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Disulfide Exchange in Hydrogen-Bonded Cyclic Assemblies: Stereochemical Self-Selection by Double Dynamic Chemistry

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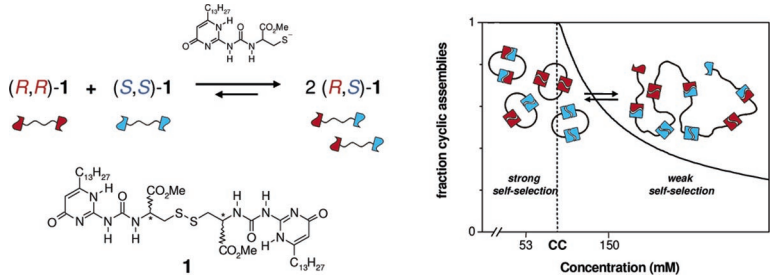

Stereoselective cyclization of cystine-based bifunctional 2-ureido-4[1H]-pyrimidinone derivatives in $CDCl_3$ solutions was demonstrated by ^1H NMR spectroscopy. Thiolate-catalyzed disulfide exchange in solution led to the equilibration of different diastereomers of **1**. At low concentrations, where formation of cyclic assemblies is the dominant mode of association, the molecules act as their own template. At these concentrations the meso diastereomer is formed preferentially, indicating a higher stability of its cyclic assemblies under the applied conditions, in comparison to the other diastereomers.

Introduction

Chiral discrimination is the basis of asymmetric catalysis, chiral chromatographic separations and many natural processes.[1] In molecular recognition, the difference in binding strength between the host and the enantiomers of the guest determines stereoselectivity. Strong binding as well as a high degree of enantioselectivity may be obtained for hydrogen-bonded host−guest complexes, due to both the strength and high directionality of hydrogen bonds.[2] Enantiomeric self-recognition—a specific example of stereoselective association—requires different association energies between molecules of equal and of opposite configuration. In the solid state, spontaneous resolution during crystallization, first described by Pasteur,[3] is a well-known phenomenon. In contrast to that, selectivities for self-recognition in solution are generally low, because the intermolecular interactions are usually quite weak. High stereoselectivities in solution require a large difference in association constant between homochiral and heterochiral assemblies,[4] and have been predicted to occur only if K_{homo} is at least an order of magnitude higher than K_{hetero}.[5] In fact, exclusive formation of homodimers by self-selection has been observed in strongly associated metal−ligand complexes[6] and assemblies based on multiple hydrogen bonding.[7] Murguly et al. described chiral discrimination in hydrogen-bonded [7]helicenes.[8] The relative positions of the hydrogen-bonding arrays in these molecules results in enantiospecific self-assembly in solution, whereas association in the solid state is diastereoselective. Homochiral supramolecular polymerization of xylylene-bridged bis(cyclic peptide)s was reported by Ishida and Aida.[9]

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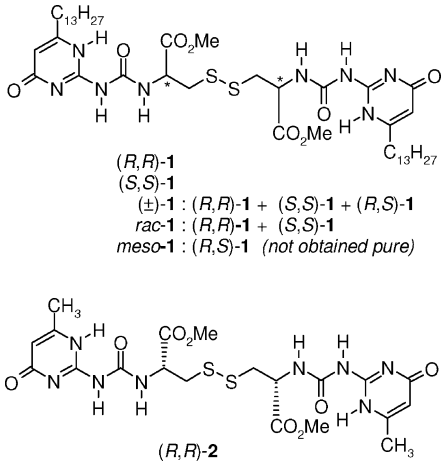
Their work points toward the fascinating possibility to translate stereoselectivity to molecular size distribution.

Recently, we have reported the association behavior of 2-ureido-4[1H]-pyrimidinone (UPy) derivatives **1** and **2**, which assemble by quadruple hydrogen bonding to form cyclic aggregates below their critical concentration (CC).[10,11] Above the CC, the amount of cyclic species becomes constant, and polymer is formed. In addition, the strong association between the UPy moieties, combined with the specific spatial arrangement of the UPy groups caused by the conformational preference of the monomer, was demonstrated to result in the selective formation of homochiral cycles in racemic solutions of bifunctional UPy derivatives. Remarkably, the CC of racemic **1** was shown to be significantly lower than that of the pure enantiomers, which was ascribed to the differential enantioselectivities with which cycles and polymers are formed. These results prompted us to include the (*R*,*S*)-diastereomer of **1** in our study and investigate the covalent fixation of stereoselectivities in **1**, employing the reversibility of the disulfide bond in this molecule.

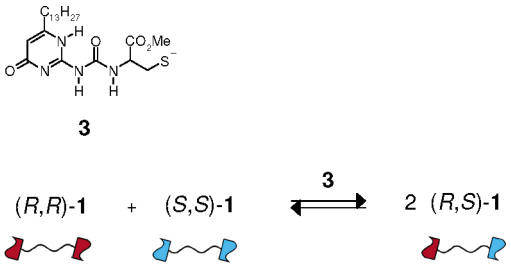
Reversibility of the disulfide bond under appropriate conditions makes it one of the most useful functional groups for "dynamic covalent chemistry".[12] For example, disulfide-thiol exchange has been employed in the study of nearest neighbor recognition in phospholipid membranes[13,14] and self-recognition of β-sheet-forming peptides,[15] as well as in the development of dynamic combinatorial libraries (DCLs).[16] Most studies using disulfide exchange have been performed in aqueous environment, but successful disulfide coupling in nonpolar solvents has been reported.[17,18] Recently, dynamic covalent chemistry was combined with the formation of hydrogen-bonded polymers, to give "double dynamic supramolecular polymers".[19]

Here we report the formation of thermodynamically controlled product mixtures of **1**, produced by disulfide exchange reactions in the presence of base and a catalytic amount of thiolate, conditions which also allow dimerization of the UPy groups of **1** by quadruple hydrogen bonding. Under these conditions, equilibration of two different types of reversible bonds (hydrogen bonds and disulfides) may take place. If the thermodynamic stability of the three monomers (*R*,*R*)-**1**, (*S*,*S*)-**1** and (*R*,*S*)-**1** is equal, the product distribution will be entirely determined by the relative energy of the assemblies. When

SCHEME 1. Structure of UPy Derivatives 1 and 2



SCHEME 2. Equilibrium between Diastereomers of 1 in Disulfide Exchange Reactions



self-assembly is stereoselective under the conditions of equilibration, each stereoisomer may act as template for its own formation during disulfide exchange (Scheme 2). This process would constitute an example of stereochemical self-selection by double dynamic chemistry. Self-selection is expected to be concentration dependent, as a result of the difference in stereoselectivity with which cyclic and linear assemblies are formed.

In the following section, before discussing dynamic chemistry via disulfide exchange, we first provide additional NMR evidence that cyclization in solutions of **1** is stereoselective, and we use the assignment of its ^{1}H NMR spectrum to identify the characteristic signals of meso-**1** in (±)-**1** mixtures.

Results and Discussion

Synthesis. Bis-UPy derivatives **1** and **2** (Scheme 1) were obtained by reaction of cystine dimethyl ester with blocked isocytosine isocyanates.[20] The synthesis of (*R*,*R*)-**1**, (*S*,*S*)-**1** and (*R*,*R*)-**2** has been reported previously.[10] Compound (±)-**1** was prepared by the same procedure, starting from d,l-cystine dimethyl ester dihydrochloride. *Rac*-**1** was obtained by mixing equimolar amounts of (*R*,*R*)-**1** and (*S*,*S*)-**1**. Selective synthesis or separation of *meso*-**1** was also attempted in a number of ways, including fractional crystallization of d,l-cystine to obtain pure *meso*-cystine and careful flash column chromatography of (±)-**1**. However, none of these methods yielded *meso*-**1** of sufficient purity, in part due to the

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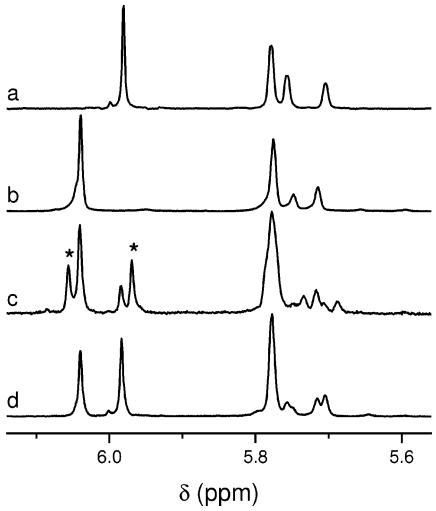

FIGURE 1. Alkylidene region of ^{1}H NMR spectra of (a) (*R*,*R*)-**1**, (b) (*R*,*R*)-**2**, (c) equimolar mixture of (*R*,*R*)-**2** and (*R*,*R*)-**1** and (d) equimolar mixture of (*R*,*R*)-**2** and (*S*,*S*)-**1**, all in dilute $CDCl_3$ solution; asterisks denote peaks assigned to cyclic heterodimer (*R*,*R*)-**1**·(*R*,*R*)-**2**.

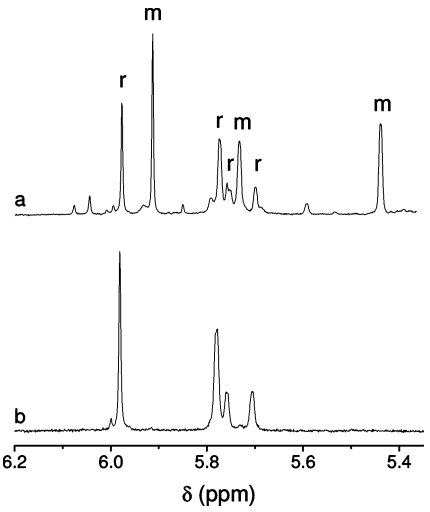

FIGURE 2. Part of the ^{1}H NMR spectra of (a) (±)-**1** and (b) *rac*-**1** in $CDCl_3$ (around 30 mM); peaks labeled "r" correspond to hydrogen-bonded assemblies of *rac*-**1**, peaks labeled "m" to assemblies of *meso*-**1**.

limited stability of *meso*-cystine toward both basic and acidic conditions.

NMR Spectroscopy of Racemic Mixture. As a result of the high association constant of the UPy groups ($K_a = 6{\cdot}10^7$ M^{-1} in $CDCl_3$),[21] monomers **1** and **2** fully associate into hydrogen-bonded assemblies in $CDCl_3$ solution, as demonstrated by the highly upfield shifts of the hydrogen-bonded proton signals in the ^{1}H NMR spectra. Because exchange is slow on the NMR time scale, UPy moieties in different assemblies give rise to separate signals, which can be assigned to either cyclic or linear species based on concentration-dependent ^{1}H NMR spectroscopy and ^{1}H NMR diffusion experiments. As reported previously,[10] the ^{1}H NMR spectrum of *rac*-**1** below the CC was found to be indistinguishable from that of the pure enantiomers, indicating that cyclization occurs enantioselectively. Previous research[11,22,23] has shown that the chemical shift of the UPy protons of hydrogen-bonded cyclic dimers is highly dependent on the conformation of monomers in the assembly, making it unlikely that homochiral and heterochiral dimers would have exactly the same chemical shift. Even more direct evidence of the enantioselectivity of cyclization could be obtained by using monomers that can be distinguished from each other, but still have the same association properties. For this purpose, we selected mixtures of derivatives **1** and **2**, which only differ in the length of the alkyl tail on the pyrimidinone ring. Comparing separate ^{1}H NMR spectra of (*R*,*R*)-**1** and (*R*,*R*)-**2** in $CDCl_3$, the sets of signals for the alkylidene protons (Figure 1a,b), as well as for the hydrogen-bonded UPy protons, were found to be very similar. This demonstrates that cyclic structures formed by the two compounds have similar conformations, which should allow the formation of cyclic heterodimers of equal enantiomers of **1** and **2**. Indeed, ^{1}H NMR spectra of mixtures of (*R*,*R*)-**1** and (*R*,*R*)-**2** were found to display signals for hydrogen-bonded assemblies of both compounds as well as some additional peaks, which were assigned to cyclic heterodimers (*R*,*R*)-**1**·(*R*,*R*)-**2** (Figure 1c). This can most clearly be seen for the alkylidene signal around 6.0 ppm; apart from the peaks also present in the spectra of the pure compounds, the ^{1}H NMR spectrum of the (*R*,*R*)-**1** and (*R*,*R*)-**2** mixture contains two additional peaks at 6.06 and 5.97 ppm, which are absent in the spectrum of a mixture of (*S*,*S*)-**1** and (*R*,*R*)-**2** (Figure 1d). This confirms the observation that cyclization of racemic **1** and **2** in deuterated chloroform solution occurs enantioselectively, i.e. a large difference in stability exists between homochiral and heterochiral assemblies.

$CDCl_3$ solutions of (±)-**1**, containing *rac*-**1** as well as *meso*-**1**, were also studied by ^{1}H NMR spectroscopy. First, the diastereomeric ratio in (±)-**1** was determined by ^{1}H NMR spectroscopy of (±)-**1** in $CDCl_3$ containing 5% of trifluoroacetic acid (TFA) to prevent the formation of hydrogen-bonded assemblies. In the ^{1}H NMR spectra thus obtained, separate peaks were observed for the UPy alkylidene proton as well as for the methyl ester protons of *rac*-**1** and *meso*-**1**. By integration of the methyl ester peaks, the mol fractions of *rac*-**1** (3.84 ppm) and *meso*-**1** (3.83 ppm) in (±)-**1** were calculated to be 0.46 ± 0.02 and 0.54 ± 0.02, respectively. In pure $CDCl_3$ solutions of (±)-**1**, in the presence of hydrogen bonding, many different assemblies are formed. Figure 2 shows the alkylidene region of ^{1}H NMR spectra of *rac*-**1** and (±)-**1** at low concentration (approximately 30 mM) in $CDCl_3$. Apart from the signals also present for *rac*-**1**, three major peaks (indicated with "m") were observed in the spectrum of (±)-**1**, which could originate either from cyclic assemblies of *meso*-**1** or mixed cycles of *meso*-**1** with the other diastereomers.[24] Deconvolution and integration of the different alkylidene signals showed that the three additional peaks represented 54±2% of the mixture of all

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assemblies. The good correspondence between this value and the mol fraction of *meso*-**1** in (±)-**1** indicates that all three peaks correspond to cyclic assemblies of *meso*-**1** and that heterocycles of *meso*-**1** and *rac*-**1** are not formed to a very large extent. This hypothesis was supported further by later experiments (see below).

Disulfide Exchange. In the first part of this paper, we have focused on the reversibility of the hydrogen-bonding interactions between molecules of **1**. In those experiments, the diastereomeric ratio between *rac*-**1** and *meso*-**1** was not affected. However, by making use of the presence of a disulfide moiety as a second reversible linkage, interconversion of different diastereomers of **1** by thiol-disulfide exchange reactions (Scheme 2) is possible. This allows us to also examine the relative stability of *meso*-**1** and *rac*-**1** in the corresponding hydrogen-bonded assemblies. To enable the described templating by the formation of cyclic assemblies, disulfide exchange experiments were performed under conditions that also allowed association by hydrogen bonding. The experiments were carried out at concentrations below as well as above the critical concentration of (±)-**1**, which was estimated to be between 65 mM and 100 mM at room temperature. Solutions of *rac*-**1** and solutions of (±)-**1** in $CDCl_3$ were treated with a catalytic amount (5 mol %) of dithiothreitol (DTT), in the presence of triethylamine. In this way, a small amount of thiolate **3** was formed, which subsequently catalyzed thiol-disulfide exchange reactions and the interconversion of different diastereomers of **1**, according to Scheme 2.

The equilibration process was monitored by ^{1}H NMR spectroscopy. In all experiments, we were interested in the position of the equilibrium depicted in Scheme 2, i.e. the ratio of *rac*-**1** and *meso*-**1** present in hydrogen-bonded assemblies. Figure 3a shows part of the ^{1}H NMR spectrum of a 53 mM solution of *rac*-**1** in $CDCl_3$, before and after treatment with 5% DTT and subsequent equilibration. Before addition of DTT, four peaks corresponding to cyclic oligomers of (*R*,*R*)-**1** and (*S*,*S*)-**1** were observed for the UPy alkylidene proton. In the presence of a catalytic amount of thiol, the intensity of these peaks decreased with time, while three peaks corresponding to *meso*-**1** appeared. In addition, the presence of **3** induced the formation of some linear hydrogen-bonded assemblies, incorporating monofunctional monomer **3** and some additional bifunctional monomers, as can be observed by the appearance of a broad peak (indicated with ∗) at 5.85 ppm. Relative integrals of the signals at different times were determined by deconvolution and are displayed in Figure 3b. Although accurate quantitative determination of the diastereomeric ratio is hampered by the presence of linear assemblies, the conversion of *rac*-**1** into *meso*-**1** is evident. Furthermore, the ratio of the three peaks assigned to *meso*-**1** remains constant during the experiment, supporting the statement that also this diastereomer associates stereoselectively and that all three peaks correspond to cyclic assemblies containing only *meso*-**1**.

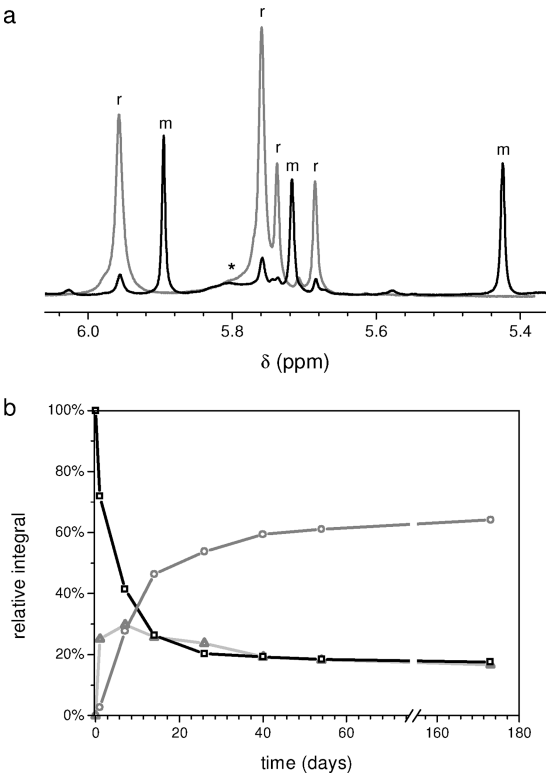

FIGURE 3. (a) Alkylidene region of ^{1}H NMR spectra of a racemic solution of **1** in $CDCl_3$ (53 mM), before (gray) and 40 days after (black) treatment with 5 mol % DTT; signals of *rac*-**1** and *meso*-**1** in cyclic assemblies are labeled with "r" and "m", respectively, and the peak marked with "∗" corresponds to thiol **3** and linear assemblies; (b) relative integrals of ^{1}H NMR signals of *rac*-**1** (squares) and *meso*-**1** (circles) in hydrogen-bonded cycles and of linear assemblies (triangles) during disulfide exchange.

After 50 days, the system had reached equilibrium, as no additional changes in the ^{1}H NMR spectrum were observed. After 173 days, the reaction mixture was quenched by addition of excess TFA. Apart from preventing further exchange reactions by protonating all thiolate present, the addition of acid inhibited formation of hydrogen-bonded aggregates and allowed accurate quantitative analysis of the diastereomeric ratio by ^{1}H NMR spectroscopy. The product was found to contain 24% of *rac*-**1** and 76% of *meso*-**1**. A similar product composition (27% and 73%, respectively) was found in a control experiment using a 53 mM solution of (±)-**1** as starting point, demonstrating that the conditions used were truly equilibrating and that the observed stereoselectivity was thermodynamic in nature. Formation of *meso*-**1** was not observed in a control experiment in which triethylamine was added to a $CDCl_3$ solution of *rac*-**1** in the absence of DTT, demonstrating that interconversion of the diastereomers indeed takes place by disulfide exchange.

Disulfide exchange in the presence of 5 mol % thiol was also performed at 150 mM, which is well above the critical concentration for (±)-**1**. In this case, equilibrium was already reached after 20 days. After quenching with TFA, 126 days after DTT addition, mole percentages of *rac*-**1** and *meso*-**1** in the product were determined to be 35% and 65%, significantly closer to the 1:1 statistical product distribution expected in the absence of self-

(24) At concentrations up to 66 mM, no significant changes in the number of alkylidene signals or the relative integrals of different signals were observed in the ^{1}H NMR spectra of (±)-**1**, indicating that only small cyclic oligomers are formed; at higher concentrations an additional peak was observed at 5.79 ppm, which was assigned to polymeric aggregates.

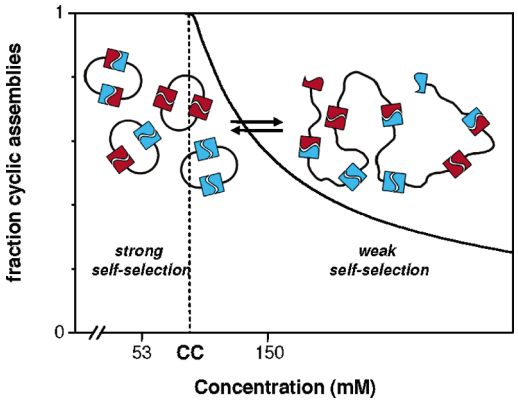

FIGURE 4. Correlation between self-selection and (critical) concentration.

selection. The concentration dependence of self-selection can be rationalized as follows: below the critical concentration, when linear aggregates are absent, the equilibrium composition of a mixture of *meso*-**1** and *rac*-**1** (both including equal amounts of (*R*)- and (*S*)-cysteine derivatives) is determined by the relative stability of their cyclic assemblies. Since association of monomers in linear aggregates is much less stereoselective, self-selection has a progressively diminishing effect on product composition as the concentration is increased above the critical concentration (Figure 4). At 150 mM, when significant amounts of the monomer are incorporated into linear polymeric aggregates, the preference for the formation of *meso*-**1** is diminished. This indicates that the observed selectivity toward *meso*-**1** indeed originates from the higher stability of its cyclic assemblies relative to those of *rac*-**1**.

Conclusions

Equilibration of $CDCl_3$ solutions of **1** by disulfide exchange in the presence of hydrogen bonding between UPy units results in enrichment of the *meso* isomer because the formation of cyclic assemblies is stereoselective and the thermodynamic stability of these assemblies are different. Above the critical concentration, the enrichment is reduced because linear supramolecular polymers are formed in which stereoselectivity is low. The diastereoselective cyclization of **1** complements the enantioselectivity of cyclization reported previously.[10] Together, these stereoselectivities substantiate the importance of strong hydrogen bonding in selective self-assembly; the disulfide exchange reaction has been shown to be a valuable tool in covalently fixing these transient selectivities.

Experimental Section

Thiol−Disulfide Exchange. All disulfide exchange experiments were carried out in NMR tubes equipped with J. Young valves and performed in an argon atmosphere. To $CDCl_3$ solutions of either *rac*-**1** or (±)-**1**, triethylamine (1 equiv.) and dithiothreitol (0.05 equiv) were added, and the solutions were shaken thoroughly. The solutions were kept at room temperature and analyzed by ^{1}H NMR spectroscopy at regular intervals.

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Supporting Information Available: Experimental details for the synthesis and characterization of (±)-**1**; detailed assignment of the ^{1}H NMR spectrum of (*R*,*R*)-**1**. This information is available free of charge via the Internet at http://pubs.acs.org.

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