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Substituent effects on the apparent p*K* of the reversible open-to-closed transition of sultams derived from sulforhodamine dyes

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Abstract

Sultams derived from sulforhodamine dyes by reaction with primary amines undergo reversible ring-chain interconversion that is pH-dependent. The apparent p*K* for the transition has been modulated in the range 4.40–8.04 by variation of the amino substituents at the 3′,6′-positions of the sulforhodamine. Alterations of the substituent on the sulfonamide nitrogen atom had much smaller effects. The pH-dependent kinetics of ring-closure after photolytic opening of the closed form of one of the compounds studied here have been measured over the pH range 6.02–8.51.

Keywords: Rhodamine sultams; Ring-chain tautomerism; pH-responsive dyes; Substituent effects; Kinetics

1. Introduction

We have previously described [1] the phenomenon of the coloured-to-colourless transition that is associated with the reversible open-to-closed, pH-dependent interconversion of sultams derived from sulforhodamine dyes (Fig. 1). More recently we have formulated a detailed kinetic scheme to explain the complex kinetics of this interconversion over the pH range 0–13 [2]. That study was conducted entirely with compound **1** (Fig. 2) that was prepared from the widely used dye sulforhodamine B chloride by reaction with methylamine. In fact, as we showed previously [1], sulforhodamine B chloride as generally available from commercial suppliers contains two sulfonyl chloride isomers and only the one with its sulfonyl chloride *ortho* to the xanthylium system can form a compound that undergoes the ring-chain process shown in Fig. 1. The isomeric sulfonamide **2** (Fig. 2) cannot interact with the xanthylium system and therefore is coloured (and fluorescent) across the pH range, except at very high acidity (pH < 1) when it can be protonated and undergo a consequent spectral change, as is known for other rhodamines and sulforhodamines [3].

The sulfonamide **1** converts from the coloured to the colourless form with a conventional Henderson–Hasselbach pH titration that is fully reversible and shows an apparent p*K* at 7.37. This value refers to a measurement in 3:1 water–ethanol solvent. In a purely aqueous solvent but containing a non-ionic detergent at a concentration above its critical micellar concentration, the apparent p*K* was reported as 5.36 [4], and we have noted [1] that the lowered p*K* value was a consequence of selective stabilisation of the colourless, closed form in the hydrophobic interior of micelles. The p*K* is an apparent value since it does not relate directly to the p*K* for ionisation of a specific proton within the molecule but rather to the overall structural change that is responsible for the colour change. The variability of the p*K* values for **1** described above shows the potential for manipulating the colour change of these compounds. Unlike conventional pH indicators, the colour change is not ‘‘instantaneous’’ in response to a change in pH, but takes place with pH-dependent kinetics that span nine orders of magnitude across the pH range 0–13 [2]. Near pH 7, observed rate constants for the colour change of **1** in stopped-flow experiments were $\sim 200\ s^{-1}$ (at 4 °C) whether approached from the coloured or colourless form [1,2].

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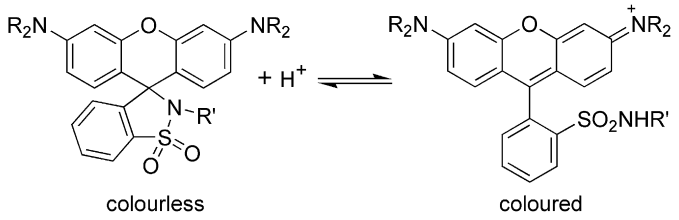

Fig. 1. Ring-chain equilibrium of sulforhodamine–amine conjugates.

A feature of the cyclic form of these compounds is that it can be opened by a pulse of near-UV light, an observation first reported ~30 years ago [5]: the open form is, as expected, coloured (and fluorescent) and this feature of the compounds prompted our interest in their chemistry, since it enables generation of a transient fluorophore by a pulse of near-UV light. Indeed, one recent paper has exploited this possibility for a related lactam derived from Rhodamine B [6]. In earlier work the colour change associated with this ring-chain interconversion has been applied in patent literature to provide permanent colour changes in either direction for thermal [7] and photographic imaging [8]. Furthermore, compounds of this type have been proposed as fluorescent pH indicators, albeit that those authors misunderstood the nature of the chemistry involved in the colour change and appear to have ascribed it to an unknown mechanism involving structures analogous to the isomer **2** [9].

Ideally, as is the case with the Rhodamine B lactam, generation of a transient fluorophore from the colourless, cyclic form would start from a blank background but for compound **1**, the apparent p*K* results in ~70% being in the open form at pH 7. Applications that could benefit from variation of the apparent p*K* for the open–closed transition can be envisaged and we therefore set out to vary this parameter by making changes to the electronic properties of substituents on the nitrogen atoms of these sultams. Thus we prepared the sulforhodamines **3**–**7** (Fig. 3) and converted them to the sultams **8**–**13** (Fig. 4). The effects of these changes are described below.

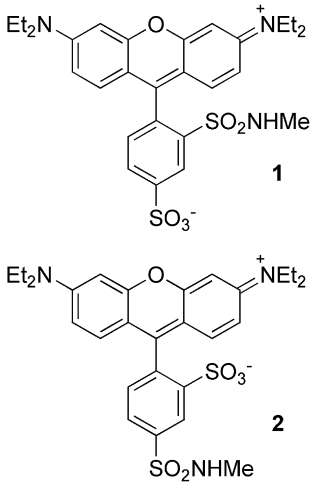

Fig. 2. Structures of isomeric monosulfonamides formed from sulforhodamine B chloride.

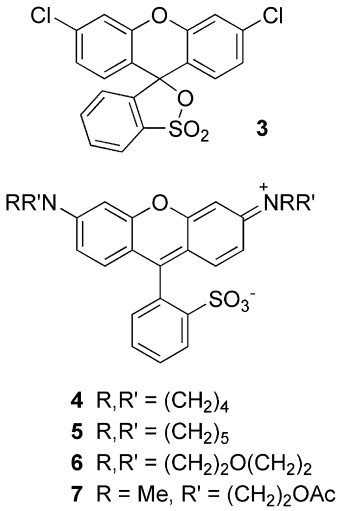

Fig. 3. Structures of compounds **3**–**7**.

2. Experimental

2.1. General methods

^{1}H NMR spectra were determined on a Varian UnityPlus 500 MHz spectrometer for solutions in $CDCl_3$ unless otherwise specified and *J* values are reported in Hz. Assignments in the sulfonated aryl ring were made from single-frequency decoupling experiments. Silica gel for flash chromatography was Merck type 9385. Solutions in organic solvents obtained after aqueous work-up were dried over anhydrous Na_2SO_4. Sulfonefluorescein dichloride **3** was synthesized as described [10] and purified by crystallisation (×2) from toluene. The dipyrrolidinylsulforhodamine **4** was available from previous work [11]. Crystals of the sulforhodamines and the derived sultams had strong tendencies to retain solvent (see examples in previous data for **4** [11] and its derived *N*-methylsultam [2]) and we were generally unable to obtain satisfactory microanalysis data. Therefore only HRMS data are reported to verify elemental composition but the NMR spectra (apart from traces of residual solvent) contained no extraneous peaks. Hence the spectroscopic data reported below are not subject to interference from other species.

2.2. Synthesis of sulforhodamines

The general method is exemplified below for the dipiperidinyl compound **5**.

2.2.1. 3′,6′-Di(1-piperidinyl)-9-(2-sulfophenyl)xanthylium, inner salt (5)

A mixture of sulfonefluorescein dichloride **3** (0.41 g, 1 mmol) and piperidine (0.40 mL, 4 mmol) in methanol (25 mL) was refluxed for 8 h, then diluted with $CHCl_3$ (100 mL), washed with 1 M HCl (2 × 25 mL) and water (25 mL), dried and evaporated. The residue was dissolved in a minimum volume of MeOH–$CHCl_3$ (4:1, ~20 mL) and diluted with Et_2O until precipitation began, then cooled in ice for 0.5 h and filtered

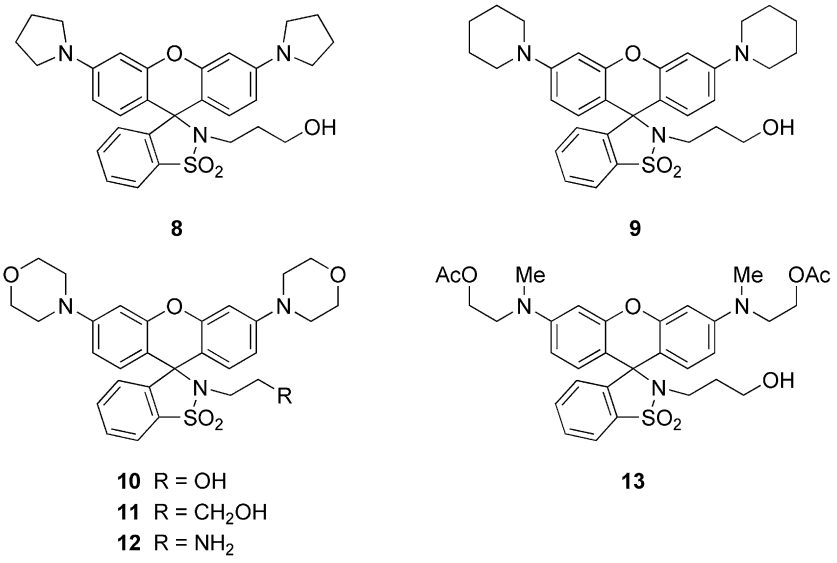

Fig. 4. Structures of the pH-responsive sultams **8**–**13**.

to give **5** as a dark, microcrystalline solid (0.34 g, 67%), >95% homogenous by TLC ($CHCl_3$–MeOH 9:1); mp > 330 °C; ^{1}H NMR δ 8.44 (1H, dd, *J* 7.9 & 1.3, Ar–H3), 7.61 (1H, dt, *J* 7.5 & 1.3, Ar–H4), 7.45 (1H, dt, *J* 7.5 & 1.3, Ar–H5), 7.39 (2H, d, *J* 9.6, H-1′,8′), 7.02 (1H, dd, *J* 7.7 & 1.3, Ar–H6), 6.90 (2H, dd, *J* 9.6 & 2.3, H-2′,7′), 6.77 (2H, d, *J* 2.3, H-4′,5′), 3.59–3.62 (8H, m, NCH_2), 1.72–1.77 (12H, m, CH_2); HRMS (FAB), *m/z* Calcd for $(C_{29}H_{30}N_2O_4S + H)^+$: 503.2005. Found: 503.2018.

2.2.2. 3′,6′-Di(4-morpholinyl)-9-(2-sulfophenyl)xanthylium, inner salt (*6*)

Prepared as for **5** above, using morpholine instead of piperidine. Yield 79%; mp > 330 °C; ^{1}H NMR ($CDCl_3$–MeOH-d_4 85:15) δ 8.31 (1H, dd, *J* 7.6 & 1.1, Ar–H3), 7.69 (1H, dt, *J* 7.8 & 1.1, Ar–H4), 7.57 (1H, dt, *J* 7.6 and 1.1, Ar–H5), 7.35 (2H, d, *J* 9.5, H-1′,8′), 7.12 (1H, dd, *J* 7.1 & 1.0, Ar–H6), 7.03 (2H, dd, *J* 9.5 & 2.4, H-2′,7′), 6.92 (2H, d, *J* 2.4, H-4′,5′), 3.88 (8H, t, *J* 4.9, OCH_2), 3.65–3.67 (8H, m, NCH_2); HRMS (FAB), *m/z* Calcd for $(C_{27}H_{26}N_2O_6S + Na)^+$: 507.1590. Found: 507.1604.

2.2.3. 3′,6′-Di[N-(2-acetoxyethyl)-N-methylamino]-9-(2-sulfophenyl)xanthylium, inner salt (*7*)

A mixture of **3** (0.41 g, 1 mmol) and 2-(methylamino)ethanol (0.32 mL, 4 mmol) in methanol (25 mL) was refluxed for 8 h. The cooled mixture was evaporated and re-evaporated from toluene (3 × 10 mL) and the residue was dissolved in dry pyridine (10 mL). Acetic anhydride (0.94 mL, 10 mmol) was added and the mixture was stirred overnight at room temperature, then diluted with $CHCl_3$ (75 mL), washed with 1 M HCl (4 × 25 mL), saturated $NaHCO_3$ (2 × 25 mL), water (25 mL) and saturated brine (25 mL), dried and evaporated. Flash chromatography (MeOH–$CHCl_3$ 15:85) gave **7** as a gum that was dissolved in a minimum volume of MeOH (~5 mL), diluted with Et_2O until a precipitate began to form, cooled in ice for 1 h and filtered to give **7** as a dark red solid (0.25 g, 44%) mp > 330 °C; ^{1}H NMR δ 8.43 (1H, dd, *J* 7.8 & 1.2, Ar–H3), 7.63 (1H, dt, *J* 7.8 & 1.2, Ar–H4), 7.48 (1H, dt, *J* 7.5 & 0.9, Ar–H5), 7.44 (2H, d, *J* 9.3, H-1′,8′), 7.04 (1H, dd, *J* 7.5 & 0.9, Ar–H6), 6.89 (2H, dd, *J* 9.6 & 2.4, H-2′,7′), 6.78 (2H, d, *J* 2.4, H-4′,5′), 4.31 (4H, t, *J* 5.8, CH_2OAc), 3.79–3.82 (4H, m, CH_2N), 3.22 (6H, s, NMe), 2.03 (6 H, s, OAc); HRMS (FAB), *m/z* Calcd for $(C_{29}H_{30}N_2O_8S + H)^+$: 567.1801. Found: 567.1791.

2.3. Synthesis of sultams

The general method is exemplified for the sultam **8**.

2.3.1. 3′,6′-Di(1-pyrrolidinyl)-2-(3-hydroxypropyl)spiro[1,2-benzisothiazole-3(2H),9′-[9H]xanthene]-1,1-dioxide (*8*)

Phosphorus pentachloride (0.272 g, 1.31 mmol) was added to a suspension of **3** (0.474 g, 1.0 mmol) in $POCl_3$ (9.5 mL) and the mixture was stirred at room temperature for 20 h. The solution was diluted with dry $CHCl_3$ (150 mL) and shaken for ~5 min with a solution of 3-amino-1-propanol (5 mL, 65 mmol) in dry $CHCl_3$ (50 mL). A further portion of 3-amino-1-propanol (5 mL, 65 mmol) in dry $CHCl_3$ (50 mL) was added and the mixture was shaken for a further 5 min. The solution was washed with H_2O (4 × 100 mL), saturated $NaHCO_3$ (100 mL), H_2O (100 mL) and saturated brine (50 mL), dried and evaporated. The residue was flash chromatographed [EtOAc–hexanes 3:7] and the recovered material was crystallised (EtOAc–hexanes) to give **8** as a pale pink solid (0.332 g, 62%), mp 256–258 °C; ^{1}H NMR δ 7.89 (1H, dd, *J* 7.4 & 1.2, Ar–H3), 7.50 (1H, dt, *J* 7.4 & 1.2, Ar–H4) 7.46 (1H, dt, *J* 7.5 & 1.2, Ar–H5), 7.00 (1H, dd, *J* 7.3 & 1.2, Ar–H6), 6.85 (2H, d, *J* 8.6, H-1′,8′), 6.26–6.28 (4H, m, H-2′,4′,5′,7′), 3.47 (2H, t, *J* 5.3, CH_2OH), 3.25–3.32 (8H, m, NCH_2), 3.06 (2H, t, *J* 6.5, SO_2NCH_2), 1.99–2.04 (8H, m, NCH_2CH_2), 1.46 (2H, quintet, *J* 6.1, CH_2CH_2OH); HRMS

(FAB), *m/z* Calcd for $(C_{30}H_{33}N_3O_4S + Na)^+$: 532.2270. Found: 532.2274.

*2.3.2. 3′,6′-Di(1-piperidinyl)-2-(3-hydroxypropyl)spiro[1,2-benzisothiazole-3(2H),9′-[9H]xanthene]-1,1-dioxide (**9**)*

Crystallised from EtOAc–hexanes (39%); mp 235–237 °C; ^{1}H NMR δ 7.89 (1H, dd, *J* 7.5 & 1.2, Ar–H3), 7.51 (1H, dt, *J* 7.5 & 1.2, Ar–H4), 7.47 (1H, dt, *J* 7.5 & 1.2, Ar–H5), 6.99 (1H, dd, *J* 7.5 & 1.2, Ar–H6), 6.87 (2H, d, *J* 9.0, H-1′,8′), 6.59–6.61 (4H, m, Ar-2′,4′,5′,7′), 3.46 (2H, t, *J* 5.7, CH_2OH), 3.20–3.22 (8H, m, NCH_2), 3.05 (2H, t, *J* 6.6, SO_2NCH_2), 1.66–1.70 (8H, m, CH_2), 1.58–1.62 (2H, m, CH_2), 1.46 (2H, quintet, *J* 6.2, CH_2CH_2OH); HRMS (FAB), *m/z* Calcd for $(C_{32}H_{37}N_3O_4S + H)^+$: 560.2583. Found: 560.2606.

*2.3.3. 3′,6′-Di(4-morpholinyl)-2-(2-hydroxyethyl)spiro[1,2-benzisothiazole-3(2H),9′-[9H]xanthene]-1,1-dioxide (**10**)*

Crystallised from EtOAc–hexanes (62%); mp 262–264 °C; ^{1}H NMR δ 7.92 (1H, dd, *J* 7.5 & 1.0, Ar–H3), 7.53 (1H, dt, *J* 7.5 & 1.0, Ar–H4), 7.49 (1H, dt, *J* 7.5 & 1.2, Ar–H5), 6.96 (2H, d, *J* 8.8, H-1′,8′), 6.95 (1H, dd, *J* 7.5 & 1.2, Ar–H6), 6.60 (2H, dd, *J* 2.6, H-4′,5′), 6.20 (2H, 8.8 & 2.6, H-2′,7′) 3.85 (8H, t, *J* 4.8, OCH_2), 3.41 (2H, q, *J* 5.6, CH_2OH), 3.19–3.21 (8H, m, NCH_2), 3.13 (2H, t, *J* 5.3, SO_2NCH_2), 2.22 (1H, t, *J* 6.8, OH); HRMS (FAB), *m/z* Calcd for $(C_{29}H_{31}N_3O_6S + H)^+$: 550.2012. Found: 550.2020.

*2.3.4. 3′,6′-Di(4-morpholinyl)-2-(3-hydroxypropyl)spiro[1,2-benzisothiazole-3(2H),9′-[9H]xanthene]-1,1-dioxide (**11**)*

Crystallised from EtOAc–hexanes (76%); mp 261–263 °C; ^{1}H NMR δ 7.91 (1H, dd, *J* 7.5 & 1.0, Ar–H3), 7.53 (1H, dt, *J* 7.5 & 1.0, Ar–H4), 7.48 (1H, dt, *J* 7.5 & 1.2, Ar–H5), 6.96 (1H, dd, *J* 7.5 & 1.2, Ar–H6), 6.94 (2H, d, *J* 8.9, H-1′,8′), 6.60–6.62 (4H, m, H-2′,4′,5′,7′), 3.85 (8H, t, *J* 4.8, OCH_2), 3.47 (2H, q, *J* 5.8, CH_2OH), 3.19–3.21 (8H, m, NCH_2), 3.06 (2H, t, *J* 6.6, SO_2NCH_2), 1.52 (1H, t, *J* 6.4, OH), 1.48 (2H, quintet, *J* 6.3, CH_2); HRMS (FAB), *m/z* Calcd for $(C_{30}H_{33}N_3O_6S + H)^+$: 564.2168. Found: 564.2194.

*2.3.5. 3′,6′-Di(4-morpholinyl)-2-(2-aminoethyl)spiro[1,2-benzisothiazole-3(2H),9′-[9H]xanthene]-1,1-dioxide (**12**)*

Pink glass (58%) which solidified when left in contact with Et_2O; ^{1}H NMR δ 7.91 (1H, dd, *J* 7.5 & 1.1, Ar–H3), 7.52 (1H, dt, *J* 7.5 & 1.1, Ar–H4), 7.48 (1H, dt, *J* 7.5 & 1.1, Ar–H5), 6.94–6.96 (3H, m, Ar–H6 & H-1′,8′), 6.60 (2H, dd, *J* 8.6 & 2.5, H-2′,7′), 6.59 (2H, d, *J* 2.5, H-4′,5′), 3.84 (8H, t, *J* 4.7, OCH_2), 3.19–3.21 (8H, m, NCH_2), 2.98 (2H, t, *J* 6.1, SO_2NCH_2), 2.55 (2H, t, *J* 6.1, CH_2NH_2), 1.47 (2H, br s, NH_2); HRMS (FAB), *m/z* Calcd for $(C_{29}H_{32}N_4O_5S + H)^+$: 549.2166. Found: 549.2156.

*2.3.6. 3′,6′-Di[N-(2-acetoxyethyl)-N-methylamino]-2-(3-hydroxypropyl)-spiro[1,2-benzisothiazole-3(2H)-9′-[9H]xanthene]-1,1-dioxide (**13**)*

Crystallised from EtOAc–hexanes (86%); mp 142–143.5 °C; ^{1}H NMR δ 7.90 (1H, dd, *J* 7.4 & 1.3, Ar–H3), 7.52 (1H, dt, *J* 7.4 & 1.3, Ar–H4), 7.48 (1H, dt, *J* 7.6 & 1.3, Ar–H5), 6.99 (1H, dd, *J* 7.6 & 1.3, Ar–H6), 6.88 (2H, d, *J* 8.7, H-1′,8′), 6.42–6.44 (4H, m, H-2′,4′,5′,7′), 4.24 (4H, t, *J* 6.0, CH_2OAc), 3.60 (4H, t, *J* 6.3, CH_2NMe), 3.46 (2H, t, *J* 6.3, CH_2OH), 3.05 (2H, t, *J* 6.3, SO_2NCH_2), 3.00 (6H, s, NMe), 2.02 (6H, s, OAc), 1.61 (1H, br s, OH), 1.47 (2H, quintet, *J* 6.3, CH_2CH_2OH); HRMS (ESI), *m/z* Calcd for $(C_{32}H_{37}N_3O_8S + H)^+$: 624.2380. Found: 624.2405.

2.4. Spectroscopic titrations

Stock solutions of sultams **8**–**13** (~1 mM in ethanol) were diluted 100-fold into buffers prepared from solutions of appropriate acids at 25 mM concentration in water–ethanol (3:1 v/v) and adjusted to the required pH values by addition of NaOH. pH values were determined with a glass electrode referenced to buffer solutions in 100% aqueous solution. The water–ethanol solvent is unlikely to cause more than 0.1 pH shift from that in water [12]. Acids used were citric acid (pH range 2.4–5.6), 2-(*N*-morpholino)ethanesulfonate (MES, pH range 6.0–6.5), 3-(*N*-morpholino)propanesulfonate (MOPS, pH range 7.0–7.5), *N*-(2-hydroxyethyl)piperazine-*N*′-3-propanesulfonate (EPPS, pH range 8.0–8.5), 2-(cyclohexylamino)-ethanesulfonic acid (CHES, pH range 9.0–9.5). Absorption values were measured at 567 nm and fitted to the Henderson–Hasselbach equation in Microsoft Excel™ to obtain pK_a values.

2.5. Flash photolysis of sultam **10**

A stock solution of **10** (~1 mM in ethanol) was diluted to 21 μM in 55 mM buffer solutions that were prepared as above in 3:1 v/v water–ethanol from MES (pH 6.02 and 6.55), MOPS (pH 7.03 and 7.51) or EPPS (pH 8.00 and 8.51). Solutions were irradiated at 4 °C in a quartz cell (path length 10 mm) using a Lambda Physik LPX 100 XeCl excimer laser producing 30 ns, 150 mJ pulses at 308 nm and focused with two orthogonally set cylindrical lenses. Absorbance data at 567 nm were obtained from a probe beam oriented at 90° with respect to the direction of the laser pulse. Typically, 10 records were averaged using a Luzchem model LFP-111 transient recorder and Tektronix digitizer (300 MHz bandwidth) and the kinetic data over the pH range studied were fitted to single exponentials using unweighted non-linear routines in Microsoft Excel™.

3. Results and discussion

Synthesis of the requisite sulforhodamines was readily achieved by heating sulfonefluorescein dichloride **3** with a four-fold molar excess of secondary amines in refluxing methanol, as previously described [8a]. At the start of our work, the sulforhodamines were converted to their sulfonyl chlorides using phosphoryl chloride alone [2] but we later found that addition of a little phosphorus pentachloride gave more efficient conversion (overnight at room temperature). In either case the crude reaction mixtures, i.e. still containing the phosphorus reagents, were diluted with chloroform and shaken with a large excess of the appropriate primary amine. This procedure avoided

isolation of sensitive sulfonyl chlorides and gave the sultams in unoptimised yields of 39–86%.

The choice of substituents on the amino groups of the xanthene was made to vary stereoelectronic effects and the variation of substituents on the sulfonamide of the di-morpholino compound was intended to probe the inductive effect operating though the carbon chain attached to this nitrogen atom. Fig. 5 shows an example of the spectrometric titrations (for compound **11**). The measured p*K* data for compounds **8**–**13** are summarised in Table 1, which also shows the absorption maxima for the parent sulforhodamines **4**–**7**. As implied by the equilibrium shown in Fig. 5, the transition for all compounds **8**–**13** is from a coloured form to a colourless one as the pH is made more alkaline. Previous workers [8a] have qualitatively described changes in the position of this equilibrium in sultams derived from a series of *N*,*N*′-diaryl sulforhodamines, but these compounds are not fluorescent [13]. In contrast, the aliphatic substituents on the compounds described here did not quench the fluorescence of the parent sulforhodamines or of the open form of the derived sultams.

The main observation from the data in Table 1 is that variation of groups on the xanthene had much greater effects on the apparent p*K* for the colour change than were caused by variations in the substituent on the sulfonamide nitrogen. For example, changing from the pyrrolidine groups of **8** to the piperidines of **9** caused the p*K* to drop by ~2 pH units with a further decrease of ~1.6 pH units for the analogous morpholino compound **11**. The equilibrium between the open and closed forms (Fig. 1) reflects the relative stabilities of the two forms and the difference between **8** and **9** most likely arises from a stereoelectronic factor. In the closed forms, the nitrogen atoms attached to the aromatic rings will tend to be pyramidal. In the open form, where the nitrogen lone pairs are extensively delocalised into the extended xanthylium system, the nitrogens will be sp^2 hybridised and planar. *Ab initio* computational studies of the ring and chain forms of a related rhodamine support this argument [11]. In going from the closed, colourless form to the open, coloured form, there is an energy cost for the alicyclic rings in flattening to accommodate the planar nitrogen atom. However, this will be lower for the pyrrolidine rings that are already much more nearly planar. The near-planarity of the pyrrolidine nitrogen atoms in models of both forms can be seen in X-ray crystallographic structures of the sulforhodamine **4** [11] (which models the open form) and its derived *N*-methylsultam [2] (closed form). In consequence, the relative energies of the open and closed forms for **8** are expected to be more closely balanced than those for **9**, i.e. implying that **8** has less propensity to close as the pH is raised than does **9**. In simple terms, the opening process could be regarded as a consequence of the extent of "push" by the amino nitrogens on the scissile C–N bond of the sulfonamide.

Table 1
Apparent p*K* values for the colour change of **8**–**13** and wavelength of the visible absorption maximum for the corresponding sulforhodamines

Sultams		Parent sulforhodamines	
Compound	Apparent p*K*	Compound	Absorption maximum (nm)[a]
8	8.04	**4**	561
9	5.97	**5**	567
10	4.58	**6**	552
11	4.35		
12	4.40		
13	6.39	**7**	549

[a] Spectra were measured for aqueous solutions.

In similar fashion, the ring oxygen in the morpholino compound **11** reduces the basicity of the amine by its inductive effect, further reducing the electronic "push" by the amino groups and favouring the closed tautomer. The strength of this inductive effect is evident from the relative p*K* values for morpholine and piperidine (8.5 and 11.3, respectively [14]): in contrast the p*K* of pyrrolidine (11.3 [14]) is essentially identical to that of piperidine, in line with the argument of stereoelectronic effects rather than simple differential basicity for the pyrrolidine and piperidine compounds. As we have studied only a single example with acyclic substituents on the amino nitrogen atoms, it is somewhat more difficult to rationalise the observed p*K* of **13**. Nevertheless, the inductively withdrawing acetoxy groups are probably the main cause of the shift compared to the value of 7.37 for sultam **1**.

The effects of the different substituents discussed above on the visible absorption spectra of the parent sulforhodamines **4**–**7** were also determined and results are shown in Table 1, as noted above. Surprisingly, there was little obvious correlation between the data for the paired sulforhodamines and their sultam derivatives, although within the sulforhodamines the effects to some extent follow logical trends. Thus the two compounds with inductively withdrawing substituents (**6** and **7**) absorb at shorter wavelengths than the pyrrolidine and piperidine compounds **4** and **5**. This could be expected from the reduced electron density on the amino nitrogens in **6** and **7**, which would reduce the electron delocalisation over the xanthylium system of these two compounds and cause a blue shift in the absorption spectrum. However, other factors must also

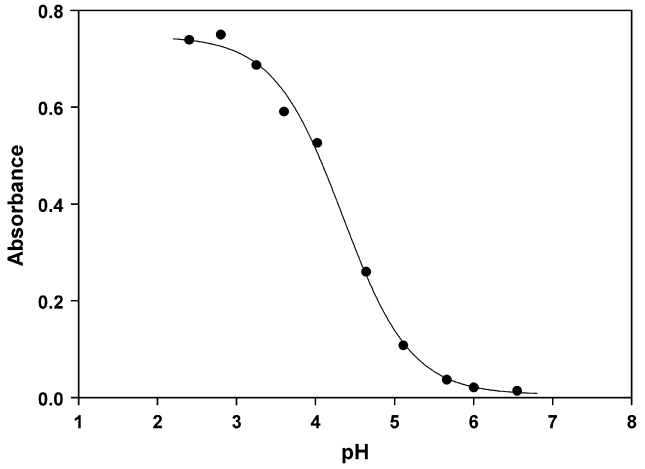

Fig. 5. pH titration of compound **11**, showing measured absorbance values (•) over the pH range and the fit (—) of these experimental points to the Henderson–Hasselbach equation.

contribute, given that **6** and **7** have almost identical visible spectra but that the p*K* values for their derived sultams **11** and **13**, respectively, differ by two pH units.

The effect of changes to substituents on the sulfonamide nitrogen was not so extensively investigated. The small difference between the hydroxyethyl and hydroxypropyl substituents in **10** and **11**, respectively, is in the expected direction, since the inductive effect of the hydroxy group will be attenuated by the additional methylene group in **11**. Thus, relative to **10**, the sulfonamide nitrogen could be expected to form a stronger C–N bond with the adjacent positive centre, so favouring the closed form. However, the p*K* value of 4.40 for the aminoethyl compound **12** was unexpected. The terminal amino group would be fully protonated at all pH values below at least 9, and so would be strongly electron-withdrawing by its inductive effect. This would be expected to weaken the C–N bond of the colourless form. On the other hand, the same effect will increase the acidity of the sulfonamide proton and thus increase the small concentration of sulfonamide ion present, so increasing the rate of the coloured-to-colourless process. In practice, the balance of kinetics and thermodynamics evidently favours the colourless form but the complex situation would require more detailed study to examine the subtleties of these interacting factors. Overall, the pragmatic result is that inductive changes in the substituent on the sulfonamide nitrogen have only a limited modulating effect on the p*K* for the colour change.

We briefly investigated the kinetics of the slow phase of ring-closure after flash photolytic opening of the sultam **10**, over the pH range 6–8.5 and the data are shown in Table 2. This compound was chosen as it had essentially no absorption in the visible region over the pH range studied, except at the lowest pH. In all cases, the data gave clean, single exponential transients, as exemplified in Fig. 6. Despite the substantial change in the apparent p*K* compared to that for sultam **1**, the rates of return to equilibrium were relatively unperturbed (factors in the range 0.55–3.7 relative to rates observed for **1** at comparable pH values). Without a full kinetic analysis of individual rate constants it is difficult to give a detailed rationalisation for the observed kinetics, but the trend to faster rates of return to the closed form as the pH increases is in line with trends in the kinetic model described elsewhere [2] for compound **1**. In this model, photolytic opening of the sultam form leads initially to a zwitterion with the coloured, positively charged rhodamine cation and the negatively charged sulfonamide anion. The sulfonamide anion then partitions between two pathways, either by direct reclosure to the starting sultam or protonation by solvent to give the neutral sulfonamide. Reclosure of the latter species is then controlled by the rate of deprotonation of the sulfonamide, a process that is pH-dependent. Thus the rate constant observed at the pH values of Table 2 becomes slower as the solution pH decreases. The rate constants are not directly related to pH because the ionisation is expected to involve both spontaneous and base-catalysed processes, as formulated by Eigen [15] for such proton-transfer reactions.

Table 2
Effects of pH on the kinetics of ring-closure after photolytic ring-opening of **10**

pH	k_{obs} [a]
8.51	3570
8.00	1230
7.51	548
7.03	313
6.55	91
6.02	66

[a] Rate constants are the mean of two determinations at each pH value.

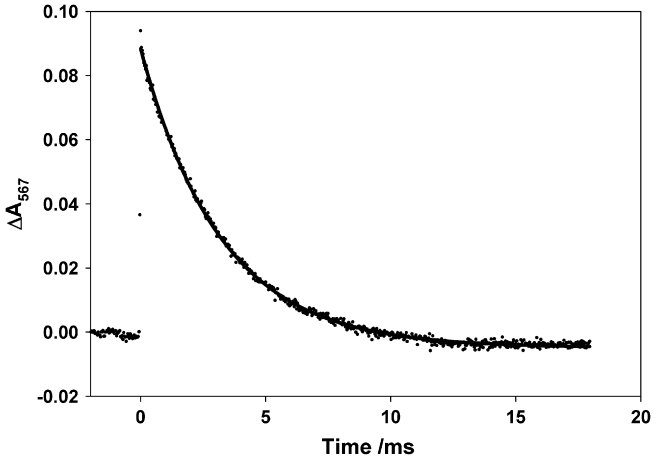

Fig. 6. Kinetics of the absorption change of **10** at pH 7.03 following laser pulse irradiation. The absorbance data (···) are overlaid by a best-fit single exponential (—).

4. Conclusion

This work demonstrates that major modulations to the apparent p*K* for the coloured-to-colourless transition of rhodamine sultams are best effected by changes to the amino group substituents. Changes to these substituents appear to have more predictable effects than the rather small changes caused by modifications to the sulfonamide substituent. We do not anticipate making further studies in this area ourselves but hope that the results presented here and elsewhere [2] may stimulate others to investigate and exploit the chemistry of the ring-chain interconversion of these sultams.

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References

[1] Corrie JET, Davis CT, Eccleston JF. Bioconjug Chem 2001;12:186–94.
[2] Corrie JET, Eccleston JF, Ferenczi MA, Moore MH, Turkenburg JP, Trentham DR. J Phys Org Chem, in press, doi:10.1002/poc.1318.
[3] (a) Ramette RW, Sandell EB. J Am Chem Soc 1956;78:872–4878;
(b) Mchedlov-Petrossyan NO. Russ J Phys Chem 1981;55:976–8;

(c) Zorov NB, Golovina AP, Alimarin IP, Khvatkova ZM. Russ J Anal Chem 1971;26:1466–70;
(d) Mchedlov-Petrossyan NO, Shapovalov SA, Egorova SI, Kleshchevnikova VN, Arias Cordova E. Dyes Pigm 1995;28:7–18.
[4] Marchesini S, Gatt S, Agmon V, Guidici ML, Monti E. Biochem Int 1992;27:545–50.
[5] (a) Knauer KH, Gleiter R. Angew Chem Int Edn Engl 1977;16:113;
(b) Willwohl H, Wolfrum J, Gleiter R. Laser Chem 1989; 10:63–72.
[6] Fölling J, Belov V, Kunetsky R, Medda R, Schönle A, Egner A, et al. Angew Chem Int Edn Engl 2007;46:6226–70.
[7] (a) Borror AL, Ellis EW, McGowan DA. European Patent 174,054;
(b) Borror AL, Ellis EW. PCT Patent 86/07313;
(c) Ellis EW. US Patent 5,393,514.
[8] (a) Foley JW, Locatell L, Zepp CM. US Patent 4,258,118;
(b) Carlier PM, Filosa MP, Lockshin MP. US Patent 5,187,282.
[9] Pitner JB, Hoke RA. US Patent 5,393,514.
[10] Orndorff WR, Vose RS. J Am Chem Soc 1924;46:1896–912.
[11] Cavallo L, Moore MH, Corrie JET, Fraternali F. J Phys Chem A 2004;108:7744–51.
[12] Bates RG. Determination of pH. Theory and practice. New York: Wiley; 1964 [chapter 8, p. 201–29].
[13] Haugland RP, editor. Data on QSY dyes. A guide to fluorescent probes and labeling technologies. 10th ed. Invitrogen Corp.; 2005. p. 73–4.
[14] Smith RM, Martell AE. Critical stability constants, vol. 2. New York: Plenum; 1975. p. 73, 79 and 81.
[15] Eigen M. Proton transfer, acid–base catalysis, and enzymatic hydrolysis. Part I: elementary processes. Angew Chem Int Edn Engl 1964;3:1–19.