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

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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.

Graphical Abstract
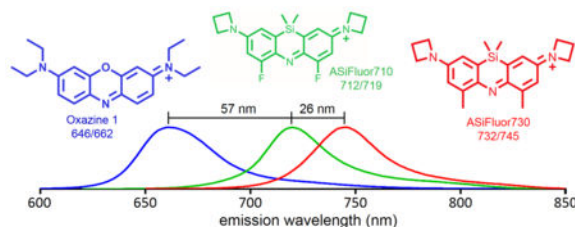
Fluorescent probes are among the simplest and most convenient chemical tools used to investigate biological processes. In particular, near infrared (NIR) fluorophores with excitation and emission between 650 nm to 900 nm are becoming increasingly popular. Unlike visible-light fluorophores, NIR light 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

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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 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).

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.

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 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 was achieved in one step using I_2 in methanol to give the final difluorinated azasiline **13** (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 60,000 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 hours 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 multi-functional 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, 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, Figure S1). Gratifyingly, HPLC and LC/MS analyses of **18** incubated in PBS for 24 hours showed no degradation (Figure S3). The quantum yield and molar extinction coefficient of **18** were calculated to be 0.11 and 121,000 M^{-1} cm^{-1} in ethanol (Table 1), which is comparable to that of Oxazine 1 (0.14 and 118,000 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, 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, 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]

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 one hour. 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]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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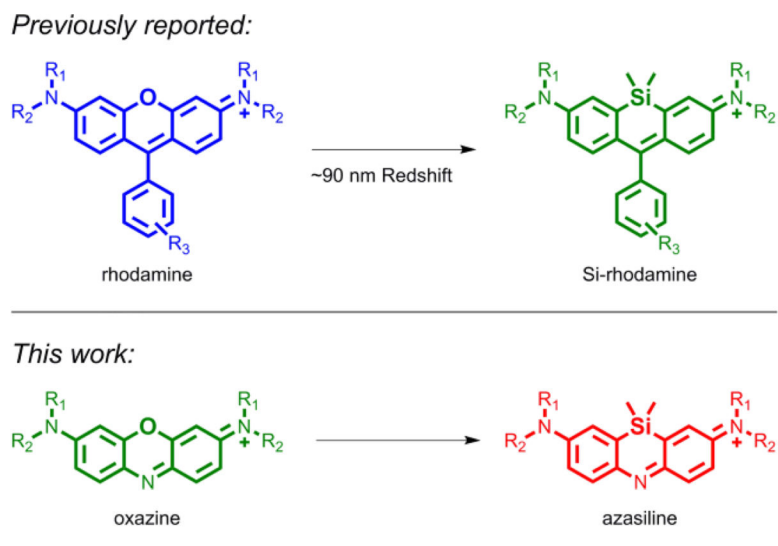
Figure 1.
Scope of this work.
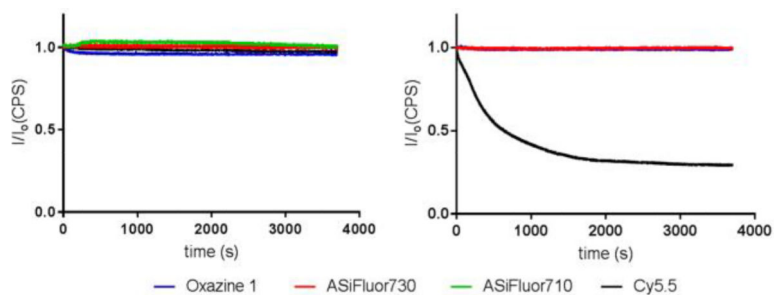
Figure 2: Photostability of Azasilines.
Each fluorophore was irradiated continuously for one hour at their max absorbance wavelength (Oxazine 1 = 646 nm; Cy5.5 = 684 nm; ASiFluor710 = 710 nm; ASiFluor730 = 730 nm) in ethanol (left panel) and PBS (right panel). ASiFluor710 was not tested in PBS due to its instability in aqueous solution.
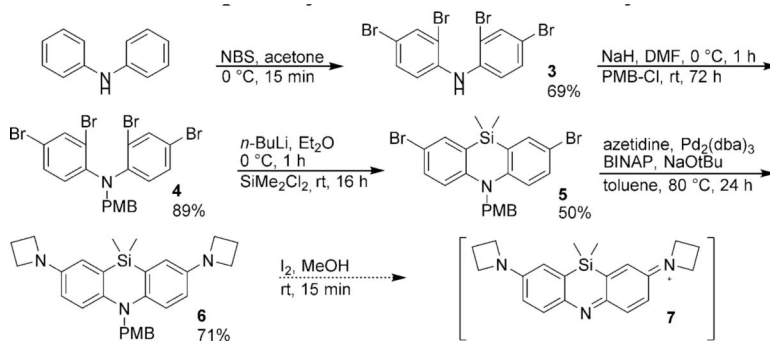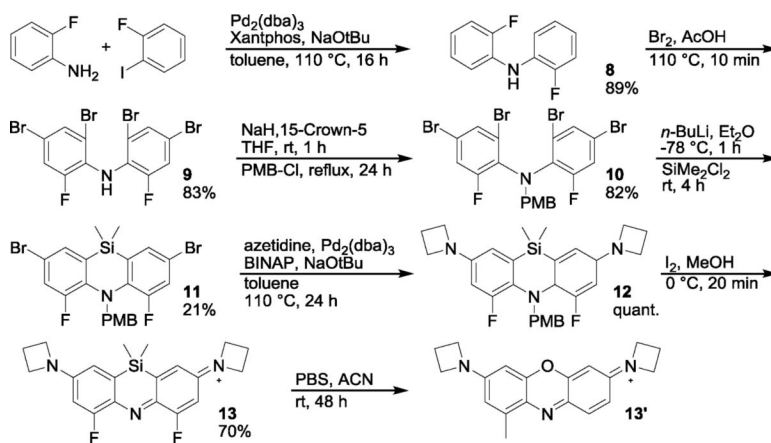
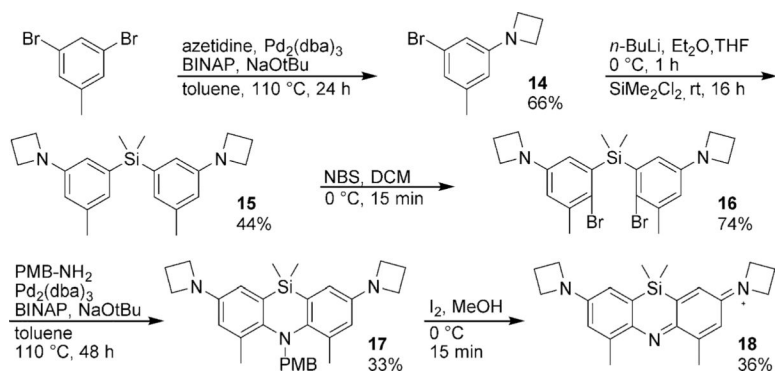Oxazine synthesis:
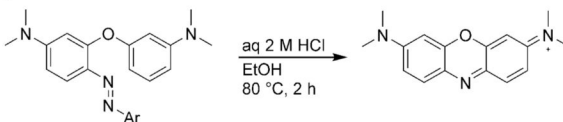
Attempted azasiline synthesis:
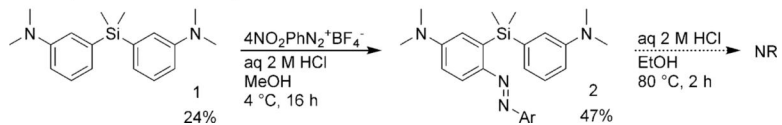
Scheme 1:
Modeling Azasiline Synthesis after Oxazines
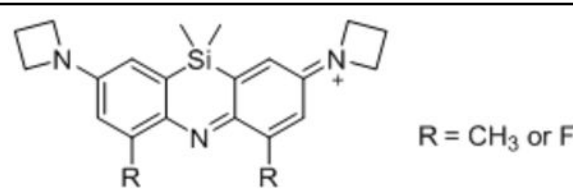
Scheme 2:
Attempted synthesis of an azetidenyl azasiline

Scheme 3:
Synthesis of Difluoro-Azasiline (ASiFluor710)

Scheme 4:
Synthesis of Dimethyl-Azasiline (ASiFluor730)

Table 1:

Photophysical Properties of Azasilines

R = CH₃ or F

R	solvent	λ_{abs} (nm)	λ_{em} (nm)	e (M⁻¹ cm⁻¹)	Φ
CH₃[a]	EtOH	732	745	121,000	0.11
	PBS	725	739	53,000	0.01
	H₂O	725	739	70,000	0.009
	0.05% Tween-20	730	745	95,000	0.06
	Ethylene Glycol	736	747	109,000	0.13
	1,4-Dioxane	701	734	46,000	0.11
F[b]	EtOH	709	719	60,000	0.11

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 e values were rounded to the nearest thousands.