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Nucleoside derived amino acids (NDA) in foldamer chemistry: synthesis and conformational studies of homooligomers of modified AZT

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Abstract

Homo short-oligomers of a novel trans-β-amino acid derived from AZT were synthesized and characterized. These adopt right-handed helical turns with their bases positioned systematically along the helix axis. These studies open up new possibilities for synthesizing nucleoside derived functional foldamers.

Keywords: Trans-β-amino acid; AZT; Oligonucleoside; NMR; Helical turn

Complex functions of proteins and peptides are strongly associated with the orientation and relative positioning of their functional groups along the structural backbone. In the recent years, oligomers of unnatural peptidic residues,[1–3] particularly with β-amino acids[4,5] and their hybrids with natural L-amino acids (α) have emerged as versatile structural templates ('foldamers') as these exhibit predictable and well-defined secondary structures such as helices, turns and strands, and offer a variety of possibilities for orienting functional side-chains.

We[6–8] and other research groups[9–11] have been engaged in the synthesis and conformational studies of oligomers based on cyclic beta sugar (furanose) amino acid building blocks. The oligomers of these amino acids both in cis and trans conformation have exhibited diverse helical patterns in solution.[6–9] These inspiring results prompted us to diversify our efforts from fused bicyclic sugar amino acids to nucleoside derived amino acids (NDA), which are more close in structure to the natural building blocks. Furthermore, oligomers of NDAs with specific conformational preferences favour well-defined backbone folding and geometrically positioned nucleobases, features that are essential for improved molecular recognition.[12,13] Chakraborty's research group has reported amide linked (δ-peptide analogues) cyclic oligomers of thymidine-based nucleoside amino acids.[14] In their classic work, Gellman et al. showed that the homooligomers of *trans*-β-ACPC or heterooligomers with *trans*-APC residues adopt robust left-handed 12-helix structures,[15–19] stabilized by periodic 12-membered (NH_i–CO_{i-2}) hydrogen bonding. Exploiting both the predictable secondary structural features of β-peptidic templates and the functional advantages of nucleosides, herein we report the synthesis and folding propensity of short homooligomers, trimer **9** and tetramer **10** of AZT derived *trans*-2,3-cyclic β-amino acid residue **2**. AZT **1**, which is a drug of choice for the treatment of AIDS, was chosen as the starting material. Through simple

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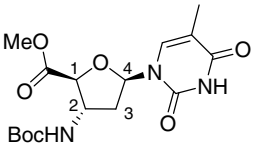
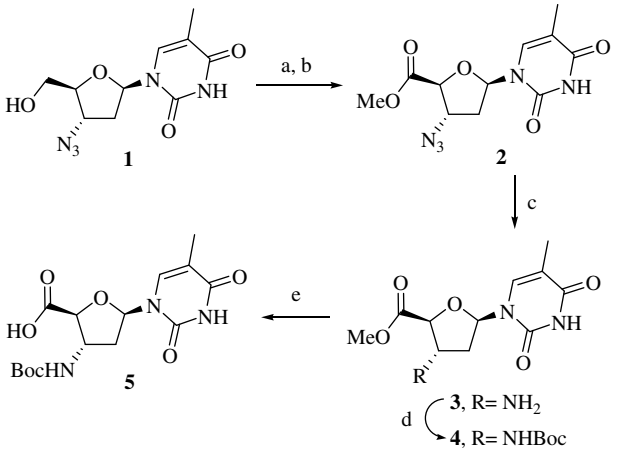
chemical transformations, **1** has furnished the *trans*-β amino acid derivative **4**, which possesses a bicyclic backbone but not the fused example as used in our earlier approaches. A detailed conformational analysis was carried out using circular dichroism (CD), NMR spectroscopy and constrained molecular dynamics (MD) simulation techniques, which have established that **9** and **10** adopt robust helical folding in solution.



NDA

Initially, we synthesised the two functionalized dimers **7** and **8** to further couple to obtain tetramer **10**. In this endeavour, trimer **9** was obtained from coupling monomeric amine **3** with dimer acid **7**. The commercially available AZT, **1** was oxidized in CH_3CN/H_2O (1:1) with BAIB and TEMPO to furnish the azido acid[20] which was immediately esterified using CH_2N_2 to obtain the azido methyl ester **2**. This azido ester **2** was reduced via hydrogenation to produce the amino ester monomer **3** in 95% yield. The second monomer **5** was obtained from **3** in two steps involving the protection of the amino group as *tert*-butyl carbamate **4** followed by mild ester hydrolysis using LiOH in THF/H_2O (3:1) (Scheme 1). The obtained monomers **3** and **5** were coupled using the well-established HOBt–EDCI protocol[21] to generate the protected dimer **6** in moderate yield (60%). The required acid functionality in **6** was released by treatment with LiOH to obtain dimer acid **7** in 88% yield.

The second dimer **8** having a free amino functionality was obtained from protected dimer **6** by treatment with trifluoroacetic acid. The two dimers **7** and **8** were again

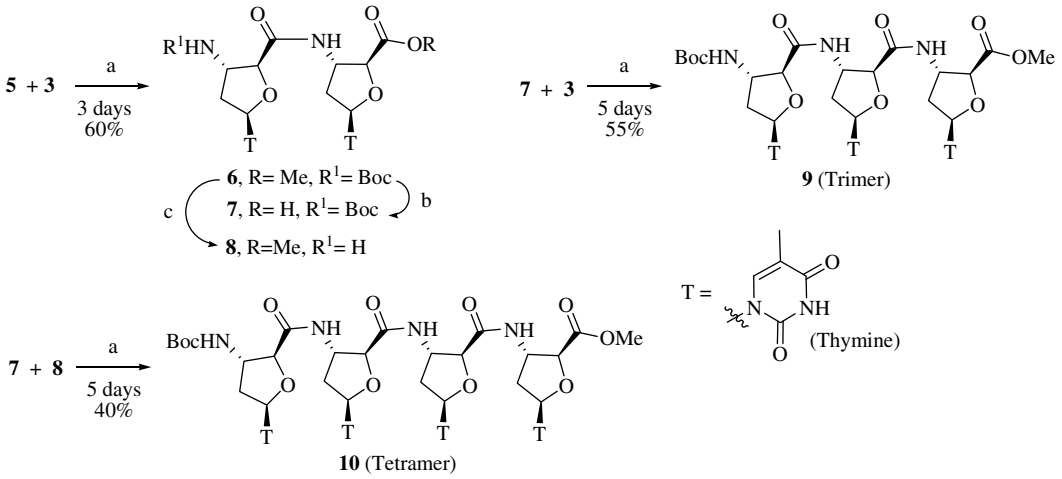


Scheme 1. Reagents and conditions: (a) TEMPO, BAIB, CH_3CN/H_2O, rt, 12 h, 80%; (b) CH_2N_2, dry ether, 5 min, 10 °C, 90%; (c) Pd/C, MeOH, H_2, rt, 5 h, 95%; (d) $(Boc)_2O$, Et_3N, MeOH, rt, 10 h, 80%; (e) LiOH, THF/H_2O (3:1), 3 h, 0 °C, 95%.

coupled under the same set of coupling conditions to obtain smoothly tetramer **10**[22] in 40% yield. The purification of this tetramer was cumbersome but could be achieved by running a slow column on silica (Finer than 230–400 mesh). The white solid thus obtained has reasonable shelf life. For our further studies, we have also synthesized the shorter trimer **9**[23] from dimer acid **7** and monomer amine **3** using HOBt–EDCI (Scheme 2).[21]

Far-UV circular dicrosim (CD) spectroscopy data are often used to derive the backbone folding pattern and handedness of oligopeptides. The CD data for 200 μM solutions of **9** and **10** (Fig. 1) in methanol exhibited distinct secondary structural patterns with a minimum, zero-crossing and maximum around 203, 212 and 224 nm, respectively. Interestingly, the observed signatures are closely comparable with those of 12-helical *trans*-β-ACPC oligomers,[15] but for the opposite handedness. The increased ellipticity from trimer to tetramer is due to the increased cooperative nature in higher oligomers in stabilizing the helical structure, with the nucleation of the secondary folding being at a trimer level.

Detailed NMR studies of **10** (1D, 2D-COSY, TOCSY, ROESY) were carried out at 298 K in structure supporting solvents, CD_3OH and a mixture of CD_3OH and $CDCl_3$. The backbone NH resonances were well dispersed (6.6–8.6 ppm) in these solvents, indicative of secondary folding. Whilst the structure was stable in methanol and 60:40 $CD_3OH/CDCl_3$ solvent mixture, complete assignment was hampered due to overlap of the α and β proton resonances of all the residues. However, they were relatively better resolved in 10:90 $CD_3OH/CDCl_3$ (50 μl CD_3OH +550 μl $CDCl_3$), in which the structure has been analyzed. Even though these oligomers dissolve in DMSO-d_6, we preferred to use methanol as solvent, since DMSO-d_6 is likely to disrupt/modify the native secondary structure. Moreover, methanol has been identified as a suitable structure supporting solvent for *trans*-β-peptidic foldamers[18] that were investigated using CD and NMR spectroscopy. Protons in the bases were assigned from the intraresidual NOEs and the sequential assignment of the residues was achieved from the NH_i–$CH\alpha_{i-1}$ NOEs. Imide NHs in the bases exhibited similar chemical shielding and all appeared around 10.4 ppm as a broad peak. The complete chemical shift assignment for **10** is given in Table 1. The temperature coefficient ($\Delta\delta/\Delta T$) studies of the NH proton resonances carried out in this solvent suggested that 3NH (−5.5 ppb/K) and 4NH (−7.0 ppb/K) are involved in moderate hydrogen bonding while 1NH (−10.5 ppb/K) and 2NH (−8.8 ppb/K) were solvent exposed. The marginally larger temperature coefficients for 3NH and 4NH compared to the usually observed values (>−4 ppb/K) are acceptable for linear oligomers with intramolecular hydrogen bonding.[24] The relatively larger value observed for 4NH could be due to a higher mobility of the less-constrained terminal residue. The large $^3J_{NH-C\beta H}$ values (≈7.00 Hz, $\phi \approx -115°$) for the trimer and tetramer show antiperiplanar arrangement between these two protons and $^3J_{C\alpha H-C\beta H}$ coupling



Scheme 2. Reagents and conditions: (a) (i) EDCI, HOBt, DIPEA, DMF, rt; (b) LiOH, THF/H_2O (3:1), 3 h, 0 °C, 88%; (c) TFA, CH_2Cl_2, 0 °C, 1 h.

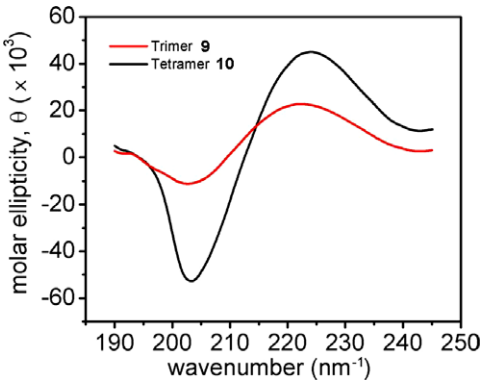


Fig. 1. CD spectra of trimer **9** and tetramer **10**.

(~5 Hz) corresponding to an N–Cβ–Cα–CO angle, θ[25,26] of ~+100°, which is consistent with that observed for 12-helical folds based on the other *trans*-β residues. The secondary structure of tetramer **10** was established by a close analysis of the ROESY data, which showed convincingly that this homooligomer adopts right-handed helical folding, as described below.

Whilst sufficient NOEs between adjacent residues could be identified to derive spatial connectivities, the assignment of long range NOEs along the backbone turned out to be ambiguous due to the overlapped resonances of the Cα and Cβ protons. However, in such cases, the NOEs involving the base methyl groups serve as suitable alternatives in the structural elucidation of oligonucleotides and biomolecules.[27,28] The observed long range inter-residue NOEs between the methyl group protons of the thymidine bases and the backbone or sugar ring protons, more specifically 1Me–4NH, 1Me–4H4, 1Me–4H1, 1Me–3H4, 2Me–3H2, 2Me–4H1 and 2Me–4H4 NOEs (Fig. 2b and a) indicate that **10** adopts helical folding that favours 12-membered NH_i–CO_{i-3} hydrogen bonding.

Further proof for this possibility comes from the presence of a characteristic NOE between 3NH-Boc.[19] The adopted folding of the backbone closely positions the base of the first residue (from the N-terminus) below the third and fourth residues. It is worth noting that for tetramer **10**, only two 12-membered hydrogen bonds involving 3NH and 4NH each are possible. It is known that short oligomers in principle exhibit various conformational possibilities which can be in equilibrium and the NMR data represent an average over these conformers. However, the present CD and NMR experimental data are suggestive

Table 1
Chemical shift assignments for tetramer **10**

Residue	NH	C1H(α)	C2H(β)	C3H(γ)	C3′H(γ′)	C4H(δ)	H_b	Me_b	NH_b
1	6.65 (d) $J_{NH,H2} = 7.0$	4.29 (d) $J_{H1,H2} = 5.0$	4.38 m	2.59 m	2.37 m	6.10 (t) $J_{H4,H3} = 6.4$ $J_{H4,H3'} = 5.0$	7.76 s	1.88 s	10.4 br s
2	8.61 (d) $J_{NH,H2} = 7.0$	4.24 (d) $J_{H1,H2} = 5.1$	4.61 m	2.55 m	2.48 m	6.20 (t) $J_{H4,H3} = 6.2$ $J_{H4,H3} = 6.2$	8.06 s	1.90 s	10.4 br s
3	8.47 (d) $J_{NH,H2} = 7.0$	4.35 (d) $J_{H1,H2} = 5.0$	4.65 m	2.49 m	2.49 m	6.26 (t) $J_{H4,H3} = 6.2$ $J_{H4,H3} = 6.2$	8.10 s	1.96 s	10.4 br s
4	8.64 (d) $J_{NH,H2} = 7.0$	4.65 (d) $J_{H1,H2} = 2.3$	4.70 $J_{NH,H2} = 7.0$ $J_{H1,H2} = 2.3$	2.27 m	2.40 m	6.52 (dd) $J_{H4,H3} = 5.6$ $J_{H4,H3} = 8.7$	8.05 s	1.96 s	10.4 br s

H_b, Me_b, and NH_b indicate the protons present in the thymine base of the NDA.

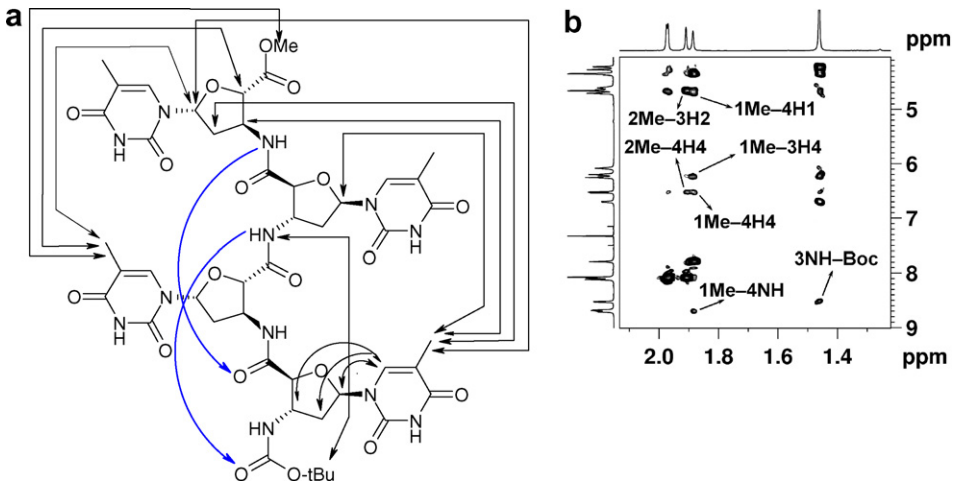


Fig. 2. (a) Schematic representation of the observed NOEs (in double headed black lines) and possible 12-membered hydrogen bonding (in single headed colour curves) for tetramer **10**. (b) Selected region of the ROESY spectrum of tetramer **10** supporting the helical fold. The cross-peaks are indicated with the atomic groups involved. Example: 2Me–3H2 indicates the cross-peak between second residue base methyl to the third residue proton at position 2 (β).

of predominant population of 12-helical conformers in the solvent medium chosen, which are also consistent with the Gellman's *trans*-β-peptidic foldamers.[15–19]

The intensities of the above mentioned inter and intra residue NOEs were converted into distances by normalizing with respect to the NOE between the geminal protons at the γ position in each residue. These NOE derived distances (Table 2) and dihedral angles are used as constraints in molecular dynamics simulations. The discover program on Insight-II with CVFF force field has been used throughout the simulations. Minimizations were determined first with steepest decent, followed by conjugate gradient methods for a maximum of 10,000 iterations each or RMS deviation of 0.001 kcal/mol, whichever was earlier. The energy minimized structures were then subjected to MD simulations using restraints, at 298 K. The molecules were initially equilibrated for 20 ps and subsequently went to a 1000 ps dynamics with 1 fs stepsize and trajectory sampling for every 10 ps. A total of 100 sampled structures were generated and energy minimized. Figure 3 shows the superimposed structures of several such lower energy structures obtained from MD simulations. The individual structures showed about 2.5 residues per turn a geometrical feature

Table 2
List of distance constraints used in MD simulations

NOEs		Distance (Å)	NOEs		Distance (Å)
1	4NH↔4H4	2.92–2.38	12	$3H_b$↔3H4	2.87–2.35
2	4NH↔4H1	2.59–2.12	13	$3H_b$↔3H2	2.87–2.35
3	4NH↔3H1	2.42–1.98	14	3H4 ↔1Me	3.28–2.68
4	$4H_b$↔4H4	3.16–2.58	15	2NH↔2H4	3.55–2.91
5	$4H_b$↔4H2	3.13–2.56	16	2NH↔1H1	2.72–2.23
6	4H4↔2Me	4.04–3.30	17	$2H_b$↔2H4	2.91–2.38
7	4H4↔1Me	3.14–2.57	18	$2H_b$↔2H2	3.15–2.57
8	4H2↔1Me	3.64–2.97	19	1NH↔1H4	3.42–2.80
9	3NH↔3H4	3.35–2.74	20	1NH↔1H1	2.88–2.35
10	3NH↔3H1	2.80–2.29	21	$1H_b$↔1H4	2.70–2.21
11	3NH↔2H1	2.51–2.05	22	$1H_b$↔1H2	3.01–2.46

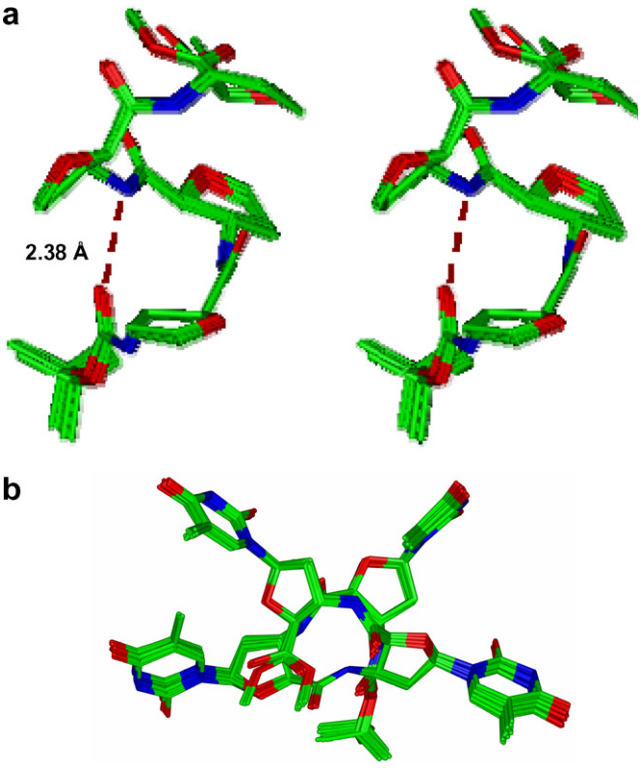


Fig. 3. (a) Stereoview of superimposed minimum energy structures of tetramer **10**, (b) top view of the same along the helical axis showing the orientation of the thymine bases with respect to the helix axis.

that is relevant to 12-helical folding and an NH_3–CO_{Boc}-hydrogen bonding distance of ~2.38 Å. The bases are radially oriented along the helix axis (Fig. 3b) thereby providing enhanced accessibility of the biofunctional surface.

It is known that a specific helical folding is associated with a cooperative nature of the constituent residues and the structure attains better stability with the increase in their population (greater than hexamer).[7,29] Hence, it is interesting and important to explore higher oligomers to

ascertain the observed conformational features. However, the synthesis of higher oligomers beyond tetramers using the conventional Boc-protected manual synthesis, was hampered due to solubility problems. In order to derive the details of specific helical folding in more populated foldamers, solid phase synthetic methods have to be employed, and work in this direction is in progress and will be reported elsewhere.

In summary, we have discussed the synthesis and conformational studies of AZT derived *trans*-β-amino acid short trimer, and tetramer homooligomers, by using CD, NMR and restrained MD techniques. The results have shown the signatures of right-handed 12-helical turns that the backbone adopts, which is consistent with Gellman's 12-helices in the oligomers of *trans*-β-amino acids. In accordance with the aim of our present work, the results encourage us to design functional oligonucleotides possessing well-defined backbone folding and having their bases positioned in a geometrically defined manner. However, detailed secondary structural features can only be obtained in hexamers and beyond, and efforts towards this are in progress.[30]

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22. Spectroscopic data for compound **10**: ^{1}H NMR data listed in Table 1. MS (ESI): *m/z* 1103 $[M+Na]^+$.
23. Spectroscopic data for compound **9**; ^{1}H NMR ($CDCl_3$, 500 MHz), δ 10.3 (br s, 3 NH), 8.57 (d, $J_{NH,2} = 7.3$ Hz, 1H), 8.50 (d, $J_{NH,2} = 7.3$ Hz, 1H), 8.06 (s, 2H), 7.91 (s, 1H), 6.63 (br s, 1H), 6.49 (t, $J_{4,3} = 8.4$ Hz, $J_{4,3'} = 5.6$ Hz, 1H), 6.20 (t, $J_{4,3} = 6.7$ Hz, $J_{4,3'} = 6.7$ Hz, 1H), 6.12 (t, $J_{4,3} = 6.5$ Hz, $J_{4,3'} = 6.5$ Hz, 1H), 4.7 (m, 1H), 4.6 (m, 1H), 4.57 (d, $J_{1,2} = 2.8$ Hz, 1H), 4.34 (d, $J_{1,2} = 4.2$ Hz, 1H), 4.30 (m, 1H), 4.29 (d, $J_{1,2} = 4.2$ Hz, 1H), 3.81 (s, 3H), 2.26–2.54 (m, 6H), 1.89–1.97 (s, 9H), 1.45 (s, 9H); HRMS (ESI): Calcd for $C_{36}H_{45}N_9O_{15}Na$: 866.2932, found: 866.2971 $[M+Na]^+$.
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