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cis-Cyclopentyl PNA (*cp*PNA) as constrained chiral PNA analogues: stereochemical dependence of DNA/RNA hybridization†

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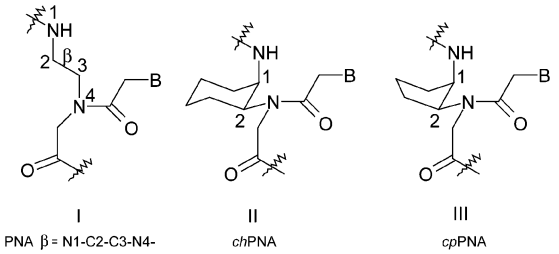
DNA/RNA hybridization studies of PNA-T oligomers with *cis*-(1*S*,2*R*/1*R*,2*S*)-cyclopentyl units in the backbone show stereochemistry dependent binding with RNA/DNA discrimination.

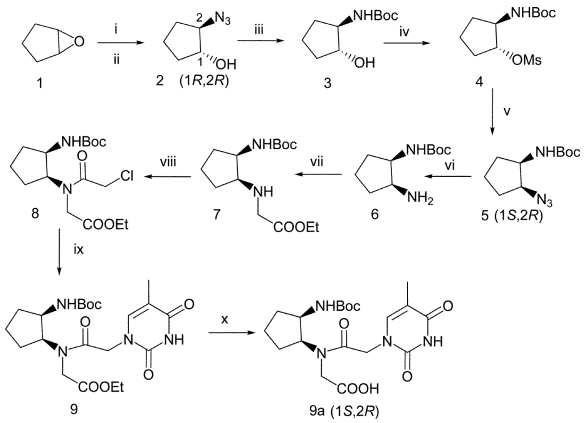
Peptide nucleic acids (PNA, **I**) are oligonucleotide mimics in which the sugar-phosphate backbone is replaced by a pseudopeptide scaffold composed of *N*-(2-aminoethyl)glycine units.[1] The nucleobases A/T/C/G are attached to the scaffold *via* carbonylmethyl spacers. PNA binds to complementary DNA/RNA with high sequence specificity and selectivity to form duplexes *via* Watson–Crick base pairs and triplexes through Watson–Crick and Hoogsteen hydrogen bonding.[1] Because of the higher thermal stability of PNA : DNA/RNA complexes compared to analogous DNA/RNA complexes and their stability to proteases and nucleases, PNAs are of great interest in medicinal chemistry, for the design of gene targeted drugs and in molecular diagnostics.[2] Attempts to understand the structure–activity relationship and thereby improve application oriented properties such as solubility, cell permeability and binding orientation, have resulted in several structural modifications of PNA.[3] These comprise ligand conjugation, introduction of chirality in the achiral PNA backbone and modifications to conformationally preorganize the PNA strand to entropically drive the complex formation.



An attribute of equal importance is imparting discrimination in PNA binding to DNA and RNA. A comparison of the available X-ray structural data of PNA_2 : DNA triplex,[4] PNA : DNA duplex[5] and NMR data of PNA : RNA duplex[6] suggests that the dihedral angle β in the PNA backbone could be a key factor. The preferred values for β in PNA_2 : DNA triplex and PNA : RNA duplex are in the range 60–70° while that for PNA : DNA duplex is about 140°, suggesting that it may be possible to impart DNA/RNA binding selectivity by tuning β through suitable modifications. In this context, we recently reported[7] cyclohexyl PNA (*ch*PNA) **II**, in which the axial–equatorial disposition of *cis*-1,2 substituents with β in the range 63–66° (opposite in sign for the two enantiomers (1*S*,2*R* and 1*R*,2*S*) showed a stereochemistry dependent preferential binding of the derived PNA-T oligomers to RNA as compared to DNA. The cyclohexyl ring is inherently too rigid as it gets locked up in either of the chair conformations. A relatively flexible system would be cyclopentane (**III**) where due to the characteristic puckering that dictates the pseudoaxial/pseudoequatorial dispositions of substituents,[8] better torsional adjustments are possible to attain the necessary hybridization competent conformations. Here we report the synthesis and X-ray structures of *cis*-(1*S*,2*R*) and (1*R*,2*S*)-cyclopentyl PNA (*cp*PNA) thymine **9**, their incorporation into PNA oligomers **10–18** and comparative DNA/RNA binding studies. While this work was in progress, Apella *et al.*[9] reported synthesis and preliminary studies on mono-substituted *trans*-(1*S*,2*S*)-cyclopentyl PNA that showed marginal stabilization of the derived PNA_2 : DNA triplex over that of the PNA : RNA duplex. Our present results on cyclopentyl PNAs having *cis* isomers suggest that these have a stereochemistry dependent stabilization effect on binding both DNA and RNA.

The synthesis of the target monomers **9a** and **9b** was achieved starting from (+)- and (−)-*trans*-2-azidocyclopentanols **2** which were obtained by enzymatic hydrolysis of the racemic 2-azidocyclopentyl butanoate using lipase[10] from *Pseudomonas cepacia* (Amano-PS) in phosphate buffer (pH = 7.2) followed by chromatography to obtain the optically pure (1*R*,2*R*)-azido cyclopentanol **2** (Scheme 1). The reduction of the azide **2** with H_2/PtO_2 and *in situ* *t*-Boc protection of the resulting amine function yielded **3** which was converted to the mesylate **4** using mesyl chloride in presence of triethylamine. This was treated with NaN_3 in dry DMF to yield the *cis* azide **5** (1*S*,2*R*), accompanied by inversion of configuration at C1. Hydrogenation of **5** using PtO_2 catalyst gave amine **6**, which was alkylated with ethyl bromoacetate in the presence of KF-celite to yield **7**.

This on acylation with chloroacetyl chloride gave **8** which was condensed with thymine in the presence of K_2CO_3 to yield *cis*-(1*S*,2*R*)-aminocyclopentyl glycyl thymine ethyl ester **9**. Upon hydrolysis using 0.5 M LiOH, ester **9** gave the required (1*S*,2*R*)-cyclopentyl PNA thymine monomer **9a**. The synthesis of the enantiomeric (1*R*,2*S*)-cyclopentyl PNA thymine monomer **9b** was accomplished starting from alcohol **2** (1*S*,2*S*) by following similar steps as above. The reported method of Apella *et al.*[9] does not allow access to the present diastereomers. All compounds were characterized by ^1H, ^{13}C NMR and mass spectroscopic analysis. The enantiomeric purity of the target monomers was established by



Scheme 1 (i) NaN_3–NH_4Cl; Bu_2O (93%), (ii) lipase, sodium phosphate buffer, pH 7.2; (iii) H_2–PtO_2; $(Boc)_2O$ (83%); (iv) MsCl, (93%); (v) NaN_3, dry DMF (92%); (vi) H_2–PtO_2, (98%); (vii) $BrCH_2COOEt$, KF-celite, dry CH_3CN (79%); (viii) $ClCH_2COCl$, Na_2CO_3, dioxane/H_2O (1 : 1) (76%); (ix) thymine, K_2CO_3 (73%); (x) 0.5 M LiOH/aq. THF (98%).

† Electronic Supplementary Information (ESI) available: Experimental procedures for the synthesis of compounds, ^1H, ^{13}C NMR, mass spectral data, crystal structural data and melting curves for triplexes. See http://www.rsc.org/suppdata/cc/b3/b317000d/

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optical rotations and the structures unambiguously confirmed by X-ray crystal data of both enantiomers (Fig. 1). The absolute configurations were derived from a knowledge of the configuration of the starting materials. The torsional angles β in both the cases were around 25°, much less than those in reported PNA_2 : DNA or PNA : RNA complexes[4–6] as well as in cyclohexane analogues.[7]

The monomers **9a** and **9b** were incorporated into *aeg*-PNA-T_8 oligomer **10** at defined positions by standard solid phase synthesis followed by cleavage with TFA–TFMSA and purification by reverse phase HPLC. All PNA oligomers (**10–18**) were characterised by MALDI-TOF. The T_m values of various PNAs hybridized with complementary DNA **19**, mismatched DNA **20** and poly rA were determined from temperature-dependent UV absorbance plots and are shown in Table 1. A Job plot generated from CD data indicated a binding stoichiometry of 2 : 1 for all DNA complexes indicating the formation of PNA_2 : DNA triplexes.

The data in Table 1 suggest that the T_m of *cp*PNA complexes (except PNA **12**) of both stereochemistry with complementary DNA **19** and poly rA were significantly higher than the corresponding complexes of control PNA **10**. Among the *cp*PNA : DNA complexes, *RS* isomers (PNA **14–16**) gave a higher T_m compared to *SR* isomers (PNA **11–13**). The C-terminal substitution stabilized the DNA and RNA complexes better than N-terminal substitution. In case of *cp*PNA : poly rA complexes (barring *cp*PNA **15**), the presence of either *SR*/*RS* isomers at the C/N terminus or internally, stabilized the complexes. Importantly, the *RS* and *SR* homooligomeric PNAs **17** and **18** exhibited enormous stabilization of both DNA and RNA complexes as compared to that of control PNA **10**. The stronger binding of *cp*PNA with DNA is achieved without losing binding selectivity as substantiated by the T_m values of mismatched *cp*PNA : DNA **20** complexes. The control PNA **10** complex was less stable by 13° due to mismatch while the destabilisation of *cp*PNA : DNA **20** complexes was larger by 18–30°. Thus the *cp*PNAs have a better selectivity (lower mismatch tolerence) and a higher binding to cDNA sequences than the unmodified PNA.

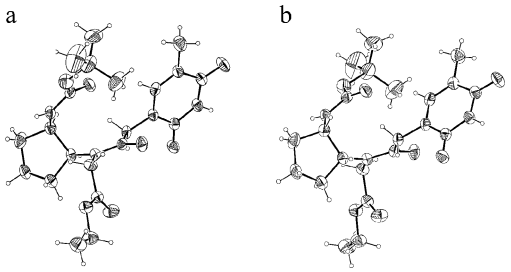

Fig. 1 ORTEP diagrams of crystal structures of **9**. **a** (1*S*,2*R*); **b** (1*R*,2*S*).‡

Table 1 UV-T_m of *cp*PNA : DNA/RNA triplexes[a]

Entry	PNA	DNA **19**	DNA **20**	Poly rA
1	PNA **10**, H-TTTTTTTTT-LysNH$_2$	45.0	34.5	62.0
2	PNA **11**, H-TTTTTTTT**t$_{SR}$**-LysNH$_2$	51.0	27.8	73.5
3	PNA **12**, H-TTTT**t$_{SR}$**TTT-LysNH$_2$	22.0	11.0	76.0
4	PNA **13**, H-**t$_{SR}$**TTTTTTT-LysNH$_2$	44.5	26.4	66.0
5	PNA **14**, H-TTTTTTTT**t$_{RS}$**-LysNH$_2$	55.0	28.0	> 85.0
6	PNA **15**, H-TTTT**t$_{RS}$**TTT-LysNH$_2$	62.0	32.0	61.0
7	PNA **16**, H-**t$_{RS}$**TTTTTTT-LysNH$_2$	48.7	27.8	69.0
8	PNA **17**, H-(**t$_{SR}$**)$_8$-LysNH$_2$	66.6	nd	> 85.0
9	PNA **18**, H-(**t$_{RS}$**)$_8$-LysNH$_2$	72.0	nd	> 85.0

[a] All values are an average of at least 3 experiments and accurate to within ±0.5°. DNA **19**, d(CGCAAAAAAAACGC); DNA **20**, d(CGCAAAA-*C*AAACGC). Buffer. Sodium phosphate (10 mM), pH 7.2 with 100 mM NaCl and 0.1 mM EDTA; nd, not determined.

The present results on *cis*-*SR*/*RS* *cp*PNAs viewed in relation to the limited data reported on *trans*-*SS* *cp*PNA[9] clearly suggest a stereochemical dependence of the stability and selectivity in DNA/RNA binding. The dihedral angle β in 1,2-disubstituted *cis*-cyclopentyl system is less than that in *ch*PNA but the relative ease of conformational adjustments in a cyclopentyl ring seems to have significant consequences for the hybridization ability of *cp*PNA oligomers. In addition to the unprecendented stabilization observed for homooligomeric, homochiral *SR*- and *RS*-*cp*PNA oligomers with cDNA (ΔT_m +21–27°, *RS* > *SR*), the binding of these *cp*PNA to poly rA was also highly improved. These results suggest that in cyclopentyl PNAs, the favourable conformational features of the monomer are co-operatively transmitted to the oligomer level and such effects are useful from an application perspective. Overall, the results presented here reinforce the idea of improving stability and DNA/RNA binding selectivity *via* rational structural modifications of PNA. Further studies on sequence dependent and RNA/DNA discriminatory effects of *cp*PNA are in progress.

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Notes and references

‡ Crystal data: (1*S*,2*R*)-**9**. $C_{21}H_{32}N_4O_7$, M = 452.51, crystal system: rectangular, crystal dimensions 0.63 × 0.60 × 0.24 mm, a = 10.6505(7), b = 10.6505(7), c = 43.402(4) Å, space group $P4_32_12_1$, V = 4923.3(6) Å^3, Z = 8, D_c = 1.221 g cm^{-3}, μ(Mo-Kα) = 0.092 mm^{-1}, T = 293(2) K, F(000) = 1936, max. and min. transmission 0.9786 and 0.9438, 34716 reflections collected, 4339 unique [$I > 2\sigma(I)$], S = 1.370, R = 0.0733, $wR2$ = 0.1537 (all data R = 0.0746, $wR2$ = 0.1543). (1*R*,2*S*)-**9**: $C_{21}H_{32}N_4O_7$, M = 452.51, crystal system: tetragonal, crystal dimensions 0.66 × 0.33 × 0.11 mm, space group $P4_12_12_1$, a = 10.6483(4), b = 10.6483(4), c = 43.318(3) Å, V = 4911.7(4) Å^3, Z = 8, D_c = 1.224 g cm^{-3}, μ(Mo-Kα) = 0.092 mm^{-1}, T = 293(2) K, F(000) = 1936, max. and min. transmission 0.9903 and 0.9416, 24647 reflections collected, 4333 unique [$I > 2\sigma(I)$], S = 1.123, R = 0.0537, $wR2$ = 0.1164 (all data R = 0.0693, $wR2$ = 0.1224). CCDC 227530 and 227531. See http://www.rsc.org/suppdata/cc/b3/b317000d/ for crystallographic data in CIF or other electronic format.

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