Articles

Binding of 5*H*-Dibenzo[*b*,*e*][1,4]diazepine and Chiral 5*H*-Dibenzo[*a*,*d*]cycloheptene Analogues of Clozapine to Dopamine and Serotonin Receptors

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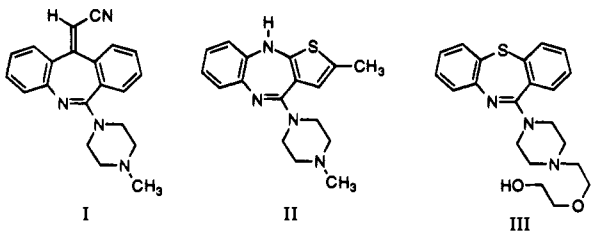
5*H*-Dibenzo[*b*,*e*][1,4]diazepine, dibenz[*b*,*f*]oxepin, and 5*H*-dibenzo[*a*,*d*]cycloheptene analogues of clozapine [8-chloro-11-(4-methylpiperazino)-5*H*-dibenzo[*b*,*e*][1,4]diazepine] were evaluated for their binding affinity to dopamine D-1, D-2, and D-4 and serotonin S-2A (5-HT_{2A}), S-2C (5-HT_{2C}), and S-3 (5-HT_3) receptors. The diazepine analogues display selective binding to the dopamine D-4 and serotonin S-2A receptors similar to that of clozapine, but none has a dopamine D-4 selectivity (K_i for the dopamine D-2A receptor/K_i for the dopamine D-4 receptor) greater than that of clozapine. All of the oxepin analogues also show substantial binding to the dopamine D-4 and serotonin S-2A receptors with 10-(4-methylpiperazino)dibenz[*b*,*f*]oxepin having a dopamine D-4 selectivity greater than that of clozapine. Some of the 5*H*-dibenzo[*a*,*d*]cycloheptene analogues also show strong binding to both the dopamine D-4 and serotonin S-2A receptors, 5-methyl-10-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cycloheptene having a dopamine D-4 selectivity of 7.8 as compared to 10 for clozapine but a serotonin S-2A selectivity (K_i for the dopamine D-2 receptor/K_i for the serotonin S-2A receptor) of 2.0 as compared to 28 for clozapine. The serotonin S-2A selectivity of 2-chloro-10-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cycloheptene is 200. As an extension of these studies, chiral 5-substituted 10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene analogues show a substantial enantiospecificity toward dopamine and serotonin receptor subtypes, (*R*)-(−)-5-methyl compound having a 2-fold higher dopamine D-4 selectivity than its (*S*)-(+) enantiomer as the result of enhanced binding to the dopamine D-4 receptor rather than diminished binding to the dopamine D-2 receptor. (pR_a,pS_b)-(+)-5-(2-Propylidene)-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene is 17 times more active in binding to the dopamine D-4 receptor than is its pS_a,pR_b enantiomer while being only 1.5 times more active in binding to the dopamine D-2 receptor.

The high-dose, atypical neuroleptic agent clozapine [8-chloro-11-(4-methylpiperazino)-5*H*-dibenzo[*b*,*e*][1,4]-diazepine, **1a**, Table 1] is an effective antipsychotic agent, but its therapeutic use has been hampered by rare incidences of blood toxicity (agranulocytosis)[4] which requires expensive monitoring of leukocytes to accompany clozapine therapy. At present, other neuroleptics are available, but no effective substitute with a clozapine-like profile and free of side effects has been found.[5] Over the past two decades, however, several new tricyclic azepines with receptor binding profiles similar to that of clozapine have been or are currently being evaluated as potential atypical antipsychotic. [6-(4-Methylpiperazino)-11*H*-dibenz[*b*,*e*]azepin-11-ylidene]acetonitrile has its antidopaminergic activity confined to the *E* isomer (I) while the *Z* isomer shows the anticholinergic (muscle relaxing) properties.[6] Olanzapine (II) was selected for clinical trials to replace its fluoro analogue flumezapine on the basis of being 4 times more potent than clozapine in blocking conditioned avoidance response in the rat.[7] Seroquel (III) combines weaker affinities for dopamine and serotonin receptors compared to clozapine with an equipotent functional antidopaminergic activity.[8]



To date, however, no replacement in the clinic for clozapine has been found, and most attempts to develop clinically useful heterocyclic isosteres of clozapine with similar biological activity have been unsuccessful because of unexpected toxicity or unacceptable side effects of these analogues.[9,10] We have speculated[11] that the presence of the heteroatoms in clozapine and its het-

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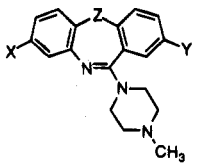
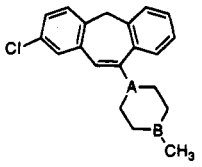
Table 1. Affinity of Clozapine and Clozapine Analogues for Dopamine and Serotonin Binding Sites



				inhibition constant, K_i, nM								
	substituents			dopamine				serotonin				
code	X	Y	Z	D-1	D-2	D-4	D-2/D-4[a]	S-2A	S-2C	S-3	D-2/S-2A[b]	D-2/S-3[c]
1a[d]	Cl	H	NH	43[e]	220	21	10	8	8	53	28	4.2
1b[f]	H	Cl	NH	14[e]	47	16	2.9	7	11	22	6.7	2.1
1c[g]	H	H	NH	200[e]	2500	420	6.0	39	84	40	64	63
1d[h]	Cl	H	NCH_3	*i*	1100	120	9.2	140	74	40	7.9	28
1e[j]	Cl	H	O	*i*	150	23	6.5	11	19	39	14	3.8
1f[k]	H	Cl	O	*i*	21	4.9	4.3	*i*	*i*	*i*	–	–

[a] Dopamine D-4 selectivity. [b] Serotonin S-2A selectivity. [c] Serotonin S-3 selectivity. [d] Clozapine. [e] Calculated (ref 1) from the IC_{50} value for the displacement of [^{3}H]SCH 23390 reported in ref 2. [f] Isoclozapine. [g] Dechloroclozapine. [h] *N*-Methylclozapine and characterized in ref 3. [i] Not determined. [j] Isoloxapine. [k] Loxapine.

Table 2. Affinity of Clozapine Analogues for Dopamine and Serotonin Binding Sites



			inhibition constant, K_i, nM							
	substituents		dopamine			serotonin				
code	A	B	D-2	D-4	D-2/D-4[a]	S-2A	S-2C	S-3	D-2/S-2A[b]	D-2/S-3[c]
2a[d,e]	CH	N	560	2700	0.21	3	3	41	200	14
2b[e]	N	CH	1400	1000	1.4	>1000	>1000	>1000	<1	<1

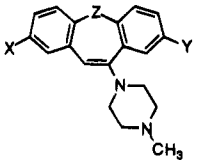
[a] Dopamine D-4 selectivity. [b] Serotonin S-2A selectivity. [c] Serotonin S-3 selectivity. [d] Hydrochloride. [e] Characterized in ref 3.

erocyclic analogues may be the origin of some of their toxicological problems. As an alternative modification of the clozapine structure, we earlier demonstrated that three of the four nitrogen atoms of clozapine may be replaced with carbon atoms giving analogues of clozapine with *in vitro* binding profiles similar to or superior to that of clozapine.[3] Thus, 2-chloro-10-(1-methyl-4-piperidinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene (**2a**, Table 2) binds twice as potently to nonmuscarinic [^{3}H]clozapine sites in rat forebrain as does clozapine while being about 50 times less anticholinergic.[3] The positional isomer of **2a**, 2-chloro-11-(4-methylpiperidino)-5*H*-dibenzo[*a*,*d*]cycloheptene (**2b**), however, was inactive at clozapine and muscarinic binding sites.[3] The analogue of clozapine in which only the two nitrogen atoms of the tricyclic system have been replaced with carbon atoms, 2-chloro-10-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cycloheptene (**3a**, Table 3), has an *in vitro* binding profile similar to that of **2a**.[3]

Clozapine and its two biologically active 5,11-dicarbo analogues **2a** and **3a** are also similar in that they exist as nonplanar conformational enantiomers. These conformational enantiomers, however, cannot be separated because of the low activation energy for conversion of one enantiomer into the other by ring inversion of their respective tricyclic systems. In order to achieve resolution into configurational enantiomers and thereby the possibility of high binding to one neuroreceptor subtype by one enantiomer and high binding to another neuroreceptor subtype by the other enantiomer, the barrier for inversion of the tricyclic system must be sufficiently high (activation energy of 20–25 kcal mol^{-1}) to prevent thermal racemization.[13]

In preliminary experiments to test the feasibility of the preparation of enantiopure clozapine analogues, dynamic proton nuclear magnetic resonance (^{1}H NMR) studies with a number of 5-substituted derivatives of 10-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cycloheptene in nitrobenzene-d_5 showed that the 5-(2-propylidene) derivative **3g** has an activation energy of greater than 23 kcal mol^{-1} at 160 °C for interconversion of its two nonplanar enantiomers.[2] This activation energy is sufficiently high for prevention of the racemization of the configurational enantiomer under normal laboratory conditions. Since **3g** and its 2-chloro analogue **3f** do not differ greatly from clozapine in their *in vitro* binding[11] to muscarinic ([^{3}H]QNB)[14] and dopamine D-1 ([^{3}H]SCH 23390)[15,16] and D-2 ([^{3}H]spiperone)[16,17] receptors, the enantiomers of these 5-isopropylidene analogues of clozapine appeared to be suitable candidates to investigate the enantioselectivity of neuroreceptor binding sites. However, because of the susceptibility of the enamine moiety in **3f** and **3g** to acid-catalyzed hydrolysis (unpublished observation), resolution of **3f** and **3g** into their enantiomers is not practical. Since the nitrogen atom at position 1 of the piperazino ring in **3f** and **3g** may not be required for biological activity[3] and the chlorine atom at C-2 in clozapine may not be crucial for a clozapine-like biological profile,[3] we have now prepared two tricarbo analogues of clozapine, (±)-5-methyl-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene [(±)-**4b**, Table 4] and (±)-

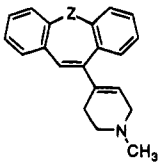
Table 3. Affinity of Clozapine Analogues for Dopamine and Serotonin Binding Sites



	substituents			inhibition constant, K_i, nM								
				dopamine				serotonin				
code	X	Y	Z	D-1	D-2	D-4	D-2/D-4[a]	S-2A	S-2C	S-3	D-2/S-2A[b]	D-2/S-3[c]
3a[d]	Cl	H	CH_2	*e*	520	2900	0.18	3	7	120	200	4.3
3b[d]	H	Cl	CH_2	*e*	1.0	1.0	1.0	2	6	49	0.5	0.020
3c	H	H	$CHCH_3$	16[f]	94	12	7.8	47	36	*e*	2.0	–
3d	H	H	$C{=}CH_2$	4.7[f]	57	47	1.2	11	18	31	5.2	1.8
3e	H	H	$C{=}CHCH_3$	68[f]	730	300	2.4	98	110	35	7.4	21
3f	Cl	H	$C{=}C(CH_3)_2$	98[g]	690	930	0.74	230	370	100	3.0	6.9
3g	H	H	$C{=}C(CH_3)_2$	120[f]	290	2700	0.11	250	390	81	1.2	3.6
3h[h]	Cl	H	O	*e*	8.7	0.90	9.7	3	6	57	3	0.15
3i[h]	H	Cl	O	*e*	2.5	0.54	4.6	3	3	84	0.8	0.030
3j[h]	H	H	O	*e*	21	2.0	11	4	6	41	5	0.51

[a] Dopamine D-4 selectivity. [b] Serotonin S-2A selectivity. [c] Serotonin S-3 selectivity. [d] Characterized in ref 3. [e] Not determined. [f] Calculated (ref 1) from the IC_{50} value for the displacement of [^{3}H]SCH 23390 reported in ref 2. [g] Calculated (ref 1) from the IC_{50} value for the displacement of [^{3}H]SCH 23390 reported in ref 11. [h] Characterized in ref 12.

Table 4. Affinity of Clozapine Analogues for Dopamine and Serotonin Binding Sites



	substituent	inhibition constant, K_i, nM					
		dopamine			serotonin		
code	Z	D-2	D-4	D-2/D-4[a]	S-2A	S-2C	D-2/S-2A[b]
4a[c]	$C{=}CH_2$	250	330	0.76	30	35	8.3
(±)-**4b**[c]	$CHCH_3$	680	320	2.1	72	89	9.4
(*R*)-(−)-**4b**	$CHCH_3$	640	190	3.4	74	70	8.6
(*S*)-(+)-**4b**	$CHCH_3$	730	400	1.8	200	250	3.7
(±)-**4c**	$C{=}C(CH_3)_2$	570	270	2.1	1200	1500	0.48
(pR_a,pS_b)-(+)-**4c**	$C{=}C(CH_3)_2$	520	150	3.5	2200	3300	0.24
(pS_a,pR_b)-(−)-**4c**	$C{=}C(CH_3)_2$	790	2500	0.32	830	830	0.95

[a] Dopamine D-4 selectivity. [b] Serotonin S-2A selectivity. [c] Hydrochloride.

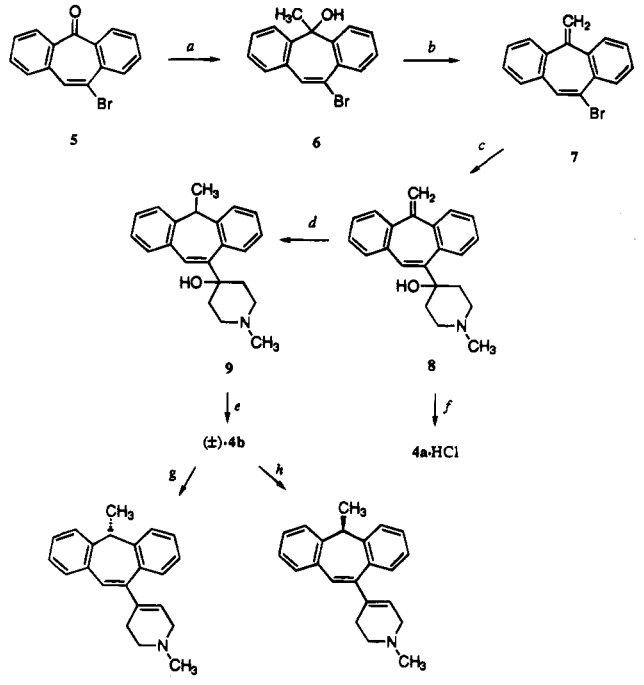
5-(2-propylidene)-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene [(±)-**4c**], capable of resolution into enantiomers. We have resolved (±)-**4b** and (±)-**4c** and have evaluated the respective enantiomers for binding to dopamine D-2[16,17] and D-4[18] and serotonin S-2A (5-HT_{2A}[19])[20,21] and S-2C (5-HT_{2C}[19])[21,22] receptors (Table 4) and compared these binding activities to the same activities as well as the serotonin S-3 (5-HT_3[19])[23-25] binding activity for clozapine (**1a**), isoclozapine (**1b**), dechloroclozapine (**1c**), and *N*-methylclozapine (**1d**) (Table 1), the two 5,10-dicarbo 10-(1-methyl-4-piperidinyl and 4-methylpiperidino) analogues of clozapine **2a** and **2b** (Table 2), and a series of 5,11-dicarbo and 5-oxy-11-carbo analogues of clozapine **3a**-**3j** (Table 3) reported earlier.[2,3,11,12]

These extensive binding studies were prompted by the general consensus that extrapyramidal side effects (EPS) are caused by blockade of limbic dopamine D-2 receptors,[26] and since all neuroleptic agents that have a proven efficacy against schizophrenia block the dopamine D-2 receptor to some degree,[26] an atypical antipsychotic profile (no EPS) must be achieved by interactions with additional neurotransmitter systems, possibly dopamine D-4,[18] dopamine D-1, and/or serotonin S-2A receptors.[27] As in earlier work with heterocyclic isosteres of clozapine which display similar behavioral and neurochemical profiles as those of clozapine,[28] we use clozapine as the model having an atypical neuroleptic receptor binding profile. Our objective is the discovery of analogues of clozapine which have carbon atoms substituted for the nitrogen atoms of clozapine and which have a dopamine and serotonin binding profile similar to that of clozapine.

Results

Synthesis. As shown in Scheme 1, the preparation of 5-methylene-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene (**4a**) and (±)-, (*S*)-(+)-, and (*R*)-(−)-5-methyl-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene [(±)-, (*S*)-(+)-, and (*R*)-(−)-**4b**] proceeded from 10-bromo-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one[29] (**5**). Treatment of **5** with methylmagnesium iodide in ether–tetrahydrofuran gave 10-bromo-5-methyl-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ol (**6**). Dehydration of **6** in acid gave 10-bromo-5-methylene-5*H*-dibenzo[*a*,*d*]cycloheptene (**7**). Reaction of the latter with *n*-butyllithium in hexanes formed the lithium salt which reacted with 1-methyl-4-piperidone. The result-

Scheme 1

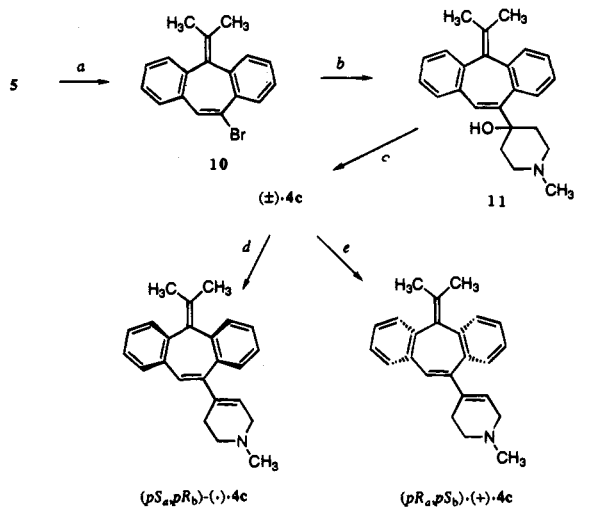


[a–h]Reagent (isolated yield): *a*, methylmagnesium iodide in ether–tetrahydrofuran and then aqueous ammonium chloride (94%); *b*, concentrated hydrochloric acid in ethanol (78%); *c*, *n*-butyllithium in ether at −78 °C and then 1-methyl-4-piperidone (61%); *d*, hydrogen over palladium on carbon in 95% ethanol (89%); *e*, concentrated hydrochloric acid in ethanol and then sodium hydroxide (16%); *f*, concentrated hydrochloric acid in ethanol (28%); *g*, (2*S*,3*S*)-*O*,*O*′-di-*p*-toluoyltartaric acid in methanol (9%); *h*, (2*R*,3*R*)-*O*,*O*′-di-*p*-toluoyltartaric acid in methanol (31%).

ing 10-(4-hydroxy-1-methyl-4-piperidinyl)-5-methylene-5*H*-dibenzo[*a*,*d*]cycloheptene (**8**) was dehydrated to give **4a**. Reduction of **8** with hydrogen afforded 10-(4-hydroxy-1-methyl-4-piperidinyl)-5-methyl-5*H*-dibenzo-[*a*,*d*]cycloheptene (**9**), which was also dehydrated to give (±)-**4b**. The latter was resolved into (*S*)-(+)-**4b** and (*R*)-(−)-**4b** by fractional recrystallization of their (2*S*,3*S*)-*O*,*O*′-di-*p*-toluoyl- and (2*R*,3*R*)-*O*,*O*′-di-*p*-toluoyltartrate hydrogen[30,31] salts, respectively.

As shown in Scheme 2, the preparation of the enantiomers of 5-(2-propylidene)-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene (**4c**) also proceeded from 10-bromo-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one[29] (**5**). Treatment of **5** with isopropyltriphenylphosphonium iodide in a Wittig reaction gave 10-bromo-5-(2-propylidene)-5*H*-dibenzo[*a*,*d*]cycloheptene (**10**). Reaction of the bromide **10** with *n*-butyllithium in ether formed the anion which reacted with 1-methyl-4-piperidone to give 10-(4-hydroxy-1-methyl-4-piperidinyl)-5-(2-propylidene)-5*H*-dibenzo[*a*,*d*]cycloheptene (**11**). Dehydration of **11** gave (±)-**4c**, and resolution of (±)-**4c** into (pS_a,pR_b)-(−)-**4c** and (pR_a,pS_b)-(+)-**4c** was accomplished by fractional crystallization, respectively, of their (2*S*,3*S*)-*O*,*O*′-di-*p*-toluoyl- and (2*R*,3*R*)-*O*,*O*′-di-*p*-toluoyltartrate hydrogen[30,31] salts. It is to be noted that (−)-**4c** and (+)-**4c** exist as configurational enantiomers by virtue of planar chirality with configurational designators assigned as outlined below. Their enantiomeric excesses (ee) were established as greater than 95% on the basis of the 300-MHz ^{1}H NMR spectrum of a 1:1 mole complex of (+)-**4c** and the chiral solvating agent (*R*)-α-(trifluoromethyl)benzyl alcohol in chloroform-*d*.

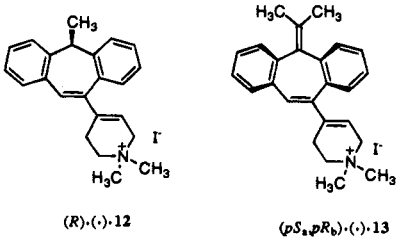
Scheme 2



[a–e]Reagent (isolated yield): *a*, 2-propylidenetriphenylphosporane in ether (76%); *b*, *n*-butyllithium in hexane–ether and then 1-methyl-4-piperidone at −78 °C (64%); *c*, concentrated hydrochloric acid in ethanol (72%); *d*, (2*S*,3*S*)-*O*,*O*′-di-*p*-toluoyltartaric acid in methanol (41%); *e*, (2*R*,3*R*)-*O*,*O*′-di-*p*-toluoyltartaric acid in methanol (52%).

This spectrum showed a singlet at 6.62 ppm downfield from TMS assigned to the vinyl proton at C-11 in (+)-**4c**. The 300-MHz ^{1}H NMR spectrum with (±)-**4c** and the chiral solvating agent showed singlets at 6.66 and 6.62 ppm assigned to the C-11 protons in the respective enantiomers. This same spectroscopic method using (*R*)α-(trifluoromethyl)benzyl alcohol and (*R*)-2,2,2-trifluoro-1-(9-anthryl)ethanol as chiral solvating agent was not successful for establishment of the ee of the enantiomers of **4b**. Using these chiral solvating agents, there was no doubling of any of the protons signals in the ^{1}H NMR spectrum of (±)-**4b**. Thus the enantiomeric excess of each enantiomer of **4b** is unknown.

Absolute Configuration Assignment. Although the circular dichroism spectra of (−)-5-methyl-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene [(−)-**4b**] and (+)- and (−)-5-(2-propylidene)-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo-[*a*,*d*]cycloheptene [(+)- and (−)-**4c**] are reported in the Experimental Section, the absolute configurations of these substances could not be established on the basis of these spectra. Thus, the methiodide salts of (−)-**4b** and (−)-**4c**, (−)-5-methyl-10-(1,2,3,6-tetrahydro-1,1-dimethylpyridinium-4-yl)-5*H*-dibenzo[*a*,*d*]cycloheptene iodide [(−)-**12**] and (−)-5-(2-propylidene)-10-(1,2,3,6-tetrahydro-1,1-dimethylpyridinium-4-yl)-5*H*-dibenzo-[*a*,*d*]cycloheptene iodide [(−)-**13**], were prepared as solid



derivatives for anomalous X-ray diffraction studies. Using (−)-**12**, X-ray diffraction clearly showed that this isomer had the *R* configuration, thus establishing the configuration of (−)-**4b** as *R*. Relevant crystal data and

Table 5. Crystal Data and Details of Structure Determination

	(pS_a,pR_b)-(−)-**4c**	(*R*)-(−)-**12**	(+)-(pS_a,pR_b)-**14**·(2*S*,3*S*)-**15**·$(CH_3OH)_2$
formula	$C_{24}H_{25}N$	$C_{23}H_{26}IN$	$C_{44}H_{43}NO_8$·$(CH_3OH)_2$
fw	327.45	443.37	777.88
cryst growth solvent	acetonitrile	acetonitrile	methanol
cryst morphology	prisms	needles	prisms
cryst color	pale yellow	yellow	colorless
cryst dimensions, mm	0.33 × 0.38 × 0.43		
space group	$P2_12_12$ (No. 18)	$P2_12_12_1$ (No. 19)	$P2_12_12_1$ (No. 19)
cell dimensions, Å	12.933(2)	9.907(8)	13.400(2)
	18.456(2)	29.885(6)	30.090(2)
	8.008(1)	7.792(7)	10.259(2)
V, Å^3	1911.5(4)	2307(2)	4136.6(9)
Z	4	4	4
d (calcd), g cm^{-3}	1.138	1.276	1.173
wavelength, Å	1.54178	0.71069	1.54178
abs coeff, cm^{-1}	4.608	13.758	6.319
no. of reflections	1668	2385	3507
no. with $F > 3.0\sigma(F)$	1348	1762	2792
$R(F)$	0.041	0.075	0.076
$R_w(F)$	0.045	0.112	0.105

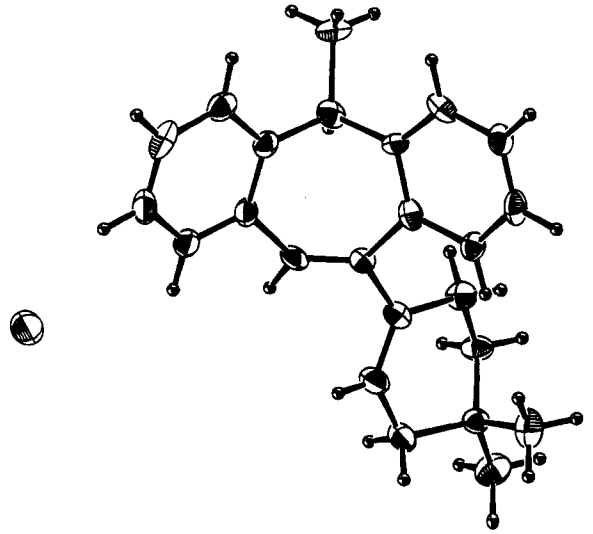


Figure 1. ORTEP view of (*R*)-(−)-5-methyl-10-(1,2,3,6-tetrahydro-1,1-dimethylpyridinium-4-yl)-5*H*-dibenzo[*a*,*d*]cycloheptene iodide [(*R*)-(−)-**12**]. Thermal ellipsoids are shown at the 30% probability level.

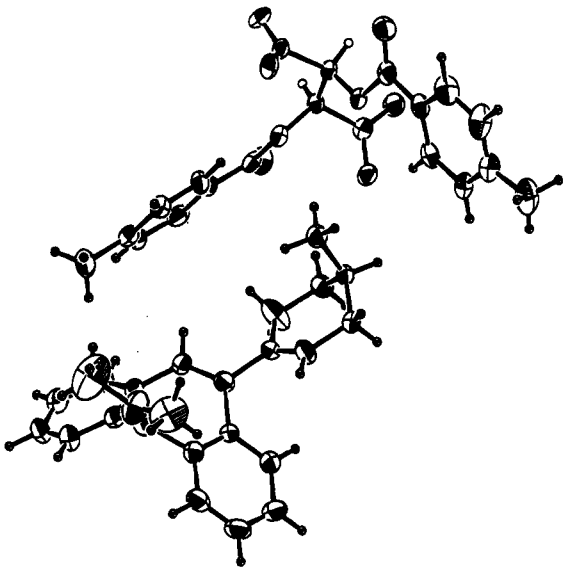


Figure 2. ORTEP view of (+)-(pS_a,pR_b)-5-(2-propylidene)-10-(1,2,3,6-tetrahydro-1-methylpyridinium-4-yl)-5*H*-dibenzo[*a*,*d*]-cycloheptene hydrogen (2*S*,3*S*)-*O*,*O*′-di-*p*-toluoyltartrate dimethanolate [(+)-(pS_a,pR_b)-**14**·(2*S*,3*S*)-**15**·$(CH_3OH)_2$]. Thermal ellipsoids are shown at the 30% probability level, and the methanol molecules are omitted for clarity.

the summaries of X-ray collection parameters are given in the Experimental Section and in Table 5, and an ORTEP view of (*R*)-(−)-**12** is shown in Figure 1.

Although a crystal for (−)-**13** appeared to be satisfactory for X-ray diffraction studies, the diffraction pattern could not be solved for the constitution and absolute configuration of (−)-**13**. It was found, however, that the salt formed from (−)-**4c** and (2*S*,3*S*)-*O*,*O*′-di-*p*-toluoyltartaric acid, (+)-5-(2-propylidene)-10-(1,2,3,6-tetrahydro-1-methylpyridinium-4-yl)-5*H*-dibenzo[*a*,*d*]cycloheptene hydrogen (2*S*,3*S*)-*O*,*O*′-di-*p*-toluoyltartrate [(+)-(pS_a,pR_b)-**14**·(2*S*,3*S*)-**15**], isolated as the dimethanolate on recrystallization from methanol, afforded a suitable crystal for X-ray studies. Similar to studies for establishment of the absolute configuration of (−)-*p*-synephrine cation on the basis of the known configuration of its (−)-3-bromocamphor-8-sulfonate anion,[32] solution of the diffraction pattern with the imposed known absolute configuration of the anion (2*S*,3*S*)-**15** as depicted in the ORTEP view in Figure 2 gives the absolute configuration of cation **14** as pS_a,pR_b. Relevant crystal and data collection parameters for the salt (+)-(pS_a,pR_b)-**14**·(2*S*,3*S*)-**15**, isolated and used as the dimethanolate, are give in Table 5. The configurational designator, using the Cahn–Ingold–Prelog system, follows from consideration of the two chiral planes a and b with C-2 of the propylidene group attached at C-5 of the 5*H*-dibenzo[*a*,*d*]cycloheptene system as the pilot atom for each plane since C-5 is common to both planes. Plane a, formed by C-5, C-5a, and C-9a, precedes plane b, formed by C-5, C-4a, and C-11a, by virtue of the attachment of the 1-methyl-1,2,3,6-tetrahydropyridinium-4-yl group at C-10 of the tricyclic system. Plane a has *S* chirality since going from C-5 to C-5a and then to C-9a describes a left-handed (counter clockwise) pattern as viewed from the pilot atom. For plane b, the chirality is *R* since the

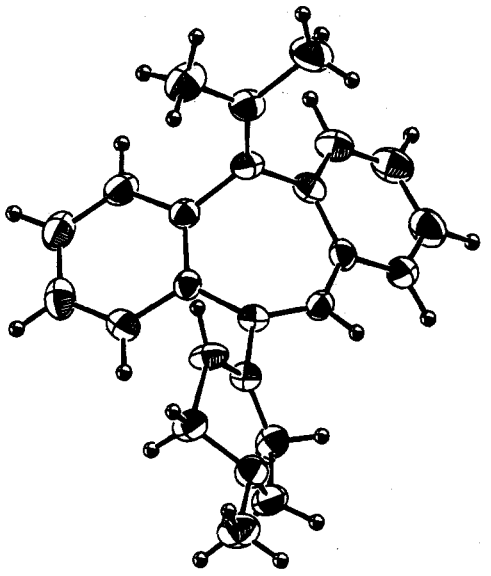


Figure 3. ORTEP view of (pS_a,pR_b)-(−)-5-(2-propylidene)-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene [(pS_a,pR_b)-(−)-**4c**]. Thermal ellipsoids are shown at the 30% probability level.

pattern from C-5 to C-4a and then to C-11a describes a right-handed (clockwise) pattern when viewed from the pilot atom.

A crystal of (−)-**4c** was also used in X-ray scattering studies, and the diffraction was solved giving the constitution and absolute configuration shown in the ORTEP view shown in Figure 3. The absolute configuration of (−)-**4c** as depicted in Figure 3 is pS_a,pR_b.

When the diffraction pattern was refined for the constitution and absolute configuration pR_a,pS_b, R_w(F) rose to 0.050. The difference between this value and that for absolute configuration pS_a,pR_b (0.045) was not significant at the $\alpha = 0.01$ level using Hamilton's *R*-test, and thus, the absolute configuration of (−)-**4c** was indeterminant on the basis of its X-ray diffraction.

Receptor Binding Studies. Affinities for dopamine D-2 and D-4 receptors were determined by inhibition of [^{3}H]spiperone (spiroperidol) binding in the presence of sodium chloride to membranes prepared from COS-7 cells transfected with a gene expressing the human dopamine D-2 (long) and D-4 receptors, respectively, as previously reported.[18] Each inhibition constant (K_i) was an average from two experiments, with the individual values from each of these experiments consistently within 10% of each other.

Affinities for serotonin S-2A (5-HT_{2A}) and S-2C (5-HT_{2C}) receptors were determined by inhibition of iodine-125-labeled lysergic acid diethylamide ([^{125}I]LSD) binding to NIH 3T3 cell line membranes containing the cloned rat serotonin S-2A receptor designated "GF-6"[21] and the cloned rat serotonin S-2C receptor designated "P_c",[21] respectively. Affinities for the serotonin S-3 (5-HT_3) receptor were determined by inhibition of [^{3}H]-GR65630 binding to the serotonin S-3 receptor prepared from NG108−15 cell membranes.[23] The inhibition constants (K_i) were calculated from the IC_{50} values in the usual way.[1]

As seen in Table 1, in comparison to the blocking of the dopamine D-2 receptor, clozapine (**1a**) is 10 and 28 times more potent in blocking the dopamine D-4 and the serotonin S-2A receptors, respectively. This serotonin S-2A selectivity is in agreement with the earlier finding that clozapine and its *N*-demethyl metabolite are potent blockers of the serotonin S-2A and S-2C receptors.[33] The dopamine D-2 and D-4 receptor binding affinities for isoclozapine (**1b**) are higher than those for clozapine (**1a**), but the dopamine D-4 selectivity for clozapine is higher than that for **1b**. The dechloro analogue **1c** is less active than clozapine for binding to the dopamine D-2 and D-4 receptors, and the dopamine D-4 selectivity for **1c** is also lower than that for clozapine but higher than that for isoclozapine (**1b**). For *N*-methylclozapine (**1d**), the affinities for both the D-2 and D-4 receptors are less than those for clozapine by about a factor of 5, but the dopamine D-4 selectivity is about the same. Increased dopamine D-2 and D-4 binding activities are seen for the oxepin series. Isoloxapine (**1e**), with its chlorine atom distal to the piperazine ring, shows lower binding affinities for the dopamine D-2 and D-4 receptors than does loxapine (**1f**), the latter with its chlorine atom proximal to the piperazine moiety. Analogue **1e**, however, has a higher dopamine D-4 selectivity than that for **1f**. Significantly, of the substances in Table 1, clozapine has the highest dopamine D-4 selectivity. Thus, shifting of the chlorine atom from the position distal to the piperazino ring in clozapine (**1a**) and isoloxapine (**1e**) to the proximal position in isoclozapine (**1b**) and loxapine (**1f**) increases affinity for the dopamine D-4 receptor but at the expense of a lower dopamine D-4 selectivity, possibly making **1b** and **1f** typical antipsychotic agents. Replacement of the bridge nitrogen atom of clozapine with an oxygen, as in isoloxapine (**1e**), loxapine (**1f**), and the three isomeric dibenz[*b*,*f*]oxepins **3h**−**3j**, results in increased affinity for the dopamine D-2 and D-4 receptors but no increase in the dopamine D-4 selectivity. For these oxepins, the presence or position of the chlorine atom has little influence on the dopamine D-4 selectivity.

As seen in Table 1, compounds **1a**−**e** all have substantial binding activities to the serotonin S-2A, S-2C, and S-3 receptors with the highest serotonin S-2A selectivity shown by dechloroclozapine (**1c**). The serotonin S-2A selectivity of clozapine is also quite substantial, a 28-fold higher binding activity for the S-2A receptor than for the D-2 receptor, but for **1c** this selectivity is even higher with a value of 64.

For the carbocyclic analogues shown in Table 2, binding activities for the dopamine D-2 and D-4 receptors and the dopamine D-4 selectivity are substantially reduced, as compared to those of clozapine. Earlier we reported that **2a** binds twice as potently to nonmuscaric [^{3}H]clozapine binding sites in rat forebrain as does clozapine while the isomer **2b** was inactive.[3] These earlier tests, then, were possibly a measure of the binding activities of **2a** and **2b** to serotonin receptors in this tissue. Since **2a** is essentially inactive for binding to the dopamine D-2 receptor, **2a** has a remarkably high serotonin S-2A selectivity of 200.

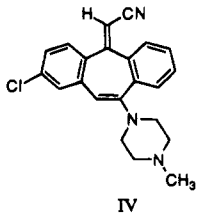
The 5,11-dicarbo analogue **3a** of clozapine (Table 3) does not bind strongly to dopamine D-2 or D-4 receptors but shows strong binding to the serotonin S-2A receptor, giving **3a** a serotonin S-2A selectivity of 200. High binding affinity for the dopamine D-2 and D-4 receptors for a 5,11-dicarbo analogue can be restored by movement of the chlorine atom proximal to the piperazino

ring (**3b**), but this change results in poor dopamine D-4 and serotonin S-2A selectivities. The dopamine D-4 selectivity is substantially enhanced for the dechloro 5-methyl derivative **3c**, paralleling the high dopamine D-4 selectivity of *N*-methylclozapine (**1d**). With a methylene group present at C-5 (**3d**), binding to either the dopamine or serotonin receptors is not greatly reduced and is similar to that of **3c**, but the dopamine D-4 selectivity is lowered and the serotonin S-2A selectivity is doubled for **3d** as compared to **3c**. A larger group at C-5 (**3e–g**) reduces binding to both the dopamine and serotonin receptors with a general decrease in dopamine D-4 selectivity as compared to **3c**. For the oxepin analogues **3h–j**, however, the dopamine D-4 selectivity is substantially enhanced without increase in the serotonin S-2A selectivity. All three oxepin analogues bind very strongly to the dopamine D-2 and D-4 receptors, the dechloro analogue **3j** having dopamine D-4 selectivity similar to that of clozapine but a serotonin S-2A selectivity decreased by a factor of 6 as compared to clozapine.

Table 4 shows the dopamine D-2 and D-4 and serotonin S-2A and S-2C binding affinities for a series 5-substituted dibenzo[*a*,*d*]cycloheptene analogues of clozapine in which the 1,2,3,6-tetrahydro-4-pyridinyl ring is in place of the piperazino ring of clozapine. Resolution of the tricarbo isosteres of clozapine **4b** and **4c** shows that the dopamine D-4 receptor displays the highest enantiospecificity of any of the receptor subtypes. Analogue (*R*)-(−)-**4b** has a 2-fold higher dopamine D-4 selectivity than its enantiomer (*S*)-(+)-**4b** as the result of enhanced binding to the dopamine D-4 receptor rather than diminished binding to the dopamine D-2 receptor. The 5-(2-propylidene) analogue [(±)-**4c**] shows substantial binding to the four receptor sites, but the dopamine D-4 and serotonin S-2A selectivities are less than those of clozapine. The analogue (pR_a,pS_b)-(+)-**4c** is 17 times more potent in binding to the dopamine D-4 receptor than is its enantiomer (pS_a,pR_b)-(−)-**4c** while being only 1.5 times more potent for binding to the dopamine D-2 receptor. In contrast to the enantiomers of **4b** for which the higher dopamine D-4 and serotonin S-2 selectivities are shown by the enantiomer with the *R* configuration, (pR_a,pS_b)-(+)-**4c** has the higher dopamine D-4 selectivity, but its enantiomer, (pS_a,pR_b)-(−)-**4c**, displays a slightly higher serotonin S-2A selectivity.

Conclusions

It has been shown that clozapine therapy causes over 80% of serotonin S-2A receptor occupancy in the frontal cortex of the human brain,[34] supporting the view of Meltzer that both serotonin S-2A and dopamine D-2 receptor blockade are required for producing an atypical antipsychotic profile.[35] Rilapine (IV), also a 5,11-dicarbo



IV

analogue of clozapine, shows a high affinity for the serotonin S-2A receptor (K_i 0.8 nM).[36] Further, autoradiographic mapping of the serotonin S-2A receptor in human postmortem brains using [^{3}H]ketanserin has shown that schizophrenic patients have elevated S-2A receptor levels (B_{max} 117 fmol/mg protein versus 62 for control) but no change in the serotonin S-1A (5-HT_{1A}) receptor levels.[37] Nevertheless, as noted by Seeman,[38] not all atypical antipsychotic agents block the serotonin S-2A receptor more potently than the dopamine D-2 receptor. To date, clozapine stands out as the only example of an atypical antipsychotic agent which also blocks the dopamine D-4 receptor more potently than the D-2 receptor.[39] In fact, a recent study of striatal tissue of postmortem schizophrenic patients found their dopamine D-4 receptor levels elevated 5-fold compared to those of matched controls.[40]

With these findings as a background, these present studies demonstrate that enhanced receptor selectivities for various subtypes of dopamine and serotonin receptors can be achieved by isosteric modification of the clozapine structure. In addition, binding studies with the enantiomers of the tricarbo isosteres (±)-**4b** and (±)-**4c** of clozapine show that the dopamine D-4 receptor displays a higher response to steric factors than do the other receptors types, and although none of the enantiomers of **4b** and **4c** has comparable potency or selectivity for the dopamine and serotonin receptors as does clozapine, these results indicate that structural modification of clozapine, not incorporating several of its heteroatoms, may produce potent and selective antagonists for either dopamine D-4 or serotonin S-2A receptors or both. Further work is in progress for the preparation and evaluation of such compounds.

Experimental Section

Solvent evaporations were done at reduced pressure using a water pump. Melting points were taken in open capillary tubes and are corrected. Rotatory powers at the sodium D line were measured with an Autopol III automatic polarimeter and a 1-dm sample tube. Electronic absorption (EA) spectra were measured in matched 1-cm cells with a Cary 2390 spectrometer operating in the auto gain mode. Circular dichroism (CD) spectra were obtained at room temperature using a Jasco J-720 spectropolarimeter and a 1-cm sample cell. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained with a JEOL FX-90Q or, as indicated, a Bruker AM-300 spectrometer operating at 90 and 300 MHz, respectively, and with tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm) downfield from the standard. Combustion analyses were done at Vanderbilt University (V) or by Galbraith Laboratories (G), Inc., Knoxville, TN, and agreed to within 0.4% of the calculated value or as otherwise noted.

5-Methylene-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene Hydrochloride (4a·HCl). A solution of 10-(4-hydroxy-1-methyl-4-piperidinyl)-5-methylene-5*H*-dibenzo[*a*,*d*]cycloheptene (**8**; 1.00 g, 3.15 mmol) in absolute ethanol (25 mL) was boiled in the presence of excess concentrated hydrochloric acid (12 M, 6.5 mL, 78 mmol) for 18 h. The reaction mixture was cooled, and the ethanol–water was evaporated. Recrystallization of the light yellow residue from isopropyl alcohol–acetone (2×) gave **4a**·HCl (0.30 g, 28%) as white needles: mp 180–185 °C (sub); ^{1}H NMR (CD_3OD) δ 2.5 (b, 2, C-3 pyridinyl H), 3.00 (s, 3, NCH_3), 3.49 (t, 2, C-2 pyridinyl H), 3.94 (b, 2, C-6 pyridinyl H), 5.24 (s, 2, $C{=}CH_2$), 5.97 (br s, 1, C-5 pyridinyl H), 7.03 (s, 1, C-11 H), 7.2–7.4 ppm (m, 8, aromatic H). Anal. (V) ($C_{22}H_{22}ClN$) C, H; N: calcd, 4.17; found, 3.53.

(±)-5-Methyl-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene [(±)-4b]. 10-(4-Hydroxy-1-methyl-4-piperidinyl)-5-methyl-5*H*-dibenzo[*a*,*d*]cyclohep-

tene (**9**; 0.90 g, 2.8 mmol) was mixed with concentrated hydrochloric acid (12 M, 2.0 mL, 24 mmol) in absolute ethanol (20 mL), and the mixture was boiled for 16 h. The ethanol was evaporated at reduced pressure, and water (25 mL) was added. The aqueous solution was made basic with 6 N sodium hydroxide and was extracted with ether (3 × 25 mL), and the ether extracts were combined and dried ($MgSO_4$). Evaporation of the ether left a light yellow solid residue (0.80 g). Recrystallization of the residue from hexane at −32 °C gave (±)-**4b** (0.14 g, 16%) as light yellow prisms: mp 89−90 °C; ^{1}H NMR ($CDCl_3$) (major conformer) δ 1.85 (d, 3, J = 7.2 Hz, C-5 CH_3), 2.6−2.8 (m, 4, C-2 and C-3 pyridinyl H), 2.43 (s, 1, NCH_3), 3.13 (q, 2, J = 3.1 Hz, C-6 pyridinyl H), 3.52 (q, 1, J = 7.2 Hz, C-5 H), 5.93 (t, 1, C-5 pyridinyl H), 7.0−7.3 (m, 9, C-11 and aromatic H); (minor conformer) 1.36 (d, 3, J = 7.2 Hz, C-5 CH_3), 2.2−2.9 (m, 4, C-4 and C-5 pyridinyl H), 2.43 (s, 1, NCH_3), 3.13 (q, 2, J = 3.1 Hz, C-3 pyridinyl H,), 4.11 (q, 1, C-5 H, J = 7.2 Hz) 5.93 (t, 1, C-4, pyridinyl H), 7.0−7.3 ppm (m, 9, C-11 and aromatic H). Anal. (V) ($C_{22}H_{23}N$) C, H, N.

(*S*)-(+)-5-Methyl-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene [(*S*)-(+)-4b]. (2*S*,3*S*)-*O*,*O*′-Di-*p*-toluoyltartaric acid (3.15 g, 8.15 mmol) in warm methanol (13 mL) was added slowly with stirring to (±)-5-methyl-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene [(±)-**4b**; 2.46 g, 8.16 mmol] in warm methanol (13 mL). The solution was warmed and stirred for 10 min. On cooling to room temperature, a white solid (3.87 g, 138%) precipitated. Recrystallization (2×) from methanol gave the pure hydrogen (2*S*,3*S*)-*O*,*O*′-di-*p*-toluoyltartrate salt of (*S*)-(+)-**4b** (1.38 g, 49%): mp 159−160 °C; $[\alpha]^{25}_D$ +132° (*c* 1.00, pyridine) unchanged on further recrystallization from methanol. The salt was added to water (30 mL), 6 N NaOH was added until basic, and the free amine was extracted with ether (3 × 30 mL). The combined ether extracts were dried ($MgSO_4$), and evaporation of the ether gave a light yellow oil. Crystallization from acetonitrile gave (*S*)-(+)-**4b** (0.11 g, 9%) as a light yellow crystalline solid: mp 90−91 °C; $[\alpha]^{25}_D$ +91° (*c* 1.16 , $CHCl_3$).

(*R*)-(−)-5-Methyl-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene [(*R*)-(−)-4b]. (2*R*,3*R*)-*O*,*O*′-Di-*p*-toluoyltartaric acid (1.93 g, 5.00 mmol) in warm methanol (10 mL) was added slowly with stirring to a partially racemic solution of (*R*)-(−)-**4b** (1.44 g, 4.78 mmol), obtained from the resolution described above, in methanol (10 mL). The solution was warmed and stirred for 10 min, and on cooling, a white solid precipitated. Recrystallization of the solid from methanol gave the pure hydrogen (2*R*,3*R*)-*O*,*O*′-di-*p*-toluoyltartrate salt of (*R*)-(−)-**4b** (1.75 g, 62%) as colorless needles: mp 160−161 °C; $[\alpha]^{25}_D$ −131° (*c* 0.97, pyridine). The salt was dissolved and the amine extracted as outlined above for the isolation of (*S*)-(+)-**4b**. Recrystallization of the solid residue from acetonitrile gave (*R*)-(−)-**4b** (0.38 g, 31%) as a light yellow solid: mp 89−90 °C; $[\alpha]^{25}_D$ −86° (*c* 1.00, $CHCl_3$); EA max 290 nm (ε 21 000), 230 (27 000) (sh), 212 (38 000); CD (*c* 0.002 56) $[\theta]_{320}$ ±0, $[\theta]_{272}$ −12 000 (sh), $[\theta]_{242}$ −27 000, $[\theta]_{236}$ −24 000 (sh), $[\theta]_{220}$ +48 000.

(±)-5-(2-Propylidene)-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene [(±)-4c]. 10-(4-Hydroxy-1-methyl-4-piperidinyl)-5-(2-propylidene)-5*H*-dibenzo[*a*,*d*]cycloheptene (**11** ; 5.97 g, 17.3 mmol) was mixed with concentrated hydrochloric acid (12 M, 4.0 mL, 48 mmol) in absolute ethanol (80 mL), and the mixture was boiled for 48 h. The solvent was evaporated at reduced pressure, and water (50 mL) and ether (25 mL) were added to the yellow residue. The mixture was made basic by the addition of 6 N sodium hydroxide. The layers were separated, and the aqueous phase was extracted with ether (3 × 50 mL). The combined ether layers were dried ($MgSO_4$), and evaporation of the ether and recrystallization of the residue from hexane gave (±)-**4c** (4.05 g, 72%) as a light yellow solid: mp 116−117 °C; ^{1}H NMR ($CDCl_3$) δ 1.67 [s, 3, C=(CH_3)CH_3], 1.69 [s, 3, C=C(CH_3)CH_3], 2.1−2.3 (m, 1, C-3 pyridinyl H), 2.40 (s, 3, NCH_3), 2.6−2.7 (m, 3, pyridinyl C-2 and C-3 H), 3.10 (q, 2, J = 1.6 Hz, pyridinyl C-6 H), 5.88 (s, 1, pyridinyl C-5 H), 6.90 (s, 1, C-11 H), 7.1−7.4 ppm (m, 8, aromatic H). Anal. (G) ($C_{24}H_{25}N$) C, H, N.

(pS_a,pR_b)-(−)-5-(2-Propylidene)-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene [(pS_a,pR_b)-(−)-4c]. (2*S*,3*S*)-*O*,*O*′-Di-*p*-toluoyltartaric acid (1.49 g, 3.86 mmol) in warm methanol (10 mL) was added dropwise with stirring to (±)-5-(2-propylidene)-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene [(±)-**4c**; 1.26 g. 3.85 mmol] in warm methanol (10 mL). The solution was warmed and stirred for 10 min. On cooling to room temperature, colorless prisms (1.22 g, 81%) were deposited: $[\alpha]^{25}_D$ +32° (*c* 1.07, pyridine). Recrystallization (2×) of the solid from methanol gave (pS_a,pR_b)-5-(2-propylidene)-10-(1,2,3,6-tetrahydro-1-methylpyridinium-4-yl)-5*H*-dibenzo[*a*,*d*]cycloheptene hydrogen (2*S*,3*S*)-*O*,*O*′-di-*p*-toluolytartrate [(pS_a,pR_b)-**14**·(2*S*,3*S*)-**15**], on the basis of its combustion analysis, isolated as the dimethanolate (0.90 g, 60%): mp 147−148 °C; $[\alpha]^{25}_D$ +25° (*c* 1.13, pyridine) unchanged on further recrystallization from methanol. Anal. (G) ($C_{46}H_{51}O_{10}N$) C, H, N. The hydrogen tartrate salt was added to water (10 mL) containing 6.0 N sodium hydroxide (2.0 mL, 12 mmol), and the mixture was extracted with ether (3 × 15 mL). The combined ether extracts were dried ($MgSO_4$), and evaporation of the ether gave an oil as residue. Crystallization of the oil from acetonitrile gave [(pS_a,pR_b)-(−)-**4c**] (0.26 g, 41%) as a light yellow solid: mp 123−124 °C; $[\alpha]^{25}_D$ −58° (*c* 1.13, $CHCl_3$); CD (*c* 0.003 44) $[\theta]_{335}$ ±0, $[\theta]_{308}$ −9000, $[\theta]_{285}$ ±0, $[\theta]_{272}$ ±0, $[\theta]_{262}$ +17 000, $[\theta]_{255}$ ±0; (*c* 0.000 688) $[\theta]_{255}$ ±0, $[\theta]_{243}$ −120 000 $[\theta]_{233}$ ±0, $[\theta]_{222}$ +180 000, $[\theta]_{210}$ +59 000.

(pR_a,pS_b)-(+)-5-(2-Propylidene)-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene [(pR_a,pS_b)-(+)-4c)]. (2*R*,3*R*)-*O*,*O*′-Di-*p*-toluoyltartaric acid (1.15 g, 2.98 mmol) in warm methanol (8 mL) was added dropwise with stirring to partially racemic (pR_a,pS_b)-(−)-**4c** (0.93 g, 2.8 mmol), isolated from the mother liquors of the resolution described above. The solution was warmed and stirred for 10 min, and on cooling, a white crystalline solid precipitated. Recrystallization of the solid from methanol gave (pR_a,pS_b)-5-(2-propylidene)-10-(1,2,3,6-tetrahydro-1-methylpyridinium-4-yl)-5*H*-dibenzo[*a*,*d*]cycloheptene hydrogen (2*R*,3*R*)-*O*,*O*′-di-*p*-toluoyltartrate dimethanolate [(pR_a,pS_b)-**14**·(2*R*,3*R*)-**15**·(CH_3OH)$_2$] (1.00 g, 67%) as colorless prisms: mp 144−146 °C ; $[\alpha]^{25}_D$ −25° (*c* 1.05, pyridine). The hydrogen tartrate salt of (pR_a,pS_b)-(+)-**4c** was dissolved in aqueous sodium hydroxide and the amine extracted into ether. The ether solution was dried ($MgSO_4$), and evaporation of the ether and recrystallization of the solid residue from acetonitrile gave pure (pR_a,pS_b)-(+)-**4c** (0.33 g, 52%) as a light yellow solid: mp 123−124 °C; $[\alpha]^{25}_D$ +54° (*c* 1.00, pyridine); ee greater than 95% on the basis of its ^{1}H NMR spectrum with an equimolar amount of (*R*)-α-(trifluoromethyl)benzyl alcohol in chloroform-*d*; EA max 290 nm (ε 11 000), 245 (17 000) (sh), 225 (24 000); CD (*c* 0.003 20) $[\theta]_{335}$ ±0, $[\theta]_{302}$ +11 000, $[\theta]_{282}$ ±0, $[\theta]_{275}$ ±0, $[\theta]_{261}$ −13 000, $[\theta]_{256}$ ±0; (*c* 0.000 640) $[\theta]_{256}$ ±0, $[\theta]_{243}$ +133 000, $[\theta]_{232}$ ±0, $[\theta]_{222}$ −150 000, $[\theta]_{210}$ −33 000.

10-Bromo-5-methyl-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ol (6). 10-Bromo-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one[29] (**5**; 20.0 g, 70.1 mmol) in tetrahydrofuran (140 mL) and ether (260 mL) was added dropwise to a stirred solution of methylmagnesium iodide (3.0 M in ether, 48 mL, 0.14 mol) in ether (600 mL) at room temperature, and after addition, the reaction mixture was stirred for 1.5 h. The mixture was cooled to 0 °C, and saturated aqueous ammonium chloride (500 mL) was added. The layers were separated, and evaporation of the dried ($MgSO_4$) ether layer gave **6** (19.9 g, 94%) as a light yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.68 (s, 3, C-5 CH_3), 2.37 (s, 1, OH), 7.2−8.0 ppm (m, 9, C-11 and aromatic H).

10-Bromo-5-methylene-5*H*-dibenzo[*a*,*d*]cycloheptene (7). 10-Bromo-5-methyl-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ol (**6**; 19.9 g. 66.1 mmol) and concentrated hydrochloric acid (12 N, 40 mL, 0.48 mol) in absolute ethanol (200 mL) were boiled overnight, and the ethanol was removed by evaporation at reduced pressure. The solid residue was mixed with water (50 mL), and the mixture was made basic with 6 N NaOH and extracted with ether (3 × 75 mL). The combined ether extracts were dried ($MgSO_4$), and evaporation of the ether gave a light brown solid residue (17.6 g). Recrystallization of this solid from 95% ethanol gave **7** (14.5 g, 78%) as light yellow solid: mp 72−73 °C; ^{1}H NMR ($CDCl_3$) δ 5.30 (s, 2, C=CH_2), 7.1−7.4

(m, 7, aromatic H), 7.54 (s, 1, C-11 H), 7.84 ppm (m, 1, C-6 H). Anal. (G) ($C_{16}H_{11}Br$) C, H, Br.

10-(4-Hydroxy-1-methyl-4-piperidinyl)-5-methylene-5*H*-dibenzo[*a*,*d*]cycloheptene (8). Under nitrogen, *n*-butyllithium (1.6 M in hexane, 35 mL, 56 mmol) was added dropwise to a stirred solution of 10-bromo-5-methylene-5*H*-dibenzo[*a*,*d*]cycloheptene (**7**; 14.5 g, 51.2 mmol) in ether (250 mL) at −78 °C. The solution was stirred at −78 °C for 2 h, and 1-methyl-4-piperidone (17.4 g, 154 mmol) in ether (130 mL) was added dropwise at −78 °C. The reaction mixture was stirred at −78 °C for 2 h and then allowed to warm to room temperature at which time a white solid precipitated. The solid was dissolved in ethyl acetate (400 mL), and water (100 mL) was added. The organic layer was separated and washed with water (4 × 100 mL). Evaporation of the dried ($MgSO_4$) organic layer gave a white solid (12.0 g) which after recrystallization from ethyl acetate gave pure **8** (9.94 g, 61%) as colorless needles: mp 184−185 °C; ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 1, OH), 2.25 (s, 3, CH_3), 1.7−2.7 (m, 8, piperidinyl H), 5.21 (s, 2, C=CH_2), 7.0−7.3 (m, 8, C-11 and aromatic H), 7.9−8.1 ppm (m, 1, aromatic H). Anal. (V) ($C_{22}H_{23}NO$) C, H, N.

10-(4-Hydroxy-1-methyl-4-piperidinyl)-5-methyl-5*H*-dibenzo[*a*,*d*]cycloheptene (9). A solution of 10-(4-hydroxy-1-methyl-4-piperidinyl)-5-methylene-5*H*-dibenzo[*a*,*d*]cycloheptene (**8**; 1.00 g, 3.15 mmol) in 95% ethanol (350 mL) was reduced with hydrogen in the presence of palladium on carbon (0.20 g) at 25 °C and atmospheric pressure. The reaction mixture consumed 99% of 1 molar equiv of hydrogen overnight. The catalyst was removed by filtration through Celite 545 (2×). Evaporation of the solvent yielded a solid residue which on recrystallization from ethyl acetate gave **9** (0.90 g, 89%) as a gray waxy solid, still contaminated with a small amount of palladium: mp 200−201 °C; ^{1}H NMR ($CDCl_3$) δ (major conformer) 1.82 (d, 3, J = 21.6 Hz, C-5 CH_3), 2.31 (s, 3, NCH_3), 1.7−2.8 (m, 8, piperidinyl H), 3.37 (q, 1, J = 21.6 Hz, C-5 H), 6.9−7.5 (m, 8, C-11 and aromatic H), 7.8−8.1 (m, 1, aromatic H); (minor conformer) 1.34 (d, 3, J = 21.6 Hz, C-5 CH_3), 2.30 (s, 3, NCH_3), 1.7−2.8 (m, 8, piperidinyl H), 4.06 (q, 1, J = 21.6 Hz, C-5 H), 6.9−7.5 (m, 8, C-11 and aromatic H), 7.8−8.1 ppm (m, 1, aromatic H). Anal. (G) ($C_{22}H_{25}NO$) H, N; C: calcd, 82.72; found, 81.47.

10-Bromo-5-(2-propylidene)-5*H*-dibenzo[*a*,*d*]cycloheptene (10). Under nitrogen, *n*-butyllithium (37 mL, 1.6 M in hexane, 59 mmol) was added dropwise to a stirred solution of isopropyltriphenylphosphonium iodide (23.3 g, 53.9 mmol) in ether (320 mL). The mixture was boiled for 3.5 h and then cooled to room temperature. 10-Bromo-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one[29] (**5**; 12.0 g, 42.1 mmol) in tetrahydrofuran (150 mL) was added dropwise, and the mixture was stirred overnight. The cooled reaction mixture was added to water (250 mL), and the layers were separated. The aqueous layer was extracted with ether (3 × 75 mL), and the combined organic layers were dried ($MgSO_4$). Evaporation of the solvent left an oil. A hexane solution of the latter was passed through a gravity column of silica gel (180 g), and evaporation of the hexane gave a white solid residue. Recrystallization from hexane gave **10** (10.0 g, 76%) as a white solid: mp 94−95 °C; ^{1}H NMR ($CDCl_3$) δ 1.68 [s, 3, C=C(CH_3)CH_3], 1.70 [s, 3, C=C(CH_3)CH_3], 7.0−7.5 (m, 7, aromatic H), 7.52 (s, 1, C-11 H), 7.7−7.8 ppm (m, 1, C-6). Anal. (V) ($C_{18}H_{15}Br$) C, H.

10-(4-Hydroxy-1-methyl-4-piperidinyl)-5-(2-propylidene)-5*H*-dibenzo[*a*,*d*]cycloheptene (11). Under nitrogen, *n*-butyllithium (1.6 M in hexane, 18 mL, 29 mmol) was added dropwise to a stirred solution of 10-bromo-5-(2-propylidene)-5*H*-dibenzo[*a*,*d*]cycloheptene (**10**; 8.50 g, 27.3 mmol) in ether (160 mL) at −78 °C. The solution was stirred at −78 °C for an additional 2 h, and 1-methyl-4-piperidone (7.69 g, 68.0 mmol) in ether (80 mL) was added dropwise to the stirred mixture at −78 °C. The solution was kept at −78 °C for 5 h and then was brought to room temperature and was stirred overnight. Water (100 mL) was added, and the layers were separated. The aqueous layer was extracted with ether (3 × 50 mL), and the combined ether layer and extracts were dried ($MgSO_4$). Evaporation of the ether left a white foam which on crystallization from ethyl acetate gave 11 (6.00 g, 64%) as a white solid: mp 164−165 °C; ^{1}H NMR ($CDCl_3$) δ 1.67 [s, 3, C=C(CH_3)CH_3], 1.70 [s, 3, C=C(CH_3)CH_3], 1.7−2.0 (m, 2, piperidinyl C-3 and C-5 H), 2.03 (s, 1, OH), 2.23 (s, 3, NCH_3), 2.2−2.7 (m, 7, piperidinyl H), 7.0−7.3 (m, 8, C-11 and aromatic H), 8.0−8.2 ppm (m, 1, C-6 H). Anal. (V) ($C_{24}H_{27}NO$) C, H, N.

(*R*)-(−)-5-Methyl-10-(1,2,3,6-tetrahydro-1,1-dimethylpyridinium-4-yl)-5*H*-dibenzo[*a*,*d*]cycloheptene Iodide [(*R*)-(−)-12]. A solution of (*R*)-(−)-5-methyl-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene [(*R*)-(−)-**4b**; 0.20 g, 0.66 mmol) and iodomethane (6.0 mL, *d* 2.280, 96 mmol) in absolute ethanol was boiled for 1.5 h. Evaporation of the solvent and excess iodomethane at reduced pressure gave a red oil. Crystallization of the oil from acetonitrile gave (*R*)-(−)-**12** (0.14 g, 48%) as light yellow prisms: mp 184−187 °C; $[\alpha]^{25}_D$ −32° (*c* 0.70, CH_3OH); 300−MHz ^{1}H NMR ($CDCl_3$) δ 1.34 (d, 3, J = 7.2 Hz, C-5 CH_3, minor conformer), 1.86 (d, 3, J = 7.2 Hz, C-5 CH_3, major conformer), 2.5−3.0 (m, 2, pyridinyl C-3 H), 3.48 (q, 1, J = 7.2 Hz, C-5 H, major conformer), 3.60 [s, 6, $N(CH_3)_2$], 4.03 (m, 2, pyridinyl C-2 H), 4.48 (q, 2, pyridinyl C-6 H), 5.96 (s, 1, pyridinyl C-5 H), 7.0−7.4 ppm (m, 9, C-11 and aromatic H).

(pS_a,pR_b)-5-(2-Propylidene)-10-(1,2,3,6-tetrahydro-1,1-dimethylpyridinium-4-yl)-5*H*-dibenzo[*a*,*d*]cycloheptene Iodide [(pS_a,pR_b)-(−)-13]. A solution in methanol (15 mL) of (pS_a,pR_b)-(−)-5-(2-propylidene)-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5*H*-dibenzo[*a*,*d*]cycloheptene [(pS_a,pR_b)-(−)-**4c**; 0.20 g, 0.61 mmol] and iodomethane (16 M in methanol, 1.0 mL, 16 mmol) was boiled for 1 h. Removal of the solvent at reduced pressure gave a yellow solid residue. Recrystallization from absolute ethanol gave (pS_a,pR_b)-(−)-**13** (0.17 g, 59%): mp 267−268 °C; $[\alpha]^{25}_D$ −22° (*c* 1.07, CH_3OH), ^{1}H NMR ($CDCl_3$) δ 1.68 [s, 3, C=C(CH_3)CH_3], 1.69 [s, 3, C=C(CH_3)CH_3], 2.4−2.8 (m, 2, pyridinyl C-3 H), 3.53 [s, 6, $N(CH_3)_2$], 3.94 (t, 2, pyridinyl C-2 H), 4.43 (b, 2, pyridinyl C-6 H), 5.88 (b, 1, pyridinyl C-5 H), 6.96 (s, 1, C-11 H), 7.0−7.4 ppm (m, 8, aromatic H). Anal. (G) ($C_{25}H_{28}IN$) C, H, I, N.

X-ray Diffraction Measurements. As shown in Table 5, crystals were grown in a suitable solvent and mounted on a glass fiber. All diffraction measurements were performed on a Rigaku AF6S diffractometer with graphite monochromatic Cu Kα (1.541 78 Å) or Mo Kα (0.710 69 Å) radiation. Data were collected at 20 ± 1 °C, and cell constants and orientation matricies for data collection were obtained from a least-squares refinement using the setting angles of 25 accurately centered reflections in the range 18° < 2θ < 25° for (pS_a,pR_b)-**4c** and (pS_a,pR_b)-**14**·(2*S*,3*S*)-**15**·$(CH_3OH)_2$ and 6.64° < 2θ < 12.98° for (*R*)-**12**. On the basis of systematic absences, packing consideration, and a statistical analysis of the intensity distributions, the space group for each crystal system was determined to be orthorhombic with a space group as shown in Table 5. Subsequent solution and refinement of the structures confirmed these choices. Data collection was performed using a continuous ω − 2θ scan with stationary backgrounds, a scan speed of 8.0° min^{-1}, and ratio of peak counting time to background counting time of 2 to 1. Limits of data collection were 6° ≤ 2θ ≤ 120°, and no decay was observed in the intensities of three representative reflections measured after every 150 reflections. Data were reduced to a unique set of intensities and associated σ values in the usual manner using the TEXSAN set of crystallographic programs (Molecular Structures Corp., 1985). The structures were solved by a combination of direct methods (SHELX-86) and Fourier techniques. All non-hydrogen atoms were refined anisotropically. As not all of the hydrogen atoms of the clusters were evident on the difference Fourier maps, their positions were calculated using idealized geometries based on packing considerations and d (C−H) = 0.95 Å. The positions were fixed for the final cycles of refinements. The final difference maps were featureless. Tables of atomic positional parameters and B (eq), atomic thermal parameters, and bond distances and angles are given in the supplementary material.

Affinity for the Serotonin S-2A (5-HT_{2A}) and S-2C (5-HT_{2C}) Receptors. Confluent GF-6 and P_o cell monolayers were dissociated in Versene and centrifuged at 1000 rpm for 5 min. The resulting pellet was homogenized in 10 volumes of 0.32 M ice-cold sucrose using a Brinkman Polytron PT10/

35 (setting 5.5, 10 s). The suspension was centrifuged at 44 000g for 15 min, and the pellet was resuspended in 5 volumes of 50 mM Tris-HCl, pH 7.6, buffer using the Polytron. The membrane homogenates were then stored at −80 °C as 1−4-mL aliquots. On the day of the binding assay, an aliquot of the membrane was thawed, diluted with 50 mM Tris-HCl, pH 7.6, buffer to an appropriate volume, and homogenized with the Polytron just prior to use.

The assay tubes, in triplicate, received 20 μL of 5 nM [^{125}I]-LSD, 20 μL of test compound (final concentration 10^{-9} to 10^{-5} M), or 10 μM mesulergine for nonspecific binding, 40 μL of membrane suspension (1−5 μg protein/assay tube, protein-concentration measured using the Bradford dye binding method) in a final volume of 100 μL of 50 mM Tris-HCl, pH 7.6, buffer. Incubation proceeded at 37 °C for 60 min and was terminated by addition of 2 mL of ice-cold assay buffer and filtration through a GF/B glass fiber filter, presoaked in 0.1% polyethylenimine. The filters were washed twice with 5 mL of ice-cold 50 mM Tris-HCl, pH 7.6, buffer and transferred to 12- × 75-mm polystyrene tubes for quantification of radioactivity with Packard Corbra Auto-gamma counter. The IC_{50} values were converted to the inhibition constants (K_i) in the usual way.[1] Data given in Tables 1−4 are the mean, rounded to no more than two significant figures, of three separate experiments, each done in tripilicate. The standard errors of the mean are given in Tables 10s−13s in the supplementary material.

Affinity for the Serotonin S-3 (5-HT$_3$) Receptor. Membranes from confluent NG108−15 cell monolayers were prepared and stored as outlined above for the preparation of those from confluent GF-6 and P_o cell monolayers except 50 mM HEPES-KOH, pH 7.4, buffer was used instead of 50 mM Tris-HCl. The assay tubes, in triplicate, received 100 μL of 1 nM [^{3}H]GR65630, 50 μL of test compound or 10 μM metoclopramide for nonspecific binding, 200 μL membrane suspension (0.1 to 0.2 mg/assay tube) in a final volume of 500 μL. Incubation proceeded at 37 °C for 30 min and was terminated by filtration of the mixture through GF/B glass fiber filters, presoaked in 0.1% polyethylenimine, using a Brandel cell harvester. The filters were rinsed with two 5-mL portions of ice-cold HEPES-KOH, pH 7.4, buffer. The filters were transferred to scintillation vials to which 4 mL of Beckman Ready Protein was added, and the radioactivity was determined by liquid scintillation spectrometry. The IC_{50} values were converted to inhibition constants (K_i) in the usual way.[1] Each value in Tables 1−4 represents one experiment done in triplicate.

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Supplementary Material Available: Tables of atomic positional parameters and B (eq), atomic thermal parameters, and bond distances and angles for (pS_a,pR_b)-(−)-5-(2-propylidene)-10-(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)-5H-dibenzo[a,d]cycloheptene [(pS_a,pR_b)-(−)-**4c**], (R)-(−)-5-methyl-10-(1,2,3,6-tetrahydro-1,1-dimethylpyridinium-4-yl)-5H-dibenzo[a,d]cycloheptene iodide [(R)-(−)-**12**], and (+)-(pS_a,pR_b)-5-(2-propylidene)-10-(1,2,3,6-tetrahydro-1-methylpyridinium-4-yl)-5H-dibenzo[a,d]cycloheptene hydrogen (2S,3S)-O,O'-di-p-toluoyltartrate dimethanolate [(+)-(pS_a,pR_b)-**14**·(2S,3S)-**15**·(CH_3OH)$_2$] and tables of the binding affinities of clozapine (**1a**) and clozapine analogues **1b**−**1e**, **2a** and **2b**, **3a**−**3j**, and **4a**−**4c** to Serotonin S-2A and S-2C binding sites, showing the mean K_i's, the number of separate experiments, and the standard errors of the mean (23 pages). Ordering information is given on any current masthead page.

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