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Sequence-Specific Alkylation of Double-Strand Human Telomere Repeat Sequence by Pyrrole-Imidazole Polyamides with Indole Linkers

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Abstract: We designed and synthesized pyrrole (Py)−imidazole (Im) hairpin polyamide 1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI) conjugates **1** and **2**, which target both strands of the double-stranded region of the human telomere repeat sequences, 5′-d(TTAGGG)$_n$-3′/5′-d(CCCTAA)$_n$-3′. High-resolution denaturing polyacrylamide gel electrophoresis demonstrated that conjugates **1** and **2** alkylated DNA at the 3′ A of 5′-ACCCT*A*-3′ and 5′-AGGGTT*A*-3′, respectively. Cytotoxicities of conjugates **1** and **2** were evaluated using 39 human cancer cell lines; averages of log IC_{50} values for conjugates **1** and **2** were −6.96 (110 nM) and −7.24 (57.5 nM), respectively. Conjugates **1** and **2** have potential as antitumor drugs capable of targeting telomere repeat sequence.

Introduction

Telomeres, which protect chromosomes from end-to-end fusion and nuclease degradation, are protein-DNA structures at the ends of eukaryotic chromosomes. These specialized structures are essential for maintaining genomic integrity. In the human genome, telomeres consist of about 4−14 kbp of double-stranded d(TTAGGG)/d(CCCTAA) repeats and 150−200 bases of single-stranded d(TTAGGG) overhangs running 5′ to 3′ toward the end of the chromosome.[1] Because of the end replication problem, telomeres in human cells decrease by about 100 bp with each cell division. Telomerase activity is present in about 90−95% of human immortal cell lines and in up to 85% of cancers that prevent telomere shortening. Because telomerase is essential for the proliferation of cancer cells, agents that target telomeres and telomerase have attracted the interest of scientists engaged in the development of anticancer drugs. To date, several agents that stabilize the G-quadruplex at the single-strand region of telomeres and inhibit telomere activity have attracted interest as potential antitumor agents.[2] Although the double-stranded telomere region is 20−50 times longer than the single-stranded region, agents that target the double-stranded telomere region have not been well investigated.

Minor groove-binding *N*-methylpyrrole (Py) and *N*-methylimidazole (Im) polyamides uniquely recognize each of the four Watson−Crick base pairs.[3] Antiparallel pairing of imidazole opposite pyrrole (Im/Py) recognizes a G−C base pair, whereas a Py/Py pair recognizes A−T or T−A base pairs. We have developed various types of sequence-specific alkylating agents by conjugation of Py−Im polyamides and alkylating moieties, including a Py−Im polyamide−cyclopropapyrroloindole (CPI) conjugate with a vinyl linker, which targets the human telomere repeat sequence.[4] Sequence-specific DNA alkylation at the target sequence of this compound was achieved, but synthetic yields and DNA alkylating efficiencies were low because of instability of CPI and the vinyl linker moiety. To overcome this, we introduced 1,2,9,9a-tetrahydrocyclopropa[1,2-*c*]benz[1,2-*e*]indol-4-one (CBI)[5] or its precursor, *seco*-CBI, as an alkylating moiety and 2-carbonyl-5-aminoindole as a linker.[6] Introduction of an indole linker greatly facilitated the synthesis of sequence-specific alkylating Py−Im polyamides. These conjugates alkylate matching sequences of DNA fragments selectively and efficiently. The reactivities of *seco*-CBI derivatives were equal to those of the corresponding CBI conjugates. Here, we report the synthesis, DNA alkylating activity, and antitumor activity of conjugates **1** and **2**, which contain indole linkers and target C-rich and G-rich strands in double-strand telomere repeat sequences (Figure 1).

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10.1021/ja0626584 CCC: $33.50

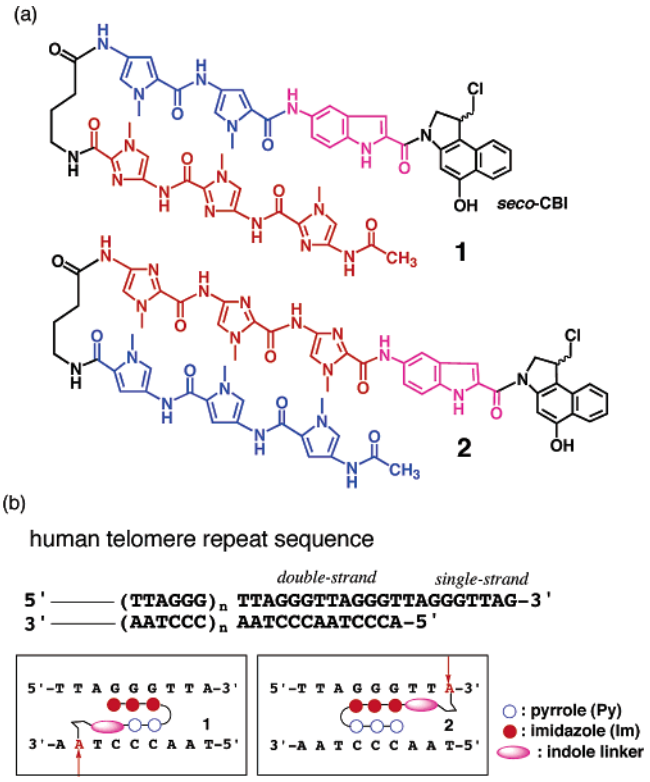

Figure 1. (a) Chemical structures of Py−Im conjugates **1** and **2**. (b) Schematic representation of sequence-specific alkylation in the human telomere repeat sequence by conjugates **1** and **2**. Arrows indicate sites of adenine N3 alkylation.

Results and Discussion

Synthesis of Conjugates 1 and 2. The synthesis of conjugate **1** is shown in Figure 2. Five-ring Py−Im polyamide **3**, which has terminal carboxylic acid groups, was prepared by Fmoc solid-phase synthesis using a Py-coupled oxime resin.[7] Indole linker **4** was synthesized by hydrolysis of commercially available ethyl 5-nitro-1*H*-indole-2-carboxylate followed by reduction using palladium−carbon and hydrogen gas. Carboxylic acid **3** was converted to an activating ester using HATU, $^{i}Pr_2NEt$, followed by coupling with **4** to produce **5**. The DNA-alkylating moiety, *seco*-CBI **6**, was prepared according to previously reported procedures. Conjugate **1** was synthesized by coupling **5** to *seco*-CBI **6** using EDCI and $NaHCO_3$.

The synthesis of conjugate **2** is shown in Figure 3. Because solid-phase synthesis of Py-Im polyamides with *N*-methylimidazole carboxylic acid termini using a oxime resin was unsuccessful, we developed a new method using Im-coupled CLEAR-acid resin. We also found that the coupling yield of *N*-methylimidazole at the C-terminals with compound **4** was low. Therefore, we synthesized compound **9** by coupling compound **7** with the dimer unit of compound **8**, which was readily synthesized from ethyl 5-nitro-1*H*-indole-2-carboxylate in three steps. The synthesis of conjugate **2** was accomplished by coupling compound **9** with *seco*-CBI **6** using EDCI and $NaHCO_3$.

The structures of conjugates **1** and **2** were confirmed by electrospray ionization time-of-flight mass spectrometry (ESI-TOF) after purification using reverse-phase HPLC. Purified

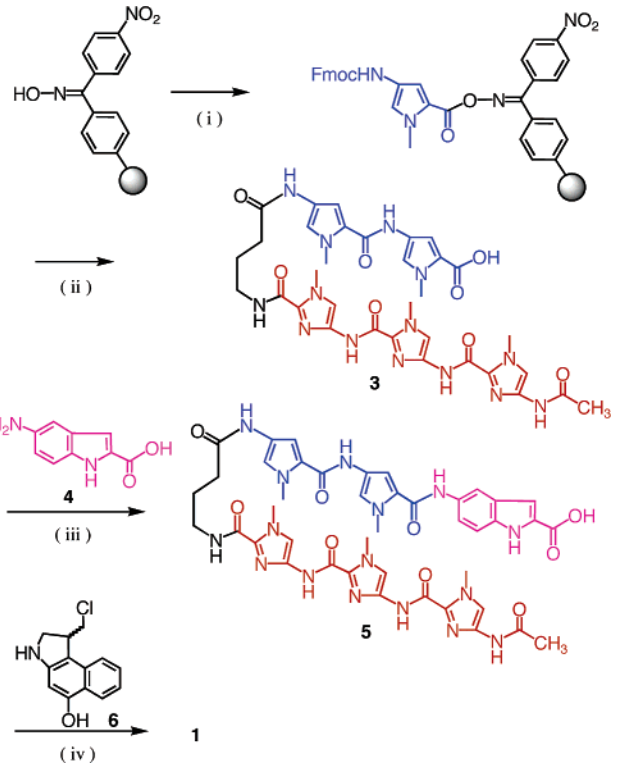

Figure 2. Synthetic scheme for the preparation of *seco*-CBI conjugate **1** with an indole linker: (i) FmocNH−Py−CO_2H, DCC, HOBt, $^{i}Pr_2NEt$ then Ac_2O DMAP, DMF; (ii) Fmoc solid-phase synthesis was followed by processing with 1 N NaOH and DMF; (iii) HATU, $^{i}Pr_2NEt$, DMF, then **4**, $^{i}Pr_2NEt$, DMF; (iv) **6**, EDCI, $NaHCO_3$, and DMF.

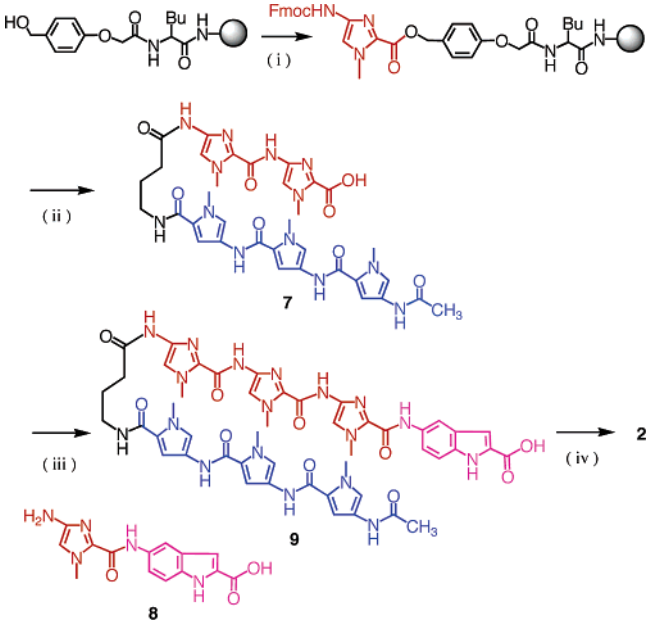

Figure 3. Synthetic scheme for the preparation of *seco*-CBI conjugate **2** with an indole linker: (i) FmocNH−Im−CO_2H, MSNT, NMI then Ac_2O DMAP, DMF; (ii) Fmoc solid-phase synthesis was followed by processing with TFA; (iii) **8**, HATU, $^{i}Pr_2NEt$, DMF; (iv) **6**, EDCI, $NaHCO_3$, DMF.

seco-CBI conjugates **1** and **2** were used for evaluation of DNA alkylation and anticancer activity.

DNA Alkylating Activities of Conjugates 1 and 2. Sequence-specific DNA alkylation by compounds **1** and **2** was examined using 5′-DNA fragments (219 bp) labeled with Texas Red. Fragments that contained four repeats of the human telomere

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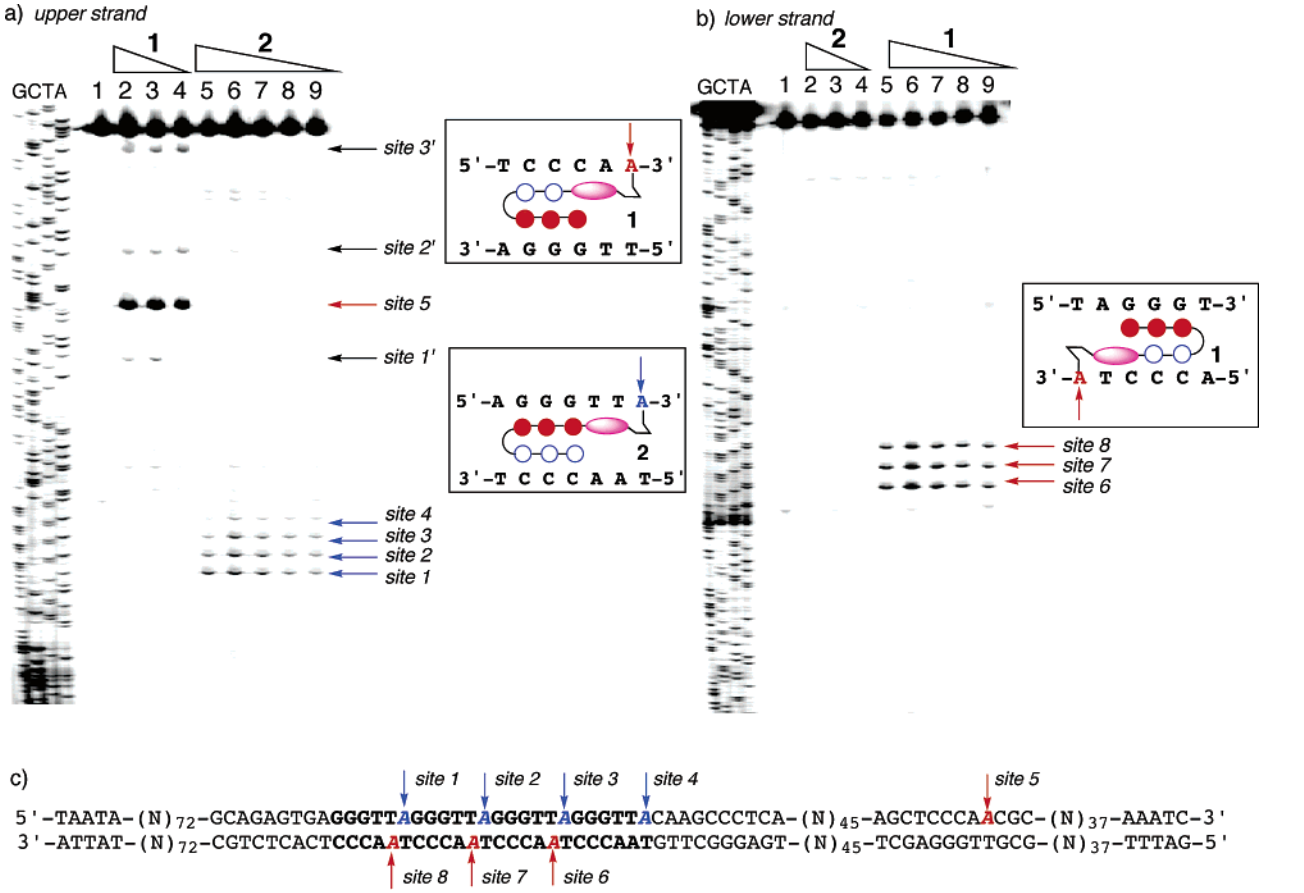

Figure 4. Thermally induced strand cleavage by conjugates **1** and **2** of DNA fragments (219 bp) that were 5′-end-labeled with Texas Red: (a) alkylation of the upper strand; (b) alkylation of the lower strand. DNA fragments included four repeats of d(GGGTTA)/d(TAACCC). These two DNA fragments are complementary. Part a: lane 1, DNA control; lanes 2−4; 5, 3, 2 μM of conjugate **1**, respectively; lanes 5−9; 5, 3, 2, 1, 0.75 μM of conjugate **2**. Part b: lane 1, DNA control; lanes 2−4; 2, 1, 0.75 μM of conjugate **2**, respectively; lanes 5−9; 2, 1, 0.75, 0.5, 0.25 μM of conjugate **1**. Telomere sequences containing alkylation sites 1−8 are represented in part c. Arrows indicate sites of DNA alkylation.

sequence were prepared by transformation into pGEM-T Easy vectors followed by PCR amplification.[8] Sequence specificities were analyzed by high-resolution denaturing polyacrylamide gel electrophoresis using an automated DNA sequencer as described previously.[9] Alkylation was carried out at 23 °C for 12 h, followed by quenching with calf thymus DNA. The samples were heated at 94 °C under neutral conditions for 20 min. The sites of N3 alkylation were visualized by thermal cleavage of the DNA strand at the alkylated sites.[10] Under these heating conditions, all alkylation sites are cleaved quantitatively; the products were observed as bands after electrophoresis. The results of analysis of the alkylated DNA fragments after heat treatment are shown in Figure 4. Conjugates **1** and **2** produced discrete cleavage bands in micromolar to nanomolar concentrations. DNA alkylation by conjugate **1** occurred mainly at three sites for which the telomere sequence was 5′-ACCCTA-3′ (sites 6−8) and at site 5 of the matched sequence 5′-TCCCAA-3′ according to the pairing rule of Py−Im polyamides. In addition to alkylation at matched sites, mismatched alkylations at 5′-ACCATA-3′ (site 1′), 5′-TGCATA-3′ (site 2′), and 5′-GGTAAA-3′ (site 3′) were observed. Densitometric analysis of the DNA cleavage bands indicated that alkylation at the match sites was about 10-fold stronger than at mismatch sites on upper strands (Supporting Information (SI), Figure S1). Similarly, conjugate **2** alkylated DNA at four sites for which the telomere sequence was 5′-AGGGTTA-3′ (sites 1−4). In contrast, DNA fragments whose sequences do not contain the target sites for conjugates **1** and **2** were not significantly alkylated by conjugates **1** and **2** (SI, Figure S2).

These results indicate that conjugates **1** and **2** precisely recognize and effectively alkylate each strand of the human telomere repeat sequence.

Antitumor Activities of Conjugates 1 and 2. To evaluate the cytotoxic potencies of conjugates **1** and **2**, we investigated the 50% cell growth inhibition (IC_{50}) values of conjugates **1** and **2** using 39 human cancer cell lines as shown in Figure 5.[11] The data shown in Figure 5 are means of IC_{50}. The mean log IC_{50} for conjugates **1** and **2** were −6.96 (110 nM) and −7.24 (57.5 nM), respectively. These results indicate that conjugates **1** and **2** have 50−100 times stronger antitumor activities than that previously reported for a CPI conjugate containing a vinyl linker (log IC_{50} = −5.24 [5.75 μM]).[4] In several human cancer

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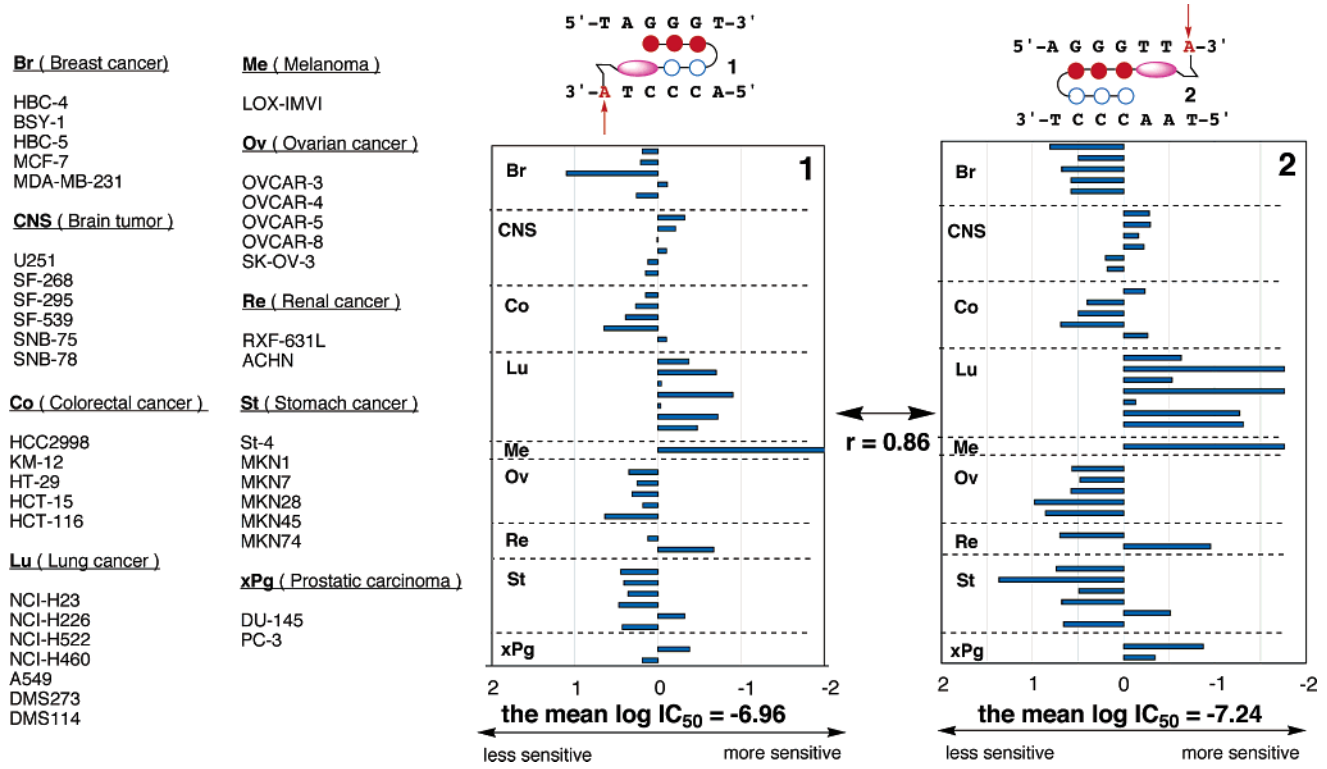

Figure 5. The log IC_{50} (50% growth inhibition) concentrations of conjugates **1** and **2** for 39 human cancer cell lines. Columns extending to the right indicate cell lines that were sensitive to the agents, and those extending to the left indicate cell lines that were less sensitive to the agents.

Table 1. Comparison of log IC_{50} Values of Conjugates **1** and **2** for Normal Cell Lines and Cancer Cell Lines

	normal cell lines[a]			cancer cell lines	
	WI-38	MRC-5	NHSF-46	lung cancer[b]	melanoma[c]
1	−6.33	−7.05	−5.69	−7.42	<−9.00
2	−6.91	−5.96	−6.25	−8.30	<−9.00

[a] WI-38 and MRC-5 were derived from normal lung tissues. NHSF-46 was derived from normal skin cell lines. [b] Lung cancer: mean value of log IC_{50} values for the seven lung cancer cell lines shown in Figure 5. [c] Melanoma: log IC_{50} value for LOX-IMVI shown in Figure 5.

cell lines, conjugates **1** and **2** were more effective than currently used anticancer drugs (e.g., log IC_{50} of mitomycin-C = −6.0; log IC_{50} of cisplatin = −5.2). We observed substantial differences in the cytotoxicities of conjugates **1** and **2**. Interestingly, conjugates **2** have relatively strong cytotoxicity against four lung-cancer cell lines (NCl-H226, NCl-H460, DMS273, DMS114). Furthermore, variation between cancer cell lines in the cytotoxicity of conjugate **2** was greater than that of conjugate **1**. This may be results of differences in alkylation sites.[13]

Because evaluation of cytotoxicities for 39 human cancer cell lines revealed that conjugates **1** and **2** had strong toxicities for cancer cell lines derived from lung and skin tissues, we investigated their cytotoxicities for human normal lung and skin cell lines. The results showed that conjugates **1** and **2** had higher cell toxicities for human cancer cell lines than for normal human skin cell lines (Table 1). In particular, the cytotoxicities of conjugates **1** and **2** were about 100-fold higher for melanoma than for normal skin cell lines: the log IC_{50} values of conjugates **1** and **2** for melanoma LOX-IMVI were both less than −9.00, whereas those of conjugates **1** and **2** for normal skin cell line NHSF-46 were −5.69 and −6.25, respectively. These results suggest that conjugates **1** and **2** have promise as new therapeutic anticancer agents for targeting double-stranded telomere sequences.

It is noteworthy that there was a strong correlation ($r = 0.86$) between mean log IC_{50} values for *seco*-CBI conjugates **1** and **2**. Correlation coefficients between log IC_{50} values of the conjugates and those of alkylating Py−Im polyamides with different sequence-specificities were lower (<0.65) than those between the conjugates; correlation coefficients between log IC_{50} of the conjugates and those of typical DNA-acting agents were significantly lower than those between the conjugates (Table 2).[12] These results suggest that DNA alkylations at the top or bottom strands of telomere repeat sequences might induce similar biological effects even though conjugates **1** and **2** have different sequence-specificities, viz., 5′-ACCCT*A*-3′ and 5′-AGGGTT*A*-3′, respectively.

Conclusions

We designed and synthesized conjugates **1** and **2**, which target human telomere duplex repeats at the 3′ ends of 5′-ACCCTA-3′ and 5′-AGGGTTA-3′, respectively. High-resolution sequencing gel electrophoresis showed that the conjugates selectively recognize and alkylate G-rich and C-rich sequences at target sites in the telomere duplex repeats at nanomolar concentrations. Evaluation of cytotoxicities using 39 human cancer cell lines and human normal cell lines demonstrated that conjugates **1** and **2** have much higher cytotoxicities than those of a previous CPI conjugate and are more toxic for cancer cell lines than for normal cell lines. As these agents selectively alkylate double-

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Table 2. Correlation Coefficients (r) between log IC_{50} Values of Conjugates **1** and **2** and Those of Various Other Compounds for Human Cancer Cell Lines

	1	2
5'-W G W C Pu-3' a / 3'-W C W G Py-5'	0.58	0.53
5'-W G C C Pu-3' a / 3'-W C G G Py-5'	0.66	0.76
MS-247 [b]	0.51	0.56
Doxorubicin [c]	0.37	0.32

[a] Py−Im polyamide derived from DU-86. [b] Nonspecific DNA alkylating agent. [c] Commercially available DNA intercalater.

stranded human telomere sequences, it is likely that they would induce telomere shortening. Examination of the biological effects of these agents is in progress.

Experimental Section

General Methods. Reagents and solvents were purchased from standard suppliers and used without further purification. Oxime resin (200−400 mesh) and CLEAR-acid resin (100−200 mesh) were purchased from Novabiochem and PEPTIDES international, respectively. 1-(Chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole **6** was synthesized and purified by reported methods.[5] Proton NMR spectra were recorded in parts per million (ppm) downfield relative to tetramethylsilane. Electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS) was produced on a BioTOF II (Bruker Daltonics) mass spectrometer. Polyacrylamide gel electrophoresis was performed on a HITACHI 5500-S DNA sequencer. Ex Taq DNA polymerase and Suprec-02 purification cartridges were purchased from Takara Co.; the Thermo Sequenase core sequencing kit and loading dye (dimethylformamide with fuschin red) from Amersham Co. Ltd; 5′-Texas Red-modified DNA oligonucleotides from Kurabo Co. Ltd; and 50% Long Ranger gel solution from FMC Bioproducts. P1 nuclease and calf intestine alkaline phosphatase (AP, 1000 units/mL) were purchased from Roche Diagnostics.

AcImImIm-γ-PyPy−Indole−*seco*-CBI (1). The five-ring Py−Im polyamide **3**, which have terminal carboxylic acid groups, was prepared by Fmoc solid-phase synthesis. To a solution of compound **3** (5.0 mg, 6.6 μmol) in DMF (0.1 mL) was added $^{i}Pr_2NEt$ (2.3 μL, 13 μmol) and HATU (2.5 mg, 6.6 μmol), and the reaction mixture was stirred for 3 h at room temperature. After the conversion from **3** to activating ester was confirmed by HPLC and ESI MS analysis, **4** (2.3 mg, 13 μmol) and $^{i}Pr_2NEt$ (2.3 μL, 13 μmol) were added to the reaction vessel. The reaction mixture was stirred for 2 h at room temperature under N_2 atmosphere. Evaporation of the solvent gave a yellow residue followed by filtration, which was washed with chloroform (2 mL × 2) and water (2 mL × 2), producing **5** as a yellow powder. ESI-TOFMS *m/e:* calcd for $C_{42}H_{45}N_{16}O_9$ $[M + H]^+$, 917.4; found, 917.5. **5** was used in the next step without further purification. To a solution of compound **5** in DMF (0.1 mL) was added *seco*-CBI **6** (3.3 mg, 14 μmol), EDCI (3.3 mg, 17 μmol), and $NaHCO_3$ (2.5 mg, 30 μmol). The reaction mixture was stirred for overnight at room temperature, and evaporation of the solvent gave a yellow residue, which was subjected to column chromatography (silica gel, 5−15% MeOH in CH_2Cl_2, gradient elution) to produce compound **1** (2.4 mg, 1.8 μmol: 27% yield for two steps) as a yellow powder. 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), conjugate **1** was used in the DNA alkylation reaction and the estimation of anticancer activity. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.67 (s, 1H; NH), 10.87 (s, 1H; NH), 10.44 (s, 1H; NH), 10.38 (s, 1H; NH), 9.64 (s, 1H; NH), 9.62 (s, 1H; NH), 8.32 (brs, 2H; CH_2), 8.20 (s, 1H; CH), 8.18 (s, 1H; CH), 8.11 (d, J = 8.0 Hz, 1H; CH), 8.08 (d, 1H; CH), 7.93 (s, 1H; CH), 7.91 (s, 1H; CH), 7.64 (s, 1H; CH), 7.54 (d, J = 6.0 Hz, 1H; CH), 7.52 (s, 1H; CH), 7.50 (s, 1H; CH), 7.46 (d, J = 5.5 Hz, 1H; CH), 7.42 (t, J = 10.0 Hz, 1H; CH), 7.39 (t, J = 8.0 Hz, 1H; CH), 7.28 (s, 1H; CH), 7.16 (s, 1H; CH), 7.10 (s, 1H; CH), 6.88 (s, 1H; CH), 4.00 (s, 3H; CH_3), 3.95 (s, 3H; CH_3), 3.86 (s, 3H; CH_3), 3.83 (s, 3H; CH_3), 3.49 (s, 3H; CH_3), 2.88 (s, 1H; CH), 2.72 (m, 2H; CH_2), 2.70 (m, 2H; CH_2), 2.26 (m, 2H; CH_2), 2.02 (s, 3H; CH_3), 1.81 (m, 2H; CH_2), 1.22 (m, 2H; CH_2). ESI-TOFMS *m/e:* calcd for $C_{55}H_{55}ClN_{17}O_9$ $[M + H]^+$, 1132.4; found, 1132.6.

AcPyPyPy-γ-ImImIm−Indole−*seco*-CBI (2). To a solution of compound **7** (10 mg, 13 μmol) in DMF (0.5 mL) was added $^{i}Pr_2NEt$ (9.2 μL, 53 μmol) and HATU (6.0 mg, 16 μmol) and compound **8** (7.9 mg, 26 μmol), and the reaction mixture was stirred for 4 h at room temperature under an Ar_2 atmosphere. Evaporation of the solvent followed by filtration, which was washed with chloroform (2 mL × 2) and water (2 mL × 2), produced **9** (7.8 mg) as a yellow powder. ESI-TOFMS *m/e:* calcd for $C_{48}H_{51}N_{18}O_{10}$ $[M + H]^+$, 1039.40; found, 1039.79. **9** was used in the next step without further purification. To a solution of compound **9** in DMF (0.1 mL) was added seco-CBI **6** (1.6 mg, 6.8 μmol), EDCI (2.6 mg, 14 μmol) and $NaHCO_3$ (1.1 mg, 13 μmol), the reaction mixture was stirred for overnight at room temperature and evaporation of the solvent gave a yellow residue, which was subjected to column chromatography (silica gel, 2−10% MeOH in CH_2Cl_2, gradient elution) to produce conjugate **2** (3.0 mg, 2.4 μmol: 19% yield for 2 steps) as a yellow powder. 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), conjugate **2** was used in the DNA alkylation reaction and the estimation of anti-cancer activity. ESI-TOF-MS *m/e*: calcd for $C_{61}H_{61}ClN_{19}O_{10}$ $[M + H]^+$, 1254.5; found, 1255.0.

AcImImIm-γ-PyPy−CO_2H (3). Fmoc−Py−CO_2H (325 mg, 0.9 mmol) and 147 mg of HOBt were dissolved in 1 mL of DMF and added to 246 mg of DCC. The reaction mixture was stirred for 10 min at room temperature and added to 500 mg of oxime resin followed by 650 μL of $^{i}Pr_2NEt$. The coupling was allowed to proceed for 12 h at room temperature. The resin was then acethylated with 115 μL of acetic anhydride (Ac_2O) and 3 mg of DMAP in 3 mL of DMF for 1 h. AcImImIm-γ-PyPy−CO_2H was synthesized in a stepwise reaction by Fmoc solid-phase methods on the Py-coupled oxime resin with Fmoc−Py−CO_2H and Fmoc−Im−CO_2H monomers. A peptide can be cleaved from oxime resin to yield primary carboxylic acid with alkali condition (1 N NaOH in DMF, 1 h, 55 °C), and purified by HPLC using a Chemcobond 5-ODS-H column (0.1% AcOH/CH_3CN 0−100% linear gradient, 0−30 min, 254 nm), to produce **3** (9.6 mg, 13%) as a yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.53 (s, 1H; OH), 10.38 (s, 1H; NH), 9.85 (s, 1H; NH), 9.84 (s, 1H; NH), 9.63 (s, 1H; NH), 9.61 (s, 1H; NH), 8.31 (brs, 1H, NH), 7.64 (s, 1H; CH), 7.51 (s, 1H; CH), 7.49 (s, 1H; CH), 7.39 (s, 1H; CH), 7.13 (s, 1H; CH), 6.85 (s, 1H; CH), 6.81 (s, 1H; CH), 4.00 (s, 3H; CH_3), 3.95 (s, 3H; CH_3), 3.94 (s, 3H; CH_3), 3.80 (s, 3H; CH_3), 3.54 (s, 3H; CH_3), 3.27 (m, 2H; CH_2), 2.02 (s, 3H; CH_3), 1.95 (m, 2H; CH_2). ESI-TOF-MS *m/e:* calcd for $C_{33}H_{39}N_{14}O_8$ $[M + H]^+$, 759.4; found, 759.3.

NH_2−Indole−CO_2H (4). Compound **4** was synthesized by 2 stepwise reaction from commercial available ethyl 5-nitroindole-2-carboxylate (hydrolysis by 1 N aq NaOH then hydrogenation by hydrogen gas catalyzed by palladium−carbon), which was used in the next coupling step without further purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.22 (s, 1H; NH), 7.11 (d, 1H, J = 8.5 Hz; CH), 6.75 (d, 1H, J = 2.0 Hz; CH), 6.67 (s, 1H; CH), 6.65 (dd, 1H, J = 2.0, 8.5 Hz; CH), 3.31 (s, 2H; NH_2).

AcPyPyPy-γ-ImIm−CO_2H (7). Fmoc−Im−CO_2H (494 mg, 1.3 mmol) and 403 mg of 1-mesitylene-2-sulfonyl-3-nitro-1H-1,2,4-triazole

(MSNT) were dissolved in 2 mL of DCM followed by 217 μL of *N*-methylimidazole (NMI) and added to 400 mg of CLEAR-acid resin and 2 mL of DMF. The coupling was allowed to proceed for 10 h at room temperature. The resin was then acethylated with 166 μL of acetic anhydride (Ac_2O), 4.3 mg of DMAP, and 3 mL of DMF for 1 h. AcPyPyPy-γ-ImIm$-CO_2H$ was synthesized in a stepwise reaction by Fmoc solid-phase methods on the Im-coupled CLEAR-acid resin with Fmoc$-$Py$-CO_2H$ and Fmoc$-$Im$-CO_2H$ monomers. A peptide can be cleaved from CLEAR-acid resin to yield primary carboxylic acid with acidic condition (TFA/dimethyl sulfide/triisopropylsilane/H_2O = 91:3:3:3, 30 min, room temperature), and purified by HPLC using a Chemcobond 5-ODS-H column (0.1% AcOH/CH_3CN 0$-$100% linear gradient, 0$-$30 min, 254 nm), to produce **7** (2.4 mg, 7%) as a yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.38 (s, 1H; NH), 9.88 (s, 1H; NH), 9.87 (s, 1H; NH), 9.80 (s, 1H; NH), 9.60 (s, 1H; NH), 8.01 (s, 1H; NH), 7.62 (s, 1H; CH), 7.51 (s, 1H; CH), 7.22 (s, 1H; CH), 7.16 (s, 1H; CH), 7.13 (s, 1H; CH), 7.03 (s, 1H; CH), 6.87 (s, 1H; CH), 6.85 (s, 1H; CH), 3.95 (s, 3H, NCH_3), 3.92 (s, 3H; NCH_3), 3.84 (s, 3H; NCH_3), 3.82 (s, 3H; NCH_3), 3.79 (s, 3H; NCH_3), 3.19 (t, 2H, J = 8.0 Hz; CH_2), 2.35 (t, 2H, J = 7.2 Hz; CH_2), 1.96 (s, 3H; $COCH_3$) 1.78 (qu, 2H, J = 9.2 Hz; CH_2). ESI-TOF-MS *m*/*e*: calcd for $C_{34}H_{40}N_{13}O_8$ $[M + H]^+$, 758.3; found, 758.4.

NH_2-Im$-$Indole$-CO_2H$ (8). To a solution of 5-aminoindole-2-carboxylate (870 mg, 4.2 mmol) in CH_2Cl_2 (50 mL) was added commercial available 1-methyl-2-trichloroacetyl-4-nitroimidazole (1.2 g, 4.2 mmol) and $^iPr_2NEt$ (1.5 mL, 8.4 mmol), and the reaction mixture was stirred for 18 h at room temperature under an H_2 atmosphere to produced compound **8** (210 mg, 0.64 mmol). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.68 (s, 1H; NH), 9.75 (s, 1H; NH), 8.10 (s, 1H; CH), 7.48 (dd, 1H, J = 2.1, 10.0 Hz; CH), 7.36 (d, 1H, J = 8.2 Hz; CH), 7.03 (s, 1H; CH), 6.51 (s, 1H; CH), 3.89 (s, 3H; CH_3), 2.88 (s, 2H; NH_2).

Preparation of 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 20 μL containing 50 μM of fragment set (5′-GCAGAGTGAGGGTTAGGGTTAGGGTTAGG-GTTACAAGCCCTCA-3′, 3′-ACGTCTCACTCCCAATCCCAATCC-CAATCCCAATGTTCGGGAG-5′). Products were identified by separation in TBE (10% native polyacrylamide gel with 0.5 μg/mL ethidium bromide using low weight DNA marker [New England BioLabs]) and visualization under UV illumination. The annealed fragments were ligated into pGEM-T easy vectors (Promega). *Escherichia coli* DH5α 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′-GATTTAGGTGACAC-TATAG-3′), 200 μM of deoxynucleotide triphosphates (Sigma Aldrich), 2 units taq DNA polymerase, and 1x ThermoPol reaction buffer (New England Bio Labs). Amplification cycles were carried out with an iCycler (BIO-RAD). The reaction mix was incubated at 95 °C for 5 min then 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) and identified by PCR (program and reaction mixtures same as above).

Preparation of 5′-Texas Red-Modified DNA Fragment and High-Resolution Gel Electrophoresis. The 5′-Texas Red-modified DNA fragments containing telomere sequence was prepared by PCR using a primer set of 5′-Texas Red labeled T7 and sp6 promoter primer or that of T7 and 5′-Texas Red labeled sp6 promoter primer; 1 ng of the telomere fragment inserted pGEM-T easy vector (program and other reagents same as above). The fragment was purified by GenElute PCR cleanup kit (Sigma Aldrich). The 5′-Texas Red labeled DNA fragments were alkylated by various concentrations of alkylation polyamides in 10 μL of 5 mM sodium phosphate buffer (pH 7.0) containing 10% DMF at 25 °C for 20 h. The reaction was quenched by the addition of calf thymus DNA (1 mM, 1 μL) and by heating the mixture for 30 min at 95 °C. The DNA was recovered by vacuum centrifugation. The pellet was dissolved in 5 μL of loading dye (formamide with fuschsin red), heated at 95 °C for 30 min, and then immediately placed on ice. The 2 μL aliquot was subjected to electrophoresis on a 6% denaturing polyacrylamide gel using a Hitachi SQ5500-E DNA Sequencer.

Analysis of Growth Inhibition against 39 Human Cancer Cell Lines. The human cancer cell lines that were composed of five breast cancer (HBC-4, BSY-1, HBC-5, MCF-7, MDA-MB-231), six brain cancer (U251, SF-268, SF-295, SF-539, SNB-75, SNB-78), colon cancer (HCC2998, KM-12, HT-29, HCT-15, HCT-116), seven lung cancer (NCI$-$H23, NCl$-$H226, NCI$-$H522, NCI$-$H460, A5449, DMS273, DMS114), one melanoma (LOX-IMVI), five ovarian cancer (OVCAR-3, OVCAR-4, OVCAR-6, OVCAR-8, SK$-$OV-3), two renal cancer (RXF-631L, ACHN), six stomach cancer (St-4, MKN1, MKN7, MKN28, MKN45, MKN74), and two prostate cancer (DU-145, PC-3) were all plated at an appropriate density in 96-well plates in RPMI1640 (sigma) containing 10% heat-inactivated fetal bovine serum (Life Technologies), penicillin (100 U/mL), and streptomycin (100 μg/mL). The cells were maintained at 37 °C in humidified atmosphere of 95% air and 5% CO_2. The cells were exposed to drugs for 48 h.[14] The cell growth was determined according to the sulforhodamine B assay.[15] Absorbance for the control well (*C*), the treated well (*T*) and the treated well at time 0 (T_0) were measured at 525 nm. The 50% growth inhibition (IC_{50}) was calculated as $100 \times [(T - T_0)/(C - T_0)] = 50$. The mean graphs, which show the differential growth inhibition of the drugs in the cell line panel, were drawn on the basis of a calculation using a set of IC_{50}.[16] Pearson correlation coefficients (r) were calculated using the following formula:

$$r = (S(x_i - x_m)(y_i - y_m))/(S(x_i - x_m)^2 S(y_i - y_m)^2)^{1/2}$$

where x_i and y_i are log IC_{50} of drug A and drug B, respectively, against each cell line, and x_m and y_m are the mean values of x_i and y_i, respectively.

Effects for Normal Human Cell Lines. The human normal cell lines used in this study were MRC-5, NHSF46, and WI-38, which are derived from male embryonic lung fibroblast, skin fibroblast, and female embryonic lung fibroblast, respectively. All normal cell lines were obtained from the RIKEN Cell Bank. MRC-5 and NHSF46 cells were maintained in MEM-alpha (Gibco), and WI-38 cells were grown in Dulbecco's modified Eagle's medium (Sigma) at 37 °C in a humidified atmosphere of 5% CO_2 in 95% air, and both of the media were supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin, and 100 mg/mL streptomycin. The growth inhibiting effects against these normal cell lines were assessed as above.

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Supporting Information Available: Full citation for ref 11, densitometric analysis of polyacrylamide gel electrophoresis shown in Figure 4, and sequencing analysis of DNA fragments whose sequences do not contain the target sites for conjugates **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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