A General Solution- and Solid-Phase Synthetic Procedure for Incorporating Three Contiguous Imidazole Moieties into DNA Sequence Reading Polyamides

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Introduction

Efforts to discover a universal set of chemical rules for the digital readout of information from double helical DNA by synthetic agents have met with encouraging success.[1,2] Small molecules that target specific DNA sequences have the potential to control gene expression.[3] Polyamides containing *N*-methylpyrrole and *N*-methylimidazole moieties, related to the naturally occurring oligopeptidic antibiotics netropsin[4] and distamycin,[5,6] are synthetic ligands that have an affinity for DNA comparable with naturally occurring DNA binding proteins.[2,7] The DNA sequence specificity of these small molecules is controlled, in part, by the linear sequence of pyrrole and imidazole units. The natural products netropsin and distamycin bind tightly and specifically to regions with four and five AT base pairs,[8] respectively. However in an attempt to recognize and bind longer sequences of DNA the empirical $n + 1$ rule[9] cannot be extended indefinitely because as the number of repeating units increases the mismatching becomes more severe (called the phasing problem) and this is manifested by a trend of weaker binding with higher homologues.[10] The NMR studies of Pelton and Wemmer[11−13] showed that at sufficiently high drug to DNA ratios two distamycin molecules can be located simultaneously in the minor groove in a highly overlapped antiparallel side by side manner. The distamycin units can be linked in two different ways as a hairpin[14] (**I**) or in a cross-linked (stapled)[15,16] (**II**) manner (Figure 1). Alternative approaches developed by Dervan[17] and Lown[18] for the design of sequence-specific DNA binding molecules that recognize longer sequences of double-helical DNA is to couple DNA binding units of similar or diverse base pair specification as extended bispolyamides tethered by linkers of appropriate shape and dimensions **III** (Figure 1). These extended bispolyamides can then address the problems of mismatching and phasing since by selecting a linker of appropriate length the semantophoric moieties may be brought into register. A number of pyrrolobispolyamides tethered by various linkers with varying geometry and length which have diverse activities have been synthesized in our group.[18] For example, a number of them were active inhibitors of HIV-1 integrase.[18b] The biological activities of pyrrolobispolyamides differed markedly depending on the geometrical configuration of the two polyamides units, and the agents bearing a para configuration were considerably more potent than those with the meta configuration. Recently it has been determined that geometrically constrained bis- distamycins are inhibitors of HIV-1 reverse transcription RNA directed DNA polymerization[18e] and bind selectively to Okazaki fragments with a specific orientation of the linkers.[18c] It was evident from results of Pommier et al.[18b] that the effective inhibitory concentrations of the tripyrrolobispyrroloamides were approximately 200-fold higher for the artificial control GC containing sequences than with the natural sequences containing the conserved AT stretch.

Dickerson[19] and Lown[20] first conceived the idea of "lexitropsin" longer chain analogues of netropsin which retain pyrrole groups at those positions where an AT base pair was to be read but substituted an imidazole group

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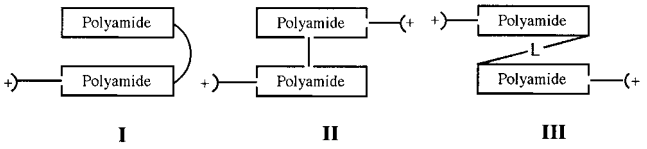


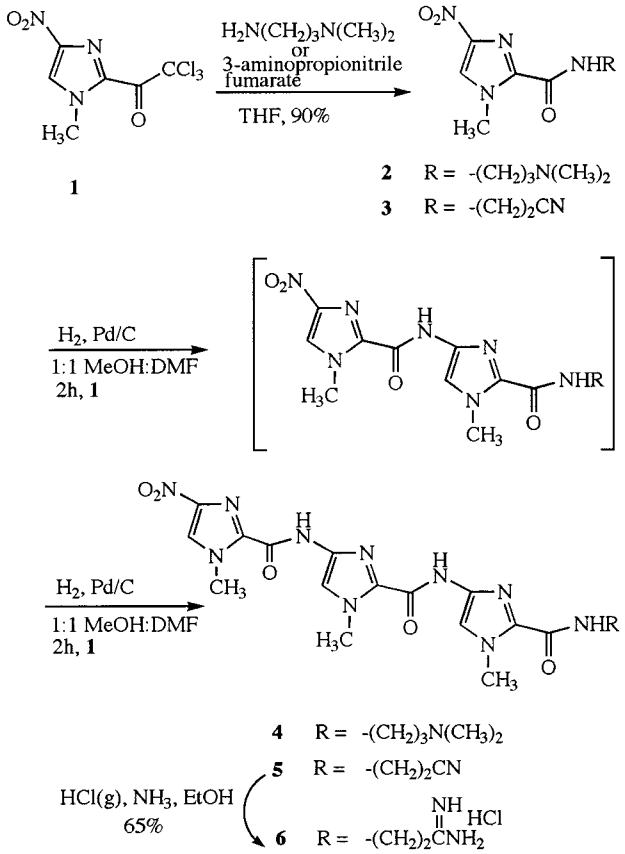
Figure 1. Hairpin (**I**), cross-linked (stapled) (**II**), extended bispolyamide (**III**).

at sites where GC pair reading was desired. Imidazole provides space for a guanine-2-amine group and provides an acceptor for a new hydrogen bond from G-2-NH_2 groups, thereby permitting a change in the base site recognition from that shown by pyrrole. It remained to be determined that given the structural requirement for accommodating the exocyclic 2-amine group of contiguous GC pairs sequences, whether one can design polyamide sequences in a such a way that these GC rich sequences could be read out with very high fidelity. Discrimination of GC from CG by the Im/Py pair requires precise positioning of the key hydrogen bond between the imidazole N3 and the exocyclic amine of guanine.[21] Dervan et al.[22] have synthesized hairpins with a maximum of four imidazole units and extended polyamides with a maximum of two contiguous imidazole units on each side of the linker. Encouraged by these and other preliminary results and with the results of the potent inhibitory effects of pyrrolobispolyamides on HIV-1 integrase (in nano molar concentrations), murine leukemic and of Okazaki fragments, we have designed a general protocol for the synthesis of imidazole polyamides which could recognize extended GC sequences with very high affinity. We have linked the three imidazole units by both flexible and rigid linkers. The problem of cellular uptake and plasma membrane permeability posed by polyamides bearing unnatural dimethyl(aminopropyl)amino termini has been addressed by incorporating the natural amidinum end group **13**−**15**. The corresponding triimidazobispolyamides bearing the unnatural end group, i.e., dimethylaminopropyl **7**−**9** were, however, also synthesized for comparison purposes against various targets. As the number of imidazole units increases in the sequence the solubility of the polyamide intermediate decreases and hence the purification of the compounds become difficult. To overcome this problem we have synthesized the triimidazole-polyamide precursor **16** for these bispolyamides by a solid-phase method.We report here a general procedure for the introduction of several contiguous imidazole moieties into the DNA reading polyamides by a combined solution and solid-phase method.

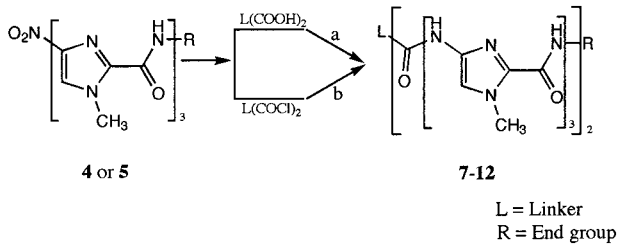
Results and Discussion

Compound **1** was synthesized by the reported procedure.[23] Subsequently, condensation with 3-(dimethylamino)propylamine or 3-aminopropionitrile fumarate in THF gave **2** and **3**, respectively (Scheme 1). A Pd/C-facilated reduction of **2** yielded an unstable amine which on coupling with **1** resulted in a diimidazole unit which was again reduced by Pd/C and coupled with **1** to give a triimidazotricarboxamide **4**. Similarly, compound **5** was

Scheme 1



Scheme 2[a]



[a] Key: (a) DCC/HOBt, rt, 24 h, 30% yield; (b) DMF/THF (1:1), Et_3N, rt, 10 h, 50% yield.

synthesized from **3** and **1**.[18g] The triimidazotricarboxamide **4** was reduced by Pd/C to give an unstable amine which was coupled with fumaric acid in 2:1 stoichiometery (Scheme 2), catalyzed by DCC/HOBt (path a, Scheme 2) to give the desired compound **7** in 30% yield in 24 h. The yields were better (50%) when the reduced amine of **4** was coupled with the fumaryl chloride with triethylamine (path b, Scheme 2). Since the yields were better with acid chloride coupling, therefore we converted the diacid 2,5-pyridine dicarboxylic acid into acid chlorides by treating them with $SOCl_2$/DMF while terephthaloyl chloride and fumaryl chlorides were used directly. These diacid chlorides were coupled with the reduced amine of **4** to afford compounds **8** and **9** (Scheme 2, Figure 2). Trimidazotricarboxamide compound **5** bearing a propionitrile terminus was converted to the amidinium terminus by Pinner reaction[24] with HCl in ethanol followed by ammonia which gave an excellent yield (70%) of the triimidazotricarboxamide **6** bearing an amidinium terminus. Attempts were made to couple the reduced amine of **6** with the acid chlorides of the linkers,

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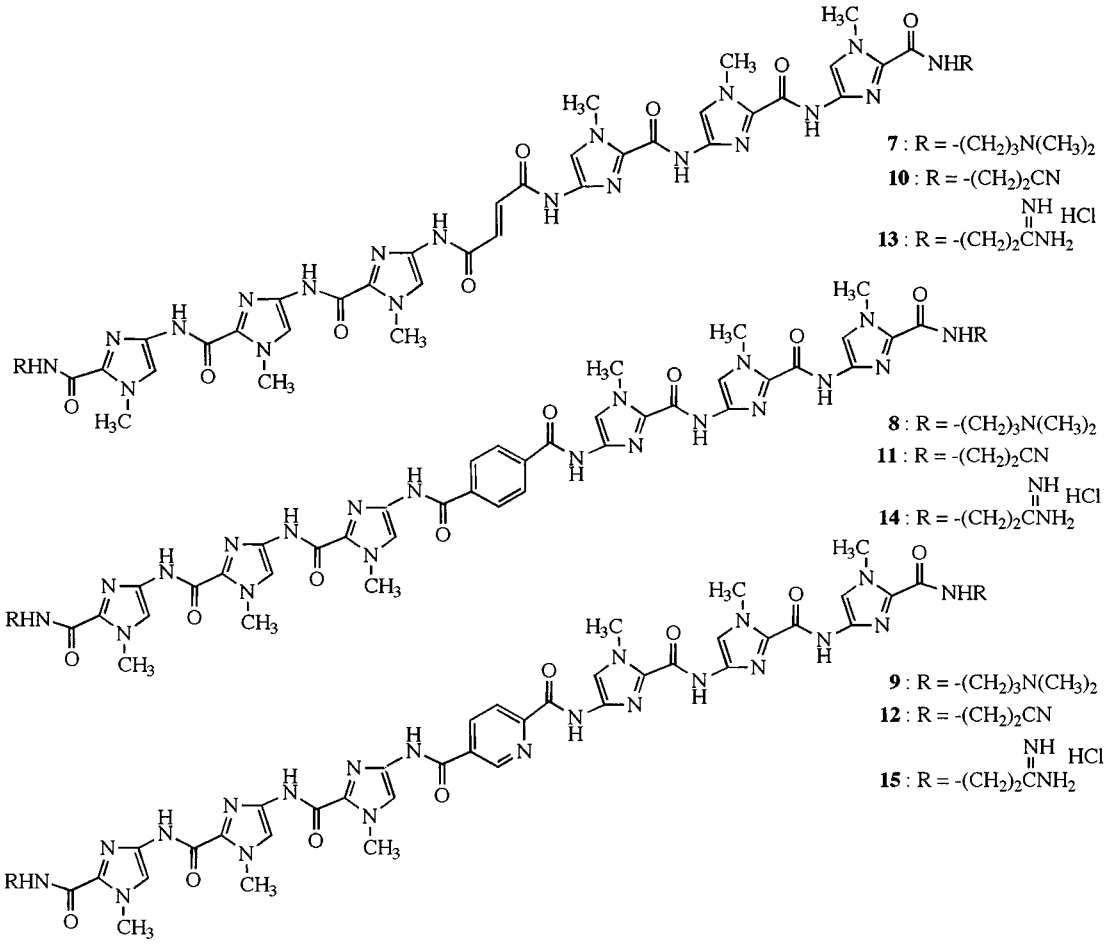

Figure 2. Triimidazolo bispolyamide.

but this did not work well because of the contamination with the inorganic salts generated by the Pinner reaction. Therefore we synthesized compounds **10**−**12** by coupling the reduced amine of **5** with the acid chlorides of the linkers in a similar way as described for compounds **7**−**9**. Finally the bispolyamides **10**−**12** with propionitrile termini were subjected to modified Pinner reaction conditions to give compounds **13**−**15**, respectively, in pure form bearing natural amidinium termini. Our observations agree with those of Baksheev et al.[14c] that the first step of the reaction of the cyano group, i.e., formation of the imino ester with an alcohol in the presence of hydrogen chloride, is completed in 90 min and that longer reaction times promote side reactions resulting in lower yields. The imino ester reacts readily with ammonia in ethanolic solution within 1h at ambient temperature to afford amidinium termini.

The triimidazole polyamide **16** having three contiguous imidazole moieties was synthesized by a solid-phase method. A considerable amount of work by Dervan's group has been reported on the synthesis of hairpins by using a Boc-*β*-alanine-Pam- resin with Boc chemistry.[25] In all the hairpins reported so far they have used a pyrrole moiety as the first unit linked to the resin and then subsequently either pyrrole or imidazole or hydroxypyrrole units are incorporated for increasing the chain length of hairpin, thereby leading to a terminus of *β*-alanine-*N*,*N*′-dimethylaminopropyl always with a pyrrole unit after cleaving the hairpin from the solid supprt with *N*,*N*′-dimethylaminopropylamine. Since we were interested in the synthesis of contiguous imidazole introduction in the DNA sequence reading polyamide we adapted the reported methodologies[25] for the synthesis of a triimidazole polyamide.

The free amine from the resin was obtained by deblocking the Boc group with 80% TFA then coupling the freshly prepared Boc-Im-COOBt ester from the Boc-Im-COOH in dry DMF with DIEA base. The reaction vessel was shaken for 2h, the resin was washed and dried, then a small sample of this resin was taken for analysis, while with the rest of the resin was used for another cycle. The shaking for the rest of the cycle was continued for 90 min. Finally the sequence was cleaved from the resin with *N*,*N*′-dimethylaminopropylamine to afford **16** (Scheme 3)**.** The terminal imidazole moiety bears a protected amino functionality which can be used for further chain elongation of the sequence of imidazole or pyrrole or could be converted into the formyl group as required.

Certain polyamides bearing contiguous imidazole groups have shown pronounced RNase A type cleavage activity at significantly low concentrations. RNase promotes hydrolysis at neutral pH by locally increased concentrations of acid and base. The optimal pH of the RNase like activity of the imidazole bearing polyamides was found to be 7.0 which is at or near the pK_a of the imidazole ring. Cleavage activity of the imidazole polyamides is significantly enhanced by salt concentrations (especially magnesium ions) in the low millimolar concentration

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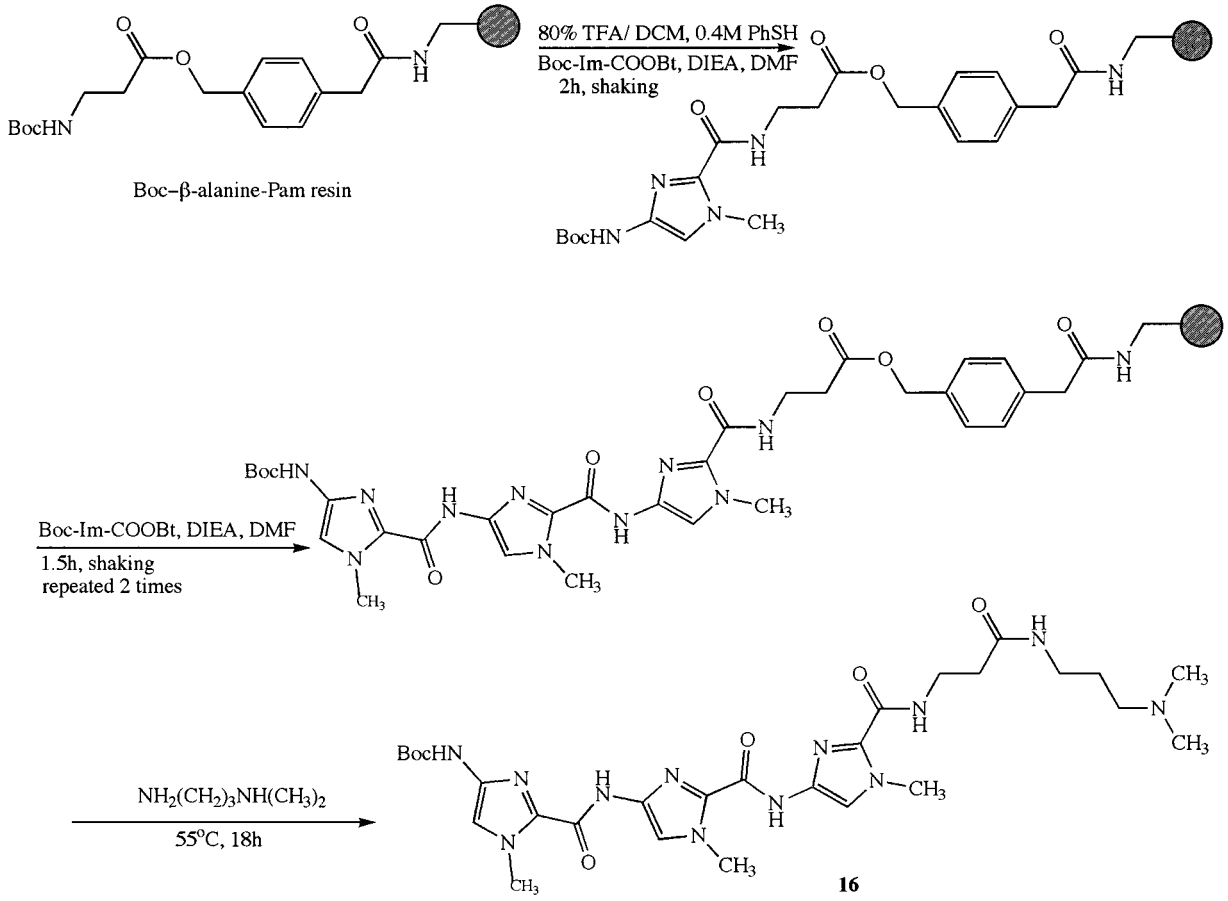
Scheme 3



range. Activity is weak in the absence of salt and inhibited at high concentrations of magnesium chloride or sodium chloride. The cleavage activity shows a bell-shaped dependence on the concentrations of the imidazole polyamide. Appreciable cleavage activity is already seen at a concentration of 0.3 mM of the compounds and activity is significantly decreased at concentration higher than 3.0 mM. RNA cleavage is structure and site specific and cleavage occurs preferentially in the single stranded regions of the different tRNA substrates.[26] The new synthetic procedure described herein will permit, inter alia, systematic exploration of this novel property of polyamides.

Experimental Section

General Methods. Melting points were determined with an electrothermal melting point apparatus and are uncorrected. All the chemicals used were of reagent grade. Dimethylformamide (DMF), methanol (MeOH), and tetrahydrofuran (THF) were of anhydrous grade procured from Aldrich Chemical Co. and were used without further purification. Freshly distilled dichloromethane (DCM) was used. The progress of the reactions was monitored by thin-layer chromatography using precoated silica gel 60F 254, E. Merck TLC plates visualizing under UV light. ^{1}H NMR spectra were recorded with a Brucker (300 MHz) spectrometer with tetramethylsilane (TMS) as internal standard on the ppm scale (δ). Multiplicity of resonance peaks are indicated as singlet (s),broad singlet (bs), doublet (d), quartet (q), triplet (t), and multiplet (m). Mass spectrometric analysis was performed by positive mode elctrospray ionization with Micromass ZapSpec Hybrid Sector -TOF.

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***N,N′*-Bis[[(3-(dimethylamino)propyl]-1-[methyl-2-[1-methyl-2-(1-methyl-2-carboxamido-4-imidazole)carboxamido-4-imidazole]carboxamido-4-imidazol-yl]]fumaryl Dicarboxamide (7). General Procedure.** Compound **4** (0.50 g, 0.99 mmol) was dissolved in DMF and methanol (10 mL, 1:1, v/v) and hydrogenated in the presence of Pd−C (10%, 0.15 g) for 2 h at room temperature. The catalyst was removed by filtration and washed with methanol. The solvent was evaporated in vacuo, and the residue was dried under high vacuum to remove traces of the solvent. The amino compound so obtained was redissolved in anhydrous DMF (10 mL) and fumaryl chloride (75 mg, 0.49 mmol) in DMF/THF (1:1, 5 mL) was added to this solution at 0 °C under an atmosphere of argon. The reaction mixture was gradually brought to room temperaure. The reaction mixture was maintained under an atmosphere of argon and stirred at 22 °C for 18 h. At this time, the TLC showed completion of the reaction. The solvent was removed in vacuo and the crude product was purified on a silica gel column using CH_2Cl_2/MeOH/NH_4OH (8:2:0.2) as eluent to afford pure **7** (158 mg, 50% yield). Mp: >300 °C. IR (film): 3386, 2956, 2714, 1672, 1538, 1473, 1335, 1125, 1019, 907, 794 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.76 (m, J = 7.0 Hz, 4H), 2.50 (s, 12H), 2.70 (t, J = 7.0 Hz, 4H), 3.32 (t, J = 6.0 Hz, 4H), 3.90 (s, 6H), 3.94 (s, 6H),4.10 (s, 6H), 7.27 (s, 2H), 7.55 (s, 2H), 7.65 (s, 2H), 7.68 (s, 2H), 8.48 (t, J = 6.0 Hz, 2H, exchanged with D_2O), 9.68 (s, 2H, exchanged with D_2O), 9.80 (s, 2H, exchanged with D_2O), 11.06 (s, 2H, exchanged with D_2O). HRMS: calcd for $C_{44}H_{59}N_{22}O_8$ 1023.488, found 1023.489 (M^+ + H, 100).

***N,N′*-Bis[[(3-(dimethylamino)propyl]-1-[methyl-2-[1-methyl-2-(1-methyl-2-carboxamido-4-imidazole)carboxamido-4-imidazole]carboxamido-4-imidazol-yl]]phenyl-1,4-dicarboxamide (8).** The title compound was synthesized similarly by coupling the reduced amine of **4** with terephthaloyl chloride to give **8** in 53% yield. Mp: 243−245 °C. IR (film): 3388, 2959, 1668, 1605, 1549, 1481, 1368, 1025, 908, 765 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.80 (m, J = 7.0 Hz, 4H), 2.56 (s, 12H), 2.78 (t, J = 7.0 Hz, 4H) 3.20 (t, J = 6.0 Hz, 4H), 3.95 (s, 6H), 4.00 (s, 6H),

4.10 (s, 6H), 7.54 (s, 2H), 7.68 (s, 2H), 7.75 (s, 2H), 8.12 (s, 4H), 8.46 (t, J = 6.0 Hz, 2H, exchanged with D_2O), 9.63 (s, 2H, exchanged with D_2O), 9.80 (s, 2H, exchanged with D_2O), 11.14 (s, 2H, exchanged with D_2O). HRMS: calcd for $C_{48}H_{61}N_{22}O_8$ 1072.504, found 1073.504 (M^+ + H, 100).

***N,N′*-Bis[[(3-(dimethylamino)propyl]-1-[methyl-2-[1-methyl-2-(1-methyl-2-carboxamido-4-imidazole)carboxamido-4-imidazole]carboxamido-4-imidazol-yl]]pyridine-2,5-dicarboxamide (9).** The title compound was synthesized in a similar manner by coupling the reduced amine of **4** with the 2,5-diacid chloride of pyridine to give **9** in 50% yield. Mp: 198−200 °C. IR (film): 3387, 2954, 1666, 1543, 1476, 1367, 1020, 908, 765 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.88 (m, J = 7.0 Hz, 4H), 2.72 (s, 12H), 3.04 (t, J = 7.0 Hz, 4H) 3.30 (t, J = 6.0 Hz, 4H), 3.92 (s, 6H), 3.97 (s, 6H),4.08 (s, 6H), 7.50 (s, 2H), 7.65 (s, 2H), 7.75 (s, 2H), 8.28 (d, J = 7.0 Hz, 1H), 8.56 (t, J = 6.0 Hz, 3H, 2H exchanged with D_2O), 8.59 (m,1H), 9.26 (d, J = 2.0 Hz, 1H) , 9.90 (s, 2H, exchanged with D_2O), 10.01 (s, 2H, exchanged with D_2O), 10.08 (s, 2H, exchanged with D_2O). HRMS: calcd for $C_{47}H_{60}N_{23}O_8$ 1074.499, found 1074.501 (M^+ + H, 100).

***N,N′*-Bis[(3-(3-propionitrile)-1-[methyl-2-[1-methyl-2-(1-methyl-2-carboxamido-4-imidazole)carboxamido-4-imidazole]carboxamido-4-imidazolyl]fumaryl Dicarboxamide (10).** Compound **5** was reduced with 10% Pd/C in DMF similarly, and the reduced amine was allowed to react with fumaryl chloride in a similar manner to give **10** in 45% yield. Mp: 259−261 °C. IR (film): 3380, 2982, 1665, 1640, 1543, 1480, 1021, 910, 764 cm^{-1}. ^1H NMR (DMSO-d_6): δ2.78 (t, J = 6.0 Hz,4H), 3.48 (q, 4H,), 3.97 (s, 6H), 4.00 (s, 6H), 4.05 (s, 6H), 7.55 (s, 3H), 7.66 (s, 2H), 7.68 (s, 2H), 7.71(s, 2H), 8.50 (t, J = 6.0 Hz, 2H, exchanged with D_2O), 9.64 (s, 2H, exchanged with D_2O), 9.72 (s, 2H, exchanged with D_2O), 11.05 (s, 2H, exchanged with D_2O). HRMS: calcd for $C_{40}H_{43}N_{22}O_8$ 959.363, found 959.362 (M^+ + H).

The following compounds were synthesized in a similar manner:

***N,N′*-Bis[(3-(3-propionitrile)-1-[methyl-2-[1-methyl-2-(1-methyl-2-carboxamido-4-imidazole)carboxamido-4-imidazole]carboxamido-4-imidazolyl]phenyl-1,4-dicarboxamide (11).** Yield: 50%. Mp: 223−225 °C. IR (film): 3380, 2980, 1661, 1642, 1601, 1543, 1475, 1020, 910, 763 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.79 (t, J = 6.0 Hz, 4H), 3.49 (q, 4H), 3.95 (s, 6H), 3.97 (s, 6H), 4.02 (s, 6H), 7.02 (s, 2H), 7.58 (s, 2H), 7.66 (s, 2H), 8.02 (s, 4H), 8.57 (t, J = 6.0 Hz, 2H, exchanged with D_2O), 9.59 (s, 2H, exchanged with D_2O), 9.90 (s, 2H, exchanged with D_2O), 10.45 (s, 2H, exchanged with D_2O). HRMS: calcd for $C_{44}H_{45}N_{22}O_8$ 1009.379, found 1009.379 (M^+ + H).

***N,N′*-Bis[(3-(3-propionitrile)-1-[methyl-2-[1-methyl-2-(1-methyl-2-carboxamido-4-imidazole)carboxamido-4-imidazole]carboxamido-4-imidazolyl]pyridine-2,5-dicarboxamide (12).** Yield: 43% yield. Mp: 225−227 °C. IR (film) 3383, 2923, 1665, 1638, 1601, 1550, 1463, 1019, 908, 764 cm^{-1}. ^1H NMR (DMSO-d_6): δ2.75 (t, J = 6.0 Hz, 4H), 3.49 (q, 4H), 3.91 (s, 6H), 3.99 (s, 6H), 4.00 (s, 6H), 7.51 (s, 2H), 7.61 (s, 2H), 7.72 (s, 2H), 8.19 (d, J = 7.0 Hz, 1H), 8.49 (t, J = 6.0 Hz, 3H, 2H exchanged with D_2O), 9.10 (m, 1H), 10.01 (s, 2H, exchanged with D_2O), 10.45 (s, 2H, exchanged with D_2O), 11.31 (s, 2H, exchanged with D_2O). HRMS: calcd for $C_{43}H_{44}N_{23}O_8$ 1010.374, found 1010.373(M^++H)

***N,N′*-Bis[(3-propiamidine)-1-[methyl-2-[1-methyl-2-(1-methyl-2-carboxami-do-4-imidazole)carboxamido-4-imidazole]carboxamido-4-imidazolyl]fumaryl dicarboxamide Dihydrochloride (13). General Procedure.** Compound **10** (500 mg, 0.52 mmol) in 25 mL of anhydrous ethanol was saturated with dry HCl with cooling.[14c] Upon saturation, the compound dissolved in the ethanol solution. After 90 min at room temperature the solvent was evaporated to dryness and the residue was washed with dry ethyl ether (2 × 50 mL) and then dried. The residue was again dissolved in dry ethanol followed by the treatment with NH_3 condensed (4.5 mL) into the reaction vessel. The reaction mixture was stirred at room temperature for 1 h. The solvent was then removed. The residue was redissolved in methanol (100 mL), and the impurities were collected by filtration. The filtrate was concentrated to a small volume (10 mL), and the pure compound was collected as the dihydrochloride salt **13** (336 mg,65% yield). Mp: >300 °C. IR (film): 3269, 2953, 2719, 1667, 1538, 1474, 1370, 1125, 1020, 967, 766 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.80 (t, J = 6.0 Hz, 4H), 3.53 (q, 4H), 4.03 (s, 6H), 4.05 (s, 6H), 4.10 (s, 6H), 7.42 (s, 2H), 7.64 (s, 2H), 7.67 (s, 2H), 8.52 (t, J = 6.0 Hz, 2H), 8.90 (brs, 4H), 9.01 (brs, 2H), 9.70 (s, 2H), 10.62 (s, 2H), 11.10 (s, 2H). HRMS: calcd for $C_{40}H_{49}N_{24}O_8$ 993.416, found 993.419 (M^+ + H, 100).

***N,N′*-Bis[(3-propiamidine)-1-[methyl-2-[1-methyl-2-(1-methyl-2-carboxamido-4-imidazole)carboxamido-4-imidazole]carboxamido-4-imidazolyl]phenyl-1,4-dicarboxamide Dihydrochloride (14).** Yield: 65%. Mp: >300 °C. IR (film): 3344, 1666, 1605, 1542, 1477, 1407, 1125, 1019, 907, 750 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.79 (t, J = 6.0 Hz, 4H), 3.50 (q, 4H), 4.05 (s, 6H), 4.08 (s, 6H), 4.13 (s, 6H), 7.67 (s, 2H), 7.70 (s, 2H), 7.80 (s, 2H), 8.25 (s, 4H), 8.56 (t, J = 6.0 Hz, 2H), 9.84 (s, 2H), 8.91 (brs, 4H), 9.15 (brs, 2H), 10.89 (s, 2H), 11.12 (s, 2H). HRMS: calcd for $C_{44}H_{51}N_{24}O_8$ 1043.432, found 1043.434 (M^+ + H).

***N,N′*-Bis[(3-propiamidine)-1-[methyl-2-[1-methyl-2-(1-methyl-2-carboxamido-4-imidazole)carboxamido-4-imidazole]carboxamido-4-imidazolyl]pyridine-2,5-dicarboxamide Dihydrochloride (15).** Yield: 65%. Mp: >300 °C. IR (film): 3338, 1671, 1603, 1542, 1474, 1367, 1125, 1019, 907, 797, 766 cm^{-1}. ^1H NMR (DMSOd$_6$): δ2.83 (t, J = 6.0 Hz,4H), 3.54 (q, 4H), 4.01 (s, 6H), 4.08 (s, 6H), 4.16 (s, 6H), 7.43 (s, 2H), 7.62 (s, 2H), 7.70 (s, 2H), 8.29 (d, J = 7.0 Hz, 1H), 8.57 (t, J = 6.0 Hz, 3H), 9.10 (brs, 5H), 9.45 (brs, 2H), 9.69 (s, 2H), 10.80 (s, 2H), 11.08 (s, 2H). HRMS: calcd for $C_{43}H_{50}N_{25}O_8$ 1044.427, found 1044.424 (M^+ + H).

Synthesis of Triimidazole Moiety by Solid-Phase Method (16). Boc-β-alanine-Pam-resin[25] (230 mg, 0.046 mmol) was packed in a 20 mL filtration column fitted with a filter and stopper at both ends. A volume of 2 mL of dry DMF was added to the resin, the mixture was shaken for 5 min, and then the solvent was drained; this procedure was repeated two more times. Then the resin was washed with dry DCM (2 × 5 mL). The resin was dried by vacuum in the column and after drying it was treated with 2 mL of 80% TFA/DCM and 1 mL of 0.5 M PhSH and shaken (2 × 30 min.). The resin was washed thoroughly with DMF (3 × 5 mL) and then with DCM (5 × 5 mL). The activated ester of imidazole was prepared[25] by stirring the mixture of 50 mg of Boc-Im-COOH (0.2 mol), HOBt (27 mg, 0.2 mmol), and DCC (41 mg, 0.2 mmol) in DMF (2 mL) for 15 min. This activated ester was then transferred to the column containing free NH_2 group on the resin with DMF (1 mL) and 0.1 mL of DIEA. This mixture was shaken for 2 h. A small portion of the resin was taken for analysis while the rest of the resin was subjected to two more cycles of coupling. After three cycles of imidazole coupling the resin was washed with DMF (3 × 5 mL) and DCM (5 × 5 mL), the resin was dried and then placed in a small glass vial (5 mL). To the resin was added 1.5 mL of 3-dimethylaminopropylamine, and this mixture was heated at 55 °C for 18 h. The resin was collected washed with DCM, and the filtrate was concentrated and purified by column chromatography.The pure compound **16** was eluted with 80:20:0.2 (CH_2Cl_2/MeOH/NH_4OH) in 55% yield as a white colored solid. Mp: 159−162 °C. IR (film): 3269, 2953, 2702, 1715, 1667, 1571, 1543, 1234, 1166, 1009, 907, 764 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 1.45 (s, 9H), 1.74 (m, 2H), 2.36 (t, J = 7.0 Hz, 2H), 2.52 (s, 6H), 2.85 (t, J = 4.9 Hz, 2H), 3.09 (m, 2H), 3.24−3.46 (m, 8H), 3.94 (s, 6H), 3.98 (s, 3H), 7.27 (s, 1H), 7.50 (s, 1H), 7.61 (s, 1H), 8.18 (2t, J = 6.0 Hz, 2H, exchanged with D_2O), 9.53 (s, 1H), 9.60 (brs, 1H, exchanged with D_2O), 9.82 (s, 1H, exchanged with D_2O). ^{13}CNMR (DMSO-d_6) δ: 170.74, 158.24, 155.46, 155.35, 152.67, 136.97, 135.01, 134.58, 134.18, 133.30, 132.75, 114.19, 113.68, 54.76, 42.43, 35.79, 35.18, 35.07, 34.95, 34.89, 28.06, 24.61, 21.46. HRMS: calcd for $C_{28}H_{43}N_{12}O_6$ 643.342, found 643.342 (M^+ + H, 100).

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Supporting Information Available: ^1H NMR spectra of compounds **4**, **5**, **7**−**12**, and **16**, HRMS of compound **10**−**16**, and ^{13}CNMR spectra of compound **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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