Article

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Synthesis of Chiral Pyrrolidine Isostere Inserted into Pyrrole Polyamide Skeleton

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An efficient and general route towards the synthesis of a series of chiral pyrrolidine pyrrole polyamide distamycin analogues starting from (L)-hydroxyproline is described. The binding abilities of these chiral pyrrolidine containing molecules to calf thymus DNA were evaluated by duplex DNA melting temperature analysis. The results revealed that both the chirality at the pyrrolidine ring and the site of incorporation plays an important role for binding at the duplex DNA.

Keywords: Polyamide; Pyrrole; Chiral pyrrolidine.

INTRODUCTION

Natural occurring DNA minor groove binding agents such as distamycin and netropsin (Fig. 1) possessing pyrrole amide moieties have been found to bind favorably at the A and T rich region of DNA.[1-3] One of the disadvantages of netropsin and distamycin is their toxicity, and this may be attributed to their extremely strong binding ability. In the past, a vast number of minor grove binding agents based on distamycin and netropsin have been synthesized with the hope of achieving the following: (i) more site selective by the incorporation of various heterocyclic ring such as N-methylimidazole, thiazole, pyridine, thiophene, triazole, furan and pyrazole[4-6] and (ii) improving fit to complement the DNA minor groove by controlling the curvature of compound.[7] Although, pyrrolidine ring is the saturated isostere of the pyrrole ring, it has not been widely incorporated into distamycin model for studying the prerequisite of having exclusively flat heterocyclic ring linked to amide for the binding to the minor groove of DNA. On the other hand, pyrrolidine with branching polyamine has been reported to stabilize DNA duplexes and triplex through strong electrostatic interaction.[8]

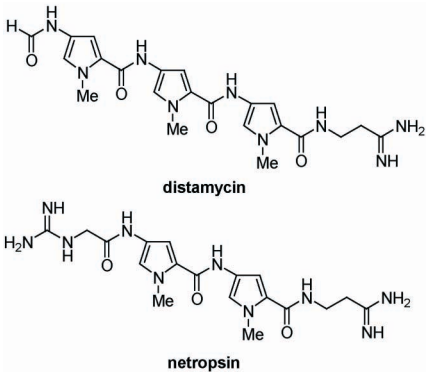

Fig. 1. Distamycin and netropsin.

Recently, Boger *et al.*[9] has investigated the replacement of all three pyrrole rings with the saturated pyrrolidine ring system, while maintaining the amide bonds. In this case, it was found that all pyrrolidine polyamide distamycin analogues showed greatly less DNA binding ability relative to distamycin. Furthermore, more basic nature of the nitrogen atom in the pyrrolidine ring which can become protonated readily was found to bind poorly to duplex DNA. As such, the N-CBz pyrrolidine derivative has been reported to be the more effective analogue for binding to duplex DNA, although the binding is still weak as compared to the pyrrole polyamide. On the other hand, *(R,R)*- and *(S,S)*-trans-cyclohexane-1,2-diamine rings linked with aliphatic spacer was found to bind to duplex DNA.[10]

So far, there has been no report on the synthesis of minor groove DNA binding agents possessing a combination of the pyrrole and pyrrolidine ring connected through an amide bond. Such a combination might be advantages as it maintain some of the rigidity of the aromatic moiety, while allowing more conformationally variable structures through the saturated pyrrolidine ring. A combination of two pyrrole rings with one pyrrolidine ring was chosen because it has been reported that a minimum of three pyrrole carboxamide units (3Py (**1**), Figure 2) are necessary for the onset of DNA binding, whereas two pyrrole caboxamide failed to exhibit any detectable binding. The pyrrolidine ring that is chiral at C-2 and C-4 hence can have two pairs of diastereo-

Dedicated to the memory of Professor Yung-Son Hon (1955–2011).

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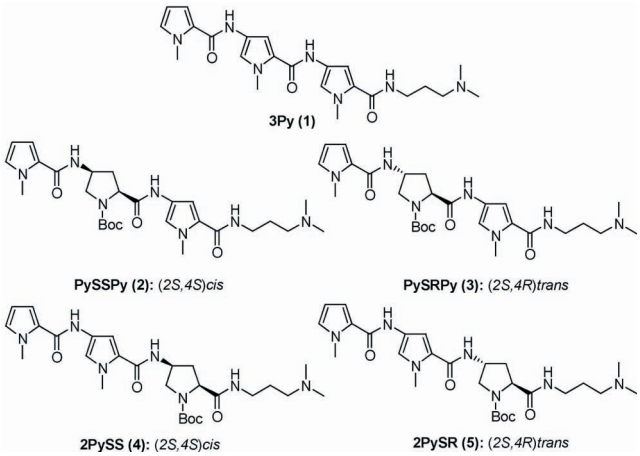


Fig. 2. Distamycin analogs bispyrrole-pyrrolidine.

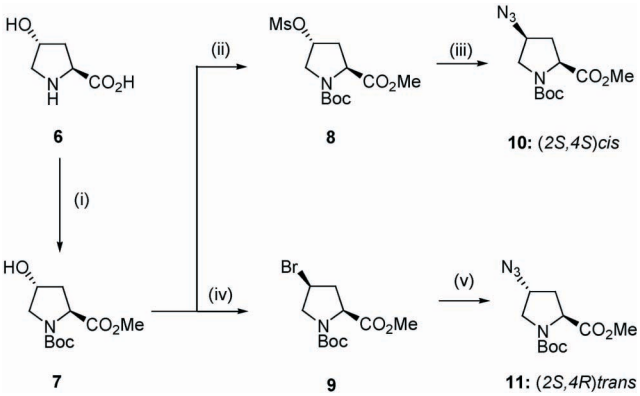
mers (*cis* 2*S,*4*S*, 2*R,*4*R* and *trans* 2*S,*4*R*, 2*R,*4*S*). Herein we report the synthesis of two of the four possible stereoisomers (*cis* 2*S,*4*S* and *trans* 2*S,*4*R*) of chiral pyrrolidine ring inserted onto different site of the bispyrrole amide, namely PySSPy (**2**), PySRPy (**3**), 2PySS (**4**) and 2PySR (**5**) (Fig. 2), and evaluate their binding ability to duplex DNA.

RESULTS AND DISCUSSION

In order to synthesize the four different combinations of pyrrolidine-pyrrole polyamides, PySSPy (**2**), PySRPy (**3**), 2PySS (**4**) and 2PySR (**5**) are derived from the appropriate chiral (*cis* 2*S,*4*S*) and (*trans* 2*S,*4*R*) 4-aminopyrrolidine-2-carboxylic acid. These were synthesized according to Scheme I starting from commercially available (*L*)-4-hydroxyproline, **6**.[11] Esterfication of **6,** followed by protecting the amine group as the N-Boc provided the common intermediate **7**. The mesylation of hydroxyl group in **7** gave **8**, which in the presence of sodium azide in DMF at 80 °C gave the inversion (2*S*,4*S*)-*cis*-azide **10**. On the other hand, the retention of configuration of azide at C-4 was achieved by conversion of the hydroxy group in **7** to the bromide **9**, followed by displacement with sodium azide give the double inversion product, or the total retention of configuration to afford (2*S*,4*R*)-*trans*-azide **11**.

We next examine the systematic incorporation of the chiral pyrrolidine into the distamycin analogues at different sites. The introduction of the pyrrolidine ring between two pyrrole amide ring was first examined and is shown in Scheme II. Accordingly, reduction of the azide **10** and **11** using Pd/C in methanol gave the chiral amino-pyrrolidine (*2S,4S*)-**13a** and (*2S,4R*)-**13b** respectively. The coupling of **13a** and **13b** with pyrrol-2-carbonyl chloride **14**, prepared

Scheme I



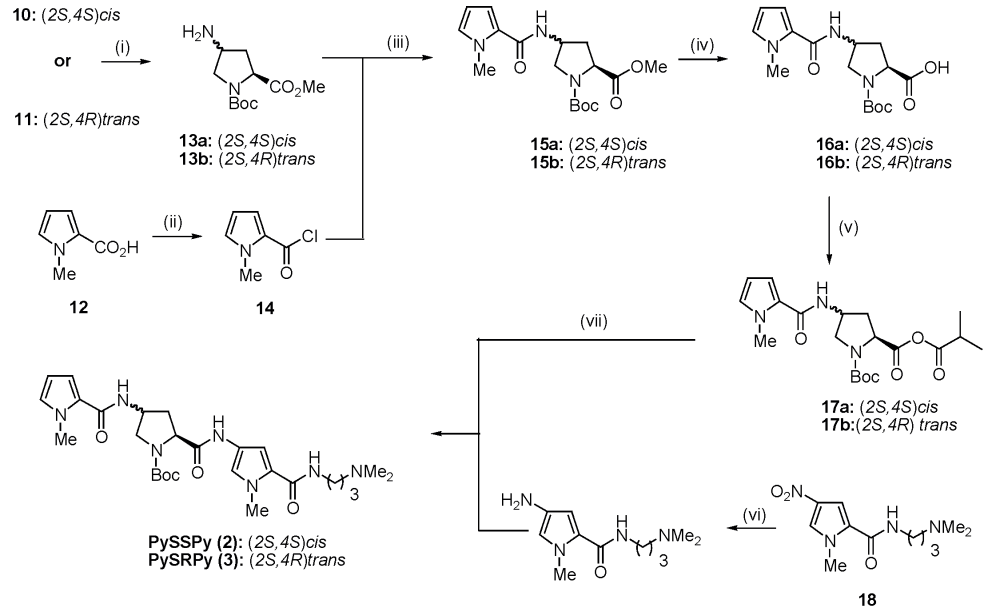
Reagents and conditions: (i) a. $SOCl_2$, MeOH b. Boc_2O, Et_3N, DCM, rt. overnight, two steps 90%; (ii) MsCl, Et_3N, DCM, 4 hr, rt. 85%; (iii) NaN_3, DMF, 80 ^oC, 3 hr. 80%; (iv) CBr_4, Ph_3P, DCM, overnight, rt. 73%; (v) NaN_3, DMF, 80 ^oC, 3 hr. 81%.

from pyrrole-2-carboxylic acid **12**[12] in the presence of Et_3N, provided the pyrrole-pyrrolidine ester **15a** and **15b** respectively. The ester group in **15a** and **15b** was hydrolyzed to the acid, followed by activation to the mix anhydride **17a** and **17b**; and was subsequently coupled with the N-dimethylpropylamine pyrrole **19** (prepared from the reduction of nitro pyrrole **18** according to a published procedure[13]) to give PySSPy (**2**) and PyRSPy (**3**) respectively.

The synthesis of the 2PySS (**4**) and 2PySR (**5**) began with the coupling of the individual chiral amino-pyrrolidine **13a** and **13b** with 4-nitro-pyrrole-2-acyl trichloride **20** to give **21a** and **21b** respectively, as shown in Scheme III. Reduction of the nitro group in **21a** and **21b** with H_2-Pd/C in methanol gave the corresponding amines. This was transformed to the desired (2*S,*4*S*)-**22a** and (2*S,*4*R*)-**22b**-bis-pyrrole-pyrrolidine ester by coupling with pyrrole-2-carbonyl chloride **14**. Hydrolysis of ester group in **22a** and **22b** to the acid **23a** and **23b** was effect by treatment with aqueous NaOH. Activation of the acid to the anhydride, followed by coupling with N,N-dimethylpropylamine gave 2PySS (**4**) and 2PyRS (**5**), respectively.

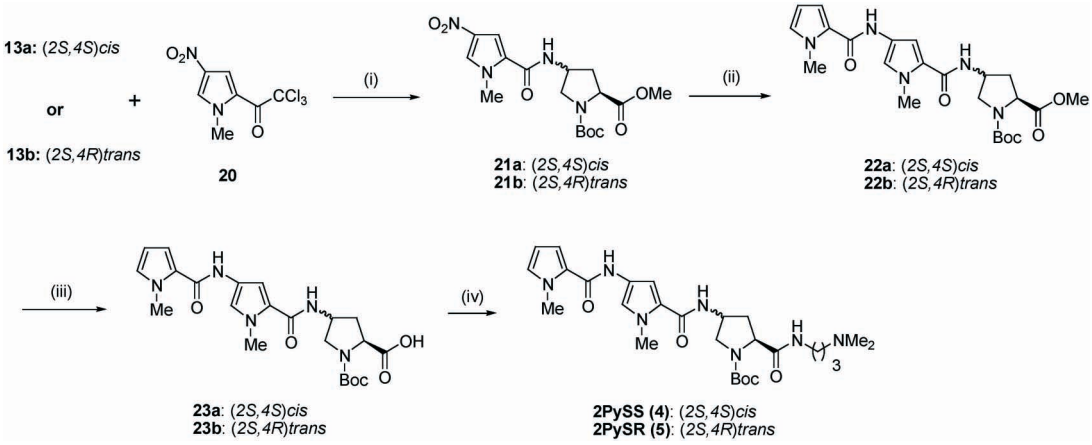
The three pyrrole carboxamide **3Py** (**1**), is known to be the minimum backbone necessary for the onset of DNA duplexes stabilization, although much less than that of distamycin. Here, the comparative thermal stabilization of calf-thymus (CT) duplex with **3Py** (**1**) and the pyrrolidyl containing analogues **PySSPy** (**2**), **PyRSPy** (**3**), **2PySS** (**4**), and **2PyRS** (**5**) were determined under identical conditions of buffer and pH by UV absorbance-temperature plot and the melting data summarized in Table 1. Almost all of the

Scheme II



Reagents and conditions: (i) H_2, Pd/C, MeOH; (ii) $SOCl_2$, THF, 0 °C, 30 min then rt 1 hour (iii) Et_3N, DCM, 0 °C, overnight, three steps **15a**: 72% , **15b**: 75%; (iv) NaOH, MeOH/H_2O, 4 hr. rt.; (v) N-methylmorpholine, isopropyl chloroformate, Et_3N, DCM; (vi) H_2, Pd/C, MeOH; (vii) Et_3N, DMF, rt. overnight, two steps from **16**, **2**: 40%, **3**: 41%.

Scheme III



Reagents and conditions: (i) Et_3N, DCM, overnight, rt. **21a**: 80% , **21b**: 80%; (ii) H_2, Pd/C, MeOH then **14**, DCM, rt. overnight, **22a**: 60%, **22b**: 55%; (iii) NaOH, MeOH/H_2O, rt. 4hr.; (iv) N-methylmorpholine, isopropyl chloroformate then N,N-dimethylpropylamine, DCM, rt. overnight, two steps **4**: 50%, **5**: 46%.

tested pyrrolidine-pyrrole polyamides stabilized the CT-DNA double helix, but to a different extent as shown by the increasing melting temperature effects (ΔTm > 0, Table 1). Among the four pyrrolidine-pyrrole diastereomers synthesized, the **2PySS (4)** *cis*-isomer appeared to be best in stabilizing the duplex (ΔTm = 2.9 °C) and superior to that of **3Py (1)** (ΔTm = 1.7 °C), followed by *cis*-**PySSPy (2)** (ΔTm = 1.0 °C). An important observation is the relatively weak binding for the **2PySR (5)** and **PySRPy (3)** *trans*-isomers. The results suggest that the degree of stabilization is dependent on the stereochemistry at the C-2 and C-4-position of the pyrrolidine ring, and the site at which it was incorporated with the bis-pyrrole units.

In summary, we have described an efficient synthesis

Table 1. Melting temperature with calf thymus DNA (2.77 μM) and compound (50 μM)

Compound	3Py(1)	PySSPy(2)	PySRPy(3)	2PySS(4)	2PySR(5)
ΔTm (°C)	1.7	1.0	0.2	2.9	0.1

of a new class of distamycin analogues by incorporating conformationally flexible chiral pyrrolidine isostere into the pyrrole polyamide backbone. Preliminary binding studies with C-T DNA showed an interesting stereochemical requirement at the C-2 and C-4 position of pyrrolidine ring for good binding. The site of incorporating the pyrrolidine ring also plays an important role. The newly synthesized distamycin analog **2PySS (4)** showed substantially improve DNA duplex stabilization over that of **3Py (1)**. Our results will add to an expanding library of methodology for the design of small molecules for DNA binding.

EXPERIMENTAL

General

Reactions were carried out under nitrogen in oven-dried glassware. Tetrahydrofuran was distilled over sodium. ^{1}H and ^{13}C NMR spectra were recorded by 200 MHz and 500 MHz spectrometer using $CDCl_3$ as solvent. Mass spectra were obtained by ESI FT-MS.

DNA melting temperature (Tm) measurement

The experiments were conducted with a Pharmacia Biotect Ultrospec 4000 UV/Visible Spectrophotometer with a temperature controller in 3 mL quartz cuvettes with a Teflon cap. The absorbance of the DNA–compound complex was monitored at 260 nm as a function of temperature and DNA without compound was used as a control. Cuvettes were mounted in a thermal block and the solution temperatures were monitored with a heating rate of 0.5 °C/min. The concentration of DNA was determined by measuring the absorbance at 260 nm. A ratio of approximately 2:1 compound/DNA (50 mM: 27.7 mM) was used in the studies. The compounds were dissolved in DMSO and kept below 1% at the final concentration in the cuvettes. All the binding experiments were carried out in an aqueous solution of 10% 1X PBS buffer (pH: 6.8) at 25 °C.

Synthesis

(2S,4S)-1-tert-butyl-2-methyl-4-(1-methyl-1H-pyrrole-2-carboxamido)pyrrolidine-1,2-dicarboxylate (15a)

To a cooled (0 °C) solution of **12** (870 mg, 7 mmole) in dry THF (30 mL) in a 100 mL flask was added thionyl chloride (3.3 g, 27.7 mmole) and the temperature (0 °C) was maintained for 30 min, then the reaction mixture was allowed to warm up to room temperature for one hour. The mixture was concentrated *in vacuo* to obtain acryl chloride **14**.

In another flask, a suspension of 5% Pd-C (150 mg) and **10** (1.5 g, 5.5 mmole) in methanol (10 mL) was stirred for four hours under 1 atm of H_2 at room temperature. The reaction was then filtered and the Pd-C catalyst wash further with methanol. The methanol filtrate was concentrated *in vacuo* to obtain amine **13** and used *in situ*.

To amine **13** prepared *in situ* was added a solution of **14** in CH_2Cl_2 (30 mL) at 0 °C and stirred for 12 hours. The reaction was quenched by the drop-wise addition of $NaHCO_{3\ (aq)}$ (30 mL) and the organic layer separated,. washed with brine, and dried over $MgSO_4$. The organic layer was concentrated to give the crude product and purified by column chromatography using EtOAc/Hexane (1/1) to obtain the product 1.4 g, 72% yield. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.125-7.271 (m, 1H), 6.67 (s, 1H), 6.58-6.63 (bs, 1H), 6.04 (bs, 1H), 4.74 (m, 1H), 4.27-4.37 (m, 1H), 3.90 and 3.89 (*diastereomer*, m, 3H), 3.75 and 3.90 (*diastereomer*, s, 3H), 3.49-3.63 (m, 2H), 2.42-2.51 (m, 1H), 1.95-2.00 (m, 1H), 1.39-1.42 (m, 9H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 174.97 and 175.08 (*diastereomer*), 160.87, 153.36 and 154.11 (*diastereomer*), 127.97, 125.21, 112.13, 107.22, 80.46, 57.75 and 57.64 (*diastereomer*), 53.02 and 53.67 (*diastereomer*), 52.37 and 52.60 (*diastereomer*), 47.16 and 48.12 (*diastereomer*), 35.70 and 36.74 (*diastereomer*), 36.59, 28.09 and 28.19 (*diastereomer*); HRMS (*m/z*, ESI): Calcd for $C_{17}H_{25}N_3O_5Na$: 374.1692, found, 374.1689.

(2S,4S)-tert-butyl-2-(5-(3-(dimethylamino)propylcarbamoyl)-1-methyl-1H-pyrrol-3-ylcarbamoyl)-4-(1-methyl-1H-pyrrole-2-carboxamido)pyrrolidine-1-carboxylate (2)

To a solution of **15** (1 g, 2.7 mmole) in MeOH/H_2O (1/1) 30 mL, sodium hydroxide was added (230 mg, 5.7 mmole) and stir for four hours at rt. The solution was acidified to pH~5 with 10% aqueous HCl and the reaction mixture was extracted with CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated to obtain crude acid compound **16a**.

To a solution of **16a** in DMF (15 mL) was added 4-methylmorpholine (600 mg, 5.9 mmole) and stirred for 20 min, followed by isopropyl chloroformate (430 mg, 3.5 mmole) and reacted for half an hour to obtain anhydride **17a**.

To the anhydride **17a** in DMF (15 mL) at 0 °C was added a solution of compound **19** in DMF (5 mL) and then left to warm to room temperature overnight. The solvent was evaporated *in vacuo* and purified by column chromatography on silica gel with CH_2Cl_2/MeOH/NH_4OH (90/10/3) to obtain the product, 588 mg, 40% yield. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.53 (m, 1H), 8.13 (m, 1H), 7.89 (m, 1H), 7.11 (s, 1H), 6.74 (m, 1H), 6.68 (s, 1H), 6.38 (m, 1H), 6.06-6.07 (m, 1H), 4.60 (m, 1H), 3.94 (s, 3H), 3.91 (s, 3H), 3.51-3.89 (m, 2H), 3.42-3.46 (m, 2H), 2.43-2.46 (m, 2H), 2.29 (m, 6H), 1.69-1.72 (m, 2H), 1.46 (m, 9H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 169.28, 161.58, 156.60, 127.82, 125.47, 123.91, 121.12, 118.30, 112.45, 107.18, 102.65, 81.31, 59.77, 59.12, 55.21, 48.96, 45.38, 39.58, 36.73, 36.62, 28.32; HRMS (*m/z*, ESI): Calcd for $C_{27}H_{42}N_7O_5$: 544.3247, found, 544.3251.

(2S,4R)-1-tert-butyl-2-methyl-4-(1-methyl-1H-pyrrole-2-carboxamido)pyrrolidine-1,2-dicarboxylate (15b)

Compound **11** and **14** were prepared in 75% yield by the same procedure as that described for **15a**, **15b**. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 6.72 (s, 1H), 6.53 (s, 1H), 6.07 (bs, 1H), 5.9-5.96 (m, 1H), 4.63-4.69 (m, 1H), 4.31-4.45 (m, 1H), 3.92 (s, 3H), 3.82-3.90 (m, 1H), 3.74 (s, 3H), 3.3-3.46 (m, 1H), δ 2.16-2.32 (m, 2H), 1.42-1.45 (m, 9H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 172.97 and 172.63 (*diastereomer*), 161.64, 153.65 and 154.3 (*diastereomer*), 128.28, 125.04, 111.87, 107.25, 80.55, 57.85 and 57.51 (*diastereomer*), 52.3 and 52.1, 52.05 and 51.49 (*diastereomer*), 48.168 and 47.76 (*diastereomer*), 37.01 and 35.82 (*diastereomer*), 36.7, 28.3 and 28.21; HRMS (*m/z*, ESI): Calcd for $C_{17}H_{25}N_3O_5Na$: 374.1692, found, 374.1689.

(2S,4R)-tert-butyl-2-(5-(3-(dimethylamino)propylcarbamoyl)-1-methyl-1H-pyrrol-3-ylcarbamoyl)-4-(1-methyl-1H-pyrrole-2-carboxamido)pyrrolidine-1-carboxylate (3)

Compound **2**, **3** are prepared in 41% yield by the same procedure as that described for **15b** and **19**. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.13 (m, 1H, pro-CONH), 7.72 (m, 1H), 7.12 (m, 1H), 6.71 (s, 1H), 6.57-6.58 (m, 1H), 6.50 (s, 1H), 6.10-6.11 (m, 1H), 6.05-6.06 (m, 1H), 4.59-4.63 (m, 1H), 4.28-4.47 (m, 1H), 3.90 (s, 3H), 3.87 (s, 3H), 3.7-3.84 (m, 2H), 3.42-3.46 (m, 2H), 2.63 (m, 2H), 2.42 (m, 6H), 1.81-1.83 (m, 2H), 1.43 (m, 9H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 161.84, 161.82, 128.23, 125.11, 123.42, 121.13, 118.63, 111.99, 107.23, 103.17, 81.04, 66.88, 57.97, 55.38, 52.34, 44.66, 38.29, 36.96, 36.55, 28.33; HRMS (*m/z*, ESI): Calcd for $C_{27}H_{42}N_7O_5$: 544.3247, found, 544.3245.

(2S,4S)-1-tert-butyl-2-methyl-4-(4-amino-1-methyl-1H-pyrrole-2-carboxamido)pyrrolidine-1,2-dicarboxylate (21a)

13a was dissolved in CH_2Cl_2 (10 mL) and a solution of compound **20** in CH_2Cl_2 (10 mL) was added with stirring overnight. The reaction mixture was added $NaHCO_3$ solution and the orgaic layer seperated and further extracted with CH_2Cl_2. The organic layers were combined and washed with $NaHCO_{3\,(aq)}$ (15 mL × 3) and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to obtain crude **16**. Purification by column chromatography on silica gel with EtOAc/Hexane (1/1) gave product **21a**, 1.2 g, 80% yield. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.53-7.59 (m, 1H), 7.51 (s, 1H), 7.09 (m, 1H), 4.64 (m, 1H), 4.23-4.32 (m, 1H), 3.89 (s, 3H), 3.72 (s, 3H), 3.44-3.62 (m, 2H), 1.93-2.50 (m, 2H), 1.32-1.36 (m, 9H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 175.00-175.07, 159.38, 153.20 and 153.91 (*diastereomer*), 134.64, 126.68, 125.82, 107.22, 80.50, 57.46 and 57.55 (*diastereomer*), 52.58, 53.12, 52.46 and 52.67 (*diastereomer*), 47.49 and 48.39 (*diastereomer*), 37.63, 35.15 and 36.05 (*diastereomer*), 27.91 and 28.03 (*diastereomer*); HRMS (*m/z*, ESI): Calcd for $C_{17}H_{24}N_4O_7Na$: 419.1543, found, 419.1547.

(2S,4S)-1-tert-butyl-2-methyl-4-(1-methyl-4-(1-methyl-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-arboxamido) pyrrolidine-1,2-dicarboxylate (22a)

Compound **12** and **21a** were prepared using the same procedure as that described for **15a**, **22a**. Purifiication by column chromatography on silica gel with EtOAc/Hexane (1/1) gave product **22a**, 1.2 g, 60% yield. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.42 (m, 1H), 8.06 (m, 1H), 7.33 (m, 1H), 6.87-6.88 (m, 1H), 6.70 (m, 1H), 6.33 (s, 1H), 6.07-6.08 (m, 1H), 6.06-6.07 (m, 1H), 4.64-4.72 (m, 1H), 4.27-4.36 (m, 1H), 3.92 (s, 3H), 3.86 (s, 3H), 3.70 (s, 3H), 3.47-3.65 (m, 2H), 2.43-2.49 (m, 2H), 1.96-2.06 (m, 2H), 1.42 (m, 9H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 174.85 and 175.02 (*diastereomer*), 160.81, 159.23 and 159.26 (*diastereomer*), 153.57 and 154.14, 128.16 and 128.22 (*diastereomer*), 125.32 and 125.40 (*diastereomer*), 122.24 and 122.45 (*diastereomer*), 121.50 and 121.60 (*diastereomer*), 119.45 and 119.58 (*diastereomer*), 111.95 and 112.15 (*diastereomer*), 107.18 and 107.21, 102.85 and 103.26 (*diastereomer*), 80.53 and 80.61 (*diastereomer*), 57.58 and 57.77 (*diastereomer*), 52.38 and 53.29 (*diastereomer*), 52.56 and 52.65 (*diastereomer*), 47.47 and 48.22 (*diastereomer*), 36.76, 36.61, 35.62, 28.15 and 28.20 (*diastereomer*); LRMS (*m/z*, ESI): 496 ($M+Na^+$); HRMS (*m/z*, ESI): Calcd

for $C_{23}H_{31}N_5O_6Na$: 496.2172, found, 496.2173.

(2S,4S)-tert-butyl-2-(3-(dimethylamino)propylcarbamoyl)-4-(1-methyl-4-(1-methyl-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido)pyrrolidine-1-carboxylate (4)

To a solution of **22a** (500 mg, 1 mmole) in MeOH/H_2O (1/1) (30 mL), sodium hydroxide was added (230 mg 5.7 mmole) and stir for four hours at rt. The solution was acidified to pH~5 with 10% aqueous HCl and the reaction mixture was extracted with CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to obtain crude **23a**.

To a solution of **23a** in CH_2Cl_2 (15 mL) was added 4-methylmorpholine (600 mg, 5.9 mmole) for 20 min, followed by isopropyl chloroformate (430 mg, 3.5 mmole) and stirred for a further half hour to obtain the anhydride.

To the anhydride in DMF (15 mL) at 0 ^oC was added N,N-dimethylpropylamine (204 mg, 2 mmole) and then left to warm to room temperature overnight. The solvent was evaporated and purified by column chromatography on silica gel with CH_2Cl_2/MeOH/NH_4OH (90/10/3) to obtain the product, 270 mg, 50% yield. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.72 (m, 1H), 8.31 (m, 1H), 7.91 (m, 1H), 7.40 (s, 1H), 7.01 (s, 1H), 6.80 (m, 1H), 6.70 (s, 1H), 6.07-6.08 (m, 1H), 4.64 (m, 1H), 4.34-4.36 (m, 1H), 3.95 (s, 3H), 3.90 (s, 3H), 3.45 (m, 2H), 3.38-3.63 (m, 2H), 2.87 (m, 2H), 2.60 (m, 6H), δ 2.18-2.29 (m, 2H), δ 1.85-1.94 (m, 2H), δ 1.43 (m, 9H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 174.03, 161.04, 159.366, 155.49, 128.09, 125.54, 122.60, 121.95, 119.27, 112.65, 107.26, 103.72, 80.70, 66.74, 59.48, 54.96 and 55.91, 55.29, 50.57, 48.43, 44.02, 36.57; HRMS (*m/z*, ESI): Calcd for $C_{27}H_{42}N_7O_5$: 544.3247, found, 544.3244.

(2S,4R)-1-tert-butyl-2-methyl4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)pyrrolidine-1,2-dicarboxylate (21b)

Compound **11** was obtained in 80% by the same procedure as that described for **21a**, **21b**.. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.56 (s, 1H), 7.17 (s, 1H), 7.09 (m, 1H), 4.64 (m, 1H), 4.35-4.46 (m, 1H), 3.98 (s, 3H), 3.82 (m, 2H), 3.75 (s, 3H), 2.33 (m, 2H), 1.42-1.46 (m, 9H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 172.88 and 172.61 (*diastereomer*), 160.20, 153.73, 134.87, 126.90 and 126.773 (*diastereomer*), 125.67, 107.51, 80.89 and 80.77 (*diastereomer*), 57.77 and 57.52 (*diastereomer*), 52.43 and 52.28 (*diastereomer*), 51.84 and 51.37 (*diastereomer*), 48.56 and 48.16 (*diastereomer*), 37.96, 36.78 and 35.6 (*diastereomer*), 28.31 and 28.22 (*diastereomer*); HRMS (*m/z*, ESI): Calcd for $C_{17}H_{24}N_4O_7Na$: 419.1543, found, 419.1547.

(2S,4R)-1-tert-butyl-2-methyl-4-(1-methyl-4-(1-methyl-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido)pyrrolidine-1,2-dicarboxylate (22b)

Compound **12** was synthesized from **23b** in 55%, yield by the same procedure as that described for **22a**, **22b**. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.62 (m, 1H), 7.11 (s, 1H), 6.76 (s, 1H), 6.87-6.88 (m, 1H), 6.70 (m, 1H), 6.33 (s, 1H), 6.64-6.66 (m, 1H), 6.62 (s, 1H), 6.11-6.12 (m, 2H), 4.64-4.66 (m, 1H), 4.33-4.34 (m, 1H), 3.97 (s, 3H), 3.89 (s, 3H), 3.84 (m, 2H), 3.74 (s, 3H), 2.31 (m, 2H), 1.42-1.46 (m, 9H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 173.09 and 172.78 (*diastereomer*), 161.4, 159.38, 154.33 and 153.69 (*diastereomer*), 128.50, 125.34, 122.8, 121.45 and 121.37 (*diastereomer*), 119.13, 111.87, 107.4, 103.9, 80.61, 57.87 and 57.55 (*diastereomer*), 52.37 and 52.22 (*diastereomer*), 52.04 and 51.5 (*diastereomer*), 48.26 and 47.79 (*diastereomer*), 36.98, 36.82 and 36.66 (*diastereomer*), 35.86, 28.36 and 28.25 (*diastereomer*); LRMS (*m/z*, ESI): 496 (M+ Na^+); HRMS (*m/z*, ESI): Calcd for $C_{23}H_{31}N_5O_6Na$: 496.2172, found, 496.2176.

(2S,4S)-tert-butyl-2-(3-(dimethylamino)propylcarbamoyl)-4-(1-methyl-4-(1-methyl-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido)pyrrolidine-1-carboxylate (5)

Compound **5** was prepared in 46% yield by the same procedure as that described for **4**. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.32-8.33 (m, 1H), 8.08 (m, 1H), 7.83 (m, 1H), 7.40 (s, 1H), 6.77 (s, 1H), 6.74 (s, 1H), 6.6 (s, 1H), 6.11-6.12 (m, 1H), 4.58-4.59 (m, 1H), 4.40-4.42 (m, 1H), 3.97 (s, 3H), 3.92 (s, 3H), 3.5-3.54 (m, 2H), 3.32-3.46 (m, 2H), 2.2-2.47 (m, 2H), 2.28 (s, 6H), 2.2-2.34 (m, 2H), 1.7-1.77 (m, 2H), 1.43 (m, 9H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 173.04, 161.10, 159.166, 156.02, 128.16, 125.6, 122.75, 121.65, 119.10, 111.94, 107.29, 103.32, 80.94, 66.74, 59.60, 56.85, 55.2, 48.72, 45.11, 38.13, 36.80 and 36.62 (*diastereomer*), 32.29, 28.33, 26.61; HRMS (*m/z*, ESI): Calcd for $C_{27}H_{42}N_7O_5$: 544.3247, found, 544.3244.

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