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Syntheses and Properties of Water-Soluble Nile Red Derivatives

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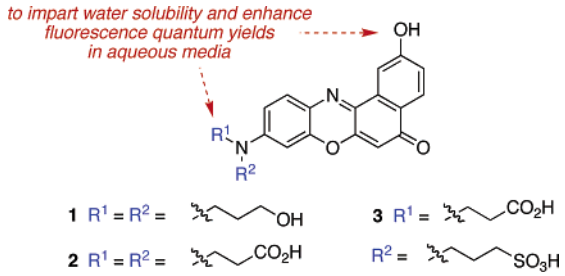

Nile Red (compound **A**) fluoresces at about 530 nm with good quantum yields in apolar solvents. In more polar ones its fluorescence emission shows a dramatic, and potentially useful, shift to about 640 nm, but its quantum yield is significantly reduced. Further, Nile Red has a very poor solubility in aqueous media. The hypothesis tested in this paper is that Nile Red derivatives that incorporate water-solubilizing groups will tend to fluoresce with *good* quantum yields in aqueous media, and in the more useful wavelength range around 640 nm. Thus three Nile Red derivatives, **1**−**3**, were prepared. Compound **1** had three hydroxyl groups more than Nile Red, but was surprisingly insoluble in aqueous media. However, the dicarboxylic acid **2** and carboxylic/sulfonic acid derivative **3** showed excellent water solubilities. Spectral data for **2** and **3** showed that they do indeed fluoresce with good quantum yields in the 640 nm region *in aqueous media*. These properties of compounds **2** and **3** might be useful in the development of fluorescent probes for biotechnology.

Introduction

Nile Red, compound **A**, is a well-known fluorescent dye with remarkable solvatochromic properties.[1−3] In apolar solvents it fluoresces with a high quantum yield in the region of 530 nm, but in polar solvents the quantum yield is dramatically reduced and the emission maximum shows bathochromic shifts of approximately 100 nm.[4] This red-shift characteristic has been used extensively as a fluorescent reporter on the environment around molecules labeled with this dye. In other contexts, however, it would be desirable to use Nile Red derivatives as labels for applications in biotechnology,[5] especially if the large bathochromic shift could be retained *without* significantly reducing the quantum yield. This is particularly true for development of probes for intracellular imaging, for instance, where emissions become increasingly more detectable with wavelengths longer than that associated with autofluorescence in the cell (typically above 550 nm).

Unfortunately, Nile Red itself has very poor solubility in aqueous media,[1] so it is not a particularly useful dye for labeling most biomolecules. There has been some effort to prepare related water-soluble derivatives of benzo[*a*]phenoxazinium, "Nile Blue", systems **B**[6,7] but very little consideration has been devoted to development of Nile Red derivatives that could be used in aqueous media.[8] The closest published research that we are aware of in this area is that by Briggs and co-workers at Amersham Co. featuring the 2-hydroxy Nile Red derivatives **C** and **D**.[9,10] Their studies did not focus on the properties of these dyes in water (the data given were in methanol), so questions

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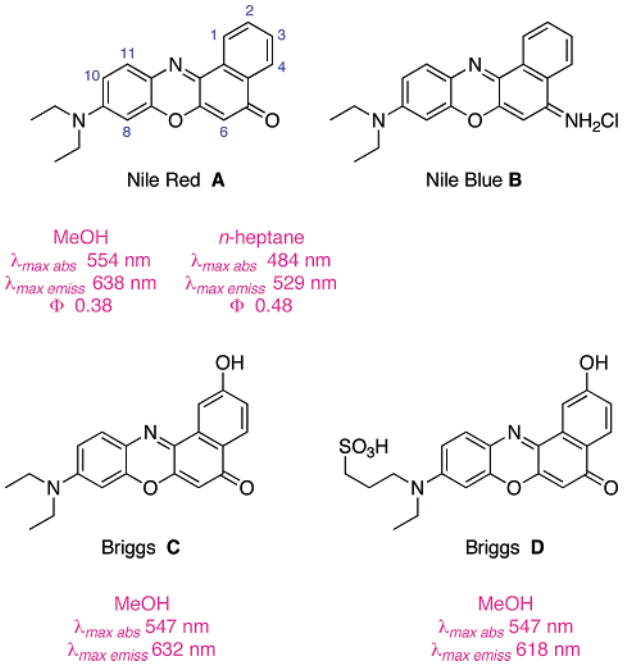
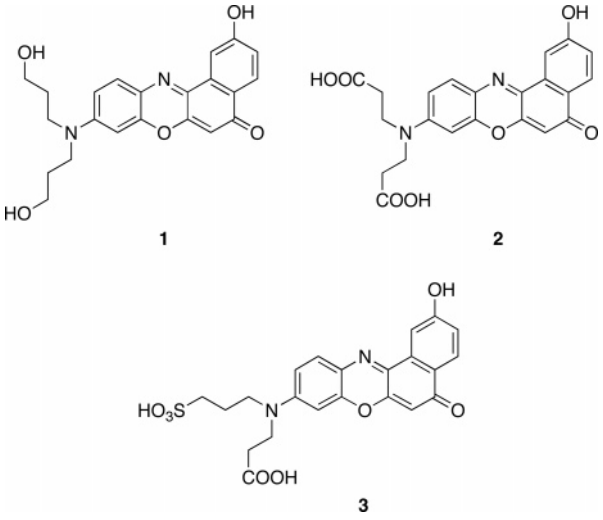


relating to bathochromic shifts and reduction of quantum yields in aqueous media were not addressed. Our own unpublished observations (made in the course of this study), however, show that one of these derivatives, 2-hydroxy Nile Red, is not significantly soluble in aqueous media.

This paper concerns development of Nile Red derivatives that have (i) water solubility, (ii) significant quantum yields in aqueous media, (iii) fluoresce over 600 nm (the more useful region for imaging in cells and tissues), and (iv) functional groups that facilitate attachment to biomolecules. Water solubility was identified as the critical factor to reach most of these goals, for the following reason. It is generally accepted that the reduced quantum yields of Nile Red in polar media are due to aggregation of the hydrophobic, flat, benzo[*a*]phenoxazinone core structures.[11] Logically, this type of aggregation could be suppressed or prevented by introducing water-solubilizing groups into the dye structure. In the context of this research, that might impart water solubility, increase the quantum yields, and facilitate the attachment of these dyes to water-soluble biomolecules. Effects on fluorescence emission maxima that occur by making the Nile Red scaffold water-soluble were unknowns that had to be tested, but we speculated that the desirable red-shift in the fluorescence maxima that is observed for Nile Red in polar media was less likely to be due to an aggregation effect than to stabilization of a relatively polarized excited state.

The considerations discussed above led us to attempt syntheses of the Nile Red derivatives **1**−**3**, and investigate their fluorescence properties in aqueous media (if possible). As stated above, the immediate objectives of this study were to identify water-soluble Nile Red derivatives that exhibit fluorescence emission maxima above 600 nm and significant quantum yields in aqueous media; dyes of this type could have general applications in biotechnology. In the more focused context of our research on fluorescent dyes, i.e., development of the



"through-bond energy transfer cassettes",[12−14] water-soluble Nile Red derivatives could be useful acceptors for syntheses of donor−acceptor cassettes.

Results and Discussion

Synthesis of Nile Red Derivatives 1−3. The synthesis developed for target dye **1** is shown in Scheme 1. 3-Aminophenol is known to undergo a facile Michael addition with acrylic acid to give a product that crystallizes out of the reaction mixture. This reaction gives good quality material on a multigram scale without necessitating chromatography. Esterification of the diacid formed in this way gives the diester **4**, which was isolated in good yields by an extraction procedure. Lithium aluminum hydride reduction of **4** gave the corresponding diol **5** that was then nitrosylated under aqueous conditions. The nitroso product **6** proved to be somewhat unstable so it was used without further purification. Assembly of the benzo[*a*]phenoxazinone skeleton was achieved via condensation of the nitroso compound **6** with 1,6-dihydroxynaphthalene. The product **1** was isolated via flash chromatography. This was the only chromatographic step used in the synthesis so it proved convenient to make this product on a several gram scale.

Scheme 2 shows the synthesis that was developed for the target dye **2**. Nitrosylation of the diester **4** (see below) afforded **7**, which was used without purification due to stability and hygroscopicity issues. Condensation of this nitroso compound with 1,6-dihydroxynaphthalene gave benzo[*a*]phenoxazinone **8**. This compound was isolated via flash chromatography (the first and only one in the sequence). Aqueous hydrolysis of the diester functionalities of **8** gave the corresponding diacid **2** that could be isolated via a simple acid base extraction procedure.

Preparation of the final target, the sulfonic acid **3**, is outlined in Scheme 3. Michael addition of acrylic acid with 3-aminophenol in near stoichiometric amounts gave the monoadduct **9**. Reaction of this amine with 1,3-propane sultone gave the ring-opened product **10**. This was nitrosylated to give the very unstable nitrosyl compound **11**, which was then condensed as above without

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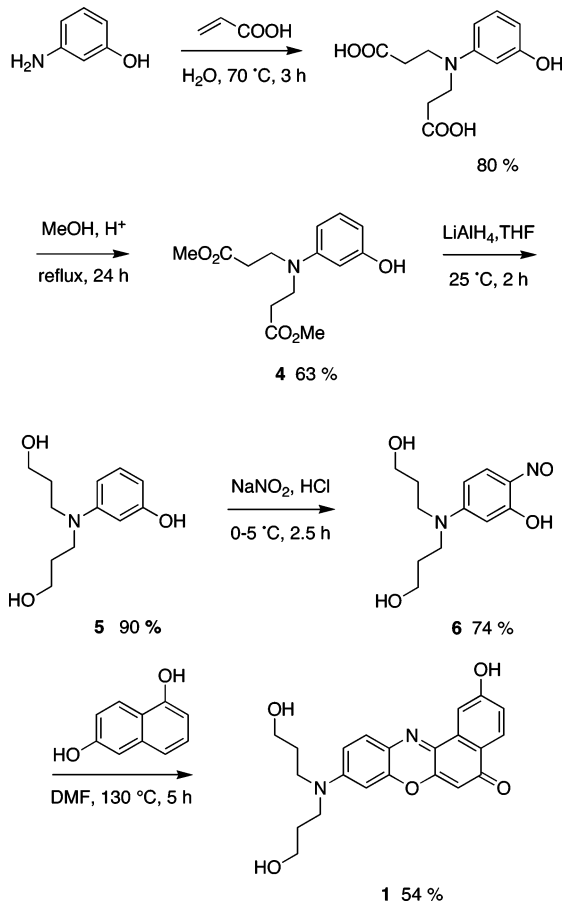
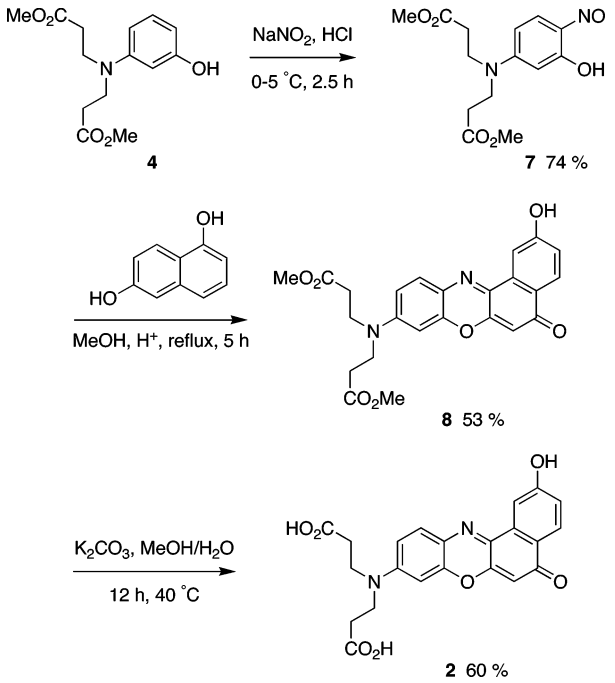
SCHEME 1



SCHEME 2



delay. Unfortunately, flash chromatography was used in each step of this procedure but, even so, the target material **3** was isolated on about a 250-mg scale. Repetition of the sequence on a larger scale was not attempted here, but we suspect that 1−3 g of material could easily be made via this approach.

SCHEME 3

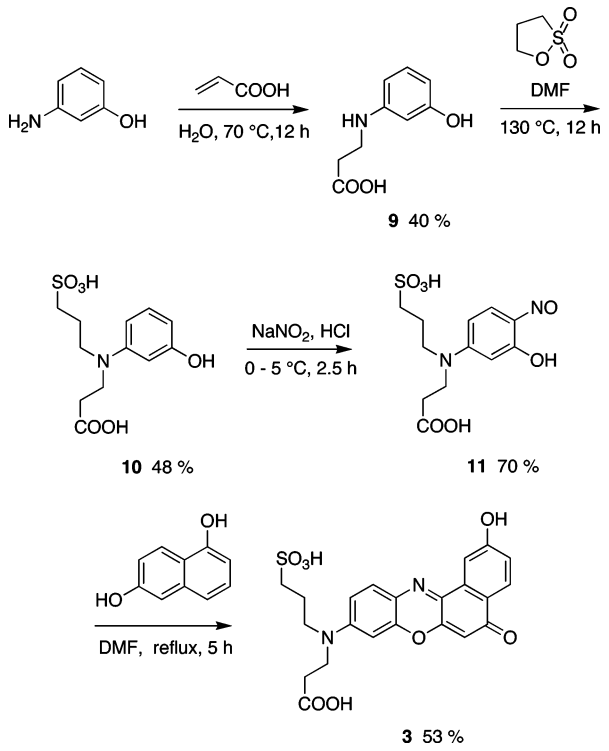


TABLE 1. Spectroscopic Properties of the Nile Red Derivatives 1−3

dye	λ_{abs} (nm)	ϵ ($M^{-1}\,cm^{-1}$)	λ_{em} (nm)	fwhm (nm)	Φ^a	solvent
1	542	24854	631	60	0.56	EtOH
2	520	4984	632	59	0.43	EtOH
2	560	7529	648	63	0.33	*b*
2	556	3400	647	70	0.07	*c*
3	548	5283	632	56	0.42	EtOH
3	558	7876	652	60	0.37	*b*
3	556	6209	650	66	0.18	*c*

[a] Measured as specified previously.[16] [b] pH 7.4 phosphate buffer. [c] pH 9.0 borate buffer. Standards used for quantum yield measurement of rhodamine B ($\Phi = 0.97$ in ethanol) and rhodamine 101 ($\Phi = 1.0$ in ethanol).

Spectroscopic Properties of Nile Red Derivatives 1−3. In water, the Nile Red derivative **1** showed hardly any solubility at all. Surprisingly, despite having a phenolic-OH, this compound was still insoluble in pH 9 borate buffer (1 M) or in sodium carbonate solution. Consequently, spectroscopic data for this compound were only recorded in EtOH (Table 1). Compound **1** was shown to have a similar absorption and emission wavelength maxima, and a slightly better quantum yield than Nile Red **A** or the hydroxy Nile Red derivative **C** in EtOH.

Compound **2** was more interesting for the purposes of this study. This dye has good solubility (for spectroscopic purposes) in EtOH, water, 1 M phosphate buffer at pH 7.4, and 1 M borate buffer at pH 9.0. In EtOH, its spectral properties are similar to those of compounds **A**, **C**, and **1** except that the molar absorptivity was measured to be approximately one-fifth that of **1**, and the Stokes shift was slightly greater (**2**, 110 nm; **1**, 90 nm). In aqueous buffers, however, the Stokes shifts for **1** and **2** were very similar. The quantum yield of **2** at pH 7.4 was quite good (0.33), but at pH 9 it reduced to 0.07. This appears to correlate with deprotonation of the phenolic hydroxyl, possibly

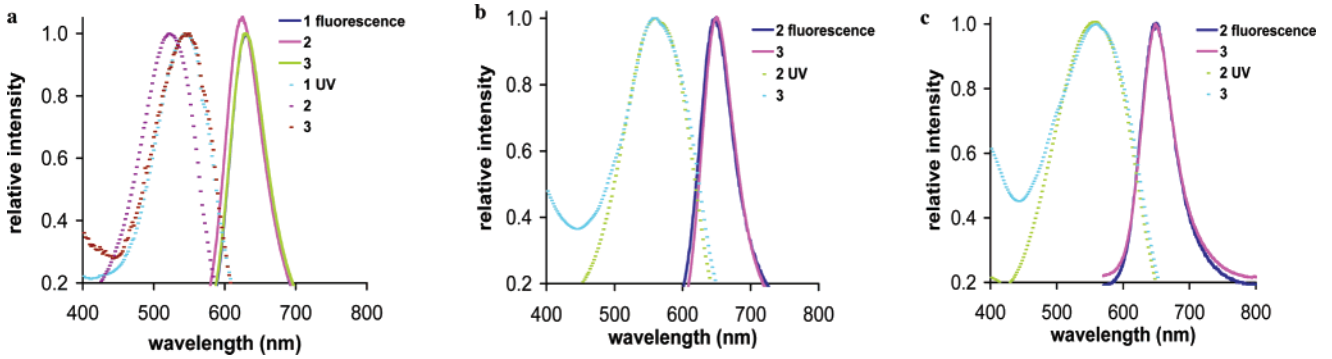


FIGURE 1. Absorption at 10^{-3} M (dashed lines) and fluorescence at 10^{-5} M (solid lines) of dyes **1**−**3** (a) in ethanol and dyes **2**−**3** (b) in phosphate buffer (pH 7.4) and (c) in borate buffer (pH 9.0).

corresponding to quenching of the fluorescence via charge transfer.[15] The full width at half-maximum height (fwhm) of the emission for **2** is 70 nm or less. Sharp fluorescence emissions facilitate resolution in experiments involving more than one probe. The fwhm for **2** compares favorably with fwhm reported for the water soluble Nile Blue derivatives mentioned earlier (they have fwhm in the range of 86−93 nm).[6]

The sulfonic acid dye **3** also had good solubility in the three media studied (Table 1). In EtOH its spectroscopic properties were unexceptional, and the quantum yield was quite good (0.42). In both the neutral and basic buffers the absorption and emission maxima were red-shifted but by only 18 nm or less relative to ethanolic solutions of the dye. Interestingly, the quantum yield of **3** was comparable in EtOH or pH 7.4 buffer, and still quite good (0.18) at pH 9, and almost double that of **2** in this medium. Moreover, the fwhm of the fluorescence emission peaks for the aqueous buffers were slightly lower than those for compound **2**.

Conclusions

All three of the dyes that are reported here are conveniently accessible via syntheses that involve about 5 steps. Compounds **1** and **2** can be produced on multigram scales since each synthesis only involves one chromatographic purification. The route to dye **3** that was developed here is less amenable to scale-up than the syntheses of the other two dyes, but, nevertheless, usable quantities are accessible.

Two of the three dyes, i.e., **2** and **3**, have solubilities that are satisfactory for applications as biological markers. They both have carboxylic acid functionalities, and these could be activated and used to conjugate the dyes to biomolecules. The fluorescence of dye **2** is significantly diminished under basic conditions, but this effect is less for **3**. Applications in biotechnology involve near-neutral pH values much more frequently than basic ones so this characteristic is not a serious drawback for dye **2**. Under near-neutral conditions, the quantum yields for **2** and **3** are good (>0.3), and they emit fluorescence as reasonably sharp peaks. Overall, dyes **2** and **3** retain the desirable bathochromic shift that Nile Red exhibits in alcoholic media relative to apolar ones, except that they can be dissolved in aqueous salt solutions. Further, the undesirable reduction of quantum yield that is observed for Nile Red on moving to more polar media is not a significant issue for the water-soluble dyes.

Experimental Section

Syntheses of Compounds 1−11: 3-[(2-Carboxyethyl)(3-hydroxyphenyl)amino]propionic Acid.[17] A solution of 3-aminophenol (109.0 g, 1 mol) in acrylic acid (185 mL, 3 mmol) and water (90 mL) was heated to 70 °C for 3 h. The reaction mixture was cooled and ethanol (180 mL) was added and kept at 5 °C for 12 h. The white precipitate that formed was filtered, washed with ethanol (50 mL), and dried to obtain dicarboxylic acid (200.0 g, 80%). Mp 153−154 °C (lit. mp 149−150 °C). ^{1}H NMR (300 MHz, acetone-d_6) δ 7.02−6.98 (m, 1H), 6.28−6.24 (m, 2H), 6.19−6.17 (m, 1H), 3.64 (t, 4H, J = 4.5 Hz), 2.59 (t, 4H, J = 4.5 Hz). ^{13}C NMR (75 MHz, acetone-d_6) δ 172.6, 158.6, 148.6, 130.0, 104.0, 104.2, 99.6, 46.8, 31.7. MS (ESI): 253.04 (M^+).

Compound 4. A solution of the dicarboxylic acid as prepared above (10.0 g, 39.5 mmol) in methanol (500 mL) along with HCl (10.0 M, 1 mL) was refluxed for 12 h. The reaction mixture was cooled and the MeOH was evaporated under reduced pressure. The residue was dissolved in EtOAc (100 mL) and the organic layer was washed with water (5 × 20 mL). The organic layer was evaporated to dryness under reduced pressure to yield **4** as a yellow semisolid (7.0 g, 63%). R_f 0.7 (50% EtOAc/hexane). ^{1}H NMR (300 MHz, CD_3OD) δ 7.05−6.95 (m, 1H), 6.25−6.15 (m, 3H), 3.65 (s, 6H), 3.59 (t, 4H, J = 7.2 Hz), 2.58 (t, 4H, J = 7.2 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 173.3, 158.4, 148.5, 130.1, 104.6, 104.2, 99.9, 51.1, 46.9, 32.1. MS (ESI): 281.36 (M^+). IR (neat) 3442, 2960, 2362, 1741 cm^{-1}.

Compound 5. A solution of **4** (1.0 g, 3.6 mmol) in THF (15 mL) was added dropwise at 0 °C under vigorous stirring to a solution of $LiAlH_4$ (820 mg, 21.6 mmol) in THF (10 mL) in a three-necked flask fitted with a reflux condenser (the reaction is exothermic); a thick white precipitate was formed almost immediately, and TLC (1:1 hexane/EtOAc) after 2 h showed complete disappearance of **4**. The reaction mixture was quenched with water and filtered to remove the solid residues. The resulting filtrate was concentrated under reduced pressure to yield **5** (730 mg, 90%) as a white solid. R_f 0.2 (1:1hexane/EtOAc). ^{1}H NMR (300 MHz, CD_3OD) δ 6.31 (t, 1H, J = 8.1 Hz), 5. 62 (s, 1H), 5.56 (d, 1H, J = 7.2 Hz), 5.52 (d, 1H, J = 8.1 Hz) 3.12 (t, 4H, J = 6.0 Hz), 2.81−2.73 (m, 4 H), 1.33 (t, 4 H, J = 6.3 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 167.5, 149.9, 128.9, 108.9, 105.1, 101.5, 60.0, 48.7, 30.3. MS (ESI): 225.13 (M^+). IR (neat) 3350, 2921, 2854 cm^{-1}.

Compound 6. Sodium nitrite (0.92 g, 13.3 mmol) in water (11.2 mL) was added, over a period of 1 h, via a syringe pump at the rate of 0.2 mL per min, to a solution of **5** (2.0 g, 8.9 mmol) in HCl (9.0 mL, 10.0 M) and water (4.5 mL) at 0 °C. The mixture was stirred for 2.5 h at 0 °C and filtered to remove residual impurities.

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The filtrate was evaporated under reduced pressure, the residue dissolved in methanol, dried with magnesium sulfate, and filtered, and methanol was evaporated to yield **6** (1.54 g, 74%). This somewhat unstable, hygroscopic nitroso compound was used in the next step without further purification. R_f 0.4 (90% EtOAc/MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 7.70 (d, 1H, J = 10.2 Hz), 7.26 (d, 1H, J = 10.2 Hz), 6.49 (s, 1H), 4.02 (d, 2H, J = 7.4 Hz), 3.98 (d, 2H, J = 7.4 Hz), 3.81−3.66 (br, 4H), 2.02−1.86 (br, 4H). ^{13}C NMR (75 MHz, CD_3OD) δ 166.1, 163.6, 144.8, 123.4, 120.0, 98.0, 58.3, 51.1, 31.8. MS (ESI): 254.24 (M^+).

Compound 1. Compound **6** (1.0 g, 3.5 mmol) was dissolved in 50 mL of dry distilled DMF and solid 1,6-dihydroxynaphthol then HCl (2 mL, 10.0 M) were added in that order. The reaction mixture was heated to 130 °C for 5 h and cooled to room temperature, then the DMF was removed under reduced pressure. The residual material was purified by flash chromatography eluting with 1:10 MeOH/EtOAc to afford **1** as a red solid (740 mg, 54%). R_f 0.3 (90%EtOAc/MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.41 (s, 1H), 7.94 (d, 1H, J = 8.4 Hz), 7.86 (d, 1H, J = 2.7 Hz), 7.56 (d, 1H, J = 9.0 Hz), 7.06 (dd, 1H, J = 6.0, 2.4 Hz), 6.82 (d, 1H, J = 8.4 Hz), 6.66 (s, 1H), 6.14 (s, 1H), 4.63 (d, 4H, J = 4.8 Hz), 3.47 (d, 4H, J = 1.5 Hz), 1.66−1.78 (br, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 182.3, 161.3, 152.3, 151.9, 147, 139.4, 134.4, 131.4, 128.2, 124.5, 119.1, 110.7, 108.7, 104.8, 97.2, 96.9, 58.9, 48.3, 30.6. MS (ESI): 395.16 $(M + H)^+$. IR (neat) 3358, 2918, 2847 cm^{-1}.

Compound 7. Sodium nitrite (1.32 g, 19.2 mmol) in water (10 mL) was added over a period of 50 min with a syringe pump at the rate of 0.2 mL per minute to a solution of **4** (5.0 g, 17.8 mmol) in HCl (6.0 mL, 10.0 M) and water (3 mL) at 0 °C. The mixture was stirred for 2.5 h at 0 °C and filtered to remove solid impurities. The filtrate was evaporated under reduced pressure to yield **7** (4.2 g, 74%) after drying. This somewhat unstable nitroso compound was directly used for the next step. R_f 0.4 (90% EtOAc/MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 7.60 (d, 1H, J = 9 Hz), 7.15(d, 1H, J = 12.0 Hz), 6.34(s, 1H), 4.09(d, 4H, J = 27.0 Hz), 3.54(s, 6H), 2.81−2.73 (m, 4H). IR (neat) 3383, 2967, 2362, 1729 cm $^{-1}$.^{13}C NMR (75 MHz, acetone-d_6) δ 182.1, 163.1, 157.5, 134.3, 104.8, 104.7, 99.7, 44.3, 39.5, 22.6. MS (ESI): 311.23 $(M + H)^+$.

Compound 8. 1,6-Dihydroxynaphthol (1.85 g, 11.6 mmol) with HCl (2.0 mL, 10.0 M) was added to a solution of **7** (4.0 g, 11.6 mmol) in MeOH (100 mL) all in one portion. The reaction mixture was refluxed for 5 h. The solvent was evaporated and the residue was purified by flash chromatography with 90% EtOAc/MeOH eluant to afford **8** as a red solid (2.8 g, 54%). R_f 0.7 (90%EtOAc/MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.94 (d, 1H, J = 9 Hz), 7.86 (s, 1H), 7.58 (d, 1H, J = 9 Hz), 7.09−7.05 (m, 1H), 6.81−6.78 (m, 1H), 6.69 (s, 1H), 6.13 (s, 1H), 3.70 (t, 4H, J = 6.0 Hz), 3.60 (s, 6H), 2.65−2.60 (t, 4H, J = 6.0 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 182.4, 172.4, 161.4, 152.1, 150.9, 146.8, 140.7, 134.3, 131.4, 128.2, 124.9, 124.5, 119.3, 110.7, 108.9, 105.1, 97.7, 52.2, 46.9, 32.1. MS (ESI): 450.14 (M^+). IR (neat) 3422, 2955, 1734 cm^{-1}.

Compound 2. A solution of K_2CO_3 (276 mg, 2 mmol) dissolved in 5 mL of water was added to a solution of **8** (100 mg, 0.2 mmol) in MeOH/water (1:1). The reaction mixture was heated to 40 °C for 36 h. The solvent was evaporated under reduced pressure; the crude mixture was then dissolved in water (10 mL) and washed with EtOAc (3 × 5 mL). The aqueous layer was acidified with HCl (4−5 drops, 10.0 M) to pH 4−5. This aqueous layer was extracted with CH_2Cl_2/2-propanol (3 × 5 mL, 1:1) and the organic layer was evaporated under reduced pressure to give **2** (60 mg, 60%) as a blue solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.95 (d, 1H, J = 9.0 Hz), 7.88−7.87 (m, 1H), 7.62 (d, 1H, J = 9 Hz), 7.09 (dd, 1H, J = 8.7, 2.7 Hz), 6.82 (d, 1H, J = 9.3 Hz), 6.69 (s, 1H), 6.16 (s, 1H), 3.76−3.65 (br, 4H), 2.58−2.51 (br, 4H). ^{13}C (75 MHz, DMSO-d_6) δ 182.3, 173.5, 161.5, 152.2, 151.1, 146.9, 140.4, 134.3, 131.5, 128.2, 125.0, 124.4, 119.3, 110.9, 108.9, 105.0, 97.5, 47.2, 32.6. MS (ESI): 421.01 $(M - H)^-$. IR (neat) 3425, 3059, 2928, 1637 cm^{-1}.

Compound 9. 3-Aminophenol (5.0 g, 4.6 mmol) in acrylic acid (4.7 mL, 6.9 mmol) and water (3 mL) was heated to 70 °C for 12 h. The reaction mixture was cooled to room temperature then the solvent was evaporated under reduced pressure. The residual material was purified by flash chromatography eluting with 1:1 hexane/EtOAc to afford **9** as a white semisolid (3.3 g, 40%). R_f 0.3 (EtOAc). ^{1}H NMR (300 MHz, CD_3OD) δ 6.93 (s, 1H), 6.21−6.12 (m, 3H), 4.31−4.27 (m, 1H), 3.34−3.30 (m, 2H), 2.64−2.52 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ 175.1, 158.1, 149.6, 129.8, 105.5, 104.8, 100.2, 39.8, 33.5. MS (ESI): 181.08 (M^+).

Compound 10. A solution of **9** (3.0 g, 16.5 mmol) and propane sultone (5.0 g, 41.2 mmol) in DMF (20 mL) was heated, with stirring, to 130 °C for 3 h. The solvent was evaporated under reduced pressure and crude residue purified by flash chromatography initially eluting with EtOAc to remove all of the unreacted sultone and then eluting with 1:1 MeOH:EtOAc to afford **10** (2.4 g, 48%) as a dark brown semisolid. R_f 0.2 (1:1 EtOAc:MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 8.47 (s, 1H), 7.04−6.94 (m, 1H), 6.31−6.22 (br, 2H), 6.10 (d, 1H, J = 9.0 Hz), 3.68−3.58 (m, 4H), 2.88 (t, 2H, J = 6.0 Hz), 2.5 (t, 2H, J = 6.0 Hz), 2.08−1.96 (br, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ 172.8, 159.2, 149.3, 130.0, 104.6, 103.8, 99.8, 60.9, 49.7, 33.8, 28.1, 23.2. MS (ESI): 302.06 $(M - H)^-$. IR (neat) 3403, 2910, 1626 cm^{-1}.

Compound 11. To a solution of **10** (0.5 g, 1.6 mmol) in HCl (2.0 mL, 10.0 M) and water (1 mL) at 0 °C was added sodium nitrite (0.13 g, 1.8 mmol) dissolved in water (2 mL) over a period of 10 min with a syringe pump at the rate of 0.2 mL per min. The mixture was stirred for 3 h at 0 °C and filtered to remove residual impurities. The filtrate was evaporated under reduced pressure to yield **11** (0.4 g, 74%). The crude product was very moisture sensitive; it was therefore used directly without further purification for the next step.

Compound 3. A solution of crude **11** (0.39 g, 1.1 mmol) and 1,6-dihydroxynaphthol (0.2 g, 1.2 mmol) in DMF (15 mL) was heated to reflux for 5 h. The DMF was evaporated under reduced pressure and the residual material was purified by flash chromatography; eluting with EtOAc removed any unreacted 1,6-dihydroxynaphthol and then eluting with 1:1 EtOAc:MeOH afforded **3** (0.25 g, 53%) as a red solid. R_f 0.15 (1:1 EtOAc: MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ 8.07 (d, 1H, J = 8.5 Hz), 8.00 (s, 1H), 7.65 (d, 1H, J = 9.0 Hz), 7.10 (d, 1H, J = 8.5 Hz), 6.97 (d, 1H, J = 9.0 Hz), 6.76 (s, 1H), 6.24 (s, 1H), 3.83 (t, 2H, J = 7 Hz), 3.69 (t, 2H, J = 8.0 Hz), 2.92−2.89 (m, 2H), 2.68 (t, 2H, J = 7.5 Hz), 2.14 (t, 2H, J = 9.0 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 184.2, 174.3, 161.3, 152.9, 151.6, 146.9, 139.6, 134.6, 131.2, 127.6, 125.4, 124.2, 118.1, 111.2, 108.6, 103.8, 96.8, 50.6, 49.6, 31.8, 22.8. MS (ESI): 471.06 $(M - H)^-$.

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Supporting Information Available: General experimental conditions and characterization data for compounds **1**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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