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Generation of Profluorescent Isoindoline Nitroxides Using Click Chemistry

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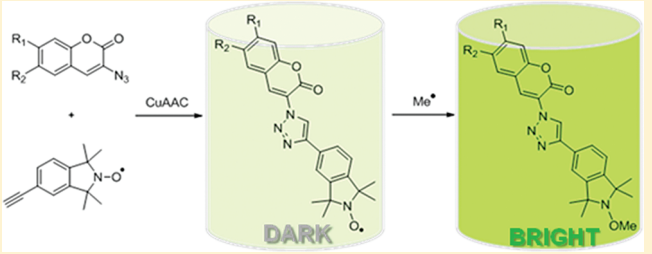
Supporting Information

ABSTRACT: Novel profluorescent nitroxides bearing a triazole linker between the coumarin fluorophore and an isoindoline nitroxide were prepared in good yields using the copper-catalyzed azide−alkyne 1,3-dipolar cycloaddition reaction (CuAAC). Nitroxides containing 7-hydroxy and 7-diethylamino substitution on their coumarin rings displayed significant fluorescence suppression, and upon reaction with methyl radicals, normal fluorescence emission was returned. The fluorescence emission for the 7-hydroxycoumarin nitroxide and its diamagnetic analogue was found to be strongly influenced by pH with maximal fluorescence emission achieved in basic solution. Solvent polarity was also shown to affect fluorescence emission. The significant difference in fluorescence output between the nitroxides and their corresponding diamagnetic analogues makes these compounds ideal tools for monitoring processes involving free-radical species.



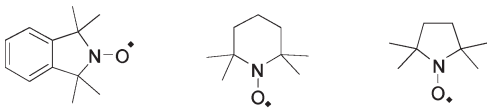
INTRODUCTION

Nitroxides (aminoxyls) are stable free-radical species which are currently employed in a broad range of applications. The isoindoline class of nitroxides (such as **1**) are finding increased use as they possess some advantages over the commercially available piperidine- and pyrrolidine-based nitroxides (such as **2** and **3**) (Chart 1). The fused aromatic ring provides structural rigidity and enhanced chemical and thermal stability in polymers.[1,2] Electron paramagnetic resonance (EPR) linewidths for isoindoline nitroxides are also often narrower,[3] and their structural diversity can easily be expanded by aromatic substitution to generate more complex structures with little impact on the reactivity or stability of the nitroxide moiety.[4,5]

One common use for nitroxides is as sensitive probes for monitoring processes involving free radicals. Profluorescent nitroxides, which contain a fluorophore tethered to a nitroxide moiety by a short covalent linkage, are effective scavengers of carbon-centered radicals and quenchers of excited electronic states.[6] They display low fluorescence due to enhanced intersystem crossing from the first excited singlet state to the triplet state via electron-exchange interactions of the nitroxide radical. However, upon redox activity or radical trapping, a diamagnetic species is formed and normal fluorophore emission is returned. Hence nitroxide−fluorophore adducts are extremely sensitive probes for the investigation of free-radical processes.

The majority of profluorescent nitroxides reported in the literature possess linkages such as esters,[7−16] amides,[17−19] or sulfonamides,[20−23] which may be susceptible to hydrolysis under certain reaction conditions. As scission of the nitroxide moiety from the fluorophore would restore fluorescence independently from any

Chart 1. Examples of Isoindoline 1, Piperidine 2, and Pyrrolidine 3 Classes of Nitroxides



1 **2** **3**

radical reactions of the nitroxide, our work has focused on the construction of nitroxide−fluorophore hybrids via carbon−carbon bond formation. We have reported the formation of an isoindoline nitroxide bearing a stilbene fluorophore[24] and an azaphenalene-based profluorescent nitroxide[25] and have also prepared molecules with nitroxides incorporated onto the fluorescent cores of phenanthrene,[26,27] naphthalene,[25] diphenylanthracene,[28] bis(phenylethynyl)anthracene,[28] and fluorescein.[29] Having previously prepared an alkyne-containing isoindoline nitroxide,[30] this provided the potential to use the copper-catalyzed azide−alkyne 1,3-dipolar cycloaddition "click" reaction to form a stable triazole linker between the nitroxide and the fluorophore.

The concept of "click chemistry" was coined in 2001 by Sharpless[31] to describe a set of "near perfect" bond-forming reactions which were very selective, high yielding, and wide in scope. The copper-catalyzed 1,3-dipolar azide, alkyne cycloaddition (CuAAC) reaction[32−35] has emerged as the premier

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example of "click chemistry" as it is virtually quantitative and easy to perform, and the triazole formed is essentially inert to reactive conditions such as oxidation, reduction, and hydrolysis. The reaction has been widely utilized in synthetic and medicinal chemistry,[36,37] surface and polymer chemistry,[38,39] and bioconjugation applications.[40,41] The versatility of the CuAAC reaction has also been exploited to incorporate nitroxide spin labels onto adenosine[42] and other biomolecules such as amino acids and carbohydrates.[43]

Herein, we describe the facile preparation of the first profluorescent nitroxides which utilize an unsaturated triazole linker to conjugatively join the isoindoline nitroxide to the coumarin fluorophore. In addition, we examine the physical properties of the prepared compounds to assess the ability of the triazole to facilitate nitroxide−fluorophore communication.

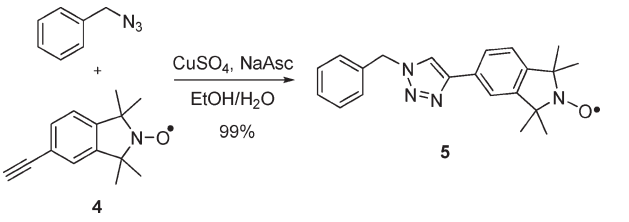
RESULTS AND DISCUSSION

In order to investigate the use of the isoindoline nitroxide, 5-ethynyl-1,1,3,3-tetramethylisoindolin-2-yloxyl **4**, as the alkyne coupling partner in Cu(I)-catalyzed [3 + 2] cycloaddition reactions, we initially undertook a model reaction with benzyl azide. Treatment of an ethanol/water solution containing **4** and benzyl azide with copper(II) sulfate and sodium ascorbate at room temperature for 16 h gave the desired "click" compound **5** in excellent yield (99%) and high purity following precipitation with water (Scheme 1). Encouraged by this result, we moved on to explore the use of the CuAAC reaction for linking alkyne nitroxide **4** to a fluorophore. Our choice of coumarin as the fluorophore for this study was based on the fact that it is small in size, biocompatible, and can be easily functionalized for use in a desired application. The fluorescence properties of coumarin dyes can also be fine-tuned by substitutions at the 3- and 7-positions,[44,45] and 3-azido coumarins are well-known coupling partners in the Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction.[46]

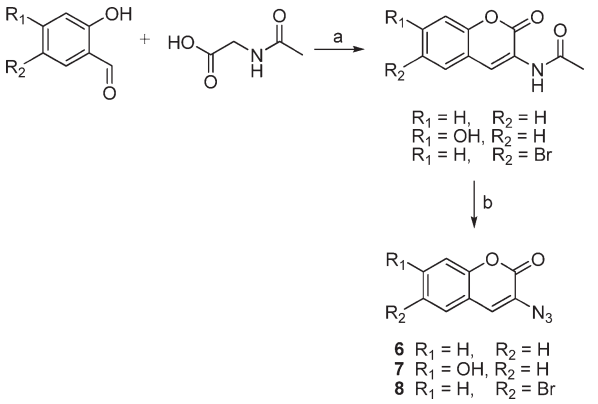
Initially, we synthesized a variety of 3-azidocoumarins **6**−**9** which would be expected to possess interesting fluorescence properties and also allow further substitution. Azides **6**−**9** were accessed using literature procedures from the corresponding deacetylated 3-aminocoumarins by in situ generation of the 3-diazonium salts upon treatment with sodium nitrite in aqueous acid, followed by the addition of sodium azide (Scheme 2).[46] The required 3-acetamidocoumarins were prepared by the condensation of the appropriately substituted 2-hydroxybenzaldehyde with *N*-acetyl−glycine in the presence of acetic anhydride.[46] Interestingly, the 3-acetamido coumarin required to synthesize 3-azido-7-diethylamine coumarin **9** could not be prepared using this method. Presumably the presence of electron donating groups disfavor coumarin formation, as the desired 3-acetamido-7-acetoxy coumarin could only be formed in low yield (phenol acetylation prior to coumarin formation would most likely weaken the electron donating effect of the phenol) and the 3-acetamido-6-bromo coumarin was obtained in much higher yield. Access to 3-azido-7-diethylamine coumarin **9** was achieved through an alternative route involving the condensation of 4-diethylamino-2-hydroxybenzaldehyde with ethyl nitroacetate, reduction of the resulting nitro coumarin with tin chloride, *in situ* diazonium formation from the amine and subsequent reaction with sodium azide (Scheme 3).[46]

With the 3-azido coumarins **6**−**9** in hand, we turned our attention toward their reaction in the copper-catalyzed 1,3-dipolar

Scheme 1. Model Copper-Catalyzed 1,3-Dipolar Cycloaddition Reaction Using Alkyne Nitroxide 4

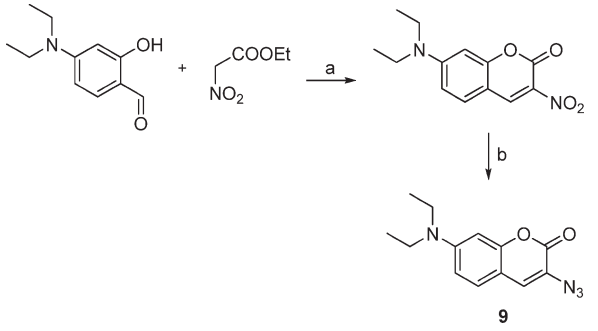


Scheme 2. Synthesis of 3-Azidocoumarins 6−8 from the Corresponding 3-Acetamidocoumarins[46]



Reagents and conditions: (a) NaAsc, Ac_2O, 120 °C, 4 h (b) (i) aq HCl, EtOH, reflux, 1 h, (ii) $NaNO_2$, 0 °C, 15 min; (iii) NaN_3, 0 °C, 30 min, yields for **6**, 87%; for **7**, 90%; for **8**, 30%.

Scheme 3. Synthesis of 3-Azidocoumarin 9 from the Corresponding Nitrocoumarin[46]



Reagents and conditions: (a) piperidine, AcOH, *t*-BuOH, reflux, 24 h (b) (i) aq HCl, $SnCl_2$, rt, 4 h, (ii) aq HCl, $NaNO_2$, 0 °C, 1 h, (iii) NaN_3, 0 °C, 5 h, 64%.

cycloaddition with alkyne nitroxide **4**. The standard 1,3-dipolar cycloaddition reaction conditions employing copper(II) sulfate and sodium ascorbate were initially explored with the unsubstituted 3-azidocoumarin **6**. Treatment of an ethanol/water solution of azide **6** and alkyne **4** with copper(II) sulfate (5 mol %) and sodium ascorbate (10 mol %) gave the desired triazole-containing compound **10** in high yield (86%) after stirring at room temperature for 16 h (Scheme 4). The presence of the triazole linkage was confirmed by X-ray crystallography.

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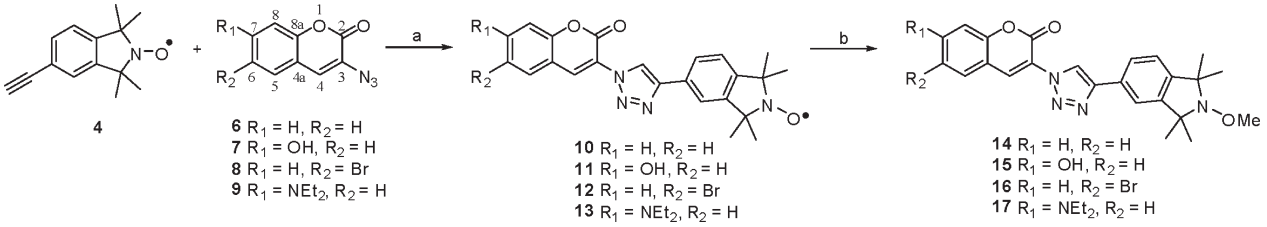
Scheme 4. Synthesis of Triazole-Linked Profluorescent Nitroxides and Their Methoxyamines



Reagents and conditions: (a) $CuSO_4$ (5 mol %), NaAsc (10 mol %), EtOH/H_2O, 16 h, rt, yields for **10**, 86%; for **11**, 90%; for **12**, 84%; for **13**, 60%; (b) H_2O_2, $FeSO_4 \cdot 7H_2O$, DMSO, 30 min, yields for **14**, 82%; for **15**, 90%; for **16**, 78%; for **17**, 84%.

The crystal structure of **10** is illustrated in Figure 1a. The N(4)−O(3) bond length is 1.2788(13) Å, which is consistent with typical bond lengths for isoindoline nitroxide radicals.[47,48] The dihedral angle between the mean plane of the atoms of the isoindoline moiety and the plane of the triazole ring is 28°. The plane of the coumarin moiety is rotated in the reverse direction making a dihedral angle of 29° with the plane of the triazole. These dihedral angles are a result of torsional twisting around the bonds connecting the isoindoline nitroxide (N(3)−C(11)−C(12)−C(13))= −26) and coumarin (N(2)−N(1)−C(9)−C(8) = 23°) moieties to the central triazole unit. As a result, the molecule has a bowlike molecular structure in the solid state, as illustrated in Figure 1b.

Click products **11**−**13** bearing OH, Br, and Et_2N substitution on the coumarin ring were also obtained in good to excellent yield (60−90%) under the same 1,3-dipolar cycloaddition reaction conditions (Scheme 4). Notably, the workup to isolate click products **10**−**13** was extremely facile, as the desired compounds precipitated from solution upon formation of the triazole linkage. Following clean up with a short silica column, products **10**−**13** were shown to be >95% pure by analytical HPLC. For additional characterization and in order to explore the impact of the nitroxide moiety on the fluorescence suppression of click products **10**−**13**, we also prepared methoxyamine versions of these compounds. The nitroxides **10**−**13** were reacted with methyl radicals generated using Fenton chemistry from dimethyl sulfoxide, ferrous ions, and hydrogen peroxide to give the desired methoxyamines **14**−**17** in high yield (78−90%).[49] A comparison of the ^{1}H NMR spectra of the nitroxides **10**−**13** with their corresponding methoxyamines **14**−**17** (see the Supporting Information) revealed the paramagnetic nature of the nitroxide radical which resulted in significant broadening of the signals for hydrogen atoms in close proximity to the nitroxide moiety.

With the new triazole-linked profluorescent nitroxides **10**−**13** and their corresponding methoxyamine adducts **14**−**17** in hand, we examined their physical properties to assess their applicability as probes for monitoring free radicals. The nitroxides **10**−**13** showed absorbance spectra which very closely resembled those of their related methoxyamine analogues **14**−**17** in THF (Figures 2-5). Substitution of the coumarin ring with electron-donating groups caused a bathochromic shift in the absorption spectra for 7-hydroxy- (**11** and **15**, λ_{max} = 354 nm) and 7-diethylamine (**13** and **17**, λ_{max} = 413 nm) substituted coumarins when compared to the unsubstituted coumarins (**10** and **14**, λ_{max} = 338 nm) and the 6-bromocoumarins (**12** and **16**, λ_{max} = 345 nm). Comparable shifts have previously been observed for similarly substituted coumarins[46,50] and are consistent with an increase in charge-transfer character[51] in the presence of electron-donating groups. The molar extinction coefficients

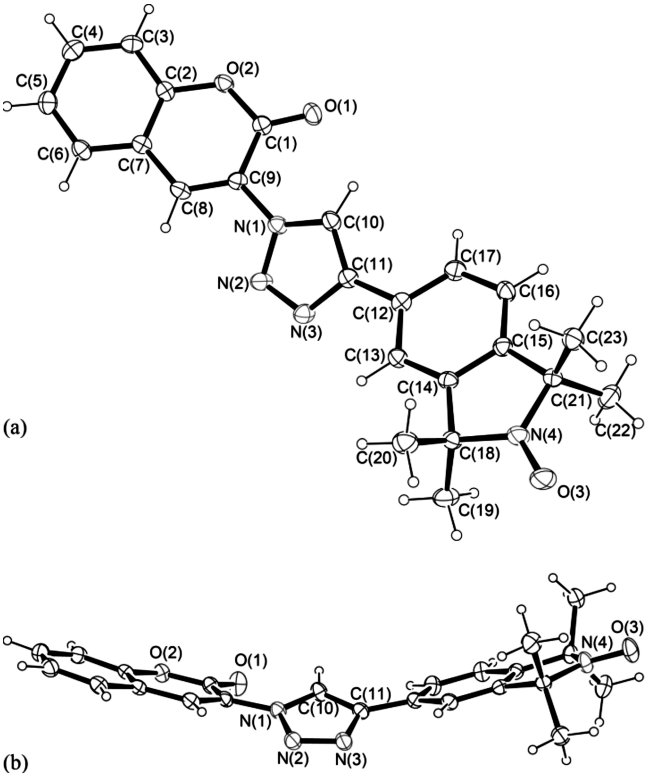


Figure 1. (a) ORTEP depiction of the crystal structure of 3-(4-(1,1,3,3-tetramethylisoindolin-2-yloxyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (**10**). (b) Alternate view of **10** illustrating the bowlike distortion from planarity.

measured for the prepared compounds **10**−**17** (Table 1) ranged from 15969 to 20057 M^{-1} cm^{-1} in THF at either 340 or 375 nm. The process of conjugating the coumarin chromophore to the nitroxide moiety via the triazole unit did not cause a significant shift in absorption maxima in comparison with maxima of parent coumarins **18** and **19** (Chart 2), which showed absorbances at 325[52] and 373 nm,[53] respectively.

A comparison of the fluorescence emission of unsubstituted coumarin nitroxide **10** and 6-bromocoumarin nitroxide **12** with their corresponding methoxyamine analogues **14** and **16** revealed modest fluorescence suppression arising from the presence of the nitroxide moiety (Figures 2 and 4). This effect was confirmed by the measured quantum yields of fluorescence (Φ_F, Table 1) for nitroxides **10** (0.001) and **12** (0.005) and methoxyamines **14** (0.01) and **16** (0.015). However, as the fluorescence intensity displayed by the diamagnetic analogues

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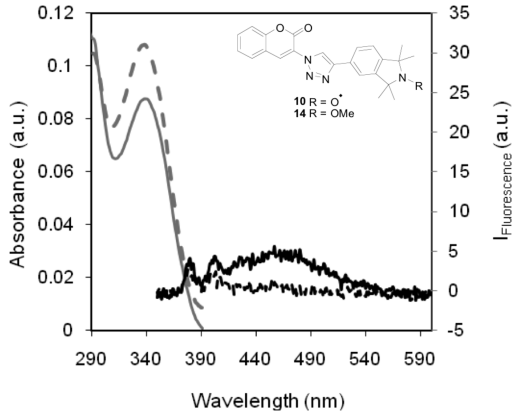

Figure 2. UV/vis spectra for compounds **10** (gray ---, 5.0 μM in THF) and **14** (gray —, 4.16 μM in THF) and fluorescence emission spectra for compounds **10** (black ---, 5.0 μM in THF) and **14** (black —, 4.16 μM in THF), following excitation at 340 nm.

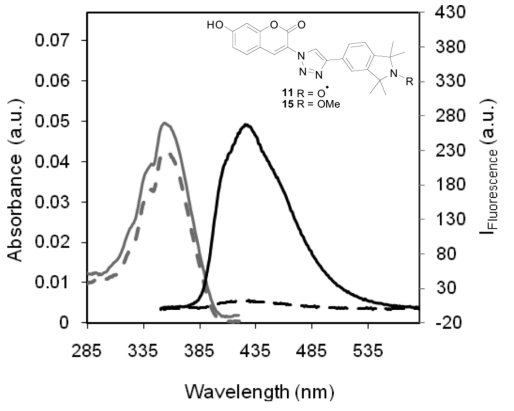

Figure 3. UV/vis spectra for compounds **11** (gray ---, 3.0 μM in THF) and **15** (gray —, 2.9 μM in THF) and fluorescence emission spectra for compounds **11** (black ---, 3.0 μM in THF) and **15** (black —, 2.9 μM in THF), following excitation at 340 nm.

14 and **16** was rather low, it can be concluded that compounds **10** and **12** would not be sufficiently sensitive to act as fluorescent probes for the measurement of radicals and reducing species. The nature and position of the substituents on the coumarin ring are well recognized to have a profound influence on the resulting fluorescence emission.[54] In particular, substitution of the coumarin ring with electron-donating groups in the 7-position and an electron acceptor in the 3-position ("push−pull" systems[55]) are known to generate strongly fluorescent species as they undergo intramolecular charge transfer.[56] Accordingly, the fluorescence intensity exhibited by diamagnetic alkoxyamines **15** and **17** (substituted with hydroxy and diethylamine groups, respectively, Figures 3 and 5) was substantially greater than that displayed by methoxyamines **14** and **16**. This observation was evident from quantum yields (Φ_F, Table 1) of 0.55 and 0.95 obtained for compounds **15** and **17**, respectively. The corresponding nitroxides **11** and **13** showed significant fluorescence suppression with measured quantum yields of 0.02 and 0.2. As the triazole does not interfere with the fluorescence suppression process, the CuAAC reaction is a very useful tool as it allows a vast array of profluorescent nitroxides possessing a robust and noncleavable covalent linker to be easily synthesized. We believe

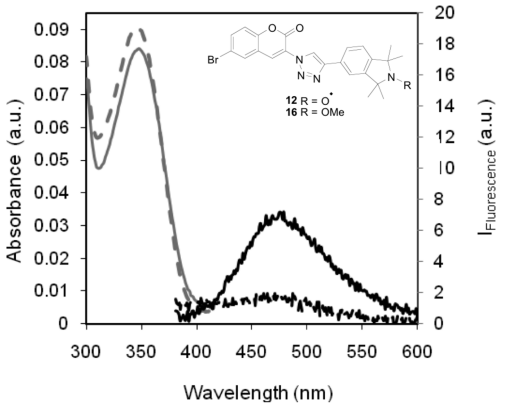

Figure 4. UV/vis spectra for compounds **12** (gray ---, 5.2 μM in THF) and **16** (gray —, 5.05 μM in THF) and fluorescence emission spectra for compounds **12** (black ---, 5.2 μM in THF) and **16** (black —, 5.05 μM in THF), following excitation at 340 nm.

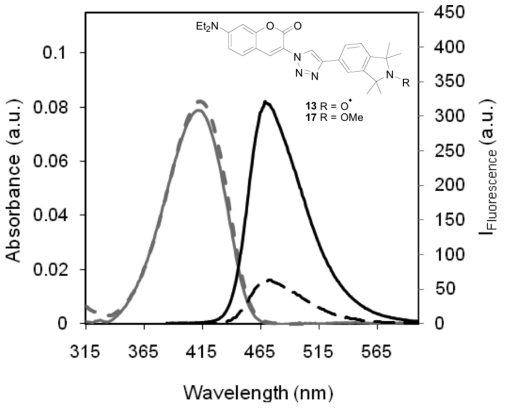

Figure 5. UV/vis spectra for compounds **13** (gray ---, 4.25 μM in THF) and **17** (gray —, 3.73 μM in THF) and fluorescence emission spectra for compounds **13** (black ---, 4.25 μM in THF) and **17** (black —, 3.73 μM in THF), following excitation at 375 nm.

Table 1. Extinction Coefficients and Quantum Yields of Fluorescence for Synthesized Nitroxide Probes 10−13 and Their Methoxyamine Analogues 14−17

entry	product	extinction coefficient[a] (M^{-1} cm^{-1})	quantum yield (Φ_F)[c]
1	**10**	17224	0.001
2	**11**	19000	0.02
3	**12**	16861	0.005
4	**13**	17519[b]	0.2[d]
5	**14**	17400	0.01
6	**15**	19972	0.55
7	**16**	15969	0.015
8	**17**	20057[b]	0.95[d]

[a] Measured in THF at 340 nm. [b] Measured in THF at 375 nm. [c] Measured in THF using anthracene as standard (340 nm excitation). [d] Measured in THF using perylene as standard (375 nm excitation).

the observed fluorescence quenching in solution to result from intramolecular communication between the nitroxide and the fluorophore via the triazole linker. If intermolecular interactions

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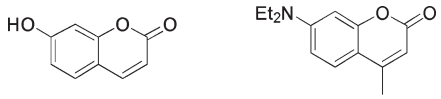
Chart 2. Parent Coumarin Structures 18 and 19



18 **19**

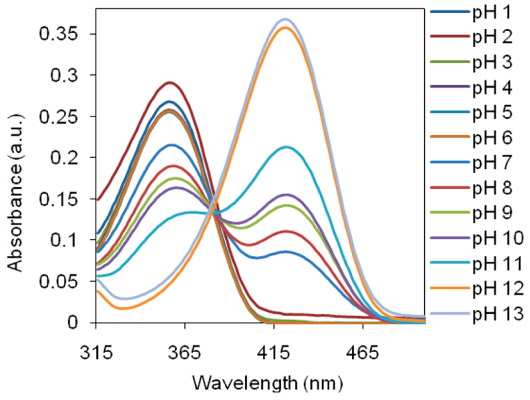


Figure 6. Absorbance spectrum of compound **15** (at 2.3 μM) at pH 1−13.

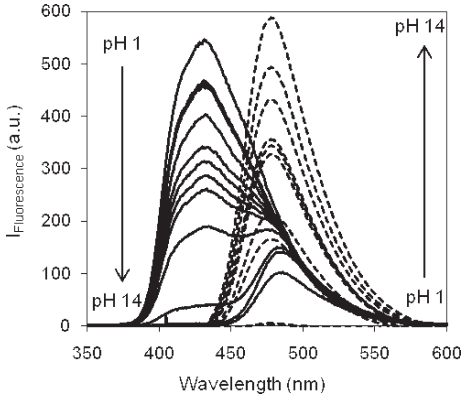


Figure 7. Fluorescence emission of compound **15** (at 2.3 μM) at pH 1−14. λ_{ex} = 340 nm (—) and λ_{ex} = 400 nm (---).

were involved in fluorescence suppression at low concentrations, the signals in the ^{1}H NMR spectrum arising from the coumarin fluorophore would presumably be broadened to the same extent as the hydrogen atoms surrounding the nitroxide moiety, and this was not observed (see the Supporting Information). The Stokes shift for the unsubstituted coumarins **10** and **14** (108 nm) and the bromocoumarins **12** and **16** (117 nm) were larger than those of the hydroxycoumarins **11** and **15** (60 nm) and diethylaminocoumarins **13** and **17** (49 nm). Hence, the presence of electron-donating groups on the coumarin ring did not cause a bathochromic shift in the observed fluorescence maxima in THF with the maxima for the hydroxy substituted compounds **11** and **15** occurring at 427 nm and the maxima for the unsubstituted coumarins **10** and **14** observed at 472 nm.

The absorbance and fluorescence emission of methoxyamine **15** was also found to be strongly influenced by the pH in aqueous solution. Under basic conditions, phenol deprotonation (the pK_a of 7-hydroxycoumarin in water is 6.18)[57] caused a bathochromic

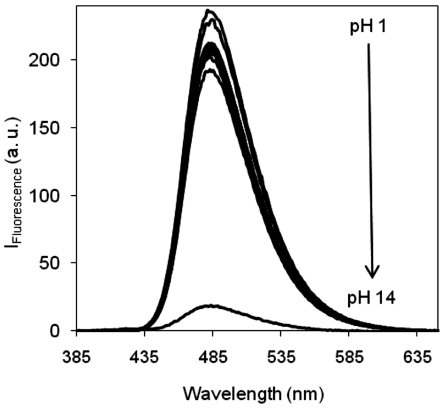


Figure 8. Fluorescence emission (λ_{ex} = 375 nm) of compound **13** (at 2.1 μM) at pH 1−14.

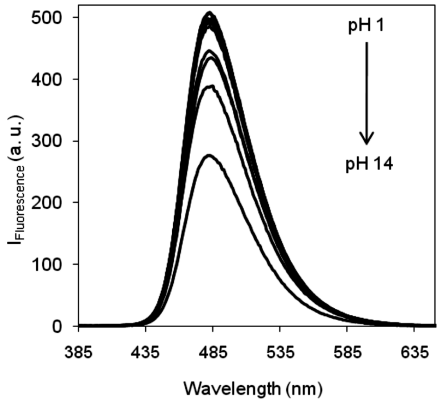


Figure 9. Fluorescence emission (λ_{ex} = 375 nm) of compound **17** (at 2.1 μM) at pH 1−14.

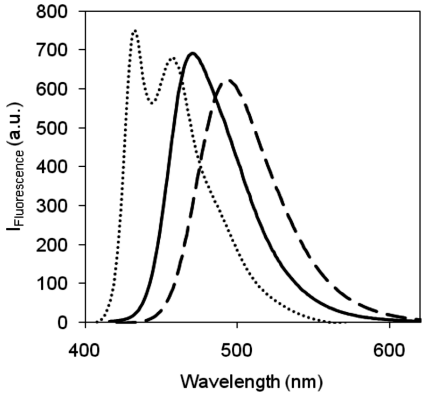


Figure 10. Fluorescence emission of compound **17** (2.6 μM) in cyclohexane (···), THF (—), and DMSO (---) following excitation at 402, 410, and 416 nm, respectively.

shift in the absorbance spectrum (Figure 6). A corresponding shift to longer wavelength in the fluorescence emission with an associated decrease in fluorescence intensity (resulting from the formation of the phenolate) was observed under basic conditions following excitation at 340 nm (Figure 7). Excitation at 400 nm showed a similar bathochromic shift in fluorescence emission for **15** with a concomitant increase in fluorescence intensity with increasing basicity (Figure 7). An analogous change in the

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fluorescence emission of the corresponding nitroxide **11** at the same concentration was difficult to detect because of its low residual fluorescence; however, the same bathochromic shift was detected in the absorbance spectrum. Similar trends in pH-dependent fluorescence have been previously observed for 7-hydroxycoumarins[58,59] with reports of maximal fluorescence at pH values above the pK_a of the phenolic proton.[60] In comparison, the fluorescence emission of the diethylamino-substituted nitroxide **13** and its methoxyamine **17** was fairly constant across a range of pH values (Figures 8 and 9). A considerable decrease in fluorescence intensity was detected at pH 14 for both **13** and **17**; however, this can be explained by significant precipitation of the compounds from the aqueous solution. The effect of solvent polarity on the fluorescence emission of methoxyamine **17** was also examined. A slight shift (~20 nm) in the absorbance spectrum to longer wavelength was observed with increasing solvent polarity for **17** with a corresponding shift in the fluorescence emission (Figure 10). The electronic spectra of coumarins are well-known to be affected by solvent polarity[61] as a change in polarizability of the surrounding medium alters the electronic distribution which results in an increase of dipole moments from the ground state into the excited state.[62]

CONCLUSIONS

The use of the copper-catalyzed azide−alkyne 1,3-dipolar cycloaddition reaction was explored as a conjugation approach to form a new type of profluorescent nitroxide bearing a triazole linker between the nitroxide and the fluorophore. The reaction of substituted coumarin azides **6**−**9** with alkyne nitroxide **4** under standard CuAAC conditions gave the desired triazole-containing compounds **10**−**13** in good to excellent yield (60−90%). The presence of the triazole subunit in compound **10** was confirmed by X-ray crystallography. The methoxyamine derivatives **14**−**17** were prepared from nitroxides **10**−**13** in high yield (79−90%) by reaction with methyl radicals formed using Fenton chemistry. The fluorescence emission intensity exhibited by diamagnetic alkoxyamines **15** and **17** (substituted with hydroxy and diethylamine groups, respectively) was substantially greater than their corresponding nitroxides **11** and **13**, which showed significant fluorescence suppression. This result indicates that the triazole subunit is an excellent linker as it allows the quenching effect to be transferred from the nitroxide to the fluorophore. The examples where the nitroxide did not strongly suppress the fluorescence were the unsubstituted coumarin nitroxide **10** and 6-bromocoumarin nitroxide **12**, where the inherent quantum yields for the corresponding methoxyamine adducts **14** and **16** were low, and therefore, other factors are interfering with the normal fluorescence behavior. The absorbance and fluorescence emission of the hydroxyl-substituted nitroxide **11** and its corresponding methoxyamine adduct **15** were shown to be strongly influenced by pH. Formation of the phenolate of **15** in basic solutions caused a bathochromic shift in both absorbance and fluorescence emission with maximal fluorescence emission in basic solutions. A shift to longer wavelength in the absorbance and fluorescence spectra of methoxyamine derivative **17** was also observed with increasing solvent polarity. This work has demonstrated the value of the copper-catalyzed azide−alkyne 1,3-dipolar cycloaddition reaction to easily prepare novel profluorescent nitroxides in high yield and shown that the presence of the triazole unit does not interfere with the fluorescence quenching process of the nitroxide moiety. This suggests that this approach can be used to conjugatively join any azide-bearing fluorophore to a nitroxide moiety to rapidly generate a diverse array of profluorescent systems.

EXPERIMENTAL SECTION

General Methods. All air-sensitive reactions were carried out under an atmosphere of ultrahigh purity argon. 5-Ethynyl-1,1,3,3-tetramethylisoindolin-2-yloxyl **4**, benzyl azide, 3-azidochromen-2-one **6**, 3-azido-7-hydroxychromen-2-one **7**, 3-azido-6-bromochromen-2-one **8**, and 3-azido-7-diethylaminochromen-2-one **9** were synthesized using established literature procedures, and the obtained NMR data matched that previously reported.[30,46,63] All other reagents were purchased from commercial suppliers and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz spectrometer and referenced to the relevant solvent peak. ESI-high-resolution mass spectra were obtained using a QTOF LC mass spectrometer which utilized electrospray ionization (recorded in the positive mode) with a methanol mobile phase. EI-high resolution mass spectra were recorded on a double-focusing magnetic sector mass spectrometer in the positive mode. Fourier transform infrared (FTIR) spectra were recorded on a Fourier transform infrared spectrometer equipped with a DTGS TEC detector and an ATR objective. Melting points were measured on a variable-temperature apparatus by the capillary method and are uncorrected. Analytical HPLC was carried out on a HPLC system using a Prep-C18 scalar column (4.6 × 150 mm, 10 μm) with a flow rate of 1 mL/min in 70% MeOH/30% H_2O. X-ray data were collected at 173(2) K on a diffractometer using Mo Kα radiation generated from a sealed tube. Data reduction was performed using CrysAlis RED. A multiscan empirical absorption correction was applied using spherical harmonics, implemented in the SCALE3 ABSPACK scaling algorithm, within CrysAlis RED, and subsequent computations were carried out using the WinGX-32[64] graphical user interface. The structure was solved by direct methods using SIR97[65] and refined with SHELXL-97.[66] Full occupancy non-hydrogen atoms were refined with anisotropic thermal parameters. C−H hydrogen atoms were included in idealized positions, and a riding model was used for their refinement. The refinement residuals are defined as $R1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ for $F_o > 2\sigma(F_o)$ and $wR2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_c^2)^2]\}^{1/2}$ where $w = 1/[\sigma^2(F_o^2) + (0.25P)^2 + 0P]$, $P = (F_o^2 + 2F_c^2)/3$.

*5-(1-Benzyl-1H-1,2,3-triazol-4-yl)-1,1,3,3-tetramethylisoindolin-2-yloxyl (**5**).* A solution of 5-ethynyl-1,1,3,3-tetramethylisoindolin-2-yloxyl **4** (50 mg, 0.23 mmol) and benzyl azide (31 mg, 0.23 mmol) in ethanol (3 mL) was diluted with water (2 mL). Sodium ascorbate (1 M solution in water, 46 μL, 0.046 mmol, 20 mol %) and copper(II) sulfate pentahydrate (1 M solution in water, 23 μL, 0.023 mmol, 10 mol %) were added. After being stirred at room temperature for 16 h, the solution was concentrated in vacuo. The resulting residue was purified by column chromatography (R_f = 0.3, EtOAc/DCM, 1:20) to afford compound **5** as a pale yellow solid (79 mg, 99%): mp 111−113 °C; IR (ATR) ν_{max} 2928 and 2975 (alkyl CH_3), 1453 and 1484 (aryl CC), 1432 (N−O$^\bullet$), 822 cm^{-1} (=C-*H*); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (br s, 1H, $H_{triazole}$), 7.36−7.51 (m, 5H), 5.68 (s, 2H) (not all signals were observable due to paramagnetic broadening by the nitroxide radical); LRMS (EI) *m*/*z* 347 (100, M^+); HRMS (EI) calcd for $C_{21}H_{23}N_4O$ 347.1872, found 347.1871. The IR data obtained for compound **5** was consistent with typical IR resonances expected for isoindoline nitroxides.[67]

General Procedure for the Copper-Catalyzed 1,3-Dipolar Cycloaddition Reaction between 3-Azidocoumarins (6−9) and 5-Ethynyl-1,1,3,3-tetramethylisoindolin-2-yloxyl (4). A mixture of 5-ethynyl-1,1,3,3-tetramethylisoindolin-2-yloxyl **4** (1 equiv) and 3-azidocoumarin **6**−**9** (1.1 equiv) was made up in ethanol and water

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(1: 1, v/v, 20 mL). To this solution were added copper(II) sulfate pentahydrate (5 mol %) and sodium ascorbate (10 mol %). The reaction was maintained with stirring for 16 h at room temperature in the absence of light and the resulting precipitate collected by vacuum filtration and washed with water.

*3-(4-(1,1,3,3-Tetramethylisoindolin-2-yloxyl)-1H-1,2,3-triazol-1-yl)-2H-chromen-2-one (**10**).* The title compound was prepared from **4** (47 mg, 0.25 mmol) according to general procedure described above and purified using silica gel chromatography (R_f = 0.22, EtOAc/*n*-hexanes, 1:1) to afford **10** as a brown solid (86 mg, 86%): mp 200−201 °C; IR (ATR) ν_{max} 2931 and 2977 (alkyl CH_3), 1733 (C═O), 1610 (*C*═*C*-COO), 1463 and 1484 (aryl CC), 1432 (N−O•), 1120 (CO), 801 cm^{-1} (=*CH*); ^1H NMR (400 MHz, DMSO-d_6) δ 9.07 (s, 1H), 8.82 (s, 1H), 7.98 (s, 1H), 7.75 (s, 1H), 7.58 (s, 1H), 7.49 (s, 1H) (not all signals were observable due to paramagnetic broadening by the nitroxide radical); LRMS (ES) *m*/*z* 424 (100, MNa^+); HRMS (EI) calcd for $C_{23}H_{21}N_4O_3Na$ [MNa^+] 424.1506, found 424.1508.

*7-Hydroxy-3-(4-(1,1,3,3-tetramethylisoindolin-2-yloxyl)-1H-1,2,3-triazol-1-yl)-2H-chromen-2-one (**11**).* The title compound was prepared from **4** (50 mg, 0.25 mmol) according to the general procedure described above and purified using silica gel chromatography (R_f = 0.10, EtOAc/*n*-hexanes, 1:1) to afford **11** as an orange solid (94 mg, 90%): mp 249−250 °C; IR (ATR) ν_{max} 3150 (O−H), 2930 and 2977 (alkyl CH_3), 1713 (C═O), 1606 (*C*═CCOO), 1460 and 1488 (aryl CC), 1416 (NO•), 1231 (OH), 1118 (CO), 853 cm^{-1} (=*CH*); ^1H NMR (400 MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.69 (s, 1H), 7.79 (s, 1H), 6.99−6.85 (m, 2H) (not all signals were observable due to paramagnetic broadening by the nitroxide radical); LRMS (ES) *m*/*z* 440 (45, MNa^+); HRMS (EI) calcd for $C_{23}H_{21}N_4O_4Na$ [MNa^+] 440.1455, found 440.1459.

*6-Bromo-3-(4-(1,1,3,3-tetramethylisoindolin-2-yloxyl)-1H-1,2,3-triazol-1-yl)-2H-chromen-2-one (**12**).* The title compound was prepared from **4** (40 mg, 0.15 mmol) according to the general procedure described above and purified using silica gel chromatography (R_f = 0.33, EtOAc/*n*-hexanes, 1:1) to afford **12** as an orange solid (61 mg, 84%): mp 246−248 °C; IR (ATR) ν_{max} 2927 and 2978 (alkyl CH_3) 1730 (C═O), 1602 (*C*═CCOO), 1462 and 1481 (aryl CC), 1418 (NO•), 1119 (CO), 815 (=*CH*), 659 cm^{-1} (CBr); ^1H NMR (400 MHz, DMSO-d_6) δ 9.11 (s, 1H), 8.77 (s, 1H), 8.24 (s, 1H), 7.89 (s, 1H), 7.52 (s, 1H) (not all signals were observable due to paramagnetic broadening by the nitroxide radical); LRMS (ES) *m*/*z* 480/482 (25, MH^+), 502/504 (25, MNa^+]; HRMS (EI) calcd for $C_{23}H_{20}N_4O_3{}^{79}BrNa$ [MNa^+] 502.0616, found 502.0604; calcd for $C_{23}H_{20}N_4O_3{}^{81}BrNa$ [MNa^+] 504.0596, found 504.0624.

*7-(Diethylamino)-3-(4-(1,1,3,3-tetramethylisoindolin-2-yloxyl)-1H-1,2,3-triazol-1-yl)-2H-chromen-2-one (**13**).* The title compound was prepared from **4** (110 mg, 0.43 mmol) according to the general procedure described above and purified using silica gel chromatography (R_f = 0.20, EtOAc/*n*-hexanes, 1:1) to afford **13** as a yellow solid (122 mg, 60%): mp 238−239 °C; IR (ATR) ν_{max} 2929 and 2978 (alkyl CH_3) 1716 (C═O), 1595 (*C*═CCOO), 1430 and 1439 (aryl CC), 1421 (NO•), 1131 (CO), 809 cm^{-1} (=*CH*); ^1H NMR (400 MHz, DMSO-d_6) δ 8.96 (s, 1H), 8.55 (s, 1H), 7.66 (s, 1H), 6.85 (s, 1H), 6.71 (s, 1H), 3.49 (s, 4H), 1.16 (s, 6H) (not all signals were observable due to paramagnetic broadening by the nitroxide radical); LRMS (ES) *m*/*z* 473 (65, MH^+), 495 (100, MNa^+); HRMS (EI) calcd for $C_{27}H_{31}N_5O_3$ [MH^+] 473.2421, found 473.2433; HRMS calcd for $C_{27}H_{30}N_5O_3Na$ [MNa^+] 495.2241, found 495.2255.

General Procedure for the Synthesis of Methoxyamines 14−17. To a solution of nitroxide **10**−**13** (1.0 equiv) in DMSO (3 mL) were added $FeSO_4 \cdot 7H_2O$ (2.0 equiv) and H_2O_2 (30% aqueous solution, 50 μL). The reaction was maintained with stirring for 30 min at room temperature under an atmosphere of argon. Water (10 mL) was added, and the resulting mixture was extracted with diethyl ether (3 × 10 mL). The combined organic layers were dried (anhydrous Na_2SO_4) and concentrated in vacuo.

*3-(4-(2-Methoxy-1,1,3,3-tetramethylisoindolin-5-yl)-1H-1,2,3-triazol-1-yl)-2H-chromen-2-one (**14**).* The title compound was prepared from **10** (21 mg, 0.05 mmol) according to the general procedure described above and purified using silica gel chromatography (R_f = 0.57, EtOAc/*n*-hexanes, 1:1) to afford **14** as a yellow solid (18 mg, 82%): mp 170−172 °C; IR (ATR) ν_{max} 2930 and 2972 (alkyl CH_3), 1726 (C═O), 1607 (*C*═CCOO), 1464 and 1488 (aryl CC), 1167 (CO), 1047 (NOC), 810 cm^{-1} (=*CH*); ^1H NMR (400 MHz, DMSO-d_6) δ 9.10 (s, 1H), 8.80 (s, 1H), 7.97 (dd, *J* = 7.6, 0.8 Hz, 1H), 7.87 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.82 (s, 1H), 7.71−7.79 (m, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.48 (t, *J* = 7.2, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 3.73 (s, 3H), 1.42 (s, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.9, 152.5, 146.9, 145.6, 144.9, 135.0, 133.0, 129.6, 129.2, 125.4, 124.7, 123.1, 122.2, 121.9, 118.8, 118.2, 116.3, 66.7, 66.6, 65.0; LRMS (ES): *m*/*z* 417 (70, MH^+); HRMS (ES) calcd for $C_{24}H_{25}N_4O_3$ [MH^+] 417.1921, found 417.1917.

*7-Hydroxy-3-(4-(2-methoxy-1,1,3,3-tetramethylisoindolin-2-yloxyl)-1H-1,2,3-triazol-1-yl)-2H-chromen-2-one (**15**).* The title compound was prepared from **11** (24 mg, 0.06 mmol) according to the general procedure described above and purified using silica gel chromatography (R_f = 0.38, EtOAc/*n*-hexanes, 1:1) to afford **15** as a yellow solid (22 mg, 90%): mp 270−272 °C; IR (ATR) ν_{max} 801 (=*CH*), 1049 (NOC), 1170 (CO), 1237 (OH), 1445 and 1461 (aryl CC), 1605 (*C*═CCOO), 1702 (C═O), 2930 and 2977 (alkyl CH_3), 3150 cm^{-1} (OH); ^1H NMR (400 MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.66 (s, 1H), 7.85 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.78−7.81 (m, 2H), 7.31 (d, *J* = 8.0 Hz, 1H), 6.92 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.87 (s, 1H), 3.73 (s, 3H), 1.42 (s, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.8, 155.2, 147.2, 146.0, 145.3, 137.0, 131.5, 129.8, 125.2, 122.7, 122.4, 119.6, 119.2, 114.9, 110.7, 102.7, 67.2, 67.1, 65.5; LRMS (ES): *m*/*z* 433 (100, MH^+), 455 (53, MNa^+); HRMS (ES) calcd for $C_{24}H_{25}N_4O_4$ [MH^+] 433.1870, found 433.1874; HRMS calcd for $C_{24}H_{24}N_4O_4Na$ [MNa^+] 455.1695, found 455.1690.

*6-Bromo-3-(4-(2-methoxy-1,1,3,3-tetramethylisoindolin-2-yloxyl)-1H-1,2,3-triazol-1-yl)-2H-chromen-2-one (**16**).* The title compound was prepared from **12** (26 mg, 0.05 mmol) according to the general procedure described above and purified using silica gel chromatography (R_f = 0.65, EtOAc/*n*-hexanes, 1: 1) to afford **16** as a white solid (21 mg, 78%): mp 254−256 °C; IR (ATR) ν_{max} 664 (CBr), 809 (=*CH*), 1070 (NOC), 1167 (CO), 1449 and 1469 (aryl CC), 1709 (C═O), 2927 and 2978 cm^{-1} (alkyl CH_3); ^1H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1H), 8.75 (s, 1H), 8.23 (d, *J* = 2.4 Hz, 1H), 7.85−7.92 (m, 3H), 7.56 (d, *J* = 8.8 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 3.74 (s, 3H), 1.42 (s, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.9, 152.0, 147.4, 146.1, 145.5, 135.6, 133.5, 131.9, 129.6, 125.2, 124.5, 122.7, 122.3, 120.7, 119.3, 119.1, 117.4, 67.2, 67.1, 65.5; LRMS (ES) *m*/*z* 495/497 (100, MH^+); HRMS (ES) calcd for $C_{24}H_{24}N_4O_3{}^{81}Br$ [MH^+] 497.1011, found 497.1009.

*7-(Diethylamino)-3-(4-(2-methoxy-1,1,3,3-tetramethylisoindolin-2-yloxyl)-1H-1,2,3-triazol-1-yl)-2H-chromen-2-one (**17**).* The title compound was prepared from **13** (20 mg, 0.04 mmol) according to the general procedure described above and purified using silica gel chromatography (R_f = 0.57, EtOAc/*n*-hexanes, 1: 1) to afford **17** as a yellow solid (17 mg, 84%): mp 228−229 °C; IR (ATR) ν_{max} 808 (=*CH*), 1053 (NOC), 1191 (CO), 1437 and 1458 (aryl CC), 1606 (*C*═CCOO), 1729 (C═O), 2926 and 2983 cm^{-1} (alkyl CH_3); ^1H NMR (400 MHz, $CDCl_3$) δ 8.83 (s, 1H), 8.49 (s, 1H), 7.79 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.70 (s, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.20 (d, *J* = 8.0 Hz, 1H), 6.71 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.60 (d, *J* = 2.0 Hz, 1H), 3.82 (s, 3H), 3.49 (q, *J* = 7.2 Hz, 4H), 1.4−1.65 (m, 12H), 1.28 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.0, 155.8, 151.6, 147.8, 146.0, 145.4, 134.5, 130.0, 129.6, 125.1, 122.0, 120.2, 119.1, 117.0, 110.1, 107.2, 97.0, 67.2, 67.1, 65.5, 45.0, 12.4; LRMS (ES) *m*/*z* 488 (10, MH^+), 510 (15, MNa^+); HRMS (ES) calcd for $C_{28}H_{33}N_5O_3Na$ 510.2476, found. 510.2476.

Quantum Yield and Extinction Coefficient Calculations. Quantum yield efficiencies of fluorescence for compounds **10**−**17** were obtained from measurements at five different concentrations in THF using the following equation

$$\Phi_{\text{F sample}} = \Phi_{\text{F standard}}(\text{Abs}_{\text{standard}}/\text{Abs}_{\text{sample}})(\Sigma[\text{F}_{\text{sample}}]/\Sigma[\text{F}_{\text{standard}}])(n^2{}_{\text{sample}}/n^2{}_{\text{standard}})$$

where Abs and F denote the absorbance and fluorescence intensity, respectively, $\Sigma[\text{F}]$ denotes the peak area of the fluorescence spectra (calculated by summation of the fluorescence intensity), and n denotes the refractive index of the solvent. Anthracene (Φ_F = 0.36 in cyclohexane) and perylene (Φ_F = 0.94 in cyclohexane) were used as standards.[68] Extinction coefficients were calculated from the obtained absorbance spectra.

pH Experiments. Solutions of 7-hydroxy-3-(4-(1,1,3,3-tetramethylisoindolin-2-yloxyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (**11**, 0.024 mM), 7-hydroxy-3-(4-(2-methoxy-1,1,3,3-tetramethylisoindolin-2-yloxyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (**15**, 0.023 mM), 7-(diethylamino)-3-(4-(1,1,3,3-tetramethylisoindolin-2-yloxyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (**13**, 0.021 mM), and 7-(diethylamino)-3-(4-(2-methoxy-1,1,3,3-tetramethylisoindolin-2-yloxyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (**17**, 0.021 mM) were prepared in THF. Aqueous solutions ranging from pH 1 to 6 were prepared by diluting 10 M HCl with deionized water. Aqueous solutions ranging from pH 8 to 14 were prepared by diluting 10 M NaOH with deionized water. Deionized water was used for the solution corresponding to pH 7. Solutions of compounds **11**, **15**, **13**, and **17** at pH 1−14 were prepared by adding 300 μL of the above solutions to aqueous solutions (2.7 mL) of either HCl (at pH 1−6), NaOH (at pH 8−14), or water (pH 7). The resulting solutions were mixed and their absorbance and fluorescence spectra measured.

Crystal data for ***10***: formula $C_{23}H_{21}N_4O_3$, M = 401.44, triclinic, $P\bar{1}$, a = 5.8079(2) Å, b = 12.9919(11) Å, c = 14.7368(9) Å, α = 112.916(7)°, β = 99.112(4)°, γ = 95.001(5)°, V = 997.66(13) Å^3, D_c = 1.336 g cm^{-3}, Z = 2, crystal size 0.30 × 0.26 × 0.12 mm, yellow prism, temperature 173(2) K, λ(Mo Kα) = 0.71073, T(multiscan)$_{min,max}$ = 0.998, 1, $2\theta_{max}$ = 57.52, *hkl* range −7 to 7, −14 to 17, −19 to 19, N = 6999, N_{ind} = 4461 (R_{merge} = 0.018), N_{obs} = 2942 ($I > 2\sigma(I)$), N_{var} = 275, residuals $R1(F, 2\sigma)$ = 0.036, $wR2(F^2$, all) = 0.062, GoF(all) = 1.032, $\Delta\rho_{min,max}$ = −0.187, 0.224 e Å^{-3}. CCDC contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

■ ASSOCIATED CONTENT

Supporting Information. (1) ^{1}H NMR spectra of compounds **5**, **10**−**17**; (2) ^{13}C NMR spectra of compounds **14**−**17**; (3) HPLC chromatograms for compounds **5** and **10**−**13**; (4) X-ray crystal structure of compound **10** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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