Supporting Information

β-Allyl carbamate fluorescent probe for vicinal dithiol proteins

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l. Materials and Instruments

1. General.

All reagents and solvents used in this study were purchased from commercial sources unless otherwise noted. *Escherichia coli* thioredoxin (E-Trx), human thioredoxin (H-Trx) and the Cys35Ser mutant of thioredoxin (E-Trx C35S) from *E. coli* were prepared as described. UV−vis spectra were recorded from an UV−vis spectrometer (Evolution 200, Thermo Scientific). Fluorescence spectroscopic studies were performed with a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies). The slit width was 5 nm for both excitation and emission. The quantum yields (Φ) of AC-green with and without rBSA were determined on an FLS920 spectrometer (Edinburgh Instruments, U.K.) with λex = 400 nm. 1H and 13C NMR spectra were recorded on Bruker Advance 400. MS spectra were recorded on Bruker Daltonics esquire 6000 mass spectrometer. HRMS was obtained on Orbitrap Elite (Thermo Scientific). The fluorescence images of the cells were acquired on a Floid cell imaging station (life technology). The fluorescence images of zebrafishes were taken with a fluorescence microscope (Olympus BX51, Japan). All procedures for in vivo experiments were carried out in accordance with the guidelines of Lanzhou University (Guidance of the Care and Use of Laboratory Animals), and were approved by the Ethics Committee of Lanzhou University. BSA and HSA were purchased from Beyotime Biotechnology (Nantong, China). The cell lines used in this study, including Hep G2 cells, HeLa cells and PC12 cells, were purchased from Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). The reduced BSA (rBSA) and reduced HSA (rHSA) were prepared by incubating the proteins with large excessive TCEP at room temperature, and the excessive TCEP was removed by a Sephadex G-25 desalting column.

2. Experimental procedures.

General procedure for the spectral measurements

For the measurements of absorption or fluorescence, probes were dissolved in DMSO to obtain a stock solution, which were diluted with PBS buffer (10 mM, pH = 7.4, containing 0.5% DMSO) to the desired concentrations. In order to get the reduced proteins, all the proteins needed to be reduced before use. In the experiment, the proteins were reduced by TCEP, then the excess amount of TCEP was removed after the reduction by a Sephadex G-25 desalting column. Then the probes were added to the protein solutions for vicinal dithiols detection. Probes were incubated with different concentrations of analytes in PBS buffer at 37 °C, and then the absorption spectra and fluorescence spectra were recorded. The fold of fluorescence increase (F/F0) was normalized to the base fluorescence intensity of the probes.

Spectral Response of AC-green to rBSA

AC-green (2 μM) was incubated with the reduced BSA (2 μM) in PBS buffer at 37 °C. The emission spectra were scanned every 5 min for 120 min. To acquire the emission spectra of AC-green toward different concentrations of rBSA, AC-green (2 μM) was incubated with increasing concentrations of rBSA in PBS buffer at 37 °C for 90 min. In addition, rBSA (2 μM) was incubated with varying concentrations of PAO (0 μM, 2 μM, 5 μM, 10 μM, 20 μM, 40 μM) for 30 min, then the probe (2 μM) was added and incubated for another 90 min in PBS buffer at 37 °C. The fluorescence change at 475 nm was determined. The fold of fluorescence increase (F/F0) was normalized to the base fluorescence intensity of the probe.

pH-Dependent Fluorescence Response of AC-green to rBSA

The experiment was performed on two groups. AC-green (2 μM) with or without the rBSA (2 μM) was added to PB solution with different pH values (pH 5.0–9.0) and the mixtures were incubated at 37 °C for 90 min. The fluorescence intensity at 475 nm was collected under 400 nm excitation. Each experiment was repeated at least three times.

Selectivity of AC-green for VDPs
For this experiment, AC-green (2 μM) was incubated with various tested species at 37 °C for 90 min in PBS buffer. The tested species included Ala (Alanine), Asp (Asparagic acid), Gin (Glutamine), Asn (Asparagin), Ile (Isoleucine), Gly (Glycine), Met (Methionine), Val (Valine), Trp (Tryptophan), Lys (Lysine), Thr (Threonine), His (Histidine), Pro (Proline), Arg (Arginine), Gly (Glycine), Phe (Phenylanine), Leu (Leucine), ZnCl\(_2\), MgCl\(_2\), KCl, Cys, Hcy, GSH (100 equiv. of each); reduced E-Trx, reduced H-Trx, reduced lysozyme, rHSA and rBSA and non-VDP proteins (oxidized E-Trx, oxidized H-Trx, lysozyme, HSA, BSA) (0.28 mg/mL of each). After co-incubation, the fluorescence intensity of AC-green was measured at 475 nm with 400 nm excitation. Each experiment was repeated at least three times.

Cytotoxicity assay

The cytotoxicity of AC-green was evaluated with Hep G2 cells and HeLa cells by the MTT assay. 1×10\(^4\) cells were incubated with varying concentrations of AC-green in triplicate in a 96-well plate at 37 °C in a final volume of 100 μL. Cells treated with DMSO alone were used as controls. At the end of the treatment (20 h), 10 μL of MTT (5 mg/mL) was added to each well and incubated for an additional 4 h at 37 °C. An extraction buffer (100 μL, 10 % SDS, 5 % isobutanol, 0.1 % HCl) was added, and the cells were incubated overnight at 37 °C. The absorbance was measured at 570 nm on Multiskan GO (Thermo Scientific).

Fluorescence imaging in living cells

Hep G2 cells were seeded in 12-well plates at 2 × 10\(^4\) cells per well in 1 mL of growth medium, and then incubated with AC-green (10 μM) for 15 min at 37 °C. For control experiment, the cells were pretreated with PAO (30 μM) for 40 min and then incubated with AC-green (10 μM) for 15 min at 37 °C. After washing the cells with PBS buffer three times, the fluorescence imaged were acquired under a Floid cell imaging station microscope (Life Technology).

Fluorescence imaging in Zebrafishes

The 6-day-old zebrafishes were incubated with AC-green (10 μM) in E3 culture medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl\(_2\), 0.33 mM MgSO\(_4\)) for 20 min at 28 °C. For control experiment, the zebrafishes were pretreated with PAO (40 μM) for 40 min and then incubated with AC-green (10 μM) for 20 min at 28 °C. After that, the larvae were washed three times with E3 culture medium and anaesthetized using 0.01 % MS 222 (Sigma, USA). The fluorescence images were taken with a fluorescence microscope at 40 x magnification.

PD model preparation and fluorescence imaging

AC-green (2 μM) was incubated with the 6-OHDA (200 μM) in PBS buffer at 37 °C for 90 min. The result demonstrated that AC-green had no response to the 6-OHDA. Then PC12 cells (2 x 10\(^5\)) were seeded in 12-well plates and cultured in DMEM supplemented with 10 % FBS, 2 mM glutamine, penicillin (100 units/mL), streptomycin (100 units/mL) at 37 °C in a humidified atmosphere of 5 % CO\(_2\) overnight. The cells were treated with varying concentrations 6-OHDA for 12 h. Then AC-green (10 μM) was added and continued incubation for another 20 min. The cells were washed with PBS and visualized and photographed under Floid cell imaging station microscope.
**Fig. S1** Time course of the fluorescence intensity of **AC-green** (2 μM) in the presence of rBSA (2 μM) at 37 °C in PBS buffer, $\lambda_{\text{ex}}/\lambda_{\text{em}} = 400/475$ nm.

**Fig. S2** Dose-dependent of the fluorescence intensity of **AC-green** (2 μM) in the presence of different concentrations rBSA (2 μM) for 90 min in PBS buffer at 37 °C, $\lambda_{\text{ex}}/\lambda_{\text{em}} = 400/475$ nm.
**Fig. S3** Fluorescence intensity of AC-green (2 μM) upon mixing with different species at 37 °C for 90 min in PBS buffer. 1, blank; 2, Ala (Alanine); 3, Asp (Asparagic acid); 4, Gln (Glutamine); 5, Asn (Asparagin); 6, Ile (Isoleucine); 7, Gly (Glycine); 8, Met (Methionine); 9, Val (Valine); 10, Trp (Tryptophan); 11, Lys (Lysine); 12, Thr (Threonine); 13, His (Histidine); 14, Pro (Proline); 15, Arg (Arginine); 16, Gly (Glycine); 17, Phe (Phenylanline); 18, Leu (Leucine); 19, ZnCl$_2$; 20, MgCl$_2$; 21, KCl; 22, Cys (Cysteine); 23, Hcy (Homocysteine); 24, GSH (Glutathione) (100 equiv. of each); 25, E-Trx (Escherichia coli thioredoxin); 26, reduced E-Trx; 27, H-Trx (Human thioredoxin); 28, reduced H-Trx; 29, lysozyme; 30, reduced lysozyme; 31, HAS (Human serum albumin); 32, rHSA (Reduced human serum albumin); 33, BSA (Bovine serum albumin); 34, rBSA (Reduced bovine serum albumin); (0.28 mg/mL of each). λ$_{ex}$/λ$_{em}$ = 400/475 nm.

**Fig. S4** Cell viability estimated by MTT assay. Hep G2 cells and Hela cells were incubated with different concentration of AC-green for 24 h.
II. Chemical synthesis

Scheme S1 Synthesis of probes

Chemical synthesis

The synthetic routes were illustrated in Scheme S1. Compounds 1, 2 were prepared by adapting the published procedures.2

Synthesis of compound 3 Compound 2 (192 mg, 0.83 mmol) and triethylamine (0.12 mL, 0.86 mmol) was dissolved in dried dichloromethane (10 mL), then acryloyl chloride (0.14 mL, 1.66 mmol) was added at 0 °C under argon. After stirring for 6 h at room temperature, the reaction mixture was diluted with dichloromethane. The dichloromethane solution was washed with a saturated aqueous NaHCO₃ solution, dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure. The residue was purified by column chromatography to afford compound 3 as a pale yellow solid (75 %).

1H NMR (400 MHz, CDCl₃) δ: 8.69 (s, 1H), 8.02 (s, 1H), 7.32 (d, \(J = 8.80\) Hz, 1H), 6.63 (d, \(J = 6.40\) Hz, 1H), 6.51 (d, \(J = 2.32\) Hz, 1H), 6.41 (m, 1H), 6.28 (m, 1H), 5.803 (m, 1H), 3.41 (q, \(J = 7.09\) Hz, 4H), 1.21 (t, \(J = 7.08\) Hz, 6H);

13C NMR (400 MHz, CDCl₃) δ: 163.94, 159.61, 152.71, 149.47, 130.72, 128.83, 126.23, 118.79, 109.63, 108.23, 97.27, 44.76, 12.46; ESI-MS (m/z): 287.2 \([M + H]^+\).

Synthesis of compound 4 Under an argon atmosphere, a mixture of compound 3 (60 mg, 0.2 mmol), formaldehyde (44 μL, 0.6 mmol), 1,4-Diazabicyclo [2.2.2] octane (DABCO) (12 mg, 0.1 mmol) and 9 mL acetonitrile was stirred at 40 °C for 24 h. Then, the mixture was diluted by 150 mL water, neutralized by sodium bicarbonate and abstracted by ethyl acetate. The organic layer was dried by Na₂SO₄, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography to afford compound 4 as a yellow solid (30 %).

1H NMR (400 MHz, CDCl₃) δ: 9.09 (s, 1H), 8.65 (s, 1H), 7.31 (d, \(J = 8.80\) Hz, 1H), 6.64 (m, 1H), 6.52 (s, 1H), 6.09 (s, 1H), 5.70 (s, 1H), 4.49 (s, 1H), 4.48 (s, 1H), 3.42 (q, \(J = -7.10\) Hz, 4H), 2.52 (t, \(J = 5.80\) Hz, 1H), 1.21 (t, \(J = 7.08\) Hz, 6H);

13C NMR (400 MHz, CDCl₃) δ: 165.83, 159.67, 152.78, 149.47, 130.72, 128.83, 128.02, 126.23, 118.79, 109.63, 108.23, 97.27, 44.76, 12.46; ESI-MS (m/z): 317.5 \([M + H]^+\).

Synthesis of compound 5a Compound 4 (24 mg, 0.075 mmol) and acetic anhydride (9 μL, 0.09 mmol) was dissolved in 2 mL toluene. After stirring at room temperature for 6 h, the reaction mixture was diluted with dichloromethane. Then the dichloromethane solution washed with a saturated
aqueous NaHCO₃ solution, dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure. The product was purified by column chromatography to afford compound 5a as a yellow solid (65%). 1H NMR (400 MHz, CDCl₃) δ: 9.10 (s, 1H), 8.64 (s, 2H), 7.30 (d, J = 8.80 Hz, 1H), 6.63 (q, J = 6.32 Hz, 1H), 6.51 (d, J = 2.40 Hz, 1H), 6.15 (s, 1H), 5.78 (s, 1H), 4.90 (s, 2H), 3.42 (q, J = 7.09 Hz, 4H), 2.17 (s, 3H), 1.21 (t, J = 7.08 Hz, 6H); 13C NMR (400 MHz, DMSO-d₆) δ: 175.11, 169.70, 163.76, 158.40, 154.75, 143.51, 135.34, 134.20, 128.90, 122.86, 114.65, 112.47, 101.81, 68.04, 49.21, 25.81, 17.51; HRMS-ESI (m/z): calcd. for 359.1599 [M + H]+, found 359.1601 [M + H]+.

Synthesis of compound 5b Compound 4 (104 mg, 0.33 mmol) and triethylamine (55 μL, 0.39 mmol) was dissolved in 6 mL dried dichloromethane. Then the benzoyl chloride (46 μL, 0.39 mmol) was dropwise into the mixture solution. After stirring at room temperature for 12 h, the reaction mixture was poured into 10 mL water and extracted with dichloromethane. The combined organic layer was washed with brine. After drying with anhydrous Na₂SO₄, the solvent was evaporated under reduced pressure. The product was collected by column chromatography to afford compound 5b as a yellow solid (47%). 1H NMR (400 MHz, CDCl₃) δ: 8.66 (s, 1H), 8.63 (s, 1H), 8.12 (s, 1H), 8.11 (s, 1H), 7.57 (m, 1H), 7.48 (s, 1H), 7.46 (s, 1H), 7.31 (d, J = 8.80 Hz, 1H), 6.63 (q, J = 6.32 Hz, 1H), 6.51 (d, J = 2.40 Hz, 1H), 6.18 (s, 1H), 5.89 (s, 1H), 5.18 (s, 2H), 3.42 (q, J = 7.09 Hz, 4H), 2.11 (t, J = 7.10 Hz, 6H); 13C NMR (400 MHz, CDCl₃) δ: 165.91, 164.51, 159.64, 154.97, 149.57, 139.00, 133.32, 129.86, 122.86, 114.65, 112.47, 101.81, 68.04, 49.21, 25.81, 17.51; HRMS-ESI (m/z): calcd. for 421.1753 [M + H]+, found 421.1758 [M + H]+.

Synthesis of compound 5c Boc-glycine (83 mg, 0.47 mmol), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) (121 mg, 0.63 mmol) and 4-dimethylaminopyridine (DMAP) (60 mg, 0.47 mmol) was dissolved in 8 mL dichloromethane, the mixture was stirred at room temperature for 1 h until the starting material disappeared. Then compound 4 (100 mg, 0.32 mmol) was added to the solution. After stirring at room temperature for 12 h, the reaction mixture was poured into 10 mL water and extracted with dichloromethane. The combined organic layer was washed with brine. After drying with anhydrous Na₂SO₄, the solvent was evaporated under reduced pressure. The product was collected by column chromatography to afford compound 5c as a yellow semisolid (32%). 1H NMR (400 MHz, CDCl₃) δ: 8.58 (s, 1H), 8.44 (s, 1H), 7.31 (d, J = 8.80 Hz, 1H), 6.82 (s, 1H), 6.53 (s, 1H), 6.02 (s, 1H), 5.59 (s, 1H), 3.43 (q, J = 7.07 Hz, 4H), 2.79 (s, 2H), 1.62 (s, 2H), 1.40 (s, 9H), 1.21 (t, J = 7.06 Hz, 6H); 13C NMR (400 MHz, DMSO-d₆) δ: 170.09, 166.42, 159.59, 155.28, 152.89, 149.71, 136.96, 128.95, 126.45, 118.28, 109.69, 109.79, 97.36, 84.19, 81.41, 63.59, 44.79, 34.93, 28.23, 28.01, 12.46; HRMS-ESI (m/z): calcd. for 496.2047 [M + Na]+, found 496.2054 [M + Na]+.

Synthesis of compound 5d Triphosgene (310 mg, 1.1 mmol), aniline (300 μL, 3.3 mmol) and triethylamine (44 μL, 0.33 mmol) was dissolved in 40 mL dichloromethane, the mixture was stirred at 0 °C for 1 h. Then added compound 4 (1.0 g, 3.2 mmol) to triethylamine (440 μL, 3.3 mmol) for 12 h. The product was collected by column chromatography to afford compound 5d as a yellow semisolid (79%). 1H NMR (400 MHz, CDCl₃) δ: 9.10 (s, 1H), 8.63 (s, 1H), 7.45 (s, 1H), 7.43 (s, 1H), 7.34 (d, J = 3.08 Hz, 1H), 7.32 (s, 1H), 7.31 (d, J = 4.00 Hz, 1H), 7.08 (t, J = 7.40 Hz, 1H), 6.66 (d, J = 7.88 Hz, 1H), 6.55 (s, 1H), 6.26 (s, 1H), 5.83 (s, 1H), 5.02 (s, 2H), 3.43 (q, J = 7.08 Hz, 4H), 2.12 (t, J = 7.08 Hz, 6H); 13C NMR (400 MHz, DMSO-d₆) δ: 165.29, 159.06, 153.76, 153.48, 150.09, 139.38, 139.32, 131.31, 129.51, 129.25, 123.87, 123.09, 118.73, 117.92, 109.94, 107.62, 96.98, 63.51, 44.49, 12.72; ESI-MS (m/z): 436.0 [M + H]+, HRMS-ESI (m/z): calcd. for 436.1867 [M + H]+, found 436.1875 [M + H]+.

Synthesis of compound 5e Compound 4 (108 mg, 0.34 mmol), triphenylphosphine (108 mg, 0.41 mmol) and carbon tetrabromide (136 mg, 0.41 mmol) was dissolved in 8 mL dichloromethane, the mixture was stirred at 0 °C for 6 h. Then remove the dichloromethane, the crude product was dissolved in 1 mL methanol, sodium methanesulfonate (40 mg, 0.2 mmol) was added to the solution. After stirring at room temperature for 12 h, the reaction mixture was poured into 20 mL water and extracted with dichloromethane. The combined organic layer was washed with brine. After drying
with anhydrous Na$_2$SO$_4$, the solvent was evaporated under reduced pressure. The product was collected by column chromatography to afford compound 5e as a yellow semisolid (43 %). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 8.58 (s, 1H), 8.54 (s, 1H), 7.31 (d, $J = 8.80$ Hz, 2H), 6.65 (d, $J = 6.65$ Hz, 2H), 6.53 (s, 1H), 6.24 (s, 1H), 6.09 (s, 1H), 4.13 (s, 2H), 3.42 (q, $J = 8.80$ Hz, 4H), 2.98 (s, 3H), 1.21 (t, $J = 7.10$ Hz, 6H); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$: 164.43, 159.55, 152.89, 149.72, 133.33, 128.90, 127.41, 126.33, 118.32, 109.74, 107.89, 97.35, 56.58, 44.82, 40.75, 12.46; HRMS-ESI (m/z): calcd. for 379.1320 [M + H]$^+$, found 379.1322 [M + H]$^+$.

**Synthesis of compound 5f** Compound 4 (108 mg, 0.34 mmol), triphenylphosphine (108 mg, 0.41 mmol) and carbon tetrabromide (136 mg, 0.41 mmol) was dissolved in 8 mL dichloromethane, the mixture was stirred at 0 °C for 6 h. Then remove the dichloromethane, the crude product was dissolved in 1 mL methanol, sodium-4-methylbenzenesulfonate (31 mg, 0.17 mmol) was added to the solution. After stirring at room temperature for 12 h, the reaction mixture was poured into 20 mL water and extracted with dichloromethane. The combined organic layer was washed with brine. After drying with anhydrous Na$_2$SO$_4$, the solvent was evaporated under reduced pressure. The product was collected by column chromatography to afford compound 5f as a yellow semisolid (32 %). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 8.42 (s, 1H), 8.27 (s, 1H), 7.79 (s, 1H), 7.77 (s, 1H), 7.32 (s, 1H), 7.29 (s, 1H), 7.25 (s, 1H), 6.63 (d, $J = 6.48$ Hz, 2H), 6.51 (s, 1H), 6.07 (s, 1H), 5.76 (s, 1H), 4.23 (s, 2H), 3.42 (q, $J = 7.08$ Hz, 4H), 2.34 (s, 3H), 1.21 (t, $J = 7.08$ Hz, 6H); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$: 164.30, 159.41, 152.81, 149.63, 145.12, 135.13, 133.68, 129.85, 128.79, 128.71, 126.78, 126.07, 118.37, 109.68, 107.93, 97.30, 58.12, 44.77, 21.59, 12.46; HRMS-ESI (m/z): calcd. for 455.1629 [M + H]$^+$, found 455.1635 [M + H]$^+$.

**Synthesis of compound 6 and 7** Compound 5d (440 mg, 1 mmol) and 2-mercaptoethanol (7.8 g, 100 mmol) was dissolved in 100 mL THF/H$_2$O (1:1), the mixture was stirred at room temperature for 30 min and extracted with dichloromethane. After drying with anhydrous Na$_2$SO$_4$, the solvent was evaporated under reduced pressure. The product was collected by column chromatography to afford compound 6 and 7. Compound 6: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 8.86 (s, 1H), 8.68 (s, 1H), 7.32 (d, $J =8.40$ Hz, 1H), 6.65 (d, $J = 6.40$ Hz, 1H), 6.52 (s, 1H), 6.00 (s, 1H), 5.72 (s, 1H), 3.84 (m, 2H), 3.59 (s, 2H), 3.42 (q, $J = 7.08$ Hz, 4H), 2.76 (t, $J = 11.3$ Hz, 2H), 1.25 (s, 1H), 1.21 (t, $J = 7.08$ Hz, 6H); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$: 165.54, 159.78, 152.77, 149.56, 140.07, 128.85, 126.48, 122.90, 118.72, 109.69, 108.16, 97.29, 60.96, 44.77, 34.75, 33.21, 12.47; ESI-MS (m/z): 377.3 [M + H]$^+$. Compound 7: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 8.86 (s, 1H), 8.65 (s, 1H), 7.29 (s, 1H), 6.63 (d, $J = 6.44$ Hz, 1H), 6.50 (d, $J = 11.2$ Hz, 1H), 3.83 (m, 4H), 3.41 (q, $J = 7.09$ Hz, 4H), 2.94 (m, 2H), 2.82 (m, 4H), 2.72 (m, 4H), 1.21 (t, $J = 7.06$ Hz, 6H); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$: 172.18, 159.78, 152.80, 149.53, 128.81, 126.83, 118.68, 109.69, 108.19, 97.22, 61.47, 48.72, 44.75, 35.77, 33.99, 12.47; ESI-MS (m/z): 456.4 [M + H]$^+$.

### III. REFERENCES


IV. Supporting figures

Fig. S5 $^1$H NMR Spectrum of compound 3 in CDCl$_3$ (400 MHz).

Fig. S6 $^{13}$C NMR Spectrum of compound 3 in CDCl$_3$ (400 MHz).
Fig. S7  ESI-Mass spectrum of compound 3 (ESI-MS).
Fig. S8 ¹H NMR Spectrum of compound 4 in CDCl₃ (400 MHz).

Fig. S9 ¹³C NMR Spectrum of compound 4 in CDCl₃ (400 MHz).
Fig. S10 ESI-Mass spectrum of compound 4 (ESI-MS).
**Fig. S11** $^1$H NMR Spectrum of compound 5a in CDCl$_3$ (400 MHz).

**Fig. S12** $^{13}$C NMR Spectrum of compound 5a in CDCl$_3$ (400 MHz).
**Fig. S13** HR-Mass spectrum of compound 5a.

**Fig. S14** $^1$H NMR Spectrum of compound 5b in CDCl$_3$ (400 MHz).
Fig. S15 $^{13}$C NMR Spectrum of compound 5b in CDCl$_3$ (400 MHz).

Fig. S16 HR-Mass spectrum of compound 5b.
Fig. S17 $^1$H NMR Spectrum of compound 5c in CDCl$_3$ (400 MHz).

Fig. S18 $^{13}$C NMR Spectrum of compound 5c in CDCl$_3$ (400 MHz).
Fig. S19  HR-Mass spectrum of compound 5c.

Fig. S20  $^1$H NMR Spectrum of compound 5d in CDCl$_3$ (400 MHz).
Fig. S21 $^{13}$C NMR Spectrum of compound 5d in CDCl$_3$ (400 MHz).
Fig. S22  ESI-Mass spectrum of compound 5d (ESI-MS).
Fig. S23 HR-Mass spectrum of compound 5d.

Fig. S24 $^1$H NMR Spectrum of compound 5e in CDCl$_3$ (400 MHz).
Fig. S25 $^{13}$C NMR Spectrum of compound 5e in CDCl$_3$ (400 MHz).

Fig. S26 HR-Mass spectrum of compound 5e.
**Fig. S27** $^1$H NMR Spectrum of compound 5f in CDCl$_3$ (400 MHz).

**Fig. S28** $^{13}$C NMR Spectrum of compound 5f in CDCl$_3$ (400 MHz).
Fig. S29  HR-Mass spectrum of compound 5f.

Fig. S30  $^1$H NMR Spectrum of compound 6 in CDCl$_3$ (400 MHz).
Fig. S31 $^{13}$C NMR Spectrum of compound 6 in CDCl$_3$ (400 MHz).
Fig. S32  ESI-Mass spectrum of compound 6 (ESI-MS).
Fig. S33  $^1$H NMR Spectrum of compound 7 in CDCl$_3$ (400 MHz).

Fig. S34  $^{13}$C NMR Spectrum of compound 7 in CDCl$_3$ (400 MHz).
Fig. S35  ESI-Mass spectrum of compound 7 (ESI-MS).