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Racemisation and rearrangement of 1,2-dihydro-1,3,5-triazines: a novel reversible thermal electrocyclic reaction†

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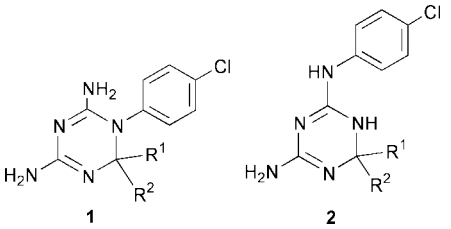
Chiral 1,2-diaryl-1,2-dihydro-4,6-diamino-1,3,5-triazines undergo facile racemisation by a reversible thermal electrocyclic reaction mechanism; the transient intermediate can lead, after tautomerisation, to rearranged racemic 2-aryl-1,2-dihydro-4-amino-6-anilino-1,3,5-triazines.

Cycloguanil **1a** (**1**, $R^1 = R^2 = Me$) is a potent inhibitor of *Plasmodium falciparum* dihydrofolate reductase (*pf*DHFR). It has been extensively employed (usually as the prodrug



1 **2**

proguanil) alone or in combination with other drugs, as a prophylactic agent and for the treatment of malaria. As a result, malaria parasites resistant to the drug have emerged and compromised its clinical utility.[1] Analysis of DHFR sequences of several resistant *P. falciparum* isolated from different geographical origins revealed that resistance to cycloguanil is associated with point mutations in the DHFR gene.[2,3] The importance of residue 16 in *pf*DHFR for binding cycloguanil has been investigated using mutants obtained *via* a synthetic gene.[1]

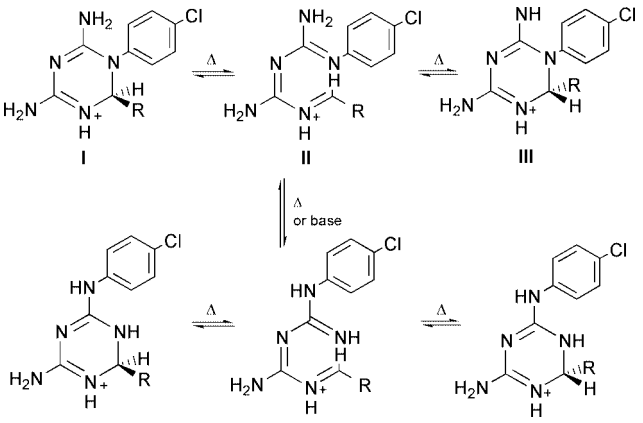
Recently, a three-dimensional homology model of *pf*DHFR was constructed to aid understanding of the structural basis of antifolate resistance in malaria.[4] This study led to the hypothesis that resistance of the A16V+S108T mutant of *pf*DHFR to cycloguanil is due to a steric clash between one of the methyl groups of cycloguanil and Val-16 of the mutant *pf*DHFR, and that mutation of residue 108 (S108T) reinforces this steric constraint. Support for this hypothesis was obtained by testing both the wild-type and A16V+S108T mutant *pf*DHFRs against cycloguanil analogues devoid of one or both methyl groups.[4] By replacing one of the C2 methyl groups in cycloguanil by hydrogen and the other by an aryl group **1b** (**1**, $R^1 = H$, $R^2 = Ph$) the inhibitory activity against the A16V+S108T mutant *pf*DHFR is restored to the level obtained with cycloguanil against the wild-type *pf*DHFR.[5] Unlike cycloguanil (**1a**), these analogues (**1b**) possess a chiral centre and only one of the enantiomers is likely to have significant inhibitory activity against the A16V+S108T mutant of *pf*DHFR.

Previous attempts to resolve racemates of **1c** (**1**, $R^1 = H$, $R^2 =$ alkyl) as diastereoisomeric salts using chiral acids have failed.[6] 1,2-Dihydro-1,3,5-triazines should be capable of thermal electrocyclic ring-opening by a disrotatory pathway; there are, however, two such pathways. Steric considerations normally determine the preferred pathway[7] and since N lacks configurational stability, enantiomer **I** will ring-open by an anticlockwise rotation about the C2–N1 bond, while enantiomer **III** will open by a clockwise rotation about the C2–N1 bond leading to the sterically least-hindered common intermediate **II** (Scheme 1). Since intermediate **II** can ring close by either a clockwise or anticlockwise disrotatory pathway at C2, the product will be racemic.

The temperature at which pericyclic reactions occur is influenced by steric factors and heteroatomic substitutents.[7,8] The electrocyclic ring closure of *trans*-2, *cis*-4, *trans*-6-octatriene to *cis*-5,6-dimethyl-1,3-cyclohexa-1,3-diene occurs at 132 °C with a $t_{0.5}$ = 4.3 h.[9] 1-Aryl-1,2-dihydro-4,6-diamino-1,3,5-triazines are synthesised from aryl biguanides and a carbonyl compound in refluxing ethanol under acidic conditions.[10] Since there is no accumulation of the Schiff base intermediate it may be reasonably assumed that the ring closure is not rate determining and could occur at or close to room temperature. If the ring-opening also occurs at or close to room temperature this would provide an explanation for the failure to resolve racemates of **1c** by the crystallisation of diastereoisomeric salts.

When α- or β-cyclodextrin was added to a solution of racemic **1d** (**1**, $R^1 = H$, $R^2 = p\text{-}ClC_6H_4$), resonances of the enantiomeric dihydrotriazines were seen to separate in the 500 MHz ^1H NMR spectrum. The effect of increasing the concentration of β-cyclodextrin on the ^1H NMR spectrum of racemic **1d** is shown in Fig. 1. This demonstrates that the enantiomers have sufficient lifetime at room temperature to be observed on the NMR time-scale and therefore in principle should be resolvable. The *p*-chlorophenyl groups are expected to bind within the β-cyclodextrin cavity and it is noticeable that their chemical shifts are most sensitive to the presence of β-cyclodextrin (Fig. 1). Only at a high molar ratio (17 : 1) of β-cyclodextrin to racemic **1d** are the enantiomeric C2–H resonances resolved.

In view of the ability of β-cyclodextrin to selectively shift the resonances of the enantiomers of **1d** in the NMR spectrum, we



Scheme 1 Proposed mechanism for the racemisation and rearrangement of 1,2-disubstituted-1,2-dihydro-4,6-diamino-1,3,5-triazines.

† Electronic supplementary information (ESI) available: Arrhenius plot for racemisation of enantiomers of **1d**. See http://www.rsc.org/suppdata/cc/b1/b101245m/

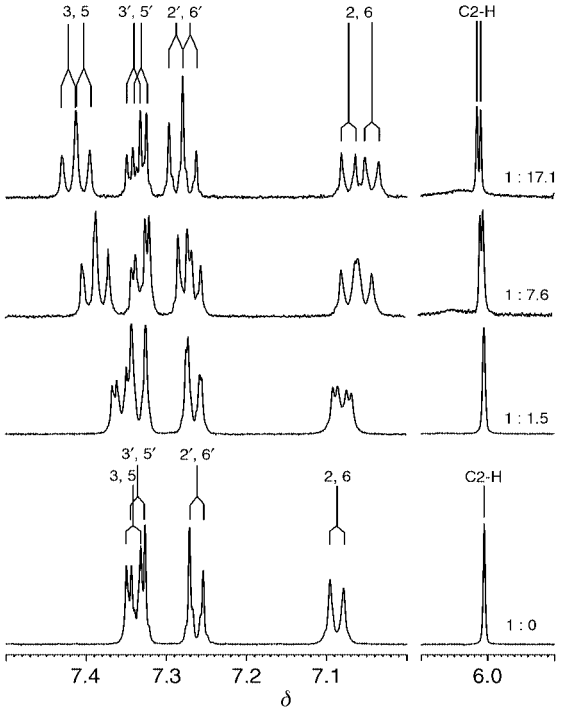


Fig. 1 ^{1}H NMR spectra of racemic **1d** hydrochloride (**1**, R^1 = H, R^2 = *p*-ClC_6H_4) in D_2O in the presence of an increasing concentration of β-cyclodextrin. The molar ratio of **1d** hydrochloride to β-cyclodextrin is shown on the right of each spectrum. 1- and 2-*p*-ClC_6H_4 substituents are designated unprimed and primed, respectively. The assignments were made by NOE experiments.

sought to separate the enantiomers of **1d** by HPLC on a column with β-cyclodextrin covalently attached to the silica support (Shodex Orpak CDBS-453; 4.6 × 150 mm). A solvent system was found[11] which would resolve the racemate and this system worked well at 10 °C. The CD spectra of the resolved enantiomers of **1d** were of similar shape but of opposite sign.

By incubating a solution of each enantiomer in a temperature controlled heating block and measuring the proportion of the two enantiomers (by HPLC on the β-cyclodextrin column) as a function of time, the rate of racemisation of the enantiomers of **1d** was determined. Racemisation of the enantiomers obeyed a first order rate law and the data were plotted using the equation $\ln x_e/(x_e - x) = 2kt$ where x is the amount of the initial enantiomer that was converted to the other enantiomer at time t, and x_e is the amount converted at equilibrium (*i.e.* $x_e = 0.5$).[12] The rate constants were determined between 40 and 60 °C at 5 °C intervals. The activation energy (E_a = 165 kJ mol^{-1}) was obtained from the slope of the Arrhenius plot (ESI†) from which the enthalpy of activation ($\Delta H^{\ddagger}$ = 162 kJ mol^{-1}) and the entropy of activation ($\Delta S^{\ddagger}$ = +176 J K^{-1} mol^{-1}) were calculated.

The relatively high enthalpy of activation is understandable since the stable 1,2-dihydro-4,6-diamino-1,3,5-triazine ring is being disrupted by breaking a single bond and forming a double bond. Moreover, the ring-opened intermediate **II** (Scheme 1) has more degrees of freedom than the cyclic 1,2-dihydro-1,3,5-triazine and hence the entropy of activation should be positive as observed. The balance of these two competing factors leads to a relatively small Gibbs function of activation and hence a fast ring-opening reaction. Thus, although it has now been possible to resolve a racemic 1,2-diaryl-1,2-dihydro-4,6-diamino-1,3,5-triazine, its half life is too short to make it worthwhile resolving these materials since as prophylactic agents they will circulate in the blood (at 37 °C) for several days.

Cycloguanil **1a** as its hydrochloride salt undergoes rearrangement to 2,2-dimethyl-4-amino-6-*p*-chloroanilino-1,2-dihydro-1,3,5-triazine **2a** (**2**, $R^1 = R^2$ = Me) on heating to its melting point (bath temperature 245 °C). As its free base in aqueous solution the rearrangement occurs at a much lower temperature (< 100 °C); the reaction is catalysed by base.[6,13] In dimethyl sulfoxide we observed rearrangement of the racemic **1d** hydrochloride (*ca.* 12 mM) at 60 °C with a half-life of *ca.* 10 days. Addition of an equimolar amount of KCl decreased the half-life to about 8 days. We propose that in dimethyl sulfoxide the chloride ion (which is essentially unsolvated in this solvent) acts as a general base and catalyses the tautomerisation of the acyclic intermediate **II** (Scheme 1). Support for this proposal was provided by the observation that addition of an equimolar amount of KF to a dimethyl sulfoxide solution of racemic **1d** hydrochloride (*ca.* 12 mM) markedly accelerates the rearrangement, the half-life at 60 °C now being about 14 min.[14] When an enantiomer of **1d** is treated under the same conditions the rearranged product **2b** (**2**, R^1 = H, R^2 = *p*-ClC_6H_4) is racemic. Thus the rearrangement and the racemisation of 1-aryl-2-substituted-1,2-dihydro-4,6-diamino-1,3,5-triazines can be envisaged as occurring by the common intermediate **II** (Scheme 1).

The reversible thermal electrocyclic reaction of 1,2-dihydro-1,3,5-triazines has not been recognised previously. Since 1,2-diaryl-1,2-dihydro-4,6-diamino-1,3,5-triazines are potent inhibitors of mutant *pf*DHFRs found in resistant strains of the malaria parasite *Plasmodium falciparum*,[5] binding to the target enzyme can be expected to perturb the enantiomer ratio in favour of the more effective inhibitor. Thus the facile reversible thermal electrocyclic reaction reported here renders unnecessary the resolution of these materials for therapeutic use.

Notes and references

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