



1-(D-Glucopyranosyl-2'-deoxy-2'-iminomethyl)-2-hydroxybenzene as chemosensor for aromatic amino acids by switch-on fluorescence

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ABSTRACT

A glucose based C2-glyco-conjugate, that is, 1-(D-glucopyranosyl-2'-deoxy-2'-iminomethyl)-2-hydroxybenzene (**L**), has been synthesized in a high yield and characterized. Titration of **L** with all the 20 naturally occurring amino acids resulted in a large fluorescence intensity enhancement only in case of aromatic amino acids, that is, Phe, Trp, His, and Tyr and not with the others. This has been attributed to the initial formation of 1:1 hydrogen bonded complex followed by π - π interactions present between the aromatic moieties of such complexes as demonstrated by absorption and computational methods. Thus **L** has been able to recognize aromatic amino acids down to 1.5–3 ppm through switch-on fluorescence behavior.

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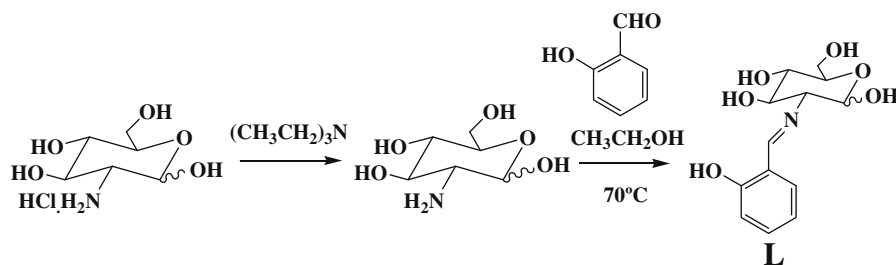
The side chains of amino acids are known to provide functional stints to the peptides and proteins that in turn drives the biological processes.^{1,2} The deficiency of some of these amino acids causes various abnormalities, for example, deficiency of cysteine results in slow growth, hair depigmentation, edema, lethargy, liver damage, muscle and fat loss, and skin lesions.³ Therefore, sensing amino acids in bio-fluids is an important task. Thus, the development of chemosensors which selectively recognizes an amino acid is of considerable current interest.⁴ Though, some of the literature-reported molecular and/or metal ion-based receptors exhibit optical response of absorption or emission type,^{5–8} these suffer from their complexity in the synthesis and their bio-compatibility. Thus the thrust continues to be in the direction of the development of bio-compatible receptors which can be synthesized with ease and in high yields. Among such molecules, those which can exhibit emission provide better sensitivity. To our knowledge, there have been no reports in the literature for the selective recognition of amino acids by carbohydrate derivatives. However, our recent work demonstrated certain non-selective amino acid recognition by C1-modified galactosyl-imine (Leu, Pro, His, Glu) and -amine derivatives respectively (Cys, Ala, Lys).⁹ Amino acid–glyco-conjugate interactions are indeed wide-spread in the field of lectin–carbohydrate (substrate) complexes, as the lectins are selective towards specific carbohydrate moiety. Therefore, in the present Letter we report, for

the first time, a glucosyl-C2-based conjugate, that is, 1-(D-glucopyranosyl-2'-deoxy-2'-iminomethyl)-2-hydroxybenzene '**L**' for the selective recognition of aromatic amino acids among the 20 naturally occurring ones by absorption, emission and mass spectrometry and the species of recognition has been modeled by computational calculations.

A carbohydrate based receptor system, **L**, was synthesized in one step of condensation reaction carried out in ethanol between glucosamine and salicylaldehyde as given in Scheme 1 to result in 87% yield.¹⁰ **L** has been characterized by a number of techniques, including, ¹H and ¹³C NMR, ESI-MS, IR, and elemental analysis (SI 01).¹¹ In order to explore the sensitivity and selectivity of **L** towards amino acids, fluorescence titrations were carried out against all 20 naturally occurring ones by adding incremental concentrations of the corresponding amino acid to **L**.

Only the aromatic amino acids exhibited appreciable enhancement in the emission intensity¹² which increases as a function of the mole ratio of amino acid added and saturates beyond 2 equiv. All the other amino acids exhibited almost no or marginal changes in the intensity. From the relative fluorescence intensity (I/I_0) versus mole ratio plot given for this titration in Figure 1a, it is clear that all the four aromatic amino acids, that is, Phe, Trp, His, and Tyr, show maximum enhancement in the fluorescence intensity. The observed that fluorescence changes can be attributed to the formation of 1:1 complex species between the amino acid and **L** initially through hydrogen bonding, however, the large enhancement observed with these amino acids can be explained by the

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Scheme 1. Synthesis of receptor **L** from glucosamine hydrochloride using triethylamine and salicylaldehyde in ethanol at 70 °C.

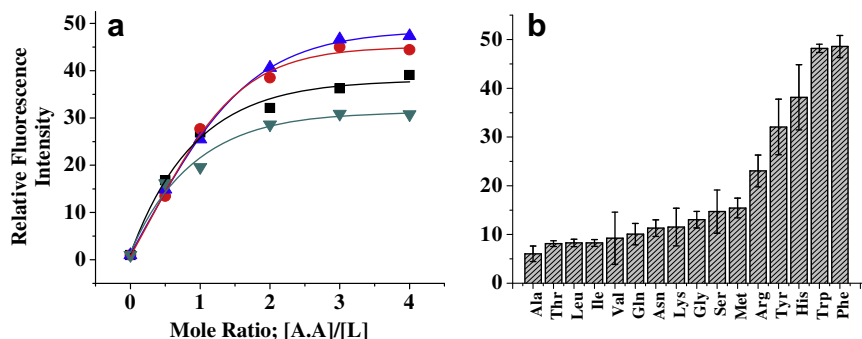


Figure 1. (a) Plot of relative fluorescence intensity (I/I_0) versus $[A.A]/[L]$ mole ratio. (b) Bar diagram indicating number of times of fluorescence enhancement observed with all the 20 amino acids. Symbols are, ■ = His; ● = Phe; ▲ = Trp; ▼ = Tyr.

additional interactions exhibited only in the case of aromatic ones and not by the others (Fig. 1b).

The association constants (K_a) have been derived based on fluorescence intensity using Bensi–Hildebrand equation, and the K_a values were found to be $\sim 19,400 \pm 600$ for the aromatic amino acids. Two other non-aromatic amino acids, that is, alanine and arginine, exhibited K_a values of 3360 ± 225 and 1930 ± 150 , respectively, and these are about one-sixth to one-tenth of that observed for the aromatic ones. Thus the aromatic amino acids combine with **L** by a factor of 5–10 times higher in affinity as compared to the non-aromatic ones. The lowest detection range for the aromatic amino acids by **L** has been found to be 1.5–3.0 ppm as studied based on fluorescence spectroscopy at various concentrations by maintaining a 1:1 ratio between **L** and the amino acid (Fig. 2).

In order to understand the involvement of the $-\text{COOH}$ function during the interaction with **L**, fluorescence titrations were carried out with amino acids where the carboxylic ($-\text{COOH}$) moiety has

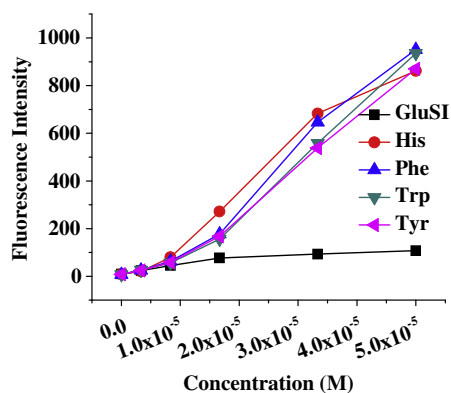


Figure 2. Concentration versus fluorescence intensity plots for aromatic amino acids measured while maintaining a 1:1 mole ratio for $[\text{amino acid}]/[\text{L}]$. Symbols are ■ = GluSI; ● = His; ▲ = Phe; ▼ = Trp; ▼ = Tyr.

been converted to the methylester ($-\text{COOCH}_3$) moiety and the corresponding results are shown in Figure 3a. In case of the esters of amino acids (viz., blocked amino acids), enhancement in the fluorescence intensity has been found to be higher as compared to the amino acids having free carboxylic moiety, clearly suggesting the involvement of the carboxylic group in bringing variation in the fluorescence intensity of **L** during the interaction. This has been clearly seen in the case of Asp and Glu where there was indeed a decrease in the fluorescence of **L** owing to the presence of two such $-\text{COOH}$ functions. The fluorescence enhancement is higher in the case of amino acids having aromatic side chain perhaps suggesting the involvement of π – π interactions during the titration of **L** with amino acids which in turn has been supported based on absorption and computational studies. In order to support that it is only the aromatic amino acid and not any aromatic carboxylic derivative that is effective in enhancing the fluorescence of **L**, the titration studies were carried out with three different aromatic carboxylic derivatives, that is, 3-phenylpropionic acid, phenylacetic acid and benzoic acid and found that there is no significant enhancement observed in the fluorescence intensity in the case of these three molecules as can be noted from the histogram given in Figure 3b (SI 05). This clearly suggested that **L** recognizes only the aromatic amino acids and not their corresponding aromatic acids.

In order to support the binding of amino acids to **L**, absorption titrations were carried out. The amino acids, that is, Asp and Glu, which exhibited almost no change in the emission intensity, also exhibited no change in the absorption spectra of the receptor molecule (SI 03). In all other cases, (Fig. 04, SI 03a and SI 03b) a new band appears at 352 nm suggesting the formation of a complex between **L** and the amino acid. The conclusions derived from the absorption were similar to that observed from the fluorescence studies. However, in the case of the aromatic amino acids, in addition to the appearance of this new band at 352 nm, the absorbance of 214 nm band diminishes as a function of the concentration of added amino acid (SI 03a) and this follows a trend, that is, $\text{Trp} \gg \text{Phe}, \text{His}, \text{Tyr}$. No change in the absorbance was observed

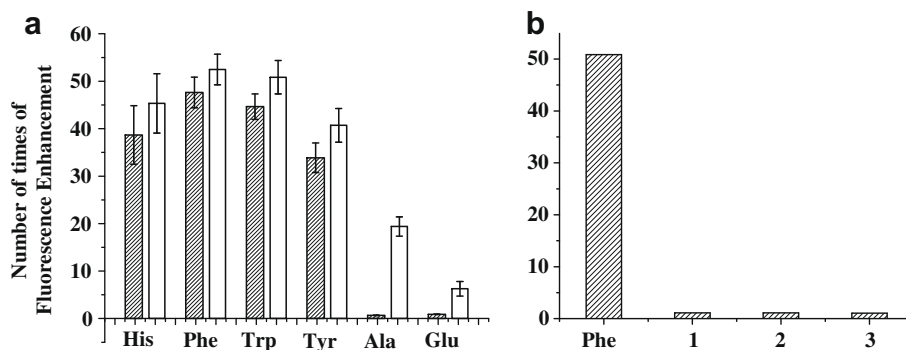


Figure 3. Histograms showing the number of times of fluorescence enhancement of **L** when titrated against: (a) amino acids (shaded) and their corresponding methyl esters (unshaded) and (b) aromatic carboxylic acid derivatives, that is, benzoic acid (1); phenylacetic acid (2); and 3-phenylpropionic acid (3).

in the 214 nm band with other amino acids (Fig. 4a), supporting the involvement of π - π interaction between **L** and the aromatic side chain of these amino acids. Recently, we have demonstrated similar absorption spectral changes to support the π - π interactions present between aromatic amino acids and naphthyl moiety of the Zn^{2+} complex of calix[4]arene-conjugate.¹³ The reversibility of this chemosensor ensemble has been demonstrated by titrating the system with ethidium bromide, which is a well known DNA intercalator, reverses the conjugated species formed by quenching the fluorescence intensity (SI 06).

The stoichiometry of the species formed between **L** and Phe or Trp has been found through MALDI-TOF mass spectra by observing the peaks at $m/z = 473$ and 472 , respectively. These peaks seem to fit well with the formation of (**L** + Phe + Na) and (**L** + Trp-H₂O + 3H⁺) species.

In order to understand the interactions present between **L** and the aromatic amino acids, computational studies were carried out in a systematic fashion by starting from semi empirical, then going through ab initio followed by DFT, in a cascade fashion. As the formation of 1:1 species was already established by MALDI-TOF-MS and supported by the Benesi-Hildebrand plot (SI 04), the 1:1 species were optimized using GAUSSIAN 03 package.¹⁴ Prior to assuming the initial guess model for computational calculations, **L** and the amino acids, that is, Phe, Trp, His, and Tyr, were independently optimized by using different theories in the said cascade fashion. The corresponding complexes of **L** with these amino acids were made by simply placing the amino acid far away from **L** in such a way that the side chain of amino acid is pointed towards the salicyl moiety of **L**. Then the complexes were also optimized in a cascade fashion by going through AM1HF/STO-3GHF/3-21GHF/6-31GB3LYP/3-21GB3LYP/6-31G and results of the com-

plexes obtained at B3LYP/6-31G were used in the discussion. At B3LYP/6-31G, the complexes of **L** with Trp, Phe, and His were mainly formed through the interaction of the carboxylic and amine moiety of the amino acid with **L**, though the interactions were present in different ways. Thus, the complex of **L** with Trp has been stabilized through two hydrogen bond interactions (Fig. 5a) formed between the carbonyl and amine functions of Trp with C1-OH and pyranosyl ring oxygen of the carbohydrate moiety respectively, forming a 9-atom ring (C=O...H-OC1 and HNH...O_{pyranose}). However, in the complex of **L** with Phe, there is only one HNH...O_{pyranose} hydrogen bond present (Fig. 5b). In the case of His complex of **L**, the -C=O and -NH₂ groups of His form hydrogen bonds with C1-OH and C3-OH, respectively (HNH...OHC₃ and C=O...HOC₁) through the formation of an 11-atom ring (Fig. 5c). On the other hand, the complex of Tyr with **L** has been stabilized through two O-H...O hydrogen bond interactions as can be seen from Figure 5d. At B3LYP/6-31G level, the stabilization energies computed using the formula = $E_c - [E_L + E_{aa}]$ {where E_c is the total energy of the complex, E_L is the total energy of the glyco-conjugate, and E_{aa} is that of the amino acid} yielded -3.9, -10.3, -7.9, and -17.3 kcal/mol, respectively, for the Phe, Trp, His, and Tyr complexes and these are commensurate with the H-bond interactions present in these complexes.

A bio-compatible glyco-conjugate, that is, 1-(D-glucopyranosyl-2'-deoxy-2'-imino-methyl)-2-hydroxybenzene [**L**], has been synthesized in one step in high yields starting from glucosyl-C2-amine and was characterized by analytical and spectral methods including ¹H and ¹³C NMR and ESI mass spectrometry. **L** has been shown to selectively recognize aromatic amino acids, that is, Phe, Trp, His, and Tyr, through several-fold fluorescence enhancement as compared to all other naturally occurring ones. The reasons for this

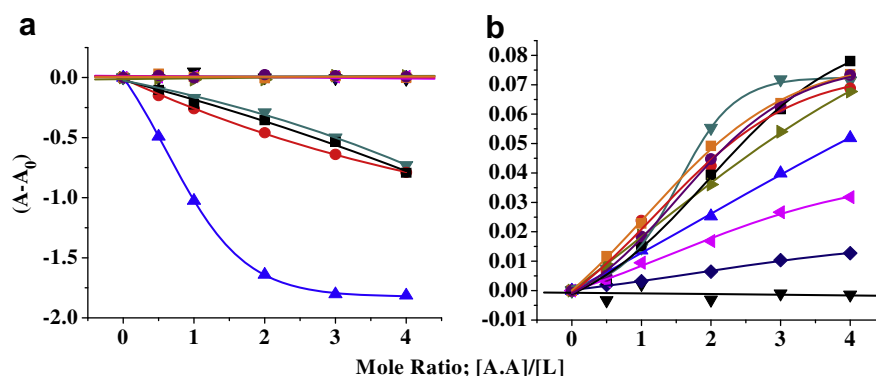


Figure 4. ($A-A_0$) versus mole ratio plots obtained based on the absorption titration of **L** with amino acids: (a) for 214 nm band; and (b) for 352 nm band. Symbols are ▼ = Asp; ◆ = Glu; ▲ = Trp; ▼ = Tyr; ● = Phe; ■ = His; ▲ = Cys; ▲ = Gln; ■ = Lys; ● = Met.

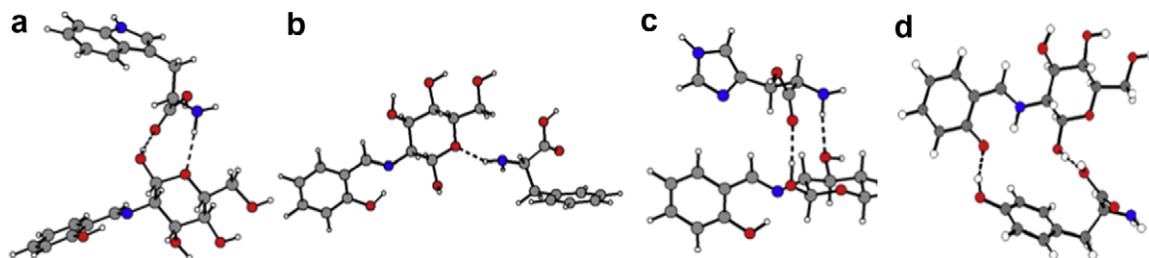


Figure 5. B3LYP optimized structures for the complexes of **L** with, (a) Trp; (b) Phe; (c) His; (d) Tyr.

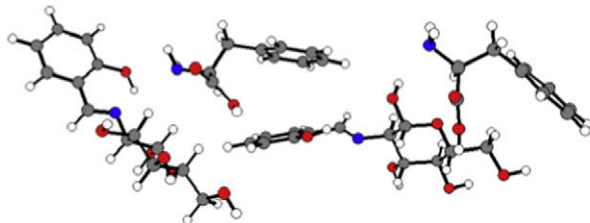


Figure 6. MM+ force field minimized structure for two complexes of Phe with **L**.

has been ascribed to the initial formation of 1:1 hydrogen bonded complex between amino acid and **L** as demonstrated by experimental (absorption and MALDI-TOF) and computational (DFT) methods. When two such 1:1 complexes of Phe with **L** were minimized in MM+ calculations, the resultant structure indeed exhibited π - π interaction between the phenyl moieties of the glyco-conjugate and the aromatic side chain of the amino acid, but the H-bond(s) present in the precursor 1:1 complex were broken, as can be seen from Figure 6. These results indeed take support of the absorption spectral changes observed at 214 nm band. Thus **L** has been shown to selectively recognize aromatic amino acids through switch-on fluorescence and its utility in the context of biomolecular recognition is currently underway in our laboratory.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.10.105.

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- Glucosamine hydrochloride (0.215 g, 1 mmol) salt has been neutralized with triethylamine in ethanol before it is used in the synthesis. To this, salicylaldehyde (0.15 ml; 1 mmol) was added. The reaction mixture was refluxed for 6 h at 60 °C. The solid product formed, **L** (0.25 gm) was filtered and washed with cold ethanol several times followed by diethyl ether at the end which was dried under a vacuum. Yield: 87%. ¹H NMR (DMSO-*d*₆, ppm): 3.25–3.80 (m, 5H, C2–H, C3–H, C4–H, C5–H), 4.54–4.95 (4d, 4H, C1–OH, C3–OH, C4–OH & C6–OH), 5.16–5.18 (d, H, C1–H, ³J_{C1–H, C2–H}, 5.5 Hz), 6.19–7.59 (2d, 2t, 4H, Ar–OH), 8.6 (s, H, CH=N), 13.2 (s, H, Ar–OH). ESI-MS *m/z* = 284 ([M+H]⁺, 100%).
- Fluorescence emission spectra were measured on Perkin-Elmer LS55 fluorescence spectrophotometer by exciting the samples at 320 nm and the emission spectra were recorded in 330–550 nm range. The bulk solution of **L** and amino acids were prepared in methanol in which 400 μ l (4%) of water was added for dissolving initially the amino acid. The bulk solution concentrations were maintained at 1×10^{-3} M. All the measurements were made in 1 cm quartz cell and maintained the effective concentration of **L** as 50 μ M. During the titration different mole ratios of amino acid were added to **L** and the emission of all the samples were measured after 24 h.
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