Substituent Effects

Substituent Effects in BODIPY in Live Cell Imaging


Abstract: Small-molecule organoselenium-based fluorescent probes possess great capacity in understanding biological processes through the detection of various analytes such as reactive oxygen/nitrogen species (ROS/RNS), biothiols (cysteine, homocysteine and glutathione), lipid droplets, etc. Herein, we present how substituents on the BODIPY system play a significant part in the detection of biologically important analytes for in vitro conditions and live cell imaging studies. The fluorescence of the probe was quenched by 2-chloro and 6-phenyl selenium groups; the probe shows high selectivity with NaOCl among other ROS/RNS, and gives a turn-on response. The maximum fluorescence intensity is attained within \( \approx 1\text{ to } 2\text{ min} \) with a low detection limit (19.6 nm), and shows a \( \approx 110\)-fold fluorescence enhancement compared to signals generated for other ROS/RNS. Surprisingly, in live cell experiments, the probe specifically located and accumulated in lipid droplets, and showed a fluorescence turn-on response. We believe this turn-on response occurred because of aggregation-induced emission (AIE), which surprisingly occurred only by introducing one lipophilic mesityl group at the meso position of the BODIPY.

Introduction

Hypochlorous acid (HOCl) is a highly reactive oxygen species (ROS) produced in the body as a means of a defense mechanism against various pathogens and plays important roles in signaling and homeostatic control.[1] HOCl is well known as a main immune system-related chemical compound that has an effect on a broad range of microorganisms during antibacterial processes.[2] Endogenous HOCl is generated through the reaction between chloride ions (Cl\(^-\)) and hydrogen peroxide (\( \text{H}_2\text{O}_2 \)), which is catalyzed by myeloperoxidase (MPO) in leukocytes including macrophages, monocytes, and neutrophils.[3] It is also known as an oxidizing agent that can react easily with biological molecules such as thiols (cysteine, homocysteine and glutathione) and thiioethers (such as methionine). Hypochlorous acid is the most well-known bacterial oxidant to be produced by the neutrophil;[4] however, the antimicrobial agent property of HOCl also has a risk of damaging tissue through the same processes that are used to eliminate invading microorganisms.[5] Thus, an excess amount of HOCl in the body can cause several human diseases such as inflammatory diseases (e.g., arthritis),[6] hepatic ischemia-reperfusion,[7] atherosclerosis,[8] neuron degeneration and death (e.g., Alzheimer’s disease),[9] lung injury,[10] and cardiovascular diseases.[11]

Lipid droplets (LDs) are well known as ubiquitous organelles that preserve and provide neutral lipids such as triglycerides, sterol esters and retinyl esters in all eukaryotic and several prokaryotic cells.[12] LDs can be found in the cytoplasm of normal cells, such as adipocytes and cells that are involved in protein trafficking and protein maturation.[13] The size of LDs varies from 20 to 100 mm (in white adipocytes).[14] These lipid probes can be used for signaling, as well as membrane components, or as a source of energy.[15] LDs have a central core of hydrophobic (neutral) lipids that are surrounded by a single layer of amphipathic lipids and proteins to separate the aqueous and organic phases.[16] It is well known that lipid droplets play important roles in the immune system such as exchanging proteins with the nucleus in the cell and helping modulate protein stability. Furthermore, LDs have sites for the synthesis of eicosanoids, which are important for defense against pathogens and cancer. These properties allow LDs to act as entities that can modulate proteins to interact with binding partners, promote assemblies of protein complexes, preserve the damaged proteins before protein decay or protein delivery.[17] Lipid droplets can also separate healthy and toxic lipids in the cells. For example, LDs can turn over abundant fatty acids into triglycerides and incorporate them into LDs which are relatively inert, stable and harmless, to protect membrane integrity. This protective

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function is possibly the reason for the high concentration of LDs in many diseases characterized by the abnormal level of lipid supply and metabolism in such cells. To date, LDs are the hallmarks of several common human diseases, such as foam cells in atherosclerotic plaques, hepatic parenchymal cells in fatty liver, and obesity which occurs because the imbalance of the lipid consumption and lipid storage. In the cells, LD accumulation is heterogeneous even between identical cells, it is well known that intracellular and extracellular stresses trigger LD formation in the cells. Thus, accumulation of LDs can occur during progression of pathologies, which are related or not to lipids, such as cardiomyopathies, neuropathies, or during viral hepatitis caused by the human immunodeficiency virus (HIV). Aberrations in neutral lipid storage in LDs have been associated with the development and progression of several common metabolic diseases, including obesity, type II diabetes, atherosclerosis, and Chanarin–Dorfman syndrome (CDS). Despite these ties with diseases, LDs have received little attention and, as a result, our knowledge of these organelles is limited. Therefore, there is a significant interest in designing highly sensitive and selective probes for the detection of analytes such as ROS/RNS, biothiols, LDs, etc.

Detection methods such as potentiometric and amperometric coulometric titrations and amperometric methods have several drawbacks, such as high detection limits (LODs) and require relatively long procedures and expensive devices to obtain results. On the other hand, fluorescence techniques offer high sensitivity, a rapid response, non-invasiveness and can be used for in vivo biological cellular imaging. To-date, there are numerous fluorescent small-molecule probes that have been synthesized for the detection of HOCl; however, only a few of these can be used for real-time detection in biological investigations. Reasons for these limitations can be low selectivity, sensitivity, poor photo-stability and/or chemostability and high molecular weight of the probe. As a well-known and widely-used fluorophore, the BODIPY (boron-dipyrromethene) class has many compelling characteristics, including an intense absorption profile, a sharp fluorescence emission spectrum, negligible triplet-state formation, high fluorescence quantum yield, good photochemical stability, good tolerance to pH, stability to a variety of physiological conditions, and chemical robustness.

Thiol compounds (thioether, internal thioester, and thiosemicarbazide), oxidation-hydrolysis of dibenzylhydrazine into dibenzoyl diimide, para-methoxyphenol, oxime, dibenzylhydrazine hydroquinone, and chalcogenide (S, Se, and Te) systems, as well as some other groups, display high oxidation properties that can be used as HOCl reactive moieties. These HOCl-reactive moieties are embedded into the fluorophore to modulate the fluorescence intensity, in accordance with the observed HOCl concentration. Oxidation of an organoselenide and telluride to their corresponding oxides by HOCl offers a rapid response and the ability for reversibility upon addition of a biothiol; thus, it is a very reliable system as a probe, since it is based on the redox cycles of selenium and tellurium, which can provide dynamic, real time detection of HOCl with turn-on signaling.

In order to synthesize novel chalcogenide (Se, and Te)-containing fluorescent probes for the detection of biologically important species (e.g. biothiols, ROS/RNS, and metal ions), our research group has discovered novel heterocyclic annulated BODIPY systems for the detection of HOCl via oxidation of E to E = O (E = Se/Te); selenium and tellurium atoms helped form a six-membered heterocycle between the 1-position of BODIPY and the ortho position of the meso phenyl ring. Recently, we reported simple BODIPY with 2-chloro, and 6-phenyl selenium groups that can be used for real-time detection of HOCl in biological systems. Incorporating a Cl atom at the 2-position in the BODIPY framework helps further decrease background fluorescence and lowers the detection limit, compared to the probe in which a proton is at this position.

Herein, we present a meso mesitylene-BODIPY embedded with a chloro and phenyl selenide at the 2- and 6-positions, respectively, and the substituent effect on the BODIPY for the detection of biologically important analyte HOCl, which involves in vitro conditions and live cells imaging studies (Figure 1). The mesityl group increases the photostability because of its electron-donating ability; this is correlated with the steric hindrance established by the two ortho-substituted methyl groups inhibiting free rotation of the BODIPY core and

![Figure 1](image-url)
meso aryl group. Thus, restricting torsional motion decreases the rate of internal conversion to non-radiative decay. The effect is then increasing its fluorescence quantum yield. Based on our previous work, we introduced chlorine at the 2-position and phenyl selenide at the 6-position, to decrease the background fluorescence of \textit{Mes-BOD-SePh}. In the absence of HOCl, the fluorescence of \textit{Mes-BOD-SePh} is quenched, owing to a photo-induced electron transfer (PET) process by substitution of the heavy atom in the 2- and 6- positions, and shows negligible fluorescence and no decrease in quantum yield of the oxidized product. As expected, the \textit{Mes-BOD-SePh} exhibited large HOCl-induced fluorescence enhancement with a rapid response, high selectivity and a comparatively wide pH detection range under in vitro conditions. Surprisingly, exposing \textit{Mes-BOD-SePh} in live cells showed a turn-on response. Through careful studies, we have demonstrated that the lipophilic mesitylene-containing probe helps target into LDs and behaves with a turn-on response due to aggregation induced emission (AIE).

**Results and Discussion**

The synthesis of \textit{Mes-BOD-SePh} is outlined in Scheme 1. Commercially available 2,4,6-trimethylbenzaldehyde \textbf{1} was treated with 2,4-dimethylpyrrole in the presence of a catalytic amount of trifluoroacetic acid (TFA) under argon; subsequent oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and then triethylamine, and finally BF$_3$·OEt$_2$ afforded the BODIPY product \textbf{2}. Reaction of \textbf{2} with N-chlorosuccinimide (NCS) in hexafluoro-2-propanol (HFIP) as the solvent provided the monochlorination product \textbf{3}. A subsequent reaction with phenylselenyl chloride delivered the probe \textit{Mes-BOD-SePh}. The characterization of the structure of \textit{Mes-BOD-SePh} was performed by using multiple spectroscopic techniques (multinuclear NMR spectral data, mass spectrometry, IR; Figure S1–S19, Supporting Information).

Results from the study of UV/Vis and fluorescent spectroscopy with the probe are shown in Figure S20. The UV/Vis spectrum (3.0 μM solution of \textit{Mes-BOD-SePh} in EtOH/10 mM phosphate-buffered saline (PBS) pH 7.4, 1:2 v/v) showed an absorbance at 523 nm. However, upon addition of NaOCl (4.0 equiv) to the \textit{Mes-BOD-SePh} solution, the absorbance peak underwent a blue shift (towards lower wavelength) at 512 nm. The non-oxidized phenylselenium group at the 6-position of the BODIPY helped quench the fluorescence of the system by a photo-induced electron transfer (PET) process. Moreover, the \textit{Mes-BOD-SePh} showed a lower background fluorescence intensity because of the added effect from chlorine at the 2-position.

\textit{Mes-BOD-SePh} selectivity was checked by screening with ROS/RNS solutions (e.g., NaOCl, H$_2$O$_2$, tBuOOH, 'OH, tBuO, O$_2$·, NO, and ONOO·) under physiological pH (EtOH/10 mM PBS, pH 7.4, 1:2 v/v) in 3.0 μM of \textit{Mes-BOD-SePh} (Figure 2). An excellent selectivity of the \textit{Mes-BOD-SePh} is also shown in Figure 2. \textit{Mes-BOD-SePh} was found to be only selective to NaOCl (4.0 equiv) and there was no change in fluorescence intensity with other ROS/RNS, even at higher concentrations (up to 100 equiv). Addition of NaOCl to the probe gives a rise in the emission to form a maximum at 526 nm with enhancement of fluorescence intensity found to be ≈110-fold. The

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure2.png}
\caption{Selectivity of \textit{Mes-BOD-SePh} with ROS/RNS; a) under visible light; b) under UV-light, $\lambda_{em}$: 365 nm; c) Fluorescence emission spectra of \textit{Mes-BOD-SePh} (3.0 μM) with ROS/RNS (NaOCl, H$_2$O$_2$, tBuOOH, 'OH, tBuO, O$_2$·, NO, and ONOO·) in the solution (EtOH/10 mM PBS, pH 7.4, 1:2 v/v) incubated for 5.0 min, $\lambda_{ex}$: 512 nm, $\lambda_{em}$: 526 nm, slit width 1.5 nm/1.5 nm; Inset: solution of \textit{Mes-BOD-SePh} (3.0 μM) with ROS/RNS (NaOCl, H$_2$O$_2$, tBuOOH, 'OH, tBuO, O$_2$·, NO, and ONOO·) in the solution (EtOH/10 mM PBS, pH 7.4, 1:2 v/v).
\end{figure}
fluorescence quantum yield ($\Phi_F$) of the Mes-BOD-SePh was 0.0016, which is related to the incorporation of chlorine and phenylselenyl in the 2- and 6-positions, respectively. However, upon addition of NaOCl, the fluorescence quantum yield ($\Phi_F$) was significantly increased to 0.45 (average of three independent experiments). An interference study of NaOCl with other ROS/RNS was performed. The result suggested that the other ROS/RNS interference studies showed no other species gave observable emission properties for Mes-BOD-SePh (Figure S21, Supporting Information).

Next, titration experiments of Mes-BOD-SePh solutions at various concentrations of NaOCl (0 to 3.3 μM) were performed; the results showed the emission intensity of Mes-BOD-SePh solution gradually increased upon the addition of NaOCl from 0 to 3.3 equiv and was found to be linearly proportional to the concentration of NaOCl (Figure 3, inset shows triplicate experiments). The Mes-BOD-SePh emission intensity reached saturation after the addition of 2.3 equiv of NaOCl. Further studies showed that the detection limit of the probe ($3\sigma/k$, where $\sigma$ stands for standard deviation of 10 blank-sample measurements and $k$ is the slope of linear equation) was 19.6 nm (Figure S22, Supporting Information).

Further time-dependent studies of Mes-BOD-SePh were carried out with the addition of 4.0 equiv of NaOCl. This probe showed very fast responses upon the addition of NaOCl and gave a full strong fluorescence within 1–2 min (Figure 4). The results of the time-dependent experiments indicated that the oxidation of selenide to selenoxide by the addition of NaOCl was very fast and could be applied to real-time sensing of NaOCl at the cellular level. To demonstrate the effect of the pH, Mes-BOD-SePh was tested under a wide range of pH values (4 to 12). The pH studies show that the pH of the solution did not have any effect on the probe under acidic conditions (Figure S23, Supporting Information). Addition of 4.0 equiv of NaOCl to the Mes-BOD-SePh solution at various pH values showed that the Mes-BOD-SePh gave a strong emission intensity under acidic conditions; however, under very basic conditions (pH 11 and 12) the emission intensity decreased significantly. These results indicated that Mes-BOD-SePh could be used to measure NaOCl in the cancer cells, which is well known to have a higher concentration of HOCl compared to normal cells.

It was previously reported that hypochlorite oxidizes selenium to selenoxide rapidly in aqueous media, as well as in organic solvents. To confirm the sensing mechanism of NaOCl based on this hypothesis (oxidation of selenium to selenoxide), the $^1$H and $^{77}$Se NMR spectra of Mes-BOD-SePh before and after treatment with NaOCl were recorded. The $^1$H NMR spectrum showed a downfield shift of the phenyl protons from $\delta = 7.11$–$7.20$ ppm to $7.45$–$7.50$ and $7.61$–$7.63$ ppm (Figure S24, Supporting Information). Also, the $^{77}$Se NMR spectrum, after addition of NaOCl, showed a sharp peak at $\delta = 205.4$ ppm that disappeared, leading to a new concomitant peak at $\delta = 822.0$ ppm (Figure S25, Supporting Information). Also, HRMS data of compound 4 was recorded. The molecular formula of the oxidized compound 4 was $\text{C}_{42}\text{H}_{38}\text{BCIF}_2\text{N}_2\text{OSeNa}$ (m/z: calcd: 595.1014; found: 595.1022, Figure S26, Supporting Information). Also, the HRMS spectroscopic and HRMS results strongly support the formation of selenoxide (Scheme 2).

In order to understand the redox cycling capacity of selenium in the Mes-BOD-SePh, the solutions of 4 (oxidized with hypochlorite) were treated with biothiols (glutathione, N-acetyl-L-cysteine, homocysteine, and L-cysteine), which are well known for their ability to revert the selenium oxide species Se=O to

![Figure 3. Emission spectral changes of Mes-BOD-SePh (3.0 μM) with various concentrations of NaOCl (0–3.3 equiv) in solution (EtOH/10 mM PBS, pH 7.4, 1:2 v/v) incubated for 5.0 min, $\lambda_{em}$: 512 nm, $\lambda_{ex}$: 526 nm, slit width 1.5 nm/1.5 nm; Inset: Plot of emission intensity of Mes-BOD-SePh (3.0 μM) at different concentrations of NaOCl in the solution (EtOH/10 mM PBS, pH 7.4, 1:2 v/v).](image-url)

![Figure 4. Time-dependent emission spectral changes of Mes-BOD-SePh (3 μM) with 4.0 equiv of NaOCl in solution (EtOH/10 mM PBS pH 7.4, 1:2 v/v); $\lambda_{ex}$: 512 nm, $\lambda_{em}$: 526 nm.](image-url)

![Scheme 2. Reaction mechanism for the detection of NaOCl.](image-url)
its original reduced state. Figure S27 (Supporting Information) shows the reaction of the oxidized product 4 with biothiols after 1 and 3 h. The results obtained showed a significant decrease in fluorescence intensity with glutathione, l-cysteine, and N-acetyl-l-cysteine after 3.0 h of incubation. This result indicated that the Mes-BOD-SePh has reversibility, which is an important attribute for monitoring the dynamic variations of hypochlorite in living systems.

To confirm the photomechanism, density functional theory (DFT) geometry optimizations and time-dependent density functional theory (TDDFT) calculations were performed. The phenylselenium moiety was nearly perpendicular to the BODIPY core in its optimized structure of Mes-BOD-SePh (Figure S28, Supporting Information). However, electron transfer between the selenium center and the BODIPY core could be efficient because of the directly attached selenium on the 6-position of the mesitylene-BODIPY. Electronic distributions of HOMO and HOMO-1 of the Mes-BOD-SePh showed very similar features occupying both BODIPY and phenylselenium (Figure S29, Supporting Information). The electronic distributions on phenylselenium were assigned with quenched fluorescence because the largest oscillator strength of the Mes-BOD-SePh was found to have a value of $f = 0.5479$ from HOMO-1 to LUMO (CI = 62.1%) and HOMO to LUMO (CI = 30.2%, Table S1, Supporting Information). On the other hand, the electron transfer from phenylselenium to the BODIPY core of the oxidized probe (compound 4) could have occurred less often because the electronic distributions seen in the HOMO and LUMO levels were present on the BODIPY core only. The largest oscillator strength was $f = 0.3459$ (HOMO–LUMO with CI = 47.7%, HOMO-1–LUMO with CI = 47.5%). From these results, the fluorescence turn-on event could be explained by a blockage of the PET that would ordinarily exist between phenylselenium and the BODIPY core by oxidation of selenium.

Finally, to examine biological applications of Mes-BOD-SePh in live cell imaging, we chose human adipose stem cells for chemosensing. Under physiological conditions (pH 7.4, PBS buffer), the selective reaction of the probe with NaOCl gave a strong green fluorescence. When human adipose stem cells were treated with the probe, followed by a 30-min incubation, the cells were expected to be non-fluorescent. Surprisingly, we observed strong green fluorescence. Compared to our previous result (meso unsubstituted BODIPY which were non-fluorescent in cells),[32k] the rigid and lipophilic mesityl moiety at the meso position of BODIPY appeared to direct the strong green fluorescence. The observed green fluorescence could exist because of aggregation of the probe; by carefully examining fluorescence images, we found that the probe appeared to selectively target lipid droplets. The selective targeting of LDs is thought to occur because of the lipophilic mesityl moiety at the meso position of BODIPY. A turn-on fluorescence response was then achieved by an aggregation induced emission process. To confirm that our probe selectively goes into lipid droplets, and shows fluorescence by AIE, we treated cells with a commercially-available lipid droplet staining dye (BODIPY 505/515).[33k] Figure 5 shows the fluorescence exhibited by the probe was consistent with the LD staining BODIPY dye.

These results suggested that the mesityl group helped carry the probe into the LD; then AIE showed green fluorescence. Interestingly, here, a single heavy atom-containing group (–SePh) was not able to suitably quench the system. Therefore, we believe that sterically bulk can override chalcogen quenching, as seen in cellular imaging (optical signal). We are assuming that the Se was not oxidized, and that the –SePh group was not cleaved.

The fluorescence intensity for most of the traditional probes is highly dependent on and sensitive to the working concentration of the probes. Low concentration causes photobleaching after several scans, whereas very high concentrations weaken the fluorescence intensity by aggregation-caused quenching (ACQ). To check the impact of the concentration, human adipose stem cells were incubated at different concentrations of Mes-BOD-SePh (1, 2.5, 5, 10, 25 μM, Figure 6). After incubating for 30 min, dot-shaped LDs with bright green emissions were observed, and the strong fluorescence made the probe clearly distinguishable from the background data. These results demonstrate that Mes-BOD-SePh can be used at
a much wider concentration range to detect LDs than other reported probes.\textsuperscript{[37]}

Furthermore, cell viability testing was performed to confirm the biocompatibility of Mes-BOD-SePh. The human adipose stem cells were pre-incubated with various concentrations of Mes-BOD-SePh (1, 2.5, 5, 7.5, 10, 25, 50 \(\mu\)M). WST-1 cell proliferation assays were carried out after cells were pre-incubated in Mes-BOD-SePh for 2 h. As shown in Figure 7, there was no substantial decrease in cell viability when using Mes-BOD-SePh. These results revealed no significant cytotoxicity of Mes-BOD-SePh at concentrations of up to 50 \(\mu\)M for 2 h and helped gain a better understanding for their potential biological applications.

![Figure 7. Concentration-dependent WST-1 cell viability assays. Human adipose stem cells were pre-incubated with various concentrations of Mes-BOD-SePh for 2 h (bar graph data is an average of three experiments).](image)

The AIE feature of Mes-BOD-SePh was investigated in a THF/water mixture, and the measured fluorescence emission spectrum is shown in Figure 8. The Mes-BOD-SePh exhibited weak emission in THF and the emission intensity was almost the same up to a THF/water mixture of 3:7, and greatly enhanced in a THF/water mixture of 1:9. Since Mes-BOD-SePh is insoluble in water, it should have aggregated in an aqueous medium. The enhanced emission intensity was thus induced by aggregate formation, demonstrating the AIE nature of the probe in an aqueous medium. The aggregate of Mes-BOD-SePh showed green fluorescence at 526 nm.

**Conclusions**

Herein, we have studied the substituent effects at the meso position on the BODIPY framework. The synthesized phenyl selenide-based BODIPY (Mes-BOD-SePh) probe showed fast and selective turn-on response to hypochlorous acid over other ROS/RNS. The probes displayed excellent selectivity, sensitivity, as well as short time responses (\(\approx1–2\) min.) for hypochlorite in physiological pH. Mes-BOD-SePh showed a fluorescence enhancement of up to \(\approx110\)-fold, with detection limits at 19.6 nm. Incorporation of a mesityl group at the meso position of BODIPY framework not only increased the fluorescence enhancement and photostability, but also selectively carried the probe into the lipid droplets. This study demonstrated how a substituent effect on the fluorophore allowed for different behaviors of the probe under physiological conditions, such as in live cell imaging experiments.

**Experimental Section**

General considerations: All chemicals were used as received from commercial suppliers (Aldrich, Tokyo Chemical Industry). \(^{1}H, ^{13}C, ^{77}Se\) NMR spectra were acquired using a Bruker Avance 400 and an Agilent-VNMRS 600 MHz spectrometer. TMS and dimethyl selenide were used as external standards. ESI-MS was performed on a BRUKER microTOF-Q II by the research support staff at KAIST. A time-of-flight mass spectrometer was operated at a resolution of 20,000. Absorption spectra were measured using a JASCO V-550 spectrophotometer. Fluorescence measurements were carried out using a Shimadzu RF-5301pc spectrofluorophotometer.

**Synthesis of 2**

To a solution of 2,4,6-trimethylbenzaldehyde 1 (148 mg, 1.0 mmol, 1 equiv) in dry CH\(_2\)Cl\(_2\) (25 mL) were added 2,4-dimethylpyrrole (0.23 mL, 2.2 mmol, 2.2 equiv) and a catalytic amount of TFA (0.37 mL, 3.0 mmol) was added to the reaction mixture and stirred for another 10 min. BF\(_3\)·OEt\(_2\) (0.37 mL, 3.0 mmol) was added to the reaction mixture and stirred for an additional 3 h at room temperature. Then, the reaction was quenched with water (25 mL) and the aqueous layer was extracted with CH\(_2\)Cl\(_2\) (3×25 mL). The combined organic layers were washed with brine, dried (Na\(_2\)SO\(_4\)) and concentrated under vacuum. The crude reaction mixture was subjected to silica gel chromatography using hexane and CH\(_2\)Cl\(_2\) (3:1) to provide 2 as an orange solid (230 mg, 62%); M.P. = 121–122 °C; IR (CHCl\(_3\)); \(\tilde{\nu}\) = 2923, 2855, 2361, 2339, 2213, 1539, 1472, 1441, 1404, 1354, 1310, 1250, 1183, 1126, 1095, 1065, 1002, 911, 854, 821, 778, 733, 705 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)/TMS): \(\delta\) = 1.38 (s, 6H, H\(_{1,13}\)), 2.09 (s, 6H, H\(_{11,13}\)), 2.33 (s, 3H, H\(_{12}\)), 2.56 (s, 6H, H\(_{1,13}\)), 2.96 (s, 2H, H\(_{12}\)), 6.95 ppm (s, 2H, H\(_{11}\)); \(^{13}C\) NMR (100 MHz, CDCl\(_3\)): \(\delta\) = 13.5 (C\(_{1,13}\)), 14.7 (C\(_{12,13}\)), 19.6 (C\(_{1}\)), 21.3 (C\(_{9}\)), 120.9 (C\(_{8}\)), 129.1 (C\(_{10}\)), 130.7 (C\(_{7}\)), 131.2 (C\(_{11}\)), 135.0 (C\(_{12}\)), 138.7 (C\(_{2}\)), 141.8 (C\(_{3}\)), 142.4 (C\(_{4}\)), 155.2 ppm (C\(_{5}\)); \(^{19}F\) NMR (128.4 MHz, CDCl\(_3\)): \(\delta\) = 0.68 ppm (t, \(J_{F,F} = 34.9\) Hz);
Synthesis of 3

To a stirred solution of 2 (183 mg, 0.5 mmol) in hexafluoro-2-propa-

col (HFIP, 4 mL) was added N-chlorosuccinimide (73 mg, 0.55 mmol, 1.1 equiv). The reaction mixture was stirred at room temperature for 15 min (reaction monitored by TLC). Then, the sol-

vent was evaporated under reduced pressure and the residue was diluted with CH₂Cl₂ (30 mL). The organic layer was washed with water (2×10 mL), dried (Na₂SO₄) and concentrated under vacuum. The crude reaction mixture was subjected to silica gel column chromatography using hexane and CH₂Cl₂ (3:1) to afford 3 as an orange solid (160 mg, 80%). M.P. = 90–91 °C; IR (CHCl₃): ν = 2923, 2855, 2363, 2213, 1611, 1539, 1472, 1441, 1404, 1354, 1310, 1250, 1183, 1126, 1095, 1065, 1002, 911, 854, 821, 778, 733, 705 cm⁻¹; ¹H NMR (400 MHz, CDCl₃/TMS): δ = 1.37 (3H, H₂a), 1.39 (3H, H₂b), 2.08 (6H, H₁a), 2.34 (3H, H₂b), 2.57 (2H, H₁b), 6.01 (1H, H₃), 6.96 ppm (2H, H₂); ¹³C NMR (100 MHz, CDCl₃): δ = 10.7 (C₁), 12.3 (C₂), 13.7 (C₃), 149 (C₄), 19.6 (C₅), 21.3 (C₆), 121.2 (C₇), 121.8 (C₈), 128.3 (C₉), 129.2 (C₁₀), 130.8 (C₁₁), 131.5 (C₁₂), 135.0 (C₁₃), 135.6 (C₁₄), 139.0 (C₁₅), 142.2 (C₁₆), 144.3 (C₁₇), 149.8 (C₁₈), 157.7 ppm (C₁₉); ¹⁹F NMR (376.4 MHz, CDCl₃): δ = 0.51 ppm (t, J₉₋₈ = 34.1 Hz); ³¹P NMR (161.9 MHz, CDCl₃): δ = 26.2 ppm (δ, J₉₋₈ = 15.1 Hz); HRMS (ESI) calculated for C₂₃H₃₅BF₂ClC₁₅(N₃)Na+: m/z 423.1587, found: m/z 423.1587 [M+Na⁺].

Synthesis of Mes-BOD-SePh

To a stirred solution of 3 (100 mg, 0.25 mmol) in dry CH₂Cl₂ (10 mL) was added PhSeCl (48 mg, 0.25 mmol, 1.1 equiv). The reaction mixture was stirred at room temperature for 30 min (reaction moni-
tored by TLC). Then, the solvent was evaporated under reduced pressure and the crude reaction mixture was subjected to silica gel column chromatography using hexane and CH₂Cl₂ (3:1) to afford Mes-BOD-SePh as a red solid (123 mg, 89%). M.P. = 161–162 °C; IR (CHCl₃): ν = 2957, 2922, 2859, 2735, 1669, 1653, 1562, 1543, 1507, 1472, 1437, 1411, 1306, 1251, 1194, 1156, 1129, 1121, 1084, 1048, 981, 850, 807, 778, 757, 704 cm⁻¹; ¹H NMR (400 MHz, CDCl₃/TMS): δ = 1.40 (3H, H₂a), 1.50 (3H, H₂b), 2.09 (2H, H₁a), 2.34 (3H, H₃a), 2.61 (3H, H₃b), 2.67 (3H, H₄a), 6.97 (2H, H₁b), 7.11–7.20 ppm (m, 5H, H₂a, H₂b, H₃a, H₃b, H₄a); ¹³C NMR (100 MHz, CDCl₃): δ = 11.0 (C₁), 12.6 (C₂), 13.5 (C₃), 13.8 (C₄), 14.5 (C₅), 19.7 (C₆), 21.4 (C₇), 118.1 (C₈), 122.6 (C₉), 126.1 (C₁₀), 128.8 (C₁₁), 129.8 (C₁₂), 129.1 (C₁₃), 129.3 (C₁₄), 129.4 (C₁₅), 130.8 (C₁₆), 132.5 (C₁₇), 134.8 (C₁₈), 137.4 (C₁₉), 139.3 (C₂₀), 142.9 (C₂₁), 147.6 (C₂₂), 152.3 (C₂₃), 159.9 ppm (C₂₄); ⁷⁷Se-NMR (76.3 MHz, CDCl₃): δ = 205.4 ppm; ²⁵²⁷F NMR (128.4 MHz, CDCl₃): δ = 0.52 ppm (t, J₉₋₈ = 33.5 Hz); ¹⁹F NMR (376.4 MHz, CDCl₃): δ = –146.1 ppm (q, J₉₋₈ = 32.0 Hz); HRMS (ESI) calculated for C₃₉H₄₅BF₂ClC₁₅(N₃)SeNa+: m/z 579.1065, found: m/z 579.1052 [M+Na⁺].

Cell viability assays

The proliferation of hADSCs was determined by WST-1 assay kit (Roche) using the manufacturer’s protocols. The hADSCs pre-treat-

ted with the Mes-BOD-SePh at various concentrations (1, 2.5, 7.5, 10, 25, 50 μM) and control cells, were seeded with 6×10⁴ cells/well in 96-well plate (n = 6). The cells proliferation reagent WST-1 was added (10 μL) to each well and the resulting cells were incubating for 2 h at 37 °C, 5% CO₂. Absorbance values were measured using a VersaMax ELISA microplate ELISA reader at 450 nm and the refer-

cence wavelength was 590 nm.

DFT calculations

Optimized structures and HOMO–LUMO distributions of the states were estimated using DFT/TDDFT calculations using the Gaussian

09 program. The B3LYP functional with a 6-31g* basis set was used, and the 6-311g* basis set was used only for Se. All calculations were performed in the gas phase. Configuration interaction (CI) coefficients were obtained from the squares of the values.

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References
