1.7 (d, J = 7.5, 3 H), 5.0 (dd, J = 7.5, 1.75, 1 H), 6.2 (d, J = 1.75, 1 H), 6.4 (s, 1H), 7.2 (m, 4 H); anal. ($C_{12}H_{10}NO_4Cl$) C, H, N]. Compound **84** [0.218 g, 4%; mp 114-116 °C; IR (KBr) 1817, 1723; NMR ($CDCl_3$) 4.5 (d, J = 5, 2 H), 5.3 (m, 2 H), 6.0 (m, 1 H), 6.4 (s, 1 H), 7.1 (m, 4 H); anal. ($C_{12}H_{10}NO_4Cl$) C, H, N].

5-[2-Chloro-6-[(cyclopropylmethyl)oxy]phenyl]oxazolidine-2,4-dione (83). A solution of compound **57** (2.00 g, 8.71 mmol), potassium *tert*-butoxide (1.95 g, 17.42 mmol), cyclopropylmethyl alcohol (10 mL), and 10 mL of Me_2SO was heated at reflux for 3 h, cooled, and poured into 200 mL of 1 N HCl. The aqueous was extracted with three portions of ethyl acetate, and the pooled organic layers were washed with water and brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to a brown oily solid, which recrystallized from ethyl acetate/hexane [1.37 g, 65%; mp 188-189 °C; IR (KBr) 1827, 1744; anal. ($C_{13}H_{12}NO_4Cl$) C, H, N].

Acknowledgment. We gratefully acknowledge Paul R. Kelbaugh, Derek Tickner, Paul DeCusati, and Harry R. Howard for their chemical contributions and Dwight MacDonald, Weldon Horner, and Kart Grizzuti for their biological expertise.

Crystal Structures of α- and β-Funaltrexamine: Conformational Requirement of the Fumaramate Moiety in the Irreversible Blockage of μ Opioid Receptors

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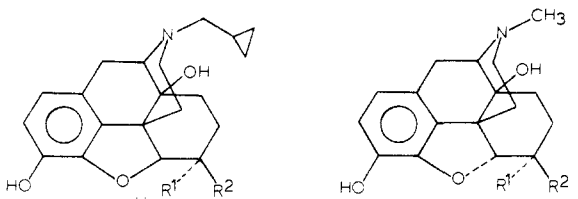
α- and β-funaltrexamine (α- and β-FNA, **1a** and **1b**) are naltrexone derivatives differing only in chirality at C(6). Both epimers bind to μ opioid receptors in GPI and MVD preparations, but only the β-epimer irreversibly blocks these receptors in both preparations. In an effort to investigate the reasons for this difference, we have determined the molecular structures of **1a** and **1b** by X-ray diffraction techniques. The two epimers have almost identical conformations in the fused ring system except for ring C, which is observed in a twist-boat conformation in α-FNA and a chair in β-FNA. As a result the electrophilic fumaramate moieties are equatorial in both structures and orthogonal to one another when the fused rings are superimposed. In the crystal structure of β-FNA there is a close intermolecular contact between a phenolic oxygen and the fumaramate double bond that can serve as a model for nucleophilic attack on the fumaramate group. When **1a** and **1b** are superimposed, the fumaramate double bond of **1a** is more than 2 Å away from that in its epimer **1b** and in the wrong orientation for nucleophilic attack from the proposed direction to take place. The results of this study are consistent with a model that postulates the involvement of two consecutive recognition steps leading to the irreversible blockage by β-FNA (Sayre, L. M.; Larson, D. L.; Fries, D. S.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1983**, *26*, 1229) and underscores the importance of the second recognition step in conferring selectivity in the Michael addition of a nucleophile to the fumaramate group.

β-Funaltrexamine (β-FNA, **1b**) is a naltrexone-derived nonequilibrium narcotic antagonist that is highly selective for the μ-type opioid receptor system.[1-5] The available evidence suggests that the nonequilibrium nature of β-FNA arises as a consequence of the reaction of the fumaramate moiety with a putative nucleophile near the recognition locus of the receptor.[6-9]



The high selectivity of β-FNA for the μ opioid receptor, despite its interaction with other opioid receptor types, has been attributed to the involvement of two consecutive recognition steps.[6-9] The first is reflected by affinity of the ligand for the recognition site; the second involves the proper alignment between the electrophilic center of the ligand with a chemically compatible receptor-based nucleophile. Because two recognition steps rather than one lead to covalent binding, enhanced receptor selectivity (recognition amplification) is obtained. Due to the high selectivity of β-FNA as a nonequilibrium antagonist at μ opioid receptors, it has been employed widely as a tool in the investigation of opioid receptor mechanisms.[10]

In contrast to β-FNA, its epimer α-FNA (**1a**) does not irreversibly block the effects of μ receptor agonists but does protect against β-FNA-induced irreversible antagonism.[9] This suggests that both α- and β-FNA interact with the same site, but the second recognition step is achieved only with β-FNA. Since there is no substantial difference between the reactivity of **1a** and **1b** in solution,[9] an obvious explanation for the observed difference in irreversible antagonism between these epimers may be related to

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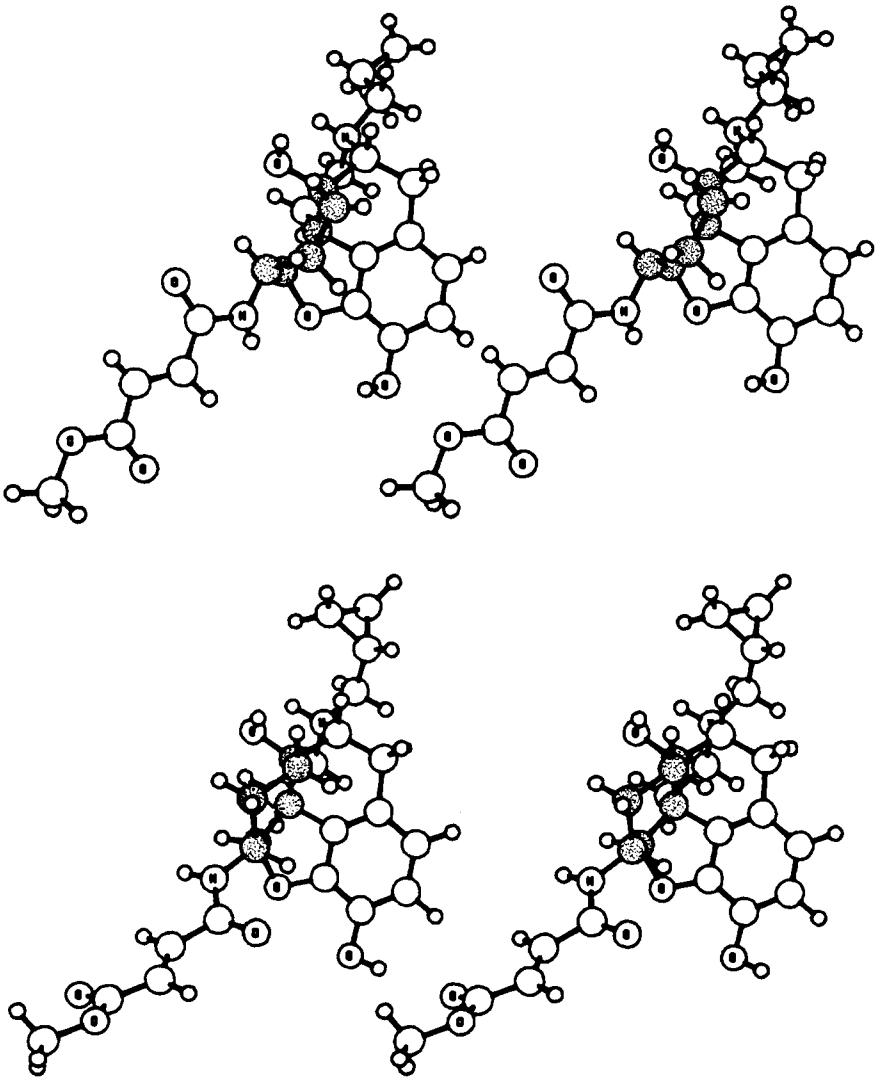

Figure 1. Stereo ORTEP views of α-funaltrexamine (top) and β-funaltrexamine (bottom). The C-ring atoms are stippled to highlight the ring conformations: a twist-boat in α-FNA and a chair in β-FNA.

different orientations of the electrophilic centers in these molecules. In order to provide experimental evidence for the preferred conformation of the fumaramate group, we have determined by X-ray diffraction studies the molecular structures of both α- and β-FNA.[11]

X-ray Results

Stereo ORTEP views of the crystallographically observed structures of **1a** and **1b** are shown in Figure 1. The two epimers have almost identical conformations in the fused ring moiety except for ring C: in α-FNA ring C is observed in a twist-boat conformation and in β-FNA ring C is in a chair conformation (see Table I). The C ring of dihydromorphine analogues has always been observed in a flattened or distorted chair in previous crystallographic studies.[12-17] The torsion angles for the C ring of naloxone[12] are given in Table I for comparison. The C ring conformations in α- and β-FNA result in the fuamaramate chain on C6 being equatorial to ring C in both compounds.

The side chains on N(1) differ in conformation: the cyclopropyl ring is trans to C(16) (see atomic numbering scheme on figure in Table I) and on the OH(14) side of the piperidyl ring in β-FNA and trans to C9 and approximately in the plane of the piperidyl ring on the C(15)–C(16) side in α-FNA. These conformations have both been observed previously, the former in cyclazocine[18] and the latter in gemazocine.[19]

The epimeric funaltrexamines **1a** and **1b** differ in hydrogen-bonding patterns even though they crystallize in the same space group with one water molecule and a halide ion per asymmetric unit. The hydrogen-bond geometry is given in Table IC. There is no hydrogen bond involving O(21) in α-FNA while all donors and acceptors are involved in hydrogen bonds with good geometry in β-FNA.

The fumaramate groups are approximately planar in both structures with the carbonyl groups at C(21) and C(24) cis to the double bond. The ester oxygen is rotated slightly out of the plane in β-FNA. The torsion angles describing the fumaramate groups are listed in Table IB.

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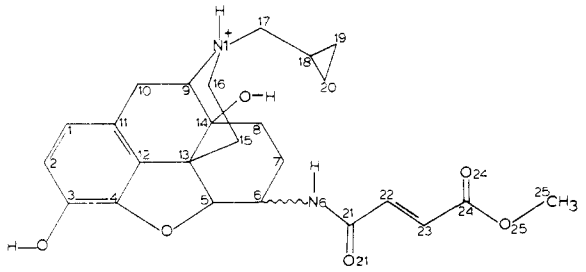
Table I. Observed Conformational Geometry from Crystallographic Studies



A. Conformation of Ring C

torsion angle	α-FNA, deg	β-FNA, deg	naloxone,[a] deg
C(13)–C(5)–C(6)–C(7)	−41.2	42.6	31.0
C(5)–C(6)–C(7)–C(8)	58.9	−59.2	−47.5
C(6)–C(7)–C(8)–C(14)	−19.3	65.3	60.6
C(7)–C(8)–C(14)–C(13)	−33.1	−55.2	−58.8
C(8)–C(14)–C(13)–C(5)	51.0	44.2	43.6
C(14)–C(13)–C(5)–C(6)	−12.8	−36.7	−28.8
av torsion angle	36.0	50.5	45.0
asymmetry parameters[b]	$\Delta C_s(6)$ = 23.5[c]	$\Delta C_s(5)$ = 10.9[d]	$\Delta C_s(6)$ = 22.5
	$\Delta C_s(7\text{–}8)$ = 19.5	$\Delta C_s(6)$ = 20.1	$\Delta C_s(7)$ = 19.7
	$\Delta C_2(5)$ = 18.8	$\Delta C_2(5\text{–}6)$ = 21.8	$\Delta C_2(5\text{–}6)$ = 17.9
	$\Delta C_2(6\text{–}7)$ = 21.1	$\Delta C_2(6\text{–}7)$ = 20.7	$\Delta C_2(6\text{–}7)$ = 29.8
conformation	twist-boat	distorted chair	flattened chair

B. Fumaramate Side-Chain Conformation

torsion angle	α-FNA, deg	β-FNA, deg
H(6)–C(6)–N(6)–C(21)	−16.1	12.4
C(5)–C(6)–N(6)–C(21)	−134.1	128.4
C(7)–C(6)–N(6)–C(21)	101.0	−107.3
C(5)–C(6)–N(6)–H(6N)	44.4	−49.9
C(7)–C(6)–N(6)–H(6N)	−80.5	74.4
C(6)–N(6)–C(21)–C(22)	179.0	−178.3
C(6)–N(6)–C(21)–O(21)	0.6	1.1
N(6)–C(21)–C(22)–C(23)	−167.9	−163.1
O(21)–C(21)–C(22)–C(23)	10.4	17.5
C(21)–C(22)–C(23)–C(24)	177.3	172.8
C(22)–C(23)–C(24)–O(24)	−5.8	17.0
C(22)–C(23)–C(24)–O(25)	173.4	−160.5
C(23)–C(24)–O(25)–C(25)	−176.3	177.6

C. Hydrogen-Bond Geometry

donor–acceptor	distance, Å	D–H···A angle, deg
α-FNA		
N(1)–O(W)	2.872	160.2
O(14)–O(24)	2.835	168.6
N(6)–Cl	3.348	160.3
O(3)–Cl	3.120	166.8
O(W)–Cl	3.340	158.1
O(W)–O14	3.045	[e]
β-FNA		
N(1)–O(21)	2.969	149.1
O(14)–O(W)	2.763	146.7
N(6)–Br	3.464	161.1
O(3)–Br	3.217	141.1
O(W)–O(24)	3.034	146.7
O(W)–Br	3.239	164.2

[a] Reference 12. [b] Duax, W. L.; Weeks, C. M.; Rohrer, D. C. In "Topics in Stereochemistry"; Allinger, N. L., Eliel, E., Eds.; Wiley: New York, 1976; Vol. 9, pp 280–286. [c] An ideal boat conformation would have $\Delta C_s(6) = \Delta C_s(7\text{–}8) = 0.0$; an ideal twist would have $\Delta C_2(5) = \Delta C_2(6\text{–}7) = 0.0$. [d] The largest asymmetry parameters are given to show distortion. An ideal chair has six asymmetry parameters equal to 0.0. See footnote *b* above. [e] Hydrogen not located.

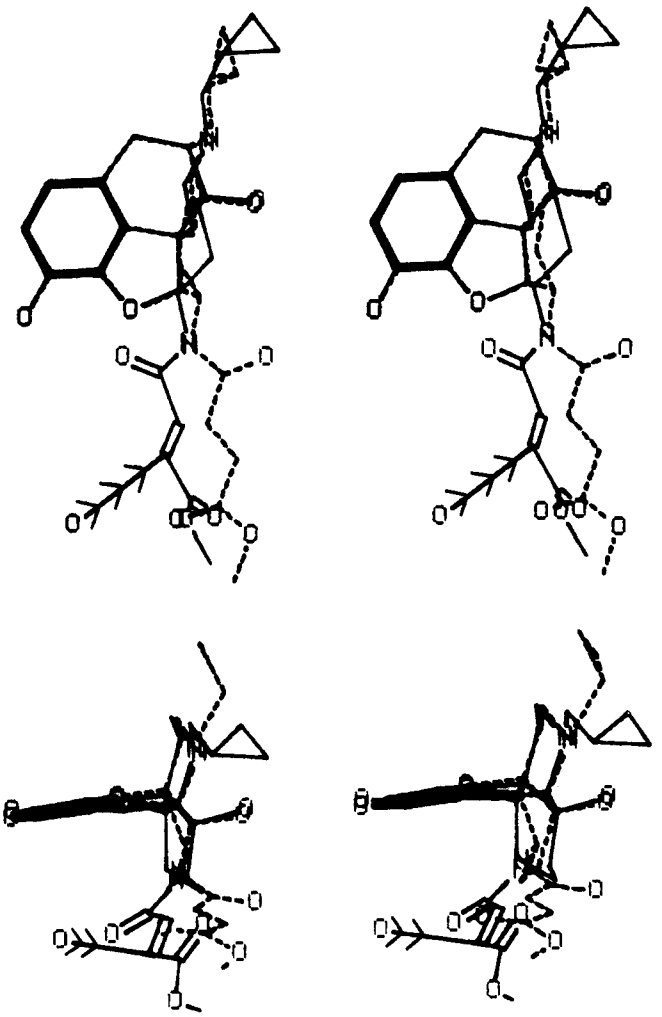


Figure 2. Stereo views of a least-squares fit of the observed structures of α- (dashed) and β-FNA (solid). The atoms fit were C1–C5, C9–C14, O3, O4, O14, N1. Bottom view is made by a 90° rotation about a horizontal axis in the top view. Note how the fumaramate side chains are oriented with respect to the morphine T-shape. The arrow denotes the proposed direction of attack by a receptor nucleophile.

The fumaramate moieties are approximately orthogonal to one another in the two structures. We have made a least-squares fit[20] of the two structures, fitting C(1) → C(5), C(9) → C(14), O(3), O(4), O(14), and N(1) (average intermolecular separation, 0.12 (6) Å). A stereo drawing of the superposition is shown in Figure 2. Crystallographic data and refinement parameters are given in Table II. Atomic coordinates for α-FNa and β-FNA are listed in Tables III and IV, respectively.

Spectral Evidence for C-Ring Conformation

Crouch[21] performed high-field ^{1}H NMR on the 6α- and 6β-epimers of oxymorphamine (**2a** and **2b**). From *J*-correlation contour plots and water-eliminated normal spectra for α- and β-oxymorphamine, they showed that in solution ring C exists in a chair conformation in the 6β-epimer but is in a twist-boat conformation in the 6α-epimer. This is precisely what is seen in the solid-state conformations of α- and β-FNA. In the latter case, it was easy to explain the observations as due to the fumaramate moiety pref-

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Table II. Crystallographic Data

	α-FNA	β-FNA
formula	$C_{25}O_6N_2H_{31}^+$, Cl^-, H_2O	$C_{25}O_6N_2H_{31}^+$, Br^-, H_2O
formula weight	510.81	554.47
crystal system	orthorhombic	orthorhombic
a, Å	10.5346 (8)	14.559 (7) Å
b, Å	31.413 (4)	17.559 (6)
c, Å	7.5347 (5)	9.413 (8)
α, β, γ, deg	90.0	90.0
volume, Å^3	2493.4 (7)	2406.(4) Å^3
ρ_{calcd}, g/cm^3	1.36	1.53
μ, mm^{-1}	1080	1156
radiation	Cu; λ = 1.5418	Mo; λ = 0.71069
space group	$P2_12_12_1$	$P2_12_12_1$
Z	4	4
no. variables	316	316
no. observations	2940	$2735 > 3\sigma$
R factor	0.068	0.102
R_w	0.071	0.079
GOF	1.493	1.870
T, K	298	83
25 reflections for cell constant refinement	$21.82° > 2\theta < 34.88°$	$15.25° > 2\theta < 21.99°$
diffractometer	Enraf-Nonius CAD4	Fortran P3
crystal size, mm	0.40 × 0.80 × 0.98	0.04 × 0.12 × 0.98
crystals grown from	aqueous solution	aqueous solution
structure solved	MULTAN[a]/NQEST[b]	MULTAN[a]/NQEST[b]
weighting scheme	experimental[c]	experimental[c]

[a] Main, P.; Lessinger, L.; Woolfson, M. M.; Germain, G.; Declercq, J. P. "MULTAN 77. A system of computer programs for the automatic solution of crystal structures from X-ray diffraction data"; Universities of York, England, and Louvain: Belgium, 1977. [b] DeTitta, G. T.; Edmonds, J. W.; Langs, D. A.; Hauptman, H. Use of negative quartet cosine invariants as a phasing figure of merit: NQEST. *Acta Crystallogr., Sect. A* **1975**, *A31*, 472. [c] Blessing, R. H.; DeTitta, G. T. Abstract 06.6-02, XIIth Congress of the IUCr, Ottawa, Ontario, Canada, Aug 1981.

erring the equatorial orientation. It is not as apparent why the amine group prefers the equatorial orientation in both epimers. For this reason we performed MM2p[22] calculations on the epimers of oxymorphamine (**2a**, **2b**).

Four structures were generated for input to the MM2p calculations. The twist-boat C-ring conformer of **2a** was generated from the crystallographic coordinates of **1a**, substituting an NH_2 group for the fumaramate in the α-position; the chair C-ring conformer of **2a** was generated from the coordinates of **1b** with an NH_2 in the α-position. Similarly the two C-ring conformers of **2b** were generated from **1a** and **1b** with NH_2 in the β-position. The structures were allowed to refine to their minimum energy. The twist-boat conformer of **2a** was calculated to be 1.10 kcal/mol lower in energy than the chair; the chair conformer of **2b** was calculated to be 4.11 kcal/mol lower in energy than the twist-boat. That is, the observed conformations are calculated to be lower in energy, but the magnitude of the difference in the α-epimer may be underestimated in light of the spectral evidence in solution.

An experimental basis for mapping the reaction coordinate (minimum energy pathway) for the nucleophilic addition to a carbonyl group by nitrogen[23] and oxygen[24] has been determined by examining the geometry of close contacts between the nucleophile and carbonyl carbon in a survey of crystal structures. The authors concluded that the nucleophile approaches along a line, not perpendicular, but forming an angle of ~107° with the C=O bond. In the structure of β-FNA a close intermolecular contact is observed between the C(23) carbon of the fumaramate double bond and the O(3) phenolic oxygen of a neighboring molecule, which appears to distort the fumaramate group. The angle formed by O(3′)—C(23)=C(22) is 118.5°; the distance from O(3′) to C(23) of the double bond is 3.19 Å. We have included O(3′) on the stereo overlap view (with arrows) and propose this as a model for nucleophilic attack on the fumaramate group in the second recognition step leading to covalent binding of the μ opioid receptor (see Figure 3). The fumaramate group on α-FNA would be positioned approximately orthogonal to the same group in β-FNA and not in an ideal position for nucleophilic attack by the receptor. From the least-squares fit of the fused ring moieties of α- and β-FNA, the C(23) of the α-epimer is more than 2 Å away from the C(23) of the β-epimer and appears to be in the wrong orientation for Michael addition to take place (see Figures 2 and 3).

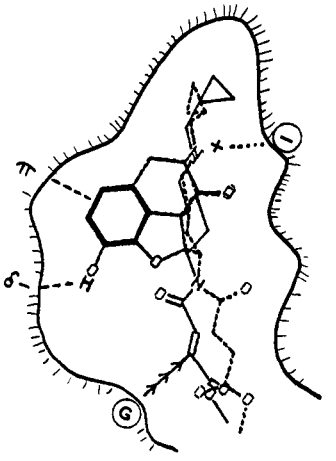

Figure 3. Schematic drawing of the proposed μ opioid receptor site. The first recognition process, common to **1a** and **1b**, involves interactions between the receptor protein and the positively charged nitrogen (–), the phenolic ring (π), the phenolic OH (δ⁻). (G) represents the second recognition site, a nucleophilic residue positioned to attack the fumaramate group of **1b** but not **1a**.

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(23) Burgi, H. B.; Dunitz, J. D.; Shefter, E. *J. Am. Chem. Soc.* **1973**, *95*, 5065.
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Table III. Atomic Coordinates (×10⁴) and Equivalent Isotropic Thermal Parameters of α-Funaltrexamine[a]

atom	x	y	z	B_{iso}
Cl	1623 (1)	6240 (1)	169 (2)	4.04
C(1)	13428 (6)	328 (2)	5288 (9)	3.73
C(2)	12154 (6)	257 (2)	5507 (9)	3.69
C(3)	11225 (6)	466 (2)	4452 (9)	3.56
C(4)	11715 (5)	738 (2)	3162 (8)	2.96
C(5)	11867 (5)	1316 (2)	1350 (7)	2.83
C(6)	11747 (5)	1737 (2)	2347 (9)	3.31
C(7)	12413 (6)	1727 (2)	4151 (9)	3.62
C(8)	13827 (6)	1629 (2)	4022 (8)	3.14
C(9)	15428 (5)	1140 (2)	2550 (8)	2.80
C(10)	15244 (6)	763 (2)	3835 (8)	3.24
C(11)	13901 (6)	609 (2)	3982 (8)	3.08
C(12)	12982 (5)	789 (1)	2908 (7)	2.70
C(13)	13198 (5)	1123 (2)	1513 (7)	2.50
C(14)	14231 (5)	1415 (1)	2257 (8)	2.61
C(15)	13642 (5)	934 (2)	−273 (8)	3.04
C(16)	14923 (5)	710 (2)	−155 (8)	3.03
C(17)	17197 (6)	807 (2)	819 (9)	3.86
C(18)	17773 (6)	716 (2)	−949 (9)	3.68
C(19)	18847 (7)	405 (2)	−990 (11)	5.56
C(20)	17639 (8)	291 (2)	−1839 (11)	5.32
C(21)	9946 (6)	2245 (2)	2248 (10)	3.68
C(22)	8591 (6)	2284 (2)	2522 (9)	3.66
C(23)	7929 (6)	2622 (2)	1986 (9)	3.63
C(24)	6577 (6)	2633 (2)	2230 (9)	3.62
C(25)	4714 (8)	3066 (3)	2099 (13)	6.17
N(1)	15879 (4)	997 (1)	710 (6)	2.83
N(6)	10416 (5)	1850 (1)	2564 (8)	3.71
O(3)	9976 (4)	404 (1)	4743 (8)	4.72
O(4)	10983 (3)	1001 (1)	2118 (6)	3.27
O(14)	14485 (4)	1730 (1)	904 (5)	3.13
O(21)	10651 (4)	2539 (1)	1724 (7)	4.43
O(24)	5923 (5)	2346 (1)	2778 (8)	5.07
O(25)	6066 (4)	3015 (1)	1794 (7)	4.18
O(W)	1300 (6)	3414 (2)	2146 (9)	7.43
H(1)[b]	1402	22	606	4.8
H(1N)	1602	122	4	3.9
H(2)	1183	7	638	4.8
H(3O)	969	63	469	5.9
H(5)	1165	131	21	3.9
H(6)	1214	195	167	4.4
H(6N)	989	172	283	4.9
H(7A)	1194	150	509	4.7
H(7B)	1224	201	479	4.7
H(8A)	1408	150	487	4.3
H(8B)	1424	194	389	4.3
H(9)	1610	132	312	3.9
H(10A)	1554	84	483	4.4
H(10B)	1581	54	337	4.4
H(14O)	1500	190	139	4.3
H(15A)	1374	117	−110	4.1
H(15B)	1301	73	−80	4.1
H(16A)	1524	63	−147	4.2
H(16B)	1482	42	61	4.2
H(17A)	1781	102	155	5.0
H(17B)	1714	50	152	5.0
H(18)	1792	93	−174	4.8
H(19A)	1954	45	−167	6.7
H(19B)	1916	27	6	6.7
H(20A)	1754	27	−329	6.5
H(20B)	1699	5	−128	6.5
H(22)	816	204	324	4.8
H(23)	833	283	133	4.7
H(25A)	448	300	345	7.4
H(25B)	443	338	170	7.4
H(25C)	422	283	125	7.4
H(OW1)	172	348	323	7.9

[a] Estimated standard deviations are given in parentheses. [b] Atomic coordinates for hydrogen atoms are ×10³.

Table IV. Atomic Coordinates (×10⁴) and Equivalent Isotropic Thermal Parameters of β-Funaltrexamine[a]

atom	x	y	z	B_{iso}
Br	466 (1)	4782 (1)	6917 (1)	1.64
C(1)	217 (6)	8030 (6)	3003 (12)	1.52
C(2)	181 (6)	8568 (7)	4070 (11)	1.74
C(3)	892 (6)	9085 (6)	4334 (11)	1.42
C(4)	1636 (6)	9059 (6)	3402 (10)	1.26
C(5)	3106 (6)	9001 (6)	2717 (9)	1.21
C(6)	3468 (7)	8437 (6)	3840 (10)	0.99
C(7)	3851 (6)	7724 (6)	3166 (13)	1.70
C(8)	3109 (6)	7303 (6)	2261 (10)	1.12
C(9)	2048 (7)	7515 (7)	111 (11)	1.08
C(10)	1140 (6)	7425 (6)	868 (11)	1.11
C(11)	976 (6)	8007 (5)	2068 (12)	1.32
C(12)	1656 (6)	8540 (5)	2283 (9)	0.88
C(13)	2558 (6)	8600 (5)	1531 (8)	0.73
C(14)	2860 (6)	7828 (6)	1015 (11)	1.10
C(15)	2452 (6)	9159 (6)	243 (10)	0.95
C(16)	1773 (7)	8857 (6)	−823 (10)	1.17
C(17)	1368 (6)	7780 (6)	−2387 (11)	1.32
C(18)	1543 (7)	6964 (6)	−2798 (10)	1.34
C(19)	1526 (7)	6792 (6)	−4387 (11)	1.56
C(20)	2431 (7)	6793 (7)	−3623 (11)	1.66
C(21)	4077 (7)	8847 (6)	6184 (10)	1.19
C(22)	4832 (6)	9330 (5)	6800 (12)	1.50
C(23)	5083 (6)	9287 (6)	8157 (13)	1.73
C(24)	5904 (6)	9720 (6)	8608 (11)	1.40
C(25)	7081 (6)	9832 (7)	10310 (11)	2.05
N(1)	2003 (5)	8033 (5)	−1182 (9)	1.26
N(6)	4128 (5)	8842 (5)	4721 (9)	1.37
O(3)	885 (5)	9604 (4)	5399 (7)	1.90
O(4)	2433 (4)	9481 (4)	3482 (7)	1.46
O(14)	3640 (4)	7920 (4)	82 (8)	1.32
O(21)	3501 (4)	8515 (4)	6874 (8)	1.49
O(24)	6242 (4)	10238 (4)	7915 (7)	1.79
O(25)	6254 (4)	9471 (4)	9797 (8)	1.81
O(W)	1051 (5)	3591 (4)	4446 (9)	2.47
H(1)[b]	−34	762	289	2.5
H(2)	−44	863	469	2.5
H(5)	366	934	228	2.1
H(6)	290	828	451	1.9
H(7A)	409	734	400	2.6
H(7B)	444	787	251	2.6
H(8A)	251	720	289	1.9
H(8B)	338	678	186	1.9
H(9)	225	696	−25	1.9
H(10A)	110	686	131	2.4
H(10B)	60	750	10	2.4
H(15A)	312	924	−26	1.8
H(15B)	222	971	63	1.8
H(16A)	180	920	−177	2.0
H(16B)	110	888	−37	2.0
H(17A)	67	783	−202	2.1
H(17B)	146	814	−329	2.1
H(18)	130	652	−210	2.2
H(19A)	136	724	−514	2.4
H(19B)	122	627	−478	2.4
H(20A)	279	626	−344	2.4
H(20B)	293	723	−381	2.4
H(22)	519	970	608	2.7
H(23)	468	897	891	2.4
H(25A)	695	1043	1048	3.0
H(25B)	728	956	1130	3.0
H(25C)	761	976	952	3.0
H(O3)	38	947	589	4.0
H(O14)	375	750	25	4.0
H(N1)	264	806	−163	3.5
H(N6)	459	912	429	3.0
H(1W)	94	406	516	3.5
H(2W)	89	382	375	4.0

[a] Estimated standard deviations are given in parentheses. [b] Atomic coordinates for hydrogen atoms are ×10³.

Other Conformations of the Fumaramate Group

The fumaramate group is seen in both α- and β-FNA structures as a planar moiety with bond distances showing evidence of delocalization through NHCOC=CCO, which is to be expected from a consideration of the chemical constraints in the side chain. This observation combined with the solution and solid-state evidence for the preferred conformation of the C ring (C(6)β-NH_2 and -fumaramate in a chair conformation and C(6)α-NH_2 and -fumaramate

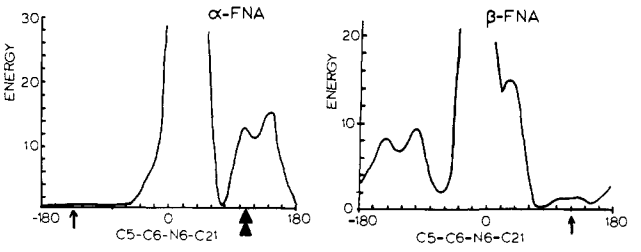

Figure 4. Potential energy vs. C5–C6–N6–C21 torsion angle in α-FNA (left) and β-FNA (right). The conformations observed in the X-ray structures are denoted by single arrows. The double arrow points to the torsion angle in α-FNA required to bring the fumaramate side chain into approximately the same orientation as observed in β-FNA.

in a twist-boat conformation) means that the main point of flexibility for motion of the fumaramate group is rotation about the C(6)–N(6) bond.

We have calculated the conformational energy profiles for rotation about the C(6)–N(6) bond in both α- and β-FNA using a version of the molecular mechanics program CAMSEQ,[25] which has been modified to be used in conjunction with the NIH PROPHET computer system.[26] CAMSEQ uses empirical potential functions for steric, electrostatic, and torsional interactions, as well as a molecule–solvent term and a molecular dipole–solvent term to evaluate relative energies associated with molecular geometry. The initial conformations for the calculations were obtained from the crystallographic coordinates of α- and β-FNA. The relative orientation of the fumaramate group with respect to the morphine fused ring system was varied by rotation about the C(6)–N(6) bond in 10° increments. The energy calculated at each increment was plotted vs. the torsion angle C(5)–C(6)–N(6)–C(21), and the resulting diagram was shifted to place the minimum energy at the zero energy level. The energy profiles are shown in Figure 4.

In each instance, the observed solid-state conformation, denoted by arrows in Figure 4, lies in approximately the center of a very flat well covering about 120° of conformational space with a very high barrier to rotation outside that region. Using PROPHET[26] we have looked at stereo drawings of the α- and β-FNA structures at the limits of their allowed conformations; twisting about C(6)–N(6) from −60° to +60° from the observed conformation of β-FNA and −40° to +80° from the observed conformation of α-FNA, that is, through the low-energy regions shown in Figure 4. Examination of these drawings shows that the regions of low energy for each structure are still approximately orthogonal to one another; throughout the 120° rotation the amide carbonyl and ester end of the double bond remain on the same side of the opiate T-frame, the 14-OH side in the case of α-FNA and the phenol 3-OH side in the case of β-FNA (see Figure 2). When the fumaramate group in α-FNA is rotated into the same orientation as in β-FNA, it is found to be in a region of high potential energy. It suggests that α-FNA cannot easily assume the proposed conformation for the second recognition step to take place and further supports the contention that the general direction of nucleophilic attack is as we have proposed (shown in Figure 3).

Summary and Conclusions

The determination of the X-ray crystal structures of α- and β-FNA (**1a**, **1b**) and the conformational energy calculations of the fumaramate moiety in these epimers have revealed a difference in preferred orientation of the electrophilic group. In this regard the π systems of the fumaramate moieties in the epimers bear an orthogonal relation to one another.

The results of these studies are consistent with the report[9] that only β-FNA (**1b**) selectively and irreversibly blocks μ opioid receptors even though both epimers (**1a**, **1b**) bind reversibly. This supports our earlier proposal that the effectiveness of covalent addition of a receptor-based nucleophile to the reversibly bound ligand is dependent upon the direction of nucleophilic attack.[6-9] Thus the lack of reactivity of α-FNA toward μ receptors is attributed to improper alignment (see Figure 3) between the nucelophile and the Michael acceptor group. It is this second recognition process that is responsible for the recognition amplification observed with β-FNA.

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(25) Weintraub, H.; Hopfinger, A. *Int. J. Quantum Chem., Quantum Biol. Symp.* **1975**, *2*, 203.

(26) This data is stored on PROPHET, which is an NIH-sponsored computer network. Information about PROPHET may be obtained from the Director, Chemical/Biological Information-Handling Program, Division of Research Resources, NIH, Bethesda, MD 20205.