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STUDIES ON THE SYNTHESIS AND PROPERTIES OF NEW PNA ANALOGS CONSISTING OF L- AND D-LYSINE BACKBONES

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Abstract: Homopentamers of PNA consisting of L- and D-lysine were synthesized. It was found that the intramolecular hydrogen bonding has no significant influence on intermolecular interaction. These pentamers show a tendency to form complex with polyA in tris(pH 8.0) solution. The L-conformer has more potential than D-isomer.

Peptide Nucleic Acid(PNA), a new class of DNA mimic, ever since its first appearance in 1991[1], has attracted much attention of chemists owing to its high hybridization affinity and biological stability. The invaluable synthetic oligomers thus have been used as labeled probes for gene detection and potential therapeutic agents[2]. Although the potential of PNA as antisense and antigene agents is actively being explored, the problems associated with creating in vivo bioefficacy, including cell membrane permeability and diverse biochemical process in delivery, are formidable. A lot of modified PNA molecules[2, 3], bearing various backbones and linkers, have been synthesized in an aim to make further improvement on properties of such kind of molecules. Ornithine, a chiral amino acid with an amino group in its side chain, has also been used as backbone in a new type of PNA. Four groups[4] have reported their studies on corresponding oligomers based on L- or D-ornithine respectively, and found that such PNAs hardly interact with complementary polynucleotide. Conformation studies by Inaki[4d] revealed that stable intramolecular hydrogen bonding was formed between thymine base and ornithine unit(Figure 1), which in turn, may inhibit the intermolecular interaction between the oligomer and complementary sequences. In this paper, we reported the synthesis of another type of PNA, with natural amino acid, L- and D-lysine as backbones, in the hope that one carbon longer in the flexible main chain will diminish the intramolecular interaction in backbone of PNA, thus facilitate interaction with complementary DNA or RNA.

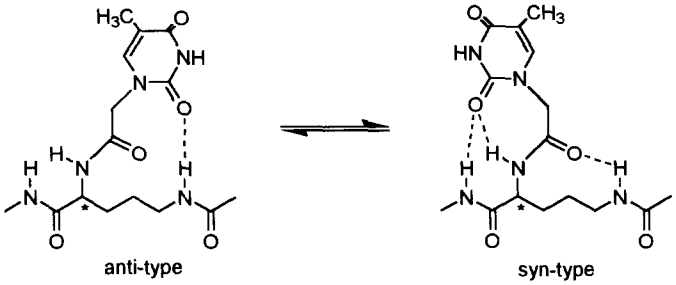

Figure 1 Intramolecular interaction in PNA with ornithine as backbone

Homo pentamers of PNA consisting of L- and D-lysine backbone(Figure 2), were synthesized respectively from corresponding blocks we previously reported[5]. Reactions in solution as peptide synthesis were used to

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elongate the chain one by one. The possible major conformation of PNA in solution was studied by 2D-NMR and molecular modeling. It was found that intramolecular hydrogen bonding only appeared between ε-NH of the lysine unit and carbonyl oxygen in the linker. It seems to have no significant influence on intermolecular interaction. Comparison of UV spectra of monomers, dimers and pentamers indicated that there's little hypochromic effect existing. Also, the results of CD illustrated little stacking was found between bases in such kind of PNA molecules. However, interaction between pentamers and complementary RNA was found and the pentamer consisting of L-lysine showed more potential than D-isomer.

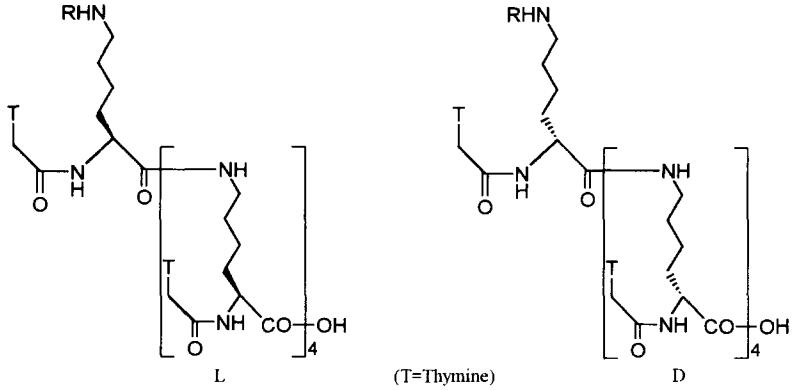
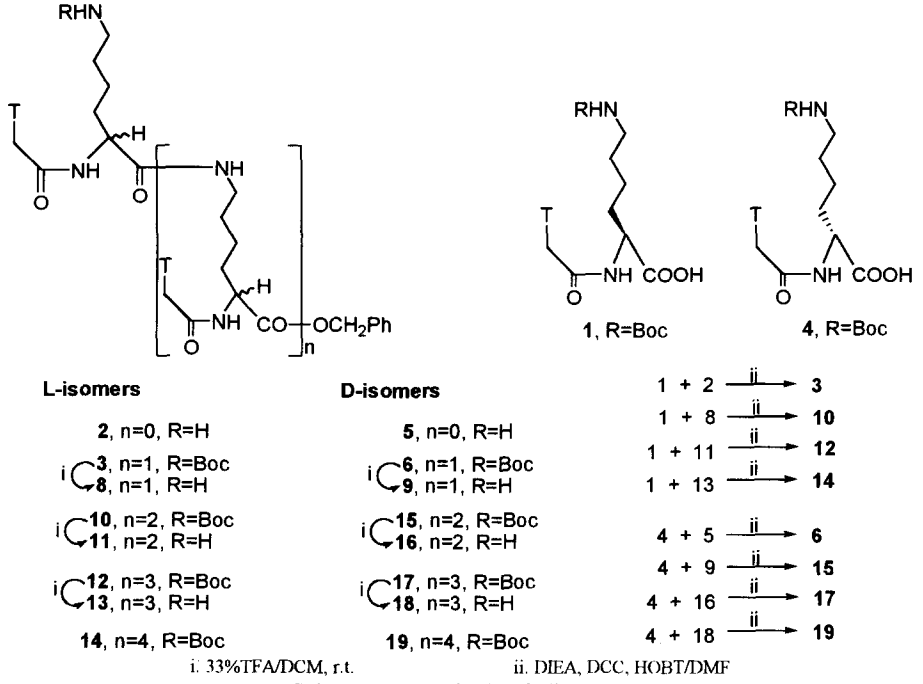


Figure 2 Homo pentamers of PNA consisting of L- and D-lysine

Protected pentamers consisting of L- and D-lysine backbones, 14 and 19, were prepared via reactions

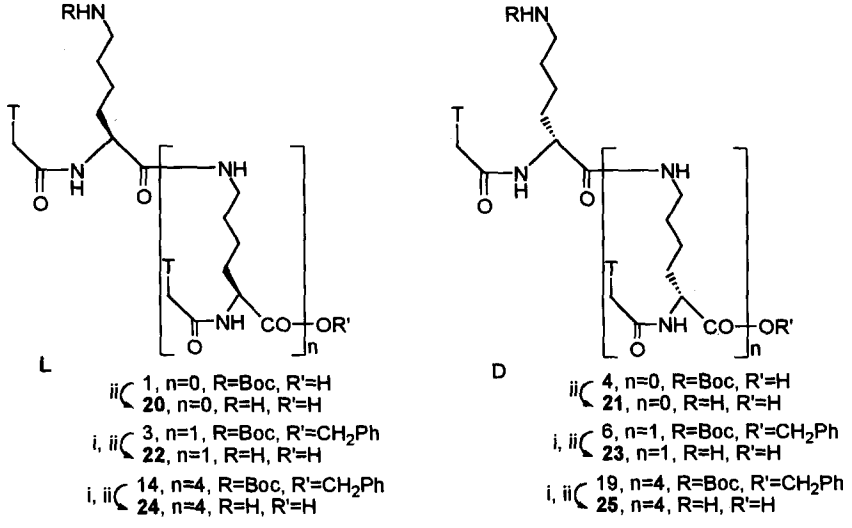


Scheme 1 Synthesis of oligomers

between the monomeric free acid component **1** or **4**[5] and free amine components to prolong the "peptide" chain one by one (Scheme 1). Peptide bond formation was achieved in presence of equimolar diisopropylethylamine (DIEA), using DCC and HOBT as coupling reagents(Scheme 1) to give oligomers in an average yield of 60%.

The optical purity of dimers was analysed by HPLC(C_{18} column, 15%CH_3CN/H_2O, 0.1%TFA), and it indicated than racemization less than 7% occurred during coupling.

Fully deprotected monomers 20, 21, dimers 22, 23 and pentamers 24, 25 were obtained from corresponding protected compounds via two steps of deprotection, with overall yield about 80% for monomers, 50% for dimers and 40% for pentamers(Scheme 2).



Scheme 2 Preparation of monomers, dimers and pentamers

The structural determination of all these compounds were confirmed by NMR and MS data[6]. The high-resolution FAB MS or TOF-MS data of the fully deprotected monomers, dimers and pentamers confirmed their composition. Peaks of fragments could also been found in TOF-MS of oligomers. For example, in TOF-MS of pentamer 25, besides peak at 1498.28 belonging to compound 25, another two peaks(901.24 and 1195.17) were assigned to trimer and tetramer, respectively. Solubility of fully deprotected monomers and dimers in water is quite good, while that of pentamers was relatively lower(<0.5mg/mL).

In ^{1}HNMR of monomer 20, the peak of methylene protons at N^{1}of thymine was split into doublet, while NOESY revealed its relation with 6-H of thymine(Figure 3). This indicated the existence of some barrier for the free rotation of thymine base. Besides, according to NOESY spectra of 20 and by the aid of molecular modeling, it was suggested that proton of the carboxylic acid could form hydrogen bond with the oxygen atom of 2-carbonyl group in thymine(Figure 3).

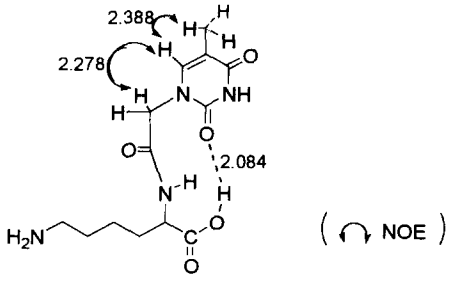


Figure 3 Molecule mechanics optimized conformation of compound 20

^{1}HNMR and NOESY of dimers(22, 23), pentamers(24, 25) showed similar characteristics as that of monomer (Fig.4). The methylene proton at N^1 of thymine, besides NOE relationship with 6-H of thymine, also showed related to α-NH of lysine unit. On the other hand, NOE signal also appeared between ε-NH and α-CH in the N-terminal lysine unit. Combined with the molecular modeling results, the oxygen atom in the methylene carbonyl linker and the proton at ε-NH are close enough to each other to form intramolecular hydrogen bonding. Such intramolecular interaction is different from the manner in the case of ornithine, for there is no intramolecular hydrogen bonding between O^2 of thymine and ε-NH in the backbone.

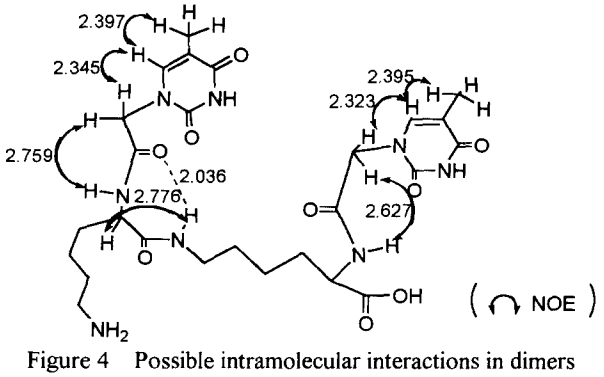


Figure 4 Possible intramolecular interactions in dimers

The intramolecular hydrogen bonding in compounds 24 and 25 was suggested in Figure 5, the longer -CH_2- chain made the backbone more flexible. It would be expected to diminish the intramolecular interaction between bases and the main chain and therefore, it may be favourable to form complex with target sequence. This means that such relatively flexible backbone made it possible for the oligomers to adopt a suitable conformation when interacting with complementary DNA or RNA.

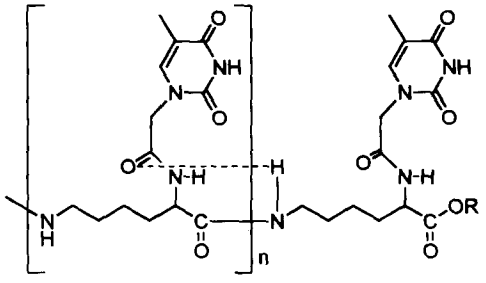


Figure 5 Possible intramolecular interactions in lysine backbone PNA

Table 1 Comparison of absorption between A-PNA molecules

Compd.	L-configuration			D-configuration		
	λ_{max}(nm)	$\varepsilon\times10^{-4}$	Hypochromism (λ_{max})	λ_{max}(nm)	$\varepsilon\times10^{-4}$	Hypochromism (λ_{max})
Monomer	268.4	0.83	0.00	268.8	0.86	0.00
Dimer	268.0	0.83	0.00	268.5	0.80	0.07
Pentamer	268.5	0.75	0.10	268.2	0.80	0.07

No significant hypochromic effect could be found comparing the absorption coefficient per residue between monomers, dimers and pentamers[7], which indicates that there's little stacking between bases. Same conclusion could be obtained from the comparison between CD of these compounds. All the compounds with same configuration studied here gave a similar CD(Figure 6). The induced Cotton effects were thought to be caused by the interaction between the transition moment of thymine and the chiral lysine unit, where the free rotation of thymine may be somewhat inhibited by intramolecular interaction mentioned above.

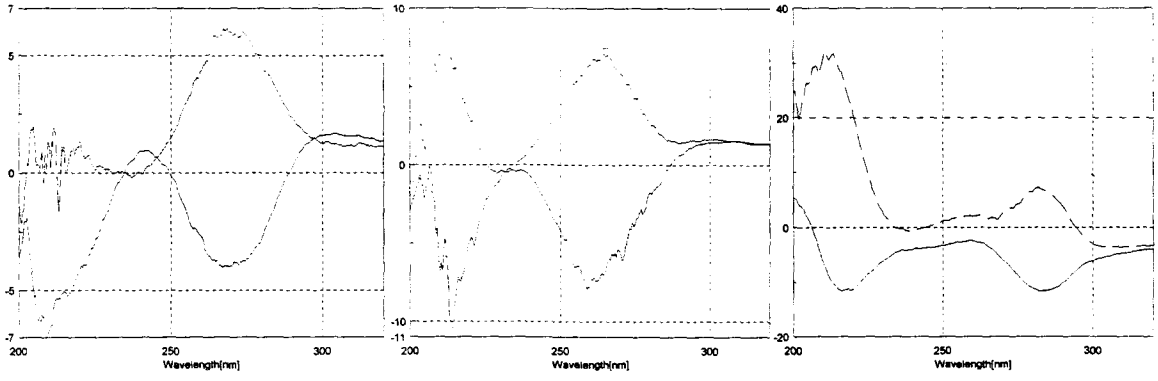


Figure 6 CD spectra of monomers(left), dimers(middle) and pentamers(right) in H_2O, all the componds of L-configuration have negative CD signals, while compounds of D-configuration have positive CD signals

In order to explore hybridizing property of PNA molecules synthesized, the CD spectra of mixture solution containing pentamers 24, 25 and complementary DNA(dA_7) or RNA(polyA) in tris buffer(pH 8.0, 100mM) were compared. The CD spectra of pentamers in the presence of dA_7 showed no intermolecular interaction between 25 and dA_7, but in the case of 24, CD spectra showed some change(Figure 7); more interestingly, the CD spectra of compound 24, 25 in presence of polyA(Figure 8) show a significant change both on shape and intensity of signal

comparing with CD of both components, especially in the case of compound 24. The changes in CD characteristics indicate that some interaction do exist between pentamers and dA_7, or between pentamers and polyA, and the pentamer consisting of L- lysine is more potential than the D-analog to form complex with polyA.

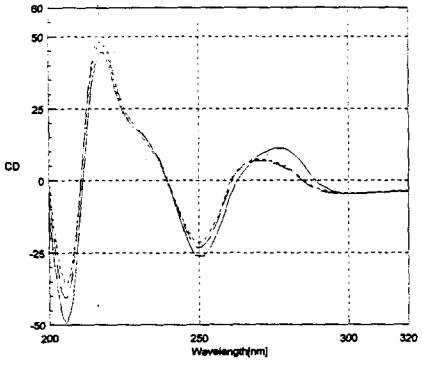


Figure 7 CD of 24-dA_7(—), 25-dA_7(...) and dA_7(---)

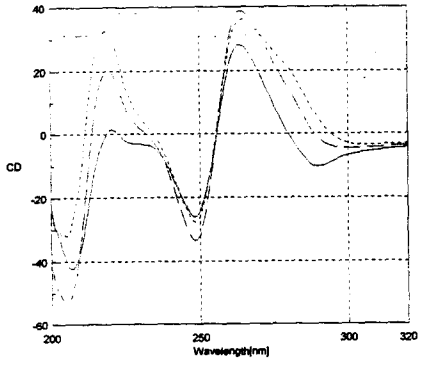


Figure 8 CD of 24-polyA(—), 25-polyA(...) and polyA(---)

In conclusion, a new class of PNA analogs consisting of L- and D-lysine were prepared from lysine units, compound 1 and 4, in solution. A new model of intramolecular interaction was suggested on the bases of UV, CD and NMR studies. Preliminary study on CD spectra of mixtures containing pentamers synthesized and complementary DNA, especially RNA, indicate that interaction between the two complementary chain may be possible. On the other hand, it's expected that the lipophilic character of the backbone would facilitate the cell permeability of these compounds. It is also encouraging that the oligomers appear soluble in aqueous solution till now. Considering the advantageous properties of such backbones, it may have the potential for the use in the design of antisense drugs.

Acknowledgements: We thank the National Science Foundation of China for the financial support.

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6. Data of **20** HRFAB-MS: 313.1506(Cacl. mass: 313.1506); ^{1}H-NMR(d_6-DMSO) δ ppm 1.343-1.749(9H, m, $C\underline{H}_2$×3, 5-$C\underline{H}_3$), 2.760(2H, m, ε-$C\underline{H}_2$), 4.211(1H, m, α-C<u>H</u>), 4.343(dd, 2H, -$C\underline{H}_2$), 7.432(1H, s, 6-<u>H</u>), 7.707 (3H, b, ε-$N\underline{H}_3^+$), 8.500(1H, d, J=8Hz, α-N<u>H</u>), 11.283(1H, b, N^3-<u>H</u>). Data of **22** HRFAB-MS: 607.2838(Cacl.: 607.2834); ^{1}H-NMR(d_6-DMSO) δ ppm 1.243-1.744(18H, m, 6×$C\underline{H}_2$, 2×5-$C\underline{H}_3$), 2.746, 3.035(2H×2, m, 2×ε-$C\underline{H}_2$), 4.173-4.391(6H, m, 2×α-C<u>H</u>, 2×-$C\underline{H}_2$T), 7.426(2H, m, 6-<u>H</u>×2), 7.660(3H, b, ε-$N\underline{H}_3^+$), 7.957(1H, t, J=5.6Hz, -N<u>H</u> in peptide bond), 8.366, 8.466(1H×2, d, J=8.4Hz, J=7.6Hz, α-N<u>H</u>×2), 11.276(2H, m, N^3-<u>H</u>×2). Data of **24** TOF-MS: 1489.59(Cacl. mass 1490.57)
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