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Ru(II) Glycodendrimers as Probes to Study Lectin−Carbohydrate Interactions and Electrochemically Measure Monosaccharide and Oligosaccharide Concentrations

Raghavendra Kikkeri,[†,⊥] Faustin Kamena,[†,⊥] Tarkeshwar Gupta,[‡] Laila H. Hossain,[†] Siwarutt Boonyarattanakalin,[§] Ganna Gorodyska,[∥] Eva Beurer,[∥] Géraldine Coullerez,[∥] Marcus Textor,[∥] and Peter H. Seeberger*[,†,⊥]

[†]*Laboratory for Organic Chemistry, ETH Zurich, Switzerland,* [‡]*Department of Chemistry, University of California, Berkeley, California 94720-1460,* [§]*Sirindhorn International Institute of Technology, Thammasat University, P.O. Box 22 Thammasat-rangsit Post Office, Pathumthani 12121, Thailand, and* [∥]*Laboratory for Organic Chemistry, Swiss Federal Institute of Technology (ETH), Zurich, Wolfgang-Pauli-Str. 10, 8093 Zurich, Switzerland.* [⊥]*Current address: Max-Planck-Institute of Colloids and Interfaces, Department of Biomolecular Systems, Am Mühlenberg 1, 14476 Potsdam, Germany.*

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We describe a novel platform on which to study carbohydrate−protein interactions based on ruthenium(II) glycodendrimers as optical and electrochemical probes. Using the prototypical concanavalin A (ConA)−mannose lectin−carbohydrate interaction as an example, oligosaccharide concentrations were electrochemically monitored. The displacement of the Ru(II) complex from lectin-functionalized gold surfaces was repeatedly regenerated. This new platform presents a method to monitor many different complex sugars in parallel.

The interaction of carbohydrates and carbohydrate-binding proteins, so-called lectins, is key to diverse processes such as cell growth, inflammatory responses, and viral infections.[1] Glycan patterns on the surface of different organisms but also between healthy and cancerous cells differ significantly. Cell-surface carbohydrates are potential diagnostic markers as well as targets for the design of carbohydrate-based vaccines.[2] Therefore, it is desirable to quantitatively analyze glycans of interest as well as their interactions with the lectins that bind them.[3] Microarrays,[4a,4b] electrochemical methods,[4c] surface plasmon resonance, and quartz crystal microbalance biosensors[4d] have been employed to analyze lectin−sugar interactions and cell−surface carbohydrates. These methods rely on multivalent carbohydrate ligand presentation because the monosaccharide−lectin binding affinity is often weak. Carbohydrate clusters on molecular templates including cyclodextrins,[5] calixarenes,[5d] dendrimers,[6] and gold nanoparticles[7] create a multivalent sugar display. Glycodendrimers have been synthesized on organic fluorescent probes, CdSe, CdS, and gold nanoparticles to monitor recognition events by electronic, optical, or microgravimetric means.[4c,7,8] However, nanoparticle−sugar conjugation requires special polymer coats to avoid nonspecific interactions.[8b] Fluorescent metallo-glycodendrimers provide an alternative to nanoparticles. Lanthanide, Ru(II), Re(II), and Ir(II) metal complexes[6b,6c] are tunable, stable, nonbleaching fluorescent probes with microsecond lifetimes.

Among these metals, the Ru(II) core is most attractive for its octahedral core symmetry and robustness. Ru(II) complexes exhibit a low excited triplet metal-to-ligand charge-transfer (^{3}MLCT) state and room-temperature ^{3}MLCT lifetimes up to 1 μs. High-emission quantum yields[9] and strong oxidizing and reducing capabilities[10] are further key characteristics. Ru(II) dendrimers have been explored as chromophoric components in light-emitting devices,[11] artificial photosynthesis,[12a] and

*Corresponding author. E-mail: peter.seeberger@mpikg.mpg.de.

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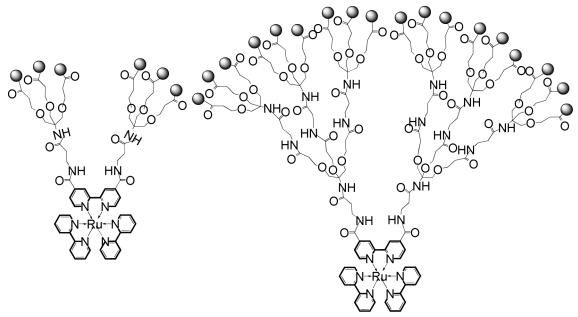

Figure 1. Mannose- and galactose-capped Ru(II)-based glycodendrimers.

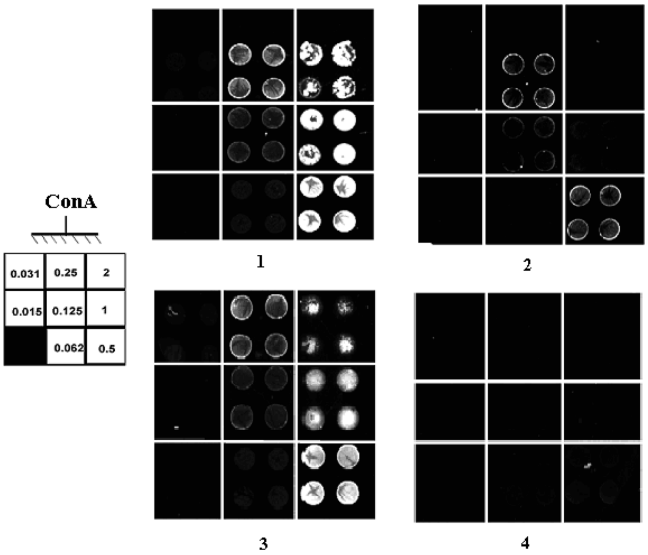

Figure 2. Incubation of Ru(II) dendrimers **1**−**4** with protein microarrays that contain different concentrations (mg/mL) of lectin ConA (excitation at 480 nm).

immunoassay[12b] and as chemosensors for phosphate,[13] oxygen,[14] and glucose.[15] Monolayers of Ru(II)-confined complexes may serve as components for memory devices and molecular switches and sensors[16] as well as electrochemical sensors for oxygen and DNA damage.[17]

Here, we report the use of robust ruthenium(II) bipyridine glycodendrimers as stable fluorescent and electrochemical probes to detect lectin−carbohydrate interactions on microarrays and gold substrates. The prototypical concanavalin A (ConA)−mannose interaction serves as a model to illustrate the new approach.

Displacement of the redox-active Ru(II) complex by mannose, dimannose, trimannose, and phosphatidylinositol mannosides (PIMs) from surface-immobilized lectin quenches the electrochemical signal. Simplicity and sensor regeneration render this

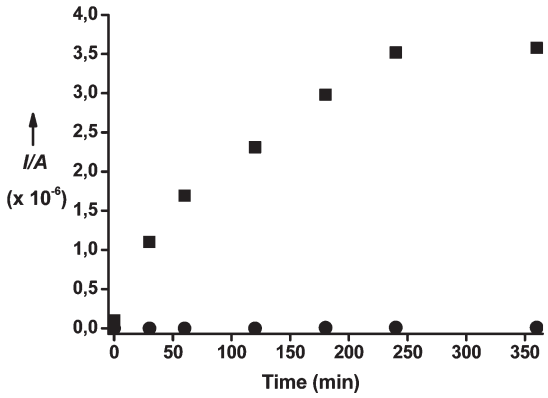

Figure 3. Square-wave voltammetry at 1.14 V following the incubation of complexes **1** (■) and **2** (•) with ConA-functionalized surfaces for 6 h.

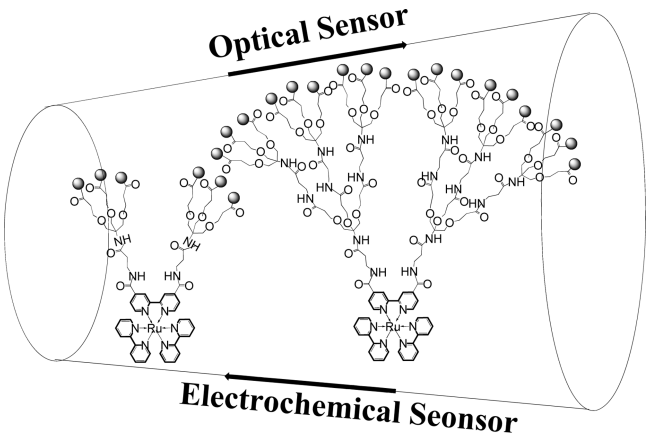

Figure 4. Schematic representation of the optical and electrochemical behavior of complexes **1** and **3**.

method attractive for monitoring even complex sugars at the micromolar level.

Carbohydrate Ru(II) dendrimers **1**−**4** (Figure 1) were prepared using methods that we reported previously.[6c] The photophysical properties of dendrimers **1**−**4** were compared to the reference compound $[Ru(bipy)_3]^{2+}$. The UV−visible spectra of metal dendrimers **1**−**4** in methanol show the characteristic metal-to-ligand charge-transfer band (MLCT) at around 450−500 nm and the intense ligand center (LC) absorption at around 300 nm. The MLCT absorptions of **1**−**4** show a bathochromic shift when compared to $[Ru(bipy)_3]^{2+}$ because of the presence of the electron-withdrawing amide groups on the bipyridines (Figure S2). The emission properties of all compounds exhibit the characteristic luminescence of the triplet metal-to-ligand charge-transfer (MLCT) excited state of the $[Ru(bipy)_3]^{2+}$ core. Minor differences related to different chemical compositions can be noted. Complexes **1**−**4** show a bathochromic shift of 30 nm compared to the reference complex due to an electron-withdrawing group on the bipyridine moiety (Figure S3). Quantum yields of all compounds were calculated by using a standard formula with $[Ru(bipy)_3]Cl_2$ as a reference. Quantum yields of complexes **1** and **2** are nearly half the value of that for **3** and **4**. This alteration in photophysical properties may be due to differences in sugar density around the ruthenium(II) core. The cyclic voltammetric (CV) response in acetonitrile using a glassy carbon (GC) electrode for **1**−**4** showed a single, metal-centered, one-electron redox process. The electrochemical behavior was similar to that of $[Ru(bipy)_3]Cl_2$ and related complexes. The redox process is

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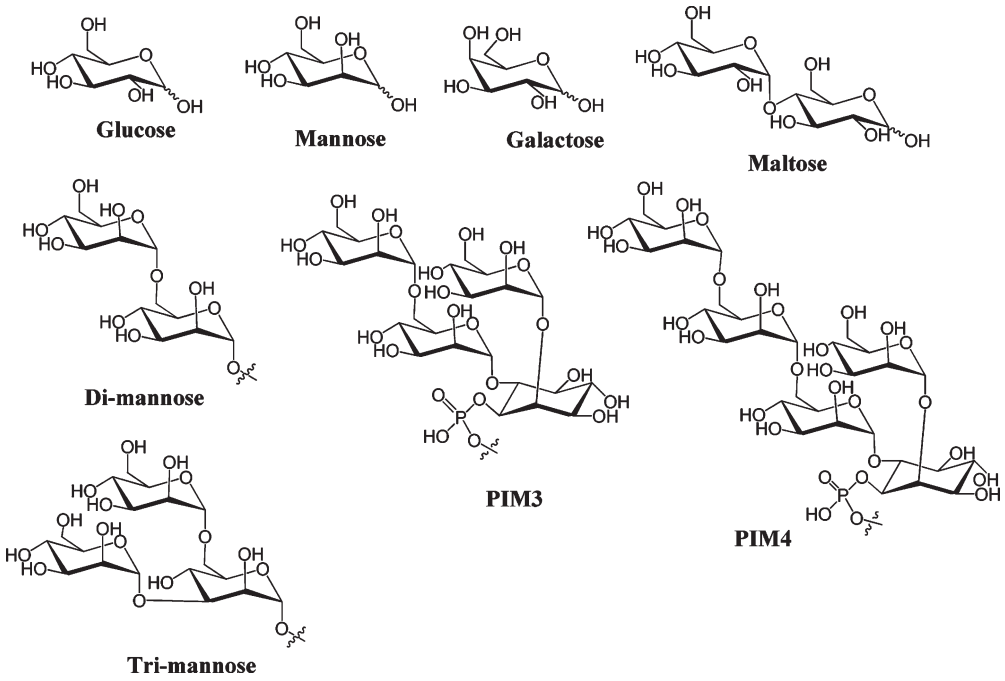

Figure 5. Structures of carbohydrates used for sensing.

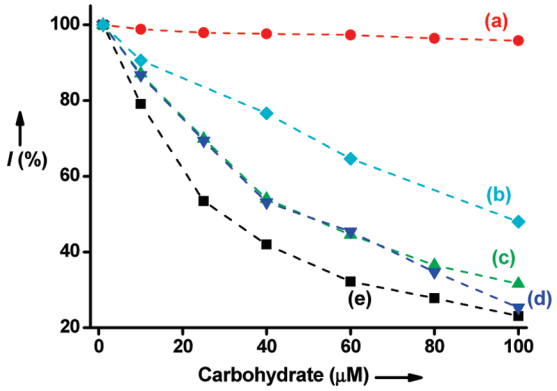

Figure 6. Response of square-wave voltammetric signals to increasing concentrations of (i) (a) D-galactose (red ●), (b) D-glucose (blue ♦), (c) D-maltose (green ▲), (d) D-mannose (blue ▲), and (e) α-D-man-(1→6)man (■).

electrochemically reversible with an $i_p^a/i_P^c \approx 1$ and $E_P^c - E_p^a \approx 80$ mV. In the case of **1**, $E_{1/2}(+2/+3) = +1.32$ V versus Ag/AgCl and 0.47 V versus Fc/Fc^+. The oxidation of $[Ru(bipy)_3]^{2+}$ occurs at a lower potential [$E_{1/2}(+2/+3) = 0.881$ V versus Fc/Fc^+ in acetonitrile], indicating that sugar substitution increases the electron-withdrawing nature of the bipyridyl groups.

Mannose-binding lectin ConA was immobilized on a microarray prior to incubation with complexes **1**−**4** (100 μM solution for 30 min), and $[Ru(bipy)_3](NO_3)_2$ served as a control. Upon fluorescence scanning of rinsed slides, strong fluorescence signals were observed on slides that were incubated with mannose complexes **1** and **3**. At high ConA concentrations (e.g., 2 mg/mL), the microarray spots appeared to be heterogeneous on the array surfaces. Protein aggregation may result in poor fluorescence. Using dendrimers **1** and **3** that contain 6 and 18 mannoses, respectively, ConA was detected at 0.125 mg/mL (620 nM). ConA does not bind galactose. Therefore, as expected, dendrimers **2** adorned with galactose showed weak nonspecific binding at high concentrations, but **4** did not show any fluorescence (Figure 2). These initial experiments demonstrated that mannose complex **3** is a more selective optical probe for lectins than **1** (Table S2).

After establishing that Ru(II) glycodendrimers can be detected visually, we wanted to establish that this detection system can also be utilized for the electrochemical detection of protein−carbohydrate interactions. ConA was immobilized on a self-assembled monolayer on a gold surface[18] prior to incubating these surfaces with Ru(II) complexes **1**−**4** or the control $[Ru(bipy)_3](Cl)_2$ for 30 min. Following incubation, the chip was transferred to a electrochemical cell containing phosphate buffer. The scanning potential of 100 mV/s in the region of 1.0−1.4 V shows a peak at 1.62 μA (Figures S7a and S8a). Repeated measurements revealed that maximal ConA/Ru(II)−complex interactions were reached after 240 min of incubation (Figure 3). Neither galactose-bearing dendrimers **2** and **4** nor $[Ru(bipy)_3](Cl)_2$ bound ConA. Interestingly, the incubation of complex **3** carrying 18 mannoses with ConA monolayers showed a very weak signal in the region of 1.0−1.4 V. An optimum current at 4.1 nA was obtained after 180 min of incubation (Figures S7b and S8b). On the basis of these findings, complex **1** is better suited for electrochemical sensing than the more complex dendrimer **3** (Figure 4).

After establishing that the lectin−glycodendrimer interactions can be measured electrochemically, we determined the detection limit when using dendrimer **1**. Different concentrations of ConA were immobilized on gold substrates and treated with 0.5 mM **1** prior to recording square-wave voltammetric (SWV) signals (Figures S9 and S10). At 2.5 nM, the detection limit for **1** is comparable to other sensors.[6c,7a,19]

Replacement of the glycodendrimer from the lectin-functionalized gold chips should allow for the detection of any sugar that is bound by the lectin. ConA-functionalized gold chips containing **1** were immersed in solutions containing varying concentrations of D-glucose, D-mannose, α-D-man-(1→6)man, D-galactose, D-maltose, or PIM glycans (Figure 5) before SWV signals for Ru(II)

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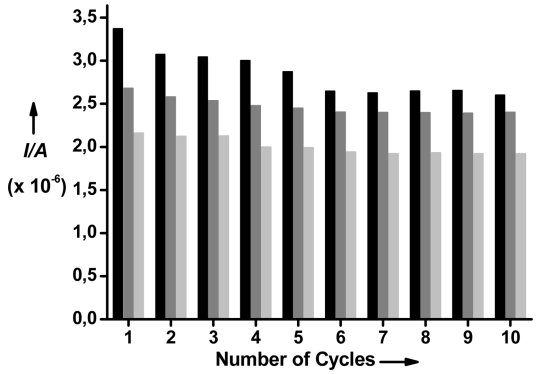
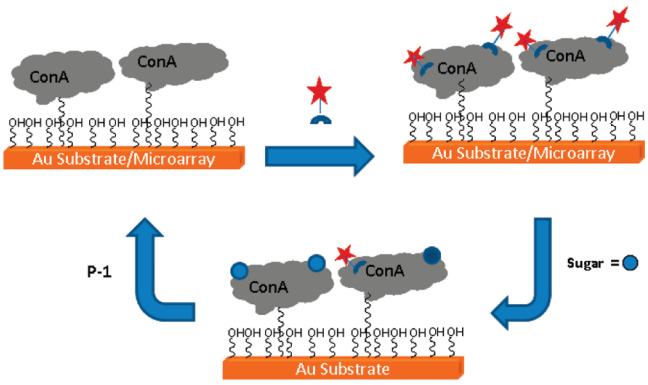


Figure 7. Maximum current signal upon regeneration of the ConA/**1** glucose detector: complex **1** on a gold substrate (black), addition of 40 μM D-glucose (gray), and addition of 80 μM D-glucose (light gray).

Scheme 1. Schematic Representation of the Ru(II)−Sugar Complex Interaction with the Lectin ConA that is Immobilized on a Gold Surface/Microarray for Use as a Sugar Sensor[a]



[a] P-1 represents the boronic acid-substituted polymer.

were recorded (Figures 6 and S11). The current decreased in a concentration-dependent manner, indicating that redox-active complex **1** is replaced in a competitive manner by the preferentially binding carbohydrate. The detection limit for glucose of 7 μM[20] compares favorably with the detection limits for other methods that are in the micromolar range.[21]

Increasing concentrations of D-mannose and disaccharide D-maltose resulted in a rapid concentration-dependent decrease in the current. The detection limit for these two sugars was in the range of 3 μM. Disaccharide α-D-man-(1→6)man was displaced most rapidly with a detection limit of 1.4 μM (Figure 6). More complex oligosaccharides trimannose, PIM3, and PIM4 rapidly quenched the electrochemical signal up to 25 μM, followed by a slowing decrease at higher concentrations. Detection limits of 0.61, 1.4, and 0.63 μM for these three mannose-containing structures were calculated (Figures S11, S12, and S13). Rapid

Table 1. Photophysical Properties of Complexes 1−4

compound	λ_{max} (nm)	τ (μs)	Φ	$E_{1/2}$ (V)
1	643	0.61	0.072	+1.32
2	643	0.63	0.071	+1.32
3	645	1.26	0.062	+1.35
4	645	1.27	0.112	+1.34
$[Ru(bipy)_3]Cl_2$	613	0.54	0.115	+0.88

quenching can be interpreted as the simultaneous displacement of weakly bound complex **1** from immobilized ConA and a high affinity of the sugar for lectin. The trend in sensitivity, PIM4 > Triman > PIM3 > α-D-man-(1→6)man > Man ≥ Mal > Glu, is consistent with the binding affinity of these glycans to ConA.[22]

Ideally, sensors can be regenerated for repeated use. Glucose served to demonstrate the regeneration of the lectin−glycodendrimer sensing platform. A gold chip exposed to 100 μM D-glucose solution was incubated with boronic acid-substituted Merrifield resin (P-1, Supporting Information)[23] for 5 min to displace any sugar attached to the immobilized ConA. Incubation with complex **1** regenerated the surface for the next measurement. To verify the quality of the readings after regenerating the electrochemical detector, the chip was exposed to solutions containing 40 and 80 μM D-glucose. The platform was regenerated 10 times using this reiterative process (Figure 7). The SWV signal decreases over the first six cycles and then remains constant for the last four regeneration cycles. Deactivation or effective hosting of glucose by ConA may be responsible for the observed decrease in the electrochemical signal after each cycle.

In conclusion, we have demonstrated that tris-bipyridyl ruthenium glycodendrimers containing defined numbers of carbohydrates enable the direct optical and electrochemical detection of carbohydrate-binding proteins at the nanomolar level. Using surface-immobilized lectin−glycodendrimer complexes, we have developed a sensitive, continuous, and inexpensive electrochemical biosensor. The sensitivity of the sensor depends on the lectin that is employed. Using ConA, we detected D-mannose, D-glucose, D-maltose, α-D-Man-(1→6)Man, PIM3, and PIM4 even at low levels. The sensitive detection of PIMs illustrates the new approach that couples the specificity of lectin−carbohydrate interactions with the sensitivity and convenience of an electrochemical readout. Regeneration of the gold substrate for continuous sugar sensing renders this detection method potentially useful for detecting bacteria and eukaryotic cells as well as any glycoconjugate. Microarrays containing multiple lectin−glycodendrimer complexes for the high-throughput detection of different sugars on a single platform are currently under investigation.

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Supporting Information Available: NMR spectral copies of all new compounds and additional related experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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