undertaking a similar study in a series of convulsant drugs that appear to share physiological sites of action despite markedly different chemical structures.

Registry No. 1a, 77-28-1; 1b, 76-74-4; 1c, 57-43-2; (S)-1d,

24016-64-6; (*R*)-1d, 24016-63-5; 2a, 2237-92-5; 2b, 125-42-8; 2c, 66968-52-3; 2d, 72961-79-6; 3a, 1952-67-6; 3b, 17013-35-3; 3c, 21149-88-2; (*S*)-3d, 86195-90-6; (*R*)-3d, 86195-91-7; 4, 86162-59-6; 5, 22173-64-4; 6, 66940-72-5; 7, 50-06-6; 8, 17013-38-6; 9, 36226-64-9; 10, 68996-50-9.

Importance of C-6 Chirality in Conferring Irreversible Opioid Antagonism to Naltrexone-Derived Affinity Labels¹

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A series of five epimeric pairs of naltrexone derivatives that contain an electrophilic substituent at the 6α - or 6β -position was synthesized and tested on the guinea pig ileal longitudinal muscle (GPI) and mouse vas deferens (MVD) preparations in order to determine if the orientation of the electrophile is important for covalent bonding to opioid receptors. In the GPI all compounds were pharmacologically active as reversible agonists, but only the 6β -isomers of the fumaramate ester 2b (β-FNA) and isothiocyanate 6b exhibited covalent reactivity, involving a selective irreversible antagonism of the μ agonist, morphine, without affecting κ agonists. The 6α -isomer 2a (α -FNA) was itself nonalkylating but was able to protect the GPI against alkylation by its epimer, β -FNA, indicating that the two epimers bind to the same receptor. These results suggest that the proper orientation of the electrophilic substituent is required for covalent bonding with a proximal nucleophile in the case of μ receptor blockade. Moreover, the lack of covalent bonding to " receptors by these or other ligands in this series indicates the possible absence of sufficiently reactive nucleophiles on this recognition site. In the MVD, 2b, but not 2a, irreversibly antagonized morphine (as in GPI), whereas neither epimer exhibited irreversible antagonism toward the δ agonist, [D-Ala²,D-Leu⁵]enkephalin (DADLE). In contrast, both of the isothiocyanate epimers ($\mathbf{6a}, \mathbf{b}$) irreversibly blocked μ and δ receptors. Evidence suggesting differences between μ receptors in the MVD and GPI was obtained with the β -iodoacetamide 5b, which was an irreversible blocker of morphine only in the MVD. When analyzed together with those of previous studies with the nitrogen must analogues, α - and β -chlornal trexamine, the data suggest that the receptor-alkylating ability of each isomer in an epimeric pair differs most when the electrophile possesses a narrow spectrum of reactivity.

Selective affinity-labeling agents are useful tools for investigating opioid receptors. The affinity labels that have been employed extensively for this purpose are β -chlornaltrexamine²⁻⁵ (β -CNA, **1b**) and β -funaltrexamine⁶⁻¹⁰ (β -FNA, **2b**). β -CNA irreversibly blocks at least three opioid receptor types (μ , κ , and δ), while β -FNA is a μ -specific, irreversible antagonist.¹¹

Implicit in the approach that we have employed in the design of affinity labels is the assumption that covalent bond formation with opioid receptors is dependent on (1) a primary recognition step of forming a reversible ligandreceptor complex, followed by (2) a secondary recognition step involving proper alignment of the electrophile with a proximal nucleophile on the receptor.

A recently reported¹ study of α -CNA [1a, the C-6 epimer of β -CNA (1b)] has suggested that each of the epimeric CNA ligands alkylates different receptor nucleophiles by virtue of the different orientation of the electrophile attached to the C-6 epimeric center. Presumably, the high reactivity of the aziridinium ion generated from α - and β -CNA has facilitated secondary recognition but has made this step less selective because of the array of nucleophiles with which this electrophile can react.

In order to provide a more rigorous test for the second recognition process, we have synthesized epimeric pairs of ligands (2-6) that contain less reactive electrophiles at the C-6 position and have evaluated them for irreversible

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opioid antagonist activity.¹² Since secondary recognition should be more sensitive to stereochemical factors when

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less reactive electrophiles are involved, it seemed possible that only one epimer (of an epimeric pair) might engage in covalent bond formation with opioid receptors. This report describes studies that demonstrate that this is indeed the case and that other types of stereodiscrimination also are important in covalent bonding to opioid receptors.

Chemistry. All of the target compounds were prepared from the corresponding epimers of naltrexamine (8), which in turn were obtained stereospecifically from naltrexone.¹³ Compounds 2, 4, and 7 were prepared by treatment of the amines with the corresponding acid chlorides in the presence of triethylamine. In these reactions, acylation of the phenolic hydroxyl competed to a minor extent with N-acylation so that complete conversion of the starting amine was not achieved without the formation of a small amount of N,O-diacylated material. However, selective solvolysis of the phenolic ester with methanolic triethylamine prior to workup permitted isolation of the desired amides in excellent yields.

The α -maleamate **3a** was obtained by reaction of the amine **8a** with the 1-hydroxybenzotriazole (HOBt) active ester (10) of methyl hydrogen maleate. Since some isomerization to the trans isomer **2a** occurred during this coupling, chromatographic separation was required. In this connection, it was demonstrated that **2a** did not form by isomerization of **3a** under either neutral, basic (dilute NH₄OH), or acidic (dilute HCl) conditions, indicating that **2a** was a direct product of the reaction. Thus, it is possible that **2a** was produced from the isomerized trans active ester **11**, which arose as a consequence of reversible conjugate addition of **3a** across the double bond of **10**.



The attempted synthesis of the β -maleamate **3b** from **8b** and **10** afforded predominantly the isomerized trans

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Scheme I



product 2b, and consequently, an alternative route to 3b was employed. Reaction of 8b with maleic anhydride, conversion of the resulting maleamic acid 9 to its cesium salt, and alkylation with methyl iodide in DMF¹⁴ afforded pure *cis*-3b.

It was found that the maleamate **3b** was unstable in aqueous buffer, ultimately being transformed to the maleamic acid **9**. Under the conditions of biological evaluation (pH 7.4), the half-life of **6b** was determined to be 18 min. In contrast, the fumaramates **2** (α - and β -FNA) were entirely stable under these same conditions.

The enhanced hydrolytic lability of the maleamate esters (3a,b) is obviously related to intramolecular catalysis due to the cis geometry of the double bond. Possible pathways leading to hydrolysis are depicted in Scheme I. Pathway A, which involves the maleimide intermediate, does not appear to be a primary contributor to the accelerated hydrolysis because the synthetically prepared maleimide was found to yield 9 more slowly than did the ester 3b. More likely possibilities are pathways involving an isomaleimide intermediate (B)¹⁵ or intramolecular general-acid catalysis by the amide NH (C). With regard to the latter mechanism, we have reported¹⁶ evidence for an internally H-bonded species.

The iodoacetamides 5 were prepared by reaction of the epimeric amines 8 with the *N*-hydroxysuccinimide active ester¹⁷ of iodoacetic acid. The isothiocyanates 6 were obtained by treatment of the amines 8 with thiophosgene in a two-phase organic-aqueous bicarbonate solvent mixture.

In order to establish that there is no substantial difference in reactivity between 6α - and 6β -epimers, we measured the relative rates of reaction of **2a** and **2b** using cysteine as a model nucleophile. The half-lives for the pseudo-first-order disappearance of these agents in the presence of a 13-fold excess of cysteine at pH 7.4 were found to be nearly identical (approximately 0.5 min). The maleamate **3b** was found to have a half-life similar to β -FNA (**2b**) under the same conditions.

Pharmacology. Guinea Pig Ileal Longitudinal Muscle (GPI). The GPI preparation was prepared by the method of Rang,¹⁸ as described previously.¹ All of the compounds (2-7) possessed agonist potencies in the range of 0.5-10 times that of morphine (Table I). Most of the α -epimers displayed full concentration-response relationships with maxima equal to that of morphine. In contrast, the β -epimers were generally less potent and displayed partial agonism with shallow concentration-response

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 		irreversible antagonist potency			
no.	agonist potency $a(n)$	concn, nM	morphine IC ₅₀ ratio ^b ± SE (n)	nalorphine (N) or EK (E) IC_{50} ratio $b \pm SE(n)$	
 2a	7.3 (4)	20	1.2 ± 0.01 (4)	$(N) 0.92 \pm 0.13 (4)$	
		200	0.87 ± 0.13 (3)	$(E) 0.86 \pm 0.08 (3)$	
26 ^c	5.0 (5)	20	6.0 ± 0.6 (6)	$(E) 1.0 \pm 0.1 (5)$	
		200		(E) 1.2 ± 0.1 (4)	
		1000	$18.8 \pm 4.3 (3)$	(E) 1.3 ± 0.1 (3)	
3a	0.8 (3)	20	$0.95 \pm 0.13(4)$	$(N) 0.49 \pm 0.1 (4)$	
		200	$1.1 \pm 0.1 (4)$	$(N) 0.98 \pm 0.33 (4)$	
3b	1.1 (3)	20	$1.5 \pm 0.6(5)$	(N) 1.0 ± 0.1 (4)	
		200	$0.63 \pm 0.02(4)$	$(N) 0.60 \pm 0.19 (4)$	
4a	1.5 (2)	20	1.2, 0.80	(N) 0.78, 0.61	
		200	$1.2 \pm 0.2 (3)$	$(N) 0.8 \pm 0.06 (3)$	
4b	40% max inhibn	20	1.9(1)	(N) 1.1 (1)	
	at $1 \mu M(3)$	200	4.2 ± 0.9 (5)	(N) 1.4 \pm 0.2 (4)	
		1000	2.9 ± 0.7 (3)	(E) 1.5 ± 0.3 (3)	
5a	0.49 (3)	20	0.97 (1)	(N) 1.2 (1)	
		200	$1.1 \pm 0.2(3)$	$(N) 0.87 \pm 0.15 (3)$	
5b	55% max inhibn	20	2.4 (1)	(N) 1.2 (1)	
	at $1 \mu M(3)$	200	$2.1 \pm 0.4 (3)$	(N) 1.2 ± 0.2 (3)	
		1000	3.4 ± 1.2 (3)	$(E) 0.7 \pm 0.1 (3)$	
6a	9.6 (4)	20	2.1 ± 0.4 (3)	(E) 0.8 (1)	
		200	$2.4 \pm 0.3(3)$	(N) 1.2 ± 0.2 (3)	
		500	2.3 ± 0.7 (3)	(E) 1.4 ± 0.4 (4)	
6b	1.9 (2)	20	6.9 ± 3.2 (3)	(N) 1.5 (1)	
	()	200	14.9 ± 3.3 (3)	(N) 1.4 \pm 0.3 (4)	
7a	2.9 (3)	20	1.1 (1)	(N) 0.7 (1)	
		200	$1.0 \pm 0.1 (3)$	$(N) 0.90 \pm 0.18 (3)$	
7b	0.81 (3)	20	$1.3 \pm 0.2(4)$	$(N) 1.0 \pm 0.1 (4)$	

Table I.	Activities of	Epimeric	Naltrexamine	Derivatives	on the	GPI
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^a Relative to morphine (=1) (IC₅₀ = $2.92 \pm 0.71 \times 10^{-7}$ M; n = 17) on the same ileum preparation; inhibition of contraction is greater than 80%. Values in parentheses are the number of determinations. ^b Agonist IC₅₀ [after 30-min incubation of the GPI with the test compound followed by washing (20 times)] divided by control agonist IC₅₀ in same preparation. Values in parentheses are the number of determinations. Nalorphine: IC₅₀ = $5.13 \pm 0.93 \times 10^{-6}$ M, n = 20. EK: IC₅₀ = $9.73 \pm 0.82 \times 10^{-10}$ M, n = 21. ^c Taken in part from data from ref 7 and 9.

200

 $1.5 \pm 0.3 (4)$

curves. The agonistic effect was *reversible* with washing in all cases.

The possibility that the compounds 2–7 would exhibit irreversible antagonism was evaluated by incubating the tissue at various concentration levels for 30 min, followed by washing (20 times). Any antagonism to morphine and ethylketazocine (EK) or nalorphine that remained was indicative of irreversible blockage of μ and κ receptors, respectively, and was expressed quantitatively as an IC₅₀ ratio (Table I). Only β -FNA (2b) and the β -isothiocyanate **6b** produced a marked concentration-dependent, irreversible antagonism to the agonist effect of the κ -selective agonists, as indicated by the fact that the IC₅₀ ratios for nalorphine or EK were close to unity and concentration independent. A nonelectrophilic epimeric pair (7a,b) was included as a control for the closely related electrophiles.

Although α -FNA (2a) did not exhibit an irreversible antagonist effect itself, its ability to inhibit the irreversible antagonist effect of β -FNA (2b) (for morphine) was evaluated in order to determine whether or not both epimers bind to common receptors. For a 20 nM, 30-min treatment with β -FNA, the morphine IC₅₀ ratio in the unprotected preparation was 6.0 ± 0.6, whereas in the presence of 200 nM α -FNA, the IC₅₀ ratio was 1.3 ± 0.02. A similar experiment using 200 nM morphine as the protector afforded an IC₅₀ ratio of 2.5 ± 0.2. These data indicate that α -FNA efficiently protects against the irreversible antagonist effect of β -FNA and is more effective in this regard than is morphine itself.

After the ileum had been pretreated with β -FNA (20 or 100 nM, 30–45 min) and washed (20 times), the *reversible* agonist activities of both α -FNA and β -FNA were not significantly different from those exhibited in the un-

treated preparation. This same behavior was observed for nalorphine and EK and suggests that the reversible agonist activities of both epimeric series are mediated through κ receptors.

 $(N) 0.85 \pm 0.17 (3)$

Mouse Vas Deferens (MVD). This assay was performed by the method of Henderson et al.¹⁹ as described previously.¹ Selected pairs of epimers (2, 5, and 6) were evaluated for agonist activity. With the exception of 5b, which produced no agonist effect up to 1 μ M, the compounds behaved as partial agonists (Table II). The agonism was reversible in all cases.

The effects of pretreatment of the MVD with the electrophiles (2, 5, and 6) on the IC_{50} values of morphine and $[D-Ala^2, D-Leu^5]$ enkephalin (DADLE) (μ - and δ -selective agonists, respectively) are listed in Table II. Pretreatment with α -FNA (2a) produced no significant changes in the IC_{50} of either morphine or DADLE. A maximal 10.6-fold rightward shift in the morphine concentration-response curve resulted when the MVD was pretreated with 200 nM β -FNA (2b) for 30 min, whereas no change was noted with DADLE. These results indicate that in the MVD, μ receptors were blocked in the presence of δ receptors, which are inert to this treatment.

The epimeric iodoacetamides (5a,b) afforded an activity profile on the MVD that was qualitatively similar to α - and β -FNA. These data (Table II) suggested that 5b irreversibly blocked μ receptors in the MVD but had no significant effect on δ receptors. It is noteworthy that no blockage of μ receptors was observed by 5b in the GPI.

Both isothiocyanate epimers (6a,b) produced concentration-dependent increases in the IC₅₀ ratios of morphine

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Table II. Activities of Epimeric Naltrexamine Derivatives on the MVD

	antagonist pote			cy
no.	agonist act. ^{a} (n)	concn, nM	morphine IC ₅₀ ratio $b \pm SE(n)$	$\begin{array}{c} \text{DADLE} \\ \text{IC}_{50} \text{ ratio}^{b} \pm \text{SE}(n) \end{array}$
2a	60% max; IC ₂₀ = 80 nM (3)	200	$0.52 \pm 0.25(4)$	0.37 ± 0.11 (3)
2b	$IC_{50} = 82 \pm 35 \text{ nM}(4)$	100	$3.9 \pm 0.8 (3)$ 10.6 ± 0.4 (3)	$1.8 \pm 0.4 (3)$ 1 1 + 0 2 (3)
5a	60% max; IC ₂₀ = 200 nM (6)	200	$2.5 \pm 0.9 (4)$	$1.2 \pm 0.3 (4)$
5b	$IC_{50} > 1 \ \mu M(2)$	200	20° (5)	$1.8^{c}(4)$
6a	45% max; IC ₃₀ = 100 nM (4)	20	3.1 [`] ± 1.0 (6)	$1.2 \pm 0.2 (3)$
		200	$10.7 \pm 5.0(5)$	6.6 ± 0.4 (3)
6b	43% max; IC ₃₀ = 100 nM (3)	20 200	13.8^{c} (4) 120^{c} (4)	1.8, 2.5 6.9 ± 3.6 (5)

^a Where the maximum inhibition of contraction was less than that required (70%) for an IC₅₀ determination, an IC₃₀ was approximated. ^b See footnote *b*, Table I, for definition of IC₅₀ ratio. Morphine IC₅₀ = $1.42 \pm 0.32 \times 10^{-6}$ M (*n* = 30); DADLE IC₅₀ = $3.57 \pm 0.47 \times 10^{-10}$ (*n* = 30). ^c Where the standard agonist did not produce a full response (>70%) following compound treatment, an estimate of an IC₃₀ ratio was made from the plotted dose-response curves.

and DADLE. The unusually large shift of the morphine concentration-response curve (~135) by **6b** indicates that other receptor populations in the MVD in addition to μ receptors are irreversibly blocked, since the selective μ -blocker β -FNA effected only an 11-fold shift to morphine.

The increased IC₅₀ ratios of DADLE after treatment with 6a or 6b are suggestive of irreversible blockade of δ receptors but could result partly from a very high degree of blockade of μ receptors, which mediate a component of DADLE agonism in MVD. In order to address this question, an MVD preparation¹⁰ possessing only δ receptors was prepared by alkylating the tissue with β -CNA (1b, 100 nM, 20 min) in the presence of a protecting concentration (2 μ M) of the δ -selective ligand Tyr-D-Ser-Gly-Phe-Leu-Thr.²⁰ The IC₅₀ ratios of DADLE obtained on this δ preparation for a 200 nM, 30-min treatment with **6a** and **6b** were 2.45 ± 0.33 and 4.56 ± 0.72 , respectively, suggesting that both epimers (especially the β) block δ receptors. A comparison of these values with those obtained on the naive tissue (Table II) indicates that almost the entire shift of DADLE exerted by **6b** is due to δ receptor blockage.

Discussion

The results of the present study are consistent with the notion that two recognition processes are involved in the affinity labeling of opioid receptors: a reversible, high-affinity binding (primary recognition) of the ligand, followed by covalent association with a proximal receptor nucleophile.^{11,12} This second step has been termed secondary recognition because it involves proper alignment between complementary reactive centers of sufficient reactivity to engage in covalent binding.

Because two consecutive recognition steps are operative, this, in principle, should afford highly selective agents. This is exemplified by β -FNA (**2b**), which specifically and irreversibly blocks the effects of morphine at μ opioid receptors without affecting κ or δ receptors. Presumably, the specificity of β -FNA is due to the narrow spectrum of reactivity of its Michael acceptor group. The fact that β -FNA (**2b**) is recognized at κ opioid receptors (as a reversible agonist) without covalent binding underscores the importance of the second recognition step in discriminating between different receptor types.

Additional evidence for the importance of secondary recognition was obtained from experiments with α -FNA (2a). This epimer behaved as a reversible κ agonist [as does β -FNA (2b)], but it also afforded efficient protection of

4	в.
[™] 0 0 II II B-NH-C-CH=CH-C-OMe	
(a) (b)	(a) (b)
t	1

Figure 1. The more reactive electrophilic carbon (a) in the fumaramate ester A and the reactive center (b) in the acrylamide B.

 μ receptors against irreversible blockade by β -FNA (2b). This indicates that both epimeric fumaramates bind to common sites, and despite possessing nearly identical intrinsic reactivities as Michael acceptors, only β -FNA is observed to effect receptor alkylation. The most likely explanation is that the Michael acceptor group of β -FNA (2b), but not of α -FNA (2a), is within covalent binding distance of a sufficiently reactive nucleophile when complexed to the receptor.

The importance of secondary recognition also is suggested from the ineffectiveness of the maleamates (3a,b)as irreversibly acting agents. Since maleamate and fumaramate esters are equally good Michael acceptors, it appears that other factors are responsible for the lack of irreversible activity. While the facile hydrolysis of 3b at pH 7.4 may contribute to an underestimate of the IC₅₀ ratio, it seems unlikely that this alone can account for the apparent absence of covalent association. The most likely explanation is that the different geometry of the maleamate group places its electrophilic center in an environment that is distant from a sufficiently reactive nucleophile on the opioid receptor.

Similarly, the inability of the epimeric acrylamides (4a,b) to react covalently with opioid receptors may be a consequence of poor secondary recognition. Two possible explanations for this relate to their lower reactivity (relative to 2) and to a different location of the electrophilic center. The greater resonance stabilization of amides compared to esters results in a reduced electrophilicity of the vinyl group conjugated to the former, relative to the latter. In this connection, it is known that conjugate addition to methyl acrylate occurs 24-29 times faster than to acrylamide.²¹ The consequences of this difference are, firstly, that the acrylamides 4 possess overall lower reactivity than the fumaramates 2 and, secondly, that the preferred reactive center (Figure 1) in the fumaramate (A) is site a rather than site b. On the other hand, nucleophilic addition to the acrylamide (B) can occur only to site b.

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Naltrexone-Derived Affinity Labels

Thus, if carbon a were closer to the receptor nucleophile than carbon b, it is conceivable that the mode of conjugate addition (site b) in the acrylamide (B) together with its lower reactivity might reduce the likelihood of covalent bond formation.

It is noteworthy that the epimeric iodoacetamides (5a,b) exhibited no significant irreversible blockade of μ or κ receptors in the GPI. Once again, lack of adequate secondary recognition may be in part responsible. The intrinsic reactivity of this electrophile relative to that of the fumaramate is not known.

Of significance is the ability of the β -iodoacetamide **5b** to irreversibly block the μ receptor-mediated response to morphine in the MVD despite the fact that no such blockage was observed in the GPI. This raises the possibility that the organization of the μ receptor in the GPI may differ from that in the MVD.²² The existence of differences between μ receptors in different organs is not wholly unexpected in view of the similar situation found with other receptor classes.

The isothiocyanates (**6a**,**b**) have a stereostructure-activity profile similar to that of the fumaramates (**2a**,**b**) in the GPI. Thus, of the two epimers, only the β -isomer **6b** irreversibly blocks the effects of morphine at μ receptors. Also, κ receptors are not affected by exposure to the isothiocyanates. These results may reflect the importance of the second recognition step that leads to the blockage of μ opioid receptors in the GPI.

However, on the MVD preparation, the isothiocyanates behave quite differently from the fumaramates in that both epimers are irreversible blockers of the agonist effects mediated by both μ and δ receptors. This decreased selectivity for these receptor types may be related to a greater reactivity of the isothiocyanate group relative to the fumaramate ester. The irreversible antagonism of μ -mediated effects by both **6a** and **6b** in the MVD again suggests possible differences between the μ receptor systems in the GPI and MVD.

Conclusions

The results of the present study provide evidence for stereocontrolled covalent binding (secondary recognition) of β -FNA (2b) after the formation of a reversible ligandreceptor complex (primary recognition). Ligands with electrophiles of intermediate reactivity and high group selectivity have greater ability to distinguish between opioid receptor types (recognition amplification), as exemplified by β -FNA (2b), which specifically and irreversibly blocks μ receptors. Its epimer, α -FNA (2a), does not react with opioid receptors because of inefficient secondary recognition.

A schematic illustration of the primary and secondary recognition processes in the action of β -FNA (2b) is shown in Figure 2A. Although an array of nucleophiles are present (G¹-G⁴), some within covalent bonding distance, the fumaramate moiety reacts only with one of these. Due to the epimeric relationship of the Michael acceptor group in α -FNA (2a), the electrophilic moiety projects into a receptor locus where the nucleophiles either are not in proper juxtaposition or are incapable of conjugate addition (Figure 2B). Similarly, β -FNA does not alkylate other receptor types (Figure 2C) because the proper nucleophile (G¹) is distal to the electrophilic center of the Michael acceptor.



Figure 2. A schematic illustration of recognition between (A) the fumaramate moiety of β -FNA (2b) and a proximal nucleophile G². No reaction (no secondary recognition) takes place with α -FNA (2a) at the same receptor type (B) or with β -FNA at a different receptor type (C) because an appropriate receptor nucleophile is not properly aligned with the electrophilic center.

A more reactive electrophile is less efficient in its ability to distinguish between different types of nucleophiles. Consequently, the chirality at C-6 becomes less important in the secondary recognition process. In the present study, the epimeric isothiocyanates (6a,b), which are presumably more reactive and less selective than the fumaramates, exhibit a pharmacological profile that is consistent with this view.

With an extremely reactive electrophile, the difference between the receptor covalent-binding capacity of the two epimers is still less pronounced. This has been demonstrated in previous studies with α -CNA (1a) and β -CNA (1b), both of which generate the aziridinium ion as the electrophilic species. It is likely that this highly reactive electrophile reacts with multiple nucleophiles within covalent bonding distance of either the β -epimer 1b (Figure 3A) or the α -epimer 1a (Figure 3B).

The model we have presented assumes that the C-6 stereochemistry does not alter the mode of binding at the receptor. Although this is a reasonable expectation, differences in the receptor-alkylating capacities of C-6 epimers could be interpreted alternatively in terms of substituent-induced alteration of binding modes.²³ It should be noted, however, that in terms of the definition of secondary recognition we have presented, the consequence of different binding modes is equivalent to the model shown in Figures 2 and 3.

Experimental Section

General. 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 were within $\pm 0.4\%$ of the theoretical values. IR spectra were obtained from KBr pellets with a Perkin-Elmer 281 instrument. NMR spectra were recorded at

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Figure 3. A schematic illustration of the interaction of β -CNA (1b) (A) and α -CNA (1a) (B) at identical receptors. Note that different nucleophiles are alkylated (secondary recognition) due to the high reactivity of the aziridinium electrophile.

ambient temperature on Varian A-60D and T-60 instruments, with tetramethylsilane and DSS as internal standard in nonaqueous media and D_2O , respectively. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Mass spectra were obtained on an AEI MS-30 instrument. All TLC data were determined with Eastman "Chromogram" 13181 plastic-backed sheets (silica gel), and the eluant EMA refers to EtOAc-MeOH-NH₄OH. Unless otherwise stated, all reagents and solvents used were reagent grade, without subsequent purification.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6-[trans-3-(methoxycarbonyl)acrylamido]morphinan Hydrochloride (2a·HCl and 2b·HCl). Both epimers were synthesized by a modification of that originally reported for 2b·HCl. A THF-CH₃CN (9:1, 10 mL) solution containing either 8a·2HCl or 8b·2HCl (1 mmol) was stirred over 3Å molecular sieves for 1 h and cooled to -25 °C. To this a solution of methyl 2-(chloroformyl)-2-butenoate (1:1 mmol) in THF (3 mL) was added dropwise with stirring over 30 min, and the mixture then was stirred for 3 h to room temperature. The reaction mixture was concentrated to 2 mL, and to this residue was added MeOH (4 mL) and 1 drop of Et₃N. After 20 h at 25 °C, the solvent was removed, and the solids were extracted with CHCl₃-MeOH (9:1, 10 mL) and washed with aqueous NaHCO₃. Back-extraction of the aqueous wash with $CHCl_3$ (2 times), evaporation of the combined organic extracts, and acidification either with HCl-MeOH or with HCl-Et₂O afforded the desired product in yields of 70-80%. 2a·HCl: mp 220 °C dec; [α]²⁵_D -166.2° (c 0.5, MeOH); $R_f 0.58$ (EMA, 95:5:1); EIMS (70 eV), m/e 454 (M⁺); NMR (D₂O) δ 6.70 and 7.05 (2 d, J = 15.4 Hz, 2 H, vinyl). Anal. (C₂₅H₃₁- ClN_2O_6) C, H, N.

 $17-(Cyclopropylmethyl)-4,5\alpha-epoxy-3,14-dihydroxy-6\alpha-$ [cis-3-(methoxycarbonyl)acrylamido]morphinan Hydrochloride (3a·HCl). Methyl hydrogen maleate (0.195 g, 1.5 mmol) was stirred with HOBt (0.141 g, 1.5 mmol) in THF (20 mL) for 90 min. To this mixture was added rapidly (30 S) 8a (0.492 g, 1.2 mmol) in THF (40 mL) and the solution was stirred for 2 h. After the solution was concentrated to 20 mL and filtered, the contents of the filtrate were subjected to two chromatographic purifications (silica gel; EtOAc-MeOH-NEt₃, 100:2:0.2) to separate the desired product 3a from its isomer 2a. The free base 3a (0.120 g) was crystallized from Et₂O-hexane: mp 192-193 °C; EIMS (70 eV), m/e 454 (M⁺). A CHCl₃ (4 mL) solution of 3a (0.075 g) was treated with Et₂O-HCl to afford 3a HCl: mp 175-180 °C dec; $[\alpha]^{25}_{D}$ -90.4° (c 0.5, MeOH); NMR (D₂O) δ 6.25 and 6.53 (2 d, J = 12 Hz, 2 H, vinyl); $R_f 0.39$ (EMA, 95:5:1). Anal. (C₂₅-H₃₀N₂O₆) C, H, N.

17-(Cyclopropylmethyl)-4,5 α -epoxy-3,14-dihydroxy-6 β -[cis-3-(methoxycarbonyl)acrylamido]morphinan Hydro-

chloride (3b-HCl). The maleamic acid 9 (0.617 g, 1.4 mmol) and Cs_2CO_3 (0.228 g, 0.7 mmol) were dissolved in MeOH (20 mL) and DMF (30 mL). The solution was concentrated slowly to a volume of 25 mL at 30 °C (reduced pressure), diluted with DMF (10 mL), and then concentrated to a volume of 30 mL. To the resulting anhydrous solution cooled to 0 °C was added a solution of MeI (0.241 g, 1.7 mmol) in DMF (3 mL), and the reaction was refrigerated for 10 h. The solution was diluted with aqueous NaHCO₃ (120 mL) and extracted with $CHCl_3$ (3 × 80 mL). The CHCl₃ was evaporated to leave a DMF concentrate, MeOH-HCl was added to pH 1.5, and the solution was again concentrated in vacuo. Dilution with 3 vol of THF and then 1 vol of Et₂O caused precipitation of 0.594 g (86%) of nearly pure 3b·HCl. Recrystallization from MeOH-acetone afforded analytically pure material: mp 257 °C dec; $[\alpha]^{25}_{D}$ -150° (c 1.3, CH₃OH); EIMS (70 eV), m/e 454 (M⁺); NMR (D₂O) δ 6.58 and 6.27 (2 d, J = 12.0Hz, 2 H, vinyl); Rf 0.66 (EMA, 80:20:2), 0.22 (EMA, 98:2:2). Anal. (C₂₅H₃₁N₂O₆Cl·H₂O) C, H, N.

 6α -Acrylamido-17-(cyclopropylmethyl)-4, 5α -epoxy-3,14dihydroxymorphinan (4a). A solution of 8a·2HOAc (0.37 g, 0.8 mmol) in 50% aqueous MeOH (25 mL) was brought to pH 10.5 with 2 N NaOH. A solution of CH_2 =CHCOCl (0.145 g, 1.6 mmol) in THF (10 mL) was added dropwise with stirring at 10 °C, keeping the pH between 10 and 11 by frequent addition of 2 N NaOH. After the addition was complete, the solution was brought to pH 11.5 for 20 h and then down to pH 10 with HOAc, concentrated, and extracted (CHCl₂). The combined organic extracts were evaporated, and the residue was chromatographed on silica gel (EtOAc-MeOH, 9:1). Fractions containing the fastest moving material were combined and evaporated. Dissolution of the residue in aqueous MeOH, concentrating until cloudy, and cooling yielded 0.246 g (75%) of 4a in two crops: decomposes without melting at 140 °C; EIMS (70 eV), m/e 396 (M⁺); R_f 0.62 (EMA, 80:20:2); $[\alpha]^{25}_{\rm D}$ -266° (c 1.2, CH₃CN). (C₂₃H₂₈N₂O₄·²/₃H₂O) C, H, N. Anal.

6β-Acrylamido-17-(cyclopropylmethyl)-4,5α-epoxy-3,14dihydroxymorphinan (4b). According to the procedure given above for 4a, 0.37 g (0.8 mmol) of 8b·2HOAc was converted to 0.266 g (79%) of 4b in two crops: decomposes without melting at 150 °C; EIMS (70 eV), m/e 396 (M⁺); R_f 0.42 (EMA, 95:5:2); $[\alpha]^{25}_{\rm D}$ -171° (c 1.3, CH₃CN). Anal. (C₂₃H₂₈N₂O₄·4/₃H₂O) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6α-(2iodoacetamido)morphinan Methanesulfonate (5a-CH₃SO₃H). According to the procedure given below for 5b, 8a free base (0.44 g, 1.3 mmol) was converted to 0.50 g (63%) of 5a-CH₃SO₃H in two crops: decomposes without melting at >200 °C, EIMS (40 eV), m/e 510 (M⁺); R_f 0.65 (EMA, 80:20:2); $[\alpha]^{25}_D$ -166° (c 1.3, CH₃OH). Anal. (C₂₃H₃₁N₂O₇SI) C, H, N.

17-(Cyclopropylmethyl)-4,5 α -epoxy-3,14-dihydroxy-6 β -(2iodoacetamido)morphinan Methanesulfonate (5b-CH₃SO₃H). A solution of 8b free base (0.38 g, 1.1 mmol) in 2-propanol (20 mL) and THF (40 mL) and a solution of N-(2-iodoacetoxy)succinimide (0.34 g, 1.2 mmol) in THF (20 mL) were each cooled to -20 °C and then mixed. The resulting solution was stored at -10 °C for 16 h and then at room temperature for 40 h. A solution of CH₃SO₃H in MeOH was added to pH 1.2, toluene (5 mL) was added, and all solvent was removed at reduced pressure. The residue was stirred for 40 h with acetone, and the solid obtained upon filtration was recrystallized from EtOH-toluene: yield of two crops of 5b-CH₃SO₃H was 0.49 g (73%); decomposes without melting at >200 °C; EIMS (70 eV), m/e 510 (M⁺); R_1 0.65 (EMA, 80:20:2); $[\alpha]^{25}_{\rm D}$ -101° (c 1.0, CH₃OH). Anal. (C₂₃H₃₁N₂O₇SI) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6αisothiocyanatomorphinan Hydrochloride (6a·HCl). A mixture of 8a·2HOAc (0.37 g, 0.8 mmol) and NaHCO₃ (0.42 g, 5 mmol) was dissolved in 15 mL H₂O-THF (2:1), and a solution of thiophosgene (0.15 g of an 85% solution in CCl₄, 1.1 mmol) in THF (10 mL) was added dropwise with stirring at 0 °C. The resulting two-phase solution was stirred to 25 °C overnight, and Et₂O was added. The organic layer was separated, and the aqueous layer was extracted with CHCl₃. The combined organic extracts were evaporated, and the residue was taken up in MeOH-CH₃CN and acidified to pH 1.8 with HCl. Concentration of the solution yielded 0.29 g (85%) of 6a·HCl, which was crystallized from MeOH-i-PrOH: mp 230 °C dec; $[\alpha]^{25}_{D}$ -265° (c 1.0, CH₃OH); R_f 0.72 (EMA, 95:5:2); EIMS (CH₃SO₃H salt, 70 eV), m/e 384 (M⁺); IR 2080 $(-N=C=S \text{ stretch}) \text{ cm}^{-1}$. Anal. $(C_{21}H_{25}N_2O_3SCl^{-2}/_3H_2O) \text{ C}, \text{ H},$ N.

 $17-(Cyclopropylmethyl)-4,5\alpha-epoxy-3,14-dihydroxy-6\beta$ isothiocyanatomorphinan Hydrochloride (6b-HCl). A mixture of 8b-2HCl (1.25 g, 3.0 mmol) and NaHCO₃ (1.68 g, 20 mmol) was dissolved in H_2O (20 mL) and THF (8 mL). A solution of thiophosgene (0.49 g of an 85% solution in CCl₄, 3.6 mmol) in THF (20 mL) was added dropwise with rapid stirring at 0 °C. The resulting two-layer solution was stirred to 25 °C overnight, Et₂O was added, and the organic layer was separated. The aqueous laver was then extracted twice with CHCl₃. The combined organic extracts were evaporated, and the residue was chromatographed rapidly on silica gel (CHCl₃-acetone-Et₃N, 90:10:1). All fastmoving material was converted into its HCl salt, yielding 0.93 g (74%) of 6b·HCl. Recrystallization from CH₃CN afforded analytically pure material: decomposes without melting at 300 °C; $[\alpha]^{25}$ –289° (c 1.2, MeOH); R_f 0.75 (EMA, 80:20:2), 0.32 (CHCl₃-Et₃N, 100:1); EIMS (CH₃ŚO₃H salt, 70 eV) 384 (M⁺); IR

2065 (-N=C=S stretch) cm⁻¹. Anal. (C₂₁H₂₅N₂O₃SCl) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6αpropanamidomorphinan (7a). A solution of 8a 2HOAc (0.46 g, 1 mmol) in 50% aqueous THF (30 mL) was brought to pH 10 with 2 N NaOH. A solution of CH₃CH₂COCl (0.32 g, 3.5 mmol) in THF (10 mL) was added dropwise with stirring at 10 °C. keeping the pH between 10 and 11 by frequent addition of 2 N NaOH. After the addition was complete, the solution was maintained at pH 10.5 for 40 h, diluted with water, concentrated, and extracted with CHCl₃. The residue obtained upon evaporation of the combined organic extracts was taken up in MeOH, and HCl was added to pH 1. Ethanol and toluene were added, and all solvent was evaporated at reduced pressure. The residue was triturated with acetone to yield 0.33 g (76%) of the HCl salt. The HCl salt was converted to the free base and crystallized from aqueous MeOH: mp 220–221 °C; EIMS (70 eV), m/e 398 (M⁺); R_f 0.56 (EMA, 90:10:2); $[\alpha]^{25}$ –225° (c 0.5 CH₃CN–CHCl₃, 1:1). Anal. $(C_{23}H_{30}N_2O_4)$ C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6βpropanamidomorphinan (7b). The propionylation was conducted with 8b-2HOAc by using the above conditions for 7a. The salt 7b-HCl (0.312 g, 72%) was converted to the free base and crystallized from 75% aqueous EtOH: mp 233-234 °C; EIMS (70 eV), m/e 398 (M⁺); R_f 0.30 (EMA, 95:5:2); $[\alpha]^{25}_{\rm D}$ -168° (c 0.5, CH₃CN-CHCl₃, 1:1). Anal. (C₂₃H₃₀N₂O₄) C, H, N.

 6β -(cis-3-Carboxyacrylamido)-17-(cyclopropylmethyl)-4,5 α -epoxy-3,14-dihydroxymorphinan (9). (a) A solution of 8b base (1.4 g, 4.1 mmol) in 20 mL of CH₂Cl₂-THF (3:2) was mixed with a solution of maleic anydride (0.417 g, 4.25 mmol) in THF (12 mL) at 0 °C and then stirred to room temperature overnight. The resulting slurry was filtered, and the product was washed with Et₂O, yielding 1.82 g (100%) of 9.

(b) To a solution of $8a \cdot 2HCl \cdot 0.75H_2O$ (2.0 g, 4.66 mmol) and Et_3N (0.980 g, 9.7 mmol) in CH_2Cl_2 (30 mL), stirred over 3Å sieves for 1 h, was added a solution of maleic anhydride (0.476 g, 4.85 mmol) in THF (30 mL) at 0 °C. The resulting mixture was allowed to stir at room temperature overnight, coarse filtered to remove the sieves, and then fine filtered. The solid (3.2 g) was taken up in 80 mL of MeOH–EtOH (8:2), heated to 60 °C with stirring, cooled, and filtered to yield 9 (19.8 g, 96%): mp >270 °C; EIMS (70 eV), m/e 440 (M⁺). Anal. ($C_{24}H_{28}N_2O_6 \cdot H_2O$) C, H, N.

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Ion-Sensitive Electrode Potentiometry of Organic Anions: Application to Quantitative Structure-Activity Relationships

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Ion-sensitive electrode potentiometry is proposed for determination of substituent constants for structural modifications of organic acids. A liquid membrane anion-sensitive electrode responds reproducibly to a wide range of carboxylate and sulfonate ions. Fragment constants for the addition of a methylene group to aromatic and aliphatic acids are -2.4 and -3.3 ± 0.15 kJ/mol, respectively. Agreement is observed between these constants and those determined by other techniques, including partitioning studies in biphasic systems, suggesting the use of potentiometry for quantitative structure-activity relationship studies. Furthermore, the electrode measurements correlate with biological effects resulting from hydrophobic interactions.

Quantitative structure-activity relationships (QSAR) have become increasingly useful in interpreting data in the fields of physical organic chemistry, biological chemistry, and new drug development. Several examples can be found for the use of these QSAR's in the study of the activity of currently available drugs.^{1,2} In some cases

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modification of a drug may be desired to increase or decrease its potency. Where lipophilicity is the determining factor, the effect may be adjusted through the addition of one or more groups.³ Substituent constants such as those

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