A Convenient Method for the Synthesis of DNA-Recognizing Polyamides in Solution

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A convenient method for the synthesis of polyamides containing *N*-methylpyrrole (Py) and *N*-methylimidazole (Im) in solution has been developed. Most of the building blocks have been prepared by a haloform reaction in a simple way that column chromatography can be avoided. By use of the DCC/HOBT coupling reaction, the building blocks prepared have been effectively connected to construct a variety of subchains and polyamides without employing amino protection and deprotection. By use of the present method, an eight-ring polyamide, PyPyPyPyγPyImImPyβDp (γ is γ-aminobutyric acid, β is β-alanine, Dp is *N*,*N*-dimethylpropyldiamine), has been synthesized by the coupling of two four-ring subchains in one step.

Introduction

Various hereditary mechanisms, e.g., gene expression, DNA replication, DNA repair, and so on, in humans are based on the recognition of specific base pairs in nucleic acids. Specific recognition, high affinity binding, and cleavage of DNA sequences by small molecules have therefore always been one of the main focuses of chemistry, biology, and pharmacology.

Inspired by natural products such as netropsin, distamycin,[1] CC-1065,[2] bleomycin,[3] and calicheamycin,[4] among others, chemists have for decades developed principles with the ultimate goal of being able to design and synthesize compounds on demand that recognize and bind to any desired sequence in double-stranded DNA.[5] Artificial oligonucleotides,[6] PNA,[7] carbohydrates,[8] and proteins[9] have been broadly studied for molecular-recognition properties.

Recently, polyamides containing *N*-methylpyrrole and *N*-methylimidazole amino acids have attracted considerable attention on the part of synthetic and biological chemists because they recognize and bind in the minor groove of predetermined DNA sequences with high affinity and specificity.[10] Since these polyamides can permeate living cell membranes, they have the potential to control specific gene expression.[11] The principal rules developed by Dervan et al. are that antiparallel pairing of Py/Im targets a C·G base pair, Im/Py targets a G·C base pair;[12] and Py/Py is degenerate, recognizing either an A·T or T·A base pair.[13] Solid-phase synthesis[14] has been used for the rapid preparation of polyamides used effectively for screening of the biological activity. The shortcomings of this synthetic strategy, however, are the following: (1) the quantities prepared are very limited; (2) an excess amount of reagents is required to facilitate coupling reactions in order to gain better yields, and thus most of the reagents are wasted; and (3) protection and deprotection of amino groups are needed for a high-yield synthesis.

For the synthesis of polyamides, as well as the analogues of netropsin and distamycin, in solution, the amide bonds have been constructed by DCC,[14,15] DECP,[16] acetyl chloride,[17] and trichloroacetyl condensation reactions,[18] and the general synthetic strategy is to introduce amino

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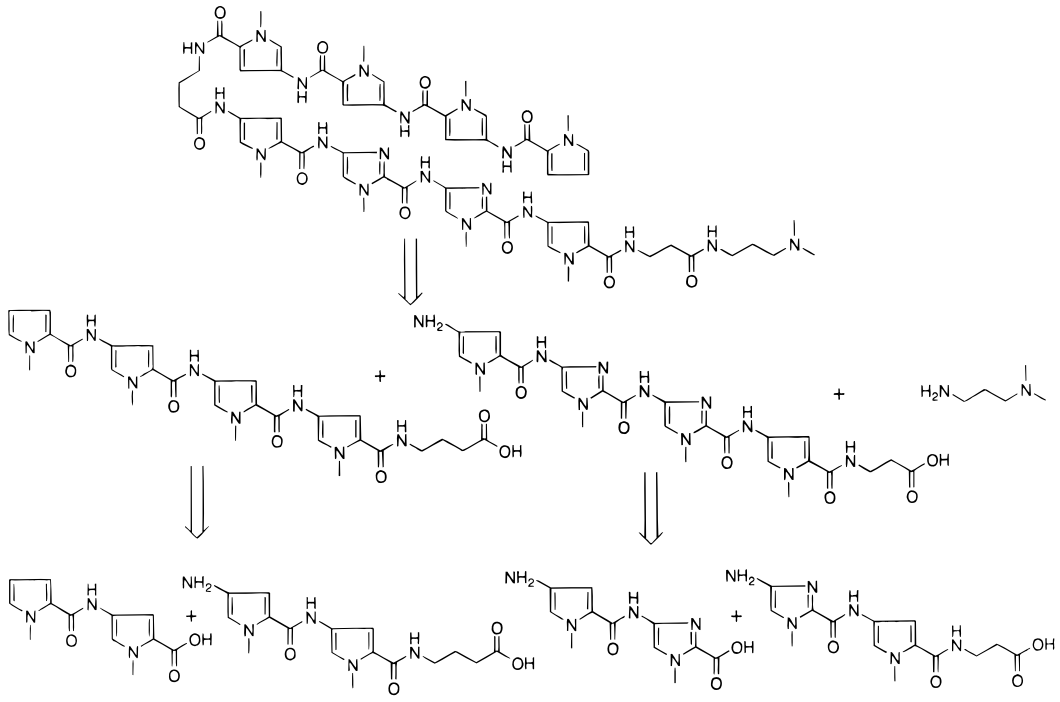
Scheme 1



acids from the N− or C− terminus sequentially. In most cases, the protection and deprotection of amino groups cannot be avoided. This synthetic route is tedious, and the yields are only marginal. In some cases, a severalfold excess of one reactant is required, and the economy of the general reaction is very poor.

To produce polyamides on a large scale and with more structural variety, we have developed a convenient method for the synthesis in the liquid phase. The polyamides synthesized by this method can easily be converted into polyamide acid derivatives, onto which other activity motifs can be attached to expand the application potentials of these polyamides.[14d, 19]

Synthetic Strategy

The binding sequence (5′-TCCT-3′) of a natural antitumor antibiotic, Calicheamicin γ^1, was chosen as the target site,[20] and a new polyamide has been designed as shown in Scheme 1.The polyamide is made up of two antiparallel subchains, and the two subchains have different carbon termini. One is a γ-aminobutyric acid, and another one is a β-alanine. According to retrosynthetic analysis (Scheme 1), the coupling of the two subchains will results in a convergent synthesis with fewer linear steps. This kind of synthesis will be advantageous over the existing methods of synthesis. The remaining step is to use the building blocks to establish the subchains. The key point in the synthesis of the polyamide was to construct the building blocks and to couple them into the subchains. In this research, the haloform reaction[18] was exploited to prepare the building blocks. By use of this kind of condensation method, dimers, trimers, and even tetramers can be obtained in a fast, simple, and convenient way without purification by column chromatography. The benefit of this kind of synthesis is that almost all kinds of different combinations of building blocks can be prepared in advance.

Results and Discussion

1. Construction of Building Block by the Haloform Reaction. For the haloform reaction, the key starting materials were 4-nitro-*N*-methyl-2-trichloroacetylpyrrole (**1**) and 4-nitro-1-methyl-2-trichloroacetylimidazole (**12**). The two compounds were easily prepared from commercially available *N*-methylpyrrole and *N*-methylimidazole through trichloroacetylation and nitration.[14,18a] Both **1** and **12** are stable at room temperature. As shown in Table 1, **12** and 1-methyl-2-trichloroacetylimidazole (**23**) can react with all the amine components in Table 1 to give the desired products without using a catalyst. **1** does not react with the amine components containing *N*-methylimidazole; however, the coupling reaction proceeds smoothly with NaH as catalyst. Nevertheless, there is no reaction between *N*-methyl-2-trichloroacetylpyrrole (**20**) and its amine components even with catalyst (NaH).

This coupling reaction was primarily governed by the electrophilicity of the carboxyl component and nucleo-

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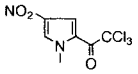
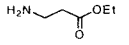
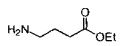
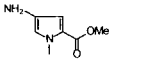
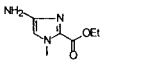
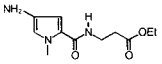
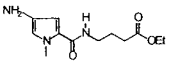
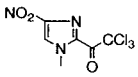
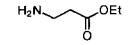
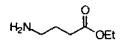
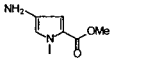
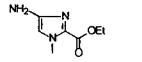
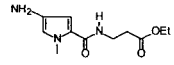
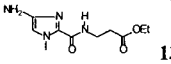
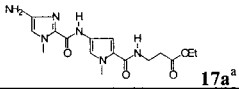
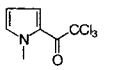
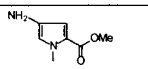
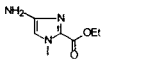
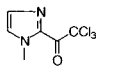
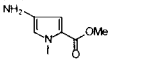
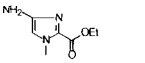
Table 1. Results of the Haloform Reaction

carboxyl component	amine component	solvent (catalyst)	product		yield (%)
 **1**	 **2**	EtOAc	$NO_2PyβOEt$	**3**	78
	 **4**	EtOAc	$NO_2PyγOEt$	**5**	75
	 **6a**[a]	EtOAc	$NO_2PyPyCOOMe$	**7**	82
	 **8a**[a]	THF (NaH)	$NO_2PyImCOOEt$	**9**	79
	 **3a**[a]	EtOAc	$NO_2PyPyβOEt$	**10**	78
	 **5a**[a]	EtOAc	$NO_2PyPyγOEt$	**11**	75
 **12**	 **2**	EtOAc	$NO_2ImβOEt$	**13**	88
	 **4**	EtOAc	$NO_2ImγOEt$	**14**	76
	 **6a**[a]	EtOAc	$NO_2ImPyCOOMe$	**15**	81
	 **8a**[a]	EtOAc	$NO_2ImImCOOEt$	**16**	71
	 **3a**[a]	EtOAc	$NO_2ImPyβOEt$	**17**	75
	 **13a**[a]	EtOAc	$NO_2ImImβOEt$	**18**	69
	 **17a**[a]	DMF	$NO_2ImImPyβOEt$	**19**	67
 **20**	 **6a**[a]	THF (NaH)	no reaction		
	 **8a**[a]	THF (NaH)	no reaction		
 **23**	 **6a**[a]	EtOAc	$ImPyCOOMe$	**24**	59
	 **8a**[a]	EtOAc	$ImImCOOEt$	**25**	61

[a] Prepared by hydrogenation of the corresponding nitro compound.

philicity of the amine component. The main differences between the four kinds of carboxyl components come from the 4-nitro or 3-nitrogen group presenting in the building block. For the carboxyl components, 4-nitro or 3-nitrogen group are electron-withdrawing, which can activate the carbonyl group and made it more easily attacked by the nucleophilic reagent. The presence of the 3-nitrogen atom in the imidazole makes **12** more electrophilic than **1**. This argument can also be used to explain the difference between **23** and its pyrrole counterpart (**20**). From Table 1, it can be observed that the activity of **23** is stronger than that of **1**. This observation is consistent with the stronger electron-withdrawing effect of the nitrogen atom in the imidazole ring than that of the nitro group attached to the pyrrole ring. The order of electrophilicity of the four kinds of carboxyl components is as shown in Figure 1.

For the amine components, on the other hand, electron-withdrawing effect of the nitrogen atom in the imidazole ring makes ethyl 4-amino-1-methylimidazole-2-carbonate

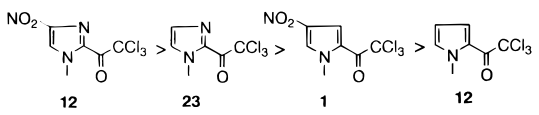


Figure 1.

(**8a**) less nucleophilic than methyl 4-amino-*N*-methylpyrrole-2-carbonate (**6a**).

In most cases, the NaH catalyst was not necessary for the coupling reaction between the carboxyl components and the amine components. The trichloroacetyl group was stable to water, and anhydrous conditions were not strictly required. Nevertheless, when the NaH catalyst was employed in the haloform reaction, dry conditions were obviously necessary.

The polyamides containing two or three heterocycles were largely insoluble in ordinary organic solvents except DMF and DMSO. The reactants with C-termini containing trichloroacetyl groups or N-termini containing amino

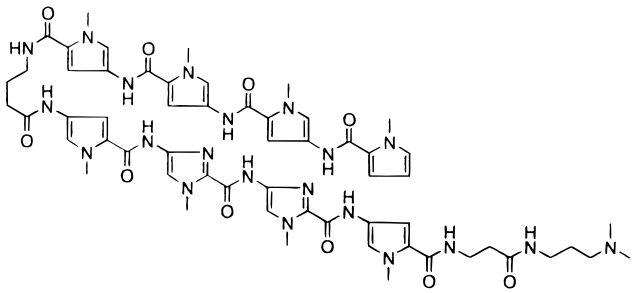
groups were more easily dissolved in $CHCl_3$, EtOAc, and EtOH. This feature greatly facilitated the purification of the product because common organic solvents could be used to wash away the reactants to leave the product after filtration.

2. Construction of Building Block by the DCC/HOBT Coupling Reaction. The main difficulty in the synthesis of building blocks is that the coupling of the **20** and the amine components was not successful even in the presence of a catalyst. To overcome this difficulty, we resorted to the DCC/HOBT coupling reaction. For example, PyPyCOOMe (**21**) was synthesized by coupling NH_2PyCOOMe (**6a**) with the active ester of PyCOOH (**20a**) in 72% yield.

The building blocks in Table 1 can be applied to the preparation of a variety of bigger building blocks by the DCC/HOBT coupling reaction. For instance, NO_2PyPyPyPyβOEt (**26**) was synthesized by the coupling of NO_2PyPyCOOH (**7a**) with NH_2PyPyβOEt (**10a**) using the DCC/HOBT coupling reaction in higher yield than results from using the haloform reaction.

The building blocks prepared by the haloform reaction cannot be applied directly to the DCC/HOBT coupling reaction. All building blocks are transformed to carboxylic acids for this coupling reaction. After saponification, an inorganic acid was used to neutralize the basic solution to give the desired product in high yield.

3. Synthesis of PyPyPyPyγPyImImPyβDp (30).



There were two crucial elements in utilizing the building blocks for the construction of tetramer subchains and polyamides by the DCC/HOBT coupling reaction. One was the quantitative reduction of the nitro compound to an amine, and the other was the preparation of active esters.

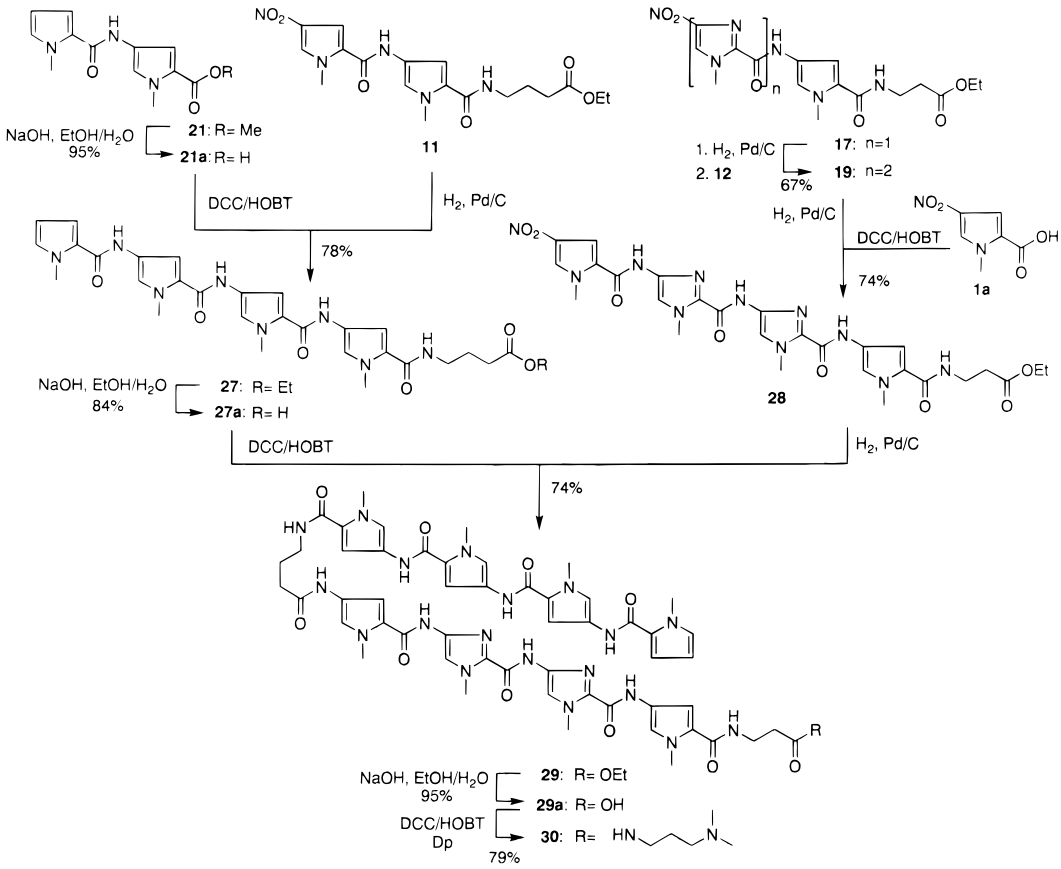
In the hydrogenation, the termination time of the hydrogenation is very important. Although the hydrogenation was almost quantitative in most cases, the amine products were rather unstable. Even under a hydrogen atmosphere, the amine compounds thus obtained gradually discomposed on long standing at room temperature. To achieve the optimum result, TLC was employed to monitor the degree of hydrogenation. Upon the completion of the hydrogenation, the reaction was terminated at once. The temperature of hydrogenation was also critical. In some cases, elevated temperature was desired. For the tetramer containing *N*-methylimidazole unit, the hydrogenation at 55 °C dramatically reduced the reaction time from days to several hours.

In the DCC/HOBT coupling reaction, the carboxyl component must first be transformed into an active ester. The solubility of the sample was an important factor for the preparation of high-quality active ester. Higher yields were usually associated with higher solubility of the starting materials. Because of the high polarity of the carboxyl component, only highly polar solvents (e.g., DMF and DMSO) can be used as reaction solvents. In the construction of the subchain **28** by the coupling of NO_2PyImCOOH (**9a**) and **17a**, the solubility of **9a** was so small, even in DMF, that the desired active ester was not generated in sufficient quantity, and the coupling was very poor. To overcome this obstacle, NO_2ImImPyβOEt (**19**) was prepared by the coupling reaction of **12** and NH_2ImPyβOEt (**17a**). Like other trimers and tetramers, **19** was not soluble in ordinary organic solvents, not even appreciably in DMF. Since all of the reactants were soluble in DMF, the precipitated product can be washed with EtOH or EtOAc to give **19** in high yield. In contrast, the solubility of NO_2PyCOOH (**1a)** was very good in DMF, and the construction of subchain **28** by the coupling of **1a** and **19** was successful.

In the preparation and use of the active ester of PyPyPyPyγCOOH (**27a**), a large amount of DMF solvent was needed to dissolve this acid which is of low solubility. The low concentration of the substrate resulted in only a small amount of active ester. Consequently, the yield of PyPyPyPyγPyImImPyβOEt (**29**) from the coupling reaction was rather low (only 16%). However, a mixed solvent of DMF and *N*-methylpyrrolidone (6:1) dissolved **27a** very well, and the yield of the coupling reaction was increased to 32%. Further experiment indicated that adding more DCC facilitated the formation of the active ester. When 3 equiv of DCC was added to the reaction solution, a significantly higher yield of **29** (74%) was achieved (see Scheme 2).When **29** was saponified with NaOH and then neutralized with hydrochloric acid, the eight-ring acid **29a** was isolated from the solvent in good yield. The hydrolysis was very fast at room temperature. It was worth noting that the control of pH after saponification was very important for the yield and for the preparation of active ester in the following step. A pH value within the range of 2−3 is optimum. When the pH value was below 2 or above 3, two spots were found in the TLC analysis of the product. One spot corresponds to product (R_f 0.8, eluant $CHCl_3/CH_3OH$ = 2:1). When the pH value was lower than 2, **29a** was partly protonated to form an ammonium salt (R_f 0.1). When the pH value was higher than 3, the desired product **29a** existed partly in the form of its conjugate base (R_f 0.1). When the pH value was kept between 2 and 3, there was only one spot in the TLC that corresponded to the desired product.

The solubility of **29a** was good in DMF. After DCC/HOBT was added, the majority of the acid was transformed into active ester. Excess *N*,*N*-dimethylpropyldiamine was added to the reaction mixture, and the final product **30** was produced in a good yield. When the coupling reaction was complete, small amounts of unreacted acid, active ester, and the excess of HOBT still remained in the solution. The R_f values of acid **29a** and the active ester were much bigger than those of HOBT and product **30** if an eluant of $CHCl_3/CH_3OH$ = 2:1 was used. However, the R_f values of HOBT and product **30** were very close (ca. 0.2∼0.3), and it was difficult to separate these compounds by gradient elution. To solve this problem, we found that the acid **29a**, the active ester, and HOBT had bigger R_f values in a solvent of higher polarity ($CH_3OH/CHCl_3$ = 2:1), whereas the R_f value of the product was very small. The best separation was achieved when a more polar solvent mixture ($CH_3OH/CHCl_3$=2:1) was employed first to elute **29a**, the active

Scheme 2



ester and the remaining HOBT and then a less polar solvent mixture ($CHCl_3/CH_3OH$=2:1) was used to elute the desired product **30**.

The final incorporation of a *N*,*N*-dimethylpropyldiamine side chain into the molecule was a crucial step in the synthesis of the polyamide. The introduction of *N*,*N*-dimethylpropyldiamine increased the polarity of the molecule. To avoid the difficulty of the separation of products with high polarities, the coupling of the amine side chain was done as the last step in the synthetic sequences. The intermediates without the amine part can easily be separated and purified by column chromatography with $CHCl_3/CH_3OH$ as eluant.

Compared with other approaches for the synthesis of polyamides, our approach will save reactants, which have been prepared through multiple steps. Because most of the acid was converted to the active ester first, the use of an excess of acid was not necessary. The equimolar amount of acid and amine component was employed in the coupling reaction using DCC/HOBT. The coupling reaction between the active ester and its amine component was convenient and fast and proceeded in high yield. On the other hand, the hydrogenation of nitro group gave 2 equiv of water, which interfered with the direct formation of the active ester using HOBT/DCC. However if most of acid is converted into the active ester before the addition of the amine component, this side reaction can be avoided.

In conclusion, the present study has demonstrated the facile and simple preparation of the building blocks, which can readily be employed for the production of all kinds of combinatorial subchains starting with *N*-methyl-4-nitro-2-trichloroacetylpyrrole and 1-methyl-4-nitro-2-trichloroacetylimidazole. This general method has been used in the synthesis of Py/Im hairpin polyamides by connection of two subchains in a single step without the need for excessive use of intermediates and amino group protecting strategies. Polyamide acid (**29a**) is a highly useful intermediate, and the attachment of polyamides to other special functional fragments to create sequence-specific molecules with unusual features is in progress. The β-alanine and γ-aminobutyric acid can alternatively substituted by other segments to adjust the properties of the desired polyamides. This will expand the scope of the polyamides and produce new hybrid molecules. They will allow us to probe deeper into the biological phenomena of interactions between small molecules and DNA, making polyamides a very useful investigation tool in gene cloning and mapping, as artificial restriction enzymes, in other operations related to DNA, and eventually in the development of drugs.

Experimental Section

Thin-layer chromatography (TLC) was performed on a silica gel 60 GF_{254} (240−400 mesh) plates and flash column chromatography was performed with silica gel 60 (240−400 mesh). Visualization was accomplished by UV light. Infrared spectra were recorded in the FT-IR mode. ^1H NMR spectra were recorded either at 400 MHz or 200 MHz, whereas ^{13}C NMR spectra were obtained at 100 MHz. Electron impact (EI) mass spectra were recorded with an ionization voltage of 70 eV, and fast-atom bombardment (FAB) mass spectra were obtained using glycerol or thioglycerol as a matrix. MALDI-TOF-MS was obtained using an α-cyano-4-hydroxycinnamic acid matrix, and accurate mass measurements (HRMS) by secondary ion mass spectrometry (SIMS) were performed using a *m*-nitrobenzyl alcohol as a matrix. HPLC analysis was performed on

a Nova-Pak HR Silica, 6 μm, 7.8 × 300 mm column in 1% or 5% CH_3OH with $CHCl_3$ as eluent and using a UV detector model at 254 nm. Elemental analyses were performed by the analytical center at Peking University. *N*,*N*-dimethylformamide, ethanol, methanol, ethyl acetate, and tetrahydrofuran were dried and purified according to standard procedures.

Synthesis of Building Blocks by the Haloform Reaction. *Ethyl β-(1-Methyl-4-nitropyrrole-2-carboxamido)alaninate ($NO_2PyβOEt$) (**3**).* Hydrochloric acid salt of ethyl β-alanine (**2**) (4.90 g, 32 mmol) was added to 150 mL of ethyl acetate, followed by the addition of TEA (8.8 mL) under ultrasonic wave. After filtering off the HCl salt of TEA, **1** (10.86 g, 40 mmol) was added. The solution was stirred overnight. Another 100 mL of ethyl acetate was added. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. After recrystallization from 60 mL of ethanol, 6.72 g of flake crystalline **3** was obtained (78% yield). IR (KBr) 3361, 3129, 1713, 1656, 1532, 1420, 1193 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.56 (d, 1H, J = 1.2 Hz), 7.08 (d, 1H, J = 0.8 Hz), 6.76 (b, 1H), 4.20 (q, 2H, J = 7.2 Hz), 3.99 (s, 3H), 3.65 (q, 2H, J = 6.2 Hz), 2.62 (t, 2H, J = 5.8 Hz), 1.29 (t, 3H, J = 7.2 Hz); MS (EI) m/z 269 (M^+); HRMS calcd for $C_{11}H_{16}N_3O_5$ (MH^+) 270.1084, found 270.1079. Anal. Calcd for $C_{11}H_{15}N_3O_5$: C, 49.07; H, 5.62; N, 15.61. Found: C, 49.14; H, 5.57; N, 15.82.

*Ethyl γ-(1-Methyl-4-nitropyrrole-2-carboxamido)butyricate ($NO_2PyγOEt$) (**5**).* A synthetic procedure similar to that for **3** was followed for the preparation of **5** (75% yield). IR (KBr) 3384, 3143, 1728, 1666, 1525, 1414, 1187 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.55 (d, 1H, J = 2.0 Hz), 7.10 (d, 1H, J = 2.0 Hz), 6.61 (b, 1H), 4.16 (q, 2H, J = 7.2 Hz), 3.99 (s, 3H), 3.44 (q, 2H, J = 6.0 Hz), 2.45 (t, 2H, J = 6.8 Hz), 1.94 (m, 2H, J = 6.8 Hz), 1.27 (t, 3H, J = 7.2 Hz); MS (EI) m/z 283 (M^+); HRMS calcd for $C_{12}H_{18}N_3O_5$ (MH^+) 284.1241, found 284.1236. Anal. Calcd for $C_{12}H_{17}N_3O_5$: C, 50.88; H, 6.05; N, 14.83. Found: C, 51.09; H, 6.01; N, 14.62.

*Methyl 1-Methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)-pyrrole-2-carbonate ($NO_2PyPyCOOMe$) (**7**).* Hydrochloric acid salt of $NH_2PyCOOMe$[14] (2.00 g, 11 mmol) was added to 30 mL of ethyl acetate, followed by TEA (3.2 mL) with ultrasonic irradiation. After filtering off the HCl salt of TEA, **1** (3.20 g, 11 mmol) was added and stirred for 2 h. After filtration, yellow solid was washed by ethyl acetate and dried. **7** (2.62 g) was obtained (82% yield). IR (KBr) 3404, 3125, 1712, 1643, 1562, 1520, 1200 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.24 (s, 1H), 8.12 (d, 1H, J = 1.7 Hz), 7.52 (d, 1H, J = 2.0 Hz), 7.42 (d, 1H, J = 2.0 Hz), 6.87 (d, 1H, J = 1.7 Hz), 3.93 (s, 3H), 3.82 (s, 3H), 3.72 (s, 3H); MS (EI) m/z 306 (M^+); HRMS calcd for $C_{13}H_{15}N_4O_5$ (MH^+) 307.1037, found 307.1045. Anal. Calcd for $C_{13}H_{14}N_4O_5$: C, 50.98; H, 4.61; N, 18.29. Found: C, 51.04; H, 4.66; N, 18.04.

*Ethyl 1-Methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)-imidazole-2-carbonate ($NO_2PyImCOOEt$) (**9**).* To a solution of $NH_2ImCOOEt$[14] (0.51 g, 3.0 mmol) in 20 mL of THF was added **1** (0.89 g, 3.3 mmol), followed by NaH (20 mg). The mixture was stirred for 6 h. The slight yellow precipitate was collected by filtration, washed with water and methanol, and dried to afford 0.76 g of yellow solid **9** (79% yield). ^1H NMR (DMSO-d_6, 200 MHz) δ 11.18 (s, 1H), 8.19 (d, 1H, J = 1.4 Hz), 7.81 (d, 1H, J = 2.0 Hz), 7.69 (s, 1H), 4.27 (q, 2H, J = 7.2 Hz), 3.95 (s, 3H), 3.93 (s, 3H), 1.29 (t, 3H, J = 7.2 Hz); MS (EI) m/z 321 (M^+); HRMS calcd for $C_{13}H_{16}N_5O_5$ (MH^+) 322.1146, found 322.1150. Anal. Calcd for $C_{13}H_{15}N_5O_5$: C, 48.60; H, 4.71; N, 21.80. Found: C, 48.59; H, 4.76; N, 21.55.

*Ethyl γ-[1-Methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxamido]butyricate ($NO_2PyPyγOEt$) (**11**).* A synthetic procedure similar to that for **17** was followed for the preparation of **11** (75% yield). IR (KBr) 3420, 3107, 1716, 1643, 1540, 1515, 1417, 1209 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.06 (s, 1H), 7.60 (d, 1H, J = 1.6 Hz), 7.28 (d, 1H, J = 2.2 Hz), 7.20 (d, 1H, J = 1.8 Hz), 6.57 (d, 1H, J = 2.2 Hz), 6.26 (t, 1H, J = 6.6 Hz), 4.15 (q, 2H, J = 7.2 Hz), 4.03 (s, 3H), 3.91 (s, 3H), 3.42 (q, 2H, J = 6.2 Hz), 2.42 (t, 2H, J = 7.0 Hz), 1.95 (m, 2H, J = 6.8 Hz), 1.26 (t, 3H, J = 7.2 Hz); MS (FAB) m/z 406 (MH^+); HRMS calcd for $C_{18}H_{24}N_5O_6$ (MH^+) 406.1721, found 406.1716. Anal. Calcd for $C_{18}H_{23}N_5O_6$: C, 53.33; H, 5.72; N, 17.27. Found: C, 53.26; H, 5.75; N, 17.09.

*Ethyl β-[1-Methyl-4-(1-methyl-4-nitroimidazole-2-carboxamido)pyrrole-2-carboxamido]alaninate ($NO_2ImPyβOEt$) (**17**).* To a solution of **3** (4.00 g, 15 mmol) in 250 mL of EtOAc was added Pd/C catalyst (10%, 0.64 g). The mixture was stirred under a slight positive pressure of H_2 overnight. The catalyst was removed by filtration through Celite, and the filtrate was concentrated in vacuo to remove the EtOAc. To the residue was added **12** (4.00 g, 15 mmol) in 6 mL of DMF immediately. The reaction mixture was stirred for 6 h and filtered to collect yellow solid, washed with ethanol and ethyl acetate, and dried to give 4.40 g of **17** (75% yield). IR (KBr) 3414, 3126,1729, 1680, 1534, 1461 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.97 (s, 1H), 7.83 (s, 1H), 7.22 (d, 1H, J = 2.5 Hz), 6.58 (d, 1H, J = 1.6 Hz), 6.53 (b, 3H), 4.21 (s, 1H), 4.19 (q, 2H, J = 7.0 Hz), 3.93 (s, 3H), 3.64 (q, 2H, J = 5.4 Hz), 2.61 (t, 2H, J = 6.0 Hz), 1.29 (t, 3H, J = 7.2 Hz); MS (FAB) m/z 393 (MH^+); HRMS calcd for $C_{16}H_{21}N_6O_6$ (MH^+) 393.1517, found 393.1516. Anal. Calcd for $C_{16}H_{20}N_6O_6$: C, 48.98; H, 5.40; N, 21.42. Found: C, 48.72; H, 5.37; N, 21.26.

Synthesis of Building Blocks by the Coupling Reaction using DCC/HOBT. *Methyl 1-Methyl-4-(1-methylpyrrole-2-carboxamido)pyrrole-2-carbonate ($PyPyCOOMe$) (**21**).* To a solution of 1-methylpyrrole-2-carboxylic acid (2.00 g, 16 mmol) in 16 mL of DMF was added HOBT (2.16 g, 16 mmol), followed by DCC (3.30 g, 16 mmol) in 16 mL of CH_2Cl_2. The reaction solution was stirred overnight to ensure the complete formation of the active ester. HCl salt of methyl 4-amino-1-methylpyrrole-2-carbonate (3.05 g, 16 mmol) was added to 16 mL of CH_2Cl_2 in a separate flask, followed by TEA (5.0 mL) with ultrasonic irradiation. After filtration, the filtrate was added to the solution of the active ester. The reaction mixture was stirred at room temperature for 4 h. DCU was removed by filtration, and the filtrate was concentrated in vacuo to remove the CH_2Cl_2 solvent. To the remaining solution was added 60 mL of ethyl acetate. The organic layer was washed with water (30 mL), saturated $NaHCO_3$ solution (30 mL × 3), 4% HCl (30 mL × 3), and brine (30 mL × 2) and was dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated in vacuo to give a white solid. Dried under an IR lamp, a white solid of **21** weighing 2.98 g was obtained (72% yield). IR (KBr) 3322, 3116, 1698, 1634, 1546, 1450, 1413 cm^{-1}; ^{1}HNMR (DMSO-d_6, 200 MHz) δ 7.52 (b, 1H), 7.43 (d, 1H, J = 2.0 Hz), 6.77 (d, 1H, J = 1.6 Hz), 6.75 (d, 1H, J = 2.4 Hz), 6.64 (q, 1H, J = 1.6), 6.12 (q, 1H, J = 2.2 Hz), 3.97 (s, 3H), 3.90 (s, 3H), 3.81 (s, 3H); MS (FAB) m/z 262 (MH^+); HRMS calcd for $C_{13}H_{16}N_3O_3$ (MH^+) 262.1186, found 262.1192. Anal. Calcd for $C_{13}H_{15}N_3O_3$: C, 59.76; H, 5.79; N, 16.08. Found: C, 59.89; H, 5.84; N, 15.73.

*$NO_2PyPyPyPyβOEt$ (**26**).* To 1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamide)pyrrole-2-carboxylic acid (0.29 g, 1.0 mmol) in 2 mL of DMF was added HOBT (0.14 g, 1.0 mmol), followed by DCC (0.21 g, 1.0 mmol) in 2 mL of CH_2Cl_2. The reaction solution was stirred overnight. DCU was removed by filtration. Separately, to a solution of $NO_2PyPyβOEt$ (0.36 g, 0.92 mmol) in 9 mL of DMF was added Pd/C catalyst (10%, 60 mg), and the mixture was stirred under a slight positive pressure of H_2 overnight. The catalyst was removed by filtration through Celite, and the filtrate was directed into the active ester solution. The mixture was stirred for 20 h. After filtration, to the filtrate was added 40 mL of $CHCl_3$. The organic layer was washed with water, saturated $NaHCO_3$ aqueous, and 4% HCl and brine and was dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated in vacuo. After purification by column chromatography with a mixture of methanol and chloroform as eluent (gradient eluate), 0.35 g of **26** was obtained (60% yield). ^1H NMR (DMSO-d_6, 400 MHz) δ 10.29 (s, 1H), 10.0 (s, 1H), 9.89 (s, 1H), 8.16 (d, 1H, J = 1.9 Hz), 8.04 (t, 1H, J = 5.6 Hz), 7.58 (d, 1H, J = 1.7 Hz), 7.27 (d, 1H, J = 1.5 Hz), 7.24 (d, 1H, J = 1.5 Hz), 7.18 (d, 1H, J = 1.7 Hz), 7.04 (t, 2H, J = 2.0 Hz), 6.84 (d, 1H, J = 1.5 Hz), 4.05 (q, 2H, J = 7.1 Hz), 4.00 (s, 3H), 3.86 (s, 3H), 3.84(s, 3H), 3.79 (s, 3H), 3.39 (m, 2H), 2.52 (t, 2H, J = 6.8 Hz), 1.17 (t, 3H, J = 7.1 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.4, 161.4,

158.5, 158.4, 157.0, 133.8, 128.2, 126.3, 123.1, 122.8, 122.7, 122.2, 122.1, 121.5, 118.7, 118.6, 118.0, 107.6, 104.8, 104.6, 104.4, 59.9, 37.5, 36.2, 36.1, 35.9, 34.8, 34.1, 14.1; MS (FAB) *m*/*z* 636 (MH^+); HRMS calcd for $C_{29}H_{34}N_9O_8$ (MH^+) 636.2525, found 636.2514.

Saponification. *1-Methyl-4-(1-methylpyrrole-2-carboxamido)pyrrole-2-carboxylic Acid (PyPyCOOH) (**21a**).* To a solution of PyPyCOOMe (2.61 g, 10 mmol) in 70 mL of ethanol was added NaOH (1.20 g in 50 mL of water). The reaction solution was stirred at room temperature overnight. After filtration, the filtrate was concentrated in vacuo to remove the ethanol solvent. The pH of the remaining aqueous solution was adjusted to about 1 by adding 6 N HCl. The precipitate was collected by filtration and was washed with water and dried under an IR lamp to offer 2.34 g of **21a** (95% yield). IR (KBr) 3463, 1665, 1581, 1418 cm^{-1}; H^1 NMR (DMSO-d_6, 200 MHz) δ 12.16 (b, 1H), 9.81 (s, 1H), 7.42 (s, 1H), 6.93 (s, 1H), 6.89 (s, 1H), 6.81 (s, 1H), 6.05 (s, 1H), 3.86 (s, 3H), 3.82 (s, 3H); MS (FAB) *m*/*z* 248 (MH^+); HRMS calcd for $C_{12}H_{14}N_3O_3$ (MH^+) 248.1030, found 248.1036. Anal. Calcd for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 17.00. Found: C, 58.27; H, 5.35; N, 16.97.

Synthesis of PyPyPyPyγPyImImPyβDp. *NO_2ImImPyβOEt (**19**).* A synthetic procedure similar to that for **17** was followed for the preparation of **19**, and 3.23 g of product was obtained (67% yield). IR (KBr) 3373, 3125, 1727, 1642, 1545, 1436 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 10.34 (s, 1H), 10.17 (s, 1H), 8.66 (s, 1H), 8.10 (t, 1H, J = 5.4 Hz), 7.58 (s, 1H), 7.24 (s, 1H), 6.97 (s, 1H), 4.08 (q, 2H, J = 7.2 Hz), 4.07 (s, 3H), 4.02 (s, 3H), 3.80 (s, 3H), 3.41 (q, 2H, J = 6.4 Hz), 2.54 (t, 2H, J = 7.2 Hz), 1.20 (t, 3H, J = 7.2 Hz); MS (FAB) *m*/*z* 516 (MH^+); HRMS calcd for $C_{21}H_{26}N_9O_7$ (MH^+) 516.1950, found 516.1966.

*PyPyPyPyγOEt (**27**).* A synthetic procedure similar to that for **26** was followed for the preparation of **27**, and 2.13 g of product was obtained (78% yield). IR (KBr) 3306, 3126, 1721, 1641, 1538, 1467 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.19 (s, 1H), 8.05 (s, 1H), 7.79 (s, 1H), 7.17 (s, 1H), 7.13 (s, 2H), 6.77 (s, 1H), 6.75 (s, 1H), 6.71 (s, 1H), 6.57 (s, 1H), 6.55 (s, 1H), 6.43 (t, 1H, J = 5.4 Hz), 6.10 (t, 1H, J = 3.2 Hz), 4.10 (q, 2H, J = 7.2 Hz), 3.95 (s, 3H), 3.86 (s, 3H), 3.82 (s, 6H), 3.39 (q, 2H, J = 6.2 Hz), 2.38 (t, 2H, J = 7.0 Hz), 1.88 (m, 2H, J = 6.8 Hz), 1.21 (t, 3H, J = 7.2 Hz); MS (FAB) *m*/*z* 605 (MH^+); HRMS calcd for $C_{30}H_{37}N_8O_6$ (MH^+) 605.2831, found 605.2833.

*PyPyPyPyγCOOH (**27a**).* A synthetic procedure similar to that for **21a** was followed for the preparation of **27a**, and 1.50 g of product was obtained (84% yield). IR (KBr) 3314, 1714, 1640, 1541, 1467, 1256 cm^{-1}; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 12.08 (s, 1H), 9.96 (s, 1H), 9.91 (s, 1H), 9.85 (s, 1H), 8.05 (t, 1H, J = 5.4 Hz), 7.25 (s, 2H), 7.19 (s, 1H), 7.06 (s, 2H), 6.95 (s, 1H), 6.93 (s, 1H), 6.88 (s, 1H), 6.07 (t, 1H, J = 5.4 Hz), 3.89 (s, 3H), 3.86 (s, 6H), 3.80 (s, 3H), 3.19 (q, 2H, J = 5.8 Hz), 2.26 (t, 2H, J = 7.4 Hz), 1.71 (m, 2H, J = 7.4 Hz); MS (FAB) *m*/*z* 577 (MH^+); HRMS calcd for $C_{28}H_{33}N_8O_6$ (MH^+) 577.2517, found 577.2485.

*NO_2PyImImPyβOEt (**28**).* To a solution of 1-methyl-4-nitropyrrole-2-carboxylic acid (0.77 g, 4.5 mmol) in 10 mL of DMF was added HOBT (0.62 g, 4.6 mmol), followed by DCC (0.93 g, 4.5 mmol). The reaction mixture was stirred overnight at room temperature. Separately, to a solution of **19** (2.33 g, 4.5 mmol) in 25 mL of DMF was added Pd/C catalyst (10%, 0.52 g), and the mixture was stirred under a slight positive pressure of H_2 at 55 °C for 12 h. The catalyst was removed by filtration through Celite, and the filtrate was directed into the active ester and stirred overnight. After filtration, 100 mL of $CHCl_3$ was added to the filtrate, and the organic layer was washed with brine (40 mL×10) and dried over anhydrous $MgSO_4$. The desiccator was removed by filtration, and the filtrate was concentrated in vacuo. After purification by column chromatography with the mixed solvent of $CHCl_3$ and CH_3OH as eluant, 2.13 g of **28** was obtained (74% yield) as a slight yellow solid. IR (KBr) 3371, 3132, 1726, 1650, 1543, 1474 cm^{-1}; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 10.90 (s, 1H), 10.26 (s, 1H), 9.43 (s, 1H), 8.23 (s, 1H), 8.11 (t, 1H, J = 5.4 Hz), 7.77 (s, 1H), 7.67 (s, 1H), 7.59 (s, 1H), 7.24 (s, 1H), 6.96 (s, 1H), 4.07 (q, 2H, J = 7.0 Hz), 4.02 (s, 3H), 4.01 (s, 3H), 3.97 (s, 3H), 3.81 (s, 3H), 3.42 (q, 2H, J = 6.4 Hz), 2.54 (t, 2H, J = 7.0 Hz), 1.19 (t, 3H, J = 7.2 Hz); MS (FAB) *m*/*z* 638 (MH^+); HRMS calcd for $C_{27}H_{32}N_{11}O_8$ (MH^+) 638.2430, found 638.2433.

*PyPyPyPyγPyImImPyβOEt (**29**).* To a mixture of **27a** (0.40 g, 0.70 mmol) in 4 mL of DMF was added 0.7 mL of *N*-methylpyrrolidone to make a clear solution. To this solution was added HOBT (0.29 g, 2.2 mmol), followed by DCC (0.42 mg, 2.0 mmol). The reaction solution was stirred overnight. Separately, to a solution of **28** (0.44 g, 0.70 mmol) in 9 mL of DMF was added Pd/C catalyst (10%, 0.13 g), and the mixture was stirred under a slight positive pressure of H_2 at 70 °C for 6 h. The catalyst was removed by filtration through Celite, and the filtrate was directed into the active ester solution and stirred overnight. The mixture was filtered to remove the DCU, and the filtrate was concentrated in vacuo. Column chromatography of the residue (gradient elate 0−5% CH_3OH in $CHCl_3$) provided 0.60 g of **29** (74% yield). IR (KBr)3305, 3125, 1710, 1647, 1533, 1437, 1252 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.19 (s, 1H), 10.04 (s, 1H), 9.77 (s, 1H), 9.73 (s, 1H), 9.71 (s, 1H), 9.67 (s, 1H), 9.36 (s, 1H), 7.92 (t, 1H, J = 5.6 Hz), 7.90 (t, 1H, J = 5.6 Hz), 7.58 (s, 1H), 7.53 (s, 1H), 7.25 (d, 1H, J = 1.6 Hz), 7.20 (s, 3H), 7.15 (d, 1H, J = 1.8 Hz), 7.05 (d, 1H, J = 1.8 Hz), 7.04 (d, 1H, J = 1.8 Hz), 6.95 (t, 2H, J = 1.7 Hz), 6.90 (d, 3H, J = 1.8 Hz), 6.05 (t, 1H, J = 2.6 Hz), 4.08 (q, 2H, J = 7.1 Hz), 4.02 (s, 3H), 4.01 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H), 3.85 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.42 (q, 2H, J = 6.5 Hz), 3.25 (q, 2H, J = 6.5 Hz), 2.53 (t, 2H, J = 7.0 Hz), 2.31 (t, 2H, J = 7.3 Hz), 1.82 (m, 2H, J = 7.2 Hz), 1.19 (t, 3H, J = 7.1 Hz); ^{13}C NMR(DMSO-d_6, 100 MHz) δ 171.4, 169.4, 161.3, 161.2, 158.7, 158.6, 158.5, 155.5, 155.2, 136.6, 134.6, 134.5, 132.7, 128.1, 125.5, 123.0, 122.8, 122.2, 122.1, 121.7, 121.2, 119.3, 118.4, 118.2, 117.8, 115.4, 113.6, 112.6, 106.6, 104.8, 104.7, 104.5, 104.3, 59.9, 38.2, 36.2, 36.1, 36.0, 35.9, 35.2, 35.0, 34.8, 34.0, 33.3, 25.7, 14.1; MS (MALDI-TOF) *m*/*z* 1188.5 (MNa^+, 1188.5 calcd for $C_{55}H_{63}N_{19}O_{11}$ + Na); HRMS calcd for $C_{55}H_{64}N_{19}O_{11}$ (MH^+)1166.5033, found 1166.5026.

*PyPyPyPyγPyImImPyβCOOH (**29a**).* A synthetic procedure similar to that for **21a** was followed for the preparation of **29a**, and 185 mg of product was obtained **(**95% yield). IR (KBr) 3393, 1643, 1538, 1437, 1256 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.20 (s, 1H), 10.02 (s, 1H), 9.78 (s, 1H), 9.74 (s, 1H), 9.72 (s, 1H), 9.68 (s, 1H), 9.38 (b, 1H), 7.91 (m, 2H), 7.58 (s, 1H), 7.53 (s, 1H), 7.26 (s, 1H), 7.20 (s, 3H), 7.15 (s, 1H), 7.05 (s, 1H), 7.04 (s, 1H), 6.95 (s, 1H), 6.94 (s, 1H), 6.90 (s, 3H), 6.05 (t, 1H, J = 3.1 Hz), 4.02 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H), 3.85 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.57 (s, 3H), 3.39 (b, 2H), 3.25 (q, 2H, J = 6.0 Hz), 2.44 (t, 2H, J = 6.8 Hz), 2.31 (t, 2H, J = 7.2 Hz), 1.82 (m, 2H, J = 7.2 Hz); ^{13}C NMR(DMSO-d_6, 100 MHz) δ 173.3, 169.4, 161.3, 161.2, 158.7, 158.6, 158.5, 155.5, 155.2, 136.6, 134.6, 134.4, 132.7, 128.1, 125.5, 123.1, 122.9, 122.8, 122.2, 122.1, 121.7, 121.2, 119.3, 118.4, 118.2, 117.8, 115.4, 113.6, 112.6, 106.6, 104.7, 104.4, 104.3, 38.2, 36.2, 36.1, 35.9, 35.1, 35.0, 34.5, 33.3, 25.7; MS (FAB) *m*/*z* 1138 (MH^+); HRMS calcd for $C_{53}H_{60}N_{19}O_{11}$ (MH^+)-1138.4719, found 1138.4683.

*PyPyPyPyγPyImImPyβDp (**30**).* To a solution of **29a** (106 mg, 0.093 mmol) in 0.5 mL of DMF was added HOBT (40 mg, 0.30 mmol), followed by DCC (58 mg, 0.28 mmol). The reaction solution was stirred overnight. *N*,*N*-Dimethylpropyldiamine (40 μL) was added to the reaction solution, and the stirring was continued for another 6 h. DCU was removed by filtration, and the filtrate was concentrated in vacuo. Flash column chromatography of the residue ($CH_3OH/CHCl_3$ = 2:1 and $CH_3OH/CHCl_3$ = 1:2) afforded 90 mg of the polyamide **30 (**79% yield). IR (KBr) 3392,1648, 1535, 1438, 1205 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.40 (s, 1H), 10.26 (s, 1H), 9.94 (s, 1H), 9.91 (d, 2H, J = 2.5 Hz), 9.84 (s, 1H), 9.43 (s, 1H), 8.06 (t, 1H, J = 5.6 Hz), 8.04 (t, 1H, J = 5.6 Hz), 7.90 (t, 1H, J = 5.4 Hz), 7.63 (s, 1H), 7.58 (s, 1H), 7.31 (d, 1H, J = 1.4 Hz), 7.24 (s, 2H), 7.23 (d, 1H, J = 1.5 Hz), 7.19 (d, 1H, J = 1.4 Hz), 7.07 (d, 1H, J = 1.7 Hz), 7.06 (d, 1H, J = 1.6 Hz), 6.96 (d, 1H, J = 1.7 Hz), 6.95 (s, 2H), 6.93 (t, 2H, J = 2.4 Hz), 6.06 (m, 1H), 4.02 (s, 3H), 4.01 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H), 3.85 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.37 (b, 2H), 3.23 (q, 2H, J = 5.6 Hz), 3.07 (m, 4H), 2.33 (t, 2H, J = 7.2 Hz), 2.26 (t, 2H, J = 7.2 Hz), 2.16 (s, 6H), 1.80 (q, 2H, J = 6.8 Hz), 1.56 (q,

2H, $J = 7.2$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.4, 169.4, 161.3, 161.2, 160.9, 158.7, 158.6, 158.5, 155.5, 155.2, 136.6, 134.6, 134.4, 132.7, 128.1, 125.5, 123.2, 123.0, 122.8, 122.2, 122.1, 121.7, 121.2, 119.3, 118.4, 118.2, 117.8, 115.4, 113.6, 112.6, 106.6, 104.8, 104.7, 104.4, 104.3, 38.2, 36.7, 36.2, 36.1, 36.0, 35.9, 335.5, 35.3, 35.2, 35.0, 33.3, 32.3, 26.8, 25.7, 24.8, 24.6; MS (MALDI-TOF) *m*/*z* 1244.5 (MNa^+, 1244.6 calcd for $C_{58}H_{71}N_{21}O_{10}$+Na). HRMS calcd for $C_{58}H_{72}N_{21}O_{10}$ (MH^+) 1222.5771, found 1222.5793.

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Supporting Information Available: HPLC analyses of **26**−**29**. This information is available free of charge via the Internet at http://acs.pubs.org.

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