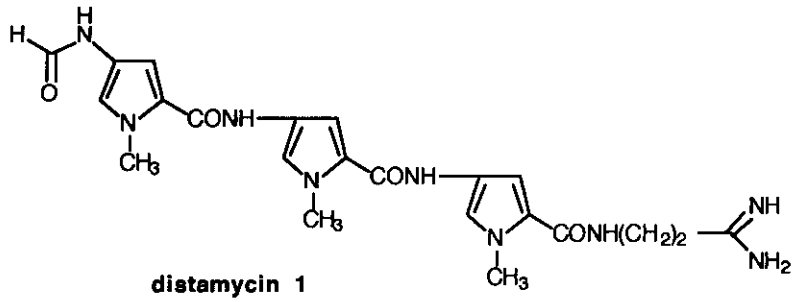
SYNTHESIS OF A NOVEL *N*-METHOXYMETHYLPYRROLE-CONTAINING DNA MINOR GROOVE BINDING OLIGOPEPTIDE RELATED TO DISTAMYCIN

Rulin Zhao and J. William Lown*

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

Abstract - A novel *N*-methoxymethylpyrrole-containing DNA minor groove binding oligo-peptide designed for increased solubility and enhanced cellular uptake and related to distamycin has been synthesized.

One approach to the general problem of developing DNA sequence specific cleaving molecules for possible application in pharmacology is by the linkage of a DNA-cleaving moiety to a sequence-specific DNA-binding molecule.[1] The naturally occurring oligopeptide, distamycin (**1**), is the most frequently used compound for the synthesis of this kind of hybrid molecule because of its strong minor groove binding ability to double stranded B-DNA at AT rich regions.[2] Targeted cleavage of double-helical polynucleotides by distamycin derivatives coupled to metal-chelating agents, isoalloxazine chromophore, propargylic sulfones and benzoyl *N*-mustard have been reported.[3]



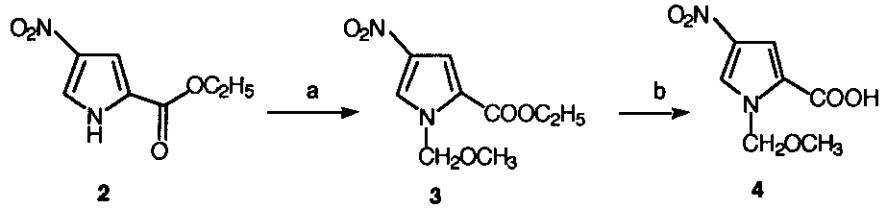
distamycin 1

It was found that certain of these functionalized hybrid molecules are potentially useful as antitumor agents.[3] To elevate its antitumor activity to a clinically useful level one of the important factors to be considered in this regard is cellular uptake.[1] Poor ability of long ligands to penetrate the cells is one of the major problems in drug development. We have reported independent evidence for the promotion of cellular uptake of oligopeptides by the incorporation of lipophilic moieties in

demonstrating rapid uptake of a spin-labeled oligopeptide by living KB cells and concentration in the nucleus.[4] In the present paper we report the synthesis of new distamycin analogues containing *N*-methoxymethyl substituted pyrrole (**13**). The methoxymethyl group in the pyrrole faces away from the DNA minor groove recognizing region and thus it should not hinder the binding interaction. The *C*-terminal amidinium group is replaced by dimethylamino which has been shown to have a similar sequence selectivity to the amidinium group of distamycin.[5] As we approach the design of groove binding agents capable of recognizing 14-16 base pair sequences (i.e. unique in the human genome), the increasing molecular weight poses problems of solubility and cell penetration. It is anticipated that the introduction of solubility-enhancing *N*-methoxymethyl groups will alleviate these problems.

The required starting material ethyl 4-nitropyrrole-2-carboxylate (**2**) was prepared following the reported procedure by condensing sodium nitromalonaldehyde and glycine ethyl ester hydrochloride in a 20% solution of sodium hydroxide.[6] Alkylation of **2** with the chloromethyl methyl ether in THF in the presence of sodium ethoxide afforded **3** in 91% yield. Alkaline hydrolysis of the ester (**3**) in aqueous methanol gave the acid (**4**) in 90% yield (Scheme I).

Scheme I



2 **3** **4**

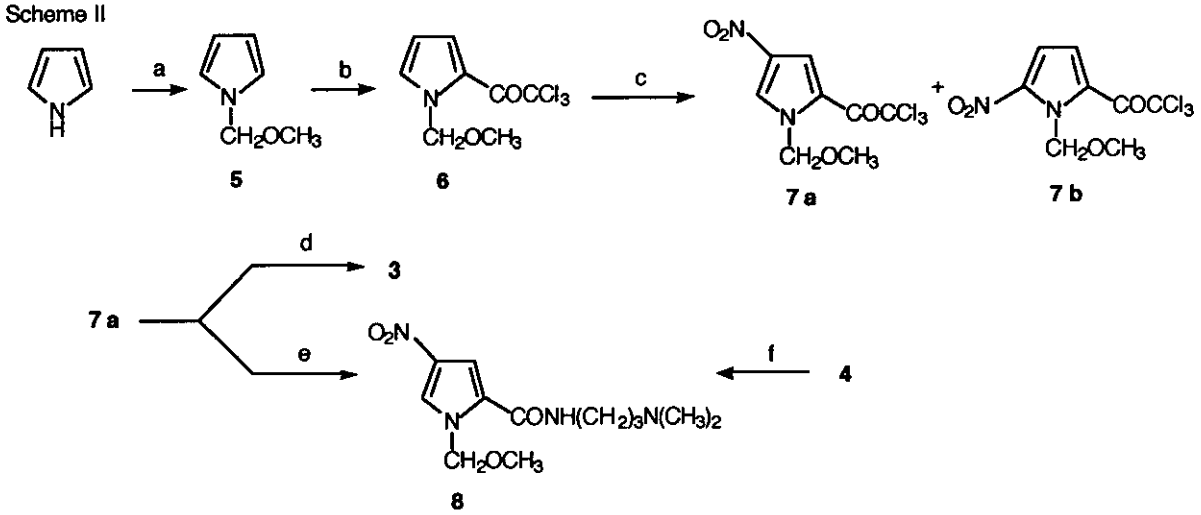
Reaction conditions: (a) C_2H_5ONa/C_2H_5OH, $ClCH_2OCH_3/THF$; (b) 1N NaOH in H_2O/CH_3OH(1:1), then IN HCl.

Intermediate (**3**) was prepared from *N*-methoxymethylpyrrole (**5**) which was, in turn, synthesized by alkylation of pyrrole with chloromethyl methyl ether in DMSO in the presence of potassium hydroxide.[7] Reaction of **5** with trichloroacetyl chloride in the CH_2Cl_2 afforded **6**.[8] Nitration of **6** afforded the desired **7a** and **7b** (3.3:1) which were separated by flash chromotography. Compound (**7a**) was allowed to condense with EtOH and 3-(*N,N*-dimethylamino)propylamine to afford readily **3** and **8** , respectively. Compound (**8**) was also prepared from the acid (**4**) which was converted into its acyl chloride with thionyl chloride and coupled with 3-(*N,N*-dimethylamino)propylamine in the presence of triethylamine (Scheme II).

Hydrogenation of **3** to the amine (**9**) is almost quantitative and required no further purification. Acylation of the 4-amino group in **9** with the acid chloride of acid (**4**) afforded the dipeptide (**10**) in 92% yield. Alkaline hydrolysis of **10** gave the acid (**11**). The nitro compound (**8**) was reduced catalytically to give the corresponding amine, which reacted with the acid chloride of **11** in the presence of triethylamine to give **12** in 78% yield. Catalytic hydrogenation of **12** to give the amine ,

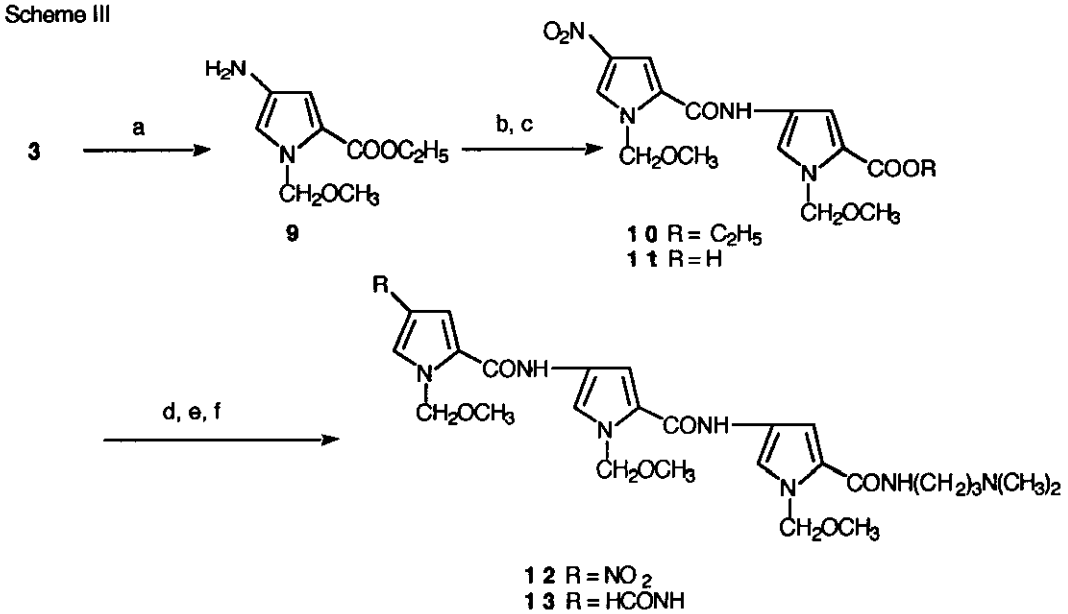
followed by *N*-formylation with formic-acetic mixed anhydride gave the desired tripeptide *N*-methoxymethyl lexitropsin (**13**) in 57% yield (Scheme III).

Scheme II



Reaction conditions: (a) KOH/DMSO,$ClCH_2OCH_3$/THF; (b) CCl_3COCl/CH_2Cl_2; (c) $HNO_3/(CH_3CO)_2O$; (d) NaH/C_2H_5OH; (e) $NH_2(CH_2)_3N(CH_3)_2$; (f) $SOCl_2$/THF, then $NH_2(CH_2)_3N(CH_3)_2$, $(C_2H_5)_3N$.

Scheme III



Reaction conditions: (a) H_2, 10% Pd/C,THF; (b) 1-methoxymethyl-4-nitropyrrole-2-carboxyl chloride $(C_2H_5)_3N$/THF; (c) 1N NaOH /H_2O + CH_3OH(1:1); (d) $SOCl_2$/THF, heat, then hydrogenation product of **8**, $(C_2H_5)_3N$; (e) H_2, Pd/C, CH_3OH; (f) $HCOOCOCH_3$/ DMF.

EXPERIMENTAL SECTION

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The ir spectra were recorded on a Nicolet magna 750 spectrophotometer with a Nic-plane microscope. The ^{1}H nmr spectra were measured on Bruker WH-200 and WH-40 spectrophotometers. FAB (fast atom bombardment) mass spectra with glycerol as the matrix were determined on a Associate Electrical Ind. (AEI) MS-9 and MS-50 focusing high resolution mass spectrometers. Kieselgel 60 (9230-400 mesh) of E. Merck and florisil (60-100 mesh) were used for chromatography, and precoated silica gel 60F-254 sheets of E. Merck were used for tlc, with the solvent system indicated in the procedure.

Ethyl 1-methoxymethyl-4-nitropyrrole-2-carboxylate (3). To a solution of sodium ethoxide (3.47 g, 0.051 mol) in absolute ethanol (200 ml) cooled to 0 °C was added **2** (9.2 g, 0.05 mol), and the resulting mixture was allowed to stir for 1 h before concentration in vacuo. The residue was dissolved in dry THF (200 ml) and chloromethyl methyl ether (4.83 g, 0.06 mol) was added over a period 10 min. The reaction mixture stirred for 2 h at room temperature.The solvent was removed in vacuo and the residue was diluted with water and then extracted with ethyl acetate (3x50 ml). The organic phase was washed with water, dried (Na_2SO_4), and evaporated to yield an oil, which was crystallized from hexane to give **3** (10.38g, 91%). mp 53°C.^{1}H Nmr ($CDCl_3$): 7.75 (d, 1H, J=2 Hz, C3-H), 7.38 (d, 1H, J=2 Hz, C5-H), 5.62 (s, 2H, NCH_2O), 4.21 (q, 2H, J=7 Hz, OCH_2CH_3), 3.28 (s, 3H, OCH_3), 1.28 (t, 3H, J=7 Hz, OCH_2CH_3). EIHRms Calcd for $C_9H_{12}N_2O_5$ 228.0750, found 228.0748(M^+, 33). Anal. Calcd for $C_9H_{12}N_2O_5$: C, 47.37, H, 5.30, N, 12.28. Found: C, 47.16, H, 5.35, N, 12.09. Ir(cast, $CHCl_3$), 3135, 2984, 2939, 2980, 1719, 1544,1512.

Ethyl 1-methoxymethyl-4-nitropyrrole-2-carboxylate (3). To a solution of **7a** (15.07 g, 0.05 mol) in absolute EtOH (50 ml) was added 80% NaH (10 mg, 0.33 mmol). The mixture was stirred for 1 h. The ethanol was removed in vacuo and the residue was diluted with water and then extracted with ethyl acetate. The organic phase was washed with water, dried (Na_2SO_4) and evaporated to yield an oil, which was crystallized from hexane to give **3** (11.18 g, 88%).

1-Methoxymethyl-4-nitropyrrole-2-carboxylic acid (4). A suspension of **3** (5.71 g, 0.025 mol) in 60 ml (0.12 mol) 2N NaOH (50% methanol and water) was stirred at room temperature overnight. Methanol was evaporated and the remaining aqueous solution was cooled to 5 °C and adjusted to pH =2 using cold 1N HCl. The precipitate was collected and crystallized from ethanol to afford **4** (4.50 g, 90%). mp 178 °C. ^{1}H Nmr (DMSO-d_6): 12.50(br s, 1H, COOH), 8.40(d, 1H, J=2 Hz, C3-H), 7.35 (d, 1H, J=2 Hz, C5-H), 5.65 (s, 2H, NCH_2O), 3.25 (s, 3H, OCH_3). EIHRms Calcd for $C_7H_8N_2O_5$ 200.04332, found 200.04318(M^+, 84). Anal. Calcd for $C_7H_8N_2O_5$: C, 42.00, H, 4.03, N, 13.99. Found: C, 42.18, H, 3.83, N, 13.77. Ir(cast, $CHCl_3$), 3152, 3129, 1701, 1500 1491.

1-Methoxymethylpyyrole (5). Dimethyl sulfoxide (300 ml) was added to potassium hydroxide(22.44 g, 0.4 mol)(crushed pellets) and the mixture was stirred for 20 min, pyrrole(13.42 g, 0.2 mol) was then added and the mixture was stirred for 1 h under the protection of Ar. To the reaction mixture cooled to 0 °C was added slowly chloromethyl metyl ether (20.13 g, 0.25 mol) over 2 h. After the addition is completed, the mixture was stirred for 1 h before water (150 ml) was added. The mixture was extracted with ether(3x100 ml). The combined ether layers were dried (Na_2SO_4) and the solvent and excess of chloromethyl methyl ether was removed under reduced pressure. The residue was distilled through a 20

cm Vigreux column to yield **5** (20.22 g, 91%). bp: 55-56 °C/20 mmHg. ^{1}H Nmr ($CDCl_3$): 6.80 (t, 2H, J=2 Hz, C2,5-H), 6.25 (t, 2H, J=2 Hz, C3,4-H), 5.18 (s, 2H, NCH_2O), 3.22 (s, 3H, OCH_3). EIHRms Calcd for C_6H_9NO: 111.0684, found 111.0685(M^+, 65). Anal. Calcd for C_6H_9NO: C, 64.83, H, 8.16, N, 12.60. Found: C, 64.30, H, 8.43, N, 12.25. Ir (neat film), 2929, 2893, 1497, 1456, 1400, 1152.

1-Methoxymethyl-2-trichloroacetylpyrrole (6). A solution of **5** (11.1 g, 0.1 mol) in dry CH_2Cl_2(100 ml) was added to a solution of trichloroacetyl chloride(18.18 g, 0.1 mol) in dry CH_2Cl_2 (100 ml) over 2 h with continuous purging of nitrogen gas to remove HCl. The reaction mixture was stirred overnight before concentration. The residue was dissolved in ethyl acetate and purified by column chromatography (SiO_2,AcOEt:hexane (1:1)) to give an oil **6** (23.85 g, 93%).^{1}H Nmr ($CDCl_3$): 7.56 (dd, 1H, J=2, 4 Hz, C3-H), 7.18 (dd, 1H, J=2, 2.5 Hz, C5-H), 6.28 (dd, 1H, J=2.5, 4 Hz, C4-H), 5.61 (s, 2H, NCH_2O), 3.32 (s, 3H, OCH_3). *EIHRms Calcd for $C_8H_8NO_2Cl_3$ 254.9621, found 254.9624 (M^+, 0.5). Ir(neat film), 3112, 2995, 2935, 2823, 1671, 1524, 1468, 1439, 1411, 1094.*

1-Methoxymethyl-4-nitro-2-trichloroacetylpyrrole (7a). To a solution of **6** (12.82 g, 0.05 mol) in acetic anhydride(50 ml) cooled to -78 °C was added fuming HNO_3(10 ml) over 30 min and then warmed up slowly to room temperature. After being stirred for 1 h, the mixture was poured into ice water and extracted with $CHCl_3$ (3x50 ml). The organic phase was washed with brine and dried (Na_2SO_4). After evaporation of the solvent, purification on a silica gel column eluting with AcOEt:hexane (1:10) *It gaves **7a** (9.98 g, 66%) and **7b** (2.98 g, 20%) as yellow oils.* **7a**:^{1}H Nmr ($CDCl_3$): 7.98 (s, 2H C3-H, C5-H), 5.68 (s, 2H, NCH_2O), 3.245(s, 3H, OCH_3). EIHRms Calcd for $C_8H_7N_2O_4Cl_3$ 299.9471, found 299.9475(M^+,4). Ir(cast, $CHCl_3$), 3122, 2980,1670, 1532, 1093. **7b**:^{1}H Nmr($CDCl_3$): 7.47 (d, 1H, J=4 Hz, C4-H), 7.18 (d, 1H, J=4 Hz, C3-H), 6.15 (s, 2H, NCH_2O), 3.30 (s, 3H, OCH_3), . EIHRms Calcd for $C_8H_7N_2O_4Cl_3$ 299.9471, found 299.9465(M^+,2). Ir(cast, $CHCl_3$), 3164, 2975, 2920, 1680, 1533.

3-(1-Methoxymethyl-4-nitropyrrole-2-carboxamido)dimethylaminopropane (8). To a solution of **7a** *(3.01g, 0.01 mol) in 100 ml of THF at 0 °C was added , dropwise, a solution of* 3-dimethylaminopropylamine (1.02 g, 0.01 mol) in 40 ml of THF. The reaction mixture was warmed up to room temperature and was stirred for an additional 1 h. The solvent was removed in vacuo. The residue was filtered through a short silica gel column, eluting with $CHCl_3$: MeOH (4:1) to give **8** (2.33 g, 82% yield) as a yellow solid. mp: 72°C. ^{1}H Nmr ($CDCl_3$): 8.75(br s, 1H, CONH) 7.80 (d, 1H, J=2 Hz, Py-H), 6.95 (d, 1H, J=2 Hz, Py-H), 5.70 (s, 2H, NCH_2O), 3.40 (m, 2H,$NH\mathbf{CH_2}CH_2$), 3.30 (s, 3H, OCH_3), 2.48 (t, 2H,J=7 Hz, $\mathbf{CH_2}N(CH_3)_2$), 2.25(s, 6H, $N(CH_3)_2$), 1.70(m, 2H $CH_2\mathbf{CH_2}CH_2$). *EIHRms Calcd for $C_{12}H_{20}N_4O_4$ 284.1495, found* 284.1494(M^+, 22). Anal. Calcd for $C_{12}H_{20}N_4O_4$: C, 50.69, H, 7.09, N, 19.71. Found: C, 50.87, H, 7.26, N, 19.53. Ir(cast,$CHCl_3$), 3314, 3129, 2943, 2862, 1653, 1553.

Coupound **(8)** was also synthesized in 91% yield from **4** (2 g, 0.01 mol) using the same procedure as that described below for **10**.

Ethyl 1-methoxymethyl-4-aminopyrrole-2-carboxylate (9). A solution of **3** (2 g, 8.76 mmol) in dry THF(100 ml) was hydrogenated over 10% Pd/C (600 mg) at atmospheric pressure and then filtered. *The filtrate was concentrated and* then dried at 40°C under vacuum to give **9** (1.65 g, 95%).^{1}H Nmr ($CDCl_3$): 6.52 (s, 2H, C3-H, C5-H), 5.53 (s, 2H, NCH_2O),

4.21 (q, 2H, J=7 Hz, OCH_2CH_3), 3.25 (s, 3H, OCH_3), 3.10(br s, 2H, NH_2), 1.30(t, 3H, J=7Hz, OCH_2CH_3). EIHRms Calcd for $C_9H_{14}N_2O_3$ 198.1004, found 198.1007(M^+,100). Ir(cast, $CHCl_3$), 3423, 3032, 2980,1680, 1546, 1514, 1436.

Ethyl 1-methoxymethyl-4-(1-methoxymethyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxylate (10) A solution of **4** (1 g, 5 mmol) was heated under reflux with $SOCl_2$ (5.95 g, 0.05 mol) in THF (50 ml) for 1 h. The solvent and excess of $SOCl_2$ were removed in vacuo and the evaporation repeated with more THF. The residue was dissolved in THF, and a solution of **8** (0.99 g, 5 mmol) and Et_3N (1.01 g, 0.01 mol) in THF was added. The mixture was stirred for 30 min and then evaporated to dryness. The residue was extracted with EtOAc. The organic phase was washed with $NaHCO_3$ and water, dried (Na_2SO_4). After evaporation of the solvent, the residue was recrystallized from EtOAc:hexane(2:1) to give **10** (1.65 g, 87%). mp 154°C. ^1H Nmr ($CDCl_3$): 7.96(br s, 1H, CONH),7.75 (d, 1H, J=2 Hz, Py-H), 7.52 (d, 1H, J=2 Hz, Py-H), 7.27(d, 1H, J=2 Hz, Py-H), 6.87 (d, 1H, J=2 Hz, Py-H), 5.65 (s, 2H, NCH_2O), 5.56 (s, 2H, NCH_2O), 4.22 (q, 2H, J=7 Hz, OCH_2CH_3), 3.36 (s, 3H, OCH_3), 3.27(s, 3H, OCH_3),1.27(t, 3H, J=7Hz, OCH_2CH_3). EIHRms Calcd for $C_{16}H_{20}N_4O_7$ 380.1340, found 380.1336(M^+, 100). Anal. Calcd for $C_{16}H_{20}N_4O_7$: C, 50.52, H, 5.30, N, 14.73. Found: C, 50.90, H, 5.27, N, 14.73. Ir(cast,$CHCl_3$), 3330, 3129, 2983, 1702, 1668, 1565.

1-Methoxymethyl-4-(1-methoxymethyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxylic acid (11). Compound **11** was synthesiized from **10** using the same procedure as that described for **7** (96%). mp 194°C. ^1H Nmr (DMSO-d_6): 10.40 (br s, 1H, CONH), 9.60 (br s, 1H, COOH), 8.38 (d, 1H, J=2 Hz, Py-H), 7.60 (s, 2H, Py-H), 6.92 (d, 1H, J=2 Hz, Py-H), 5.72(s, 2H, NCH_2O), 5.58(s, 2H, NCH_2O), 3.36 (s, 3H, OCH_3), 3.25(s, 3H, OCH_3). EIHRms Calcd for $C_{14}H_{16}N_4O_7$ 352.1019, found 352.1017(M^+, 100). Anal. Calcd for $C_{14}H_{16}N_4O_7$: C, 47.73, H, 4.57, N, 15.90. Found C, 47.86, H, 4.38, N, 15.74. Ir(cast,MeOH), 3420, 3300, 2980,1672, 1562 1506, 1468.

3-{1-Methoxymethyl-4-[1-methoxymethyl-4-(1-methoxymethyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}dimethylaminopropane (12). A solution of **8** (284 mg, 1 mmol) in 100 ml of dry THF was hydrogenated over 10% Pd/C(600 mg) at atmospheric pressure and then filtered. To a solution of **11** (352 mg, 1 mmol) in 40 ml of THF was added $SOCl_2$ (2.38 g, 0.02 mol). The reaction mixture was stirred for 2 h at 70 °C and was evaporated in vacuo and the evaporation repeated with more THF(10 ml). The residue was dissolved in THF(10 ml), and a solution of the above reaction product of **8** in THF(10 ml) and Et_3N (1.01 g, 0.01 mol) was introduced at 0 °C. The solution was stirred for 1 h and then evaporated to dryness. The organic phase was washed with 10% $NaHCO_3$ solution and H_2O, then dried (Na_2SO_4). After evaporation of the solvent, the residue was recrystallized from EtOAc: Et_2O(1:1) to give **12** (447 mg, 76%). mp108°C. ^1H Nmr ($CDCl_3$): 9.30(br s, 1H, CONH) 8.56(br s, 1H, CONH), 7.95(t, 1H, J=7 Hz, $CONHCH_2$), 7.75 (d, 1H, J=2 Hz, Py-H), 7.64 (d, 1H, J=2 Hz, Py-H), 7.56 (d, 1H, J=2 Hz, Py-H), 7.47 (d, 1H, J=2 Hz, Py-H), 6.87 (d, 1H, J=2 Hz, Py-H), 6.82 (d, 1H, J=2 Hz, Py-H), 5.72 (s, 2H, NCH_2O), 5.56 (s, 2H, NCH_2O), 5.51 (s, 2H, NCH_2O), 3.40 (s and m, 5H, OCH_3 and $CONHCH_2CH_2$) , 3.28 (s, 3H, OCH_3), 3.22(s, 3H, OCH_3), 2.65 (t, 2H, J=7 Hz, $CH_2N(CH_3)_2$,) 2.44(s, 6H, $N(CH_3)_2$),1.82(m, 2H $CH_2CH_2CH_2$). EIHRms Calcd for $C_{26}H_{36}N_8O_8$ 588.2656, found 588.2642(M^+, 0.7). Ir(cast,$CHCl_3$ /MeOH), 3277, 3124, 2943, 2864, 2781,1644, 1541.

3-{1-Methoxymethyl-4-[1-methoxymethyl-4-(1-methoxymethyl-4-formylaminopyrrole-2-carboxamido)-pyrrole-2-carboxamido]pyrrole-2-carboxamido}dimethylaminopropane (13). A solution of **12** (112 mg,

0.2 mmol) and 10% Pd/C (100 mg) in MeOH(50 ml) was stirred in H_2 (55 psi) on a Paar shaker apparatus for 4 h. The catalyst was collected and washed with MeOH, then the filtrate was concentrated and dried under vacuum. The amine residue was cooled to 0°C, then THF (20 ml), and $HCOOCOCH_3$(0.88 g, 0.01 mol) were added. The solution was allowed to warm up to room temperature and stirred under argon overnight then evaporated to dryness. The residue was purified on a silica gel column eluting with $CHCl_3$: MeOH(4:1) to give **13** (77.4 mg, 66%) as a yellow solid, tlc Rf. 0.20(MeOH:$CHCl_3$:Et_3N = 4:1:0.1).^{1}H Nmr (DMSO-d_6): 10.22(br s,1H, CONH) ,10.15(br s, 1H, CONH), 10.08(br s, 1H, CONH), 8.19(t, 1H, J=7 Hz, $CONHCH_2$), 8.12(s, 1H, HCONH), 7.42 (s, 1H, Py-H), 7.38 (s, 1H, Py-H), 7.35 (s, 1H, Py-H), 7.12(s, 1H, Py-H), 7.00 (s, 1H, Py-H), 6.90 (s, 1H, Py-H), 5.70 (s, 4H, 2xNCH_2O), 5.62 (s, 2H, NCH_2O), 3.20 (s and m, 11H, 3 x OCH_3 and $CONHCH_2CH_2$) , 2.25 (t, 2H, J=7 Hz, $CH_2N(CH_3)_2$), 2.18(s, 6H, $N(CH_3)_2$), 1.61(m, 2H, $CH_2CH_2CH_2$). EIHRms Calcd for $C_{27}H_{38}N_8O_7H$ 587.2941, found 587.2936(MH^+). Ir(cast, $CHCl_3$ /MeOH),3276, 3094, 2940, 2826, 1649, 1584.

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