A water soluble glucopyranosyl conjugate as selective and reactive probe for cysteine in buffer and its application to living cells

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Contents

Page no:

S01. Synthesis and characterization of P2 02
S02. Synthesis and characterization of P4 02
S03. Synthesis and characterization of C1 03
S04. Synthesis and characterization of P5 04
S05. Synthesis and characterization of P6 05
S06. Synthesis and characterization of L 07
S07. Characterization of L1 08
S08. Fluorescence studies of L with amino acids 09
S09. Determination of Limit of Detection (LOD) of Cys by L 10
S10. Comparison of the detection limits of recently developed fluorescent probes for Cys in the literature. 10
S11. Histogram for the competitive amino acid titrations of [L] with amino acids 11
S12. Fluorescence spectra for the titration of [L] with molecular weight thiols 12
S13. Fluorescence spectra for the titration of [L] with oxidized state of BSA, HSA and GSH 12
S14. Fluorescence spectra for the titration of [L] with reduced state of BSA, HSA and GSH 12
S15. Absorption spectra obtained during the titration [L] with different amino acids 13
S16. Absorption spectra for the titration of [L] with oxidized forms of GSH 14
S17. ESI MS spectrum for the titration of [L] with Cys 15

$\text{P}_2$ was obtained as a yellow solid (85 %). \(^1\)HNMR (400 MHz, CDCl$_3$, $\delta$ ppm): 1.9 (t, $^1\text{J}=2.2$ Hz and $^2\text{J}=2.2$ Hz, 1H), 2.8 (s, 6H), 3.8 (d, $\text{J}=7.4$, 2H), 4.62 (t, $^1\text{J}=9.8$ Hz and $^2\text{J}=8.2$ Hz, 1H), 7.18 (d, $\text{J}=7.8$ Hz, 1H), 7.50-7.58 (m, 2H), 8.23-8.29 (m, 2H), 8.53 (d, $\text{J}=8.4$ Hz, 1H).


The yield is 69 % as white crystals. \(^1\)HNMR (400 MHz, CDCl$_3$, $\delta$ ppm): 2.0 (S, 3H), 2.08 (S, 3H), 2.12 (S, 3H), 2.16 (S, 3H), 3.8-3.9 (m, 1H), 4.14 (d, $\text{J}=6.4$ Hz, 1H), 4.28-4.3 (dd, $^1\text{J}=2.2$ Hz and $^2\text{J}=2.2$ Hz 1H), 4.65 (d, $\text{J}=7.6$ Hz, 1H), 4.95 (t, $^1\text{J}=7.2$ Hz and $^2\text{J}=6.8$ Hz,1H), 5.15 (t, $^1\text{J}=7.8$ Hz and $^2\text{J}=5.6$ Hz, 1H) 5.25 (t, $\text{J}=8.2$ Hz and $^2\text{J}=6.4$ Hz 1H).
Fig. S02. (b) $^1$H NMR (CDCl$_3$, 400 MHz) for P$_4$.

S03. Characterization of C$_1$
Fig. S03: (c) $^1$H NMR (CDCl$_3$, 400 MHz) (d) $^{13}$C NMR (CDCl$_3$, 400 MHz) (e) HRMS for C$_1$. 
S04. Characterization of P₅

Fig. S04. (f) ¹H NMR (CDCl₃, 400 MHz) (g) ¹³C NMR (CDCl₃, 400 MHz) (h) HRMS for P₅.
S05. Characterization of P₆

Fig. S05. (i) ¹H NMR (CDCl₃, 400 MHz) (j) ¹³C NMR (CDCl₃, 400 MHz) (k) ESI MS for P₆.
S06. Characterization of L

Fig. S06: (l) $^1$H NMR (CD3OD, 400 MHz) (m) $^{13}$C NMR (CD3OD, 400 MHz) (n) HRMS for L.
Fig. S07: (p) $^1$H NMR (CD$_3$OD, 400 MHz) (q) $^{13}$C NMR (D$_2$O, 400 MHz) (r) HRMS for L$_1$.  

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S07. Characterization of L$_1$
S08. Fluorescence studies of L with amino acids

Fig. S08 Fluorescence spectra obtained for the titration of L [5 µM, λ_ex = 360 nm] with different amino acids in HEPES buffer pH at 7.4.
**S09** Determination of Limit of Detection (LOD) of Cys by L

![Image](image_url)

**Fig. S09** (a) Fluorescence spectral traces of L during titration with Cys to determine LOD. (b) The linear dynamic fluorescence response for the titration of L with Cys to determine the detection limit (LOD). The LOD was derived by using the formula $3\sigma/k$ where $\sigma$ = standard deviation of the blank (10 blank samples) and $k$ = is the slope of linear calibration curve.

**S10. Comparison of the detection limits of recently developed fluorescent probes for Cys in the literature.**

<table>
<thead>
<tr>
<th>Probe</th>
<th>Detection medium</th>
<th>Detection Limit (M)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image_url" alt="Probe1" /></td>
<td>Tris-HCl buffer</td>
<td>$100\times10^{-6}$</td>
<td><em>Bioorg. Med. Chem. Lett</em>. <strong>2008</strong>, <em>18</em>, 2246</td>
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<td><img src="image_url" alt="Probe2" /></td>
<td>HEPES Buffer:DMSO (20:80)</td>
<td>$2.13\times10^{-5}$</td>
<td><em>Org. Lett.</em>, <strong>2013</strong>, <em>15</em>, 3630–3633</td>
</tr>
<tr>
<td><img src="image_url" alt="Probe3" /></td>
<td>CH$_3$CN:H$_2$O: DMSO (79:20:1)</td>
<td>$5\times10^{-7}$</td>
<td><em>Org. Biomol. Chem.</em>, <strong>2012</strong>, <em>10</em>, 1966</td>
</tr>
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<td><img src="image_url" alt="Probe4" /></td>
<td>CH$_3$CN:H$_2$O</td>
<td>$4.19\times10^{-7}$</td>
<td><em>Analyst</em>, <strong>2013</strong>, <em>138</em>, 7169–7174</td>
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<tr>
<td></td>
<td>CH$_3$OH/H$_2$O (4:1)</td>
<td>4×10$^{-7}$</td>
<td>Org. Biomol. Chem., 2011, 9, 3844</td>
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<tr>
<td></td>
<td>HEPES buffer: CH$_3$CN (70:30)</td>
<td>7×10$^{-8}$</td>
<td>RSC Adv., 2013, 3, 11543–11546</td>
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<td></td>
<td>HEPES buffer (100 %)</td>
<td>2.5×10$^{-7}$</td>
<td>Present work</td>
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S11. Histogram for the competitive amino acid titrations of [L] with amino acids

**Fig. S11.** Fluorescence spectra obtained for the competitive titration of L (5µM) with Cys in presence of different amino acids (200µM). (a) Histogram showing the fluorescence response of L at 550 nm band when titrated with different amino acids. (b) Visual fluorescent color change of {L+Amino acids} +Cys} with different amino acids under 365 nm UV-light.
S12. Fluorescence spectra for the titration of [L] with different molecular weight thiols

Fig. S12: Fluorescence spectra obtained for the titration of L [5 µM, \( \lambda_{\text{ex}} = 360 \text{ nm} \)] with molecular weight thiols in HEPES buffer at pH 7.4; (a) MPA, (b) TAA, (c) Hcy, (d) DTT_{red} and (e) GSH_{red}.

S13. Fluorescence spectra for the titration of [L] with oxidized state of BSA, HSA and GSH

Fig. S13: Fluorescence spectra obtained for the titration of L (5 µM, \( \lambda_{\text{ex}} = 360 \text{ nm} \)) in HEPES buffer at pH 7.4 with oxidized state of (a) BSA, (b) HSA and (c) GSH_{OX}.

S14. Fluorescence spectra for the titration of [L] with reduced state of BSA, HSA and GSH

Fig. S14: Fluorescence spectra obtained for the titration of L (5 µM, \( \lambda_{\text{ex}} = 360 \text{ nm} \)) in HEPES buffer at pH 7.4 with reduced state of (a) BSA, (b) HSA and (c) GSH.
S15. Absorption spectra obtained during the titration [L] with different amino acids

**Fig. S15.** UV-Visible spectral traces obtained during the titration of **L** (10µM) with different amino acids in HEPES buffer at pH 7.4.
S16. Absorption spectra for the titration of [L] with oxidized forms of GSH

**Fig. S16.** UV-Visible spectral traces obtained during the titration of L (10µM) with GSH$_{ox}$ in HEPES buffer at pH 7.4.
Fig. S17. ESI-MS spectral titration of L with Cys in CD$_3$OD-D$_2$O (1:1): (a) [L] followed by ‘n’ equivalents of Cys, where, (a) n=0, (b) n= 1, (c) n = 2.5, (d) n = 5 and (e) n = 10.