ELECTRONIC SUPPLEMENTARY INFORMATION

On surface *O*–glycosylation by catalytic microcontact printing

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Materials and methods

Instrumentation

Contact angle analysis was performed using a *DSA 100* (KRÜSS) in combination with *Drop Shape Analysis v1.90.0.14.* Static contact angles were measured with 7 µl drops of ultrapure water. **X-Ray photoelectron spectroscopy** was performed with a *Kratos Axis Ultra* (KRATOS) using monochromated Al Kα irradiation with an excitation energy of 1486.6 eV. For region scans a pass energy of 0.02 eV was employed. The obtained data was analyzed with *CasaXPS Software Suite* v2315. All spectra were calibrated to the binding energy of the C-1s-orbital in aliphatic carbon-carbon chains (285 eV). **Fluorescence microscopy** was performed with a BX 53 microscope (OLYMPUS) operated with an XC 10 camera (OLYMPUS) and a X-Cite® Series 120Q (LUMEN DYNAMICS) as the irradiation source. For **light microscopy**, a CKX 41 (OLYMPUS) microscope with an XC 30 (OLYMPUS) camera and a X-Cite® Series120Q (LUMEN DYNAMICS) as the irradiation source were used. Data processing was carried out with the software *Olympus Stream Start 1.8*, *Photoshop CS* and *ImageJ v1.50.* **ToF-SIMS** measurements were carried out using a TOF V (IONTOF GmbH, Münster) compatible instrument equipped with a 30 keV liquid metal ion gun (IONTOF GmbH, Münster). As primary ions Bi_3^+ clusters with a pulsed current of 0.05 pA were used.

Preparation of 11-hydroxyundecenyltricholosilane substrates

Hydroxyl-terminated SAMs was prepared according to a literature procedure.[1] Silicon and glass slides were cut in suitable sizes and cleaned by treatment in an ultra-sonic bath first in acetone then in ethanol and afterwards in distilled water for 10 min each time. To activate the surface the substrates were exposed to a mixture of conc. sulfuric acid and 30% hydrogen peroxide (3:1 vol.) for 30 min. After cleaning the activated substrates and removal of the activation reagent by extensively rinsing the substrates with distilled water, they were carefully dried in an argon stream. The activated cleaned and dried glass or silicon slides were put in a solution of 11-acetoxyundecenyltricholosilane (0.10 vol%) in toluene for about 1 h. Afterwards the substrates were rinsed with acetone and ethanol and the success of the SAM generation was verified by contact angle measurements. For the removal of the acetyl protecting group the substrates were stirred for 2.5 h and 85 °C in 2 M HCl. Afterwards the substrates were rinsed with water and acetone and dried properly.

Microcontact printing of methylthiomannosides

Hydroxyl-terminated substrates were incubated with a solution of **1** or **2** (25 mM) and NIS (25 mM) in dry N,N-dimethylformamide (DMF) for 5 min. For the combined printing of both methylthiomannosides, a solution of **1** (20 mM), **2** (5 mM) and NIS (25 mM) in dry DMF was drop casted to the substrates. Structured or homogenous stamps were incubated with a solution of TMSOTf (2.5 mM) for 1 min. The excess of the solution was removed and the stamps were brought into contact with the surfaces. The TMSOTf incubated stamps were used in the printing process directly after drying. After 2 h, the substrates were rinsed with acetone and ethanol. For the removal of the benzoyl group the substrates were stirred in 50 mM sodium methoxide overnight at room temperature. The success of the procedure was monitored via contact angle measurements.

Incubation of proteins at surface bound ligands

To verify the successful immobilisation of mannose to the surfaces, the substrates were stained with fluorescein modified concanavalin A (ConA-FITC) or rhodamine modified concanavalin A (ConA-Rh). To reduce unspecific binding of the proteins to the surface the dried substrates were incubated with a 3 wt% solution of BSA in HEPES buffer (20 mM HEPES, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$, pH 7.5) for 30 min.

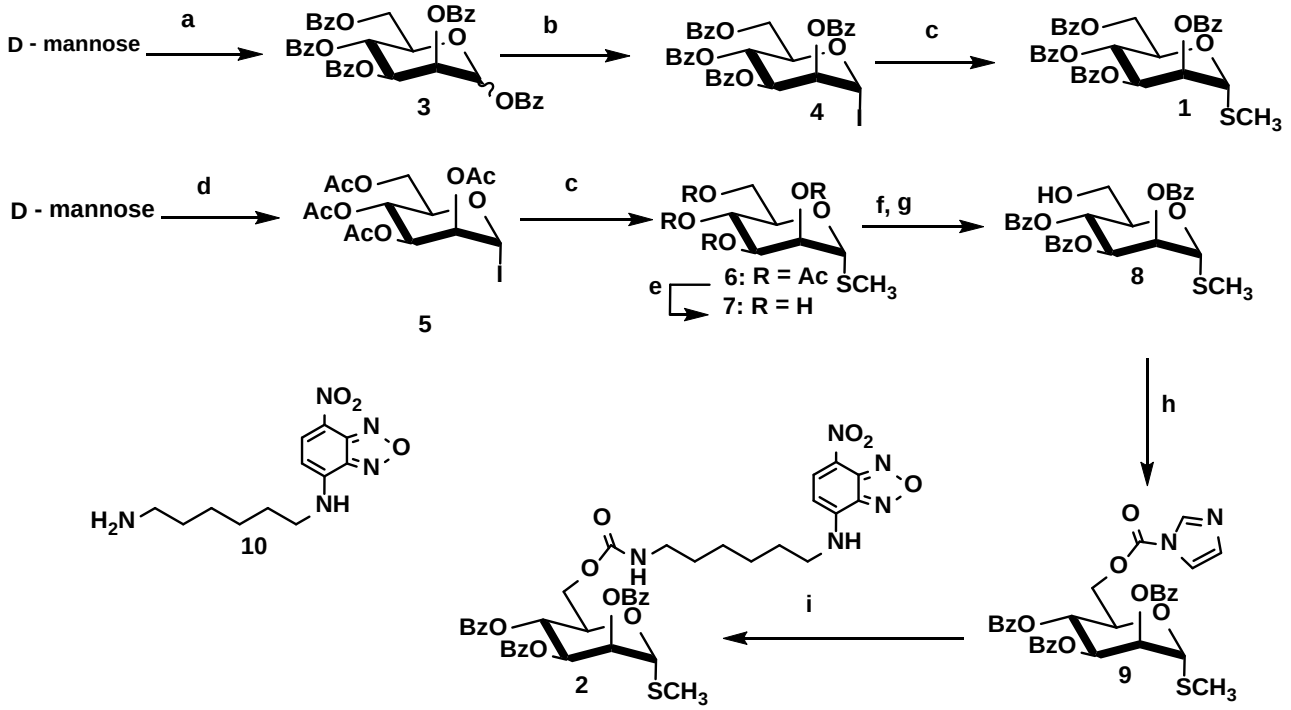
[1] Kettling, F.; Vonhören, B.; Krings, J. A.; Saito, S.; Ravoo, B. J. *Chem. Commun.* **2015**, *51*, 1027-1030.

and washed with HEPES buffer (2 x 5 min.) afterwards. The substrates were then incubated with a solution of ConA-FITC (100 µg/mL in HEPES buffer) or ConA-Rh (100 µg/mL in HEPES buffer) for 30 min. Prior to fluorescence microscopy, the substrates were washed with HEPES buffer and dried carefully.

Preparation of PDMS stamps

PDMS stamps were prepared using *Sylgard® 184 Silicone Elastomer Kit* (DOW CORNING). PDMS was mixed with the curing agent in a 10:1 ratio and agitated with a glass bar for 5 min. The mixture was put on a silicon master and residual gas in the mixture was removed in vacuum using a desiccator. The PDMS mixture was cured at 80°C overnight. If not mentioned otherwise, the stamps were cut into suitable pieces (ca 1 x 1 cm) and treated in a UV ozonizer (*PSD-UV*, *Novascan Technologies Inc.*) for 55 min. The freshly oxidized stamps were immediately stored in distilled water. Prior to use, the stamps were carefully dried in an argon stream.

Synthesis



Scheme S1. Synthesis of thioglycoside donors. Reagents and conditions: a) BzCl, pyridine, rt, overnight, extractive work-up, quantitative; b) I_2, PMHS, DCE reflux, 15 min then extractive work-up, quantitative; c) thiourea, CH_3CN, 60°C, 30 min then CH_3I, Et_3N, rt, 1h, 83%; d) I_2, Ac_2O, rt, 30 min then further I_2, PMHS, DCM reflux, 10 min then extractive work-up, quantitative; e) NaH, MeOH, rt, 2h, quantitative; f) TrCl, pyridine, 100°C, 1h then further pyridine, BzCl, rt, overnight then extractive work-up; g) AcOH/ H_2O 5:1, 70°C, 2h, 52% over three steps; h) carbonyldiimidazole, DCM, rt, 2h, 89%; i) compound **10,** THF/DMSO 5:1, 80°C, 3h, 64%

1,2,3,4,6, per-O-benzoyl-D-mannopyranoside (3): To a suspension of D-mannose (314 mg, 1.74 mmol) in pyridine (4 mL) was added benzoyl chloride (1.2 mL, 10.4 mmol) at 0°C. After 10 min the reaction was allowed to warm to rt and stirred overnight. The mixture was diluted with ethyl acetate and sequentially washed with aq. NaOH and a saturated solution of aq. $CuSO_4$. The organic phase was then dried over anhydrous Na_2SO_4 and concentrated under vacuum to yield crude compound **3** which was submitted to the subsequent thioglycoside synthesis as illustrated below.

Methyl 2,3,4,6-tetra-O-benzoyl-1-thio-α-D-mannopyranoside (1):[2,3,4] Polymethylhydrosiloxane (114 µL, 1.91 mmol) was added to a solution of the crude per-O-benzoylated compound **3** and iodine (486 mg, 1.91 mmol) in anhydrous 1,2-dichloroethane (8 mL) and refluxed under stirring until complete consumption of the starting material was observed by TLC (15 min). The mixture was then diluted with dichloromethane (DCM) and washed with aq. Na_2CO_3 containing a slight amount of sodium thiosulfate (sufficient to reduce residual iodine in the organic phase). The organic phase was then dried over anhydrous Na_2SO_4 and concentrated under vacuum. Subsequently, thiourea (199 mg, 2.61 mmol) was added to the obtained crude iodide **4** and the mixture was suspended in dry CH_3CN (8 mL) and stirred at 60°C until the quantitative generation of a polar product (30 min). After

cooling to rt, CH_3I (217 µL, 3.48 mmol) and Et_3N (970 µL, 7.0 mmol) were sequentially added. After stirring for 1 h, the mixture was diluted with DCM and washed with water. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude residue was purified by chromatography to yield compound **1** (900 mg, 83% overall yield) as a white foam. ^1H NMR ($CDCl_3$, 600 MHz) δ 8.13 – 7.28 (Ar), 6.17 (1H, t, J = 10.0 Hz, H-4), 5.87 (1H, dd, J = 3.0 and 10.0 Hz, H-3), 5.85 (1H, bd, J = 3.0 Hz, H-2), 5.49 (1H, bs, H-1), 4.84 – 4.81 (1H, m, H-5), 4.71 (1H, dd, J = 2.6 Hz and 12.0 Hz, H-6a), 4.56 (1H, dd, J = 4.5 Hz and 12.0 Hz, H-6b), 2.28 (3H, s, -SCH_3). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 166.7, 166.2, 165.6, 165.5, 134.8 (x2), 134.5, 134.4, 131.1 – 129.6, 84.9, 73.1, 71.8, 70.5, 68.4, 64.2, 15.1

Methyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside (6):[2,3,4] To a suspension of D-mannose (994 mg, 5.52 mmol) in acetic anhydride (2.66 mL, 28.1 mmol) was added iodine (98.1 mg, 0.39 mmol) and the mixture was stirred at room temperature until completion of the acetylation (30 min). After dilution with DCM (4 mL), further iodine (1.44 g, 5.68 mmol) and polymethylhydrosiloxane (363 µL, 6.07 mmol) were sequentially added and the mixture was refluxed until TLC displayed quantitative iodination of the per-acetylated compound (10 min). The mixture was then diluted with DCM and washed with aq. Na_2CO_3 containing a slight amount of sodium thiosulfate (sufficient to reduce residual iodine in the organic phase). The organic phase was then dried over anhydrous Na_2SO_4 and concentrated under vacuum. Subsequently, thiourea (630 mg, 8.28 mmol) was added to the obtained crude iodide **5** and the mixture was suspended in dry CH_3CN (8 mL) and stirred at 60°C until the quantitative generation of a polar product (30 min). After cooling to rt, CH_3I (687 µL, 11.04 mmol) and Et_3N (3.07 mL, 22.08 mmol) were sequentially added. After stirring for 1 h, the mixture was diluted with DCM and washed with water. The organic phase was dried and concentrated under vacuum. The crude residue was purified by chromatography to yield compound **6** (1.753 g, 84% overall yield) as a white foam.

Methyl 1-thio-α-D-mannopyranoside (7): A 0.5 M solution of NaH in methanol (400 µL) was added to a solution of compound **6** (1.753 g, 4.64 mmol) in methanol/DCM 9:1 (15 mL). The mixture was stirred at room temperature until TLC displayed complete de-O-acetylation and then neutralized with Amberlyst resin. After filtration, the product was concentrated under vacuum to yield deprotected thioglycoside **7** (974 mg, 100% yield) as a white solid, which was directly submitted to the subsequent step.

Methyl 2,3,4-tri-O-benzoyl-1-thio-α-D-mannopyranoside (8): A mixture of compound **7** (974 mg, 4.64 mmol) and trityl chloride (1.42 g, 5.1 mmol) was suspended in pyridine (2 mL) and stirred at 100°C for 1.5 h. After cooling to room temperature, further pyridine (3.75 mL, 46.4 mmol) and benzoyl chloride (2.96 mL, 25.5 mmol) were sequentially added and the mixture was stirred until complete per-O-benzoylation of the tritylated compound (TLC). The reaction was quenched with methanol (2 mL), then the mixture was diluted with DCM and washed with water. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude residue was

[2] Valerio, S.; Iadonisi, A.; Adinolfi, M.; Ravidà, A. *J. Org. Chem.* **2007**, *72*, 6097–6106

[3] Adinolfi, M.; Iadonisi, A.; Ravidà, A.; Schiattarella, M. *Tetrahedron Lett.* **2003**, *44*, 7863–7866

[4] Giordano, M.; Iadonisi, A. *Eur. J. Org. Chem.* **2013**, 125–131

suspended in acetic acid/H_2O 5:1 (40 mL) and stirred for 3h at 70 °C. The mixture was then diluted with DCM and sequentially washed with water and aq. Na_2CO_3. The organic phase was dried and concentrated under vacuum and the crude residue was purified by chromatography to afford compound **8** (1.26 g, 52% yield) as a white foam. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.10 – 7.26 (Ar), 5.94 (1H, dd, J = 3.0 and 10.0 Hz, H-3), 5.90 (1H, t, J = 10.0 Hz, H-4), 5.82 (1H, bd, J = 3.0 Hz, H-2), 5.49 (1H, bs, H-1), 4.45 – 4.43 (1H, m, H-5), 3.86 (1H, dd, J = 2.1 Hz and 12.8 Hz, H-6a), 3.84 (1H, dd, 3.8 Hz and 12.8 Hz, H-6b), 2.26 (3H, s, -SCH_3).

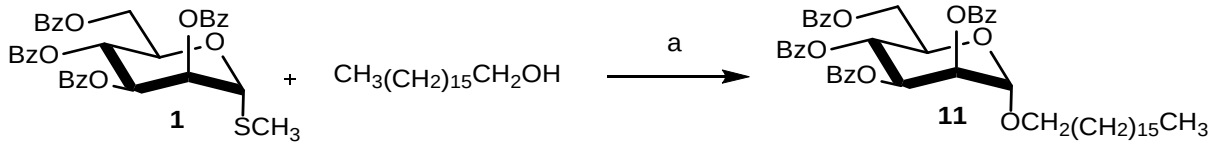
Methyl 2,3,4-tri-O-benzoyl-6-O-imidazoyl-1-thio-α-D-mannopyranoside (9):

Carbonyldiimidazole (781 mg, 4.82 mmol) was added to a solution of compound **8** (1.26 g, 2.41 mmol) in dry DCM (10 mL) and the mixture was stirred at room temperature until completion of the reaction (2 h). The mixture was diluted with DCM and washed with water. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under vacuum and the product was purified by chromatography, yielding compound **9** (1.33 g, 89% yield) as a white foam. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.32 – 7.14 (Ar), 6.09 (1H, t, J = 10.0 Hz, H-4), 5.86 (1H, dd, J = 3.0 and 10.0 Hz, H-3), 5.84 (1H, dd, J = 1.2 and 3.0 Hz, H-2), 5.46 (1H, bs, H-1), 4.82 – 4.80 (2H, overlapped signals, H-5, H-6a), 4.61 (1H, dd, J = 4.2 and 12.0 Hz, H-6b), 2.26 (3H, s, -SCH_3).

Compound (2): A mixture of compound **9** (1.33 g, 2.15 mmol) and amine **10** was suspended in tetrahedrofurane/dimethylsulfoxide 5:1 (18 mL) and heated at 80°C. After 3 h, the mixture was cooled to room temperature, diluted with ethyl acetate and washed with water. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude residue was purified by chromatography to yield compound **2** (1.14 g, 64% yield) as a deep orange foam. ^{1}H NMR ($CDCl_3$, 600 MHz)δ d, J = 8.6 Hz, NBD aromatic proton), 8.11 – 7.26 (Ar), 6.51 (1H, s, -NH-NBD), 6.14 (1H, d, J = 8.6 Hz, NBD aromatic proton), 5.95 (1H, t, J = 10.0 Hz, H-4), 5.82 (1H, dd, J = 3.2 and 10.0 Hz, H-3), 5.77 (1H, bs, H-2), 5.48 (1H, bs, H-1), 4.76 (1H, t, J = 6.0 Hz, -NH), 4.69 – 4.66 (1H, m, H-5), 4.39 (2H, m, H-6a, H-6b), 3.44-3.48 (2H, m, -CH_2-NH-), 3.20 – 3.17 (2H, m, - m, -CH_2-NH-), 2.26 (3H, s, -SCH_3), 2.06 – 1.27 (alkyl chain protons). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 166.1, 165.5, 165.3, 156.0, 144.2, 143.8, 136.4, 133.6, 133.5, 133.2 – 128.3, 83.6, 71.9, 70.3, 69.3, 67.3, 63.3, 43.5, 40.4, 29.8, 28.2, 26.0, 25.7, 13.8

Compound (10): The synthesis of NBD-conjugated hexamethylenediamine **10** was performed according to literature.[5]

[5] Yamaguchi, T.; Asanuma, M.; Nakanishi, S.; Saito, Y.; Okazaki, M.; Dodo, K.; Sodeoka, M. *Chem. Sci.* **2014**, *5*, 1021-1029

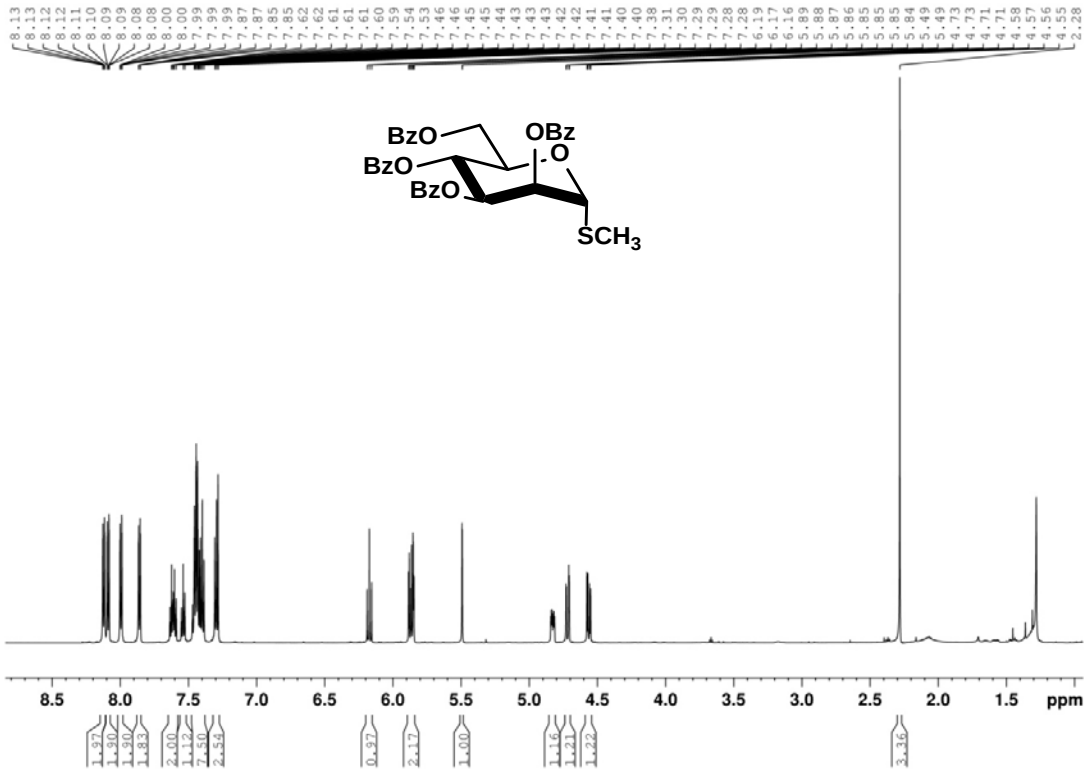


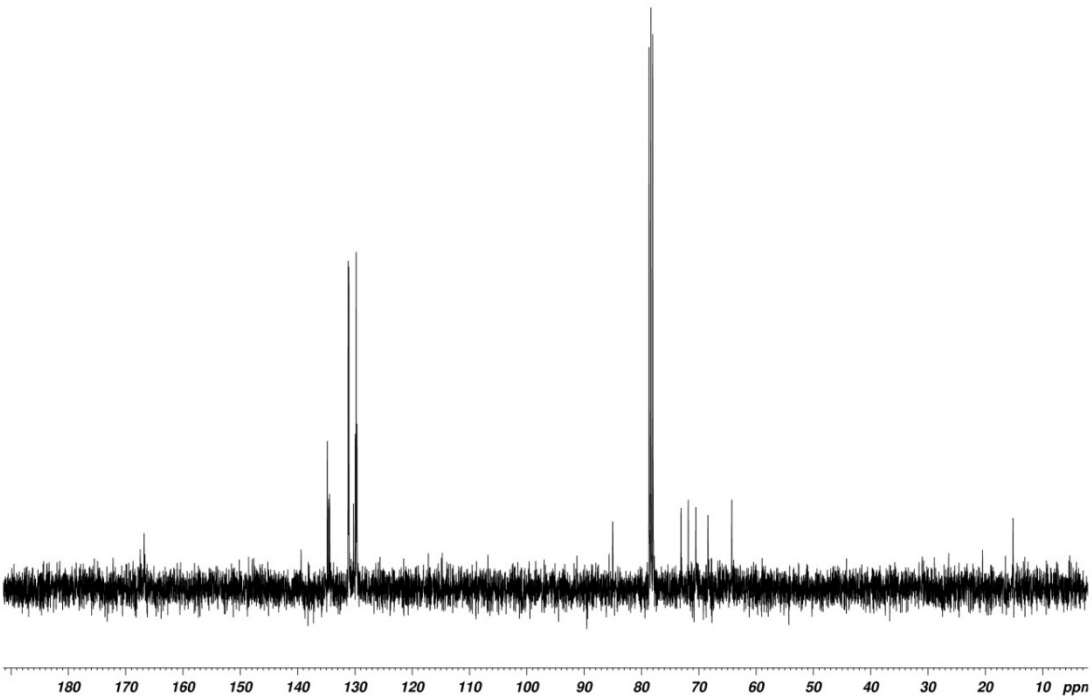
Scheme S2. Glycosylation of a primary alcohol in solution. Reagents and conditions: a) DMF, NIS, TMSOTf, AW-300 4 Å molecular sieves, DCM, 0°C to rt, 2 h, 37%

1–Heptadecyl 2,3,4,6–tetra–O–benzoyl-α-D-mannopyranoside (11): 1-Heptadecanol (6 mg, 0.0223 mmol) and mannosyl thioglycoside **1** (21 mg, 0.0335 mmol) were coevaporated three times with dry toluene. Freshly activated AW-300 4 Å molecular sieves and then NIS (16 mg, 0.0711 mmol) were sequentially added to the mixture under argon. DCM (0.4 mL) and then DMF (26 µL, 0.335 mmol), freshly dried over 4 Å molecular sieves, were added and the mixture was stirred at 0°C for 5 min before the addition of TMSOTf in DCM (0.67 M, 100 µL, 0.067 mmol). After 10 min the mixture was allowed to warm to rt and stirred for 2 h. The reaction was quenched with triethylamine and then the mixture was diluted with DCM and washed with water. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under vacuum and the crude product was purified by chromatography to yield compound **11** (7 mg, 37% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.12 – 7.27 (Ar), 6.12 (1H, t, J = 10.1 Hz, H-4), 5.94 (1H, dd, J = 3.4 Hz and 10 Hz, H-3), 5.71 (1H, dd, J = 1.7 Hz and 3.4 Hz, H-2), 5.10 (1H, d, J = 1.7 Hz, H-1), 4.71 (1H, dd, J = 2.5 Hz and 12.1 Hz, H-6a), 4.51 (1H, dd, J = 4.6 Hz and 12.1 Hz, H-6b), 4.46 – 4.43 (1H, m, H-5), 3.86 – 3.82 (1H, bd, J = 9.5 Hz -O-CH_aH$_b$- aglycone), 3.61 – 3.57 (1H, bd, J = 9.5 Hz -O-CH$_a$$H_b$- aglycone), 1.73 – 1.27 (alkyl chain CH_2 protons), 0.92 (3H, t, J = 6.2 Hz, -CH_3).

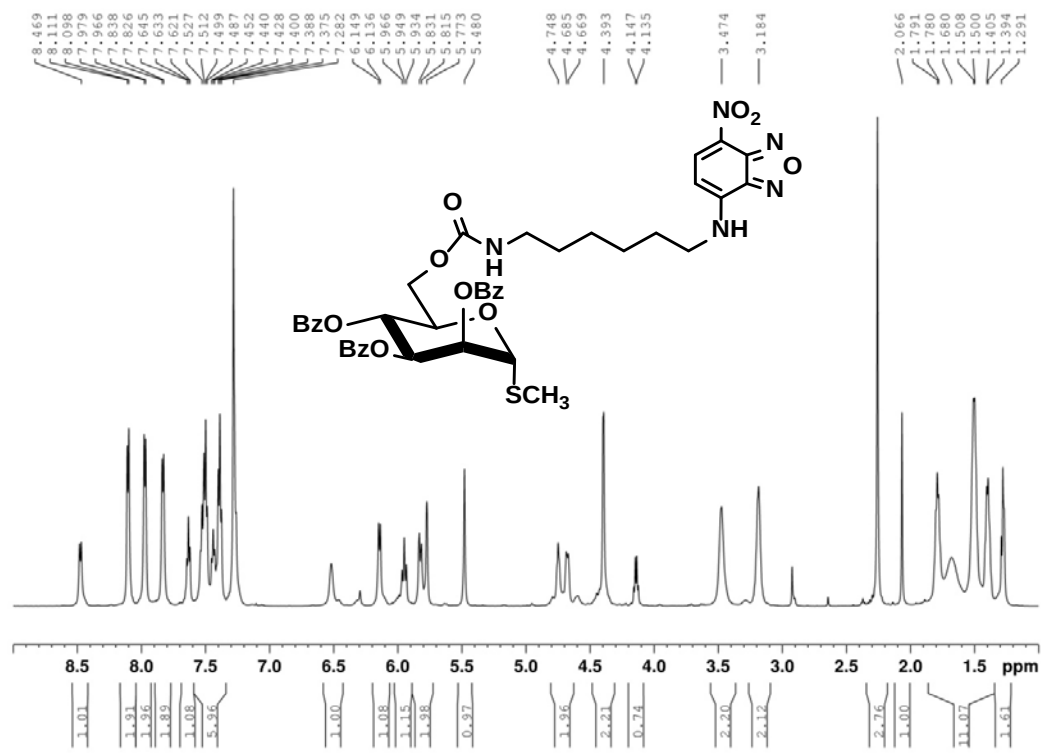
^{1}H NMR and ^{13}C NMR spectra of 1 and 2

Compound 1





Compound 2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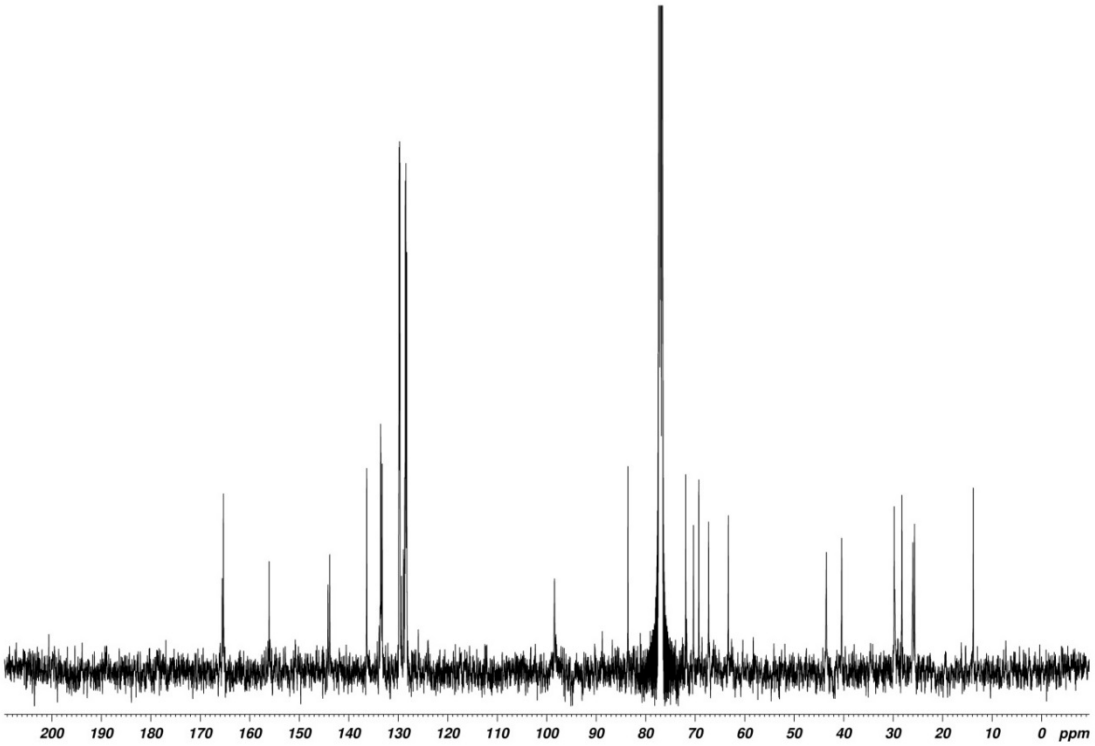
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MALDI–TOF mass spectrum of 2

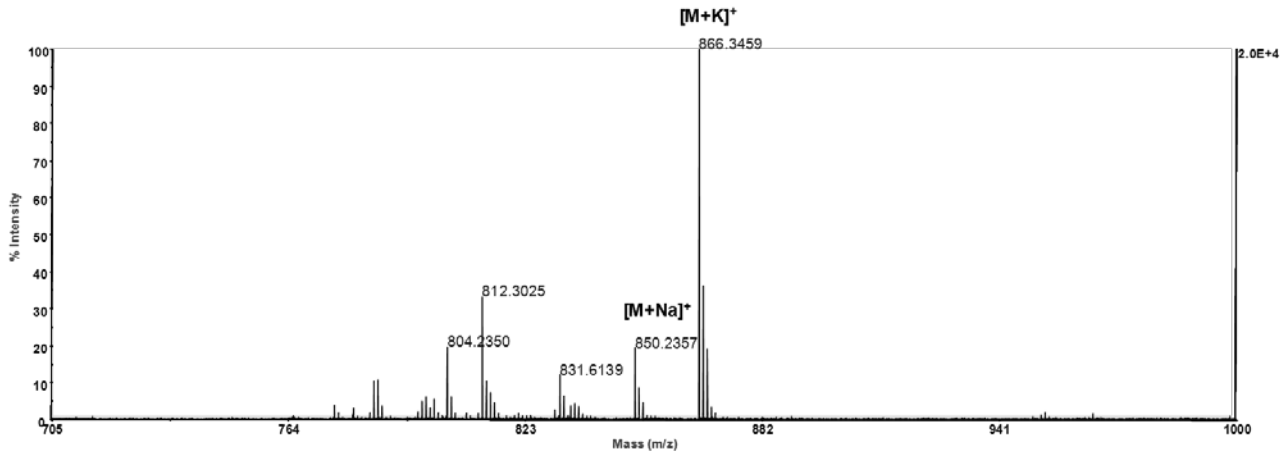

Figure S1. MALDI-TOF mass spectrum (positive ion mode) of **2**.

Absorbance and emission spectra of 2

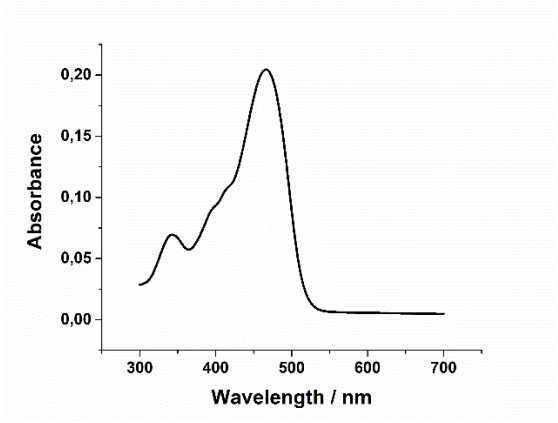

Figure S2. UV/vis spectrum of **2** (10 µM) in DMF

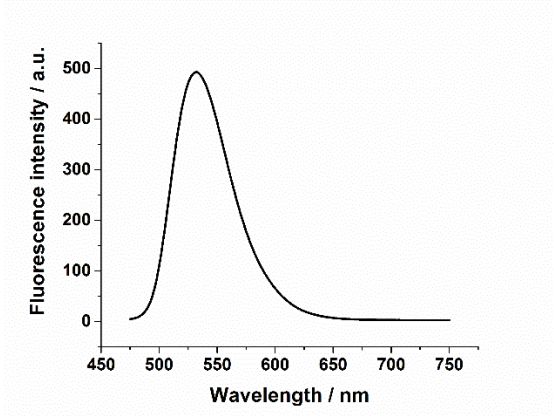

Figure S3. Fluorescence emission spectrum of **2** (10 µM) in DMF