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Solid-Phase Catch, Activate, and Release Synthesis of Cyanine Dyes

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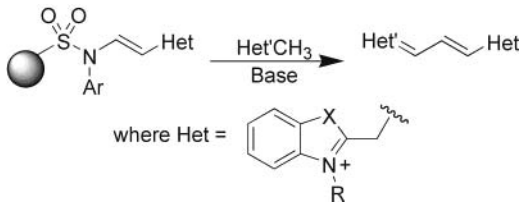
ABSTRACT



Trimethine cyanine dye was synthesized by capture and activation of a hemicyanine intermediate on sulfonyl chloride resin followed by reaction and concomitant cleavage by a heterocyclic carbon nucleophile. A small array of dyes were synthesized and characterized to demonstrate the versatility of this chemistry for a number of hemicyanines and heterocyclic nucleophiles.

Solid-phase organic synthesis and combinatorial chemistry have been used largely for the synthesis of peptides[1] and druglike molecules.[2] However, such approaches are finding broader applications in areas such as catalyst and materials discovery. In particular there are a number of recent examples of solid-phase synthetic routes to dyes.[3−5] These syntheses take advantage of solid-phase techniques to generate compounds in high purity with minimal purification. As such, they lend themselves well to the synthesis of large libraries of compounds that can be rapidly screened for molecules with desired properties.

Our focus has been to explore novel solid-phase synthetic routes toward cyanine dyes with a view to carrying out discovery chemistry. These dyes comprise a class of fluorescent compounds with the general structure shown in Figure 1. They consist of two nitrogen-containing heterocycles linked to each other by an odd-numbered polyene chain. Alkylation of both nitrogens imparts a net positive charge to this delocalized system. Structural diversity is possible by varying the polyene chain, the nitrogen substituents, or the heterocycles themselves. This allows the solubility, reactivity, and spectroscopic properties of these compounds to be fine-tuned for specific applications. As a result these compounds have found applications in various roles such as photographic sensitizers,[6] nonlinear optical materials,[7] and probes for biomolecular labeling.[8] In particlar their use in genetic analysis, DNA sequencing, and proteomics[9] is growing. There is considerable scope for discovery chemistry within this dye class given the new application areas. This endeavor would undoubtably benefit from combinatorial chemical approaches.

Solution synthesis of unsymmetrical trimethine dyes (n = 1), where the two heterocycles differ, is generally by the

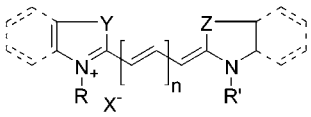


Figure 1. Cyanine dye structure. For dyes in general use Y, Z = CR_2, NH, O, S, $(CH)_2$. R = alkyl; n = 0−3.

(1) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149−2154.
(2) Andres, C. J.; Denhart, D. J.; Deshpande, M. S.; Gillman, K. W. *Comb. Chem. High Throughput Screening* **1999**, *2*, 191−210.
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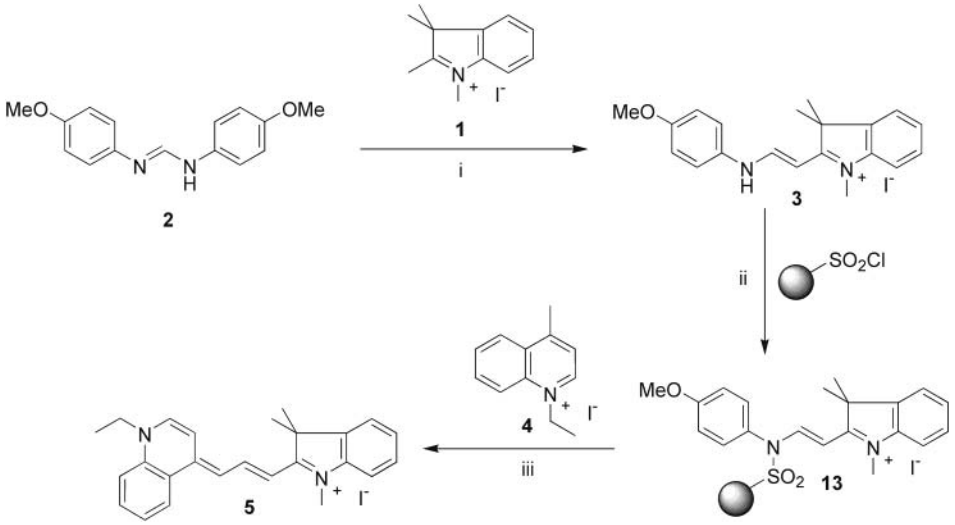
Scheme 1. Synthetic Route to Unsymmetrical Cyanine Dyes[a]



[a] (i) $(EtO)_3CH$, EtOH, 80 °C, 2 h; (ii) DIEA, DCM, rt, 4 h; (iii) DIEA, pyridine, rt, 30 min.

reaction of a methylene base such as **1** with a single-carbon synthon at the ester oxidation level, such as *N*,*N*′-diarylformamidine **2**, to give a hemicyanine intermediate of the form **3**.[10] However, intermediates such as **3** are susceptible to reaction with a second molecule of heterocycle to form contaminating symmetrical dye, which can be nontrivial to separate from the hemicyanine**.** The hemicyanine normally requires activation, for example, by N-acylation, to ensure efficient reaction with a second methylene base such as **4** to form the final product **5**. In solution, unsymmetrical dyes such as **5** can be nontrivial to separate from hemicyanines such as **3**.

In this letter we present a solid-phase synthetic route to unsymmetrical trimethine cyanine dyes. The chemistry is designed to minimize requirements for purification steps and enable discovery chemistry. The strategy employs hemicyanine intermediate prepared in solution using amidine chemistry. Simple washing is adequate to remove residual aniline and amidine. We envisaged activating the hemicyanine carbon toward a second nucleophilic attack by N-sulfonylation as demonstrated in solution by Peng et al.,[11] rather than the more traditional acylation step. This is consistent with the findings of Brooker et al. that the less available the nitrogen lone pair is for delocalization, the more reactive the hemicyanine derivative is to nucleophiles.[12] Furthermore, hemicyanine capture by polymer-bound sulfonyl chloride should selectively immobilize hemicyanine but not contaminating symmetrical dye, which is an important purification step. Reaction of bound hemicyanine with a second heterocyclic carbon nucleophile should lead to concomitant product cleavage to release pure cyanine dye product into solution. Indeed, an attractive feature of this strategy is the potential to visually or spectroscopically screen dye products directly as they form in free solution. Use of a substoichiometric quantity of the second heterocyclic nucleophile would minimize the level of contaminating starting material in the product mixture. Since such compounds are of high value and used in less than milligram quantities, consideration of purity and ease of purification is highly relevant to the synthetic process and also for discovery chemistry.

To explore and optimize the proposed chemical strategy, we first studied the synthesis of unsymmetrical dye **5** as summarized in Scheme 1. The precursor hemicyanine **3** was synthesized in solution from *N*,*N*′-bis(4-methoxy-phenyl)-formamidine **2** and heterocycle **1**. A variety of solvents, bases, and reaction times for hemicyanine capture were investigated. Reaction in DCM solution with DIEA gave by far the best result, with loadings of 91% and 89% by mass increase and nitrogen elemental analysis, respectively. Solvents, bases, reactant stoichiometries, reaction times, and temperatures were investigated for the reaction of captured hemicyanine. Crude products were analyzed by HPLC with evaporative light scattering detection and purities evaluated. Quantitation by this method avoids problems associated with differences in molar extinction coefficients for such compounds and impurities and is an appropriate analytical method for nonvolatile compounds.[13] For the dye formation/

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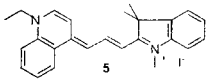
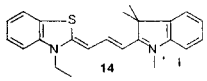
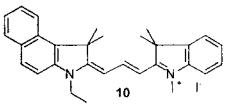
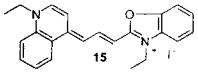
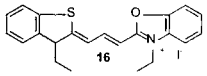
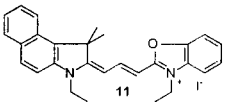
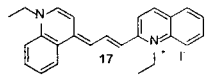
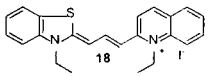
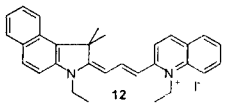
Table 1. Results of Dye Array Synthesis

compound	yield[a]	purity[b]	λmax abs / nm	ε / $M^{-1}cm^{-1}$	λmax em / nm
 5	79%	89%	599	4.8×10^4	647
 14	60%	91%	542	5.6×10^4	565
 10	-[c]	<1%	565	7.3×10^4	581
 15	43%	>95%	593	6.9×10^4	616
 16	23%	>95%	519	4.8×10^4	534
 11	32%[c]	50%	528	6.8×10^4	554
 17	42%	93%	653	1.1×10^5	665
 18	19%	84%	577	1.1×10^4	610
 12	-[c]	<1%	581	7.0×10^4	605

[a] Yield of crude product. [b] Crude purities measured by HPLC with evaporative light scattering detection. [c] See also ref 15.

release step we found that 30 min at room temperature in 1:9 DIEA/pyridine was sufficient to completely react 0.3 equiv of lepidinium salt **4**. This contrasts with the 45 min at reflux in 1:5 DIEA/pyridine used by Peng et al.[11] After being washed twice with water, the product **5** was obtained in 89% purity and 79% crude yield.

The synthesis of one compound having been demonstrated, the versatility of this strategy was tested by its application to the synthesis of a 3 × 3 array of unsymmetrical trimethine cyanine dyes. The six heterocycles shown in Figure 2 were chosen as a diverse set of building blocks. In addition to products with absorbances spanning a large part of the visible spectrum, we anticipated that their chemical diversity would test the robustness of the chemistry. Heterocycles **1**, **6**, and **7** were first reacted with *N*,*N*′-bis(4-methoxy-phenyl)-formamidine to form their respective hemicyanines analogous to **3**. While the compounds derived from **1** and **6** were readily purified by washing with acetone and diethyl ether, that derived from **7** could not be easily separated from contaminating symmetrical dye byproduct. Traces of amidine and aniline were removed by acetone washes, and this compound was used crude in the loading reaction, exploiting the selectivity of the loading reaction to remove the full-dye contaminant.

Resin loading of hemicyanine was carried out in DCM solution with DIEA base, and the products were reacted with 0.3 equiv of heterocycles **4**, **8**, and **9**. Crude product purities were analyzed by HPLC. Products were purified by simple flash column chromatography prior to analysis by FTIR, ^{1}H NMR, UV−vis, and fluorescence spectroscopic methods.[14] This last purification step was carried out for detailed product characterization but may not be necessary for discovery chemistry. The purity of crude products were generally high with the exception of compounds derived from heterocycle **9**, for which we have devised an alternative set of reaction conditions.[15]

(14) Known products had properties comparable to those in the literature (details in Supporting Information). Products **10**−**12** have not been previously described in the literature and were also characterized by ^{13}C NMR spectroscopy and HRMS.

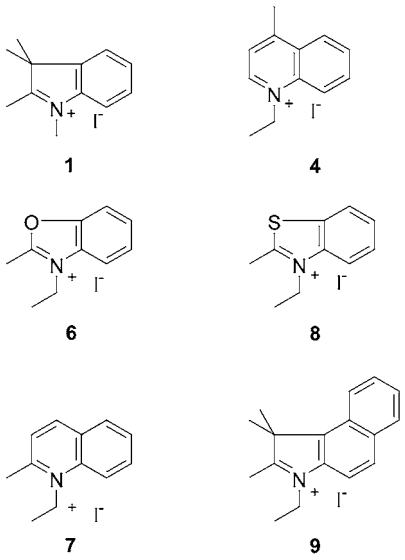


Figure 2. Heterocycles for array synthesis.

An earlier report by Isacsson et al.[3] describes an alternative solid-phase synthesis of cyanine dye. However, the approach we report herein is traceless with automatic product cleavage, thus eliminating a synthetic step and allowing rapid and direct screening of the resultant dye solution. In conclusion, we have demonstrated a novel catch, activate, and release approach to trimethine cyanine dyes. Subsequent synthesis of an array of such compounds has shown that high product purities are possible for a variety of compounds by this method. Work is underway to synthesize a larger library of dyes for discovery chemistry. The application of this strategy to other classes of cyanine dye, including pentamethine dyes, and more hydrophilic sulfonated compounds is also currently being explored.

Acknowledgment. This work was supported by a CASE award from Amersham Biosciences UK and the BBSRC. We thank James Nairne and Jon Cummins for valuable discussions and proof reading the manuscript.

Supporting Information Available: General experimental procedures and full characterization of new compounds **10**−**12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Improved yields and purities of compounds derived from **9** were obtained by prior deprotonation with NaH followed by reaction with immobilized hemicyanine in DCM (details in Supporting Information), although these conditions are less general.