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Design and synthesis of regioisomeric triazole based peptidomimetic macrocycles and their dipole moment controlled self-assembly†

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Two peptidomimetic macrocycles, regioisomeric in terms of the position of triazole/amide, have been synthesized. Both undergo self-assembly in a parallel manner but in solvents of opposite polarity, ascribed to (β, β) and (β-D, β-L) hydrogen bonding leading to formation of two different unique classes of organic nanostructures.

Among numerous concepts for nanotube design, self-assembly of peptides has fascinated people most owing to the diversity in size and shape achievable for the products which are easy to access, biocompatible, find application in biology, and prove to be useful in materials science for constructing pores of transmembrane ion channels.[1,2] In particular, cyclic D,L-α-peptides as well as cyclic β^3-peptides can self-assemble into extended hollow tubular structures due to their complementary structure and function. Modifications of physical properties such as interior property as well as polarity of peptide nanotubes can be accomplished by peptide main chain backbone alteration such as insertion of an alicyclic ring.[3] For the design of cyclic peptides as well as peptidomimetic macrocycles with enhanced selectivity for molecular recognition, ion transport as well as the separation process, the direction of growth of the nanotube is an important consideration.[3,4]

Triazole, an amide bond surrogate, is an attractive modification for building up cyclic-β-tetrapeptides.[3a] It is a perfect mimic of the amide bond in terms of planarity, polarity, as well as hydrogen bond donating and accepting ability.[5] But it has the capacity to impart higher macrodipole than obtained by normal cyclic β^3-tetra peptides, as it possesses a larger dipole moment than the amide bond. It can also influence the conductance property through voltage gating and rectification of a transmembrane ion channel as well as anion macrodipole interaction that responds to membrane polarisation.[6] We have additionally been interested in constructing carbohydrate-containing triazole/amide-based peptidomimetic macrocycles which undergo self-assembly to form hollow-tubular structures. Recently we reported[7] a carbohydrate based triazole/amide macrocycle which is conformationally homologous to D,L-α-amino acids in terms of functional groups while retaining the backbone chirality. This class of macrocycles displays two rotameric conformations which undergo both parallel and antiparallel backbone to backbone H-bonding in the solution phase. We therefore set ourselves the task of synthesising two stable rotameric conformations of this class of macrocycles individually. This communication describes our success in designing two peptidomimetic macrocycles (**2a,b**) regioisomeric in terms of the position of triazole/amide bonds, where the presence of two different rotamers could be successfully demonstrated. Of the two classes of macrocycles one is parallel (**2a**) and the other is antiparallel (**2b**), but both undergo self-assembly in only a parallel manner. Studies on these isomeric macrocycles bring out that (i) unidirectionality of functional group (*i.e.* amide and triazole) arrangement can be maintained in these systems also as in cyclic β^3-peptides;[3a] (ii) replacement of two different amide bonds by 1,4-linked triazole moieties can control the polarity of the peptide nanotube ensemble due to different backbone conformation; and (iii) 1,4-linked triazole modification in cyclic β-peptide systems can bridge between two different classes of peptide nanotube ensembles, for example cyclic β-peptides with unidirectional functional groups as well as

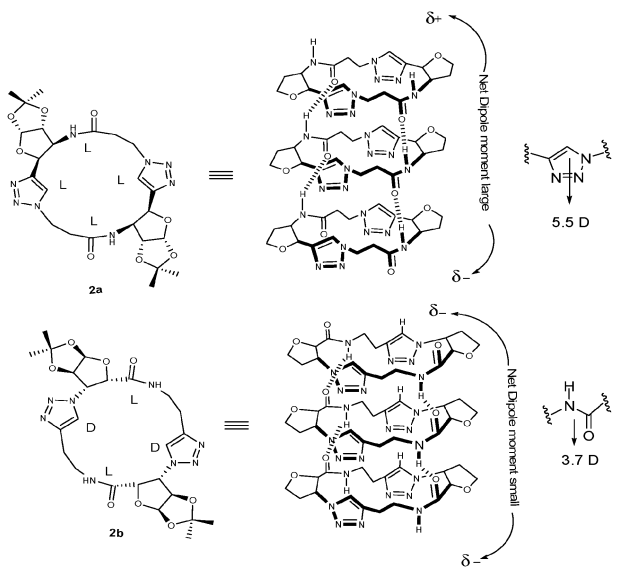

Fig. 1 Compounds **2a** and **2b** are the two regioisomeric (based on the location of carbohydrate and β-alanine) stable rotameric peptidomimetic macrocycles undergoing self-assembly in solution (carbohydrate substituents excluded to facilitate visualisation of assembly).

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alternative orientation of functional groups (D,L-homologous conformation) (Fig. 1).

Peptidomimetic macrocycle **2a** was synthesized[8] using a standard organic protocol previously reported from our laboratory. Thus the triazolo amino protected ester intermediate was prepared by Cu-catalyzed azide alkyne cycloaddition reaction using azido β-alanine methyl ester (synthesized by Cu(I) catalyzed diazo-transfer reaction of β-alanine with triflic azide) and Cbz-protected sugar homopropargyl amine. Triazole/amide macrocycle **2b** was on the other hand synthesised from the basic intermediate **10** *via* Cu(I) catalyzed cycloaddition between 3-azido-(1,2:5,6)-diisopropylidene glucose and homopropargyl alcohol. This intermediate was converted to its azide derivative through the corresponding mesyl ester. The reduction of azide to amine by stannous chloride and subsequent protection by Cbz–Cl yielded the Cbz protected triazolyl sugar **12**. After smooth deprotection of the isopropylidene derivative, oxidation furnished the Cbz protected sugar triazole amino acid. This product was converted to its linear tetramer by iterative protection and deprotection *via* acid treatment and then amine coupling by the standard EDC·HCl, HOBt protocol. The linear tetramer was hydrolyzed and then hydrogenolysed to yield the free amino acid which was cyclised by DPPA–Et_3N to peptidomimetic macrocycle **2b**.[8]

The two macrocycles were subjected to transmission electron microscopy (TEM) to investigate the possibility of nanotube formation. Interestingly, **2a** forms nanotubes only in acetonitrile : water (4 : 1) whereas **2b** forms them in nonpolar solvents such as (2 : 3) $CDCl_3$: CCl_4. Besides TEM, AFM morphology was also used to get insight into molecular association and self-assembly of both the macrocycles **2a** and **2b** in solvent systems of differing polarity, which revealed the same shape of nanorods as observed from TEM analysis. The selected area electron diffraction patterns of the two macrocycles also signify nanotube formation (Fig. 2).

This difference in polarity of the two nanotubes encouraged us to study their homo-assembly property by analysing their backbone conformation *via* molecular modelling coupled with multi-dimensional NMR studies, as well as by FT-IR. The FTIR spectra recorded for **2a** and **2b** as KBr pellets and in $CHCl_3$ solution showed, respectively, bands at 3280 and 3327 cm^{-1} for amide A, 1673 and 1668 cm^{-1} for amide I, and 1568 and 1533 cm^{-1} for amide II, which reveals substantial evidence for self-assembly of the two macrocycles *via* β-sheet like hydrogen bonding.[4c,7,9] ESI MS contained the pseudo molecular ion peak for the dimeric species at *m*/*z* 1143 $(2M + Na)^+$ and for the monomeric species at 583 $(M + Na)^+$ for both **2a** and **2b** in (4 : 1) CH_3CN–H_2O and (2 : 3) $CDCl_3$–CCl_4 respectively.

The ^1H NMR spectrum of macrocycle **2a** in a polar solvent mixture such as CD_3CN and H_2O (2%) is well resolved and shows a high degree of C_2 symmetry of the molecule. The observed coupling constant $J_{NH,S\beta H}$ is 7.8 Hz which establishes the flatness of the peptide backbone. The weak intensity of ROE cross-peaks between signals of triazole and adjacent C_α protons indicates that the conformation is analogous to that of L-α-amino acid residues in cyclic D,L-α-peptides as well as typical cyclo-β-peptide structures. The dramatic consequence of replacement of the triazole ring by amide was observed in macrocycle **2b**. In polar solvents like CD_3CN all the proton signals are well resolved, which reflects high order C_2 symmetry. However, the intensity of ROE correlation for the triazole proton signal was strong with the peak for the C_β proton and weak with that for the C_α proton, testifying to a *cis*-relationship which is generally accessible by D-α-amino acids. The observed $J_{C\alpha H,C\beta H}$ of furanoid sugar components (around 3 Hz) and $J_{NH,CH2}$ for the β-alanine moiety (around 3, 9 Hz) indicate the presence of a *gauche* conformation of the C_α–C_β unit in compound **2b**. On the basis of molecular modelling studies *via* NMR, the peptide backbone conformation is likely to be composed of all D,L-α-amino acid residues as the triazole hydrogen is equivalent to NH of amide and N2–N3 of the carbonyl group of an amide bond. The study also reveals that the macrocyle is stable as the "clockwise rotamer" keeping the direction of all triazole and amide bonds in the same direction, whereas regioisomer **2b** is stabilised as the "anticlockwise rotamer" by their alternative orientation. These anticipated structures can be formed due to intramolecular hydrogen bonding[10] between amide NH and the N2–N3 unit of the triazole ring in **2b**, which is ruled out in **2a** owing to conformational restriction of the sugar moiety. As a result macrocycle **2a** is capable of developing large dipole moment in the nanotube ensemble and is organised as nanorods in polar solvent, whereas **2b** underwent molecular organisation in nonpolar medium (Fig. 3).

Regarding the self-assembly property, investigation of concentration dependent ^1H NMR spectra revealed marginal downfield

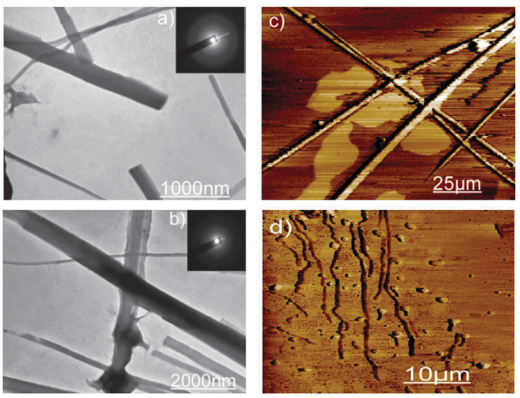

Fig. 2 TEM images of rodlike assemblies of **2a** (a) and **2b** (b); the AFM images are in CH_3CN : H_2O (4 : 1) for **2a** (c) and (2 : 3) $CDCl_3$: CCl_4 for **2b** (d). The selected area electron diffraction patterns are shown as insets in (a) and (b).

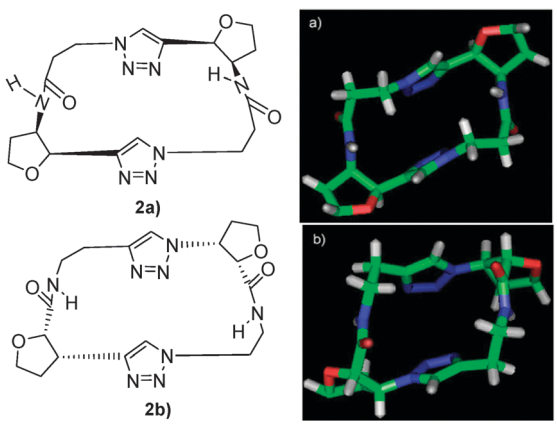

Fig. 3 Energy minimized structure of peptidomimetic macrocycles **2a** and **2b** using CVFF force field in insightII silicon O_2 work station 98.0 *in vacuo* and in $CHCl_3$ respectively: (a) resembling typical cyclo-β-peptide structure; (b) resembling cyclic D,L-α-peptides.

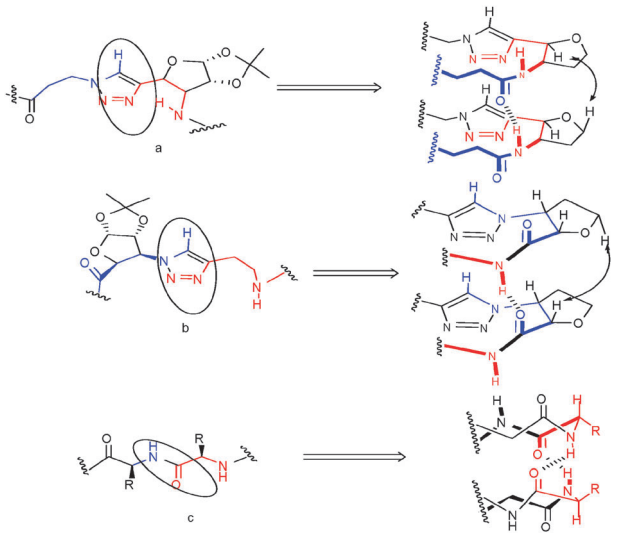

Fig. 4 Comparison between triazole/amide β- and β-D,L-conformation and D,L-α-peptides: (a) triazole/amide β-conformation and their parallel stacking *via* (β,β) H-bonding (right side), (b) parallel stacking *via* (β-D, β-L) H-bonding in D,L-homologous triazole/amide macrocycle, (c) antiparallel (α-D, α-D) H-bonding in cyclic D,L-α-peptides. Blue indicates L-amino acid or equivalent while red indicates the D-counterpart.

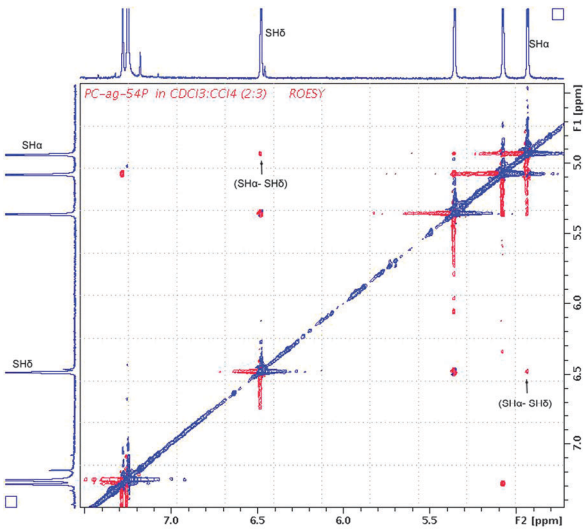

Fig. 5 Selected region of the ROESY spectrum of the self-assembled macrocycle **2b**, showing parallel-homostacking interaction by cross-peaks between SH_α–SH_δ (600 MHz, 298 K, 2 : 3 $CDCl_3$: CCl_4).

shift of an amide proton signal, which demonstrates the strong H-bonding of higher order aggregation for C_2 symmetric planar β-sheet structure of macrocycle **2b**.[7–9] Retention of a unique set of all proton signals of **2b** at 243 K also indicates fast equilibrium (in the NMR time scale) between a monomer and a dimer (or higher order aggregates).[8]

In analogy to the typical cyclo β-peptides, the peptidomimetic macrocycle **2a** underwent self-assembly in a parallel fashion which was attested by the existence of Roesy interaction between H_α and H_δ proton signals of sugar components[7,9a] in acetonitrile–water. Surprisingly, the macrocycle **2b**, although featuring an alternative conformation of amide and triazole, showed a similar kind of stacking interaction in a non-polar solvent like (2 : 3) $CDCl_3$: CCl_4 as deduced by Roesy interaction between H_α and H_δ of sugar moieties, but there was no evidence of an anti-parallel mode of hydrogen bonding as in our previous results.[7] From a conformational point of view as discussed earlier, triazole linked pseudo di-β-peptide and normal D,L-α-dipeptide are the same. But due to the presence of one more carbon in β-peptide structure the existence of (β-D, β-L) hydrogen bonding, which occurs by parallel stacking, is more likely. This is unlike normal cyclic D,L-α-peptides and (α,γ)-cyclic peptides where only antiparallel hydrogen bonding takes place by (α-D, α-D) or (γ-L, γ-L) bonding (Fig. 4 and 5).

In summary we have designed, synthesized and characterized two different regioisomeric triazole/amide based peptidomimetic macrocycles consisting of *cis*-β-furanoid sugar, β-alanine moiety and also triazole modification, which can effectively control the polarity of the nanotube due to different orientation of functional groups. These macrocycles can be used as model systems for different classes of artificial ion channels as their unidirectional assembly pattern allows them to possess different dipole moments. NMR, ESI-MS, FTIR, TEM, AFM, and SAED studies have been employed to characterize the hierarchical organisation of these new classes of peptidomimetic macrocycles. We have also established that the two macrocycles do undergo a similar type of parallel homo-stacking *via* amide NH and amide carbonyl oxygen H-bonding although their functional group orientations are different. The anion binding property as well as X-ray crystallography study of their larger analogues by including one α-amino acid will be taken up in future.

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