

Self-organization and chirality in the high dilution solutions of glycoluril enantiomers with (*R*)- and (*S*)-methionine moieties

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The high dilution (1×10^{-15} – 1×10^{-5} mol dm⁻³) solutions of glycolurils containing an (*S*)-methionine moiety are capable of space-time self-organization to give nanoassociates, unlike the solutions of glycolurils with an (*R*)-methionine moiety. The formation of supra-molecular domains in the solutions at concentrations of 1×10^{-3} – 1×10^{-2} mol dm⁻³ is responsible for a transition from molecular to supramolecular chirality.

The high dilution solutions of biologically active compounds are of considerable interest for solution chemistry, biosphere evolution, ecology, pharmacology, toxicology, medicine and agriculture.^{1,2} Recently, a phenomenon of the formation of large-scale nanosized molecular ensembles (up to 400 nm) called nanoassociates was discovered in a study³ of the self-organization of high dilution aqueous solutions of biologically active compounds with concentrations of 10^{-20} – 10^{-6} mol dm⁻³. The formation and rearrangement of nanoassociates give rise to non-monotonic concentration plots of the physicochemical properties of high dilution solutions and correlate with their bioeffects.³ The self-organization of high dilution solutions is initiated by a solute under certain conditions depending on its structure, the presence of external geomagnetic and electromagnetic fields and preparation procedures.

We studied the effect of the spatial structure of solutes on the self-organization and physicochemical properties of the high dilution aqueous solutions of glycolurils, namely, 2-(3,7-dioxo-2,4,6,8-tetraazabicyclo[3.3.0]oct-2-yl)-4-methylthiobutanoic acid derivatives containing chiral methionine amino acid moieties, viz., (*R*)- or (*S*)-Met: **1**, racemate (**1a** + **1b**); **1a**, **1a'**, diastereomers with the 2(*S*)-(1*R*,5*S*) and 2(*S*)-(1*S*,5*R*)-configurations of asymmetric carbon atoms, respectively; **1b**, **1b'**, diastereomers with the 2(*R*)-(1*S*,5*R*) and 2(*R*)-(1*R*,5*S*)-configuration of asymmetric

carbon atoms, respectively; the pairs of **1a** and **1b**, **1a'** and **1b'** enantiomers.

The test compounds are characterized by a broad spectrum of biological activity, including neurotropic action.^{4–8} In addition to a pharmacophoric glycoluril moiety, the molecules contain (*R*)- or (*S*)-Met moieties. (*S*)-Met is involved in ribosomal protein and choline synthesis, it activates the action of hormones, vitamins, enzymes, etc., whereas these functions are not inherent in (*R*)-Met.⁴ Studies on the pharmacological activity of glycolurils revealed a neurotropic effect only for glycoluril **1a** with an (*S*)-methionine moiety.⁵ Thus, the self-organization and properties of dilute solutions of glycolurils **1**, **1a**, **1a'**, **1b**, **1b'** might give a key to understanding the specificity of amino acid enantiomers.⁹

Glycolurils **1**, **1a**, **1a'**, **1b** and **1b'** were synthesized by diastereoselective reactions of (*S,R*)-, (*S*)- and (*R*)-*N*-carbamoyl-methionine **2**, **2a** and **2b**, respectively, with 4,5-dihydroxyimidazolidin-2-one **3**^{5,10,11} (Scheme S1, Online Supplementary Materials). An XRD study of the glycolurils showed that the configuration of an amino acid moiety remains the same.^{5,10} Racemate **1** is crystallized as a conglomerate of **1a** and **1b**.⁵

The preparation and characterization of solutions under natural and hypoelectromagnetic conditions were carried out as described previously^{3,12} (see Online Supplementary Materials). The effect of reduced external fields was studied using a screening cylindrical three-layer heat-treated permalloy container with a screen factor

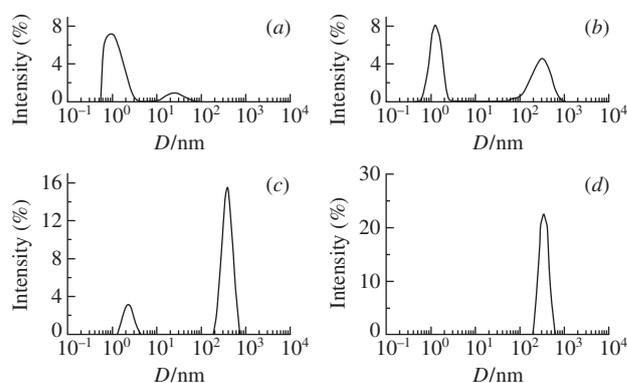
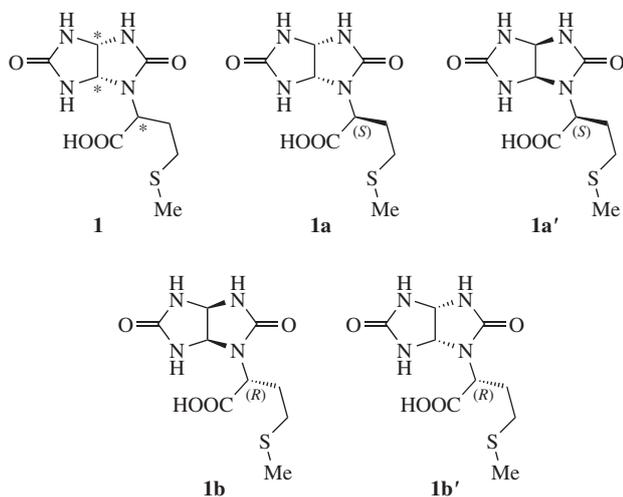


Figure 1 Size distribution of particles in an aqueous solution of **1a** kept for 24 h on a laboratory bench: (a) 7×10^{-2} , (b) 1×10^{-2} , (c) 1×10^{-4} and (d) 1×10^{-11} mol dm⁻³.

of ~ 1000 .¹³ The working solutions were simultaneously kept for 24 h on a laboratory bench (natural conditions, series A) and in the screening container (hypoelectromagnetic conditions, series B). The particle parameters and solution properties were compared for series A and B of the same solution.

Studies of the solutions of **1**, **1a**, **1a'**, **1b**, **1b'** in a broad concentration range from 7×10^{-2} (2%) to 1×10^{-18} mol dm⁻³ by dynamic light scattering (DLS) and microelectrophoresis showed that three groups of glycolurils can be distinguished. The first comprises diastereomers **1a** and **1a'** with the (*S*)-Met moiety, the second, diastereoisomers **1b** and **1b'** with the (*R*)-Met moiety, and the third, racemate **1** (**1a** + **1b**).

At a concentration of 7×10^{-2} mol dm⁻³, the solutions of all of the test glycolurils kept under ambient conditions contained only hydrated molecules about 1 nm large [Figure 1(a)], in agreement with the data obtained in studies on the solutions of 2,4,6,8-tetramethyl-2,4,6,8-tetraazobicyclo[3.3.0]octane-3,7-dione (Mebicar tranquillizer) by microwave spectroscopy and DLS.^{3,12} In a concentration range of 1×10^{-2} – 1×10^{-4} mol dm⁻³, supramolecular domains¹⁴ appear in the solutions of **1**, **1a**, **1a'**, **1b** and **1b'** [Figure 1(b),(c)], similarly to previous results.^{3,12}

The subsequent dilution of glycoluril solutions results in dramatic differences in their self-organization. In the solutions of diastereomers **1a** and **1a'**, molecular ensembles hundreds of nanometers in size (nanoassociates^{3,12}) are formed [Figure 1(d)] at 1×10^{-5} – 1×10^{-15} mol dm⁻³ (for example, see Figure S1, Online Supplementary Materials).

If the solutions of **1a** and **1a'** are diluted to 1×10^{-16} mol dm⁻³ or lower, no particles are detected due to a polymodal distribution. The formation of nanoassociates in a solution of racemate **1** occurs similarly to that in a solution of **1a**, but only down to a concentration of 1×10^{-9} mol dm⁻³. In the solutions of diastereomers **1b** and **1b'**, particles are not determined by the DLS method, starting from a concentration of 1×10^{-5} mol dm⁻³ and below due to a polymodal distribution (Figure S2).

In the solutions of **1**, **1a**, **1a'**, **1b** and **1b'**, which were kept in the shielding container, particles hundreds of nanometers in size are only formed in a concentration range of 1×10^{-4} – 1×10^{-1} mol dm⁻³, but they are not formed at 1×10^{-6} mol dm⁻³ and below.

Thus, supramolecular domains are produced in the solutions of all the test glycolurils in a concentration range from 1×10^{-4} to 1×10^{-1} mol dm⁻³ regardless of the presence of external fields. The formation of nanoassociates in a calculated concentration range from 1×10^{-5} to 1×10^{-15} mol dm⁻³ is only possible with external physical fields and occurs only in the solutions of diastereomers **1a** and **1a'** rather than diastereomers **1b** and **1b'**. The ability of the solutions of racemate **1** to form nanoassociates in a range from 1×10^{-5} to 1×10^{-9} mol dm⁻³ is probably due to the presence of the (*S*)-Met moiety in compound **1a**. Hence, upon dilution, particles with varying sizes are formed in the solutions of diastereomers **1a** and **1a'**: hydrated molecules with sizes of about 1 nm (7×10^{-2} mol dm⁻³), supramolecular domains of 200–300 nm (1×10^{-4} – 1×10^{-2} mol dm⁻³) and nanoassociates of 220–370 nm (1×10^{-15} – 1×10^{-5} mol dm⁻³). The DLS method detects

only hydrated molecules and supramolecular domains in the solutions of **1b** and **1b'**.

An analysis of the concentration dependences of the size (*D*) (Figure S3) and ζ -potential (Figure S4) of nanoassociates formed in the solutions of **1a** and **1a'** indicates that the non-monotonic plots of nanoassociate parameters are symbatic in the entire range of dilutions, except for 1×10^{-12} and 1×10^{-5} mol dm⁻³ for *D*. The nanoassociate size in the solutions of **1a'** is smaller and the ζ -potentials are larger nearly in the entire concentration range than in solutions of **1a**. Thus, the self-organization of high dilution solutions of diastereomers **1a** and **1a'** occurs similarly in many respects.

Figure 2 shows the parameters of nanoassociates formed in **1a** solutions as a function of concentration. An increase in the nanoassociate size at 1×10^{-5} , 1×10^{-10} and 1×10^{-13} mol dm⁻³ to 320–370 nm is accompanied by an increase in the ζ -potential to –12, –7 and –9 mV, respectively. For the parameters of nanoassociates formed in the solutions of **1a'**, a similar behavior was observed in a narrower concentration range of 1×10^{-13} – 1×10^{-7} mol dm⁻³ (see Figure S5). Such a correlated variation of nanoassociate parameters is typical of high dilution solutions of many compounds.³

Figure 3 (curves 1 and 2) depicts the non-monotonous concentration plots of specific electric conductivity (χ) for the solutions of **1a** and **1a'**. The shapes of these curves are nearly the same, like those for nanoassociate parameters (Figure S3). However, the χ values in solutions of **1a'** are higher than those in **1a** solutions, in conformity with the higher ζ -potential for the nanoassociates in **1a'** solution (see Figure S4).

The existence of a relationship between the parameters of nanoassociates and the physicochemical properties of high dilution solutions is a characteristic feature inherent in solutions of those compounds whose structures favour the formation of nanoassociates.³ For example, Figure 4 shows a relationship between the concentration plots of the ζ -potential of nanoassociates formed in solutions of **1a'** and the χ values of the solutions in a range of 1×10^{-13} – 1×10^{-7} mol dm⁻³. Generally, the most considerable variation in the parameters of nanoassociates and in the physicochemical properties of solutions occurs in the same concentration ranges. In particular, as can be seen in Figures 2–4 and S3–S8 (see Online Supplementary Materials), these ranges for high dilution solutions of **1a** and **1a'** are 1×10^{-13} – 1×10^{-12} , 1×10^{-10} – 1×10^{-9} and 1×10^{-7} – 1×10^{-5} mol dm⁻³.

The unusual physicochemical properties of high dilution solutions caused by the formation and rearrangement of nanoassociates are evident from the concentration plots of the properties of the solutions of diastereomers **1b'** and **1b** (Figure 3, curves 3, 4, Figure 5 and Figure S9), where nanoassociates cannot be reliably detected by DLS. The specific electric conductivity and pH of the solutions of compound **1b** are almost indistinguishable from analogous properties of distilled water ($3 \mu\text{S cm}^{-1}$, pH 6) at concentrations lower than 1×10^{-5} mol dm⁻³ (Figure 5), unlike the solutions of **1a** (e.g. Figure S8), where the values of χ at 1×10^{-13} , 1×10^{-10} and 1×10^{-7} mol dm⁻³ are 10, 7

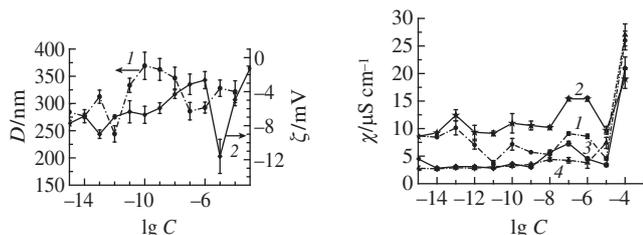


Figure 2 (1) Size (*D*) and (2) ζ -potential of nanoassociates in the solutions of **1a** as functions of concentration.

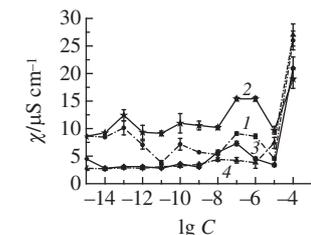


Figure 3 Electrical conductivity (χ) vs. concentration of the aqueous solutions of (1) **1a**, (2) **1a'**, (3) **1b'** and (4) **1b**.

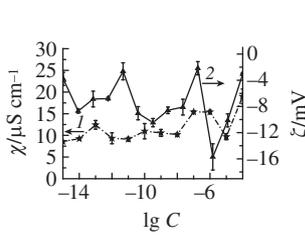


Figure 4 (1) Electrical conductivity (χ) of the solutions of **1a'** and (2) ζ -potential of nanoassociates as functions of concentration.

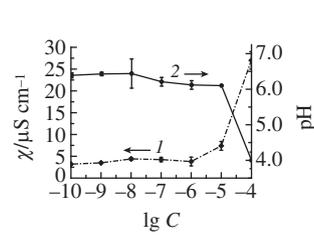


Figure 5 (1) Electrical conductivity (χ) and (2) pH of the solutions of **1b** as functions of concentration.

and $6 \mu\text{S cm}^{-1}$, respectively. In other words, the properties of the high dilution solutions of **1b** that cannot form nanoassociates are indistinguishable from the properties of the solvent (water).

In the solutions of **1b'**, the values of χ become indistinguishable from that of distilled water at $< 1 \times 10^{-8} \text{ mol dm}^{-3}$ (Figure 3, curve 3, Figure S9). At $1 \times 10^{-11} \text{ mol dm}^{-3}$, a small increase in $\Delta\epsilon$ (where $\Delta\epsilon = \epsilon_{\text{solution}} - \epsilon_{\text{solvent}}$) is observed, which is smaller by a factor of 3.5 than the maximum value of $\Delta\epsilon$ in a $1 \times 10^{-12} \text{ mol dm}^{-3}$ solution of **1a'** (Figures S7 and S9). Hence, though it was impossible to detect particles in high dilution solutions of **1b'** or **1b** with DLS the physicochemical properties of the solutions indirectly indicate that structurization occurs in the solutions of **1b'**, but it is much less pronounced than that in the solutions of **1a** and **1a'**.

The concentration plots of χ and $\Delta\epsilon$ for the solutions of enantiomers **1a** and **1b**, **1a'** and **1b'** in a range of 1×10^{-15} – $1 \times 10^{-4} \text{ mol dm}^{-3}$ (Figures S7 and S9) indicate that the χ and $\Delta\epsilon$ values are nearly the same in the region where supramolecular domains are formed. This fact shows that their physicochemical properties are identical.¹⁶ However, the properties of high dilution solutions (1×10^{-15} – $1 \times 10^{-6} \text{ mol dm}^{-3}$) of enantiomers **1a** and **1b**, **1a'** and **1b'** differ considerably (*cf.* Figures S10, S11 and S7, S9).

This difference is explained by the fact that the high dilution solutions of glycolurils **1a**, **1a'** are capable of space-time self-organization, which results in nanoassociates and non-monotonic changes in the physicochemical properties, whereas this capability is missing or very weak in the solutions of diastereomers **1b** and **1b'**.

We studied the optical activity of the solutions of enantiomers **1a** and **1b**, **1a'** and **1b'** in the course of dilution. Table 1 summarizes the optical rotation angles α of the aqueous solutions of glycolurils at 7×10^{-2} (2%) and $1 \times 10^{-2} \text{ mol dm}^{-3}$. These concentrations correspond to the solutions containing hydrated glycolurils molecules [Figure 1(a)] and supramolecular domains [Figure 1(b)], respectively. The values of $[\alpha]_{\text{D}}^{25}$ for the molecular solutions of **1a** and **1b**, **1a'** and **1b'** are higher than those of supramolecular systems by factors of 280 and 90, respectively. Thus, the Biot law is not observed upon a transition from the molecular solutions to supramolecular systems of **1a**, **1b**, **1a'** and **1b'**.

The molecules of chiral compounds can assemble the surrounding achiral solvent molecules into chiral aggregates that possess optical activity; in this case, the contribution of the chiral compound itself to the total optical activity of the solution is insignificant.^{16,17} The specific rotation of the aqueous solutions of D-levoglucosan varies in a concentration range of 0.03 – 4.0 mol dm^{-3} due to the existence of domains with different structures in different concentration ranges.¹⁸

The idea that the observed phenomenon is owing to the formation of chiral supramolecular domains in solution in a range of 1×10^{-3} – $1 \times 10^{-2} \text{ mol dm}^{-3}$ is supported by the fact that the Biot law is observed in this concentration range in the solutions of **1a'** and **1b'**: the values of $[\alpha]_{\text{D}}^{25}$ are constant and equal to -0.85 and $+0.85$, respectively. The optical rotation angles α for these enantiomers are -0.013° and $+0.013^\circ$, respectively, at $1 \times 10^{-3} \text{ mol dm}^{-3}$. The value of α for enantiomers **1a** and **1b** cannot be measured at

1×10^{-3} – $1 \times 10^{-2} \text{ mol dm}^{-3}$ because it is smaller than the determination limit of the instrument upon a tenfold dilution of the solution. At $\leq 1 \times 10^{-4} \text{ mol dm}^{-3}$, the angle α for both enantiomer pairs does not exceed the instrument accuracy. In the solutions of **1a** and **1b**, **1a'** and **1b'** kept in a permalloy container, the values of α in a concentration range of 7×10^{-2} – $1 \times 10^{-3} \text{ mol dm}^{-3}$, where supramolecular domains are formed, are the same as those in the solutions kept under natural conditions. Hence, the formation of supramolecular domains in the solutions of **1a** and **1b**, **1a'** and **1b'** in a concentration range of 1×10^{-3} – $1 \times 10^{-2} \text{ mol dm}^{-3}$ is responsible for the appearance of supramolecular chirality, which differs from molecular chirality at higher concentrations.

Thus, we demonstrated that the capability of glycolurils containing chiral (*S*)- and (*R*)-methionine moieties to undergo space-time self-organization with the formation of nanoassociates in high dilution aqueous solutions essentially depends on (*S*)- or (*R*)-configuration of the methionine moiety. The high dilution solutions of glycolurils with an (*S*)-Met moiety are capable of space-time self-organization, unlike those with an (*R*)-Met moiety. The observed effect can be useful for understanding the functional specialization of amino acid enantiomers in living nature.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2015.01.027.

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Table 1 Optical rotation angles of the solutions of test glycolurils.

Compound	Concentration/ mol dm^{-3}	Optical rotation angle $\alpha/^\circ$	Specific rotation $[\alpha]_{\text{D}}^{25}$
1a	7×10^{-2} (2%)	$(+18.50$	$+18.50$
	1×10^{-2}	$+0.01$	$+0.065$
1b	7×10^{-2} (2%)	$(-18.50$	-18.50
	1×10^{-2}	$(-0.01$	-0.065
1a'	7×10^{-2} (2%)	$(-77.78$	-77.78
	1×10^{-2}	$(-0.13$	-0.85
1b'	7×10^{-2} (2%)	$(+77.78$	$+77.78$
	1×10^{-2}	$(+0.13$	$+0.85$