Opioid ligands with mixed properties from substituted enantiomeric *N*-phenethyl-5-phenylmorphans. Synthesis of a μ-agonist δ-antagonist and δ-inverse agonists†‡

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Enantiomeric *N*-phenethyl-*m*-hydroxyphenylmorphans with various substituents in the *ortho*, *meta* or *para* positions of the aromatic ring in the phenethylamine side-chain (chloro, hydroxy, methoxy, nitro, methyl), as well as a pyridylethyl and a indolylethyl moiety on the nitrogen atom, were synthesized and their binding affinity to the μ-, δ-, and κ-opioid receptors was examined. The higher affinity ligands were further examined in the [^{35}S]GTPγS assay to study their function and efficacy. 3-((1*R*,5*S*)-(−)-2-(4-Nitrophenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-**10m**) was found to be a μ-agonist and δ-antagonist in that functional assay and was about 50 fold more potent than morphine *in vivo*. 3-((1*R*,5*S*)-(−)-2-(4-Chlorophenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-**10i**) and several other ligands displayed inverse agonist activity at the δ-opioid receptor. The absolute configuration of all of the reported compounds was established by chemical conversion of (−)-**6** to 1*R*,5*S*-(−)-**8b**·HBr.

Introduction

The synthesis of compounds that act stereoselectively at each of the opioid receptors and as potent, pure opioid agonists or antagonists, has been a long-sought goal, and several non-peptidic ligands with those properties have been found.[2–10] More recently, it has been suggested that ligands that display μ-agonist and δ-antagonist activity may be useful as pharmacological tools or medications. Theoretically, a μ-agonist should induce analgesia and the δ-antagonist activity of the drug could reduce or eliminate the tolerance and dependence caused by the μ-agonist.[11,12] The dual effect could conceivably occur through a ligand that is fortuitously able to fit to a binding site in both receptors, or perhaps to a receptor that, when activated by a suitable ligand, can effect the signaling properties of a related receptor.[13,14] Interestingly, it has been found that μ- and δ-receptors can exist as heterodimers.[13–15] Further, Emmerson *et al.*,[16] have noted that compounds with a δ-receptor inverse agonist profile may be useful as anorectants. Thus, the interest in new ligands that interact with opioid receptors has not abated, although the goals of the search have evolved.

In an attempt at dissociating the undesirable effects of morphine from its analgesic action in the 1950's, May and his collaborators examined the effect of simplification of the structure of the rigid 5-ring morphine-like compounds (4,5-epoxymorphinans).[17] One of the first of the molecularly less complex 5-phenylmorphan compounds that they synthesized and examined,[18] [(±)-5-*m*-hydroxyphenyl-*N*-methylmorphan, ((±)-3-(2-methyl-2-aza-bicyclo[3.3.1]nonan-5-yl)-phenol, (±)-**1**] was found to be as potent as the well-known analgesics in the multicyclic rigid epoxymorphinan (*e.g.*, morphine), morphinan (*e.g.*, levorphanol), and 6,7-benzomorphan (*e.g.*, metazocine) classes of antinociceptives. When the enantiomers of (±)-**1** (Fig. 1) were examined by May *et al.*,[19,20] they found that the (+)-enantiomer showed potent antinociceptive activity in mice (*ca.* 3 or 4 times more potent than morphine) and the (−)-enantiomer showed both antinociceptive (morphine-like) and narcotic antagonist activity (80 fold less potent than naloxone as an antagonist in morphine-dependent Rhesus monkeys).

This is in sharp contrast to the epoxymorphinan opioids where only the enantiomer with morphine's absolute configuration, that is, the natural product or a compound derived from it, interacts

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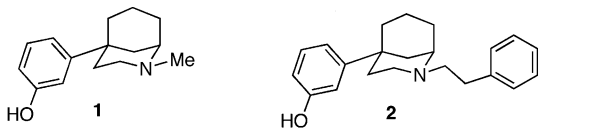


(-)-**1**: antinociceptive and narcotic antagonist
(+)-**1**: potent antinociceptive
(+)-**2**: μ and κ-opioid antagonist
(-)-**2**: μ, κ, and δ-opioid antagonist

Fig. 1 *N*-Methyl- and *N*-phenethyl-phenylmorphans.

well with opioid receptors and has significant analgesic activity. Single-crystal X-ray analysis[21] subsequently determined that the (−)-enantiomer ((−)-5-*m*-hydroxyphenyl-2-methylmorphan hydrobromide) had the 1*R*,5*S* configuration. Prior to the work of May *et al.*, opioid antagonist activity had not been found in any enantiomer from any opioid with a so-called "agonist" side-chain on the nitrogen atom (*e.g.*, *N*-methyl, *N*-phenethyl, *N*-pentyl), nor have they often been found since that time among any of the derivatives from the molecularly rigid series of opioids (*e.g.*, in the epoxymorphinans, morphinans, or benzomorphans). This novel and unexpected effect in the 5-phenymorphans has been ascribed to the lack of rigidity of the aromatic ring, as well as to its equatorially-oriented link to the piperidine ring rather than the more common axial arrangement found in the rigid multicyclic analgesics.[20]

In that context, it was interesting to note that racemic *N*-phenethyl-9β-methyl-5-(3-hydroxyphenyl)morphan (3-(2-phenethyl-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol) was reported by Thomas *et al.*,[22] to have high affinity for the μ opioid receptor and to have only opioid antagonist activity in the [^{35}S]GTP-γ-S assay. The conversion to a pure μ-opioid antagonist was said to be due to the circumscribed rotation of the aromatic ring by a properly situated, sterically interfering alkyl moiety. We subsequently reported that one of the *N*-phenethyl-substituted 5-phenylmorphan enantiomers ((−)-**2**) without a 9β-methyl substituent that was said to limit the rotation of the aromatic ring, was also a selective μ-opioid antagonist,[23–25] as determined in the same [^{35}S]GTP-γ-S assay as that used by Thomas *et al.*[22]

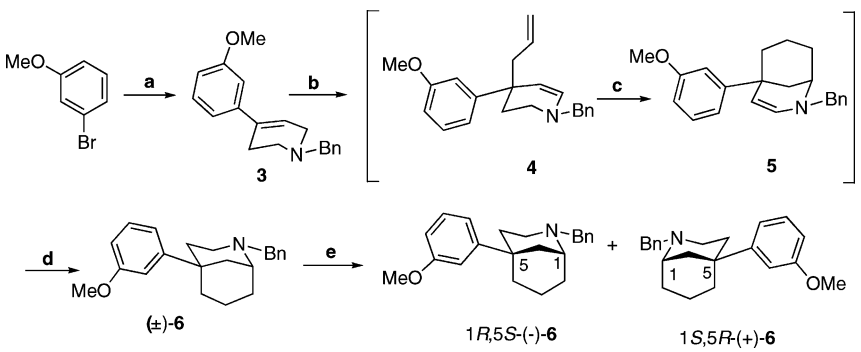
An *N*-phenethyl substituent apparently influences the activity of the 5-phenylmorphan molecule[26] much differently than in the more rigid cyclic morphinan-type of opioids. We have now explored this unusual effect of an *N*-phenethyl moiety, both with the hope of finding 5-phenylmorphan ligands that might be capable of interacting with both μ- and δ-opioid receptors and that would act as a μ-agonist and δ-antagonist. Also, differently substituted *N*-phenethyl phenylmorphans with varying affinity for opioid receptors and varying efficacy might provide greater insight into how that moiety exerts its unusual antagonist effect. We substituted various electron-withdrawing or donating groups (*e.g.*, methoxy, chloro, nitro, *etc.*) on the aromatic ring in the *N*-phenethyl side-chain, and, as well, used a different heteroaromatic ring (Table 1). We determined the affinity of these ligands to the μ-, δ-, and κ-opioid receptors and observed whether the electronic character of the aromatic ring was as important for receptor interaction in the 5-phenylmorphans as we have previously postulated.[27] The higher affinity analogues were further evaluated for their efficacy using the [^{35}S]GTP-γ-S assay (Tables 1 and 2).

Results and discussion

The synthesis of a key intermediate, 2-benzyl-5-(3-methoxyphenyl)-2-aza-bicyclo[3.3.1]nonane ((±)-**6**), was achieved as shown in Scheme 1.

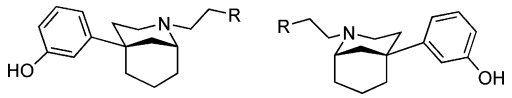
Lithiation of 3-bromoanisole by *n*-BuLi followed by its addition to 1-benzyl-4-piperidone gave a hydroxypiperidine intermediate that, without purification, was dehydrated to tetrahydropyridine **3** in good yield (93% over 2 steps). The metalated enamine resulting from lithiation of **3** by *sec*-BuLi was treated with allyl bromide to give endocyclic enamine **4**, which upon treatment with a 1 : 1 mixture of HCO_2H and H_3PO_4 at room temperature for 72 h gave an intermediate enamine **5**. This enamine was immediately reduced with $NaBH_4$ to 2-benzyl-5-(3-methoxyphenyl)-2-aza-bicyclo[3.3.1]nonane ((±)-**6**). The desired enantiomeric salts were obtained through resolution of (±)-**6** with (+)- and (−)-di-*O*,*O*′-*p*-toluyl-D-tartaric acid. The base (−)-**6** obtained from the (−)-**6**. di-*O*,*O*′-*p*-toluyl-L-tartaric acid salt showed slight levorotation at the sodium D line (589 nm), and was dextrorotatory at 365 nm. Its hydrobromide salt ((−)-**6**·HBr) was levorotatory. The enantiomeric purities of the corresponding bases ((−)-**6** and (+)-**6** at $[a]_{589}$ nm) were determined by chiral HPLC using a Daicel Chiralcel OD column. The optical purity of both enantiomers was shown to be greater than 99.8%. The experimental details are presented in the Supplementary Information‡.

The absolute configuration of (−)-**6** was determined to be 1*R*,5*S* by chemical conversion to the known 3-((1*R*,5*S*)-2-methyl-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol·HBr ((−)-**8b**.HBr, Scheme 2), an



Scheme 1 *Reagents and conditions*: a) i) THF, −78 °C, *n*-BuLi, then 1-benzyl-4-piperidone, to −20 °C; ii) toluene, *p*-toluenesulfonic acid monohydrate, reflux, 93% over 2 steps: b) THF, −40 °C, *sec*-BuLi, then allyl bromide, to 0 °C; c) H_3PO_4 : HCO_2H (1 : 1), rt; d) MeOH, 0 °C, $NaBH_4$, then rt, 60% over 3 steps; e) diastereomeric optical resolution with (+)-di-*O*,*O*′-*p*-toluyl-D-tartaric acid and (−)-di-*O*,*O*′-*p*-toluyl-L-tartaric acid, then NH_4OH.

Table 1 Binding affinity of *N*-substituted 5-phenylmorphans to opioid receptors



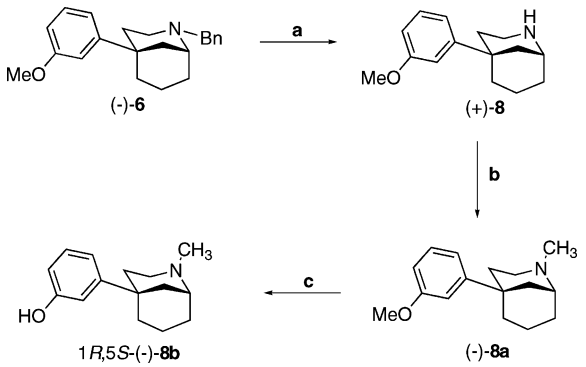
1*R*,5*S*-(-)-**10** 1*S*,5*R*-(+)-**10**

		K_i (nM ± SD)[a]		
Cmpd.[b]	R	[^{3}H]DAMGO (μ)	[^{3}H]DADLE (δ)	[^{3}H]U69593 (κ)
(+)-**10a**	2-Me-phenyl	68 ± 3	510 ± 24	96 ± 4
(−)-**10a**	2-Me-phenyl	19 ± 0.9	310 ± 24	190 ± 17
(+)-**10b**	3-Me-phenyl	63 ± 2	880 ± 32	190 ± 7
(−)-**10b**	3-Me-phenyl	9.8 ± 0.1	86 ± 5	320 ± 22
(+)-**10c**	4-Me-phenyl	38 ± 2	270 ± 9	440 ± 15
(−)-**10c**	4-Me-phenyl	17 ± 1.3	36 ± 2	900 ± 88
(+)-**10d**	2-MeO-phenyl	52 ± 2	680 ± 47	170 ± 22
(−)-**10d**	2-MeO-phenyl	25 ± 2	390 ± 13	320 ± 24
(+)-**10e**	3-MeO-phenyl	104 ± 4	1110 ± 79	280 ± 25
(−)-**10e**	3-MeO-phenyl	20 ± 1.1	160 ± 6	300 ± 17
(+)-**10f**	4-MeO-phenyl	107 ± 9	510 ± 30	1100 ± 68
(−)-**10f**	4-MeO-phenyl	20 ± 1.3	55 ± 4	880 ± 76
(+)-**10g**	2-Cl-phenyl	43 ± 1.0	135 ± 3	180 ± 14
(−)-**10g**	2-Cl-phenyl	13 ± 0.8	200 ± 13	330 ± 30
(+)-**10h**	3-Cl-phenyl	87 ± 3	1140 ± 63	200 ± 4
(−)-**10h**	3-Cl-phenyl	4.1 ± 0.4	160 ± 15	290 ± 21
(+)-**10i**	4-Cl-phenyl	65 ± 2	300 ± 13	500 ± 19
(−)-**10i**	4-Cl-phenyl	6.3 ± 0.5	103 ± 13	180 ± 17
(+)-**10j**	2,4-di-Cl-phenyl	79 ± 3	170 ± 5	1060 ± 59
(−)-**10j**	2,4-di-Cl-phenyl	50 ± 3	178 ± 23	1063 ± 68
(+)-**10k**	2-NO_2-phenyl	83 ± 3	390 ± 13	220 ± 8
(−)-**10k**	2-NO_2-phenyl	5.2 ± 0.5	250 ± 24	750 ± 61
(+)-**10l**	3-NO_2-phenyl	69 ± 3	1400 ± 65	130 ± 4
(−)-**10l**	3-NO_2-phenyl	1.9 ± 0.1	117 ± 11	45 ± 4
(+)-**10m**	4-NO_2-phenyl	28 ± 0.6	500 ± 23	240 ± 24
(−)-**10m**	4-NO_2-phenyl	0.9 ± 0.1	35 ± 4	140 ± 9
(+)-**10n**	2-Pyridyl	149 ± 12	500 ± 53	300 ± 10
(−)-**10n**	2-Pyridyl	34 ± 3	2400 ± 127	1400 ± 130
(+)-**10o**	3-Pyridyl	42 ± 3	1825 ± 185	380 ± 22
(−)-**10o**	3-Pyridyl	13 ± 1.0	1040 ± 92	1340 ± 133
(+)-**10p**	4-Pyridyl	75 ± 6	710 ± 35	1820 ± 169
(−)-**10p**	4-Pyridyl	51 ± 4	1900 ± 240	310 ± 32
(+)-**10q**	3-Indolyl	118 ± 9	1100 ± 71	190 ± 5
(−)-**10q**	3-Indolyl	40 ± 2	260 ± 12	720 ± 53
(+)-**10r**	2-OH-phenyl	65 ± 3	510 ± 23	93 ± 4
(−)-**10r**	2-OH-phenyl	21 ± 2	540 ± 39	250 ± 17
(+)-**10s**	3-OH-phenyl	76 ± 8	350 ± 45	350 ± 17
(−)-**10s**	3-OH-phenyl	26 ± 2	550 ± 27	390 ± 27
(+)-**10t**	4-OH-phenyl	40 ± 3	430 ± 23	1000 ± 45
(−)-**10t**	4-OH-phenyl	148 ± 12	470 ± 41	1800 ± 109
(+)-**10u**	Vinyl	35 ± 3	1050 ± 150	53 ± 5
(−)-**10u**	Vinyl	320 ± 13	2800 ± 250	1370 ± 108
(+)-**2**	Phenyl	19 ± 2	600 ± 60	54 ± 4
(−)-**2**	Phenyl	11 ± 0.9	540 ± 48	75 ± 5

[a] [^{3}H]DAMGO (D-Ala^2,MePhe^{4}Gly-ol^5)enkephalin; [^{3}H]DADLE (D-Ala^2,D-Leu^5)enkephalin; [^{3}H]U69593 *trans*-3,4-dichloro-*N*-methyl[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide. Rat brain membranes were used for μ and δ binding, and guinea pig brain membranes were used for κ binding. All results, $n = 3$. [b] The dextrorotatory and levorotatory compounds were 1*S*,5*R* and 1*R*,5*S*, respectively. All of the compounds were tested in salt form except for the **10n**, **10p** and **10q** enantiomers that were tested as bases.

N-methyl-*m*-hydroxy-5-phenylmorphan salt that had previously had its configuration established by X-ray crystallographic analysis.[21]

Thus, the (−)-**6** base (levorotatory at 589 nm) from the (−)-**6**.di-*O*,*O*′-*p*-toluyl-L-tartaric acid salt was converted to the secondary amine base (+)-**8** ($[\alpha]_D^{20}$ +2.9 (*c* 0.81, EtOH)) by catalytic hydrogenation with H_2/Pd. The hydrochloride salt of (+)-**8** was levorotatory. The (+)-**8** base was *N*-methylated through reductive formylation (H_2/Pd and HCHO) to (1*R*,5*S*)-(−)-5-(3-methoxyphenyl)-2-methyl-2-aza-bicyclo[3.3.1]nonane (**8a**) ($[\alpha]_D^{20}$ −10.9 (*c* 0.70, EtOH), $[\alpha]_{365}^{20}$ −21.1 (*c* 0.70, EtOH)), that, like the known[20] 1*S*,5*R*-*N*-methyl base, 1*S*,5*R*-(+)-5-(3-methoxyphenyl)-2-methyl-2-aza-bicyclo[3.3.1]nonane, was an oil. The (−)-*N*-methyl compound was converted to its hydrobromide salt, (1*R*,5*S*)-(−)-5-(3-methoxyphenyl)-2-methyl-2-aza-bicyclo[3.3.1]nonane·HBr (**8a**·HBr, mp 195–197 °C; $[\alpha]_D^{20}$ −5.1 (*c* 0.82, MeOH), $[\alpha]_{365}^{20}$ −15.4 (*c* 0.82, MeOH)). The melting point was comparable to the known 1*S*,5*R*-(+)-hydrobromide salt



Scheme 2 *Reagents and conditions*: a) H_2/Pd; b) H_2/Pd–HCHO; c) 48% HBr.

(199–201 °C).[20] The base **8a** was *O*-demethylated to 3-((1*R*,5*S*)-(−)-2-methyl-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol (**8b**)[19] (mp 151–153 °C, $[\alpha]_D^{20}$ −11.7 (*c* 0.775, EtOH), $[\alpha]_{365}^{20}$ −26.8 (*c* 0.775, EtOH); lit.,[19] 153–154 °C, $[\alpha]_D^{20}$ −12.7) and **8b** was converted to its hydrobromide salt (mp 228–229 °C, $[\alpha]_D^{20}$ −4.6 (*c* 0.81, H_2O); lit.,[21] 232–233 °C, $[\alpha]_D^{20}$ −4.2 (H_2O)), with the known 1*R*,5*S* configuration.[21] Thus, through the chemical conversion (Scheme 2) of (−)-**6** to 3-((1*R*,5*S*)-(−)-2-methyl-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol hydrobromide (**8b**·HBr) we were able to assign the absolute configuration of all of the compounds reported herein.

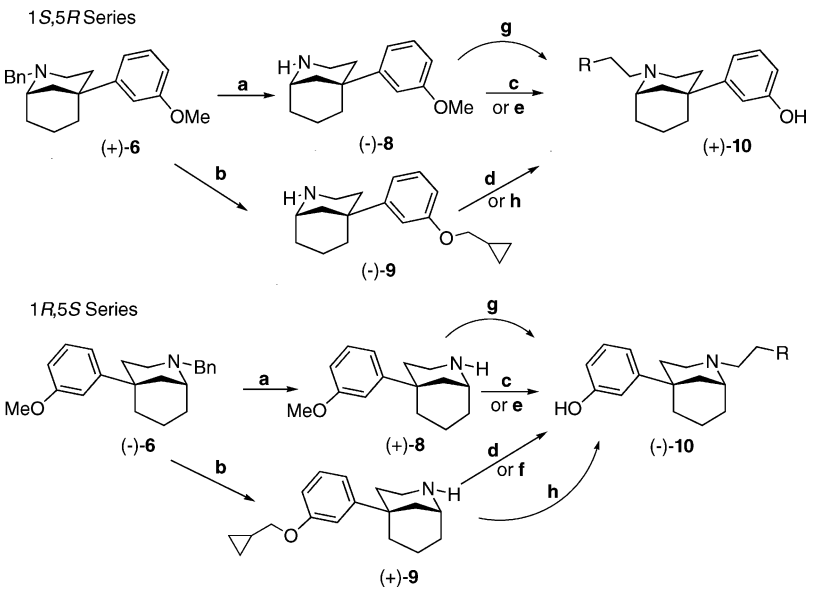
As noted above, hydrogenation of (+)-**6** in the presence of 10% Pd–C gave the methoxyphenylamine base ((−)-**8**), which was converted to (+)-**8**·HCl in high yield (Scheme 3).

The cyclopropylmethoxyphenylamine (+)-**9** was prepared for selective protection during some *N*-derivatizations *via* demethylation of (+)-**6** followed by reprotection with (bromomethyl)cyclopropane and dehydrogenation. The amines 1*R*,5*S*-(+)-**8** and (−)-**9** were also prepared from (−)-**6** in the same way. The arylethyl-*O*-tosylate **7a–n** (Scheme 4), a very useful intermediate for some of the *N*-arylethyl substituents, was synthesized by the reaction of the corresponding arylethyl alcohol and *p*-toluenesufonyl chloride in the presence of triethylamine.[28]

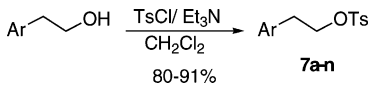
Table 2 Agonist and antagonist activity of selected ligands at opioid receptors

Compound	μ % S[a]	δ % I[b]	κ % I[b]
(−)-**10a**	1.0	100	97
(−)-**10b**	15	94	89
(−)-**10c**	39	82	82
(−)-**10d**	30	92	91
(−)-**10e**	36	86	88
(−)-**10f**	50	74	74
(−)-**10g**	19	89	93
(−)-**10h**	47	98	93
(−)-**10i**	41	100	93
(−)-**10k**	184	60	83
(−)-**10l**	142	87	84
(+)-**10m**	−8.3	100	96
(−)-**10m**	124	99	90
(−)-**10o**	164	57	62
(−)-**10r**	23	98	96
(−)-**10s**	9.0	99	84
DAMGO	500		

[a] % Stimulation (agonist efficacy) compared with DAMGO (μ). Negative numbers indicate inverse agonist effect. [b] % Inhibition (antagonist efficacy) after stimulation with SNC80 (δ), or U50499 (κ). Each value is the mean of 3 independent determinations. Compounds were screened at a 10 μM concentration.



Scheme 3 *Reagents and conditions*: a) AcOH, 10% Pd–C, H_2 (60 psi), rt, 93%; b) i) CH_2Cl_2, BBr_3·SMe_2 in CH_2Cl_2, 50 °C; ii) dry DMF, *t*-BuOK, 0 °C, (bromomethyl)cyclopropane, 50 °C; iii) AcOH, 10% Pd–C, H_2 (70 psi), 50 °C, 54–57% over 3 steps; c) i) dry DMF, **7**, K_2CO_3, 50 °C; ii) 1,2-dichloroethane, BBr_3·SMe_2 in CH_2Cl_2, reflux, 17–35% over 2 steps; d) i) dry DMF, **7**, K_2CO_3, 50 °C; ii) MeOH, conc. HCl, reflux, 27–38% over 3 steps; e) i) CH_2Cl_2, indole-3-acetic acid, EDCI·HCl, rt; ii) THF, $LiAlH_4$, 0 °C; iii) 1,2-dichloroethane, BBr_3·SMe_2 in CH_2Cl_2, reflux, ~20% over 3 steps; f) i) CH_2Cl_2, 3-pyridylacetic acid, EDCI·HCl, rt; ii) THF, $LiAlH_4$, 0 °C; iii) MeOH, conc. HCl, reflux, 22% over 3 steps; g) i) dry DMF, 4-bromo-1-butene, K_2CO_3, 50 °C; ii) 1,2-dichloroethane, BBr_3·SMe_2 in CH_2Cl_2, reflux, ~20% over 2 steps; h) i) dry DMF, 4-(2-bromoethyl)-pyridine, K_2CO_3, 50 °C; ii) MeOH, conc. HCl, reflux, 25–58% over 2 steps.



Scheme 4

Experimental details for all of the above are given in the Supplementary Information‡.

The target compounds, enantiomeric **10a–u** were prepared using one of several methods from the reaction of the corresponding enantiomeric amines and *N*-arylethyl precursor (See Scheme 3 and Table 1). The reaction of enantiomeric amines **8** or **9** with arylethyl-*O*-tosylate **7a–n** in the presence of potassium carbonate gave *N*-arylethyl derivatives, which were subsequently deprotected to provide the enantiomeric phenolic compounds **10a–10n** or **10r–10t** (Method A or B). Enantiomeric compounds **10o** and **10q** were prepared from the reaction of enantiomeric amines **9** or **8**, respectively, and 3-pyridylacetic acid (for **10o**) or indole-3-acetic acid (for **10q**) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) followed by reduction and demethylation (Method C or E). Also, 1*S*,5*R*-(+)-**10p** and 1*R*,5*S*-(−)-**10u** were synthesized from the reaction of 1*S*,5*R*-(+)-**9** or 1*R*,5*S*-**8**, respectively, and 4-(2-bromoethyl)-pyridine (for (+)-**10p**) or 4-bromo-1-butene (for (−)-**10u**) followed by deprotection to give the phenolic compound.

In general, the compounds with the highest affinity for the μ-opioid receptor were levorotatory and had the 1*R*,5*S* configuration (except for **10t**, Table 1, the *p*-hydroxyphenethyl derivative). This was also usually true at the δ-receptor, but there were many more exceptions (*e.g.*, **10g**, **10j**, **10n**, **10r**, **10s**, and **10t** in Table 1). At the κ-receptor, all of the compounds with the 1*S*,5*R* configuration (except **10f**), had higher affinity than those with the 1*R*,5*S* configuration, as did the parent *N*-phenethyl compound **2**. This difference probably reflects the very different binding sites for these compounds in the κ- *vs.* μ- or δ-opioid receptors.

The compound that had the highest affinity for the μ-opioid receptor, 1*R*,5*S*-(−)-3-{2-[2-(4-nitrophenyl)-ethyl]-2-aza-bicyclo[3.3.1]non-5-yl}-phenol (1*R*,5*S*-(−)-**10m**), was the 4-nitrophenylethyl substituted phenylmorphan (K_i = 0.9 nM, Table 1). It also showed fair affinity for the δ-receptor (K_i = 35 nm), and less affinity for the κ-receptor (K_i = 140 nm). The entire set of compounds was screened, at a 10 μM concentration, for μ agonism and δ antagonism in the [^{35}S]-GTP-γ-S binding assay (Table 2). Four compounds ((−)-**10k**, (−)-**10l**, (−)-**10m** and (−)-**10o**) showed the greatest degree of μ agonism, although the stimulation was considerably less than that produced by DAMGO. Among these four compounds, (−)-**10m** showed the greatest degree of δ antagonism, and was therefore selected for additional study.

To characterize the interaction of (−)-**10m** with μ and δ receptors in greater detail, we determined the effects of (−)-**10m** on basal [^{35}S]GTP-γ-S binding. As reported in Fig. 2, (−)-**10m** reduced basal [^{35}S]-GTP-γ-S binding by ~30%, indicating that this compound is an inverse δ agonist.

Interestingly, naltrindole (NTI) also showed inverse δ agonist activity, suggesting that in this cell line the δ receptors are constitutively active. Also, (−)-**10m** was a partial μ agonist. As reported in Fig. 3, (−)-**10m** potently stimulated [^{35}S]GTP-γ-S binding to μ receptors with an EC_{50} value of 2.39 nM and an E_{MAX}

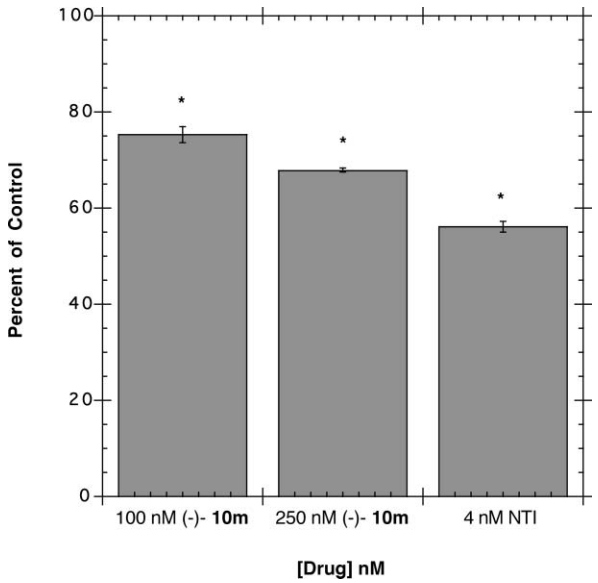


Fig. 2 Effect of test drugs on basal [^{35}S]GTP-γ-S binding in hDOR-CHO cells. [^{35}S]GTP-γ-S binding assays were conducted as described in Methods. Each point is the mean ± SD ($n = 3$). *$p < 0.05$ when compared to control (Students t-test).

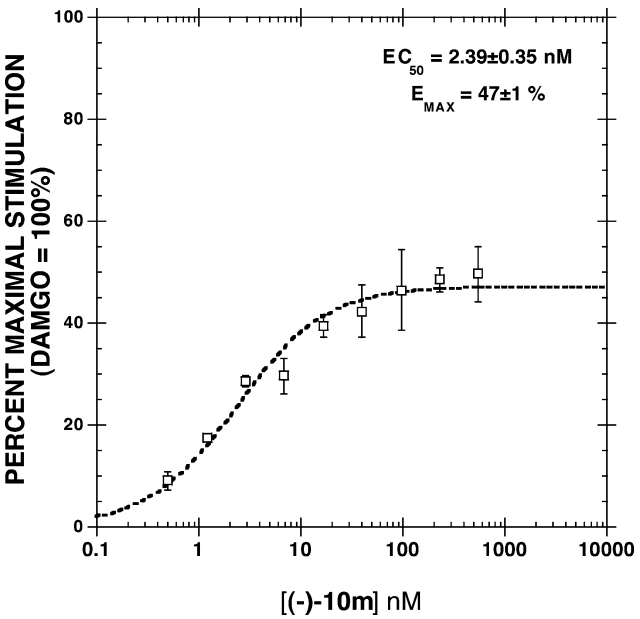


Fig. 3 Effect of (−)-**10m** on [^{35}S]GTP-γ-S binding in hMOR-CHO cells. [^{35}S]GTP-γ-S binding assays were conducted as described in Methods. The data were fit to a dose-response equation for the best-fit estimates of the EC_{50} and E_{MAX}. Maximal stimulation was determined with 1 μM DAMGO. Each point is the mean ± SD (n = 3).

value of 47 ± 1%, where the 100% is defined as the [^{35}S]GTP-γ-S stimulation produced by 1 μM DAMGO.

The EC_{50} of DAMGO at the μ receptor in recent [^{35}S]GTP-γ-S assays was 11 ± 1 nM, indicating that (−)-**10m** is a more potent μ agonist than DAMGO. In order to determine the K_e (antagonist K_i) value of (−)-**10m** at the δ receptor, we generated SNC80 dose-response curves in the absence and presence of two concentrations of (−)-**10m**. As expected for a δ antagonist, (−)-**10m** produced a

dose-dependent rightward shift in the SNC80 dose-response curve without significantly altering the E_{MAX} values (Fig. 4).

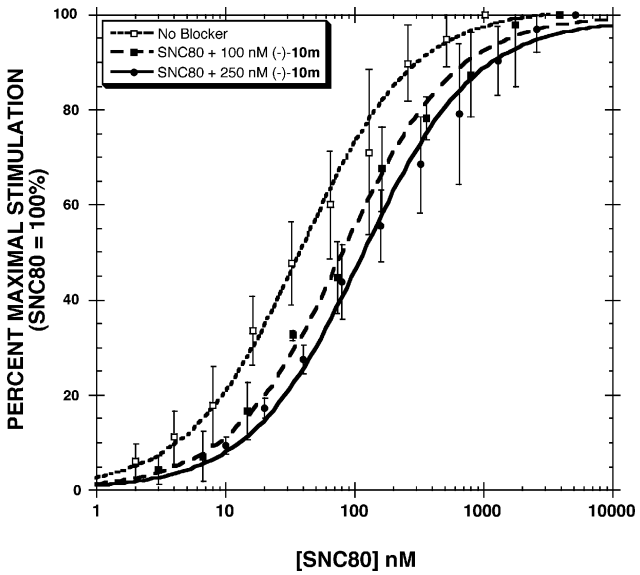


Fig. 4 Effect of (−)-**10m** on SNC80-stimulated [^{35}S]GTP-γ-S binding in hDOR-CHO cells. [^{35}S]GTP-γ-S binding assays were conducted as described in the Methods section. The data were fit to a dose-response equation for the best-fit estimates of the EC_{50} and E_{MAX} (see Table 3). Each point is the mean ± SD (n = 4–10).

These data, reported in Table 3, show that the K_e value of (−)-**10m** is ∼122 nM, indicating that (−)-**10m** is a much weaker δ antagonist than NTI (K_e = 0.23 nm).

The data indicate that (−)-**10m** is much more potent as a μ agonist than as a δ antagonist. This profile is the opposite of that reported for 4-chlorophenylpyridomorphinan, which was much more potent as a δ antagonist than as a μ agonist.[11] Despite having lower affinity at κ receptors than δ receptors (Table 1), the *K*e value of (−)-**10m** at κ receptors was similar to that observed for the δ receptor (∼106 nm). As observed with the δ receptor, (−)-**10m** is a weak κ antagonist as compared to nor-binaltorphimine (nor-BNI) (*K*e = 0.1 nm).

We also examined the compound *in vivo* (Fig. 5). It was found to be approximately 50-times more potent than morphine in the 55 °C tail flick assay (in mice, in comparison of A_{50} values).

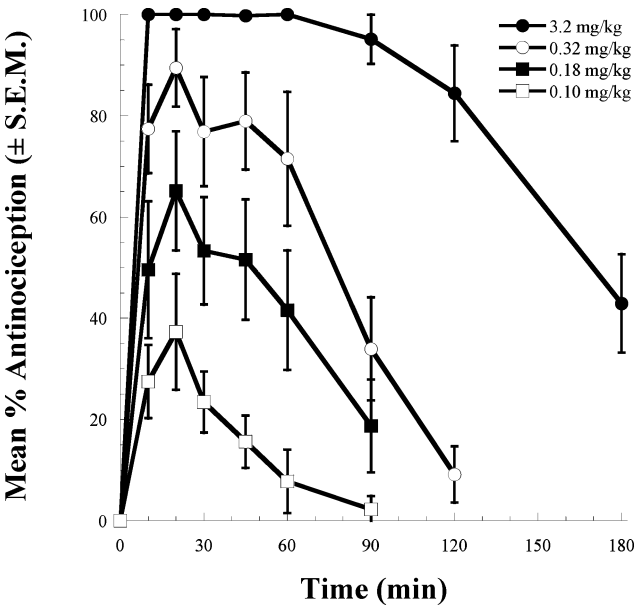


Fig. 5 Dose and time-response curve for subcutaneous (−)-**10m** in the 55 °C tail-flick test.

The time of peak effect was determined to be 20 min and the A_{90} dose was 0.32 mg kg^{-1}. The calculated A_{50} value (95% confidence interval) was 0.131 mg kg^{-1} (0.1016–0.1699 mg kg^{-1}). Robust increases in locomotion and Straub-tail were observed during testing of the higher doses. The (−)-**10m** was further evaluated in other antinociceptive assays in the mouse. It was found to be 18 times more potent than morphine in the hot plate assay, 6 times more potent in the phenylquinone assay, 43 times more potent in the tail flick assay (comparison *via* standard literature assays[29]), and was inactive as an opioid antagonist in the tail flick *vs.* morphine assay. Straub tail and increased locomotor activity were also observed in these assays.

Three analogues with K_i values *ca.* 5 nM at the μ-receptor, the 3-chlorophenyl (1*R*,5*S*-(−)-**10h**), 2-nitrophenyl (1*R*,5*S*-(−)-**10k**),

Table 3 Characterization of (−)-**10m** at δ and κ receptors in the [^{35}S]GTP-γ-S binding assay[a]

Conditions	EC_{50}/nM ± SD	E_{MAX} (% maximal stimulation ±SD)	K_e/nM
Delta receptors			
SNC80 (n = 10)	37.8 ± 3.0	100 ± 2	
SNC80 + 100 nM (−)-**10m** (n = 5)	60.2 ± 7.9	97 ± 3	169
SNC80 + 250 nM (−)-**10m** (n = 4)	115 ± 10	99 ± 2	122
SNC80 + 4 nM naltrindole (n = 3)	701 ± 28	114 ± 2	0.23
Kappa receptors			
(−)-U40488 (n = 7)	56.2 ± 3.1	97 ± 1.1	
(−)-U40488 +50 nM (−)-**10m** (n = 3)	321 ± 29	105 ± 3	106
(−)-U40488 +300 nM (−)-**10m** (n = 4)	1140 ± 63	123 ± 3	155
(−)-U40488 +0.1 nM norBNI (n = 7)	111 ± 7.3	102 ± 2	0.10

[a] [^{35}S]-GTP-γ-S binding was conducted as described in the Methods section. Agonist dose–response curves (ten points/curve) were generated in the absence and presence of the indicated concentrations of test agents. Since (−)-**10m** decreased basal [^{35}S]-GTP-γ-S binding in the hDOR-CHO cells, SNC80 dose response curves were calculated relative to the depressed baseline. The data for each experimental condition were pooled and the best-fit estimates of the EC_{50} and E_{MAX} values determined using MLAB-PC. The K_e values were calculated according to the equation: [Test Drug]/($EC_{50\text{-}2}$/$EC_{50\text{-}1}$ − 1), where $EC_{50\text{-}2}$ is the EC_{50} value in the presence of the test drug and $EC_{50\text{-}1}$ is the value in the absence of the test drug. Each parameter value is ±SD.

and the 3-nitrophenyl compound (1*R*,5*S*-(−)-**10l**) (K_i = 4, 5, and 2 nM, respectively), were all partial μ-agonists, and a fourth compound, the 3-methylphenyl analogue (1*R*,5*S*-(−)-**10b**), with a K_i value *ca.* 10 nM for the μ-receptor was found to be a selective μ-antagonist (Table 2). Neither the 2-nitrophenyl nor the 3-nitrophenyl analogue was found to have δ-antagonist activity in the [^{35}S]GTP-γ-S assay, nor did any of the methoxyphenyl compounds. The desired μ-agonist δ-antagonist activity was only found in a compound bearing a 4-nitro substituent. That substituent is bulky and together with its well-known inductive and electronic effects could modify the aromatic ring's ability to interact with amino acids in receptors, suggesting that this mixture of pharmacological activities might be sensitive to a combination of electronic and steric effects in the *N*-phenethyl-5-phenylmorphans.

Three other compounds, besides the aforementioned 4-nitro derivative, had fair (K_i <100 nm) affinity for the δ-receptor, the 4-methylphenyl (1*R*,5*S*-(−)-**10c**), 4-methoxyphenyl (1*R*,5*S*-(−)-**10f**), and the 4-chlorophenyl (1*R*,5*S*-(−)-**10i**) analogues. 3-((1*R*,5*S*)-(−)-2-(4-Chlorophenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-**10i**) was of special interest, since it decreased [^{35}S]GTP-γ-S binding to δ receptors 18% below baseline (data not shown), indicating that it is an inverse δ agonist. In antinociceptive assays, (−)-**10i** was equipotent with morphine in the hot plate assay, 5 times more potent in the phenylquinone assay, and 4 times more potent in the tail flick assay. It had no opioid antagonist activity in the tail flick *vs.* morphine assay, presumably an assay for μ-opioid antagonists. The 4-chlorophenethyl analogue (−)-**10i** might be a leading candidate for further research that might provide a potent anorectant,[16] and it is also interesting because of its unusual combination of properties, a μ-agonist and δ-inverse agonist.

In the substituted phenyl series, the 3-nitrophenyl compound (−)-**10l** had fair affinity, and the 2-methylphenyl and 2-hydroxyphenyl analogues had a little less affinity for the κ-receptor (K_i = 45, 68 and 93 nM, respectively). Both the ((+)-**10a** and (+)-**10r**, were in the 1*S*,5*R* enantiomeric series. Their 1*R*,5*S* counterparts had some, but less, affinity for the κ-receptor (K_i = *ca.* 190 and 250 nM, respectively). The higher affinity 2-methylphenyl analogue (1*S*,5*R*-(+)-**10a**) was noted to have some efficacy as a κ-antagonist in the [^{35}S]GTP-γ-S binding assay.

The efficacies and affinities of the newly synthesized ligands at the μ-receptor were obviously influenced by the substituent on the phenyl ring. These substituents are relatively distant from the nitrogen atom, and would not be likely to have much direct influence on a nitrogen atom known to be important for interaction with opioid receptors. The properties of the aromatic ring itself must, then, influence receptor affinity and/or efficacy. The steric bulk of an indolyl ((−)-**10q**) and a nitrophenethyl (−)-**10k**, (−)-**10l**, or (−)-**10m**) moiety was unlikely to be the predominant factor in receptor interaction, since (−)-**10q** had considerably less affinity than the phenethyl analogue (−)-**2**, and, as noted above, the 4-nitrophenyl analogue ((−)-**10m**) had the highest affinity at the μ-opioid receptor. The electronic influence of the substituent on binding was also not obvious. The compounds with the next highest affinities for the μ-receptor were analogues with a 3-chlorophenyl ((−)-**10h**, K_i = 4 nm) moiety and a 3-nitrophenyl moiety ((−)-**10l**, K_i = 1.9 nm). It is likely that a 3-chloro, a 3-nitro and a 4-nitro substituent would affect the electronic character of the aromatic ring differently, yet all had high affinity. Also, both the *ortho*- and the *meta*-phenolic compounds (−)-**10r** and (−)-**10s** had similar, moderately high affinities for the μ-receptor, but the *para*-analogue (−)-**10t** had much less affinity. Thus, although it is clear that the substituent on the *N*-phenethyl group affected affinity, the mechanism underlying that effect is unclear.

Conclusions

Unlike previously synthesized *N*-phenethyl substituted phenylmorphans that were found to be promiscuous opioid antagonists (Fig. 1), some of the new analogues were selective μ-agonists with good affinity at the μ- and others had moderate affinity at the δ-opioid receptor. Several compounds with the 1*R*,5*S* absolute configuration were found, in the [^{35}S]GTP-γ-S binding assay, to have efficacy as partial μ-agonists ((−)-**10m**, (−)-**10h**, (−)-**10k**, and (−)-**10l**), and one was a μ-antagonist ((−)-**10b**. Most useful and interesting was the discovery that the 4-nitrophenyl analogue (−)-**10m** had affinity and efficacy at μ and δ receptors; it was found to have both μ-agonist and δ-antagonist activity. Although much more potent than morphine as an antinociceptive, it also showed some signs that it might have undesired side-effects (Straub-tail at higher doses). Also, the 4-chlorophenyl analogue (−)-**10i**, which had lower affinity than the former compound at μ and δ receptors, was found to be an inverse agonist at the δ opioid receptor and was morphine-like in antinociceptive assays. Both of these analogues will serve as model compounds for the synthesis of more efficacious ligands.

Experimental

All melting points were determined on a Thomas-Hoover melting-point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR, 300 MHz) and carbon nuclear magnetic resonance (^{13}C NMR, 75 MHz) spectra were recorded on a Varian Gemini-300 instrument in $CDCl_3$ (unless otherwise noted) with the values given in ppm (TMS as internal standard) and *J* (Hz) assignments of ^{1}H resonance coupling. The high-resolution electrospray ionization (ESI) mass spectra were obtain on a Waters LCT Premier time-of-flight (TOF) mass spectrometer. Thin-layer chromatography (TLC) was performed on 0.25 mm Analtech GHLF silica gel. Flash column chromatography was performed with Bodman silica gel LC 60 A. Elemental analyses were performed by Atlantic Microlabs, Inc., Norcross, GA. Enantiomeric purity was assessed by HPLC (Shimadzu LC-6A with a Shimadzu SPD-6AV UM detector (at 254 nm) using Daicel's Chiralcel OD column (250 × 4.6 mm). The mobile phase was hexanes–2-propanol–diethylamine (95:5:0.25) at a flow rate of 0.6 mL min^{-1}.

The experimental details for compounds **3**, (±)-**6** (and the resolution to (+)- and (−)-**6**), **7**, **7a** through **7l**, (+)- and (−)-**8** and (+)- and (−)-**9** are included in the Supplementary Information‡.

General procedure for syntheses of 3-((1*S*,5*R*)-(+)-2-(2-(or 3- or 4-)arylethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10) and 3-((1*R*,5*S*)-(−)-2-(2-(or 3- or 4)-arylethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10)

Method A. A mixture of enantiomeric amine **8** (2–3 mmol), corresponding arylethyl tosylate **7** (1.5 equiv.) and K_2CO_3 (3 equiv) in dry DMF (~0.2 M concentration) were heated at 50 °C for 6 h.

After the cooled mixture was filtered and the solvent evaporated, the residue was diluted with 5% MeOH in CH_2Cl_2, washed with water and brine, dried over Na_2SO_4, passed through a pad of silica gel and concentrated to dryness. The product was directly used for demethylation, which used 1.0 M $BBr_3{\cdot}SMe_2$ in CH_2Cl_2 (*vide supra*). Column chromatography of crude product with 1–2% MeOH in CH_2Cl_2 gave 10 (17–35% yield over 2 steps).

Method B. A mixture of enantiomeric amine **9** (2–3 mmol), the corresponding arylethyl tosylate **7** (2 equiv.) and K_2CO_3 (3 equiv.) in dry DMF (~0.15 M concentration) was heated at 60 °C for 4 h. After the cooled mixture was filtered and the solvent evaporated, the residue was diluted with ethyl acetate, washed with water and brine, dried over Na_2SO_4, passed through a pad of silica gel and concentrated to dryness. The product was used without further purification in the demethylation procedure, in which the reaction mixture in concentrated HCl–MeOH (1 : 1, 0.05 M solution) was refluxed for 2 h. After cooling, it was basified with NH_4OH (to ~pH 10), extracted with 10% MeOH in CH_2Cl_2, washed with water and brine, dried over Na_2SO_4 and concentrated to give a crude product. Column chromatography of this crude product with 2–5% MeOH in CH_2Cl_2 gave **10** (27–38% yield over 2 steps).

Method C. A mixture of enantiomeric amine (−)-**9** (250 mg, 0.92 mmol), 3-pyridylacetic acid (209 mg, 1.2 mmol) and EDCI (230 mg, 1.2 mmol) in CH_2Cl_2 (6 mL) was stirred at room temperature overnight. The reaction was diluted with 5% 2-propanol in CH_2Cl_2, washed with aqueous Na_2CO_3 solution (to ~pH 10) and brine, dried over Na_2SO_4 and passed through a pad of silica gel. After evaporation, the residue was used without further purification in the following reduction. $LiAlH_4$ (42 mg, 1.1 mmol) was added to a solution of amide (~0.36 mmol) in dry THF (3 mL) at 0 °C and the mixture was stirred for 2 h. The reaction was quenched by addition of saturated NH_4Cl solution, filtered through a pad of celite, extracted with ethyl acetate, washed with water and brine, dried over Na_2SO_4 and concentrated to give a crude amine, which was subjected to deprotection using concentrated HCl–MeOH (see method B) to provide (−)-**10k** (60 mg, 22% over 3 steps).

Method D. A mixture of enantiomeric amine **9** (~2 mmol), 4-(2-bromoethyl)pyridine (~3 equiv.) and K_2CO_3 (~6 equiv.) in dry DMF (3 mL) was heated at 50 °C for 2 h. After the cooled mixture was filtered and the solvent evaporated, the residue was diluted with ethyl acetate, washed with water and brine, dried over Na_2SO_4, passed through a pad of silica gel and concentrated to dryness. The crude product was subjected to deprotection using concentrated HCl–MeOH (see method B) to provide **10** (25–58% over 2 steps).

Method E. A mixture of enantiomeric amine **8** (2–3 mmol), indole-3-acetic acid (1.1 equiv.) and EDCI (1.3 equiv.) in CH_2Cl_2 (~2 M concentration) was stirred at room temperature overnight. The reaction was diluted with 5% 2-propanol in CH_2Cl_2, washed with aqueous Na_2CO_3 solution (to ~pH 10) and brine, dried over Na_2SO_4 and passed through a pad of silica gel. The crude product was used for the reduction (see method C) and demethylation (see method A) to give **10** (~20% yield over 3 steps).

Method F. A mixture of enantiomeric amine **8** (~2 mmol), 4-bromobut-1-ene (2 equiv.) and K_2CO_3 (4 equiv.) in dry DMF (4 mL) was heated at 50 °C for 2 h. After the cooled mixture was filtered and the solvent evaporated, the residue was diluted with ethyl acetate, washed with water and brine, dried over Na_2SO_4, passed through a pad of silica gel and concentrated to dryness. The crude product was used for the demethylation (see method A) to give **10** (~20% yield over 2 steps).

3-((1*S*,5*R*)-(+)-2-(2-methylphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10a) and 3-((1*R*,5*S*)-(−)-2-(2-methylphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10a)

(+)-**10a** was obtained from the reaction of 1*S*,5*R*-**8** and **7a** by method A. Mp 260–261.5 °C as HCl salt; $[\alpha]_D^{20}$ +16.6 (*c* 0.42, MeOH); ^{1}H NMR ($CDCl_3$, free base): δ 7.18–7.09 (m, 5H), 6.81 (d, 1H, $J = 8$ Hz), 6.76 (br-t, 1H, $J = 2$ Hz), 6.63 (dd, 1H, $J =$ 8 and 2 Hz), 3.31 (br, 1H), 3.10–3.00 (m, 2H), 2.90–2.70 (m, 4H), 2.29 (s, 3H), 2.18 (br-t, 2H, $J = 12$ Hz), 2.04–1.82 (m, 5H), 1.70–1.54 (m, 2H), 1.47–1.36 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 156.68, 153.21, 138.25, 136.09, 130.22, 129.35, 129.24, 126.26, 126.01, 116.32, 113.58, 112.70, 56.28, 52.28, 49.55, 38.08, 34.70, 30.88, 24.30, 22.78, 19.22; HRMS (ESI): Found 336.2318 $[MH]^+$, $C_{23}H_{30}NO$ requires 336.2327. Found: C, 73.58; H, 8.13; N, 3.70. Calc. for $C_{23}H_{29}NO{\cdot}HCl{\cdot}0.25H_2O$: C, 73.38; H, 8.17; N, 3.72%.

(−)-**10a** was obtained from the reaction of 1*R*,5*S*-**8** and **7a** by method A. Mp 263–264 °C as HCl salt; $[\alpha]_D^{20}$ −16.7 (*c* 0.42, MeOH); HRMS (ESI): Found 336.2304 $[MH]^+$, $C_{23}H_{30}NO$ requires 336.2327. Found: C, 74.10; H, 8.24; N, 3.73. Calc. for $C_{23}H_{29}NO{\cdot}HCl$: C, 74.27; H, 8.13; N, 3.77%.

3-((1*S*,5*R*)-(+)-2-(3-Methylphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10b) and 3-((1*R*,5*S*)-(−)-2-(3-methylphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10b)

(+)-**10b** was obtained from the reaction of 1*S*,5*R*-**8** and **7b** by method A. Mp 248–249 °C as HCl salt; $[\alpha]_D^{20}$ +13.8 (*c* 0.38, MeOH); ^{1}H NMR (CD_3OD, free base): δ 7.26–7.07 (m, 5H), 6.84 (dq, 1H, $J = 8$ and 1 Hz), 6.80 (br-t, 1H, $J = 2$ Hz), 6.66 (ddd, 1H, $J = 8$, 2 and 1 Hz), 3.95 (br, 1H), 3.74–3.52 (m, 2H), 3.47–3.04 (m, 2H), 2.34 (s, 3H), 2.40–2.02 (m, 7H), 1.92–1.70 (m, 3H); ^{13}C NMR (d_6-DMSO, HCl salt): δ 157.36, 151.04, 137.72, 136.94, 129.39, 129.30, 128.51, 127.44, 125.85, 115.13, 113.04, 111.61, 54.23, 54.16, 48.56, 36.69, 34.95, 33.89, 33.23, 29.80, 21.40, 20.96, 20.43; HRMS (ESI): Found 336.2335 $[MH]^+$, $C_{23}H_{30}NO$ requires 336.2327. Found: C, 74.19; H, 8.21; N, 3.73. Calc. for $C_{23}H_{29}NO{\cdot}HCl$: C, 74.27; H, 8.13; N, 3.77%.

(−)-**10b** was obtained from the reaction of 1*R*,5*S*-**8** and **7b** by method A. Mp 205–206 °C as HBr salt; $[\alpha]_D^{20}$ −13.6 (*c* 0.38, MeOH); HRMS (ESI): Found 336.2299 $[MH]^+$, $C_{23}H_{30}NO$ requires 336.2327. Found: C, 66.17; H, 7.34; N, 3.35. Calc. for $C_{23}H_{29}NO{\cdot}HBr$: C, 66.34; H, 7.26; N, 3.36%.

3-((1*S*,5*R*)-(+)-2-(4-Methylphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10c) and 3-((1*R*,5*S*)-(−)-2-(4-methylphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10c)

(+)-**10c** was obtained from the reaction of 1*S*,5*R*-**8** and **7c** by method A. Mp 237–238 °C as HCl salt; $[\alpha]_D^{20}$ +13.4 (*c* 0.32, MeOH); ^{1}H NMR ($CDCl_3$, free base): δ 7.18–7.09 (m, 5H), 6.82 (d, 1H, $J = 8$ Hz), 6.78 (br-t, 1H, $J = 2$ Hz), 6.62 (dd, 1H, $J = 8$ and 2 Hz), 3.31 (br, 1H), 3.10–3.00 (m, 2H), 2.90–2.70 (m, 4H), 2.31

(s, 3H), 2.19 (br-t, 2H, J = 12 Hz), 2.04–1.82 (m, 5H), 1.72–1.54 (m, 2H), 1.42–1.30 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 156.41, 153.32, 137.00, 135.61, 129.25, 129.09, 128.62, 116.57, 113.30, 112.54, 57.79, 52.30, 49.62, 38.17, 38.08, 36.83, 34.73, 33.26, 24.32, 22.70, 21.10; HRMS (ESI): Found 336.2329 $[MH]^+$, $C_{23}H_{30}NO$ requires 336.2327. Found: C, 74.03; H, 8.33; N, 3.81. Calc. for $C_{23}H_{29}NO{\cdot}HCl$: C, 74.27; H, 8.13; N, 3.77%.

(−)-**10c** was obtained from the reaction of 1*R*,5*S*-**8** and **7c** by method A. Mp 218–219 °C as HBr salt; $[\alpha]_D^{20}$ −12.7 (*c* 0.37, MeOH); HRMS (ESI): Found 336.2343 $[MH]^+$, $C_{23}H_{30}NO$ requires 336.2327. Found: C, 66.15; H, 7.37; N, 3.43. Calc. for $C_{23}H_{29}NO{\cdot}HBr$: C, 66.34; H, 7.26; N, 3.36%.

3-((1*S*,5*R*)-(+)-2-(2-Methoxyphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10d) and 3-((1*R*,5*S*)-(−)-2-(2-methoxyphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10d)

(+)-**10d** was obtained from the reaction of 1*S*,5*R*-**9** and **7d** by method B. Mp 244–245 °C as HCl salt; $[\alpha]_D^{20}$ +20.0 (*c* 0.42, MeOH); ^{1}H NMR ($CDCl_3$, free base): δ 7.23–7.12 (m, 3H), 6.90–6.82 (m, 4H), 6.68 (dd, 1H, J = 8 and 2 Hz), 3.84 (s, 3H), 3.35 (br, 1H), 3.10–3.02 (m, 2H), 2.94–2.72 (m, 4H), 2.18 (br 2H), 2.04–1.88 (m, 5H), 1.70–1.56 (m, 2H), 1.43–1.36 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 157.72, 156.80, 153.43, 130.66, 129.40, 128.52, 127.70, 120.74, 116.64, 113.62, 112.94, 110.52, 55.82, 55.49, 52.30, 49.93, 38.33, 38.19, 36.91, 34.92, 28.22, 24.47, 22.90; HRMS (ESI): Found 352.2274 $[MH]^+$, $C_{23}H_{30}NO_2$ requires 352.2277. Found: C, 70.73; H, 7.75; N, 3.62. Calc. for $C_{23}H_{29}NO_2{\cdot}HCl{\cdot}0.25H_2O$: C, 70.39; H, 7.83; N, 3.57%.

(−)-**10d** was obtained from the reaction of 1*R*,5*S*-**9** and **7d** by method B. Mp 241–242 °C as HCl salt; $[\alpha]_D^{20}$ −20.2 (*c* 0.42, MeOH); HRMS (ESI): Found 352.2292 $[MH]^+$, $C_{23}H_{30}NO_2$ requires 352.2277. Found: C, 71.11; H, 7.78; N, 3.59. Calc. for $C_{23}H_{29}NO_2{\cdot}HCl$: C, 71.21; H, 7.79; N, 3.61%.

3-((1*S*,5*R*)-(+)-2-(3-Methoxyphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10e) and 3-((1*R*,5*S*)-(−)-2-(3-methoxyphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10e)

(+)-**10e** was obtained from the reaction of 1*S*,5*R*-**9** and **7e** by method B. Mp 226–227 °C as HCl salt; $[\alpha]_D^{20}$ +13.9 (*c* 0.52, MeOH); ^{1}H NMR ($CDCl_3$, free base): δ 7.23–7.12 (m, 2H), 6.84 (d, 1H, J = 8 Hz), 6.82–6.72 (m, 4H), 6.64 (dd, 1H, J = 8 and 2 Hz), 3.79 (s, 3H), 3.26 (br, 1H), 3.07–2.98 (m, 2H), 2.88–2.76 (m, 4H), 2.18 (d, 2H, J = 12 Hz), 2.08–1.84 (m, 5H), 1.72–1.56 (m, 2H), 1.43–1.32 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 159.62, 156.31, 153.55, 142.01, 129.33, 129.26, 121.13, 116.68, 114.51, 113.24, 112.53, 111.38, 57.62, 55.14, 52.25, 49.58, 38.36, 38.18, 37.02, 34.82, 34.01, 24.46, 22.80; HRMS (ESI): Found 352.2264 $[MH]^+$, $C_{23}H_{30}NO_2$ requires 352.2277. Found: C, 70.99; H, 7.83; N, 3.56. Calc. for $C_{23}H_{29}NO_2{\cdot}HCl$: C, 71.21; H, 7.79; N, 3.61%.

(−)-**10e** was obtained from the reaction of 1*R*,5*S*-**9** and **7e** by method B. Mp 223–224 °C as HCl salt; $[\alpha]_D^{20}$ −13.7 (*c* 0.52, MeOH); HRMS (ESI): Found 352.2271 $[MH]^+$, $C_{23}H_{30}NO_2$ requires 352.2277. Found: C, 71.04; H, 7.90; N, 3.53. Calc. for $C_{23}H_{29}NO_2{\cdot}HCl$: C, 71.21; H, 7.79; N, 3.61%.

3-((1*S*,5*R*)-(+)-2-(4-Methoxyphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10f) and 3-((1*R*,5*S*)-(−)-2-(4-methoxyphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10f)

(+)-**10f** was obtained from the reaction of 1*S*,5*R*-**9** and **7f** by method B. Mp 214–215 °C as HCl salt; $[\alpha]_D^{20}$ +15.2 (*c* 0.33, MeOH); ^{1}H NMR ($CDCl_3$, free base): δ 7.19–7.10 (m, 3H), 6.90–6.80 (m, 3H), 6.78 (t, 1H, J = 2 Hz), 6.64 (dd, 1H, J = 8 and 2 Hz), 3.78 (s, 3H), 3.25 (br, 1H), 2.86–2.75 (m, 4H), 2.16 (d, 2H, J = 12 Hz), 2.08–1.84 (m, 5H), 1.72–1.56 (m, 2H), 1.43–1.32 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 157.94, 156.09, 153.74, 132.52, 129.63, 129.27, 116.87, 113.81, 113.07, 112.43, 58.01, 55.26, 52.19, 49.59, 38.49, 38.22, 37.13, 34.88, 33.16, 24.55, 22.81; HRMS (ESI): Found 352.2274 $[MH]^+$, $C_{23}H_{30}NO_2$ requires 352.2278. Found: C, 71.05; H, 7.80; N, 3.54. Calc. for $C_{23}H_{29}NO_2{\cdot}HCl$: C, 71.21; H, 7.79; N, 3.61%.

(−)-**10f** was obtained from the reaction of 1*R*,5*S*-**9** and **7f** by method B. Mp 215–216 °C as HCl salt; $[\alpha]_D^{20}$ −15.0 (*c* 0.33, MeOH); HRMS (ESI): Found 352.2274 $[MH]^+$, requires $C_{23}H_{30}NO_2$ requires 352.2296. Found: C, 71.01; H, 7.96; N, 3.63. Calc. for $C_{23}H_{29}NO_2{\cdot}HCl$: C, 71.21; H, 7.79; N, 3.61%.

3-((1*S*,5*R*)-(+)-2-(2-Chlorophenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10g) and 3-((1*R*,5*S*)-(−)-2-(2-chlorophenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10g)

(+)-**10g** was obtained from the reaction of 1*S*,5*R*-**8** and **7g** by method A. Mp 204–205 °C as HCl salt; $[\alpha]_D^{20}$ +18.7 (*c* 0.33, MeOH); ^{1}H NMR ($CDCl_3$, free base): δ 7.35–7.30 (m, 1H), 7.22–7.08 (m, 4H), 6.83 (d, 1H, J = 8 Hz), 6.77 (m, 1H), 6.64 (dd, 1H, J = 8 and 2 Hz), 3.30 (br, 1H), 3.10–2.95 (m, 3H), 2.90–2.70 (m, 2H), 2.24–2.10 (m, 2H), 2.08–1.80 (m, 5H), 1.72–1.56 (m, 2H), 1.43–1.32 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 156.46, 153.40, 137.83, 133.95, 130.98, 129.41, 129.24, 127.64, 126.89, 116.52, 113.37, 112.61, 55.50, 52.28, 49.52, 38.16, 36.88, 34.74, 31.53, 24.52, 22.75; HRMS (ESI): Found 356.1785 $[MH]^+$, $C_{22}H_{27}ClNO$ requires 356.1781. Found: C, 67.23; H, 7.13; N, 3.55. Calc. for $C_{22}H_{26}ClNO{\cdot}HCl$: C, 67.34; H, 6.94; N, 3.57%.

(−)-**10g** was obtained from the reaction of 1*R*,5*S*-**8** and **7g** by method A. Mp 215–216 °C as HBr salt; $[\alpha]_D^{20}$ −17.2 (*c* 0.39, MeOH); HRMS (ESI): Found 356.1801 $[MH]^+$, $C_{22}H_{27}ClNO$ requires 356.1781. Found 356.1801. Found: C, 60.48; H, 6.22; N, 3.20. Calc. for $C_{22}H_{26}ClNO{\cdot}HBr$: C, 60.49; H, 6.23; N, 3.21%.

3-((1*S*,5*R*)-(+)-2-(3-Chlorophenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10h) and 3-((1*R*,5*S*)-(−)-2-(3-chlorophenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10h)

(+)-**10h** was obtained from the reaction of 1*S*,5*R*-**8** and **7h** by method A. Mp 227–229 °C as HBr salt; $[\alpha]_D^{20}$ +11.4 (*c* 0.33, MeOH); ^{1}H NMR ($CDCl_3$, free base): δ 7.20–7.10 (m, 4H), 7.04 (dt, 1H, J = 6 and 2 Hz), 6.83 (d, 1H, J = 8 Hz), 6.75 (t, 1H, J = 2 Hz), 6.63 (dd, 1H, J = 8 and 2 Hz), 3.25 (br, 1H), 3.02 (dd, 2H, J = 9 and 3 Hz), 2.84–2.76 (m, 4H), 2.20–2.10 (m, 2H), 2.08–1.80 (m, 5H), 1.74–1.54 (m, 2H), 1.43–1.32 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 156.46, 153.31, 142.24, 134.04, 129.60, 129.30, 128.78, 126.96, 126.26, 116.50, 113.42, 112.58, 57.29, 52.34, 49.48, 38.16, 38.09, 36.87, 34.71, 33.42, 24.35, 22.72; HRMS (ESI): Found 356.1783

$[MH]^+$, $C_{22}H_{27}ClNO$ requires 356.1781. Found: C, 60.54; H, 6.37; N, 3.19. Calc. for $C_{22}H_{26}ClNO{\cdot}HBr$: C, 60.49; H, 6.23; N, 3.21%.

(−)-**10h** was obtained from the reaction of 1*R*,5*S*-**8** and **7h** by method A. Mp 239–241 °C as HCl salt; $[\alpha]_D^{20}$ −13.8 (*c* 0.44, MeOH); HRMS (ESI): Found 356.1806 $[MH]^+$, $C_{22}H_{27}ClNO$ requires 356.1781. Found: C, 66.99; H, 7.03; N, 3.46. Calc. for $C_{22}H_{26}ClNO{\cdot}HCl$: C, 67.34; H, 6.94; N, 3.57%.

3-((1*S*,5*R*)-(+)-2-(4-Chlorophenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10i) and 3-((1*R*,5*S*)-(−)-2-(4-chlorophenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10i)

(+)-**10i** was obtained from the reaction of 1*S*,5*R*-**8** and **7i** by method A. Mp 251–252 °C as HCl salt; $[\alpha]_D^{20}$ +13.5 (*c* 0.39, MeOH); ^{1}H NMR ($CDCl_3$, free base): δ 7.24 (d, 2H, J = 8 Hz), 7.20–7.10 (m, 3H), 6.82 (d, 1H, J = 8 Hz), 6.79 (br, 1H), 6.63 (dd, 1H, J = 8 and 2 Hz), 3.24 (br, 1H), 3.05–3.00 (m, 2H), 2.85–2.75 (m, 4H), 2.22–2.10 (m, 2H), 2.05–1.80 (m, 5H), 1.74–1.54 (m, 2H), 1.43–1.32 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 156.29, 153.28, 138.53, 131.89, 130.08, 129.30, 128.49, 116.65, 113.24, 112.45, 57.34, 52.42, 49.54, 38.16, 38.05, 36.80, 34.70, 33.08, 24.36, 22.64; HRMS (ESI): Found 356.1784 $[MH]^+$, $C_{22}H_{27}ClNO$ requires 356.1781. Found: C, 67.32; H, 7.03; N, 3.56. Calc. for $C_{22}H_{26}ClNO{\cdot}HCl$: C, 67.34; H, 6.94; N, 3.57%.

(−)-**10i** was obtained from the reaction of 1*R*,5*S*-**8** and **7i** by method A. Mp 239–241 °C as HCl salt; $[\alpha]_D^{20}$ −13.6 (*c* 0.39, MeOH); HRMS (ESI): Found 356.1790 $[MH]^+$, $C_{22}H_{27}ClNO$ requires 356.1781. Found: C, 67.30; H, 7.03; N, 3.52. Calc. for $C_{22}H_{26}ClNO{\cdot}HCl$: C, 67.34; H, 6.94; N, 3.57%.

1*S*,5*R*-(+)-3-{2-[2-(2,4-Dichlorophenyl)-ethyl]-2-aza-bicyclo[3.3.1]non-5-yl}-phenol ((+)-10j) and 1*R*,5*S*-(−)-3-{2-[2-(2,4-Dichlorophenyl)-ethyl]-2-aza-bicyclo[3.3.1]non-5-yl}-phenol ((−)-10j)

(+)-**10j** was obtained from the reaction of 1*S*,5*R*-**8** and **7j** by method A. Mp 249–250 °C as HBr salt; $[\alpha]_D^{20}$ +20.0 (*c* 0.32, MeOH); ^{1}H NMR ($CDCl_3$, free base): δ 7.35 (br-s, 1H), 7.18–7.12 (m, 3H), 6.85 (d, 1H, J = 8 Hz), 6.77 (t, 1H, J = 2 Hz), 6.63 (dd, 1H, J = 8 and 2 Hz), 3.25 (br, 1H), 3.10–2.90 (m, 4H), 2.88–2.66 (m, 2H), 2.15 (d, 2H, J = 12 Hz), 2.08–1.84 (m, 5H), 1.72–1.55 (m, 2H), 1.43–1.32 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 156.22, 153.55, 136.56, 134.60, 132.50, 131.71, 129.29, 129.16, 127.12, 116.75, 113.24, 12.51, 59.88, 55.34, 52.35, 49.46, 38.31, 38.18, 36.99, 34.79, 31.14, 24.64, 22.75; HRMS (ESI): Found 390.1382 $[MH]^+$, $C_{22}H_{26}Cl_2NO$ requires 390.1391. Found: C, 61.84; H, 6.15; N, 3.25. Calc. for $C_{22}H_{25}Cl_2NO{\cdot}HCl$: C, 61.91; H, 6.14; N, 3.28%.

(−)-**10j** was obtained from the reaction of 1*R*,5*S*-**8** and **7j** by method A. Mp 242–243 °C as HCl salt; $[\alpha]_D^{20}$ −19.7 (*c* 0.32, MeOH); HRMS (ESI): Found 390.1394 $[MH]^+$, $C_{22}H_{26}Cl_2NO$ requires 390.1391. Found: C, 61.67; H, 6.28; N, 3.21. Calc. for $C_{22}H_{25}Cl_2NO{\cdot}HCl$: C, 61.91; H, 6.14; N, 3.28%.

3-((1*S*,5*R*)-(+)-2-(2-Nitrophenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10k) and 3-((1*R*,5*S*)-(−)-2-(2-nitrophenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10k)

(+)-**10k** was obtained from the reaction of 1*S*,5*R*-**8** and **7k** by method A. Mp 218–219 °C as HCl salt; $[\alpha]_D^{20}$ +2.5 (*c* 0.48, MeOH); ^{1}H NMR ($CDCl_3$, free base): δ 7.89 (dd, 1H, J = 8 and 2 Hz), 7.52–7.46 (m, 1H), 7.38–7.30 (m, 2H), 7.15 (t, 1H, J = 8 Hz), 6.88–6.82 (m, 1H), 6.78 (t, 1H, J = 2 Hz), 6.65 (dd, 1H, J = 8 and 2 Hz), 3.22 (br, 1H), 3.19–2.76 (m, 5H), 2.22–2.10 (m, 2H), 2.08–1.82 (m, 4H), 1.74–1.55 (m, 2H), 1.48–1.35 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 156.29, 153.58, 149.43, 135.41, 133.02, 132.73, 129.29, 127.23, 124.62, 116.69, 113.28, 112.56, 56.34, 52.60, 49.31, 38.25, 38.20, 36.93, 34.75, 31.18, 24.67, 22.71; HRMS (ESI): Found 367.2037 $[MH]^+$, $C_{22}H_{27}N_2O_3$ requires 367.2022. Found: C, 65.09; H, 6.74; N, 6.77. Calc. for $C_{22}H_{26}N_2O_3{\cdot}HCl{\cdot}0.25H_2O$: C, 64.86; H, 6.80; N, 6.88%.

(−)-**10k** was obtained from the reaction of 1*R*,5*S*-**8** and **7k** by method A. Mp 217–218 °C as HCl salt; $[\alpha]_D^{20}$ −2.2 (*c* 0.48, MeOH); HRMS (ESI): Found 367.2030 $[MH]^+$, $C_{22}H_{27}N_2O_3$ requires 367.2022. Found: C, 65.24; H, 6.75; N, 6.84. Calc. for $C_{22}H_{26}N_2O_3{\cdot}HCl$: C, 65.58; H, 6.75; N, 6.95%.

3-((1*S*,5*R*)-(+)-2-(3-Nitrophenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10l) and 3-((1*R*,5*S*)-(−)-2-(3-nitrophenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10l)

(+)-**10l** was obtained from the reaction of 1*S*,5*R*-**8** and **7l** by method A. Mp 270–272 °C as HCl salt; $[\alpha]_D^{20}$ +13.6 (*c* 0.35, MeOH); ^{1}H NMR ($CDCl_3$, free base): δ 8.12–8.03 (m, 2H), 7.56–7.52 (m, 1H), 7.43 (t, 1H, J = 8 Hz), 7.17 (t, 1H, J = 8 Hz), 6.88 (d, 1H, J = 8 Hz), 6.79 (t, 1H, J = 2 Hz), 6.65 (dd, 1H, J = 8 and 2 Hz), 3.20 (br, 1H), 3.10–2.76 (m, 6H), 2.18–1.84 (m, 7H), 1.74–1.55 (m, 2H), 1.48–1.35 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 156.12, 153.54, 148.24, 142.42, 135.14, 129.33, 129.17, 123.54, 121.26, 116.78, 113.11, 112.34, 56.94, 52.53, 49.39, 38.37, 38.16, 37.02, 34.79, 33.53, 24.64, 22.70; HRMS (ESI): Found 367.2009 $[MH]^+$, $C_{22}H_{27}N_2O_3$ requires 367.2022. Found: C, 65.58; H, 6.95; N, 6.55. Calc. for $C_{22}H_{26}N_2O_3{\cdot}HCl$: C, 65.58; H, 6.75; N, 6.95%.

(−)-**10l** was obtained from the reaction of 1*R*,5*S*-**8** and **7l** by method A. Mp 269–270 °C as HCl salt; $[\alpha]_D^{20}$ −13.1 (*c* 0.35, MeOH); HRMS (ESI): Found 367.2012 $[MH]^+$, $C_{22}H_{27}N_2O_3$ requires 367.2022. Found: C, 64.93; H, 6.84; N, 6.63. Calc. for $C_{22}H_{26}N_2O_3{\cdot}HCl{\cdot}0.25H_2O$: C, 64.86; H, 6.80; N, 6.88%.

3-((1*S*,5*R*)-(+)-2-(4-Nitrophenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10m) and 3-((1*R*,5*S*)-(−)-2-(4-nitrophenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10m)

(+)-**10m** was obtained from the reaction of 1*S*,5*R*-**8** and **7m** by method A. Mp 242–243 °C as HCl salt; $[\alpha]_D^{20}$ +15.9 (*c* 0.42, MeOH); ^{1}H NMR ($CDCl_3$, free base): δ 8.16–8.10 (m, 2H), 7.38–7.33 (m, 2H), 7.17 (t, 1H, J = 8 Hz), 6.88 (d, 1H, J = 8 Hz), 6.78 (t, 1H, J = 2 Hz), 6.63 (dd, 1H, J = 8 and 2 Hz), 3.21 (br, 1H), 3.10–2.78 (m, 6H), 2.18–1.84 (m, 7H), 1.74–1.55 (m, 2H), 1.48–1.34 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 155.96, 153.66, 148.47, 146.49, 129.56, 129.35, 123.58, 116.93, 113.00, 112.30, 56.82, 52.52, 49.39, 38.47, 38.19, 37.08, 34.83, 34.02, 24.72, 22.70; HRMS (ESI): Found 367.2034 $[MH]^+$, $C_{22}H_{27}N_2O_3$ requires 367.2022. Found: C, 65.37; H, 6.89; N, 6.90. Calc. for $C_{22}H_{26}N_2O_3{\cdot}HCl$: C, 65.58; H, 6.75; N, 6.95%.

(−)-**10m** was obtained from the reaction of 1*R*,5*S*-**8** and **7m** by method A. Mp 244–245 °C as HCl salt; $[\alpha]_D^{20}$ −16.0 (*c* 0.33, MeOH); HRMS (ESI): Found 367.2008 $[MH]^+$, $C_{22}H_{27}N_2O_3$ requires 367.2022. Found: C, 65.47; H, 6.82; N, 7.00. Calc. for $C_{22}H_{26}N_2O_3{\cdot}HCl$: C, 65.58; H, 6.75; N, 6.95%.

3-((1*S*,5*R*)-(+)-2-(2-(Pyridin-2-yl)ethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10n) and 3-((1*R*,5*S*)-(−)-2-(2-(pyridin-2-yl)ethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10n)

(+)-**10n** was obtained from the reaction of 1*S*,5*R*-**8** and **7n** by method A. Mp 103–104 °C as free base; $[\alpha]_D^{20}$ +14.7 (*c* 0.33, MeOH, HCl salt); ^{1}H NMR ($CDCl_3$, free base): δ 8.52 (d, 1H, $J = 5$ Hz), 7.61 (dt, 1H, $J = 8$ and 2 Hz), 7.20 (d, 1H, $J = 8$ Hz), 7.18–7.08 (m, 2H), 6.84–6.78 (m, 2H), 6.65 (dd, 1H, $J = 8$ and 2 Hz), 3.20 (br, 1H), 3.10–2.90 (m, 6H), 2.18–1.78 (m, 7H), 1.68–1.52 (m, 2H), 1.42–1.26 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 160.36, 156.78, 153.62, 148.74, 136.76, 129.15, 123.62, 121.41, 116.22, 113.16, 112.57, 55.68, 52.60, 49.22, 38.34, 38.25, 36.94, 36.09, 34.79, 24.83, 22,62; HRMS (ESI): Found 323.2148 $[MH]^+$, $C_{21}H_{27}N_2O$ requires 323.2123. Found: C, 75.84; H, 8.21; N, 8.36. Calc. for $C_{21}H_{26}N_2O{\cdot}0.5H_2O$: C, 76.10; H, 8.21; N, 8.45%.

(−)-**10n** was obtained from the reaction of 1*R*,5*S*-**8** and **7n** by method A. Mp 181–182 °C as HCl salt; $[\alpha]_D^{20}$ −14.7 (*c* 0.33, MeOH, HCl salt); HRMS (ESI): Found 323.2128 $[MH]^+$, $C_{21}H_{27}N_2O$ requires 323.2123. Found: C, 73.83; H, 8.17; N, 8.20. Calc. for $C_{21}H_{26}N_2O{\cdot}H_2O$: C, 74.08; H, 8.29; N, 8.23%.

3-((1*S*,5*R*)-(+)-2-(2-(Pyridin-3-yl)ethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10o) and 3-((1*R*,5*S*)-(−)-2-(2-(pyridin-3-yl)ethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10o)

(+)-**10o** was obtained from the reaction of 1*S*,5*R*-**9** and **7o** by method C. Mp 243–245 °C as HCl salt; $[\alpha]_D^{20}$ +8.0 (*c* 0.60, MeOH, free base); ^{1}H NMR ($CDCl_3$, free base): δ 8.50–8.42 (m, 2H), 7.57 (dt, 1H, $J = 8$ and 2 Hz), 7.28–7.22 (m, 1H), 7.14 (t, 1H, $J = 8$ Hz), 6.86–6.80 (m, 2H), 6.67 (dd, 1H, $J = 8$ and 2 Hz), 3.18 (br, 1H), 3.10–2.94 (m, 2H), 2.88–2.72 (m, 4H), 2.16–1.82 (m, 7H), 1.72–1.52 (m, 2H), 1.42–1.26 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 156.83, 153.55, 149.48, 146.89, 136.80, 136.27, 129.21, 123.54, 116.22, 113.01, 112.39, 57.04, 52.56, 49.33, 38.43, 38.27, 37.06, 34.81, 31.20, 24.81, 22.70; HRMS (ESI): Found 323.2143 $[MH]^+$, $C_{21}H_{27}N_2O$ requires 323.2123. Found: C, 76.08; H, 8.27; N, 8.54. Calc. for $C_{21}H_{26}N_2O{\cdot}0.5H_2O$: C, 76.10; H, 8.21; N, 8.45%.

(−)-**10o** was obtained from the reaction of 1*R*,5*S*-**9** and **7o** by method C. Mp 237–241 °C as HCl salt; $[\alpha]_D^{20}$ −7.9 (*c* 0.60, MeOH, free base); HRMS (ESI): Found 323.2138 $[MH]^+$, $C_{21}H_{27}N_2O$ requires 323.2123. Found: C, 76.17; H, 8.15; N, 8.39. Calc. for $C_{21}H_{26}N_2O{\cdot}0.5H_2O$: C, 76.10; H, 8.21; N, 8.45%.

3-((1*S*,5*R*)-(+)-2-(2-(Pyridin-4-yl)ethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10p) and 3-((1*R*,5*S*)-(−)-2-(2-(pyridin-4-yl)ethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10p)

(+)-**10p** was obtained from the reaction of 1*S*,5*R*-**9** and **7p** by method D. Mp 170–174 °C as HCl salt; $[\alpha]_D^{20}$ +7.4 (*c* 0.60, MeOH, free base); ^{1}H NMR ($CDCl_3$, free base): δ 8.54–8.46 (m, 2H), 7.20–7.12 (m, 3H), 6.86 (d, 1H, $J = 8$ Hz), 6.83 (t, 1H, $J = 2$ Hz), 6.60 (dd, 1H, $J = 8$ and 2 Hz), 3.17 (br, 1H), 3.10–2.92 (m, 2H), 2.88–2.76 (m, 4H), 2.16–1.82 (m, 7H), 1.76–1.54 (m, 2H), 1.46–1.32 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 156.47, 153.70, 150.17, 149.20, 129.25, 124.40, 116.52, 112.89, 112.28, 56.30, 52.57, 49.32, 38.50, 38.29, 37.13, 34.85, 33.60, 24.90, 22.70; HRMS (ESI): Found 323.2115 $[MH]^+$, $C_{21}H_{27}N_2O$ requires 323.2123. Found: C, 76.24; H, 8.02; N, 8.41. Calc. for $C_{21}H_{26}N_2O{\cdot}0.5H_2O$: C, 76.10; H, 8.21; N, 8.45%.

(−)-**10p** was obtained from the reaction of 1*R*,5*S*-**9** and **7p** by method D. Mp 168–172 °C as HCl salt; $[\alpha]_D^{20}$ −7.8 (*c* 0.60, MeOH, free base); HRMS (ESI): Found 323.2104 $[MH]^+$, $C_{21}H_{27}N_2O$ requires 323.2123. Found: C, 76.84; H, 8.01; N, 8.47. Calc. for $C_{21}H_{26}N_2O{\cdot}0.25H_2O$: C, 77.15; H, 8.17; N, 8.57%.

3-(1*S*,5*R*)-(+)-(2-(2-(1*H*-Indol-3-yl)ethyl)-2-aza-bicyclo-[3.3.1]nonan-5-yl)phenol ((+)-10q) and 3-(1*R*,5*S*)-(−)-(2-(2-(1*H*-indol-3-yl)ethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10q)

(+)-**10q** was obtained from the reaction of 1*S*,5*R*-**8** and **7q** by method E. Mp 203–205 °C as free base; $[\alpha]_D^{20}$ +13.4 (*c* 0.38, MeOH, free base); ^{1}H NMR (d_6-DMSO, free base): δ 10.76 (s, 1H), 9.19 (s, 1H), 7.52 (d, 1H, $J = 8$ Hz), 7.33 (d, 1H, $J = 8$ Hz), 7.16 (d, 1H, $J = 2$ Hz), 7.13–6.94 (m, 3H), 6.77 (d, 1H, $J = 8$ Hz), 6.74 (d, 1H, $J = 2$ Hz), 6.57 (dd, 1H, $J = 8$ and 2 Hz), 3.15 (br, 1H), 3.04–2.94 (m, 2H), 2.88–2.71 (m, 4H), 2.12–2.00 (m, 2H), 1.98–1.70 (m, 5H), 1.67–1.44 (m, 2H), 1.40–1.28 (m, 1H); ^{13}C NMR (d_6-DMSO, free base): δ 157.16, 153.72, 136.17, 128.97, 127.34, 122.42, 120.76, 118.27, 118.10, 115.23, 112.89, 112.36, 111.71, 111.30, 56.32, 51.90, 48.74, 38.44, 38.29, 37.09, 34.52, 24.77, 23.46, 22.42; HRMS (ESI): Found 361.2299 $[MH]^+$, $C_{24}H_{29}N_2O$ requires 361.2280. Found: C, 77.97; H, 7.90; N, 7.44. Calc. for $C_{24}H_{28}N_2O{\cdot}0.5H_2O$: C, 78.01; H, 7.91; N, 7.58%.

(−)-**10q** was obtained from the reaction of 1*R*,5*S*-**8** and **7q** by method E. Mp 201–203 °C as free base; $[\alpha]_D^{20}$ −13.6 (*c* 0.38, MeOH, free base); HRMS (ESI): Found 361.2287 $[MH]^+$, $C_{24}H_{29}N_2O$ requires 361.2280. Found: C, 77.94; H, 7.86; N, 7.54. Calc. for $C_{24}H_{28}N_2O{\cdot}0.25H_2O$: C, 78.98; H, 7.86; N, 7.54%.

3-(1*S*,5*R*)-(+)-(2-(2-Hydroxyphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10r) and 3-(1*R*,5*S*)-(−)-(2-(2-hydroxyphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10r)

(+)-**10r** was obtained from the reaction of 1*S*,5*R*-**8** and **7d** by method A. Mp 186–188 °C as HBr salt; $[\alpha]_D^{20}$ +14.3 (*c* 0.38, MeOH); ^{1}H NMR ($CDCl_3$, free base): δ 7.16–7.08 (m, 2H), 6.99 (d, 1H, $J = 7$ Hz), 6.89–6.80 (m, 3H), 6.73 (d, 1H, $J = 7$ Hz), 6.69 (d, 1H, $J = 8$ Hz), 3.27 (br, 1H), 3.15–3.02 (m, 1H), 2.98–2.95 (m, 1H), 2.77–2.29 (m, 2H), 2.18–1.90 (m, 8H), 1.77–1.64 (m, 2H), 1.57–1.50 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 157.68, 156.80, 152.48, 130.94, 129.22, 128.53, 127.91, 118.88, 117.85, 116.35, 112.93, 112.35, 56.92, 54.15, 48.93, 37.87, 37.75, 36.43, 34.51, 31.35, 24.07, 22.64; HRMS (ESI): Found 338.2123 $[MH]^+$, $C_{22}H_{28}NO_2$ requires 338.2120. Found: C, 63.24; H, 7.23; N, 2.89. Calc. for $C_{22}H_{27}NO_2{\cdot}HBr{\cdot}0.75THF$: C, 63.56; H, 7.25; N, 2.96%.

(−)-**10r** was obtained from the reaction of 1*R*,5*S*-**8** and **7d** by method A. Mp 187–188 °C as HBr salt; $[\alpha]_D^{20}$ −14.7 (*c* 0.38, MeOH); HRMS (ESI): Found 338.2135 $[MH]^+$, $C_{22}H_{28}NO_2$ requires 338.2120. Found: C, 62.99; H, 7.16; N, 2.92. Calc. for $C_{22}H_{27}NO_2{\cdot}HBr{\cdot}0.25THF$: C, 63.30; H, 6.93; N, 3.21%.

3-(1*S*,5*R*)-(+)-(2-(3-Hydroxyphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10s) and 3-(1*R*,5*S*)-(−)-(2-(3-hydroxyphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10s)

(+)-**10s** was obtained from the reaction of 1*S*,5*R*-**8** and **7e** by method A. Mp 250–252 °C as HCl salt; $[\alpha]_D^{20}$ +16.7 (*c* 0.30, MeOH, free base); ^{1}H NMR (d_6-acetone free base): δ 7.27–7.16 (m, 2H), 6.99–6.93 (m, 2H), 6.88–6.85 (m, 1H), 6.82 (d, 1H, J = 8 Hz), 6.79–6.73 (m, 2H), 3.27 (br, 1H), 3.21–3.02 (m, 2H), 2.98–2.78 (m, 4H), 2.28–1.92 (m, 7H), 1.80–1.64 (m, 2H), 1.57–1.44 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 158.31, 158.22, 155.04, 143.49, 130.08, 129.99, 120.78, 116.74, 116.64, 113.73, 113.35, 112.83, 58.56, 53.63, 49.87, 39.53, 39.44, 38.27, 35.72, 35.16, 26.00, 23.51; HRMS (ESI): Found 338.2105 $[MH]^+$, $C_{22}H_{28}NO_2$ requires 338.2120. Found: C, 70.42; H, 7.59; N, 3.58. Calc. for $C_{22}H_{27}NO_2$·HCl: C, 70.67; H, 7.55; N, 3.75%.

(−)-**10s** was obtained from the reaction of 1*R*,5*S*-**8** and **7e** by method A. Mp 242–243 °C as HBr salt; $[\alpha]_D^{20}$ −11.7 (*c* 0.36, MeOH); HRMS (ESI): Found 338.2124 $[MH]^+$, $C_{22}H_{28}NO_2$ requires 338.2120. Found: C, 63.41; H, 6.87; N, 3.44. Calc. for $C_{22}H_{27}NO_2$·HBr: C, 63.16; H, 6.75; N, 3.35%.

3-(1*S*,5*R*)-(+)-(2-(4-Hydroxyphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10t) and 3-(1*R*,5*S*)-(−)-(2-(4-hydroxyphenethyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10t)

(+)-**10t** was obtained from the reaction of 1*S*,5*R*-**8** and **7f** by method A. Mp 265–267 °C as HCl salt; $[\alpha]_D^{20}$ +14.9 (*c* 0.38, MeOH); ^{1}H NMR (d_6-acetone free base): δ 7.27–7.16 (m, 3H), 6.99–6.93 (m, 2H), 6.88–6.83 (m, 2H), 6.79–6.73 (m, 1H), 3.25 (br, 1H), 3.21–3.02 (m, 2H), 2.96–2.76 (m, 4H), 2.28–1.90 (m, 7H), 1.80–1.60 (m, 2H), 1.56–1.40 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 158.22, 156.45, 155.13, 132.62, 130.54, 129.98, 116.75, 115.95, 113.32, 112.82, 59.07, 53.65, 49.85, 39.59, 39.51, 38.37, 35.76, 34.42, 26.11, 23.51; HRMS (ESI): Found 338.2113 $[MH]^+$, $C_{22}H_{28}NO_2$ requires 338.2120. Found: C, 70.44; H, 7.58; N, 3.76. Calc. for $C_{22}H_{27}NO_2$·HCl: C, 70.67; H, 7.55; N, 3.75%.

(−)-**10t** was obtained from the reaction of 1*R*,5*S*-**8** and **7f** by method A. Mp 265–266 °C as HCl salt; $[\alpha]_D^{20}$ −15.1 (*c* 0.38, MeOH); HRMS (ESI): Found 338.2102 $[MH]^+$, $C_{22}H_{28}NO_2$ requires 338.2120. Found: C, 70.56; H, 7.70; N, 3.73. Calc. for $C_{22}H_{27}NO_2$·HCl: C, 70.67; H, 7.55; N, 3.75%.

3-((1*S*,5*R*)-(+)-2-(But-3-enyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((+)-10u) and 3-((1*R*,5*S*)-(−)-2-(but-3-enyl)-2-aza-bicyclo[3.3.1]nonan-5-yl)phenol ((−)-10u)

(+)-**10u** was obtained from the reaction of 1*S*,5*R*-**8** and **7u** by method F. Mp 226–227 °C as HCl salt; $[\alpha]_D^{20}$ +6.6 (*c* 0.35, MeOH); ^{1}H NMR ($CDCl_3$, free base): δ 8.46 (br-s, 1H), 7.08 (t, 1H, J = 8 Hz), 6.76 (d, 1H, J = 8 Hz), 6.70 (m, 1H), 6.56 (dd, 1H, J = 8 and 2 Hz), 5.85–5.70 (m, 1H), 5.10–4.95 (m, 2H), 3.21 (br, 1H), 3.04–2.92 (m, 2H), 2.73–2.24 (m, 2H), 2.38–2.25 (m, 2H), 2.20–2.07 (m, 2H), 2.02–1.95 (m, 5H), 1.72–1.47 (m, 2H), 1.45–1.29 (m, 1H); ^{13}C NMR ($CDCl_3$, free base): δ 156.69, 153.14, 136.16, 129.09, 116.06, 115.82, 113.42, 112.71, 54.82, 51.98, 49.51, 38.06, 37.90, 36.64, 34.60, 31.32, 24.22, 22.67; HRMS (ESI): Found 272.2022 $[MH]^+$, $C_{18}H_{26}NO$ requires 272.2014. Found: C, 69.99; H, 8.46; N, 4.49. Calc. for $C_{18}H_{25}NO$·HCl: C, 70.22; H, 8.51; N, 4.55%.

(−)-**10u** was obtained from the reaction of 1*R*,5*S*-**8** and **7u** by method F. Mp 225–227 °C as HCl salt; $[\alpha]_D^{20}$ −6.3 (*c* 0.35, MeOH); HRMS (ESI): Found 272.2010 $[MH]^+$, $C_{18}H_{26}NO$ requires 272.2014. Found: C, 69.57; H, 8.51; N, 4.45. Calc. for $C_{18}H_{25}NO$·HCl·0.25H_2O: C, 69.21; H, 8.55; N, 4.48%.

Pharmacological methods

Antinociceptive (hot plate, tail-flick, phenylquinone) assays in mice. These assays were run as previously noted.[29]

Cell culture and membrane preparation

The recombinant CHO cells (hMOR-CHO, hDOR-CHO and hKOR-CHO) were produced by stable transfection with the respective human opioid receptor cDNA, and provided by Dr Larry Toll (SRI International, CA). The cells were grown on plastic flasks in DMEM (100%) (hDOR-CHO and hKOR-CHO) or DMEM–F-12 (50%–50%) medium (hMOR-CHO) containing 10% FBS, and G-418 (0.10-0.2 mg/ml) under 95% air–5% CO_2 at 37 °C. Cell monolayers were harvested and frozen in −80 °C.

[^{35}S]GTP-γ-S binding assays

On the day of the assay, cells were thawed on ice for 15 min and homogenized using a polytron in 50 mM Tris-HCl, pH 7.4, containing 4 μg mL^{-1} leupeptin, 2 μg mL^{-1} chymostatin, 10 μg mL^{-1} bestatin and 100 μg mL^{-1} bacitracin, The homogenate was centrifuged at 30000*g* for 10 min at 4 °C, and the supernatant discarded. The membrane pellets were resuspended in binding buffer and used for [^{35}S]GTP-γ-S binding assays. [^{35}S]GTP-γ-S binding was determined as described previously.[30] Briefly, test tubes received the following additions: 50 μL buffer A (50 mM Tris-HCl, pH 7.4, containing 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA), 50 μL GDP in buffer A–0.1% BSA (final concentration = 10 μM), 50 μL drug in buffer A–0.1% BSA, 50 μL [^{35}S]GTP-γ-S in buffer A–0.1% BSA (final concentration = 50 pM), and 300 μL of cell membranes (50 μg of protein) in buffer B. The final concentrations of reagents in the [^{35}S]GTP-γ-S binding assays were: 50 mM Tris-HCl, pH 7.4, containing 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 10 μM GDP and 0.1% BSA. Incubations proceeded for 3 h at 25 °C. Nonspecific binding was determined using GTP-γ-S (40 μM). Bound and free [^{35}S]-GTP-γ-S were separated by vacuum filtration through GF/B filters. The filters were punched into 24-well plates to which was added 0.6 mL LSC-cocktail (Cytoscint). Samples were counted, after an overnight extraction, in a Trilux liquid scintillation counter at 27% efficiency.

Opioid binding assays

Opioid binding assays proceeded according to published procedures.[31,32] μ-Receptors were labeled with [^{3}H]DAMGO. Rat membranes for μ- and δ-receptor binding assays were prepared each day using a partially thawed frozen rat brain that was homogenized with a polytron in 10 mL per brain of ice-cold 10 mM Tris-HCl, pH 7.0. Membranes were then centrifuged twice at 30000*g* for 10 min and resuspended with ice-cold buffer following each

centrifugation. After the second centrifugation, the membranes were resuspended in 50 mM Tris-HCl, pH 7.4 (60 mL per brain), at 25 °C. Incubations proceeded for 2 h at 25 °C in 50 mM Tris-HCl, pH 7.4, along with a protease inhibitor cocktail [bacitracin (100 μg mL^{-1}), bestatin (10 μg mL^{-1}), leupeptin (4 μg mL^{-1}) and chymostatin (2 μg mL^{-1})]. The nonspecific binding was determined using 20 μM of levallorphan. Delta binding sites were labeled using [^{3}H]DADLE (2 nm) and rat brain membranes. Rat membranes were prepared each day as described above. After the second centrifugation, the membranes were resuspended in 50 mM Tris-HCl, pH 7.4 (50 mL per brain), at 25 °C. Incubations proceeded for 2 h at 25 °C in 50 mM Tris-HCl, pH 7.4, containing 100 mM choline chloride, 3 mM $MnCl_2$, 100 nM DAMGO to block binding to μ-sites, and the protease inhibitor cocktail. Nonspecific binding was determined using 20 μM levallorphan. κ-Binding sites were labeled using [^{3}H]U69593 (2 nm). Guinea pig brain membranes were prepared each day using partially thawed guinea pig brain that was homogenized with a polytron in 15 mL per brain of ice-cold 10 mM Tris-HCl, pH 7.0. The membranes were then centrifuged twice at 30000*g* for 10 min and resuspended with ice-cold buffer following each centrifugation. After the second centrifugation, the membranes were resuspended in 50 mM Tris-HCl, pH 7.4 (85 mL per brain), at 25 °C. Incubations proceeded for 2 h at 25 °C in 50 mM Tris-HCl, pH 7.4, containing 1 μg mL^{-1} of captopril and the protease inhibitor cocktail. Nonspecific binding was determined using 1 μM U69593. Each radioligand was displaced by 8–10 concentrations of test drug. Compounds were stored as a 10 mM stock solution in 100% DMSO. All drug dilutions were done in 10 mM Tris-HCl, pH 7.4, containing 1 mg mL^{-1} bovine serum albumin. All washes were done with ice-cold 10 mM Tris-HCl, pH 7.4.

Data analysis and statistics

For [^{35}S]GTP-γ-S binding experiments, the percent stimulation of [^{35}S]GTP-γ-S binding was calculated according to the following formula: (S − B)/B × 100, where B is the basal level of [^{35}S]GTP-γ-S binding and S is the stimulated level of [^{35}S]GTP-γ-S binding.[33] EC_{50} values (the concentration that produces fifty percent maximal stimulation of [^{35}S]GTP-γ-S binding) and E_{max} (% of maximal stimulation in the [^{35}S]-GTP-γ-S binding) were determined using either the program MLAB-PC (Civilized Software, Bethesda, MD) or KaleidaGraph (Version 3.6.4, Synergy Software, Reading, PA). For opioid binding experiments, the data were fit to the two-parameter logistic equation for the best-fit estimates of the IC_{50} and N values: $Y = 100/(1 + ([\text{INHIBITOR}]/IC_{50})^N)$, where "$Y$" is the percent of control value.

Sources

[^{35}S]GTP-γ-S (SA = 1250 Ci $mmol^{-1}$) was obtained from DuPont NEN (Boston, MA). Various opioid peptides were provided by Multiple Peptide System *via* the Research Technology Branch, NIDA. The sources for the reagents used in the opioid binding assays have been described.[31,32]

Warm water tail-flick test

Antinociception was assessed at 55 °C. The latency to the first sign of a rapid tail-flick was taken as the behavioral endpoint. Each mouse was first tested for baseline latency by immersing its tail in the water and recording the time to response. Mice not responding within 5 s were excluded from further testing. Mice were then administered the test compound and tested for antinociception at 10, 20, 30, 45, 60, 90, 120, 150 and 180 min post-injection. A cutoff of 10 s was used to avoid tissue damage. Antinociception was calculated by the following formula: % antinociception = 100 × (test latency − control latency)/(10 − control latency). Dose–response lines were constructed at times of peak agonist effect and analyzed by linear regression using FlashCalc software (Dr Michael Ossipov, University of Arizona, Tucson, AZ). All A_{50} values (95% confidence limits) shown are calculated from the linear portion of the dose–response curve. A minimum of three doses per curve and 10 mice per dose were used.

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