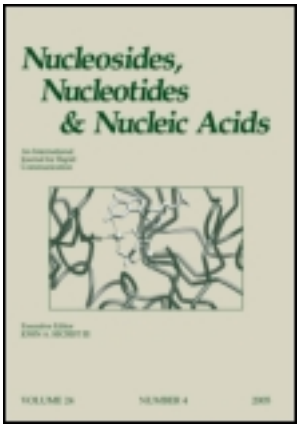

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Synthesis and Evaluation of Pyrrole Polyamide- 2′-Deoxyguanosine 5′-Phosphate Hybrid

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SYNTHESIS AND EVALUATION OF PYRROLE POLYAMIDE-2′-DEOXYGUANOSINE 5′-PHOSPHATE HYBRID

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□ *Pyrrole polyamide-2′-deoxyguanosine 5′-phosphate hybrid (Hybrid **4**) was synthesized and evaluated in terms of the inhibition of mouse mammary carcinoma FM3A cell growth. Hybrid **4** was found to exhibit dose-dependent inhibition of cell growth.*

Keywords Pyrrole polyamide-2′-deoxyguanosine 5′-phosphate hybrid; minor groove binder; anticancer agent

INTRODUCTION

The precise control of specific gene expression represents the ultimate aim of gene therapy. We previously designed and synthesized nucleosides (Hybrid **1** and Hybrid **2**) linked to a pyrrole polyamide[1] minor groove binder (MGB) comprising modified distamycin A, which possess high affinity for the AAATT sequence, as lead compounds for potential application as gene therapy agents (Figure 1).[2] The pyrrole polyamide moiety of 2′-deoxyguanosine hybrids was linked at the 2-exocyclic amino group, which is positioned in the minor groove of a DNA duplex. The ds-DNA binding ability of hybrids was investigated by analysis[3] of circular dichroism (CD) spectra and melting temperature (T_m) values using several DNA duplexes. It was shown that Hybrid **2** possessed specific DNA binding ability and stabilized DNA duplexes [e.g., 5′-d(CGCAAATTGGC)-3′/3′-d(GCGTTTAACCG)-5′ duplex : Hybrid **2** = 1 : 1, $\Delta T_m = 5.6^{\circ}$C].[2]

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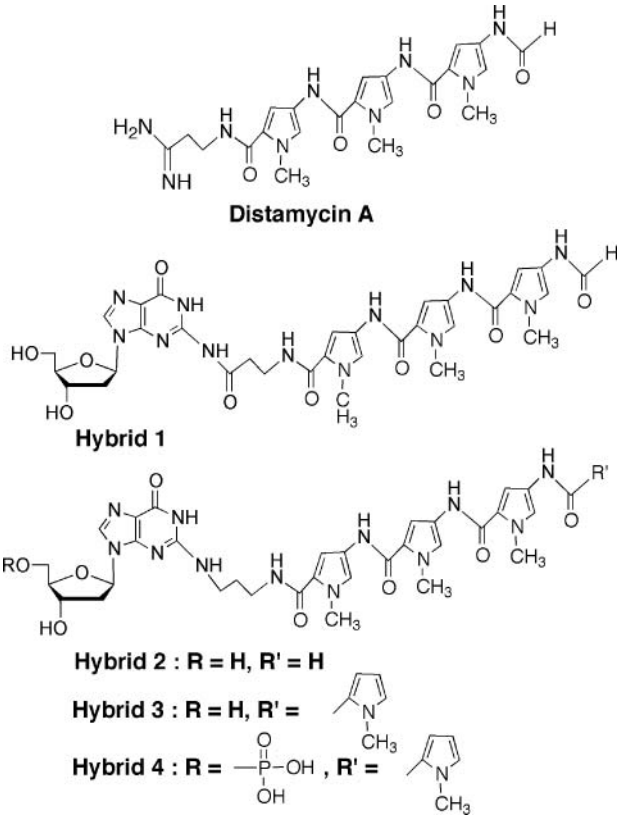


FIGURE 1 Pyrrole polyamide-2′-deoxyguanosine hybrids.

Furthermore, we synthesized oligonucleotide-conjugated pyrrole polyamide-2′-deoxyguanosine hybrids and subsequently examined the DNA recognition and binding ability of modified dsDNA. The formyl functional group of Hybrid **2** was unstable under conditions employed for DNA oligonucleotide solid phase synthesis. Oligonucleotides **5** conjugated to Hybrid **3**

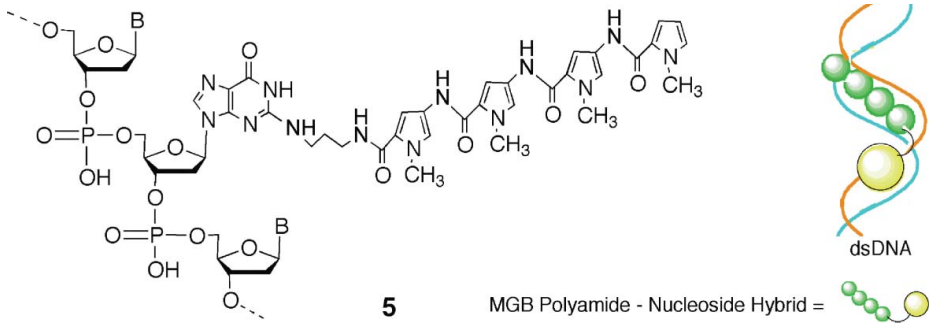


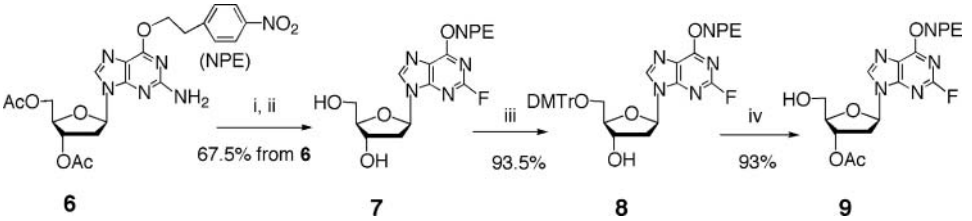
FIGURE 2 Oligonucleotide-conjugated pyrrole polyamide-2′-deoxyguanosine hybrid. (Color figure available online).

were therefore synthesized and investigated for specific binding to ssDNA (Figure2).[4] From the T_m values and CD spectral analyses, it was found that oligonucleotides **5** possessed high specific binding to ssDNA oligonucleotides containing the pyrrole polyamide binding sequence, and that the generated dsDNAs possessed high stability [e.g., 5′-d(CG**M**AATTTGGC)-3′/3′-d(GCCTTAAACCG)-5′, **M** = Hybrid **3**, $\Delta T_m = 23.0°C$].[4] These results suggest that Hybrids **2** and **3** may potentially be used to control the expression of target genes through interaction with the DNA duplex and/or incorporation into the DNA during biosynthesis. Based on the studies described above, we extended our studies to evaluating the biological activity of the hybrids synthesized. In terms of incorporation of the hybrids into DNA during biosynthesis, hybrids possessing a phosphate group at the 5′ position of the sugar moiety were expected to show high biological activity. Introduction of a phosphate group also offers solubility advantages for drugs used in water-based solutions. Herein, we report on the synthesis of Hybrid **4** bearing a 5′-phosphate group and evaluation of the inhibition of mouse mammary carcinoma FM3A cell growth by Hybrids **1–4** (Figure 1).

RESULTS AND DISCUSSION

Hybrid **4** was efficiently synthesized by condensation of 2′-deoxy-2-fluoroinosine derivative **9** with 3-(Py$_4$-amino)propylamine from **10**[4] and conversion to the 5′-phosphate derivative via 5′-phosphoramidite derivative **12**.

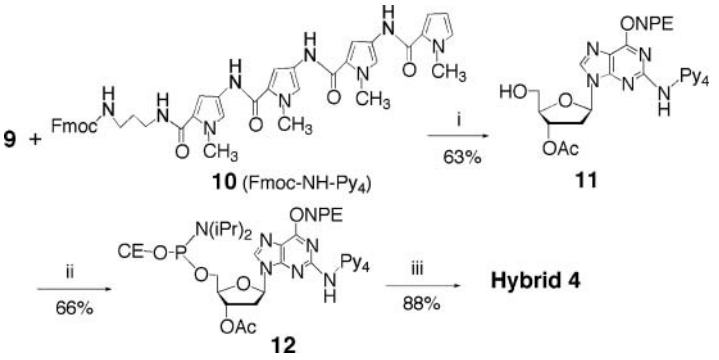
3′-Acetyl-2′-deoxy-2-fluoro-O^6-[2-(4-nitropheny)ethylinosine (**9**) was prepared from 3′,5′-di-*O*-acetyl-2′-deoxy-O^6-[2-(4-nitropheny)ethylguanosine (**6**)[4] as shown in Scheme 1. Compound **6** was deacetylated with 2:1 conc. NH_4OH/1,4-dioxane and then treated with 35% HF/pyridine and *tert*-butyl nitrite to give 2-fluoroinosine derivative **7**.[5] After conversion of **7** to 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2-fluoro-O^6-[2-(4-nitrophenyl)-ethyl]inosine (**8**),[5] 3′-*O*-acetylaion of **8** followed by removal of the DMTr group afforded **9** in good yield.



6 **7** **8** **9**

SCHEME 1 Synthesis of 2′-deoxy-2-fluoroinosine derivative **9**. Reaction conditions: (i) 2:1 conc. NH_4OH/1,4-dioxane, rt, 12 hours. (ii) 35% HF-pyridine, *tert*-butyl nitrite, −30°C ∼rt, 2 hours. (iii) DMTr-Cl (1.1 eq.), pyridine, rt, 4 hours. (iv) 1. Ac_2O, pyridine, rt, 3 hours; 2. 2% Cl_3CCOOH, 1:3 CH_3OH/CH_2Cl_2, rt, 1 hour.

Treatment with **9** and **10** in the presence of Et_3N afforded **11** in 63% yield through removal of the Fmoc group of **10** and condensation with the amine derivative (Py_4–NH_2). Compound **11** was converted to 5′-phosphoramidite derivative **12** using a general method.[6] Phosphoramidite **12** was hydrolyzed with 0.35 M acetic acid in H_2O–CH_3CN and oxidized with 0.02 M I_2 in H_2O-pyridine-THF. The resulting phosphate derivative was treated with 1 M NaOH in H_2O–CH_3OH to effect removal of the 2-cyanoethyl, acetyl (Ac), and (4-nitrophenyl)ethyl (NPE) protecting groups and finally neutralized using an ion exchange resin. Hybrid **4** was obtained as a precipitate from the neutralized solution in 88% yield based with **12** (Scheme 2).



SCHEME 2 Synthesis of Hybrid **4**. reaction conditions: (i) 1:5 Et_3N/DMF, 60°C, 10 hours. (ii) $[(iPr)_2N]_2POCE$ (1.5 eq.), 1*H*-tetrazole (1.1 eq.), CH_2Cl_2, rt, 1 hour. (iii) 1. 0.35 M AcOH/H_2O-CH_3CN, rt, 30 min; 2. 0.02 M I_2/H_2O-pyridine-THF, rt, 20 minutes; 3. 1 M NaOH/H_2O-CH_3OH, rt, 17 hours; 4. Dowex 50 W × 8 (H^+ form).

Inhibition of mouse mammary carcinoma FM3A cell growth by pyrrole polyamide compounds (Hybrids **1–4** and distamycin A) was then evaluated.[7] FM3A cells (3×10^4 cells/mL) were cultured in ES medium supplemented with 2% heat-inactivated fetal calf serum at 37°C in an atmosphere of 5% CO_2. Cells were grown for 2 days in the absence (control) or presence of pyrrole polyamide compounds (Hybrids **1–4** and distamycin A) (10 and 30 μM). Cell viability (percent of control) is shown in Figure 3. Hybrid **1**, a 2′-deoxyganosine derivative bearing pyrrole polyamide through an amide bond (–N^2HCO–) using an aminopropionyl linker, and distamycin A had no effect on cell growth. On the other hand, Hybrids **2–4**, comprising 2′-deoxyganosine bearing pyrrole polyamides through an N^2-alkyl bond using an aminopropyl linker, induced dose-dependent inhibition of cell growth. In particular, Hybrid **4** exhibited the highest inhibition compared with the other Hybrids. We previously reported that oligonucleotides **5** conjugated to Hybrid **3** possessed high specific binding to ssDNA oligonucleotides containing the pyrrole polyamide binding sequence, and that the generated dsDNAs possessed high stability.[4] These results suggest that Hybrids **2–4** bearing

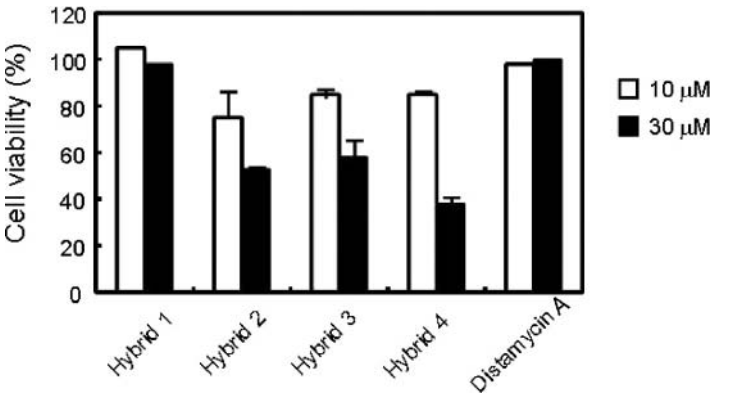


FIGURE 3 Effect of pyrrole polyamide compounds (Hybrids **1–4** and distamycin A) on the growth of mouse mammary carcinoma FM3A cells.Cells were grown for 2 days in the absence or presence of pyrrole polyamide compounds.

pyrrole polyamide using an aminopropyl linker inhibited cell growth by incorporation into DNA during DNA replication and biosynthesis through a process involving binding of the pyrrole polyamide moieties to the generated DNA duplexes, and that Hybrid **4** bearing a 5′-phosphate group is a suitable substrate for biosynthesis. Further studies investigating the effect of linker length and hybrids involving imidazole polyamide[1] are currently underway.

CONCLUSION

We synthesized Hybrid **4** bearing a 5′-phosphate group and evaluated the biological activity of Hybrids **1–4**. Hybrid **4** showed marked inhibition of mouse mammary carcinoma FM3A cell growth. Consequently, Hybrid **4** might potentially be useful as a lead compound in the development of novel anticancer agents.

EXPERIMENTAL

General

Column chromatography was performed on silica gel (Silica gel N60, purchased from Kanto Chemical Co., Inc. Japan) using methanol/chloroform and hexane/ethyl acetate as eluent. Melting points were determined using a Yanaco Micro-melting-point apparatus, and are uncorrected. ^{1}H-NMR and ^{31}P-NMR spectra were recorded on a Brucker DRX 400 spectrometer. ^{19}F-NMR spectra were recorded on a Varian MERCURY 300 spectrometer. Mass spectra were recorded on a Micromass Q-Tof Ultima API spectrometer. Elemental analyses were determined using an Elemental Vavio EL apparatus. 3′,5′-Di-*O*-acetyl-2′-deoxy-O^6-[2-(4-nitrophenyl)ethylguanosine (**6**),

pyrrole amide tetramer **10**, and Hybrids **1–3** were prepared as previously described.[2,4]

2′-Deoxy-2-fluoro-*O*^{6}-[2-(4-nitrophenyl)ethyl]inosine (7)

3′,5′-Di-*O*-acetyl-2′-deoxy-*O*^{6}-[2-(4-nitrophenyl)ethyl]guanosineime (**6**) (0.892 g, 1.78 mmol) was dissolved in 1,4-dioxane (16 mL) and 28% ammonia/water (19.5 mL) was added to the solution. After stirring for 20 hours, the solution was evaporated to dryness to give 2′-deoxy-*O*^{6}-[2-(4-nitrophenyl)ethyl]guanosine, which was used in the next reaction without purification; ^{1}H-NMR (ca. 5% CD_3OD-$CDCl_3$) δ 2.25 (dd, 1H, $J_{1',2''}$ = 5.7 Hz, $J_{2',2''}$ = 13.5 Hz, *H*-2″), 2.95–3.04 (m, 1H, *H*-2′), 3.26 (t, 2H, J = 6.9 Hz, –$OCH_2CH_2PhNO_2$), 3.65–3.69 (m, 1H, *H*-5″), 3.96 (dd, 1H, $J_{4',5'}$ = 1.5 Hz, $J_{5',5''}$ = 12.6 Hz, *H*-5′), 4.18–4.20 (m, 1H, *H*-4′), 4.72 (t, 2H, J = 6.9 Hz, –$OCH_2CH_2PhNO_2$), 4.72–4.74 (m, 1H, *H*-3′), 4.95 (s, 2H, NH_2), 6.22 (dd, 1H, $J_{1',2'}$ = 9.3 Hz, $J_{1',2''}$ = 5.7 Hz, *H*-1′), 7.48 (d, 2H, J = 9.0 Hz, Ar-*H* of the NPE group ×2), 7.62 (s, 1H, *H*-8), 8.16 (d, 2H, J = 9.0 Hz, Ar-*H* of the NPE group × 2).

A stirred solution of 2′-deoxy-*O*^{6}-[2-(4-nitrophenyl)ethyl]guanosine (0.629 g, 1.51 mmol) in 35% HF/pyridine (7.5 mL) was prepared in an ice-bath and *tert*-butyl nitrite (0.54 mL, 4.53 mmol) was added. After stirring for 2 hours, the solution was diluted with ethyl acetate (150 mL) and then poured slowly over 5% aqueous sodium hydrogen carbonate solution (100 mL). The organic layer was washed with water (100 mL), dried over anhydrous magnesium sulfate, and evaporated to dryness. The residue was subjected to chromatographic separation on a column of silica gel using ethyl acetate/hexane as eluent to give **7** (0.428 g, 67.5% yield) as a white powder; m.p. 143–145°C; ^{1}H-NMR ($CDCl_3$) δ2.36 (dd, 1H, $J_{1',2''}$ = 5.7 Hz, $J_{2',2'''}$ = 13.1 Hz, *H*-2″), 2.97 (m, 1H, *H*-2′), 3.32 (t, 2H, J = 6.9 Hz, –$OCH_2CH_2PhNO_2$), 3.81 (m, 1H, *H*-5″), 3.97 (m, 1H, *H*-5′), 4.20 (m, 1H, *H*-4′), 4.80 (m, 1H, *H*-3′), 4.85 (t, 2H, J = 6.9 Hz, –$OCH_2CH_2PhNO_2$), 6.32 (dd, 1H, $J_{1',2'}$ = 9.3 Hz, $J_{1',2''}$ = 5.7 Hz, *H*-1′), 7.50 (d, 2H, J = 8.4 Hz, Ar-*H* of the NPE group × 2), 7.98 (s, 1H, *H*-8), 8.18 (d, 2H, J = 8.4 Hz, Ar-*H* of the NPE group × 2); ^{19}F-NMR ($CDCl_3$) δ –48.95 (s); ESI-TOF MS calcd for $C_{18}H_{18}N_5O_6$ $(M+H)^+$ 420.1319, found 420.1313; Anal. calcd for $C_{18}H_{18}FN_5O_6$; C, 51.55; H, 4.33; N, 16.70, found. C, 51.35; H, 4.39: N, 16.85.

2′-Deoxy-5′-*O*- (4,4′-dimethoxytrityl) -2-fluoro-*O*^{6}-[2-(4-nitrophenyl)ethyl]inosine (8)

Compound **7** (1.49 g, 3.54 mmol), after azeotropic evaporation from pyridine (5 mL × 3), was dissolved in dried pyridine (18 mL), and 4,4′-dimethoxytrityl chloride (1.32 g, 3.9 mmol) was added to the solution. After stirring for 12 hours, the mixture was quenched with water (2 mL), diluted

with chloroform (70 mL), and washed with 5% aqueous sodium hydrogen carbonate solution (50 mL × 2) and water (50 mL). After drying over anhydrous magnesium sulfate, the organic layer was evaporated to dryness, and the residue was subjected to chromatographic separation on a column of silica gel using methanol/chloroform as eluent to give **8** (2.39 g, 93.5% yield) as a white glass; ^{1}H-NMR ($CDCl_3$) δ 2.54 (m, 1H, *H*-2″), 2.77 (m, 1H, *H*-2′), 3.32 (t, 2H, $J = 6.9$ Hz, –$OCH_2CH_2PhNO_2$), 3.35–3.47 (m, 2H, *H*-5′ and –5″), 3.78 (s, 6H, –OCH_3 × 2 of the DMTr group), 4.12 (m, 1H, *H*-4′), 4.64–4.68 (m, 1H, *H*-3′), 4.83 (t, 2H, $J = 6.9$ Hz, –$OCH_2CH_2PhNO_2$), 6.37 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.9$ Hz, *H*-1′), 6.79 (d,4H, $J = 10.5$ Hz, Ar-*H* of the DMTr group), 7.17–7.29 (m, 9H, Ar-H of the DMTr group × 9), 7.38 (d, 2H, $J = 6.9$ Hz, Ar-*H* of the NPE group × 2), 8.03 (s, 1H, *H*-8), 8.17 (d, 2H, $J =$ 6.9 Hz, Ar-*H* of the NPE group × 2); ^{19}F-NMR ($CDCl_3$) δ –49.29 (s); ESI-TOF MS calcd for $C_{39}H_{36}FN_5O_8$ $(M+H)^+$ 722.2626, found 722.2597; Anal. calcd for $C_{39}H_{36}FN_5O_8 + 0.5\ H_2O$; C, 64.10; H, 5.10; N, 9.58, found. C, 63.94; H, 5.21: N, 9.97.

3′-*O*-Acetyl-2′-deoxy-2-fluoro-*O*^{6}-[2-(4-nitrophenyl)ethyl]inosine (9)

Compound **8**[4,5] (0.375 g, 0.52 mmol), after azeotropic evaporation from pyridine (5 mL × 3), was dissolved in dried pyridine (18 mL), and acetic anhydride (0.30 mL, 3.1 mmol) was added to the solution. After stirring for 3 hours, the solution was evaporated to dryness to give 3′-*O*-acetyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2-fluoro-*O*^{6}-[2-(4-nitrophenyl)ethyl]inosine (0.397 g), which was used in the next reaction without purification; ^{1}H-NMR ($CDCl_3$) δ 2.16 (s, 3H, CH_3 of the Ac group), 2.63 (m, 1H, *H*-2″), 2.87 (m, 1H, *H*-2′), 3.32 (t, 2H, $J = 6.9$ Hz, –$OCH_2CH_2PhNO_2$), 3.36–3.48 (m, 2H, *H*-5′ and –5″), 3.78 (s, 6H, –OCH_3 × 2 of the DMTr group), 4.26 (m, 1H, *H*-4′), 4.83 (t, 2H, $J = 6.9$ Hz, –$OCH_2CH_2PhNO_2$), 5.48 (m, 1H, *H*-3′), 6.40 (dd, 1H, $J_{1',2'} = 8.4$ Hz, $J_{1',2''} = 5.7$ Hz, *H*-1′), 6.93 (d,4H, $J = 11.1$ Hz, Ar-*H* of the DMTr group), 7.20–7.44 (m, 9H, Ar-H of the DMTr group × 9), 7.50 (d, 2H, $J = 8.7$ Hz, Ar-*H* of the NPE group × 2), 8.11 (s, 1H, *H*-8), 8.18 (d, 2H, $J = 8.7$ Hz, Ar-*H* of the NPE group × 2).

3′-*O*-Acetyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2-fluoro-*O*^{6}-[2-(4-nitrophenyl)ethyl]inosine (0.397 g, 0.52 mmol) was treated with a 2% Cl_3CCOOH/CH_2Cl_2 (6 mL)-methanol (2 mL) solution by stirring for 1 hour at room temperature. The mixture was neutralized with 5% aqueous sodium hydrogen carbonate solution (50 mL), diluted with chloroform (50 mL), and washed with water (50 mL). After drying over anhydrous magnesium sulfate, the organic layer was evaporated to dryness, and the residue was subjected to chromatographic separation on a column of silica gel using methanol/chloroform as eluent to give **9** (0.223 g, 93%) as a white glass; ^{1}H-NMR ($CDCl_3$) δ 2.13 (s, 3H, CH_3 of the Ac group), 2.46 (dd, 1H,

$J_{1',2''}$ = 5.1 Hz, $J_{2',2''}$ = 13.8 Hz, *H*-2″), 3.05 (m, 1H, *H*-2′), 3.33 (t, 2H, J = 6.9 Hz, –OCH$_2$C*H*$_2$PhNO$_2$), 3.88–4.00 (m, 2H, *H*-5′ and –5″), 4.25 (m, 1H, *H*-4′), 4.85 (t, 2H, J = 6.9 Hz, –OC*H*$_2$CH$_2$PhNO$_2$), 5.53 (d, 1H, $J_{2',3'}$ = 5.7 Hz, *H*-3′), 6.28 (dd, 1H, $J_{1',2'}$ = 9.6 Hz, $J_{1',2''}$ = 5.1 Hz, *H*-1′), 7.50 (d, 2H, J = 8.4 Hz, Ar-*H* of the NPE group × 2), 8.00 (s, 1H, *H*-8), 8.18 (d, 2H, J = 8.4 Hz, Ar-*H* of the NPE group × 2); ^{19}F-NMR (CDCl$_3$) δ –49.18 (s); ESI-TOF MS calcd for $C_{20}H_{21}FN_5O_7$ $(M+H)^+$ 462.1425, found 462.1442; Anal. calcd for $C_{20}H_{20}FN_5O_7 + 0.5\ H_2O$; C, 51.07; H, 4.50; N, 14.89, found. C, 50.92; H, 4.35: N, 14.82.

Pyrrole Amide Tetramer-2′-*O*-acetyl-2′-deoxy-*O*^{6}-[2-(4-nitrophenyl)ethyl]guanosine Hybrid Derivative 11

Compound **9** (0.242 g, 0.525 mmol) and **10** (0.385 g, 0.5 mmol) were dissolved in DMF (2 mL), and triethylamine (0.4 mL) was added to the solution. After stirring for 10 hours at 60°C, the solution was evaporated. The residue was subjected to chromatographic separation on a column of silica gel using methanol/ethyl acetate as eluent to give **11** (0.311 g, 63%) as a yellow glass; ^{1}H-NMR (CDCl$_3$) δ 1.86 (m, 2H, –CH$_2$C*H*$_2$CH$_2$–), 2.06 (s, 3H, C*H*$_3$ of the Ac group), 2.33 (dd, 1H, $J_{1',2''}$ = 5.7 Hz, $J_{2',2''}$ = 13.8 Hz, *H*-2″), 3.18–3.24 (m, 1H, *H*-2′), 3.22 (t, 2H, J = 6.6 Hz, –OCH$_2$C*H*$_2$PhNO$_2$), 3.46–3.58 (m, 4H, –C*H*$_2$CH$_2$C*H*$_2$–), 3.80–3.90 (m, 2H, *H*-5′ and –5″), 3.86 (s, 3H, C*H*$_3$), 3.90 (s, 3H, C*H*$_3$), 3.93 (s, 3H, C*H*$_3$), 3.97 (s, 3H, C*H*$_3$), 4.20–4.22 (m, 1H, *H*-4′), 4.70 (t, 2H, J = 6.6 Hz, –OC*H*$_2$CH$_2$PhNO$_2$), 5.53 (d, 1H, $J_{2',3'}$ = 5.4 Hz, *H*-3′), 6.12 (dd, 1H, J = 2.7 Hz, J = 3.9 Hz, Py-*H*), 6.24 (dd, 1H, $J_{1',2'}$ = 9.6 Hz, $J_{1',2''}$ = 5.7 Hz, *H*-1′), 6.38 (s, 1H, N^2-*H*), 6.71–6.77 (m, 5H, Py-*H* × 5), 7.12 (m, 2H, Py-*H* × 2), 7.14 (s, 1H, Py-*H*), 7.26 (m, 1H, CH$_2$N*H*CO-Py), 7.43 (d, 2H, J = 8.7 Hz, Ar-*H* of the NPE group × 2), 7.67 (s, 1H, *H*-8), 7.73 (s, 1H, –N*H*CO–), 7.82 (s, 1H, –N*H*CO–), 8.06 (s, 1H, –N*H*CO–), 8.11 (d, 2H, J = 8.7 Hz, Ar-*H* of the NPE group × 2); ESI-TOF MS calcd for $C_{47}H_{53}N_{14}O_{11}$ $(M+H)^+$ 989.4018, found 989.3993; Anal. Calcd for $C_{47}H_{52}N_{14}O_{11}$+ 1.5 H_2O: C, 55.56; H, 5.45; N, 19.30, found. C, 55.49; H, 5.43; N, 19.50.

Pyrrole Amide Tetramer-2′-deoxyguanosine 5′-phoasphate Hybrid (Hybrid 4)

Compound **11** (0.198 g, 0.2 mmol) was dissolved in dichloromethane (1.6 mL), and cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (96 μL, 0.3 mmol) and 0.5 M 1*H*-tetrazole/acetonitrile solution (0.44 mL, 0.22 mmol) were added to the solution. After stirring for 30 min, triethylamine (31 μL, 0.22 mmol) was added. The mixture was evaporated to dryness and the residue was subjected to chromatographic separation on a

column of silica gel using 0.1% triethylamine/ethyl acetate/hexane as eluent to give phosphoramidite derivative **12** (a diastereomer mixture) (0.156 g, 66%) as a slightly yellow glass; ^{31}P-NMR ($CDCl_3$) δ 149.83 and 149.94.

Compound **12** (20 mg, 0.168 mmol) was treated with 0.5 M acetic acid/acetonitrile solution (0.5 mL) and H_2O (0.2 mL). After stirring for 30 minutes, the mixture was evaporated. The residue was treated with 0.02 M I_2/H_2O-pyridine-THF solution (3 mL). After stirring for 20 minutes, 1 M NaS_2O_3/H_2O solution was added, and the mixture was evaporated. The residue was treated with 2 M $NaOH/H_2O$ solution (2 mL) and methanol (3 mL) at room temperature for 17 hours. The solution was neutralized with Dowex 50 W × 8 (H^+ form) and the resin was instantly removed by filtration. The filtrate was allowed to stand for 1 hour in an ice-bath and Hybrid **4** was separated by centrifugation as a white powder in 88% yield (13 mg); ^{1}H-NMR (CD_3OD) δ 1.90 (m, 2H, –$CH_2CH_2CH_2$–), 2.27 (m, 1H, *H*-2″), 2.81 (m, 1H, *H*-2′), 3.39 (m, 2H, –$CH_2CH_2CH_2$–), 3.50 (m, 2H, –$CH_2CH_2CH_2$–), 3.87 (m, 1H, *H*-5″), 3.86 (s, 3H, CH_3), 3.91 (s, 6H, CH_3 × 2), 3.93 (s, 3H, CH_3), 4.04–4.09 (m, 2H, *H*-4′ and –5′), 4.62 (m, 1H, *H*-3′), 6.09 (m, 1H, Py-*H*), 6.28 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.9$ Hz, *H*-1′), 6.83 (s, 1H, Py-*H*), 6.85 (s, 1H, Py-*H*), 6.87 (m, 1H, Py-*H*), 6.93 (m, 2H, Py-*H* × 2), 7.22 (m, 2H, Py-*H* × 2),7.26 (s, 1H, Py-*H*), 8.04 (s, 1H, *H*-8), 7.73; ^{31}P-NMR (CD_3OD) δ 0.47; ESI-TOF MS calcd for $C_{37}H_{44}N_{13}O_{11}P$ $(M + H)^+$ 878.3099, found 878.3096.

Biological Activity of Pyrrolepolyamide Compounds

The effect of pyrrole polyamide compounds [Hybrids **1–4** and distamycin A hydrochloride (Sigma)] on the growth of mouse mammary carcinoma FM3A[7] cells (Japan Health Science Foundation) was examined. Mouse mammary carcinoma FM3A cells (3×10^4 cells/mL) were cultured in ES medium (Wako Pure Chemical Industries, Ltd.) supplemented with 2% calf serum at 37°C in an atmosphere of 5% CO_2, and containing 0 μM (control), 10 or 30 μM pyrrole polyamide compound (Hybrids **1–4** or distamycin A). After 2 days, cell viability was determined according to the method of Jones et al.[8] (Figure 3).

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