Supporting Information

Glycosylation enhances the aqueous sensitivity and lowers the cytotoxicity of a naphthalimide zinc ion probe

Lei Dong, a1 Yi Zang, b1 Dan Zhou, ab Xiao-Peng He,a* Guo-Rong Chen,a Tony D James, c and Jia Li,b*

a Key Laboratory for Advanced Materials & Institute of Fine Chemicals, East China University of Science and Technology, 130 Meilong Rd., Shanghai 200237, PR China, Email: xphe@ecust.edu.cn (X.-P. He)

b National Center for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 189 Guo Shoujing Rd., Shanghai 201203, PR China, Email: jli@simm.ac.cn (J. Li)

c Department of Chemistry, University of Bath, Bath, BA2 7AY, UK

1 Equal contribution

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**Figure S1.** Fluorescence spectra of (a) DL1 (10 μM) and (d) DL2 (10 μM) in the absence and presence of different metal cations including Zn$^{2+}$, Cd$^{2+}$, Co$^{2+}$, Ba$^{2+}$, Mg$^{2+}$, K$^+$, Na$^+$, Mn$^{2+}$, Pd$^{2+}$, Pb$^{2+}$, Ca$^{2+}$, Ag$^+$, Ni$^{2+}$, Hg$^{2+}$ and Cu$^{2+}$. Fluorescence spectra of (b) DL1 (10 μM) and (e) DL2 (10 μM) in the absence and presence of different anions including F$^-$, Cl$^-$, Br$^-$, I$^-$, SO$_4^{2-}$, SO$_3^{2-}$, CO$_3^{2-}$, PO$_4^{3-}$ and NO$_3^-$.

Fluorescence spectra of (c) DL1 (10 μM)/Cd$^{2+}$ (20 μM) complex and (f) DL2 (10 μM)/Cd$^{2+}$ (20 μM) complex in the absence and presence of increasing Zn$^{2+}$ (0-20 μM). All the spectroscopic analyses were carried out in an aqueous solution (0.05 M Tris-HCl, pH 7.4) with an excitation at 365 nm.
Figure S2. Fluorescence spectra of (a) DL1 (10 μM) and (c) DL2 (10 μM) in the absence and presence of Zn^{2+} (50 μM) and then TPEN (N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine, a zinc chelator) (50 μM). Job plot analysis of (b) DL1 and (d) DL2 in complexation with Zn^{2+}. All the spectroscopic analyses were carried out in an aqueous solution (0.05 M Tris-HCl, pH 7.4) with an excitation at 365 nm.
S2. Experimental section

General

All chemicals and regents are of high commercially available grade, and were used as received. NMR spectra were recorded on a Bruker AM-400 spectrometer using tetramethyl silane (TMS) as the internal standard ($\delta = 0$). High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier XE spectrometer using standard conditions (ESI, 70 eV). All UV-vis absorption spectra were measured on a Varian Cary 500 UV-vis spectrophotometer and fluorescence spectra measured on a Varian Cary Eclipse Fluorescence spectrophotometer.

Synthesis of Compound 2

![Chemical structure](image)

A solution of 2-chloroacetyl chloride (1.1 g, 9.6 mmol) in 10 mL dry CH$_2$Cl$_2$ was added dropwise to a solution of 1 (2 g, 8.0 mmol)$^1$ and 4-dimethylaminopyridine (DMAP, 1.0 g, 8.0 mmol) in 40 mL dry CH$_2$Cl$_2$ with an ice bath. After stirring for 2 h at room temperature, the resulting mixture was removed under reduced pressure to obtain a pale-yellow solid. The crude product was purified by silica-gel column chromatography using dichloromethane as eluent to afford 2 (2.4 g, 92%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 3.16 (t, $J = 4.0$ Hz, 1H), 4.56 (s, 2H), 4.78 (d, $J = 4.0$ Hz, 2H), 7.85 (dd, $J = 12.0$ Hz, 8.0Hz, 1H), 8.29 (d, $J = 8.0$ Hz, 1H), 8.51-8.60 (m, 2H), 8.67-8.72 (m, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 29.0, 43.6, 72.9, 79.3, 117.8, 120.1, 121.9, 124.3, 126.7, 128.2, 129.5, 131.2, 131.8, 139.7, 162.0, 162.6, 166.0. HR-ESI-MS m/z: [M + H]$^+$ calcd. for 325.0380, found 325.0383.
Synthesis of Compound 5

To a solution of 2 (500 mg, 1.5 mmol) and azide 3 (630 mg, 1.6 mmol) in CH₂Cl₂/H₂O (20 mL/10 mL) were added sodium ascorbate (594 mg, 3.0 mmol) and CuSO₄·5H₂O (560 mg, 2.2 mmol). The mixture was stirred over night at room temperature. The resulting mixture was then diluted with dichloromethane and washed successively with water and brine. The combined organic layer was dried over MgSO₄, filtered and concentrated in vacuum. The resulting residue was purified by column chromatography on silica gel (CH₂Cl₂/EtOAc = 5:1, v/v) to afford 5 (836 mg, 80%) as a yellow solid. 

¹H NMR (400 MHz, CDCl₃): δ 1.84 (s, 3H), 2.01 (s, 3H), 2.01 (s, 3H), 2.26 (s, 3H), 4.09-4.29 (m, 3H), 4.47 (s, 2H), 5.23-5.26 (m, 1H), 5.35-5.41 (m, 1H), 5.54-5.61 (m, 3H), 5.85-5.89 (m, 1H), 7.51 (t, J = 8.0 Hz, 1H), 8.03 (d, J = 8.0 Hz, 1H), 8.08 (s, 1H), 8.30 (d, J = 8.0 Hz, 1H), 8.36 (d, J = 8.0 Hz, 1H), 8.47 (d, J = 8.0 Hz, 1H), 9.49 (s, 1H); 

¹³C NMR (100 MHz, CDCl₃): δ 20.3, 20.5, 20.6, 20.8, 34.8, 43.6, 61.2, 66.8, 67.9, 70.7, 74.1, 86.4, 118.4, 119.3, 122.4, 122.5, 123.7, 126.5, 128.3, 131.0, 132.2, 138.3, 144.1, 163.5, 165.1, 169.0, 169.8, 170.0, 170.3. HR-ESI-MS m/z: [M + H]⁺ calcd. for 698.1501, found 698.1505.
**Synthesis of Compound 6**

To a solution of 2 (500 mg, 1.5 mmol) and azide 4 (630 mg, 1.6 mmol) in CH\(_2\)Cl\(_2\)/H\(_2\)O (20 mL/10 mL) were added sodium ascorbate (594 mg, 3.0 mmol) and CuSO\(_4\)·5H\(_2\)O (560 mg, 2.2 mmol). The mixture was stirred overnight at room temperature. The resulting mixture was then diluted with dichloromethane and washed successively with water and brine. The combined organic layer was dried over MgSO\(_4\), filtered and concentrated in vacuum. The resulting residue was purified by column chromatography on silica gel (CH\(_2\)Cl\(_2\)/EtOAc = 5:1, v/v) to afford 6 (773 mg, 74%) as a yellow solid. 

\[\delta 1.81 (s, 3H), 2.03 (s, 3H), 2.06 (s, 3H), 2.07 (s, 3H), 4.00-4.06 (m, 1H), 4.08-4.18 (m, 2H), 4.26-4.34 (m, 1H), 4.47 (s, 2H), 5.23-5.31 (m, 1H), 5.38-5.46 (m, 2H), 5.48-5.54 (m, 2H), 5.93 (d, J = 8.0 Hz, 1H), 7.51 (t, J = 8.0 Hz, 1H), 8.05-8.16 (m, 2H), 8.23 (d, J = 8.0 Hz, 1H), 8.30 (d, J = 8.0 Hz, 1H), 8.35 (d, J = 8.0 Hz, 1H), 9.60 (s, 1H); \] 

\[\delta 20.2, 20.5, 20.5, 20.7, 34.8, 43.6, 53.4, 61.5, 67.6, 70.4, 72.6, 75.1, 85.8, 85.8, 118.2, 119.4, 122.2, 123.7, 126.4, 126.7, 128.1, 130.8, 132.1, 138.4, 162.9, 163.4, 165.3, 168.9, 169.3, 169.9, 170.5.\]

HR-ESI-MS \(m/z\): [M + H]\(^+\) calcd. for 698.1501, found 698.1500.
Synthesis of DL1

To a solution of 5 (150 mg, 0.21 mmol) and di-(2-picolyl)amine (DPA) (56 mg, 0.28 mmol) in acetonitrile (40 mL) were added \(N,N\)-diisopropylethylamine (DIPEA) (1 mL) and potassium iodide (50 mg). The mixture was stirred and refluxed overnight under nitrogen atmosphere. Then the mixture was cooled to room temperature and the solvent removed under reduced pressure. The resulting mixture was then diluted with dichloromethane and washed successively with water and brine. The combined organic layer was dried over MgSO\(_4\), filtered and concentrated in vacuum. Then to the solution of the resulting residue re-dissolved in EtOH/H\(_2\)O (30 mL/10 mL) were added triethylamine (3 mL) and ammonium hydroxide (3 mL). After stirring for 6 h at room temperature, solvent was removed under reduced pressure and the resulting residue purified by column chromatography on silica gel (CH\(_2\)Cl\(_2\)/MeOH = 5:1, v/v) to afford DL1 (103 mg, 71%) as a yellow solid. \(^1\)H NMR (400 MHz, CD\(_3\)OD): δ 3.35-3.47 (m, 2H), 3.49-3.54 (m, 1H), 3.55-3.64 (m, 4H), 3.67-3.73 (m, 1H), 3.85 (d, \(J = 4.0\) Hz, 1H), 3.98 (t, \(J = 8.0\) Hz, 1H), 4.14-4.26 (m, 2H), 4.30-4.42 (m, 2H), 5.35 (d, \(J = 8.0\) Hz, 2H), 5.42 (d, \(J = 8.0\) Hz, 1H), 7.21 (d, \(J = 8.0\) Hz, 1H), 7.41 (t, \(J = 8.0\) Hz, 1H), 7.53-7.63 (m, 4H), 7.89 (d, \(J = 8.0\) Hz, 1H), 8.04 (dd, \(J = 8.0\) Hz, 8.0 Hz, 2H), 8.11-8.19 (m, 2H), 8.38-8.45 (m, 2H), 8.86 (d, \(J = 8.0\) Hz, 1H), 8.94 (d, \(J = 4.0\) Hz, 1H); \(^1^3\)C NMR (100 MHz, DMSO-\(d_6\)): δ 35.2, 48.6, 58.5, 59.9, 60.4, 68.4, 69.2, 73.6, 78.4, 88.0, 116.6, 116.8, 121.8, 122.2, 122.5, 122.9, 123.4, 126.6, 128.4, 128.5, 131.0, 132.1, 136.7, 139.8, 143.2, 149.1, 157.9, 162.6, 163.3, 170.5. HR-ESI-MS \(m/z\): [M + H]\(^+\) calcd. for 695.2578, found 695.2582. HPLC: \(t_R = 5.377\) min of 80% methanol/20% water (0.6 mL/min), purity 95%. 
Synthesis of DL2

To a solution of 6 (150 mg, 0.21 mmol) and di-(2-picolyl)amine (DPA) (56 mg, 0.28 mmol) in acetonitrile (40 mL) were added N,N-diisopropylethylamine (DIPEA) (1 mL), and potassium iodide (50 mg). The mixture was stirred and refluxed overnight under nitrogen atmosphere. Then the mixture was cooled to room temperature and the solvent removed under reduced pressure. The resulting mixture was then diluted with dichloromethane and washed successively with water and brine. The combined organic layer was dried over MgSO₄, filtered and concentrated in vacuum. Then to the solution of the resulting residue re-dissolved in EtOH/H₂O (30 mL/10 mL) were added triethylamine (3 ml) and ammonium hydroxide (3 ml). After stirring for 6 h at room temperature solvent was removed under reduced pressure and the resulting residue purified by column chromatography on silica gel (CH₂Cl₂/MeOH = 5:1, v/v) to afford DL2 (107 mg, 74%) as a yellow solid.

¹H NMR (400 MHz, CD₃OD): δ 3.46-3.52 (m, 2H), 3.49-3.57 (m, 4H), 3.58-3.62 (m, 2H), 3.64-3.69 (m, 2H), 3.82-3.88 (m, 1H), 4.28 (t, J = 16.0 Hz, 2H), 4.41-4.52 (m, 2H), 5.46 (s, 2H), 5.57 (d, J = 8.0 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.51 (t, J = 8.0 Hz, 1H), 7.63-7.73 (m, 5H), 7.99 (d, J = 8.0 Hz, 1H), 8.11-8.18 (m, 3H), 8.49-8.51 (m, 3H), 8.95 (d, J = 4.0 Hz, 1H), 9.03 (d, J = 4.0 Hz, 1H); ¹³C NMR (100 MHz, DMSO-d₆): δ 25.1, 34.8, 60.5, 69.4, 71.8, 76.8, 79.9, 87.4, 107.2, 108.1, 119.3, 121.6, 122.0, 123.9, 129.8, 131.1, 134.1, 143.5, 153.0, 158.0, 162.6, 163.6, 163.8, 166.8, 175.4, 178.7. HR-ESI-MS m/z: [M + H]⁺ calcd. for 695.2578, found 695.2573. HPLC: t_R = 5.819 min of 80% methanol/20% water (0.6 mL/min), purity 96%.
**Spectroscopic measurements**

The stock solution of probes (1 mM) was prepared in DMSO. Stock solutions of 10 mM of Zn(ClO$_4$)$_2$, Cd(ClO$_4$)$_2$, Co(ClO$_4$)$_2$, Mg(ClO$_4$)$_2$, KClO$_4$, Mn(ClO$_4$)$_2$, Cd(ClO$_4$)$_2$, Ag(ClO$_4$)$_2$, Ni(ClO$_4$)$_2$, Hg(ClO$_4$)$_2$ and Cu(ClO$_4$)$_2$ were prepared in a deionized water. The fluorescence measurements were carried out with a path length of 10 mm and an excitation wavelength at 365 nm by scanning the spectra between 400 nm and 750 nm. The bandwidth for both excitation and emission spectra was 5 nm. Unless otherwise mentioned, all the spectra were recorded in 0.05 M Tris- HCl buffer at 25 °C.

**Determination of the Spiked Zn$^{2+}$ concentration in environmental water samples**

Water samples were collected from Huangpu River of Shanghai and Tai Lake of Wuxi. The samples were passed through a microfiltration membrane before use. Prior to detection, the fluorescence of DL1 and DL2 (5 μM) in the presence of various concentrations of Zn$^{2+}$ was recorded and plotted as a function of concentration as the calibration curve. Then the samples spiked with different concentrations of Zn$^{2+}$ were quantified by fitting to the curves.

**Cell imaging assay**

HepG2 and HeLa were cultured in DMEM-HG supplemented with 10% FBS, and A549 cells cultured in Ham's F-12 supplemented with 10% FBS. Then, cells (2.0 × 10$^4$/well) were seeded on a black 96-well microplate with optically clear bottom (Greiner bio-one, Germany) overnight. After incubation with 20 μM DL1 or 1 in 0.05 M Tris-HCl for 30 min, the cells were treated with or without 20 μM Zn$^{2+}$ in 0.05 M Tris-HCl for another 30 min (followed by three rinses using Tris-HCl).

Then the cells on the microplate were fixed by 4% paraformaldehyde in Tris-HCl for 10 min at room temperature. After three rinses in Tris-HCl the fluorescence was eventually detected and photographed with Operetta high content imaging system (Perkinelmer, US). The quantitative results were obtained from Columbus analysis system (Perkinelmer, US). Based on different textures in bright field channel, regions of an image were divided into cells and background using an interactive training mode. Then, the fluorescence intensity properties of cells and background were calculated.
**Cell viability assay**

Cells were plated overnight on 96-well plates at 5000 cells per well in growth medium. After seeding, cells were treated at increasing concentrations (10 μM, 20 μM, 50 μM, 100 μM, 200 μM) of probe (dissolved in DMSO, diluted to final concentration with PBS) for 24 h. 20 μL per well of MTS/PMS (20:1, Promega Corp) solution was added. The absorbance was measured on a SpectraMax 340 microplate reader (Molecular Devices, USA) at 490 nm with a reference at 690 nm after incubation of 2 h at 37 °C. The optical density of the result in MTS assay was directly proportional to the number of viable cells. Each experiment was done in triplicate.
S3. Original NMR copies of new compounds

$^1$H NMR of Compound 2:

$^{13}$C NMR of Compound 2:
$^1$H NMR of Compound 5:

$^{13}$C NMR of Compound 5:
$^1$H NMR of Compound 6:

$^{13}$C NMR of Compound 6:
$^1$H NMR of DL1:

$^{13}$C NMR of Compound DL1:
$^1$H NMR of DL2:

$^{13}$C NMR of Compound DL2:
Reference