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SYNTHESIS AND NMR CHARACTERIZATION OF SELECTIVELY ^{15}N-LABELLED *TRANS*- AND *CIS*-BUTENEDIAMIDO-LINKED BIS-NETROPSINS

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SUMMARY

The synthesis of specifically ^{15}N labelled *trans*- and *cis*-isomers of bis-netropsins derived from 2-butene-1,4-dicarboxylate as the central linker, namely the (*E*)- and (Z)-forms of N-[2-[2-[(3-amino-3-iminopropyl)(^{15}N)aminocarbonyl]-1-methylpyrrole-4-aminocarbonyl]-1-methylpyrrol-4-yl], N'-[2-[2-[(3-amino-3-iminopropyl)aminocarbonyl]-1-methylpyrrole-4-aminocarbonyl]-1-methylpyrrol-4-yl]-2-butenediamide (**18** & **28**) are described. A highly convergent synthetic route was adopted for the preparation of the *trans*-derivative starting from N-methyl-4-nitro-2-trichloroacetylpyrrole and fumaroyl chloride. The most efficient methods of coupling aminopyrrole intermediates with activated carboxylic acid derivatives were utilized and the ^{15}N-labelled moieties were incorporated in the later stages of the synthesis. The preparation of the *cis*-derivative, starting with maleic anhydride, required a modification of the above strategy due to the nature of products formed upon initial coupling with aminopyrrole derivatives. ^{1}H- and ^{15}N-NMR were used to fully characterize the final products. The ^{1}H NMR experiment allowed the assignment of an intramolecular hydrogen bond for the *cis* bis-netropsin **28**.

KEYWORDS: bis-netropsin, butenedioate-linked, ^{15}N labelling, DNA binding agents.

INTRODUCTION

The natural products netropsin and distamycin (Figure 1) are crescent shaped di- and tripeptides, respectively, which exhibit a wide spectrum of antitumor, antimicrobial and antiviral properties, largely due to interactions with DNA (1-3). These compounds bind in the minor groove of DNA at sites of four or five contiguous AT base pairs. In an effort to expand the binding site size to 15-16 base pairs, that is expected to comprise a unique sequence site in the human genome, synthetic and DNA binding studies on a series of dimeric analogs bearing netropsin moieties joined by polymethylene linkers, $(CH_2)_n$ of variable length, or by rigid linkers have been reported by us (for review see references 2,3). One significant advantage of such compounds is the convergence of twice as many useful functional groups, e.g., the periodically

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spaced amide NH groups which are involved in hydrogen bonding with AT base pairs along an extended length of the minor groove. Compared with the flexible polymethylene chain-linked bis-netropsins where both the mono- and bidentate binding modes prevail, our use of conformationally constrained cycloalkane and olefenic linkers proved more effective (in *trans* configuration) for the desired high-affinity bidentate binding of dimeric oligopeptides to duplex DNA (2-4).

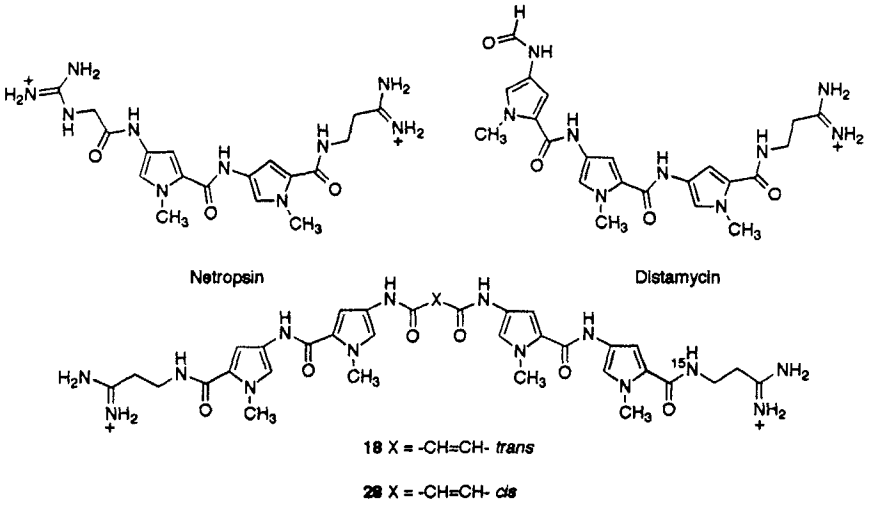


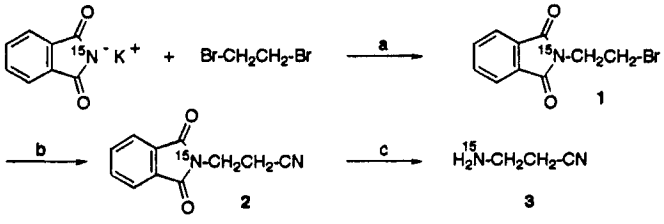
Figure 1: Structural formulae of netropsin, distamycin and the target ^{15}N-labelled bis-netropsins **18** and **28** described in this study.

Structural studies, primarily using proton NMR and X-ray methods, have been employed to gain a fairly detailed understanding of the contributing factors to highly specific molecular recognition of the DNA minor groove by netropsin, distamycin, and structurally related compounds. However, two important questions, the exact nature of intermolecular ligand-DNA hydrogen bonds and the dynamic processes such as kinetics of exchange of netropsin-like ligands between equivalent DNA binding sites, have not been addressed adequately. In these aspects, the use of ^{15}N NMR studies employing netropsin/distamycin fragments specifically labelled with ^{15}N may provide valuable information regarding structure and dynamics of ligand-DNA interactions. Towards these ends and to investigate further the mono- and bidentate binding modes for the dimeric analogs, we present the synthetic routes for obtaining the isomeric pair of *cis* and *trans*-butenediamido-linked bis-netropsins, shown in Figure 1.

SYNTHESIS

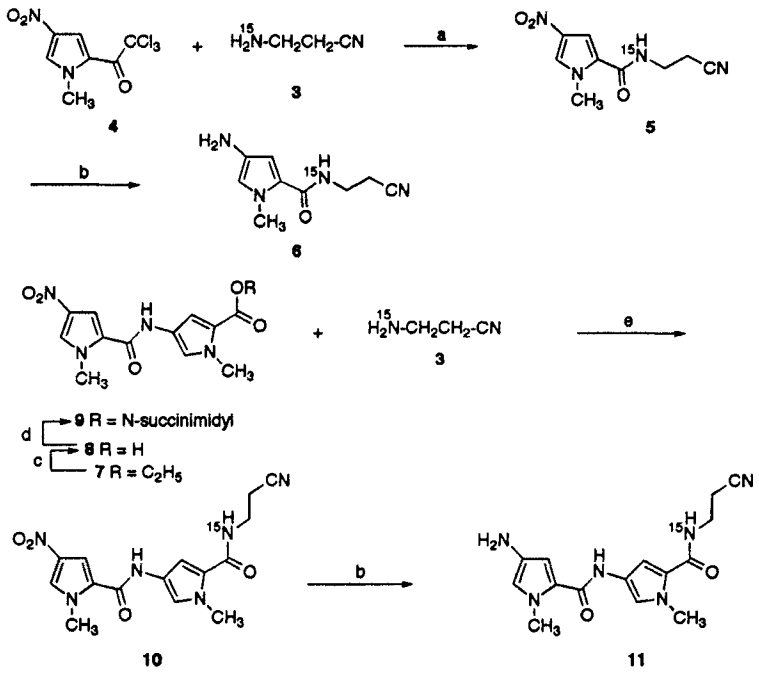
Our choice of incorporating the ^{15}N labels in the terminal aliphatic head groups (propanamidine) was dictated primarily by the fact that such molecules are presumably tightly anchored within the DNA minor groove via the positively charged termini. The primary synthon, ^{15}N-labelled β-aminopropionitrile **3**, was

prepared in 3 steps from potassium (^{15}N) phthalimide, a low cost commercially available material, as shown in Scheme 1. In the second step, substitution reaction with potassium (^{15}N)cyanide was also carried out to afford a doubly ^{15}N-labelled 3-aminopropionitrile. However, the use of rather stringent Pinner reaction conditions (ethanolic HCl followed by excess NH_3), to convert the nitrile to amidine functionality (5) in the later stages of the synthesis of target compounds **18** and **28**, makes the doubly labelled derivatives rather inaccessible due to the exchange of the nitrile ^{15}N-label with NH_3 (6).



Scheme 1: (a) DMF, 90 °C, 1 h; (b) KCN/DMSO, 65 °C, 6 h; (c) hydrazine monohydrate, MeOH, 25 °C, 20 h.

Further elaboration of ^{15}N-labelled amine **3** to aminopyrrolecarboxamide **6** and aminobis(pyrrolecarboxamide) **11** is shown in Scheme 2. Thus, N-methyl-4-nitro-2-trichloroacetylpyrrole **4**, prepared according to the method described by Nishiwaki *et al.* (7), was condensed with **3** and further



Scheme 2: (a) Et_3N/THF, 70 °C, 20 h; (b) 10% Pd-C, CF_3COOH, MeOH-DMF (4:1 v/v), H_2 (60 psi), 30 °C, 3 h; (c) 1 N aq. NaOH, EtOH, 70 °C, 2 h; (d) TSTU, Et_3N, DMF, 25 °C, 20 h; (e) Et_3N, DMF, 25 °C, 20 h.

reduced to afford **6**, which was isolated and stored as the trifluoroacetate salt to prevent any oxidative/photoiytic degradation. The bispyrrole derivative **7** was also prepared according to Nishiwaki *et al.* (7) and hydrolysed to the acid **8**, followed by activation to the N-succinimidyl ester **9** by reaction with N,N,N',N'-tetramethyl-O-(N-succinimidyl)uronium tetrafluoroborate (TSTU), according to the general procedure of Knorr *et al.* (8). The condensation reaction with amine **3** gave **10** which afforded the intermediate **11** upon catalytic hydrogenation.

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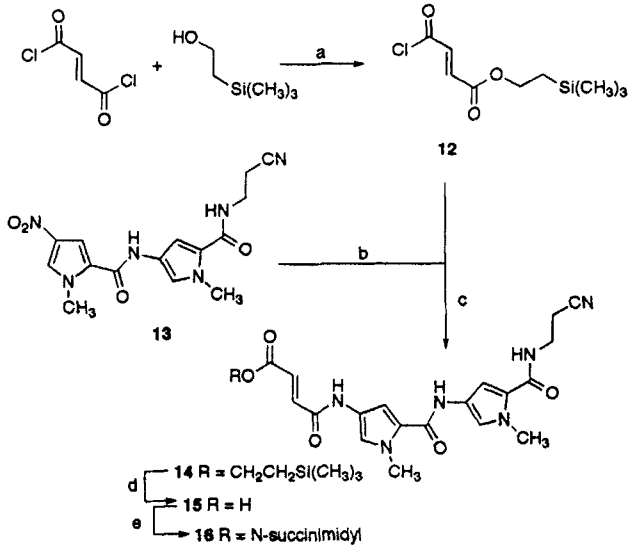
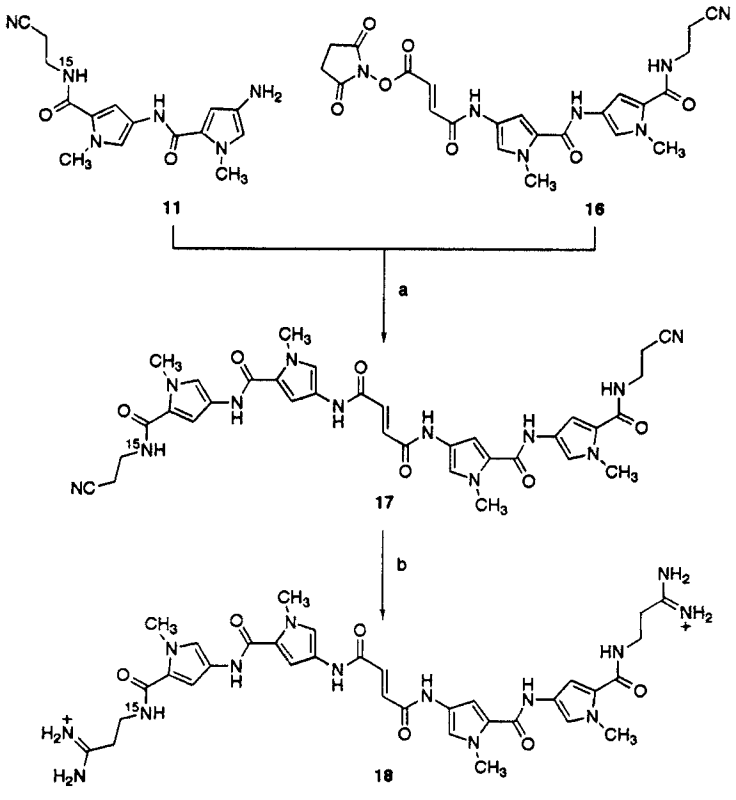
The preparation of the target ^{15}N-labelled derivative is outlined in Schemes 3 and 4. The asymmetric monoactivated chloride **12**, prepared from fumaroyl chloride and β-trimethylsilylethanol, was allowed to react with the aminobis(pyrrolecarboxamide) derivative obtained by reduction of **13**. The condensation product **14** was treated with tetrabutylammonium fluoride (TBAF) to afford the acid **15** (9), which was converted to the active N-succinimidyl ester form **16**.



Scheme 3: (a) Benzene, 25 °C, 48 h ; (b) 10% Pd-C, MeOH-DMF (4:1 v/v), H_2 (60 psi), 30 °C, 3 h; (c) Et_3N, DMF-THF(2:1 v/v), 20 h; (d) TBAF, THF, 20 h; (e) TSTU, $(^iPr)_2EtN$, 25 °C, 20 h.

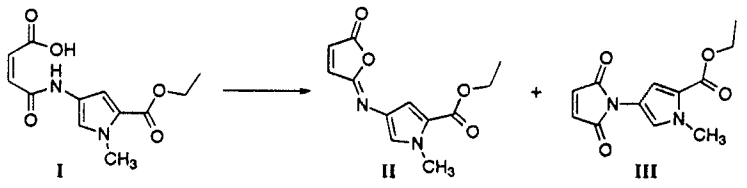
Condensation of **16** with the ^{15}N-labelled amine **11** provided the covalently linked bis-netropsin framework in the form of the dinitrile **17** which, under Pinner reaction conditions described in detail by Lown (5), provided the target diamidinium compound **18** (10).



Scheme 4 : (a) Et_3N, DMF, 25 °C, 20 h; (b) Ethanolic HCl, -78 °C, 3 h ; followed by liq. NH_3, dry EtOH, 25 °C, 20 h.

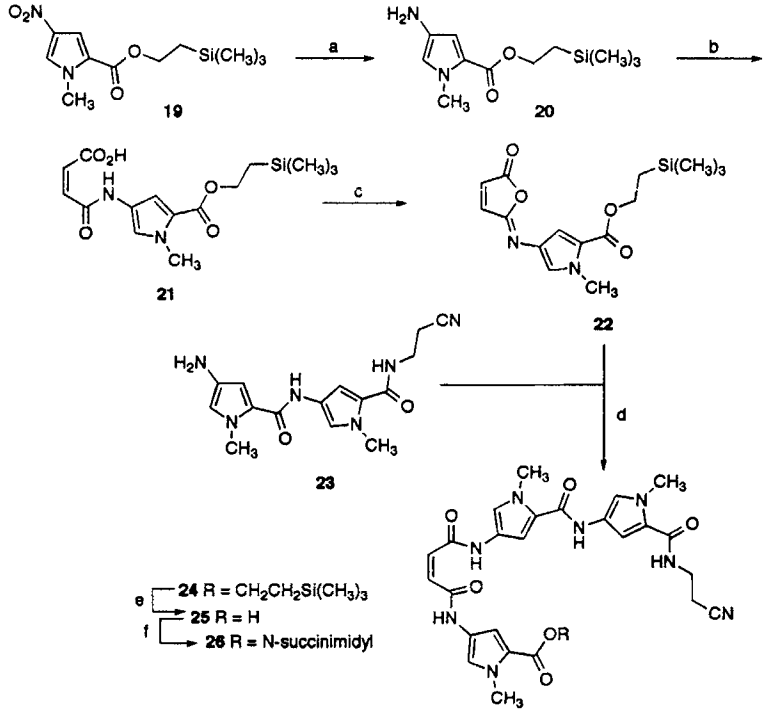
cis-Butenediamido-linked bis-netropsin 28

In our attempts to extend the above strategy to the preparation of the *cis* derivative, it was observed that although the *cis*-isomer of acid **15** could be obtained from **13** and maleic anhydride, it was quite resistant to further activation to the succinimidyl ester form analogous to **16**. The model active ester formation reactions, carrried out on a pyrrolecarboxamide derived from maleic acid, compound **I** (Scheme 5), indicated the nature of two relatively "inactive" products, namely the cyclic isoimide **II** and the imide form **III**. Fortunately, the isoimide form **II** was found to be useful for further elaboration to the final product.



Scheme 5: TSTU, Et_3N, dichloromethane, 30 °C, 3 h

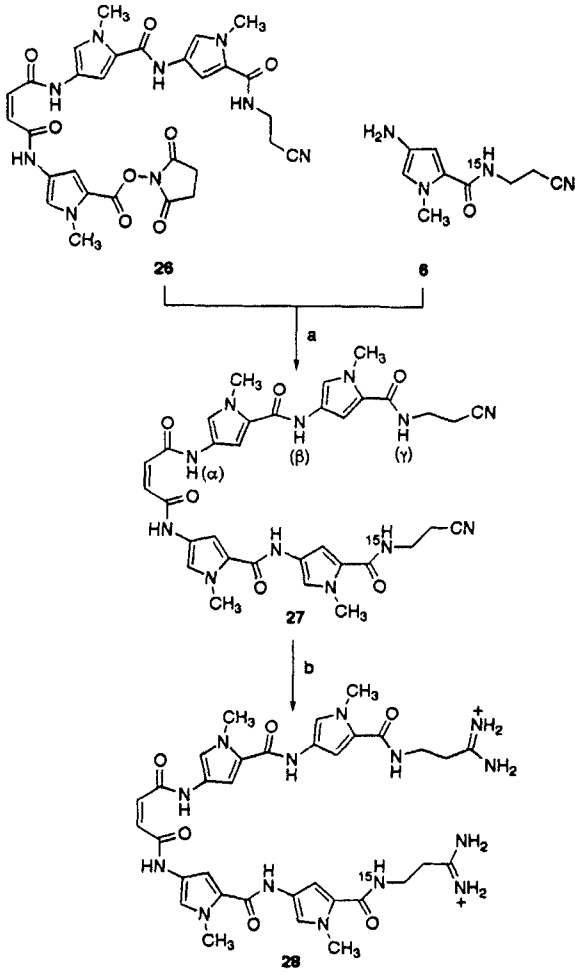
The conversion of maleamic acids to maleic isoimides has also been reported previously (11,12) along with their further reactivity towards primary amines to afford N,N-disubstituted maleamides. As both the isoimide and the isomeric imide forms were formed during our attempts at activation with TSTU, we sought alternative routes to suppress the contamination by the imide form. A number of methods for activation of carboxylic acids were tried, including DCC, $(CF_3CO)_2O$ and acetyl chloride, which all gave an excessive formation of the imide forms over the desired isoimide derivatives.



Scheme 6: (a) 10% Pd-C, CF_3COOH, MeOH, H_2 (60 psi), 30 °C, 3 h; (b) maleic anhydride, Et_3N, THF, 20 h; (c) Et_3N, ClCOOEt, THF, 20 h; (d) Et_3N, DMF-THF(1:9, v/v), 70 °C, 20 h; (e) 1.0 M TBAF in THF, 25 °C, 20 h; (f) TSTU, Et_3N, DMF, 20 h.

The best method for preparing selectively the isoimide derivative **22** (Scheme 6) was determined to be a reaction of the acid **21** with ethyl chloroformate. It is important to note the use of the β-trimethylsilylethyl group in the reactions leading from **19** to **22**, due to its facile cleavage with fluoride ions (9); ethyl esters, under saponification conditions, were observed to give a side reaction of hydrolytic breakdown of the maleiamide functionality.

The N-succinimidyl active ester **26** was successfully condensed with the ^{15}N-labelled aminopyrrole **6**, as depicted in Scheme 7, and the dinitrile compound **27** was converted to the target diamidinium product **28** via Pinner reaction (5,10).



Scheme 7: (a) CF_3COOH, DMF, 25 °C, 20 h; (b) Ethanolic HCl, -78 °C, 3 h; followed by liq. NH_3, dry EtOH, 25 °C, 20 h.

NMR CHARACTERIZATION

All the ^{15}N-labelled precursors and the final target products were characterized fully by ^{1}H- and ^{15}N-NMR. The amide ^{15}NH resonance signals showed characteristic ^{15}N-^{1}H splitting ($^{1}J_{NH}$ = 90 Hz) in the proton NMR spectra. An interesting feature of the N,N-disubstituted maleamides **24**, **27** and **28** is the presence of two distinct forms in equillibrium, driven by the formation of an intramolecular hydrogen bond, as illustrated in Figure 2 for compound **28**.

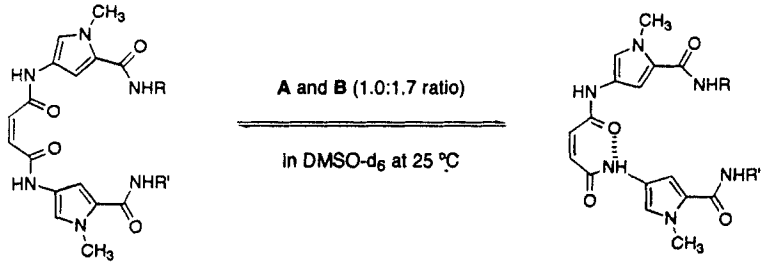


Figure 2: Two distinct conformational forms in equillibrium, observed as a result of intramolecular hydrogen bonding in the case of the *cis*-maleic diamide linked bis-netropsin analog **28**.

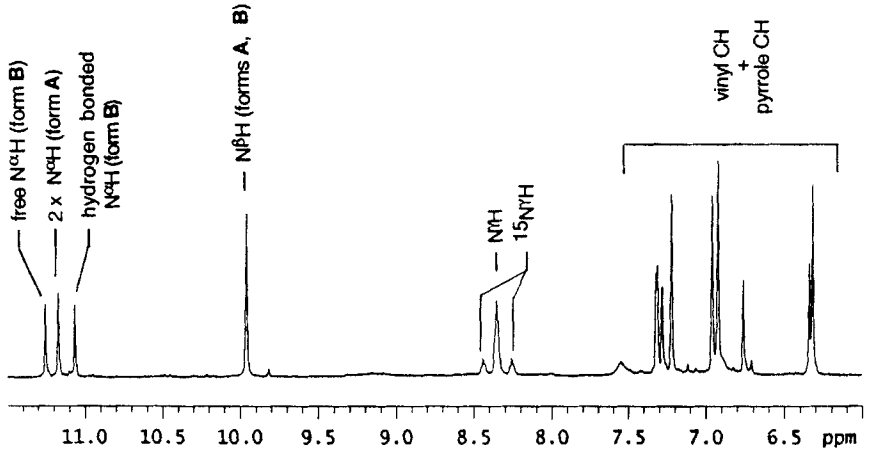


Figure 3: The ^{1}H-spectrum (6-11.5 ppm region) of compound **27** in DMSO-d_6 showing the assignments of selected signals for forms **A** and **B**. See structure **27** in Scheme 7 for the notations α, β and γ.

The low field region of the ^{1}H-NMR spectrum of compound **27** is shown in Figure 3, showing the appearance of three distinct signals corresponding to the NH protons directly linked to the central double bond. One of these signals is attributed to the NH groups of the symmetrical form **A**, while the other two were assigned, on the basis of characteristic NOE cross peaks in a two dimensional ROESY experiment (data not shown), to the individual NH protons (free and hydrogen bonded) of the asymmetrical form **B**.

The asymmetry induced by the intramolecular hydrogen bond in form **B** is also manifested in the magnetic inequivalence of the vinylic CH protons and of the vicinal pyrrole CH protons. Quantitatively, the molar ratio of the two species in equillibrium was estimated from the integration areas of the characteristic signals described above.

We previously observed that the *trans* isomer (fumaramide) exhibited binding at longer segments of DNA than either the corresponding $(CH_2)_2$-linked flexible dimer or the *cis* isomer (maleamide) which binds at the same 4-5 base pairs long sites as does the monomeric netropsin itself (13). These results can now be rationalized on the basis of the above NMR experiments which show that for the free *cis*-isomer of the ligand itself, the intramolecular hydrogen bonding at the central maleamide functionality forces an inward folding of the netropsin units relative to each other. This situation is far less favorable than that required for a bidentate binding of the *cis*-isomer to DNA. Further ^{1}H- and ^{15}N-NMR studies on the DNA complexes formed with the bis-netropsins **18** and **28** are currently underway and the results will be reported shortly.

EXPERIMENTAL

General Comments. In the procedures described below, all the reactions were carried out under Ar atmosphere and the evaporations were performed in vacuo using a rotary evaporator. All starting organic chemicals were obtained from Aldrich Chemical Co., unless otherwise indicated, and were used without further purification. HPLC grade solvents were used for chromatography. Anhydrous DMF was distilled under reduced pressure from CaH_2. Melting points were recorded on a Fisher-Johns capillary apparatus, and are uncorrected. The ^{1}H NMR spectra for characterizing the reaction products were recorded on a Bruker WH-300 or on a Varian Unity-500 spectrometer. All ^{1}H chemical shifts are reported in parts-per-million downfield relative to tetramethylsilane (TMS) as internal standard. High resolution mass spectra were determined using the electron ionization technique on Associated Electrical Industries (AEI) MS-9 and MS-50 focusing mass spectrometers. Kieselgel 60 (230-400 mesh) obtained from E. Merck was used for flash chromatography.

The ^{15}N NMR spectra were recorded on a Varian Unity-500 spectrometer at 50.65 MHz ^{15}N frequency using a multinuclear 5-mm probe. All experiments were performed at 30 °C, the temperature being regulated by the spectrometer-interfaced computer and monitored via a thermocouple implant in the probe. Unless otherwise noted, all spectra were acquired with full NOE effect (i.e., full ^{1}H noise decoupling) using a pulse flip angle of 60° and a 2-s relaxation delay. Typical spectral width was 20 KHz, and satisfactory signal-to-noise ratios were normally obtained after *ca* 200 transients. Acquisition time was 1.8-s and 64 K data points were employed. A line broadening of 1 Hz per data point was applied before Fourier-transformation of the collected FID's. Chemical shift values were measured relative to external ^{15}N-benzamide solution in DMSO and then converted to the ^{15}N scale in ppm relative to NH_3 using a conversion constant of +133.1 ppm (14).

N-(2-Bromoethyl)(^{15}N)phthalimide (1). A mixture of potassium (^{15}N)phthalimide (1.0 g, 5.37 mmol) and 1,2-dibromoethane (3.0 g, 15 mmol) in DMF (10 ml) was heated at 90 °C for 2 h. After removal of the solvent under reduced pressure, the residue was taken up in water (20 ml) and the product extracted with CH_2Cl_2 (3 x 20 ml). The pooled organic phases were dried (anhydrous Na_2SO_4), filtered and the solvent removed. The pasty residue was triturated in petroleum ether (20 ml) and the resulting solid was dried, giving 1.12 g (81.8% yield) of nearly colorless crystals. m.p. 76-78 °C; ^{1}H NMR ($CDCl_3$) δ 3.62 (dt, 2 H, $^3J_{NH}$ = 2 Hz & J = 6.5 Hz, CH_2-Br), 4.10 (dt, 2 H, $^2J_{NH}$ = 1 Hz & J = 6.5 Hz, ^{15}N-CH_2), 7.72 & 7.87 (2 m, 2 H each, Ar-H); ^{15}N NMR ($CDCl_3$) δ 185.17; EI-MS m/z 255 (M^+, 12.46 %).

N-(2-Cyanoethyl)(^{15}N)phthalimide (2). KCN (369 mg, 5.67 mmol) was added to a stirred solution of N-(2-bromoethyl)(^{15}N)phthalimide **1** (965 mg, 3.78 mmol) in dry DMSO (5 ml), and the mixture was stirred at 65 °C for 6 h.

After cooling, the mixture was poured into ice water (100 ml), and the precipitate was collected, washed with water, and dried. The product was purified by silica gel flash chromatography (hexane-acetone, 3:1 v/v eluent) to afford 389 mg (51.2% yield) of a colorless solid. m.p. 137-139 °C; ^{1}H NMR ($CDCl_3$) δ 2.82 (dt, 2 H, $^3J_{NH}$ = 2 Hz & J = 6.5 Hz, CH_2-CN), 4.00 (dt, 2 H, $^2J_{NH}$ = 1 Hz & J = 6.5 Hz, ^{15}N-CH_2), 7.77 & 7.90 (2 m, 2 H each, Ar-H); ^{15}N NMR ($CDCl_3$) δ 182.76; HR-MS (EI) m/z 201.20 (M^+, 4.36 %).

3-(^{15}N)Aminopropionitrile hemifumarate (3). A mixture of **2** (234 mg, 1.16 mmol) and hydrazine monohydrate (69 mg, 1.38 mmol) in MeOH (10 ml) was stirred at 25 °C for 20 h. The white precipitate was collected and the filtrate concentrated, then the residue was suspended in 1 N aq. NaOH solution (10 ml) and further extracted with $CHCl_3$ (3 x 20 ml). The combined organic layers were dried (anhydrous Na_2SO_4) and the solvent was evaporated. The resulting free amine was isolated as a hemifumarate by dissolution in water (10 ml) containing fumaric acid (54 mg, 0.462 mmol) and lyophilized to give 96 mg (64% yield). (*Note*: Conversion to the fumarate salt helps in isolation, purification and safe storage of the aminonitrile, which, like other primary amines, is subject to oxidative decomposition over long periods of time). m.p. 140-142 °C; ^{1}H NMR (DMSO-d_6) δ 2.80 (dt, 2 H, $^3J_{NH}$ = 2 Hz & J = 6.5 Hz, CH_2-CN), 3.03 (t, 2 H, J = 6.5 Hz, $^{15}NH_2$-CH_2), 6.30 (exch s, 3 H, $^{15}NH_3^+$), 6.50 (s, 1 H, CH-fumarate); ^{15}N NMR (DMSO-d_6) δ 60.75; HR-MS (EI) m/z 71.09 (M^+, 46.33 %).

1-Methyl-4-nitro-2-trichloroacetylpyrrole (4) was prepared according to Nishikawa *et al.* (7).

3-[1-Methyl-4-nitro-2-pyrrolecarbox(^{15}N)amido]propionitrile (5). To an ice-cold (0 °C) solution of amine **3** (142 mg, 1.1 mmol) and Et_3N (112 mg, 1.1 mmol) in dry THF (5 ml) was added a solution of **4** (271 mg, 1 mmol) in dry THF (5 ml). After completion of the addition, the mixture was heated at 70 °C for 20 h. Removal of the solvent afforded the crude product which was purified by silica gel flash chromatography; the first elution with CH_2Cl_2 allowed the elimination of the unreacted 1-methyl-4-nitro-2-trichloroacetylpyrrole and a second elution with a mixture CH_2Cl_2-EtOAc (2:3 v/v) provided 140 mg of the labelled compound **5** (63% yield). m.p. 109-111 °C; ^{1}H NMR ($CDCl_3$) δ 2.73 (dt, 2 H, $^3J_{NH}$ = 2 Hz & J = 6.5 Hz, CH_2-CN), 3.67 (qd, 2 H, $^2J_{NH}$ = 1 Hz & J = 6.5 Hz, ^{15}N-CH_2), 4.00 (s, 3 H, Py-NCH_3), 6.30 & 6.62 (2 exch t, 1 H, $^1J_{NH}$ = 90 Hz & J = 6.5 Hz, CO^{15}NH), 7.14 & 7.58 (2 d, 1 H each, J = 2 Hz, 2 x Py-CH); ^{15}N NMR ($CDCl_3$) δ 128.82; HR-MS (EI) m/z 223.20 (M^+, 30.96 %).

3-[4-Amino-1-methyl-2-pyrrolecarbox(^{15}N)amido]propionitrile trifluoroacetate (6). A solution of the nitro compound **5** (130 mg, 0.583 mmol), CF_3COOH (67 mg, 0.583 mmol) and a catalytic amount of 10% Pd-C in methanol (50 ml) was hydrogenated in a Parr shaker at 30 °C for 3 h. The mixture was filtered through Celite and the filtrate was evaporated. The residue obtained was lyophilized to afford 180 mg of the amine **6** (99.5 % yield). ^{1}H NMR ($CDCl_3$) δ 2.71 (dt, 2 H, $^3J_{NH}$ = 2 Hz & J = 6.5 Hz, CH_2-CN), 3.40 (q, 2 H, J = 6.5 Hz, ^{15}N-CH_2), 3.82 (s, 3 H, Py-NCH_3), 6.79 & 7.08 (2 d, 1 H each, J = 2 Hz, 2 x Py-CH), 8.38 & 8.68 (2 exch t, 1 H, $^1J_{NH}$ = 90 Hz & J = 6.5 Hz, CO^{15}NH), 9.70 (exch s, 2 H, NH_2); ^{15}N NMR ($CDCl_3$) δ 137.64; HR-MS (EI) m/z 193.22 (M^+, 90.74 %).

Ethyl 1-methyl-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxylate (7). This was prepared according to the procedure of Nishiwaki *et al.* (7). (68% yield); m.p. 224-226 °C; ^{1}H NMR (DMSO-d_6) δ 1.27 (t, 3 H, J = 8.5 Hz, CH_3), 3.84 (s, 3 H, Py-NCH_3), 3.94 (s, 3 H, Py-NCH_3), 4.20 (q, 2 H, J = 8.5 Hz, CH_2), 6.90 & 7.42 & 7.55 & 8.20 (4 d, 1 H each, J = 2 Hz, 4 x Py-CH), 10.25 (exch s, 1 H, CONH); EI-MS m/z 320 (M^+, 100 %).

1-Methyl-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxylic acid (8). A solution of compound **7** (320 mg, 1 mmol) in 1 N aq. NaOH (4 ml, 4 mmol) and EtOH (10 ml) was heated at 70 °C for 2 h. The hot solution was then acidified with concentrated HCl to give 291 mg of the microcrystalline yellow compound **8** (99.6% yield). ^{1}H NMR (DMSO-d_6) δ 3.84 & 3.95 (2 s, 3 H each, 2 x Py-NCH_3), 6.30 & 7.40 & 7.55 & 8.20 (4 d, 1 H each, J = 2 Hz, 4 x Py-CH), 10.22 (exch s, 1 H, CONH), 12.10 (exch s, 1 H, COOH); EI-MS m/z 292 (M^+, 27.9 %).

N-Succinimidyl 1-methyl-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxylate (9). A mixture of **8** (532 mg, 1.8 mmol), N,N,N',N'-tetramethyl-O-(N-succinimidyl)uronium tetrafluoroborate (662 mg, 2.2 mmol) and Et_3N (223 mg, 2.2 mmol) in DMF (20 ml) was stirred at 25 °C for 20 h in the dark. After removal of the solvent under reduced pressure, the residue was taken up in water and the resulting precipitate collected to afford

665 mg of the pure compound **9** (95% yield). ^{1}H NMR (DMSO-d_6) δ 2.84 (s, 4 H, CH_2CO), 3.87 & 3.97 (2 s, 3 H each, 2 x Py-NCH_3), 7.18 & 7.57 & 7.73 & 8.20 (4 d, 1 H each, J = 2 Hz, 4 x Py-CH), 10.40 (exch s, 1 H, CONH); EI-MS m/z 389 (M^+, 12.55 %).

3-[1-Methyl-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarbox(^{15}N)amido]propionitrile (10). A mixture of activated ester **9** (143 mg, 0.368 mmol), amine **3** (96 mg, 0.737 mmol) and Et_3N (89 mg, 0.885 mmol) in DMF (10 ml) was stirred at 25 °C for 20 h. After removal of the solvent under reduced pressure, the crude product was purified by silica gel flash chromatography (CH_2Cl_2-EtOAc, 3:2 v/v) to provide 95 mg of the labelled compound **10** (74.5% yield). (*Note*: This product is the same as compound **13** with the exception of the ^{15}N-label. The overall modified sequence of **7** → **8** → **9** → **10** thus represents a slight departure from that used for the preparation of **13** according to reference 7). m.p. 231-233 °C; ^{1}H NMR (DMSO-d_6) δ 2.72 (dt, 2 H, $^3J_{NH}$ = 2 Hz & J = 6.5 Hz, CH_2-CN), 3.40 (q, 2 H, J = 6.5 Hz, ^{15}N-CH_2), 3.82 & 3.95 (2 s, 3 H each, 2 x Py-NCH_3), 8.24 & 8.54 (2 exch t, 1 H, $^1J_{NH}$ = 90 Hz & J = 6.5 Hz, CO^{15}NH), 6.91 & 7.23 & 7.58 & 8.18 (4 d, 1 H each, J = 2 Hz, 4 x Py-CH), 10.27 (exch s, 1 H, CONH); ^{15}N NMR (DMSO-d_6) δ 136.77; HR-MS (EI) m/z 345.12 (M^+, 91.35 %).

3-[1-Methyl-4-(4-amino-1-methyl-2-pyrrolecarboxamido)-2-pyrrolecarbox(^{15}N)amido]propionitrile trifluoroacetate (11). A solution of the nitro compound **10** (87 mg, 0.252 mmol) with a catalytic amount of 10% Pd-C and CF_3COOH (30 mg, 0.263 mmol) in a mixture of DMF-MeOH (1:4, v/v, 20 ml) was hydrogenated in a Parr shaker (60 psi) at 30 °C for 3 h. The solution was filtered trough Celite and the resulting filtrate was concentrated under reduced pressure. The residue was dissolved in water and lyophilized to give 98 mg of the pure amine (90.6% yield). ^{1}H NMR (DMSO-d_6) δ 2.71 (dt, 2 H, $^3J_{NH}$ = 2 Hz & J = 6.5 Hz, CH_2-CN), 3.40 (q, 2 H, J = 6.5 Hz, ^{15}N-CH_2), 3.82 & 3.88 (2 s, 3 H each, 2 x Py-NCH_3), 8.20 & 8.50 (2 exch t, 1 H, $^1J_{NH}$ = 90 Hz & J = 6.5 Hz, CO^{15}NH), 6.87 & 6.90 & 7.00 & 7.21 (4 d, 1 H each, J = 2 Hz, 4 x Py-CH), 9.15 (exch s, 2 H, NH_2), 9.97 (exch s, 1 H, CONH).

(E) 4-(2-Trimethylsilylethyloxy)-4-oxo-2-butenoyl chloride (12). To a solution of fumaroyl chloride (7.34 g, 48 mmol) in dry benzene (20 ml) stirred at 15 °C was added dropwise a solution of β-trimethylsilylethanol (11.23 g, 95 mmol) in dry benzene (25 ml). After stirring for 48 h the resulting white precipitate was collected and the filtrate was evaporated to afford an oily residue. Fractional distillation under reduced pressure (bp = 81 °C, 5.2 mm Hg) afforded 6.8 g of the monochloride **12** (60.3% yield). ^{1}H NMR ($CDCl_3$) δ 0.00 (s, 9 H, $Si(CH_3)_3$), 1.00 (t, 2 H, J = 8.5 Hz, CH_2-$Si(CH_3)_3$), 4.25 (t, 2 H, J = 8.5 Hz, OCH_2), 6.90 (q, 2 H, J = 15.5 Hz, vinyl CH).

3-[1-Methyl-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]propionitrile (13). This was prepared as described previously by Nishiwaki *et al.* (7). (51.3% yield) m.p. 214-217 °C; ^{1}H NMR (DMSO-d_6) δ 2.73 (t, 2 H, J = 6.5 Hz, CH_2CN), 3.40 (q, 3 H, J = 6.5 Hz, CONH-CH_2), 3.82 & 3.96 (2 s, 3 H each, 2 x Py-NCH_3), 6.93 & 7.24 & 7.60 & 8.19 (4 d, 1 H each, J = 2 Hz, 4 x Py-CH), 8.40 (exch t, 1H, J = 6.5 Hz, CH_2NH), 10.27 (exch s, 1 H, CONH).

(E) N-[2-[2-[(2-Cyanoethyl)aminocarbonyl]-1-methylpyrrole-4-aminocarbonyl]-1-methylpyrrol-4-yl]-4-oxo-4-(2-trimethylsilylethyloxy)-2-butenamide (14). A solution of the nitro compound **13** (344 mg, 1 mmol) with a catalytic amount of 10% Pd-C in a mixture of DMF-MeOH (3:7, v/v, 50 ml) was hydrogenated in a Parr shaker (60 psi) at 30 °C for 3 h. The catalyst was removed by filtration through Celite and the filtrate was evaporated in the dark (in order to preclude the decomposition of the free amine). The residue was dissolved in DMF (10 ml) and cooled to -78 °C. To this solution of amine was added dropwise diisopropylethylamine (142 mg, 1.1 mmol) followed by a solution of the monochloride **12** (258 mg, 1.1 mmol) in dry THF (5 ml). After the addition was complete, the reaction mixture was allowed to warm up to 25 °C and stirred for 20 h. The solvent was evaporated and the resulting brown oil was purified by flash chromatography to afford the title product as a yellow solid, 372 mg (72.6% yield). m.p. 109-111 °C; ^{1}H NMR (DMSO-d_6) δ 0.00 (s, 9 H, $Si(CH_3)_3$), 1.02 (t, 2 H, J = 8.5 Hz, CH_2-$Si(CH_3)_3$), 2.73 (t, 2 H, J = 6.5 Hz, CH_2CN), 3.40 (q, 2 H, J = 6.5 Hz, CONH-CH_2), 3.82 & 3.86 (2 s, 3 H each, 2 x Py-NCH_3), 4.26 (t, 2 H, J = 8.5 Hz, OCH_2), 6.90 (q, 2 H, J = 15.5 Hz, vinyl CH), 6.92 & 6.97 & 7.22 & 7.33 (4 d, 1H each, J = 2 Hz, 4 x Py-CH), 8.35 (exch t, 1H, J = 6.5 Hz, CH_2NH), 9.96 (exch s, 1 H, CONH), 10.22 (exch s, 1 H, CONH); FAB-MS m/z 513 (M^+, 5.9%).

(E) 4-[N-[2-[2-[(2-Cyanoethyl)aminocarbonyl]-1-methylpyrrole-4-aminocarbonyl]-1-methylpyrrol-4-yl]amino]-4-oxo-2-butenoic acid (15). To a solution of **14** (372 mg, 0.726 mmol) in DMF (5 ml) was added dropwise a 1.0 M solution of tetrabutylammonium fluoride in THF (3 ml) and the mixture was stirred for 20 h. Evaporation of the solvents afforded a brown oil which was dissolved in CH_2Cl_2 (20 ml) and washed with 1 N aq. HCl (20 ml). The organic extract was then evaporated and the residue taken up in 1 N aq. NaOH (5 ml). The title carboxylic acid was precipitated by careful acidification with ice-cold conc. HCl to afford 293 mg of the desired product (98% yield). m.p. 156-158 °C; ^{1}H NMR (Acetone-d_6) δ 2.75 (t, 2 H, J = 6.5 Hz, CH_2CN), 3.56 (q, 2 H, J = 6.5 Hz, CONH-CH_2), 3.90 & 3.94 (2 s, 3 H each, 2 x Py-NCH_3), 6.92 (q, 2 H, J = 15.5 Hz, vinyl CH), 6.88 (2 d, 2 H, J = 2 Hz, 2 x Py-CH), 7.22 & 7.30 (2 d, 1 H each, J = 2 Hz, 2 x Py-CH), 7.71 (exch m, 1 H, CH_2NH), 9.23 (exch s, 1 H, CONH), 9.64 (exch s, 1 H, CONH); FAB-MS m/z 413 (M^+, 2.11%).

(E) 4-[N-[2-[2-[(2-Cyanoethyl)aminocarbonyl]-1-methylpyrrole-4-aminocarbonyl]-1-methylpyrrol-4-yl]amino]-4-oxo-4-(N-succinimidyl)-2-butenoic acid (16). A mixture of carboxylic acid **15** (226 mg, 0.548 mmol), diisopropylethylamine (78 mg, 0.603 mmol) and N,N,N',N'-tetramethyl-O-(N-succinimidyl)uronium tetrafluoroborate (TSTU) (182 mg, 0.603 mmol) in DMF (20 ml) was stirred at 25 °C in the dark for 20 h. After removal of the solvent under reduced pressure, the residue was taken up in water and the resulting precipitate filtered to afford 275 mg of the activated ester **16** (98% yield). m.p. 158-162 °C; ^{1}H NMR (DMSO-d_6) δ 2.72 (t, 2 H, J = 6.5 Hz, CH_2CN), 2.86 (s, 4 H, CH_2CO), 3.39 (q, 2H, J = 6.5 Hz, CONH-CH_2), 3.81 & 3.88 (2 s, 3 H each, 2 x Py-NCH_3), 6.92 & 6.98 (2 d, 1 H each, J = 2 Hz, 2 x Py-CH), 7.19 (q, 2 H, J = 15.5 Hz, vinyl CH), 7.22 & 7.37 (2 d, 1 H each, J = 2 Hz, 2 x Py-CH), 8.35 (exch t, 1 H, CH_2NH), 9.96 (exch s, 1H, CONH), 10.78 (exch s, 1 H, CONH); FAB-MS m/z 410 (M^+, 1.37%).

(E) N-[2-[2-[(2-Cyanoethyl)(^{15}N)aminocarbonyl]-1-methylpyrrole-4-aminocarbonyl]-1-methylpyrrol-4-yl], N'-[2-[2-[(2-cyanoethyl)aminocarbonyl]-1-methylpyrrole-4-aminocarbonyl]-1-methylpyrrol-4-yl]-2-butenediamide (17). A mixture of activated ester **16** (86 mg, 0.169 mmol), amine **11** (108 mg, 0.247 mmol) and Et_3N (25 mg, 0.247 mmol) in DMF (10 ml) was stirred at 25 °C in the dark for 20 h. After removal of the solvent under reduced pressure, the residue was purified by silica gel flash chromatography ($CHCl_3$-MeOH, 9:1 v/v, eluent) to afford 47 mg of the pure compound **17** (39% yield). m.p. 185-188 °C; ^{1}H NMR (DMSO-d_6) δ 2.70 (t, 4 H, J = 6.5 Hz, CH_2CN), 3.39 (q, 4 H, J = 6.5 Hz, CONH-CH_2), 3.80 & 3.86 (2 s, 6 H each, 4 x Py-NCH_3), 6.92 & 6.94 & 7.22 & 7. 33 (4 d, 2 H each, J = 2 Hz, 8 x Py-CH), 7.08 (s, 2 H, vinyl CH), 8.19 & 8.50 (2 exch t, 1 H, $^1J_{NH}$ = 90 Hz & J = 6.5 Hz, CO^{15}NH), 8.34 (exch t, 1 H, J = 6.5 Hz, CONH), 9.96 (exch s, 1 H, CONH), 10.49 (exch s, 1 H, CONH); ^{15}N NMR (DMSO-d_6) δ 136.47; FAB-MS m/z 709.74 (M^++1, 0.15%).

(E) N-[2-[2-[(3-Amino-3-iminopropyl)(^{15}N)aminocarbonyl]-1-methylpyrrole-4-aminocarbonyl]-1-methylpyrrol-4-yl], N'-[2-[2-[(3-amino-3-iminopropyl)aminocarbonyl]-1-methylpyrrole-4-aminocarbonyl]-1-methylpyrrol-4-yl]-2-butenediamide (18). A suspension of compound **17** (33 mg, 0.0465 mmol) in dry EtOH (5 ml) was cooled to -78 °C and dry HCl gas was passed through it until saturation at which point a clear solution resulted. This solution was evaporated to dryness and the residue was lyophilized several times from dry EtOH to remove any excess of HCl. The residue was then washed with anhydrous Et_2O and this imino ester intermediate was collected by filtration. To this solid redissolved in dry EtOH (2 ml) was added freshly distilled liq. NH_3 (3 ml) and the resulting mixture was stirred at 25 °C for 20 h. The solvent was evaporated and the residue was lyophilized twice from dry EtOH. The final residue was washed with anhydrous Et_2O to afford 19 mg of the title product **18** (55% yield). m.p. > 270 °C; ^{1}H NMR (DMSO-d_6; 500 MHz) δ 2.62 (t, 4 H, J = 6.5 Hz, CH_2CN), 3.51 (q, 4 H, J = 6.5 Hz, CONH-CH_2), 3.80 & 3.87 (2 s, 6 H each, 4 x Py-NCH_3), 6.94 & 6.96 & 7.17 & 7.33 (4 s, 8 H, 8 x Py-CH), 7.09 (s, 2 H, vinyl CH), 8.10 & 8.28 (2 exch t, 1 H, $^1J_{NH}$ = 90 Hz & J = 6.5 Hz, CO^{15}NH), 8.19 (exch t, 1 H, J = 6.5 Hz, CONH), 8.52 & 8.93 (2 exch m, 8 H, NH), 9.92 & 10.47 (2 exch s, 2 H each, 2 x CONH); ^{15}N NMR (DMSO-d_6-CD_3OD,4:1,v/v; 500 MHz) δ 136.02 (CO^{15}NH); *Note*: In this case, the ^{15}N NMR spectrum was acquired using an inverse gated decoupling sequence to suppress any possible ^{15}N-^{1}H nuclear Overhauser effects since normal fully decoupled spectra failed to show any resonance signals, evidently due to one or several circumstances when the signal is nulled (15,16); FAB-MS m/z 743.79 (M^++1, 0.21%).

2-Trimethylsilylethyl 1-methyl-4-nitro-2-pyrrolecarboxylate (19). This was prepared as described previously by Nishiwaki *et al.* (7). (99.5% yield); m.p. 61-63 °C; ^{1}H NMR ($CDCl_3$) δ 0.10 (s, 9 H, $Si(CH_3)_3$), 1.10 (t, 2 H, J = 8.5 Hz, $CH_2Si(CH_3)_3$), 3.99 (s, 3 H, Py-NCH_3), 4.36 (t, 2 H, J = 8.5 Hz, CH_2O), 7.40 & 7.59 (2 d, 1 H each, J = 2 Hz, 2 x Py-CH); HR-MS (EI) m/z 270.37 (M^+, 4 %).

2-Trimethylsilylethyl 4-amino-1-methyl-2-pyrrolecarboxylate (20). A mixture of **19** (3.4 g, 12.57 mmol), CF_3COOH (1.48 g, 13 mmol) and a catalytic amount of 10% Pd-C in MeOH (100 ml) was hydrogenated in a Parr shaker (60 Psi) at 30 °C for 3 h. The mixture was filtered through Celite and the filtrate was evaporated to provide 4.02 g of the title compound (90.2% yield). m.p. 145-150 °C; ^{1}H NMR (DMSO-d_6) δ 0.00 (s, 9 H, $Si(CH_3)_3$), 1.00 (t, 2 H, J = 8.5 Hz, $CH_2Si(CH_3)_3$), 3.86 (s, 3 H, Py-NCH_3), 4.28 (t, 2 H, J = 8.5 Hz, CH_2O), 6.76 & 7.22 (2 d, 1 H each, J = 2 Hz, 2 x Py-CH), 9.90 (exch s, 2 H, Py-NH_2); HR-MS (EI) m/z 240.38 (M^+, 2.94 %).

(Z) 4-[N-[1-Methyl-2-[(2-trimethylsilylethyl)oxycarbonyl]pyrrol-4-yl]amino]-4-oxo-2-butenoic acid (21). To an ice-cold mixture of **20** (4.00 g, 11.28 mmol) and Et_3N (1.14 g, 11.30 mmol) in THF (50 ml) was added dropwise a solution of maleic anhydride (1.10 g, 11.28 mmol) in THF (10 ml). After the addition was complete, the mixture was allowed to warm up to 25 °C and stirring was continued for 20 h. The solvent was evaporated and the residue was extracted between CH_2Cl_2 (50 ml) and 1 N aq. HCl (25 ml). The organic extracts were dried (anhydrous Na_2SO_4) and concentrated to afford 2.71 g of a yellow solid (71% yield). m.p. 175-177 °C; ^{1}H NMR (Acetone-d_6) δ 0.05 (s, 9 H, $Si(CH_3)_3$), 1.10 (t, 2 H, J = 8.5 Hz, $CH_2Si(CH_3)_3$), 3.95 (s, 3 H, Py-NCH_3), 4.33 (t, 2 H, J = 8.5 Hz, CH_2O), 6.47 (q, 2 H, J = 13 Hz, vinyl CH), 6.90 & 7.52 (2 d, 1 H each, J = 2 Hz, 2 x Py-CH), 10.61 (exch s, 1 H, CONH), 15.45 (exch s, 1 H, COOH); HR-MS (EI) m/z 338.44 (M^+, 22.84 %).

N-[1-Methyl-2-[(2-trimethylsilylethyl)oxycarbonyl]pyrrol-4-yl]maleisoimide (22). To an ice-cold mixture of **21** (1.01 g, 3 mmol) and Et_3N (303 mg, 3 mmol) in dry THF (40 ml) was added dropwise a solution of ethyl chloroformate (326 mg, 3 mmol) in dry THF (5 ml). After the addition was complete, the mixture was stirring was stirred at 25 °C for 20 h. The mixture was filtered and the filtrate was evaporated to afford 886 mg of the title compound (92.2% yield). m.p. 91-93 °C; ^{1}H NMR ($CDCl_3$) δ 0.09 (s, 9 H, $Si(CH_3)_3$), 1.10 (t, 2 H, J = 8.5 Hz, $CH_2Si(CH_3)_3$), 3.94 (s, 3 H, Py-NCH_3), 4.33 (t, 2 H, J = 8.5 Hz, CH_2O), 6.54 (d, 1 H, J = 5.5 Hz, vinyl CH), 7.20 & 7.22 (2 d, 1 H each, J = 2 Hz, 2 x Py-CH), 7.34 (d, 1 H, J = 5.5 Hz, vinyl CH); HR-MS (EI) m/z 320.43 (M^+, 47.23 %).

3-[1-Methyl-4-(4-amino-1-methyl-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]propionitrile trifluoroacetate (23). A mixture of the nitro compound **13** (1 g, 2.9 mmol) and CF_3COOH (331 mg, 2.9 mmol) with a catalytic amount of 10% Pd-C in a solvent mixture of MeOH-DMF (1:4, v/v) was hydrogenated as described above for **11**. A similar work up procedure provided 1.05 g of the title compound (84.6% yield). ^{1}H NMR (DMSO-d_6) δ 2.70 (t, 2 H, J = 6.5 Hz, CH_2CN), 3.37 (q, 2 H, J = 6.5 Hz, CONH-CH_2), 3.70 & 3.80 (2 s, 3 H each, 2 x Py-NCH_3), 6.25 & 6.35 & 6.88 & 7.17 (4 d, 1H each, J = 2 Hz, 4 x Py-CH), 8.30 (exch t, 1 H, J = 6 Hz, CH_2NH), 9.60 (exch s, 1 H, CONH).

(Z) N-[-1-Methyl-2-[[(2-trimethylsilylethyl)oxycarbonyl]pyrrol-4-yl], N'-[2-[2-[(2-cyanoethyl)-aminocarbonyl]-1-methylpyrrole-4-aminocarbonyl]-1-methylpyrrol-4-yl]-2-butenediamide (24). A mixture of compound **22** (243 mg, 0.758 mmol), amine **23** (325 mg, 0.758 mmol) and Et_3N (77 mg, 0.758 mmol) in a mixture of DMF-THF (1:9, v/v) was stirred at 70 °C for 20 h. The solvent was removed under reduced pressure and the residue was purified by silica gel flash chromatography ($CHCl_3$-MeOH, 9:1 v/v) to afford 304 mg of **24** (63% yield). m.p. 120-123 °C; ^{1}H NMR (DMSO-d_6) δ 0.05 (s, 9 H, $Si(CH_3)_3$), 1.02 (t, 2 H, J = 8.5 Hz, $CH_2Si(CH_3)_3$), 2.72 (t, 2 H, J = 6.5 Hz, CH_2CN), 3.37 (q, 2 H, J = 6.5 Hz, CONH-CH_2), 3.80 & 3.83 & 3.85 (3 s, 3 H each, 3 x Py-NCH_3), 4.26 (t, 2 H, J = 8.5 Hz, CH_2-O), 6.31 (s, 2 H, vinyl CH), 6.77 & 6.92 & 6.95 & 7.20 & 7.29 & 7.43 (6 d, 1 H each, J = 2 Hz, 6 x Py-CH), 8.33 (exch t, 1 H, CH_2NH), 9.93 (exch s, 1 H, CONH), 10.94 (exch s, 1 H, CONH), 11.03 (exch s, 1 H, CONH); FAB-MS (EI) m/z 634.77 (M^++ 1, 2.27 %).

(Z) N-[2-(Hydroxycarbonyl)-1-methylpyrrol-4-yl], N'-[2-[2-[(2-cyanoethyl)aminocarbonyl]-1-methylpyrrole-4-aminocarbonyl]-1-methylpyrrol-4-yl]-2-butenediamide (25). To a solution of compound **24** (304 mg, 0.478 mmol) in THF (20 ml) was added dropwise a 1.0 M solution of tetrabutylammonium fluoride in THF (1 ml) and stirring was continued for 20 h. The solution was then cooled to 5 °C and acidified to pH 3

with 6 N aq. HCl. The mixture was evaporated to dryness and the solid residue was washed with water and dried to afford 237 mg of **25** (93% yield). m.p. 167-170 °C; ^{1}H NMR (DMSO-d_6) δ 2.70 (t, 2 H, J = 6.5 Hz, CH_2CN), 3.35 (q, 2 H, J = 6.5 Hz, CONH-CH_2), 3.79 & 3.81 & 3.85 (3 s, 3 H each, 3 x Py-NCH_3), 6.30 (s, 2 H, vinyl CH), 6.74 & 6.91 & 6.95 & 7.20 & 7.30 & 7.40 (6 d, 1 H each, J = 2 Hz, 6 x Py-CH), 8.33 (exch t, 1 H, CH_2NH), 9.93 (exch s, 1 H, CONH), 11.00 (exch s, 1 H, CONH), 11.16 (exch s, 1 H, CONH); FAB-MS (EI) m/z 534.54 (M^++ 1, 0.55 %).

(Z) N-[1-Methyl-2-[(N-Succinimidyloxy)carbonyl]pyrrol-4-yl], N'-[2-[2-[(2-cyanoethyl)amino-carbonyl]-1-methylpyrrole-4-aminocarbonyl]-1-methylpyrrol-4-yl]-2-butenediamide (26). To a mixture of **25** (235 mg, 0.44 mmol) and Et_3N (53 mg, 0.53 mmol) in DMF (10 ml) was added in small portions N,N,N',N'-tetramethyl-O-(N-succinimidyl)uronium tetrafluoroborate (TSTU) (160 mg, 0.53 mmol) and stirring was continued at 25 °C in the dark for 20 h. After removal of the DMF under reduced pressure, the residue was purified by silica gel flash chromatography ($CHCl_3$-MeOH, 9:1 v/v) to afford 205 mg (74% yield) of the title compound. m.p. 147-149 °C; ^{1}H NMR (DMSO-d_6) δ 2.71 (t, 2 H, J = 6.5 Hz, CH_2CN), 2.83 (s, 4 H, CH_2CO), 3.88 (q, 2 H, J = 6.5 Hz, CONH-CH_2), 3.79 & 3.82 & 3.85 (3 s, 3 H each, 3 x Py-NCH_3), 6.33 (s, 2 H, vinyl CH), 6.90 & 6.95 & 7.10 & 7.20 & 7.30 & 7.70 (6 d, 1 H each, J = 2 Hz, 6 x Py-CH), 8.32 (exch t, 1 H, CH_2NH), 9.94 (exch s, 1 H, CONH), 10.85 (exch s, 1 H, CONH), 11.03 (exch s, 1 H, CONH); FAB-MS (EI) m/z 631.61 (M^++ 1, 0.61 %).

(Z) N-[2-[2-[(2-Cyanoethyl)(^{15}N)aminocarbonyl]-1-methylpyrrole-4-aminocarbonyl]-1-methyl-pyrrol-4-yl], N'-[2-[2-[(2-cyanoethyl)aminocarbonyl]-1-methylpyrrole-4-aminocarbonyl]-1-methylpyrrol-4-yl]-2-butenediamide (27). A mixture of **26** (255 mg, 0.404 mmol), amine **6** (205 mg, 0.668 mmol) and Et_3N (68 mg, 0.670 mmol) in DMF (10 ml) was stirred at 70 °C for 20 h. The solvent was removed under reduced pressure and the residue was purified by silica gel flash chromatography ($CHCl_3$-MeOH, 9:1 v/v) to afford 113 mg of the title compound (39.4% yield). m.p. 152-154 °C; ^{1}H NMR (DMSO-d_6) δ 2.70 (t, 4 H, J = 6.5 Hz, CH_2CN), 3.39 (q, 4H, J = 6.5 Hz, CONH-CH_2), 3.80 & 3.85 (2 s, 6 H each, Py-NCH_3); 6.30 & 6.32 (2 s, 2 H, vinyl CH_{asym} & CH_{sym}), 6.76 & 6.92 & 6.96 & 7.21 & 7.27 & 7.30 & 7.31 (7 d, 8 H, J = 2 Hz, 8 x Py-CH), 8.18 & 8.49 (2 exch t, 1 H, $^1J_{NH}$ = 90 Hz & J = 6.5 Hz, CO^{15}NH), 8.34 (exch t, 1 H, J = 6.5 Hz, CONH), 9.96 (exch s, 2 H, CONH), 11.06 & 11.16 & 11.24 (3 exch s, 2 H, $CONH_{asym}$ & $CONH_{sym}$); ^{15}N NMR (DMSO-d_6) δ 136.76; FAB-MS m/z 709.74 (M^++1, 0.29%).

(Z) N-[2-[2-[(3-Amino-3-iminopropyl)(^{15}N)aminocarbonyl]-1-methylpyrrole-4-aminocarbonyl]-1-methylpyrrol-4-yl], N'-[2-[2-[(3-amino-3-iminopropyl)aminocarbonyl]-1-methylpyrrole-4-amino-carbonyl]-1-methylpyrrol-4-yl]-2-butenediamide (28). A solution of the dinitrile **27** (35 mg, 0.05 mmol) was treated under Pinner reaction conditions as described above for the preparation of compound **18** to afford 20 mg of the title compound (54% yield). m.p. > 270 °C; ^{1}H NMR (DMSO-d_6; 500 MHz) δ 2.62 (t, 4 H, J = 6.5 Hz, CH_2CN), 3.51 (q, 4 H, J = 6.5 Hz, CONH-CH_2), 3.80 & 3.87 (2 s, 6 H each, Py-NCH_3), 6.33 & 6.36 (2 s, 2 H, vinyl CH_{asym} & CH_{sym}), 6.77 & 6.95 & 6.98 & 7.06 & 7.18 & 7.27 & 7.29 & 7.31 (8 s, 8 H, 8 x Py-CH), 8.10 & 8.28 (2 exch t, 1 H, $^1J_{NH}$ = 90 Hz & J = 6.5 Hz, CO^{15}NH), 8.19 (exch m, 1H, CONH), 8.56 & 8.94 (2 exch m, 8 H, NH), 9.91 & 9.93 (2 exch s, 2 H each, CONH), 11.07 & 11.21 & 11.30 (3 exch s, 2 H, $CONH_{asym}$ & $CONH_{sym}$); ^{15}N NMR (DMSO-d_6-CD_3OD,4:1,v/v; 500 MHz) δ 135.99 (CO^{15}NH); *Note*: In this case too, as for compound **18**, the normal full ^{1}H noise decoupling led to a complete loss of the ^{15}N resonance intensity, probably due to unfavorable conditions of a long correlation time leading to a suppressed signal. A fairly good quality spectrum with satisfactory signal-to-noise ratio was obtained by employing the inverse gated ^{1}H decoupling sequence and by changing the solvent system to a mixture of DMSO-d_6 and methanol-d_4 (4:1 v/v) to alter the correlation time (15,16); FAB-MS m/z 743.79 (M^++1, 0.21%).

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REFERENCES & FOOTNOTES

1. Zimmer, C. and Wahnert, U. *Prog. Biophys. Mol. Biol.* 47: 31 (1986)
2. Lown, J.W. *Chemtracts—Org. Chem.* 6: 205 (1993)
3. Singh, M.P. and Lown, J.W. In "*Studies in Medicinal Chemistry*"; Atta-ur-Rahman (Ed.), Harwood Acad. Publishers, 1994, Amsterdam.
4. Singh, M.P., Plouvier, B., Hill, G.C., Gueck, J., Pon, R.T., and Lown, J.W. *J. Am. Chem. Soc.* 116, 7006 (1994) and references cited therein.
5. Lown, J.W. *Org. Prep. Proced. Int.* 21: 1 (1989)
6. The problems with the use of $^{15}NH_3$ in the Pinner reactions for **17** → **18** and **27** → **28** are (i) nonselective incorporation of the ^{15}N-labels in both the netropsin subunits and (ii) the attendant complications due to exchange of the nitrile nitrogens with $^{15}NH_3$.
7. Nishiwaki, E., Tanaka, S., Lee, H., and Shibuya, M. *Heterocycles* 27: 1945 (1988)
8. Knorr, R., Trzeciak, A., Bannwarth, W., and Gillessen, D. *Tetrahedron Lett.* 30: 1927 (1989)
9. Greene, T.W. and Wuts, P.G.M., Eds., "*Protective Groups in Organic Synthesis*", Wiley-Interscience Pub., 1991, New York, pp. 242.
10. It is not intended to imply any conclusions on the shapes/conformations of the extended molecules in the synthetic Schemes 4 and 7, which are illustrated only for the purpose of clarity.
11. Capraro, H.-G., Winkler, T., and Martin, P. *Helv. Chim. Acta* 66: 362 (1983)
12. Ismail, M.F., Enayat, E.I., Aziz El Bassiouny, F.A., and Younis, H.A. *Acta Chim. Hung.* 128: 365 (1991)
13. Rao, K.E., Zimmermann, J., and Lown, J.W. *J. Org. Chem.* 56: 786 (1991)
14. Levy, G.C. and Lichter, R.L., Eds., "*Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy*", Wiley-Interscience Pub., 1979, New York.
15. Hawkes, G.E., Litchman, W.M., and Randall, E.W. *J. Magn. Reson.* 19: 255 (1975)
16. Hawkes, G.E., Randall, E.W., and Hull, W.E. *J. Chem. Soc. Perkin Trans.* II, 1268 (1977)