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Stereoselective self-sorting in the self-assembly of a Phe–Phe extended guanidiniocarbonyl pyrrole carboxylate zwitterion: formation of two diastereomeric dimers with significantly different stabilities†

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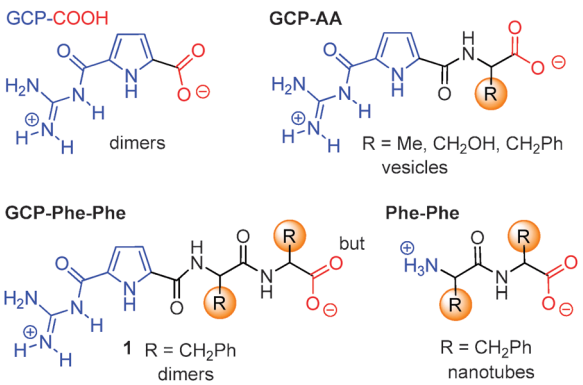
The 'dipeptide extended' guanidiniocarbonyl pyrrole carboxylate zwitterion GCP–Phe–Phe 1 forms stable dimers in DMSO. However, dimerization is highly stereoselective. Only homochiral dimers are formed and the (L,L)·(L,L) dimer (K_{dim} > 10^5 M^{-1}) is significantly more stable by a factor of 10^3 than the diastereomeric (D,L)·(D,L) dimer (K_{dim} = 120 M^{-1}).

Self-assembly of self-complementary building blocks in polar solutions is still difficult to achieve.[1] Hydrogen bonds which are often used for self-assembly due to their directionality and complementarity are not strong enough in competing solvents such as DMSO, alcohols or even water.[2] Additional noncovalent interactions such as electrostatic and hydrophobic interactions or π-stacking are needed to ensure stable complexation in these solvents. We have a long standing expertise in zwitterion induced self-assembly in polar solutions.[3] About ten years ago we reported a rigid guanidiniocarbonyl pyrrole carboxylate zwitterion which forms dimers held together by two H-bond assisted ionpairs (Scheme 1). The stability of the dimers is very high (K_{dim} > 10^8 M^{-1} in DMSO and 10^2–10^3 M^{-1} in water).[4] Recently, we found that more flexible 'amino acid extended' guanidiniocarbonyl pyrrole carboxylate zwitterions in which the carboxylate and the guanidiniocarbonyl pyrrole cation (GCP) are separated by one amino acid also self-assemble in DMSO but not into dimers but into vesicles instead.[5] This finding was in line with other reports on self-assembly of small peptides, which often form large nanostructures such as tubes, fibers or spherical particles.[6] For example the zwitterionic dipeptide H–Phe–Phe–OH self-assembles into nanotubes which are several micrometers in length and *ca.* 50–300 nm in diameter.[7,8] The cationic dipeptide H–Phe–Phe–NH_2 (as hydrochloride salt) forms nanotubes which upon dilution then rearrange into vesicles.[9] We were therefore quite surprised when we now discovered that the dipeptide Phe–Phe when derivatized with our GCP cation at the N-terminus again self-assembles only into discrete dimers with no formation of any larger aggregates. Furthermore, dimerization of this 'dipeptide extended' guanidiniocarbonyl pyrrole carboxylate is highly stereoselective. Only homochiral dimers are formed and their stability differs by a factor of at least >10^3 for the two diastereomeric dimers, the (L,L)·(L,L) and the (D,L)·(D,L) dimer, respectively.

The synthesis of the four stereoisomers of GCP–Phe–Phe–OH **1a–d** followed a standard protocol (SuppInfo†). At millimolar concentrations in DMSO none of the four stereoisomers gave any experimental indication for the formation of larger nanostructures either in DLS nor AFM (data not shown). Instead only formation of discrete and well defined dimers was observed (*vide infra*).

However, the two diastereomers **1a**/**b** (L,L and D,D) and **1c**/**d** (D,L and L,D) self-assemble very differently as immediately evident from the NMR spectra (Fig. 1). The shifts in both spectra are characteristic for ionpair formation between the GCP cation and the carboxylate.[4] For example, in the spectrum of the L,L stereoisomer **1a** the guanidinio amide NH gives a signal at δ = 14.5 and the four guanidinium NH protons give two signals at δ = 10.1 and δ = 8.0, respectively. These shifts are similar to those observed previously in related zwitterions which dimerize in solution[4] and they are diagnostic for ionpair formation. Stereosiomer **1c** also shows similar shifts, however, in contrast to **1a** the signals for the D,L stereoisomer **1c** are rather broad and appear at higher field. For example, the guanidinio amide NH gives a broad signal at δ = 13.5 for **1c**, a shift of nearly 1 ppm to higher field compared to the sharp signal at δ = 14.5 for **1a**.



Scheme 1 The self-assembly of guanidiniocarbonyl pyrrole carboxylate zwitterions depends on the linker between the carboxylate and the cation.

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† Electronic supplementary information (ESI) available: Details for the synthesis of **1**, NMR spectra, UV/Vis dilution study and details on the DFT calculations. See DOI: 10.1039/c1cc12520f

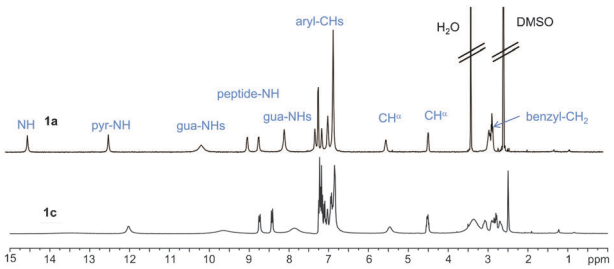

Fig. 1 ^{1}H NMR spectra of the two diastereomers of the GCP–Phe–Phe zwitterions **1a** (15 mM, top) and **1c** (80 mM, bottom) in DMSO-d6.

Also the signals for the pyrrole NH and the two signals for the four guanidinium NH protons appear at higher field. A NMR dilution-study of **1d** in DMSO (Fig. 2) showed concentration-dependent shift-changes in the range from 0.5 to 100 mM, which indicated an intermolecular aggregation. Formation of a dimer **1d**·**1d** was confirmed in a DOSY-NMR experiment (c = 30 mM in DMSO-d6). A hydrodynamic radius r_h = 0.87 nm and hence a particle diameter of 1.74 nm was obtained, which compares well with the molecular dimensions of a dimer as estimated from molecular modeling studies (the calculated molecular surface of the dimer of 725 Å^2 corresponds to a sphere with *ca.* 1.5 nm diameter). A quantitative analysis of the NMR shift changes provides a dimerisation constant of K_{dim} = 120 M^{-1}. Hence, the stability of the dimers is similar to other flexible zwitterions in such a highly polar solvent.[10]

Quite different results were obtained for the two enantiomeric zwitterions **1a**,**b** (L,L and D,D stereoisomer). As explained above the NMR spectrum also clearly confirms the formation of ionpairs (Fig. 1). However, at least in the range from 0.1–100 mM in DMSO no concentration dependent shift changes are observed. DOSY NMR (c = 50 mM) provided a diameter of 1.82 nm confirming again the presence of dimers in solution of similar size as the dimers formed from **1c**. The lack of concentration dependent shift changes in the NMR spectrum indicates a very high stability of the dimers ($K_{dim} > 10^4$ M^{-1}) in sharp contrast to the stability of the dimers formed from the diastereomer **1c** (K_{dim} = 120 M^{-1}). Due to their high stability the dissociation

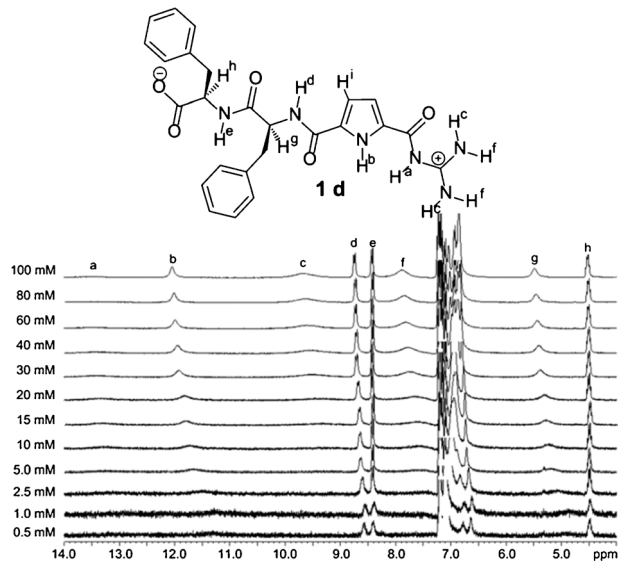

Fig. 2 ^{1}H NMR dilution study of zwitterion **1d** in DMSO-d6.

of the dimers formed from **1a** requires much lower concentrations than accessible in a NMR experiment. Concentration dependent UV/Vis studies in the concentration range from 0.8 to 0.005 mM allowed to follow this dissociation. The molar absorptivity ε of the pyrrole moiety at around λ = 300 nm increases with decreasing concentrations. We know from other studies that binding of a carboxylate to the guanidiniocarbonyl pyrrole cation causes a decrease in this absorption band.[11] Therefore, the increase of ε in more dilute solutions reflects the dissociation of the dimers into non ionpaired monomers. A quantitative analysis of this concentration dependent UV change provides a dimerization constant of K_{dim} = 3 × 10^5 M^{-1}.

Hence, the enantiomers **1a**,**b** (L,L and D,D) form significantly more stable dimers than their diastereomers **1c**,**d** (D,L and L,D). The dimer stabilities differ by a factor of 10^3. Dimerization is also highly stereoselective. When we mixed any of the four stereoisomers **1a–d** no indication for the formation of mixed dimers was observed in the NMR spectra (SuppInfo†). Even though in principle 10 different dimers could form, each stereoisomer **1a–d** in a self-sorting process exclusively interacts with itself forming only the four homochiral dimers

The calculated energy minimized structures of the two dimers of **1a** and **1c** are shown in Fig. 3. In both dimers the typical H-bond assisted ion pairing motif between the carboxylate of the one dipeptide and the guanidiniocarbonyl pyrrole cation of the other is seen (in agreement with the observed downfield shifts in the NMR). Furthermore, the adjacent pyrrole carbonyl oxygen forms an additional H-bond to the C-terminal amide NH next to the carboxylate. In this respect both dimers are similar. However, the change in stereochemistry of one of the Cα within the dipeptide has a profound effect on how these two ion pairs are arranged within the dimer. In the dimer **1a**·**1a** the two molecules are facing each other and are aligned side by side. One benzyl group of each monomer points upwards and the other downwards from the plane of the two ion pairs. In the diastereomeric dimer **1c**·**1c** the two ion pairs are on top of each other and all four benzyl groups point to one side of the dimer.

A DFT calculation (m052x/6-311+G**) confirmed the observed difference in stabilities of the two dimers. The geometry optimized structure of dimer **1a**·**1a** is 5.7 kcal mol^{-1} more stable than dimer **1c**·**1c** and the calculated dimerization energies going from the optimized monomers to the dimers differ by 2.7 kcal/mol. These values are in good agreement with the observed experimental difference in stability of $\Delta K \geq 10^3$, which corresponds to an energy difference of $\Delta E \geq$ 4.2 kcal mol^{-1}. The difference in stability of the two dimers stems most likely from a combination of two major effects: First, a more compact structure which allows probably more extensive aromatic interactions and a more favorable solvation and second a significantly more efficient charge interaction within dimer **1a**·**1a** as evident from the changes of the molecular dipoles. Both monomers **1a** and **1c** have a molecular dipole of *ca.* μ = 13 D. However, whereas the side-by-side alignment of the two monomers within dimer **1a**·**1a** nearly completely cancels the dipole (μ = 1.8 D), the on-top orientation within dimer **1c**·**1c** actually causes an increase to μ = 19 D. Hence, relative to the monomers the charge interaction is more favorable within dimer **1a**·**1a** than within dimer **1c**·**1c**.

According to the calculated structure of dimer **1a**·**1a** the two amide NH protons of the dipeptide moiety are bound in different

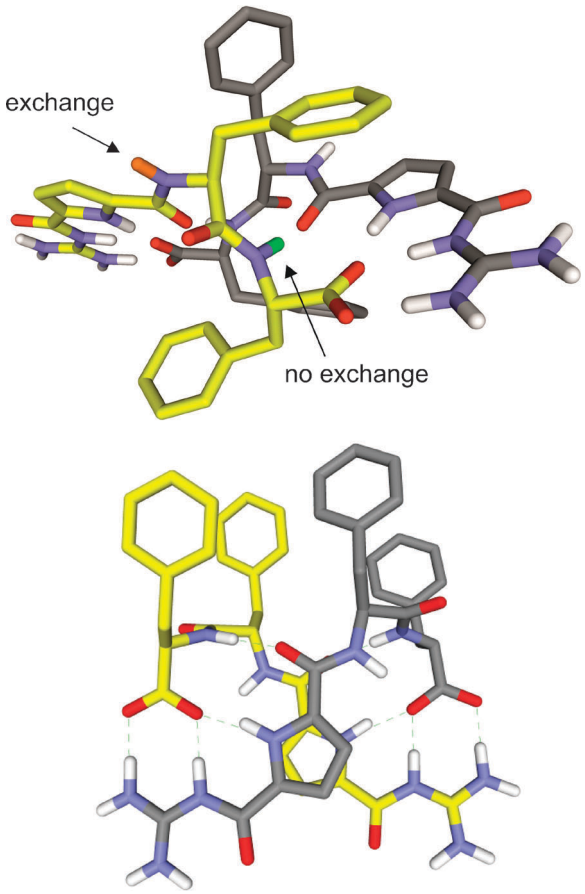

Fig. 3 Energy minimized structures of the dimer of **1a** (top) and **1c** (bottom) as obtained from force field calculations. Nonpolar hydrogens are omitted for clarity.

chemical environments. Whereas the N-terminal amide NH^d is solvent exposed, the C-terminal amide NH^e forms an intermolecular H-bond to the pyrrole carbonyl oxygen of the second molecule. This difference in the binding situation of the amide NH protons could be confirmed by a H/D exchange experiment (Fig. 4). After addition of 10 μl D_2O to a 10 mM solution of **1a** in DMSO-d6, the intensity of the NMR signal of the amide NH^d proton decreases very fast within minutes. The signal of the amide NH^e proton which is involved in the intermolecular H-bond to the second zwitterion remains however unchanged. Even after several hours only very little H/D exchange is observed for this proton. This experiment therefore confirms the proposed structure and also the extraordinary high stability of this dimer. No significant amount of monomer is present because otherwise exchange of both amide NH protons would occur, which is the case for the dimer of **1c**. Both amide NH protons exchange with similar rates and within one hour both signals have disappeared. Even though the two amide NH protons also have a different binding environment within dimer **1c**·**1c**, the significantly lower stability of this dimer means that always a substantial fraction of monomers is present in which both amide NH protons can exchange with the solvent with equal rates.

In conclusion, we have shown here that dimerization of zwitterion **1** is highly stereospecific as of all possible ten stereoisomeric dimers only the four homochiral dimers are

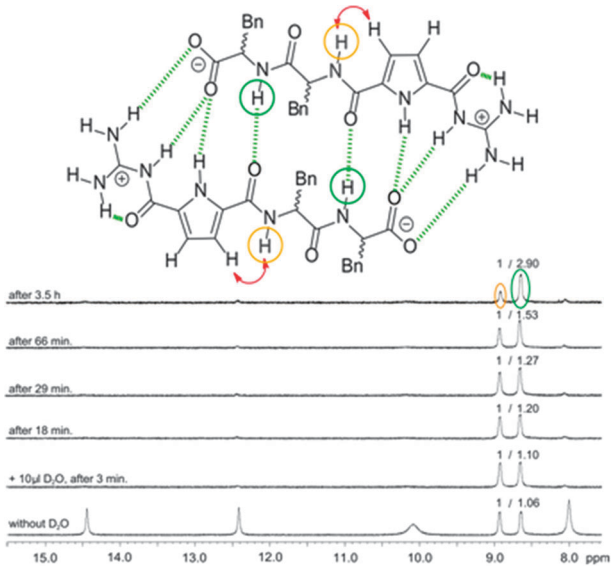

Fig. 4 H/D exchange experiment of zwitterion **1a** in DMSO-d6 (10 mM). The two amino acid amide NH protons show significantly different exchange rates. The C-terminal amide NH^e (circled green) which is involved in the dimerization does not exchange even within 3.5 h. The N-terminal amide NH^d (circled orange) which is exposed to the solvent does show significant H/D exchange within this time. The red arrows indicate a diagnostic nOe which also confirms this structure.

formed which furthermore differ significantly in their stability by at least a factor of 10^3. Even the switch of the configuration of one stereocentre can thus have a tremendous effect on the self-assembling properties of the molecule.

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