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NOVEL SYNTHESIS OF XANTHENE AND BENZOXANTHENE DYES

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by

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Undergraduate honors thesis under the direction of

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Novel Synthesis of Xanthene and Benzoxanthene Dyes

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Abstract

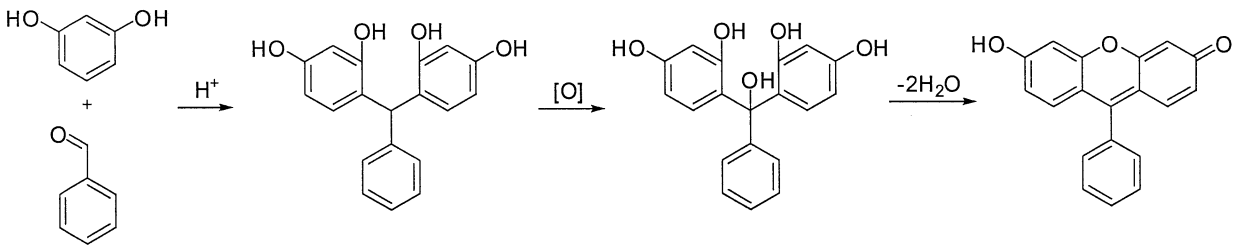
A facile synthetic route utilizing readily available reagents affords a series of regioisomerically pure xanthene dye derivatives. Advantages include relatively mild conditions and good to excellent yields. Non-polar, highly crystalline intermediates are isolable by standard chromatographic techniques. The intermediates are in the requisite xanthene oxidation state, thus avoiding the need for relatively inefficient oxidation chemistry and/or harsh conditions. The methods described herein have been successfully utilized in our lab towards the synthesis of new benzoxanthene dye architectures currently unattainable via other methodology.

** The research contained in this thesis was conducted with Dr. Youjun Yang during 2005-2006 and are published in Yang, Y.; Escobedo, J. O.; Wong, A.; Schowalter, C. M.; Touchy, M. C.; Jiao, L.; Crowe, W. E.; Fronczek, F. R.; Strongin, R. M. A Convenient Preparation of Xanthene Dyes. *J. Org. Chem.* **2005**, 70(17), 6907-6912 and Yang, Y.; Lowry, M.; Schowalter, C. M.; Fakayode, S. O.; Escobedo, J. O.; Xu, X.; Zhang, H.; Jensen, T. J.; Fronczek, F. R.; Warner, I. M.; Strongin, R. M.; An Organic White Light-Emitting Fluorophore. *J. Am. Chem. Soc.* **2006**, 128(43), 14081-14092.

Introduction

Research has led to numerous applications for fluorone derivatives as agents for molecular recognition and inhibitors for biological systems. Among these applications include the detection of metal ions,[1] sialic acid,[2] homocysteine,[3] HIV-1 nucleocapsid protein,[4] and applications for screening assays in mitochondrial permeability,[5] and acetylcholinesterase inhibition.[6]

Classic fluorone synthesis was first reported by Mohlau and Koch in 1894. It involves condensation of resorcinol with an aldehyde under thermal and acid-catalyzed conditions to form the carbinol leuco base followed by oxidation and dehydration of the carbinol leuco base to produce the dye (Scheme 1).[7]



Scheme 1. Classic fluorone dye synthesis reported by Mohlau and Koch.

Complications involved with the Mohlau and Kock methodology include low-yields, by-product formation and troublesome purifications resulting from high polarity of the products.[7] Various improvements of this method were reported by Neckers et al., Lippard et al., and Bergess et al., however, harsh conditions, low-yields and troublesome purifications were retained in general.[8,9,10]

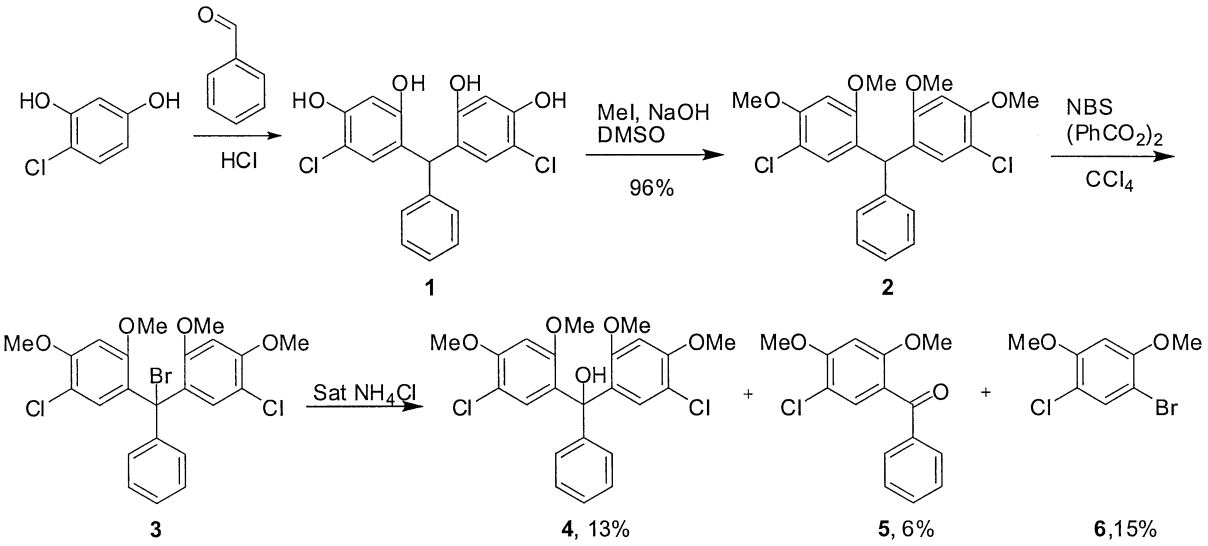
Benzofluoresceins, fluorescein derivatives, are greatly desired for use in ratiometric intracellular pH measurement due to their near neutral pH, long wavelength absorption and emission and clear isosbestic and iso-emmisive points. These properties eliminate complications arising from absorption and emission of the biological media interfering with the fluorescence

signal. Intracellular pH measurement is useful on the cellular level for the detection of pH variation in endocytic events, calcium regulation, cell growth, chemotaxis, cell adhesion, and other cellular processes. [11]

Results and Discussion

Xanthene-dye Synthesis[12]

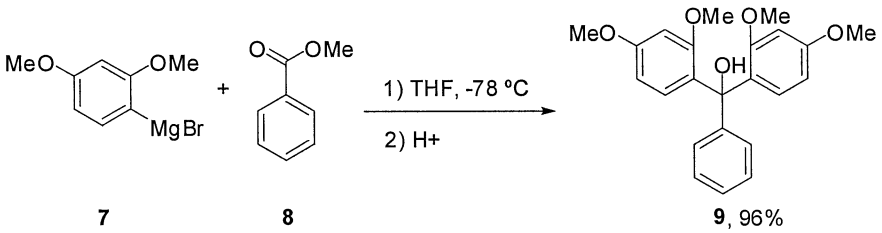
It was proposed that if the methylated form of the oxidation product, carbinol, could be prepared, the dye could be obtained through a simple demethylation. During the initial synthesis, benzylic bromination of the fully methylated carbinol leuco base was applied using N-bromosuccinimide (NBS). However, the carbinol dye precursor **4** was only obtained in a 13% yield after hydrolysis due to the existing side-reactions (Scheme 2).



Scheme 2. Synthesis of the methylated carbinol **4**. Compound **1** is synthesized by known methodology to afford **2**. Benzylic bromination of **2** by NBS in the presense of benzoyl peroxide generates **3**, which is hydrolyzed *in situ* to yield **4**. **5** and **6** are undesired by-products isolated.

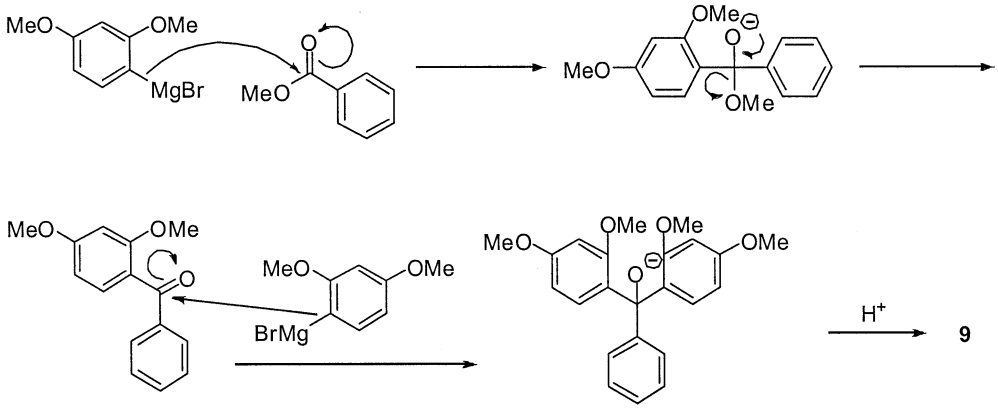
To optimize the yield, an alternative synthesis of the methylated carbinol precursor to the xanthene dye was developed involving a simple Grignard addition to benzoates. By this method, the carbinol precursor **9** is synthesized directly from a one-step process, reacting 2,4-

dimethoxybenzenemagnesium bromide **7** with methyl benzoate **8** in tetrahydrofuran (THF). THF is a preferred solvent for Grignard reactions as it does not contain acidic hydrogens that can interact with the Grignard reagent. The Grignard addition is followed by an acid work-up. Compound **9** is obtained in excellent yield, 96% (Scheme 3). Importantly, it can be easily purified via a variety of standard techniques such as recrystallization and column chromatography.



7 **8** **9**, 96%

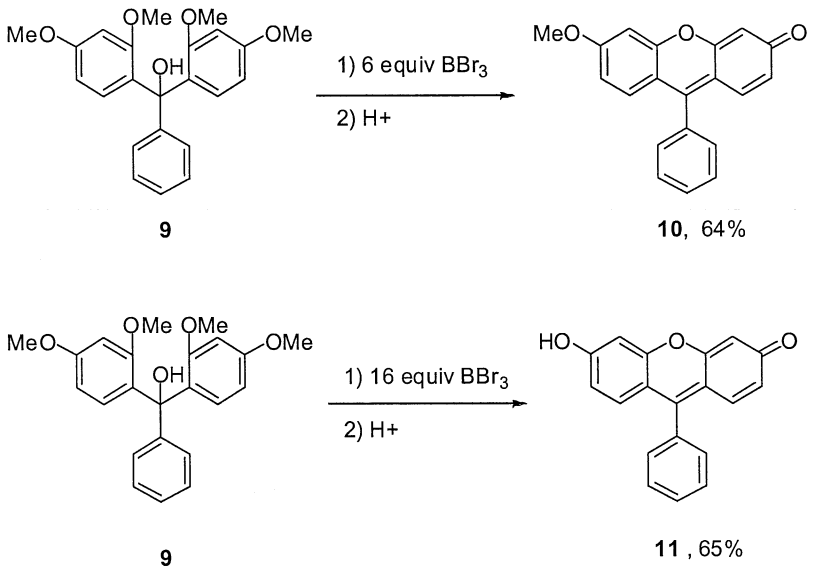
Scheme 3. Preparation of the methylated carbinol dye precursor by Grignard addition.



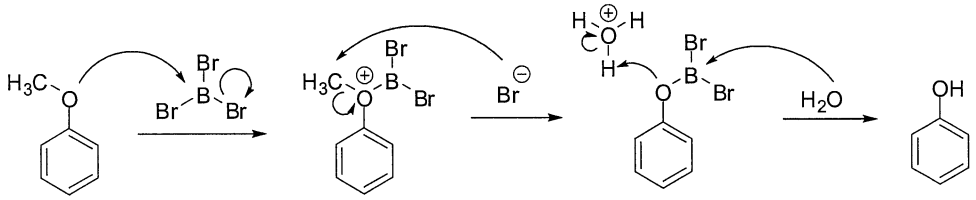
Scheme 4. Arrow-pushing mechanism of the synthesis of **9** via Grignard addition.

Upon treatment of **9** with six equivalents of boron tribromide (BBr_3), monomethyl ether **10** is obtained in a 64% yield. When sixteen equivalents of BBr_3 are used, the fully demethylated xanthene dye **11** is obtained in a 65% yield (Scheme 5). The appropriate number of equivalents of BBr_3 required for complete demethylation of the carbinol precursor was determined through trial and error. The arrow pushing mechanism for the demethylation via BBr_3 is shown in Scheme 6. Xanthene dye **11** does not dissolve in either CH_2Cl_2, the solvent for

demethylation or H_2O, it will precipitate from the solution when hydrolyzed. Good purity dye can be isolated by a simple suction filtration and washed with distilled H_2O. Column chromatography is able to provide analytically pure sample for further studies.



Scheme 5. Synthesis of fluorone dye **11** via demethylation of the carbinol precursor **9** by 16 equivalents of BBr_3 to achieve total demethylation.

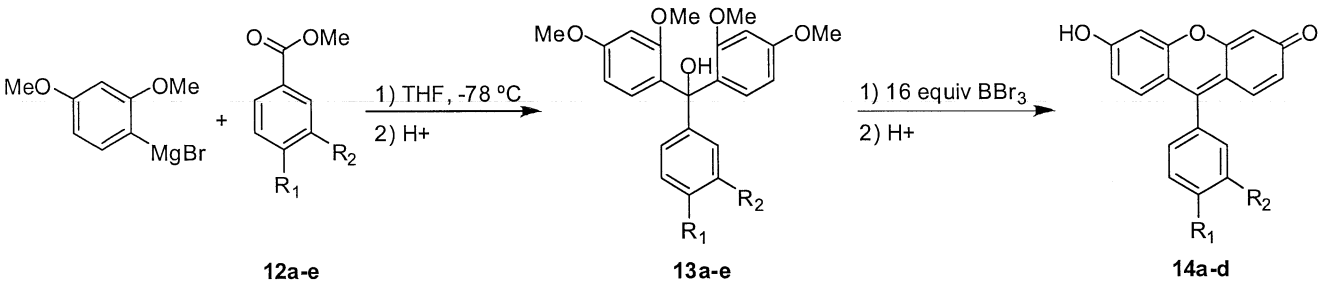


Scheme 6. Arrow pushing mechanism for the demethylation by BBr_3.

The simple method of fluorone dye synthesis via Grignard addition and demethylation sequence illustrated in Scheme 3 and Scheme 5 was applied to a series of different benzoates (Table 1). Corresponding carbinols are obtained and easily purified via standard flash chromatography in good to excellent yields (83-99%). Corresponding fluorone dyes are furnished by demethylation of carbinols by BBr_3 in good yield (70-96%). An important feature of this reaction is that the products collected via suction filtration after hydrolysis and purified

simply by washing with distilled water are already of sufficient purities for most of the analytically studies.

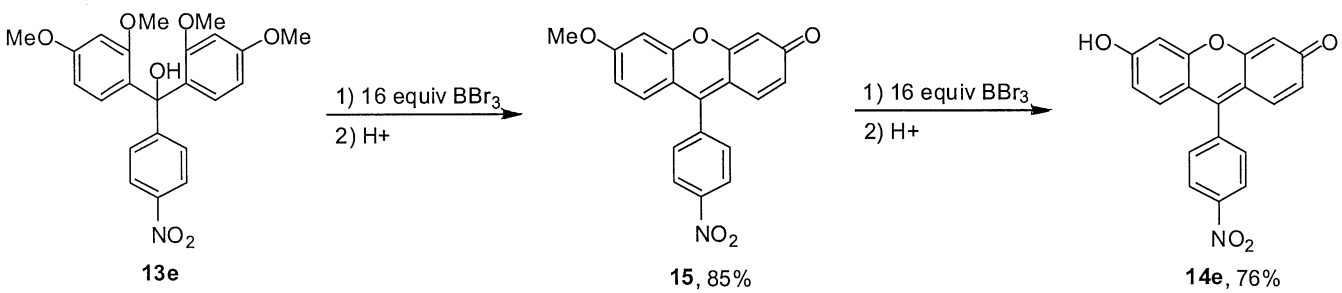
Table 1. Preparation of fluorone dyes followed Grignard addition/demethylation sequence.



12a-e **13a-e** **14a-d**

Entry	Substrate	R_1	R_2	Carbinol	Carbinol yield (%)	Fluorone	FluoroneYield (%)
1	**12a**	Br	H	**13a**	92	**14a**	70
2	**12b**	Ph	H	**13b**	99	**14b**	87
3	**12c**	OMe	H	**13c**	83	**14c**	96
4	**12d**	H	NO_2	**13d**	91	**14d**	73
5	**12e**	NO_2	H	**13e**	95	**14e**	

The demethylation of xanthene dye precursor **13e** is a special case in the series of xanthene dye syntheses by the novel method. Addition of sixteen equivalents of BBr_3 leads to monomethyl ether **15**, which is not fully demethylated. A second demethylation of **15** by BBr_3 is performed in order to synthesize **14e** (Scheme 7).

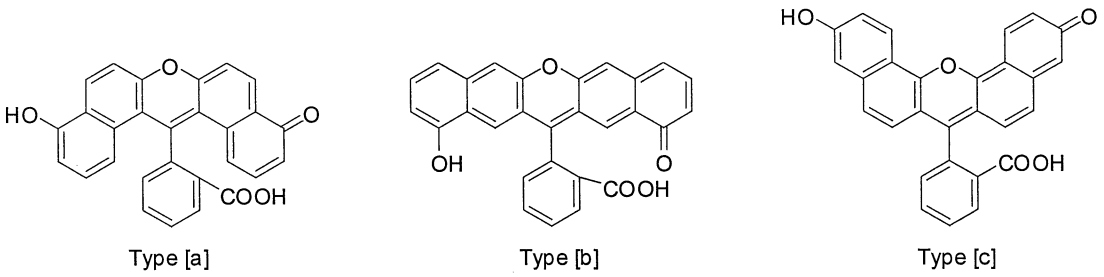


13e **15**, 85% **14e**, 76%

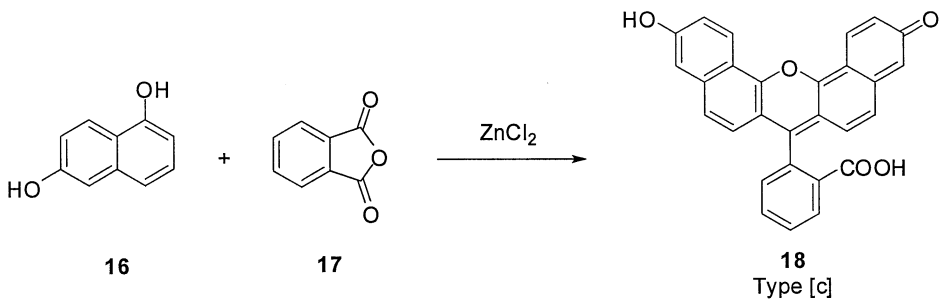
Scheme 7. Two step demethylation of **13e** by BBr_3.

Benzoxanthene Dye Synthesis[13, 14]

There are three possible regio-isomers of naphthofluoresceins (Scheme 8).[11] However, only the synthesis of type [c] regio-isomer is reported via condensation of 1,6-dihydroxylnaphthalene and phthalic anhydride under acid catalyzed conditions at high temperature (Scheme 9). Type [a] and [b] isomers cannot be synthesized via classical condensation under thermal conditions and their synthesis is not reported.



Scheme 8. Three regio-isomers of naphthofluorescein.

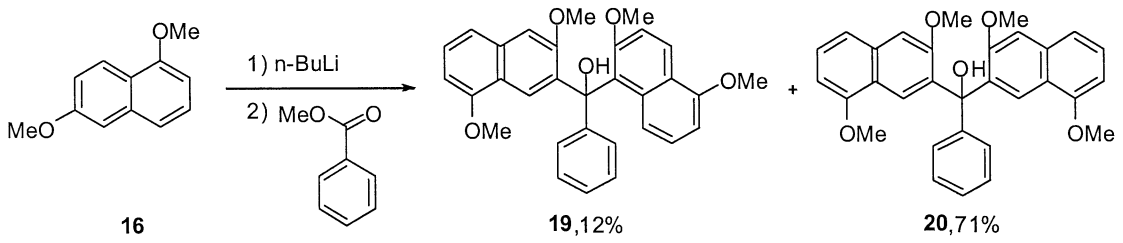


Scheme 9. Classical synthesis of naphthofluorescein under thermal conditions.

The synthesis of novel type [a] and [b] naphthofluorescein dye precursor frameworks are mainly motivated by theoretical studies performed by Wolfbeis et al., who predicted that they would display considerably longer absorption and emission wavelengths compared to their type [c] isomer.[11]

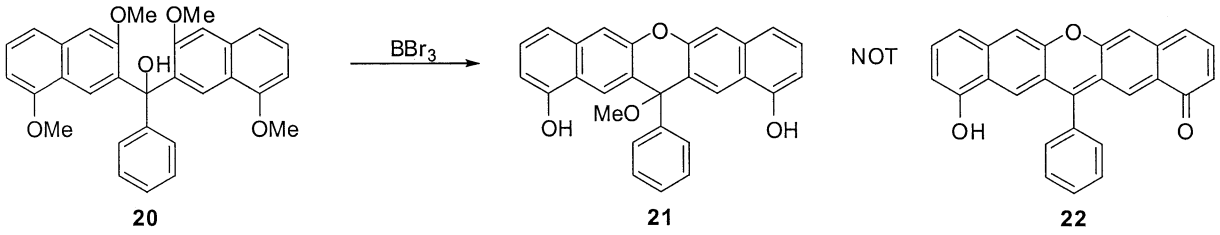
Grignard reagent of 1,6-dimethoxynaphthalene could not be synthesized readily. Thus, lithiation of 1,6-dimethoxynaphthalene was used instead to generate the initial nucleophilic

attack for the synthesis of carbinol compounds. Reaction with methyl benzoate furnishes **19** and **20**, which are precursors of Type [a] and Type [b] benzoxanthene (Scheme 10).



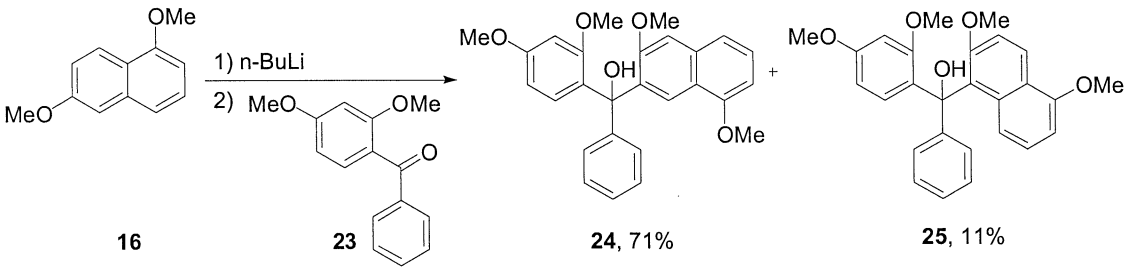
Scheme 10. Methylated naphthofluorone precursors for Type [a] and Type [b] isomers are obtained in 12% and 71% yields respectively.

Complications were retained in pursuit of the novel naphthofluorone dye **22** by demethylation of the corresponding carbinol precursor **20** due to the ease of forming the solvent adduct (Scheme 11).



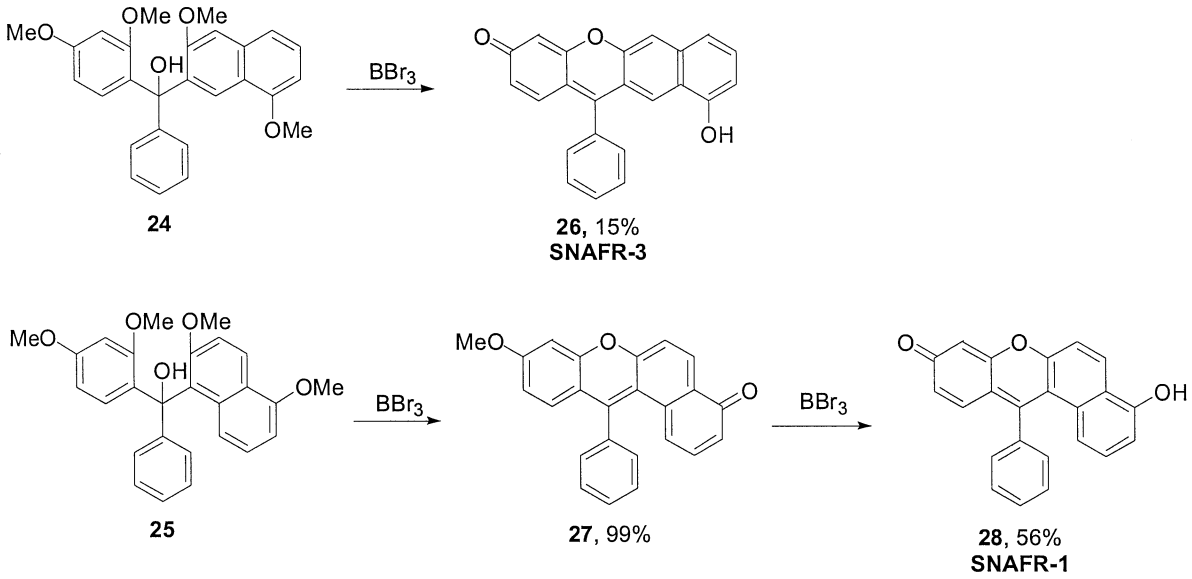
Scheme 11. Demethylation of the Type [b] naphthofluorone dye precursor failed to produce the desired naphthofluorone dye **22** due to solvent addition.

Carbinol precursors **24** and **25** to the type [b] and type [a] seminaphthofluorones respectively are synthesized by the reaction between lithiated 1,6-dimethoxynaphthalene and 2,4-dimethoxybenzophenone (Scheme 12). Reduced degree of conjugation is chosen in order to reduce the formation of solvent adduct. The structures of the precursors are confirmed via single crystal X-Ray diffraction (Figure 1).



Scheme 12. Novel synthesis of seminaphthofluorone carbinol precursors.

Subsequent demethylation of **24** by BBr_3 yields **26**, seminaphthofluorone-3 (**SNAFR-3**), in 15% yield. Two-step demethylation of **25** is necessary to obtain **28**, the seminaphthofluorone-1 (**SNAFR-1**) in 56% yield.



Scheme 13. Synthetic Scheme for Type [a] and Type [b] seminaphthofluorones.

Benzoxanthene dye precursors **24** and **25** as well as the demethylated benzoxanthene dye **26** were crystallized and ORTEP drawings are shown in Figure 1.

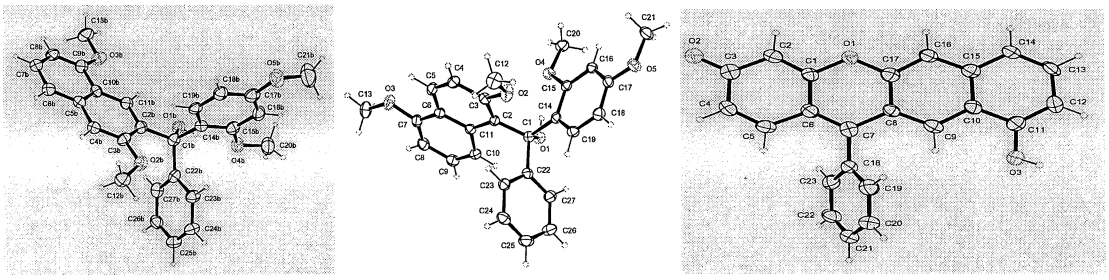
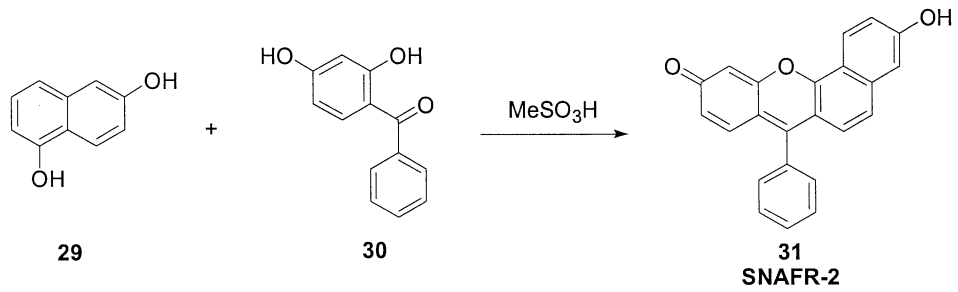


Figure 1. From left to right: ORTEP drawings of **24**, **25**, and benzoxanthene dye **26**.

To study the effect of different benzannulation on the photophysical properties, **31**, seminaphthofluorone-2 (**SNAFR-2**) is synthesized also via traditional acid-catalyzed condensation (Scheme 14).



Scheme 14. Synthesis of type [c] seminaphthofluorone (**SNAFR-2**) for comparison.

Conclusions

A simple synthetic method utilizing readily available reagents affording a series of regioisomerically pure xanthene dye derivatives is developed. Advantages include relatively mild conditions and good to excellent yields. Non-polar, highly crystalline carbinol leuco bases, xanthene dye intermediates, are isolable by standard chromatographic techniques. These intermediates are in the appropriate xanthene oxidation state, avoiding the need for oxidation chemistry and/or harsh conditions. This same methodology is utilized towards the synthesis of new benzoxanthene dye architectures, previously unattainable via other methodology.

Experimental

5,5'-Dichloro-2,2',4,4'-tetramethoxytrityl Alcohol (4). In a 50-mL round-bottom flask, compound 2 (0.500 g, 1.18 mmol) and NBS (0.315 g, 1.77 mmol) are dissolved in 20 mL of CCl4. A catalytic amount of benzoyl peroxide (10 mg) is added. The reaction mixture is heated at reflux for 1 h with vigorous stirring. It is cooled to room temperature and quenched with 50 mL of saturated NH_4Cl (aq). The mixture is extracted with CH_2Cl_2 (3 x 15 mL). The combined extracts are dried over $MgSO_4$ and filtered, and the filtrate is evaporated to dryness.The solid residue is purified by flash column chromatography (silica gel; CH_2Cl_2) to give 72 mg (14%) of **4** along with 18 mg (6%) of **5** and 43 mg (15%) of **6**. The structures of **5** and **6** were verified via independent syntheses (vide infra). Data for **4**: ^{1}H NMR (DMSO-d_6, 250 MHz) δ 7.23-7.16 (m, 5H), 7.05 (s, 2H), 6.76 (s, 2H), 5.52 (s, 1H), 3.88 (s, 6H); ^{13}C NMR (DMSO-d_6, 62.9 MHz) δ 156.6, 154.4, 146.0, 129.0, 127.3, 127.3, 127.0, 126.4, 111.3, 98.8, 78.0, 56.2, 55.9. MALDI-TOF m/z 431.517 $[M - OH]^+$, 447.544 $[M]^+$.

2,2',4,4'-Tetramethoxytrityl Alcohol (9). Magnesium turnings (0.543 g, 22.3 mmol) and a few crystals of I_2 are placed in a 250-mL three-neck round-bottom flask fitted with a dropping funnel and a condenser. A solution of 2,4-dimethoxybromobenzene (5.0 g, 23.0 mmol) in 20 mL of anhydrous THF is added dropwise to the magnesium. The mixture is stirred for 20-30 min. The resulting Grignard reagent (2,4-dimethoxyphenylmagnesium bromide) is cooled in a dry ice/acetone bath before a solution of methyl benzoate (1.25 g, 9.38 mmol) in 40 mL of dry THF is added dropwise. The mixture is stirred overnight and then quenched with 100 mL of distilled water and neutralized with 2 N HCl. The unreacted 2,4-dimethoxybromobenzene as well as THF are removed by steam distillation. The resulting mixture is extracted with CH_2Cl_2 (3 x 30 mL). The combined extracts are dried over $MgSO_4$ and filtered, and the filtrate is evaporated to

dryness. The residue is purified by flash chromatography (silica gel; EtOAc-hexane, 20:80) to afford 3.49 g (96%) of **9**. Compound **9** was prepared previously via other methodology. NMR data is in agreement with the assigned structure: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.21-7.10 (m, 5H), 6.86 (d, J = 8.6 Hz, 2H), 6.52 (d, J = 2.2 Hz, 2H), 6.42 (dd, J = 8.6, 2.2 Hz, 2H), 5.17 (s, 1H), 3.73 (s, 6H), 3.40 (s, 6H); ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ 159.7, 157.9, 147.4, 129.2, 127.4, 126.7, 127.9, 104.1, 99.5, 79.1, 55.4, 55.1.

9-Phenyl-6-methoxy-3-fluorone (10). A solution of 8 (0.178 g, 0.468 mmol) in 10 mL of dry CH_2Cl_2 is cooled to -78 °C using a dry ice/acetone bath before BBr_3 (0.935 g, 3.74 mmol) is added dropwise. The mixture is allowed to warm to room temperature gradually before being quenched with 20 mL of distilled H_2O. The mixture is extracted with CH_2Cl_2 (3 x 10 mL). The combined extracts are dried over $MgSO_4$ and filtered, and the filtrate is concentrated under reduced pressure. The residue is purified by flash chromatography (silica gel, EtOAc), affording 90 mg (64%) of **10**. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 7.63-7.61 (m, 3H), 7.47-7.43 (m, 2H), 7.21 (d, J = 2.4 Hz, 1H), 7.08 (d, J = 9.0 Hz, 1H), 7.01 (d, J = 9.7 Hz, 1H), 6.94 (dd, J = 9.0, 2.4 Hz, 1H), 6.42 (dd, J = 9.7, 1.8 Hz, 1H), 6.21 (d, J = 1.8 Hz, 1H), 3.90 (s, 3H); ^{13}C NMR (DMSO-d_6, 62.9 MHz) δ 183.8, 164.1, 158.4, 154.0, 149.2, 132.4, 130.8, 129.7, 129.6, 129.5, 129.4, 128.8, 117.0, 113.9, 113.6, 104.7, 100.6, 56.3. MALDI-TOF m/z 303.154 $[M + H]^+$.

9-Phenyl-6-hydroxy-3-fluorone (11). To a stirred solution of **8** (0.400 g, 1.05 mmol) in 10 mL of dry CH2Cl2 at -78 °C, BBr_3 (4.20 g, 16.8 mmol) is added dropwise. The mixture is warmed to room temperature gradually before being quenched with 20 mL of distilled H_2O. After being stirred for 20 min, filtration leads to a collection of a red precipitate (**11**). A sample for analytical purposes is obtained by flash chromatography (silica gel, EtOAc-MeOH 9:1). A quantity of 197 mg (65%) of **11** is collected. Compound **11** is known; however, no

characterization data was previously reported via the older synthetic methods. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 7.63-7.60 (m, 3H), 7.46-7.43 (m, 2H), 7.01 (d, J = 9.2 Hz, 2H), 6.60 (dd, J = 9.2, 2.1 Hz, 2H) 6.60 (d, J = 2.1 Hz, 2H); ^{13}C NMR (DMSO-d_6, 62.9 MHz) δ 156.3, 149.8, 132.5, 130.5, 129.5, 129.3, 128.8, 114.6, 103.4.

2,2',4,4'-Tetramethoxy-4''-bromotrityl Alcohol (13a). This compound was prepared following the protocol described above for compound **9** except that 4-bromo methyl benzoate **12a** (2.01 g, 9.38 mmol) was used as the ester. The yield of **13a** (3.97 g) is 92%. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.25 (d, J = 8.5 Hz, 2H), 6.94 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 8.6 Hz, 2H), 6.42 (d, J = 2.0 Hz, 2H), 6.34 (dd, J = 8.6, 2.0 Hz, 2H), 5.17 (s, 1H), 3.63 (s, 6H), 3.32 (s, 6H); ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ 159.9, 157.8, 147.2, 129.7, 129,4, 129.1, 125.9, 118.9, 104.2, 99.4, 78.5, 55.3, 55.1. MALDI-TOF m/z 457.880 $[M]^+$, 441.521 $[M - OH]^+$, 481.532 $[M + K]^+$.

2,2',4,4'-Tetramethoxy-4''-phenyltrityl Alcohol (13b). This compound is prepared following the procedure described above for compound **9** except that 4-phenyl methyl benzoate **12b** (1.13 g, 5.33 mmol) is used. The yield of **13b** (2.43 g) is 99%. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 7.64 (d, J = 7.6 Hz, 2H), 7.47 (d, J = 8.4 Hz, 2H), 7.43 (t, J = 7.8 Hz, 2H), 7.32 (t, J = 7.1 Hz, 1H), 7.19 (d, J = 8.4 Hz, 2H), 6.93 (d, J = 8.6 Hz, 2H), 6.55 (d, J = 2.3 Hz, 2H), 6.45 (dd, J = 8.6, 2.3 Hz, 2H); ^{13}C NMR (DMSO-d_6, 62.9 MHz) δ 159.7, 157.9, 146.7, 140.0, 137.6, 129.2, 128.9, 128.1, 127.1, 126.6, 126.5, 125.0, 104.1, 99.5, 78.9, 55.4, 55.1. MALDI-TOF m/z 439.654 $[M - OH]^+$, 479.599 $[M + Na]^+$.

2,2',4,4'-Tetramethoxy-4''-methoxytrityl Alcohol (13c). This compound is prepared following the procedures described above for compound **9** except that 4-methoxy methyl benzoate **12b** (1.55 g, 9.38 mmol) is used. The yield of **13c** (3.2 g) is 83%. ^{1}H NMR ($CDCl_3$,

300 MHz) δ 7.16 (d, J = 6.9 Hz, 2H), 6.83 (d, J) 8.6 Hz, 2H), 6.79 (d, J = 6.9 Hz, 2H), 6.49 (d, J = 2.4 Hz, 2H), 6.37 (dd, J = 8.6, 2.4 Hz, 2H), 3.79 (s, 9H), 3.54 (s, 6H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 160.3, 158.7, 158.3, 139.7, 130.4, 129.1, 127.6, 112.7, 103.8, 100.2, 80.6, 55.9, 55.5, 55.4. MALDI-TOF *m/z* 409.490 $[M]^+$.

2,2',4,4'-Tetramethoxy-3"-nitrotrityl Alcohol (13d). This compound is prepared following the procedure described above for compound **9** except that 3-nitro methyl benzoate **12d** (1.70 g, 9.38 mmol) is used. The yield of **13d** (3.86 g) is 95%. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.00-7.98 (m, 2H), 7.55-7.42 (m, 2H), 7.08 (d, J = 8.5 Hz, 2H), 6.54 (d, J = 1.9 Hz, 2H), 6.50 (dd, J = 8.5, 1.9 Hz, 2H), 5.59 (s, 1H), 3.75 (s, 6H), 3.39 (s, 6H); ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ 160.2, 157.6, 150.3, 146.7, 134.2, 129.1, 127.8, 125.0, 122.0, 120.7, 104.4, 99.4, 78.1, 55.1. MALDI-TOF *m/z* 424.481 $[M]^+$, 448.565 $[M + Na]^+$, 464.538 $[M + K]^+$.

2,2',4,4'-Tetramethoxy-4"-nitrotrityl Alcohol (13e). This compound is prepared following the procedure described above for compound **9** except that 4-nitro methyl benzoate **12e** (1.70 g, 9.38 mmol) is used. The yield of **13e** (3.51 g) is 91%. ^{1}H NMR (DMSO-d_6, 300 MHz) *ä* 8.04 (d, J = 8.9 Hz, 2H), 7.35 (d, J = 8.9 Hz, 2H), 7.06 (d, J = 8.4 Hz, 2H), 6.54 (d, J = 2.4 Hz, 2H), 6.49 (dd, J = 8.4, 2.4 Hz, 2H), 5.53 (s, 1H), 3.75 (s, 6H), 3.40 (s, 6H); ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ 160.2, 157.6, 155.9, 145.4, 129.0, 128.6, 124.9, 121.7, 104.4, 99.4, 78.2, 55.1. MALDI-TOF *m/z* 424.701 [M]+, 408.624 [M - OH]+.

9-(4-Bromophenyl)-6-hydroxy-3-fluorone (14a). This compound was prepared following the procedure above for compound **11** except that compound **13a** (0.400 g, 0.871 mmol) is used. The yield of **14a** (223 mg) is 70%. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 7.88 (d, J = 7.5 Hz, 2H), 7.47 (d, J = 7.5 Hz, 2H), 7.29 (d, J = 9.4 Hz, 2H), 6.90 (d, J = 2.1 Hz, 2H), 6.88 (dd

J = 9.4, 2.1 Hz, 2H); ^{13}C NMR (DMSO-d_6, 62.9 MHz) δ 172.1, 158.0, 132.5, 131.9, 131.7, 124.2, 120.9, 115.6, 102.8. MALDI-TOF *m/z* 367.228 $[M + H]^+$.

9-(4-Biphenyl)-6-hydroxy-3-fluorone (14b). This compound is prepared following the procedure above for compound **11** except that compound **22** (0.500 g, 1.09 mmol) is used. The yield of **14b** (330 mg) is 87%. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 7.91 (d, J = 7.8 Hz, 2H), 7.79 (d, J = 7.9 Hz, 2H), 7.55-7.49 (m, 5H), 7.09 (d, J = 9.0 Hz, 2H), 6.59 (dd, J = 9.0, 2.2 Hz, 2H), 6.54 (d, J = 2.2 Hz, 2H); ^{13}C NMR (DMSO-d_6, 62.9 MHz) δ 157.2, 150.4, 141.9, 140.1, 132.6, 131.4, 131.0, 130.0, 128.9, 127.8, 127.7, 122.6, 115.0, 104.3. MALDI-TOF *m/z* 365.312 $[M + H]^+$, 387.322 $[M + Na]^+$.

9-(4-Hydroxyphenyl)-6-hydroxy-3-fluorone (14c). This compound is prepared following the procedure above for compound **11** except that compound **13c** (1.26 g, 3.07 mmol) is used. The yield of **14c** (900 mg) is 96%. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 10.07 (s, 1H), 7.25 (d, J = 8.2 Hz, 2H), 7.14 (d, J = 9.2 Hz, 2H), 6.98 (d, J = 8.2 Hz, 2H), 6.59 (d, J = 9.4 Hz, 2H), 6.52 (s, 2H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 158.6, 156.4, 150.4, 131.1, 130.8, 122.6, 115.5, 114.6, 105.5, 103.3. MALDI-TOF *m/z* 305.127 $[M + H]^+$, 327.100 $[M + Na]^+$, 341.221 $[M + K]^+$.

9-(3-Nitro-phenyl)-6-hydroxy-3-fluorone (14d). This compound is prepared following the procedure above for compound **11** except that compound **14d** (0.480 g, 1.13 mmol) is used. The yield of **14d** (274 mg) is 73%. 1H NMR (DMSO-*d*6, 300 MHz) δ 8.42 (d, J = 7.0 Hz, 1H), 8.26 (s, 1H), 7.89 (m, 2H), 6.96 (d, J = 9.1 Hz, 2H), 6.55 (dd, J = 9.1, 2.9 Hz, 2H), 6.53 (d, J = 2.9 Hz, 2H); ^{13}C NMR (DMSO-d_6, 62.9 MHz) δ 156.3, 138.0, 147.0, 136.0, 134.3, 130.5, 130.3, 124.3, 114.3, 103.5. MALDI-TOF *m/z* 334.186 $[M + H]^+$, 356.167 $[M + Na]^+$.

9-(4-Nitrophenyl)-6-hydroxy-3-fluorone (14e). This compound was prepared following the procedure above for compound **11** except that compound **15** (30 mg) is used. The

eluent for flash chromatography is EtOAc-MeOH 8:2. The yield of **14e** (21 mg) is 76%. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 8.44 (d, J = 8.6 Hz, 2H), 7.46 (d, J = 8.6 Hz, 2H), 6.90 (dd, J = 8.9, 1.1 Hz, 2H), 6.54 (d, J = 8.9 Hz, 2H), 6.52 (d, J = 1.1 Hz, 2H); ^{13}C NMR (DMSO-d_6, 62.9 MHz) δ 174.8, 156.3, 148.0, 147.3, 139.7, 131.0, 130.1, 123.4, 122.1, 113.5, 103.6. MALDI-TOF *m/z* 334.273 $[M + H]^+$, 356.301 $[M + Na]^+$.

9-(4-Nitrophenyl)-6-methoxy-3-fluorone (15). This compound is prepared following the procedure above for compound **11** except that compound **13e** (0.500 g, 1.18 mmol) is used. The yield of **15** (346 mg) is 85%. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.46(d, J = 8.6 Hz, 2H), 7.25 (d, J = 2.3 Hz, 1H), 7.07 (d, J = 9.4 Hz, 1H), 6.98(d, J = 9.8 Hz, 1H), 6.93 (dd, J = 9.4, 2.3 Hz, 1H), 6.44 (dd, J = 9.8, 1.8 Hz, 1H), 6.26 (d, J = 1.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ 183.8, 164.3, 158.2, 154.0, 148.2, 139.3, 131.1, 130.5, 129.8, 129.5, 127.1, 124.0, 123.2, 117.2, 113.68, 113.4, 105.0, 100.8, 56.4. MALDI-TOF *m/z* 348.419 $[M + H]^+$.

Compounds 19 and 20. 1,6-Dimethoxynaphthalene (5 g) was lithiated according to a known procedure in a 100 ml three-neck round bottom flask. The lithiated 1,6-dimethoxynaphthalene was cooled to -78 °C. Methyl benzoate (1.7 g) in 20 mL THF was added dropwise over 20 min. The mixture was allowed to warm to rt over 6 h. The reaction mixture was quenched with deionized water and neutralized with 2 N HCl. THF was removed *in vacuo*. The resulting mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were dried over $MgSO_4$ and filtered, and evaporated to dryness. The residue was purified via flash chromatography (silica gel; EtOAc:hexane, 20:80) to afford 0.77 g (11%) of **19** and 4.5 g (71%) of **20**. Data for compound **19**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.30 (d, J = 9.2 Hz, 1H), 7.94 (d, J = 9.2 Hz, 1H), 7.49 (s, 2H), 7.34 – 7.28 (m, 7H), 7.14 – 7.06 (m, 4H), 6.61 (dd, J = 7.8, 2.9 Hz, 2H), 5.38 (s, 1H), 3.97 (s, 3H), 3.76 (s, 3H), 3.68 (s, 3H), 3.14 (s, 3H). ^{13}C NMR ($CDCl_3$,

75MHz) δ (ppm): 156.7, 155.8, 155.4, 155.2, 146.4, 138.5, 134.3, 127.7, 126.7, 126.3, 124.9, 123.7, 122.7, 121.4, 121.1, 120.2, 118.3, 113.9, 105.4, 101.3, 101.3, 81.7, 56.5, 55.4, 55.3, 55.2. ESI $[M\text{-}OH]^+$ calcd 463.1904, found 463.1664. Data for compound **20**: ^1H NMR ($CDCl_3$, 250 MHz) δ (ppm): 8.06 (s, 2H), 7.32-7.40 (m, 7H), 7.22 – 7.17 (m, 4H), 6.68 (dd, J = 6.3, 2.3 Hz, 2H), 5.38 (s, 1H), 3.88 (s, 6H), 3.69 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 156.8, 156.0, 146.7, 135.3, 134.0, 127.9, 126.6, 126.4, 123.3, 120.2, 118.4, 106.8, 102.1, 81.4, 55.4, 55.3. ESI $[M\text{-}OH]^+$ calcd 463.1904, found 463.3515.

Compound 21. To a stirred solution of **20** (0.200 g) in 30 mL anhydrous CH_2Cl_2 at -78 °C, BBr_3 (1.5 mL) is added dropwise. The mixture is warmed to rt slowly before quenching with 20 mL distilled H_2O. After stirring for 20 min and filtration, a red precipitate is collected. The red precipitate is washed and transferred into a round bottom flask with acetone and dried *in vacuo*. The resulting red powder is purified by flash chromatography (silica gel, Hexane: EtOAc 6:4) to give 107 mg (61%) of compound **21**. ^1H NMR (DMSO-d_6, 300 MHz) δ (ppm): 10.24 (s, 2H), 8.07 (s, 2H), 7.70 (s, 2H), 7.36 – 7.12 (m, 10 H), 6.72 (d, J = 7 Hz, 2H). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ (ppm): 154.1, 150.7, 150.4, 149.6, 135.9, 135.5, 130.8, 129.1, 128.9, 128.3, 127.5, 126.2, 125.9, 124.4, 124.3, 122.6, 119.2, 118.2, 112.0, 111.7, 107.3 77.2. MALDI-TOF $[M\text{-}OMe]^+$ calcd 339.117, found 338.982.

Compounds 24 and 25. The preparation procedure is the same as for compounds **19** and **20** except that 2, 4-dimethoxybenzophenone is used instead of methyl benzoate. The crude product is purified via flash chromatography (silica gel; EtOAc:hexane, 20:80) to afford 3.43 g (71%) of **24** and 0.52 g (11%) of **25**. Data for compound **24**: ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.06 (s, 1H), 7.27-7.38 (m, 7H), 7.14 (s, 1H), 6.84 (d, J = 8.6 Hz, 1H), 6.68 (d, J = 5.1 Hz, 1H), 6.55 (d, J = 2.0 Hz, 1H), 6.40 (dd, J = 8.6, 2.0 Hz, 1H), 5.29 (s, 1H), 3.88 (s, 3H), 3.82 (s, 3H), 3.63 (s,

3H), 3.56 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 160.1, 158.6, 156.7, 156.0, 146.9, 135.2, 134.3, 130.5, 127.8, 127.1, 126.7, 126.6, 126.4, 122.9, 120.1, 118.4, 106.8, 103.6, 102.2, 100.0, 81.0, 55.6, 55.3, 55.2. ESI $[M\text{-}OH]^+$ calcd 413.1753, found 413.1549. Data for compound **25**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.31 (d, J = 9.3 Hz, 1H), 7.92 (d, J =8.9 Hz, 1H), 7.45-7.09 (m, 7H), 6.63 (d, J = 7.6 Hz, 1H), 6.52 (d, J = 2.4 Hz, 1H), 6.46 (d, J = 8.6 Hz, 1H), 6.33 (dd, J = 8.6, 2.4 Hz, 1H), 5.38 (s, 1H), 3.97 (s, 3H), 3.81 (s, 3H), 3.56 (s, 3H), 3.28 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 159.5, 158.0, 155.2, 147.3, 130.4, 129.3, 128.2, 127.6, 126.6, 124.8, 123.7, 122.6, 121.0, 114.3, 103.4, 101.4, 99.4, 81.4, 56.7, 55.4, 55.3. ESI $[M\text{-}OH]^+$ calcd 413.1753, found 413.1223.

Compound 26.(SNAFR-3) To a stirred solution of **24** (0.500 g) in 30 mL anhydrous CH_2Cl_2 at -78 °C, BBr_3 (4.65 g) is added dropwise. The mixture is warmed to rt slowly before quenching with 20 mL distilled H_2O. After stirring for 20 min and filtration, a red precipitate is collected. The red precipitate is washed with acetone, transferred into a round bottom flask and dried *in vacuo*. The resulting red powder is purified by flash chromatography (silica gel, EtOAc-MeOH 9:1) to give 60 mg (15%) of compound **26**. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 10.68 (bs, 1H), 7.99 (s, 1H), 7.91 (s, 1H), 7.69-7.40 (m, 7H), 7.03 (d, J = 9.8 Hz, 1H), 6.82 (d, J = 6.6 Hz, 1H), 6.43 (d, J = 9.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 195.7, 158.9, 155.1, 149.5, 149.3, 137.4, 133.5, 132.4, 131.5, 130.5, 130.2, 129.7, 125.5, 122.5, 120.5, 120.4, 118.2, 112.4, 108.3, 106.5. HRMS $[M+H]^+$ calcd 339.1021, found 339.1016.

Compound 27 was prepared with the same method as compound **26** except that 500 mg of compound **25** are used and the temperature is raised to 50 °C before adding BBr_3 affording 390 mg (99%) of **27**. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.97 (s, 1H), 8.17 (d, J = 9.2 Hz), 7.77 (d, J = 9.0 Hz), 7.36 – 7.25 (m, 5H), 7.19 (t, J = 7.5 Hz, 2H), 7.04 (t, J = 6.6 Hz, 2H), 6.98

(d, J = 8.5 Hz, 1H), 6.70 (d, J = 2.1 Hz, 1H), 6.63 (dd, J = 7.0, 3.4 Hz, 2H), 3.75 (s, 3H). HRMS $[M+H]^+$ calcd 353.1172, found 353.1171.

Compound 28 (**SNAFR-1**) was prepared in the same manner as compound **26** except that 25 mg of compound **27** are used and the temperature is raised to 50 °C before the addition of BBr_3 affording 16 mg (56%) of **SNAFR-1**. Data for **SNAFR-1**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 8.58 (d, J = 9.3 Hz, 1H), 7.68-7.69 (m, 3H), 7.66 (d, J = 9.3 Hz, 1H), 7.44-7.48 (m, 2H), 7.00 (d, J = 9.9 Hz, 1H), 6.98 (dd, J = 8.4, 7.2 Hz, 1H), 6.84 (d, J = 7.2 Hz, 1H), 6.51 (dd, J = 9.9, 1.8 Hz, 1H), 6.43 (d, J = 9.3 Hz, 1H), 6.32 (d, J = 1.8 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75MHz) δ (ppm): 183.1, 157.2, 154.0, 149.2, 136.4, 130.8, 130.2, 130.1, 129.7, 128.9, 129.0, 128.1, 120.0, 115.9, 115.2, 108.5, 103.3. MALDI-TOF $[M+H]^+$ calculated 339.102, found 339.319.

Compound 31 (**SNAFR-2**). 1,6-dihydroxynaphthalene (**29**, 1.5 g, 9.3 mmol) and 2,4-dihydroxybenzophenone (**30**, 2.0 g, 9.3 mmol) are added into a 100 mL round bottom flask containing 25 mL CH_3SO_3H. The mixture is heated to reflux for 24 h. The resulting dark-red liquid is then poured into 200 mL distilled H_2O and neutralized by addition of $NaHCO_3$ until the solution turns almost colorless. Then, the liquid is decanted and the resulting residue dissolved in MeOH and dried with Na_2SO_4. The solution is filtrated and evaporated to dryness. The remaining red residue is purified by flash chromatography (EtOAc:MeOH=9.5:0.5). 18.6 mg (3 % yield) of **SNAFR-2** are obtained. Data for compound **SNAFR-2**:^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 8.52 (d, J = 9.3 Hz, 1H), 7.65-7.70 (d, 3H), 7.56 (d, J = 9.0, 1H), 7.49-7.53 (m, 2H), 7.32 (dd, J = 9.3, 2.7 Hz, 1H), 7.23 (d, J = 2.7 Hz, 1H), 7.09 (d, J = 9.3 Hz, 1H), 7.04 (d, J = 9.0, 1H), 6.52 (dd, J = 9.3, 1.5 Hz, 1H), 6.50 (d, J = 1.5 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75MHz) δ (ppm): 183.5, 159.6, 158.3, 150.0, 137.7, 132.7, 130.3, 130.0, 129.4, 128.8, 124.6,

123.5, 123.2, 119.9, 116.0, 113.5, 110.0, 104.7. MALDI-TOF $[M+H]^+$ calculated 339.102, found 339.431.

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