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Cysteine Cyclic Pyrrole−Imidazole Polyamide for Sequence-Specific Recognition in the DNA Minor Groove

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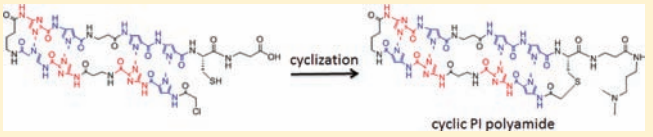
Supporting Information

ABSTRACT: Pyrrole−imidazole (PI) polyamides are small DNA-binding molecules that can recognize predetermined DNA sequences with high affinity and specificity. Hairpin PI polyamides have been studied intensively; however, cyclic PI polyamides have received less attention, mainly because of difficulties with their synthesis. Here, we describe a novel cyclization method for producing PI polyamides using cysteine and a chloroacetyl residue. The cyclization reaction is complete within 1 h and has a high conversion efficiency. The method can be used to produce long cyclic PI polyamides that can recognize 7 bp DNA sequences. A cyclic PI polyamide containing two β-alanine molecules had higher affinity and specificity than the corresponding hairpin PI polyamide, demonstrating that the cyclic PI polyamides can be used as a new type of DNA-binding molecule.



INTRODUCTION

Small DNA-binding molecules have been assessed as antitumor and antibacterial drugs.[1−4] Dervan et al. demonstrated the molecular design of *N*-methylpyrrole (P)−*N*-methylimidazole (I) polyamides as specific minor-groove-binding molecules.[5,6] PI polyamides can recognize each of the four Watson−Crick base pair sequences through side-by-side pairing along the 5′−3′ axis of the DNA helix.[7] An antiparallel pairing of I opposite P (I/P) recognizes a G·C base pair, whereas a P/P pair recognizes A·T or T·A base pairs.

Hairpin PI polyamides with a γ-aminobutyric acid turn (γ-turn) have been demonstrated as useful DNA-binding units with respect to both sequence specificity and DNA binding affinity.[4] These attributes are similar to those of transcription factors. Whereas hairpin PI polyamides with fewer than 10 rings can bind to the minor groove of B-DNA without any substitution, those with 10 rings or more require β-alanines for efficient binding.[8] β-Alanine acts as an aliphatic substitute for a P ring to match the curvature of the minor groove surfaces at longer binding sites.[9,10] Recently, in vivo studies have shown that hairpin PI polyamides can inhibit the expression of specific genes by competitive binding to regulatory sequences.[11−14] In addition, hairpin polyamides conjugated with various compounds, such as alkylating agents,[15,16] fluorescent dyes,[17−21] or SAHA,[22] have been developed to add different functions to PI polyamides. The polyamides have also been used for the construction of nanodevices.[23]

Cyclic PI polyamides are alternatives to hairpin PI polyamides as specific DNA-binding molecules. Cyclic PI polyamides have been constructed by adding a second γ-turn at the C and N termini of hairpin PI polyamides.[24] An eight-ring cyclic PI polyamide was shown to bind to a corresponding DNA sequence with higher affinity than the alternative hairpin PI polyamide.[25] In terms of mismatched DNA sequences, the specificity of the eight-ring cyclic PI polyamide was also better than that of the alternative hairpin PI polyamide.[25,26] The high-resolution crystal structure of the cyclic PI polyamide−DNA complex provided a molecular basis for disruption of transcription factor−DNA interfaces.[27,28] Additionally, cyclic PI polyamides targeted to androgen response elements (AREs) modulated gene expression in cell culture.[29] The cyclic polyamides were shown to inhibit androgen receptor-mediated expression of prostate-specific antigen by targeting the DNA sequence found in the ARE. However, studies on the biological activities of cyclic PI polyamides are limited, mainly because of the low yield of cyclization. So far, only eight-ring cyclic PI polyamides have been used in biological studies.[27,28] Therefore, more efficient cyclization procedures for PI polyamides are needed for intensive studies on cyclic PI polyamides.

Here, we describe a novel cyclization reaction for PI polyamides using cysteine (Cys) and a chloroacetyl residue. This method is more efficient than the conventional PI polyamide cyclization method[24] and can be applied to longer PI polyamides. We also examined the DNA binding affinities and sequence specificities of Cys cyclic PI polyamides. Some had higher affinities for the target DNA sequence than the corresponding

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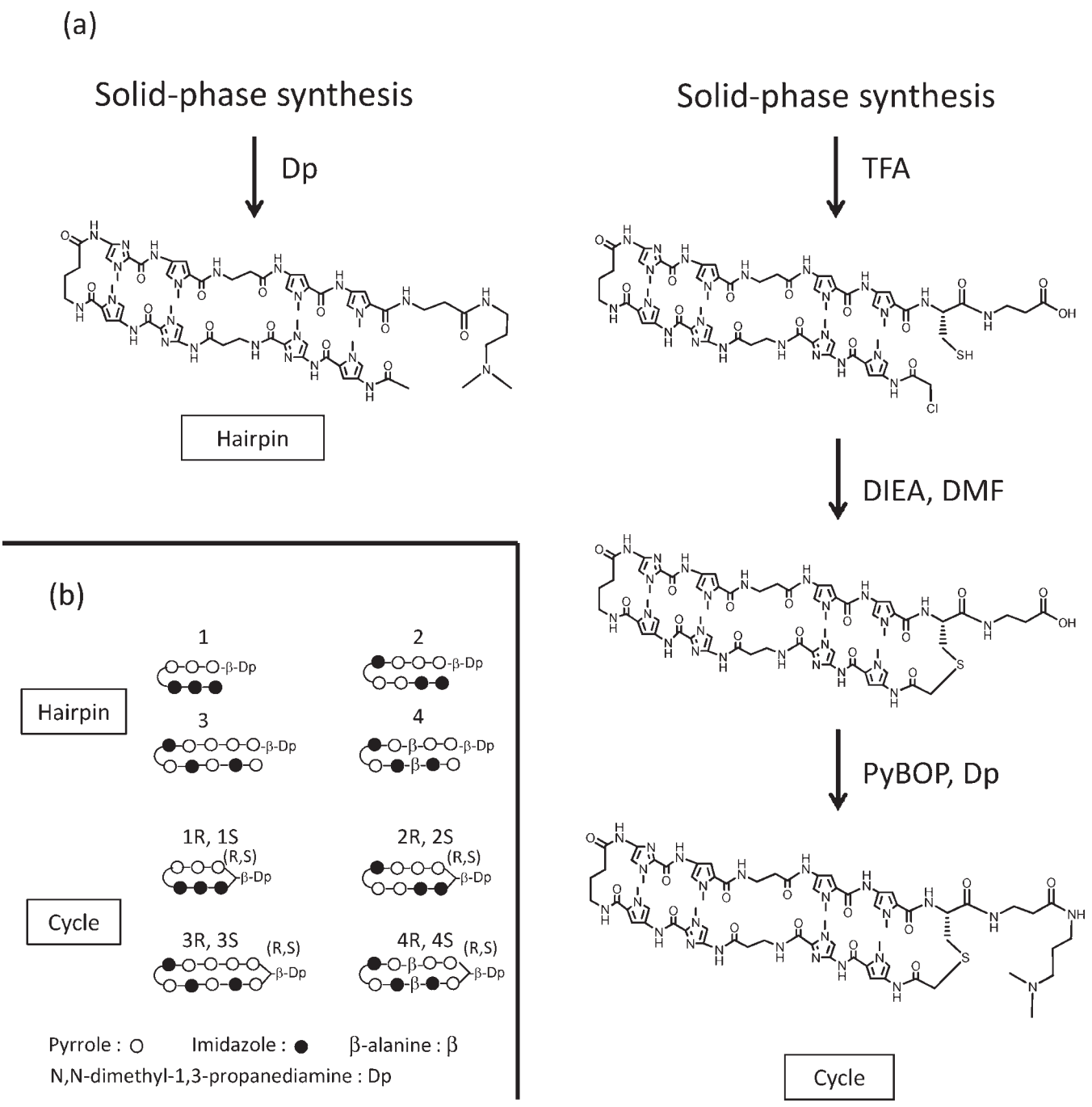

Figure 1. (a) Syntheses of hairpin (**4**) and Cys cyclic (**4R**) PI polyamides. (b) Structures of 12 PI polyamides used in this study.

hairpin PI polyamides. These Cys cyclic PI polyamides have potential for biological applications.

RESULTS AND DISCUSSION

Cys Cyclic PI Polyamide Synthesis. Cyclization of PI polyamides is accomplished by a reaction between the N-terminal chloroacetyl group and the sulfhydryl group on Cys.[30,31] This cyclization method was used by Suga et al.[32] to produce cyclic polypeptides but has not yet been applied to the PI polyamide. After completion of machine-assisted solid-phase synthesis, the hairpin PI polyamides containing Cys and a chloroacetyl group at the N-terminal were cleaved from resins using trifluoroacetic acid (TFA). The resulting crude extract was dissolved in

Table 1. Binding Affinities of Six-Ring PI Polyamides

	5′-biotin-G C G C T A A C C C T A A C T_T β-Dp 3′-C G C G A T T G G G A T T G T^T		
polyamide	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
1 β-Dp	2.6×10^5	1.6×10^{-2}	6.4×10^{-8}
1R ^{R}β-Dp	7.7×10^3	1.8×10^{-3}	2.3×10^{-7}
1S ^{S}β-Dp	9.9×10^3	1.8×10^{-3}	1.9×10^{-7}

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dimethylformamide (DMF) and diisopropylethylamine (DIEA) for 30 min. (Benzotriazol-1-yloxy)trispyrrolidinophosphonium hexafluorophosphate (PyBOP) and N,N-dimethyl-1,3-propanediamine (Dp) were added to a reaction solution at room temperature. After the resulting solution was stirred for 40 min, the solvent was removed in vacuo. After purification by high-performance liquid chromatography (HPLC), the desired cyclic PI polyamides were obtained (**1R, 1S, 2R, 2S, 3R, 3S, 4R,** and **4S**) (Figure 1b). To examine the chiral effects of Cys on the affinity of PI polyamides, we made two types of cyclic PI polyamides, *R*-form and *S*-form, which are produced by L-Cys and D-Cys, respectively. All cyclization reactions of the eight types of cyclic PI polyamides were completed within 1 h with a high conversion efficiency. This Cys cyclization method is thought to be more useful than the previously proposed method using amine condensation.[24]

Table 2. Binding Affinities of Eight-Ring PI Polyamides

5′-biotin-G C T A G T C C A G G C T_T-β-Dp
3′-C G A T C A G G T C C G T^T

polyamide	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
2 1-β-Dp	9.8×10^5	5.7×10^{-3}	5.8×10^{-9}
2R 1-(R)β-Dp	3.0×10^4	1.9×10^{-3}	6.2×10^{-8}
2S 1-(S)β-Dp	1.0×10^4	1.1×10^{-3}	1.1×10^{-7}

Table 3. Binding Affinities of Ten-Ring PI Polyamides

5′-biotin-G C C T G C A C T A G C C T_T-β-Dp
3′-C G G A C G T G A T C G G T^T

polyamide	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
3 1-β-Dp	1.1×10^4	3.3×10^{-3}	2.9×10^{-7}
3R 1-(R)β-Dp	5.6×10^3	3.2×10^{-2}	5.7×10^{-6}
3S 1-(S)β-Dp	1.4×10^3	1.9×10^{-2}	1.4×10^{-5}

Hairpin PI Polyamide Synthesis. To compare the DNA-binding abilities of hairpin PI polyamides to identify the same target sequence, four hairpin PI polyamides were synthesized using the Fmoc solid-phase synthesis method, starting with Fmoc-β-Ala-Wang resin and using the Fmoc monomers according to the previous procedure.[33] After completion of machine-assisted solid-phase synthesis, the hairpin PI polyamides were cleaved from resins by Dp to produce PI polyamides **1**−**4** (Figure 1). Products were purified by reversed-phase HPLC, and the structures were identified by ^{1}H NMR and electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS).

Binding Affinities of Six-Ring, Eight-Ring, and Ten-Ring Cyclic PI Polyamides. The binding affinities of six-, eight-, and ten-ring cyclic PI polyamides were measured using a surface plasmon resonance (SPR) method by a Biacore X instrument. Each PI polyamide was passed to a 5′-biotinylated hairpin DNA immobilized on a sensor chip by a biotin−avidin system. The sensorgrams of six-ring PI polyamides (**1, 1R**, or **1S**) are shown in Figure S1 (Supporting Information), and the rates of association (k_a) and dissociation (k_d) and the dissociation constants (K_D) are shown in Table 1. Compared with the six-ring hairpin PI polyamide, the lower k_a rates of the six-ring cyclic PI polyamides indicate a weaker association and the lower k_d rates indicate that they dissociate more slowly than their hairpin counterpart. The higher K_D value shows that the total affinities of the six-ring cyclic PI polyamides are lower than that of the six-ring hairpin PI polyamide. There was a similar relationship

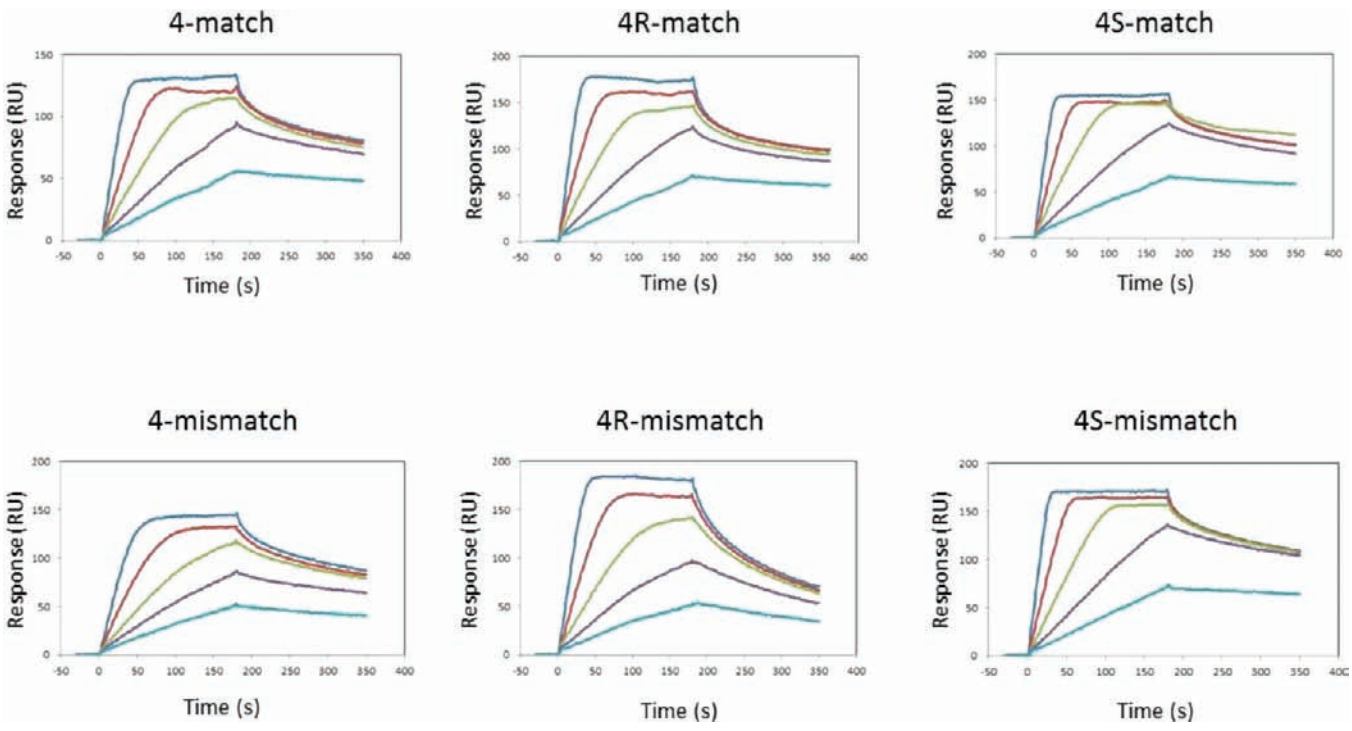

Figure 2. SPR sensorgrams for the interactions of β-alanine-containing PI polyamides. The concentrations of PI polyamides were 120 nM (blue), 60 nM (red), 30 nM (green), 15 nM (purple), and 7.5 nM (light blue).

Table 4. Binding Affinities of β-Alanine-Containing PI Polyamides

	match			1-bp mismatch			
	5'-biotin-G C C T G C A C T A G C C T_T -β-Dp 3'-C G G A C G T G A T C G G T_T			5'-biotin-G C C T G G A C T A G C C T_T -β-Dp 3'-C G G A C C T G A T C G G T_T			specificity
polyamide	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	
4 -β-Dp	3.5×10^5	1.1×10^{-3}	3.1×10^{-9}	3.0×10^5	1.1×10^{-3}	3.7×10^{-9}	1.2
4R ^{R}-β-Dp	3.5×10^5	6.9×10^{-4}	2.0×10^{-9}	3.5×10^5	2.9×10^{-3}	8.2×10^{-9}	4.0
4S ^{S}-β-Dp	5.6×10^5	1.6×10^{-3}	2.8×10^{-9}	5.7×10^5	1.5×10^{-3}	2.8×10^{-9}	1.0

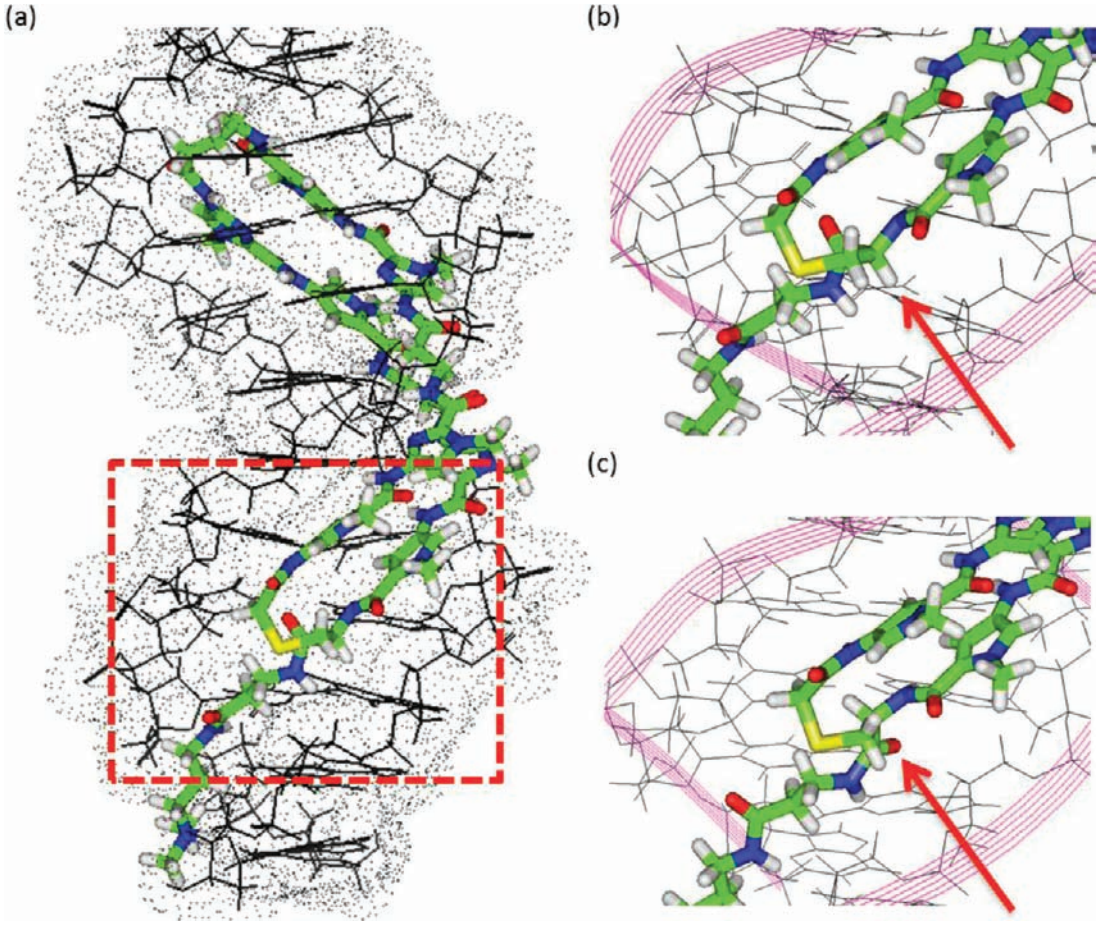

Figure 3. Energy-minimized model of the cyclic PI polyamide−DNA 13-mer duplex. (a) Overall structure of cyclic PI polyamide (**4R**) binding with the corresponding DNA. (b, c) Positions of the Cys moieties in **4R** (b) and **4S** (c) and the corresponding DNA.

between the eight-ring hairpin (**2**) and eight-ring cyclic (**2R** or **2S**) PI polyamides (Figure S2, Supporting Information, and Table 2). Eight-ring cyclic PI polyamides have weaker associations and slower dissociation rates than eight-ring hairpin PI polyamide, and their total affinities are also lower than those of hairpin PI polyamides (Table 2). It should be noted that the affinities of eight-ring PI polyamides were much stronger than those of six-ring PI polyamides. On the other hand, the K_D data of ten-ring cyclic PI polyamides (**3R** or **3S**) show that ten-ring PI polyamides have much lower affinities than the six- and eight-ring PI polyamides (Figure S3, Supporting Information, and Table 3). This may be because the inherent curvature of a ten-ring PI polyamide does not precisely match the minor groove of B-DNA. Therefore, some conformational change is required for efficient recognition of longer DNA.

Binding Affinities of β-Alanine-Containing Cyclic PI Polyamides. For recognition of sequences longer than 6 bp, Dervan et al. successfully introduced β-alanine to add flexibility to long hairpin PI polyamides.[7] We applied this method to cyclic PI polyamides, producing β-alanine-containing cyclic PI polyamides

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that are able to recognize 7 bp DNA. The binding affinities of β-alanine-containing cyclic PI polyamides (**4R** or **4S**) to corresponding DNA were stronger than that of the alternative hairpin PI polyamide (**4**) (Figure 2 and Table 4, left column). In particular, **4R** showed the best affinity and the slowest dissociation. To compare the sequence specificities of β-alanine-containing PI polyamides, we investigated their binding affinities to 1 bp mismatched DNA. In 1 bp mismatched DNA, a Py/Im would recognize G/C, while in matched-DNA, it recognizes C/G. The **4R** polyamide had better sequence specificity than the alternative hairpin PI polyamide (Figure 2 and Table 4, right column). Specificity is calculated by dividing the K_D of the mismatched DNA by that of the matched DNA. Taken together, the cyclic PI polyamides tend to have relatively slower dissociation rates and better affinities, especially in the *R*-form, suggesting its potential use for in vivo studies. However, the affinities between cyclic and hairpin polyamides are not strikingly different. Hence, the employment of these two types of polyamides is dependent upon the need and the situation. In addition, other factors, including cell permeability and time of degradation in living cells, would need to be investigated before in vivo studies.

Molecular Modeling Studies on Cys Cyclic PI Polyamides. To investigate the manner in which cyclic PI polyamides bind to DNA, we carried out molecular modeling studies. The overall structure of **4R** binding in the minor groove of matching DNA is shown in Figure 3 (left panel). The structure is similar to the previously determined crystal structure of a cyclic PI polyamide produced by another method.[27,28] In **4S** (Figure 3, upper right panel), the carbonyl group of Cys is positioned close to the phosphate group of the DNA, whereas the carbonyl group is positioned on the inside of the DNA in **4R** (Figure 3, lower right panel). In **4S**, there may be electrostatic repulsion between the carbonyl group of Cys and the phosphate group of DNA. Even though the orientations of these enantiomers differ, they bind snugly to the minor groove of the duplex, as shown in Figure 3.

CONCLUSIONS

In this study, we developed a new type of cyclic PI polyamide and assessed its affinity to the corresponding DNA. The six-, eight-, and ten-ring cyclic PI polyamides had weaker affinities for the corresponding DNA than hairpin PI polyamides, whereas β-alanine-containing cyclic PI polyamides had stronger affinities and specificities than the hairpin PI polyamides. In particular, the *R*-form cyclic PI polyamide with β-alanine had the best affinity and specificity. These cyclic PI polyamides may be used as a new type of DNA-binding motif to deliver various functional molecules to the minor groove of duplex DNA.

MATERIALS AND METHODS

General Procedures. ^{1}H NMR spectra were recorded on a JEOL JNM-FX400 superconducting NMR spectrometer (400 MHz for ^{1}H) and JEOL JNM ECA-600 spectrometer (600 MHz for ^{1}H), with chemical shifts reported in parts per million relative to residual solvent and coupling constants in hertz. The following abbreviations were applied to spin multiplicity: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). SPR assays were performed on a Biacore X system (GE Healthcare), and processing of data was carried out using BIAevaluation, version 4.1. HPLC analysis was performed on a Jasco engineering PU-2080 plus series system using a 150 × 10 mm Chemco-Pak Chemcoband 5-ODS-H reversed-phase column in 50 mM ammonium formate in water with acetonitrile as the eluent at a flow rate of 1.0 mL/min and a linear gradient elution of 0 to 100% acetonitrile in 20 min with detection at 254 nm. Collected fractions were analyzed by ESI-TOF-MS (Bruker). Reversed-phase flash chromatography was performed on CombiFlash Rf (Teledyne Isco, Inc.) using a 4.3 g reversed-phase flash column (C18 RediSep Rf) in 0.1% trifluoroacetic acid in water with acetonitrile as the eluent at a flow rate of 18.0 mL/min and a linear gradient elution of 0 to 35% acetonitrile in 5 to 30 min with detection at 254 nm. Fmoc-β-Wang resin (0.36 mmol/g), *O*-(1*H*-6-chlorobenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU), Fmoc-Cys(Trt), and Fmoc-D-Cys(Trt) were purchased from Peptide International. Fmoc-I-COOH, Fmoc-P-COOH, DMF, 1-methyl-2-pyrrolidone (NMP), and piperidine were purchased from Wako, and Fmoc-β-Ala-OH, Fmoc-γ-Abu-COOH, and PyBOP were from Novabiochem. DIEA, acetic anhydride, and Dp were from Nacalai Tesque, Inc. Trifluoroacetic acid was from Kanto Chemical Co., Inc. Chloroacetic anhydride was from Aldrich. All other solvents and materials were from standard suppliers (highest quality available).

Fmoc Solid-Phase Syntheses of PI Polyamides. All PI polyamide syntheses were performed on a PSSM-8 (Shimadzu) with a computer-assisted operation system on a 0.03 mmol scale (80 mg of Fmoc-β-Wang resin, 0.36 mmol/g) by using Fmoc chemistry. An Fmoc unit (0.20 mmol) in each step was set up to solve by NMP on the synthetic line. The following conditions were used in all PI polyamide solid-phase syntheses for each cycle: twice deblocking for 4 min with 20% piperidine/DMF (0.6 mL), activating for 2 min with HCTU (88 mg, 0.21 mmol) in DMF (1 mL) and 10% DIEA/DMF (0.4 mL), coupling for 60 min, and washing with DMF. All couplings were carried out with a single-couple cycle. Fmoc-PI-CO_2H as a dimer-coupling unit was employed for the difficulty of coupling an NH_2—I moiety to Fmoc-P-CO_2H. Fmoc-L-Cys(Trt) and Fmoc-D-Cys(Trt) were employed under the same coupling conditions. At the last capping process, the samples were washed for 15 min with 20% acetic anhydride/DMF for **1**—**4** and with 20% chloroacetic anhydride/DMF for **1R**—**4R** and **1S**—**4S**. All lines were purged with solution transfers and bubbled by N_2 gas for stirring the resin. After the completion of the synthesis, the resin was washed with DMF (2 mL) and methanol (2 mL) and then dried in a desiccator at room temperature in vacuo.

AcPI-β-IP-γ-IP-β-PP-β-Dp. The resin (109 mg, 0.019 mmol) was placed in a 2 mL plastic vial, and 0.5 mL of Dp (1 mL) was added following stirring for 4 h at 55 °C. The resin was removed by filtration and washed thoroughly with dichloromethane. The resultant filtrate was concentrated in vacuo. The residue was triturated by diethyl ether and dried in vacuo. The resulting crude polyamide (23.5 mg) as a light-yellow powder was purified by reversed-phase HPLC. After purification, appropriate fractions were collected under freeze-dry conditions to give PI polyamide **4** (8.0 mg, 30%) for the evaluation of DNA binding affinity. Analytical HPLC: t_R = 9.7 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.35 (s, 1H, NH), 10.31 (s, 1H, NH), 10.24 (s, 1H, NH), 9.89 (s, 1H, NH), 9.88 (s, 1H, NH), 9.84 (s, 1H, NH), 9.80 (s, 1H, NH), 8.08—8.02 (m, 4H, NH), 7.89 (m, 1H, NH), 7.50 (s, 1H, NH), 7.45 (s, 1H, CH), 7.44 (s, 1H, CH), 7.22 (s, 1H, CH), 7.20 (s, 1H, CH), 7.17 (s, 1H, CH), 7.15 (s, 1H, CH), 7.10 (s, 1H, CH), 6.97 (s, 1H, CH), 6.96 (s, 1H, CH), 6.93 (s, 1H, CH), 6.91 (s, 1H, CH), 6.87 (s, 1H, CH), 6.86 (s, 1H, CH), 3.95—3.80 (24H, NMe), 3.54 (m, 2H, CH_2), 3.44 (m, 2H, CH_2), 3.37 (m, 2H, CH_2), 3.19 (m, 2H, CH_2), 3.11 (m, 2H, CH_2), 3.01 (m, 2H, CH_2), 2.75 (s, 3H, NMe), 2.74 (s, 3H, NMe), 2.62 (m, 2H, CH_2), 2.50 (m, 2H, CH_2), 2.46 (m, 2H, CH_2), 2.35 (m, 2H, CH_2), 1.96 (s, 3H, Ac), 1.79 (m, 4H, CH_2). ESI-TOF-MS: *m*/*z* calcd for $C_{65}H_{84}N_{25}O_{13}$ $[M + H]^+$ 1422.66, found 1422.68.

AcIII-γ-PPP-β-Dp (**1**), AcIIPP-γ-IPPP-β-Dp (**2**), and AcPIPIP-γ-IPPPP-β-Dp (**3**) were synthesized according to the above procedure and purified by reversed-phase flash chromatography under similar conditions. The following are data for **1**. Analytical HPLC: t_R = 9.7 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H, NH), 9.88 (s, 1H,

NH), 9.87 (s, 1H, NH), 9.83 (s, 1H, NH), 9.61 (s, 1H, NH), 8.33−8.28 (m, 1H, NH), 8.08−7.99 (m, 2H, NH), 7.65 (s, 1H, NH), 7.52 (s, 1H, CH), 7.50 (s, 1H, CH), 7.23 (s, 1H, CH), 7.21 (s, 1H, CH), 7.15 (s, 1H, CH), 7.10 (s, 1H, CH), 7.05 (s, 1H, CH), 6.97 (s, 1H, CH), 6.88 (s, 1H, CH), 4.02 (s, 3H, NMe), 3.97 (s, 6H, NMe), 3.84 (s, 6H, NMe), 3.80 (s, 3H, NMe), 3.43−3.35 (m, 2H, CH_2), 3.16−3.07 (m, 2H, CH_2), 3.05−2.97 (m, 2H, CH_2), 2.75 (s, 3H, NMe), 2.74 (s, 3H, NMe), 2.56−2.50 (m, 2H, CH_2), 2.49−2.45 (m, 2H, CH_2), 2.35 (t, J = 7.5 Hz, 2H, CH_2), 2.03 (s, 3H, Ac) 1.82 (t, J = 7.5 Hz, 2H, CH_2), 1.75 (t, J = 8.0 Hz, 2H, CH_2). ESI-TOF-MS: m/z calcd for $C_{47}H_{62}N_{19}O_9$ $[M + H]^+$ 1036.49, found 1036.57. The following are data for **2**. Analytical HPLC: t_R = 9.7 min. ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.33 (s, 1H, NH), 10.29 (s, 1H, NH), 10.25 (s, 1H, NH), 9.96 (s, 1H, NH), 9.93 (s, 1H, NH), 9.92 (s, 1H, NH), 9.88 (s, 1H, NH), 9.32 (s, 1H, NH), 8.05−8.01 (m, 3H, NH), 7.57 (s, 1H, CH) 7.51 (s, 1H, CH), 7.46 (s, 1H, CH), 7.27 (d, J = 1.0 Hz, 1H, CH), 7.26 (d,J = 0.5 Hz, 1H, CH), 7.22 (d, J = 0.5 Hz, 1H, CH), 7.18 (d, J = 0.5 Hz, 1H, CH), 7.16−7.15 (m, 1H, CH), 7.08 (s, 1H, CH), 7.06 (d, J = 0.5 Hz, 1H, CH), 7.00 (s, 1H, CH), 6.90 (d, J = 1.0 Hz, 1H, CH), 6.87 (d, J = 1.0 Hz, 1H, CH), 4.01 (s, 3H, NMe), 3.98 (s, 3H, NMe), 3.95 (s, 3H, NMe), 3.87−3.78 (m, 12H, NMe), 3.38 (q, J = 10.0 Hz, 2H, CH_2), 3.20 (q, J = 10.0 Hz, 2H, CH_2), 3.11 (q, J = 10.0 Hz, 2H, CH_2), 3.03−2.98 (m, 2H, CH_2), 2.75 (s, 3H, NMe), 2.74 (s, 3H, NMe), 2.38−2.34 (m, 4H, CH_2), 1.80 (t, J = 6.5 Hz, 2H, CH_2), 1.73 (t, J = 8.5 Hz, 2H, CH_2). ESI-TOF-MS: m/z calcd for $C_{59}H_{74}N_{23}O_{11}$ $[M + H]^+$ 1280.59, found 1280.67. The following are data for **3**. Analytical HPLC: t_R =10.1 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.25 (s, 1H, NH), 10.24 (s, 1H, NH), 10.21 (s, 1H, NH), 9.98−9.85 (m, 6H, NH), 9.82 (s, 1H, NH), 8.08−7.97 (m, 3H, NH), 7.55 (s, 1H, CH), 7.46 (s, 1H, CH), 7.40 (s, 1H, CH), 7.27 (d, J = 1.5 Hz, 1H, CH), 7.25−7.13 (m, 8H, CH), 7.09 (d, J = 1.5 Hz, 1H, CH), 7.07 (d, J = 1.5 Hz, 1H, CH), 6.99 (d, J = 1.5 Hz, 1H, CH), 6.93 (d, J = 1.5 Hz, 1H, CH), 6.88 (d, J = 1.5 Hz, 1H, CH), 4.02−3.93 (m, 9H, NMe), 3.88−3.77 (m, 21H, NMe), 3.39 (m, 2H, CH2), 3.22 (m, 2H, CH_2), 3.11 (m, 2H, CH_2), 3.02 (m, 2H, CH_2), 2.74 (s, 3H, NMe), 2.73 (s, 3H, NMe), 2.37−2.33 (m, 4H, CH_2), 1.97 (s, 3H, Ac), 1.80 (t, J = 7.2 Hz, 2H, CH_2), 1.74 (t, J = 7.6 Hz, 2H, CH_2). ESI-TOF-MS: m/z calcd for $C_{71}H_{86}N_{27}O_{13}$ $[M + H]^+$ 1524.68, found 1524.81.

Cyclo-(AcPI-*β*-IP-*γ*-IP-*β*-PP-(*R*)-Cys)-*β*-Dp (4R). The resin (117 mg, 0.020 mmol) was placed in a 2 mL plastic vial, trifluoroacetic acid (0.95 mL), triisopropylsilane (0.02 mL), and water (0.02 mL) were added, and the solution was stirred at room temperature for 1 h. The resin was removed by filtration and washed thoroughly with dichloromethane. The resultant filtrate was removed in vacuo. The residue was triturated by diethyl ether and dried in vacuo. The resulting crude product (12.7 mg) as a light-yellow powder was checked by HPLC and ESI-TOF-MS. Analytical HPLC: t_R = 10.4 min. ESI-TOF-MS: m/z calcd for $C_{63}H_{76}ClN_{24}O_{15}S$ 1475.53 $[M + H]^+$, found 1475.59 $[M + H]^+$. The crude product was dissolved in DMF (1 mL) and DIEA (0.02 mL) and used for the next step without further purification. Following stirring for 30 min, the peak was consumed and efficiently converted to a new peak by HPLC analysis. The cyclic product **4R** or **4S** was checked by HPLC and ESI-TOF-MS. Analytical HPLC: t_R = 9.2 min. ESI-TOF-MS: m/z calcd for $C_{63}H_{75}N_{24}O_{15}S$ $[M + H]^+$ 1439.55, found 1439.52. PyBOP (2 mg, 0.004 mmol) and Dp (0.001 mL, 0.01 mmol) were added to a reaction solution at room temperature. The coupling reaction was continuously conducted following stirring for another 40 min, and the solvent was removed in vacuo. The residue was triturated by diethyl ether and diethyl ether−dichloromethane and dried in vacuo. The resulting polyamide crude (14.5 mg) was purified by reversed-phase flash chromatography. After purification, appropriate fractions were collected under freeze-dry conditions to give Cys cyclic PI polyamide **4R** (2.0 mg, 7% yield for 16 steps) for the evaluation of DNA binding affinity. Analytical HPLC: t_R = 9.7 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H, NH), 10.33 (s, 1H, NH), 10.21 (s, 1H, NH), 10.0 (s, 1H, NH), 9.88 (s, 1H, NH), 9.87 (s, 1H, NH), 9.85 (s, 1H, NH), 8.13−7.76 (m, 7H, NH), 7.50 (s, 1H, CH), 7.46 (s, 1H, CH), 7.43 (s, 1H, CH), 7.29 (s, 1H, CH), 7.24 (s, 1H, CH), 7.21 (s, 1H, CH), 7.18 (s, 1H, CH), 7.17 (s, 1H, CH), 6.99 (s, 1H, CH), 6.97 (s, 1H, CH), 6.94 (s, 1H, CH), 6.90 (s, 1H, CH), 6.81 (s, 1H, CH), 4.51 (m, 1H, CH), 3.95−3.90 (12H, NMe), 3.83−3.78 (12H, NMe), 3.53 (m, 2H, CH_2), 3.37 (m, 2H, CH_2), 3.30 (m, 2H, CH_2), 3.24 (m, 2H, CH_2), 3.20 (m, 2H, CH_2), 3.10 (s, 2H, CH_2), 3.00 (s, 2H, CH_2), 2.75 (s, 3H, NMe), 2.74 (s, 3H, NMe), 2.62 (m, 2H, CH_2), 2.54 (m, 2H, CH_2), 2.50 (m, 2H, CH_2), 2.34 (m, 2H, CH_2), 2.28 (m, 2H, CH_2), 1.79 (m, 2H, CH_2), 1.73 (m, 2H, CH_2). ESI-TOF-MS: m/z calcd for $C_{68}H_{87}N_{26}O_{14}S$ $[M + H]^+$ 1523.65, found 1523.62.

Cyclo-(AcIII-γ-PPP-(*R*)-Cys)-β-Dp (**1R**), cyclo-(AcIIPP-γ-IPPP-(*R*)-Cys)-β-Dp (**2R**), and cyclo-(AcPIPIP-γ-IPPPP-(*R*)-Cys)-β-Dp (**3R**) were synthesized according to the above procedure and purified by a reversed-phase fraction collector under similar conditions. The following are data for **1R**. Analytical HPLC: t_R = 9.8 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.83 (s, 1H, NH), 9.89 (s, 1H, NH), 9.87 (s, 1H, NH), 9.82 (s, 1H, NH), 9.73 (s, 1H, NH), 9.59 (s, 1H, NH), 8.31 (t, J = 6.0 Hz, 1H, NH), 8.06−8.00 (m, 3H, NH), 7.65 (s, 1H, CH), 7.56 (s, 1H, CH), 7.52 (s, 1H, CH), 7.48−7.45 (m, 2H, CH), 7.21 (d, J = 1.5 Hz, 1H, CH), 7.16 (d, J = 1.5 Hz, 1H, CH), 7.15 (d, J = 1.5 Hz, 1H, CH), 7.06 (d, J = 1.5 Hz, 1H, CH), 5.33 (s, 2H, CH_2), 4.01 (s, 3H, NMe), 3.96 (s, 6H, NMe), 3.84 (s, 3H, NMe), 3.83 (s, 3H, NMe), 3.81 (s, 3H, NMe), 3.42−3.24 (m, 7H, CH_2, CH), 3.12 (q, J = 12.0 Hz, 2H, CH_2), 3.01 (m, 2H, CH_2), 2.76 (s, 3H, NMe), 2.75 (s, 3H, NMe), 2.36 (t, J = 7.0 Hz, 2H, CH_2), 2.28 (t, J = 7.0 Hz, 2H, CH_2), 1.82 (m, 2H, CH_2), 1.75 (m, 2H, CH_2). ESI-TOF-MS: m/z calcd for $C_{50}H_{65}N_{20}O_{10}S$ $[M + H]^+$ 1137.48, found 1137.59. The following are data for **2R**. Analytical HPLC: t_R = 9.7 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.40 (s, 1H, NH), 10.28 (s, 1H, NH), 10.26 (s, 1H, NH), 9.92−9.60 (m, 4H, NH), 9.37 (s, 1H, NH), 8.11−7.96 (m, 4H, NH), 7.56 (s, 1H, CH), 7.53 (s, 1H, CH), 7.47 (s, 1H, CH), 7.41−7.38 (m, 2H, CH), 7.36 (d, J = 0.5 Hz, 1H, CH), 7.32 (d, J = 0.5 Hz, 1H, CH), 7.19 (d, J = 0.5 Hz, 1H, CH), 7.00 (d, J = 0.5 Hz, 1H, CH), 6.92 (d, J = 0.5 Hz, 1H, CH), 6.90 (d, J = 0.5 Hz, 1H, CH), 6.88 (d, J = 0.5 Hz, 1H, CH), 6.86 (d, J = 0.5 Hz, 1H, CH), 4.51 (m, 1H, CH), 4.00 (s, 3H, NMe), 3.99 (s, 3H, NMe), 3.94 (s, 3H, NMe), 3.85−3.84 (m, 9H, NMe), 3.82 (s, 3H, NMe), 3.80 (s, 3H, NMe), 3.36−3.16 (m, 6H, CH_2), 3.12−3.06 (m, 2H, CH_2), 3.02−2.96 (m, 2H, CH_2), 2.76 (s, 3H, NMe), 2.75 (s, 3H, NMe), 2.36 (t, J = 7.5 Hz, 2H, CH_2), 2.28 (t, J = 7.5 Hz, 2H, CH_2), 1.83−1.76 (m, 2H, CH_2), 1.74−1.68 (m, 2H, CH_2). ESI-TOF-MS: m/z calcd for $C_{62}H_{77}N_{24}O_{12}S$ $[M + H]^+$ 1381.58, found 1381.68. The following are data for **3R**. Analytical HPLC: t_R = 10.2 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.30 (s, 1H, NH), 10.23 (s, 1H, NH), 10.19 (s, 1H, NH), 10.08−9.86 (m, 7H, NH), 8.17−8.12 (m, 1H, NH), 8.04−7.97 (m, 3H, NH), 7.55 (s, 1H, CH), 7.45 (s, 1H, CH), 7.39 (s, 1H, CH), 7.37 (s, 1H, CH), 7.34 (s, 1H, CH), 7.31 (s, 1H, CH), 7.23 (s, 1H, CH), 7.20 (s, 1H, CH), 7.08 (s, 1H, CH), 7.05 (s, 1H, CH), 6.99−6.87 (m, 7H, CH), 4.56−4.50 (m, 1H, CH), 3.98 (s, 3H, NMe), 3.97 (s, 3H, NMe), 3.95 (s, 3H, NMe), 3.88−3.78 (m, 21H, NMe), 3.40 (m, 2H, CH_2), 3.22 (m, 2H, CH_2), 3.13−3.05 (m, 4H, CH_2), 3.04−2.96 (m, 4H, CH_2), 2.76 (s, 3H, NMe), 2.74 (s, 3H, NMe), 2.40−2.24 (m, 4H, CH_2), 1.84−1.77 (m, 2H, CH_2), 1.75−1.68 (m, 2H, CH_2). ESI-TOF-MS: m/z calcd for $C_{74}H_{89}N_{28}O_{14}S$ $[M + H]^+$ 1625.68, found 1625.80.

(*S*)-Polyamides **1S, 2S, 3S**, and **4S** were synthesized by using Fmoc-D-Cys(Trt) instead of Fmoc-Cys(Trt) through a solid-phase synthetic protocol. After the purification by reversed-phase flash chromatography and ESI-TOF-MS confirmation, they were evaluated for DNA binding affinity.

SPR Assays. We performed SPR assays as described in previous studies.[33,34] Briefly, the assays were performed using a BIACORE X instrument. Biotinylated hairpin DNAs were purchased from JBioS (Tokyo, Japan), and the sequences are shown in the tables. Hairpin

biotinylated DNAs are immobilized to a streptavidin-coated sensor chip SA to obtain the desired immobilization level (approximately 1200 RU rise). SPR assays were carried out using HBS-EP buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, and 0.005% surfactant P20) with 0.1% DMSO at 25 °C. A series of sample solutions with various concentrations were prepared in HBS-EP buffer with 0.1% DMSO and injected at a flow rate of 20 mL/min. To measure the rates of association (k_a) and dissociation (k_d) and dissociation constant (K_D), data processing was performed with an appropriate fitting model using the BIAevaluation 4.1 program. The 1:1 Langmuir binding model or 1:1 Langmuir binding model with drifting baseline was used for fitting the sensorgrams to give better fitting. All sensorgrams are shown in the Supporting Information, and all values are shown in the tables.

Molecular Modeling Studies. Minimizations were performed with the Discover (MSI, San Diego, CA) program using CVFF force-field parameters. The starting structure was constructed using the builder module of Insight using standard bond lengths and angles. A total of 24 Na cations were placed at the bifurcating position of the O−P−O angle at a distance of 2.21 Å from the phosphorus atom. The resulting complex was soaked in a 10 Å layer of water. The whole system was minimized without constraints to the stage where the rms was less than 0.001 kcal/(mol Å).

■ ASSOCIATED CONTENT

Supporting Information. SPR sensorgrams for six-, eight-, and ten-ring PI polyamides. This material is available free of charge via the Internet at http://pubs.acs.org.

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