JOC Article

Solid-Phase Methods for the Synthesis of Cyanine Dyes

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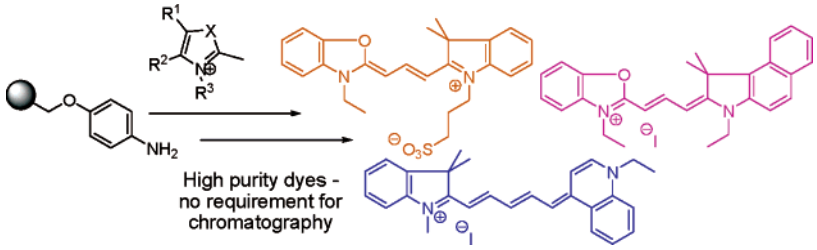
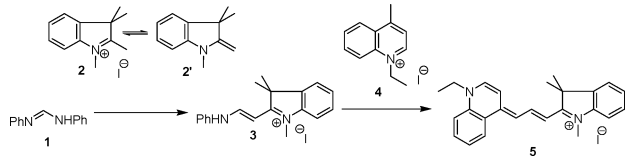


We report here a series of studies that explore solid-phase methodologies for the synthesis of various cyanine dyes. The scope of the previously reported catch-and-release method using sulfonyl chloride resin[1] has now been extended to include pentamethine and water-soluble cyanine dyes. We also report a new and chemically distinct synthetic strategy, employing the stepwise attack of heterocyclic carbon nucleophiles on immobilized polyene-chain precursors, allowing the clean synthesis of hydrophobic and hydrophilic trimethine and pentamethine dyes from more easily obtained starting materials. Overall, both approaches appear to be robust and versatile strategies to delivering a wide range of cyanine-based dyes in high purity.

Introduction

While solid-phase organic synthesis and combinatorial chemistry are commonly used for the synthesis of peptides[2] and druglike molecules,[3] such techniques have found broader applications in areas such as material science and catalysis. We have been interested in the application of solid-phase chemistry for the synthesis and discovery chemistry of fluorescent organic dyes. There have been a number of recent examples of solid-phase synthetic routes to dyes.[4] The cyanine family of dyes (e.g., **5**, Scheme 1) are of particular interest[5] due to their relative stability, high molar extinction coefficients, high fluorescence intensity, and narrow spectrum width. They comprise an important class of compounds having many applications, including photography,[6] optical data storage[7] and more recently biomolecular labeling[8] and proteomics.[9]

SCHEME 1. General Synthesis of a Cyanine Dye



Synthesis of cyanine dyes is generally accomplished by the stepwise reaction of nucleophilic heterocycles such as **2** and **4** with a polyene-chain precursor such as amidine **1**. The quaternized heterocycles behave as

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(1) Mason, S. J.; Balasubramanian, S. *Org. Lett.* **2002**, *4*, 4261−4264.

(2) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149−2154.

(3) Franzén, R. G. *J. Comb. Chem.* **2000**, *2*, 195−214.

(4) (a) Isacsson J.; Westman, G. *Tetrahedron Lett.* **2001**, *42*, 3207−3210. (b) Calderelli, M.; Baxendale, I. R.; Ley, S. V. *Green Chem.* **2000**, *2,* 43−45. (c) Merrington, J.; James, M.; Bradley, M. *Chem Commun.* **2002**, *33*, 140−141.

(5) Hamer, F. M. In *The Cyanine Dyes and Related Compounds*; Weissberger, A., Ed.; Interscience: New York, London, 1964.

(6) James, T. H. *Adv. Photochem.* **1986**, *13*, 329−425.

(7) Spitler, M. T.; Ehret, A.; Kietzmann, R.; Willig, F. *J. Phys. Chem. B* **1997**, *101*, 2552−2557.

(8) Mujumdar, R. B.; Ernst, L. A.; Mujumdar, S. R.; Lewis, C. J.; Waggoner, A. S. *Bioconjugate Chem.* **1993**, *4*, 105−111.

(9) Tonge, R.; Shaw, J.; Middleton, B.; Rowlinson, R.; Rayner, S.; Young, J.; Pognan, F.; Hawkins, E.; Currie, I.; Davison, M. *Proteomics* **2001**, *1*, 377−396.

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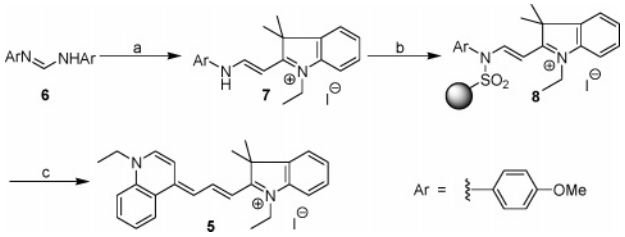
SCHEME 2. Catch-and-Release Synthesis of Trimethine Cyanine Dye[a]



[a] Reagents and conditions: (a) **2**, EtOH, triethylorthoformate, reflux; (b) dichloromethane (DCM), diisopropylethylamine (DIEA), polystyrene (PS)-SO_2Cl; (c) **4**, pyridine, DIEA.

nucleophiles in their methylene base form, **2′** (Scheme 1). Isolation of intermediate hemicyanine **3** is vital for the synthesis of unsymmetrical dyes in which the two heterocycles differ. However, purification of such intermediates and the final dye products is a particular problem in the synthesis of these dyes, often requiring nontrivial chromatographic separation of similar compounds. The use of solid-phase techniques to cleanly synthesize cyanine dyes with minimal purification is attractive and two approaches have been described in the literature.[1,4] However, both published strategies require significant solution-phase synthesis and purification of dye precursors. A more direct means of synthesizing combinatorial arrays of these dyes in high purity from easily available components would be desirable for future discovery chemistry.

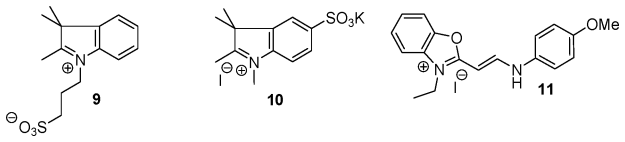
In our previously published strategy,[1] hemicyanine **7** was synthesized from the reaction of heterocycle **2** with amidine **6**. Hemicyanine intermediates such as **7** are relatively unreactive at room temperature but can be activated by functionalization of the aniline nitrogen with an electron-withdrawing group, for example, by acetylation or sulfonylation.[10,11] By reaction of hemicyanine **7** with sulfonyl chloride polystyrene, we demonstrated its activation via concurrent immobilization onto the solid phase (Scheme 2).

In the subsequent step immobilized activated hemicyanine **8** is reacted with a substoichiometric quantity of a second heterocycle such as **4** (0.3 equiv), so that all **4** is consumed. Unreacted hemicyanine would remain on the resin and could be removed by filtration to leave, after an aqueous wash to remove salt byproduct, pure trimethine cyanine dye in solution. Products were analyzed by HPLC with UV detection and evaporative light-scattering detection (ELSD) to obtain purity values independent of UV/vis extinction coefficients.[12]

In the work presented here, we show that this method can be extended to include water soluble dyes using sulfonated heterocycles, and furthermore that the approach can be employed for the synthesis of a pentamethine dye, using an extended hemicyanine. We also report a new strategy to prepare trimethine cyanines without the need for initial solution-phase synthesis of hemicyanines, but rather using the quaternized heterocycles, yielding dyes of high purity. This second route could also be used to prepare pentamethine dyes.

Results and Discussion

Water-Soluble Dyes. We first sought to explore whether the polymer-supported sulfonyl chloride methodology was suitable for the synthesis of water-soluble dyes. Water solubility is required for dyes that are to be used in biological applications, for example, the labeling of proteins[8,13,14] and DNA.[15,16] Solubility can be achieved by appending a charged solubilizing functional group to one or both heterocyclic halves of a dye. Sulfonated heterocycles are investigated in our current work. Heterocycles **9** and **10** were synthesized by modification of literature procedures.[13]

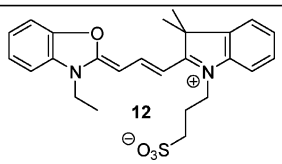
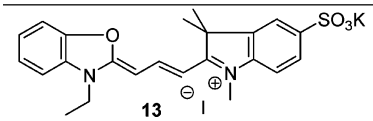


Hemicyanine **11** was loaded onto sulfonyl chloride resin using our published method[1]. Reaction of 0.3 equiv of heterocycles **9** and **10** in pyridine/DIEA yielded dyes that were contaminated only with the DIEA salt formed during the reaction. As aqueous extraction of the salt is not possible with a water-soluble dye, the salt was completely avoided by addition of 3.8 equiv of polymer-supported DIEA to the cleavage reaction. Any salts formed are now bound to resin and are removed by filtration. A summary of the data of sulfonated dyes **12** and **13** is shown in Table 1.

Imidate Route to Trimethine Cyanine Dyes. As an alternative route to trimethine dyes, we set out to design and synthesize an electrophilic single-carbon synthon of the form **14** (Scheme 3) bearing two orthogonal leaving groups (X and Y), of which one is connected to the polymer support. By judicious choice of these leaving groups, reaction with a solution-phase heterocycle **2** would selectively lead to formation of a substituted intermediate **16**. Should any of the less favored leaving group (resin-bound X) leave from the proposed tetrahedral intermediate **15**, the resulting byproduct would be automatically cleaved into solution and may be removed by filtration. Similarly, any symmetrical impurity formed by reaction of **14** with two equivalents of nucleophile would be automatically cleaved from the resin. Subsequent reaction of intermediate **16** with a substoichiometric quantity of a second nucleophile **4** would cleave pure unsymmetrical product **5** into solution, allowing facile purification by filtration.

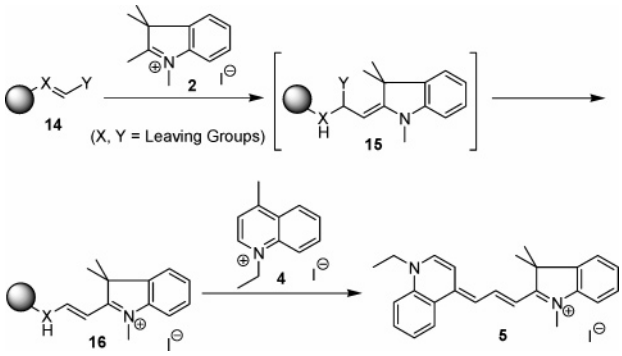
(10) Brooker, L. G. S.; White, F. L.; Keyes, G. H.; Smyth, E. P.; Oesper, P. F. *J. Am. Chem. Soc.* **1941**, *63*, 3192−3203.
(11) Peng, Z.; Geise, H. J.; Zhou, X.; Peng, B.; Carleer, R.; Dommisse, R. *Liebigs Ann./Recl.* **1997**, 27−33.
(12) Mourey, T. H.; Oppenheimer, L. E. *Anal. Chem*. **1994**, *56*, 2427−2434.
(13) Mujumdar, R. B.; Ernst, L. A.; Mujumdar, S. R.; Waggoner, A. S. *Cytometry* **1989**, *10*, 11−19.
(14) (a) Lipowska, M.; Patonay, G.; Strekowski, L. *Synth. Commun.* **1993**, *23*, 3087−3094. (b) Gruber, H. J.; Hahn, C. D.; Kada, G.; Reiner, C. K.; Harms, G. S.; Ahrer, W.; Dax, T. G.; Knaus, H.-G. *Bioconjugate Chem.* **2000**, *11*, 696−704. (c) Lin, Y.; Weissleder, R.; Tung, C.-H. *Bioconjugate Chem.* **2002**, *13*, 605−610.
(15) Ying, L.; Green, J. J.; Li, H.; Klenerman, D.; Balsubramanian, S. *Proc. Nat. Acad. Sci. U.S.A.* **2003**, *100*, 14629−14634.
(16) McKinney, S. A.; Tan, E.; Wilson, T. J.; Nahas, M. K.; Déclais, A.-C.; Clegg, R. M.; Lilley, D. M. J.; Ha, T. *Biochem. Soc. Trans.* **2004**, *32*, 41−45.

TABLE 1. Results of Sulfonated Trimethine Dye Synthesis

Compound	Purity[a]	Yield[b]	λ_{abs} / nm[c]	ε/ $M^{-1}cm^{-1}$	λ_{em} / nm[c]
 **12**	63%	51%	510	7.2×10^4	544
 **13**	85%	42%	507	8.6×10^4	543

[a] Determined by HPLC with ELSD quantitation. [b] Crude yield (based on amount of heterocycle in cleavage step). [c] Taken in MeOH.

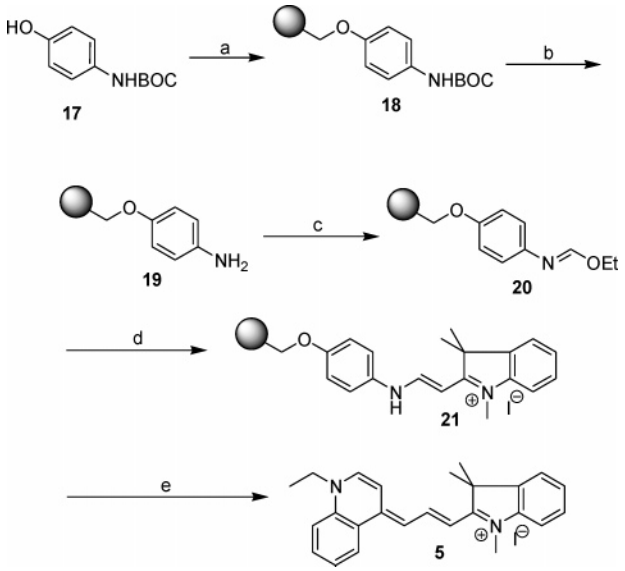
SCHEME 3. Cyanine Dye Synthesis from Immobilized Single-Carbon Synthon



Furth et al. have described an immobilized amidine linker,[17] which would be expected to react with **2** to form a suitable hemicyanine intermediate **21** as the kinetic product, by loss of dimethylamine in preference to a less basic resin-bound aniline. However, the work of Oszczapowicz et al. suggests that rapid re-attack of this lost aliphatic amine would occur, leading to the cleavage of hemicyanine into solution.[18] Instead, polystyrene-bound imidate **20** (Scheme 4) was chosen as a suitable immobilized single-carbon synthon for this synthesis. Okuyama et al. have shown that, in the absence of acid, hydrolysis of analogous electron-rich imidates is by preferential loss of alcohol to form amides.[19] Indeed, in the work of Knott,[20] reaction of a solution-phase analogue of **20** with heterocyclic carbon nucleophiles results in formation of hemicyanines by selective loss of ethanol. Once formed, we anticipated activating **21** to further nucleophilic attack by *N*-acetylation. Subsequent reaction with a substoichiometric quantity of a second heterocycle **4** should give unsymmetrical dye in solution, contaminated only by DIEA salt which may be easily removed by aqueous washing.

Immobilized aniline **19** was synthesized by an improvement of the strategy of Gordon et al. in which *N*-BOC-protected *p*-aminophenol **17** was loaded onto merrifield resin using Cs_2CO_3 rather than NaH as originally reported (Scheme 4).[21] This was converted to the imidate by reaction with triethylorthoformate in the presence of $BF_3{\cdot}OEt_2$ in a variation of the solution-phase method of Roberts,[22] in preference to the recently reported method of Martínez-Teipel et al.[23] Imidate formation was confirmed by characteristic peaks at 13.4, 61.5, 69.4, 114.6, 121.4 in the ^{13}C gel-phase NMR and sharp peaks at 1601 cm^{-1} (Ar stretch) and 1645 cm^{-1} (C=N stretch) in the FTIR spectrum.

SCHEME 4. Trimethine Cyanine Dye Synthesis from Immobilized Imidate[a]

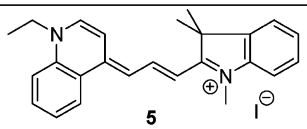
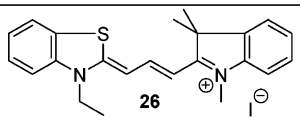
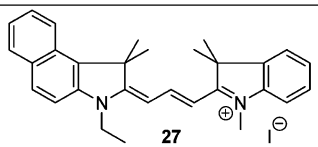
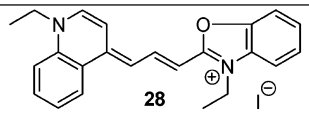
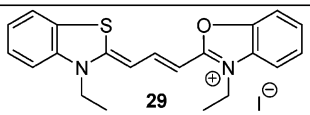
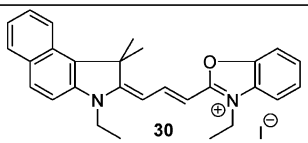
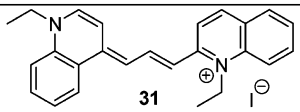
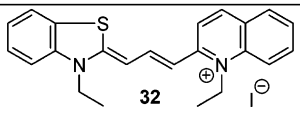
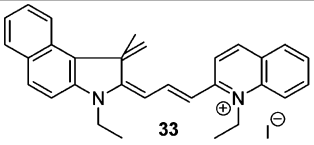


[a] Reagents and conditions: (a) Merrifield Resin, DMF, Cs_2CO_3, 50 °C; (b) DCM, TFA; (c) DCM, $BF_3{\cdot}OEt_2$, $HC(OEt)_3$; (d) DMF, **2**, 80 °C; (e) DIEA, pyridine, Ac_2O, **4**.

Subsequent reaction to form immobilized hemicyanine was investigated at 80 °C in DMF, varying the number of equivalents of heterocycle **2** used. Since the extent of reaction was unclear from ^{13}C gel-phase NMR and FTIR spectra, the resulting loadings of hemicyanine were quantified indirectly by the purity of the cleaved dye **5** formed on reacting the product resins with 0.45 equiv (based on maximum possible loading) lepidinium iodide **4** in the presence of Ac_2O, DIEA, and pyridine. Purities

(17) Furth, P. S.; Reitman, M. S.; Cook, A. F. *Tetrahedron Lett.* **1997**, *38*, 5403−5406.
(18) Oszczapowicz, J.; Orlinski, R.; Jaroszewska, J. *Polish J. Chem.* **1981**, *55*, 102−110.
(19) Okuyama, T.; Pletcher, T. C.; Sahn, D. J.; Schmir, G. L. *J. Am. Chem. Soc.* **1973**, *95*, 1253−1265.
(20) (a) Knott, E. B. *J. Chem. Soc.* **1946**, 120−122. (b) Knott, E. B. U.S. Patent 2487882, 1949.

TABLE 2. Results of Trimethine Dye Array Synthesis

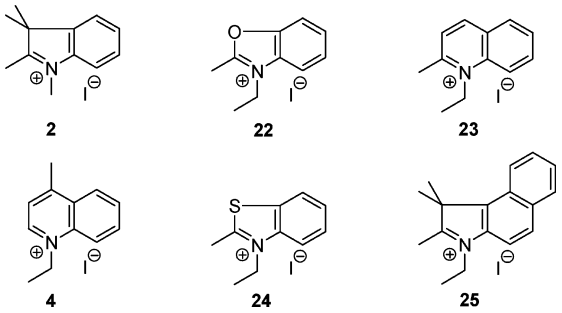
Compound	Monomer 1[a]	Monomer 2[b]	Purity[c]	Yield[d]	λ_{abs} / nm[e]	ε/ $M^{-1}cm^{-1}$	λ_{em} / nm[e]
 **5**	**2**	**4**	>95% (89%)	19% (79%)	599	4.8×10^4	647
 **26**	**2**	**24**	>95% (91%)	26% (60%)	542	5.6×10^4	565
 **27**	**2**	**25**	89% (<1%)	54% (-)	565	7.3×10^4	581[3a]
 **28**	**22**	**4**	>95% (>95%)	58% (43%)	593	6.9×10^4	616
 **29**	**22**	**24**	>95% (>95%)	30% (23%)	519	4.8×10^4	534
 **30**	**22**	**25**	>95% (50%)	76% (32%)	528	6.8×10^4	554[3a]
 **31**	**23**	**4**	>95% (93%)	70% (42%)	653	1.1×10^5	665
 **32**	**23**	**24**	>95% (84%)	23% (19%)	577	1.1×10^4	610
 **33**	**23**	**25**	50% (<1%)	53% (-)	581	7.0×10^4	605[3a]

[a] Used in hemicyanine formation. [b] Used in dye formation and cleavage. [c] Determined by HPLC with ELSD quantitation. [d] Crude yield (based on amount of heterocycle in cleavage step). [e] Taken in MeOH; values in parentheses are corresponding purities and yields for the sulfonyl chloride catch−activate−release method.[1]

were measured by HPLC with evaporative light-scattering detection as before. Reaction of imidate **20** with 5 equiv of heterocycle **2** for 4 h resulted in the best observed dye purity of 63% with the major impurity being unreacted **4**. The dye formation and cleavage step was then investigated, employing 0.30 and 0.15 equivalents of heterocycle **4**. These resulted in improved purities of 86% and >95% respectively, with the latter being chosen as the optimum stoichiometry for product purity.

Having optimized conditions for the synthesis of a single dye in high purity, we tested our strategy by the synthesis of a 3 × 3 array of unsymmetrical dyes (Table 2). The same monomers were used as described for the sulfonamide activation strategy,[1] allowing a direct comparison of the two synthetic routes. These heterocycles represent a chemically diverse set of reactants as well as giving products with absorption wavelengths spanning a large region of the visible spectrum. Heterocycles **2**, **22**

and **23** were used in hemicyanine formation while **4**, **24** and **25** were used in the final dye-forming step. ^{1}H NMR, FTIR, UV/vis, and fluorescence spectroscopic analyses of the products were consistent with the previously obtained data.[24]



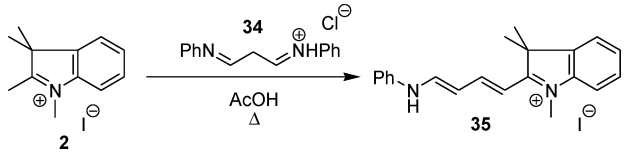
Reactions of heterocycle **25** appeared slow, resulting in lower purities, consistent with our previous observations when using the sulfonyl-chloride capture strategy.[1] However, the results in Table **3** show consistently higher purities than our previous synthesis.

The synthesis of water-soluble dyes was investigated using heterocycles **9** and **10**. Heterocycle **22** was loaded onto the imidate derivatized resin **20**. Subsequent reaction with sulfonated heterocycles **9** and **10** using 5.4 equiv of PS-DIEA and 5% Ac_2O in pyridine, followed by filtration through a plug of silica (DCM, then 10% MeOH/DCM), yielded both dyes **12** and **13** in >95% purity and 73% and 59% yield, respectively, with spectroscopic data as previously determined.

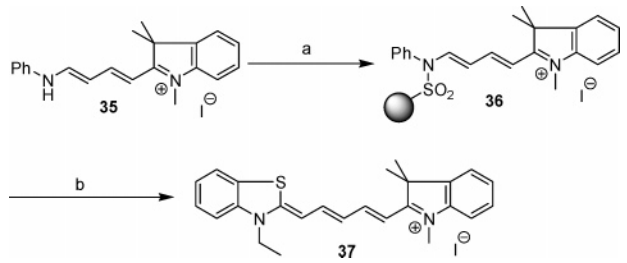
However, the approach of first loading the sulfonated heterocycles onto the imidate resin, **20** was not fruitful, as judged by the negligible mass increase, the absence of new peaks in the gel-phase ^{13}C NMR spectra and lack of any characteristic SO_3 peaks in the FT-IR spectrum of the resin after loading. Furthermore, changing time and the temperature of the loading reaction resulted in no improvement, and negligible dye was formed on subsequent reaction with quaternary salt **24**. Thus we concluded that the sulfonated heterocycles do not react well in the loading reaction, but react well when used as the nucleophile in the dye formation/cleavage step.

Synthesis of Pentamethine Cyanine Dyes. The trimethine series of cyanines are useful and important, but there are distinct advantages to the pentamethine class of cyanine dyes. These dyes absorb light at ~650 nm, wavelengths ~100 nm longer than the trimethine counterparts, which means that for biological applications they will be competing with less background cellular autofluorescence, allowing for more sensitive readings. We explored both the sulfonyl chloride capture route and imidate route for pentamethine dye synthesis.

SCHEME 5. Synthesis of Tetramethine Hemicyanine

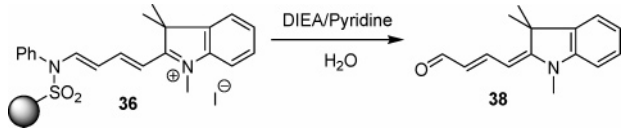


SCHEME 6. Solid-Phase Catch-and-Release Synthesis of Pentamethine Dye[a]



[a] Reagents and conditions: (a) DCM, DIEA, PS-SO_2Cl; (b) **24**, pyridine, DIEA.

SCHEME 7. Hydrolysis of Immobilized Tetramethine Hemicyanine



In solution, pentamethine cyanine dyes may be synthesized by routes analogous to those for trimethine dyes.[24] In particular, reaction of heterocycles such as **2** with malonaldehyde bisphenylimine salt **34** (Scheme 5) yields extended analogues of the hemicyanines used in our trimethine dye synthesis. Tetramethine hemicyanine **35** was obtained in 24% yield on refluxing heterocycle **2** with malonaldehyde bisphenylimine hydrochloride **34**, in acetic acid.

It was anticipated that hemicyanine **35** would be captured by sulfonyl chloride resin, in a manner similar to the shorter analogues such as **7**, and be subsequently activated to attack by a second heterocycle. Using identical conditions to those for the capture of dimethine hemicyanines, **35** was loaded onto the resin in 96% yield as judged by nitrogen elemental analysis (Scheme 6). However, reaction with a substoichiometric quantity of heterocycle **24** for 1 h under conditions optimized for trimethine dye synthesis gave the desired product in only 27% purity, despite complete consumption of heterocycle from solution. The same major impurity was found to also form in the absence of **24** and had a molecular mass of 227, consistent with the hydrolysis product **38** (Scheme 7).

Decreasing the reaction time from 1 h to 30 min significantly improved product purity to 92%. By reducing the concentration of base used from 10% to 1%, the reaction gave product **37** in >95% purity (see Table 3 for characterization) and 64% crude yield after 1 h, demonstrating the potential applicability of the catch-and-release strategy to the synthesis of extended dyes. Having proven this concept, we focused attention on the alternative malonaldehyde-based route for the remainder of our

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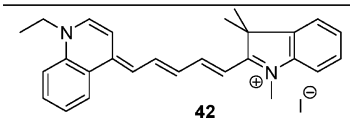
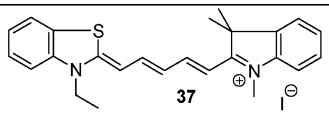
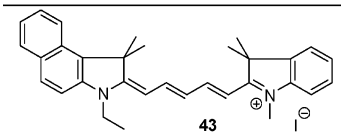
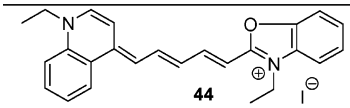
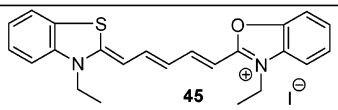
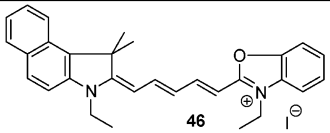
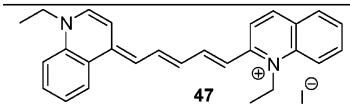
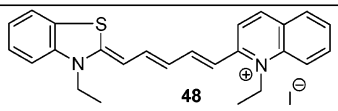
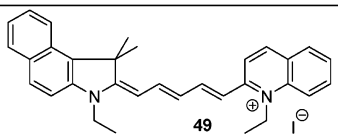
TABLE 3. Results of Pentamethine Dye Array Synthesis

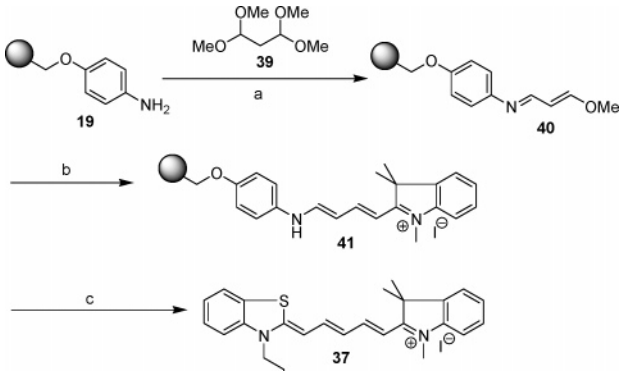
Compound	Monomer 1[a]	Monomer 2[b]	Purity[c]	Yield[d]	λ_{abs} / nm[e]	ε / $M^{-1}cm^{-1}$	λ_{em} / nm[e]
 42	**2**	**4**	91%	60%	640	0.5×10^4	657
 37	**2**	**24**	>95%	92%	643	1.8×10^5	664
 43	**2**	**25**	>95%	96%	656	2.6×10^4	679
 44	**22**	**4**	>95%	12%	578	0.1×10^4	599
 45	**22**	**24**	>95%	72%	614	6.6×10^4	636
 46	**22**	**25**	92%	46%	622	1.1×10^4	652
 47	**23**	**4**	66%[f]	-	-	-	-
 48	**23**	**24**	50%[f]	-	-	-	-
 49	**23**	**25**	76%	57%	674	1.3×10^4	702

[a] Used in hemicyanine formation. [b] Used in dye formation and cleavage. [c] Determined by HPLC with ELSD quantitation. [d] Crude yield (based on amount of heterocycle in cleavage step). [e] Taken in MeOH. [f] Dyes not clearly separated by HPLC; purities estimated by ^1H NMR integration.

work which has the clear advantage of not requiring any prior synthesis of components in solution.

The sulfonyl chloride capture route requires the synthesis of hemicyanines for resin loading, which was a particular problem for the pentamethine dye studied due to difficulties in purification. We reasoned that a strategy involving synthesis of the hemicyanines directly on the solid support would minimize the effort required to purify these intermediates, delivering a route better suited to the rapid generation of dye libraries. We reasoned that malonaldehyde diacetal **39** would react with aniline resin **19** in an analogous manner to the ortho ester used previously to give the immobilized polyene-bridge precursor **40** (Scheme 8). In species **40**, the aniline and alcohol leaving groups are on different carbons, so the substitution reaction products of this immobilized reagent were expected to be dependent on the site of nucleophilic attack as well as relative leaving group abilities. In this

SCHEME 8. Solid-Phase Synthesis of Pentamethine Cyanine Dye[a]



[a] Reagents and conditions: (a) DCM, $BF_3{\cdot}OEt_2$; (b) DMF, **2**, 80 °C; (c) DIEA, pyridine, Ac_2O, **24**.

respect, we anticipated that the attack adjacent to the electronegative oxygen would promote formation of bound hemicyanines such as **41**.

Reaction of PS-aniline **19** with diacetal **39** in the presence of a Lewis acid gave a product which showed strong peaks at δ 57.0, 70.3, 116.0, 121.8 ppm in its gel-phase ^{13}C NMR spectrum, which we attributed to the presence of the methoxy group, benzyloxy connection to the resin backbone, and two pairs of aniline carbons, respectively. In addition, an IR spectroscopic peak at 1630 cm^{-1}, indicative of the conjugated C=N bond, was observed. Reaction with heterocycle **2** in DMF at 80 °C, as in the trimethine dye synthesis, resulted in loss of this FTIR signal and subsequent reaction with 0.13 equivalents of heterocycle **24**, led to formation of the desired pentamethine dye **37** in solution. The sample was filtered through a plug of silica, eluting with DCM, and 5% MeOH/DCM to yield dye **37** in >95% purity and 92% crude yield. A 3 × 3 array using the six heterocycles described for the trimethine imidate route was synthesized to demonstrate the ease and effectiveness of the pentamethine route, the results of which are shown in Table 3. It was noted that when forming the on-bead hemicyanine with 1-ethyl-2-methylquinolinium iodide, **23** the subsequent dye-forming reaction generally led to reduced purity than for either of the other heterocycles. In these two cases, products were contaminated with symmetrical dyes, presumably caused by reversal of the cyanine formation step.

Synthesis of Water-Soluble Pentamethine Dyes. To show that this synthetic strategy is amenable to the use of sulfonated heterocycles to form water-soluble pentamethine dyes, we loaded heterocycle **2** onto the resin, as previously described. The cleavage step involved the use of 4.4 equiv of PS-DIEA, 0.1 equiv of heterocycles **9** and **10**, in 5% Ac_2O in pyridine. The crude dye was filtered through a plug of silica with DCM then 10% MeOH/DCM, to yield the dyes as described in Table 4.

Conclusions

In conclusion, we have developed and compared two novel approaches to the solid-phase synthesis of trimethine and pentamethine cyanine dyes, which afford products in high purity without the need for nontrivial column chromatography. The versatility of both routes has been demonstrated by the synthesis of arrays of trimethine and pentamethine cyanine dyes that include hydrophilic sulfonated dyes, which are of great importance in biological labeling applications. The imidate and malonaldehyde routes are the preferred methods for the synthesis of trimethine and pentamethine dyes respectively, over the sulfonyl chloride route. This is because there is no longer a need to synthesise the hemicyanine before resin loading, which can be tricky in itself. These strategies lend themselves well to the rapid synthesis of libraries of dyes for discovery chemistry and work is currently underway to identify compounds with novel biological activity.

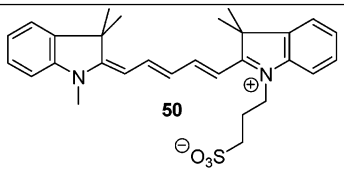
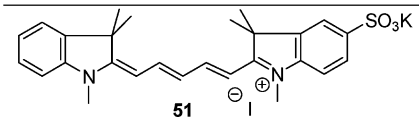
Experimental Section

N,N′-Bis-(4-methoxy-phenyl)-formamidine (**6**) was synthesized by the method of Van Dormael et al.[25] (4-Hydroxyphenyl)carbamic acid *tert*-butyl ester (**17**) was prepared by the method of Vigroux et al.[26]

Representative Dimethine Hemicyanine Synthesis. A mixture of *N,N′*-bis-(4-methoxyphenyl)formamidine **6** (1.280 g, 5 mmol), 1,2,3,3-tetramethyl-3*H*-indolium iodide, **2** (1.505 g, 5 mmol) and triethyl orthoformate (0.83 mL, 5 mmol) in ethanol (3 mL) was heated under reflux for 2 h. The mixture was cooled to room temperature and then on ice and the product isolated by filtration under reduced pressure, washed with ice-cold acetone and diethyl ether, and dried in vacuo. The product **7** (1.931 g, 89%) was isolated as yellow/orange crystals (mp 229−230 °C).

2-[(*E*)-2-(4-Methoxyphenylamino)vinyl]-1,3,3-trimethyl-3*H*-indolium iodide (7):[27] ^1H NMR (400 MHz, $CDCl_3$) δ

TABLE 4. Results of Sulfonated Pentamethine Dye Synthesis

Compound	Purity[a]	Yield[b]	λ_{abs} / nm[c]	ε/ $M^{-1}cm^{-1}$	λ_{em} / nm[c]
 **50**	>95%	64%	639	1.2×10^4	664
 **51**	>95%	86%	638	1.5×10^4	666

[a] Determined by HPLC with ELSD quantitation. [b] Crude yield. [c] Taken in MeOH.

1.69 (s, 6H), 3.66 (s, 3H), 3.82 (s, 3H), 6.95 (d, J = 9 Hz, 2H), 7.13 (d, J = 7 Hz, 1H), 7.29 (d, J = 8 Hz, 1H), 7.36−7.45 (m, 5H), 8.42 (d, J = 12 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.1, 31.9, 49.1, 55.7, 91.3, 110.8, 115.2, 119.5, 122.1, 125.7, 129.0, 131.5, 140.0, 142.2, 150.2, 158.5, 176.9; FTIR (DCM) 1508, 1589, 1633 cm^{-1}; EI HRMS $C_{20}H_{22}N_2O^+$ 306.1732 calcd, 306.1739 found; UV/vis λ_{abs} = 412 nm, $\epsilon = 6.9 \times 10^4$ M^{-1} cm^{-1} (MeOH).

3-Ethyl-2-[(*E*)-2-(4-methoxyphenylamino)vinyl]benzooxazol-3-ium iodide (11):[25] ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (t, J = 7 Hz, 3H), 3.73 (s, 3H), 4.15 (q, J = 7 Hz, 2H), 6.81 (d, J = 9 Hz, 2H), 6.85 (d, J = 12 Hz, 1H), 7.34−7.44 (m, 5H), 7.54 (d, J = 8 Hz, 1H), 8.48 (t, J = 12 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.1, 40.1, 55.6, 110.7, 111.5, 115.0, 119.0, 120.7, 125.9, 126.4, 130.2, 131.8, 146.6, 149.0, 157.9, 163.1; FTIR (DCM) 1512, 1584, 1636 cm^{-1}; ESI HRMS $C_{18}H_{19}N_2O_2^+$ 295.1447 calcd, 295.1437 found; UV/vis (MeOH) λ_{abs} = 385 nm, $\epsilon = 1.1 \times 10^5$ M^{-1} cm^{-1}.

1-Ethyl-2-[(*E*)-2-(4-methoxyphenylamino)vinyl]quinolinium iodide:[24] ^{1}H NMR (400 MHz, CD_3OD) δ 1.59 (t, J = 7 Hz, 3H), 3.63 (m, 2H), 3.82 (s, 3H), 6.23 (d, J = 12 Hz, 1H), 7.00 (d, J = 9 Hz, 2H), 7.33 (d, J = 9 Hz, 2H), 7.59 (t, J = 8 Hz, 1H), 7.86−7.94 (m, 2H), 8.03 (d, J = 9 Hz, 1H), 8.15 (m, 2H), 8.75 (d, J = 12 Hz, 1H); FTIR (DCM) 1506, 1567, 1589, 1615 cm^{-1}; UV/vis (MeOH) λ_{abs} = 448 nm, $\epsilon = 8.4 \times 10^4$ M^{-1} cm^{-1}.

Synthesis of 2,3,3-Trimethyl-1-(3-sulfonatepropyl)-3*H*-indolium (9). To 2,3,3-trimethylindolenine (500 mg, 3.14 mmol) was added a solution of 1,3-propane sultone (422 mg, 3.45 mmol) in 1,2-dichlorobenzene (3 mL). The reaction mixture was stirred at 120 °C for 18 h. The purple precipitate (804 mg, 91%) was collected by filtration and washed with cold diethyl ether and dried in vacuo: ^{1}H NMR (400 MHz, D_2O) δ 1.49 (s, 6H), 2.31 (q, J = 7 Hz, 2H), 3.02 (t, J = 7 Hz, 2H), 4.56 (t, J = 7 Hz, 2H), 7.53−7.57 (m, 2H), 7.63−7.66 (m, 1H), 7.72−7.77 (m, 1H); ^{13}C NMR (DEPT 135°) (100 MHz, DMSO-d_6) δ 14.2 (CH_3), 22.4 (CH_3), 24.1 (CH_2), 47.0 (CH_2), 47.8 (CH_2), 115.8 (CH), 123.8 (CH), 129.3 (CH), 129.7 (CH), 141.6 (q), 142.3 (q), 196.9 (q); ESI HRMS $C_{14}H_{19}NSO_3^+$ 304.0983 calcd, 304.0984 found.

Potassium 5-Sulfono-1,2,3,3-tetramethylindolenium Iodide (10). Potassium 5-sulfono-2,3,3-trimethylindolenine (500 mg, 1.8 mmol) was dissolved in EtOH (3.5 mL). To this solution was added MeI (561 μL, 9.02 mmol), and the mixture was refluxed for 18 h. The deep pink precipitate (491 mg, 65%) was collected by filtration, washed with diethyl ether, and dried in vacuo: ^{1}H NMR (400 MHz, D_2O) δ 1.52 (s, 6H), 3.96 (s, 3H), 7.77 (d, J = 9 Hz, 1H), 7.95 (dd, J = 9, 1 Hz, 1H), 8.04 (d, J = 1 Hz, 1H); ^{13}C NMR (DEPT 135°) (100 MHz, DMSO-d_6) δ 15.9 (CH_3), 23.2 (CH_3), 36.5 (CH_3), 55.7 (q), 116.2 (CH), 122.2 (CH), 129.6 (CH), 142.9 (q), 143.6 (q), 151.0 (q), 198.5 (q); ESI HRMS $C_{12}H_{15}NSO_3Na^+$ 276.0670 calcd, 276.0660 found.

Representative Procedure for the Capture of Hemicyanines by Sulfonyl Chloride Resin. Hemicyanine **7** (792 mg, 1.82 mmol) was stirred at room temperature with sulfonyl chloride-derivatized polystyrene (400 mg, 0.61 mmol) in dry DCM (4 mL) for 4 h in the presence of DIEA (106 μL, 0.61 mmol). The resin was isolated by filtration, washed with DMF and DCM, and dried in vacuo to give the product (616 mg, 89% by mass increase, 91% by N elemental analysis) as orange beads.

Representative Procedure for the Trimethine Dye Formation from Captured Hemicyanine. Quaternary salt **4** (12.6 mg, 42 μmol) and polymer-bound hemicyanine **8** (150 mg, 0.14 mmol) were stirred in dry 9:1 pyridine/DIEA (1.5 mL) at room temperature for 30 min. The mixture was filtered and the resin washed with dry DCM. The solvent was removed from the filtrate in vacuo. The resulting solid was redissolved in DCM and washed twice with water.

Representative Procedure for the Sulfonated Trimethine Dye Formation from Captured hemicyanine. Sulfonated quaternary salt **9** (12.6 mg, 42 μmol) and polymer-bound hemicyanine of heterocycle **22** (150 mg, 0.14 mmol) and PS-DIEA (150 mg, 0.53 mmol) were stirred in dry pyridine (1.5 mL) at room temperature for 30 min. The mixture was filtered and the resin washed with dry DCM. The solvent was removed from the filtrate in vacuo.

2-[3-(3-Ethyl-3*H*-benzooxazol-2-ylidene)propenyl]-3,3-dimethyl-1-(3-sulfonatepropyl)-3*H*-indolium (12): ^{1}H NMR (400 MHz, MeOD) δ 1.51 (t, J = 7 Hz, 3H), 1.73 (s, 6H), 2.23 (q, J = 8 Hz, 2H), 2.98 (t, J = 8 Hz, 2H), 4.26 (t, J = 8 Hz, 2H), 4.37 (q, J = 7 Hz, 2H), 6.34 (d, J = 13 Hz, 1H), 6.42 (d, J = 13 Hz, 1H), 7.21 (td, J = 7, 1 Hz, 1H), 7.34 (dd, J = 7, 1 Hz, 1H), 7.45−7.37 (m, 1H), 7.53−7.45 (m, 3H), 7.66 (dd, J = 7, 1 Hz, 1H), 7.74 (dd, J = 7, 1 Hz, 1H), 8.55 (t, J = 13 Hz, 1H); ^{13}C NMR (DEPT 135°) (100 MHz, MeOD) δ 13.4 (CH_3), 19.3 (CH_3), 23.8 (CH_2), 40.8 (CH_2), 50.4 (CH_2), 66.6 (CH_2), 91.4 (CH), 100.2 (CH), 111.5 (CH), 112.4 (CH), 112.6 (CH), 123.3 (CH), 125.6 (CH), 127.3 (CH), 127.7 (CH), 129.9 (CH), 132.3 (q), 141.9 (q), 143.6 (q), 148.8 (q), 150.4 (CH, 163.7 (q), 169.3 (q), 174.1 (q); ESI HRMS $C_{25}H_{29}N_2O_4S^+$ 453.1848 calcd, 453.1844 found; UV/vis (MeOH) λ_{abs} = 510 nm, $\epsilon = 7.2 \times 10^4$ M^{-1}cm^{-1}, λ_{em} = 544 nm; HPLC (Phe-hex) t_R = 21.97 min, > 95%.

Potassium 3-ethyl-2-[3-(1,3,3-trimethyl-5-sulfono-1,3-dihydroindol-2-ylidene)propenyl]benzooxazol-3-ium iodide (13): ^{1}H NMR (400 MHz, MeOD) δ 1.51 (t, J = 7 Hz, 3H), 1.74 (s, 6H), 3.55 (s, 3H), 4.39 (q, J = 7 Hz, 2H), 6.24 (d, J = 13 Hz, 1H), 6.36 (d, J = 13 Hz, 1H), 7.23 (d, J = 7 Hz, 1H), 7.56−7.53 (m, 2H), 7.70−7.69 (m, 1H), 7.79−7.76 (m, 1H), 7.87−7.85 (m, 2H), 8.56 (t, J = 13 Hz, 1H); ^{13}C NMR (DEPT 135°) (126 MHz, MeOD) δ 13.5 (CH_3), 19.3 (CH_3), 47.5 (CH_3), 66.7 (CH_2), 92.6 (CH), 100.2 (CH), 110.5 (CH), 112.6 (CH), 112.8 (CH), 121.2 (CH), 127.6 (CH), 129.9 (CH), 132.0 (CH), 132.4 (q), 133.6 (q), 142.5 (q), 145.5 (q), 148.8 (q), 150.4 (CH), 163.8 (q), 169.3 (q), 174.6 (q); ESI HRMS $C_{23}H_{25}N_2O_4S^+$ 425.1535 calcd, 425.1526 found; UV/vis (MeOH) λ_{abs} = 507 nm, $\epsilon = 8.6 \times 10^4$ M^{-1} cm^{-1}, λ_{em} = 543 nm; HPLC (Phe-hex) t_R = 18.65 min, >95%.

PS-Bound *N*-BOC-aniline (18).[21] A mixture of (4-hydroxyphenyl)carbamic acid *tert*-butyl ester **17** (752 mg, 3.6 mmol), Cs_2CO_3 (1.174 g, 3.6 mmol), and 2% DVB cross-linked chloromethyl polystyrene (1 g, 1.2 mmol) in DMF (7.5 mL) was heated at 50 °C for 32 h. The resin was isolated by filtration, washed with water, DMF, and DCM and then dried in vacuo, giving the product (quantitative by N analysis, 95% by Cl analysis) as a beige resin: ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4, 70.3, 115.2, 120.5; FTIR (DCM) 1601, 1725 cm^{-1}. Anal. Calcd: N, 1.30; Cl, 0. Found: N, 1.52; Cl, 0.16.

PS-Bound Aniline (19).[21] Resin **21** (500 mg, 0.46 mmol) was shaken at room temperature with a 20% solution of TFA in DCM (4 mL) for 2 h, filtered, and washed with DCM. The resin was then shaken with a 10% solution of triethylamine in DCM (4 mL) for 15 min, filtered, washed with DCM, and dried in vacuo to give the product as a beige resin: ^{13}C NMR (100 MHz, $CDCl_3$) δ 70.7, 116.1, 116.4; FTIR (ATR) 1509, 1601 cm^{-1}. Anal. Calcd: N, 1.47. Found: N, 1.20.

PS-Bound Imidate (20). To PS-aniline **19** (400 mg, 0.34 mmol) was added a solution of $(EtO)_3CH$ (0.6 mL, 4.6 mmol) and $BF_3{\cdot}OEt_2$ (60 μL, 0.38 mmol) in DCM (6 mL) and the solution stirred at room temperature for 6 h. Dry DIEA (100 μL, 0.56 mmol) was added and the mixture stirred for a further 5 min. The resin was isolated by filtration, washed with DCM, and dried in vacuo to give the product as a beige resin: ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.4, 61.5, 69.4, 114.6, 121.4; FTIR (ATR) 1601, 1645 cm^{-1}. Anal. Calcd: N, 1.16. Found: N, 1.12.

(25) Van Dormael, A.; Jaeken, J.; Nys, J. *Bull. Soc. Chim. Belg.* **1949**, *58*, 477−486.

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Representative Procedure for Preparation of PS-Bound Dimethine Hemicyanines. To PS-imidate **20** (1.00 g, 0.8 mmol) and 1,2,3,3-tetramethyl-3*H*-indolium iodide (1.20 g, 4.0 mmol) was added DMF (10 mL), and the mixture stirred at 80 °C for 4 h. On cooling, the resin was isolated by filtration, washed with DMF and DCM and dried in vacuo to give the product as an orange resin.

Representative Procedure for Formation and Cleavage of Trimethine Cyanine Dyes. To PS-bound hemicyanine **21** (200 mg, 0.13 mmol) was added a solution of quaternary salt **4** (5.8 mg, 20 μmol), Ac_2O (0.1 mL), and DIEA (0.2 mL) in pyridine (1.85 mL) and the mixture stirred at room temperature for 1 h. The resin was removed by filtration and washed with DCM. The solvent was removed from the filtrate in vacuo and the resulting solid redissolved in DCM, washed twice with water, and then precipitated with diethyl ether to give the product in >95% purity as a dark blue solid (1.8 mg, 19% crude yield).

Representative Procedure for Formation and Cleavage of Sulfonated Trimethine Cyanine Dyes. To PS-bound hemicyanine **21** (200 mg, 0.13 mmol) and PS-DIEA (200 mg, 0.7 mmol) was added a solution of quaternary salt **9** (5.6 mg, 20 μmol) and Ac_2O (0.1 mL) in pyridine (1.9 mL) and the mixture stirred at room temperature for 1 h. The resin was removed by filtration and washed with DCM. The solvent was removed from the filtrate in vacuo. The resulting crude product was filtered through a short plug of silica, eluting with 2% MeOH/DCM, then 10% MeOH/dicloromethane. The orange product was collected in >95% purity and 73% yield.

2-{(*E*)-3-[1-Ethyl-1*H*-quinolin-(4*E*)-ylidene]propenyl}-1,3,3-trimethyl-3*H*-indolium iodide (5):[24a] ^{1}H NMR (400 MHz, CD_3OD) δ 1.60 (t, J = 7 Hz, 3H), 1.73 (s, 6H), 3.49 (s, 3H), 4.71 (q, J = 7 Hz, 2H), 6.22 (d, J = 13 Hz, 1H), 7.12 (t, J = 8 Hz, 2H), 7.22 (d, J = 13 Hz, 1H), 7.33 (t, J = 8 Hz, 1H), 7.40 (d, J = 7 Hz, 1H), 7.78 (t, J = 7 Hz, 1H), 7.83 (d, J = 7 Hz, 1H), 8.03 (t, J = 7 Hz, 1H), 8.15 (d, J = 9 Hz, 1H), 8.43 (t, J = 13 Hz, 1H), 8.46 (d, J = 7 Hz, 1H), 8.61 (d, J = 8 Hz, 1H); FTIR (DCM) 1525 cm^{-1}; UV/vis (MeOH) λ_{abs} = 599 nm, ϵ = 4.8 × 10^4 M^{-1} cm^{-1}, λ_{em} = 647 nm; HPLC (Phe-hex) t_R = 25.76 min, >95%.

2-{(*E*)-3-[3-Ethyl-6,7-dihydro-3*H*-benzothiazol-(2*E*)-ylidene]propenyl}-1,3,3-trimethyl-3*H*-indolium iodide (26):[24b] ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (t, J = 7 Hz, 3H), 1.71 (s, 6H), 3.59 (s, 3H), 4.53 (q, J = 7 Hz, 2H), 6.30 (d, J = 13 Hz, 1H), 6.74 (d, J = 13 Hz, 1H), 7.23−7.26 (m, 2H), 7.40 (t, J = 8 Hz, 1H), 7.48−7.52 (m, 2H), 7.67 (t, J = 6 Hz, 1H), 7.78 (d, J = 8 Hz, 1H), 7.94 (d, J = 8 Hz, 1H), 8.22 (t, J = 13 Hz, 1H); FTIR (DCM) 1556, 1655 cm^{-1}; UV/vis (MeOH) λ_{abs} = 542 nm, ϵ = 5.6 × 10^4 M^{-1} cm^{-1}, λ_{em} = 565 nm; HPLC (Phe-hex) t_R = 25.06 min, >95%.

3-Ethyl-2-{(*E*)-3-[1-ethyl-1*H*-quinolin-(4*E*)-ylidene]-propenyl}benzooxazol-3-ium iodide (28):[24c] ^{1}H NMR (400 MHz, CD_3OD) δ 1.46 (t, J = 7 Hz, 3H), 1.55 (t, J = 7 Hz, 3H), 4.19 (q, J = 7 Hz, 2H), 4.55 (q, J = 7 Hz, 2H), 5.94 (d, J = 13 Hz, 1H), 7.03 (d, J = 13 Hz, 1H), 7.32 (t, J = 7 Hz, 1H), 7.36−7.43 (m, 2H), 7.53 (d, J = 8 Hz, 1H), 7.63−7.67 (m, 2H), 7.92 (t, J = 7 Hz, 1H), 7.97 (d, J = 8 Hz, 1H), 8.17 (d, J = 7 Hz, 1H), 8.47 (d, J = 8 Hz, 1H), 8.52 (t, J = 13 Hz, 1H); FTIR (DCM) 1519, 1550 cm^{-1}; UV/vis (MeOH) λ_{abs} = 593 nm, ϵ = 6.9 × 10^4 M^{-1} cm^{-1}, λ_{em} = 616 nm, HPLC (Phe-hex) t_R = 24.3 min, >95%.

3-Ethyl-2-{(*E*)-3-[3-ethyl-6,7-dihydro-3*H*-benzothiazol-(2*E*)-ylidene]propenyl}benzooxazol-3-ium iodide (29):[24c] ^{1}H NMR (400 MHz, CD_3OD) δ 2.27 (t, J = 7 Hz, 3H), 2.33 (t, J = 7 Hz, 3H), 4.28 (q, J = 7 Hz, 2H), 4.41 (q, J = 7 Hz, 2H), 6.04 (d, J = 13 Hz, 1H), 6.50 (d, J = 13 Hz, 1H), 7.40−7.49 (m, 3H), 7.55−7.59 (m, 2H), 7.64 (d, J = 8 Hz, 2H), 7.83 (d, J = 8 Hz, 1H), 8.28 (t, J = 13 Hz, 1H); FTIR (DCM) 1558 cm^{-1}; UV/vis (MeOH) λ_{abs} = 519 nm, ϵ = 4.8 × 10^4 M^{-1} cm^{-1}, λ_{em} = 534 nm, HPLC (Phe-hex) t_R = 24.45 min, >95%.

1-Ethyl-2-{(*E*)-3-[1-ethyl-1*H*-quinolin-(4*E*)-ylidene]-propenyl}quinolinium iodide (31):[24c] ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50−1.55 (m, 6H), 4.44−4.55 (m, 4H), 6.46 (d, J = 13 Hz, 1H), 7.08 (d, J = 13 Hz, 1H), 7.38 (t, J = 7 Hz, 1H), 7.60 (t, J = 6 Hz, 1H), 7.63 (d, J = 7 Hz, 1H), 7.67−7.71 (m, 2H), 7.74−7.76 (m, 2H), 7.85−7.86 (m, 2H), 7.98 (d, J = 7 Hz, 1H), 8.04 (d, J = 10 Hz, 1H), 8.43 (d, J = 9 Hz, 1H), 8.61 (t, J = 13 Hz, 1H); FTIR (DCM) 1518, 1531 cm^{-1}; UV/vis (MeOH) λ_{abs} = 653 nm, ϵ = 1.1 × 10^5 M^{-1} cm^{-1}, λ_{em} = 665 nm; HPLC (Phe-hex) t_R = 19.79 min, >95%.

1-Ethyl-2-{(*E*)-3-[3-ethyl-6,7-dihydro-3*H*-benzothiazol-(2*E*)-ylidene]propenyl}quinolinium iodide (32):[24c,d] ^{1}H NMR (400 MHz, CD_3OD) δ 1.44 (t, J = 7 Hz, 3H), 1.56 (t, J = 7 Hz, 3H), 4.35 (q, J = 7 Hz, 2H), 4.59 (q, J = 7 Hz, 2H), 6.52 (d, J = 13 Hz, 1H), 6.57 (d, J = 13 Hz, 1H), 7.23−7.53 (m, 5H), 7.76 (d, J = 8 Hz, 1H), 7.82 (t, J = 7 Hz, 1H), 8.01 (d, J = 10 Hz, 1H), 8.05 (d, J = 10 Hz, 1H), 8.25 (t, J = 13 Hz, 1H); FTIR (DCM) 1526, 1557, 1607 cm^{-1}; UV/vis (MeOH) λ_{abs} = 577 nm, ϵ = 1.1 × 10^4 M^{-1} cm^{-1}, λ_{em} = 610 nm; HPLC (Phe-hex) t_R = 25.63 min, >95%.

1,3,3-Trimethyl-2-((1*E*,3*E*)-4-phenylaminobuta-1,3-dienyl)-3*H*-indolium Iodide (35).[28] A mixture of 1,2,3,3-tetramethyl-3*H*-indolium iodide (602 mg, 2.0 mmol) and malonaldehyde bisphenylimine hydrochloride (568 mg, 2.2 mmol) was heated at 120 °C in acetic acid (20 mL) for 6 h. On cooling, the solvent was removed in vacuo and the product isolated by flash column chromatography ($CHCl_3$/MeOH) as a green solid (208 mg, 24%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.66 (s, 6H), 3.44 (s, 3H), 5.85 (d, J = 12 Hz, 1H), 7.00 (t, J = 12 Hz, 1H), 7.01 (d, J = 8 Hz, 1H), 7.13 (t, J = 7 Hz, 1H), 7.21 (t, J = 7 Hz, 1H), 7.27−7.37 (m, 4H), 7.58 (d, J = 8 Hz, 2H), 8.0 (t, J = 12 Hz, 1H), 8.44 (d, J = 12 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2, 30.9, 48.5, 100.1, 109.4, 114.6, 118.8, 122.0, 124.1, 125.9, 128.5, 129.5, 140.2, 141.5, 143.0, 153.4, 156.2, 171.4; FTIR (DCM) 1531, 1567 cm^{-1}; EI HRMS $C_{21}H_{23}N_2^+$ 303.1861 calcd, 303.1848 found; UV/vis (MeOH) λ_{abs} = 515 nm, ϵ = 1.1 × 10^5 M^{-1}cm^{-1}, λ_{em} = 545 nm.

2-{(1*E*,3*E*)-5-[3-Ethyl-3*H*-benzo[*b*]thiophen-(2*E*)-ylidene]-penta-1,3-dienyl}-1,3,3-trimethyl-3*H*-indolium Iodide (37).[29] 1-Ethyl-2-methylbenzothiazolium iodide (8.7 mg, 29 μmol) and PS-bound hemicyanine **36** (100 mg, 95 μmol) were stirred in 99:1 pyridine/DIEA (1 mL) at room temperature for 1 h. The mixture was filtered and the resin washed with DCM. The solvent was removed from the filtrate in vacuo. The resulting solid was redissolved in DCM, washed twice with water and precipitated with diethyl ether to give the product (9.5 mg, 64% crude yield) as a dark blue solid in >95% purity: ^{1}H NMR (400 MHz, CD_3OD) δ 1.47 (t, J = 7 Hz, 3H), 1.68 (s, 6H), 3.49 (s, 3H), 4.51 (q, J = 7 Hz, 2H), 6.05 (d, J = 13 Hz, 1H), 6.57 (t, J = 13 Hz, 1H), 6.67 (d, J = 13 Hz, 1H), 7.15 (t, J = 7 Hz, 2H), 7.36 (t, J = 8 Hz, 1H), 7.40 (d, J = 7 Hz, 1H), 7.50 (t, J = 8 Hz, 1H), 7.64 (t, J = 7 Hz, 1H), 7.77 (d, J = 8 Hz, 1H), 7.92−8.03 (m, 3H); ^{13}C NMR (DEPT 135°) (100 MHz, MeOD) δ 13.2 (CH_3), 27.9 (CH_3), 28.4 (CH_3), 68.2 (CH_2), 107.5 (CH), 109.6 (CH), 113.5 (CH), 121.7 (CH), 122.5 (CH), 123.1 (CH), 124.3 (CH), 126.5 (CH), 128.8 (CH), 129.0 (CH), 131.1 (CH), 132.7 (q), 140.7 (q), 141.3 (q), 143.4 (q), 151.0 (CH), 152.8 (CH), 166.7 (q), 167.8 (q), 171.2 (q); ESI HRMS $C_{25}H_{27}N_2S^+$ 387.1895 calcd, 387.1893 found; UV/vVis (MeOH) λ_{abs} = 643 nm, ϵ = 1.8 × 10^5 M^{-1} cm^{-1}, λ_{em} = 664 nm, HPLC (Phe-hex) t_R = 27.19 min, >95%.

PS-Bound 4-(3-Methoxyallylideneamino)phenol (40). To PS-aniline **19** (400 mg, 0.34 mmol) was added a solution of malonaldehyde bis-dimethyl acetal (0.6 mL, 3.6 mmol) and BF_3·OEt_2 (60 μL, 0.38 mmol) in DCM (6 mL) and the solution stirred at room temperature for 6 h. Dry DIEA (100 μL, 0.56 mmol) was added and the mixture stirred for a further 5 min. The resin was isolated by filtration, washed with DCM, and dried in vacuo to give the product as a brown resin.

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Representative Procedure for Preparation of PS-Bound Tetramethine Hemicyanines. To PS-bound 4-(3-methoxyallylideneamino)phenol **40** (300 mg, 0.3 mmol) and 1,2,3,3-tetramethyl-3*H*-indolium iodide (451 mg, 1.5 mmol) was added DMF (3 mL) and the mixture stirred at 80 °C for 4 h. On cooling, the resin was isolated by filtration, washed with DMF and DCM, and dried in vacuo to give the product as a brown resin.

Representative Procedure for Formation and Cleavage of Pentamethine Cyanine Dyes. To PS-bound hemicyanine **41** (200 mg, 0.16 mmol) was added a solution of 1-ethyl-2-methylbenzothiazolium iodide (7.3 mg, 20 μmol), Ac_2O (0.1 mL), and DIEA (0.2 mL) in pyridine (1.7 mL) and the mixture stirred at room temperature for 1 h. The resin was removed by filtration and washed with DCM. The solvent was removed from the filtrate in vacuo and the resulting solid redissolved in DCM and washed twice with water. The crude product was filtered through a short plug of silica eluting with DCM then 5% MeOH/DCM. The product (9.5 mg, 92% crude yield) was collected as a dark blue solid in >95% purity.

Sulfonated dyes were prepared in the same manner, using PS-DIEA (200 mg, 0.7 mmol) in 95:5 pyridine/Ac_2O. Filtration through silica was achieved using with 2% MeOH/dicloromethane then 10% MeOH/DCM.

2-[5-(1-Ethyl-1*H*-quinolin-4-ylidene)penta-1,3-dienyl]-1,3,3-trimethyl-3*H*-indolium iodide (42): ^{1}H NMR (400 MHz, CD_2Cl_2) δ 1.67 (t, J = 7 Hz, 3H), 1.74 (s, 6H), 3.35 (s, 3H), 4.80 (q, J = 7 Hz, 2H), 5.78 (d, J = 13 Hz, 1H), 6.22 (d, J = 13 Hz, 1H), 6.56 (t, J = 13 Hz, 1H), 6.89 (d, J = 8 Hz, 1H), 7.10−7.06 (m, 2H), 7.31−7.27 (m, 1H), 7.43−7.39 (m, 1H), 7.78−7.75 (m, 1H), 8.10−7.98 (m, 2H), 8.14 (t, J = 13 Hz, 2H), 8.45 (d, J = 7 Hz, 1H), 8.99 (d, J = 7 Hz, 1H); ^{13}C NMR (DEPT 135°) (126 MHz, CD_2Cl_2) δ 15.2 (CH_3), 27.3 (CH_3), 28.2 (CH_3), 52.1 (CH_2), 104.3 (CH), 109.2 (CH), 111.7 (CH), 113.3 (CH), 115.5 (CH), 123.4 (CH), 126.2 (CH), 127.2 (CH), 128.9 (CH), 129.3 (CH), 129.7 (CH), 135.4 (CH), 136.7 (CH), 139.6 (q), 141.0 (q), 144.3 (q), 146.9 (CH), 149.7 (CH), 154.4 (q), 155.5(q), 173.0 (q), 175.3 (q); ESI HRMS $C_{27}H_{29}N_2^+$ 381.2331 calcd, 381.2340 found; UV/vis (MeOH) λ_{abs} = 640 nm, ϵ = 0.5 × 10^4 M^{-1} cm^{-1}, λ_{em} = 657 nm; HPLC (Phe-hex) t_R = 27.29 min, 91%.

2-[5-(3-Ethyl-1,1-dimethyl-1,3-dihydrobenz[*e*]indol-2-ylidene)penta-1,3-dienyl]-1,3,3-trimethyl-3*H*-indolium iodide (43): ^{1}H NMR (400 MHz, MeOD) δ 1.45 (t, J = 7 Hz, 3H), 1.73 (s, 6H), 2.00 (s, 6H), 3.61 (s, 3H), 4.31 (q, J = 7 Hz, 2H), 6.26 (d, J = 13 Hz, 1H), 6.40 (d, J = 13 Hz, 1H), 6.69 (t, J = 13 Hz, 1H), 7.28−7.23 (m, 2H), 7.39−7.37 (m, 1H), 7.50−7.46 (m, 2H), 7.64−7.63 (m, 2H), 8.03−7.97 (m, 2H), 8.27−8.23 (m, 2H), 8.36 (t, J = 13 Hz, 1H); ^{13}C NMR (DEPT 135°) (126 MHz, MeOD) δ 13.3 (CH_3), 17.4 (CH_3), 18.8 (CH_3), 43.9 (CH_2), 55.9 (CH_3), 104.1 (CH), 111.6 (CH), 112.0 (CH), 123.3 (CH), 123.4 (CH), 125.9 (CH), 126.2 (CH), 126.7 (CH), 128.7 (CH), 129.5 (q), 129.6 (CH), 131.1 (CH), 131.8 (CH), 133.5 (q), 135.5 (q), 140.5 (q), 142.4 (q), 144.4 (q), 154.8 (CH), 155.1 (CH), 174.5 (q), 176.2 (q); ESI HRMS $C_{32}H_{35}N_2^+$ 447.2800 calcd, 447.2802 found; UV/vis (MeOH) λ_{abs} = 656 nm, ϵ = 2.6 × 10^4 M^{-1} cm^{-1}, λ_{em} = 679 nm; HPLC (Phe-hex) t_R = 28.93 min, >95%.

2-[5-(1-Ethyl-1*H*-quinolin-4-ylidene)penta-1,3-dienyl]-1,3,3-trimethyl-3*H*-indolium iodide (44): ^{1}H NMR (400 MHz, CD_2Cl_2) δ 1.67 (t, J = 7 Hz, 3H), 1.74 (s, 6H), 3.35 (s, 3H), 4.80 (q, J = 7 Hz, 2H), 5.78 (d, J = 13 Hz, 1H), 6.22 (d, J = 13 Hz, 1H), 6.56 (t, J = 13 Hz, 1H), 6.89 (d, J = 8 Hz, 1H), 7.10−7.06 (m, 2H), 7.31−7.27 (m, 1H), 7.43−7.39 (m, 1H), 7.78−7.75 (m, 1H), 8.10−7.98 (m, 2H), 8.14 (t, J = 13 Hz, 2H), 8.45 (d, J = 7 Hz, 1H), 8.99 (d, J = 7 Hz, 1H); ^{13}C NMR (DEPT 135°) (126 MHz, CD_2Cl_2) δ 15.2 (CH_3), 27.3 (CH_3), 28.2 (CH_3), 52.1 (CH_2), 104.3 (CH), 109.2 (CH), 111.7 (CH), 113.3 (CH), 115.5 (CH), 123.4 (CH), 126.2 (CH), 127.2 (CH), 128.9 (CH), 129.3 (CH), 129.7 (CH), 135.4 (CH), 136.7 (CH), 139.6 (q), 141.0 (q), 144.3 (q), 146.9 (CH), 149.7 (CH), 154.4 (q), 155.5(q), 173.0 (q),175.3 (q); ESI HRMS $C_{27}H_{29}N_2^+$ 381.2331 calcd, 381.2340 found; UV/vis (MeOH) λ_{abs} = 640 nm, ϵ = 0.1 × 10^4 M^{-1} cm^{-1}, λ_{em} = 657 nm; HPLC (Phe-hex) t_R = 20.82 min, >95%.

3-Ethyl-2-[5-(3-ethyl-6,7-dihydro-3*H*-benzothiazol-2-ylidene)penta-1,3-dienyl]benzooxazol-3-ium iodide (45): ^{1}H NMR (400 MHz, MeOD) δ 1.42 (t, J = 7 Hz, 3H), 1.45 (t, J = 7 Hz), 4.25 (q, J = 7 Hz, 2H), 4.36 (q, J = 7 Hz, 2H), 5.98 (d, J = 13 Hz, 1H), 6.39 (d, J = 13 Hz, 1H), 6.51 (t, J = 13 Hz, 1H), 7.20−7.46 (m, 3H), 7.53−7.73 (m, 5H), 7.78 (d, J = 8 Hz, 1H), 7.95 (t, J = 13 Hz, 1H); ^{13}C NMR (DEPT 135°) (126 MHz, CD_2Cl_2) δ 12.86 (CH_3), 13.24 (CH_3), 40.38 (CH_2), 42.5 (CH_2), 111.7 (CH), 113.8 (CH), 113.9 (CH), 123.7 (CH), 126.2 (CH), 126.4 (CH), 127.1 (q), 127.3 (CH), 129.2 (CH), 132.4 (q), 142.5 (q), 148.6 × 2 (q), 162.8 (q); ESI HRMS $C_{23}H_{23}N_2OS^+$ 375.5166 calcd, 375.5152 found; UV/vis (MeOH) λ_{abs} = 613 nm, ϵ = 6.6 × 10^4 M^{-1} cm^{-1}, λ_{em} = 636 nm; HPLC (Phe-hex) t_R = 31.89 min, >95%.

3-Ethyl-2-[5-(3-ethyl-1,1-dimethyl-1,3-dihydrobenz[*e*]-indol-2-ylidene)penta-1,3-dienyl]benzooxazol-3-ium iodide (46): ^{1}H NMR (400 MHz, CD_2Cl_2) δ 1.45 (t, J = 7 Hz, 3H), 1.54 (t, J = 7 Hz), 4.12 (q, J = 7 Hz, 2H), 4.36 (q, J = 7 Hz, 2H), 6.13 (d, J = 14 Hz, 1H), 6.25 (d, J = 14 Hz, 1H), 6.68 (t, J = 14 Hz, 1H), 7.36 (d, J = 9 Hz, 1H), 7.44−7.50 (m, 4H), 7.58−7.63 (m, 2H), 7.92−7.98 (m, 3H), 8.06 (t, J = 14 Hz, 1H), 8.13 (d, J = 9 Hz, 1H); ^{13}C NMR (DEPT 135°) (126 MHz, CD_2Cl_2) δ 12.5 (CH_3), 13.5 (CH_3), 27.6 (CH_3), 29.7 (CH_2), 40.6 (CH_2), 90.6 (CH), 100.5 (CH), 110.4 (CH), 111.2 (CH), 111.4 (CH), 121.9 (CH), 122.3 (CH), 124.8 (CH), 126.3 (CH), 126.8 (CH), 127.8 (CH), 128.5 (q), 130.2 (CH), 130.7 (CH), 131.0 (q), 131.8 (q), 133.2 (q), 139.7 (q), 147.5 (q), 152.0 (CH), 152.9 (CH), 161.8 (q), 172.6 (q), 174.2 (q); ESI HRMS $C_{30}H_{31}N_2O^+$ 435.2436 calcd, 435.2439 found; UV/vis (MeOH) λ_{abs} = 622 nm, ϵ = 1.1 × 10^4 M^{-1} cm^{-1}, λ_{em} = 652 nm; HPLC (Phe-hex) t_R = 29.26 min, 92%.

1-Ethyl-2-{(1*E*,3*E*)-5-[3-ethyl-1,1-dimethyl-1,3-dihydrobenz[*e*]indol-(2*E*)-ylidene]penta-1,3-dienyl}quinolinium iodide (49): ^{1}H NMR (400 MHz, MeOD) δ 1.39 (t, J = 7 Hz, 3H), 1.59 (t, J = 7 Hz, 3H), 4.10 (q, J = 7 Hz, 2H), 4.73 (q, J = 7 Hz, 2H), 6.03 (d, J = 13 Hz, 1H), 6.36 (d, J = 13 Hz, 1H), 6.68 (t, J = 13 Hz, 1H), 7.41 (t, J = 7 Hz, 1H), 7.42 (d, J = 9 Hz, 1H), 7.56 (t, J = 7 Hz, 1H), 7.63 (t, J = 7 Hz, 1H), 7.96−7.90 (m, 5 H), 8.08 (d, J = 9 Hz, 1H), 8.16 (d, J = 9 Hz, 2H), 8.26−8.21 (m, 2H); ^{13}C NMR (DEPT 135°) (126 MHz, MeOD) δ 12.4 (CH_3), 13.3 (CH_3), 27.8 (CH_3), 45.4 (CH_2), 50.9 (CH_2), 110.6 (CH), 111.2 (CH), 118.0 (CH), 120.8 (CH), 123.0 (CH), 125.0 (CH), 125.1 (CH), 127.7 (q), 127.8 (CH), 128.3 (CH), 129.9 (q), 130.9 (CH), 131.0 (CH), 131.4 (CH), 132.6 (q), 132.9 (q), 134.8 (CH), 140.0 (CH), 140.1 (q), 141.4 (q), 149.9 (CH), 153.5 (CH), 153.6 (CH), 155.4 (q), 155.5 (q), 170.6 (q); ESI HRMS $C_{32}H_{33}N_2^+$ 445.2644 calcd, 445.2660 found; UV/vis (MeOH) λ_{abs} = 674 nm, ϵ = 1.3 × 10^4 M^{-1} cm^{-1}, λ_{em} = 702 nm; HPLC (Phe-hex) t_R = 29.15 min, 76%.

2-{5-[3,3-Dimethyl-1-(3-sulfonatepropyl)-1,3-dihydroindol-2-ylidene]penta-1,3-dienyl}-1,3,3-trimethyl-3*H*-indolium (50): ^{1}H NMR (400 MHz, MeOD) δ 1.70 (s, 6H), 1.72 (s, 6H), 2.24 (q, J = 7 Hz, 2H), 2.98 (t, J = 7 Hz, 2H), 3.62 (s, 3H), 4.32 (t, J = 7 Hz, 2H), 6.28 (d, J = 13 Hz, 1H), 6.40 (d, J = 13 Hz, 1H), 6.66 (t, J = 13 Hz, 3H), 7.30−7.22 (m, 3H), 7.42−7.38 (m, 3H), 7.49−7.46 (m, 2H), 8.25 (t, J = 13 Hz, 1H); ^{13}C NMR (DEPT 135°) (126 MHz, MeOD) δ 19.3 (CH_3), 24.1 (CH_2), 27.8 (CH_3), 43.9 (CH_2), 50.7 (CH_3), 66.7 (CH_2), 104.3 (CH), 104.6 (CH), 111.9 (CH), 112.0 (CH), 123.3 (CH), 123.4 (CH), 126.1 (CH), 126.3 (CH), 127.0 (CH), 129.7 (CH), 129.9 (CH), 132.4 (CH), 133.6 (q), 142.4 (q), 142.6 (q), 143.6 (q), 144.3 (q), 155.7 (CH), 169.3 (q), 174.4 (q), 175.5 (q); ESI HRMS $C_{29}H_{34}N_2O_3SNa^+$ 513.2188 calcd, 513.2194 found; UV/vis (MeOH) λ_{abs} = 639 nm, ϵ = 1.2 × 10^4 M^{-1} cm^{-1}, λ_{em} = 664 nm; HPLC (Phe-hex) t_R = 24.26 min, >95%.

Potassium 1,3,3-trimethyl-2-[5-(1,3,3-trimethyl-5-sulfo-1,3-dihydroindol-2-ylidene)penta-1,3-dienyl]-3*H*-indolium iodide (51): ^{1}H NMR (400 MHz, MeOD) δ 1.72 (s, 6H), 1.73 (s, 6H), 3.57 (s, 3H), 3.68 (s, 3H), 6.20 (d, J = 13 Hz, 1H), 6.37 (d, J = 13 Hz, 1H), 6.64 (t, J = 13 Hz, 1H), 7.24 (d, J =

9 Hz, 1H), 7.37−7.31 (m, 1H), 7.45−7.42 (m, 1H), 7.52 (d, J = 7 Hz, 1H), 7.87−7.85 (m, 2H), 8.22 (t, J = 13 Hz, 1H), 8.29 (t, J = 13 Hz, 1H); ^{13}C NMR (DEPT 135°) (126 MHz, MeOD) δ 27.6 (CH_3), 27.9 (CH_3), 51.2 (CH_3), 54.4 (CH_3), 104.0 (CH), 105.7 (CH), 110.7 (CH), 112.4 (CH), 121.2 (CH), 123.4 (CH), 126.9 (CH), 127.2 (CH), 128.0 (CH), 129.8 (CH), 132.4 (CH), 133.6 (q), 142.1 (q), 142.6 (q), 142.9 (q), 144.1 (q), 145.9 (q), 154.9 (CH), 156.6 (CH), 169.3 (q), 174.2 (q), 176.9 (q); ESI HRMS $C_{27}H_{30}N_2O_3S^+$ 463.2055 calcd, 463.2056 found; UV/vis (MeOH) λ_{abs} = 638 nm, $\epsilon = 1.5 \times 10^4$ M^{-1} cm^{-1}, λ_{em} = 666 nm; HPLC (Phe-hex) t_R = 22.15 min, >95%

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Supporting Information Available: HPLC traces for all dyes and ^{1}H NMR for compounds **9**, **10**, **47**, and **48**. This material is available free of charge via the Internet at http://pubs.acs.org.

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