Acyl, N-Protected α-Aminoacyl, and Peptidyl Derivatives as Prodrug Forms of the Alcohol Deterrent Agent Cyanamide

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Cyanamide ($H_2NC{\equiv}N$), a potent aldehyde dehydrogenase (AlDH) inhibitor that is used therapeutically as an alcohol deterrent agent, is known to be rapidly metabolized and excreted in the urine as acetylcyanamide (1). On the basis of our observation that 1 is deacetylated to cyanamide in vivo, albeit very slightly, thereby serving as a precursor or prodrug form of the latter, several acyl derivatives of cyanamide were synthesized specifically as prodrugs, including benzoylcyanamide (2), pivaloylcyanamide (3), and 1-adamantoylcyanamide (4), as well as long- and medium-chain fatty acyl derivatives such as palmitoyl- (6), stearoyl- (7), and *n*-butyrylcyanamide (5). N-Protected α-aminoacyl and peptidyl derivatives of cyanamide were also synthesized, and these include *N*-carbobenzoxyglycyl- (10), hippuryl- (13), *N*-benzoyl-L-leucyl- (14), *N*-carbobenzoxyglycyl-L-leucyl- (18), *N*-carbobenzoxy-L-pyroglutamyl- (22), L-pyroglutamyl-L-leucyl- (19), and L-pyroglutamyl-L-phenylalanylcyanamide (20). All of these prodrugs of cyanamide raised ethanol-derived blood acetaldehyde levels in rats significantly over controls 3 h after ip drug administration, and some of these were still capable of elevating blood acetaldehyde 16 h post drug administration. A selected group of cyanamide prodrugs were also evaluated by the oral route of administration and showed nearly equivalent activity as the ip route in elevating ethanol-derived blood acetaldehyde. These results suggest potential utility of these prodrugs as deterrent agents for the treatment of alcoholism.

Cyanamide, in the form of its citrated calcium salt[1] or as a 1% aqueous solution,[2] is used therapeutically as an alcohol deterrent agent in Europe, Canada, and Japan. Cyanamide is a potent inhibitor of the low K_m mitochondrial aldehyde dehydrogenase (AlDH),[3] the isozyme primarily responsible for the bulk of the metabolism of ethanol to acetaldehyde (AcH) by the liver.[4] Inhibition of AlDH in vivo by alcohol deterrent agents such as cyanamide and disulfiram results in the elevation of circulating blood AcH, which triggers an unpleasant and sometimes toxic physiological reaction known as the disulfiram–ethanol reaction (DER).[5] This adverse pharmacological effect is presumed to serve as a deterrent to further consumption of ethanol.

Cyanamide itself does not inhibit AlDH, but must be enzymatically activated by catalase to an as yet unidentified active metabolite.[6] The use of cyanamide as an alcohol deterrent agent is therapeutically advantageous when other drugs are coadministered, since, unlike disulfiram, cyanamide does not inhibit enzymes such as dopamine-β-hydroxylase or cytochrome P-450,[7] although catalase itself is inhibited.[8] Cyanamide has a rapid onset of action that is short-lived[3d,9] with maximal pharmacological effect occurring at 1–4 h and lasting up to 24 h, due to its facile metabolic conversion to and rapid excretion in the urine as acetylcyanamide (1).[10] Thus, bioreversible prodrug forms of cyanamide that prevent rapid catabolism and hence premature loss of biological activity and that permit the sustained release of optimal amounts of cyanamide in vivo over extended periods would be highly desirable as second generation alcohol deterrent agents. Such protracted drug action should also overcome the problem of patient compliance since multiple dose regimen over short intervals would be obviated.

Although 1 does not inhibit AlDH in vitro,[11] doses of 1.0 mmol/kg administered to rats raised ethanol-derived blood AcH levels 25-fold over control values, a result that constitutes pharmacological evidence that some cyanamide must have been liberated in vivo.[12] Indeed, administration of [1-^{14}C]acetyl-labeled 1 to rats gave rise to the excretion of nearly 2% of the administered radioactive dose as [^{14}C]CO_2 within 8 h, thereby providing corroborative biochemical evidence that finite deacetylation of 1 to cyanamide had taken place.[12] On the basis of these results, several acyl derivatives of cyanamide were synthesized specifically as prodrug forms, including benzoylcyanamide (2), the sterically bulky pivaloylcyanamide (3) and 1-adamantoylcyanamide (4) ostensibly to retard the rate of enzymatic hydrolysis of the acylcyanamide linkage, and lipophilic long-chain fatty acyl derivatives such as palmitoylcyanamide (6) and stearoylcyanamide (7) to prevent rapid renal excretion, with *n*-butyrylcyanamide (5) and phenylacetylcyanamide (Na salt, 23) representing those of intermediate carbon chain length. N-Protected α-aminoacyl and peptidyl derivatives of cyanamide were also synthesized, and these include *N*-carbobenzoxyglycyl-

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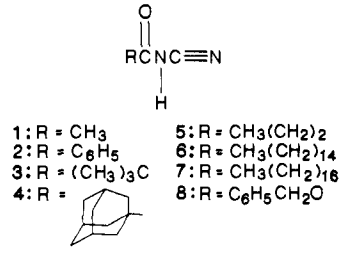
cyanamide (Z-Gly-NHCN, **10**), hippurylcyanamide (Bz-Gly-NHCN, **13**), *N*-benzoyl-L-leucylcyanamide (Bz-L-Leu-NHCN, **14**), *N*-carbobenzoxyglycyl-L-leucylcyanamide (Z-Gly-L-Leu-NHCN, **18**), *N*-carbobenzoxy-L-pyroglutamylcyanamide (Z-L-pGlu-NHCN, **22**), L-pyroglutamyl-L-leucylcyanamide (L-HpGlu-L-Leu-NHCN, **19**), and L-pyroglutamyl-L-phenylalanylcyanamide (L-HpGlu-L-Phe-NHCN, **20**), all of which are based on the potential for peptidase action for the ultimate cleavage of the acylcyanamide linkage in vivo.

Chemistry

Acylcyanamides. Acetylcyanamide (**1**) and benzoylcyanamide (**2**) were prepared by acylating sodium cyanamide with the corresponding acid chlorides by procedures described previously.[10] The other aliphatic acylcyanamides were prepared in like manner and in good yields by reacting 3 equiv of sodium cyanamide in freshly distilled dry tetrahydrofuran (THF) with the appropriate acyl chlorides. All of the acylcyanamides were stable crystalline solids, with the exception of **1** and **5**, which were unstable colorless liquids; **1**, however, was stable as its sodium salt. *N*-Carbobenzoxycyanamide (Z-NHCN, **8**), prepared by treating excess cyanamide in aqueous base with benzyloxycarbonyl chloride, was unstable at room temperature and was therefore isolated also as its sodium salt.



N-Phthaloylglycylcyanamide (**9**), Z-Gly-NHCN (**10**), Z-L-Leu-NHCN (**11**), and Boc-L-Leu-NHCN (**12**) were prepared by condensation of the N-protected amino acids via their *N*-hydroxysuccinimide activated esters with excess cyanamide in the presence of varying concentrations of aqueous sodium hydroxide. Interestingly, the urethane-protected (α-aminoacyl)cyanamides were unstable; for example, attempted recrystallization of **10** from hot ethyl acetate, or allowing of **11** and **12** to stand at room temperature, resulted in an intramolecular cyclization reaction.[13] Compound **10**, however, was successfully recrystallized from THF/petroleum ether. Attempted deblocking of the protective groups, for example, by hydrogenolysis of the Z group of **10** or hydrazinolysis of the phthaloyl group in **9**, led to mixtures of intractable products that lacked C≡N absorptions in their IR spectra and, therefore, likely represent intramolecular cyclization products.

Unlike the urethane-protected (aminoacyl)cyanamides, the benzoyl-protected Bz-Gly-NHCN (**13**) and Bz-L-Leu-NHCN (**14**) were stable, crystalline solids.

Peptidylcyanamides. On the basis of the above finding that Bz-Gly-NHCN (**13**) was stable and did not undergo intramolecular cyclization, the synthesis of dipeptidylcyanamides was considered. Amino protective groups used here were the Z, (2-bromoethoxy)carbonyl (Beoc), (2-phosphonioethoxy)carbonyl (Peoc), and L-pyroglutamyl (L-HpGlu) groups.

Beoc-Gly-L-Leu-NHCN (**15**) was obtained as a thick, clear liquid. To deblock, the compound, as its sodium salt, was treated with excess $AgClO_4$ in aqueous acetone at room temperature,[14] but the desired product, Gly-L-Leu-NHCN (**16**), could not be obtained pure. Conversion of **15** to Peoc-Gly-L-Leu-NHCN (**17**) with triphenylphosphine in refluxing acetone followed by treatment of the latter in methanol with 1% NaOH at room temperature, a deblocking method developed by Kunz,[15] gave a hygroscopic white solid whose IR and NMR spectral properties suggested the presence of **16**. However, the latter could not be isolated in pure enough state for pharmacological studies.

An alternative synthetic route to **16** involved the deblocking of the Z group in Z-Gly-L-Leu-NHCN (**18**). By catalytic transfer hydrogenation[16] of **18** with use of ammonium formate, the removal of the Z group was complete in 40 min as monitored by TLC. Although the elemental analysis of the product fell outside the accepted theoretical values, the chemical and spectral data were consistent with structure **16**; for example, the compound spotted on TLC plates gave a yellow color when sprayed with ninhydrin reagent and an orange color with a ferricyanide/nitroprusside spray reagent,[17] indicative of the presence of α-amino and cyanamide groups, respectively. Its IR spectrum displayed a band at 2160 cm^{-1} (N=C=NH), and its NMR spectrum (Experimental Section) was fully compatible with the assigned structure. Ion exchange chromatographic purification of **16** from a repeat run gave an amorphous solid that contained the solvent used for elution, as well as water of hydration, which could not be removed by washing or prolonged drying.

L-HpGlu-L-Leu-NHCN (**19**) and L-HpGlu-L-Phe-NHCN (**20**) were readily prepared by coupling of L-HpGlu-L-Leu and L-HpGlu-L-Phe with sodium cyanamide via their respective *N*-hydroxysuccinimide activated esters. However, **19** and L-pyroglutamylcyanamide itself (**21**) could not be obtained in pure form. That **21** might be unstable was indicated by the observation that *N*-carbobenzoxy-L-pyroglutamylcyanamide (**22**), a stable crystalline solid, when subjected to catalytic hydrogenolysis with H_2/Pd gave a mixture of multiple products, which discouraged further attempts to prepare **21**.

Pharmacological Results

Those derivatives of cyanamide that could be unequivocably characterized were evaluated in rats for their ability to elevate ethanol-derived blood AcH—a pharmacological consequence of the inhibition of hepatic AlDH—according to the protocol outlined in the Experimental Section. The prodrugs were administered as single doses, followed by a subsequent dose of ethanol at 2 or 15 h, with blood AcH levels being measured 1 h after each dose of ethanol. Elevation of blood AcH measured at 16 h post drug administration would therefore indicate a prolonged effect of the prodrug. The results are presented in Figures 1 and 2.

Cyanamide, a known AlDH inhibitor used as positive control, elevated blood AcH over 100-fold (1047 ± 147 μM) compared to controls receiving vehicle alone at 3 h, and this elevation was still significant at 16 h post drug administration (Figure 1). Acetylcyanamide (**1**), the major urinary metabolite of cyanamide, as its stable sodium salt,

(13) To iminohydantoin derivatives. A manuscript describing this cyclization reaction is in preparation for publication elsewhere.

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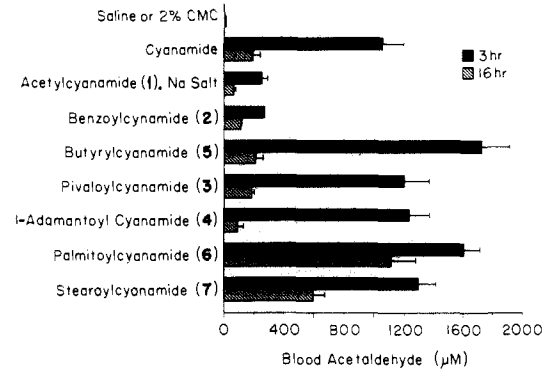

Figure 1. Effect of acyl derivatives of cyanamide on ethanol-derived blood AcH levels in rats. Dose: 1.0 mmol/kg. The drug administration protocols and blood AcH assay methods are described in the Experimental Section. The results are given as average ± SE from duplicate determinations of blood AcH from four or more animals. Where no error bars are indicated, n = 2. Blood AcH and ethanol levels for the saline/CMC controls were, respectively, 7.54 ± 1.69 μM and 49.1 ± 2.30 mM (n = 10) at 3 h and 11.7 ± 2.75 μM and 49.5 ± 3.06 mM (n = 11) at 16 h.

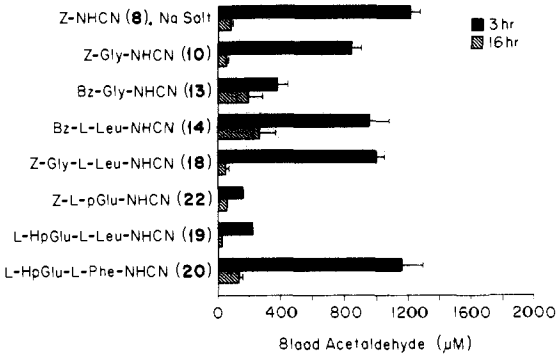

Figure 2. Effect of N-protected α-aminoacyl derivatives of cyanamide on ethanol-derived blood AcH in rats. See legend to Figure 1 for further details. Measured blood ethanol concentrations after prodrug and ethanol administration were (cumulative for Figures 1 and 2) 53.8 ± 1.45 mM (n = 45) at 3 h and 52.6 ± 1.18 mM (n = 63) at 16 h.

was, as expected, much weaker than cyanamide in elevating ethanol-derived blood AcH; however, the compound still significantly raised blood AcH at 3 h. Benzoylcyanamide (**2**) displayed activity similar to that of **1**.

n-Butyrylcyanamide (**5**), a medium-chain aliphatic acyl cyanamide, was found to be, like cyanamide, a short-acting AlDH inhibitor in vivo but even more potent. This compound raised blood AcH levels to 1732 ± 181 μM at 3 h, and the elevation in blood AcH levels at 16 h was a moderate 215 ± 47 μM. Pivaloylcyanamide (**3**) and 1-adamantoylcyanamide (**4**), which were designed to retard the rate of hydrolysis of the acylcyanamide linkage by placing sterically bulky substituents on the carbonyl group, were found to be highly active but, surprisingly, also short acting. This finding suggests that either a rapid hydrolysis to cyanamide and/or a single-pass elimination via the kidneys was taking place. Palmitoylcyanamide (**6**) and stearoylcyanamide (**7**), the two long-chain aliphatic acylcyanamides, were superior to cyanamide at 3 h, and they were still effective at 16 h, as indicated by AcH levels that were elevated 100-fold and 50-fold, respectively, over controls.

Treatment of rats with Z-NHCN (**8**) followed by ethanol gave rise to blood AcH levels of 1212 ± 62 μM at 3 h (Figure 2). Similarly, Z-Gly-NHCN (**10**) and Z-Gly-L-Leu-NHCN (**18**) elevated blood AcH in excess of 100-fold

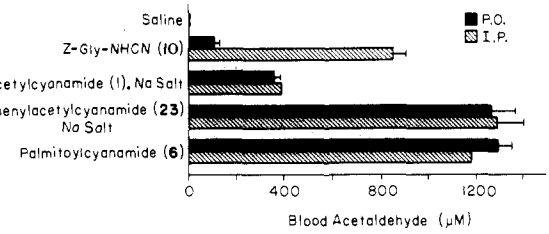

Figure 3. Comparison of oral (po) vs. intraperitoneal (ip) routes of administration for selected cyanamide prodrugs on ethanol-derived blood AcH in rats. Dose: 1.0 mmol/kg; blood AcH levels were measured 4 h after prodrug administration (1 h after ethanol). Blood ethanol levels at 4 h were 53.6 ± 1.50 mM (po, n = 16) and 46.7 ± 1.39 mM (ip, n = 11).

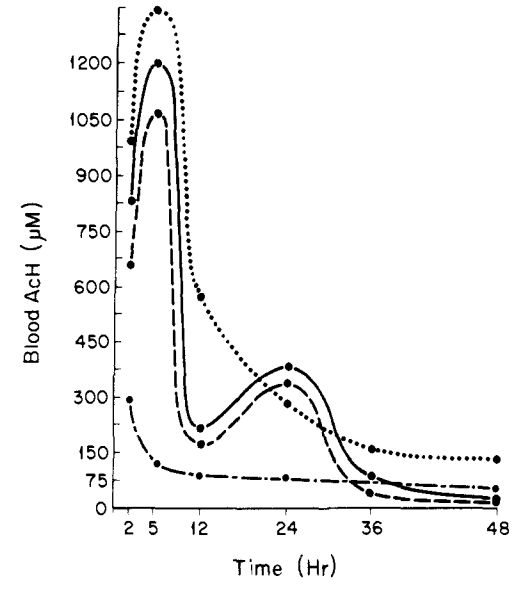

Figure 4. Blood AcH levels after ethanol measured over a 48-h time course after a single oral dose (1.0 mmol/kg) of representative cyanamide prodrugs to rats. Each point represents the averaged values from two animals. The study protocol is described in the Experimental Section. Cyanamide (control) (···); benzoylcyanamide (**2**) (-·-); palmitoylcyanamide (**6**) (—); Bz-L-Leu-NHCN (**14**) (---).

over controls. Bz-Gly-NHCN (**13**) and Bz-L-Leu-NHCN (**14**) not only elevated blood AcH levels at 3 h but also were longer acting, with ethanol-derived blood AcH significantly elevated even at 16 h (Figure 2).

L-HpGlu-L-Leu-NHCN (**19**) and Z-L-pGlu-NHCN (**22**) were not nearly as active as the other cyanamide prodrugs. However, L-HpGlu-L-Phe-NHCN (**20**) was found to be very effective over the short term, giving rise to blood AcH levels of 1155 tu 137 μM at 3 h.

The oral route of administration of selected cyanamide prodrugs was directly compared to the ip route in a side-by-side experiment (Figure 3). It can be seen that except for Z-Gly-NHCN (**10**), which was more effective by the ip route in raising blood AcH, palmitoylcyanamide (**6**), as well as phenylacetylcyanamide (**23**) and acetylcyanamide (**1**) as their sodium salts, had equivalent activity using the two different modes of drug administration.

When examined over a 48-h time course, Bz-L-Leu-NHCN (**14**) and palmitoylcyanamide (**6**) still retained activity at 24 h, although a trough was apparent at 12 h (Figure 4). This surge in activity at 24 h, also observed to a lesser degree with stearoylcyanamide (**7**) when administered intraperitoneally at 0.5 mmol/kg,[12] may reflect metabolic recycling of the liberated cyanamide. Benzoylcyanamide (**2**) was no more effective orally than by the ip route.

Discussion and Conclusions

Our original drug design called for the preparation of—among others—free α-aminoacyl and peptidyl derivatives of cyanamide with the protective groups on the amino nitrogen removed. However, the instability of the (α-aminoacyl)cyanamides, indeed, even their Z- and Boc-protected derivatives, viz., **10**, **11**, and **12**, precluded their preparation. Also, samples of the dipeptidyl glycyl-L-leucylcyanamide (**16**) could not be obtained in sufficiently pure state from its amino-protected precursors **15**, **17**, or **18** for pharmacological evaluation.

When Z-NHCN (**8**) was found to be active in elevating blood AcH after ethanol administration, suggesting that the Z group was very likely being hydrolytically cleaved in vivo, we opted to test the α-aminoacyl and peptidyl derivatives of cyanamide *with their protective groups intact*. Pharmacological activity appears not to have been compromised, as most of these N-protected (α-aminoacyl)cyanamides were quite active in raising ethanol-derived blood AcH (Figure 2).

The biotransformation of the carbobenzoxy group has not been accorded much attention despite its widespread use as an amine-protecting group for a variety of amino acids and peptides. Investigation of the enzymatic stability of a number of carbobenzoxy and acyl derivatives of L-amino acids to acylase I isolated from hog kidney indicated that, with the exception of the acyl derivatives of L-aspartic acid, L-tyrosine, and L-tryptophan, all of the compounds were hydrolyzed irrespective of the nature of the amino acid or of the acyl group.[18] Hydrolysis of the carbamate moiety was the major metabolic route for the lower *n*-monoalkyl carbamates in rats,[19] and this was thought to be due almost entirely to extrahepatic metabolic reactions.[20] Similarly, cleavage of aromatic carbamates has been shown to be catalyzed by an esterase in the plasma albumin fraction from several mammalian and avian sources.[21]

Thus, although the precise metabolic fate of the carbobenzoxy group in vivo is still subject to conjecture, the available published data suggest that the carbobenzoxy group on the cyanamide derivatives described here could be expected to be hydrolyzed by one or more of the enzyme systems alluded to above. This can be followed by peptidase action to liberate the cyanamide moiety. Alternatively, the hydrolysis of the acylcyanamide linkage may precede the enzymatic removal of the carbobenzoxy group.

The benzoyl-protected (α-aminoacyl)cyanamides, viz., Bz-Gly-NHCN (**13**) and Bz-L-Leu-NHCN (**14**), were presumed to undergo enzymatic debenzoylation followed by cleavage of the liberated (aminoacyl)cyanamide by leucine aminopeptidase. Accordingly, the leucine derivative **14** would be expected to be more active than the glycine derivative **13**, an expectation that was fully realized (Figure 2).

Although the pyrrolidonecarboxamide bond is not hydrolyzed by classical peptidyl hydrolases, pyrrolidonecarboxamido peptidase (pyrrolidonyl peptidase; pyrrolidonyl carboxypeptidase), an exo peptidase, is present in a variety of animal, plant, and human tissues,[22] and this enzyme catalyzes the hydrolysis of L-pyroglutamated amino acids and peptides.[23,24] The relative rates of cleavage by the enzyme isolated from *Pseudomonas fluorescens* were L-HpGlu-L-Ala > L-HpGlu-L-Val > L-HpGlu-L-Ile > L-HpGlu-L-Leu > L-HpGlu-L-Phe > L-HpGlu-L-Tyr,[25] and the enzyme exhibited stringent requirement for the L-optical antipodes of the amino acids. Similar relative hydrolysis rates were found with the enzyme isolated from rat liver,[26] except that the hydrolysis of L-HpGlu-L-Ile and L-HpGlu-L-Phe proceeded at very similar rates. The rationale for the preparation of L-HpGlu-L-Leu-NHCN (**19**) and L-HpGlu-L-Phe-NHCN (**20**) was based accordingly. However, **19** had only a modicum of activity in elevating ethanol-derived blood AcH in rats, whereas the phenylalanyl analogue **20** was one of the most active of the aminoacyl and peptidyl series (Figure 2).

Acylcyanamides such as **1** and **2** are inactive in inhibiting AlDH in vitro[12] and appear to require prior hydrolysis to the parent cyanamide and subsequent oxidation of the latter by catalase to generate the active enzyme inhibitor.[6] Although other mechanisms may exist for the direct activation of these acylated cyanamides, the present observation that most of these compounds, including the N-protected (α-aminoacyl)- and peptidylcyanamides, were nearly uniformly active in elevating ethanol-derived blood AcH in rats provides deductive evidence that finite quantities of cyanamide must have been liberated in vivo, thereby fulfilling their designation as prodrugs of cyanamide. Indeed, since the released cyanamide must still be further bioactivated before the inhibition of AlDH is manifest, that is, a cascade of two sequential enzymatic steps is necessary for activity, these acyl, N-protected α-aminoacyl and peptidyl derivatives of cyanamide can be considered to be *pro*-prodrugs of the as yet unidentified ultimate inhibitory species from cyanamide. The quantities liberated need not be large; for example, >90% of the administered dose of cyanamide is known to be excreted as the inactive acetylated conjugate within 6 h;[12] yet, cyanamide is the most potent in vivo AlDH inhibitor known.[27] This suggests that the relatively small fraction of cyanamide that is bioactivated by catalase is sufficient to exert a profound inhibitory action on AlDH.[10]

To the extent that some of the acyl derivatives of cyanamide—especially, the long-chain fatty acylcyanamides—are highly lipophilic and may deposit in adipose tissues from where they may be slowly released into the circulation via the lymphatic system, these prodrugs are

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(27) A referee has suggested that the blood AcH levels of 1–2 mM that we are observing for the most active cyanamide derivatives (Figures 1–3) are abnormally high, on the basis of equilibrium considerations and assuming nearly total inhibition of AlDH but a functional alcohol dehydrogenase (ADH). Using the reported K_{eq} of 1.94×10^{-4} for the reversible oxidation of ethanol to AcH by ADH at 38 °C, pH 7.0 (Higgins, J. J. In *Biochemistry and Pharmacology of Ethanol*; Majchrowicz, E. Noble, E. P., Eds.; Plenum: New York, 1979; Vol. 1, p 199), EtOH concentration of 50 mM, and liver NAD^+:NADH ratios of 100:1 to 200:1 during ethanol metabolism, our calculations show AcH concentrations of 0.97–1.94 mM in reasonable agreement with the observed values. The rate of ethanol clearance from the blood of rats given cyanamide is considerably delayed as a result of the inhibition of AlDH by this agent (ref 2b; see also: DeMaster, E. G.; Shirota, F. N.; Nagasawa, H. T. *Alcohol* **1985**, *2*, 117).

longer acting.[12] Coupled with other physicochemical methods for controlled and sustained drug delivery,[28] for example, by means of liposomes and other polymeric matrix microencapsulation, or transdermal drug delivery methods, and so on, these cyanamide prodrugs may have the potential for wide applicability in the deterrent treatment of alcoholism.

Experimental Section

Melting points were determined on a Fisher-Johns melting apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer Model 141 optical polarimeter. Microanalyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, NY, or Galbraith Laboratories, Knoxville, TN. The following spectrophotometers were used: IR, Beckman IR-10 and Perkin-Elmer Model 281; NMR, Varian T-60D and JEOL-FX90Q Fourier transform spectrometer (Me_4Si or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) used as internal standard); CI-MS, Finnigan 4000 mass spectrometer equipped to collect both positive and negative ions using methane or ammonia as the ionization gas (Mass Spectrometry Service Laboratory, Department of Chemistry, University of Minnesota). Headspace gas chromatography was carried out on a Packard Model 419 Becker flame ionization gas chromatograph equipped with an Autolab 6300 digital integrator. Statistical results were analyzed with use of a Hewlett-Packard Model 9830A desk-top computer. Unless otherwise noted, silica gel GF plates (Analtech, Inc., Newark, DE) were used for thin-layer chromatography (TLC), and ion-exchange columns were prepared with analytical-grade ion-exchange resins from Bio-Rad Laboratories, Richmond, CA. Cyanamide and its derivatives were detected on TLC plates with use of a ferricyanide/nitroprusside spray reagent.[17] Pyroglutamic acid derivatives spotted on TLC plates were detected (modification of Mazur et al.[29]) by spraying the plates successively with an aqueous solution of 5% sodium hypochlorite (commercial bleach), potassium iodide (1%), and soluble starch (1%). The plates were placed on a hot plate and heated maximally for 3–5 min. Susceptible compounds were detectable as blue-black spots against a white or very light blue background. All chemicals and solvents were reagent grade unless specified otherwise and were purchased from commercial vendors.

General Procedure for Acylcyanamides. Pivaloylcyanamide (3). Pivaloyl chloride (2.41 g, 2.46 mL, 0.020 mol) in 50 mL of THF was added dropwise to a suspension of sodium cyanamide (3.84 g, 0.060 mol) in 100 mL of THF at ice bath temperature with stirring. The reaction was allowed to proceed at room temperature overnight. The reaction mixture was then extracted with EtOAc (2 × 50 mL). The aqueous layer (pH 10.5) was separated, acidified to pH 1.5 with 10% HCl, and extracted with $CHCl_3$ (3 × 50 mL). The $CHCl_3$ extract was dried (Na_2SO_4) and evaporated in vacuo to give 2.15 g (85.2% yield) of crude product. This was crystallized from EtOAc and petroleum ether (bp range 30–60 °C) to yield 1.4 g of white crystalline **3**, mp 112–118 °C. TLC: R_f 0.56 in EtOAc/petroleum ether/AcOH (50:100:1) (orange color with ferricyanide spray reagent). IR (Nujol, cm^{-1}): 3180 (NH), 2240 (C≡N), 1730 (C=O). NMR ($CDCl_3$, δ from Me_4Si): 1.26 (s, 9 H, $(CH_3)_3C$). Anal. ($C_6H_{10}N_2O$) C, H, N.

1-Adamantoylcyanamide (4) was prepared with use of 1-adamantoyl chloride on a 0.01-mol scale in the above procedure to give **4** in 51% yield, mp 168–170 °C. TLC as above: R_f 0.58 (UV quenching (weak) and reddish orange color with ferricyanide). IR (Nujol, cm^{-1}): 3210 (NH), 2230 (C≡N), 1710 (C=O). NMR ($CDCl_3$, δ from Me_4Si): 1.62–2.2 (m, aliphatic CH_2's and CH's). Anal. ($C_{12}H_{16}N_2O$) C, H, N.

***n*-Butyrylcyanamide (5)** was prepared by reacting *n*-butyryl chloride (3.20 g, 0.030 mol) with sodium cyanamide (5.76 g, 0.090 mol) for 24 h. The reaction mixture was then evaporated to dryness in vacuo. The resulting yellow solid residue was dissolved in distilled water (50 mL), the solution was adjusted to pH 6.5 with 10% HCl, and the mixture was extracted with EtOAc (2 × 50 mL). The aqueous portion (pH 7.8) was acidified to pH 1.5 with 10% HCl and extracted with CH_2Cl_2 (3 × 30 mL). The separated organic layer was dried (Na_2SO_4) and evaporated in vacuo to give 3.33 g of crude **5** as a pale yellow liquid. A portion of the crude product (2.00 g, 17.8 mmol) was applied to a dry silica gel column (22 × 2.5 cm, 230–240 mesh, EM Reagent) in EtOAc/petroleum ether (1:3) and eluted with the same solvent at 15 psi with use of the flash chromatography technique.[30] A total of 32 20-mL fractions were collected. The fractions containing the desired compound were pooled and evaporated in vacuo to give 1.77 g (87.6% yield) of pure **5** as a clear colorless liquid. This compound is unstable and decomposes on standing, even at 5 °C when stored for prolonged periods. TLC as above: R_f 0.60 (UV quenching and orange color with ferricyanide). IR (neat, cm^{-1}): 3100–3250 (NH), 2860–2960 (alkyl), 2260 (C≡N), 1725 (C=O). NMR ($CDCl_3$, δ from Me_4Si): 8.2–8.5 (br, 1 H, NH), 2.3–2.6 (t, 2 H, CH_2CO), 1.5–1.9 (sextet, 4 H, CH_2CH_2), 0.8–1.1 (t, 3 H, CH_3). CI-MS (NH_3; positive ion): *m/z* (rel intensity) 147 (12.6) [(M + 1) + 2 NH_3], 130 (100.0) [(M + 1) + NH_3], 105 (5.2) [(M + 1) + 2 NH_3 − H_2NCN]; CI-MS (NH_3; negative ion): *m/z* (rel intensity) 111 (100.0) [M − 1], 68 (2.0) [(M − 1) − $CH_3CH_2CH_2$], 41 (16.7) [(M − 1) − $CH_3CH_2CH{=}C{=}O$].

Palmitoylcyanamide (6) was prepared by reacting palmitoyl chloride (5.5 g, 0.020 mol) with sodium cyanamide (3.84 g, 0.060 mol) for 43 h. The solid product that had precipitated was collected (8.48 g of waxy residue), ground to a fine powder, and stirred in 150 mL of distilled water at ice bath temperature. The resulting soapy suspension (pH 11) was acidified to pH 2 with 10% HCl, and the precipitated product was collected and air-dried to give 5.4 g of crude **6**. This product was dissolved in warm THF, the mixture was filtered, and the filtrate was concentrated in a rotating evaporator to turbidity. The product that precipitated was recrystallized from THF/petroleum ether to yield 3.6 g (crop 1) of white powder. An additional 0.6 g (crop 2) was obtained by workup of the mother liquor (74.8% total yield), mp 62–65 °C. TLC: R_f 0.68 in EtOAc/petroleum ether/HOAc (10:20:1). IR (Nujol, cm^{-1}): 3230 (NH), 2250 (C≡N), 1740 (C=O). NMR ($CDCl_3$, δ from Me_4Si): 2.3–2.6 (t, 2 H, CH_2CO), 1–1.8 (m, $(CH_2)_{13}$), 0.8–1 (t, 3 H, CH_3). Anal. ($C_{17}H_{32}N_2O$) C, H, N.

Stearoylcyanamide (7) was prepared on a 0.01-mol scale with use of stearoyl chloride by the general procedure to give **7** in 83.3% yield, mp 74–75 °C. TLC as for **3**: R_f 0.69 (weak UV quenching). IR (Nujol, cm^{-1}): 3210 (NH), 2250 (C≡N), 1735 (C=O). NMR ($CDCl_3$, δ from Me_4Si): 2.3–2.6 (t, 2 H, CH_2CO), 1.0–1.8 (fused s, $(CH_2)_{14}$), 0.8–0.9 (t, 3 H, CH_3). Anal. ($C_{19}H_{36}N_2O$) C, H, N.

***N*-Carbobenzoxycyanamide (8), Sodium Salt (8a).** To a stirred solution of cyanamide (6.3 g, 0.15 mol) in 60 mL of distilled water at ice bath temperature were added, simultaneously, carbobenzoxy chloride (8.5 g, 0.050 mol) and 10% NaOH (40 mL, 0.10 mol) dropwise. After 3 h, the reaction mixture (pH 10.1) was extracted with EtOAc (2 × 50 mL) followed by Et_2O (2 × 50 mL). The aqueous layer was then acidified with 10% HCl to pH 1.5 and extracted with Et_2O (4 × 50 mL). The ether extract was dried (Na_2SO_4) and evaporated in vacuo to give a clear pale pink liquid. The liquid was dissolved in 50 mL of methanol at ice bath temperature, and the solution (pH 1.8) was titrated with 5% methanolic NaOH to pH 5.9. The solution was then filtered, and the filtrate was evaporated in vacuo to near dryness to give a pale yellow liquid. Et_2O (100 mL) was added to the liquid with occasional stirring. On scratching with a glass rod, a white solid cake formed, which was collected and washed with Et_2O to give 5.17 g (52.2% yield) of pure **8a** as a white powder, mp 216–217 °C. TLC: R_f 0.56 in CH_2Cl_2/CH_3OH (5:1) (UV quenching and orange color with ferricyanide). IR (Nujol, cm^{-1}): 3100–3040 (Ar CH), 2150 (N=C=NNa), 1640 (C=O). NMR (D_2O/DSS, δ from DSS): 7.38 (s, 5 H, C_6H_5), 5.03 (s, 2 H, $C_6H_5CH_2O$). Anal. ($C_9H_6N_2O_2$·Na) C, H, N.

***N*-Carbobenzoxyglycine Succinimido Ester.** A mixture of Z-Gly (4.18 g, 0.020 mol), dicyclohexylcarbodiimide (DCC, 4.13 g, 0.020 mol), and *N*-hydroxysuccinimide (2.30 g, 0.020 mol) was

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stirred in 100 mL of acetonitrile at ice bath temperature for 5 h. After filtration to remove dicyclohexylurea (4.12 g), the filtrate was evaporated in vacuo. The semisolid residue was recrystallized from $CHCl_3$/petroleum ether (bp range 60–70 °C) to give 4.99 g (81.5% yield) of crystalline product, mp 111 °C. IR (Nujol, cm^{-1}): 3300 (NH), 3020 and 3060 (Ar CH), 1820, 1780, 1740, and 1690 (C=O's). Anal. ($C_{14}H_{14}N_2O_6$) C: calcd, 54.90; found, 54.15; H, N.

***N*-Carbobenzoxyglycylcyanamide (10).** A solution of the activated ester above (1.53 g, 5.00 mmol) in 50 mL of THF was added dropwise simultaneously with 10% NaOH (4 mL, 0.4 g, 0.010 mol), with stirring, to a reaction vessel containing cyanamide (0.63 g, 0.015 mol) in 100 mL of distilled water at ice bath temperature. After stirring overnight at room temperature, the reaction mixture (pH 9.5) was extracted with EtOAc (2 × 50 mL), and the separated aqueous layer was acidified with 10% HCl to pH 1.5. The oil that formed was extracted into Et_2O (2 × 50 mL), and the ether layer was dried (Na_2SO_4) and evaporated in vacuo to give 1.0 g of solid residue. This product was recrystallized from THF/petroleum ether (bp range 60–70 °C) to give 0.50 g (43% yield) of crystalline **10**, mp 198 °C dec. IR (Nujol, cm^{-1}): 3300, 3100 (NH), 2250 (C≡N), 1725 and 1680 (C=O's). Anal. ($C_{11}H_{11}N_3O_3$) C, H, N.

***N*-Phthaloylglycylcyanamide (9).** *N*-Phthaloylglycine succinimido ester (2.85 g, 9.44 mmol; mp 160–164 °C, prepared from *N*-phthaloylglycine[31] and *N*-hydroxysuccinimide following the procedure above for the corresponding Z-Gly ester) in 50 mL of THF was added to a solution of sodium cyanamide (0.725 g, 1.33 mmol) in 100 mL of H_2O with vigorous stirring at ice bath temperature. After 5 h, the reaction mixture (pH 6) was made alkaline (pH 10) with 10% NaOH (ice bath) and worked up as for **10** to give 1.6 g of crude **9** as a solid. A portion (300 mg) of this product was recrystallized from hot absolute EtOH to give 280 mg of crystalline **9**, mp 176–178 °C dec. TLC: R_f 0.54 in EtOAc/AcOH (100:1) (UV quenching and orange color (weak) with ferricyanide). IR (Nujol, cm^{-1}): 3350–3520 (NH), 3050 (Ar CH), 2250 (C≡N), 1770 and 1725 (C=O's). NMR ($CDCl_3$/Me_2SO-d_6 (10:1), δ from Me_4Si): 7.7–8.0 (m, 4 H, C_6H_4), 4.5 (s, 2 H, CH_2). Anal. ($C_{11}H_6N_3O_3$) C, H, N.

***N*-Carbobenzoxyglycyl-L-leucylcyanamide (18).** The activated ester prepared from Z-Gly-L-Leu (4.84 g, 0.015 mol), DCC (3.09 g, 0.015 mol), and *N*-hydroxysuccinimide (1.73 g, 0.015 mol) in 50 mL of THF was added (after removal of the urea) to a solution of sodium cyanamide (2.88 g, 0.045 mol) in 30 mL of distilled water at ice bath temperature with stirring. After 24 h at room temperature, the reaction mixture was concentrated in vacuo to half its original volume and filtered, and the filtrate was acidified to pH 2.5 with 10% HCl. After extraction with CH_2Cl_2 (4 × 50 mL), the combined CH_2Cl_2 extract was dried (Na_2SO_4) and the solvent evaporated in vacuo to give 4.86 g of crude **18** as a thick semisolid. This product was dissolved in CH_2Cl_2 and the solution washed with 5% citric acid. The separated organic layer was dried (Na_2SO_4) and decolorized with activated charcoal, and the solvent was evaporated in vacuo to give 4.10 g (78.9% yield) of **18**, mp 50–55 °C. $[\alpha]^{23}$ −34.0° (*c* 1.0, MeOH). TLC: R_f 0.54 in CH_2Cl_2/MeOH/AcOH (90:10:5) (UV quenching and orange color with ferricyanide). IR (Nujol, cm^{-1}): 3300 (NH), 3060 (Ar CH), 2260 (C≡N), 1650–1730 (C=O's). NMR ($CDCl_3$, δ from Me_4Si): 7.35 (s, 5 H, C_6H_5), 5.7–6.1 (NH), 5.1 (s, 2 H, OCH_2), 4.3–4.7 (m, 1 H, NHC*H*CO), 3.8–4.0 (fused s, 2 H, NHCH_2CO), 1.4–1.8 (m, 2 H, CH_2CH), 0.8–1.0 (fused d, 6 H, (CH_3)$_2$CH). Anal. ($C_{17}H_{22}N_4O_4$) C, H, N.

***N*-Benzoylglycylcyanamide (hippurylcyanamide) (13)** was prepared on a 0.020-mol scale from hippuric acid and cyanamide following the procedure for **10** to give **13** in 56.1% yield, mp >155 °C dec (turns to brown oil at 160 °C). TLC: R_f 0.36 in EtOAc/AcOH (100:1) (UV quenching and orange color with ferricyanide). IR (Nujol, cm^{-1}): 3400 (NH), 3080 (Ar CH), 2220 (C≡N), 1710 and 1620 (C=O's). NMR ($CDCl_3$/Me_2SO-d_6 (1:1), δ from Me_4Si): 8.2–8.4 (br, NH), 7.3–8.0 (m, 5 H, C_6H_5), 4.1–4.2 (d, 2 H, NHCH_2). Anal. ($C_{10}H_9N_3O_2$) C, H, N.

***N*-Benzoyl-L-leucylcyanamide (14)** was prepared by coupling Bz-L-Leu with cyanamide on a 0.012-mol scale following the procedure for the preparation of **10** to give 2.80 g of crude product as a tacky solid. This product was triturated with Et_2O and collected to give 1.30 g (42.9% yield) of amorphous **14**, mp 136–137 °C. $[\alpha]^{23}$ +0.7° (*c* 1.0, CH_3OH). TLC: R_f 0.93 in EtOAc/petroleum ether/THF/CH_2Cl_2/AcOH (50:50:10:50:4) and 0.24 in EtOAc/petroleum ether/AcOH (50:100:1) (UV quenching and orange color with ferricyanide). IR (Nujol, cm^{-1}): 3300, 3100 (NH), 2230 (C≡N), 1750 and 1630 (C=O's). NMR (Me_2SO-d_6, δ from Me_4Si): 7.4–8.0 (d, 5 H, C_6H_5), 4.2–4.6 (m, 1 H, NHC*H*CO), 1.4–1.9 (m, 3 H, C*H*CH_2), 0.8–1.1 (d, 6 H, (CH_3)$_2$CH). Anal. ($C_{14}H_{17}N_3O_2$) C, H, N.

***N*-[(2-Bromoethoxy)carbonyl]glycyl-L-leucylcyanamide (15).** 2-Bromoethyl chloroformate (1.87 g, 0.010 mol) and, separately, 10% NaOH (4 mL, 0.01 mol) were added simultaneously, dropwise over 10 min, to a solution of Gly-L-Leu (1.78 g, 0.010 mol) in 100 mL of water and 4 mL of 10% NaOH. After 18 h at room temperature, the reaction mixture was acidified to pH 2 with 10% HCl and extracted with EtOAc (3 × 50 mL). The separated organic layer was dried (Na_2SO_4) and evaporated in vacuo to give 3.26 g (96.1% yield) of Beoc-Gly-L-Leu as a clear liquid. This product was activated with DCC (2.06 g, 0.010 mol) and *N*-hydroxysuccinimide (1.15 g, 0.010 mol) in THF at ice bath temperature for 2 h. After removal of the urea, the THF solution of the activated ester was added dropwise to a solution of sodium cyanamide (1.28 g, 0.020 mol) in 60 mL of H_2O at ice bath temperature. After 18 h at room temperature, the reaction was worked up by the usual procedure, except that $CHCl_3$ was used for final extraction of the product. Evaporation in vacuo gave **15** as a clear thick liquid. TLC: R_f 0.29 in EtOAc/petroleum ether/AcOH (50:100:1) (orange color with ferricyanide). IR (neat, cm^{-1}): 3300 (NH), 2860–2960 (alkyl), 2260 (C≡N), 1650–1750 (C=O's). NMR ($CDCl_3$, δ from Me_4Si): 4.4–4.7 (m, NHC*H*CO), 4.3–4.5 (t, CH_2CH_2O), 3.8–4.0 (fused d, NHCH_2CO), 3.4–3.6 (t, CH_2CH_2Br), 1.4–2.0 (m, CH_2CH), 0.8–1.1 (fused d, (CH_3)$_2$CH).

Attempts to deblock **15** to Gly-L-Leu-NHCN (**16**) by the $AgClO_4$ method[14] or by conversion to Peoc-Gly-L-Leu-NHCN (**17**) followed by alkaline hydrolysis of the latter did not give **16** that was considered pure enough for biological studies.

Glycyl-L-leucylcyanamide (16). Z-Gly-L-Leu-NHCN (**18**) (1.6 g, 4.6 mmol) was subjected to catalytic transfer hydrogenation with 9% Pd/C (200 mg) and ammonium formate (450 mg) in 30 mL of methanol for 40 min. The reaction mixture was filtered through a bed of Celite filter aid, and the filtrate was evaporated in vacuo to give 1.3 g of crude **16**. This product was dissolved in 100 mL of water, and the solution was adjusted to pH 7 with 10% NaOH and then applied to a column of AG 1 × 8 anion-exchange resin (2 × 23 cm, 100–200 mesh, acetate form). The column was eluted with a linear pH gradient consisting of equal volumes of 0.5 N acetic acid (50 mL) in the reservoir and water (500 mL) in the mixing flask. The ninhydrin-positive fractions were combined and lyophilized to give 0.17 g of a white powder, mp >135 °C dec, (gradually turns yellow and finally melts at 170–171 °C to a brown liquid). TLC: R_f 0.60 in *i*-PrOH/H_2O/NH_4OH (100:10:5) and 0.93 in *t*-BuOH/AcOH/H_2O (4:1:5) (yellow color with ninhydrin; orange color with ferricyanide). IR (Nujol, cm^{-1}): 2140 (N=C=N), 1680 and 1600 (C=O's). NMR (MeOH-d_4, δ from Me_4Si): 4.2–4.5 (m, 1 H, NHC*H*CO), 3.6 (s, 2 H, H_2NCH_2CO), 1.9 (s, CH_3COOH), 1.4–1.8 (m, 3 H, CH_2C*H*), 0.8–1.0 (d, 6 H, (CH_3)$_2$CH). Anal. ($C_9H_{16}N_4O_2$·0.51CH_3COOH·H_2O) C, H, N.

L-Pyroglutamyl-L-leucine Benzyl Ester. L-Pyroglutamic acid succinimido ester (4.52 g, 0.020 mol) (prepared in the usual manner from L-pyroglutamic acid and *N*-hydroxysuccinimide, recrystallized from CH_2Cl_2, mp 136–137 °C) was added to a solution of L-leucine benzyl ester (4.43 g, 0.020 mol) in 60 mL of THF at room temperature. After 15 h, the reaction mixture was evaporated in vacuo and the pale yellow residual liquid was dissolved in CH_2Cl_2 (50 mL). The solution was successively washed with 10% citric acid (50 mL) and 5% $NaHCO_3$ (50 mL), dried (Na_2SO_4), and evaporated in vacuo to give 6.37 g of crude product as a thick pale yellow liquid. Crystallization from hot CH_2Cl_2/petroleum ether gave 5.42 g (81.51% yield) of the title ester, mp 127–128 °C. TLC: R_f 0.58 in EtOAc/AcOH (100:1) (UV quenching). NMR ($CDCl_3$, δ from Me_4Si): 7.34 (s, 5 H, C_6H_5), 5.14 (s, 2 H, $OCH_2C_6H_5$), 4.5–4.9 (m, 1 H, NHC*H*CO), 3.9–4.3 (m, 1 H, NHC*H*CO), 2.0–2.7 (m, 4 H, cyclic CH_2CH_2), 1.4–1.8 (m, 2

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H, CH_2), 0.7–1.1 (fused d, 6 H, $(CH_3)_2CH$). Anal. ($C_{18}H_{24}N_2O_4$) C, H, N.

L-Pyroglutamyl-L-leucine. L-Pyroglutamyl-L-leucine benzyl ester from above (6.65 g, 0.020 mol) in 100 mL of MeOH was subjected to hydrogenation with H_2/9% Pd/C in a Parr apparatus for 1 h. The hydrogenation mixture was filtered through a bed of Celite filter aid, and the filtrate was evaporated in vacuo to give 4.78 g of solids, which were crystallized from MeOH/Et_2O to give 4.63 g (95.6% yield) of product, colorless crystals, mp 152–154 °C (lit.[25] mp 151–152 °C). $[\alpha]^{23}$ −19.4° (c 1.0, MeOH). TLC: R_f 0.57 in $CHCl_3$/MeOH/AcOH (80:20:5) (brown color with hypochlorite spray reagent). NMR (MeOH-d_4, δ from Me_4Si): 4.3–4.6 (m, 1 H, NHC*H*CO), 4.1–4.3 (q, 1 H, NHC*H*CO), 1.9–2.6 (m, 4 H, cyclic CH_2CH_2), 1.5–1.8 (m, 2 H, CH_2CH), 0.7–1.1 (m, 6 H, $(CH_3)_2CH$). Anal. ($C_{11}H_{18}N_3O_4$) C, H, N.

L-Pyroglutamyl-L-leucylcyanamide (19). The activated ester prepared from L-pyroglutamyl-L-leucine (above) (1.21 g, 5.0 mmol), DCC (1.03 g, 5.0 mmol), and *N*-hydroxysuccinimide (0.58 g, 5.0 mmol) in 150 mL of THF was added (after removal of the urea) dropwise to a solution of sodium cyanamide (0.64 g, 10 mmol) in 70 mL of distilled water at ice bath temperature. After 4 h, the reaction mixture was concentrated in vacuo to half its original volume at room temperature and filtered. The filtrate was neutralized to pH 7 with 10% HCl and applied to a column of AG 1 × 2 anion-exchange resin (15 × 2 cm, 100–200 mesh, acetate form). The column was washed with water (380 mL) until no more cyanamide was detected (by spotting the effluent on TLC plates and spraying with the ferricyanide reagent; cyanamide gives a purple color) and then eluted with a linear pH gradient consisting of 0.15 N HCl in the reservoir and water in the mixing flask in equal volumes. A total of 45 20-mL fractions were collected. The fractions containing the desired product (orange color with ferricyanide reagent) were pooled and extracted with EtOAc/THF (5:1) (2 × 30 mL) and then EtOAc (4 × 30 mL). The organic extract was dried (Na_2SO_4) and evaporated in vacuo to dryness. The resulting film was triturated with anhydrous Et_2O, and the solids that formed were collected to give 1.29 g (97% yield) of crude product. A portion of the product (140 mg) was dissolved in MeOH and purified by preparative TLC (Preadsorbent, Analtech, 1000-μm thickness) with use of $CHCl_3$/MeOH/AcOH (80:20:5) as developing solvent. The bands that corresponded to the desired product were scraped off and extracted with EtOAc/absolute EtOH (2:1). The extract (filtered) was evaporated in vacuo to dryness and the residue triturated with Et_2O to give solids, which were collected and dried in vacuo, 79 mg, mp >175 °C dec (gradually turns yellow, then to a dark brown residue at 205 °C). TLC: R_f 0.36 in $CHCl_3$/MeOH/AcOH (80:20:5) (UV quenching (weak), orange color with ferricyanide, and brown with the hypochlorite spray reagent). IR (Nujol, cm^{-1}): 3300 (NH), 2170 (C≡N), 1670–1690 (C=O's). NMR (MeOH-d_4, δ from Me_4Si): 4.1–4.6 (m, NHC*H*CO), 2.1–2.6 (m, cyclic CH_2CH_2), 1.4–1.8 (m, CH_2CH), 0.8–1.1 (m, $(CH_3)_2CH$). Anal. ($C_{12}H_{18}N_4O_3$) C, N: calcd, 54.12, 21.04; found, 50.29, 17.64; H. (This sample was found to contain HpGlu-L-Leu as revealed by TLC.)

L-Pyroglutamyl-L-phenylalanylcyanamide (20). A procedure similar to that for the preparation of **10** was followed with use of L-pyroglutamyl-L-phenylalanine[32] (4.10 g, 14.8 mmol), DCC (3.37 g, 16.3 mmol), *N*-hydroxysuccinimide (1.88 g, 16.3 mmol), and sodium cyanamide (1.90 g, 29.7 mmol) to give 4.20 g of crude product. Recrystallization from absolute EtOH/Et_2O gave 1.36 g (crop 1) of **20**. The filtrate was concentrated and crystallized in the same manner to give 0.53 g (crop 2) (total yield of **20**, 42.5%), mp 163–165 °C. $[\alpha]^{23}$: +7.81° (c 1.0, MeOH). TLC: R_f 0.29 in $CHCl_3$/MeOH/AcOH (80:20:5) (UV quenching and orange color with ferricyanide). IR (Nujol, cm^{-1}): 3350 (NH), 3020 (Ar CH), 2160 (C≡N), 1650–1690 (C=O's). NMR (MeOH-d_4, δ from Me_4Si): 7.22 (s, 5 H, C_6H_5), 4.5–4.7 (q, 1 H, NHC*H*CO), 4.0–4.2 (m, 1 H, NHC*H*CO), 2.8–3.2 (m, 2 H, $CH_2C_6H_5$), 1.7–2.6 (m, 4 H, cyclic CH_2CH_2). Anal. ($C_{15}H_{16}N_4O_3$) C, H, N.

***N*-Carbobenzoxy-L-pyroglutamylcyanamide (22).** Z-L-Pyroglutamic acid succinimido ester[33] (1.8 g, 5.0 mmol) in 40 mL of THF was added dropwise to a solution of sodium cyanamide (0.96 g, 15 mmol) in 30 mL of distilled water at ice bath temperature. After 4 h the reaction mixture was worked up in the usual manner. The precipitate that appeared on final acidification was collected and dried to give 0.66 g (46% yield) of amorphous solids, mp 185–186 °C (turns slightly yellow >170 °C). $[\alpha]^{23}$ −31.6° (c 1.0, CH_3CN). TLC: R_f 0.50 in CH_2Cl_2/MeOH/AcOH (80:20:5) (UV quenching and gradual development of yellow color with ferricyanide). IR (Nujol, cm^{-1}): 3160 (NH), 3050 (Ar CH), 2240 (C≡N), 1775 and 1725 (C=O's). NMR (acetone-d_6, δ from Me_4Si): 7.4 (s, 5 H, C_6H_5), 5.3 (s, 2 H, CH_2O), 4.8–5.0 (m, 1 H, NCHCO), 2.2–3.0 (m, 4 H, cyclic CH_2CH_2). Anal. ($C_{14}H_{13}N_3O_4$) C, H, N.

(Phenylacetyl)cyanamide, sodium salt (23) was prepared from phenylacetyl chloride (3.09 g, 0.020 mol) and sodium cyanamide (2.56 g, 0.040 mol) according to the general procedure except that anhydrous diethyl ether was used as solvent. The solid product was dissolved in distilled water (80 mL), the mixture filtered, and the filtrate acidified with 10% HCl to pH 1.5 at ice bath temperature. After removal of a small amount of gummy residue, the sodium salt was prepared as for **8a** above. The crude salt was dissolved in a minimal amount of warm absolute ethanol and filtered. Addition of anhydrous diethyl ether to the filtrate gave 2.12 g (58.2% yield) of white crystalline **23**, mp 107–109 °C. TLC: R_f 0.81 in isopropyl alcohol/H_2O/concentrated NH_4OH (20:2:1) (UV quenching and yellow-orange color with ferricyanide). IR (Nujol, cm^{-1}): 3100–3040 (Ar CH), 2160 (N=C=NNa). NMR (D_2O/DSS, δ from DSS): 3.58 (s, 2 H, CH_2CO), 7.32 (s, 5 H, C_6H_5). Anal. ($C_9H_7N_2ONa \cdot 0.7H_2O$) C, H. N: calcd, 14.38; found, 13.90.

Pharmacological Evaluation. These studies were performed in adherence with guidelines established in the "Guide for the Care and Use of Laboratory Animals" published by the U.S. Department of Health and Human Resources (NIH Publication 85-23, revised 1985). Animals were housed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC), and the research protocol was approved by the Animal Study Subcommittee of the Minneapolis VA Medical Center.

Drug Administration Protocol. The prodrugs were dissolved in physiological saline or suspended in 2% aqueous (carboxymethyl)cellulose (CMC) (for the water-insoluble prodrugs) and administered at a dose of 1.0 mmol/kg, ip, at zero time to fasted male rats of Sprague–Dawley descent (BioLab, Inc., St. Paul, MN), weighing 185–225 g. Ethanol (2.0 g/kg, ip) was given either at 2 h or at 15 h (Figures 1 and 2). The animals were sacrificed 1 h following each ethanol dose for measurement of blood acetaldehyde. Cyanamide-treated animals served as positive control. Four animals were used for each drug protocol except where noted. For the oral route of administration, the prodrugs (1.0 mmol/kg) were administered by stomach tube at zero time, and the ethanol dose (2.0 g/kg, ip) was always given 1 h prior to sacrifice. Thus, for the 48-h time-course studies (Figure 4), the ethanol doses were at 1, 4, 11, 23, 35, and 47 h, with each time period being represented by two animals.

Blood Acetaldehyde Determination. Blood AcH levels were measured 1 h after the administration of ethanol in treated and control animals. The animal was stunned, and blood was immediately withdrawn by open chest cardiac puncture. Aliquots (0.2 mL) were placed in 20-mL serum vials containing 1.0 mL of 5 mM sodium azide (to minimize the artifactual generation of AcH from ethanol), and the vials were immediately capped with rubber septums. The vials were frozen on dry ice and kept frozen at −20 °C until assayed. AcH was determined in duplicate by a headspace gas chromatographic procedure[34] and quantitated by reference to a standard curve based on standards with known concentrations of AcH.

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Registry No. 1·Na, 84946-07-6; **2**, 15150-25-1; **3**, 97585-08-5; **4**, 103818-47-9; **5**, 5634-65-1; **6**, 100551-52-8; **7**, 79316-84-0; **8a**,

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57324-57-9; **9**, 103818-48-0; **10**, 103818-49-1; **13**, 103818-50-4; **14**, 100551-53-9; **15**, 103818-51-5; **16**, 103818-52-6; **18**, 103818-53-7; **19**, 103818-54-8; **20**, 103818-55-9; **22**, 103818-56-0; **23**, 103818-57-1; Z-Gly-L-Leu, 1421-69-8; Bz-L-Leu, 1466-83-7; Gly-L-Leu, 869-19-2; Beoc-Gly-L-Leu, 103818-58-2; ethanol, 64-17-5; pivaloyl chloride, 3282-30-2; sodium cyanamide, 19981-17-0; *n*-butyryl chloride, 141-75-3; palmitoyl chloride, 112-67-4; 1-adamantoyl chloride, 2094-72-6; stearoyl chloride, 112-76-5; cyanamide, 420-04-2; carbobenzoxy chloride, 501-53-1; *N*-carbobenzoxyglycine succinimido ester, 2899-60-7; *N*-hydroxysuccinimide, 6066-82-6; *N*-phthaloylglycine *N*-hydroxysuccinimide ester, 3397-29-3; sodium cyanamide, 19981-17-0; hippuric acid, 495-69-2; 2-bromoethyl chloroformate, 4801-27-8; L-pyroglutamyl-L-leucine benzyl ester, 103818-59-3; L-pyroglutamic acid succinimido ester, 59850-85-0; L-leucine benzyl ester, 1738-69-8; L-pyroglutamyl-L-leucine, 21282-11-1; L-pyroglutamyl-L-phenylalanine, 21282-12-2; Z-L-pyroglutamic acid succinimido ester, 40291-26-7; phenylacetyl chloride, 103-80-0.

Preparation and Analgesic Activity of (−)-11α-Substituted 1,2,3,4,5,6-Hexahydro-6α,7-(methyleneoxy)-2,6-methano-3-benzazocines[1]

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Dihydrocodeinone oxime (**1**) under Beckmann rearrangement conditions gave a product (**2**) that facilitated the preparation of (−)-11α-substituted 1,2,3,4,5,6-hexahydro-6α,7-(methyleneoxy)-2,6-methano-3-benzazocines, a hitherto little-examined series of morphine partial structures. Compounds **7a** and **12** gave good levels of agonist antinociceptive activity. Masking of the 8-oxygen function, as in **6** and **8**, dramatically reduced mouse hot-plate activity, as did its loss (**9**).

Derivatives of 1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocine (the benzomorphans) constitute a class of opiate analgesics with a broad spectrum of pharmacological actions.[3,4] They include agents with μ or κ agonist and mixed agonist/antagonist properties. Numerous variants have been synthesized not only in an attempt to develop a potent analgesic with minimal undesired properties but also as a means of exploring the nature of the opiate receptor.

(−)-1,2,3,4,5,6-Hexahydro-2,6-methano-3-benzazocines are configurationally related to morphine and as such are responsible predominantly for the antinociceptive properties of the racemate. Some (−)-benzomorphans lack a physical dependence capacity (PDC) in rhesus monkeys, and in addition, they may precipitate or exacerbate the abstinence syndrome.[5-7] In particular, the demonstration[8] that (−)-3,6-dimethyl-8-hydroxy-11α-propyl-2,6-methano-3-benzazocine and the corresponding 11β-propyl derivative appeared to have minimal PDCs and the latter also exhibited antagonist properties led us to investigate stereospecific routes to related series.[9]

During the course of the study we synthesized (−)-1,2,3,4,5,6-hexahydro-8-hydroxy-6α,7-(methyleneoxy)-3-methyl-11α-propyl-2,6-methano-3-benzazocine (**7**) and a series of related compounds that we report here. Previously Sargent and Ager[10] had converted codeine via *N*-phenethyl-7,8-dihydroxydihydrodesoxycodeine into a 6α,7-(methyleneoxy)-2,6-methano-3-benzazocine analogue of phenazocine that had twice the mouse hot-plate antinociceptive potency of morphine. In addition, the ozonolysis of thebaine[11] afforded a low yield of furanobenzomorphan, but no biological data were presented. More recently[12] an oxide-bridged 5-(*m*-hydroxyphenyl)-morphan was reported to lack in vivo opiate activity.

The main structural difference between 1,2,3,4,5,6-hexahydro-6α,7-(methyleneoxy)-2,6-methano-3-benzazocines and 4,5-epoxymorphinans (morphine and related compounds) is the absence of a C ring in the former. A vestigial C ring together with an intact furan moiety has been shown in a furanobenzomorphan analogue of phenazocine[10] to maintain good analgesic properties. Reden et al.[13] have indicated that in morphine series, although a phenolic hydroxyl group aids receptor binding, it is not a prerequisite. Masking of the 3-OH as in codeine or heroin, however, does reduce receptor affinity considerably.

Morphinan-6-ones do not require an oxygen function in the aromatic ring to elicit analgesic responses, but oxygen at C-3 or C-4 enhanced both receptor binding and antinociceptive activity.[14] *N*-Methyl-4-methoxymorphinan-6-one, however, is a more potent analgesic than its phenolic counterpart (cf. levorphanol and its methyl ether[15]).

The investigation of furanobenzomorphans such as those described here may shed further light on the role of both the C ring and aromatic oxygenation in opiate antinociceptive responses.

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(1) The correct nomenclature for this ring system is 3*H*-2a,6-methano-2*H*-furo[4,3,2-*f*,*g*][3]benzazocine, but for purposes of comparison with other benzomorphan studies, the 2,6-methano-3-benzazocine name and numbering have been adopted in this paper.
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