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A Cyclopalladated BODIPY Construct as a Fluorescent Probe for Carbon Monoxide

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Dedicated to the memory of Prof. Dr. Ayhan S. Demir (1950–2012).

By introducing a palladium ion into the backbone of BODIPY, we devised a cyclopalladated BODIPY construct that was almost non-emissive in the absence of any analyte but became highly fluorescent upon interacting with carbon monoxide (CO) in solution and in living cells. A process of *ortho*-carbonylation and depalladation mediated by the specific binding of CO to palladium, promoted the release of the heavy atom from the fluorophore and consequently generated a fluorescence signal with an exceptionally high (60-fold) enhancement ratio.

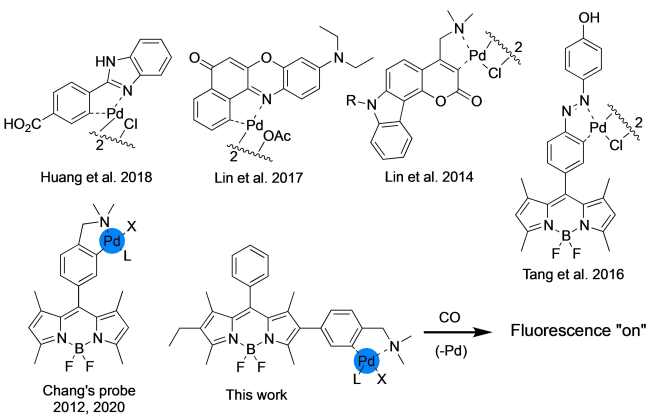
Introduction

Historically acknowledged as a toxic gas due to its strong binding affinity towards hemoglobin,[1] carbon monoxide (CO) is now recognized as an important signaling molecule in the family of gasotransmitters.[2] Produced endogenously from the enzymatic breakdown of heme,[3] CO plays key regulatory roles in many physiological processes.[4] Despite its potential toxicity at pathological concentrations,[5] multiple streams of emerging research and pre-clinical studies suggest that CO exhibits anti-inflammatory as well as cyto- and tissue-protective effects in living systems at low doses.[6–13] The therapeutic effects of exogenous CO in living systems have mostly been studied with CO releasing molecules (CORMs) that allow the controlled, precise delivery of CO to the molecular targets.[14,15] Because assessing CO levels in cellular milieux is vital to investigating CO's diverse biological roles and therapeutic effects, research on developing fluorescent probes able to monitor transient CO in living systems is greatly needed.

In the last few years, numerous types of fluorescent CO probes have been devised along those lines,[16–21] most of which are smart extensions of a sensing strategy pioneered by Chang's research group.[22] Chang's probe design, COP-1, involves integrating palladium into the periphery of a fluorophore (e.g., meso-phenyl position of BODIPY), thereby generating a fluorophore-palladacycle, that remains non-emissive until it interacts with CO (Scheme 1). The binding of CO to the palladium center mediates an *ortho*-carbonylation reaction, and consequently generates a palladium-free fluorophore with a strong fluorescence emission. By contrast, another family of CO-based probes operate via the deallylation of allyl ether-based pre-fluorescent compounds but require adding exogenous palladium for their operation, which poses a critical limitation for biological applications.[23–28]

As past studies have shown, the position of the metal center and its distance to the fluorophore core may dramatically affect the quenching of probe's fluorescence emission. For an ideal probe system with a large turn-on response, the metal center should be as close to the fluorophore's core as possible in order to intensify the heavy atom effect.[29–32] For those reasons, we examined whether changing the position of the palladacycle on BODIPY's skeleton would affect or even improve the probe's analytical performance compared with its congeners in terms of CO selectivity, analyte specificity, response time and signal-to-background ratio.

With all of the above in mind, for the first time ever we designed and constructed a probe, BOD-MRV, by building a



Scheme 1. Structures of turn-on CO probes with a palladacycle skeleton.

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palladacycle on the 2-phenyl backbone of a BODIPY core (Scheme 1). We envisioned that placing palladium at the 2-position of BODIPY core would efficiently quench the fluorescence emission due to the heavy-atom effect. As a result, the CO-triggered depalladation of the probe skeleton was expected to allow us to recognize CO's presence as indicating an increase in fluorescence intensity.

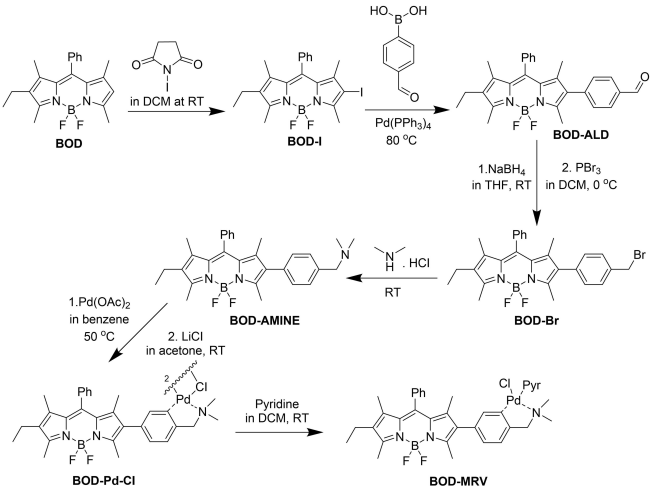
Results and Discussion

First, we prepared BOD-MRV according to the synthetic route outlined in Scheme 2. Following a Suzuki coupling protocol, iodo-BODIPY was coupled with 4-formylphenylboronic acid to yield BOD-ALD, which was subsequently reduced with $NaBH_4$ to an alcohol derivative, brominated and treated with dimethyl amine to generate BOD-AMINE. The complexation of BOD-AMINE with $Pd(OAc)_2$ at 50 °C in benzene and subsequent ligand exchange with LiCl in acetone yielded the dimeric-μ-chloro BODIPY derivative, BOD-Pd-CI. Next, to lower the probe's molecular weight and improve its hydrophilicity,[33] a bridge-splitting reaction with pyridine was performed to furnish the monomeric form of the probe, BOD-MRV. The structure of the title compound was clearly confirmed by NMR spectroscopy and HRMS.

Second, we systematically investigated the spectroscopic behavior of BOD-MRV towards CO with the aid of UV (ultraviolet) and fluorescence spectroscopy. After a stream of CO gas under balloon pressure was passed through the solution with the probe (wet DCM, 5 μM) for 30 min, the addition of CO gas to the solution immediately prompted an off-on type optical response that was easily observable with the naked eye. With the addition of CO, the solution became distinctly orange with a bright green emission (see Supporting Information).

Third, encouraged by our preliminary observations, we conducted a systematic spectral investigation of the sensing process by using CORM-2 as a safe, convenient source of exogenous CO that releases 0.7 moles of CO per mol at 37 °C.[34,35] The absorption and emission spectra of the probe in the absence and presence of CO appear in Figure 1. As shown, free BOD-MRV (PBS:CH_3CN, 8:2, pH 7.4) displayed an absorption band at 517 nm as a characteristic band of a BODIPY core. Meanwhile, upon excitation at 460 nm, the probe was nearly non-emissive ($\Phi_{F\ (BOD\text{-}MRV)} = 0.035$, $\varepsilon_{517} = 6600\ M^{-1}\,cm^{-1}$), which clearly indicates that quenching effect of palladium is remarkably strong when it is positioned at the 2-position of BODIPY core. Nonetheless, the systematic addition of CORM-2 to the solution significantly elevated the intensity of the emission band at 537 nm in linear fashion and across a wide range of concentrations (0–500 μM), and within 120 min the probe emission intensity plateaued after rising by more than 60-fold when 50 equiv. of CORM-2 was added. Using analytical data collected from fluorescence measurements, we determined the limit of detection to be 263 nM provided that signal-to-noise ratio (S/N) equals 3 (see Supporting Information).

The selectivity test for BOD-MRV with biological relevant analytes including anions, reactive oxygen species and sulfur species (HOCl, H_2O_2, OH, BuOOH, NO^-, NO_2^-, NO_3^-, ROO^-, O_2^- and H_2S), revealed no obvious spectral changes and thus indicated the probe's explicit selectivity towards CO (Figure 2).



Scheme 2. Synthesis of BOD-MRV.

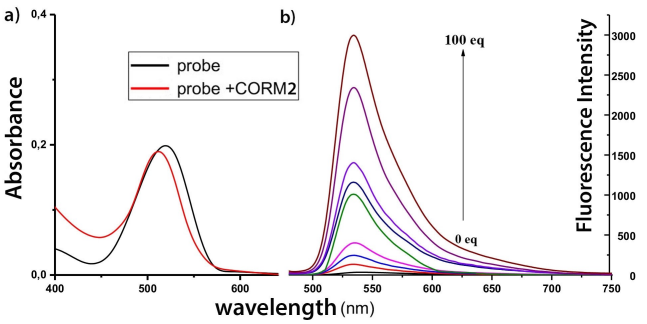


Figure 1. (a) Absorbance spectra of BOD-MRV in the absence and presence of 50 equiv. CORM-2 (250 μM). (b) Fluorescence spectra of BOD-MRV (5 μM) with increasing concentrations of CORM-2 in PBS: CH_3CN (pH 7.4, v/v,8:2), (λ_{ex}: 460 nm).

Prof. Dr. Mustafa Emrullahoğlu received his doctorate in 2009 from Middle East Technical University (METU, Turkey) under the supervision of Prof. Dr. Ayhan S. Demir. In 2010, he started his independent academic career with a tenure position at İzmir Institute of Technology (IZTECH), where he launched into research areas at the interface of Organic, Inorganic, Biological and Physical Chemistry. In 2022, he joined the Photonics Department at IZTECH as a full Professor. His research focuses on finding new ways for manipulating photo-physical processes to obtain useful signals for fluorescence sensing and imaging applications. He has been actively involved in the development of new families of multi-halogenated and metal-based photosensitizers for Photodynamic Therapy (PDT).

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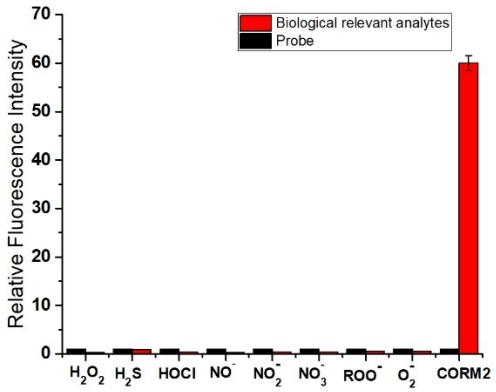


Figure 2. Fluorescence intensities of BOD-MRV (5 μM) in PBS:CH_3CN (pH 7.4, v/v, 8:2), emission at 537 nm: in the presence of CORM-2 and other reactive species/anions (250 μM, 50 equiv.) (λ_{ex}: 460 nm).

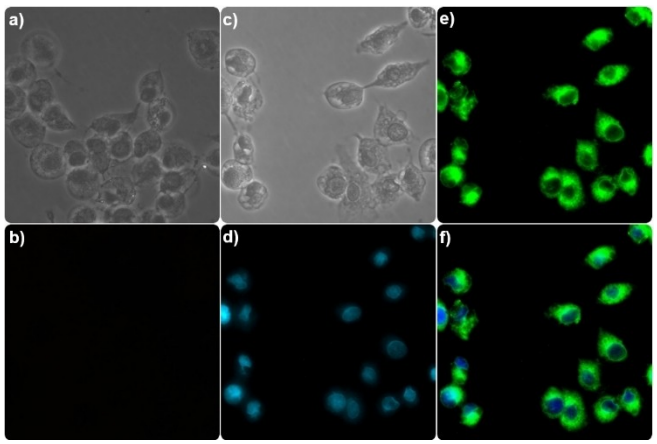
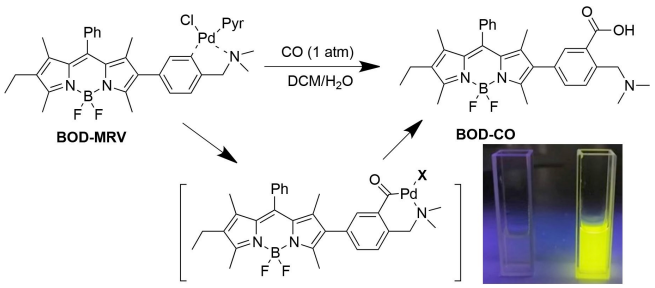


Figure 3. Fluorescence images of human lung adenocarcinoma cells (A549). (a) Bright field image of A549 cells treated with only BOD-MRV (4 μM); (b) fluorescence image of cells treated with only BOD-MRV (4 μM) (control); (c) bright field image of A549 cells treated with BOD-MRV (4 μM) and CORM-2 (120 μM); (d, e) fluorescence image of A549 cells treated with BOD-MRV (4 μM), DAPI and CORM-2 (120 μM); (f) merged images of frames (d and e). (λ_{ex}: 460 nm) scale bar: 100 μm.

In further investigations into how fluctuations in pH affected the fluorescence behavior of the sensing system, the probe was sufficiently stable in the pH range of 4.0–9.0, and BOD-MRV's response to the addition of CO remained unaffected when the pH of the sensing medium was in the physiological range (see Supporting Information).

To shed light on the sensing mechanism, the crude product formed by passing a stream of CO gas into the solution with the probe for 2 hours was subjected to HRMS. The green emissive product ($\Phi_{F\ (BOD\text{-}CO)} = 0.86$, $\varepsilon_{517} = 12900\ M^{-1}cm^{-1}$) was unambiguously confirmed as a carboxylated BODIPY derivative (HRMS: $[M+H^+]$: found, 530.2748, see Supporting Information) thereby unambiguously showing that fluorescence activation proceeded via CO-mediated *ortho*-carbonylation-depalladation, as aligns with past findings in the literature (Scheme 3).[33]

The outstanding performance BOD-MRV, including its unique specificity, low detection limit, low background fluorescence and high turn-on ratio, among other qualities, prompted us to assess its feasibility to detect CO released by CORM-2 in living cells. To that end, we first incubated A549 human lung adenocarcinoma cells with CORM-2 (120 μM) for 20 min and then with BOD-MRV (4 μM) for 20 min, after which we stained them with a nucleus staining dye, DAPI, for another 10 min and ultimately captured fluorescence images. As Figure 3 shows, the cells incubated with BOD-MRV did not display any fluorescence in the absence of CORM-2 but began to emit strongly after incubation with CORM-2. The results of such preliminary cell imaging suggest that the probe can permeate cell membranes and be efficiently used to image CO in living cells.



Scheme 3. Proposed mechanism for CO sensing. Inset: Probe's fluorometric response to CO (gas) or CORM-2.

Conclusion

In summary, we have reported the synthesis, spectral properties, and imaging applications of BOD-MRV, a new cyclopalladated BODIPY dye, which showed a turn-on fluorescence response towards CO. In an *ortho*-carbonylation/depalladation reaction sequence mediated by CO, the non-emissive probe structure rapidly transformed into a new structure by releasing palladium, a heavy atom, which allowed the recognition of CO as an increase in emission intensity. Apart from the sensitive (263 nM) response in solution with an exceptionally high turn-on ratio and very low detection limit, the probe proved highly successful in imaging CO in living cells. We thus believe that integrating heavy atoms to the 2-phenyl position of a BODIPY core can aid the efficient development of new probes for CO and other analytes of interest.

Experimental Section

All reagents were purchased from commercial suppliers (Acros Organics and Sigma-Aldrich) and used without further purification. All reactions were performed under argon atmosphere. ^1H NMR and ^{13}C NMR were measured on a Varian V NMRJ 400 Nuclear Magnetic Resonance Spectrometer. UV absorption spectra and fluorescence emission spectra were obtained on Horiba-Duetta fluorescence and absorbance spectrometer. Samples were measured in a 10.0 mm path length quartz cuvette (2.0 mL volume. Upon excitation at

460 nm, the emission spectra were integrated over the range 480 nm to 750 nm (Both excitation and emission slit width 5 nm/5 nm). Cell imaging was performed with Zeiss Axio Observer inverted fluorescence microscope. pH was recorded by HI-8014 instrument (HANNA). Mass spectra were recorded on Agilent 6530 Accurate-Mass QTOF LC/MS. All measurements were conducted at least in triplicate.

Synthesis of BOD. To a solution of kryptopyrrole (1,35 mL, 10.0 mmol) and Et_3N (1,95 mL, excess) in THF (10 mL) at room temperature was added benzoyl chloride (0,86 mL, excess) over few minutes. The mixture was heated under reflux for 6 h and cooled to room temperature. Triethylamine hydrochloride precipitate was removed by filtration and solvent was removed under vacuum. The resultant residue was purified by column chromatography with n-hexane-EtOAc (20:1) to afford as (4-ethyl-3,5-dimethyl-1H-pyrrol-2-yl(phenyl)methanone.[36] Phosphorous oxychloride (0.5 mL, 5 mmol) was added to a stirred solution of 4-ethyl-3,5-dimethyl-1H-pyrrol-2-yl)(phenyl)methanone (1.135 g, 5 mmol) in dichloromethane (25 mL) at room temperature and under argon atmosphere. After 30 min, 2,4-dimethylpyrrole (0.5 mL, 5 mmol) in dichloromethane (10 mL) was added, and the mixture was further stirred at 40 °C for 2 h. Et_3N (3.5 mL, 25 mmol) was then added and, after 15 min, boron trifluoride diethyl etherate was added (6 mL, 35 mmol). The mixture was stirred at room temperature for 2 h. The resultant residue was purified by column chromatography with hexane-ethyl acetate (30:1) to afford BOD (1.2 g, 68%) as orange solid.[37] ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.42–7.38 (m, 3H), 7.22–7.19 (m, 2H), 5.87 (s, 1H), 2.47 (s, 6H), 2.23 (q, 2H), 1.27 (s, 3H), 1.23 (s, 3H), 0.91 (t, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 155.54, 153.95, 142.05, 141.01, 139.49, 135.52, 133.60, 131.38, 131.04, 129.20, 128.95, 128.22, 120.67, 17.21, 14.69, 14.62, 14.41, 12.77, 11.83.

Synthesis of BOD-I. To a stirred solution of BODIPY (935 mg, 2.79 mmol) in 20 mL DCM, N-iodosuccinimide (1.57 g, 7 mmol) in 20 mL DCM was added partially at room temperature. After 2 hours, the reaction was extracted with DCM, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography with n-hexane-EtOAc (20:1) and afforded BOD-I (1.067 g, 80%) as red crystals. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.52–7.47 (m, 3H), 7.28–7.25 (m, 2H), 2.62 (s, 3H), 2.56 (s, 3H), 2.31 (q, 2H), 1.35 (s, 3H), 1.30 (s, 3H), 0.99 (t, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 157.99, 152.92, 141.87, 140.91, 140.49, 135.17, 134.65, 131.71, 130.46, 129.19, 129.08 127.99, 17.07, 16.47, 15.67, 14.36, 12.85, 11.87.

Synthesis of BOD-ALD. BOD-I (613 mg, 1.28 mmol) was dissolved in toluene (50 mL), EtOH (26 mL) and K_2CO_3 (2 M, 26 mL). 4-formylphenylboronic acid (480 mg, 3.2 mmol) and $Pd(PPh_3)_4$ (135 mg, 0.117 mmol) was added to this solution at RT under argon atmosphere and stirred for 40 minutes. The reaction mixture was refluxed for 0.5 hour at 80 °C. After completion of reaction, solvent was removed under vacuum and the resulting residue was extracted three times with DCM (3×30 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. The resultant residue was purified by column chromatography with n-hexane-EtOAc (20:1) to afford BOD-ALD (409 mg, 70% yield) as orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 10.01 (s, 1H), 7.90–7.87 (m, 2H), 7.52–7.46 (m, 3H), 7.34–7.30 (m, 4H), 2.59 (s, 3H), 2.53 (s, 3H), 2.33 (q, 2H), 1.32 (s, 3H), 1.29 (s, 3H), 1.01 (t, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 192.02, 157.50, 150.95, 141.43, 141.10, 140.46, 137.52, 135.47, 134.87, 134.51, 132.25, 131.35, 130.94, 130.52, 129.79, 129.36, 129.18, 128.18, 17.24, 14.60, 13.31, 12.97, 12.72, 12.00.

Synthesis of BOD-OH. To a stirred solution of BOD-ALD (390 mg, 0.85 mmol) in 40 mL THF, $NaBH_4$ (32 mg, 0.85 mmol) was added by one portion. After 90 minutes, solvent was removed under reduced pressure and the residue was purified by column chromatography with n-hexane-EtOAc (10:1) to afford BOD-OH (234 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.44–7.39 (m, 3H), 7.31–7.29 (m, 2H), 7.25–7.23 (m, 2H), 7.08–7.06 (m, 2H), 4.64 (s, 2H), 2.50 (s, 3H), 2.43 (s, 3H), 2.25 (q, 2H), 1.24 (s, 3H), 1.19 (s, 3H), 0.93 (t, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 155.96, 152.22, 141.19, 139.68, 139.48, 138.11, 135.66, 135.15, 133.81, 133.52, 131.73, 131.34, 130.52, 129.96, 129.27, 129.03, 128.23, 127.09, 65.30, 17.23, 14.69, 13.33, 12.84, 12.72, 11.91.

Synthesis of BOD-Br. BOD-OH (234 mg, 0.51 mmol) was dissolved in 25 mL DCM at 0 °C. To this solution, PBr_3 (51 μL, 0.51 mmol) was added dropwise within 10 minutes. After completion of the reaction, solvent was evaporated and the residue was purified by column chromatography with n-hexane-EtOAc (20:1) to afford BOD-Br (174 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.43–7.39 (m, 3H), 7.33–7.31 (m, 2H), 7.25–7.23 (m, 2H), 7.06–7.04 (m, 2H), 4.45 (s, 2H), 2. 50 (s, 3H), 2.43 (s, 3H), 2.25 (q, 2H), 1.24 (s, 3H), 1.19 (s, 3H), 0.93 (t, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 156.15, 151.79, 141.09, 139.70, 137.85, 136.14, 135.45, 134.28, 133.82, 131.98, 131.69, 130.56, 129.13, 128.94, 128.91, 128.06, 33.50, 17.08, 14.52, 13.20, 12.72, 12.57, 11.78.

Synthesis of BOD-AMINE. To a stirred solution of dimethylamine hydrochloride (135 mg, 1.65 mmol) in 30 mL DCM and 0.4 mL MeOH at RT, Et_3N (250 μL, 1.65 mmol) was added. BOD-Br (174 mg, 0.33 mmol) in 20 mL DCM was added to this solution. After 2.5 hours, solvent was removed under reduced pressure and the residue was purified by column chromatography with DCM-MeOH (20:1) mixture and afforded BOD-AMINE.[38] ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.49–7.44 (m, 5H), 7.31–7.29 (m, 2H), 7.17–7.15 (m, 2H), 3.82 (s, 2H), 2.56 (s, 3H), 2.51 (s, 6H), 2.49 (s, 3H), 2.31 (q, 2H), 1.30 (s, 3H), 1.25 (s, 3H), 0.99 (t, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 156.34, 151.78, 141.26, 139.89, 137.94, 135.56, 134.77, 133.99, 132.05, 131.96, 131.86, 130.74, 130.56, 130.19, 129.27, 129.06, 128.19, 62.60, 43.87, 17.21, 14.62, 13.32, 12.84, 12.70, 11.90.

Synthesis of BOD-Pd-OAc. To a solution of BOD-AMINE (179 mg, 0.37 mmol) in 10 mL benzene, $Pd(OAc)_2$ (89 mg 0.4 mmol) was added. The reaction mixture was sonicated for 1 minute, then it was stirred for 14 hours at 50 °C under argon atmosphere in dark. The reaction mixture was then cooled to room temperature and 40 mL hexane was added to obtain orange precipitate, BOD-Pd-OAc.[22] ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.50–7.43 (m, 3H), 7.31–7.29 (m, 2H), 6.86–6.84 (m, 1H), 6.76–6.73 (m, 2H), 3.70 (d, J = 14 Hz, 1H), 3.13 (d, J = 14 Hz, 1H), 2.79 (s, 3H), 2. 56 (s, 3H), 2.44 (s, 3H), 2.31 (q, 2H), 2.00 (s, 3H), 1.30–1.21 (m, 9H), 0.99 (t, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 180.97, 155.25, 152.68, 145.73, 143.82, 140.97, 139.30, 138.22, 135.72, 133.82, 133.60, 133.52, 131.53, 130.65, 130.24, 129.21, 129.17, 128.97, 128.28, 128.23, 126.39, 120.83, 72.33, 52.64, 51.46, 29.82, 24.64, 17.22, 14.70, 13.39, 12.73, 11.85.

Synthesis of BOD-Pd-Cl. The BOD-Pd-OAc was dissolved in acetone that was saturated with LiCl. The reaction mixture was stirred at room temperature for 4 h under argon atmosphere in dark. After completion of reaction, solvent was removed under vacuum and the resulting residue was dissolved in DCM and filtered through celite, solvent was removed under vacuum. Finally, the product was recrystallized with hexane to afford BOD-Pd-Cl as orange solid (89%).[22] ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.44–7.32 (m, 3H), 7.27–7.19 (m, 2H), 6.88–6.82 (m, 1H), 6.77 (d, J = 7.4 Hz, 1H), 6.65 (d, J = 7.5 Hz, 1H), 3.84 (s, 2H), 2.85–2.69 (m, 6H), 2.48 (s, 3H), 2.43 (s, 3H), 2.29–2.17 (m, 2H), 1.22 (s, 3H), 1.17 (s, 3H), 0.92 (t, J = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 154.74, 153.50, 145.80, 142.75, 140.97, 139.00, 138.59, 135.77, 134.45, 133.73, 133.29, 131.38, 130.75, 130.54, 129.17, 128.90, 128.30, 126.71, 121.13, 73.18, 29.83, 17.23, 14.71, 14.26, 13.56, 12.84, 12.73, 11.81.

Synthesis of BOD-MRV. To a stirred solution of BOD-Pd-Cl (48 mg, 0.077 mmol) in 9 mL DCM, pyridine (0.093 mmol, 7.5 μL) was added. The reaction was maintained 4 hours, under argon atmosphere in dark. After completion of the reaction, solvent was removed and the residue was crystallized with $CHCl_3$- n-hexane system.[33] ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.87–8.79 (m, 2H), 7.77 (tt, J = 7.7, 1.6 Hz, 1H), 7.48–7.41 (m, 3H), 7.31 (ddd, J = 7.6, 5.1, 1.4 Hz, 2H), 7.24–7.19 (m, 2H), 6.94 (d, J = 7.6 Hz, 1H), 6.73 (dd, J = 7.6, 1.6 Hz, 1H), 5.72 (d, J = 1.5 Hz, 1H), 3.97 (s, 2H), 2.92 (s, 6H), 2.51 (s, 3H), 2.35–2.22 (m, 5H), 1.25 (s, 3H), 1.13 (s, 3H), 0.96 (t, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 155.43, 153.61, 153.44, 152.12, 148.51, 146.01, 145.93, 140.77, 139.32, 137.83, 137.72, 135.50, 133.77, 133.53, 133.09, 131.42, 130.59, 130.38, 129.02, 128.84, 128.04, 126.39, 125.19, 121.10, 73.91, 52.79, 31.56, 17.05, 14.51, 13.28, 12.61, 11.70. HRMS: m/z: Calcd. for ($C_{35}H_{38}BClF_2N_4Pd$) [M + H$^+$]: 705.1959; found, 705.1907)

Cell Imaging. A549 Human Lung Adenocarcinoma cell lines were grown in DMEM supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 at 37 °C. The cells were plated on 12 mm cover glasses in 6-well plate and allowed to grow for 24 h. Before the experiments, the cells were washed with PBS buffer, and then the cells were incubated with CORM-2 (120 μM) for 20 min at 37 °C, then washed with PBS three times. After incubating with BOD-MRV (4 μM) for 20 min at 37 °C, cells were rinsed with PBS three times, and DAPI was added for 10 min at 37 °C, and it was washed with PBS three times. Then, the fluorescence images were acquired through a Zeiss Axio Observer inverted fluorescence microscope.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: BODIPY · Carbon monoxide · Chemosensors · Fluorescent probes · Palladacycles

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