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Substituted 9-Diethylaminobenzo[*a*]phenoxazin-5-ones (Nile Red Analogues): Synthesis and Photophysical Properties

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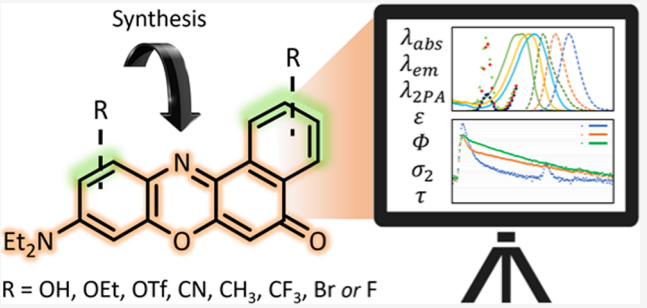
ABSTRACT: Nile Red is a benzo[*a*]phenoxazone dye containing a diethylamino substituent at the 9-position. In recent years, it has become a popular histological stain for cellular membranes and lipid droplets due to its unrivaled fluorescent properties in lipophilic environments. This makes it an attractive lead for chemical decoration to tweak its attributes and optimize it for more specialized microscopy techniques, e.g., fluorescence lifetime imaging or two-photon excited fluorescence microscopy, to which Nile Red has never been optimized. Herein, we present synthesis approaches to a series of monosubstituted Nile Red derivatives (9-diethylbenzo[*a*]phenoxazin-5-ones) starting from 1-naphthols or 1,3-naphthalenediols. The solvatochromic responsiveness of these fluorophores is reported with focus on how the substituents affect the absorption and emission spectra, luminosity, fluorescence lifetimes, and two-photon absorptivity. Several of the analogues emerge as strong candidates for reporting the polarity of their local environment. Specifically, the one- and two-photon excited fluorescence of Nile Red turns out to be very responsive to substitution, and the spectroscopic features can be finely tuned by judiciously introducing substituents of distinct electronic character at specific positions. This new toolkit of 9-diethylbenzo[*a*]-phenoxazine-5-ones constitutes a step toward the next generation of optical molecular probes for advancing the understanding of lipid structures and cellular processes.



INTRODUCTION

Benzophenoxazines represent an interesting scaffold for chemical modification. Not only have the structures shown potent anticancer,[1−4] antimalaria,[5] antibiotic,[1,6] and antifungal activities,[7] but also they have been widely applied as dyes and pigments due to their intense colors.[8−11] While the unsubstituted ring system is not particularly fluorescent, this situation may be drastically altered by the addition of substituents that can donate or accept electron density.[8] A selection of such dyes based on the benzo[*a*]phenoxazine core have been reported in the literature;[8,12] the most notable ones being Meldola's blue,[13] Nile Blue,[14] and Nile Red[10] (Scheme 1a). These bear dialkylamino groups at the 9-position that are vital to their luminescence by donating electron density into the π ring system. Nile Red (9-diethylbenzo[*a*]phenoxazin-5-one) stands out by being electrically neutral and water-insoluble since it features a carbonyl group at the 5-position that readily accepts the electron density. This makes it a strong push−pull chromophore (Scheme 1b), which underlies its distinct solvatochromicity[15,16] and ability to indicate e.g., cellular events in live-cell imaging.[17−19] In addition, Nile Red's inherent lipophilicity, near-infrared (NIR) emission, large Stokes shift, two-photon absorptivity (2PA),[20,21] and low background fluorescence[16,22] (as the luminosity is quenched in aqueous (aq) media) render it a near-ideal histochemical stain for studying membranes[23,24] and lipid droplets in cells.[25−28] The increase of its exquisite solvent sensitivity and steep emission when residing in hydrophobic environments has also been employed in various forms of stochastic super-resolution localization microscopy.[29−31]

The synthesis of Nile Red (**2**) starts with the thermal condensation of 5-diethylamino-2-nitrosophenol (**1**) with 1-naphthol (Scheme 1c),[10,32] and the resulting *p*-benzoquinoneimine intermediate then undergoes rapid oxidative cyclization.[33] This condensation−cyclization sequence is inherently inefficient as the cyclization step squanders the nitroso reagent **1** in the process (for oxidation),[10,33] and surplus loading of **1** mostly instigates additional byproducts. This issue—as well as the scarcity of synthetically accessible nitrosophenol and 1-naphthol derivatives—is likely to have discouraged the development of de novo synthesized analogues.[8] A few

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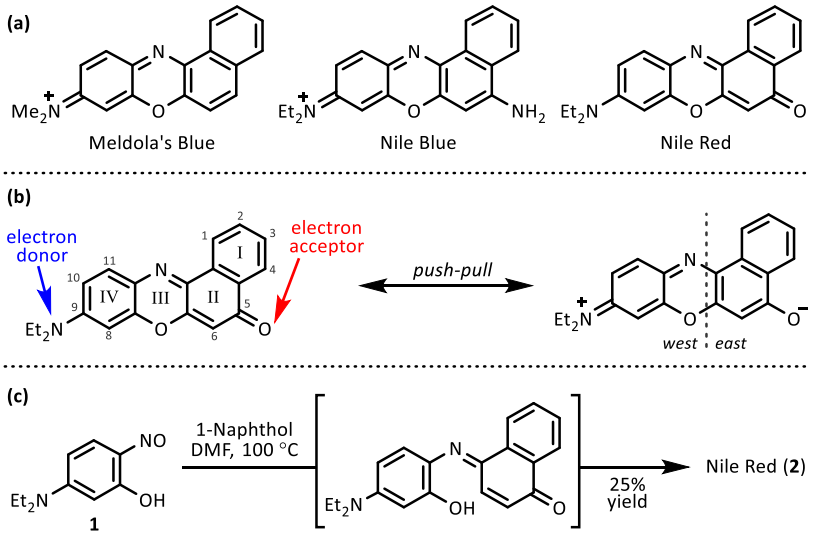
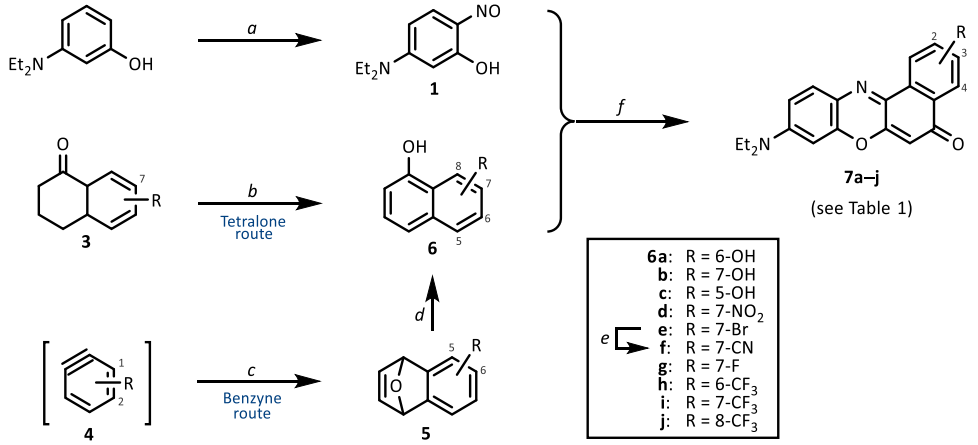
Scheme 1



Scheme 2. Synthesis of 9-Diethylbenzo[*a*]phenoxazine-5-one Derivatives from *o*-Nitrosophenols and Naphthols[a]



[a]Reagents and conditions: (a) $NaNO_2$, H_2O, 0 °C, 76% yield. (b) Pd/C, K_2CO_3, *N*,*N*-dimethylacetamide (DMA), 165 °C, 66% yield (**6d**), or Pd/C, tetraglyme, 260 °C, 72% yield (**6g**). (c) furan, tetrahydrofuran (THF) or 1,2-dimethoxyethane (DME). (d) HCl, EtOH, 50 °C, yield over two steps: 41% (**6e**), 19% (**6h**), 16% (**6i**), 48% (**6j**). (e) CuCN, dimethylformamide (DMF), 160 °C, 88% yield. (f) See Table 1. See the Experimental Details for details.

examples of substituted Nile Red analogues have been reported in the literature, including 6-fluoro,[34] 6-bromo,[35] and 6-acyloxy[36] derivatives and a collection of 2- and 3-alkoxy derivatives.[37−41] Indeed, the 6-position is the site of the highest frontier electron density,[42] and the 2- or 3-oxy derivatives are straightforward due to the commercial availability of 1,6- and 1,7-naphthalenediols.

We have realized that a detailed understanding of the effects of introducing small electron-withdrawing or -donating groups to the peripheral rings (I, IV) of Nile Red is missing. In fact, peripheral substitutions appear to have the greatest effect on the photophysical properties according to our preceding computational study.[20] In the present work, we have explored the chemical and optical nature of a new series of substituted 9-diethylbenzo[*a*]phenoxazin-5-ones. Such new analogues of Nile Red represent an interesting expansion of the fluorescent probe toolbox and for the first time offer a systematic account of the optical consequences of substituents. To this end, we report novel synthetic routes that led to this library and present full spectroscopic characterization (absorption spectra, emission spectra, quantum yields (QYs), fluorescence lifetimes, two-photon absorption (2PA) spectra, and one- and two-photon brightnesses). Independently of our work, the synthesis of the 3-fluoro and 3-methoxy derivatives has very recently been reported;[43] however, the authors did not study their fluorescent properties in detail.

RESULTS AND DISCUSSION

Synthesis. Introduction of substituents on the easternmost ring (I) of benzo[α]phenoxazin-5-ones requires 1-naphthols bearing 5-, 6-, 7-, or 8-substituents for the synthesis. However, remarkably few such 1-naphthol derivatives have been reported in the literature as it requires substitution on the deactivated half of naphthalene.[44] 1,5-, 1,6-, and 1,7-Naphthalenediols (**6a−c**) are commercially available, and we successfully synthesized a small library of nitro-, bromo-, cyano-, fluoro-, and trifluoromethyl-substituted 1-naphthols (**6d−j**) using either α-tetralones or benzynes as starting materials (Scheme 2). In the tetralone approach to naphthols, tetralones (**3**) were prompted to undergo Pd/C-catalyzed dehydrogenative aroma-

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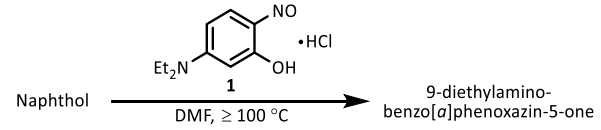
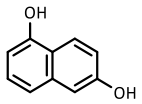
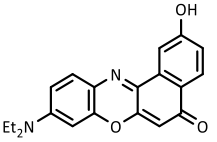
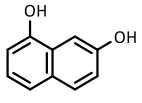
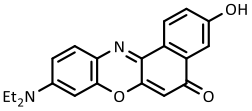
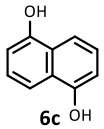
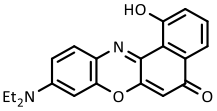
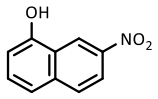
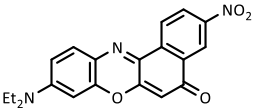
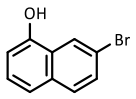
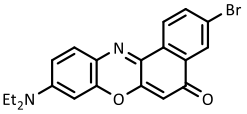
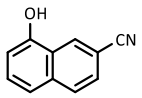
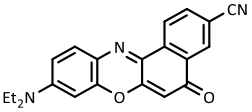
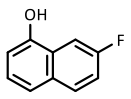
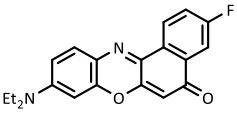
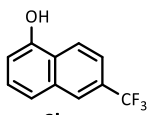
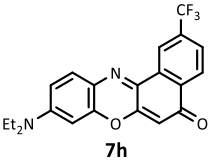
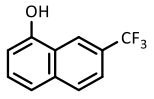
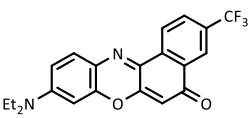
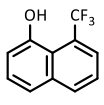
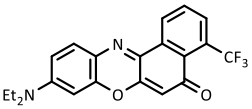
tization.[45,46] In the benzyne route, furan was mixed with dehydrobenzene intermediates (**4**) that were generated in situ when either chlorobenzenes were treated with *n*-BuLi,[47] anthranilic acids were treated with nitrite,[48] or diaryliodonium salts were treated with lithium hexamethyldisilazane (LHMDS).[49] The resulting Diels–Alder cycloadducts—1,4-dihydro-1,4-epoxynaphthalenes (**5**)—then isomerized with acid to 1-naphthols.[50] This approach was prone to yield regioisomeric mixtures of 1-naphthol products that needed chromatographic separation (e.g., **6h** and **6i**). In specific cases where the regioisomers were totally inseparable, the tetralone approach was preferable (e.g., in the case of **6d**). Yet, the tetralone approach necessitated scalding temperatures and starting materials that were not easily accessible. The 7-nitro and 7-bromo-1-tetralones (for the syntheses of **6d** and **6e**) were successfully obtained by nitration and bromination of α-tetralone,[51,52] respectively. In the case of 7-cyano-1-naphthol (**6f**), this novel compound was successfully synthesized from 7-bromo-1-naphthol (**6e**) upon the action of CuCN under Rosenmund–von Braun conditions.[53]

The naphthol derivatives **6a–j** were mixed with 5-diethylamino-2-nitrosophenol (**1**) at 100 °C in DMF to produce the corresponding 9-diethylaminobenzo[*a*]-phenoxazin-5-ones (Table 1). The reactions were monitored by thin-layer chromatography (TLC) and occasionally electrospray ionization (ESI)–mass spectrometry. In those cases where no reaction had occurred after 4–12 h at 100 °C, incremental heating up to 160 °C had no effect and slowly resulted in the decomposition of nitrosophenol **1**. As shown in Table 1, the nature of the substituent has a remarkable effect on the reaction. The hydroxy Nile Red derivatives (**7a–c**) are formed in modest (47%, **7c**) to high yields (69–72%, **7a** and **7b**), whereas the yields of the fluoro- and trifluoromethyl derivatives (**7g–j**) are comparable to the unsubstituted case (**2**), i.e., 14–32 vs 25%. Thus, the β-hydroxy substituents of **6a** and **6b** appear to favorably increase the nucleophilic strength of the 4-position on the naphthol ring, which in turn promotes the reaction and minimizes side reactions. To our surprise, none of the 3-nitro, 3-cyano, and 3-bromo Nile Red derivatives (**7d–f**) were produced at quantifiable levels. In these cases, it appears that the nitro, cyano, and even bromo substituents are too deactivating for the reaction to proceed. This clearly shows the limited scope of the original synthesis approach to substituted Nile Red derivatives.

To combat this synthetic obstacle, we launched a search for novel, alternative synthetic routes to the benzo[*a*]phenoxazine-5-one heterocycle. In this process, we for instance examined the coupling of 2-amino-5-(diethylamino)phenol with naphthoquinone (Scheme 3);[54,55] however, this only gave trace production of Nile Red (**2**), possibly due to the oxidative instability of the former starting material[56] as well as the formation of side products. We also examined the impact of replacing the nitroso functionality with an arylazo group (Scheme 3), which has successfully been used in the synthesis of Nile Blue.[57,58] However, this led to slower reactions even at elevated temperatures (150 °C) and a poorer yield of **2** (15% yield), thus not constituting an improvement of the original procedure. Finally, we examined the idea that the nitroso and/or the hydroxy functionality could be transferred to the naphthol moiety (Table 2) to interconvert the electrophile and nucleophile center. Indeed, ethyl 1,3-dihydroxy-2-naphthoate has previously been used for the synthesis of 6-acyloxy 9-diethylbenzo[*a*]phenoxazin-5-ones,[36] and Nile Blue has previously been synthesized using 4-nitroso-1-naphtylamine.[57] The original synthesis yields 25% of Nile Red (route A). When the nitroso group was relocated to the naphthol ring (routes C and D), no reaction was found to take place. However, upon

Table 1. Synthesized 9-Diethylaminobenzo[*a*]phenoxazine-5-ones from 1-Naphthols



Naphthol	Benzo[α]phenoxazin-5-one	Yield
 **6a**	 **7a**	72%
 **6b**	 **7b**	69%
 **6c**	 **7c**	47%
 **6d**	 **7d**	0%
 **6e**	 **7e**	0%
 **6f**	 **7f**	0%
 **6g**	 **7g**	32%
 **6h**	 **7h**	21%
 **6i**	 **7i**	14%
 **6j**	 **7j**	27%

https://dx.doi.org/10.1021/acs.joc.0c02346
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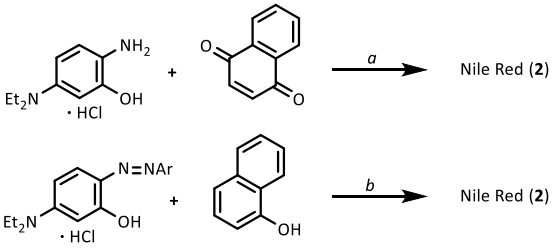
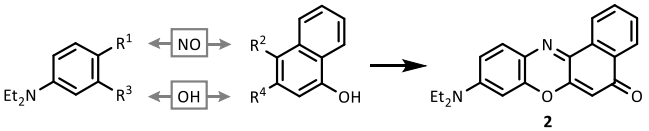
Scheme 3. Synthesis of Nile Red from 2-Amino- or 2-Aryldiazenyl-5-(diethylamino)phenols[a]



[a]Reagents and conditions: (a) DMF, 100 °C, trace yield. (b) DMF, 150 °C, 15% yield. Ar = *p*-nitrophenyl.

Table 2. Benzo[*a*]phenoxazinone Formation after Transfer of the Nitroso or the Hydroxy Group



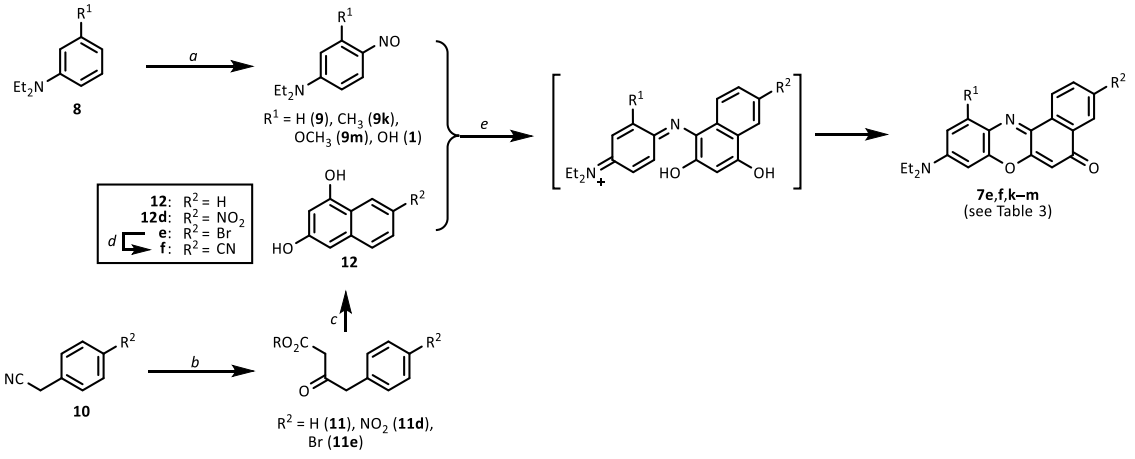
2

#	R^1	R^2	R^3	R^4	conditions	yield (%)
A	NO	H	OH	H	DMF, 100 °C	25
B	NO	H	H	OH	DMF, 80 °C	80
C	H	NO	OH	H	DMF, ≥100 °C	0
D	H	NO	H	OH	DMF, ≥100 °C	0

coupling 1,3-naphthalenediol with *N*,*N*-diethyl-*p*-nitrosoaniline (route B), we noticed remarkably improved reactivity, chemoselectivity, and isolated yield of Nile Red (80% yield). We attribute this to the presumed increase in the nucleophilicity of C-4 in 1,3-naphthalenediol relative to 1-naphthol[59,60] and possibly the increased electrophilicity of the nitroso group in the absence of the hydroxyl group. The high yield also implies that the reaction uses ambient air for the oxidative cyclization rather than consuming the nitroso material.

The beneficial outcome of the condensation of 1,3-naphthalenediol with *N*,*N*-diethyl-*p*-nitrosoaniline (route B) prompted us to explore the scope of this reaction. Scheme 4 outlines the synthesis of selected nitrosoanilines (**9**) and 1,3-naphthalenediols (**12**) and their coupling to produce 9-diethylbenzo[*a*]phenoxazine-5-ones. The novel 7-bromo-1,3-naphthalendiol (**12e**) was neatly synthesized by a sulfuric acid-promoted cyclocondensation[61] of ethyl γ-4-bromophenyl acetoacetate (**11e**), which in turn was prepared using a Blaise reaction,[62] i.e., Zn-promoted reaction of 4-bromobenzonitrile with ethyl bromoacetate.[63] 7-Cyano-1,3-naphthalenediol (**12f**) was obtained by a Cu-catalyzed ipso-cyanation[53] of the 7-bromo derivative **12e**. Unfortunately, the Blaise reaction did not proceed with a 4-nitro substituent (this has also been noted elsewhere[64]). Rather, acetoacetate **11d** was produced in good yield by hydrolysis of nitrile **10** (R^2 = NO_2) and imidazolide activation and then $MgCl_2$/Et_3N-mediated condensation with monomethyl malonate[65,66] followed by decarboxylation. Nevertheless, all attempts to cyclize this β-keto ester failed and compound **12c** was not realized. When 7-bromo and 7-cyano-1,3-naphthalenediols (**12e** and **12f**) were each treated with *N*,*N*-diethyl-*p*-nitrosoaniline (**9**), only the 3-bromo Nile Red derivative (**7e**) was formed (57% yield), and no reaction took place with the nitrile (Table 3). This indicates that the cyano group still deactivates the ring positions too much despite two activating hydroxyls. We also gauged the possibility of having methyl, hydroxy, and methoxy substituents on the nitrosoaniline to synthesize the 11-methyl, 11-hydroxy, and 11-methoxy benzo[*a*]phenoxazinones **7k**–**m** (Table 3). 11-Methyl derivative **7k**, which emerged from the condensation of 1,3-naphthalenediol (**12**) with *N*,*N*-diethyl-3-methyl-4-nitrosoaniline (**9k**), was isolated in 46% yield. Formation of the corresponding 11-hydroxy and 11-methoxy derivatives **7l** and **7m** was never observed, however. Instead, these reactions yielded unsubstituted Nile Red (**2**) in high yields (78–84%). Hence, it appears that the aromaticity after electrocyclization is spontaneously restored by elimination (of water or methanol) in lieu of oxidative dehydrogenation. It follows that any substituent that is also a leaving group (even a poor leaving group like methoxy) cannot be introduced at the 11-position of Nile Red in this way.

Scheme 4. Synthesis of 9-Diethylbenzo[*a*]phenoxazin-5-one Derivatives from *p*-Nitrosoanilines and 1,3-Naphthalenediols[a]



[a]Reagents and conditions: (a) $NaNO_2$, H_2O, 0 °C, 69% yield (**9**), 56% yield (**9k**), 71% yield (**9m**), 76% yield (**1**). (b) Ethyl bromoacetate, Zn, THF, 84% yield (**11**), 90% yield (**11e**), or (i) H_2SO_4, H_2O. (ii) Carbonyldiimidazole (CDI), THF, 65 °C. (iii) Methyl potassium malonate, $MgCl_2$, Et_3N (**11d**), 83% yield over three steps. (c) H_2SO_4, 60 °C, 77% (**12**), 0% yield (**12d**), 67% yield (**12e**). (d) CuCN, DMF, 160 °C, 90% yield. (e) See Table 3. R = Et (**11**, **11e**)/Me (**11d**).

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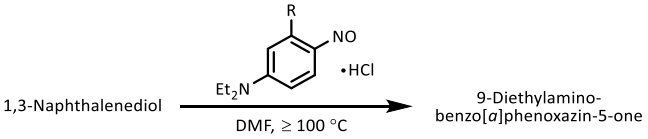
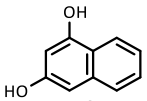
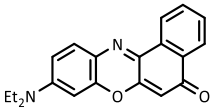
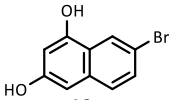
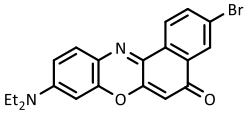
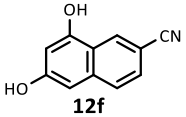
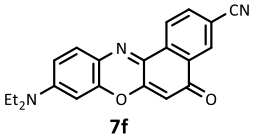
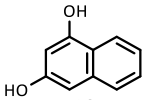
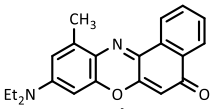
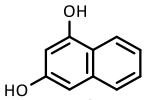
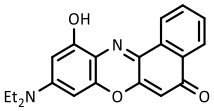
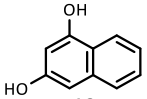
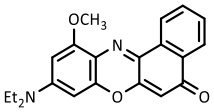
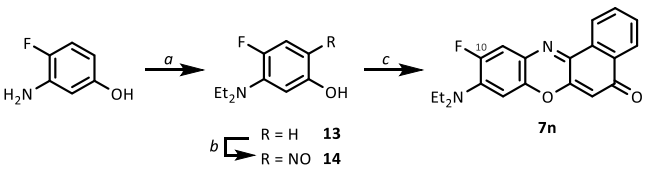
Table 3. Synthesis of 9-Diethylaminobenzo[*a*]phenoxazin-5-ones from 1,3-Naphthalenediols



1,3-Naphthalenediol	R	Benzo[α]-phenoxazin-5-one	Yield
 **12**	H	 Nile Red (**2**)	80%
 **12e**	H	 **7e**	57%
 **12f**	H	 **7f**	0%
 **12**	CH_3	 **7k**	46%
 **12**	OH	 **7l**	0% (84% of **2**)
 **12**	OCH_3	 **7m**	0% (78% of **2**)

Next, we examined the potential of using route A or route B of Table 2 for introducing substituents at positions 8 or 10 (i.e., ortho to the diethylamino group). Substitution at these sites has not been reported in the literature. Using route A, we successfully nitrosylated 5-diethylamino-4-fluorophenol (**13**) at the 2-position in 56% yield and coupled this with 1-naphthol (or alternatively 1,3-naphthalene-diol) to obtain the 10-fluoro Nile Red derivative **7n** (Scheme 5). However, all other attempts to *p*-nitrosylate other o-substituted *N*,*N*-diethylanilines (*o*-CH_3, $-CF_3$, $-OH$, $-OEt$, $-OCH_3$, $-NEt_2$) were unsuccessful due to the domination of *N*-nitroso-*N*-dealkylated products and/or *C*-nitro products that hindered isolation of the desired *p*-nitrosoaniline (reactions are listed in Table S1). Formation of these known side products has previously only been detected sporadically[67−70] and was associated with electron-rich aromatic amines.[71] The current observation indicates that with *o*-substituted *N*,*N*-diethylanilines their production is prevalent, which could be due to steric hindrance diminishing the sp^2 character of nitrogen. Indeed, steric hindrance from *o*-substituents has previously been reported to block nitrosation,[72] and no successful p-nitrosations of o-substituted *N*,*N*-dialkylanilines appears to have been reported in the literature.

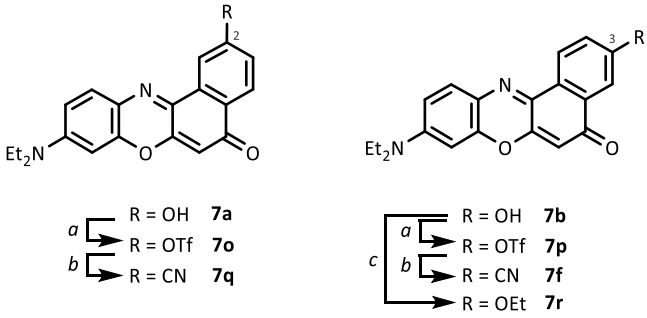
Scheme 5. Synthesis of 10-Fluoro-Substituted 9-Diethylbenzo[*a*]phenoxazin-5-one[a]



[a]Reagents and conditions: (a) C_2H_5I, Na_2CO_3, *i*PrOH, H_2O, 85 °C, 59% yield. (b) $NaNO_2$, H_2O, 0 °C, 56% yield. (c) 1-Naphthol, DMF, 100 °C, 10% yield, or 1,3-naphthalenediol, DMF, 80 °C, 23% yield.

Finally, we explored the potential of converting the 2- and 3-hydroxy substituents of benzo[*a*]phenoxazinones **7a** and **7b** into other functional groups (Scheme 6). First, triflates **7o** and **7p** were produced by treating the hydroxy derivatives with *N*-phenyl bistriflimide and triethylamine.[73] Subsequent ipso-cyanation of the triflates into nitriles **7f** and **7q** proved very challenging since even advanced cyanation systems (e.g., CuCN, $KCN/Pd_2(dba)_3$/dppf,[74] $Zn(CN)_2/Pd(PPh_3)_4$,[75] $K_4[Fe(CN)_6]/Pd(OAc)_2$/XPhos,[76] or *n*-PrCN/$Ni(COD)_2$/$AlMe_3$/Xantphos[77]) had either no effect or triggered hydrolysis and/or reductive detriflation. However, the novel use of CuCN in combination with a Buchwald catalyst system[78] comprising $Pd_2(dba)_3$, SPhos, and Na_2CO_3 was found to successfully interconvert the triflates into nitriles **7f** and **7q** in high yields (76−87%). Finally, the 3-ethoxy-substituted Nile Red analogue **7r** was obtained separately by alkylation of 3-hydroxy derivative **7b** with iodoethane and K_2CO_3.

Scheme 6. Synthesis of Triflate-, Cyano-, and Ethoxy-Substituted 9-Diethylbenzo[*a*]phenoxazin-5-ones[a]



[a]Reagents and conditions: (a) $PhN(OTf)_2$, THF, rt, 77% yield (**7o**), 70% yield (**7p**). (b) CuCN, Na_2CO_3, $Pd_2(dba)_3$, SPhos, toluene, EtOH, 80 °C, 87% yield (**7q**), 76% yield (**7f**). (c) C_2H_5I, K_2CO_3, DMF, 50 °C, 90% yield.

Photophysical Properties. The new set of Nile Red variants (Figure 1) enable us to survey the optical consequences of appending substituents on the top face of Nile Red. Indeed, such experimental insight into how different electron-withdrawing and -donating substituents influence the chromophore's push−pull character is pivotal to the design of new fluorescent dyes with superior characteristics for fluorescence imaging techniques, for example, fluorescence lifetime imaging microscopy (FLIM), two-photon excited fluorescence (2PEF) microscopy as well as for stimulated emission depletion (STED) and localization-based fluorescence nanoscopy.

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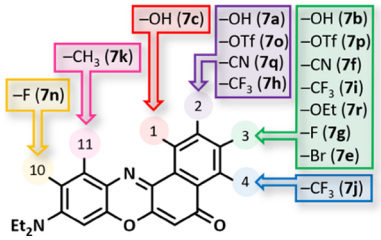

Figure 1. Overview of the synthesized 1-, 2-, 3-, 4-, 10-, or 11-substituted 9-diethylbenzo[*a*]phenoxazin-5-ones.

Nile Red (**2**) has peak absorption at ∼530 nm and emits yellow-gold fluorescence at ∼600 nm.[79] These wavelengths are blue-shifted in apolar solvents and red-shifted in polar solvents.[15,16] In addition, Nile Red absorbs near-infrared (NIR) photons at ∼880 and ∼1060 nm through a two-photon absorption (2PA) process.[21] Such two-photon excited fluorescence (2PEF)[80] is induced by near-simultaneous (∼10^{-15} s) absorption of two photons within the cross-sectional area of the molecule (3.4 × 10^{-15} cm^2). This necessitates large photon flux densities (>10^{30} photons/cm^2), which is attainable using ultrafast pulsed laser sources (e.g., 880 nm excitation using Ti:sapphire femtosecond lasers). Practical benefits to using 2PEF-based microscopy include diminished background fluorescence from fluorophores outside the focal volume (∼0.1 μm^3), reduced photobleaching, less photodamage to sensitive tissues, increased penetration depth,[81] and intrinsic three-dimensional resolution in laser scanning microscopy.[82] The 2PA cross section for Nile Red, i.e., the efficiency of two-photon excitation (usually expressed in Goeppert−Mayer units: GM = 10^{-50} cm^4 s/photon), is ∼50 GM at 880 nm.[21]

Our present spectroscopic data for Nile Red and the 15 analogues are summarized in Table 4. To establish the ability of the fluorophores to sense the polarity of their environment, we measured the compounds in three isoviscous organic solvents: apolar aprotic toluene (PhMe), polar aprotic chloroform ($CHCl_3$), and protic methanol (MeOH) with dielectric constants (ε_r) of 2.38, 4.81, and 32.7, respectively.[83] Table 4 includes the one-photon absorption maximum (λ_{max}^{1PA}), the emission maximum (λ_{max}^{em}), the 2PA maximum in the first near-infrared (NIR-I) spectral window (i.e., from 800 to 1000 nm), the peak one-photon molar absorptivity (ε), the peak two-photon cross section (σ_2), the quantum yield (Φ), and the one-photon excited fluorescence lifetime (τ). To probe their applicability for microscopy, we also list their brightnesses $\Phi\varepsilon$ and two-photon action cross sections $\Phi\sigma_2$ (also called two-photon brightness). The high brightness of a fluorophore is a clear advantage in cell biology to secure a high signal-to-noise ratio at low concentrations and to limit the irradiation intensity to the tissue.

Figure 2 illustrates the one-photon absorption (1PA) and emission bands of the substituted Nile Red analogues in chloroform. Full UV−vis and fluorescence spectra are included in the SI (Figures S64−S79). Nile Red and its analogues all show a single intense absorption band (assigned to the $\pi \rightarrow \pi^*$ transition[21]) that peaks between 527 and 570 nm in chloroform. As shown in Table 4, increasing the solvent polarity greatly shifts the absorption maxima to longer wavelengths for all of the Nile Red analogues: toluene (502−549 nm) < $CHCl_3$ (527−570 nm) < MeOH (530−578 nm). The largest red shift occurs between toluene and chloroform, which could indicate that dipole−dipole interactions and dielectric effects, rather than strong hydrogen bonding (as in methanol), accounts for most of the red shift. Yet, some degree of hydrogen bonding does seem to play a role in the solvation of Nile Red.[21,84] As shown in Figure 2, the substituents generally increase the wavelength of absorption (up to +33 nm) in chloroform. The exceptions are the 10-fluoro and 2-hydroxy substituents (compounds **7n** and **7a**), which induce blue-shifted absorption bands. The fluorescence of compound **7a** has in fact been studied elsewhere,[85] but it is included in this study for reference. The substituents that red shift the absorption the most are the cyano (by 33−28 nm), triflate (by 19−21 nm), and trifluoromethyl groups (by 17−18 nm), all placed at the 2- and 3-positions. These groups are strongly electron-withdrawing, and the order aligns closely with their withdrawal power.[86] It follows that pull substituents on the northeastern part of Nile Red (ring I) effectively reduce the energy required for excitation. This behavior was predicted in our computational work[20] and is consistent with the presumed stabilizing effect of the electron-withdrawing groups in the excited charge-separated state, such that the transition energy is decreased with respect to Nile Red. The substituents' conjugation with the π system (as in the case of cyano and triflate) may also contribute to the red shift.

It would be natural to assume that a substituent with hydrogen bond-donating ability (i.e., hydroxy) should behave very differently from the others. However, this does not seem to be the case since, e.g., the 3-hydroxy and 3-ethoxy derivatives (compounds **7b** and **7r**) behave remarkably similar, not only in chloroform, as shown in Figure 2, but also in methanol (see Table 4).

In general, the absorption wavelengths are similar for identical substituents on the northeastern ring, except for the hydroxy substituents (i.e., analogues **7a**−**c**). While the 1- and 3-hydroxy substituents (compounds **7b** and **7c**) cause red shift of 17 and 2 nm, respectively, the 2-hydroxy substituent (compound **7a**) induces a blue shift of 7 nm. Under the framework of valence bond theory, this discrepancy can be explained by the fact that only at the 2-position is the hydroxy group able to mesomerically donate electron density to the 5-carbonyl group, which would counteract the original dipole moment. In line with this logic, the 1- and 3-hydroxy groups might in fact be largely electron-withdrawing (by virtue of the inductive effect). The pronounced red shift of the 1-hydroxy derivative can be further explained by its ability to form a hydrogen bond to the nitrogen atom of the oxazine ring.[87] Similarly, a reduction in the dipole moment possibly also explains why the 10-fluoro derivative absorbs at lower wavelengths (527 nm) than the corresponding 3-fluoro derivative (542 nm) because in the former case the C−F bond is aligned against the direction of the dipole moment.

The molar absorptivity of Nile Red (ε ∼ 30 mM^{-1} cm^{-1}) is only very weakly affected by substitution (see Table 4). Slightly improved absorption is recognizable when Nile Red is substituted with the cyano (ε = 35.3−47.4 mM^{-1} cm^{-1}) and triflate (ε = 31.8−41.0 mM^{-1} cm^{-1}) groups, as well as 2-trifluoromethyl and 2-hydroxy groups (ε = 32.3−37.2 mM^{-1} cm^{-1}). There is no clear trend with solvent as a variable since the variation is small enough to be within the error of the measurement. Indeed, the molar absorptivities of the Nile Red analogues are largely independent of the solvent polarity, like Nile Red.

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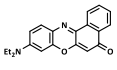
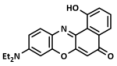
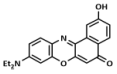
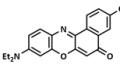
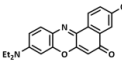
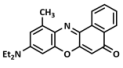
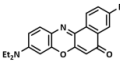
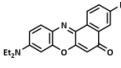
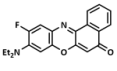
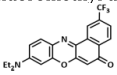
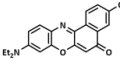
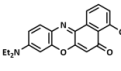
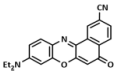
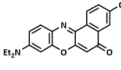
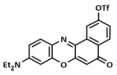
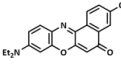
Table 4. Photophysical Parameters of 1, 7a−c, 7e−k, and 7n−r in Toluene, Chloroform, and Methanol[a,b]

Structure	#	Solvent	Band maxima λ_{max}^{1PA} [nm]	λ_{max}^{em} [nm]	λ_{max}^{2PA} [c] [nm]	Stokes shift [cm^{-1}]	ε [mM^{-1}cm^{-1}]	σ_2 [GM]	Φ [d]	τ [ns]	Brightness[e] $\Phi\varepsilon$ [mM^{-1}cm^{-1}]	$\Phi\sigma_2$ [GM]
	**2**	PhMe	521	566	875	1526	28.9	44	0.83	4.06	24.0	36.5
		$CHCl_3$	537	594	875	1787	32.6	50	0.64	4.41	20.9	32.3
		MeOH	550	636	873	2459	32.6	21	0.34	2.90	11.1	7.0
Oxy derivatives												
	**7c**	PhMe	538	583	866	1435	20.3	105	0.008	1.21	0.1	0.63
		$CHCl_3$	554	608	870	1603	35.2	48	0.006	0.69	0.3	0.38
		MeOH	561	659	869	2651	14.2	15	0.005	0.27	0.1	0.077
	**7a**	PhMe	502	571	875	2407	32.3	140	0.56	3.82	18.1	78.6
		$CHCl_3$	530	603	878	2284	36.1	170	0.44	4.24	15.9	74.6
		MeOH	544	634	878	2609	34.9	49	0.28	3.32	9.8	13.6
	**7b**	PhMe	517	572	875	1860	28.2	44	0.65	3.91	18.3	28.5
		$CHCl_3$	539	611	877	2186	29.0	51	0.50	4.50	14.5	25.3
		MeOH	545	642	877	2772	24.0	18	0.28	3.23	6.7	5.1
	**7r**	PhMe	522	574	878	1735	22.5	41	0.63	3.83	14.2	25.6
		$CHCl_3$	538	605	878	2058	23.3	37	0.49	4.50	11.4	18.1
		MeOH	548	641	878	2648	22.6	14	0.28	3.19	6.3	3.8
Methyl derivative												
	**7k**	PhMe	520	580	872	1989	39.1	96	0.50	4.13	19.6	48.1
		$CHCl_3$	540	608	865	2071	27.3	65	0.49	4.47	13.4	31.9
		MeOH	556	637	870	2287	35.5	36	0.31	3.60	11.0	11.2
Halogen derivatives												
	**7e**	PhMe	533	573	873	1310	24.7	31	0.78	3.78	19.3	24.2
		$CHCl_3$	550	602	872	1571	27.8	26	0.78	4.09	21.7	20.3
		MeOH	561	637	873	2127	27.6	19	0.36	2.71	9.9	6.8
	**7g**	PhMe	527	567	873	1339	21.2	28	0.87	3.94	18.4	24.4
		$CHCl_3$	542	596	874	1672	24.0	41	0.76	4.29	18.2	31.2
		MeOH	551	636	872	2426	23.8	19	0.37	2.67	8.8	7.0
	**7n**	PhMe	503	595	874	3074	22.9	18	0.52	3.83	11.9	9.2
		$CHCl_3$	527	607	876	2501	25.2	21	0.45	4.39	11.3	9.4
		MeOH	530	629	876	2970	26.9	70	0.04	0.38	1.1	2.8
Trifluoromethyl derivatives												
	**7h**	PhMe	541	579	871	1213	36.3	60	0.81	3.93	29.4	48.6
		$CHCl_3$	554	601	863	1412	37.2	39	0.80	4.23	29.8	31.2
		MeOH	565	638	869	2025	36.4	31	0.35	2.36	12.7	10.9
	**7i**	PhMe	541	578	872	1183	17.4	27	0.84	3.94	14.6	22.7
		$CHCl_3$	555	603	863	1434	20.9	24	0.76	4.26	15.9	18.2
		MeOH	568	632	867	1783	20.8	16	0.45	2.44	9.4	7.0
	**7j**	PhMe	529	571	874	1390	21.2	74	0.76	3.91	16.1	56.2
		$CHCl_3$	542	595	873	1643	24.0	46	0.78	4.23	18.7	35.9
		MeOH	553	631	873	2235	23.8	35	0.43	3.04	10.2	15.1
Cyano derivatives												
	**7q**	PhMe	545	594	868	1514	42.1	82	0.49	4.24	20.6	40.3
		$CHCl_3$	565	619	871	1544	35.3	91	0.49	4.40	17.3	44.8
		MeOH	573	644	869	1924	37.6	59	0.19	2.34	7.1	11.2
	**7f**	PhMe	549	598	866	1493	47.4	110	0.49	3.99	23.2	53.9
		$CHCl_3$	570	619	871	1389	42.6	126	0.49	4.01	20.9	61.7
		MeOH	578	642	870	1725	36.8	76	0.22	2.50	8.1	16.7
Triflate derivatives												
	**7o**	PhMe	540	591	868	1598	38.5	78	0.53	4.20	20.4	41.2
		$CHCl_3$	558	613	871	1608	32.9	78	0.49	4.40	16.1	38
		MeOH	567	640	870	2012	38.1	50	0.19	2.55	7.2	9.5
	**7p**	PhMe	538	589	869	1609	39.2	73	0.54	4.03	21.2	39.3
		$CHCl_3$	556	612	872	1646	41.0	89	0.53	4.09	21.7	47.4
		MeOH	566	640	870	2043	31.8	42	0.20	2.45	6.4	8.3

[a]Full steady-state 1PA, 2PA, and fluorescence spectra are included in the Supporting Information (SI). [b]Values for **2**, **7e**, and **7g−j** are taken from our preceding publication (ref 21). [c]Two-photon absorption maximum in the NIR-I window (800−1000 nm). Two-photon absorption (2PA) values were obtained indirectly using two-photon excited fluorescence (TPEF) measurements from 780 to 1020 nm in 10 nm increments using a tunable Ti:sapphire laser. The precise λ_{max}^{2PA} and σ_{max}^{2PA} values were extracted using a Gaussian fit to the data points of nine independent runs. [d]Quantum yields were determined by the relative method using rhodamine B in EtOH (Φ 0.5) or Nile Red in the respective solvents as the

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Table 4. continued

reference standard(s). [e]The fluorophore "brightness" corresponds to the molar absorptivities (1PA) or absorption cross sections (2PA) multiplied by the quantum yields.

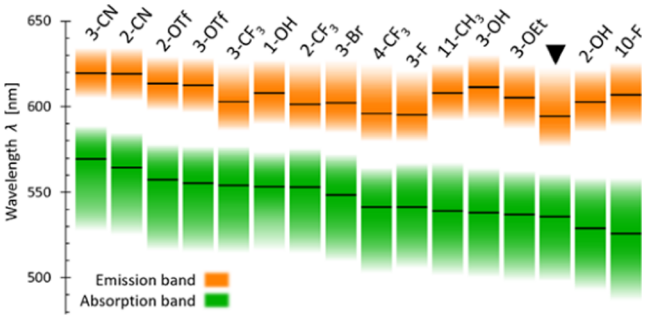

Figure 2. Graphical representation of the absorption and emission bands of the Nile Red derivatives in chloroform. The substituents are listed in order of their effect on the absorption wavelength. Unsubstituted Nile Red is indicated with ▼. Full absorption and fluorescence spectra are included in the Supporting Information. The black lines designate the peak maximum (100%), and the shown bands reflect the width at 70% of the maximum.

The Nile Red analogues produce fluorescence with peak emission from 596 to 619 nm in chloroform (Figure 2). Thus, all substituents evoke a red shift in the emission maximum of 2−25 nm compared to Nile Red (594 nm). The amount of red shift generally follows the trend seen in absorption, i.e., the cyano and triflate derivatives are the reddest fluorophores. However, relative to absorption, the emission spectrum is less sensitive to the substituents. As a result, the relative sizes of the Stokes shifts mirror, in reverse, the absorption wavelength of the fluorophores (Figure 3), i.e., the fluorophores with

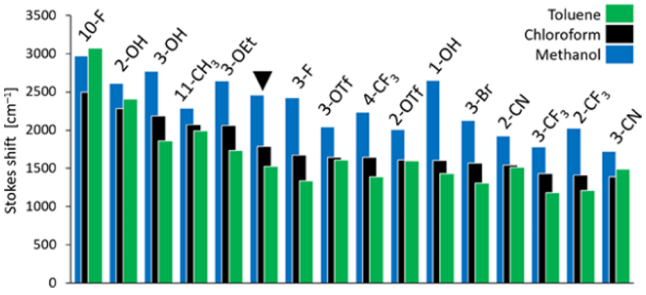

Figure 3. Comparison of the Stokes shifts (in cm^{-1}) of the Nile Red derivatives. The substituents are listed in order of their effect on the Stokes shift in chloroform. Unsubstituted Nile Red is indicated with ▼.

strongest electron-withdrawing groups exhibit the lowest Stokes shifts. This seems to be a logic consequence of the substituents' stabilizing effect on the highly dipolar excited state.

Like Nile Red, the derivatives' Stokes shifts are generally slightly larger in methanol than in the apolar solvents as the polar medium and hydrogen bonding seem to comfort the extremely dipolar twisted intramolecular charge transfer (TICT) state of Nile Red.[42,88] The 10-fluoro substituted derivative (**7n**) exhibit the largest Stokes shifts (>2500 cm^{-1} or >80 nm) in all solvents. It is not inconceivable that the proximity of the 10-fluoro substituent to the diethylamino group causes the molecule to rotate around the C−NEt_2 bond and basically assume a TICT state already in the ground state. This would lead to a high dipole moment difference between the ground- and excited-state structures and possibly significant solvent reorganization.

The effect of solvent on the wavelength of the fluorescence, i.e., solvatofluorochromicity, is depicted in Figure 4. This is a

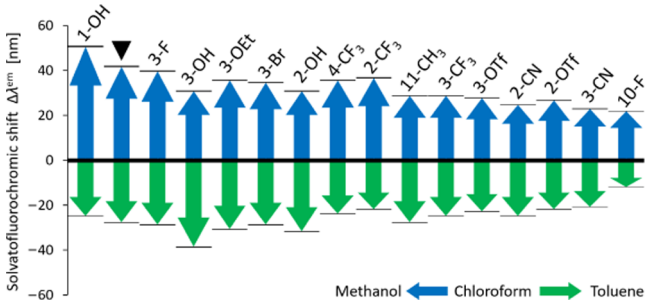

Figure 4. Polarity-induced shifts (in nm) in the emission band of the Nile Red derivatives. The substituents are listed in order of their effect on the solvatofluorochromism. The black center line indicates the compounds' peak emission in chloroform (see Table 4), and arrows represent the polarity-dependent shift in the maxima $\Delta\lambda_{max}^{em}$. Unsubstituted Nile Red is indicated with ▼.

direct measure of the dyes' power to indicate—by the color of their fluorescence—the polarity of their local environment in fluorescence microscopy. In the case of Nile Red, changing the solvent from chloroform to toluene and methanol causes shifts in the peak emission of −28 and +42 nm, respectively, giving a total solvatofluorochromic span of 70 nm. This is consistent with values seen in the literature,[16,21,89] and the fluorescence is thus more sensitive to the solvent polarity compared to absorption. As seen, decorating Nile Red with substituents generally reduces its solvatofluorochromic span. A 10-fluoro substituent (as in compound **7n**) evokes the least polarity-sensitive emission with a sensitivity of only 34 nm, which is half that of Nile Red. Apart from compound **7n**, the fluorophores that have the reddest absorption are also the least solvatofluorochromic. This may reflect the fact that the strong electron-withdrawing groups (such as the cyano and triflate groups) stabilize the relaxed excited state to a degree where the molecules are not as reliant on the stabilizing contribution of a polar environment. Indeed, the dominant contribution to the drop in the solvatofluorochromism across the series is seen from methanol to chloroform (Figure 4).

The quantum yields (Φ) and the fluorescence lifetimes (τ) of the fluorophores are shown in Figures 5 and 6. Nile Red and halogenated derivatives (F, CF_3, and Br) exhibit higher quantum yields (Φ = 0.64−0.84 in chloroform and toluene) than the rest of the fluorophores (Φ < 0.65). In the case of the 2-CF_3-substituted derivative (compound **7i**), the combination of its high molar extinction coefficient ε and high quantum yield makes this fluorophore the brightest Nile Red derivative ($\varepsilon\Phi \sim 30$ mM^{-1} cm^{-1} in chloroform and toluene; $\varepsilon\Phi$ = 12.7 mM^{-1} cm^{-1} in methanol). In general, the quantum yields are largely similar in chloroform and toluene but are more or less

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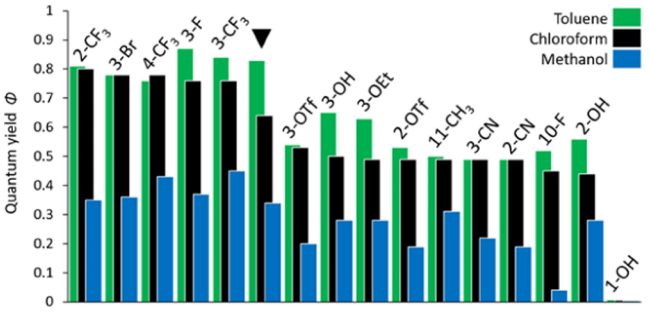

Figure 5. Comparison of the quantum yields of the Nile Red derivatives. The substituents are listed in order of their effect on the quantum yield in chloroform. Unsubstituted Nile Red is indicated with ▼.

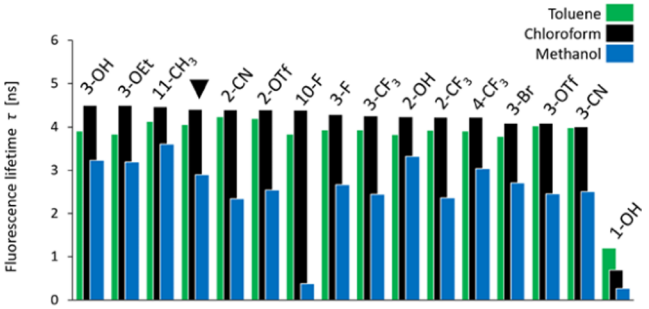

Figure 6. Comparison of the fluorescence lifetimes of the Nile Red derivatives. The substituents are listed in order of their effect on the fluorescence lifetime in chloroform. Unsubstituted Nile Red is indicated with ▼. The lifetimes were determined using the time-correlated single-photon count (TCSPC) method.

halved in polar methanol. This is consistent with the invariantly shorter fluorescence lifetimes in methanol ($\tau \leq$ 3.6 ns) given that short lifetimes are usually indicative of nonradiative de-excitation of the fluorophore.[90] Indeed, polar environments are often said to foster solute relaxation into the nonemissive TICT state.[42,91,92] Introduction of substituents only scarcely affects the fluorescence lifetime of Nile Red in toluene and chloroform with τ mostly above 4 ns. The 1-hydroxy derivative (**7c**) stands out as essentially nonemissive, and this is not seen with the other hydroxy derivatives, which indicates that the intramolecular hydrogen bond might facilitate nonradiative decay pathways, possibly by excited-state intramolecular proton transfer (ESIPT).[93] We note that the fluorescence decay of the 1-hydroxy derivative (**7c**) has a very clear biexponential nature, which seems to conflict with an earlier report.[87] The biexponential decays are most evident in chloroform and toluene (see Figure S91). The 10-fluoro derivative (compound **7n**) emerges as being moderately emissive in toluene and chloroform (Φ = 0.45−0.52) but very dark when taken in methanol (Φ = 0.04). This incongruity is also apparent in the fluorescence lifetime analysis of **7n**, in which it shows a monoexponential decay with τ above 3.8 ns in chloroform and toluene but a biexponential decay (Figure S92) with components having lifetimes τ of 0.26 ns (98%) and 2.19 ns (2%). The sub-nanosecond lifetime can be traced back to the formation of a charge-localized TICT state in methanol. This solvent-dependent fluorescence lifetime of **7n** makes it an efficacious tool for interpreting the polarity of the fluorophore's local microenvironment in FLIM. It is noteworthy that the photophysical behavior of **7n** differs distinctly from that of its 3-substituted isomer, i.e., compound **7g**.

The fluorophores' peak two-photon cross sections σ_2 in the NIR-I window (800−1000 nm) are tabulated in Table 4, and their derived two-photon brightnesses $\Phi\sigma_2$ (in GM) in each of the three solvents are illustrated in Figure 7. 2PA spectra are

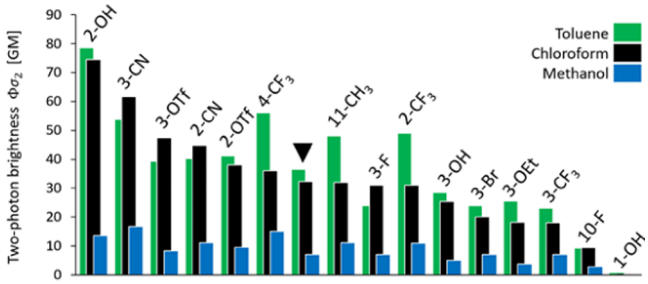

Figure 7. Comparison of the two-photon brightnesses $\Phi\sigma_2$ of the Nile Red derivatives. The fluorophores are listed in order of decreasing two-photon brightness in chloroform. Unsubstituted Nile Red is indicated with ▼.

included in the SI (Figures S80−S90). For all of the fluorophores, the peak two-photon excitation lies within a very narrow interval of 763−778 nm (see Table 4). Indeed, the wavelength of this 2PA band is rather robust to substitution and solvent polarity and has no obvious relation to the 1PA bands. However, similar to the one-photon excitation, this transition is most likely of $\pi \rightarrow \pi^*$ character to an intramolecular charge transfer (ICT) state.[21] The most luminous molecule for 2PEF microscopy is the 2-hydroxy Nile Red derivative (compound **7a**), followed by the derivatives that contain strongly withdrawing cyano and triflate groups (compounds **7f** and **7o−q**) at the 2- and 3-positions. These are all brighter than Nile Red itself, primarily due to substantial increases in the absorption component (σ_2) rather than the emissivity (Φ). The high two-photon brightness of 2-hydroxy derivative **7a**, which is due to its large two-photon cross section (σ_2 up to 170 GM), is somewhat puzzling given that this derivative holds a strong electron-donating substituent on the eastern side, which in the context of the few-state model[94,95] ought to diminish the difference and transition dipole moments, and therefore reduce the two-photon transition probability.[20] The molecules are generally 4−5-fold brighter in toluene and chloroform ($\Phi\sigma_2$ = 18.2−78.6) than in methanol ($\Phi\sigma_2$ = 2.9−13.6) due to similar percentwise reductions in the σ_2 and Φ values. The 10-fluoro and 1-hydroxy derivatives (compounds **7n** and **7c**) are special in this regard in that they are inherently dark and therefore unfit for 2PEF-based microscopy—especially the latter.

■ CONCLUSIONS

This study demonstrates the utility of synthetic chemistry to generate a family of Nile Red analogues that offer characteristics that may aid in the time-resolved and super-resolution imaging of polar and neutral lipids in cells. This study, for the first time, highlights the limited scope of the original synthetic approach toward new 9-diethylbenzo-[*a*]phenoxazine-5-ones, i.e., analogues of Nile Red. This is especially true when it comes to the introduction of electron-withdrawing groups (such as nitro and cyano) on the eastern side of the molecule. A cyano substituent was instead successfully installed via the hydroxy and triflate substituents. In addition, we present a new

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convenient approach for the synthesis of Nile Red in unprecedented high yields (≥80%) using commercial 1,3-naphthalenediol and *p*-nitroso-*N*,*N*-diethylanilines. This approach offers a second line of attack for the development of Nile Red analogues, which was exemplified by the introduction of a bromine substituent.

In summary, we present the successful synthesis of 15 fluorescent 9-diethylbenzo[*a*]phenoxazine-5-ones that feature a variety of different substituents (hydroxy, ethoxy, methyl, trifluoromethyl, fluoro, bromo, cyano, triflate). With an emphasis on probing their applicability to fluorescence microscopy, we have ranked the fluorophores on the basis of, e.g., their responsiveness to polarity, two-photon absorptivity, and luminosity. Our results reinforce the presumption that the introduction of strongly electron-withdrawing groups (such as cyano, triflate, and trifluoromethyl) on the eastern side allows excitation at lower energies as well as endows the fluorophores with enhanced one- and two-photon brightness. However, this comes at the expense of reductions in the Stokes shifts and solvatofluorochromicities. Indeed, electron-donating groups (such as hydroxy, ethoxy, and methyl) increase the Stokes shift of Nile Red and efficiently report the polarity of their environment. Our results also show that the location of the substituent may have a detrimental impact on spectroscopic properties. This is for instance seen in the case of the hydroxy substituents, where the 1-, 2-, and 3-positions show diverse and sometimes contrasting behavior, as well as in the case of a fluoro substituent, which has a different character on the eastern and western sides. Clearly, there is not the best probe, but rather the choice of probe rests on the exact requirements and conditions of a given assay. For example, analogue **7f** (with a 3-cyano substituent) comes off as a brilliant probe for boosting the molar absorptivity while lowering the excitation energy, analogue **7n** (with a 10-fluoro atom) appears superb for high-contrast imaging of polar and nonpolar interfaces using FLIM, and compound **7a** (with a 2-hydroxy substituent) enhances the signal-to-noise ratio for 2PEF microscopy.

It is our hope that this work nicely illustrates how the decoration of the Nile Red skeleton with minor substituents evokes substantial changes in its photophysical properties. This makes the Nile Red motif a very attractive scaffold for the design and development of useful biophysical and biomedical probes. Work is currently in progress to study the in vivo efficacy of these new Nile Red analogues in cells.

EXPERIMENTAL DETAILS

General Details. All chemicals were used as supplied except CH_2Cl_2 that was distilled. Dry chemicals were prepared using a selection of methods: CH_3OH was freshly distilled from Mg and stored over 3 Å molecular sieves. THF was freshly distilled from Na and stored over 4 Å sieves. CH_2Cl_2 was freshly distilled and stored over 4 Å sieves. Triethylamine and toluene were passed over a column of silica and stored over 4 Å sieves. Petroleum ether was dried over NaH, filtered, and stored over 4 Å sieves. 1,2-Dichloroethane (DCE) was dried over Al_2O_3, filtered, and stored over 4 Å sieves. CH_3CN was dried over 3 Å sieves, and pyridine was dried over 4 Å sieves. DMF and dimethyl sulfoxide (DMSO) were bought in anhydrous form. All reactions were monitored by TLC using silica gel 60 F_{254}-coated aluminum-backed plates, which were visualized at 254 or 365 nm. Flash chromatography was performed using silica gel 60 (particle size 0.040−0.063 mm). After chromatography, appropriate fractions were pooled and concentrated. All products were dried under high vacuum (<1 mmHg) overnight to give products of high analytical purity. High-resolution mass spectrometry (HRMS)-ESI was recorded on a Bruker micrOTOF-Q II (ESI) quadrupole-time-of-flight instrument in the positive ion mode with an accuracy of ±5 ppm. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on a Bruker Avance III 400 instrument at 400, 101, and 376 MHz, respectively. Chemical shifts are reported in ppm relative to tetramethylsilane ($\delta_{H,C}$ 0 ppm) or the deuterated solvents (DMSO-d_6 δ_c 39.5 ppm, $CDCl_3$ δ_c 77.2 ppm, CD_3OD δ_c 49.0). Two-dimensional (2-D) experiments (heteronuclear single quantum coherence (HSQC), correlation spectroscopy (COSY), and heteronuclear multiple bond correlation (HMBC)) have been used in assigning ^{1}H and ^{13}C NMR signals.

*5-Diethylamino-2-nitrosophenol Hydrochloride (**1**).*[56] 3-Diethylaminophenol (10.0 g, 60.5 mmol) was dissolved in a mixture of concentrated HCl (25 mL, 0.29 mol) and H_2O (15 mL), and the reaction mixture was cooled to 0 °C. A solution of $NaNO_2$ (4.18 g, 60.5 mmol) in H_2O (25 mL) was added dropwise over 60 min, and the reaction mixture was stirred for 2 h at 0 °C (ice bath). This resulted in a green-yellow precipitate of 5-diethylamino-2-nitrosophenol hydrochloride (**1**), which crystallized from EtOH/Et_2O (1:1) in dark green crystals (10.2 g, 46.0 mmol). Yield: 76%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.57 (d, J = 10.4 Hz, 1H, H-3), 7.23 (dd, J = 10.4, 2.4 Hz, 1H, H-4), 6.71 (d, J = 2.4 Hz, 1H, H-6), 3.83 (br, 4H CH_2), 1.29 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 166.0 (C-5), 161.3 (C-2), 144.8 (C-1), 123.1 (C-3), 119.7 (C-4), 97.6 (C-6), 48.0 (CH_2), 13.6 (CH_3). The crystal form contained 1/4 parts of the free base: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.31 (d, J = 10.0 Hz, 1H, H-3), 6.91 (dd, J = 10.0, 2.6, 1H, H-4), 5.77 (d, J = 2.6 Hz, 1H, H-6), 3.62 (br, 4H, CH_2), 1.19 (t, J = 8.0, 3H, CH_3). HRMS (ESI): calcd for $C_{10}H_{14}N_2O_2Na^+$ [M + Na]$^+$, m/z 217.0947, found: m/z 217.0954. This mixture was used for subsequent reactions.

*Nile Red (**2**) Using the 1,3-Naphthalenediol Approach (Route B).* To a solution of 1,3-naphthalenediol (**12**, 0.50 g, 3.12 mmol) in anhydrous DMF (25 mL) was added 4-nitroso-*N*,*N*-diethylaniline (**9**, 0.56 g, 3.12 mmol). This reaction mixture was stirred at 80 °C (DrySyn heating block) for 16 h and concentrated under reduced pressure. The crude product was purified by flash chromatography (0−50% EtOAc in hexanes) to obtain Nile Red (**2**), which crystallized from hexanes in green crystals (0.80 g, 2.50 mmol). Yield: 80%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.63 (dd, J = 7.7, 1.5 Hz, 1H, H-4), 8.30 (dd, J = 7.7, 1.5 Hz, 1H, H-1), 7.71 (td, J = 7.7, 1.5 Hz, 1H, H-3), 7.64 (td, J = 7.7, 1.5 Hz, 1H, H-2), 7.58 (d, J = 9.1 Hz, 1H, H-11), 6.64 (dd, J = 9.1, 2.8 Hz, 1H, H-10), 6.44 (d, J = 2.8 Hz, 1H, H-8), 6.37 (s, 1H, H-6), 3.45 (q, J = 7.1 Hz, 4H, CH_2), 1.25 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 183.7 (C-5), 152.2 (C-6a), 150.8 (C-9), 146.8 (C-7a), 140.0 (C-12a), 132.1 (C-4a), 131.8 (C-12b), 131.3 (C-3), 131.1 (C-11), 129.9 (C-2), 125.7 (C-1), 125.0 (C-11a), 123.8 (C-4), 109.7 (C-10), 105.8 (C-6), 96.3 (C-8), 45.1 (CH_2), 12.6 (CH_3). HRMS (ESI): calcd for $C_{20}H_{18}N_2O_2Na^+$ [M + Na]$^+$, m/z 341.1260, found: m/z 341.1263.

4-Nitroso-1,3-naphthalenediol. To a solution of lawsone (1.00 g, 5.74 mmol) in NaOH (2.0 M, 10 mL) was slowly added hydroxylamine hydrochloride (400 mg, 5.74 mmol). The reaction mixture was stirred at 60 °C (DrySyn heating block) for 1 h, cooled, and then neutralized with HCl (2.0 M). This resulted in a precipitate of 4-nitroso-1,3-naphthalenediol, which was filtered off, washed with ice-cold water, dried, and crystallized from boiling H_2O as yellow crystals (0.96 g, 5.06 mmol). Yield: 88%; mp 196−197 °C (dec). In solution, this product manifests itself as its *E*/*Z* naphthoquinoneoxime tautomers[96] in 4:1 ratio. *E*: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 13.74 (br, 1H, OH), 9.03 (dd, J = 8.0, 1.3 Hz, 1H, H-8), 8.06 (d, J = 7.5, 1.3 Hz, 1H, H-5), 7.70 (ddd, J = 8.0, 7.5, 1.3 Hz, 1H, H-7), 7.63 (td, J = 7.5, 1.3 Hz, 1H, H-6), 5.95 (s, 1H, H-3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 182.1, 173.8, 166.1, 140.5, 132.3, 130.3, 129.6, 126.7, 125.5, 105.4. HRMS (ESI): calcd for $C_{10}H_7NO_3Na^+$ [M + Na]$^+$, m/z 212.0318, found: m/z 212.0312.

4-Nitroso-1-naphthol. To a solution of 1,4-naphthoquinone (2.00 g, 12.7 mmol) in EtOH (95%, 10 mL) were added hydroxylamine hydrochloride (0.88 g, 12.7 mmol) and two drops of HCl (37%). This reaction mixture was refluxed at 80 °C (DrySyn heating block) for 1

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h, cooled to room temperature, and diluted with H_2O (50 mL). This resulted in a precipitate of 4-nitroso-1-naphthol, which crystallized from boiling H_2O in greyish crystals (1.60 g, 9.23 mmol). Yield: 73%; mp 194−195 °C (dec). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 13.30 (s, 1H, OH), 8.21 (ddd, J = 8.0, 1.3, 0.6 Hz, 1H, H-8), 8.04 (ddd, J = 7.8, 1.5, 0.6 Hz, 1H, H-5), 8.03 (d, J = 10.4 Hz, 1H, H-3), 7.75 (ddd, J = 8.0, 7.3, 1.5 Hz, 1H, H-7), 7.64 (ddd, J = 7.8, 7.2, 1.3 Hz, 1H, H-6), 6.66 (d, J = 10.4 Hz, 1H, H-2). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 184.3 (C-1), 145.3 (C-4), 133.1 (C-4a), 132.9 (C-7), 130.5 (C-2), 129.6 (C-8a), 129.4 (C-6), 126.3 (C-3), 125.6 (C-5), 122.2 (C-8). HRMS (ESI): calcd for $C_{10}H_7NO_2Na^+$ [M + Na]$^+$, m/z 196.0369, found: m/z 196.0357.

*7-Nitro-1-naphthol (**6d**).* To a flask containing concentrated sulfuric acid (40 mL) at 0 °C was added α-tetralone (5.00 g, 34.2 mmol). To this stirred solution was added dropwise a solution of $NaNO_2$ (2.71 g, 39.3 mmol) in sulfuric acid (98%, 15 mL). After the addition over 30 min, the reaction mixture was stirred at 0 °C for 1 h and then poured onto crushed ice (100 g). After thawing, the precipitate was filtered off, washed with H_2O, and purified by flash chromatography (0−20% EtOAc in hexanes) to give 7-nitro-1-tetralone (**3d**) as a pale yellow solid (3.82 g, 20.0 mmol). Yield: 58%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.84 (d, J = 2.5 Hz, 1H, H-8), 8.29 (dd, J = 8.5, 2.5 Hz, 1H, H-6), 7.47 (d, J = 8.5 Hz, 1H, H-5), 3.09 (t, J = 6.0 Hz, 2H, H-4), 2.74 (dd, J = 7.3, 6.0 Hz, 2H, H-3), 2.25−2.17 (m, 2H, H-2). HRMS (ESI): calcd for $C_{10}H_9NO_3Na^+$ [M + Na]$^+$, m/z 214.0475, found: m/z 214.0468. 7-Nitro-1-tetralone (**3d**, 3.00 g, 15.7 mmol) was dissolved in *N*,*N*-dimethylacetamide (30 mL), followed by the addition of K_2CO_3 (0.43 g, 3.14 mmol) and Pd/C (10 wt % loading, 0.83 g, 0.78 mmol). The reaction mixture was stirred at 165 °C (DrySyn heating block) for 2 h, cooled to room temperature, and filtered through a pad of celite. The filtrate was partitioned between CH_2Cl_2 (50 mL) and H_2O (50 mL). The basic aqueous layer was neutralized with concentrated HCl, upon which the organic layer was isolated, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography (0−30% EtOAc in hexanes) to obtain 7-nitro-1-naphthol (**6d**) as a white solid (1.96 g, 10.4 mmol). Yield: 66%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.96 (br, 1H, OH), 9.02 (d, J = 2.4 Hz, 1H, H-8), 8.19 (dd, J = 9.1, 2.4 Hz, 1H, H-6), 8.07 (d, J = 9.1 Hz, 1H, H-5), 7.60 (dd, J = 8.3, 7.6 Hz, 1H, H-3), 7.53 (d, J = 8.3, 1H, H-4), 7.06 (dd, J = 7.6, 1.1 Hz, 1H, H-2). ^{13}C{^{1}H} NMR (DMSO-d_6, 101 MHz): δ 155.2 (C-1), 144.0 (C-7), 136.7 (C-4a), 131.2 (C-3), 129.4 (C-5), 122.9 (C-8a), 119.2 (C-8), 119.1 (C-6), 118.3 (C-4), 110.0 (C-2). HRMS (ESI): calcd for $C_{20}H_{14}N_2O_6Na^+$ [2M+Na]$^+$, m/z 401.0744, found: m/z 401.0732.

*7-Bromo-1-naphthol (**6e**).* To a stirred solution of furan (7.88 g, 115 mmol) in 20 mL of 1,2-dimethoxyethane (DME) at 70 °C were slowly infused a solution of isoamyl nitrite (3.17 mL, 23.6 mmol) in DME (10 mL) and separately a solution of 2-amino-5-bromobenzoic acid (5.00 g, 23.1 mmol) in DME (10 mL). After the infusion over 30 min, the reaction mixture was stirred at 70 °C (DrySyn heating block) for another 50 min, upon which it was cooled down to room temperature. The reaction mixture was concentrated under reduced pressure, and the crude product was purified by flash chromatography (30% CH_2Cl_2 in hexanes) to give 6-bromo-1,4-epoxy-1,4-dihydronaphthalene (**5e**) as a pale yellow liquid (1.80 g, 8.05 mmol). Yield: 67%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.36 (s, 1H, H-5), 7.09−7.08 (m, 2H, H-7, H-8), 7.01 (dd, J = 5.5, 1.8 Hz, 1H, vinyl CH), 6.98 (dd, J = 5.5, 1.7 Hz, 1H, vinyl CH), 5.70 (dd, J = 1.7 Hz, 2H, allyl CH). 6-Bromo-1,4-epoxy-1,4-dihydronaphthalene (**5e**, 1.87 g, 8.38 mmol) was dissolved in a HCl solution (1.25 M in EtOH, 25 mL, 31.3 mmol) and stirred at 50 °C (DrySyn heating block) for 20 h. The mixture was concentrated under reduced pressure, and the crude product was purified by flash chromatography to give 7-bromo-1-naphthol (**6e**) as a white solid (1.14 g, 5.11 mmol). The product remained contaminated with 6-bromo-1-naphthol, but it was used without further purification. Yield: 61%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.37 (d, J = 2.0 Hz, 1H, H-8), 7.67 (d, J = 8.8 Hz, 1H, H-5), 7.55 (dd, J = 8.8, 2.0 Hz, 1H, H-6), 7.40 (d, J = 8.3 Hz, 1H, H-4), 7.32 (dd, J = 8.3, 7.5 Hz, 1H, H-3), 6.82 (dd, J = 7.5, 1.0 Hz, 1H, H-2), 5.28 (s, 1H, OH). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 150.6 (C-1), 133.2 (C-4a), 129.9 (C-6), 129.3 (C-5), 126.3 (C-3), 125.5 (C-8a), 124.4 (C-8), 120.6 (C-4), 119.3 (C-7), 109.5 (C-2). HRMS (ESI): calcd for $C_{10}H_7Br_2ONa^+$ [M + Na]$^+$, m/z 244.9572 (^{79}Br), 246.9552 (^{81}Br), found: m/z 244.9567 (^{79}Br), 246.9550 (^{81}Br).

*7-Cyano-1-naphthol (**6f**).* To a solution of 7-bromo-1-naphthol (**6e**, 200 mg, 0.90 mmol) in anhydrous DMF (1.00 mL) was added CuCN (96 mg, 1.08 mmol), and the reaction mixture was stirred at 160 °C (DrySyn heating block) for 3 h in a sealed vessel. The solution was cooled to room temperature, diluted with H_2O (5 mL), and extracted with EtOAc (2 × 5 mL). The combined organic phases were concentrated under reduced pressure, and the resulting crude product was recrystallized from EtOH to give 7-cyano-1-naphthol (**6f**) as a white solid (134 mg, 0.79 mmol). Yield: 88%; mp 175−185 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.30 (d, J = 8.7 Hz, 1H, H-5), 8.19 (d, J = 1.6 Hz, 1H, H-8), 7.60 (dd, J = 8.7, 1.6 Hz, 1H, H-6), 7.48 (dd, J = 8.3, 1.2 Hz, 1H, H-4), 7.43 (dd, J = 8.3, 7.2 Hz, 1H, H-3), 6.98 (dd, J = 7.2, 1.2 Hz, 1H, H-2), 5.71 (s, 1H, OH). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 151.6 (C-1), 135.9 (C-4a), 133.8 (C-8), 127.8 (C-3), 126.9 (C-8a), 125.6 (C-6), 123.6 (C-5), 120.9 (C-4), 120.5 (CN), 111.6 (C-2), 110.1 (C-7). HRMS (ESI): calcd for $C_{11}H_7NONa^+$ [M + Na]$^+$, m/z 192.0420, found: m/z 192.0420.

*7-Fluoro-1-naphthol (**6g**).*[46] A suspension of 7-fluoro-1-tetralone (**3g**, 1.00 g, 6.09 mmol) and Pd/C (10 wt %, 0.36 g, 0.34 mmol) in anhydrous tetraglyme (10 mL) was refluxed at 260 °C (DrySyn heating block) for 24 h. Hereafter, the mixture was cooled to room temperature, filtered through a pad of celite into ice water (100 mL), and extracted with EtOAc (100 mL). Subsequent extraction with KOH (25% solution in H_2O/MeOH, 1:1, 100 mL) and neutralization with conc. hydrochloric acid gave a precipitate of 7-fluoro-1-naphthol (**6g**), which crystallized from petroleum ether in white needles (0.71 g, 4.37 mmol). Yield: 72%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.83−7.73 (m, 2H, H-5, H-8), 7.43 (dt, J = 8.2, 1.0 Hz, 1H, H-4), 7.32−7.21 (m, 2H, H-3, H-6), 6.83 (dt, J = 7.5, 1.0 Hz, 1H, H-2), 5.22 (s, 1H, OH). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 159.4 (d, $^1J_{C-F}$ = 245 Hz, C-7), 149.9 (d, $^4J_{C-F}$ = 5 Hz, C-1), 130.8 (d, $^4J_{C-F}$ = 1 Hz, C-4a), 129.0 (d, $^3J_{C-F}$ = 9 Hz, C-5), 124.1 (d, $^3J_{C-F}$ = 9 Hz, C-8a), 123.9 (d, ^6J = 2 Hz, C-3), 119.6 (d, $^5J_{C-F}$ = 1 Hz, C-4), 115.8 (d, $^2J_{C-F}$ = 25 Hz, C-6), 108.3 (C-2), 104.6 (d, $^2J_{C-F}$ = 22 Hz, C-8). MS (EI): calcd for $C_{10}H_7FO^+$ (M^+), m/z 162.05, found: m/z 162.1 (M^+), 144.1 ($C_{10}H_8O^+$), 115.0 ($C_9H_7^+$).

*6-Trifluoromethyl-1-naphthol (**6h**) and 7-Trifluoromethyl-1-naphthol (**6i**).*[47] To a stirred solution of 4-chlorobenzotrifluoride (2.96 mL, 22.2 mmol) in anhydrous THF (30 mL) at −78 °C was added dropwise a *n*-butyllithium solution (2.5 M in hexanes, 8.86 mL, 22.2 mmol). The reaction mixture was stirred at −78 °C for 60 min, after which it was cannulated slowly through Teflon tubing into a flask containing stirred furan (16.1 mL, 0.22 mol) at 25 °C. The reaction mixture was stirred overnight and then portioned between Et_2O (50 mL) and H_2O (50 mL). The organic layer was isolated, washed with H_2O (50 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography (0−5% EtOAc in hexanes) to afford 1,4-epoxy-1,4-dihydro-6-(trifluoromethyl)naphthalene (**5h**) as a colorless oil (3.29 g, 15.5 mmol). Yield: 70%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.45 (s, 1H, H-5), 7.31 (d, J = 7.5 Hz, 1H, H-8), 7.27 (d, J = 7.5 Hz, 1H, H-7), 7.04 (dd, J = 5.5, 1.7 Hz, 1H, vinyl CH), 7.01 (dd, J = 5.5, 1.7 Hz, 1H, vinyl CH), 5.75 (d, J = 1.7 Hz, 1H, allyl CH), 5.74 (d, J = 1.7 Hz, 1H, allyl CH). 1,4-Epoxy-1,4-dihydro-6-(trifluoromethyl)-naphthalene (3.27 g, 15.4 mmol) was dissolved in a HCl solution (1.25 M in EtOH, 25 mL, 31.3 mmol) and stirred at 50 °C (DrySyn heating block) for 20 h. The mixture was evaporated to dryness, and 6-trifluoromethyl-1-naphthol (**6h**) was isolated by fractional crystallization from pentanes in colorless needles (0.88 g, 4.16 mmol). Yield: 27%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.31 (dd, J = 8.8, 0.9 Hz, 1H, H-8), 8.11 (s, 1H, H-5), 7.64 (dd, J = 8.8, 1.7 Hz, 1H, H-7), 7.52 (d, J = 8.3 Hz, 1H, H-4), 7.40 (dd, J = 8.3, 7.5 Hz, 1H, H-3), 6.92 (dd, J = 7.5, 1.0 Hz, 1H, H-2), 5.33 (s, 1H, OH). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 151.4 (C-1), 133.6 (C-4a), 128.4 (q, $^2J_{C-F}$ = 32 Hz, C-6), 127.3 (C-3), 125.6 (C-8a), 125.3 (q, $^3J_{C-F}$ = 5 Hz, C-5), 124.4 (q,

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$^{1}J_{C-F}$ = 272 Hz, CF_3), 123.1 (C-8), 121.5 (C-4), 120.8 (q, $^{3}J_{C-F}$ = 3 Hz, C-7), 110.6 (C-2). The mother liquid was concentrated to give 2/3 parts of 7-trifluoromethyl-1-naphthol (**6i**) and 1/3 parts of **6h**. Further isolation of **6i** by recrystallization was unsuccessful, and it was used without further purification (0.78 g). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.58 (dd, J = 1.8, 0.9 Hz, 1H, H-8), 7.91 (d, J = 8.7 Hz, 1H, H-5), 7.67 (dd, J = 8.7, 1.8 Hz, 1H, H-6), 7.50 (d, J = 8.4 Hz, 1H, H-4), 7.44 (dd, J = 8.4, 7.3 Hz, 1H, H-3), 6.88 (dd, J = 7.3, 1.0 Hz, H-2), 5.44 (s, 1H, OH). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 152.0 (C-1), 135.9 (C-4a), 128.6 (C-5), 128.2 (C-3), 127.1 (q, $^{2}J_{C-F}$ = 34 Hz, C-7), 124.6 (q, $^{1}J_{C-F}$ = 272 Hz, CF_3), 123.3 (C-8a), 122.1 (q, $^{3}J_{C-F}$ = 3 Hz, C-6), 120.6 (C-4), 120.1 (q, $^{3}J_{C-F}$ = 5 Hz, C-8), 109.8 (C-2).

*8-Trifluoromethyl-1-naphthol (**6j**).*[47] To a stirred solution of 2-chlorobenzotrifluoride (1.45 mL, 11.1 mmol) in anhydrous THF (20 mL) at −78 °C was added dropwise a *n*-butyllithium solution (2.5 M in hexanes, 4.43 mL, 11.1 mmol). The reaction mixture was stirred at −78 °C for 60 min, after which it was cannulated slowly through Teflon tubing into a flask containing stirred furan (8.1 mL, 0.11 mol) at 25 °C. The reaction mixture was stirred overnight and then portioned between Et_2O (50 mL) and H_2O (50 mL). The organic layer was isolated, washed with H_2O (50 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography (0−5% EtOAc in hexanes) to afford 1,4-epoxy-1,4-dihydro-5-(trifluoromethyl)-naphthalene (**5j**) as a colorless oil (1.31 g, 6.18 mmol). Yield: 56%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.39 (d, J = 6.8 Hz, 1H, H-7), 7.18 (d, J = 6.8 Hz, 1H, H-5), 7.09 (t, J = 6.8 Hz, 1H, H-6), 7.07 (dd, J = 5.6, 1.8 Hz, 1H, vinyl CH), 7.05 (dd, J = 5.6, 1.8 Hz, 1H, vinyl CH), 6.00 (d, J = 1.8 Hz, 1H, allyl CH), 5.78 (d, J = 1.8 Hz, 1H, allyl CH). 1,4-Epoxy-1,4-dihydro-5-(trifluoromethyl)naphthalene (**5j**, 1.30 g, 6.13 mmol) was dissolved in a HCl solution (1.25 M in EtOH, 25 mL, 31.3 mmol) and stirred at 50 °C (DrySyn heating block) for 20 h. The mixture was evaporated to dryness to give 8-trifluoromethyl-1-naphthol (**6j**), which crystallized from pentanes in colorless needles (1.12 g, 5.27 mmol). Yield: 86%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.99 (d, J = 8.2 Hz, 1H, H-7), 7.96 (d, J = 7.5 Hz, 1H, H-4), 7.53 (dd, J = 8.2, 1.2 Hz, 1H, H-5), 7.47 (t, J = 8.2 Hz, 1H, H-6), 7.42 (t, J = 7.5 Hz, 1H, H-3), 7.01 (dd, J = 7.5, 1.2 Hz, 1H, H-2), 5.50 (s, 1H, OH). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 150.6 (C-1), 136.5 (C-4a), 133.6 (q, J = 2 Hz, C-6), 127.1 (C-5), 126.4 (q, J = 8 Hz, C-7), 125.0 (q, J = 272 Hz, CF_3), 124.5 (C-3), 124.2 (q, J = 32 Hz, C-8), 122.5 (C-4), 120.5 (C-8a), 114.2 (C-2).

*9-Diethylamino-2-hydroxy-5H-benzo[a]phenoxazin-5-one (**7a**).* To a solution of 1,6-naphthalenediol (**6a**, 1.00 g, 6.24 mmol) in anhydrous DMF (25 mL) was added 5-diethylamino-2-nitrosophenol hydrochloride (**1**, 1.44 g, 6.24 mmol). This reaction mixture was stirred at 100 °C (DrySyn heating block) for 16 h and concentrated under reduced pressure. The crude product was purified by flash chromatography (0−50% EtOAc in hexanes) to obtain 9-diethylamino-2-hydroxy-5*H*-benzo-[*a*]phenoxazin-5-one (**7a**), which was purified by flash chromatography (0−50% EtOH in EtOAc) and crystallized from absolute EtOH in dark green crystals (1.51 g, 4.51 mmol). Yield: 72%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.41 (s, 1H, OH), 7.96 (d, J = 8.5 Hz, 1H, H-4), 7.87 (d, J = 2.5 Hz, 1H, H-1), 7.54 (d, J = 9.0 Hz, 1H, H-11), 7.08 (dd, J = 8.5, 2.5 Hz, 1H, H-3), 6.76 (dd, J = 9.0, 2.6 Hz, 1H, H-10), 6.60 (d, J = 2.6 Hz, 1H, H-8), 6.13 (s, 1H, H-6), 3.47 (q, J = 6.9 Hz, 4H, CH_2), 1.16 (t, J = 6.9 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 181.5 (C-5), 160.5 (C-2), 151.4 (C-6a), 150.6 (C-9), 146.3 (C-7a), 138.7 (C-12a), 133.7 (C-4a), 130.7 (C-11), 127.3 (C-4), 123.81 (C-11a), 123.78 (C-12b), 118.2 (C-3), 109.7 (C-10), 108.1 (C-1), 104.0 (C-6), 95.9 (C-8), 44.4 (CH_2), 12.4 (CH_3). HRMS (ESI): calcd for $C_{20}H_{19}N_2O_3^+$ [M + H]$^+$, m/z 335.1390, found: m/z 335.1405.

*9-Diethylamino-3-hydroxy-5H-benzo[a]phenoxazin-5-one (**7b**).*[97] In a similar way, 1,7-naphthalenediol (**6b**, 1.00 g, 6.24 mmol) was mixed with 5-diethylamino-2-nitrosophenol hydrochloride (**1**, 1.44 g, 6.24 mmol) to form 9-diethylamino-3-hydroxy-5*H*-benzo[*a*]-phenoxazin-5-one (**7b**), which was purified by flash chromatography (0−20% EtOH in EtOAc) and crystallized from absolute EtOH in dark green crystals (1.44 g, 4.30 mmol). Yield: 69%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.11 (br, 1H, OH), 8.40 (d, J = 8.7 Hz, 1H, H-1), 7.58 (d, J = 9.1 Hz, 1H, H-11), 7.46 (d, J = 2.6 Hz, 1H, H-4), 7.23 (dd, J = 8.7, 2.6 Hz, 1H, H-2), 6.80 (dd, J = 9.1, 2.7 Hz, 1H, H-10), 6.64 (d, J = 2.7 Hz, 1H, H-8), 6.25 (s, 1H, H-6), 3.48 (q, J = 7.0 Hz, 4H, CH_2), 1.16 (t, J = 7.0 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 181.8 (C-5), 159.6 (C-3), 151.3 (C-6a), 150.0 (C-9), 145.9 (C-7a), 138.7 (C-12a), 132.9 (C-4a), 130.2 (C-11), 125.6 (C-1), 124.0 (C-11a), 123.2 (C-12b), 120.0 (C-2), 110.0 (C-10), 109.9 (C-4), 104.5 (C-6), 95.9 (C-8), 44.3 (CH_2), 12.3 (CH_3). HRMS (ESI): calcd for $C_{20}H_{19}N_2O_3^+$ [M + H]$^+$, m/z 335.1390, found: m/z 335.1379. The data is in agreement with previous reports.[43,97]

*9-Diethylamino-1-hydroxy-5H-benzo[a]phenoxazin-5-one (**7c**).* In a similar way, 1,5-dihydroxynaphthalene (**6c**, 2.40 g, 16.24 mmol) was mixed with 5-diethylamino-2-nitrosophenol hydrochloride (**1**, 2.30 g, 10.50 mmol) to form 9-diethylamino-1-hydroxy-5*H*-benzo[*a*]phenoxazin-5-one (**7c**), which was purified by flash chromatography (0−20% EtOH in EtOAc) and crystallized from absolute EtOH in dark green crystals (1.70 g, 4.30 mmol). Yield: 47%. ^{1}H NMR (DMSO/$CDCl_3$, 400 MHz): δ 13.12 (br, 1H, OH), 7.69 (d, J = 7.9 Hz, 1H, H-4), 7.58 (d, J = 9.0 Hz, 1H, H-11), 7.53 (t, J = 7.9 Hz, 1H, H-3), 7.22 (d, J = 7.9 Hz, 1H, H-2), 6.75 (dd, J = 9.0, 2.7 Hz, 1H, H-10), 6.58 (d, J = 2.7 Hz, 1H, H-8), 6.30 (s, 1H, H-6), 3.51 (q, J = 7.1 Hz, 4H, CH_2), 1.24 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR (DMSO/$CDCl_3$, 101 MHz): δ 182.1 (C-5), 159.0 (C-1), 151.4 (C-6a), 150.7 (C-9), 146.5 (C-7a), 141.5 (C-12a), 132.0 (C-4a), 131.3 (C-3), 129.2 (C-2), 121.2 (C-11a), 120.1 (C-11), 116.8 (C-4), 114.2 (C-12b), 110.0 (C-10), 106.2 (C-6), 96.5 (C-8), 44.7 (CH_2), 12.4 (CH_3). HRMS (ESI): calcd for $C_{20}H_{19}N_2O_3^+$ [M + H]$^+$, m/z 335.1390, found: m/z 335.1406. The data is in agreement with previous reports.[37,87]

*3-Bromo-9-diethylamino-5H-benzo[a]phenoxazin-5-one (**7e**).* In a similar way, 7-bromo-1,3-naphthalenediol (**12e**, 150 mg, 0.63 mmol) was mixed with 5-diethylamino-2-nitrosophenol hydrochloride (**1**, 145 mg, 0.63 mmol) to obtain 3-bromo-9-diethylamino-5*H*-benzo[*a*]phenoxazin-5-one (**7e**), which crystallized from hexanes in dark green crystals (143 mg, 0.36 mmol). Yield: 57%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.48 (d, J = 8.5 Hz, 1H, H-1), 8.42 (d, J = 2.1 Hz, 1H, H-4), 7.78 (dd, J = 8.5, 2.1 Hz, 1H, H-2), 7.57 (d, J = 9.1 Hz, 1H, H-11), 6.67 (dd, J = 9.1, 2.7 Hz, 1H, H-10), 6.44 (d, J = 2.7 Hz, 1H, H-8), 6.36 (s, 1H, H-6), 3.47 (q, J = 7.1 Hz, 4H, CH_2), 1.27 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 182.2 (C-5), 152.3 (C-6a), 151.1 (C-9), 146.9 (C-7a), 138.9 (C-12a), 134.3 (C-2), 133.0 (C-4a), 131.3 (C-11), 130.8 (C-3), 128.6 (C-4), 125.6 (C-1), 125.2 (C-11a), 124.9 (C-12b), 110.1 (C-10), 105.6 (C-6), 96.3 (C-8), 45.2 (CH_2), 12.6 (CH_3). HRMS (ESI): calcd for $C_{20}H_{18}BrN_2O_2^+$ [M + H]$^+$, m/z 397.0546 (^{79}Br), 399.0526 (^{81}Br), found: m/z 397.0540 (^{79}Br), 399.0524 (^{81}Br).

*3-Cyano-9-diethylamino-5H-benzo[a]phenoxazin-5-one (**7f**).* To a solution of 9-diethylamino-3-(((trifluoromethyl)sulfonyl)oxy)-5*H*-benzo[*a*]phenoxazin-5-one (**7p**, 200 mg, 0.43 mmol) in toluene/EtOH (25 mL, 4:1) were added CuCN (192 mg, 2.14 mmol), SPhos (14 mg, 34 μmol), $Pd_2(dba)_3$ (16 mg, 17 μmol), and Na_2CO_3 (2.0 M, 5.0 mL, 10.0 mmol). The reaction mixture was flushed with N_2 and stirred at 80 °C (DrySyn heating block) overnight. This resulted in a purple precipitate of crude 3-cyano-9-diethylamino-5*H*-benzo[*a*]-phenoxazin-5-one (**7f**), which was filtered off, extracted with acetone (100 mL), concentrated under reduced pressure, and recrystallized from boiling toluene in dark green crystals (112 mg, 0.33 mmol). Yield: 76%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.75 (d, J = 8.3 Hz, 1H, H-1), 8.63 (d, J = 1.7 Hz, 1H, H-4), 7.91 (dd, J = 8.3, 1.7 Hz, 1H, H-2), 7.64 (d, J = 9.2 Hz, 1H, H-11), 6.73 (dd, J = 9.2, 2.7 Hz, 1H, H-10), 6.50 (d, J = 2.7 Hz, 1H, H-8), 6.43 (s, 1H, H-6), 3.51 (q, J = 7.1 Hz, 4H, CH_2), 1.29 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 181.5 (C-5), 152.8 (C-6a), 151.8 (C-9), 147.3 (C-7a), 137.5 (C-12a), 135.1 (C-4a), 133.2 (C-2), 131.9 (C-11), 131.8 (C-12b), 130.6 (C-4), 125.6 (C-11a), 124.7 (C-1), 118.5 (C-3), 113.0 (CN), 110.5 (C-10), 105.7 (C-6), 96.3 (C-8), 45.3 (CH_2), 12.6 (CH_3). HRMS (ESI): calcd for $C_{21}H_{18}N_3O_2^+$ [M + H]$^+$, m/z 344.1394, found: m/z 344.1407.

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*9-Diethylamino-3-fluoro-5H-benzo[a]phenoxazin-5-one (**7g**).* To a solution of 6-fluoro-1-naphthol (**6g**, 200 mg, 1.23 mmol) in anhydrous DMF (25 mL) was added 5-diethylamino-2-nitrosophenol hydrochloride (**1**, 285 mg, 1.23 mmol). This reaction mixture was stirred at 100 °C (DrySyn heating block) for 16 h and concentrated under reduced pressure. The crude product was purified by flash chromatography (0−50% EtOAc in hexanes) to obtain 9-diethylamino-3-fluoro-5*H*-benzo[*a*]phenoxazin-5-one (**7g**), which crystallized from toluene in dark green crystals (132 mg, 0.39 mmol). Yield: 32%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.66 (dd, J = 8.8, 5.3 Hz, 1H, H-1), 7.95 (dd, J = 9.3, 2.7 Hz, 1H, H-4), 7.61 (d, J = 9.1 Hz, 1H, H-11), 7.41 (ddd, J = 8.8, 8.0, 2.7 Hz, 1H, H-2), 6.69 (dd, J = 9.1, 2.8 Hz, 1H, H-10), 6.49 (d, J = 2.8 Hz, 1H, H-8), 6.41 (s, 1H, H-6), 3.48 (q, J = 7.1 Hz, 4H, CH_2), 1.27 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 182.4 (C-5), 164.2 (d, $^1J_{C-F}$ = 251 Hz, C-3), 152.2 (C-6a), 150.9 (C-9), 146.8 (C-7a), 139.2 (C-12a), 134.1 (C-4a), 131.1 (C-11), 128.4 (d, $^4J_{C-F}$ = 3 Hz, C-12b), 126.6 (d, $^3J_{C-F}$ = 8 Hz, C-1), 125.1 (C-11a), 119.2 (d, $^2J_{C-F}$ = 23 Hz, C-2), 111.6 (d, $^2J_{C-F}$ = 23 Hz, C-4), 110.0 (C-10), 105.7 (C-6), 96.3 (C-8), 45.1 (CH_2), 12.6 (CH_3). ^{19}F NMR ($CDCl_3$, 376 MHz): δ −109.05. HRMS (ESI): calcd for $C_{20}H_{18}FN_2O_2^+$ [M + H]$^+$, m/z 337.1347, found: m/z 337.1355.

*9-Diethylamino-2-trifluoromethyl-5H-benzo[a]phenoxazin-5-one (**7h**).* In a similar way, 6-trifluoromethyl-1-naphthol (**6i**, 200 mg, 0.94 mmol) was mixed with 5-diethylamino-2-nitrosophenol hydrochloride (**1**, 218 mg, 0.94 mmol) to obtain 9-diethylamino-2-trifluoromethyl-5*H*-benzo[*a*]-phenoxazin-5-one (**7h**), which crystallized from hexanes in dark green crystals (77 mg, 200 μmol). Yield: 21%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.93 (d, J = 1.9 Hz, 1H, H-1), 8.42 (d, J = 8.2 Hz, 1H, H-4), 7.85 (dd, J = 8.2, 1.9 Hz, 1H, H-3), 7.64 (d, J = 9.1 Hz, 1H, H-11), 6.70 (dd, J = 9.1, 2.8 Hz, 1H, H-10), 6.48 (d, J = 2.8 Hz, 1H, H-8), 6.43 (s, 1H, H-6), 3.49 (q, J = 7.1 Hz, 4H, CH_2), 1.28 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 182.4 (C-5), 152.6 (C-6a), 151.4 (C-9), 147.0 (C-7a), 138.4 (C-12a), 133.7 (C-4a), 132.3 (C-12b), 131.5 (C-11), 126.6 (C-4), 125.8 (q, $^3J_{C-F}$ = 3 Hz, C-3), 125.2 (C-11a), 121.3 (q, $^3J_{C-F}$ = 4 Hz, C-1), 110.3 (C-10), 105.9 (C-6), 96.3 (C-8), 45.2 (CH_2), 12.6 (CH_3). HRMS (ESI): calcd for $C_{21}H_{18}F_3N_2O_2^+$ [M + H]$^+$, m/z 387.1315, found: m/z 387.1322.

*9-Diethylamino-3-trifluoromethyl-5H-benzo[a]phenoxazin-5-one (**7i**).* In a similar way, a batch (0.36 g) consisting of 2/3 parts of 7-trifluoromethyl-1-naphthol (**6i**, 1.41 mmol) and 1/3 parts of 6-trifluoromethyl-1-naphthol (**6h**, 0.71 mmol) was mixed with 5-diethylamino-2-nitrosophenol hydrochloride (**1**, 0.49 g, 2.12 mmol) to obtain 9-diethylamino-3-(trifluoromethyl)-5*H*-benzo[*a*]-phenoxazin-5-one (**7i**), which crystallized from hexanes in dark green crystals (78 mg, 202 μmol). Yield: 14%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.76 (d, J = 8.5 Hz, 1H, H-1), 8.61 (d, J = 2.0 Hz, 1H, H-4), 7.92 (dd, J = 8.5, 2.0 Hz, 1H, H-2), 7.64 (d, J = 9.1 Hz, 1H, H-11), 6.71 (dd, J = 9.1, 2.8 Hz, 1H, H-10), 6.50 (d, J = 2.8 Hz, 1H, H-8), 6.44 (s, 1H, H-6), 3.50 (q, J = 7.1 Hz, 4H, CH_2), 1.28 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 182.3 (C-5), 152.6 (C-6a), 151.5 (C-9), 147.1 (C-7a), 138.4 (C-12a), 134.8 (C-12b), 131.8 (C-4a), 131.7 (C-11), 127.3 (q, $^3J_{C-F}$ = 3 Hz, C-2), 125.3 (C-11a), 124.6 (C-1), 123.3 (q, $^3J_{C-F}$ = 4 Hz, C-4), 110.2 (C-10), 105.8 (C-6), 96.3 (C-8), 45.2 (CH_2), 12.6 (CH_3). ^{19}F NMR ($CDCl_3$, 376 MHz): δ −62.48 (CF_3). HRMS (ESI): calcd for $C_{21}H_{18}F_3N_2O_2^+$ [M + H]$^+$, m/z 387.1315, found: m/z 387.1320.

*9-Diethylamino-4-trifluoromethyl-5H-benzo[a]phenoxazin-5-one (**7j**).* In a similar way, 8-trifluoromethyl-1-naphthol (**6j**, 200 mg, 0.94 mmol) was mixed with 5-diethylamino-2-nitrosophenol hydrochloride (**1**, 218 mg, 0.94 mmol) to obtain 9-diethylamino-4-(trifluoromethyl)-5*H*-benzo[*a*]phenoxazin-5-one (**7j**), which crystallized from hexanes in dark green crystals (98 mg, 252 μmol). Yield: 27%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.00 (dd, J = 8.0, 1.3 Hz, 1H, H-1), 8.04 (dd, J = 8.0, 1.3 Hz, 1H, H-3), 7.77 (t, J = 8.0 Hz, 1H, H-2), 7.59 (d, J = 9.1 Hz, 1H, H-11), 6.67 (dd, J = 9.1, 2.8 Hz, 1H, H-10), 6.46 (d, J = 2.8 Hz, 1H, H-8), 6.38 (s, 1H, H-6), 3.48 (q, J = 7.1 Hz, 4H, CH_2), 1.27 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 181.5 (C-5), 151.2 (C-9), 150.7 (C-6a), 146.9 (C-7a), 139.1 (C-12a), 134.6 (C-12b), 131.4 (C-11), 130.2 (C-2), 129.8 (C-4a), 129.4 (q, $^3J_{C-F}$ = 7 Hz, C-3), 128.6 (q, $^2J_{C-F}$ = 33 Hz, C-4), 128.2 (C-1), 124.9 (C-11a), 123.9 (q, $^1J_{C-F}$ = 273 Hz, CF_3), 109.8 (C-10), 107.2 (C-6), 96.3 (C-8), 45.2 (CH_2), 12.6 (CH_3). ^{19}F NMR ($CDCl_3$, 376 MHz): δ −58.03 (CF_3). HRMS (ESI): calcd for $C_{21}H_{18}F_3N_2O_2^+$ [M + H]$^+$, m/z 387.1315, found: m/z 387.1317.

*9-Diethylamino-11-methyl-5H-benzo[a]phenoxazin-5-one (**7k**).* In a similar way, 1,3-naphthalenediol (**12**, 167 mg, 1.04 mmol) was mixed with *N*,*N*-diethyl-3-methyl-4-nitrosoaniline (**9k**, 200 mg, 1.04 mmol) to obtain 9-diethylamino-11-methyl-5*H*-benzo[*a*]phenoxazin-5-one (**7k**), which crystallized from toluene in dark green crystals (152 mg, 0.48 mmol). Yield: 46%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.65 (dd, J = 7.6, 1.5 Hz, 1H, H-1), 8.30 (dd, J = 7.6, 1.5 Hz, 1H, H-4), 7.70 (td, J = 7.6, 1.5 Hz, 1H, H-2), 7.62 (td, J = 7.6, 1.5 Hz, 1H, H-3), 6.48 (d, J = 2.3 Hz, 1H, H-10), 6.35 (s, 1H, H-6), 6.30 (d, J = 2.7 Hz, 1H, H-8), 3.42 (q, J = 7.1 Hz, 4H, CH_2), 2.63 (s, 3H, CH_3), 1.24 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 183.7 (C-5), 152.2 (C-6a), 150.2 (C-9), 147.1 (C-7a), 140.3 (C-11), 138.2 (C-12a), 132.4 (C-12b), 131.8 (C-4a), 131.1 (C-2), 129.6 (C-3), 125.6 (C-4), 124.2 (C-11a), 123.6 (C-1), 110.5 (C-10), 105.2 (C-6), 94.5 (C-8), 44.9 (CH_2), 17.3 (CH_3), 12.7 (CH_3). HRMS (ESI): calcd for $C_{21}H_{21}N_2O_2^+$ [M + H]$^+$, m/z 333.1598, found: m/z 333.1594.

*9-Diethylamino-10-fluoro-5H-benzo[a]phenoxazin-5-one (**7n**).* In a similar way, 1-naphthol (90 mg, 0.63 mmol) was mixed with 5-diethylamino-4-fluoro-2-nitrosophenol (**14**, 156 mg, 0.63 mmol) to obtain 9-diethylamino-10-fluoro-5*H*-benzo[*a*]phenoxazin-5-one (**7n**), which crystallized from hexanes in dark green crystals (26 mg, 65 μmol). Yield: 10%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.61 (dd, J = 7.5, 1.4 Hz, 1H, H-1), 8.28 (dd, J = 7.5, 1.4 Hz, 1H, H-4), 7.72 (td, J = 7.5, 1.4 Hz, 1H, H-2), 7.67 (td, J = 7.5, 1.4 Hz, 1H, H-3), 7.36 (d, J = 14.3 Hz, 1H, H-11), 6.58 (d, J = 8.0 Hz, 1H, H-8), 6.37 (s, 1H, H-6), 3.44 (qd, J = 7.1, 1.3 Hz, 4H, CH_2), 1.26 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 183.7 (C-5), 151.9 (C-6a), 150.0 (d, $^1J_{C-F}$ = 243 Hz, C-10), 142.4 (C-12a), 142.3 (C-7a), 141.4 (d, $^2J_{C-F}$ = 10 Hz, C-9), 131.9 (C-4a), 131.6 (C-12b), 131.5 (C-2), 130.6 (C-3), 125.8 (C-4), 124.9 (C-11a), 124.1 (C-1), 116.2 (d, $^2J_{C-F}$ = 25 Hz, C-11), 106.2 (C-6), 101.4 (d, $^3J_{C-F}$ = 5 Hz, C-8), 46.5 (d, $^4J_{C-F}$ = 6 Hz, CH_2), 12.6 (d, $^5J_{C-F}$ = 2 Hz, CH_3). ^{19}F NMR ($CDCl_3$, 376 MHz): δ −127.00. HRMS (ESI): calcd for $C_{20}H_{17}FN_2O_2Na^+$ [M + Na]$^+$, m/z 359.1166, found: m/z 359.1178.

*9-Diethylamino-2-(((trifluoromethyl)sulfonyl)oxy)-5H-benzo[a]-phenoxazin-5-one (**7o**).* To a solution of 9-diethylamino-2-hydroxy-5*H*-benzo[*a*]phenoxazin-5-one (**7a**, 800 mg, 2.39 mmol) in anhydrous THF (20 mL) were added *N*-phenyl-bis-(trifluoromethanesulfonimide) (2.02 g, 7.18 mmol) and Et_3N (1.00 mL, 7.18 mmol). This reaction mixture was stirred for 17 h at room temperature, concentrated under reduced pressure, and purified by flash chromatography (0−100% EtOAc in hexanes) to obtain 9-diethylamino-2-(((trifluoromethyl)sulfonyl)oxy)-5*H*-benzo[*a*]-phenoxazin-5-one (**7o**), which crystallized from toluene in green crystals (0.86 g, 1.84 mmol). Yield: 77%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.53 (d, J = 2.5 Hz, 1H, H-1), 8.40 (d, J = 8.8 Hz, 1H, H-4), 7.63 (d, J = 9.1 Hz, 1H, H-11), 7.49 (dd, J = 8.8, 2.6 Hz, 1H, H-3), 6.70 (dd, J = 9.1, 2.7 Hz, 1H, H-10), 6.47 (d, J = 2.7 Hz, 1H, H-8), 6.39 (s, 1H, H-6), 3.49 (q, J = 7.1 Hz, 4H, CH_2), 1.28 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 181.9 (C-5), 152.7 (C-6a), 151.53 (C-9), 151.49 (C-2), 147.2 (C-7a), 137.9 (C-12a), 134.3 (C-4a), 131.7 (C-11), 131.2 (C-12b), 128.7 (C-4), 125.2 (C-11a), 122.2 (C-3), 116.4 (C-1), 110.3 (C-10), 105.6 (C-6), 96.3 (C-8), 45.3 (CH_2), 12.6 (CH_3). ^{19}F NMR ($CDCl_3$, 376 MHz): δ −72.75 (Tf). HRMS (ESI): calcd for $C_{21}H_{18}F_3N_2O_5S^+$ [M + H]$^+$, m/z 467.0883, found: m/z 467.0891.

*9-Diethylamino-3-(((trifluoromethyl)sulfonyl)oxy)-5H-benzo[a]-phenoxazin-5-one (**7p**).* In a similar way, 9-diethylamino-3-hydroxy-5*H*-benzo[*a*]phenoxazin-5-one (**7b**, 1.00 g, 3.00 mmol) was mixed with *N*-phenyl-bis(trifluoromethanesulfonimide) (3.21 g, 9.00 mmol) and Et_3N (1.25 mL, 9.00 mmol) to obtain 9-diethylamino-3-(((trifluoromethyl)sulfonyl)oxy)-5*H*-benzo[*a*]phenoxazin-5-one (**7p**), which crystallized from toluene in dark green crystals (0.98 g,

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2.11 mmol). Yield: 70%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.74 (d, J = 8.8 Hz, 1H, H-1), 8.18 (d, J = 2.7 Hz, 1H, H-4), 7.61 (d, J = 9.1 Hz, 1H, H-11), 7.59 (dd, J = 8.8, 2.7 Hz, 1H, H-2), 6.71 (dd, J = 9.1, 2.7 Hz, 1H, H-10), 6.49 (d, J = 2.7 Hz, 1H, H-8), 6.42 (s, 1H, H-6), 3.48 (q, J = 7.1 Hz, 4H, CH_2), 1.28 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 181.4 (C-5), 152.4 (C-6a), 151.4 (C-9), 150.6 (C-3), 147.0 (C-7a), 137.9 (C-12a), 133.6 (C-4a), 131.8 (C-12b), 131.6 (C-11), 126.5 (C-1), 125.3 (C-11a), 124.1 (C-2), 118.2 (C-4), 110.3 (C-10), 105.6 (C-6), 96.3 (C-8), 45.2 (CH_2), 12.6 (CH_3). ^{19}F NMR ($CDCl_3$, 376 MHz): δ −72.70 (Tf). HRMS (ESI): calcd for $C_{21}H_{18}F_3N_2O_5S^+$ [M + H]$^+$, m/z 467.0883, found: m/z 467.0886.

*2-Cyano-9-diethylamino-5H-benzo[a]phenoxazin-5-one (**7q**).* To a solution of 9-diethylamino-2-(((trifluoromethyl)sulfonyl)oxy)-5*H*-benzo[*a*]phenoxazin-5-one (**7o**, 200 mg, 0.43 mmol) in toluene/EtOH (25 mL, 4:1) were added CuCN (192 mg, 2.14 mmol), SPhos (14 mg, 34 μmol), $Pd_2(dba)_3$ (16 mg, 17 μmol), and Na_2CO_3 (2.0 M, 5.0 mL, 10.0 mmol). The reaction mixture was flushed with N_2 and stirred at 80 °C (DrySyn heating block) overnight. This resulted in a purple precipitate of crude 2-cyano-9-diethylamino-5*H*-benzo[*a*]-phenoxazin-5-one (**7q**), which was filtered off, extracted with acetone (100 mL), concentrated under reduced pressure, and recrystallized from boiling toluene in dark green crystals (128 mg, 0.37 mmol). Yield: 87%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.96 (d, J = 1.4 Hz, 1H, H-1), 8.39 (d, J = 8.2 Hz, 1H, H-4), 7.83 (dd, J = 8.2, 1.4 Hz, 1H, H-3), 7.62 (d, J = 9.1 Hz, 1H, H-11), 6.72 (dd, J = 9.1, 2.7 Hz, 1H, H-10), 6.48 (d, J = 2.7 Hz, 1H, H-8), 6.43 (s, 1H, H-6), 3.50 (q, J = 7.1 Hz, 4H, CH_2), 1.29 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 181.8 (C-5), 152.5 (C-6a), 151.6 (C-9), 147.2 (C-7a), 137.4 (C-12a), 133.9 (C-4a), 132.5 (C-12b), 131.71 (C-11), 131.67 (C-3), 128.5 (C-1), 126.7 (C-4), 125.4 (C-11a), 118.4 (C-2), 114.7 (CN), 110.5 (C-10), 106.1 (C-6), 96.3 (C-8), 45.3 (CH_2), 12.6 (CH_3). HRMS (ESI): calcd for $C_{21}H_{18}N_3O_2^+$ [M + H]$^+$, m/z 344.1394, found: m/z 344.1399.

*3-Ethoxy-9-diethylamino-5H-benzo[a]phenoxazin-5-one (**7r**).* To a solution of 9-diethylamino-3-hydroxy-5*H*-benzo[*a*]phenoxazin-5-one (**7b**, 100 mg, 0.30 mmol) in anhydrous DMF (3.0 mL) were added iodoethane (36 μL, 0.45 mmol) and K_2CO_3 (64 mg, 0.46 mmol). This reaction mixture was stirred at 50 °C (DrySyn heating block) for 16 h and concentrated under reduced pressure. The crude product was purified by flash chromatography (0−50% EtOAc in hexanes) to obtain 3-ethoxy-9-diethylamino-5*H*-benzo[*a*]phenoxazin-5-one (**7r**) as a dark purple solid (98 mg, 0.27 mmol). Yield: 90%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.56 (d, J = 8.9 Hz, 1H, H-1), 7.72 (s, 1H, H-4), 7.59 (d, J = 9.1 Hz, 1H, H-11), 7.25 (d, J = 8.9 Hz, 1H, H-2), 6.67 (d, J = 9.1 Hz, 1H, H-10), 6.48 (s, 1H, H-8), 6.39 (s, 1H, H-6), 4.21 (q, J = 7.1 Hz, 2H, OCH_2), 3.45 (q, J = 7.3 Hz, 4H, CH_2), 1.47 (t, J = 7.1 Hz, 3H, CH_3), 1.25 (t, J = 7.3 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 183.6 (C-5), 160.9 (C-3), 151.9 (C-6a), 150.3 (C-9), 146.5 (C-7a), 140.1 (C-12a), 133.5 (C-4a), 130.8 (C-11), 125.8 (C-1), 125.3 (C-12b), 125.1 (C-11a), 120.7 (C-2), 109.7 (C-10), 107.8 (C-4), 105.7 (C-6), 96.4 (C-8), 64.0 (OCH_2), 45.0 (CH_2), 14.8 (CH_3), 12.6 (CH_3). HRMS (ESI): calcd for $C_{22}H_{23}N_2O_3^+$ [M + H]$^+$, m/z 363.1703, found: m/z 363.1707.

*N,N-Diethyl-3-methyl-4-nitrosoaniline (**9k**).* *N*,*N*-Diethyl-*m*-toluidine (**8k**, 0.50 g, 3.06 mmol) was dissolved in a mixture of concentrated HCl (2.00 mL, 23.2 mmol) and H_2O (1.20 mL), and the reaction mixture was cooled to 0 °C. A solution of $NaNO_2$ (212 mg, 72.6 mmol) in H_2O (10 mL) was added dropwise over 60 min, and the reaction mixture was stirred for 2 h at 0 °C (ice bath). This resulted in a yellow precipitate of *N*,*N*-diethyl-3-methyl-4-nitrosoaniline (**9k**), which crystallized from MeOH/H_2O (1:1) in green crystals (0.33 g, 1.71 mmol). Yield: 56%; mp 58−59 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.77 (d, J = 10.2 Hz, 1H, H-5), 7.42 (d, J = 10.2 Hz, 1H, H-6), 7.36 (s, 1H, H-2), 4.01 (q, J = 7.0 Hz, 4H, CH_2), 2.45 (s, 3H, 3-CH_3), 1.32 (t, J = 7.0 Hz, 6H, CH_3). HRMS (ESI): calcd for $C_{11}H_{17}N_2O^+$ [M + H]$^+$, m/z 193.1335, found: m/z 193.1327.

*N,N-Diethyl-3-methoxy-4-nitrosoaniline (**9m**).* *N*,*N*-Diethyl-3-methoxyaniline (**8m**, 0.30 g, 1.67 mmol) was dissolved in a mixture of concentrated HCl (1.00 mL, 11.6 mmol) and H_2O (0.60 mL), and the reaction mixture was cooled to 0 °C. A solution of $NaNO_2$ (115 mg, 1.67 mmol) in H_2O (0.50 mL) was added dropwise over 15 min, and the reaction mixture was stirred for 2 h at 0 °C (ice bath). The reaction mixture was diluted with ice water, and conc. aq NaOH was slowly added to give a neutral solution. The product was extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic extracts were dried over $MgSO_4$ and concentrated to give *N*,*N*-diethyl-3-methoxy-4-nitrosoaniline (**9m**) as a green-red oil (0.25 g, 1.19 mmol). Yield: 71%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.83 (dd, J = 9.0, 2.6 Hz, 1H, H-6), 7.68 (d, J = 2.6 Hz, 1H, H-2), 6.76 (d, J = 9.0, 1H, 1H, H-5), 3.91 (s, 3H, OCH_3), 3.37 (q, J = 7.1 Hz, 4H, CH_2CH_2), 1.22 (t, J = 7.0 Hz, 3H, CH_2CH_3). HRMS (ESI): calcd for $C_{11}H_{16}N_2O_2Na^+$ [M + Na]$^+$, m/z 231.1104, found: m/z 231.1106.

*Methyl 4-(4-Nitrophenyl)-3-oxobutanoate (**11d**).*[98] 4-Nitrophenylacetonitrile (4.1 g, 25.3 mmol) was added to a mixture of conc. sulfuric acid (10 mL, 187 mmol) and H_2O (10 mL). This reaction mixture was refluxed for 30 min and then diluted with H_2O (100 mL). The mixture was cooled to 0 °C, upon which a solid precipitated. The solid was filtered off, washed with ice-cold water, and dried to yield 4-nitrophenylacetic acid as colorless crystals (4.49 g, 24.8 mmol). Yield: 98%; mp 150−151 °C. To a solution of 4-nitrophenylacetic acid (4.40 g, 24.3 mmol) in anhydrous THF (50 mL) was added 1,1′-carbonyldiimidazole (4.33 g, 26.7 mmol), and the mixture was stirred at room temperature for 1 h. Monomethyl potassium malonate (4.55 g, 29.2 mmol) and anhydrous $MgCl_2$ (3.47 g, 36.4 mmol) were added, and the reaction mixture was stirred at room temperature overnight. A solution of HCl (1.0 M, 50 mL) was added, and the phases were separated. The aqueous phase was extracted with EtOAc (50 mL). The combined organic phases were washed with brine (50 mL), H_2O (50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography (0−30% EtOAc in hexanes) to give methyl 4-(4-nitrophenyl)-3-oxobutanoate (**11d**) as a yellow solid (4.90 g, 20.7 mmol). Yield: 57%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.21 (d, J = 8.7 Hz, 2H, *m*-CH), 7.38 (d, J = 8.7 Hz, 2H, *o*-CH), 3.99 (s, 2H, γ-CH_2), 3.76 (s, 3H, CH_3), 3.54 (s, 2H, α-CH_2). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 197.4 (CO), 166.2 (CO_2Me), 139.4 (aromatic C), 129.6 (aromatic CH), 129.1 (aromatic C), 122.8 (aromatic CH), 51.6 (CH_3), 48.0 (CH_2), 47.6 (CH_2). Note that **11d** exists in $CDCl_3$ as a mixture of keto (4/5 parts) and enol (1/5 parts) isomers. This is evident by the bridging hydrogen at δ 12.06 ppm and the enol vinyl hydrogen at δ 5.00 ppm. Only the signals associated with the keto isomer are reported. HRMS (ESI): calcd for $C_{11}H_{11}NO_5Na^+$ [M + Na]$^+$, m/z 260.0529, found: m/z 260.0537.

*Ethyl 4-(4-Bromophenyl)-3-oxobutanoate (**11e**).*[63] To a suspension of activated Zn (3.33 g, 51 mmol) and a few crystals of I_2 in refluxing anhydrous THF (100 mL) was added ethyl bromoacetate (0.1 mL, 0.83 mmol), and the suspension was refluxed for 60 min. Hereafter, 4-bromophenylace-tonitrile (1.00 g, 5.10 mmol) and ethyl bromoacetate (2.35 mL, 19.6 mmol) were coadded dropwise, and the reaction mixture was refluxed for 30 min, upon which it was cooled to room temperature and a saturated solution of K_2CO_3 (100 mL) was added. The aqueous layer was extracted with THF (3 × 25 mL), and the combined organic extracts were concentrated under reduced pressure. HCl (1.0 M, 25 mL) was added, and the solution was stirred at room temperature for 30 min and diluted with CH_2Cl_2 (25 mL). The organic layer was isolated, washed with saturated aqueous $NaHCO_3$ (25 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography (0−10% EtOAc in hexanes) to obtain ethyl 4-(4-bromophenyl)-3-oxobutanoate (**11e**) as a white solid (1.31 g, 4.59 mmol). Yield: 90%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.46 (d, J = 8.4 Hz, 2H, *m*-CH), 7.08 (d, J = 8.4 Hz, 2H, *o*-CH), 4.18 (q, J = 7.1 Hz, 1H, CH_2CH_3), 3.80 (s, 2H, γ-CH_2), 3.46 (s, 2H, α-CH_2), 1.26 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 198.7 (CO), 165.9 (CO_2Et), 130.9 (aromatic CH), 130.3 (aromatic CH), 130.0 (aromatic C), 120.4 (aromatic C), 60.5 (CH_2CH_3), 48.1 (CH_2), 47.5 (CH_2), 13.1 (CH_3). MS (ESI): calcd for $C_{12}H_{14}BrO_3^+$ [M + H]$^+$, m/z 306.9940 (^{79}Br), 308.9920 (^{81}Br), found: m/z 306.9934 (^{79}Br), 308.9915 (^{81}Br).

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*7-Bromo-1,3-naphthalenediol (**12e**).* Ethyl 4-(4-bromophenyl)-3-oxobutanoate (**11e**, 1.25 g, 4.38 mmol) was mixed with sulfuric acid (98%, 5.0 mL, 93 mmol), and the mixture was stirred at 60 °C (DrySyn heating block) for 3 days. The reaction mixture was cooled and poured onto ice. After thawing, the supernatant was decanted off, and the residue was dissolved in Et_2O (10 mL) and washed with saturated aqueous $NaHCO_3$ (10 mL). The organic phase was concentrated to a sticky mass, which was treated with cold petroleum ether to precipitate 7-bromo-1,3-naphthalenediol (**12e**), which crystallized from toluene in off-white crystals (0.70 g, 2.93 mmol). Yield: 67%; mp 172−174 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.30 (s, 1H, OH), 9.66 (s, 1H, OH), 8.06 (d, J = 2.2 Hz, 1H, H-8), 7.54 (d, J = 8.8 Hz, 1H, H-5), 7.42 (dd, J = 8.8, 2.2 Hz, 1H, H-6), 6.61 (d, J = 2.1 Hz, 1H, H-4), 6.54 (d, J = 2.1 Hz, 1H, H-2). HRMS (ESI): calcd for $C_{10}H_8BrO_2H^+$ $[M + H]^+$, m/z 238.9702 (^{79}Br), 240.9682 (^{81}Br), found: m/z 238.9692 (^{79}Br), 240.9656 (^{81}Br).

*6,8-Dihydroxy-2-naphthonitrile (**12f**).* To a solution of 7-bromo-1,3-naphthalenediol (**12e**, 174 mg, 0.72 mmol) in anhydrous DMF (1 mL) was added CuCN (78 mg, 0.87 mmol), and the reaction mixture was stirred at 160 °C (DrySyn heating block) for 3 h in a sealed vessel. The solution was cooled to room temperature, diluted with H_2O (5 mL), and extracted with EtOAc (2 × 5 mL). The combined organic phases were concentrated under reduced pressure, and the resulting crude product was recrystallized from EtOH−H_2O to give 6,8-dihydroxy-2-naphthonitrile (**12f**) as a white solid (121 mg, 0.66 mmol). Yield: 90%. ^{1}H NMR ($(CD_3)_2CO$:$CHCl_3$, 400 MHz): δ 9.11 (br, 2H, OH), 8.49 (d, J = 1.7 Hz, 1H, H-1), 7.65 (d, J = 8.5 Hz, 1H, H-4), 7.47 (dd, J = 8.5, 1.7 Hz, 1H, H-3), 6.78 (d, J = 2.0 Hz, 1H, H-5), 6.72 (d, J = 2.0 Hz, 1H, H-7). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 159.1 (C-6), 155.1 (C-8), 137.6 (C-4a), 129.2 (C-1), 127.2 (C-4), 127.1 (C-3), 120.1 (CN), 119.6 (C-8a), 104.4 (C-2), 102.5 (C-7), 101.7 (C-5). HRMS (ESI): calcd for $C_{22}H_{13}N_2O_4^-$ $[2M - H]^-$, m/z 369.0881, found: m/z 369.0881.

*3-Diethylamino-4-fluorophenol (**13**).* To a solution of 3-amino-4-fluorophenol (0.90 g, 4.91 mmol) in isopropanol/H_2O (1:1, 20 mL) were added Na_2CO_3 (1.15 g, 10.8 mmol) and iodoethane (1.97 mL, 24.6 mmol), and the reaction mixture was refluxed at 85 °C (DrySyn heating block) for 10 days and monitored by ^{19}F NMR spectroscopy. Hereafter, the isopropanol was evaporated by air flow and brine (10 mL) was added. The product was extracted with CH_2Cl_2 (3 × 25 mL), and the combined organic extracts were dried over $MgSO_4$ and concentrated. The crude product was purified by flash chromatography (0−10% EtOAc in hexanes) to give 3-diethylamino-4-fluorophenol (**13**) as a volatile colorless liquid (0.61 g, 2.87 mmol). Yield: 59%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.84 (dd, J = 12.6, 8.6 Hz, 1H, H-5), 6.40 (dd, J = 7.3, 3.0 Hz, 1H, H-2), 6.25 (dt, J = 8.6, 3.0 Hz, 1H, H-6), 4.64 (br, 1H, OH), 3.19 (dq, J = 7.1, 0.7 Hz, 4H, CH_2), 1.09 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 101 MHz): δ 150.7 (d, $^4J_{C-F}$ = 2 Hz, C-1), 149.3 (d, $^1J_{C-F}$ = 237 Hz, C-4), 138.0 (d, $^2J_{C-F}$ = 10 Hz, C-3), 115.4 (d, $^2J_{C-F}$ = 24 Hz, C-5), 106.4 (d, $^3J_{C-F}$ = 4 Hz, C-2), 105.3 (d, $^3J_{C-F}$ = 8 Hz, C-6), 44.9 (d, $^4J_{C-F}$ = 4 Hz, CH_2), 11.5 (CH_3). ^{19}F NMR ($CDCl_3$, 376 MHz): δ −133.04. HRMS (ESI): calcd for $C_{10}H_{15}NOF^+$ $[M + H]^+$, m/z 184.1132, found: m/z 184.1128.

*5-Diethylamino-4-fluoro-2-nitrosophenol (**14**).* 5-Diethylamino-4-fluorophenol (**13**, 0.30 g, 1.64 mmol) was dissolved in a mixture of concentrated HCl (1.00 mL, 11.6 mmol) and H_2O (0.60 mL), and the reaction mixture was cooled to 0 °C. A solution of $NaNO_2$ (113 mg, 1.64 mmol) in H_2O (0.50 mL) was added dropwise over 15 min, and the reaction mixture was stirred for 2 h at 0 °C (ice bath). The resulting precipitate was filtered off and washed with small amounts of chilled H_2O and EtOH to give 5-diethylamino-4-fluoro-2-nitrosophenol (**14**) as a dark green solid (194 mg, 0.91 mmol). Yield: 56%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.04 (d, J = 16.3 Hz, 1H, H-5), 5.97 (s, 1H, H-2), 3.58 (qd, J = 7.0, 2.0 Hz, 4H, CH_2), 1.22 (t, J = 7.0 Hz, CH_3). ^{13}C{^{1}H} NMR (DMSO-d_6, 101 MHz): δ 114.5 (d, $^2J_{C-F}$ = 24 Hz, C-3), 99.2 (d, $^3J_{C-F}$ = 7 Hz, C-6), 48.0 (d, $^4J_{C-F}$ = 7 Hz, CH_2), 12.8 (CH_3). Quaternary carbons are not sufficiently resolved from the baseline. HRMS (ESI): calcd for $C_{10}H_{13}FN_2O_2Na^+$ $[M + Na]^+$, m/z 235.0853, found: m/z 235.0843.

Absorption Spectra. 1PA spectra (800−400 nm) were recorded at room temperature on a PerkinElmer Lambda 35 UV/vis spectrophotometer. Fresh 10 mM stock solutions of the Nile Red analogues in DMSO were diluted with toluene, chloroform, or methanol to obtain solute concentrations of 20 μM. Baseline correction was achieved by subtracting the absorbance of the solvent from the absorption spectra of the samples. Quartz cuvettes with an optical path length of 10 mm were used.

Emission Spectra. The steady-state emission spectra (500−800 nm) were recorded on a PerkinElmer LS 55 luminescence spectrometer attached with FL WinLab software at 3.0 μM concentration in 10 × 5 mm^2 fluorescence quartz cuvettes. Measurements were based on digital averages of five scans at room temperature. The emission spectra were recorded by exciting the samples at 500 nm. An excitation slit of 4.0 nm and a scan speed of 120 nm min^{-1} were used.

Quantum Yield Measurements. QYs were measured at room temperature using the relative method.[99] Rhodamine B in EtOH (Φ 0.50)[100] and Nile Red in the respective solvents (Φ 0.34 in MeOH, Φ 0.64 in $CHCl_3$, and Φ 0.83 in toluene) were used as standards. Samples in MeOH, $CHCl_3$, and toluene were excited at 558, 546, and 526 nm, respectively.

Fluorescence Lifetime Measurements. Fluorescence lifetimes were measured on an Abberior Instruments 2C Facility Line STED instrument fitted with an Olympus IX83 microscope system. An SPC-150N time-correlated single-photon counting (TCSPC) module from Becker & Hickl GmbH was used for fluorescence lifetime imaging (FLIM). Sample concentrations of 3.0 μM were used, and all samples were excited at 561 nm. Fluorescence decay data were analyzed using SPCImage (version 8.1, Becker & Hickl GmbH), which allows single/multiexponential curve fitting on a pixel-by-pixel basis using a weighted least-squares numerical approach.

2PA Measurements. 2PA spectra were obtained using TPEF measurements from 780 to 1020 nm in 10 nm increments on a Nikon TI Eclipse TI-S-E microscope. Excitation was delivered using a Ti:sapphire tunable laser (Mai Tai HP DeepSee) with sub-100 fs pulse generation. Emission was detected using a Princeton monochromator (ARC-SP 2155). Sample concentrations of 20.0 μM were used except for compound **7a**, where concentrations of 500 μM were used in chloroform and methanol. The 20.0 μM solutions of Nile Red in toluene, chloroform, and methanol were used as the two-photon standard.[21]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c02346.

Synthetic procedures, NMR spectra, optical spectra, and fluorescence decay traces (PDF)

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All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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