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Synthesis of Quinoline Dicarboxylic Esters as Biocompatible Fluorescent Tags

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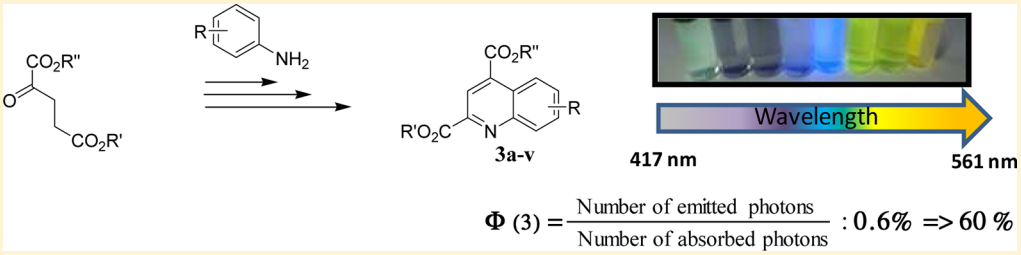
Supporting Information



ABSTRACT: A series of dicarboxylic quinoline derivatives bearing electron-releasing or -withdrawing substituents have been synthesized using mono- or/and biphasic methodologies. By controlling the regioselectivity of addition into our electrophilic intermediate, we also characterized by which mechanism the Doebner−Miller cyclization step occurred. As anticipated, electron-releasing substituents induce a red shift of the low-energy absorption allowing excitation in the visible region. In addition, by playing on the strength and position of the electron-releasing substituents, chromophore having interesting fluorescent properties such as large Stoke shifts, good fluorescent quantum yields, emission in the visible green-yellow region and reasonable two-photon absorption in the NIR region have been obtained. These small-size fluorophores, which can be made water-soluble and have been shown to be non-toxic, can be hetero- and/or polyfunctionalized and thus represent promising key units for fluorescence-based physiological experiments with low background interactions.

INTRODUCTION

Fluorescent probes and among them quinoline-based structures play a crucial role in bioimaging studies.[1,2] The use of fluorescent dyes easily functionalizable and compatible with biological experiments represents a key feature for physiological research.[3,4] Recent elaborated studies used coumarin or quinoline scaffolds as central caged or fluorescent chemical cores to investigate small endogenous molecule functions.[5−8] These experiments remain limited and are highly dependent on both intrinsic biological activity and fluorescent properties of fluorophore (low tissue penetration, probe biological side effects, decrease of natural function of studied molecules when labeled, etc.). The use of small and biological inactive fluorophore coupled to the molecule of interest appears crucial to observe both its localization and its function in physiological conditions.[9,10]

To be efficient, these fluorescent probes should be biocompatible (i.e., have low side effects at typical concentrations used for bioapplications) and have optimal fluorescent properties. In this context, quinolines appear quite attractive due to their relative synthetic versatility. The quinoline heterocyclic scaffold allows facile introduction of substituents and subsequent tuning of physicochemical properties of molecules (fluorescence, solubility, etc.). Synthesis of substituted quinolines have been reported using various mechanisms and strategies: Conrad−Limpach−Knorr,[11,12] Skraub−Doebner−Von Miller,[13−15] Frielaender,[16,17] halogen mediated,[18] copper catalyzed[19] or miscellaneous.[14,20] Classical Skraup procedure involves the use of a large amount of sulfuric acid at high temperature. Other methods are not fully satisfactory with regards to yields, reaction conditions, and operational simplicity. The Doebner−Miller method allows mild conditions, but yields remain low and reactions involve tedious isolation procedures from complex reaction mixtures.[13−15] In this context, we present herein an improved methodology that allows generating a large range of derivatives which are liable for further derivatization. Their absorption and fluorescent properties, as well as biocompatibility, have been studied in order to identify the most promising structures as fluorescent tags.

RESULTS AND DISCUSSION

Synthesis. Here, we report the Doebner−Miller synthesis and fluorescent properties of a novel series of quinoline dicarboxylic esters generated in improved synthetic conditions and better yields compare with those previously described.[21−23] To synthetize our series of compounds, we selected two

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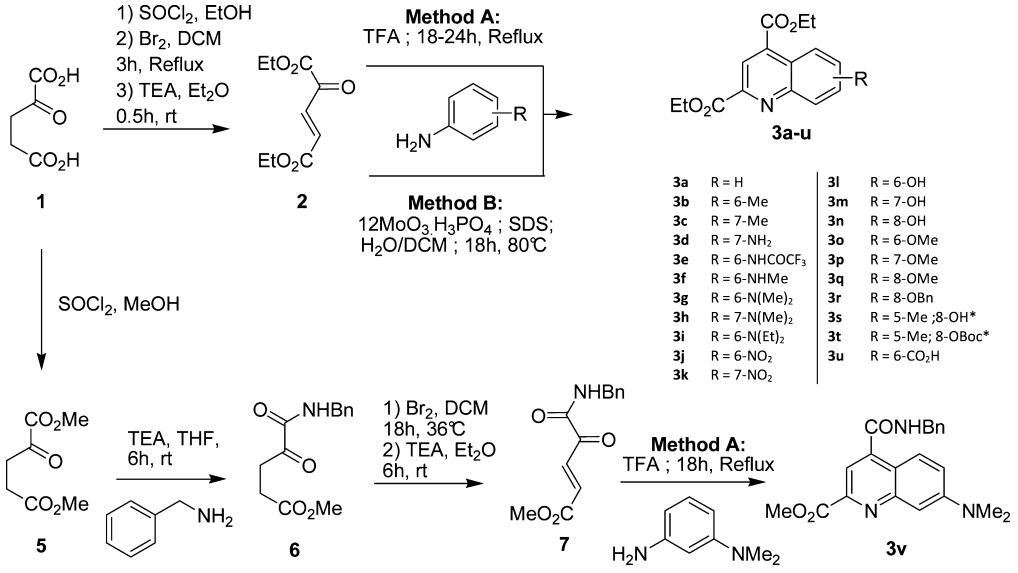
Scheme 1. Synthetic Methodology Used for Quinoline Diester (3) Synthesis Using Monophasic (A) or Biphasic (B) Methods



*Specific conditions used for these compounds.

Table 1. Yields of Quinoline Cyclization Step

		yields[a] (%)				yields[a] (%)	
entry	R	method A	method B	entry	R	method A	method B
3a	H	47	11	**3l**	6-OH	13	34
3b	6-Me	55	32	**3m**	7-OH	8	20
3c	7-Me	20	48	**3n**	8-OH	30	10
3d	6-NH_2	**3e**	5	**3o**	6-OMe	39	28
3e	6-$NHCOCF_3$	8	–	**3p**	7-OMe	21	33
3f	6-NHMe	15	–	**3q**	8-OMe	46	13
3g	6-$N(Me)_2$	25	–	**3r**	8-OBn	30	1
3h	7-$N(Me)_2$	23	–	**3s**	5-Me; 8-OH	60	–
3i	6-$N(Et)_2$	15	–	**3t**	5-Me; 8-OBoc	23	traces
3j	6-NO_2	33	–	**3u**	6-CO_2H	32	–
3k	7-NO_2	nr	nr				

[a]nr, no reaction; dashes, not experimented.

synthetic conditions, using either classical TFA as Brønsted acid, or biphasic phosphomolybdic acid and sodium dodecyl sulfate (SDS) as surfactant (Scheme 1).

Ketoglutaric acid is first diesterified in the presence of thionyl chloride in ethanol. The unsaturated ester **2** is then generated in two successive steps, alpha bromination of ketone followed by elimination of HBr. The three steps give only the *E*-isomer of **2** in very good global yield (80%). Intermediate **2** is then condensed with various anilines using classical method A or/and biphasic method B leading to the desired quinolines **3**.

As shown in Table 1, yields of the condensation step remained low and appeared to be highly dependent on the substituent position present in the aniline. Surprisingly, electron-donating or electron-withdrawing groups did not impact the cyclization. For example, compounds with substituents at position 6, **3j** (R = NO_2), **3o** (R = OMe) or **3u** (R = CO_2H) afforded similar yields, respectively 33, 39 and 32%. On the other hand, the effect of group position is crucial when using Method A and B and highlights that position 7 is notably unfavorable compared to the others (compounds **3l–n** or **3o–q** or **3b,c**). Nevertheless, we could underline that the classical methodology (Method A) is less efficient when aniline is substituted in this position 7 (**3c**, **3m**, **3p**) and yields the attempted product in very low proportion. In contrast, even if reaction with 7-sublttituted aniline remained difficult with both methodologies, method B gives the corresponding product in better yield and with less side product. Indeed, when *m*-aminoaniline was condensed with intermediate **2**, Method B provided the desired product **3d** whereas Method A gave only the side compound **3e**.

Altogether, these results led us to investigate the influence of various parameters involved in the cyclization mechanism (e.g., amine pK_a, regioselectivity). Predicted pK_a values of anilines with SPARC[24,25] confirmed that no correlation could be evidenced between basicity and condensation yield (data not shown). Indeed, starting with anilines having distant pK_a's (R = NO_2 and R = OMe) gave the corresponding quinolines **3j** and **3o** with similar yields (33 and 39%, respectively).

Regarding the cyclization, two different reaction mechanisms have been postulated for the first step in the literature. The aniline may attack either directly the ketone group to form the imine intermediate[26] or through a 1–4 Michael addition leading to the corresponding enolate adduct.[14]

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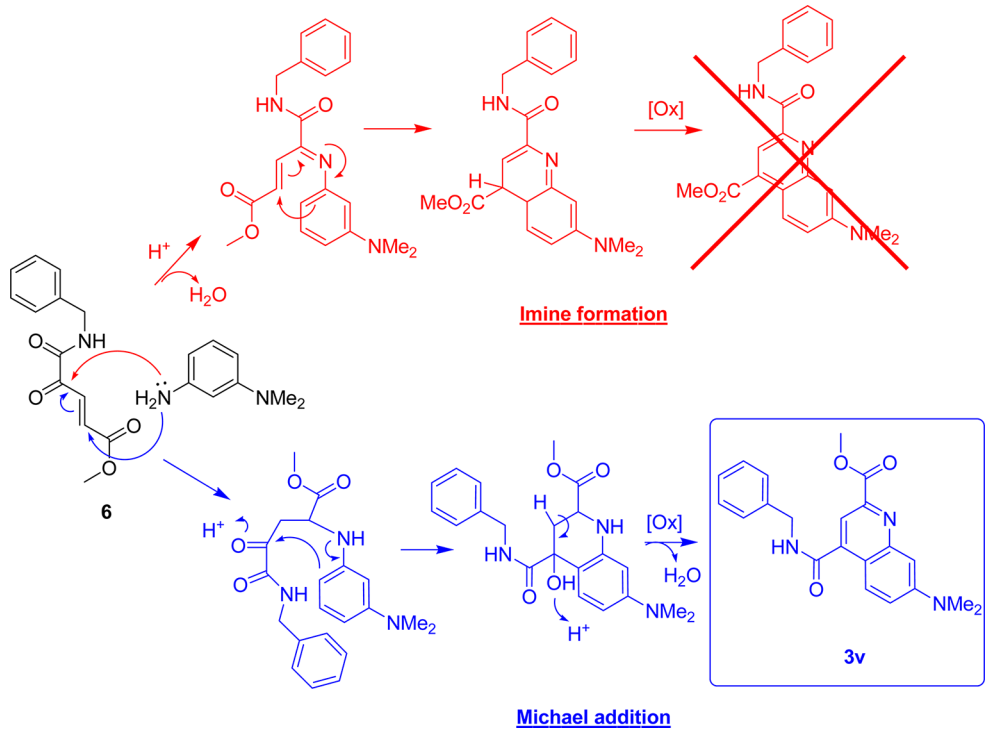
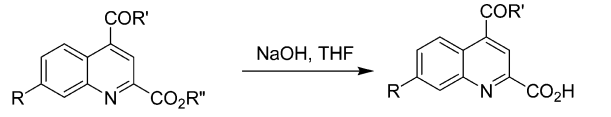
Scheme 2. Cyclization Mechanism of Quinoline Diester



To confirm their propositions, authors succeeded in isolating each key intermediate (imine or enolate). The mechanism appears to be correlated to both electrophilic character of the unsaturated moiety and the nucleophilicity of the amine.[14,26] In order to clarify this question, we decided to replace the α-keto-ester of our electrophilic intermediate by a α-keto benzyl amide **7**. This reactant, when condensed with *m*-*N*,*N* dimethylamino aniline led to a quinoline unsymetrically substituted on the two carbonyl groups (**3v**) (Scheme 2). Surprisingly, while authors previously characterized an imine adduct for similar quinolines, our cyclization is 1−4 Michael addition mediated according to the C4 benzamide position in compound **3v** (Supporting Information). It can be noted that even if the reaction appears difficult and incomplete, no enolate intermediate as expected for our 1−4 Michael addition or, of course, any imine adduct (C2 benzamide attack intermediate) have been isolated.[14]

Biocompatibility. To confirm the potentiality of these structures as a multifunctionalizable and biocompatible scaffold, we performed solubility and cytoxicity assays of two monoacid derivatives **4p** and **4v**. (Scheme 3) These were obtained by basic treatment of compounds **3p** and **3v** with 1 equiv of sodium hydroxide in THF.[23] Regioselectivity of the deprotection was characterized by 1D and 2D NMR (Section 2, Supporting Information). It allows the access to a free acid that may be subsequently functionalized by a reactive group (hydroxyl, amine, thiol or halide) present on the bioactive compounds of interest.[6−8] Compounds **4p** and **4v** exhibited high aqueous solubility up to 50 mM compared to the poor solubility of compounds **3a**−**v**. In addition, no cytotoxic effects were observed even at 1 mM in cell culture.

Scheme 3. Classical and Regioselective Deprotection of Compounds 3p and 3v



3p: R= OMe; R'= -OEt; R''=Et
3v: R= N(Me)2; R'= -NHBn; R''= Me

4p: R= OMe; R'= -OEt
4v: R= N(Me)2; R'= -NHBn

Photophysical Properties. In order to assess the potentialities of newly synthesized quinoline derivatives as fluorescent probes, their photophysical properties were investigated in ethanol or pure water (for water-soluble compounds) solutions. All compounds show significant absorption in UV−visible range and, in some cases, fluorescence emission depending on the nature and position of the substituents on the quinoline ring (Table 2). Compounds **3a**−**c**, **3j** and **3u**, which bear alkyl or electron-withdrawing groups in the sixth (or seventh) position, show a medium intensity absorption band in the UV region and almost no fluorescence. In contrast, diester derivatives **3d**, **3f**−**i**, **3l**−**s** bearing electron-donating (ED) substituents instead show medium intensity absorption band in the near UV region or blue-visible region as a result of the bathochromic shift induced by ED substituents while their fluorescence strongly depends on the ED substituents' strength and positions. When the ED groups are in the sixth or eighth position, significant fluorescence is obtained, whereas the substitution on the seventh position leads to a sizable reduction of the fluorescence quantum yield. Comparison of chromophores **3o**−**r** (as well as **3l**−**n** and **3g**,**h**) indicate that the sixth position substitution leads to the strongest absorption and emission, as clearly exemplified in Figure 1A,B, whereas the eighth position substitution induces a major bathochromic shift of the emission band (which is shifted to the green-yellow region). Replacement of O-alkyl by OH induces a marked hypochromic and slight bathochromic shift of absorption, as well as a red shift and significant reduction of fluorescence except for the eighth position substitution (Table 2). As illustrated in Figure 2C,D,

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Table 2. Photophysical Properties and Two-Photon Absorption of New Quinoline Derivatives

entry	R	λ_{abs}^{max} (nm)	ε^{max} (10^3 M^{-1} cm^{-1})	λ_{em}^{max} (nm)	Stokes shift (cm^{-1})	Φ^a (%)	λ_{TPA}^{max} (nm)	σ_2^{max} (GM)[d]
3a	H	319	3.5	420	7 500	0.6[b]	/	/
3b	6-Me	330	4.7	417	6 300	2.2[b]	/	/
3c	7-Me	321	4.8	422	7 500	1.9[b]	/	/
3d	6-NH_2	421	5.6	537	5 100	40[c]	820	10.5
3e	6-$NHCOCF_3$	346	3.4	436	6 000	34[b]	/	/
3f	6-NHMe	429	11	539	4 800	47[c]	840	11
3g	6-$N(Me)_2$	443	12	564	4 800	59.5[c]	920	12
3h	7-$N(Me)_2$	430	5.0	541	4 800	41[c]	840	13
3i	6-$N(Et)_2$	453	9.5	561	4 200	49[c]	920	20.5
3j	6-NO_2	304	9.3	/	/	/	/	/
3l	6-OH	364	6.4	466	6 000	16[b]	/	/
3m	7-OH	373	1.7	474	5 700	0.7[b]	/	/
3n	8-OH	369	0.7	527	8 100	17[c]	/	/
3o	6-OMe	355	8.2	441	5 500	53[b]	/	/
3p	7-OMe	362	2.8	451	5 500	9.8[b], 9.9[c]	/	/
3q	8-OMe	368	1.4	526	8 200	17[b,c]	/	/
3r	8-OBn	366	1.7	512	7 800	20[b]	/	/
3s	5-Me; 8-OH	369	2.0	528	8 200	26[c]	/	/
3t	5-Me; 8-OBoc	312	2.6	505	12 200	22[c]	/	/
3u	6-CO_2H	316	3.5	/	/	/	/	/
3v	7-$N(Me)_2$	426	2.3	586	6 400	6[c]	/	/
4p[e]	7-OMe	349	3.9	462	5 100	15[c]	/	/
4v[e] (sodium Salt)	7-$N(Me)_2$	403	1.7	603	7 100	1.3[c]	/	/

[a]Fluorescence quantum yield. [b]Standard: quinine bisulfate. [c]Standard: fluorescein. [d]GM = 10^{-50} cm^4 s photon $^{-1}$. [e]In water.

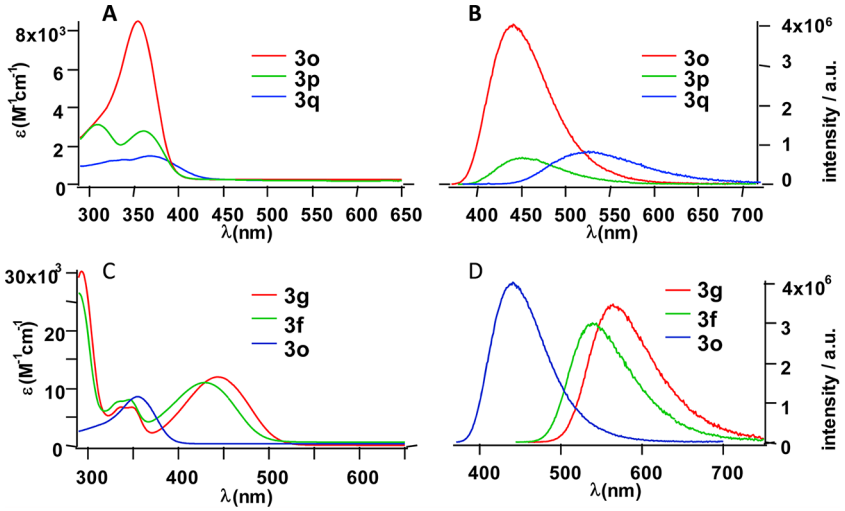

Figure 1. Effect of substitution position: Absorption (A) and emission (B) spectra of quinoline derivatives **3o−q** in ethanol. Effect of substituent donating strength: Absorption (C) and emission (D) spectra of quinoline derivatives **3f**, **3g**, **3o**.

increasing the ED strength of the substituents in the sixth position induces a red-shift of both absorption and emission. The same stands for substitution by ED groups in the seventh position, with additional marked hyperchromic effect and fluorescence enhancement, as indicated by comparison of **3h** and **3p** characteristics (Table 2). As a result, substitution by strong ED groups (such as NR_2) in the seventh position also leads to strong fluorescence. Interestingly, investigation of the fluorescence properties of biocompatible water-soluble derivatives **4p** and **4v** (Table 2) provides evidence that ester-acid quinoline derivatives are indeed promising fluorescent tags for biomolecules. Indeed compound **4p** shows a 50% fluorescence enhancement in water as compared to its diester precursor **3p** in ethanol. In contrast, comparison of **3h**, **3v** (in ethanol) and **4v** (in water) fluorescence reveals that the benzamide connection has deleterious effect on fluorescence.

Hence the ester linkage in the fourth position should be preferred to amide linkage for grafting the new quinoline tags on biomolecules. On the basis of the above results, the newly synthesized diester quinoline derivatives bearing strong ED substituents in the sixth or seventh position were selected as the most promising as fluorescent tags for biomolecules.

Comparisons with structures already described give also information and highlight the potentiality of our structures. For example, when in the second position, the ester of compounds **3o−q** was replaced by a sulfonyl group ($-SO_2Me$), the less functionalizable resulting quinolines showed similar quantum yields of 69 vs 53, 19 vs 10, and 5 vs 17%, respectively, for

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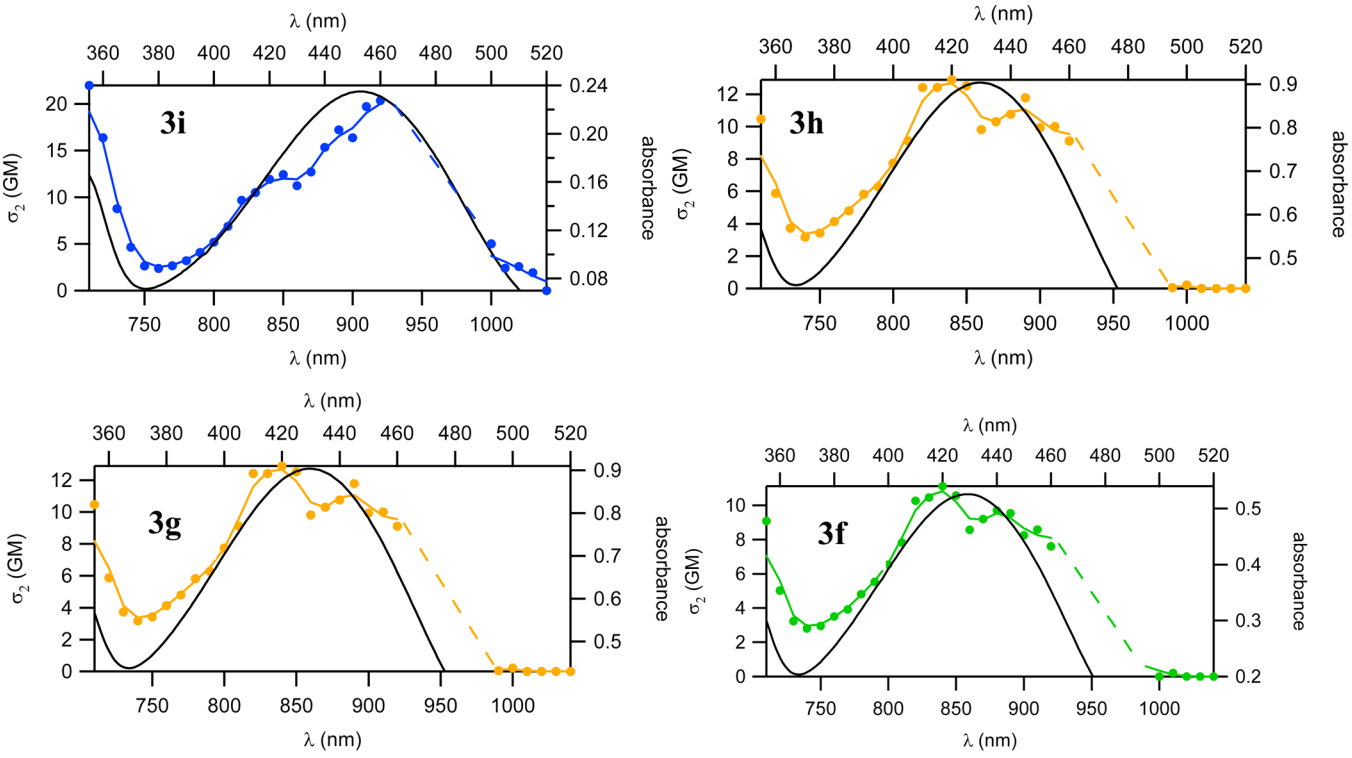

Figure 2. Two-photon absorption data in absolute ethanol for quinoline **3i**, **3h**, **3g** and **3f** (solid lines indicate rescaled one-photon absorption).

methoxy group in position sixth, seventh, and eighth.[27] Another example has been described by Ito et al. with an analogue of **3g** (where the ester in the fourth position was replaced by a hydroxyl group). The quantum yield of this derivative (6%) is one log lower than with our structure (60%).[28] Other studies showed as for our structures that electron-withdrawing substituent induced a loss of fluorescence.[29−32]

Two-Photon Absorption Properties. In order to assess the interest of these red-shifted fluorescent derivatives as potential probes for biphotonic imaging, we have investigated their TPA responses in the spectral region of interest for bioimaging (i.e., 700−1000 nm region) by measuring their two-photon excited fluorescence in solution.[33] These small-size chromophores show a broad TPA band located in the 750−1000 nm region, which matches the low-energy one-photon absorption band, indicating that the lowest one-photon excited state is also two-photon allowed (Figure 2).

We note that chromophores **3g** and **3h**, which bear identical ED substituent in the sixth or seventh position, show similar TPA maximum cross-section (12 and 13 GM, respectively), but the TPA band of compound **3g** is red-shifted in agreement with the bathochromic shift of the one-photon absorption (Table 2). On the other hand, slight increase of ED strength (**3d** → **3f** → **3g** → **3i**) induces either a red-shift of the TPA bands (**3d** → **3f** → **3g**) or a marked increase (by almost a factor of 2) for **3g** → **3i**. As a result, chromophore **3i** has a TPA maximum action cross-section at 920 nm, which is about 2 orders of magnitude larger than that of flavines[34,35] (i.e., common endogenous chromophores having the largest TPA action maximum cross sections in the NIR region). We stress that this is not the case for one-photon excitation in the near UV−visible region; flavines having larger maximum absorption coefficient than the quinoline derivatives investigated in the present work. This indicates that two-photon excitation would allow more selective excitation of the newly synthesized fluorescent derivatives in biological conditions thanks to the large contrast between their two-photon absorption cross-section and that of endogenous fluorophores.[36]

■ CONCLUSION

The present study has demonstrated that the implemented synthetic methodologies (classical or biphasic) give access to a large variety of substituted quinoline diesters in reasonable to good yields. Moreover, the elucidation of cyclization mechanism allows the control of substitution either in the phenyl or in the heterocyclic ring of quinolines, paving the way to a wide range of substitutions.

Compounds **3f**−**3i**, which bear a strong ED substituent in the sixth or seventh position, represent promising moieties for use as new fluorescent tags for monitoring of biomolecules due to their reduced size, suitable fluorescence, much larger TPA cross sections than endogenous chromophores at 900 nm, biocompatibility and possibility for heterofunctionalization. We are currently investigating this route.

■ EXPERIMENTAL SECTION

Materials and Methods. All air-sensitive manipulations were performed under a positive pressure of nitrogen or argon using standard Schlenk line. Solvents were degassed prior to use when necessary. THF was freshly distilled before use. Column chromatography was conducted on silica gel 60. NMR spectra were recorded on a 250 or 500 MHz (^{1}H frequency) spectrometer. Chemical shifts are reported using tetramethylsilane or the residual solvent peak as internal reference for ^{1}H or for ^{13}C. Product visualization was achieved with 2% (w/v) ninhydrin in ethanol. Thin layer chromatography (TLC) system for routine monitoring the course of certain reactions and confirming the purity of analytical samples employed aluminum-backed silica gel plates: cyclohexane/ethyl acetate or CH_2Cl_2/MeOH were used as developing solvents, and detection of spots was made by UV light and/or by iodine vapors.

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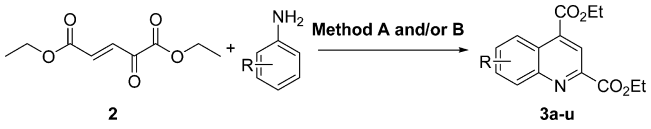

General Procedure for the Synthesis of Quinoline-2,4-dicarboxylic Acid Diethyl Ester. *Method A.* A mixture of diethyl 2-ketoglutaconate **2** (2.0 mmol) and aniline (1.0 mmol) in 2 mL of TFA was stirred at reflux for 18−24 h; after TFA removal by evaporation, the residue was dissolved in 20 mL of AcOEt, and the solution was washed with 5 mL of saturated aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The desired products were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate).

Method B. Phosphomolybdic acid (0.02 mmol) and sodium dodecylsulfate (0.02 mmol) were dissolved in water (5 mL). A solution of aniline (1.0 mmol) and diethyl 2-ketoglutaconate **2** (1.5 mmol) in CH_2Cl_2 (5 mL) was then added to the previous mixture and vigorously stirred at 80 °C for 18 h. After completion of the reaction, the lower layer was separated and slighly basified using sodium bicarbonate solution, and the product was extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated to get a crude product, which was further purified by column chromatography over silica gel.

*Diethyl quinoline-2,4-dicarboxylate (**3a**).* Aniline affords the unsubstituted quinoline-2,4-dicarboxylic acid diethyl ester, which was chromatographed (cyclohexane/EtOAc, 6:1) to isolate a light beige amorphous solid (yield method A, 47% (128 mg); method B, 11% (30 mg)): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.78 (d, J = 8.2 Hz, 1H), 8.61 (s, 1H), 8.34 (d, J = 8.2 Hz, 1H), 7.67−7.83 (m, 2H), 4.55 (q, J = 7.2 Hz, 2H), 4.50 (q, J = 7.2 Hz, 2H), 1.47 (t, J = 7.2 Hz, 3H), 1.45 (t, J = 7.2 Hz, 3H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 165.9, 165.0, 148.7, 148.0, 136.8, 131.4, 130.6, 130.3, 126.4, 125.7, 122.2, 62.7, 62.3, 14.5, 14.4; HRMS (ESI) calcd. for $C_{15}H_{16}NO_4$ [M + H] 274.1079, found 274.1072.

*Diethyl 6-methylquinoline-2,4-dicarboxylate (**3b**).* 4-Methylaniline affords the corresponding quinoline-2,4-dicarboxylic acid diethyl ester, which was chromatographed (cyclohexane/EtOAc, 5:1) to isolate a yellow amorphous solid (yield method A, 55% (158 mg); method B, 32% (92 mg)): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.61 (s, 1H), 8.59 (brs, 1H), 8.27 (d, J = 8.7 Hz, 1H), 7.67 (dd, J = 8.7, 2 Hz, 1H), 4.58 (q, J = 7.2 Hz, 2H), 4.53 (q, J = 7.2 Hz, 2H), 2.61 (s, 3H), 1.50 (t, J = 7.2 Hz, 3H), 1.49 (t, J = 7.2 Hz, 3H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 165.8, 164.9, 147.2, 146.7, 140.9, 135.6, 132.8, 130.8, 126.4, 124.3, 122.1, 62.4, 62.0, 22.3, 14.4, 14.3; HRMS (ESI) calcd. for $C_{16}H_{18}NO_4$ [M + H] 288.1236, found 288.1238.

*Diethyl 7-methylquinoline-2,4-dicarboxylate (**3c**).* 3-Methylaniline affords the corresponding quinoline-2,4-dicarboxylic acid diethyl ester, which was chromatographed (cyclohexane/EtOAc, 4:1) to isolate a yellow amorphous solid. (yield method A, 20% (57 mg); method B, 48% (138 mg)): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.70 (d, J = 8.7, 1H), 8.58 (s, 1H), 8.17 (s, 1H), 7.58 (dd, J = 8.7, 1.3 Hz, 1H), 4.57 (q, J = 7.2 Hz, 2H), 4.51 (q, J = 7.2 Hz, 2H), 2.58 (s, 3H), 1.50 (t, J = 7.2 Hz, 3H), 1.48 (t, J = 7.2 Hz, 3H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 165.9, 165.0, 148.9, 147.8, 141.2, 136.4, 132.7, 130.2, 125.2, 124.5, 121.5, 62.6, 62.2, 21.8, 14.5, 14.4; HRMS (ESI) calcd. for $C_{16}H_{18}NO_4$ [M + H] 288.1236, found 288.1231.

*Diethyl 6-aminoquinoline-2,4-dicarboxylate (**3d**).* 1-4-Phenylenediamine affords the diethyl 6-aminoquinoline-2,4-dicarboxylate which was chromatographed (cyclohexane/EtOAc, 4:1) to isolate a yellow amorphous solid (yield method B, 5% (14.4 mg)): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.59 (s, 1H), 8.12 (d, J = 9.1 Hz, 1H), 7.98 (d, J = 2.5 Hz, 1H), 7.22 (dd, J = 9.1, 2.6 Hz, 1H), 4.54 (q, J = 7.0 Hz, 2H), 4.48 (q, J = 7.0 Hz, 2H), 4.35 (brs, 2H), 1.48 (t, J = 7.1 Hz, 3H), 1.46 (t, J = 7.1 Hz, 3H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 166.3, 165.4, 148.5, 144.3, 143.2, 133.1, 132.3, 129.0, 123.4, 122.4, 104.6, 62.2, 61.8, 14.6, 14.5; HRMS (ESI) calcd. for $C_{15}H_{17}N_2O_4$ [M + H] 289.1188, found 289.1180.

*Diethyl 6-(2,2,2-trifluoroacetamido)quinoline-2,4-dicarboxylate (**3e**).* 1-4-Phenylenediamine affords the diethyl 6-(2,2,2-trifluoroacetamido)quinoline-2,4-dicarboxylate which was chromatographed (cyclohexane/EtOAc, 3:1) to isolate a orange amorphous solid (yield method A, 8% (31 mg): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 9.18 (d, J = 2.4 Hz, 1H), 8.71 (s, 1H), 8.38 (d, J = 9.5 Hz, 1H), 8.15 (dd, J= 9.5, 2.4 Hz, 1H), 4.70 (brs, 1H), 4.58 (q, J = 7.2 Hz, 2H), 4.55 (q, J = 7.2 Hz, 2H), 1.51 (t, J = 7.2 Hz, 6H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 165.6, 164.8, 147.9, 155.3 (d, J = 37.9 Hz), 146.7, 136.8, 135.9, 132.8, 127.0, 124.0, 123.5, 115.7 (d, J = 288.9 Hz), 115.4, 62.8, 62.6, 14.5, 14.4; HRMS (ESI) calcd. for $C_{17}H_{16}F_3N_2O_5$ [M + H] 385.1011, found 385.1013.

*Diethyl 6-(methylamino)quinoline-2,4-dicarboxylate (**3f**).* *N*-Methyl-*p*-phenylenediamine dihydrochloride affords the corresponding quinoline-2,4-dicarboxylic acid diethyl ester, which was chromatographed (cyclohexane/EtOAc, 2:1) to isolate a red amorphous solid (yield method A, 15% (45 mg)): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.60 (s, 1H), 8.16 (d, J = 9.2 Hz, 1H), 7.78 (d, J = 2.4 Hz, 1H), 7.20−7.23 (m, 1H), 4.52 (q, J = 7.2 Hz, 2H), 4.49 (q, J = 7.2 Hz, 2H), 3.00 (s, 3H), 1.47 (t, J = 7.2 Hz, 6H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 166.2, 164.9, 150.5, 143.5, 141.0, 132.0, 131.6, 129.83, 123.6, 123.1, 99.8, 62.3, 61.8, 30.3, 14.5, 14.4; HRMS (ESI) calcd. for $C_{16}H_{19}N_2O_4$ [M + H] 303.1345, found 303.1340.

*Diethyl 6-(dimethylamino)quinoline-2,4-dicarboxylate (**3g**).* 4-(Dimethylamino)aniline affords the corresponding quinoline-2,4-dicarboxylic acid diethyl ester, which was chromatographed (cyclohexane/EtOAc, 5:1) to isolate a orange amorphous solid (yield method A, 25% (79 mg)): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.59 (s, 1H), 8.14 (d, J = 9.4 Hz, 1H), 7.89 (d, J = 2.6 Hz, 1H), 7.39 (dd, J= 9.4, 2.6 Hz, 1H), 4.51 (q, J = 7.2 Hz, 2H), 4.46 (q, J = 7.2 Hz, 2H), 3.14 (s, 6H), 1.46 (t, J = 7.2 Hz, 3H), 1.45 (t, J = 7.2 Hz, 3H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 166.6, 165.6, 151.1, 143.3, 142.1, 132.6, 131.5, 129.0, 123.8, 119.7, 101.9, 62.1, 61.9, 40.6 (2C), 14.6, 14.5; HRMS (ESI) calcd. for $C_{17}H_{21}N_2O_4$ [M + H] 317.1501, found 317.1496.

*Diethyl 7-(dimethylamino)quinoline-2,4-dicarboxylate (**3h**).* *N,N*-Dimethyl-1,3-phenylenediamine dihydrochloride affords the corresponding quinoline-2,4-dicarboxylic acid diethyl ester, which was chromatographed (cyclohexane/EtOAc, 5:1) to isolate a brown amorphous solid (yield method A, 23% (72 mg)): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.57 (d, J = 9.6 Hz, 1H), 8.25 (s, 1H), 7.24−7.30 (m, 2H), 4.49 (q, J = 7.2 Hz, 2H), 4.43 (q, J = 7.2 Hz, 2H), 3.04 (s, 6H), 1.42 (t, J = 7.2 Hz, 3H), 1.40 (t, J = 7.2 Hz, 3H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 166.3, 165.3, 151.3, 150.8, 147.9, 135.9, 126.0, 119.8, 119.0, 117.9, 107.7, 62.3, 61.9, 40.3 (2C), 14.5, 14.4. ; HRMS (ESI) calcd. for $C_{17}H_{21}N_2O_4$ [M + H] 317.1501, found 317.1494.

*Diethyl 6-(diethylamino)quinoline-2,4-dicarboxylate (**3i**).* *N,N*-Diethyl-*p*-phenylenediamine affords the corresponding quinoline-2,4-dicarboxylic acid diethyl ester, which was chromatographed (cyclohexane/EtOAc, 5:1) to isolate a orange amorphous solid (yield method A, 15% (51 mg)): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.60 (s, 1H), 8.14 (d, J = 9.5 Hz, 1H), 7.96 (d, J = 2.3 Hz, 1H), 7.38 (dd, J= 9.5, 2.3 Hz, 1H), 4.53 (q, J = 7.2 Hz, 2H), 4.48 (q, J = 1.45 = 7.2 Hz, 2H), 3.55 (q, J = 7.2 Hz, 4H), 1.47 (t, J = 7.2 Hz, 3H), 1.45 (t, J = 7.2 Hz, 3H), 1.28 (t, J = 7.2 Hz, 6H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 166.6, 165.6, 148.7, 143.3, 141.8, 132.8, 132.1, 129.3, 123.8, 119.6, 101.5, 62.0, 61.6, 45.2 (2C), 14.6, 14.5, 12.7 (2C); HRMS (ESI) calcd. for $C_{19}H_{25}N_2O_4$ [M + H] 345.1814, found 345.1807.

*Diethyl 6-nitroquinoline-2,4-dicarboxylate (**3j**).* 4-Nitroaniline affords the corresponding quinoline-2,4-dicarboxylic acid diethyl ester, which was chromatographed (cyclohexane/EtOAc, 9:1) to isolate a orange amorphous solid (yield method A, 33% (105 mg)): ^{1}H NMR (δ, ppm) ($CDCl_3$, 500 MHz) 9.84 (d, J = 2.4 Hz, 1H), 8.77 (s, 1H), 8.55 (dd, J = 9.4, 2.4 Hz, 1H), 8.47 (d, 9.4 Hz, 1H), 4.59 (q, J = 7.2 Hz, 2H), 4.57 (q, J = 7.2 Hz, 2H), 1.52 (t, 7.2 Hz, 3H), 1.50 (t, J = 7.2 Hz, 3H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 500 MHz) 164.7, 164.2, 151.3, 150.4, 148.0, 138.3, 133.1, 125.5, 123.9 (2C), 123.8, 63.1, 63.0, 14.4, 14.3; HRMS (ESI) calcd. for $C_{15}H_{15}N_2O_6$ [M + H] 319.0930, found 319.0936.

*Diethyl 6-hydroxyquinoline-2,4-dicarboxylate (**3l**).* 4-Hydroxyaniline affords the corresponding quinoline-2,4-dicarboxylic acid diethyl

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ester, which was chromatographed (cyclohexane/EtOAc, 2:1) to isolate a light yellow amorphous solid (yield method A, 13% (37 mg); method B, 34% (98 mg)): ^{1}H NMR (δ, ppm) (CD_3OD, 250 MHz) 8.48 (s, 1H), 8.18−8.03 (m, 2H), 7.43 (dd, J = 9.3, 2.7 Hz, 1H), 4.50 (q, J = 7.1 Hz, 2H), 4.49 (q, J = 7.1 Hz, 2H), 1.47 (t, J = 7.1 Hz, 6H); ^{13}C NMR (δ, ppm) (MeOD, 250 MHz) 166.9, 166.0, 161.0, 145.3, 145.0, 135.1, 133.1, 129.7, 124.72, 123.4, 107.6, 63.2, 63.0, 14.6, 14.5; HRMS (ESI) calcd. for $C_{15}H_{16}NO_5$ [M + H] 290.1028, found 290.1028.

*Diethyl 7-hydroxyquinoline-2,4-dicarboxylate (**3m**).* 3-Hydroxyaniline affords the corresponding quinoline-2,4-dicarboxylic acid diethyl ester, which was chromatographed (cyclohexane/EtOAc, 1:1) to isolate a yellow amorphous solid (yield method A, 8% (23 mg); method B, 20% (58 mg)): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.35 (s, 1H), 8.27 (d, J = 9.3 Hz, 1H), 7.57 (d, J = 1.7, 1H), 6.94 (dd, J = 9.3, 1.7 Hz, 1H), 4.58 (q, J = 7.2 Hz, 2H), 4.36 (q, J = 7.2 Hz, 2H), 1.57 (t, J = 7.2 Hz, 3H), 1.15 (t, J = 7.2 Hz, 3H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 165.5, 164.2, 159.9, 148.9, 146.04, 136.8, 126.7, 123.3, 120.8, 119.2, 109.7, 62.6, 62.2, 14.4, 14.0; HRMS (ESI) calcd. for $C_{15}H_{16}NO_5$ [M + H] 290.1028, found 290.1031.

*Diethyl 8-hydroxyquinoline-2,4-dicarboxylate (**3n**).* 2-Aminophenol affords the corresponding quinoline-2,4-dicarboxylic acid diethyl ester, which was chromatographed (cyclohexane/EtOAc, 5:1) to isolate a orange amorphous solid (yield method A, 30% (86 mg); method B, 10% (29 mg)): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.63 (s, 1H), 8.24 (d, J = 8.7 Hz, 1H), 7.66 (t, J = 8.2 Hz, 1H), 7.26 (d, J = 7.7 Hz, 1H), 4.53 (dd, J=14.2, 7.2 Hz, 2H), 4.51 (d, J=14.3, 7.2 Hz, 2H), 1.48 (t, J = 7.2 Hz, 3H), 1.47 (t, J=7.2 Hz, 3H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 165.7, 164.5, 153.5, 145.2, 139.0, 136.7, 132.1, 126.9, 123.0, 116.1, 111.3, 62.5, 62.3, 14.5, 14.4; HRMS (ESI) calcd. for $C_{15}H_{16}NO_5$ [M + H] 290.1028, found 290.1025.

*Diethyl 6-methoxyquinoline-2,4-dicarboxylate (**3o**).* *p*-Anisidine affords the corresponding quinoline-2,4-dicarboxylic acid diethyl ester, which was chromatographed (cyclohexane/EtOAc, 6:1) to isolate a light beige amorphous solid (yield method A, 39% (118 mg); method B, 28% (85 mg)): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.67 (s, 1H), 8.21−8.226 (m, 2H), 7.45 (dd, J = 9.3, 2.8 Hz, 1H), 4.56 (q, J = 7.2 Hz, 2H), 4.51 (q, J = 7.2 Hz, 2H), 3.98 (s, 3H), 1.49 (t, 7.2 Hz, 3H), 1.48 (t, J = 7.2 Hz, 3H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 166.0, 165.1, 161.1, 145.2, 145.0, 133.8, 132.8, 128.5, 123.9, 123.1, 103.1, 62.4, 62.0, 55.8, 14.5, 14.4; HRMS (ESI) calcd. for $C_{16}H_{18}NO_5$ [M + H] 304.1185, found 304.1179.

*Diethyl 7-methoxyquinoline-2,4-dicarboxylate (**3p**).* *m*-Anisidine affords the corresponding quinoline-2,4-dicarboxylic acid diethyl ester, which was chromatographed (cyclohexane/EtOAc, 4:1) to isolate a brown amorphous solid (yield method A, 21% (63 mg); method B, 33% (100 mg)): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.71 (d, J = 9.2 Hz, 1H), 8.50 (s, 1H), 7.69 (brs, 1H), 7.39 (dd, J = 9.5, 2.6 Hz, 1H), 4.57 (q, J = 7.2 Hz, 2H), 4.51 (q, J = 7.2 Hz, 2H), 4.97 (s, 3H), 1.49 (t, 7.2 Hz, 3H), 1.45 (t, J = 7.2 Hz, 3H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 165.9, 165.0, 161.3, 150.7, 148.0, 136.5, 126.6, 123.9, 121.9, 120.1, 108.6, 62.6, 62.2, 55.9, 14.5, 14.4; HRMS (ESI) calcd. for $C_{16}H_{18}NO_5$ [M + H] 304.1185, found 304.1188.

*Diethyl 8-methoxyquinoline-2,4-dicarboxylate (**3q**).* 2-Methoxyaniline affords the corresponding quinoline-2,4-dicarboxylic acid diethyl ester, which was chromatographed (cyclohexane/EtOAc, 2:1) to isolate a light yellow amorphous solid (yield method A, 46% (139 mg); method B, 13% (39 mg)): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.66 (s, 1H), 8.33 (dd, J = 8.8, 0.6 Hz, 1H), 7.67 (dd, J = 8.8, 7.8 Hz, 1H), 7.13 (dd, J = 7.8, 0.6 Hz, 1H), 4.54 (q, J = 7.0 Hz, 2H), 4.51 (q, J = 7.0 Hz, 2H), 4.09 (s, 3H), 1.50 (t, J = 7.0 Hz, 3H), 1.45 (t, J = 7.0 Hz, 3H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 165.9, 165.1, 156.3, 146.5, 140.8, 136.6, 130.9, 127.6, 122.8, 117.1, 108.4, 62.7, 62.3, 56.3, 14.4, 14.4; HRMS (ESI) calcd. for $C_{16}H_{18}NO_5$ [M + H] 304.1185, found 304.1182.

*Diethyl 8-(benzyloxy)quinoline-2,4-dicarboxylate (**3r**).* 2-Benzyloxyaniline affords the corresponding quinoline-2,4-dicarboxylic acid diethyl ester, which was chromatographed (cyclohexane/EtOAc, 8:1) to isolate a yellow amorphous solid (yield method A, 30% (113 mg)): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.67 (s, 1H), 8.36 (d, J = 8.2 Hz, 1H), 7.56−7.62 (m, 3H), 7.32−7.43 (m, 3H), 7.19 (d, J = 7.9 Hz, 1H), 5.44 (s, 2H), 4.56 (q, J = 7.2 Hz, 2H), 4.50 (q, J = 7.2 Hz, 2H), 1.46−1.52 (m, 6H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 165.9, 165.1, 155.4, 146.6, 141.3, 136.5, 136.5, 130.7, 128.6 (2C), 127.9, 127.6, 127.2 (2C), 122.5, 117.6, 111.2, 71.2, 62.3, 62.2, 14.3, 14.3; HRMS (ESI) calcd. for $C_{22}H_{22}NO_5$ [M + H] 380.1498, found 380.1494.

*Diethyl 8-hydroxy-5-methylquinoline-2,4-dicarboxylate (**3s**).* To a solution of diethyl 8-(*tert*-butoxycarbonyloxy)-5-methylquinoline-2,4-dicarboxylate (**3t**) (1 mmol) in CH_2Cl_2 was added piperidine (2 mmol). The reaction mixture was stirred at room temperature for 18 h, and then the solvent was evaporated, and the residue was filtered through a pad of silica gel (cyclohexane/EtOAc, 2:1). The solvent was evaporated to give a yellow oil (yield 60%, 182 mg): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.48 (brs, 1H), 8.13 (s, 1H), 7.42 (d, J = 7.8 Hz, 1H), 7.17 (d, J = 7.8 Hz, 1H), 4.52 (q, J = 7.2 Hz, 2H), 4.52 (q, J = 7.2 Hz, 2H), 2.53 (s, 3H), 1.48 (t, J = 7.2 Hz, 3H), 1.45 (t, J = 7.2 Hz, 3H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 169.2, 164.3, 151.9, 144.4, 140.9, 138.9, 133.3, 124.4, 123.8, 120.5, 111.1, 62.7, 62.4, 20.5, 14.5, 14.2; HRMS (ESI) calcd. for $C_{16}H_{18}NO_5$ [M + H] 304.1185, found 304.1181.

*Diethyl 8-(tert-butoxycarbonyloxy)-5-methylquinoline-2,4-dicarboxylate (**3t**).* A solution of 2-amino-4-methylphenol (1.0 mmol) and diethyl 2-ketoglutaconate (2.0 mmol) in 2 mL of TFA was stirred at reflux for 24 h, after which TFA was distilled off. The residue was dissolved in 20 mL of EtOAc, and the solution was washed with 5 mL of saturated aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product (yellow oil) was used in the next step without purification. The residue (1.0 mmol) was solubilized in anhydrous CH_2Cl_2 (10 mL), and both 4-dimethylaminopyridine (1.2 mmol) and di-*tert*-butyl dicarbonate (1.0 mmol) were added at 0 °C under nitrogen. The mixture was first stirred for 5 min at 0 °C and then for 18 h at room temperature. The reaction mixture was evaporated under reduced pressure, and the residue was purified through a silica gel column (cyclohexane/EtOAc, 5:1) to yield 190 mg (23%) of **3t** as a yellow amorphous solid: ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.16 (s, 1H), 7.45−7.53 (m, 2H), 4.52 (q, J = 7.2 Hz, 2H), 4.49 (q, J = 7.2 Hz, 2H), 2.61 (s, 3H), 1.60 (s, 9H), 1.45 (t, 7.2 Hz, 3H), 1.45 (t, J = 7.2 Hz, 3H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 169.0, 164.6, 152.0, 147.0, 146.9, 141.9, 140.8, 132.1, 131.3, 125.1, 121.7, 120.6, 83.7, 62.7, 62.3, 27.8 (3C), 21.1, 14.5, 14.1; HRMS (ESI) calcd. for $C_{21}H_{26}NO_7$ [M + H] 404.1709, found 404.1713.

*2,4-Bis(ethoxycarbonyl)quinoline-6-carboxylic acid (**3u**).* 4-Aminobenzoic acid affords the corresponding quinoline-2,4-dicarboxylic acid diethyl ester, which was chromatographed (CH_2Cl_2/MeOH, 9.5:0.5) to isolate an orange amorphous solid (yield method A, 32% (101 mg)): ^{1}H NMR (δ, ppm) (DMSO-d_6, 500 MHz) 9.28 (s, 1H), 8.42−8.44 (m, 2H), 8.25 (m, 1H), 4.46−4.52 (m, 4H), 1.39−1.43(m, 6H); ^{13}C NMR (δ, ppm) (DMSO-d_6, 500 MHz) 169.0, 165.0, 164.1, 148.8, 148.4, 137.2, 131.1, 130.1, 126.9, 126.2, 124.6, 121.4, 62.1, 61.9, 14.2, 14.1; HRMS (ESI) calcd. for $C_{16}H_{16}NO_6$ [M + H] 318.0978, found 318.0973.

*4-(Ethoxycarbonyl)-7-methoxyquinoline-2-carboxylic acid (**4p**).* To a solution of diethyl 7-methoxyquinoline-2,4-dicarboxylate (**3p**) (275 mg, 1 mmol) in of THF/H_2O (5:5, 10 mL) was added NaOH (40 mg, 1 mmol). The reaction mixture was monitored with TLC (cyclohexane/EtOAc, 5:5). After the completion of reaction, the sovent was evaporated, and the crude residue was purified by preparative RP-HPLC (Preparative C_{18} column, λ 240 nm, solvent system: H_2O (A), CH_3CN (B), 8 mL/min, gradient: 0′-5′: 80% (A)/20% (B); 20′-30′: 80% (A)/20% (B); 40′-60′: 80% (A)/20% (B)) to yield a light yellow amorphous solid 4-(ethoxycarbonyl)-7-methoxyquinoline-2-carboxylic acid (30%, 82 mg): ^{1}H NMR (δ, ppm) (D_2O, 250 MHz) 8.25 (d, J = 9.2 Hz, 1H), 8.17 (s, 1H), 7.36 (d, J= 2.7 Hz, 1H), 7.23 (dd, J = 9.4, 2.7 Hz, 1H), 4.54 (q, J = 7.2 Hz, 2H), 3.98 (s, 3H), 1.49 (t, J = 7.2 Hz, 3H); ^{13}C NMR (δ, ppm) (D_2O, 500 MHz) 172.1, 168.6, 161.4, 154.4, 149.6, 137.5, 127.0, 122.6, 121.0, 120.1, 107.8, 64.1, 56.5, 14.2; HRMS (ESI) calcd. for $C_{14}H_{14}NO_5$ [M + H] 276.0872, found 276.0866.

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*Methyl 4-(benzylcarbamoyl)-7-(dimethylamino)quinoline-2-carboxylate (**3v**). Methyl 5-(benzylamino)-4,5-dioxopent-2-enoate (**7**).* Dimethyl 2-oxoglutarate (174 mg, 1.0 mmol) was dissolved in 2 M benzylamine in THF (5 mL, 1.5 mmol) and stirred for 6 h at room temperature. The solvent was then evaporated in vacuo, and the remaining oily residue was purified by column chromatography (cyclohexane/EtOAc, 1:3), affording 206 mg (82%) of methyl 5-(benzylamino)-4,5-dioxopentanoate as a colorless oil (**6**): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 7.26−7.32 (m, 5H), 4.48 (d, J = 6.1 Hz, 2H), 3.68 (s, 3H), 3.27 (t, J = 6.5 Hz, 2H), 2.65 (t, J = 6.5 Hz, 2H). To a solution of methyl 5-(benzylamino)-4,5-dioxopentanoate (250 mg, 1.0 mmol)) dissolved in 15 mL of CH_2Cl_2 was added bromine (0.077 mL, 1.5 mmol) dissolved in 1 mL of CH_2Cl_2. The solution was stirred at 35 °C for 18 h, after which the solvent and residual HBr were evaporated to yield methyl 5-(benzylamino)-3-bromo-4,5-dioxopentanoate as an yellow oil (yield, quantitative): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 7.21−7.23 (m, 5H), 5.56 (dd, J = 9.2, 6.0 Hz, 1H), 4.43 (d, J= 6.1 Hz, 2H), 3.59 (s, 3H), 3.22 (dd, J = 17.2, 9.2 Hz, 1 H), 3.02 (dd, J = 17.2, 6.1 Hz). Triethylamine (2.0 mmol, 0.278 mL) was added to a solution of 5-(benzylamino)-3-bromo-4,5-dioxopentanoate (327 mg, 1 mmol) in 10 mL of diethyl ether. After being stirred for 6 h, the solvent was then evaporated in vacuo, and the oily residue was purified by column chromatography (cyclohexane/EtOAc, 4:1), affording the methyl 5-(benzylamino)-4,5-dioxopent-2-enoate as a yellow amorphous solid (**7**) (61%, 151 mg): ^{1}H NMR (δ, ppm) ($CDCl_3$, 250 MHz) 8.00 (d, J = 16.4 Hz, 1H), 7.30−7.32 (m, 5H), 7.04 (d, J = 16.4 Hz, 1H), 4.51 (d, J = 6.1 Hz, 2H), 3.83 (s, 3H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 185.5, 165.4, 159.9, 135.1, 133.0, 128.9 (2C), 128.0, 127.9 (2C), 127.9, 52.6, 43.6.

*Methyl 4-(benzylcarbamoyl)-7-(dimethylamino)quinoline-2-carboxylate (**3v**).* A mixture of methyl 5-(benzylamino)-4,5-dioxopent-2-enoate **7** (1.5 mmol) and *N*,*N*-dimethyl-1,3-phenylenediamine dihydrochloride (1.0 mmol) in 3 mL of TFA affords the corresponding quinoline-2,4-dicarboxylic acid diethyl ester, which was chromatographed (cyclohexane/EtOAc, 3:1) to isolate an orange amorphous solid (yield 19%, 69 mg): ^{1}H NMR (δ, ppm) ($CDCl_3$, 500 MHz) 8.12 (d, J = 10 Hz, 1H), 7.83 (s, 1H), 7.20−7.41 (m, 7H), 6.75 (brs, 1H), 4.72 (d, J = 5.6 Hz, 2H), 4.01 (s, 3H), 3.08 (s, 6H); ^{13}C NMR (δ, ppm) ($CDCl_3$, 250 MHz) 167.2, 165.9, 151.7, 150.3, 147.5, 142.3, 137.8, 129.0 (2C), 128.2 (2C)., 127.9, 125.9, 119.3, 118.3, 114.3, 107.5, 53.2, 44.3, 40.4 (2C); HRMS (ESI) calcd. for $C_{21}H_{22}N_3O_3$ [M + H] 364.1661, found 364.1659.

*4-(Benzylcarbamoyl)-7-(dimethylamino)quinoline-2-carboxylic acid (**4v**).* To a solution of methyl 4-(benzylcarbamoyl)-7-(dimethylamino)quinoline-2-carboxylate (**3v**) (100 mg, 0.28 mmol) in of THF/H_2O (5:5, 10 mL) was added NaOH (7 mg, 0.28 mmol). The reaction mixture was monitored with TLC (cyclohexane/EtOAc, 1:1). The solvent was removed under reduced pressure, the solid was washed with chloroform (20 mL), dissolved in water (3 mL) and then acidified with 0.1 N HCl solution to give a yellow precipitate. The precipitate was filtered to give **4v** (68%, 66 mg) as a yellow amorphous solid: ^{1}H NMR (δ, ppm) (DMSO-d_6, 500 MHz) 9.36 (brs, 1H), 7.95 (d, J = 9.3 Hz, 1H), 7.92 (s, 1H), 7.46−7.34 (m, 5H), 7.31−7.23 (m, 2H), 4.53 (d, J = 5.5 Hz, 2H), 3.02 (s, 6H); ^{13}C NMR (δ, ppm) (DMSO-d_6, 250 MHz) 167.9, 167.5, 157.3, 150.7, 149.2, 141.4, 139.5, 128.3 (2C), 127.2 (2C), 126.8, 125.3, 116.9, 116.5, 115.3, 107.6, 42.5, 39.8 (2C); HRMS (ESI) calcd. for $C_{20}H_{20}N_3O_3$ [M + H] 350.1505, found 350.1503.

Photophysical Properties. Emission spectra were obtained using a spectrofluorometer, for each compound at $\lambda_{ex} = \lambda_{max}$ (abs) with $A_{\lambda ex}$ = 0.1 to minimize internal absorption. Fluorescence quantum yields were measured on samples at rt; fluorescein in 0.1 N NaOH (Φ = 0.90 at λ_{exc} = 470 nm)[37,38] and quinine bisulfate in 1 N H_2SO_4 (Φ = 0.54 at λ_{exc} = 347 nm) were used as standards depending on the emission spectral range.

Two-photon absorption cross sections (σ_2) were obtained by measuring the two-photon excited fluorescence (TPEF) cross sections ($\sigma_2\phi$) and fluorescence emission quantum yield (Φ). TPEF cross sections of 10^{-4} M solutions were measured relative to known reference compounds fluorescein in 0.01 M aqueous NaOH for 700−980 nm) according to the experimental protocol established by Xu and Webb,[39,40] and using the appropriate solvent-related refractive index corrections.[41] The quadratic dependence of the fluorescence intensity on the excitation intensity was verified for all investigated compounds and wavelengths, indicating that the measurements were carried out in intensity regimes in which saturation or photodegradation did not occur. Measurements were conducted using excitation sources delivering fs pulses. This is preferred in order to avoid excited state absorption during the pulse duration, a phenomenon that has been shown to lead to overestimated TPA cross-section values. To span the 700−980 nm range, a Nd:YLF-pumped Ti:sapphire oscillator was used generating 150 fs pulses at a 76 MHz rate. To span the 1000−1400 nm range, an OPO (PP-BBO) was added to the setup to collect and modulate the output signal of the Ti:sapphire oscillator. The excitation was focused into the cuvette through a microscope objective (10X, NA 0.25). The fluorescence was detected in epifluorescence mode via a dichroic mirror and a barrier filter by a compact CCD spectrometer. Total fluorescence intensities were obtained by integrating the corrected emission.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Nuclear magnetic resonance spectra 1D and 2D for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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