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Reactivity Tournament of Isothiocyanato-Functionalized Saccharides with 1,6-Diamino-3,6-oxaoctane

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Summary. Thiourea-bridged glycoclusters and glycodendrimers are described in the literature as mimetics of (oligoantennary) oligosaccharides to serve as high-affinity lectin ligands. In extension of this concept, the assembly of different, structurally varied isothiocyanato-functionalized sugar derivatives on an oligoamine scaffold would lead to novel mixed glycoclusters. To control this approach, the relative reactivities of the isothiocyanates used in the thiourea-bridging reaction have to be known. Therefore, competition experiments with six different sugar isothiocyanates were carried out using 1,8-diamino-3,6-dioxaoctane as a symmetrical difunctionalized core molecule. Reactivities were ranked on the basis of integration ratios in the ^{1}H NMR spectra. A first mixed thiourea-bridged glycocluster was successfully prepared.

Keywords. Sugar isothiocyanates; Thiourea bridging; Carbohydrates; Bioorganic chemistry.

Introduction

The oligosaccharide portions found in glycoproteins and glycolipids have important functions in cell biology [1]. Due to their structural diversity they can serve to store biological information, *e.g.* in cell-cell recognition and adhesion processes, or to assist the control of biochemical events [2, 3]. Molecular recognition of carbohydrate ligands is mediated by specialized proteins called lectins and selectins [4]. Lectins may contain one or more carbohydrate recognition domains (CRDs) on a single peptide or protein chain, respectively, or they may occur as clusters, *e.g.* on cell surfaces [5]. Multivalent binding of the receptor protein CRDs to multiple copies of carbohydrate ligands as presented in oligoantennary glycoconjugates can lead to high avidities, a finding which has been named the cluster effect [6, 7]. Because multivalency has been recognized as an important characteristic of carbohydrate-protein interactions [8–10], numerous synthetic multivalent neoglycoconjugates of different architecture have been introduced [11–13], for example to prevent early adhesion of neutrophiles to endothelial surfaces [14] or to block microbial adhesion [15, 16].

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Representative classes of mimetics of oligosaccharides and oligoantennary glycoconjugates have been called glycoclusters and glycodendrimers [17–19]. A feasible approach for their synthesis includes a coupling reaction between an oligovalent core molecule and a sugar derivative. As glycosylation reactions suffer from the fact that they have to be optimized for every individual case, alternative approaches have been sought to link a carbohydrate derivative to a scaffold molecule, among which the thiourea-bridging strategy has become a very successful one [20–23]. This method combines an isothiocyanato-functionalized carbohydrate derivative [24] with a branched or dendritic oligoamine. Accordingly, thiourea-bridged glycodendrimers and glycoclusters have been prepared with glycosyl isothiocyanates and *p*-NCS-phenyl glycosides, and 6-deoxy-6-isothiocyanato-functionalized saccharides have been employed for the synthesis of thiourea-bridged glycoclusters [25, 26]. Usually, such reactions proceed with high yields and do not require tedious optimization of reaction conditions.

One of our projects involves the clustering of structurally different NCS-functionalized sugar derivatives to develop high-affinity ligands for various lectins. Using derivatives with different NCS-functionalized spacers would allow to vary the resulting glycoclusters with respect to their conformational flexibility. Furthermore, combination of different carbohydrate ligands in one thiourea-bridging reaction can eventually lead to highly diversified glycoclusters of a mixed type.

To allow for a rational synthesis of mixed glycoclusters of this kind in one pot, a grading of the reactivities of the reaction partners is necessary [27]. This paper describes a reactivity tournament of sugar isothiocyanates of different structural characteristics to obtain their relative reactivities in thiourea-bridging reactions.

Results and Discussion

Six different sugar isothiocyanates (**1**–**6**, Fig. 1) were chosen for comparison of their reactivities in thiourea-bridging: glycosyl isothiocyanates **1** and **2**, alkyl isothiocyanates **3** and **4**, and aryl isothiocyanates **5** and **6**. As we are especially interested in the inhibition of mannose-specific bacterial adhesion [28], mannose was chosen as starting material for all derivatives except for **1** which was included to obtain a hint about unexpected reactivity differences of analogous derivatives of different sugar series. Compounds **1** [29], **2** [29], **3** [30], **4** [25], and **6** [22] were synthesized according to standard procedures. A new route was followed for the preparation of **5**, involving direct glycosylation of 4-isothiocyanatobenzylalcohol instead of glycosylation of 4-nitrobenzylalcohol and subsequent derivatization of the *p*-nitro group. This method allowed to exclude the possibility of O → N-acetyl migration.

For the design of the competition experiments it has to be taken into account that in the thiourea-bridging reaction of sugar isothiocyanates with oligoamines the reactivity of the second amino group is influenced by reaction of the first one. Therefore, a diamine instead of a monoamine was chosen for the reactivity tournament to be closer to the real case scenario of the preparation of thiourea-bridged glycoclusters and glycodendrimers. The reaction between two different sugar isothiocyanates (**X** and **Y**, Fig. 2) and a diamine can lead to three products:

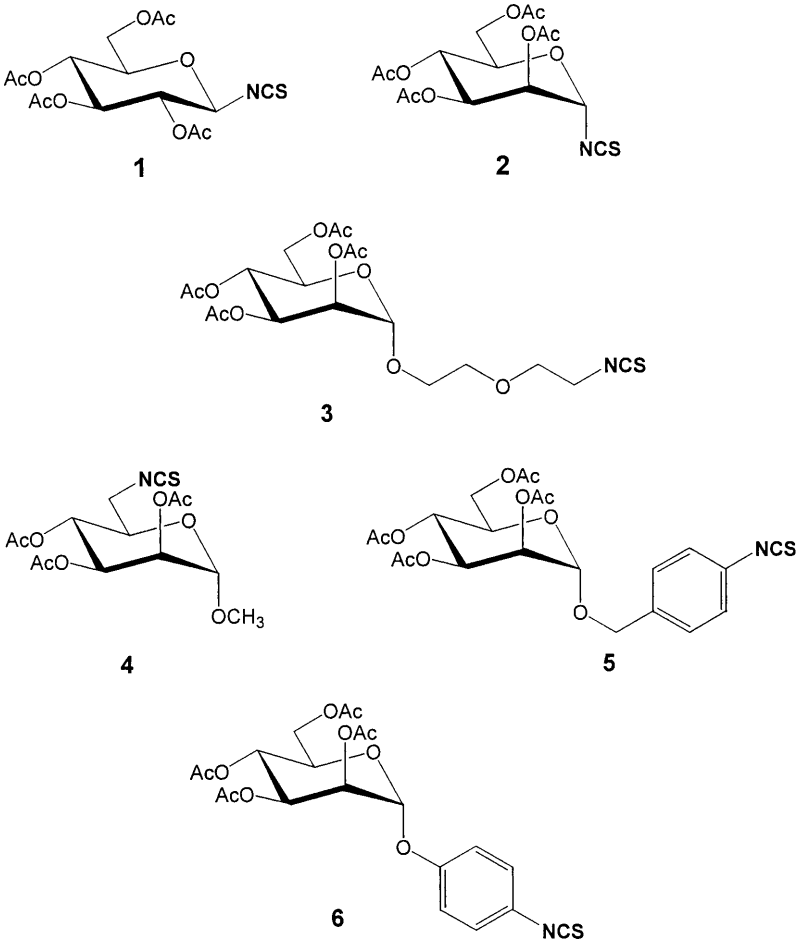

Fig. 1. Sugar isothiocyanates **1**–**6**

two homodimers and one heterodimer (Fig. 2). The diamine used in the reaction has to be soluble in an inert solvent. Because our first choice, 1,6-diaminohexane, had to be dissolved in protic solvents such as ethanol, 1,8-diamino-3,6-dioxaoctane was chosen instead. It is a symmetrical difunctionalized core molecule soluble in dichloromethane.

First, all homodimers (**1a**–**6a**) were prepared by thiourea-bridging with the above diamine (Fig. 3), mainly to be used as standards in NMR analysis (Table 1). Then, for each competition experiment a pair of sugar isothiocyanates chosen from compounds **1**–**6** (Fig. 1) was selected. Two equivalents of each relative to the diamine core molecule were dissolved in boiling dichloromethane, and the diamine was added dropwise as a solution in dichloromethane. The reaction mixture was stirred under reflux for three hours; then the solvent was removed under reduced pressure. Flash chromatography or HPLC did not allow the complete separation of the three different reaction products. Therefore, it was decided to use flash chromatography to separate the excess of educts from the product mixture. The two fractions thus obtained were analyzed by NMR spectroscopy, and the NMR spectra were used to estimate the relative reactivities of the isothiocyanates employed.

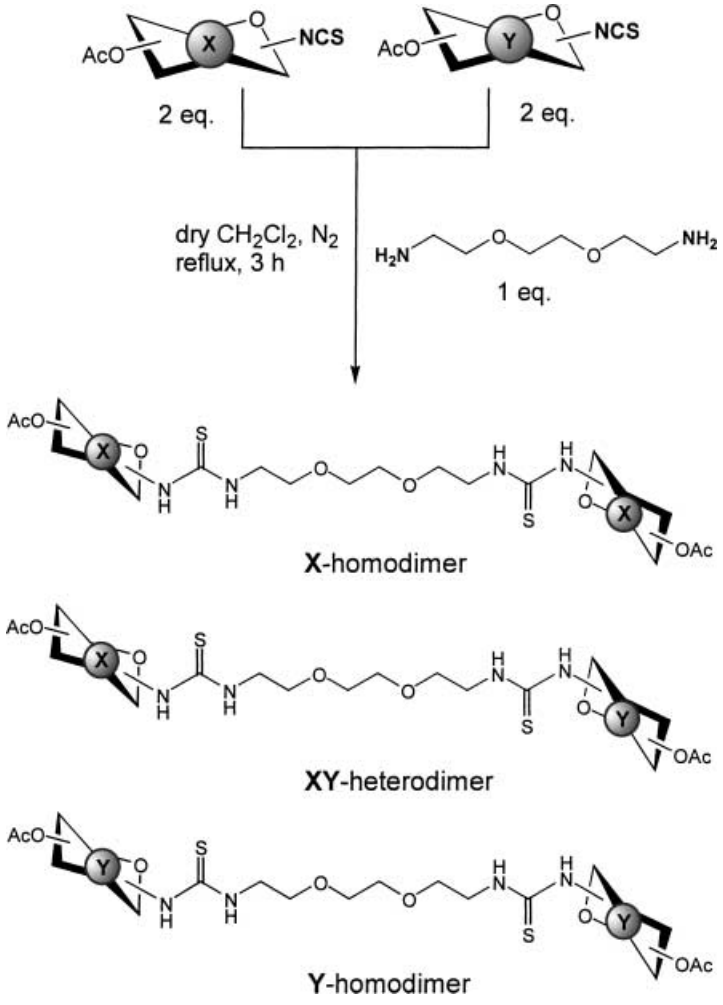

Fig. 2. The competition experiment: reaction of two different sugar isothiocyanates in the thiourea-bridging reaction

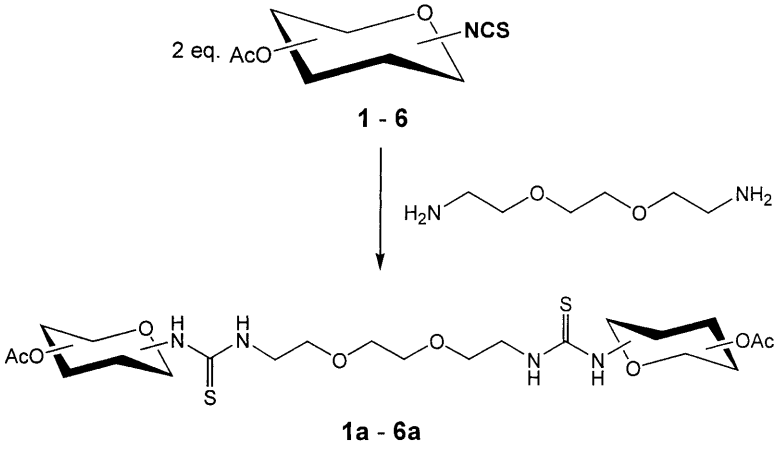

Fig. 3. Thiourea-bridging reaction to obtain the homodimers **1a**–**6a**

Table 1. ^{1}H NMR shifts of H-1 signals of the employed sugar isothiocyanates and their respective dimers

	$\delta(^1H)$/ppm	B_0/MHz, solvent		$\delta(^1H)$/ppm	B_0/MHz, solvent
1	4.99	400, $CDCl_3$	**1a**	5.87	500, acetone-d_6
2	5.53	400, $CDCl_3$	**2a**	5.99	500, acetone-d_6
3	4.86	400, $CDCl_3$	**3a**	4.88	400, $CDCl_3$
4	4.72	400, $CDCl_3$	**4a**	4.65	400, $CDCl_3$
5	4.83	400, $CDCl_3$	**5a**	4.88	500, $CDCl_3$
6	5.47	400, $CDCl_3$	**6a**	5.52	500, $CDCl_3$

The ratio of unreacted starting materials (educts **X** and **Y**) as well as the ratio of glycosyl moieties **X** and **Y** present in the dimer mixtures could be obtained from the ^{1}H NMR spectra of the respective fractions. Two redundant informations were extracted from the spectra in each case: From the spectrum of the excess educts, the turnover of each educt isothiocyanate could be estimated, and from the spectrum of the product mixture it could be deduced which derivative had reacted with the amine to which extent. As in most cases the sugar H-1 signals were well separated from the other resonances, the integration of these peaks (Table 1) formed the basis for the determination of relative reactivities. The integration of the relevant signals of the NMR spectra of both fractions provided the same information for an individual competition experiment as exemplified for the competition reaction between **4** and **5** (C(**4**/**5**) in Table 2; Fig. 4).

The competition experiments clearly showed tremendous differences in the reactivity of different isothiocyanato-functionalized sugars when examined in the thiourea-bridging reaction (Table 2). The glycosyl isothiocyanates **1** and **2** were by far the most reactive derivatives due to the special reactivity of the anomeric

Table 2. Results of the reactivity tournament employing competition experiments between two different sugar isothiocyanates **X** and **Y**

C(**X**/**Y**)[1]	Educts		Products		Major product
	Turnover/ % **X**	Turnover/ % **Y**	% **X** in dimer mixture	% **Y** in dimer mixture	
C(**1**/**2**)	44	56	49	51	**2**
C(**1**/**5**)	83	17	80	20	**1**
C(**2**/**3**)	94	6	≥ 99	≤ 1	**2**
C(**2**/**4**)	97	3	99	1	**2**
C(**2**/**5**)	91	9	86	14	**2**
C(**2**/**6**)	88	12	–[2]	–[2]	**2**
C(**3**/**4**)	37	63	38	62	**4**
C(**3**/**5**)	14	86	–[2]	–[2]	**5**
C(**4**/**5**)	16	84	18	82	**5**
C(**5**/**6**)	54	46	56	44	**5**

[1] Competition reaction of sugar isothiocyanates **X** and **Y**; [2] Percentage in dimer mixture could not be determined because of overlapping signals in the ^{1}H NMR spectrum

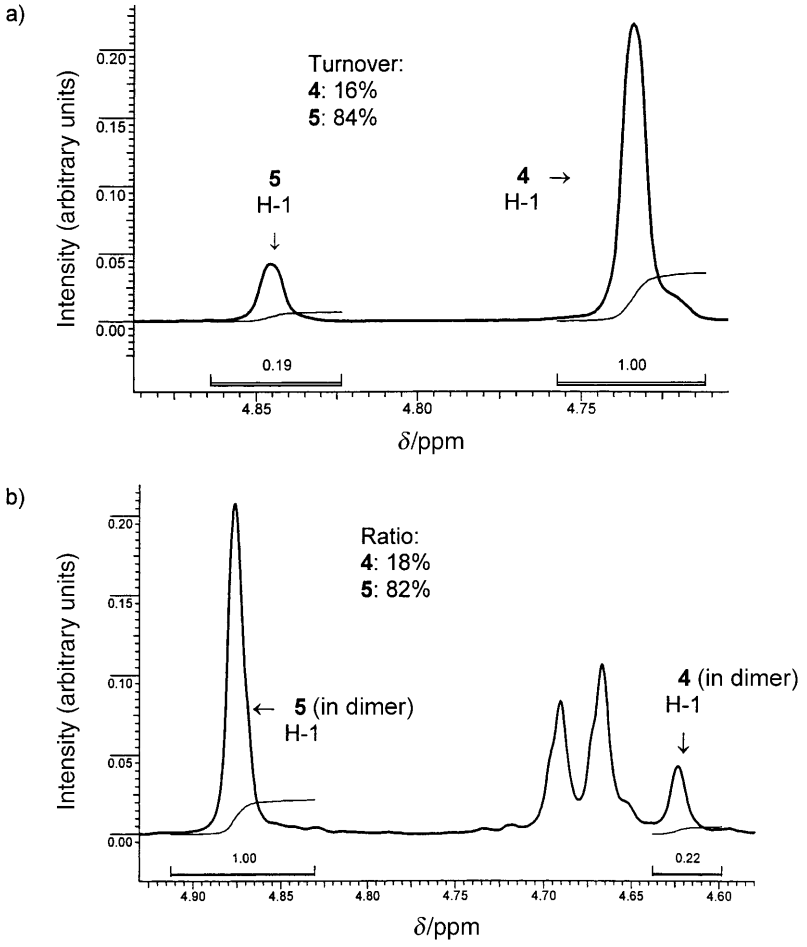

Fig. 4. (a) ^{1}H NMR signals of the H-1 protons of excess educts obtained in the competition experiment between **4** and **5**; (b) ^{1}H NMR signals of the H-1 protons of the product mixture obtained in the competition experiment between **4** and **5**; the signals were often broad, especially in the cases of **1a** and **2a** where acetone-d_6 was used as solvent for better resolved signals

position. Significant differences of the reactivities of the α-mannosyl (**2**) and β-glucosyl isothiocyanate (**1**) were not found. The derivatives with an aromatic aglycone formed a second group with similar reactivity. In this case, the mesomeric effect provided by the phenyl moiety may decrease the electrophilicity of the NCS-carbon. It could be shown that the *p*-NCS-benzyl glycoside **5** was slightly more reactive than the *p*-NCS-phenyl mannoside **6**. An explanation for this difference might be given on the basis of competing mesomeric effects. Among the alkyl isothiocyanates, the 6-NCS-modified derivative **4** is clearly more reactive than **3** which carries the NCS-group on the aliphatic spacer aglycone.

From integration of the broad H-1 signals in the measured ^{1}H NMR spectra the relative reactivities of the sugar isothiocyanates tested could be unequivocally deduced, leading to an order of reactivity of $\mathbf{2} \geq \mathbf{1} \gg \mathbf{5} > \mathbf{6} \gg \mathbf{4} > \mathbf{3}$.

Based on this ranking, a first rational synthesis of a mixed thiourea-bridged glycocluster was carried out. *Tris*-(2-aminoethyl)-amine was selected as branched

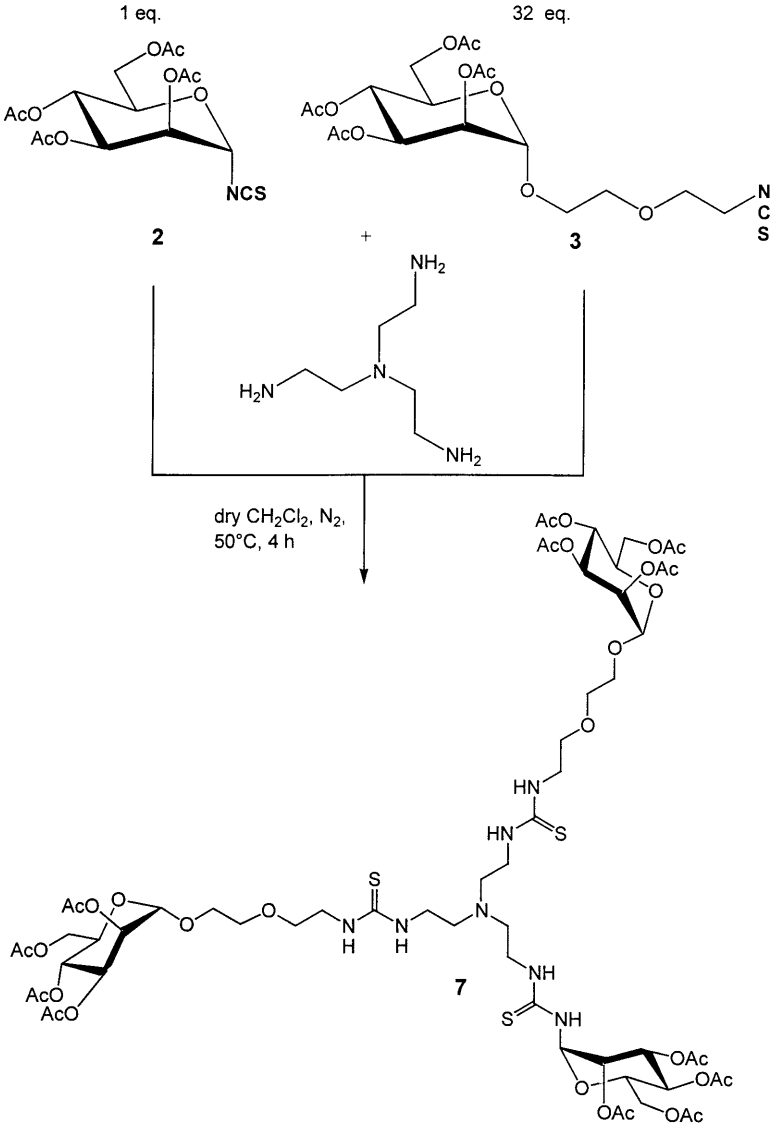

Fig. 5. Preparation of the defined mixed glycocluster $[(\mathbf{2})_1(\mathbf{3})_2]$ (**7**)

triamine to be reacted with the most reactive isothiocyanate **2** and the least reactive derivative **3** in one pot (Fig. 5). A molecule of the composition $[(\mathbf{2})_1(\mathbf{3})_2]$ (**7**) was selected as the target glycocluster. As **2** was shown to be 16 times more reactive than **3**, one equivalent of **2** and 32 equivalents of **3** were therefore reacted with one equivalent of the core molecule. The obtained product mixture was analyzed by MALDI-TOF mass spectrometry, revealing three different mass peaks at $m/z = 1401.44$ (corresponding to $[(\mathbf{2})_2(\mathbf{3})_1]$), $m/z = 1489.49$ (corresponding to the desired cluster $[(\mathbf{2})_1(\mathbf{3})_2]$), and $m/z = 1577.54$ (corresponding to $(\mathbf{3})_3$). The mass peak for the target cluster was clearly predominating over the others. Even though integration of MALDI-TOF peaks cannot necessarily form the basis of a

quantitative analysis, there is not doubt that the target glycocluster **7** was formed as the main product in this experiment.

In conclusion, we have determined the relative reactivities of six structurally different sugar isothiocyanates in a thiourea-bridging reaction using an aliphatic diamine as the core molecule. The determined reactivities can form the basis of a controlled synthesis of mixed glycoclusters as shown on the example of **7**.

Experimental

Melting points were determined in capillary tubes in an Apotec apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter (20°C, 589 nm, length of cuvette: 1 dm). Reactions were monitored by TLC on silica gel GF_{254} (Merck) with detection under UV and by charring with 10% H_2SO_4 in EtOH and subsequent heating. Flash column chromatography was performed on silica gel 60 (40–63 μm, Merck). NMR spectra were recorded on Bruker AMX 400 or Bruker DRX 500 instruments. Chemical shifts are given relative to the solvent peaks of $CDCl_3$ (7.24 ppm for ^{1}H, 77.0 ppm for ^{13}C) or acetone-D_6 (2.04 ppm for ^{1}H, 29.3 ppm for ^{13}C). Where necessary, assignments were based on COSY experiments. IR spectra were taken with an ATI Mattson instrument, Genesis Series (KBr). MALDI-TOF mass spectra were measured with a Bruker Biflex III with 19 kV acceleration voltage. DHB ($c = 10\,\mu g/mm^3$ in 40% CH_3CN/H_2O) was used as the matrix. Ionization was effected with a nitrogen laser at 337 nm.

4-Isothiocyanatobenzyl 2,3,4,6-tetra-O-acetyl-mannopyranoside (**5**; $C_{22}H_{25}NO_{10}S$)

To a solution of 1-O-(2,3,4,6-tetra-O-acetyl-α-*D*-mannopyranosyl)-trichloroacetimidate (2.8 g, 5.85 mmol) and 4-isothiocyanatobenzylalcohol (1.0 g, 6.14 mmol) in 25 cm^3 dry CH_2Cl_2, two drops of *TMS-OTf* were added under an Ar atmosphere. The reaction mixture was then stirred for two days at room temperature. The reaction was stopped by addition of 1 cm^3 triethylamine. After evaporation of the solvent, the crude product was purified by flash column chromatography to give an amorphous yellowish solid.

Yield: 43%; IR (KBr): $\nu_{NCS} = 2123\,cm^{-1}$; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.30$ (d, 2H, H_{ar}), 7.20 (d, 2H, H_{ar}), 5.33 (dd, 1H, H-3), 5.27 (t, 1H, H-4), 5.24 (dd, 1H, H-2), 4.83 (d, 1H, H-1), 4.67 (d, 1H, benzyl-C*H*H′), 4.51 (d, 1H, benzyl-CH*H*′), 4.25 (dd, 1H, H-6), 4.04 (dd, 1H, H-6′), 3.95 (ddd, 1H, H-5), 2.11, 2.08, 2.01, 1.96 (each s, each 3H, 4 OAc) ppm; $^3J_{1,2} = 1.5$, $^3J_{2,3} = 3.6$, $^3J_{3,4} = 9.6$, $^3J_{4,5} = 9.6$, $^3J_{5,6} = 5.1$, $^3J_{5,6'} = 2.5$, $^2J_{6,6'} = 12.2$ Hz; ^{13}C NMR (100.61 MHz, $CDCl_3$): $\delta = 170.54$, 169.97, 169.88, 169.65 (C=O), 135.78 (NCS), 135.47 (OCH_2C_{ar}), 131.09 (N–C_{ar}), 129.13 ($OCH_2C_{ar}(C_{ar})_2$), 125.87 (N–$C_{ar}(C_{ar})_2$), 96.73 (C-1), 69.42, 68.96, 68.77, 66.02 (C-2, C-3, C-4, C-5), 68.81 (CH_2), 62.36 (C-6), 20.81, 20.70, 20.62 (4 C(O)CH_3) ppm.

General procedure for the preparation of thiourea-bridged homodimers (**1a**–**6a**)

A solution of 2.2 equivalents of the sugar isothiocyanate in 10 cm^3 dry CH_2Cl_2 was heated to reflux temperature under a N_2 atmosphere. Then, a solution of 1,8-diamino-3,6-oxaoctane (1 equivalent in the 500-fold volume of dry CH_2Cl_2) was added dropwise. The course of the reaction was monitored by TLC (toluene/ethylacetate mixtures). After the reaction was complete (2–6 h) the solvent was removed *in vacuo* and the crude product was purified by flash column chromatography.

1a ($C_{36}H_{54}N_4O_{20}S_2$): ^{1}H NMR (500 MHz, acetone-d_6): $\delta = 7.45$ (s, 2H, sugar-NH), 7.33 (s, 2H, NH), 5.87 (s, 2H, H-1), 5.35 (t, 2H, H-3), 5.06–4.92 (m, 4H, H-2, H-4), 4.25 (d, 2H, H-6), 4.05 (dd, 2H, H-6′), 3.97 (bd, 2H, H-5), 3.83–3.66 (bd, 4H, HN–CH_2), 3.66–3.52 (bm, 8H, $HNCH_2CH_2OCH_2$, $HNCH_2CH_2$), 2.00, 1.99, 1.98, 1.94 (each s, each 6H, 8 OAc) ppm; $^3J_{2,3} = 9.8$, $^3J_{3,4} = 9.8$, $^3J_{4,5} = 9.8$, $^3J_{5,6'} = 2.2$, $^2J_{6,6'} = 12.3$ Hz; ^{13}C NMR (125.76 MHz, acetone-d_6): $\delta = 184.69$ (C=S), 170.18, 170.10, 169.62, 169.54 (C=O), 82.48 (C-1), 73.38, 73.29, 71.09, 68.70 (C-2, C-3, C-4, C-5),

70.30 ($HNCH_2CH_2O$), 69.16 ($HNCH_2CH_2OCH_2$), 62.09 (C-6), 44.75 ($HNCH_2$), 20.15, 20.10, 20.06, 19.99 (8 C(O)CH_3) ppm; MALDI-TOF-MS: $m/z = 927.35$ $[M+H]^+$ (calcd.: 927.29), 949.47 $[M+Na]^+$ (calcd.: 949.27), 965.47 $[M+K]^+$ (calcd.: 965.24).

2a ($C_{36}H_{54}N_4O_{20}S_2$): ^{1}H NMR (500 MHz, acetone-d_6): $\delta = 8.06$ (d, 2H, sugar-NH), 7.34 (s, 2H, NH), 5.99 (s, 2H, H-1), 5.36–5.30 (m, 4H, H-2, H-3), 5.24 (t, 2H, H-4), 4.27 (dd, 2H, H-6), 4.08 (dd, 2H, H-6′), 4.00 (bd, 2H, H-5), 3.84–3.69 (bs, 4H, $HNCH_2$), 3.69–3.56 (bm, 8H, $HNCH_2CH_2OCH_2$, $HNCH_2CH_2$), 2.12, 2.02, 2.01, 1.96 (s, each 6H, 8 OAc) ppm; $^3J_{2,3} = 3.2$, $^3J_{3,4} = 9.2$, $^3J_{4,5} = 9.2$, $^3J_{5,6} = 5.1$, $^3J_{5,6'} = 2.5$, $^2J_{6,6'} = 12.0$ Hz; ^{13}C NMR (125.76 MHz, acetone-d_6): $\delta = 184.36$ (C=S), 170.30, 169.81, 169.73, 169.55 (C=O), 80.45 (C-1), 70.36 ($HNCH_2CH_2O$), 70.11, 69.57, 69.21, 66.81, (C-2, C-3, C-4, C-5), 69.21 ($HNCH_2CH_2OCH_2$), 62.56 (C-6), 44.88 ($HNCH_2$), 20.20, 20.14, 20.05 (8 C(O)CH_3) ppm; MALDI-TOF-MS: $m/z = 927.29$ $[M+H]^+$ (calcd.: 927.29), 949.34 $[M+Na]^+$ (calcd.: 949.27), 965.31 $[M+K]^+$ (calcd.: 965.24).

3a ($C_{44}H_{70}N_4O_{20}S_2$): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.91$ (bs, 4H, NH), 5.32–5.18 (m, 6H H-2, H-3, H-4), 4.88 (d, 2H, H-1), 4.24 (dd, 2H, H-6), 4.09 (dd, 2H, H-6′), 4.01 (ddd, 2H, H-5), 3.82–3.51 (m, 28H, spacer-CH_2), 2.13, 2.07, 2.02, 1.96 (s, each 6H, 8 OAc) ppm; $^3J_{1,2} = 1.5$, $^3J_{2,3} = 3.1$, $^3J_{3,4} = 10.2$, $^3J_{4,5} = 10.2$, $^3J_{5,6} = 5.1$, $^3J_{5,6'} = 2.6$, $^2J_{6,6'} = 12.2$ Hz; ^{13}C NMR (100.61 MHz, $CDCl_3$): $\delta = 170.73$, 170.39, 170.04, 169.73 (C=O), 97.49 (C-1), 70.00, 69.91, 69.82 (spacer-OCH_2), 69.66, 69.00, 68.43, 66.11 (C-2, C-3, C-4, C-5), 67.02 (sugar-OCH_2), 62.48 (C-6), 44.34 ($CH_2NHCSNHCH_2$), 20.93, 20.74, 20.69 (8 C(O)CH_3) ppm; MALDI-TOF-MS: $m/z = 1103.31$ $[M+H]^+$ (calcd.: 1103.39), 1125.35 $[M+Na]^+$ (calcd.: 1125.37), 1141.34 $[M+K]^+$ (calcd.: 1141.35).

4a ($C_{34}H_{54}N_4O_{18}S_2$): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.30$ (dd, 2H, H-3), 5.19 (dd, 2H, H-2), 5.13 (t, 2H, H-4), 4.65 (d, 2H, H-1), 4.08 (bd, 2H, H-6), 4.00 (bd, 2H, H-6′), 3.92 (ddd, 2H, H-5), 3.66–3.61 (bq, 4H, $HNCH_2$), 3.61–3.47 (bm, 8H, $HNCH_2CH_2OCH_2$, $HNCH_2CH_2$), 3.36 (s, 6H, OCH_3), 2.12, 2.06, 1.96 (s, each 6H, 6 OAc) ppm; $^3J_{1,2} = 1.5$, $^3J_{2,3} = 3.6$, $^3J_{3,4} = 9.7$, $^3J_{4,5} = 9.7$, $^3J_{5,6} = 6.6$, $^3J_{5,6'} = 2.0$ Hz; ^{13}C NMR (100.61 MHz, $CDCl_3$): $\delta = 170.28$, 170.05, 169.83 (C=O), 98.37 (C-1), 69.95 ($HNCH_2CH_2O$), 69.87 ($HNCH_2CH_2OCH_2$), 69.59, 69.13, 68.79, 67.15 (C-2, C-3, C-4, C-5), 55.33 (OCH_3), 45.33 (C-6), 44.37 ($HNCH_2$), 20.88, 20.83, 20.66 (6 C(O)CH_3) ppm; MALDI-TOF-MS: $m/z = 871.10$ $[M+H]^+$ (calcd.: 871.30), 893.13 $[M+Na]^+$ (calcd.: 893.28), 909.10 $[M+K]^+$ (calcd.: 909.25).

5a ($C_{50}H_{66}N_4O_{22}S_2$): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.33$ (d, 4H, H_{ar}), 7.14 (d, 4H, H_{ar}), 5.33 (dd, 2H, H-3), 5.29–5.24 (m, 4H, H-2, H-4), 4.88 (d, 2H, H-1), 4.69 (d, 2H, benzyl-CHH′), 4.51 (d, 2H, benzyl-CHH′), 4.26 (dd, 2H, H-6), 4.08 (dd, 2H, H-6′), 4.00 (ddd, 2H, H-5), 3.82–3.68 (bs, 4H, $HNCH_2$), 3.66–3.52 (m, 8H, $HNCH_2CH_2O$, $HNCH_2CH_2OCH_2$), 2.12, 2.09, 2.01, 1.96 (each s, each 6H, 8 OAc) ppm; $^3J_{1,2} = 1.5$, $^3J_{2,3} = 3.5$, $^3J_{3,4} = 10.1$, $^3J_{4,5} = 10.1$, $^3J_{5,6} = 5.1$, $^2J_{5,6'} = 2.2$ Hz; ^{13}C NMR (125.76 MHz, $CDCl_3$): $\delta = 170.59$, 170.04, 169.94, 169.64 (C=O), 129.39 ($C_{ar}N'H$), 128.98 ($C_{ar}CH_2O$), 128.17 ($C_{a,a'}C_{ar}CH_2O$), 124.41 ($C_{x,x'}C_{ar}NH$), 96.82 (C-1), 70.17 ($HNCH_2CH_2O$), 69.48 ($HNCH_2CH_2OCH_2$), 69.10 ($C_{ar}CH_2$), 69.48, 69.00, 68.73, 66.08 (C-2, C-3, C-4, C-5), 62.42 (C-6), 44.93 ($HNCH_2$), 20.84, 20.73, 20.65 (8 C(O)CH_3) ppm; MALDI-TOF-MS: $m/z = 1139.11$ $[M+H]^+$ (calcd.: 1139.37), 1161.18 $[M+Na]^+$ (calcd.: 1161.35), 1177.14 $[M+K]^+$ (calcd.: 1177.32).

6a ($C_{48}H_{62}N_4O_{22}S_2$): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.12$ (s, 2H, ar-NH), 6.40 (s, 2H, NH), 7.12 (d, 4H, H_{ar}), 7.05 (d, 4H, H_{ar}), 5.52–5.46 (m, 4H, H-1, H-3), 5.38 (dd, 2H, H-2), 5.33 (t, 2H, H-4), 4.22 (dd, 2H, H-6), 4.07–3.99 (m, 4H, H-5, H-6′), 3.71 (bs, 4H, $HNCH_2$), 3.55 (bs, 4H, $HNCH_2CH_2OCH_2$), 3.50 (bs, 4H, $HNCH_2CH_2O$), 2.15, 2.01, 1.99, 1.98 (each s, each 6H, 8 OAc) ppm; $^3J_{3,4} = 9.8$, $^3J_{4,5} = 9.8$, $^3J_{5,6} = 5.4$, $^2J_{6,6'} = 12.6$ Hz; ^{13}C NMR (100.61 MHz, $CDCl_3$): $\delta = 180.81$ (C=S), 170.37, 169.90, 169.83, 169.52 (C=O), 153.95 (C_{ar}–O), 131.36 (C_{ar}–NH), 126.53 (HN–$C_{ar}C_{x,x'}$), 117.45 (O–$C_{ar}C_{a,a'}$), 95.79 (C-1), 70.02 ($HNCH_2CH_2O$), 69.20 ($HNCH_2CH_2OCH_2$), 69.12, 69.08, 68.63, 65.61 (C-2, C-3, C-4, C-5), 61.87 (C-6), 44.69 ($HNCH_2$), 20.73, 20.53 (8 C(O)CH_3) ppm; MALDI-TOF-MS: $m/z = 1111.25$ $[M+H]^+$ (calcd.: 1111.34), 1133.09 $[M+Na]^+$ (calcd.: 1133.32), 1149.01 $[M+K]^+$ (calcd.: 1149.29).

General procedure for the competition experiments

Under a N_2 atmosphere, 2 equivalents of each of the two competing sugar isothiocyanates were dissolved in 10 cm^3 dry CH_2Cl_2 at reflux temperature. Then, a solution of 1,8-diamino-3,6-oxaoctane (1 equivalent in a 500-fold volume of dry CH_2Cl_2) was added dropwise. Progress of the reaction was monitored by TLC (toluene/ethylacetate mixtures). After the reaction was complete (2–6 h) the solvent was removed *in vacuo* and the crude product was purified by flash column chromatography.

Mixed glycocluster (**7**; $C_{59}H_{91}N_7O_{31}S_3$)

Under a N_2 atmosphere, **2** (28 mg, 0.07 mmol, 0.33 equiv. per amino group) and **3** (1.06 g, 2.20 mmol, 16 equiv. per amino group) were dissolved in 15 cm^3 dry CH_2Cl_2. To this solution, *tris*-(2-aminoethyl)-amine (10 mg, 0.07 mmol, dissolved in 0.5 cm^3 of dry CH_2Cl_2) was added dropwise. This mixture was then heated under reflux for 3 h. After the removal of the solvent, the excess educts were separated from the products by flash column chromatography. The product mixture was then analyzed by MALDI-TOF-MS.

MALDI-TOF-MS: $m/z = 1402.75$ ($[(\mathbf{2})_2(\mathbf{3})_1\text{-cluster} + H]^+$, 30%, calcd.: 1402.45 for $C_{55}H_{83}N_7O_{29}S_3$ ($M = 1401.44$)), 1490.86 ($[(\mathbf{2})_1(\mathbf{3})_2\text{-cluster} + H]^+$, 57%, calcd.: 1490.50 for $C_{59}H_{91}N_7O_{31}S_3$ ($M = 1489.49$)), 1578.98 ($[(\mathbf{3})_3\text{-cluster} + H]^+$, 13%, calcd.: 1578.55 for $C_{53}H_{99}N_7O_{33}S_3$ ($M = 1577.54$)).

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