°C during 15 min, was stopped by the addition of 3.6 mL of *N,N*-diisopropylethylamine (21 mmol). After warming up to room temperature, the solution was diluted with 13.5 mL of H_2O, stirred for 10 min, and decanted.

The organic phase was dried over Na_2SO_4 filtered, and evaporated. The residue was chromatographed on a silica column (CH_2Cl_2–EtOH, 99:1): 225 mg of pure **26** was obtained (62%); mp 241 °C; TLC (CH_2Cl_2–EtOH, 97:3) R_f 0.54; IR 1725, 1630, 1520 cm^{-1}; $[\alpha]_D$ −212.5° (*c* 0.16, $CHCl_3$). Anal. ($C_{15}H_{16}O_6N_4$) C, H, N.

2-(6′-Deoxy-α-L-*lyxo*-hexopyranos-4′-ulosyl)-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (27). **26** (200 mg, 0.5 mmol) was stirred in 10 mL of 99% formic acid for 1.5 h. The solution was evaporated and the residue crystallized in methanol: yield 165 mg (93%); mp 180 °C (EtOH); TLC (CH_2Cl_2–EtOH, 8:2) R_f 0.69; MS, *m/e* 308 (1) ($M^{+\cdot}$), 290 (7) ($M^{+\cdot} - H_2O$), 235 (15) [HBC(O-H)=CHCHO], 207 (100) (BCH=CHCHO), 205 (16), 193 (96) (HBCHO), 191 (30) (BCH=CH_2), 178 (11), 177 (16), 164 (9), 163 (6) ($B^{+\cdot}$); IR 1730, 1630, 1520 cm^{-1}; UV ϵ_{225} 8700, ϵ_{327} 4800; $[\alpha]_D$ −102.7° (*c* 0.13, $CHCl_3$). Anal. ($C_{12}H_{12}O_6N_4$) C, H, N.

2-(6′-Deoxy-α-L-*lyxo*-hexopyranos-4′-ulosyl)-8-amino[1,2,4]triazolo[1,5-*a*]pyridine (28). **27** (375 mg, 1.2 mmol) dissolved in 100 mL of MeOH was hydrogenated over 10% Pd/C at room temperature and 1.5 kg/cm^2 of pressure during 4 h. After filtration, the solution was evaporated and the residue was chromatographed on Bio-Gel. Pure fractions crystallized in methanol: yield 220 mg (65%); mp 170 °C (EtOH–n-C_5H_{12}); TLC (CH_2Cl_2–EtOH, 25:15) R_f 0.58; MS, *m/e* 278 (11) ($M^{+\cdot}$), 260 (33) ($M^{+\cdot} - H_2O$), 205 (12), 177 (100) (BCH=CHOH), 175 (20), 163 (87) (HBCHO), 161 (52) (BCH=CH_2), 148 (14), 147 (10), 134 (24), 133 (5), ($B^{+\cdot}$); IR 1730, 1625 cm^{-1}; UV ϵ_{270} 7300, ϵ_{297} 5700; $[\alpha]_D$ −59.9° (*c* 0.10, H_2O). Anal. ($C_{12}H_{14}O_4N_4$) C, H, N.

2-(6′-Deoxy-2′,3′-*O*-isopropylidene-α-L-*tallo*-hexopyranosyl)-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (29). To a stirred suspension of 1.02 g of **26** (2.9 mmol) and 1.08 g (2.9 mmol) of $CeCl_3$ (7 H_2O) in 24 mL of MeOH, kept at 0 °C, was added in portions, in 2 h, 200 mg of $NaBH_4$ (5.3 mmol). The solution was diluted with 50 mL of CH_2Cl_2 and washed with water saturated with NaCl. The organic phase, dried over Na_2SO_4, was evaporated and the residue chromatographed on silica (CH_2Cl_2–EtOH, 98.5:1.5): 424 mg of pure **29** (41%) was obtained; mp 115 °C (Et_2O–n-C_5H_{12}); TLC (CH_2Cl_2–EtOH, 96:4) R_f 0.50; IR 1635, 1530 cm^{-1}; $[\alpha]_D$ −150.9° (*c* 0.11, $CHCl_3$). Anal. ($C_{15}H_{18}O_6N_4$) C, H, N.

2-(6′-Deoxy-α-L-*tallo*-hexopyranosyl)-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (30). **29** (220 mg, 0.63 mmol) was treated with HCOOH as described above. The residue obtained by evaporation was chromatographed on Bio-Gel. The pure product, after lyophilization, crystallized in methanol: yield 141 mg (72%); mp 159 °C (AcOEt–n-C_5H_{12}); TLC (CH_2Cl_2-EtOH, 8:2) R_f 0.44; MS, *m/e* 310 (<1) ($M^{+\cdot}$), 293 (6) ($M^{+\cdot} - 17$), 235 (2), 221 (6), 207 (51) (BCH=CHOH), 205 (9), 193 (100) (HBCHO), 178 (7), 177 (14) (B=CH_2), 163 (5) ($B^{+\cdot}$); IR 1650, 1540 cm^{-1}; UV ϵ_{227} 9800, ϵ_{328} 5100; $[\alpha]_D$ −109.1° (*c* 0.13, H_2O). Anal. ($C_{12}H_{14}O_6N_4$) C, H, N.

2-(2′,6′-Dideoxy-L-*erythro*-hex-1′-enopyranos-3′-ulosyl)-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (31). To a stirred solution of 500 mg (1.7 mmol) of **13** in 15 mL of DMF was added 5 g (58 mmol) of activated MnO_2.[26] After 16 h the solvent was evaporated. The powder was treated with CH_2Cl_2 and then with a mixture CH_2Cl_2–EtOH (1:1) and decanted. The organic solutions were brought to dryness and chromatographed on a silica column (CH_2Cl_2-EtOH, 99.5:0.5); pure fractions crystallized: yield 201 mg (40%); mp 211 °C (EtOH); TLC (CH_2Cl_2–EtOH, 95:5); R_f 0.53; CIMS (isobutane, 160 °C, 5.5 eV), *m/e* 291 (MH^+), 261 ($MH^+ - NO$); IR 1675, 1640, 1540 cm^{-1}. Anal. ($C_{12}H_{10}O_5N_4 \cdot 0.25C_2H_5OH$) C, H, N.

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Preparation and Analgesic Properties of Amino Acid Derivatives of (−)-5,9α-Diethyl-2′-hydroxybenzomorphan

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The *N*-arginyl derivative of methionine-enkephalin (fragment 60–65 of β-lipotropin) has been shown to be equiactive with the parent pentapeptide, despite the fact that the tyrosine amino group in this compound has been neutralized by the formation of an amide linkage. A series of *N*-(amino acid) derivatives of (−)-5,9α-diethyl-2′-hydroxybenzomorphan was prepared and evaluated for analgesic activity. In vitro activities were found to vary greatly, depending on the nature of the amino acid used. The *N*-arginyl derivative was found to be equipotent to (−)-5,9α-diethyl-2′-hydroxybenzomorphan and also to methionine-enkephaline in the naloxone binding assay.

The discovery of the "endogenous opioid substances", the enkephalins[1] and the endorphins,[2] as proposed neurotransmitters or neuroregulators involved in the perception of pain was rekindled interest in the field of opioid analgesics. It has strengthened the belief that agents can be found which will be both potent analgesics and free of the deleterious side effects (tolerance, physical dependance, etc.) normally associated with opioid analgesics. In the past several years, a massive body of evidence has been accumulated on structure–activity relationships of the enkephalins[3] such that a fairly clear picture has emerged

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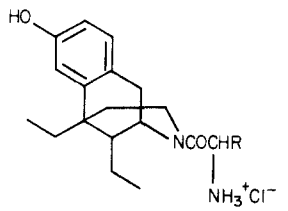
Table I. Inhibition of [^{3}H]Naloxone Binding by N-(Amino Acid) Derivatives of (−)-5,9α-Diethyl-2′-hydroxy-6,7-benzomorphan



2a-f

compd	R	inhibition of [^{3}H]naloxone binding (IC_{50}, nM)[a] −Na$^+$	Na$^+$	ratio
1		20	360	18.0
2a	H	287	8 184	28.0
2b	$CH_2CH_2SCH_3$	301	5 586	19.0
2c	$CH_2CH(CH_3)_2$	1 503	6 384	4.2
2d[b]	$CH_2C_6H_5$	1 540	3 350	2.2
2e[c]	$CH_2C_6H_5$	>10 000	>10 000	
2f	$(CH_2)_3NH(C{=}NH)NH_2$	15.5	660	42.6
Met-enkephalin		18	240	13.3

[a] The results are the mean of three determinations which did not vary by more than 10%. [b] Derived from the (−)-phenylalanine. [c] Derived from the (+)-phenylalanine.

as to the effects of changes in and additions to the basic pentapeptide structures.

One striking piece of reported data is that the *N*-arginyl-methionine-enkephalin has activity comparable to that of the parent system.[4] Thus, although the basic amine center on the tyrosine residue has been neutralized by formation of an amide linkage and the new basic centers present on the arginine residue are of necessity considerably displaced in space relative to the tyrosine nitrogen atom, the activity is retained. Such activity is difficult to explain if one considers the tyramine unit present in the enkephalins (and in the morphinoid analgesics) as a primary requirement for activity.[5]

On consideration of this seemingly unusual retention of activity in *N*-Arg-Met-enkephalin, we were prompted to investigate the possibility that a similar activity pattern might hold for the benzomorphans, since they have been assumed to be rigid analogues of the Tyr-Gly-Gly part of the enkephalins.[5] On examination of the literature, we were unable to find examples of *N*-(amino acid) substituted benzomorphan derivatives. Thus, in addition to the *N*-arginyl derivative, we have prepared a series of amino acid amide derivatives of (−)-5,9α-diethyl-2′-hydroxy-6,7-benzomorphan[6] (Table I), and they have been tested for opiate receptor binding activity.

Results and Discussion

The preparation of these derivatives was carried out using the usual conditions for peptide bond formation. Thus, the reaction of the nor-base (−)-5,9α-diethyl-2′-hydroxy-6,7-benzomorphan (1) with *t*-Boc derivatives of amino acids in methylene chloride was carried out at room temperature using dicyclohexylcarbodiimide as the dehydrating agent. 1-Hydroxybenzotriazole was also added in some cases to minimize the racemization of the amino acid and the formation of acyl urea.[7] The resulting amide was isolated via chromatography on silica gel. The *t*-Boc group was readily removed by treatment with hydrogen chloride gas in a chloroform solution, yielding the desired amides **2a–e** as amorphous products after evaporation of the solvent. The preparation of the arginine derivative **2f** was slightly different. After coupling with DCC, the ω-nitro group, used to block the guanidine portion of this molecule, was removed by hydrogenation over palladium on charcoal prior to removal of the *t*-Boc.

These derivatives, **2a–f**, were tested in a binding assay as developed by Pert et al.[8] using homogenized brain tissue and tritiated naloxone.

Examination of the data in Table I shows that, indeed, activity is retained in vitro in this series of compounds. It is also notable that the L-argininyl derivative is the most active member of the series, followed by the simple *N*-glycyl derivative, with the others being considerably less active. Within the series can be found compounds with sodium ratios[9] ranging in vitro from 42.6, indicating a pure agonist, to 2.2, indicative of a partial antagonist. The range of sodium ratios observed is difficult to rationalize and appears not to follow any discernable pattern based either on bulk or lipophilicity of the side chains.

The D-phenylalanine analogue was prepared for two reasons. Firstly, it was viewed as a derivative of D-phenylalanine, claimed to be analgesic by Ehrenpreiss[10] due to its inhibition of the enkephalinases. As can be seen from Table I, this D-phenylalanine derivative does not bind at all to the opiate receptors. Moreover, this derivative was shown to be inactive as an enkephalinase inhibitor[11] and as a peptidase inhibitor.[11] Secondly, the D analogue was also conceived as a probe to assess the possibility of amide hydrolysis by proteases that could be present in the assay. Consequential formation of the benzomorphan base during the assay was excluded due to the wide variability of sodium ratios observed and because of the similar results obtained when the assay was performed in the presence of bacitracin at concentrations sufficient to inhibit protease activity.

The fundamental activity of the series is also difficult to reconcile with a strict molecular comparison of the parent benzomorphan structure. Thus, when, for example, **2d** is superimposed with the parent benzomorphan, the amino function of the N-(amino acid) amides is displaced considerably (at least 3.3 Å based on model studies[12]) relative to the basic center in **1**. Based on classical structure–activity relationships in the benzomorphans,[13] such a displacement produces a considerable reduction, if not elimination, of opiate binding activity. This clearly is not the case in this series. A possible rationalization[14] of this binding activity involves the hypothesis of Galt.[15]

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Table II. Amino Acid Derivatives of (-)-5,9α-Diethyl-2'-hydroxybenzomorphan

no.	% yield	mp, °C	$[\alpha]_D$ (c 1, MeOH), deg	formula	anal.[a]
2a	37	105-110	-167	$C_{18}H_{26}N_2O_2 \cdot H_2O$	C, H, N
2b	14	63-67	-140	$C_{21}H_{32}N_2O_2S$	C, H, N
2c	38	98-100	-154	$C_{22}H_{34}N_2O_2$	C, H, N
2d	75	95-98	-169	$C_{25}H_{32}N_2O_2 \cdot H_2O$	C, H, N
2e	78	235-238	-61	$C_{25}H_{32}N_2O_2 \cdot HCl$	C, H, N, Cl
2f	61	220-222	-106	$C_{22}H_{35}N_5O_2 \cdot 2HCl$	C, H, N, Cl

[a] Analytical results were within ±0.4% of the theoretical values.

Galt theorized that the receptor binding surface interacting with the phenyl portion of the tyramine unit is quite large and nonspecific, thus allowing for some considerable lateral displacement of the aromatic ring. If such a displacement is allowed in comparing 1 with the derivatives **2a–f**, while keeping the basic amino functions and the phenol hydroxyls as key points of overlap, then a good fit can be achieved. Furthermore, this fit is improved if one allows the piperidine ring of **2a–f** to adopt a twist-boat conformation. This conformation should be accessible, since the computed energy difference between the twist-boat and the chair conformations was found to be 3.8 kcal.

In order to attempt to explain the observed activity of the L-phenylalanyl derivative (**2d**) and the lack of activity of the D-phenylalanyl derivative (**2e**), we have studied the relative energies of the rotamers of these two structures which best match benzomorphan.[11] This study showed that the best-fit rotamer of **2e** has an energy of some 40 kcal higher than the similar rotamer for **2d**. It thus appears that serious nonbonded interactions make it essentially impossible for the D-phenylalanyl derivative to take up the required conformation.

Conclusion

A series of amino acid amide derivatives of (-)-5,9α-diethyl-2'-hydroxy-6,7-benzomorphan has been prepared and, as in the recently reported case of *N*-Arg-Met-enkephalin, opiate receptor binding activity is retained in this series, with the arginyl derivative possessing activity essentially unchanged from the parent system. The derivatives have been found to be inactive when tested in in vivo assays (tail flick, hot plate).

Experimental Section

Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were measured on a Perkin-Elmer 267 grating spectrophotometer. A Varian EM-360 instrument was used to record NMR spectra in deuteriochloroform using tetramethylsilane as an internal standard. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. The low-resolution mass spectra were obtained from the Morgan-Schaffer Corp., Montreal. Preparative HPLC separations were performed on a Waters prepLC/System 500 using a prep-PAK silica cartridge and eluting at a flow rate of 100 mL/min. Optical roations were measured on a Zeiss polarimeter Model LEP-A2. All reactions, as well as chromatographic separations, were monitored routinely with the aid of thin-layer chromatography (TLC) using precoated 0.25-mm silica gel GF plates (Analtech). Spots were visualized by UV and with charring by sulfuric acid.

Biology. [^{3}H] Naloxone Binding to Rat Brain Membranes. (A) Membrane Preparation. Suspensions of rat brain membranes were prepared by the method of Pert et al.[8] as follows. Whole rat brain, minus the cerebellum, was placed on ice immediately after decapitation. Each brain was minced separately in 20 mL of cold 0.05 M Tris-Cl, pH 7.7, and homogenized on ice with a Polytron PT10, at setting 4 for 30 s. Homogenates were combined, diluted 100-fold (w/v), and centrifuged at 49000*g* for 15 min at 4 °C. The pellets were suspended in 100 mL of the ice-cold Tris-Cl buffer and rehomogenized with a Polytron PT10. This homogenate was diluted to the original volume, incubated at 37 °C for 40 min, and cooled on ice, with gentle stirring for 15 min. This stirred suspension was used for binding assays and protein determinations.[16]

(B) Binding Assay. Incubations were carried out in triplicate in 12 × 75 mm disposable polystyrene tubes. The assay contained, in a final volume of 2 mL, the following: 1.5 nM [^{3}H]naloxone (38000 cpm), 1.0 mL of brain membrane suspension (0.7–0.8 mg of protein), with or without 100 mM NaCl, with or without 10^{-6} M levallorphan tartrate, 0.05 M Tris-Cl, pH 7.7.

The incubation mixtures were made up at 4 °C with all components except [^{3}H]naloxone. After 10 min, the radioligand was added, the tubes were vortexed, and the mixture was incubated at 25 °C for 30 min and then cooled at 4 °C for 15 min. Each incubation mixture was then filtered through a Whatman GF/B 2.4-cm filter, under vacuum, in a 45 cavity Sandbek filtration manifold. The tubes and filters were quickly washed twice with 2 mL of cold 0.05 M Tris-Cl, pH 7.7. The damp filters were placed in Beckman Biovials, 3 mL of Biofluor was added, and the filters were counted after a 3-h stabilization period.

Nonspecific binding of radioligand was measured in the presence of 10^{-6} M levallorphan tartrate. Specific binding is defined as the difference between the binding in the absence of levallorphan (total binding) and the binding in the presence of 10^{-6} M levallorphan.

Drugs to be tested were dissolved in Me_2SO at 10^{-3} M, with subsequent dilutions in 0.05 M Tris-Cl, pH 7.7. Drugs were tested at five concentrations around the expected IC_{50}, and the percent inhibition of binding was determined for each drug concentration. The IC_{50} for inhibition of binding was determined by log-probit analysis of the dose–response data and by linear-regression analysis on a Hewlett-Packard 9815A calculator.

Chemistry. General Procedure for the Preparation of 2a–f. Equimolar equivalents of (-)-5,9α-diethyl-2'-hydroxy-6,7-benzomorphan and the *tert*-butoxycarbonyl derivative of the amino acid were stirred in dichloromethane (6 mL per milliequivalent) at 0 °C. An equimolar amount of dicyclohexylcarbodiimide was added, and the mixture was stirred for 2 days at 0 °C. After the addition of acetic acid (10 drops), the mixture was stirred for 2 h. The mixture was filtered; the filtrate was washed with 20% citric acid and saturated $NaHCO_3$ and then reduced to dryness. The mixture was taken up in ether–light petroleum ether (1:1), cooled for 1 h, filtered, and reduced to dryness to give the essentially pure *N*-*t*-Boc protected amide. This intermediate was taken up in chloroform (12 mL per milliequivalent). The solution was saturated with anhydrous HCl and let to stand at room temperature for 2 h. The excess HCl was blown off with N_2, and the solution was reduced to dryness. The residue was taken up in water, washed with chloroform, basified with saturated $NaHCO_3$, and extracted with chloroform. The extracts were dried and reduced to dryness to yield a residue, which was purified by preparative HPLC using $CH_3CN/MeOH/Et_3N$, 95:4:1, as eluent to yield the major peak, as a foam which could not be induced to crystallize. In some cases, the material, thus purified, was converted to the HCl salt (HCl in MeOH). The derivatives prepared according to this procedure (**2a–f**) are listed in Table II.

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