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NEW STRATEGY TO ANTIVIRAL AGENTS FROM PEPTIDE NUCLEIC ACID DERIVATIVES

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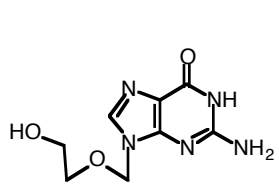
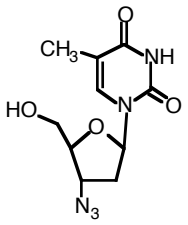
Abstract –New strategy to develop antiviral agents against HIV and herpes virus from the derivatization of peptide nucleic acid monomers has been described.

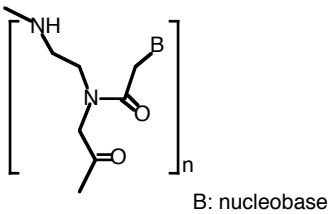
Many antivirals are focused on the inhibition of viral polymerases and reverse transcriptases, which are the key enzymes in the replicative cycle of any virus for chemotherapy.[1] Most available drugs of aciclovir, and AZT are nucleoside analogues, and extensive research towards derivatisation on the sugar moiety of natural nucleoside to enhance antiviral activity has been carried out in numerous laboratories.[2] On the other hand, aminoethylglycine peptide nucleic acids (aegPNAs),[3] reported in 1991 by Nielsen, is a nucleic acid analogue in which the sugar-phosphate backbone is replaced by peptide linkage. Many kinds of nucleotides have been synthesized in order to improve the binding specificity to DNA and RNA, solubility and uptake into cells.[4] However there is no report on the derivatization of PNA monomers, which are potent isosters of natural nucleoside, in order to explore new antivirals. In this communication, we report on the preparation of PNA monomer derivatives (**3a**,**b**) and (**15**) with hydroxyl group in backbone, which is an important group for the phosphorylation by cellular kinases in the activation mechanism of antiviral nucleoside analogues, of isogaPNA[5] and proPNA[6] developed in our laboratory.



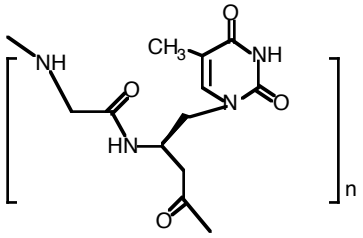
Aciclovir



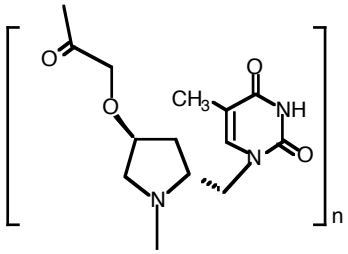
AZT



aegPNA



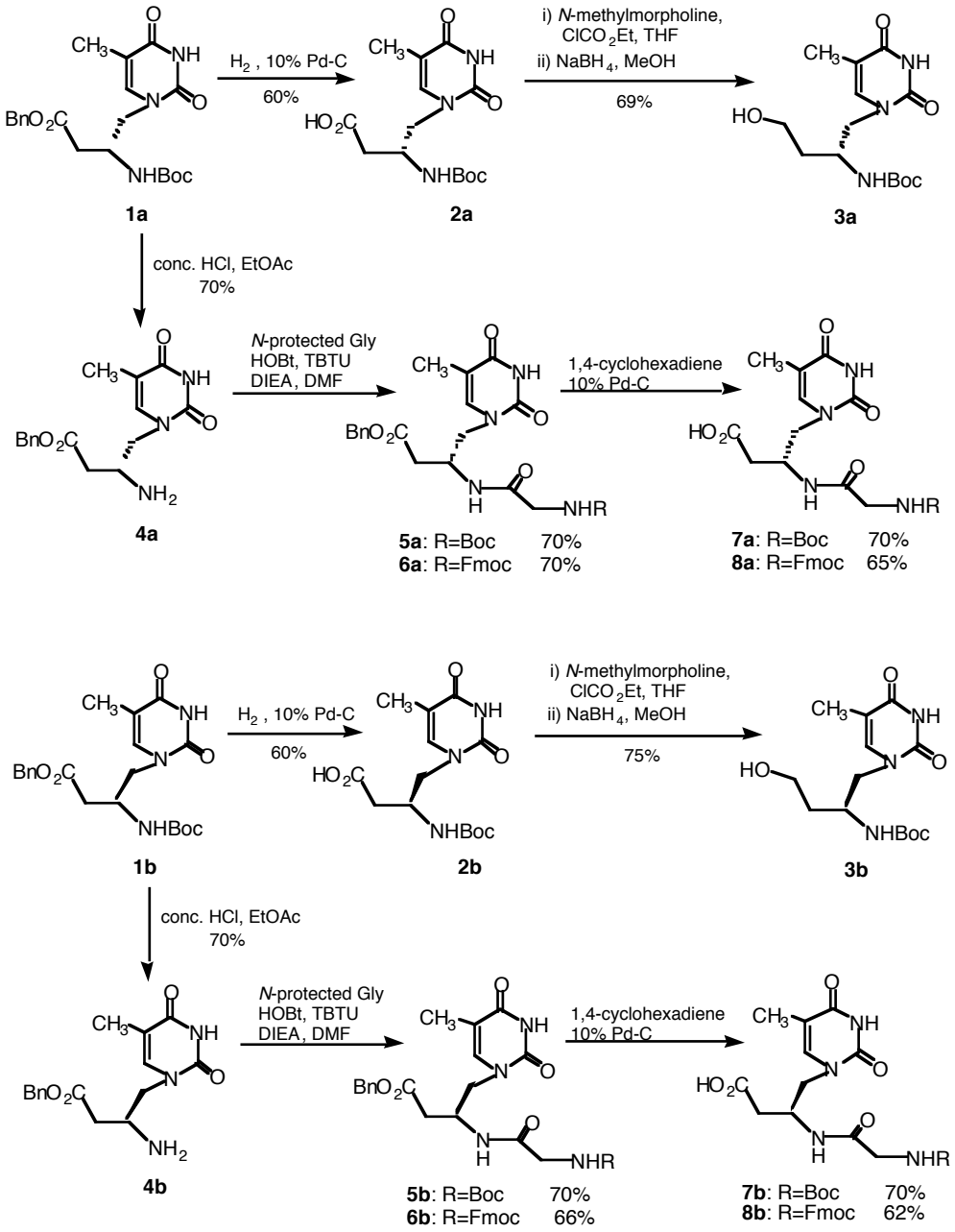
IsogaPNA



ProPNA

The synthesis of 3-hydroxy-1-(thymin-1-ylmethyl)propylamine (**3a**,**b**) proceeds through benzyl ester compounds (**1a**,**b**),[5] which are intermediates of isogaPNA monomers (Scheme 1). Removal of benzyl group of **1a**,**b** with H_2 in the presence of 10% Pd-C followed by reduction of carboxylic compounds (**2a**,**b**) with ethyl chloroformate and sodium borohydride provided our objective compounds (**3a**,**b**).[7,8] We also prepared Boc- and Fmoc-protected isogaPNA monomers (**7a**,**b**) and (**8a**,**b**) for the comparison with hydroxyl compounds (**3a**,**b**). After removal of Boc group of **1a**,**b** with conc. HCl, coupling with *N*-protected glycine followed by deprotection of benzyl group by use of 1, 4-cyclohexadiene and 10% Pd-C afforded **7a**,**b** and **8a**,**b**.

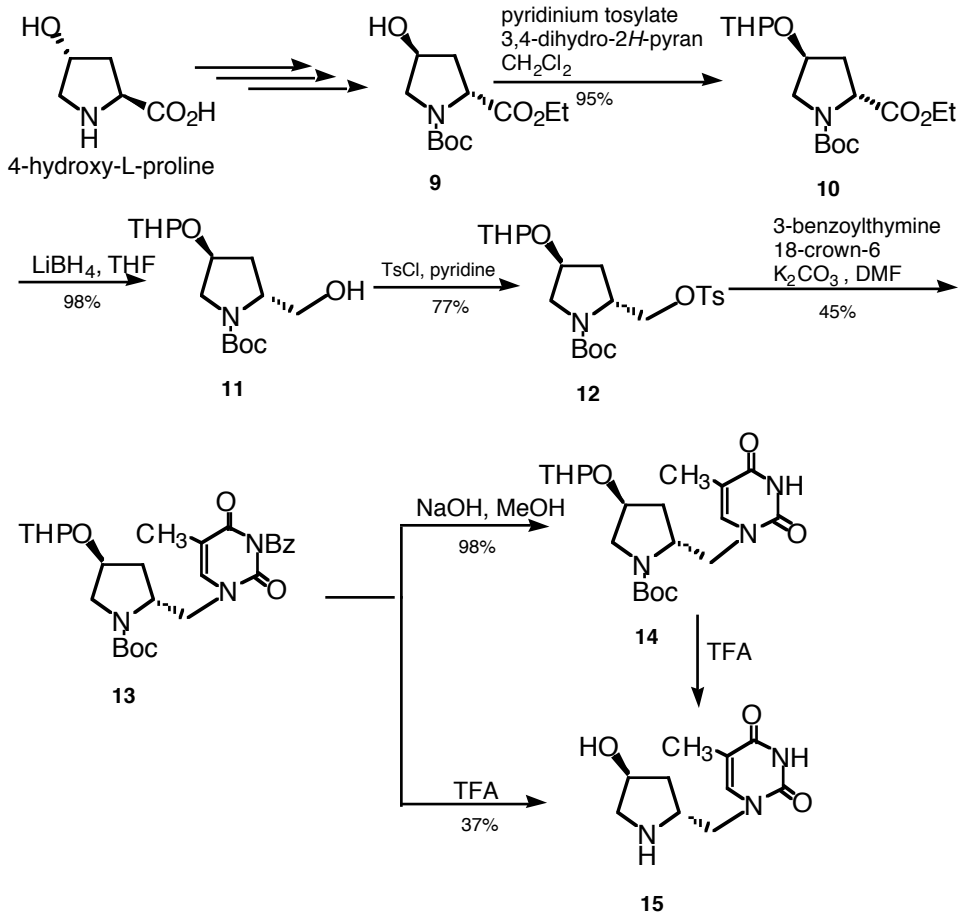
4-Hydroxy-L-proline was used as a starting material for the preparation of 1-(4-hydroxypyrrolidin-2-ylmethyl)thymine (**15**) (Scheme 2). After conversion from 4-hydroxy-L-proline to compound (**9**) according to the method of Lowe and Stille,[9] THP protection of secondary hydroxyl group of **9**, reduction of ethyl ester of compound (**10**) with $LiBH_4$ and tosylation of primary hydroxyl group of compound (**11**) followed by coupling between **12** and 3-benzoylthymine provided THP and Boc protected compound (**13**). After debenzoylation of **13** under basic condition, treatment of compound (**14**) with TFA gave hydroxyl compound (**15**).[10] Alternatively, compound (**15**) was prepared by treatment of **13** with TFA.



Scheme 1

AntiHIV-1 and antiHSV-1 activities of hydroxyl compounds (**3**) and (**15**), isogaPNA monomers (**7**) and (**8**), and the intermediate (**2**, **4**, **6**, **7**, and **14**) were examined by use of Lenti RT activity kit of CAViDiTECH and plaque reduction assay with HSV-1 and Vero cells,[11] respectively. Unfortunately, the antiviral activities against HIV-1 and HSV-1 were not observed in these assays. However, interestingly compound (**8b**) (50 □g/mL) derived from L-aspartic acid only showed potent cytotoxicity on Vero cells that was not shown by compound (**8a**) derived from D-aspartic acid. The chilarity of molecules is one of the important factors to show pharmacological activities. Therefore, it seems to be a merit to develop new

antiviral drugs that the construction of chiral backbone of PNA monomers is relatively easy because of derivatization from chiral amino acids. Versatile derivatization from PNA monomers is worthy of further exploration.



Scheme 2

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8. To a stirred solution of **2** (1.96 g, 6.0 mmol) in THF (30 mL) under argon at -10^{0}C, *N*-methylmorpholine (0.66 mL, 6 mmol) was added, followed by ethyl chloroformate (0.57 mL, 6 mmol). After 10 min, sodium borohydride (0.68 g, 18 mmol) was added in one portion. Methyl alcohol (30 mL) was then added dropwise to the mixture over a period of 10 min at 0°C. The solution was stirred for additional 10 min, and then neutralized with 1*N* HCl (12 mL). The organic solvents were evaporated under reduced pressure and the product was extracted with ethyl acetate (3 x 20 mL). The organic phase was washed consecutively with 1*N* HCl (30 mL), H_2O (30 mL), 5% aq. $NaHCO_3$ (30 mL), H_2O (2 x 30 mL), dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel with dichloromethane : methyl alcohol (15 : 1) and then recrystallization from ethyl acetate – n-hexane to give **3**. **3a** (69%) mp 180°C; $[\alpha]_D^{25}$ +8.5°(*c* 0.1, methanol): ^{1}H NMR (DMSO-d_6) δ: 1.27 (s, 9H, CMe_3), 1.49-1.51 (m, 2H, CH_2), 1.74 (s, 3H, Me), 3.59-3.62 (m, 2H, CH_2), 3.89-3.92 (m, 2H, CH_2), 4.35-4.36 (m, 1H, CH), 6.39 (d, *J*= 8.4 Hz, 1H, NH), 7.22 (s, 1H, CH-6 of thymine), 10.76 (br, 1H, OH); FAB-MS m/z: 314 (M^++1); Anal. Calcd for $C_{14}H_{23}N_3O_5$: C, 53.66; H, 7.40; N, 13.41. Found: C, 53.59; H, 7.51; N, 13.44. **3b** (75%) mp:180°C; $[\alpha]_D^{25}$ -8.0°(*c* 0.1, methanol): ^{1}H NMR (DMSO-d_6) δ: 1.27 (s, 9H, CMe_3), 1.50-1.51 (m, 2H, CH_2), 1.74 (s, 3H, Me), 3.59-3.62 (m, 2H, CH_2), 3.89-3.92 (m, 2H, CH_2), 4.34-4.38 (m, 1H, CH), 6.41 (d, *J*= 8.4 Hz, 1H, NH), 7.23 (s, 1H, CH-6 of thymine), 10.75 (br, 1H, OH); FAB-MS m/z: 314 (M^++1); Anal. Calcd for $C_{14}H_{23}N_3O_5$: C, 53.66; H, 7.40; N, 13.41. Found: C, 53.59; H, 7.51; N, 13.44.
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10. The solution of **13** (0.18 g, 0.33 mmol) in TFA (5 mL) was stirred for 2 h. Ether (20 mL) was added into the reaction mixture. The precipitated crystals were collected by filtration with suction and

washed with ether (50 mL) to give TFA salt of **15** 0.07 g (37 %). mp 185°C; $[\alpha]_D^{25}$ +7.5°(*c* 0.1, methanol): ^{1}H NMR (CD_3OD) δ: 1.78 (s, 3H, Me), 1.66-1.91 (m, 1H, CHH-3), 2.04-2.15 (m, 1H, CHH), 3.09 (d, *J*= 13.7 Hz, 1H, CHH-5), 3.43 (dd, *J*=13.7 Hz and *J*= 5.0 Hz, 1H, CHH-5), 3.89-4.17 (m, 3H, CH-4 and CH_2), 4.42-4.50 (m, 1H, CH-2), 7.36 (s, 1H, CH=); FAB-MS m/z: 340 (M^++1); Anal. Calcd for $C_{12}H_{16}N_3O_5F_3$: C, 42.48; H, 4.75; N, 12.39. Found: C, 42.48; H, 4.69; N, 12.03.

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