0.0029 mol), KOH (0.40 g, 0.007 mol), MeOH (5 mL), and H_2O (5 mL) was heated under reflux for 3 h and then evaporated. Workup as for method C gave 71, yield 0.70 g (92%): mp 280–282 °C. Anal. ($C_{13}H_{10}N_2O_2S$) C, H, N.

2-(1*H*-Imidazol-1-ylmethyl)-3-methylbenzo[*b*]thiophene-5-carboxylic Acid (72) (Method F). A mixture of **34** (1.0 g, 0.0033 mol) and 6 N HCl (80 mL) was heated on a steam bath for 6 h and then cooled. The solid was filtered off, dried, and crystallized from EtOH/Et_2O to give **72** HCl, yield 0.72 g (70%): mp 298–299 °C. Anal. ($C_{14}H_{12}N_2O_2S$·HCl) C, H, N.

Acknowledgment. We thank M. J. Pitcher, R. F. Gammon, P. H. Koza, E. Hawkeswood, G. Land, R. I. R. Wilding, and the late D. J. Tims for their able technical assistance. We also thank P. F. Wadsworth and his staff for analytical and spectral data and Dr. T. J. Carty, Pfizer, Groton, for the cyclooxygenase results.

Registry No. 1, 78218-09-4; 3, 85624-79-9; 4, 82788-51-0; 5, 82788-48-5; 7, 16238-12-3; 8, 86793-16-0; 9, 86793-20-6; 10, 102697-27-8; 11, 86793-17-1; 12, 86793-21-7; 13, 102724-26-5; 14, 86793-18-2; 15, 86793-22-8; 16, 50638-12-5; 17, 102697-28-9; 18, 102697-29-0; 19, 7312-07-4; 20, 86792-71-4; 21, 86792-72-5; 22, 86792-75-8; 23, 86792-78-1; 24, 86792-73-6; 25, 86792-76-9; 26, 86792-79-2; 27, 86792-74-7; 28, 86792-77-0; 29, 86792-80-5; 30, 31310-28-8; 31, 86792-83-8; 32, 86792-65-6; 33, 86792-84-9; 34, 86792-66-7; 35, 86792-85-0; 36, 1423-62-7; 37, 86792-81-6; 38, 86792-82-7; 39, 86792-86-1; 40, 86793-54-6; 41, 86792-87-2; 42, 86793-55-7; 43, 86792-88-3; 44, 86793-56-8; 45, 86792-89-4; 46, 86793-57-9; 47, 34586-66-8; 48, 75212-27-0; 49, 21523-62-6; 50, 86793-31-9; 51, 86793-32-0; 52, 86793-33-1; 53, 86793-35-3; 54, 86793-36-4; 56, 86793-72-8; 58, 32281-97-3; 59, 86793-75-1; 59·HCl, 86793-74-0; 60, 86793-76-2; 61, 102697-30-3; 62, 86793-77-3; 63, 102697-31-4; 64, 86793-78-4; 65, 102697-32-5; 66, 86793-79-5; 67, 102697-33-6; 68, 86793-45-5; 69, 86793-19-3; 70, 86793-23-9; 71, 86793-37-5; 72, 86792-67-8; 73, 86792-90-7; 74, 86793-38-6; 75, 86793-58-0; 76, 86793-39-7; 77, 86793-40-0; 78, 86793-34-2; 79, 86793-46-6; 80, 86793-73-9; 81, 102697-34-7; 82, 86793-80-8; 4-$BrC_6H_4CH{=}CHCO_2H$, 50663-21-3; $CH_3S_2CH_3$, 624-92-0; $(C_6H_5)_3COH$, 76-84-6; imidazole, 288-32-4; thromboxane synthetase, 61276-89-9.

Synthesis and Opioid Antagonist Potencies of Naltrexamine Bivalent Ligands with Conformationally Restricted Spacers[1]

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Bivalent ligands 1–4 with naltrexamine pharmacophores and spacers of different lengths containing a fumaryl moiety were synthesized and evaluated for μ and κ opioid antagonist activity on the electrically stimulated guinea pig ileal longitudinal muscle (GPI). The fumaryl moiety was incorporated into the spacer in order to determine the effect of conformational restriction of the spacer on the relationship between spacer length and opioid antagonist potency. While it was found that the fumaryl and succinyl series (11) possessed a very similar structure–potency profile with respect to antagonism at μ opioid receptors, the interaction of these two series at κ receptors differed substantially from one another. This difference was manifested by the longer spacer requirement for peak κ antagonist potency in the fumaryl relative to the succinyl series. It is concluded that the conformational restriction imposed by the fumaryl group in a short spacer ($n = 0$) prevents effective interaction of both pharmacophores with vicinal recognition sites of the κ receptor system; as the spacer is lengthened ($n = 2$) and becomes more flexible, the simultaneous occupation of vicinal recognition sites occurs with greater facility.

Bivalent ligands are defined[2,3] as structures that contain two pharmacophores joined through a connecting unit (spacer). Such compounds have attracted attention as opioid receptor probes.[2-13] Of considerable interest is the use of this approach in the design of opioid antagonists that are selective for specific receptor types. In this regard, we have reported that a short spacer between β-naltrexamine pharmacophores favors κ antagonist activity.[2,4,6] It was proposed that the vicinal sites which recognize κ-selective bivalent ligands are closer to one another than those that recognize μ- or δ-selective bivalent ligands.

This paper describes studies designed to test this proposal. We have investigated the effect of conformational restriction of the spacer on κ and μ opioid receptor antagonist activity of the bivalent ligand series 1–4. The results of these studies lend additional support to the proximity of the vicinal recognition sites for κ-selective bivalent ligands.

Table I. Physical Properties of *N,N'*-Fumaroylbis(oligoglycine)naltrexamine

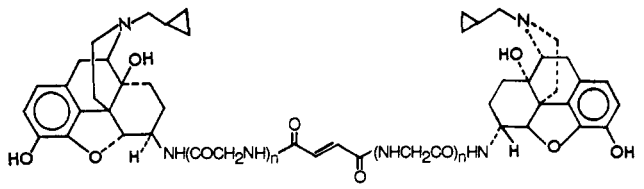
compd no.	R_f	% yield	formula[a]
1	0.76[b]	55	$C_{44}H_{52}N_4O_8·5H_2O$
2	0.26[c]	97	$C_{48}H_{58}N_6O_{10}·3H_2O$
3	0.57[d]	83	$C_{52}H_{64}N_8O_{12}·3H_2O$
4	0.86[e]	45	$C_{60}H_{76}N_{12}O_{16}·6H_2O$

[a] C, H, N analyses were within 0.4% of theory. Melting point for 1–4 was >270 °C. [b] EtOAc–MeOH–NH_4OH (80:20:1). [c] EtOAc–MeOH–NH_4OH (75:25:1). [d] EtOAc–MeOH–NH_4OH (8:8:0.2). [e] EtOAc–MeOH–H_2O–NH_4OH (8:8:8:1).

† Department of Medicinal Chemistry.
‡ Department of Pharmacology.

(1) Stereochemical Studies on Medicinal Agents, 31. For paper 30, see: Sugg, E. E.; Portoghese, P. S. *J. Med. Chem.* in press.
(2) Erez, M.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1982**, *25*, 847.
(3) Portoghese, P. S.; Ronsisvalle, G.; Larson, D. L.; Yim, C. B.; Sayre, L. M.; Takemori, A. E. *Life Sci.* **1982**, *31*, 1283.

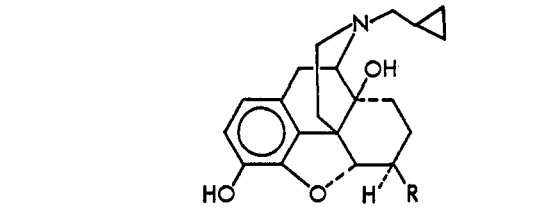
0022-2623/86/1829-1650$01.50/0



1, n = 0; 2, n = 1; 3, n = 2; 4, n = 4

Design Considerations and Chemistry. The previous investigation of bivalent antagonists utilized the succinyl group to produce symmetry in the spacer of the bivalent ligands.[6] In order to reduce the conformational mobility of the spacer, we replaced the succinyl group with a fumaryl group. The resulting bivalent ligands **1–4** are therefore very similar to the corresponding succinyl series, except that the trans double bond in the fumaryl moiety confers conformational rigidity to that segment of the spacer. Presumably, the glycyl units in the spacer should be as flexible as those in the succinyl-containing spacers. The monovalent ligand **5** also was prepared in order to factor out the contribution of the spacer to opioid antagonist activity.

The bivalent ligands (Table I) were prepared by using a standard peptide coupling procedure where the *N*-hydroxysuccinimide ester of fumaric acid or the corresponding bis(oligoglycyl)fumaramide, (**7**, n = 1, 2, 4) was allowed to react with 2 equiv of β-naltrexamine (**6**) (Scheme I). The symmetrical spacers **7** (Table II) were obtained by reaction of fumaryl chloride with 2 equiv of the corresponding oligoglycine under Schotten–Baumann conditions. The large differences in solubility between the bivalent products (**1–4**) and the reactants facilitated the purification procedure.



5, R = $NH(COCH_2NH)_2COC{=}CCO(NHCH_2CO)_2NH_2$

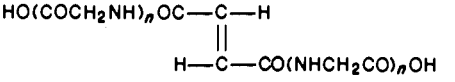
6, R = NH_2

10, R = $NH(COCH_2NH)_2H$

Preparation of the monomer **5** was accomplished by allowing 1 equiv of glycylglycinamide to react with monomethylfumaryl chloride under Schotten–Baumann conditions to give the intermediate fumaramide ester **8**, which was hydrolyzed by base to the monocarboxylic acid **9** (Scheme II). This acid was coupled to 6β-(glycylglycyl)naltrexamine[14] **10** via the HOSu active ester to give the desired monomeric ligand **5**.

Pharmacology. The bivalent ligands **1–4** and the corresponding monomer **5** were tested on the guinea pig ileal longitudinal muscle preparation (GPI) (Table III).[15]

Table II. *N,N'*-Fumaroylbis(oligoglycine) Spacers (7)



compd no.	n	R_f	% yield	formula[a]
7a	1	0.38[b]	52	$C_8H_{10}N_2O_6$
7b	2	0.57[c]	78	$C_{12}H_{16}N_4O_8$
7c	4	0.76[d]	85	$C_{20}H_{28}N_8O_{12}\cdot 0.75H_2O$[e]

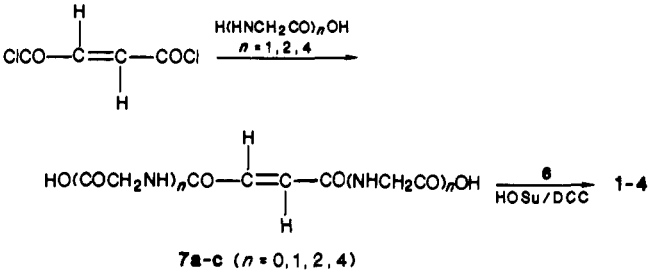
[a] Where otherwise indicated, C, H, N analyses were within 0.4% of theory. Melting point for **7a–7c** was >270 °C. [b] EtOAc–MeOH–HOAc–H_2O (75:22:1:0.5). [c] EtOAc–HOAc (8:1). [d] EtOAc–MeOH–H_2O (8:8:4:1). [e] N: calcd 19.12; found 18.65.

Table III. Antagonist Potencies of Bivalent Ligands at μ and κ Opioid Receptors of the GPI

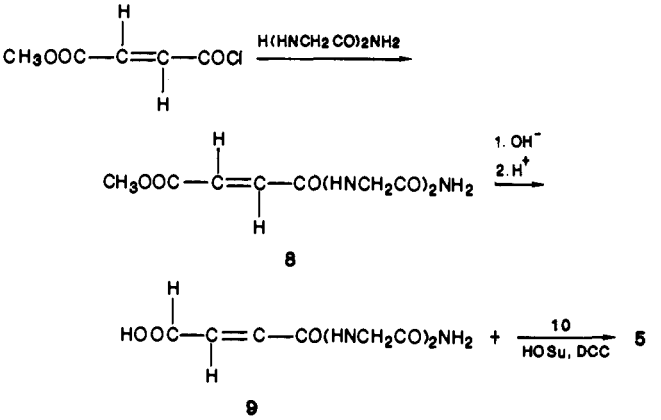
	antagonism[a]			
compd no.	morphine IC_{50} ratio[b]	rel potency[c]	EK IC_{50} ratio[b]	rel potency[c]
1	4.8 ± 0.7	2.1	3.4 ± 1.1	2.3
2	8.2 ± 1.9	3.6	6.6 ± 0.9	4.4
3	100[d]	43	19.6 ± 6.8	13.1
4	22.7 ± 7.2	9.9	3.3 ± 1.2	2.2
5 (monomer)	2.3 ± 0.5	1.0	1.5 ± 0.4	1.0

[a] Values represent the mean ± SE of three determinations. [b] IC_{50} in presence of antagonist (5 nM) divided by control IC_{50} in same GPI preparation. [c] Relative to that of monovalent ligand **5**. [d] Maximum response was 58% (n = 3).

Scheme I



Scheme II



(4) Portoghese, P. S.; Takemori, A. E. *Life Sci.* **1985**, *36*, 802.
(5) Portoghese, P. S.; Larson, D. L.; Yim, C. B.; Sayre, L. M.; Ronsisvalle, G.; Lipkowski, A. W.; Takemori, A. E.; Rice, K. C.; Tam, S. W. *J. Med. Chem.* **1985**, *28*, 1140.
(6) Portoghese, P. S.; Larson, D. L.; Sayre, L. M.; Yim, C. B.; Ronsisvalle, G.; Tam, S. W.; Takemori, A. E. *J. Med. Chem.*, in press.
(7) Hazum, E.; Chang, K.-J.; Leighton, H. J.; Lever, O. W.; Cuatrecases, P. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 347.
(8) Shimohigashi, Y.; Costa, T.; Chen, H.-C.; Rodbard, D. *Nature (London)* **1982**, *297*, 333.
(9) Krumins, S. A.; Costa, T.; Rodbard, D. *Life Sci.* **1982**, *32*, 511.
(10) Costa, T.; Shimohigashi, Y.; Krumins, S. A.; Munson, P. J.; Rodbard, D. *Life Sci.* **1982**, *31*, 1625.
(11) Shimohigashi, Y.; Costa, T.; Matsuura, S.; Chen, H.-C.; Rodbard, D. *Mol. Pharmacol.* **1982**, *21*, 558.
(12) Lipkowski, A. W.; Konecka, A. M.; Sroczynska, I. *Peptides* **1982**, *3*, 697.
(13) Lipkowski, A. W.; Konecka, A. M.; Sadowski, B. *Pol. J. Pharmacol. Pharm.* **1982**, *34*, 69.
(14) Rein, M. M.S. Thesis, University of Minnesota, 1984.
(15) Rang, H. P. *Brit. J. Pharmacol.* **1964**, *22*, 356.

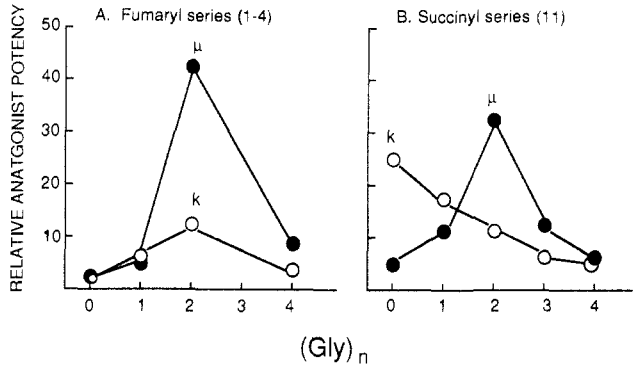


Figure 1. Relationship between opioid receptor antagonist potency (μ and κ) and the number of glycyl units, $(Gly)_n$ in each half of the spacer. Data points from panels A and B are from Table III and from ref 6, respectively. Note the different profiles for antagonism at κ receptors in the fumaryl and succinyl series of bivalent ligands.

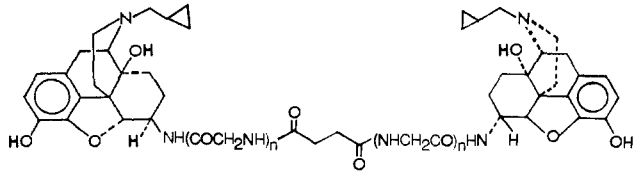
All ligands (5 nM) were incubated with the GPI for a period of 30 min prior to testing with morphine (μ selective) or with ethylketazocine (EK) (κ selective). In the presence of **1–5** the concentration–response curves of morphine and EK were shifted in parallel fashion to higher concentration relative to the controls. In the case of compound **3**, the shift in the morphine IC_{50} was accompanied by a decline of the maximum response (58%), and therefore its IC_{50} ratio is an estimated value. The antagonism to morphine and to EK by **1–5** was capable of being reversed by washing. No agonism was observed for **2–4** at concentrations up to 1 μM. Bivalent ligand **1** and monovalent analogue **5** displayed very weak agonism (35–40% maximum response) at this concentration.

Members of this series (**1–4**) exhibited qualitatively similar structure–activity profiles for antagonism to morphine and to EK. In this regard, maximum shifts were observed with bivalent ligand **3**. All the bivalent ligands were more effective as morphine antagonists than monomer **5**. Potency increases over that of the monomer **5** amounted to factors of about 43 for morphine antagonism and 13 for EK antagonism.

Discussion

The structure–activity relationships of the series containing the fumaryl moiety (**1–4**) and the corresponding series[6] **11** with a succinyl group in the spacer show a striking difference with respect to the antagonism of ethylketazocine (EK), a κ opioid agonist. In the fumaryl series, peak antagonism was observed for **3** (n = 2), while the succinyl series displayed greatest potency at shortest spacer length (n = 0) (Figure 1). On the other hand, the structure–activity profiles for morphine (μ-selective) antagonism are qualitatively similar.

These data suggest that the conformational flexibility of the spacer chain plays an important role in the interaction of the pharmacophores with κ opioid receptors. Since the fumaryl moiety possesses a trans double bond, the spacers of compounds **1–4** should be less capable of assuming a folded "hairpin" type of conformation when compared to the corresponding succinyl analogues **11**.



11, n = 0–4

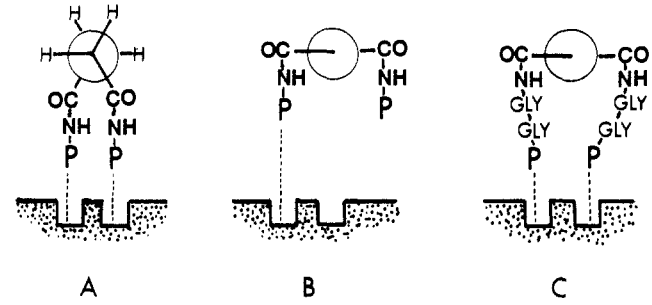
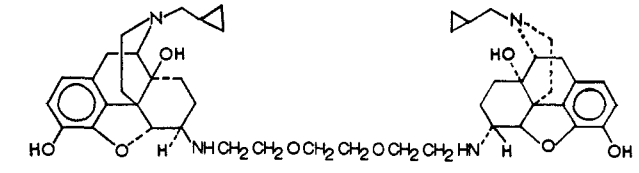


Figure 2. A schematic illustration of how conformational flexibility of the spacer may permit interaction with vicinal recognition sites associated with κ receptors (P = pharmacophore and GLY = glycine). The succinyl spacer in **11** (n = 0) is capable of bridging the recognition sites (A) while the corresponding bivalent ligand with the fumaryl spacer **1** is not capable of a similar interaction because of the conformational restriction (B). Lengthening the spacer **3** confers flexibility and permits bridging to occur (C). The vicinal recognition sites are not necessarily identical.

Thus, at short spacer length (n = 0) the two pharmacophores attached to the fumaryl spacer are restricted with respect to their relative orientation to one another. This in turn can reduce the effectiveness of interaction with vicinal recognition sites. On the other hand, the succinyl analogue (n = 0) is considerably more flexible, and it is conceivable that such vicinal sites can more easily accommodate the ligand. Upon adding glycyl units to a spacer containing the fumaryl moiety, greater conformational flexibility is introduced, and the pharmacophores of the bivalent ligand can more easily "dock" at these vicinal recognition sites (Figure 2). The peak κ antagonism of **3** suggests that this occurs when the spacer contains four glycyl units (n = 2). Additional increases in the number of glycyl units into the spacer (n = 4) introduce unfavorable entropic factors that reduce antagonist potency.

The fact that antagonism at μ receptors in the fumaryl and succinyl series are qualitatively similar suggests that conformational restriction plays less of a role. We propose that this is because the spacer chain is in a more extended conformation when the pharmacophore interact simultaneously with vicinal μ recognition sites. This suggests that the vicinal recognition sites involved in μ antagonism are separated by a greater distance than the vicinal sites that mediate κ antagonism. In this connection, the report[5] that each of the vicinal sites prefer the active (−)-opiate pharmacophore over the inactive (+)-opiate suggests that these recognition sites are similar in the μ receptor system.

Finally, the results of the present study are consistent with the high κ antagonist potency and selectivity of a related bivalent ligand, TENA[4] **12**, which contains a spacer



12 (TENA)

of quite different constitution from that employed in **1–4**. As it was found[2] that a bivalent homologue of TENA with a longer spacer was not a selective κ antagonist, this also implicates a shorter interrecognition site distance requirement for κ antagonism. Whether such vicinal recognition sites represent discrete κ opioid receptors or a κ receptor and a neighboring accessory binding site has not yet been determined. The lack of significant antagonist κ selectivity among monovalent ligands related to TENA tends to implicate the former possibility.[2,16]

Experimental Section

Melting points were determined in open capillary tubes by using a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by M-H-W, Phoenix, AZ, and are within 0.4% of the theoretical values unless otherwise specified. TLC data were obtained by using Analtech silica gel plates. IR spectra were obtained with a KBr pellet on a Perkin-Elmer 281 infrared spectrometer. NMR spectra were recorded at ambient temperature on a JEOL 90-MHz spectrometer using Me_2SO-d_6 as solvent and Me_4Si as internal standard. All reagents and solvents were reagent grade and were used without purification. Abbreviations employed are DCC, dicyclohexylcarbodiimide, HOSu, *N*-hydroxysuccinimide; DCU, dicyclohexylurea; Me_2SO, dimethyl sulfoxide; THF, tetrahydrofuran; EK, ethylketazocine; DADLE, [D-Ala^2,D-Leu^5]enkephalin.

Bis(6β-naltrexaminooligoglycyl)fumaramides (1–4). To a solution of fumaric acid or bis(oligoglycyl)fumaramide (**7**, n = 1, 2, 4) (1.0 mmol) and HOSu (242 mg, 2.1 mmol) in 5 mL of THF or Me_2SO was added DCC (433 mg, 2.1 mmol). The mixture was stirred at 23 °C for 2 days, then the DCU which had formed was removed by filtration, and β-naltrexamine[17,18] **6** (719 mg, 2.1 mmol) was added to the filtrate. This mixture was stirred for an additional 48 h at 23 °C and then poured into Et_2O to yield a precipitate that was collected by filtration and thoroughly washed with $CHCl_3$ and EtOAc. All compounds exhibited characteristic IR amide carbonyl stretching at 1610–1680 and 1500–1565 cm^{-1}. Characteristic NMR absorptions for the glycyl methylene, naltrexamine C-5, and fumaryl vinyl protons were observed at δ 3.61–3.83, 4.41–4.52, and 6.76–6.97, respectively. Additional physical constants for these compounds are listed in Table I.

***N*,*N*′-Fumaroylbis(oligoglycines) (7a–c).** A solution of fumaryl chloride (2.3 g, 15 mmol) in acetone (2 mL) was added dropwise to a solution of the oligoglycine (33 mmol) in 1.5 N NaOH (22 mL) at 20 °C. During the addition, the pH was maintained between 11 and 12 by the frequent addition of 1.5 N NaOH. The reaction mixture was stirred for an additional 30 min and then acidified to pH 1 with 1 N HCl. The precipitated product was collected by filtration, washed with H_2O, and employed in the synthesis of **2–4**. The compounds exhibited the physical properties listed in Table II.

***N*-[[(Carbamoylmethyl)carbamoyl]methyl]fumaramic Acid (9).** To a solution of glycylglycinamide (3.3 g, 20 mmol) in 1.5 N NaOH (13 mL) was added a solution of fumaroyl chloride monomethyl ester (3.0 g, 20 mmol) in acetone (5 mL) dropwise with stirring at 20 °C over 1 h. During the addition, the pH was maintained between 10 and 11 by the frequent addition of 1.5 N NaOH. This intermediate **8** was collected by filtration and washed with H_2O: yield 52%, mp 220 °C; TLC R_f 0.34 (EtOAc–MeOH–H_2O 8:3:2); NMR (D_2O) δ 3.82 (s, CH_3), 3.92, 4.08 (2 s, CH_2), 6.91, 7.21 (2 d, J = 13.9 Hz, vinyls). Anal. ($C_9H_{13}N_3O_5$·0.25H_2O) C, H, N.

A solution of **8** (0.5 g, 2.1 mmol) in 1.5 N NaOH (50 mL) was stirred at 24 °C for 2 h and then acidified with concentrated HCl. The resulting product **9** was collected by filtration, washed thoroughly with H_2O, and employed in the synthesis of **5** without further purification: yield 95%; mp 250 °C; TLC R_f 0.27 (EtOAc–MeOH–HOAc–H_2O 75:25:1:0.5); NMR (Me_2SO-d_6) δ 3.63 and 3.85 (2 d, CH_2), J = 5.7 Hz), 6.50 and 6.97 (2 d, J = 15.4 Hz vinyls), 7.06 and 7.23 (2 s, $CONH_2$), 8.18 and 8.83 (2 t, J = 5.7 Hz, CONH).

[*N*-[[*N*-[[(Carbamoylmethyl)carbamoyl]methyl]fumaramido]acetyl]glycinamido]naltrexamine (5). The monoacid **9** (440 mg, 1.9 mmol) was dissolved in Me_2SO (10 mL) and treated with HOSu (236 mg, 2.0 mmol) and DCC (419 mg, 2.0 mmol). The mixture was stirred at 25 °C for 3 days, the precipitated DCU was removed by filtration, and 6β-*N*-(glycylglycyl)naltrexamine[14] (**10**) (927 mg, 2.0 mmol) was dissolved in the filtrate. The solution was stirred at 25 °C for 1 day and then poured into Et_2O (200 mL). The precipitated product was isolated by filtration and washed several times with $CHCl_3$ and EtOAc to give **5**: yield 55%; mp 205–215 °C; TLC R_f 0.33 (EtOAc–MeOH–NH_4OH, 8:8:1); NMR (Me_2SO-d_6) δ 3.61 and 3.69 (2 s, CH_2), 3.83 (s, CH_2), 4.47 (d, J = 7.7 Hz, C-5 H), 6.53 (br s, Ar H), 6.90 (s, vinyl). Anal. Calcd for ($C_{32}H_{41}N_7O_9$·5H_2O) C 6.78, found 6.16.

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Registry No. 1, 103304-74-1; 2, 103304-75-2; 3, 103304-76-3; 4, 103321-09-1; 5, 103304-82-1; 6, 67025-97-2; 7a, 103304-77-4; 7b, 103304-78-5; 7c, 103304-79-6; 8, 103304-80-9; 9, 103304-81-0; 10, 103304-83-2; H(NHCH$_2$CO)$_2$OH, 556-50-3; H(NHCH$_2$CO)$_4$OH, 637-84-3; fumaric acid, 110-17-8; glycine, 56-40-6; fumarylchloride, 627-63-4; glycylglycinamide, 20238-94-2; fumaroyl chloride monomethyl ester, 17081-8-9.

(16) Botros, S.; Lipkowski, A. W.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1986**, *29*, 874.
(17) Jiang, J. B.; Hanson, R. M.; Portoghese, P. S.; Takemori, A. E. *J. Med. Chem.* **1977**, *20*, 1100.
(18) Sayre, L. M.; Portoghese, P. S. *J. Org. Chem.* **1980**, *45*, 3366.