Synthesis and σ Binding Properties of 2′-Substituted 5,9α-Dimethyl-6,7-benzomorphans

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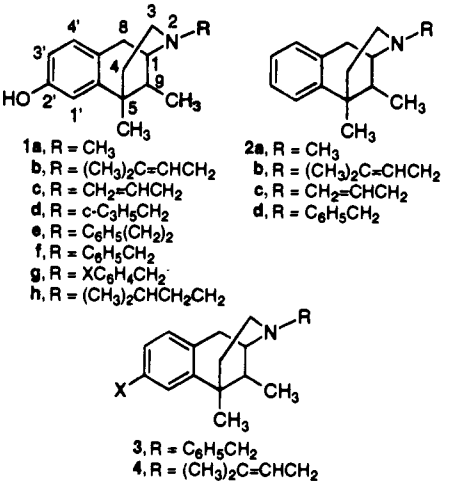
The synthesis and σ1 and σ2 binding properties of several (+)- and (−)-2-benzyl- and 2-dimethylallyl-2′-substituted-5,9α-dimethyl-6,7-benzomorphans (**3** and **4**) are presented. In agreement with previously reported binding data for 2-substituted 5,9α-dimethyl-2′-hydroxy-6,7-benzomorphans (*N*-substituted-*N*-normetazocine), all (1*S*,5*S*,9*S*)-(+)-isomers showed higher affinity for the σ1 site than the corresponding (1*R*,5*R*,9*R*)-(−)-isomers. Replacement of the 2′-hydroxy group of (+)-2-benzyl-5,9α-dimethyl-2′-hydroxy-6,7-benzomorphan [(+)-**1f**] with a 2′-NH_2 and 2′-$N(CH_3)_2$ [(+)-**3b** and (+)-**3c**, respectively] had only a small effect on the σ1 K_i values. Changing the 2′-hydroxy group of (+)-**1f** to an H, F, Cl, Br, I, NHAc, or $NHSO_2CH_3$ resulted in a 5-fold or greater loss in potency. In contrast, replacement of the 2′-hydroxy group of (+)-2-(dimethylallyl)-5,9α-dimethyl-2′-hydroxy-6,7-benzomorphan [(+)-**1b**, (+)-pentazocine] with a 2′-H or 2′-F group resulted in a 2-fold increase in potency. Conversion of (+)-**1f** to its 2′-desoxy analogue (+)-**2d** resulted in a 27.5-fold loss in affinity. This suggests that (+)-**1f** and other *N*-substituted benzomorphan analogues may be binding to single σ1 receptors in a different way or to different σ1 receptors. (−)-Pentazocine [(−)-**1b**] and its 2′-fluoro analogue, (−)-2-(dimethylallyl)-5,9α-dimethyl-2′-fluoro-6,7-benzomorphan [(−)-**4a**] showed the highest potency for the σ2 binding site.

Metazocine (**1a**) and the *cis*-*N*-substituted N-normetazocine analogues, pentazocine (**1b**), *N*-allyl-*N*-normetazocine (**1c**), cyclazocine (**1d**), and phenazocine (**1e**), are (1*RS*,5*RS*,9*RS*)-(±)-2-substituted 2′-hydroxy-5,9α-dimethyl-6,7-benzomorphans which are well-known for their opioid activity.[1,2] The (1*R*,5*R*,9*R*)-(−)-isomers[3] of the racemic drugs are responsible for their opioid activity,[1,2] the (1*S*,5*S*,9*S*)-(+)-isomers[3] have proven extremely useful in the characterization of the σ1 binding site,[4] and both isomers have proven valuable in the characterization of the σ2 binding site. (+)-Pentazocine [(+)-**1b**] and (+)-*N*-allyl-*N*-normetazocine [(+)-**1c**] have preference for the σ1 site, whereas the enantiomers (−)-pentazocine [(−)-**1b**] and (−)-*N*-allyl-*N*-normetazocine [(−)-**1c**] have a slight preference for the σ2 site.[4]

As part of a structure–activity relationship study of the benzomorphan class of compounds, we reported that the σ1 binding potency of a number of (+)- and (−)-*N*-substituted *N*-normetazocines was sensitive to changes in the *N*-substituent as well as the absolute stereochemistry of **1**.[5,6] Some analogues with larger, more lipophilic *N*-substituents possessed high affinity for the σ1 site. However, the overall SAR suggested that other factors were involved in the relative σ1 binding potency of these compounds. (+)-*N*-Benzyl-*N*-normetazocine [(+)-**1f**] with a K_i of 0.67 nM possessed the highest affinity for the σ1 site and was highly selective for the σ1 site relative to binding at the μ opioid and PCP receptors. In a more recent study, we reported that the σ1 binding affinity of a series of (+)-*cis*-*N*-(2-, 3-, and 4-substituted benzyl)-*N*-normetazocines [(+)-**1g**] was highly dependent on the size and location of the substituent.[7]



In a preliminary communication, we also reported that *cis*-2-substituted 5,9α-dimethyl-6,7-benzomorphans (**2a–c**, *cis*-*N*-substituted 2′-deoxy-*N*-normetazocines) possessed binding affinity for the σ1 site almost identical to the corresponding *cis*-*N*-substituted *N*-normetazocines (**1a–c**), showing that replacement of the 2′-hydroxyl group of these analogues had only small effects on binding potency.[8] In a separate study, we reported that (−)-2-benzyl-5,9α-dimethyl-6,7-benzomorphan [(−)-**2d**] was also a useful ligand for studying the σ2 binding site.[9]

In the present study, details on our preliminary report on the synthesis and binding affinity of **2a–d** and the synthesis and σ1 and σ2 binding properties of a series

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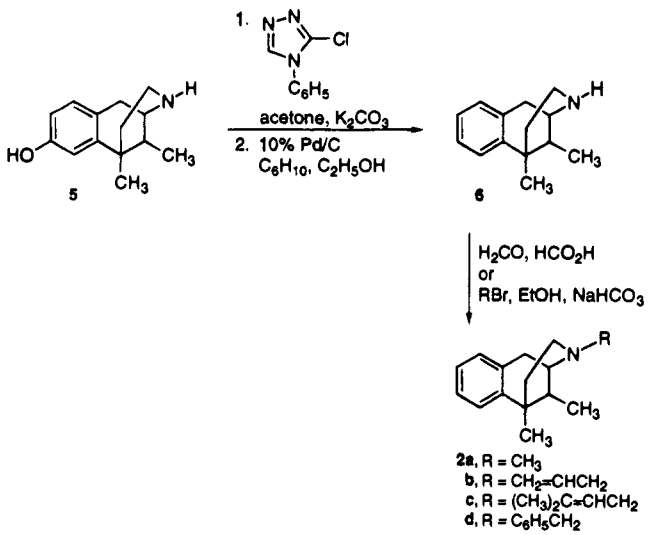
Scheme 1



of (+)- and (−)-2-benzyl- and 2-dimethylallyl-2′-substituted-5,9α-dimethylbenzomorphans (**3** and **4**, respectively) are presented.

Chemistry

Scheme 1 shows the method used to prepare the (+)- and (−)-isomers of the 2-substituted 5,9α-dimethyl-6,7-benzomorphans (**2a**−**d**). Refluxing an acetone solution of *N*-normetazocine (**5**) with 5-chloro-1-phenyl-1*H*-tetrazole in the presence of potassium carbonate followed by hydrogenolysis of the intermediate 2-phenyltetrazoyl ether using 10% palladium on carbon catalyst in a refluxing mixture of cyclohexene and ethanol gave the desired 5,9α-dimethyl-6,7-benzomorphan (**6**). Treatment of (+)-**6** with a refluxing solution of formic acid and formaldehyde gave (+)-2,5,9α-trimethyl-6,7-benzomorphan [(+)-**2a**]. Alkylation of (+)- and (−)-**6** with the appropriate alkyl bromide gave the 2-substituted 5,9α-dimethyl-6,7-benzomorphans (**2b**−**d**). The physical properties of the compounds are listed in Table 1.

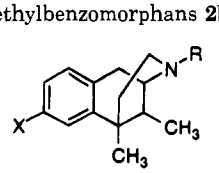
(+)- and (−)-2′-Amino-2-benzyl-5,9α-dimethyl-6,7-benzomorphans (**3b**) are key intermediates for the synthesis of the other 2′-substituted analogues of the (+)- and (−)-isomers of **3** and **4**. Compound **3b** was prepared by modification of a procedure reported by Wentland and co-workers for the synthesis of (±)-2′-amino-2-allyl-5,9α-dimethyl-6,7-benzomorphans.[10] The reaction sequence is outlined in Scheme 2. *O*-Methylation of the appropriate **1f** enantiomer with methyl iodide in tetrahydrofuran using sodium hydride as the base in the presence of tetrabutylammonium iodide followed by catalytic debenzylation using 10% palladium on carbon catalyst in methanol gives 5,9α-dimethyl-2′-methoxy-6,7-benzomorphan (**7**). Sodium metal reduction of **7** in liquid ammonia−tetrahydrofuran−isopropyl alcohol solution followed by *N*-benzylation with benzyl bromide in dimethylformamide containing potassium carbonate yielded the *N*-benzyl enol ether (**8**). Oximination of **8** with hydroxylamine hydrochloride in refluxing pyridine and ethanol mixture gave the *N*-benzyl oxime **9**. Dehydration of **9** in refluxing acetic anhydride and acetic acid containing hydrogen chloride gave the 2′-acetanilide **3a**. Hydrolysis of **3a** in 6 N hydrochloric acid yielded the desired (+)- and (−)-**3b** isomers depending on the stereochemistry of the starting **1f**. Hydrolysis of (+)-**9** gave the ketone (+)-**10**.

Subjection of (+)-**3b** to reductive alkylation using formaldehyde and sodium cyanoborohydride gave the 2′-dimethylamino analogue, (+)-**3c** (Scheme 3). Mesylation of (+)-**3b** with methanesulfonyl chloride in ethanol catalyzed with potassium hydrogen carbonate yielded 2′-methanesulfonamide, (+)-**3d**. Diazotization of (+)- and (−)-**3b** followed by treatment with pyridine polyhydrogen fluoride and cuprous chloride yielded the 2′-fluoro and 2′-chloro analogues, (+)-**3e**−**f** and (−)-**3e**−**f**, respectively. The diazonium salt from the (+)-isomer was also treated with cuprous bromide and iodine−potassium iodide to give the 2′-bromo- and 2′-iodo analogues, (+)-**3g**−**h**, respectively. Catalytic debenzylation of (+)- and (−)-**3e** followed by reductive alkylation of the intermediate *N*-nor compound using 3-methyl-2-butenal and sodium cyanoborohydride afforded (+)- and (−)-**4a**. In the case of the (−)-isomer, a small amount of the 2-(3-methylbutyl) analogue **4b** was isolated.

Results and Discussion

2-Substituted 5,9α-dimethyl-2′-hydroxy-6,7-benzomorphans were the first structural class of σ ligands with the 2-allyl analogue, referred to as *N*-allyl-*N*-normetazocine, NANM, or SKF 10,047 by various investigators, being the first σ ligand.[4] Since these initial studies, many classes of compounds have been reported to possess σ binding properties.[11] The structural diversity of these high-affinity σ ligands has complicated attempts to define a unique pharmacophore for the σ binding site. In order to gain additional information on this pharmacophore, we have continued to conduct SAR studies on the 6,7-benzomorphan class of compounds. Even though *N*-allyl-*N*-normetazocine was the ligand used to originally classify the σ binding site,[4] little was known about the structure−activity relationship (SAR) of this class of compounds prior to our recent SAR reports.[5−8] Our SAR studies on this

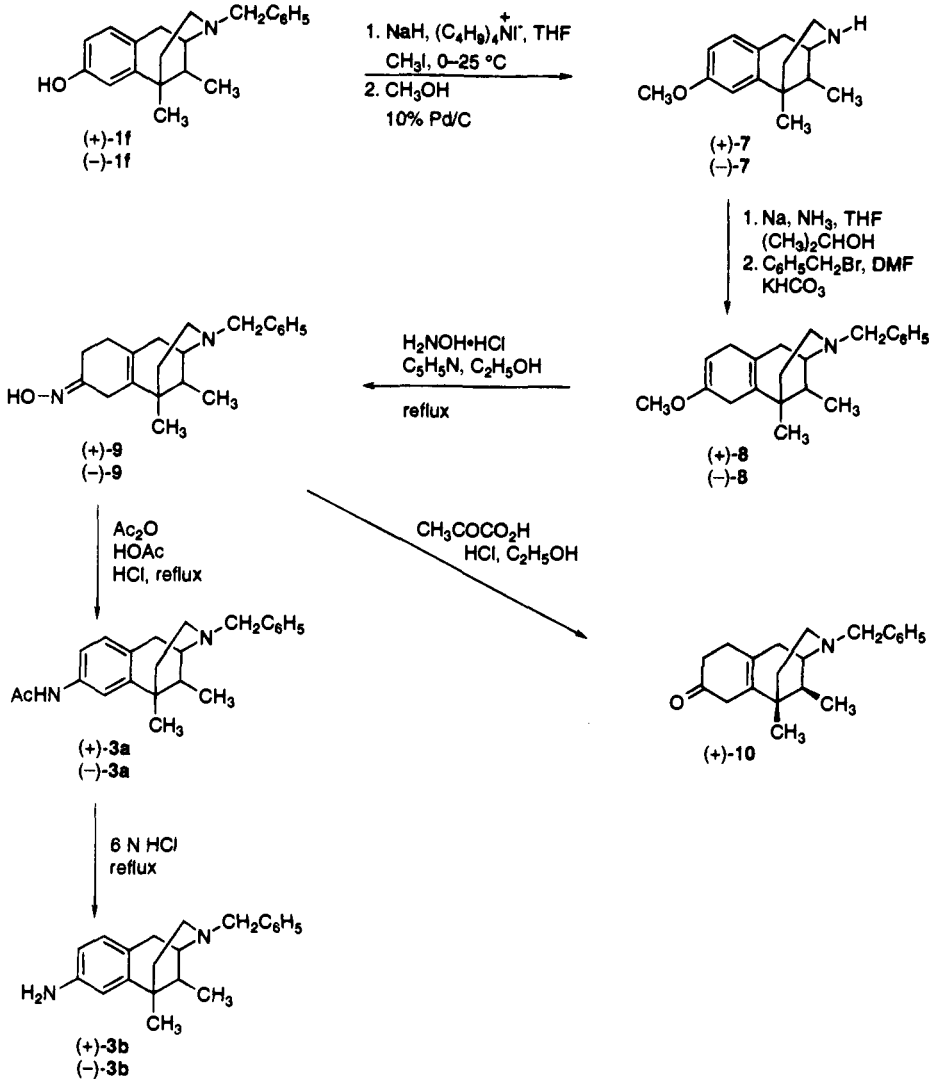
Table 1. Physical Properties for 2-Substituted 5,9-Dimethylbenzomorphans **2b**−**d**



compd	X	R	molecular formula[a]	% yield	mp, °C	$[\alpha]^{25}_D$, deg (*c*, C_2H_5OH)
(+)-**2b**	H	CH_2=$CHCH_2$	$C_{17}H_{24}ClN$	28	230 dec	+82.6 (1.15)
(+)-**2c**	H	$(CH_3)_2C$=$CHCH_2$	$C_{19}H_{27}N$	71	oil	+102.4 (1.08)
(−)-**2c**	H	$(CH_3)_2C$=$CHCH_2$	$C_{19}H_{27}N$	70	oil	−106 (1.0)
(+)-**2d**	H	$C_6H_5CH_2$	$C_{21}H_{26}ClN$	50	215−216	+88 (0.115)
(−)-**2d**	H	$C_6H_5CH_2$	$C_{21}H_{26}ClN$	53	214−215	−95.5 (0.85)

[a] Elemental analyses were within ±0.4% of the actual values for C, H, and N.

Scheme 2



class of compounds can be divided into three benzomorphan zones which include: the aromatic ring (A), the saturated or morphan segment (B), and the nitrogen substituent (C) (Figure 1). In previously published studies of zone C analogues, (+)- and (−)-2-benzyl-5,9α-dimethyl-2′-hydroxy-6,7-benzomorphan [(+)- and (−)-**1f**] and (+)- and (−)-pentazocine [(+)- and (−)-**1b**] have been shown to be highly useful ligands for studying the σ1 and σ2 binding sites.[4-6] In the present study (Table 2), the effects of changing the 2′-substituent X in zone A were investigated.

We first investigated the replacement of 2′-hydroxyl by a hydrogen (2′-deoxy analogues). Thus, conversion of (+)- and (−)-pentazocine [(+)-**1b** and (−)-**1b**] as well as (+)-metazocine [(+)-**1a**], (+)-*N*-allyl-*N*-normetazocine [(+)-**1c**], and (−)-*N*-benzyl-*N*-normetazocine [(−)-**1f**] to the 2′-deoxy analogues (+)-**2b**, (−)-**2b**, (+)-**2a**, and (+)-**2c** results in only small changes in K_i values for the σ1 binding site. In contrast, conversion of the (+)-*N*-benzyl-*N*-normetazocine [(+)-**1f**] to its 2′-deoxy analogue (+)-**2d** resulted in a 27.5-fold loss in affinity.

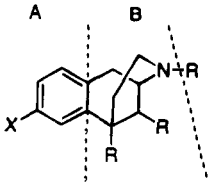


Figure 1. Structure–activity relationship zones for the benzomorphan structure.

A comparison of the K_i values for the (+)-2-benzyl analogues (+)-**1f** and (+)-**3a**–**h** show that the 2′-group in this series has a substantial effect on σ1 binding with the K_i values spanning a 194-fold range. In contrast, the same compounds showed only a 7-fold range in σ2 K_i values. Comparison of the 2′-F, 2′-Cl, 2′-Br, and 2′-I analogues (+)-**3e**–**h** showed that σ1 affinity decreases as the size and electronegativity of the 2′-substituent increases and decreases, respectively. However, the SAR is not straightforward since (+)-**3g** and (+)-**2d** which have a 2′-Br and 2′-H substituent, respectively, possess almost identical K_i values (18.53 vs 18.44 nM) even though the latter compound has the smallest size and lowest electronegativity of all the substituents. A similar pattern exists in the (−)-series with the 2′-fluoro analogue (−)-**3e** having a σ1 affinity about 2-fold greater than the 2′-chloro analogue (−)-**3f**. However, the unsubstituted (2′-H) analogue (−)-**2d** has a lower potency (larger K_i) than either (−)-**3e** or (−)-**3f**.

Based on a previously determined quantitative structure–activity relationship (QSAR) between substituent volume and the binding affinity of a series of *N*-

Scheme 3

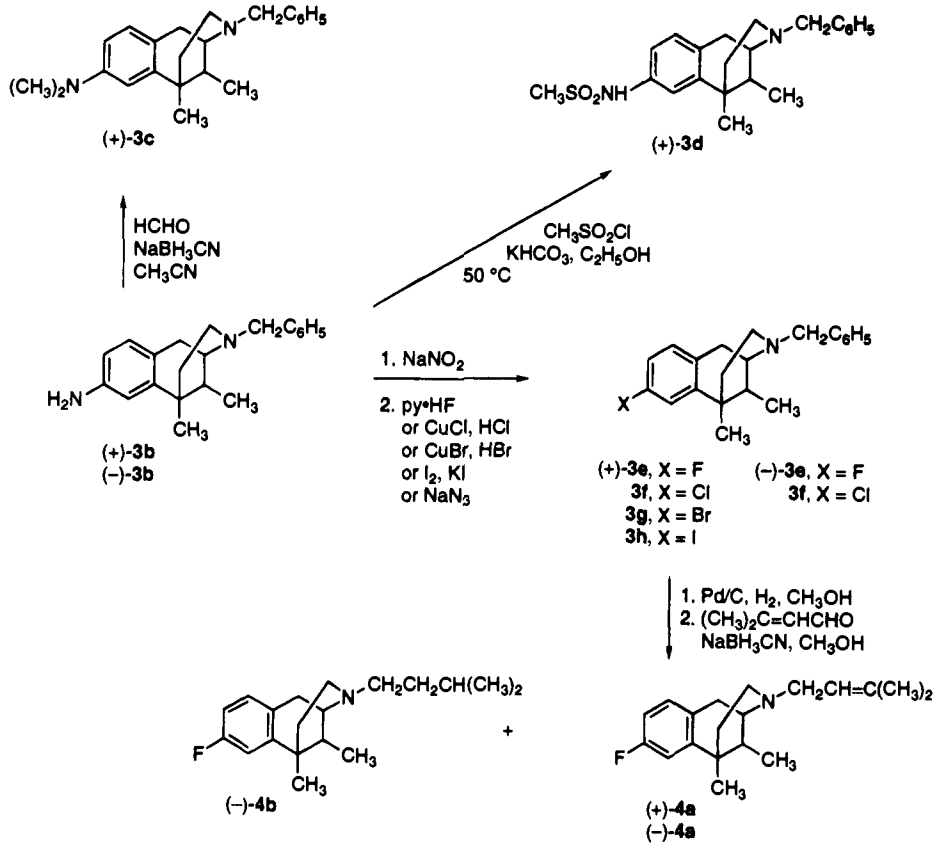
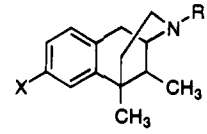


Table 2. σ1 and σ2 Binding Data for 2′-Substituted 5,9α-Dimethyl-6,7-benzomorphans



			K_i (nM)[a,b]				
compd	X	R	σ1 [^{3}H]pentazocine	±SEM	σ2 [^{3}H]-DTG	±SEM	σ2/σ1 ratio[c]
(+)-**1a**	OH	CH_3	2100	269	–	–	–
(+)-**1b**	OH	$(CH_3)_2C{=}CHCH_2$	3.1	0.3	1540	313	500
(−)-**1b**	OH	$(CH_3)_2C{=}CHCH_2$	83.1	6.2	36.5	5.76	0.44
(+)-**1c**	OH	$CH_2{=}CHCH_2$	59.7	2.5	>10,000		>168
(+)-**1f**	OH	$C_6H_5CH_2$	0.67	0.1	1710	407	2600
(−)-**1f**	OH	$C_6H_5CH_2$	36.5	6.3	192	35	5.3
(+)-**2a**	H	CH_3	482	41	–	–	–
(+)-**2b**	H	$(CH_3)_2C{=}CHCH_2$	1.57	0.15	–	–	–
(−)-**2b**	H	$(CH_3)_2C{=}CHCH_2$	26.4	2.9	295	4	11
(+)-**2c**	H	$CH_2{=}CHCH_2$	82.5	10.4	–	–	–
(+)-**2d**	H	$C_6H_5CH_2$	18.4	2.13	487	65.6	26
(−)-**2d**	H	$C_6H_5CH_2$	58.6	7.5	230	3	3.9
(+)-**3a**	$NHCOCH_3$	$C_6H_5CH_2$	5.68	0.05	1410	221	250
(+)-**3b**	NH_2	$C_6H_5CH_2$	1.47	0.04	832	5.14	570
(+)-**3c**	$(CH_3)_2N$	$C_6H_5CH_2$	1.75	0.18	258	18.8	150
(+)-**3d**	$NHSO_2CH_3$	$C_6H_5CH_2$	10.4	0.96	1900	263	180
(+)-**3e**	F	$C_6H_5CH_2$	3.81	0.82	264	36.4	69
(−)-**3e**	F	$C_6H_5CH_2$	19.6	1.15	43	0.71	2.2
(+)-**3f**	Cl	$C_6H_5CH_2$	7.16	0.28	429	20	60
(−)-**3f**	Cl	$C_6H_5CH_2$	40.1	5.55	381	1.98	9.5
(+)-**3g**	Br	$C_6H_5CH_2$	18.5	1.96	501	38.1	27
(+)-**3h**	I	$C_6H_5CH_2$	130.6	16.6	2037	84.2	16
(+)-**4a**	F	$(CH_3)_2C{=}CHCH_2$	1.48	0.15	154	3.41	104
(−)-**4a**	F	$(CH_3)_2C{=}CHCH_2$	30.5	3.59	51.8	6.51	1.7
(−)-**4b**	F	$(CH_3)_2CHCH_2CH_2$	4.68	0.36	56.4	2.03	12
10	O=	$C_6H_5CH_2$	11.3	1.91	691	101	61

[a] rValues are the average ± SEM of two to three experiments. Each experiment was carried out in duplicate. [b] ND = not determined. [c] σ2/σ1 are ratios of K_i values.

(substituted benzyl) *N*-normetazocines,[7] a similar correlation was sought for a closely related group of (+)-*N*-benzyl-2′-substituted-benzomorphans (+)-**1f**, (+)-**2d**, and (+)-**3a**–**h**. In contrast to the *N*-(subsituted benzyl)

N-normetazocines, no statistically significant correlation of substituent volume (alone or in combination with the classical QSAR molecular descriptors π and MR), and σ receptor binding affinity was apparent. A three-dimensional QSAR study based on CoMFA[12] steric and electrostatic and HINT[13] lipophilicity fields also failed to produce any useful structure–activity correlations. This suggests that effects from changes in region A are more complicated than that found for region C. One or more of the analogues used in the QSAR studies could be binding to the same σ1 site in a different orientation or binding to different subtypes of σ1 receptor.

It is interesting to note that the 2′-hydroxyl group of (+)-**1f** can be changed to an NH_2 or $N(CH_3)_2$ group (analogues (+)-**3b** and (+)-**3c**, respectively) with little loss in binding affinity for the σ1 binding site. This data, along with the fact that all the (+)-**3** analogues listed in Table 2 with the exception of the 2′-iodo analogue (+)-**3h** possess K_i values lower than 19 nM show that the 2′-hydroxyl group, is not essential for good σ1 binding affinity. The fact that (+)-**10**, which does not possess an A region aromatic ring, has a K_i value of 11 nM shows that reasonable affinity can be achieved in its absence. However, it should be noted that (+)-**1f** which possesses a 2′-hydroxyl group is by far the most selective ligand for the σ1 site relative to the σ2 site ($\sigma2/\sigma1$ ratio = 2546). This ligand also has the highest potency of any reported benzomorphan analogue (K_i = 0.67 nM) and is also highly selective for the σ1 site relative to binding at the μ-opioid and PCP (NMDA uncompetitive) receptors.[5,6]

In our previous study, we reported that changing the *N*-dimethylallyl group of (+)-pentazocine (+)-**1b** to the reduced *N*-(3-methylbutyl) [(+)-**1h**] moiety had very little effect on σ1 binding affinity.[5,6] In contrast, reduction of the *N*-dimethylallyl group of 2′-fluoro pentazocine analogue (−)-**4a** to give the 3-methylbutyl analogue (−)-**4b** gave a 6-fold increase in σ1 binding but had little effect on σ2 binding.

In agreement with previously reported σ1 binding data for *N*-substituted *N*-normetazocines, the (+)-isomers of *N*-benzyl 2′-F and 2′-Cl analogues, **3e** and **3f**, respectively, and the 2′-F analogue of pentazocine, **4a**, showed higher affinity for σ1 than the corresponding (−)-isomer. The high $\sigma2/\sigma1$ K_i ratios for the (+)-isomer is typical of other (+)-benzomorphans and (+)-morphans.[4,14,15] Conversely, the (−)-isomers of **3e** and **4a** have greater affinity for σ2 than the (+)-isomers. This adds additional support to the hypothesis that σ1 and σ2 binding sites are structurally distinct entities.[14] None of the compounds were highly selective for the σ2 site. In fact, none of the (+)- and (−)-2′-substituted 5,9α-dimethyl-6,7-benzomorphans bind with high affinity to the σ2 site. (−)-Pentazocine [(−)-**1b**] with a K_i = 36.5 nM is the most potent analogue and shows slightly greater affinity for σ2 relative to σ1.

The possible involvement of the σ2 binding subtype in the motor effect of neuroleptics[9,16] has been proposed. In one study, the results showed that 2′-deoxy analogues (−)-**2b** and (−)-**2d** exhibited such motor effects in rats.[9] The data in Table 2 suggest that (−)-**3e** and (−)-**4a** would be better σ2 ligands for in vivo motor effect studies.

In summary, the 2′-group of 6,7-benzomorphan analogues has a significant effect on σ1 binding but little effect on σ2 binding. In cases where both the (+)- and (−)-isomers were prepared, the (+)-isomer showed higher affinity for the σ1 receptor and the (−)-isomer showed the higher affinity for the σ2 receptor. The 2′-phenolic group of (+)-metazocine [(+)-**1a**], (+)-pentazocine [(+)-**1b**], (+)-*N*-allyl-*N*-normetazocine [(+)-**1c**], and (−)-*N*-benzyl-*N*-normetazocine [(−)-**1f**] does not contribute significantly to σ1 binding affinity. In contrast, the 2′-phenolic group of (+)-*N*-benzyl-*N*-normetazocine [(+)-**1f**] is essential for its potent σ1 affinity. This suggests that (+)-**1f** and other *N*-substituted benzomorphan analogues may be binding to single σ1 receptors in a different way or to different σ1 receptors. Heterogeneity of σ1 receptors has been suggested by multiple K_d values for [^{3}H]-(+)-pentazocine binding in various human and rodent cell lines.[17]

Experimental Section

Melting points were determined on a Thomas-Hoover capillary tube apparatus. All optical rotations were determined at the sodium D line using a Rudolph Research Autopol III polarimeter (1 dm cell). NMR spectra were recorded on a Bruker WM-250 or AMX-500 spectrometer using tetramethylsilane as internal standard. Thin-layer chromatography was carried out on Whatman silica gel 60 plates using hexane–Et_2O–Et_3N (10:9:1). Visualization was accomplished under UV illumination or in an iodine chamber. Microanalyses were carried out by Atlantic Microlab, Inc., Norcross, GA.

(+)- and (−)-5,9α-Dimethyl-6,7-benzomorphan [(+)- and (−)-6]. A mixture of 1.85 g (0.005 mol) of (+)-**5**,[5] 0.905 g (0.005 mol) of 5-chloro-1-phenyl-1*H*-tetrazole, and 2.3 g of potassium carbonate in 100 mL of acetone was refluxed for 25 h. The sodium chloride was removed by filtration and washed with acetone. The combined filtrate and washings were concentrated and dried under vacuum to give the 2-phenyltetrazoyl ether. The crude product was dissolved in 100 mL of a 1:1 mixture of ethanol and cyclohexane and 3.5 g of 10% Pd/C added, and the mixture was refluxed for 16 h. The catalyst was removed by filtration and the solvent removed on a rotary evaporator under vacuum. The resulting residue was purified by flash chromatography on silica gel 60 using ethyl acetate–hexane (1:1) as eluent. Concentration of the product fraction gave 994 mg (94%) of (+)-**6**.

Subjection of (−)-**5**[5] to a similar reaction scheme gave 57% of (−)-**6**. The ^{1}H NMR ($CDCl_3$) of (+)- and (−)-**6** showed δ 0.82 (d, J = 7.0 Hz, 3), 1.11–1.36 (m, 1), 1.38 (s, 3), 1.61–1.84 (m, 2), 2.15–2.80 (m, 4), 3.11–3.27 (m, 2), 7.06–7.26 (m, 4).

These samples of (+)- and (−)-**6** were used to prepare (+)-**2a–b** and (+)- and (−)-**2c–d** without further purification.

(+)-2,5,9α-Trimethyl-6,7-benzomorphan [(+)-2a]. A solution of 994 mg (4.95 mmol) of (+)-**6** in 6 mL of formic acid containing 4.5 mL of 37% formaldehyde was heated for 2 h at 90–100 °C. After the solution was cooled to 25 °C, 30 mL of 10% hydrochloric acid was added. The mixture was made basic with 10% sodium hydroxide solution and extracted with ethyl ether. The ethyl ether extracts were washed with brine followed by water and dried (Na_2SO_4). The residue obtained was purified by flash chromatography on silica gel 60 using ethyl acetate–hexane (1:1) as eluent. Concentration of the product fraction gave 945 mg (89%) of (+)-**2a**. The hydrochloride salt was prepared by adding an ethereal solution of hydrogen chloride to an ethyl ether solution of (+)-**2a**. The resulting salt was separated by filtration and recrystallized from an ethanol and ethyl ether mixture: mp 167–168.6 °C; $[\alpha]^{25}_D$ +55 (*c* 0.8, C_2H_5OH). Anal. ($C_{15}H_{22}ClN\cdot0.25H_2O$) C, H, N.

General Procedure for the Synthesis of 2-Substituted 5,9α-Dimethyl-6,7-benzomorphans (2b–d). A mixture of 140 mg (0.70 mmol) of (+)- or (−)-**6**, 0.77 mmol of the appropriate alkyl halide, and 210 mg (2.5 mmol) of sodium hydrogen carbonate in 8 mL of ethanol was heated to reflux for 4 h. The mixture was concentrated and the residue extracted with diethyl ether. The ethereal extract was con-

centrated under vacuum to give the crude product which was purified by flash chromatography on silica gel (hexane–ethyl acetate–methanol, 8:1:1). (+)- And (−)-**2c** were characterized as their free bases; the other samples were characterized as their hydrochloride salts. The hydrochloride salts were formed by adding 1 N hydrogen chloride in diethyl ether solution to an ethereal solution of the (+)- and (−)-**2** free base. The precipitated salts were collected by filtration and thoroughly washed with diethyl ether. The physical data for each compound is listed in Table 1.

(+)- and (−)-5,9α-Dimethyl-2′-methoxy-6,7-benzomorphan (7). To a stirred solution of (+)-**1f**[5] (2.5 g, 0.008 mol) in THF (50 mL) under nitrogen at 0 °C was added tetrabutylammonium iodide (100 mg) and 60% sodium hydride in oil (0.5 g, 0.0012 mol) followed by iodomethane (1.7 g, 0.012 mol). After 1 h at 0 °C the stirred reaction mixture was allowed to warm to 25 °C and remain for 16 h. After neutralization with 10% aqueous ammonium chloride, the THF was removed on a rotary evaporator, and the remaining solution was extracted with chloroform (4 × 20 mL) and dried (Na_2SO_4). Removal of the solvent on a rotary evaporator, followed by flash chromatography of the residue using ethyl acetate–hexane (1:1) as the eluent, gave 2.5 g of crude (+)-2-benzyl-5,9α-dimethyl-2′-methoxy-6,7-benzomorphan. This sample and 250 mg of 10% Pd/C in 50 mL of methanol was stirred under a hydrogen atmosphere for 24 h. The catalyst was removed by filtration and washed with methanol. Removal of the solvent followed by vacuum drying gave 1.86 g (100%) of crude (+)-**7**. A 0.046 mol synthesis provided 7.4 g (70%) of (+)-**7**.

Subjection of (−)-**1f**[5] to a similar reaction sequence provided 8.5 g (80%) of (−)-**7**.

The samples of (+)-**7** and (−)-**7** were used in the next step without further purification.

(+)- and (−)-2′-Amino-2-benzyl-5,9α-dimethyl-6,7-benzomorphan (3b). A procedure analogous to that reported by Wentland[10] and co-workers to prepare other 2-substituted analogues was used to prepare the optical isomers, (+)- and (−)-**3b**. Thus, a solution of 1.97 g (0.0085 mol) of (+)-**7** in 40 mL of THF and 40 mL of isopropyl alcohol was added with stirring to 80 mL of liquid ammonia cooled in a dry ice–acetone bath. Sodium (2.5 g, 0.11 g at) was added in small pieces over 0.5 h. After the blue color disappeared, 40 mL of methanol was added, and the ammonia was allowed to evaporate. The residue was diluted with water and extracted with ethyl ether (3 × 30 mL). The extracts were dried (Na_2SO_4) and concentrated to give 1.83 g of solid which was heated in 5 mL of DMF containing 1 g of $KHCO_3$ and 1.2 g (0.007 mol) of benzyl bromide to give 1.82 g (66%) of (+)-**8**. The crude sample of (+)-**8**, NH_2OH·HCl (572 mg, 0.008 mmol), pyridine (5 mL), and 95% EtOH (20 mL) were refluxed with stirring for 16 h, concentrated, diluted with water (50 mL), extracted with $CHCl_3$ (5 × 30 mL), and dried (Na_2SO_4). Removal of the solvent on a rotary evaporator followed by flash chromatography of the residue on silica gel using $CHCl_3$–CH_3OH–NH_4OH (90:9:1) as the eluent provided 1.5 g of (+)-**9**. This sample was dissolved in 20 mL of acetic acid containing 1.4 g (0.0014 mol) of acetic anhydride. Hydrogen chloride gas was passed into the solution, and the resulting mixture was stirred at reflux for 1.5 h, concentrated, diluted with aqueous NH_4OH (50 mL), extracted with $CHCl_3$ (3 × 20 mL), and dried (Na_2SO_4). Flash chromatography of the residue obtained, after removal of the solvent, on silica gel using $CHCl_3$–CH_3OH–NH_4OH (90:9:1) as the eluent gave 1.31 g of (+)-**3a**.

A small sample of (+)-**3a** was converted to the dihydrochloride salt: mp 170–171 °C; $[\alpha]^{25}_D$ +92.4° (*c* 1.0, C_2H_5OH). Anal. ($C_{23}H_{29}ClN_2O$·0.25H_2O) C, H, N.

A 477 mg (1.4 mmol) sample of (+)-**3a** was refluxed in 6 N hydrochloric acid for 2 h. The cooled solution was washed with chloroform and made basic with concentrated ammonium hydroxide, extracted with $CHCl_3$ (3 × 20 mL), dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography on silica gel using $CHCl_3$–CH_3OH–NH_4OH (90:9:1) as eluent to give 418 mg of (+)-**3b** free base. This sample of (+)-**3b** was used without further purification for the synthesis of other 2′-substituted (+)-**3** analogues.

A small sample of (+)-**3b** was converted to the dihydrochloride salt: mp 205 °C dec; $[\alpha]^{25}_D$ +90.4° (*c* 1.0, C_2H_5OH). Anal. ($C_{21}H_{28}Cl_2N_2$·H_2O) C, H, N.

The enantiomer (−)-**3b** was prepared by a procedure identical to that for (+)-**3b**. From 4.0 g (0.0017 mol) of (−)-**7** was obtained 2.0 g of (−)-**3a**. Hydrolysis of a 1.0 g (0.0029 mol) sample of (−)-**3a** provided 737 mg of (−)-**3b** which was used for the preparation of other (−)-**3** analogues.

(+)-2-Benzyl-5,9α-dimethyl-2′-oxo-1′,3′,4′-trihydro-6,7-benzomorphan [(+)-10]. A mixture of (+)-**9** (66 mg, 0.2 mmol) and pyruvic acid (37.5 mg, 0.4 mmol) in 95% ethanol (4 mL) containing concentrated hydrochloride (50 μL) was heated to reflux for 6 h. The solvent was removed, and the product was basified with aqueous ammonium hydroxide, extracted with chloroform (4 × 20 mL), dried, and evaporated to give 33 mg (53%) of pure free base: ^{1}H NMR ($CDCl_3$) δ 0.84 (d, *J* = 7.0 Hz, 3, $CHCH_3$), 0.79 (s, 3, CH_3), 1.26–1.36 (m, 1), 1.75–2.81 (m, 13), 3.59 (d, 2, *J* = 6.1 Hz, $CH_2C_6H_5$), 7.20–7.35 (m, 5, $CH_2C_6H_5$). The hydrochloride salt: mp 123–125 °C dec; $[\alpha]^{25}_D$ +50° (*c* 0.16, EtOH). Anal. ($C_{21}H_{28}NClO$·0.75H_2O) C, H; N: calcd, 3.90; found, 4.33.

(+)-2-Benzyl-2′-(dimethylamino)-5,9α-dimethyl-6,7-benzomorphan [(+)-3c]. To a stirred solution of (+)-**3b** (100 mg, 0.3 mmol) and 37% formaldehyde (49 mg, 1.6 mmol) in acetonitrile (10 mL) was added sodium cyanoborohydride (35 mg, 0.5 mmol). After stirring for 15 min, glacial acetic acid was added until the solution was neutral to pH paper and was further stirred for 2 h. The solvent was evaporated, aqueous ammonium hydroxide added, the product extracted with chloroform (4 × 30 mL), dried (Na_2SO_4), and evaporated. Purification by flash chromatography on silica gel using ethyl acetate–hexane (1:1) as eluent gave pure (+)-**3c** (93.8 mg, 86%): ^{1}H NMR ($CDCl_3$, free base) δ 0.82 (d, 3, *J* = 7.0 Hz, CH_3), 1.27–1.30 (m, 1), 1.37 (s, 3, CH_3), 1.80 (dt, 1, *J* = 4.7, 12.5 Hz), 1.89–1.93 (m, 1), 2.16 (dt, 1, *J* = 3.0, 12.1 Hz), 2.62 (dd, 1, *J* = 5.8, 18.1 Hz), 2.87–3.16 (m, 2), 2.89 (s, 6, $(NCH_3)_2$), 3.59, 3.71 (2, *J* = 14.4 Hz, $CH_2C_6H_5$, AB), 6.57–6.64 (m, 2, aromatic H), 6.97 (d, 1, *J* = 8.2 Hz, aromatic H), 7.18–7.37 (m, 5, benzylic H). The dihydrochloride salt: mp 200 °C dec; $[\alpha]^{25}_D$ +88.7° (*c* 1, EtOH). Anal. ($C_{23}H_{32}Cl_2N_2$·H_2O) C, H, N.

(+)-2-Benzyl-2′-methanesulfonamido-5,9α-dimethyl-6,7-benzomorphan [(+)-3d]. A mixture of (+)-**3b** (220 mg, 0.72 mmol), potassium bicarbonate (200 mg), methanesulfonyl chloride (124 mg, 1.08 mmol), and ethanol (10 mL) was heated to reflux overnight under nitrogen. The mixture was cooled and diluted with water (50 mL), and the product was extracted with chloroform (3 × 40 mL) and dried (Na_2SO_4). After removal of solvent, the crude product was purified by flash chromatography on silica gel using $CHCl_3$–CH_3OH–NH_4OH (90:9:1) as eluent to give 202 mg (73%) of (+)-**3d**: ^{1}H NMR ($CDCl_3$) δ 0.80 (d, 3, *J* = 70 Hz, CH_3), 1.23–1.28 (m, 1), 1.34 (s, 3, CH_3), 1.80 (dt, 1, *J* = 4.7, 12.5 Hz), 1.88–1.95 (m, 1), 2.06 (dt, 1, *J* = 3.0, 12.1 Hz), 2.43–2.49 (m, 1), 2.66 (dd, 1, *J* = 5.8, 18.1 Hz), 2.89–3.08 (m, 2H), 3.00 (s, 3, CH_3SO_2), 3.59, 3.72 (2, $CH_2C_6H_5$, AB, *J* = 14.4 Hz), 7.03–7.37 (m, H, benzylic H). The hydrochloride salt: mp 170–171 °C; $[\alpha]^{25}_D$ +86.2° (*c* 1, EtOH). Anal. ($C_{22}H_{29}ClN_2O_2S$·0.5H_2O) C, H, N.

(+)- and (−)-2-Benzyl-2′-fluoro-5,9α-dimethyl-6,7-benzomorphan (3e). To (+)-**3b** (110 mg, 0.36 mmol) dissolved in pyridinium poly(hydrogen fluoride) (3 mL) was slowly added sodium nitrite (82 mg, 1.1 mmol). The solution was stirred at room temperature under nitrogen for 1 h and an additional 1 h at 85 °C. The reaction mixture was quenched with ice water and the product extracted with chloroform (4 × 20 mL). The extract was neutralized with aqueous ammonium hydroxide, dried (Na_2SO_4), and evaporated. The residual product was purified by flash chromatography on silica gel using ethyl acetate–hexane (1:1) as eluent to afford 72 mg (65%) of (+)-**3e**: ^{1}H NMR ($CDCl_3$) δ 0.80 (d, 3, ^{1}H NMR ($CDCl_3$) δ 0.80 (d, 3, *J* = 7.0 Hz, CH_3), 1.24–1.31 (m, 1), 1.35 (s, 3, CH_3), 1.82 (dt, 1, *J* = 4.7, 12.5 Hz), 1.92–1.96 (m, 1), 2.07 (dt, 1, *J* = 3.0, 12.1 Hz), 2.43–2.49 (m, 1), 2.65 (dd, 1, *J* = 5.8, 18.1 Hz), 2.89–3.12 (m, 2), 3.60, 3.72 (2, $CH_2C_6H_5$, AB, *J* = 14.4 Hz), 6.81 (dt, 1, *J* = 8.3, 2.7 Hz aromatic H), 6.92 (dd, 1, *J* = 8.1, 2.7 Hz aromatic H), 7.03–7.09 (m, 1, aromatic H), 7.21–7.38 (m, 5,

benzylic H). The hydrochloride salt: mp 143–145 °C; $[\alpha]^{25}_D$ +93° (c 0.1, EtOH). Anal. ($C_{21}H_{25}ClFN\cdot0.75H_2O$) C, H, N.

The (−)-enantiomer, (−)-**3e**·HCl: prepared by a similar procedure; mp 132–134 °C; $[\alpha]^{25}_D$ −94° (c 0.09, EtOH). Anal. ($C_{21}H_{25}ClFN\cdot0.75H_2O$) C, H, N.

(+)- and (−)-2-Benzyl-2′-chloro-5,9α-dimethyl-6,7-benzomorphan (3f). Sodium nitrite (73 mg, 1.1 mmol) was added to concentrated sulfuric acid (2 mL) at 0 °C under nitrogen. The mixture was allowed to warm to room temperature, stirred further for 15 min, and cooled to 0 °C, and a solution of (+)-**3b** (216 mg, 0.7 mmol) in glacial acetic acid (2 mL) was added dropwise. The diazonium solution was stirred at room temperature for 1 h and added to a cooled solution of copper(I) chloride (109 mg, 1.1 mmol) in concentrated hydrochloride acid (2 mL). The resulting mixture was heated at 70 °C for 1 h, cooled in an ice bath, and basified with 10% ammonium hydroxide. The product was extracted with chloroform (3 × 20 mL), dried, evaporated, and purified by flash chromatography on silica gel using hexane–ethyl acetate (2:1) as eluent to give 36 mg (16%) of (+)-**3f**: ^{1}H NMR ($CDCl_3$) δ 0.78 (d, 3, J = 7.0 Hz, CH_3), 1.24–1.31 (m, 1), 1.35 (s, 3, CH_3), 1.87 (dt, 1, J = 4.7, 12.5 Hz), 1.95–1.96 (m, 1), 2.06 (dt, 1, J = 3.0, 12.1 Hz), 2.43–2.50 (m, 1), 2.64 (dd, 1, J = 5.8, 18.1 Hz), 2.88–3.06 (m, 2), 3.59, 3.71 (2, $CH_2C_6H_5$, AB, J = 14.4 Hz), 6.99–7.69 (m, 8, aromatic and benzylic H). The hydrochloride salt had mp 135–137 °C, $[\alpha]^{25}_D$ +88° (c 0.1, C_2H_5OH). Anal. ($C_{21}H_{25}Cl_2N\cdot0.75H_2O$) C, H, N.

The (−)-enantiomer, (−)-**3f**·HCl was prepared by a similar procedure. The hydrochloride salt: mp 135–137 °C; $[\alpha]^{25}_D$ −90° (c 0.1, EtOH). Anal. ($C_{21}H_{25}Cl_2N\cdot0.25H_2O$) C, H, N.

(+)-2-Benzyl-2′-bromo-5,9α-dimethyl-6,7-benzomorphan [(+)-3g]. A solution of **3b** (248 mg, 0.8 mmol) in glacial acetic acid (3 mL) was added dropwise to a cooled solution of sodium nitrite (84 mg, 1.2 mmol) in concentrated sulfuric acid (3 mL), the temperature being held at 15–20 °C. The resulting diazonium solution was stirred for 45 min at room temperature, added over a 5 min period to a solution of copper(I) bromide (174 mg, 1.2 mmol), 48% hydrobromic acid (2 mL), and water (2 mL) at room temperature, and stirred further at 70–80 °C for 45 min. The reaction mixture was cooled, basified with 10% ammonium hydroxide, the product extracted with chloroform (4 × 30 mL), dried (Na_2SO_4), and evaporated. Flash chromatography using hexane–ethyl acetate (4:1) as eluent gave 35 mg (11%) of (+)-**3g**: ^{1}H NMR ($CDCl_3$) δ 0.78 (d, 3, J = 7.0 Hz, CH_3), 1.26–1.31 (m, 1), 1.35 (s, 3, CH_3), 1.82 (dt, 1, J = 4.7, 12.5 Hz), 1.90–1.98 (m, 1), 2.04 (dt, 1, J = 3.0, 12.1 Hz), 2.43–2.49 (m, 1), 2.62 (dd, 1, J = 5.8, 18.1 Hz), 2.88–3.05 (m, 2), 3.59, 3.67 (2, $CH_2C_6H_5$, AB, J = 14.4 Hz), 6.98 (d, 1, aromatic H, J = 8.2), 7.21–7.43 (m, 7, benzylic H). The hydrochloride salt: mp 150–152 °C; $[\alpha]^{25}_D$ +97.6° (c 0.125, EtOH). Anal. ($C_{21}H_{25}BrClN\cdot0.75H_2O$) C, H, N.

(+)-2-Benzyl-5,9α-dimethyl-2′-iodo-6,7-benzomorphan [(+)-3h]. A solution of (+)-**3b** (114 mg, 0.37 mmol) in glacial acetic acid (1 mL) was added dropwise to a stirred solution of sodium nitrite (39 mg, 0.56 mmol) in concentrated sulfuric acid (1 mL) at 0 °C under nitrogen. The resulting diazonium solution was stirred for 1 h at 15–20 °C and quenched with iodine (113 mg, 0.45 mmol) in sodium iodide (150 mg) in water (1 mL) at 0 °C. The excess iodine was discharged with sodium thiosulfite and the reaction mixture basified with aqueous ammonia. The product was extracted with chloroform (3 × 20 mL), dried (Na_2SO_4), and evaporated. Flash chromatography on silica gel using hexane–ethyl acetate (2:1) as the eluent yielded 45 mg (29%) of (+)-**3h**: ^{1}H NMR ($CDCl_3$) δ 0.78 (d, 3, J = 7.1 Hz, CH_3), 1.24–1.31 (m, 1), 1.34 (s, 3, CH_3), 1.81 (dt, 1, J = 4.9, 12.5 Hz), 1.90–1.94 (m, 2), 2.03 (dt, 1, J = 3.0, 12.1 Hz), 2.43–2.47 (m, 1), 2.63 (dd, 1, J = 5.8, 18.1 Hz), 2.87–3.04 (m, 2), 3.59, 3.67 (s, $CH_2C_6H_5$, AB, J = 14.4 Hz), 6.85 (d aromatic H, 1, J = 8.1 Hz), 7.15–7.53 (m, 7, benzylic H). The hydrochloride salt: mp 178–180 °C; $[\alpha]^{25}_D$ +96.4° (c 0.11, EtOH). Anal. ($C_{21}H_{25}ClIN$) C, H, N.

(+)- and (−)-*cis*-2-(3,3-Dimethylallyl)-5,9α-dimethyl-2′-fluoro-6,7-benzomorphan (4a). A solution of 170 mg (0.55 mmol) of (+)-**3e** in 40 mL of methanol containing 60 mg of 10% Pd/C was kept under a hydrogen atmosphere for 16 h. The catalyst was removed by filtration and the filtrate concentrated to give 95 mg of crude (+)-2′-fluoro-5,9α-dimethyl-6,7-benzomorphan. This material along with 73 mg (0.87 mmol) of 3-methyl-2-butenal and 55 mg (0.87 mmol) of sodium cyanoborohydride in 20 mL of methanol was stirred at 25 °C under nitrogen for 16 h. The reaction mixture was concentrated, dissolved in water (20 mL), extracted with $CHCl_3$ (3 × 30 mL), and dried (Na_2SO_4). The residue obtained from removal of the solvent was purified by flash chromatography on silica gel using ethyl acetate–hexane (1:4) as the eluent to give 32 mg (26%) of (+)-**4a**: ^{1}H NMR ($CDCl_3$) δ 0.82 (d, 3, J = 7.1 Hz, CH_3), 1.27–1.34 (m, 1), 1.34 (s, 3, CH_3), 1.66 (s, 3), 1.73 (s, 3), 1.73–2.03 (m, 3), 2.51–2.68 (m, 2), 2.83–3.12 (m, 4), 5.26 (tt, 1, J = 1.3, 7.0 Hz, $CH_2CH[C(CH_3)_2]$), 6.80 (dt, 1, J = 2.7, 8.4 Hz, aromatic H), 6.92 (dd, 1, J = 2.7, 10.7 Hz, aromatic H), 7.04 (t, 1, J = 7.2 Hz, aromatic H). The hydrochloride salt: mp 190–192 °C; $[\alpha]^{25}_D$ +73 (c 0.055, EtOH). Anal. ($C_{19}H_{27}ClFN\cdot0.25H_2O$) C, H, N.

The enantiomer (−)-**4a**·HCl: prepared by a similar procedure; mp 185–187 °C dec; $[\alpha]^{25}_D$ −70° (c 0.1, EtOH). Anal. ($C_{19}H_{27}ClFN\cdot0.25H_2O$) C, H, N.

In this case, a 24 mg sample of (−)-**4b** was obtained: ^{1}H NMR ($CDCl_3$, free base) δ 0.83 (d, 3, J = 7.0 Hz, CH_3), 0.91 (d, 6, J = 6.6 Hz), 1.23–1.99 (m, 9), 1.34 (s, 3, CH_3), 2.44–2.67 (m, 3), 2.90–2.97 (m, 2), 5.79 (dt, 1, J = 1.7, 8.4 Hz, aromatic H), 6.92 (dd, 1, J = 2.3, 10.9 Hz, aromatic H), 6.99–7.05 (m, 1, aromatic H). The hydrochloride salt: mp 220–222 °C dec; $[\alpha]^{25}_D$ −41.2° (c 0.085, EtOH). Anal. ($C_{19}H_{29}ClFN\cdot0.25H_2O$) C, H, N.

Biological Materials and Methods. σ Binding Assays. σ1 binding sites were labeled using the σ1-selective ligand, [^{3}H]-(+)-pentazocine[18] and guinea pig brain membranes, as described previously.[15] Rat liver membranes have been shown previously to be a rich source of σ2 sites[19–21] and are labeled using [^{3}H]DTG in the presence of dextrallorphan to mask σ1 sites.[15,22,23]

Various concentrations of the test ligand ranging from 0.005 to 1000 nM or from 0.05 to 10000 nM were incubated with guinea pig brain membranes (σ1) or rat liver membranes (σ2) and radioligand. Assays were carried out using the conditions described below: σ1, 3 nM [^{3}H]-(+)-pentazocine, σ2, 3 nM [^{3}H]-DTG + 1 μM dextrallorphan. IC_{50} values were derived using the computerized iterative curve fitting program GraphPAD InPlot (San Diego, CA). K_i values were calculated from IC_{50} values using the Cheng–Prusoff equation[24] and K_d values that were predetermined in independent experiments.

Membrane Preparation. Crude P_2 membrane fraction was prepared from frozen guinea pig brains (Pel-Freeze, Rogers, AK) minus cerebellum. Brains were allowed to thaw slowly on ice before homogenization. Crude P_2 membrane fraction was also prepared from the livers of male Sprague–Dawley rats (150–200 g, Taconic Farms). Animals were killed by decapitation and the livers removed and minced before homogenization.

Tissue homogenization was carried out at 4 °C in 10 mL/g tissue weight of 10 mM Tris-HCl/0.32 M sucrose, pH 7.4 using 10 motor-driven strokes in a Potter-Elvehjem Teflon–glass homogenizer. The crude homogenate was centrifuged for 10 min at 1000*g* and the pellet discarded. The resultant supernatant was centrifuged at 31000*g* for 15 min. The pellet was resuspended in 3 mL/g 10 mM Tris-HCl, pH 7.4, by vortexing, and the suspension was allowed to incubate at 25 °C for 15 min. Following centrifugation at 31000*g* for 15 min, the pellet was resuspended to 1.53 mL/g in 10 mM Tris-HCl, pH 7.4, and aliquots were stored at −80 °C until use. Protein concentration of the suspension was determined by the method of Lowry[25] and was 20–25 mg of protein/mL.

σ1 Binding Assay. Guinea pig brain membranes (325–500 μg of protein) were incubated with 3 nM [^{3}H]-(+)-pentazocine (51.7 Ci/mmol) in 0.5 mL of 50 mM Tris-HCl, pH 8.0, for 120 min at 25 °C. Nonspecific binding was determined in the presence of 10 μM (+)-pentazocine. Test compounds were added in concentrations ranging from 0.005 to 1000 or from 0.05 to 10000 nM. Assays were terminated by the addition of 5 mL of ice-cold 10 mM Tris-HCl, pH 8.0, followed by rapid filtration through glass fiber filters using a Brandel cell harvester (Gaithersburg, MD). Filters were then washed

twice with 5 mL of ice-cold buffer. Prior to use, filters were soaked in 0.5% polyethylenimine for at least 30 min at 25 °C.

σ2 Binding Assay. Rat liver membranes (160–200 μg of protein) were incubated with 3 nM [^{3}H]DTG (39.4 Ci/mmol) in the presence of 1 μM unlabeled dextrallorphan. Incubations were carried out in 0.5 mL of 50 mM Tris-HCl, pH 8.0 for 120 min at 25 °C. Nonspecific binding was determined in the presence of 5 μM haloperidol. Test compounds were added in concentrations ranging from 0.005 to 1000 or from 0.05 to 10000 nM. Assays were terminated by the addition of 5 mL of ice-cold 10 mM Tris-HCl, pH 8.0 followed by rapid filtration through glass fiber filters using a Brandel cell harvester (Gaithersburg, MD). Filters were then washed twice with 5 mL of ice-cold buffer. Prior to use, filters were soaked in 0.5% polyethylenimine for at least 30 min at 25 °C.

All scintillation counting was carried out in Ecoscint (National Diagnostics, Manville, NJ) after an overnight extraction of counts.

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