

The Isomerisation of Meso-amine Substituted Heptamethine Dyes

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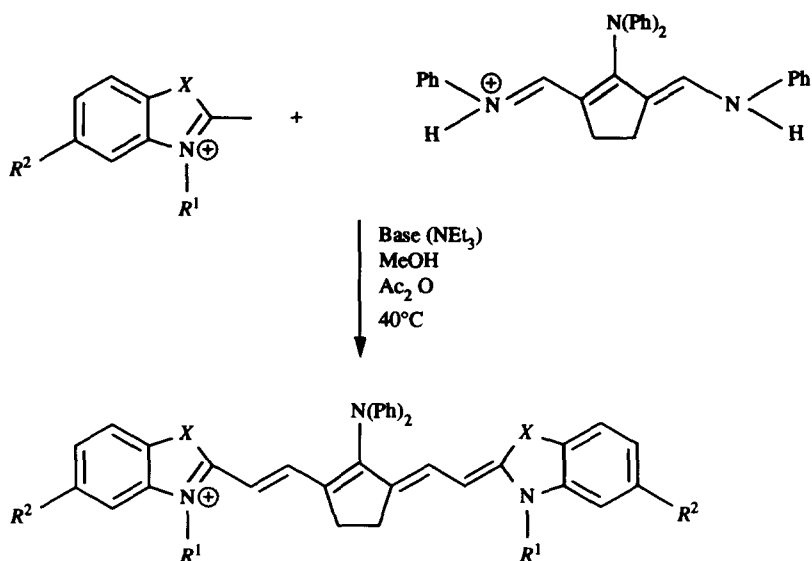
ABSTRACT

From investigations into the absorption and fluorescence characteristics of several new meso-amine substituted heptamethine cyanine dyes and the commercially available laser dyes IR140, IR132 and IR144, it appears that these dyes exist in at least two interconvertible ground state isomeric conformations in organic solvents. From the position and nature of absorption and fluorescence characteristics, it is concluded that these conformers differ in the contribution to the chromophore of the meso-amine substituent, which either acts as an electron-donor by resonance or else withdraws by induction. Fluorescence emission below 900 nm can only be attributed to the conformer in which the meso-amine groups acts as an electron-donor by resonance. This gives rise to a system that is analogous to the allopolar isomerism exhibited by holomeropolar cyanine dyes.

1 INTRODUCTION

The absorbance, fluorescence, and photosensitising characteristics of a large variety of cyanine dyes have been studied.^{1,2} The near-infrared absorption and emission of some heptamethine dyes, including partially rigidised meso-amine substituted dyes such as IR140 (Fig. 1), IR132 (Fig. 2) and IR144 (Fig. 3), has enabled them to be used as near-infrared laser dyes,³ and more recently a variant of IR144 has been used as a fluorescent label in DNA sequencing.⁴ This paper describes a newly discovered isomerism of these

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Dye	X	R ¹	R ²
i) IR140	S	CH ₂ CH ₃	Cl
ii) propanoyl-cya	S	(CH ₂) ₂ CO ₂ ⁻	H
iii) oxapropanoyl-cya	O	(CH ₂) ₂ CO ₂ ⁻	CH ₃
iv) dimethylindolinium-cya	C(CH ₃) ₂	CH ₃	H

Fig. 1. Reaction to give a meso-diphenylamine substituted heptamethine cyanine dye.

meso-amine substituted heptamethine dyes, which is analogous to the allopolar isomerism exhibited by holomeropolar cyanine dyes.¹

Heptamethine dyes with small meso-amine substituents, such as piperidine in IR144 (Fig. 3), possess a hypsochromically shifted absorption maximum relative to the unsubstituted analogue, consistent with an electron-donor group (the meso-amine group) being present at an inactive position (the meso-carbon atom), in accordance with the Dewar-Knott rules.⁵ In comparison, heptamethine dyes substituted with a larger meso-amine group, such as diphenylamine in IR140 (Fig. 1) and IR132 (Fig. 2), possess bathochromically

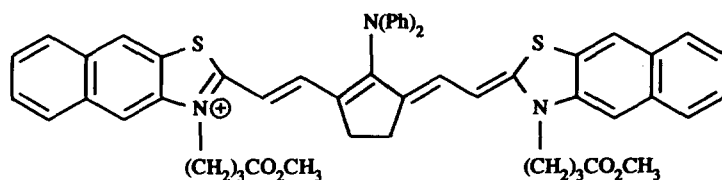


Fig. 2. IR132.

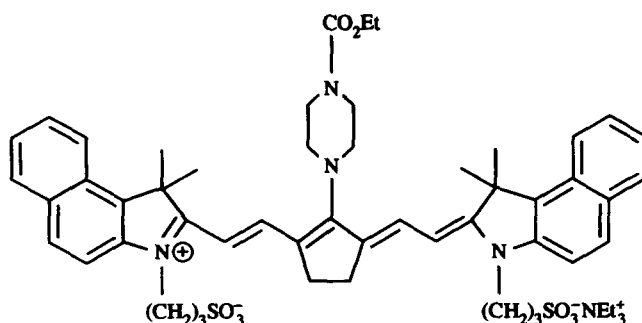


Fig. 3. IR144.

shifted absorption maxima relative to their unsubstituted analogues, due to steric hindrance restricting the meso-amine nitrogen atom from achieving coplanarity so that it cannot donate its lone pair of electrons by resonance into the chromophoric chain,⁶ which is thus exposed only to the inductive electron-withdrawing effect of the nitrogen atom. The bathochromic shift of the absorption maximum of meso-diphenylamine substituted dyes is consistent with an electron-withdrawing group being present at an inactive position, in accordance with the Dewar-Knott rules.⁵ Therefore, from the position of the absorption maxima of these dyes, they have previously been thought to exist in single conformations, the nature of which depends upon the size of the meso-amine group.

Fluorescence investigations have shown that only low fluorescence outputs (quantum yield <0.1) are observed from these dyes.^{7,8}

Further investigations reported here into the absorbance and fluorescence characteristics of meso-amine substituted heptamethine dyes in solution reveal, however, that two ground state isomeric forms of each dye are present, only one of which gives fluorescence emission (below 900 nm). This partly explains the low fluorescence quantum yield of meso-diphenylamine substituted heptamethine dyes, since only one isomeric form of these dyes is fluorescent and only a minority of dye molecules are in this isomeric form. This finding has implications for lasing and fluorophore technologies in which these dyes are used.

2 EXPERIMENTAL

IR140 (Fig. 1), IR132 (Fig. 2) and IR144 (Fig. 3) are commercially available laser grade dyes from Eastman Kodak Ltd. The dyes propanoyl-cya, oxapropanoyl-cya and dimethylindolinium-cya (Fig. 1) are new dyes synthesized by variation of a general procedure as outlined in the Eastman Kodak patent,⁹ involving the condensation of the respective quaternised nitrogen

heterocycles with a vinylogous diamidine in a molar ratio of 2:1 at reflux temperature for ten minutes in the presence of acetic anhydride as acetylating reagent and triethylamine as base, as shown in (Fig. 1). The syntheses of propanoyl-cya and oxapropanoyl-cya (Fig. 1) required the use of methanol as solvent to aid dissolution and excess triethylamine to ionise the carboxylic acid groups. A relatively low temperature of 40°C was employed during the synthesis of oxapropanoyl-cya, as higher temperatures caused excessive dye breakdown. Dyes were precipitated from the reaction mixture by addition of hexane, and purified by dissolution of the crude solid mixture in methanol (50 ml) followed by reprecipitation using 1 M HCl (20 ml), and then further purified by silica gel chromatography with methanol as eluent. Oxapropanoyl-cya could only be purified by silica gel column chromatography with methanol due to its susceptibility to hydrolytic breakdown on contact with aqueous acid.

¹H NMR spectra were recorded on a Bruker AC250 with Me₄Si as an internal reference. FAB mass spectra were obtained by the MS80 service at the University of Warwick. Fluorescence spectroscopy was performed on a Perkin Elmer LS50 spectrofluorimeter. Absorption spectroscopy was performed on a Shimadzu UV2100.

IR140 (Fig. 1), supplied by Kodak, ¹H NMR, CD₃OD, 50°C: δ 1.35(t, 6H, CH₃), 2.88(s, 4H, CH₂), 4.23(q, 4H, CH₂), 5.95(d, 2H, vinyl), 6.91(d, 2H, vinyl), 7.17–7.28(m, 8H, diphenylamine H), 7.37–7.59(m, 6H, aromatic), 7.71(d, 2H, diphenylamine H).

IR132 (Fig. 2), supplied by Kodak, ¹H NMR, d₆DMSO, 20°C: δ 2.07(s, 6H, OCH₃), 2.27(quin, 4H, CH₂), 3.03(s, 4H, CH₂), 4.26(t, 4H, CH₂), 4.55(t, 4H, NCH₂), 6.27(d, 2H, vinyl), 6.99(d, 2H, vinyl), 7.31–7.38(m, 6H aromatic), 7.53–7.66(m, 8H, aromatic), 7.98–8.08(m, 6H, aromatic), 8.39(s, 2H, aromatic). *m/z* 854, C₅₃H₄₈N₃O₄S₂ requires 854.

IR144 (Fig. 3), supplied by Kodak, ¹H NMR, CD₃OD, 47°C: δ 1.30(t, 9H, triethylamine CH₃), 1.36(t, 3H, CH₃), 1.98(s, 12H, CH₃), 2.29(quin, 4H, CH₂), 2.88(s, 4H, CH₂), 2.97(t, 4H, CH₂), 3.18(q, 6H, triethylamine CH₂), 3.82–3.85(m, 4H, CH₂), 3.92–3.87(m, 4H, CH₂), 4.27(q, 2H, CH₂), 4.33(t, 4H, CH₂), 5.99(d, 2H, vinyl), 7.37–7.42(m, 2H, aromatic), 7.53–7.60(m, 4H, aromatic), 7.88–7.96(m, 6H, aromatic and vinyl), 8.17–8.20(d, 2H, aromatic), *m/z* 907, C₁₂H₅₉N₄O₈S₂ requires 907.

Propanoyl-cya (Fig. 1), 3,3-di(2-carboxyethyl)-11-diphenylamino-10,12-ethylenebenzothiatricarbocyanine chloride. Yield after purification 20%, ¹H NMR, CD₃OD, 20°C: δ 2.76(t, 4H, CH₂), 3.10(s, 4H, CH₂), 4.62(t, 4H, CH₂), 7.54–7.84(m, 8H, aromatic), 7.34–7.46(m, 8H, diphenylamine H), 6.29(d, 2H, vinyl), 7.10(d, 2H, vinyl), 7.89–7.92(d, 2H diphenylamine H).

Dimethylindolinium-cya (Fig. 1), 3,3-dimethyl-11-diphenylamino-10,12-ethylenedimethylindolinetricarbocyanine perchlorate. Yield after purification 28%, ^1H NMR, CD_3OD , 20°C : δ 1.88(s, 12H, CH_3) 2.72(s, 6H, CH_3), 2.93(s, 4H, CH_2), 5.77(d, 2H, vinyl), 7.07–7.23(m, 10H, aromatic and vinyl), 7.26–7.60(m, 10H, diphenylamine H). m/z 602, $\text{C}_{43}\text{H}_{44}\text{N}_3$ requires 602.

Oxapropanoyl-cya (Fig. 1), 3,3-di(3-carboxypropyl) -5,5'-methyl-11-diphenylamino-10,12-ethylenebenzoxatricarbocyanine perchlorate. Yield after purification 10%, No NMR data obtained due to reactivity of oxapropanoyl-cya with trace H_2O in the atmosphere and solvent.

3 RESULTS AND DISCUSSION

3.1 Absorbance and fluorescence characteristics of large meso-amine substituted heptamethine dyes

In previous studies of the fluorescence emission from the meso-diphenylamine substituted heptamethine dye IR140 (Fig. 1) it has been assumed that this dye exists in a single conformation in organic solvents.^{7,8} Moreover, the use of laser pulse excitation in these fluorescence studies did not permit the measurement of excitation spectra. It is apparent from our measurements that the excitation maxima of all the meso-diphenylamine substituted heptamethine dyes studied are substantially hypsochromically shifted from the absorption maxima (Table 1). This implies that a different

TABLE 1

Absorbance and Fluorescence Characteristics of Various Meso-amine Substituted Heptamethine Cyanine Dyes in Methanol

Dye	Absorption maximum (nm)	Molar extinction coefficient. ($\times 10^{-5}$) (solvent) (litre $\text{mol}^{-1} \text{cm}^{-1}$)	Excitation maximum (nm)	Emission maximum (nm)	Fluorescence intensit ^a (%)
IR132 (Fig. 2)	811	1.7 (DMSO)	708	837	3.51
IR140 (Fig. 1)	800	1.5 (DMSO)	712	830	7.03
Propanoyl-cya (Fig. 1)	805	1.48 (MeOH)	700	825	8.2
Oxapropanoyl-cya (Fig. 1)	738	1.5 (MeOH) ^b	640	785	100.0
Dimethylindolinium-cya (Fig. 1)	780	1.45 (MeOH)	700	815	1.17
IR144 (Fig. 3)	745	1.24 (DMSO)	712	825	46.87

^aFluorescence output (%) relative to the output of oxapropanoyl-cya.

^bDue to the rapid reaction with trace H_2O in the atmosphere and solvent, the value given is a minimum value for the excitation coefficient.

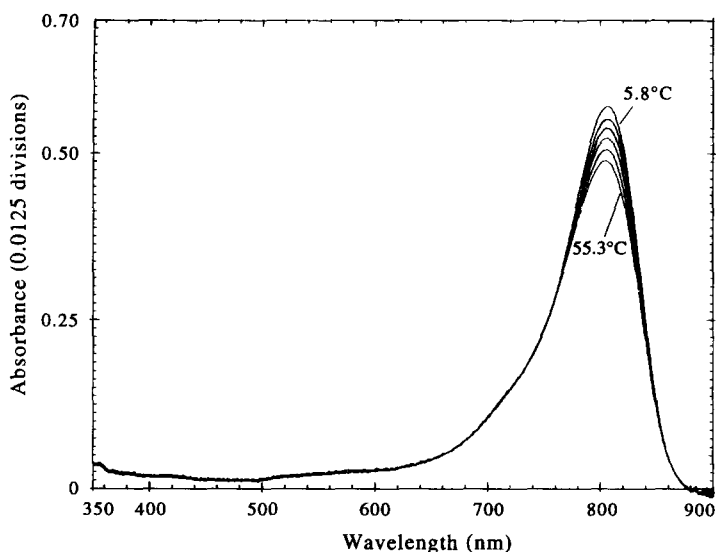


Fig. 4. The temperature dependence of the absorption spectrum of IR140 (Fig. 1) in methanol.

species is responsible for the fluorescence emission than that which is responsible for absorbance at the absorption maximum. Therefore at least two isomeric species exist in organic solvents, one of which possesses a hypsochromically shifted absorption maximum and is responsible for the fluorescence emission previously attributed to the predominant isomer. The predominant isomer is responsible for absorbance at the absorption maximum and has no fluorescence emission below 900 nm.

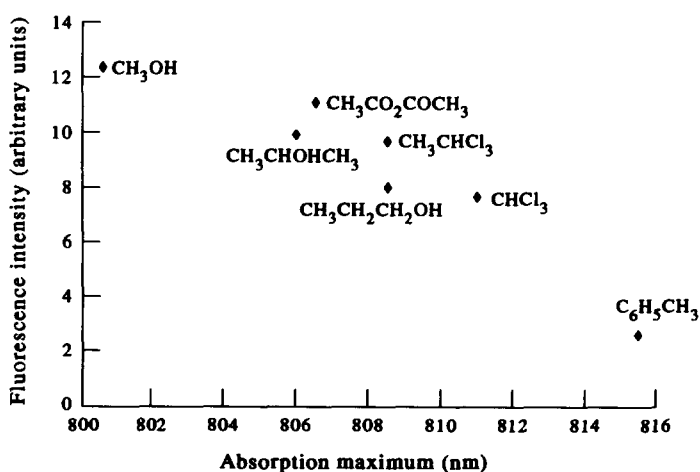


Fig. 5. Fluorescence intensity versus absorption maximum of IR140 in various solvents.

The excitation maximum of the fluorescent species coincides with the region of the hypsochromic shoulder in the absorption spectrum (Fig. 4). However, not all of the absorption intensity in this region can be attributed to this fluorescent species, because the perpendicularly polarised transition of the non-fluorescent species gives rise to absorption in this region also.¹⁰

The fluorescence output of the fluorescent species appears to coincide with the region of absorption of the non-fluorescent species (Table 1), resulting in a lower measurable fluorescence output due to internal absorption. The extent of internal absorption should depend upon the position of the absorption maximum of the non-fluorescent species, and indeed solvents which cause a bathochromic shift of the absorption maximum result in a greater overlap of the absorption spectrum of the non-fluorescent species with the emission spectrum of the fluorescent species and, consequently, a lower fluorescence output is apparent, as for IR140 (Fig. 5).

The extinction coefficient of the fluorescent species cannot be calculated, and in turn this prevents the calculation of the fluorescence quantum yield. However, despite this and the uncertainty caused by internal absorption, the relative emission intensity (Table 1) can be seen to vary according to the heterocyclic endgroup in the same order as previously unsubstituted cyanine dyes, i.e. dimethylindolinium < benzothiazolium < benzoxazolium.¹²

The absorption spectra of meso-diphenylamine substituted heptamethine dyes are temperature-sensitive; increasing temperature causes a decrease in absorbance at the absorption maximum and a corresponding, but smaller, increase in the region of the hypsochromic shoulder, as shown for IR140 and IR132 in Figs 4 and 6. These absorption intensity changes are reversible with decreasing temperature over the range shown, although the use of excessively high temperature (>60°C) results in the loss of some absorption intensity upon reversal, possibly due to dye breakdown. This temperature dependence is consistent with two species in equilibrium, a non-fluorescent species converting to the hypsochromically absorbing fluorescent species as the temperature is increased. It is likely that the extinction coefficient of the fluorescent species is lower than that of the non-fluorescent species because the decrease in absorbance near the absorbance maximum is not matched by the increase in absorbance near the hypsochromic shoulder (Figs 4 and 6). Although fluorescence decrease with increasing temperature is expected for all chromophores,¹³ this might not be the case for these dyes since a more fluorescent species is formed by the isomerization process as the temperature is increased. Indeed, the fluorescence output of these dyes appear to be constant from 0°C to 60°C in methanol, suggesting a competition between the fluorescence-enhancing and fluorescence-diminishing processes.

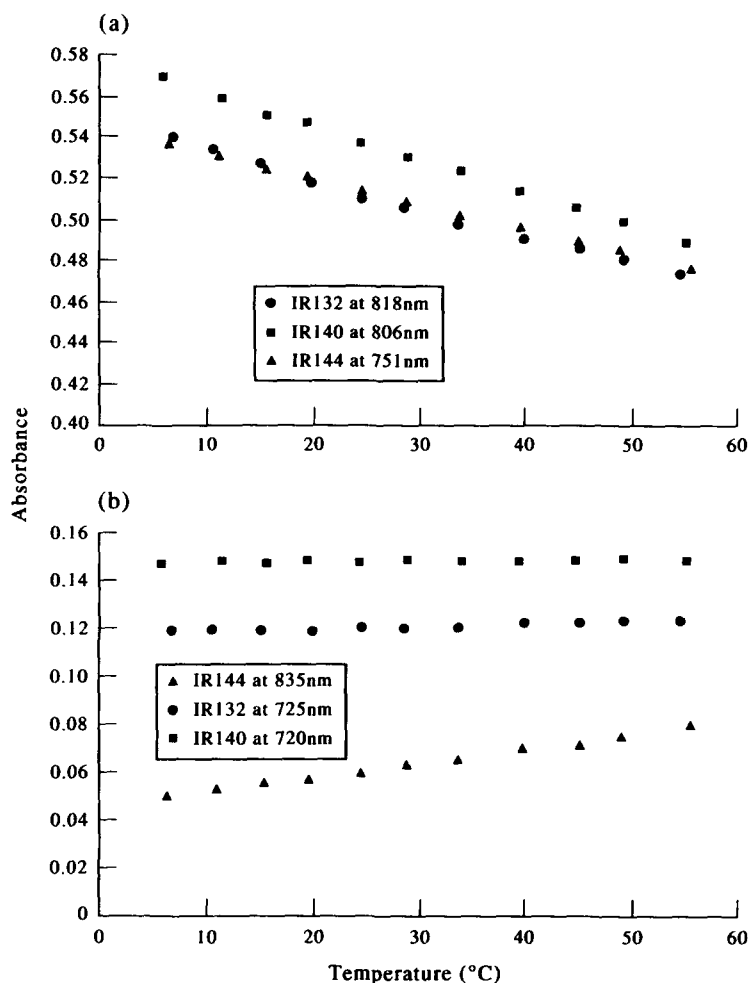


Fig. 6. (a) The variation of absorbance of the predominant species with temperature; (b) the variation of the absorbance of the minor dye species with temperature.

3.2 Absorbance and fluorescence characteristics of small meso-amine substituted heptamethine dyes

Small meso-amine substituted heptamethine dyes, such as IR144 (Fig. 3), have been proposed to exist in a single conformation in which the small meso-amine substituent can donate electron density into the chromophoric chain by resonance.⁶ However, the excitation maximum for the small meso-amine substituted dye, IR144 (Fig. 3) is slightly hypsochromically shifted from its absorption maximum (Table 1), and a reversible temperature-dependent absorption spectrum is apparent, a bathochromic shoulder

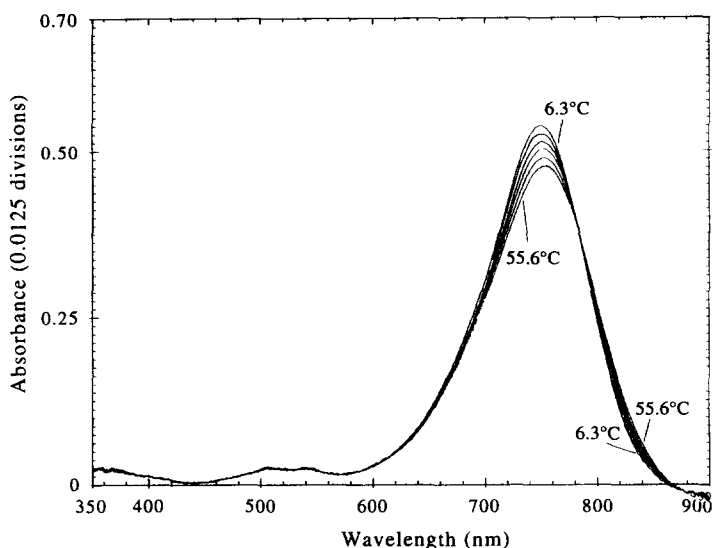


Fig. 7. The temperature dependence of the absorption spectrum of IR144 (Fig. 3) in methanol.

related to a non-fluorescent species increasing with increasing temperature at the expense of the absorption maximum (Figs 6 and 7). Consequently, for small meso-amine substituted heptamethine dyes also, two interconverting species are present in solution. However, the hypsochromic fluorescent species is predominant at room temperature, and therefore the absorption maximum (Fig. 7) is more representative of the hypsochromic fluorescent species as opposed to being more representative of the bathochromic non-fluorescent species in the meso-diphenylamine substituted dyes. Fluorescence output is higher in IR144 (Fig. 3) than in the closely related meso-diphenylamine substituted analogue dimethylindolinium-cya (Fig. 1), not only because in IR144 solutions the hypsochromic fluorescent species is predominant, but also because the smaller proportion of non-fluorescent bathochromic species present in IR144 solutions results in substantially less internal absorption of the fluorescence output. The extinction coefficient of the non-fluorescent bathochromic species of IR144 is reasonably high, such that the increase in the bathochromic shoulder with temperature (Fig. 7) is much more apparent when compared to the much smaller increase with temperature of the absorption relating to the fluorescent species of IR140 (Fig. 4), which probably has a lower extinction coefficient.

3.3 Variable-temperature NMR studies

If two rapidly interconverting dye species are present, an NMR spectrum will show the average δ value for a given H atom, this average δ value

TABLE 2

Characteristics of Trinuclear and Dinuclear Conformations of Meso-amine Substituted Heptamethine Dyes

<i>Trinuclear conformation</i>	<i>Dinuclear conformation</i>
Resonance donating meso-amine group.	Non-resonance donating meso-amine group.
Predominant conformation for dyes with small meso-amine groups.	Predominant conformation for dyes with large meso-amine groups.
Low fluorescence quantum yield* and large Stokes shift*.	No fluorescence emission below 900 nm.
Lower wavelength absorption maximum* in comparison to dinuclear conformation.	Higher wavelength absorption maximum in comparison to trinuclear conformation.
Lower extinction coefficient* in comparison to dinuclear conformation.	Higher extinction coefficient in comparison to trinuclear conformation.

*Characteristics shared with trinuclear dyes.¹⁴

being weighted according to the proportion of each species present at a given temperature. However, the changes in δ values for IR144 (Fig. 3) and IR140 (Fig. 1) in CD₃OD over the temperature range, -30°C to 50°C were not significant ($\pm 0.02 \delta$) with a general downfield shift (0.03δ) of all the δ values of these dyes with decreasing temperature. Precipitation of dyes at temperatures lower than -30°C in a variety of deuterated solvents prevented the measurement of NMR spectra at lower temperatures.

4 CONCLUSIONS

Meso-amine substituted heptamethine dyes have previously been thought to be in a single conformation, the nature of which depended on the size of the meso-amine group.⁶ The dyes were considered to possess either a dinuclear chromophore (involving the two terminal heterocyclic nitrogen atoms only) when the meso-amine group was large and could only act by inductively electron withdrawal; or a trinuclear chromophore (involving all three nitrogen atoms) when the meso-amine group was small and could donate by resonance. However, our findings indicate that a minor proportion of the unfavoured conformation of each of these dyes is also present in organic solvents. Thus, a minor proportion of dye is in the trinuclear conformation when the meso-amine group is large, and a minor proportion of dye is in the dinuclear conformation when the meso-amine is small. Many

characteristics of the dyes in the trinuclear and dinuclear conformations are different (Table 2), the characteristics of the trinuclear conformation being more similar to those of trinuclear dyes.¹⁴ This is not unexpected, because both the trinuclear conformation of meso-amine substituted heptamethine dyes and conventional trinuclear dyes possess branched π electron systems.

The dinuclear/trinuclear isomerism reported here is closely related to allopolar isomerism exhibited by holomeropolar cyanine dyes, where a similar twisting of a meso-substituent takes place in a chromophore with a branched π electron system and a high degree of steric crowding giving rise to two isomeric species with different chromophoric properties.^{15,16,17} The dinuclear conformation reported here is analogous to the holopolar isomeric form and the trinuclear conformation is analogous to the meropolar isomeric form.

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