Title	Sequence-specific DNA binding by long hairpin pyrrole–imidazole polyamides containing an 8-amino-3,6-dioxaoctanoic acid unit
Author(s)	Sawatani, Yoshito; Kashiwazaki, Gengo; Chandran, Anandhakumar; Asamitsu, Sefan; Guo, Chuanxin; Sato, Shinsuke; Hashiya, Kaori; Bando, Toshikazu; Sugiyama, Hiroshi
Citation	Bioorganic and Medicinal Chemistry (2016), 24(16): 3603-3611
Issue Date	2016-08-15
URL	http://hdl.handle.net/2433/230868
Right	© 2016. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/; The full-text file will be made open to the public on 15 August 2018 in accordance with publisher's 'Terms and Conditions for Self-Archiving'.; This is not the published version. Please cite only the published version. この論文は出版社版でありません。引用の際には出版社版をご確認ご利用ください。
Type	Journal Article
Textversion	author

Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

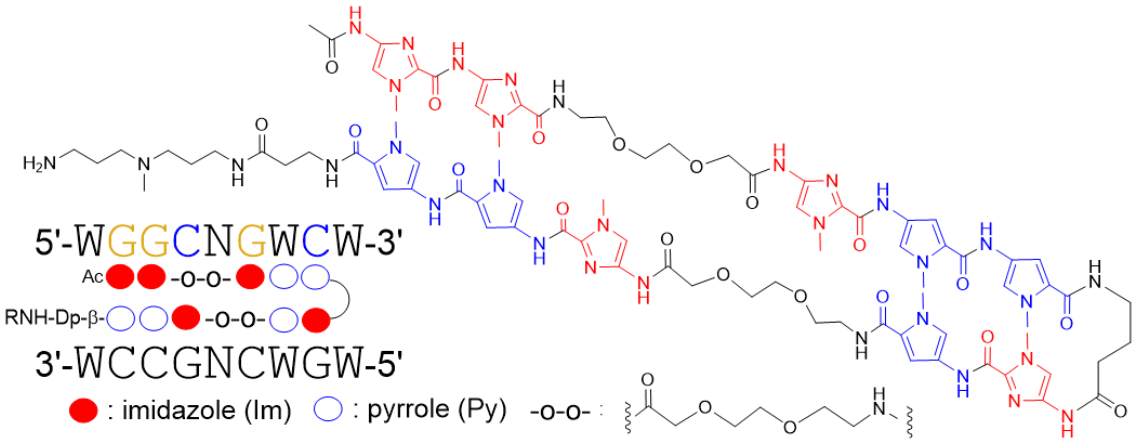
Sequence-specific DNA binding by long hairpin pyrrole-imidazole polyamides containing an 8-amino-3,6-dioxaoctanoic acid unit

Leave this area blank for abstract info.

Yoshito Sawatani[a], Gengo Kashiwazaki[a], Anandhakumar Chandran[a], Sefan Asamitsu[a], Chuanxin Guo[a], Shinsuke Sato[b], Kaori Hashiya[a], Toshikazu Bando[a] and Hiroshi Sugiyama[a, b]

[a]*Department of Chemistry, Graduate School of Science, Kyoto University, Kitashirakawaoiwakecho, Sakyo, Kyoto, 606-8502, Japan*

[b]*Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Yoshida-Ushinomiyacho, Sakyo, 606-8501, Japan*



Bioorganic & Medicinal Chemistry
journal homepage: www.elsevier.com

Sequence-specific DNA binding by long hairpin pyrrole-imidazole polyamides containing an 8-amino-3,6-dioxaoctanoic acid unit

Yoshito Sawatani[a], Gengo Kashiwazaki[a], Anandhakumar Chandran[a], Sefan Asamitsu[a], Chuanxin Guo[a], Shinsuke Sato[b], Kaori Hashiya[a], Toshikazu Bando[a, *] and Hiroshi Sugiyama[a, b, *]

[a]*Department of Chemistry, Graduate School of Science, Kyoto University, Kitashirakawaoiwakecho, Sakyo, Kyoto, 606-8502, Japan*
[b]*Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Yoshida-Ushinomiyacho, Sakyo, Kyoto, 606-8501, Japan*

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
DNA binder
Py-Im polyamide
ethylene glycol unit
SPR
Bind-n-Seq

ABSTRACT

With the aim of improving aqueous solubility, we designed and synthesized five *N*-methylpyrrole (Py)-*N*-methylimidazole (Im) polyamides capable of recognizing 9-bp sequences. Their DNA-binding affinities and sequence specificities were evaluated by SPR and Bind-n-Seq analyses. The design of polyamide **1** was based on a conventional model, with three consecutive Py or Im rings separated by a β-alanine to match the curvature and twist of long DNA helices. Polyamides **2** and **3** contained an 8-amino-3,6-dioxaoctanoic acid (AO) unit, which has previously only been used as a linker within linear Py-Im polyamides or between Py-Im hairpin motifs for tandem hairpin. It is demonstrated herein that AO also functions as a linker element that can extend to 2-bp in hairpin motifs. Notably, although the AO-containing unit can fail to bind the expected sequence, polyamide **4**, which has two AO units facing each other in a hairpin form, successfully showed the expected motif and a K_D value of 16 nM was recorded. Polyamide **5**, containing a β-alanine-β-alanine unit instead of the AO of polyamide **2**, was synthesized for comparison. The aqueous solubilities and nuclear localization of three of the polyamides were also examined. The results suggest the possibility of applying the AO unit in the core of Py-Im polyamide compounds.

*This is an author version based on a template provided by Elsevier.

1. Introduction

Long hairpin motifs composed of *N*-methylpyrrole (Py) and *N*-methylimidazole (Im) recognize and bind DNA in the minor groove in a sequence-specific manner. An antiparallel pairing of Im opposite Py (Im/Py pair) distinguishes G–C from C–G, whereas a Py/Py pair binds both A–T and T–A base pairs.[1-6] Py-Im polyamides have strong DNA binding affinity and sequence specificity for target match sequences. Various types of sequence-specific Py-Im polyamides have been developed to control gene expression by competitive binding against DNA-binding proteins such as transcription factor.[7-18] The introduction of a β-alanine moiety within the hairpin motif allows the crescent-shaped Py-Im polyamides to fit into the minor groove of dsDNA, and antiparallel pairings of β/β, Py/β-pair recognize both T–A and A–T.[19] In general, the β-linker was set in the hairpin motif to extend the DNA sequence recognition. Although long hairpin Py-Im polyamides have interesting biological properties, they tend to have limited aqueous solubility and can precipitate by aggregation in the aqueous phase.[20]

To address this issue, we investigated the use of the 8-amino-3,6-dioxaoctanoic acid (AO) unit, which Laemmli et al. originally used as a hydrophilic and flexible linker for compounds targeting the long satellites I and III and SARs (scaffold associated regions).[21] Later, they developed Py-Im polyamides with an AO linker between the two hairpin motifs for binding insect and vertebrate telomeric repeats.[22] Recently, our group also reported a tandem dimer[23] and a tandem trimer that can be used to target human telomere sequences.[24] However, herein we used the same group within hairpin motifs to enhance aqueous solubility.

To conduct a large-scale evaluation of the DNA-binding characteristics of Py-Im polyamides and other DNA-binding molecules, Ansari et al. established a cognate site identification (CSI) microarray platform and developed a visualization method called a sequence-specificity landscape (SSL) display.[25-29]

* Corresponding author. e-mail: bando@kuchem.kyoto-u.ac.jp (T. Bando)
* Corresponding author. e-mail: hs@kuchem.kyoto-u.ac.jp (H. Sugiyama)

Sequence specificities of Py-Im polyamides have also been investigated by using high-throughput sequencing.[30-32]

Here, we designed and synthesized thirteen Py-Im polyamides (Fig. 1) to investigate the utility of the AO unit with respect to affinity toward a DNA sequence, sequence specificities of DNA binding, aqueous solubilities, and cell permeabilities. Polyamide **1** is a standard design with one β-alanine inserted within the structure to relax the distortion between three rings.[19] Polyamides **2** and **3** each has one AO unit in either the N- or C-side strand, respectively. Notably, the introduction of the AO unit can decrease the number of coupling steps because the moiety covers two base pairs. Polyamide **4** has two AO units, one in each strand. To examine whether the AO unit can be replaced by a common aliphatic combination motif, instead of the AO in polyamide **2**, polyamide **5** contained one β-alanine-β-alanine unit. Although several Py-Im polyamides containing a β-alanine-β-alanine moiety within linear motifs have been developed in attempts to alkylate human telomere sequences,[33, 34] to our knowledge, the group has not been used within a hairpin motif.

First, the K_D values of polyamides **1**–**5** were obtained by SPR experiments. Second, the biotinylated polyamides were applied to Bind-n-Seq analysis,[31] which is a high-throughput technique to determine the binding specificities of Py-Im polyamides: treatment of ODN containing random sequences by a biotinylated Py-Im polyamide of interest; pull-down of the ODN bound by the polyamide using magnetic Streptavidin beads; elution and amplification; emulsion PCR then sequencing on a chip. Third, saturating concentrations for three of the polyamides were measured. Finally, the FITC-labeled polyamides were applied to a breast cancer cell line, MDA-MB-231, to establish their nuclear localization. The inclusion of the AO unit was found to enhance the solubility of the polyamide without leading to loss of cell permeability; however, it was revealed that DNA-binding affinity depended significantly on its position.

2. Results and Discussion

2.1. Synthesis of Py-Im polyamides 1–13

The Py-Im hairpin polyamides **1**–**5**, with an amino group at the C-terminus, were prepared by Fmoc solid-phase synthesis starting from a Py-coupled oxime resin followed by treatment with 3,3′-diamino-*N*-methyldipropylamime. The purities and structures of Py-Im polyamides **1**–**5** were confirmed by ^{1}H NMR spectroscopic analysis and by electrospray ionization time-of-flight mass spectrometry (ESI-TOFMS) after purification by reverse-phase flash chromatography. Purified **1**–**5** were used for SPR assays and to evaluate their solubility in aqueous media. Biotin conjugates **6**–**10** were synthesized by coupling with PEG-linked biotin *N*-hydroxysuccinimide and purified by reverse-phase HPLC for Bind-n-Seq. Similarly, fluorescein conjugates **11**–**13** were synthesized by coupling with fluorescein isothiocyanate (FITC) and the products were purified by reverse-phase HPLC for cell-permeability assays. Given that the AO unit can extend to two base pairs, the coupling time can be reduced by its introduction.

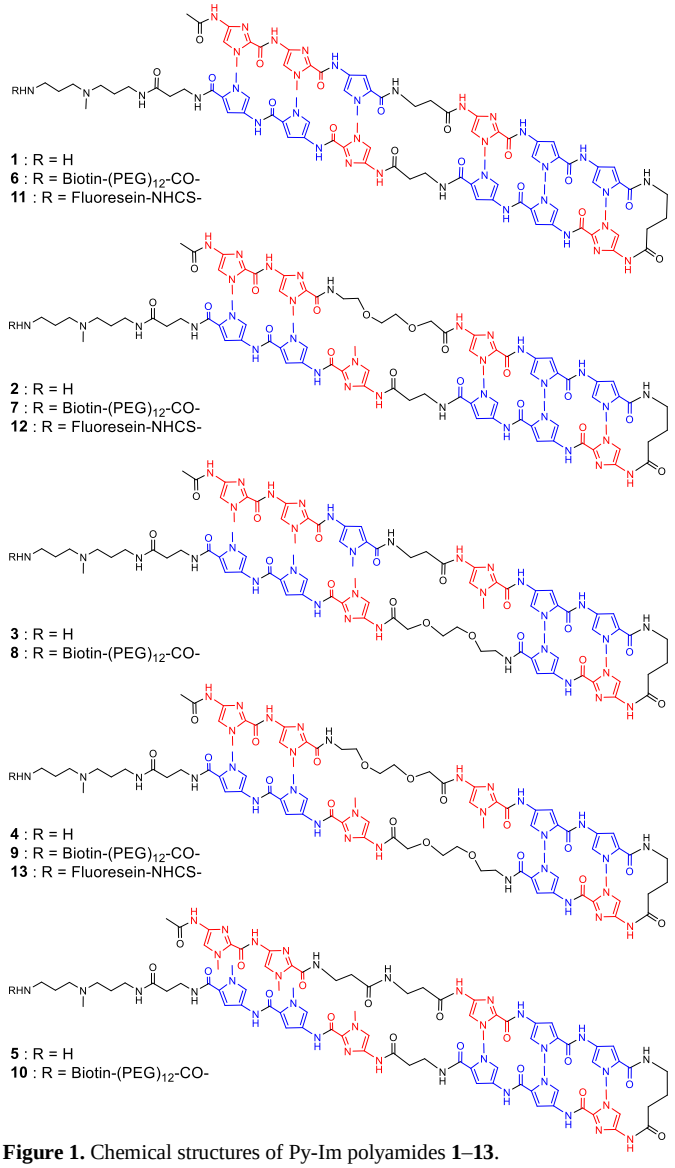


Figure 1. Chemical structures of Py-Im polyamides **1**–**13**.

2.2. Binding affinities of 1–5 determined by SPR analysis

SPR assays were performed to evaluate the influence on DNA-binding affinities of Py-Im polyamides **1**–**5** by the insertion of an AO unit. The sequence of the immobilized ODN was 5′-biotin labeled GCG**AGGCAGACA**CGCTTTTGCG**TGTCTGCCT**CGC-3′. This sequence was determined based on the top 1 sequence in Bind-n-Seq analysis of polyamide **1**, which had already been elucidated. The sensorgrams of polyamides **1**–**5** are shown in Fig. 2, and the rates of association (k_a) and dissociation (k_d), and the dissociation constants (K_D) are shown in Table 1. The K_D values of polyamides **1**–**5** were 5.9, 18, 250, 16, and 15 nM, respectively. Notably, the binding affinity of Py-Im polyamide **1** was the strongest.

One of the reasons why polyamides **2** and **4** had lower affinities than polyamide **1** can be the loss of a hydrogen bond. However, even though the number of hydrogen bonds of polyamides **1** and **5** is the same, the affinity of polyamide **1** was higher. This suggests that the hydrogen bond is not the only determinant and that excess flexibility of the AO or β-alanine-β-alanine moiety may reduce the affinity. Although it is difficult to explain the large difference in K_D between polyamides **2** and **3**, the low affinity of the latter was recovered by inserting another AO unit to obtain polyamide **4**. This observation indicates that the affinity of the Py-Im polyamide is sensitive to the environment around the AO unit.

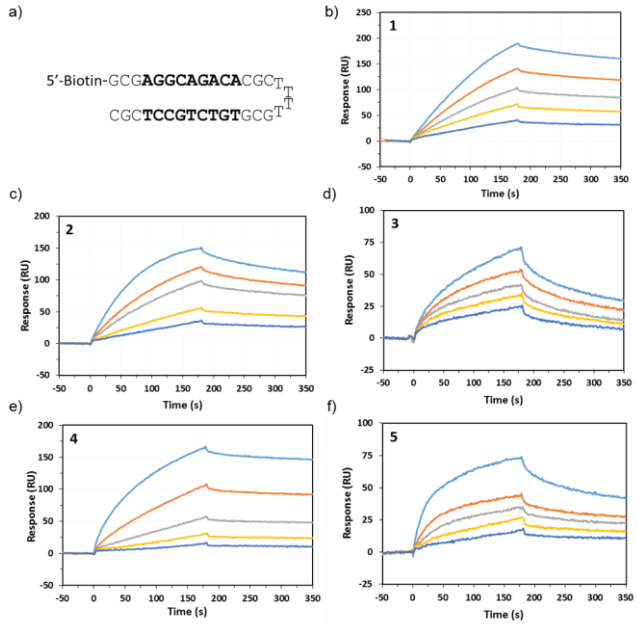


Figure 2. SPR-binding assays to evaluate the binding properties of polyamides **1**-**5**. (a) A biotinylated DNA including a target sequence. The target sequence for the polyamides is shown in bold. (b) SPR sensorgrams for interactions of **1**. The concentrations were 100 nM (blue), 85 nM (orange), 70 nM (gray), 55 nM (yellow), and 40 nM (dark blue). (c) SPR sensorgrams for interactions of **2**. The concentrations were 250 nM (blue), 167 nM (orange), 111 nM (gray), 74 nM (yellow), and 49 nM (dark blue). (d) SPR sensorgrams for interactions of **3**. The concentrations were 250 nM (blue), 200 nM (orange), 150 nM (gray), 100 nM (yellow), and 50 nM (dark blue). (e) SPR sensorgrams for interactions of the **4**. The concentrations were 625 nM (blue), 313 nM (orange), 156 nM (gray), 78 nM (yellow), and 39 nM (dark blue). (f) SPR sensorgrams for interactions of **5**. The concentrations were 156 nM (blue), 78 nM (orange), 39 nM (gray), 20 nM (yellow), and 10 nM (dark blue).

Table 1. The values of the association rates (k_a) and dissociation rates (k_d) obtained from curve fittings of sensorgrams, and dissociation constants (K_D).

polyamide	**1** [a]	**2**	**3**	**4**	**5**
k_a [1/Ms]	2.4×10^{5}	1.0×10^{5}	1.0×10^{4}	1.8×10^{4}	1.3×10^{5}
k_d [1/s]	1.4×10^{-3}	1.8×10^{-3}	4.5×10^{-3}	3.0×10^{-4}	1.9×10^{-3}
K_D [M]	5.9×10^{-9}	1.8×10^{-8}	2.5×10^{-7}	1.6×10^{-8}	1.5×10^{-8}

2.3. Bind-n-Seq

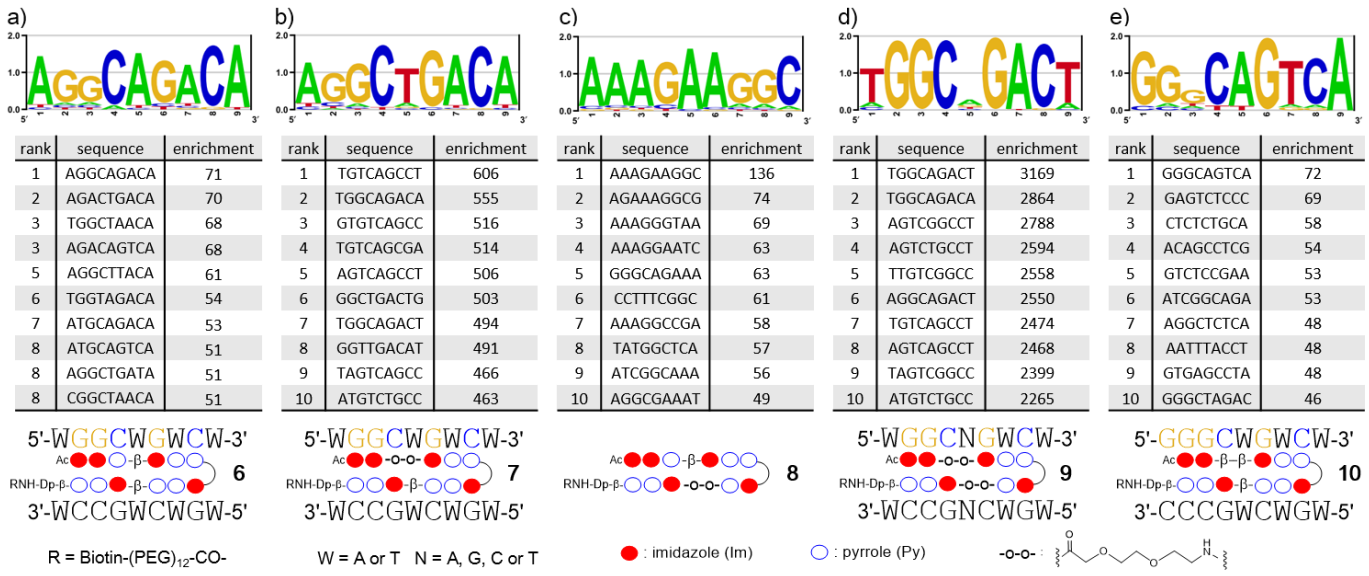
For the large-scale analyses of DNA sequence specificities, we performed Bind-n-Seq[30, 31, 35] experiments with a next-generation sequencer, Ion PGM, with a library of oligomers containing randomized 21-mer. Polyamides **1**–**5** were biotinylated for this assay to yield polyamides **6**–**10** by following a previously reported procedure.[31]

The results revealed sequences pulled down by each polyamide **6**–**10**; their top-10 sequences in the order of enrichment values are shown in Fig. 3 together with their motifs, which were processed by using enoLOGOS (http://www.benoslab.pitt.edu/cgi-bin/enologos/enologos.cgi).

Generally, polyamides **6**, **7**, **9**, and **10** bind oligomers according to their expected sequences (Fig. 3). However, the binding motif from polyamide **8** was so unspecific that it even raised doubts about its hairpin formation. Nonetheless, the Bind-n-Seq results support the results of SPR analysis, which showed that the K_D value for polyamide **3** was one order of magnitude lower than those of polyamides **2**, **4**, and **5**. Again, although it is challenging to establish the cause of these differences from these data alone, it can be concluded that the position of the AO unit should be selected so that it does not disturb the binding to the predetermined sequence based on the pairing rules.

Looking in more detail at the results obtained with polyamide **9** revealed that the sequences contained an interesting "N (aNy base)" at site 5 (Fig. 3d). This phenomenon is easily explained. The pairing rules of Py-Im polyamide are based on hydrogen bonds formed mainly between amide NH and a proton acceptor of the nucleobase in the minor groove. As an antiparallel slipped pair of AO units accrues one base pair facing no proton donor, that site would be "recognized" as N. This recognition has never been reported because it could not be achieved by using the common elements in Py-Im polyamide, *N*-methylpyrrole, *N*-methylimidazole, β-alanine and γ-turn. This feature might be useful in designing long Py-Im polyamide probes containing a site that should accommodate any of the bases. Similar to the results obtained by SPR analysis, it is notable that inserting the second AO unit in polyamide **8** can lead to a recovery of both sequence specificity and DNA-binding affinity.

Another point that can be noted from the Bind-n-Seq results is that site 1 of the binding motif of polyamide **10** (Fig. 3e) had G specificity, whereas W (A or T) was expected. It is difficult to rationalize this result but β-alanine-β-alanine is the apparent trigger for this unfavorable recognition. The result that polyamide **7** motif demonstrated the expected target sequence might imply an advantage of using the AO unit over β-alanine-β-alanine.



rank	sequence	enrichment
1	AGGCAGACA	71
2	AGACTGACA	70
3	TGGCTAACA	68
3	AGACAGTCA	68
5	AGGCTTACA	61
6	TGGTAGACA	54
7	ATGCAGACA	53
8	ATGCAGTCA	51
8	AGGCTGATA	51
8	CGGCTAACA	51

rank	sequence	enrichment
1	TGTCAGCCT	606
2	TGGCAGACA	555
3	GTGTCAGCC	516
4	TGTCAGCGA	514
5	AGTCAGCCT	506
6	GGCTGACTG	503
7	TGGCAGACT	494
8	GGTTGACAT	491
9	TAGTCAGCC	466
10	ATGTCTGCC	463

rank	sequence	enrichment
1	AAAGAAGGC	136
2	AGAAAGGCG	74
3	AAAGGGTAA	69
4	AAAGGAATC	63
5	GGGCAGAAA	63
6	CCTTTCGGC	61
7	AAAGGCCGA	58
8	TATGGCTCA	57
9	ATCGGCAAA	56
10	AGGCGAAAT	49

rank	sequence	enrichment
1	TGGCAGACT	3169
2	TGGCAGACA	2864
3	AGTCGGCCT	2788
4	AGTCTGCCT	2594
5	TTGTCGGCC	2558
6	AGGCAGACT	2550
7	TGTCAGCCT	2474
8	AGTCAGCCT	2468
9	TAGTCGGCC	2399
10	ATGTCTGCC	2265

rank	sequence	enrichment
1	GGGCAGTCA	72
2	GAGTCTCCC	69
3	CTCTCTGCA	58
4	ACAGCCTCG	54
5	GTCTCCGAA	53
6	ATCGGCAGA	53
7	AGGCTCTCA	48
8	AATTTACCT	48
9	GTGAGCCTA	48
10	GGGCTAGAC	46

Figure 3. Motif analyses, the top 10 sequences pulled down by Bind-n-Seq, and schematic representation of the sequence-specific recognition of conjugate **6** (a), **7** (b), **8** (c), **9** (d), and **10** (e).

2.4. Solubility and cell permeability

Given that one of the motivations for introducing an AO unit was to enhance aqueous solubility and to circumvent aggregation of Py-Im polyamide in aqueous media, we measured the saturating concentration in water as previously reported.[36] To this end, an aqueous solution (2.0 μL) was diluted in DMF (198 μL). The absorbance of the peak around 310 nm of each sample was measured and the concentration of each conjugate was calculated. Saturating concentrations of polyamides **1**, **2**, and **4** were 9.1, 12.2, and 13.9 mM, respectively. As expected, the aqueous solubilities of polyamides increased as the number of AO units increased. Polyamide **3** was excluded from this solubility and cell-permeability assay because it has exactly the same components as polyamide **2** and can be assumed to have nearly the same properties. Polyamide **5** was not tested because the aim of this study was to investigate the characteristics of AO-incorporating Py-Im polyamide

The situation regarding hydrophilicity is finely balanced: whereas poor solubility of Py-Im polyamide is problematic because it can lead to the formation of aggregates, high hydrophilicity can inhibit cell permeability. We therefore also examined the cell permeability of FITC-polyamides **11**–**13**. Thus, human breast cancer cells (MDA-MB-231) were cultured and FITC-polyamides **11**–**13** were introduced to the cell. Subsequent confocal microscopy studies revealed localized fluorescence as shown in Fig. 4 and Fig. S1 (Fig. 4 shows a detailed view of a single cell in which FITC-polyamide and Hoechst were introduced; Fig. S1 shows the whole pictures). The images demonstrate that the nuclear localization of polyamides **12** and **13** were comparable with that of polyamide **11**.

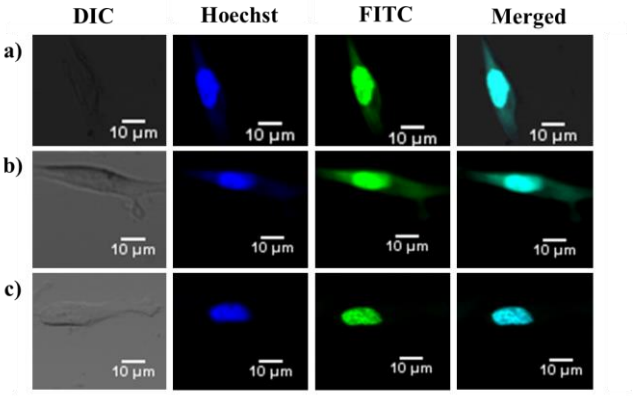


Figure 4. Confocal images of MDA-MB-231 cells after 72 h incubation.

3. Conclusion

We have studied the function and effects of incorporating an AO unit in hairpin Py-Im polyamides from five types of Py-Im polyamide: a normal polyamide **1**; polyamides **2** and **3**, with one AO unit; polyamide **4**, with two AO units; and polyamide **5**, with β-alanine-β-alanine. SPR and Bind-n-Seq analyses revealed that polyamides **2** and **7** had a significantly better binding affinity and sequence specificity than polyamides **3** and **8**, although the components of the Py-Im polyamides were same. Notably, polyamides **4** and **9** recovered both characteristics and gained an unprecedented "recognition" of base N (Fig. 3d). However, it became clear that the position of the AO unit affects the DNA-binding features significantly. Nevertheless, incorporation of an AO unit was shown to lead to greater aqueous solubility without loss of cell permeability. Thus, the possibility of using the AO unit as a linker within long hairpin Py-Im polyamides in biological settings has been established.

4. Experimental

4.1. General information

The reagents for polyamide synthesis such as Fmoc-Py-OH, Fmoc-Im-OH, Fmoc-Py-Im-OH, Piperidine 98%, NMP (Super Dehydrated), DMF (for Peptide Synth.) were purchased from Wako. Acetic Anhydride (Guaranteed Reagent) was from nacalai tesque. Fmoc-β-Ala-COOH and Fmoc-β-Ala-Wang resin (100-200 mesh) were obtained from Novabiochem. Fmoc-mini-PEG™ was purchased from PEPTIDE INTERNATIONAL.

The reagents for coupling reaction such as Fluorescein-5-isothiocyanate and DIEA was obtained from TCI, DMF was from KANTO CHEMICAL. NHS-PEG_{12}-Biotin was purchased from Thermo Scientific. HPLC grade acetonitrile (nacalai tesque) was used for both analytical and preparative HPLC. Water was prepared by a Milli-Q apparatus (MERCK MILLIPORE). ESI-TOF MS was performed on a Bio-TOF II (Bruker Daltonics) mass spectrometer by using positive ionization mode. NMR spectra were recorded on a JEOL JNM ECA-600 NMR spectrometer with tetramethylsilane as an internal standard. The following abbreviations apply to spin multiplicity: s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad).

4.2. Synthesis of polyamide 1

Fmoc solid-phase synthesis of Py-Im polyamide was performed on a PSSM-8 with a computer-assisted operation system. 56 mg of β-Wang resin (0.55 mmol/g) was used on the solid-phase synthesis. The resulting crude polyamide (14.4 mg, 7.36 μmol) was purified with reversed-phase column chromatography. Purification was performed on CombiFrash Rf using a 4.3 g C18 RediSep Rf reversed-phase flash column (Teledyne Isco, Inc) with 0.1% TFA in water and acetonitrile as the eluent at a flow rate of 18.0 mL/min and a linear gradient elution of 0 to 35% acetonitrile from 5 to 40 min. Collected appropriate fractions were concentrated on an evaporator and lyophilized. Then we obtained the polyamide **1** (4.2 mg, 2.2 μmol, 7.0% yield). Analytical HPLC: t_R = 10.4 min (0.1% TFA/MeCN, linear gradient 0–50%, 0–20 min). ESI-TOFMS m/z calcd for $C_{89}H_{113}N_{36}O_{17}^{3+}$ $[M + 3H]^{3+}$ 652.6356, found 652.6333. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 10.31 (s, 1H), 10.29 (s, 2H), 10.28 (s, 1H), 10.25 (s, 1H), 10.01 (s, 1H), 10.00 (s, 1H), 9.98 (s, 1H), 9.89 (s, 3H), 9.42 (s, 1H), 8.09 (m, 2H), 8.04 (m, 3H), 7.56 (s, 1H), 7.49(s, 1H), 7.46 (s, 2H), 7.45 (s, 1H), 7.25 (s, 3H), 7.22 (s, 1H), 7.18 (s, 1H), 7.16 (s, 1H), 7.15 (s, 1H), 7.12 (m, 3H), 6.97 (s, 1H), 6.89 (s, 1H), 6.88 (s, 1H), 6.86 (s, 1H), 3.99 (s, 3H), 3.97 (s, 3H), 3.95 (s, 6H), 3.94 (s, 1H), 3.84 (s, 9H), 3.81 (m, 6H), 3.80 (s, 6H), 3.19 (m, 4H), 3.13 (m, 4H), 3.05 (m, 4H), 2.86 (s, 2H), 2.74 (d, J = 4.1 Hz, 3H), 2.59 (m, 4H), 2.36 (m, 4H), 2.04 (s, 3H), 1.89 (m, 4H), 1.77 (m, 4H).

4.3. Synthesis of polyamide 2

Fmoc solid-phase synthesis of Py-Im polyamide was performed on a PSSM-8 with a computer-assisted operation system. 49 mg of β-Wang resin (0.55 mmol/g) was used on the solid-phase synthesis. The resulting crude polyamide (13.2 mg, 6.92 μmol) was purified with reversed-phase column chromatography. Purification was performed on CombiFlash Rf using a 4.3 g C18 RediSep Rf reversed-phase flash column (Teledyne Isco, Inc) with 0.1% TFA in water and acetonitrile as the eluent at a flow rate of 18.0 mL/min and a linear gradient elution of 0 to 45% acetonitrile from 5 to 50 min. Collected appropriate fractions were concentrated on an evaporator and lyophilized. Then we obtained the polyamide **2** (3.3 mg, 1.7 μmol, 6.4% yield). Analytical HPLC: t_R = 10.3 min (0.1% TFA/MeCN, linear gradient 0–50%, 0–20 min). ESI-TOFMS m/z calcd for $C_{86}H_{113}N_{34}O_{18}^{3+}$ $[M + 3H]^{3+}$ 636.6319, found 636.6272. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 10.31 (s, 1H), 10.28 (s, 1H), 10.26 (s, 1H), 10.06 (s, 1H), 10.00 (s, 1H), 9.98 (s, 1H), 9.90 (s, 2H), 9.88 (s, 1H), 9.61 (s, 2H), 9.35 (s, 1H), 8.32 (t, J = 5.5 Hz, 1H), 8.09 (t, J = 5.5 Hz,1H), 8.04 (m, 3H), 7.51 (s, 1H), 7.48 (s, 1H), 7.48 (s, 1H), 7.47 (s, 1H), 7.46 (s, 1H), 7.26 (s, 2H), 7.18 (s, 1H), 7.16 (s, 3H), 7.13 (m, 2H), 7.09 (s, 1H), 6.89 (s, 1H), 6.88 (s, 1H), 6.87 (s, 1H), 4.11 (s, 2H), 3.97 (s, 3H), 3.96 (s, 6H), 3.95 (s, 3H), 3.91 (s, 3H), 3.84 (s, 6H), 3.81 (m, 6H), 3.80 (s, 6H), 3.69 (m, 2H), 3.63 (m, 2H), 3.60 (m, 2H), 3.51 (m, 2H), 3.20 (m, 4H), 3.13 (m, 2H), 3.06 (m, 4H), 2.86 (s, 2H), 2.74 (d, J = 3.4 Hz, 3H), 2.60 (m, 2H), 2.36 (m, 4H), 2.03 (s, 3H), 1.92 (m, 2H), 1.79 (m, 4H).

4.3. Synthesis of polyamide 3

Fmoc solid-phase synthesis of Py-Im polyamide was performed on a PSSM-8 with a computer assisted operation system. 83 mg of β-Wang resin (0.55 mmol/g) was used on the solid-phase synthesis. The resulting crude polyamide (33.5 mg) was purified with reversed-phase column chromatography. Purification was performed on CombiFlash Rf using a 4.3 g C18 RediSep Rf reversed-phase flash column (Teledyne Isco, Inc) with 0.1% TFA in water and acetonitrile as the eluent at a flow rate of 18.0 mL/min and a linear gradient elution of 0 to 45% acetonitrile from 5 to 50 min. Collected appropriate fractions were concentrated on an evaporator and lyophilized. Then we obtained the polyamide **3** (6.8 mg, 3.6 μmol, 7.9% yield). ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.30 (s, 1H), 10.28 (s, 1H) 10.27 (s, 1H), 10.25 (s, 1H), 10.09 (s, 1H), 9.97 (s, 1H), 9.89 (s, 3H), 9.60 (s, 1H), 9.32 (s, 1H), 8.08 (m, 3H), 8.02 (m, 2H), 7.56 (s, 1H), 7.503 (s, 1H), 7.499 (s, 1H), 7.46 (s, 1H), 7.44 (s, 1H), 7.26 (d, 1H, J = 1.4 Hz), 7.22 (d, 1H, J = 1.4 Hz), 7.20 (s, 2H), 7.17 (s, 2H), 7.15 (d, 1H, J = 2.0 Hz), 7.13 (d, 1H, J = 1.4 Hz), 6.97 (d, 1H, J = 2.0 Hz), 6.93 (d, 1H, J = 2.0 Hz), 6.89 (d, 1H, J = 2.0 Hz), 6.88 (d, 1H, J = 2.0 Hz), 4.11 (s, 2H), 3.99 (s, 3H), 3.98 (s, 3H), 3.96 (s, 3H), 3.95 (s, 3H), 3.93 (s, 3H), 3.84 (s, 3H), 3.81 (s, 6H), 3.79 (s, 6H), 3.77 (s, 3H), 3.62 (m, 4H), 3.42 (m, 2H), 3.19 (m, 2H), 3.13 (m, 2H), 3.05 (m, 4H), 2.85 (br, 2H), 2.74 (d, 3H, J = 4.8 Hz), 2.60 (m, 4H), 2.35 (m, 8H), 2.03 (s, 3H), 1.88 (br, 2H), 1.78 (m, 4H). ESI-TOFMS: m/z calcd for $C_{86}H_{112}N_{34}O_{18}^{2+}$: 954.4441 $[M + 2H]^{2+}$, found: 954.4453; HPLC: t_R= 17.0 min (0.1% TFA/MeCN, linear gradient 0–50%, 0–20 min).

4.4. Synthesis of polyamide 4

Fmoc solid-phase synthesis of Py-Im polyamide was performed on a PSSM-8 with a computer assisted operation system. 50 mg of β-Wang resin (0.55 mmol/g) was used on the solid-phase synthesis. The resulting crude polyamide (14.1 mg, 7.58 μmol) was purified with reversed-phase column chromatography. Purification was performed on CombiFlash Rf using a 4.3 g C18 RediSep Rf reversed-phase flash column (Teledyne Isco, Inc) with 0.1% TFA in water with acetonitrile as the eluent at a flow rate of 18.0 mL/min and a linear gradient elution of 0 to 45% acetonitrile from 5 to 50 min. Collected appropriate fractions were concentrated on evaporator and lyophilized. Then we obtained the polyamide **3** (7.4 mg, 4.0 μmol, 15% yield). Analytical HPLC: t_R = 10.4 min (0.1% TFA/MeCN, linear gradient 0–50%, 0–20 min). ESI-TOFMS *m/z* calcd for $C_{86}H_{113}N_{34}O_{18}^{3+}$ $[M + 3H]^{3+}$ 636.6319, found 636.6272. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 10.31 (s, 1H), 10.25 (s, 1H), 10.11 (s, 1H), 10.06 (s, 1H), 9.90 (s, 1H), 9.89 (s,1H), 9.88 (s, 1H), 9.62 (s, 1H), 9.61 (s, 1H), 9.33 (s, 1H), 8.32 (t, J = 6.9 Hz, 1H), 8.08 (m, 2H), 8.03 (m, 2H), 7.51 (s, 1H), 7.50 (s, 1H), 7.48 (s, 1H), 7.48 (s, 1H), 7.44 (s, 1H), 7.21 (m, 2H), 7.16 (m, 3H), 7.15 (m, 1H), 7.09 (m, 1H), 6.93 (m, 1H), 6.89 (m, 2H), 4.11 (s, 4H), 3.97 (s, 3H), 3.96 (s, 3H), 3.95 (s, 3H), 3.93 (s, 3H), 3.90 (s, 3H), 3.81 (m, 6H), 3.79 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H), 3.68 (m, 4H), 3.62(m, 4H), 3.60 (t, J = 5.5 Hz, 2H), 3.57 (t, J = 6.2 Hz, 2H), 3.51 (m, 4H), 3.42 (m, 4H), 3.19 (m, 2H), 3.12 (m, 2H), 3.02 (m, 4H), 2.84 (m, 2H), 2.74 (d, J = 5.5 Hz, 3H), 2.61 (m, 3H), 2.38 (m, 2H), 2.35 (m, 2H), 2.03 (s, 3H), 1.78 (m, 4H).

4.3. Synthesis of polyamide 5

Fmoc solid-phase synthesis of Py-Im polyamide was performed on a PSSM-8 with a computer assisted operation system. 80 mg of β-Wang resin (0.55 mmol/g) was used on the solid-phase synthesis. The resulting crude polyamide (21.8 mg) was purified with reversed-phase column chromatography. Purification was performed on CombiFlash Rf using a 4.3 g C18 RediSep Rf reversed-phase flash column (Teledyne Isco, Inc) with 0.1% TFA in water and acetonitrile as the eluent at a flow rate of 18.0 mL/min and a linear gradient elution of 0 to 45% acetonitrile from 5 to 50 min. Collected appropriate fractions were concentrated on an evaporator and lyophilized. Then we obtained the polyamide **5** (6.1 mg, 3.2 μmol, 7.3% yield). ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.39 (s, 1H), 10.28 (s, 1H) 10.27 (s, 1H), 10.25 (s, 1H), 9.98 (s, 1H), 9.96 (s, 1H), 9.94 (s, 1H), 9.89 (s, 3H), 9.37 (s, 1H), 8.30 (t, 1H, J = 5.9 Hz), 8.09 (t, 1H, J = 5.9 Hz), 8.04 (m, 4H), 7.50 (s, 1H), 7.49 (s, 1H), 7.47 (s, 1H), 7.46 (s, 1H), 7.44 (s, 1H), 7.26 (s, 3H), 7.18 (d, 1H, J = 1.4 Hz), 7.17 (d, 1H, J = 1.4 Hz), 7.16 (d, 1H, J = 1.4 Hz), 7.14 (d, 1H, J = 1.4 Hz), 7.13 (d, 2H, J = 1.4 Hz), 6.90 (d, 1H, J = 2.0 Hz), 6.89 (d, 1H, J = 2.0 Hz), 6.87 (d, 1H, J = 2.0 Hz), 3.963 (s, 3H), 3.957 (s, 3H), 3.951 (s, 3H), 3.948 (s, 3H), 3.945 (s, 3H), 3.85 (s, 3H), 3.84 (s, 6H), 3.81 (s, 3H), 3.80 (s, 6H), 3.42 (m, 2H), 3.39 (m, 2H), 3.34 (m, 2H), 3.20 (m, 2H), 3.13 (m, 2H), 3.04 (m, 4H), 2.86 (br, 2H), 2.74 (d, 3H, J = 4.8 Hz), 2.61 (m, 4H), 2.37 (m, 8H), 2.02 (s, 3H), 1.89 (br, 2H), 1.79 (m, 4H). ESI-TOFMS: m/z calcd for $C_{86}H_{111}N_{35}O_{17}^{2+}$: 952.9449 $[M + 2H]^{2+}$, found: 952.9472; HPLC: t_R= 16.1 min (0.1% TFA/MeCN, linear gradient 0–50%, 0–20 min).

4.5. Synthesis of polyamide 6

To a solution of the crude Py-Im polyamide (2.3 mg) in DMF (150 μL), DIEA (0.8 μL, 4.7 μmol) and NHS-PEG$_{12}$-Biotin (4.0 mg, 4.2 μmol, ThermoScientific) were added. The reaction mixture was incubated overnight at room temperature. Evaporation of the solvent gave a yellow oil, which was purified by PU-2089 Plus HPLC (JASCO) using 10 ID ×150 mm 5C$_{18}$-MS-II COSMOSIL Packed Column (Nacalai Tesque, Inc.) with 0.1% TFA in water and acetonitrile as the eluent at a flow rate of 3.0 mL/min and a linear gradient elution to afford the hairpin Py-Im Polyamide-biotin conjugate (0.8 mg, 0.29 μmol, 9.3%) as a light yellow powder. Analytical HPLC: t_R = 18.2 min (0.1% TFA/MeCN, linear gradient 0–50%, 0–20 min). ESI-TOF-MS m/z calcd for $C_{126}H_{177}N_{39}O_{32}S^{3+}$ $[M + 3H]^{3+}$ 927.7787, found 927.7815.

4.6. Synthesis of polyamide 7

1.0 mg of polyamide **2** (0.53 μmol) was dissolved in 4.9 μL DMF solution of NHS-PEG$_{12}$-Biotin (0.11 mg/μL, 0.52 μmol in 4.9 μL), and then 0.53 μL of DIEA (3.1 μmol) was added. After the mixture was stirred at r.t. for 2 h, it was triturated with diethyl ether and purified by PU-2089 Plus HPLC (JASCO) using 10 ID ×150 mm 5C$_{18}$-MS-II COSMOSIL Packed Column (Nacalai Tesque, Inc.) with 0.1% TFA in water and acetonitrile as the eluent at a flow rate of 3.0 mL/min and a linear gradient elution of 30 to 50% acetonitrile in 30 min. The collected fractions were concentrated on evaporator and lyophilized. Then we obtained the polyamide **7** (0.52 mg, 0.18 μmol, 33% yield). Analytical HPLC: t_R = 11.1 min (0.1% TFA/MeCN, linear gradient 0–50%, 0–20 min). ESI-TOFMS *m/z* calcd for $C_{123}H_{181}N_{37}O_{33}S^{4+}$ $[M + 4H]^{4+}$ 684.0830, found 684.0834.

4.7. Synthesis of polyamide 8

2.0 mg of polyamide **3** (1.0 μmol) was dissolved in 9.4 μL DMF solution of NHS-PEG$_{12}$-Biotin (0.10 mg/μL, 1.0 μmol in 9.4 μL), and then 1.0 μL of DIEA (6.0 μmol) was added. After the mixture was stirred at r.t. for 10 min, it was triturated with diethyl ether and purified by HPLC to obtain the polyamide **8** (0.3 mg, 0.1 μmol, 10% yield). Analytical HPLC: t_R = 13.1 min (0.1% TFA/MeCN, linear gradient 0–50%, 0–20 min). ESI-TOFMS *m/z* calcd for $C_{123}H_{180}N_{37}O_{33}S^{3+}$ $[M + 3H]^{3+}$ 911.7750, found 911.7706.

4.8. Synthesis of polyamide 9

1.0 mg of polyamide **3** (0.54 μmol) was dissolved in 5.4 μL DMF solution of NHS-PEG$_{12}$-Biotin (0.10 mg/μL, 0.57 μmol), and then 0.55 μL of DIEA (3.2 μmol) was added. After the mixture was stirred at r.t. for 2 h, it was triturated with diethyl ether and purified by PU-2089 Plus HPLC (JASCO) using 10 ID x 150 mm 5C$_{18}$-MS-II COSMOSIL Packed Column (Nacalai Tesque, Inc.) in 0.1 % TFA with water and acetonitrile as the eluent at a flow rate of 3.0 mL/min and a linear gradient elution of 30 to 50% acetonitrile in 30 min. The collected fractions were concentrated on evaporator and lyophilized. Then we obtained the polyamide **9** (0.5 mg, 0.2 μmol, 35% yield). Analytical HPLC: t_R = 11.2 min (0.1% TFA/MeCN, linear gradient 0–50%, 0–20 min). ESI-TOFMS *m/z* calcd for $C_{120}H_{181}N_{35}O_{34}S^{4+}$ $[M + 4H]^{4+}$ 672.0802, found 672.0787.

4.9. Synthesis of polyamide 10

2.0 mg of polyamide **5** (1.0 μmol) was dissolved in 9.4 μL DMF solution of NHS-PEG$_{12}$-Biotin (0.10 mg/μL, 1.0 μmol in 9.4 μL), and then 1.0 μL of DIEA (6.0 μmol) was added. After the mixture was stirred at r.t. for 40 min, it was triturated with diethyl ether and purified by HPLC to obtain the polyamide **10** (0.4 mg, 0.1 μmol, 10% yield). Analytical HPLC: t_R = 12.7 min (0.1% TFA/MeCN, linear gradient 0–50%, 0–20 min). ESI-TOFMS *m/z* calcd for $C_{123}H_{179}N_{38}O_{32}S^{3+}$ $[M + 3H]^{3+}$ 910.7751, found 910.7645.

4.10. Synthesis of polyamide 11

0.90 mg of polyamide **1** (0.46 μmol) and 0.36 mg of fluorescein-5-isothiocyanate (0.92 μmol) were dissolved in 6.0 μL DMF, and then 0.88 μL of DIEA (0.92 μmol) was added. After the mixture was stirred at r.t. for 3 h, it was triturated with diethyl ether and purified by PU-2089 Plus HPLC (JASCO) using 10 ID x 150 mm 5C$_{18}$-MS-II COSMOSIL Packed Column (Nacalai Tesque, Inc.) with 0.1 % TFA in water and acetonitrile as the eluent at a flow rate of 3.0 mL/min and a linear gradient elution of 30 to 50% acetonitrile in 30 min. The collected fractions were concentrated on evaporator and lyophilized. Then we obtained the polyamide **11** (1.0 mg, 0.43 μmol, 93% yield). Analytical HPLC: t_R = 11.4 min (0.1% TFA/MeCN, linear gradient 0–50%, 0–20 min). ESI-TOFMS *m/z* calcd for $C_{123}H_{180}N_{37}O_{33}S^{3+}$ $[M + 3H]^{3+}$ 782.3142, found 782.3176.

4.11. Synthesis of polyamide 12

1.0 mg of polyamide **2** (0.53 μmol) and 0.82 mg of fluorescein-5-isothiocyanate (1.1 μmol) were dissolved in 6.0 μL DMF, and then 0.45 μL of DIEA (1.1 μmol) was added. After the mixture was stirred at r.t. for 3 h, it was triturated with diethyl ether and purified by PU-2089 Plus HPLC (JASCO) using 10 ID x 150 mm 5C$_{18}$-MS-II COSMOSIL Packed Column (Nacalai Tesque, Inc.) in 0.1 % TFA in water and acetonitrile as the eluent at a flow rate of 3.0 mL/min and a linear gradient elution of 30 to 50% acetonitrile in 30 min. The collected fractions were concentrated on evaporator and lyophilized. Then we obtained the polyamide **8** (1.0 mg, 0.44 μmol, 83% yield). Analytical HPLC: t_R = 11.5 min (0.1% TFA/MeCN, linear gradient 0–50%, 0–20 min). ESI-TOFMS *m/z* calcd for $C_{107}H_{124}N_{35}O_{23}S^{3+}$ $[M + 3H]^{3+}$ 766.3105, found 766.3124.

4.12. Synthesis of polyamide 13

1.0 mg of polyamide **4** (0.54 μmol) and 0.21 mg of fluorescein-5-isothiocyanate (0.54 μmol) were dissolved in 6.0 μL DMF, and then 0.23 μL of DIEA (0.54 μmol) was added. After the mixture

was stirred at r.t. for 2 h, it was triturated with diethyl ether and purified by PU-2089 Plus HPLC (JASCO) using 10 ID x 150 mm 5C_{18}-MS-II COSMOSIL Packed Column (Nacalai Tesque, Inc.) with 0.1 % TFA in water and acetonitrile as the eluent at a flow rate of 3.0 mL/min and a linear gradient elution of 30 to 50% acetonitrile in 30 min. The collected fractions were concentrated on evaporator and lyophilized. Then we obtained the polyamide **13** (0.8 mg, 0.4 μmol, 66% yield). Analytical HPLC: t_R = 11.7 min (0.1% TFA/MeCN, linear gradient 0–50%, 0–20 min). ESI-TOFMS m/z calcd for $C_{104}H_{123}N_{33}O_{24}S^{3+}$ [M + 3H]$^{3+}$ 750.3067, found 750.3055.

4.13. SPR binding assays

SPR experiments were performed on a Biacore X instrument (GE healthcare) according to previous reports.[37, 38] A biotinylated hairpin DNA was purchased from JBioS and its sequence is shown in Figure 2a. The biotinylated DNA was immobilized to a streptavidin-functionalized SA sensor chip to obtain the desired immobilization level (approximately 900 RU rise). SPR measurements were carried out using degassed and filtered HBS buffer (10 mM HPES 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 a wide range of concentrations were prepared in the buffer with 0.1% DMSO and injected at a flow rate of 20 μL/min. Optimized concentration range was then selected for the subsequent quantitative analysis. The resulting sensorgrams were fitted with 1:1 binding fitting model using BIAevaluation 4.1 program in order to obtain the values of binding affinities (K_D) and kinetics parameters (k_a and k_d).

4.14. Bind-n-Seq Analysis

Bind-n-Seq analysis were proceeded as previously reported.[31] Briefly, oligonucleotides consisting of Ion torrent sequencing library adaptor A1, Ion Express Barcode, 21-mer randomized sequence and another adaptor P1 were purchased from Sigma-Aldrich and duplexed via primer extension; 50 nM of Py-Im polyamide-biotin conjugates were allowed to bind 1.5 μM of the library in 50 μL for 16 h at rt; enrichment by Streptavidin M-280 Dynabeads and elution from on the beads; PCR amplification, quantification by 2100 Bioanalyzer (Agilent Technologies) and emulsion PCR on Ion OneTouch 2 system (Thermo Fisher Scientific); sequencing by Ion Torrent PGM 318 chip (Thermo Fisher Scientific). The results were primarily analyzed by Bind-n-Seq analysis pipeline and the motifs were processed by enoLOGOS[39] (URL: http://www.benoslab.pitt.edu/cgi-bin/enologos/enologos.cgi).

4.15. Measurement of aqueous solubility

Saturated solutions of a polyamide **1**, **2** and **3** in Milli-Q were prepared. The solution (2.00 μL) was diluted in DMF (198 μL). The absorbance of the peak around 310 nm of each sample was measured and concentration of each conjugate was calculated. Absorbance was measured with Nanodrop 1000 (LMS, Inc). Molar extinction coefficients ε of Py-Im polyamides in DMF solution at around 305 nm were calculated as follows:[43]

$$\varepsilon = 9900 \times (\text{sum number of P and I})$$

Concentrations of Py-Im polyamides were determined by the Lambert-Beer law.

$$\text{Abs} = \varepsilon c l$$

where Abs, c and l are absorbance, molar concentration and the path length, respectively.

4.16. Cell culture and confocal microscopy[11, 41]

MDA-MB-231 human breast cancer cell line was purchased from European collection of cell cultures and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in humidified atmosphere of 5% CO_2.

Each MICRO COVER GLASS (MATSUNAMI, Inc) was coated by 500 μL of StemSure 0.1 w/v % Gelatin Solution (Wako, Inc) before seeding. Cells were seeded at a final density of 5 x 10^3 cells on each cover glass in a 24-well plate and precultured for 24 h at 37 °C. After the medium was replaced with a fresh one containing 0.5% DMSO and FITC polyamides (340 nM), the cells were incubated for 48 h at 37 °C. Each well was then washed twice with 500 μL of PBS to remove the excess compound. Hoechst 33342 (5 μM) was added to the medium 1 h before the PBS washing. After that, the cells were fixed by 480 μL 3.5% formaldehyde solution (SIGMA ALDLICH) in each well for 20 min, and washed by 500 μL of PBS twice for 20 min. After that, the cover glass was mounted in FLUOROMOUNT/PLUS (COSMO BIO CO., LTD) and sealed with a nailpolish. Imaging was performed with FV1200 Laser Scanning Microscopes (OLYMPUS, Inc). FITC-Polyamide **11**-**13** were excited using 473 nm laser at 6% transmissivity with a pinhole of 80 μm and a standard fluorescein filter set. Hoechst was excited using 405 nm laser lines at 10% transmissivity with pinhole of 80 μm. Image analysis was performed using FV10-ASW. PMT voltage in the observation of **11** is 424 V on Hoechst, 363 V on fluorescein, 86 V on DIC. PMT voltage in the observation of **12** is 501 V on Hoechst, 430 V on fluorescein, 100 V on DIC. PMT voltage in the observation of **13** is 478 V on Hoechst, 473 V on fluorescein, 105 V on DIC. Objective lens magnification is 20X.

Acknowledgments

This work was supported by JSPS KAKENHI (grant number 24225005 to H. S. and 24310155 to T. B. Basic Science and Platform Technology Program for Innovative Biological Medicine from Japan Agency for Medical Research and Development, AMED to H. S.

References and notes

1. Trauger, J.W.; Baird, E.E.; Dervan, P.B. *Nature* **1996**, *382*, 559.
2. White, S.; Szewczyk, J.W.; Turner, J.M.; Baird, E.E.; Dervan, P.B. *Nature* **1998**, *391*, 468.
3. Dervan, P.B. *Bioorg. Med. Chem* **2001**, *9*, 2215.
4. Dervan, P.B.; Edelson, B.S. *Curr. Opin. Struct. Biol.* **2003**, *13*, 284.
5. Dervan, P.B.; Doss, R.M.; Marques, M.A. *Curr. Med. Chem. - Anti-Cancer Agents* **2005**, *5*, 373.
6. Blackledge, M.S.; Melander, C. *Bioorg. Med. Chem.* **2013**, *21*, 6101.
7. Nickols, N.G.; Dervan, P.B. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 10418.
8. Nickols, N.G.; Jacobs, C.S.; Farkas, M.E.; Dervan, P.B. *ACS Chem. Biol.* **2007**, *2*, 561.
9. Chenoweth, D.M.; Harki, D.A.; Phillips, J.W.; Dose, C.; Dervan, P.B. *J. Am. Chem. Soc.* **2009**, *131*, 7182.
10. Raskatov, J.A.; Meier, J.L.; Puckett, J.W.; Yang, F.; Ramakrishnan, P.; Dervan, P.B. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 1023.
11. Raskatov, J.A.; Nickols, N.G.; Hargrove, A.E.; Marinov, G.K.; Wold, B.; Dervan, P.B. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 16041.
12. Pandian, G.N.; Nakano, Y.; Sato, S.; Morinaga, H.; Bando, T.; Nagase, H.; Sugiyama, H. *Sci. Rep.* **2012**, *2*, 544.

13. Nickols, N.G.; Szablowski, J.O.; Hargrove, A.E.; Li, B.C.; Raskatov, J.A.; Dervan, P.B. *Mol. Cancer Ther.* **2013**, *12*, 675.
14. Syed, J.; Pandian, Ganesh N.; Sato, S.; Taniguchi, J.; Chandran, A.; Hashiya, K.; Bando, T.; Sugiyama, H. *Chem. Biol.* **2014**, *21*, 1370.
15. Han, L.; Pandian, G.N.; Chandran, A.; Sato, S.; Taniguchi, J.; Kashiwazaki, G.; Sawatani, Y.; Hashiya, K.; Bando, T.; Xu, Y.; Qian, X.; Sugiyama, H. *Angew. Chem. Int. Ed.* **2015**, *54*, 8700.
16. Hiraoka, K.; Inoue, T.; Taylor, R.D.; Watanabe, T.; Koshikawa, N.; Yoda, H.; Shinohara, K.; Takatori, A.; Sugimoto, H.; Maru, Y.; Denda, T.; Fujiwara, K.; Balmain, A.; Ozaki, T.; Bando, T.; Sugiyama, H.; Nagase, H. *Nat. Commun.* **2015**, *6*, 6706.
17. Igarashi, J.; Fukuda, N.; Inoue, T.; Nakai, S.; Saito, K.; Fujiwara, K.; Matsuda, H.; Ueno, T.; Matsumoto, Y.; Watanabe, T.; Nagase, H.; Bando, T.; Sugiyama, H.; Itoh, T.; Soma, M. *PLoS One* **2015**, *10*, e0125295.
18. Syed, J.; Chandran, A.; Pandian, G.N.; Taniguchi, J.; Sato, S.; Hashiya, K.; Kashiwazaki, G.; Bando, T.; Sugiyama, H. *ChemBioChem* **2015**, *16*, 1497.
19. Turner, J.M.; Swalley, S.E.; Baird, E.E.; Dervan, P.B. *J. Am. Chem. Soc.* **1998**, *120*, 6219.
20. Hargrove, A.E.; Raskatov, J.A.; Meier, J.L.; Montgomery, D.C.; Dervan, P.B. *J. Med. Chem.* **2012**, *55*, 5425.
21. Janssen, S.; Durussel, T.; Laemmli, U.K. *Mol. Cell* **2000**, *6*, 999.
22. Maeshima, K.; Janssen, S.; Laemmli, U.K. *EMBO J.* **2001**, *20*, 3218.
23. Kawamoto, Y.; Bando, T.; Kamada, F.; Li, Y.; Hashiya, K.; Maeshima, K.; Sugiyama, H. *J. Am. Chem. Soc.* **2013**, *135*, 16468.
24. Kawamoto, Y.; Sasaki, A.; Hashiya, K.; Ide, S.; Bando, T.; Maeshima, K.; Sugiyama, H. *Chem. Sci.* **2015**, *6*, 2307.
25. Warren, C.L.; Kratochvil, N.C.; Hauschild, K.E.; Foister, S.; Brezinski, M.L.; Dervan, P.B.; Phillips, G.N.; Jr., Ansari, A.Z. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 867.
26. Puckett, J.W.; Muzikar, K.A.; Tietjen, J.; Warren, C.L.; Ansari, A.Z.; Dervan, P.B. *J. Am. Chem. Soc.* **2007**, *129*, 12310.
27. Carlson, C.D.; Warren, C.L.; Hauschild, K.E.; Ozers, M.S.; Qadir, N.; Bhimsaria, D.; Lee, Y.; Cerrina, F.; Ansari, A.Z. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 4544.
28. Tietjen, J.R.; Donato, L.J.; Bhimisaria, D.; Ansari, A.Z. In *Methods in Enzymology*; Chris, V. Ed.; Academic Press, 2011; Vol. 497, pp 3-30.
29. Erwin, G.S.; Bhimsaria, D.; Eguchi, A.; Ansari, A.Z. *Angew. Chem. Int. Ed.* **2014**, *53*, 10124.
30. Meier, J.L.; Yu, A.S.; Korf, I.; Segal, D.J.; Dervan, P.B. *J. Am. Chem. Soc.* **2012**, *134*, 17814.
31. Chandran, A.; Li, Y.; Kizaki, S.; Pandian, G.N.; Hashiya, K.; Bando, T.; Sugiyama, H. *ChemBioChem* **2014**, *15*, 2647.
32. Chandran, A.; Kizaki, S.; Bando, T.; Pandian, G.N.; Sugiyama, H. *ChemBioChem* **2015**, *16*, 20.
33. Kashiwazaki, G.; Bando, T.; Shinohara, K.-i.; Minoshima, M.; Nishijima, S.; Sugiyama, H. *Bioorg. Med. Chem.* **2009**, *17*, 1393.
34. Kashiwazaki, G.; Bando, T.; Shinohara, K.-i.; Minoshima, M.; Kumamoto, H.; Nishijima, S.; Sugiyama, H. *Bioorganic & Medicinal Chemistry* **2010**, *18*, 2887.
35. Kang, J.S.; Meier, J.L.; Dervan, P.B. *J. Am. Chem. Soc.* **2014**, *136*, 3687.
36. Takagaki, T.; Bando, T.; Kitano, M.; Hashiya, K.; Kashiwazaki, G.; Sugiyama, H. *Bioorg. Med. Chem.* **2011**, *19*, 5896.
37. Lacy, E.R.; Le, N.M.; Price, C.A.; Lee, M.; Wilson, W.D. *J. Am. Chem. Soc.* **2002**, *124*, 2153.
38. Asamitsu, S.; Kawamoto, Y.; Hashiya, F.; Hashiya, K.; Yamamoto, M.; Kizaki, S.; Bando, T.; Sugiyama, H. *Bioorg. Med. Chem.* **2014**, *22*, 4646.
39. Workman, C. T.; Yin, Y.; Corcoran, D. L.; Ideker, T.; Stormo, G. D.; Benos, P. V. *Nucleic Acids Res.* **2005**, *33*, W389-W392.
40. Kashiwazaki, G.; Bando, T.; Yoshidome, T.; Masui, S.; Takagaki, T.; Hashiya, K.; Pandian, G.N.; Yasuoka, J.; Akiyoshi, K.; Sugiyama, H. *J. Med. Chem.* **2012**, *55*, 2057.
41. Lai, Y.-M.; Fukuda, N.; Ueno, T.; Matsuda, H.; Saito, S.; Matsumoto, K.; Ayame, H.; Bando, T.; Sugiyama, H.; Mugishima, H.; Serie, K. *J. Pharmacol. Exp. Ther.* **2005**, *315*, 571.

Supplementary Material

Supplementary material associated with this article can be found, in the online version at URL.