Phosphonic acid appended naphthalenediimide molecular receptor for saccharides and aminoglycoside antibiotics recognition

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Abstract: Design of symmetric phosphonic acid functionalized naphthalene diimide bolaamphiphile (**NDI 1**) is reported. **NDI 1** based molecular recognition of saccharides and aminoglycoside antibiotics in aqueous media was investigated. UV-vis and fluorescence measurements revealed an efficient protocol for **NDI 1** as molecular receptor. The sensor successfully recognises saccharides and aminoglycoside antibiotics kanamycin and neomycin in terms of both absorbance intensity and binding affinity. This protocol provides new platform for the design and synthesis of phosphonic acid appended NDI sensor for recognition of multifunctional biomolecules.

Keywords: aminoglycoside antibiotics; bolaamphiphile; molecular recognition; saccharides; naphthalene diimide

1. Introduction

Biologically important molecular system such as carbohydrates or saccharides plays a significant role in the metabolic pathways of living systems [1-3]. Carbohydrates are placed on the cell surface to act as sensors and as biochemical signals [4a] The structural diversity of the carbohydrates on the level of complexity far exceeds those of nucleic acids and proteins. Carbohydrates or saccharides are the chemical storage in the plant and is the source as a for

human body. Among the carbohydrates, monosaccharides possesses several stereocentres around each carbon atom creating individual stereoisomers with unique chemical and physical characteristics and are biologically important. In recent years, recognition of carbohydrates by synthetic molecular architecture has gained momentum. In the metabolic pathways of living systems, saccharides playa a key role. Therefore detecting of the biologically important saccharide presence and their concentration in aqueous media is essential in industrial and also medicinal fields [4b,c]. Various synthetic receptors for saccharide detection (galactose, glucose, fructose, ribose, and xylose) [13-15] was used various techniques such as electrochemical [8], colorimetric [10], UV-vis [6], fluorescence emission [5], circular dichroism [7], polymeric [9], surface functionalised sensor [11], and also photoinduced electron transfer [12]. But these methods display some serious drawback in aqueous media. The recognition of saccharides by small molecular receptor based on boronic acid show tremendous growth during the last decade [16]. The molecular architecture with boronic functional group form complexes with saccharides through covalent interaction and represent an important binding force in the detection of saccharides [17]. Recently, the design, synthesis and application of novel chromophores with different functional groups [16-19] for the recognition of saccharides and aminoglycoside antibiotics [22] in aqueous media has gained great importance.

In aqueous media recognition of saccharides and aminoglycoside antibiotics remains an important challenge due to competitive hydrogen bonding by a solvent [23] Literature search revealed that, to date very less examples of saccharide recognition based on phosphonic acid functionalized chromophores are reported [24-26]. It is well documented that P=O functional groups are strong hydrogen bond acceptor and plays a key role in saccharide recognition [27-28]. Rao *et al.* reported the complex formation between phosphate and phosphonate with 1,2-diols in solid state [29]. The binding of fructose to DNA via phosphonate and 1,2-diol

interaction was proposed by Pelmore and co-workers [30]. Molecular receptor based on phosphonic acid functionalised naphthalene diimide for saccharide and aminoglycoside antibiotics are not reported till date [31]. With the aid of better chromophore with hydrogen bonding phosphonic acid functional group, it will possible to develop sensitive sensors to detect saccharides and aminoglycoside antibiotics in aqueous media. Herein, we report design of symmetric bolaamphiphile **NDI 1** as a molecular receptor towards saccharides and aminoglycoside antibiotics. The **NDI 1** bolaamphiphile shows complex formation with monosaccharides and aminoglycosides via non-covalent H-bonding interactions. In hydrophilic environment **NDI 1** exhibits intermolecular π-π stacking interactions, which lead to change in UV-vis absorption and fluorescence emission spectral peak intensities.

2. Experimental Section

A stock solution of bolaamphiphile NDI **1** (2.0 mM) was prepared in H_2O and 20 μL stock solution added to a quartz cuvette and further diluted in 2 mL H_2O (path length = 1 cm) then UV-vis absorption and fluorescence emission was measured by manipulating pH by using either 0.1 N HCl) or NaOH, respectively. The receptor then further titrated with various saccharides and aminoglycoside antibiotics (1×10^{-2} M).

2.1. UV-vis measurements

The UV-vis absorption spectra were recorded using a Shimadzu spectrophotometer (model: UV 1800) at r.t. in the quartz cell with 1.0 cm path length. Furthermore, the spectra's **NDI 1** were recorded in H_2O with saccharides, kanamycin and neomycin at pH 9.0.

2.2. Fluorescence measurements

The fluorescence (FL) was recorded using Schimadzu Fluorescence Spectrophotometer. (model: RF-6000) in H_2O at pH 9 at room temperature with excitation wavelength (λ_{ex} = 384 nm).

3. Result and discussion

In aqueous media the molecular recognition of carbohydrates and aminoglycoside antibiotics leads to give important information. The interaction between **NDI 1** bolaamphiphile with water soluble phosphonic acid head groups offer an important platform for recognition of saccharides and aminoglycoside antibiotics. The recognition study was performed at pH 9, since phosphonic functional group completely deprotonated at this state. The deprotonated phosphonic acid able to interact with carbohydrates and aminoglycoside antibiotics *via* non-covalent H-bonding. Herein, we investigated the molecular recognition characteristics of the bolaamphiphile **NDI 1** against the various monosaccharides such as D- form of galactose, glucose, fructose, lyxose, ribose, xylose as well as aminoglycosides antibiotics (kanamycin and neomycin) as illustrated in Fig. 1. The molecular recognition properties were examined using UV-visible and fluorescence emission spectroscopic techniques.

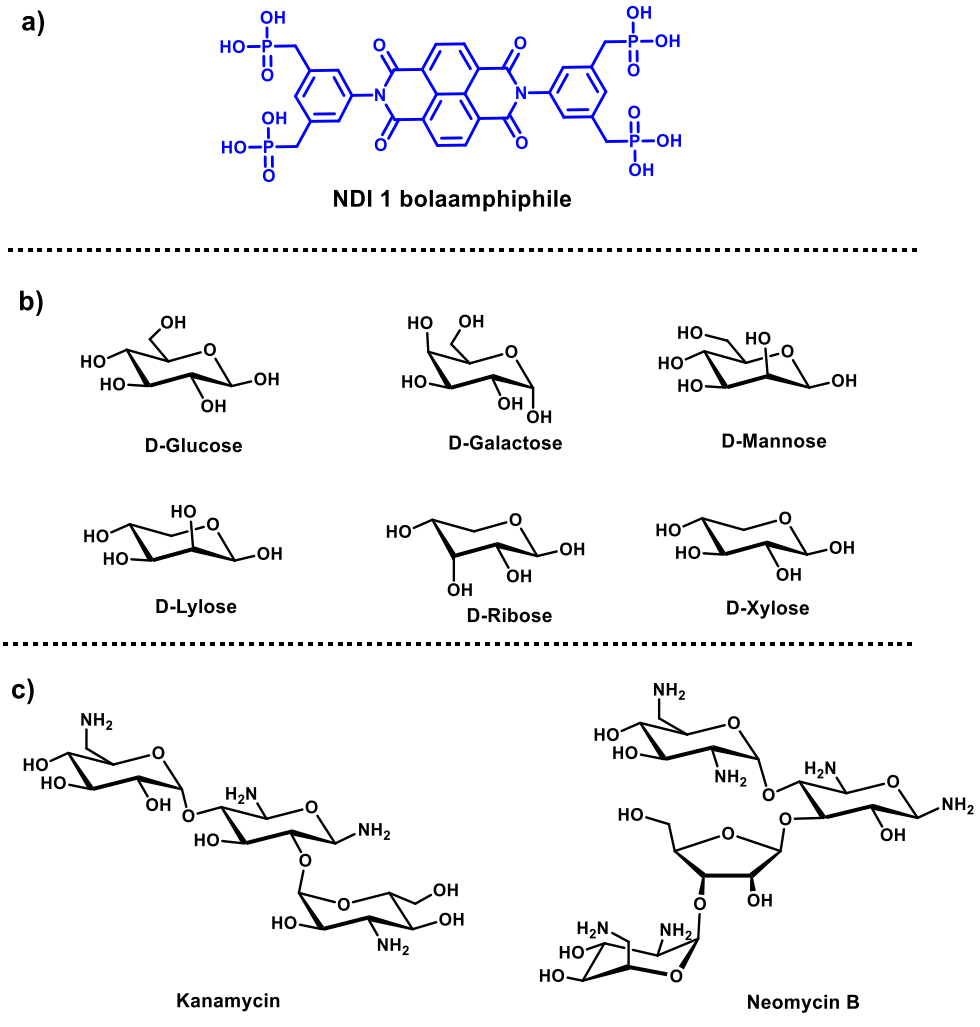

Fig. 1 Molecular structures used in this study: (a) **NDI 1,** (b) monosaccharides and (c) aminoglycosides antibiotics.

3.1. *UV-Visible spectroscopy*

The UV-vis spectroscopic measurements of **NDI 1** bolaamphiphile was performed in aqueous media at pH 9. At room temperature, **NDI 1** bolaamphiphile (1 x 10^{-5} M) exhibits strong two absorption maxima one at 363 nm and second one at 384 nm with small peak at 347 nm which attributed to π–π∗ transition [32]. The titration experiments of **NDI 1** with carbohydrates such as D- form of galactose, glucose, fructose, lyxose, ribose, and xylose and also aminoglycoside antibiotics e.g. kanamycin and neomycin in aqueous media. The concentration of the saccharide is 1 × 10^{-2} M. As illustrated in Fig. 2a, upon addition of D-galactose, peak intensity of the absorption maxima at 363 nm and 384 nm decreases and stabilized with the addition of 20 equiv. of the D-galactose. Furthermore, the addition of other carbohydrate as well as aminoglycoside moieties display the same trend with respective change in absorption at 363 nm and 384 nm (Fig. 2b-f). The changes of the bolaamphiphile **NDI 1** complexes with these sugars *via* non-covalent H-bonding interactions and in water media **NDI 1** undertakes strong π-π intermolecular interactions which lead to change in absorption peak intensities.

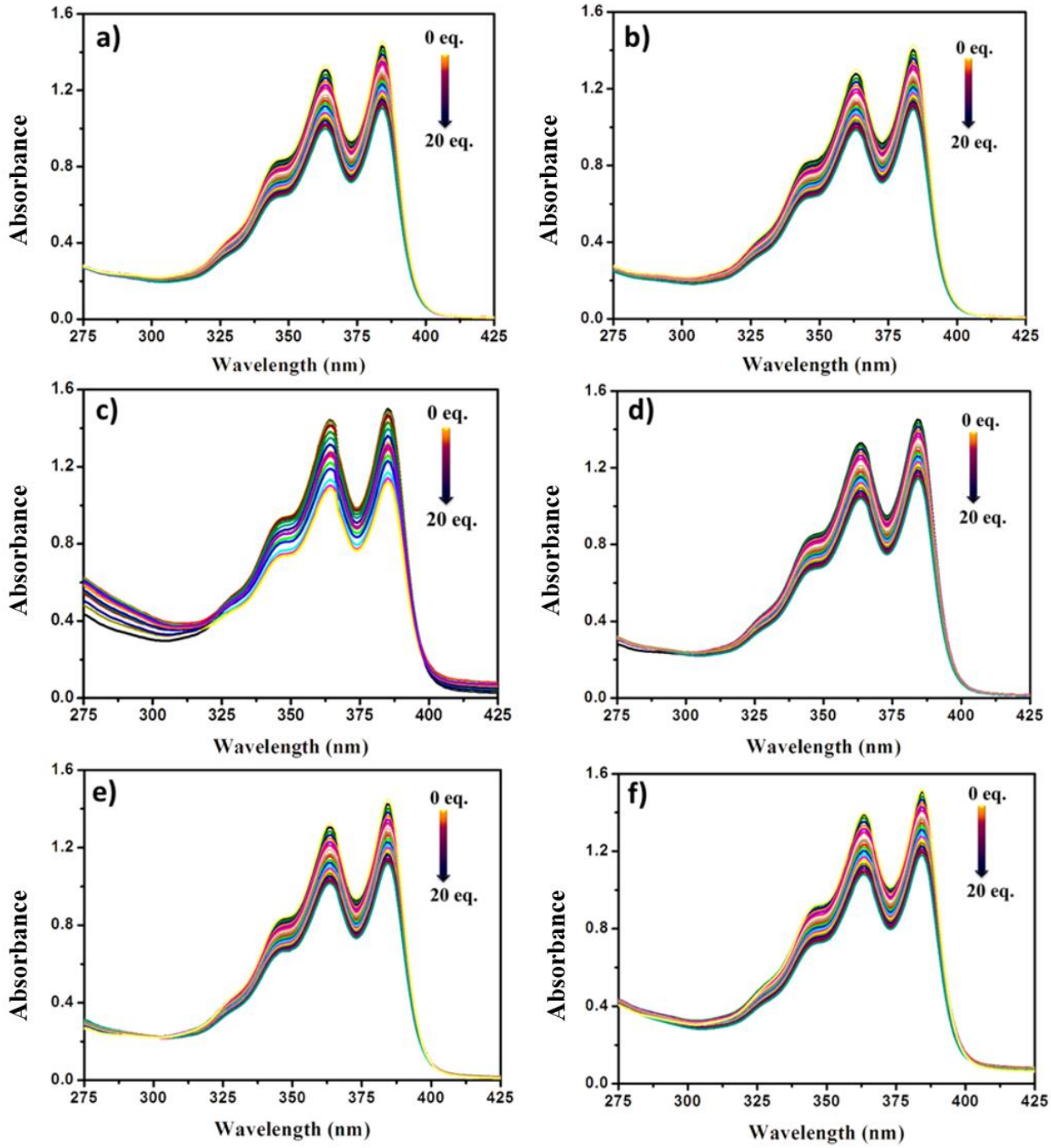

Fig. 2 UV-visible absorption spectra of bolaamphiphile **NDI 1** (1 x 10^{-5} M) at pH 9 in H_2O upon incremental addition of D- form of sugars (1 x 10^{-2} M) as: a) galactose, b) glucose, c) mannose, d) lyxose, e) ribose and f) xylose

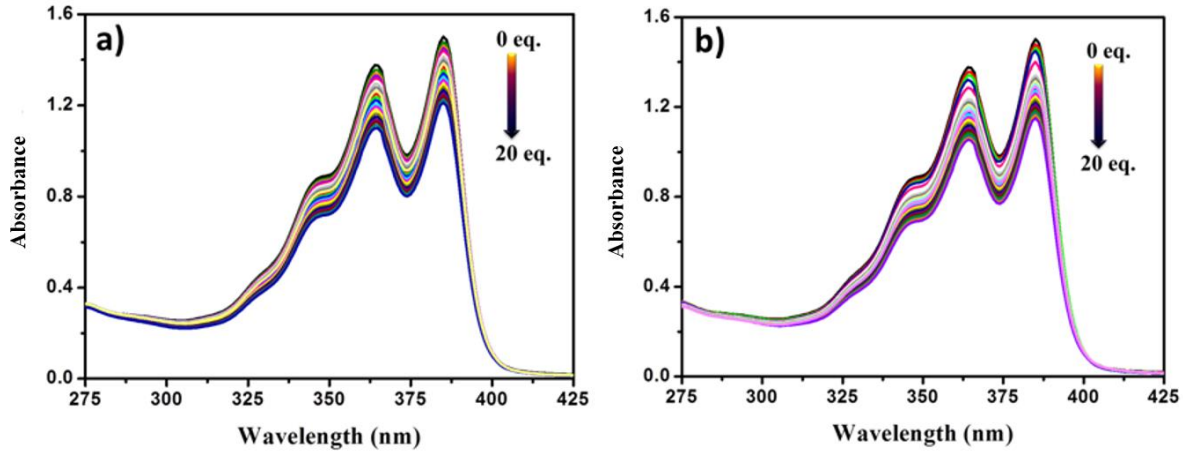

Fig. 3 UV-visible spectrums of **NDI 1** (1 x 10^{-5} M) in H_2O at pH 9 upon addition of a) kanamycin (0-20 equiv., 1 x 10^{-2} M), b) neomycin (0-20 equiv., 1 x 10^{-2} M).

To generalise the recognition ability of NDI 1 bolaamphiphile, we further examined the UV-*via* absorption changes upon addition of kanamycin (0-20 equiv.) and neomycin (0-20 equiv.) at pH 9. The absorption maxima at 363 nm and 384 nm of **NDI 1** with the addition of kanamycin and neomycin display the decrease in intensity as illustrated in Fig. 3a and 3b, respectively. We presume that the deprotonated phosphonic head group may interact with –OH and $–NH_2$ functional groups via non-covalent hydrogen bonding present in kanamycin and neomycin. Thus, UV-vis absorption study indicates the presence of phosphonic head group lead to recognise these carbohydrates and aminoglycosides. Furthermore, we employed UV-vis absorption experiments to determine the binding (association) constant of NDI 1 towards these sugars and aminoglycosides

The binding constant (K_a) of the bolaamphiphile **NDI 1** with the examined carbohydrates and aminoglycoside antibiotics were calculated using UV-vis measurements. As illustrated in Fig. 4a-h, the plot between 1/(A0-A) versus 1/[monosaccharide or aminoglycoside antibiotics) was utilized to calculate the association constant. As summarized in Table 1, the binding constant K_a were obtained from the linear fitted data to the Benesi-Hildebrand equation.

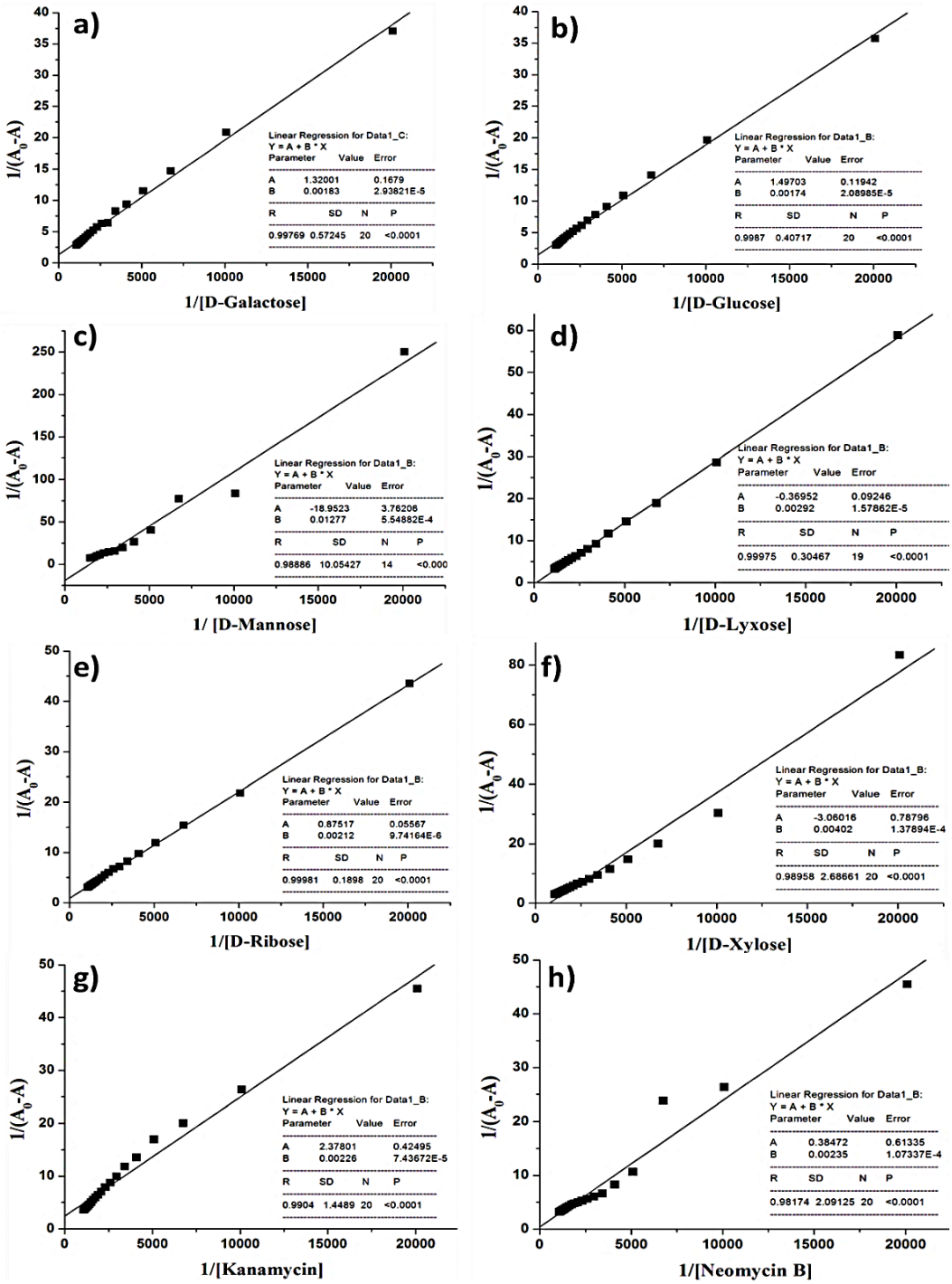

Fig. 4 Benesi-Hilderbrand plot of bolaamphiphile **NDI 1** with D- form of sugars as: a) galactose, b) glucose, c) mannose, d) lyxose, e) ribose, f) xylose as well as antibiotics: g) kanamycin and h) neomycin.

Table 1: Binding constant of bolaamphiphile **NDI 1** with saccharides and aminoglycoside antibiotics in H_2O.

Sr. No.	Carbohydrates/Aminoglycoside antibiotics	Association Constant (K_a)
1	D-galactose	4.13×10^{-2} M
2	D-glucose	3.85×10^{-2} M
3	D-mannose	4.39 x 10 M
4	D-lyxose	9.26×10^{-2} M
5	D-ribose	5.39×10^{-2} M
6	D-xylose	8.12×10^{-2} M
7	kanamycin	1.86×10^{-2} M
8	neomycin A	1.10×10^{-3} M

3.2. *Fluorescence spectroscopy*

The fluorescence emission spectroscopic technique was utilized to study the binding interactions of bolaamphiphile **NDI 1** with monosaccharides at pH 9 in H_2O. The fluorescence emission spectroscopy results of the bolaamphiphile **NDI 1** and their non-covalent interaction with different carbohydrates is illustrated in Fig. 5a-f. The molecular receptor **NDI 1** (1×10^{-5} M) at pH 9 in aqueous media, displays the two strong emission peaks at 414 nm and 547 nm (λ_{ex} = 384 nm). This is attributed to the monomeric form of the bolaamphiphile NDI-1. At first emission spectral changes of **NDI 1** was recorded with the addition of various monosaccharides (20 equiv.). The addition of various monosaccharides to a solution of bolaamphiphile **NDI 1** show significant changes in fluorescence emission intensity (Fig. 5a-f) was resulted. As illustrated in Fig. 5a, with the addition of D-galactose to **NDI 1,** the emission peak at 414 nm increases significantly, whereas, the peak at 547 nm decreases. This changes in fluorescence peak intensity resulted into isosbestic point at 525 nm, indicating the formation of complex.

Similar trend of emission intensity changes was observed for **NDI 1** with the addition of other sugars (Fig. 5b-f). Such change in emission spectra is attributed to the complex formation between **NDI 1** and tested monosaccharides via non-covalent H-bonding interactions.

Furthermore, to generalise the molecular recognition of **NDI 1**, we tested kanamycin and neomycin aminoglycosides. As illustrated in Fig. 6a and 6b, with the addition of kanamycin and neomycin, respectively, **NDI 1** display the change in emission peak, at 414 nm increase in intensity and at 547 nm decrease in peak intensity was observed. Herein, as shown in Fig. 6a and Fig 6b, at 525 nm isosbestic point formation was observed. This suggests that bolaamphiphile **NDI 1** interacts with kanamycin and neomycin via complex formation. This complexation is attributed to the non-covalent hydrogen bonding formation. Thus, the similar trend for molecular recognition of monosaccharides and aminoglycoside antibiotics was observed.

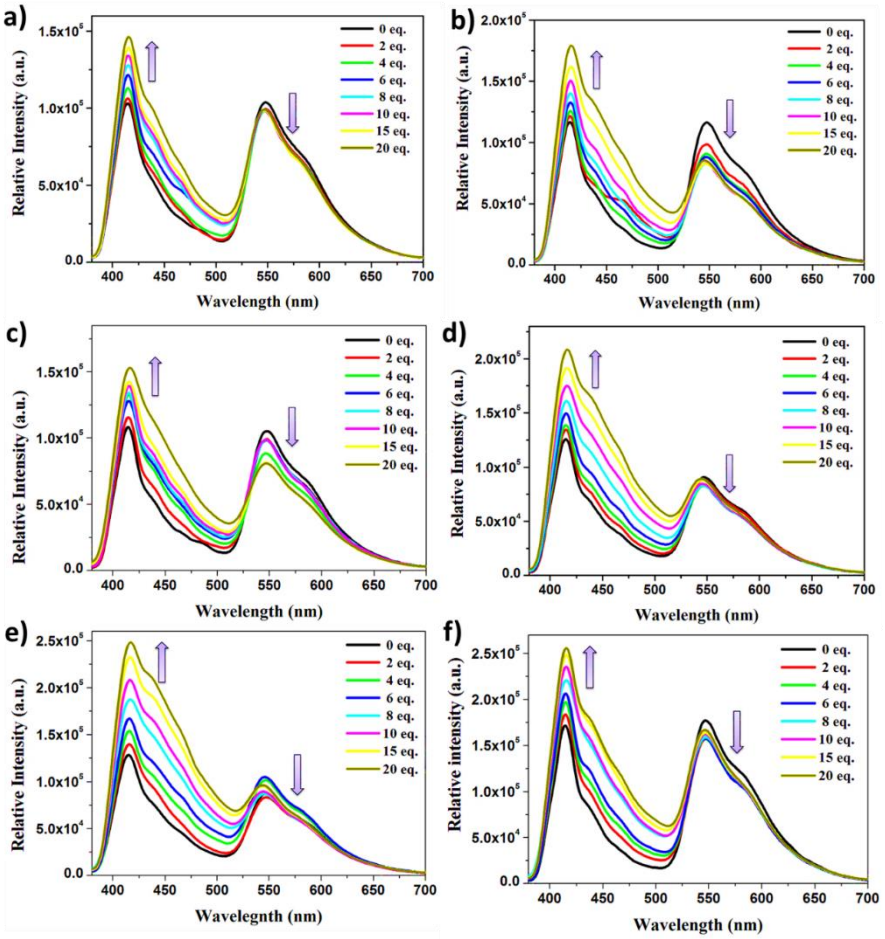

Fig 5 Fluorescence of **NDI 1** (c = 1 x 10^{-5} M) with addition of various saccharides in the D-form such as: a) galactose, b) glucose, c) mannose, d) lyxose, e) ribose and f) xylose (0-20 equiv., 1 x 10^{-2} M)) at pH 9 in H_2O.

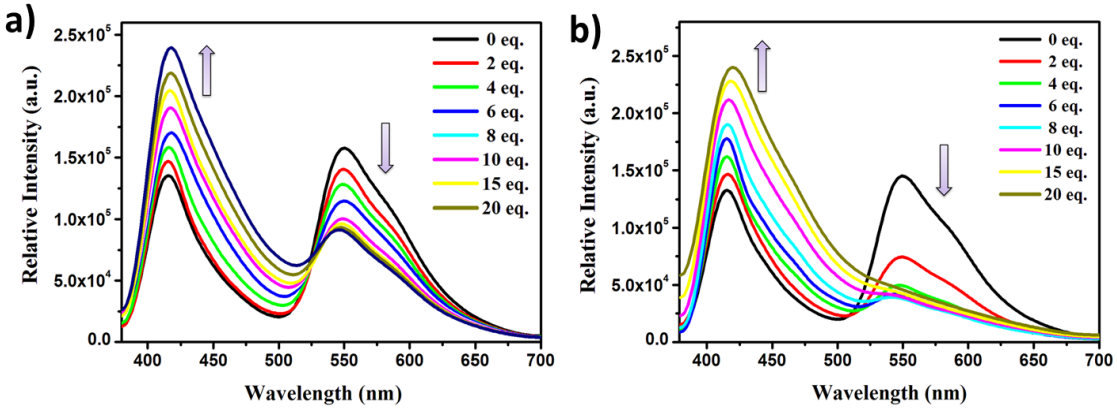

Fig. 6 Fluorescence of bolaamphiphile **NDI 1** (1 x 10^{-5} M) upon excitation at λ_{ex} = 380 nm) with the addition of a) kanamycin (0-20 equiv., 1 x 10^{-2} M) and b) neomycin (0-20 equiv., 1 x 10^{-2} M)) at pH 9 in H_2O.

In Fig 7, we demonstrated that the plausible binding mode of phosphonic appended bolaamphiphile **NDI 1** with monosachharides/aminoglycosides. Herein, we presume that at pH 9, the fully deprotonated phosphonic functional group interacts with monosachharides/aminoglycosides through non-covalent hydrogen bonding. The change in UV-vis and fluorescence emission spectra was observed due to intermolecular π-π interactions of core of NDIs.

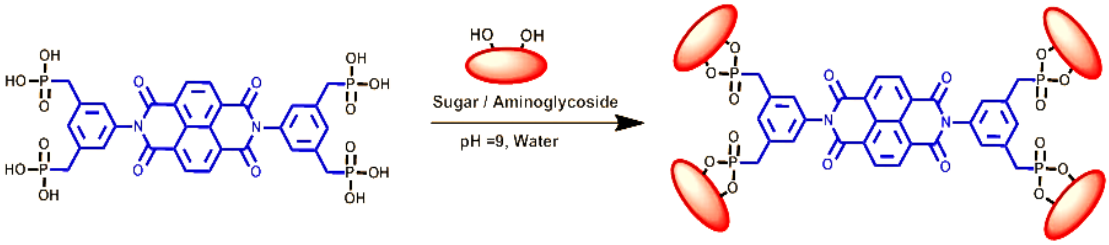

Fig. 7 Schematic presentation displaying the plausible binding mode between bolaamphiphile **NDI 1** with monosachharides/aminoglycosides.

4. Conclusion

In summary, we developed phosphonic acid appended **NDI 1** bolaamphiphile for molecular recognition of monosachharides of the D- form such as galactose, glucose, fructose, lyxose, ribose, and xylose and aminoglycoside antibiotics e.g. kanamycin and neomycin with association constant ranging from 10^{-2} M to 10^{-3} M. The binding between fully deprotonated phosphonic acid with –OH and/or –NH2 is attributed to the non-covalent H-bonding. We believe that **NDI 1** receptor with phosphonic head group undergoes molecular recognition for monosachharides/aminoglycosides could be valuable tools to recognise the specific molecular entities and opens new avenue in the field of research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

CRediT role(s) Authorship contribution statement

Kamalakar P. Nandre: Methodology; manuscript corrections; **Sidhanath V. Bhosale:** PI, conceptualization, manuscript writing & corrections.

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