g) were used in groups of six. Arthritis was induced by subcutaneous injection into the plantar surface of the right hind paw of 0.125 mg of dead tubercle bacillus (comprising human strains PN, DT, and C) homogenized in 0.05 mL of liquid paraffin.

The course of the subsequent inflammatory response was monitored over 18 days by measurement of the volumes of the hind paws by mercury plethysmography. The volume of the right injected paw was measured initially and then every 2 or 3 days over the 18 days of the test. Volume of the left uninjected paw was measured every 2 or 3 days from the 9th day onward. Compounds were administered in 14 daily oral doses (33 mg/kg) beginning on the day before adjuvant injection. Indomethacin was used (3 mg/kg po) as a standard drug.

Areas under the time-course curves were calculated and represented the progress and the severity of the inflammatory response. Following administration of drugs, the difference in the areas under the time-course curves of drug-treated animals from the corresponding areas for the controls was statistically evaluated by analysis of variance.

Joint involvement was assessed by measuring the angle through which the hind paws could be moved easily and expressed as a percentage change over nontreated controls.

Assay of Cyclooxygenase and Lipoxygenase Metabolites of Arachidonic Acid. The method used for detecting inhibitors of arachidonic acid (AA) metabolism was described in a previous paper.[17] Guinea pig peritoneal PMNs, incubated with [^{14}C]AA, were stimulated with the calcium ionophore A23187. Compounds were used at 30 μM concentration in 0.1 N NaOH–Krebs Ringer bicarbonate buffer solution except for indomethacin (3 μM). Indomethacin and BW755C were used as standards. The radiolabeled products, as cyclooxygenase and lipoxygenase metabolites, were identified by using HPLC and GC–MS. A TLC system was used to separate these metabolites, which were then assayed by automatic quantitative scanning. Results were expressed as a percentage change of the radioactivity in each metabolite compared with the stimulated controls. The results in terms of $PGF_{2\alpha}$, PGE_2, and TxB_2 as cyclooxygenase products and 5-HETE and 5,12-diHETE as lipoxygenase metabolites are shown in Table III.

Acknowledgment. We are grateful to David Rackham, Sarah Morgan, and Juliet Brown for spectroscopic data. Elemental analyses were carried out at the Lilly Microanalytical Lab, Indianapolis, IN. We also thank Sue Donovan for her secretarial help in preparation of the manuscript.

Registry No. **1**, 109217-20-1; **2**, 109217-21-2; **3**, 109217-22-3; **4**, 109217-23-4; **5**, 109241-76-1; **6**, 109217-24-5; **7**, 109217-25-6; **8**, 109217-26-7; **9**, 109217-27-8; **10**, 109217-28-9; **11**, 109217-29-0; **12**, 109217-30-3; **13**, 109217-31-4; **14**, 109217-32-5; **15**, 109217-33-6; **16**, 109217-34-7; **17**, 109217-35-8; **18**, 109217-36-9; **19**, 109217-37-0; **20**, 109217-38-1; **21**, 109217-39-2; **22**, 109217-40-5; **23**, 109217-41-6; **24**, 109217-42-7; **25**, 109217-43-8; **26**, 109217-44-9; **27**, 109217-45-0; **28**, 109217-46-1; **29**, 109217-47-2; **30**, 109217-48-3; **31**, 109217-49-4; **32**, 109217-50-7; **33**, 109217-51-8; **34**, 109217-52-9; IV, 109217-53-0; methyl (2-methoxyphenyl)acetate, 27798-60-3; thiophene-2-carbonyl chloride, 5271-67-0; 2-[2-methoxy-5-(2-thienylcarbonyl)phenyl]-2-methylpropanoic acid, 109241-77-2; 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde, 1620-98-0; phenyl isocyanate, 103-71-9.

Synthesis and Evaluation of Melphalan-Containing *N,N*-Dialkylenkephalin Analogues as Irreversible Antagonists of the δ Opioid Receptor[1]

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N,N-Dialkylated leucine enkephalin analogues containing melphalan (Mel) in place of Phe^4 were synthesized as potentially irreversible antagonists of the δ opioid receptor. These compounds, along with the corresponding Phe^4 peptides, were tested for both agonist and antagonist activity in the GPI and MVD smooth muscle preparations. All but two of the eight compounds showed antagonist activity at 1 μM against [D-Ala^2,D-Leu^5]enkephalin (DADLE) in the MVD when tested under reversible conditions; in all cases the Mel^4 peptide had lower activity against DADLE than did the corresponding Phe^4 peptide. At higher concentrations (10 μM) the two active Mel^4 analogues, (benzyl)$_2$Tyr-Gly-Gly-Mel-Leu (**2a**) and (allyl)$_2$Tyr-Aib-Aib-Mel-Leu (**3a**), both showed weak irreversible antagonism at the δ receptor. Compound **2a** was a selective irreversible δ opioid antagonist while **3a** was an irreversible antagonist at both the μ and δ opioid receptors.

Nonequilibrium opioid receptor antagonists are valuable pharmacological tools. Since they do not dissociate from the receptor, they have advantages over reversible ligands in studies such as receptor isolation. They can also be useful in sorting out various opioid receptor types and in determining their relative importance in various opiate-induced pharmacological effects. The ligands that have been employed extensively for these purposes are β-chlornaltrexamine[3,4] (β-CNA) and β-funaltrexamine[4,5] (β-FNA). In vitro, β-CNA irreversibly blocks three opioid receptor types, whereas β-FNA's irreversible antagonism is highly μ-selective. Both ligands show similar activity in vivo, displaying ultralong-acting opioid antagonist activity.

Several compounds have been prepared as potential affinity labels for the δ opioid receptor. Most of these are enkephalin derivatives.[4,6] In addition, the nonpeptide ligands fentanyl isothiocyanate (FIT) and fumaramido-

(1) Presented, in part, at the International Narcotic Research Conference, San Francisco, CA, July 7–11, 1986. Lovett, J. A.; Portoghese, P. S. In *Progress in Opioid Research: Proc. 1986 International Narcotics Research Conference*; Holaday, J. W., Law, P.-Y., Herz, A., Eds.; National Institute on Drug Abuse Research Monograph 75, DHEW Pub. No. (ADM) 87-1507; Supt, of Docs., U.S. Government Printing Office: Washington, DC, 1986; pp 185–188.

(2) Present address: Department of Chemistry, California State University Sacramento, Sacromento, CA 95819.

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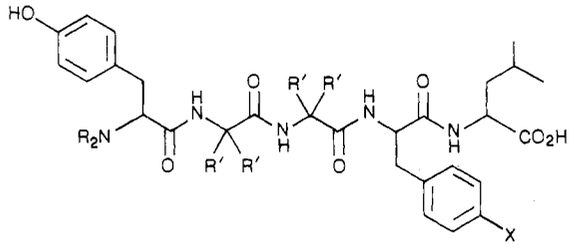
oripavine (FAO) have been reported to be highly selective nonequilibrium blockers of δ opioid receptor binding sites.[7]

All of these affinity labels for the δ opioid receptor were derived from agonists. To date only one compound, a derivative of *N*-(cyclopropylmethyl)-*endo*-ethenotetrahydronororipavine, has been reported to possess δ opioid receptor antagonist selectivity.[8,9] We decided to explore the modification of selective δ opioid antagonists as an alternative approach to designing affinity labels with demonstrable irreversible δ opioid antagonist activity. In this paper we report the chemical modification and biological activity of δ antagonist peptides in an effort to obtain such ligands.

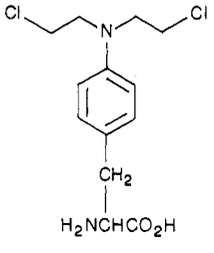
Design Rationale and Chemistry

The compounds chosen for synthesis, **1a–4a**, were based on *N,N*-dialkyl leucine enkephalin analogues, compounds that are selective reversible antagonists of the δ opioid receptor.[10–14] Since changes in the peptide chain or the



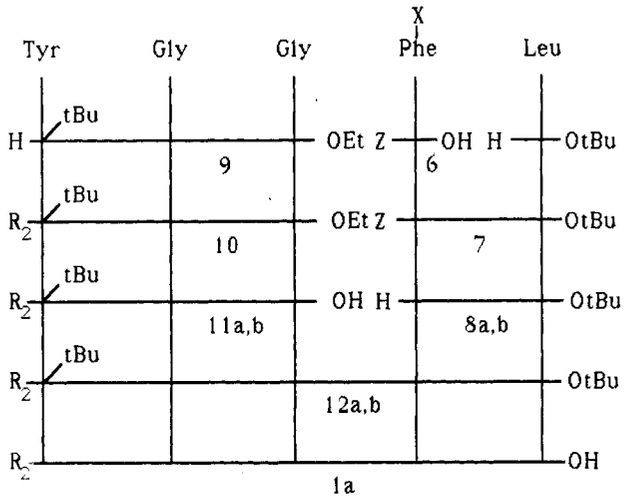
1a: R = $CH_2CH{=}CH_2$, R′ = H, X = $N(CH_2CH_2Cl)_2$(Mel)
1b: R = $CH_2CH{=}CH_2$, R′ = H, X = H
2a: R = CH_2Ph, R′ = H, X = $N(CH_2CH_2Cl)_2$(Mel)
2b: R = CH_2Ph, R′ = H, X = H
3a: R = $CH_2CH{=}CH_2$, R′ = CH_3(Aib), X = $N(CH_2CH_2Cl)_2$(Mel)
3b: R = $CH_2CH{=}CH_2$, R′ = CH_3(Aib), X = H
4a: R = CH_2Ph, R′ = CH_3(Aib), X = $N(CH_2CH_2Cl)_2$(Mel)
4b: R = CH_2Ph, R′ = CH_3(Aib), X = H

amine terminus could alter the alignment of the affinity label with potential alkylation sites on the receptor, derivatives of several reversible antagonists were prepared. The reactive functional group was incorporated in these derivatives at the para position of the phenylalanine ring; the nitrogen mustard moiety was chosen as the alkylating group for this initial series of compounds, since the corresponding amino acid melphalan (Mel, **5**) could be in-



5

Scheme I



6 X = $-N(CH_2CH_2Cl)_2$ (Mel)
7 X = $-N(CH_2CH_2Cl)_2$ (Mel)
8a X = $-N(CH_2CH_2Cl)_2$ (Mel)
8b X = −H
10 R = $-CH_2Ph$
11a R = $-CH_2CH{=}CH_2$
11b R = $-CH_2Ph$
12a R = $-CH_2CH{=}CH_2$, X = $-N(CH_2CH_2Cl)_2$ (Mel)
12b R = $-CH_2Ph$, X = $-N(CH_2CH_2Cl)_2$ (Mel)

corporated directly into the peptide by standard peptide synthetic techniques. The corresponding reversible ligands containing phenylalanine, **1b–4b**, were also evaluated for opioid activity.

Both the Gly- and Aib-containing peptides were synthesized by a 3 + 2 coupling of the desired N-terminal tripeptide with one of two C-terminal dipeptides. Mel-LeuOtBu (**8a**), the C-terminal dipeptide used to prepare the potentially irreversible δ antagonists **1a–4a**, was synthesized as shown in Scheme I. *N*-(Benzyloxycarbonyl)melphalan (**6**) was obtained by the standard method and coupled as its isobutyric carbonic mixed anhydride[15,16] to HCl·LeuOtBu to give **7**. The benzyloxycarbonyl protecting group was then removed by catalytic transfer hydrogenation[17] to give **8a**.

The Gly-containing peptides **1a** and **2a** were prepared by coupling the N,N-dialkylated N-terminal tripeptides **11a**[14] and **11b**, respectively, with either Mel-LeuOtBu (**8a**) or Phe-LeuOtBu (**8b**) followed by deprotection. The *N,N*-dibenzyl derivative **11b** was prepared in a manner similar to **11a**,[14] namely by alkylation of the tripeptide **9**[14] with benzyl bromide to give **10** followed by hydrolysis of the ethyl ester. The tripeptides **11a**[14] and **11b** were coupled to **8a** by using the isobutyric carbonic mixed anhydride procedure to give **12a** and **12b**, respectively. Deprotection using 50% trifluoroacetic acid in CH_2Cl_2 containing anisole as a scavenger gave **1a** and **2a**, respectively.

The Aib-containing peptides were prepared in a similar manner (Scheme II). The synthesis of the N-terminal tripeptides involved coupling ZTyr(OtBu)[14,18] via the iso-

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Table I. Reversible Agonist and Antagonist Activities of *N,N*-Dialkyl Leucine Enkephalin Analogues in the Mouse Vas Deferens (MVD) and Guinea Pig Ileum (GPI)

	MVD[a]			GPI[b]		
	agonism:[c]	antagonism: IC_{50} ratio[d]		agonism:[c]	antagonism: IC_{50} ratio[d]	
compd	% inhibn	DADLE	morphine	% inhibn	morphine	EK
1a[e]	4.2 ± 2.7	1.2 ± 0.1	1.1 ± 0.2	2.3, 10.0	0.6, 1.1	0.6, 1.0
1b[f]	10.0 ± 4.1	2.7 ± 0.6	0.8 ± 0.1	−6.7, 0	0.8, 1.1[g]	0.6, 1.1[g]
2a[e]	16.2 ± 1.5[h]	3.0 ± 0.5[h,i]	1.0 ± 0.5[i]	4.8, 10.0	0.5, 0.7	0.4, 0.4
2b[f]	11.4 ± 5.9	6.6 ± 1.4[h]	0.6 ± 0.2	1.9, 2.9	1.0, 1.0	0.8, 1.2
3a[e]	20.6 ± 6.5	5.0 ± 0.9	2.2 ± 0.7[h]	8.1, 14.7	0.6, 1.0	0.3, 0.6
3b	8.4 ± 7.0	15.6 ± 2.4[g,j]	0.8 ± 0.1[g,k]	16.2, 37.8	0.5, 0.6	0.7, 0.9
4a[e]	22.5 ± 5.2	1.2 ± 0.2	1.0 ± 0.3	9.4, 12.5	0.4, 1.0	0.2, 0.3
4b	25.7 ± 4.9	5.6 ± 1.7	1.0 ± 0.1	68.7 ± 6.7[l]	0.7, 1.3[m]	1.0 ± 0.5[m,n]

[a] Compounds were tested at 1 μM. Results are mean ± standard error of the mean (SEM); $n = 3$ unless otherwise indicated. [b] Compounds were tested at 1 μM. Results are individual values ($n = 2$) unless otherwise indicated. [c] Percent inhibition of the twitch after a 10-min incubation of the tissue with the test compound. [d] The IC_{50} of the agonist after a 10-min incubation (unless otherwise indicated) with the test compound divided by the IC_{50} of the agonist in the same tissue in the absence of the test compound. [e] Melphalan-containing compounds were added to the tissues as a solution in methanol (10–100 μL). In the concentrations used methanol had no effect on the tissue. [f] Reference 14. [g] Thirty-minute incubation. [h] $n = 4$. [i] Five-minute incubation. [j] Ke against DTLET and Leu[5] enkephalin in the MVD is 30.6 and 30.1 nM, respectively. Reference 13. [k] Ke against normorphine in the MVD is >5000 nM. Reference 13. [l] $IC_{50} = 0.3 \pm 0.1$ μM, $n = 4$. [m] At 0.02 μM. [n] Mean ± SEM, $n = 3$.

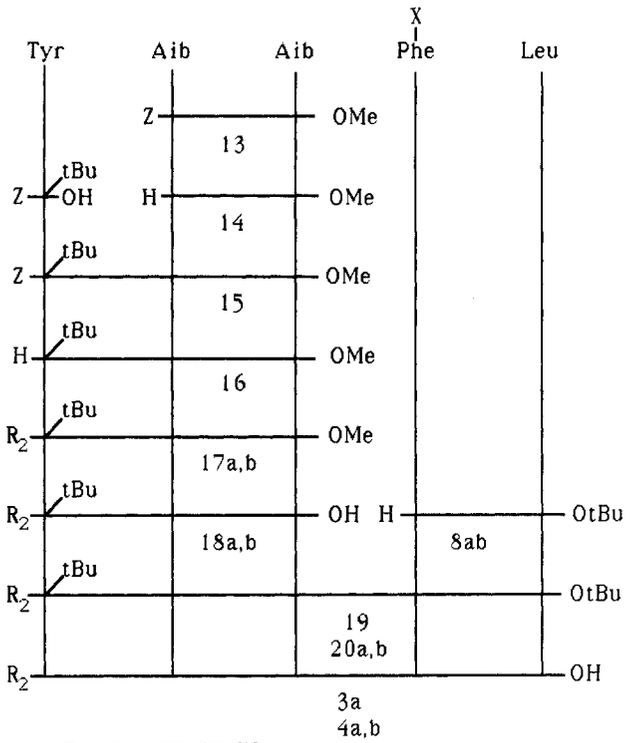
butyric carbonic mixed anhydride with the dipeptide **14**, which had been obtained from the protected precursor **13**[19] by catalytic hydrogenation. The resulting tripeptide **15**, was then deprotected by catalytic transfer hydrogenation to give the analogue **16** containing a free amino group. Alkylation of **16** with allyl bromide or benzyl bromide gave **17a** and **17b**, respectively. These methyl esters were then hydrolyzed to the acids **18a** and **18b**. Because of the steric hindrance of the Aib residue, the couplings of **18a** and **18b** to the C-terminal dipeptides **8a** and **8b** were performed by the pivaloyl mixed anhydride procedure.[19] These reactions proceeded in only moderate yields (48–78%) to the pentapeptides (**19**, **20a**, and **20b**) with *N*-pivaloyl-Phe-(X)-LeuOtBu as the major side product. The deprotected pentapeptides **3a**, **4a** and **4b**, respectively, were obtained by using 50% trifluoroacetic acid in CH_2Cl_2 containing anisole.

Pharmacological Results

The compounds were initially tested for reversible opioid agonist and antagonist activities in the electrically stimulated mouse vas deferens (MVD)[20] and guinea pig ileum (GPI)[21] (Table I). Antagonist activity was evaluated with morphine, ethylketazocine (EK), and [D-Ala^2,D-Leu^5]-enkephalin (DADLE) as agonists for the μ, κ, and δ opioid receptors, respectively.[22] This activity is expressed as the ratio of the IC_{50} values and represents the factor by which the agonist dose–response curve is shifted to higher concentrations in the presence of the antagonist.

All but two of the compounds were reversible antagonists in the MVD when tested at 1 μM against the δ receptor agonist DADLE. Of the Phe^4 peptides (**1b–4b**), all of which possessed δ opioid antagonist activity, **3b** (ICI 174,864) exhibited the highest activity while the corresponding Gly-containing compound **1b** showed the lowest antagonist activity. Thus the effect of N-substitution on the rank order of δ antagonist activity was reversed for the Aib-containing compounds as compared to the Gly-containing peptides. Replacement of phenylalanine by melphalan resulted in decreased antagonist activity at the δ receptor. For two compounds, **1a** and **4a**, this decrease

Scheme II



17a R = -CH_2CH=CH_2
17b R = -CH_2Ph
18a R = -CH_2CH=CH_2
18b R = -CH_2Ph
19 R = -CH_2CH=CH_2, X = -$N(CH_2CH_2Cl)_2$ (Mel)
20a R = -CH_2Ph, X = -$N(CH_2CH_2Cl)_2$ (Mel)
20b R = -CH_2Ph, X = -H

yielded compounds that were inactive as δ receptor antagonists at 1 μM. For analogues **2a** and **3a** the 3-fold decrease in activity yielded derivatives that still possessed reversible δ receptor antagonist activity. Only one compound, **3a**, showed any antagonist activity against morphine in the MVD.

Compounds **2a–3b** were also tested at 10 μM in the MVD (Table II). As expected, the reversible IC_{50} ratios against DADLE increased for all compounds, but the increase was significantly greater for the Phe-containing analogs (9.4- to 14.6-fold increase) than for the Mel-containing peptides (2.6- to 3.9-fold increase). One compound,

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Table II. Reversible and Irreversible Antagonism of **2a–3b** at 10 μM in the Mouse Vas Deferens[a,b]

compd	agonism: % inhibn[c]	antagonism: DADLE IC_{50} ratio		antagonism: morphine IC_{50} ratio	
		rever[d]	irrev[e]	rever[d]	irrev[e]
2a[f]	18.3 ± 7.7[g]	7.7 ± 3.3[g]	2.4 ± 0.8[h,i]	1.7 ± 0.2[j]	1.2 ± 0.2[j]
2b	32.0 ± 9.0	96.9 ± 24.0	1.9 ± 0.3	2.5 ± 0.2	1.0 ± 0.1
3a[f]	16.8 ± 1.5	19.4 ± 4.7[g]	2.9 ± 0.9[h,k]	16.3 ± 8.5[j]	3.0 ± 0.4[j]
3b	15.8 ± 6.3	147 ± 30	5.5 ± 1.2	1.6 ± 0.4	0.8 ± 0.1

[a] At 1 μM the irreversible ratios for **2a**, **3a**, and **3b** were not significantly greater than 1. [b] Results are ±SEM, $n = 3$ unless otherwise indicated. [c] Agonism: see Table I, footnote *c*. [d] Reversible IC_{50} ratio: see Table I, footnote *d*. [e] Irreversible IC_{50} ratio: the IC_{50} of the agonist in a tissue that was exposed to the antagonist for 10 min (unless otherwise indicated) and then washed 30 times divided by the IC_{50} of the agonist in the same tissue prior to expossre to the antagonist. [f] See Table I, footnote *e*. [g] $n = 4$. [h] $n = 5$. [i] Irreversible IC_{50} ratio = 1.2 ± 0.2 after a 30-min incubation. [j] Thirty-minute incubation. [k] Irreversible IC_{50} ratio = 3.0 ± 0.4 after a 30-min incubation.

3a, showed substantial reversible antagonist activity against morphine, with no selectivity for antagonism against DADLE over morphine.

In the GPI one compound, **4b**, showed significant agonist activity (IC_{50} = 0.3 μM), even though it did not show such activity in the MVD (Table I). All of the compounds were inactive as antagonists in the GPI against both morphine and EK.

The two melphalan-containing peptides that showed reversible δ antagonist activity at 1 μM were tested for irreversible antagonist activity at both 1 and 10 μM. The compounds were also tested as irreversible antagonists against morphine in order to determine the selectivity for the δ receptor of any observed irreversibility. The corresponding Phe-containing compounds **2b** and **3b** were evaluated under similar conditions to verify that the washing procedure effectively removed noncovalently bound ligand. Irreversible antagonism was assessed as follows: the tissue was incubated with the test compound for 10 min and then washed 30 times with fresh buffer to remove any of the test compound that was not covalently bound. The IC_{50} of the agonist was then determined and compared to the IC_{50} of the agonist in the same tissue before exposure to the antagonist; the results are expressed as the ratio of these two values.

At 1 μM the compounds **2a**, **3a**, and **3b** showed no significant irreversible antagonism toward DADLE in the MVD. At 10 μM both **2a** and **3a** showed irreversible antagonist activity of marginal significance (Table II). Longer incubation times did not increase the degree of irreversible antagonism. For the reversible Phe-containing compounds **2b** and **3b**, the IC_{50} ratios decreased substantially (a 51- and 27-fold decrease, respectively) after washing, but were still greater than one. Under irreversible conditions, compound **3a** showed significant antagonist activity toward morphine. In the GPI at 10 μM neither **2a** nor **3a** showed any significant reversible or irreversible antagonist activity against either morphine or ethylketazocine (reversible IC_{50} ratios ≤ 1.4, irreversible IC_{50} ratios ≤ 1.2).

Discussion and Conclusions

Three major factors determine the efficiency of receptor alkylation by affinity labels:[4] (1) the reversible affinity of the ligand for the receptor, (2) the proximity of the electrophile on the affinity label to the nucleophile on the receptor, and (3) the intrinsic reactivity of the electrophile toward the available nucleophile.

Decreased reversible affinity is the most likely reason that the ligands **2a** and **3a** were only marginally effective as irreversible antagonists. The lower reversible antagonism of the Mel-containing analogues **1a–4a** relative to the corresponding Phe-containing peptides **1b–4b** suggests that the bulky nitrogen mustard might be causing steric interference at the subsite on the receptor that binds the phenylalanine aromatic ring.

The irreversible antagonism of **2a** and **3a** was also affected, although probably to a lesser extent, by the orientation of the nitrogen mustard moiety and by its reactivity. The similar irreversible δ antagonist activity for **2a** and **3a** at 10 μM in spite of the 2.5-fold higher reversible antagonism of **3a** indicates that the orientation of the alkylating group may be different in the two analogues. The aromatic nitrogen mustard of melphalan is less reactive than other alkylating agents, such as the aliphatic *N*-methyl nitrogen mustard,[23] which may be a factor in why only weak irreversible antagonism was observed for **2a** and **3a**, even at 10 μM.

The introduction of the nitrogen mustard moiety also affected the receptor selectivity of one analogue. Although the other peptides were selective δ receptor antagonists, even at high concentrations, the introduction of the nitrogen mustard into **3b** to give **3a** increased the compound's antagonist activity at the μ receptor and thus decreased its δ receptor selectivity. The similar irreversible antagonism of DADLE and morphine by **3a** may be a reflection of the indiscriminate nature of nitrogen mustard in its reaction with nucleophiles. The lack of antagonist activity of **3a** toward morphine in the GPI suggests there may be some differences in the μ receptor in these two tissues.[24]

Since washing did not completely remove 10 μM of the reversible Phe-containing peptides **2b** and **3b**, the question arises of whether the weak irreversible antagonism of **2a** and **3b** was actually due to covalent binding or only to incomplete removal of noncovalently bound compound. The reversible IC_{50} ratios for **2b** and **3b** at 10 μM, however, are 7–13 times higher than the reversible IC_{50} ratios for the corresponding Mel-containing peptides **2a** and **3a**, indicating that the Phe-containing compounds probably have higher affinity for the receptor and therefore may be harder to remove by washing than **2a** and **3a**. At 1 μM, where the reversible IC_{50} ratios for **2b** and **3b** are more comparable to those seen at 10 μM for **2a** and **3a**, the antagonist **3a** was completely removed by washing (irreversible IC_{50} ratio = 0.5 ± 0.1). This suggests that the irreversible antagonism observed for **2a** and **3a** at 10 μM was probably not due to incomplete removal of noncovalently bound compound.

In conclusion, the melphalan-containing *N*,*N*-dialkyl enkephalin analogues **2a** and **3a** were weak irreversible antagonists at the δ opioid receptor. Analogues **3a** also showed weak irreversible antagonist activity at the μ receptor in the MVD, but not in the GPI. The poor irreversible activity is probably due to steric interference of the initial recognition step by the bulky nitrogen mustard.

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Future derivatives will incorporate less bulky functional groups at the para position on the phenylalanine ring and will hopefully have greater reversible affinity for the receptor and increased irreversible antagonist activity.

Experimental Section

The types of sources of equipment, chromatographic materials, reagents, and solvents were as given previously[14] with the following changes: (1) the solvent system used for high-performance liquid chromatography (HPLC), unless otherwise indicated, was a mixture of 0.1% trifluoroacetic acid (TFA) and MeOH (HPLC grade) used at a flow rate of 1 mL/min, t_m = 3.14–3.36 min; and (2) reverse-phase (RP) thin-layer chromatography was performed on either octyl (C_8) or octadecyl (C_{18}) plates (KC8F and KC18F, respectively, 200 μm) obtained from Whatman, Clifton, NJ. Melphalan was obtained from Sigma Chemical Co., St. Louis, MO; it and all other amino acids were of the L configuration. Only ^{1}H NMR spectra for selected compounds with characteristic signals are reported below; the remaining compounds gave the expected spectra.

***N*-(Benzyloxycarbonyl)melphalan (6).** Benzyl chloroformate (360 mg, 2.10 mmol) was added slowly (10 min) to a cold (0–5 °C) mixture of melphalan (500 mg, 1.64 mmol) and sodium carbonate (215 mg, 2.03 mmol) in 1.0 N NaOH (1.80 mL, 1.80 mmol) and the reaction mixture stirred at 0–5 °C for 4.5 h. During the course of the reaction, H_2O (6.1 mL) and additional benzyl chloroformate (120 mg, 0.70 mmol) were added. The reaction mixture was diluted with H_2O (50 mL) and extracted with Et_2O (3 × 20 mL), the Et_2O extracts were washed with H_2O and 0.25 N NaOH, and the combined aqueous layers were acidified to pH 4–5 and 1 N HCl and extracted with EtOAc (3 × 50 mL). The EtOAc extract was washed with 1 N HCl (2×), H_2O, and saturated NaCl (10 mL each), dried (Na_2SO_4), and evaporated to give 588 mg (81.6%) of crude product. This was purified by silica gel column chromatography (CH_2Cl_2/0–20% MeOH step gradient) to give 506 mg (70.4%) of **6**: $[\alpha]^{25}_D$ +10.5° (*c* 0.51, MeOH); R_f (CH_2Cl_2/10% MeOH) 0.41, (*n*-BuOH/AcOH/H_2O, 4/1/5, upper layer) 0.87; ^{1}H NMR (Me_2SO-d_6, 300 MHz) δ 2.695 (dd, J_1 = 10.88 Hz, J_2 = 13.84 Hz, 1 H, Mel β CH), 2.914 (dd, J_1 = 4.57 Hz, J_2 = 14.39 Hz, 1 H, Mel β CH), 3.681 (s, 8 H, $N(CH_2CH_2Cl)_2$), 4.043 (m, 1 H α CH), 4.963 (s, 2 H, OCH_2Ph), 6.623 (d, J = 7.82 Hz, 2 H, Mel aromatic), 7.070 (d, J = 7.60 Hz, 2 H, Mel aromatic), 7.200–7.289 (m, 5 H, benzyl aromatic), 7.54 (br d, J ~ 6.5 Hz, 1 H, NH). Anal. ($C_{21}H_{24}N_2O_4Cl_2 \cdot {}^1/_2H_2O$) C, H, N, Cl.

***N*-(Benzyloxycarbonyl)melphalanylleucine *tert*-Butyl Ester (7).** *N*-Benzyloxycarbonylmelphalan (**6**) (352 mg, 0.80 mmol) was reacted with *N*-methylmorpholine (81 mg, 0.80 mmol) and isobutyl chloroformate (111 mg, 0.81 mmol) in freshly distilled THF (4.0 mL) at −18 to −12 °C for 1.8 min under standard conditions,[15,16] and a cold solution of leucine *tert*-butyl ester hydrochloride (180 mg, 0.80 mmol) and *N*-methylmorpholine (81 mg, 0.80 mmol) in DMF (2.0 mL) added. After the mixture was stirred under N_2 at −15 °C for 30 min and at 25 °C for an additional 1.5 h, isolation[25] yielded 478 mg (98.0%) of the dipeptide **7** as a glass: $[\alpha]^{25}_D$ −12.3° (*c* 1.0, MeOH); R_f (CH_2Cl_2/5% MeOH) 0.71, (EtOAc) 0.73. Anal. ($C_{31}H_{43}N_3O_5Cl_2$) C, H, N, Cl.

Melphalanylleucine *tert*-Butyl Ester (8a). The protected dipeptide **7** (300 mg, 0.49 mmol) was reacted with ammonium formate (125 mg, 1.98 mmol) and 10% palladium on carbon (75 mg, 0.25 w/w peptide) in MeOH (2.5 mL). The usual isolation procedure[14] yielded 232 mg (98.0%) of crude product, which was purified by silica gel column chromatography (CH_2Cl_2/0–5% MeOH gradient) to give 198 mg (83.5%) of **8a** as an oil: $[\alpha]^{25}_D$ −25.5° (*c* 0.48, MeOH); R_f (CH_2Cl_2/5% MeOH) 0.38, (EtOAc) 0.32. Anal. ($C_{23}H_{37}N_3O_3Cl_2$) C, H, N, Cl.

***N*,*N*-Dibenzyl-*O*-*tert*-butyltyrosylglycylglycine Ethyl Ester (10).** The tripeptide **9**[14] (380 mg, 1.00 mmol) was treated with *N*,*N*-diisopropylethylamine (0.36 g, 2.80 mmol) and benzyl bromide (1.44 g, 8.41 mmol) in dry acetonitrile (2.0 mL) for 18 h at 25 °C, as described previously for *N*,*N*-dibenzyl-*O*-*tert*-butyltyrosylglycylglycylphenylalanylleucine methyl ester.[14] The reaction mixture was evaporated, and the residue was dissolved in EtOAc (100 mL), washed with H_2O and saturated NaCl, dried (Na_2SO_4), and evaporated. Silica gel column chromatography (CH_2Cl_2/0–5% EtOH gradient) of the residue gave 523 mg (93.4%) of **10** as a glass: $[\alpha]^{25}_D$ −7.2° (*c* 1.1, absolute EtOH); R_f (CH_2Cl_2/5% EtOH) 0.41, (EtOAc) 0.53. Anal. ($C_{33}H_{41}N_3O_5 \cdot {}^1/_2H_2O$) C, H, N.

***N*,*N*-Dibenzyl-*O*-*tert*-butyltyrosylglycylglycine (11b).** Ethyl ester **10** (407 mg, 0.727 mmol) was hydrolyzed in 0.25 N NaOH (3.2 mL, 0.80 mmol) and THF (7.0 mL) at 0–5 °C for 5 min and at 25 °C for 1.5 h. The reaction mixture was neutralized with 1 N HCl and the THF evaporated in vacuo. The aqueous solution was applied to a C_8 reverse-phase column and eluted with a H_2O/50–100% MeOH gradient to give 361 mg (93.4%) of the product **11b**: $[\alpha]^{25}_D$ −4.4° (*c* 1.0, MeOH); R_f (CH_2Cl_2/10% MeOH) 0.28, (RP, C_{18}, 70% MeOH/H_2O) 0.42.

***N*,*N*-Diallyl-*O*-*tert*-butyltyrosylglycylglycyl-melphalanylleucine *tert*-Butyl Ester (12a).** The acid **11a**[14] (47 mg, 0.11 mmol) in THF (0.5 mL) was converted to the isobutyric carbonic acid mixed anhydride according to the procedure described above for compound **7**, and a cold solution of the dipeptide **8a** (52 mg, 0.11 mmol) in THF (0.35 mL) was added. After being stirred under N_2 at −15 °C for 30 min and at 25 °C for 1.5 h, the reaction mixture was evaporated, the residue dissolved in EtOAc (50 mL), and the organic layer washed with 5% $NaHCO_3$, H_2O (twice), and saturated NaCl (15 mL each), dried (Na_2SO_4), and evaporated to give 90.5 mg (93.6%) of product, which was purified by silica gel column chromatography (CH_2Cl_2/0–5% MeOH gradient) to give 78 mg (80.7%) of the pentapeptide **12a**: $[\alpha]^{25}_D$ −10.0° (*c* 0.51, MeOH); R_f (CH_2Cl_2/5% MeOH) 0.30, (EtOAc) 0.44. Anal. ($C_{46}H_{68}N_6O_7Cl_2$), C, H, N, Cl.

***N*,*N*-Dibenzyl-*O*-*tert*-butyltyrosylglycylglycyl-melphalanylleucine *tert*-Butyl Ester (12b).** The tripeptide **11b** (53 mg, 0.10 mmol) was coupled to **8a** (47 mg, 0.10 mmol) in THF (1.0 mL) by the isobutyric carbonic acid mixed anhydride method, as described for compound **7** above. After 40 min at −15 °C and 3.25 h at 25 °C, the product was isolated as described for **12a** above to give 80 mg (81.0%) of the pentapeptide **12b** without column chromatography: $[\alpha]^{25}_D$ −5.5° (*c* 1.0, MeOH); R_f (CH_2Cl_2/5% MeOH) 0.43, (EtOAc) 0.59. Anal. ($C_{54}H_{72}N_6O_7Cl_2$) C, H, N, Cl.

***N*,*N*-Diallyltyrosylglycylglycylmelphalanylleucine ([*N*,*N*-Diallyl,Mel^4,Leu^5]enkephalin, 1a).** The protected pentapeptide **12a** (60 mg, 0.068 mmol) was treated with trifluoroacetic acid (0.75 mL) and anisole (150 μL) in CH_2Cl_2 (0.60 mL) under N_2 for 25 min at 0–5 °C and 3 h at 25 °C. Evaporation and C_8 reverse-phase column chromatography (H_2O/50–100% MeOH gradient) yielded 40.5 mg (77.3%) of **1a** as a solid: mp 106.5–112 °C; $[\alpha]^{25}_D$ +2.1° (*c* 0.50, MeOH); HPLC (80% MeOH/0.1% TFA) t_R = 6.87 min, (70–90% MeOH/0.1% TFA gradient over 10 min) t_R = 8.93 min. Anal. ($C_{38}H_{52}N_6O_7Cl_2$) C, H, N, Cl.

***N*,*N*-Dibenzyltyrosylglycylglycylmelphalanylleucine ([*N*,*N*-Dibenzyl,Mel^4,Leu^5]enkephalin, 2a).** The protected pentapeptide **12b** (56 mg, 0.057 mmol) was stirred for 15 min at 0–5 °C and for 3 h at 25 °C under N_2 in a solution of trifluoroacetic acid (0.625 mL) and CH_2Cl_2 (0.5 mL) containing anisole (0.125 mL). After evaporation the residue was purified by C_8 reverse-phase column chromatography (H_2O/50–100% MeOH gradient) to give 33 mg (66.5%) of **2a**: mp 101–105 °C; $[\alpha]^{25}_D$ −11.1° (*c* 0.50, MeOH); R_f (RP, C_{18}, 70% MeOH/H_2O) 0.33; HPLC (80% MeOH/0.01 M NH_4OAc, pH 4.0) t_R = 9.21 min, (70–90% MeOH/0.01 M NH_4OAc, pH 4.0, gradient over 10 min) t_R = 15.2 min, (80% MeOH/0.1% TFA) t_R = 8.08 min, (70–90% MeOH/0.1% TFA gradient over 10 min) t_R = 15.8 min. Anal. ($C_{46}H_{56}N_6O_7Cl_2 \cdot H_2O$) C, H, N, Cl.

Methyl α-Aminoisobutyryl-α-aminoisobutyrate Hydrochloride (14). A mixture of **13**[19] (2.90 g, 8.62 mmol), 10% palladium on carbon (290 mg, 0.10 w/w peptide), and concentrated HCl (0.72 mL, 8.64 mmol) in MeOH (45 mL) was hydrogenated for 45 min at 1 atm. After the catalyst was filtered and washed with additional MeOH, the filtrate was evaporated and dried in vacuo. The gummy solid was triturated several times with Et_2O and dried in vacuo over NaOH pellets to give 1.794 g (87.2%) of amine **14** as the hydrochloride salt: mp 175.5–178.5 °C C; R_f (CH_2Cl_2/10% MeOH) 0.27, (*n*-BuOH/AcOH/H_2O, 3/1/1) 0.60;

(25) The illustrative procedure in ref 16 was followed except that cold 10% citric acid was used to wash the EtOAc extract rather than 1 N HCl.

^{1}H NMR (DMSO-d_6, 90 MHz) δ 1.078 (s, 3 H, Aib CH_3), 1.402 (s, 6 H, 2 Aib CH_3), 1.486 (s, 3 H, Aib CH_3), 3.577 (s, 3 H, OCH_3), 8.251 (br s, 3 H, NH_3^+), 8.487 (s, 1 H, NH).

Methyl *N*-(Benzyloxycarbonyl)-*O*-*tert*-butyltyrosyl-α-aminoisobutyryl-α-aminoisobutyrate (15). *N*-(Benzyloxycarbonyl)-*O*-*tert*-butyltyrosine[14,18] (343 mg, 0.92 mmol) in THF (5.0 mL) was converted to the isobutyric carbonic acid mixed anhydride according to the procedure described above for compound **7** and then treated with a cold solution of the amine hydrochloride **14** (219 mg, 0.92 mmol) and *N*-methylmorpholine (93 mg, 0.92 mmol) in DMF (3.0 mL). The reaction mixture was stirred under N_2 at −15 °C for 45 min and at 25 °C for an additional 2–2.5 h and then diluted with H_2O (35–50 mL). Extraction with EtOAc (3 × 50 mL) and product isolation according to the procedure described for compound **7** gave 480 mg (94.2%) of the tripeptide **15**, which was purified by silica gel column chromatography (CH_2Cl_2/0–5% MeOH gradient) to give 385 mg (75.5%) of product as a glass: $[\alpha]^{25}_D$ +31.2° (*c* 1.0, MeOH); R_f (CH_2Cl_2/5% MeOH) 0.41, (EtOAc) 0.50. Anal. ($C_{30}H_{41}N_3O_7$) C, H, N.

Methyl *O*-*tert*-Butyltyrosyl-α-aminoisobutyryl-α-aminoisobutyrate (16). The protected tripeptided **15** (350 mg, 0.63 mmol) was treated with ammonium formate (160 mg, 2.54 mmol) and 10% palladium on carbon (85 mg, 0.24 w/w peptide) in MeOH (3 mL) for 15 min at 25 °C. The usual workup[14] yielded 253 mg (95.3%) of **16**, which was recrystallized from EtOAc/hexane (196 mg, 73.8%): mp 119–121 °C; $[\alpha]^{25}_D$ +27.7° (*c* 1.0, MeOH); R_f (CH_2Cl_2/10% MeOH) 0.45. Anal. ($C_{22}H_{35}N_3O_5$) C, H, N.

Methyl *N*,*N*-Diallyl-*O*-*tert*-butyltyrosyl-α-aminoisobutyryl-α-aminoisobutyrate (17a). The amine **16** (421 mg, 1.00 mmol) was treated with *N*,*N*-diisopropylethylamine (362 mg, 2.80 mmol) and allyl bromide (1.22 g, 10.0 mmol) in dry acetonitrile (2.0 mL) under N_2 at 25 °C for 18 h, as described above for **10**. Isolation and silica gel column chromatography (CH_2Cl_2/0–5% MeOH gradient) yielded 427 mg (85.1%) of **17a** as an oil: $[\alpha]^{25}_D$ −1.9° (*c* 1.0, MeOH); R_f (CH_2Cl_2/5% MeOH) 0.42, (EtOAc) 0.55. Anal. ($C_{28}H_{43}N_3O_5$) C, H, N.

Methyl *N*,*N*-Dibenzyl-*O*-*tert*-butyltyrosyl-α-aminoisobutyryl-α-aminoisobutyrate (17b). The amine **16** (150 mg, 0.36 mmol), *N*,*N*-diisopropylethylamine (127 mg, 0.98 mmol), and benzyl bromide (575 mg, 3.63 mmol) in dry acetonitrile (0.75 mL) were stirred under N_2 at 25 °C for 18 h, as described for the preparation of **10**. Isolation of the product as described above for **10** and silica gel column chromatography (CH_2Cl_2/0–5% MeOH gradient) yielded 210 mg (98.3%) of **17b**, which was recrystallized from EtOAc/hexane (180 mg, 84.3%): mp 102–104.5 °C; $[\alpha]^{25}_D$ −6.7° (*c* 1.0, MeOH); R_f (CH_2Cl_2/5% MeOH) 0.58, (CH_2Cl_2/10% MeOH) 0.75. Anal. ($C_{36}H_{47}N_3O_5$) C, H, N.

***N*,*N*-Diallyl-*O*-*tert*-butyltyrosyl-α-aminoisobutyryl-α-aminoisobutyric Acid (18a).** Methyl ester **17a** (250 mg, 0.500 mmol) was hydrolyzed in 4 N NaOH (0.30 mL, 1.20 mmol) and a MeOH (0.90 mL)/THF (1.20 mL) mixture for 15 min at 0–5 °C and 2.5 h at 25 °C. Isolation as described for **11b** yielded 208 mg (85.5%) of the **18a**: $[\alpha]^{25}_D$ −6.2° (*c* 0.40, MeOH); R_f (CH_2Cl_2/10% MeOH) 0.33, (RP, C_8, 70% MeOH/H_2O) 0.56.

***N*,*N*-Dibenzyl-*O*-*tert*-butyl-L-tyrosyl-α-aminoisobutyryl-α-aminoisobutyric Acid (18b).** Methyl ester **17b** (150 mg, 0.249 mmol) in a MeOH (0.45 mL)/THF (0.60 mL) mixture was treated at 0–5 °C with 4 N NaOH (0.15 mL, 0.60 mmol). After the mixture was stirred for 18 h at 25 °C, the product was isolated as described above for **11b** to give 139 mg (94.9%) of **18b**: $[\alpha]^{25}_D$ −1.1° (*c* 1.0, MeOH); R_f (RP, C_8, 70% MeOH/H_2O) 0.37.

***N*,*N*-Diallyl-*O*-*tert*-butyltyrosyl-α-aminoisobutyryl-α-aminoisobutyrylmelphalanylleucine *tert*-Butyl Ester (19).** A cold (−5 °C) solution of the acid **18a** (73 mg, 0.15 mmol) and triethylamine (15 mg, 0.15 mmol) in dry toluene (150 μL) was treated with trimethylacetyl chloride (18 mg, 0.15 mmol). After the mixture was stirred under N_2 for 1 h at −5 °C and 1.25 h at 25 °C, a solution of the amine **8a** (72 mg, 0.15 mmol) in toluene (0.45 mL) was added and the reaction mixture stirred at 25 °C for 2 days. The reaction mixture was diluted with EtOAc, washed with 5% $NaHCO_3$ (twice), H_2O, and saturated NaCl, dried (Na_2SO_4), and evaporated to give 114 mg of crude product, which after silica gel column chromatography (CH_2Cl_2/0–30% EtOAc gradient) gave 67.5 mg (47.7%) of **19**: $[\alpha]^{25}_D$ −35.7° (*c* 0.52, MeOH); R_f (CH_2Cl_2/5% MeOH) 0.32, (CH_2Cl_2/20% EtOAc) 0.45. Anal. ($C_{50}H_{76}N_6O_7Cl_2$) C, H, N, Cl.

***N*,*N*-Dibenzyl-*O*-*tert*-butyltyrosyl-α-aminoisobutyryl-α-aminoisobutyrylmelphalanylleucine *tert*-Butyl Ester (20a).** The acid **18b** (62 mg, 0.11 mmol) was treated with triethylamine (11 mg, 0.11 mmol) and trimethylacetyl chloride (13 mg, 0.11 mmol) in dry toluene (100 μL) under N_2 for 2 h at −5 °C and 2.5 h at 25 °C, as described for the preparation of **19**. After addition of a solution of the amine **8a** (50 mg, 0.105 mmol) in toluene (0.30 mL), the reaction mixture was stirred at 25 °C under N_2 for 18 h. Isolation as described above yielded 112 mg of crude product, which was purified by silica gel column chromatography (CH_2Cl_2/0–20% EtOAc gradient) to give 75 mg (68.1%) of **20a**: $[\alpha]^{25}_D$ −21.7° (*c* 0.52, MeOH); R_f (CH_2Cl_2/5% MeOH) 0.51, (CH_2Cl_2/20% EtOAc) 0.14. Anal. ($C_{58}H_{80}N_6O_7Cl_2$) C, H, N, Cl.

***N*,*N*-Dibenzyl-*O*-*tert*-butyltyrosyl-α-aminoisobutyryl-α-aminoisobutyrylphenylalanylleucine *tert*-Butyl Ester (20b).** A cold (0–5 °C) solution of the acid **18b** (59 mg, 0.10 mmol) and triethylamine (10 mg, 0.10 mmol) in dry toluene (50 μL) was treated with trimethylacetyl chloride (12 mg, 0.10 mmol). After the mixture was stirred for 2 h at −5 °C and at 25 °C for 4 h (additional toluene (50 μL) was added during the course of the reaction), a solution of the amine **8b** (33 mg, 0.10 mmol) in toluene (0.4 mL) was added and the mixture stirred at 25 °C for 2 days. The product (86 mg, 95.1%) was isolated as described for **19** and purified by silica gel column chromatography (CH_2Cl_2/0–5% MeOH gradient) to yield **20b** (71 mg, 78.5%): $[\alpha]^{25}_D$ −16.2° (*c* 0.50, MeOH); R_f (CH_2Cl_2/5% MeOH) 0.47, (EtOAc) 0.65. Anal. ($C_{54}H_{73}N_5O_7$) C, H, N.

***N*,*N*-Diallyltyrosyl-α-aminoisobutyryl-α-aminoisobutyrylmelphalanylleucine ([*N*,*N*-Diallyl,-Aib^2,Aib^3,Mel^4,Leu^5]enkephalin 3a).** The protected pentapeptide **19** (47.5 mg, 0.050 mmol) in CH_2Cl_2 (0.4 mL) was treated with anisole (100 μL) and trifluoroacetic acid (0.50 mL) for 25 min at 0–5 °C and for 3 h at 25 °C. Evaporation and C_8 reverse-phase column chromatography (H_2O/50–100% MeOH gradient) yielded 30 mg (72.1%) of **3a**: mp 96–100 °C; $[\alpha]^{25}_D$ −28.4° (*c* 0.44, MeOH); HPLC (80% MeOH/0.1% TFA) t_R = 7.53 min, (70–90% MeOH/0.1% TFA gradient over 10 min) t_R = 10.7 min. Anal. ($C_{42}H_{60}N_6O_7Cl_2$) C, H, N, Cl.

***N*,*N*-Dibenzyltyrosyl-α-aminoisobutyryl-α-aminoisobutyrylmelphalanylleucine ([*N*,*N*-Dibenzyl,-Aib^2,Aib^3,Mel^4,Leu^5]enkephalin, 4a).** The protected pentapeptide **20a** (45 mg, 0.043 mmol) was treated with trifluoroacetic acid (0.50 mL) and anisole (100 μL) in CH_2Cl_2 (0.40 mL) under N_2 at 0–5 °C for 15 min and at 25 °C for 3 h, as described above for **3a**. Evaporation and reverse-phase column chromatography as described above yielded 38 mg (94.6%) of **4a**: mp 101–106 °C; $[\alpha]^{25}_D$ −23.4° (*c* 0.50, MeOH); HPLC (80% MeOH/0.1% TFA) t_R = 11.3 min, (90% MeOH/0.1% TFA) t_R = 5.38 min, (70–90% MeOH/0.1% TFA gradient over 10 min) t_R = 16.5 min. Anal. ($C_{50}H_{64}N_6O_7Cl_2$) C, H, N, Cl.

***N*,*N*-Dibenzyltyrosyl-α-aminoisobutyryl-α-aminoisobutyrylphenylalanylleucine ([*N*,*N*-Dibenzyl,-Aib^2,Aib^3,Leu^5]enkephalin, 4b).** The protected pentapeptide **20b** (50 mg, 0.055 mmol) and anisole (125 μL) in CH_2Cl_2 (0.5 mL) were treated at 0 °C with trifluoroacetic acid (0.625 mL), as described above for **3a**. After the mixture was stirred under N_2 for 15 min at 0 °C and at 25 °C for 2 h, it was evaporated and the residue purified by C_8 reverse-phase column chromatography as described above to give 39.5 mg (90.2%) of **4b**: mp 128–134 °C; $[\alpha]^{25}_D$ −22.4° (*c* 0.47, MeOH); R_f (RP, C_8, 70% MeOH/H_2O) 0.33; HPLC (70% MeOH/0.1% TFA) t_R = 23.3 min, (80% MeOH/0.1% TFA) t_R = 8.04 min, (90% MeOH/0.1% TFA) t_R = 5.14 min, (70–90% MeOH/0.1% TFA gradient over 10 min) t_F = 14.2 min. Anal. ($C_{46}H_{57}N_5O_7 \cdot 1.5H_2O$) C, H, N.

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Registry No. **1a**, 109363-32-8; **1b**, 85835-41-2; **2a**, 109334-88-5; **2b**, 107941-76-4; **3a**, 109334-89-6; **3b**, 92535-15-4; **4a**, 109334-90-9; **4b**, 109335-03-7; **5**, 148-82-3; **6**, 109334-91-0; **7**, 109334-92-1; **8a**, 109334-93-2; **8b**, 28635-78-1; **9**, 107941-82-2; **10**, 109334-94-3; **11a**, 88282-65-9; **11b**, 109363-33-9; **12a**, 109334-95-4; **12b**, 109335-04-8; **13**, 6671-25-6; **14**, 109334-96-5; **15**, 109334-97-6; **16**, 109334-98-7; **17a**, 109334-99-8; **17b**, 109363-34-0; **18a**, 109335-00-4; **18b**, 109335-05-9; **19**, 109335-01-5; **20a**, 109335-02-6; **20b**, 109335-06-0; CbzCl, 501-53-1; H-Leu-OBu-*t*·HCl, 2748-02-9; $PhCH_2Br$, 100-39-0; Cbz-Tyr(Bu-*t*)-OH, 5545-54-0; $H_2C{=}CHCH_2Br$, 106-95-6.

Notes

3-Carbonylacrylic Derivatives as Potential Antimicrobial Agents. Correlations between Activity and Reactivity toward Cysteine

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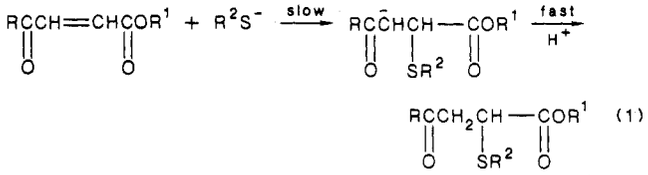
A number of 3-carbonylacrylic acid derivatives were prepared, with a view to varying systematically the stereoelectronic environment of the conjugated double bond. The rates of reaction with cysteine were measured spectrophotometrically when possible or by stopped flow when very fast. Some of the final reaction products were isolated. Other properties examined were partition substituent constants and antimicrobial activity. On the basis of published data and these studies, the activity appears to be the combined effect of at least two mechanisms, one probably related to the effect of these structures on surface tension, the other to the electrophilic properties of the unsaturated system.

The potential antimicrobial activity of α,β-unsaturated carbonyl compounds continues to receive attention, and several substances containing this function, obtained either from natural or synthetic sources, are currently used in therapy.[1] It is generally assumed and confirmed by experimental evidence that the activity of this class of compounds is due to alkylation of nucleophilic groups, such as amino groups, or, preferably, sulfhydryls of essential enzymes.[2-8] The reaction involves a Michael-type addition of the nucleophile to the activated double bond of 3-carbonylacrylates, according to the scheme



(1)

It should be noted that Michael-type additions are often reversible, meaning that these substances can yield reversible macromolecular complexes and, hence, cannot be strictly considered irreversible alkylating agents.[2,9] On the other hand, the studies of Lee et al.[10,11] established that these unsaturated structures exert their biological effect by inhibiting enzyme activities, which control cell division without alkylating or impairing DNA template function. These two aspects make compounds containing the α,β-unsaturated carbonyl moiety attractive among alkylating agents. Unfortunately, they share with the latter the lack of selectivity, certainly because their high degree of reactivity leads to indiscriminate reactions with many cell constituents. In this respect, the extensive synthetic efforts to date have led to only minimal improvements over the prototype drugs.

Several systematic studies on unsaturated carbonyl derivatives failed to establish definite reactivity–activity relationships.[12-14] This lack of correlation may mean that differences in reactivity toward nucleophiles, in themselves, do not necessarily have a determining influence upon biological activity, when considered independently from other structural parameters. Furthermore, any substitution in these highly reactive molecules creates a marked structural diversity, precluding the determination of most of the free-energy parameters required for a rigorous Hansch analysis.[15]

As far as aroylacrylates are concerned, few studies have assessed activity–structure relationships. In 1949 Kirchner and Cavallito[2] found a good correlation between antimicrobial activity and chain length of alkyl substituents in the para position of 3-benzoylacrylic acid, and in 1979 Bowden et al.[16] correlated activities and partition sub-

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