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Integrating glucosyl moiety with the reporting group through linker resulting in conjugates suitable for ion and molecular recognition: Critical analysis of our own demonstrations

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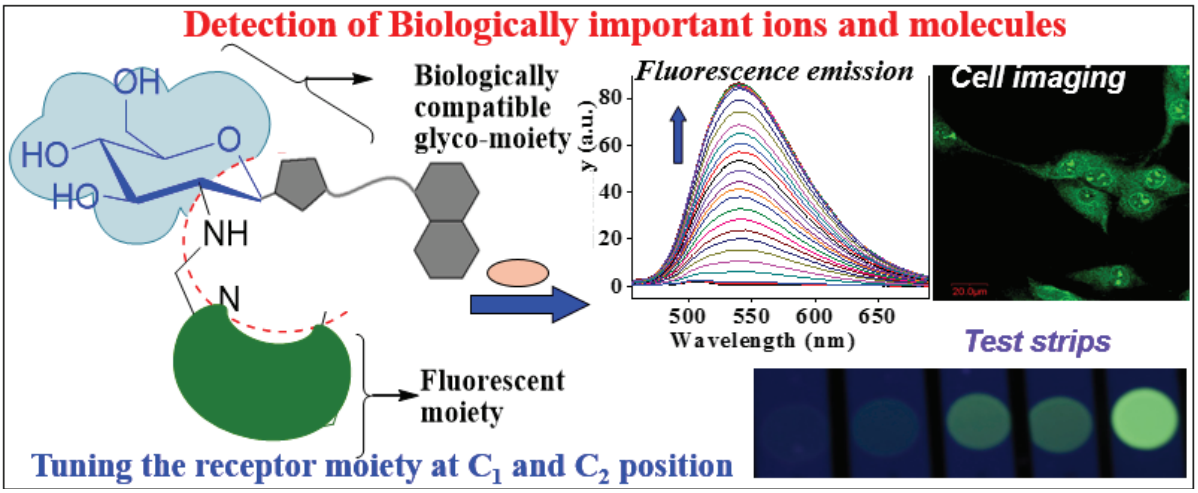
Graphical Abstract



Abstract

This perspective article prepared based on the doctoral thesis work of one of the authors mainly focuses to provide a comprehensive and comparative view of designing glucosyl-based molecular systems possessing binding cores to act as receptors for ions and molecules in solution and on solid surface and to provide cellular imaging in demonstrating their practical applicability.

Key words: Glucosyl-conjugates; control molecules; selective recognition; cation…π interaction; recognition on solid support; DFT computations; fluorescence imaging of cells.

Introduction

Carbohydrates are an important class of biomolecules which received tremendous focus in the recent times due to their involvement in a number of biological functions in living organisms.[1-5] Hence, synthetically modified glyco-moieties resulting in glyco-conjugates would be of great relevance and importance owing to their water solubility and biological compatibility in the selective recognition of ions and molecules. [6-11] In the recent times, the researchers were involved in introducing various functional moieties to generate binding cores suitable for ions and molecular species.[12-17] Moreover, only limited receptors based on carbohydrate are known in the literature for ion and molecular recognition.

Since carbohydrate derivatives have active roles in chemistry and biology,[18-20] our aim is to explore some of

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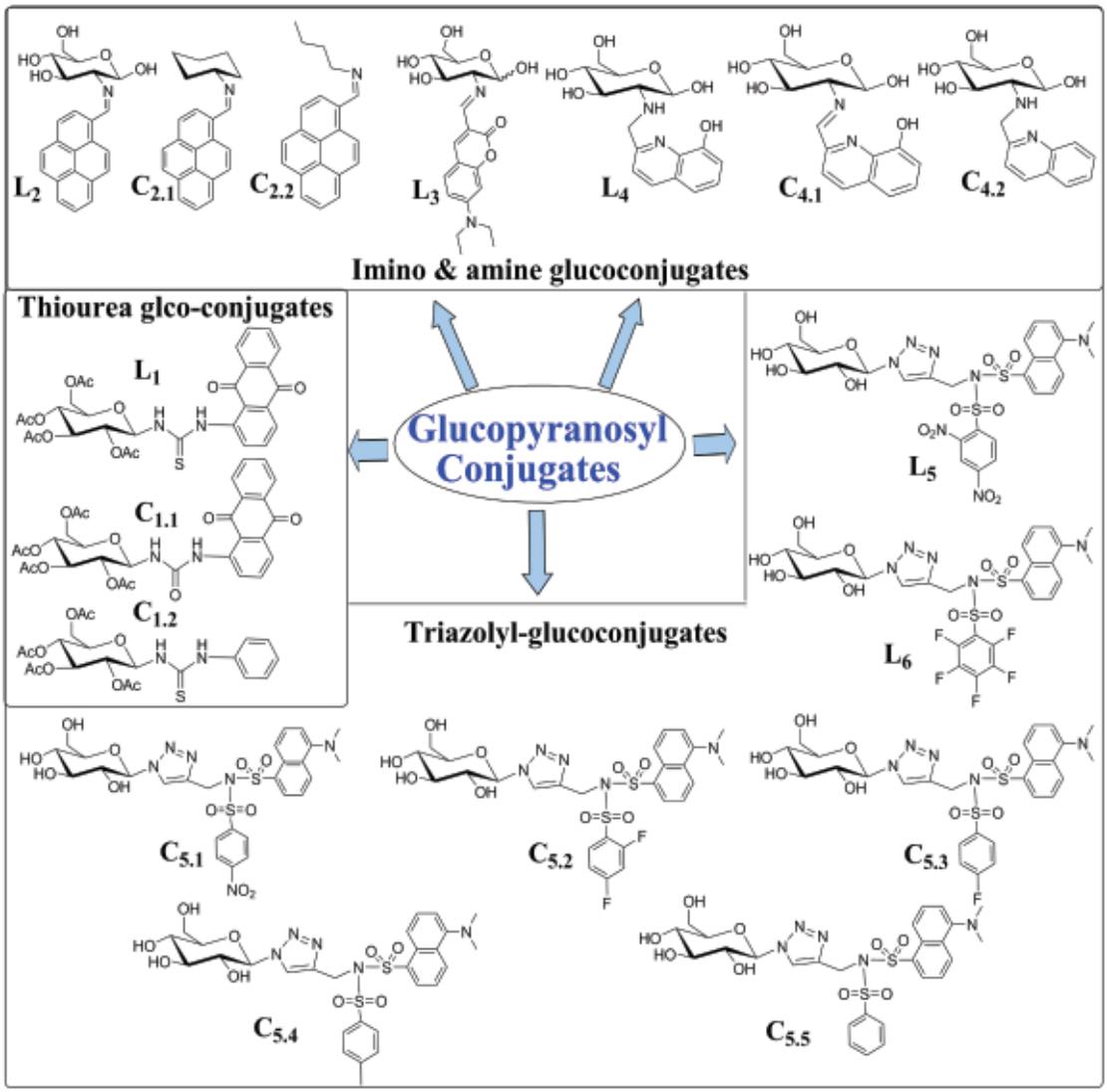
the interesting characteristics of appropriately functionalized carbohydrates. Therefore, C-1 and C-2 derivatives of glucose were chosen for the studies. The advantage of the carbohydrate conjugates over the other derivatives is their ability to have water solubility, easy derivatizability at various positions and capability to provide a pre-organized binding core towards various guest species or molecules. The feasibility of the synthesis and high yield of C-1 and C-2 derivatives of glucose make them easy to synthesize these derivatives in larger quantities and hence all the present studies were based on such aspects.

Need for the functionalization of carbohydrates: The presence of high pK_a OH groups, the stereo-centers, and the existence of anomeric equilibria in carbohydrates are unfavourable for the complexation.[21] Further, these have least affinity to coordinate to ions and to interact with molecular species.[22] Therefore, the modification of the glyco-moiety is necessary to enhance its binding affinity towards ions and molecular species. Our recent efforts resulted in the design and synthesis of several derivatives of glucose possessing variety of binding cores, a reactive center and a moiety to provide appropriate signal while all these are connected to either C-1 and C-2 position of the glucosyl moiety through imine, amine and triazole linkers, so that the resulting conjugates would act on the guest species. The necessity and the importance of the glucosyl-moiety and the receptor group have been addressed in comparison with that of the control molecules, where the latter are those which do not possess either the glucosyl-moiety or the receptor moiety as can be noticed from Scheme 1.

Characterization: All the glucopyranosyl conjugates and its precursor molecules have been well characterized by several techniques, such as, ^{1}H and ^{13}C NMR, FT-IR and ESI MS. The anomerization of all the conjugates (α- or β-) were established by ^{1}H and ^{13}C NMR, and in some cases by single crystal XRD studies. The coupling constants obtained from ^{1}H NMR spectra further supported the anomeric nature.



Scheme 1. Schematic representation of all the glucosyl-conjugates discussed in this article.

Results and discussions

All the corresponding glucosyl conjugates reported in this article have been divided into three different categories for convenience based on the nature of their connectivity to the glucosyl moiety. These are, the thiourea based gluco-conjugates, imino/amino-gluco-conjugates and the triazole based gluco-conjugates. The utility of each of these categories in ion and molecular recognition was addressed.

Gluco-conjugates based on the thiourea moiety: The receptor molecule, $\mathbf{L_1}$ and the control molecules, *viz*., $\mathbf{C_{1.1}}$ and $\mathbf{C_{1.2}}$ possesses glucose moiety but differ in their linker or aromatic group as anthraquinone.[23] These are integrated into the gluocose *via* thiourea or urea link as shown in Scheme 1. Therefore, the results obtained from the receptors are interpretable in comparison with the studies carried out using the control molecules, $\mathbf{C_{1.1}}$ and $\mathbf{C_{1.2}}$. Such comparisons will ascertain the role of the glucose moiety, thiourea and the anthraquinone group (Figure 1a-c). The F^- titrations of the control molecules, viz., $\mathbf{C_{1.1}}$ and $\mathbf{C_{1.2}}$ exhibit fluorescence increase of about 4 and 1.5 fold respectively and hence their sensitivity is much lower than that observed for $\mathbf{L_1}$ (Figure 1b). Thus the sensitivity of $\mathbf{L_1}$ towards F^- is higher by >3 and >10 times when compared to $\mathbf{C_{1.1}}$ and $\mathbf{C_{1.2}}$ respectively, supporting that both the thiourea and anthraquinone are important in the detection.

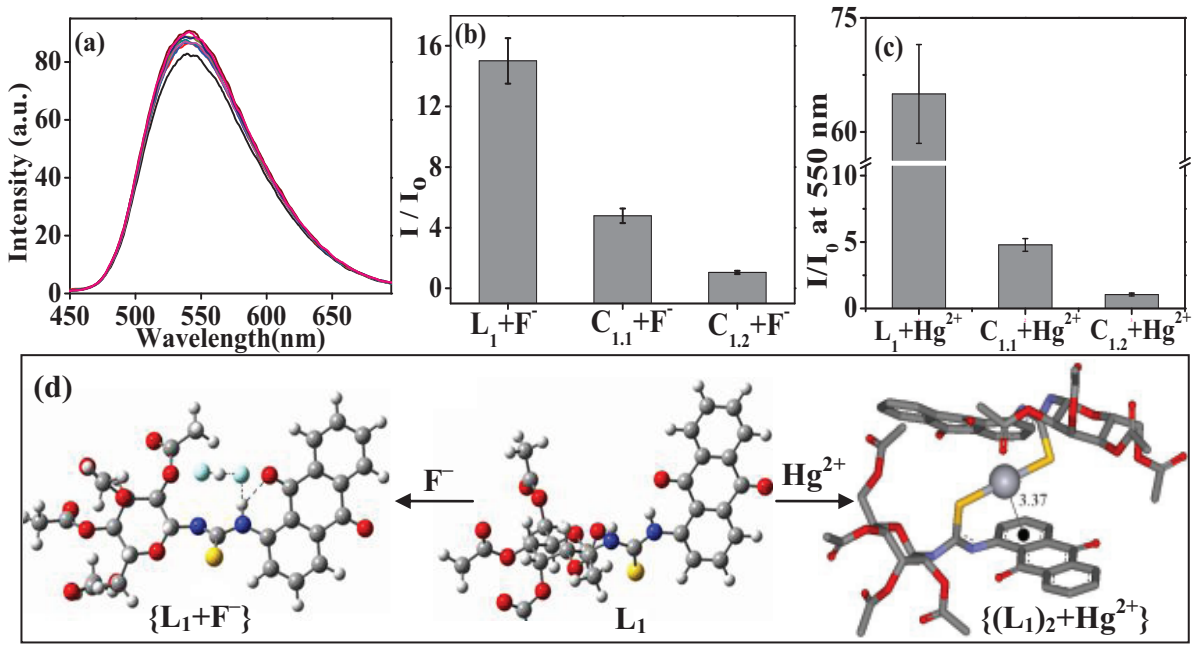


Figure 1. (a) Fluorescence spectral traces for the titration of $\mathbf{C_{1.2}}$ (5 μM) with Hg^{2+} in *HEPES buffer* (λ_{ex} = 430 nm). Comparative histogram of relative fluorescence intensity (I/I_0) at 510 nm for the titration of $\mathbf{L_1}$, $\mathbf{C_{1.1}}$ and $\mathbf{C_{1.2}}$ with (b) F^- and (c) Hg^{2+}. (d) DFT optimized structures of $\mathbf{L_1}$ and its complexes with F^- and Hg^{2+}.

Similar fluorescence titrations carried out between the control molecules ($\mathbf{C_{1.1}}$ and $\mathbf{C_{1.2}}$) and Hg^{2+} (λ_{ex} = 430 nm) did not exhibit any significant change in their fluorescence intensity, suggesting that $\mathbf{C_{1.2}}$ is not a suitable receptor for this ion and hence the thiourea unit present in $\mathbf{L_1}$ is essential for the recognition.[24] All the results indeed provide an in-depth information regarding the functioning of $\mathbf{L_1}$ in the detection of F^- and Hg^{2+}. There is a mechanistic difference of $\mathbf{L_1}$ in sensing F^- *vs* Hg^{2+}. In the case of fluoride, the F^- initially interacts through H-bonding with the –NH groups and finally goes as HF_2^- species. However, at lower mole ratios of Hg^{2+}, it interacts with thiourea (C=S) of $\mathbf{L_1}$ to give 1:2 complex (Figure 1d).[24] Thus the interaction, complexation and mechanistic features of Hg^{2+} sensing differs from that of F^- altogether, suggesting that $\mathbf{L_1}$ act as a dual receptor that extends to both the cation and anion recognition as can be understood from Figure 1.

Receptor molecules based on imine/amine moiety: The glucopyranosyl conjugates possessing imine and amine moieties have been synthesized and their schematic structures are given in Scheme 1. All these derivatives exhibit different binding cores and can be used as receptors for various metal ions. For example the binding cores present in $\mathbf{L_3}$ are 'NO_2' which includes imine nitrogen (imine-N), coumarine oxygen (O^-) and glucose C-1-OH and hence act as a tridentate ligand. Similarly, the available binding cores in $\mathbf{L_2}$ and $\mathbf{L_4}$ are different from that of $\mathbf{L_3}$.

Three glucosyl-conjugates, *viz.*, $\mathbf{L_2}$, $\mathbf{L_3}$ and $\mathbf{L_4}$ differ either in their aromatic moiety or in their linker moiety or both (Scheme 1). These have been studied for their metal ion recognition using fluorescence and absorption spectroscopy in aqueous *HEPES buffer*. The

conjugate **L**$_3$ recognizes Cu^{2+} in aqueous *HEPES buffer* by exhibiting a 95% fluorescence quenching in pH range 7 to 10 even in the presence of several biologically and ecologically relevant metal ions (Figure 2a).[25] In the complex, each **L**$_3$ uses imine nitrogen, keto oxygen of coumarine and C3-O^- group of glucosyl-moiety for Cu^{2+} binding. Each copper center is tetra-coordinated to result in a distorted square planar geometry bound by 'NO_3' core as determined by the DFT computational study (Figure 2d).

However, the **L**$_3$ and **L**$_4$ are non-selective towards Hg^{2+} in aqueous *HEPES buffer*, whereas **L**$_2$ showed ratiometric enhancement in the fluorescence emission at 500 nm selectively towards Hg^{2+} (Figure 2b).[26] The selectivity is due to the stacking of two pyrenyl groups present in the complex followed by exhibiting the excimer emission. Thus the results of ^{1}H-NMR titration and computational studies suggest that the Hg^{2+} interacts with both the imine moiety (by binding) as well as the aromatic moiety (through cation…π interaction). All this clearly supports a sandwiched Hg^{2+} between the pyrene moieties of two receptor molecules through cation…π interactions as shown in Figure 2e.

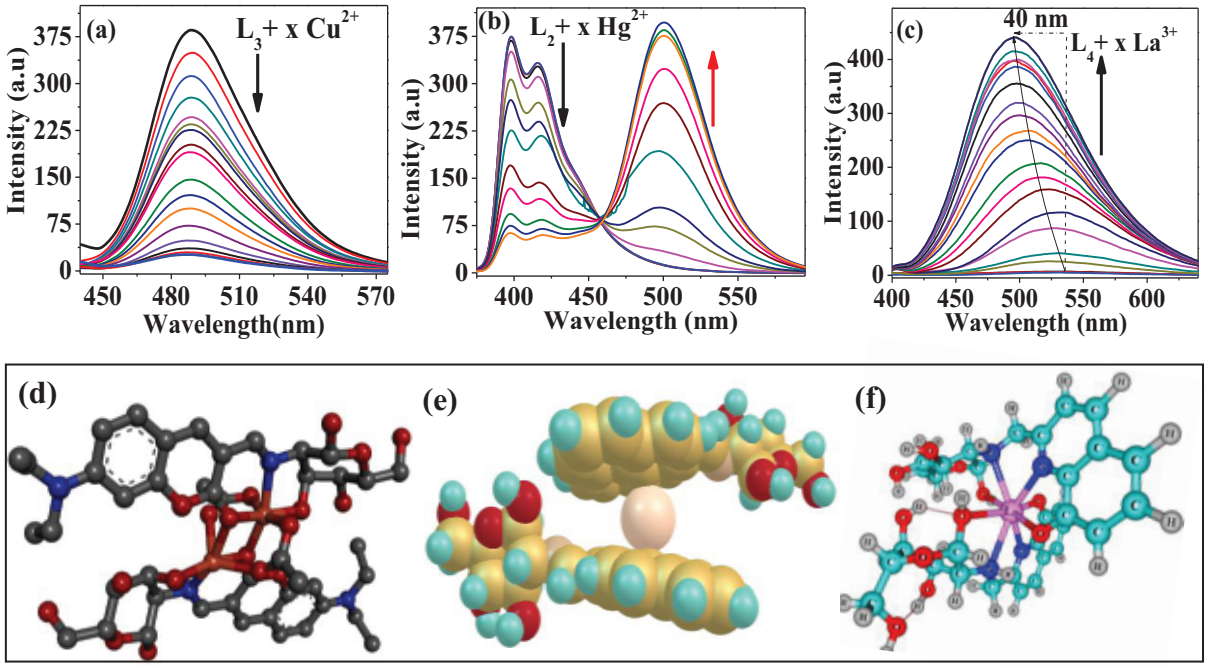


Figure 2. Fluorescence spectra obtained during the titration of (a) **L**$_3$ with Cu^{2+} (x = 0 to 12 mole ratio), (b) **L**$_2$ with Hg^{2+} (x = 0 to 10 mole ratio) and (c) **L**$_4$ with La^{3+} (x = 0 to 20 mole ratio) in aqueous HEPES buffer at pH = 7.4. Optimized structures of (d) {Cu+(**L**$_3$)$_2$}, (e) {Hg(**L**$_2$)$_2$} and (f) {La(**L**$_4$)$_2$} by DFT method.

The receptor **L**$_4$ showed selectivity towards La^{3+} ion based on the fluorescence study that is associated with a 40 nm blue shift in the λ_{em} and about 70 fold enhancement in the intensity (Figure 2c).[27] However, the other lanthanide and transition metal ions exhibited no significant enhancement. The selectivity could be attributed to the presence of several donor atoms in **L**$_4$ to coordinate the La^{3+} ion. The optimized structure exhibits octa-coordination about La^{3+} with 'N_4O_4' binding core arising from two **L**$_4$ ligands and it fits to a distorted trigonal dodecahedral geometry (Figure 2f). In this, each **L**$_4$ acts as a tetradentate ligand by extending an 'N_2O_2' core where the bound oxygen centers are from quinoline and C1-OH of the carbohydrate moiety.

The control molecules, viz., **C**$_{2.1}$ and **C**$_{2.2}$ differ from **L**$_2$ by having a non-coordinating cyclohexyl or *n*-butyl moiety in place of the carbohydrate unit. The presence of the carbohydrate moiety imparts more sensitivity in terms of fold and biocompatibility to **L**$_2$. Therefore, the sensitivity of **L**$_2$ towards Hg^{2+} is twice as that of the control molecules as demonstrated using fluorescence spectroscopy (Figure 3a).

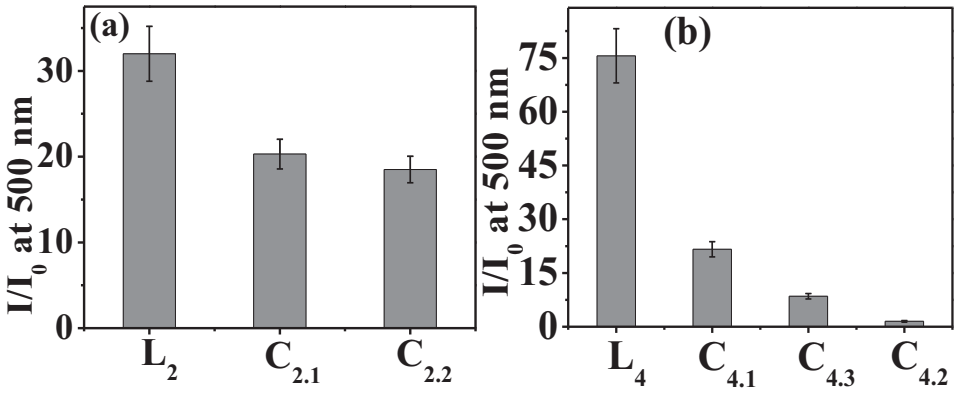


Figure 3. (a) Histogram showing the number of folds of fluorescence enhancement (I/I_0) in the titration of $\mathbf{L_2}$, $\mathbf{C_{2.1}}$ and $\mathbf{C_{2.2}}$ with Hg^{2+}. (b) Histogram showing the relative fluorescence intensity of $\mathbf{L_4}$, $\mathbf{C_{4.1}}$, $\mathbf{C_{4.2}}$ and $\mathbf{C_{4.3}}$ with La^{3+}.

In order to demonstrate the importance of the functional groups and the glucosyl moiety present in $\mathbf{L_4}$, the control molecules $\mathbf{C_{4.1}}$, $\mathbf{C_{4.2}}$ and $\mathbf{C_{4.3}}$ were synthesized in order to understand the binding nature of La^{3+} ion with $\mathbf{L_4}$. Fluorescence titration of $\mathbf{C_{4.2}}$ and $\mathbf{C_{4.3}}$ with La^{3+} shows about 1.5 and 10±1 times increase in the fluorescence intensity (Figure 3b). Similar fluorescence studies were also carried out using $\mathbf{C_{4.1}}$, the sensitivity of $\mathbf{L_4}$ towards La^{3+} is twice as that of the control molecule, $\mathbf{C_{4.1}}$. Comparison of all these results supports the necessity of both the quinoline-hydroxyl moiety and the amine group for binding, and the glucose moiety to impart water solubility and biocompatibility in the sensitive recognition of La^{3+}.

Gluco-conjugates based on the triazole moiety: Two receptor molecular systems, *viz.*, $\mathbf{L_5}$, $\mathbf{L_6}$, and five control systems, viz., $\mathbf{C_{5.1}}$, $\mathbf{C_{5.2}}$, $\mathbf{C_{5.3}}$, $\mathbf{C_{5.4}}$, and $\mathbf{C_{5.5}}$ (Scheme 1) all possessing triazole link were synthesized and characterized.[28-29] All these derivatives have been designed to possess different electrophilic centers and further were connected by a glucosyl- moiety that is derivatized with a fluorogenic center possessing dansyl group through triazole moiety. The presence of differently substituted benzenesulfonyl moieties in the derivatives provides an efficient reactive center for ions and molecules for nucleophilic substitution which results in fluorescence enhancement. All the three derivatives exhibit weak fluorescence because of intramolecular charge transfer (ICT) that involves the fluorophore to the electron deficient moiety. The electrophilicity decreases as the number of electron withdrawing groups present on the aromatic moiety of the receptor decrease.

The conjugate $\mathbf{L_5}$ recognizes Cys selectively by exhibiting enhancement in the fluorescence intensity by 125 times, among all the naturally occurring amino acids in HEPES buffer at physiological pH (Figure 4a).[28] Both the nitro-derivatives, viz., $\mathbf{C_{5.1}}$ and $\mathbf{L_5}$ were studied for CN^- reactivity and found enhancement in the fluorescence intensity by 15±2 and 200±25 times (Figure 4b). When one looks at the reactivity of $\mathbf{L_5}$ towards other anions, such as the molecules possessing thiol moiety, the Cys shows higher sensitivity. However, the $\mathbf{L_6}$ does not undergo any reaction by Cys or other -SH molecules, supporting that the $\mathbf{L_6}$ is a better probe for CN^- possessing high selectivity.[29] In addition, other derivatives such as $\mathbf{C_{5.2}}$, $\mathbf{C_{5.3}}$, and $\mathbf{C_{5.4}}$ which have neither the fluorine nor the nitro group were also studied. The study also includes a derivative possessing an electron releasing $-CH_3$ group ($\mathbf{C_{5.5}}$).

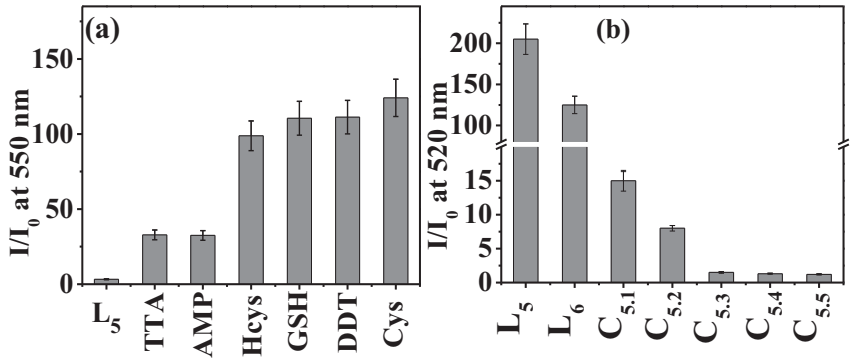


Figure 4. (a) Histogram showing the relative fluorescence intensity at 550 nm for the reaction different thiol molecules and proteins with $\mathbf{L_5}$ (5 μM, at λ_{ex} = 360 nm, pH = 7.4). (b) Histogram showing the relative fluorescence intensity of different molecular probes at 520 nm in presence of CN^- (5 μM, at λ_{ex} = 360 nm, pH = 7.4).

In $\mathbf{C_{5.2}}$, only 6±1 times fluorescence enhancement was observed with CN^- ion because of the presence of semi fluorinated moiety (2, 4 di-fluorobenzene) (Figure 4b). Similar titrations were carried out with the control molecules possessing one and no carbon bound fluorine, viz., $\mathbf{C_{5.3}}$ and $\mathbf{C_{5.4}}$ and found no significant change in the fluorescence intensity during the addition of Cys or CN^- ion. All this supports that by tuning electron withdrawing groups one can achieve the selective recognition of biologically and environmentally important analytes (Figure 4b).

Ion recognition on solid support: The conjugates are used as molecular sensors for various analytes in solution where they form a complex or the reaction triggers resulting in an enhancement in the fluorescence intensity. In order to use such receptors for the detection of analytes in various samples routinely, an easy, sensitive, inexpensive and *use-and-throw* method has been developed by coating silica gel sheets or Whatman cellulose filter paper strips with the conjugate. For example, increasing concentration of Hg^{2+} were added to $\mathbf{L_1}$ coated on silica gel sheets using a micropipette to result in different mole ratios $[Hg^{2+}]/[\mathbf{L_1}]$ in the range 0 to 4 and the sheets were allowed to dry. The corresponding fluorescence spectra and color exhibited by these samples under UV light are shown in Figure 5a. The receptor $\mathbf{L_1}$ exhibits weak fluorescence emission centered at 540 nm similar to that observed in solution. The presence of Hg^{2+} shifts the emission peak by 8 nm towards blue and shows an overall enhancement by 11±1 times (Figure 5b).[24] The minimum detection limit of 285 ± 15 and 345 ± 17 ppb were observed with $\mathbf{L_1}$ on silica gel sheets for the Hg^{2+} present in *HEPES buffer* and in blood serum solutions respectively.

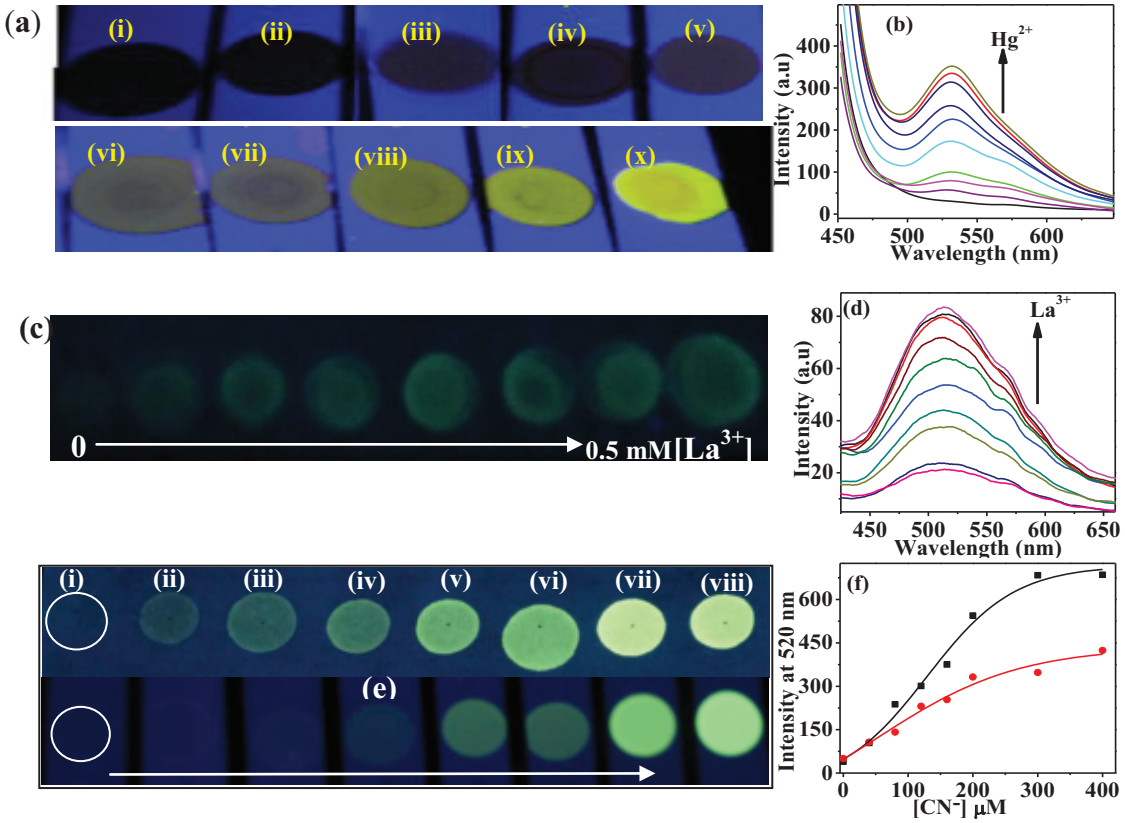


Figure 5. (a) Fluorescence titration carried out using the $\mathbf{L_1}$ with increasing concentration of Hg^{2+} from (i)-(x) coated silica gel sheets support under 365 nm light. (b) Fluorescence spectra obtained from these under (a). (c) Photograph of all the samples used in the titration on Whatman filter paper strips under 365 nm UV light. (d) Fluorescence spectra obtained from these under (c). (e) Photographs taken under UV light (365 nm) of $\mathbf{L_6}$ coated Whatman No.1 cellulose filter paper (upper panel) and silica gel strips (lower panel) upon addition of increasing concentration of CN^- from (i) to (viii). (f) Plot of emission intensity *vs*. concentration of CN^- at 520 nm of those given under (e) (black corresponds to Whatman cellulose filter paper and red corresponds to the silica gel strips).

The corresponding fluorescence spectra and color exhibited by the samples using $\mathbf{L_4}$ under UV light are shown in Figure 5c. The conjugate $\mathbf{L_4}$ exhibits increase in the emission intensity at 510 nm as the La^{3+} concentration increases (Figure 5d). As the fluorescence intensity ratio plot is fairly linear in the range of 10 – 100 µM of La^{3+} it can provide a lowest detection of 10 ± 1 µM (1.3 ppm) for La^{3+} on a Whatman filter paper.[26] Further we have

demonstrated the detection of CN^- using **L_6** coated Whatman No.1 cellulose filter paper and silica gel strips with different sensitivities.

The fluorescence intensity ratio plot is fairly linear in 10 – 250 μM of CN^-, the detection of CN^- by fluorescence is practically feasible using Whatman No.1 cellulose filter paper strips and silica gel sheet in this concentration range (Figure. 5e).[29] By using these **L_6** coated Whatman No.1 cellulose filter paper and silica gel strips, the concentrations as low as 10 μM (270 ppb) and 25 μM (670 ppb) respectively of CN^- is detectable under UV light by naked eye (Figure 5f).

Cellular imaging: Complexation or nucleophilic trigger results in the fluorescence enhancement or quenching in solution. Further studies were extended to biological cells in order to find the utility of gluco-conjugates in imaging Hep G2 or HeLa cells by fluorescence microscopy. For example, Hep G2 cells incubated with **L_3** [10 μM] showed high intracellular green fluorescence and the presence of Cu^{2+} showed quenching in fluorescence emission suggesting that Cu^{2+} [20 μM] is responsible for the same in the cells (Figure 6a,b).[25] Similarly the Hep G2 or HeLa cells treated with 10 μM of the conjugate **L_4** displayed feeble intracellular fluorescence as can be seen from Figure 6c. To the same, when [20 μM] concentrations of exogenous La^{3+} were added, the cells exhibited intense green fluorescence emission (Figure d), suggesting that La^{3+} is responsible for enhancing the fluorescence intensity of **L_4** in living cells. [26]

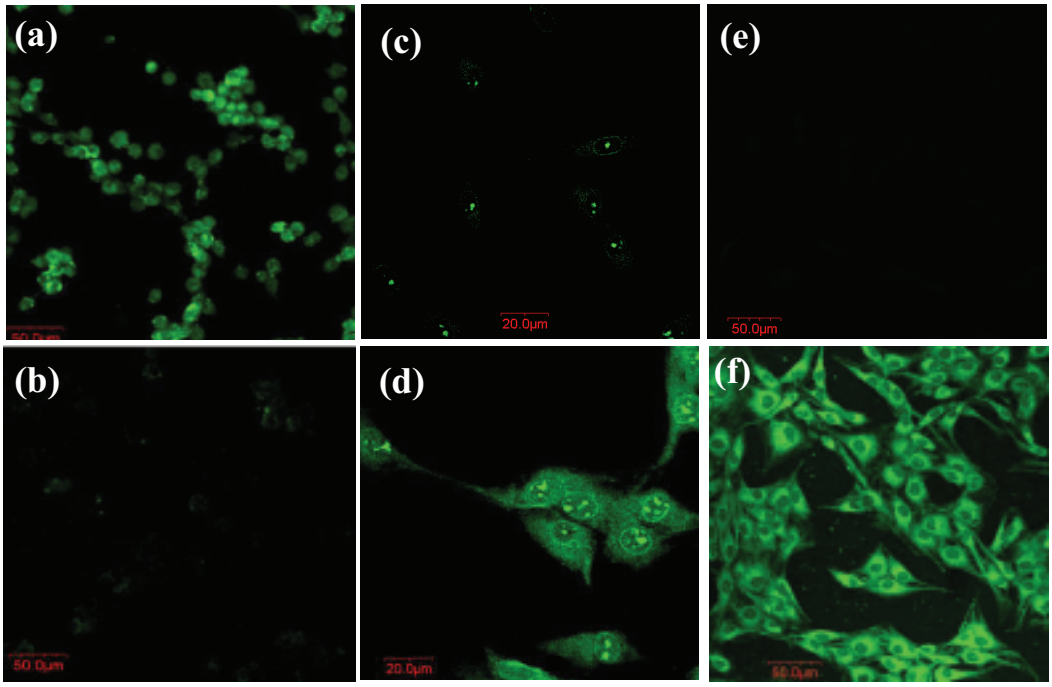


Figure 6. Fluorescence images obtained from the Hep G2 cells (excitation at ~430 nm and emission at ~490 nm) upon treatment in PBS buffer at pH = 7.4: (a) treated with **L_3**(10 μM); (b) cells treated with **L_3** followed by 20 μM of Cu^{2+} solution; (c) cells incubated with probe **L_4** (10 μM) for 30 min; (d) cells treated with **L_4** (10 μM) followed by La^{3+} (15 μM) for 1 h; (e) HepG2 cells and (f) upon treatment with the probe **L_5** (10 μM).

The conjugate probe, **L_5** exhibited fluorescence intensity upon reaction with Cys, since cellular concentration of Cys is higher and hence this can be detected *in cells* by fluorescence microscopy. The HepG2 cells were incubated in PBS buffer (pH = 7.4) containing 10 μM of the conjugate **L_5** for 30 min at 37 ºC, the cells exhibited effective intracellular green fluorescence emission owing to the reaction of intracellular thiols on **L_5** (Figure 6e,f).[28] In a control experiment, the cells did not exhibit any significant fluorescence emission upon pre-treatment with an excess of thiol- consuming reagent, viz., *N*-ethylmaleimide (NEM). All these results clearly support that gluco-pyranosyl conjugates are effective cellular imaging agents and are useful in the detection of different analytes. The HepG2 and HeLa cells are viable up to 50 μM and 12 hr and were affected to only a smaller extent beyond this and hence the gluco-pyranosyl conjugates are biologically compatible probes.

Critical analysis and perspectives

This article addresses some interesting features of **C-1** or **C-2**-glucopyranosyl conjugates obtained by connecting the glucosyl- moiety that is derivatized with a fluorophore having ion binding core or electrophilic center through different linkers. The recognition properties of these derivatives have been found to change abruptly upon slight modification of the ligating groups and thereby

suggesting that the control lies on the chemical modification. All the conjugates were extensively characterized by various techniques. Metal ion recognition properties of $\mathbf{L_1}$, $\mathbf{L_2}$, $\mathbf{L_3}$ and $\mathbf{L_4}$ have been explored. Upon doing so, the detection of several bio-relevant metal ions was achieved and this includes, naked eye detection of La^{3+} in *HEPES* buffer by $\mathbf{L_4}$, fluorescence sensing of Cu^{2+} by $\mathbf{L_3}$ (over $\mathbf{L_1}$ and $\mathbf{L_2}$), Hg^{2+} by $\mathbf{L_1}$ and $\mathbf{L_2}$. The stoichiometry of the species formed *in situ* were established by ESI MS.

The conjugate $\mathbf{L_1}$ exhibits selective chromogenic as well as fluorescent chemosensor property towards F^- by a fluorescence enhancement of 13 times upon binding with F^-. $\mathbf{L_5}$ and $\mathbf{L_6}$ have been studied for amino acid and anion recognition property and achieved selective detection of Cys and CN^- respectively among naturally occurring amino acid and several anions supporting their specific nucleophilic attack. The reactivity of $\mathbf{L_5}$ towards Cys shows 120±10 times higher sensitivity among the amino acids studied. It also it exhibits fluorescence enhancement by 210 times towards CN^- ion. However, the $\mathbf{L_6}$ does not undergo any reaction by Cys or other -SH molecules and exhibits only selectively towards CN^- with a fluorescence enhancement of 125 times, supporting that $\mathbf{L_6}$ is a better probe for CN^- possessing high selectivity. Thus appropriate chemical modification(s) brought on the derivative can alter the selectivity and hence acts as a crucial parameter to play in order to tune the receptor properties. Therefore, the glycoconjugates have been successfully designed for the selective detection of several biologically relevant and/or toxic metal ions as well as anions. The probe molecules, viz., $\mathbf{L_2}$, $\mathbf{L_4}$ and $\mathbf{L_6}$ have been demonstrated for their selective sensing of various analytes in *HEPES buffer* solution, on thin layer silica gel and Whatman filter paper by fluorescence spectroscopy. The utility of these conjugates was also demonstrated through cellular imaging study. The importance of the glucosyl unit, fluorescent moiety and the linker group in the conjugates for the selective recognition of analytes was explored by carrying out the studies with appropriate control molecular systems.

Thus, this article deals with some new contributions made from our research group in the field of ion and molecular recognition and supramolecular chemistry by appropriately derivatizing either at the C1- or at C2- position of the glucosyl moiety and by studying its ion and molecular recognition properties. The utility of these conjugates has been demonstrated to detect various analytes on thin layer silica gel, Whatman cellulose filter paper strips and in living cells. Thus, all these conjugates generated by chemical medication brought using the glucosyl platform are potentially useful in detecting various analytes even in biologically relevant medium by supporting the chemical group fine tuning as the game changer in their receptor properties.

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References

1. Varki, A. *Glycobiology*, **1993**, *3*, 97–130.
2. Bertozzi, C. R.; Kiessling, L. L. *Science*, **2001**, *291*, 2357–2364.
3. Paulson, J. C.; Blixt, O.; Collins, B. E. *Nat Chem Bio.,* **2006**, *2*, 238–248.
4. Hart, G. W.; Copeland, R. J. Cell, **2010**, *143*, 672–676.
5. He, X-P.; Zeng, Y-L.; Zang, Y.; Li, J.; Field, R. A.; Chen, G-R. *Carbohydr. Res.*, **2016**, *429*, 1-22.
6. Shi, D-T.; Zhou, D.; Zang, Y.; Li, J.; Chen, G-R.; James, T. D.; He, X-P.; Tian, H. *Chem. Commun*., **2015**, *51*, 3653-3655.
7. Ke, B.; Chen, W.; Ni, N.; Cheng, Y.; Dai, C.; Dinh, H.; Wang, B. *Chem. Commun.,* **2013**, *49*, 2494-2496.
8. Dong, L.; Zang, Y.; Zhou, D.; He, X-P.; Chen, G-R.; James, T. D.; Li, J. *Chem. Commun*., **2015**, *51*, 11852-11855
9. Mitra, A.; Mittal, A. K.; Rao, C. P. *Chem. Commun*., **2011**, *47,* 2565–2567.
10. Shi1, D-T.; Wei, X-L.; Sheng, Y.; Zang, Y.; He, X-P.; Xie, J.; Liu, G.; Tang, Y.; Li, J.; Chen, G-R. *Sci. Rep.*, **2014**, *4*, 4252-4258.
11. Li, K-B.; Zang, Y.; Wang, H.; Li, J.; Chen, G-R.; James, T. D.; He, X-P.; Tian, H. *Chem. Commun*., **2014**, *50*, 11735-11737.
12. Singhal, N. K.; Mitra, A.; Rajsekhar, G.; Shaikh, M. M.; Kumar, S.; Guionneau, P.; Rao, C. P. *Dalton Trans*., **2009**, 8432–8442.
13. Tang,Y-H.; Qu, Y.; Song, Z.; He, X-P.; Xie, J.; Hua, J.; Chen, G-R. *Org. Biomol. Chem*, **2012**, *10*, 555-560.
14. Diwan, U.; Kumar, A.; Kumar, V.; Upadhyay, K. K.; Roychowdhury, P. K. *Sensors and Actuators B*., **2014**, *196*, 345–351.
15. Hemamalinia, A.; Das, T. M. *New J. Chem*., **2013**, *37*, 2419-2425.
16. Mitra, A.; Hinge, V. K.; Mittal, A.; Bhakta, S.; Guionneau, P.; Rao, C. P. *Chem. Eur. J*., **2011**, *17*, 8044 – 8047.
17. Yang, Y-K.; Shim, S.; Tae, J. *Chem. Commun*., **2010**, *46*, 7766–7768.
18. Varki, A. C.; Esko, J. D.; Freeze, H. H.; Stanley, P.; Bertozzi, C. R.; Hart, G. W.; Etzler, *Cold Spring Harbor*, New York, **2009**
19. Lee, Y. C.; Lee, R. T. *Acc. Chem. Res*., **1995**, *28*, 321−327.
20. Albersheim, P.; Valent, B. S. *J. Cell Biol*., **1978**, *78*, 627–643.
21. Bowen, M. L.; Orvig, C. *Chem. Commun.,* **2008**, 5077-5091.
22. Ozker, K.; Collier, B. D. *Nucl. Med. Commun*., **1999**, *20*, 1055–1058.
23. Areti, S.; Khedkar, J. K.; Chilukula, R.; Rao, C. P. *Tetrahedron Lett*., **2013**, *54*, 5629-5634.
24. Areti, S.; Yarramala, D. S.; Samanta, K.; Hinge, V. K.; Khedkar, J. K.; Rao, C. P. *RSC Advances*., **2014**, *4*, 16290-16297.
25. Areti, S.; Khedkar, J. K.; Bandaru, S.; Teotia, R.; Bellare, J.; Rao, C. P. *Anal. Chim. Acta*., **2015**, *873*, 80-87.
26. Areti, S.; Hinge, V. K.; Rao, C. P. *Carbohydr. Res*., **2014**, *399*, 64-69.
27. Areti, S.; Bandaru, S.; Teotia, R.; Rao, C. P. *Anal. Chem*., **2015**, *87*, 12348–12354.
28. Areti, S.; Bandaru, S.; Teotia, R.; Rao, C. P. *Analyst*, **2015**, *140*, 7391–7398.

29. Areti, S.; Bandaru, S.; Yarramala, D. S.; Rao, C. P. *Anal*. *Chem*., **2015**, *87*, 12396–12403.