α,γ-Cyclic peptide ensembles with a hydroxylated cavity†

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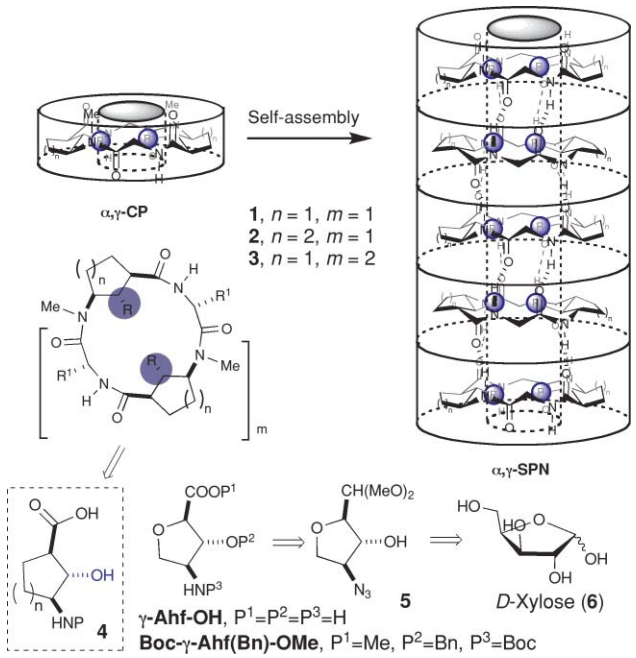
Here we describe a self-assembling α,γ-cyclic tetrapeptide that contains the 4-amino-3-hydroxytetrahydrofuran-2-carboxylic acid, in which the hydroxy group is pointing towards the inner cavity of the resulting dimers.

Peptides have found applications in a variety of areas in science, ranging from drug design to nanomaterials, because of their ease of synthesis, introduction of chemical diversity, and large diversity and ease modulation of their 3D structures.[1] One of the simplest peptide modifications to improve biological properties or induce conformational changes is backbone N-methylation.[2] Cyclic peptides (CPs) that contain tertiary amides have also been used to change the pharmacological properties of peptides, as simple models for β-sheets, turn structures, or peptide nanotubes.[3] For example, self-assembling peptide nanotubes (SPNs)[4] are formed by stacks of CPs stabilized by β-sheet-type hydrogen-bonding interactions and dimer-forming homochiral N-methylated CPs have been used as simple models for the basic structure of nanotubes.[5] We describe here a new member of the dimer-forming CP family that is characterized by a novel structure and internal cavity, in which the use of a β-hydroxy-γ-amino acid is able to stabilize the dimer but also to induce the formation of one major topoisomeric dimer. In recent years we have explored the properties of CPs composed of α-amino acids (α-Aas) alternated with γ-amino acids, more specifically a *cis*-3-aminocycloalkanecarboxylic acid (γ-Aca).[6,7] In these α,γ-CPs (*e.g.* **1–3**, Scheme 1) the relative rigidity of the cycloalkane ring, besides creating a hydrophobic cavity as a result of the projection of one of the cycloalkane methylenes into the lumen,[7a] ensures that the peptide backbone has the all-*trans* conformation required for the CP ring to be flat and stackable. Furthermore, α,γ-CPs have the largest association constant reported for CPs that form SPNs.[7a,8] We report here our synthetic studies towards a C2-modified γ-amino acid, namely 4-amino-3-hydroxytetrahydrofuran-2-carboxylic acid (**γ-Ahf-OH**), and its application in dimer-forming α,γ-CPs.

In general, we envisaged that the 3-amino-2-hydroxycycloalkanecarboxylic acid derivatives (**4**) should have the hydroxy group in a *trans* orientation relative to the α-carboxylic and γ-amino groups, so in the flat peptide conformation the hydroxy group would be projected into the cavity in a pseudoequatorial orientation (Scheme 1). In contrast, the all *cis*-isomer of **4** would direct the OH group in a pseudoaxial orientation and thus it would be perpendicular to the plane of the ring.



Scheme 1 Model for nanotube formation and synthetic strategy for C2-modified γ-amino acid (**γ-Ahf-OH**) and cyclic peptide precursor.

Given the above structural requirements, our synthetic objective was the fully protected derivative **Boc-γ-Ahf(Bn)-OMe** (Scheme 1). We envisioned that this compound could be prepared from *D*-xylose (**6**) by means of previously described intermediate **5**,[9] which could then be transformed into **Boc-γ-Ahf(Bn)-OMe** by benzylation, acetal/aldehyde oxidation, azide reduction and protection. The selection of appropriate orthogonal protecting groups for the amino, hydroxy and carboxylic groups would allow the use of this compound in either Fmoc or Boc solid and solution phase synthesis.[10] For our synthetic purposes, Boc, benzyl and methyl groups were selected, respectively, for solution phase synthesis.

According to the proposed synthetic sequence, *D*-xylose was transformed into compound **5** in five steps and 75% overall yield (Scheme 2).[9] Benzylation of the free hydroxy group by treatment of **5** with sodium hydride, benzyl bromide and tetrabutylammonium iodide provided compound **7** in 77% yield. In order to carry out the acetal oxidation, we initially attempted direct transformation to the methyl ester by several methods, including treatment with chromium salts,[11] DDQ,[12] oxone[13] or hydrogen peroxide and hydrogen chloride in methanol.[14] However, the use of these conditions did not give the desired ester in a reasonable yield. It was then decided to transform the acetal in a stepwise fashion

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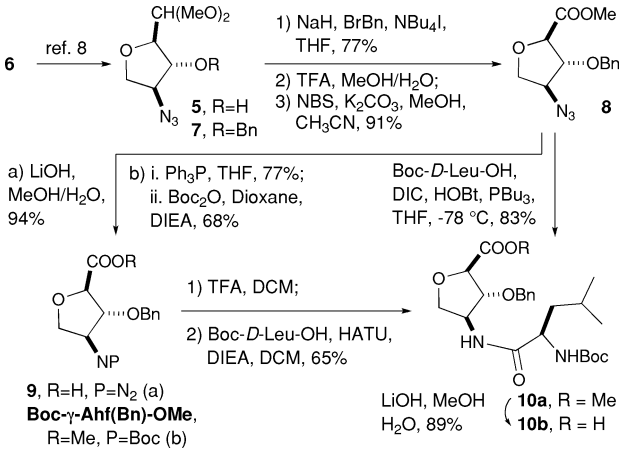
† Electronic supplementary information (ESI) available: Detailed descriptions of the synthesis of the key compounds and their complete characterization, atomic cartesian coordinates for the stationary points calculated with basis set [B3LYP/6-31G(d)], and interaction energies for dimers $\mathbf{D}_{c\text{-}16}$ and $\mathbf{D}_{t\text{-}16}$. See DOI: 10.1039/b911247m



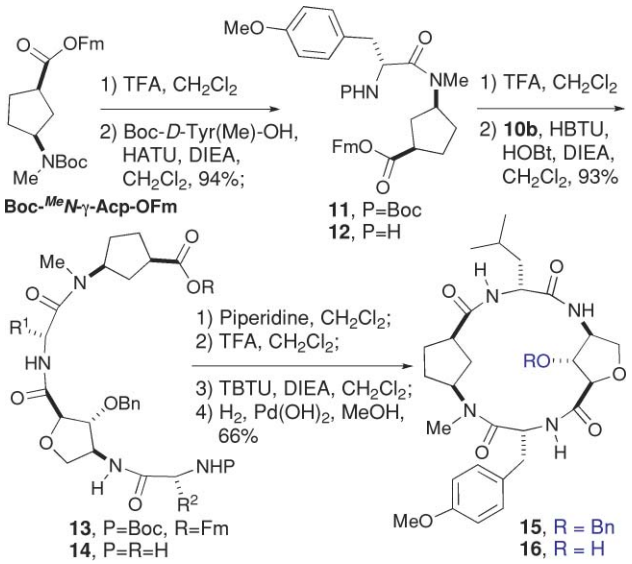
Scheme 2 Synthesis of **Boc-γ-Ahf(Bn)-OMe** and dipeptide **10b**.

to the aldehyde and later to the carboxylic acid derivative. The best conditions involved heating a 1:1 mixture in methanol in the presence of trifluoroacetic acid. The resulting aldehyde was oxidized with N-bromosuccinimide and potassium carbonate to give ester **8** in 91% yield.[15] Hydrolysis of this ester with lithium hydroxide gave the corresponding acid **9** in almost quantitative yield. The 4-azidotetrahydrofurancarboxylic acid derivative can be used directly in peptide synthesis on a solid support,[16] where the coupling of each amino acid can be carried out by means of a Staudinger-type reaction.[17] Additionally, the Staudinger reduction with tributylphosphine in THF, followed by reaction with Boc anhydride and diisopropylethylamine in dioxane (route *b* in Scheme 2), provided the desired **Boc-γ-Ahf(Bn)-OMe** in seven steps and 27% overall yield.

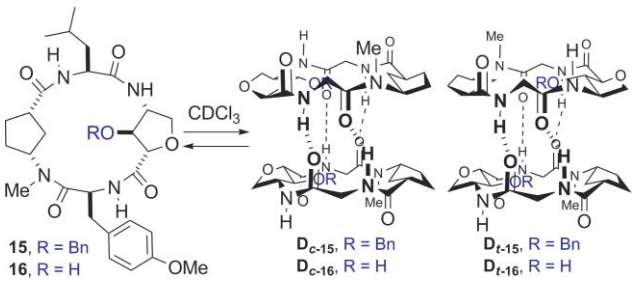
Treatment of **Boc-γ-Ahf(Bn)-OMe** with trifluoroacetic acid followed by coupling with Boc-*D*-Leu-OH in the presence of HATU and diisopropylethylamine in CH_2Cl_2 gave dipeptide **10a** in 65% yield.[18] This dipeptide was also obtained in similar overall yield by *in situ* Staudinger coupling of compound **8** with Boc-*D*-Leu-OH in the presence of tributylphosphine, diisopropylcarbodiimide and N-hydroxybenzotriazole.

Hydrolysis of **10a** with lithium hydroxide in a methanol/water mixture provided acid **10b** in 89% yield. The resulting compound was coupled with dipeptide **12** to provide tetrapeptide **13** in 93% yield (Scheme 3). Dipeptide **12** was obtained by TFA treatment of compound **11**, which was prepared from **Boc-^{Me}N-γ-Acp-OFm**.[7d,e] The use of *N*-methyl γ-Acp was selected to block nanotube formation by introducing steric impediments and removing hydrogen-donors on the γ-face of the ring.[7] Additionally, *D*-Tyr was selected to simplify peptide characterization and purification because of its spectroscopic properties. Finally, removal of N- and C-terminal protecting groups, by subsequent treatment with piperidine and TFA, provided the corresponding unprotected peptide **14**. The reaction of this tetrapeptide with TBTU and DIEA in CH_2Cl_2 in concentrations between 5–15 mM provided cyclic tetrapeptide **15** in 66% yield.[19] Finally, the benzyl-protecting group was removed by hydrogenolysis with palladium hydroxide in methanol to give **16** in almost quantitative yield.

The self-assembly process of the resulting peptides should provide two non-equivalent dimers depending on the relative orientation between the two β-sheet-forming CPs (Scheme 4). We



Scheme 3 Synthesis of CP **16**.



Scheme 4 Self-assembling properties of CPs **15** and **16** and the structure of the expected dimer registers, which differ in the relative orientation of the γ-Ahf, only CPs **16** is able to give the corresponding dimer ($\mathbf{D_{16}}$).

have denoted as *cis*-dimers ($\mathbf{D}_{c\text{-}15}$ and $\mathbf{D}_{c\text{-}16}$) those in which the two **γ-Ahf** residues are on the same side of the dimer, while the *trans*-dimers ($\mathbf{D}_{t\text{-}15}$ and $\mathbf{D}_{t\text{-}16}$) are those in which the xylose-derived γ-Aa are on opposite sides. The ^{1}H NMR spectrum of protected CP **15** suggests that it does not undergo dimer formation ($\mathbf{D}_{t\text{-}15}$) as the N–H signals are not shifted downfield and are concentration independent. On the other hand, the ^{1}H NMR spectrum of the unprotected peptide **16** (Fig. 1) clearly shows that the NH groups [8.1 (NH_{Tyr}) and 7.4 (NH_{Leu}) ppm] are shifted downfield, suggesting their participation in hydrogen-bonding interactions, while the NH_{Ahf} proton that is exposed to solvent in the dimeric form gives a signal at 6.6 ppm. The formation of dimers is also reflected

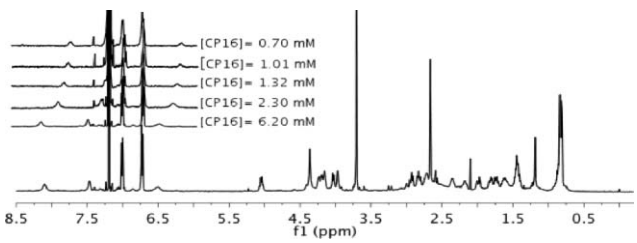


Fig. 1 ^{1}H NMR spectrum of a 6.2 mM solution of peptide **16** in $CDCl_3$ with the 6.0–8.5 ppm region of the spectra of 6.20, 2.30, 1.32, 1.01 and 0.70 mM solutions, showing the downfield shift of NH_{Tyr} and NH_{Leu}.

by the downfield shift of the N–H resonances on increasing the concentration. The association constant, determined at 298 K by dilution experiments, was estimated to be 1.2×10^4 M^{-1} in $CDCl_3$, almost two orders of magnitude larger than previously reported tetrapeptides.[20] So, the presence of the equatorially oriented hydroxy group pointing inwards into the ensemble is clearly participating in an intramolecular hydrogen-bonded interaction between both peptides in the dimer that is stabilizing it. Van't Hoff plots for the range 273–313 K afford values of −39.31 KJ/mol for ΔH°_{298} and −53.38 J/K.mol for ΔS°_{298} and these are consistent with dimerization being essentially an enthalpy-driven[21] hydrogen-bonding process. Dimer formation was also supported by MS, which gave a peak arising from a singly charged species corresponding to sodium dimer $\mathbf{D_{16}}$ (1111), suggesting self-assembly properties and perhaps a good propensity for cation coordination.

Unfortunately, we were unable to establish the type ($\mathbf{D}_{c\text{-}16}$ and $\mathbf{D}_{t\text{-}16}$) or ratio of the topoisomeric dimers formed, either by dimensional NMR or by X-ray crystallography.[22] Computational modelling provided additional insights into the structural properties of peptide nanotubes and helped in the interpretation of experimental observations. These studies were performed using density functional theory (DFT) calculations, with the B3LYP functional and the standard 6-31G(d) basis set for geometry optimizations,[23] in order to evaluate the influence of the OH groups and to explore the dimer preference. As a starting point, we used the X-ray crystallographic data for the homodimer *c*-[(*D*-Phe-*L*-^{Me}*N*-γ-Ach)$_2$-].[7d] From this structure, one of the *N*-methyl groups and the side chains of the α-amino acids were removed, with the former change based on the low effect on the global stability of the dimer.[24] The hydroxylated structures *c*-[*D*-Ala$_1$-γ-Ahf-*D*-Ala$_2$-^{Me}*N*-γ-Acp-] were prepared by replacing the corresponding aliphatic hydrogen by an OH group. The final energies were refined by single-point calculations using two different functionals: B3LYP and Truhlar functional M05-2x,[25] both with the 6-31+G(d,p) basis set.[26] The calculations were carried out with the GAUSSIAN03 package.[27] The most stable structure found was $\mathbf{D}_{c\text{-}16}$, in which both hydroxy groups form a hydrogen bond and at the same time the one acting as the acceptor also interacts with the carbonyl group present in the monomer (Fig. 2). On the other hand in $\mathbf{D}_{t\text{-}16}$ both OH groups form respective hydrogen bonds with the carbonyl groups of the opposite monomer, with N···O distances slightly longer than in $\mathbf{D}_{c\text{-}16}$. This dimer is between 1.53 (B3LYP functional) and 1.93 kcal/mol (Truhlar's functional) less stable than $\mathbf{D}_{c\text{-}16}$, suggesting that the strength of the hydrogen bond between the two hydroxyl groups is responsible for the preferential formation of the *cis*-dimer ($\mathbf{D}_{c\text{-}16}$). These results are consistent with those observed experimentally based on the above-mentioned lack of NOE cross-peaks between the Ahf and Acp α- and γ-protons.

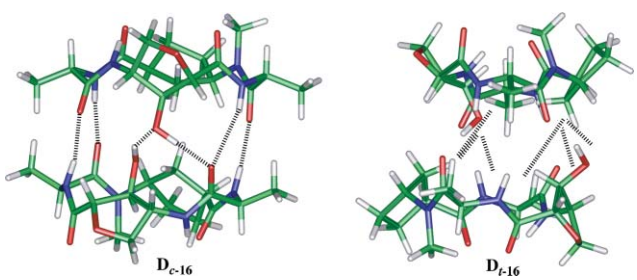


Fig. 2 Structure of two topoisomeric dimers of **16** ($\mathbf{D}_{c\text{-}16}$ and $\mathbf{D}_{t\text{-}16}$) optimized at the B3LYP/6-31+G(d.p).

Interestingly, the analogous dehydroxylated dimers preferred the opposite *trans* conformation (1.59 or 3.21 kcal/mol more stable, using the B3LYP or Truhlar's functional, respectively) and present intersubunit interaction energies that are 11.5 and 10.6 kcal/mol lower than the corresponding *cis* and *trans* hydroxylated dimers, respectively. In other words, the two additional hydrogen bonds formed in the cavity of the hydroxylated dimer are responsible for the higher stability for the ensemble.

Conclusions

We have successfully prepared from *D*-xylose a new cyclic γ-amino acid (4-amino-3-hydroxytetrahydrofuran-2-carboxylic acid, **γ-Ahf**) with appropriate protecting groups that can be used in both solution and solid phase synthesis methods. Furthermore, we used this amino acid in the synthesis of a novel α,γ-CP that form dimers that is simple model for α,γ-SPNs, in which the cavity properties and dimer structure are modulated by the hydroxyl group of the **γ-Ahf**. This group, as a result of the flat conformational disposition of the CP and its equatorial orientation, points inwards into the ensemble and directs the self-assembly process not only to stabilize the dimer ensemble but also the topoisomeric dimer ($\mathbf{D}_c$ *versus* $\mathbf{D}_t$) formed in solution. This cyclic peptide functionalization should lead to nanotubes with greater selectivity as ion channels, catalysts, receptors or molecule containers. Work is in progress to address these possibilities.

Acknowledgements

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