The Synthesis of some Water Insoluble Dyes for the Measurement of pH in Water Immiscible Solvents

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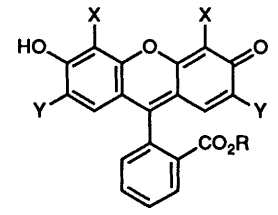
A series of water insoluble dyes for the measurement of pH in an inaccessible water phase in water immiscible solvents have been designed and synthesised based upon the fluorescein system. The solubilities of the anionic forms of the dyes depend strongly upon the nature of the ester substituent and the degree of substitution of the aromatic rings. For dye molecules with long chain alkyl esters and with bromine substituents in the rings, the sodium or potassium salts are soluble in organic solvents such as diethyl ketone or ethyl acetate and the anions do not partition measurably into aqueous solution. Other less highly substituted dyes can be solubilised as their anions by the addition of quaternary ammonium salts or crown ethers. Titration curves for the dyes show that they are able to respond reproducibly to the pH of a water phase in contact with the organic phase although the detailed physical chemistry of the ion exchange is complex. Certain of the dyes are suitable for monitoring the pH in reaction systems using enzymes as catalysts in water immiscible solvents.

The significance of non-aqueous solvents as media for reactions catalysed by enzymes has been emphasised strongly in recent years.[1] Although many transformations of substrates have been successfully demonstrated, the underlying physical chemical principles that control the kinetics or equilibria of such reactions are poorly understood. One of the parameters that is difficult to measure in such reaction media is the pH, control of which is important for the success of a biotransformation. There are several cases in which biotransformations in water immiscible solvents have failed for reasons that may be related to pH changes.[2,3] In this paper we describe the design and synthesis of molecules capable of reporting the pH in water immiscible solvents.

It is insufficient to take a water soluble indicator in a mixed solvent system and to relate a colour change that may occur to a change of conditions in the organic phase.[3] What is required is a molecule that not only has the chromophoric properties of an indicator but also both neutral and ionic forms remain virtually exclusively in the organic phase. Such a molecule must also avoid interfering with the enzyme-catalysed reaction by binding to the protein. Further, the indicator must be devoid of surfactant properties. Ideally, it should also be possible to vary the pH at which the chromophore changes by means of substituents through their electronic effects. In order to obtain such a compound, an indicator molecule was required such that its solubility and acid–base properties could be manipulated independently, to a first approximation. The fluorescein dyes and their relatives **1** possess a large conjugated chromophore; the pH range of their response is known to be very sensitive to the ring substituents and there is a carboxylate substituent which is a suitable site for the introduction of further substituents in order to control solubility.



Compound	X	Y	R
1a	H	H	Pr^i
1b	H	H	Neopentyl
1c	H	H	2 - Phenylethyl
1d	H	H	Cyclohexyl
1e	H	H	n - Octyl
2	Br	H	n - Octyl
3	Br	Br	n - Octyl
4	H	H	3, 7, 11 - Trimethyldodecyl
5	Br	H	3, 7, 11 - Trimethyldodecyl
6	Br	Br	3, 7, 11 - Trimethyldodecyl

Table 1. Solubility of fluorescein esters in water and 1,1,1-trichloroethane.

Ester	R	Water	Solubility/μmol dm^{-3} 1,1,1-Trichloroethane
1a	Pr^i	32	240
1b	Neopentyl	17	290
1c	2-Phenylethyl	14	120
1d	Cyclohexyl	11	46
1e	n-Octyl	0	400

Results and Discussion

Initially we prepared a series of simple alkyl esters (**1a–e**) of fluorescein by acid catalysed esterification. The solubilities of the esters in 1,1,1-trichloroethane, as a representative water immiscible solvent, were investigated in comparison with solubilities in water (Table 1). The electronic spectra of the anion and neutral form of the indicators were well separated (Fig. 1) and the determination of the concentration of each species by spectrophotometery was therefore straightforward. The indicator molecules also exhibit intense fluorescence but

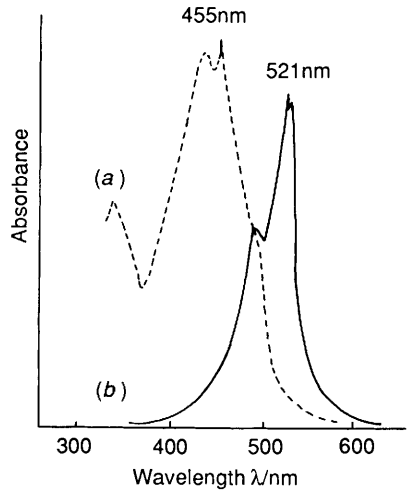


Fig. 1. Visible absorption spectrum of **1e** in 1,1,1-trichloroethane: (*a*), neutral form; (*b*), anionic form.

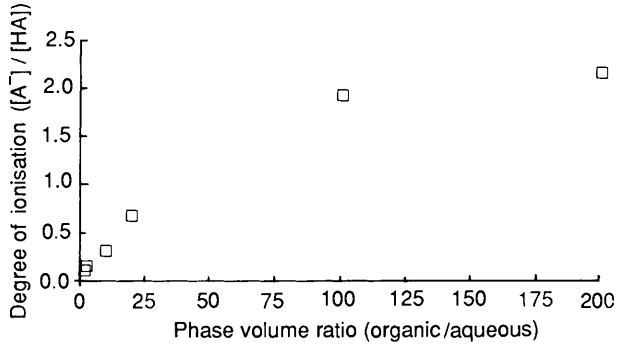


Fig. 2. Degree of ionisation of **3** in 1,1,1-trichloroethane at constant pH (2.6), as a function of aqueous phase volume, in the presence of tetrabutylammonium ions (0.6 mol dm^{-3}).

this property has not been exploited in the present measurements. The hydrogen ion released on ionisation of the indicator will partition into the adjacent aqueous phase. It is therefore necessary to provide a positively charged counter-ion to maintain electroneutrality in the organic phase and prevent partition of the anion form of the indicator into the aqueous phase at high pH. These counter-ions should have low solubility in the organic phase in the company of an inorganic anion but high solubility with the indicator anion as partner. Such systems involving added counter-cations did not allow us to establish directly a practical system for the investigation of enzyme-catalysed reactions but did permit the study of some of the structural parameters that control ionisation and solubility of the indicators.

Studies using Fluorescein n-Octyl Esters as Indicators.—Solutions of fluorescein n-octyl ester in 1,1,1-trichloroethane containing tetra-n-butylammonium iodide as a counter-ion source were equilibrated with buffered solutions over a range of pH values. Fluorescein n-octyl ester was found to be too weakly acidic; its anionic form was not produced in significant concentration even at alkaline aqueous pH. In addition, there was a detectable partition of the indicator into the aqueous phase at high pH; fluorescein n-octyl ester was clearly too polar for the system also.

The acidity of the indicator was increased by bromination. Two brominated derivatives of **1e** were synthesised, 4,5-dibromofluorescein n-octyl ester **2** and 2,4,5,7-tetrabromofluorescein n-octyl ester **3**. Both **2** and **3** were dissolved in 1,1,1-trichloroethane containing tetra-n-butylammonium counter-ions and tested against buffered solutions as before. These molecules responsed as indicators with the anionic forms increasing in concentration as the aqueous pH rises. As expected, the more bromine atoms present in the molecule, the more acidic the indicator. The pH for half-neutralisation of the dibromo indicator **2** in the presence of 0.1 mol dm^{-3} sodium pyrophosphate buffer and 0.75 mmol dm^{-3} tetra-n-butylammonium iodide was 6.1 and for the tetrabromo analogue **3** 2.8. It must be emphasised that these pH values for half-neutralisation are not constants, they depend upon counter-ion concentration. Both of these molecules could in principle be used as pH indicators in a two-phase system involving 1,1,1-trichloroethane, tetra-n-butylammonium counter-ions and the aqueous phase whose pH is to be measured. However the titration equilibria were found to be sensitive both to the nature of the counter-ion and to its concentration. Tetraethylammonium counter-ions increased the effective pK_a of the indicator and promoted some partition into the aqueous phase at high pH. Small counter-ions such as tetramethylammonium led to precipitation of the indicator at the phase interface. The titration equilibria were also found to be sensitive to phase volume ratio, an undesirable feature for the intended application in which small volumes of water, inaccessible to conventional pH measurement techniques, must be examined. These phenomena can be understood in terms of a theoretical model of the equilibria that will be discussed in a subsequent paper. Fig. 2 illustrates the behaviour observed; the degree of ionisation of the indicator increased at constant pH as the volume of the aqueous phase was decreased. The above properties of the fluorescein–n-alkylammonium systems dictated that practical systems could only be obtained after careful calibration. We therefore sought alternative methods of providing the counter-ion required for electroneutrality of the organic phase.

Metal Cation Crown Ether Complexes as Counter-ions.—In the previous experiments, the counter-ions needed to preserve electroneutrality in the organic phase were provided by tetraalkylammonium salts. Tetraalkylammonium ions appeared to partition freely between the phases resulting in the indicators' pH response depending upon the phase volume ratio in the system. Crown ethers enable metal ions to be solvated in organic media and should therefore enable metal cations from the buffer solution to enter the organic phase and act as counter-ions for the anionic form of the indicator as it is produced. Solutions of 2,4,5,7-tetrabromofluorescein n-octyl ester **3** in 1,1,1-trichloroethane containing dibenzo-18-crown-6 were equilibrated with buffered solutions over a range of pH values. In both experiments using crown ethers, the equilibrium between the neutral and anionic forms of the indicator was very easily altered when solutions were transferred to the spectrometer cells for analysis. It was found that such effects were minimised but not removed by the use of polypropylene pipettes to transfer the solutions. Although titration equilibria could again be established in these systems, the sensitivity to anions made them impractical as systems for studying enzyme-related phenomena. Problems with dependence upon the phase volume ratio also existed with these crown ether systems because the crown ether partitions into both phases. A solution to these practical problems resided in the preparation of fluorescein analogues containing more hydrophobic esters.

Fluorescein Hexahydrofarnesyl Esters.—The complexities with the above binary systems implied a requirement for much more hydrophobic indicators. If the indicator molecule were made less polar then its anionic form would be able to obtain the necessary counter-ion from the aqueous phase directly.

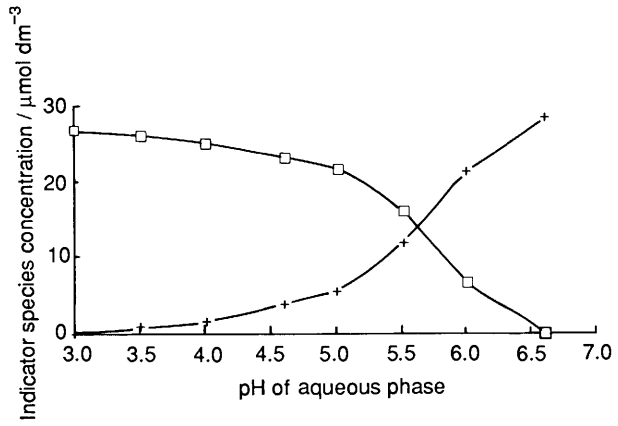


Fig. 3. Ionisation curves for **6** in ethyl acetate with 0.1 mol dm^{-3} tetrasodium pyrophosphate aqueous phase: □, [HA]; +, $[A^-]$.

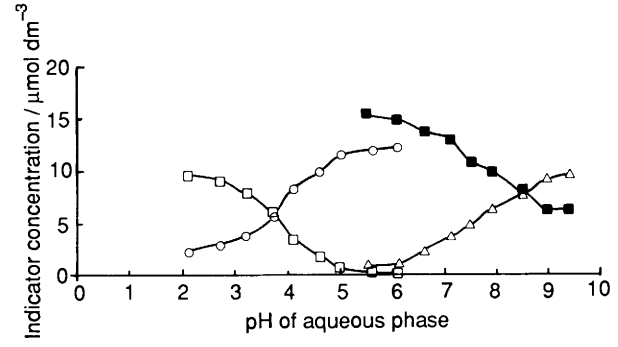


Fig. 4. Effect of bromination on ionisation of indicators: ionisation curves for **5b** and **6** in diethyl ketone with 0.1 mol dm^{-3} tetrasodium pyrophosphate as aqueous phase: ■, **5b** [HA]; △, **5b** $[A^-]$; □, **6** [HA]; ○, **6** $[A^-]$.

Increasing the number of carbon atoms in the alkyl chain of the fluorescein ester would make the ester less polar and the risk of surfactant behaviour in the two-phase system would be reduced by using a branched chain alcohol in the esterification. Fluorescein 3,7,11-trimethyldodecyl ester **4** was prepared in the usual way using 3,7,11-trimethyldodecanol (hexahydrofarnesol) as the alcohol. After bromination 4,5-dibromofluorescein-(3,7,11-trimethyldodecyl) ester **5** and 2,4,5,7-tetrabromofluorescein 3,7,11-trimethyldodecyl ester **6** were obtained and used in titration experiments.

When 1,1,1-trichloroethane was used as solvent for titration experiments with the tetrabromo derivative **6** precipitation of the sodium salt of the indicator occurred. However, when more polar solvents, ethyl acetate or diethyl ketone or n-butanol, were used, satisfactory titration curves were obtained. Figs.3–5 show the results obtained for di- and tetra-bromo indicators in the three solvents. The solubility of both anion and neutral form of the indicator **6** appeared to be well balanced for measurements to be made without additional additives. It would, however, be expected that the concentration of sodium ions in the aqueous phase might influence the degree of ionisation of the indicator at constant pH. This point can readily be appreciated by considering the equilibrium diagram Fig. 6 in which both ionisation and phase transfer equilibria are represented. The results in Table 2 are consistent with this expectation. The sensitivity of the indicators to the concentration of monovalent cations in the aqueous phase raised the question of whether divalent cations that are commonly required in enzyme-catalysed reactions would also influence the titration behaviour. Experiments showed that there was no

Table 2.

Aqueous Na^+/mol dm^{-3}	Aqueous pH	Solvent	Degree of ionisation of indicator $[A^-]/[HA]$
0.5	6	EtOAc	3.45
0.05	6	EtOAc	0.73
0.005	6	EtOAc	0.35
0.4	5.0	Et_2CO	3.5
0.04	5.0	Et_2CO	2.77
0.004	5.0	Et_2CO	0.39

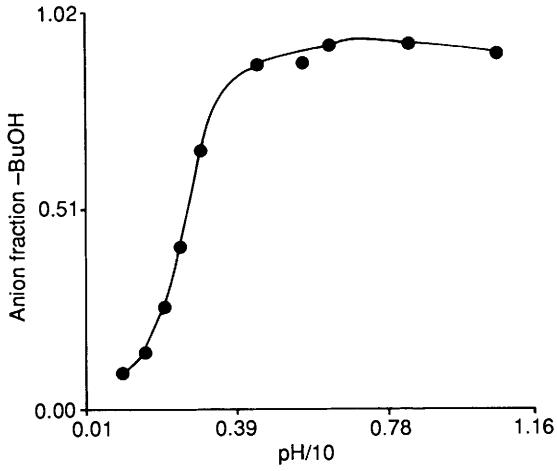


Fig. 5. Ionisation curve for **6** in n-butanol with 0.1 mol dm^{-3} tetrasodium pyrophosphate as the aqueous phase.

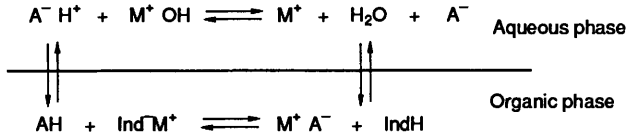


Fig. 6. Ionic equilibria in two-phase systems involving indicators and other acid-base pairs: AH = an organic acid; IndH = indicator acid form; M = metal ion.

significant effect on the titration curve when magnesium ions were included; calcium ions caused partial precipitation of the indicator.

Although ethyl acetate and diethyl ketone were similar solvents in many aspects, ethyl acetate was unsuitable for extended experiments at high pH because it suffers hydrolysis at an appreciable rate leading to a fall in the aqueous pH. Diethyl ketone, on the other hand, was found to be a robust solvent and with it, several important properties of the indicators relevant to practical use were established experimentally.

Using the di- and tetra-bromohexahydrofarnesyl indicators **5** and **6** it was possible to cover a wide pH range from 3 to 9 encompassing all the values usually relevant to enzyme-catalysed reactions. The most important result, however, was the demonstration that the ionisation of **6** was independent of the phase volume ratio. Low water volumes were achieved by adsorbing water from a micropipette on silica and, to avoid surface effects of glass, experiments at low water volume were carried out using polypropylene tubes. Fig. 7 for the dibromo-indicator shows that the ionisation ratio was essentially constant when polypropylene tubes were used.

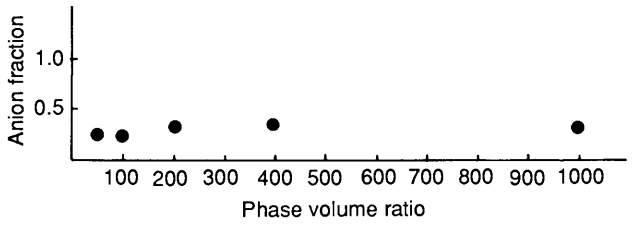


Fig. 7. Effect of the phase volume ratio (pentan-3-one/0.1 mol dm^{-3} tetrasodium pyrophosphate) on degree of ionisation of **5** in the organic phase at constant pH (7.0).

Conclusions

The last results above establish a system suitable for the direct measurement of the pH of a small volume aqueous phase in contact with an organic phase in large excess. Detailed analysis of the equilibria for all the systems has been carried out and an equation deduced to model their behaviour. The curves to which the points on the figures are fitted derive from this analysis which will be discussed in following papers. We have used this indicator successfully to demonstrate pH related phenomena in reactions catalysed by chymotrypsin[4] and a lipase.[5]

Experimental

NMR spectra were obtained at 250 MHz using a Bruker WH-280 spectrometer with tetramethylsilane as internal standard. All reactants were reagent grade. Preparative chromatography was carried out on silica gel MFC using gravity elution. Electron impact mass spectra were obtained on a modified MS902 spectrometer at Strathclyde and FAB spectra at the SERC service facility at the University of Wales, Swansea.

Fluorescein Propan-2-yl ester **1a**.—A solution of fluorescein (0.5 g) in propan-2-ol (20 cm^3) containing sulphuric acid (1 cm^3) was heated under reflux for 3 days. Excess of solvent was removed under reduced pressure and the residual red liquid diluted with water. Neutralisation with dilute aqueous sodium hydroxide afforded an orange solid which was recrystallised from ethyl acetate and aqueous methanol to give the required *ester* (125 mg), m.p. 256–259 °C; $\delta_H[(CD_3)_2SO]$ 0.78 (6 H, d), 4.74 (1, H, septet), 6.57 (1 H, s), 6.62 (3 H, s), 6.83 (2 H, d), 7.51 (1 H, d), 7.82 (2 H, m) and 8.14 (1 H, d).

Fluorescein 2,2-Dimethylpropanyl Ester **1b**.—Fluorescein (1 g) and 2,2-dimethylpropanol (2 g) were fused and sulphuric acid (1 cm^3) added. The mixture was heated at 150 °C for 5 h and the volatile components removed by distillation under reduced pressure. The residue was dissolved in methanol from which the crude product was precipitated by adding water; the main impurity was shown to be unesterified fluorescein by TLC. Chromatography on silica gel (65 g) eluting with methanol–ethyl acetate (1:3) afforded the required *ester* (136 mg), m.p. 260–263 °C; $\delta_H[(CD^3)_2SO]$ 0.70 (9 H, s), 3.68 (2 H, s), 6.80 (2 H, d), 6.88 (2 H, s), 7.02 (2 H, d), 7.30 (1 H, d), 7.70 (2 H, quintet) and 8.25 (1 H, d).

Fluorescein 2-Phenylethyl Ester **1c**.—Fluorescein (1 g) and 2-phenylethanol (25 cm^3) were mixed with sulphuric acid (2 cm^3) and heated at 130 °C for 5.5 h. The cooled mixture was washed with pH 9 aqueous buffer 600 cm^3) and the washings extracted with ether (200 cm^3). The ether extracts and the residual alcohol solution were combined and dried (Na_2SO_4). From this solution an orange solid precipitated which was recrystallised from methanol to give the required *ester* (574 mg), m.p. 246 °C (Found: C, 76.7; H, 4.6. $C_{29}H_{19}O_5$ requires C, 77.1; H, 4.6%); $\delta_H[(CD_3)_2SO]$ 2.65 (2 H, t), 4.17 (2 H, t), 6.50 (2 H, s), 6.54 (2 H, s), 6.75 (2 H, d), 7.05 (2 H, d), 7.18 (3 H, m), 7.47 (1 H, d), 7.80 (2 H, m) and 8.10 (1 H, d).

Fluorescein Cyclohexyl Ester **1d**.—Fluorescein (1 g) was suspended in cyclohexanol (30 cm^3) and sulphuric acid (2 cm^3) added. The mixture was heated under reflux for 7 h. The cooled solution was diluted with ether (100 cm^3), and washed with pH 9 buffer (800 cm^3); the buffer extracts were back extracted with ether (500 cm^3). The ether solution was dried (Na_2SO_4) and evaporated to dryness. The residue was purified by chromatography on silica gel (100 g) eluting with methanol–ethyl acetate (3:7). The product was recrystallised from methanol to give the required *ester* (331 mg), m.p. 258–259 °C (Found: C, 75.1; H, 5.4. $C_{26}H_{22}O_5$ requires C, 75.5; H, 5.1%); $\delta_H[(CD_3)_2]$ 1.20 (10 H, m), 4.60 (1 H, s), 6.52 (2 H, s), 6.55 (2 H, s), 6.80 (2 H, d), 7.49 (1 H, d), 7.80 (2 H, m) and 8.18 (1 H, d).

Fluorescein n-Octyl ester **1e**.—Fluorescein (5 g) was suspended in n-octanol (30 cm^3) and sulphuric acid (3 cm^3) added. The mixture was heated at 140 °C for 5 h. The resulting solution was allowed to cool and ether (300 cm^3) added to precipitate the crude product which contained a mixture of unchanged fluorescein and the required ester. Multiple recrystallisation from ethyl acetate afforded the *n-octyl ester* (0.9 g) (Found: C, 75.9; H, 6.5. $C_{28}H_{28}O_5$ requires C, 75.7; H, 6.4%); $\delta_H[(CD_3)_2]$ 0.85 (3 H, t), 1.15 (12 H, m), 3.90 (2 H, t), 6.56 (4 H, d), 6.80 (2 H, d), 7.47 (1 H, d), 7.82 (2 H, m) and 8.17 (1 H, d) (Found: M^+, 444.1934. $C_{28}H_{28}O_5$ requires M, 444.1937); λ_{max}/nm: $[CH_3CCl_3]$ 459.9 (ε 15 990 dm^3 mol^{-1} cm^{-1}) acid, 521 (42 680) anion.

4,5-Dibromofluorescein n-Octyl Ester **2**.—Fluorescein n-octyl ester (0.9 g) was suspended in dry methanol (10 cm^3) and bromide (270 mm^3) added dropwise with stirring at room temperature to give a clear orange solution. The solution was evaporated to dryness under reduced pressure and the residue digested by heating with ethyl acetate (20 cm^3). The residue was filtered off and dried affording the required *dibromo ester* (0.79 g), m.p. 218–222 °C (Found: M^+, 602.0073. $C_{28}H_{26}O_5{}^{79}Br^{81}Br$ requires M, 602.0126; λ_{max}/nm: $[CH_3CCl_3]$ 463.9 (ε 11 370 dm^3 mol^{-1} cm^{-1}) acid, 534.2 (50 230) anion; $\delta_H[(CD_3)_2SO]$ 0.83 (3 H, t), 1.12 (12 H, m), 3.86 (2 H, t), 6.85 (4 H, q), 7.48 (1 H, d), 7.84 (2 H, m) and 8.20 (1 H, d).

2,4,5,7-Tetrabromofluorescein n-Octyl Ester **3**.—Fluorescein n-octyl ester (0.1 g) was suspended in dry methanol (2 cm^3) and bromide (60 cm^3) added in two equal portions at room temperature with stirring. On addition of the second portion of bromine, a red precipitate formed. Reaction was allowed to continue for 3 h. The red solid was filtered off and washed with dry methanol. This material proved to be the required *tetrabromo ester*, m.p. 110–115 °C. The filtrate yielded an evaporation of the dibromo ester **2** [Found: ($M^+ + H^+$), 760.839. $C_{28}H_{24}O_5{}^{79}Br_2{}^{81}Br_2$ requires M, 760.839]; λ_{max}/nm $[CH_3CCl_3]$ 473.5 (ε 18 430 dm^3 mol^{-1} cm^{-1}) acid, 547 (93 891); $\delta_H[(CD_3)_2SO]$ 0.85 (3 H, t), 1.15 (12 H, m), 3.95 (2 H, t), 7.13 (2 H, s), 7.53 (1 H, d), 7.87 (2 H, m) and 8.22 (2 H, d).

Fluorescein 3,7,11-Trimethyldodecyl Ester **4**.—To 3,7,11-trimethyldodecan-1-ol (5 g) was added solid fluorescein (6.5 g). The mixture was stirred at 130 °C for 9 h, under an inert atmosphere, in the presence of concentrated sulphuric acid (0.5 cm^3). After the reaction mixture had been cooled, it was dissolved in dilute aqueous sodium hydroxide. The alkaline solution was thoroughly extracted with ethyl acetate. The ethyl acetate extracts were then washed with dilute aqueous sodium hydroxide, dried over anhydrous sodium sulphate and evaporated to yield a red oil (5.3 g). Analysis of the oil using TLC

(eluant, ethyl acetate) showed product at R_f 0.4 together with unchanged material. Silica gel column chromatography of the oil with a gradual increase in solvent polarity from 20% ethyl acetate in hexane to 100% ethyl acetate produced unchanged alcohol, unchanged fluorescein and finally crude product ester (1.1 g). The crude product was purified using preparative TLC (silica gel GF_{254}; eluant, ethyl acetate). The pure product ester (R_f 0.4) was extracted from silica using methanol, the methanol being evaporated to give the required *ester* as a powder (0.33 g, 41%) (Found: M^+, 542.2999. $C_{35}H_{42}O_5$ requires M, 542.3032); δ_H[$(CD_3)_2SO$] 0.71 (3 H, d), 0.79 (3 H, d), 0.81 (6 H, d), 1.10 (15 H, m), 1.55 (2 H, t), 3.90 (2 H, t), 6.20 (2 H, dd), 6.24 (2 H, dd), 6.56 (2 H, dd), 7.39 (1 H, dd), 7.64 (2 H, m) and 8.07 (1 H, dd).

4,5-*Dibromofluorescein*-(3,7,11-*trimethyldodecyl*) *Ester* **5**.—Fluorescein 3,7,11-trimethyldodecyl ester (0.05 g) was dissolved in dry methanol (5 cm^3) and the solution was stirred at room temperature. To this solution was added bromine (1 equiv.) and the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was evaporated to dryness, taken up in chloroform and separated using preparative TLC (silica gel GF_{254}) eluting with ethyl acetate. Component at R_f 0.3 was extracted using methanol, evaporated to dryness and re-dissolved in hot ethyl acetate. After filtering, the filtrate was evaporated to give a red solid. The solid was triturated with hexane to give a red crystalline *dibromo ester* (0.025 g, 39%) [Found: $(M + H^+)$, 701.148. $C_{35}H_{42}O_5{}^{79}Br_2$ requires $(M + H^+)$ 701.147]; λ_{max}/nm: (pentan-3-one) 536.1 (ε 40 347 dm^3 mol^{-1} cm^{-1}) anion, 460.9 (9942) acid; δ_H[$(CD_3)_2SO$] 0.61 (3 H, d), 0.79 (3 H, d), 0.85 (6 H, d), 1.10 (15 H, m), 1.50 (2 H, m), 3.94 (2 H, m), 6.25 (2 H, d), 6.61 (1 H, dd), 7.41 (1 H, d), 7.76 (2 H, m) and 8.09 (1 H, d).

2,4,5,7-*Tetrabromofluorescein*-(3,7,11-*trimethyldodecyl*) *Ester* **6**.—Fluorescein 3,7,11-trimethyldodecyl ester (0.05 g) was dissolved in dry methanol and the solution was stirred at room temperature. To the stirred solution was added bromine (5 equiv.) and the mixture was stirred at room temperature. After 1 h a red solid precipitate was formed. Stirring was continued for a further hour. The reaction medium was evaporated to dryness, the product taken up in chloroform and filtered and the filtrate analysed by TLC [eluant ethyl acetate–hexane (3:1), plus one drop glacial acetic acid]. A chromatogram showed one component of R_f 0.6. The product was purified by preparative TLC (silica gel GF_{254}) using ethyl acetate and a few drops of acetic acid as eluting solvent.

The product was extracted from silica using methanol and the solvent evaporated to give a dark red powder. This powder was taken up in chloroform, the solution filtered and evaporated to dryness to give a red powder. A small volume of hexane was added to the powder and scratched from which the required *tetrabromo ester* was obtained as a crystalline solid (0.056 g, 71%) [Found: $(M + H^+)$, 858.949. $C_{35}H_{38}O_5{}^{79}Br_2{}^{81}Br_2$ requires $(M + H^+)$, 858.948]; λ_{max}/nm: (pentan-3-one) 545.9 (ε 79 592 dm^3 mol^{-1} cm^{-1}) anion, 475.7 (15 646) acid; δ_H[$(CD_3)_2SO$] 0.59 (3 H, t), 0.79 (3 H, t), 0.84 (6 H, t), 1.10 (15 H, m), 1.49 (2 H, m), 3.97 (2 H, m), 6.90 (1 H, s), 6.91 (1 H, s), 7.49 (1 H, dd), 7.71 (2 H, m) and 8.12 (1 H, dd).

Acknowledgements

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