyashchitskii, B. A.; Shvets, V. I.; Evstigneeva, R. P.; Preobrazhenskii, N. A. *Zh. Obshch. Khim.* **1970**, *40* (1), 248.

0022-2623/90/1833-0641$02.50/0