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Report

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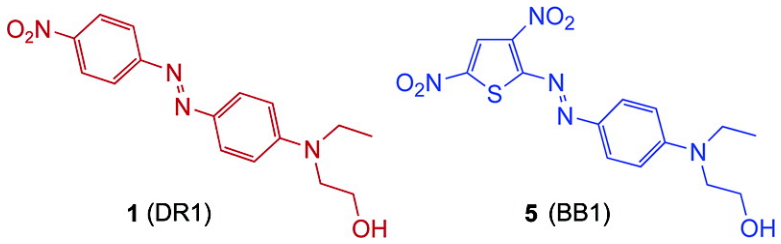

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Reports

A Blue Dye for Substrate Tagging in the Two-Color Screening of Combinatorial Libraries

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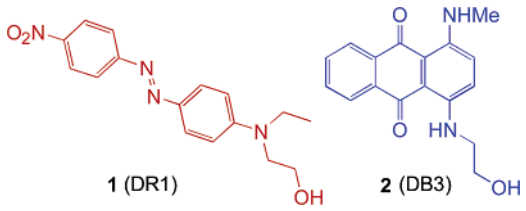
Split-and-mix combinatorial chemistry has made important contributions to the study of molecular recognition.[1] In systems for which fully rational design is unrealistic, the method allows large libraries of potential receptors or substrates to be tested in parallel. Screening is typically visual, employing colored targets that concentrate in beads bearing the most effective binding partners. Dye-labeling of targets is, therefore, an important part of the methodology. For studies in nonpolar solvents, such as chloroform, the most popular label has been the commercially available azo-dye Disperse Red 1 (**1**, DR1).[1a,j,2] This compound is hydrophobic, robust, and furnished with a hydroxyl group for attachment to a target species (typically via an ester linkage).

An elegant extension of the simple screening experiment is the two-color assay, capable of highlighting beads with exceptional selectivity (as opposed to affinity). For example, two substrates are labeled with complementary chromophores, and a library of receptors is exposed to a 1:1 mixture. Beads are now selected according to the purity of the color they accumulate. The group of Still have demonstrated the protocol by labeling a first substrate with DR1 and a second with Disperse Blue 3 (**2**, DB3). The method was used to optimize selectivity between peptide binding substrates in one case[3] and "pseudoenantiomeric" reactants in another.[4]



Despite its potential, the two-color screen has not been widely adopted. A major reason is that, in contrast to **1**, the blue label **2** has significant disadvantages. First, although commercially available, it is supplied in just 20% purity. Purification is complex and arduous, including a chromatographic step, and is difficult to perform on a large scale.[5] Second, the anthraquinone chromophore is less robust than the diazo system. It is vulnerable to oxidation, reduction, and deprotonation and is, thus, difficult to carry through a synthesis. Finally, DB3 is not an ideal partner for DR1 in the screening experiments. In the dye-label screening protocol, there is always a danger that a library member will bind the chromophore in the colored conjugate, rather than the "substrate" and, thus, generate a false positive. For the two-color screen, it is therefore advantageous if the labels are chemically and structurally similar; if binding to the chromophore does then occur, it should not be selective and should not register as a "hit".

A blue dye with more favorable properties would greatly increase the practicality of the two-color screening method. We now report such a compound, the azo-dye **5** (BB1).[6] Although **5** is structurally similar to **1**, the electron-poor dinitrothiophene unit shifts the absorption maximum to longer wavelength. This results in an intense blue color,

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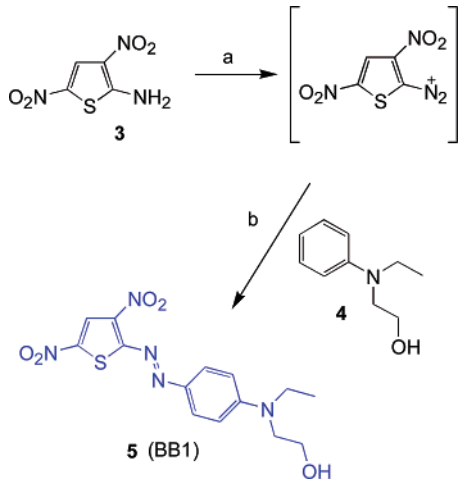
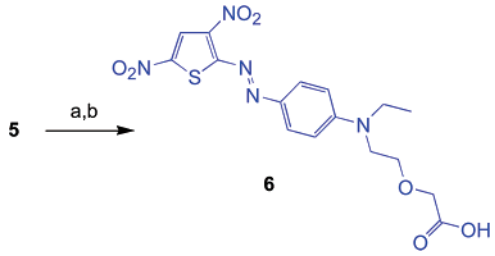
Scheme 1. Synthesis of Blue Diazo Dye **5**[a]



[a] (a) Nitrosylsulfuric acid, acetic acid, propionic acid, 0 °C, 90 min; (b) HCl (aq), 0 °C, 2 h, then 6 N NaOH; 45−49% yield.

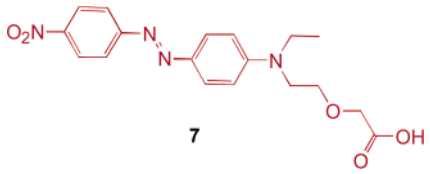
Scheme 2. Synthesis of Blue-Labeled Carboxylic Acid **6**[a]



[a] (a) *tert*-Butyl diazoacetate, $Rh_2(OAc)_4$ (cat.), DCM-toluene 40 °C, 2 h, then RT, 24 h; 43% yield; (b) TFA, DCM, 0 °C to RT, 5 h; 98% yield.

clearly differentiable from the red of **1**. As shown in Scheme 1, BB1 may be prepared in acceptable yield from commercially available starting materials **3** and **4**.[5] The procedure is adapted from that of Shen et al. for a related diazo compound.[7]

As a nitro-substituted azo-compound, BB1 is clearly a much-improved structural match for DR1; however, it needs to fulfill a number of further requirements in order to be useful in the two-color assay. First, it must be readily attached to potential substrates. The hydroxyl group in BB1 proved somewhat less nucleophilic than that in DR1, but it could be acylated without complication.[8] In addition, treatment of BB1 with *tert*-butyl diazoacetate in the presence of a rhodium (II) catalyst, followed by deprotection with TFA, gave the blue carboxylic acid **6**, as indicated in Scheme 2. This molecule can be used to label nucleophilic centers, serving as a counterpart for the red **7**.[9]



Second, the dye must have suitable optical properties. On one hand, the color must be clearly different from that of DR1; on the other, the extinction coefficients should be fairly similar to ensure that a mixture of the two dyes is

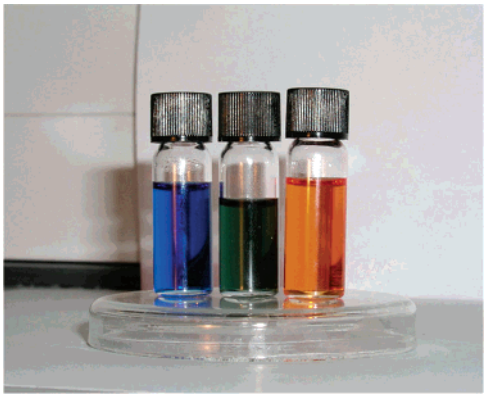


Figure 1. 0.1 mM chloroform solutions of (from left to the right): BB1 (**5**); 1:1 equimolar mixture of BB1 and DR1; and DR1 (**1**).

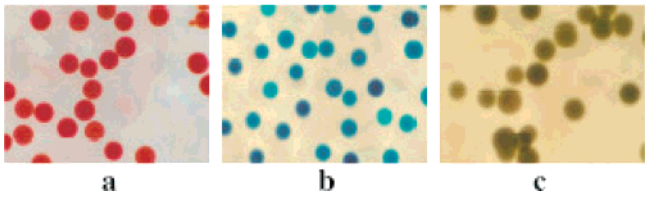
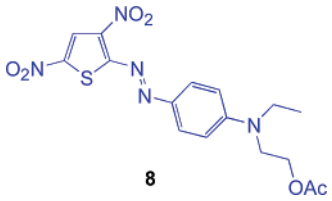


Figure 2. Acid-functionalized dyes absorbed onto aminomethyl Tentagel resin (0.4 mmol g^{-1}): a, DR1 derivative **7**; b, BB1 derivative **6**; c, equimolar mixture of **7** and **6**. The resin was agitated with solutions containing excess colored substrates in $CHCl_3$. They were then washed thoroughly with $CHCl_3$, filtered, and suspended in hexane before being observed under the microscope.

distinguishable from pure samples of either. DR1 shows λ_{max} at 483 nm (ϵ 38 200 $M^{-1}\cdot cm^{-1}$) in ethanol, whereas the corresponding absorption for BB1 is at 633 nm (ϵ 31 800).[5] As shown in Figure 1, chloroform solutions of **1** and **5** contrasted strongly with each other and were clearly differentiable from the deep green-gray equimolar mixture.

A similar result was obtained when the dyes were absorbed on resin beads. Thus, high-loading aminomethyl polystyrene or Tentagel resins were exposed to chloroform solutions containing (a) blue acid **6**, (b) red acid **7**, and (c) an equimolar mixture of the two. As shown in Figure 2 the dyes concentrated in the beads, presumably through ammonium carboxylate formation. Again, the mixed and pure dyes were clearly distinguishable. These images should mimic the appearance of "hits" (blue or red) and of strong-binding but unselective beads (grey-green) in the two-color screening application. As expected, the blue chromophore of **6** gives a usefully intense color, sufficient for detection at moderate binding levels but not so extreme that the beads appear black (Figure 2).

Finally, the chromophore should not exhibit any noncovalent affinity for the polymeric matrix of the beads under the proposed screening conditions (nonpolar organic solvents). To test this possibility, the aminomethyl polystyrene and Tentagel resins were N-acetylated, and **5** was O-acetylated to give **8**. Addition of **8** in chloroform to the two resins, agitation for 30 min, filtration, and brief washing (chloroform/hexane, 70:30) gave colorless beads. As expected,[3] repetition of the experiment with O-acetylated DR1[10] gave the same result.



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In conclusion, BB1 (**5**) seems ideally suited to partner DR 1 (**1**) in 2-color screening methodology. It is readily accessible and chemically similar to **1**, so that parallel syntheses of substrate pairs should proceed without difficulty. The two dyes are also structurally similar, reducing the chance of false positives in library screening. In the future, we plan to exploit these compounds in further applications of the methodology, in particular, the discovery of enantioselective receptors from split-and-mix combinatorial libraries.

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Supporting Information Available. Experimental details for the syntheses of **5**, **6**, and **8** and for the purification of **2**. UV spectra of **1** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) For further details, see the Supporting Information.

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(8) For example, treatment of **5** with Ac_2O/DMAP gave **8** in ~4 h. Under the same conditions, acetylation of **1** was complete in ~40 min.

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