^{1}H NMR 7.00–7.15 (m, 4), 6.19 (s, 1), 4.28 (dd, 1, J = 7.5, 7.5), 2.79 (t, 2, J = 8.1), 2.52 (m, 2), 2.21 (m, 2), 1.5–2.0 (m, 7); ^{13}C NMR 147.6, 134.7, 134.4, 127.1, 126.4, 126.1, 125.5, 118.3, 70.0, 61.2, 35.9, 32.9 (2 C), 31.1, 28.4, 26.1, 25.4; IR ($CHCl_3$) 3615, 2920 cm^{-1}. Anal. Calcd for $C_{17}H_{20}O$: C, 84.96; H, 8.39. Found: C, 84.82; H, 8.32.

Rearrangement of 32. Alcohol **32** (0.128 g, 0.52 mmol) was stirred with KH (0.24 g of 35% dispersion in mineral oil, 2.08 mmol) in 10 mL of THF under N_2 at 0 °C for 2 h. Water was added, and the solution was extracted with ether. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to give 0.141 g of crude product containing mineral oil. Flash chromatography of 0.120 g (7:1 hexane–EtOAc) gave 22.0 mg (20%) of **34a**, followed by 83.9 mg (77%) of **34b** that was 90% pure, containing chromatographically inseparable impurities.

The data for **34a**: ^{1}H NMR 7.1–7.4 (m, 4), 4.27 (br s, 1, $w_{1/2} \approx 10$), 3.32 (br s, 1, $w_{1/2} \approx 14$), 2.80 (m, 3), 2.1–2.5 (m, 5), 1.2–1.9 (m, 5) [addition of D_2O simplified the peak at δ 4.27 (ddd, 1, J = 2.9, 6.1, 6.1), decoupling at δ 3.32 removed a coupling from the peak at δ 4.27 (dd, J = 2.9, 6.1)]; ^{13}C NMR 128.5 (CH), 128.4 (CH), 126.6 (CH), 125.9 (CH), 73.3 (CH), 45.2 (CH), 37.4 (CH), 36.3 (CH_2), 34.9 (CH_2), 29.8 (CH_2), 28.3 (CH_2), 27.2 (CH_2), 25.0 (CH_2) (the 4 quaternary carbons were not observed); IR ($CHCl_3$) 3580, 2940 cm^{-1}; UV (EtOH) λ_{max} (ϵ) 213.4 (10 335), 265.1 (2056), 271.8 (2000).

The data for **34b**: ^{1}H NMR 7.0–7.3 (m, 4), 4.47 (ddd, 1, $J \approx$ 4, 4, 4), 3.27 (br s, 1, $w_{1/2} \approx 7$), 2.72 (m, 2), 1.2–2.60 (m, 10), 1.05 (m, 1) [decoupling at δ 3.27 removed a coupling from the peak at δ 4.47 (dd, 1, J = 4, 4)]; ^{13}C NMR 128.2 (CH), 126.1 (CH), 125.8 (CH), 125.0 (CH), 69.4 (CH), 46.6 (CH), 36.1 (CH), 33.9 (CH_2), 33.6 (CH_2), 29.7 (CH_2), 28.4 (CH_2), 27.3 (CH_2), 24.3 (CH_2) (the 4 quaternary carbons were not observed); IR ($CHCl_3$) 3620, 2980 cm^{-1}; UV (EtOH) λ_{max} (ϵ) 220 (10 650), 266 (3520), 270 (3510).

Isomerization of 34b to 35. Treatment of **34b** with 10 equiv of KH at 0 °C for 2 h gave complete conversion to **35**. Alcohol **35** was recrystallized from hexane: mp 118–119 °C; ^{1}H NMR 7.54 (d, 1, J = 7.6), 7.10–7.25 (m, 3), 464 (br s, 1, $w_{1/2}$ = 8), 2.75 (m, 2), 1.4–2.5 (m, 12); ^{13}C NMR 141.1 (C), 135.5 (C), 134.4 (C), 128.0 (C), 127.4 (CH), 126.7 (CH), 126.1 (CH), 122.6 (CH), 64.6 (CH), 45.1 (CH), 35.2 (CH_2), 31.8 (CH_2), 31.6 (CH), 30.7 (CH_2), 28.3 (CH_2), 28.2 (CH_2), 24.2 (CH_2); IR ($CHCl_3$) 3610, 2960 cm^{-1}; UV (EtOH) λ_{max} (ϵ) 219 (18 640), 264 (11 177), 320 (180).

Spiro[cyclohexane-1,1′(2*H*)-3′,4′,5′,6′-tetrahydropentalen]-2′-one (39). Ketone **8** (62.2 mg, 0.33 mmol) was added to neat boron trifluoride etherate (0.5 mL, 4.07 mmol) at 25 °C. The mixture was stirred for 45 min at 25 °C. The reaction was quenched by addition of 2 mL of saturated sodium bicarbonate solution and extracted with two portions of ether. The combined ether layers were washed with brine, dried ($MgSO_4$), and concentrated to give 55.2 mg of crude **39**. Flash chromatography on silica gel (24:1 hexane–EtOAc) gave 47.4 mg (76%) of pure **39**: ^{1}H NMR 2.78 (br s, 2), 2.51 (m, 2), 2.38 (m, 2), 2.14 (m, 2), 1.75 (m, 2), 1.39–1.58 (m, 8); ^{13}C NMR 150.7, 137.9, 40.6, 32.5, 30.9, 30.6, 25.5, 25.1, 22.7 (the carbonyl and quaternary carbons were not observed); IR (neat) 2930, 2850, 1740, 1445 cm^{-1}.

Acknowledgment. We thank the National Institutes of Health for financial support.

Registry No. **1a**, 7766-48-5; **1b**, 113404-49-2; **2a**, 38267-20-8; **2b**, 113404-50-5; **3a**, 113404-50-5; **3b**, 113404-52-7; **3c**, 113404-53-8; **3d**, 113404-55-0; **5a**, 104923-37-7; **5b**, 113472-65-4; **5c**, 113472-66-5; **6a**, 113404-57-2; **6b**, 113404-58-3; **6c**, 113404-59-4; **8**, 113404-54-9; **9a**, 113404-60-7; **9b**, 113404-61-8; **9c**, 113404-82-3; (2*Z*,4*E*)-**11**, 113404-56-1; (2*Z*,4*Z*)-**11**, 113404-83-4; **12b**, 113404-62-9; **12c**, 113404-63-0; **14**, 113430-72-1; **15a**, 113404-64-1; **15b**, 97961-71-2; **15c**, 113404-84-5; (2*Z*,4*E*)-**17**, 54716-14-2; (2*Z*,4*Z*)-**17**, 54716-13-1; (2*E*,4*Z*)-**17**, 54716-16-4; (2*E*,4*E*)-**17**, 54716-17-5; **21a**, 113404-69-6; **21b**, 113404-72-1; **21c**, 113404-86-7; **22a**, 113404-73-2; **22b**, 113404-75-4; **22c**, 113404-76-5; **25**, 113404-65-2; **26**, 113404-66-3; **27**, 113404-67-4; **28**, 113404-68-5; **29a**, 113404-77-6; **29b**, 113404-79-8; **29c**, 113404-89-0; **31**, 113404-70-9; **32** (α-isomer), 113404-71-0; **32** (β-isomer), 113472-67-6; **34**, 113404-80-1; **35**, 113404-74-3; **39**, 113404-78-7; methyl crotonate, 18707-60-3; mesyl chloride, 124-63-0; 3-methyl-4-penten-1-ol, 51174-44-8; 3-methyl-4-penten-1-yl mesylate, 113404-81-2; methyl 1-cyclohexeneacetate, 53723-52-7; ethyl (*E*)-2-hexenoate, 27829-72-7; ethyl 3,3-dimethylacrylate, 638-10-8; ethyl (*E*)-3-methyl-2-pentenoate, 24410-84-2; ethyl (*Z*)-3-methyl-2-pentenoate, 27805-84-1; ethyl 3-ethyl-3,8-nonadienoate, 113404-85-6; 3,4-dimethyl-2-(4-pentenyl)-2-cyclobutenone, 113404-87-8; (1-methyl-1-propenyl)bicyclo[3.2.0]heptan-6-one, 113404-88-9; ethyl 3,4-dihydronaphthalene-2-acetate, 63625-94-5.

Total Synthesis and Absolute Configuration of the Antibiotic Oligopeptide (4*S*)-(+)-Anthelvencin A and Its 4*R*-(−) Enantiomer

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Received August 14, 1987

The total syntheses of the two enantiomers of anthelvencin A [the naturally occurring isomer (4*S*)-(+)-**1a** and its enantiomer (4*R*)-(−)-**1b**] with enantiomeric excess of 80 ± 4% are described. The absolute configuration of natural anthelvencin A is thereby unambiguously assigned. The two enantiomers, (+)-**1a** and (−)-**1b**, bind to duplex calf thymus DNA with constants of (1.46 ± 0.01) × 10^7 and (1.35 ± 0.01) × 10^7 M^{-1}, respectively, as determined by an ethidium displacement assay.

Anthelvencin A (**1**) is a naturally occurring oligopeptide isolated from the culture of *Streptomyces venezuelae*.[1] It has antibiotic and anthelmintic activity.[1] Anthelvencin A (**1**) is a member of a modest class of oligopeptides, all of which are biologically active. This class of natural products includes kikumycin B (**2**),[2,3] noformycin (**3**),[4] netropsin (**4**),[5] and distamycin A (**5**)[5b,6] (see Scheme I). The biological activities of **4** and **5** arise, in part, from their ability to bind to the minor groove of B-DNA at A·T-rich sequences.[7] The firm and site-specific binding of these sequence-reading oligopeptides and DNA is a net result

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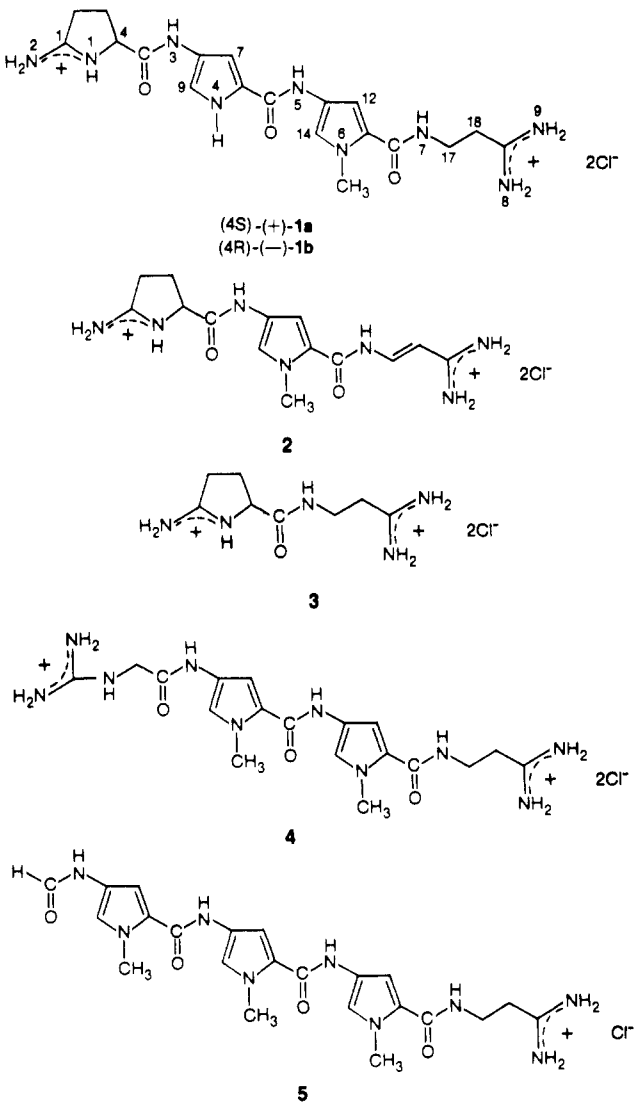
0022-3263/88/1953-1855$01.50/0

Scheme I[a]



[a] Anthelvencin A (1), kikumycin B (2), norformycin (3), netropsin (4), distamycin A (5).

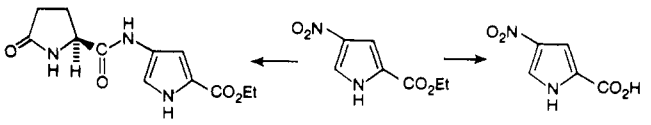
of specific hydrogen bonding, electrostatic attraction, and van der Waals interactions.[8] The sequence specificities of 1–3 have not, as yet, been reported.

The sequence-specific binding property of these natural products and DNA has stimulated much work in studying the interactions of netropsin (4)[8b,9] and distamycin A (5)[10] with B-DNA. Our interest in molecular recognition has led to the design of lexitropsins, or information-reading oligopeptides, that can read DNA sequences. For example, the lexitropsin formylimidazolylimidazolylamidinium chloride has been shown to read the squence 5′-CCGT-3′ uniquely.[11] This is a result, inter alia, of hydrogen-bond formation between guanine 2-NH_2 in the minor groove and N3 of the imidazole moiety.[12]

We have recently studied the molecular recognition of chiral lexitropsins, such as (4*S*)-(+)- and (4*R*)-(−)-dihydrokikumycin B,[3a] and DNase I footprinting studies show that the natural (+) enantiomer binds more strongly to the sequence $(A{\cdot}T)_4$ of the *Nci*I/*Hin*dIII fragment of pBR322 DNA than the (−) enantiomer.[3b] We now report studies aimed at synthesizing chiral lexitropsins that possess three heterocyclic moieties and assessing the effects of chirality on DNA binding and, possibly, on biological activity.

Results and Discussion

The synthetic route to anthelvencin A (1) is outlined in Scheme II and follows our recent synthesis of (+)- and (−)-dihydrokikumycin B.[3] In the synthesis of anthelvencin A, a 4-aminopyrrole-2-carboxylate synthon is needed. The required starting nitropyrrole ester **6**[13] can be readily prepared from glycine ethyl ester hydrochloride and sodium nitromalonic aldehyde.[14] Only a limited amount of information is available on the condensation of β-aminopyrroles with carboxylic acid derivatives.[15] This has been due, in part, to the limited availability of β-aminopyrroles.[15] Therefore, we have examined the condensation of (4*S*)-(−)-2-pyrrolidone-5-carboxylic acid with the β-aminopyrrole derived from catalytic reduction of ester **6**. This condensation reaction was effected with dicyclohexylcarbodiimide (DCC) and a catalytic amount of 4-(dimethylamino)-pyridine (DMAP) to afford ester **7** in 71% yield. The coupling reaction did not proceed without DMAP, and only tar was obtained. This is presumably because of the low nucleophilicity of the aminopyrrole combined with its high instability.



7 **6** **8**

Alkaline hydrolysis of ester **6** followed by acidification provided acid **8** in quantitative yield.[13] Nitrile **9** can be readily prepared in high yield from 1-methyl-2-pyrrolecarboxylic acid.[5b] Catalytic reduction of the nitro substituent in **9** provided an unstable amine intermediate, which was coupled with the acid chloride of **8**[16] in the presence of triethylamine to afford **10** in 70% yield. Pinner reaction of **10** with HCl in dry ethanol[17] followed by treatment of the imidate ester intermediate with ammonia gave the amidine hydrochloride **11** in quantitative yield. The presence of the amidinium moiety in **11** is corroborated by the appearance of two exchangeable ^1H NMR signals (two protons each) at 8.78 and 9.10 ppm and the disappearance of the infrared nitrile stretch of **10** at 2247 cm^{-1}. The nitro group of **11** was reduced catalytically to give amine **12**, which is unstable in solution. The amine **12**, when allowed to react with the optically active (4*S*)-(−)-2-amino-1-pyrroline-5-carboxylic acid (**13a**)[3] (84 ± 4% ee) in the presence of DCC and DMAP, gave (+)-anthelvencin A (**1a**) in 49% yield. The structure of **1a** is con-

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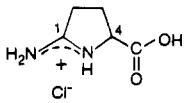
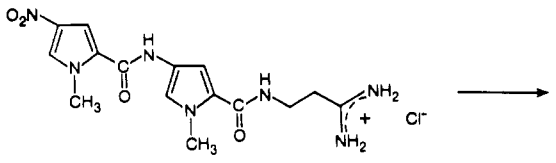
firmed by the presence of a M − H − 2Cl ion at m/z 428 in the FAB-MS anlaysis, by the appearance of the ^1H NMR NH3 signal at 10.52 ppm, and by comparison with the published IR and UV data of the natural product.[1] ^1H NMR analysis of **1a** in the presence of a chiral shift reagent [tris[3-[(trifluoromethyl)hydroxymethylene]-(+)-camphorato]europium(III)] in a 1:1 DMSO-d_6/CD_3CN solution and measurement of the resulting unequal diastereotopic amide NH5 signals at about 10 ppm provided the enantiomeric excess (ee) of **1a** to be 80 ± 4%. Therefore, judging by the small differences in ee values of **1a** (80 ± 4%) and the starting acid **13** (84 ± 4%), racemization in the coupling reaction effected by DMAP is minimal, i.e., ~2%.



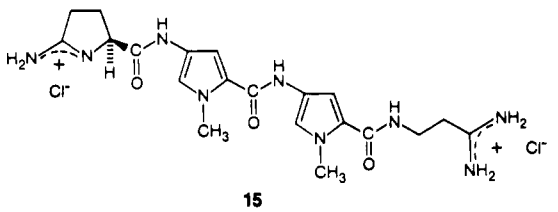
(4S)-(−)-**13a**
(4R)-(+)-**13b**

Comparison of the specific rotation of the synthetic material **1a** with that of the natural antibiotic[1] establishes the absolute configuration of (+)-anthelvencin as 4*S*.

The unnatural (4*R*)-(−)-anthelvencin (**1b**) was synthesized by using the procedure described above from acid (4*R*)-(+)-**13b**.



14



15

We have also attempted to prepare both enantiomers of *N*-methylanthelvencin A (**15**). Reduction of the nitro moiety in **14**[5b] followed by coupling with acid (4*S*)-(−)-**13a** gave **15**. However, **15** is highly hygroscopic and unstable; thus a pure sample of *N*-methylanthelvencin A cannot be obtained. This high instability may be the reason why the normethyl form of **15** (i.e., the natural product **1a**) is selected for by the producing microorganism.[1]

Binding of (+)- and (−)-Anthelvencin A to Calf Thymus DNA. Both enantiomers of anthelvencin A, (+)-**1a** and (−)-**1b**, bind to duplex native DNA. Their binding constants to calf thymus DNA determined by the ethidium binding assay which gives relative values[18] are: 1.46×10^7 M^{-1} for **1a** and 1.35×10^7 M^{-1} for **1b**. The binding constant for netropsin under these conditions is 1.86×10^6 M^{-1}.[18] The uncertainty for the binding constants is $\pm 0.01 \times 10^6$ M^{-1}. The DNA sequence specificity and the effects of chirality on DNA binding at individual sites determined by quantitative DNase and MPE footprinting, together with the biological activities of **1a** and **1b**, will be reported in due course.

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Experimental Section

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. The IR spectra were recorded on a Nicolet 7199 FT spectrophotometer, and only the principal bands are reported. The ^1H NMR spectra were recorded on Bruker WH-200 and WH-400 spectrometers. FAB (fast atom bombardment) mass spectra using glycerol as the matrix were determined on an Associate Electrical Industries (AEI) MS-9 and MS-50 focusing high-resolution mass spectrometer. Kieselgel 60 (230–400 mesh) of E. Merck was used for flash chromatography, and precoated silica gel 60F-254 sheets (Merck) were used for TLC, with the solvent system indicated in the procedure. TLC plates were visualized by using UV light or 2.5% phosphomolybdic acid in methanol with heating.

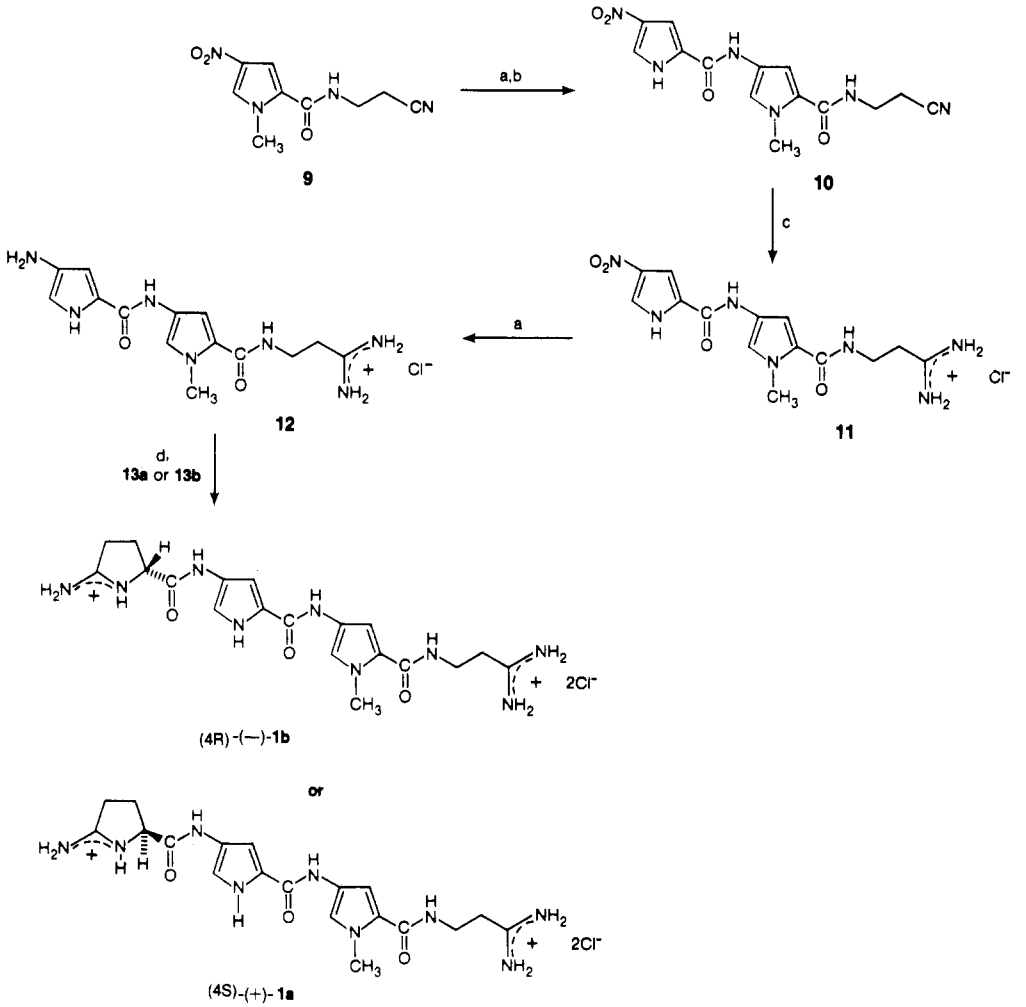
All compounds obtained commercially were used without further purification unless otherwise stated. Ethanol and methanol were freshly distilled from magnesium turnings; tetrahydrofuran was distilled from sodium/benzophenone under an atmosphere of dry argon; ether was dried over sodium; methylene chloride was distilled from phosphorus pentoxide and stored over molecular sieves, 3A; triethylamine was treated with potassium hydroxide, then distilled from barium oxide, and stored over molecular sieves, 3A; dimethylformamide was distilled from barium oxide and stored over molecular sieves, 3A.

Ethyl 4-Nitropyrrole-2-carboxylate (6).[13] A warm (50 °C) solution of sodium nitromalonaldehyde (5.0 g, 35.7 mmol) and glycine ethyl ester hydrochloride (4.4 g, 29.4 mmol) in 65% ethanol (18.5 mL) was treated with an aqueous solution of 20% sodium hydroxide (2.7 mL, dropwise). After the solution was stirred at 50 °C for 1 h, the reaction mixture was chilled (5 °C) and the precipitate collected. The crude product was recrystallized from methanol to give ester **6** as fine needles (2.60 g, 48% yield): mp 174–175 °C (lit.[13] mp 174 °C); TLC (5:45:55 methanol/hexane/ethyl acetate) R_f 0.85; IR (Nujol) 3270, 3154, 1701, 1687, 1508, 1467, 1458, 1329, 1208 cm^{-1}; ^1H NMR (DMSO-d_6) δ 13.1 (s br, 1 H), 8.08 (d, 2.0, 1 H), 7.26 (d, 2.0, 1 H), 4.29 (q, 6.6, 2 H), 1.31 (t, 6.6, 3 H).

Ethyl 4-(2-Oxopyrrolidine-5-carboxamido)pyrrole-2-carboxylate (7). A solution of ester **6** (859 mg, 4.67 mmol) and 5% palladium on charcoal (253 mg) in methanol (20 mL) was hydrogenated at atmospheric pressure and room temperature, until TLC analysis indicated complete reduction of the nitro compound. The catalyst was removed by filtration. Concentration of the filtrate gave an oily product, which was coevaporated with dry methylene chloride (twice). Owing to the instability of the amine intermediate, it was used directly in the next step. The amine, (*S*)-2-pyrrolidone-5-carboxylic acid (920 mg, 7.13 mmol), 4-(dimethylamino)pyridine (DMAP; 77.8 mg, 0.64 mmol), and dicyclohexylcarbodiimde (DCC; 1.44 g, 6.97 mmol) were dissolved in dry DMF (25 mL) at 0 °C. After 5 min at 0 °C, the solution was stirred under an atmosphere of argon for 17 h at room temperature. The urea was removed by filtration, and concentration of the filtrate in vacuo gave an oil, which was dissolved in a small amount of chloroform and set aside at 5 °C for 16 h. The resulting white crystalline material was collected and dried under high vacuum (about 80 °C) to afford **7** (881 mg, 71% yield): mp 198–202 °C; TLC (10% MeOH/$CHCl_3$) R_f 0.44; IR (Nujol) 3298, 1733, 1662, 1558, 1450 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.67 (s br, 1 H), 10.08 (s, 1 H), 7.87 (s, 1 H), 7.26 (s, 1 H), 6.76 (s, 1 H), 4.23 (q, 8.1, 2 H), 4.12 (dd, 4.0, 5.0, 1 H), 2.31 (m, 1 H), 2.14 (m, 2 H), 1.96 (m, 1 H), 1.29 (t, 8.1, 3 H); exact MS, m/z (relative intensity) for $C_{12}H_{15}N_3O_4$ 265.1063 (M^+, 28).

4-Nitropyrrole-2-carboxylic Acid (8).[13] A solution of ester **6** (2.0 g, 10.9 mmol) in 15% KOH solution (31 mL) was heated to gentle reflux for 4 h. The solution was chilled (about −20 °C) and acidified to about pH 1 with concentrated HCl. The resulting pale yellow precipitate was extracted with ether (4 times). The combined ether extracts were dried ($MgSO_4$), and removal of the drying agent and solvent gave a pale yellow solid, which was dried in vacuo (80 °C) for 2 h to afford acid **8** (1.70 g, 99% yield): mp 210 °C dec (CO_2 gas liberated) (lit.[13] mp 217 °C); IR (Nujol) 3640, 3200, 1700, 1575, 1464, 1377, 1310 cm^{-1}; ^1H NMR (DMSO-d_6) δ

Scheme II[a]



[a] Reaction conditions: (a) H_2, Pd/C, MeOH room temperature; (b) acid chloride of 8, Et_3N in THF; (c) HCl in dry EtOH, then dry NH_3; (d) DCC, DMAP, DMF, room temperature, 16 h, (4*S*)-(−)-**13a** or (4*R*)-(+)-**13b**.

13.21 (s br, 1 H), 12.9 (s br, 1 H), 7.86 (d, 1.7, 1 H), 7.06 (d, 1.7, 1 H).

3-[1-Methyl-4-(4-nitropyrrole-2-carboxamido)pyrrole-2-carboxamido]propionitrile (10). A suspension of acid 8 (761 mg, 5.59 mmol) in dry dimethoxyethane (DME) (12 mL) and dry triethylamine (1.3 mL) was stirred at ambient temperature for 10 min. The reaction mixture was concentrated, resuspended in dry DME (13 mL), and chilled (0 °C). Thionyl chloride (8.5 mL) in dry DME (8.5 mL) was then added slowly. After 15 min, the resulting solution was concentrated to dryness, and the acid chloride product was coevaporated with dry CH_2Cl_2 (twice).

A suspension of nitrile **9** (1.01 g, 5.16 mmol) and 5% Pd on charcoal (360 mg) in methanol (29.0 mL) was hydrogenated at room temperature and atmospheric pressure to give the unstable amine intermediate. The oily amine was coevaporated with dry CH_2Cl_2 (twice) to give a foamy material.[5b] The amine was suspended in dry THF (22.0 mL) containing dry triethylamine (1.3 mL) and chilled (−20 °C). A solution of the acid chloride prepared from 8 in dry CH_2Cl_2 (13 mL) was added dropwise. After 15 min at −20 °C, the reaction mixture was stirred under argon at room temperature for 16 h. The solvent was removed under reduced pressure to give a solid, which was washed with water (twice), carbon tetrachloride (once), and hexane (once) to give a tan crystalline product **10** (1.20 g, 70% yield), which was homogeneous by TLC and NMR analyses: mp 255 °C dec; TLC (5:45:55 MeOH/hexane/ethyl acetate) R_f 0.32; IR (Nujol) 3388, 3270, 2247, 1637, 1465, 1376, 1310 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.88 (s br, 1 H), 8.42 (t, 6.3, 1 H), 7.96 (s, 1 H), 7.61 (s, 1 H), 7.26 (s, 1 H), 6.87 (s, 1 H), 3.83 (s, 3 H), 3.40 (q, 6.3, 2 H), 2.74 (t, 6.3, 2 H); exact MS, *m/z* (relative intensity) for $C_{14}H_{14}N_6O_4$ 330.1069 (M^+, 46).

3-[1-Methyl-4-(4-nitropyrrole-2-carboxamido)pyrrole-2-carboxamido]propionamidine Hydrochloride (11). A chilled (0 °C) suspension of nitrile **10** (994.6 mg, 3.01 mmol) in dry ethanol (4 mL) was bubbled with dry hydrogen chloride for 15 min. The resulting solution was stirred at 0 °C for an additional hour and at room temperature for 30 min. Removal of the solvent under reduced pressure gave a solid residue, which was washed with dry ether (once). The residue was redissolved in dry EtOH (42 mL) and chilled (0 °C). Dry ammonia was condensed into the reaction mixture. After 50 min at 0 °C, the solvent was removed to give a pale brown solid, which was washed with carbon tetrachloride and filtered. The residue was washed with 2-propanol and CCl_4 and then dried under high vacuum at 80 °C to give amidine **11** (1.15 g, 96% yield): NMR and TLC analyses showed that **11** is pure; mp 275 °C dec; TLC (10:85:5 MeOH/$CHCl_3$/HOAC) R_f 0.33; IR (Nujol) 3200 (br), 1696, 1639, 1580, 1553, 1500, 1466, 1370, 1312 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.88 (s br, 1 H), 10.49 (s, 1 H), 9.10 (s br, 2 H), 8.78 (s br, 2 H), 8.36 (t, 6.4, 1 H), 7.97 (s, 1 H), 7.67 (s, 1 H), 7.29 (s, 1 H), 6.94 (s, 1 H), 3.84 (s, 3 H), 3.53 (q, 6.4, 2 H), 2.67 (t, 6.4, 3 H), after D_2O exchange 7.95 (s, 1 H), 7.57 (s, 1 H), 7.22 (s, 1 H), 6.85 (s, 1 H), 3.77 (s, 3 H), 3.50 (t, 6.2, 2 H), 2.60 (t, 6.2, 3 H); FAB-MS, *m/z* (relative intensity) $(C_{14}H_{17}N_7O_4)H^+$ 348, found 348 (M − Cl, 31). Anal. Calcd for $C_{14}H_{18}N_7O_4Cl$ (383.45): C, 43,8; H, 4.7; N, 25.6. Found: C, 43.5; H, 4.6; N, 26.0.

3-[1-Methyl-4-[4-(2-amino-1-pyrroline-5-carboxamido)-pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine Dihydrochloride. (4*S*)-(+)-Anthelvencin A (1a). A suspension of amidine **11** (250 mg, 0.65 mmol) in methanol (60 mL) was hydrogenated over 10% Pd on charcoal (125 mg) at room temperature and atmospheric pressure. The reaction was stopped

when TLC analysis showed complete disappearance of the starting material 11. Removal of the catalyst and concentration of the filtrate gave a residue, which was coevaporated with dry CH_2Cl_2 (twice) to give the amine intermediate **12** as a glassy green solid: ^{1}H NMR (DMSO-d_6) δ 10.67 (s, 1 H), 9.65 (s, 1 H), 8.97 (s br, 2 H), 8.61 (s br, 2 H), 8.21 (t, 6.0, 1 H), 7.18 (s, 1 H), 6.84 (s, 1 H), 6.39 (s, 1 H), 6.27 (s, 1 H), 3.80 (s, 3 H), 3.50 (q, 6.0, 2 H), 3.20 (s br, 2 H), 2.61 (t, 6.0, 3 H), and after D_2O exchange 7.13 (s, 1 H), 6.79 (s, 1 H), 6.40 (s, 2 H), 3.76 (s, 3 H), 3.50 (t, 6.0, 2 H), 2.60 (t, 6.0, 3 H).

Owing to the instability of **12**, it was used directly in the next step. The mixture of amine **12**, (4*S*)-(−)-2-amino-1-pyrroline-5-carboxylic acid hydrochloride (**13a**) (145.6 mg, 0.89 mmol), and DMAP (11.4 mg, 0.10 mmol) was dissolved in dry DMF (3.5 mL) and chilled (0 °C). A solution of DCC (185.5 mg, 0.90 mmol) in dry DMF (3.5 mL) was added. After 15 min at 0 °C, the solution was stirred under an atmosphere of argon at ambient temperature for 16 h. The urea was removed by filtration, and removal of the solvent under reduced pressure gave a solid residue. Water (10 mL) was added, and the solid that precipitated was removed by filtration. Concentration of the aqueous extract gave a solid residue, which was redissolved in methanol and the hot solution decolorized with activated charcoal. The filtrate was concentrated to a small volume, and acetone was added to crystallize (4*S*)-(+)-anthelvencin A (**1a**) as a white solid. The product was collected by filtration, and the residue was quickly dried in vacuo (80 °C), to avoid decomposition, to afford **1a** (158 mg, 49% yield) which is homogeneous by TLC analysis: mp 170–175 °C dec; TLC (90:5:5 MeOH/$CHCl_3$/HOAC) R_f 0.17; $[\alpha]^{24}_D$ + 7.7° (*c* 0.0030, H_2O) [lit.[1] $[\alpha]^{22}_D$ +9.7° (H_2O)]; UV (H_2O) 238 (ϵ 3.95), 292 nm (4.03) (lit.[1] 235, 300 nm); IR (Nujol) 3127 (br), 1689, 1642, 1584, 1527, 1405 cm^{-1} [lit.[1] (mineral oil) 3120 (br), 1690, 1635, 1580, 1530, 1400 cm^{-1}]; ^{1}H NMR (DMSO-d_6) δ 11.44 (s br, NH4), 10.52 (s, NH3), 10.09 (s, NH5), 9.04 and 9.01 (both s, 7 amidine H's), 8.37 (t, 5.8, NH7), 7.23, 7.16, 6.97, 6.89 (4 d, 1.2, H14, H9, H7, H12), 4.60 (dd, 4.9, 8.6, H4), 3.81 (s, N6-CH_3), 3.51 (q, 5.8, H17), 2.85 (t, 7.7, H2), 2.64 (t, 5.8, H18), 2.07 (m, H3), and after D_2O exchange 7.17, 7.13, 6.88, 6.79 (4 d, 1.6, H7, H9, H12, H14), 4.51 (dd, 4.9, 8.6, H4), 3.73 (s, N6-CH_3), 3.66 (t, 5.0, H17), 2.85 (m, H2, H3), 2.64 (t, 5.0, H18), 2.04 (m, H3); FAB-MS, *m/z* (relative intensity) $(C_{19}H_{25}N_9O_3)H^+$ 428, found 428 (M − H − 2Cl, 2), 315 (17), 209 (8), 123 (100).

The preparation of (4*R*)-(−)-**1b** followed the procedure given above. The characterization data for (−)-**1b** are identical with those of (+)-**1a** except for $[\alpha]^{24}_D$ −8.0° (*c* 0.0063, H_2O).

***N*-Methylanthelvencin A (15).** A suspension of amidine **14** (200 mg, 0.54 mmol) and 5% Pd on carbon (307 mg) in methanol (25 mL) was hydrogenated at atmospheric pressure and room temperature. Removal of the catalyst and solvent gave a residue, which was coevaporated with dry CH_2Cl_2 (twice) to give a foamy amine intermediate. The amine, (4*S*)-(−)-2-amino-1-pyrroline-5-carboxylic acid hydrochloride (**13a**) (119.2 mg, 0.72 mmol), and DMAP (8.9 mg, 0.07 mmol) were dissolved in dry DMF (8 mL) and chilled (0 °C). A solution of DCC (167.9 mg, 0.82 mmol) in dry DMF (4 mL) was added. After 15 min at 0 °C, the ice bath was removed, and the solution was stirred at room temperature for 16 h. The urea was removed by filtration, and concentration of the filtrate gave an oily residue. The residue was washed with CCl_4, and the product was extracted with water (10 mL). The resulting precipitate was removed by filtration, and removal of the solvent gave a brown oily residue, which was redissolved in MeOH and decolorized with charcoal. The filtrates was concentrated to a small volume, and acetone was added to precipitate **15** as a pale brown solid. All attempts to isolate the product by filtration failed to give any good-quality material. The product is hygroscopic and unstable in air and decomposes readily during the filtration procedure: IR (Nujol) 3120 (br), 1688, 1648, 1584, 1522, 1402 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.39 (s, 1 H), 9.96 (s, 1 H), 8.38 (t, 5.5, 1 H), 7.23, 7.22, 6.92, 6.89 (4 s, 1 H each), 4.48 (dd, 4.5, 8.0, 1 H), 3.83, 3.81 (2 s, 3 H each), 3.48 (q, 5.5, 2 H), 2.80 (m, 2 H), 2.55 (t, 5.5, 3 H), 2.45 (m, 1 H), 2.00 (m, 1 H), and after D_2O exchange 7.14 (s, 2 H), 6.81 (s, 1 H), 6.78 (s, 1 H), 4.51 (dd, 4.5, 8.0, 1 H), 3.76, 3.72 (2 s, 3 H each), 3.50 (t, 5.7, 2 H), 2.80 (m, 2 H), 2.57 (t, 5.7, 3 H), 2.45 (m, 1 H), 2.00 (m, 1 H); FAB-MS, *m/z* (relative intensity) $(C_{20}H_{27}N_9O_3)H^+$ 451, found 451 (M − H − 2Cl, 1).

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