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Requirement of β-alanine components in sequence-specific DNA alkylation by pyrrole–imidazole conjugates with seven-base pair recognition

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Abstract—To investigate the effect of incorporation of β-alanine in alkylating *N*-methylpyrrole (Py)–*N*-methylimidazole (Im) polyamide, *seco*-CBI conjugates **2**–**8** were synthesized by an Fmoc solid-phase method and subsequent coupling with an alkylating moiety. DNA-alkylating activities of conjugates **2**–**8** were evaluated by high-resolution denaturing gel electrophoresis with 202-base pair (bp) DNA fragments. Alkylation by conjugates **2** and **3**, which have antiparallel pairings of β-alanine (β) opposite β (β/β) and Py/β, occurred mainly at the adenine (A) of the matching sequences, 5′-AGCTCCA-3′ (site 1) and 5′-AGCACCA-3′ (site 3). However, conjugate **4**, with β/Py, did not show any DNA-alkylating activities. Similarly, conjugate **5**, which possessed a Py/Py pair, weakly alkylated the matching sites at micromolar concentrations. Conjugates **6** and **7**, which possessed β/β and Py/β pairs, respectively, alkylated at the A of the matching sequences, 5′-ACTACCA-3′ (site 2) and 5′-ACAACCA-3′ (site 4). In contrast, conjugated **8**, with a Py/Py pair, showed lower activity and less alkylated DNA at sites 2 and 4 with mismatched alkylation at site 1 at a higher concentration than that of **6** and **7**. These results demonstrate that incorporation of β-alanine is required for the sequence-specific alkylation by *seco*-CBI Py–Im conjugates with a seven-base pair sequence.

1. Introduction

Minor groove-binding hairpin polyamides containing *N*-methylpyrrole (Py) and *N*-methylimidazole (Im) recognize each of the four Watson–Crick base pairs.[1] Sequence-specific DNA recognition depends on the sequence of side-by-side Py and Im components oriented in the amino-carboxyl (N–C) direction with respect to the 5′–3′ direction of the DNA duplex. Antiparallel pairing of Im opposite Py (Im/Py) recognizes a G–C base pair, whereas a Py/Im pairing recognizes a C–G base pair.[2] Antiparallel pairing of Py/Py recognizes A–T or T–A base pairs. Polyamides comprising more than five contiguous Py or Im are overcurved compared with the DNA minor groove. Thus, introduction of flexible β-alanine (β) in the polyamides allows the crescent-shaped polyamides to fit into the DNA minor groove, and β/β, β/Py, and Py/β pairings effectively recognize T–A and A–T pairs.[3,4]

Py–Im hairpin polyamides have strong binding affinity and sequence-specificity for targeting DNA sequences.[5] We have developed various DNA sequence-specific alkylating agents using Py–Im as a DNA-binding unit, and have investigated their DNA-alkylating activities and biological activities.[6] Using Fmoc solid-phase synthesis to assist the synthesis of Py–Im polyamides, we recently developed conjugate **1**, which is a hybrid between *seco*-CBI,[7–9] a precursor of 1,2,9,9a-tetrahydrocyclopropa[1,2-*c*]benz[1,2-*e*]indol-4-one (CBI), and a Py–Im hairpin polyamide with a 5-amino-1*H*-indole 2-carbonyl (indole) linker. Conjugate **1** efficiently alkylates at the adenine (A) of the matching sequences, 5′-WGWCCA-3′ (W = A or T), in DNA fragments (Fig. 1).[10] A ring-opened *seco*-CBI moiety readily converts to CBI under neutral pH, which alkylates at the

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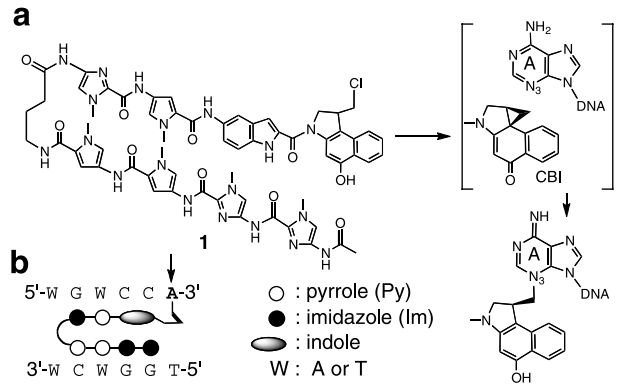

Figure 1. (a) Chemical structures of Py–Im *seco*-CBI conjugate **1** and the DNA-alkylating mechanism at N3 of adenine. (b) Schematic representation of sequence-specific alkylation in the specific DNA sequence by **1**. Arrows indicate sequence-specific sites of adenine N3 alkylation.

N3 of adenine in the DNA minor groove.[11] To expand the recognition sequence of the alkylating Py–Im polyamides, we introduced a β/β unit in the hairpin polyamide with nine-base pair recognition.[10] Although the β/β unit incorporating Py–Im polyamides caused sequence-specific DNA alkylation,[10] the general principles of the introduction of the β unit in the DNA-alkylating polyamides are not understood completely. Here we describe a comparative study of DNA alkylation by seven different alkylating Py–Im conjugates **2**–**8**, which possess β/β, Py/β, β/Py, or Py/Py pairs. The conjugates with β/β or Py/β specifically alkylated at the A of the targeted matching sequences at nanomolar concentrations. Although conjugates **5** and **8**, with Py/Py pairs, showed weak and inaccurate alkylating activities, the incorporation of β-alanine at an appropriate position greatly facilitated sequence-specific DNA alkylation.

2. Molecular design and synthesis

To evaluate the effects of incorporating β-alanine in the Py–Im polyamides on sequence-specific DNA alkylation, seven types of alkylating polyamide were designed, as shown in Figure 2. The *seco*-CBI conjugates **2**–**8** have different pairings of β/β, Py/β, β/Py, and Py/Py, which are shown in the rectangles in Figure 2. Py–Im hairpin polyamide *seco*-CBI conjugates **2**–**8** were synthesized by Fmoc solid-phase synthesis using an oxime resin or β-alanine CLEAR-acid resin, and treated to cause the cleavage and then the coupling with indole-*seco*-CBI unit **9** using HCTU as described previously.[12–14] Conjugates **2**–**8** were purified by reverse-phase HPLC and then used to evaluate the specific DNA alkylation. The purity and identity of conjugates **2**–**8** were verified by reversed-phase analytical HPLC and ESI-TOF mass spectrometry.

3. Evaluation of specific DNA alkylation

We investigated the DNA alkylation by conjugates **2**–**8** at four targeting sites 1–4 on 202-bp DNA fragments labeled with Texas Red at the 5′ end.[15,16] Sites 1 and 3 were designed as putative alkylation sites for conjugates **2**–**5**, and sites 2 and 4 were matching sites for conjugates **6**–**8**. DNA alkylation was conducted at 23 °C for 15 h and quenched by the addition of calf thymus DNA. The sites of DNA alkylation were visualized by thermal cleavage of the alkylated DNA strands using high-resolution denaturing polyacrylamide gel electrophoresis as described previously.[17–20] Under these heating conditions, all purine N3 alkylated sites in the DNA produced cleavage bands almost quantitatively on the polyacrylamide gel. Sequencing analysis of the alkylated DNA fragments by conjugates **2**–**5** is shown in Figure 3. DNA alkylation by conju-

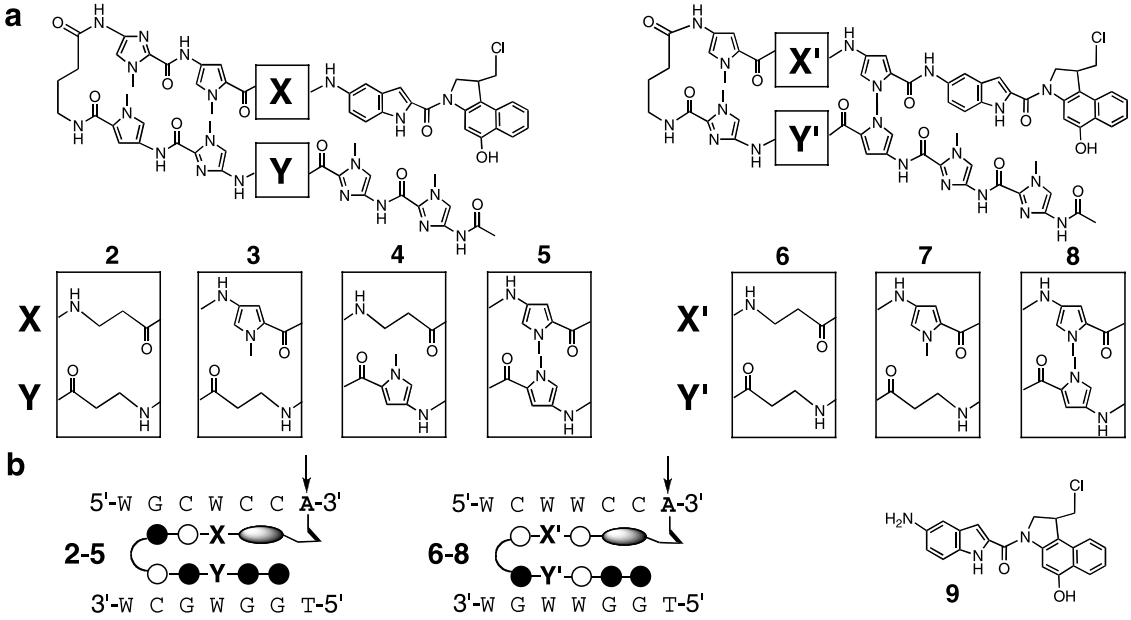

Figure 2. (a) The chemical structures of the Py–Im hairpin polyamide *seco*-CBI conjugates **2**–**8** and indole-*seco*-CBI unit **9**. (b) Schematic representation of sequence-specific alkylation by conjugates **2**–**8**. Arrows indicate sites of adenine N3 alkylation.

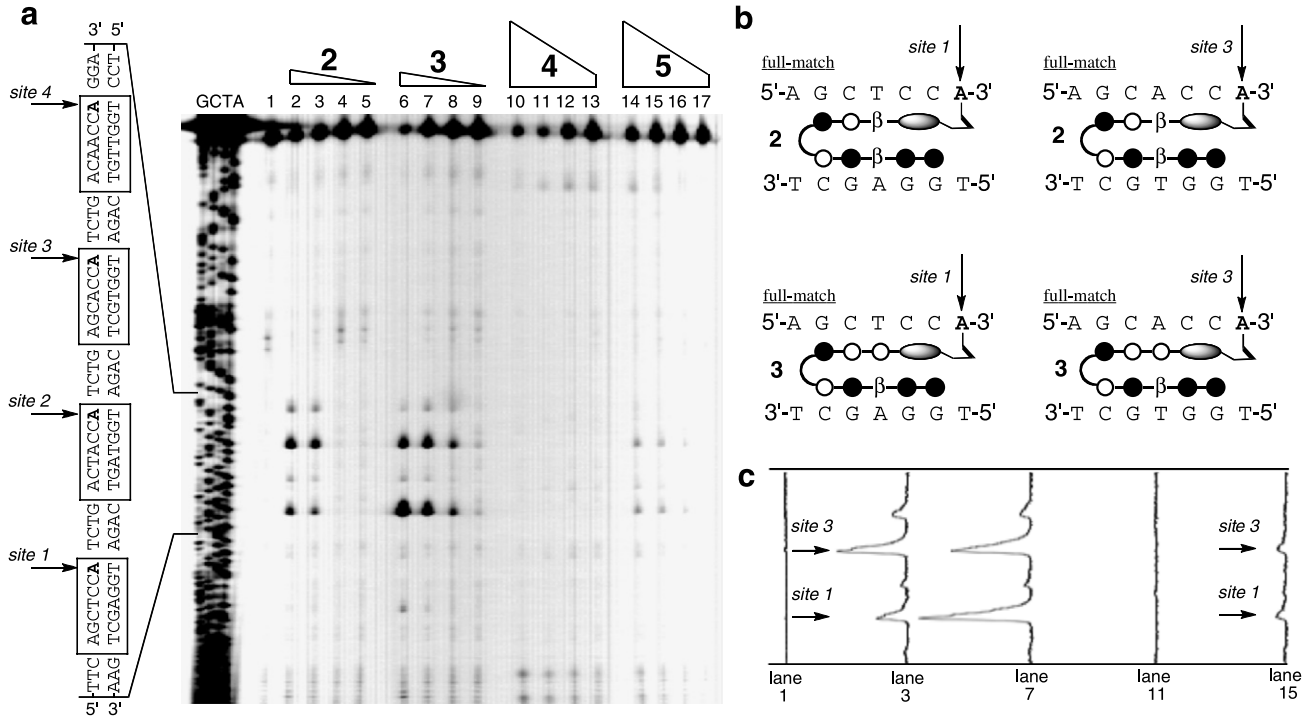

Figure 3. Thermally induced strand cleavages of the 5′-Texas Red labeled 202-bp DNA fragment (6 nM) by conjugates **2**, **3**, **4**, and **5** incubated for 15 h at 23°C; lane 1 = DNA control; lanes 2–5 = 500, 250, 100, and 50 nM of **2**; lanes 6–9 = 500, 250, 100, and 50 nM of **3**; lanes 10–13 = 5, 2, 1, and 0.5 μM of **4**; lanes 14–17 = 5, 2, 1, and 0.5 μM of **5**. The induced specific sequences containing alkylation sites 1–4 are represented on the left side of the sequencing lanes. (b) Schematic representation of sequence-specific alkylation in the specific sequence by conjugates **2** and **3**. Arrows indicate sites of adenine N3 alkylation. (c) Densitometric analysis of specific DNA alkylation at sites 1 and 3 in lanes 1, 3, 7, 11, and 15.

gates **2** and **3** occurred mainly at the A of sequences 5′-AGCTCCA-3′ (site 1) and 5′-AGCACCA-3′ (site 3) with minor alkylations at the A of 5′-ACTACCA-3′ (site 2) and 5′-ACAACCA-3′ (site 4), which are 2-bp mismatch sites according to the recognition rule of Py–Im polyamides (lanes 2–9). DNA alkylation by *seco*-CBI conjugate **3** occurred more efficiently at the matching sites (sites 1 and 3) compared with the reaction by conjugate **2**, which showed a faint 2-bp mismatch alkylation at sites 2 and 4 in the densitometric analysis (Fig. 3c). In contrast to the reactivities of **2** and **3**, alkylation by **4** was not observed at concentrations up to 5 μM (lanes 10–13). Similarly, conjugate **5**, with Py/Py pairing, very weakly alkylated the A of 5′-AGCTCCA-3′ (site 1) and 5′-AGCACCA-3′ (site 3) at a concentration of micromolar (lanes 14–15). It is not clear why conjugate **4**, possessing β-alanine in the *N*-terminal of the indole linker, lacks reactivity. One possibility is that a large conformational change of β-alanine prevents the proper positioning of the alkylating moiety in the minor groove. The results indicate clearly that incorporation of β-alanine in an appropriate manner significantly increased the alkylation activity of Py–Im polyamides.

Sequencing analysis of the alkylated DNA fragments by conjugates **6**–**8** is shown in Figure 4. DNA alkylation by conjugates **6** and **7** occurred predominantly at the A of sequences 5′-ACTACCA-3′ (site 2) and 5′-ACAACCA-3′ (site 4) (lanes 2–9). In contrast, alkylation by **8** was observed at target sites (sites 2 and 4) with minor 2-bp mismatch alkylation at site 1 even at a higher concentration than that of **6** and **7** (lanes 10–13).

These results indicate clearly that the incorporation of a β-alanine dramatically improves the reactivity and recognition ability of the *seco*-CBI conjugates and that low reactivity of conjugates **5** and **8** exhibit overcurvature of polyamides in a hairpin conformation as DNA sequence-specific alkylating agents. These results also indicate that conjugates **3** and **7** effectively alkylate according to Dervan's pairing rule and that they exhibit an appropriate molecular design for sequence-specific DNA-alkylating polyamides with seven-base pair recognition.

4. Conclusions

To investigate the effects of incorporating β-alanine in Py–Im polyamide *seco*-CBI conjugates, we synthesized compounds **2**–**8** and measured their reactivities as DNA-alkylating agents. Introducing the β-alanine significantly increased sequence-specific DNA alkylation. Consistent with the previous observation of the binding of Py–Im polyamides,[3,4] β/β and Py/β pairings recognized T–A or A–T base pairs in the alkylation by Py–Im polyamide *seco*-CBI conjugates **2**, **3**, **6**, and **7**, which alkylate A in specific seven-base pair sequences. These results demonstrate that the incorporation of β-alanine is required for the sequence-specific alkylation by *seco*-CBI Py–Im conjugates with seven-base pair recognition

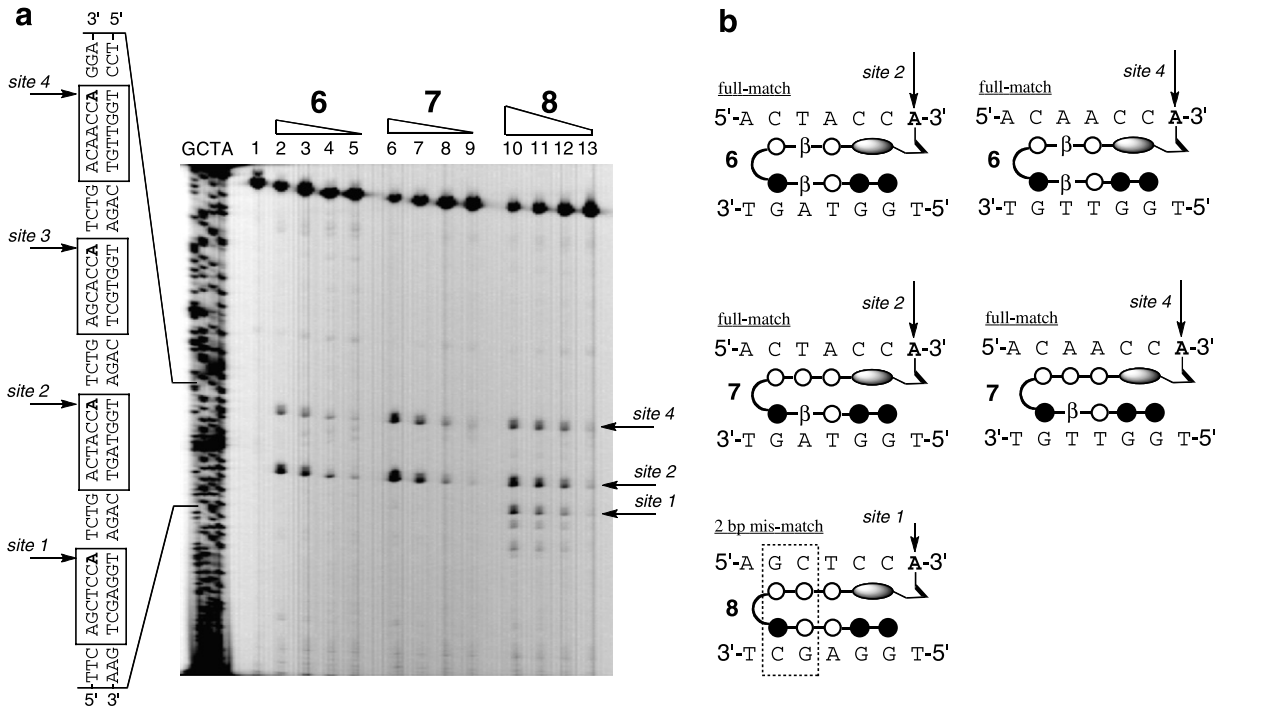

Figure 4. Thermally induced strand cleavages of 5′-Texas Red labeled 202-bp DNA fragment (6 nM) by conjugates **6**, **7,** and **8** incubated for 15 h at 23 °C; lane 1 = DNA control; lanes 2–5 = 250, 100, 50, and 25 nM of **6**; lanes 6–9 = 250, 100, 50, and 25 nM of **7**; lanes 10–13 = 1000, 500, 250, and 100 nM of **8**. The induced specific sequences containing alkylation sites 1–4 are represented on the left side of the sequencing lanes. (b) Schematic representation of sequence-specific alkylation in the specific sequence by conjugates **6**–**8**. Arrows indicate sites of adenine N3 alkylation.

and that incorporation of β-alanine at an appropriate position will provide important information for the design of *seco*-CBI conjugates with expanded recognition sequences.

5. Experiments

5.1. General

Reagents and solvents were purchased from standard suppliers and used without further purification. ^{1}H NMR spectra were recorded on a JEOL JNM-AL400 Superconducting NMR spectrometer 400 MHz, with chemical shifts reported in parts per million relative to residual solvent and coupling constants in Hz. The following abbreviations were applied to spin multiplicity: s (singlet), d (doublet), and m (multiplet). High-performance liquid chromatography (HPLC) analysis was performed with a JASCO PU-2080 Plus HPLC pump, a JASCO 807-IT HPLC UV/vis detector also performed and a Chemcobond 5-ODS-H reversed-phase column (4.6 × 150 mm) in 0.1% AcOH in water with acetonitrile as eluent at a flow rate of 1.0 mL/min with detection at 254 nm. Electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS) were produced on a Bio-TOF II (Bruker Daltonics) mass spectrometer using a positive ionization mode. Machine-assisted polyamide syntheses were performed on a Microwave Peptide Synthesizer (CEM) with computer-assisted operation system at a 0.10 mmol scale by using Fmoc chemistry. All DNA fragments, 5′-Texas Red labeled primers, and cold primers were purchased from Sigma–Aldrich. Ex Taq DNA polymerase was purchased from Takara. Thermo Sequence core sequencing kit and loading dye (formamide with fuchsin red) were purchased from GE Healthcare. Polymerase Chain Reaction (PCR) was performed on an iCycler (BIO-RAD). Long Ranger^{TM} gel solution (50%) was purchased from FMC bioproducts. Polyacrylamide gel electrophoresis was performed on a HITACHI 5500-S DNA sequencer, and data were analyzed by FLAGLYS version 2 software (HITACHI). NH_2-Indole-*seco*-CBI (**9**) was prepared by already published methods.[14] CLEAR-acid resin (100–200 mesh) and oxime resin (200–400 mesh) were purchased from PEPTIDES international and Novabiochem, respectively.

5.2. Solid-phase synthesis of polyamides

Py–Im polyamides supported by oxime resin (for the synthesis of the conjugates **1**, **3**, and **5**–**8**) and β-alanine CLEAR-acid resin (for the synthesis of the conjugates **2** and **4**) were prepared in a stepwise reaction by reported fmoc solid-phase protocol (FmocPyCO_2H, FmocIm-CO_2H, Fmoc-β-CO_2H, HATU, DIEA, DMF).[12] Products using oxime resin were cleaved with alkali condition (1 N NaOH in DMF, 1 h, 55 °C).[14] Products using CLEAR-acid resin were cleaved with acidic condition (trifluoroacidic acid, triisopropylsilane in DMS–H_2O, 1 h, rt). The purification by HPLC using a Chemcobond 5-ODS-H column (0.1% AcOH/CH_3CN 0–100% linear gradient, 0–30 min, 254 nm) produced the Py–Im polyamides as a yellow powder.

5.2.1. AcImIm-β-ImPy-γ-ImPyPy-CO_2H (10). 22 mg, 21 μmol, 21% for 10 steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.40 (s, 1H; NH), 10.36 (s, 2H; NH), 10.27 (s, 1H; NH), 9.95 (s, 1H; NH), 9.93 (s, 1H; NH), 9.79 (s, 1H; NH), 8.25 (m, 1H; NH), 8.05 (m, 1H; NH), 7.52 (s, 1H; Im–H), 7.49 (s, 1H; Im–H), 7.45 (s, 1H; Im–H), 7.44 (s, 1H; Im–H), 7.26 (s, 1H; Py–H), 7.21 (s, 2H; Py–H), 7.09 (s, 1H; Py–H), 6.96 (s, 2H; Py–H), 3.96 (s, 9H; NCH_3), 3.95 (s, 3H; NCH_3), 3.84 (s, 3H; NCH_3), 3.82 (s, 3H; NCH_3), 3.80 (s, 3H; NCH_3) 3.50 (m, 2H; CH_2), 3.19 (m, 2H; CH_2), 2.64 (m, 2H; CH_2), 2.34 (m, 2H; CH_2), 2.02 (s, 3H; $COCH_3$), 1.79 (m, 2H; CH_2); ESI-TOF-MS *m/e* calcd for $C_{47}H_{55}N_{20}O_{11}$ $[M+H]^+$ 1075.44; found 1075.76.

5.3. Synthesis of indole *seco*-CBI conjugates

To a solution of compound **10** (9.2 mg, 8.6 μmol) in DMF (80 μL) were added ^{i}Pr$_2$NEt (9.0 μL, 52 μmol) and HCTU (4.3 mg, 10 μmol). The reaction mixture was stirred for 2 h at room temperature. After the conversion from **10** to activated ester was confirmed by HPLC and ESI-TOF-MS analysis, **9** (4.4 mg, 11 μmol) was added to the reaction vessel. The reaction mixture was stirred overnight at room temperature under N_2 atmosphere. Evaporation of the solvent gave a yellow residue by filtration, which was washed with chloroform (2 mL) and water (2 mL) and was subjected to column chromatography (silica gel 5–10% MeOH in CH_2Cl_2, gradient elution) to produce conjugate **3** (4.7 mg, 3.2 μmol, 38%) as a yellow powder.

5.3.1. AcImIm-β-ImPy-γ-ImPyPy-indole-*seco*-CBI (3). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.68 (s, 1H; NH), 10.47 (s, 1Hm; OH), 10.39 (s, 2H; NH), 10.37 (s, 1H; NH), 10.28 (s, 1H; NH), 10.00 (s, 1H; NH), 9.93 (s, 1H; NH), 9.83 (s, 1H; NH), 9.43 (s, 1H; NH), 8.25 (m, 1H; NH), 8.11 (d, J = 8.0 Hz, 1H; CH), 8.09 (s, 1H; CH), 8.07 (m, 1H; NH), 7.97 (s, 1H; CH), 7.86 (d, J = 8.0 Hz, 1H; CH), 7.58 (m, 2H; CH), 7.56 (s, 1H; Im–H), 7.53 (s, 1H; Im–H), 7.47 (s, 2H; Im–H), 7.38 (m, 1H; CH), 7.30 (s, 1H; Py–H), 7.29 (s, 1H; CH), 7.21 (s, 1H; Py–H), 7.17 (s, 2H; Py–H), 7.14 (s, 1H; Py–H), 7.09 (m, 1H; CH), 6.97 (s, 1H; Py–H), 4.81 (m, 1H; CH_2), 4.56 (m, 1H; CH_2), 4.23 (m, 1H; CH), 4.00 (m, 2H; CH_2), 3.95 (s, 12H; NCH_3), 3.87 (s, 6H; NCH_3), 3.80 (s, 3H; NCH_3), 3.49 (m, 2H; CH_2), 3.20 (m, 2H; CH_2), 2.70 (m, 2H; CH_2), 2.34 (m, 2H; CH_2), 2.02 (s, 3H; $COCH_3$), 1.80 (m, 2H; CH_2); ESI-TOF-MS *m/e* calcd for $C_{69}H_{71}ClN_{23}O_{12}$ $[M+H]^+$ 1448.53; found 1448.74.

Similar synthetic and analytic protocols used for conjugates **2** and **4**–**8** were followed to prepare conjugate **3**. After further purification by HPLC using a Chemcobond 5-ODS-H column (0.1% AcOH/CH_3CN 0–50% linear gradient, 0–40 min, 254 nm), **2**–**8** were used in the DNA alkylation reaction.

Compound **2**: ESI-TOF-MS *m/e* calcd for $C_{66}H_{70}ClN_{22}O_{12}$ $[M+H]^+$ 1397.52; found 1397.72; compound **4**: ESI-TOF-MS *m/e* calcd for $C_{69}H_{71}ClN_{23}O_{12}$ $[M+H]^+$, 1448.53; found 1449.12; compound **5**: ESI-TOF-MS *m/e* calcd for $C_{72}H_{72}ClN_{24}O_{12}$ $[M+H]^+$ 1499.55; found 1499.78; compound **6**: ESI-TOF-MS *m/e* calcd for $C_{67}H_{71}ClN_{21}O_{12}$ $[M+H]^+$ 1396.53; found 1396.75; compound **7:** ESI-TOF-MS *m/e* calcd for $C_{70}H_{72}ClN_{22}O_{12}$ $[M+H]^+$ 1447.54; found 1447.76; compound **8:** ESI-TOF-MS *m/e* calcd for $C_{73}H_{73}ClN_{23}O_{12}$ $[M+H]^+$ 1498.55; found 1498.84.

5.4. Cloning of plasmid DNA

DNA fragments were annealed in a final volume of 50 μL containing 25 μM of fragment set (5′-TCAGCTCC ATCTGACTACCATCTGAGCACCATCTGACAAC CAGGA-3′ and 3′-AAGTCGAGGTAGACTGATGG TAGACTCGTGGTAGACTGTTGGTCC-5′). Products were identified by separation in TBE (10% native polyacrylamide gel with 0.5 μg/mL ethidium bromide using low weight DNA marker) and visualization under UV illumination. The annealed fragments were ligated into pGEM-T easy vectors (Sigma–Aldrich). *Escherichia coli* DH5R competent cells (Toyobo) were transformed and cultured on LB plates with 100 μg/mL ampicillin and 32 μg X-gal/400 μg IPTG overnight at 37 °C. White colonies were identified by colony direct PCR in 20 μL of the reaction mixtures containing 250 nM of primer set (T7: 5′- TAATACGACTCACTATAGGG-3′, sp6: 5′-GATTTAGGTGACACTATAG-3′), 200 μM of deoxynucleotide triphosphates (Sigma–Aldrich), 2 U Taq DNA polymerase, and 1× ThermoPol reaction buffer (New England Bio Labs). Amplification cycles were carried out. The reaction mix was incubated at 95 °C for 5 min followed by 30 incubation cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, with a final extension step of 72 °C for 7 min. The appropriate colony was selected for transfer to 5 mL of LB medium with 100 μg/mL ampicillin and cultured overnight at 37 °C. The plasmids with inserts were extracted using GenElutePlasmid miniprep kit (Sigma–Aldrich).

5.5. High-resolution gel electrophoresis

The 5′-Texas Red labeled 202-bp DNA fragment was prepared by PCR using plasmid with inserts described above and the following primers; 5′-Texas Red labeled sp6: 5′-G ATTTAGGTGACACTATAG-3′ and T7: 5′- TAATAC GACTCACTATAGGG-3′. Obtained 5′-Texas Red labeled DNA fragments were purified using PCR-Clean Up Kit (Sigma) and their concentration was determined by UV absorption.

A reaction mixture (10 μL) containing each of alkylating conjugates, a 5′-Texas Red labeled DNA fragment (6 nM, duplex concentration), and 5 mM sodium phosphate buffer (pH 7.0) was incubated at 23 °C for 15 h. Then, the rest of the alkylating conjugate was quenched by addition of 1 mM calf thymus DNA (1 μL) and the samples were heated at 95 °C for 10 min to cleave DNA strands at its specific alkylating sites. After the removal of solvents under reduced pressure, loading dye (6 μL) was added. The samples were heated at 95 °C for 20 min and immediately cooled on ice. Samples (2 μL) were subjected to electrophoresis on 6% denaturing polyacrylamide gel using Hitachi 5500-S DNA Sequencer.

Acknowledgments

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References and notes

1. Dervan, P. B.; Edelson, B. S. *Curr. Opin. Struct. Biol.* **2003**, *13*, 284–299.
2. Dervan, P. B. *Bioorg. Med. Chem.* **2001**, *9*, 2215–2235.
3. Turner, J. M.; Swalley, S. E.; Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1998**, *120*, 6219–6226.
4. Trauger, J. W.; Baird, E. E.; Mrksich, M.; Dervan, P. B. *J. Am. Chem. Soc.* **1996**, *118*, 6160–6166.
5. Denison, C.; Kodadek, T. *Chem. Biol.* **1998**, *5*, R129–R145.
6. Bando, T.; Sugiyama, H. *Acc. Chem. Res.* **2006**, *39*, 935–944.
7. Boger, D. L.; Ishizaki, T.; Kitos, P. A.; Suntornwat, O. *J. Org. Chem.* **1990**, *55*, 5823–5832.
8. Boger, D. L.; Yun, W.; Teegarden, B. R. *J. Org. Chem.* **1992**, *57*, 2873–2876.
9. Boger, D. L.; McKie, J. A. *J. Org. Chem.* **1995**, *60*, 1271–1275.
10. Bando, T.; Sasaki, S.; Minoshima, M.; Dohno, C.; Shinohara, K.; Narita, A.; Sugiyama, H. *Bioconjug. Chem.* **2006**, *17*, 715–720.
11. Bando, T.; Narita, A.; Sasaki, S.; Sugiyama, H. *J. Am. Chem. Soc.* **2005**, *127*, 13890–13895.
12. Wurtz, N. R.; Turner, J. M.; Baird, E. E.; Dervan, P. B. *Org. Lett.* **2001**, *3*, 1201–1203.
13. Belitsky, J. M.; Nguyen, D. H.; Wurtz, N. R.; Dervan, P. B. *Bioorg. Med. Chem.* **2002**, *10*, 2767–2774.
14. Minoshima, M.; Bando, T.; Sasaki, S.; Shinohara, K.; Shimizu, T.; Fujimoto, J.; Sugiyama, H. *J. Am. Chem. Soc.* **2007**, *129*, 5384–5390.
15. Shinohara, K.; Sasaki, S.; Minoshima, M.; Bando, T.; Sugiyama, H. *Nucleic Acids Res.* **2006**, *34*, 1189–1195.
16. Sasaki, S.; Bando, T.; Minoshima, M.; Shimizu, T.; Shinohara, K.; Takaoka, T.; Sugiyama, H. *J. Am. Chem. Soc.* **2006**, *128*, 12162–12168.
17. Bando, T.; Narita, A.; Saito, I.; Sugiyama, H. *Chem. -Eur. J.* **2002**, *8*, 4781–4790.
18. Bando, T.; Narita, A.; Asada, K.; Ayame, H.; Sugiyama, H. *J. Am. Chem. Soc.* **2004**, *126*, 8948–8955.
19. Boger, D. L.; Johnson, D. S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1438–1474.
20. Boger, D. L.; Munk, S. A. *J. Am. Chem. Soc.* **1992**, *114*, 5487–5496.