# Ru(II)-Glycodendrimers as Probes to Study Lectin-Carbohydrate Interactions and Electrochemically Measure Mono- and Oligosaccharide Concentrations

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## Supporting Information

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## 1. General Information

All chemicals used were reagent grade and used as supplied except where noted. Dichloromethane ($CH_2Cl_2$) was purified by a Cycle-Tainer Solvent Delivery System. Triethylamine was distilled over $CaH_2$ prior to use. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 $F_{254}$ plates (0.25 mm). Compounds were visualized by UV irradiation or dipping the plate in CAN solution followed by heating. Flash column chromatography was carried out using force flow of the indicated solvent on Fluka Kieselgel 60 (230-400 mesh).

$^1H$ and $^{13}C$ NMR spectra were recorded on a Varian VXR-300 (300 MHz) or Bruker DRX500 (500 MHz) spectrometer. High-resolution mass spectra (HR MALDI MS) were performed by the Mass Spectrometry-service at the Laboratory for Organic Chemistry (ETH Zurich). ESI-MS were run on an Agilent 1100 Series LC/MSD instrument. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotation measurements were conducted using a Perkin-Elmer 241 polarimeter.

$RuCl_3 \cdot x\ H_2O$ and 2,3,4,5,6-pentafluorophenol were purchased from Fluka. Acrylonitrile was purchased from Alfa Aesar and used directly in the reaction. ConcanavalinA was purchased from Appli Chem (Axon Lab AG). Slides were scanned using a LS400 scanner from Tecan and quantified using Scan Array Express Software. Absorption spectra were recorded using a Varian CARY 50 spectrophotometer fitted with Hellma optical fibers (Hellma, 041.002-UV) and an immersion probe made of quartz suprazil (Hellma, 661.500-QX). Fluorescence emission spectra were recorded on a Perkin-Elmer LS-50B spectrofluorometer. Confocal microscopy was performed on a SP1 Leica confocal microscope (Leica Germany). Synthesis of 2,2'-bipyridine-4,4'-dicarboxylic acid and *cis*-$Ru(bipy)_2Cl_2$ was carried out as described previously.[1]

## 2. Synthesis of Complexes 2 and 4

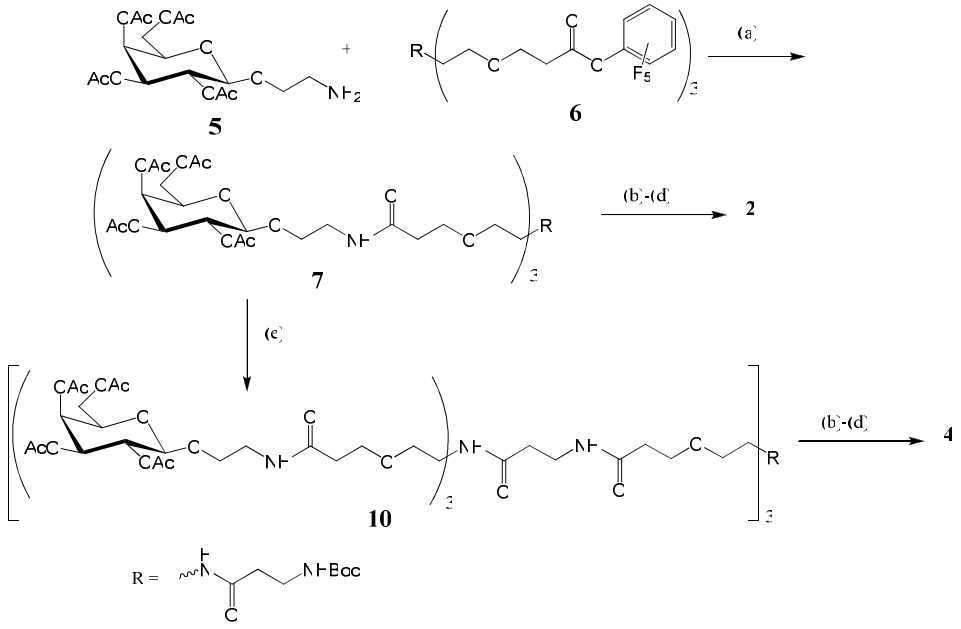


***Fig S1.*** Synthesis of Ru(II)-bispyridyl dendrimers **2** and **4**. (a) TEA/DCM, 12 h, 52% (b) TFA, 2,2'-bipyridine 4,4'-dicarboxylic acyl chloride, DCM, TEA, 12 h, 35% (c) *cis*-$Ru(bipy)_2Cl_2$, EtOH, 6 h, 52% (d) NaOMe, MeOH, 2 h, 65% (e) **6**, TEA, DCM, 12 h, 52%

**General Procedure A: Synthesis of Sugar-tripods:** The Boc-protected amino-sugar (4.0 eq) was dissolved in 10 mL dichloromethane/trifluoroacetic acid (3:1) and stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure and the resulting oil was dissolved in anhydrous dichloromethane (20 mL). To this mixture, was added *tert*-butoxycarbonyl-3-{*N*-{tris[3-[pentafluoro-phenyl-carboxyl-ethoxy)methyl]} methyl amine}-3-*β*-alanine (1.0 eq), the pH was adjusted to 8 with triethylamine (TEA) and the mixture was stirred at room temperature for 12 h. The solvent was evaporated *in vacuo* and purified by flash silica column chromatography.

**General Procedure B: Synthesis of Bipyridine Derivatives:** 2,2'Bipyridine-4,4'-dicarboxylic acid (1.0 eq) was dissolved in $SOCl_2$ (1 mL) and refluxed under nitrogen for 12 h. Excess $SOCl_2$ was removed in *vacuo* and the crude 2,2'bipyridine-4,4'-dicarboxylic acyl chloride was used directly in

the next step. Boc-protected amino-sugar-tripod (3.0 eq) was dissolved dichloromethane/trifluoroacetic acid (10 mL, 3:1 resp.) and stirred at room temperature for 1 h. The mixture was concentrated *in vacuo* and then redissolved in dichloromethane (20 mL). To this mixture was added 2,2'bipyridine-4,4'-dicarboxylic acyl chloride (1 eq) and the pH adjusted using TEA to pH 8. The reaction mixture was stirred for 12 h, the solvent removed *in vacuo* and the mixture purified by silica column flash chromatography.

**General Procedure C: Synthesis of Ruthenium(II)-Complexes:** The bipyridine-sugar derivative (1.0 eq) and *cis*-ruthenium(II)bis(bipyridine)dichloride (1.1 eq) were dissolved in de-oxygenated ethanol (30 mL) and the mixture was refluxed for 6-8 h. The compound was then purified by silica column flash chromatography.

**General Procedure D: Synthesis of Ruthenium(II)-sugar Complexes:** Ruthenium(II) complex (1.0 eq) and sodium methoxide (0.2 eq) were dissolved in methanol (10 mL) and stirred at room temperature for 2 h. The solvent was then evaporated *in vacuo*, the residue was redissolved in water and dialyzed against water using 500 molecular weight cut-off resin. After two days of dialysis, the sample was lyophilized.

***tert*-Butoxycarbonyl-3-{tris[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside-ethoxy]methyl]methylamide}-3-*β*-alanine (7).** General procedure A with 2-(*tert*-butoxycarbonylamino)ethoxy-2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside **5** (0.85 g, 1.73 mmol), *tert*-butoxycarbonyl-3-{*N*-{tris[3-[pentafluoro-phenyl-carboxyl-ethoxy)methyl]}methyl amine}-3-*β*-alanine **6** (0.43 g, 0.43 mmol) and flash silica column chromatography yielded *tert*-butoxycarbonyl-3-{tris[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside-ethoxy]methyl]methylamide}-3-*β*-alanine (0.26 g, 52%). $R_f$ = 0.45 ($CH_2Cl_2$/MeOH, 93:7); $[\alpha]_D^{r.t}$ = +10.2 (c =1.0, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 6.90 (br.s, 1H), 6.47 (br.s, 3H), 5.37 (d, $J$ = 3.3 Hz, 4H), 5.14 (t, $J$ = 7.8 Hz, 3H), 4.99 (dd, $J$ = 3.3, 4.2 Hz, 3H), 4.49 (d, $J$ = 7.8 Hz, 3H), 4.12-4.10 (m, 6H), 3.92 (t, $J$ = 6.2 Hz, 3H), 3.84-3.83 (m, 3H), 3.70 (t, $J$ = 5.4 Hz, 8H), 3.65 (s, 9H), 3.42 (t, $J$ = 5.7 Hz, 6H), 3.33 (q, $J$ =

5.7 Hz, 2H), 2.39 (t, *J* = 5.4 Hz, 6H), 2.14 (s, 9H), 2.04 (s, 9H), 2.02 (s, 9H), 1.96 (s, 9H), 1.40 (s, 9H), $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 171.1, 170.0, 169.8, 169.7, 155.7, 101.1, 70.6, 69.1, 68.7, 67.2, 67.0, 66.9, 61.2, 59.7, 45.65, 41.67, 39.1, 37.0, 36.4, 28.3, 20.6; FTIR($CHCl_3$): 3343, 2945, 1751, 1560, 1458, 1350 cm$^{-1}$; HRMS (MALDI-ToF) (*m/z*): $[M+Na]^+$ calcd. for $C_{69}H_{105}N_5O_{39}Na$ 1650.6284; found: 1650.6252.

**(v) 1,1'-(2,2'-Bipyridine-4,4'-diyl)bis-3-*β*-propane-{-3-{tris[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside-ethoxy]methyl]methylamide (8).** General procedure B with *tert*-butoxycarbonyl-3-{tris[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-*α*-D-galactopyranoside-ethoxy]methyl]methylamide}-3-*β*-alanine **7** (0.25 g, 0.15 mmol), 2,2'bipyridine-4,4'-dicarboxylic acid (12.5 mg, 0.52 mmol) and flash silica column chromatography yielded 1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-propane-{-3-{tris[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside-ethoxy] methyl]methylamide (96 mg, 35%). $R_f$ = 0.5 ($CH_2Cl_2$/MeOH, 92:8); $[\alpha]_D^{r.t}$ = +12.8 (c = 1.0, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.85 (d, *J* = 4.5 Hz, 2H), 8.85 (br.s, 2H), 7.83 (d, *J* = 4.5 Hz, 2H), 5.24 (d, *J* = 3.6 Hz, 6H), 5.13-5.10 (m, 14H), 4.68 (d, *J* = 7.2 Hz, 6H), 4.13 (br.s, 24H), 3.85-3.83 (m, 6H), 3.68 (br.s, 29H), 3.42-3.39 (m, 18H), 2.60 (t, *J* = 6.6 Hz, 4H), 2.42 (t, *J* = 5.4 Hz, 12H), 2.13 (s, 18H), 2.05 (s, 18H), 2.01 (s, 18H), 1.94 (s, 18H), $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 173.7, 171.8, 171.0, 170.9, 167.3, 163.3, 162.9, 162.4, 161.9, 155.4, 149.1, 143.5, 125.7, 115.7, 101.8, 71.9, 71.5, 69.9, 69.7, 68.9, 68.4, 68.2, 62.2, 54.4, 40.2, 36.9, 20.2; FTIR($CHCl_3$): 3684, 3489, 1752, 1675, 1454, 1442, 1065 cm$^{-1}$; HRMS-MALDI: Calcd for $C_{140}H_{197}N_{12}O_{76}Na$ 3286.189; Found : 3286.199.

**(vii) *Cis*-Ruthenium(II)bis(bipyridine){1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-*β*-propane-{tris-[3-4-ethoxy-2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside-ethoxy}methyl]methylamide (9).** General procedure C with 1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-propane-{tris-[3-4-ethoxy-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside-ethoxy}methyl]methylamide (90 mg, 0.027 mmol), *cis*-ruthenium(II)bis(bipyridine)dichloride (16 mg, 0.03 mmol) purification by flash silica column

chromatography by using acetronitrile/water/saturated $KNO_3$ (7.5:1:1.5-7:3) as eluent yielded *cis*-ruthenium(II)bis(bipyridine){1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-propane-{tris-[3-4-ethoxy-2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside-ethoxy}methyl] methyl amide (48 mg, 52%). $R_f$ = 0.5 (acetonitrile/Sat $KNO_3$, 80:20); $[\alpha]_D^{r.t}$ = +8.9 (c = 1.0, $H_2O$); $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ 9.16 (s, 2H), 8.72 (d, *J* = 7.1 Hz, 4H), 8.14 (t, *J* = 4.8 Hz, 6H), 8.01 (d, *J* = 5.7 Hz, 4H), 7.92 (t, *J* = 5.4 Hz, 4H), 7.85 (dd, *J* = 5.7, 4.8 Hz, 6H), 7.53 (t, *J* = 4.5 Hz, 4H), 7.30 (br.s, 1H), 5.37 (d, *J* = 3.0 Hz, 6H), 5.13-5.08 (m, 14H), 4.71 (d, *J* = 4.5 Hz, 6H), 4.13 (br.s, 18H), 3.88-3.85 (m, 6H), 3.65 (br.s, 36H), 3.37 (t, *J* = 4.2 Hz, 9H), 2.62 (t, *J* = 6.3 Hz, 4H), 2.41 (t, *J* = 5.1 Hz, 12H), 2.12 (s, 18H), 2.05 (s, 18H), 2.00 (s, 18H), 1.95 (s, 18H), $^{13}$C NMR (75 MHz, $CD_3OD$): $\delta$ 173.5, 173.1, 171.6, 171.4, 170.8, 165.2, 162.8, 158.5, 157.7, 153.0, 151.7, 143.4, 139.1, 132.8, 128.7, 128.4, 125.3, 101.8, 72.0. 71.5, 70.0, 69.6, 69.0, 68.5, 68.3, 62.3, 61.3, 59.5,54.5, 40.3, 38.2, 36.7, 20.4; HRMS-MALDI (*m/z*): Calcd for $C_{160}H_{215}N_{16}O_{76}Ru$ 3677.242; Found: 3678.244.

**(viii) *Cis*-Ruthenium(II)bis(bipyridine){1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-*β*-propane-{tris-[3-4-ethoxy-*β*-D-galactopyranosyl-ethoxy}methyl] methyl amide (2).** General procedure D with *cis*-ruthenium(II)bis(bipyridine){1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-β-propane-{tris-[3-4-ethoxy-2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside-ethoxy}methyl]methylamide (42 mg, 11.3 µmol) sodium methoxide (10 mg, 2.2 µmol) yielded 17 mg, (65%) of *cis*-ruthenium(II)bis(bipyridine){1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-*β*-beta-propane-{tris-[3-4-ethoxy-*β*-D-galactopyranosyl-ethoxy}methyl] methylamide. $[\alpha]_D^{r.t}$ = +1.8 (c = 1.0, $H_2O$); $^1$H NMR (300 MHz, $D_2O$/MeOD): $\delta$ 8.95 (br.s, 2H), 8.56 (d, *J* = 7.8 Hz, 4H), 8.04 (dd, *J* = 7.8, 6.0 Hz, 6H), 7.70 (t, *J* = 5.4 Hz, 4H), 7.65 (d, *J* = 6.0 Hz, 2H), 7.39 (t, *J* = 6.0 Hz, 4H), 4.36 (d, *J* = 7.8 Hz, 6H), 3.96-3.94 (m, 4H), 3.90 (d, *J* = 2.7 Hz, 12H), 3.75-3.65 (m, 34H), 3.61 (br.s, 24H), 3.49-3.46 (m, 12H), 3.43-3.41 (m, 2H), 2.57 (t, *J* = 6.0 Hz, 4H), 2.35 (t, *J* = 5.7 Hz, 12H), $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ 181.7. 174.9, 173.2, 172.6, 158.6. 157.9, 152.1, 138.6, 131.6, 129.2, 126.7, 122.4, 110.8, 104.3, 77.8,

73.5, 71.8, 71.8, 68.5, 67.6, 66.6, 62.4, 61.4, 40.0, 37.3, MALDI-ToF (*m/z*): $[M-1]^+$ Calcd for $C_{112}H1_{166}N_{16}O_{52}Ru$ 2668.988; Found: 2667.978.

**(i) *tert*-Butoxycarbonyl-3-{tris[3-carboxyl ethoxy]methyl] 3'-{tris[2'-ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside-ethoxy]methyl]methylamide}-3-β-alanine (10).** General procedure A with *tert*-butoxycarbonyl-3-{tris[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside-ethoxy]methyl]methylamide}-3-β-alanine (0.45 g, 0.27 mmol), *tert*-butoxycarbonyl-3-{*N*-{tris[3-[pentafluoro phenyl carboxyl-ethoxy)methyl]}methylamine}-3-β-alanine (92 mg, 0.091 mmol) and flash silica column chromatography by $CH_2Cl_2/CH_3OH$ (12-13%) as eluent yielded 0.26 g (52%) of *tert*-butoxycarbonyl-3-{tris[3-carboxylethoxy]methyl]3'-{tris[2'-ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside-ethoxy]methyl]methylamide}-3-β-alanine. $R_f$ 0.55 ($CH_2Cl_2$:MeOH = 90:10); $[\alpha]_D^{r.t}$ = +2.7 (c =1.0, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$): δ 6.84 (br.s, 1H), 6.63 (br.s, 1H), 5.37 (d, *J* = 3.3 Hz, 9H), 5.12 (t, *J* = 7.8 Hz, 9H), 4.99 (dd, *J* = 3.3, 4.2 Hz, 9H), 4.51-4.45 (m, 9H), 4.14-4.08 (m, 18H), 3.95-3.92 (m, 9H), 3.84-3.78 (m, 9H), 3.66-3.56 (m, 64H), 3.41-3.36 (m, 34H), 2.41 (t, *J* = 5.4 Hz, 32H), 2.14 (s, 27H), 2.04 (s, 27H), 2.02 (s, 27H), 1.96 (s, 27H), 1.41 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ171.3, 170.2, 170.0, 169.8, 169.6, 101.2, 70.7, 69.0, 68.9, 68.7, 67.4, 67.3, 67.0, 39.3, 36.4, 28.9, 20.9,FTIR($CHCl_3$): 3385, 3019, 1749, 1658, 1522, 1232, 1205 $cm^{-1}$; HRMS-MALDI (m/z): $[M+ Na]^+$ Calcd for $C_{215}H_{321}N_{17}O_{120}$ 5036.9538; Found : 5060.9534.

**(iii) 1,1'-(2,2'-Bipyridine-4,4'-diyl)bis-3-beta-propane-{*tris*-[3-carboxylethoxy]methyl]3'-{*tris*[2'-ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside-ethoxy]methyl]methylamide (11).** General procedure B with *tert*-butoxycarbonyl-3-{tris[3-carboxyl ethoxy]methyl]3'-{tris[2'-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside-ethoxy]methyl]methylamide}-3-β-alanine (0.15 g, 0.029 mmol), 2,2'bipyridine-4,4'-dicarboxylic acid (2.4 mg, 0.0098 mmol) and flash silica column chromatography by $CH_2Cl_2/CH_3OH$ (15-16%) as eluent gave 36 mg (14 %) of 1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-propane-{tris-[3-carboxylethoxy]methyl]3'-{tris[2'-ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside-ethoxy]methyl]methylamide. $R_f$ 0.35 ($CH_2Cl_2$:MeOH =

86:14); $[\alpha]_D^{r.t}$ = +5.8 (c =1.0, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$): δ 8.85 (d, *J* = 4.5 Hz, 4H), 7.86 (br.s, 2H), 7.24 (br.s, 2H), 5.39 (d, *J* = 3.6 Hz,18H), 5.12-5.07 (m, 48H), 4.68 (d, *J* = 7.2 Hz, 18H), 4.14-4.06 (m, 48H), 3.86-3.81 (m, 18H), 3.68-3.52 (m, 118H), 3.40-3.31(m, 56H), 3.12-3.07 (m, 14H), 2.73 (t, *J* = 5.2 Hz. 2H), 2.45-2.38 (m, 64H), 2.13 (s, 54H), 2.05 (s, 54H), 2.01 (s, 54H), 1.94 (s, 54H); $^{13}$C NMR (125MHz, $CDCl_3$): δ 173.5, 171.5, 171.4, 170.9, 163.2, 162.8, 162.3, 161.8, 153.1, 147.6, 144.2, 123.4, 111.8, 101.8, 71.9, 71.6, 70.1, 69.0, 68.5, 68.3, 62.3, 54.6, 39.0, 34.5, 20.5; FTIR($CHCl_3$): 3404, 2944, 2478, 1752, 1680, 1543, 1455, 1405, 1333, 1264, 1232 $cm^{-1}$; MALDI-HRMS (m/z): $[M+1]^+$ Calcd for $C_{428}H_{630}N_{36}O_{238}$ 10081.8301; Found : 10082.831.

**(iv) *Cis*-Ruthenium(II)bis(bipyridine)1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-propane-{tris-[3-carboxylethoxy]methyl]3'-{tris[2'-ethoxy2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside-ethoxy]methyl]methylamide (12).** General procedure C with 1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-propane-{tris-[3-carboxyl-ethoxy]methyl]3'-{tris[2'-ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside-ethoxy] methyl]methyl amide (35 mg, 3.47 μmol), ***cis***-ruthenium(II)bis(bipyridine)dichloride (3.4 mg, 6.8 μmol) and flash silica column chromatography by acetronitrile/saturated $KNO_3$ (7:3) as eluent gave 21 mg (58%) of *cis*-ruthenium(II) bis(bipyridine)1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-propane-{tris-[3-carboxyl ethoxy]methyl]3'-{tris[2'-ethoxy2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside-ethoxy]methyl] methylamide. $R_f$ 0.3 (acetonitrile:Sat $KNO_3$ = 7.5:2.5); $[\alpha]_D^{r.t}$ = +1.9 (c =1.0, $H_2O$); $^1$H NMR (300MHz, $CD_3OD$): δ = 9.1 (s, 2H), 8.75 (d, *J* = 7.1 Hz, 4H), 8.14 (br.s, 6H), 8.01 (d, *J* = 5.7 Hz, 6H), 7.95-7.92 (m, 6H), 7.85 (br.s, 4H), 7.5 (t, *J* = 4.5 Hz, 4H), 7.30 (br.s, 1H), 5.39 (d, *J* = 3.6 Hz, 18H), 5.12-5.07 (m, 36H), 4.68 (d, *J* = 7.2 Hz, 18H), 4.14 (br.s, 58H), 3.85-3.81 (m, 18H), 3.67-3.57(br, 130H), 3.41-3.36 (m, 51H), 2.43-2.38 (m, 68H), 2.12 (s, 54H), 2.06 (s, 54H), 2.03 (s, 54H), 1.95 (s, 54H); $^{13}$C NMR (125MHz, $CD_3OD$): δ 174.1; 172.0, 171.8, 171.4, 171.3, 165.1, 162.8, 158.5, 157.7, 153.0, 151.7, 143.4, 139.1, 132.8, 128.7, 128.4, 125.3, 103.0, 72.2. 71.7, 70.1, 69.8, 69.1, 68.7, 68.6, 68.5, 62.4,

61.5, 54.7, 40.4, 36.2, 20.5; MALDI-HRMS (m/z): $[M+1]^+$ Calcd for $C_{448}H_{646}N_{40}O_{238}Ru$ 10495.871; Found: 10495.8731.

(v) ***Cis*-Ruthenium(II)bis(bipyridine)1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-propane-{tris-[3-carboxyl-ethoxy]methyl]3'-{tris[2'-ethoxy-β-D-galactopyranosyl-ethoxy]methyl]methyl amide (4).** General procedure D with *cis*-ruthenium(II) bis(bipyridine)1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-propane-{tris-[3-carboxylethoxy]methyl]3'-{tris[2'-ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside-ethoxy]methyl]methylamide (16 mg, 1.54 μmol) and sodium methoxide (3 mg, 20%) gave 9 mg (65%) of *cis*-ruthenium(II) bis(bipyridine) 1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-beta-propane-{tris-[3-carboxyl-ethoxy]methyl]3'-{tris[2'-ethoxy-β-D-galactopyranosyl-ethoxy]methyl]methylamide. $[\alpha]_D^{r.t}$ = -5.3 (c =1.0, $H_2O$); $^1H$ NMR (300MHz, MeOD): δ 9.05 (s, 2H), 8.65 (d, *J* = 7.8 Hz, 4H), 8.45 (s, 6H), 8.03 (br.s, 6H), 7.8 (d, *J* = 6.0 Hz, 6H), 7.39 (d, *J* = 6.0 Hz, 2H), 4.39 (d, *J* = 7.8 Hz, 18H), 3.95 (q, *J* =4.8 Hz, 8H), 3.91-3.85 (m, 36H), 3.76-3.60 (m, 186H), 3.47-3.45 (m, 4H), 3.41-3.35 (m, 58H), 2.47-2.42 (m, 64H); $^{13}C$ NMR (125MHz, $CD_3OD$): δ 181.1, 174.4, 173.3, 172.7, 158.8. 157.0, 152.1, 138.6, 131.6, 129.2, 126.7, 122.3, 110.8, 104.3, 77.8, 73.5, 71.8, 71.8, 68.5, 67.6, 66.6, 62.4, 61.4, 40.1, 37.3. MALDI-HRMS (m/z): $[M-1]^+$ Calcd for $C_{304}H_{502}N_{40}O_{166}Ru$ 7471.1113; Found: 7471.112.

**3. Photophysical and electrochemical properties**

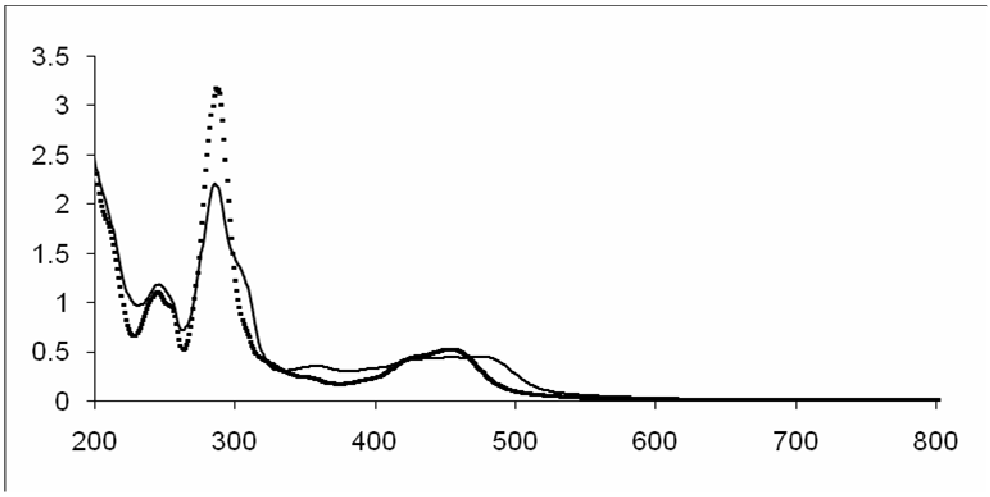


***Fig S2.*** UV-Visible spectra of **1** (solid line) and **$Ru(bipy)_3(NO_3)_2$** (dotted line)

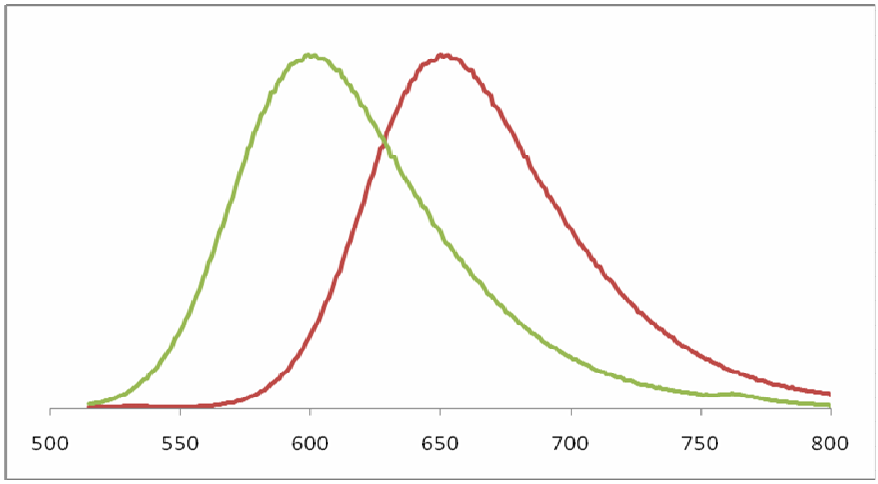


***Fig S3.*** Normalized fluorescence spectra of **1** (red line) and $Ru(bipy)_3(NO_3)_2$ (green line)

**Electrochemical Measurements.** Cyclic voltammetry experiments were carried out using a CHI-660A potentiostat. A three electrode setup was used for measurements consisting of a glassy carbon working electrode, a platinum wire counter electrode and an Ag/AgCl, KCl(sat'd) reference electrode. The measurements were performed using methanol solutions of compounds ($2 \times 10^{-3}$ M) under nitrogen bubbling with $N_2$ layer of blanket over the sample at room temperature (22°C). $^tBu_4NPF_6$ (0.1 M) was used as supporting electrolyte. The setup was calibrated with ferrocenium/ferrocene couple for which the $E_{1/2}$ was observed at 0.45 V. Square-wave voltammetry (SWV) was carried out using modified gold surface as working electrode, platinum wire as a counter and Ag/AgCl as a reference electrode. All measurements were performed at room temperature (22°C).

**Cyclic Voltametry of Ru-complexes in Solution.** The cyclic voltammetry measurements for the Ru-complex were carried out in methanol using $^tBu_4NPF_6$ as supporting electrolyte. The Ru-complex exhibits reversible redox processes in positive potential ($E_{1/2}$ = 1.32 V *vs* Ag/AgCl and 0.87 V *vs* $fc/fc^+$) (Fig S4). The analysis of the redox waves at the scan rates 100-900 $mVs^{-1}$ provides clear evidence that an oxidation process occurs for the Ru(II) to Ru(III) couple. Each couple is diffusion controlled as evidenced by the constant current function ($i_p$ *vs* $\nu^{-1/2}$) over the scan rates for 100-900

$mVs^{-1}$. The ΔE value (80 mV) for the redox process was in the range expected for one-electron couples. The $i_c/i_a$ is found to be unity indicating this process is reversible.

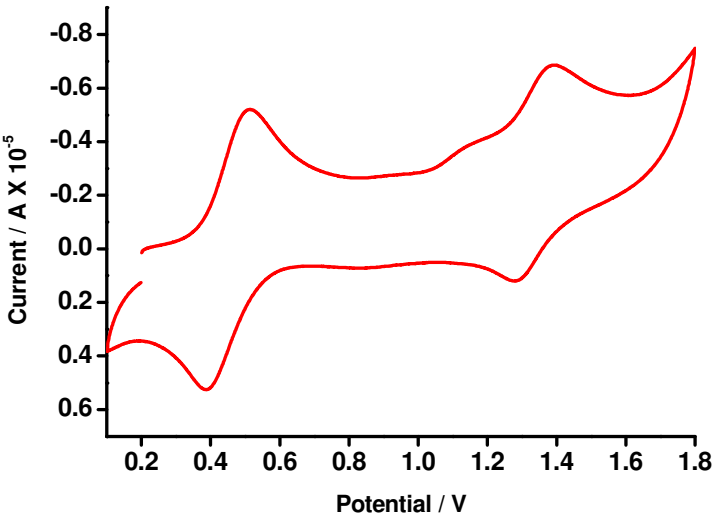


***Fig S4.*** Cyclic voltammeter of the complex **1** in acetonitrile solution (2 mM) using glassy carbon as working, Pt as counter and Ag/AgCl as reference electrode. The measurement was also calibrated with fc/fc+.

**4. Optical Lectin Sensor.**

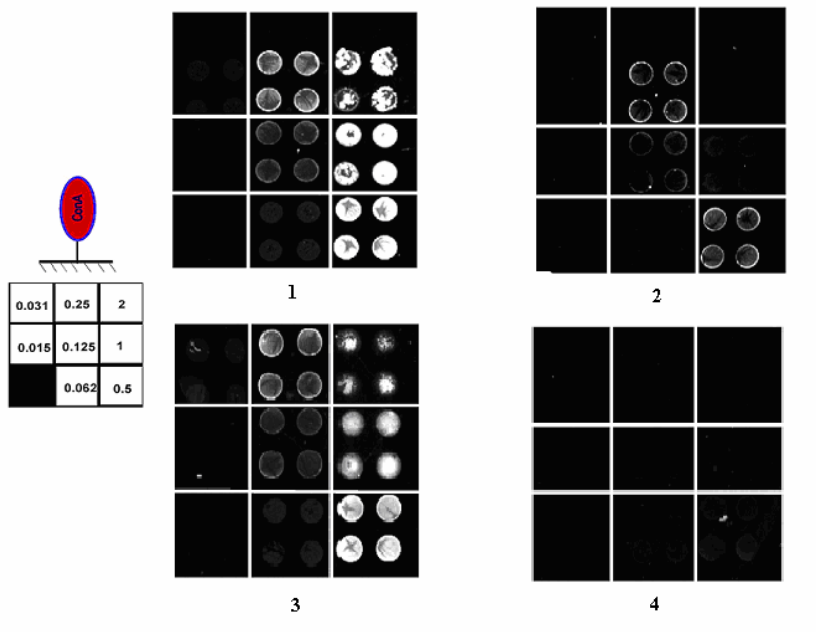


***Fig. S5.*** Incubation of Ru(II) dendrimers **1-4** with protein microarrays that contain different concentrations (mg/mL) of the lectin ConA (excitation at 480 nm).

**Lectin Microarray Construction.** Concanavalin A was diluted to 2, 1, 0.5, 0.25, 0.125, 0.062, 0.031 and 0.015 mg/mL in PBS buffer. Each sample was printed in quadruplicate on the surface of aldehyde-derivatized glass slides. The printed slides were incubated for 24 h in a humid chamber then quenched with 1% BSA in PBS for 24h at room temperature. The slides were washed three times with PBS before use.

**Microarray Binding Assay.** Microarray slides were incubated with 10 μM solution of Ru-sugar complexes dissolved in lectin binding buffer (10 mM Hepes pH 6.5, 1mM $MgCl_2$, 1mM $CaCl_2$, 1% BSA) for 1h at room temperature. The slides were subsequently washed three times with PBS and three times with water, then visualised with Perking Elmer scanner. Excitation was done at 488 nM and the detection at 633 nM.

**5. Electrochemical Lectin Sensor**

**Preparation of Mixed SAM Monolayers and Immobilization of ConA Lectin and Ru(II) Complex.**

**Materials.** Gold coated glass slides modified with coupling layer, lectin, ethanol amine, *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide (EDAC), phosphate buffer (20 mM, pH: 8.6), Trsi-HCl (pH: 7.4, 50 mM), D(+)glucose. D(+)mannose, D(+)galactose, D(+)maltose, D(+)manα(1-6)man, PIM3, PIM4, Tri-mannose. Coupling buffer: 20 mM phosphate buffer (pH: 8.6) containing 100 mM NaCl. Washing buffer: 50 mM Tris-HCl (pH: 7.4) containing 0.1 M NaCl.

**Atomic Force Microscopy (AFM) Measurement**

Topography of the modified surfaces were investigated in the dry state with a multimode instrument (Digital Instruments, Santa Barbara, CA) operating in the tapping mode. Silicon tips (OMCL-AC160TS from Olympus Corporation, Japan) with a radius less then 7 nm, spring constant of 42 N/m, and resonance frequency of 300 kHz were used.

The gold substrate appears on AFM images as stack of relatively small (up to 700 nm long) atomically flat platelets (plates/scales/flakes) with a root-mean-square (rms) roughness of about 0.2 nm. Modification with thiol self assembled monolayer was not changing the morphology of the surface noticeably (See SI, Fig S4). Overall rms roughness of the 2x2 μm image of the gold surface modified by SAM is 0.67 nm mostly due to the gaps between the plates. In contrast to SAM, ConA immobilization changes surface morphology significantly revealing well-defined, globular features with a mean height of about 2.2 nm that can be attributed to the presence of ConA. One can notice only a slight increase in rms roughness (0.82 nm for 2x2 μm image) that is mostly due to the ConA globules since gaps in the surface are almost closed and not contributing to the surface roughness.

**Gold Samples Used for CV**

Glass slides (approx. 1x4 cm) were washed with EtOH. The glass slides used for SAM films were prepared by evaporating gold (purity >99.99%, Umicore, Balzers, Liechtenstein). The silicon wafers were coated with a 10 nm chromium adhesion layer, followed by an 50-nm gold film in an evaporation chamber (MED 020 coating system, BALTEC, Balzers, Liechtenstein) at a pressure of about $2x10^{-5}$ mbar. Samples were then immediately immersed into a solution of 6-mercaptohexan-1-ol and 11-mercaptoundecanoic acid (9:1 by volume, of 0.1M solution of both compounds, all solutions were made up using 4:1 EtOH/$H_2O$) for 16 h.[1] If not functionalized immediately, samples were washed in Pirhana solution (7:3 conc. $H_2SO_4$, 30 % $H_2O_2$, respectively) for 15 min, rinsed exhaustively with water, then with EtOH (absolute). Samples were then immersed into a mixture of solutions of 6-mercaptohexan-1-ol and 11-mercaptoundecanoic acid (as described above). The samples were then rinsed with EtOH and dried under a stream of nitrogen.

**Gold Samples Used for AFM**

The freshly cleaned (Pirhana solution, 10 min) silicon wafers were coated with a 170 nm gold film in an evaporation chamber (MED 020 coating system, BALTEC, Balzers, Liechtenstein) at a pressure of about $2x10^{-5}$ mbar. Onto these surfaces, was deposited a small drop of Norland Optical

adhesive 61 (NOA 61) and these were then covered with pre-cleaned glass. The samples were cured using a UV lamp (Radium Sanolux, HRC 300-280, 300W, 230V AC) and then the gold layer was transferred onto the glass, by means of mechanical separation of the silicon wafer and glass slides.

**Ellipsometry.** Single-sided polished silicon wafers (approx 1x1 cm) for VASE measurements were prepared like the samples for CV. However, polished silicon wafers were used instead of glass pieces.

**VASE.** The monolayer thickness was measured using a VASE ellipsometer (M-2000FTM, J.A. Wollam, Inc., Lincoln, NE). Data were evaluated using the software WVASE32 (WexTech Systems Inc., New York). The measurement was conducted in the spectral range of 370-995 nm at angle of incidence of 65°C, 70°C and 75°C under ambient conditions (in air), immediately after monolayer formation (average thickness of 10.4 Å). The changes observed on the SAM after immobilization of ConA were monitored by VASE ellipsometry measurements (average value of approx. 20.6 Å).

**Lectin Immobilization.** The gold substrate (modified with a mixed monolayer) was washed twice with EtOH. The substrate was then placed in PBS (10 mL) containing NHS (1 mg) and EDAC (1 mg). After approximately 30 min mixing the supernatant was removed. Lectin solution (1 mL of 10 mg/100 mL) was added to PBS (20 mM, 100 mL, pH 8.6); then 10 mL of this solution was distributed to each of the six different vials, containing SAM-coated gold substrates. After 6 h mixing, the solution was removed and the substrate was placed in aqueous solution of ethanolamine (0.1 mL in 10 mL $H_2O$) for 10 min. After removing the solution, the gold substrates were washed with 10 mL of 50 mM Tris-HCl buffer (3 x containing 100 mM NaCl, pH 7.4).

**Immobilization of Ru-Complex.** The ConA functionalized gold substrates were placed in a solution of Ru-complex (**1-4**) (0.5 mM in Tris-HCl containing 10 mm NaCl) at room temperature. After 30 min immersion, the substrate was removed and washed three times with Tris-HCl buffer and square wave voltammetry reading were recorded in Tris-HCl (containing 10 mM NaCl). The substrate was placed again in the same Ru-complex solution. The square-wave voltammetry reading was recorded

on the same substrate after 30 min, 1h, 2h, 3h, and 4h immersion time in the same solution and washing.

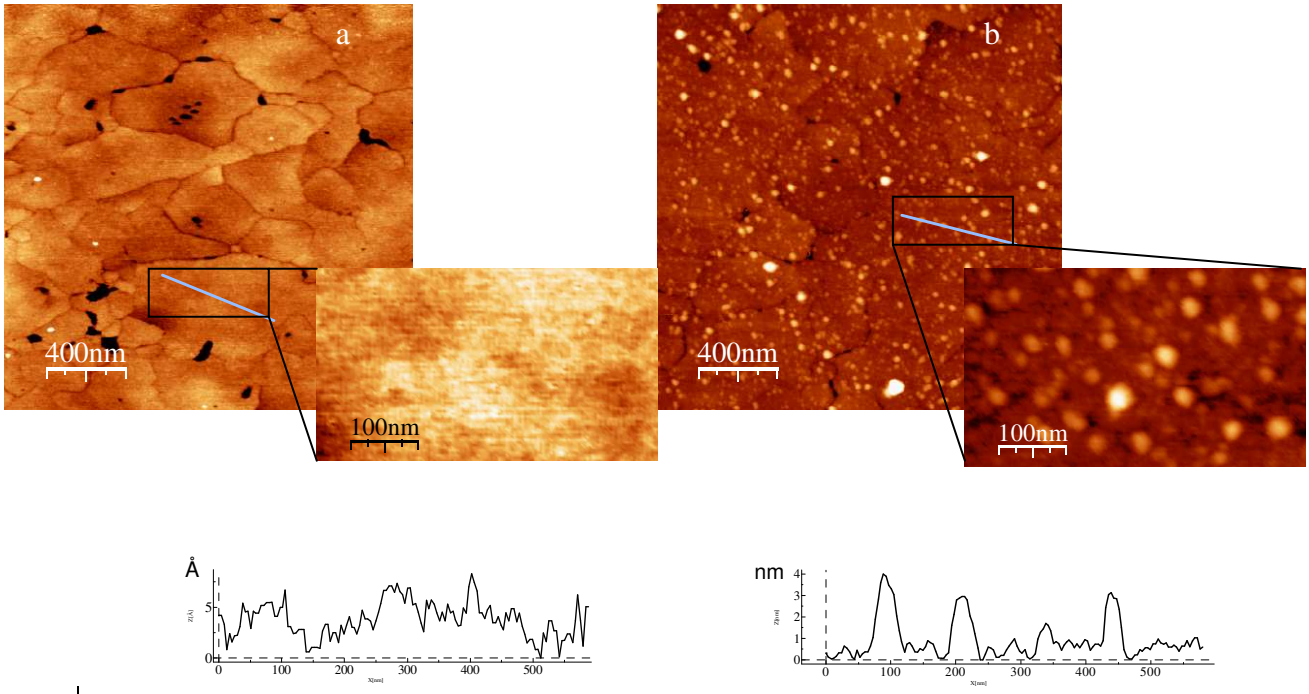


***Fig S6.*** AFM topographical images (2 µm × 2 µm) of (a) mixed SAM (z – range 4 nm), (b) immobilized ConA (z – range 10 nm) with corresponding cross-sections.

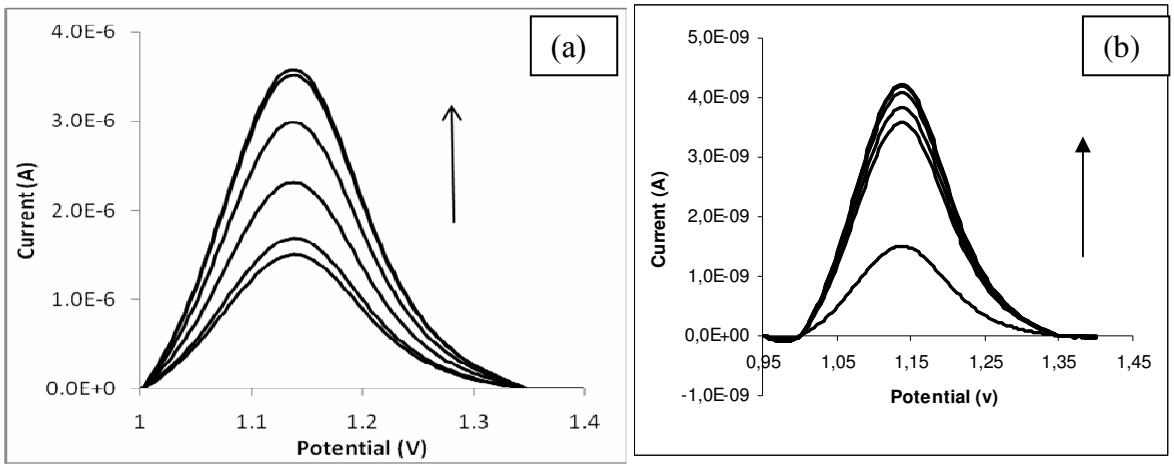


***Fig S7.*** **(a)** Square-wave voltammetric signals of the complex **1**-based monolayer formation after 30 min, 60 min, 120 min, 180 min, 240 min and 360 min immersion time in 0.5 mM ; **(b)** SWV signals of complex 3-based monolayer formation.

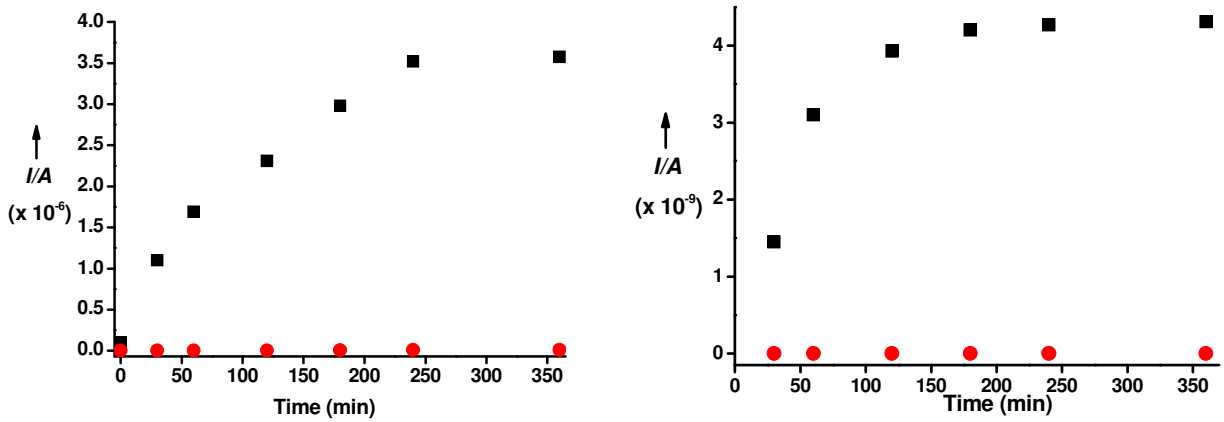


***Fig. S8***. Square-wave voltammetric measurements at 1.14 V following incubation of (a) complexes **1** (■) and **2** (●) with ConA-functionalized surfaces for six hours; (b) complex **3** (■) and **2** (●) with ConA-functionalized surfaces for six hours.

Different concentrations of ConA were immobilized on gold substrates and treated with 0.5 mM of complex **1** and **3** and SWV single was recorded. Complex **1** showed a steady and linear decrease in current single at $10^{-6}$ to $10^{-10}$ M of ConA lectin. In contrast, complex **3** showed a modest and relatively variable current response (Fig S9). The detection limits for the complex **1** calculated by this results showed comparable sensitivity than other sensors described in the literature (Table 2). These results indicate that a high degree of carbohydrate density around the ruthenium core allows for efficient encapsulation of the Ru(II) core and alter the rates of electron transfer between complex **3**[20,8c] and Au-surface results less sensitive biosensors.

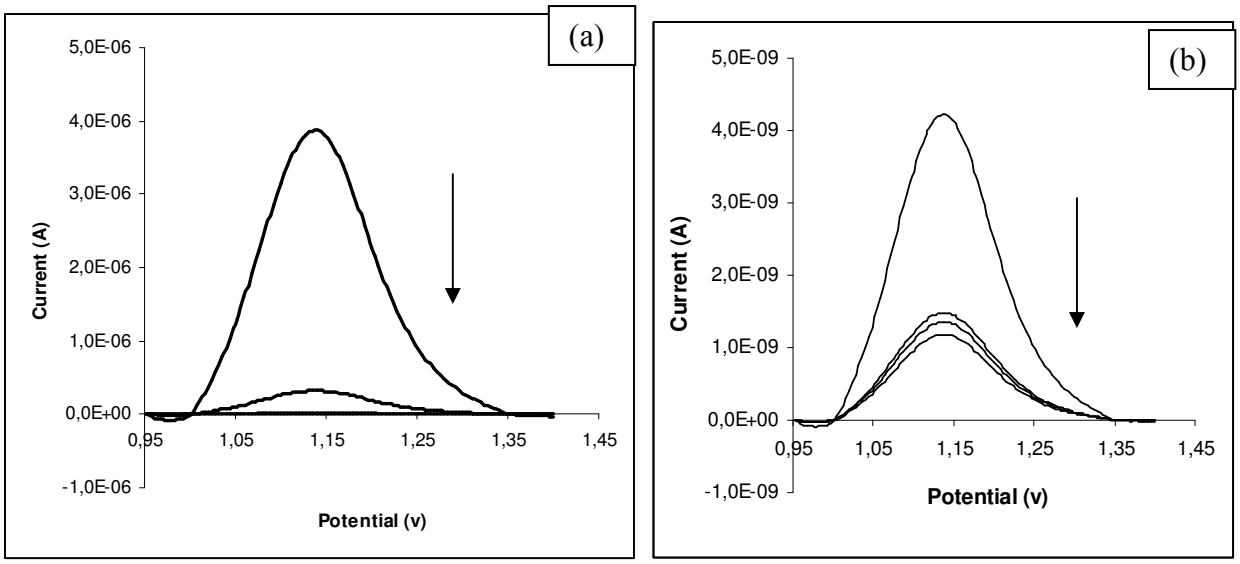


***Fig. S9.*** (a) Current signal with complex **1;** (b) Current response with complex **3** at $10^{-6}$, $10^{-7}$, $10^{-8}$, $10^{-10}$ M concentration of ConA immobilized on the gold substrate;

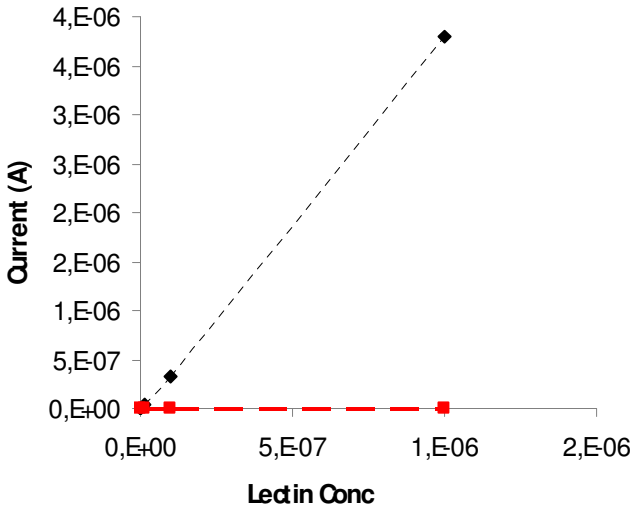


***Fig. S10.*** Current signal with complex **1** (black line) **3** (red line) at different concentrations of ConA immobilized on a gold substrate.

| Methods | Detection limits | Ref |
|---|---|---|
| Optical detection by Ru(II)-carbohydrate coated dendrimers and BBV by photoinduced electron transfer process | 28 ± 3 nM | 2a |
| Optical detection by fluorescent carbohydrate protected Au nanodots and ConA interactions | 0.7 nM | 2b |
| Optical detection by gold nanoparticles chips with a self-assembled sugar bilayer | 0.1 nM | 2c |
| Optical detection by ConA microarray with Ru(II)-carbohydrate dendrimers | 620 nM | Current method |
| Electrochemical detection by immobilizing ConA-Ru(II) dendrimers | 2.5 ± 0.12 nM | Current method |

***Table S2.*** Detection limits of ConA by different sensor systems.

**6. Sugar Detection**

1 mM stock solution of sugars were made in millipore water and diluted to desired concentrations using millipore water. The Con A-functionalized gold substrate was treated with a series of aqueous solutions of glucose ($1 \times 10^{-6}$ - $1 \times 10^{-4}$ M). The substitution of the Ru-mannose complex by the sugar was monitored using square-wave voltammetry. In a set of experiments, gold substrate modified wit Ru-complex was immersed in aqueous solutions containing $1 \times 10^{-6}$ M of sugar for 5 min. The sample was rinsed with water, then with Tris-HCl buffer and dried under $N_2$ before recording SWV.

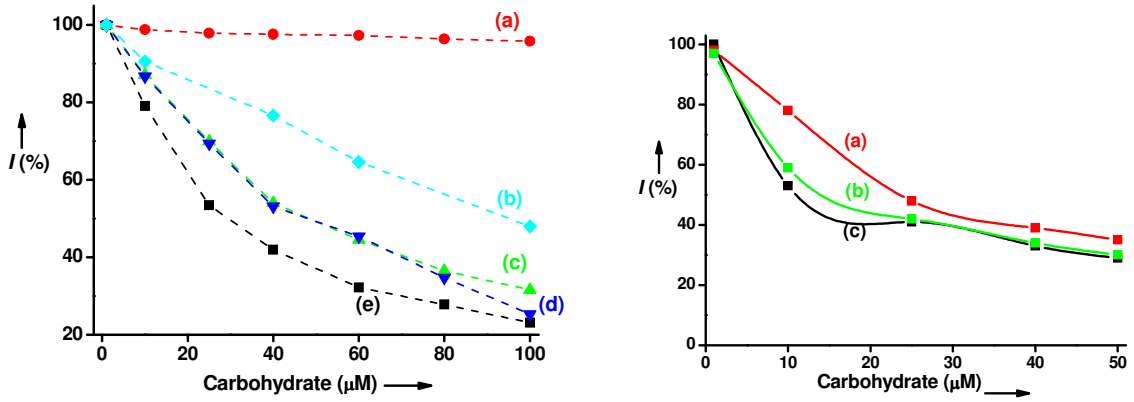


***Fig. S11:*** Response of square-wave voltammetric signals to increasing concentrations of **(i)** (a) D-galactose (●) (b) D-glucose (♦)(c) D-maltose(▲) (d) D-mannose(▲) and (e) D-manα(1-6)man (■); **(ii)** (a) PIM3 (■); (b)Tri-mannose(■); (c) PIM4 (■).

| Methods | Detection limits (μM) |
|---|---|
| D-glucose | 7 ± 0.12 |
| D-mannose | 3 ± 0.11 |
| D-maltose | 3 ± 0.06 |
| D-galactose | - |
| D-manα(1-6)man | 1.4 ± 0.12 |
| PIM3 | 1.4 ± 0.11 |
| Tri-mannose | 0.61 ± 0.07 |
| PIM4 | 0.61 ± 0.11 |

***TableS 3***. Detection limits of differents free sugars by electrochemical ConA/Ru(II)-glycodendrimer method.

| Methods | Detection limits (M) | Ref |
|---|---|---|
| Multilayer Displacement method (ConA/ferrocene) | $10^{-3}$ | 3a, 3b |
| AuNPs/Glucose oxidase (direct wiring) | $10^{-4}$ | 3c |
| PtNPs/Glucose oxidase(analysis of $H_2O_2$) | $10^{-2}$ | 3d |
| Molecular Imprinting method | $10^{-3}$ | 3e |
| Quantum dots/lectin interactions method to detect of GalNH, Gal, $\beta$-D-Gal-[1-3]-D-GalNAc | 2.7 x$10^{-6}$, $10^{-6}$, $10^{-7}$ | 3f |
| Carbon nanotubes/Glucose oxidase (direct wiring) | 3.0 x $10^{-9}$ | 3g |

***Table S4***. Detection limits of glucose and different free sugars by electrochemical methods.

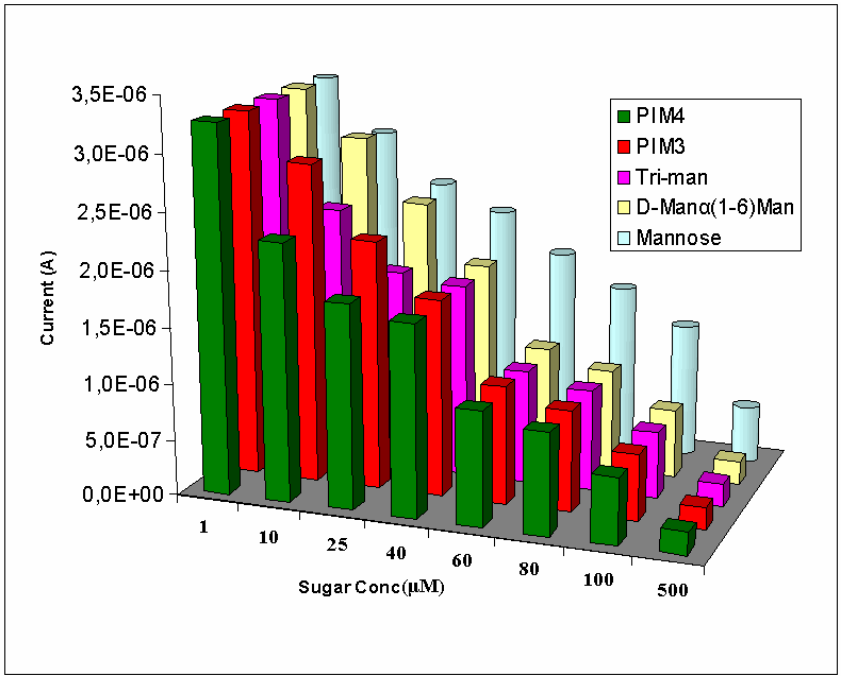


***Fig S12.*** Square wave voltammetric signals in the presence of 1- 500 µM concentration of different mannose structures.

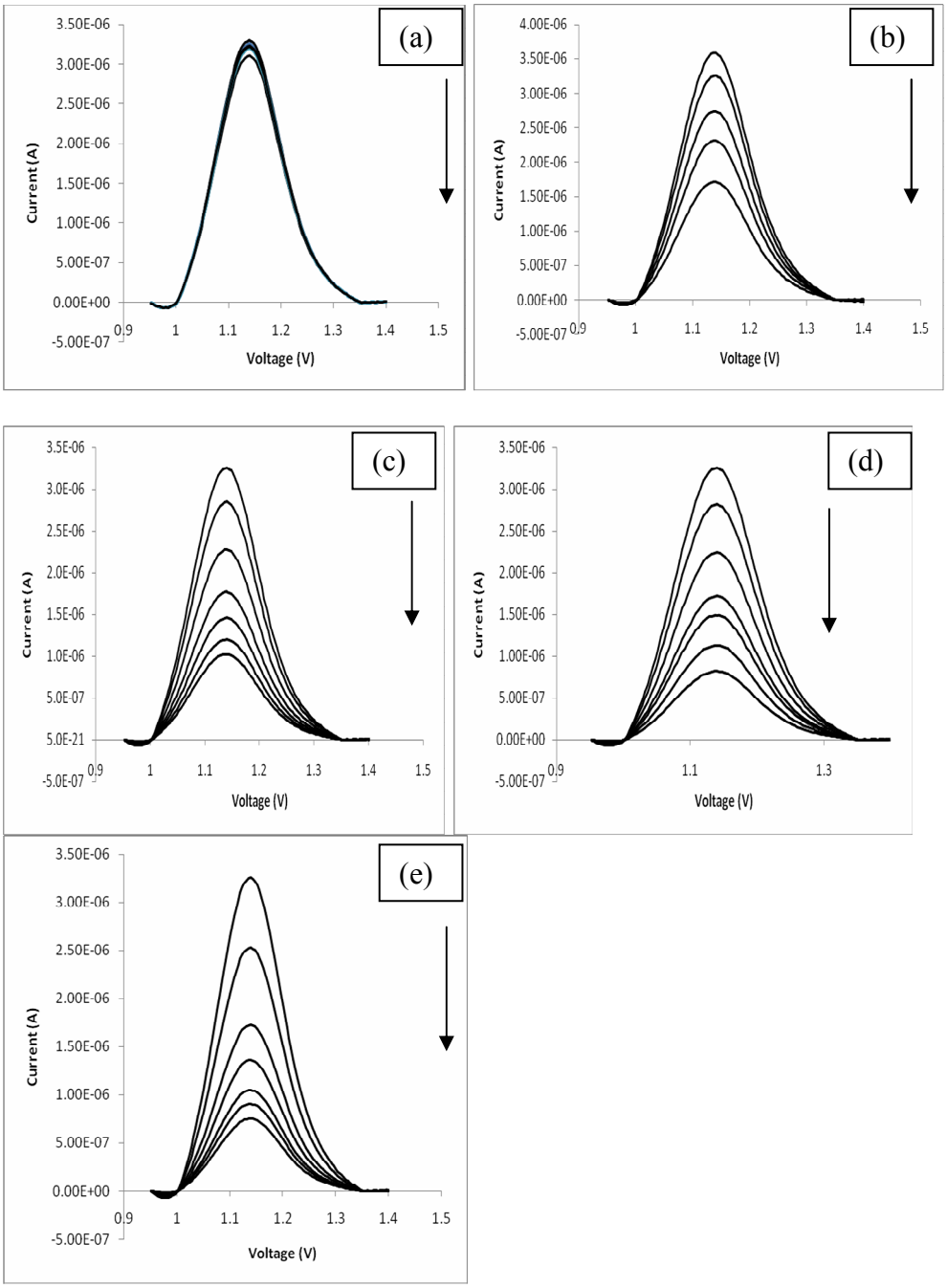


***Fig S13.*** Square wave voltammetric signals in the presence of 1- 100 µM concentration of (a) D-Galactose (b) D-Glucose (c) D-Mannose (d) D-Maltose (e) D-Manα(1-6)Man.

**Procedure for Regeneration of ConA-Au Substrate.** A stock solution of 50 mg boronic acid confined Merrifield resin[26] was swelled in a 6:4 mixture of DMF and 0.1M of phosphate buffer at pH 9.8 (3 mL). The gold substrate was immersed into the aqueous solution for 2-3 min. The sample was rinsed with phosphate buffer (0.1 M, pH 7.5), deionized water and then dried under a stream of

$N_2$. This substrate was once again incubated in a solution of complex **1** (0.5 mM) for 4 h, to obtain the regenerated substrate used for sugar sensing.

**7. References.**

**1.** Yavin, E.; Weiner, L.; Arad-Yellin, R.; Shanzer, A., *J. Phy. Chem A,* **2001**, 34, 8018

**2. (a)** Kikkeri, R.; Garcia-Rubio, I.; Seeberger, P. H., *Chem Comm.* **2009**, 235; (b) Huang, C-C.; Chen, C-T.; Shiang, Y-C.; Lin, Z-H.; Chang, H-T, *Anal. Chem*. **2009**, 81, 875. (c) Guo, C.; Boullanger, P.; Jiang, L.; Liu, T, *Biosens. Bioelectron*. **2007**, 22, 1830

**3. (a)** Xiao, Y.; Patolsky, F.; Katz, E.; Hainfeld, J. F.; Willner, I. *Science*. **2003**, 299, 1877. (b) Zayats, M.; Katz, E.; Baron, R.; *J. Am. Chem. Soc*. **2005**, 127,12400. (c) Bahshi, L.; Frasconi, M.; Telvered, R.; Yehezkeli, O.; Willner, I. *Anal. Chem*. **2008**, 80, 8253. (d) Sato, K.; Kodama, D.; Anzai, J. -I., *Anal. Bioanal. Chem.* **2006**, 386, 1899. (e) Malitesta, c.; Losito, L.; Zambonin, P. G. *Anal. Chem*. **1999**, 71, 1366. (f) Dai, Z.; Kawde, A.-N.; Xiang, Y.; La Belle, J. T.; Gerlach, J.; Bhavanandan, V. P.; Joshi. L.; Wang, J., *J. Am. Chem. Soc* **2006,** 128, 10018. (g) Jazkumar, d. R. S.; Narayanan, S. S. *Carbon*, **2009**, 47, 957.