Electronic Supplementary Material (ESI) for ChemComm.

CDy14: A novel biofilm probe targeting exopolysaccharide Psl

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Experimental

General Procedure for the Synthesis of CDy14.

To a solution of compound (BDN)[1] (x equiv) in anhydrous acetonitrile was added with corresponding aldehyde (4x equiv), followed by acetic acid (6x equiv) and pyridine (6x equiv), then heated at 85 °C for 5 min. After solvent evaporation, the crude residue was purified by flash column chromatography to afford condensed product as a solid.

Characterization of CDy14.

CDy14. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.72-7.64 (m, 2H), 7.46-7.41 (m, 1H), 7.17-7.08 (m, 3H), 6.93 (s, 2H), 6.73-6.70 (m, 2H), 6.58-6.57 (m, 1H), 6.49-6.47 (m, 1H), 5.72 (S, 2H), 3.86 (s, 6H), 3.74 (s, 3H), 1.78 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 156.34, 153.64, 151.33, 145.46, 144.24, 139.93, 139.75, 137.52, 134.91, 134.45, 131.70, 131.25, 126.41, 120.34, 119.84, 117.43, 116.50, 113.63, 105.21, 60.59, 56.30, 16.03; HRMS m/z : calc for $C_{27}H_{26}BF_2N_3NaO_3$ (M+Na) 512.1932, found: 512.1935; Extinction coefficient (ε): 46680 $M^{-1}cm^{-1}$ (Solvent: Ethanol, Wavelength (λ): 555 nm).

Quantum Yield Measurements.

Quantum yield were calculated by measuring the integrated area of emission spectra for CDy14 compound in comparison to the same measurement for Rhodamine B (Φst = 0.5) as reference compound in ethanol. CDy14 Rhodamine B were excited at 520 nm and emission spectra were collected from 550 to 700 nm.

$$\Phi x = \Phi st(Ix/Ist)(Ast/Ax)(\eta x2/\eta st2) \quad (1)$$

Quantum yields were calculated using eq 1, Where "Φst" is the reported quantum yield of the standard, "I" is the integrated emission spectrum, "A" is the absorbance at the excitation wavelength, and "η" is the refractive index of the solvents used. The subscript "x" denotes unknown and "st" denotes standard. Emission was integrated between 550 and 700 nm, and quantum yield for CDy14 was found to be 0.0014.

Screening for biofilm binding compound using mutant *P. aeruginosa*.

Two *P. aeruginosa* strains, *wspF* and *pYhiH* were used for 96 well-based screening. Two strains were cultured at 37˚C for 20 hours in 100 µL LB media and the fluorescent intensity between *wspF* and *pYhjH* biofilms was analyzed under EVOS fluorescent microscope (AMG, Mill Creek, WA) after 1 hour incubation with DOFL. In repetitive experiment, 2 pieces of cover glass were incubated in 50 mL conical tube by adding 5 mL LB media and two mutants (*wspF* and *pYhjH*) for

testing compound on cover glass where biofilm was formed. The compounds showed stronger fluorescent intensity in *wspF* biofilm than *pYhjH* biofilm were chosen as *wspF* biofilm staining probe.

Bacterial strains and growth conditions.

P. aeruginosa strains used in this research are PAO1, PAO1-GFP, *PAO1Δpsl-GFP*, *PAO1Δpel-GFP*, P_{BAD}-Psl strains were cultured at 37˚C incubator in LB media.

Confirmation of CDy14 target.

PAO1Δpel-GFP, PAO1Δpsl-GFP strains were inoculated in chamber slide with ABTG media after pre-culture with LB media. After 3 days, each of compounds treated to biofilm and incubated for 30 min.

PAO1 was inoculated in 50 mL tube with LB media and added cover glass. Next day, the biofilm on cover glass was washed with PBS for remove planktonic cells and stained HHA-FITC for 30 min. After washing with PBS, it was stained CDy14 for 30 min.

Image of biofilm was taken after 30 min staining. All biofilm images were acquired under confocal laser scanning microscope

Super resolution images.

PAO1 (O.D.600 = 0.1) strain was inoculated in 8-well chamber with ABTG media and incubated for 20 hours at 37˚C condition to get biofilm. CDy14 (1 μM) and HHA-FITC were treated to PAO1 biofilm for 1 hour at 37˚C incubator before analyzing two different fluorescent signals with super resolution structure illumination microscopy (SR-SIM). The images of the CDy14 and HHA-FITC treated PAO1 biofilm were captured using LSM 780 ELYRA PS.1 system (Carl Zeiss, Germany) using 100× oil lens. Fluorescent signal from the GFP channel represents the distribution of Psl in PAO1 biofilm matrix and fluorescent signals from TRITC channel represents localization of CDy14. Two images from GFP and TRITC channels were processed and two images were merged for analyzation of CDy14 localization in biofilm matrix.

Localization of CDy14 on biofilm during development.

Compounds were treated to different stage of biofilm depending incubation time; 1, 3, 5 days. For imaging of living biofilm, PAO1-GFP strains were inoculated in chamber slide for 1, 3, 5 days with ABTG media at room temperature and supplied fresh media after remove old media, every day. CDy14 was treated to each stage of biofilm at chamber slide and incubated for 30 minutes. Biofilm images were acquired under confocal laser scanning microscope.

Generation of corneal infection model.

PAO1-GFP and PAO1*Δpsl*-GFP were cultured at 37˚C with 100 rpm shaking overnight. 500 μL of cultured bacteria was transferred to 1.5 mL ependorf tube and centrifuge at 14,000 rpm for 2 minutes then pellet was harvested by discarding supernatant. Pellet was resuspended with same volume with PBS and centrifuged at 14,000 rpm two more times. Finally, pellets were resuspended in 250 μL of PBS buffer. C57BL/6 black mice were anesthetized with ketamine/xylazine by subcutaneous injection. Under anesthesia conditions, light scratches were generated using mini blade (bvi Beaver®) in corneal region of eye. Re-suspended *P. aeruginosa* (*PAO1-GFP* and *PAO1Δpsl-GFP*) in PBS buffer was dropped in scratched corneal and mice were maintained for 1 day for inducing biofilm in corneal regions. All animal experiment procedures were performed in accordance with a protocol approved by the Institutional Animal Care and Use Committee.

Strain	Genotype	Main matrix composition and biofilm formation	Reference
PAO1-GFP	Green fluorescent protein (gfp)-tagged wild type PAO1 strain	proper biofilm formation	(2)
PAO1 *ΔwspF*	wspF mutation	forming proper biofilm with increased polysaccharide	(3)
PAO1 *p-yhjH*	*yhjH gene encoding*	Unable to form proper biofilm	(3)
PAO1Δpsl-GFP	*Psl* gene mutation	forming proper biofilm excluding Psl polysaccharide	(4)
PAO1Δpel-GFP	*Pel* gene mutation	forming proper biofilm excluding Pel polysaccharide	(4)
P_{BAD}-Psl	psl operon promoter with an L-arabinose-inducible promoter	biofilm formation with Psl production by L-arabinose	(5)

Table S1. Strains used in this study

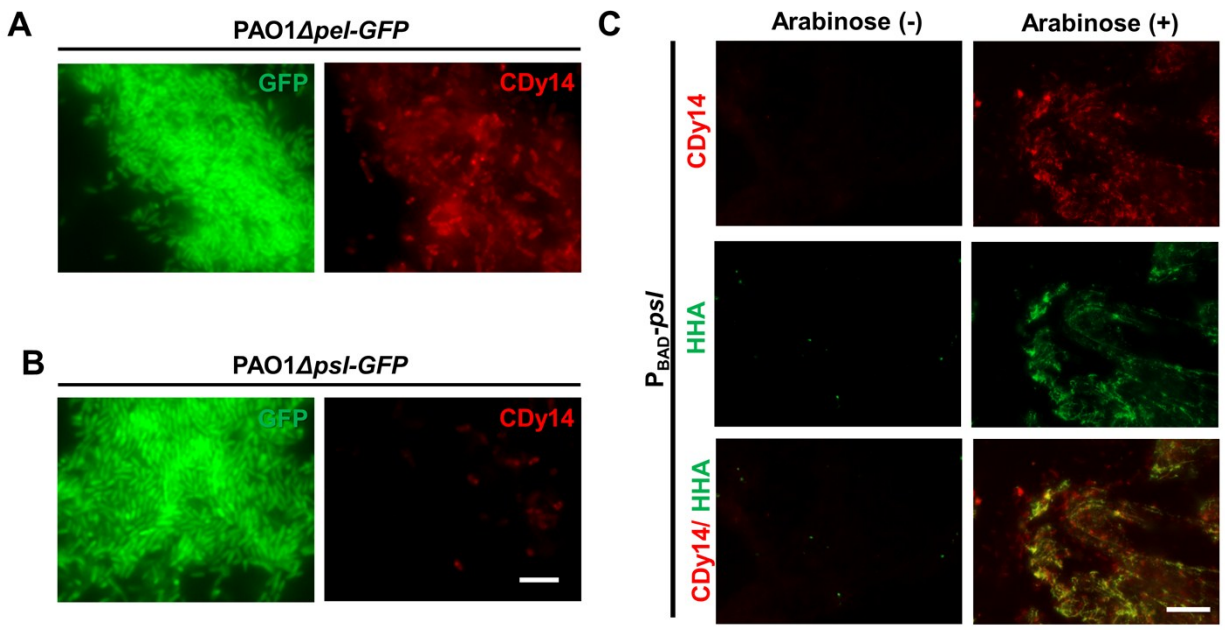


Fig. S1 Target validation of CDy14. (A, B) CDy14 was treated on biofilm of PAO1Δpsl; Psl-deficient *P. aeruginosa* and PAO1Δpel; Pel-deficient *P aeruginosa*, which were formed on slide glasses. **(C)** CDy14 target was examined with P_{BAD}-Psl strain regulating expression of psl by arabinose. Scale bars, 10 μm.

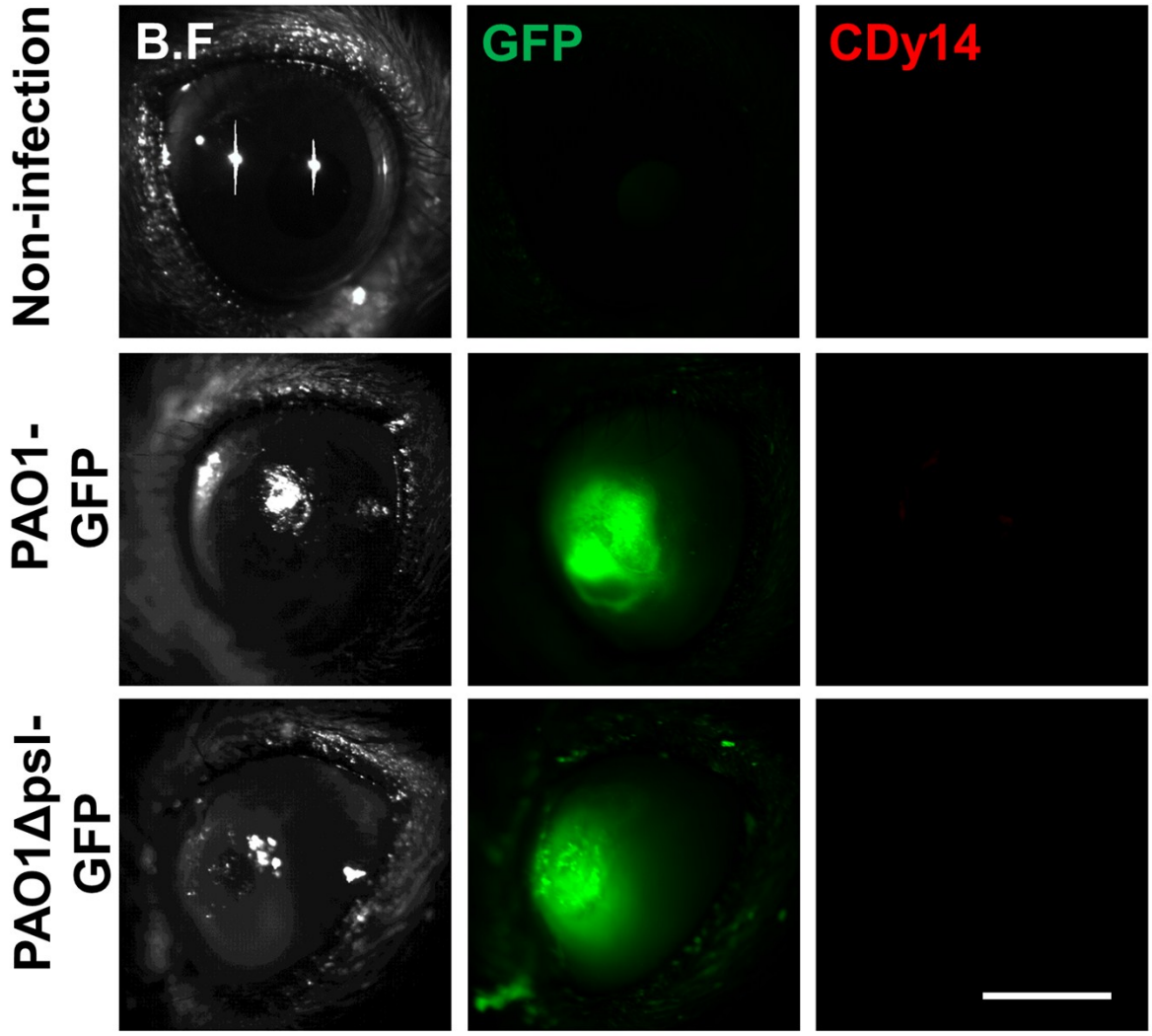
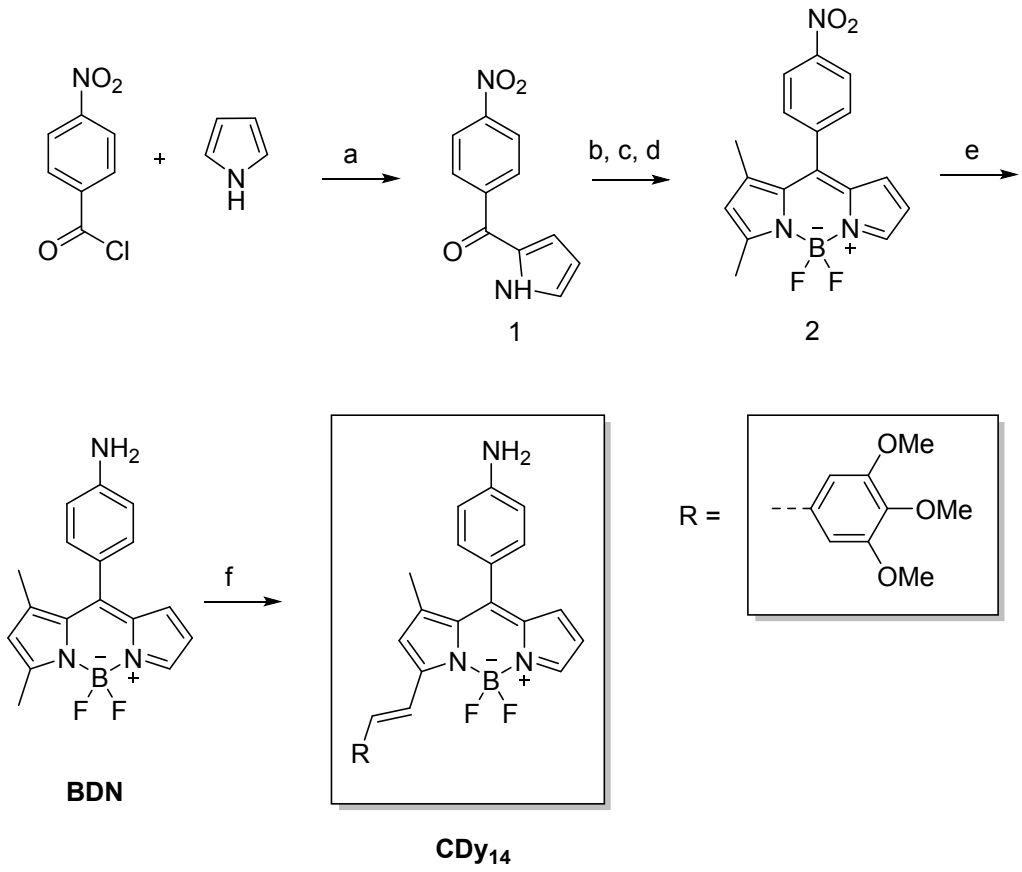


Fig. S2 No signal on the infected corneal region without CDy14. No signal was detected in the non-infected control eye (upper row), infected eye by PAO1-GFP (middle row), *PAO1Δpsl*-GFP (Psl deficient) without CDy14. Scale bars: 2 mm.

Compound information

General Procedure for the Synthesis of CDy14.

To a solution of compound (BDN)[1] (x equiv) in anhydrous acetonitrile was added with corresponding aldehyde (4x equiv), followed by acetic acid (6x equiv) and pyridine (6x equiv), then heated at 85 °C for 5 min. After solvent evaporation, the crude residue was purified by flash column chromatography to afford condensed product as a solid.



Scheme. S1: Synthesis of CDy14. (a) MeMgBr, THF, -78 ˚C to RT, overnight; (b) $NaBH_4$, THF/H_2O (10:1), 0 ˚C to RT, 0.5h; (c) 2,4-dimethyl pyrrole, $InCl_3$, CH_2Cl_2, RT, 4hr; (d) i. DDQ, CH_2Cl_2, RT, 15min. ii. $BF_3.Et_2O$, Et_3N, 0 ˚C to RT, 8h; (e) Pd/C, hydrazine monohydrate, EtOH/THF (5:1), reflux, 2h; (f) aldehyde, AcOH, pyrrolidine, MeCN, 85 ˚C, 5 min.

Quantum Yield Measurements.

Quantum yield were calculated by measuring the integrated area of emission spectra for CDy14 compound in comparison to the same measurement for Rhodamine B (Φst = 0.5) as reference compound in ethanol. CDy14 Rhodamine B were excited at 520 nm and emission spectra were collected from 550 to 700 nm.

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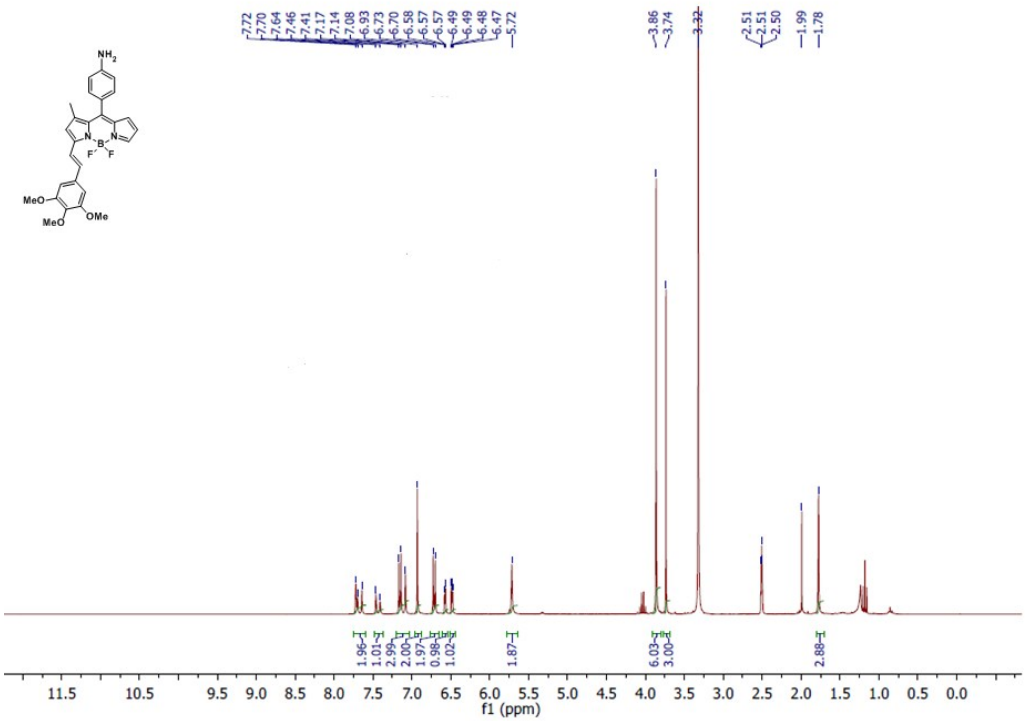
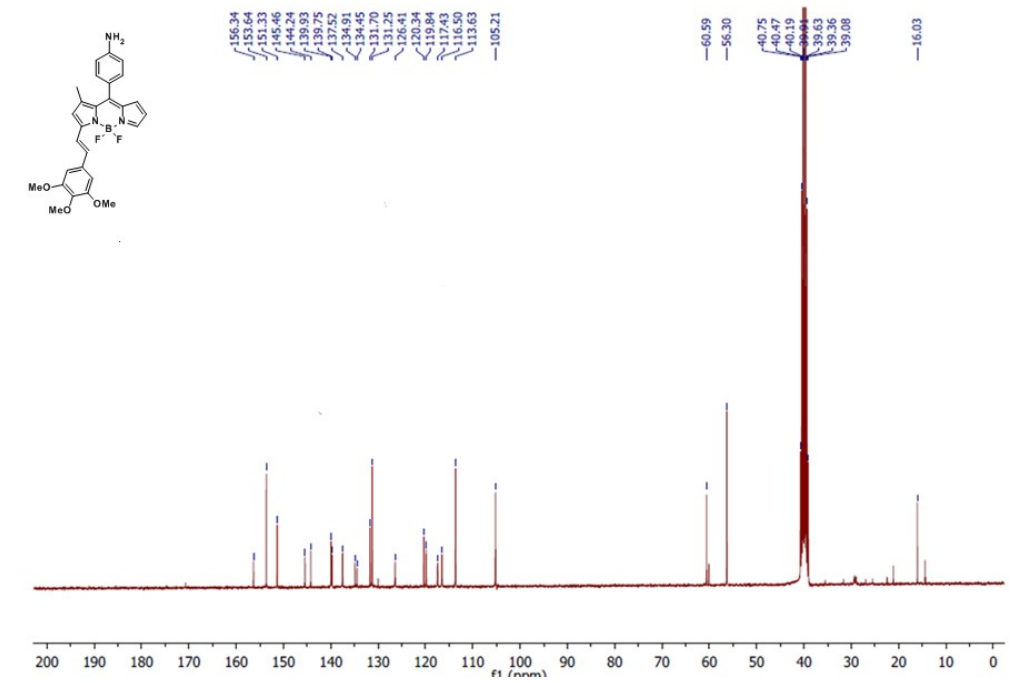
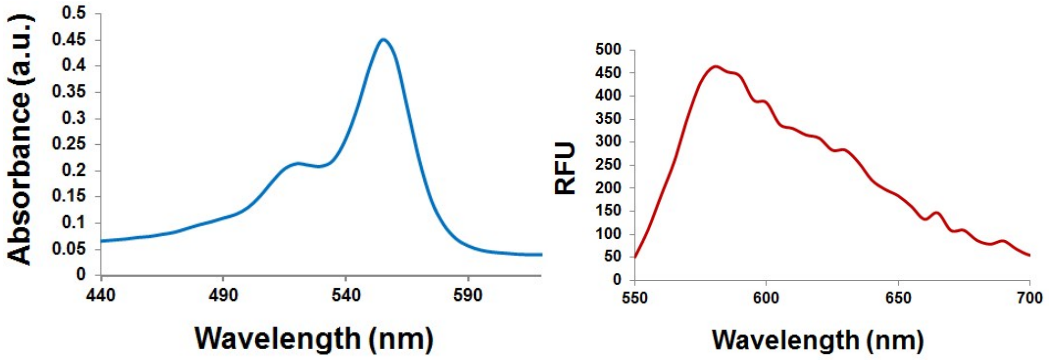
^{1}H NMR (300 MHz, DMSO-d_6) spectrum of CDy14



^{13}C NMR (75 MHz, DMSO-d_6) spectrum of CDy14



Absorption and emission spectra of CDy14

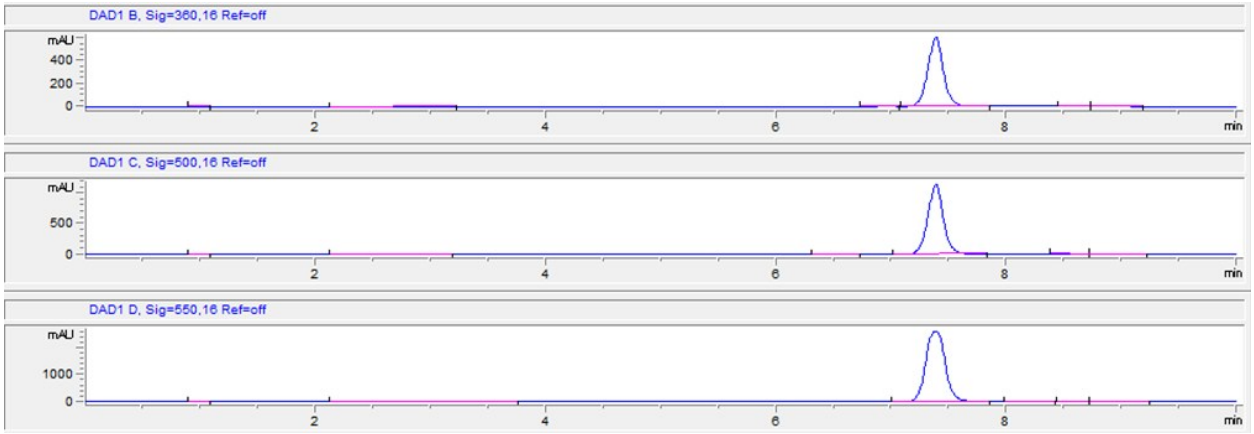


Absorption and emission spectra of **CDy14** were measured at the concentration of 10 μM in Ethanol, Excitation wavelength (λ_{ex}) = 520 nm.

Table. S2 Spectroscopic properties and purity table for **CDy14**: absorbance maximum (λ_{abs}), fluorescent emission maximum (λ_{em}), and quantum yield (QY) in ethanol.

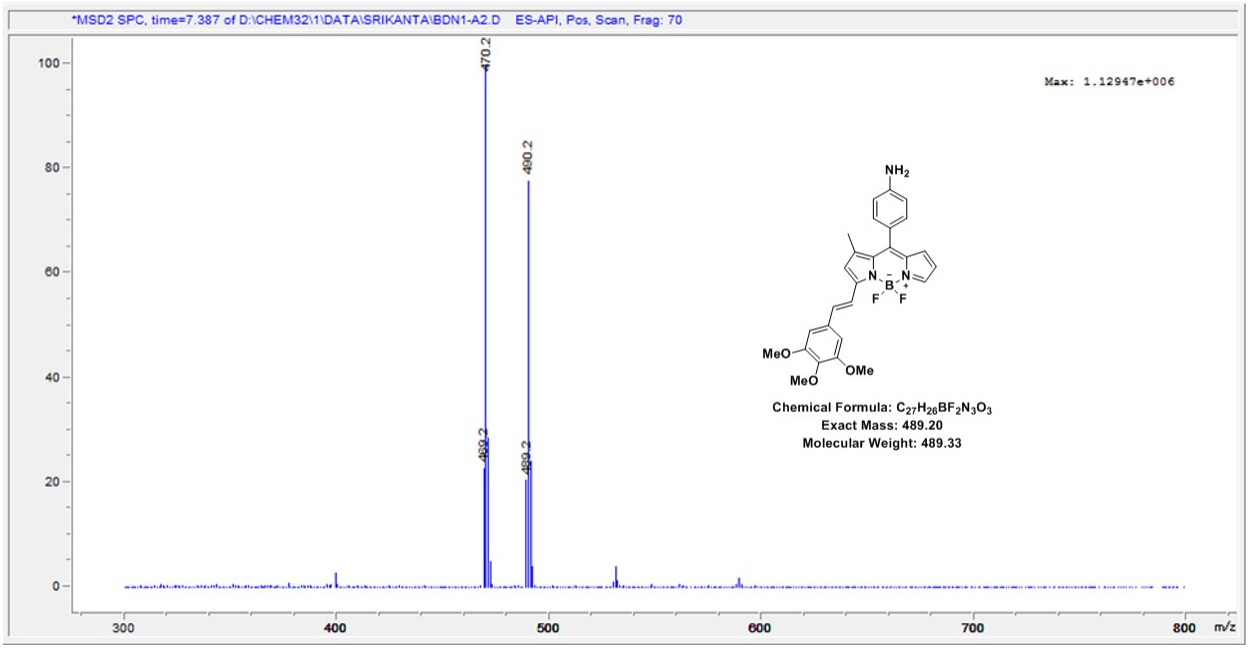
Compound	M^+ (cal)	M^+ 1(exp)	Abs (nm)	Em (nm)	QY	Purity (%)
CDy14	489.20	490.2	555	580	0.0014	98

LC Spectrum of CDy14



LC spectrum **CDy14**. LC trace in 550 nm, 500 nm and 360 nm. (Eluent: water (0.1% formic acid), acetonitrile (MeCN) with 0.1% formic acid; Gradient: 5% to 95% MeCN in 10 min.)

MS spectrum of CDy14



MS spectrum **CDy14**. ESI mass spectrum that showed 490.2 [M+H], 470.2 [M-F]

Reference:

1. Kang, N. Y, et al. (2013) Visualization and Isolation of Langerhans Islets by a Fluorescent Probe. *Angew. Chem. Int. Edit*. 52(33):8557-8560.
2. H. M. Allesen, K.B. Barken, K. M. Yang L, J. S. Webb, S. Kjelleberg, S. Molin, M. Givskov, N. T. Tolker, *Mol Microbiol*. 2006, **59**, 1114.
3. S. L. Chua, Y. Ding, Y. Liu, Z. Cai, J. Zhou, S. Swarup, Dl. Drautz-Moses, S. C. Schuster, S. Kjelleberg, M. Givskov, L. Yang, *Open Biol*. 2016, **11**, 160162.
4. Y. Liu, Y. Hu, Y. Liu, J. Zhang, J. Ulstrup, S. Molin, *Environ Microbiol*. 2011, **7**, 1705.
5. L. D. Christensen, V. G. Maria, M. T. Rybtke, H. Wu, W. C. Chiang, M. Alhede, N. Høiby, H. Niels, T. E. Nielsen, M. Givskov, T. T. N. Nielsen, *Infect Immun*. 2013, **81**, 2705.