Supplementary Material (ESI) for Chemical Communications

SUPPORTING INFORMATION

Phosphine-Mediated Disulfide Metathesis in Aqueous Media

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General Methods

All commercial reagents and solvents were used as received. 1-thio-β-D-glucopyranose sodium salt and Concanavalin A (L7647) were from Sigma-Aldrich. 1-Thio-β-D-galactopyranose[1] and 1-thio-α-D-mannopyranose[2] and thiocholine[3] were synthesized following procedures previously described. Chemical reactions were monitored with thin-layer chromatography using precoated silica gel 60 (0.25 mm thickness) plates. Flash column chromatography was performed on silica gel 60 (0.040-0.063 mm). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400 (100) MHz and/or Bruker Avance DMX 500 at 500 (125) MHz, respectively. Buffer solutions were prepared from D_3PO_4 (85% w/w in D_2O) and NaOD (40% w/w in D_2O) using an Ecoscan pH meter equipped with a Ross Combination pH Semi Microelectrode.

References

1. Pei, Z.; Dong, H.; Caraballo, R.; Ramström, O. *Eur. J. Org. Chem.* **2007**, 4927-4934.
2. Pei, Z.; Larsson, R.; Aastrup, T.; Anderson, H.; Lehn, J.-M.; Ramström, O. *Biosens. Bioelectron.* **2006**, *22*, 42-48.
3. Larsson, R; Ramström, O. *Eur. J. Org. Chem.* **2006**, 285-291.

General Synthesis of the symmetrical disulfide derivatives

To an aqueous solution of thiol compound was added a saturated ethanolic solution of iodine until a permanent yellow color appeared. The mixture was then concentrated to yield the crude disulfide. Pure disulfide derivatives were subsequently obtained by crystallization in ethanol or by precipitation in methanol/ethyl acetate mixtures.

1,1'-Dithio-α-D-dimannopyranoside (**1-1**)

^{1}H NMR (D_2O, 500 MHz): δ (ppm) 5.35 (d, 2H, J = 0.9 Hz, H-1, H'-1), 4.18 (dd, 2H, J = 1.5, 2.9 Hz, H-2, H'-2), 3.87-3.93 (m, 4H, H-5, H'-5, H-6, H'-6), 3.78-3.83 (m, 4H, H-3, H'-3, H-6', H'-6'), 3.72 (t, 2H, J = 9.5 Hz, H-4, H'-4); ^{13}C NMR (D_2O, 125 MHz): δ (ppm) 90.1, 74.7, 70.78 (x2), 66.8, 60.7. MS (ESI) measured for $C_{12}H_{22}O_{10}S_2$ ($[M+Na]^+$), *m/z*: 413.07. Found: 413.07.

1,1'-Dithio-β-D-digalactopyranoside (**2-2**)

^{1}H NMR (D_2O, 500 MHz): δ (ppm) 4.54 (d, 2H, J = 9.7 Hz, H-1, H'-1), 3.97 (d, 2H, J = 3.26 Hz, H-4, H'-4), 3.83 (t, 2H, J = 9.7 Hz, H-2, H'-2), 3.72-3.80 (m, 4H, H-5, H'-5, H-6, H'-6), 3.72 (dd, 2H, J = 3.1, 12.3 Hz, H-6', H'-6'), 3.69 (dd, 2H, J = 3.3, 9.5 Hz, H-3, H'-3); ^{13}C NMR (D_2O, 125 MHz): δ (ppm) 89.9, 79.6, 73.9, 68.8, 68.7, 61.1. MS (ESI) measured for $C_{12}H_{22}O_{10}S_2$ ($[M+Na]^+$), *m/z*: 413.07. Found: 413.13.

1,1'-Dithio-β-D-diglucopyranoside (**3-3**)

^{1}H NMR (D_2O, 500 MHz): δ (ppm) 4.59 (d, 2H, J = 9.6 Hz, H-1, H'-1), 3.92 (d, 2H, J = 11.7 Hz, H-6, H'-6), 3.74 (dd, 2H, J = 5.6, 12.4 Hz, H-6', H'-6'), 3.60 (t, 2H, J = 9.4 Hz, H-2, H'-2), 3.53 (t, 2H, J = 9.2 Hz, H-3, H'-3), 3.49 (m, 2H, H-5, H'-5), 3.43 (t, 2H, J = 9.5 Hz, H-4, H'-4); ^{13}C NMR (D_2O, 125 MHz): δ (ppm) 89.4, 80.3, 77.0, 71.2, 69.2, 60.8. MS (ESI) measured for $C_{12}H_{22}O_{10}S_2$ ($[M+Na]^+$), *m/z*: 413.07. Found: 413.13.

Thiocholine disulfide (**4-4**)

^{1}H NMR (D_2O, 500 MHz): δ (ppm) 3.67-3.73 (m, 4H, CH_2-N), 3.14-3.20 (m, 4H, CH_2-S), 3.17 (s, 18H, N-CH_3); ^{13}C NMR (D_2O, 125 MHz): δ (ppm) 64.9, 52.9, 29.6. MS (ESI) measured for $C_{10}H_{26}N_2S_2^{2+}$ ($[M]^{2+}$), *m/z*: 119.07. Found: 120.00.

General Synthesis of the non-symmetrical disulfide derivatives (control experiments)

To an aqueous solution of thiol compounds (from 2 to 4) was added a saturated ethanolic solution of iodine until a permanent yellow color appeared. The mixture was then concentrated to yield the disulfide mixtures. Pure non-symmetrical disulfide derivatives **2-3** and **3-4** were obtained after purification by flash chromatography (ethyl acetate/methanol/water). The other non-symmetrical disulfides revealed impossible to separate.

1,1'-S,S'-β-D-galactopyranosyl-D-glucose (2-3)

^{1}H NMR (D_2O, 500 MHz): δ (ppm) 4.58 (d, 1H, J = 13.6 Hz, H'-1), 4.56 (d, 1H, J = 13.6 Hz, H-1), 3.97 (d, 1H, J = 3.3 Hz, H-4), 3.91 (dd, 1H, J = 2.0,12.5 Hz, H'-6), 3.64-3.82 (m, 7H, H-2, H'-2 H-3, H-5, H-6, H-6', H'-6'), 3.55 (t, 1H, J = 8.9 Hz, H'-3), 3.47-3.52 (m, 1H, H'-5), 3.44 (t, 1H, J = 9.3 Hz, H'-4); ^{13}C NMR (D_2O, 125 MHz): δ (ppm) 90.6, 88.7, 80.2, 79.5, 77.0, 73.8, 70.9, 69.1, 68.9, 68.8, 61.1, 60.8. MS (ESI) measured for $C_{12}H_{22}O_{10}S_2$ ($[M+Na]^+$), *m/z*: 413.07. Found: 413.13.

1-S-β-D-glucopyranosyl-thiocholine (3-4)

^{1}H NMR (D_2O, 500 MHz): δ (ppm) 4.58 (d, 1H, J = 9.5 Hz, H-1), 3.92 (dd, 1H, J = 2.1, 12.2 Hz, H-6), 3.70-3.77 (m, 3H, H-6', CH_2-N), 3.66 (t, 1H, J = 9.3 Hz, H-2), 3.58 (t, 1H, J = 9.0 Hz, H-3), 3.51-3-55 (m, 1H, H-5), 3.44 (t, 1H, J = 9.4 Hz, H-4); ^{13}C NMR (D_2O, 125 MHz): δ (ppm) 89.1, 80.3, 77.0, 70.5, 69.4, 65.2, 60.8, 53.0, 30.7. MS (ESI) measured for $C_{11}H_{24}O_5N_1S_2$ ($[M]^+$), *m/z*:314.11. Found: 314.07.

^{1}H-NMR and ^{13}C-NMR spectra of disulfide 2-3

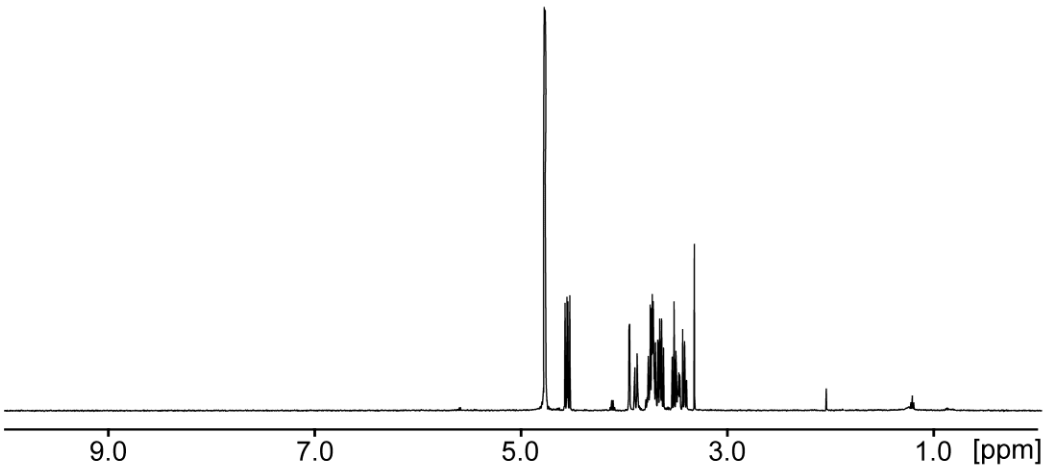

Figure S1. ^{1}H-NMR spectrum of disulfide **2-3** in D_2O.

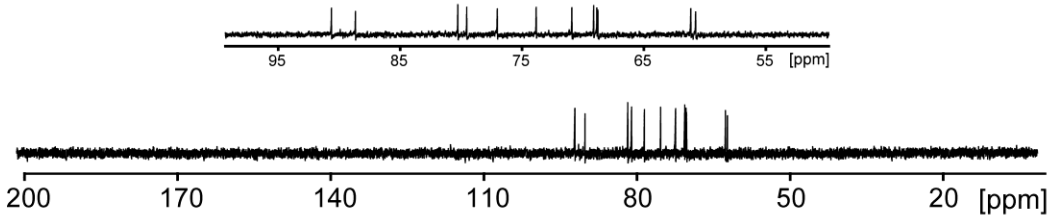

Figure S2. ^{13}C-NMR spectrum of disulfide **2-3** in D_2O.

^{1}H-NMR and ^{13}C-NMR spectra of disulfide 3-4

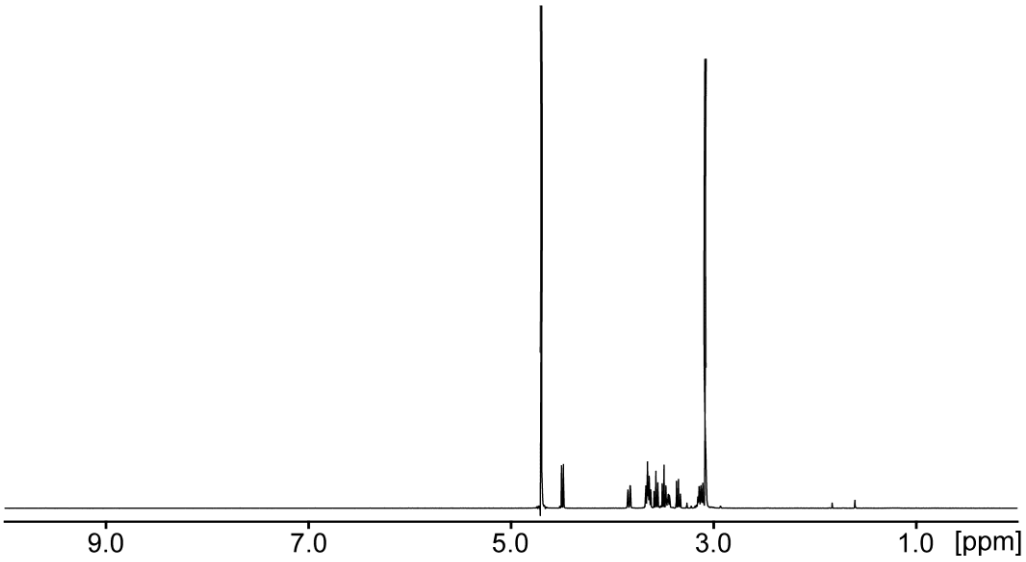

Figure S3. ^{1}H-NMR spectrum of disulfide **3-4** in D_2O.

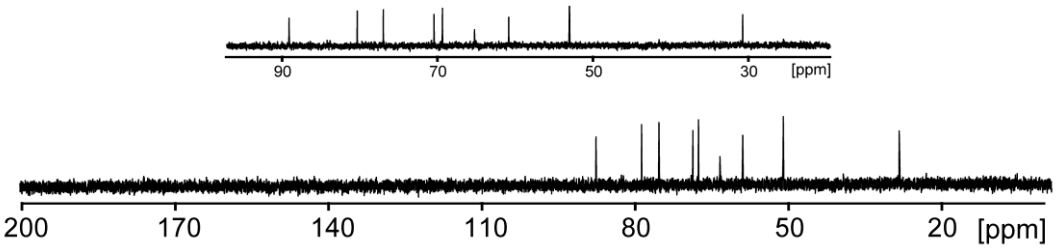

Figure S4. ^{13}C-NMR spectrum of disulfide **3-4** in D_2O.

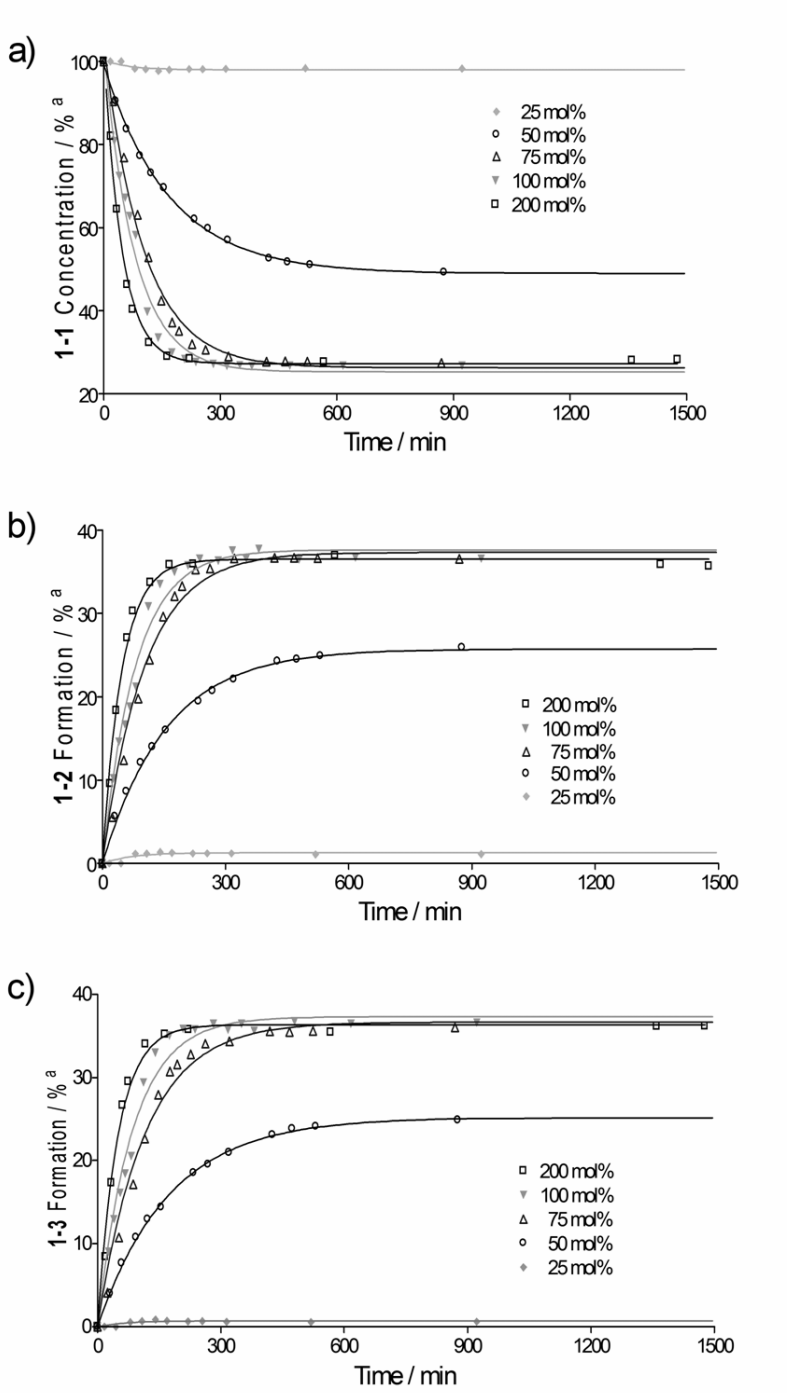

Figure S5. Concentration of **1-1** and formation of **1-2** and **1-3** at different phosphine concentrations. Experimental conditions: **1-1**, **2-2** and **3-3** (15 mM each), r.t., *d*-buffer/ CD_3CN (90:10), pD 8. [a]Yield calculated relatively to the mannose species in solutions.

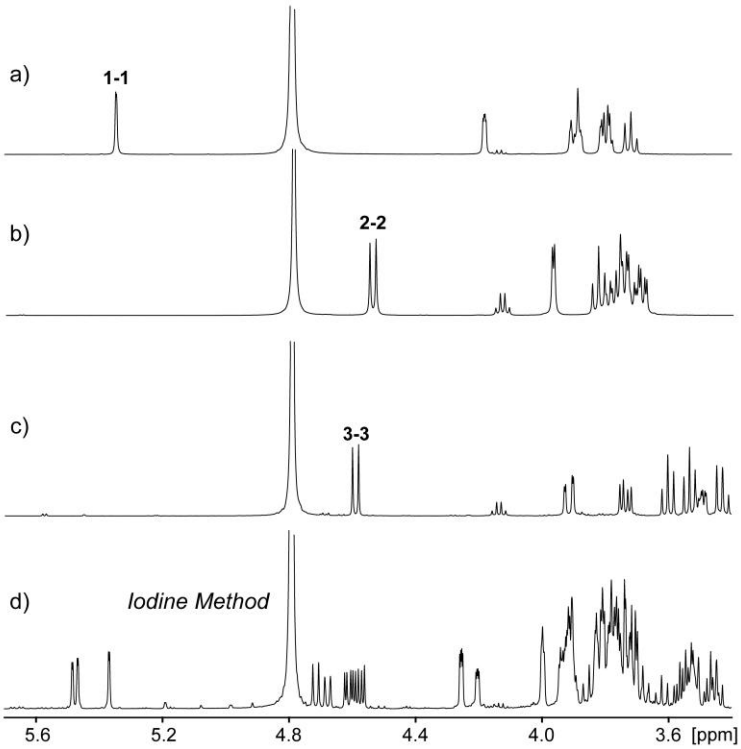

Figure S6. Control experiment for system **1-1**, **2-2** and **3-3**: a) ^{1}H NMR reference spectra of disulfide **1-1**, b) ^{1}H NMR reference spectra of disulfide **2-2** c) ^{1}H NMR reference spectra of disulfide **3-3** and d) disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **1**, **2** and **3**).

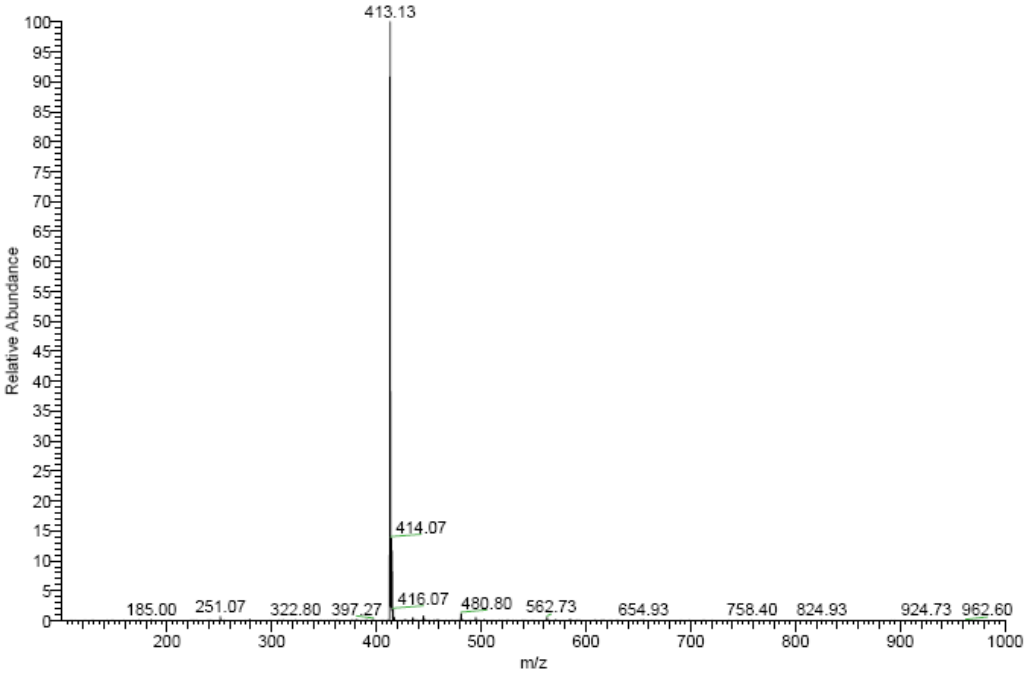

Figure S7. Mass spectrum (ESI) of the disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **1**, **2** and **3**).

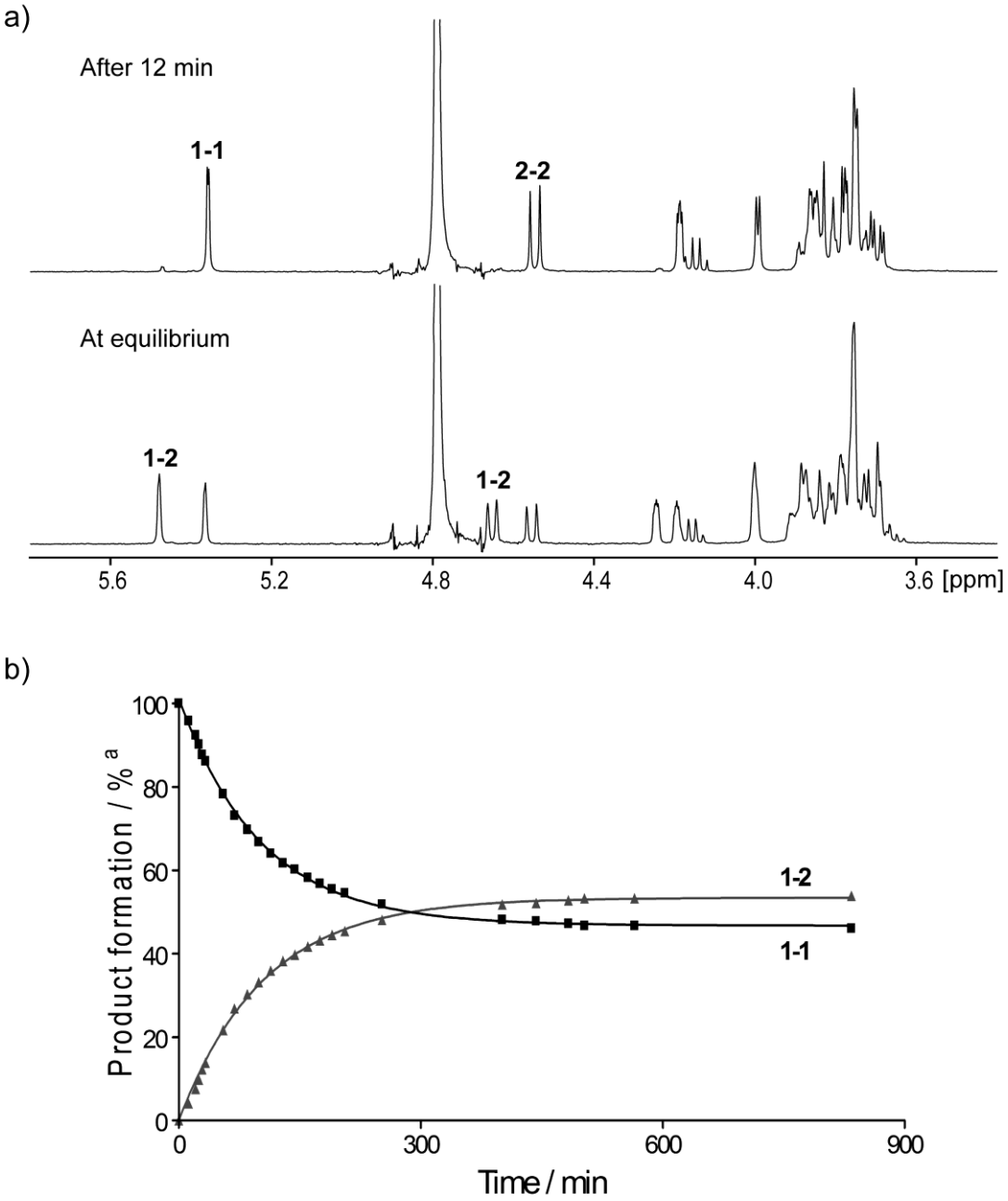

Figure S8. Disulfide metathesis in dynamic system (**1-1**, **2-2**) generation: a) ^{1}H NMR spectra of the reaction mixture and b) kinetic profile of the disulfide metathesis. Experimental conditions: **1-1** and **2-2** (15 mM each), HEPA (100 mol%), r.t., *d*-buffer/ CD_3CN (90:10), pD 8. [a]Yield calculated relatively to the mannose species in solutions.

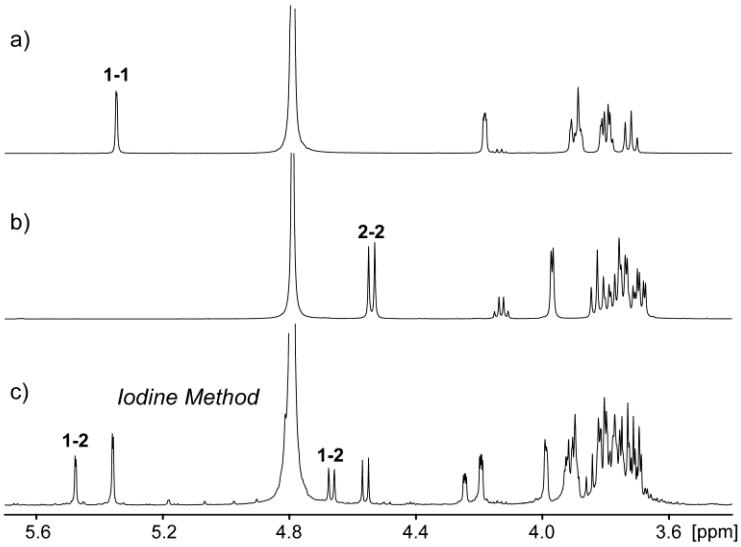

Figure S9. Control experiment for system **1-1** and **2-2**: a) ^{1}H NMR reference spectra of disulfide **1-1**, b) ^{1}H NMR reference spectra of disulfide **2-2** and c) disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **1** and **2**).

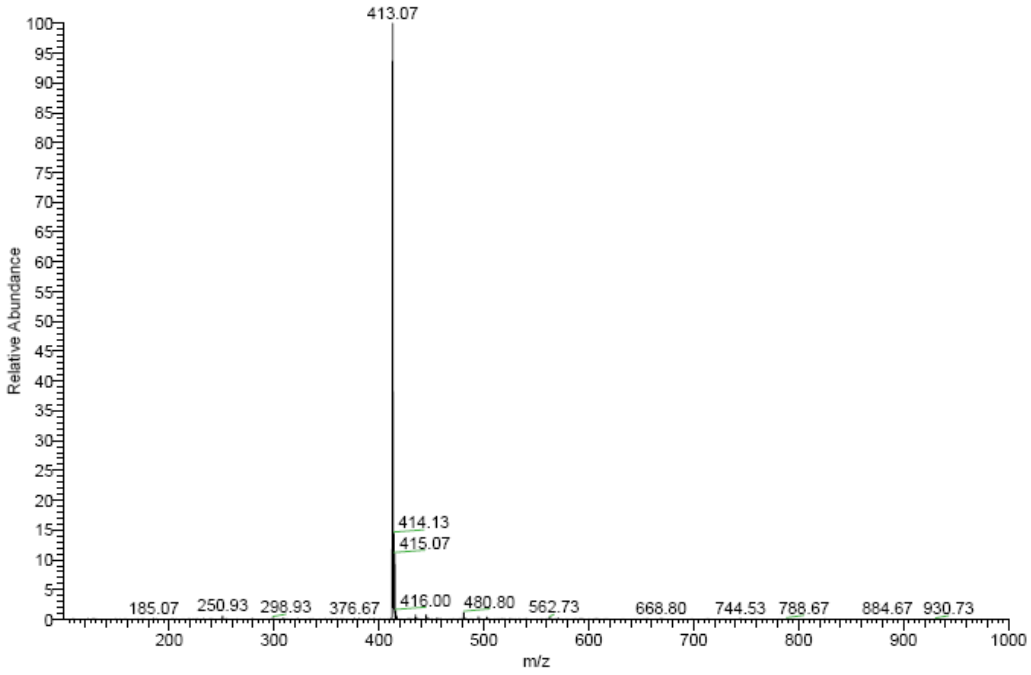

Figure S10. Mass spectrum (ESI) of the disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **1** and **2**).

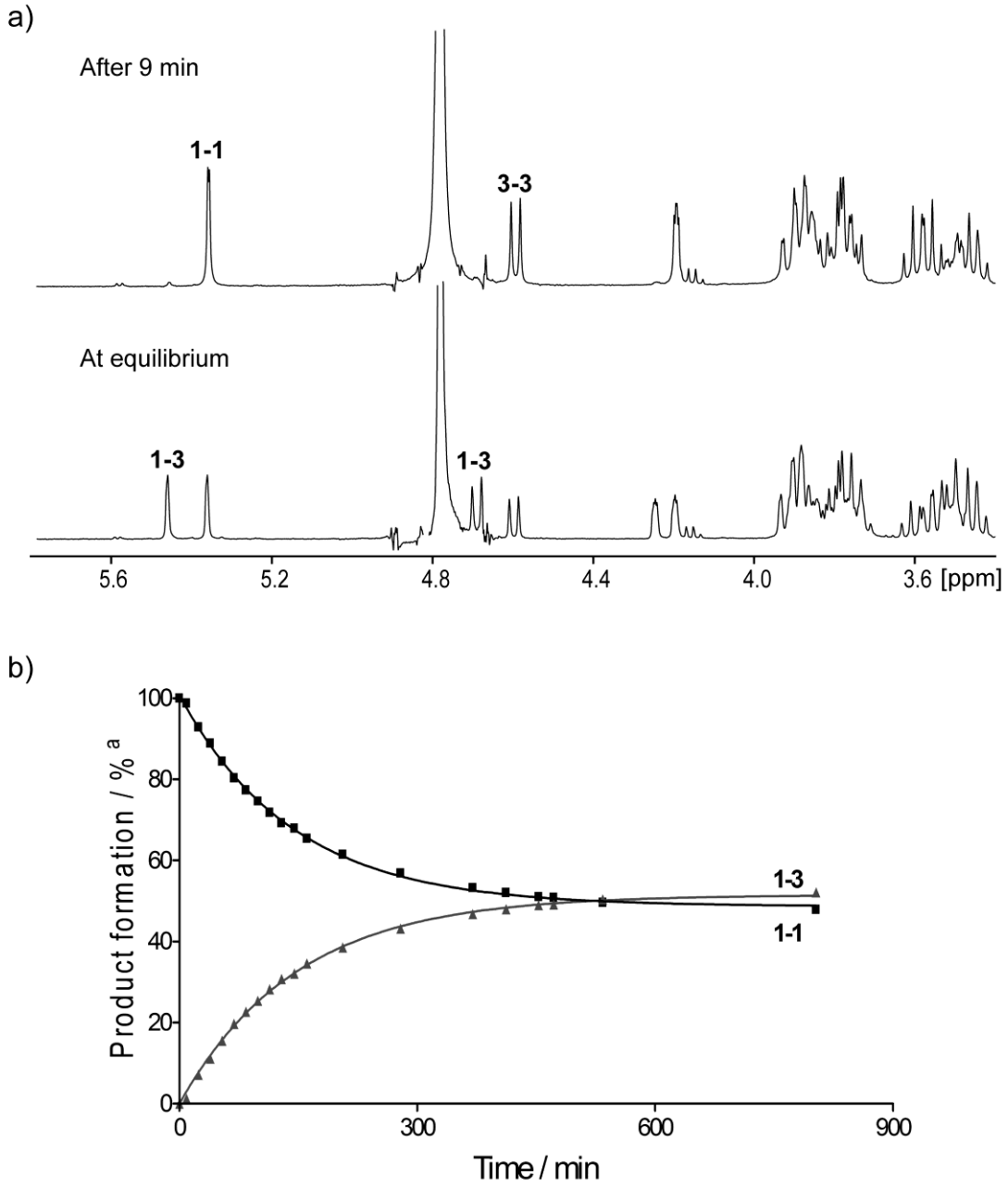

Figure S11. Disulfide metathesis in dynamic system (**1-1**, **3-3**) generation: a) ^{1}H NMR spectra of the reaction mixture and b) kinetic profile of the disulfide metathesis. Experimental conditions: **1-1** and **3-3** (15 mM each), HEPA (100 mol%), r.t., *d*-buffer/ CD_3CN (90:10), pD 8. [a]Yield calculated relatively to the mannose species in solutions.

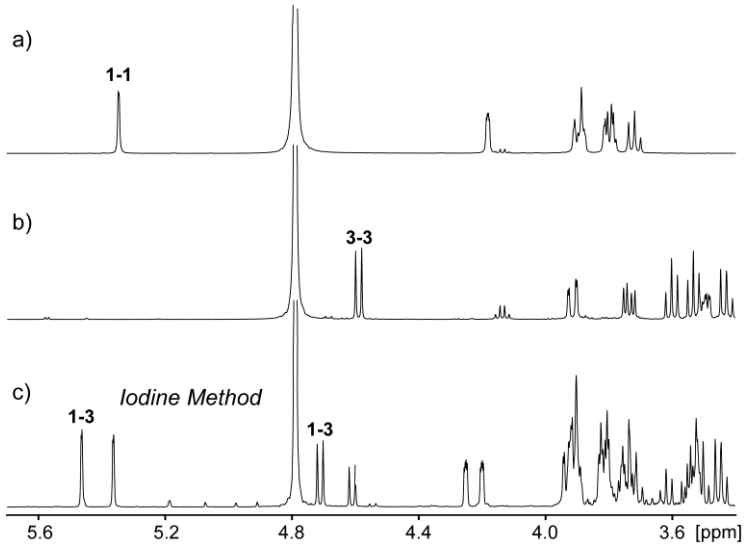

Figure S12. Control experiment for system **1-1** and **3-3**: a) ^{1}H NMR reference spectra of disulfide **1-1**, b) ^{1}H NMR reference spectra of disulfide **3-3** and c) disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **1** and **3**).

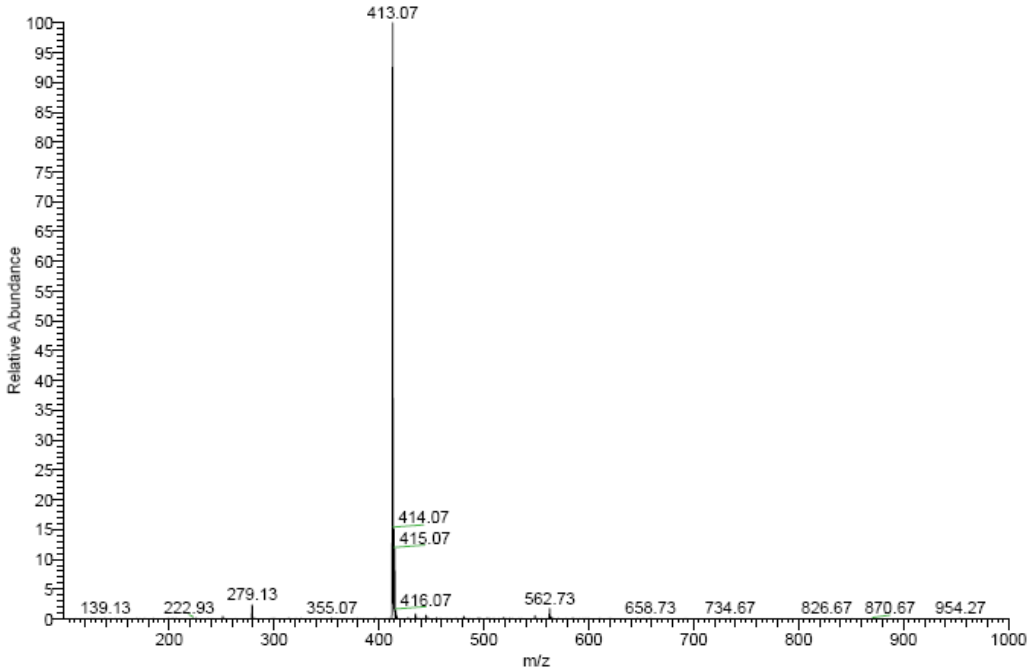

Figure S13. Mass spectrum (ESI) of the disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **1** and **3**).

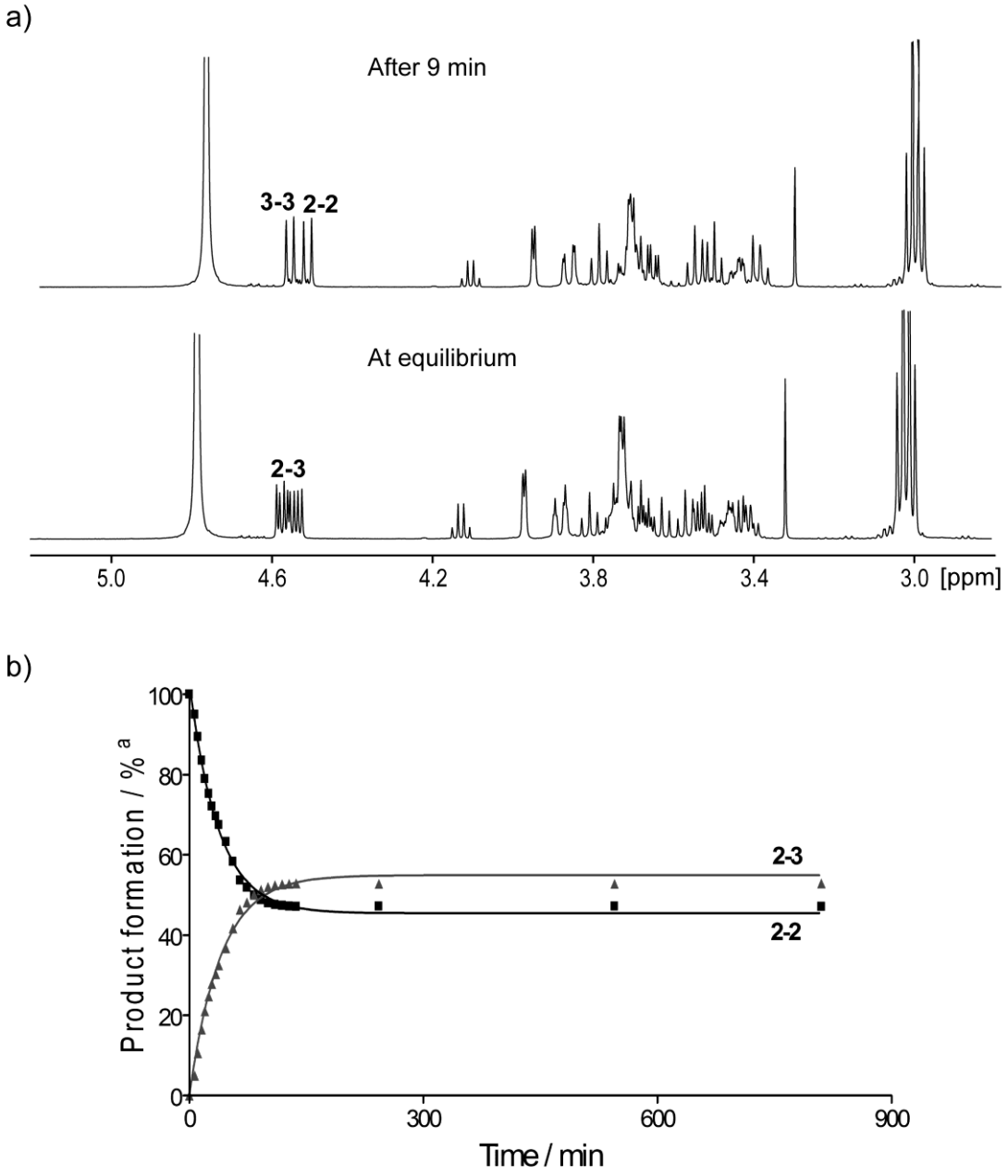

Figure S14. Disulfide metathesis in dynamic system (**2-2**, **3-3**) generation: a) ^{1}H NMR spectra of the reaction mixture and b) kinetic profile of the disulfide metathesis. Experimental conditions: **2-2** and **3-3** (15 mM each), HEPA (100 mol%), r.t., *d*-buffer/ CD_3CN (90:10), pD 8. [a]Yield calculated relatively to the galactose species in solutions.

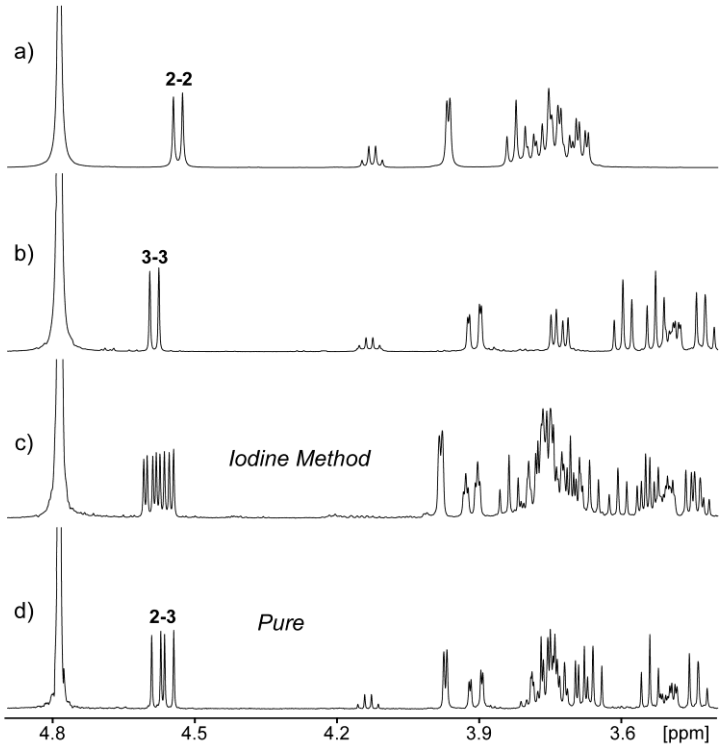

Figure S15. Control experiment for system **2-2** and **3-3**: a) ^{1}H NMR reference spectra of disulfide **2-2**, b) ^{1}H NMR reference spectra of disulfide **3-3**, c) disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **2** and **3**) and d) ^{1}H NMR spectra of disulfide **2-3**.

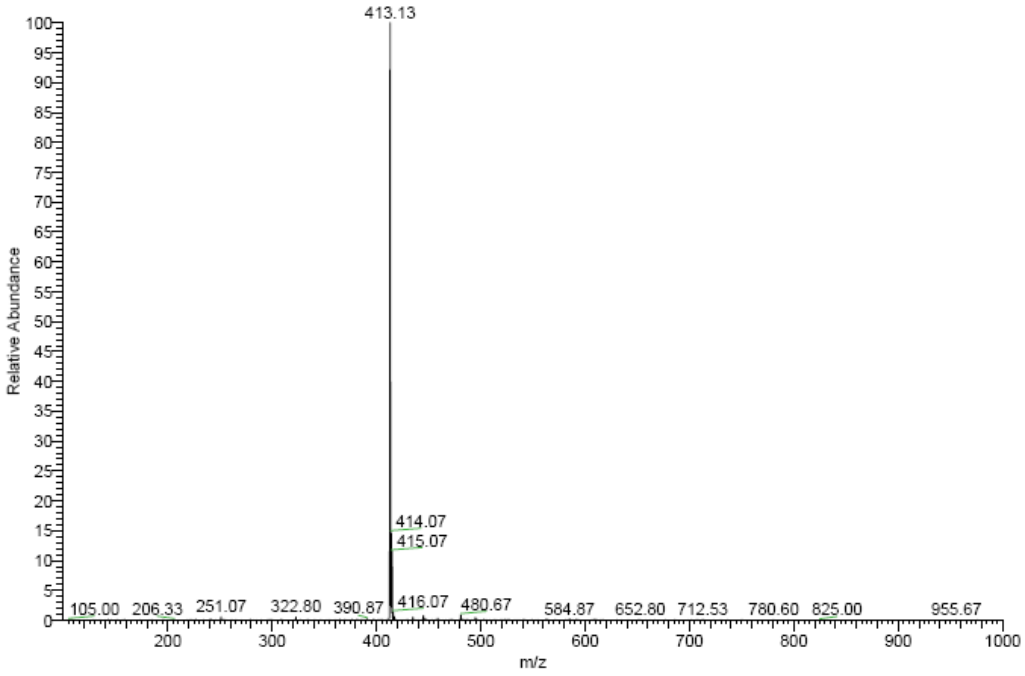

Figure S16. Mass spectrum (ESI) of the disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **2** and **3**).

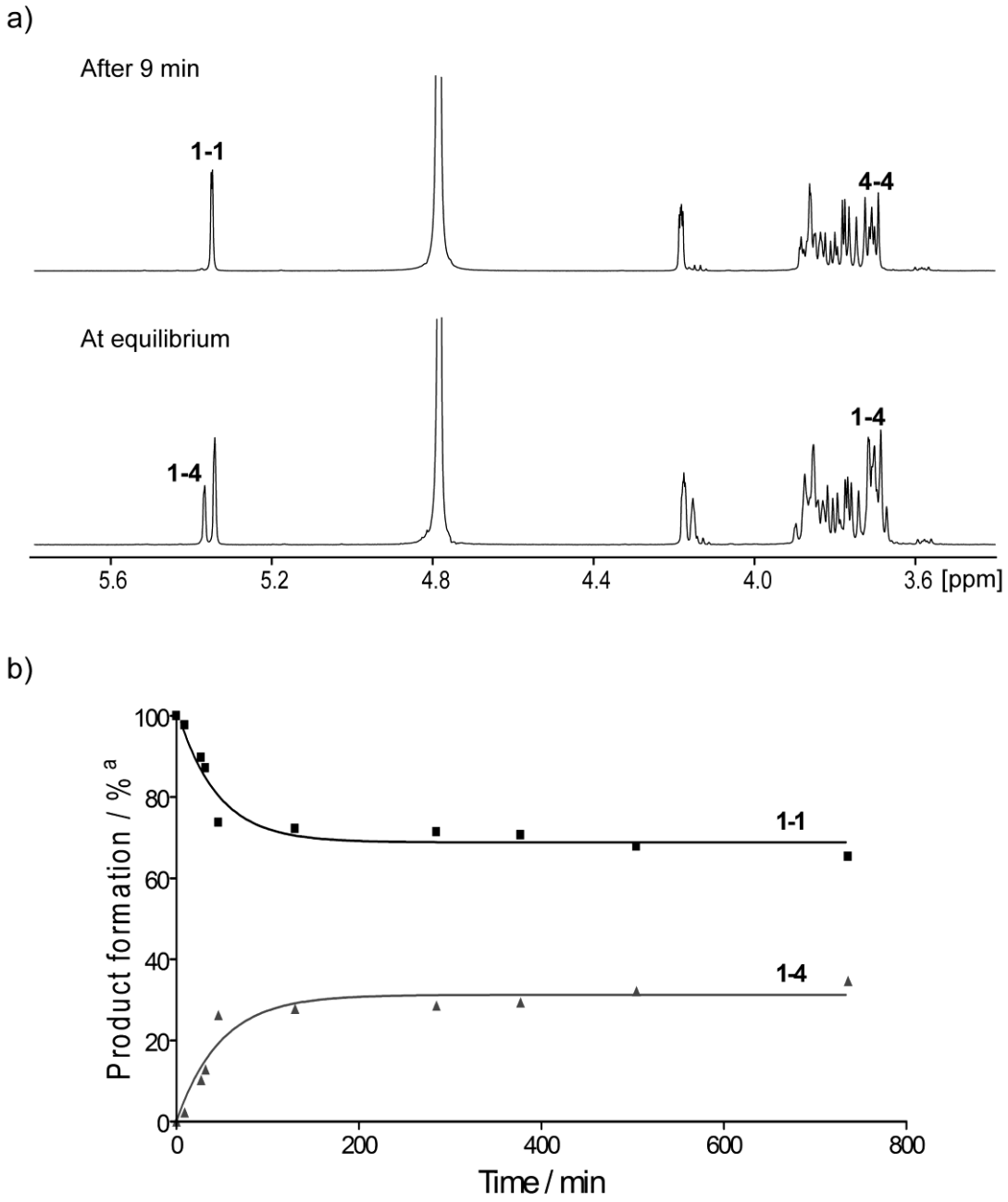

Figure S17. Disulfide metathesis in dynamic system (**1-1**, **4-4**) generation: a) ^{1}H NMR spectra of the reaction mixture and b) kinetic profile of the disulfide metathesis. Experimental conditions: **1-1** and **4-4** (15 mM each), HEPA (100 mol%), r.t., *d*-buffer/ CD_3CN (90:10), pD 8. [a]Yield calculated relatively to the mannose species in solutions.

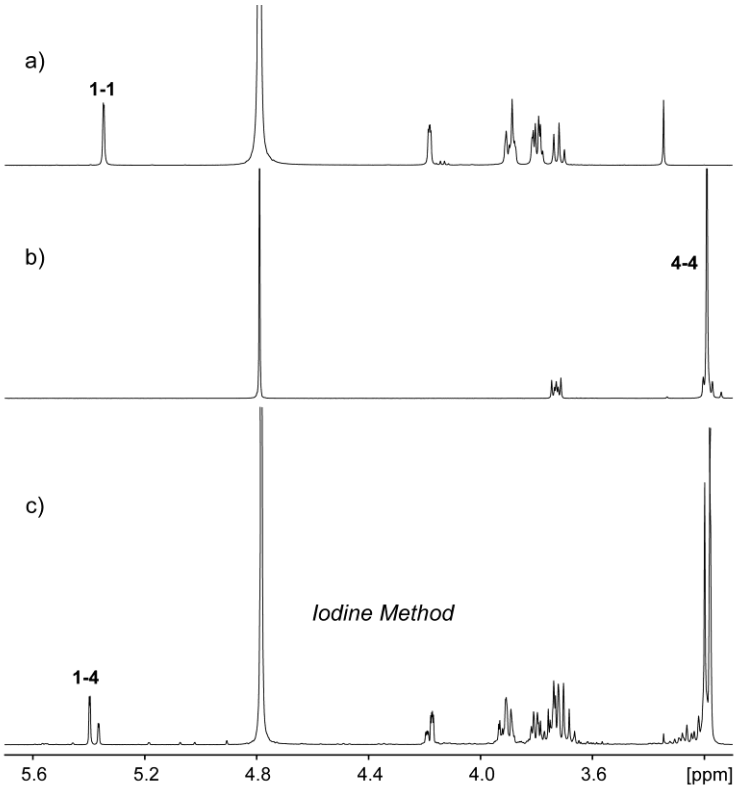

Figure S18. Control experiment for system **1-1** and **4-4**: a) ^{1}H NMR reference spectra of disulfide **1-1**, b) ^{1}H NMR reference spectra of disulfide **4-4** and c) disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **1** and **4**).

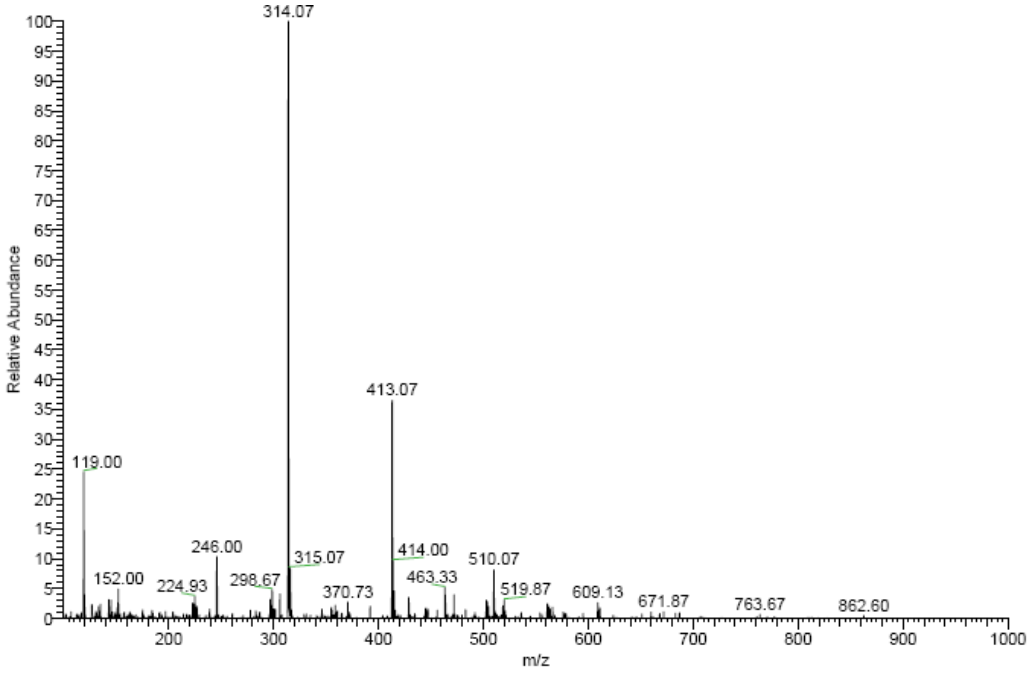

Figure S19. Mass spectrum (ESI) of the disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **1** and **4**).

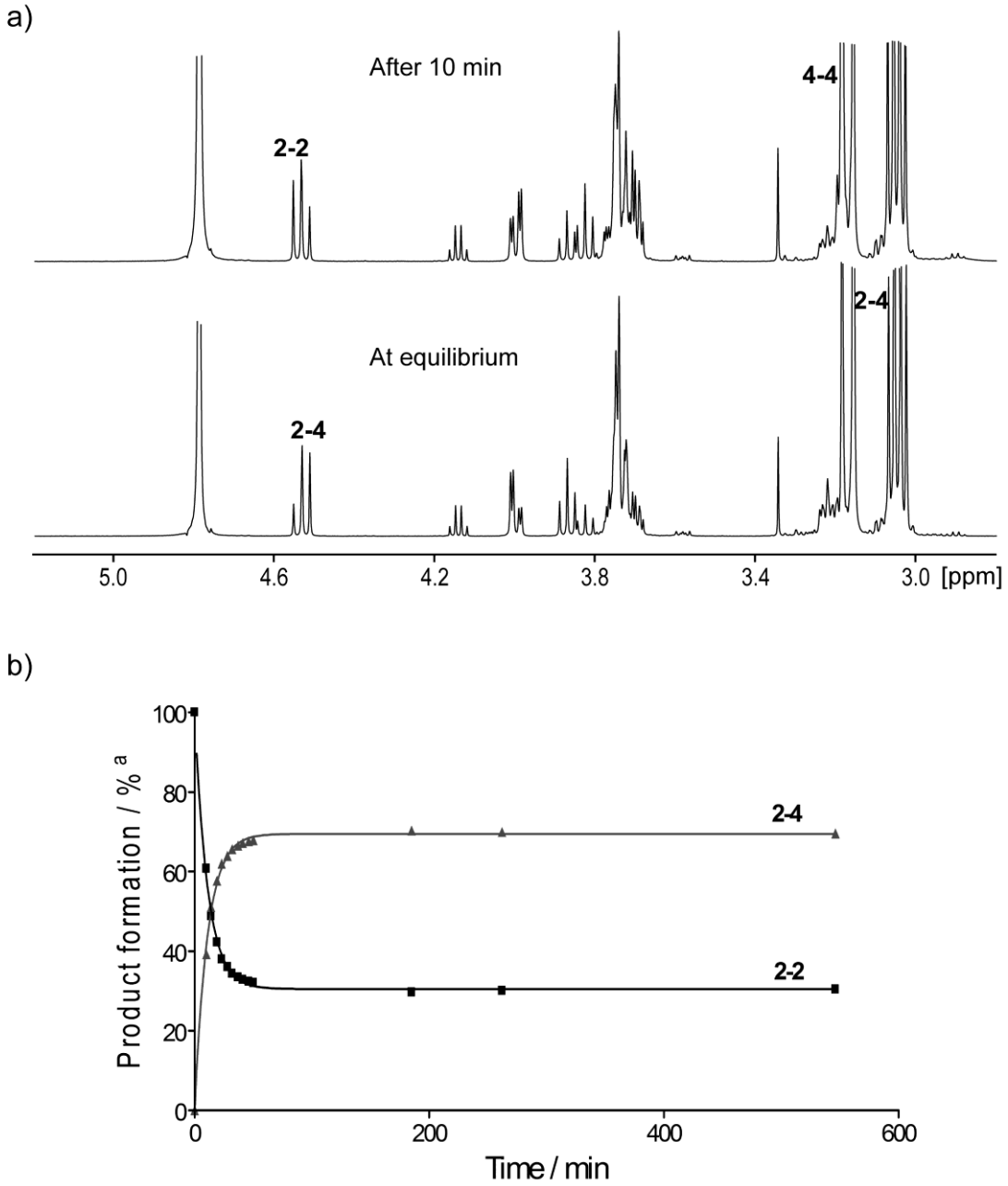

Figure S20. Disulfide metathesis in dynamic system (**2-2**, **4-4**) generation: a) ^{1}H NMR spectra of the reaction mixture and b) kinetic profile of the disulfide metathesis. Experimental conditions: **2-2** and **4-4** (15 mM each), HEPA (100 mol%), r.t., *d*-buffer/ CD_3CN (90:10), pD 8. [a]Yield calculated relatively to the galactose species in solutions.

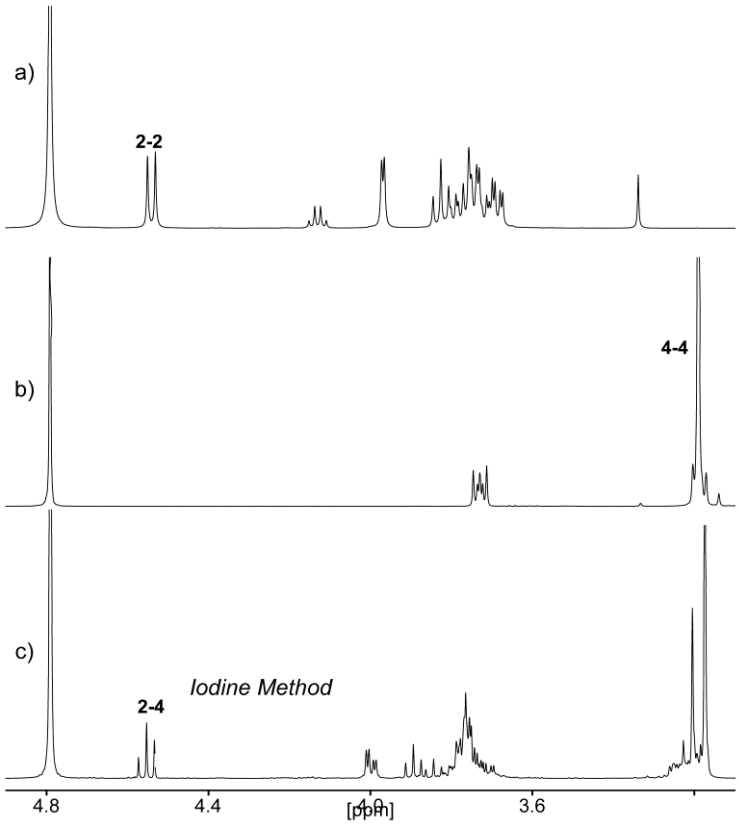

Figure S21. Control experiment for system **2-2** and **4-4**: a) ^{1}H NMR reference spectra of disulfide **2-2**, b) ^{1}H NMR reference spectra of disulfide **4-4** and c) disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **2** and **4**).

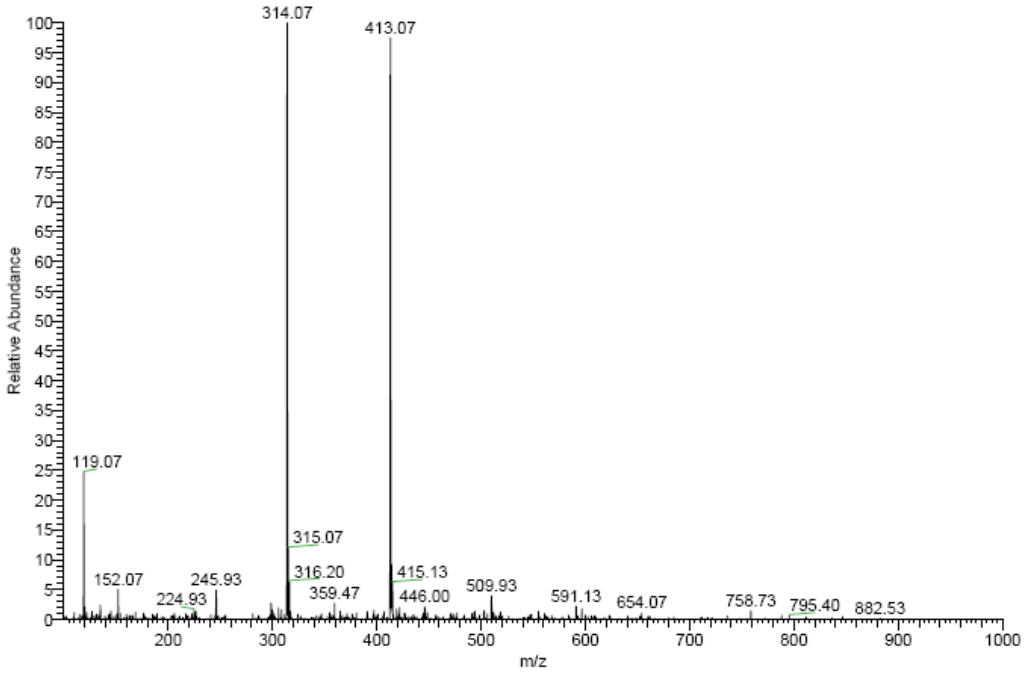

Figure S21. Mass spectrum (ESI) of the disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **2** and **4**).

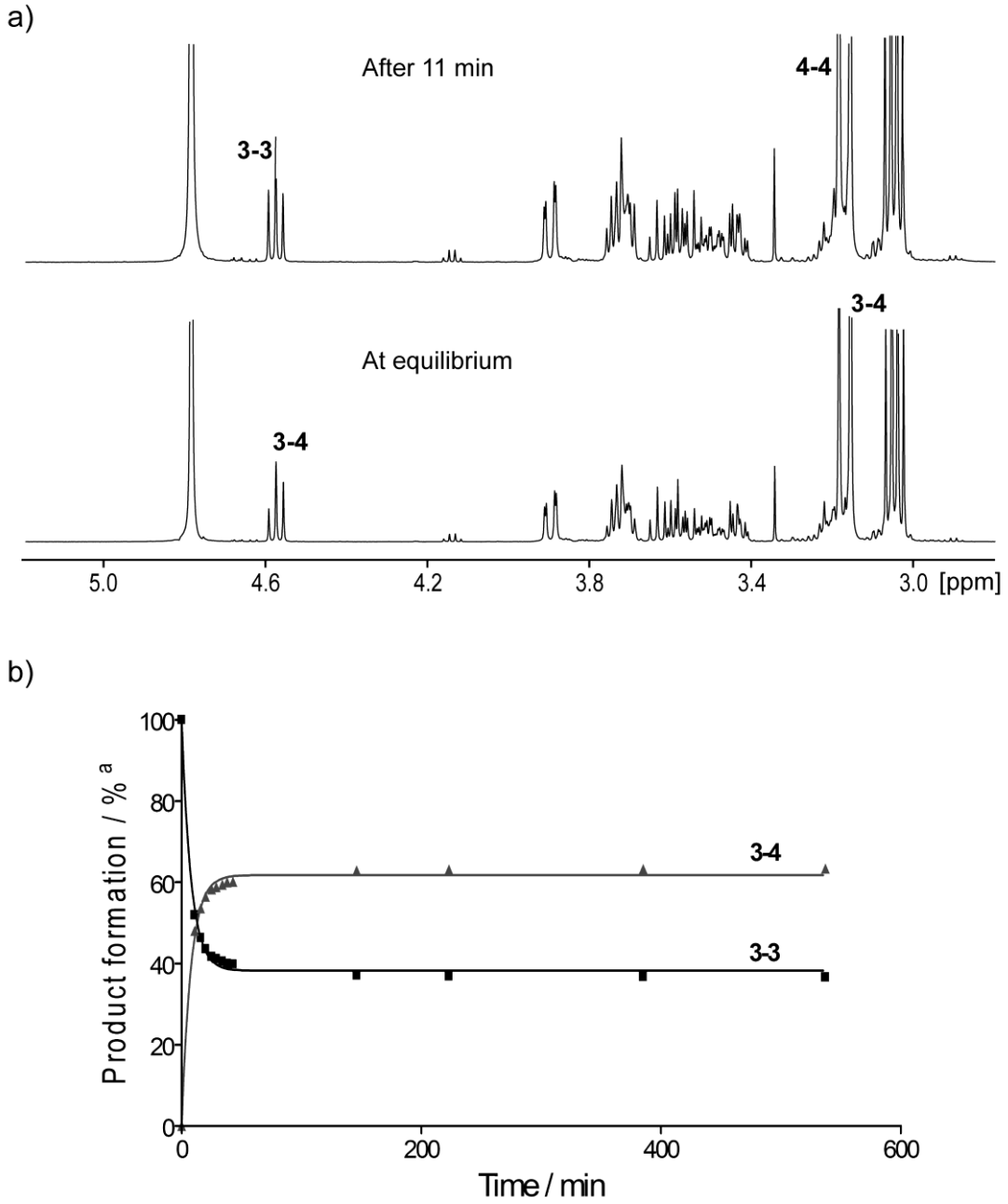

Figure S22. Disulfide metathesis in dynamic system (**3-3**, **4-4**) generation: a) ^{1}H NMR spectra of the reaction mixture and b) kinetic profile of the disulfide metathesis. Experimental conditions: **3-3** and **4-4** (15 mM each), HEPA (100 mol%), r.t., *d*-buffer/ CD_3CN (90:10), pD 8. [a]Yield calculated relatively to the glucose species in solutions.

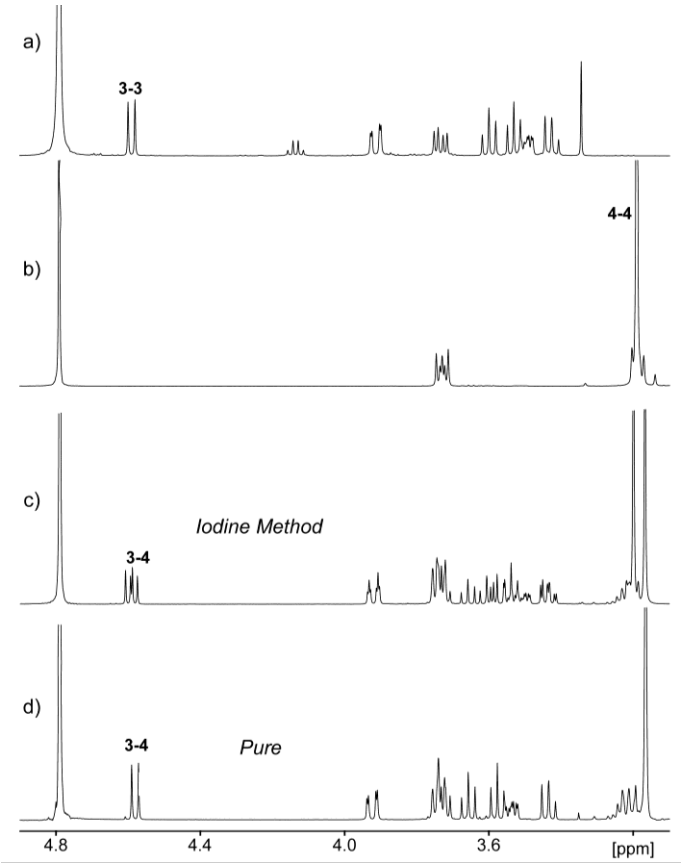

Figure S23. Control experiment for system **3-3** and **4-4**: a) ^{1}H NMR reference spectra of disulfide **3-3**, b) ^{1}H NMR reference spectra of disulfide **4-4**, c) disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **2** and **4**) and d) ^{1}H NMR spectra of disulfide **3-4**.

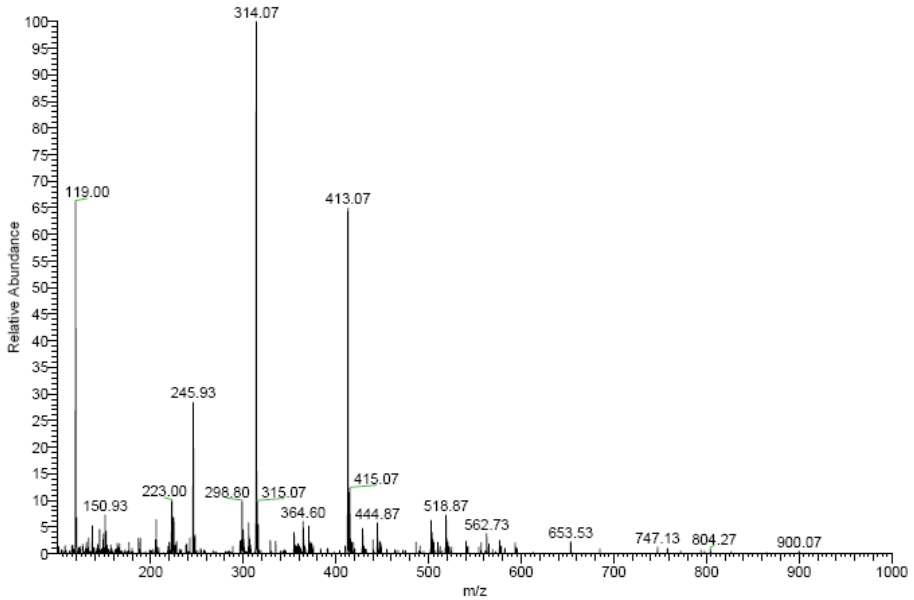

Figure S24. Mass spectrum (ESI) of the disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **3** and **4**).

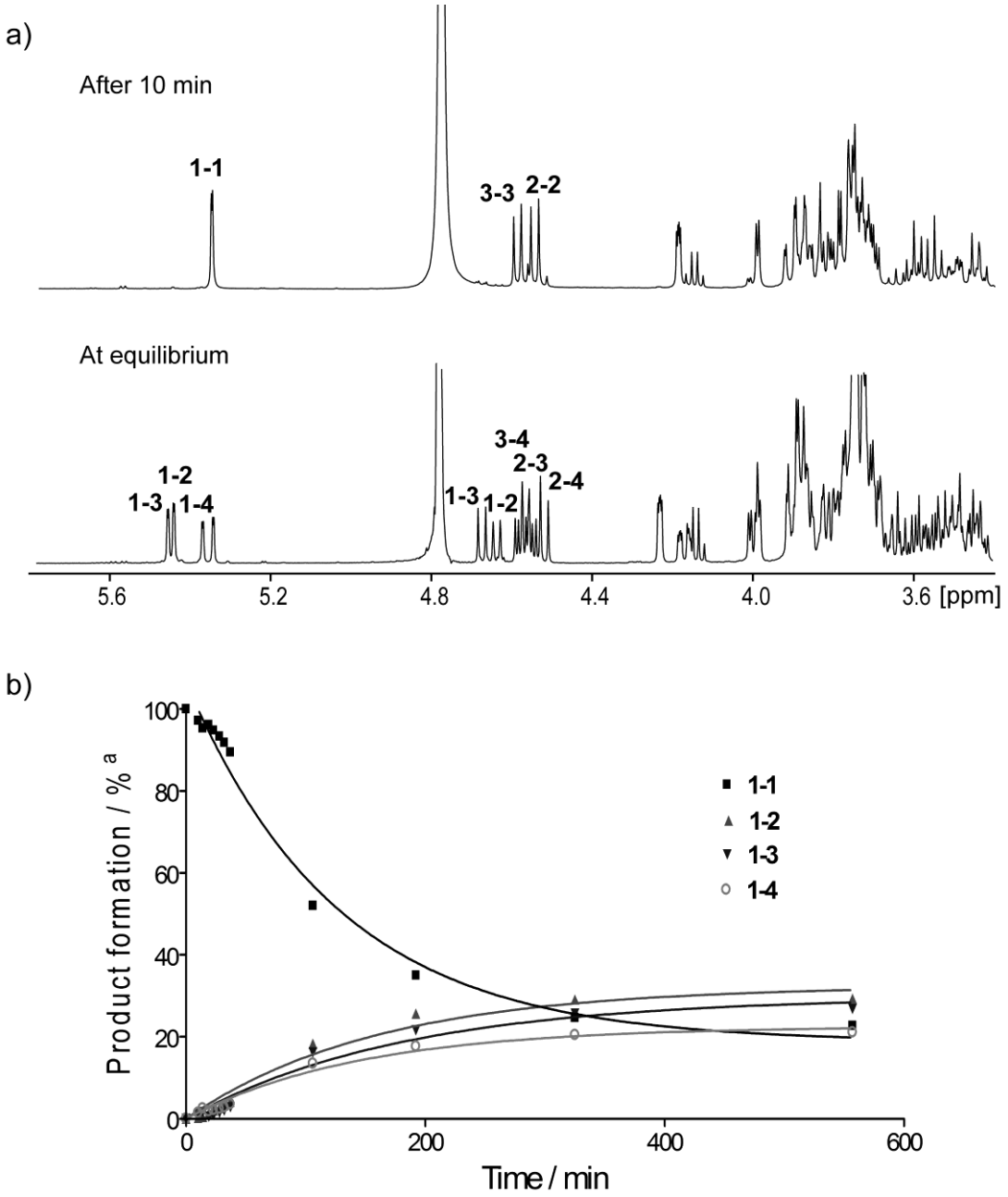

Figure S25. Disulfide metathesis in dynamic system (**1**-**1**, **2**-**2**, **3**-**3** and **4**-**4**) generation: a) ^{1}H NMR spectra of the reaction mixture and b) kinetic profile of the disulfide metathesis. Experimental conditions: **1**-**1**, **2**-**2**, **3**-**3** and **4**-**4** (15 mM each), HEPA (100 mol%), r.t., *d*-buffer/ CD_3CN (90:10), pD 8. [a]Yield calculated relatively to the mannose species in solutions.

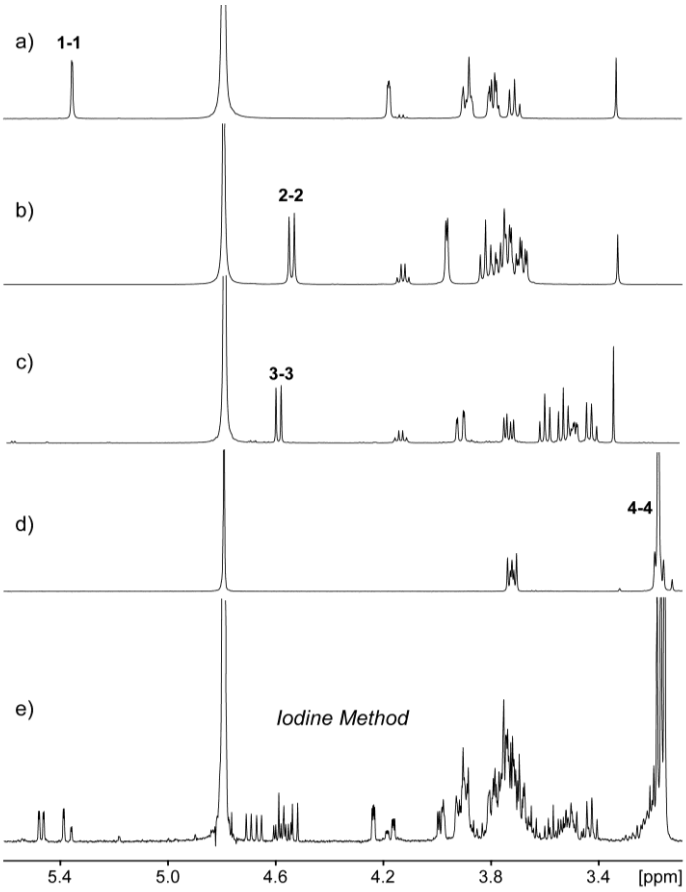

Figure S26. Control experiment for system **1-1**, **2-2**, **3-3** and **4-4**: a) ^{1}H NMR reference spectra of disulfide **1-1**, b) ^{1}H NMR reference spectra of disulfide **2-2**, c) ^{1}H NMR reference spectra of disulfide **3-3**, d) ^{1}H NMR reference spectra of disulfide **4-4** and) disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **1**, **2**, **3** and **4**).

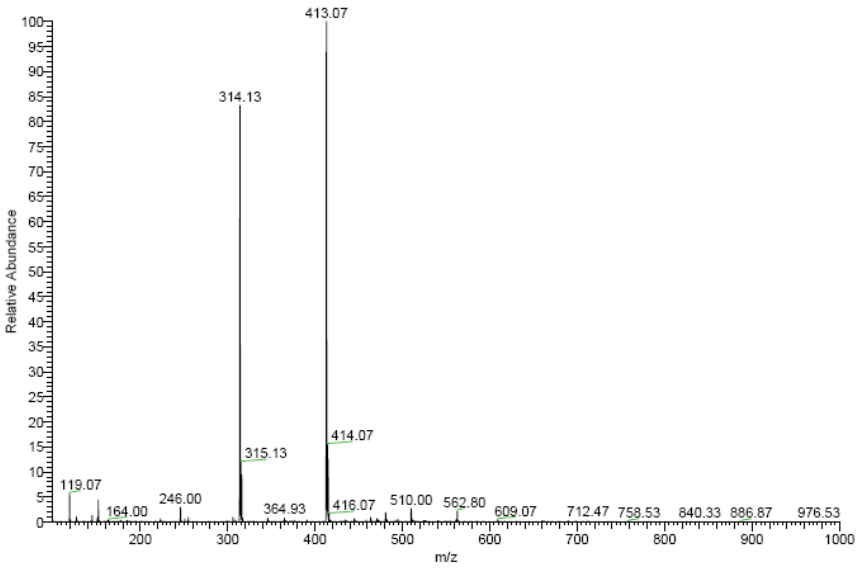

Figure S27. Mass spectrum (ESI) of the disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **1**, **2**, **3** and **4**).

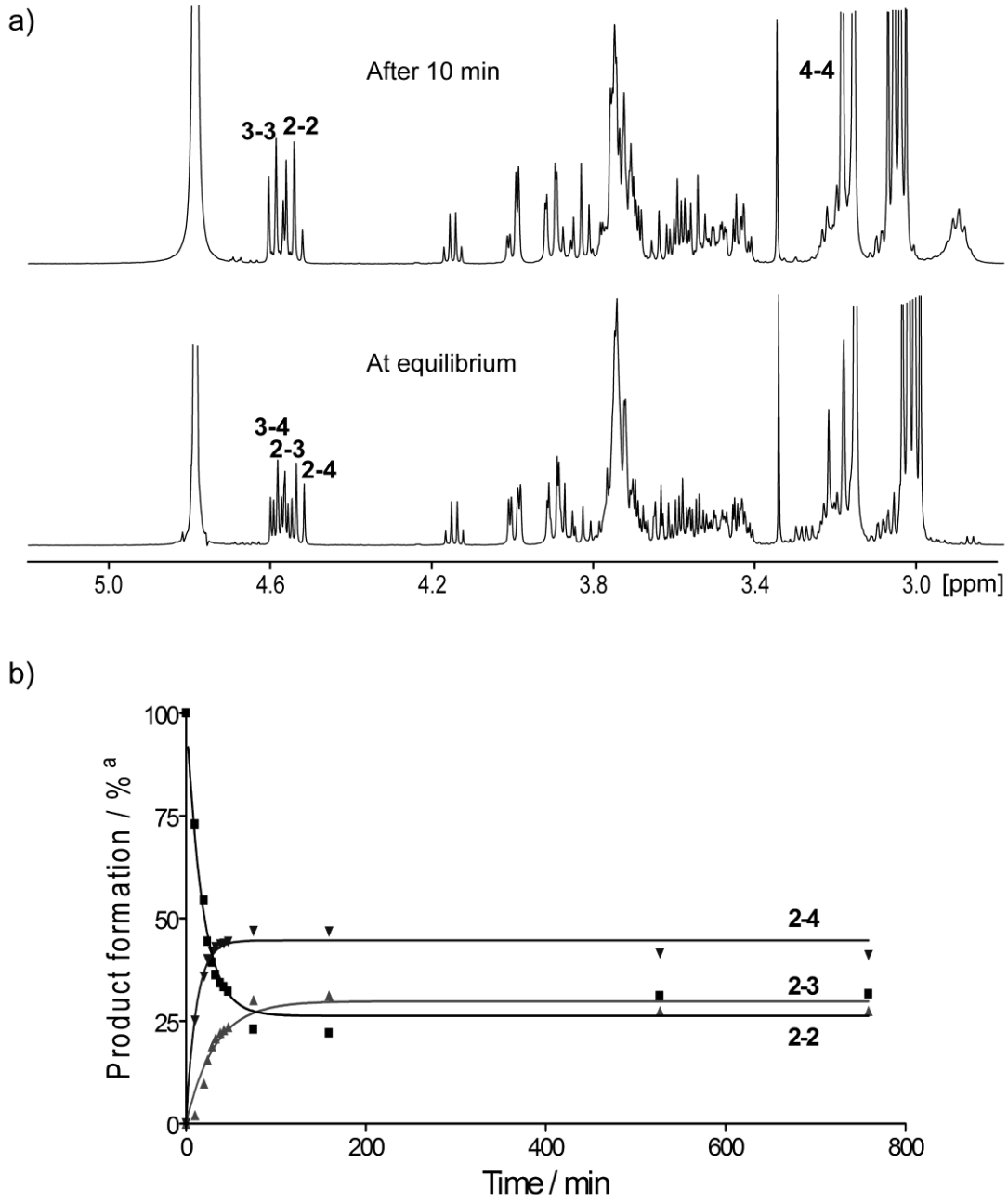

Figure S28. Disulfide metathesis in dynamic system (**2-2**, **3-3** and **4-4**) generation: a) ^{1}H NMR spectra of the reaction mixture and b) kinetic profile of the disulfide metathesis. Experimental conditions: **2-2**, **3-3** and **4-4** (15 mM each), HEPA (100 mol%), r.t., *d*-buffer/ CD_3CN (90:10), pD 8. [a]Yield calculated relatively to the galactose species in solutions.

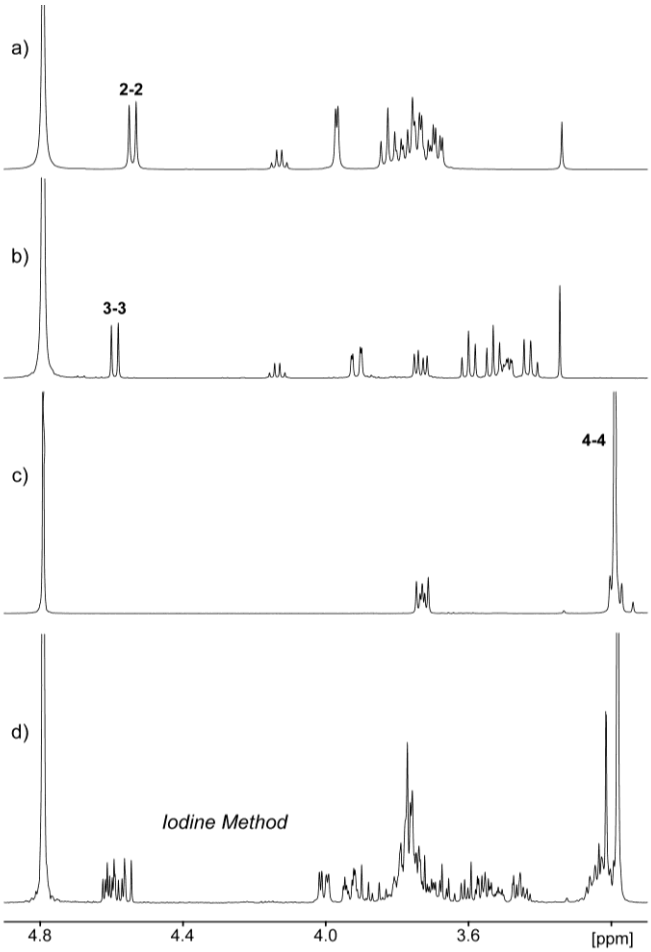

Figure S29. Control experiment for system **2-2**, **3-3** and **4-4**: a) ^{1}H NMR reference spectra of disulfide **2-2**, b) ^{1}H NMR reference spectra of disulfide **3-3** c) ^{1}H NMR reference spectra of disulfide **4-4** and d) disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **2**, **3** and **4**).

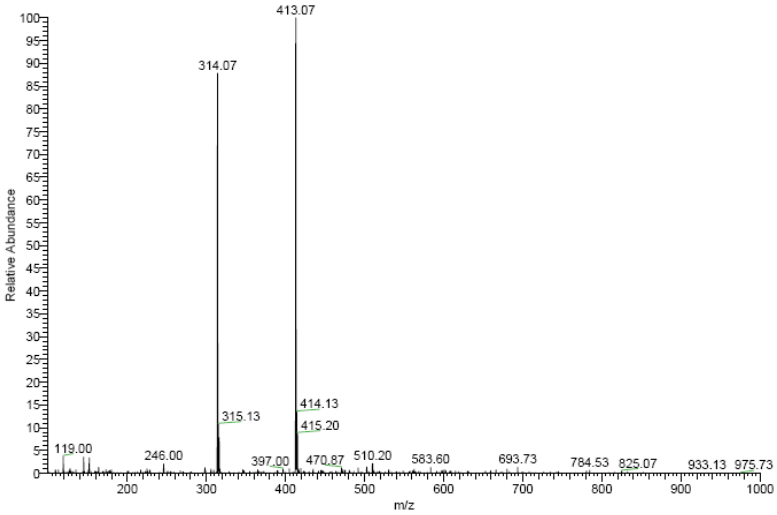

Figure S30. Mass spectrum (ESI) of the disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **2**, **3** and **4**).

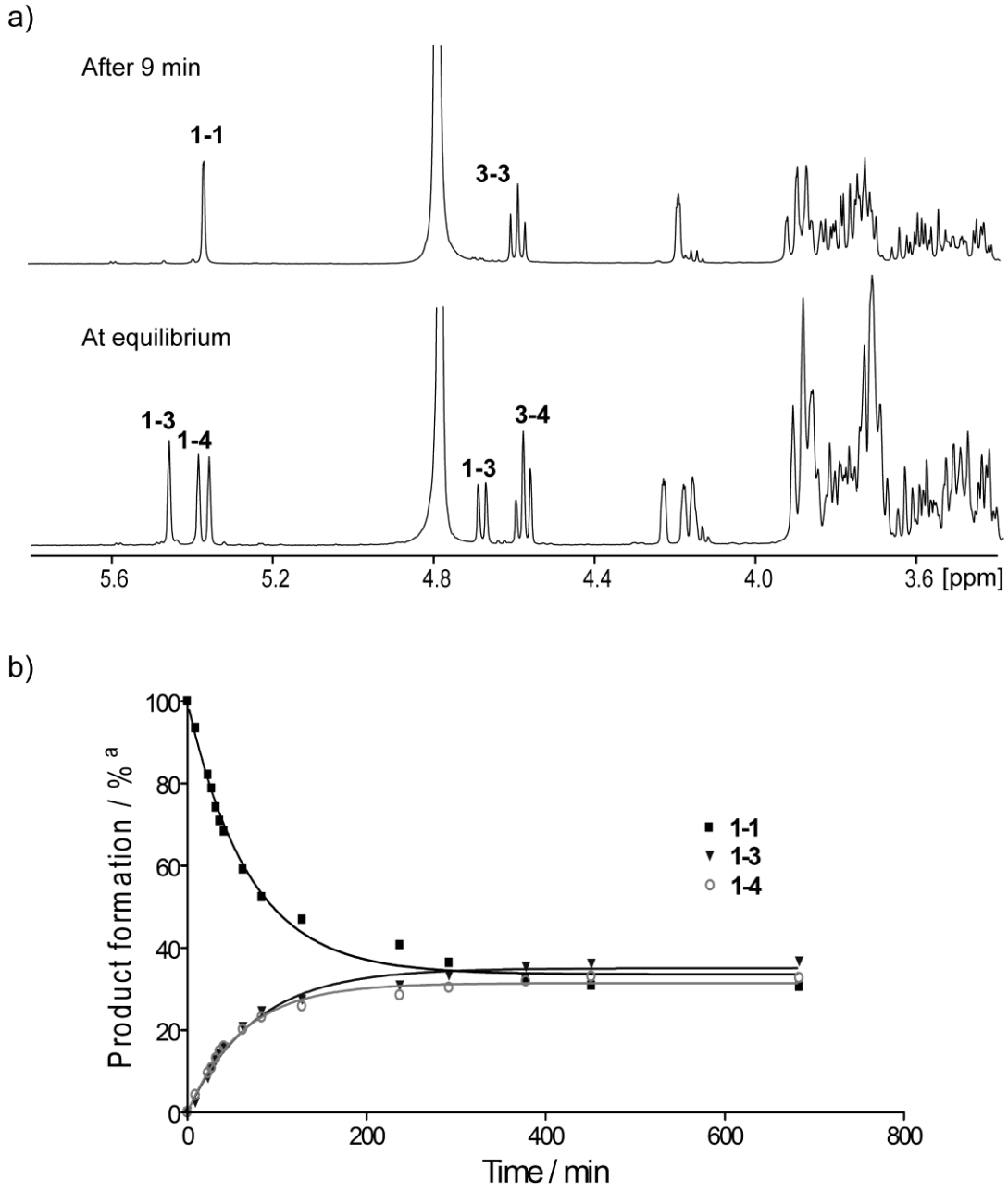

Figure S31. Disulfide metathesis in dynamic system (**1-1**, **3-3** and **4-4**) generation: a) ^{1}H NMR spectra of the reaction mixture and b) kinetic profile of the disulfide metathesis. Experimental conditions: **1-1**, **3-3** and **4-4** (15 mM each), HEPA (100 mol%), r.t., *d*-buffer/ CD_3CN (90:10), pD 8. [a]Yield calculated relatively to the mannose species in solutions.

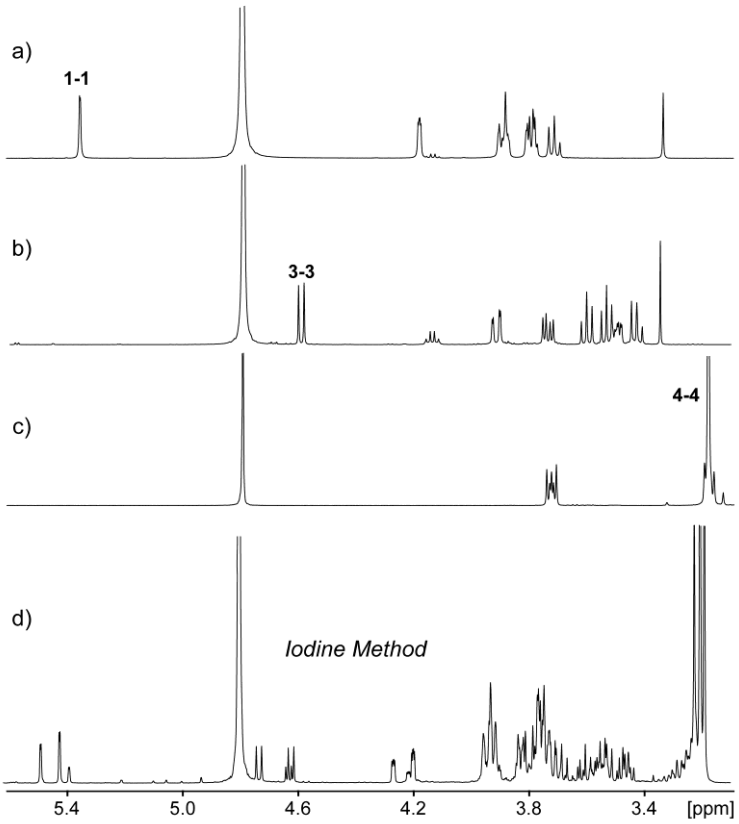

Figure S32. Control experiment for system **1-1**, **3-3** and **4-4**: a) ^{1}H NMR reference spectra of disulfide **1-1**, b) ^{1}H NMR reference spectra of disulfide **3-3** c) ^{1}H NMR reference spectra of disulfide **4-4** and d) disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **1**, **3** and **4**).

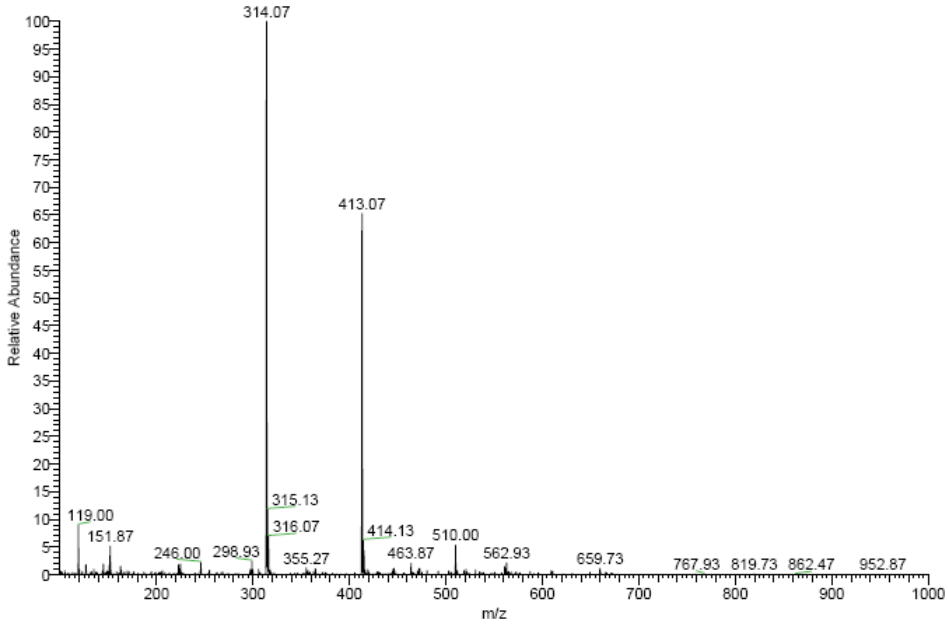

Figure S33. Mass spectrum (ESI) of the disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **1**, **3** and **4**).

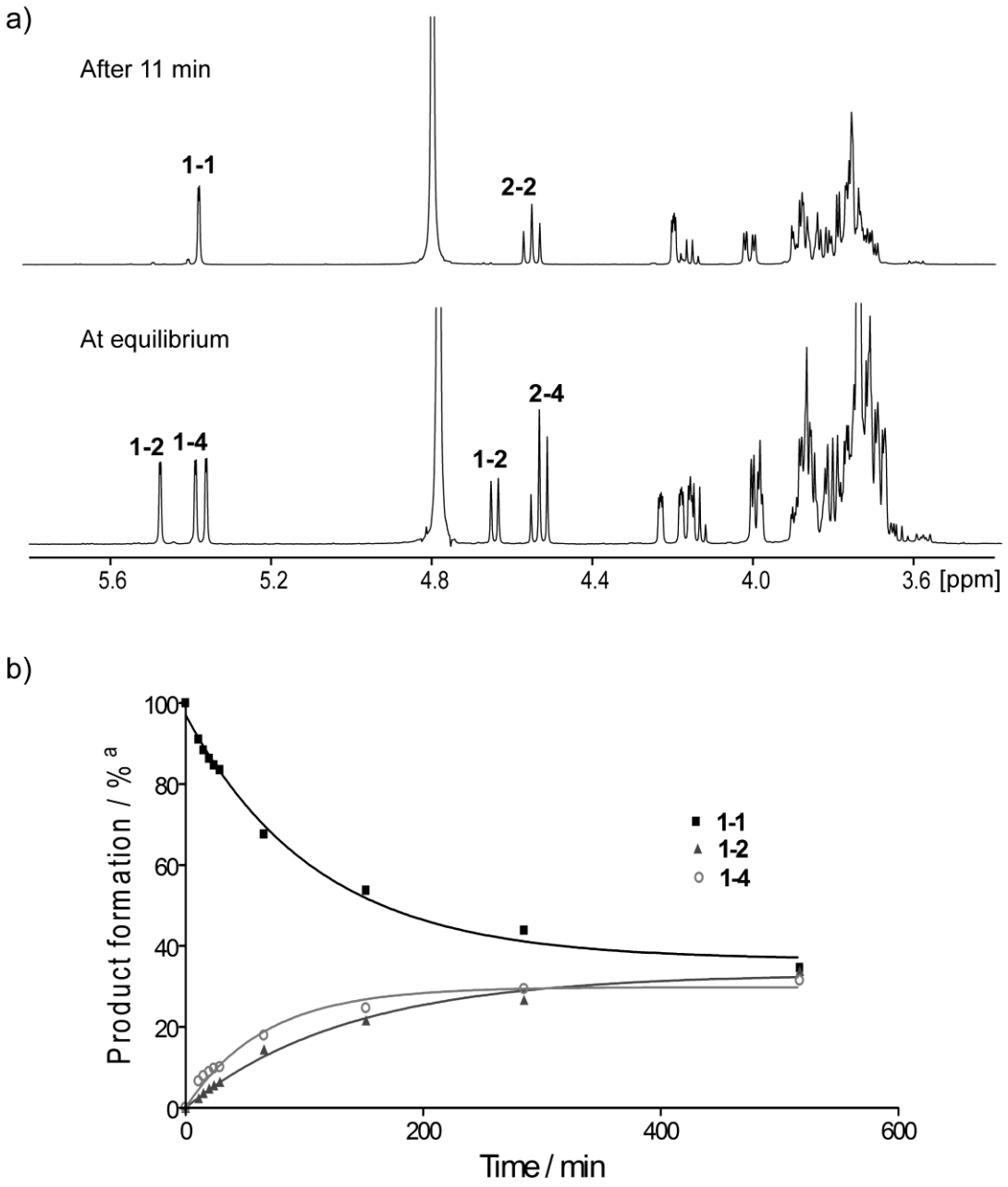

Figure S34. Disulfide metathesis in dynamic system (**1-1**, **2-2** and **4-4**) generation: a) ^{1}H NMR spectra of the reaction mixture and b) kinetic profile of the disulfide metathesis. Experimental conditions: **1-1**, **2-2** and **4-4** (15 mM each), HEPA (100 mol%), r.t., *d*-buffer/ CD_3CN (90:10), pD 8. [a]Yield calculated relatively to the mannose species in solutions.

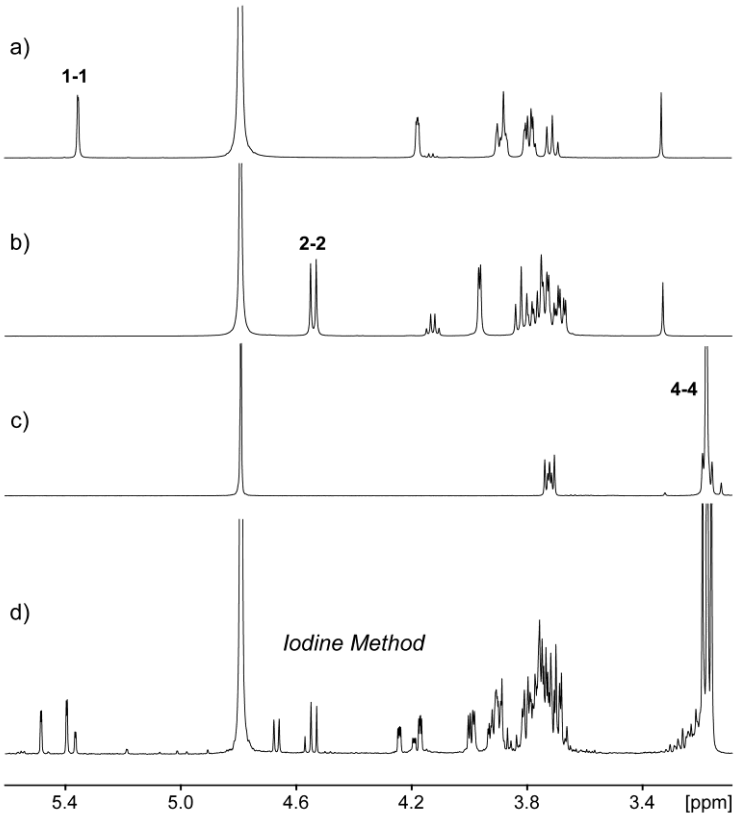

Figure S35. Control experiment for system **1-1**, **2-2** and **4-4**: a) ^{1}H NMR reference spectra of disulfide **1-1**, b) ^{1}H NMR reference spectra of disulfide **2-2** c) ^{1}H NMR reference spectra of disulfide **4-4** and d) disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **1**, **2** and **4**).

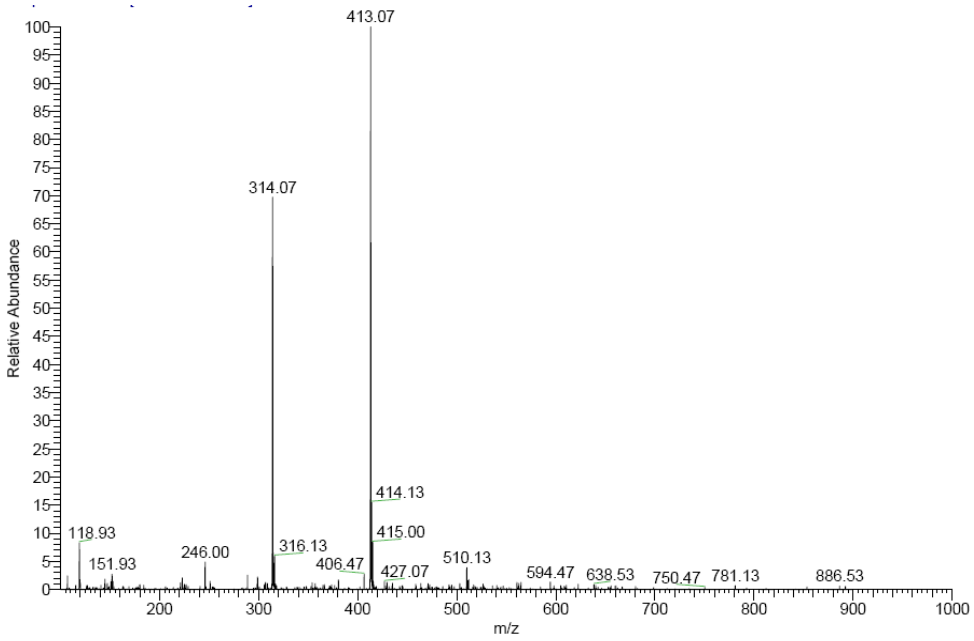

Figure S36. Mass spectrum (ESI) of the disulfide mixture prepared by thiol oxidation with iodine (starting thiols scaffold **1**, **2** and **4**).

STD-NMR experiments:

All NMR experiments were performed at a temperature of 298K on a Bruker Avance DMX 500 at 500 (125) MHz. Selective saturation was achieved by a train of Gauss-shaped pulses of 49 ms each, separated by a 1 ms delay. A number of 40 selective pulses were applied, leading to a total length of the saturation train of 2 s. The *on*-resonance irradiation on the enzyme was performed at a chemical shift of 8 ppm. *Off*-resonance irradiation was set to 100 ppm, where no protein signals were present.

Total number of scans used in STD experiments changed from 520 to 1040. Spectra processing was performed on a PC station using Topspin 2.0 software (Bruker). Each sample contained Concanavalin A in a 100 μM active site concentration. The glycosyl disulfides from the dynamic system were in a 100:1 ratio regarding the lectin. HEPA, when present, was of 75 mol% of the disulfide concentration. No incubation time of the ligands with ConA was needed.

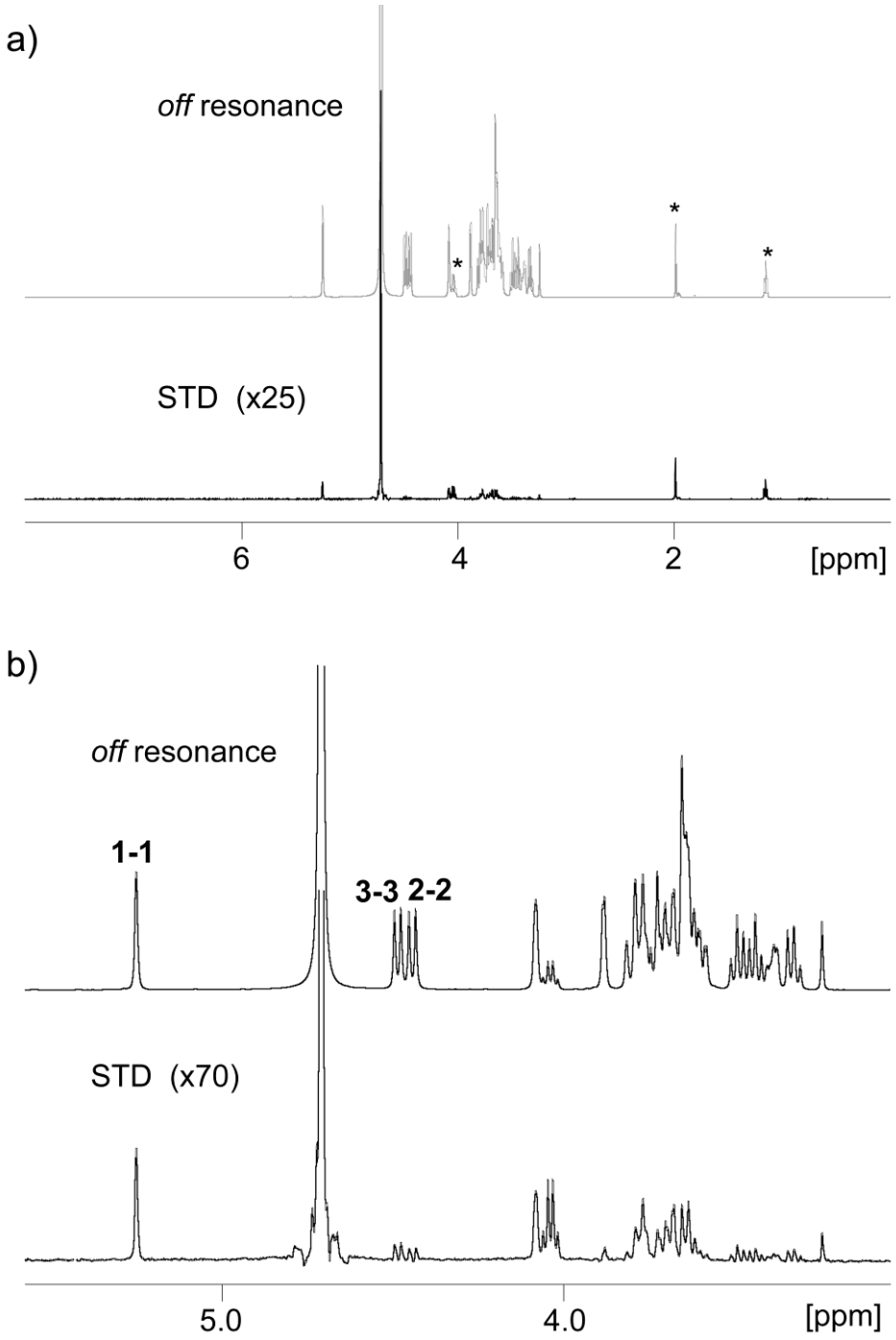

Figure S37. ^{1}H STD NMR binding studies of the carbohydrate system in absence of HEPA with Con A: a) full system spectra; enlarged area of b) the carbohydrate region. * Signals from the remaining solvent.