Supplementary Material for Chemical Communications

Supplementary data

Electrochemical detection of *Arachis hypogaea* (peanut) agglutinin binding to monovalent and clustered lactosyl motifs immobilized on a polypyrrole film

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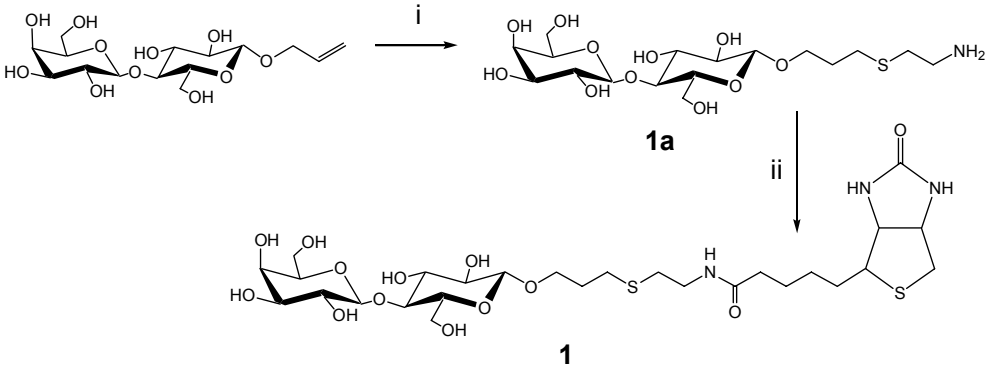
General methods

Reactions were monitored by TLC using silica gel 60 F254 precoated plates (E. Merck, Darmstadt) with CH_3CN/H_2O 8:2 (v/v) or AcOEt/AcOH/H_2O 3:3:1 (v/v/v) as eluting solvents. The detection was realized by charring with either a sulfuric acid solution (H_2SO_4/MeOH/H_2O 3:45:45) or with a 1:1 (v/v) mixture of a 2% (v/v) H_2SO_4/EtOH solution and a 0.2% (w/v) solution of *p*-dimethylaminocinnamaldehyde in EtOH. For flash chromatography, Merck silica gel 60 was used. NMR spectra were recorded on Bruker AC 300. Proton chemical shifts are reported in ppm relative to external $SiMe_4$ (0 ppm). Low-resolution FAB mass spectra were recorded in the positive mode of an R1010C quadripolar mass spectrometer (model 2000, Nermag, Reuil-Malmaison, France). High-resolution FAB mass spectra were recorded on a Micromass Zabspec TOF mass spectrometer. Protected amino acids and Sasrin resin used for the synthesis of the RAFT molecule were purchased from France Biochem S.A. or Advanced ChemTech

Europe. Reverse phase HPLC analyses were performed on Waters equipment using C_{18} columns. The analytical (Nucleosil 120 Å 3 μm C_{18} particles, 30 x 4.6 mm^2) was operated at 1.3 mL/min and the preparative column (Delta-Pak 300 Å 15 μm C_{18} particles, 200 x 25 mm^2) at 22 mL/min with UV monitoring at 214 nm and 250 nm using a linear A-B gradient (buffer A: 0.09% TFA in water; buffer B: 0.09% TFA in 90% acetonitrile). Mass spectra were obtained by electron spray ionization (ES-MS) on a VG Platform II in the positive mode. Avidin, NHS-biotin, *Arachis hypogaea* lectin (peanut agglutinin PNA) and horseradish peroxidase labeled PNA were purchased from Sigma.

Synthesis of lactosyl-conjugates

Biotin-lactoside (1)



Reagents and conditions: i) $HCl.NH_2$-$(CH_2)_2$-SH (6.4 eq), H_2O, hν (254 nm), RT overnight, 95%; ii) NHS-biotin (1.5 equiv.), DMF, RT overnight, 80%

3-[2-[Aminoethyl]-thio]-propyl β-D-galactopyranosyl-(1➠4)-β-D-glucopyranoside (1a)

A solution of allyl β-lactoside[1] (104 mg, 0.27 mmol) and cysteamine hydrochloride (199 mg, 6.4 equiv.) in distilled water (1 mL) was degassed and flushed under argon. The

mixture was irradiated at 254 nm under a UV lamp for 14 hours. The resulting amino derivative was purified by ion exchange chromatography on acidic Dowex 50WX4 resin and eluted with a gradient of aqueous ammonia (0.05N to 0.3N). After evaporation and lyophilisation, **1a** was obtained with 95% yield (120mg).

^{1}H NMR (300MHz, D_2O): δ 4.36 (d, 1H, *J* = 8Hz), 4.32 (d, 1H, *J* = 7.6Hz), 3.88-3.79 (m, 3H), 3.70-3.41 (m, 7H), 3.20-2.55 (m, 10H), 1.79 (q, 2H, *J* = 6.7Hz).

^{13}C NMR (75MHz, D_2O): δ 103.3, 102.4, 78.8, 75.7, 75.1, 74.8, 73.2, 72.9, 71.3, 69.2, 68.9, 61.3, 60.4, 39.5, 31.8, 29.2, 27.6.

3-[4-Biotinylamido-2-[aminoethyl]-thio]-propyl β-D-galactopyranosyl-(1➠4)-β-D-glucopyranoside (1)

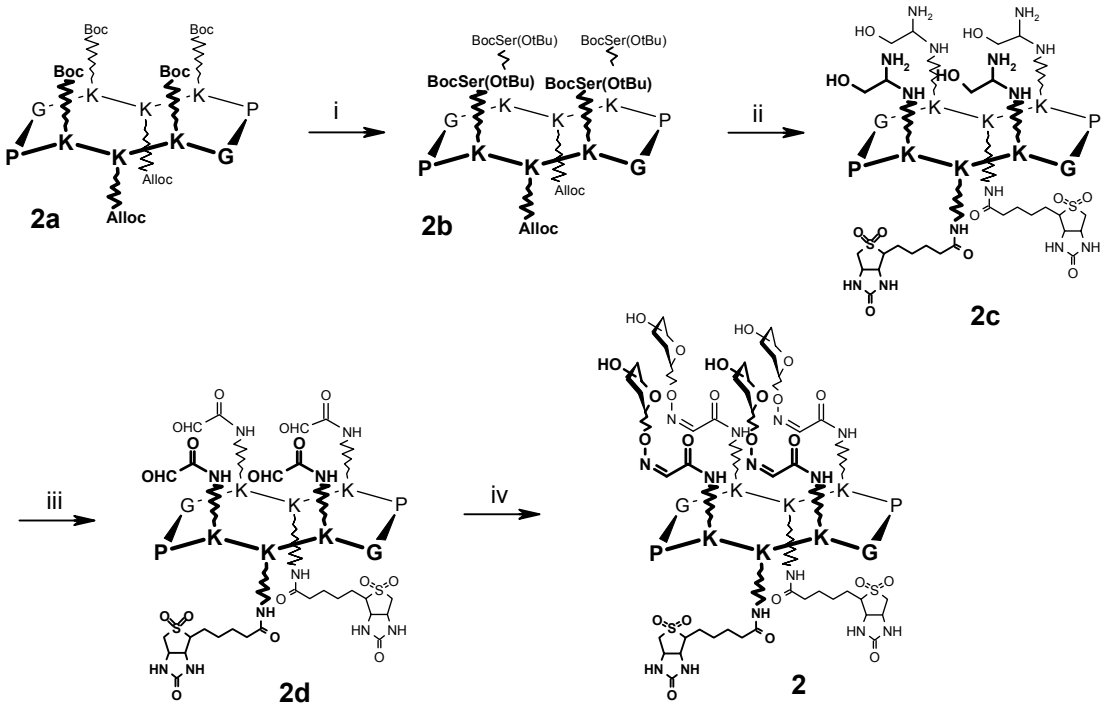
A solution of the **1a** (64mg, 0.14mmol) and NHS-biotin (72mg, 1.5 equiv.) in a minimum of anhydrous DMF was stirred overnight at room temperature and concentrated. After purification by flash chromatography with silica gel (CH_3CN/H_2O 8:2), **1** is isolated with 80% yield (120 mg).

^{1}H NMR (300MHz, D_2O): δ 4.5(dd, 1H, *J* = 4.7Hz, *J* = 8Hz), 4.38-4.33(m, 3H), 3.89-3.81(m, 3H), 3.72-3.40(m, 12H) 3.32-3.17(m, 4H) 2.89(dd, 1H, *J* = 5Hz, *J* = 13Hz), 2.66(d, 1H, *J* = 13Hz), 2.59(q, 2H, *J* = 7Hz), 2.17(t, 1H, *J* = 7.3Hz), 1.80(q, 2H, *J* = 6.7Hz), 1.64-1.46(m, 4H), 1.32(q, 2H, *J* = 7.3Hz)

^{13}C NMR (75MHz, D_2O): δ 184, 177.2, 103.3, 102.5, 78.8, 75.7, 75.1, 74.8, 73.2, 72.9, 71.3, 69.2, 68.9, 62.4, 61.4, 60.6, 60.5, 55.7, 40.1, 38.8, 35.8, 30.9, 29.2, 28.2, 28.0, 27.8, 25.5

HRMS (FAB+) calcd for $C_{27}H_{47}N_3O_{13}S_2$: 708.244 [$M+Na^+$], found: 708.245.

RAFT-lactosyl conjugate (2)



Reagents and conditions: i) 1: TFA/CH_2Cl_2 (1/1), 1 h; 2: BocSer(O*t*Bu), PyBOP, DIPEA, DMF, 2 h; ii) 1: $PhSiH_3$, $Pd(Ph_3P)_4$, CH_2Cl_2, 30 min; 2: Biotin sulfone,[4] DIPEA, DMF, 2 h; 3: TFA/TIS/H_2O (95/2.5/2.5), 2 h; iii) $NaIO_4$, H_2O, 1 h; iv) Lactose-β-ONH_2, AcOH/H_20 (1/1), 12 h.

$(Alloc)_2$RAFT(BocSerO*t*Bu)$_4$ (2b).

Removal of Boc moieties from $(Alloc)_2RAFT(Boc)_4$ **2a**[3] (570 mg, 0.45 mmol) was carried out in a solution containing 50% TFA in CH_2Cl_2 (50 mL) for 1 h at room temperature. The crude was concentrated, precipitated with diethyl ether to yield

$(Alloc)_2RAFT(NH_2)_4$ (476 mg, 85%) as a white powder (R_t = 6.7 min; 5 to 100% B in 15 min).

To this compound (476 mg, 0.38 mmol) dissolved in DMF (20 mL) were added BocSer(*t*Bu)OH (600 mg, 2.28 mmol), and PyBOP (1.18 g, 2.28 mmol) and the pH was adjusted to 8-9 with DIPEA (1.32 mL, 7.6 mmol). The solution was stirred 2 h at room temperature and the $(Alloc)_2RAFT(BocSerO tBu)_4$ **2b** (480 mg, 57%) was obtained after RP-HPLC purification (R_t = 14.6 min, 5 to 100% B in 15 min).

MS (ESI+) calcd for $C_{106}H_{185}N_{20}O_{30}$: 2218.35 [$M+H^+$], found: 2218.15.

$(Biotin)_2RAFT(Ser)_4$ (2c).

Alloc groups were removed from **2b** (409 mg, 0.18 mmol) under argon gas by adding $PhSiH_3$ (1.14 mL, 9.22 mmol) and $Pd(PPh_3)_4$ (85 mg, 0.07 mmol) in dry CH_2Cl_2. After 30 min, the solvent was evaporated under reduced pressure and the $(NH_2)_2RAFT(BocSerO tBu)_4$ (324 mg, 88%) obtained by precipitation in diethyl ether (R_t = 11.7 min; 5 to 100% B in 15 min).

This product (74 mg, 0.036 mmol) was dissolved in DMF (5 mL) then biotin sulfone[4] (30 mg, 0.11 mmol) and PyBOP (56 mg, 0.11 mmol) were added. The pH was adjusted to 8-9 with DIPEA (38 μL, 0.22 mmol) and the mixture was stirred at room temperature for 2 h. After evaporation of the solvent, the product **2c** (65 mg, 70%) was purified by semi-preparative RP-HPLC (R_t = 12.1 min; 5 to 100% B in 15 min).

The removal of Boc and *t*Bu protecting groups from $(Biotin)_2RAFT(BocSerO tBu)_4$ (65 mg, 0.025 mmol) was achieved by treatment with 10 mL of a solution of TFA/TIS/H_2O (95/2.5/2.5). After 2 h at room temperature, evaporation and precipitation from diethyl

ether, the product **2c** (43 mg, 95%) was obtained as a white powder (R_t = 6.8 min; 5 to 60% B in 15 min).

MS (ESI+) calcd for $C_{82}H_{140}N_{24}O_{26}S_2$: 1941.98 [M+H$^+$], found: 1941.59.

(Biotin)$_2$RAFT(CHO)$_4$ (2d).

The serine oxidation of **2c** (43 mg, 0.022 mmol) was carried out in with sodium periodate (189 mg, 0.088 mmol) in 5 mL of water. After 1 h at room temperature, the solution was purified by RP-HPLC to afford **2** (28 mg, 69%) (R_t = 7.3 min; 5 to 60% B in 15 min).

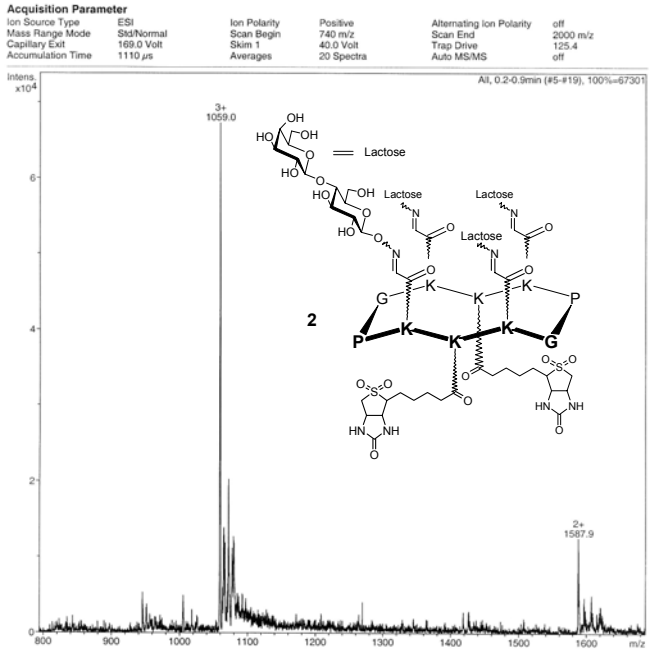
MS (ESI+) calcd for $C_{78}H_{121}N_{20}O_{26}S_2$: 1817.82 [M+H$^+$], found: 1817.58.

(Biotin)$_2$RAFT(Lactose)$_4$ (2).

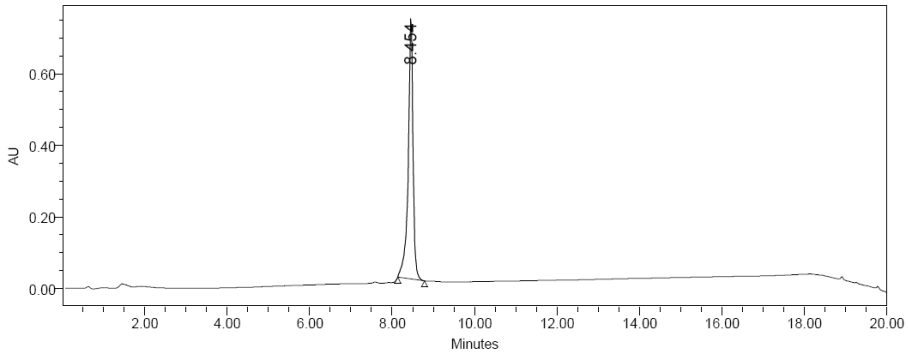
To a solution of **2d** (28 mg, 0.015 mmol) in 1 mL of AcOH/$H_2$0 (1/1) was added β-aminoxylated lactose[2] (55 mg, 0.17 mmol). The solution was stirred at room temperature overnight. The biotinylated lactoconjugate **2** (24 mg, 50%) was finally obtained after RP-HPLC purification (R_t = 8.5 min; 5 to 60% B in 15 min).

MS (ESI+) calcd for $C_{126}H_{205}N_{24}O_{66}S_2$: 3174.28 [M+H$^+$], found: 3173.81.

ES-MS (positive mode) spectrum of compound (2)



RP-HPLC profile of pure compound (2) (5 to 60% B in 15 min, 214 nm)



Synthesis of pyrrole monomers (3,4)

Biotin [12(1-pyrrolyl) dodecyl] ester **3**[5] and the amphiphilic pyrrole derivative [12(1-pyrrolyl) dodecyl] trimethylammonium tetrafluoroborate **4**[6] were synthesized following the previously reported procedures.

Preparation of lactose functionalized polypyrrole assembly

Copolymer biotin-pyrrole film realization

The electropolymerization of monomers **3, 4** and electrochemical characterization of copolymer film were run at room temperature under argon atmosphere in a conventional three-electrode cell with an EG&G Princeton Applied Research 175 potentiostat in conjunction with a Sefram TGV 164 recorder. A 10mM Ag/Ag^+ double junction electrode in CH_3CN was used as reference electrode and a platinum electrode as counter electrode. The working electrodes were glassy carbon rotating disk (diameter 3mm) polished with 1-μm diamond paste. The electrogeneration of the copolymer film on glassy carbon disk was performed by electro-oxidation of monomer's mixing (4mM/4mM) at 0.80 V in CH_3CN containing 0.1M tetrabutylammonium perchlorate (TBAP) as a supporting electrolyte. The characterization of the copolymer film and the thickness of the film were controlled cycling the potential between -0.1 and +0.85 V and integrating the electrical charge passed during this cyclic voltammogram.

Molecular assembly preparation

Deposition of the avidin layer was realized by incubating the polypyrrole coated electrodes in an avidin solution (20µl, 0.5mg/mL) in freshly prepared phosphate buffer (0.1 M, pH 7.2) for 30 min at room temperature. The obtained electrodes were carefully rinsed with phosphate buffer (0.1 M, pH 7.2), and then incubated in a solution of biotin-lactoside **1** (20µL, 2µg/mL i.e 3.10^{-6} M) or RAFT-lactosyl conjugate **2** (20µL, 8µg/mL i.e. $2.5.10^{-6}$ M) during 30min at room temperature. The electrodes were then rinsed thoroughly with phosphate buffer (0.1 M, pH 7.2) to remove the weakly adsorbed biotinylated substrates.

Supplementary Material for Chemical Communications

The binding of PNA lectin was studied by incubating 20µl of PNA (10µg/ml) in phosphate buffer (0.1 M, pH 7.2) containing 1mM $CaCl_2$ and 1mM $MnCl_2$, during 45 min at room temperature. Electrodes were then rinsed with phosphate buffer (0.1 M, pH 7.2). The peroxidase conjugate was used with the same way.

Electrochemical measurements

The impedance spectroscopy, permeability and amperometric measurements were performed with a multipurpose potensiostat/galvanostat Autolab (PGSTAT 100 and FRA module). A conventional three-electrode cell with a saturated calomel electrode (SCE) as reference and a platinum electrode as counter electrode was used in water media. The impedance spectroscopy measurements were carried out by scanning the frequency from 10^{-1} to $5\ 10^5$ Hz, acquiring sixteen points from $5\ 10^5$ to $5\ 10^4$ Hz, thirty three points from $5\ 10^4$ to 5 Hz and height points from 5 to 10^{-1} Hz, in aid of logarithmic distribution. An alternating voltage of $5mV_{rms}$ was over imposed with DC potential 0V. These measurements were treated with Z View (Scribner Associates, Inc).

The permeability measurements were obtained between 0 and -0.4V, scanning to 5mV/s. Ru(III)hexamine trichloride 2mM was used as redox permeation probe.

Amperometric measurements were carried out in phosphate buffer (0.1 M, pH 7.2) containing hydroquinone (2mM). For the response measurements, the HRP-PNA modified electrodes were first conditioned at 0V to reach a stable background current. After stabilization, 80µL of H_2O_2 (0.5mM) were injected into the electrolyte. The current intensity for the quinone reduction was monitored as indicator of the amount of anchored lectin.

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