Enantiomers of Diastereomeric *cis*-*N*-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamides: Synthesis, X-ray Analysis, and Biological Activities

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Received December 15, 1994[⊗]

(±)-*cis*-*N*-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide (**1**) is a mixture of four stereoisomers [(2*S*,3*R*,4*S*)-**1a**, (2*R*,3*R*,4*S*)-**1b**, (2*R*,3*S*,4*R*)-**1c**, and (2*S*,3*S*,4*R*)-**1d**], which together constitute two diastereoisomeric pairs of optical isomers. These four stereoisomers were prepared from optically active intermediates of known absolute configuration by procedures which had no effect on the configurations of the piperidine 3- and 4-carbons. The configuration of the phenylethyl 2-carbon in the final products was determined by X-ray analysis of (2*S*,3*S*,4*R*)-**1d**. A ^{1}H NMR comparison of the final products to ohmefentanyl established that the racemic pair previously known as ohmefentanyl was a mixture of (2*S*,3*R*,4*S*)-**1a** and (2*R*,3*S*,4*R*)-**1c**. The individual activities of **1a**, **1b**, **1c**, and **1d** were evaluated in a variety of binding and pharmacological assays. The binding data revealed that isomers **1b** and **1c** had the highest affinity and selectivity for the μ site labeled with [^{3}H]DAMGO. In contrast, the four isomers displaced [^{3}H]etorphine in the order **1a** ≈ **1b** > **1c** ≈ **1d**. Evaluation of the four isomers on the mouse vas deferens (MVD) preparation revealed a potency order of **1a** > **1b** > **1c** > **1d** with concentrations of **1a** and **1b** in the femtomolar range causing inhibition. Experiments using the antagonists naltrexone (μ), ICI 174864 (δ), and norbinaltorphimine (κ) demonstrated that the effects of **1a** were mediated largely by the μ receptor while both δ and κ agonist effects contributed to the actions of **1b** and **1c**. Isomer **1d** acted as a weak μ antagonist in the MVD preparation. The same potency order was observed in a mouse analgesic assay and a rhesus monkey single dose suppression study. From the latter study the potency of **1a** was estimated to be 20 000–50 000 times that of morphine, making this isomer one of the most potent opiates known. In the rhesus monkey study, isomer **1d** failed to substitute for morphine and seemed to exacerbate withdrawal at doses of 0,6, 3.0, and 6.0 mg/kg. On the basis of the mouse data, isomer **1a** was 21 000 times more potent than **1d**, whereas isomers **1b** and **1c** were similar in their opiate activity in vivo. Using the optical isomers of *cis*-3-methylfentanyl as reference compounds, we analyzed the effects on the pharmacological activities of introducing a phenylethyl 2-hydroxyl group into the molecule. From this analysis we drew the following conclusions regarding structure: (a) the (3*R*,4*S*)-piperidine stereochemistry found in the more potent *cis*-3-methylfentanyl isomer was required for potent opiate agonist activity; (b) the introduction of a phenylethyl 2-hydroxyl with the *S* configuration had an enormous impact on this activity as demonstrated by the extraordinary μ agonist properties of **1a** and the weak μ agonist/antagonist properties of **1d**; and (c) the introduction of a 2-hydroxyl with the *R* configuration had a much smaller impact on the opiate agonist activity. Finally, our findings demonstrated the importance of the combination of 2-hydroxyl and 3-methyl substituents to the pharmacological properties of the four isomers.

(±)-*cis*-*N*-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide (**1**), also known as ohmefentanyl and as *cis*-β-hydroxy-3-methylfentanyl, is an extremely potent analgesic agent with a high selectivity for the opioid μ receptor.[1-4] Indeed, compound **1** is one of the "super potent" opioids,[5,6] a description given to several analogs of fentanyl which are more potent in producing antinociception than predicted on the basis of their affinity for the μ receptor.

Due to the presence of three asymmetric carbon atoms, eight stereoisomers of *N*-[1-(2-hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide are theoretically possible. Eliminating the four stereoisomers in which the piperidine C3 and C4 substituents have a trans relationship leaves the four cis isomers depicted in Chart 1. The four cis stereoisomers constitute two diastereoisomeric pairs of optical isomers (**1a**, **1c** and **1b**, **1d**). In an earlier study these pairs were separated, and one pair was found to be 5.3 times more potent than the other;[7] however, the composition of the more active pair was not reported. Test data from mice indicated that the more active pair was 6300 times more potent than morphine.[1,7] This more active pair was later referred to as ohmefentanyl.[1,3,4,7]

We later synthesized a sample of **1** (designated as RTI-4614-4) which we determined to be a mixture of the four cis isomers shown in Chart 1.[8] Subsequent testing of this material revealed that it possessed potent analgesic activity.[9,10] In particular, test data from

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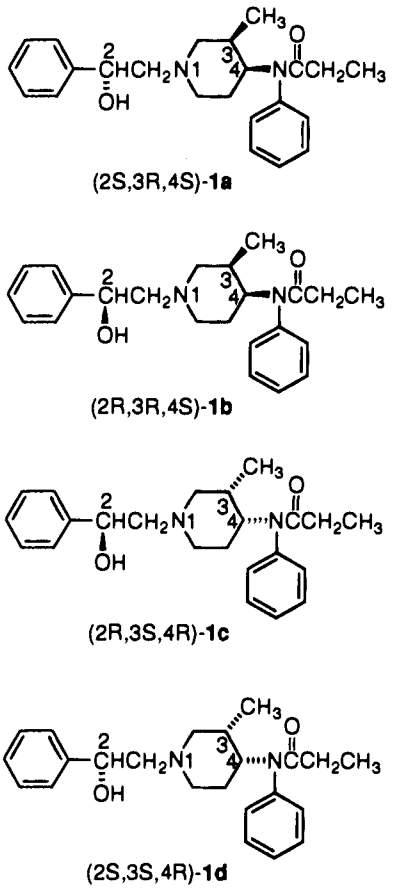
[⊗] Abstract published in *Advance ACS Abstracts*, April 1, 1995.

0022-2623/95/1838-1547$09.00/0

Chart 1



monkeys indicated that our sample was about 25 000 times more potent than morphine,[9] which put it into the same potency level as carfentanil. In vitro ligand binding studies revealed that our synthetic sample had a 27 000-fold binding selectivity for μ versus δ opioid receptors.[6]

A direct comparison of ohmefentanyl with our synthetic sample[8] demonstrated that whereas our sample was a mixture of both of the possible diastereoisomeric pairs (cf. Chart 1), ohmefentanyl was composed of just one of these diastereoisomeric pairs.[6,11] Assuming that the NIH sample of ohmefentanyl used in this comparison was prepared as reported in the earlier study,[7] our finding that it was composed of only one diastereoisomeric pair is consistent with the method of preparation. It was clear from this comparison that ohmefentanyl was a subset of our sample (RTI-4614-4).

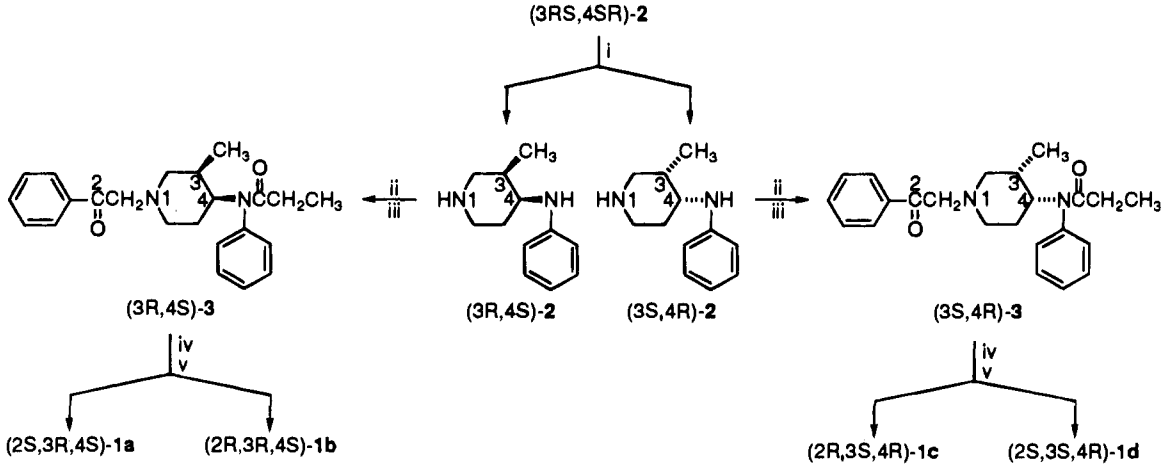
In view of the above reports and the different isomeric compositions of ohmefentanyl and our synthetic sample of **1**, there is a clear need to resolve (±)-*cis*-*N*-[1-(2-hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide (**1**) into its four stereoisomers (cf. Chart 1). We report in this paper the synthesis, absolute configuration, and biological activities of these individual isomers. In addition, we compare our findings to other recently reported results.[12]

Results

Chemical. The starting material was (±)-1-benzyl-3-methyl-4-piperidone, which was prepared in three steps from benzylamine using slight modifications of literature procedures.[13] Condensation of (±)-1-benzyl-3-methyl-4-piperidone with aniline followed by sodium borohydride reduction of the intermediate Schiff base afforded a mixture of (±)-*cis*-1-benzyl-3-methyl-*N*-phenyl-4-piperidinamine and (±)-*trans*-1-benzyl-3-methyl-*N*-phenyl-4-piperidinamine.[14] After chromatographic separation, the isomeric products were further purified by vacuum distillation and crystallization of their respective oxalate salts. Subsequent hydrogenolysis of (±)-*cis*-1-benzyl-3-methyl-*N*-phenyl-4-piperidinamine provided (±)-*cis*-3-methyl-*N*-phenyl-4-piperidinamine [(3*RS*,4*SR*)-**2**] (cf. Chart 2). The tartaric acid resolution of (3*RS*,4*SR*)-**2** was accomplished using reported procedures[15,16] to obtain enantiomerically pure samples of (3*R*,4*S*)-(+)-**2** and (3*S*,4*R*)-(−)-**2**. The absolute configurations of these isomers had been determined earlier by an X-ray crystallographic study.[15]

Starting with (3*R*,4*S*)-(+)-**2**, alkylation with 2-bromoacetophenone followed by acylation with propionyl chloride afforded (3*R*,4*S*)-*cis*-(−)-*N*-[3-methyl-1-(2-oxo-2-phenylethyl)-4-piperidyl]-*N*-phenylpropanamide [(3*R*,4*S*)-(−)-**3**] (cf. Chart 2). The ketone was reduced with sodium borohydride to generate a diastereoisomeric mixture of alcohols (**1a** and **1b**), which were separated by fractional crystallization. A similar sequence starting with (3*S*,4*R*)-(−)-**2** provided the other pair of alcohols (**1c** and **1d**). As established by previous

Chart 2[a]



[a] Reagents: (i) tartaric acid resolution; (ii) $C_6H_5COCH_2Br$, K_2CO_3, KI; (iii) CH_3CH_2COCl, Et_3N; (iv) $NaBH_4$; (v) fractional crystallization.

Table 1. Four Stereoisomers of (±)-*cis*-*N*-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide (1)

					^{1}H NMR (free base) δ ($CDCl_3$)[b]				
compound	mp, °C	$[\alpha]^{25}_D$ (*c*, MeOH)	salt[a]	mp, °C (salt)	pip 2He	pip 2Ha	pip 6He	pip 6Ha	ArCH(OH)CH_2N
(2*S*,3*R*,4*S*)-**1a**	121–122	+22.3°, 0.77	A	198–200	2.60 (dt)	2.66 (dd)	3.06 (br d)	2.06 (dt)	2.34 (dd), 2.42 (dd)
(2*R*,3*R*,4*S*)-**1b**	138–140	−32.6°, 1.32	B	128–130	2.96 (br d)	2.34–2.42	2.70 (br d)	2.34–2.42	2.34–2.42
(2*R*,3*S*,4*R*)-**1c**	121–122	−22.6°, 0.65	A	199–201	2.60 (dt)	2.66 (dd)	3.06 (br d)	2.06 (dt)	2.34 (dd), 2.42 (dd)
(2*S*,3*S*,4*R*)-**1d**	140–141	+33.7°, 0.85	B	128–130	2.96 (br d)	2.34–2.42	2.70 (br d)	2.34–2.42	2.34–2.42
ohmefentanyl[c]			A	188–194	2.62 (br d)	2.65 (dd)	3.06 (br d)	2.07 (dt)	2.36 (dd), 2.43 (dd)

[a] Salt: A, HCl; B, oxalate. [b] ^{1}H NMR abbreviations: a = axial, e = equatorial, d = doublet, br = broad, dd = doublet of doublets, dt = doublet of triplets. [c] Supplied by Dr. Heng Xu, NIDA, NIH.

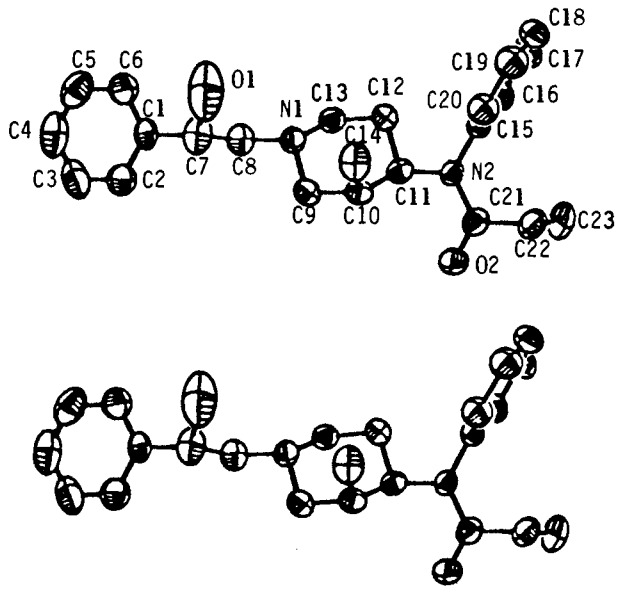


Figure 1. A stereodiagram of (2*S*,3*R*,4*R*)-*cis*-(+)-*N*-[1-(2-hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide [(2*S*,3*S*,4*R*)-*cis*-(+)-**1d**] showing 50% thermal ellipsoids. The hydrogen atoms are omitted for clarity. The carbons are numbered consecutively in the drawing, with C7, C10, and C11 (drawing) corresponding to the phenylethyl C2, the piperidine C3, and the piperidine C4, respectively.

syntheses,[15,16] these transformations from intermediates **2** to the final products had no effect on the configurations of the piperidine 3- and 4-carbons. The melting point and optical rotation data on the four stereoisomers are summarized in Table 1. Similar melting point and optical rotation values (free bases) were obtained by Zhu and co-workers.[12]

On the basis of previous synthetic work,[15,16] our synthetic route to the four stereoisomers established the stereochemistries at the piperidine 3- and 4-carbons for all four synthetic products. An X-ray analysis of the more soluble isomer (free base) derived from (3*S*,4*R*)-(−)-**2** showed that the alcohol had the *S* configuration. A stereodiagram[17] of this isomer, (2*S*,3*S*,4*R*)-**1d**, is shown in Figure 1. This finding established that the phenylethyl 2-carbon of isomer **1c**, the less soluble (free base) isomer derived from (3*S*,4*R*)-(−)-**2**, had the *R* configuration. Knowing the configurations at the phenylethyl 2-carbons of isomers **1c** and **1d** also established the configurations of their respective optical isomers, **1a** and **1b**. Isomers **1a** and **1b** were readily identified by comparison of their physical and spectral properties to those of **1c** and **1d**.

As expected, optical isomers **1a** and **1c** had identical ^{1}H NMR spectra as did optical isomers **1b** and **1d**. The major differences between the **1a**, **1c** pair and the **1b**, **1d** pair were in the chemical shift values observed for the hydrogens bonded to the piperidine 2- and 6-carbons (cf. Table 1). Indeed, the broadened downfield doublet at δ 3.06 assigned to the piperidine 6He in the spectra of **1a** and **1c** was diagnostic for the presence of these isomers. Likewise, the doublet at δ 2.96 assigned to the piperidine 2He was diagnostic for the presence of isomers **1b** and **1d**. Moreover, all four resonances (2He, 2Ha, 6He, 6Ha) were clearly discernible in the spectra of isomers **1a** and **1c**. However, in the spectra of isomers **1b** and **1d**, the 2Ha and 6Ha resonances were part of a four-proton multiplet that included the ArCH(OH)CH_2N signals. The assignments of these resonances followed from our earlier studies on the mixture of isomers.[8]

Also presented in Table 1 are the corresponding ^{1}H NMR assignments for ohmefentanyl. In this case, we collected the NMR data on a sample of the hydrochloride salt in deuteriochloroform which had been treated with an equivalent amount of sodium deuterioxide to generate the free base in situ. Nevertheless, comparison of the chemical shift data indicates that ohmefentanyl gave the ^{1}H NMR resonances diagnostic of the **1a**, **1c** pair. This finding indicated that ohmefentanyl was a mixture of (2*S*,3*R*,4*S*)-**1a** and (2*R*,3*S*,4*R*)-**1c**.

Biological. The four stereoisomers were evaluated in reversible binding assays at the μ, δ, and κ opioid binding sites. Isomers **1a** and **1c** were assayed as hydrochloride salts; **1b** and **1d** as oxalate salts. The assays were carried out utilizing previously described procedures.[18–20] The apparent K_i values and slope factors (*B*) of the four isomers at the three opioid binding sites are presented in Table 2. In addition, the μ/δ and μ/κ ratios are presented for isomers **1b** and **1c**. Since isomers **1a** and **1d** had low affinities for the δ and κ binding sites, the μ/δ and μ/κ ratios were not calculated. Also given in Table 2 are the corresponding K_i value and slope factor for the mixture of isomers (RTI-4614-4)[6] as well as values for fentanyl, (3*R*,4*S*)-*cis*-3-methylfentanyl, (3*S*,4*R*)-*cis*-3-methylfentanyl, ohmefentanyl, and morphine.[5] Our findings for the μ and δ binding sites have been published in preliminary form.[21]

In addition to the binding experiments summarized in Table 2, the four stereoisomers were tested in an opioid receptor binding assay involving the displacement of [^{3}H]etorphine in rat brain cerebral membranes.[22,23] Isomers **1a** and **1c** were again assayed as hydrochloride salts; **1b** and **1d** as oxalate salts. From this assay the following EC_{50} values (nM) were determined: **1a**, 5.9; **1b**, 6.8; **1c**, 102; and **1d**, 380.

The four stereoisomers were also evaluated on the isolated, electrically-stimulated mouse vas deferens (MVD) preparation using a reported procedure.[22] Each isomer was evaluated alone (n = 9) and in the presence of the following antagonists (n = 3): naltrexone (100 nM), which is used to block μ receptors in this preparation, ICI 174864 (100 nM), a δ receptor antagonist,[24]

Table 2. In Vitro Ligand Binding Results

compound	K_i (nM ± SD) [B ± SD] μ	δ	κ	μ/δ[d]	μ/κ[e]
RTI-4614-4	0.0055 ± 0.0006	148 ± 12	84.8 ± 12.0	26 909	15 418
(HCl salt)	[0.69 ± 0.05]	[0.88 ± 0.06]	[0.69 ± 0.05]		
(2*S*,3*R*,4*S*)-**1a**	47.07 ± 7.21	>1.5 μM	>0.5 μM	ND[g]	ND[g]
(HCl salt)	[0.98 ± 0.05]				
(2*R*,3*R*,4*S*)-**1b**	0.013 ± 0.002	103.42 ± 13.20	122.2 ± 7.3	7955	9400
(oxalate salt)	[0.94 ± 0.28]	[0.77 ± 0.08]	[1.05 ± 0.06]		
(2*R*,3*S*,4*R*)-**1c**	0.005 ± 0.002	84.06 ± 10.74	41.7 ± 1.4	16 812	8340
(HCl salt)	[1.11 ± 0.05]	[0.96 ± 0.10]	[1.03 ± 0.03]		
(2*S*,3*S*,4*R*)-**1d**	16.47 ± 1.07	>3 μM	>0.5 μM	ND[g]	ND[g]
(oxalate salt)	[0.92 ± 0.02]				
fentanyl[f]	3.97 ± 0.60	1,035 ± 115	196.5 ± 8.3	260	49.5
(3*R*,4*S*)-*cis*-3- methylfentanyl[f]	0.020 ± 0.005	77.3 ± 6.7	57.4 ± 6.1	3865	2870
(3*S*,4*R*)-*cis*-3- methylfentanyl[f]	30.60 ± 5.13	>1 μM	ND[g]	ND[g]	ND[g]
ohmefentanyl[f,h]	0.83 ± 0.09	182.82 ± 20.37	ND[g]	220	ND[g]
morphine[f]	8.14 ± 1.24	684 ± 83	69.1 ± 4.1	84	8.5

[a] The μ sites were labeled with [^{3}H]DAMGO ([D-Ala^2-MePhe^4-Gly-ol^5]enkephalin). [b] The δ sites were labeled with [^{3}H]DADLE ([D-Ala^2-D-Leu^5]enkephalin) in the presence of LY164929 (see the Experimental Section). [c] The κ sites were labeled with [^{3}H]U69,593 [(5α,7α,8β)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]benzeneacetamide]. [d] K_i [^{3}H]DADLE/K_i [^{3}H]DAMGO. [e] K_i [^{3}H]U69,593/K_i [^{3}H]DAMGO. [f] Reference 5. In this study the μ sites were labeled with [^{3}H]-6β-fluoro-6-desoxyoxymorphone ([^{3}H]FOXY). [g] Not determined. [h] Ohmefentanyl was found to be a mixture of (2*S*,3*R*,4*S*)-**1a** and (2*R*,3*S*,4*R*)-**1c**.

Table 3. Analgesic and Dependence-Liability Results

compound	mouse assay[a] ED_{50} (mg/kg sc)	monkey SDS test[b] effect (mg/kg)	potency (×M)
RTI-4614-4	0.0002	CS (0.0005)	25 000
(HCl salt)	(0.0001−0.0003)		
(2*S*,3*R*,4*S*)-**1a**	0.0001	CS (0.00015)	20 000−50 000
(HCl salt)	(0.00005−0.0004)		
(2*R*,3*R*,4*S*)-**1b**	0.0013	CS (0.002)	1500
(oxalate salt)	(0.0004−0.0046)		
(2*R*,3*S*,4*R*)-**1c**	0.08	CS (0.1)	30
(HCl salt)	(0.003−0.02)		
(2*S*,3*S*,4*R*)-**1d**	2.1	NS (12.0)	–
(oxalate salt)	(0.6−7.8)		
(3*R*,4*S*)-*cis*-3-methylfentanyl[c]	0.00058		
	(0.00049−0.00068)		
(3*S*,4*R*)-*cis*-3-methylfentanyl[c]	0.068		
	(0.051−0.091)		

[a] HP assay. [b] SDS = single dose suppression, CS = complete suppression, NS = no suppression, M = morphine. [c] Reference 16. The data are for warm-water tail withdrawal, rats, iv administration.

and norbinaltorphimine (norBNI) (10 nM), a κ receptor antagonist.[25] The complex findings for isomers **1a−c** are presented graphically in Figure 2 in which the percent inhibition is plotted against the log molar concentration for each compound. Both **1a** and **1b** produced inhibitory actions at very low concentrations (femtomolar) in an apparently biphasic manner. In addition, both **1a** and **1b** inhibited the MVD over a wide concentration range (seven log units). Naltrexone shifted the **1a** inhibition curve orders of magnitude to the right and also produced a classical two log inhibition curve. ICI 174864 also significantly altered the **1a** inhibition curve by shifting the entire curve to the right without substantially altering the biphasic nature of the inhibition curve. The κ antagonist norBNI had no significant effect on the **1a** curve. Naltrexone, ICI 174864, and norBNI all exerted prominent effects at the lower concentrations of isomer **1b**, while only naltrexone exerted major effects at the higher concentrations of **1b**. The results observed with isomer **1c** were qualitatively similar to those observed with **1b** except that the **1c** dose−response curve was less biphasic and isomer **1c** was less potent than **1b**.

Since isomer **1d** behaved as a weak partial agonist in the MVD assay (data not shown), it was evaluated as an antagonist against the following agonists: sufentanil (μ), DSLET (δ), and U50,488 (κ).[22] Isomer **1d** was found to be a weak antagonist at the μ receptor (pA_2 6.62 ± 0.31 versus sufentanil). In contrast, it did not block the actions of DSLET or U50,488 in concentrations up to 3 μM.

In addition, the four stereoisomers were evaluated for analgesic activity in the mouse hot plate assay using a reported procedure.[26] The ED_{50} values (confidence limits) for the four isomers and the mixture of isomers (RTI-4614-4)[9] are presented in Table 3. Also shown in Table 3 are the corresponding values for (3*R*,4*S*)-*cis*-3-methylfentanyl and (3*S*,4*R*)-*cis*-3-methylfentanyl.[16]

Finally, the four stereoisomers as well as the mixture (RTI-4614-4)[9] were evaluated in a substitution for morphine (single dose suppression) assay in morphine-dependent rhesus monkeys.[26] These results are also presented in Table 3. Both the mixture of isomers and isomers **1a**, **1b**, and **1c** all substituted completely for morphine (i.e., completely suppressed withdrawal) at the dosage shown in Table 3. In each case the onset of action was rapid and the duration of action was approximately 90 min. Both the mixture and isomer **1a** demonstrated considerable activity at a dosage level equivalent to one-fifth that shown in Table 3 (i.e., 0.0001 and 0.000 03 mg/kg, respectively) while isomers **1b** and **1c** had good activity at 0.0005 and 0.025 mg/kg, respectively. Due to its extremely high activity, the potency of isomer **1a** could only be estimated to 20 000−

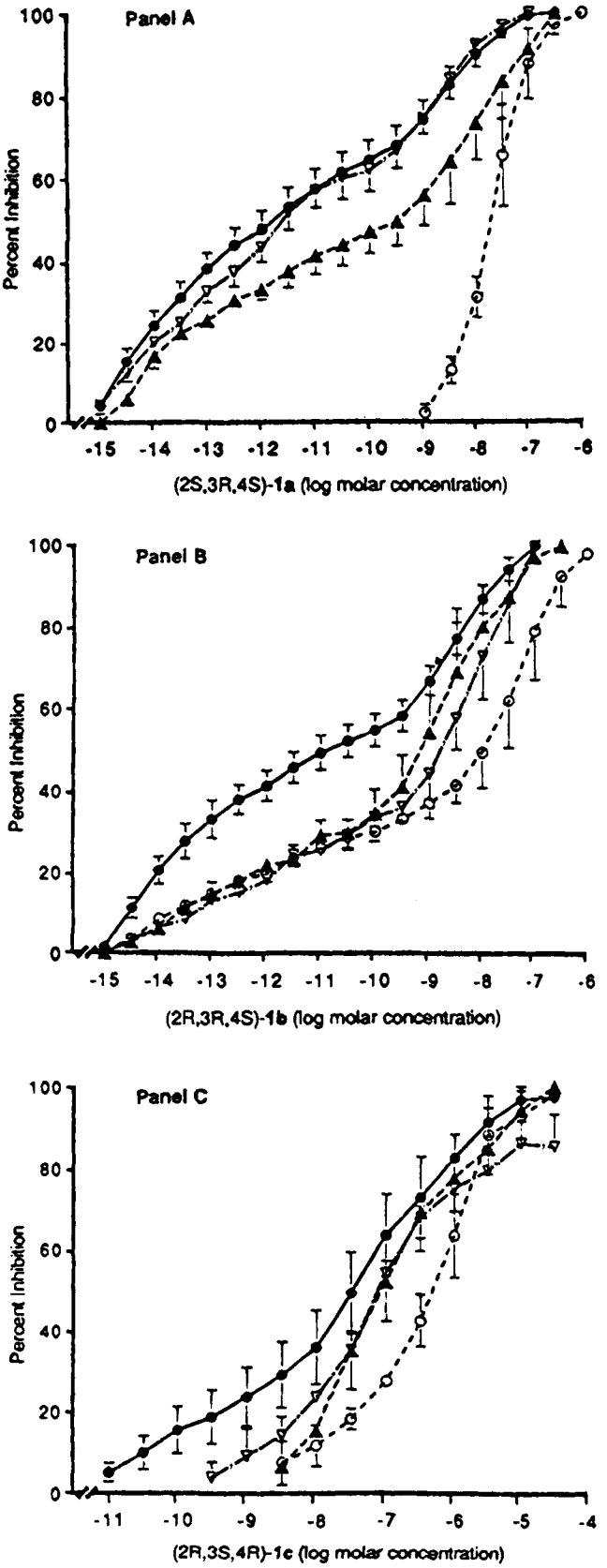


Figure 2. Results in the mouse vas deferens preparation for (2*S*,3*R*,4*S*)-1a (panel A), (2*R*,3*R*,4*S*)-**1b** (panel B), and (2*R*,3*S*,4*R*)-**1c** (panel C). In each panel, the data are plotted for the test compound alone (●) and in the presence of naltrexone (○), ICI 174864 (▲), and norbinaltorphimine (▽), respectively.

50 000 times that of morphine. In contrast, isomer **1d** did not substitute for morphine over a dosage range of 0.6–12.0 mg/kg; as a consequence, no potency estimate could be made. Interestingly, at doses of 0.6, 3.0 and 6.0 mg/kg, isomer **1d** appeared to exacerbate withdrawal in a dose-related manner.

Discussion

After their initial synthesis of (±)-*cis*-*N*-[1-(2-hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide (**1**),[1] the original investigators synthesized a mixture of the four cis isomers (cf. Chart 1) and a mixture of the four trans isomers.[7] Both mixtures were separated into two diastereoisomeric pairs by fractional crystallization, with each pair being a racemic mixture. The biological test data showed that both cis pairs were more potent than both trans pairs, a finding which paralleled the known greater potency of (±)-*cis*-3-methylfentanyl over (±)-*trans*-3-methylfentanyl.[16] In addition, the test data demonstrated that the less soluble (petroleum ether) cis pair was 5.3 times more potent than the more soluble cis pair. The less soluble cis pair was identified[7] as the product isolated from the initial synthesis[1] and was later referred to as ohmefentanyl.[3,4]

Our initial synthesis of (±)-*cis*-*N*-[1-(2-hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide (**1**) involved the reaction of (±)-*cis*-*N*-(3-methyl-4-piperidyl]-*N*-phenylpropanamide[1,14] with styrene oxide, a procedure which afforded a mixture of the four possible cis stereoisomers.[6,8] Later we obtained the same mixture[8] utilizing a reported route[7] [alkylation of (±)-*cis*-*N*-(3-methyl-4-piperidyl]-*N*-phenylpropanamide[1,14] with 2-bromoacetophenone followed by sodium borohydride reduction]. Efforts to fractionate this mixture into diastereoisomeric pairs were only partially successful. Although we isolated a small amount of the less soluble fraction which contained no detectable amount of the more soluble fraction by ^{1}H NMR analysis, we were unable to isolate a sample of the more soluble fraction of similar purity.

We felt that the separation problem was complicated by the need to crystallize apart pairs of isomers. We reasoned that incorporation of an optical resolution step at an intermediate point in the synthesis would simplify the problem because only two diastereoisomeric final products would be generated from each optically pure intermediate. We chose (±)-*cis*-3-methyl-*N*-phenyl-4-piperidinamine (**2**) as the key intermediate since both the optical resolution of this compound and the absolute configurations of the optical isomers were reported.[15,16] The subsequent conversions of (3*R*,4*S*)-(+)-**2** and (3*S*,4*R*)-(−)-**2** to the target compounds were accomplished using modifications of reported procedures.[1,7,8] From each optical isomer of **2** we obtained a diastereoisomeric mixture of two isomers of **1** (cf. Chart 2). As we had hoped, these two-component mixtures were easily separated by fractional crystallization, thereby enabling pure samples of each isomer shown in Chart 1 to be obtained.

We stated earlier that we had found ohmefentanyl to be a subset of our initially prepared sample (RTI-4614-4) in terms of its isomeric composition. As shown in Table 1, the ^{1}H NMR spectrum of ohmefentanyl (NIH sample) contained the same key resonances as isomers **1a** and **1c**, which resonances clearly distinguish them from isomers **1b** and **1d**. From this comparison we concluded that ohmefentanyl, which corresponded to the

Table 4. Binding Affinities of Selected Peptide Ligands at Rat Brain μ Receptors Labeled by [^{3}H]DAMGO or [^{125}I]IOXY-AGO[a]

compound	[^{3}H]DAMGO		[^{125}I]IOXY-AGO	
	IC_{50} (nM ± SD)	slope (B ± SD)	IC_{50} (nM ± SD)	slope (B ± SD)
[Met^5]enkephalin	15.3 ± 1.8	1.05 ± 0.11	110 ± 19	0.70 ± 0.08
[Leu^5]enkephalin	20.6 ± 2.3	0.83 ± 0.07	221 ± 25	0.52 ± 0.03
α-neoendorphin	39.0 ± 2.9	0.83 ± 0.05	308 ± 76	0.71 ± 0.13
β-endorphin$_{(1-31)}$	2.93 ± 0.28	1.13 ± 0.11	64.0 ± 14.4	0.42 ± 0.04

[a] Data from ref 27. Binding assays were conducted as described in the Experimental Section using [^{3}H]DAMGO (3.1 nM) or [^{125}I]IOXY-AGO (0.016 nM) and rat brain membranes. The data from two independent experiments were pooled and fit to the two parameter logistic equation for the best fit estimates of the IC_{50} and Hill slope.

less soluble racemic pair isolated in earlier studies,[1,3,4,7] was a mixture of (2*S*,3*R*,4*S*)-**1a** and (2*R*,3*S*,4*R*)-**1c**. The fact that these were the less soluble isomers in each synthetic mixture which we obtained supported this conclusion.

In a recent abstract the preparation of the four stereoisomers shown in Chart 1 by a different route was reported.[12] In this latter route the (3*R*,4*S*)-(+)-**2** and (3*S*,4*R*)-(−)-**2** isomers were reacted with (*R*)-(+)-styrene oxide and (*S*)-(−)-styrene oxide to produce four optically active intermediates which were subsequently converted to the target compounds. The melting point and optical rotation values for the four cis isomers prepared by this latter route[12] agreed closely with our values.

The in vitro ligand binding results (cf. Table 2) revealed that the two stereoisomers with the highest affinity for the μ binding site had the *R* configuration at the phenylethyl 2-carbon, which finding was reported previously.[21] Thus, isomers **1b** and **1c** had about a 3000-fold higher affinity for the μ binding site than isomers **1a** and **1d**, respectively. The stereochemistries of the piperidine 3- and 4-carbons exerted a small effect on the affinity of the molecule for the μ site: the (3*R*,4*S*) configuration lowered the affinity of the molecule for the μ site by a factor of two relative to the (3*S*,4*R*) configuration. The observed order of binding affinity (μ site, [^{3}H]DAMGO) was **1c** > **1b** > **1d** > **1a**.

Isomers **1b** and **1c** displayed much weaker binding affinities for the δ and κ sites. The μ/δ selectivities were 7900 (**1b**) and 16 800 (**1c**); the μ/κ selectivities were 9400 (**1b**) and 8300 (**1c**) (cf. Table 2). These differences were observed because changing the configurations of the piperidine 3- and 4-carbons from (3*S*,4*R*) to (3*R*,4*S*) increased the K_i for the μ site (2.6-fold) and κ site (2.9-fold) more than it increased the K_i for the δ site (1.2-fold).

It is important to note that isomers **1a** and **1d** are so much weaker than **1b** and **1c** that they do not contribute to the inhibition of [^{3}H]DAMGO binding by the mixture of isomers (RTI-4614-4). This is because **1b** and **1c** have completely inhibited [^{3}H]DAMGO binding (about 0.1 nM) before **1a** and **1d** achieve concentrations high enough to inhibit binding (about 5 nM). Similarly, isomers **1b** and **1c** would dominate the [^{3}H]DADLE and [^{3}H]U69,593 inhibition curves.

An interesting contrast was provided by the EC_{50} values for displacement of specific equilibrium binding of [^{3}H]etorphine. While the four isomers were weak inhibitors of [^{3}H]etorphine in this assay, the order of potency was **1a** ≈ **1b** > **1c** ≈ **1d**. This corresponded to an observed order of binding affinities at the μ and δ sites obtained using [^{3}H]ohmefentanyl (μ) and [^{3}H]-DPDPE (δ) as ligands.[12] This latter data paralleled our findings for isomers **1b** and **1d** using [^{3}H]DAMGO and [^{3}H]DADLE; however, the latter investigators observed that **1a** was equivalent to **1b** in binding affinity whereas **1c** had affinities which were similar to those of **1d**. Since no other experimental details were given,[12] there was no way to compare our experimental procedures to theirs. Nevertheless, these different findings utilizing different radioligands suggested that the binding of these different isomers to the receptor binding sites was a complex phenomenon involving many factors.

An illustration of the dependence of binding affinities on the radioligand used to label the receptor is provided by a recent study in which substantial differences were observed in the IC_{50} values of selected opioid peptide ligands at the μ opioid binding site depending on the radioligand employed (cf. Table 4). Thus, the binding affinities of [Met^5]enkephalin, [Leu^5]enkephalin, α-neoendorphin, and β-endorphin$_{(1-31)}$ appeared 10−20 times greater using [^{3}H]DAMGO than using [^{125}I]IOXY-AGO.[27] These observations point to the need to examine the interaction of **1a**, **1b**, **1c**, **1d**, and ohmefentanyl with cloned μ receptors using several different radioligands.

In a separate study we evaluated the four stereoisomers as pseudoirreversible inhibitors of μ receptor binding.[28] We observed that isomers **1b** and **1c**, in addition to the mixture (RTI-4614-4), acted as pseudoirreversible inhibitors of the μ receptor labeled with [^{3}H]-DAMGO. This finding was consistent with the binding affinities which we observed with the same radioligand (cf. Table 2) since pseudoirreversible inhibition was only produced by those isomers having a high-affinity interaction with the receptor. Isomers **1b** and **1c** also behaved as pseudoirreversible inhibitors of the μ receptor as labeled with [^{3}H]fentanyl or [^{3}H]etorphine.[28] However, the effects of **1b** and **1c** on the [^{3}H]fentanyl and [^{3}H]etorphine binding were somewhat different from those on the [^{3}H]DAMGO binding. In particular, the [^{3}H]etorphine binding was less sensitive to the pseudoirreversible effects of **1b** and **1c**.[28] These data provided evidence that certain μ ligands bind to different binding domains of the drug recognition site of the μ receptor, a factor which could affect their apparent binding affinities.

The MVD data (cf. Figure 2) were complex and difficult to interpret. Nevertheless, it was clear that **1a** and **1b** were the most potent compounds with concentrations in the femtomolar range causing inhibition. They also caused inhibition over a wide concentration range in an apparently biphasic manner. The effects of ICI 174864 and naltrexone on the **1a** inhibition curve suggested that it is a relatively weak δ agonist and that its activity at μ receptors is responsible for its potent actions and biphasic inhibition curve. In contrast, naltrexone, ICI 174864, and norBNI all noticeably blocked the activity of **1b** at lower concentrations while only naltrexone exerted a major effect at higher concentrations of **1b**. These findings suggested that both

δ and κ agonist effects contribute to the actions of **1b** in the MVD. This is consistent with the moderate affinity of **1b** for the δ and κ receptor binding sites (cf. Table 2). Isomer **1c** was less potent than **1a** and **1b**. The effects of naltrexone, ICI 174864, and norBNI on the activity of **1c** were similar to those on **1b**, findings which were again consistent with the binding affinity (cf. Table 2). Viewed collectively, the MVD data suggested that isomers **1a** and **1b** in particular are likely to have complex pharmacologies, with both δ and κ receptors contributing to their in vivo effects.

We also noted that both **1a** and **1b**, the most active compounds in the MVD preparation, had the same piperidine stereochemistry as (3*R*,4*S*)-*cis*-3-methylfentanyl;[15,16] the configuration of the phenylethyl 2-carbon seemed to have little effect on the biological activity. Interestingly, isomer **1a**, a highly active compound in the smooth muscle preparation, had the lowest binding affinity (cf. Table 2) whereas **1c**, the isomer with the highest binding affinity, was third in potency in the MVD preparation. The weak partial agonist/antagonist activity observed for isomer **1d** was also noteworthy since it was unusual activity for a fentanyl analog.

The in vivo findings from both mice and rhesus monkeys (cf. Table 3) established that **1a** was the most active compound in these assays. In fact, the estimated potency of 20 000–50 000 times morphine for this isomer places it among the most potent opiates known. The same order of potency (**1a** > **1b** > **1c** > **1d**) observed by us was found by Zhu and co-workers[12] in mice (hot plate, ip). There was also a clear parallel with the order of potency observed in the MVD preparation. In addition, similar ED_{50} values were observed for the four stereoisomers in the mouse tail flick assay (data not shown). Since **1a** was extremely potent and since it seemed unusually sensitive to naloxone in the tail flick assay, a pA_2 test was conducted. The result (pA_2 7.21 ± 0.10 versus naloxone) indicated that **1a** interacts competitively with naloxone at the μ receptor. Also noteworthy was the observation that **1d**, which displayed some analgesic activity in the mouse assay, appeared to act as a weak μ antagonist at the lower dosage levels in the rhesus monkey assay,[26] an observation which was also consistent with the MVD finding.

Since the mixture of two racemic diastereoisomers (RTI-4614-4) is composed of almost equal amounts of all four isomers, one would expect it to have a somewhat lower in vivo potency than the most active isomers. Although the mixture was in fact quite potent,[9] its potency was approximately one-half to one-third that displayed by isomer **1a** (cf. Table 3). This comparison implied that the extraordinary in vivo activity of isomer **1a** was in large measure responsible for the activity of the mixture.

An interesting observation was that isomer **1a** was the most potent compound in vivo and in the MVD preparation, yet it had the lowest binding affinity of the four stereoisomers for the μ binding site as labeled by [^{3}H]DAMGO. Since the biological effects of **1a** appear to be mediated largely by the μ opioid receptor, a possible explanation is that **1a** is the most efficacious isomer. It is known that the ability of a drug to produce its effect is determined not only by its affinity for its receptor but also by its receptor reserve or intrinsic efficacy.[29] Thus, isomer **1a**, despite having a considerably lower affinity for the μ receptor, could have a much lower ED_{50} if it activated the μ receptors at a much lower fractional occupancy than the other stereoisomers. The experiments reported in this paper do not address the issue of receptor reserve. It would be of interest in future studies to examine this issue directly using irreversible antagonists such as clocinnamox.[30]

We note that the binding data of Zhu and co-workers,[12] obtained using [^{3}H]ohmefentanyl to label the μ binding site, provide a more consistent picture in the sense that there is a more direct correlation between binding affinity and μ agonist activity. As discussed above, whether the differences between our binding data and their data were due to the choice of radioligand, differences in experimental procedures, the effects of pseudoirreversible inhibition,[28] or to other factors is undetermined. However, we note further that even the μ binding data of Zhu and co-workers[12] does not correlate with the extraordinary potency of isomer **1a** as demonstrated by the MVD and the rhesus monkey data (cf. Figure 2 and Table 3).

Using the optical isomers of *cis*-3-methylfentanyl as reference compounds, we also examined the effects on the biological activities of introducing a phenylethyl 2-hydroxyl group into the molecule. Beginning with (3*R*,4*S*)-*cis*-3-methylfentanyl, the more potent of the two isomers,[16] the introduction of a 2-hydroxyl group with *S* configuration to obtain isomer **1a** enhanced the μ opioid agonist properties of the molecule as revealed not only by the mouse analgesic data (cf. Table 3) but also by the MVD and rhesus monkey data. It also produced the large decrease noted above in the binding affinity for the μ site labeled with [^{3}H]DAMGO (cf. Table 2). The introduction of a 2-hydroxyl group with *R* configuration (isomer **1b**) had essentially no effect on the binding affinity and smaller, but nevertheless significant, effects on the μ opioid agonist properties. Beginning with (3*S*,4*R*)-*cis*-3-methylfentanyl, the introduction of a 2-hydroxyl group with *R* configuration (isomer **1c**) seemed to have little effect on the μ opioid agonist properties but greatly increased the affinity for the μ binding site labeled with [^{3}H]DAMGO (cf. Tables 2 and 3). In contrast, the introduction of a 2-hydroxyl group with *S* configuration (isomer **1d**) had essentially no effect on the binding but produced a molecule which displayed weak μ agonist and weak μ antagonist properties.

From the above comparison, some conclusions concerning structure and opioid activity are possible. First, the same (3*R*,4*S*)-piperidine stereochemistry present in the more active isomer of *cis*-3-methylfentanyl is required for potent opioid agonist activity. Isomers **1a** and **1b**, which have this (3*R*,4*S*)-piperidine stereochemistry, are more potent compounds than isomers **1c** and **1d**, which have the (3*S*,4*R*) stereochemistry found in the less active isomer of *cis*-3-methylfentanyl. Second, the introduction of the 2-hydroxyl group with the *S* configuration has the most pronounced effect on these agonist properties. This is most clearly illustrated by the extraordinary agonist activity of (2*S*,3*R*,4*S*)-**1a**. However, the weak opioid antagonist properties of (2*S*,3*S*,4*R*)-**1d** also represent a significant change from the agonist properties of (3*S*,4*R*)-*cis*-3-methylfentanyl.[16] In addition, isomer **1a** is 21 000 times more potent than **1d** based on the mouse data (cf. Table 3). This difference is orders of magnitude greater than the 117-fold differ-

ence between the two *cis*-3-methylfentanyl isomers (cf. Table 3). Third, the introduction of the 2-hydroxyl group with the *R* configuration has a much smaller effect on the observed pharmacological properties of the molecule. The properties of (2*R*,3*S*,4*R*)-**1c** seem to be quite similar to those of (3*S*,4*R*)-*cis*-3-methylfentanyl while those of (2*R*,3*R*,4*S*)-**1b** appear to be somewhat enhanced over those of (3*R*,4*S*)-*cis*-3-methylfentanyl.

Earlier structure/activity studies have shown that the introduction of a phenylethyl 2-hydroxyl group into the structure of a potent fentanyl analog generally results in a decrease in potency.[31,32] However, isomer **1a** is an obvious exception to this trend, and to a lesser extent so are isomers **1b** and **1c**. The trend is observed only with isomer **1d**. Thus, the combination of the 2-hydroxyl group and the 3-methyl group is clearly important to the pharmacological properties of these four stereoisomers. At one extreme, isomer **1a**, this combination is highly favorable for causing a biological response; at the other, isomer **1d**, it is highly unfavorable. From this it is also evident that the effects of the stereochemistry of the 2-hydroxyl group on the conformation(s) allowed to the parent 3-methyl isomers are important. Moreover, it is likely that, in the interaction of these compounds with the receptor, the positions of the 2-hydroxyl and 3-methyl groups relative to each other are important. However, this latter hypothesis requires further testing.

During this study a new model of conformation–activity relationships for μ-selective opioids was published which was based on an investigation of several different classes of opiates.[33] Included among the compounds studied were fentanyl, carfentanil, and ohmefentanyl. The authors considered six important spatial positions of these (and other) molecules which were assumed to be directly implicated in their interaction with the μ opioid receptor. The authors found that the various compounds may bind to the μ receptor with their protonated nitrogen atom, assuming one or the other of two different orientations. In the case of ohmefentanyl, a possible role of the 3-methyl group was stabilization of the anilido group in the correct orientation for an interaction of the aromatic ring with a corresponding group on the receptor while the 2-hydroxyl group was thought to stabilize an interaction with a hydrophobic area of the receptor by either hydrogen bonding or proton donation. On the basis of their model, the authors predicted the order of potency for the four isomers of RTI-4616-4 to be **1b** > **1a** > **1c** > **1d**.[33] Our observed order of potency (MVD and in vivo data) was **1a** > **1b** > **1c** > **1d**. The source of the discrepancy may be the relationship between the 3-methyl group and the phenylethyl 2-hydroxyl group in the authors' postulated essential recognition area for ohmefentanyl.[33]

Experimental Section

Melting points (uncorrected) were determined on a Hoover capillary apparatus. ^{1}H NMR spectra were recorded using either a Bruker Am-250 MHz or a Bruker AMX-500 MHz spectrometer. All compounds gave ^{1}H NMR spectra consistent with their assigned structures. The ^{1}H NMR chemical shifts shown in Table 1 are given in δ values relative to tetramethylsilane.

Refractive indices were measured at the sodium D line on a Bausch & Lomb Abbe-3L refractometer. Optical rotations were determined at the sodium D line on a Rudolph Research Autopol III polarimeter. Analytical HPLC was performed utilizing two Waters model 510 pumps, a model 680 automated gradient controller, a model 481 Lamba-Max spectrophotometer, and a model 745 data module. Unless otherwise noted, anhydrous Na_2SO_4 was used to dry organic solutions. Short-path distillations were done using an Aldrich Kugelrohr apparatus. Elemental analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA, and by Galbraith Laboratories, Inc., Knoxville, TN.

(±)-1-Benzyl-3-methyl-4-piperidone. The three-step preparation of (±)-1-benzyl-3-methyl-4-piperidone from benzylamine was carried out as described in the literature[13] with three exceptions. In the Dieckmann condensation/decarboxylation step, 80% NaH was used as the base, toluene as the reaction solvent, and Et_2O as the extraction solvent. The title compound was isolated as a yellow oil which was sufficiently pure for use in the next reaction. Distillation of a typical crude sample afforded a clear, pale yellow oil (41% from benzylamine), bp 93–99 °C/0.05 mm (lit.[13] bp 110–115 °C/0.3 mm); η^{23}_D 1.5332 (lit.[13] η_o 1.5286, 26 °C).

(±)-*cis*-1-Benzyl-3-methyl-*N*-phenyl-4-piperidinamine and (±)-*trans*-1-Benzyl-3-methyl-*N*-phenyl-4-piperidinamine. The condensation of crude (±)-1-benzyl-3-methyl-4-piperidone with aniline followed by in situ $NaBH_4$ reduction of the intermediate imine was accomplished as described in the literature[14] with three exceptions. Toluene and EtOH were the reaction solvents, and the imine was not isolated. Chromatography on silica gel 60 with an EtOAc/hexane (20% → 40%) solvent gradient was used to separate the isomers and perform an initial cleanup. The recovered cis isomer was further purified by short-path distillation (typical boiling range, 150–165 °C/0.06 mm) followed by conversion to an oxalate salt (44%): mp 200–202 °C (lit.[14] mp 176.5–177.5 °C). Anal. ($C_{40}H_{50}N_4O_4$) C, H, N. The analysis result indicated a 2:1 salt rather than the 1:1 salt reported previously.[14]

The recovered trans isomer was similarly purified by short-path distillation (typical bp 150–160 °C/0.16 mm) followed by conversion to an oxalate salt (5%): mp 98–99 °C (lit.[14] mp 150–152 °C). Anal. ($C_{21}H_{26}N_2O_4$·0.75 EtOAc) C, H, N. The solvation by EtOAc of the 1:1 salt was also apparent from the ^{1}H NMR spectrum.

(±)-*cis*-3-Methyl-*N*-phenyl-4-piperidinamine [(3*RS*,4*SR*)-2]. A solution of (±)-*cis*-1-benzyl-3-methyl-*N*-phenyl-4-piperidinamine in MeOH was adjusted to pH 4 by addition of dioxane saturated with HCl gas, and then $Pd(OH)_2$/C was added. The resultant mixture was shaken 5 d at 40 psi of H_2 on a Parr apparatus and then filtered. The filtrate was evaporated, and the residue was dissolved in H_2O. The solution was adjusted to pH 10 using concentrated NH_4OH and extracted with several portions of EtOAc. The combined organic extracts were dried and evaporated to provide **2** (105%) as an amber oil. Material from several runs was combined and vacuum-distilled (short-path) to obtain pure **2** as a clear oil, typical boiling range 108–130 °C/0.15 mm.

Resolution of (3*RS*,4*SR*)-2. The tartaric acid resolution of (3*RS*,4*SR*)-**2** was accomplished using reported procedures.[15,16] Each tartrate salt was recrystallized three times, affording white crystals. The (3*R*,4*S*)-(+)-**2** (−)-tartrate had mp 165–166 °C and $[\alpha]^{25}_D$ +21.8° (*c* 0.76, MeOH) (lit.[16] $[\alpha]^{25}_D$ +20.3°), and the (3*S*,4*R*)-(−)-**2** (+)-tartrate had mp 165–166 °C and $[\alpha]^{25}_D$ −21.3° (*c* 0.95, MeOH) (lit.[16] $[\alpha]^{25}_D$ −19.7°). A determination of enantiomeric purity was accomplished by HPLC analysis of the diastereoisomeric 2-benzyl-2-methyl carbamates using a reported method.[15] The analysis results showed that the enantiomeric purities of (3*R*,4*S*)-(+)-**2** and (3*S*,4*R*)-(−)-**2** were 98.5% and 99.1%, respectively. The resolved isomers were stored as their tartrate salts until needed. An earlier X-ray crystallographic analysis had established the absolute configuration of (3*S*,4*R*)-(−)-**2**.[15]

(3*R*,4*S*)-*cis*-(−)-*N*-[3-Methyl-1-(2-oxo-2-phenylethyl)-4-piperidyl]-*N*-phenylpropanamide [(3*R*,4*S*)-(−)-3] Hydrochloride. To a solution of (3*R*,4*S*)-(+)-**2** (6.1 g, 0.032 mol) in

absolute EtOH (200 mL) were added K_2CO_3 (20 g) and several crystals of KI. After stirring for 5 min, 2-bromoacetophenone (6.4 g, 0.32 mol) was added. The resultant mixture was stirred 1 h at room temperature. The EtOH was removed and the residue partitioned between toluene (300 mL) and 5% $NaHCO_3$ (300 mL). The organic layer was then extracted with 10% citric acid (4 × 150 mL). The combined acidic extracts were adjusted to pH 10 with concentrated NH_4OH and extracted with $CHCl_3$ (4 × 125 mL). The combined $CHCl_3$ extracts were dried and evaporated to obtain (3*R*,4*S*)-*cis*-(+)-3-methyl-1-(2-oxo-2-phenylethyl)-*N*-phenyl-4-piperidinamine as an amber oil (9.9 g, 100%). This was used without further purification. In a separate experiment the alkylation product was characterized as the HCl salt: mp 227–229 °C; $[\alpha]^{23}_D$ +65.9° (*c* 1.05, MeOH). Anal. ($C_{20}H_{25}ClN_2O$) C, H, Cl, N.

A solution of propionyl chloride (5.1 g, 0.055 mol) in CH_2Cl_2 (50 mL) was added dropwise to an ice-cold solution of (3*R*,4*S*)-*cis*-3-methyl-1-(2-oxo-2-phenylethyl)-*N*-phenyl-4-piperidinamine (9.9 g, 0.032 mol) and Et_3N (6.0 g, 0.059 mol) in CH_2Cl_2 (200 mL). Following the addition, the ice bath was removed and the resultant mixture stirred overnight. Afterward, it was washed with saturated $NaHCO_3$ (4 × 100 mL), dried, and concentrated. The residue was dissolved in MeOH and treated with dioxane saturated with HCl gas. Subsequent solvent evaporation gave 12.5 g [97% from (+)-**2**] of crude (3*R*,4*S*)-(−)-**3**·HCl as an off-white solid. Recrystallization from EtOAc/MeOH returned white crystals (9.8 g, 75%): mp 208–210 °C; $[\alpha]^{25}_D$ −4.5° (*c* 0.64, MeOH). Anal. ($C_{23}H_{29}ClN_2O_2$) C, H, Cl, N.

(3*S*,4*R*)-*cis*-(+)-*N*-[3-Methyl-1-(2-oxo-2-phenylethyl)-4-piperidyl]-*N*-phenylpropanamide [(3*S*,4*R*)-(+)-3] Hydrochloride. In a manner analogous to the synthesis of (3*R*,4*S*)-(−)-**3**·HCl, (3*S*,4*R*)-(+)-**3**·HCl was prepared from (3*S*,4*R*)-(−)-**2** in 97% crude yield. Recrystallization from EtOAc/MeOH yielded white crystals: mp 210–212 °C; $[\alpha]^{25}_D$ +4.7° (*c* 1.10, MeOH). Anal. ($C_{23}H_{29}ClN_2O_2$) C, H, Cl, N. The intermediate (3*S*,4*R*)-*cis*-(−)-3-methyl-1-(2-oxo-2-phenylethyl)-*N*-phenyl-4-piperidinamine was also characterized as the HCl salt: mp 227.5–229 °C; $[\alpha]^{23}_D$ −66.5° (*c* 1.05, MeOH). Anal. ($C_{20}H_{25}ClN_2O$) C, H, Cl, N.

(2*S*,3*R*,4*S*)-*cis*-(+)-*N*-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide [(2*S*,3*R*,4*S*)-1a] Hydrochloride and (2*R*,3*R*,4*S*)-*cis*-(−)-*N*-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide [(2*R*,3*R*,4*S*)-1b] Oxalate. Solid $NaBH_4$ (0.91 g, 0.024 mol) was added in portions to a solution of (3*R*,4*S*)-(−)-**3**·HCl (9.8 g, 0.024 mol) in MeOH (200 mL). The resultant mixture was refluxed 2 h and then cooled. The MeOH was evaporated and the residue partitioned between EtOAc (250 mL) and 10% $NaHCO_3$ (250 mL). The EtOAc layer was dried and evaporated to obtain a mixture of 1a and 1**b** free bases (9.6 g, 97%). Fractional crystallization from diisopropyl ether afforded a less soluble base and a more soluble base. The less soluble base crystallized pure from the mixture while the more soluble base required three additional crystallizations before isomeric purity was achieved. The mother liquors from the fractional crystallization were evaporated to recover the balance of the reduction product as a mixture of isomers.

The less soluble isomer [(2*S*,3*R*,4*S*)-1**a**] formed fine white crystals (2.07 g): data in Table 1 (lit.[12] mp 117–119 °C; $[\alpha]_D$ +19.79°, *c* 6.0). Conversion of the base to the HCl salt followed by recrystallization from EtOAc/MeOH gave (2*S*,3*R*,4*S*)-1**a**·HCl as fine white needles, mp 198–200 °C. Anal. ($C_{23}H_{31}ClN_2O_2$) C, H, Cl, N.

The more soluble isomer [(2*R*,3*R*,4*S*)-1**b**] formed compact white crystals (1.75 g): data in Table 1 (lit.[12] mp 135–137 °C; $[\alpha]_D$ −31.91°, *c* 4.7). Conversion of the base to the oxalate salt followed by recrystallization from 2-PrOH/diisopropyl ether afforded (2*R*,3*R*,4*S*)-1**b** oxalate as a fine white powder, mp 128–130 °C. Anal. ($C_{25}H_{32}N_2O_6 \cdot 0.25H_2O$) C, H, N.

(2*R*,3*S*,4*R*)-*cis*-(−)-*N*-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide [(2*R*,3*S*,4*R*)-1c] Hydrochloride and (2*S*,3*S*,4*R*)-*cis*-(+)-*N*-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide [(2*S*,3*S*,4*R*)-1d] Oxalate. Starting with (3*S*,4*R*)-(+)-**3**·HCl and following the $NaBH_4$ reduction procedure described above afforded a mixture of 1**c** and 1**d** free bases (97%). Fractional crystallization as before provided a less soluble base and a more soluble base. The less soluble base [(2*R*,3*S*,4*R*)-1**c**] again crystallized as fine white crystals: data in Table 1 (lit.[12] mp 117–119 °C; $[\alpha]_D$ −20.54°, *c* 3.1). Conversion of the base to the HCl salt followed by recrystallization from EtOAc/MeOH gave (2*R*,3*S*,4*R*)-1**c**·HCl as fine white needles, mp 199–201 °C. Anal. ($C_{23}H_{31}ClN_2O_2 \cdot 0.25H_2O$) C, H, Cl, N.

The more soluble isomer was again obtained as compact white crystals: data in Table 1 (lit.[12] mp 135–137 °C; $[\alpha]_D$ +33.15°, *c* 3.6). A sample of this base was submitted for X-ray crystallographic analysis. Conversion of the base to the oxalate salt followed by recrystallization from 2-PrOH/diisopropyl ether afforded (2*S*,3*S*,4*R*)-1**d** oxalate as a fine white powder, mp 128–130 °C. Anal. ($C_{25}H_{32}N_2O_6 \cdot 0.25H_2O$) C, H, N.

X-ray Analysis. Crystal data: orthorhombic; a = 7.607(2) Å, b = 11.744(5) Å, c = 23.230(11) Å; V = 2075(1) Å^3; $\alpha = \beta = \gamma = 90°$; D_{calc} = 1.17 g cm^{-1}; Z = 4, space group $P2_12_12_1$. Colorless crystals. Dimension of crystal used for data collection: 0.65 × 0.43 × 0.40 mm; λ(Mo Kα) = 0.710 69 Å.

Data Collection. The cell constants were obtained by refining the setting angles of 25 reflections with 2θ values between 20 and 35°. The intensity data were collected in two batches by the ω-scan method at variable scan speeds between 3.9 and 29.3 deg min^{-1}, depending on intensity. The first batch was up to 35° in 2θ; all reflections of the type hkl and $hk\bar{l}$ were collected (h, 0–6; k, 0–9; l, −19 to 19). The second batch was from 35° ≤ 2θ ≤ 62°, and intensities of type hkl only were collected (h, 6–11; k, 9–17; l, 19–33). Stationary backgrounds were measured on both sides of a peak each for one-half of the scan time. A total of 4240 reflections were measured in the two batches, which were merged, and the usual corrections were applied (no absorption correction was necessary, μ being 0.70 cm^{-1}), yielding 2560 unique observed reflections, R_{int} = 0.035. A Siemans/Nicolet P3/F automatic diffractometer was used for all measurements.

Structure Solution and Refinement. The structure was solved by direct methods and difference Fourier techniques and refined by the blocked-cascade least squares refinement technique[34] utilizing 2267 data with intensities $I \geq 2.0\sigma(I)$. The function minimized was $\sum w(|F_o| - |F_c|)^2$, where $w = 1/(\sigma^2F + 0.0002F^2)$. The hydrogens attached to the phenyl rings were placed in calculated positions and refined in the riding mode; all others were located from a difference Fourier map and were refined with individual isotropic displacement parameters, except for the OH hydrogen. The latter hydrogen could not be refined properly and was, therefore, fixed with an isotropic displacement parameter, U, of 0.10 Å^2. A secondary extinction correction was applied to the data near the end of the refinement, the extinction coefficient being 7.3(6) × 10^{-6}. The final discrepancy indices were R = 0.0533 and R_w = 0.0467. The final difference Fourier was featureless with maximum and minimum electron densities of +0.34 and −0.22 e Å^{-3}, respectively. The scattering factors were taken from the *International Tables for X-ray Crystallography*.[35] All computations were performed using the program package SHELXTL[36] on a Data General microeclipse computer. The atomic coordinates and isotropic thermal parameters are given in Table 5.

In Vitro Ligand Binding Assays. Assays for μ, δ, and κ binding sites followed published procedures. The μ binding sites were labeled using [^{3}H][D-Ala^2-MePhe^4-Gly-ol^5]enkephalin ([^{3}H]DAMGO) and rat-lysed P2 membranes as previously described.[19] Briefly, incubations proceeded for 4–6 h at 25 °C in 50 mM Tris-HCl, pH 7.4, containing a protease inhibitor cocktail (PIC = bacitracin [100 μg/mL], bestatin [10 μg/mL], leupeptin [4 μg/mL], and chymostatin [2 μg/mL]). The nonspecific binding was determined using 20 μM levallorphan. The δ binding sites were labeled using [^{3}H][D-Ala^2-D-Leu^5]enkephalin ([^{3}H]DADLE) and rat-lysed P2 membranes as previously described.[18] Briefly, incubations proceeded for 4–6 h at 25

Table 5. Atomic Coordinates ($\times 10^4$) and Isotropic Thermal Parameters ($Å^2 \times 10^3$)

	x	y	z	U[a]
C(1)	11650(4)	11204(2)	−275(1)	48(1)
C(2)	10771(5)	11901(3)	−657(2)	67(1)
C(3)	11631(7)	12379(3)	−1119(2)	86(2)
C(4)	13347(7)	12192(4)	−1202(2)	88(2)
C(5)	14243(5)	11516(4)	−831(2)	82(2)
C(6)	13393(5)	11014(3)	−365(2)	70(1)
C(7)	10670(5)	10655(3)	217(1)	67(1)
O(1)	11737(5)	10486(3)	689(1)	142(2)
C(8)	9812(4)	9547(3)	42(1)	51(1)
N(1)	8892(3)	9033(2)	526(1)	44(1)
C(9)	7149(4)	9515(2)	603(1)	48(1)
C(10)	6290(4)	9093(2)	1153(1)	46(1)
C(11)	6124(4)	7806(3)	1105(1)	40(1)
C(12)	7920(4)	7273(2)	1001(1)	43(1)
C(13)	8773(4)	7796(3)	475(1)	46(1)
C(14)	7245(5)	9507(3)	1688(1)	65(1)
N(2)	5145(3)	7262(2)	1590(1)	43(1)
C(15)	6033(4)	6600(3)	2024(1)	43(1)
C(16)	6422(4)	5470(3)	1927(1)	52(1)
C(17)	7218(4)	4828(3)	2343(1)	68(1)
C(18)	7634(4)	5291(4)	2861(2)	71(2)
C(19)	7271(4)	6416(3)	2968(1)	68(1)
C(20)	6465(4)	7079(3)	2549(1)	56(1)
C(21)	3343(4)	7334(3)	1571(1)	50(1)
O(2)	2594(3)	7864(2)	1194(1)	69(1)
C(22)	2300(4)	6665(3)	2011(1)	64(1)
C(23)	1720(5)	5531(4)	1761(2)	87(2)

[a] Equivalent isotropic U defined as one third of the trace of the orthogonalized $\mathbf{U}_{ij}$ tensor.

°C in 50 mM Tris-HCl, pH 7.4, containing 3 mM $MnCl_2$, 100 mM choline chloride, and a protease inhibitor cocktail. The nonspecific binding was determined using 20 μM levallorphan. To block binding to the μ binding site, 100 nM of the highly μ-selective peptide LY164929 was included.[18] The κ binding sites were labeled using [^{3}H]-(5α,7α,8β)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]benzeneacetamide ([^{3}H]-U69,593) and guinea pig brain membranes depleted of μ and δ binding sites by pretreatment with the irreversible ligands 2-(*p*-ethoxybenzyl)-1-[(diethylamino)ethyl]-5-isothiocyanatobenzimidazole hydrochloride (BIT) and *N*-[1-(2-(4-isothiocyanato)phenylethyl)-4-piperidyl]-*N*-phenylpropanamide hydrochloride (FIT) as previously described,[20] except that the incubation temperature was 25 °C. Briefly, incubations proceeded for 4−6 h at 25 °C in 50 mM Tris-HCl, pH 7.4, containing a protease inhibitor cocktail and 1 μg/mL captopril. Nonspecific binding was determined using 1 μM U69,593. Each ^{3}H-labeled ligand was displaced by nine concentrations of test drug, two or three times, each time utilizing a different dilution of test drug. The data were pooled, and the IC_{50} values and slope factors were obtained using the program ALLFIT.[37] The K_i values were calculated utilizing standard equations.

Details on the binding assay involving the displacement of [^{3}H]etorphine in rat brain cerebral membranes have been described previously.[23] Briefly, aliquots of a membrane preparation suspended in 50 nM-Tris HCl, pH 7.4, were incubated 40 min at 25 °C with [^{3}H]etorphine in the presence of 150 nM NaCl and in the presence of different concentrations of test compound. The specific, or opioid receptor related, interaction of [^{3}H]etorphine was determined as the difference in binding obtained in the absence or presence of an appropriate excess of unlabeled etorphine. The potency of the test compound (EC_{50} value) in displacing the specific binding of [^{3}H]etorphine was determined from log-probit plots of the data. (The EC_{50} value is the concentration of test compound which caused 50% inhibition of specific binding of [^{3}H]etorphine.)

Mouse Vas Deferens, Rhesus Monkey Substitution for Morphine (SDS) and Mouse Antinociception Assays. These assays were performed utilizing previously described procedures.[22,26,38] Isomers **1a** and **1c** were tested as hydrochloride salts; **1b** and **1d** as oxalate salts. Due to the complex nature of the data obtained in the mouse vas deferens preparation for isomers **1a**, **1b**, and **1c**, the calculation of EC_{50} values was difficult. Consequently, the findings are presented graphically.

Acknowledgment. The synthesis and in vitro ligand binding (μ, δ, κ) studies were supported by Grant DA06317 from the National Institute on Drug Abuse (NIDA). The authors thank Mr. D. K. Sawyer (synthesis), Mr. K. G. Boldt (synthesis), and Mr. K. D. Gaetano (NMR analyses) for their technical assistance. The X-ray diffractometer at North Carolina State University was upgraded with the help of Grant CHE 8307022 from the National Science Foundation. The [^{3}H]etorphine binding, the mouse vas deferens, mouse antinociception, and rhesus monkey substitution for morphine (SDS) studies, performed under the auspices of The College on Problems of Drug Dependence, Inc. (CPDD), were supported by NIDA and CPDD. The authors thank Dr. A. E. Jacobson for his assistance in coordinating these studies.

Supplementary Material Available: Tables of bond lengths, bond angles, anisotropic thermal parameters, H-atom coordinates, and their isotropic thermal parameters (4 pages). Ordering information is given on any current masthead page.

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JM9408373