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Rotaxane-encapsulated cyanine dyes: enhanced fluorescence efficiency and photostability

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Two isomers of a cyanine dye α-cyclodextrin rotaxane have been synthesised in aqueous solution, and structurally characterised by 2D NMR spectroscopy; they exhibit enhanced fluorescence and photostability, compared with the free dye.

Cyanine dyes are used as photographic sensitisers, laser dyes and biological fluorescence probes, and in optical data recording.[1,2] Their main limitation is low photochemical stability.[3] Here we report the first synthesis of a cyanine dye rotaxane, in which the chromophore is locked inside the cavity of a cyclodextrin.[4] Two diastereomers of the rotaxane have been isolated; one of these isomers exhibits substantially enhanced fluorescence quantum yield and photostability, in oxygen-saturated dioxane, relative to the unencapsulated dye.

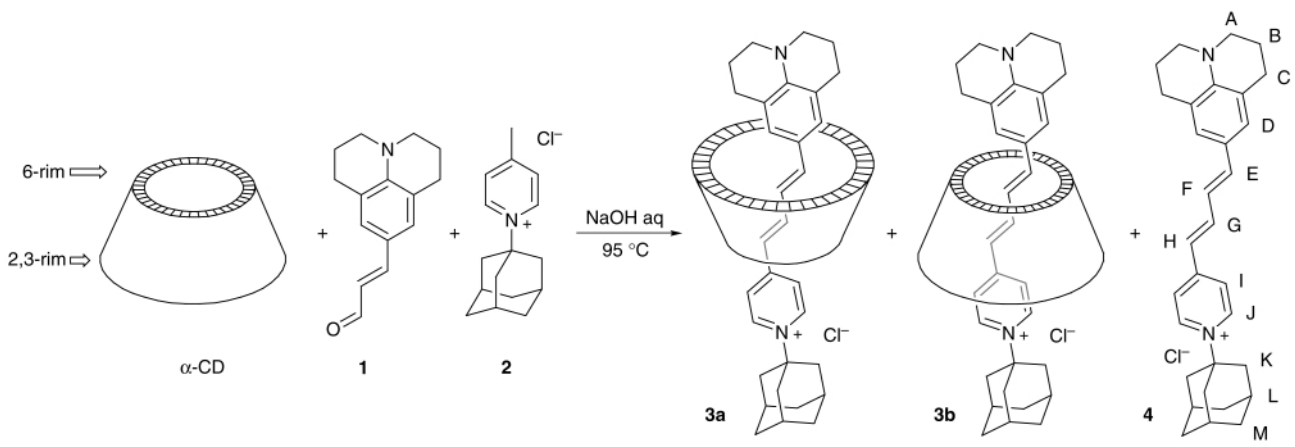
Many dyes form inclusion complexes with cyclodextrins in aqueous solution, due to hydrophobic binding. These complexes often exhibit enhanced fluorescence[5] and photostability,[5g,6] but they can only be studied in water, in the presence of excess cyclodextrin, because they readily dissociate; the equilibrium constants are typically 10^2–10^3 M^{-1} in water, and much lower in other solvents. Rotaxane-encapsulated dyes[7] are similar to inclusion complexes except that they cannot dissociate, because the ends of the dye are too bulky to pass through the macrocycle, so they can be studied in a wide range of solvents.

Our strategy for preparing these rotaxanes is to synthesise a dumbbell-shaped dye in water, in the presence of a macrocycle; hydrophobic binding between the dye-precursors and the macrocycle results in rotaxane formation.[7] Cyanine dyes are not usually synthesised in aqueous solution, but we have found that reaction of 3-(9-julolidinyl)prop-2-en-1-al[8] **1** with *N*-(1-adamantyl)-4-methylpyridinium chloride[9] **2** and α-cyclodextrin in aqueous sodium hydroxide gives cyanine dye rotaxanes **3a** and **3b** as well as the free dye **4** (Scheme 1).† α-Cyclodextrin is cone-shaped; it has a narrow 6-rim (with primary OH groups) and a wide 2,3-rim (with secondary OH groups). So there are two possible orientations of the cyclodextrin unit, giving rise to the two stereoisomers **3a** and **3b**. The yield of **3a** and **3b** is low (6%, based on re-isolated aldehyde **1**), which is not surprising since the reaction involves dehydration in aqueous solution. ^{1}H NMR showed that this product was a 1:2 mixture of two stereoisomers **3a** and **3b**, which were separated by reverse phase chromatography. There is only one previous report of the separation of rotaxane stereoisomers of this type.[10]

The two pure cyanine dye rotaxanes **3a** and **3b** were both thoroughly authenticated by ^{1}H and ^{13}C NMR spectroscopy, and mass spectrometry, and their ^{1}H NMR spectra were fully assigned using 2D techniques. The vicinal ^{1}H–^{1}H coupling constants across each alkene unit in **3a** and **3b** (H_E–H_F and H_G–H_H) are in the range 15.1–15.4 Hz, proving that both isomers have the *trans–trans* geometry. NOESY experiments confirmed that the isomers have different orientations of the cyclodextrin unit. In rotaxane **3a**, NOEs were observed from H3 of the cyclodextrin (the wide rim) to both the aromatic and benzylic julolidine signals (H_C and H_D), and from H5 (near the narrow rim) to H_I at the other end of the dye. The opposite pattern of NOEs was observed in **3b**: from H3 to H_I and H_J; from H5 to H_D and from H6 to H_C. These NOE measurements not only elucidate the isomerism in **3a** and **3b**, but also show that the cyclodextrin is sitting round the reactive polymethine region in both rotaxanes. In both cases the NOEs are not consistent with a single static position of the cyclodextrin, showing that each isomer is dynamic in solution.

Encapsulation results in a bathochromic shift in the electronic absorption and emission spectra of both rotaxanes (for **3a**, **3b** and **4** in water λ_{max}(abs.) = 525, 535 and 477 nm; λ_{max}(em.) = 710, 718 and 678 nm respectively). The relative fluorescence quantum yields[11] of these three compounds in water, and a range of other solvents, are listed in Table 1. In water, the fluorescence quantum yields of both of the rotaxanes are lower than that of the free dye,[12] whereas in other solvents, especially dioxane, rotaxane **3b** is spectacularly more fluorescent. The fluorescence behaviour of the two isomers **3a** and **3b** are surprisingly different, with **3a** behaving more like the free dye **4**.

The higher fluorescence efficiency of **3b** in dioxane is not consistent with several common explanations for enhanced



Scheme 1

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Table 1 Relative fluorescence quantum yields of rotaxanes **3a** and **3b**, and free dye **4** in a range of solvents[11]

Solvent	ϕ_f **3a**	ϕ_f **3b**	ϕ_f **4**
H_2O	0.71	0.71	1.0
MeCN	1.6	4.8	2.0
Me_2CO	2.6	11	2.0
Me_2SO	4.8	9.1	4.3
MeOH	2.5	4.0	2.1
EtOH	4.8	10	4.4
Pr^iOH	6.8	21	10
THF	16	46	18
Dioxane	81	124	22

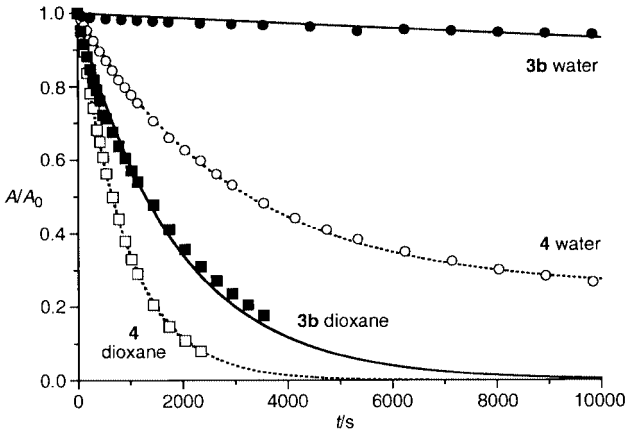

Fig. 1 Photo-bleaching curves for rotaxane **3b** in water, free dye **4** in water, **3b** in dioxane and **4** in dioxane, fitted to first-order decay curves with rate constants of 7.1×10^{-6}, 3.3×10^{-4}, 5.4×10^{-4} and 1.1×10^{-3} s^{-1}, respectively. Photolyses were carried out in 10 mm quartz cuvettes and O_2-saturated solvents with light from a tungsten filament bulb, using a 35 mm slide projector; A is the absorbance at time t and A_0 is the initial absorbance.

fluorescence in aqueous cyclodextrin–dye inclusion complexes, such as the exclusion of water from the dye surface and the changes in polarity around the dye when it enters the cyclodextrin.[5b,e,f] Here the fluorescence is enhanced in a range of non-aqueous solvents, and especially in dioxane which has a similar polarity to the cyclodextrin cavity. Cyclodextrin-encapsulation has been viewed as a way of reducing the quenching effect of an aqueous environment, whereas here encapsulation dramatically increases the fluorescence efficiency in a solvent in which the dye is already highly fluorescent. The enhanced fluorescence of **3a** and **3b**, compared to **4**, in dioxane is probably due to the reduced flexibility of the encapsulated chromophore.[5i] The possibility that this fluorescence enhancement is due to reduced aggregation[5c,d,i] can be discounted since **4** shows no sign of aggregation under these conditions.

The photostability of rotaxane **3b** was compared with that of the free dye **4**, in both water and dioxane, by monitoring the decrease in absorbance during irradiation of oxygen-saturated solutions with visible white light.[3c,6] Pseudo first order photo-bleaching curves were obtained in all cases, as shown in Fig. 1 (see caption for rate constants). The rotaxane **3b** exhibits higher photostability in both solvents (40-fold in water and 2-fold in dioxane, compared to the free dye **4**), and both compounds fade much faster in dioxane than in water. The mechanism of photo-bleaching has not yet been elucidated, but whatever process is involved, the faster bleaching in dioxane is probably related to the stronger fluorescence in this solvent, since a lower rate of quenching allows excited states to undergo more photo-chemistry. Matsuzawa *et al.*[6a] have shown that inclusion of cyanine dyes in β-cyclodextrin can enhance the photostability both by reducing the rate of singlet oxygen formation and by reducing the rate of reaction between singlet oxygen and the dye; both these factors may be involved here. It is remarkable that, in dioxane, **3b** exhibits a 5-fold fluorescence enhancement simultaneously with a 2-fold photostability enhancement.

The discovery that cyclodextrin encapsulation can increase the fluorescence and photostability of dyes under solvent conditions which already favour fluorescence indicates that this may be a valuable way of improving the stability and brightness of luminescent and electro-luminescent materials.

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Notes and references

† *Experimental:* 3-(9-Julolidinyl)prop-2-en-1-al **1** (1.0 mmol, 0.22 g), *N*-(1-adamantyl)-4-methylpyridinium chloride **2** (1.0 mmol, 0.26 g), α-cyclodextrin (4 mmol, 3.9 g) and aqueous NaOH (0.1 M, 20 ml) were stirred under N_2 at 95 °C for 24 h. The mixture was extracted with CH_2Cl_2, from which unreacted **1** (0.15 g, 67%) was recovered, together with **4** (14 mg, 2.9%). The aqueous phase was chromatographed (Sephadex CM25, eluting with NH_4HCO_3 aq) and lyophilized to yield 29 mg (6% based on re-isolated **1**) of a mixture of **3a** and **3b**. These isomers were separated by reverse-phase C18 silica chromatography, eluting with Pr^iOH–H_2O–0.01 M NH_4HCO_3 1:3:1, to give, in order of elution, rotaxanes **3a** and **3b**. Each fraction was anion-exchanged to the chloride and dried to give a purple powder.

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