Supplementary Information

Live cell labeling of native intracellular bacterial receptors using aniline-catalyzed oxime ligation

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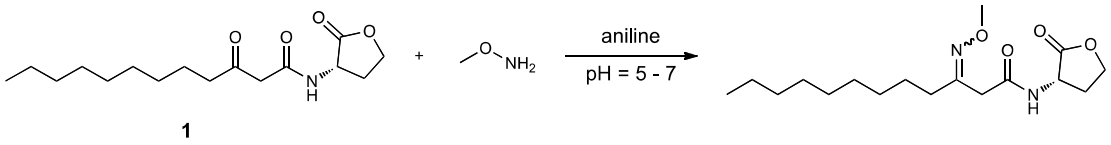


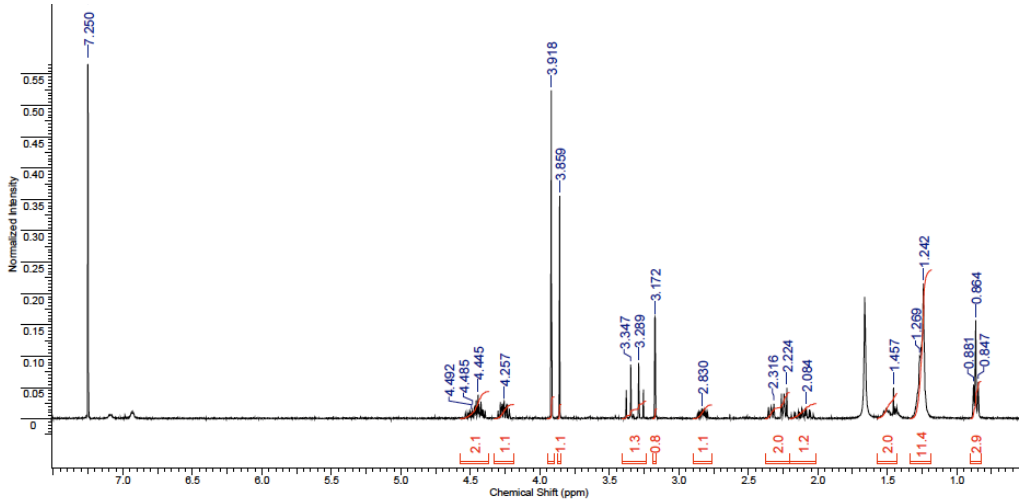
1

Table S1. Optimization of conditions for the model ligation reaction between 3-oxo-C_{12}-HSL and methoxyamine.

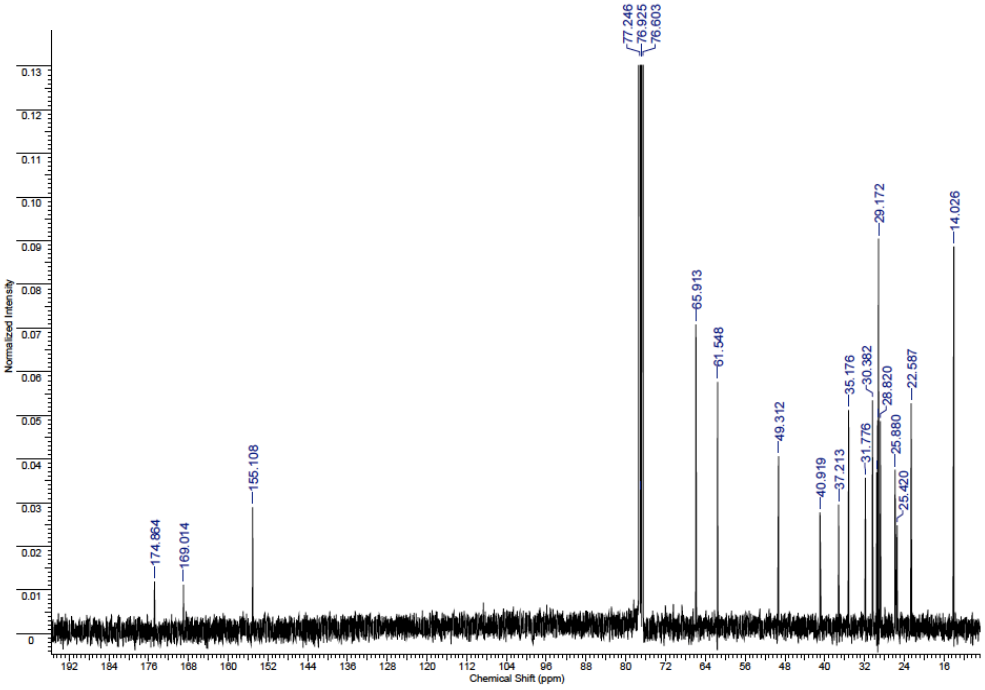
Entry	pH	time (h)	Conversion (%[a])
1 [b]	4.5 [d]	2.5	50
2 [b]	4.5 [d]	8	100
3 [b]	5.5 [d]	2.5	25
4 [b]	5.5 [d]	8	71
5 [b]	7 [e]	2.5	trace
6 [b]	7 [e]	8	15
7 [c]	7 [e]	8	60

[a] Mesured by electrospray ionization mass spectrometry (ESI-MS).
[b] Conditions: 100 µM 3-oxo-C_{12}-HSL, 1 mM methoxyamine, 10 mM aniline, 100 mM buffer.
[c] Conditions: 10 µM 3-oxo-C_{12}-HSL, 1 mM methoxyamine, 10 mM aniline, 100 mM buffer.
[d] Sodium acetate buffer
[e] Sodium phosphate buffer.

a


7.250
3.918
3.859
3.347
3.289
3.172
4.492
4.485
4.445
4.257
2.830
2.316
2.224
2.084
1.457
1.269
1.242
0.881
0.847
0.864
Normalized Intensity
Chemical Shift (ppm)

b


77.246
76.925
76.603
65.913
61.548
49.312
40.919
37.213
35.176
31.776
30.382
29.172
28.820
25.880
25.420
22.587
14.026
174.864
169.014
155.108
Normalized Intensity
Chemical Shift (ppm)

c

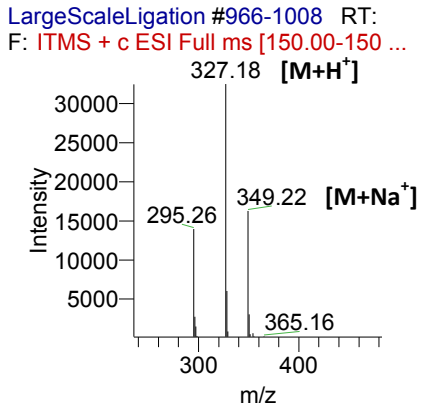

LargeScaleLigation #966-1008 RT:
F: ITMS + c ESI Full ms [150.00-150 ...
327.18 [M+H+]
349.22 [M+Na+]
295.26
365.16
Intensity
m/z

Figure S1. Characterization of 3-methoxyimino-C_{12}-HSL. To a solution of 3-oxo-C_{12}-HSL (100 µM, in 100 mL sodium acetate buffer, pH 5.1) was added 1 mM aniline and 100 µM methoxyamine. and After 12 h, the solution was extracted with 3*100 mL $CHCl_3$, and dried over Na_2SO_4. The mixture was filtered, and the solvent removed in vacuo. Finally, the product was purified by RP-HPLC. **a,** ^{1}H-NMR spectrum. **b,** ^{13}C-NMR spectrum. **c,** ESI mass spectrum.

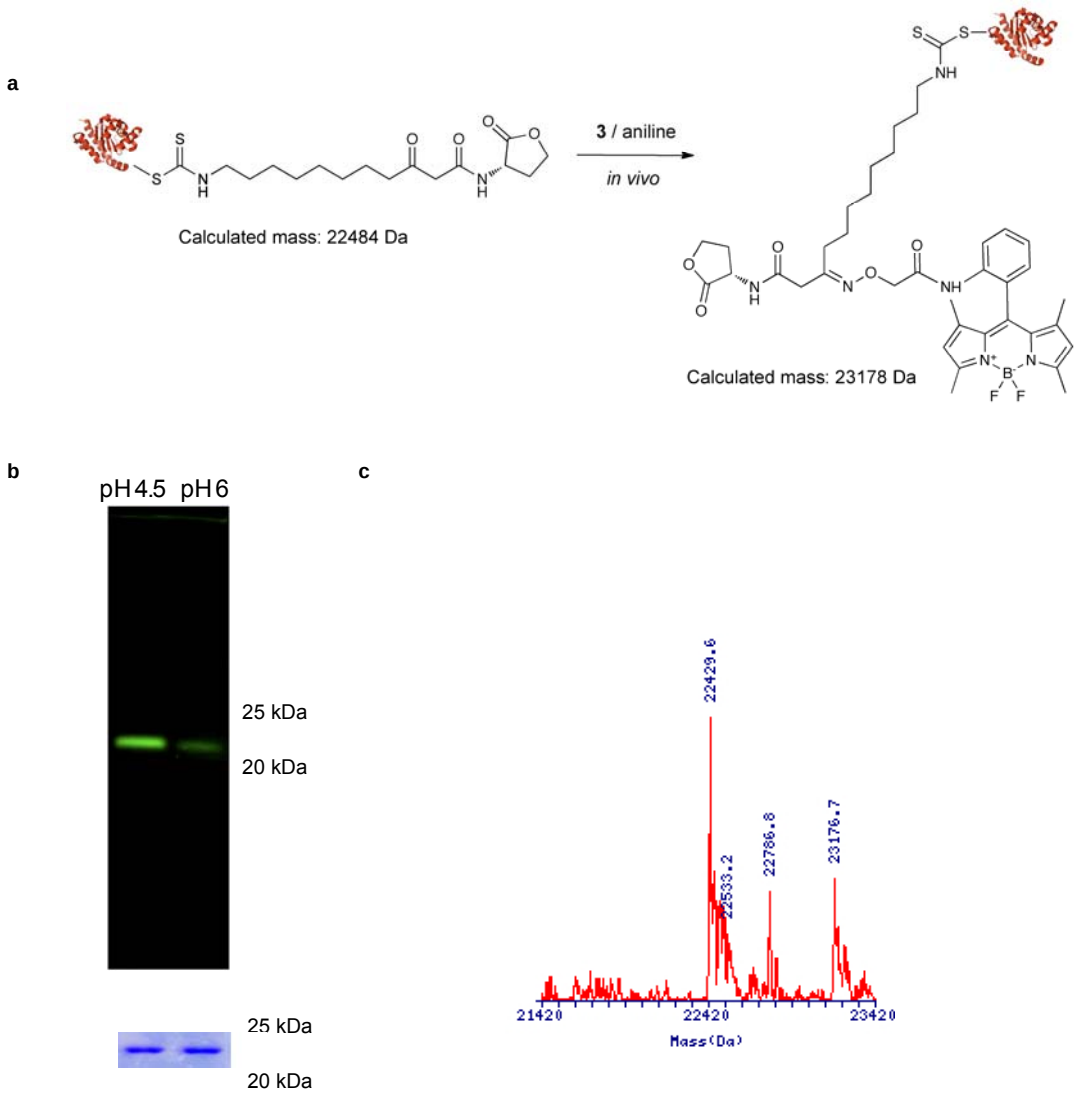


Figure S2. LasR labeling using aniline-catalyzed oxime chemistry. a, Scheme of the condensation reaction between LasR-ITC-12 and BODIPY-ONH_2 (**3)**. Reactions were carried out with 1 mM aniline, 100 µM aminooxy-BODIPY, and 1 µM LasR-ITC-12 for 12 h at pH 5 or 6.6. **b**, Fluorescence readout of a 12% SDS-PAGE (top) and Coomassie blue staining (bottom). Alternatively, reaction were also analyzed by LC-MS. **c,** Decon voluted ESI mass spectra of the *in vitro* reaction at pH 6.6. See Figure 1 for deconvoluted ESI mass spectra at pH 5. Note: product with a mass of 22430 corresponds to free LasR-LBD.

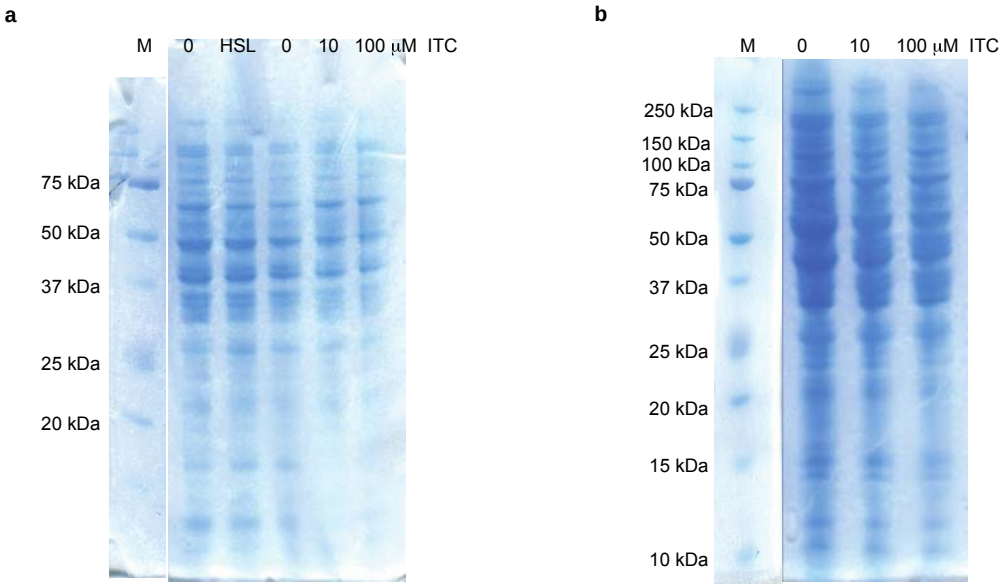


Figure S3. Coomassie blue-stained gel for the *in vivo* and *in vitro* labeling of LasR by BODIPY-ONH$_2$ (3). Ligation reactions were carried out at pH 6.6 (*in vivo*) or 5.1 (*in vitro*), with 100 μM of aminooxy-BODIPY and 1 mM aniline, for 12 h at 25 ºC (*in vitro*) or 8 ºC (*in vivo*). **a**, *in vitro*. **b**, *in vivo*. M: molecular weight markers.

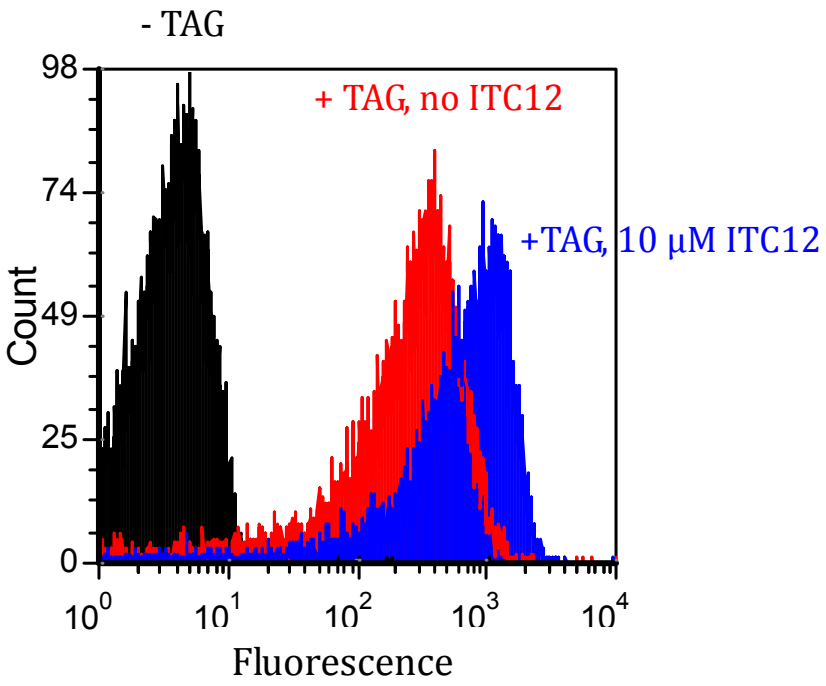


Figure S4. Analysis of QS receptor *in vivo* labeling by flow cytometry (without washing the cells with LB media). *Pseudomonas aeruginosa* cells were incubated in the presence ((blue) 10 µM) or the absence (red) of ITC-12. After removing the unreacted ITC-12, cell numbers were normalize to an OD_{600} of 2 and treated at 8 ºC and pH 6.6 with 100 µM of aminooxy-BODIPY, and 1 mM aniline for 12 h. (black) Without addition of aminooxy-BODIPY.

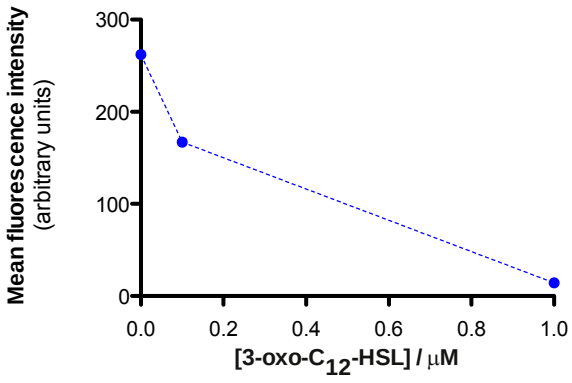


Figure S5. Effect on BODIPY labeling of LasR by ITC-12 in the presence of its cognate ligand as determined by flow cytometry. *Pseudomonas aeruginosa* cells were incubated in the presence of ITC-12 (100 μM) and 3-oxo-C_{12}-HSL (0, 0.1, and 1 μM). After removing the unreacted ligands, cell numbers were normalized to an OD_{600} of 2 and treated at 8 ºC and pH 6.6 with 100 μM of aminooxy-BODIPY, and 1 mM aniline for 12 h.

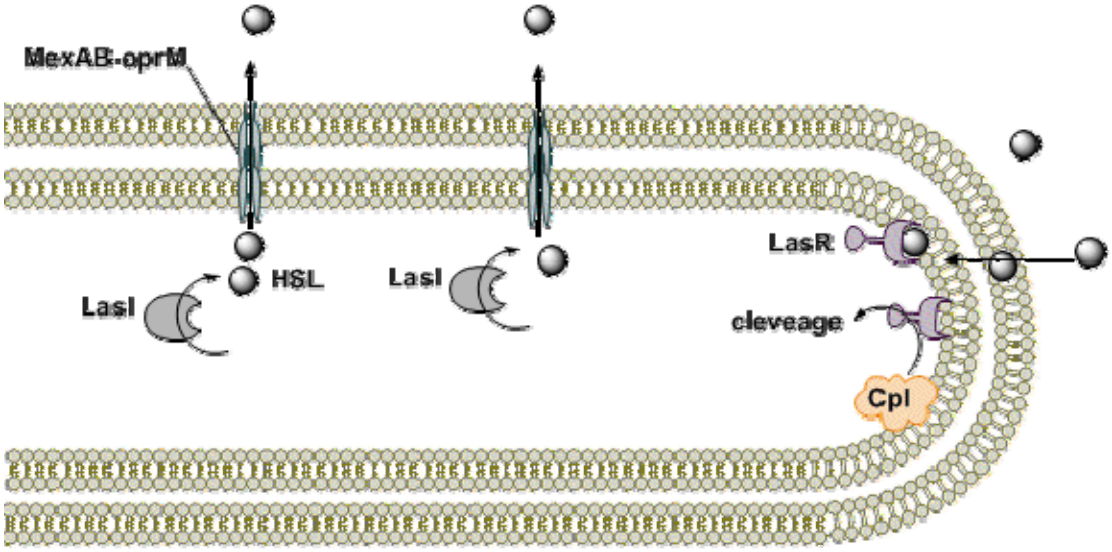


Figure S6. Proposed model for noise control in *P. aeruginosa*. The localization of LasR, as observed by fluorescence microscopy, can be rationalized by considering reports that newly synthesized autoinducers undergo active secretion from *P. aeruginosa* to the environment via the MexAB-OprM efflux pump,[1] possibly localized at the central part of the cell.[2] LuxR-type family proteins, such as LasR, are membrane-associated monomers, which, in the absence of their cognate ligand, misfold and are rapidly degraded by endogenous proteases.[3] Is it likely that through their association with the membrane, these proteins allow bacteria to specifically detect extracellular autoinducers that passively diffuse into the cell, especially if the autoinducers are secreted from a location in the cell that is far removed from the poles, where the receptors appear to be located. In a previous study that offer support for hypothesis, Zhu and Winans determined that Clp proteases are the primary proteins responsible for the turnover of TraR, a LuxR-type protein in *Agrobacterium tumefaciens*.[3] In addition, it has been proposed that Clp proteases are deployed mainly at the bacterial poles, and that LasR undergoes degradation at low

[1] Pearson, J.P., Van Delden, C. & Iglewski, B.H. *J. Bacteriol.* **181**, 1203-1210 (1999).
[2] Tashiro, Y. et al. *J. Bacteriol.* **190**, 3969-3978 (2008); Xu, X.-H.N., Brownlow, W.J., Kyriacou, S.V., Wan, Q. & Viola, J.J. *Biochemistry* **43**, 10400-10413 (2004).
[3] Zhu, J. & Winans, S.C. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 1507-1512 (2001).

cell densities.[4] The benefit for bacteria to separate the localizations of autoinducer secretion and sensing would lay in reducing the chance that secreted autoinducers bind receptors on the cell that secretes them, thereby increasing the sensitivity of the cell in gauging the presence and number of other cells.

[4] McGrath, P.T., Iniesta, A.A., Ryan, K.R., Shapiro, L. & McAdams, H.H. *Cell* **124**, 535-547 (2006).

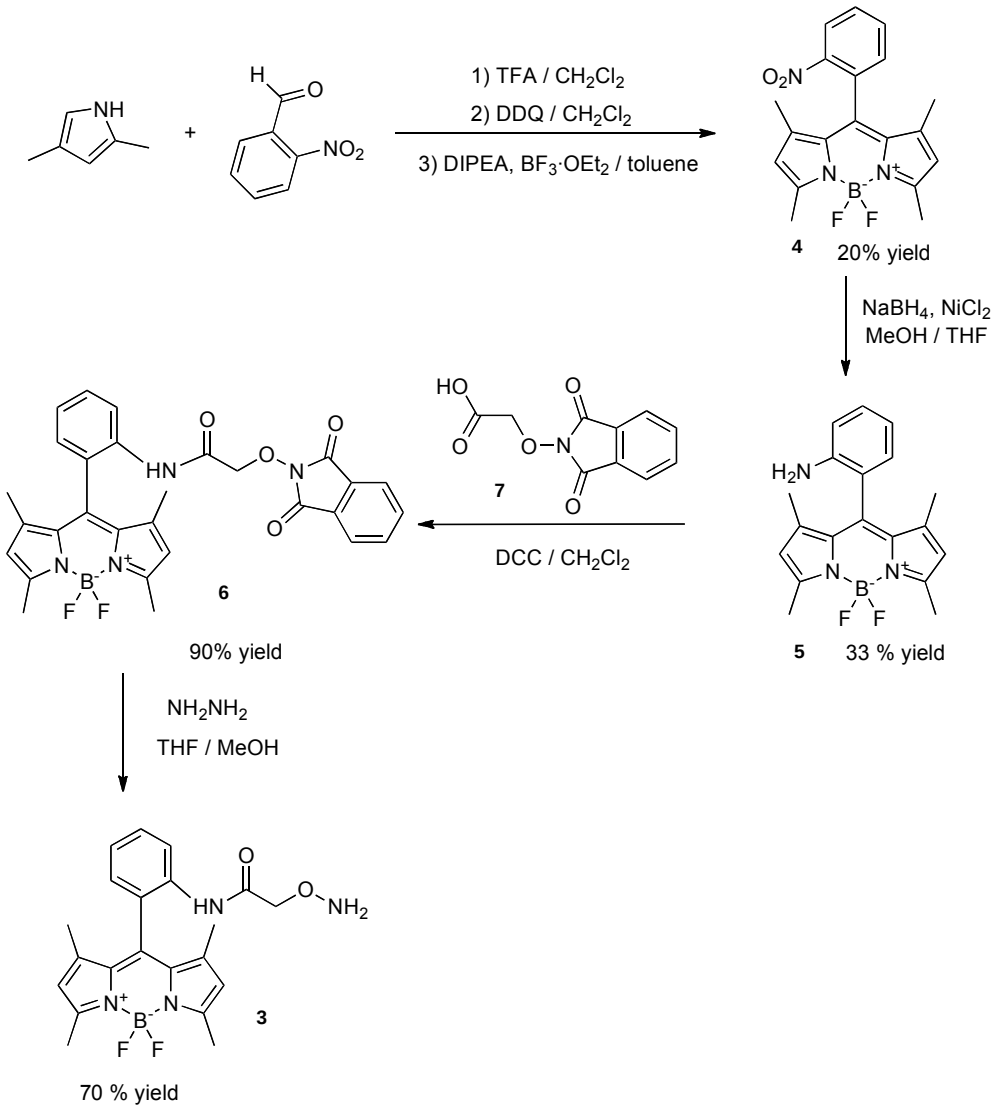
Supplementary methods

General

All chemical reagents were purchased from Sigma-Aldrich or Acros and used without further purification, unless noted. Solvents were dried and purged using an MBraun solvent purification system. Thin-layer chromatography was performed on TLC aluminum sheets silica gel 60 with F254 indicator (Merck). Flash chromatography was performed on Merck 40-63 μm silica gel. Solvent ratios for the purification of compounds by flash chromatography are reported as percent volume (v/v). NMR analysis weas performed using a Bruker Avance DPX400 or, alternatively, a Bruker Avance DMX500 spectrometer. Analytical HPLC analysis was performed using a Surveyor Plus HPLC System (Thermo Scientific) with a Luna C18.5 μm (150 x 4.6 mm) column at a flow rate of 1 mL/min. Preparative HPLC was performed using a Sapphire 600 instrument (ECOM) with a Luna C18 column, 10 μm (250 x 21.20 mm), at a flow rate of 20 mL/min. All runs used linear gradients of 0.1% aqueous TFA (solvent A) and acetonitrile containing 0.1% TFA (solvent B). Compounds were identified by UV detection with dual wavelengths (230 nm, 260 nm). All MS analyses were performed on a LCQ Fleet mass spectrometer (Thermo Scientific) with an ESI source. Spectra were collected in the positive ion mode and analyzed by Xcalibur software (Thermo Scientific). Compounds 3-oxo-C_{12}-HSL and ITC-12 were synthesized following modifications of procedures described by Chhabra et al.[5] and by us,[6] respectively.

[5] Chhabra, S. R.; Harty, C.; Hooi, D. S. W.; Daykin, M.; Williams, P.; Telford, G.; Pritchard, D. I.; Bycroft, B. W. *J. Med. Chem.* **2002**, *46*, 97-104.
[6] Amara, N. et al. *J. Am. Chem. Soc.* **131**, 10610-10619 (2009).

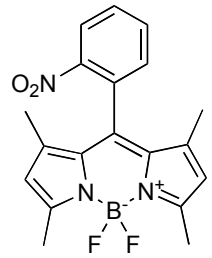
Scheme S1: Synthesis of BODIPY-ONH$_2$ (3)



Synthesis of aminooxy-BODIPY (3)

The synthesis of compound **3** is outlined in Scheme S1. Nitro-BODIPY **4** was prepared following a reported procedure.[7] Reduction of the nitro moiety, followed by coupling with the DCC activated acid **7** provided the phthalimido protected BODIPY. Cleavage of the protecting group with hydrazine completed the BODIPY scaffold.

4,4-Difluoro-8-(2-nitrophenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (4). This compound was prepared following procedures described by Sunahara et al.[7] To a solution, under Ar atmosphere, of 2-nitrobenzaldehyde (871.6 mg, 5.7 mmol) and 2,4-dimethylpyrrole (1.76 mL, 11.43 mmol) in 500 mL anhydrous CH_2Cl_2, one drop of trifluoroacetic acid was added. The solution was stirred for 20 h at RT at which time DDQ (1.3 g, 5.7 mmol) was added, and stirring continued for 30 min at RT. The reaction mixture was washed with 300 mL H_2O, dried over Na_2SO_4, filtered and concentrated in vacuo. The crude mixture was then purified through a short pad of neutral alumina (CH_2Cl_2), providing a brown solid. This compund and DIPEA (5 mL, 28 mmol) were dissolved in 200 mL of anhydrous toluene, and the resulting solution was stirred for 5 min at RT, upon which $BF_3{\cdot}Et_2O$ (5 mL, 40 mmol) was added dropwise, and stirring continued for another 30 min at RT. The reaction was quenched by addition of 200 mL of H_2O. The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The compound was purified by flash chromatography on silica gel (DCM/hexane 2:1), affording the nitro-BODIPY **5** as a wine-red power.

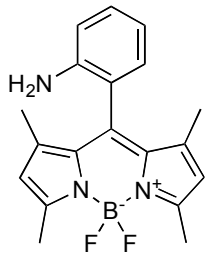


Yield: 20 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.17 (dd, J = 8.08, 1.22, Hz, 1H), 7.78 (td, J = 7.47, 1.22 Hz, 1H), 7.69 (td, J = 7.78, 1.52 Hz, 1H), 7.45 (dd, J = 7.62, 1.52 Hz, 1H), 5.98 (s, 2H), 2.55 (s, 6H), 1.35 (s, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 156.1, 148.3, 141.7, 134.0, 131.0, 130.5, 130.2, 124.9, 121.5, 14.6, 13.8; **MS (ESI)** m/z: calc: $[M-F]^+$ 350.17, measured: $[M-F]^+$ 350.24.

[7] Sunahara, H., Urano, Y., Kojima, H. & Nagano, T. *J. Am. Chem. Soc.* **129**, 5597-5604 (2007).

4,4-Difluoro-8-(2-aminophenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (5).

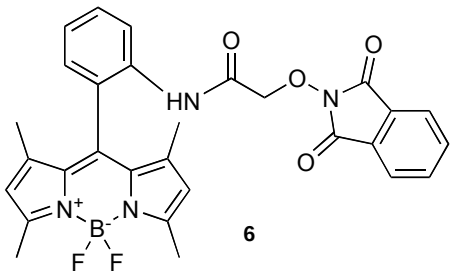
Compound **4** (105 mg, 0.45 mmol) and $NiCl_2 \cdot 6H_2O$ (212 mg, 0.89 mmol) were dissolved in 3 mL of a mixture of THF and MeOH (1:1) and cooled to -25 °C. Then, sodium borohydride (67.3 mg, 1.78 mmol) was added in small portions (during 30 min) at this temperature. The reaction was allowed to stir for 20 min at -15 °C, and was quenched with sat. aq. NH_4Cl (2 mL) and extracted with DCM (3*10 mL). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated in vacuo. Purification of the resulting brown solid by flash chromatography on silica gel (DCM/hexane 1:1) afforded amine **5** as an orange powder.



Yield: 33 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.23 (td, J = 7.33, 1.68 Hz, 1H), 6,98 (dd, J = 7.55, 1.60 Hz, 1H), 6.85 (td, J = 7.48, 1.07 Hz, 1H), 6.74 (dd, J = 8.09, 0.61 Hz, 1H), 5.98 (s, 2H), 2.54 (s, 6H), 1.55 (s, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$):): δ 155.6, 143.2, 138.3, 131.05, 130.2, 128.6, 121.0, 119.2, 115.6, 14.5, 13.6; **MS(ESI)** m/z: calc: $[M-F]^+$ 320.1, measured: $[M-F]^+$ 320.3.

4,4-Difluoro-8-(2-(2-((1,3-dioxoisoindolin-2-yl)oxy)acetamido)phenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (6).

DCC (150 mg, 0.73 mmol) was added to a solution of **5** (58.7 mg, 0.147 mmol) and **7** (161.3 mg, 0.73 mmol) in 2 mL of a anhydrous DCM. The suspension was stirred overnight at RT, filtered, and the solvent was evaporated in vacuo. The crude reaction mixture was purified by flash chromatography on a column of silica gel (DCM) affording compound **6** as an orange powder.



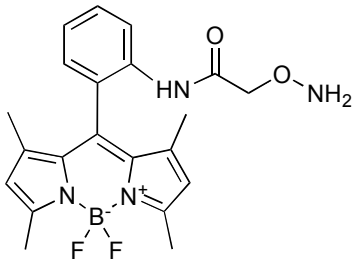
6

Yield: 90 %. **^{1}H NMR** (400 MHz, $(CD_3)_2CO$): δ 9.29 (s, 1H), 8.23 (m, 1H), 7.90-7.80 (m, 4H), 7.57 (td, J = 7.2, 2 Hz, 1H), 7.43-7.36 (m , 2H), 6.08 (s, 2H), 4.72 (s, 2H), 2.41 (s, 2H), 1.49 (s, 2H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 165.6, 162.6, 154.9, 142.6, 134.8, 134.5, 129.8, 129.0, 128.3, 127.0, 126.2, 125.0, 123.4, 123.3, 121.3, 75.6, 14.2, 13.5; **MS(ESI)** m/z: cald: $[M-F]^+$ 523.3, measured: $[M-F]^+$ 523.4.

4,4-Difluoro-8-(2-(2-(aminooxy)acetamido)phenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (3)

Hydrazine monohydrate (13.68 mg, 0.276 mmol) was added to a solution of **6** (50 mg, 0.09 mmol) in 1 mL of mixture of THF and MeOH (1:1), and the reaction was stirred for 2 h at RT. The reaction was quenched upon addition of 5 mL of H_2O, and the product was extracted with 3*10 mL of $CHCl_3$. The combined organic extracts

were dried over anhydrous Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The crude material was purified by RP-HPLC (50% ACN/50% water to 100% ACN over 40 min) affording an orange solid.

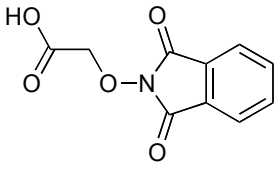


Yield: 70 %. **^{1}H NMR** (400 MHz, MeOD): δ 8.1 (d, J = 8.4 Hz, 1H), 7.55 (t, J = 7.4, 1.5 Hz, 1H), 7.42 (td, J = 7.4, 0.9 Hz, 1H), 7.31 (dd, J = 7.6, 1.6 Hz, 1H), 6.09 (s, 2H), 4.35 (s, 2H), 2.50 (s, 6H), 1.45 (s, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 169.9, 167.1, 156.2, 143.2, 136.7, 134.5, 133.9, 132.3, 130.7, 130.3, 129.8, 128.5, 125.3, 122.5, 122.4, 121.2, 73.3, 13.1, 12.6. **MS(ESI)** m/z: cald: $[M-F]^+$ 393.2, measured: $[M-F]^+$ 393.2.

Phthalimido-aminooxyacetic acid (7)

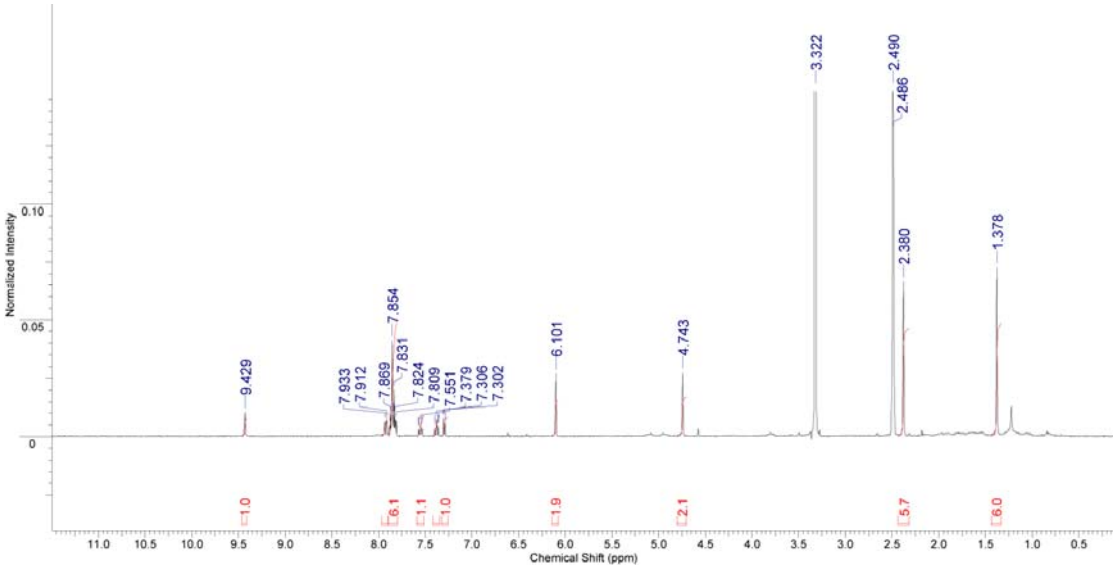
This compound was synthesized using a previously published procedure.[8] NaH (480 mg, 7.5 mmol) was added portionwise to a solution of N-hydroxyphthalimide (0.8 g, 5 mmol) in 20 mL of anhydrous DMF at 0 °C under Ar atmosphere. The resulting red suspension was stirred for 10 min, and a solution of bromoacetic acid in 10 mL anhydrous DMF was added dropwise (30 min) at 0 °C. The crude mixture was then stirred overnight at RT, and the reaction was quenched by pouring it into crushed ice and acidified with HCl (1M). The resulting white solid was then filtered and washed with cold hexane, and the crude material was finally purified by RP-HPLC (60% ACN/40% water to 100% ACN over 40 min) affording a white solid.

[8] Katritzky, A. R.; Avan, I.; Tala, S. R. *J. Org. Chem.*, *74*, 8690-8694 (2009).

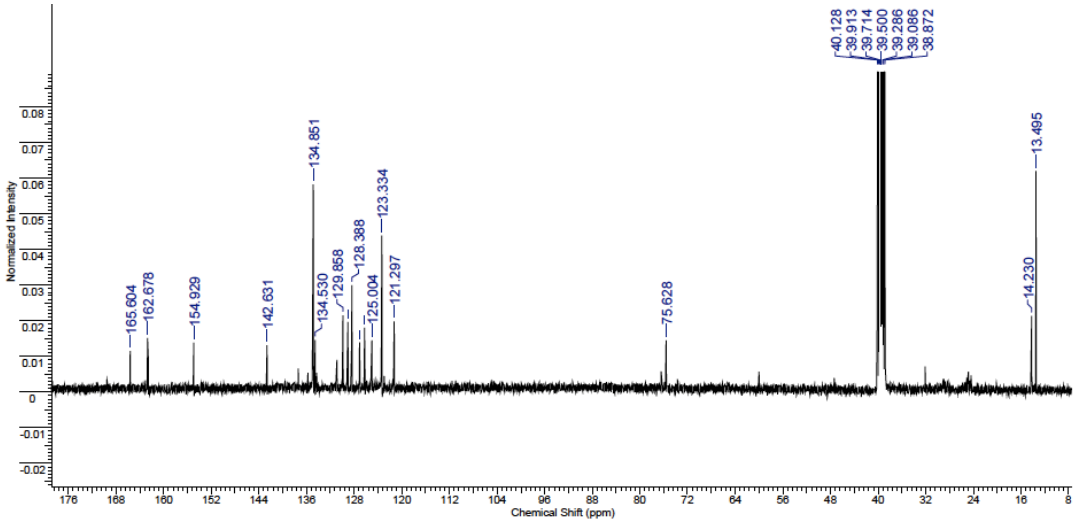


Yield: 70 %. **^{1}H NMR** (400 MHz, MeOD): δ 7.90-7.80 (m, 4H), 4.85 (s, 2H). **MS(ESI)** m/z: cald: $[M+H]^+$ 222,1, measured: $[M+H]^+$ 222,1

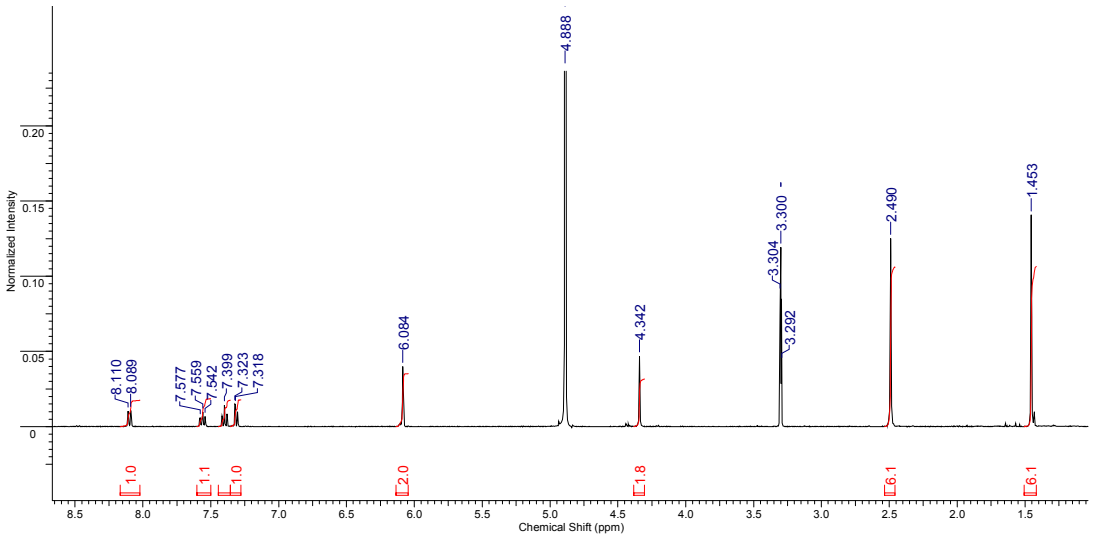
^{1}H-NMR for compund **6**



^{13}C-NMR for compound **6**



^{1}H-NMR for compund **3**



^{13}C-NMR for compound **3**