# Probes for Narcotic Receptor Mediated Phenomena. 19.[1] Synthesis of (+)-4-[(αR)-α-((2S,5R)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide (SNC 80): A Highly Selective, Nonpeptide δ Opioid Receptor Agonist

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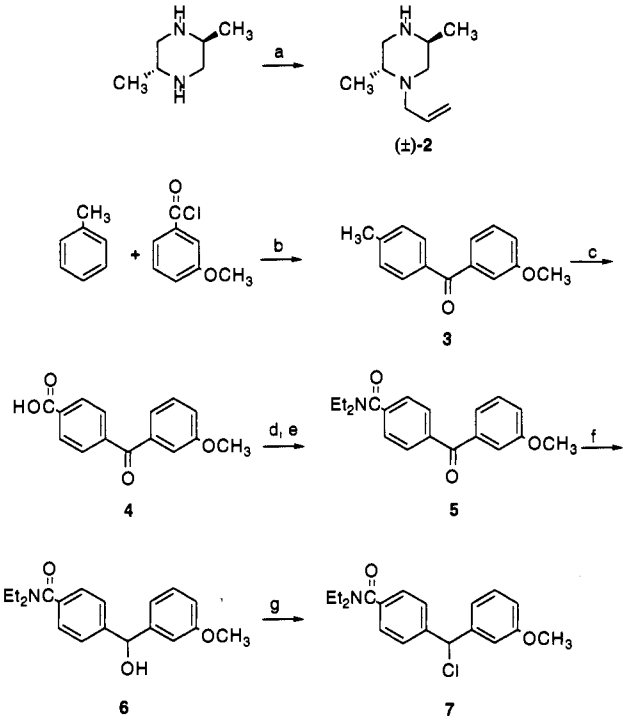
Extensive research during the last two decades has provided unequivocal evidence for the existence of μ, δ, and κ opioid receptor types and substantial evidence for the existence of subtypes of each.[2-9] These advances were largely dependent on the development of highly selective receptor ligands which continue to play a centrally important role in receptor subtype identification and in the elucidation of their function. The latter includes recent recognition that opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway and control reinforcing behavior by mediation of dopamine release in reward circuits of the brain and that the "opiatergic tone of the CNS" can be elevated or depleted by exogenous opioids.[10,11] Recent studies have shown human cocaine addicts have depleted enkephalin mRNA and μ opioid receptors associated with euphoria and elevated dynorphin mRNA and κ opioid binding associated with dysphoria;[12] however, chronic cocaine administration in the rat elevates μ opioid receptor density,[13] suggesting species-dependent chemical alteration of the opiatergic tone of the CNS by cocaine. Remarkably, other studies have shown that δ receptor antagonists prevent cocaine seeking behavior,[14] cocaine facilitation of reward,[15] and development of morphine tolerance and dependence.[16] In addition, the δ receptor agonist DPLPE produces cocaine-appropriate responding for reward which is not observed with the μ agonist DAMGO,[17] and other studies have shown that δ receptor agonists can modulate μ receptor mediated antinociception.[18] New, highly potent and selective nonpeptide δ opioid receptor agonists, antagonists, affinity labels, and imaging agents for positron emission tomography (PET) and single photon emission computed tomography (SPECT) studies are now required to optimally advance the understanding of these effects as well as the development of new medications which act on these sites. PET and SPECT imaging agents offer opportunities for study of the conscious human CNS in normal, abnormal, and drug-altered states and may permit the development of clinical correlates of opioid receptor dysfunction with disease states.[1] Such drugs thus hold potential as new agents for diagnosis of CNS disorders and for monitoring drug therapy involving changes in the opioid receptor–endorphin system. In such studies, nonpeptide ligands are advantageous over peptides in that they are generally less subject to metabolism and also can penetrate the blood–brain barrier and therefore can be administrated peripherally *in vivo*.[19]

Recently, a novel nonpeptide δ opioid receptor racemic agonist, BW373U86 [(±)-1], was reported[20] and appears to be a prime template for the discovery of new probes for the δ receptor system. Studies with this compound *in vitro*,[20-22] *in vivo*,[20,22-27] and in *ex vivo* functional assays[20,22] collectively indicate it is a δ-selective agonist which exerts some of its effects through μ opioid receptors. Studies with the optically pure enantiomers of (±)-1 and related compounds are now required to best utilize this important lead since it is well established that drug enantiomers can show distinctly different and in some cases opposite pharmacological effects.[28]

We now report the synthesis and absolute configuration of the optically pure enantiomers of phenolic 1, its benzylic epimer 10, and their methyl ethers 8 and 9, respectively. Evaluation of these compounds have shown that one compound in this series, the nonphenolic (+)-8 (SNC 80), exhibits the remarkable μ/δ selectivities in both receptor binding and bioassays of approximately 2000-fold.

Our synthetic analysis involved assembly of these molecules from two components: (a) the appropriate

**Scheme 1**[a]




[a] (a) Allyl bromide (50%);[29] (b) $AlCl_3$ (84%); (c) $KMnO_4$ (53%); (d) $SOCl_2$; (e) 44% $Et_2NH$ (90%); (f) $NaBH_4$ (70%); (g) 36% HCl (90%).

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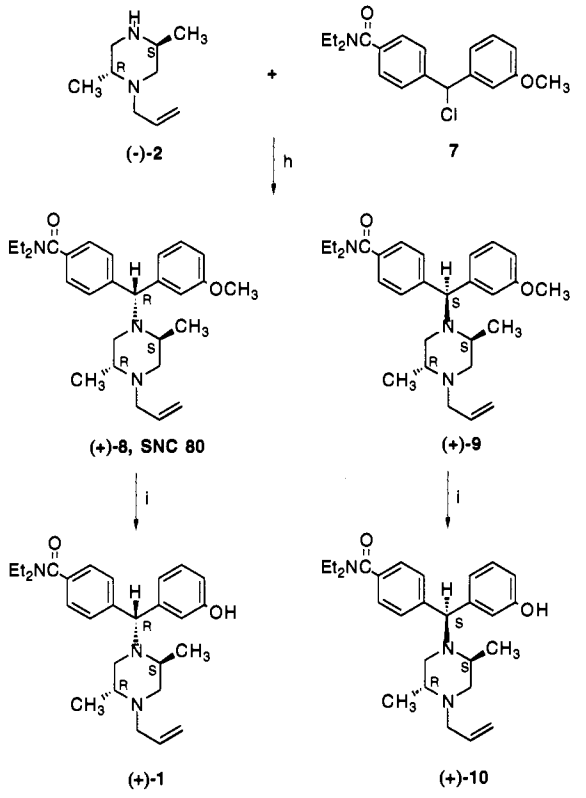
**Scheme 2**[a]




[a] (h) $K_2CO_3$/acetonitrile (65%); (i) $BBr_3$ (45%).

**Table 1.** Inhibition of Radioligand Binding to Rat Brain $\mu$ Receptors and Mouse Brain $\delta$ Receptors by BW373U86, Its Enantiomers 1, Benzylic Epimers 10, and Their Methyl Ethers 8 and 9

| compound (configuration) | $IC_{50}$, nM ± SD | | $IC_{50}$ ratio: $\mu/\delta$ |
|---|---|---|---|
| | $\mu$ binding[a] | $\delta$ binding[b] $IC_{50}$, nM ± SD | |
| (±)-1 (BW373U86) | 46.3 ± 4.42 | 0.92 ± 0.06 | 50.3 |
| (-)-1 ($\alpha S$, 2R, 5S) | 2322 ± 199 | 9.58 ± 0.85 | 242 |
| (+)-1 ($\alpha R$, 2S, 5R) | 9.71 ± 0.37 | 0.31 ± 0.02 | 31 |
| (-)-8 ($\alpha S$,2R,5S) | 9366 ± 798 | >2000 | nd |
| (+)-8 ($\alpha R$,2S,5R) (SNC 80) | 2467 ± 200 | 1.06 ± 0.14 | 2327 |
| (-)-9 ($\alpha R$,2R,5S) | 9138 ± 823 | 3.50 ± 0.39 | 2611 |
| (+)-9 ($\alpha S$,2S,5R) | 5712 ± 457 | 56.5 ± 3.10 | 101 |
| (-)-10 ($\alpha R$,2R,5S) | 167 ± 42 | 0.49 ± 0.07 | 341 |
| (+)-10 ($\alpha S$,2S,5R) | 426 ± 53 | 5.96 ± 0.53 | 71 |

[a] Binding against [$^3$H]DAMGO in rat brain membranes.[32] [b] Binding against [$^3$H]DADLE[33] in mouse brain membranes depleted of functional $\mu$ receptors by pretreatment with BIT.[34]

enantiomer of 1-allyl-*trans*-2,5-dimethyl-1,4-piperazine, 2, and (b) the benzhydryl chloride 7 as shown in Schemes 1 and 2. This route offers the advantage that it requires only one optical resolution, that of the 1-allyl-*trans*-2,5-dimethyl-1,4-piperazine (±)-2,[29] to obtain the enantiomers of 1 and its benzylic epimer 10. Optical resolution of (±)-2[30] with (+)- and (-)-camphoric acids provided the enantiomers of 2. Optical purity of (+)- and (-)-2 was determined to be >98% by NMR of the ureas formed with optically pure $\alpha$-methylbenzyl isocyanate[31] and was determined to be >99% by HPLC of the ureas formed with 1-naphthylisocyanate on a Chiralcel OD chiral column. The optically pure (+)-enantiomer of 1, its benzylic epimer (+)-10, and their methyl ethers (+)-8 and (+)-9 were prepared as shown in Scheme 2. Repetition of Scheme 2 with piperazine (+)-2 provided the corresponding (-)-enantiomers of these compounds. The absolute configuration of (+)-2 was determined as 2S,5R by single-crystal X-ray analysis of the salt with (+)-camphoric acid. This result and the X-ray determination of relative configuration of (-)-8 allowed the assignment of the absolute configuration of the compounds shown in Scheme 2 and their enantiomers.

The affinities of the enantiomers and immediate synthetic precursors of 1 for $\mu$ opioid receptors and $\delta$ receptors (Table 1) were determined by inhibition of binding of [$^3$H]DAMGO to rat brain membranes[32] and [$^3$H]DADLE[33] to mouse brain membranes depleted of $\mu$ binding sites by the pretreatment with the irreversible ligand BIT,[34] respectively. Nonspecific binding was determined using 20 $\mu$M levallorphan.

The enantiomers of 1 and their benzylic epimers 10 show high affinity for $\delta$ receptors with less affinity for $\mu$ receptors as shown in Table 1. The most potent compounds at $\delta$ receptors in the binding assays were (-)-10 and (+)-1 which showed subnanomolar affinity, but unfortunately these compounds also showed significant $\mu$ receptor binding which limited their selectivity. In contrast, striking results were obtained with the corresponding methyl ethers of these compounds in which $\mu$ binding was virtually eliminated with little effect of $\delta$ binding. The resulting (+)-8 and (-)-9 showed $\mu/\delta$ selectivity ratios in binding ranging of 2327- and 2611-fold, respectively. These compounds are thus the most $\delta$ selective (vs $\mu$) nonpeptide agonists reported and rival some of the most selective peptide ligands. It should be pointed out that in each of the optical pairs 1, 8, 9, and 10, the enantiomer with the $\alpha R$ absolute configuration is the most potent and $\delta$ receptor selective in the binding assays. The two compounds with the highest binding selectivity, (+)-8 and (-)-9, are thus of the *R* benzylic configuration but have the opposite piperazine configuration, suggesting that the absolute configuration of the benzylic position is the most important stereochemical determinant of $\delta$ receptor binding selectivity. The opioid activity (Table 2) of these two compounds was next evaluated in the isolated mouse vas deferens (MVD) and in guinea pig ileum (GPI) bioassays.[35] These studies revealed that the $\delta$ selectivity of 1996 fold found

**Table 2.** Agonist Activity of Selected Compounds in the Mouse Vas Deferens (MVD) and Guinea Pig Ileum (GPI) Bioassays and Antagonism of (+)-8 (SNC 80) by ICI174.864 ($\delta$ Antagonist, 1 $\mu$M) and CTAP ($\mu$ Antagonist, 1 $\mu$M)

| compound | $IC_{50}$ ± SEM, nM | | $IC_{50}$ ratio: GPI ($\mu$)/MVD ($\delta$) |
|---|---|---|---|
| | GPI ($\mu$ receptors) | MVD ($\delta$ receptors) | |
| (±)-1 (BW373U86)[a] | 143 ± 16 | 0.2 ± 0.02 | 715 |
| (+)-8 (SNC80) | 5457 ± 2052 | 2.73 ± 0.50 | 1996 |
| (-)-9 | 1517 ± 214 | 30.9 ± 4.0 | 49 |
| DPDPE | 7300 ± 1700 | 5.1 ± 0.5 | 1800 |
| [D-Ala$^2$,Glu$^4$]deltorphin | 15000 ± 1000 | 0.85 ± 0.07 | 17,000 |
| (+)-8 + ICI174864 (SR)[b] | - | 3250 ± 1830 (1190) | - |
| (+)-8 + CTAP (SR) | - | 5.34 ± 1.6 (1.9) | - |

[a] Data from ref 22. [b] Shift ratio, $IC_{50}$ in the presence of the antagonist/$IC_{50}$ in the absence of the antagonist.

for (+)-8 paralleled that observed in the binding assays and exceeded that of (±)-1, but this was not the case for (−)-9, which only showed 49-fold selectivity. In this case, the αR configuration in (−)-9 does not confer the same level of selectivity in the bioassays as it did in the binding assays. Further evidence for activity of (+)-8 at δ opioid receptors was obtained from $IC_{50}$ values determined in the presence of 1 μM of the δ antagonist ICI 174,864[36] and separately in the presence of 1 μM of the μ antagonist CTAP.[37,38] In these assays, ICI174,864 produced an $IC_{50}$ shift of 1190-fold while CTAP only shifted the $IC_{50}$ 1.9-fold, indicating that the δ antagonist was 626 times more effective in shifting the $IC_{50}$ of (+)-8. These results collectively suggest that (+)-8 is a highly selective and potent nonpeptide δ agonist, which will be of substantial value in further elucidation of δ receptor function. *In vivo* studies with (+)-8 are in progress and will be reported in due course.

In conclusion, a practical synthesis and initial biological characterization of optically pure isomers of (±)-1 and related compounds are described. From this series of compounds, two of the methyl ethers demonstrate high affinity and selectivity toward δ receptors in binding assays. These data together with our results in the MVD and in GPI indicate that (+)-8 is a highly selective and potent nonpeptide δ agonist with about 2000-fold δ/μ selectivity in both the binding and bioassays. Since the cDNA of μ, δ, and κ opioid receptors have now been cloned,[39-45] and their amino acid sequences expressed, (+)-8 and related compounds can be utilized to compliment and extend initial studies on a molecular basis[46] of the δ receptor interaction with (±)-1. These and subsequent investigations will provide valuable insight into the role of opioid receptor subtypes in drug-seeking behavior, antinociception, and the development of tolerance and dependence as well as other aspects of addictive diseases. Additional structure–activity relationship studies with (+)-8 as a template are in progress to develop highly selective affinity labels, imaging agents, and other research tools.

**Supplementary Material Available:** X-ray diffraction data for (+)-2·(+)-camphorate and (−)-8 including ORTEP drawings, crystal coordinates, bond distances and bond angles (12 pages). Ordering information is given on any current masthead page.

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