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Silicon Substitution in Oxazine Dyes Yields Near-Infrared Azasiline Fluorophores That Absorb and Emit beyond 700 nm

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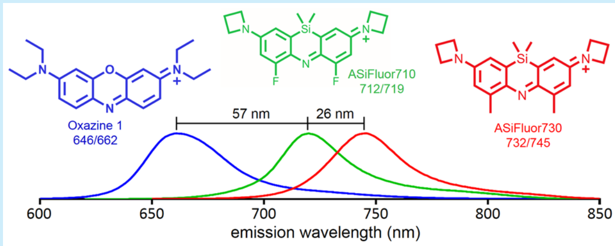
Supporting Information

ABSTRACT: Exchanging the bridging oxygen atom in rhodamine dyes with a dimethylsilyl group red-shifts their excitation and emission spectra, transforming orange fluorescent rhodamines into far-red Si-rhodamines. To study the effect of this substitution in other dye scaffolds, synthetic approaches to incorporate silicon into the bridging position of oxazine dyes were developed. The fluorescence of the compact azasiline dyes ASiFluor710 and ASiFluor730 is red-shifted by 57−83 nm from that of Oxazine **1**.



Fluorescent probes are among the simplest and most convenient chemical tools used to investigate biological processes. In particular, near-infrared (NIR) fluorophores with excitation and emission between 650 and 900 nm are becoming increasingly popular. Unlike visible-light fluorophores, NIR light can effectively penetrate live tissues, lessening phototoxicity and improving contrast.[1] Although the benefits of employing NIR fluorophores are well recognized, NIR scaffolds have been dominated by just a few structural classes, particularly cyanines.[2] Expanding the repertoire of scaffolds for constructing NIR fluorophores and chromophores can improve our ability to fine-tune spectral properties and develop novel chemical sensors.

Substitution of the bridging oxygen in xanthene fluorophores with silicon or other group 14 elements has been shown to dramatically red-shift their excitation and emission wavelengths and, thus, has received much attention in the past decade (Figure 1).[3,4] Among the group 14 elements, silicon produces the most red-shifted fluorophores.[5] For example, the excitation and emission wavelengths of tetramethylrhodamine (548_{ex}/572_{em}) are red-shifted by ~90 nm in Si-tetramethylrhodamine (643_{ex}/662_{em}).[6] Moreover, Si-rhodamines (Figure 1) retain the high photostability of rhodamines with quantum yields comparable to those of popular near-IR cyanine fluorophores.[7,8] Hence, numerous Si-rhodamines have been reported with advantageous properties for live cell and in vivo imaging techniques.[9−12]

Clearly, Si incorporation has expanded the applications and potential of rhodamine fluorophores, and the incorporation of other elements at this position is a burgeoning area of research.[13−16] Less well studied is the effect of these modifications on other dye scaffolds. Oxazine dyes already have typical excitation and emission wavelengths around 640−660 nm.[17] We hypothesized that silicon substitution in oxazines would yield analogously red-shifted "Si-oxazine" (azasiline) dyes (Figure 1).

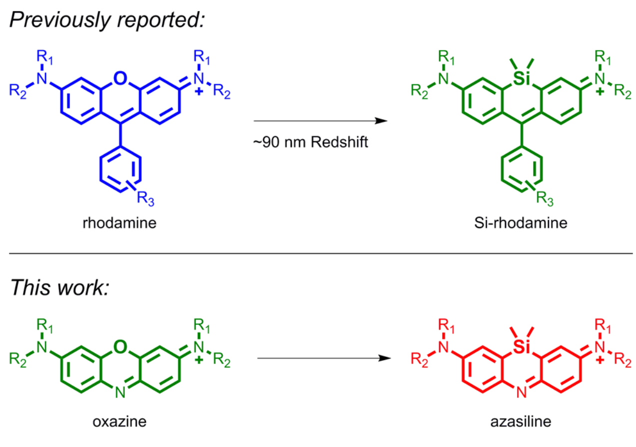


Figure 1. Scope of this work.

Here, we report the synthesis and photophysical characteristics of two azasiline fluorophores with excitation and emission wavelengths over 700 nm.

On the basis of the structural similarities between oxazines and the envisioned azasilines, we first designed a synthetic scheme modeled after known synthetic routes to oxazine dyes[17,18] (Scheme 1). Silane **1** was obtained by treating 3-bromo-*N*,*N*-dimethylaniline with *n*-BuLi and dichlorodimethylsilane.[19] Analogously to oxazine dye synthesis, a diazonitrobenzene moiety was added to yield **2**. However, acid-catalyzed cyclization conditions that readily form oxazine dyes failed for the corresponding azasilines. The lack of reaction could reflect the lowered nucleophilicity of the aryl silane compared to the aryl ether and/or the longer C−Si bond length.

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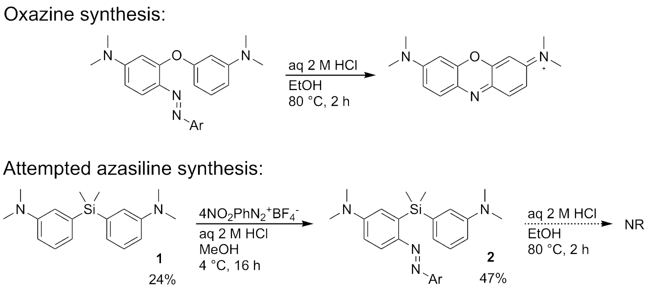
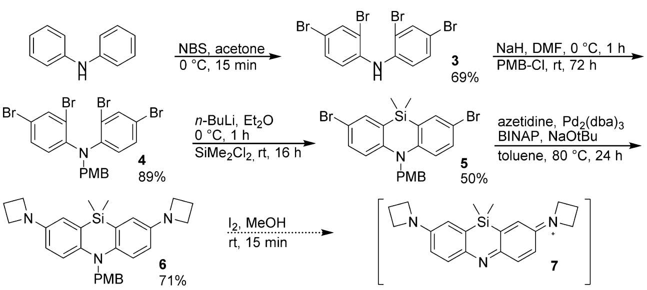
Scheme 1. Modeling Azasiline Synthesis after Oxazines



We next adopted a synthetic route predicated on the synthesis of azasilines as blue fluorescent emitters for OLEDs.[20,21] In this revised scheme, diphenylamine was tetrabrominated with NBS to yield **3** (Scheme 2). The

Scheme 2. Attempted Synthesis of an Azetidenyl Azasiline



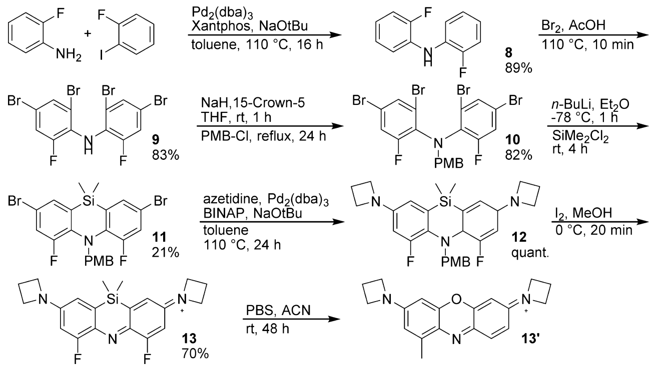
nitrogen was then protected with a PMB group using NaH to yield **4**. Lithium−halogen exchange with *n*-BuLi followed by dichlorodimethylsilane treatment afforded the cyclized product **5**.

Azetidines, which have been shown to increase the quantum yields of rhodamines,[6] were then installed using Buchwald−Hartwig coupling to yield the protected *leuco*-dye **6**. However, attempted deprotection of **6** with TFA to yield azasiline **7** resulted in decomposition after solvent removal. In an alternative method for deprotection, iodine treatment of **6** in methanol yielded the crude putative azasiline fluorophore as a green compound, and HPLC purification encouragingly revealed a peak with an absorption maxima of 730 nm. However, upon solvent removal, decomposition again occurred to yield a possibly polymeric product that was insoluble in acetone, chloroform, methanol, water, and DMSO.

Stymied by the apparent instability of **7**, we next tested whether the stability of the azasiline dye could be modulated by altering the sterics and/or electronics of the azasiline with different substituents. Two different derivatives were designed: one that includes inductively withdrawing but π-donating fluorine groups[10] (**13**) and one that contains bulkier and electron-donating methyl groups (**18**).

To access the difluoro azasiline **13**, bis(2-fluorophenyl)-amine **8** was synthesized through Buchwald−Hartwig coupling of 2-fluoroaniline with 1-bromo-2-fluorobenzene (Scheme 3). As with diphenylamine in Scheme 2, **8** was tetrabrominated, protected with PMB and cyclized to yield the core brominated azasiline. Transformation to the azetidine *leuco*-dye **12** was then performed by Buchwald−Hartwig amination. Deprotection and oxidation were achieved in one step using I_2 in methanol to give the final difluorinated azasiline **13** (ASiFluor710).

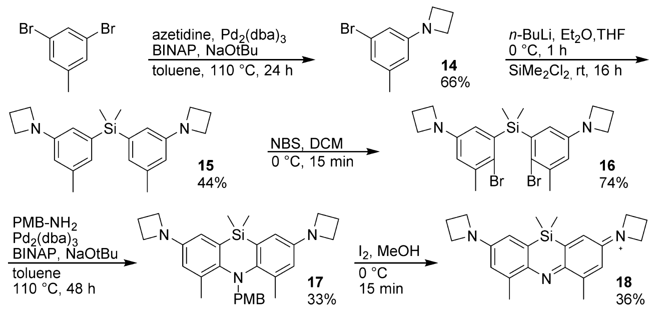
Scheme 3. Synthesis of a Difluoroazasiline (ASiFluor710)



To our delight, **13** readily oxidized and precipitated out of solution during I_2 treatment. Simple filtering and washing of the precipitant with hexanes and methanol afforded pure product. In ethanol, the dye exhibited absorption and fluorescence at wavelengths >700 nm (Figure S1), with an extinction coefficient of 60000 M^{-1} cm^{-1} and quantum yield of 0.11 (Figure S2). Unfortunately, the dye slowly degraded in PBS (Figure S3). LC/MS analysis of ASiFluor710 incubated in PBS for 48 h revealed a new peak with absorbance maxima of ~640 nm and a mass of 306, compared to 370 for the parent dye (Figure S3b). ^{1}H and ^{19}F NMR indicated the loss of both fluorines and the dimethylsilyl group. The data are consistent with the unexpected rearrangement to oxazine dye **13′**, highlighting the need to further explore the unique chemistry of silicon, especially in the context of multifunctional heterocycles.[22]

Although the incorporation of the π-donor fluorine allowed the synthesis of an azasiline dye, its reactivity in S_NAr reactions likely leads to fluorine substitution and subsequent fluoride-mediated reaction with the silyl group. Methyl groups are more benign electron donors that may help stabilize the oxidized azasiline dye without this liability. To access the dimethyl azasiline derivative, we first followed the same general synthetic route used to synthesize **13**. However, that scheme failed at the PMB protection step, presumably due to the increased steric hindrance posed by the methyl groups. We therefore revisited our original synthetic scheme and postulated that cyclization could be achieved via Buchwald−Hartwig coupling (Scheme 4). First, Buchwald−Hartwig coupling of azetidine with 3,5-dibromotoluene yielded **14**. The symmetric silane **15** was then formed by lithium−halogen exchange and treatment with dichlorodimethylsilane. Subsequent dibromination with NBS produced silane **16**, which underwent Buchwald−Hartwig coupling with 4-methoxybenzylamine to effect ring closure,

Scheme 4. Synthesis of a Dimethylazasiline (ASiFluor730)



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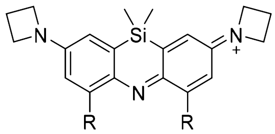
yielding the protected *leuco* dye **17**. Iodine treatment of **17** yielded the final product **18** (ASiFluor730).

ASiFluor730 (**18**) fluoresces at >730 nm in both organic and aqueous solvents (Table 1 and Figure S1). Gratifyingly, HPLC and LC/MS analyses of **18** incubated in PBS for 24 h showed no degradation (Figure S3). The quantum yield and molar extinction coefficient of **18** were calculated to be 0.11 and 121000 M^{-1} cm^{-1} in ethanol (Table 1), which is comparable to that of oxazine 1 (0.14 and 118000 M^{-1} cm^{-1}).[23,24] In ethylene glycol and 1,4-dioxane, **18** has quantum yields of 0.13 and 0.11, respectively. However, the quantum yield of **18** dropped to approximately 0.01 in deionized water and PBS, which is 11-fold lower than in ethanol (Table 1 and Figure S2). The addition of 0.05% Tween-20 increased the quantum yield to 0.06, suggesting that the reduced quantum yield in aqueous solvent is due in part to aggregation.[25] (Table 1 and Figure S2). Future incorporation of polar groups such as sulfonates should increase aqueous solubility and may, in turn, increase quantum yields in water.[26,27]

Table 1. Photophysical Properties of Azasilines



R = CH_3 or F

R	solvent	λ_{abs} (nm)	λ_{em} (nm)	ε^{a} (M^{-1} cm^{-1})	Φ^{b}
CH_3[a]	EtOH	732	745	121000	0.11
	PBS	725	739	53000	0.01
	H_2O	725	739	70000	0.009
	0.05% Tween-20	730	745	95000	0.06
	ethylene glycol	736	747	109000	0.13
	1,4-dioxane	701	734	46000	0.11
F[b]	EtOH	709	719	60000	0.11

[a]ε values were rounded to the nearest thousands. [b]Φ (relative quantum yields) were calculated with 1,1′,3,3,3′,3′-hexamethylindotricarbocyanine iodide (HITCI, a) and Cy5.5 (b) as standards.[2,15,16].

Lastly, we compared the photostability of the ASiFluors to Oxazine **1** and Cy5.5. Each fluorophore was subjected to continuous irradiation at its peak absorption wavelength for 1 h. All fluorophores were photostable in ethanol. However, in PBS, Cy5.5 lost about 60% of its initial fluorescence, whereas Oxazine **1** and ASiFluor730 were unaffected (Figure 2).

In conclusion, we have developed synthetic approaches to a new class of near IR-fluorophores based on azasilines, the Si analogues of oxazine dyes. Although not as bright as cyanines, ASiFluor730 is one of the most compact near-IR fluorophores that absorbs beyond 700 nm (Table S1). The NIR excitation and emission wavelength, photostability, and compact size show promise for the future development of azasiline-based optical probes and suggests that silicon can be incorporated more broadly into chromophore scaffolds to further expand the diversity of NIR fluorophores, chromophores, and luminescent molecules.[28−30]

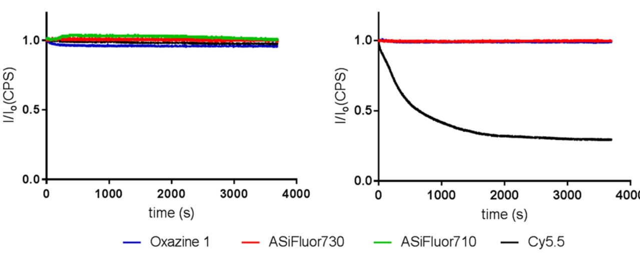


Figure 2. Photostability of azasilines. Each fluorophore was irradiated continuously for 1 h at their max absorbance wavelength (Oxazine 1 = 646 nm; Cy5.5 = 684 nm; ASiFluor710 = 710 nm; ASiFluor730 = 730 nm) in ethanol (left panel) and PBS (right panel). ASiFluor710 was not tested in PBS due to its instability in aqueous solution.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b01786.

Experimental procedures, supplemental tables and figures, and full spectroscopic data for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Hong, G.; Antaris, A. L.; Dai, H. Near-Infrared Fluorophores for Biomedical Imaging. *Nature Biomedical Engineering* **2017**, *1*, 0010.

(2) Pansare, V. J.; Hejazi, S.; Faenza, W. J.; Prud'homme, R. K. Review of Long-Wavelength Optical and NIR Imaging Materials: Contrast Agents, Fluorophores, and Multifunctional Nano Carriers. *Chem. Mater.* **2012**, *24*, 812−827.

(3) Fu, M.; Xiao, Y.; Qian, X.; Zhao, D.; Xu, Y. A Design Concept of Long-Wavelength Fluorescent Analogs of Rhodamine Dyes: Replacement of Oxygen with Silicon Atom. *Chem. Commun.* **2008**, *0*, 1780−1782.

(4) Umezawa, K.; Citterio, D.; Suzuki, K. New Trends in Near-Infrared Fluorophores for Bioimaging. *Anal. Sci.* **2014**, *30*, 327−349.

(5) Koide, Y.; Urano, Y.; Hanaoka, K.; Terai, T.; Nagano, T. Evolution of Group 14 Rhodamines as Platforms for Near-Infrared Fluorescence Probes Utilizing Photoinduced Electron Transfer. *ACS Chem. Biol.* **2011**, *6*, 600−608.

(6) Grimm, J. B.; English, B. P.; Chen, J.; Slaughter, J. P.; Zhang, Z.; Revyakin, A.; Patel, R.; Macklin, J. J.; Normanno, D.; Singer, R. H.; et al. A General Method to Improve Fluorophores for Live-Cell and Single-Molecule Microscopy. *Nat. Methods* **2015**, *12*, 244−250.

(7) Ikeno, T.; Nagano, T.; Hanaoka, K. Silicon-substituted Xanthene Dyes and Their Unique Photophysical Properties for Fluorescent Probes. *Chem. - Asian J.* **2017**, *12*, 1435−1446.

(8) Koide, Y.; Urano, Y.; Hanaoka, K.; Piao, W.; Kusakabe, M.; Saito, N.; Terai, T.; Okabe, T.; Nagano, T. Development of NIR Fluorescent Dyes Based on Si−Rhodamine for in Vivo Imaging. *J. Am. Chem. Soc.* **2012**, *134*, 5029−5031.

(9) Wang, T.; Zhao, Q.-J.; Hu, H.-G.; Yu, S.-C.; Liu, X.; Liu, L.; Wu, Q.-Y. Spirolactonized Si-Rhodamine: A Novel NIR Fluorophore

DOI: 10.1021/acs.orglett.8b01786
Org. Lett. XXXX, XXX, XXX−XXX

Utilized as a Platform to Construct Si-Rhodamine-Based Probes. *Chem. Commun.* **2012**, *48*, 8781−8783.
(10) Grimm, J. B.; Brown, T. A.; Tkachuk, A. N.; Lavis, L. D. General Synthetic Method for Si-Fluoresceins and Si-Rhodamines. *ACS Cent. Sci.* **2017**, *3*, 975−985.
(11) Lukinavičius, G.; Umezawa, K.; Olivier, N.; Honigmann, A.; Yang, G.; Plass, T.; Mueller, V.; Reymond, L., Jr; I, R. C.; Luo, Z.-G.; et al. A Near-Infrared Fluorophore for Live-Cell Super-Resolution Microscopy of Cellular Proteins. *Nat. Chem.* **2013**, *5*, 132−139.
(12) Kolmakov, K.; Hebisch, E.; Wolfram, T.; Nordwig, L. A.; Wurm, C. A.; Ta, H.; Westphal, V.; Belov, V. N.; Hell, S. W. Far-Red Emitting Fluorescent Dyes for Optical Nanoscopy: Fluorinated Silicon-Rhodamines (SiRF Dyes) and Phosphorylated Oxazines. *Chem. - Eur. J.* **2015**, *21*, 13344−13356.
(13) Liu, J.; Sun, Y.-Q.; Zhang, H.; Shi, H.; Shi, Y.; Guo, W. Sulfone-Rhodamines: A New Class of Near-Infrared Fluorescent Dyes for Bioimaging. *ACS Appl. Mater. Interfaces* **2016**, *8*, 22953−22962.
(14) Zhou, X.; Lai, R.; Beck, J. R.; Li, H.; Stains, C. I. Nebraska Red: A Phosphinate-Based near-Infrared Fluorophore Scaffold for Chemical Biology Applications. *Chem. Commun. (Cambridge, U. K.)* **2016**, *52*, 12290−12293.
(15) Zhou, X.; Lesiak, L.; Lai, R.; Beck, J. R.; Zhao, J.; Elowsky, C. G.; Li, H.; Stains, C. I. Chemoselective Alteration of Fluorophore Scaffolds as a Strategy for the Development of Ratiometric Chemodosimeters. *Angew. Chem., Int. Ed.* **2017**, *56*, 4197−4200.
(16) Chai, X.; Cui, X.; Wang, B.; Yang, F.; Cai, Y.; Wu, Q.; Wang, T. Near-Infrared Phosphorus-Substituted Rhodamine with Emission Wavelength above 700 Nm for Bioimaging. *Chem. - Eur. J.* **2015**, *21*, 16754−16758.
(17) Pauff, S. M.; Miller, S. C. Synthesis of Near-IR Fluorescent Oxazine Dyes with Esterase-Labile Sulfonate Esters. *Org. Lett.* **2011**, *13*, 6196−6199.
(18) Anzalone, A. V.; Wang, T. Y.; Chen, Z.; Cornish, V. W. A Common Diaryl Ether Intermediate for the Gram-Scale Synthesis of Oxazine and Xanthene Fluorophores. *Angew. Chem., Int. Ed.* **2013**, *52*, 650−654.
(19) Wang, B.; Chai, X.; Zhu, W.; Wang, T.; Wu, Q. A General Approach to Spirolactonized Si-Rhodamines. *Chem. Commun. (Cambridge, U. K.)* **2014**, *50*, 14374−14377.
(20) Suzuki, R.; Tada, R.; Hosoda, T.; Miura, Y.; Yoshioka, N. Synthesis of Ester-Substituted Dihydroacridine Derivatives and Their Spectroscopic Properties. *New J. Chem.* **2016**, *40*, 2920−2926.
(21) Sun, J. W.; Baek, J. Y.; Kim, K.-H.; Moon, C.-K.; Lee, J.-H.; Kwon, S.-K.; Kim, Y.-H.; Kim, J.-J. Thermally Activated Delayed Fluorescence from Azasiline Based Intramolecular Charge-Transfer Emitter (DTPDDA) and a Highly Efficient Blue Light Emitting Diode. *Chem. Mater.* **2015**, *27*, 6675−6681.
(22) Barraza, S. J.; Denmark, S. E. Synthesis, Reactivity, Functionalization, and ADMET Properties of Silicon-Containing Nitrogen Heterocycles. *J. Am. Chem. Soc.* **2018**, *140*, 6668−6684.
(23) Rurack, K.; Spieles, M. Fluorescence Quantum Yields of a Series of Red and Near-Infrared Dyes Emitting at 600−1000 Nm. *Anal. Chem.* **2011**, *83*, 1232−1242.
(24) Würth, C.; Grabolle, M.; Pauli, J.; Spieles, M.; Resch-Genger, U. Relative and Absolute Determination of Fluorescence Quantum Yields of Transparent Samples. *Nat. Protoc.* **2013**, *8*, 1535−1550.
(25) Buschmann, V.; Weston, K. D.; Sauer, M. Spectroscopic Study and Evaluation of Red-Absorbing Fluorescent Dyes. *Bioconjugate Chem.* **2003**, *14*, 195−204.
(26) Mujumdar, R. B.; Ernst, L. A.; Mujumdar, S. R.; Lewis, C. J.; Waggoner, A. S. Cyanine Dye Labeling Reagents - Sulfoindocyanine Succinimidyl Esters. *Bioconjugate Chem.* **1993**, *4*, 105−111.
(27) Panchuk-Voloshina, N.; Haugland, R. P.; Bishop-Stewart, J.; Bhalgat, M. K.; Millard, P. J.; Mao, F.; Leung, W.-Y.; Haugland, R. P. Alexa Dyes, a Series of New Fluorescent Dyes That Yield Exceptionally Bright, Photostable Conjugates. *J. Histochem. Cytochem.* **1999**, *47*, 1179−1188.
(28) Adams, S. T.; Miller, S. C. Beyond D-Luciferin: Expanding the Scope of Bioluminescence Imaging in Vivo. *Curr. Opin. Chem. Biol.* **2014**, *21*, 112−120.
(29) Sharma, D. K.; Adams, S. T.; Liebmann, K. L.; Miller, S. C. Rapid Access to a Broad Range of 6′-Substituted Firefly Luciferin Analogues Reveals Surprising Emitters and Inhibitors. *Org. Lett.* **2017**, *19*, 5836−5839.
(30) Miller, S. C.; Mofford, D. M.; Adams, S. T. Lessons Learned from Luminous Luciferins and Latent Luciferases. *ACS Chem. Biol.* **2018**, DOI: 10.1021/acschembio.7b00964.

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Org. Lett. XXXX, XXX, XXX−XXX