Scalable Synthesis of Lissamine Rhodamine B Sulfonyl Chloride and Incorporation of Xanthene Derivatives onto Polymer Supports

Hua Yang,[a] Sundar Vasudevan,[b] Christopher O. Oriakhi,[b] Jay Shields,[b] Rich G. Carter*[a]

[a] Department of Chemistry, Oregon State University, 153 Gilbert Hall, Corvallis, OR 97331, USA
Fax +1(541)7379496; E-mail: rich.carter@oregonstate.edu

[b] Hewlett–Packard Corporation, 1000 NE Circle Blvd, Corvallis, OR 97330, USA

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Abstract: A scalable synthetic route to lissamine rhodamine B sulfonyl chloride has been developed and a series of monomeric derivatives of this xanthene dye have been synthesized. Their subsequent incorporation into polymer supports has been accomplished and led to improved thermal stability for the conjugates as compared to the free dye.

Key words: heterocycles, polymers, dyes

Lissamine rhodamine B (Acid Red 52, C.I. 45100) has been extensively used in the materials and biological communities as a fluorescent and photoluminescent tag.[1,2] While xanthene **1** is commercially available from Acros Chemicals, the cost of even modest amounts of this material is quite high.[3] Interestingly, no reliable synthetic procedure for its production has been reported in the chemical literature. The synthesis of new xanthene dyes is also an area of considerable importance for the printing and fabric industries.[4] In many cases, a trade-off between improved stability and color intensity exists due to the inherent requirements for each property. For example, xanthene dyes such as **2** are among the most chromatic magenta dyes but they are also among the most fugitive – particularly in regard to thermal stability.[5] One intriguing possibility through which to address these limitations was the incorporation of structurally modified xanthene dye compounds into a polymer support. The linkage of the dye via a covalent bond to the polymer backbone should increase the overall stability of the dye by minimizing degradation pathways. Such covalent bonding of the dye derivative to a polymer matrix might provide better protection of the chromophore than simple mixing of the dye with a polymer. In the former case, the protective polymer would be in intimate contact with the dye molecules, whereas in the latter case, the dye molecules might separate from the protective polymer once the colorant is applied onto media such as paper and fabric. Consequently, we required a low cost and scalable route to synthesize the sulfonyl chloride **1**. In this paper, we describe the successful development of a scalable route to chloride **1**, derivatization to a series of xanthene dye monomers and their successful conjugation to polymers.

One possible cost-effective route would be via the readily available sodium salt **2**.[6] The chlorination of the *p*-sulfonate should be favored over the more sterically congested *o*-sulfonate moiety. Unfortunately, initial screening of a series of traditional reagents for this transformation, such as $SOCl_2$[7] and $POCl_3$,[8] gave disappointing results. After considerable experimentation, we discovered that use of oxalyl chloride (**3**) with catalytic amounts of dimethylformamide (DMF)[9] (Scheme 1) led to clean conversion of the sodium salt **2** to the sulfonyl chloride **1** in excellent yield. We have prepared 100 grams of the product **1** via this oxalyl chloride route.

With a viable route now in hand for the synthesis of the key intermediate **1**, the subsequent derivatization of **1** was explored (Scheme 2). Treatment of the sulfonyl chloride **1** with the acrylate derivative **4**, in the presence of triethylamine, led to the clean formation of sulfonamide **5** in good yield. A similar transformation could be accomplished with the acrylamide analogue **6**, to yield the adduct **7** in 66% yield. The use of allyl amine **8** as the nucleophile was

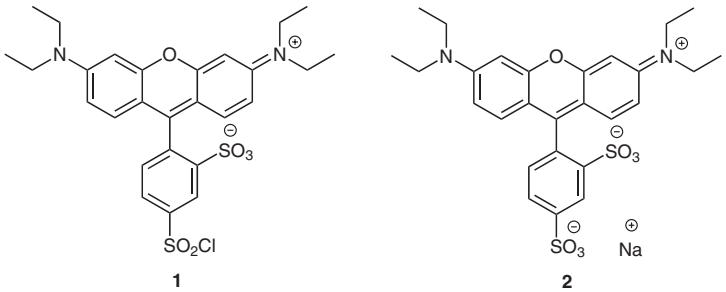

Figure 1 Xanthene dyes

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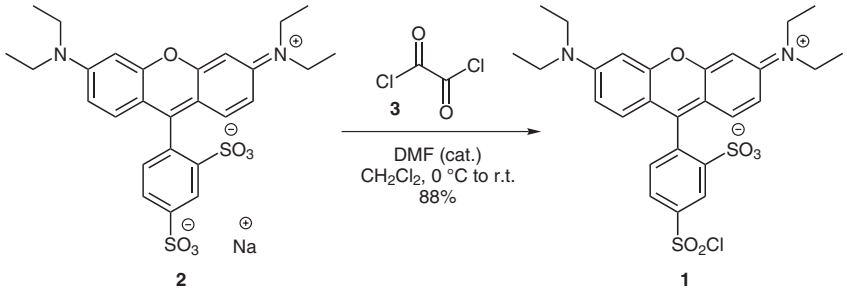
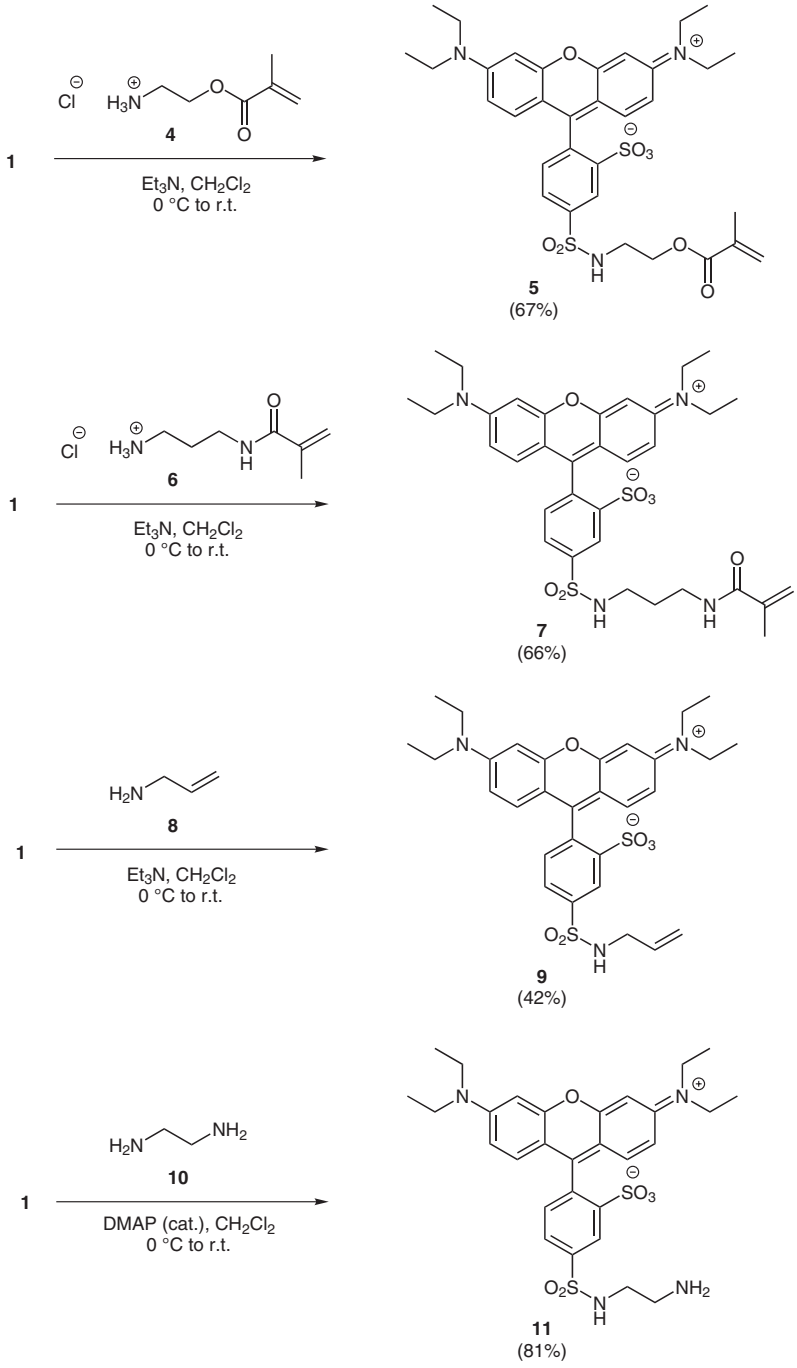


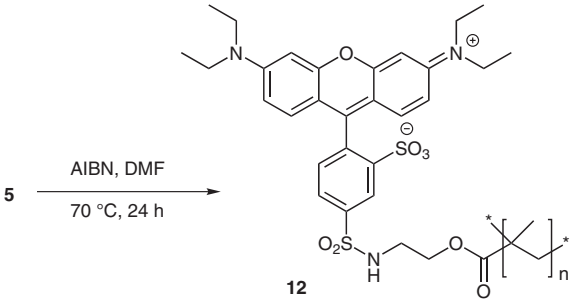
Scheme 1 Scalable synthesis of sulfonyl chloride **1**



Scheme 2 Representative derivatizations of sulfonyl chloride **1**

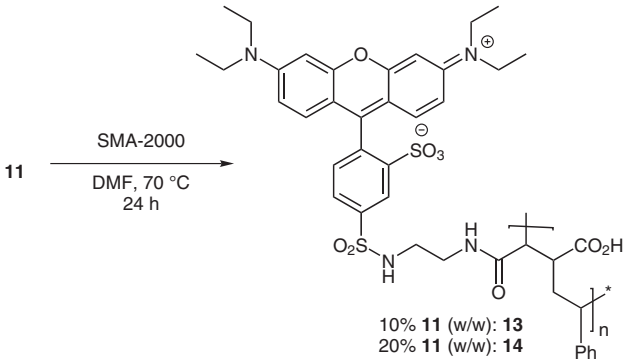
also explored; this yielded allyl sulfonamide **9** in a modest 42% yield. Selective monosulfonylation of the diamine **10** was feasible in excellent yield (81%). It is important to note that the dye monomers were prepared as single isomers and no additional electrolyte contaminants appeared to be present.

Next, attention was turned to the incorporation of the synthesized rhodamine derivatives into a polymer matrix (Scheme 3). Treatment of acrylate derivative **5** under standard polymerization conditions (AIBN, DMF, 70 °C, 24 h), led to clean formation of the polymerized dye **12** [PDI = 1.4, M_w = 52,400]. While formation of this dye had occurred cleanly, the polymer **12** proved only slightly soluble in aqueous media. A similar outcome was observed with co-polymerization of **5** with methyl methacrylate and/or acrylic acid. Only at high acrylic acid loadings could some reasonable solubility in aqueous media be observed; however, the level of solubility was insufficient for subsequent experiments. Interestingly, attempted polymerization of the acrylamide derivative **7** under identical conditions (AIBN, DMF, 70 °C, 24 h), led to no observed polymer formation. Co-polymerization of **7** with other monomers was not attempted based on this initial result from the homo-polymerization experiment.



Scheme 3 Polymerization of methacrylate derivatives

The primary amine dye derivative **11** offered alternate strategies for its attachment to a polymer (Scheme 4). The possibility of attaching this dye derivative to a preformed polymer matrix containing a suitably reactive electrophilic group such as an anhydride was particularly interesting. In addition, any remaining anhydride moiety could be saponified under basic conditions to improve the water solubility of the dye–polymer conjugate. The polymer that was selected for study was styrene/maleic anhydride resin developed by Sartomer (codenamed SMA-2000). Heating of the amine **11** with SMA-2000 in DMF at 70 °C (24 h), led to clean incorporation of the dye onto the resin. After precipitation by addition of water, the dye was washed with ice-cold hydrochloric acid and water with minimal loss of color from the solid. Dye loading ratios of 10% and 20% (w/w) were obtained by this process, to yield conjugates **13** and **14** respectively. UV/Vis data for compounds **13** and **14**, as provided in the experimental section, were similar to those of the monomeric dye **11**.



Scheme 4 Polymer incorporation of amine **11**

These dye–polymer conjugates were first analyzed using Gel Permeation Chromatography (GPC). The polydispersity index (PDI = M_W/M_N) of the starting SMA-2000 was reported to be 2.5. The PDI for the 20% dye/SMA-2000 conjugate **14** was shown to be 3.67, indicating a non-uniform incorporation of the dye onto the polymer. The median molecular weight of the conjugate was determined to be 11482 mAU. In addition, only 1.8% of free dye was detected in the polymer. TGA analysis of the amine **11** indicated that a sizable mass loss was detected at 190 °C and complete decomposition was observed at 350 °C. A similar pattern was observed with the parent dye **1**. More importantly, TGA analysis of the conjugate **14** did *not* yield significant loss at approximately 190 °C; instead, no decomposition was observed until approximately 370 °C. This result is similar to that observed for the unmodified SMA-2000 resin. A similar result was observed using isothermal TGA at 170 °C for 12 hours. This data appears to support the conclusion that the polymer successfully stabilizes the dye from decomposition.

In conclusion, a viable route for the synthesis of sulfonyl chloride **1** has been developed. This key intermediate has been converted into a series of novel dye monomers **5**, **7**, **9** and **11**. The subsequent polymerization has been accomplished with the methacrylate derivative **5**. In addition, the amine **11** has been successfully attached to commercially available SMA-2000, with up to 20% dye loading. TGA of the dye–polymer conjugate **14** indicates that the polymer is able to stabilize the dye from thermal decomposition.

Melting points were measured on a Mel-Temp apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet Nexus 470 FT-IR spectrophotometer, as neat samples unless otherwise indicated. ^{1}H NMR spectra were recorded on a Brüker 300 spectrometer at 300 MHz or a Brüker 400 spectrometer at 400 MHz in the indicated solvent and are reported in ppm relative to TMS and referenced internally to the residually protonated solvent. ^{13}C NMR spectra were recorded on Brüker 300 spectrometer at 75 MHz or a Brüker 400 spectrometer at 100 MHz in the solvent indicated and are reported in ppm relative to TMS and referenced internally to the residually protonated solvent. Absorbance spectra were measured using a Cary 50 UV-Vis spectrophotometer; samples were dissolved in 15% 2-pyrrolidone and 1.25% KOH to give a 1% stock solution that was further diluted with deionized H_2O as necessary for absorbance

measurements. Thermogravimetric analyses were carried out on a TGA2950 from TGA Instruments.

Sulfonyl Chloride (1)

To a stirred solution of Acid red 52 sodium salt (**2**; 49.5 g, 85.3 mmol) in CH_2Cl_2 (600 mL) at 0 °C, was added, sequentially, oxalyl chloride (**3**; 54.1 g, 36.6 mL, 0.426 mol) slowly and DMF (1.02 mL). The resulting mixture was stirred at r.t. for 16 h then the reaction was concentrated in vacuo. To the slurry, benzene (500 mL) was added and the solvent was removed in vacuo. Et_2O (400 mL) was added to the resultant solid, which was filtered and washed with EtOAc (100 mL) and dried under vacuum to yield **1**.

Yield: 43.4 g (88%); pink solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.27 (s, 1 H), 7.73 (d, *J* = 7.5 Hz, 1 H), 7.15 (d, *J* = 7.8 Hz, 1 H), 7.05–6.91 (m, 5 H), 3.75–3.55 (m, 8 H), 1.34–1.10 (m, 12 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 160.0, 157.6, 155.4, 149.5, 147.0, 133.3, 129.5, 129.4, 126.0, 125.6, 114.2, 113.9, 95.7, 45.7, 12.9.

Methacrylate (5)

To a stirred solution of sulfonyl chloride **1** (85.0 g, 147 mmol) in CH_2Cl_2 (735 mL) at 0 °C was added, sequentially, 2-aminoethyl methacrylate hydrochloride (29.2 g, 177 mmol) and DMAP (0.899 g, 7.37 mmol). Et_3N (38.3 g, 53 mL, 295 mmol) was added dropwise and the reaction was allowed to warm to r.t. After 13 h, the reaction was concentrated in vacuo and the resulting solid was washed with EtOAc to give **5**.

Yield: 65.9 g (67%); pink solid; mp 258 °C (dec.).

IR (KBr): 2968, 1647, 1596, 1527, 1471, 1419, 1342, 1277, 1247, 1183, 1075, 916 cm^{-1}.

^{1}H NMR (300 MHz, CD_3OD): δ = 8.67 (s, 1 H), 8.13 (dd, *J* = 7.8, 1.8 Hz, 1 H), 7.52 (d, *J* = 7.8 Hz, 1 H), 7.17–6.95 (m, 6 H), 6.18 (s, 1 H), 5.66 (s, 1 H), 4.19 (t, *J* = 5.7 Hz, 1 H), 3.75–3.65 (m, 8 H), 3.40–3.36 (m, 2 H), 1.96 (s, 3 H), 1.34–1.30 (m, 12 H).

^{13}C NMR (75 MHz, CD_3OD): δ = 167.2, 158.0, 156.4, 155.8, 145.8, 142.9, 136.0, 134.1, 132.3, 131.2, 127.8, 126.2, 125.4, 113.9, 113.6, 95.6, 63.1, 45.4, 41.6, 17.0, 11.4.

HRMS (ES, +ve): *m*/*z* [M + H]$^+$ calcd for $C_{33}H_{40}N_3O_8S_2$: 670.2257; found: 670.2236.

UV/Vis: λ_{max} (ε) = 567 nm (44500) (shoulder around 530 nm).

Methacrylamide (7)

To a stirred solution of sulfonyl chloride **1** (209.4 mg, 0.363 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added, sequentially, *N*-(3-aminopropyl)methacrylamide hydrochloride (65 mg, 0.363 mmol) and Et_3N (0.109 g, 0.15 mL, 1.07 mmol). The reaction was allowed to warm to r.t. and, after 13 h, the reaction was concentrated in vacuo and purified by chromatography over silica gel column (MeOH–CH_2Cl_2, 1→5%) to give **7**.

Yield: 0.162 g (65%); pink solid; mp 260 °C (dec.).

IR (KBr): 2976, 2942, 2619, 1651, 1596, 1475, 1338, 1174, 1075, 920, 680 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.45 (s, 1 H), 7.96–7.90 (m, 3 H), 7.49 (d, *J* = 8.0 Hz, 1 H), 7.06–6.96 (m, 4 H), 5.65 (s, 1 H), 5.32 (s, 1 H), 3.67–3.65 (m, 8 H), 3.18–3.13 (m, 2 H), 2.94–2.89 (m, 2 H), 1.86 (s, 3 H), 1.68–1.64 (m, 2 H), 1.25–1.22 (m, 12 H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 168.0, 158.1, 157.6, 155.5, 148.6, 142.0, 140.5, 133.5, 133.2, 131.1, 126.9, 126.2, 119.3, 114.1, 95.9, 45.7, 37.0, 29.8, 19.1, 13.0.

HRMS (ES, +ve): *m*/*z* [M + H]$^+$ calcd for $C_{34}H_{43}N_4O_7S_2$: 683.2573; found: 683.2537.

Allyl Sulfonamide (9)

To a stirred solution of sulfonyl chloride **1** (0.363 g, 0.628 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added, sequentially, allylamine (39.6 mg, 52 μL, 0.693 mmol) and Et_3N (0.176 mL). The reaction was allowed to warm to r.t. and, after 16 h, the reaction was concentrated in vacuo and purified by chromatography over silica gel column (MeOH–CH_2Cl_2, 1→5%) to give major isomer **9**.

Yield: 0.158 g (42%); mp 256 °C (dec.).

IR (KBr): 3070, 2981, 2929, 1647, 1591, 1531, 1419, 1342, 1282, 1178, 1071, 1024, 920, 860, 684 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.45 (s, 1 H), 8.12 (t, *J* = 6.0 Hz, 1 H), 7.95 (dd, *J* = 8.0, 2.0 Hz, 1 H), 7.48 (d, *J* = 8.0 Hz, 1 H), 7.09–6.95 (m, 7 H), 5.80–5.72 (m, 1 H), 5.23–5.10 (m, 2 H), 3.71–3.58 (m, 10 H), 1.25–1.22 (m, 12 H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 158.1, 157.6, 155.6, 148.6, 142.2, 134.5, 133.5, 133.2, 131.0, 127.0, 126.3, 117.1, 114.2, 114.0, 95.9, 45.7, 13.0.

HRMS (ES, +ve): *m*/*z* [M + H]$^+$ calcd for $C_{30}H_{36}N_3O_6S_2$: 598.2046; found: 598.2020.

Amine (11)

To a stirred solution of ethylenediamine (2.50 g, 2.80 mL, 41.6 mmol) in CH_2Cl_2 (250 mL) at 0 °C was added, sequentially, Et_3N (1.40 g, 1.93 mL, 13.9 mmol) and DMAP (0.250 g, 2.05 mmol). Then, sulfonyl chloride **1** (8.00 g, 13.9 mmol) was added over 2 h. The reaction was allowed to warm to r.t. then, after 13 h, the reaction was concentrated in vacuo and the resulting solid was washed with CH_2Cl_2–EtOAc (1:2) to give amine **11**.

Yield: 6.74 g (81%); pink solid; mp 248 °C (dec.).

IR (KBr): 2968, 2912, 1647, 1587, 1470, 1415, 1338, 1252, 1174, 1080, 684 cm^{-1}.

^{1}H NMR (300 MHz, CD_3OD): δ = 8.67 (s, 1 H), 8.17 (d, *J* = 8.1 Hz, 1 H), 7.58 (d, *J* = 7.8 Hz, 1 H), 7.14–6.96 (m, 6 H), 6.18 (s, 1 H), 5.66 (s, 1 H), 4.19 (t, *J* = 5.7 Hz, 1 H), 3.73–3.66 (m, 8 H), 3.51–2.97 (m, 4 H), 1.40–1.15 (m, 12 H).

^{13}C NMR (75 MHz, CD_3OD): δ = 158.0, 155.8, 145.9, 141.6, 132.2, 131.4, 128.0, 126.4, 113.9, 113.6, 95.6, 45.4, 44.0, 39.6, 11.4.

HRMS (ES, +ve): *m*/*z* [M + H]$^+$ calcd for $C_{29}H_{37}N_4O_6S_2$: 601.2155; found: 601.2124.

UV/Vis: λ_{max} (ε) = 566 (39400), 534 nm (32500; minor peak).

Monomeric Polymer (12)

To a stirred solution of methacrylate **5** (2.12 g, 3.16 mmol) in DMF (32 mL) was added AIBN (38 mg, 0.232 mmol). The reaction was heated at 70 °C for 24 h then cooled to r.t. and poured into EtOAc (120 mL) at 0 °C. The solid was filtered and washed with EtOAc (200 mL), CH_2Cl_2 (200 mL) and dried in vacuo to yield the polymer **12**.

Yield: 1.71 g (81%); pink solid.

IR (KBr): 2981, 2938, 2675, 1647, 1591, 1475, 1337, 1183, 1071, 1032, 920, 804, 675 cm^{-1}.

^{1}H NMR (300 MHz, CD_3OD): δ = 8.67 (s, 1 H), 8.12–7.99 (m, 1 H), 7.51–7.49 (m, 1 H), 7.12–6.95 (m, 5 H), 4.13–4.11 (m, 2 H), 3.70–3.68 (m, 8 H), 1.45–1.30 (m, 12 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 161.9, 157.6, 155.5, 148.5, 147.1, 142.2, 133.6, 133.4, 133.2, 131.2, 127.0, 126.7, 126.1, 125.7, 114.1, 114.0, 95.9, 63.0, 45.8, 17.2, 13.0.

UV/ Vis: λ_{max} = 567 nm (shoulder around 530 nm).

Dye Polymer Conjugate (14)

To a solution of DMF (200 mL) was added SMA 2000 (32 g) under Argon. The reaction mixture was then heated at 50 °C to give a clear solution. To this solution was added, sequentially, amine **11** (8.0 g, 13.3 mmol), DMAP (0.16 g, 1.31 mmol) and Et_3N (2.69 g, 26.6 mmol). The reaction was heated at 70 °C for 13 h then the reaction was cooled to r.t. and poured into ice-cold aq HCl (1 N, 200 mL). The solid was filtered and washed with ice-cold aq HCl (1 N, 200 mL), deionized H_2O (400 mL) and dried in vacuo to yield the dye–polymer conjugate **14**.

Yield: 34 g (85%); pink solid.

IR (KBr): 3411, 3058, 3028, 2929, 1776, 1733, 1647, 1587, 1497, 1454, 1342, 1178, 1067, 916, 761, 701 cm^{-1}.

^1H NMR (300 MHz, CD_3OD): δ (partial signals) = 8.40 (s), 7.22–6.93 (m), 3.70–3.55 (m), 1.21–1.16 (m).

UV/ Vis: λ_{max} = 571 nm (minor peak at 536 nm).

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