Synthesis of New Peptide Nucleic Acid Monomer with Glycylglycine Backbone

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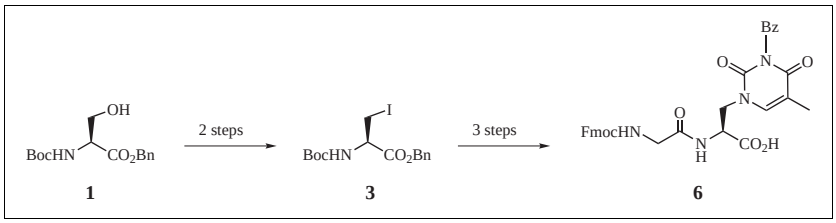

New thymine peptide nucleic acid (PNA) monomer with glycylglycine backbone was prepared. This involved a key step of the coupling between iodinated serine (**3**) and 3-benzoylthymine.

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Introduction.

Aminoethylglycine peptide nucleic acid (aegPNA) is an analogue with the deoxyribose phosphate replaced by a polyamide backbone [1-3]. In spite of such a dramatic structural change, aegPNA shows a high affinity to DNA and RNA in a sequence-specific fashion. As polyamide based nucleic acid analogues have the great potential as tools in many biological applications, development of novel analogues of PNA became an attractive area of research soon after aegPNA was reported by Nielsen and co-workers [4-8].

We previously reported on the binding activity to DNA and RNA with aegPNAs including PNA monomers with glycylalanine backbone (isogaPNA monomers) [9]. IsogaPNA is 2',5'-isoDNA mimic chiral peptide nucleic acid that is one of interesting antisense compounds having the preferential binding activity to RNA [10]. However, unfortunately the incorporation of isogaPNA monomers to aegPNAs resulted in decrease of hybridization properties to DNA and RNA. The decreased Tm values compared to unmodified aegPNAs indicate that the glycylalanine backbone of isogaPNA may not be optimal in length to hybridize with DNA and RNA. In this context, we examined the preparation of PNA monomer with glycylglycine backbone (isoggPNA monomer), in which length is different from that of isogaPNA, in order to investigate the hybridization property of isoggPNA with DNA and RNA (Figure 1).

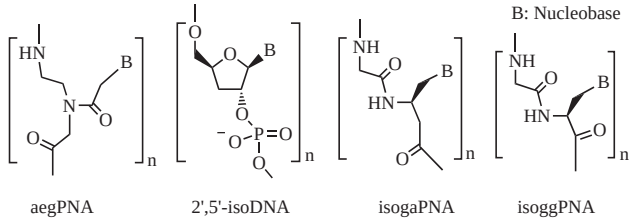


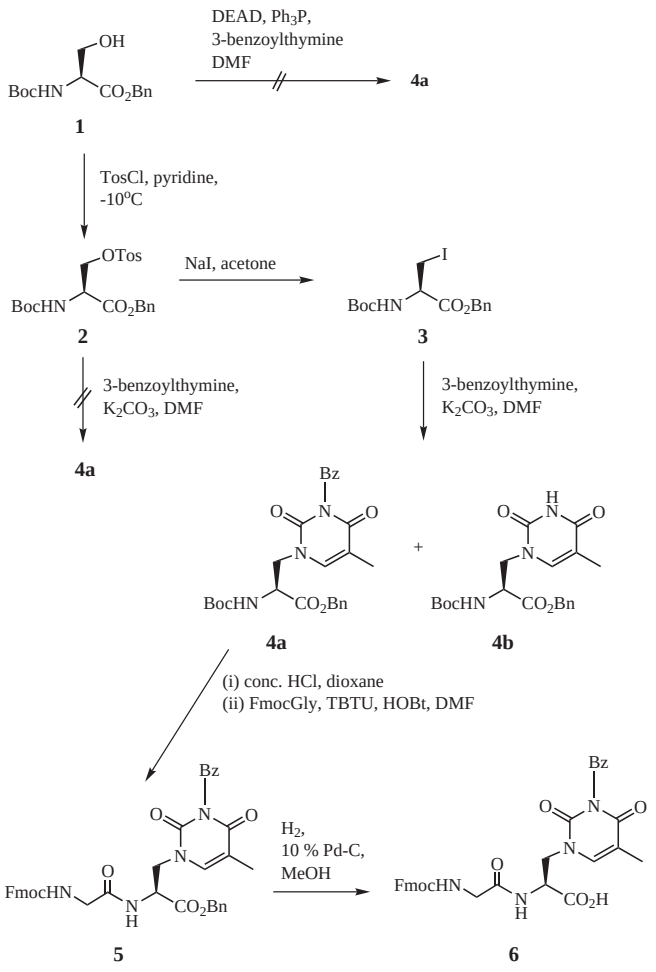
Figure 1. Structures of aegPNA, 2',5'-isoPNA, isogaPNA and isoggPNA.

Results and Discussion.

The synthesis of thyminylmethylglycine (**4**) is the key step for the preparation of thymine isoggPNA monomer (**6**). The coupling reaction of L-serine with 3-benzoylthymine [11] under the Mitsunobu conditions by the same method as that for the coupling between hydroxymethylalanine and 3-benzoylthymine in the preparation of isogaPNA monomers resulted in failure (the starting material 3-benzoylthymine was recovered). The next attempt of the coupling with tosylated compound (**2**) [12] and 3-benzoylthymine under basic conditions did not proceed either (tosylation of BocSer methyl ester underwent facile β elimination at the tosylated position to give methyl acrylate compound). To this end, compound (**2**) was converted to the corresponding iodide (**3**), that was further reacted with 3-benzoylthymine, affording compounds (**4a**) and (**4b**) in 68 % and 11 % yields successfully after flash silica gel chromatography and subsequent recrystallyzation. Removal of the Boc group of **4a** followed by the coupling with FmocGly in the presence of TBTU, finally reductive cleavage of benzyl ester of **5** by the treatment with 10% Pd-C and H_2 gas gave thymine isoggPNA monomer (**6**) (Scheme 1).

In summary, we have achieved the synthesis of novel thymine isoggPNA monomer with the different length of backbone from that of isoga PNA monomers. It's

Scheme 1



Synthesis of 3-(3-Benzoyl-5-methyl-2,4-dioxo-3,4-dihydro-2*H*- pyrimidin-1-yl)-2-[2-(9*H*-fluoren-9-ylmethoxycarbonylamino)acetyl-amino]propionic acid (**6**).

incorporation into aegPNA oligomers and binding studies with DNA and RNA are currently under progress.

EXPERIMENTAL

All the melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR and IR spectra were taken with a JEOL JNM-AL 300 and a JASCO JIR-6500W. Chemical shifts were determined using tetramethylsilane as an internal standard. Mass spectra were obtained from a JEOL JNS-DX303HF Mass Spectrometer. Optical rotation was measured on a JASCO DIP-1000 KUY digital polarimeter. All flash column chromatography was performed using flash-grade silica gel (Kanto Kagaku, 40-100, 60N).

Benzyl 2(*S*)-(*tert*-butoxycarbonylamino)-3-*p*-toluenesulfonyl-propionate (**2**) and Benzyl 2(*R*)-(*tert*-butoxycarbonylamino)-3-iodopropionate (**3**).

These compounds were prepared according to the literature methods [11].

Tosylated compound (**2**): ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.34(s, 9H, CMe3), 2.35(s, 3H, Me), 4.17-4.26(m, 1H, C*H*H), 4.30-4.37(m, 1H, CH*H*), 4.44-4.51(m, 1H, CH), 5.02(d, *J*=12.0 Hz, 1H, PhC*H*H), 5.10((d, *J*=12.0 Hz, 1H, PhCH*H*), 5.24(d, *J*=7.6 Hz, 1H, NH), 7.10-7.36(m, 7H, Ph and arom), 7.64(d, *J*=8.3 Hz, 2H, arom).

Iodide compound (**3**): ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.37(s, 9H, CMe_3), 3.29-3.38(m, 1H, C*H*H), 3.47-3.55(m, 1H, CH*H*), 3.47-3.55(m, 1H, CH), 5.13(s, 2H, CH_2), 7.35(s, 5H, Ph), 7.45(d, *J*=8.1 Hz, 1H, NH).

3-(3-Benzoyl-5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-2-*tert*-butoxycarbonylaminopropionic acid benzyl ester (**4a**) and 2-*tert*-Butoxycarbonylamino-3-(5-methyl-2,4-dioxo-3,4-dihydro-*2H*- pyrimidin-1-yl)propionic acid benzyl ester (**4b**).

To a solution of iodide compound (**3**) (4.06 g, 10 mmol) and 3-benzoylthymine (4.60 g, 20 mmol) in DMF (30 mL) was added anhydrous K_2CO_3 (2.76 g, 20 mmol) at rt. After stirring for 12 h, the solvent was evaporated to dryness. The resulting residue was dissolved in ethyl acetate (200 mL), washed with water (200 mL), and then dried ($MgSO_4$). After concentration of the solution, the crude products were purified by flash chromatography on silica gel with ethyl acetate:hexane (1:2) to give **4a** (68 %) and **4b** (11%). Analytical samples were further purified by recrystalization from ethyl acetate - hexane.

4a: mp 133-134ºC; $[\alpha]_D^{25}$ -3.2º (c 0.1, methanol); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.43(s, 9H, CMe_3), 1.90(s, 3H, Me), 3.70-3.85(m, 1H, C*H*H), 4.34-4.42(m, 1H, CH*H*), 4.59-4.70(m, 1H, CH), 5.07(d, *J*=12.0Hz, 1H, PhC*H*H), 5.19(d, *J*=12.0 Hz, 1H, PhCH*H*), 5.47(d, *J*=6.6 Hz, 1H, NH), 7.02(s, 1H, CH-6 of Thymine), 7.33(s, 5H, Ph), 7.48(dd, *J*=7.8 and 7.8, 2H, PhC=O), 7.63(dd, *J*=7.8 and 7.8 Hz, 1H, PhC=O), 8.04(d, *J*=7.8 Hz, 2H, PhC=O); ^{13}C-NMR(75 MHz, $CDCl_3$) δ: 12.30(Me), 28.21(Me), 50.47(CH_2), 52.26(CH), 68.08(CH_2), 80.68(C), 110.41(C), 128.53(CH),128.63(CH), 129.01(CH), 130.68(CH), 131.59(C), 134.68(C), 134.89(CH), 140.46(CH), 150.06(C), 155.22(C), 163.04(C), 168.86(C), 169.39(C); FAB-MS *m/z*: 508 (M^++1); *Anal.* Calcd for $C_{27}H_{29}N_3O_7$: C, 63.89; H, 5.76; N, 8.28. Found: C, 63.98; H, 5.73; N, 8.30.

4b: mp 191-192ºC; $[\alpha]_D^{23}$ 1.0º (c 0.1, methanol); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.41(s, 9H, CMe_3), 1.85(s, 3H, Me), 4.02(dd, *J*=14.2 and 6.8 Hz, 1H, C*H*H), 4.20(dd, *J*=14.2 and 5.6 Hz, 1H, CH*H*), 4.52(dd, *J*=6.8 and 5.6 Hz, 1H, CH), 5.16(d, *J*=12.0Hz, 1H, PhC*H*H), 5.22(d, *J*=12.0 Hz, 1H, PhCH*H*), 5.46(br, 1H, NH), 6.92(s, 1H, CH-6 of Thymine), 7.34(s, 5H, Ph), 8.25(brs, 1H, NH); ^{13}C-NMR(75 MHz, $CDCl_3$) δ: 11.95(Me), 27.98(Me), 48.12(CH_2), 51.70(CH), 66.38(CH_2), 78.72(C), 108.03(C), 127.89(CH),128.11(CH), 128.39(CH), 135.58(C), 141.80(CH), 150.87(C), 155.26(C), 164.20(C), 169.76(C); FAB-MS *m/z*: 404 (M^++1); *Anal.* Calcd for $C_{27}H_{29}N_3O_7$: C, 59.54; H, 6.25; N, 10.42. Found: C, 59.65; H, 5.95; N, 10.23.

3-(3-Benzoyl-5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-2-[2-(9*H*-fluoren-9-ylmethoxycarbonylamino)acetylamino] propionic acid benzyl ester (**5**).

To a solution of compound (**4a**) (0.51 g, 1 mmol) in 1,4-dioxane (10 mL) was added conc. HCl (8 mL). The reaction

mixture was stirred at rt till the spot of **4a** disappeared on TLC. The solution was neutralized with saturated $NaHCO_3$, extracted with CH_2Cl_2 (3 x 100 mL), and then dried ($MgSO_4$). The organic solvent was evaporated to get the crude deprotected compound of Boc as an oil. After drying under reduced pressure for 24 h, the oil compound was used in the subsequent coupling without purification. Diisopropylethylamine (0.35 mL, 2 mmol) was added to a stirred solution of the crude oily compound, FmocGly (0.30 g, 1 mmol), HOBt (0.15 g, 1 mmol), and TBTU (0.32 g, 1 mmol) in DMF (6 ml) under argon at rt. After stirring for 1.5 h, the solvent was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (100 mL) and the solution was washed consecutively with H_2O (80 mL), 4% aq. $NaHCO_3$ (3 x 80 mL), 5% aq. $KHSO_4$ (3 x 80 mL), H_2O (2 x 80 mL), dried ($MgSO_4$), and the organic solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel with ethyl acetate:*n*-hexane (3:1) and then recrystallization from ethyl alcohol-diethyl ether to give **5** (74% from **4a**). mp 194-195ºC; $[\alpha]_D^{25}$ 5.0º (c 0.1, methanol); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.87(s, 3H, Me), 3.78-3.85(m, 2H, CH_2), 4.13-4.28(m, 2H, CH_2), 4.39(d, *J*=6.9 Hz, 2H, CH_2), 4.82-4.91(m, 1H, CH), 5.08(d, *J*=12.0 Hz, 1H, PhC*H*H), 5.17(d, *J*=12.0 Hz, 1H, PhCH*H*), 5.30-5.38(m, 1H, CH), 7.02(d, *J*=5.2 Hz, 1H, NH), 7.28-7.48(m, 7H, arom), 7.33(s, 5H, Ph), 7.52-7.62(m, 5H, arom and NH), 7.76(d, *J*=7.4 Hz, 2H, arom), 7.93(d, *J*=7.4 Hz, 2H, arom); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 12.24(Me), 44.54(CH_2), 47.04(CH), 49.51(CH_2), 52.11(CH), 67.33(CH_2), 68.28(CH_2), 111.37(C), 120.00(CH), 125.05(CH), 127.08(CH), 127.76(CH), 128.63(CH), 128.72(CH), 128.81(CH), 129.10(CH), 130.60(CH), 131.44(C), 134.58(C), 135.07(CH), 140.21(CH), 141.29(C), 143.67(C), 150.52(C), 156.67(C), 162.88(C), 168.77(C), 169.49(C); FAB-MS *m/z*: 687 (M^++1); *Anal.* Calcd for $C_{39}H_{34}N_4O_8$: C, 68.21; H, 4.99; N, 8.16. Found: C, 67.92; H, 4.95; N, 8.18.

3-(3-Benzoyl-5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-2-[2-(9*H*-fluoren-9-ylmethoxy-carbonylamino)acetyl-amino]propionic acid (**6**).

To a solution of **5** (0.85 g, 1.2 mmol) in methyl alcohol (20 mL) was added 10% palladium on carbon (0.6 g) under argon. After stirring for 0.5-1 h under atmospheric pressure of hydrogen gas, the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (ethyl acetate-methyl alcohol gradient) to give **6** (87%) as a white solid. Analytical sample was purified by recrystallization from CH_2Cl_2 - *n*-hexane. mp 183-184ºC; $[\alpha]_D^{25}$ -9.4º (c 0.1, methanol); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.78(s, 3H, Me), 3.55-3.70(m, 3H, CH_2 and C*H*H), 4.18-4.28(m, 3H, CH_2 and CH*H*), 4.37-4.45(m, 2H, CH x 2), 7.25-7.41(m, 4H, arom), 7.27(s, 1H, CH-6 of Thymine), 7.42-7.56(m, 3H, Ph), 7.61-7.75(m, 3H, Ph and NH), 7.81(br, 1H, NH), 7.87(d, *J*=7.5 Hz, 2H, arom), 8.04(d, *J*=7.5 Hz, 2H, arom); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 11.74(Me), 43.67(CH_2), 46.60(CH), 51.27(CH_2), 52.28(CH), 65.80(CH_2), 107.63(C), 120.09(CH), 125.21(CH), 127.09(CH), 127.61(CH), 129.24(CH), 130.56(CH), 131.32(C), 135.13(CH), 140.69(C), 142.78(CH), 143.80(C), 149.45(C), 156.47(C), 163.03(C), 168.94(C), 170.01(C), 171.12(C); FAB-MS *m/z*: 596 (M^++1); *Anal*. Calcd for $C_{32}H_{28}N_4O8$: C, 64.42; H, 4.73; N, 9.39. Found: C, 64.44; H, 4.88; N, 9.12.

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