the general procedure as a yellow oil, which, after chromatography, was slurried with ether to afford a white solid: mp 163–164 °C; 50% yield.

Anal. Calcd for $C_{15}H_{13}F_3NOP$: C, 57.88; H, 4.21; N, 4.50. Found: C, 57.77; H, 4.19; N, 4.47.

1-Methyl-2-(4-bromophenyl)-2,3-dihydro-1*H*-benzazaphosphole 1-oxide (1e) was prepared according to the general procedure in essentially quantitative yield as white crystals (CCl_4–ether–ligroin): mp 135–136 °C; mass spectrum, *m/e* (relative intensity) 321 (100), 323 (91), 306 (76), 308 (70), 180 (40), 121 (42).

Anal. Calcd for $C_{14}H_{13}BrNOP$: C, 52.21; H, 4.04; N, 4.35. Found: C, 52.10; H, 4.08; N, 4.32.

1-Methyl-2-(4-phenoxyphenyl)-2,3-dihydro-1*H*-2,1-benzazaphosphole 1-oxide (1g) was prepared according to the general procedure as a white, crystalline solid: 40% yield; mp 176–177 °C; mass spectrum, *m/e* (relative intensity) 355 (100), 320 (30).

1-Methyl-2-(2-methoxyphenyl)-2,3-dihydro-1*H*-2,1-benzazaphosphole 1-oxide (1h) was prepared according to the general procedure in 75% yield as an uncrystallizable yellow glass: mass spectrum, *m/e* (relative intensity) 273 (32), 258 (24), 113 (28), 108 (22), 84 (100), 86 (69).

***P*-(α-Chloro-2-tolyl)-*P*-methylphosphinamide (19).** A four-necked, round-bottom flask equipped with an addition funnel, a gas inlet, a mechanical stirrer, and a cold finger condenser was dried with a hot gun under a N_2 atmosphere. Ammonia (~25 mL) was condensed at the cold finger, and 100 mL of precooled anhyd ether was added. The phosphinyl chloride (**16**) was added dropwise over a period of 15 min under a N_2 atmosphere. After the mixture was stirred an additional 0.5 h, the cold finger was removed, and the system was swept with N_2. Sunction filtration yielded a white solid which was extracted with ethyl acetate in a Soxhlet extractor. The ethyl acetate solution was cooled in an ice bath to yield white plates in several crops: 6.2 g (62% yield); mp 137–138 °C; NMR (Me_2SO-d_6) δ 1.55 (d, 14, 3, *J* = 14 Hz), 4.7 (br s, 2), 5.2 (AB pattern, 2, *J* = 11 Hz), 7.4–8.1 (m, 4); mass spectrum, *m/e* (relative intensity) 205 (17), 203 (45), 152 (42), 151 (100), 143 (11).

Anal. Calcd for $C_8H_{11}ClNOP$: C, 47.19; H, 5.45; Cl, 17.41; N, 6.88. Found: C, 46.95; H, 5.54; Cl, 17.29; N, 6.81.

Registry No. **1a**, 78089-50-6; **1b**, 78089-51-7; **1c**, 78089-52-8; **1d**, 78089-53-9; **1e**, 78089-54-0; **1f**, 78089-55-1; **1g**, 78089-56-2; **1h**, 78089-57-3; **8a**, 78089-58-4; **8b**, 78108-43-7; **8c**, 78108-44-8; **9a**, 78089-59-5; **9b**, 78089-60-8; **9c**, 78089-61-9; **10**, 78089-62-0; **11**, 78108-45-9; **12**, 78089-63-1; **13**, 615-37-2; **14**, 61820-30-2; **15**, 78089-64-2; **16**, 78089-65-3; **17**, 78089-66-4; **18a**, 78089-67-5; **18b**, 78089-68-6; **18c**, 78089-69-7; **18d**, 78089-70-0; **18e**, 78089-71-1; **18f**, 78089-72-2; **18g**, 78108-46-0; **18h**, 78108-47-1; **19**, 78089-73-3; formic acid, 64-18-6; acetic anhydride, 108-24-7; trifluoroacetic anhydride, 407-25-0; diethyl methylphosphonite, 15715-41-0; *o*-iodobenzylamine, 39959-51-8; benzenamine, 62-53-3; 4-methoxybenzenamine, 104-94-9; 3,4-dichlorobenzenamine, 95-76-1; 3-(trifluoromethyl)benzenamine, 98-16-8; 4-bromobenzenamine, 106-40-1; 4-phenoxybenzenamine, 139-59-3; 2-methoxybenzenamine, 90-04-0; 2,6-dimethylbenzenamine, 87-62-7; *N*-phenyl-*p*-(α,α-dichloro-2-tolyl)-*p*-methylphosphinamide, 78089-74-4.

Novel Efficient Total Synthesis of Antiviral Antibiotic Distamycin A

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In connection with an attempt to design a flexible synthesis of analogues of distamycin A (**14**) for a structure–activity study, by starting from 4-[[(*tert*-butyloxy)carbonyl]amino]-1-methylpyrrole-2-carboxylic acid (**3**) and the corresponding formyl derivative (**9**), the distamycin A precursor **12** was prepared. The versatility of **12** is demonstrated by direct attachment, after activation, of preformed β-aminopropionamidine dihydrobromide to give **14** in fair yield. We conclude that N- and/or ring-substituted derivatives of **3** and **9** may lead to the corresponding analogues of **12** and thus serve as useful precursors, to which the amino amidine or derivatives thereof can be attached. After hydrogenation of the corresponding nitro compound, **3** and **9** were prepared with (*tert*-butyloxy)carbonyl fluoride and formic anhydride, respectively. Amide bond formations were accomplished with carbodiimides, occasionally via intermediary active esters (**8**, **13**).

Distamycin A (**14**) is an antibiotic with pronounced antiviral and oncolytic properties. It probably exerts its action by binding to A-T rich regions in DNA. Some aspects of the chemistry and biology of this compound have been reviewed recently.[1-4] The substance was isolated by extracting the fermentation broth of *Streptomyces distallicus*, which also contained other antibiotics of similar structure.[1,5,6] The structure of **14** was established from spectroscopic studies of the parent compound and some of its degradation products and proved by synthesis.[5,7] Since there are relatively few antiviral drugs with favorable therapeutic indices available today, distamycin A and selected analogues have received considerable attention, and this has brought about the development of improved modifications of the original synthesis.[8,9] Although several derivatives of **14** have been prepared and screened for their biological activities, the structural modifications have been largely restricted to the N-terminal acyl group and, preferentially, the C-terminal amidine moiety.[1,10,11] A few derivatives containing *N*-propylpyrrole residues have, however, been synthesized and examined with respect to DNA-binding properties.[12-15]

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It is interesting to note that the cytotoxicity, which is characteristic of compounds with high affinity for DNA, can apparently be separated from the antiviral activity by increasing the number of pyrrole residues in the molecule.[16]

This paper presents a novel approach to the synthesis of distamycin A, characterized by direct application of stable versatile derivatives of the unstable monomer 4-amino-1-methylpyrrole-2-carboxylic acid (2) as well as of the preformed amino amidine side chain. Further it relies heavily on the use of some mild and efficient reagents and procedures, hitherto mainly utilized in the field of peptide synthesis. One advantage of this strategy is that powerful reagents such as hydrogen chloride and ammonia are eliminated from steps involving pyrroles, thus allowing the incorporation of sensitive fragments into the molecule. Moreover, it can also be employed in the preparation of analogues with unsaturated and/or sulfur-containing amino amidine residues and is presently being applied in the synthesis of a number of distamycin A analogues as part of a structure–activity study now in progress in this laboratory.

Results and Discussion

As starting material, 1-methyl-4-nitropyrrole-2-carboxylic acid (1) was selected. It was previously prepared by a relatively laborious five-step route, starting from furane-2-carboxylic acid.[8,12] The disadvantages of this old route have now been circumvented by a simplified and more efficient procedure starting from the corresponding crude ethyl ester of 1 as obtained by nitration of ethyl 1-methylpyrrole-2-carboxylate.[17]

Catalytic hydrogenation of 1 was effected under standard conditions. It should, however, be pointed out here that this reaction proceeded more rapidly in aqueous medium than in either ethanol or dimethylformamide (DMF). The resulting unstable air-sensitive amino acid 2 (Scheme I) was directly converted into the stable carboxylic acid 3 in high yield. Relatively little information on (*tert*-butyloxy)carbonyl (Boc) derivatives of aromatic amino compounds is available in the literature. The reduced nucleophilicity of such species in comparison with that of ordinary amino acids may limit the use of some reagents otherwise applied in the preparation of Boc amino acids, and it has been claimed that 4-[[(*tert*-butyloxy)-carbonyl]amino]benzoic acid is rather difficult to synthesize by standard methods.[18] Since this compound, however, was readily obtained from *p*-aminobenzoic acid in good yield by using (*tert*-butyloxy)carbonyl fluoride under aqueous conditions, we decided to use the latter reagent for conversion of 2 into 3, which was obtained in high yield with only traces of impurities present in the crude product. A more general use of BocF was previously limited by difficulties in its preparation, which requires access to carbonyl chloride fluoride and carefully controlled conditions.[19] It can also be mentioned that in our hands the use of aqueous sodium hydroxide in place of sodium carbonate in this hydrogenation and subsequent acylation gave a significantly lower yield of a darker, less pure product. Purified 3 is a well-defined white solid, which like normal Boc amino acids can be stored at room temperature for months without any detectable deterioration, making it a convenient precursor for synthesis of different oligomers.

Due to the presence of the Boc group, acid conditions were, in principle, precluded in the esterification of 3. As expected, however, it was found that 3 could be smoothly converted into its benzyl ester 5 in essentially quantitative yield by treating the cesium salt 4 with benzyl bromide in anhydrous DMF at ambient temperature.[20] The benzyl ester 5 is also a stable compound, which can be handled without any special precautions.

The protecting Boc group in 5 was conveniently cleaved off according to a standard procedure with trifluoroacetic acid (TFA) at room temperature. Many pyrrole derivatives are notoriously acid labile, especially compounds containing electron-donating substituents. In this case, however, the resulting amino analogue 6 was formed in satisfactory yield with very little decomposition, provided air was excluded during workup.

The carbodiimide-mediated condensation of 3 and 6 to 7 was performed in DMF, and the yield was satisfactory, especially taking into account the reduced nucleophilicity of the amino group in 6 compared to that of aliphatic amines. The reactivity of 1-ethyl-3-[3-(dimethylamino)-propyl] carbodiimide hydrochloride (EDC) did not seem to be inferior to *N,N'*-dicyclohexylcarbodiimide (DCC) in this coupling, and the workup of the reaction mixture was much simplified. Amide 7 could also be prepared in excellent yield by reaction of active ester 8 with 6 in dry dimethyl sulfoxide (Me_2SO) in the presence of 2 equiv of tetramethylguanidine (TMG). The 1-hydroxy-1,2,3-benzotriazole ester 8 was readily synthesized from equimolar amounts of 3 and 1-hydroxy-1,2,3-benzotriazole (HOBT) by employing DCC as coupling reagent. Careful examination of the crude reaction mixture in this case revealed the presence of an impurity (~1%), and it was possible to isolate small amounts of this compound by chromatographic methods. Spectroscopic properties suggest that this yellow compound may be the 3-*N*-acyl isomer of 8.[21] In this context it is also worth mentioning that, in contrast to the behavior of 8, the corresponding *N*-hydroxysuccinimide ester of 3 does not appear to possess sufficient reactivity to acylate 6. Thus, when equimolar amounts of 3 and 6 were allowed to react with a slight excess of DCC in dichloromethane in the presence of 1 equiv of *N*-hydroxysuccinimide (HOSu) for 20 h, a complex mixture was formed. From this crude mixture substantial quantities of the *N*-hydroxysuccinimide ester of 3 could be isolated. When the corresponding reaction was conducted in DMF and HOBT was used in place of HOSu, the desired product 7 was obtained, but the yield was considerably increased when HOBT was omitted from the reaction mixture.

In the hitherto described preparations of distamycin A the N-terminal amino group was formylated in the last step of the synthetic routes, and the yields in this reaction were not very impressive. Therefore, methods were sought of accomplishing the formylation at an early stage of the synthesis, and formyl derivative 9 was prepared. The condensation of 9 with amine 10 using EDC furnished the protected trimer 11 in reasonable yield. This derivative was largely insoluble in all ordinary organic solvents except DMF and Me_2SO, and this feature greatly facilitated the

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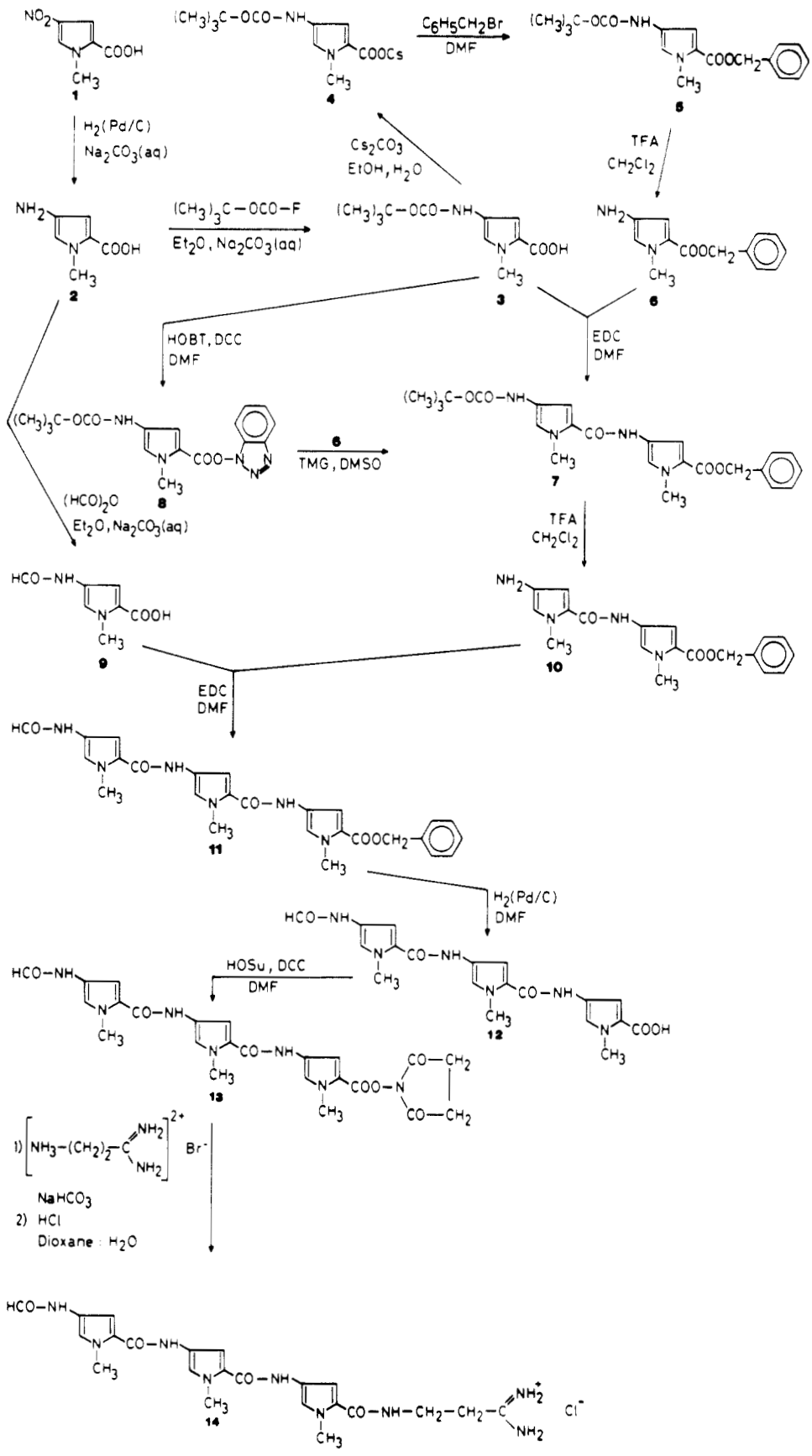
Scheme I



workup, since the more soluble contaminants present in the crude product could simply be washed away. The amine component **10** was conveniently obtained from **7** by using the method of deprotection described above for the conversion of **5** into **6**. Acid **9**, mentioned above, was prepared by direct formylation of **2** under aqueous conditions. Preliminary experiments using mixed formic acetic anhydride as the formylating agent showed that acetylation of **2** occurred to a considerable extent. In fact, the resulting crude product consisted of equimolar amounts of **9** and the corresponding acetyl analogue. Consequently, the yield of pure **9** was rather low. This

obstacle was, however, overcome by employing formic anhydride instead in this reaction. Formic anhydride is an unstable species and cannot be isolated in a pure state. This elusive compound which can only be handled in solution at relatively low temperature has not been satisfactorily characterized until recently.[22] It was prepared from formic acid with thionyl chloride in the presence of pyridine, and this method was employed in our case with good results. Since the use of formic anhydride eliminates the possibility of acetylation, it appears to be a safer reagent of wide applicability. Apparently, the potential of this convenient formylation procedure remains to be explored.

Catalytic hydrogenation of 11 in the presence of palladium on carbon with DMF as solvent afforded 12 in high yield and purity. Often the formyl group is rather sensitive to catalytic hydrogenation.[23] It was therefore satisfactory that the formyl group in 11 resisted these reaction conditions and remained largely unaffected. The product 12 was converted into the active ester 13 by using a standard procedure. This ester was stable provided that moisture was excluded.

The final incorporation of the amidine side chain in the molecule constituted a crucial step in the synthesis of 14 and presented certain difficulties at the outset. Thus, when an equimolar mixture of 12 and β-aminopropionamidine dihydrobromide in dry DMF was treated with DCC in the presence of triethylamine, very little of 14 was produced as judged from TLC on the crude mixture. Instead a new substance appeared in the mixture, and ^{1}H NMR of a purified sample indicated that it was probably the *N*-acyldicyclohexylurea analogue of 12. When the active ester 13 was used in place of 12 and carbodiimide in the same solvent and with the same base, TLC showed that minor amounts of 14 were produced, but it was severely contaminated with several other substances. The same impurities were produced, but to a lower degree, when dry pyridine was utilized as the solvent, with or without the versatile acylation catalyst 4-(dimethylamino)pyridine.[24] It was finally discovered that the reaction of 13 with β-aminopropionamidine dihydrobromide proceeded satisfactorily in dioxane–water in the presence of sodium bicarbonate and that a surprisingly small amount of side products was formed. Considerable amounts of 13 still remained unreacted, however, but this was partly remedied by using an excess of the amidine in the coupling. Working under anhydrous conditions with DMF as solvent was not encouraging since in this case too, a variety of side products occurred in the crude mixture. With our best procedure the yield of purified 14 did not exceed 40%. Continued efforts will therefore be made to optimize this reaction step. Synthetic 14 as obtained by this route was in all respects identical with an authentic sample of distamycin A.

Experimental Section

All melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. TLC analyses were performed on 0.25-mm-thick precoated UV-sensitive silica gel plates (Merck DC-Fertigplatten Kieselgel 60 F_{254}), generally in (A) $CHCl_3$–EtOH–H_2O,100:50:4 (v/v/v) or (B) CH_2Cl_2–acetone, 4:1 (v/v). Spots were visualized by inspection under UV light at 254 nm or, preferentially, after exposure to vaporized I_2. All compounds described were chromatographically pure unless otherwise stated. In the work with the sensitive amino derivatives 2, 6, and 10, solvents and liquid reagents were flushed with nitrogen before use. ^{1}H NMR spectra were obtained on a JEOL FX 60 instrument at 59.8 MHz, and shifts are given in parts per million relative to tetramethylsilane. The mass spectra were recorded on a LKB 9000 gas chromatograph–mass spectrometer at the lowest possible volatilization temperature. In all cases the mass spectra were compatible with the proposed structures, and the molecular ion peaks are given in the appropriate cases. Elemental analyses were performed by the Analytical Department, Institute of Chemistry, University of Uppsala, and by the Swedish University of Agricultural Sciences, Uppsala.

1-Methyl-4-nitropyrrole-2-carboxylic acid (1). Simplified Procedure. Crude ethyl 1-methyl-4-nitropyrrole-2-carboxylate[17] (83.0 g, 0.419 mol) in 2 M aqueous sodium hydroxide–EtOH (10:1, ~900 mL) was refluxed for 1.5 h, and simultaneously most of the ethanol was allowed to evaporate. The dark reaction mixture was cooled and filtered through glass wool to remove insoluble contaminants. The filtrate was extracted with CH_2Cl_2 (3 × 100 mL, extracts discarded). The aqueous phase was cooled in ice, cautiously acidified with concentrated HCl, and left in a cool place for a few hours. The precipitated acid was collected and rinsed with small portions of ice–water. The moist product was recrystallized from water (~1 L, decolorizing carbon). Pure 1 was obtained as small tan needles. The yield of dry acid was 49.1 g (69%). Workup of the filtrate and mother liquor only afforded about 5 g of a darker, less pure product which was discarded: mp 201–201.5 °C (lit.[25] mp 200 °C); R_f^A 0.25; mass spectrum (70 eV), *m/e* 170 (M^+); ^{1}H NMR (acetone-d_6) δ 8.00 (m, 1 H), 7.35 (d, 1 H), 4.04 (d, 3 H), $J_{3,5}$ = 2.1 Hz, $J_{5,1\text{-}CH_3}$ = 0.5 Hz.

(*tert*-Butyloxy)carbonyl Fluoride. An ethereal solution containing 8–10% of (*tert*-butyloxy)carbonyl fluoride was prepared essentially by analogy with a previously described procedure for [(2-phenyl isopropyl)oxy]carbonyl fluoride.[19] The content of reagent was conveniently measured by ^{1}H NMR (~10% solution in acetone-d_6). Integration was performed over the (CH_3)$_3$C signal of (*tert*-butyloxy)carbonyl fluoride (δ 1.54, d, $J_{H,F}$ = 1.47 Hz) and the CH_2 signal of diethyl ether (δ 3.41, q, *J* = 7.06 Hz).

4-[[(*tert*-Butyloxy)carbonyl]amino]-1-methylpyrrole-2-carboxylic Acid (3). Recrystallized 1 (8.50 g, 50 mmol), dissolved in 1.0 M aqueous sodium carbonate (150 mL), was hydrogenated in a Parr apparatus at 60 psi of H_2 at room temperature over a Pd catalyst (5% on carbon) until the hydrogen absorption ceased (3–4 h). The catalyst was filtered off in a stream of nitrogen. The light yellow filtrate containing the unstable amino acid 2 was cooled in ice under a blanket of nitrogen, and tetrahydrofurane (50 mL) was added, followed, dropwise, for 1 h by a solution of (*tert*-butyloxy)carbonyl fluoride in ether (120 mL, 8% w/v, 80 mmol) under vigorous stirring and cooling. The resulting slurry was stirred for additional 2 h at ambient temperature, the colorless ether layer was then separated, and the yellow aqueous phase, which should be distinctly alkaline (pH 9–10), was extracted with ether (2 × 100 mL, extracts discarded). The aqueous phase was then cautiously acidified with solid $KHSO_4$ (pH 3–4) and extracted with ether (3 × 100 mL), and the combined yellow extracts were washed with 1M aqueous KHSO4 until washings essentially colorless (3 × 100 mL), water (2 × 100 mL), and brine (3 × 100 mL). After being dried ($MgSO_4$) and treated with a spoonful of decolorizing carbon, this extract was run through a short silica gel column (5 × 5 cm), which was washed with dry ether as long as the eluate remained colorless. On evaporation almost pure 3 remained as a white, solid, crisp foam. The yield of crude product was 9.12 g (76%), which was sufficiently pure for synthesis, but contained trace amounts of an impurity with R_f^A 0.45 (blue fluorescence at 254 nm). This could be removed by chromatography on a silica gel column with dry ether as the mobile phase. The analytical specimen was obtained by dissolving the chromatographed material in a small volume of dry ether, followed by addition of this to a large volume of cold pentane under rapid stirring, whereupon the product precipitated as a white powder. After being filtered and washed with small portions of cold pentane, it was dried over paraffin: mp 151–151.5 °C dec; R_f^A 0.64; mass spectrum (70 eV), *m/e* 240 (M^+); ^{1}H NMR (acetone-d_6)

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δ 8.14 (br s, ~1 H), 7.11 (d, 1 H), 6.80 (d, 1 H), 3.87 (s, 3 H), 1.46 (s, 9 H), $J_{3,5}$ = 2.1 Hz.

Anal. Calcd for $C_{11}H_{16}N_2O_4$: C, 55.0; H, 6.7; N, 11.7. Found: C, 55.2; H, 6.7; N, 11.5.

Benzyl 4-[[(*tert*-Butyloxy)carbonyl]amino]-1-methylpyrrole-2-carboxylate (5). (a) Cesium Salt 4. A solution of **3** (14.76 g, 61.5 mmol) in EtOH–water (3:1 v/v, 300 mL) was treated dropwise under stirring with a solution of Cs_2CO_3 (10.03 g, 30.8 mmol) in water (75 mL). After being filtered, the solution was taken to complete dryness in vacuo (below 60 °C), giving a semisolid residue, which was dissolved in 99% EtOH (200 mL), and this solution was again taken to dryness. The EtOH procedure was repeated twice, giving, after being dried in vacuo at 60 °C for several h, **4** as a solid pale yellow foam, suitable for preparation of **5**.

(b) Benzyl Ester 5. The crude **4** was dissolved in dry DMF (150 mL), and the clear well-stirred solution was immediately treated dropwise with benzyl bromide (10.6 g, 62.0 mmol). When allowed to stand, this salt solution sometimes formed a jelly which was difficult to handle. During the addition the reaction mixture became lukewarm, and a precipitate appeared. The resulting slurry was stirred at 40 °C overnight and then quenched in ice–water (~1 L), giving a precipitate that was collected, rinsed with plenty of water, and dried in vacuo over P_2O_5. The dry material was triturated with cold petroleum ether, and the insoluble part was washed with small portions of cold petroleum ether until the pungent smell of benzyl bromide was hardly detectable. The resulting crude benzyl ester (20.1 g, 99%) was sufficiently pure and could be used directly for synthesis. An analytical specimen could be obtained as white, shiny crystals by successive recrystallizations from EtOH (8 mL/g, decolorizing carbon) and cyclohexane (70 mL/g): mp 143–143.5 °C; R_f^B 0.65; mass spectrum (70 eV), *m/e* 330 (M^+); ^1H NMR (acetone-d_6) δ 8.14 (br s, ~1 H), 7.39 (perturbed s, 5 H), 7.13 (d, 1 H), 6.83 (d, 1 H), 5.26 (s, 2 H), 3.88 (s, 3 H), 1.45 (s, 9 H).

Anal. Calcd for $C_{18}H_{22}N_2O_4$: C, 65.4; H, 6.7; N, 8.5. Found: C, 65.7; H, 6.8; N, 8.2.

1,2,3-Benzotriazol-1-yl 4-[[(*tert*-Butyloxy)carbonyl]amino]-1-methylpyrrole-2-carboxylate (8). To a rapidly stirred mixture of **3** (2.40 g, 10 mmol) and 1-hydroxy-1,2,3-benzotriazole (1.26 g, 10.1 mmol) in dry DMF (50 mL) was added DCC (2.20 g, 10.7 mmol) in small portions at ambient temperature. After the mixture was stirred for 20 h, the precipitated urea was filtered off and the yellow solution poured into ice–water (~1 L). After a few hours in a refrigerator the resulting fine-grained precipitate was collected by filtration and rinsed with abundant water. After air-drying, the crude material was dissolved in CH_2Cl_2 (20 mL), and the insoluble part was filtered off. The filtrate was chromatographed on a silica gel column with CH_2Cl_2–acetone 9:1 (v/v) as the mobile phase, giving pure **8** as a colorless, slightly sticky mass. It was dissolved in CH_2Cl_2 (15 mL) and slowly added to a large volume (~600 mL) of cold pentane under vigorous stirring. The fine-grained white precipitate was collected, washed with pentane, and dried. The yield was 2.52 g (71%). An analytical sample was obtained as white lustrous flakes from CH_2Cl_2–petroleum ether (1:4 v/v, 80 mL/g): mp 138–138.5 °C; R_f^B 0.60; mass spectrum (70 eV), *m/e* 357 (M^+); ^1H NMR (acetone-d_6) δ 8.49 (br s, ~1 H), 7.4–8.2 (m, 5 H), 7.33 (d, 1 H), 3.94 (s, 3 H), 1.50 (s, 9 H), $J_{3,5}$ = 1.9 Hz.

Anal. Calcd for $C_{17}H_{19}N_5O_4$: C, 57.1; H, 5.4; N, 19.6. Found: C, 57.2; H, 5.4; N, 19.4.

Continued elution afforded a yellow fraction (~15 mg), which was recrystallized from CH_2Cl_2–petroleum ether 1:5 (v/v, 1 mL/mg): mp 175–177 °C dec; R_f^B 0.49; mass spectrum (70 eV), *m/e* 357 (M^+); ^1H NMR (acetone-d_6) δ 8.44 (br s, 1 H), 7.47–7.95 (m, 6 H), 4.05 (s, 3 H), 1.49 (s, 9 H).

Benzyl 4-[[[4-[[(*tert*-Butyloxy)carbonyl]amino]-1-methylpyrrol-2-yl]carbonyl]amino]-1-methylpyrrole-2-carboxylate (7). (a) Amine Component 6. A solution of **5** (3.63 g, 11.0 mmol) in CH_2Cl_2 (20 mL) was treated (under nitrogen) with TFA (10 mL), the resulting yellowish solution was left at ambient temperature in a stoppered flask for 1 h and then partioned between 30% aqueous K_2CO_3 (100 mL) and CH_2Cl_2 (25 mL), and the lower yellow layer was collected. The aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL), and the combined organic extracts, after being washed with brine (50 mL), were dried over $MgSO_4$. Removal of the solvent left a yellow viscous oil containing **6** together with traces of impurities (R_f^B 0.21). The yield was 2.29 g (91%).

(b) Preparation of 7 by Carbodiimide Coupling. Crude **6** as obtained above was dissolved in DMF (15 mL, previously flushed with nitrogen), and **3** (2.40 g, 10.0 mmol) was added, giving a clear solution. Solid EDC (2.03 g, 10.6 mmol) in small portions was introduced with stirring for 10 min under nitrogen and the brownish mixture allowed to react overnight at 40 °C. It was diluted with ice-cold 1 M aqueous $KHSO_4$ (500 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The yellow-brown extract was washed in turn with 1 M aqueous $KHSO_4$ (3 × 100 mL), 1 M aqueous $NaHCO_3$ (3 × 100 mL), and brine (100 mL), dried over $MgSO_4$, and treated with a spoonful of decolorizing carbon. Evaporation at reduced pressure afforded essentially pure **7** as a solid golden brown foam. The yield of crude product was 3.67 g (80%). An analytical sample was prepared by chromatography on silica gel with CH_2Cl_2–acetone (20:1 v/v) as the mobile phase and subsequent crystallization from CH_2Cl_2–petroleum ether (1:2 v/v, 250 mL/g), giving straw-colored woolly needles: mp 160–160.5 °C dec; R_f^B 0.54; mass spectrum (70 eV), 452 (M^+); ^1H NMR (acetone-d_6) δ 9.21 (br s, 1 H), 8.09 (br s, 1 H), 7.49 (d, 1 H), 7.40 (perturbed s, 5 H), 6.98 (d, 1 H), 6.93 (d, 1 H), 6.80 (d, 1 H), 5.26 (s, 2 H), 3.91 (s, 6 H), 1.45 (s, 9 H), both $J_{3,5}$ = 1.9 Hz.

Anal. Calcd for $C_{24}H_{28}N_4O_5$: C, 63.7; H, 6.2; N, 12.4. Found: C, 63.4; H, 6.2; N, 12.2.

(c) Preparation of 7 from Active Ester 8. A mixture of crude **6**, obtained from **5** (1.815 g, 5.50 mmol) as described above, **8** (1.785 g, 5.00 mmol), and tetramethylguanidine (1.15 g, 10.0 mmol) in dry dimethyl sulfoxide (25 mL) was stirred at 50 °C for 20 h. The resulting brownish mixture was quenched in ice–water (~1 L), and the yellow fine-grained precipitate was collected, rinsed with large quantities of water, and dried. The yield of crude, essentially pure **7** was 2.10 g (93%). Traces of dark polar impurities were conveniently removed by chromatography as outlined above.

4-(Formylamino)-1-methylpyrrole-2-carboxylic Acid (9). (a) Formic Anhydride in Ether Solution. A solution of formic acid (4.60 g, 100 mmol) in dry ether (100 mL) was chilled to −78 °C, and dry pyridine (7.90 g, 100 mmol) was added slowly with stirring. The resulting slurry was treated with thionyl chloride (5.99 g, 50 mmol) dropwise at −50 to −60 °C with vigorous stirring for 15 min. The resulting thick suspension was stirred at −78 °C for another 15 min, after which the temperature was allowed to rise to −40 °C, and was immediately used as such in the next step.

(b) Formylation of 2. A solution of **1** (4.25 g, 25 mmol) in 2 M aqueous Na_2CO_3 (150 mL) was hydrogenated as described in the preparation of **3**, and, after removal of the catalyst, the resulting light yellow filtrate containing the unstable **2** was cooled in ice. In a nitrogen atmosphere, with vigorous stirring, half of the formic anhydride slurry was added in small portions for 15 min by using a Pasteur pipet. After this addition, the resulting two-phase system was stirred in ice-bath for 15 min, and then the remainder of the formic anhydride batch was transferred to the reaction mixture similarly. After the addition was completed, the light yellow suspension was agitated for 2 h more, whereupon it was allowed to warm up to room temperature. Water was cautiously added with shaking until the lower aqueous phase became clear, and the ether phase was separated. The yellow aqueous solution, which should be distinctly alkaline, was extracted with ether (2 × 50 mL, extracts discarded) and cautiously acidified with formic acid to pH 3.5. After one night in a refrigerator, the precipitated acid was collected and washed with small portions of ice–water followed by cold methanol and dry ether. The crude product weighed 2.30 g after being dried. Concentration of the aqueous filtrate and washings to one-third of the volume and chilling overnight afforded a second crop of pure **9** (0.88 g). The total yield of **9** was 76%. Recrystallization from methanol (80 mL/g, decolorizing carbon) gave a white microcrystalline product: mp 200–202 °C dec; R_f^A 0.39; mass spectrum (70 eV), *m/e* 168 (M^+); ^1H NMR (dimethyl-d_6 sulfoxide) δ 12.21 (br s, ~1 H), 10.02 (br s, ~1 H), 8.13 (perturbed d, 1 H), 7.32 (d, 1 H), 6.71 (d, 1 H), 3.82 (s, 3 H).

Anal. Calcd for $C_7H_8N_2O_3$: C, 50.0; H, 4.8; N, 16.7. Found: C, 50.1; H, 4.9; N, 16.5.

Benzyl 4-[[[4-[[[4-(Formylamino)-1-methylpyrrol-2-yl]carbonyl]amino]-1-methylpyrrol-2-yl]carbonyl]amino]-1-

methylpyrrole-2-carboxylate (11). (a) Amine Component 10. Crude **7** (4.52 g, 10.0 mmol) was deprotected in CH_2Cl_2 with TFA essentially as described for preparation of **6**. After a similar workup, the brown CH_2Cl_2 extract was evaporated at reduced pressure, giving crude **10** in almost quantitative yield as a brownish solid (R_f^B 0.07). After a thorough drying, this product was used in the coupling step.

(b) Preparation of 11 by Carbodiimide Coupling. Crude **10** from above was dissolved in dry DMF (50 mL), and **9** (1.68 g, 10.0 mmol) was added. This mixture was then treated with EDC (2.00 g, 10.4 mmol) in small portions with shaking. The resulting brown solution was stirred at 40 °C overnight and then quenched in ice-cold 1.0 M aqueous $KHSO_4$ (500 mL). The light brown, fine-grained precipitate was collected, washed thoroughly in turn with 1 M $KHSO_4$, 1 M $NaHCO_3$, and water, and dried in air. The slightly brown, somewhat sticky powder was carefully triturated with cold methanol (~10 mL) and the insoluble part washed with cold methanol (3 × 10 mL) followed by dry ether. The yield of dry crude **11** obtained as a tan powder was 4.51 g (90%), and it was pure enough for most purposes. The analytical sample was prepared by dissolving the crude material in warm DMF (~80 °C, 10 mL/g) and cautiously diluting the solution with refluxing EtOH (~100 mL/g). After clarification with decolorizing carbon, the solution was cooled to room temperature and seeded. Pure **11** precipitated as a cream-colored microcrystalline solid after 2 days in the cold: mp 258–260 °C dec; R_f^A 0.69; mass spectrum (70 eV), 502 (M^+); ^1H NMR (dimethyl-d_6 sulfoxide) δ 10.05 (br s, ~1 H), 9.92 (br s, ~2 H), 8.13 (perturbed d, 1 H), 7.49 (d, 1 H), 7.41 (perturbed s, 5 H), 7.23 and 7.21 (overlapping doublets, 2 H), 7.07 (perturbed d, 1 H), 6.98 (d, 1 H), 6.92 (d, 1 H), 5.25 (s, 2 H), 3.85 (s, 9 H), all $J_{3,5}$ = 1.9 Hz.

Anal. Calcd for $C_{26}H_{26}N_6O_5$: C, 62.1; H, 5.2; N, 16.7. Found: C, 61.7; H, 5.3; N, 16.4.

4-[[[4-[[[4-(Formylamino)-1-methylpyrrol-2-yl]carbonyl]amino]-1-methylpyrrol-2-yl]carbonyl]amino]-1-methylpyrrole-2-carboxylic Acid (12). Crude **11** (1.255 g, 2.50 mmol), dissolved in dry DMF (30 mL), was shaken with hydrogen at 60 psi at room temperature for 2 h in the presence of a Pd catalyst (5% on carbon). The mixture was filtered through low-porosity filter paper, and the yellow filtrate was evaporated to complete dryness at reduced pressure. The semisolid brownish residue was dissolved in EtOH (~10 mL), and the solution was filtered. The clear filtrate was then added to 250 mL of cold dry ether with rapid stirring. The resulting suspension was chilled overnight, and the light yellow precipitated powder was collected and washed with dry ether. The yield of crude **12** was 886 mg (86%). Workup of the filtrate gave a further ~30 mg of darker, less pure product. Compound **12** is hygroscopic and was dried for several days in vacuo over P_2O_5: mp ~235 °C dec; R_f^A 0.30; no molecular ion peak was present in the 12-eV mass spectrum; ^1H NMR (dimethyl-d_6 sulfoxide) δ 12.09 (br s, 1 H), 10.06 (br s, 1 H), 9.91 (br s, 2 H), 8.13 (perturbed d, 1 H), 7.43 (d, 1 H), 7.24 and 7.21 (overlapping d, 2 H), 7.06 (perturbed d, 1 H), 6.92 (d, 1 H), 6.87 (d, 1 H), 3.85 (s, 9 H), all $J_{3,5} \approx 1.9$ Hz.

Anal. Calcd for $C_{19}H_{20}N_6O_5$: C, 55.3; H, 4.9; N, 20.4. Found: C, 55.1; H, 5.1; N, 20.0.

***N*-Succinimidyl 4-[[[4-[[[4-(Formylamino)-1-methylpyrrol-2-yl]carbonyl]amino]-1-methylpyrrol-2-yl]carbonyl]amino]-1-methylpyrrole-2-carboxylate (13).** A mixture of **12** (1.15 g, 2.79 mmol) and *N*-hydroxysuccinimide (0.33 g, 2.87 mmol), dissolved in dry DMF (10 mL), was cooled in ice-bath. To this well-stirred solution was added solid DCC (0.60 g, 2.91 mmol) in small portions for 10 min, and stirring was continued for an additional 1 h at 0 °C and then at 40 °C overnight. After the mixture cooled, the dicyclohexylurea formed was filtered off and washed with small portions of cold DMF. The combined brownish filtrate and washings were evaporated to dryness at low pressure (temperature below 40 °C), and the semisolid residue was dissolved in acetone (10 mL). After filtration it was diluted with CH_2Cl_2 (10 mL) and applied to a silica gel column with CH_2Cl_2–acetone 2:1 (v/v) as the mobile phase. First was eluted a small quantity of dicyclohexylurea together with trace amounts of the *N*-acyldicyclohexylurea. Continued elution with CH_2Cl_2–acetone 1:1 (v/v) afforded the desired product **13** (1.26 g, 88%) as a white solid. This product could be reprecipitated by dissolving in a small quantity of acetone, filtering, and slowly diluting with cold ether with rapid stirring. This material, obtained as a white powder, was very hygroscopic and could not be analyzed satisfactorily: mp 195–198 °C dec; R_f^A 0.59; no molecular ion peak was present in the 12-eV mass spectrum; ^1H NMR spectrum (dimethyl-d_6 sulfoxide) δ 10.08 (br s, ~2 H), 9.96 (br s, 1 H), 8.14 (perturbed t, 1 H), 7.77 (d, 1 H), 7.26 and 7.22 (overlapping signals, 3 H), 7.13 (d, 1 H), 6.93 (d, 1 H), 3.87 (s, 9 H), 2.87 (s, 4 H), all $J_{3,5} \approx 1.5$–2.0 Hz.

Distamycin A (14). To an ice-cooled, well-stirred solution of β-aminopropionamidine dihydrobromide[26] (94 mg, 0.38 mmol) and $NaHCO_3$ (31 mg, 0.37 mmol) in dioxane–water 1:2 (v/v, 15 mL) was added dropwise a fresh solution of **13** (127 mg, 0.25 mmol) in dioxane (5 mL) for 15 min. When the addition was complete, the reaction mixture was stirred at room temperature overnight. The resulting light yellow solution was cautiously acidified to pH ~4.0 with a few drops of dilute HCl and filtered to remove trace amounts of insoluble impurities present in some runs. The filtrate was applied to a silica gel column (5 × 30 cm) with MeOH–water (2:1 v/v) as the mobile phase. First was obtained some starting material **13** together with *N*-hydroxysuccinimide and traces of other contaminants. Continued elution with MeOH–0.01 M aqueous HCl 2:1 (v/v) afforded a fraction containing pure **14**. This fraction (~500 mL) was concentrated to ~20 mL at low pressure below 25 °C. The residual almost colorless solution was filtered, and the pH was adjusted to 4.0 with dilute HCl. After the mixture was refrigerated for several days, pure **14** precipitated as an almost colorless voluminous solid. This material was collected, rapidly washed with small portions of ice-water, and dried in vacuo over P_2O_5. The yield of dry **14** was 41.5 mg. Further concentration of the combined filtrate and washings to 5 mL and subsequent chilling for a few days gave a second crop of 11.5 mg of a somewhat darker product. The total yield of **14** was 41%: mp ~190 °C dec; R_f 0.22 (ethyl acetate–acetone–acetic acid–water, 5:3:1:1 v/v/v/v); trace amounts of an impurity with R_f 0.14 were present in some batches; no molecular ion peak was present in the 12-eV mass spectrum[9], but at 70 eV and 125 °C a slight peak with *m/e* 464 ($M^+ - NH_3$) was obtained. ^1H NMR (dimethyl-d_6 sulfoxide) δ 10.19 (br s, ~1 H), 9.95 (br s, ~2 H), 8.8–9.1 (perturbed m, ~4 H), 8.26 (br s, ~1 H), 8.13 (perturbed s, 1 H), 7.23 (overlapping signals, 3 H), 7.07 (d, 1 H), 6.95 (d, 2 H), 3.85 (s, 6 H), 3.83 (s, 3 H), 3.51 (perturbed m, 2 H), 2.65 (perturbed t, 2 H).

Anal. Calcd for $C_{22}H_{27}N_9O_4 \cdot HCl$: C, 51.0; H, 5.5; N, 24.3; Cl, 6.8. Found: C, 50.8; H, 5.5; N, 23,6; Cl, 6.2.

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Registry No. **1**, 13138-78-8; **2**, 45776-13-4; **3**, 77716-11-1; **4**, 77716-12-2; **5**, 77716-13-3; **6**, 77716-14-4; **7**, 77716-15-5; **8**, 77716-16-6; **8** (3-*N*-acyl isomer), 77716-17-7; **9**, 77716-18-8; **10**, 77716-19-9; **11**, 77716-20-2; **12**, 77716-21-3; **13**, 77727-43-6; **14**, 6576-51-8; ethyl 1-methyl-4-nitropyrrole-2-carboxylate, 2853-29-4; (*tert*-butyloxy)-carbonyl fluoride, 18595-34-1; HOBT, 2592-95-2; HOSu, 123-56-8; β-aminopropionamidine dihydrobromide, 77152-88-6.

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