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Synthesis of a Near-Infrared Fluorescent Probe for Imaging Catecholamines via a Tandem Nucleophilic Aromatic Substitution

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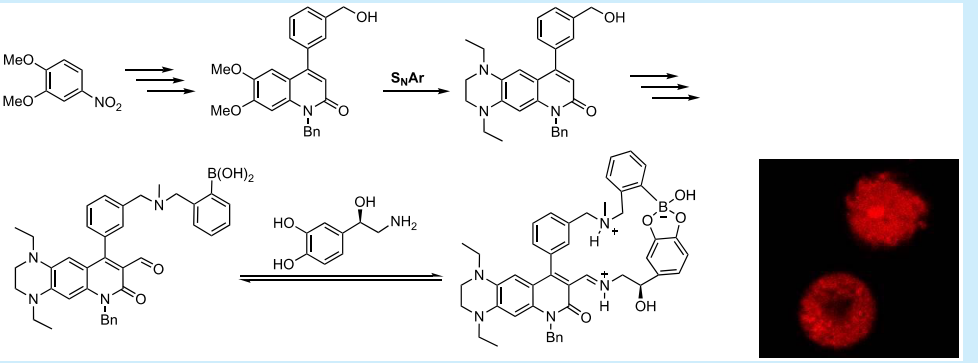

ABSTRACT: A near-infrared (NIR) fluorescent probe NS667 was developed using a novel synthetic strategy by integrating an electron-rich 1,2,3,4-tetrahydroquinoxaline (THQ) into the scaffold from NS510, which binds to catecholamines with high affinity. The fluorophore core was constructed with a tandem nucleophilic aromatic substitution. Upon binding to catecholamines, the fluorescence of this probe shifted, with the emission in the NIR region. Live cell imaging results demonstrate that NS667 can effectively image norepinephrine in chromaffin cells with shifted fluorescence, which highlights the potential of the probe for neuroimaging in tissues.

Catecholamines (epinephrine, norepinephrine, and dopamine), serving as major neurotransmitters, play an important role in regulating brain function and are of central importance for communication between neurons in the sympathetic nervous system.[1] They are chemical messengers that transmit electrical signals across synapses and are implicated in critical functions that include movement, learning, memory, emotion, sleep, and attention.[2] Irregular levels of catecholamine neurotransmitters are associated with various neurological diseases.[3] Therefore, measurements of the distribution, uptake, storage, release, and utilization of catecholamine neurotransmitters are of substantial significance.[1]

Fluorescent imaging for the detection of catecholamine neurotransmitters offers clear advantages compared to other methods like chromatography and electrochemistry.[4] Fluorescence is less invasive and provides better spatial resolution. For this reason, a number of fluorescent probes for imaging catecholamines were developed, some of which contribute to the exploration of the norepinephrine signaling pathway and its connection to various disease states.[5,6] Additionally, due to their structural similarity to neurotransmitters, fluorescent false neurotransmitters can be loaded into secretory vesicles with genuine neurotransmitters to effectively monitor the fluorescence loss associated with the release of neurotransmitters.[7]

In our research group, we have been dedicated to the development of fluorescent probes for imaging neurotransmitters, with the achievement of creating a toolbox of such probes (Figure 1).[8,9] Most of these probes fluoresce in the green, except for NS715, which is a NIR probe. Unfortunately, NS715 is not selective for any particular amine.[9] Thus, this toolbox lacks a high-affinity NIR fluorescent probe capable of sufficiently imaging neurotransmitters in tissue samples. Inspired by our previous work (NS715 and NS510), herein we present a high-affinity NIR fluorescent probe NS667 for imaging catecholamines in live cells, with a potential application in tissues.

During the development of probe NS510 (Figure 1), a boronic acid group is the predominant factor contributing to significantly high affinity of NS510 for catecholamines.[9] Additionally, NS510 possesses the scaffold that aligns favorably with catecholamines due to the well-suited geometry.[9]

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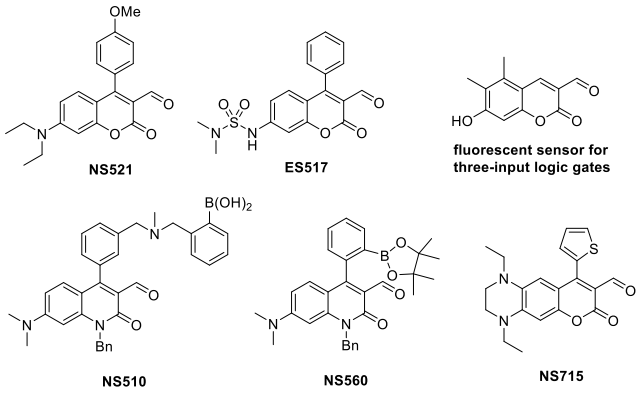


Figure 1. Tool box of fluorescent probes developed in our lab.

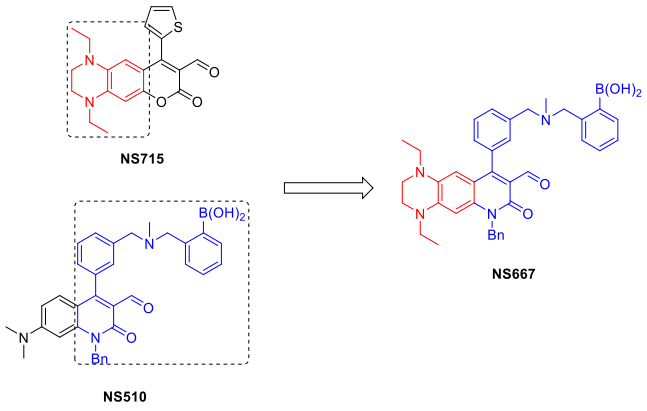


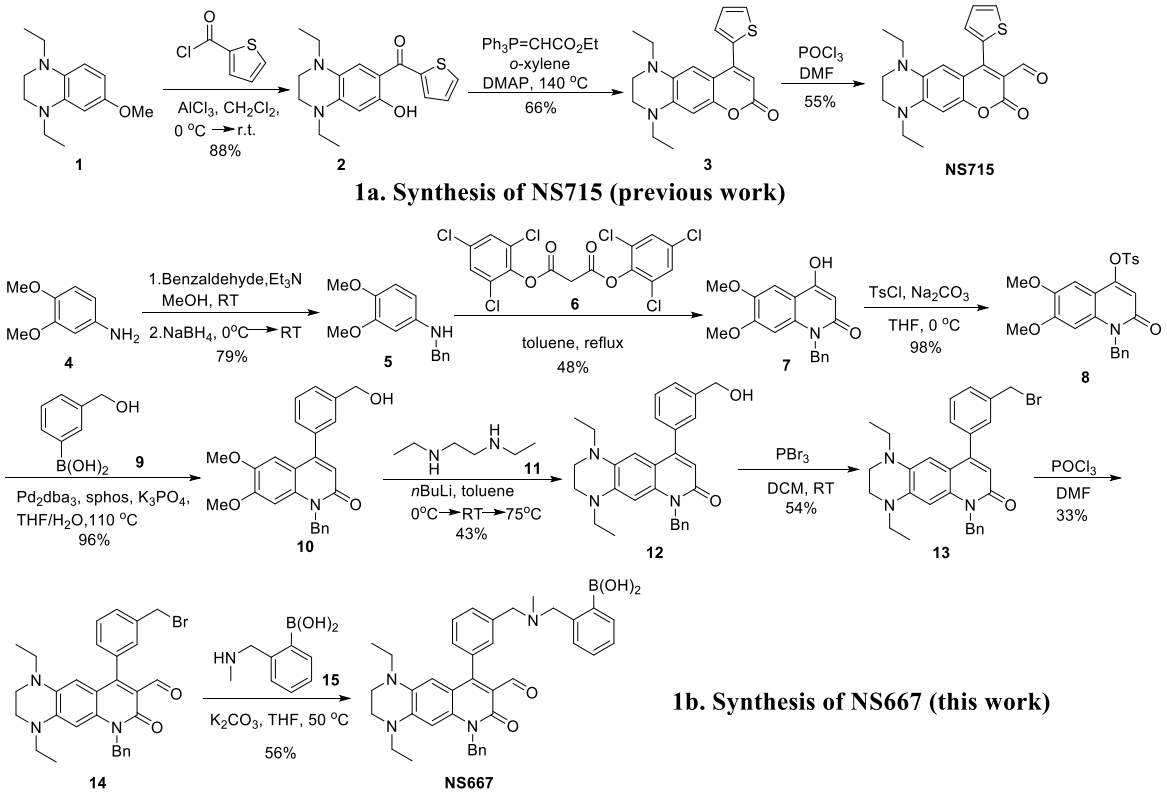
Figure 2. Design of near-infrared fluorescent probe NS667.

Furthermore, the incorporation of electron-rich diethyl-substituted THQ (1,2,3,4-tetrahydroquinoxaline) leads to the emission of NS715 (Figure 1) in the NIR region.[10] For these reasons, we decided to incorporate both the scaffold of NS510 (highlighted in blue) and the diethyl-substituted THQ (highlighted in red) from NS715 to develop our NIR high-affinity fluorescent probe NS667 for imaging catecholamines (Figure 2).

We first sought to synthesize NS667 in the same manner as NS715 (Scheme 1a).[10] However, the quinolone fluorophore could not be prepared using this method. Therefore, we conducted tests and screenings of various synthetic pathways in the pursuit of successfully synthesizing NS667. Among these approaches, the one presented in Scheme 1b proved to be the most effective.

To begin, the commercially available starting material **4** was subjected to reductive amination, resulting in the formation of the substituted aniline compound **5**. The quinolone core compound **7** was furnished via a Pechmann cyclization after the reaction with compound **6**. Next, the benzyl alcohol substituent **9** was appended on tosylated compound **8** using a Suzuki coupling reaction to produce compound **10** in a high yield. The next step is the key to achieve this synthesis: the dimethoxy substituent compound **10** underwent a tandem nucleophilic aromatic substitution with the dilithium amide intermediate generated by deprotonation of compound **11** with *n*-butyllithium, leading to the production of the diamine-substituted compound **12** in a moderate yield.[11] Furthermore, fomylated compound **14** was produced with brominated compound **13** using a Vilsmeier−Haack reaction. The phenylboronic acid moiety **15** was appended on compound **14** to complete the synthesis of target probe NS667.

Scheme 1. Syntheses of NS715 and NS667



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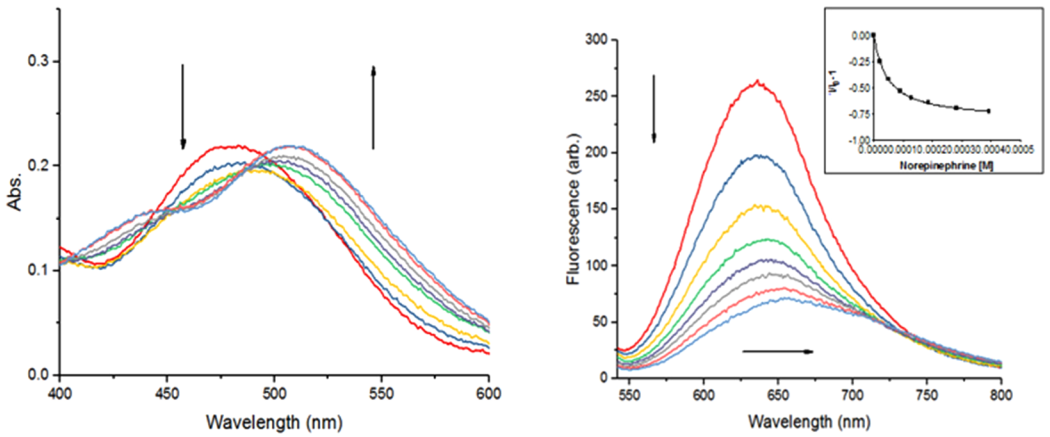
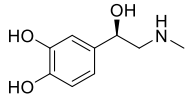
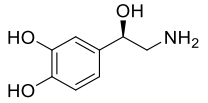
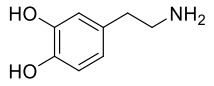
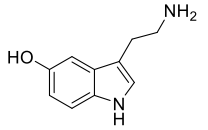
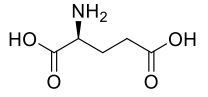


Figure 3. UV−vis and fluorescence titration of NIR probe NS667 (20 μM) with 10 mM norepinephrine in buffer (25 mM HEPES, 50 mM $Na_2S_2O_3$, pH 7.4, 2% DMSO). λ_{ex} = 532 nm, λ_{ex} = 654 nm. Inset is the fit of the fluorescence to a binding isotherm.

Table 1. Association Constants (K_a) and Spectroscopic Parameters for the Binding of NS667 and NS510[9] to Several Neurotransmitters

	NS667[d]			NS510[e]		
neurotransmitter	K_a(M^{-1})[a]	I_{sat}/I_0[b]	$\Delta\lambda_{em}$ (nm)	K_a(M^{-1})[a]	I_{sat}/I_0[b]	λ_{em} (nm)
 epinephrine	16,906	0.58	637 → 638	35,900[c]	0.80	510
 norepinephrine	20,186	0.15	636 → 654	13,500	1.9	510
 dopamine	17,428	0.23	635 → 667	17,900	0.88	510
 serotonin	2,619	0.23	635 → 659	N/A	N/A	N/A
 glutamate	109	0.31	634 → 640	32	26	510

[a]K_a measured by fluorescence spectroscopy. Errors in K_a values are ±10% based on triplicate titrations. [b]I_{sat} is the fluorescence intensity at saturation taken from the theoretical fit to the binding. [c]K_a measured by UV−vis spectroscopy. [d]This work. [e]Reference 9.

NS667 was screened with a number of relevant neurotransmitters using both absorption and fluorescence spectroscopy. As observed with the probe NS510 developed by our research group, NS667 bound to catecholamines bearing a primary amine (dopamine and norepinephrine) via the formation of an iminium ion as well as a boronate ester, which produced a red shift in absorption from approximately 480 to 520 nm upon the addition of catecholamine. Excitation at 532 nm afforded a fluorescence shift from 636 to 654 nm for norepinephrine (Figure 3) and that from 635 to 667 nm for dopamine (Figure S1). Remarkably, NS667 produced a large Stokes shift (160 nm) and fluorescence, which is well in the NIR spectral region. However, the probe binding to epinephrine produced a negligible shift (from 637 to 638 nm) in fluorescence upon excitation at 532 nm due to the absence of an interaction between the secondary amine of epinephrine and the aldehyde of the probe (Figure S3). Although the fluorescence of the probe is reduced upon

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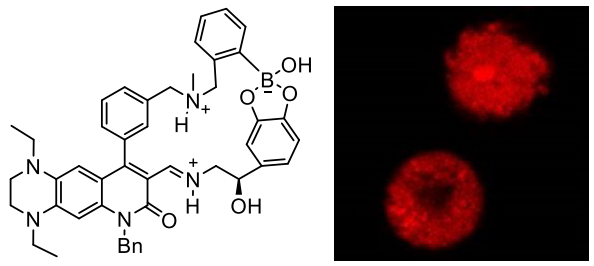

Figure 4. Structure of NS667 bound to norepinephrine and confocal image of norepinephrine-enriched chromaffin cells incubated with NS667 (0.5 μM NS667; λ_{ex} = 515 nm).

binding analytes, the high binding affinity for catecholamines and the substantial Stokes shift are distinctive features, allowing the differentiation of fluorescence upon the interaction of catecholamine with NS667 as compared to other biogenic amines. Moreover, the significant decrease in background autofluorescence within the NIR region ensures that NS667 remains functional even though its fluorescence is reduced.

Table 1 summarizes the binding and spectroscopic data for the association of NS667 and NS510 with relevant vesicular neurotransmitters that include the monoamine catecholamines (dopamine, norepinephrine, and epinephrine), serotonin, and the amino acid glutamate. NS667 showed a weak affinity for glutamate along with a modest fluorescence reduction. NS667 exhibited a much higher affinity (~6.4-fold) for serotonin (2,619 M^{-1}), compared to the binding affinity for serotonin with NS715 (409 M^{-1}), which is presumably attributed to the primary interaction between the hydroxyl of serotonin and the boronic acid of the probe besides the secondary interaction between the primary amine and the aldehyde via iminium formation. Notably, NS510 is completely quenched by serotonin, making NS667 the best serotonin sensor in the series.

More importantly, NS667 exhibited a remarkable binding affinity for catecholamines. Norepinephrine and dopamine bound to NS667 with high affinity due to the interaction of the boronic acid and the catechol via boronate ester formation, leading to moderate fluorescence quenching. The binding of epinephrine to NS667 produced a milder fluorescence quenching, as anticipated, owing to the single interaction between catechol and boronic acid. This high binding affinity toward catecholamines is very similar to that of NS510. In the pursuit of achieving a high affinity, a significantly large Stokes shift, and NIR-region emission for NS667, there is a trade-off with a considerable reduction in fluorescence intensity. Hence, based on our reasoning, NS667 is adept at sufficiently imaging catecholamines. Consequently, we opted to validate the utility of NS667 using norepinephrine-enriched chromaffin cells.

We isolated norepinephrine-enriched chromaffin cells from bovine adrenal glands.[12] Next, norepinephrine-enriched population chromaffin cells were plated and incubated with a 0.5 μM solution of NS667 at 37 °C for 30 min, then the excess probe was washed off. The cells were examined by confocal fluorescence microscopy using 515 nm excitation. The norepinephrine-enriched cells were brightly stained with NS667 (Figure 4). Punctate staining of the chromaffin cells suggested that the probe labels the secretory vesicles found throughout the cell. The cell imaging data also indicate this NIR fluorescent probe NS667 could label catecholamines in the secretory vesicles in live cells.

In conclusion, a near-infrared fluorescent probe was designed and synthesized with exceptionally high affinity and notable selectivity. Upon association with catecholamines, it exhibits a reduced fluorescence intensity in the near-infrared region, along with a significant Stokes shift. NS667 gave punctate staining of secretory vesicles in norepinephrine-enriched chromaffin cells using a confocal microscope. Our initial findings in norepinephrine-enriched chromaffin cells show great promise for potential applications in live biological systems, and we expect to further explore the practical utility of this probe in live cells and tissue.

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.3c03343.

UV−vis and fluorescence spectra, cell studies, synthetic procedures, and ^{1}H NMR and ^{13}C NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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