removed, and the crude sulfoxide (3.31 g, 0.010 mol; 90% yield) was refluxed in chloroform (100 mL). The reaction was complete after 1.5 h (as shown by TLC). After removal of the solvent and column chromatography on silica gel (eluent ethyl ether/hexane (75:25), products **12a** (1.69 g, 8.00 mmol) and **12b** (0.100 g, 0.500 mmol) were obtained as liquids: total yield 80% (based on the starting sulfide **10**); IR ($CHCl_3$) same functional groups absorptions at 3500–3600, 1740, 1710, 1630; ^1H NMR ($CDCl_3$) (**12b**) δ 1.30 (t, 3 H, CH_2, CH_3, J = 7.0), 2.10 (s, 3 H, OAc), 3.10–3.40 (m, 2 H, $-CH_2(OH)$), 5.82 (s, 1 H, H_b), 6.32 (s, 1 H, H_a). Spectral data for **12a** were identical with those of an authentic sample previously prepared in our laboratory by another method.[7] Compounds **12a** and **12b** were transformed by acid catalysis into lactone **1c**.[7]

β-Hydroxy-α-methyl-α-(phenylthio)-γ-butyrolactone (14). Sulfide **10** (0.85 g, 2.7 mmol) was dissolved in a mixture of THF (10 mL) and a saturated aqueous $Ba(OH)_2$ solution (30 mL). The reaction mixture was left for 4 h at room temperature; a solution of 2 N HCl was then added in order to adjust to pH 4. The THF was distilled off, and the aqueous phase wsa extracted with CH_2Cl_2. The organic layer was dried over magnesium sulfate, the solvent was removed, and the crude dihydroxyacid **13** was refluxed for a few minutes in $CHCl_3$ (50 mL) containing a catalytic amount of *p*-TsOH. Removal of the solvent and column chromatography on silica gel (eluent ethyl ether/hexane (75:25)) afforded lactone **14** (0.37 g, 1.6 mmol) in a 60% yield (from the starting sulfide **10**): mp 84–85 °C; IR ($CDCl_3$) 3400–3600, 1775; ^1H NMR ($CDCl_3$) δ 1.43 (s, 3 H, CH_3), 4.0–4.6 (m, 3 H, OCH_2, $-CH(OH)$), 7.3–7.8 (m, 5 H, SPh); MS, m/e 224 (M^+). Anal. ($C_{11}H_{12}O_3S$) C, H, S.

β-Hydroxy-α-methylene-γ-butyrolactone (1b, Tulipalin B). *m*-CPBA oxidation of lactone **14** (0.300 g, 1.32 mmol) and pyrrolysis of the crude sulfoxide was conducted under the same conditions as used for the sulfide **10**. Purification by silica gel column chromatography (eluent ethyl ether/hexane (75:25)) afforded lactone **1b** (0.135, 1.19 mmol; 90% yield from lactone **14**). The IR and NMR spectra were identical in all respects with data of the literature[10] (and with a sample prepared by another method[7]).

Biological Assays. Albino Himalayan spotted (Hoffmann La Roche, Füllingsdorf) female guinea pigs weighing from 300 to 500 g were sensitized by the FCAT method[13] on alternate days. The hapten, emulsified in Freund's complete adjuvant (FCA), was injected intradermally (0.1 mL) in the shaved nuchal region of the animal (a total of three injections were given). Six groups of six guinea pigs each were sensitized by an emulsion of a 1:1 FCA/saline mixture containing 0.22 mol/L of the haptens (compounds **1a–c** and **6a–c**).

After a 15-day rest, the elicitation reaction was achieved by an open epicutaneous test (OET): 25 μL of a 0.088 M (~2% w/v) solution of the hapten in a 1:1 mixture of ethanol and methylene chloride was deposited on the shaved flank of the animal (on a 2-cm^2 area delimited by a calibrated circular stamp). Skin reactions were read 24 h later against the following scale: 0 = no reaction; 0.5 = slight erythema not covering the whole test area; 1 = erythema covering all the test area; 2 = erythema plus swelling of the test area; 3 = erythema plus swelling going well beyond the test area.

Before any sensitization, irritation thresholds (primary toxicity) were determined (same procedure as above for elicitation) on FCA-injected controls. All compounds were nonirritating at a 2% concentration.

Acknowledgment. We thank the CNRS for a Bourse d'Ingénieur-Docteur to P.B. (1977–1980) and Hoffmann La Roche through its Institut für Biologische und Medizinische Forschung A.G. (Füllingsdorf) for the generous gifts of guinea pigs.

Etodolac (1,8-Diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic Acid): A Potent Antiinflammatory Drug. Conformation and Absolute Configuration of Its Active Enantiomer

Leslie G. Humber,*† Christopher A. Demerson,† Panchanadam Swaminathan,† and Peter H. Bird‡

Ayerst Laboratories Research, Inc., Princeton, New Jersey 08540, and Concordia University, Montreal, Canada.
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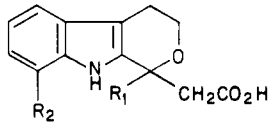
The active (+) enantiomer of the antiinflammatory agent etodolac (1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]-indole-1-acetic acid) has been assigned an *S* absolute configuration on the basis of a crystallographic analysis of the (*S*)-(−)-borneol ester of (−)-etodolac, and the conformation of etodolac has been determined by a crystallographic analysis of (±)-etodolac. Analyses of the solid-state conformation, as well as energy-minimized conformations obtained by molecular mechanics calculations, have failed to provide a basis for identifying a probable receptor-site conformation.

Etodolac (Ultradol) (1, 1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acid), has been shown to be a potent antiinflammatory drug with analgesic and antipyretic activity in animal models.[1,2] These properties have



1 (etodolac). $R_1 = R_2 = C_2H_5$
2 (prodolic acid). $R_1 = n\text{-}C_3H_7$. $R_2 = H$

also been demonstrated in man. Thus, etodolac is as efficacious as aspirin in rheumatoid arthritis patients at doses of 100 and 200 mg given twice daily.[3] In doses of 100 mg or higher, etodolac has demonstrated significant analgesia in patients following gynecologic, urologic, orthopedic, and oral surgery.[4–6] Etodolac has also been shown to reverse the skeletal changes associated with Freund's adjuvant arthritis in rats[7,8] and to retard the

† Ayerst Laboratories Research, Inc.
‡ Concordia University.

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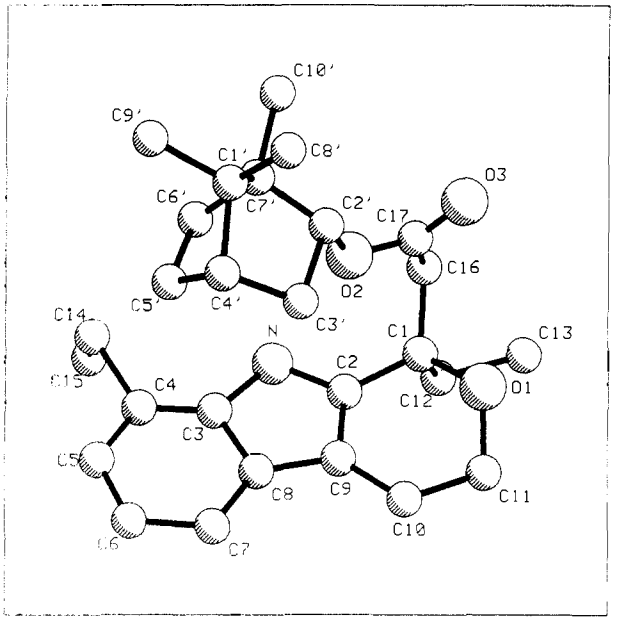

Figure 1. PLUTO drawing of (−)-etodolac (S)-(−)-borneol ester.

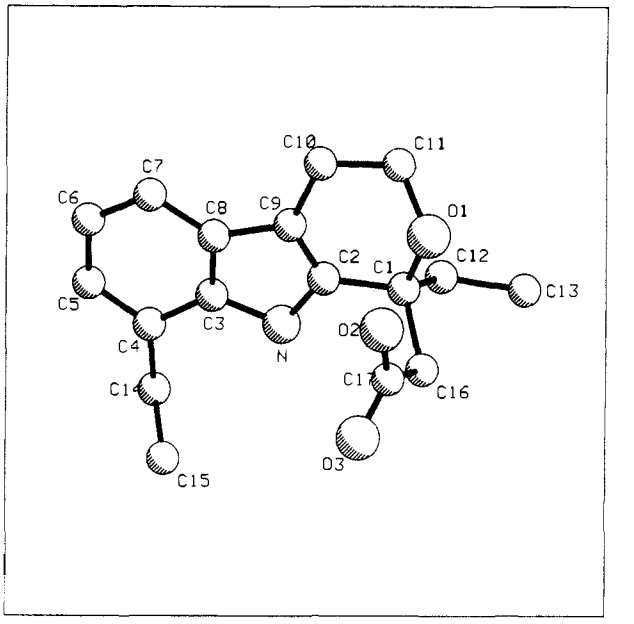

Figure 2. PLUTO drawing of (±)-etodolac.

progression of osseous defects and joint space narrowing in hands and wrists of patients with active rheumatoid arthritis treated with 300 mg/day.[9] Unlike other antiinflammatory drugs, etodolac has a minimal effect on rat gastric mucosal prostaglandin levels[10] and in man is indistinguishable from placebo when one considers the degree of gastrointestinal micro bleeding.[11,12]

We have recently reported that the antiinflammatory and prostaglandin synthetase inhibiting properties of etodolac reside almost exclusively in its (+) enantiomer.[13] An objective of the present study was to determine the absolute configuration of (+)-etodolac. To this end, a crystallographic study was conducted on the (S)-(−)-borneol ester of (−)-etodolac,[13] as this derivative formed superior crystals to the diastereoisomeric ester with (+)-etodolac.

A receptor-site conformation of the 1-alkyldihydropyranoacetic acid moiety of prodolic acid, **2**, an analogue of etodolac, has been proposed on the basis of structure–activity considerations,[14] and another objective of this study was to compare the proposed receptor-site conformation with that of etodolac in the solid state, as well as with a low-energy conformation obtained by molecular mechanics calculations. Since it was feared that because of the presence of the bulky borneol moiety in the ester the dihydropyranoacetic acid moiety might adopt a conformation different from that in etodolac itself, the crystal structure of (±)-etodolac was also determined.

Results and Discussion

A single-crystal X-ray diffractometric analysis of the (S)-(−)-borneol ester of (−)-etodolac established its structure to be as illustrated in Figure 1, that is with the etodolac moiety having an *R* absolute configuration. Since this crystal was derived from the inactive (−)-etodolac, (+)-etodolac could now be assigned the *S* configuration.

Table I. Total Energies and Carboxyl Group Locations of 1-Alkyldihydropyranoindole-1-acetic Acid Conformers

conformer[a]	C_2–C_1–C_{16}–C_{17} torsion angle, deg	energy, kcal/mol	ht,[b] Å	dist,[c] Å
I	306	16.26	2.34	5.40
II	78	17.57	0.79	5.78
III	72	18.65	2.18	5.94
IV	219	21.55	1.05	6.93
V	297.6		2.21	5.53

[a] See text for description of conformers. [b] Height of carboxyl carbon above the plane of the indole nucleus. [c] Distance of carboxyl carbon from center of benzene ring.

The conformation of etodolac as established by the crystallographic analysis of (±)-etodolac is shown in Figure 2 and as a stereoscopic pair in Figure 3. It shows that both the oxygen of the pyrano ring and the acetic acid chain lie above the plane of the indole ring. The torsion angle about C_2–C_1–C_{16}–C_{17} (see Figure 1 for numbering) is 297.6°, and as a result, the carbon atom of the carboxyl group of etodolac is situated 5.53 Å from the center of the benzene ring and lies in a plane 2.21 Å above that of the indole ring. In contrast, the previously suggested[14] receptor-site conformation of 1-alkyldihydropyranoindole-1-acetic acids was one in which the pyrano oxygen was below the plane of the indole ring, with the carboxyl carbon lying over the center of the dihydropyran ring (see Figure 4). This conformer, as well as the crystal structure conformation, was minimized to completion by using the SIMPLEX algorithm as defined in the SYBYL molecular modeling system.[15] Energy profiles were then generated by rotating the carboxyl group through 360° about the C_1–C_{16} bond, in 3° increments, to generate plots of angle vs. total energy. In each case, two valley points were found. Table I contains data on these four low-energy conformers. Those derived from profiling the minimized X-ray structure are designated conformers I and II, while those derived from the proposed receptor-site conformation are designated conformers III and IV. The data in Table I define the

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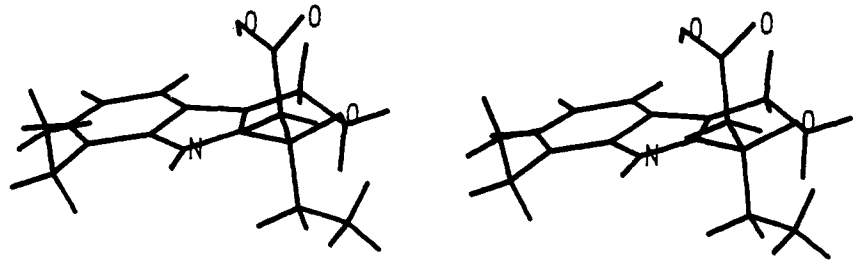

Figure 3. Stereoview of X-ray structure of (±)-etodolac.

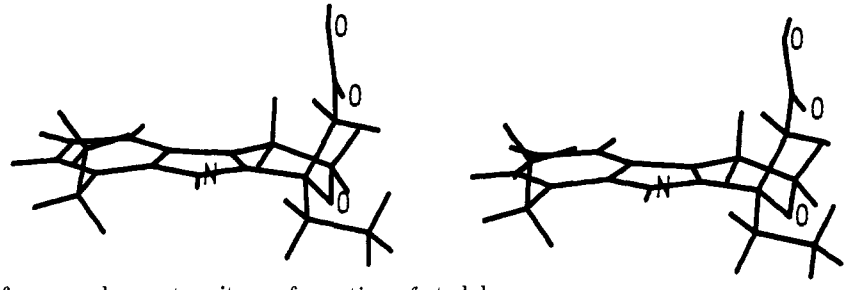

Figure 4. Stereoview of proposed receptor-site conformation of etodolac.

locations of the carboxyl groups of conformers I–IV and of the X-ray structure V, with respect to the plane of the indole ring, and the values of the C_2–C_1–C_{16}–C_{17} torsion angles, the heights of the carboxyl carbons above the plane of the indole ring, and the distance of the carboxyl carbons from the center of the benzene ring are given. Total energies for conformers I–IV are also included.

It is seen that the total energies of conformers I–IV lie within a 5 kcal/mol range. With the inexactitudes of molecular mechanics calculations, we do not feel that the total energies provide a basis for identifying either of conformers I–IV as a probable receptor-site conformation. The values of the parameters in Table I that describe the orientation of the carboxyl carbon with respect to the plane of the indole ring are presented graphically in Figure 5, where the plane of the indole ring lies in the *XZ* plane, the origin corresponds to the center of the benzene ring, and the *X* and *Y* axes are calibrated in arbitrary units corresponding to angstroms. Inspection of this graph shows that conformers of equivalent low energy can have their carboxyl groups lying in a variety of locations with respect to the plane of the indole ring.

While conformers I and III have their carboxyl groups clustered close to that of the crystal structure conformation V, those of conformers II and IV are ~1.5 Å away in different directions. If the carboxyl group plays a role in the binding of etodolac to its receptor, a unique location for this group would probably be critical. The analysis presented above unfortunately does not provide a basis for the identification of a probable receptor-site conformation.

Experimental Section

Single-Crystal X-ray Analysis of (±)-Etodolac. The crystal data for racemic etodolac are as follows: molecular formula $C_{17}H_{21}NO_3$; molecular weight 272.31; orthorhombic *Pbca*; a = 8.60 (8), b = 18.59 (1), c = 19.06 (1) Å; V = 3046.5 Å^3; Z = 8; ρ_{calcd} = 1.188 g cm^{-3}, ρ_{obsd} = 1.20 (5) g cm^{-3} (flotation in aqueous ZnI_2 solution); $|F_{000}|$ = 1159.83 (20 °C, Mo Kα, λ = 0.71069 Å, μ = 0.77 cm^{-1}).

Crystals of racemic etodolac were obtained by recrystallization from benzene–petroleum ether. Trial photographs taken using Weissenberg and precession cameras revealed that the crystals belong to the space group *Pbca*, with the unit cell dimensions of a = 8.602 (8), b = 18.586 (10), and c = 19.055 (10) Å. A small brick-shaped sample of approximate dimensions 0.2 × 0.2 × 0.3 mm was used for collecting three-dimensional intensity data on a computer-controlled Picker FACS-I four-circle diffractometer

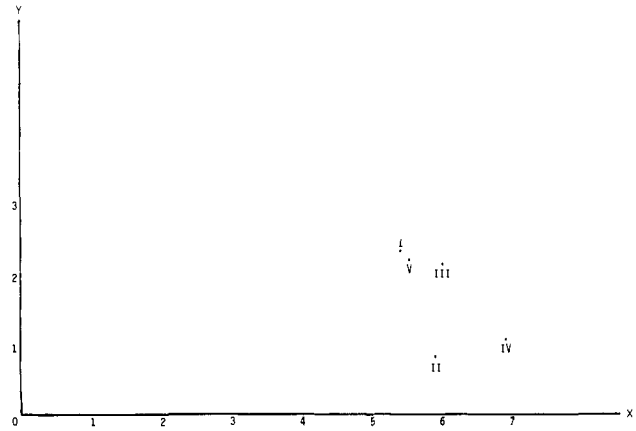

Figure 5. Locations of carboxyl carbon of conformers I–V of etodolac with respect to the plane of the indole ring. See Table I for data used and text for calibratioin units used for axes.

with a graphite monochromator. Due to weak diffraction by these crystals, the number of useful reflections was restricted to be low. The X-ray intensity data were collected by using multiple-scan technique[16] in the range 3.5° < 2θ < 35.0°, employing graphite-monochromated Mo Kα radiation (λ = 0.7107 Å). A reflection scan measurement was terminated and the intensity accepted when the estimated error based on counting statistics was less then 1%. Similarly a reflection was not recorded when the error in the counting, after approximately 12 scans, was predicted to be greater than 5%. A total of 956 unique reflections was collected out of which 416 with $I > 2\sigma(I)$ were considered observed reflections. These were used in structure determination and least-squares refinement of the structure.

The structure was solved by direct methods using the MULTAN program,[17] employing 104 reflections having $|E| > 1.60$. The positional and the isotropic temperature factors of the non-hydrogen atoms were refined by full-matrix least-squares refinement to a R_F = 0.116 and R_{wF} = 0.149 where $R_F = [\sum||F_o| - k|F_c||]/\sum|F_o|$ and $R_{wF} = [[\sum w(|F_o| - k|F_c|)^2]/\sum w|Fo|^2]^{1/2}$. A final difference Fourier map revealed no significant peaks of electron density.

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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 = 0.71069 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.

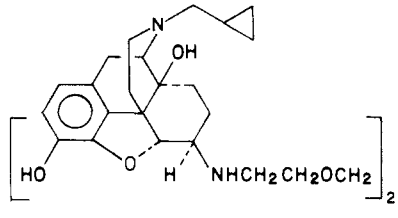
Investigation of the Structural Requirements for the κ-Selective Opioid Receptor Antagonist, 6β,6β′-[Ethylenebis(oxyethyleneimino)]bis[17-(cyclopropylmethyl)-4,5α-epoxymorphinan-3,14-diol] (TENA)

S. Botros,† A. W. Lipkowski,† A. E. Takemori, and P. S. Portoghese*†

Department of Medicinal Chemistry, College of Pharmacy, and Department of Pharmacology, Medical School, University of Minnesota, Minneapolis, Minnesota 55455. *Received July 23, 1985*

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.

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