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Specific Alkylation of Human Telomere Repeat Sequences by a Tandem-Hairpin Motif of Pyrrole−Imidazole Polyamides with Indole-*seco*-CBI

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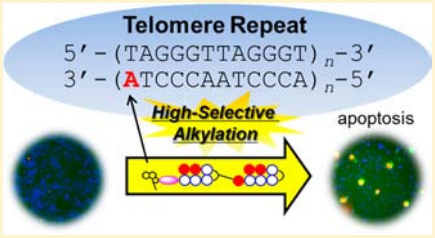
Supporting Information

ABSTRACT: We designed and synthesized a tandem-hairpin motif of pyrrole (P)—imidazole (I) polyamide 1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz-[e]indole (*seco*-CBI) conjugates (**1**) that targets the human telomere repeat sequence 5′-d(CCCTAA)$_n$-3′. As a control, conjugate **2** (hairpin PI polyamide with *seco*-CBI), which also targets the human telomere repeat sequence, was synthesized. High-resolution denaturing polyacrylamide gel electrophoresis (PAGE) using 5′ Texas Red-labeled 219-bp DNA fragments revealed the outstandingly high sequence selectivity of **1**, with no mismatch alkylation. Furthermore, an evaluation performed in human cancer cell lines demonstrated that conjugate **1** has low cytotoxicity compared with conjugate **2**. In addition, a cell-staining analysis indicated that conjugate **1** induced apoptosis moderately by DNA damage. This study demonstrated that conjugate **1** can be used as an effective alkylator for telomere repeat sequences or as an apoptotic inducer.



INTRODUCTION

Telomeres are protein−DNA structures that are located at each end of chromatids and play vital roles in several life processes, such as genome protection, cancer, and aging.[1−3] In the human genome, telomeres are composed of double-stranded d-(TTAGGG)/d(CCCTAA) repeats and single-stranded d-(TTAGGG) regions running from the 5′ to the 3′ end. The structures of telomeres cause the end-replication problem; the last primer that initiates lagging-strand synthesis leaves a gap that cannot be filled. Because of this problem, the lengths of telomeres decrease by 50−150 bp with each cell division, which relates to the aging process.[4,5] In most human cancer cells, telomere repeats are elongated by highly expressed telomerase, leading to unlimited proliferation.[6−8] Therefore, the development of agents that target this process has been expected as anticancer therapy and studied keenly.[9−11] Alkyl agents with *N*-methylpyrrole (P)-*N*-methylimidazole (I) polyamides are one such example, as they have the potential to target telomere sequences selectively.

PI polyamides are minor-groove binders that bind selectively to predetermined DNA sequences in accordance with the unique binding rule; the 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.[12−19] Our group has developed PI polyamides as alkylating agents that selectively target several DNA sequences, including human telomere sequences.[20−34] An alkyl agent with a hairpin PI polyamide targeting human telomere sequences was first reported by our group in 2003 (Figure 1a).[31] After some optimizations of the linker and alkylation agent, conjugates with strong alkylation ability for human telomere sequences were developed (Figure 1b).[32] However, because of its short base recognition (6−7 bp), the conjugates exhibited some inaccuracy, with mismatch alkylations. Some experiments were also performed to obtain PI polyamide conjugates with a long base recognition: we reported the recognition of a 10 bp sequence using the strategy of heterodimer formation.[20] Using this approach, linear-type conjugates were developed that exhibited higher specificity for a human telomere sequence with 12 bp recognition (Figure 1c).[33] However, in this concerted binding system, each monomer can interact with several sequences via another binding mode (hairpin formation[20]), which may cause unexpected effects. The generation of long-sequence recognition by single molecular, tandem-type PI polyamides has also been reported.[29,30,35−37] Among those studies, tandem-hairpin PI polyamide probes were developed to target the human telomere, and it was revealed that the probes localized selectively at the human telomeric region (Figure 1d).[38,39]

Here, we used this tandem-hairpin PI polyamide motif that targets human telomere repeat sequences (5′-d(AACCCT)$_n$-

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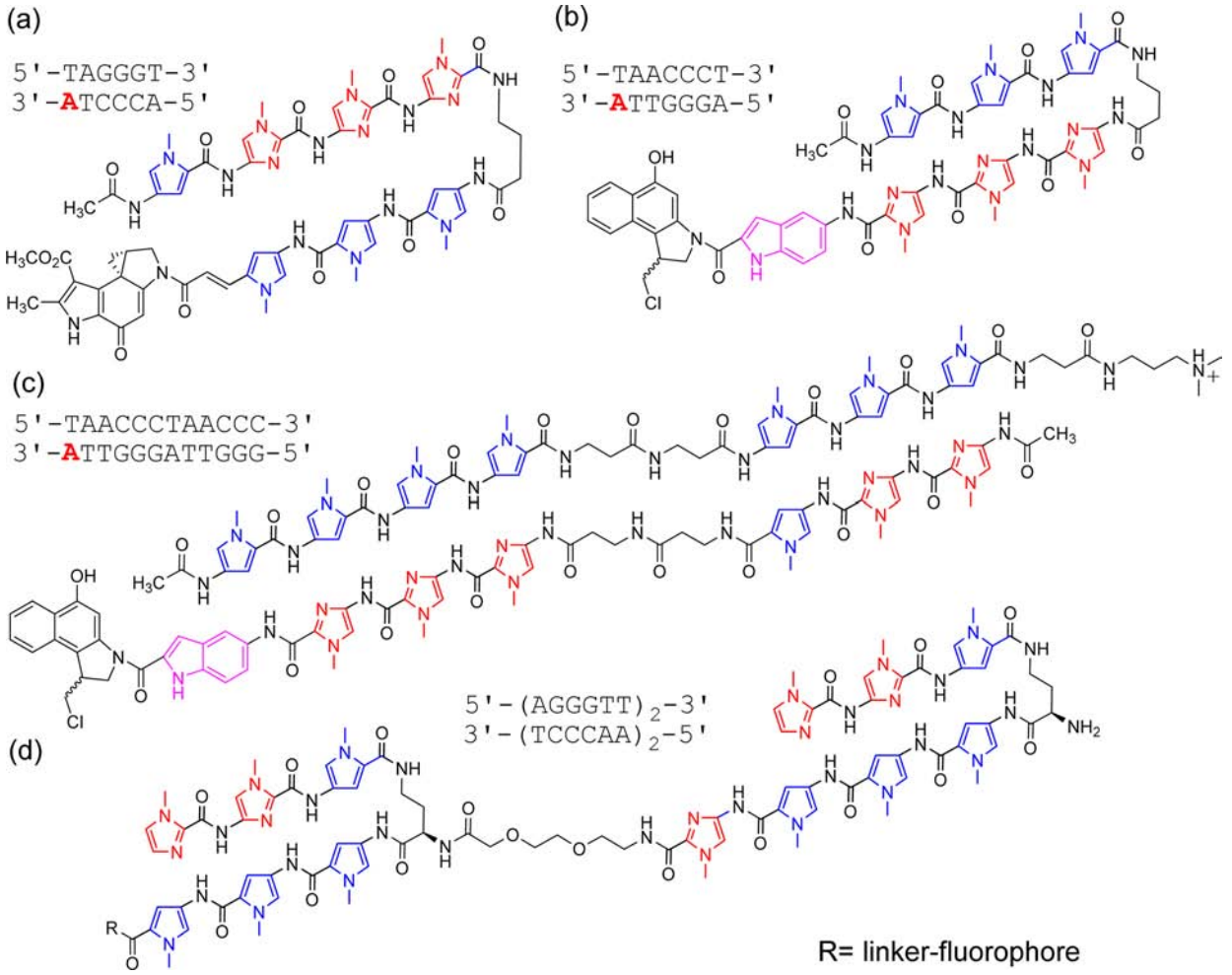

Figure 1. Studies of PI polyamides targeting a human telomere sequence. (a,b) Hairpin and (c) heterodimer-type alkylators and (d) tandem-hairpin-type probes have been reported. The DNA sequences described here indicate the targeting sequence and red-colored bases indicate alkylation sites.

3′) as a DNA alkylation agent to realize the high telomere-selective alkylator. As described above, telomeres in cancer cells are elongated by telomerase, which leads to infinite cell divisions. Therefore, it is expected that alkylation of telomere sequences would inhibit this process and induce cell death, especially programmed cell death. Although some studies of telomere-targeting PI polyamides reported high cytotoxicity in cancer cell lines, the process of cell death has not been discussed. Moreover, if this inhibition works, the alkylator of telomeric DNA can be applied to various cancer cell lines. As an alkylation compound, 1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[e]indole (*seco*-CBI)[40−44] was conjugated with the PI polyamide and its effects were analyzed. Compared with a conventional conjugate, it was found that the tandem-hairpin PI polyamide with *seco*-CBI yielded highly selective alkylation without mismatch DNA alkylation. In addition, using cancer cell lines, it was revealed that the tandem-hairpin PI polyamide conjugate had low cytotoxicity and induced apoptotic cell death moderately.

■ MATERIALS AND METHODS

General. HCTU was purchased from Peptide International. Fmoc-I-COOH, Fmoc-P-COOH, DMF, 1-methyl-2-pyrrolidone (NMP), and piperidine were purchased from Wako, and Fmoc-Py-oxime resin (200−400 mesh), Fmoc-*β*-Ala-OH, Fmoc-*γ*-Abu-COOH, PyBOP were from Novabiochem. Diisopropylethylamine (DIEA) was from Nacalai Tesque, Inc. All other reagents and solvents were purchased from standard suppliers and without further purification. High-performance liquid chromatography (HPLC) purification was performed with a JASCO PU-2089 Plus HPLC pump, a JASCO UV2075 HPLC UV/vis detector, and a COSMOSIL $5C_{18}$-MS-II reversed phase column (10 × 150 mm, Nacalai) in 0.1% TFA in water with CH_3CN as eluent at a flow rate of 3.0 mL/min, and a linear gradient elution of 20−60% CH_3CN over 40 min with detection at 254 nm. The analytical HPLC was performed with a COSMOSIL $5C_{18}$-MS-II reversed phase column (4.6 × 150 mm, Nacalai) in 0.1% TFA in water with CH_3CN as eluent at a flow rate of 1.0 mL/min, and a linear gradient elution of 0−100% CH_3CN over 20 min with detection at 254 nm. Collected fractions were analyzed by ESI-TOF-MS (Bruker). UV spectra were measured on a Nanodrop ND-1000 spectrophotometer.

Fmoc Solid-Phase Syntheses of PI Polyamides. Solid phase syntheses were performed on a PSSM-8 (Shimadzu) with a computer-assisted operation system at a 0.02 mmol scale (45 mg of Fmoc-Py-oxime resin, 0.38 mmol/g) by using Fmoc chemistry. 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)

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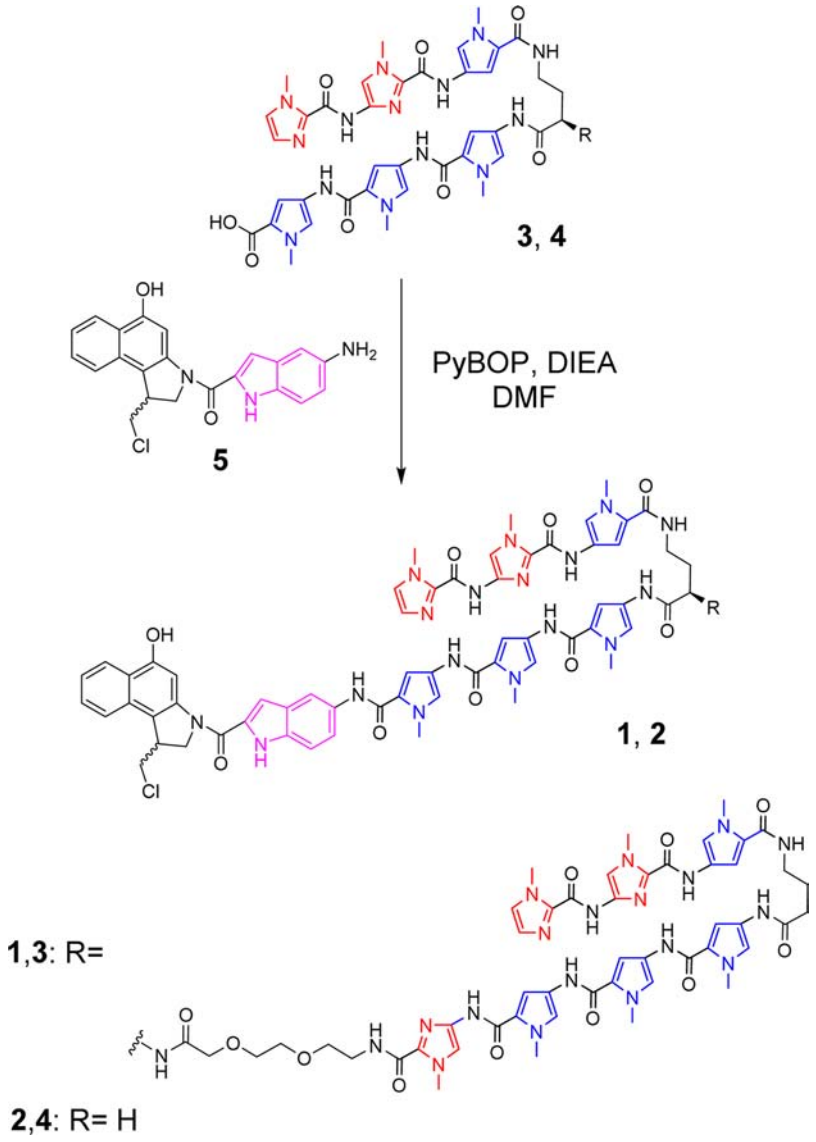

Figure 2. Synthetic scheme for the preparation of *seco*-CBI conjugate **1** and **2** with an indole linker.

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 single-couple cycle. Fmoc-PI-CO_2H, IIP-CO_2H, and Fmoc-$_D$-Dab(IIP)-OH as units were employed for the coupling difficulty.[39] All lines were purged with solution transfers and bubbled by N_2 gas for stirring 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.

Synthesis of IIP-γ-PPPI-PEG-D-Dab(IIP)-PPP-CO_2H (3). PI polyamide was synthesized according to the previously reported solid-phase methods.[39] IIP-γ-PPPI-PEG-D-Dab(IIP)-PPP-CO_2-oxime-resin was cleaved from oxime-resin to yield primary carboxylic acid with alkali condition (1 N NaOH in 1,4-dioxane, 3 h, 55 °C). The solution was evaporated and white crude was obtained. 1.3 mg of this crude was purified by HPLC to produce **3** as a white powder (0.3 mg, 0.2 μmol). Compound **3** was used for synthesis of conjugate **1** without further purification (Figure 2). Analytical HPLC: t_R = 10.2 min. ESI-TOF-Mass: *m*/*z* calcd. for $C_{87}H_{99}N_{33}O_{19}$: $[M+2H]^{2+}$ 955.8976; found: 955.8934.

Synthesis of IIP-γ-PPP-CO_2H (4). IIP-γ-PPP-CO_2H was synthesized in a stepwise reaction by Fmoc solid-phase methods on the Py-coupled oxime resin. A peptide was cleaved from oxime-resin to yield primary carboxylic acid with alkali condition (1 N NaOH in 1,4-dioxane, 3 h, 55 °C), and purified by HPLC to produce **4** as a white powder (4.1 mg, 5.0 μmol). Compound **4** was used for synthesis of conjugate **2** without further purification (Figure 2). Analytical HPLC: t_R = 9.4 min. ^{1}H NMR (600 MHz, [D_6]DMSO) δ 10.30 (s, 1H; NH), 9.90 (s, 1H; NH), 9.89 (s, 1H; NH), 9.86 (s, 1H; NH), 9.84 (s, 1H; NH), 8.08−8.07 (t, *J* = 5.1 Hz, 1H; NH), 7.57 (s, 1H; CH), 7.46 (s, 1H; CH), 7.42 (s, 1H; CH), 7.23 (s, 1H; CH), 7.22 (s, 1H; CH), 7.17 (s, 1H; CH), 7.10 (s, 1H; CH), 7.04 (s, 1H; CH), 7.00 (s, 1H; CH), 6.87 (s, 1H; CH), 6.84 (s, 1H; CH), 4.00 (m, 6H; CH_3), 3.84 (s, 3H; CH_3), 3.83 (s, 3H; CH_3), 3.82 (s, 3H; CH_3), 3.81 (s, 3H; CH_3), 3.23−3.20 (q, *J* = 6.4 Hz, 2H; CH_2), 2.29−2.27 (t, *J* = 7.2 Hz, 2H; CH_2), 1.80−1.78 (quin, *J* = 7.2 Hz, 2H; CH_2). ESI-TOF-Mass: *m*/*z* calcd. for $C_{38}H_{42}N_{14}O_8$: $[M+H]^+$ 823.3388; found: 823.3389.

Synthesis of H_2N-indole-*seco*-CBI (5). Compound **5** was prepared as previously reported.[20]

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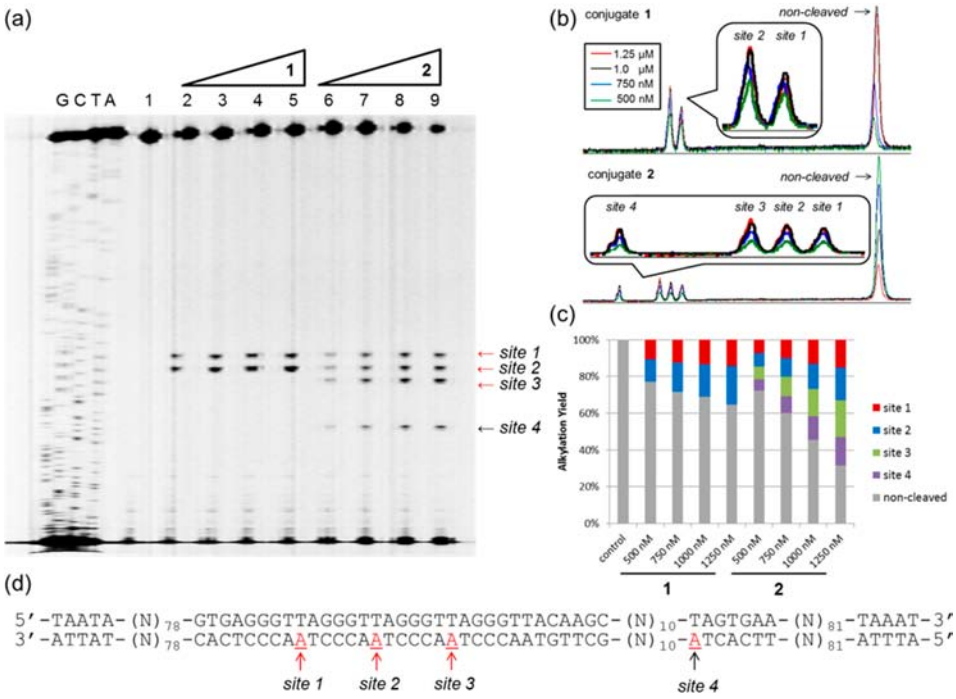

Figure 3. (a) Thermally induced strand cleavage of a 5′ Texas Red-labeled 219 bp DNA fragment (27 nM) incubated for 18 h at 23 °C; Lane 1, DNA control; Lanes 2−5, 500, 750, 1000, 1250 nM of conjugate **1**; Lanes 6−9, 500, 750, 1000, 1250 nM of conjugate **2**. (b) Densitometric analysis of the high-resolution gel electrophoresis. (c) The alkylation yield was estimated from the averaged yield of DNA alkylation at site 1, 2, 3, and 4 by conjugates **1** and **2** at 500 nM, 750 nM, 1000 nM, and 1250 nM. Yields were based on the amount of starting substrate DNA. Detailed values are shown in Table S1. (d) Telomere sequences containing alkylation sites 1−4. Arrows indicate sites of DNA alkylation.

Synthesis of IIP-γ-PPPI-PEG-D-Dab(IIP)-PPP-indole-*seco*-CBI (1) and IIP-γ-PPP-indole-*seco*-CBI (2). Polyamide-indole-*seco*-CBI conjugates were synthesized as previously described.[20,23−30] Conjugates were purified by HPLC. Electrospray-ionization time-of-flight mass spectrometry was performed to confirm the polyamide-*seco*-CBI conjugates.

1: Analytical HPLC: t_R = 13.2 min. ESI-TOF-Mass: *m*/*z* calcd. for $C_{109}H_{115}ClN_{36}O_{20}$: $[M+2H]^{2+}$1142.4467; found: 1142.4447.

2: Analytical HPLC: t_R = 13.9 min. ESI-TOF-Mass: *m*/*z* calcd. for $C_{60}H_{58}ClN_{17}O_9$: $[M+H]^+$ 1196.4370; found: 1196.4181.

Preparation of 5′ Texas Red-Modified DNA Fragments Containing Four Repeats of the Human Telomere Sequence. All DNA fragments and primers for cloning or DNA amplification were purchased from Proligo. The DNA fragments were annealed in a final volume of 100 μL containing 10 μM of fragment set (5′-GCAGAGTGAGGGTTAGGGTTAGGGTTAGGGTTACAAGCCCTCA-3′, 3′-ACGTCTCACTCCCAATCCCAATCCCAATCCCAATGTTCGGGAG-5′). The annealed fragments were ligated into pGEM-T easy vectors (Promega). *Escherichia coli* DH5R competent cells (Toyobo) were transformed and cultured on LB plates with 100 μg/mL ampicillin, 25 μL of X-gal at 20 mg/mL, and 25 μL of IPTG at 100 mM overnight at 37 °C. White colonies were identified by colony direct PCR in 10 μL of the reaction mixtures containing 500 nM of primer set (T7: 5′-TAATACGACTCACTATAGG-3′, sp6: 5′-CATACGATTTAGGTGACACTATAG-3′) and GoTaq Master Mix (Promega). Amplification cycles were carried out with an iCycler (BIO-RAD). The reaction mix was incubated at 95 °C for 3 min, then followed by 40 incubation cycles of 95 °C for 30 s, 60 °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 GenElute Plasmid Miniprep Kit (Sigma Aldrich) and identified by PCR (program and reaction mixtures same as above). The fragment was purified by GenElute PCR cleanup kit (Sigma-Aldrich). The sequence of this PCR product (219 bp, Figure 3d) is 5′ Texas Red-ATTTAGGTGACACTATAGAATACTCAAGCTATGCATCCAACGCGTTGGGAGCTCTCCCATATGGTCGACCTGCAGGCGGCCGCGAATTCACTAGTGATTGAGGGCTTGTAACCCTAACCCTAACCCTAACCCTCACTCTGCAATCGAATTCCCGCGGCCGCCATGGCGGCCGGGAGCATGCGACGTCGGGCCCAATTCGCCCTATAGTGAGTCGTATTA-3′ (219 bp).

High-Resolution Gel Electrophoresis. The 5′ Texas Red-labeled DNA fragments (final concentration: 27 nM) were alkylated by alkylating polyamide conjugate **1** and **2** (final concentration: 500, 750, 1000, 1250 nM) in 10 μL of 3.0 mM sodium phosphate buffer (pH 7.0) containing 10% DMF at room temperature for 18 h. The reaction was quenched by adding calf thymus DNA (10 mg/mL, 1 μL) and heated at 95 °C for 5 min. The DNA was recovered by centrifugal concentrator (TAITEC). The pellet was dissolved in 5 μL of loading dye (contents: 10 mL of formamide, 200 μL of water, 300 μL of 0.5 M aqueous solution of disodium dihydrogen ethylenediaminetetraacetate dihydrate, and 2.5 mg of New fuchsin) and heated at 95 °C for 25 min, and 1.2 μL aliquot was loaded on a 6% denaturing polyacrylamide gel containing 6.0 M of urea and electrophoresed using SQ-5500-S (HITACHI). For preparation of 500 mL of this gel, 183 g of urea and 60 mL of 50% Long Ranger gel solution (Lonza Rockland, Inc.) were added to ca. 200 mL of water and stirred for 30 min with 6 g of

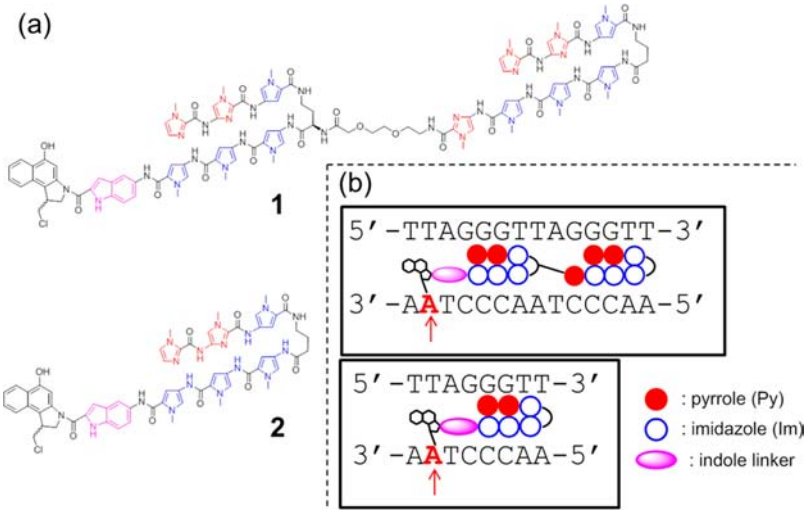

Figure 4. (a) Chemical structures of PI conjugate **1** and **2**. (b) Schematic representation of sequence-specific alkylation in the human telomere repeat sequence by conjugate **1** and **2**. Arrows indicate sites of adenine N3 alkylation.

anion and cation exchange resin (AG 501-X8 Resin, Bio-Rad Laboratories, Inc.). After filtration, the resin was rinsed with 50 mL of 10× TBE, and water was added to the filtrate to a 500 mL volume. Electrophoresis was conducted under 1.5 kV, ca. 25 mA, and 40 °C (Figure 3).

Cell Culture. The human cancer cell lines were cultured in Dulbecco's modified Eagle's medium (Sigma) containing 10% heat-inactivated fetal bovine serum (JRH Biosciences). The cells were maintained at 37 °C in humidified atmosphere of 95% air and 5% CO_2.

Cytotoxicity Assay. Colorimetric assays using Cell Count Reagent SF (Nacalai) were performed on 96-well plates. A 50 μL amount of each cell suspension was added at a density of 1 × 10^4 cells/well. PI polyamide conjugates **1** and **2** were dissolved in DMSO, and 50 μL of each solution in the medium (final DMSO concentration was 0.1%) was added. After treatment for 48 h, 10 μL of WST-8 reagent was added to each well and incubated at 37 °C. Absorbance was then measured at 450 and 600 nm using an MPR-A4i microplate reader (Tosoh).

Cell Staining. Cell staining using Apoptotic/Necrotic/Healthy Cells Detection Kit (PromoKine)[45] was performed on 8-well chambers. A 200 μL amount of each cell suspension was added at a density of 1 × 10^4 cells/well. PI polyamide conjugate **1** and **2** were dissolved in DMSO, and 100 μL of each solution in the medium (final DMSO concentration was 0.1%) was added. After treatment for 48 h, 100 μL of staining solution (mixture of FITC-Annexin V, Ethidium Homodimer III, and Hoechst 33342 in Binding Buffer) was replaced to each well and incubated at r.t. for 15 min. Cell images were recorded with BIOREVO BZ-9000 (Keyence).

■ RESULTS AND DISCUSSION

Preparation of the PI Polyamide Conjugates. Two PI polyamides conjugated with the alkylating agent *seco*-CBI at the C-terminal end were designed to target human telomere sequence d(CCCTAA)$_n$ (Figure 4a). The molecular design of conjugate **1** was based on a former study that reported that the unpaired imidazole in conjugate **1** recognizes both G-C and C-G base pairs.[38,46] Therefore, conjugate **1** recognizes 5′-WWCCSWWWCCNNA-3′ (W = A or T; S = G or C; and N = A, T, C, or G) and conjugate **2** recognizes 5′-WWCCNNA-3′ (Figure 4b). The PI polyamide conjugate **1** and **2** were synthesized according to procedures reported previously (Figure 2).[31−34,39] Tandem-hairpin PI polyamide **3**, which contained terminal carboxylic acid groups, was prepared by Fmoc solid-phase synthesis using a Py-coupled oxime resin.[47] Subsequently, the amino-indole-*seco*-CBI dimer (**5**) was coupled with carboxylic acid (**3**) in dimethylformamide (DMF) using benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and ^{i}Pr$_2$NEt to produce the desired polyamide-indole-*seco*-CBI conjugate **1**. Using the method described above, the hairpin PI polyamide **4** and desired conjugate **2** were also synthesized as control experiments. The structures of conjugate **1** and **2** were confirmed by electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS) after purification using reverse-phase HPLC.

DNA Alkylating Activities of Conjugates 1 and 2. Sequence-specific DNA alkylation by conjugate **1** and **2** was examined using DNA fragments (219 bp) labeled with Texas Red (27 nM). Fragments containing four 5′-d(AACCCT)$_4$-3′ human telomere repeats were prepared by transformation into pGEM-T easy vectors followed by PCR amplification.[32] Sequence specificities were analyzed by high-resolution denaturing polyacrylamide gel electrophoresis (PAGE) using an automated DNA sequencer, as described previously.[20−30] Alkylation was carried out at 23 °C for 18 h, followed by quenching with calf thymus DNA. The samples were heated at 95 °C under neutral buffer conditions for 5 min. The sites of N3 alkylation were visualized by thermal cleavage of the DNA strand at the alkylated sites.[48−51] Under these heating conditions, all alkylation sites were cleaved quantitatively; the products were observed as bands after electrophoresis. Figure 3a shows the results of PAGE using conjugates **1**, **2**, and 219 bp PCR products. From the design of the conjugates, it was predicted that conjugate **1** would alkylate two adenine sites in the four repeated telomere sequences (sites 1 and 2, Figure 3d) and that conjugate **2** would alkylate three adenine sites (sites 1−3). As a result, DNA alkylation by conjugate **1** occurred at only two sites, which were located in the targeted telomere sequence 5′-AACCCTAACCCTAACCCTA-3′ (sites 1 and 2). In the case of conjugate **2**, however, mismatch alkylation was observed at 5′-TTCACTA-3′ (site 4; 1 bp mismatch) in addition to three match alkylation sites (sites 1−3). A previous study also reported a tandem-hairpin PI polyamide with *seco*-

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CBI targeting telomere sequence that showed some mismatch alkylations of DNA srands.[29,30] This report supports that conjugate **1** is a remarkably specific DNA alkylator of its targeting site.

The densitometric analysis of the PAGE was also performed (Figure 3b,c). The alkylation yield of each band increased in a dose-dependent way, and the total alkylation yield of conjugate **2** was higher than that of conjugate **1**. However, focusing on their match alkylation sites (sites 1−3) at the lower concentration of the conjugates (500 and 750 nM), the total alkylation yield was almost identical (Table S1). Above all, it is most notable that no mismatch alkylation site was detected about conjugate **1**. The results described above indicate that conjugate **1** alkylates its targeted site precisely with sufficient alkylating efficiency, and may be a telomere alkylating agent.

Effects of Conjugates 1 and 2 in Living Cells. To evaluate the effects of conjugate **1** and **2** in living cells, conjugate-associated cytotoxicity was investigated using MDA-MB-231 breast cancer cells, DU145 prostatic cancer cells, and NCI-H460 lung cancer cells. Conjugates **1** and **2** were administered to the three cancer cell lines for 48 h. Cytotoxicity was determined as a function of concentration of the conjugates via a standard WST cell viability assay (Figure 5). The results of this assay revealed that the cytotoxicity of conjugate **1** was lower than that of conjugate **2** in every case. The sensitivity of conjugate **2** was the highest in NCI-H460, followed by DU145 and MDA-MB-231 cells, in this order, which is consistent with the former report.[32] The morphologies of the cells were observed directly using the same concentration (2 μM, 0.1% DMSO), to supplement the cytotoxic difference. The results of this experiment revealed that the morphology of cells changed dramatically under treatment with conjugate **2** compared with conjugate **1** (Figures 6 and S1). This difference may be derived from the nonspecific alkylation of conjugate **2** and cell permeability.

To confirm that conjugate **1** actually affects living cells, cell death was also investigated by fluorescent staining. Conjugates **1** and **2** were administrated to the three cancer cell lines for 48 h. Subsequently, apoptotic, necrotic, and healthy cells treated with conjugate **1** or **2** were stained with three different fluorophores (FITC-Annexin V, Ethidium Homodimer III, and Hoechst 33342),[45] as reported in Figure 7 (MDA-MB-231) and S2 (DU145 and NCI-H460). In this observation, different concentrations of conjugates **1** and **2** were applied based on their cytotoxicity (Figure 5), to observe the process of cell death. In the control experiment (0.1% DMSO), most cells were healthy (blue staining), as only a few necrotic cells (red staining) were detected, suggesting that the main cell death pathway was necrosis. Conversely, the cells treated with conjugate **1** or **2** showed more abundant cell death, which is consistent with the results of the cytotoxicity assay described above. In particular, apoptotic cells (green or both green and red staining) were detected in cells treated with the conjugates. As the telomere region of DNA has important roles in DNA replication, these results suggest that it is likely that the conjugates alkylate the telomere sequence and induce programmed cell death.

CONCLUSION

We designed and synthesized a tandem-hairpin-type PI polyamide conjugate (**1**) and a hairpin-type conjugate (**2**), both of which targeted human telomere repeats. High-resolution sequencing gel electrophoresis showed that con-

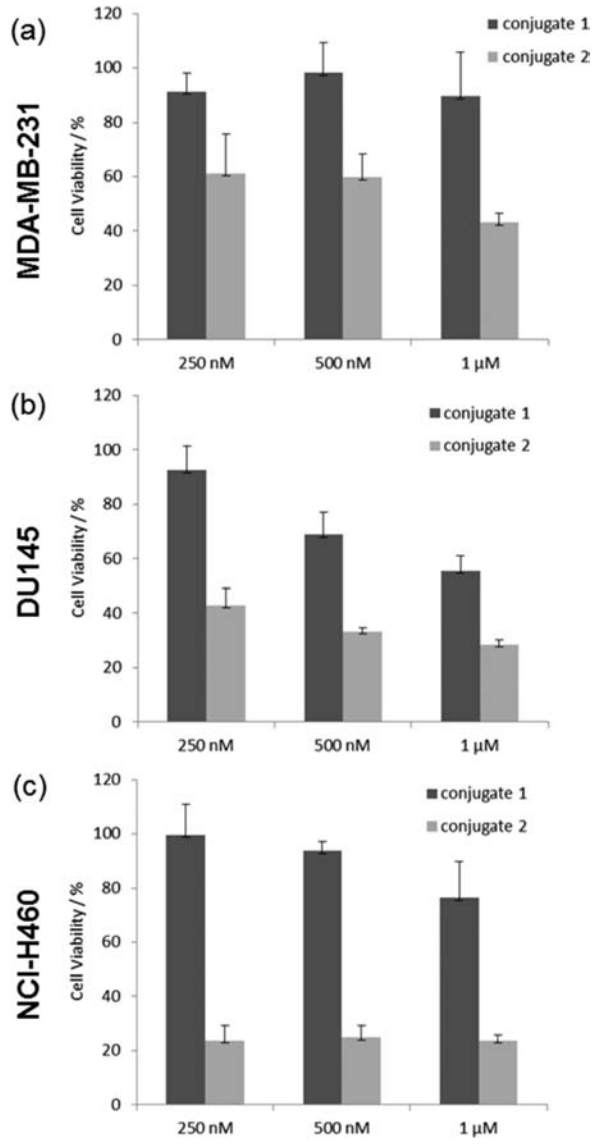

Figure 5. Cell viabilities of conjugate **1** and **2** with (a) MDA-MB-231, (b) DU145, and (c) NCI-H460. Cells were treated with the conjugates for 48 h. Black and gray columns indicate conjugates **1** and **2**, respectively.

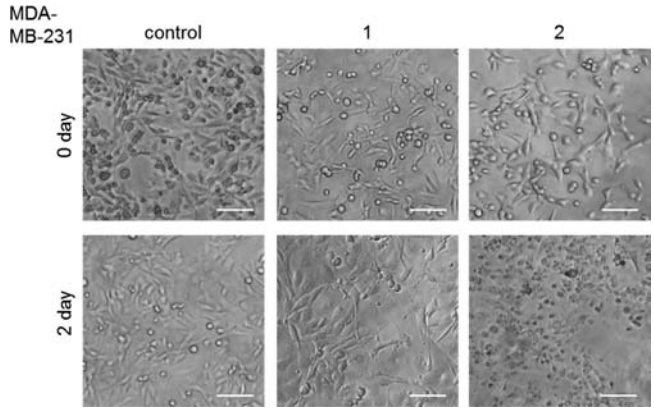

Figure 6. Morphologies of MDA-MB-231 before and after the conjugate treatment (2 μM, 0.1% DMSO). White bars in the images indicate 100 μm.

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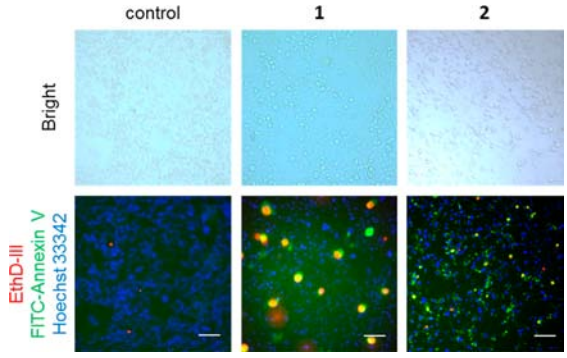

Figure 7. Images of MDA-MB-231 in fluorescence microscope. Cells were imaged after 2 days treatment with only 0.1% DMSO (control), conjugate **1** (**1**; 2 μM, 0.1% DMSO), and conjugate **2** (**2**; 150 nM, 0.1% DMSO). Healthy cells are stained blue, necrotic cells are stained red, and green or double colors red and green (yellow) are dead cells processing from apoptotic cell population. White bars in the images indicate 100 μm.

jugate **1** selectively recognized and alkylated the target telomere repeat sequence 5′-AACCCTAACCCTA-3′ at nanomolar concentrations, whereas conjugate **2** alkylated some mismatch sequences. A cytotoxicity assay performed using three human cancer cell lines demonstrated that conjugate **1** has lower cytotoxicity than conjugate **2**. Cell staining using three fluorophores also showed that conjugates **1** and **2** induced apoptotic cell death. These results demonstrate that conjugate **1** is a potential high-sequence-specific alkylator and can be used as a biotool for DNA cleavage at the telomere site with low cytotoxicity or as a moderate inducer of apoptosis in cells.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Original data of the densitometric analysis in PAGE and cell images (DU145, NCI-H460). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

seco-CBI, 1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[e]-indole; PI, pyrrole-imidazole; ESI, electrospray ionization; HPLC, high performance liquid chromatography; DMF, dimethylformamide; NMP, 1-methyl-2-pyrrolidone; DIEA, diisopropylethylamine; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; HCTU, 1*H*-benzotriazolium 1-[bis(dimethylamino)methylene]-5-chlorohexafluorophosphate (1-),3-oxide; DMSO, dimethyl sulfoxide; Fmoc, 9-fluorenylmethyloxycarbonyl; PEG, polyethylene glycol

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