97634-72-5; **22**, 97634-62-3; **22** (tosylate), 97634-71-4; **22** (chloride), 97644-31-0; **23** (isomer 1), 97634-63-4; **23** (isomer 2), 97673-13-7; **24**, 97634-64-5; $(EtO)_2P(O)CH(CH_3)CO_2Et$, 3699-66-9; $Ph_3PMe^+I^-$, 2065-66-9; PhSLi, 2973-86-6; ethyl 2,7-dimethyl-(*E*)-2,6-octadienoate, 73658-21-6; geranyl phenyl sulfide, 35162-74-4; 9-(diethylamino)-9-cyano-1-(phenylthio)-3,7-dimethylnona-2,6-diene, 97634-68-9; (diethylamino)acetonitrile, 3010-02-4; geranyl acetate, 105-87-3.

Efficient Total Syntheses of the Oligopeptide Antibiotics Netropsin and Distamycin

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New and efficient total syntheses of the natural oligopeptide antiviral antibiotics netropsin and distamycin are described. These procedures feature a different strategy of introduction of the terminal groups from that used hitherto, high yield coupling steps, improvements in the Pinner reaction for introducing the amidine moiety, and the novel use of *N*-formylimidazole for introduction of the formyl moiety in distamycin. The methods also avoid column chromatography with the attendant contamination of the oligopeptide hydrochlorides with inorganic salts eluted from adsorbents. The synthetic procedures are general and may be adapted to the synthesis of related oligopeptide structures.

The family of naturally occurring oligopeptides includes netropsin,[1] distamycin,[2,3] anthelvencin,[4] kikumycin B,[5] amidinomycin,[6] and norformycin.[7] They have attracted considerable attention on the part of synthetic chemists and pharmacists because some representatives exhibit antiviral, antibacterial, and anticancer activities.[3,8,9] The first two agents are also of interest in molecular biology because their biological properties appear to arise, in part, from the unique XTTT and XTTTT sequence preferential and minor groove selective binding to DNA of netropsin and distamycin, respectively.[3,10,11] Our interest in molecular recognition has led us to explore the synthesis of related "lexitropsins" based on the oligopeptide structures but designed to recognize and bind to alternative and unique sequences in the minor groove of duplex DNA.[12] The consequent requirement for an adaptable and general synthesis of such oligopeptides directed our attention to existing synthetic procedures of some of the natural lead antibiotics. We report efficient and general total syntheses of netropsin and distamycin. The methods developed offer several advantages of yield and purity on individual steps over reported procedures and avoid the chromatographic separations generally used hitherto.

Synthetic Strategy. A number of elegant total syntheses of natural oligopeptide antibiotics have been reported.[2,13–16] All except one[16] have been based essentially on the method introduced by Julia and Préau-Joseph. The latter consists of introducing the amidino group early, carrying forward the nitro heterocyclic intermediate which is then reduced catalytically prior to coupling of the guanidino group.[13] The one exception to this general plan, due to Grehn and Ragnarrson[16] cannot be evaluated from the viewpoint of efficiency because all the yields are given on crude products. The synthesis of distamycin starting from *N*-methyl-4-nitropyrrole-2-carboxylic acid requires twelve steps in this procedure. This approach also used uncommon reagents like (*tert*-butyloxy)carbonyl fluoride and formic anhydride.

Thus the existing methods[2,13–16] do not lend themselves to the development of an adaptable general synthetic procedure and suffer from several disadvantages including the following: (i) The coupling reactions employ unsatisfactory methods, e.g., the reaction of an amine with an acyl chloride in the presence of aqueous sodium bicarbonate or triethylamine in ethanol gives unsatisfactory yields in our hands; one procedure also uses uncommon protecting groups for coupling.[16] (ii) Existing methods of introduction of the formyl group in distamycin[14–16] are unsatisfacotry with regard to yield and the required purification. (iii) The sequence of attachment of the end groups is inconvenient in that the products require column chromatography, which is also undesirable because of contamination with inorganic salts (from the adsorbents) of the final oligopeptides that are customarily isolated as hydrochloride salts. (iv) The reaction conditions for the Pinner reaction were not optimized.

Thus our objectives were to try to improve existing methods in not only increasing the yields but also to avoid chromatographic methods of separation.

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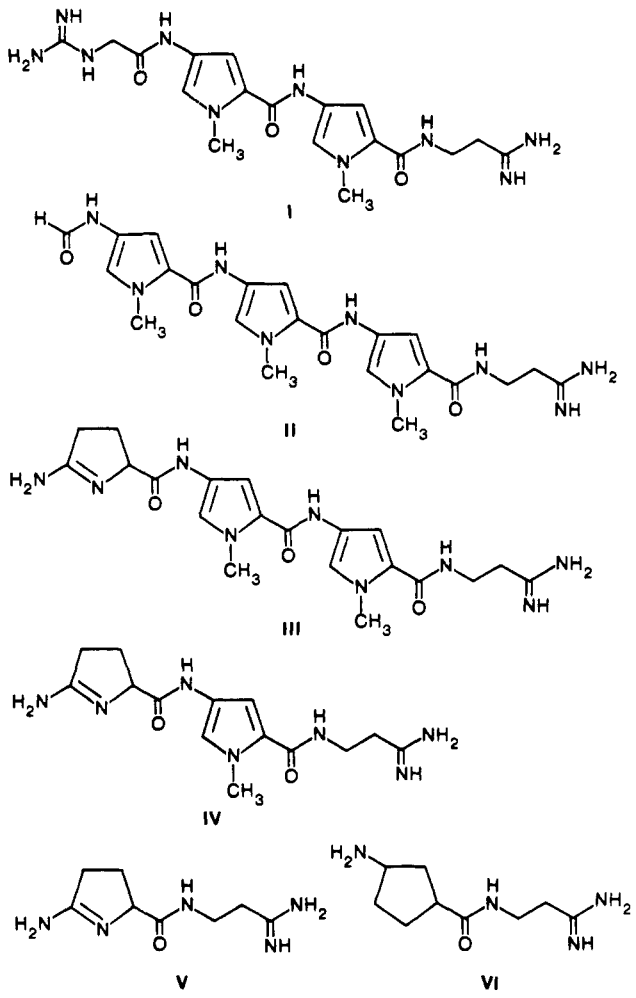


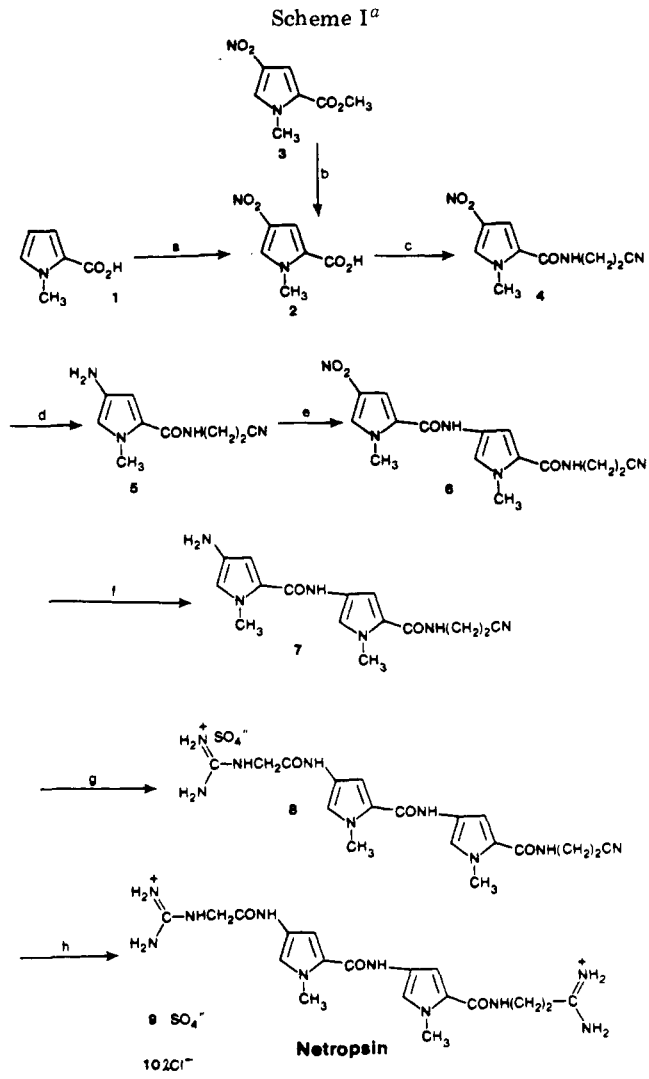
Figure 1. Structures of oligopeptide antibiotics, netropsin (I), distamycin (II), anthelvencin (III), kikumycin B (IV), amidinomycin (V), and noformycin (VI).

Synthesis of Netropsin. Nitration of 1-methyl-2-pyrrolecarboxylic acid (**1**)[15] or that of its ethyl ester[17] gives a mixture of the desired 4-nitro derivative **2** and other nitro derivatives. Existing methods employ column chromatography for the separation of the nitro esters.[15] We found that when **1** is nitrated with nitric acid–acetic anhydride mixture the 4-nitropyrrole acid **2** crystallizes from the reaction mixture at low temperature as a pure isomer in fair yield of 43%. Consequently **2** may be obtained directly and in a procedure that avoids chromatography. The acid **2** was converted into its acyl chloride with thionyl chloride and coupled with aminopropionitrile in the presence of Hunig's base to give **4** in 95% yield (Scheme I). The latter was reduced by catalytic hydrogenation to give the amine **5** which was isolated as the hydrochloride in 46% yield. The free amine of **5** in common with the other 4-aminopyrroles is extremely unstable. In contrast the corresponding hydrochloride salt is more stable and may be stored and consequently was used in some steps of our syntheses. Hydrogenation of **4** to **5** is almost quantitative and often **5** was used as formed immediately without isolation. Acylation of the 4-amino group in **5** with the acid chloride of **2** afforded **6** in 91% yield. The yield of the latter was improved to 95% by acylating **5** directly without isolation as the hydrochloride.

The nitro compound **6** was reduced catalytically to give the amine **7**, which is stable in the solid form but not in solution. The amine **7**, when allowed to react with guanidineacetic acid in the presence of dicyclohexylcarbodiimide afforded **8** isolated as the sulfate. Pinner reaction[18] on **8** with HCl in ethanol, followed by ammonia gave an excellent yield (77%) of netropsin **9** isolated as the sulfate. We found that the reaction of the nitrile group with ethanol in the presence of HCl is completed after 90 min and that longer reaction times give more side products and decomposition.[19] In addition reaction of the imino ether with NH_3 is almost instantaneous and there is no need to run the reaction overnight as previously claimed.[19]

Our procedure introduces the oligopeptide end groups in the reverse order from that reported hitherto.[13] Among the advantages are that compound **8** can be readily isolated in pure form and, since the last step is virtually quantitative by TLC examination, the final product **9** is almost pure. Compound **9** was then treated with barium chloride to obtain the hydrochloride **10**, the form in which netropsin was originally isolated.[13] In order to avoid contamination

Scheme I[a]



[a] Reaction conditions: (a) HNO_3, Ac_2O, ambient temp; (b) aqueous NaOH, heat; (c) $SOCl_2$, Δ, then add 3-aminopropionitrile; (d) H_2, Pd/C in MeOH; (e) 1-methyl-4-nitropyrrole-2-acyl chloride and Hunig's base in THF; (f) H, Pd/C in MeOH; (g) guanidineacetic acid hydrochloride, DCC in Me_2SO, and then Na_2SO_4 to give **8**; (h) HCl in EtOH, then dry NH_3, EtOH, and then Na_2SO_4 to give **9**. $BaCl_2$ gives **10**.

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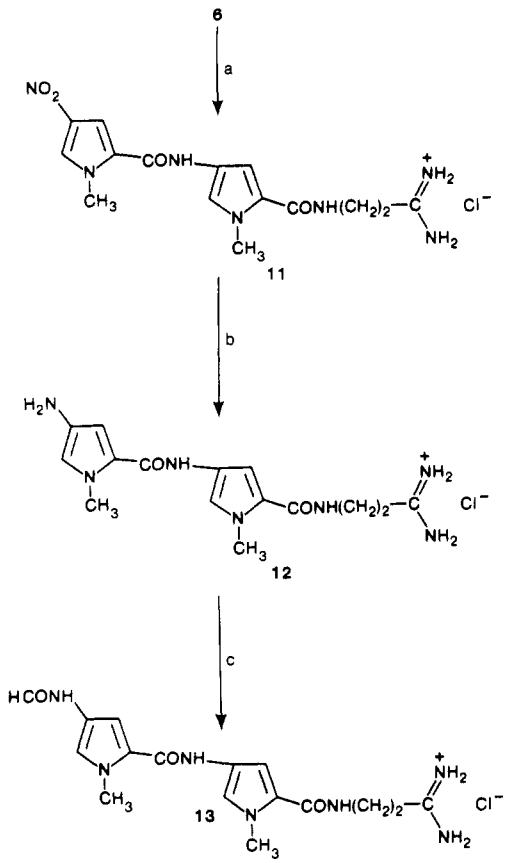
Scheme II[a]



[a] Reaction conditions: (a) HCl in EtOH, then dry NH_3, EtOH; (b) H_2, Pd/C in MeOH; (c) *N*-formylimidazole in THF.

of **10** with barium chloride, a slight excess of **9** was used and the hydrochloride **10** was extracted with methanol in which **9** is insoluble. The netropsin hydrochloride thus obtained was identical with an authentic sample of the antibiotic.

Synthesis of Distamycin. In order to adapt our procedure for the synthesis of distamycin an effecient means of introducing the terminal *N*-formyl group was necessary. N-Formylation has been the least satisfactory step in all the synthetic procedures developed for distamycin and related structures. Acetic–formic anhydride,[14] formic acid, and dicyclohexylcarbodiimide[15] or formamide and ethyl formate[20] rarely give yields up to 40% and rather more often are in the range 10–20%. An additional disadvantage has been the necessity to purify the product by column chromatography.[15,16]

The model amino compound **12** and **18** were found to be unstable in the presence of formic acid (accounting for the poor yields of some previous methods[14,15]) so a milder neutral method of N-formylation was sought. We examined Staab's reagent *N*-formylimidazole for this purpose.[21] When the reagent is pure it is very hygroscopic and unstable. However we found that it can be used without isolation and it reacts with amino heterocycles such as **12** in methanolic solution to give a quantitative yield of the *N*-formyl product as judged by TLC examination. The excess of the reagent reacts with methanol to give volatile methyl formate and imidazole the latter of which is readily removed by extraction with nonpolar solvents. This efficient method was then applied to the synthesis of distamycin.

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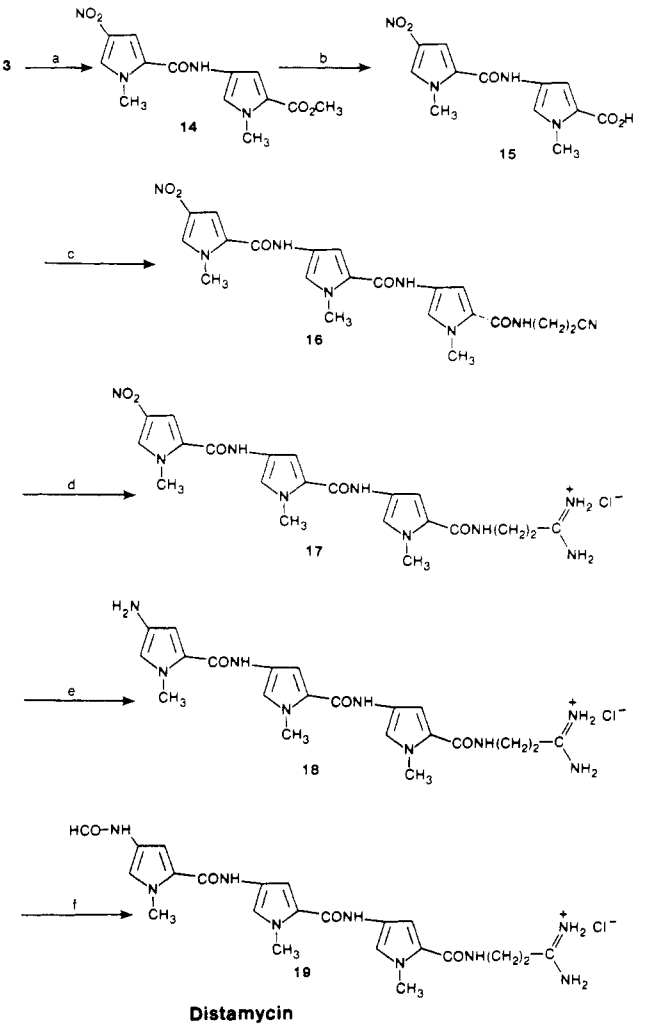
Scheme III[a]



Distamycin

[a] Reaction conditions: (a) H_2, Pd/C in MeOH, then 1-methyl-4-nitropyrrole-2-acyl chloride in THF; (b) aqueous ethanolic NaOH heat; (c) compound **5** hydrochloride, 1,8-bis(dimethylamino)naphthalene in DMA, then DCC; (d(HCl, EtOH, then dry NH_3, EtOH; (e) H_2, Pd/C, in MeOH; (f) *N*-formylimidazole in THF/MeOH.

Starting from ester **3** the nitro dipyrrole peptide **14** was prepared using the chemistry described previously and summarized in Scheme II. Hydrolysis of the ester and condensation of **15** with compound **5** in the presence of a proton sponge and DCC afforded **16** in 64% yield. This procedure has the advantage of avoiding DCC in the introduction of the side chains with the attendant problems of separation and reduced yield resulting from side reaction of DCC with the amine.[15] Application of our modified Pinner reaction conditions to **16** gave the amidine **17**. Catalytic reduction of **17** gave the stable amine **18** in 70% yield. Treatment of the amine **18** with *N*-formylimidazole gave pure distamycin, in 71% yield, identical with an authentic sample.

These polar peptidic antibiotics containing guanidyl and/or amidine groups are readily analyzed for purity by TLC on silica gel with methanol as eluent.

Acetic acid is necessary as a cosolvent when only one strongly polar group is present and formic acid is used when two polar groups are present in the molecule. Also for such polar compounds FAB-MS[22] proved satisfactory for determining the molecular composition. When one

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polar group is present peaks corresponding to $(M - HSO_4)^+$, $(M - Cl)^+$, $(2M - Cl - HCl)^+$, and $(2M - Cl)^+$ are detected. When two polar groups are present in the molecule the ions $(M - HSO_4)^+$, MH^+, $(2M - HSO_4)^+$, and M_2H^+ are detected for sulfates and $(M - Cl - HCl)^+$ and $(M - Cl)^+$ for hydrochlorides.

Conclusion

In conclusion efficient and adaptable synthetic routes have been developed to the oligopeptide antibiotics netropsin and distamycin. The application of these methods to produce "lexitropsins",[11,12] i.e., oligopeptides designed to recognize specific DNA sequences in the minor groove, as well as novel oligopeptides which exhibit antiviral and anticancer properties will be reported in due course.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The IR spectra were recorded on Nicolet 7199 FT spectrophotometer, and only the principal peaks are reported.

The ^1H NMR spectra were recorded on Bruker WH-200 and WH-400 spectrometers. FAB (fast atom bombardment) mass spectra were determined on an Associated Electrical Industries (AEI) MS-9 and MS-50 focusing high resolution mass spectrometers. Kieselgel 60 (230–400 mesh) of E. Merck was used for flash chromatography and precoated sheets Silica gel 60F-254 of E. Merck were used for TLC. TLC system: (i) covalent peptidic compounds was chloroform–methanol 9:1; (ii) ionic compounds with one ionic pair was methanol with some AcOH; (iii) ionic compounds with two ionic pairs was methanol with some formic acid.

1-Methyl-4-nitropyrrole-2-carboxylic Acid (2). Acetic anhydride (8 mL) was treated with 1.6 mL of 70% nitric acid and the mixture heated to 50 °C for 15 min, then cooled to room temperature, and slowly added to a suspension of 2 g of **1** in 12 mL of Ac_2O cooled to −25 °C. The mixture was stirred at −15 °C for 0.5 h, then the temperature was allowed to rise to ambient, and stirring was continued for 20 min. The mixture was again cooled to −25 °C and the resulting precipitate collected in a funnel cooled in dry ice. The solid was washed with a small quantity of cold Ac_2O (−25 °C) followed by Ac_2O–CCl_4 1:1 (−25 C) and then CCl_4 and hexane. The crystalline solid was taken up in water containing 1 g of NaOH. Acidification with HCl precipitated pure **2**: 1.18 g (43% yield); mp 204–205 °C (lit.[17] mp 201–201.5 °C).

Alternatively **2** was obtained from methyl 1-methyl-4-nitropyrrole-2-carboxylate by treatment with hot aqueous alkaline solution in 92% yield.

1-Methyl-4-nitropyrrole-2-carboxamidopropionitrile (4). A solution of 7 g (41 mmol) of **2** and a slight excess of $SOCl_2$ in 20 mL of THF was heated under reflux for 5 min (longer heating should be avoided because side products are formed). The excess of $SOCl_2$ and solvent were removed under reduced pressure and the evaporation was repeated with some anhydrous THF. The residue was dissolved in THF and cooled to −20 °C. A solution of 3.12 mL of aminopropionitrile and 7.8 mL of Hunig's base in 10 mL of THF was added. The temperature was allowed to rise to ambient, then the solvent was removed in vacuo, water was added, and the resulting crystalline solid collected affording **4**: 8.71 g (95% yield); mp 132–133 °C (lit.[13] mp 135 °C); ^1H NMR (Me_2SO-d_6) δ 2.74 (t, 2 H, ^3J = 6.5 Hz), 3.45 (q, 2 H, $J_{HH} = J_{HNH}$ = 6.5 Hz), 3.90 (s, 3 H), 7.44 and 8.13 (2 d, 2 H, J = 2 Hz aromatic), 8.74 (t, 1 H, J_{HNH} = 6.5 Hz, NH); IR ν_{max} (Nujol) 1529, 1556, 1647, 2240 cm^{-1}.

1-Methyl-4-aminopyrrole-2-carboxamidopropionitrile Hydrochloride (5). Methanol cooled to −10 °C was added to 2.5 g of 10% Pd on charcoal (cooling prevents spontaneous combustion of the methanol vapors in the presence of the catalyst) and 9.1 g of **4** was added and hydrogenated at 40 °C and atmospheric pressure. After the calculated amount of hydrogen was taken up, the catalyst was removed by filtration, the methanol evaporated under reduced pressure, isopropyl alcohol was added, and the mixture cooled to −30 °C and treated with anhydrous HCl. The crude product was precipitated with ethyl acetate, redissolved in isopropyl alcohol, and filtered through charcoal. The filtrate was concentrated to a small volume in vacuo, and the product precipitated with ethyl acetate, collected, washed with hot acetonitrile, and then dried at 100 °C under vacuum to give **5**: 4.3 g (46% yield); mp 195 °C; ^1H NMR (Me_2SO-d_6) δ 2.70 (t, 2 H, ^3J = 7 Hz, CH_2), 3.30 (bs, ~2 H, NH_2, H_2O), 3.38 (q, 2 H, $^3J = J_{HNH}$ = 7 Hz, CH_2), 3.80 (s, 3 H, CH_3), 6.83 and 7.07 (2 d, 2 H, J = 2 Hz, aromatic), 8.58 (t, 1 H, ^3J = 7 Hz, NH), 10.07 (bs, 1 H, NH^+). Anal. Calcd for $C_9H_{13}ClN_4O$ (228.68): C, 47.3; H, 5.7; N, 24.5; Cl, 15.5. Found: C, 46.0; H, 5.8; N, 23.9; Cl, 15.8.

1-Methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)-pyrrole-2-carboxamidopropionitrile (6). (a) A solution of 510 mg (3 mmol) of **2** was heated under reflux with 2 mL of $SOCl_2$ in 10 mL of THF until the solid had dissolved. The solvent and excess $SOCl_2$ were removed in vacuo and the evaporation repeated with more THF. The residue was dissolved in anhydrous THF and cooled to −20 °C, and a solution of 684 mg of **5** and 1.2 mL of Hunig's base in 10 mL of anhydrous CH_3CN added. The mixture was stirred at ambient temperature for 30 min and then evaporated to dryness, after which water was added and the product collected and washed with isopropyl alcohol and hexane to give pure **6**, 946 mg (91.4% yield).

(b) In an alternative procedure, 222 mg (1 mmol) of **4** was hydrogenated over 60 mg of 10% Pd on charcoal in methanol as described above. After hydrogenation the catalyst was removed by filtration, the methanol removed in vacuo, and the evaporation was repeated with some THF. The residue was dissolved in anhydrous THF and 180 μL of Hunig's base were added. The mixture was cooled to −20 °C and the solution of the acid chloride of **2** in THF (prepared from 170 mg of **2** as described in **a**) was added. After 30 min at room temperature the solvent was removed in vacuo and water added. The resulting yellow precipitate was collected and washed with isopropyl alcohol and hexane to give **6**: 330 mg (95.6% yield); mp 230–232 °C (lit. mp 248 °C,[13] mp 254–255 °C[14]); ^1H NMR (Me_2SO-d_6) δ, 2.76 (t, 2 H, ^3J = 6.5 Hz), 3.43 (q, 2 H, $^3J = J_{HNH}$ = 6.5 Hz), 3.85 and 3.98 (2 s, 6 H, CH_3), 6.95, 7.25, 7.60, 8.20 (4 d, 4 H, J = 2 Hz, aryl), 8.40 (t, 1 H, J_{HNH} = 6.5 Hz, NH), 10.28 (s, 1 H, NH); IR ν_{max} (Nujol) 1523, 1533, 1560, 1576, 1653, 1672, 2270, 3140, 3402 cm^{-1}.

1-Methyl-4-(1-methyl-4-aminopyrrole-2-carboxamido)-pyrrole-2-carboxamidopropionitrile (7). A solution of 2 g (5.8 mmols) of **6** in 20 mL of DMF and 20 mL of CH_3OH was hydrogenated at atmospheric pressure over 400 mg of 10% Pd on charcoal until TLC (SiO_2 gel, $CHCl_3$ with 10% CH_3OH) indicated only a trace of starting material. The catalyst was removed by filtration, the solvents removed in vacuo, and the residual solid treated with CH_3CN and CCl_4. The resulting crystalline solid was collected and washed with a 1:1 mixture of CH_3CN and EtOAc and then with hexane to give **7**: 1.28 g (70% yield); mp 175–180 °C; ^1H NMR (Me_2SO-d_6) δ 2.74 (t, 2 H, J = 5.5 Hz), 3.74 and 3.82 (2 s, 6 H), 6.27, 6.37, 6.91 and 7.20 (4 d, 4 H, J = 2.2 Hz), 8.33 (t, 1 H, J = 5.5 Hz), 9.62 (s, 1 H), D_2O exchange results in disappearance of the peaks at δ 8.33 and 9.62 and reveals a triplet at δ 3.50 (2 H).

1-Methyl-4-[1-methyl-4-(guanidineacetamido)pyrrole-2-carboxamido]pyrrole-2-carboxamidopropionitrile Sulfate (8). A solution of 105 mg (0.33 mmole) of **7** and 76 mg (0.33 mmol) of guanidineacetic acid hydrochloride in 2 mL of anhydrous Me_2SO was treted with a solution of 103 mg (0.5 mmol) of DCC in 1 mL of Me_2SO in portions during 2 h at room temperature under argon. After stirring for an additional 1 h the solution was concentrated to dryness in vacuo. Water (5 mL) was added and the solid which precipitated was removed by filtration. The filtrate was concentrated to a small volume in vacuo and a small volume of a concentrated aqueous solution of sodium sulfate was added. The resulting crystalline solid was collected by filtration, recrystallized from water, washed with ethanol, and dried at 110 °C under reduced pressure to give **8**: 80 mg (52% yield); mp 193–195 °C; FAB-MS (glycerol) $(C_{18}H_{23}N_9O_3)H^+$ 414, found 414; IR ν_{max} (Nujol) 1665, 2245, 3194, 3320 cm^{-1}.

1-Methyl-4-[1-methyl-4-(guanidineacetamido)pyrrole-2-carboxamido]pyrrole-2-carboxamidopropionamidine Sulfate. Netropsin Sulfate (9). A solution of 80 mg (0.17 mmole) of **8** in 8 mL of anhydrous EtOH was treated with dry HCl gas with cooling. After saturation the reaction mixture was set aside at room temperature for 2 h. The EtOH was removed in vacuo

and the residue was washed with dry ether and decanted. Anydrous EtOH (8 mL) was added and dry NH_3 gas was condensed into the vessel. After stirring for 1 h at room temperature the solvents were removed in vacuo. The residue was dissolved in water and an aqueous solution of Na_2SO_4 added. The resulting gel was collected, washed with cold water, recrystallized from water, washed with ethanol and hexane, and dried at 110 °C under reduced pressure to give **9**: 70 mg (76.6% yield); mp 235–240 °C dec (lit.[23] mp 224–225 °C); FAB-MS (glycerol) 431 $(M - HSO_4)^+$, 529 MH^+, 959 $(2M - HSO_4)^+$, 1057 M_2H^+. Anal. Calcd for $C_{18}H_{28}N_{10}O_7S$ (528.55): C, 40.9; H, 5.3; N, 26.5; S, 6.1. Found: C, 40.8; H, 5.4; N, 26.0; S, 5.9 (see next section for spectral characterizaton).

1-Methyl-4-[1-methyl-4-(guanidineacetamido)pyrrole-2-carboxamido]pyrrole-2-carboxamidopropionamidine Dihydrochloride. Netropsin Chloride (10). A solution of 145 mg (0.27 mmol, 10% excess) of **9** in boiling water was treated with a solution of 61 mg (0.25 mmol) of $BaCl_2 \cdot 2H_2O$. The precipitated $BaSO_4$ was removed by centrifugation and the supernatant was evaporated to dryness. The residue was treated with methanol and the solution filtered. The methanolic solution was evaporated to dryness, the residue was dissolved in a small volume of water and then treated with ethanol and ethyl acetate to give **10** which was dried at 110 °C under reduced pressure: 128 mg (almost 100% based on the amount of $BaCl_2 \cdot 2H_2O$ used). An analytical sample of **10** was prepared by dissolving it in water and precipitating with acetone: mp 215 °C (lit.[23] mp 168–172 °C); ^1H NMR (Me_2SO-d_6) δ 2.65 (t, 2 H), 3.52 (q, 2 H), 3.83 and 3.85 (2 s, 6 H), 4.03 (s, 2 H), 6.95 (m, 2 H), 7.20 (s, 2 H), 7.40 (bs, 4 H), 7.62 (s, 1 H), 8.25 (t, 1 H), 8.65, 8.99 (2 bs, 4 H), 9.95 (s, 1 H), 10.28 (s, 1 H); FAB MS (glycerol/sulfolane) 431 $(M - HCl - Cl)^+$, 467 $(M - Cl)^+$, 861 $(2M - 3HCl - Cl)^+$; IR ν_{max} (Nujol) 1662, 1702, 3200, 3275, 3358 cm^{-1}. Anal. Calcd for $C_{18}H_{28}Cl_2N_{10}O_3 \cdot H_2O$ (521.42): C, 41.5; H, 5.8; Cl, 13.6; N, 26.9. Found: C, 41.9; H, 5.8; Cl, 13.5; N, 26.1.

The synthetic netropsin chloride shows a ΔTm of 19.5° with calf thymus DNA in 20 mM potassium sodium phosphate buffer pH 6.9 at a D/P ratio of 1.0, identical with an authentic sample (Lederle Labs batch 1264-123-1). Compound **10** also exhibited the characteristic wide spectrum antiviral activity of authentic netropsin in tests courtesy of Prof. Erik De Clercq, Leuven, Belgium.

1-Methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)-pyrrole-2-carboxamidopropionamidine Hydrochloride (11). A suspension of 3.2 g (9.3 mmol) of **6** in 50 mL of anhdrous EtOH was saturated with anhydrous HCl gas with efficient cooling with dry ice and acetone. The cooling bath was removed and when the reaction temperature rose to the ambient value the solid had dissolved. The solution was stirred at room temperature for 1.5 h, after which time a solid had precipitated. The solvent was removed in vacuo and the residual solid was washed with dry ether and then decanted. Dry EtOH (100 mL) and MeOH (50 mL) were added and dry NH_3 gas condensed into the vessel. The mixture was heated up to 55 °C and the disappearance of the imino ester was followed by TLC on silica gel using MeOH containing a little acetic acid as eluent. The hot solution was filtered to give pure **11**: 3.32 g (90% yield); mp 315 °C dec (lit.[13] mp 324–325); ^1H NMR (Me_2SO-d_6) δ 2.68 (t, 2 H), 3.54 (q, 2 H), 3.83 and 3.97 (2 s, 6 H), 6.98, 7.27, 7.69, 8.20 (4 d, 1 H each) 8.37 (t, 1 H), 7.3–8.6 (b s, 4 H), 10.39 (s, 1 H), after D_2O exchange 2.66 (t, ^3J = 6.5 Hz, 2 H), 3.60 (t, ^3J = 6.5 Hz, 2 H), 3.80 and 3.93 (2 s, 6 H) 6.94, 7.24, 7.55, 8.10 (4 d, J = 1.8 Hz, 1 H each); IR ν_{max} (Nujol) 1513, 1537, 1654, 1694, 3280 cm^{-1}.

1-Methyl-4-(1-methyl-4-aminopyrrole-2-carboxamido)-pyrrole-2-carboxamidopropionamidine Hydrochloride (12). A solution of 132.8 mg (0.33 mmol) of **11** in 10 mL of methanol was hydrogenated over 60 mg of 10% Pd on charcoal at 40 °C for 3 h. The catalyst was removed by filtration, the filtrate concentrated to a very small volume, and acetonitrile was added to precipitate **12** as a white solid, 93 mg (76% yield), which was dried at 80 °C under reduced pressure; it darkens at 188 °C and melts at 205° C: ^1H NMR (DMF-d_7) δ 2.9 (obscured by solvent peak) 3.70 (q, J = 6 H, 2 H), 3.89 and 3.91 (2 s, 6 H), 6.94, 7.06, 7.11, 7.38 (4 d, J = 2 Hz, 4 H), 8.55 (t, J = 6 Hz, 1 H), 9.50 (bd, 4 H), 10.6 (s, 1 H); FAB-MS (glycerol) 332 $(M - Cl)^+$, 663 $(2M - Cl - HCl)^+$, 699 $(2M - Cl)+$; IR ν_{max} (Nujol) 1689, 3247 cm^{-1}.

1-Methyl-4-[1-methyl-4-(formylamino)pyrrole-2-carboxamido]pyrrole-2-carboxamidopropionamidine Hydrochloride (13). A solution of 76 μL (2 mmol) of 98% formic acid in 1 mL of THF was added to 325 mg (2 mmol) of carbonyldiimidazole in 2 mL of THF. After 15 min the solution was added to 147 mg (0.4 mmol) of **12** in 5 mL of MeOH cooled to −40 °C. The mixture was kept at −40 °C for 15 min then, after concentration to a small volume, ethyl acetate was added to precipitate the product. This was collected and taken up in a small volume of isopropyl alcohol (this operation must be fast because the product is hygroscopic). The product was reprecipitated with ethyl acetate. The solid was collected, rapidly washed with ethyl acetate and hexane, and immediately dried under reduced pressure at 80 °C to give **13**: 110 mg (70% yield); no distinct melting point. TLC on silica gel with methanol and a drop of acetic acid indicated a pure product of complete formylation R_f for **12** = 0.1 and for **13** = 0.3; ^1H NMR (Me_2SO-d_6) δ 2.62 (t, J = 6.5 Hz, 2 H), 3.51 (q, J = 6.5 Hz, 2 H), 3.78 and 3.80 (2 s, 6 H), 6.92 (2 d, J = 1.8 Hz, 2 H), 7.18 (s, 2 H), 8.11 (s, 1 H), 8.24 (t, J = 6.5 Hz, 1 H), 8.72 and 9.02 (2 s, 4 H), 9.93 (s, 1 H), 10.14 (s, 1 H); FAB-MS (glycerol, sulfolane) 360 $(M - Cl)^+$; IR ν_{max} (Nujol) 1638, 1680, 3270 cm^{-1}.

Methyl 1-Methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxylate (14). A solution of the methyl ester **3** (1.85 g, 10 mmol, prepared by treatment of **2** with $SOCl_2$ then with MeOH) was hydrogenated over 600 mg of 10% Pd on charcoal in 10 mL of MeOH at atmospheric pressure. The catalyst was removed by filtration, the solvent evaporated under reduced pressure, and then 1.8 mL of Hunig's base in 3 mL of THF was adeed. The mixture was cooled to −20 °C and treated with a solution of the acid chloride from 1.71 g of **2** in 5 mL of THF. After 30 min of stirring at room temperature the mixture was evaporated to dryness, water was added, and the resulting yellow solid collected. The latter was purified by dissolving in hot DMF and precipitating with EtOH to give **14**: 2.45 g (80% yield); mp 225 °C (lit.[15] mp 262 °C); ^1H NMR (Me_2SO-d_6) 3.74, 3.84, and 3.95 (35, 9 H), 6.88, 7.43, 7.55 and 8.14 (4d, J = 2 Hz, 4 H), 10.78 (s, 1 H).

1-Methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)-pyrrole-2-carboxylic Acid (15). A solution of 614 mg (2 mmol) of **14** and 400 mg (10 mmol) of NaOH in 15 mL of water and 15 mL of EtOH was heated under reflux until the solid dissolved. If this solution is acidified when cold, a gel is formed which is separable only by centrifugation. Therefore the hot solution was acidified with concentrated HCl to give the microcrystalline yellow **15**: 554 mg (95% yield); mp 254–255 °C dec; ^1H NMR (Me_2SO-d_6) δ 3.82 (s, 3 H), 3.94 (s, 3 H), 6.83, 7.40, 7.55, 8.16 (4d; J = 2 Hz, 1 H each), 10.22 (s, 1 H, NH) 12.25 (bs, 1 H, CO_2H); IR ν_{max} (Nujol) 1522, 1588, 1672, 3418 cm^{-1}; MS, m/z (relative intensity) for $C_{12}H_{12}N_4O_5$ 292.0812 (61, M^+), 248.0915 [35, $(M - CO_2)^+$].

1-Methyl-4-[1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxamido]pyrrole-2-carboxamidopropionitrile (16). A solution of 146 mg (0.5 mmol) of **15**, 160 mg (0.7 mmol) of **5**, and 150 mg of 1,8-bis(dimethylamino)naphthalene in 1 mL of *N,N*-dimethylacetamide was treated with 154 mg of dicyclohexylcarbodiimide in 1 mL of THF during 2 h at room temperature. After an additional 1 h of stirring at room temperature the solvents were removed in vacuo, water was added, and the mixture decanted. Trituration of the gummy product with MeOH gave a yellow microcrystalline substance which was collected. The solid was redissolved in a small amount of DMF and filtered, and the product reprecipitated with EtOH to give **16**: 150 mg (64% yield); mp 300 °C (lit. mp 282–285 °C,[14] mp 257–260 °C[15]); ^1H NMR (Me_2SO-d_6), 2.60 (t, J = 6 Hz, 2 H), 3.42 (q, J = 6 Hz, 2 H), 3.82, 3.86 and 3.97 (3s, 9 H), 6.95, 7.05, 7.25, 7.30, 7.61 and 8.20 (6s, 1 H each), 8.36 (t, J = 6 Hz, 1 H), 10.00 (s, 1 H), 10.30 (s, 1 H); IR ν_{max} (Nujol) 1524, 1540, 1581, 1642, 1670, 2245, 3130, 3295, 3367 cm^{-1}; MS, m/z (relative intensity) for $C_{21}H_{22}N_8O_5$ 466.1714 (33, M^+).

1-Methyl-4-[1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxamido]-2-carboxamidopropionamidine Hydrochloride (17). A suspension of 500 mg (1.07 mmol) of **16** in 5 mL of anhydrous EtOH was treated with dry HCl gas with efficient cooling (dry ice–acetone) until saturated.

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The mixture was stirred for 1.5 h at room temperature then the solvent was removed in vacuo and the residue washed with dry ether and decanted. Dry ethanol (5 mL) was added and dry NH_3 gas condensed into the vessel. After 1 h at room temperature the solvents were removed under reduced pressure, ethanol was added, and the yellow precipitate collected to give pure **17**: 497 mg (89% yield); no distinct melting point; decomposition starts at 190 °C (lit. mp 199–207 °C[14] mp 204–207 °C[15]); ^{1}H NMR (Me_2SO-d_6) δ 2.64 (t, 2 H), 3.50 (q, 2 H), 3.82, 3.85 and 3.96 (3s, 9 H), 6.96, 7.09, 7.23, 7.30, 7.66, 8.20 (6d, 1 H each), 8.0–8.8 (bs, 5 H), 10.01 and 10.40 (2s, 1 H each); FAB-MS (glycerol) 484 $(M - Cl)^+$; IR ν_{max} (Nujol) 1649, 1695, 3300 cm^{-1}.

1-Methyl-4-[1-methyl-4-(1-methyl-4-aminopyrrole-2-carboxamido)pyrrole-2-carboxamido]pyrrole-2-carboxamidopropionamidine Hydrochloride (18). A solution of 300 mg (0.58 mmol) of **17** in 10 mL of MeOH was hydrogenated over 130 mg of 10% Pd on charcoal at 40 °C. The catalyst was removed by filtration and the solvent removed in vacuo. The residue was extracted with 100 mL of cold isopropyl alcohol. The combined extracts were concentrated in vacuo to dryness, the residue was dissolved in a small volume of ethanol, and then ethyl acetate was added to precipitate the product as a white solid. The product was collected and transferred rapidly, while still moist, to a drying apparatus. Once the material is solid and dry it is less hygroscopic and represents pure **18**: 200 mg (70% yield); no distinct melting point; ^{1}H NMR (DMF-d_7) δ 3.4–3.8 (bs), 3.83, 3.90, 3.93 (3s, 9 H), 6.37, 6.55 (2d, J = 2 Hz, 1 H each), 7.10, 7.18, 7.32, 7.36 (4d, J = 2 Hz, 1 H each), 8.55 (t, J = 6 Hz, 1 H), 9.4–9.7 (bs, 4 H), 9.72 and 10.10 (2s, 1 H each); IR ν_{max} (Nujol) 1640, 1690, 3290 cm^{-1}; FAB-MS (glycerol) 454 $(M - Cl)^+$.

1-Methyl-4-[1-methyl-4-[1-methyl-4-(formylamino)-pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamidopropionamidine Hydrochloride (Distamycin Hydrochloride) (19). A solution of 240 mg (0.46 mmol) of **17** in 30 mL of methanol was hydrogenated over 120 mg of 10% Pd on charcoal during 2.5 h. The catalyst was removed by filtration, the filtrate concentrated to 15 mL and cooled to −40 °C, and a solution of *N*-formylimidazole, prepared from 2 mmol of carbonylimidazole as described for **13**, was added. After 30 min at −40 °C the solution was concentrated to a small volume and ethyl acetate was added to precipitate the product. The latter was collected, then extracted with cold isopropyl alcohol, and filtered through charcoal. The filtrate was concentrated to a small volume and EtOAc was added to precipitate the product. The latter was collected, washed with EtOAc and hexane, and dried in vacuo. The substance is amorphous without a distinct melting point. **19**: 170 mg (71% yield); ^{1}H NMR (Me_2SO-d_6) δ 2.62 (t, J = 6 Hz, 2 H), 3.51 (q, J = 6 Hz, 2 H), 3.82 (s, 3 H), 3.85 (s, 6 H), 6.93 and 6.96 (2d, J = 1.7 Hz, 2 H), 7.06 (d, J = 1.7 Hz, 1 H), 7.19 (m, 2 H), 7.23 (d, J = 1.7 Hz, 1 H), 8.13 (s, 1 H), 8.22 (t, J = 6 Hz, 1 H), 8.73 and 8.98 (2bs, 4 H), 9.92 and 9.94 (2s, 2 H), 10.12 (s, 1 H); IR ν_{max} (Nujol) 1642, 1690, 3280 cm^{-1}; FAB-MS 482 $(M - Cl)^+$.

The synthetic distamycin hydrochloride shows a ΔTm of 15.5° with calf thymus DNA in 20 mM potassium sodium phosphate buffer pH 6.9 at a D/P ratio of 1.0, identical with an authentic sample from Boehringer-Mannhiem, cat. no. 10442, batch 1417211. Compound **19** also exhibited the characteristic wide spectrum antiviral activity of authentic distamycin, in tests courtesy of Prof. Erik De Clercq, Leuven, Belgium.

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The Absolute Structure of (+)-Strigol

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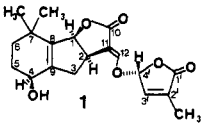
The absolute structure of the potent seed germination stimulant (+)-strigol has been established by resolution of racemic strigol via the corresponding *N*-[(*R*)-1-(1-naphthyl)ethyl]carbamate and X-ray crystallographic analysis.

Strigol (1) was isolated from root exudates of cotton (*Gossypium hirsutum* L.), and the relative structure was established by Cook and co-workers.[1] Strigol exhibited



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potent activity as a seed germination stimulant for *Striga* species and similar parasitic plants in the genus *Orobanche*.[2] It remains the most potent germination stimulant for seeds of witchweed (*Striga asiatica* (L.) Kuntze) which causes considerable damage to crops of the Gramineae family such as corn, sorghum, and sugarcane.[3] Witchweed seeds can remain dormant in the soil for several years until favorable conditions prevail including exposure to some type of chemical germination stimulant. The concept of invoking a chemical signal to break dormancy and stimulate germination of weed seeds is relevant to weed control. For parasitic weeds of the *Striga* type, inducing germination in the absence of a host plant would result in starvation of the seedling and hence offers an alternative to herbicide treatment.

Two total syntheses[4,5] of (±)-strigol were reported shortly after the structure determination. More recently, we have developed an improved synthesis of strigol,[6] and the

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