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Structure–Activity Relationship of N17′-Substituted Norbinaltorphimine Congeners. Role of the N17′ Basic Group in the Interaction with a Putative Address Subsite on the κ Opioid Receptor

P. S. Portoghese,*,† C.-E. Lin,† F. Farouz-Grant,† and A. E. Takemori‡

Department of Medicinal Chemistry, College of Pharmacy, and Department of Pharmacology, Medical Sch University of Minnesota, Minneapolis, Minnesota 55455

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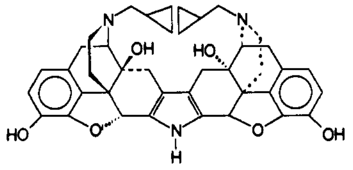
A series of norbinaltorphimine congeners (**2–12**) which contain different groups at the N17′-position have been synthesized in order to evaluate the role of N17′ in conferring κ opioid antagonist selectivity at opioid receptor sites. The compounds that contain a basic N17′ nitrogen (**2–9**) were found to be selective κ antagonists. Amidation of N17′ afforded congeners **10–12** with feeble κ antagonist potency and low selectivity. The fact that potent antagonism and selectivity were observed only when members of the series contain a basic N17′ nitrogen suggests that it interacts with extracellular domains of the κ receptor that contain acidic amino acid residues. The N-terminal domain and extracellular loop 2, both of which contain acidic residues, are candidates for this interaction and may be components of the κ address subsite of the receptor.

It is now widely accepted that there are at least three major types of opioid receptors (μ, δ, and κ) that modulate numerous central and peripheral effects of endogenous mediators.[1] In order to sort out the multiple types of opioid receptors that mediate these effects, highly selective antagonists have been developed as pharmacological probes.[2] The prototypical opioid antagonist that is diagnostic for actions mediated by κ-receptors is norbinaltorphimine[3] (**1**, norBNI). Structure–activity studies[4,5] have revealed that only one of the two (−)-naltrexone-derived pharmacophores is required for the κ-antagonist activity of **1**. Moreover, we have demonstrated that the geometry of the spacer which connects the pharmacophores plays a role in modulating the potency and selectivity in this series, presumably by acting as a rigid scaffold to orient the second basic nitrogen (N17′) of **1**.[6] In this connection, a very recent study has confirmed the necessity of a properly oriented second basic nitrogen for κ antagonist selectivity.[7]



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It has been suggested[5] that the N17′ basic nitrogen of **1** may mimic the guanidine moiety of Arg[7] of dynorphin A,[8] a κ-selective opioid peptide. The protonated form of the guanidine group of Arg[7] is believed to be a key part of the dynorphin address.[9] This guanidine group was hypothesized to interact with a subsite which contains acidic residues that are unique to the κ receptor system.[6] A series of novel κ antagnoists based on this model has been recently reported.[10] Moreover, the very recent cloning of the κ opioid receptor has provided support for this model because this G protein coupled receptor contains a substantial number of acidic residues on its extracellular domains.[11]

In order to investigate the role of the N17′ basic nitrogen in **1**, and its requirement as the primary recognition component for κ receptor selectivity, we have synthesized norBNI analogus **2–12** which contain different substituents on the second basic nitrogen. The results of this study emphasize the critical importance of a basic address mimic in the recognition of κ-selective opioid antagonists.

Chemistry

Bimorphinan **2** was obtained by refluxing in glacial acetic acid equivalent amounts of naltrexone (**13**), noroxymorphone (**14**), and hydrazine dihydrochloride (Scheme 1). Compounds **3–7** were obtained from **2** by reductive alkylation with sodium cyanoborohydride and the appropriate aldehydes in a methanolic pH 6.5 KOAc–HOAc buffer. Hydrogenolysis of **7** afforded the ethylamine **8**, which upon treatment with formamidinesulfinic acid in the presence of triethylamine, yielded the corresponding guanidine **9**. The acetamide **10** was prepared by reaction of **2** with acetic anhydride. Coupling of **2** with Z-Gly or Z-Gly-Gly afforded **15** and **16**, respectively, which upon hydrogenolysis gave the corresponding amides **11** and **12**.

Biological Results

Smooth Muscle Preparations. Compounds **2–12** were tested on the electrically stimulated guinea pig ileal longitudinal muscle (GPI) and mouse vas deferens (MVD) preparations as described previously.[12] Test compounds (100 nM) were incubated for 15 min with the preparation prior to the testing with standard agonists. Morphine (M), ethylketazocine (EK), and [D-Ala^2,D-Leu^5]enkephalin[13] (DADLE) were employed as μ-, κ-, and δ-selective agonists, respectively. Morphine and EK were employed in the GPI, and DADLE was used in the MVD preparation. A minimum of three replicate determinations were carried out for each compound. The antagonist potency is expressed as the IC_{50} ratio which is the IC_{50} of the agonist in the presence of antagonist divided by the control IC_{50} of the agonist in the same preparation. Antagonist

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* Author to whom correspondence may be addressed.

† Department of Medicinal Chemistry.

‡ Department of Pharmacology.

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Scheme 1

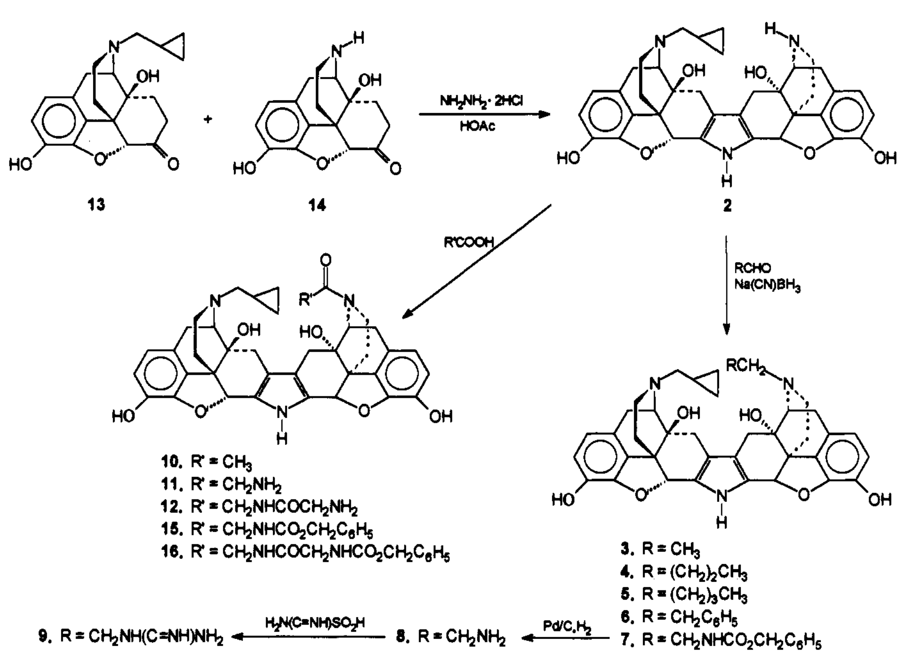
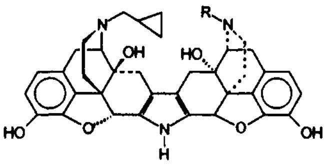


Table 1. Opioid Antagonist Potencies of Norbinaltorphimine Analogues in the GPI and MVD Preparations



	R^a	EK^b IC_{50} ratio	EK^b K_e (nM)	M^b IC_{50} ratio	$DADLE^c$ IC_{50} ratio	selectivity ratio κ/μ	selectivity ratio κ/δ
1	$CH_2CH(CH_2)_2$(norBNI)	181 ± 7	0.55	8.3 ± 1.8	10.4 ± 2.9	22	17
2	H	41 ± 10	2.5	1.9 ± 0.4	8.5 ± 1.7	22	4.8
3	CH_2CH_3	22 ± 5	4.7	1.6 ± 0.5	6.3 ± 0.5	14	3.5
4	$(CH_2)_3CH_3$	61 ± 16	1.70	1.8 ± 0.6	1.9 ± 0.9	34	32
5	$(CH_2)_4CH_3$	27 ± 8	3.8	3.1 ± 1.5	3.1 ± 0.7	8.7	8.7
6	$(CH_2)_2$Ph	44 ± 13	2.4	1.8 ± 0.4	2.4 ± 0.5	24	18
7	$(CH_2)_2$NHCbz	26 ± 9	4.0	4.5 ± 1	4.6 ± 1.2	5.7	5.7
8	$(CH_2)_2NH_2$	20 ± 5	5.1	1.6 ± 0.6	1.9 ± 0.6	12.5	10
9	$(CH_2)_2NH(C{=}NH)NH_2$	27 ± 6	3.8	1.7 ± 0.5	1.0 ± 0.2	15	27
10	$COCH_3$	4.3 ± 1	30.3	1.8 ± 0.5	2.8 ± 0.5	2.4	1.5
11	$COCH_2NH_2$	11.8 ± 3.1	9.3	1.4 ± 0.3	2.2 ± 0.6	8.4	5.4
12	$COCH_2NHCOCH_2NH_2$	9.6 ± 2.5	11.7	2.0 ± 0.4	0.67 ± 0.09	4.8	*d*

[a] The concentration of the antagonist was 100 nM. [b] GPI preparation. [c] MVD preparation. [d] Not calculated because IC_{50} ratio was <1.

potencies expressed as K_e values are derived from the equation K_e = [antagonist]/(IC_{50} ratio-1).

The compounds **2–9**, which contain two basic piperidine nitrogens (N17 and N17′), were found to be selective κ opioid receptor antagonists with K_e values ranging from 1.7 to 5.1 nM (Table 1). The most potent compound (**4**) in this group possessed one-third the antagonist activity of norBNI (**1**). However, the selectivity of **4** surpassed that of **1**. Amides **10–12** exhibited substantially lower κ potency than compounds (**2–9**), the N17′ atom of which is basic. Introduction of a basic nitrogen in the substituent (**11**, **12**) appeared to offset the detrimental effect of amidation, but these compounds were generally less selective than compounds **1–9**.

None of the ligands possessed significant agonism at 1 μM in the GPI or MVD and, in fact, one compound (**8**) enhanced the twitch by 107% in the GPI.

Binding

Opioid receptor binding assays using guinea pig brain membranes were carried out on selected compounds (Table 2) by competition with selective radioligands using a modification of the procedure of Werling et al.[14] Binding to κ, μ, and δ sites was determined by displacement of tritiated (−)-(5α,7α,8β)-*N*-methyl[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide[15] (U69593), [D-Ala^2,MePhe^4,Gly-ol^6]enkephalin[16] (DAMGO), and [D-Pen^2,D-Pen^5]enkephalin[17] (DPDPE), respectively.

The K_i values for **7** and **9** at κ sites were comparable to that of norBNI (**1**). However, the most potent compound (**4**) in the present series possessed 7-fold greater affinity relative to **1**. Because the binding curve of **4** was bell-shaped and did not reach a maximum, its K_i value was calculated from the descending portion of the curve. The amide **12** has >2 orders of magnitude less affinity for κ sites than other members of the series.

Discussion

The results of the present study are consistent with the message–address model[18,19] discussed in connection with prior structure–activity studies on norBNI-related κ opioid antagonists.[4–7,10] Thus, one of the antagonist pharma-

Table 2. Binding of Norbinaltorphimine Analogues to Guinea Pig Brain Membranes

compound	K_i (nM)[a] μ	K_i (nM)[a] κ	K_i (nM)[a] δ	K_i selectivity ratio μ/κ	K_i selectivity ratio δ/κ
1[b](norBNI)	47	0.28	43	168	154
4	73 (43–122)	0.04 (0.004–0.37)[c]	15 (11–22)	1825	375
7	70 (19–260)	0.36 (0.02–8.4)[c]	117 (23–585)	194	325
8	46 (37–580)	3.4 (1.6–7.0)	137 (28–663)	14	41
9	50 (13–202)	0.59 (0.08–4.7)	37 (20–68)	85	63
12	66 (27–160)	21 (13–35)	>1000	3	>15

[a] The geometric mean (95% confidence interval) of three replicate determinations for competition with [^{3}H]DAMGO (μ), [^{3}H]U69593 (κ), and [^{3}H]DPDPE (δ). [b] Data for norBNI is taken from Takemori, A. E.; Ho, B. Y.; Naeseth, J. S.; Portoghese, P. S. *J. Pharmacol. Exp. Ther.* 1988, *246*, 255–258. [c] Calculated from the descending portion of a bell-shaped binding curve.

cophores of norBNI 1 was envisages to interact with a message subsite, and the N17′ basic nitrogen of the second pharmacophore was postulated to associate with an acidic amino acid residue that is part of the address subsite. In view of the acidic residues which are present on the extracellular domains of the κ receptor,[11] this appears to be a reasonable location for the address subsite.

The fact that modification of the N17′-alkyl substituent in congeners **2**–**9** does not produce a large change of κ-antagonist potency is consistent with the view that the cationic form of N17′ associates with one or more of the many acidic residues in the N-terminus and/or extracellular loop 2 of the κ receptor. We consider these domains to be important selectivity determinants because the corresponding extracellular domains of the δ and μ receptors contain fewer acidic residues.[11,20–22] The fact that the transmembrane domains of the δ, μ, and κ receptors are highly homologous makes it unlikely that these domains contribute significantly to the selection of different opioid ligands.

Norbinaltorphimine (**1**) is 3-fold more potent than the most potent member (**4**) of the series, but this may be related more to the C_2 symmetry of **1**, as it possesses two identical antagonist pharmacophores.

Significantly, the N17′-acylated congeners **10**–**12** are generally less potent and less selective as κ antagonists. Apparently, elimination of the basicity of N17′ by amidation hinders association with the acidic residues in the address subsite. It is noteworthy that the *N*-glycyl congeners **11** and **12** were found to be somewhat more potent than the acetamide **10**. One possible reason for this is the presence of an amino group which may weakly associate with acidic residues in the locus of the address subsite. The apparent reduction in the association may be related to the greater distance between the antagonist pharmacophore and basic nitrogen when compared to norBNI (**1**). In this regard, we have shown that the relative position of the basic nitrogens are important in the recognition process.[6]

The rank order binding affinities (**4** > **7** > **9** > **12**) for κ sites parallels that of the pharmacologic antagonist potencies. However, the ligand **4** with the lowest K_i in the present series appears to have a 7-fold higher affinity than that of norBNI **1**, even though it is one-third as potent as a κ antagonist. As **4** exhibited a bell-shaped binding curve, it is possible that multiple interacting binding sites are involved.

The possibility of multiple binding sites on a single opioid receptor was proposed nearly 30 years ago from structure–activity studies.[23] Since that time, additional evidence has suggested that this may be a common occurrence with opioid ligands.[24,25] Particularly noteworthy are reports that suggest different binding sites for opioid agonists and antagonists.[26,27] Very recently, evidence for different binding sites on the δ opioid receptor was obtained from the point mutation of Asp95 to Asn (TM2), in that it afforded a mutant with reduced binding for agonists but not antagonists.[28]

Although the message–address concept is of heuristic value in the design of selective opioid ligands, it may not be suitable for a detailed comparison of the interaction of opioid agonists and antagonists with opioid receptors. The modeling of mutant opioid receptors should provide greater insight into the question of multiple binding of opioid ligands to a single type of opioid receptor.

Experimental Section

Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and are within ±0.4% of the theoretical values. IR spectra were obtianed on a Perkin-Elmer 281 infrared spectrometer and peaks positions are expressed in cm^{-1}. NMR spectra were recorded at ambient temperature on Ge-300 300-MHz and Bruker AC-200 200-MHz instruments and chemical, shifts are reported as δ values (ppm) relative to TMS. Mass spectra were obtained on a VG 7070E-HF instrument. All TLC data were determined with E. Merck Art. 5554 DC-Alufolien Kieselgel 60 F$_{254}$. Column chromatography was carried out on E. Merck silica gel 60 (230–400 mesh). The eluents used during column chromatography and reverse-phase preparative HPLC (21.1 mm × 50 cm C$_{18}$, 10 μm), $CHCl_3$–MeOH–NH_4OH and MeOH–H_2O–CH_3CN, are denoted by CMA and MWA, respectively. The flow rate used during reverse-phase HPLC was 10 mL/min. Dimethylformamide was distilled from calcium hydride, and tetrahydrofuran was distilled from Na/benzophenone. All other solvents and reagents were used without any further purifications unless specified. Naltrexone hydrochloride salt and noroxymorphone were provided by Mallinckrodt.

17-(Cyclopropylmethyl)-6,6′,7,7′-tetradehydro-4,5α:4′,5α′-diepoxy-6,6′-imino-7,7′-bimorphinan-3,3′,14,14′-tetrol (2). Noroxymorphone **14** (1.00 g, 3.48 mmol), naltrexone **13** (1.0 equiv, 1.18 g), and hydrazine dihydrochloride (1.02 equiv, 373 mg) were dissolved in 100 mL of glacial acetic acid. The reaction mixture was refluxed for 17 h. The solvent was evaporated under reduced pressure. Purification of the crude material was accomplished by column chromatography (silica gel), eluted with CMA 95:5:0.5, to provide the desired material in 51% yield (1.18 g). The product **2** was recrystallized with MeOH–Et_2O and isolated as a solid: mp >230 °C; ^{1}H NMR (300 MHz, D_2O) δ 6.78 (m, 4H, H_2 $H_{2'}$ H_1 $H_{1'}$), 5.61 (s, 1H, H_5), 5.59 (s, 1H, $H_{5'}$), 4.11 (d, 1H, J = 6.30 Hz, H_9), 3.84 (d, 1H, J = 6.30 Hz, $H_{9'}$), 3.43–3.14 (m, 7H), 3.05–2.95 (m, 3H), 2.57–2.23 (m, 6H, H_{15} $H_{15'}$ H_8 $H_{8'}$), 1.85 (m, 2H, H_{15} $H_{15'}$), 1.08 (m, 1H, H_{19}), 0.53 (m, 2H, H_{20} H_{21}), 0.16 (m, 2H, H_{20} H_{21}); ^{13}C NMR (50 MHz, D_2O–methanol-d_4) δ 145.38, 145.29, 141.82, 141.49, 131.67, 131.56, 127.30, 127.28, 125.50, 125.14, 124.79, 122.54, 120.60, 117.06, 117.02, 86.70, 86.48, 75.73, 74.58, 64.21, 60.41, 59.90, 50.39, 48.93, 48.91, 48.55, 39.32, 31.38, 30.96, 30.70, 29.58, 26.29, 8.03, 7.66, 5.05; IR (KBr) 3142, 3050, 1640, 1620 (w), 1507, 1405, 1324 cm^{-1}; HRMS (FAB) 608 (M + H$^+$), calcd 608.2760, obsd 608.2758. Anal. ($C_{36}H_{37}O_6N_3 \cdot 2CH_3OH$) C, H, N: calcd 6.25, found 5.75.

17-(Cyclopropylmethyl)-17′-ethyl-6,6′,7,7′-tetradehydro-4,5α:4′,5α′-diepoxy-6,6′-imino-7,7′-bimorphinan-3,3′,14,14′-tetrol (3). Compound **2** (316.1 mg, 0.52 mmol) was dissolved in 8 mL of buffer (KOAc–HOAc in methanol, pH 6.8). Acetaldehyde (1.79 mmol, 0.1 mL, 78 mg) and sodium cyanoborohydride (196 mg, 3.12 mmol) were added, and the mixture was stirred at room temperature for 16 h. The solvent was removed under vacuum and the residue was treated with water–ethyl acetate. Ammonium hydroxide was then added and the organic phase was separated, washed with brine, dried, and evaporated to afford a solid residue which was purified on a Chromatotron (CMA 90:9:1) to give **3** (110.1 mg, 67%). Compound **3** was further purified by reverse-phase preparative HPLC (MWA 30:19:50 + 1% NH_4OH; t_R = 18.4 min), followed by normal-phase preparative HPLC (CMA 92:8:0.5) to give pure **3** (R_f = 0.15 in CMA 92:8:0.5; R_f = 0.78 in CMA 84:15:1): mp >220 °C; ^1H NMR (300 MHz, methanol-d_4) δ 6.58 (m, 4H, H_1 H_2 $H_{1'}$ $H_{2'}$), 5.36 (s, 2H, H_5 $H_{5'}$), 3.21 (m, 1H), 3.10 (m, 3H), 2.65–2.85 (m, 3H), 2.40 (m, 4H), 2.30 (m, 4H), 2.11 (m, 7H), 1.65 (m, 2H, H_{15} $H_{15'}$), 1.08 (q, 3H), 0.92 (m, 1H, H_{19}), 0.48 (m, 2H, H_{20} H_{21}), 0.13 (m, 2H, H_{20} H_{21}); ^{13}C NMR (75 MHz, methanol-d_4) δ 144.73 (C_3 and $C_{3'}$), 141.11 (C_4 and $C_{4'}$), 132.16 (C_6 and $C_{6'}$), 126.48 (C_{12} and $C_{12'}$), 125.01 (C_{11} and $C_{11'}$), 119.41 (C_1 and $C_{1'}$), 118.48 (C_2 and $C_{2'}$), 116.34 (C_7 and $C_{7'}$), 85.83 (C_5 and $C_{5'}$), 74.62 (C_{14}), 74.52 ($C_{14'}$), 63.45 (C_9), 60.37 (C_{18}), 48.86 (C_{13} and $C_{13'}$), 44.55 (CH_2), 44.31 ($C_{16'}$ and C_{16}), 32.67 (C_8 and $C_{8'}$), 29.85 (C_{10} and $C_{10'}$), 23.91 (C_{15} and $C_{15'}$), 13.36 (CH_3), 10.16 (C_{19}), 4.54 (C_{20}), 4.08 (C_{21}); IR (KBr) 3395, 1632, 1615 cm^{-1}; HRMS (FAB) 636 (M + H^+), calcd 636.3074, obsd 636.3083. Anal. ($C_{38}H_{41}O_6N_3\cdot H_2O$) C, H, N.

17-(Cyclopropylmethyl)-17′-butyl-6,6′,7,7′-tetradehydro-4,5α:4′,5α′-diepoxy-6,6′-imino-7,7′-bimorphinan-3,3′,14,14′-tetrol (4). Compound **2** (271 mg, 0.45 mmol) was reacted with butyraldehyde (0.15 mL, 120 mg, 1.66 mmol) and sodium cyanoborohydride (203 mg, 3.23 mmol) as described for **3**. Purification of the crude material by reverse-phase preparative HPLC (MWA 35:19:45 + 1% NH_4OH; t_R = 40.1 min) and normal-phase preparative HPLC (CMA 85:15:0.5; t_R = 11.3 min) led to **4** (75 mg, 25%): mp >220 °C; ^1H NMR (300 MHz, methanol-d_4) δ 6.67 (m, 4H, H, H′, H_2, H_2'), 5.55 (s, 2H, H_5 $H_{5'}$), 3.44 (m, 1H, H_9), 3.36 (d, 1H, J = 6.60 Hz, $H_{9'}$), 3.24 (d, 1H, J = 9.21 Hz, H_{10}), 3.18 (m, 1H, $H_{10'}$), 2.94 (m, 1H), 2.85 (M, 1H), 2.79 (m, 1H), 2.69 (m, 1H), 2.63 (m, 2H), 2.54 (m, 2H), 2.49 (m, 1H), 2.37 (m, 6H, H_{15} H_{15}' H_8 $H_{8'}$), 1.78 (m, 2H, H_{15} $H_{15'}$), 1.64 (m, 2H, CH_2), 1.49 (m, 2H, CH_2), 1.04 (m, 4H, CH_3, H_{19}), 0.66 (m, 2H, H_{20} H_{21}), 0.29 (m, 2H, H_{20} H_{21}); ^{13}C NMR (75 MHz, methanol-d_4) δ 144.65, 141.02, 132.03, 126.42, 126.79, 125.72, 119.50, 117.99, 116.30, 86.01, 74.60, 74.50, 64.13, 63.35, 60.21, 55.08, 48.83, 44.90, 44.66, 32.58, 32.47, 30.58, 29.77, 29.51, 23.99, 21.41, 14.31, 9.92, 4.66, 4.05; IR (KBr) 3392, 2927, 1621, 1613 cm^{-1}; HRMS (FAB) 664 (M + H^+), calcd 664.3387, obsd 664.3365. Anal. ($C_{40}H_{45}O_6N_3\cdot H_2O$) C, H, N.

17-(Cyclopropylmethyl)-17′-pentyl-6,6′,7,7′-tetradehydro-4,5α:4′,5α′-diepoxy-6,6′-imino-7,7′-bimorphinan-3,3′,14,14′-tetrol (5). Compound **2** (135 mg, 0.22 mmol) was reacted with valeraldehyde (0.2 mL, 162 mg, 1.88 mmol) and sodium cyanoborohydride (204.6 mg, 3.26 mmol) in 7 mL of KOAc–HOAc in MeOH as described for **3**. Purification of the crude material by reverse-phase preparative HPLC (MWA 35:14:50 + 1% NH_4OH), and by normal-phase preparative HPLC (CMA 85:14:1, t_R = 8.04 min), afforded **5** (80.3 mg, 53%) as a solid (R_f = 0.30 in MCA 90:9:1): mp >220 °C; ^1H NMR (300 MHz, methanol-d_4) δ 6.68 (m, 4H, H, H′, H_2, $H_{2'}$), 5.50 (2, 1H, H_5), 5.52 (s, 1H, $H_{5'}$), 3.11–3.52 (m, 5H), 2.30–3.04 (m, 11H), 2.17 (m, 1H), 1.83 (m, 2H), 1.68 (m, 1H), 1.53 (m, 5H), 1.06 (m, 4H), 0.67 (m, 2H, H_{20} H_{21}), 0.31 (m, 2H, H_{20} H_{21}); ^{13}C NMR (75 MHz, methanol-d_4) δ 145.10, 142.54, 131.90, 126.54, 124.97, 119.43, 118.51, 116.35, 85.86, 74.71, 74.62, 64.36, 63.55, 60.43, 55.49, 48.96, 44.88, 44.66, 32.79, 30.66, 29.90, 28.32, 24.12, 23.99, 23.59, 14.42, 10.22, 4.55, 4.14; IR (KBr) 3395, 2924, 1637, 1616 cm^{-1}; HRMS (FAB) 678 (M + H^+), calcd 678.3543, obsd 678.3527. Anal. ($C_{41}H_{47}O_6N_3\cdot H_2O$) C, H, N.

17-(Cyclopropylmethyl)-17′-phenethyl-6,6′,7,7′-tetradehydro-4,5α:4′,5α′-diepoxy-6,6′-imino-7,7′-bimorphinan-3,3′,14,14′-tetrol (6). Compound **2** (135 mg, 0.22 mmol) was reacted with phenylacetaldehyde (0.2 mL, 205 mg, 1.71 mmol) and sodium cyanoborohydride (201.6 mg, 3.21 mmol) in 5 mL of KOAc–HOAc in MeOH as described for **3**. Purification of the crude material by reverse-phase HPLC (MWA 40:19:50 + 1% NH_4OH, t_R = 17.5 min) and by normal-phase preparative HPLC (CMA 85:14:1, t_R = 7.67 min) gave **6** (95 mg, 60%) as a solid: mp >220 °C (start dec 195 °C); ^1H NMR (300 MHz, methanol-d_4) δ 7.35 (m, 5H, Ph), 6.64 (m, 4H, H_1 $H_{1'}$ H_2 $H_{2'}$), 5.53 (s, 1H, $H_{5'}$), 5.50 (s, 1H, H_5), 3.49 (m, 1H, $H_{9'}$), 3.35 (d, 1H, J = 6.30 Hz, H_9), 3.26 (d, 1H, J = 7.80 Hz, H_{10}), 3.21 (d, 1H, J = 7.80 Hz, $H_{10'}$), 2.95 (d, 1H), 2.75–3.00 (m, 8H), 2.22–2.61 (m, 9H), 1.83 (m, 2H), 1.04 (m, 1H, H_{19}), 0.65 (m, 2H, H_{20} H_{21}), 0.27 (m, 2H, H_{20} H_{21}); ^{13}C NMR (75 MHz, methanol-d_4) δ 145.04, 141.95, 141.96, 141.78, 132.20, 132.14, 129.90, 129.57, 127.26, 126.61, 125.61, 125.56, 119.62, 118.34, 116.48, 86.13, 74.89, 74.73, 65.01, 63.59, 57.32, 48.88, 45.01, 44.28, 35.18, 32.81, 30.05, 29.89, 24.83, 24.10, 10.35, 4.75, 4.30; IR (KBr) 3395, 2924, 1735, 1707 cm^{-1}; HRMS (FAB) 712 (M + H^+), calcd 712.3387, obsd 712.3378. Anal. ($C_{44}H_{45}O_6N_3$) C, H, N.

17-(Cyclopropylmethyl)-17′-[2-[(carbobenzyloxy)amino]ethyl]-6,6′,7,7′-tetradehydro-4,5α:4′,5α′-diepoxy-6,6′-imino-7,7′-bimorphinan-3,3′,14,14′-tetrol (7). Compound **7** (500 mg, 0.73 mmol) was dissolved in 40 mL of a KOAc–HOAc buffer solution in MeOH (pH 6.5). To this was added *N*-(carbobenzyloxy)glycinal[29] (1.0 equiv, 150 mg) and $NaCNBH_3$ (10 equiv, 450 mg). The reaction mixture was stirred overnight at room temperature. The solvent was evaporated under reduced pressure. Ethyl acetate was added and the organic layer was washed with saturated $NaHCO_3$ and dried over $MgSO_4$. A TLC plate eluted with CMA 99:1:0.5 showed a fairly clean product. Upon filtration of $MgSO_4$, the solvent was evaporated under reduced pressure. The crude product, isolated as an oil, was purified on column chromatography (silica gel), eluted with CMA 95:5:0.5. The desired product was isolated (411 mg, 73%) as a solid: mp 151–154 °C; ^1H NMR (300 MHz, methanol-d_4) δ 7.28 (m, 4H, Ph), 6.47 (m, 5H, H_1 $H_{1'}$ H_2 $H_{2'}$ Ph), 5.37 (s, 1H, $H_{5'}$), 5.35 (s, 1H, H_5), 5.02 (m, 2H, CH_2Ph), 3.22–3.03 (m, 6H, H_9 $H_{9'}$ H_{10} $H_{10'}$ CH_2), 2.74–2.51 (m, 6H, H_{18} H_{10} $H_{10'}$ H_{16} $H_{16'}$), 2.40–2.19 (m, 10H, H_{16} $H_{16'}CH_2$ H_8 $H_{8'}$ H_{15} $H_{15'}$), 1.61 (m, 2H, H_{15} $H_{15'}$), 0.85 (m, 1H, H_{19}), 0.50 (m, 2H, H_{20} H_{21}), 0.13 (m, 2H, H_{20} H_{21}); HRMS (FAB) 785 (M + H^+), calcd 785.3550, obsd 785.3586. Anal. ($C_{46}H_{48}O_8N_4\cdot 3.5H_2O$) C, H, N.

17-(Cyclopropylmethyl)-17′-(ethylamino)-6,6′,7,7′-tetradehydro-4,5α:4′,5α′-diepoxy-6,6′-imino-7,7′-bimorphinan-3,3′,14,14′-tetrol Hydrochloride Salt (8). Compound **7** (164 mg, 0.20 mmol) was dissolved in 10 mL of MeOH. To this was added 1 N HCl (2.0 equiv, 400 μL) and a catalytic amount of 10% Pd on C. The hydrogenation reaction was run for 90 min at atmospheric pressure and room temperature. Upon completion of the reaction, the catalyst was filtered over Celite and the Celite washed several times with MeOH. The solvent was evaporated under reduced pressure and the isolated solid was recrystallized (MeOH–Et_2O) to afford **7** (140 mg, 89%). This compound was further purified by elution on preparative plate (silica gel, 1 mm) with CMA 80:20:0.5 to provide the desired material: mp >240 °C; ^1H NMR (300 MHz, methanol-d_4) δ 6.61 (bs, 2H, H_2 $H_{2'}$), 6.51 (m, 2H, H_1 $H_{1'}$), 5.50 (s, 1H, $H_{5'}$), 5.40 (s, 1H, H_5), 4.04 (d, 1H, J = 4.80 Hz, $H_{9'}$), 3.40–3.29 (m, 3H, H_9 H_{10} $H_{10'}$), 3.19–2.59 (m, 12H), 2.43–2.25 (m, 6H, H_{15} H_8 $H_{8'}$ $H_{15'}$), 1.84 (bd, 1H, J = 12.30 Hz, $H_{15'}$), 1.67 (bd, 1H, J = 8.40 Hz, H_{15}), 1.11 (m, 1H, H_{19}), 0.70 (m, 2H, H_{20} H_{21}), 0.48 (m, 2H, H_{20} H_{21}); ^{13}C NMR (75 MHz, methanol-d_4) δ 144.18, 143.89, 141.27, 140.58, 131.06, 129.75, 126.09, 125.25, 121.89, 119.53, 119.06, 118.27, 117.43, 115.62, 114.23, 84.87, 84.52, 74.33, 72.92, 65.05, 62.79, 58.03, 52.23, 47.80, 47.20, 46.78, 36.65, 30.63, 30.01, 29.67, 29.43, 29.09, 24.78, 24.18, 6.06, 5.43, 2.60; HRMS (FAB) 651 (M + H^+), calcd 651.3182, obsd 651.3214. Anal. ($C_{38}H_{42}O_6N_4\cdot HCl$) C, H, N.

17-(Cyclopropylmethyl)-17′-(2-guanidinoethyl)-6,6′,7,7′-tetradehydro-4,5α:4′,5α′-diepoxy-6,6′-imino-7,7′-bimorphinan-3,3′,14,14′-tetrol Sulfonate Salt (9). Compound **8** (26.5 mg, 0.036 mmol) was dissolved in DMF (2 mL) with Et_3N (2.0 equiv, 17 μL) and formamidinesulfinic acid[30] (1.2 equiv, 6.1 mg). The reaction mixture was stirred at room temperature overnight, the precipitate was filtered, and the solvent was evaporated under reduced pressure. Addition of either to the crude material led to the isolation of the desired product (25 mg, 90%). Purification of this salt was accomplished through multiple recrystallizations (MeOH–Et_2O and MeOH–EtOAc): mp >250 °C; ^1H NMR (300 MHZ, DMSO-d_6) δ 8.53 (bs, 1H), 7.55 (bs, 1H), 6.72 (bs, 2H, H_2

$H_{2'}$), 6.50 (m, 2H, H_1 $H_{1'}$), 5.52 (s, 1H, $H_{5'}$), 5.41 (s, 1H, H_5), 4.14 (d, 1H, *J* = 4.80 H, $H_{9'}$), 3.40–3.29 (m, 3H, H_9 H_{10} $H_{10'}$), 3.11–2.48 (m, 12H), 2.43–2.25 (m, 6H), 1.84 (bd, 2H, H_{15} $H_{15'}$), 1.01 (m, 1H, H_{19}), 0.68 (m, 2H, H_{20} H_{21}), 0.53 (m, 2H, H_{20} H_{21}); HRMS (FAB) 693 (M^+), calcd 693.3400, obsd 693.3401. Anal. Calcd. ($C_{39}H_{44}O_6N_6 \cdot HSO_3 \cdot H_2O$) C, H, N.

17-(Cyclopropylmethyl)-17'-acetyl-6,6',7,7'-tetradehydro-4,5α:4',5α'-diepoxy-6,6'-imino-7,7'-bimorphinan-3,3',14,14'-tetrol (10). Compound **2** (154 mg, 0.25 mmol) in MeOH (10 mL) was added to acetic anhydride (0.5 mL) and stirred at 23 °C for 5 min. Upon evaporation of the solvent, the residue was dried under vacuum, purified by flash chromatography (silica gel), and eluted with CMA 91:8:1, to give **10** (68 mg, 42%). Compound **10** was crystallized from MeOH–$CHCl_3$: mp >290 °C; ^{1}H NMR (300 MHz, methanol-d_4) δ 6.49 (m, 4H, H_1, H_2, $H_{1'}$, $H_{2'}$), 5.45 (s, 1H, H_5), 5.42 (s, 1H, $H_{5'}$), 5.41 (s, 1H, $H_{5'}$), 4.17 (d, 1H, *J* = 7.00 Hz, $H_{9'}$), 3.71 (dd, 1H, *J* = 4.70, 14.50 Hz, H_9), 3.11–3.42 (m, 5H), 2.61–3.11 (m, 5H), 2.20–2.61 (m, 6H), 2.14 (s, Me), 2.09 (s, Me), 1.73 (m, 1H, H_{15}), 1.60 (m, 1H, $H_{15'}$), 0.96 (m, 1H, H_{19}), 0.60 (m, 2H, H_{20} H_{21}), 0.29 (m, 2H, H_{20} H_{21}); ^{13}C NMR (75 MHz, methanol-d_4) δ 172.76, 172.57, 145.04, 144.76, 141.56, 141.12, 132.27, 131.81, 131.63, 126.71, 126.66, 126.40, 126.24, 126.01, 125.04, 124.83, 119.91, 119.77, 119.50, 118.44, 117.94, 116.70, 116.17, 86.18, 85.91, 74.67, 74.63, 74.48, 63.52, 61.01, 60.46, 55.18, 44.95, 41.11, 35.94, 33.64, 33.41, 32.72, 30.84, 30.45, 29.94, 24.05, 21.85, 10.19, 4.62, 4.14; IR (KBr) 3302, 1641, 1617 cm^{-1}; HRMS (FAB) 650 (M + H^+), calcd 650.2866, obsd 650.2849. Anal. ($C_{38}H_{39}O_7N_3 \cdot 4H_2O$) C, H, N.

17-(Cyclopropylmethyl)-17'-[*N*-(carbobenzyloxy)glycyl]-6,6'-7,7'-tetradehydro-4,5α:4',5α'-diepoxy-6,6'-imino-7,7'-bimorphinan-3,3'-14,14'-tetrol (15). To a solution of Cbz-glycine (138 mg, 3.0 eq) in DMF (10 mL) was added benzotriazol-l-yloxytris(dimethylamino)phosphonium hexafluorophosphate (Bop reagent) (3.0 equiv, 293 mg) and Et_3N (3.0 equiv, 100 μL). The reaction mixture was stirred at room temperature for 15 min. To this was added **2** (150 mg, 0.22 mmol) and Et_3N (5.0 equiv, 146 μL) in DMF (2 mL). The reaction mixture was stirred overnight at room temperature. Ethyl acetate was added and the organic layer was washed with brine and saturated $NaHCO_3$ solution and dried over $MgSO_4$. Upon filtration of $MgSO_4$, the solvent was evaporated under reduced pressure. The crude material was dissolved in MeOH (10 mL) containing K_2CO_3 (2.0 equiv, 88 mg) and the reaction mixture was stirred overnight at room temperature. Upon evaporation of the solvent, the crude mixture was chromatographed (silica gel) with CMA 99:1:0.5. The desired material **15** (100 mg, 56%) was isolated as an oil which was crystallized (chloroform–hexanes) (100 mg): mp 128–131 °C (start dec at 113 °C); ^{1}H NMR (300 MHz, methanol-d_4) δ 7.30 (m, 5H, Ph), 6.48 (m, 4H, H_2 $H_{2'}$ H_1 $H_{1'}$), 5.38 (m, 2H, H_5 $H_{5'}$), 5.06 (d, 2H, CH_2Ph), 4.37 (dd, 1H, *J* = 3.90, 13.50 Hz, $H_{9'}$), 4.10 (bd, 2H, CH_2), 3.95 (bs, ^{H}, NH), 3.60 (dd, 1H, *J* = 4.80, 13.20 Hz, H_9), 3.43–3.12 (m, 4H), 2.78–2.69 (m, 3H), 2.45–2.32 (m, 9H), 1.65–1.53 (m, 2H, H_{15} $H_{15'}$), 0.85 (m, 1H, H_{19}), 0.49 (m, 2H, H_{20} H_{21}), 0.10 (m, 2H, H_{20} H_{21}); ^{13}C NMR (75 MHz, methanol-d_4) δ 169.61, 169.43, 158.23, 144.16, 143.94, 140.79, 140.34, 137.46, 131.45, 131.42, 130.92, 130.79, 128.72, 128.28, 128.25, 128.20, 128.14, 125.88, 125.83, 125.55, 125.37, 125.13, 124.16, 123.99, 119.14, 119.01, 118.75, 117.62, 117.14, 115.88, 115.31, 115.15, 85.31, 85.12, 85.08, 73.81, 73.66, 66.93, 62.61, 59.59, 58.38, 55.07, 44.15, 43.05, 42.94, 38.46, 35.70, 32.68, 31.85, 29.98, 29.71, 29.58, 29.14, 23.23, 9.33, 3.89, 3.31; HRMS (FAB) 799 (M + H^+), calcd 799.3424, obsd 799.3425.

17-(Cyclopropylmethyl)-17'-[[*N*-(carbobenzyloxy)glycyl]glycinamido]-6,6',7,7'-tetradehydro-4,5α:4',5α'-diepoxy-6,6'-imino-7,7'-bimorphinan-3,3',14,14'-tetrol (16). Compound **2** (150 mg, 0.22 mmol) was added to a solution of Cbz-glycylglycine (3.5 equiv, 206 mg), Bop reagent (3.5 equiv, 342 mg), and Et_3N (8.0 equiv, 262 μL), in DMF (10 mL). The experimental conditions and workup procedure were similar to that reported for **15**. The desired product **16** was isolated as an oil (85 mg, 45%): ^{1}H NMR (300 MHz, methanol-d_4) δ 7.31 (m, 5H, Ph), 6.50 (m, 4H, H_2 $H_{2'}$ H_1 $H_{1'}$), 5.40 (s, 1H, H_5), 5.37 (s, 1H, $H_{5'}$), 5.07 (bs, 2H, CH_2Ph), 4.35 (m, 1H, $H_{9'}$), 4.12 (bs, 1H, NH), 4.03 (bd, 2H, CH_2), 3.76 (d, 2H, *J* = 4.80 Hz, CH_2), 3.57 (dd, 1H, *J* = 7.50, 14.70 Hz, H_9), 3.37–3.09 (m, 3H, H_{10} $H_{10'}$ H_{18}), 2.85–2.65 (m, 2H), 2.50–2.23 (m, 11H), 1.57 (m, 2H, H_{15} $H_{15'}$), 0.86 (m, 1H, H_{19}), 0.51 (m, 2H, H_{20} H_{21}), 0.18 (m, 2H, H_{20} H_{21}); ^{13}C NMR (75 MHz, methanol-d_4) δ 171.97, 171.79, 171.66, 171.59, 168.82, 168.70, 144.18, 144.13, 143.97, 140.74, 140.71, 140.48, 131.14, 131.08, 130.96, 130.90, 130.82, 128.74, 128.30, 128.17, 125.76, 125.73, 125.68, 125.52, 124.55, 124.18, 124.00, 119.16, 119.04, 118.90, 117.62, 117.33, 115.57, 115.34, 115.18, 85.15, 85.08, 85.04, 73.94, 73.76, 73.65, 67.12, 62.63, 60.82, 60.11, 59.30, 58.49, 57.56, 55.05, 54.30, 44.62, 44.15, 41.58, 38.52, 35.73, 35.02, 32.79, 32.68, 32.48, 31.35, 30.00, 29.69, 29.59, 29.21, 23.37, 8.67, 4.15, 3.16; HRMS (FAB) 856 (M + H^+), calcd 856.3557, obsd 856.3577.

17-(Cyclopropylmethyl)-17'-glycyl-6,6',7,7'-tetradehydro-4,5α:4',5α'-diepoxy-6,6'-imino-7,7'-bimorphinan-3,3',14,14'-tetrol Hydrochloride Salt (11). Compound **15** (100 mg, 0.12 mmol) was dissolved in 10 mL of MeOH, in the presence of 2 N HCl (2.0 equiv, 100 μL) and a catalytic amount of 10% Pd on C. The hydrogenation reaction was run for 45 min at room temperature and atmospheric pressure. The solution was filtered thoroughly over Celite and the Celite washed with MeOH. The solvent was evaporated under reduced pressure. The crude product **15** was redissolved in a minimum amount of MeOH and to this was added some Et_2O. The solid product (60 mg, 65%) was purified by reverse-phase preparative HPLC (MAW 5:2:3 + 0.5% NH_4OH): mp >230 °C; ^{1}H NMR (300 MHz, methanol-d_4) δ 6.51 (m, 4H, H_1 H_2 $H_{1'}$ $H_{2'}$), 5.47 (s, 1H, H_5), 5.42 (s, 1H, $H_{5'}$), 5.39 (s, 1H, $H_{5'}$), 4.37 (dd, 1H, *J* = 4.80, 14.70 Hz, $H_{9'}$), 3.98 (m, 1H, NH), 3.84 (m, 2H, CH_2), 3.56 (dd, 1H, *J* = 3.60, 13.50 Hz, H_9), 3.39–3.29 (m, 2H, H_{10} $H_{10'}$), 3.14–2.98 (m, 5H), 2.87–2.66 (m, 5H) 2.57–2.23 (m, 4H), 1.77 (d, 1H, *J* = 12.30 Hz, $H_{15'}$), 1.67 (dd, 1H, *J* = 14.70, 20.70 Hz, H_{15}), 1.01 (m, 1H, H_{19}), 0.68 (m, 2H, H_{20} H_{21}), 0.39 (m, 2H, H_{20} H_{21}); HRMS (FAB) 665 (M + H^+), calcd 665.2975, obsd 665.2936. Anal. ($C_{38}H_{40}O_7N_4 \cdot HCl$) C, H, N.

17-(Cyclopropylmethyl)-17'-(glycylglycinamido)-6,6',7,7'-tetradehydro-4,5α:4',5α'-diepoxy-6,6'-imino-7,7'-bimorphinan-3,3',14,14'-tetrol Hydrochloride Salt (12). Compound **16** (33 mg, 0.04 mmol) was dissolved in 5 mL of methanol with a catalytic amount of Pd 10% on C, and 1 NHCl (3.0 equiv, 120 μL). The experimental conditions, workup, and purification procedures were similar to those described for compound **15**. The desired product **12** was isolated (25 mg, 90%) as a solid: mp > 250 °C; ^{1}H NMR (300 MHz, methanol-d_4) δ 6.50 (m, 4H), 5.45 (s, 1H, H_5), 5.41 (s, 1H, $H_{5'}$), 5.38 (s, 1H, $H_{5'}$), 4.42 (dd, 1H, *J* = 3.60, 13.20 Hz, $H_{9'}$), 4.20 (bs, 2H, CH_2), 4.11 (bs, 2H, CH_2), 3.73 (m, 1H, H_9), 3.40–3.29 (m, 2H, H_{10} $H_{10'}$), 3.22–2.96 (m, 5H), 2.88–2.64 (m, 5H), 2.51–2.24 (m, 4H), 1.78 (d, 1H, *J* = 12.30 Hz, $H_{15'}$), 1.63 (dd, 1H, *J* = 12.30, 19.50 Hz, H_{15}), 0.97 (m, 1H, H_{19}), 0.62 (m, 2H, H_{20} H_{21}), 0.35 (m, 2H, H_{20} H_{21}); HRMS (FAB) 722 (M + H^+), calcd 722.3189, obsd 722.3191. Anal. ($C_{40}H_{43}O_8N_5 \cdot HCl$) C, H, N.

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