Supporting Information

Turn-on and Turn-off Fluorescent Probes for Carbon Monoxide Detection and Blood Carboxyhemoglobin Determination

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CONTENTS

presence of 200 μM of chemical species (indicated) and 1% CO balanced by airunder 5% MeCN in PBS. All data were taken after 30 min of incubation. Species used: (2~5) NH_4F, NaBr, NaI without $PdCl_2$, NaI; (6~10) Na_2S without $PdCl_2$, Na_2S, L-cysteine, Na_2SO_3, formalin; (11~15) NaCN, $NaNO_2$, HCOONa, KSCN, NaH_2PO_2; (16~20) $K_3[Fe(CN)_6]$, H_2O_2, NaClO, $NaNO_3$, NaN_3; (21~23) K_2CO_3, NaOAc, 1% CO in air.

Figure S13. Visual fluorescence features **(a)** of **MPVC-I** (10 μM) in the presence of 200 μM of cationic species (indicated) under standard condition; Visual fluorescence features **(b)** of **MPVC-I** (10 μM) in the presence of 200 μM of cationic species (indicated) and 1 atm of CO under standard condition. All data were taken after 30 min of incubation. Species used: (left to right) blank, $Cr(NO_3)_3$, $MnCl_2$, $FeCl_3$, $CoCl_2$, $NiCl_2$, $CuSO_4$, $Zn(OAc)_2$, $CdCl_2$, $HgCl_2$, gaseous CO.

Figure S14. Visual fluorescence features **(a)** of **MPVC-II** (2 μM) in the presence of 20 μM of $PdCl_2$ and 200 μM of cationic species (indicated) under 5% MeCN in PBS; Visual fluorescence features **(b)** of **MPVC-II** (2 μM) in the presence of 200 μM of cationic species (indicated) and 1% CO balanced by airunder 5% MeCN in PBS. All data were taken after 30 min of incubation. Species used: (left to right) blank, $Cr(NO_3)_3$, $MnCl_2$, $FeCl_3$, $CoCl_2$, $NiCl_2$, $CuSO_4$, $Zn(OAc)_2$, $CdCl_2$, $HgCl_2$, 1% CO in air.

4. Liquid Chromatography and Mass Spectrometry

5. Two-photon Excited Fluorescence (2PEF) Measurements

Figure S15. Irradiation power dependent 2PEF emission intensity (left) and emission spectra (right) upon 800 nm laser excitation of **(a, b)** Azidocarboylation resultant, **(c, d)** Carboxylation resultant of **MPVC-I** under standard condition.

Figure S16. Irradiation power dependent 2PEF emission intensity (left) and emission spectra (right) upon 800 nm laser excitation of **(a, b) MPVC-I** (10 μM), **(c, d) MPVC-II** (2 μM) in pH 7.4 1xPBS buffer.

Figure S17. Irradiation power dependent 2PEF emission intensity (left) and emission spectra (right) upon 800 nm laser excitation of **(a, b) MPVC**-COO^- (10 μM, pH 7.4), **(c, d) MPVC**-COOH (10 μM, pH 2.7).

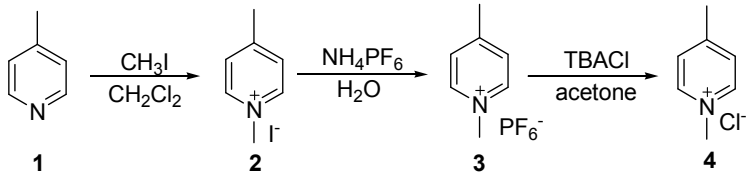
6. Quantum Yields and Two-photon Absorption Cross Sections of Samples

Table S2. Generalization of apparent quantum yields and two photon absorption cross sections. (**a~e**) refer to **MPVC-I** sensing system. **(a)**: absence of azide; **(c)**: 1 atm of CO for 30 min; **(d)**: absence of azide, 1 atm of CO for 30 min; **(e)**: solution **(d)** acidified to pH 2.7; **(f) MPVC-II** (2 μM).

7. Cell Viability and Cytotoxicity Test

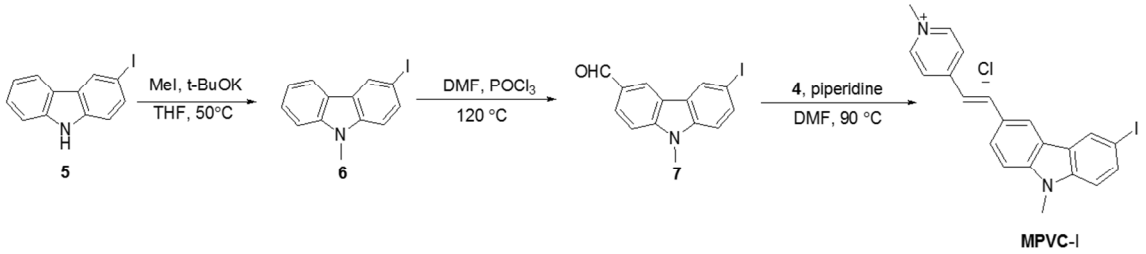
Figure S18. (a, b) Cell viability and **(c, d)** cytotoxicity test with varying concentrations of **MPVC-I** and **MPVC-II**.

1. Synthesis



Synthesis of 1,4-dimethylpyridinium iodide (2). 4-methylpyridine (**1**, 2.0 mL, 21 mmol) was slowly added iodomethane (2.0 mL, 32 mmol) at room temperature with stirring. The mixed liquids immediately became yellow and solidified. After 5 minutes the mixture was added hexane, sonicated and the solid was collected by filtration before subjection to vacuum drying. The off-white oily solid product was used without further purification.

Synthesis of 1,4-dimethylpyridinium chloride (4). 4-methylpyridine (**1**, 2.0 mL, 21 mmol) was slowly added iodomethane (2.0 mL, 32 mmol) at room temperature with stirring. The mixed liquids immediately became yellow and solidified. After 5 minutes the mixture was partitioned between hexane and water, and the water layer with **1,4-dimethylpyridinium iodide (2)** was subjected to addition of saturated $NH_4PF_{6(aq)}$ (30 mmol). The precipitated **1,4-dimethylpyridinium hexafluorophosphate (3)** was collected by filtration and washed with water. After drying in the air, **3** was dissolved in minimum amount of acetone and added saturated tetrabutylammonium chloride (TBACl) in acetone (30 mmol). The ionic liquid layer was washed with excess acetone and finally dried in vacuum to afford **4** as oily liquid, which solidified upon standing. In the above procedure, compound **2** and **3** were not further purified and chacterized. Yield: 2.4 g, 80%. **^{1}H NMR** (D_2O, 400MHz): δ 8.42-8.44 (d, 2H), 7.70-7.71 (d, 2H), 4.17 (s, 3H), 2.50 (s, 3H).



Synthesis of 3-iodo-9-methylcarbazole (6). Iodomethane (0.75 mL, 12 mmol) was slowly added to a solution of 3-iodocarbazole (**5**, 2.93 g, 10.0 mmol) and potassium *tert*-butoxide (1.68 g, 15.0 mmol) in THF at 60 °C. After stirring the mixture at 60 °C for 1 h, the residual solvent was evaporated under reduced pressure. The addition of water facilitated the precipitation of solids, which were subsequently collected via filtration and washed with cold methanol. Yield: >99%. Spectroscopic data were in agreement with literature values.[1] **^{1}H NMR** ($CDCl_3$, 400 MHz): 8.39 (s, 1H), 8.02-8.04 (dd, 1H), 7.70-7.73 (dd, 1H) 7.48-7.52 (t, 1H), 7.38-7.40 (d, 1H), 7.23-7.27 (t, 1H), 7.18-7.20 (d, 1H), 3.83 (s, 3H, -CH_3).

Synthesis of 3-formyl-6-iodo-9-methylcarbazole (7). Compound **6** (3.07 g, 10.0 mmol) was dissolved in DMF (7.25 mL) and $POCl_3$ (1.82 mL, 20.0 mmol) was slowly added at 0 °C. The resulting solution was stirred at 120 °C for 3 hours before quenched with ice water followed by extraction with EtOAc. The combined organic layer was washed by water and saturated Na_2CO_3 solution, and then dried with anhydrous Na_2SO_4. The resultant was finally purified by silica gel chromatography (Eluent: hexane/EtOAc = 2/1 in volume) to afford pale yellow powder. Yield: 2.07 g, 62 %. **^{1}H NMR** ($CDCl_3$, 400MHz): δ 10.09 (s, 1H, -CHO), 8.54 (s, 1H), 8.45 (s, 1H), 8.04-8.06 (d, 1H), 7.78-7.81 (d, 1H), 7.46-7.49 (d, 1H), 7.23-7.25 (d, 1H), 3.88 (s, 3H, -CH_3). **^{13}C NMR** ($CDCl_3$, 125.7 MHz), δ 191.5, 144.2, 140.8, 135.0, 129.5, 129.0, 127.5, 125.2, 124.1, 121.6, 111.1, 109.0, 83.0, 29.5. **LRMS** (ESI^+, acetone): *m/z* calcd. for $[M+H]^+$ 357.9699, found 358.468.

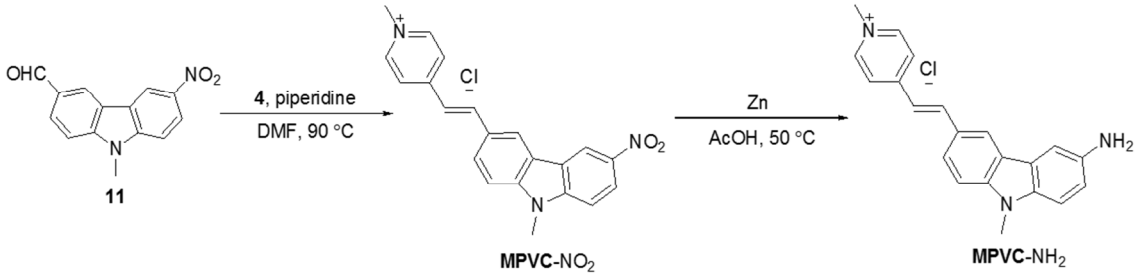
Synthesis of (E)-3-iodo-9-methyl-6-(2-(*N*-methyl-4-pyridinium)vinyl)carbazole chloride (MPVC-I). Compound **7** (167 mg, 0.500 mmol) and **4** (108 mg, 0.750 mmol)) were dissolved in 2 mL of DMF and stirred at 90 °C for 13 hours. The reaction mixture was diluted with acetone, centrifuged and the remaining orange solid was washed with acetone and EtOAc successively before dried in vacuum. Yield: 84.7 mg, 37%. **^{1}H NMR** (*d_6*-DMSO, 400MHz): δ 8.79-8.80 (d, 2H), 8.64 (s, 1H), 8.57 (s, 1H), 8.13-8.17 (m, 3H), 7.88-7.91 (d, 1H), 7.77-7.79 (d, 1H), 7.70-7.72 (d, 1H), 7.53 (s, 1H), 7.49-7.51 (d, 1H), 4.23 (s, 3H), 3.89 (s, 3H). **^{13}C NMR** (*d_6*-DMSO, 125.7

MHz), δ 153.4, 145.3, 142.5, 142.4, 140.9, 134.7, 129.3, 127.5, 127.1, 126.6, 125.0, 123.3, 121.8, 121.7, 120.8, 112.8, 110.6, 83.2, 47.1, 29.8. **LRMS** (ESI^+, H_2O): *m/z* calcd. for $[M\text{-}Cl]^+$ 425.0509, found 426.064.

Synthesis of 9-methylcarbazole (9). Carbazole (**8**, 8.8 g, 50 mmol) and potassium *tert*-butoxide (8.4 g, 75 mmol) were dissolved in THF at 50 °C, and then iodomethane (3.7 mL, 60 mmol) was added. The mixture was kept at this temperature for 2 hours before most of solvents were evaporated under reduced pressure and water added. The solid was collected by filtration and washed with cold methanol. Yield: 9.0 g, 99%. **^{1}H NMR** ($CDCl_3$, 400MHz): δ 7.94-7.97 (m, 2H), 7.31-7.35 (m, 2H), 7.07-7.11 (m, 2H), 3.69 (s, 3H, CH_3).

Synthesis of 9-methylcarbazole-3-carbaldehyde (10). Compound **9** (1.8 g, 10 mmol) was dissolved in 7 mL of DMF and slowly added $POCl_3$ (1.8 mL, 20 mmol) at 0 °C. The mixture was heated up to 125 °C for 3 hours before cooled down and quenched with cold water. After neutralized with aqueous NaOH, the mixture was extracted with EtOAc multiple times and the organic combination was washed with saturated aqueous $NaHCO_3$. The organic layer was dried with anhydrous Na_2SO_4, evaporated and purified by silica-gel column chromatography (eluent: EtOAc/hexane). Yield: 2.1 g, quantitative. **^{1}H NMR** ($CDCl_3$, 400MHz): δ 10.06 (s, 1H, CHO), 8.58 (s, 1H), 8.08-8.10 (d, 1H), 7.93-7.95 (d, 1H), 7.55-7.58 (m, 1H), 7.43-7.46 (m, 2H), 7.33-7.35 (d, 1H), 3.88 (s, 3H, CH_3).

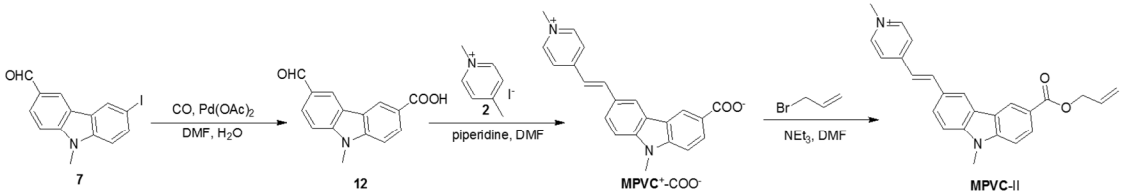
Synthesis of 9-methyl-6-nitrocarbazole-3-carbaldehyde (11). Compound **10** (2.09 g, 10.0 mmol) was dissolved in 15 mL of AcOH in ice bath and fuming nitric acid (0.478 mL, 11.0 mmol) in AcOH (5 mL) was slowly added. Then the reaction was brought to room temperature and stirred for 1 hour. Water was added and the resulting solid was collected by filtration, followed by washing with water, ethanol and EtOAc. Yield: 1.9 g, 76%. **^{1}H NMR** ($CDCl_3$, 400MHz): δ 10.16 (s, 1H, CHO), 9.09-9.10 (d, 1H), 8.68-8.69 (d, 1H), 8.46-8.49 (dd, 1H), 8.14-8.16 (dd, 1H), 7.58-7.60 (d, 1H), 7.50-7.52 (d, 1H), 4.00 (s, 3H, CH_3).



Synthesis of (E)-3-nitro-9-methyl-6-(2-(*N*-methyl-4-pyridinium)vinyl)carbazole chloride (MPVC-NO_2). Compound **11** (127 mg, 0.500 mmol), **4** (72.0 mg, 0.500 mmol)) and 1 drop of piperidine were dissolved in 4 mL of DMF and stirred at 90 °C for 4 hours. The reaction mixture was added acetone, centrifuged and the remaining orange solid was washed with acetone and EtOAc successively before dried in vacuum. Yield: 100 mg, 53%. **^{1}H NMR** (*d_6*-DMSO, 400MHz): δ 9.17-9.18 (d, 1H), 8.86 (s, 1H), 8.83-8.84 (d, 2H), 8.37-8.40 (dd, 1H), 8.18-8.19 (d, 2H), 8.14-8.17 (d, 1H), 7.94-7.97 (dd, 1H), 7.83-7.83 (d, 1H), 7.80-7.81 (d, 1H), 7.56-7.60 (d, 1H), 4.25 (s, 3H), 3.99 (s, 3H). **^{13}C NMR** (*d_6*-DMSO, 125.7 MHz), δ 153.2, 145.4, 144.9, 143.7, 141.9, 140.9, 128.5, 128.4, 123.5, 123.1, 122.3, 122.2, 121.8, 121.8, 117.7, 111.5, 110.6, 47.2, 30.3. **LRMS** (ESI^+, H_2O): *m/z* calcd. for $[M\text{-}Cl]^+$ 344.1394, found 344.51.

Synthesis of (E)-3-amino-9-methyl-6-(2-(*N*-methyl-4-pyridinium)vinyl)carbazole chloride (MPVC-NH_2). Compound MPVC-NO_2 (100 mg, 0.264 mmol) was dissolved in AcOH/H_2O = 2/3 mixed solvent and zinc powder (52.0 mg, 0.792 mmol) was added in one portion. The reaction mixture was stirred at 50°C for 5 hours. After unreacted zinc filtered, the remainder was diluted with water, neutralized with $NH_3{\cdot}H_2O_{(aq)}$, precipitated with NH_4PF_6 and ion-exchanged back to Cl^- with TBACl in acetone. Yield: 61.0 mg, 66%. **^{1}H NMR** (*d_6*-DMSO, 400MHz): δ 8.74-8.76 (d, 2H), 8.34 (s, 1H), 8.18 (d, 1H), 8.12-8.14 (d, 2H), 7.55-7.57 (d, 1H), 7.47 (s, 1H), 7.43 (s, 1H), 7.28 (s, 1H), 6.85-6.88 (d, 1H), 5.03 (s, 2H, br), 4.20 (s, 3H), 3.80 (s, 3H). **^{13}C NMR** (*d_6*-DMSO, 125.7

MHz), δ 153.6, 145.0, 143.1, 142.7, 142.5, 134.8, 125.9, 125.5, 123.2, 123.0, 122.7, 121.8, 119.5, 116.2, 110.5, 110.0, 104.6, 47.0, 29.6. **LRMS** (ESI^+, H_2O): *m/z* calcd. for $[M\text{-}Cl]^+$ 314.1652, found 314.430.



Synthesis of 6-formyl-9-methylcarbazole-3-carboxylic acid (12). Compound **7** (1.99 g, 5.94 mmol), K_2CO_3 (3.28 g, 23.7 mmol) and $Pd(OAc)_2$ (13.3 mg, 59.4 μmol) were suspended in a mixture of 1 mL DMF and 1 mL water. The atmosphere was changed from air to nitrogen using a Schlenk line and then CO (from a balloon) was introduced. The resulting suspension was then stirred at 60 °C overnight. The insoluble materials that formed were removed by filtration and washed by 0.1 M NaOH solution. The filtrate was then combined with the mother liquid and then acidified to pH 1 with concentrated HCl. The precipitate was collected via filtration, washed with 0.1 M HCl and water successively, and dried under vacuum to afford a grey solid. Yield: 1.45 g, 96 %. **^{1}H NMR** (d_6-DMSO, 400MHz): δ 12.74 (bs, 1H, -COOH), 10.07 (s, 1H, -CHO), 8.89 (s, 1H), 8.88 (s, 1H), 8.11-8.14 (d, 1H), 8.03-8.05 (d, 1H), 7.80-7.82 (d, 1H), 7.73-7.76 (d, 1H), 3.97 (s, 3H, $-CH_3$). **^{13}C NMR** (d_6-DMSO, 125.7 MHz): δ 192.4, 168.2, 145.2, 144.4, 129.4, 128.3, 127.2, 125.1, 123.2, 123.0, 122.6, 122.4, 110.7, 110.2, 30.1. **LRMS** (ESI^+, acetone): *m/z* calcd. for $[M+Na]^+$ 276.0631, found 276.176.

Synthesis of (E)-3-carboxyl-9-methyl-6-(2-(*N*-methyl-4-pyridinium)vinyl)carbazole (MPVC$^+$-COO$^-$). Compound **12** (1.27 g, 5.00 mmol), **2** (1.29 g, 5.50 mmol) and 2 drops of pyperidine were dissolved in 5 mL of DMF and stirred at 80 for 6 hours. The reaction mixture was added acetone, centrifuged and the remaining red-orange solid was washed with dilute $NaOH_{(aq)}$ under sonication. The solid was collected from resulting suspension by centrifugation, washed with minimum amount of water and dried in vacuum. Yield: 1.10 g, 64 %. **^{1}H NMR** (d_6-DMSO, 400MHz): δ 8.79-8.80 (d, 2H), 8.69 (s, 1H), 8.61 (s, 1H), 8.16-8.22 (m, 3H), 8.07-8.09 (d, 1H), 7.82-7.85 (d, 1H), 7.65-7.68 (d, 1H), 7.55-7.59 (d, 1H), 7.44-7.46 (d, 1H), 4.23 (s, 3H), 3.90 (s, 3H). **LRMS** (ESI^+, H_2O): *m/z* calcd. for $[M+H]^+$ 343.1441, found 343.2.

Synthesis of (E)-3-allyloxycarbonyl-9-methyl-6-(2-(*N*-methyl-4-pyridinium)vinyl)carbazole bromide (MPVC-II). Compound **MPVC**$^+$-COO^- (0.500 mmol, 171 mg), allyl bromide (1.50 mmol, 181.5 mg) and (i-Pr)$_2$NEt (1.50 mmol, 258 μL) were dissolved in 2 mL of DMF and stirred at room temperature for 20 hours. The solution was added acetone and the precipitation was collected by centrifugation, washed with acetone and dried in vacuum to afford red-orange solid. Yield: 185 mg, 80%. The product was further purified by washing with acetonitrile and dried in vacuum (net yield 38%). **^{1}H NMR** (d_6-DMSO, 400MHz): δ 8.81-8.88 (m, 4H), 8.14-8.22 (m, 4H), 7.91-7.95 (m, 1H), 7.75-7.80 (t, 2H), 7.60-7.64 (d, 1H), 6.08-6.17 (m, 1H), 5.45-5.50 (d, 1H), 5.31-5.34 (d, 1H), 4.87-4.89 (d, 2H), 4.25 (s, 3H), 3.98 (s, 3H). **^{13}C NMR** (d_6-DMSO, 125.7 MHz), δ 165.8, 152.9, 144.8, 143.9, 142.6, 141.8, 133.0, 127.4, 122.8, 122.6, 122.3, 121.8, 120.8, 120.6, 117.9, 110.4, 109.7, 64.9, 46.7, 29.6. **LRMS** (ESI^+, H_2O): *m/z* calcd. for $[M\text{-}Br]^+$ 383.1754, found 383.3.

2. NMR Spectra

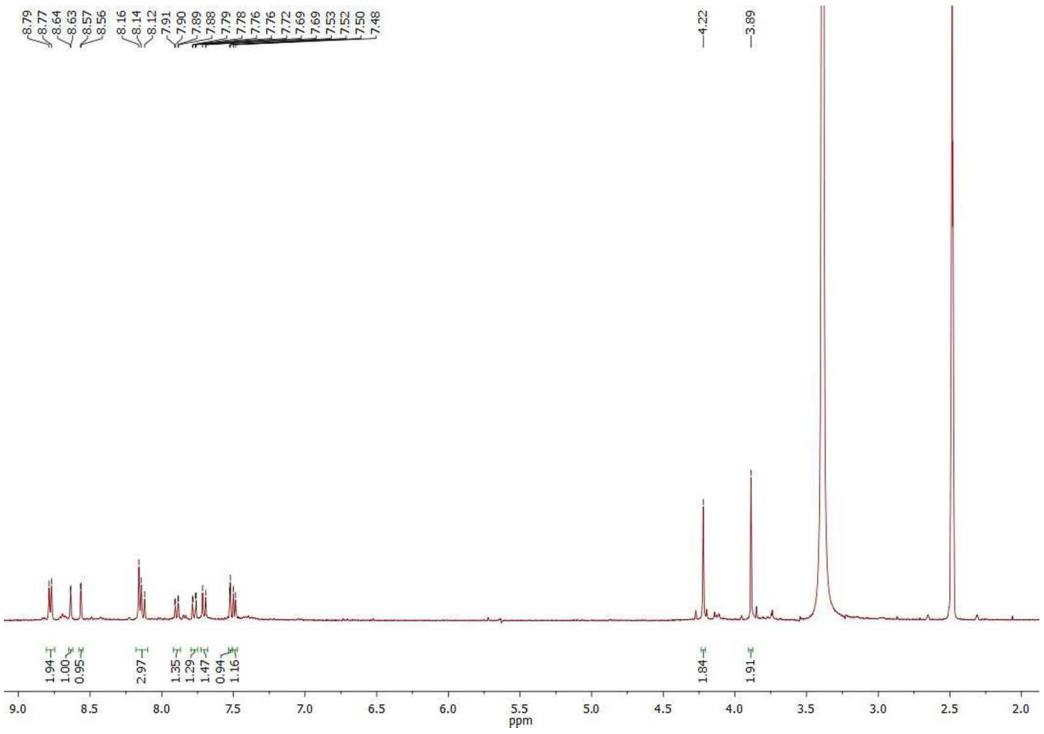


Figure S1. [1]H NMR spectrum of **MPVC**-I in d_6-DMSO.

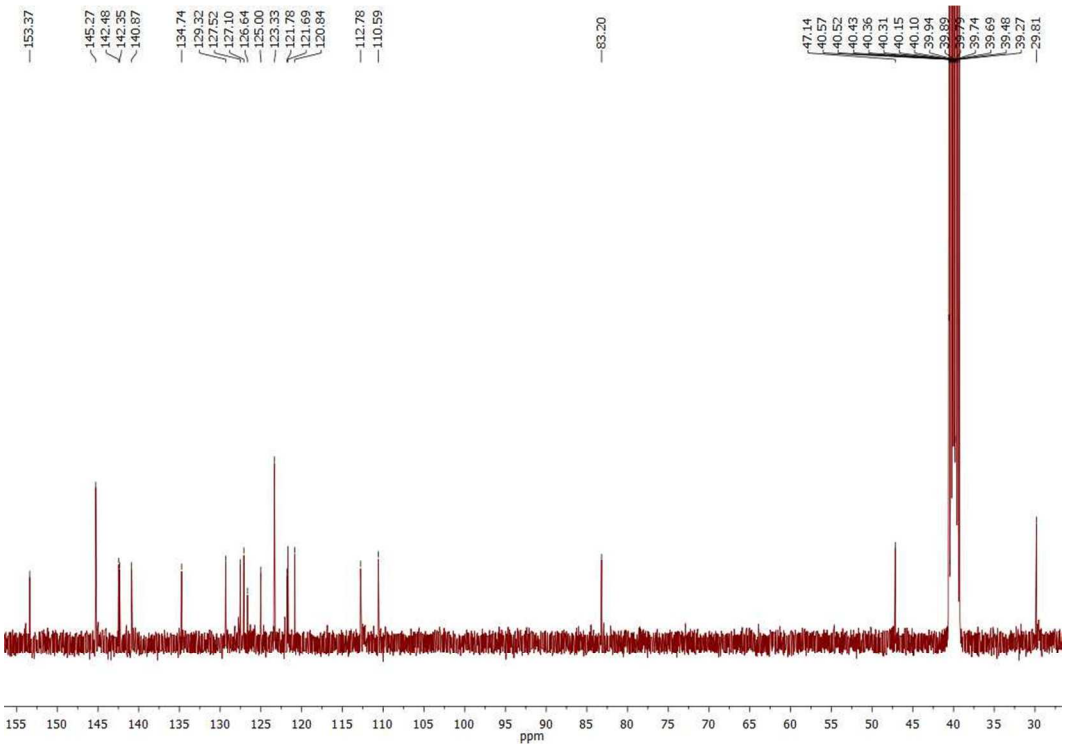


Figure S2. [13]C NMR spectrum of **MPVC**-I in d_6-DMSO.

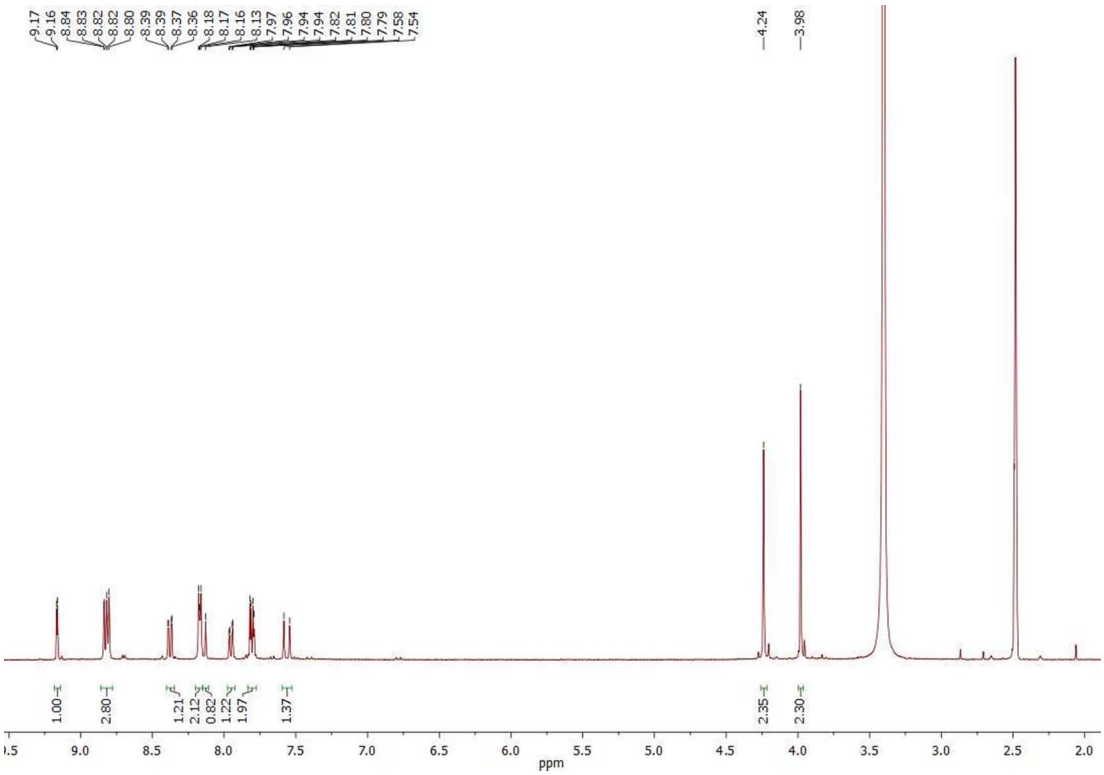


Figure S3. ^{1}H NMR spectrum of **MPVC**-NO_2 in d_6-DMSO.

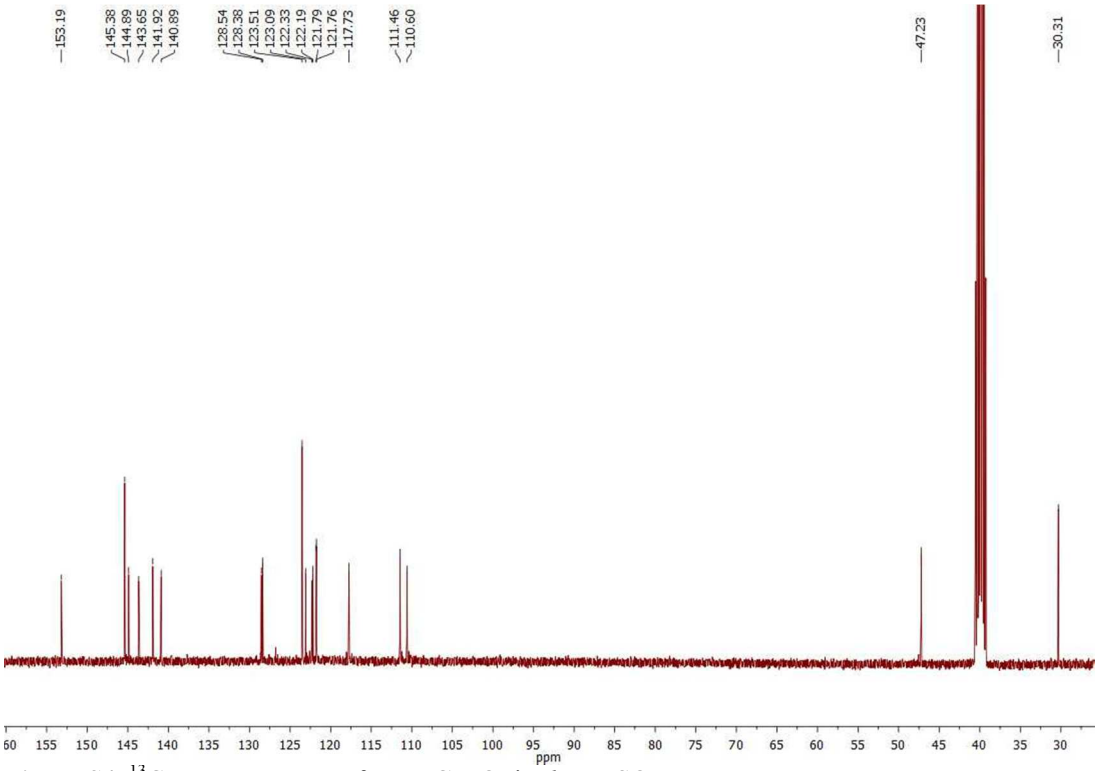


Figure S4. ^{13}C NMR spectrum of **MPVC**-NO_2 in d_6-DMSO.

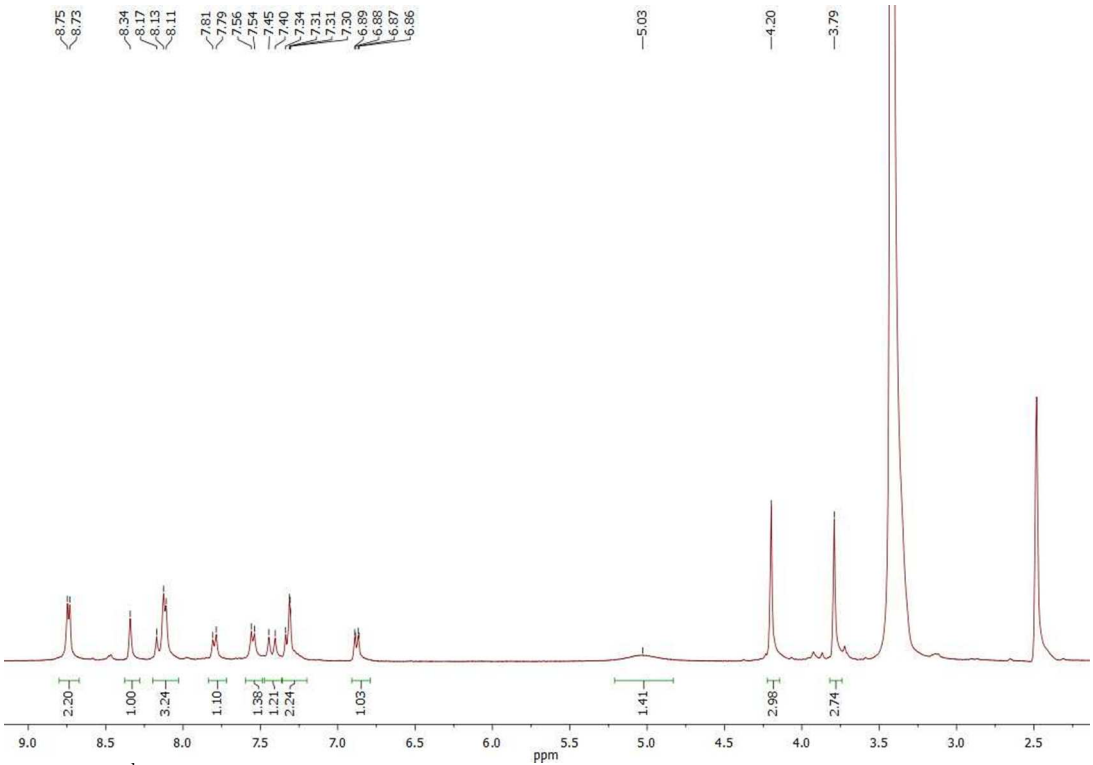


Figure S5. ^{1}H NMR spectrum of **MPVC**-NH_2 in d_6-DMSO.

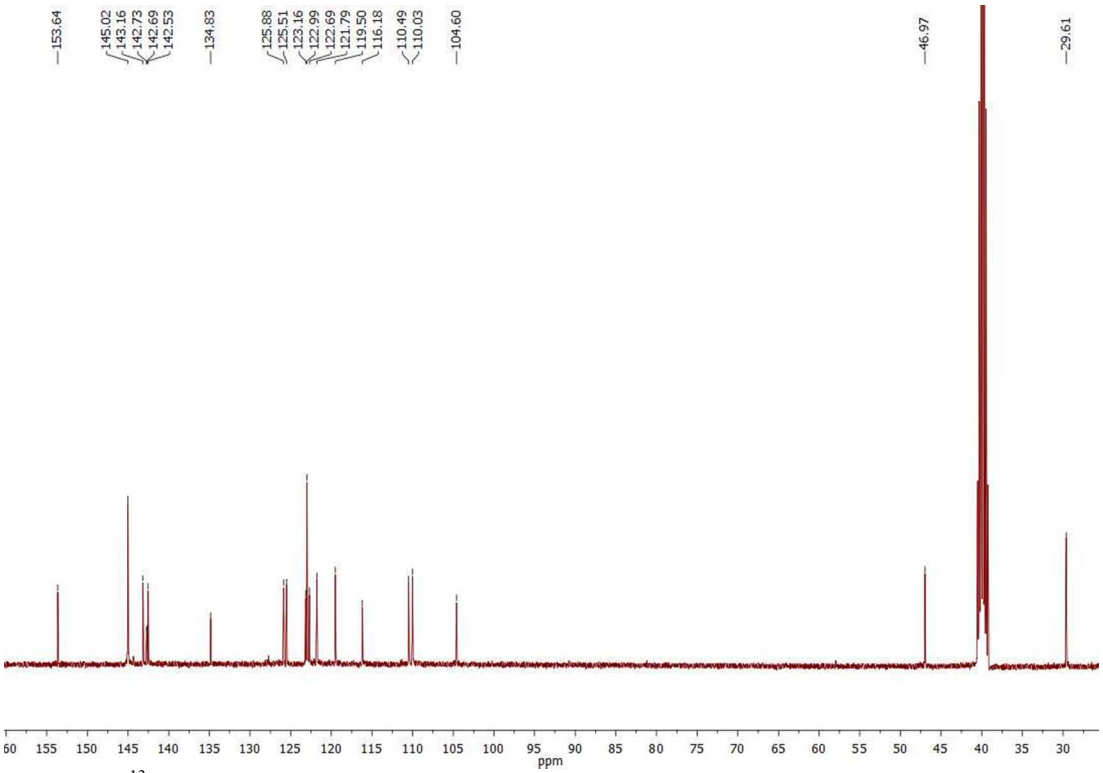


Figure S6. ^{13}C NMR spectrum of **MPVC**-NH_2 in d_6-DMSO.

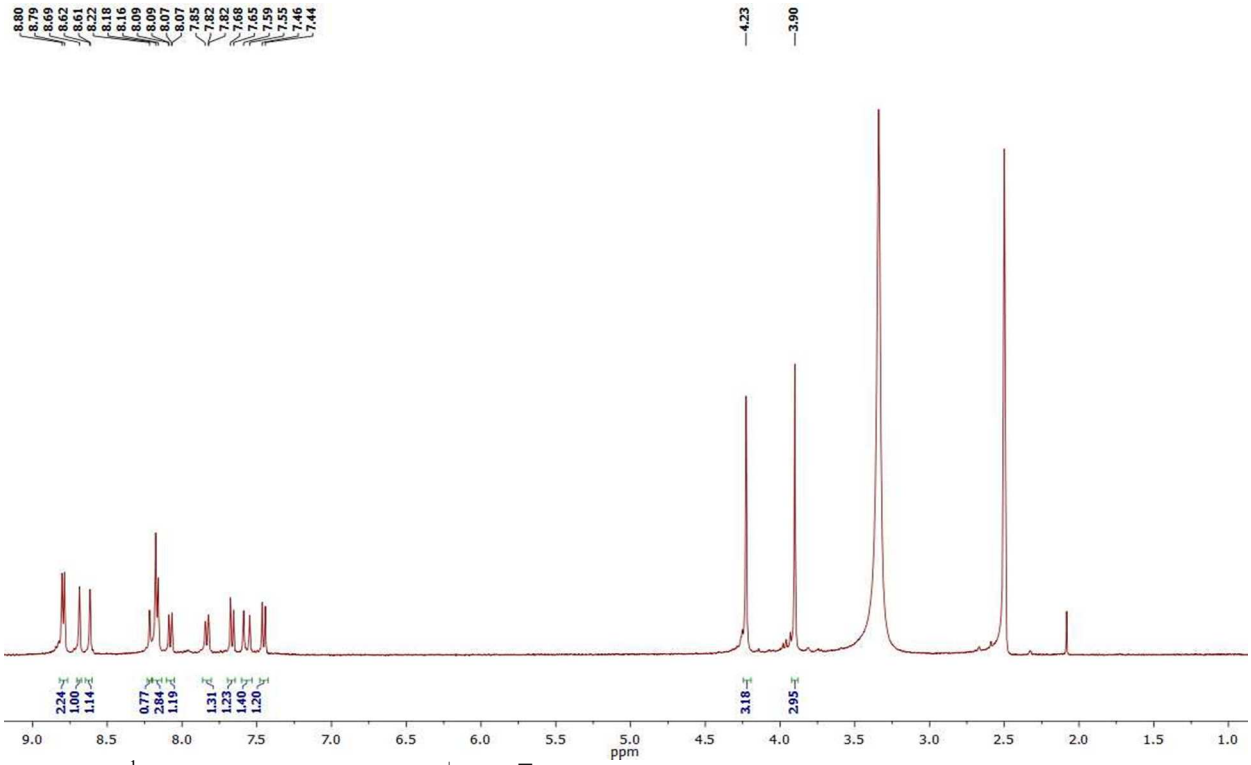


Figure S7. ^{1}H NMR spectrum of **MPVC**$^{+}$-COO^{-} in d_6-DMSO.

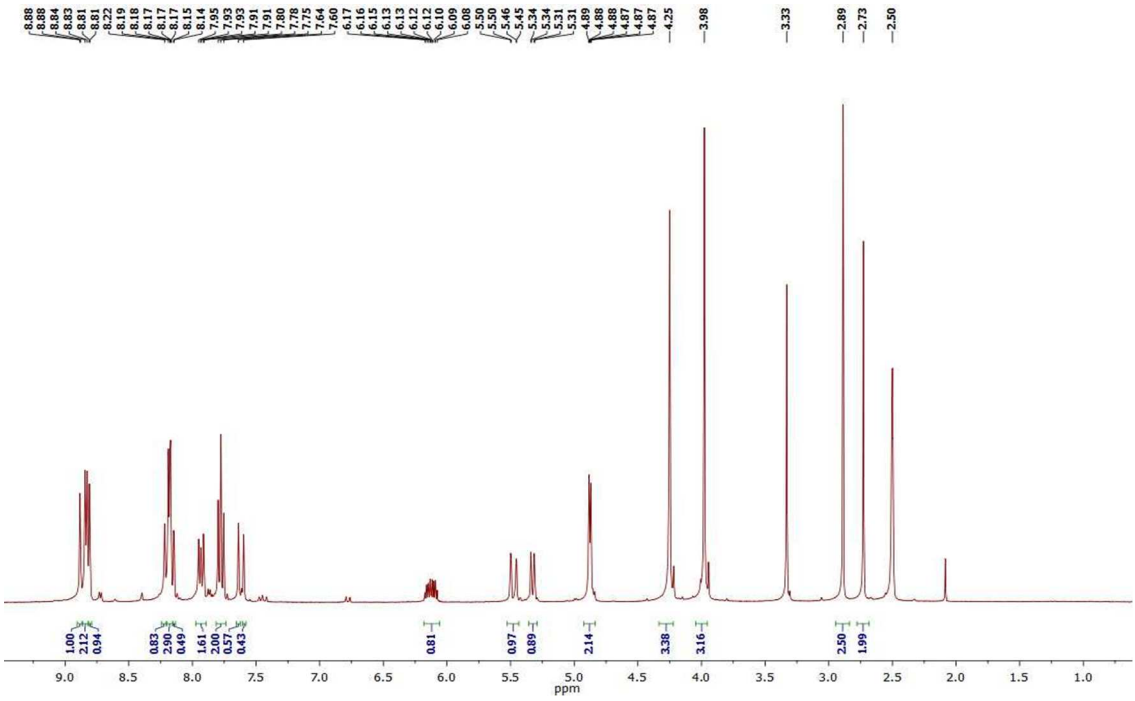


Figure S8. ^{1}H NMR spectrum of **MPVC**-II in d_6-DMSO.

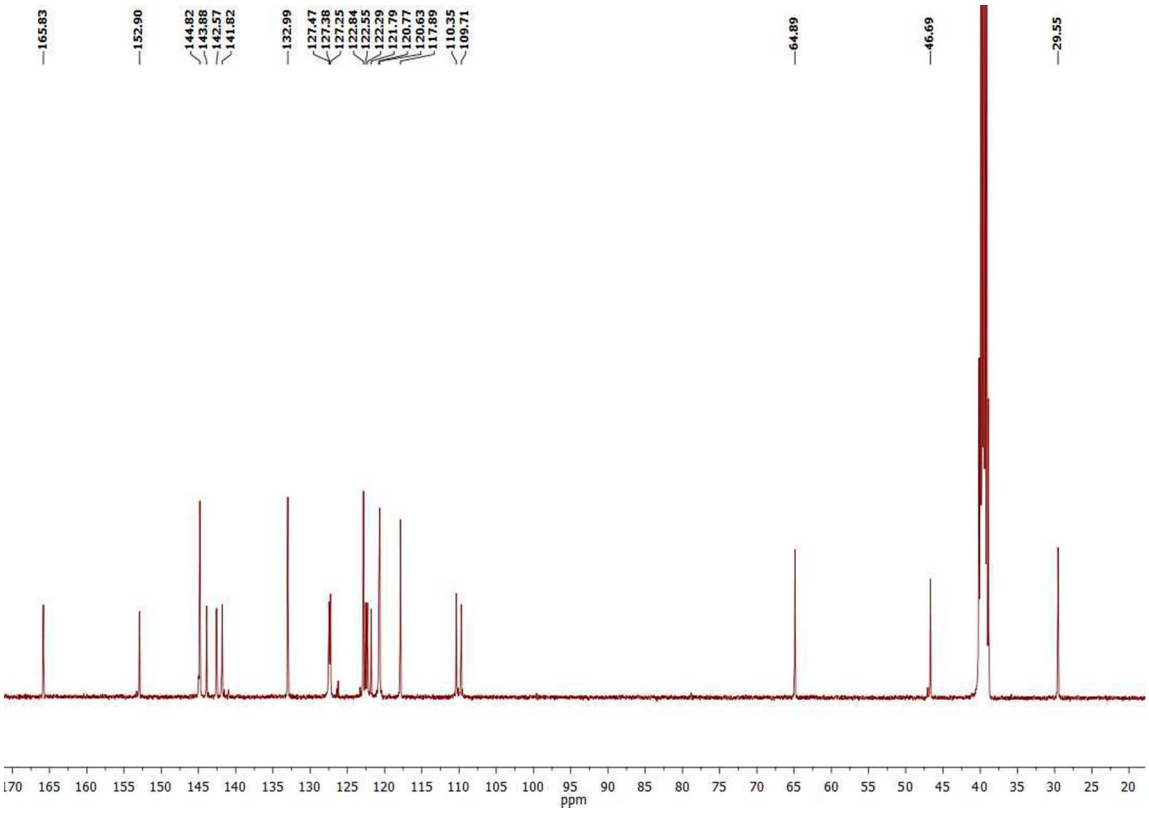


Figure S9. ^{13}C NMR spectrum of **MPVC**-II in d_6-DMSO.

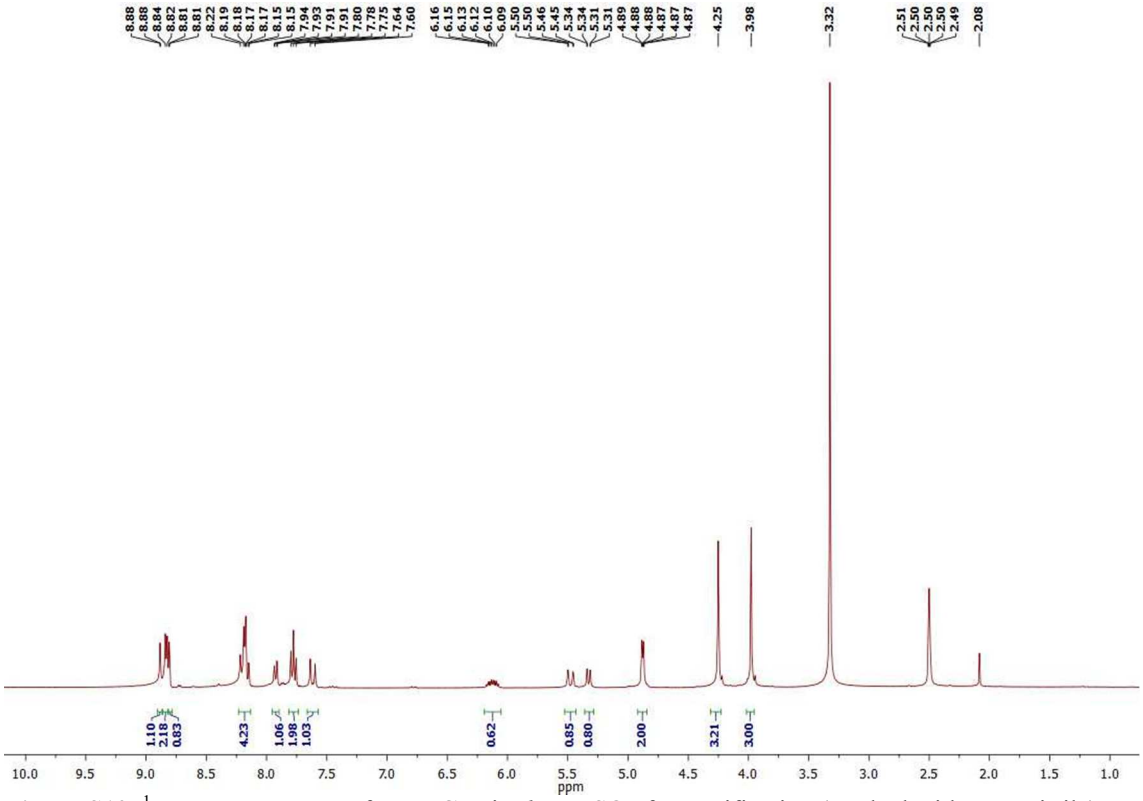


Figure S10. ^{1}H NMR spectrum of **MPVC**-II in d_6-DMSO after purification (washed with acetonitrile).

3. Fluorescence Studies

Table S1. Apparent rate constants of palladium catalyzed azidocarbonylation in various conditions calculated from fluorescence.

#	Solvent [a]	Catalyst [a]	Ligand [a]	Additive [a]	k (R^2) [b]
1	toluene/DMSO [c]	$Pd(OAc)_2$	Xantphos	NaOAc	n.d. [m]
2	PBS	$PdCl_2$			0.0683 (0.995)
3	PBS/DMSO	$Pd(OAc)_2$		NaOAc	0.120 (0.991)
4	PBS/DMSO	$Pd(OAc)_2$	Xantphos		0.0999 (0.975)
5	**PBS/DMSO**	**$Pd(OAc)_2$**	**Xantphos**	**NaOAc**	**0.240 (0.998)**
6	PBS/DMSO&DMF [d]	$Pd(OAc)_2$	Xantphos	NaOAc	0.129 (0.995)
7	PBS/DMSO&THF [d]	$Pd(OAc)_2$	Xantphos	NaOAc	0.0985 (0.995)
8	PBS/DMSO [e]	$Pd(OAc)_2$	Xantphos	NaOAc	0.214 (0.993)
9	PBS/DMSO [f]	$Pd(OAc)_2$	Xantphos	NaOAc	0.141 (0.994)
10	PBS/DMSO	$Pd(OAc)_2$ [g]	Xantphos	NaOAc	0.142 (0.996)
11	PBS/DMSO	$Pd(OAc)_2$ [h]	Xantphos	NaOAc	0.113 (0.968)
12	PBS/DMSO	$Pd(OAc)_2$	Xantphos [i]	NaOAc	0.139 (0.976)
13	PBS/DMSO	$Pd(OAc)_2$	Xantphos [j]	NaOAc	0.191 (0.991)
14	PBS/DMSO	$Pd(OAc)_2$	PPh_3	NaOAc	0.330 (0.989)
15	PBS/DMSO	$Pd(OAc)_2$	P(*o*-MeC_6H_4)$_3$	NaOAc	0.267 (0.990)
16	PBS/DMSO	$Pd(OAc)_2$	dppe	NaOAc	n.d
17	PBS/DMSO	$Pd(OAc)_2$	dppp	NaOAc	n.d
18	PBS/DMSO	$Pd(OAc)_2$	dppf	NaOAc	0.222 (0.989)
19	PBS/DMSO	$Pd(OAc)_2$	dpephos	NaOAc	0.221 (0.993)
20	PBS/DMSO	$Pd(OAc)_2$	R-BINAP	NaOAc	0.274 (0.996)
21	PBS/DMSO	$Pd(OAc)_2$	Xantphos	Na_2CO_3	0.201 (0.970)
22	PBS/DMSO	$Pd(OAc)_2$	Xantphos	4-DMAP	0.0428 (0.994)
23	PBS/DMSO	$Pd(OAc)_2$	Xantphos	Aliquat-336	n.d
24	PBS/DMSO	$Pd(OAc)_2$	Xantphos	n-$C_{12}H_{25}SO_4Na$	n.d
25 [k]	PBS/DMSO	$Pd(OAc)_2$	Xantphos	NaOAc	0.144 (0.991)
26 [l]	PBS/DMSO	$Pd(OAc)_2$	Xantphos	NaOAc	0.155 (0.993)
27	PBS/DMSO	$Pd(OAc)_2$	Xantphos	NaOAc/Na_2CO_3	0.154 (0.991)
28	PBS/DMSO	$Pd(dba)_2$	Xantphos	NaOAc	n.d

[a] Standard condition is as follows. Room temperature (25 ºC). Probe concentration: 10 μM. Solvent: 0.5% of assisting solvent (right) in main solvent (left); PBS refers to 1X (10 mM) phosphate buffered saline. Catalyst: 10 μM. Ligand: 20 μM. Additive: 1 mM. NaN_3: 2 mM. CO: 1 atm. Ligand abbreviation annotations are as follows. Xantphos: 4,5-bis-(diphenylphosphino)-9,9-dimethylxanthene; dppe: 1,2-bis-(diphenylphosphino)ethane; dppp: 1,3-bis-(diphenylphosphino)ethane; dppf: 1,1'-bis-(diphenylphosphino)ferrocene; dpephos: (oxydi-2,1-phenylene)bis-(diphenylphosphine). [b] k: apparent rate constant of 1st order reaction kinetics, unit: min^{-1}. R^2: linear regression coefficient of determination. [c] Tetrabutylammonium azide ($TBAN_3$) used as azide sourse. [d] DMSO: 0.1%, DMF or THF, 0.4%, PBS balancing. [e] 1% DMSO as assisting solvent. [f] 10% DMSO as assisting solvent. [g] 5 μM of catalyst was applied. [h] 20 μM of catalyst applied. [i] 10 μM of ligand applied. [j] 40 μM of ligand applied. [k] 1 mM of NaN_3 applied. [l] 4 mM of NaN_3 applied. [m] not detected because of small fluorescence change.

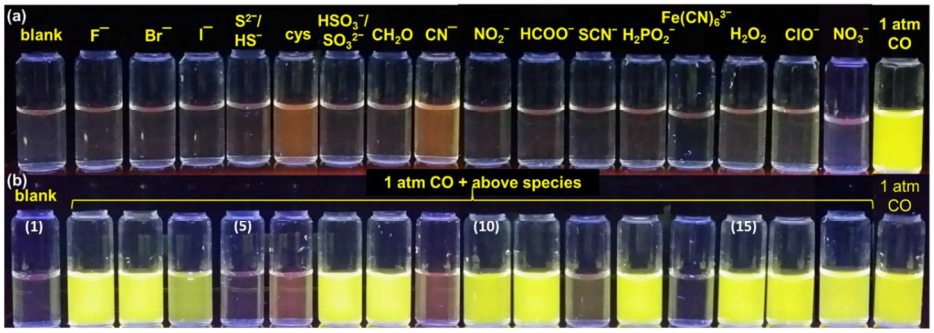


Figure S11. Visual fluorescence features **(a)** of **MPVC-I** (10 μM) in the presence of 200 μM of chemical species (indicated) under standard condition; Visual fluorescence features **(b)** of **MPVC-I** (10 μM) in the presence of 200 μM of chemical species (indicated) and 1 atm of CO under standard condition. All data were taken after 30 min of incubation. Species used: (2~5) NH_4F, NaBr, NaI, Na_2S; (6~10) L-cysteine, Na_2SO_3, formalin, NaCN, $NaNO_2$; (11~15) HCOONa, KSCN, NaH_2PO_2, $K_3[Fe(CN)_6]$, H_2O_2; (16~18) NaClO, $NaNO_3$, gaseous CO.

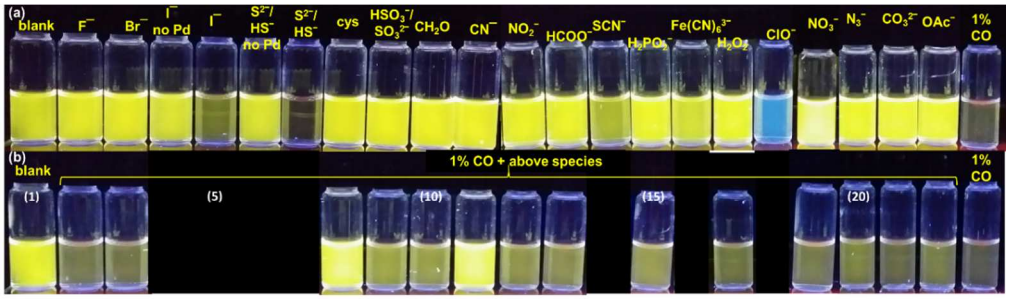


Figure S12. Visual fluorescence features **(a)** of **MPVC-II** (2 μM) in the presence of 20 μM of $PdCl_2$ and 200 μM of chemical species (indicated) under 5% MeCN in PBS; Visual fluorescence features **(b)** of **MPVC-II** (2 μM) in the presence of 200 μM of chemical species (indicated) and 1% CO balanced by airunder 5% MeCN in PBS. All data were taken after 30 min of incubation. Species used: (2~5) NH_4F, NaBr, NaI without $PdCl_2$, NaI; (6~10) Na_2S without $PdCl_2$, Na_2S, L-cysteine, Na_2SO_3, formalin; (11~15) NaCN, $NaNO_2$, HCOONa, KSCN, NaH_2PO_2; (16~20) $K_3[Fe(CN)_6]$, H_2O_2, NaClO, $NaNO_3$, NaN_3; (21~23) K_2CO_3, NaOAc, 1% CO in air.

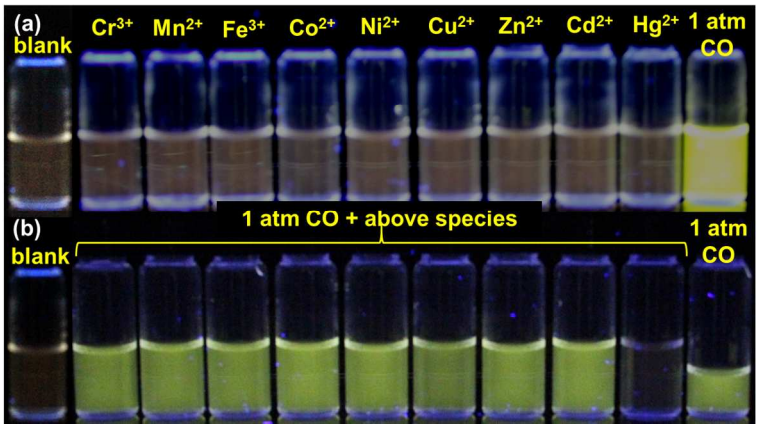


Figure S13. Visual fluorescence features **(a)** of **MPVC-I** (10 μM) in the presence of 200 μM of cationic species (indicated) under standard condition; Visual fluorescence features **(b)** of **MPVC-I** (10 μM) in the presence of 200 μM of cationic species (indicated) and 1 atm of CO under standard condition. All data were taken after 30 min of incubation. Species used: (left to right) blank, $Cr(NO_3)_3$, $MnCl_2$, $FeCl_3$, $CoCl_2$, $NiCl_2$, $CuSO_4$, $Zn(OAc)_2$, $CdCl_2$, $HgCl_2$, gaseous CO.

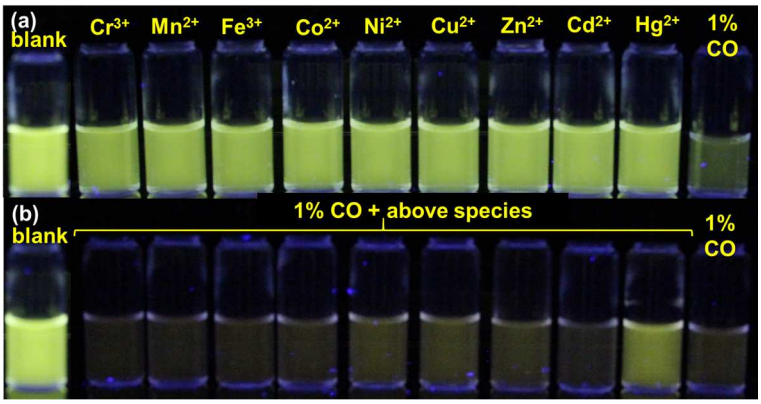


Figure S14. Visual fluorescence features **(a)** of **MPVC-II** (2 μM) in the presence of 20 μM of $PdCl_2$ and 200 μM of cationic species (indicated) under 5% MeCN in PBS; Visual fluorescence features **(b)** of **MPVC-II** (2 μM) in the presence of 200 μM of cationic species (indicated) and 1% CO balanced by airunder 5% MeCN in PBS. All data were taken after 30 min of incubation. Species used: (left to right) blank, $Cr(NO_3)_3$, $MnCl_2$, $FeCl_3$, $CoCl_2$, $NiCl_2$, $CuSO_4$, $Zn(OAc)_2$, $CdCl_2$, $HgCl_2$, 1% CO in air.

4. Liquid Chromatography and Mass Spectrometry

In order to elucidate the real reaction process, we subjected these two probes to liquid chromatography (LC) /mass spectrometry (MS). In the case of azidocarbonylation of **MPVC-I**, after introduction of 1 atm CO for 10 min under standard condition, signals of [**MPVC**$^+$-NCO] and [**MPVC**$^+$-CON_3] appeared at retention time of 7.5 min, although the Curtius rearrangement might occur either in solution or on the column. For **MPVC-II**, exposure to 1% CO for 10 min lead to significant conversion to [**MPVC**$^+$-COOH].[2] Moreover, the final product of isocyanate hydrolysis and subsequent decarboxylation: [**MPVC**-NH_2] was synthesized separately and gave no fluorescence in pH 7.4 buffer. The above results confirmed that azidocarbonylation of **MPVC-I** and deallylation of **MPVC-II** were responsible for the enhancement and quenching of fluorescence, respectively.

5. Two-photon Excited Fluorescence (2PEF) Measurements

Femtosecond laser pulses from a Ti:sapphire oscillator (Coherent, Vitesse, 800 nm, 80 MHz, 100 fs) were used to two-photon excitable fluorescence (2PEF) measurement. The irradiation power of laser pulses was controlled by a combination of polarizer and half-wave plate and focused by a lens (f = 75 mm) in reflection geometry. 2PEF was dispersed in spectrograph (Andor Shamrock 193i) and detected by CCD (Andor DV-420A-BU2). Samples for laser induced 2PEF experiments were prepared in the same way as fluorescence studies. A combination with polarizer and half-wave plate was used to control the irradiation power.

The relationship between irradiation power and emission intensity of all **MPVC-** related compounds finely agreed with the quadratic power dependence of 2PEF[3] (**Figures S15~S17a, c**). Upon 800 nm femtosecond laser excitation, all resultant solutions of **MPVC-I** after CO treatment exhibited peak emission at around 580 nm (**Figures S15b, d**) similar to the one-photon excited fluorescence spectra. In the case of **MPVC-I**, the much weaker emission was at around 615 nm (**Figure S16b**). **MPVC-II** gave considerably high fluorescence at as low as 2 μM concentration (**Figure S16d**). The byproduct of azidocarbonylation **MPVC**-COO^-/**MPVC**-COOH exhibited pH dependent fluorescence behavior, resulting in 4 folds enhancement from high (anionic) to low (neutral carboxylic acid) pH (**Figures S17b, d**).

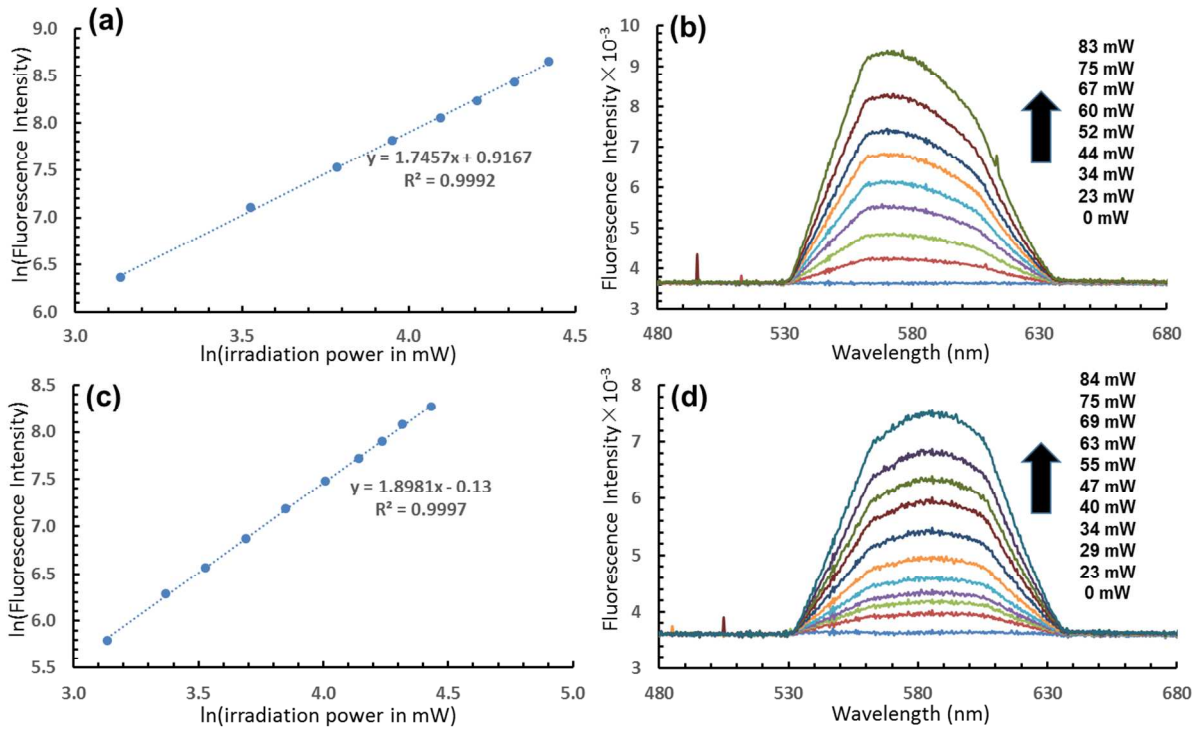


Figure S15. Irradiation power dependent 2PEF emission intensity (left) and emission spectra (right) upon 800 nm laser excitation of **(a, b)** Azidocarboylation resultant, **(c, d)** Carboxylation resultant of **MPVC-I** under standard condition.

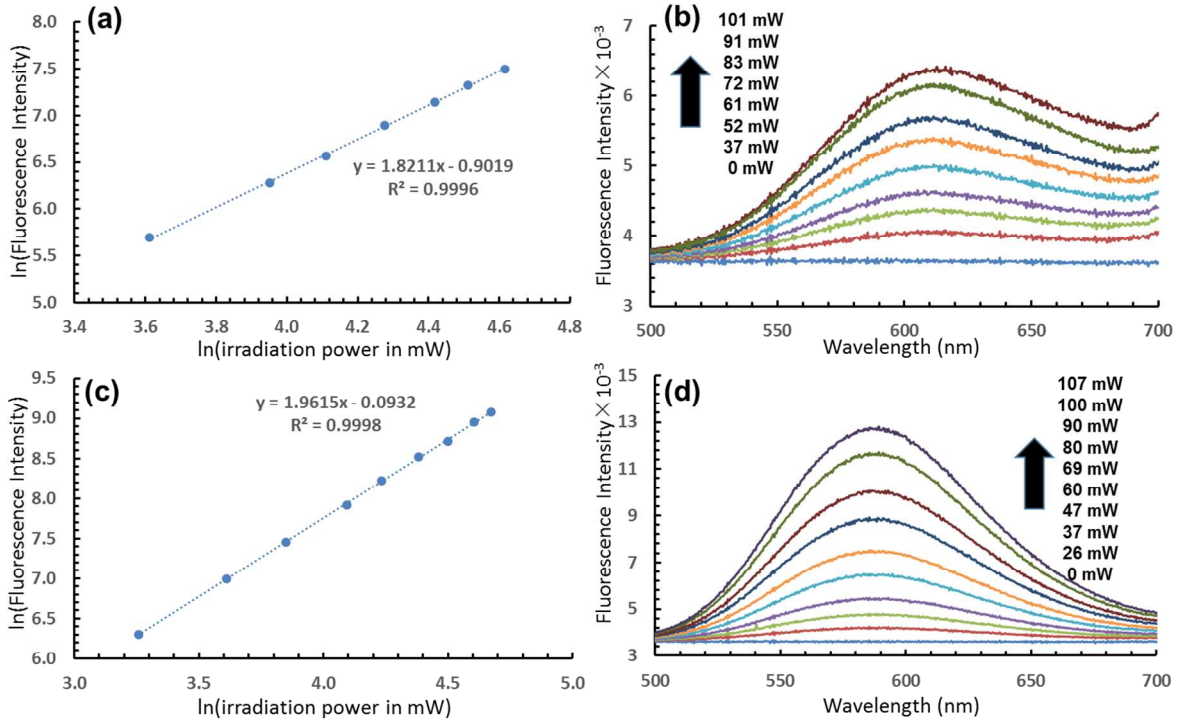


Figure S16. Irradiation power dependent 2PEF emission intensity (left) and emission spectra (right) upon 800 nm laser excitation of **(a, b) MPVC-I** (10 μM), **(c, d) MPVC-II** (2 μM) in pH 7.4 1xPBS buffer.

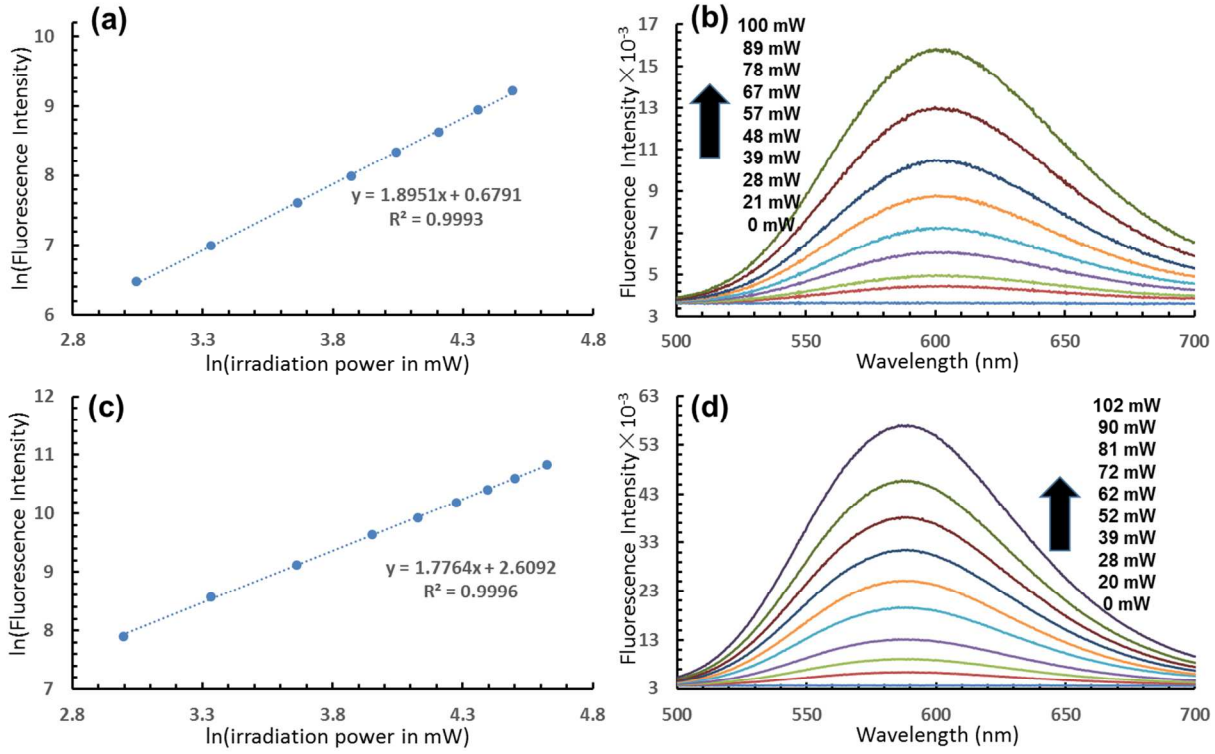


Figure S17. Irradiation power dependent 2PEF emission intensity (left) and emission spectra (right) upon 800 nm laser excitation of **(a, b) MPVC**-COO^- (10 μM, pH 7.4), **(c, d) MPVC**-COOH (10 μM, pH 2.7).

6. Quantum Yields and Two-photon Absorption Cross Sections of Samples

Anthracene as a standard (Φ_s = 0.28 in ethanol at λ_{ex} = 340 nm) was used for quantum yield measurement of probe **MPVC**-I related resultant solutions. Typically, the quantum yield was obtained by integrating the photons emitted by the analyte from 450 nm to 700 nm and calculated according to the following formula:[4]

$$\Phi_u = \Phi_s \frac{A_s F_u n_u^2}{A_u F_s n_s^2}$$

where A is absorbance at the excitation wavelength, F integrated emission area across the band, Φ quantum yield, n refraction index of the solvent, and subscript u and s denote "unknown" and "standard", respectively.

Fluorescein dissolved in pH = 11 aqueous solution was used as the reference molecular (Φ_s = 0.92 at λ_{em} = 500-600 nm, σ_s = 32 GM at λ_{ex} = 810 nm[5]) for two-photon cross section measurements. 2PEF spectra of the analytes were integrated from 450 nm to 700 nm and the cross section was calculated according to the following formula:

$$\sigma_u = \sigma_s \frac{F_u n_u^2 \Phi_s C_s}{F_s n_s^2 \Phi_u C_u}$$

Apparent quantum yields and two-photon absorption cross sections are summarized in **Table S2**.

Table S2. Generalization of apparent quantum yields and two photon absorption cross sections. (**a~e**) refer to **MPVC-I** sensing system. **(a)**: absence of azide; **(c)**: 1 atm of CO for 30 min; **(d)**: absence of azide, 1 atm of CO for 30 min; **(e)**: solution **(d)** acidified to pH 2.7; **(f) MPVC-II** (2 μM).

#	Quantum Yield Φ (%)	Cross Section σ (GM)
(a)	0.33	25.0
(b)	0.066	16.2
(c)	2.4	251
(d)	1.1	143
(e)	4.4	130
(f)	8.3	199

7. Cell Viability and Cytotoxicity Test

The mouse fibroblast NIH 3T3 cells were cultured in high glucose Dulbecco's Modified Eagle Medium (DMEM) with 10 % fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/L streptomycin. Then, cells were grown till 70 % confluence under a humidified atmosphere with 5 % CO_2 at 37 °C. The confluent cells were harvested with 0.05 % (w/v) Trypsin EDTA, and then 5×10^3 cells/cm^2 were re-plated into 48-well culture plates. After 1 day, **MPVC-I** and **MPVC-II** were dissolved in culture medium and added into each well with varying concentrations of 1, 5, 10, and 20 μM, respectively.

The cell viability is measured with Live/Dead Assay kit. After 48 hours with treatment of **MPVC-I** and **MPVC-II**, the cells incubated with Calcein-AM and Ethidium homodimer-1 for 30 min at 37 °C were observed by fluorescence microscope. The number of live and dead cells were counted with Leica Application Suite X software and cell viability was calculated.

The cell cytotoxicity test was performed by using the alamar blue® assay (Invitrogen). For normalized data, the cytotoxicity test was analyzed at 1 and 3 days, respectively. The alamar blue solution was added into each well and incubated for 2 hours after removal of cell medium. Then, the fluorescence was measured with 570 nm of wavelength.

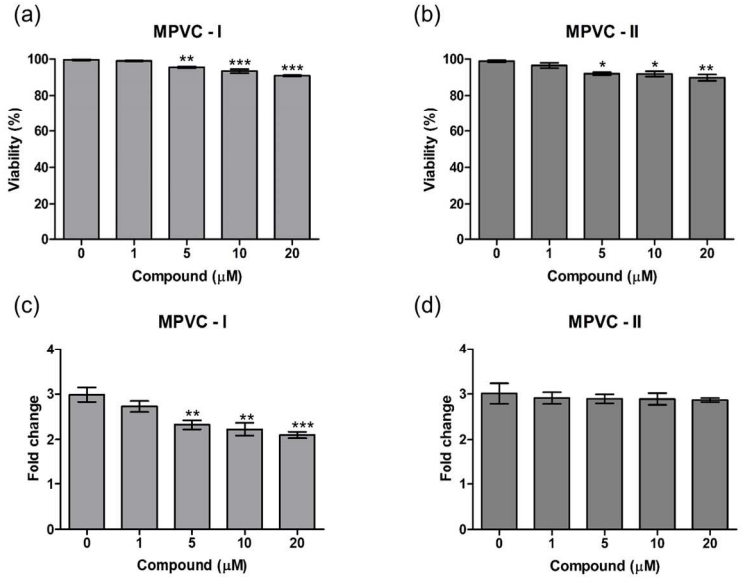


Figure S18. (a, b) Cell viability and **(c, d)** cytotoxicity test with variable concentration of **MPVC-I** and **MPVC-II**. The viability of NIH 3T3 cells with 1 μM of **MPVC-I** and **MPVC-II** showed no obvious change compared with

untreated group. Although cell viability was slightly reduced when concentration of **MPVC-I** and **MPVC-II** increased, the cell viability still observed over 85 %. The normalized alamar blue fluorescence intensity for treatment of **MPVC-II** indicated non-toxicity in cell. Although the **MPVC-I** induced mild cell toxicity when using the higher concentration, 1 μM of **MPVC-I** allowed to proliferate the cell. All graphs were represented with standard error of mean (denoted as *$p<0.05$; **$p<0.01$; ***$p<0.001$, compared to untreated group by One-way ANOVA with Tukey's Multiple Comparison post-hoc test).

References

(1) Alcaide, B.; Almendros, P.; Alonso, J. M.; Busto, E.; Fernandez, I.; Ruiz, M. P.; Xiaokaiti, G., Versatile Synthesis of Polyfunctionalized Carbazoles from (3-Iodoindol-2-yl)butynols via a Gold-Catalyzed Intramolecular Iodine-Transfer Reaction. *Acs. Catal.* **2015,** *5* (6), 3417-3421.

(2) To protect instrumentation, deionized water was used as main solvent instead of PBS.

(3) Yang, W.; Chan, P. S.; Chan, M. S.; Li, K. F.; Lo, P. K.; Mak, N. K.; Cheah, K. W.; Wong, M. S., Two-photon fluorescence probes for imaging of mitochondria and lysosomes. *Chem. Commun.* **2013,** *49* (33), 3428-3430.

(4) Eaton, D. F., Reference Materials for Fluorescence Measurement. *Pure. Appl. Chem.* **1988,** *60* (7), 1107-1114.

(5) Makarov, N. S.; Drobizhev, M.; Rebane, A., Two-photon absorption standards in the 550-1600 nm excitation wavelength range. *Opt. Express.* **2008,** *16* (6), 4029-4047.