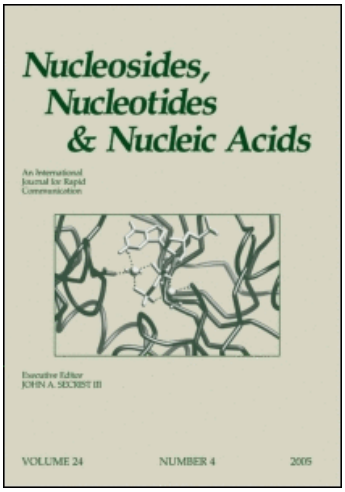

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SYNTHESIS AND EVALUATION OF PROLYL CARBAMATE NUCLEIC ACIDS (PrCNA)

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SYNTHESIS AND EVALUATION OF PROLYL CARBAMATE NUCLEIC ACIDS (PrCNA)

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ABSTRACT

Carbamate linked prolyl nucleic acids are obtained in high yield and purity under mild conditions in solution and solid phase. *p*-Nitrophenylchloroformate is used as the activating reagent for alcohol. Homooligomers of PrCNA do not bind to DNA. The introduction of this modification in PNA sequences destabilizes the triplxes, inspite of enhancement in the base stacking.

Carbamate linked nucleic acids are known to bind with complementary DNA strands with higher thermal stability (1,2). Carbamate linkage being uncharged these are expected to be capable of penetrating the cell membrane and are more stable towards enzymes (3). Earlier, prolyl nucleic acids having peptide backbone were prepared in our laboratory and it was found that the homooligomers of prolyl PNA did not bind to complementary DNA strand (4). This was probably because of the constraint in the flexible PNA backbone (5). We replace the peptide linkages in prolyl nucleic acids with carbamate linkages thus increasing the number of atoms in the backbone while keeping the stereogenic centers intact. Here, we report the synthesis and the binding studies of the carbamate linked prolyl nucleic acids.

The activated monomer building blocks **4a** and **4b** were synthesized from *trans*-L-hydroxy proline **1** (Scheme 1). After stepwise protection of ring nitrogen as benzylcarbamate and acid function as methyl ester the hydroxy group was converted to the corresponding azide in two steps. The azide was then selectively hydrogenated using Raney Ni and the resultant amine was protected as *t*-butoxycarbamate. The

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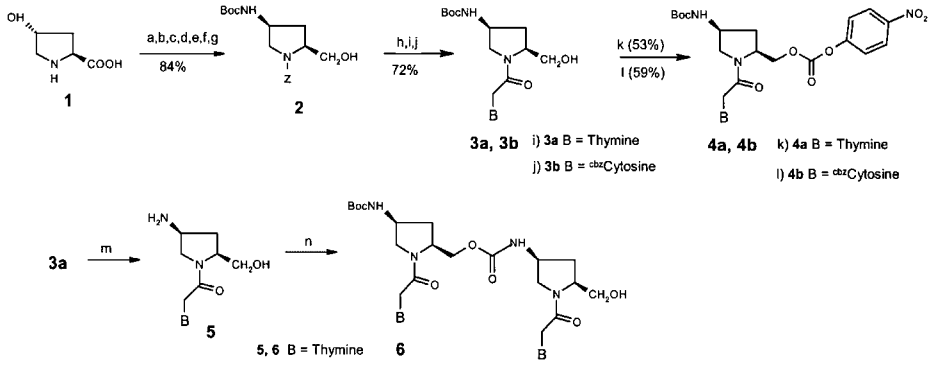

Scheme 1. (a) CBzCl, $NaHCO_3$ Water/Dioxane (b) $SOCl_2$, MeOH (c) MsCl/py (d) NaN_3, DMF, 70°C (e) H_2, Raney Ni, MeOH (f) $BocN_3$, TEA, DMSO, 50°C (g) $LIBH_4$, THF (h) H_2, Pd-C, MeOH (i)Thyminylacetic acid, DCC, HOBt, DMF (j) 4-N-CBz-cytosinylacetic acid, DCC, HOBt, DMF (k) *p*-nitrophenylchloroformate, Dioxane, Py (l) *p*-nitrophenylchloroformate, Dioxane, TEA (m) 50% TFA./DCM, DIPEA (n) DMF/DIPEA.

resulting protected ester was reduced using $LiBH_4$/THF to get **2**. The Ring nitrogen was then deprotected followed by coupling with nucleobaseacetic acid in presence of DCC and HOBt to obtain **3a** (B = Thymine, T) and **3b** (B = Cytosine, C^{CBz}). The Boc protected amino alcohols **3a** and **3b** were activated to give the corresponding *p*-nitrophenylcarbonates (6) **4a** and **4b** respectively. All the new compounds were characterized by ^1H, ^{13}C NMR and mass spectrometry (7).

The activated monomer synthons **4a** and **4b** along with standard PNA monomers (8) were used to synthesize the oligomer sequences **6** (in solution phase) (1,2) and **7–13** (on solid support) using standard protocols. The sequences **7–13** were cleaved from the support using standard conditions used for the cleavage of peptides (8). Stability of the carbamate linkage under these conditions was established by independent treatment of **6** with TFMSA/TFA and methanolic ammonia and HPLC and mass spectral analysis of the samples. The carbamate linkage was found to be stable in acidic condition but degradation was observed under basic condition. The oligomers **7–13** were purified by FPLC and purity was checked by HPLC. The homogeneity of each of the oligomer was established by MALDI-TOF

PrCNA/PNA sequences

6 Boc-T*T–OH*
7 H-T*T*T*-(β-ala)-OH
8 H-T T T T T T T T-(β-ala)-OH
9 H-T T T T T T T*T-(β-ala)-OH
10 H-T T T T* T T T T-(β-ala)-OH
11 H-T* T T T T T T T-(β-ala)-OH
12 H-T* T* T* T* T* T* T* T*-(β-ala)-OH
13 H-T* C* T* C* T* T* T* T*-(β-ala)-OH

DNA sequences

14 5′-G C A A A A A A A A C G-3′
15 5′-A A A A A A G A G A-3′

Scheme 2.

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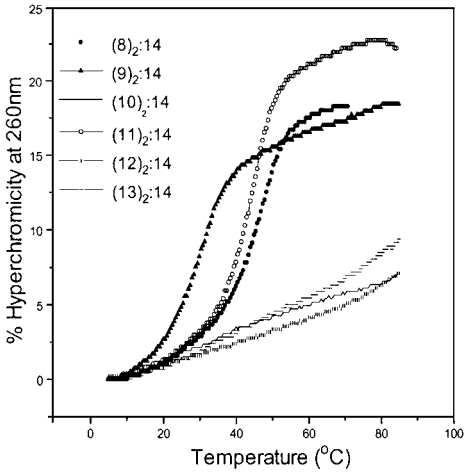

Figure 1. Melting curves for $(PrCNA/PNA)_2$: DNA complexes in Sodium phosphate buffer at pH 7.3. Concentration of DNA strand was taken as 1.5 μM.

mass spectrometry (7). The effect of the carbamate linked prolyl units on the structural features of the backbone of the oligomers was studied using CD spectroscopy. Compounds **5**, **6** and **7** showed sequential enhancement in the CD signal at 260 nm with increasing number of three prolyl thymine units and plateaued in the prolyl octamer.

The UV melting studies of the complexes of oligomers sequences **8**–**13** with complementary DNA strands **14**–**15** were performed to evaluate the thermal stability (Fig. 1). The homooligomers **12** and **13** did not bind to DNA. PNA sequence, **10**, with T^* at the center of the sequence did not bind to DNA. When the modification is at the N-terminus as in PNA **11**, marginal destabilization compared to PNA_2:DNA

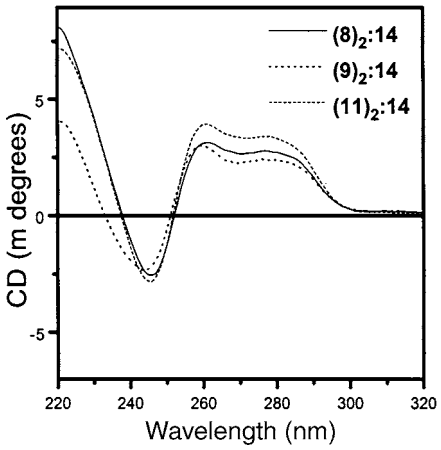

Figure 2. CD curves for triplexes in Sodium phosphate buffer at pH 7.3.

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Table 1. UV Melting Temperatures of Triplexes

Complexes	$(\mathbf{8})_2$:**14**	$(\mathbf{9})_2$:**14**	$(\mathbf{10})_2$:**14**	$(\mathbf{11})_2$:**14**	$(\mathbf{12})_2$:**14**	$(\mathbf{13})_2$:**15**
Tm °C	44.5	31.5	—	44	—	—
% Hyperchromicity	18.2	18.4	6.8	22.2	7.1	9.3

complex was observed whereas the C-terminal modification in **9** destabilized the triplex by 13°C. The reassociation process of DNA hybridization was monitored by decrease in absorbance with lowering temperature. Absorbance Vs temperature profiles for PrCNA:DNA complexes showd that the rate of reassociation to form triplexes is slower than the rate of dissociation and is similar to DNA:PNA complexes. CD curves obtained for complexes $(\mathbf{9})_2$:**14** and $(\mathbf{11})_2$:**14** were similar in pattern to $(\mathbf{8})_2$:**14** (Fig. 2).

In conclusion, we have successfully developed solid phase synthesis chemistry for carbamate linked oligomers and the insertion of prolyl carbamate linkage into PNA. The results from DNA binding studies show that carbamate linked prolyl homooligomers do not bind to DNA possibly due to incompatible internucleobase distances in the PrCNA. Placement of carbamate linkage either at C-terminal or N-terminal in PNA causes destabilization of triplexes.

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7. Compound **4a** ^{1}H NMR in $CDCl_3$ δ ppm 8.25 (d 2H phenyl J = 0.2), 7.4 (d 2H phenyl J = 0.2), 7 (s 1H thy 6H), 4.5 (m 5H OMe & Pro C5), 1.9 (3H Thy Me), 1.4 (d 9H Boc). Compound **4b** ^{1}H NMR in $CDCl_3$ δ ppm 8.25 (d 2H nitroPhe), 7.6 (d 1H Cyt C6 J = 0.15), 7.25 (m 7H cbz, nitroPhe, Cyt C5), 5.2 (s 2H benzyl CH2), 4.5 (m 5H benzyl CH2, Proline C2-mehyl, C5), 1.4 (s 9H t-Boc). Mass (FAB) m/z = 667(M+1), 689(M+Na) calculated mass 666. Compound **6** Mass(FAB) m/z = 691 (M+1), 713(M+Na) Calculated mass 690. Compound **9** Mass (MALDI TOF) m/z = 2268.8 calculated mass 2259 and **10** m/z = 2266.5 calculated mass 2259.
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