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Kanamycin A 6′-pyrenylamide: a selective probe for heparin detection

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ABSTRACT

We have synthesized a novel derivative of kanamycin A bearing a pyrenyl fluorophore which can be used for the specific recognition of heparin in EtOH:H_2O (1:3) through formation of a 2:1 complex. The receptor shows specific optical signalling for heparin, but exhibits no significant changes on addition of various other biological molecules.

Fluorescent molecules have been central to the development of highly sensitive and selective sensors for the detection of cations, anions and neutral molecules, particularly in bioimaging and biomolecule detection.[1,2] Heparin is a widely used pharmacological agent for anticoagulation during surgery.[3] Heparin works as a modulator and effector, disrupting important signalling through binding to molecules such as antithrombin III leading ultimately to the inactivation of factor Xa.[4] Heparin, a highly sulfated aminoglycan, is also a highly negatively-charged biopolymer at physiological pH. Several methods for heparin detection have been described including the use of surface plasmon resonance, colorimetric changes and indicator displacement assay approaches.[5,6] In addition, there have been several reports on heparin binding to various synthetic receptors[7] and polymeric conjugates.[8–10] The drawbacks associated with many of these approaches, such as poor selectivity and sensitivity and the use of short emission wavelengths, mean that simple, practical sensors for the detection of heparin are still needed.

On the other hand, aminoglycoside antibiotics and their derivatives act as key components for new applications. Examples including kanamycin A are a group of clinically important antibacterial drugs used for the treatment of Gram-negative infections.[11] Their widespread use over the past decade has been significantly compromised by oto- and nephrotoxicity and the rapid emergence of bacterial resistance. However, their propensity to bind strongly to negatively-charged species has led to studies in gene transfection, micelle formation and in forming fibres on DNA, amongst others.[12–14]

Herein, we report that a new kanamycin A derivative **1** bearing a pyrenyl fluorophore chromophore is highly selective and sensitive for heparin detection by both colorimetric and fluorometric analyses. Derivative **1** contains two kanamycin A moieties that could function as binding sites and a built-in pyrene component which should act as a colorimetric reporter of any binding events (Fig. 1). The conjugate kanapy-6′ (kanamycin A-pyrene) **1** bears three important features: (1) polar hydroxy head groups at the periphery for hydrogen bonding, (2) four amino functional groups for interaction with anionic species and (3) a pyrenyl moiety which plays the role of both colorimetric and fluorometric reporter upon binding. It is the polyamine unit (kanamycin, with its pyrene fluorophore, i.e. kanapy-6′) that aggregates around the polyanionic heparin, bringing them into close proximity.

Kanamycin A 6′-pyrenylamide was prepared in 65% yield by condensation of kanamycin A disulfate (**2**) with pyrene butyric acid **3** in the presence of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-bis(tetramethylene)uranium hexafluorophosphate (HBPyU) and sodium bicarbonate in a 1:1 mixture of distilled deionized water and THF at room temperature (Scheme 1). The major product shows coupling at the 6′-position only, in line with the earlier results.[15,16]

The ability of **1** to complex with biological molecules was explored with UV–vis absorption and fluorescence spectrometry.

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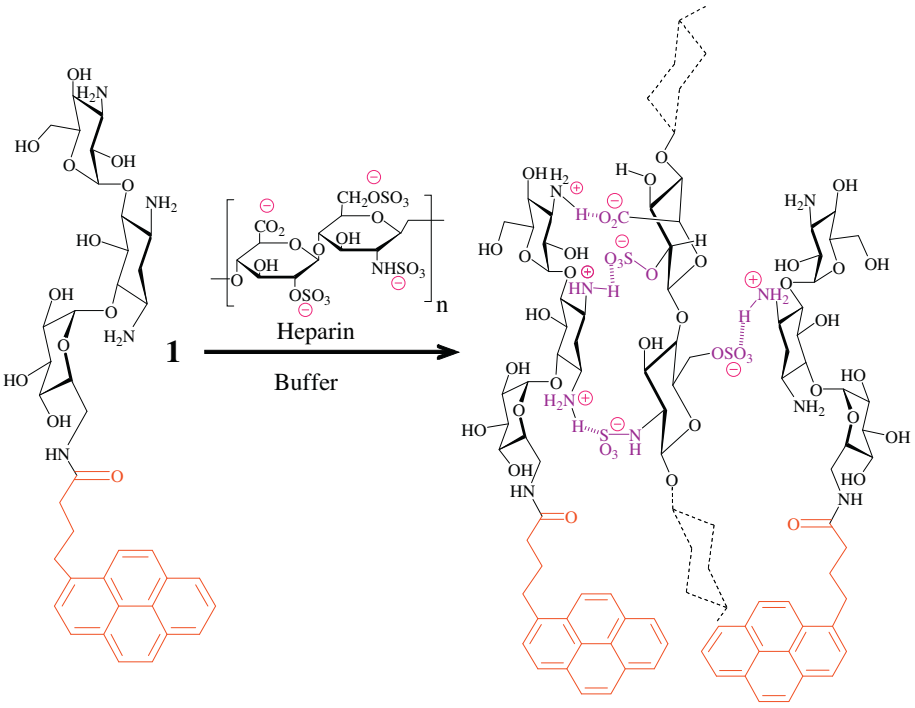
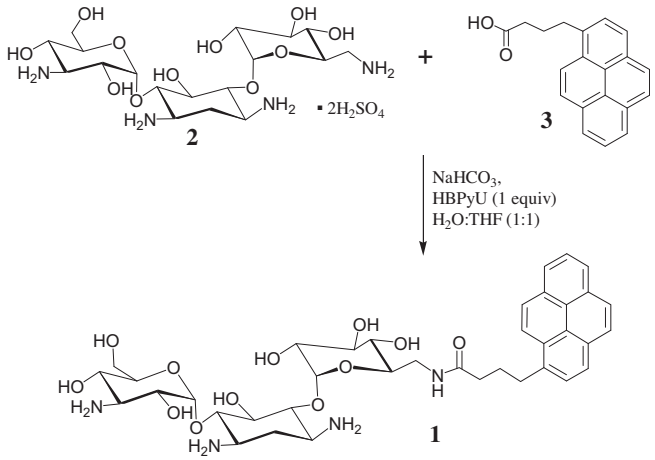


Figure 1. Schematic illustration of the proposed interactions of heparin with kanapy-6′ (**1**).



Scheme 1. Synthetic route for the synthesis of kanamycin A 6-pyrenylamide (**1**).

The conjugate kanapy-6′ (**1**) displays two absorption bands at 342 and 328 nm and one weak band at 308 nm in the UV spectrum in EtOH:H_2O (1:3),[17] as shown in Figure 2. Upon addition of heparin, concentration-dependent changes were observed occurring in intensity and bathochromic shifts leading to a clear isosbestic point at 348 nm. Figure 2b illustrates the gradual enhancement in absorption at 358 nm upon interaction of heparin with **1** and the apparent association constant (K_a) was determined to be 2.1×10^{-4} M. The band broadening and red shift in the absorption of **1** upon the accumulation of heparin can be attributed to intermolecular π–π interactions between two or more pyrene residues in the complex.

The formation of a pyrene excimer was also supported by fluorescence titration of **1** with heparin. The fluorescence emission spectrum of kanapy-6′ (**1**) (λ_{exc} = 342 nm) consists of two strong bands at 376 and 400 nm, corresponding to the emission of the

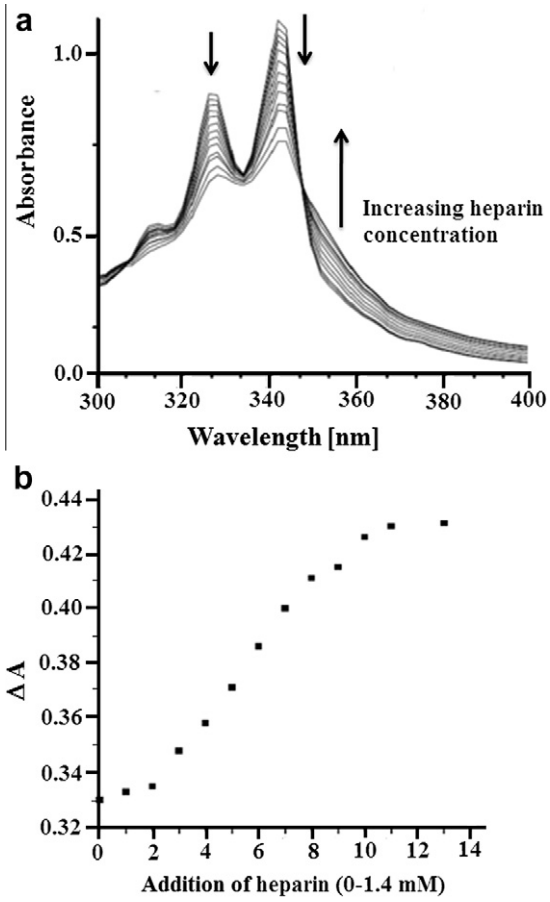


Figure 2. Overall changes in the absorption spectra of **1** (10 μM) upon addition of heparin (0–1.4 mM) in 10 mM HEPES buffer solution (EtOH:H_2O = 1:3). (b) Changes in the conjugate **1** at relative absorption intensity (I_{358}) on addition of 10 μl aliquots of heparin.

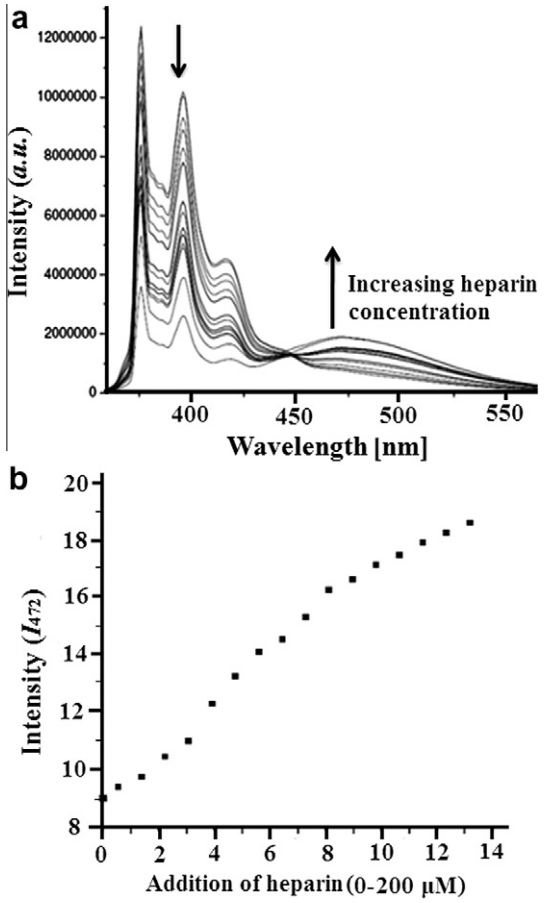


Figure 3. (a) Changes in the fluorescence spectra of conjugate **1** (10 μM) upon titration with heparin (0–200 μM) in 10 mM HEPES buffer solution (EtOH:H_2O = 1:3). (b) Changes in conjugate **1** at relative emission intensity (I_{472}) upon addition of 10 ml aliquots of heparin.

pyrene monomer (Fig. 3). The addition of heparin (0–200 μM) led to quenching of the monomer fluorescence and the appearance of a new broad band at 472 nm, which is typical of excimer formation.[2] To substantiate the role of kanamycin A, pyrene **3** failed to show any marked difference in its fluorescence spectrum under the same conditions. The apparent association constant (K_a) was determined to be 1.7×10^{-4} M using a linear-regression analysis (see graph displayed in Fig. 3b).

The selectivity of conjugate **1** for heparin in EtOH:H_2O (1:3) was apparent from an investigation of the emission response of other important biomolecules such as adenosine triphosphate (ATP), adenosine monophosphate (AMP) and amino acids including triglycine, aspartic acid, ascorbic acid and glutamic acid and also DNA, pimelic acid, dodecanedioic acid and octadodecanoic acid (Fig. 4). Such selectivity in a semi-aqueous environment is promising for the use of kanapy-6′ in practical applications.

In summary, we have reported a novel kanamycin A based fluorescent neutral sensor for optical signalling of heparin in which the pyrene unit shows optical for both UV/vis-absorption and fluorescence. The receptor **1** displayed changes in UV/vis absorption and fluorescence emission intensities selectively for heparin in buffer over various biologically active molecules. The sensor should have potential in various sensing applications such as the study of heparin transport or purification, where the availability of cheap and easy-to-prepare heparin receptors would be advantageous. We are currently tailoring these and other kanamycin A based receptors to be specific for other binding events of choice.

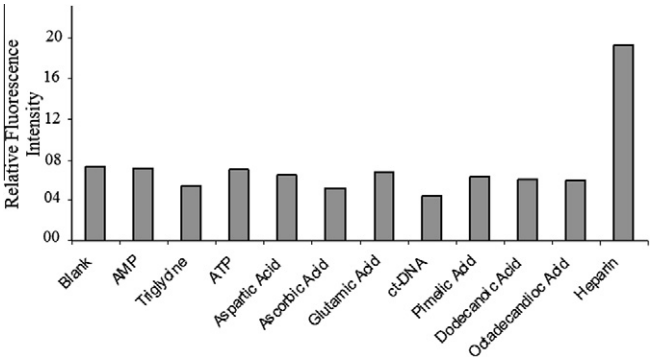


Figure 4. Selectivity of conjugate **1** for heparin upon addition of various biomolecules at a concentration of 200 μM in 10 μM HEPES buffer solution (EtOH:H_2O = 1:3) to a 10 μM solution of conjugate **1** in 10 μM HEPES buffer solution (EtOH:H_2O = 1:4) at pH 7.4. Excitation wavelength was at λ_{max} = 342 nm, with a slit width of 2 nm.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.03.123.

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16. Synthesis of kanapy-6′ (**1**)**:** Kanamycin A disulfate (**2**) (300 mg, 0.44 mmol) and $NaHCO_3$ (185 mg, 2.20 mmol) were dissolved in 15 ml of distilled H_2O with stirring. Pyrene butyric acid **3** (128 mg, 0.44 mmol) in 12.5 ml of THF was added at room temperature, followed by addition of HBPyU (190 mg, 0.44 mmol) in small portions. The mixture was stirred for 6 h at room temperature, the THF evaporated in vacuo, and the aqueous solution lyophilized. Flash column chromatography on silica gel ($CHCl_3$: MeOH: aq. NH_3) gave the kanapy-6′ (**1**) adduct in 65% yield. ^{1}H NMR (300 MHz; CD_3OD;

25 °C) δ: 8.48 (m, 1H, aromatic-H of pyrene), 8.31 (m, 1H, aromatic-H of pyrene), 7.82–8.16 (m, 1H, aromatic-H of pyrene), 7.60–7.66 (m, 1H, aromatic-H of pyrene), 7.18–7.25 (m, 3H, aromatic-H of pyrene), 5.54 (anomeric proton, 1H), 5.04–5.30 (4H, from sugar rings), 3.60–3.95 (m, 14H, from sugar rings), 3.11–3.79 (m, 3H, from sugar rings), 2.08–2.36 (m, 8H, from sugar rings and pyrene chain), 1.71–1.85 (m, 4H, from sugar rings); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 27.7, 27.8, 27.9, 28.1, 32.8, 33.0, 35.3, 35.4, 35.8, 36.0, 46.4, 48.3, 50.6, 56.2, 61.2, 70.0, 72.2, 72.7, 73.0, 73.2, 73.3, 73.5, 88.0, 91.2, 112.5, 118.2, 122.4, 123.7, 123.8, 123.9, 124.0, 124.1, 124.6, 124.7, 126.5, 126.6, 126.9, 127.7, 128.6, 129.7, 130.8, 131.3, 131.4, 136.9, 137.2, 137.5, 172.4, 173.0; FT-IR (KBr): ν_{max}/cm^{-1} 3271, 3037, 2875, 1641, 1546, 1409, 1350, 1145, 1045, 840; LC–MS (m/z) calcd for $C_{38}H_{50}N_4O_{12}$ [M^+] 754.8, found: 777 ($M+Na^+$), 755 $(M+H)^+$; HRMS (m/z) calcd for $C_{38}H_{51}N_4O_{12}$ 754.8031, found: 755.8029 $(M+H)^+$. Calculated elemental composition: 60.47% C, 6.68% H, 7.42% N; measured elemental composition: 60.41% C, 6.64% H, and 7.47% N.

17. Preparation of stock solutions: All samples were freshly prepared from stock solutions. The stock solution of kanapy-6′ (**1**) (10 μM) was prepared in 10 mM HEPES buffer solution (EtOH:H_2O = 1:4) at pH 7.4 for spectral measurements. The stock solution of heparin (200 mM) was prepared in 10 mM HEPES buffer solution (EtOH:H_2O = 1:3).