Electronic Supplementary Information

Dynamic glycosylation of liposomes by thioester exchange

Johanna Moratz, Florian Klepel and Bart Jan Ravoo*

Organic Chemistry Institute, Westfälische Wilhelms-Universität Münster, Corrensstrasse 40,

48149 Münster, Germany, Email: b.j.ravoo@uni-muenster.de

Table of contents

1. Additional measurements

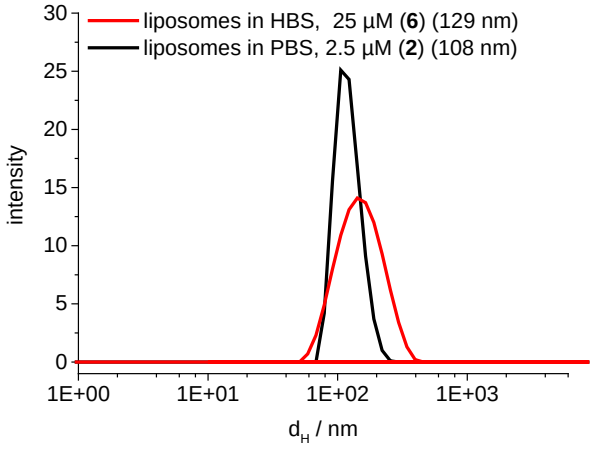


Fig. S1: Average hydrodynamic diameter of liposomes determined by dynamic light scattering in different buffer solutions. Conditions: 100 µM lecithin; 25 µM (**6**) or 2.5 µM (**2**); 20 mM HBS (pH 8.0, 150 mM NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$) or 20 mM phosphate buffer (pH 8.0); 20 °C.

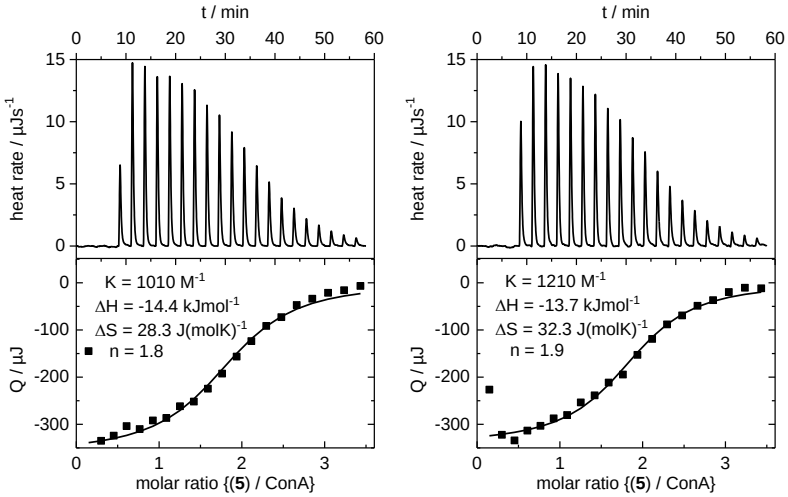


Fig. S2: Thermodynamic parameters for binding of ConA to thiol (**5**) determined by isothermal titration calorimetry. The fact that a 2:1 interaction instead of 4:1 is observed can be attributed to partial oxidation of the thiol or pH-dependent dissociation of ConA into dimers. Conditions: 10 mM (**5**); 1 mM ConA; HBS (pH 8.0, 150 mM NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$); 25 °C.

2. Experimental methods

Materials and methods

Chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany), Acros Organics (Nidderau, Germany), Alfa Aesar (Karlsruhe, Germany), Iris Biotech GmbH (Marktredwitz, Germany) and were used without further purification. Thin layer chromatography was performed on Merck (Darmstadt, Germany) analytical TCL plates (60 F254 silica gel plates). Compound Visualization was realized either by UV light irradiation at 254 nm or by dipping in basic permanganate solution. Silica gel with a grain size of 40 – 65 µm (Merck, Darmstadt, Germany) was used for preparative silica gel chromatography. For measurements in aqueous solutions dd H_2O was prepared with a PureLab UHQ purification unit (ELGA, High Wycombe, UK) with a resistance greater than 18 MΩ was used. All pH values were adjusted with a freshly calibrated S220 SevenCompactTM pH/ion meter (Mettler Toledo GmbH, Gießen, Germany). Untrasonication was performed with a Sonorex RK 510 Transistor (BANDELIN electronic GmbH & Co. KG, Berlin, Germany) with a working frequency of 35 kHz at 25 °C. Photometry was performed in PMMA cuvettes (Brand GmbH & Co. KG, Wertheim, Germany) with a V-650 spectrophotometer from JASCO (Gross-Umstadt, Germany) equipped with a temperature controlled PAC-743 automatic 6-position cell changer from JASCO. Fluorescence anisotropy measurements were carried out in semi-micro quartz-glass cuvettes (Hellma Analytics, Müllheim, Germany) with a FP-6500 spectrofluorimeter (JASCO, Gross-Umstadt, Germany) and manual FDP-223 polarization equipment from JASCO. DLS measurements were performed with a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). All data was plotted and analyzed using Origin Pro (version 8.5.0G, OriginLab Corporation, Northampton, USA). Mass spectrometry measurements were recorded with a LTQ Orbitrap XL (Thermo-Fisher Scientific, Bremen, Germany) or a MicroTof (Bruker Daltronics, Bremen, Germany) system. NMR spectroscopic measurements were obtained using a Bruker AV 300 or AV400 (Bruker Analytische Messtechnik, Karlsruhe, Germany). Chemical shifts were referenced to the residual solvent peak and the data was analyzed using MestReNova (Mestrelab Research S.L., Santiago de Compostela, Spain). IR Spectra were recorded using a Fourier transformation IR spectrometer from Varian (Type 310). The data was analyzed using the Resolution Pro software and all spectral data was corrected by subtracting the background signal. The signals were described according to their intensity (w = weak, m = medium, s = strong, br = broad).

Preparation of buffer solutions

Phosphate buffered saline (PBS) was obtained by mixing 100 mM stock solutions of $NaH_2PO4{\cdot}2H_2O$ and $Na_2HPO_4{\cdot}12H_2O$ in dd H_2O until the desired pH of 8.00 was reached (approx. 1:19, v:v). The buffers was then degassed by sonication for 30 min and purged with argon gas. HBS (20 mM, 150 mM NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$) was prepared by dissolving hydroxyethylpiperazine-1-ethanesulfonic acid (477 mg) in dd H2O (100 mL). The pH was adjusted with NaOH (2 mM in dd H_2O) to 8.00. Subsequently, NaCl (877 mg) and a stock solution of $MnCl_2$ und $CaCl_2$ (100 µL, 1 M in dd H_2O) were added.

Liposome preparation

To limit weighing errors soy bean lecithin was used as 1 mM stock solution in chloroform. In a small round bottom glass flask the appropriate amount of stock solution was dried under a gentle stream of argon while rotating. The resulting thin film of amphiphiles was desorbed from the glass flask by sonicating in buffer (35 kHz, 30 min), giving a slightly turbid suspension with a concentration of 100 µM amphiphile. Unilamellar vesicles of defined size were obtained by pressing the solution back and forth between two 2.5 ml gastight Hamilton® syringes (Hamilton Messtechnik GmbH, Höchst, Germany) on opposing sites of a metal casing enclosing a polycarbonate membrane of 100 nm pore size. Extrusion was performed nine times and vesicle size distribution was monitored by DLS.

Optical density experiments

Optical density measurements (OD600) were performed on a V-650 double-beam spectrophotometer (JASCO Germany GmbH, Gross-Umstadt, Germany) with a PAC-743 automatic 6-position peltier cell changer (JASCO Germany GmbH, Gross-Umstadt, Germany). Samples were handled in semi-micro quartz-glass or disposable PMMA cuvettes (BRAND GmbH & Co. KG, Wertheim, Germany) with a path length of 1 cm and a sample volume of 1 mL, equipped fight tightly fitting caps. To avoid lectin denaturation, measurements were carried out in samples equilibrated to 25 °C for 10 min. Approximately 4 min after starting the measurement, stock solutions of thioesters (**1**) or (**6**) and/or thiomannose (**5**) respectively were added with Eppendorf® pipettes, agitating and mixing the solution thoroughly in the process and capping the cuvettes afterwards. ConA was added from stock solution in the same manner at the stated time points.

Fluorescence polarization measurements

To a liposome suspension (100 µM lecithin) a stock solution of thioester (**2**) in methanol was added resulting in a concentration of 2.5 µM and fluorescence polarization was measured immediately. Subsequently stock solutions of thiomannose (**5**) in PBS were added to final concentrations from 0.2 mM to 20 mM and the anisotropy was determined again. The samples were then stirred in capped

cuvettes at 200 rpm at 25 °C or 45 °C respectively. Before measuring the anisotropy the samples were equilibrated to ambient temperature for 5 min. All measurements were performed five times, neglecting the highest and lowest values obtained and determining the statistic average from the three remaining results.

Extraction and monitoring of thioester exchange by mass spectrometry

To determine the conversion and yield of dynamic thiol-thioester exchange, liposome suspensions (100 µM lecithin) were prepared in HBS with different compositions (see table S1). Thioester was performed at pH 8 and and aliquots (500 µL) were taken at different time points and acidified with HCl (2 M, 10 µL) to pH 2 prior to analysis. The solvent was removed immediately on a rotary evaporator, dioxane (500 µL) was added and the samples were sonicated for 10 min to extract the relevant species. The solute was immediately dried under vacuum and analyzed by ESI mass spectrometry.

For interpretation the relative peak intensities of the ion peaks of the educt (I_E), product (I_P) und hydrolysis product (I_H) were evaluated graphically. These relative intensities were used to calculate the molar ratios of the components (X_E, X_P und X_H) in the reaction mixture:

$$X_E = \frac{1}{I_{tot}} \cdot \frac{I_E}{I_P} \cdot K_{EP} \qquad X_P = \frac{1}{I_{tot}} \cdot \frac{I_P}{I_E \cdot K_{EP}} \qquad X_H = \frac{1}{I_{tot}} \cdot \frac{I_H}{I_E \cdot K_{EP}}$$

$$I_{tot} = \frac{I_E}{I_P} \cdot K_{EP} + \frac{I_P}{I_E \cdot K_{EP}} + \frac{I_H}{I_E \cdot K_{EP}}$$

The correcting parameters K_{EP} and K_{EH} were determined by preparation of a concentration standard with equimolar concentrations of the three components and analogous extraction, analysis and evaluation. From the relative peak intensities of this standard (i_E, i_P and i_H) the correcting parameters were calculated as following:

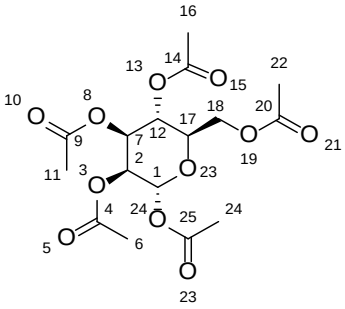
$$K_{EP} = \frac{i_E}{i_P} \qquad K_{EH} = \frac{i_E}{i_H}$$

Table S1 Concentrations and signals for monitoring thioester exchange via mass spectrometry.

	Concentration	**Exchange**	**Detected species**	***m/z***
Educt (6)	5 µM	15 µM	$(M+Na)^+$	849.45
Product (1)	5 µM	-	$(M+Na)^+$	673.39
Hydrolysis (3)	5 µM	-	$(M+Na)^+$	583.37
Thioglycerol	-	100 µM		
Lecithin	100 µM	100 µM		

3. Synthesis of thiol-tagged mannose (5)

Mannose pentaacetate (5-1)[1]



D-Mannose (10.1 g, 56 mmol) was slowly added to a solution of iodine (142 mg, 0.56 mmol, 0.01 eq) in acetic anhydride (50 mL) at 18 °C, then refluxed at 145 °C for 90 minutes. The mixture was diluted with DCM (100 mL) and washed with $Na_2S_2O_3$ aq. (150 mL), neutralized with $NaHCO_3$ aq. and washed with $Na_2S_2O_3$ aq. (100 mL), $NaHCO_3$ (2·100 mL), H_2O (2·100 mL) and brine (100 mL). The organic phase was dried over Na_2SO_4, concentrated and purified by column chromatography (EtOAc:cyclohexane, 2:1)

Molecular Formula: $C_{16}H_{22}O_{11}$.

Yield: 14.8 g (37.9 mmol, 68%).

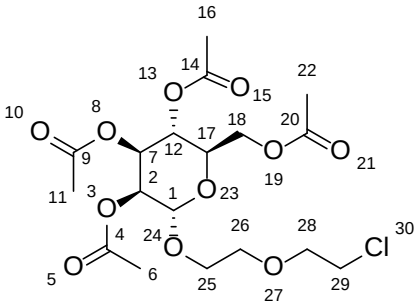
Rf (EtOAc:cyclohexane, 2:1): 0.55

^{1}H-NMR (300 MHz, $CDCl_3$): δ/ppm = 6.02 (d, *J* = 1.9 Hz, 1H, H-1), 5.32 – 5.25 (m, 2H, H-2, H-7), 5.20 (t, *J* = 2.2 Hz, 1H, H-12), 4.27 – 4.17 (m, 1H, H-18), 4.03 (dd, *J* = 12.4, 2.4 Hz, 1H, H-17), 3.98 (m, 1H, H-18'), 2.11 – 1.94 (5·s, 15H, H-6, H-11, H-16, H-22, H-24).

^{13}C-NMR (101 MHz, $CDCl_3$): δ/ppm = 170.61-168.04 (C-4, C-9, C-14, C-20, C-25), 90.56 (C-1), 70.57 (C-17), 68.71 (C-2), 68.30 (C-7), 65.48 (C-12), 62.06 (C-18), 20.84-20.62 (C-6, C-11, C-16, C-22, C-24).

ESI-MS$^+$ (MeOH): $[M+Na]^+$ calc.: *m/z* = 413.1054; exp.: 413.1038.

1-((2-Chloroethoxy)ethoxy)mannose tetraacetate (5-2)[2]



A solution of **5-1** (1.91 g, 5.12 mmol) and chloro(ethoxy)ethanol (810 µL, 7.68 mmol, 1.5 eq) in DCM (25 mL) was cooled to 0 °C. After addition of $BF_3{\cdot}Et_2O$ (3.2 mL, 25.6 mmol, 5 eq) the solution was stirred at r.t. for 150 h. The mixture was washed with $NaHCO_3$ aq. (50 mL) and the aqueous phase was extracted with DCM (2·30 mL). The combined organic phases were dried over $MgSO_4$, concentrated and purified by column chromatography (EtOAc:pentane, 1:2→1:1).

Molecular Formula: $C_{18}H_{27}ClO_{11}$.

Yield: 1.17 g (2.58 mmol, 53%).

Rf (EtOAc:cyclohexane, 1:1): 0.33.

---

[1] K.P. Ravindranathan Kartha, R. A. Field, *Tetrahedron* **1997**, *53*, 11753-11766.

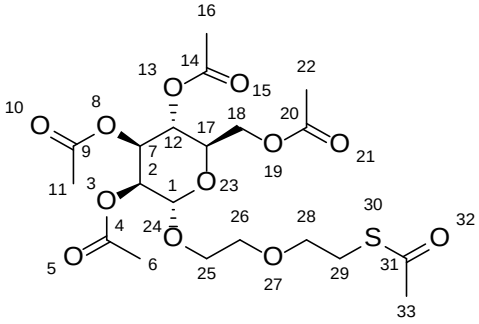
[2] Adapted sequence: E. Mahon, T. Aastrup, M. Barboiu *Chem. Commun.* **2010**, 46, 5491-5493.

^{1}H-NMR (300 MHz, $CDCl_3$): δ/ppm = 5.36 – 5.19 (m, 3H, H-2, H-7, H-12), 4.86 (d, *J* = 1.3 Hz, 1H, H-1), 4.25 (dd, *J* = 12.8, 5.5 Hz, 1H, H-18), 4.12 – 4.02 (m, 2H, H-17, H-18'), 3.76 – 3.55 (m, 8H, H-25, H-26, H-28, H-29), 2.12 – 1.95 (4·s, 12H, H-6, H-11, H-16, H-22).

^{13}C-NMR (75 MHz, $CDCl_3$): δ/ppm = 170.64 – 169.72 (C-4, C-9, C-14, C-20), 97.56 (C-1), 71.33 – 69.47 (C-25, C-26, C-28), 69.04 – 66.06 (C-2, C-7, C-12, C-17), 62.39 (C-18), 42.84 (C-29), 20.86 – 20.67 (C-6, C-11, C-16, C-22).

ESI-MS$^+$ (MeOH): $[M+Na]^+$ calc.: *m/z* = 477.1140; exp.: 477.1134.

1-((2-Acetylthioethoxy)ethoxy)mannose tetraacetate (5-3)[2]



A solution of **5-2** (1.17 g, 2.58 mmol) and potassium thioacetate (589 mg, 5.16 mmol, 2 eq) in DMF (40 mL) was stirred at r.t. for 5 days. The solution was diluted with EtOAc (100 mL), washed with H_2O (2·70 mL), $HaHCO_3$ aq. (70 mL) and brine (70 mL). The organic phase was concentrated and dried over $MgSO_4$.

Molecular Formula: $C_{20}H_{30}O_{12}S$.

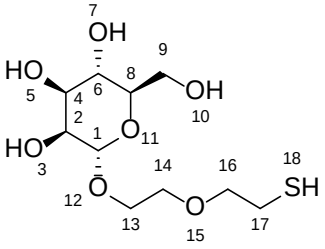
Yield: 1.28 g (2.58 mmol, quant).

^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm = 5.34 – 5.27 (m, 1H, H-2), 5.25 – 5.18 (m, 2H, H-7, H-12), 4.83 (dd, *J* = 4.6, 1.8 Hz, 1H, H-18), 4.28 – 4.20 (m, 1H), 4.07 – 4.02 (m, 2H, H-17, H-18'), 3.68 – 3.48 (m, 6H, H-25, H-26, H-28), 3.06 – 2.99 (m, 2H, H-29), 2.29 - 1.93 (5·s, 12H, H-6, H-11, H-16, H-22, H-33)

^{13}C-NMR (101 MHz, $CDCl_3$): δ/ppm = 194.41 (C-31), 170.57 – 169.66 (C-4, C-9, C-14, C-20), 97.55 (C-1), 69.66 – 69.44 (C-25, C-26, C-28), 69.01 – 66.06 (C-2, C-7, C-12, C-17), 62.36 (C-18), 30.18 (C-33), 28.82 (C-29), 20.83 – 20.62 (C-6, C-11, C-16, C-22).

ESI-MS$^+$ (MeOH): $[M+Na]^+$ calc.: *m/z* = 517.1356; exp.: 517.1327.

1-((2-Mercaptoethoxy)ethoxy)mannose (5)[2]



Sodium methoxide (203 mg, 3.75 mmol, 1.5 eq) was added under argon atmosphere to a solution of **5-3** (1.24 g, 2.5 mmol) in degassed dry methanol (30 mL). The solution was stirred at r.t. for 6 h and neutralized with Amberlite. After filtration the solvent was removed under reduced pressure.

Molecular Formula: $C_{10}H_{20}O_7S$.

Yield: 900 mg (3.17 mmol, quant.).

^{1}H-NMR (300 MHz, MeOH-d4): δ/ppm = 4.79 (d, *J* = 1.7 Hz, 2H, H-1), 3.92 – 3.47 (m, 16H, H-2 – H-17), 2.62 (t, *J* = 6.7 Hz, 2H, H-17).

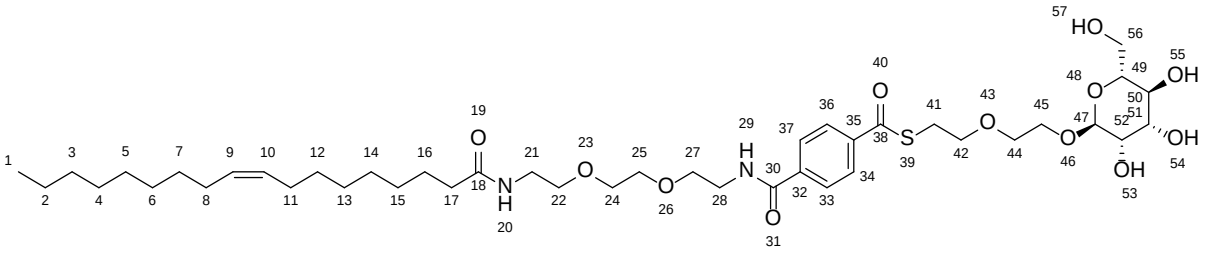
^{13}C-NMR (75 MHz, MeOH-d4): δ/ppm = 101.64 (C-1), 74.64 – 74.40 (C-13, C-14), 72.30 (C-16), 71.94 – 70.87 (C-2, C-4, C-6, C-8), 68.24 – 67.85 (C-8, C-9), 62.26 (C-17).

IR (neat): ν/cm^{-1} = 664 (m), 764 (m), 810 (w), 880 (w), 972 (m), 1026 (s), 1095 (s), 1250 (w), 1350 (m), 1605 (m), 1651 (m), 1736 (w), 2778 (w), 2878 (w), 2924 (m), 3271 (br).

ESI-MS^{+} (MeOH): [M+Na]$^{+}$ calc.: *m/z* = 307.0822; exp.: 307.0838.

4. Synthesis of thioester (6)

(Z)-S-(((2-Mannosyl)ethoxy)ethyl) 4-((2-(2-(2-oleamidoethoxy)ethoxy)ethyl)-amino)benzothioate



(Z)-Perfluorphenyl 4-((2-(2-(2-oleamidoethoxy)ethoxy)ethyl)amino)benzoate[3] (47 mg, 65 µmol) and thiomannose (**5)** (57 mg, 201 µmol, 3.1 eq) were dissolved in DMF. After addition of DIPEA (66.3 µL, 390 µmol, 6.0 eq) the solution was stirred at r.t. over night. The solvent was removed under reduced pressure and the product was purified by column chromatography (EtOAC:CH_2Cl_2:MeOH, 5:5:1.5 → 1:1:1).

Molecular Formula: $C_{42}H_{70}N_2O_{12}S$.

Yield: 12 mg (14.5 µmol, 22%).

Rf (EtOAC:CH_2Cl_2:MeOH, 5:5:1.5): 0.06.

^{1}H-NMR (300 MHz, MeOH-d4): δ/ppm = 8.18 – 7.71 (m, 4H, H-33, H-34, H-36, H-37), 5.41 – 5.27 (m, 2H, H-9, H-10), 4.84 – 4.74 (m, 1H, H-47), 3.82 (dt, *J* = 8.2, 3.6 Hz, 3H, H-49 – H-51), 3.76 – 3.55 (m, 20H, 18H, H-21, H-22, H-24, H-25, H-27, H-41, H-42, H-44, H-45, H-56), 3.52 (t, *J* = 5.5 Hz, 3H, H-28, H-52), 2.22 – 2.10 (m, 2H, H-17), 2.07 – 1.89 (m, 2H, H-11), 1.56 (t, *J* = 13.2 Hz, 2H, H-8), 1.27 (bs, 22H, H-2 – H-7, H-12 – H-16), 0.88 (t, *J* = 6.7 Hz, 3H, H-1).

^{13}C-NMR (151 MHz, MeOH-d4): δ/ppm = 192.34 (C-38), 176.42 (C-18), 169.10 (C-30), 140.45 (C-32), 140.17 (C-15) 128.87 (C-36, C-34), 128.29 (C-34, C-37), 101.79 (C-47), 72.14 and 62.89 (C-49, C-51), 70.64 (C-21), 74.60 – 67.80 and 29.83 (C-22, C-24, C-25, C-27, C-4, C-44, C-52, C-58, C-60), 41.04 (C-28), 40.26 (C-41), 38.43 (C-8), 37.07 (C-17), 33.61 – 30.27 and 23.73 (C-2 – C-7, C12 – C16), 27.01 (C-11), 26.81 (C-8), 14.45 (C-1).

IR (neat): ν/cm^{-1} = 571 (w), 679 (w), 772 (w), 810 (w), 864 (w), 918 (m), 972 (m), 1034 (s), 1096 (s), 1211 (w), 1242 (w), 1296 (w), 1358 (m), 1458 (w), 1543 (m), 1636 (s), 2855 (m), 2924 (m), 3295 (br).

ESI-MS$^+$ (MeOH): $[M+Na]^+$ calc.: *m/z* = 849.4542; exp.: 849.4515.

[3] S.A. Berg, B.J. Ravoo, *Soft Matter* **2014**, *10*, 69-74.

5. NMR spectra

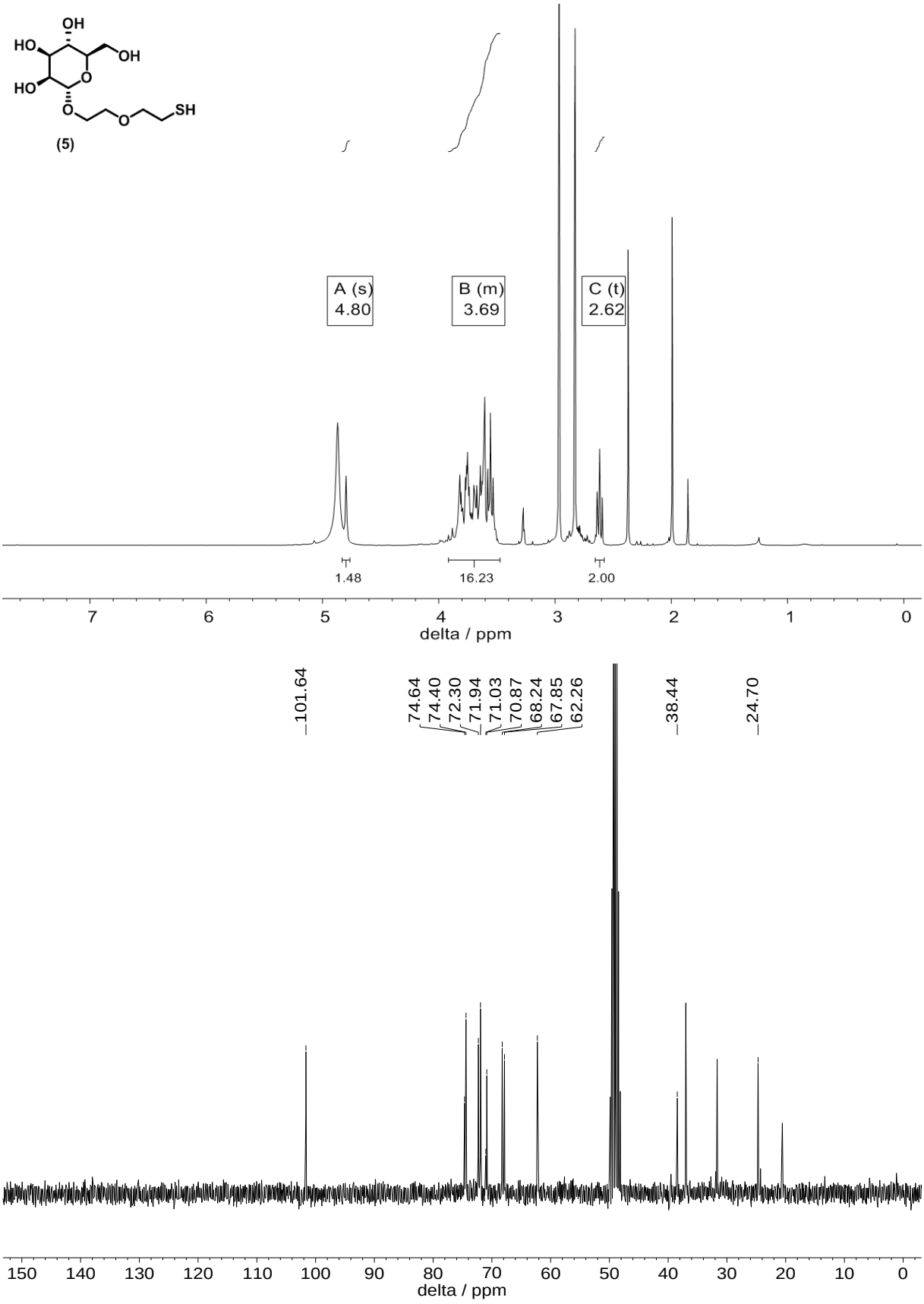


Fig. S3 ^{1}H-NMR and ^{13}C-NMR of thiol-tagged mannose (**5**), 300 MHz and 101 MHz MeOH-d4.

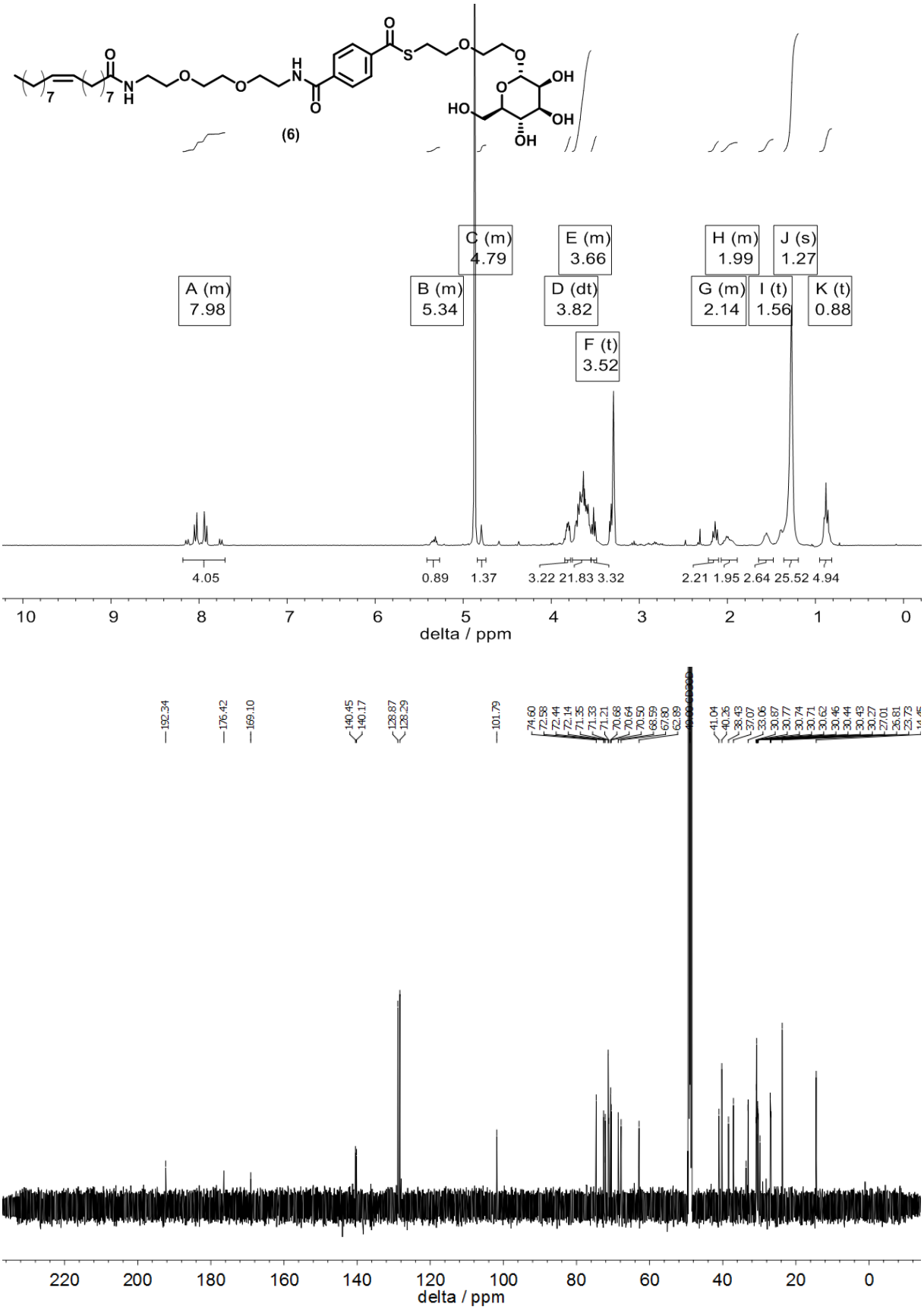


Fig. S4 ^{1}H-NMR and ^{13}C-NMR of thioester (**6**), 300 MHz and 151 MHz MeOH-d4.

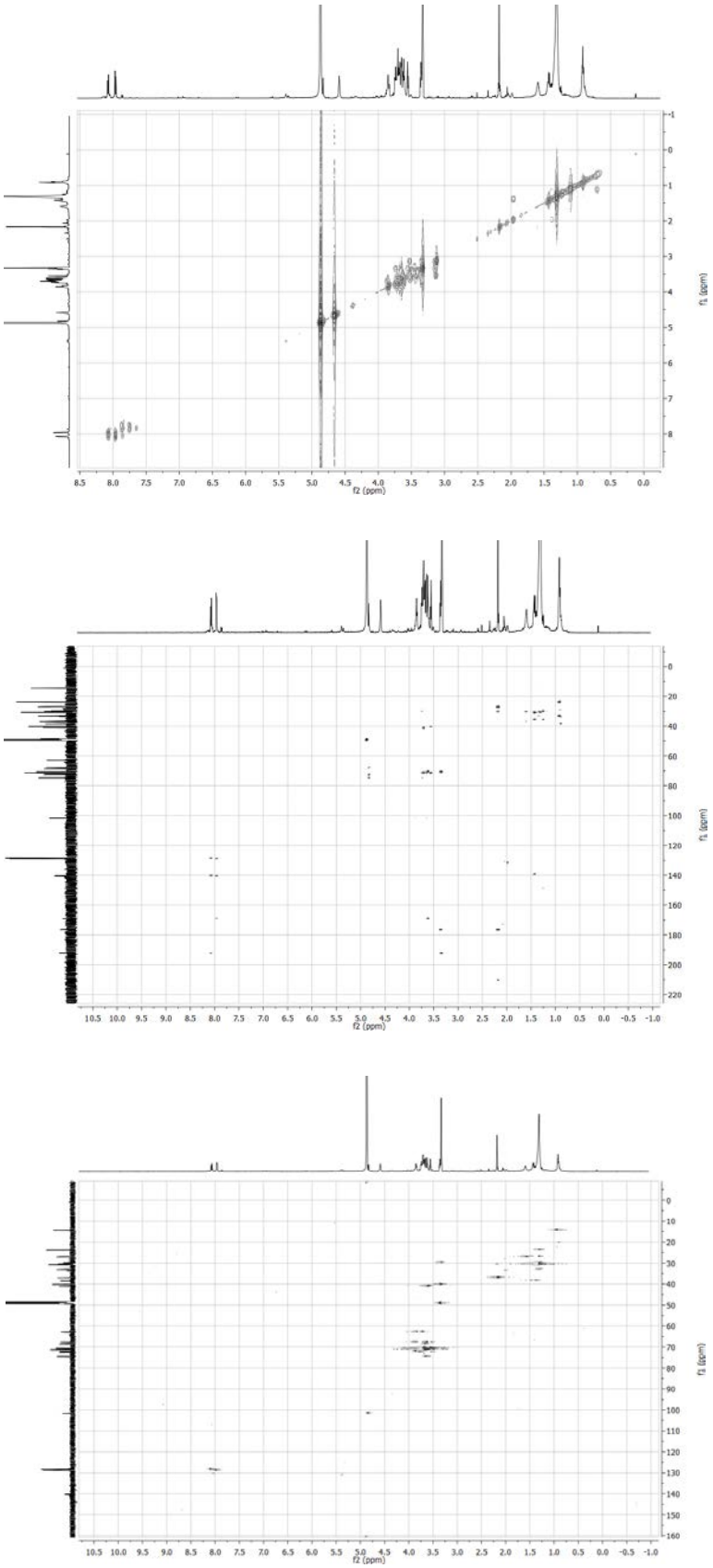


Fig. S5 COSY, HMBC and HSQC NMR of of thioester (**6**), 600 MHz and 151 MHz, MeOH-d4.

6. IR spectra

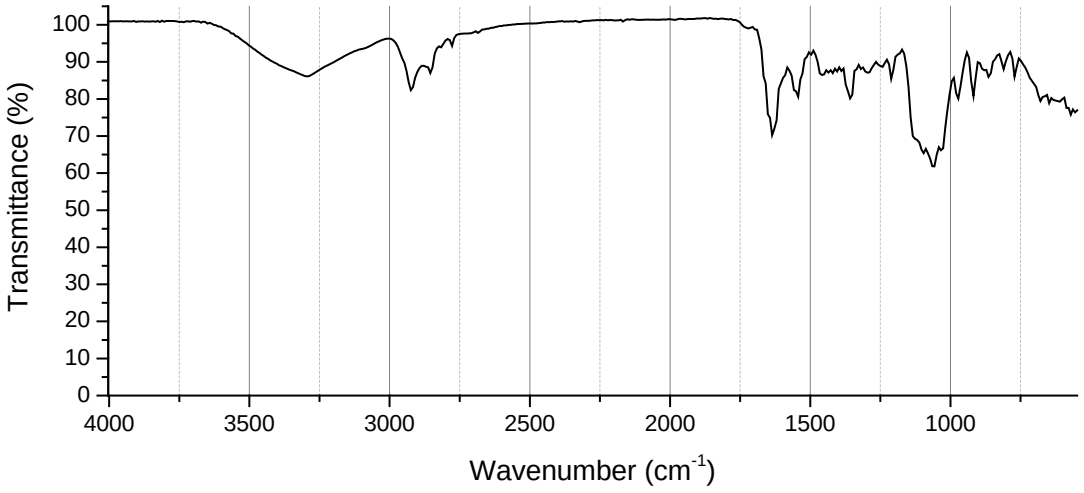


Fig. S6 IR spectrum of thiol-tagged mannose (**5**).

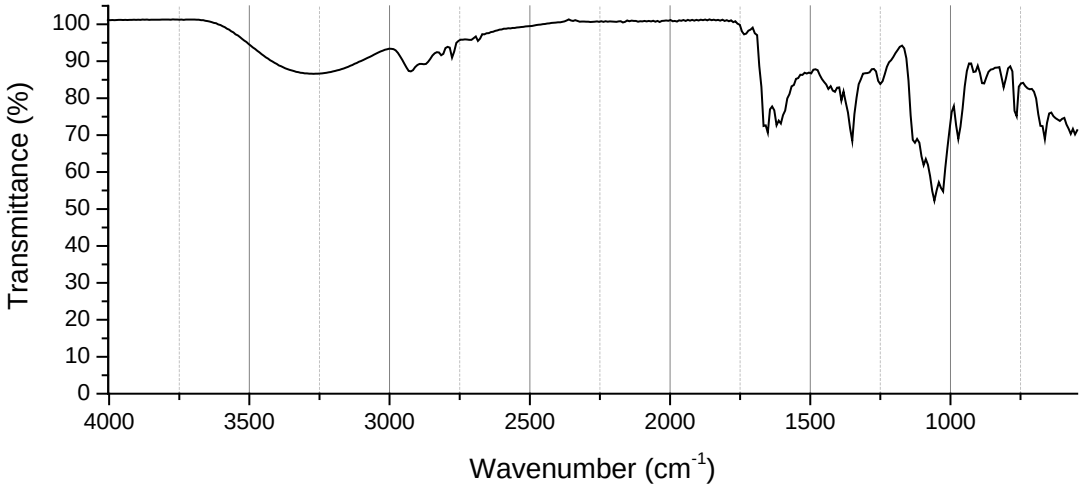


Fig. S7 IR spectrum of thiol-tagged thioester (**6**).