Effect of chirality of L/D-proline and prochiral glycine as the linker amino acid in five-atom linked thymidinyl-(α-amino acid)-thymidine dimers†

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The chirality of the amide linker in dimer blocks was found to have a profound effect on the orientation of base stacking interactions as studied by CD and NMR spectroscopy.

The constant quest for a simple, straightforward peptide backbone superior to peptide nucleic acids (PNA)[1] has been a major research activity in our and other laboratories in the last decade.[2] The ever-increasing span of therapeutic interventions by oligonucleotides in antisense RNA,[3] siRNA,[4] miRNA,[5] splice correction,[6] *etc.* has further emphasized the need for the development of modified oligonucleotides. Because of its uncharged and achiral nature, PNA remains hydrophobic and sparingly soluble in water. It also shows aggregation properties and very low cell-uptake that necessitate conjugation with cationic peptides or negatively charged DNA to attain its optimum therapeutic value.[7] Although PNA posed quite a few problems for its application as a therapeutic agent, the amide linker in the PNA backbone promised key advantages over several other backbone modifications as linkers in oligomers, most obvious being the applicability of the well-established solid-phase peptide synthesis methodology.[8] Over the last decade, we and others have been working towards improving PNA properties by introduction of chirality,[9] positive charges,[10] peptide conjugation,[11] *etc.* with remarkable success in terms of novelty and creativity. The very recent and striking example is that of prolyl-(ACPC)-PNA consisting of alternating α-amino acid proline as a nucleobase carrier and chiral β-aminocyclopentane carboxylic acid.[2h] We later reported a similar concept but a synthetically more straightforward (α- + β-) amino acid backbone in which the β-amino acid derived from thymidine alternated with naturally occurring α-L-amino acids such as proline, sarcosine, lysine and methionine, yielding amide-linked oligomers[12] (Fig. 1). It is known that in DNA, the phosphate group connecting the two nucleosides is prochiral and when rendered chiral due to substitution at phosphorus, affects DNA/RNA recognition processes.[13] The partial replacement of the DNA/RNA phosphodiester linker by five-atom amide linkers has been found to be very useful for RNA recognition.[2b,c] Also, in earlier studies, chirality of amide linkers was found to affect the binding strength of modified oligomers to target sequences.[2b] In the thyminyl homo-oligomers recently reported by us, L-amino acids were used alternating with thyminyl-β-amino acids to form oligomers that recognized both DNA and RNA.[12] The implications of amino acid chirality in this backbone are not yet studied. In the case of partial replacement of phosphodiester linkages with the amide dimer units, where it is necessary to keep the continuity of the backbone for synergistic stacking interactions, such studies may be important. To delineate structural differences, if any, that L- or D-amino acids in the backbone would assert on the geometry of the derived oligomers, T-(amino acid)-T dimers are the simplest units where such effects can be investigated. In this communication, we present synthesis of T-(amino acid)-T dimer blocks with L-proline (**Ia**)/D-proline (**Ib**) and prochiral glycine units (**Ic**) (Fig. 2). We chose to use the prochiral glycine and L/D-prolines for our study, which can be extended to other amino acids. L/D-Prolines were chosen because they are devoid of amide N–H and hence remove the influence of hydrogen bonds on the overall structural features.[2h] The effect of the backbone chirality on the base stacking in the dimer building blocks is studied by CD spectroscopy[14] as reported earlier for TpT,[14a,b] 2′-*O*-methyl-TpT[14e] and LNA dimer blocks.[15] Temperature-dependent NMR studies[14b] were carried out in support of the CD results.

The synthesis of the dimeric building blocks **Ia** using L-proline as a representative example is depicted in Scheme 1. 5′-Dimethoxytrityl-3′-deoxy-3′-aminothymidine **1** was synthesized by literature-reported procedures.[2c] Acylation of **1** using Fmoc-protected L-proline (**2a**), D-proline (**2b**) or glycine (**2c**) yielded **3a**, **3b** and **3c**, respectively. The intermediate free amino compounds **4a**, **4b** and **4c** obtained by removing the Fmoc protecting group in **3a**/**b**/**c** were *N*-acylated using 3′-*O*-*tert*-butyldimethylsilylthymidine-4′-carboxylic acid **5**. Compound **5** was obtained by TEMPO–BAIB

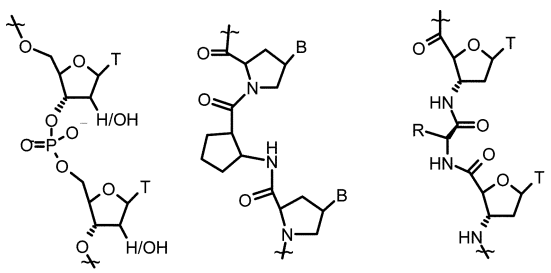

Fig. 1 DNA, RNA and modified PNA analogues.

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† Electronic supplementary information (ESI) available: ^{1}H NMR, mass spectral data for compounds **Ia**, **Ib**, **Ic**, N/S conformational analysis of **Ia**, **Ib**, **Ic**, temperature dependent change in H-6 ^{1}H NMR signals. See DOI: 10.1039/b913546d

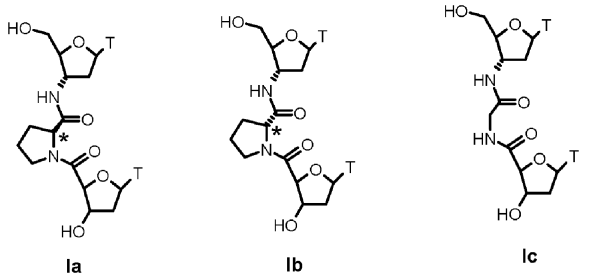
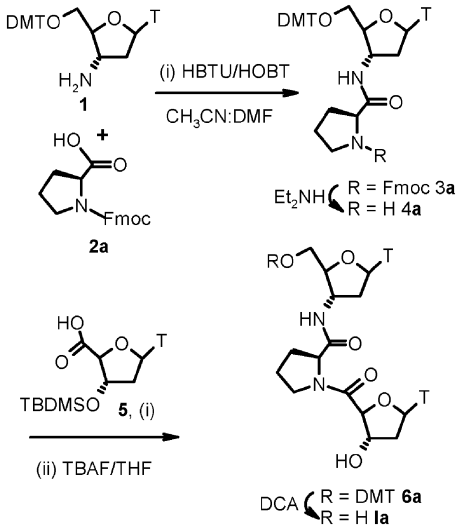


Fig. 2 Proposed dimer blocks containing L-Pro **Ia**, D-Pro **Ib** and glycine **Ic** as linker amino acids.

oxidation of the 5′-alcohol of 3′-*O*-*tert*-butyldimethylsilyl-thymidine using a reported procedure.[16] The individual products **6a**, **6b** and **6c** obtained after desilylation using TBAF in dry THF, were purified by silica-gel column chromatography and characterized using NMR and mass spectral analyses. For the present studies **6a**, **6b**, **6c** were detritylated to give **Ia**, **Ib**, **Ic**, respectively. All the three dimer units were extensively characterized by NMR, and confirmed by mass spectral analysis. The ^{1}H assignments were confirmed by 2D-COSY studies. α-Amino acid peptides prefer to be *trans* oriented with respect to the amide bond because of steric reasons and the $Z : E$ ratio is about 1000 : 1 except in the case of proline. In prolines, the *Z*-form is only slightly favored over the *E*-form because of steric interactions involving the ω-carbon of the proline ring.[17] This preference was also observed in the case of proline-linked nucleoside dimer building blocks **Ia** and **Ib**. The remote sensing of *trans*/*cis* isomers at the prolyl amide bond is evident from the major : minor (83 : 17 in **Ia** and 87 : 13 in **Ib**) H1′ NMR signals corresponding to the 5′- and 3′-thyminyl units in dimers where the internucleoside amino acid linker is either L- or D-proline. This difference is absent in **Ic** where glycine is used as the internucleoside amino acid. We further studied the conformational preferences of the sugar residues in the dimer units by using the Sum rule (ESI†).[18] In all the three dimers **Ia**, **Ib** and **Ic**, the 3′-sugar exhibited higher N-type geometry uniformly as compared to the 5′-thyminyl units for the major isomer. The percentage of N character was found to be higher where sugar ring is substituted by nitrogen in the ring B (50–64%) as compared to 3′-OH sugar (18–28%) in ring A.

The chirality of the internucleoside linker such as prochiral natural phosphate, R_p and S_p chiral phosphorothioates[13] or *R*/*S* amides[2b] is known to exert large effects on the binding efficiency of the oligomers but the origin of this binding discrimination has not been studied on the molecular level. In these cases also the sugar conformations do not drastically differ in the diastereomers formed because of the chirality of the internucleoside linker as in the case of the dimers **Ia**, **Ib** and **Ic**. Conversely, the N-type conformational preference of the sugar ring in 2′-OMe or LNA type oligomers is known to dramatically improve the binding efficiency of the oligomers and in these cases, effective base stacking is one of the well-established contributing factors to the duplex stability. Increase in the positive CD band at 275 nm is ascribed to the effective base stacking interactions even at the dimer level.[15] We therefore carried out CD studies on the dimer blocks **Ia**, **Ib**



Scheme 1 Synthesis of dimer building blocks.

and **Ic** to evaluate any contributions of chirality of the α-amino acid towards stacking interactions. Indeed, the chirality of the amino acid in the dimers was found to exert profound effects on the CD signals. The D-proline containing T-(D-proline)-T **Ib** showed a strong positive band at 275 nm [Fig. 3(a)] and a negative band at 240 nm. In the T-(L-proline)-T dimer **Ia**, along with the negative band at 240 nm, two distinct low intensity CD bands were observed at 265 and 290 nm along with a broad minimum at 275–280 nm [Fig. 3(a)]. The T-(glycine)-T dimer **Ic** showed low intensity broad bands at 240 and 280 nm [Fig. 4(c)]. The high intensity of CD signal at ~280 nm leads us to believe that **Ib** is well stacked even at the dimer level and the orientation of stacked species is similar to the LNA dimer blocks.[15] The minimum at ~275 nm in **Ia** is comparable to the negative CD signal at ~275 nm in the case of the homogeneous amide-backbone T-[(L-pro-T)]$_7$ octamer [Fig. 3(b)] earlier reported by us.[12] Thus, the stacking interactions in **Ia** and **Ib** could be arising from different adopted conformations due to opposite chirality in the internucleoside linker giving rise to opposite CD signals at ~275 nm. Glycine, being prochiral, could either result in the existence of both chiral components in equal measure, which cancel each other, or may not contribute to the base-stacked structure, and eventually lead to only a weak maximum at ~280 nm. To our knowledge, this is the

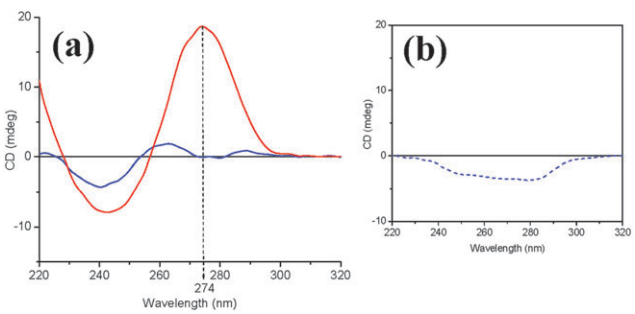


Fig. 3 (a) CD spectra of T-(L-Pro)-T **Ia** (blue) and T-(D-Pro)-T **Ib** (red) at a concentration of 100 μM in water; (b) CD spectrum of T-[(L-Pro)-T]$_7$.[12]

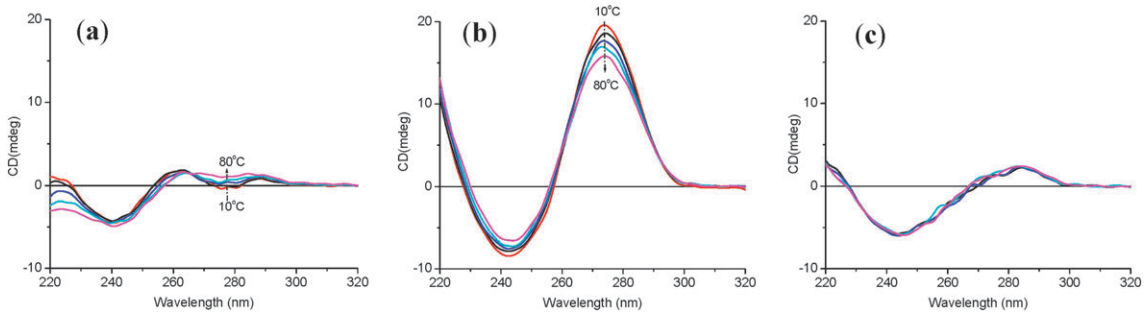


Fig. 4 Temperature-dependent CD studies of thymine dimers containing (a) L-proline **Ia**, (b) D-proline **Ib** and (c) glycine **Ic** at 100 μM concentration, in water at 10 (red), 20 (black), 40 (blue), 60 (light blue) and 80 °C (purple).

first report where such profound effects of backbone linker chirality on the resulting dimer CD signals are reported.

The strong positive band at ~275 nm in the T-(D-proline)-T dimer **Ib** displayed significant temperature dependence from 10–80 °C [Fig. 4(b)] as reported earlier for the TpT dimer block.[14b] For the T-(L-proline)-T dimer **Ia**, increasing temperature resulted in the reduction of the amplitude of CD minimum band at ~275 nm [Fig. 4(a)]. These changes in intensities of CD maxima and minima imply the presence of higher fractions of stacked species in **Ib** and **Ia**. No temperature-dependent change in CD intensity was observed in the case of T-(glycine)-T dimer **Ic** [Fig. 4(c)] and thus fractions of stacked species are less significant at the dimer level in **Ic**. The downfield shift in the ^{1}H NMR signal corresponding to H-6 protons in **Ia** (δ 8.08 and 7.66) and **Ib** (δ 8.01 and 7.56) compared to **Ic** (δ 7.69 and 7.64) also implies the presence of stacked species in **Ia** and **Ib** as against **Ic**.[14b] Temperature-dependent NMR studies in **Ib** confirmed the increasing fraction of unstacked species at higher temperatures, evident by an upfield shift of the H-6 resonance signal in ^{1}H NMR (ESI†). We further observed that the change in H-6 proton chemical shift depends on temperature and confirmed that the unstacked species in the dimer at higher temperature reduces the magnitude of the downfield shift. Though the north/south conformational equilibria of the sugar rings are known to affect the base stacking interactions,[15] these (ESI†) could not be directly correlated to the CD intensity corresponding to the dimer blocks **Ia**,**b**,**c** in the present case. The change in the chirality of linker amino acid does not drastically alter the N/S sugar conformations in the case of the three dimers reported in this paper. Thus we arrive at the conclusion that the different adopted stacked conformations due to internucleoside chirality could be the reason for opposite CD signals in **Ia** and **Ib**. To the best of our knowledge, this is the first report regarding the control of base stacking interactions by internucleoside linker chirality.

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