Organic & Biomolecular Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

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Journal Name

COMMUNICATION

Conformational promiscuity in triazolamers derived from quaternary amino acids mimics peptide behaviour

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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1,4-substituted triazole oligomers made from quaternary amino acid derivatives present a conformational behaviour with similarities to that of natural peptides. Twisted strand and zig-zag type conformations have been both obtained in the crystal state. In solution signs of weak ordered structures have been detected depending on the substituents and the solvents used.

Since the late 90's artificial folded structures (foldamers) that can mimic the naturally occurring architectures present in some macromolecules (peptides, proteins, DNA) have received growing interest.[1] Several structures such as oligoureas,[2] different classes of peptides[3] or aromatic oligoamides[4] have been described to fold in regular stable helical conformations. Foldamers are intriguing molecules and they have the potential to bind to biomacromolecules due to their ability to interact with surfaces, provided they have the appropriate side chains[5] and spatial distribution.[6] In this regard peptides seem a unique platform in which to build large molecules with tailored properties.[3] However, peptides suffer from proteolytic degradation and different alternatives, like the use of β-peptides[7] have been employed. Another possibility would be the use of an amide bond surrogate[8] to replace the labile peptidic linkage. One of the proposed options is the use of a 1,2,3-triazole linkage.[9] The copper-catalysed azide-alkyne [3 + 2] cycloaddition (CuAAC) has been used to synthesise or modify peptide oligomers and peptidomimetics[10] or to generate anion-responsive foldamers,[11] Regarding peptide-like foldamers, some years ago, Arora described the synthesis and conformational preferences of a series of triazole tetramers, so called clickamers (from the 'click' CuAAC reaction) or triazolamers, derived from natural amino acids.[12] They calculated the relative energies of triazole dimers and concluded that they can adopt mainly four different conformations, the two anti-conformations (with respect to the triazole dipole) being the preferred ones. NMR studies showed that one of the anti-conformations is preferred leading to a zig-zag structure similar to the β-strands found in peptides. 1-5 triazole linkage has also been used in the synthesis of peptide-like foldamers but they adopt mixed conformations.[13]

Encouraged by the previous results we decided to study the possible conformations of quaternary derivatives. Peptides derived from α,α-disubstituted amino acids have shown to adopt regular helical conformations.[14] The simplest quaternary amino acid, dimethylglycine (Aib) is achiral and therefore forms racemic mixtures of helices.[15] However, the screw sense of Aib oligomers can be controlled by only one terminal residue.[16,17] We envisioned that by adding another substituent in the carbon centre positioned between triazole rings we could induce a turn-like conformation in the dimer moieties as a consequence of the Thorpe-Ingold effect.[18] This effect would induce the whole structure to adopt a well-arranged organization and short oligomers would mimic the conformational behaviour of short peptide derivatives. To prove our hypothesis we synthesised and studied a series of short clickamers.

Our synthesis starts with the CuAAC reaction of *N*-Boc protected 2-methyl-3-butyn-2-amine **1a** to a benzylic azide (Scheme 1). Thus, the alkyne end is blocked and the chain can be grown from the 'C-terminus' to the 'N-terminus' in analogy with peptide synthesis. After amine deprotection and azide transfer reaction[19] a new monomer can be linked. Following an iterative process we could build azide terminated trimers **8** in good overall yields. Finally we coupled a chiral quaternary amino alkyne derivative **1b** to yield tetramers **9**. If the overall structures were to form stable helices then the chiral residue

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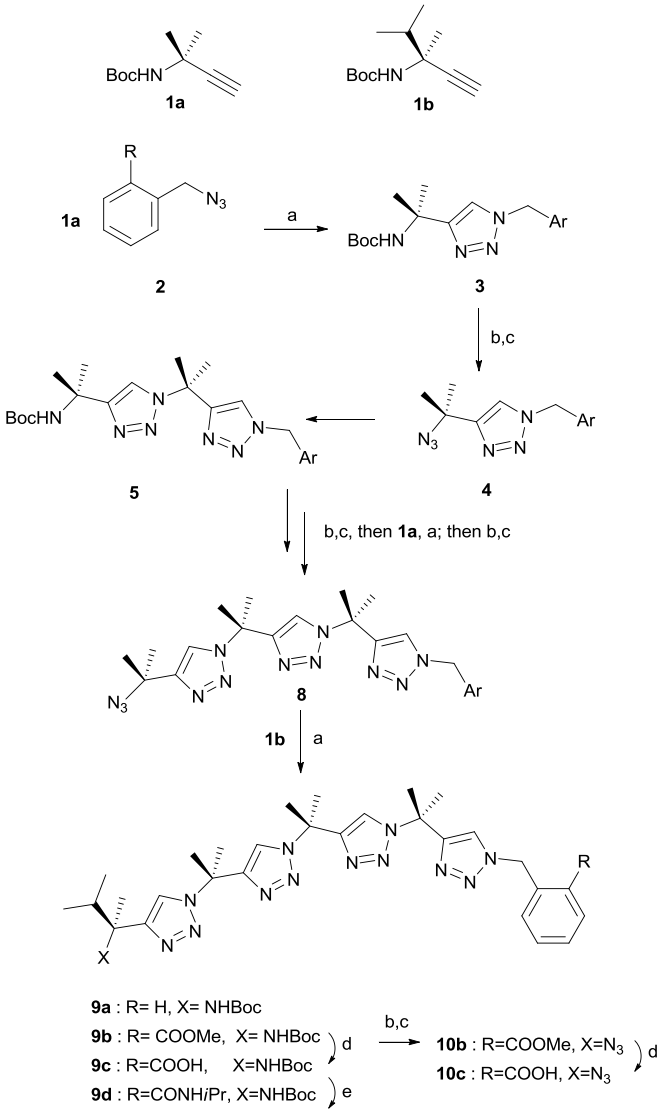
Electronic Supplementary Information (ESI) available: General experimental procedures and analytical data for all new compounds and X-ray data for compound **8a** and **10c** (CIF) [CCDC numbers for the crystal structures: CCDC 1406855 (**8a**), 1406856 (**10c**) See DOI: 10.1039/c000000x/

may favour the formation of a preferred screw sense. If these two conditions are obeyed the symmetry around the two benzylic protons will be broken and the protons will be placed in an asymmetric environment becoming anisochronous, therefore displaying an AB quartet in the ^{1}H-NMR spectra. This NMR method has been widely used by Clayden in the study of helical compounds and provides a rapid tool for the detection of helical conformations in solution.[17,20] In this particular case it seems reasonable to assume that the influence of the chiral centre would be negligible in a zig-zag conformation. We investigated compounds **9-10** by means of ^{1}H-NMR. Compound **9a** gave a sharp singlet for the benzylic protons. This would indicate that an ordered conformation is not achieved in the solvents tested ($CDCl_3$, CD_2Cl_2, CD_3OD or CD_3CN). However, another possibility is that a certain degree of order is established along the structure but the rotational freedom around the benzyl moiety renders the two protons identical in the NMR time scale.



Scheme 1. Synthesis of triazol oligomers. a) $CuSO_4$·5H20, Na ascorbate, THF:$H_2$0 1:1. b) TFA:CH_2Cl_2 1:1. c) Imidazole-1-sulfonyl azide hydrochloride, K_2CO_3, $CuSO_4$·$5H_2$0 (cat), MeOH. d) LiOH, THF:H_2O 3:1. e) EDCI, DIPEA, isopropylamine, DMF.

To induce a certain rotational constriction we prepared and studied the *ortho* substituted derivatives **9b-d**, envisioning that the residue placed in the aromatic ring could establish a hydrogen bond interaction with the triazoles in the main chain. Whereas the ester compound **9b** did not show any signal that would indicate an ordered structure, the ^{1}H-NMR spectrum of compound **9d** in $CDCl_3$ already shows a narrow AB quartet for the benzylic protons (see ESI). This quartet is conserved over a rank of concentrations (1-10 mM). A more pronounced effect is observed with the acid derivative **9c**. In $CDCl_3$ an AB system is clearly observed, overlapped with an additional signal, probably arising from a different conformation (Figure 1). Most signals of the ^{1}H-NMR of this compound in chloroform are affected by the concentration which indicates the formation of aggregates. Moreover, the signals get broader and more complex at lower temperatures (-7 ^{o}C). It seems reasonable to argue that at lower temperatures the aggregation is favoured. At higher temperatures (45 ^{o}C) the signals are sharper and the AB system is clearer. From these experiments we conclude that the aggregation works against long-range order, giving rise to other conformations which show a signal that overlaps with the AB quartet of the ordered product. NMR experiments in more polar solvents further confirmed this hypothesis. In CD_3CN at 25 ^{o}C a very narrow but still visible AB quartet is seen, suggesting a very weak predominance of an ordered structure. In this solvent the ^{1}H-NMR is independent on the concentration in a 20 fold range (1-20 mM) meaning that the transfer of chiral information from the MeVal moiety to the benzylic protons exclusively arises from an intramolecular process. The AB signal is still visible at 45 ^{o}C and very faint at 60 ^{o}C. At lower temperatures (-7 ^{o}C) the AB quatriplet is much clearer, giving a good indication for the predominance of a helical structure. In methanol, the benzylic protons appear as a sharp singlet, a further proof that the restriction of rotation through an intramolecular hydrogen bonding is necessary for an efficient transfer of structural information readable by NMR methods.

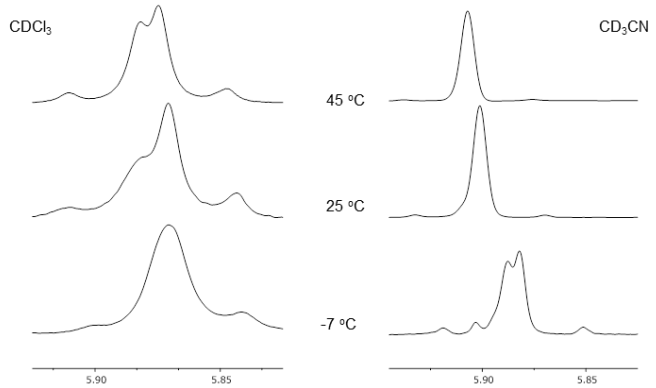


Figure 1. ^{1}H-NMR (500 MHz) expansions of **9c** (10 mM) in $CDCl_3$ (left) or CD_3CN (right) at the given temperatures.

However, compounds **10b-c** did not show any sign of folded structure by NMR. We believe that a bulky group, such as the BOC-protecting group, is also necessary to effectively induce a

single screw sense in the molecule. We carried out further solution analysis by 2D-NMR experiments (NOESY), but they resulted inconclusive as no unambiguous non-trivial contacts could be detected. In addition, the narrow chemical shift difference hampers the observation of such contacts and the lack of scalar couplings prevents unambiguous assignment for each of the repeating monomeric unit. Circular dicroism (CD) did not give any further information due to the lack of suitable chromophores in the molecules. Fortunately, we were able to grow suitable crystals for X ray analysis of compound **8a** and **10c** (N_3-trimer, Ar= Ph).

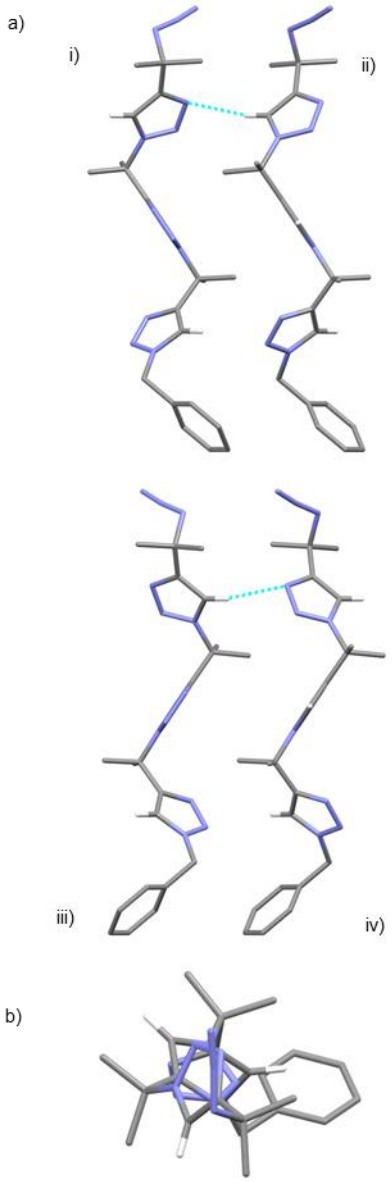


Figure 2. X-ray structure of trimer **8a** (Ar= Ph). a) View of the four molecules found in the unit cell along the crystallographic b axis: i and iv are (*P*) helices; ii and iii have (*M*) helicity. b) top view of a (*P*) molecule, showing the 120° disposition between triazole units . Only hydrogens of the triazole units are depicted for clarity

The crystals were obtained by slow cooling of a hot acetonitrile solution. Azide trimer **8a** crystallises in an orthorhombic cell with four molecules per cell, containing two pairs of helical-like enantiomers. As can be seen in Figure 2, the X-ray structure of compound **8a** shows a regular twisted-strand arrangement with a turn of 120° for each monomer respect to the axis of the molecule. The regularity of the twist can be clearly seen from the disposition of the CH of the triazole linkers as well as from the gem-dimethyl groups (Figure 2b). The molecule therefore completes a full turn and the two different screw-senses can be found in the crystallographic cell. Intermolecular hydrogen bonding between the first triazole linkers from the azide end are formed between pairs of enantiomeric molecules. This structure confirms that oligomers of clickamers made from quaternary substituted building blocks can adopt regular structures, in consonance with the results observed by NMR spectroscopy.

Interestingly, a different structure was found by X-ray diffraction for **10c**, crystallised in conditions similar to that of **8a** (Figure 3). In this case the compound crystallises in a monoclinic system, with two molecules in the cell. The oligomer displays a zig-zag structure with two antiparallel molecules in the unit cell.

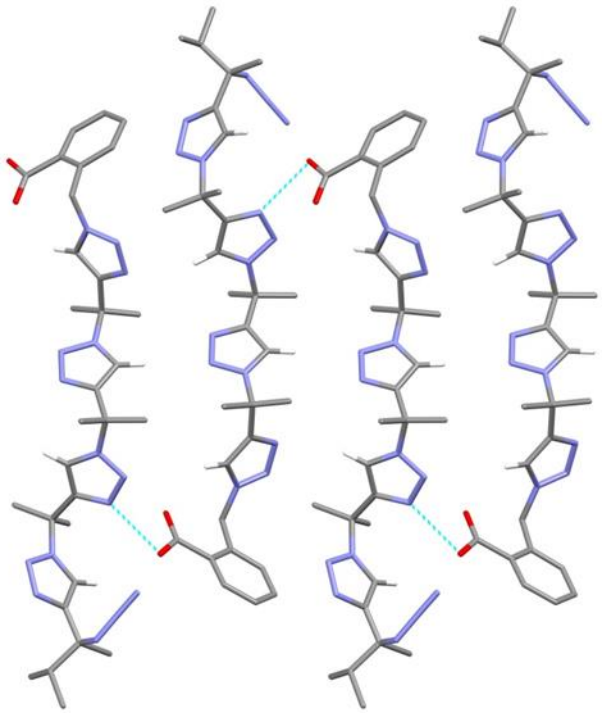


Figure 3. X-ray structure of a tetramer of **10c**. Zig-zag structures are aligned in a antiparallel manner. Stacking occurs in the direction perpendicular to the figure. Only hydrogens of the triazole units are depicted for clarity.

A hydrogen bond between the carboxylic acid and the second triazole starting from the azide end is formed in a *head-to-tail* arrangement between molecules in adjacent cells. Thus, antiparallel strands form a structure somehow resembling the natural β-sheets found in peptides (see figure ESI1).[21] The antiparallel disposition allows the stabilization of the structure by dipole-dipole interactions within the triazole rings.

These interactions could explain the alignment of the strand. In the *a* axis the molecules stack perfectly among each other forming layers of strands comparable to amyloid aggregation in peptides which is responsible for several pathologies including Alzheimer's disease.[22] This disposition explicates the tendency of **10c** to aggregate and would explain the loss of anisochrony in the ^{1}H-NMR studies.

To better compare the differences between the conformations we superimposed the resulting X-ray structures. In fig. 4 we overlaid the first carbon and first triazole ring. As can be seen in the figure whereas compound **10c** adopts a more or less regular zig-zag conformation (solid bold line) compound **8a** (empty line) starts turning around the molecule axis. Thus, **8a** diverges from the zig-zag conformation and the CH from triazole linkers point in different directions as compared to **10c**, completing a 360 turn around the molecular axis. This conformation would be responsible for bringing a certain amount of local order (as inferred by the NMR experiments) although would not be translated into the establishment of long conformational order in higher oligomers.[23]

In non-polar solvents like chloroform there must be an equilibrium between twisted conformation and the zig-zag strand responsible for aggregation. Thus aggregation shifts the equilibrium towards β-sheet structures that cannot transfer the chiral information along the scaffold, leaving the benzylic protons in isochronous environment. Therefore aggregation works against observation of anisochrony in the benzylic protons in these oligomers as previously suggested.

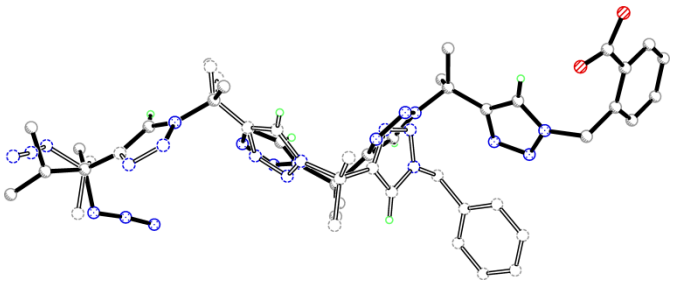


Figure 4. Overlay of structures **8a** (hollow bonds) and **10c** (solid bold bonds). The superimposition has been realized by aligning the quaternary carbon with the azide functionality and the first triazole linker.

We believe that the formation of the intermolecular hydrogen bonds and dipole-dipole interactions may be decisive to discriminate between the two possible overall conformations. Bulky groups that prevent aggregation may favour helical-like ordered conformations, which would explain the differences in the NMR for **9c** and **10c**. We therefore conclude that both, restricted rotation through intramolecular hydrogen bonding and the presence of a bulky protecting group are necessary to observe a certain anisochrony resulting from transfer of chiral information in this kind of oligomers.

Conclusions

We have successfully synthesised a series of short triazolamers derived from quarternary amino acids that show a conformational behaviour that parallels peptide oligomers. NMR studies indicate that several conformations may co-exist in solution together with aggregation. The formation of ordered structures depends on the nature of the solvent but also on the residues present in the molecule. Evidence of folded structures have been found for residues with bulky N-groups and rotationally restricted benzylic protons in solvents such as chloroform and acetonitrile. Moreover, X-ray diffraction studies provide conclusive evidence that the oligomers can exist as twisted strands or zig-zag structures, depending on the compound substituents. The study of folded systems is becoming increasingly important to target protein-protein interactions and we think that our investigations provide a new insight to design new molecules with defined conformations.

Aknowledgements

This work was supported by the Spanish Ministry of Economy and Competitiveness (MINECO, CTQ2012-38543-C03-03). J.S. acknowledges MINECO (Ramon y Cajal contract) and EU (FP7-PEOPLE-2012-CIG-321659) for funding fellowship. We thank Dr. Yolanda Pérez for helpful assistance with NMR experiments.

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