The final atomic parameters, bond lengths and angles, and the observed and calculated structure amplitudes are submitted as supplementary material.

Single-Crystal X-ray Analysis of (-)-Etodolac (*S*)-(-)-Borneol Ester. The crystal data for (+)-*endo*-(1*S*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl 1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetate are as follows: molecular formula $C_{27}H_{35}NO_3$; molecular weight 421.56; orthorhombic $P2_12_12_1$; a = 22.75 (4), b = 10.66 (2), c = 9.77 (2) Å; V = 2369.4 Å^3; Z = 4; ρ_{calcd} = 1.182 g cm^{-3}, ρ_{obsd} = 1.19 (3) g cm^{-3} (flotation in aqueous ZnI_2 solution); $|F_{000}|$ = 911.88 (20 °C, Mo Kα, λ = 0.710 69 Å, μ = 0.71 cm^{-1}).

Thin platelike crystals of etodolac borneol ester were obtained by crystallization of the compound from hexane. A crystal of dimensions 0.15 × 0.05 × 0.20 mm was used for X-ray diffraction data collection. The method of data collection was the same as that described for (±)-etodolac. A total of 2595 reflections were collected corresponding to Miller indices hkl and $h\bar{k}\bar{l}$, which were then averaged to give 1298 unique reflections. Out of these, only 955 with $I > 2\sigma(I)$ were considered observed and used during structure determination.

The structure was solved by direct methods using the MULTAN program,[17] employing 152 reflections having $|E| > 1.50$. The positional and isotropic temperature factors of the non-hydrogen atoms were refined by least-squares technique to an R_F = 0.123 and R_{wF} = 0.174 where $R_F = [\sum||F_o| - k|Fc||]/\sum|F_o|$ and $R_{wF} = [[\sum w(|F_o| - k|F_c|)^2]/\sum w|F_o|^2]^{1/2}$. A final difference Fourier map revealed no significant peaks of electron density.

Acknowledgment. We acknowledge valuable discussions with Dr. A. Treasurywala on certain aspects of this work.

Supplementary Material Available: Listings of bond lengths, bond angles, observed and calculated structure factors, and atomic coordinates and thermal parameters for (±)-etodolac and etodolac borneol ester (16 pages). Ordering information is given on any current masthead page.

Investigation of the Structural Requirements for the κ-Selective Opioid Receptor Antagonist, 6β,6β′-[Ethylenebis(oxyethyleneimino)]bis[17-(cyclopropylmethyl)-4,5α-epoxymorphinan-3,14-diol] (TENA)

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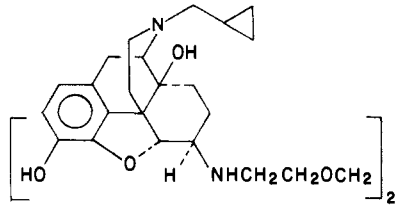
In an effort to determine whether or not the basic nitrogens in the spacer of the bivalent ligand 6β,6β′-[ethylenebis(oxyethyleneimino)]bis[17-(cyclopropylmethyl)-4,5α-epoxymorphinan-3,14-diol] (TENA, **1**) is responsible for its selective κ opioid antagonist activity, we have synthesized monovalent analogues **2**–**4** that contain a C-6 side chain with basic nitrogens. Analogue **2** behaved as a potent opioid agonist in the guinea pig ileum preparation (GPI) and possessed no significant κ opioid antagonist activity (IC_{50} ratio = 1) relative to TENA (IC_{50} ratio = 20). The agonist activity of **3** and **4** interfered with the opioid antagonist assay and therefore did not permit evaluation of antagonist activity in a concentration range where TENA is effective. Although the results obtained with **2** are consistent with the requirement of a second opiate pharmacophore (rather than a second basic nitrogen in the spacer) for the κ antagonist activity of TENA, the potent agonism associated with these monomers do not allow a firm conclusion in this regard.

Molecules that consist of two pharmacophores connected by a spacer chain have been termed bivalent ligands.[1-3] Such molecules[4-10] are of interest as probes for opioid receptors because of the possiblity of bridging a subpopulation of vicinal receptors when the spacer is a specific length. The bivalent ligand 6β,6β′-[ethylenebis(oxyethyleneimino)]bis[17-(cyclopropylmethyl)-4,5α-epoxymorphinan-3,14-diol] (TENA, **1**) was designed on this basis and is an opioid antagonist that has relatively high selectivity for receptors of the κ type.[3] Of the reported opioid antagonists, TENA possesses the greatest potency and selectivity toward κ receptors.



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Since closely related monovalent analogues were not κ selective, the pharmacologic profile of TENA was attributed to the bivalent nature of this ligand.[1] However, the fact that there was no basic nitrogen atom attached to the terminus of the 3,6-dioxaoctane side chain in the monovalent analogues of TENA raised the possibility that this high κ selectivity might be due to the association of a κ receptor subsite with the protonated nitrogen in the spacer, rather than with the second pharmacophore. In an effort to address this possibility, we have synthesized and tested several monovalent analogues (**2**–**4**) that contain two basic nitrogens in the side chain.

† Department of Medicinal Chemistry.

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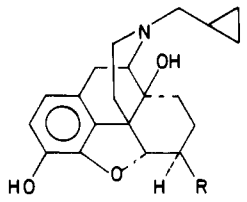
Table I. Activities of β-Naltrexamine Derivatives on the GPI

					EK antagonism	
no.[a]	morphine IC_{50}, nM (control)	compd IC_{50}, nM	N^b	rel agonist potency	N^b	IC_{50} ratio[c]
2	123 ± 60	21.1 ± 9.3	3	5.8	3	1.1 ± 0.6[d]
3	104 ± 35	11.4 ± 4.5	5	9.1	4	1.0 ± 0.2[e]
4	107 ± 64	20.6 ± 8.0	3	5.2	3	3.1 ± 0.9[e]

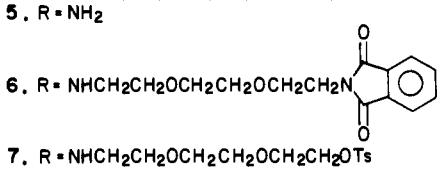
[a] Tested in the form of the salts specified in the Experimental Section. [b] Number of replicate determinations. [c] IC_{50} of EK in the presence of antagonist divided by IC_{50} of EK in absence of antagonist; ethylketazocine (EK) control $IC_{50} = 4.3 \pm 1.7 \times 10^{-10}$ M ($N = 10$); TENA IC_{50} ratio at 2 nM was 1.3 ± 0.3 ($N = 3$). [d] Concentration of 2 was 20 nM. [e] Concentration of 3 or 4 was 2 nM.

Design Rationale and Chemistry

In the present study, we prepared the monovalent ligands 2–4 in order to investigate further the concept of the bivalent ligand approach for developing κ-selective opioid antagonists. These compounds are related to the bivalent ligand TENA in that they contain the same pharmacophore, β-naltrexamine (5),[11] which is attached via the 6-amino group to the same 3,6-dioxaoctane spacer. They differ from TENA in that, instead of a second pharmacophore, they contain a nonopiate amine moiety.



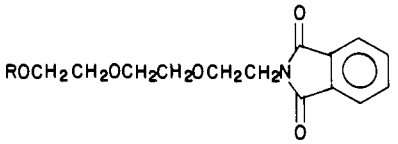
2. R = $NHCH_2CH_2OCH_2CH_2OCH_2CH_2NH_2$
3. R = $NHCH_2CH_2OCH_2CH_2OCH_2CH_2NH(C{=}NH)NH_2$
4. R = $NHCH_2CH_2OCH_2CH_2OCH_2CH_2NHCH_2C_6H_5$
5. R = NH_2



6. R = $NHCH_2CH_2OCH_2CH_2OCH_2CH_2N$

7. R = $NHCH_2CH_2OCH_2CH_2OCH_2CH_2OTs$

Compound 2 was synthesized by reacting β-naltrexamine (5) with 1-phthalimino-8-(tosyloxy)-3,6-dioxaoctane (9) in toluene–diglyme to give intermediate 6. Dephthalylation of 6 with ethanolic hydrazine yielded the free amine 2. The intermediate 9, in turn, was prepared by reacting triethylene glycol monochloride with potassium phthalimide, followed by treatment of the resulting phthalimido derivative 8 with tosyl chloride in pyridine. The monovalent ligand 3 was prepared by reacting 2 with methyl isothiourea sulfate in aqueous ethanol. The benzylamino target compound 4 was prepared by reacting 5 with 3 equiv of triethylene glycol ditosylate,[12] followed by treatment of the resulting monotosyl intermediate 7[1] with benzylamine.



8. R = H
9. R = Ts

Pharmacology

Compounds 2–4 were evaluated on the electrically stimulated longitudinal muscle of the guinea pig ileum[13] (GPI). The agonist potency of each target compound was determined relative to a control morphine IC_{50}. All compounds were potent agonists, with activities 5–10 times that of morphine (Table I). It was not possible to determine whether or not significant κ antagonist activity was present because of the potent agonism. In this regard, no significant decrease in the ileal responses to μ (morphine) and κ [ethylketazocine (EK)] agonists in the presence of 20 nM (2) or 2 nM (3, 4) concentrations were observed.

Discussion

Recently, we have provided evidence that the enhanced μ opioid receptor antagonist activity of bivalent ligands depends on the presence of two opiate pharmacophores.[14] The report[3] that 20 nM TENA (1) afforded an IC_{50} ratio of 20 for the κ agonist EK, while the monomer 2 in the present study is ineffective as an antagonist at identical concentration, is consistent with the idea that two pharmacophores also are required for the κ antagonist activity of TENA. However, the fact that monomers 3 and 4 did not behave as κ antagonists does not necessarily provide additional support for the requirement of a second pharmacophore because, at their IC_{20} concentration (2 nM), the antagonist activity of these ligands may not be detectable. In fact, TENA itself at 2 nM did not produce significant EK antagonism. Unfortunately, the agonist responses at higher concentrations of 3 and 4 interfered with the evaluation of antagonist activity. By comparison, no such interference is associated with TENA, since its maximum agonist response in the GPI is only 10%.[1]

Experimental Section

General Procedures. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and were within ±0.4% of the theoretical values. IR spectra were obtained from KBr pellets with a Perkin-Elmer 281 spectrophotometer. NMR spectra were recorded at ambient temperature on JNM-FX 90 Q FT NMR spectrometer and Nicolet 300-MHz NMR spectrophotometer with tetramethylsilane as internal standard. Mass spectra were obtained on an AE1 MS-30 (EI, 20 eV) or Finnigan 4000 (CI, NH_3, positive or negative) instrument. All TLC data were determined with Analtech uniplate thin-layer chromatography plates (silica gel), and the eluant EMA refers to EtOAC–MeOH–NH_4OH. Unless otherwise stated, all reagents and solvents used were reagent grade without subsequent purification.

1-Hydroxy-8-phthalimido-3,6-dioxaoctane (8). A mixture of 2-[2-(2-chloroethoxy)ethoxy]ethanol (Aldrich Chemical Co.) (5.5 g, 0.33 mol), potassium phthalimide (5.58 g, 0.03 mol), and dimethylformamide (5 mL) was stirred at 110 °C for 5 h. The mixture was filtered, the filtrate was diluted with ether (50 mL), and the separated oily product was chromatographed on silica gel with EtOAC–hexane–MeOH (14:14:2). The product (6 g, 71%) was recrystallized from ether–heptane: mp 54–55 °C; R_f 0.31 (silica gel, EtOAC–hexane–MeOH, 14:14:2); CIMS, m/e 279 (M^+); NMR ($CDCl_3$) δ 7.85 and 7.72 (4 H, d and m, phthalimide, Ar), 3.93–3.45 (12 H, t and m, CH_2O), 2.50 (1 H, br, OH). Anal. ($C_{14}H_{17}NO_5$) C, H, N.

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1-Phthalimido-8-(tosyloxy)-3,6-dioxaoctane (9). To a solution of **8** (5.0 g, 0.018 mol) in dry pyridine (20 mL) was added over a 1-h period *p*-toluenesulfonyl chloride (6.82 g, 0.036 mol) at 0–5 °C. After stirring for 3 h at 23 °C, the mixture was poured onto a mixture of HCl (25 mL) and ice, and the product was extracted with EtOAc (25 mL × 4). After removal of the solvent in vacuo, the crude product was chromatographed on silica gel with EtOAc–hexane (1:1) to give an oil (6 g, 77%), which solidified upon cooling: mp 80–81 °C; R_f 0.33 (silica gel, EtOAc–hexane, 1:1); EIMS, *m/e* 432 (M − 1); NMR ($CDCl_3$) δ 7.84 and 7.71 (4 H, d and m, phthalimide, Ar), 7.77 and 7.44 (4 H, q, Ts, Ar), 4.18–3.48 (12 H, t and m, CH_2O), 2.44 (3 H, s, CH_3). Anal. ($C_{21}H_{23}NO_7S$), C, H, N.

1-Phthalimido-8-(β-naltrexamino)-3,6-dioxaoctane Dihydrochloride (6·2HCl). A mixture of **9** (650 mg, 1.5 mmol), β-naltrexamine **5** (342 mg, 1 mmol), and $NaHCO_3$ (840 mg) in toluene–diglyme (15 mL, 2:1) was heated under N_2 at 110 °C for 10 h. After filtration and removal of solvents in vacuo, the crude product was dissolved in 0.5 N HCl (10 mL) and extracted with EtOAc (25 mL × 3). The aqueous phase was rendered basic (pH 9) with NH_4OH, and the liberated free base was extracted with EtOAc (25 mL × 4). After removal of the solvent in vacuo, the crude product was chromatographed on silica gel with EtOAc–MeOH–NH_4OH (85:15:0.5) to give 300 mg (50%) of **6**, R_f 0.31 (EMA, 85:15:1), which was converted to the HCl salt (hygroscopic); mp 105–110 °C; CIMS, *m/e* 603 (M^+); NMR (Me_2SO-d_6) δ 6.85 and 6.76 (2 H, q, Ar), 7.85 (4 H, m, phthalimide, Ar), 4.92 (1 H, d, C-5 H), 3.81–3.50 (12 H, m, CH_2O); IR (KBr) cm^{-1} 1777 and 1715 (C=O, phthalimide). Anal. ($C_{34}H_{41}N_3O_7$·2HCl·0.5H_2O) C, H, N.

1-Amino-8-(β-naltrexamino)-3,6-dioxaoctane Trihydrochloride (2·3HCl). A solution of 6·2HCl (340 mg, 0.563 mmol) and hydrazine (96 mg, 3 mmol) in EtOH (5 mL) was stirred at 23 °C for 2 days. The solvent was removed in vacuo and the residue was stirred at 23 °C with HCl (1 N, 10 mL) for 5 h. After removal of the insoluble phthalhydrazide by filtration and evaporation of the solvent in vacuo, the crude product was chromatographed on silica gel with EtOAc–MeOH–NH_4OH (80:20:1) to give **2** an oil (229 mg, 0.48 mmol, 96%); R_f 0.35 (EMA, 50:50:5). The free base was converted to the 3HCl salt with 1 N HCl in 2-propanol; mp 190–193 °C dec; EIMS, *m/e* 472.9 (M^+); NMR (Me_2SO-d_6) δ 9.58 (1 H, s, OH phenolic), 9.37 (1 H, br s, H^+N), 8.96 (2 H, br, nal-N^+H_2), 8.12 (3 H, br s, N^+H_3), 6.80 and 6.67 (2 H, q, *J* = 8.2 Hz, Ar), 6.43 (1 H, br s, C-14 OH), 5.00 (1 H, d, *J* = 7.1 Hz, C-5 H), 3.78–3.58 (12 H, t and m, CH_2O). Anal. ($C_{26}H_{39}N_3O_5$·3HCl·3H_2O) C, N; H: calcd, 7.1; found, 8.09.

1-Guanidino-8-(β-naltrexamino)-3,6-dioxaoctane Sulfate (3·1.5H_2SO_4). A solution of **2** (46 mg, 0.097 mmol) and methyl isothiourea sulfate (28 mg, 0.1 mmol) in aqueous ethanol (50%, 3 mL) was stirred at 100 °C for 25 h. Sulfuric acid (1.8 N, 2 mL) then was added to the cold mixture. After removal of solvents in vacuo, the product was purified by gel filtration on Sephadex (LH-20) with MeOH to give the product, mp 155–160 °C (foaming); R_f 0.19 (EMA, 50:50:10); NMR (Me_2SO-d_6) δ 9.45 (1 H, s, OH phenolic), 7.30 (5 H, br s, $NHC(NH_2)_2^+NH_2$), 6.68 and 6.61 (2 H, q, *J* = 8 Hz, Ar), 4.96 (1 H, d, C-5 H), 3.88–3.45 (12 H, m, CH_2O); ^{13}C NMR 157.1 ($NHC(NH_2)_2^+$), 69.80, 69.64, and 69.23 ppm (CH_2O). Anal. ($C_{27}H_{41}N_5O_5$·1.5H_2SO_4·1.5H_2O) C, H.

1-(Benzylamino)-8-(β-naltrexamino)-3,6-dioxaoctane Trihydrochloride (4·3HCl). To a refluxing toluene (15 mL) solution of triethylene glycol ditosylate[12] (4.0 g, 8.76 mmol) containing $NaHCO_3$ (1 g) was added over a 2-h period 1 g of β-naltrexamine **5** in 15 mL of toluene–diglyme (2:1). The reaction was conducted under N_2 at 110 °C for an additional 5 h. After filtration and removal of solvents in vacuo, the excess of ditosylate was removed by dissolving the crude product in 1 N HCl (10 mL) and extracted with EtOAc (30 mL × 3). The aqueous phase containing the product was basified with NH_4OH (pH 9) and extracted with EtOAc (25 mL × 5). After removal of the solvent in vacuo, the crude intermediate compound **7**[1] was purified by gradient elution chromatography using silica gel and EtOAc–MeOH–NH_4OH (99:1:0.5 to 80:20:1). The product (440 mg, 0.7 mmol) was isolated as a glass: R_f 0.67 (EMA, 70:30:4); CIMS, *m/e* 455 (M − TsOH); NMR ($CDCl_3$) δ 7.83 and 7.40 (4 H, q, Ts, Ar), 6.71 and 6.54 (2 H, q, *J* = 8.2 Hz, Ar), 4.51 (1 H d, *J* = 7.97 Hz, C-5 H), 3.98–3.57 (12 H, m, CH_2O). To a solution of **7** (160 mg, 0.28 mmol) in toluene–diglyme (5 mL, 2:1) containing $NaHCO_3$ (120 mg) was added a solution of benzylamine (61 mg, 0.57 mmol) in toluene (1 mL). The reaction that was conducted under N_2 was stirred at 110 °C for 6 h. After filtration and removal of solvent in vacuo, the crude product was chromatographed on silica gel with EtOAc–MeOH–NH_4OH (85:15:1) to give the 4 as an oil (30 mg, 0.053 mmol), which was converted to the 3HCl salt (hygroscopic); R_f 0.25 (EMA, 80:20:2); CIMS, *m/e* 563 (M^+); NMR (D_2C exchanged in Me_2SO-d_6) δ 7.47 (5 H, m, Ar), 6.82 (2 H, Ar), 4.98 (1 H, d, C-5 H), 3.92–3.52 (12 H, m, CH_2O). Anal. ($C_{33}H_{45}N_3O_5$·3HCl·3H_2O) C, H; N: calcd, 5.80; found, 5.16.

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Additions and Corrections

1986, Volume 29

Reinhard Sarges,* Harry R. Howard, Kathy M. Donahue, Williard M. Welch, Beryl W. Dominy, Albert Weissman, B. Kenneth Koe, and Jon Bordner: Neuroleptic Activity of Chiral *trans*-Hexahydro-γ-carbolines.

Page 19. The supplementary material paragraph was inadvertently omitted. It should read as follows: Listings of coordinates and bond angles and distances, anisotropic temperature factors, hydrogen coordinates, and observed and calculated structure factors and a stereoscopic view of molecule **8** are available as supplementary material (26 pages). Ordering information is given on any current masthead page.