Supplementary Information

Photogenerated Lectin Sensors Produced by Thiol-Ene/Yne Photo-Click Chemistry in Aqueous Solution

Oscar Norberg[‡, #], *Irene H. Lee* [†], *Teodor Aastrup*[#], *Mingdi Yan*[†,]*, and *Olof Ramström*[‡,]*

[‡]KTH Royal Institute of Technology, Department of Chemistry, Teknikringen 30, S-10044, Stockholm, Sweden. [#]Attana AB, Björnnäsvägen 21, S-11347, Stockholm, Sweden. [†]Department of Chemistry, University of Massachusetts, Lowell, 1 University Avenue, Lowell, MA 01854, USA. Contact: ramstrom@kth.se, Fax: +46 8 7912333; mingdi_yan@uml.edu, Fax: +1 978-934-3013

1-(2-Bromoethyl)-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (2)

1,2,3,4,6-Penta-*O*-acetyl-D-mannopyranose (**1**) was synthesized using acetic anhydride starting from D-mannose.(Norberg et al. 2011) A solution of 2-bromoethanol (247 µL, 3.50 mmol) in DCM (40 mL) was subsequently added to compound **1** (1.14 g, 2.92 mmol). The mixture was stirred at 0 °C and $BF_3·Et_2O$ (2.20 mL, 17.5 mmol) was added slowly. After 1 h, the mixture was allowed to reach rt, and after 23 h added to ice water (20 mL). The resulting mixture was extracted with DCM (2 × 30 mL), and the combined organic phase was washed with ice water, saturated $NaHCO_3$ (aq), and ice water. The organic phase was dried over $MgSO_4$ and the solvent evaporated under reduced pressure yielding product **2** with more than 95% purity according to ^1H-NMR (1.25 g, 94%). Compound **2** was used in the next step without further purification. ^1H NMR (500 MHz, $CDCl_3$): δ 5.35 (dd, 1 H, *J* = 3.3 and 10.0 Hz, H-3), 5.31-5.27 (m, 2 H, H-2, H-

4), 4.88 (d, 1 H, *J* = 1.5 Hz, H-1), 4.27 (dd, 1 H, *J* = 5.9 and 12.7 Hz, H-6), 4.15-4.12 (m, 2 H, H-5, H-6’), 3.98 (dt, 1 H, *J* = 6.4, and 11.1 Hz, CH_2CH_2Br), 3.89 (dt, 1 H, *J* = 5.7 and 11.4 Hz, CH_2CH_2Br), 3.52 (t, 1 H, *J* = 6.0 Hz, CH_2Br), 2.16 (s, 3 H, Ac), 2.11 (s, 3 H, Ac), 2.05 (s, 3 H, Ac), 2.00 (s, 3 H, Ac). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.8, 170.2, 170.0, 169.9, 97.9, 69.5, 69.0, 69.0, 67.2, 66.2, 62.6, 50.5, 21.0, 20.9, 20.9, 20.8. The spectroscopic data are in agreement with the literature.(Davis et al. 2000)

1-(2-Acetylthioethyl)-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (3)

1-(2-Bromoethyl)-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**2**, 1.23 g, 2.70 mmol) was dissolved in DMF (40 mL). Potassium thioacetate (617 mg, 5.41 mmol) was added and the mixture was stirred at 45 °C for 17 h until TLC indicated full conversion. The solvent was then evaporated under reduced pressure and the residue was dissolved in diethyl ether and water. The organic phase was washed with saturated $NaHCO_3$ (aq), and ice water. The organic phase was dried over $MgSO_4$ and the solvent evaporated under reduced pressure yielding 1.11 g of crude product. The crude product was separated by flash column chromatography with solvent system hexanes/ethyl acetate (Hex/EtOAc) 1:1 giving pure compound **3** (909 mg, 72% over two steps). ^1H NMR (500 MHz, $CDCl_3$): δ 5.33 (dd, 1 H, *J* = 3.5 and 10.0 Hz, H-3), 5.29-5.23 (m, 2 H, H-2, H-4), 4.83 (d, 1 H, *J* = 1.3 Hz, H-1), 4.27 (dd, 1 H, *J* = 5.7 and 12.4 Hz, H-6), 4.11 (dd, 1 H, *J* = 2.4 and 12.3 Hz, H-6’), 4.03 (ddd, 1 H, *J* = 2.2, 5.6 and 9.7 Hz, H-5), 3.77 (dt, 1 H, *J* = 6.5 and 10.1 Hz, CH_2CH_2SAc), 3.63 (dt, 1 H, *J* = 6.4 and 10.1 Hz, CH_2CH_2SAc), 3.12 (t, 1 H, *J* = 6.4 Hz, CH_2SAc), 2.36 (s, 3 H, SAc), 2.16 (s, 3 H, Ac), 2.10 (s, 3 H, Ac), 2.05 (s, 3 H, Ac), 2.00 (s, 3 H, Ac). ^{13}C NMR (125 MHz, $CDCl_3$): δ 195.2, 170.8, 170.2, 170.0, 169.9, 97.7, 77.4, 77.2, 76.9, 69.6, 69.1, 68.9, 67.1, 66.3, 62.6, 30.7, 28.7, 21.0, 20.9, 20.8. The spectroscopic data are in agreement with the literature.(Revell et al. 1998)

1-(2-mercaptoethyl)-α-D-mannopyranoside (4)

1-(2-Acetylthioethyl)-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**3**, 1.45 g, 3.30 mmol) was dissolved in methanol (35 mL). Sodium methoxide (196 mg, 3.63 mmol) was added and the mixture was stirred at rt for 3 h until TLC indicated full conversion. The solvent was then evaporated under reduced pressure giving the sodium salt of compound **4** (851 mg, quant.) with less than 5% disulfide product. ^1H NMR (500 MHz, D_2O): δ 4.90 (d, 1 H, *J* = 1.5 Hz, H-1), 3.95 (dd, 1 H, *J* = 1.7 and 3.4 Hz, H-2), 3.89 (dd, 1 H, *J* = 2.0 and 12.0 Hz, H-6), 3.81 (dd, 1 H, *J* = 3.5

and 9.5 Hz, H-3), 3.77-3.68 (m, 3 H, H-5, H-6’, C*H*$_2$CH$_2$SH), 3.66-3.57 (m, 2 H, H-4, C*H*$_2$CH$_2$SH), 2.69 (at, 2 H, *J* = 7.5 Hz, C*H*$_2$SH). ^{13}C NMR (125 MHz, D$_2$O): δ 99.6, 72.7, 71.0, 70.5, 70.1, 66.8, 61.0 23.2. HRMS (ESI) m/z calcd. for $C_8H_{16}O_6S$ [M+Na]+ 263,05655, found 263.05634. The spectroscopic data are in agreement with the literature.(Revell et al. 1998)

1-(2-Bromoethyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (6)

A solution of 2-bromoethanol (361 µL, 5.11 mmol) in DCM (50 mL) was subsequently added to 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose (**5**, 1.66 g, 4.26 mmol). The mixture was stirred at -40 °C (acetonitrile/carbon dioxide bath) and BF_3•Et_2O (3.21 mL, 25.6 mmol) was added slowly. After 1 h, the mixture was allowed to slowly reach rt, and after 22 h added to ice water (30 mL). The resulting mixture was extracted with DCM (2 × 30 mL), and the combined organic phase was washed with ice water, saturated $NaHCO_3$ (aq), and ice water. The organic phase was dried over $MgSO_4$ and the solvent evaporated under reduced pressure yielding product 1.90 g of crude product. The crude product was separated by flash column chromatography with solvent system hexanes/ethyl acetate (Hex/EtOAc) 2:1 giving pure compound **6** (1.29 g, 67%).^{1}H NMR (500 MHz, CDCl$_3$): δ 5.40 (bd, 1 H, *J* = 3.2 Hz, H-4), 5.24 (dd, 1 H, *J* = 7.9 and 10.4 Hz, H-2), 5.03 (dd, 1 H, *J* = 3.4 and 10.5 Hz, H-3), 4.54 (d, 1 H, *J* = 8.0 Hz, H-1), 4.20-4.16 (m, 3 H, H-6, H-6’, C*H*$_2$CH$_2$Br), 3.92 (at, 1 H, *J* = 6.70 Hz, H-5), 3.85-3.80 (m, 1 H, C*H*$_2$CH$_2$Br), 3.49-3.46 (m, 2 H, C*H*$_2$Br), 2.16 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.06 (s, 3 H, Ac), 2.00 (s, 3 H, Ac). ^{13}C NMR (125 MHz, CDCl$_3$): δ 170.6, 170.4, 170.2, 169.7, 101.6, 71.0, 70.8, 69.8, 68.8, 68.0, 67.1, 66.9, 61.4, 30.5, 21.1, 20.8, 20.7, 20.6. The spectroscopic data are in agreement with the literature.(Davis et al. 2000)

1-(2-Acetylthioethyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (7)

1-(2-Bromoethyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**6**, 1.26 g, 2.78 mmol) was dissolved in DMF (50 mL). Potassium thioacetate (634 mg, 5.55 mmol) was added and the mixture was stirred at 45 °C for 7 h until TLC indicated full conversion (very similar R_f-values between starting material and product). The solvent was then evaporated under reduced pressure and the residue was dissolved in diethyl ether and water. The organic phase was washed with saturated $NaHCO_3$ (aq), and ice water. The organic phase was dried over $MgSO_4$ and the solvent evaporated under reduced pressure yielding 1.14 g of crude product. The crude product was separated by flash column chromatography with solvent system hexanes/ethyl acetate

(Hex/EtOAc) 3:1 giving pure compound **6** (680 mg, 56%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.39 (bd, 1 H, *J* = 3.5 Hz, H-4), 5.21 (dd, 1 H, *J* = 7.9 and 10.5 Hz, H-2), 5.02 (dd, 1 H, *J* = 3.4 and 10.5 Hz, H-3), 4.49 (d, 1 H, *J* = 8.0 Hz, H-1), 4.17 (dd, 1 H, *J* = 6.7 and 11.3 Hz, H-6), 4.13 (dd, 1 H, *J* = 6.8 and 11.3 Hz, H-6'), 4.00 (ddd, 1 H, *J* = 1.2, 5.5 and 10.4 Hz, CH_2CH_2Br), 3.91 (dt, 1 H, *J* = 1.1 and 6.8 Hz, H-5), 3.61 (ddd, 1 H, *J* = 1.1, 6.5 and 10.4 Hz, CH_2CH_2Br), 3.14 (dt, 1 H, *J* = 5.8 and 13.9 Hz, CH_2Br), 3.05 (dt, 1 H, *J* = 7.0 and 13.9 Hz, CH_2Br), 2.34 (s, 3 H, SAc), 2.16 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.05 (s, 3 H, Ac), 1.99 (s, 3 H, Ac). ^{13}C NMR (125 MHz, DMSO): δ 194.9, 169.9, 169.9, 169.5, 169.1, 99.9, 70.1, 69.9, 68.5, 67.7, 67.3, 61.2, 30.5, 28.4, 20.5, 20.5, 20.4, 20.3. The spectroscopic data are in agreement with the literature.(Schofield et al. 2008)

1-(2-Mercaptoethyl)-β-D-galactopyranoside (8)

1-(2-Acetylthioethyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**7**, 420 mg, 0.959 mmol) was dissolved in of methanol (40 mL). Sodium methoxide (57 mg, 1.05 mmol) was added and the mixture was stirred at rt for 4 h until TLC indicated full conversion. The solvent was then evaporated under reduced pressure giving 268 mg of crude product. The crude product was separated by flash column chromatography with solvent system DCM/methanol 6:1 giving pure compound **7** (154 mg, 70%). ^{1}H NMR (500 MHz, D_2O): δ 4.43 (d, 1 H, *J* = 7.9 Hz, H-1), 4.01 (dt, 1 H, *J* = 6.3 and 10.7 Hz, CH_2CH_2SH), 3.91 (bd, 1 H, *J* = 3.4 Hz, H-4), 3.84-3.72 (m, 3 H, H-6, H-6', CH_2CH_2SH), 3.68 (ddd, 1 H, *J* = 1.0, 4.3 and 7.8 Hz, H-5), 3.64 (dd, 1 H, *J* = 3.4 and 10.0 Hz, H-3), 3.52 (dd, 1 H, *J* = 7.9 and 10.0 Hz, H-2), 2.76 (t, 2 H, *J* = 6.5 Hz, CH_2SH). ^{13}C NMR (125 MHz, D_2O): δ 102.0, 74.4, 71.9, 70.8, 69.9, 67.8, 60.1, 22.6. HRMS (ESI) m/z calcd. for $C_8H_{16}O_6S$ [M+Na]+ 263,05655, found 263.05623. The spectroscopic data are in agreement with the literature.(Schofield et al. 2008)

2-(2-(2-(Allyloxy)ethoxy)ethoxy)ethanol (11)

2-(2-(2-Chloroethoxy)ethoxy)ethanol (**9**, 1.87 g, 11.1 mmol) and allyl alcohol (**10**, 3.0 mL, 44.4 mmol) were dissolved in DMF (40 mL). The mixture was stirred at 45 °C and ground sodium hydroxide (1.33 g, 33.3 mmol) was added. The mixture was then stirred for 2 h until TLC indicated full conversion. The mixture was diluted with diethyl ether (70 mL) and washed with 1 M HCl and water. The collected aq. phase was then extracted with DCM (3 × 50 mL), and the combined organic phases were dried over $MgSO_4$ and the solvent evaporated under reduced pressure yielding product 1.04 g of crude product. The crude product was separated by flash column chromatography with ethyl acetate as solvent giving pure compound **11** (633 mg, 30%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.92 (ddt, 1 H, *J* = 5.7, 10.4 and 17.2 Hz, $OCH_2C\mathit{H}CH_2$), 5.28 (adq, 1 H, *J* = 1.6 and 17.2 Hz, $OCH_2CHC\mathit{H_2}$), 5.19 (adq, 1 H, *J* = 1.6 and 10.4 Hz, $OCH_2CHC\mathit{H_2}$), 4.03 (bd, 2 H, *J* = 5.7 Hz, $OC\mathit{H_2}CHCH_2$), 3.74 (bd, 2 H, *J* = 4.5 Hz, $OC\mathit{H_2}$), 3.71-3.66 (m, 6 H, $OC\mathit{H_2}$), 3.65-3.61 (m, 4 H, $OC\mathit{H_2}$), ^{13}C NMR (125 MHz, $CDCl_3$): δ 134.8, 117.4, 72.6, 72.5, 70.8, 70.8, 70.5, 69.5, 61.9. The spectroscopic data are in agreement with the literature.(Lim et al. 2007)

2-(2-(2-(Allyloxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (12)

2-(2-(2-(Allyloxy)ethoxy)ethoxy)ethanol (**11**, 366 mg, 1.92 mmol) was dissolved in DCM (40 mL). The mixture was stirred at 0 °C and *p*-toluenesulfonyl chloride (402 mg, 2.12 mmol) and ground potassium hydroxide (432 mg, 7.70 mmol) were added slowly. After 1 h, the mixture was allowed to slowly reach rt, and after 4 h TLC indicated full conversion. The mixture was poured on ice water and extracted with DCM (3 × 50 mL). The combined organic phases were dried over $MgSO_4$ and the solvent evaporated under reduced pressure giving pure compound **12** (663 mg, quant.). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.80 (bd, 2 H, *J* = 8.2 Hz, Ar-H), 7.34 (bd, 2 H, *J* = 8.3 Hz, Ar-H), 5.91 (ddt, 1 H, *J* = 5.7, 10.4 and 17.2 Hz, $OCH_2C\mathit{H}CH_2$), 5.27 (adq, 1 H, *J* = 1.6 and 17.2 Hz, $OCH_2CHC\mathit{H_2}$), 5.18 (adq, 1 H, *J* = 1.6 and 10.4 Hz, $OCH_2CHC\mathit{H_2}$), 4.16 (at, 2 H, *J* = 4.9 Hz, $C\mathit{H_2}OTs$), 4.01 (dt, 2 H, *J* = 1.4 and 5.6 Hz, $OC\mathit{H_2}CHCH_2$), 3.69 (at, 2 H, *J* = 4.9 Hz, $OC\mathit{H_2}$), 3.64-3.57 (m, 8 H, $OC\mathit{H_2}$), 2.45 (s, 3 H, $ArC\mathit{H_3}$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 144.9, 134.2, 133.2, 130.0, 128.2, 116.7, 70.9, 70.7, 70.6, 69.4, 69.2, 68.9, 58.6, 21.8. The spectroscopic data are in agreement with the literature.(Lim et al. 2007)

3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)prop-1-ene (13)

2-(2-(2-(Allyloxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**12**, 663 mg, 1.92 mmol), sodium azide (138 mg, 2.12 mmol) and tetrabutylammonium iodide (71 mg, 0.192 mmol) were dissolved in DMF (12 mL). The mixture was stirred at 45 °C for 22 h until TLC indicated full conversion. The mixture was diluted with diethyl ether (30 mL) and washed twice with saturated $NaHCO_3$ (aq), and ice water. The organic phase was then dried over $MgSO_4$ and the solvent evaporated under reduced pressure giving pure compound **13** (316 mg, 77%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.92 (ddt, 1 H, *J* = 5.6, 10.4 and 17.3 Hz, $OCH_2C\mathit{H}CH_2$), 5.28 (adq, 1 H, *J* = 1.6 and 17.3 Hz, $OCH_2CHC\mathit{H_2}$), 5.18 (adq, 1 H, *J* = 1.5 and 10.4 Hz, $OCH_2CHC\mathit{H_2}$), 4.03 (bd, 2 H, *J*

= 5.7 Hz, OC*H*$_2$CHCH$_2$), 3.69-3.67 (m, 8 H, OC*H*$_2$), 3.62-3.60 (m, 2 H, OC*H*$_2$), 3.39 (t, 2 H, *J* = 5.1 Hz, C*H*$_2$N$_3$). ^{13}C NMR (125 MHz, CDCl$_3$): δ 134.2, 116.7, 70.9, 70.7, 70.6, 69.5, 69.2, 58.4, 50.7. The spectroscopic data are in agreement with the literature.(Lim et al. 2007)

2-(2-(2-(Allyloxy)ethoxy)ethoxy)ethanamine (14)

3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)prop-1-ene (**13**, 316 mg, 1.47 mmol) was dissolved in dry THF (35 mL) and stirred at 0 °C. Triphenyl phosphine (1.39 g, 5.29 mmol) was added in aliquots. After 23 h, TLC indicated full conversion. Ten equivalents of water were added and the mixture was stirred at rt for 1 h. The solvent was then evaporated under reduced pressure giving 1.92 g of crude product. The crude product was separated by flash column chromatography with solvent system ethanol/ammonia solution (30%) (EtOH/NH$_3$) 9:1 giving pure compound **14** (90 mg, 32%).^{1}H NMR (500 MHz, CDCl$_3$): δ 5.92 (ddt, 1 H, *J* = 5.7, 10.3 and 17.2 Hz, OCH$_2$C*H*CH$_2$), 5.28 (adq, 1 H, *J* = 1.6 and 17.3 Hz, OCH$_2$CHC*H*$_2$), 5.18 (adq, 1 H, *J* = 1.5 and 10.4 Hz, OCH$_2$CHC*H*$_2$), 4.03 (dt, 2 H, *J* = 1.3 and 5.7 Hz, OC*H*$_2$CHCH$_2$), 3.68-3.60 (m, 8 H, OC*H*$_2$), 3.51 (t, 2 H, *J* = 5.2 Hz, C*H*$_2$NH$_2$). ^{13}C NMR (125 MHz, CDCl$_3$): δ 134.9, 117.3, 73.7, 72.4, 70.8, 70.8, 70.5, 69.6, 42.0. HRMS (ESI) m/z calcd. for $C_9H_{19}NO_3$ [M+H]+ 190.14440, found 190.14354. The spectroscopic data are in agreement with the literature.(Lim et al. 2007)

References

Davis, B.G., Maughan, M.A.T., Green, M.P., Ullman, A., Jones, J.B., 2000. Glycomethanethiosulfonates: powerful reagents for protein glycosylation. Tetrahedron: Asymmetry 11(1), 245-262.

Lim, Y.-b., Park, S., Lee, E., Jeong, H., Ryu, J.-H., Lee, M.S., Lee, M., 2007. Glycoconjugate Nanoribbons from the Self-Assembly of Carbohydrate-Peptide Block Molecules for Controllable Bacterial Cell Cluster Formation. Biomacromolecules 8(5), 1404-1408.

Norberg, O., Deng, L., Aastrup, T., Yan, M., Ramström, O., 2011. Photo-Click Immobilization on Quartz Crystal Microbalance Sensors for Selective Carbohydrate-Protein Interaction Analyses. Anal. Chem. 83(3), 1000-1007.

Revell, D.J., Knight, J.R., Blyth, D.J., Haines, A.H., Russell, D.A., 1998. Self-Assembled Carbohydrate Monolayers: Formation and Surface Selective Molecular Recognition. Langmuir 14(16), 4517-4524.

Schofield, C.L., Mukhopadhyay, B., Hardy, S.M., McDonnell, M.B., Field, R.A., Russell, D.A., 2008. Colorimetric detection of Ricinus communis Agglutinin 120 using optimally presented carbohydrate-stabilised gold nanoparticles. Analyst 133(5), 626-634.