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Visual Monitoring of Solid-Phase Extraction Using Chromogenic Fluorous Synthesis Supports

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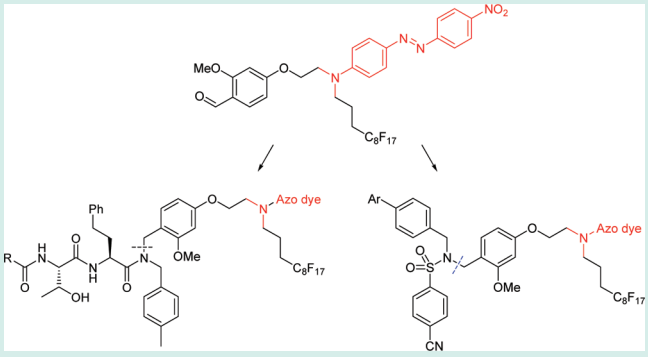
Supporting Information

ABSTRACT: Reductive aminations and further transformations of an azo dye and fluorous tagged aldehyde are described. The intensely colored 2,4-dialkoxybenzyl protected amines undergo Fmoc-based peptide coupling, Suzuki reactions, and sulfonamide formation with product isolation facilitated by visual monitoring of fluorous solid phase extraction. Target compounds are released from the supports in high yields and purities by treatment with trifluoroacetic acid (TFA).



KEYWORDS: *fluorous, synthesis support, azo dye, solid-phase extraction*

Since its introduction by Curran and co-workers,[1] light fluorous tagging (usually defined as a single tag with no more than 21 fluorine atoms) of reagents and substrates coupled with fluorous solid-phase extraction (F-SPE)[2] has become a valuable component of solution phase parallel synthesis.[3] The technique takes advantage of the selective interactions between highly fluorinated molecules to effect partitioning of the fluorous tagged components into the fluorous stationary phase leading to rapid isolation and purification without the need for laborious conventional extraction and chromatography procedures. In cases where the substrate carries the fluorous tag, reaction mixtures are applied to fluorous silica gel cartridges and the unretained byproduct diverted to waste. The desired fluorous tagged product is then eluted from the cartridge using a fluorophilic solvent and advanced to the next step in the synthesis. Ideally, the SPE process (as distinct from chromatography) affords only one fraction for collection and analysis, and detailed experimental procedures have been published describing loading capacities, optimal flow rates, solvent compatibilities, and eluent strength.[4] Optimization of the elution step may be required, however, to prevent leaving compound behind on the cartridge because of low solubilities or, in the case of basic molecules, adsorption at uncapped silanol residues of the base silica gel.[5] Moreover, solubilities and basicities may not be uniform across the members of a parallel synthesis library and, when using conventional tags, it is only after evaporation and gravimetric analysis that product recoveries are determined. Retaining cartridges for further elution and waste tubes to guard against losses in the loading and first pass elution steps can be inconvenient especially when processing multiple samples in parallel.

In principle, the partition and subsequent elution of a fluorous-tagged substrate from an SPE cartridge can be monitored, instantaneously, using a colored fluorous tag. Thus, in a manual or automated fluorous facilitated synthesis the operator can intervene if not all the colored material is captured in the loading step or does not fully elute from the cartridge in the expected volume of solvent. For example, if the SPE cartridge remains colored after the elution step additional solvent(s) can be added to elute all the color. While fluorous dyes have been reported previously,[6−10] there do not appear to be any reports of derivatives that also incorporate a reactive functional group for use as a synthesis support or linker (to use solid-phase terminology). Although there are many permutations for a trifunctional molecule comprising a fluorous tag, chromophore, and linker, it is important to retain the fluorous character and high solubility in organic solvents that characterize conventional tags. Permanently charged or readily ionized dyes and composite molecules with a large number of hydrogen bond donors were therefore avoided in the prototype design illustrated in Figure 1. Thus, the nitrogen atom of aniline **1** is substituted with a fluorous tag via electronically insulating methylene groups and with a dialkoxybenzaldehyde linker analogous to those used in solid-phase chemistry[11,12] and previously incorporated into aldehyde **3**,[13] and related fluorous compounds.[14] Chromophores can then be appended by electrophilic substitution in the aniline ring of **1**. In this Note, the synthesis and properties of azo dye derivative **2** are described as well as applications in two synthetic schemes facilitated by visual monitoring of the F-SPE process.

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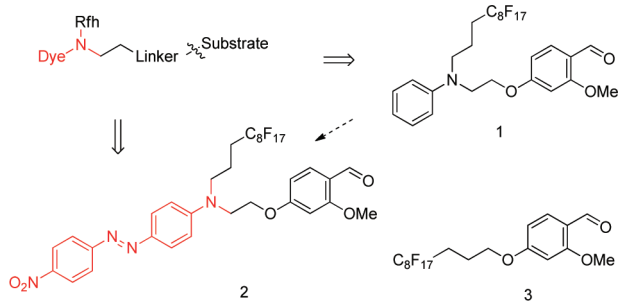
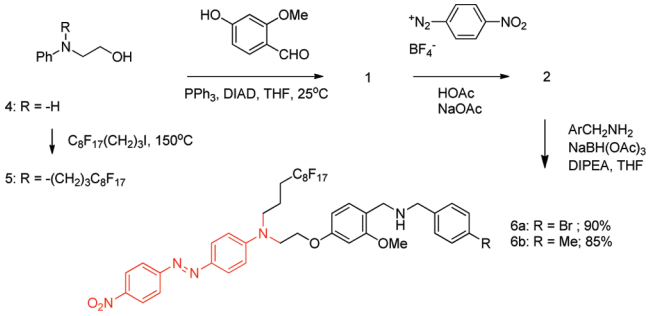


Figure 1. Design of chromogenic fluorous dialkoxybenzaldehyde **2** and comparison to previously reported linker **3**.[13] Rfh denotes a fluorous tag with intervening methylene groups.

Reaction of *N*-hydroxyethylaniline (**4**) with 3-(perfluorooctyl)propyl iodide in a high temperature melt afforded **5** which was subjected to a Mitsunobu reaction with 4-hydroxy-2-methoxybenzaldehyde to give **1** in high purity after F-SPE (Scheme 1). On a larger scale this compound was purified by normal phase chromatography on silica gel. Reaction of **1** with 4-nitrobenzene diazonium tetrafluoroborate in aqueous acetic acid gave derivative **2** in high yield and purity after recrystallization from acetonitrile. These reactions can readily be conducted on a multigram scale. Compound **2** exhibits typical light absorption properties for a donor–acceptor azo dye with λmax 463 nm (ε = 33,000 M^{-1} cm^{-1}) in tetrahydrofuran (THF) solution. Unlike aldehydes **1** and **3**, azo derivative **2** is only sparingly soluble in dichloromethane, methanol, or acetonitrile but has acceptable solubility in THF (25 mM solutions can be prepared by gentle heating). In this solvent, clean reductive amination with substituted benzylamines was accomplished affording amines **6a–b** with generally higher solubility. Although amines **6a–b** have short retention times in reverse phase chromatography (C-18), when applied in dimethylformamide (DMF) solution to fluorous silica gel that had been equilibrated with aqueous methanol, the red band was retained in the stationary phase. Subsequent treatment with methanol did not effectively elute the red product band because of solubility limitations. Addition of THF resulted in an intensely red solution but failed to remove all the color from the stationary phase, even after multiple elutions. Further elution with 2 M methanolic ammonia-THF mixtures did, however, recover the remainder of the colored material suggesting that the secondary amino group of **6** was adsorbing to the base silica gel (which would not have been apparent using a conventional tag). UV spectroscopic analysis suggested that when 110 mg (110 μmol) of **6b** was subjected to F-SPE on a 5 g cartridge some 35% was retained by adsorption rather than partition into the fluorous phase (see Supporting Information). It was also apparent from this experiment that a solution of **6b** at a concentration of 5 μM eluting from the base of the cartridge was the lower limit visible to the naked eye.

The utility of fluorous-chromogenic amine **6b** for the synthesis of C- and N-capped peptides was investigated (Scheme 2). HATU mediated coupling between a slight excess of Fmoc-S-homophenylalanine and **6b** in DMF-THF solution

Scheme 1. Synthesis and Reductive Amination of Chromogenic Fluorous Aldehyde



Scheme 2. Synthesis of Capped Peptides on Chromogenic Fluorous Support

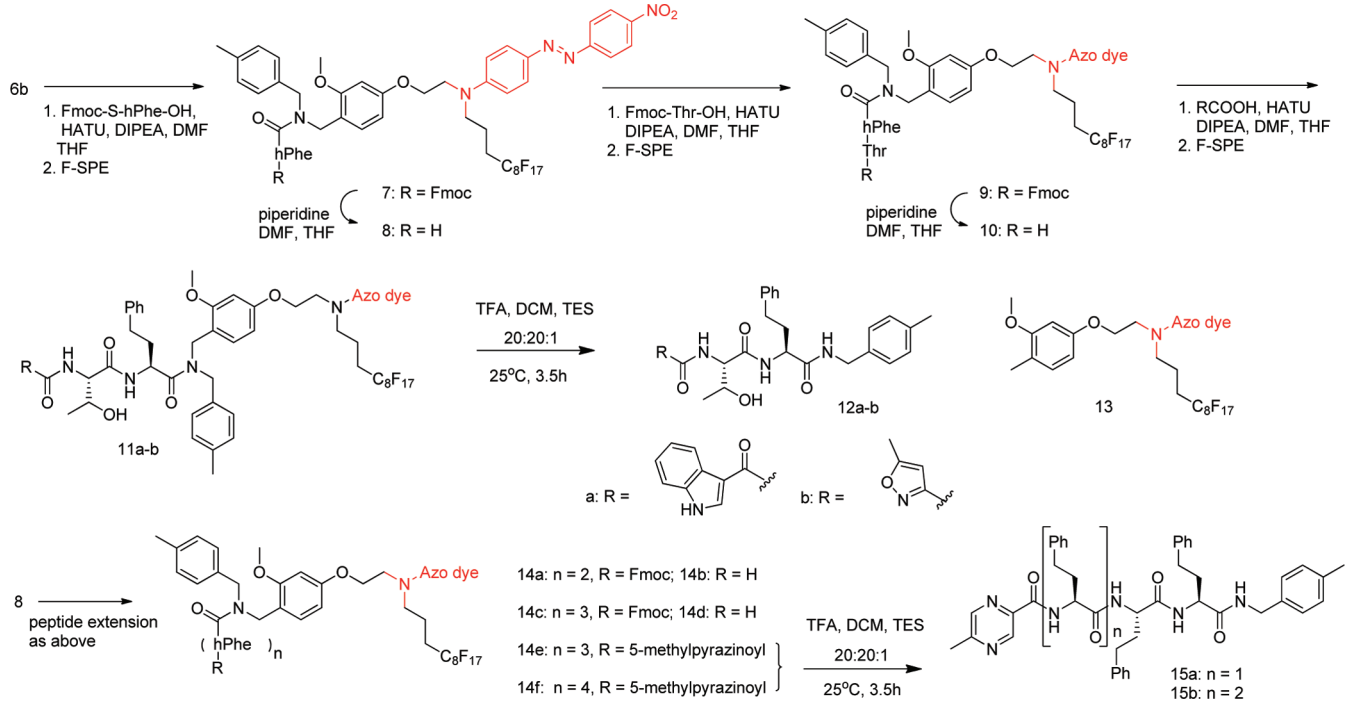


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Table 1. Synthesis of Capped Peptides on Chromogenic Fluorous Support[a]

compound	yield (%)	purity (%)	compound	yield (%)	purity (%)	compound	yield (%)	purity (%)
7	96	90	**12a**	91	70	**14e**	86	95
8	87	>95	**12b**	60	80	**14f**	70	95
9	75	>95	**14a**	85	95	**15a**	72	89
10	95	95	**14b**	95	95	**15b**	55	80
11a	88	85	**14c**	92	95			
11b	69	>95	**14d**	95	90			

[a]Yields and purities refer to products isolated by F-SPE; purities determined by HPLC-MS from DAD trace as detailed in Supporting Information.

(0.1 M in **6b**) was complete within 10 min as monitored visually by reverse phase tlc (conducted on C-18 plates eluting with 95:5 MeCN:water). The reaction mixture was subjected to F-SPE eluting the colored band quantitatively with THF to give **7** in high yield and purity according to LC-MS analysis (Table 1). Treatment of **7** with piperidine in DMF led to rapid removal of the Fmoc protecting group; solvent, piperidine, and byproduct were removed in the first pass of the F-SPE process in aqueous methanol and subsequent elution with THF afforded the desired product **8** in high yield and purity. Extending the sequence using Fmoc-Thr-OH gave **9** which was deprotected to give fluorous tagged dipeptide **10**.[15] N-terminal capping of **10** was next conducted with two carboxylic acids and the products **11a–b** again isolated by F-SPE facilitated by visual monitoring (Figure 2) which confirmed quantitative retention

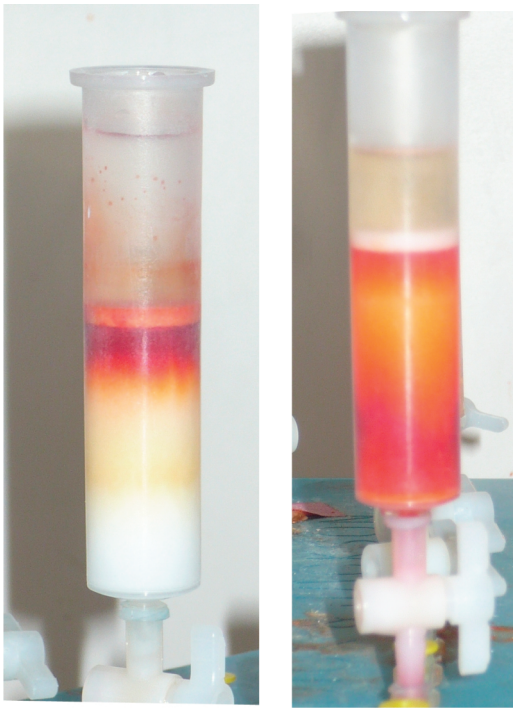

Figure 2. (a) Fluorous SPE capture of **11a** (reaction scale 40 mg (32 μmol) of amino acid **10**), on 5 g *Fluoroflash* cartridges and elution of impurities with MeOH-water (9:1). (b) Elution of **11a** by addition of fluorophilic solvent THF.

of the desired fraction in the first pass elution and complete elution by addition of the fluorophilic solvent THF. The purities of the fluorous tagged compounds are satisfactory and readily assessed by LC-MS (Table 1).

Treatment of **11a–b** with trifluoroacetic acid (TFA) in dichloromethane in the presence of triethylsilane led to pronounced bathochromic shifts consistent with formation of an azonium ion and released the peptides from the chromogenic tag within 4 h. Subsequent filtration of these mixtures in aqueous methanol over fluorous silica gel afforded the proteasome inhibitors[16,17] **12a–b** in moderate purity. Peptides of purity >95% were obtained in the case of **12b** by trituration with ether while **12a** required reverse-phase chromatography. Further elution of the cartridge with THF gave a colored fraction consisting of one major product, **13**, isolated in 80% yield, corresponding to hydride transfer from the silane scavenger to the carbocation-like species formed in the detagging process.

To test if the fluorous character of these constructs is retained for higher molecular weight substrates, tagged amino acid **8** was extended to the Fmoc-protected hPhe trimer **14c**, deprotected to give **14d** and then capped with a pyrazine carboxylic acid to give **14e**. All three of these derivatives were successfully isolated by F-SPE as for their dipeptide analogues. Elaboration of **14d** to tetramer **14f** was also accomplished[18] and, although only 18.5% of the mass of **14f** is contributed by fluorine, isolation by F-SPE was still effective. Subsequent TFA induced detagging of **14e** and **14f** afforded proteasome inhibitors **15a** and **15b** in moderate purity which could be improved to >95% by trituration with ether. It is also of interest that analytical and preparative normal or reverse phase chromatography can be conducted on the tagged peptides shown in Scheme 2, processes that would be tedious in the absence of the tags because of low solubilities and the need for tlc stains or UV detectors.

A second demonstration case involved the conversion of colored fluorous aryl bromide **6a** to biaryl sulfonamides **16** as depicted in Scheme 3. Suzuki reaction of **6b** with excess 2-fluorophenylboronic acid proceeded readily in THF solution under microwave irradiation to give **17a** in high yield and purity after F-SPE (Table 2). Subsequent treatment with 4-cyanobenzenesulfonyl chloride and DMAP gave **18a** in high purity after F-PSE. Acid induced detagging of **18a** afforded the biarylsulfonamide **16a** isolated in high yield and purity in the void volume from F-SPE with the colored fluorous tag (**13**) remaining in the fluorous stationary phase. The results from reaction sequences starting with two additional boronic acids were similar and comparable to the same reactions conducted on fluorous linker **3** reported previously by Ladlow and coworkers[13] indicating that the azo dye is fully compatible with the synthetic sequence.

After multiple use of F-SPE cartridges for isolation of the products shown in Schemes 2 and 3, a red residue built up on the base support; while this affects the ability to discern small (microgram) amounts of compound remaining on the cartridge, it does not affect observations of solutions eluting from the cartridge, which as noted previously, has a visual detection limit of about 5 μM.

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Scheme 3. Suzuki and Sulfonylation Reactions on a Chromogenic Fluorous Support

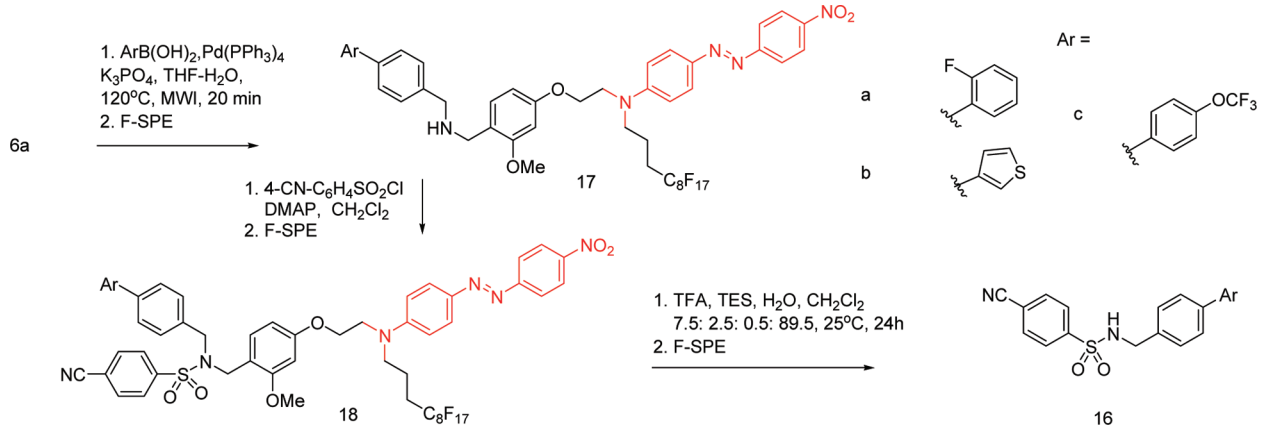

Table 2. Suzuki and Sulfonylation Reactions on a Chromogenic Fluorous Support[a]

compound	yield (%)	purity (%)	compound	yield (%)	purity (%)	compound	yield (%)	purity (%)
17a	82	90	**18a**	95	90	**16a**	79	94
17b	88	90	**18b**	95	88	**16b**	75	95
17c	85	85	**18c**	92	95	**16c**	82	95

[a]Yields and purities refer to products isolated by F-SPE; purities determined by HPLC-MS from DAD trace as detailed in Supporting Information.

In summary, a synthesis support consisting of a fluorous tag, azo dye, and dialkoxybenzaldehyde linker has been prepared in three steps from commercially available starting materials and subjected to reductive amination, peptide and sulfonamide formation with comparable efficiency to reactions involving conventional tags. The intense color of the azo dye, which does not adversely affect the fluorous character of tagged products, permits simple real-time optimization of the capture and elution steps in F-SPE without the need to collect and analyze fractions. Reaction progress and product purities are conveniently monitored by normal or reverse phase tlc or by LC-MS. In addition, hydrophobic peptides that are difficult to handle under conventional solution phase conditions because of low solubility and UV absorption are rendered soluble in solvents such as toluene, dichloromethane, and THF and visible to the naked eye. This design can be generalized by appending alternative synthesis linkers, such as ones for which the original functional group is regenerated in the detagging process, to the side arm of aniline **1**. Attachment of other dyes to the aryl ring as appropriate for compatibility with the synthesis scheme can be envisioned.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and characterization data for new compounds. ^{1}H and ^{13}C spectra for representative compounds. Images of capture and elution of **6b** from a fluorous SPE cartridge. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(15) See Supporting Information for synthetic details. Satisfactory elemental analyses were obtained for **6a** and **6b** after filtration of DCM solutions over a short column of neutral alumina eluting with EtOAc-hexane mixtures and for **7** and **9** after recrystallization from MeCN. Further purification of **8**, **10**, and **11** is readily effected by filtration of MeCN solutions over a C-18 cartridge while compounds **14** can be purified further by filtration over silica gel.
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