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(1*S*,2*R*/1*R*,2*S*)-Aminocyclohexyl Glycyl Thymine PNA: Synthesis, Monomer Crystal Structures, and DNA/RNA Hybridization Studies

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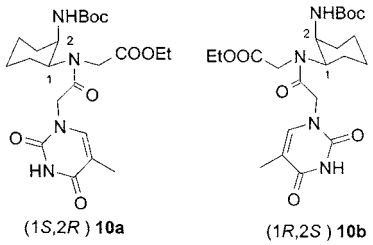
ABSTRACT



The synthesis of ethyl *cis*-(1*S*,2*R*/1*R*,2*S*)-2-aminocyclohex-1-yl-*N*-(thymin-1-yl-acetyl) glycinate (10a and 10b) via enzymatic resolution of the key racemic intermediate *trans*-2-azido cyclohexanols 3 is reported. The crystal structures of 10 show equatorial disposition of the tertiary amide group, with the torsion angle β in the range 60−70°. The PNA oligomers incorporating these show differential effects in hybridizing with complementary DNA and RNA.

Peptide nucleic acids (PNA, **I**) are acyclic, achiral DNA mimics, in which nucleobases are attached via an acetyl linker to a pseudopeptide backbone composed of *N*-(2-aminoethyl) glycyl units.[1] PNA hybridizes through Watson−Crick hydrogen bonding to complementary oligonucleotides. PNA:DNA and PNA:RNA duplexes exhibit thermal stability higher than that of the corresponding DNA:DNA and DNA:RNA duplexes.[2] PNAs are of great interest in medicinal chemistry, with potential for the design of gene-targeted drugs.[3] Several rationale modifications have been reported in order to understand the structure−activity relationship.[1b] The objectives of these modifications are to overcome the ambiguity in binding orientations of cDNA/RNA, to restrict conformational flexibility in backbone and impart features for selective RNA and DNA binding.[4] The approaches include introduction of chirality in the achiral PNA backbone to influence the orientation of cDNA binding and designing of cyclic analogues to preorganize the PNA structure, an attribute that could entropically drive the duplex formation.[4]

One such earlier modification was to constrain the flexibility in the aminoethyl segment of the PNA monomer by introducing a cyclohexyl ring as in **II**.[5] The *trans*-(1*S*,2*S*)-cyclohexyl PNA **II** hybridizes with the complementary DNA similarly to unmodified PNA, while *trans*-(1*R*,2*R*)-cyclohexyl PNA lacked such a property.[5] Thermodynamic data

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indicated that the DNA binding of the (1*S*,2*S*) cyclohexyl PNA is accompanied by a decrease in the entropy loss and a reduced gain in enthalpy. Molecular Dynamics simulations performed on model structures of (1*S*,2*S*)- and (1*R*,2*R*)-cyclohexyl PNAs showed the torsion angle β (N−CH_2−CH_2−NHCO) to be close to 180°, corresponding to a *trans*-(1,2-diaxial) disposition[5] (Figure 1). An analysis of

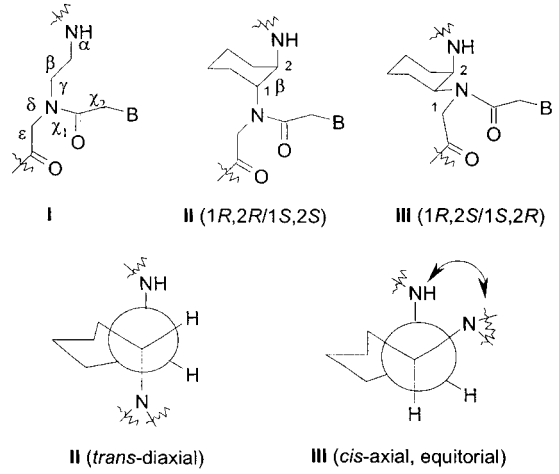


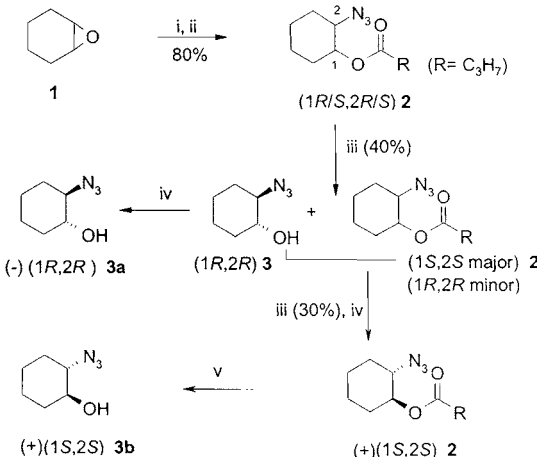
Figure 1. PNA analogues and chiral cyclohexyl PNA.

PNA:DNA and PNA:RNA duplexes by solution NMR studies and crystal structure have revealed distinctive differences in the PNA conformations between the two duplexes.[6] The most notable difference is in the torsion angle β; in a PNA:RNA duplex it is in the range of 60−70°, whereas in a PNA:DNA duplex, it is approximately 140°. This suggests that the *trans* diaxial geometry of the (1*S*,2*S*/1*R*,2*R*)-cyclohexyl PNA **II** is far from the required PNA conformation and not suitable for facile hybridization with either DNA or RNA targets.

Classical PNA is very flexible and can attain different conformations to accommodate binding to both DNA and RNA. In view of results on *trans*-(1*S*,2*S*/1*R*,2*R*)-cyclohexyl PNAs and PNA-DNA/RNA duplex structural data, it occurred to us that if torsion angle β can be restricted to values in the range 60−70°, the resulting PNA may bind to RNA selectively over DNA. This is possible in a *cis*-1,2-disubstituted cyclohexyl system wherein the substituents are in axial-equitorial disposition. In cyclohexyl PNAs, this corresponds to (1*S*,2*R*/1*R*,2*S*) configuration **III**, wherein the torsion angle β would be around 65°. These may be better suited for incorporation into the PNA backbone to impart DNA/RNA discriminating properties. In this paper, we describe a chemoenzymatic synthesis of *cis*-(1*S*,2*R*/1*R*,2*S*)-aminocyclohexyl glycyl PNA thymine monomers for incorporation into PNA oligomers and the crystal structure of the title monomers. The torsion angle β in these corresponds to −63° and +66°, which is close to the desired value supporting the postulate of initial design. To our knowledge, this is the first report of a crystal structure of any backbone-constrained PNA monomer.

Synthesis of (1*S*,2*R*/1*R*,2*S*)-Aminocyclohexyl Glycyl PNA Thymine Ethyl Ester Monomers (10a/10b). The synthesis of title compounds was achieved starting from (+)- and (−)-*trans*-2-azido cyclohexanols **3a** and **3b**. These were obtained by enzymatic hydrolysis of racemic 2-azido cyclohexanoate **2** using lipases, well-known in the literature.[7] The butyrate ester of racemic *trans*-2-azido cyclohexanols was synthesized by oxirane ring opening of the commercially available *meso*-epoxide **1** with sodium azide[8] followed by esterification with butyric anhydride (Scheme 1). The racemic

Scheme 1[a]



[a] Reagents and conditions: (i) NaN_3, NH_4Cl, 80% aq ethanol, reflux, 18 h, 89%; (ii) *n*-butyric anhydride, dry pyridine, DMAP (cat.), rt, 16 h, 91%; (iii) *Pseudomonas cepacia* lipase, phosphate buffer, pH 7.2, 2.5 h; (iv) column chromatography; (v) NaOMe in MeOH, rt, 0.5 h.

butyrate ester **2** was resolved by enzymatic enantioselective hydrolysis using the lipase from *Pseudomonas cepacia* (Amano-PS) in sodium phosphate buffer (pH = 7.2, 40% conversion), followed by chromatography, to obtain optically pure (1*R*,2*R*)-azido alcohol **3a**. Amano-PS lipase was found to be much better than the earlier reported lipase from *Candida cylindracea* for this resolution step and gave better enantiomeric purity for the azido alcohol **3** under identical reaction conditions and in less reaction time. Further enzyme treatment of the mixture of (1*R*,2*R*) (minor) and (1*S*,2*S*) (major) butyrate ester **2** for the second time, column purification, and subsequent methanolysis of the (1*S*,2*S*)-**2** ester using NaOMe in methanol gave pure (1*S*,2*S*)-**3b** in 30% overall yield. The enantiopurity of both (*1R*,2*R*)-**3a** and (1*S*,2*S*)-**3b** isomers was about 98−99% by comparing with known values for the optical rotations reported in the

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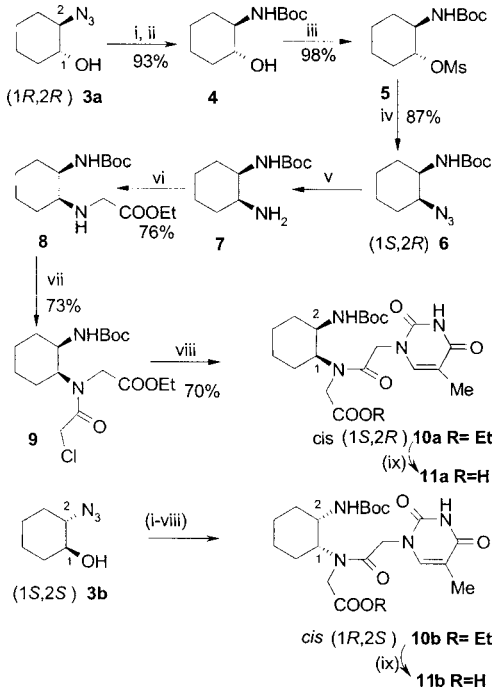
literature[7] and by ^{13}C NMR using the chiral chemical shift reagent Eu(hfc)$_3$.

The reduction of the azide function in (1*R*,2*R*)-**3a** by hydrogenation using Adam's catalyst[9] and in situ *t*-Boc protection of the resulting amine function yielded the Boc-protected amino alcohol (1*R*,2*R*)-**4** (Scheme 2), which was

Scheme 2[a]



[a] Reagent and conditions: (i) PtO_2, dry EtOAc, H_2, 35−40 psi, rt, 3.5 h; (ii) $(Boc)_2O$; (iii) MsCl, dry pyridine, DMAP, 0−5 °C, 5 h; (iv) NaN_3, dry DMF, 72 °C, 18 h; (v) PtO_2, MeOH, H_2, 35−40 psi, rt, 3.5 h; (vi) $BrCH_2COOEt$, KF-Celite, dry CH_3CN, rt, 4 h; (vii) $ClCH_2COCl$, Na_2CO_3, dioxan/H_2O (1:1), 0 °C, 0.5 h; (viii) thymine, K_2CO_3 dry DMF, 65 °C, 3.5 h; (ix) 0.5 M LiOH/aq THF, rt, 0.5 h.

then converted to corresponding mesylate (1*R*,2*R*)-**5**. The mesylate was treated with NaN_3 in dry DMF to yield the azide (1*S*,2*R*)-**6** with inversion of configuration at C1. The azide (1*S*,2*R*)-**6** was hydrogenated using Adam's catalyst to give the amines (1*S*,2*R*)-**7**, which without further purification was alkylated with ethyl bromoacetate in the presence of KF-Celite. This resulted in monosubstituted *cis*-1,2-diamines (1*S*,2*R*)-**8,** which on acylation with chloroacetyl chloride gave the compound (1*S*,2*R*)-**9**. The condensation of (1*S*,2*R*)-**9** with thymine in the presence of K_2CO_3 in DMF yielded the desired (1*S*,2*R*)-aminocyclohexyl (thymin-1-yl-acetyl) glycyl PNA monomer ethyl ester (1*S*,2*R*)-**10a**, which on hydrolysis gave the monomer (1*S*,2*R*)-**11a**. The synthesis of other enantiomer (1*R*,2*S*)-**11b** was accomplished starting from (1*S*,2*S*)-**3b** following same steps as described above. All compounds were characterized by ^1H,^{13}C NMR spectroscopy and mass spectrometry analysis.

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X-ray Crystal Structures of (1*S*,2*R*/1*R*,2*S*)-Aminocyclohexyl PNA Thymine Monomers 10a/10b. X-ray quality crystals of both (1*S*,2*R*)-**10a** and (1*R*,2*S*)-**10b** were obtained only from chloroform,[10] and the ORTEP diagrams are shown in Figure 2. The torsion angles for (1*S*,2*R*)-**10a** and (1*R*,2*S*)-**10b** are shown in Table 1. The torsion angle β in both isomers are around 65° in magnitude, but with opposite signs.

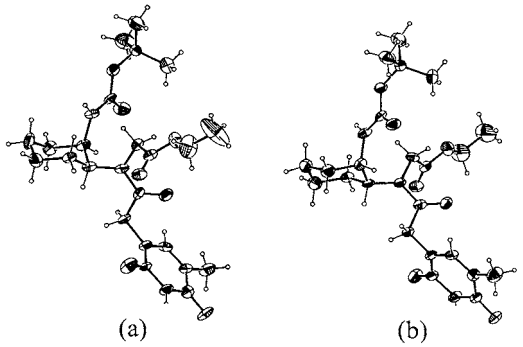

Figure 2. ORTEP[13] view of (a) (*1S*,2*R*)- and (b) (1*R*,2*S*)-aminocyclohexyl glycyl PNA thymine monomer ethyl ester **10**.

Table 1. Torsion Angles of PNA Monomers and PNA-DNA/PNA-RNA Duplexes

compound	α	β	γ	δ	χ_1	χ_2
(1*S*,2*R*)-**10a**	128	−63	76	119	1.02	−175
(1*R*,2*S*)-**10b**	−129	66	−78	−119	−0.87	174
PNA-DNA[a]	105	141	78	139	−3	151
PNA-RNA[b]	170	67	79	85	4	−171

[a] Reference 6b. [b] Reference 6a.

In the isomer (1*S*,2*R*)-**10a**, β is negative and all other angles are closer to the values found in PNA:RNA complex both in sign and magnitude.[6] Though in solution, the tertiary amide bond exists as a rotameric mixture,[6] in crystal structures the amide bond is *trans*, with the carbonyl pointing toward the glycyl component.[11] The crystal structure data, particularly with reference to the angle β, suggest that the PNA oligomers derived from *cis*-disubstituted (1*S*,2*R*)-cyclohexyl PNA monomers, with axial−equatorial dispositions, are better preorganized for a favorable interaction with RNA than with DNA. In the PNA monomer **10** with the backbone imposed on the cyclohexyl ring, the N-terminus substituent occupies the axial position, while the more bulky tertiary amide substituent takes up the equatorial disposition.

PNA T_{10} oligomers **12** and **13** incorporating the modified monomers and the unmodified control **14** were synthesized using Boc chemistry on L-lysine-derivatized MBHA resin[5] and characterized by mass spectrometry analysis.[12] These sequences having single modifications are identical to the reported[5] ones in order to make appropriate comparisons.

The T_m values of PNAs **12** and **13**, hybridized with complementary RNA (poly rA) and DNA (poly dA), were obtained from temperature-dependent UV absorbance data (Figure 3) and are shown in Table 2. From the data it is

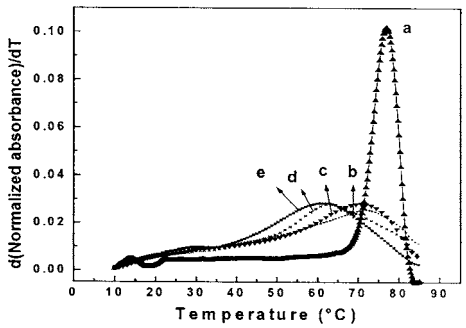

Figure 3. Derivative UV−temp curves. Poly rA with (a) PNA **12**, (b) PNA **13**. Poly dA with (c) PNA **14**, (d) PNA **12**, (e) PNA **13**.

seen that the PNA oligomer **12** with cyclohexyl (1*S*,2*R*) geometry is preferred for binding to RNA over the PNA **13** having *cis* (1*R*,2*S*) geometry ($\Delta T_m = +7$°C). The observed transition was also very sharp compared to the broad nature in other cases. In comparison, the two isomers had no significant differences in binding with complementary DNA ($\Delta T_m = -2$ °C). The preferential binding to RNA over DNA by one of the enantiomers was not observed with the reported *trans* isomers,[5] although the *trans*-(1*S*,2*S*) was the preferred geometry for binding to both DNA and RNA. These preliminary studies suggest that preorganization of backbone geometry using monomers with appropriate torsion angles may lead to induction of substantial selectivity in DNA/RNA binding.

Table 2. Thermal Stability (T_m, °C) of PNA-DNA/RNA Hybrids[a]

PNA oligomer	poly rA	poly dA
H-TTTTT$_{SR}$TTTTT-Lys-NH_2 (**12**)	77.2	61.5
H-TTTTT$_{RS}$TTTTT-Lys-NH_2 (**13**)	70.7	63.5
H-TTTTTTTTTT-Lys-NH_2 (**14**)	>80	72.4

[a] Buffer: 10 mM sodium phosphate, pH 7.0, 100 mM NaCl, 0.1 mM EDTA. The T_m values reported are the average of 3 independent experiments and are accurate to ±0.5 °C, PNA:DNA/RNA (2:1).

On the basis of a structural rationale, we have synthesized [(1*S*,2*R*)/(1*R*,2*S*)]-aminocyclohexyl (thymin-1-yl-acetyl) glycyl PNA monomers **10**, followed by their incorporation into PNA oligomers. Preliminary binding data with cDNA/RNA have suggested stereochemical discrimination in binding of the derived PNA oligomers with RNA that is not significant in DNA. Further studies on the synthesis and properties of mixed PNA and homochiral oligomers are currently in progress. The synthetic route described here also provides an easy access for *cis*-cyclohexyl 1,2-diamines **7**, which are useful ligands for applications in chiral catalysis[14] and peptidomimetics.

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(10) Single crystals of both **10a** (1*S*,2*R*) and **10b** (1*R*,2*S*) were obtained by crystallization from chloroform only. X-ray intensity data were collected on a Bruker SMART APEX CCD diffractometer at low temperature. Crystal data for **10(a)**: $C_{23}H_{33.25}Cl_{4.50}N_4O_7$, $M = 637.31$, crystal dimensions 0.75 × 0.34 × 0.02 mm^3; crystal system orthorhombic, space group $P2_12_12_1$, $a = 6.201(4)$, $b = 22.599(14)$ $c = 23.835(14)$ Å, $V = 3340(3)$ Å^3, $Z = 4$, $D_c = 1.267$ g cm^{-3}, μ(Mo Kα) = 0.436 mm^{-1}, $T = 150(2)$ K, $F(000) = 1327$, Max and min transmission 0.9913 and 0.7370, 31159 reflections collected, 5864 unique [$I > 2\sigma(I)$], $S = 1.164$, R value 0.1043, wR2 = 0.2477 (all data $R = 0.1197$, wR2 = 0.2567). Data for **10(b)**: $C_{23.50}H_{35}Cl_{5.25}N_4O_7$, $M = 671.67$, crystal dimensions 1.21 × 0.27 × 0.05 mm^3, crystal system orthorhombic, space group $P2_12_12_1$, $a = 6.2353(9)$, $b = 22.617(3)$ $c = 23.988(3)$ Å, $V = 3382.8(8)$ Å^3, $Z = 4$, $D_c = 1.319$ g cm^{-3}, μ(Mo Kα) = 0.492 mm^{-1}, $T = 150(2)$ K, $F(000) = 1397$, Max and min transmission 0.9773 and 0.5888, 43174 reflections collected, 5932 unique [$I > 2\sigma(I)$], $S = 1.046$, R value 0.0723, wR2 = 0.2074 (all data $R = 0.0847$, wR2 = 0.2147). All data were corrected for Lorentzian, programs. SHELX-97 (Sheldrick, G. M. *SHELX-97 Program for Crystal Structure Solution and Refinement*; University of Gottingen: Germany, 1997) was used for structure solution and full polarization and absorption effects using Bruker's SAINT and SADABS matrix least squares refinement on F2. Hydrogen atoms were included in the refinement as per the riding model. Both enantiomers contain one molecule of ordered chloroform with full occupancy and another molecule of highly disordered chloroform with occupancy less than unity. The high R values despite data collection at low temperature could be attributed to the extensive disorder of the solvent molecule in **10a** and **10b** and extensive disorder of the end methyl group of the side chain in **10a**.

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