scopically and the positions and intensities of the visible chromophores recorded (see Tables I and II).

Acetylation of human serum albumin was achieved by adding 20 mg (194 mmol) of acetic anhydride to a stirring solution of 200 mg (3×10^{-3} mmol) of human serum albumin in 40 mL of 0.01 M pH 7.0 phosphate buffer. After stirring for 1.5 h at room temperature, the solution was dialyzed against 2 L of distilled water overnight, after which the protein solution was lyophilized.

Acknowledgment. We thank Dr. H. Baer for the ring methyl analogues of PDC provided to him by C. R. Dawson. This work was supported in part by National Institutes of Health Grants AI 14752 and RO 1 AI 12947 and Food and Drug Administration Grant 223-77-1201.

Permethyl Analogue of the Pyrrolic Antibiotic Distamycin A

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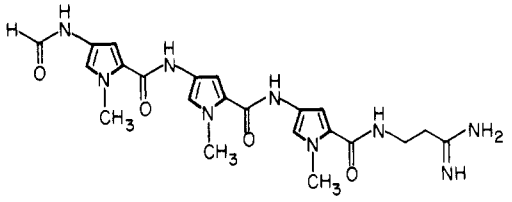
The synthesis of an analogue of distamycin A, a pyrrolic oligopeptide possessing antiviral and antibiotic activity, is described in which each of the three pyrrole rings is fully methylated. This structural modification results in pyrrole rings which are extraordinarily electron rich and required the development of a new synthetic approach to these polypyrrolic amides. The key reactions involved development of a general method for the synthesis of 3-aminopyrroles and for formation of an amide bond between a pyrrole-2-carboxylic acid and these 3-aminopyrroles. Since the acid is hindered, a poor electrophile, and acid sensitive, while the amine is unstable and a hindered, weak nucleophile, amide bond formation under the usual conditions was poor. A very efficient method, however, was developed involving the isolation of 1-hydroxybenzotriazole active ester prepared in situ from another active ester. Neither the mono-, di-, nor tripyrrolic permethyl analogues were effective antimalarials, and none showed anticancer activity.

Distamycin A (1), a naturally occurring substance isolated from *Streptomyces distallicus*,[1] has been the subject of numerous chemical[1–6] and biological investigations.[7,8] It has antiviral, antitumor, and antibacterial activity and forms complexes with various types of DNA, thus inhibiting DNA-dependent syntheses.[7] It is a member of a modest class of oligopeptides, all of which are biologically active; this class includes anthelvencin A (2),[9] congocidin (3),[10] and kikumycin B (4),[11] which contain a common pyrrole subunit, and amidinomycin (5)[12] and noformycin (6),[13] which are nonaromatic. Their biological activity has stimulated much work in several fields, including molecular biological endeavors, since the interactions of distamycin A and congocidin with DNA reveal substantial information about the DNA. This, in turn, has led to syntheses of two (1 and 3) of the natural products,[2,6a,10] and the syntheses of homologues and analogues[3–5,6b,8,14–16] soon followed.



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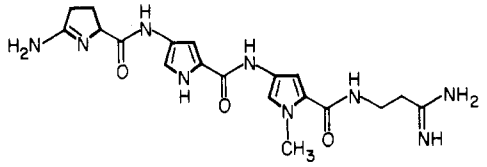

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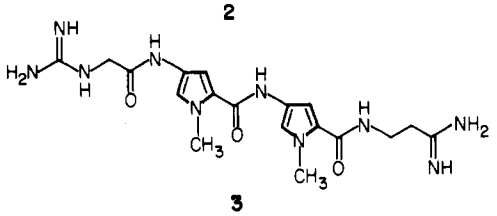

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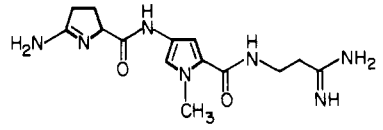

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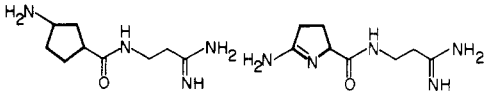

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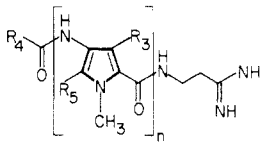
From these synthetic efforts it became clear that substitution of a benzenoid ring[14,15] for the pyrrole nucleus led to loss of biological activity, while some side-chain modifications retained activity.[3-5,6b,8,16] The syntheses of all of the distamycin A and congocidin derivatives, as well as the subsequent total syntheses, follow very closely the method developed for the original distamycin A synthesis.[2] We sought a more general synthesis and for the permethyl analogues needed to develop a new approach, since the original method is severely limited as to the type of pyrrole ring that can be carried through the synthetic sequence. This report presents the synthesis of distamycin A and two lower homologues in which the pyrrole ring has methyl groups at the 3 and 5 positions (7, $R_3 = R_5 = CH_3$; R_4 = H; n = 1–3), along with their evaluation as antimalarials.



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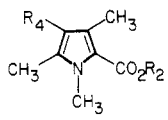
Our rationale for considering these compounds primarily as candidate antimalarials results from the nature of distamycin's mode of action. Distamycin is known to intercalate into DNA and interfere with DNA-dependent syntheses. Similar mechanisms pertain to a number of antimalarials, e.g., chloroquine, which are active by virtue of their binding to DNA and inhibiting DNA-dependent syntheses.[7] Thus, we sought to join this commonality and devise effective antimalarial compounds.

Discussion

Our choice of the permethyl homologue is based on the fact that pyrrole is an electron-rich aromatic ring[17] and seems to be critical for biological activity in these systems. The introduction of methyls would substantially increase this electron richness;[18,19] the pK_a difference between N-methyl- and 2,4-dimethylpyrrole is about 10^5. Introduction of the methyls should also make the compounds more lipophilic, and a correlation between DNA binding and/or activity with lipophilicity and electron density could be made. The steric situation would be significantly different since the pyrrole ring is now fully substituted with each group diortho substituted; this might effect the conformation of these compounds, which appears to be important.[8] Also the ring methyls might block certain metabolic pathways such as those involving pyrrole oxidases. In addition, two of the natural products occur as a pyrrolic N-methyl and N–H pair,[9,11] and in each case the N-methyl derivative is more active.

Introduction of these two ring methyls into the pyrrole nucleus immediately imposes development of a new synthetic approach, since the known methods are not flexible enough to accommodate such a change. All previous syntheses of distamycin A,[2,6a] congocidin,[10] and their analogues[3-5,6b,8] employ a nitro group as the amine synthon. Introduction of the nitro group by ring synthesis to produce an ester of 4-nitro-1,3,5-trimethylpyrrole-2-carboxylic acid 8 (R_2 = alkyl; R_4 = nitro) would require 3-nitropentanedione (9, R = CH_3), analogous to the sodium salt



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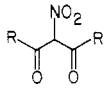

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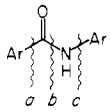
of nitromalondialdehyde (9, R = H) used in the synthesis of the demethylpyrrole ring.[2] 3-Nitropentane-2,4-dione exists only as a metal chelate.[20,21] Attempts to prepare the parent molecule by nitration of 2,4-pentanedione with acetone cyanohydrin nitrate[22] or acetyl nitrate[23] failed.

Introduction of a nitro group by electrophilic substitution into a preformed pyrrole ring initially seems to be a straightforward process, since the pyrrole ring is an excellent substrate for electrophilic substitution. However, formation of the desired nitro compound 8 ($R_4 = NO_2$) was a poor-yield process and suffered as a general procedure[24] due to the isomer problem should more than one position on the ring be available.

Thus, we turned to development of a general process applicable to this series of compounds, the key feature of which is the interpyrrolic amide bond. Three approaches to this linkage are evident, as shown by general structure 10. Formation of 10 via bond "a" would require an α-free



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pyrrole and a β-pyrroleisocyanate, a reasonable combination since Friedel–Crafts acylation with an isocyanate into an α-free pyrrole seemed attractive. In spite of the precedence,[25] however, it was not very successful, and a maximum yield of 25% was obtained using many modifications. An explanation for this poor yield could involve the pyrrole N-substituent. The reaction is base catalyzed, and our attempts made with N-CH_3 groups were not very productive, in spite of the increased electron density and in contrast with N–H compounds.[25] Formation of 10 via bond "b" would require a peptide bond to be forged between an α-pyrrole acid and β-pyrrole amine, and this is the method we turned to. Forming bond "c" would require an aromatic amide acting as a nucleophile to an aromatic ring, a rather unique situation.

Synthetic Plan

The approach to these compounds was thus defined but it proved to be more difficult than we had anticipated. Specifically, we needed the combination of a protected amine, an activated acyl, an amine, and a protected carboxylic acid. This array is typical for peptide synthesis: one amide bond is formed and the incipient bonds are carried along as protected amines and acids.

The limitations for the protected carboxylic acid were defined by the properties of pyrrolecarboxylic acids. They are subject to acid-catalyzed decarboxylation;[26] therefore,

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their genesis should be under nonacidic conditions, and a benzyl ester, to be removed by hydrogenolysis, was the method of choice. Thus, the desired compound would be benzyl 4-amino-1,3,5-trimethylpyrrole-2-carboxylate (8, $R_2 = C_6H_5CH_2$; $R_4 = NH_2$).

Even though β-aminopyrroles are relatively obscure,[27-29] they might be stronger bases and nucleophiles than the usual aromatic amine because of the electron density within the pyrrole ring and, thus, be effective in the proposed amide bond formation. This is not the case; the pK_a of 8 ($R_2 = C_2H_5$; $R_4 = NH_2$) is 5.5. We did, however, have an efficient process for the preparation of the required β-aminopyrroles via β-isocyanatopyrroles, themselves readily convertible both to the β-aminopyrroles and to their protected derivatives.

The other moiety, containing the protected amine and activated acyl, proved more formidable to devise. The active ester component was the major difficulty. Pyrrolecarboxylic acids, besides being very sensitive to decarboxylation, are quite insensitive to nucleophilic attack at the carbonyl, behaving as vinylogous amides. This property is reflected in their low energy carbonyl stretch in the IR,[17] as well as their resonance at higher field in ^{13}C NMR spectra.[30] The majority of peptide coupling reagents proved ineffective. Pyrrolecarboxylates are strong nucleophiles, and attempts to prepare many active acyl derivatives usually gave the symmetrical pyrrole anhydride as a major byproduct, accompanied by decarboxylation, incomplete consumption of starting materials, and side-products.

Synthesis of Permethyldistamycins (7, $R_3 = R_5 = CH_3$; $R_4 = H$; $n = 1$–3). The introduction of the amine group via a mild selective procedure proceeded through a Curtius rearrangement. This method is quite versatile since, in addition to satisfying the above two criteria, any pyrrolecarboxylate group becomes a potential amine and pyrrolecarboxylates are a broadly available class of compounds. This rearrangement had to occur before any amide bonds were present in the permethyl substrates (see following). We started with ethyl 4-carboxy-1,3,5-trimethylpyrrole-2-carboxylate[31] (8, $R_2 = C_2H_5$; $R_4 = CO_2H$), which was transesterified to the corresponding benzyl ester. This was transformed via the azide 8 ($R_2 = C_6H_5CH_2$; $R_4 = CON_3$) to the isocyanate ($R_4 = NCO$), which was isolated in about 75% yield from the acid. The conversion through acyl azide to isocyanate is a very quick, mild procedure made possible by use of diphenylphosphoryl azide (DPPA)[32] in acetonitrile/triethylamine (TEA) at 50 °C for an hour, conveniently monitored by IR.

Although compound 8 ($R_2 = C_6H_5CH_2$; $R_4 = NCO$) added primary and secondary alcohols rapidly and in excellent yields by simply mixing with an excess of the dry alcohol, the addition of *tert*-butyl alcohol under the same conditions gave no conversion to carbamate. Many catalysts exist for just such a conversion,[33,34] but in our hands most were capricious, *tert*-butoxide[35] was ineffective, and irradiation[36] was impractical. Cuprous chloride was found to be the catalyst of choice, in analogy with CuCl-catalyzed additions of alcohols to diimides.[37] The isocyanate smoothly added *tert*-butyl alcohol in the presence of CuCl at 60 °C in a few hours to give the corresponding carbamate (8, $R_4 = t\text{-}BuO_2CNH$) in nearly quantitative yield.

Although an amine salt probably could have been obtained by treatment of the *tert*-butyl carbamate with aqueous TFA,[38] another route to the free amine was available. The action of DPPA on the corresponding ethyl ester (8, $R_2 = C_2H_5$; $R_4 = CO_2H$) proceeded through the azide to isocyanate, which was quenched with benzyl alcohol to give the benzyl carbamate (8, $R_2 = C_2H_5$; $R_4 = C_6H_5CH_2O_2CNH$). This was subsequently hydrogenolyzed to the amine ethyl ester and finally transesterified with benzyl alcohol to the desired compound (8, $R_2 = C_6H_5CH_2$; $R_4 = NH_2$). The overall yield for these five steps is 35%. Acylation of the free amine could be accomplished by a variety of reagents either as a protective measure or to produce an interpyrroyl amide bond. This later step requires an activated pyrrole acyl group and is discussed in the following.

Most of the reported methods for ester activation prior to amide bond formation were ineffective. Finally, a multistep but extremely efficient process was devised, consisting of using one active ester to produce another active ester that was isolable and thereby purifyable and was at the same time highly reactive. A solution of the pyrrole-2-carboxylic acid in methylene chloride was prepared at 0 °C by the use of excess TEA. Addition of 1-hydroxybenzotriazole (HOBt)[39,40] was followed by 2-chloro-1-methylpyridinium iodide,[41] each in slight excess, the reaction being complete in 5 min at 0 °C. The product, after simple liquid–liquid partition, was obtained in quantitative yield as the active ester of 1-hydroxybenzotriazole. The presumed intermediate is the 2-pyridinium ester from which the 2-pyridinone is displaced with HOBt.

The obvious changes, either omitting HOBt, or including the amine component, or both, were not successful. The above combination of reagents gave comparable results in each permethyl species (i.e., one to three pyrrole rings) examined, and the group in the 4 position, whether an acid derivative or acylated amine, had no effect. The success of this scheme is undoubtedly due to the strong nucleophilic properties[40] of HOBt, since no symmetrical pyrrole anhydride was ever observed in the presence of HOBt but was often the major product in its absence with any other type of active ester formation.

For acylation of the hindered and weak pyrrole amines (8, R_2 = alkyl; $R_4 = NH_2$) with HOBt active esters, acetic acid catalysis with warming to 40 °C was necessary. For acylation of any other amine studied, no heat or catalyst was used. The mild acid catalysis was suggested by some hydrolysis studies of active esters of various pyrrole-2-carboxylic acids.[42] These derivatives were hydrolyzed in

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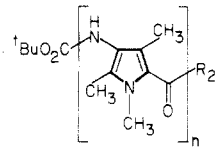
alkali with much more difficulty than in acid, indicative of the unwillingness of pyrrole carbonyls to undergo nucleophilic attack.

With the development of the HOBt active ester procedure, the construction of multiple interpyrrolic amides became quite facile. The sequence CO_2Bn (e.g., 8, R_2 = $C_6H_5CH_2$; R_4 = t-BuO_2CNH) → CO_2H → CO_2Bt and coupling with the appropriate amine (8, R_2 = $C_6H_5CH_2$; R_4 = NH_2) gave two pyrroles joined by an amide bond, one ring bearing a protected amine and the other a protected acid (11, R_2 = OBn; n = 2). It was simply repeated to give



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three pyrroles joined in the same manner and bearing the same synthons (11, R_2 = OBn; n = 3). Another cycle of this process brought us to an intermediate (11, R_2 = OBt; n = 3) which could be used to introduce any 2-carboxyl side chain we wished with no commitment as to the identity of the group on the 4′-amine, and this process is discussed below.

Introduction of the 2-Carboxyl and 4′-Amino Side Chains. The reported processes for introduction of the carboxyl side chain, β-aminopropionamidine, found in most of the natural products (1–3, 5, and 6) uses a nitrile as an amidine synthon. After the nitrile is introduced, it is converted in a two-step Pinner[43] reaction, through an imidate, to an amidine. Thus, acylation of β-aminopropionitrile with a pyrroylhydroxybenzotriazolide (11, n = 1; R_2 = OBt) readily afforded the analogous derivative for our synthesis (11, R_2 = $NHCH_2CH_2CN$; n = 1). However, the subsequent Pinner sequence, anhydrous HCl in alcohol followed by anhydrous NH_3 in alcohol, gave a mixture of pyrrolic products. Clearly, the permethylpyrrole ring is too electron rich to survive strong acid treatment. Also, it would be more efficient if the aminoamidine side chain could be introduced as an intact unit. β-Aminopropionamidine,[44] a readily obtainable compound, reacts smoothly with the series of HOBt active esters (11, R_2 = OBt; n = 1–3) in DMF with 100 mol % triethylamine to give excellent yields of products acylated on the amine.[45]

The last conversion in the synthesis required the removal of the *tert*-butyloxycarbonyl group protecting the amine and subsequent formylation. This was accomplished in one step in excellent yields by simply refluxing the compounds (11, R_2 = $NHCH_2CH_2C(=NH)NH_2 \cdot HCl$; n = 1–3) in formic acid for 1.5 h; the reported use of acetic formic anhydride and formic acid/DCC was not as convenient. The efficiency of the iterative coupling procedure (benzyl ester → acid → HOBt active ester → coupling) and the subsequent side-chain introductions is indicated by the overall yield of 54% for the 10-step process starting from the monomeric protected amine ester (11, R_2 = OBn; n = 1) to the final product, permethyldistamycin A_3 (7, R_3 = R_5 = CH_3; R_4 = H; n = 3). Permethyldistamycin A_1 and permethyldistamycin A_2 were likewise formed by treating the appropriate HOBt active ester (11, R_2 = OBt; n = 1, 2) with β-aminopropionamidine dihydrochloride, followed by hot formic acid.

Biological Results. Evaluation of potential antimalarials was carried out by the Rane assay.[46] PMD_1, PMD_2, and PMD_3, as their salts, were nontoxic and did not increase the mean survival time at the highest level tested (640 mg/kg) in the blood schizonticidal screen (*P. berghei*, mouse). These compounds were also nontoxic to mouse 3T3 fibroblasts and Maloney sarcoma transformed 3T3 fibroblasts at the highest level tested (25 μg/mL culture medium).[47]

Experimental Section

All reactions were carried out under a nitrogen blanket with magnetic stirring, and evaporations were done in vacuo on a Berkeley rotary evaporator. ^1H NMR spectra were taken in $CDCl_3$ with internal Me_4Si on a Varian T-60 spectrophotometer, and IR spectra were obtained on a Perkin-Elmer 337 instrument. Mass spectra were done on CEC 103 and 110B instruments with percent base peak given, field desorption mass spectra (FD, m/e) were performed on a modified Kratos/AEI MS902 mass spectrometer at the Space Sciences Laboratory, Lawrence Radiation Laboratory, Berkeley, Calif., and melting points were determined on a Buchi apparatus and are uncorrected. UV spectra were taken on a Cary 14 in 95% ethanol unless otherwise noted. Thin-layer chromatographies (TLC) were performed on commercial silica unless indicated thusly: acidic alumina (HAL) or neutral alumina (NAL) in solvent systems A, $CHCl_3$; B, 0.25% HOAc, 50% EtOAc, 50% $CHCl_3$; C, EtOH.

Ethyl 4-Isocyanato-1,3,5-trimethylpyrrole-2-carboxylate (8, R_2 = C_2H_5; R_4 = NCO). DPPA (100 mol %), ethyl 4-carboxy-1,3,5-trimethylpyrrole-2-carboxylate[31] (8, R_2 = C_2H_5; R_4 = CO_2H, 100 mol %), acetonitrile (1 mL/mmol), and triethylamine (TEA, 100 mol %) were combined and heated for 90 min at 50 °C. After evaporation, the residue was dissolved in CH_2Cl_2 (1 mL/mmol), diluted with hexane (25 mL/mmol), and allowed to stand for 30 min. The solution phase was removed, and the oily precipitate was treated again with CH_2Cl_2/hexane to remove more product. The organic phase was evaporated to give 70% yield of the isocyanate as a clear oil which solidified: mp 35 °C; UV λ_{max} (isooctane) 280 nm, sh 248; IR (neat) 2275, 1690 cm^{-1}; NMR δ 4.21 (q, J = 7 Hz, 2 H), 3.70 (s, 3 H), 2.03 (s), 1.98 (s, 6H, total), 1.35 (t, J = 7 Hz, 3 H); MS, m/e 223 (14), 222 (100), 194 (41), 193 (72), 177 (58), 165 (17), 150 (65), 149 (52), 121 (14). Anal. ($C_{11}H_{14}N_2O_3$) N, H; C: calcd, 59.5; found, 59.0.

The material readily adds water to produce the corresponding symmetrical urea as an insoluble white solid, mp 295–297 °C.

Ethyl 4-[(Benzyloxycarbonyl)amino]-1,3,5-trimethylpyrrole-2-carboxylate (8, R_2 = C_2H_5; R_4 = $C_6H_5CH_2O_2CNH$). The previous procedure was modified adding dry benzyl alcohol (1 mL/mmol) to the original reaction mixture after 90 min. Heating was continued for 5 h at 50 °C until the isocyanate was consumed, at which time the solvents were removed [70 °C (0.5 μm)]. The phosphonium salts were removed by washing a solution of the product with several portions of 10% Na_2CO_3, dilute HCl, and water to give a quantitative yield of crude product sufficiently pure for the next step. A pure sample, crystallized from ether, had mp 123–124 °C; TLC (A) R_f 0.49; NMR δ 7.35 (s, 5 H), 6.13 (s, 1 H), 5.15 (s, 2 H), 4.27 (q, J = 7 Hz, 2 H), 3.73 (s, 3 H), 2.17

(43) S. Patai, Ed., "The Chemistry of Amidines and Imidates", Wiley, New York, 1975.

(44) G. Hilgetag, M. Paul, J. Gunther, and M. Witt, *Ber. Dtsch. Chem. Ges.*, **97**, 704 (1964).

(45) In principle, the dibasic β-aminopropionamidine might be acylated on either the amine or amidine, and this point is almost totally overlooked in the literature. However, under the reaction conditions with 100 mol % base, the unprotonated amine ($pK_a \approx 8.9$) should have about 10^3 times the concentration of unprotonated amidine ($pK_a \approx 11$–12), but amidines acylate about 10^4 as fast as amines (see ref 43, p 373) and a possible problem arises. Other evidence clearly supports the identity of the products from our reaction as being acylated amines. This question of amine vs. amidine acylation is addressed in detail by P. L. Barker, P. L. Gendler, and H. Rapoport, submitted for publication.

(46) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967). We thank Dr. T. Sweeney of the Division of Medicinal Chemistry, Walter Reed Army Institute of Research, for these data.

(47) Performed by Dr. J. Bartholomew, Laboratory of Chemical Biodynamics, University of California, Berkeley.

(s, 3 H), 2.07 (s, 3 H), 1.33 (t, J = 7 Hz, 3 H). Anal. ($C_{18}H_{22}N_2O_4$) C, H, N.

Ethyl 4-Amino-1,3,5-trimethylpyrrole-2-carboxylate (8, R_2 = C_2H_5; R_4 = NH_2). A solution of the corresponding benzyloxycarbonylamine 8 (R_2 = C_2H_5; R_4 = $C_6H_5CH_2O_2CNH$) in methanol (10 mL/g) and 10% (w/w) of 10% Pd/C was shaken with hydrogen overnight. The mixture was filtered, the catalyst was washed with methanol, and the solvent was evaporated to give a quantitative yield of crude product: TLC (A) R_f 0.13. Sublimation gave pure material in 85% yield: mp 58–59 °C; NMR δ 4.18 (q, J = 7.5 Hz, 2 H), 3.69 (s, 3 H), 2.37 (s, 2 H), 2.11 (s), 2.07 (s, 6 H total). Anal. ($C_{10}H_{16}N_2O_2$) C, H, N. The compound is air sensitive, turning yellow, and has a pK_a of 5.5 (H_2O). The HCl salt was crystallized from ethanol: mp 210–227 °C dec; NMR (Me_2SO-d_6) δ 9.71 (br s, 3 H), 4.21 (q, J = 7 Hz, 2 H), 3.70 (s, 3 H), 2.32, 2.27 (2 s, 6 H), 1.30 (q, J = 7.2 Hz, 3 H). Anal. ($C_{10}H_{17}ClN_2O_2$) C, H, N.

Benzyl 4-Amino-1,3,5-trimethylpyrrole-2-carboxylate (8, R_2 = $C_6H_5CH_2$; R_4 = NH_2). Sodium metal (100 mol %) was dissolved in benzyl alcohol (2500 mol %) to which ethyl 4-amino-1,3,5-trimethylpyrrole-2-carboxylate (100 mol %) was added. The solution was heated at 100 °C (10 mm) for 4 h, after which time the benzyl alcohol was removed by lowering the pressure to 1 mm. The residue was partitioned between ether and water, washed with H_2O (2×), dried (Na_2SO_4), and evaporated, and the residue was sublimed [80 °C (20 μm)] to give a quantiative yield of the benzyl ester, mp 61–62 °C. Anal. ($C_{15}H_{18}N_2O_2$) C, H, N. The preparation of this compound from ethyl 4-carboxy-1,3,5-trimethylpyrrole-2-carboxylate (8, R_2 = C_2H_5; R_4 = CO_2H) can be performed without isolation of any intermediates.

Benzyl 4-Carboxy-1,3,5-trimethylpyrrole-2-carboxylate (8, R_2 = $C_6H_5CH_2$; R_4 = CO_2H). Benzyl alcohol was degassed by distilling a portion at 90 °C (50 μm), to the cooled alcohol was added an NaH/oil dispersion (140 mol %) in portions, followed by ethyl 4-carboxy-1,3,5-trimethylpyrrole-2-carboxylate (100 mol %), and the solution was kept at 90 °C (10 mm) until the transesterification was complete as determined by NMR analysis for the presence of ethyl ester. The reaction product was isolated by evaporating excess benzyl alcohol, dissolving the residue in water, adjusting the pH to 2 with 0.5 M HCl in the cold, extracting with $CHCl_3$, drying (Na_2SO_4), and evaporating the $CHCl_3$. Trituration with hexane to remove mineral oil left a quantitative yield of benzyl ester: mp 182–184 °C; TLC (B) R_f 0.55; NMR δ 9.26 (br, 1 H), 7.6 (s, 5 H), 5.58 (s, 2 H), 3.98 (s, 3 H), 2.65 (s, 3 H), 2.62 (s, 3 H).

Benzyl 4-isocyanato-1,3,5-trimethylpyrrole-2-carboxylate (8, R_2 = $C_6H_5CH_2$; R_4 = NCO) was prepared in the same manner as the analogous isocyanato ethyl ester in 75% yield: mp 64 °C; IR (KBr) 2275, 1681 cm^{-1}; NMR δ 7.27 (s, 5 H), 5.17 (s, 2 H), 3.72 (s, 3 H), 2.21, 2.13 (2 s, 6 H).

Benzyl 4-[(*tert*-Butyloxycarbonyl)amino]-1,3,5-trimethylpyrrole-2-carboxylate (11, R_2 = $C_6H_5CH_2O$; n = 1). To the corresponding 4-isocyanatopyrrole 8 (R_2 = $C_6H_5CH_2$; R_4 = NCO) was added dry *tert*-butyl alcohol (5 mL/mmol) and CuCl (5 mol %), and the mixture was heated for 5 h at 60 °C. The residue was recrystallized from CH_3OH/H_2O to give a 95% yield of product: mp 116–118 °C; NMR δ 7.40 (s, 5 H), 5.65 (s, 1 H), 5.14 (s, 2 H), 3.80 (s, 3 H), 2.20, 2.15 (2 s, 6 H). Anal. ($C_{20}H_{26}N_2O_4$) C, H, N. This compound was also prepared by treating the corresponding amine with *tert*-butyloxycarbonyl azide.

4-[(*tert*-Butyloxycarbonyl)amino]-1,3,5-trimethylpyrrole-2-carboxylic Acid (11, R_2 = OH; n = 1). The corresponding 2-benzyl ester 11 (R_2 = $C_6H_5CH_2O$; n = 1) was dissolved in dioxane, 10% Pd/C (10% w/w) was added, and the mixture was shaken with hydrogen for 12 h: TLC (B) R_f 0.50. The catalyst was removed by filtration and washed with methanol, and the filtrate was evaporated to give crude product, which was used immediately.

Preparation of HOBt Active Esters. General Procedure. To 100 mol % of the pyrrolecarboxylic acid, as a crude residue from hydrogenolysis, and 105 mol % HOBt·H_2O in CH_2Cl_2 (4 mL/mmol) at 0 °C was added TEA (400 mol %). To the resulting solution was added 2-chloro-1-methylpyridinium iodide (110 mol %). The reactions were rapid, 15 min sufficing, and were conveniently followed by TLC (B). The active esters have higher R_f values than the acids, and their spots also develop a red color, given more time, than the acids.

The reaction mixture, usually containing a precipitate, was poured into 0.05 M HCl diluted with CH_2Cl_2, washed with 0.05 M HCl (2×) and H_2O (1×), dried (Na_2SO_4), and evaporated to give crude active ester in quantitative yield contaminated by 1-methylpyridinone (N-CH_3 at δ 3.6). The active esters are stable neat or in CH_2Cl_2 solution (0.1 M) for several weeks in the refrigerator.

HOBt ester of 4-[(*tert*-butyloxycarbonyl)amino]-1,3,5-trimethylpyrrole-2-carboxylic acid (11, R_2 = OBt; n = 1) was prepared as a clear oil according to the general procedure: TLC (B) R_f 0.79; NMR δ 8.05 (m, 1 H), 7.51 (m, 3 H), 5.93 (br, 1 H), 3.80 (s, 3 H), 2.45 (s, 3 H), 2.21 (s, 3 H), 1.51 (s, 9 H).

***N*-(2-Cyanoethyl)-4-(*tert*-butoxycarbonyl)-1,3,5-trimethylpyrrole-2-carboxamide (11, R_2 = $NHCH_2CH_2CN$; n = 1).** The crude active ester (100 mol %) in CH_2Cl_2 (1 M) was treated with excess β-aminopropionitrile (400 mol % freshly prepared from the commercial fumarate salt) and the reaction was followed by TLC (B), product R_f 0.49. The reaction mixture was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$, the organic phase was washed with H_2O (1×), dried (Na_2SO_4), and evaporated, and the residue was crystallized from benzene: mp 180–181 °C; NMR δ 6.12 (br, 1 H), 5.73 (br, 1 H), 3.73, 3.73 (s, t, J = 6.5 Hz, 5 H), 2.68 (t, J = 6.5 Hz, 2 H), 2.17, 2.10 (2 s, 6 H), 1.48 (s, 9 H). Anal. ($C_{16}H_{24}N_4O_3$) C, H, N.

***N*-(3-Amino-3-iminopropyl)-4-[(butyloxycarbonyl)amino]-1,3,5-trimethylpyrrole-2-carboxamide Hydrochloride (11, R_2 = $NHCH_2CH_2C(=NH)NH_2$·HCl; n = 1).** To 100 mol % of the HOBt ester of 4-[(*tert*-butyloxycarbonyl)amino]-2-carboxy-1,3,5-trimethylpyrrole (11, R_2 = OBt; n = 1) in DMF (2 mL/mmol) at ice-bath temperature were added β-aminopropionamidine dihydrochloride (150 mol %) and TEA (150 mol %). After 3 h at room temperature, the DMF was evaporated and the residue was boiled with $CHCl_3$ to remove the TEA salts and then chromatographed on acidic alumina (100:1, w/w, Merck) with ethanol to give a quantitative yield of product: mp 220–222 °C dec; NMR (D_2O) δ 3.73 (t, J = 6.5 Hz, 2 H), 3.54 (s, 3 H), 2.78 (t, J = 6.5 Hz, 2 H), 2.08, 2.03 (2 s, 6 H), 1.50 (s, 9 H); UV λ_{max} 278 nm; FD MS, m/e 339 (37), 338 (100), M^+ 337 (31), 320 (9). Anal. ($C_{16}H_{28}ClN_5O_3$) C, H, N.

***N*-(3-Amino-3-iminopropyl)-4-(formylamino)-1,3,5-trimethylpyrrole-2-carboxamide Hydrochloride (PMD_1·HCl, 7, R_3 = R_5 = CH_3; n = 1).** The corresponding 4-[(*tert*-butyloxycarbonyl)amino]pyrrole 11 (R_2 = 3-aminopropionamidine hydrochloride; n = 1) was dissolved in HCO_2H (97%, 10 mL/g), and the solution was refluxed for 2 h. The solvent was evaporated and the residue was recrystallized from CH_3OH/2-propanol to give a quantitative yield of PMD_1·HCl: mp 225 °C; NMR (D_2O) δ 8.26 (s, 1 H), 7.63 (br, 1 H), 4.15 (t, J = 6.5 Hz, 2 H), 3.60 (s, 3 H), 2.85 (t, J = 6.5 Hz, 2 H), 2.13, 2.08 (2 s, 6 H); UV δ_{max} 275 nm; IR (KBr) 3311, 3049, 1692, 1661, 1623, 1552 cm^{-1}; MS, m/e 265 (1), 248 (26), 219 (26), 195 (5); FD MS, m/e 266 (100), M^+ 265 (58), 248 (33); TLC (HAL C) R_f 0.81. Anal. ($C_{12}H_{20}ClN_5O_2$) C, H, Cl, N.

***N*-[5-(Benzyloxycarbonyl)-1,2,4-trimethylpyrrol-3-yl]-4-[(*tert*-butyloxycarbonyl)amino]-1,3,5-trimethylpyrrole-2-carboxamide (11, R_2 = $C_6H_5CH_2O$; n = 2).** To 100 mol of each of the corresponding HOBt active ester 11 (R_2 = OBt; n =1) and pyrrole amine 8 (R_2 = $C_6H_5CH_2$; R_4 = NH_2) in dry DMF (5 mL/mmol) was added glacial HOAc (5 mol %). The mixture was stored at 20 °C for 2 days, followed by 40 °C overnight. Addition of CH_3OH/H_2O (2:1; (100 mL/mmol) to the DMF solution gave amide in 70% yield: TLC (B) R_f 0.58; mp 200–202 °C dec; NMR δ 7.47 (s, 5 H), 6.75 (br, 1 H), 5.83 (br, 1 H), 5.40 (s, 2 H), 3.92 (s, 3 H), 3.82 (s, 3 H), 2.37, 2.32, 2.25 (3 s, 1:1:2, 12 H), 1.60 (s, 9 H); MS, m/e M^+ 508 (0.5), 436 (2), 434 (57), 408 (9), 284 (5), 257 (9), 193 (5), 177 (100). Anal. ($C_{28}H_{36}N_4O_5$) C, H, N.

***N*-(5-Carboxy-1,2,4-trimethylpyrrol-3-yl)-4-[(*tert*-butyloxycarbonyl)amino]-1,3,5-trimethylpyrrole-2-carboxamide (R_2 = OH; n = 2).** The corresponding 2-(benzyloxycarbonyl)pyrrole 11 (R_2 = $C_6H_5CH_2O$; n = 2), 10% Pd/C (10% w/w), and a 2:1 mixture of dioxane/ethanol (6–7 mL/mmol) were refluxed briefly to effect solution, and the cooled solution was shaken with hydrogen for 4 h. The solution was filtered, the catalyst was washed, and the solvent was evaporated to give a quantitative yield of acid.

HOBt Ester of 11 (R = OH; n = 2) was prepared according to the general procedure and obtained as a clear oil: TLC (B) R_f 0.51; NMR δ 8.03 (m, 1 H), 7.47 (m, 3 H), 6.87 (br, 1 H), 5.85 (br, 1 H), 3.85, 3.75 (2 s, 6 H), 2.47 (s, 3 H), 2.32 (s, 3 H), 2.23 (s, 3 H), 2.13 (s, 3 H), 1.50 (s, 9 H).

***N*-[5-[[(3-Amino-3-iminopropyl)amino]carbonyl]-1,2,4-trimethylpyrrol-3-yl]-4-[(*tert*-butyloxycarbonyl)amino]-1,3,5-trimethylpyrrole-2-carboxamide hydrochloride (11, R = $NHCH_2CH_2C(=NH)NH_2 \cdot HCl$; n = 2)** was prepared in an analogous manner to 11 ($R_2 = HNCH_2CH_2C(=NH)NH_2 \cdot HCl$; n = 1): mp 250 °C dec from CH_3OH/isopropanol; NMR (D_2O) δ 3.77 (t, J = 6 Hz), 3.57 (s), 2.82 (t, J = 6 Hz, 2 H), 2.17, 2.13, 2.08 (3 s, 1:2:1, 12 H), 1.52 (s, 9 H); UV λ_{max} 282 nm (ϵ 21 000), sh 247 (9660). Anal. ($C_{24}H_{38}ClN_7O_4$) H, N; C: calcd, 55.0; found, 55.5.

***N*-[5-[[(3-Amino-3-iminopropyl)amino]carbonyl]-1,2,4-trimethylpyrrol-3-yl]-4-(formylamino)-1,3,5-trimethylpyrrole-2-carboxamide hydrochloride ($PMD_2 \cdot HCl$, 7, $R_3 = R_5 = CH_3$; n = 2)** was prepared in the same manner as $PMD_1 \cdot HCl$, except that the residue from evaporation of the formic acid was dissolved in boiling methanol and filtered; evaporation of the methanol left pure material in quantitative yield: mp 245 °C dec; UV λ_{max} 280 nm (ϵ 27 000), sh 240 (12 400); NMR (D_2O) δ 8.27 (s, 1 H), 3.79 (t, J = 6 Hz), 3.62 (s), 2.83 (t, J = 6 Hz, 2 H), 2.20, 2.17, 2.12 (3 s, 1:2:1, 12 H). Anal. ($C_{20}H_{30}ClN_7O_3$) C, H, N.

***N*-[5-(Benzyloxycarbonyl)-1,2,4-trimethylpyrrol-3-yl]-4-[[[4-[(*tert*-butyloxycarbonyl)amino]-1,3,5-trimethylpyrrol-2-yl]carbonyl]amino]-1,3,5-trimethylpyrrole-2-carboxamide (11, $R_2 = C_6H_5CH_2O$; n = 3)** was prepared in the same manner as the corresponding compound 11 ($R_2 = C_6H_5CH_2O$; n = 2), except that the reaction was in DMF (0.5 M solution) with 4-aminopyrrole 8 ($R_2 = C_6H_5CH_2$; $R_4 = NH_2$) in slight excess (105 mol %) and 1 mol % HOAc for 48 h at 20 °C and then for 24 h at 40 °C. The residue from evaporation of the DMF was boiled with ethanol (20 mL/mmol), cooled, and filtered to give product in 85% yield: mp 250–252 °C dec; TLC (B) R_f 0.37; NMR (TFA) δ 8.42 (br, 4 H), 7.45 (s, 5 H), 5.47 (s, 2 H), 3.88, 3.85, 3.82 (3 s, 9 H), 2.52, 2.47, 2.42, 2.37, 2.32 (5 s, 18 H), 1.67 (s, 9 H). Anal. ($C_{36}H_{46}N_6O_6$) C, H, N.

***N*-(5-Carboxy-1,2,4-trimethylpyrrol-3-yl)-4-[[[4-[(*tert*-butyloxycarbonyl)amino]-1,3,5-trimethylpyrrol-2-yl]carbonyl]amino]-1,3,5-trimethylpyrrole-2-carboxamide (11, R_2 = OH; n = 3).** The benzyl ester 11 (n = 3) was hydrogenolyzed in a similar manner to benzyl esters 11 (n = 1, 2), except for modifications due to the insolubility when n = 3. Dioxane/CH_3OH (25 mL, 1:1) and 0.1 mmol of 11 ($R_2 = C_6H_5CH_2O$; n = 3) were refluxed (24 h) until solution was effected; then 30% Pd/C (10%, w/w) was added and the mixture shaken with hydrogen for 2 h. Similar isolation as above gave quantitative yield of acid.

HOBt ester of 11 (R_2 = OH; n = 3) was prepared according to the general procedure, except that active ester formation was allowed to proceed for 30 min: TLC (B) R_f 0.22; NMR δ 8.05 (m, 1 H), 7.43 (s, 3 H), 6.91 (s, 1 H), 6.77 (s, 1 H), 5.85 (s, 1 H), 3.78, 3.74, 3.70 (3 s, 9 H), 2.45 (s, 3 H), 2.32, 2.27, 2.22, 2.17, 2.13 (5 s, 15 H), 1.50 (s, 9 H).

***N*-[5-[[(3-Amino-3-iminopropyl)amino]carbonyl]-1,2,4-trimethylpyrrol-3-yl]-4-[[[4-[(butyloxycarbonyl)amino]-1,3,5-trimethylpyrrol-2-yl]carbonyl]amino]-1,3,5-trimethylpyrrole-2-carboxamide hydrochloride (11; $R_2 = HNCH_2CH_2C(=NH)NH_2 \cdot HCl$; n = 3)** was prepared in the same manner as the corresponding compound 11 ($R_2 = HNCH_2CH_2C(=NH)NH_2 \cdot HCl$; n = 1), except that the compound with n = 3 could not be dissolved for chromatography. Purification was effected by refluxing the DMF residue with ethanol (25 mL/mmol), cooling, and filtering, which gave quantitative yield of product: TLC (CH_3OH/NAL) R_f 0.50; mp decomposes up to 275 °C; UV λ_{max} 284 nm (ϵ 42 700), sh 250 (19 600); NMR (TFA) δ 8.42 (m), 8.27 (br s), 7.92 (br), 7.72 (br, 8 H, total), 4.07 (m), 3.79 (br, 11 H, total), 3.70 (br, 2 H), 2.50, 2.34, 2.27 (3 s, 18 H), 1.66 (s, 9 H). Anal. ($C_{32}H_{48}ClN_9O_5 \cdot 0.5H_2O$) C, H, N.

***N*-[5-[[(3-Amino-3-iminopropyl)amino]carbonyl]-1,2,4-trimethylpyrrol-3-yl]-4-[[[4-(formylamino)-1,3,5-trimethylpyrrol-2-yl]carbonyl]amino]-1,3,5-trimethylpyrrole-2-carboxamide hydrochloride ($PMD_3 \cdot HCl$, 7, $R_3 = R_5 = CH_3$; n = 3)** was prepared in the same manner as the corresponding compound 7 ($R_3 = R_5 = CH_3$; n = 1), except that the formic acid residue was not totally soluble in methanol. Several crops were obtained from methanol or by trituration with hot ethanol to give a quantitative yield of product: TLC (CH_3OH/HAL) R_f 0.75 (streak); mp 248–254 °C dec with gradual darkening >200 °C; NMR (TFA) δ 8.55 (br, 1 H), 8.13 (s), 8.13 (m), 7.90 (br), 7.73 (br, 8 H, total), 4.07 (m, 2 H), 3.81 (br, 9 H), 3.13 (br 2 H), 2.38 (m), 2.30 (s, 18 H, total); UV λ_{max} 284 nm (ϵ 40 000), sh 240 (18 600); IR (KBr) 3205, 2899, 1669, 1626, 1575, 1531, 1479, 1425, 1376, 1282, 1136, 935, 752, 725 cm^{-1}. FD MS, m/e M + 1 566 (100), 548 (22), 495 (14). Anal. ($C_{28}H_{40}ClN_9O_4$) C, H; N: calcd, 20.9; found, 20.5.

Ethyl 4-(formylamino)-1,3,5-trimethylpyrrole-2-carboxylate (8, $R_2 = C_2H_5$; R_4 = HCONH) was readily prepared by the action of hot formic acid on the corresponding 4-isocyanatopyrrole 8 ($R_2 = C_2H_5$; R_4 = NCO) either as a crude oil or directly in the reaction mixture in which the isocyanate was formed. It was also readily available by formylation, via a variety of agents, of the 4-aminopyrrole 8 ($R_2 = C_2H_5$; $R_4 = NH_2$): mp 155–156 °C, resolidifying, mp 167–168 °C (from benzene); NMR δ 7.20 (br, 1 H), 4.30 (q, J = 7.5 Hz), 4.27 (q, J = 7.5 Hz, 2 H, total), 3.80, 3.75 (2 s, 3 H), 2.21, 2.19, 2.10 (3 s, 1:2:1), 6 H), 1.35 (t, J = 7.5 Hz), 1.32 (t, J = 7.5 Hz, 3 H, total); IR (KBr) 3257, 1689, 1664 cm^{-1}. Anal. ($C_{11}H_{16}N_2O_3$) C, H, N.

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