JOC The Journal of Organic Chemistry

Article

Cite This: J. Org. Chem. 2020, 85, 726−744

pubs.acs.org/joc

Two-Photon Excitable Photoremovable Protecting Groups Based on the Quinoline Scaffold for Use in Biology

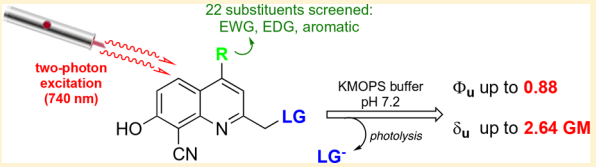
Anna-Lisa K. Hennig,[†,§] Davide Deodato,[†,§] Naeem Asad,[†,§] Cyril Herbivo,[†] and Timothy M. Dore*[,†,‡]

[†]New York University Abu Dhabi, P.O. Box 129188, Abu Dhabi, United Arab Emirates

[‡]Department of Chemistry, University of Georgia, Athens, Georgia 30602, United States

Supporting Information

ABSTRACT: Photoremovable protecting groups (PPGs) are powerful tools for physiological studies, harnessing light as an on/off switch to provide tight spatio-temporal control over the release of biological effectors through two-photon excitation (2PE) in tissue culture and whole-animal studies. We carried out a series of systematic structural modifications to the (8-cyano-7-hydroxyquinolin-2-yl)methyl (CyHQ) chromophore to conduct an SAR study with the aim of enhancing its photochemical properties, especially its two-photon uncaging action cross section (δ_u). The best results were obtained when substituents were added at the C4 position, which improved δ_u for release of acetate up to 7-fold, while retaining all the other excellent properties of the CyHQ PPG, including high quantum yield (Φ_u), low susceptibility to spontaneous hydrolysis in the dark, and good aqueous solubility. Hammett correlation analysis suggested that photolysis efficiency is favored by electron-rich substituents at C4, giving important insights into the mechanism of the photolysis reaction. The four best CyHQ derivatives were used to mediate the efficient release of homopiperonylic acid in high yield under simulated physiological conditions. Our efforts have led to the development of 2PE-sensitive PPGs with remarkable δ_u values (up to 2.64 GM), excellent quantum yields (up to 0.88), and high-yielding effector release (up to 92%).



INTRODUCTION

In recent years, therapeutic agents and biologically active molecules have been increasingly employed to study biological systems and understand the physiological processes involved therein. Nevertheless, these probes can have unwanted side effects and reactions caused by activation of off-target receptors resulting from the systemic application of and poor control over the diffusion of an agent in tissue preparation. To address this issue, there is a need to develop new tools that can turn "off" the biological activity of these molecules during administration and delivery to the desired location within the biological sample and turn it back "on" with an external stimulus. Light (especially in the IR region) is an ideal exogenous, non-chemical stimulus that can be employed to turn on the bioactivity, while causing negligible harm to the biological system. In this regard, light-sensitive probes such as photoremovable protecting groups (PPGs)[1−3] are extremely powerful tools for studying physiological processes. PPGs (also referred to as "caging" groups or phototriggers) are useful because of their ability to render a bioactive agent inactive by masking its biological function while being simple to use. They offer the means of delivering bioactive molecules such as neurotransmitters,[4,5] Ca^{2+},[6] second messengers,[7] etc. to small addressable targets and enable experiments that follow physiological events in real time. These tools have been proven useful for studying physiological processes in cell cultures,[8,9] tissues,[10,11] and whole animals.[12,13]

For use in biological systems, "good caging groups" possess several atributes:[1−3] (i) optimal solubility at physiological pH; (ii) rapid release kinetics; (iii) high quantum yields (Φ_u) of the photolysis reaction; (iv) stability against hydrolysis in the dark; (v) low or no toxicity of the PPG and its photoproducts; and (vi) good absorption at long wavelengths to avoid photodamage, allow deeper tissue penetration, and minimize the undesirable photochemical reactions that occur at high-energy wavelengths. Over the last 30 years, the library of biologically useful PPGs has significantly increased and many of those currently employed satisfy most of the aforementioned conditions. Nevertheless, there is still a void in the development of PPGs that can efficiently release the active component through excitation in the IR region. Any PPGs that have been designed to absorb near-IR light tend to suffer from various limitations, such as inefficient photolysis, lower excited state energy, or limited solubility in the case of extended chromophores.[14,15] Two-photon excitation (2PE) is an attractive method for releasing PPGs at longer wavelengths.[16] This photophysical phenomenon, first described theoretically by Maria Göppert-Mayer,[17] occurs when two photons are absorbed simultaneously by the chromophore and excite it, which triggers a photolysis reaction. The efficiency of a PPG toward 2PE is defined by the two-photon uncaging action cross

Received: October 14, 2019
Published: December 6, 2019

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726−744

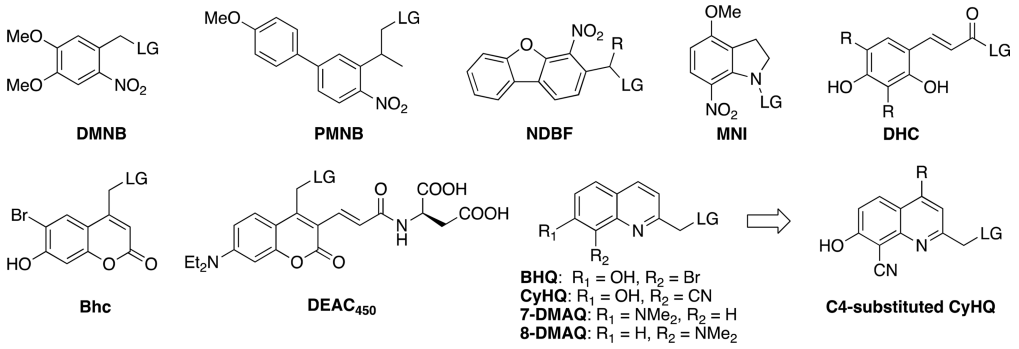


Figure 1. Examples of PPGs photolyzed through two-photon excitation. LG, leaving group.

section (δ_u), expressed in Göppert-Mayer (GM), and described by the following equation:

$$\delta_u = \delta_a \Phi_u \quad (1)$$

where δ_a is the two-photon absorbance cross section and Φ_u is the quantum yield of the photochemical reaction. Photochemistry driven by 2PE not only provides precise temporal control but is also highly localized, conferring much better spatial control by releasing the biological effector in femtoliter-sized volumes at the focal point of the laser beam.[18,19] This strategy affords red-shifting of the light-induced photorelease[20] into the therapeutic window (~650−950 nm) resulting in deeper tissue penetration, reduced photodamage to the biological system, and enhanced three-dimensional resolution of biological effector activation.

It is evident that 2PE-sensitive PPGs have numerous differences compared to those excited by traditional one-photon excitation (1PE). Nevertheless, chromophores with adequate properties for use in biology are extremely rare with most of those that exist exhibiting low sensitivity to 2PE (δ_u < 1 GM), slow photorelease kinetics, or biological incompatibility (low aqueous solubility, poor cellular uptake, and toxicity)[10,20−26] (Figure 1). Based on bleaching experiments and diffusion rates, a δ_u value of nearly 4 GM is required to achieve a large steady-state concentration of activated effectors within the focal volume of the laser.[27] Coumarin-based PPGs represent the most successful example to date, possessing high δ_u values (>1 GM) and fast kinetics.[19,28,29] Coumarin PPGs, however, display a high level of fluorescence upon excitation, which particularly limits their applicability when fluorescent indicators are used to observe the physiological event, as is common. Thus, there is a need to develop PPGs with better 2PE properties that can be used in experiments that require precise timing and location of photoactivation in tissue culture, ex vivo organs, and whole animals.

A promising direction toward the discovery of PPGs with improved sensitivity to 2PE without compromising biological compatibility is to turn toward quinoline-based PPGs, which possess high photolysis efficiencies, extremely fast release kinetics, excellent aqueous solubilities, and low cellular toxicities and are able to release a wide variety of functional groups (carboxylic acids, phenols, aldehydes, diols, amines, and phosphates).[18,30−34] These compounds also exhibit low levels of fluorescence, enabling them to be used alongside fluorescent dyes.[32,35] One drawback to quinoline-based PPGs is the low values of δ_u (generally <0.6 GM),[30,36,37] which limit their application in tissues or whole animal studies. A high δ_u value (2.3 GM) was reported for a quadrupolar structure in which a fluorene group was inserted between the C5 positions of two 8-dimethylaminoquinoline (8-DMAQ, Figure 1) PPGs,[38] but this value was measured in a 1:1 acetonitrile/TRIS buffer, which could never be used in a biological preparation. Furthermore, at 366 nm (1PE), a powerful 8 W lamp required 1 to 5 h to deplete 1 mL of a 0.1 mM solution (i.e., 100 μmol of the substrate) of the PPG, and the δ_u measurements were conducted by exposing a 45 μL sample for 5 h to 100 mW, 730 nm laser irradiation without any evaporation of the solvent. These observations run counter to the argument that these PPGs photolyze efficiently. A 5-*para*-carboxyphenyl substituent on 8-DMAQ resulted in a more water-soluble PPG, and the authors reported δ_u = 2.0 GM but provided insufficient information on how the cross-section measurement for the photolysis reaction was made.[39]

The 8-bromo- (BHQ) and (8-cyano-7-hydroxyquinolin-2-yl)methyl (CyHQ) PPGs (Figure 1), which have δ_u = 0.59 and 0.32 GM, respectively, for their corresponding protected acetates,[30] represent interesting scaffolds that have been used successfully for the photoactivation of biological effectors to study different physiological processes.[13,33,40−42] BHQ and CyHQ rapidly photolyze on the nanosecond timescale[31,43,44] and 3 mL of a 0.1 mM solution (i.e, 300 μmol of substrate) fully cleaves in approximately 2 min or less with 8−12 mW of 365 nm light from an LED.[31,45,46] In the present study, we introduced chemical modifications to these chromophores to enhance the photochemical properties, especially the δ_u values. Our efforts led to the identification of a set of C4-substituted chromophores (Figure 1) that showed almost an order of magnitude of an increase in δ_u, while retaining the other excellent properties of the quinoline-based PPGs.

RESULTS AND DISCUSSION

Despite having a slightly higher cross section, BHQ suffers a secondary photo-debromination reaction when irradiated that limits its utility,[43] and when we placed a phenyl group in the C4 position, debromination was the exclusive outcome. For this reason, we chose CyHQ, a PPG with clean and robust photochemistry, as a model compound for an optimization campaign. Acetate was selected as a model leaving group to simplify the synthesis and for comparison with literature data.

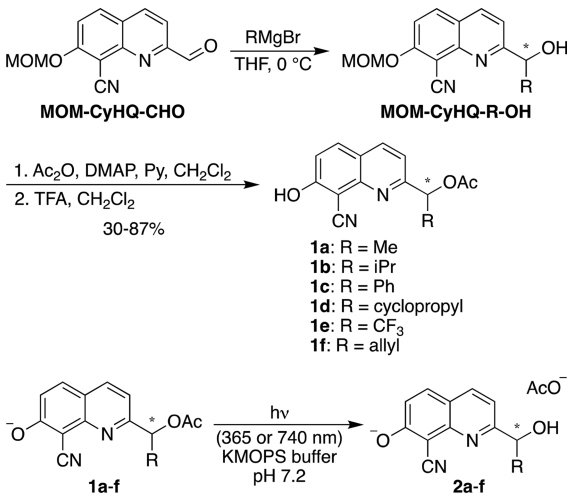
The value of δ_u is affected by two parameters: δ_a and Φ_u. Enhancing either parameter will increase δ_u. Enhancing δ_a of organic molecules has proved to be an extremely difficult challenge and has been the subject of many review articles.[16,47−50] General strategies include extending conjugation,[51] adding planarity,[52] introducing molecular symmetry or multibranched oligomers,[49] or inserting strong donor/acceptor

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726−744

pairs.[53] All these modifications tend to require the introduction of multiple large, lipophilic, aromatic rings that negatively impact the solubility of the chromophore. Alternatively, Φ_u depends on the rate constants of the bond reorganization events happening after light absorption and can be significantly affected by small structural modifications that have only a slight impact on chromophore solubility.

Taking these considerations into account, we introduced point modifications on the CyHQ PPG with the aim of increasing δ_u by modulating Φ_u. We investigated the effects of a primary vs secondary carbon α to the leaving group since this modification has been shown to increase the photolysis rate (and ultimately, Φ_u) for CyHQ-protected anilines.[46] Six derivatives (**1a**−**1f**), bearing substituents that would impart different electronic and steric effects at the 2-methyl position, were designed and synthesized (Scheme 1). The secondary

Scheme 1. Synthesis and Photolysis Reaction of 2-Methyl-Substituted Acetates 1a−1f



alcohols used as starting materials (MOM-CyHQ-R-OH) were obtained through a Grignard reaction on the corresponding aldehyde (MOM-CyHQ-CHO[30,31]) using a protocol described previously.[46] The modified CyHQ-protected acetates **1a**−**1f** were prepared by acetylation of the alcohols followed by MOM deprotection. All compounds were isolated as racemic mixtures.

The photochemistry induced by 1PE and 2PE of the modified CyHQ-protected acetates **1a**−**1f** was investigated by irradiating 0.1 mM solutions of each of them in simulated physiological buffer (potassium 3-morpholinopropane-1-sulfonate (KMOPS), pH 7.2) with 365 nm light (1PE) from an LED or 740 nm light from a Ti:sapphire laser (2PE). The photochemical reactions were sampled at different time intervals to monitor the course of the photolysis reaction by HPLC (see the Supporting Information for full details). The photochemical and photophysical properties determined are shown in Table 1.

These data showed no improvement in the 2PE-mediated photolysis reaction; the δ_u values measured were in most cases lower than those of the parent compound CyHQ-OAc, except for the isopropyl derivative **1b**, which showed a slightly higher value (0.35 GM). The cyclopropyl derivative **1d** displayed an improved 1PE quantum yield and sensitivity ($\varepsilon\cdot\Phi_u$), a measure of the efficacy of a PPG at a given wavelength, but was less

Table 1. Photophysical and Photochemical Data for 2-Methyl-Substituted CyHQ-Protected Acetates[a]

Compound	λ_{abs} (nm)	ε_{365} (M^{-1} cm^{-1})	Φ_u	sensitivity (ε Φ_u)	δ_u (GM)[b]
CyHQ-OAc[c]	364	7700	0.31	2387	0.32
1a	362	7010	0.28	1977	0.29
1b	362	10,000	0.16	1600	0.35
1c	365	11,240	0.13	1461	n.p.[d]
1d	363	5900	0.47	2773	0.19
1e	367	5750	0.35	2013	0.14
1f	345	5250	0.39	2048	0.06

[a]0.1 mM solution in KMOPS buffer, pH 7.2. [b]GM = 10^{-50} cm^4 s/photon. [c]Taken from lit. 30. [d]No photolysis.

sensitive to 2PE than CyHQ-OAc. Taken together, these results demonstrate that altering the electronic and steric properties of the methyl group at the C2 position of the quinoline ring has no positive effect on the 2PE-mediated photolysis of CyHQ-OAc.

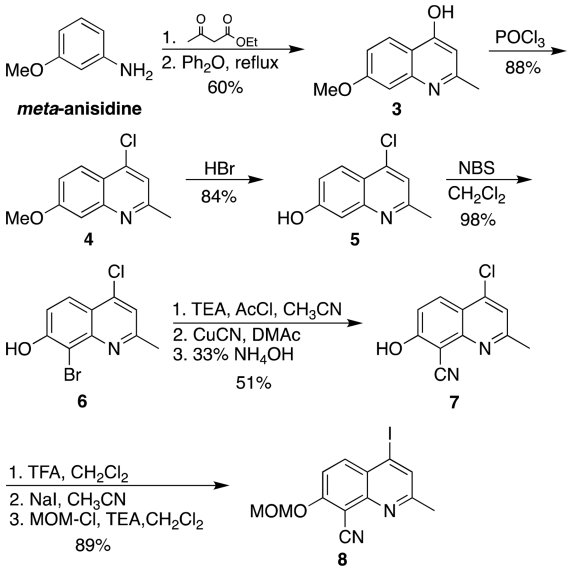
The next modification was carried out on the C4 position to exploit the so-called "meta-effect" first described by Zimmerman and co-workers.[54−57] This effect arises from the selective transmission of electron density to the meta position of an aromatic ring in the first excited state (in contrast to the ortho/para transmission in the ground state) and has been shown to increase the rate of light-induced heterolysis of various PPGs.[58−60] Furthermore, adding chlorine to the 4-position of 7-DMAQ-OAc (a previously reported quinoline-based PPG, Figure 1) improved its δ_u by more than 3-fold,[30] suggesting a positive impact of a 4-substituent on the photochemical properties of this family of PPGs. Several CyHQ-OAc derivatives with C4 modifications were synthesized to investigate the influence of meta substitution on the δ_u and Φ_u values. The effects of electron-withdrawing groups (EWG) (chloro and cyano), electron-donating groups (EDG) (dimethyl amino, morpholino, and methyl), and aromatic (phenyl and pyrrole) substituents at C4 were explored. A series of derivatives with substituted phenyl groups at the C4 position was also synthesized to modulate the electronics of the quinoline ring, and a 4-ethynylbenzene derivative was prepared to extend the conjugation of the quinoline.

The synthesis of this new series of CyHQ-based PPGs required the preparation of the C4-activated compounds **7** and **8**, which were obtained according to a high-yielding pathway (Scheme 2). The synthesis began with a condensation reaction between *meta*-anisidine and ethyl acetoacetate, and the corresponding imine was then cyclized by refluxing with diphenyl ether to afford the 4-hydroxyquinoline **3**. Chlorination with $POCl_3$ yielded the 4-chloro derivative **4**, which was then deprotected with HBr, resulting in the formation of 7-hydroxyquinoline, **5**. Subsequent bromination with NBS afforded 8-bromo-7-hydroxyquinoline, **6**, which was converted to the corresponding 8-cyano compound **7** using a previously described method.[31] The protocol involved protection of the phenol with an acetyl group followed by subsequent cyanation with copper(I) cyanide and further treatment with ammonium hydroxide solution to remove copper complexes. To facilitate the subsequent Suzuki reaction, the 4-iodo intermediate **8** was prepared from the chloride **7** by nucleophilic aromatic substitution and subsequent MOM protection.

The key step toward the synthesis of the target 4-substituted CyHQ derivatives **9a**−**9t** was a Michael addition or Suzuki or

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726−744

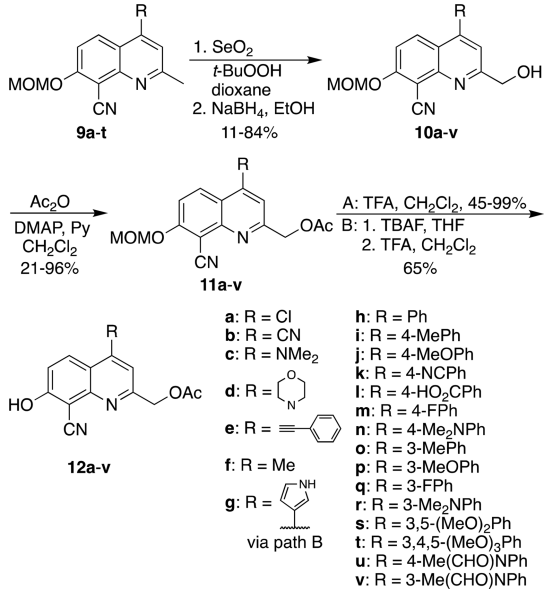
Scheme 2. Synthesis of Common Intermediates 7 and 8



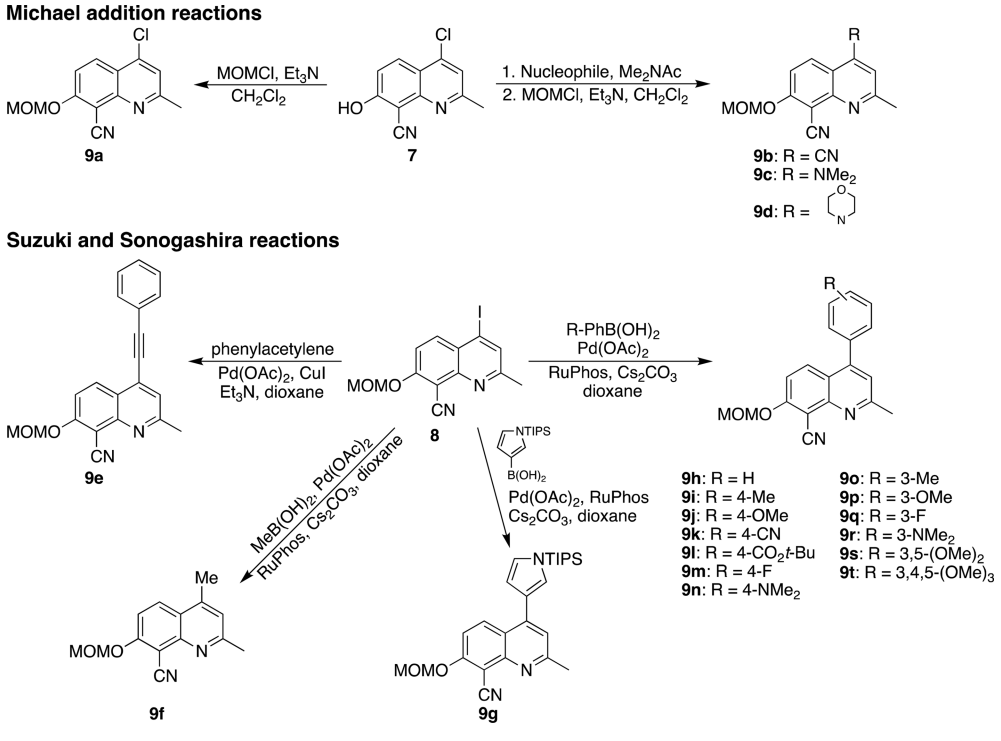
Sonogashira coupling at C4 of the 4-chloro and 4-iodo intermediates 7 and **8**, respectively (Scheme 3). The Michael addition reactions were performed on substrate **7** in refluxing *N*,*N*′-dimethylacetamide with the addition of the nucleophile (copper cyanide for **9b**, dimethylamine generated by decomposition of the solvent for **9c**, or morpholine for **9d**). A Sonogashira coupling reaction was used to prepare the phenylacetylene derivative **9e** from iodide **8**. Suzuki coupling reactions on iodide **8** under typical conditions (boronic acid, palladium acetate, and phosphine ligand in anhydrous dioxane) afforded 4-substituted compounds **9f**–**9t** in good to high yields (Scheme 3).

The pathway leading to 4-substituted CyHQ-protected acetates **12a**–**12v** began with a Riley oxidation reaction of **9a**–**9t** followed by reduction of the intermediate aromatic aldehydes with $NaBH_4$ to yield benzylic alcohols **10a**–**10v**. During the first step of the process, the two dimethylaminophenyl derivatives **9n** and **9r** partially underwent an over-oxidation reaction generating, together with the expected derivatives **10n** and **10r**, the N-formylated products **10u** and **10v**, which were isolated and carried forward separately (Scheme 4). The acetate leaving group was added by

Scheme 4. Synthesis of 4-Substituted CyHQ-Protected Acetates 12a–12v



Scheme 3. Synthesis of 4-Substituted Quinolines 9a–9t



DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726–744

acetylation followed by deprotection of the MOM group with TFA, affording the 4-substituted CyHQ-protected acetates **12a**–**12v**. For the 4-pyrrole derivative **12g**, an additional step was necessary: treatment with tetra-butyl ammonium fluoride solution to cleave the triisopropylsilyl protecting group (Scheme 4).

The photolysis reactions proceeded cleanly, generating alcohols **13a**–**13v** and acetate as the only photoproducts (Figure 2). The time course for each photolysis reaction

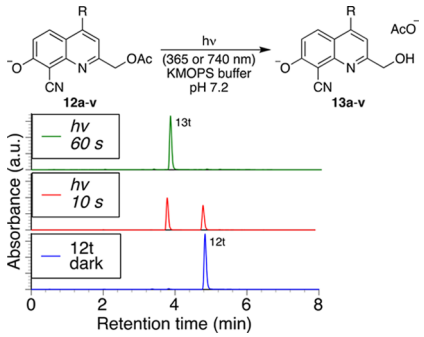


Figure 2. Photolysis of 4-substituted CyHQ acetates. (top) Photochemical reaction. (bottom) HPLC traces for the photolysis of **12t** at 365 nm (LED source, 1PE) at different time intervals. Absorbance was monitored at 320 nm.

(through 1PE or 2PE) was monitored by HPLC (selected examples are shown in Figure 3; all of the time courses are reported in the Supporting Information). At 365 nm (1PE), the reactions are extremely fast since in most cases the starting material is completely consumed within a minute of

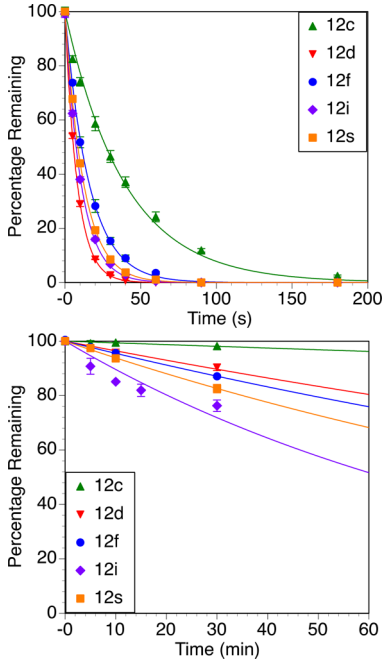


Figure 3. Time courses for the photolyses of **12c**, **12d**, **12f**, **12i**, and **12s** mediated by (top) 1PE (LED, 365 nm) and (bottom) 2PE (Ti:sapphire laser, 740 nm). The percentage remaining was determined by HPLC analysis and is the average of three runs. Lines are least-squares fits of the data to a simple exponential decay. All fits for the photolyses via 2PE approach zero asymptotically. Error bars represent the standard deviation of the mean.

irradiation. The time courses of the photolysis at 740 nm (2PE) are, as expected, considerably slower due to the small illumination volume, but we could reach up to 25% consumption of the starting material within 30 min of irradiation (250–350 mW average laser power) for compound **12i**. From these curves, values of δ_u and Φ_u could be calculated as previously described (Table 2).[28,30,31,45] The average power used to measure δ_u is larger than what would be used in biological studies on a microscope (<10 mW) in order to detect changes to the concentration of the starting material and products by HPLC in a reasonable amount of time because the laser focal volume is smaller than the sample volume. The value of δ_u measured with this technique does not depend on the average laser power.

The photophysical and photochemical properties of the library of 4-substituted CyHQ-protected acetates were compared with the literature data for BHQ-OAc and CyHQ-OAc (Table 2). All the derivatives tested exhibited good solubility (>0.1 mM) in the simulated physiological buffer KMOPS used for the photochemical experiments. The values of molar absorptivity (ε) at 365 nm (the wavelength used to perform 1PE experiments) ranged from 4150 to 8200 M^{-1} cm^{-1}, in line with those of other CyHQ-based PPG–effector conjugates.[30,31,45,46]

Substitution at the 4-position of the quinoline core had a large impact on the photochemical behavior of the chromophore. Strong electron-withdrawing groups (e.g., cyano **12b**) and extended conjugation (e.g., ethynylbenzene **12e**) resulted in red-shifted absorption and emission wavelengths (33 and 19 nm, respectively), but the photolysis quantum yield was low (Φ_u = 0.00025 and 0.04, respectively). This can be explained by the larger Stokes shift. The Stokes shift is the difference between the wavelengths of emission and absorbance of the same electronic transition and gives an account of the energy lost through vibrational relaxation and solvent reorganization processes.[61] High Stokes shift values correspond to a lower energy of the S_1 singlet excited state, which can result in a decreased reaction quantum yield. The introduction of strong electron-donating substituents (e.g., dimethylamino, **12c**) also negatively impacted the photochemical properties, whereas weaker electron-donating groups (e.g., morpholino, **12d**, and methyl, **12f**) had a positive effect. In particular, the 4-methyl derivative **12f** exhibited a 4-fold increase of δ_u (1.21 GM, Table 2) compared to unsubstituted CyHQ-OAc (0.32 GM), while retaining a good quantum yield. Furthermore, this compound had the lowest Stokes shift for the whole series (80 nm) reinforcing the hypothesis that this parameter is an important predictor of an efficient photolysis reaction.

Modulating the electronic properties of the C4 substituent had dramatic effects on the photochemical behavior of the CyHQ PPG. For this reason, we synthesized a series of CyHQ derivatives with a range of C4-phenyl groups bearing different substituents (EWG, EDG) at the para or meta positions (**12h**–**12v**). Electron-deficient C4 substituents (**12 k** and **12l**) resulted in a marked decrease of the photolysis quantum yield (Table 2), attributable to high Stokes shift values (153 and 134 nm, respectively). The introduction of a para or meta dimethylamino group (**12n** and **12r**) also dramatically reduced the quantum yield, and these compounds exhibited no reactivity through 2PE, as similarly observed for the dimethylamino derivative **12c**. Changing the aromatic group to 3-pyrrole (**12g**) resulted in an increase of the quantum yield

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726–744

Table 2. Photophysical and Photochemical Data for 4-Substituted CyHQ Acetates[a]

compound	C4 substituent	λ_{abs} (nm)	λ_{em} (nm)	Stokes shift (nm)	ε_{365} (M^{-1} cm^{-1})	Φ_u	sensitivity (ε Φ_u)	δ_u (GM)[b]	τ_d (h)[c]
BHQ-OAc[d]	H	369	500	131	2600	0.29	754	0.59	71
CyHQ-OAc[d]	H	364	449	85	7700	0.31	2387	0.32	484
12a	Cl	369	460	91	6650	0.30	1981	0.48	217
12b	CN	397	523	126	4560	0.00025	1	n.p.[e]	1283
12c	NMe_2	348	443	95	4670	0.22	1016	0.10	213
12d	morpholine	355	447	92	5860	0.48	2791	1.10	338
12e	ethynylbenzene	383	504	121	5580	0.04	198	n.p.[e]	262
12f	Me	365	445	80	5840	0.24	1424	1.21	373
12g[f]	3-pyrrole	364	458	94	4800	0.44	2119	n.p.[e]	379
12h	Ph	371	470	99	4480	0.44	1979	0.67	405
12i	4-Me-Ph	369	465	96	5020	0.50	2779	2.12	400
12j	4-MeO-Ph	371	465	94	6710	0.42	2869	1.43	572
12k	4-CN-Ph	363	516	153	6750	0.04	262	0.04	1199
12l	4-CO_2H-Ph	361	495	134	6480	0.19	1247	0.07	1421
12m	4-F-Ph	370	467	97	8200	0.14	1164	0.96	672
12n	4-Me_2N-Ph	363	454	91	7930	0.002	20	n.p.[e]	402
12o	3-Me-Ph	370	474	104	5220	0.44	2282	1.11	393
12p	3-MeO-Ph	370	485	115	6260	0.23	1443	0.73	787
12q	3-F-Ph	370	489	119	7810	0.14	1071	0.99	592
12r	3-Me_2N-Ph	369	473	104	4890	0.003	13	n.p.[e]	126
12s	3,5-$(MeO)_2$-Ph	370	476	106	5000	0.31	1520	2.07	339
12t	3,4,5-$(MeO)_3$-Ph	370	476	106	6000	0.40	2412	1.83	1356
12u	4- Me(CHO)N-Ph	378	462	84	4150	0.39	1614	0.55	616
12v	3-Me(CHO)N-Ph	370	485	115	4960	0.35	1733	0.50	538

[a]0.1 mM solution in KMOPS buffer, pH 7.2. [b]GM = 10^{-50} cm^4 s/photon. [c]Time constant of dark hydrolysis in KMOPS buffer at room temperature. [d]Data from lit. 30 except for λ_{em}, which was measured in this work. [e]No photolysis. [f]Secondary photoproducts detected. See Table S1 in the Supporting Information for additional data.

(Φ_u = 0.44) and a complete loss of sensitivity to 2PE. When a weak EWG or EDG was attached to the aromatic ring, we observed an increase in the value of δ_u (0.49−2.12 GM) compared to CyHQ-OAc. Compounds bearing a weak electron-withdrawing group (e.g., 4-fluoro **12m**, 3-methoxy **12p**, and 3-fluoro **12q**) despite having high δ_u values suffer from low quantum yields, possibly caused by the increased Stoke shift values. The introduction of a second methoxy group (**12s**) restored the Φ_u value to that of 4-unsubstituted CyHQ (0.31) and enhanced δ_u by more than 6-fold (2.07 GM). The 3,4,5-trimethoxy derivative **12t** showed a high δ_u value (1.83 GM) and excellent quantum yield (Φ_u = 0.40). The best PPG of the series was derivative **12i**, bearing a *para*-tolyl group at the 4-position, which exhibited the highest values of δ_u (2.12 GM) and Φ_u (0.50). The former presents a 7-fold increase from the value of δ_u for CyHQ-OAc.

An important requirement for a good PPG for biological use is the stability toward spontaneous hydrolysis in the dark to avoid the activation of the target before light exposure. The time constants of dark hydrolysis (τ_d) for each derivative (Table 2) were obtained by incubating the compounds in the KMOPS buffer at room temperature in the dark, monitoring the degradation at different time intervals over 7 days by HPLC. All compounds displayed excellent stability in the dark with values of τ_d typically above 10 days.

From the evaluation of the photophysical and photochemical properties of the C4-substituted acetates, several compounds stand out as good PPGs with enhanced sensitivity to 2PE compared to unsubstituted CyHQ. The 4-methylphenyl derivative **12i** represents the best compound in terms of photolysis efficiency and sensitivity to 2PE, together with the 3,5-dimethoxyphenyl derivative **12s**, which exhibits the second highest value of δ_u. Compound **12t** is interesting because it is extremely stable toward dark hydrolysis (τ_d = 56 days) while retaining an excellent quantum yield and 2PE sensitivity; therefore, it would be useful for protecting buffer-labile molecules. The 4-methyl derivative **12f** has good potential despite having slightly inferior photolysis performance because it displays high solubility (data not shown) in aqueous media and would be suitable for mediating the photolytic release of lipophilic leaving groups.

Hammett analysis was used to verify the correlation between the electronic effects on position C4 and the photochemical properties. The sensitivity ($\varepsilon \cdot \Phi_u$) and δ_u values of compounds **12h**−**12v** were plotted against the Hammett constants (σ) of the substituents[62] and fitted to a linear curve (Figure 4). A good correlation (R^2 = 0.81) was found when comparing σ values with sensitivity, demonstrating the positive effect of an electron-rich C4-phenyl group on the photochemistry. This result corroborated previous mechanistic studies that suggested the development of positive charges on the C2 methylene group during the course of the cleavage reaction.[43] The enhanced transmission of electron density from the meta position results in stabilization of the cation, increasing its lifetime and enabling more efficient photolysis. A similar observation was made when δ_u versus the Hammett constant was plotted, albeit with a lower value of R^2.

We prepared an additional set of derivatives bearing a leaving group with a strong UV absorbance to monitor and quantify the released product because acetate release cannot be monitored by HPLC. Homopiperonylic acid (ε = 1870 M^{-1} cm^{-1} at 280 nm) was selected as the leaving group, and it was conjugated to those 4-substituted CyHQ PPGs that showed the best overall photochemical properties, generating com-

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726−744

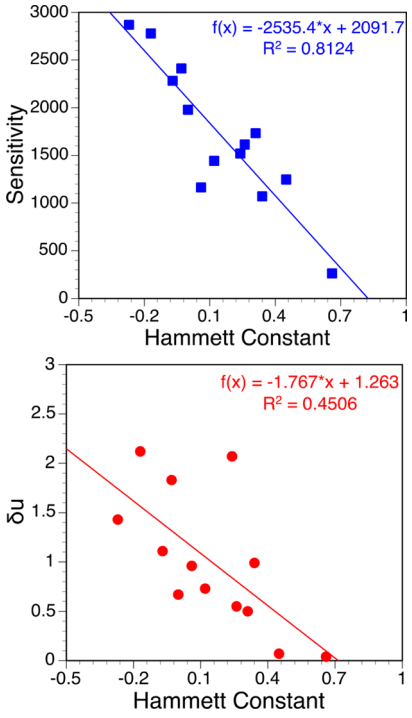
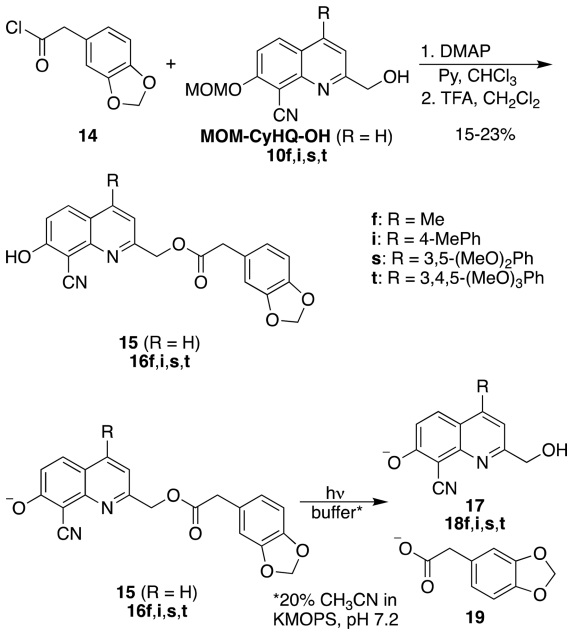


Figure 4. Hammett correlation plots for compounds **12h**–**12v**. (top) Sensitivity values were plotted against the Hammett constant[62] and fitted to a linear curve (blue line). (bottom) The values of δ_u were plotted against the Hammett constant[62] and fitted to a linear curve (red line). Compounds **12m** and **12r** were omitted because they do not photolyze through 2PE.

pounds **16f**, **16i**, **16s**, and **16t** (Scheme 5). CyHQ-protected homopiperonylate **15** was prepared for comparison. The synthesis of this set of derivatives required the preparation of acyl chloride **14** from homopiperonylic acid with oxalyl chloride and DMF (cat.) in toluene (97% yield).[63]

Scheme 5. Synthesis and Photolysis Reaction of 4-Substituted CyHQ-Protected Homopiperonylic Acids 15 and 16f, 16i, 16s, and 16t



Subsequently, **14** was reacted with MOM-CyHQ-OH and each primary alcohol, **10f**, **10i**, **10s**, and **10t**, followed by MOM group deprotection in TFA, affording the target compounds **15** and **16f**, **16i**, **16s**, and **16t** (Scheme 5).

The homopiperonylate derivatives were subjected to the same photochemical evaluation performed for compounds **12a**–**12v**. In this case, the solubility of the constructs was affected by the lipophilicity of the leaving group, so acetonitrile (20% v/v) was added to the KMOPS buffer to facilitate complete solubilization. The photophysical constants (λ_{abs}, λ_{em}, Stokes shift, and ε_{365}) were equivalent to those of the corresponding acetates (Table 3), suggesting that the introduction of the homopiperonylate group did not impact the photophysical properties of the 4-substituted CyHQ PPG. The photochemical properties were greatly enhanced by this new leaving group. The new constructs were exceptionally stable toward hydrolysis in the dark, showing almost no spontaneous decomposition for up to 7 days of incubation in the KMOPS buffer (Table 3). The photolysis reaction was extremely efficient at 365 nm (1PE) and 740 nm (2PE) and high yielding (Figure 5). The photocleavage was generally completed within 30–40 s at 365 nm (1PE). Through 2PE (740 nm), the 4-substituted CyHQ derivatives **16f**, **16i**, **16s**, and **16t** released homopiperonylate faster and more effectively than the parent CyHQ derivative **15**.

Compounds **16f**, **16i**, **16s**, and **16t** exhibited the highest values of Φ_u and δ_u ever recorded for quinoline-based PPGs (Φ_u = 0.62–0.88 and δ_u = 1.84–2.64 GM). It is evident that the high values of Φ_u and δ_u are determined by the 4-substitution since the unsubstituted control derivative **15** displays only modest increases of Φ_u and δ_u (0.4 and 0.66 GM, respectively) compared to CyHQ-OAc. We monitored and quantified by HPLC the release of homopiperonylate, and the chemical yields obtained were 63–92% (Table 3 and Figures S34 and S35, Supporting Information), confirming the efficiency of these 4-substituted CyHQ PPGs and the broad applicability of the strategy. A systematic evaluation of the solubility of the derivatives in pure KMOPS buffer was carried out to check this aspect of the biocompatibility of the probes (Table 3). With the exception of compound **16s**, the PPG constructs display solubility values compatible with the typical concentrations used for photoactivation experiments in biological preparations (1–10 μM).[64,65]

To demonstrate that the photochemistry was driven by 2PE at 740 nm, we conducted a power-dependence 2PE-mediated photolysis experiment on compounds **16i** and **16s**. The photolysis of PPGs through 2PE will depend quadratically on the average power of the laser.[17,66] Solutions of **16i** and **16s** were each irradiated for 15 min using different laser powers (250–650 mW), and the resulting percentage of photolysis was plotted against the laser power (Figure 6). The data for compound **16s** fit to a power curve ($y = ax^b$) with b = 2.09 (R^2 = 0.999), confirming a 2PE mechanism. Derivative **16i** fit less well (b = 1.28, R^2 = 0.996). Both compounds exhibited a quadratic dependence of the photolysis reaction on the laser power, giving R^2 = 0.999 for the corresponding curve fits, which also support a 2PE mechanism.

CONCLUSIONS

We have created a series of PPGs that are efficiently photolyzed through 2PE and are well suited for physiological studies. Functionalizing the C4 position of the CyHQ PPG with electron-donating substituents remarkably improved the

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726–744

Table 3. Photophysical and Photochemical Data for 4-Substituted CyHQ-Protected Homopiperonylic Acids 15 and 16f, 16i, 16s, and 16t[a]

cmp	C4 substituent	λ_{abs} (nm)	λ_{em} (nm)	Stokes shift (nm)	ε_{365} (M^{-1} cm^{-1})	Φ_u	sensitivity (ε Φ_u)	δ_u (GM)[b]	τ_d (h)[c]	yield (%)[d]	solubility (μM)[e]
15	H	367	449	82	6560	0.40	2605	0.66	2568	78	109
16f	Me	367	444	77	3500	0.88	3097	1.84	1783	73	98
16i	4-Me-Ph	373	463	90	4530	0.74	3346	2.25	n.h.[f]	74	24
16s	3,5-$(MeO)_2$Ph	374	481	107	4310	0.81	3494	2.64	n.h.	63	n.s.[g]
16t	3,4,5-$(MeO)_3$-Ph	374	471	97	5240	0.62	3260	2.37	n.h.	92	18

[a]0.1 mM solution in KMOPS buffer, pH 7.2, with 20% of CH_3CN added. [b]GM = 10^{-50} (cm^4 s)/photon. [c]Time constant of hydrolysis in KMOPS buffer (with 20% CH_3CN added) in the dark at room temperature. [d]Chemical yield of released homopiperonylate under 1PE. [e]In KMOPS buffer without acetonitrile cosolvent. [f]No hydrolysis (<2% hydrolysis detected after 7 days). [g]Not soluble. See Table S2 in the Supporting Information for additional data.

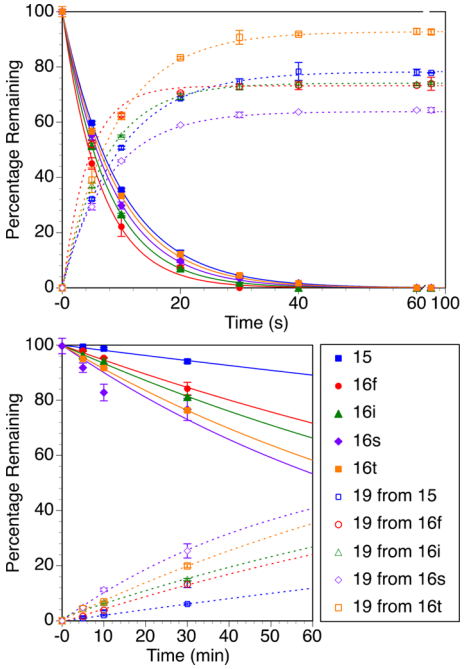


Figure 5. Time courses for the photolyses of **15** and **16f**, **16i**, **16s**, and **16t** through (top) 1PE (LED, 365 nm) and (bottom) 2PE (Ti:sapphire laser, 740 nm)). The percentage remaining was determined by HPLC analysis and is the average of three runs. The percent yield of homopiperonylic acid **19** is also given. Lines are least-squares fits of a simple exponential decay (solid lines) and an exponential rise to max (dotted lines). All fits for the photolyses via 2PE approach zero asymptotically. Error bars represent the standard deviation of the mean.

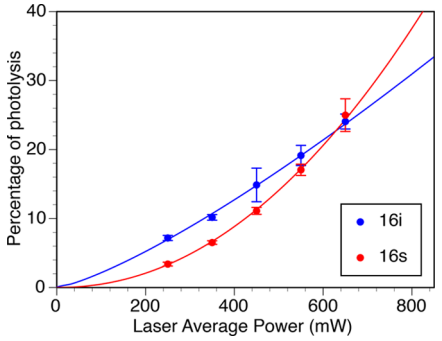


Figure 6. Dependence of the photolysis rate on average laser power. Compounds **16i** (blue dots) and **16s** (red dots) were photolyzed at different laser powers for 15 min. The remaining concentrations were determined by HPLC analysis and are the average of three runs. Lines are least-squares fits of a power equation ($y = ax^b$). For **16i**, $a = 5.86 \times 10^{-3}$ and $b = 1.28$. For **16s**, $a = 3.36 \times 10^{-5}$ and b = 2.09. The data also fit a quadratic equation ($y = ax^2 + bx + c$). For **16i**, $a = 2.29 \times 10^{-5}$, $b = 2.21 \times 10^{-2}$, $c = 1.21 \times 10^{-2}$, and $R^2 = 0.999$. For **16s**, $a = 6.51 \times 10^{-5}$, $b = 4.43 \times 10^{-2}$, $c = 1.01 \times 10^{-2}$, and $R^2 = 0.999$. Error bars represent the standard deviations of the mean.

photochemical properties, in particular, Φ_u (0.50) and δ_u (2.12 GM). Moreover, these 4-substituted-CyHQ PPGs exhibited good solubility at physiological pH and stability toward spontaneous hydrolysis in the dark and underwent rapid, clean photochemical reactions without generating harmful byproducts. Hammett analysis showed a good correlation between the electronic effects and the photolysis efficiency, demonstrating the positive influence on the photochemistry of an electron-rich group at the 4-position of CyHQ. This result was in accordance to our previous mechanistic investigations suggesting a positive charge development on the C2 methylene group during the course of the cleavage reaction. Studies on the photocleavage of several 4-substituted CyHQ PPG–homopiperonylic acid conjugates demonstrated a high chemical yield of a model biological effector (63–92%). The photochemical efficiency of these derivatives was higher than that of the corresponding acetates: Φ_u = 0.62–0.88 and δ_u = 1.84–2.64 GM. Taken together, these factors will make these quinoline-based PPGs useful for photoactivation experiments in vitro as well as in tissue and whole animals where irradiation at long wavelengths is required to ensure low photodamage and deeper tissue penetration. Results of experiments that demonstrate the effectiveness of these probes in physiology will be reported in due course.

EXPERIMENTAL SECTION

Synthesis. *General Procedure.* Reagents and solvents were purchased from commercial sources and used without purification. For reactions carried out above room temperature, an oil bath was used as the source of heating. The UV spectra were recorded on a Lambda25 UV–vis–NIR spectrophotometer (PerkinElmer). Emission spectra were obtained with a PerkinElmer LS 55 fluorescence spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Avance III HD 500 or 600 MHz NMR spectrometer. uHPLC analysis and preparative HPLC purifications were carried on an Agilent Infinity series system with an autosampler and diode array detector using Zorbax eclipse C-18 reverse phase columns, having a mobile phase composed of water with 0.1% TFA and acetonitrile. HRMS was performed on an Agilent 6540 HD Accurate Mass QTOF/LC/MS with electrospray ionization (ESI). Purification was carried out using flash chromatography on an Isolera Spektra 4 with Biotage SNAP cartridges packed with KPSIL silica. KMOPS buffer consisted of 100 mM KCl and 10 mM MOPS (3-(*N*-morpholino)-propanesulfonic acid) titrated to pH 7.2 with 0.1 N NaOH.

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726–744

General Procedure for the Preparation of 2-Methyl-Substituted Acetates ***1a−1f.*** To a solution of MOM-CyHQ-R-OH[46] (30 mg, 1 equiv) in CH_2Cl_2 (5 mL), pyridine (5 equiv) and 4-dimethylaminopyridine (1 equiv) were added. The mixture was cooled to 0 °C with an ice bath and acetic anhydride (4 equiv) was added dropwise. The mixture was stirred at 0 °C for 30 min and then at room temperature for 6 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL), and the resulting solution was washed with a saturated ammonium chloride solution (10 mL) and H_2O (2 × 10 mL), dried over $MgSO_4$, and concentrated to dryness. The resulting residue was purified by column chromatography (hexanes/EtOAc gradient) to yield the corresponding MOM-protected acetate, which was dissolved in CH_2Cl_2 (2 mL). TFA (0.2 mL) was added dropwise and the reaction was stirred for up to 5 h until HPLC showed complete consumption of the starting material. After evaporation of the solvent, the resulting residue was purified either by trituration with tetrahydrofuran or by column chromatography with $MeOH/CH_2Cl_2$, affording the respective acetates **1a−1f**.

*1-(8-Cyano-7-hydroxyquinolin-2-yl)ethyl Acetate (**1a**).* (26 mg, 87% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.26 (d, J = 8.4 Hz, 1H), 8.01 (d, J = 9.0 Hz, 1H), 7.47 (d, J = 8.3 Hz, 1H), 7.27 (d, J = 9.0 Hz, 1H), 6.02 (q, J = 6.8 Hz, 1H), 2.20 (s, 3H), 1.68 (d, J = 6.8 Hz, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.0, 164.13, 162.9, 148.3, 137.3, 133.7, 121.5, 117.7, 116.4, 114.7, 94.5, 73.2, 19.7, 19.4; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{14}H_{13}N_2O_3$, 257.0921; found, 257.0913.

*1-(8-Cyano-7-hydroxyquinolin-2-yl)-2-methylpropyl Acetate (**1b**).* (22 mg, 75% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.24 (d, J = 8.4 Hz, 1H), 8.01 (d, J = 9.0 Hz, 1H), 7.41 (d, J = 8.3 Hz, 1H), 7.26 (d, J = 9.0 Hz, 1H), 5.75 (d, J = 5.5 Hz, 1H), 2.54−2.35 (m, 1H), 2.22 (s, 3H), 0.99 (dd, J = 18.9, 6.8 Hz, 6H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.2, 164.0, 161.7, 148.3, 136.7, 133.7, 121.4, 117.6, 117.3, 114.8, 94.5, 81.1, 32.5, 19.5, 18.1; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{16}H_{17}N_2O_3$, 285.1234; found, 285.1234.

*(8-Cyano-7-hydroxyquinolin-2-yl)(phenyl)methyl Acetate (**1c**).* (20 mg, 69% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.16 (d, J = 8.4 Hz, 1H), 7.93 (d, J = 9.1 Hz, 1H), 7.56−7.50 (m, 2H), 7.42 (d, J = 8.4 Hz, 1H), 7.36 (dd, J = 8.3, 6.6 Hz, 2H), 7.33−7.27 (m, 1H), 7.24 (d, J = 9.0 Hz, 1H), 6.93 (s, 1H), 2.29 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 170.7, 164.1, 161.3, 148.3, 138.4, 137.2, 133.7, 128.3, 128.1, 127.2, 121.4, 117.8, 117.1, 114.8, 94.6, 78.3, 19.7; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{19}H_{15}N_2O_3$, 319.1077; found, 319.1081.

*(8-Cyano-7-hydroxyquinolin-2-yl)(cyclopropyl)methyl Acetate (**1d**).* (9 mg, 30% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.26 (d, J = 8.4 Hz, 1H), 8.01 (d, J = 9.1 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.27 (d, J = 9.0 Hz, 1H), 5.34 (d, J = 8.7 Hz, 1H), 2.20 (s, 3H), 1.44 (dddd, J = 12.9, 8.5, 6.5, 4.9 Hz, 1H), 0.73−0.58 (m, 4H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.0, 164.0, 161.7, 148.3, 137.1, 133.7, 121.6, 117.6, 117.0, 114.7, 94.6, 80.6, 19.7, 15.0, 3.1, 2.2; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{16}H_{15}N_2O_3$, 283.1077; found, 283.1079.

*1-(8-Cyano-7-hydroxyquinolin-2-yl)-2,2,2-trifluoroethyl Acetate (**1e**).* (15 mg, 49% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.38 (d, J = 8.4 Hz, 1H), 8.09 (d, J = 9.1 Hz, 1H), 7.62 (d, J = 8.4 Hz, 1H), 7.35 (d, J = 9.1 Hz, 1H), 6.46 (q, J = 6.9 Hz, 1H), 2.30 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 168.8, 164.5, 153.1, 148.3, 137.7, 133.8, 123.1 (q, J = 287.3 Hz), 122.3, 118.9, 117.6, 114.3, 94.7, 73.0 (q, J = 32.3 Hz), 19.0; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{14}H_{10}F_3N_2O_3$, 311.0638; found, 311.0605.

*1-(8-Cyano-7-hydroxyquinolin-2-yl)but-3-en-1-yl Acetate (**1f**).* (20 mg, 68% yield). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 8.37 (d, J = 8.5 Hz, 1H), 8.11 (d, J = 9.1 Hz, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.35 (d, J = 9.1 Hz, 1H), 5.92−5.86 (m, 1H), 5.86−5.77 (m, 1H), 5.12 (dq, J = 17.1, 1.6 Hz, 1H), 5.06 (ddd, J = 10.3, 2.1, 1.1 Hz, 1H), 2.81 (dddt, J = 14.5, 6.5, 5.0, 1.4 Hz, 1H), 2.76−2.66 (m, 1H), 2.15 (s, 3H); ^{13}C NMR{1H} (126 MHz, DMSO-d_6, δ): 170.5, 164.6, 161.5, 148.3, 138.1, 134.6, 133.9, 121.58, 118.8, 118.6, 117.7, 115.7, 94.5, 75.7, 38.7, 21.3; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{16}H_{15}N_2O_3$, 283.1077; found, 283.1066.

Preparation of Intermediates ***7*** *and* ***8.*** *7-Methoxy-2-methylquinolin-4-ol (**3**).* A mixture of *meta*-anisidine (2.24 mL, 20.00 mmol, 1 equiv) and ethyl acetoacetate (3.04 mL, 24.00 mmol, 1.2 equiv) was stirred at 100 °C for 16 h. The reaction mixture was cooled to room temperature and added dropwise to a refluxing (260 °C) solution of diphenyl ether (25 mL). After stirring at reflux for 1.5 h, the mixture was cooled to room temperature and stirred overnight. The resulting precipitate was collected by filtration, washed with diethyl ether (3 × 30 mL), and dried under vacuum to yield **3** as a dark brown, powdery solid (1.05 g, 5.55 mmol, 60%). ^{1}H NMR (500 MHz, chloroform-d, δ): 10.43 (s, 1H), 8.25 (d, J = 8.9 Hz, 1H), 6.92 (dd, J = 9.0, 2.3 Hz, 1H), 6.88 (s, 1H), 6.11 (s, 1H), 3.83 (s, 3H), 2.41 (s, 3H); ^{13}C NMR{1H} (126 MHz, DMSO-d_6, δ): 162.1, 157.1, 130.5, 127.1, 123.9, 119.1, 113.0, 108.6, 99.3, 55.8, 19.8; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{11}H_{12}NO_2$, 190.0863; found, 190.0860.

*4-Chloro-7-methoxy-2-methylquinoline (**4**).* A solution of **3** (6.88 g, 36.36 mmol, 1 equiv) in $POCl_3$ (17 mL) was heated to reflux (110 °C) and stirred for 3 h. The reaction mixture was cooled to room temperature and then added dropwise to a flask containing H_2O (250 mL) on ice. The pH of the solution was raised to 7 with 2 M NaOH. The resulting precipitate was collected by vacuum filtration and washed with H_2O to yield **4** as a pink, flaky solid (6.66 g, 32.07 mmol, 88%). ^{1}H NMR (500 MHz, methanol-d_4, δ): 7.99 (d, J = 9.2 Hz, 1H), 7.33 (s, 1H), 7.26 (d, J = 2.6 Hz, 1H), 7.21 (dd, J = 9.2, 2.6 Hz, 1H), 3.94 (s, 3H), 2.63 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 161.8, 159.4, 149.7, 142.6, 124.6, 119.6, 119.4, 119.3, 105.7, 54.7, 23.1; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{11}H_{11}ClNO$, 208.0524; found, 208.0529.

*4-Chloro-2-methylquinolin-7-ol (**5**).* A solution of **4** (6.66 g, 32.07 mmol, 1 equiv) in 48% HBr (133.25 mL) was heated to reflux (125 °C) under a N_2 atmosphere and stirred overnight. The reaction mixture was then cooled to room temperature, placed in an ice bath, and diluted with H_2O (50 mL). K_2CO_3 (powder) was added until the mixture reached pH 7. The mixture was stirred for a few hours to ensure all of the product had precipitated, and the precipitate was collected by filtration, washed with H_2O (4 × 100 mL), and dried under vacuum to yield **5** as a brown powder (5.19 g, 26.80 mmol, 84%). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.09 (d, J = 9.1 Hz, 1H), 7.39 (s, 1H), 7.28 (d, J = 2.3 Hz, 1H), 7.24 (dd, J = 9.0, 2.4 Hz, 1H), 2.67 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 160.3, 159.3, 149.6, 143.1, 125.0, 119.4, 119.0, 118.8, 108.5, 22.8; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{10}H_9ClNO$, 194.0367; found, 194.0378.

*8-Bromo-4-chloro-2-methylquinolin-7-ol (**6**).* To a solution of **5** (3.00 g, 15.49 mmol, 1 equiv) in CH_2Cl_2 (38 mL), acetic acid (0.93 mL, 16.32 mmol, 1.05 equiv) was added slowly, followed by NBS (3.3 g, 18.53 mmol, 1.2 equiv). The mixture was stirred at room temperature overnight, then diluted with CH_2Cl_2 (500 mL), and washed with H_2O (3 × 200 mL). The aqueous layers were combined and back-extracted with CH_2Cl_2 (3 × 200 mL). The organic layers were combined and the solvent was evaporated under vacuum to yield **6** as a dark brown solid (4.14 g, 15.19 mmol, 98% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.08 (d, J = 9.1 Hz, 1H), 7.43 (s, 1H), 7.32 (d, J = 9.1 Hz, 1H), 2.72 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 160.4, 157.0, 147.0, 143.0, 123.8, 119.7, 118.4, 110.4, 106.0, 23.3; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{10}H_8BrClNO$, 271.9472; found, 271.9486.

*4-Chloro-7-hydroxy-2-methylquinoline-8-carbonitrile (**7**).* To a solution of **6** (4.43 g, 16.34 mmol, 1 equiv) in acetonitrile (35 mL), triethylamine (5.7 mL, 40.40 mmol, 2.5 equiv) was added while stirring. The mixture was cooled to 0 °C with an ice bath and a solution of acetyl chloride (1.4 mL, 19.56 mmol, 1.2 equiv) in acetonitrile (7 mL) was added dropwise. The ice bath was removed and the mixture was allowed to reach room temperature and stirred for 1 h. The solvent was evaporated under vacuum, and the resulting residue was partitioned between EtOAc (100 mL) and H_2O (100 mL). The layers were separated and the organic layer was washed with a saturated $NaHCO_3$ solution (50 mL) and H_2O (2 × 50 mL).

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726−744

The organic layer was then evaporated to dryness. The resulting crude product was dissolved in *N*,*N*-dimethylacetamide (20 mL) and added dropwise to a refluxing (160 °C) solution of copper(I) cyanide (2.93 g, 32.70 mmol, 2 equiv) in *N*,*N*-dimethylacetamide (15 mL). The reaction mixture was stirred for 1 h and then cooled. Once the temperature had reached 50 °C, H_2O (100 mL) was slowly added. The resulting slurry was stirred for 1 h at room temperature, and then the precipitate was collected by filtration, washed with H_2O (75 mL), and dried under vacuum. The crude product (copper adducts) was suspended in 33 wt % ammonium hydroxide solution (100 mL) and stirred at room temperature for 1 h. The mixture was filtered through alumina. The pH of the filtrate was adjusted to 3 using 36% HCl and then extracted with 2-butanol (3 × 100 mL). The organic layers were combined, washed with H_2O (50 mL), and then concentrated to dryness. The resulting solid was stirred in acetonitrile (100 mL) and filtered to remove any insoluble salts. The filtrate was concentrated to dryness to yield **7** as a brown solid (1.83 g, 8.37 mmol, 51%). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 8.13 (d, J = 9.3 Hz, 1H), 7.47 (s, 1H), 7.35 (d, J = 9.3 Hz, 1H), 2.62 (s, 3H); ^{13}C NMR{1H} (126 MHz, DMSO-d_6, δ): 164.9, 162.0, 149.7, 142.0, 129.9, 120.9, 118.9, 118.1, 115.6, 94.8, 25.1; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{11}H_8ClN_2O$, 219.0320; found, 219.0316.

*4-Iodo-7-(methoxymethoxy)-2-methylquinoline-8-carbonitrile (**8**).* To a suspension of **7** (460 mg, 2.10 mmol, 1 equiv) in CH_2Cl_2 (6 mL), TFA (0.96 mL, 12.57 mmol, 6 equiv) was added and the reaction mixture was stirred at room temperature for 30 min. The solvent was removed under vacuum to yield the corresponding pyridinium salt, which was suspended in anhydrous acetonitrile (30 mL) under a N_2 atmosphere. NaI (3.15 g, 21.04 mmol, 10 equiv) was added and the mixture was heated to reflux and stirred for 6 h. The reaction mixture was then cooled to room temperature, and the solvent evaporated under vacuum. The remaining solid was dissolved in EtOAc (100 mL) and washed with 5% $NaHSO_3$ (2 × 20 mL) and H_2O (3 × 50 mL). The organic layer was then evaporated to dryness. The residue was suspended in CH_2Cl_2 (50 mL) and cooled to 0 °C with an ice bath. Triethylamine (1.17 mL, 8.40 mmol, 4 equiv) was added, followed by a 2 M solution of MOM-Cl (3.15 mL, 6.30 mmol, 3 equiv) in CH_2Cl_2. The ice bath was removed and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and then washed with a saturated $NaHCO_3$ solution (50 mL) and H_2O (2 × 50 mL). The organic layer was dried over $MgSO_4$ and concentrated to dryness. The crude product was purified by column chromatography (hexanes/EtOAc gradient) to yield **8** as a white solid (662 mg, 1.87 mmol, 89% over three steps). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.10 (d, J = 9.4 Hz, 1H), 7.86 (s, 1H), 7.52 (d, J = 9.3 Hz, 1H), 5.47 (s, 2H), 3.59 (s, 3H), 2.74 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 162.4, 162.0, 148.1, 137.4, 133.0, 123.8, 115.8, 114.4, 111.2, 99.5, 95.1, 56.9, 24.9; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{13}H_{12}IN_2O_2$, 354.9938; found, 354.9945.

*Preparation of Compounds **9a**–**9e**. 4-Chloro-7-(methoxymethoxy)-2-methylquinoline-8-carbonitrile (**9a**).* A suspension of **7** (261 mg, 1.2 mmol, 1 equiv) in CH_2Cl_2 (30 mL) was cooled to 0 °C with an ice bath. Triethylamine (0.67 mL, 4.8 mmol, 4 equiv) was added, followed by a 2 M solution of MOM-Cl (1.8 mL, 3.6 mmol, 3 equiv) in CH_2Cl_2. The ice bath was removed and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with a saturated $NaHCO_3$ solution (30 mL) and H_2O (2 × 30 mL). The organic layer was dried over $MgSO_4$ and concentrated to dryness. The crude product was purified by column chromatography (hexanes/EtOAc gradient) to yield **9a** as a white solid (298 mg, 1.14 mmol, 95%). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.33 (d, J = 9.4 Hz, 1H), 7.57 (d, J = 9.4 Hz, 1H), 7.38 (s, 1H), 5.47 (s, 2H), 3.60 (s, 3H), 2.78 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 162.5, 162.5, 149.6, 142.6, 130.0, 121.7, 120.0, 115.4, 114.5, 99.7, 95.1, 57.0, 25.5; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{13}H_{12}ClN_2O_2$, 263.0582; found, 263.0578.

*7-(Methoxymethoxy)-2-methylquinoline-4,8-dicarbonitrile (**9b**).* Compound **7** (300 mg, 1.37 mmol, 1 equiv) was dissolved in *N*,*N*-dimethylacetamide (5 mL) and added dropwise to a refluxing (160 °C) solution of copper(I) cyanide (490 mg, 5.48 mmol, 4 equiv) in *N*,*N*-dimethylacetamide (5 mL). The reaction mixture was stirred overnight and then cooled. Once the temperature had reached 50 °C, H_2O (100 mL) was slowly added. The resulting slurry was stirred for 1 h at room temperature, and then the precipitate was collected by filtration, washed with H_2O (75 mL), and dried under vacuum. The crude product (copper adducts) was suspended in 33 wt % ammonium hydroxide solution (100 mL) and stirred at room temperature for 1 h. The mixture was filtered through alumina. The pH of the filtrate was adjusted to 3 using 36% HCl and then extracted with 2-butanol (3 × 100 mL). The organic layers were combined, washed with H_2O (50 mL), and then concentrated to dryness. The resulting solid was stirred in acetonitrile (100 mL) and then filtered to remove any insoluble salts. The filtrate was concentrated to dryness, and the resulting residue was subjected to the procedure described for converting **7** into the MOM ether **9a**, purifying by column chromatography (hexanes/EtOAc gradient) to yield **9b** as a dark solid (120 mg, 0.47 mmol, 35%). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.27 (d, J = 9.3 Hz, 1H), 7.70 (d, J = 9.3 Hz, 1H), 7.62 (s, 1H), 5.50 (s, 2H), 3.61 (s, 3H), 2.87 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 163.0, 162.0, 148.6, 130.4, 125.3, 119.1, 119.0, 117.0, 114.9, 113.9, 100.3, 95.2, 57.1, 25.5; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{14}H_{12}N_3O_2$, 254.0924; found, 254.0913.

*4-(Dimethylamino)-7-(methoxymethoxy)-2-methylquinoline-8-carbonitrile (**9c**).* A solution of **7** (300 mg, 1.37 mmol, 1 equiv) in *N*,*N*-dimethylacetamide (10 mL) was stirred at reflux (160 °C) overnight. After cooling, a saturated solution of ammonium chloride (20 mL) was added and the resulting slurry was extracted with EtOAc (3 × 50 mL). The combined EtOAc extracts were dried over $MgSO_4$ and concentrated to dryness. The resulting residue was subjected to the procedure described for converting **7** into the MOM ether **9a**, purifying by column chromatography (hexanes/EtOAc gradient) to yield **9c** as a dark solid (180 mg, 0.66 mmol, 48%).^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.15 (d, J = 9.4 Hz, 1H), 7.33 (d, J = 9.4 Hz, 1H), 6.63 (s, 1H), 5.43 (s, 2H), 3.59 (s, 3H), 3.03 (s, 6H), 2.70 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 162.5, 161.3, 157.7, 150.9, 130.5, 116.5, 115.4, 112.0, 107.6, 99.6, 94.9, 56.8, 43.9, 29.3; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{15}H_{18}N_3O_2$, 272.1394; found, 272.1409.

*7-(Methoxymethoxy)-2-methyl-4-morpholinoquinoline-8-carbonitrile (**9d**).* To a solution of **7** (100 mg, 0.45 mmol, 1 equiv) in *N*,*N*-dimethylacetamide (2 mL), K_2CO_3 (190 mg, 1.37 mmol, 3 equiv) and morpholine (78 μL, 0.90 mmol, 2 equiv) were added. The mixture was stirred overnight at 100 °C. After cooling, a saturated solution of ammonium chloride (20 mL) was added, and the resulting slurry was extracted with EtOAc (3 × 50 mL). The combined EtOAc extracts were dried with $MgSO_4$ and concentrated to dryness. The resulting residue was subjected to the procedure described for converting **7** into the MOM ether **9a**, purifying by column chromatography (hexanes/EtOAc gradient) to yield **9d** as a dark yellow solid (58 mg, 0.18 mmol, 39%). ^{1}H NMR (500 MHz, chloroform-*d*, δ) 8.07 (d, J = 9.4 Hz, 1H), 7.35 (d, J = 9.3 Hz, 1H), 6.70 (s, 1H), 5.39 (s, 2H), 3.94 (t, J = 4.6 Hz, 4H), 3.54 (s, 3H), 3.23–3.09 (m, 4H), 2.67 (s, 3H).; ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 166.9, 161.1, 155.5, 142.6, 132.6, 115.8, 112.0, 111.8, 106.5, 88.1, 66.0, 52.5, 19.3; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{17}H_{20}N_3O_3$, 314.1499; found, 314.1497.

*7-(Methoxymethoxy)-2-methyl-4-(phenylethynyl)quinoline-8-carbonitrile (**9e**).* To a solution of **8** (60 mg, 0.17 mmol, 1 eq) in anhydrous dioxane (5 mL) under a N_2 atmosphere, triethylamine (0.12 mL, 0.8 mmol, 4.5 equiv) and phenylacetylene (0.04 mL, 0.34 mmol, 2 equiv) were added dropwise, followed by dropwise addition of a solution of RuPhos (7 mg, 0.017 mmol, 0.1 equiv), palladium acetate (2 mg, 0.008 mmol, 0.05 eq), and copper(I) iodide (2 mg, 0.008 mmol, 0.05 equiv) in anhydrous dioxane (2 mL). The mixture was heated to 80 °C and stirred under a N_2 atmosphere for 1 h. After cooling, the mixture was diluted with EtOAc (50 mL) and filtered through celite. The filtrate was washed with H_2O (2 × 50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$,

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726–744

concentrated to dryness, and purified by column chromatography (hexanes/EtOAc gradient) to yield **9e** as a clear oil (48 mg, 0.15 mmol, 87%). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.40 (d, J = 9.2 Hz, 1H), 7.66−7.61 (m, 2H), 7.55−7.51 (m, 1H), 7.48−7.40 (m, 4H), 5.46 (s, 2H), 3.59 (s, 3H), 2.78 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 162.1, 161.9, 148.6, 132.0, 131.8, 129.9, 129.6, 128.7, 124.0, 121.8, 121.1, 114.9, 114.8, 99.6, 98.9, 95.1, 84.3, 56.9, 25.5; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{21}H_{17}N_2O_2$, 329.1285; found, 329.1274.

General Procedure for Suzuki Coupling Reaction. Compound **8** (100 mg, 0.28 mmol, 1 equiv), the boronic acid (0.42 mmol, 1.5 equiv) and cesium carbonate (184 mg, 0.56 mmol, 1 eq) were dissolved in anhydrous dioxane (7 mL) under a N_2 atmosphere. A solution of RuPhos (13 mg, 0.028 mmol, 0.1 equiv) and palladium acetate (4 mg, 0.017 mmol, 0.05 equiv) in anhydrous dioxane (2 mL) was added dropwise. The mixture was heated to 80 °C and stirred under a N_2 atmosphere. Upon completion of the reaction (4−12 h), the mixture was cooled, diluted with EtOAc (50 mL), and filtered through celite. The filtrate was washed with H_2O (50 mL), saturated $NaHCO_3$ solution (50 mL), and brine (50 mL). The organic layer was dried over $MgSO_4$, concentrated to dryness, and purified by column chromatography (hexanes/EtOAc gradient) to yield the pure C4-substituted compound.

*7-(Methoxymethoxy)-2,4-dimethylquinoline-8-carbonitrile (**9f**).* (40 mg, 59% yield). ^{1}H NMR (500 MHz chloroform-*d*, δ): 8.08 (d, J = 9.3 Hz, 1H), 7.45 (d, J = 9.3 Hz, 1H), 7.11 (s, 1H), 5.44 (s, 2H), 3.58 (s, 3H), 2.72 (s, 3H), 2.64 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 162.2, 161.4, 148.8, 144.4, 129.7, 122.6, 121.8, 115.2, 114.0, 99.8, 95.0, 56.8, 25.5, 18.4; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{14}H_{15}N_2O_2$, 243.1189; found, 243.1186.

*7-(Methoxymethoxy)-2-methyl-4-(1-(triisopropylsilyl)-1H-Pyrrol-3-yl)quinoline-8-carbonitrile (**9g**).* (56 mg, 67% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.46 (d, J = 9.4 Hz, 1H), 7.40 (d, J = 9.4 Hz, 1H), 7.25 (s, 1H), 7.05 (t, J = 1.7 Hz, 1H), 6.92 (t, J = 2.4 Hz, 1H), 6.59 (dd, J = 2.8, 1.4 Hz, 1H), 5.43 (s, 2H), 3.58 (s, 3H), 2.77 (s, 3H), 1.52 (hept, J = 7.5 Hz, 3H), 1.16 (d, J = 7.5 Hz, 18H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 161.9, 161.4, 149.7, 143.6, 132.4, 125.5, 124.5, 122.4, 120.7, 120.6, 115.5, 113.7, 111.8, 99.5, 94.9, 56.8, 25.6, 17.8, 11.7; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{26}H_{36}N_3O_2Si$, 450.2571; found, 450.2571.

*7-(Methoxymethoxy)-2-methyl-4-phenylquinoline-8-carbonitrile (**9h**).* (36 mg, 50% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.01 (d, J = 9.4 Hz, 1H), 7.57−7.49 (m, 3H), 7.48−7.42 (m, 2H), 7.41 (d, J = 9.4 Hz, 1H), 7.23 (s, 1H), 5.43 (s, 2H), 3.58 (s, 3H), 2.82 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 161.9, 161.7, 149.4, 148.7, 137.2, 131.9, 129.4, 128.7, 121.9, 120.4, 115.2, 114.4, 107.3, 99.7, 95.0, 56.8, 25.6; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{19}H_{17}N_2O_2$, 305.1285; found, 305.1272.

*7-(Methoxymethoxy)-2-methyl-4-(p-tolyl)quinoline-8-carbonitrile (**9i**).* (90 mg, 99% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 7.98 (d, J = 9.5 Hz, 1H), 7.44 (d, J = 9.5 Hz, 1H), 7.36−7.28 (m, 4H), 7.22 (s, 1H), 5.46 (s, 2H), 3.55 (s, 3H), 2.71 (s, 3H), 2.43 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 162.0, 161.9, 149.2, 148.9, 138.8, 133.9, 131.9, 129.1, 129.1, 121.6, 120.1, 114.5, 114.3, 98.2, 94.9, 55.7, 23.9, 19.9; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{20}H_{19}N_2O_2$, 319.1441; found, 319.1443.

*7-(Methoxymethoxy)-4-(4-methoxyphenyl)-2-methylquinoline-8-carbonitrile (**9j**).* (59 mg, 63% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.13 (d, J = 9.4 Hz, 1H), 7.54 (d, J = 9.5 Hz, 1H), 7.50−7.40 (m, 2H), 7.32 (s, 1H), 7.17−7.07 (m, 2H), 5.51 (s, 2H), 3.90 (s, 3H), 3.58 (s, 3H), 2.77 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 163.2, 158.7, 149.6, 137.5, 135.5, 133.5, 130.7, 129.5, 127.8, 116.0, 114.8, 114.2, 113.9, 113.5, 77.3, 55.2, 53.5, 29.7; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{20}H_{19}N_2O_3$, 335.1390; found, 335.1398.

*4-(4-Cyanophenyl)-7-(methoxymethoxy)-2-methylquinoline-8-carbonitrile (**9k**).* (50 mg, 54% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 7.90−7.83 (m, 3H), 7.63−7.57 (m, 2H), 7.46 (d, J = 9.4 Hz, 1H), 7.22 (s, 1H), 5.45 (s, 2H), 3.59 (s, 3H), 2.85 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 162.0, 161.9, 149.4, 146.5, 141.9, 132.5, 130.9, 130.2, 121.8, 119.6, 118.3, 115.1, 114.8, 112.9, 100.03, 95.0, 56.9, 25.7; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{20}H_{16}N_3O_2$, 330.1237; found, 330.1236.

*tert-Butyl 4-(8-Cyano-7-(methoxymethoxy)-2-methylquinolin-4-yl)benzoate (**9l**).* (68 mg, 60% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.16 (d, J = 7.9 Hz, 2H), 7.94 (d, J = 9.4 Hz, 1H), 7.52 (d, J = 7.9 Hz, 2H), 7.42 (d, J = 9.4 Hz, 1H), 7.23 (s, 1H), 5.44 (s, 2H), 3.59 (s, 3H), 2.84 (s, 3H), 1.66 (s, 9H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 165.2, 162.0, 161.8, 149.3, 147.8, 141.2, 132.4, 131.5, 129.8, 129.3, 121.8, 120.0, 115.0, 114.7, 99.8, 95.0, 81.6, 56.9, 28.2, 25.7; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{24}H_{25}N_2O_4$, 405.1809; found, 405.1814.

*4-(4-Fluorophenyl)-7-(methoxymethoxy)-2-methylquinoline-8-carbonitrile (**9m**).* (90 mg, 99% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 7.97 (d, J = 9.4 Hz, 1H), 7.48−7.38 (m, 3H), 7.23 (dd, J = 16.4, 7.8 Hz, 3H), 5.44 (s, 2H), 3.58 (s, 3H), 2.81 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 164.1 ($^1J_{C-F}$ = 249.5 Hz), 162.0, 161.7, 149.4, 147.7, 133.1 ($^4J_{C-F}$ = 3.8 Hz), 131.6, 131.1 ($^3J_{C-F}$ = 8.6 Hz), 122.0, 120.3, 115.9 ($^2J_{C-F}$ = 21.6 Hz), 115.1, 114.6, 99.7, 95.0, 56.9, 25.6; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{19}H_{16}FN_2O_2$, 323.1190; found, 323.1188.

*4-(4-(Dimethylamino)phenyl)-7-(methoxymethoxy)-2-methylquinoline-8-carbonitrile (**9n**).* (49 mg, 51% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.24 (d, J = 9.5 Hz, 1H), 7.54 (d, J = 9.5 Hz, 1H), 7.46−7.37 (m, 2H), 7.31 (s, 1H), 6.97−6.90 (m, 2H), 5.51 (s, 2H), 3.59 (s, 3H), 3.06 (s, 6H), 2.77 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 166.0, 159.9, 157.6, 151.2, 146.2, 132.5, 130.2, 127.3, 125.8, 119.8, 114.0, 112.1, 104.5, 98.3, 94.9, 55.7, 39.1, 23.8; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{21}H_{22}N_3O_2$, 348.1935; found, 348.1933.

*7-(Methoxymethoxy)-2-methyl-4-(m-tolyl)quinoline-8-carbonitrile (**9o**).* (81 mg, 91% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.03 (d, J = 9.4 Hz, 1H), 7.46−7.38 (m, 2H), 7.33 (d, J = 7.7 Hz, 1H), 7.30−7.23 (m, 2H), 7.22 (s, 1H), 5.44 (s, 2H), 3.59 (s, 3H), 2.82 (s, 3H), 2.46 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 161.9, 161.7, 149.3, 148.9, 138.5, 137.1, 132.0, 130.0, 129.5, 128.6, 126.5, 121.9, 120.4, 115.2, 114.3, 99.6, 95.0, 56.8, 25.6, 21.5; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{20}H_{19}N_2O_2$, 319.1441; found, 319.1441.

*7-(Methoxymethoxy)-4-(3-methoxyphenyl)-2-methylquinoline-8-carbonitrile (**9p**).* (93 mg, 99% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.04 (d, J = 9.4 Hz, 1H), 7.51−7.34 (m, 2H), 7.23 (s, 1H), 7.10−7.00 (m, 2H), 6.98 (dd, J = 2.6, 1.6 Hz, 1H), 5.44 (s, 2H), 3.88 (s, 3H), 3.58 (s, 3H), 2.82 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 161.9, 161.7, 159.8, 149.3, 148.6, 138.5, 131.9, 129.8, 122.5, 121.8, 120.4, 115.2, 115.1, 114.4, 114.2, 99.6, 95.0, 56.8, 55.4, 25.6; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{20}H_{19}N_2O_3$, 335.1390; found, 335.1399.

*4-(3-Fluorophenyl)-7-(methoxymethoxy)-2-methylquinoline-8-carbonitrile (**9q**).* (83 mg, 92% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 7.96 (d, J = 9.4 Hz, 1H), 7.51 (td, J = 7.9, 5.7 Hz, 1H), 7.42 (d, J = 9.4 Hz, 1H), 7.23 (d, J = 8.1 Hz, 2H), 7.20−7.12 (m, 2H), 5.43 (s, 2H), 3.57 (s, 3H), 2.80 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 162.7 ($^1J_{C-F}$ = 247.6 Hz), 162.0, 161.7, 149.28, 147.3 ($^7J_{C-F}$ = 1.8 Hz (position 4 of quinoline)), 139.2 ($^4J_{C-F}$ = 7.3 Hz), 131.5, 130.5 ($^3J_{C-F}$ = 8.4 Hz), 125.2 ($^5J_{C-F}$ = 3.25 Hz), 121.8, 120.0, 116.5 ($^2J_{C-F}$ = 21.6 Hz), 115.7 ($^6J_{C-F}$ = 20.8 Hz), 115.0, 114.7, 99.7, 95.0, 56.8, 25.6; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{19}H_{16}FN_2O_2$, 323.1190; found, 323.1197.

*4-(3-(Dimethylamino)phenyl)-7-(methoxymethoxy)-2-methylquinoline-8-carbonitrile (**9r**).* (79 mg, 82% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.11 (d, J = 9.3 Hz, 1H), 7.42−7.34 (m, 2H), 7.25 (s, 1H), 6.86 (dd, J = 8.5, 2.7 Hz, 1H), 6.76 (t, J = 5.9 Hz, 2H), 5.44 (s, 2H), 3.59 (s, 3H), 3.03 (s, 6H), 2.82 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 161.9, 161.6, 150.6, 149.8, 149.3, 138.0, 132.3, 129.3, 121.8, 120.7, 117.5, 115.3, 114.2, 113.2, 112.6, 99.5, 95.0, 56.8, 40.5, 25.7; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{21}H_{22}N_3O_2$, 348.1707; found, 348.1685.

*4-(3,5-Dimethoxyphenyl)-7-(methoxymethoxy)-2-methylquinoline-8-carbonitrile (**9s**).* (92 mg, 90% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.07 (d, J = 9.4 Hz, 1H), 7.41 (d, J = 9.5 Hz, 1H),

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726−744

7.23 (s, 1H), 6.63−6.57 (m, 2H), 6.57 (s, 1H), 5.44 (s, 2H), 3.86 (s, 6H), 3.59 (s, 3H), 2.82 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 161.9, 161.7, 160.9, 149.3, 148.7, 139.1, 132.0, 121.6, 120.3, 115.2, 114.4, 111.0, 108.4, 107.6, 100.5, 99.5, 95.0, 94.3, 56.8, 55.6, 25.6; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{21}H_{21}N_2O_4$, 365.1496; found, 365.1478.

*7-(Methoxymethoxy)-2-methyl-4-(3,4,5-trimethoxyphenyl)-quinoline-8-carbonitrile (**9t**).* (80 mg, 72% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.09 (d, *J* = 9.3 Hz, 1H), 7.44 (d, *J* = 9.4 Hz, 1H), 7.24 (s, 1H), 6.66 (s, 2H), 5.45 (s, 2H), 3.97 (s, 3H), 3.91 (s, 6H), 3.60 (s, 3H), 2.83 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 162.0, 161.7, 153.8, 153.4, 149.4, 148.8, 138.4, 132.7, 131.9, 121.7, 120.4, 115.2, 114.4, 106.6, 99.6, 95.0, 93.0, 61.1, 56.9, 56.3, 56.0, 25.7; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{22}H_{23}N_2O_5$, 395.1601; found, 395.1608.

*General Procedure for the Preparation of Primary Alcohols **10a**−**10v**.* To a suspension of selenium dioxide (0.9 mmol, 3 equiv) in dioxane (8 mL), a 70% *tert*-butyl hydroperoxide solution (0.3 mmol, 1 eq) in H_2O was added and the mixture was stirred at 50 °C for 15 min. A solution of one of the quinolines **9a**−**9t** (0.3 mmol, 1 equiv) in dioxane (2 mL) was added dropwise. The mixture was heated to 70 °C and stirred until competition of the reaction (1−12 h), which was monitored by LC−MS. After cooling, the reaction mixture was diluted with EtOAc (60 mL) and filtered through celite. The filtrate was washed with H_2O (30 mL), saturated $NaHCO_3$ solution (30 mL), and brine (30 mL). The organic layer was dried over $MgSO_4$ and concentrated to a dry residue that was dissolved in ethanol (10 mL). Sodium borohydride (0.9 mmol, 3 equiv) was added in small portions, and the mixture was stirred at room temperature for 6 h. The solvent was evaporated under vacuum, the residue was dissolved in EtOAc (50 mL), and the resulting solution was washed with H_2O (3 × 30 mL) and brine (30 mL), dried over $MgSO_4$, concentrated to dryness, and purified by column chromatography (hexanes/EtOAc gradient) to yield the respective pure primary alcohol **10a**−**10v**.

*4-Chloro-2-(hydroxymethyl)-7-(methoxymethoxy)quinoline-8-carbonitrile (**10a**).* (130 mg, 42% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.30 (d, *J* = 9.4 Hz, 1H), 7.59 (d, *J* = 9.5 Hz, 1H), 7.44 (s, 1H), 5.48 (s, 2H), 4.93 (s, 2H), 3.59 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 163.0, 162.7, 148.3, 143.5, 130.2, 120.7, 118.2, 116.0, 114.1, 99.4, 95.2, 64.2, 57.0; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{13}H_{12}ClN_2O_3$, 279.0531; found, 279.0534.

*2-(Hydroxymethyl)-7-(methoxymethoxy)quinoline-4,8-dicarbonitrile (**10b**).* (10 mg, 11% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.35 (d, *J* = 9.3 Hz, 1H), 7.81−7.74 (m, 2H), 7.47−7.39 (m, 1H), 5.53 (s, 2H), 5.06 (d, *J* = 3.2 Hz, 2H), 3.62 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 163.2, 162.7, 147.6, 130.7, 122.0, 120.3, 119.8, 117.7, 114.7, 113.5, 113.2, 95.3, 64.5, 57.2; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{14}H_{12}N_3O_3$, 270.0873; found, 270.0859.

*4-(Dimethylamino)-2-(hydroxymethyl)-7-(methoxymethoxy)-quinoline-8-carbonitrile (**10c**).* (50 mg, 31% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.19 (d, *J* = 9.4 Hz, 1H), 7.40 (d, *J* = 9.4 Hz, 1H), 6.60 (s, 1H), 5.46 (s, 2H), 4.85 (s, 2H), 4.46 (s, 1H), 3.60 (s, 3H), 3.08 (s, 6H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 162.1, 161.5, 158.3, 149.6, 130.8, 117.4, 114.8, 112.5, 103.4, 99.6, 94.9, 67.1, 64.1, 53.4, 43.9; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{15}H_{18}N_3O_3$, 288.1343; found, 288.1365.

*2-(Hydroxymethyl)-7-(methoxymethoxy)-4-morpholinoquinoline-8-carbonitrile (**10d**).* (12 mg, 30% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.15 (d, *J* = 9.4 Hz, 1H), 7.46 (d, *J* = 9.5 Hz, 1H), 6.75 (s, 1H), 5.46 (s, 2H), 4.88 (s, 2H), 4.38 (s, 1H), 3.99 (t, *J* = 4.6 Hz, 4H), 3.60 (s, 3H), 3.24 (t, *J* = 4.6 Hz, 4H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 162.9, 161.8, 157.8, 149.2, 129.8, 117.9, 114.6, 113.7, 105.4, 99.8, 95.0, 66.7, 64.3, 56.9, 52.8; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{17}H_{20}N_3O_4$, 330.1448; found, 330.1437.

*2-(Hydroxymethyl)-7-(methoxymethoxy)-4-(phenylethynyl)-quinoline-8-carbonitrile (**10e**).* (30 mg, 59% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.52 (d, *J* = 9.3 Hz, 1H), 7.70−7.60 (m, 3H), 7.50−7.45 (m, 4H), 5.50 (d, *J* = 2.8 Hz, 2H), 4.98 (s, 2H), 4.25 (s, 1H), 3.61 (d, *J* = 2.6 Hz, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 162.4, 161.8, 147.5, 132.1, 132.0, 131.0, 129.9, 128.7, 122.3, 121.6, 120.4, 115.6, 114.3, 99.9, 99.6, 95.1, 84.1, 64.1, 57.0; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{21}H_{17}N_2O_3$, 345.1234; found, 345.1207.

*2-(Hydroxymethyl)-7-(methoxymethoxy)-4-methylquinoline-8-carbonitrile (**10f**).* (81 mg, 47% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.15 (d, *J* = 9.4 Hz, 1H), 7.54 (d, *J* = 9.4 Hz, 1H), 7.13 (s, 1H), 5.47 (s, 2H), 4.90 (d, *J* = 4.4 Hz, 2H), 3.60 (s, 3H), 2.71 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 161.9, 161.7, 147.4, 145.7, 123.0, 122.8, 118.7, 114.8, 114.6, 99.7, 95.0, 64.0, 56.9, 18.7; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{14}H_{15}N_2O_3$, 259.1077; found, 259.1048.

*2-(Hydroxymethyl)-7-(methoxymethoxy)-4-(1-(triisopropylsilyl)-1H-pyrrol-3-yl)quinoline-8-carbonitrile (**10g**).* (12 mg, 43% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.54 (dd, *J* = 9.5, 3.8 Hz, 1H), 7.50 (dd, *J* = 9.4, 3.8 Hz, 1H), 7.29−7.25 (m, 1H), 7.10 (s, 1H), 6.96 (s, 1H), 6.63 (s, 1H), 5.48 (d, *J* = 3.8 Hz, 2H), 4.96 (d, *J* = 3.8 Hz, 2H), 4.51 (s, 1H), 3.62 (d, J = 3.9 Hz, 3H), 1.54 (dq, *J* = 14.9, 6.8, 5.4 Hz, 3H), 1.18 (dd, *J* = 7.6, 3.8 Hz, 18H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 161.7, 161.5, 148.4, 144.7, 132.7, 125.7, 124.7, 122.3, 121.6, 116.8, 114.9, 114.4, 111.8, 99.4, 94.9, 64.1, 56.9, 17.8, 11.6; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{26}H_{36}N_3O_3Si$, 466.2520; found, 466.2520.

*2-(Hydroxymethyl)-7-(methoxymethoxy)-4-phenylquinoline-8-carbonitrile (**10h**).* (23 mg, 40% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.08 (d, *J* = 9.5 Hz, 1H), 7.61−7.51 (m, 3H), 7.51−7.43 (m, 3H), 7.26 (s, 1H), 5.47 (s, 2H), 5.00 (s, 2H), 3.60 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 162.0, 161.8, 149.8, 148.1, 136.9, 132.2, 129.4, 129.1, 128.9, 121.5, 118.2, 115.1, 114.5, 99.6, 95.0, 64.2, 56.9; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{19}H_{17}N_2O_3$, 321.1234; found, 321.1232.

*2-(Hydroxymethyl)-7-(methoxymethoxy)-4-(p-tolyl)quinoline-8-carbonitrile (**10i**).* (28 mg, 84% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.13 (d, *J* = 9.5 Hz, 1H), 7.61−7.54 (m, 2H), 7.44−7.38 (m, 4H), 5.51 (s, 2H), 4.92 (s, 2H), 3.58 (s, 3H), 2.47 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 164.2, 162.2, 150.0, 148.5, 138.9, 134.2, 132.2, 129.2, 129.1, 121.0, 118.2, 114.9, 114.4, 98.4, 94.9, 64.8, 55.7, 19.91; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{20}H_{19}N_2O_3$, 335.1390; found, 335.1390.

*2-(Hydroxymethyl)-7-(methoxymethoxy)-4-(4-methoxyphenyl)-quinoline-8-carbonitrile (**10j**).* (22 mg, 41% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.13 (d, *J* = 9.5 Hz, 1H), 7.61−7.54 (m, 2H), 7.44−7.38 (m, 4H), 5.51 (s, 2H), 4.92 (s, 2H), 3.58 (s, 3H), 2.47 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 164.2, 162.2, 150.0, 148.5, 138.9, 134.2, 132.2, 129.2, 129.1, 121.0, 118.2, 114.9, 114.4, 98.4, 94.9, 64.8, 55.7, 19.91; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{20}H_{19}N_2O_4$, 351.1339; found, 351.1330.

*4-(4-Cyanophenyl)-2-(hydroxymethyl)-7-(methoxymethoxy)-quinoline-8-carbonitrile (**10k**).* (17 mg, 25% yield). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 8.11−8.06 (m, 2H), 8.01 (d, *J* = 9.5 Hz, 1H), 7.80−7.75 (m, 2H), 7.66 (d, *J* = 9.6 Hz, 1H), 7.63 (s, 1H), 5.55 (s, 2H), 4.82 (s, 2H), 3.48 (s, 3H); ^{13}C NMR{1H} (126 MHz, DMSO-d_6, δ): 165.8, 162.4, 148.5, 147.6, 142.0, 133.3, 132.3, 130.9, 120.3, 119.1, 119.0, 116.5, 115.2, 112.2, 98.7, 95.3, 65.1, 56.8; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{20}H_{16}N_3O_3$, 346.1186; found, 346.1186.

*tert-Butyl 4-(8-Cyano-2-(hydroxymethyl)-7-(methoxymethoxy)-quinolin-4-yl)benzoate (**10l**).* (16 mg, 30% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.21−8.15 (m, 2H), 8.00 (d, *J* = 9.4 Hz, 1H), 7.56−7.47 (m, 3H), 7.26 (s, 1H), 5.47 (s, 2H), 5.02 (d, *J* = 4.6 Hz, 2H), 4.33 (t, *J* = 4.8 Hz, 1H), 3.60 (s, 3H), 1.67 (s, 9H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 165.1, 162.1, 161.8, 148.8, 148.1, 140.8, 132.7, 131.8, 129.9, 129.3, 121.1, 118.1, 115.4, 114.4, 99.8, 95.0, 81.7, 64.3, 57.0, 28.2; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{24}H_{25}N_2O_5$, 421.1758; found, 421.1790.

*4-(4-Fluorophenyl)-2-(hydroxymethyl)-7-(methoxymethoxy)-quinoline-8-carbonitrile (**10m**).* (16 mg, 25% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.03 (d, *J* = 9.4 Hz, 1H), 7.53−7.43 (m, 3H),

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726−744

7.27 (dd, J = 13.6, 5.1 Hz, 3H), 5.46 (s, 2H), 4.99 (s, 2H), 4.36 (s, 1H), 3.59 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 163.2 ($^{1}J_{C-F}$ = 249.4 Hz), 162.0, 161.9, 148.71, 148.11, 132.8 ($^{4}J_{C-F}$ = 3.7 Hz), 131.9, 131.2 ($^{3}J_{C-F}$ = 8.6 Hz), 121.4, 118.3, 116.1 ($^{2}J_{C-F}$ = 21.8 Hz), 115.3, 114.5, 99.6, 95.0, 64.3, 56.9; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{19}H_{16}FN_2O_3$, 339.1139; found, 339.1140.

*4-(4-(Dimethylamino)phenyl)-2-(hydroxymethyl)-7-(methoxymethoxy)quinoline-8-carbonitrile (**10n**).* (10 mg, 32% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.24 (d, J = 9.4 Hz, 1H), 7.46 (d, J = 9.5 Hz, 1H), 7.39 (d, J = 8.5 Hz, 2H), 7.20 (s, 1H), 6.86 (d, J = 8.7 Hz, 2H), 5.47 (s, 2H), 4.97 (s, 2H), 3.61 (s, 3H), 3.08 (s, 6H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 161.8, 161.5, 150.9, 150.2, 148.3, 132.7, 130.7, 130.5, 124.1, 121.8, 117.7, 114.8, 114.5, 112.1, 99.4, 95.0, 64.2, 56.9, 40.3; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{21}H_{22}N_3O_3$, 364.1656; found, 364.1654.

*2-(Hydroxymethyl)-7-(methoxymethoxy)-4-(m-tolyl)quinoline-8-carbonitrile (**10o**).* (18 mg, 60% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.09 (d, J = 9.4 Hz, 1H), 7.48 (d, J = 9.5 Hz, 1H), 7.44 (t, J = 7.6 Hz, 1H), 7.35 (d, J = 7.7 Hz, 1H), 7.30−7.23 (m, 3H), 5.46 (s, 2H), 4.99 (s, 2H), 3.59 (s, 3H), 2.47 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 161.9, 161.7, 150.0, 148.1, 138.7, 136.8, 132.4, 130.0, 129.8, 128.7, 126.5, 121.5, 118.2, 115.0, 114.6, 99.5, 95.0, 64.2, 56.9, 21.5; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{20}H_{19}N_2O_3$, 335.1390; found, 335.1390.

*2-(Hydroxymethyl)-7-(methoxymethoxy)-4-(3-methoxyphenyl)-quinoline-8-carbonitrile (**10p**).* (20 mg, 38% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.10 (d, J = 9.4 Hz, 1H), 7.51−7.43 (m, 2H), 7.26 (s, 1H), 7.10−7.01 (m, 2H), 6.99 (t, J = 2.1 Hz, 1H), 5.46 (s, 2H), 5.00 (d, J = 4.0 Hz, 2H), 4.39 (t, J = 4.9 Hz, 1H), 3.89 (s, 3H), 3.60 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 162.0, 161.8, 159.8, 149.7, 148.0, 138.2, 132.3, 129.9, 121.7, 121.4, 118.1, 115.1, 115.1, 114.6, 114.4, 99.5, 95.0, 64.3, 56.9, 55.5; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{20}H_{19}N_2O_4$, 351.1339; found, 351.1331.

*4-(3-Fluorophenyl)-2-(hydroxymethyl)-7-(methoxymethoxy)-quinoline-8-carbonitrile (**10q**).* (30 mg, 34% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.03 (d, J = 9.4 Hz, 1H), 7.53 (dt, J = 14.2, 8.5 Hz, 2H), 7.30−7.15 (m, 4H), 5.46 (s, 2H), 4.99 (s, 2H), 4.35 (s, 1H), 3.58 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 162.7 ($^{1}J_{C-F}$ = 248.1 Hz), 162.1, 162.0, 148.3 ($^{7}J_{C-F}$ = 1.7 Hz (position 4 of quinoline)), 148.1, 138.8 ($^{4}J_{C-F}$ = 7.6 Hz), 131.82, 130.6 ($^{3}J_{C-F}$ = 8.4 Hz), 125.2 ($^{5}J_{C-F}$ = 2.8 Hz), 121.1, 118.2, 116.5 ($^{2}J_{C-F}$ = 22.4 Hz), 116.1 ($^{6}J_{C-F}$ = 20.9 Hz), 115.4, 114.5, 99.6, 95.1, 64.3, 56.9; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{19}H_{16}FN_2O_3$, 339.1139; found, 339.1140.

*4-(3-(Dimethylamino)phenyl)-2-(hydroxymethyl)-7-(methoxymethoxy)quinoline-8-carbonitrile (**10r**).* (15 mg, 29% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.17 (d, J = 9.4 Hz, 1H), 7.47 (d, J = 9.4 Hz, 1H), 7.39 (dd, J = 8.4, 7.4 Hz, 1H), 7.26 (s, 1H), 6.88 (dd, J = 8.3, 2.6 Hz, 1H), 6.80−6.73 (m, 2H), 5.47 (s, 2H), 5.00 (d, J = 4.3 Hz, 2H), 4.44 (t, J = 4.8 Hz, 1H), 3.61 (s, 3H), 3.03 (s, 6H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 161.9, 161.6, 150.9, 150.6, 148.0, 137.7, 132.6, 129.4, 121.8, 118.0, 117.4, 114.9, 114.7, 113.0, 112.8, 99.5, 95.0, 64.2, 56.9, 40.5; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{21}H_{22}N_3O_3$, 364.1656; found, 364.1622.

*4-(3,5-Dimethoxyphenyl)-2-(hydroxymethyl)-7-(methoxymethoxy)quinoline-8-carbonitrile (**10s**).* (22 mg, 45% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.13 (d, J = 9.4 Hz, 1H), 7.48 (d, J = 9.5 Hz, 1H), 7.26 (s, 1H), 6.61 (t, J = 2.3 Hz, 1H), 6.58 (d, J = 2.3 Hz, 2H), 5.46 (s, 2H), 4.99 (s, 2H), 4.38 (s, 1H), 3.86 (s, 6H), 3.59 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 162.0, 161.8, 161.0, 149.8, 148.0, 138.7, 132.3, 121.4, 118.0, 115.1, 114.6, 107.6, 100.7, 99.5, 95.0, 64.3, 56.9, 55.6; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{21}H_{21}N_2O_5$, 381.1445; found, 381.1441.

*2-(Hydroxymethyl)-7-(methoxymethoxy)-4-(3,4,5-trimethoxyphenyl)quinoline-8-carbonitrile (**10t**).* (55 mg, 45% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.14 (d, J = 9.5 Hz, 1H), 7.51 (d, J = 9.5 Hz, 1H), 7.27 (s, 1H), 6.67 (s, 2H), 5.47 (s, 2H), 4.99 (s, 2H), 3.96 (s, 3H), 3.91 (s, 6H), 3.60 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 162.0, 161.7, 153.5, 149.8, 148.1, 138.6, 132.4, 132.3, 121.5, 118.1, 115.1, 114.6, 106.6, 99.5, 95.0, 64.3, 61.1, 57.0, 56.4; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{22}H_{23}N_2O_6$, 411.1551; found, 411.1557.

*N-(4-(8-Cyano-2-(hydroxymethyl)-7-(methoxymethoxy)-quinolin-4-yl)phenyl)-N-methylformamide (**10u**).* (10 mg, 23% yield). The crude product was carried forward to the next step without further purification. HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{21}H_{20}N_3O_4$, 378.1448; found, 378.1427.

*N-(3-(8-Cyano-2-(hydroxymethyl)-7-(methoxymethoxy)-quinolin-4-yl)phenyl)-N-methylformamide (**10v**).* (8 mg, 16% yield). The crude product was carried forward to the next step without further purification. HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{21}H_{20}N_3O_4$, 378.1448; found, 378.1414.

*General Procedure for the Preparation of MOM-Protected Acetates **11a**−**11v**.* To a solution of one of the primary alcohols **10a**−**10v** (0.1 mmol, 1 equiv) in CH_2Cl_2 (5 mL), pyridine (0.5 mmol, 5 equiv) and 4-dimethylaminopyridine (0.1 mmol, 1 equiv) were added. The mixture was cooled to 0 °C with an ice bath and acetic anhydride (0.4 mmol, 4 equiv) was added dropwise. The mixture was stirred at 0 °C for 30 min and then at room temperature for 6 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL), and the resulting solution was washed with a saturated ammonium chloride solution (10 mL) and H_2O (2 × 10 mL), dried over $MgSO_4$, and concentrated to dryness. The resulting crude product was purified by column chromatography (hexanes/EtOAc gradient) to yield the respective MOM-protected acetate **11a**−**11v**.

*(4-Chloro-8-cyano-7-(methoxymethoxy)quinolin-2-yl)methyl Acetate (**11a**).* (15 mg, 45% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 9.09 (d, J = 9.3 Hz, 1H), 8.41 (s, 1H), 8.29−8.24 (m, 1H), 6.12 (s, 2H), 3.96−3.95 (m, 3H), 2.98 (s, 3H), 2.03 (s, 2H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 170.6, 162.8, 159.5, 149.1, 143.7, 130.1, 120.9, 118.6, 116.4, 114.1, 100.1, 95.1, 66.2, 57.0, 21.0; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{15}H_{14}ClN_2O_4$, 321.0637; found, 321.0632.

*(4,8-Dicyano-7-(methoxymethoxy)quinolin-2-yl)methyl Acetate (**11b**).* (8 mg, 64% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.34 (d, J = 9.4 Hz, 1H), 7.78 (t, J = 4.7 Hz, 2H), 5.52 (s, 2H), 5.50 (s, 2H), 3.61 (s, 3H), 2.30 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 170.5, 163.2, 159.3, 148.2, 130.5, 122.1, 120.2, 119.8, 118.1, 114.8, 113.5, 100.6, 95.3, 66.0, 57.2, 20.8; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{16}H_{14}N_3O_4$, 312.0979; found, 312.0966.

*(8-Cyano-4-(dimethylamino)-7-(methoxymethoxy)quinolin-2-yl)methyl Acetate (**11c**).* (30 mg, 96% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.17 (d, J = 9.4 Hz, 1H), 7.38 (d, J = 9.4 Hz, 1H), 6.74 (s, 1H), 5.44 (s, 2H), 5.36 (s, 2H), 3.59 (s, 3H), 3.08 (s, 6H), 2.27 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 170.9, 161.5, 159.0, 158.4, 150.6, 130.6, 117.1, 115.1, 112.7, 104.3, 100.0, 94.9, 67.1, 56.8, 43.9, 21.0; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{17}H_{20}N_3O_4$, 330.1448; found, 330.1464.

*(8-Cyano-7-(methoxymethoxy)-4-morpholinoquinolin-2-yl)-methyl Acetate (**11d**).* (14 mg, 96% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.14 (d, J = 9.4 Hz, 1H), 7.46 (d, J = 9.4 Hz, 1H), 6.87 (s, 1H), 5.45 (s, 2H), 5.39 (s, 2H), 3.99 (t, J = 4.6 Hz, 4H), 3.58 (s, 3H), 3.24 (t, J = 4.6 Hz, 4H), 2.28 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 170.9, 161.8, 159.7, 157.9, 150.1, 129.6, 117.7, 114.8, 114.0, 106.1, 100.3, 94.9, 66.9, 66.7, 56.9, 52.7, 21.0; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{19}H_{22}N_3O_5$, 372.1554; found, 372.1557.

*(8-Cyano-7-(methoxymethoxy)-4-(phenylethynyl)quinolin-2-yl)-methyl Acetate (**11e**).* (7 mg, 62% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.51 (dd, J = 9.4, 1.1 Hz, 1H), 7.69−7.59 (m, 4H), 7.49−7.44 (m, 3H), 5.49 (s, 2H), 5.48 (s, 2H), 3.61 (s, 3H), 2.30 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 170.7, 162.5, 158.9, 148.3, 132.0, 131.9, 131.1, 129.8, 128.7, 122.2, 121.7, 120.9, 116.0, 114.4, 100.1, 99.8, 95.1, 84.2, 66.5, 56.9, 21.0; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{23}H_{19}N_2O_4$, 387.1339; found, 387.1376.

*(8-Cyano-7-(methoxymethoxy)-4-methylquinolin-2-yl)methyl Acetate (**11f**).* (19 mg, 77% yield). ^{1}H NMR (500 MHz, chloroform-*d*, δ): 8.15 (d, J = 9.3 Hz, 1H), 7.54 (d, J = 9.3 Hz, 1H), 7.27 (d, J =

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726−744

12.5 Hz, 1H), 5.46 (s, 2H), 5.42 (s, 2H), 3.59 (s, 3H), 2.72 (s, 3H), 2.28 (d, J = 0.9 Hz, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-d, δ): 170.9, 161.8, 158.9, 148.4, 145.8, 129.8, 122.7, 119.3, 115.1, 114.8, 100.1, 94.9, 66.7, 56.9, 21.0, 18.8; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{16}H_{17}N_2O_4$, 301.1183; found, 301.1185.

*(8-Cyano-7-(methoxymethoxy)-4-(1-(triisopropylsilyl)-1H-pyrrol-3-yl)quinolin-2-yl)methyl Acetate (**11g**).* (14 mg, 92% yield). ^{1}H NMR (500 MHz, chloroform-d, δ): 8.51 (d, J = 9.4 Hz, 1H), 7.49 (d, J = 9.5 Hz, 1H), 7.29 (s, 1H), 7.09 (s, 1H), 6.96 (t, J = 2.5 Hz, 1H), 6.65−6.61 (m, 1H), 5.47 (s, 4H), 3.60 (s, 3H), 2.28 (s, 3H), 1.55 (p, J = 7.5 Hz, 3H), 1.19 (d, J = 7.5 Hz, 18H); ^{13}C NMR{1H} (126 MHz, chloroform-d, δ): 170.9, 161.7, 158.5, 149.3, 144.8, 132.5, 125.7, 124.7, 122.4, 121.5, 117.5, 115.1, 114.7, 111.9, 99.9, 94.9, 66.9, 56.8, 29.7, 21.0, 17.8, 11.6; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{28}H_{38}N_3O_4Si$, 508.2626; found, 508.2586.

*(8-Cyano-7-(methoxymethoxy)-4-phenylquinolin-2-yl)methyl Acetate (**11h**).* (15 mg, 21% yield). ^{1}H NMR (600 MHz, chloroform-d, δ): 8.06 (d, J = 9.4 Hz, 1H), 7.60−7.53 (m, 3H), 7.52−7.45 (m, 3H), 7.37 (s, 1H), 5.51 (s, 2H), 5.46 (s, 2H), 3.59 (s, 3H), 2.28 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-d, δ): 170.8, 162.0, 158.7, 149.8, 148.9, 137.0, 132.0, 129.4, 129.0, 128.8, 121.4, 118.7, 115.5, 114.7, 100.1, 95.0, 66.7, 56.9, 21.0; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{21}H_{19}N_2O_4$, 363.1339; found, 363.1353.

*(8-Cyano-7-(methoxymethoxy)-4-(p-tolyl)quinolin-2-yl)methyl Acetate (**11i**).* (10 mg, 74% yield). ^{1}H NMR (500 MHz, chloroform-d, δ): 8.09 (d, J = 9.5 Hz, 1H), 7.47 (d, J = 9.5 Hz, 1H), 7.36 (d, J = 12.1 Hz, 5H), 5.50 (s, 2H), 5.46 (s, 2H), 3.59 (s, 3H), 2.49 (s, 3H), 2.27 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-d, δ): 170.8, 161.9, 158.7, 149.9, 149.0, 139.1, 134.1, 132.1, 129.5, 129.4, 121.5, 118.6, 115.3, 114.8, 100.0, 95.0, 67.0, 56.9, 29.3, 21.0; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{22}H_{21}N_2O_4$, 377.1496; found, 377.1515.

*(8-Cyano-7-(methoxymethoxy)-4-(4-methoxyphenyl)quinolin-2-yl)methyl Acetate (**11j**).* (10 mg, 70% yield). ^{1}H NMR (500 MHz, chloroform-d, δ): 8.11 (d, J = 9.5 Hz, 1H), 7.48 (d, J = 9.5 Hz, 1H), 7.45−7.39 (m, 2H), 7.34 (s, 1H), 7.12−7.06 (m, 2H), 5.50 (s, 2H), 5.46 (s, 2H), 3.93 (s, 3H), 3.60 (s, 3H), 2.27 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-d, δ): 170.8, 161.9, 160.3, 158.7, 149.6, 149.1, 132.1, 130.8, 129.3, 121.5, 118.6, 115.3, 114.8, 114.3, 100.1, 95.0, 66.8, 57.0, 55.5, 21.0; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{22}H_{21}N_2O_5$, 393.1445; found, 393.1458.

*(8-Cyano-4-(4-cyanophenyl)-7-(methoxymethoxy)quinolin-2-yl)-methyl Acetate (**11k**).* (6 mg, 66% yield). ^{1}H NMR (500 MHz, chloroform-d, δ): 7.93−7.85 (m, 3H), 7.64−7.58 (m, 2H), 7.53 (d, J = 9.5 Hz, 1H), 7.34 (s, 1H), 5.52 (s, 2H), 5.47 (s, 2H), 3.60 (s, 3H), 2.28 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-d, δ): 170.7, 162.2, 158.9, 148.9, 147.6, 141.7, 132.6, 131.0, 130.2, 120.5, 118.5, 118.2, 116.1, 114.4, 113.1, 100.4, 95.0, 66.6, 57.0, 20.9; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{22}H_{18}N_3O_4$, 388.1292; found, 388.1309.

*tert-Butyl 4-(2-(Acetoxymethyl)-8-cyano-7-(methoxymethoxy)-quinolin-4-yl)benzoate (**11l**).* (5 mg, 60% yield). ^{1}H NMR (500 MHz, chloroform-d, δ): 8.21−8.15 (m, 2H), 7.98 (d, J = 9.4 Hz, 1H), 7.56−7.51 (m, 2H), 7.49 (d, J = 9.5 Hz, 1H), 7.36 (s, 1H), 5.52 (s, 2H), 5.46 (s, 2H), 3.60 (s, 3H), 2.28 (s, 3H), 1.67 (s, 9H); ^{13}C NMR{1H} (126 MHz, chloroform-d, δ): 170.8, 165.1, 162.17, 158.8, 148.9, 148.8, 141.0, 132.6, 131.6, 129.9, 129.3, 121.0, 118.5, 115.7, 114.6, 100.2, 95.0, 81.7, 66.7, 56.9, 28.2, 21.0; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{26}H_{27}N_2O_6$, 463.1864; found, 463.1864.

*(8-Cyano-4-(4-fluorophenyl)-7-(methoxymethoxy)quinolin-2-yl)-methyl Acetate (**11m**).* (20 mg, 95% yield). ^{1}H NMR (500 MHz, chloroform-d, δ): 8.01 (d, J = 9.5 Hz, 1H), 7.51−7.44 (m, 3H), 7.34 (s, 1H), 7.29−7.23 (m, 2H), 5.49 (s, 2H), 5.46 (s, 2H), 3.59 (s, 3H), 2.27 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-d, δ): 170.8, 163.2 ($^1J_{C-F}$ = 249.3 Hz), 162.0, 158.7, 148.9, 148.7, 133.0 ($^4J_{C-F}$ = 3.7 Hz), 131.7, 131.2 ($^3J_{C-F}$ = 8.3 Hz), 121.3, 118.7, 116.0 ($^2J_{C-F}$ = 21.8 Hz), 115.6, 114.7, 100.1, 95.0, 66.7, 56.9, 21.0; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{21}H_{18}FN_2O_4$, 381.1245; found, 381.1240.

*(8-Cyano-4-(4-(dimethylamino)phenyl)-7-(methoxymethoxy)-quinolin-2-yl)methyl Acetate (**11n**).* (8 mg, 73% yield). ^{1}H NMR (500 MHz, chloroform-d, δ): 8.22 (d, J = 9.5 Hz, 1H), 7.46 (d, J = 9.5 Hz, 1H), 7.41−7.37 (m, 2H), 7.34 (s, 1H), 6.90−6.84 (m, 2H), 5.49 (s, 2H), 5.47 (s, 2H), 3.60 (s, 3H), 3.09 (s, 6H), 2.27 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-d, δ): 170.8, 161.8, 158.6, 150.9, 150.2, 149.2, 132.5, 130.6, 124.3, 121.7, 118.2, 115.0, 114.9, 112.1, 99.9, 94.9, 66.9, 56.9, 40.3, 21.0; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{23}H_{24}N_3O_4$, 406.1761; found, 406.1747.

*(8-Cyano-7-(methoxymethoxy)-4-(m-tolyl)quinolin-2-yl)methyl Acetate (**11o**).* (15 mg, 74% yield). ^{1}H NMR (500 MHz, chloroform-d, δ): 8.07 (d, J = 9.5 Hz, 1H), 7.50−7.41 (m, 2H), 7.35 (d, J = 4.7 Hz, 2H), 7.30−7.24 (m, 2H), 5.50 (s, 2H), 5.45 (s, 2H), 3.59 (s, 3H), 2.48 (s, 3H), 2.27 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-d, δ): 170.8, 161.9, 158.7, 150.0, 148.9, 138.7, 137.0, 132.1, 130.0, 129.7, 128.7, 126.5, 121.4, 118.6, 115.4, 114.8, 100.0, 95.0, 66.7, 56.9, 21.5, 21.0; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{22}H_{21}N_2O_4$, 377.1496; found, 377.1517.

*(8-Cyano-7-(methoxymethoxy)-4-(3-methoxyphenyl)quinolin-2-yl)methyl Acetate (**11p**).* (19 mg, 85% yield). ^{1}H NMR (500 MHz, chloroform-d, δ): 8.08 (d, J = 9.5 Hz, 1H), 7.47 (t, J = 9.1 Hz, 2H), 7.29 (s, 1H), 7.06 (ddd, J = 17.1, 7.9, 2.0 Hz, 2H), 6.99 (t, J = 2.0 Hz, 1H), 5.50 (s, 2H), 5.46 (s, 2H), 3.89 (s, 3H), 3.59 (s, 3H), 2.27 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-d, δ): 170. 8, 162.0, 159.8, 158.7, 149.7, 148.9, 138.3, 132.0, 129.9, 121.8, 121.3, 118.5, 115.5, 115.2, 114.7, 114.3, 100.0, 95.0, 66.7, 56.9, 55.5, 21.0; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{22}H_{21}N_2O_5$, 393.1445; found, 393.1425.

*(8-Cyano-4-(3-fluorophenyl)-7-(methoxymethoxy)quinolin-2-yl)-methyl Acetate (**11q**).* (29 mg, 86% yield). ^{1}H NMR (500 MHz, chloroform-d, δ): 8.02 (d, J = 9.5 Hz, 1H), 7.58−7.50 (m, 2H), 7.50 (s, 1H), 7.30−7.21 (m, 2H), 7.19 (ddd, J = 9.2, 2.6, 1.6 Hz, 1H), 5.49 (s, 2H), 5.46 (s, 2H), 3.58 (s, 3H), 2.27 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-d, δ): 170.8, 162.8 ($^1J_{C-F}$ = 248.4 Hz), 162.1, 158.8, 148.9, 148.3 ($^7J_{C-F}$ = 1.8 Hz (position 4 of quinoline)), 139.5 ($^4J_{C-F}$ = 7.6 Hz), 131.6, 130.6 ($^3J_{C-F}$ = 8.5 Hz), 125.2 ($^5J_{C-F}$ = 3.2 Hz), 121.0, 118.6, 116.5 ($^2J_{C-F}$ = 22.2 Hz), 116.0 ($^6J_{C-F}$ = 21.4 Hz), 115.8, 114.6, 100.1, 95.0, 66.6, 56.9, 20.9; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{21}H_{18}FN_2O_4$, 381.1245; found, 381.1231.

*(8-Cyano-4-(3-(dimethylamino)phenyl)-7-(methoxymethoxy)-quinolin-2-yl)methyl Acetate (**11r**).* (5 mg, 62% yield). ^{1}H NMR (500 MHz, chloroform-d, δ): 8.15 (d, J = 9.4 Hz, 1H), 7.46 (d, J = 9.5 Hz, 1H), 7.43−7.37 (m, 2H), 6.89 (dd, J = 8.9, 2.6 Hz, 1H), 6.81−6.73 (m, 2H), 5.51 (s, 2H), 5.46 (s, 2H), 3.60 (s, 3H), 3.04 (s, 6H), 2.27 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-d, δ): 170.8, 161.9, 158.7, 150.9, 150.6, 140.1, 140.0, 137.9, 132.4, 129.4, 118.4, 117.5, 115.2, 114.9, 113.1, 112.7, 95.0, 90.2, 66.8, 56.9, 40.5, 21.0; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{23}H_{24}N_3O_4$, 406.1761; found, 406.1759.

*(8-Cyano-4-(3,5-dimethoxyphenyl)-7-(methoxymethoxy)-quinolin-2-yl)methyl Acetate (**11s**).* (22 mg, 90% yield). ^{1}H NMR (500 MHz, chloroform-d, δ): 8.10 (d, J = 9.5 Hz, 1H), 7.48 (d, J = 9.5 Hz, 1H), 7.36 (s, 1H), 6.62 (t, J = 2.3 Hz, 1H), 6.58 (d, J = 2.2 Hz, 2H), 5.50 (s, 2H), 5.46 (s, 2H), 3.87 (s, 6H), 3.59 (s, 3H), 2.27 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-d, δ): 170.8, 162.0, 161.0, 158.7, 149.8, 148.8, 138.9, 132.1, 121.3, 118.3, 115.4, 114.7, 107.7, 100.6, 100.0, 95.0, 66.7, 56.9, 55.6, 21.0; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{23}H_{23}N_2O_6$, 423.1551; found, 423.1558.

*(8-Cyano-7-(methoxymethoxy)-4-(3,4,5-trimethoxyphenyl)-quinolin-2-yl)methyl Acetate (**11t**).* (15 mg, 92% yield). ^{1}H NMR (500 MHz, chloroform-d, δ): 8.11 (d, J = 9.5 Hz, 1H), 7.50 (d, J = 9.5 Hz, 1H), 7.36 (s, 1H), 6.66 (s, 2H), 5.50 (s, 2H), 5.46 (s, 2H), 3.97 (s, 3H), 3.91 (s, 6H), 3.59 (s, 3H), 2.28 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-d, δ): 170.8, 162.0, 158.7, 153.5, 149.8, 148.9, 138.5, 132.5, 132.0, 121.4, 118.4, 115.5, 114.8, 106.6, 100.0, 95.0, 66.7, 61.1, 56.9, 56.4, 21.0; HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{24}H_{25}N_2O_7$, 453.1656; found, 453.1653.

*(8-Cyano-7-(methoxymethoxy)-4-(4-(N-methylformamido)-phenyl)quinolin-2-yl)methyl Acetate (**11u**).* HRMS (ESI-QTOF) m/z: [M + H]$^+$ calcd for $C_{23}H_{22}N_3O_5$, 420.1554; found, 420.1555. Not isolated. The solid obtained was carried directly to the next step.

*(8-Cyano-7-(methoxymethoxy)-4-(3-(N-methylformamido)-phenyl)quinolin-2-yl)methyl Acetate (**11v**).* (4 mg, 43% yield). ^{1}H

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726−744

NMR (500 MHz, chloroform-*d*, δ): 8.62 (s, 1H), 8.00 (d, *J* = 9.5 Hz, 1H), 7.64−7.59 (m, 1H), 7.55−7.50 (m, 1H), 7.38−7.35 (m, 2H), 7.28 (d, *J* = 2.2 Hz, 2H), 5.52 (s, 2H), 5.47 (s, 2H), 3.60 (d, *J* = 1.8 Hz, 3H), 3.41 (s, 3H), 2.28 (s, 3H); ^{13}C NMR{1H} (126 MHz, chloroform-*d*, δ): 170.8, 162.1, 162.0, 158.8, 148.9, 148.5, 142.8, 138.7, 131.4, 130.2, 127.2, 122.8, 122.4, 121.0, 118.6, 115.8, 114.5, 100.3, 95.0, 66.7, 56.9, 32.1, 21.0; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{23}H_{22}N_3O_5$, 420.1554; found, 420.1551.

General Procedure for the Preparation of Acetates ***12a−12v***. To a solution of one of the acetates **11a−11v** (0.05 mmol, 1 equiv) in CH_2Cl_2 (2 mL), TFA (0.2 mL) was added dropwise. The reaction was stirred for up to 5 h until HPLC showed complete consumption of the starting material and then concentrated to dryness. The product was purified either by trituration with tetrahydrofuran or by reverse-phase preparative chromatography (water/CH_3CN), affording the respective protected acetate **12a−12v**.

*(4-Chloro-8-cyano-7-(hydroxy)quinolin-2-yl)methyl Acetate (**12a**).* (12 mg, 90% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 9.07 (d, *J* = 9.3 Hz, 1H), 8.39 (s, 1H), 8.24 (d, *J* = 9.4 Hz, 1H), 6.11 (s, 2H), 3.96 (p, *J* = 1.7 Hz, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.4, 160.2, 150.4, 143.8, 130.7, 120.8, 119.8, 118.6, 115.9, 95.8, 66.7, 30.2, 21.3; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{13}H_{10}ClN_2O_3$, 277.0374; found, 277.0380.

*(4,8-Dicyano-7-(hydroxy)quinolin-2-yl)methyl Acetate (**12b**).* (3 mg, 45% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.28−8.21 (m, 1H), 7.90−7.85 (m, 1H), 7.52−7.45 (m, 1H), 5.48−5.42 (m, 2H), 2.26 (t, *J* = 3.9 Hz, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.0, 165.2, 159.0, 148.6, 130.2, 121.1, 120.4, 119.5, 119.0, 114.6, 113.8, 95.6, 65.6, 19.3; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{14}H_{10}N_3O_3$, 268.0717; found, 268.0715.

*(8-Cyano-4-(dimethylamino)-7-(hydroxy)quinolin-2-yl)methyl Acetate (**12c**).* (21 mg, 99% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.46 (d, *J* = 9.6 Hz, 1H), 7.24 (d, *J* = 9.5 Hz, 1H), 7.00 (s, 1H), 5.37 (s, 2H), 3.54 (s, 6H), 2.22 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 170.4, 166.5, 160.1, 149.4, 142.5, 133.8, 114.7, 111.9, 110.6, 102.3, 87.8, 61.7, 43.7, 19.1; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{15}H_{16}N_3O_3$, 286.1186; found, 286.1205.

*(8-Cyano-7-(hydroxy)-4-morpholinoquinolin-2-yl)methyl Acetate (**12d**).* (14 mg, 99% yield). ^{1}H NMR (500 MHz, acetonitrile-d_3, δ): 8.10 (d, *J* = 9.4 Hz, 1H), 7.35 (d, *J* = 9.5 Hz, 1H), 6.97 (s, 1H), 5.29 (s, 2H), 3.91−3.86 (m, 4H), 3.67 (t, *J* = 4.7 Hz, 4H), 2.20 (s, 3H); ^{13}C NMR{1H} (126 MHz, acetonitrile-d_3, δ): 170.7, 166.7, 160.1, 152.4, 144.6, 132.2, 116.7, 113.4, 112.5, 104.9, 89.9, 66.1, 63.1, 52.6, 20.0; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{17}H_{18}N_3O_4$, 328.1292; found, 328.1293.

*(8-Cyano-7-(hydroxy)-4-(phenylethynyl)quinolin-2-Yl)methyl Acetate (**12e**).* (5 mg, 81% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.40 (dd, *J* = 9.2, 2.1 Hz, 1H), 7.71 (dt, *J* = 7.9, 2.0 Hz, 2H), 7.57 (d, *J* = 2.1 Hz, 1H), 7.49 (h, *J* = 5.2, 4.8 Hz, 3H), 7.35 (dd, *J* = 9.2, 2.2 Hz, 1H), 5.40 (d, *J* = 2.2 Hz, 2H), 2.26 (d, *J* = 2.0 Hz, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.1, 164.4, 158.5, 148.6, 131.7, 131.5, 130.8, 129.6, 128.5, 121.6, 120.6, 119.5, 118.4, 114.5, 99.1, 95.0, 83.6, 66.0, 19.4; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{21}H_{15}N_2O_3$, 343.1077; found, 343.1058.

*(8-Cyano-7-(hydroxy)-4-methylquinolin-2-yl)methyl Acetate (**12f**).* (18 mg, 99% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.18 (d, *J* = 9.2 Hz, 1H), 7.31 (s, 1H), 7.26 (d, *J* = 9.3 Hz, 1H), 5.36 (s, 2H), 2.71 (s, 3H), 2.24 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.1, 163.9, 158.2, 148.0, 147.2, 130.2, 121.3, 118.2, 117.3, 114.7, 94.5, 65.9, 19.4, 17.3; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{14}H_{13}N_2O_3$, 257.0921; found, 257.0919.

*(8-Cyano-7-hydroxy-4-(1H-Pyrrol-3-yl)quinolin-2-yl)methyl Acetate (**12g**).* To a solution of **11 g** (10 mg, 0.02 mmol, 1 equiv) in THF (2 mL) at 0 °C, a 1 M solution of tetrabutylammonium fluoride (0.022 mL, 0.022 mmol, 1.1 equiv) in THF was added dropwise. The mixture was stirred at 0 °C for 30 min and then at room temperature for 1 h. When the reaction was completed, as indicated by LC−MS analysis, the solvent was evaporated. The resulting residue was subjected to the procedure described for converting **11a** into the MOM ether **12a**, affording **12g** (4 mg, 65% yield). ^{1}H NMR (500 MHz, acetonitrile-d_3, δ): 9.73 (s, 1H), 8.50 (d, *J* = 9.3 Hz, 1H), 7.42 (s, 1H), 7.33 (d, *J* = 9.3 Hz, 1H), 7.24 (dt, *J* = 3.2, 1.8 Hz, 1H), 6.99 (q, *J* = 2.5 Hz, 1H), 6.55 (td, *J* = 2.7, 1.6 Hz, 1H), 5.34 (s, 2H), 2.20 (s, 3H); ^{13}C NMR{1H} (126 MHz, acetonitrile-d_3, δ): 170.6, 163.2, 158.2, 149.5, 145.1, 132.6, 119.9, 119.5, 119.3, 116.8, 115.4, 109.1, 95.2, 66.4, 52.5, 25.1, 20.1; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{17}H_{14}N_3O_3$, 308.1030; found, 308.1024.

*(8-Cyano-7-hydroxy-4-phenylquinolin-2-yl)methyl Acetate (**12h**).* (12 mg, 93% yield). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 12.06 (s, 1H), 7.95 (d, *J* = 9.3 Hz, 1H), 7.64−7.51 (m, 5H), 7.39 (s, 1H), 7.35 (d, *J* = 9.3 Hz, 1H), 5.38 (s, 2H), 2.19 (s, 3H); ^{13}C NMR{1H} (126 MHz, DMSO-d_6, δ): 170.8, 164.4, 158.5, 149.6, 149.3, 137.2, 132.2, 129.9, 129.4, 129.3, 119.6, 119.1, 118.3, 115.9, 95.0, 66.5, 21.2; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{19}H_{15}N_2O_3$, 319.1077; found, 319.1083.

*(8-Cyano-7-(hydroxy)-4-(p-tolyl)quinolin-2-yl)methyl Acetate (**12i**).* (10 mg, 99% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 7.99 (d, *J* = 9.3 Hz, 1H), 7.38 (s, 4H), 7.34 (s, 1H), 7.21 (d, *J* = 9.3 Hz, 1H), 5.42 (s, 2H), 2.46 (s, 3H), 2.23 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.2, 164.0, 158.1, 150.3, 149.0, 138.9, 134.1, 131.9, 129.1, 129.1, 119.8, 117.6, 117.5, 114.8, 94.7, 66.1, 19.9, 19.4; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{20}H_{17}N_2O_3$, 333.1234; found, 333.1250.

*(8-Cyano-7-(hydroxy)-4-(4-methoxyphenyl)quinolin-2-Yl)methyl Acetate (**12j**).* (9 mg, 95% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.04 (d, *J* = 9.3 Hz, 1H), 7.49−7.42 (m, 2H), 7.35 (s, 1H), 7.22 (d, *J* = 9.3 Hz, 1H), 7.16−7.10 (m, 2H), 5.43 (s, 2H), 3.91 (s, 3H), 2.23 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.2, 164.0, 160.6, 158.1, 150.2, 149.1, 131.9, 130.5, 129.2, 119.9, 117.6, 117.5, 114.8, 114.0, 94.7, 66.1, 54.5, 19.4; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{20}H_{17}N_2O_4$, 349.1183; found, 349.1176.

*(8-Cyano-4-(4-cyanophenyl)-7-(hydroxy)quinolin-2-yl)methyl Acetate (**12k**).* (6 mg, 99% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 7.99−7.93 (m, 2H), 7.90 (d, *J* = 9.3 Hz, 1H), 7.76−7.70 (m, 2H), 7.42 (s, 1H), 7.27 (d, J = 9.3 Hz, 1H), 5.46 (s, 2H), 2.24 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.2, 164.1, 158.4, 149.2, 148.0, 141.9, 132.4, 131.1, 130.3, 130.2, 119.1, 118.2, 117.9, 117.5, 114.6, 112.5, 95.2, 70.1, 66.1, 19.4; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{20}H_{14}N_3O_3$, 344.1030; found, 344.1034.

*4-(2-(Acetoxymethyl)-8-cyano-7-hydroxyquinolin-4-yl)benzoic Acid (**12l**).* (5 mg, 99% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.27−8.21 (m, 2H), 7.95 (d, *J* = 9.3 Hz, 1H), 7.68−7.59 (m, 2H), 7.42 (s, 1H), 7.26 (d, *J* = 9.3 Hz, 1H), 5.46 (s, 2H), 2.24 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.2, 167.8, 164.1, 158.4, 149.2, 149.0, 141.7, 131.4, 131.1, 129.8, 129.4, 119.4, 118.0, 117.5, 114.7, 95.1, 66.1, 19.4; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{20}H_{15}N_2O_5$, 363.0975; found, 363.0976.

*(8-Cyano-4-(4-fluorophenyl)-7-(hydroxy)quinolin-2-yl)methyl Acetate (**12m**).* (18 mg, 94% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 7.96 (d, *J* = 9.3 Hz, 1H), 7.59−7.53 (m, 2H), 7.37 (s, 1H), 7.36−7.30 (m, 2H), 7.24 (d, *J* = 9.3 Hz, 1H), 5.44 (s, 2H), 2.24 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.21, 163.97, 163.2 ($^1J_{C-F}$ = 248.0 Hz), 158.3, 149.2, 149.0, 137.3, 133.3, 131.5, 131.2 ($^3J_{C-F}$ = 8.2 Hz), 119.7, 117.8, 117.7, 115.3 ($^2J_{C-F}$ = 22.1 Hz), 95.0, 66.2, 19.4; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{19}H_{14}FN_2O_3$, 337.0983; found, 337.0970.

*(8-Cyano-4-(4-(dimethylamino)phenyl)-7-(hydroxy)quinolin-2-yl)methyl Acetate (**12n**).* (7 mg, 99% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.06 (d, *J* = 9.4 Hz, 1H), 7.56−7.48 (m, 2H), 7.36 (s, 1H), 7.27−7.19 (m, 3H), 5.42 (s, 2H), 3.18 (s, 6H), 2.24 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.2, 164.0, 158.0, 149.9, 149.2, 148.6, 132.8, 131.9, 130.6, 119.7, 117.5, 117.3, 114.9, 94.7, 66.1, 53.4, 41.3, 19.4; HRMS (ESI-QTOF) *m*/*z*: [M + H]$^+$ calcd for $C_{21}H_{20}N_3O_3$, 362.1499; found, 362.1479.

*(8-Cyano-7-(hydroxy)-4-(m-tolyl)quinolin-2-yl)methyl Acetate (**12o**).* (14 mg, 99% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 7.95 (d, *J* = 9.3 Hz, 1H), 7.43 (t, *J* = 7.6 Hz, 1H), 7.37−7.29 (m, 3H), 7.26 (dt, *J* = 7.4, 1.5 Hz, 1H), 7.19 (d, *J* = 9.3 Hz, 1H), 5.41 (s, 2H), 2.45 (s, 3H), 2.22 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.2, 164.0, 158.1, 150.4, 149.0, 138.5, 137.0, 131.9, 129.7, 129.3, 128.4, 126.3, 119.8, 117.6, 117.5, 114.8, 94.7, 66.1, 20.0, 19.4; HRMS

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726−744

(ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{20}H_{17}N_2O_3$, 333.1234; found, 333.1259.

*(8-Cyano-7-(hydroxy)-4-(3-methoxyphenyl)quinolin-2-yl)methyl Acetate (**12p**).* (18 mg, 99% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 7.96 (d, J = 9.3 Hz, 1H), 7.46 (t, J = 8.1 Hz, 1H), 7.34 (s, 1H), 7.20 (d, J = 9.3 Hz, 1H), 7.11−7.07 (m, 1H), 7.05−7.00 (m, 2H), 5.41 (s, 2H), 3.87 (s, 3H), 2.22 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.2, 164.0160.0, 158.1, 150.0, 149.0, 138.4, 131.8, 129.6, 121.4, 121.3, 119.7, 117.7, 117.5, 114.8, 114.0, 94.8, 66.1, 54.5, 19.4; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{20}H_{17}N_2O_4$, 349.1183; found, 349.1181.

*(8-Cyano-4-(3-fluorophenyl)-7-(hydroxy)quinolin-2-yl)methyl Acetate (**12q**).* (27 mg, 99% yield). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 12.11 (s, 1H), 7.94 (dd, J = 9.3, 2.4 Hz, 1H), 7.64 (tdt, J = 11.1, 7.7, 3.8 Hz, 1H), 7.47−7.32 (m, 5H), 5.38 (t, J = 3.6 Hz, 2H), 2.19 (t, J = 3.5 Hz, 3H); ^{13}C NMR{1H} (126 MHz, DMSO-d_6, δ): 170.7, 164.50, 162.6 ($^1J_{C-F}$ = 244.8 Hz), 158.5, 149.2, 148.1, 139.4 ($^3J_{C-F}$ = 7.8 Hz), 132.1, 131. 4 ($^4J_{C-F}$ = 8.3 Hz), 126.17, 119.35, 119.26, 118.3, 116.9 ($^2J_{C-F}$ = 22.5 Hz), 116.3 ($^6J_{C-F}$ = 20.6 Hz), 115.8, 95.0, 66.4, 21.2; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{19}H_{14}FN_2O_3$, 337.0983; found, 337.0969.

*(8-Cyano-4-(3-(dimethylamino)phenyl)-7-(hydroxy)quinolin-2-yl)methyl Acetate (**12r**).* (3 mg, 71% yield). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 7.45 (d, J = 9.5 Hz, 1H), 7.31 (t, J = 7.9 Hz, 1H), 6.82 (t, J = 4.1 Hz, 2H), 6.71−6.64 (m, 2H), 6.61 (d, J = 9.7 Hz, 1H), 5.19 (s, 2H), 2.94 (s, 6H), 2.14 (s, 3H); ^{13}C NMR{1H} (126 MHz, DMSO-d_6, δ): 170.8, 155.2, 153.2, 150.8, 149.2, 142.9, 139.4, 129.4, 129.2, 127.3, 127.0, 121.2, 117.4, 115.5, 113.5, 113.2, 112.5, 91.6, 67.2, 21.2; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{21}H_{20}N_3O_3$, 362.1499; found, 362.1493.

*(8-Cyano-4-(3,5-dimethoxyphenyl)-7-(hydroxy)quinolin-2-yl)-methyl Acetate (**12s**).* (18 mg, 92% yield). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 8.01 (d, J = 9.4 Hz, 1H), 7.39 (s, 1H), 7.35 (d, J = 9.4 Hz, 1H), 6.68 (d, J = 2.4 Hz, 1H), 6.64 (d, J = 2.4 Hz, 2H), 5.37 (s, 2H), 3.81 (s, 6H), 2.19 (s, 3H); ^{13}C NMR{1H} (126 MHz, DMSO-d_6, δ): 170.7, 164.6, 161.1, 158.4, 149.5, 149.2, 139.1, 132.3, 119.5, 119.1, 118.0, 115.9, 108.0, 101.1, 94.9, 66.5, 55.9, 21.2; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{21}H_{19}N_2O_5$, 379.1288; found, 379.1286.

*(8-Cyano-7-(hydroxy)-4-(3,4,5-trimethoxyphenyl)quinolin-2-yl)-methyl Acetate (**12t**).* (14 mg, 99% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.05 (d, J = 9.3 Hz, 1H), 7.39 (s, 1H), 7.24 (d, J = 9.4 Hz, 1H), 6.81 (s, 2H), 5.42 (s, 2H), 3.90 (s, 6H), 3.87 (s, 3H), 2.24 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.2, 164.0, 158.1, 153.4, 150.2, 149.0, 138.3, 132.9, 131.9, 119.8, 117.7, 117.5, 114.8, 106.7, 94.7, 66.1, 59.8, 55.4, 19.4; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{22}H_{21}N_2O_6$, 409.1394; found, 409.1398.

*(8-Cyano-7-(hydroxy)-4-(4-(N-methylformamido)phenyl)-quinolin-2-yl)methyl Acetate (**12u**).* (3 mg, 26% yield, over two steps). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.65 (s, 1H), 8.00 (d, J = 9.3 Hz, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.57−7.48 (m, 2H), 7.40 (s, 1H), 7.26 (d, J = 9.3 Hz, 1H), 5.45 (s, 2H), 3.42 (s, 3H), 2.24 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.2, 164.0, 162.9, 158.3, 149.3, 149.0, 143.3, 142.8, 135.1, 131.5, 130.5, 122.0, 119.6, 117.8, 117.6, 95.0, 66.2, 30.8, 19.4; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{21}H_{18}N_3O_4$, 376.1292; found, 376.1295.

*(8-Cyano-7-(hydroxy)-4-(3-(N-methylformamido)phenyl)-quinolin-2-yl)methyl Acetate (**12v**).* (4 mg, 99% yield). ^{1}H NMR (500 MHz, methanol-d_4, δ): 8.62 (s, 1H), 7.99 (d, J = 9.3 Hz, 1H), 7.69−7.64 (m, 1H), 7.55−7.49 (m, 2H), 7.49−7.38 (m, 2H), 7.26 (dd, J = 9.3, 1.4 Hz, 1H), 5.45 (s, 2H), 3.39 (s, 3H), 2.24 (s, 3H); ^{13}C NMR{1H} (126 MHz, methanol-d_4, δ): 171.2, 164.0, 163.1, 158.3, 149.2, 149.0, 142.7, 138.8, 131.5, 129.8, 127.2, 123.0, 122.4, 119.6, 118.0, 117.7, 114.7, 95.0, 66.1, 30.9, 19.4; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{21}H_{18}N_3O_4$, 376.1292; found, 376.1296.

*3,4-(Methylenedioxy)phenylacetyl Chloride (**14**).*[63] To a solution of homopiperonylic acid (500 mg, 2.77 mmol, 1 equiv) in toluene (20 mL), oxalyl chloride (0.28 mL, 3.33 mmol, 1.2 equiv) and DMF (one drop) were added dropwise. The reaction was stirred at room temperature for 1 h. The solvent was then evaporated furnishing pure acetyl chloride **14** (533 mg, 97% yield). ^{1}H NMR (500 MHz, chloroform-d, δ): 6.82 (dd, J = 8.0, 1.4 Hz, 1H), 6.79−6.70 (m, 2H), 5.99 (s, 2H), 4.07 (s, 2H); ^{13}C NMR{1H} (126 MHz, chloroform-d, δ): 172.1, 148.1, 147.6, 124.7, 123.1, 109.8, 108.6, 101.4, 52.7; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_9H_8ClO_3$, 199.0156; found, 199.0157.

*Preparation of Protected Homopiperonylates **15** and **16f**, **16i**, **16s**, and **16t**.* To a solution of MOM-CyHQ-OH or one of the primary alcohols **10f**, **10i**, **10s**, and **10t** (0.1 mmol, 1 equiv) in chloroform (3 mL), pyridine (0.5 mmol, 5 equiv) and 4-dimethylaminopyridine (0.1 mmol, 1 equiv) were added, followed by the dropwise addition of a solution of **14** (0.3 mmol, 3 equiv) in chloroform (2 mL). The mixture was stirred at 60 °C for 12 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL), and the resulting solution was washed with H_2O (2 × 10 mL) and brine (10 mL), dried over $MgSO_4$, and concentrated to dryness. The crude product was dissolved in CH_2Cl_2 (2 mL) and TFA (0.2 mL) was added dropwise. The reaction was stirred for up to 5 h until HPLC showed complete consumption of the starting material. The product was purified by reverse-phase preparative chromatography (water/CH_3CN), affording **15** or the respective 4-substituted CyHQ-protected homopiperonylate **16f**, **16i**, **16s**, and **16t**.

*(8-Cyano-7-hydroxyquinolin-2-yl)methyl 2-(Benzo[d][1,3]dioxol-5-yl)acetate (**15**).* (22 mg, 15% yield). ^{1}H NMR (500 MHz, acetonitrile-d_3, δ): 8.26 (s, 1H), 8.05−8.00 (m, 1H), 7.39 (s, 2H), 6.86 (d, J = 29.4 Hz, 3H), 5.97 (s, 2H), 5.40 (s, 2H), 3.78 (s, 2H); ^{13}C NMR{1H} (126 MHz, acetonitrile-d_3, δ): 171.4, 163.4, 158.8, 148.4, 147.7, 146.7, 137.4, 134.0, 128.0, 122.7, 121.8, 118.1, 117.9, 115.0, 109.8, 108.0, 101.3, 95.0, 66.7, 40.0; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{20}H_{15}N_2O_5$, 363.0975; found, 363.0965.

*(8-Cyano-7-hydroxy-4-methylquinolin-2-yl)methyl 2-(Benzo[d]-[1,3]dioxol-5-yl)acetate (**16f**).* (3 mg, 19% yield). ^{1}H NMR (500 MHz, acetonitrile-d_3, δ): 8.17 (d, J = 9.2 Hz, 1H), 7.43 (d, J = 9.3 Hz, 1H), 7.18 (s, 1H), 6.90 (s, 1H), 6.81 (d, J = 7.1 Hz, 1H), 6.75 (d, J = 7.9 Hz, 1H), 5.97 (s, 2H), 5.34 (s, 2H), 3.77 (s, 2H), 2.66 (s, 3H); ^{13}C NMR{1H}{1H} (126 MHz, acetonitrile-d_3, δ): 171.4, 163.7, 158.4, 148.6, 147.7, 146.7, 146.6, 130.4, 128.0, 122.7, 122.5, 121.4, 118.3, 115.4, 109.8, 109.7, 108.0, 101.3, 66.6, 40.1, 17.8; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{21}H_{17}N_2O_5$, 377.1132; found, 377.1117.

*(8-Cyano-7-hydroxy-4-(p-tolyl)quinolin-2-yl)methyl 2-(Benzo[d]-[1,3]dioxol-5-yl)acetate (**16i**).* (8 mg, 32% yield). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 12.08 (s, 1H), 7.97 (d, J = 9.3 Hz, 1H), 7.43−7.33 (m, 5H), 7.21 (s, 1H), 6.91 (s, 1H), 6.81 (t, J = 6.3 Hz, 2H), 5.96 (s, 2H), 5.41 (s, 2H), 3.79 (s, 2H), 2.43 (s, 3H); ^{13}C NMR{1H}{1H} (126 MHz, DMSO-d_6, δ): 171.6, 164.4, 158.5, 149.6, 149.3, 147.6, 146.6, 139.0, 134.2, 132.3, 129.9, 129.7, 128.2, 123.1, 119.6, 118.9, 117.8, 116.0, 110.4, 108.6, 101.3, 94.9, 66.7, 30.9, 21.3; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{27}H_{21}N_2O_5$, 453.1445; found, 453.1437.

*(8-Cyano-4-(3,5-dimethoxyphenyl)-7-hydroxyquinolin-2-yl)-methyl 2-(Benzo[d][1,3]dioxol-5-yl)acetate (**16s**).* (4 mg, 22% yield). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 12.12 (s, 1H), 8.00 (d, J = 9.3 Hz, 1H), 7.36 (d, J = 9.4 Hz, 1H), 7.29 (s, 1H), 6.90 (s, 1H), 6.80 (s, 2H), 6.68 (t, J = 2.2 Hz, 1H), 6.61 (d, J = 2.2 Hz, 2H), 5.95 (s, 2H), 5.41 (s, 2H), 3.81 (s, 6H), 3.80 (s, 2H); ^{13}C NMR{1H} (126 MHz, DMSO-d_6, δ): 171.6, 164.5, 161.0, 158.4, 158.3, 149.6, 149.2, 147.6, 146.6, 139.1, 132.4, 128.2, 123.1, 119.5, 119.1, 117.8, 116.0, 110.4, 108.5, 107.9, 101.3, 101.1, 94.8, 66.7, 55.9; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{28}H_{23}N_2O_7$, 499.1500; found, 499.1489.

*(8-Cyano-7-hydroxy-4-(3,4,5-trimethoxyphenyl)quinolin-2-yl)-methyl 2-(Benzo[d][1,3]dioxol-5-yl)acetate (**16t**).* (4 mg, 23% yield). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 12.12 (s, 1H), 8.08 (d, J = 9.3 Hz, 1H), 7.39−7.31 (m, 2H), 6.91 (s, 1H), 6.79 (d, J = 6.8 Hz, 4H), 5.95 (s, 2H), 5.41 (s, 2H), 3.83 (s, 6H), 3.80 (s, 2H), 3.76 (s, 3H); ^{13}C NMR{1H} (126 MHz, DMSO-d_6, δ): 171.6, 164.5, 158.4, 153.5, 149.7, 149.2, 147.6, 146.5, 138.3, 132.6, 132.6, 128.2, 123.1, 119.6, 119.0, 118.6, 117.9, 116.1, 110.4, 108.5, 107.3, 101.3, 94.8, 66.7, 60.6, 56.5; HRMS (ESI-QTOF) m/z: $[M + H]^+$ calcd for $C_{29}H_{25}N_2O_8$, 529.1605; found, 529.1614.

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726−744

Spectroscopy. *Measurement of UV Spectra and the Molar Extinction Coefficient (ε).* UV−vis spectra were obtained from a 0.1 mM solution of compound in KMOPS buffer (for compounds **15** and **16f**, **16i**, **16s**, and **16t**, 20% acetonitrile was used as a cosolvent). A blank solution of KMOPS or 20% acetonitrile in KMOPS was used to subtract baseline absorption. The spectra were recorded between 250 and 500 nm. Each measurement was repeated in triplicate and the absorbance values were averaged. ε values at λ = 365 nm were calculated using the Beer−Lambert law: $\varepsilon = A(cl)^{-1}$, were A is the absorbance value measured at 365 nm, c the concentration of the sample, and l the cuvette length (1 cm).

Measurement of Emission Spectra. Fluorescence spectra were recorded from solutions of compounds in 0.001 N NaOH. The high background fluorescence of MOPS did not permit measurements in KMOPS buffer. Concentrations ranged from 0.25 to 10 μM depending of the responsiveness of the substrates. A blank solution of 0.01 N NaOH was used to subtract baseline emission. The spectra were recorded between 400 and 700 nm. Each measure was repeated in duplicate and the emission values were averaged.

Determination of the Time Constant (τ) for Dark Hydrolysis. Substrates were dissolved in KMOPS buffer (for compounds **15** and **16f**, **16i**, **16s**, and **16t**, 20% acetonitrile was used as a cosolvent) to a final concentration of 0.1 mM. The solutions were kept in the dark and sampled at different time intervals over 7 days. The percentage of starting material remaining was determined by HPLC analysis (see section describing the analysis of the photochemical reactions), and the time constant value (τ) was obtained from the following equation

$$\tau = \frac{t_{1/2}}{\ln(2)}$$

where $t_{1/2}$ represents the half-life expressed in hours.

Determination of the Solubility of 15 and 16f, 16i, 16s, and 16t. A 15 μL aliquot of a stock solution of **15** or **16f**, **16i**, **16s**, and **16t** (10 mM) in DMSO was diluted in 1 mL of KMOPS buffer. The mixture was sonicated at 45 °C in the dark for 1 h and then equilibrated at room temperature over 24 h. The mixture was sampled and filtered and the concentration was determined by HPLC. Experiments were repeated in triplicate.

Photolysis Reactions. *One-Photon Excitation (1PE).* Stock solutions (10 mM) of substrates in DMSO were diluted with KMOPS buffer (KCl 100 mM, MOPS 10 mM, pH 7.2) to a final concentration of 0.1 mM (for compounds **15** and **16f**, **16i**, **16s**, and **16t**, 20% acetonitrile was used as a cosolvent). Solutions were placed in a 3 mL quartz cuvette together with a stirring bar and irradiated with a LED lamp (Cairn OptoLED Lite) at 365 nm with stirring. Aliquots (70 μL) were sampled at different time intervals and analyzed by reverse-phase uHPLC, using an external standard calibration method for quantification. All experiments were repeated in triplicate. HPLC analyses were performed on an Agilent 1290 Infinity Series uHPLC using a Zorbax Eclipse Plus C18 column, monitoring the AUC at 320 nm. Separations were obtained with a gradient elution (flux rate of 0.3 mL/min) using a mobile phase composed of A = 0.1% trifuoroacetic acid in water and B = acetonitrile (starting from 5% B to 100% over 10 min and re-equilibrating to 5% B before the next run). The quantification of the percentage of the starting material remaining was obtained by the comparison of the AUC measured with calibration curves generated from known concentrations of the substrate. The percentages remaining were plotted versus time, and the $t_{90\%}$ values (time in seconds for 90% of reaction) were obtained by fitting a single exponential decay curve to the data using the software DeltaGraph (Red Rock Software). The quantum efficiency (Φ_u) of the photolysis reaction was calculated from the following equation

$$\Phi_u = (I\sigma t_{90\%})^{-1}$$

where I represents the lamp intensity in Einstein cm^{-2} s^{-1} (measured by ferrioxalate actinometry)[67] and σ is the decadic extinction coefficient (1000 × ε, molar extinction coefficient).[28,30,31] The release of 3,4-(methylenedioxy)phenylacetate (for compounds **15** and **16f**, **16i**, **16s**, and **16t**) was quantified, following an external standard calibration method (monitoring the AUC at 280 nm) and plotted versus time, fitting an exponential rise to the max curve to the data.

Two-Photon Excitation (2PE). Working solutions were prepared as described for photolysis reactions mediated by 1PE. For compound **15** and **16f**, **16i**, **16s**, and **16t**, an internal standard (7-hydroxy-2-methylquinoline-8-carbonitrile, 50 μM final concentration) was added to account for solvent evaporation during the experiment. Solutions (25 μL) were placed into a microcuvette (26.10F-Q-10, Starna, 10 × 1 × 1 mm illuminated dimensions) and irradiated for different time intervals (typically 5, 10, and 30 min) with 740 nm light from a femtosecond-pulsed and mode-locked Ti:sapphire laser (Mai Tai HP DeepSee, Spectra-Physics) focused on the center of the cuvette chamber. The average power used was 250−350 mW (depending on the experiment) measured after passing through the cuvette. Samples were analyzed by reverse-phase uHPLC to quantify the percentage of starting material remaining, as described for the photolysis mediated by 1PE, which was plotted versus time. The resulting data were plotted using DeltaGraph (Red Rock Software) software and fitted to a single exponential decay curve. The two-photon uncaging action cross-section (δ_u) values were determined, following a previously reported procedure,[28,30,31] using fluorescein as an external standard and the following equation

$$\delta_u = \frac{N_p \phi Q_{f2} \delta_{aF} C_f}{\langle F(t) \rangle C_s}$$

where N_p is the number of product molecule formed per second determined by HPLC analysis, ϕ is the collection efficiency of the fluorescence detector positioned at a right angle to the excitation beam, Q_{f2} is the two-photon fluorescence quantum yield of fluorescein (0.9),[68,69] δ_{aF} is the fluorescein absorbance cross section (30 GM at 740 nm),[70] C_f is the concentration of fluorescein, $\langle F(t) \rangle$ is the time-averaged fluorescent photon flux (photon/s) from the emission of the fluorescein standard measure by the detector, and C_s is the concentration of substrate.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.9b02780.

Spectroscopic and analytical data, Figures S1−S35, and Tables S1 and S2 (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: timothy.dore@nyu.edu.

ORCID

Davide Deodato: 0000-0001-6715-0503

Naeem Asad: 0000-0001-6871-5270

Timothy M. Dore: 0000-0002-3876-5012

Author Contributions

§A.-L.K.H., D.D., and N.A. contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by New York University Abu Dhabi. Part of this work was carried out using Core Technology Platform resources at New York University Abu

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726−744

Dhabi. This work is dedicated to the memory of Prof. Maurizio Botta from University of Siena.

REFERENCES

(1) Klán, P.; Šolomek, T.; Bochet, C. G.; Blanc, A.; Givens, R.; Rubina, M.; Popik, V.; Kostikov, A.; Wirz, J. Photoremovable Protecting Groups in Chemistry and Biology: Reaction Mechanisms and Efficacy. *Chem. Rev.* **2013**, *113*, 119−191.

(2) Pelliccioli, A. P.; Wirz, J. Photoremovable protecting groups: reaction mechanisms and applications. *Photochem. Photobiol. Sci.* **2002**, *1*, 441−458.

(3) Mayer, G.; Heckel, A. Biologically active molecules with a "light switch". *Angew. Chem., Int. Ed.* **2006**, *45*, 4900−4921.

(4) Salierno, M.; Marceca, E.; Peterka, D. S.; Yuste, R.; Etchenique, R. A fast ruthenium polypyridine cage complex photoreleases glutamate with visible or IR light in one and two photon regimes. *J. Inorg. Biochem.* **2010**, *104*, 418−422.

(5) Matsuzaki, M.; Hayama, T.; Kasai, H.; Ellis-Davies, G. C. Two-photon uncaging of gamma -aminobutyric acid in intact brain tissue. *Nat. Chem. Biol.* **2010**, *6*, 255−257.

(6) Momotake, A.; Lindegger, N.; Niggli, E.; Barsotti, R. J.; Ellis-Davies, G. C. The nitrodibenzofuran chromophore: a new caging group for ultra-efficient photolysis in living cells. *Nat. Methods* **2006**, *3*, 35−40.

(7) Mentel, M.; Laketa, V.; Subramanian, D.; Gillandt, H.; Schultz, C. Photoactivatable and Cell-Membrane-Permeable Phosphatidylinositol 3,4,5-Trisphosphate. *Angew. Chem., Int. Ed.* **2011**, *50*, 3811−3814.

(8) Nakanishi, J.; Kikuchi, Y.; Takarada, T.; Nakayama, H.; Yamaguchi, K.; Maeda, M. Spatiotemporal control of cell adhesion on a self-assembled monolayer having a photocleavable protecting group. *Anal. Chim. Acta* **2006**, *578*, 100−104.

(9) Schäfer, F.; Wagner, J.; Knau, A.; Dimmeler, S.; Heckel, A. Regulating angiogenesis with light-inducible AntimiRs. *Angew. Chem., Int. Ed.* **2013**, *52*, 13558−13561.

(10) Matsuzaki, M.; Ellis-Davies, G. C. R.; Nemoto, T.; Miyashita, Y.; Iino, M.; Kasai, H. Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nat. Neurosci.* **2001**, *4*, 1086−1092.

(11) Smith, M. A.; Ellis-Davies, G. C. R.; Magee, J. C. Mechanism of the distance-dependent scaling of Schaffer collateral synapses in rat CA1 pyramidal neurons. *J. Physiol.* **2003**, *548*, 245−258.

(12) Zheng, G.; Cochella, L.; Liu, J.; Hobert, O.; Li, W.-h. Temporal and Spatial Regulation of MicroRNA Activity with Photoactivatable Cantimirs. *ACS Chem. Biol.* **2011**, *6*, 1332−1338.

(13) O'Connor, M. J.; Beebe, L. L.; Deodato, D.; Ball, R. E.; Page, A. T.; VanLeuven, A. J.; Harris, K. T.; Park, S.; Hariharan, V.; Lauderdale, J. D.; Dore, T. M. Bypassing Glutamic Acid Decarboxylase 1 (Gad1) Induced Craniofacial Defects with a Photoactivatable Translation Blocker Morpholino. *ACS Chem. Neurosci.* **2019**, *10*, 266−278.

(14) Ellis-Davies, G. C. Caged compounds: photorelease technology for control of cellular chemistry and physiology. *Nat. Methods* **2007**, *4*, 619−628.

(15) Šolomek, T.; Wirz, J.; Klán, P. Searching for Improved Photoreleasing Abilities of Organic Molecules. *Acc. Chem. Res.* **2015**, *48*, 3064−3072.

(16) Bort, G.; Gallavardin, T.; Ogden, D.; Dalko, P. I. From One-Photon to Two-Photon Probes: "Caged" Compounds, Actuators, and Photoswitches. *Angew. Chem., Int. Ed.* **2013**, *52*, 4526−4537.

(17) Göppert-Mayer, M. Über Elementarakte mit zwei Quantensprüngen. *Ann. Phys.* **1931**, *9*, 273−294.

(18) Zhu, Y.; Pavlos, C. M.; Toscano, J. P.; Dore, T. M. 8-Bromo-7-hydroxyquinoline as a Photoremovable Protecting Group for Physiological Use: Mechanism and Scope. *J. Am. Chem. Soc.* **2006**, *128*, 4267−4276.

(19) Olson, J. P.; Banghart, M. R.; Sabatini, B. L.; Ellis-Davies, G. C. Spectral Evolution of a Photochemical Protecting Group for Orthogonal Two-Color Uncaging with Visible Light. *J. Am. Chem. Soc.* **2013**, *135*, 15948−15954.

(20) Aujard, I.; Benbrahim, C.; Gouget, M.; Ruel, O.; Baudin, J.-B.; Neveu, P.; Jullien, L. *o*-Nitrobenzyl photolabile protecting groups with red-shifted absorption: syntheses and uncaging cross-sections for one- and two-photon excitation. *Chem. - Eur. J.* **2006**, *12*, 6865−6879.

(21) Specht, A.; Thomann, J.-S.; Alarcon, K.; Wittayanan, W.; Ogden, D.; Furuta, T.; Kurakawa, Y.; Goeldner, M. New photoremovable protecting groups for carboxylic acids with high photolytic efficiencies at near-UV irradiation. Application to the photocontrolled release of L-glutamate. *ChemBioChem* **2006**, *7*, 1690−1695.

(22) Gug, S.; Charon, S.; Specht, A.; Alarcon, K.; Ogden, D.; Zietz, B.; Leonard, J.; Haacke, S.; Bolze, F.; Nicoud, J.-F.; Goeldner, M. Photolabile glutamate protecting group with high one- and two-photon uncaging efficiencies. *ChemBioChem* **2008**, *9*, 1303−1307.

(23) Gagey, N.; Neveu, P.; Benbrahim, C.; Goetz, B.; Aujard, I.; Baudin, J.-B.; Jullien, L. Two-Photon Uncaging with Fluorescence Reporting: Evaluation of the *o*-Hydroxycinnamic Platform. *J. Am. Chem. Soc.* **2007**, *129*, 9986−9998.

(24) Gagey, N.; Neveu, P.; Jullien, L. Two-photon uncaging with the efficient 3,5-dibromo-2,4-dihydroxycinnamic caging group. *Angew. Chem., Int. Ed.* **2007**, *46*, 2467−2469.

(25) Picard, S.; Genin, E.; Clermont, G.; Hugues, V.; Mongin, O.; Blanchard-Desce, M. Octupolar chimeric compounds built from quinoline caged acetate moieties: a novel approach for 2-photon uncaging of biomolecules. *New J. Chem.* **2013**, *37*, 3899−3913.

(26) Korzycka, K. A.; Bennett, P. M.; Cueto-Diaz, E. J.; Wicks, G.; Drobizhev, M.; Blanchard-Desce, M.; Rebane, A.; Anderson, H. L. Two-photon sensitive protecting groups operating via intramolecular electron transfer: uncaging of GABA and tryptophan. *Chem. Sci.* **2015**, *6*, 2419−2426.

(27) Kiskin, N. I.; Chillingworth, R.; McCray, J. A.; Piston, D.; Ogden, D. The efficiency of two-photon photolysis of a "caged" fluorophore, *o*-1-(2-nitrophenyl)ethylpyranine, in relation to photodamage of synaptic terminals. *Eur. Biophys. J.* **2002**, *30*, 588−604.

(28) Furuta, T.; Wang, S. S. H.; Dantzker, J. L.; Dore, T. M.; Bybee, W. J.; Callaway, E. M.; Denk, W.; Tsien, R. Y. Brominated 7-hydroxycoumarin-4-ylmethyls: photolabile protecting groups with biologically useful cross-sections for two photon photolysis. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 1193−1200.

(29) Klausen, M.; Dubois, V.; Clermont, G.; Tonnele, C.; Castet, F.; Blanchard-Desce, M. Dual-wavelength efficient two-photon photorelease of glycine by π-extended dipolar coumarins. *Chem. Sci.* **2019**, *10*, 4209−4219.

(30) Davis, M. J.; Kragor, C. H.; Reddie, K. G.; Wilson, H. C.; Zhu, Y.; Dore, T. M. Substituent Effects on the Sensitivity of a Quinoline Photoremovable Protecting Group to One- and Two-Photon Excitation. *J. Org. Chem.* **2009**, *74*, 1721−1729.

(31) Asad, N.; Deodato, D.; Lan, X.; Widegren, M. B.; Phillips, D. L.; Du, L.; Dore, T. M. Photochemical Activation of Tertiary Amines for Applications in Studying Cell Physiology. *J. Am. Chem. Soc.* **2017**, *139*, 12591−12600.

(32) Li, Y.-M.; Shi, J.; Cai, R.; Chen, X.-Y.; Guo, Q.-X.; Liu, L. Development of new quinoline-based photo-labile groups for photoregulation of bioactive molecules. *Tetrahedron Lett.* **2010**, *51*, 1609−1612.

(33) Li, Y. M.; Shi, J.; Cai, R.; Chen, X.; Luo, Z. F.; Guo, Q. X. New quinoline-based caging groups synthesized for photo-regulation of aptamer activity. *J. Photochem. Photobiol., A* **2010**, *211*, 129−134.

(34) Narumi, T.; Miyata, K.; Nii, A.; Sato, K.; Mase, N.; Furuta, T. 7-Hydroxy-N-Methylquinolinium Chromophore: A Photolabile Protecting Group for Blue-Light Uncaging. *Org. Lett.* **2018**, *20*, 4178−4182.

(35) Ma, J.; Cheng, S. C.; An, H.; Li, M.-D.; Ma, C.; Rea, A. C.; Zhu, Y.; Nganga, J. L.; Dore, T. M.; Phillips, D. L. Comparison of the Absorption, Emission, and Resonance Raman Spectra of 7-Hydroxyquinoline and 8-Bromo-7-Hydroxyquinoline Caged Acetate. *J. Phys. Chem. A* **2011**, *115*, 11632−11640.

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726−744

(36) Dunkel, P.; Tran, C.; Gallavardin, T.; Dhimane, H.; Ogden, D.; Dalko, P. I. Quinoline-derived two-photon sensitive quadrupolar probes. *Org. Biomol. Chem.* **2014**, *12*, 9899−9908.
(37) Petit, M.; Tran, C.; Roger, T.; Gallavardin, T.; Dhimane, H.; Palma-Cerda, F.; Blanchard-Desce, M.; Acher, F. C.; Ogden, D.; Dalko, P. I. Substitution Effect on the One- and Two-photon Sensitivity of DMAQ "Caging" Groups. *Org. Lett.* **2012**, *14*, 6366−6369.
(38) Tran, C.; Berqouch, N.; Dhimane, H.; Clermont, G.; Blanchard-Desce, M.; Ogden, D.; Dalko, P. I. Quinoline-Derived Two-Photon Sensitive Quadrupolar Probes. *Chem. − Eur. J.* **2017**, *23*, 1860−1868.
(39) Tran, C.; Gallavardin, T.; Petit, M.; Slimi, R.; Dhimane, H.; Blanchard-Desce, M.; Acher, F. C.; Ogden, D.; Dalko, P. I. Two-Photon "Caging" Groups: Effect of Position Isomery on the Photorelease Properties of Aminoquinoline-Derived Photolabile Protecting Groups. *Org. Lett.* **2015**, *17*, 402−405.
(40) Ouyang, X.; Shestopalov, I. A.; Sinha, S.; Zheng, G.; Pitt, C. L. W.; Li, W.-H.; Olson, A. J.; Chen, J. K. Versatile Synthesis and Rational Design of Caged Morpholinos. *J. Am. Chem. Soc.* **2009**, *131*, 13255−13269.
(41) Zhang, Z. P.; Li, Y. M.; Chen, X. Y.; Guo, Q. X. Photoregulation of protein plasmid expression in vitro and in vivo using BHQ caging group. *Chin. Chem. Lett.* **2011**, *22*, 338−341.
(42) Rea, A. C.; Vandenberg, L. N.; Ball, R. E.; Snouffer, A. A.; Hudson, A. G.; Zhu, Y.; McLain, D. E.; Johnston, L. L.; Lauderdale, J. D.; Levin, M.; Dore, T. M. Light Activated Serotonin for Exploring Its Action in Biological Systems. *Chem. Biol.* **2013**, *20*, 1536−1546.
(43) Ma, J.; Rea, A. C.; An, H.; Ma, C.; Guan, X.; Li, M.-D.; Su, T.; Yeung, C. S.; Harris, K. T.; Zhu, Y.; Nganga, J. L.; Fedoryak, O. D.; Dore, T. M.; Phillips, D. L. Unraveling the Mechanism of the Photodeprotection Reaction of 8-Bromo- and 8-Chloro-7-hydroxyquinoline Caged Acetates. *Chem.—Eur. J.* **2012**, *18*, 6854−6865.
(44) Huang, J.; Muliawan, A. P.; Ma, J.; Li, M. D.; Chiu, H. K.; Lan, X.; Deodato, D.; Phillips, D. L.; Dore, T. M. A spectroscopic study of the excited state proton transfer processes of (8-bromo- 7-hydroxyquinolin-2-yl)methyl-protected phenol in aqueous solutions. *Photochem. Photobiol. Sci.* **2017**, *16*, 575−584.
(45) McLain, D. E.; Rea, A. C.; Widegren, M. B.; Dore, T. M. Photoactivatable, biologically-relevant phenols with sensitivity toward 2-photon excitation. *Photochem. Photobiol. Sci.* **2015**, *14*, 2141−2158.
(46) Deodato, D.; Asad, N.; Dore, T. M. Photorearrangement of Quinoline-Protected Dialkylanilines and the Photorelease of Aniline-Containing Biological Effectors. *J. Org. Chem.* **2019**, *84*, 7342−7353.
(47) He, G. S.; Tan, L.-S.; Zheng, Q.; Prasad, P. N. Multiphoton Absorbing Materials: Molecular Designs, Characterizations, and Applications. *Chem. Rev.* **2008**, *108*, 1245−1330.
(48) Terenziani, F.; Katan, C.; Badaeva, E.; Tretiak, S.; Blanchard-Desce, M. Enhanced two-photon absorption of organic chromophores: theoretical and experimental assessments. *Adv. Mater.* **2008**, *20*, 4641−4678.
(49) Pawlicki, M.; Collins, H. A.; Denning, R. G.; Anderson, H. L. Two-Photon Absorption and the Design of Two-Photon Dyes. *Angew. Chem., Int. Ed.* **2009**, *48*, 3244−3266.
(50) Abe, M.; Chitose, Y.; Jakkampudi, S.; Thuy, P. T. T.; Lin, Q.; Van, B. T.; Yamada, A.; Oyama, R.; Sasaki, M.; Katan, C. Design and Synthesis of Two-Photon Responsive Chromophores for Near-Infrared Light-Induced Uncaging Reactions. *Synthesis* **2017**, *49*, 3337−3346.
(51) Lee, W.-H.; Lee, H.; Kim, J.-A.; Choi, J.-H.; Cho, M.; Jeon, S.-J.; Cho, B. R. Two-Photon Absorption and Nonlinear Optical Properties of Octupolar Molecules. *J. Am. Chem. Soc.* **2001**, *123*, 10658−10667.
(52) Kurotobi, K.; Kim, K. S.; Noh, S. B.; Kim, D.; Osuka, A. A quadruply azulene-fused porphyrin with intense near-IR absorption and a large two-photon absorption cross section. *Angew. Chem., Int. Ed.* **2006**, *45*, 3944−3947.
(53) Rumi, M.; Ehrlich, J. E.; Heikal, A. A.; Perry, J. W.; Barlow, S.; Hu, Z.; McCord-Maughon, D.; Parker, T. C.; Roeckel, H.; Thayumanavan, S.; Marder, S. R.; Beljonne, D.; Bredas, J.-L. Structure-Property Relationships for Two-Photon Absorbing Chromophores: Bis-Donor Diphenylpolyene and Bis(styryl)benzene Derivatives. *J. Am. Chem. Soc.* **2000**, *122*, 9500−9510.
(54) Zimmerman, H. E.; Sandel, V. R. Mechanistic organic photochemistry. II. Solvolytic photochemical reactions. *J. Am. Chem. Soc.* **1963**, *85*, 915−922.
(55) Zimmerman, H. E.; Somasekhara, S. Mechanistic organic photochemistry. III. Excited state solvolyses. *J. Am. Chem. Soc.* **1963**, *85*, 992−927.
(56) Zimmerman, H. E. The Meta Effect in Organic Photochemistry: Mechanistic and Exploratory Organic Photochemistry. *J. Am. Chem. Soc.* **1995**, *117*, 8988−8991.
(57) Zimmerman, H. E. Meta-Ortho Effect in Organic Photochemistry: Mechanistic and Exploratory Organic Photochemistry. *J. Phys. Chem. A* **1998**, *102*, 5616−5621.
(58) Havinga, E.; De Jongh, R. O.; Dorst, W. Photochemical acceleration of the hydrolysis of nitrophenyl phosphates and nitrophenyl sulfates. *Recl. Trav. Chim. Pays-Bas* **1956**, *75*, 378−383.
(59) Ding, X.; Wang, P. Photochemical Cleavage of Benzylic C-O Bond Facilitated by an Ortho or Meta Amino Group. *J. Org. Chem.* **2017**, *82*, 7309−7316.
(60) Wang, P.; Devalankar, D. A.; Lu, W. Photochemical Cleavage of Benzylic C-N Bond To Release Amines. *J. Org. Chem.* **2016**, *81*, 6195−6200.
(61) Vollmer, F.; Rettig, W.; Birckner, E. Photochemical mechanisms producing large fluorescence Stokes shifts. *J. Fluoresc.* **1994**, *4*, 65−69.
(62) Hansch, C.; Leo, A.; Taft, R. W. A survey of Hammett substituent constants and resonance and field parameters. *Chem. Rev.* **1991**, *91*, 165−195.
(63) Schrittwieser, J. H.; Resch, V.; Sattler, J. H.; Lienhart, W.-D.; Durchschein, K.; Winkler, A.; Gruber, K.; Macheroux, P.; Kroutil, W. Biocatalytic Enantioselective Oxidative C-C Coupling by Aerobic C-H Activation. *Angew. Chem., Int. Ed.* **2011**, *50*, 1068−1071.
(64) Inlay, M. A.; Choe, V.; Bharathi, S.; Fernhoff, N. B.; Baker, J. R.; Weissman, I. L.; Choi, S. K. Synthesis of a photocaged tamoxifen for light-dependent activation of Cre-ER recombinase-driven gene modification. *Chem. Commun.* **2013**, *49*, 4971−4973.
(65) Duc, T. N.; Huse, M. A Generalizable Platform for the Photoactivation of Cell Surface Receptors. *ACS Chem. Biol.* **2015**, *10*, 2435−2440.
(66) Houk, A. L.; Givens, R. S.; Elles, C. G. Two-Photon Activation of p-Hydroxyphenacyl Phototriggers: Toward Spatially Controlled Release of Diethyl Phosphate and ATP. *J. Phys. Chem. B* **2016**, *120*, 3178−3186.
(67) Hatchard, C. G.; Parker, C. A. A new sensitive chemical actinometer. II. Potassium ferrioxalate as a standard chemical actinometer. *Proc. R. Soc. London, Ser. A* **1956**, *235*, 518−536.
(68) Xu, C.; Webb, W. W. Measurement of two-photon excitation cross sections of molecular fluorophores with data from 690 to 1050 nm. *J. Opt. Soc. Am. B* **1996**, *13*, 481−491.
(69) Xu, C.; Guild, J.; Webb, W. W.; Denk, W. Determination of absolute two-photon excitation cross sections by *in situ* second-order autocorrelation. *Optics Lett.* **1995**, *20*, 2372−2374.
(70) Albota, M. A.; Xu, C.; Webb, W. W. Two-photon fluorescence excitation cross sections of biomolecular probes from 690 to 960 nm. *Appl. Opt.* **1998**, *37*, 7352−7356.

DOI: 10.1021/acs.joc.9b02780
J. Org. Chem. 2020, 85, 726−744