peritoneally implanted L1210 lymphoid leukemia in $C_3D_2F_1/J$ mice (C_3H/HEJ female × DBA/2J male mice, supplied by Jackson Labs) with following schedules qd 1–5 and qd 1–9 according to the procedures outlined in the NCI Protocol[24] with some modification.[5-7] Each drug was tested over a wide range of doses. The active dose ranges are those giving ILS values ≥ 25%. Optimum dose is a dose producing greatest increase in life span on a particular treatment schedule.

Enzymatic Hydrolysis. Enzymatic cleavage of the pyrophosphate diester bond of the conjugate by phosphodiesterase I (EC 3.1.4.1), 5′-nucleotidase (EC 3.1.3.5), acid phosphatase (EC 3.1.3.2), and alkaline phosphatase (EC 3.1.3.1) was studied according to the procedures described previously.[6] Hydrolysis in normal human serum was also studied as described previously.[6]

Determination of Resistance to Cytidine Deaminase. Human liver cytidine deaminase (EC 3.5.4.5) was prepared from tissue which has been removed at autopsy according to the published procedure.[25] Assays for cytidine and ara-C were carried out spectrophotometrically at 290 nm.[25] The specific activity of the cytidine deaminase used in this study was 4.27×10^{-5} mU/mg of protein and protein concentration was 19.95 mg/mL. For assay of deamination of the conjugates, a mixture of compound (10 μmol), 0.2 mL of the enzyme preparation, and 0.8 mL of 0.1 M Tris-HCl (pH 8.0) was incubated at 25 °C for 24 h. During the incubation, aliquots (0.1 mL) were streaked on TLC plate (0.1 × 20 × 20 cm) followed by developing with solvent A. Each band was extracted with 50% ethanol and quantitated by UV. The band matching with the conjugate was further incubated with 5′-nucleotidase (EC 3.1.3.5) from *Crotalus adamanteus* (Sigma Chemical Co.) in 0.1 M Tris-HCl (pH 9.0) and 0.005 M $MgSO_4$ at 37 °C for 24 h, and the products were separated by paper chromatography and characterized and quantitated by UV as described previously.[6]

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Registry No. 4-*N*,*N*′-dicyclohexyl-4-morpholinecarboxamidine, 69467-87-4; **6a**, 93604-93-4; **6c**, 93644-70-3; **6d**, 93604-94-5; **6e**, 93604-95-6; **6g**, 93604-96-7; **7a**, 73532-89-5; **7b**, 93604-97-8; **7c**, 93604-98-9; **7d**, 93604-99-0; **7e**, 93605-00-6; **8** ($R_2 = C_{16}H_{33}$), 3539-43-3; **8** ($R_2 = C_6H_{11}CH_2$), 33026-79-8; **9a**, 93605-01-7; **9b**, 93605-02-8; ara-CMP, 7075-11-8; morpholine, 110-91-8; 2-cyanoethyl phosphate, 2212-88-6; 11-deoxy-21-iodocorticosterone, 20576-46-9; 21-iodocortisone, 5758-63-4; 21-iodocorticosterone, 35500-25-5; 21-iodocortexolone, 4470-79-5; 21-iodopregnisone, 55786-16-8; 1-adamantanemethanol, 770-71-8; N^4,2′,3′-triacetyl-ara-CMP, 71778-96-6; 2-chloroethanol, 107-07-3; corticosterone 21-monophosphate, 10589-81-8.

Synthesis, Stereochemistry, and Analgesic Activity of 4-Mono- and 4,4-Disubstituted 1,2,3,4,5,6-Hexahydro-2,6-methano-3-benzazocines

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The synthesis of 4-alkyl-, 4-aralkyl-, and 4-alkenyl-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocines is described together with some 4,4-disubstituted and 8-hydroxy derivatives. Evidence of the stereochemistry of the 4-substituent was from ^1H and ^{13}C NMR. In the 4-methyl series the equatorial epimer **1b** has a higher analgesic (hot-plate) potency than **1a**, and **10a**, **10c**, and **10f** are also good agonists. **5a** afforded analgesic properties without an antagonist component. Surprisingly **10d**, bearing an 8-OH function, was without analgesic activity, contrasting with the significant hot-plate activity exhibited by 1,2,3,4,5,6-hexahydro-3,5,6-trimethyl-2,6-methano-3-benzazocine. If the assumption is made that the more active enantiomorph in members of this series is configurationally related to (−)-morphine, then it may be that the enantiotopic edge in hexahydro-2,6-methano-3-benzazocines has a narrow steric requirement for analgesic responses.

In spite of the current interest in possible relationships between opioid peptides and synthetic analgesics related to morphine, much work remains to be done in the area of structure–activity relationships in analgesic series such as the 1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocines (6,7-benzomorphans).[1] Steric crowding about nitrogen[2] has been suggested as being important in the analgesic response of such compounds, and Belleau[3,4] and Kolb[5] have proposed that nonbonding electron directionality might influence activity both qualitatively and quantitatively.

Here, the synthesis and stereochemistry of a series of 4-substituted hexahydro-2,6-methano-3-benzazocines with and without an 8-position phenolic hydroxyl group are described, and the effect of steric crowding about nitrogen on analgesic response is explored. Alkyl, aralkyl, and alkenyl substitution α to nitrogen in the piperidine ring of benzomorphans does not unduly influence analgesic properties.

Chemistry. Key intermediates to our objective compounds (**1a–c**) were prepared by the conventional Grewe synthesis of 2,6-methano-3-benzazocines.[1] In the absence of an 11-methyl substituent, both the α-cis (**1a**) and β-trans (**1b**) epimers were isolated. The predominant α-cis epimer **1c** is formed when an 11-methyl group is present. These structures and their stereochemistry were established by examination of ^1H and ^{13}C NMR spectra.[6]

Insertion of alkyl, aralkyl, or alkenyl groups α to nitrogen in **1a**, **1b**, and **1c** may be effected by means of the mediation of the benzazocinium ion (**2**). Mercury(II) acetate oxidation of **1a** or **1b**, according to the method of Leonard,[7,8] afforded 50% of 1,2,5,6-tetrahydro-3,4,6-tri-

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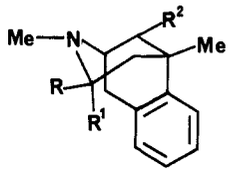


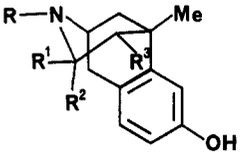
1a	R = H,	R' = Me,	R^2 = H
1b	R = Me,	R' = H,	R^2 = H
1c	R = H,	R' = Me,	R^2 = Me
1d	R = Me,	R' = H,	R^2 = Me
1e	R = Me,	R' = Me,	R^2 = H

methyl-2,6-methano-3-benzazocinium perchlorate (**2**).[9] Treatment of **2** with aqueous KCN gave 4-cyano-4-methylhexahydromethanobenzazocine, **3**. An identical product was obtained in rather higher yield, exploiting a modified Polonovski reaction. Compound **1b** was treated with hydrogen peroxide and trifluoroacetic anhydride to afford the intermediate **6**, which was then reacted with aqueous KCN.[10,11] The latter method was employed for the conversion of compounds lacking a 4-methyl substituent (**7**) to the corresponding cyanohexahydro-2,6-methano-3-benzazocines (**8**). In this case the cyano function was assigned as equatorial on the piperidine chair from ^{13}C and ^1H considerations reported previously.[6] Either the iminium perchlorate **2** or cyanohexahydromethanobenzazocines **3** or **8** may be reacted with lithium alkyls or Grignard reagents[8,12-14] to insert appropriate substituents in the 4-position; however, nitrile displacement offered a cleaner, higher yielding route.

Reaction of 4-cyano-1,2,3,4,5,6-hexahydro-3,4,6-trimethyl-2,6-methano-3-benzazocine (**3**) with methyllithium gave only traces of crude 3,4,4,6-tetramethyl-2,6-methano-3-benzazocine (**1e**) with a high recovery of starting material. ^1H NMR singlets at δ 0.31 and 1.05 corresponding to the 4-position geminal methyl groups suggested its formation. In contrast, benzyl- and allylmagnesium halides reacted rapidly with **3** to afford the corresponding diastereoisomers epimeric about the 4-position (**4a** and **5a**; **4b** and **5b**). The major isomer (48%) from attack on **3** by benzylmagnesium chloride was isolated after column chromatography. It was shown[6] to have an equatorial 4-benzyl configuration (**4b**) from the 4-methyl ^1H NMR signal at δ 0.38 (s). Attempts to isolate the minor axial benzyl isomer (**5b**) were unsuccessful. Treatment of **3** with allylmagnesium bromide gave each 4-allyl isomer (**4a** and **5a**) in the ratio of 1:1. In **5a** the 4-methyl ^1H NMR signal occurs at δ 0.31 (s) (Table III).

The predominance of a single axial benzyl isomer (**5a**) in the former reaction may be explained by stabilization of the transition complex, but the result contrasts markedly with the behavior of **8**. When 4-cyano-1,2,3,4,5,6-hexahydro-3,6,11-trimethyl-2,6-methano-3-benzazocine (**8**) is reacted with an appropriate Grignard reagent, the corresponding compounds **9a**, **9b**, **9c**, or **9d** result. In each case attack occurs from the more hindered side of the molecule and 4-axial substitution results exclusively.[6]

In general, hexahydro-2,6-methano-3-benzazocines bearing an hydroxyl function in the 8-position exhibit a higher level of analgesic activity than the corresponding nonphenolic analogues. To extend earlier studies,[2] therefore, we have prepared a series of derivatives related to those above. The phenolic derivatives **10a** and **10b** were prepared by a Grewe synthesis.[2] Although **10c** was detected by ^1H NMR in the crude **10b**, it could not be isolated. The *N*-alkyl (**10e**) and *N*-cyclopropylmethyl (**10f**) analogues of **10b** were prepared by unexceptional methods.



	R	R^1	R^2	R^3
10a	Me	H	H	H
10b	Me	Me	H	H
10c	Me	H	Me	H
10d	Me	H	H	Me
10e	$CH_2CH{=}CH_2$	Me	H	H
10f	CH_2-cyclopropyl	Me	H	H
10g	H	Me	H	H

Biological Results and Discussion

In an earlier communication[2] a tentative suggestion was made that differences in analgesic activity between the 4- and 5-methylhexahydromethanobenzazocines **1a** and **12** might be explained by hindrance of the tertiary nitrogen pharmacophore. Isolation of the 4-methyl (eq) (**1b**) and 4-methyl (ax) (**1a**) epimers and the observed higher degree of hot-plate activity in the former (Table I) indicated that this explanation was unlikely. In the 4-methyl (eq) series, cyclopropylmethyl and allyl N-substituents (**10e** and **10f**) afford good agonists, surprisingly without antagonist activity in the mouse tail-flick assay vs. morphine.[15] Any increase in the size of the 4-axial substituent beyond methyl, e.g., **4a**, **9b**, **9c**, **9d**, eliminated analgesic activity. However, a 4-allyl (eq) group, as in **5a**, afforded a compound with good analgesic properties without an antagonist component,[15] suggesting that here the allyl group does not substitute spatially for an *N*-allyl function. The corresponding 4(e)-benzylhexahydromethanobenzazocine (**5b**) was more toxic and without analgesic responses.

Perhaps the most surprising observation in these series was the absence of analgesic activity in **10d**, contrasting with the corresponding compound without an 8-OH group but which had a significant hot-plate activity (ED_{50}, 4.2). Clearly, the relationship between the level and type of pharmacological response in 4-substituted hexahydro-2,6-methano-3-benzazocines with and without an 8-OH group requires further study.

It would be reasonable to assume that, as in other benzomorphan series where resolution of racemates has been effected, the enantiomorph configurationally related to (−)-morphine is responsible for much of the antinociceptive activity of the racemate. If this is the case, then it may

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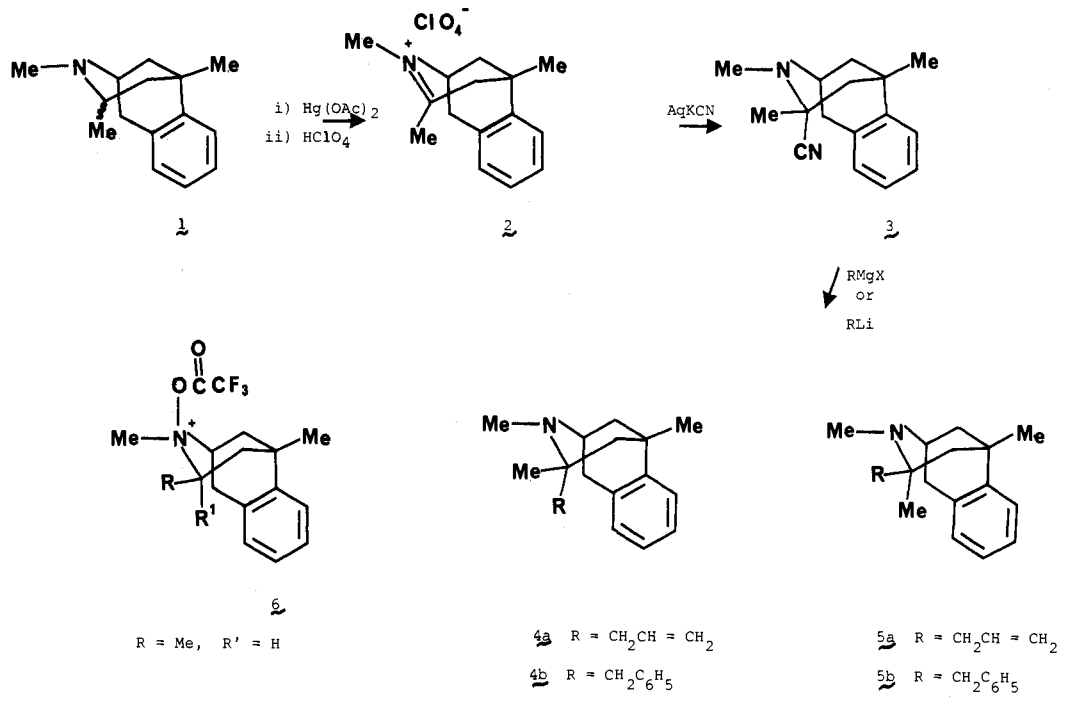
Scheme I



Scheme II

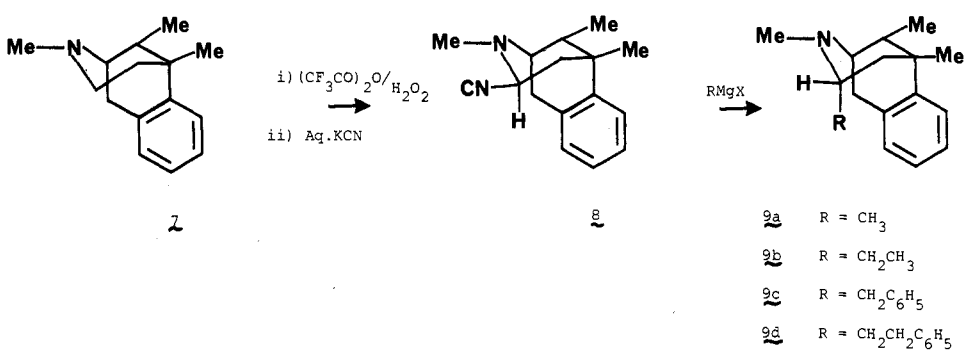
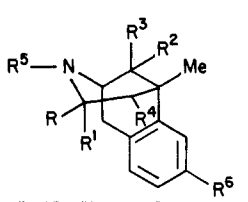


Table I. Analgesic Activities of 4-Substituted 1,2,3,4,5,6-Hexahydro-2,6-methano-3-benzazocines



compd	R	R^1	R^2	R^3	R^4	R^5	R^6	ED_{50},[a] mg/kg
1a	H	Me	H	H	H	Me	H	24.7 (20.5–29.7)
1b	Me	H	H	H	H	Me	H	10.6 (8.0–14.1)
1c	H	Me	Me	H	H	Me	H	14.8 (9.8–22.2)
4a	Me	allyl	H	H	H	Me	H	IA[d] (20)
5a	allyl	Me	H	H	H	Me	H	3.7 (2.7–5.2)
5b	$PhCH_2$	Me	H	H	H	Me	H	*b*
9b	H	Et	Me	H	H	Me	H	IA[d] (20)
9c	H	$PhCH_2$	Me	H	H	Me	H	*b*
9d	H	$PhCH_2CH_2$	Me	H	H	Me	H	*b*
10b	Me	H	H	H	H	Me	OH	1.1 (0.85–1.5)
10d	H	H	H	H	Me	Me	OH	IA[d] (50)
10e	Me	H	H	H	H	allyl	OH	4.2 (2.8–6.2)
10f	Me	H	H	H	H	CPM[c]	OH	2.4 (1.9–3.2)
morphine sulfate								1.2

[a] Tested as hydrochlorides (sc) in water. Mouse hot-plate test[18] employing caesarian-derived general purpose mice at NIH, Bethesda, MD. [b] Low level of activity exhibited which did not follow a dose–response relationship. [c] Cyclopropylmethyl. [d] Inactive at the dose level (mg/kg) indicated.

Table II. 4-Mono- and 4,4-Disubstituted 1,2,3,4,5,6-Hexahydro-2,6-methano-3-benzazocines

compd	method[a]	yield, %	molecular formula	anal.	mp, °C	purification
1b[2] methiodide			$C_{16}H_{24}NI$	C, H, N	268–269[b]	
1c·HCl	B	50	$C_{16}H_{24}NCl$	C, H, N	268–270[b]	150 °C (0.2 mmHg)[d]
3		73	$C_{16}H_{20}N_2$	C, H, N	102–104[c]	
4a·HCl	A, B	39	$C_{18}H_{26}NCl$	C, H, N	252–253[b]	silica gel column
5a·HCl	A, B	20	$C_{18}H_{26}NCl$	C, H, N	246–247[b]	$CHCl_3$/EtOAc
5b·HCl	A, B	90	$C_{22}H_{28}NCl$	C, H, N	246–249[b]	170 °C (0.5 mmHg)[d]
8			$C_{16}H_{20}N_2$	C, H, N	102–103[c]	
9b·HCl	B	46	$C_{17}H_{28}NCl$	C, H, N	271–273[b]	130 °C (0.5 mmHg)[d]
9c·HCl	B	90	$C_{22}H_{28}NCl$	C, H, N	217–219[b]	170 °C (0.2 mmHg)[d]
9d·HCl	B	40	$C_{23}H_{30}NCl\ H_2O$	C, H, N	237–239	160 °C (1 mmHg)[d]

[a] See Experimental Section. [b] Recrystallization solvent EtOH/Et_2O. [c] Recrystallization solvent petroleum ether (60–80 °C). [d] Short-path distillation.

Table III. ^1H NMR Chemical Shifts of Principal Signals[a]

compd	*N*-Me	4-Me	6-Me	11-Me
3	2.52	1.37 (s)	1.32	
4a	2.38	0.31[b] (s)	1.32	
5a	2.48	1.06[c] (s)	1.32	
5b	2.56	0.38 (s)	1.24	
8	2.58		1.38	0.84 (d) (J = 6 Hz)
9b	2.50		1.29	0.80
9c	2.64		1.23	0.81
9d	2.50		1.35	0.84

[a] $CDCl_3$ solutions, values in ppm from Me_4Si. [b] Vinylic protons of 3-allyl group resonances at 5.00 and 5.80 ppm. [c] Vinylic protons of 3-allyl group resonances at 5.20, 4.10, and 4.65 ppm.

be concluded, tentatively, that the enantiotopic edge in 1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocines, Pro 6S, equivalent to Pro 4S in 4-phenylpiperidines,[16,17] has a narrow steric requirement for analgesic responses. Futher studies are required in optically pure series with C-4 substituents.

Experimental Section

The infrared spectra (liquids as films and solids as Nujol mulls) were recorded with a Unicam SP 1025 spectrometer, and melting points (uncorrected) were taken on a Townson and Mercer melting point apparatus.

Proton noise and off-resonance-decoupled ^{13}C NMR spectra[6] were recorded with a JEOL FX 90Q spectrometer operating at 22.5 MHz, and ^1H NMR spectra on a JEOL PS 100 spectrometer operating at 100 MHz. Samples were prepared in 5 mm o.d. tubes as approximately 10% solutions in $CDCl_3$ or $(CD_3)_2SO$ with Me_4Si as reference and deuterium of the solvents provided the lock signal for ^{13}C NMR. All short-path distillations were carried out in a Buchi GKR-50. Mass spectra were measured on a VG Micromass 7070E mass spectrometer operating at 70 eV (EI) and with Xe/glycerol for fast atom bombardment (FAB).

Physical data on compounds are shown in Table II. C, H, N values are within ±0.4% of theory unless otherwise indicated.

1,2,3,4,5,6-Hexahydro-3,4,6-trimethyl-2,6-methano-3-benzazocine (1a and 1b). The isomeric mixture was prepared by the method of Parfitt and Walters.[2] **1a** was isolated as the hydrochloride, mp 152–154 °C, and gave the methiodide, mp 268–269 °C (lit.[2] mp 264–265.5 °C). Compound **1b** was isolated from the mother liquors as the oxalate that gave the hydrochloride, mp 222–224 °C. The methiodide had mp 242–243 °C. Anal. ($C_{16}H_{24}NI$) C, H, N.

1,2,3,4,5,6-Hexahydro-3,6,11-trimethyl-2,6-methano-3-benzazocine (13) was prepared by the method of May and Fry.[21] The hydrochloride had mp 202–204 °C (lit.[21] 203–205 °C).

1,2,5,6-Tetrahydro-3,4,6-trimethyl-2,6-methano-3-benzazocinium Perchlorate (2). Mercuric oxide (52.40 g, 240 mmol) was added gradually over 15 min to a stirred solution of 1,2,3,4,5,6-hexahydro-3,4,6-trimethyl-2,6-methano-3-benzazocine (mixed **1a** and **1b**) (10.42 g, 50 mmol) in 40% acetic acid (200 mL) heated under reflux. The mixture was heated for a further 2 h, and cooled, and the precipitated mercuric acetate was collected and washed with 5% acetic acid solution (50 mL). The combined filtrate and washings were saturated with H_2S, and the resultant suspension filtered through Celite. After basification with potassium carbonate, the filtrate was extracted with $CHCl_3$ (5 × 200 mL). The $CHCl_3$ extract was acidified with ether previously saturated with $HClO_4$ and the solvent removed to give the iminium perchlorate (7.59 g, 50%); mp 164–166 °C (EtOH); ν_{max} (Nujol) 1680 cm^{-1} (>C=N<); EIMS, m/z 213 (M^+); FAB MS, m/z 214 $(M + 1)^+$. Anal. ($C_{15}H_{20}N_4ClO_4$) Calcd: C, 57.4; H, 6.4; N, 4.7; Found: C, 56.9; H, 6.4; N, 4.7.

1,2,3,4,5,6-Hexahydro-3,4,6-trimethyl-2,6-methano-3-benzazocine-4-nitrile (3). To a solution of KCN (1.20 g, 18.0 mmol) in water (50 mL) in a separatory funnel was added **2** (2.0 g, 6.0 mmol) and diethyl ether (50 mL). The mixture was shaken vigorously and the ether layer removed. The aqueous layer was extracted further with ether (3 × 100 mL), and the combined ether extracts were dried (Na_2SO_4) and concentrated to give the product, 1.05 g (73%), after recrystallization from petroleum ether (60–80 °C); mp 102–104 °C; ν_{max} (Nujol) 2220 cm^{-1} (C≡N). Anal. ($C_{16}H_{20}N_2$) C, H, N.

3 was also prepared by the procedure of Groutas, Essawi, and Portoghese.[10]

1,2,3,4,5,6-Hexahydro-3,6,11-trimethyl-2,6-methano-3-benzazocine-4-nitrile (8) was prepared by the method of Groutas, Essawi, and Portoghese.[10]

4-Mono- and 4,4-Disubstituted 1,2,3,4,5,6-Hexahydro-2,6-methano-3-benzazocines (Table II). Methods A and B below are exemplified for 1,2,3,4,5,6-hexahydro-4-benzyl-2,6-methano-3-benzazocine (**9c**); other derivatives were prepared according to Table II.

Method A. To a stirred ice-cooled suspension of the iminium perchlorate **2** (2.50 g, 0.008 mol) in dry ether (40 mL) was added an ethereal solution of benzyl magnesium chloride (1.2 molar equiv). The mixture was stirred at 5 °C for 2 h, poured onto crushed ice, basified with strong NH_4OH, and extracted with ether (3 × 100 mL). The combined ether extracts were dried (Na_2SO_4), and evaporating the solvent gave crude **9c** base, which was distilled over a short path [170 °C (0.5 mmHg)] to give **9c** base (2.19 g, 90%). The hydrochloride separated as colorless plates from ethanol–ether.

Method B. A solution of the nitrile **3** (2.50 g, 0.0104 mol) in dry ether (100 mL) was added slowly to a stirred, ice-cooled solution of benzylmagnesium chloride (1.2 molar equiv). The mixture was stirred at room temperature for 3 h (15 h for 11-methyl derivatives) and then poured onto crushed ice. The ethereal layer was collected and the aqueous phase extracted with ether (2 × 100 mL). The combined ether extracts were dried (Na_2SO_4) and evaporation of the solvent gave crude **9c**, which was treated as for method A.

1,2,3,4,5,6-Hexahydro-3,4,6-trimethyl-8-hydroxy-2,6-methano-3-benzazocine (10b). An ethereal solution of (4-methoxybenzyl)magnesium chloride (from 46.9 g of 4-methoxy-

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benzyl chloride) was added to a stirred suspension of 2,4-lutidinium methiodide (25 g, 0.1 mol) in dry ether (100 mL) during 15 min at ambient temperature. The mixture was stirred for a further 2 h and then poured into a mixture of crushed ice (500 g) and 60% $HClO_4$ (60 mL). Crude 2-(4-methoxybenzyl)-1,4,6-trimethyl-1,2-dihydroxypyridine perchlorate (28.0 g) was collected from the center layer by filtration after 0.5 h, washed with EtOH, and dried. This intermediate was suspended in a mixture of MeOH (150 mL) and 2 N NaOH (200 mL) and treated with $NaBH_4$ (7.50 g) during 15 min. The mixture was kept at 55–60 °C overnight, diluted with water (200 mL), cooled, and extracted with ether (4 × 100 mL). The combined ether extracts were dried (Na_2SO_4), and the solvent was evaporated to give the corresponding tetrahydropyridine (19.20 g). This was dissolved in 47% aqueous HBr (150 mL), was heated under reflux for 22 h, and then poured into ice/water. The solution was basified with strong NH_4OH and extracted with $CHCl_3$ (4 × 100 mL). The combined extracts were dried (Na_2SO_4), and the solvent was removed to give a crude oil, which on washing with ether gave the product (6.9 g, 33%), mp 234–236 °C. Anal. ($C_{15}H_{21}NO$) C, H, N.

1,2,3,4,5,6-Hexahydro-4,6-dimethyl-8-hydroxy-2,6-methano-3-benzazocine (10g). A solution of **10b** (4.10 g, 0.018 mol) in acetic anhydride (10 mL) was heated at 100 °C for 3 h, and the excess acetic anhydride was removed in vacuo. The residue was dissolved in ethyl acetate (100 mL), the solution was washed with 5% aqueous Na_2CO_3 (3 × 50 mL) and dried (anhydrous K_2CO_3), and the solvent was removed to give the crude *O*-acetate (4.0 g). A solution of this intermediate in $CHCl_3$ (25 mL) was added gradually to a solution of CNBr (1.60 g) in $CHCl_3$ (25 mL) at room temperature with stirring. The mixture was heated under reflux, for 3 h, cooled, washed with 5% HCl (50 mL), and dried (Na_2SO_4). The solvent was removed to give crude *N*-cyano compound (4.07 g), which was dissolved in 7% HCl (80 mL), and the soluton was heated under reflux overnight. The cooled solution was made alkaline with aqueous ammonia and extracted with $CHCl_3$ (4 × 50 mL), and the combined, dried (anhydrous K_2CO_3) extracts were evaporated to give solid **10g** (2.27 g, 59%); mp 241–243 °C after recrystallization from 2-propanol. Anal. ($C_{14}H_{19}NO$) C, H, N.

1,2,3,4,5,6-Hexahydro-4,6-dimethyl-3-allyl-8-hydroxy-2,6-methano-3-benzazocine (10e). A mixture of **10g** (1.5 g, 6.9 mmol), allyl bromide (0.84 g, 6.9 mmol), and K_2CO_3 (0.5 g) in ethanol (50 mL) was heated under reflux for 24 h and then the solvent was removed. The residue was dissolved in water and extracted with $CHCl_3$ (3 × 100 mL), and the combined, dried (Na_2SO_4) extracts were concentrated to give the product (**10e**) (0.84 g, 48%). The hydrochloride crystallized from 2-butanone/MeOH and had mp 263–265 °C. Anal. ($C_{17}H_{24}NOCl$) C, H, N.

1,2,3,4,5,6-Hexahydro-4,6-dimethyl-3-(cyclopropylmethyl)-8-hydroxy-2,6-methano-3-benzazocine (10f). A solution of cyclopropylcarbonyl chloride (1.88 g, 18 mmol) in dry THF (30 mL) was added gradually to a solution of **10g**, (2.0 g, 9.2 mmol) and triethylamine (1.81 g, 19 mmol) in THF (50 mL) and the mixture stirred at room temperature for 3 h when the precipitated triethylamine hydrochloride was removed. Evaporation of the solvent gave crude cyclopropylamide (3.04 g), which was dissolved in THF (40 mL) and added dropwise to a suspension of LAH (0.97 g) in dry THF (50 mL). The mixture was heated under reflux for 8 h after which the excess LAH was destroyed cautiously. After removal of the precipitate, the filtrate was dried (Na_2SO_4) and the solvent evaporated to give the product (1.41 g, 61%), which solidified on standing. The hydrochloride crystallized from $EtOH/Et_2O$ and had mp 280–282 °C. Anal. ($C_{18}H_{26}NOCl$) C, H, N.

1,2,3,4,5,6-Hexahydro-3,5,6-trimethyl-8-hydroxy-2,6-methano-3-benzazocine (10d) was prepared by the procedure of Parfitt and Walters[2] for the nonphenolic derivative. The hydrochloride crystallized from ethanol–ether and had mp 283–285 °C dec. Anal. ($C_{15}H_{22}NOCl$) C, H, N.

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Structure–Activity Studies on the N-Terminal Region of Growth Hormone Releasing Factor

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In previous reports illustrating the effects of conformational restriction of the N-terminal region of human pancreatic growth hormone releasing factor, we demonstrated that D-amino acid substitutions in either of positions 1, 2, or 3 resulted in greatly increased growth hormone releasing activity both in vivo and in vitro. The most active compound, [D-Ala-2]GRF(1–29)NH_2, was 51 times more active than the parent 29 amino acid peptide in the sodium pentobarbital anesthetized rat. These observations have now been extended to analogues containing multiple D-amino acid replacements in these three positions. Once again, peptides with superagonist potencies ranging from 1200% to 3800% were obtained after solid-phase synthesis and purification by medium-pressure reverse-phase liquid chromatography. In addition, it was found that [D-Asn-8]- and [D-Ala-4]GRF(1–29)NH_2 were, respectively, 2.43 and 1.1 times more active than GRF(1–29)NH_2 itself. In contrast, [D-Phe-6] and [D-Thr-7] analogues were virtually inactive. Chou–Fasman structural predictions suggest that the first three residues of the peptide assume no fixed type of conformation but that a reverse turn could be present between residues 6 and 10. Attempts are made to rationalize the biological results with these calculations. The effects of other side chains on the D-amino acid in position 2 were also investigated. Both the Ac-[D-Phe-2]- and Ac-[D-Arg-2]peptides had very low activity. Several of the inactive peptides were tested as possible antagonists of GRF; however, none was able to block the stimulatory effects of GRF(1–29)NH_2 after combined administration.

Although the human pancreatic tumor,[2,3] rat,[4] and bovine[5] growth hormone releasing factors, possessing 40 or 44 amino acids, are well within range of rapid solid-phase techniques, it is much preferrable to work with as short

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