Dendritic Galactosides Based on a β-Cyclodextrin Core for the Construction of Site-Specific Molecular Delivery Systems: Synthesis and Molecular Recognition Studies

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Abstract: In order to evaluate the ability of multivalent glycosides based on a β-cyclodextrin core as site-specific molecular carriers, a study on both the inclusion complexation behaviour and lectin binding affinity of branched and hyperbranched β-cyclodextrins is presented. A series of cluster galactosides constructed on β-cyclodextrin scaffolds containing seven 1-thio-β-lactose or β-lactosylamine bound to the macrocyclic core through different spacer arms were synthesised. In addition, the first synthesis of three first-order dendrimers based on a β-cyclodextrin core containing fourteen 1-thio-β-D-galactose, 1-thio-β-lactose and 1-thio-β-melibiose residues was performed. Calorimetric titrations performed at 25 °C in buffered aqueous solution (pH 7.4) gave the affinity constants and the thermodynamic parameters for the complex formation of these β-cyclodextrin derivatives with guests sodium 8-anilino-1-naphthalenesulfonate (ANS) and 2-naphthalenesulfonate, and lectin from peanut (*Arachis hypogaea*) (PNA). The persubstitution of the primary face of the β-cyclodextrin with saccharides led to a slight increase of the binding constant values for the inclusion complexation with ANS relative to the native β-cyclodextrin. However, the increase of the steric congestion due to the presence of the saccharide residues on the narrow rim of the β-cyclodextrin may cause a decrease of the binding ability as shown for sodium 2-naphthalenesulfonate. The spacer arms are not passive elements and influence the host binding ability according to their chemical nature. PNA forms soluble cross-linked complexes with cluster galactosides and lactosides scaffolded on β-cyclodextrin but not with cluster galactopyranosylamines or melibiose. Both, perbranched and hyperbranched β-cyclodextrins, form stronger complexes with PNA than the monomeric analogues. However, the use of hyperbranched CDs does not contribute to the improvement of the complex stability relative to heptakis-glycocyclodextrin derivatives. Finally, a titration experiment with PNA and a complex formed by a heptakis lactose β-cyclodextrin derivative with sodium 2-naphthalenesulfonate showed the formation of a soluble cross-linked complex with stronger affinity constant and higher stoichiometry than those observed for the complex formation of PNA with the same heptakis-lactose β-cyclodextrin derivative, suggesting the formation of a three component complex.

Keywords: calorimetry · cluster compounds · cyclodextrins · host–guest systems · molecular recognition

Introduction

Cyclodextrin (CD) derivatives bearing saccharides may be useful as carriers for transporting drugs to membrane-containing specific sugar receptors.[1] In addition, the well-defined torus-shaped structures of CDs provide a versatile scaffold for the construction of branched structures of bioactive molecules such as glycosides. This latter feature would allow the use of the so-called cluster effect[2] as a means to increase the protein–saccharide binding[3] and therefore could improve the effectiveness of the drug delivery system. In this context, we have reported[4] the synthesis of a variety of persubstituted β-CD derivatives branched with O-, S-glycosides and glycopyranosylamines with, in some cases, enhanced

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lectin binding affinity. Other groups have recently reported alternative strategies for the synthesis of glyco-β-CDs per-substituted onto the primary face,[5] secondary face and both primary and secondary faces simultaneously.[6] Working on the same concept, other research groups have reported the synthesis of cluster glycosides based on different cores such as calixarenes[7] and calix[4]resorcarenes.[8] In particular, Aoyama and co-workers[8] have demonstrated that the latter type of compounds can deliver guest molecules to polar solid surfaces such as quartz, but also to biological targets such as lectins.

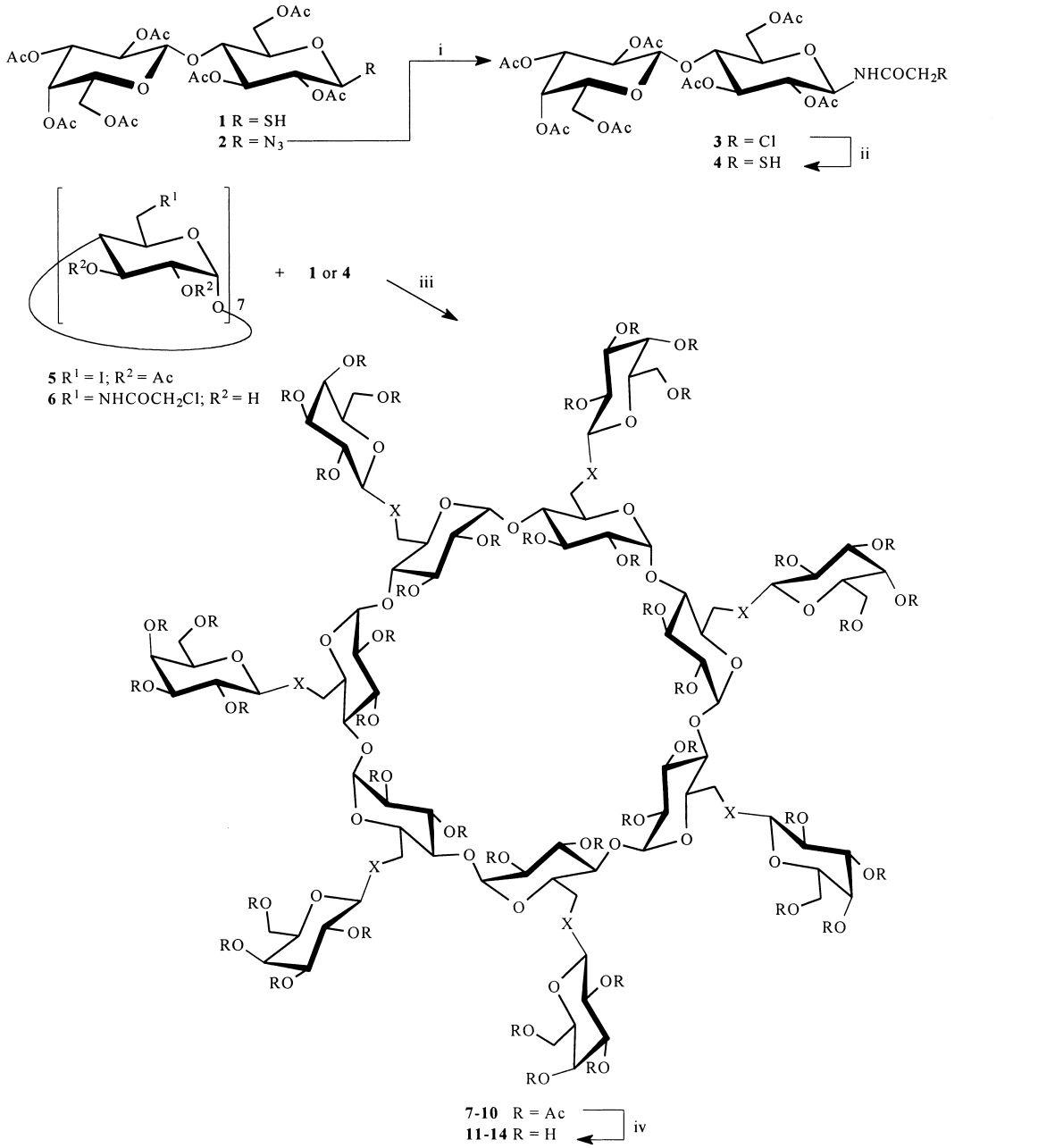
The use of CDs as a scaffold of cluster glycosides offers several advantages over other macrocyclic compounds: It is more readily available and affordable, more biocompatible, and has the ability to form inclusion compounds with a large variety of guests in aqueous solution.[9]

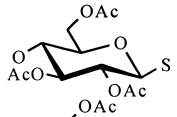
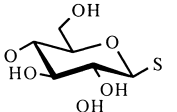
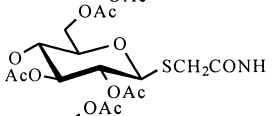
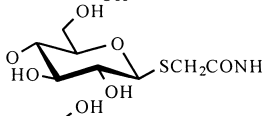
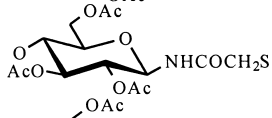
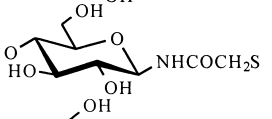
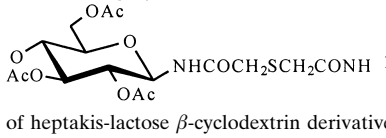
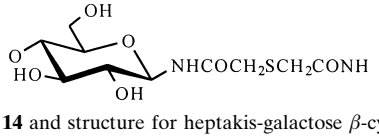
In this paper, we describe the synthesis of cluster galactosides constructed on β-CD scaffolds containing seven β-lactoses through different spacer arms, as well as three first-order dendrimers, also based on a β-CD core, containing fourteen β-D-galactoses, β-lactoses and β-melibioses. In order to evaluate the potential of these multivalent macrocyclic structures as site-specific molecular carriers we studied both their affinities for a biological target and the inclusion complexation behaviour with some representative guest molecules by using microcalorimetric titrations. To our knowledge, this is the first time that a calorimetric study has been carried out on this kind of glycosyl β-CDs. As a biological target the plant lectin from peanut (*Arachis hypogaea*) was chosen. Peanut lectin (PNA) is a homotetrameric protein with a molecular weight of 110 kDa that has one saccharide-binding site per subunit and binds with high affinity D-galactosyl residues through specific binding interactions.[10]

Abstract in Spanish: *Con el objetivo de evaluar la capacidad de glicósidos multivalentes, basados sobre núcleos de β-ciclodextrinas, como transportadores moleculares con especificidad por el sitio de unión, se presenta un estudio del comportamiento complejante y de la afinidad por lectinas de β-ciclodextrinas ramificadas e hiperramificadas. Así, se han sintetizado una serie de clusters de galactósidos construidos sobre una base de β-ciclodextrina conteniendo siete residuos de 1-tio-β-lactosa o β-lactosilamina unidos al núcleo macrocíclico a través de diferentes brazos espaciadores. Asimismo, se han realizado las primeras síntesis de tres dendrímeros de primer orden basados en núcleos de β-ciclodextrinas con catorce residuos de 1-tio-β-D-galactosa, 1-tio-β-lactosa y 1-tio-β-melibiosa. Se han realizado valoraciones calorimétricas a 25 °C en disolución acuosa tamponada (pH 7.4) para obtener las constantes de afinidad y los parámetros termodinámicos de los complejos formados por los derivados de β-ciclodextrinas con los huéspedes 8-anilino-1-naftalensulfonato (ANS) y 2-naftalensulfonato sódico y la lectina del cacahuete (Arachis hypogaea) (PNA). La persubstitución de la cara primaria de la β-ciclodextrina con sacáridos, produjo un ligero incremento de los valores de las constantes de afinidad de los complejos de inclusión formados con ANS con respecto a la β-ciclodextrina nativa. Sin embargo, el incremento de la congestión estérica debida a la presencia de los residuos sacarídicos en el lado estrecho de la β-ciclodextrina puede causar una disminución de la capacidad de unión con la molécula huésped, como se observó en el caso del 2-naftalenosulfonato sódico. Los brazos espaciadores no son elementos pasivos e influyen en la capacidad de unión del anfitrión en función de su naturaleza química. La PNA forma complejos entrecruzados solubles con los clusters de galactósidos y lactósidos sobre anillos de β-ciclodextrina pero no con los clusters de galactopiranosilaminas o melibiosa. Tanto las β-ciclodextrinas ramificadas como las hiperramificadas forman complejos más fuertes con PNA que los análogos monoméricos. No obstante, el uso de β-ciclodextrinas hiperramificadas no mejora la estabilidad de los complejos con relación al uso de los derivados de heptakis-glicociclodextrinas. Finalmente, un experimento de valoración realizado con PNA y un complejo formado por un derivado de heptakis-lactosa-β-ciclodextrina con el 2-naftalenosulfonato sódico permitió observar la formación de un complejo soluble entrecruzado con una constante de afinidad y estequiometría mayores que las observadas en la formación del complejo de PNA con el mismo derivado de heptakis-lactosa-β-ciclodextrina, sugiriendo la formación de un complejo con tres componentes.*

Results and Discussion

Synthesis: We first carried out the synthesis of perlactosylated β-CDs in which the anomeric position of the D-glucose unit of the lactosyl moiety is scaffolded onto the primary face of the CD core through a direct bond as well as through different spacer arms. Nucleophilic displacement of per-6-halo-6-deoxy-β-CDs by sugar-containing thiol derivatives has been shown to be a very useful strategy for the attachment of sugar units onto CDs.[1b, 4] Thus, compounds **1**[11a] and **4** were used as nucleophile precursors which in combination with CD electrophiles **5** and **6** will allow an easy access to the series of heptavalent lactosyl CDs **11**–**14** (Scheme 1). First, the lactosyl azide derivative **2**[11b] was sequentially treated with 1,3-propanedithiol and triethylamine in anhydrous methanol, followed by treatment with chloroacetic anhydride; this affords glycosyl amide **3** in 58% yield in a one-pot reaction. Alternatively, compound **2** was transformed into **3**, also in one-pot reaction, in a Staudinger reaction by sequential treatment with nBu$_3$P at room temperature and chloroacetic anhydride at −80 °C in dry CH_2Cl_2, isolating the amide **3** in 88% yield, after chromatographic purification.[4b, 12] The *N*-chloroacetyl lactosylamine **3** was then treated with thiourea in dry acetone followed by addition of aqueous Na_2SO_3 to give the thiol **4** in 80% yield. Coupling of the lactosyl thiols **1** and **4** with β-CD derivatives **5**[13] and **6**[4a] in the presence of Cs_2CO_3 for seven days, followed by addition of acetic anhydride, pyridine and *N*,*N*′-dimethylaminopyridine (DMAP) afforded the branched β-CDs **7**–**10** in 76–86% yields. Zemplén de-O-acetylation of compounds **7**–**10** furnished the β-CD derivatives branched with seven β-lactosyl residues through sulphur



R	X	R	X	R	X
7 Ac		**11** H		**35** H	S
8 Ac		**12** H		**36** H	SCH_2CONH
9 Ac		**13** H		**37** H	$NHCOCH_2S$
10 Ac		**14** H		**38** H	$NHCOCH_2SCH_2CONH$

Scheme 1. Synthesis of heptakis-lactose β-cyclodextrin derivatives **11**–**14** and structure for heptakis-galactose β-cyclodextrin derivatives **35**–**38**. i) Bu_3P/CH_2Cl_2, 1 h, RT, then $(ClCH_2CO)_2O/CH_2Cl_2$, $-80\,^{\circ}C \rightarrow$ RT: **3** (88%); or Et_3N/1,3-propanedithiol/MeOH, 3 h, then $(ClCH_2CO)_2O$, 6 h, RT: **3** (58%); ii) a) $(NH_2)_2CS/(CH_3)_2CO$, 12 h, RT; b) Na_2SO_3/H_2O, 30 min, RT: **4** (80%); iii) a) Cs_2CO_3/DMF, 7 d, 60 °C; b) Ac_2O/py, 48 h, 40 °C: **7** (79%), **8** (81%), **9** (86%), **10** (76%); iv) NaOMe/MeOH, 12 h, RT: **11** (95%), **12** (96%), **13** (98%), **14** (98%).

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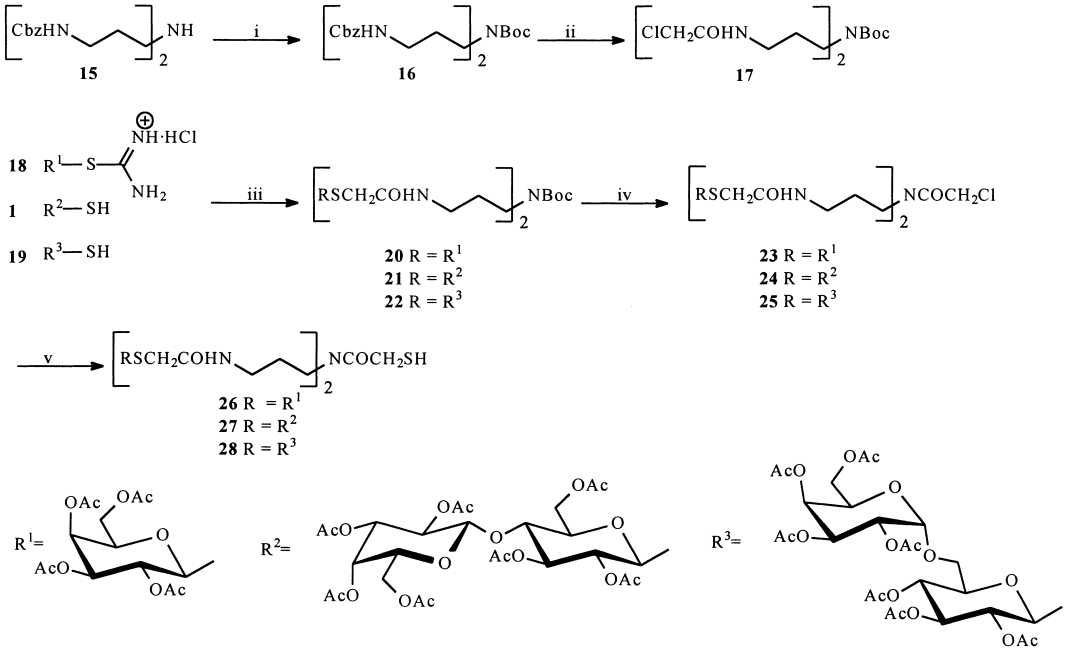
and through the spacer chains SCH_2CONH, $NHCOCH_2S$ and $NHCOCH_2SCH_2CONH$ **11**–**14** in 79–98% yields.

The synthesis of the hyperbranched β-CDs started with the treatment of 3,3′-bis(*N*,*N*′-benzyloxycarbonyl)-3,3′-bis(propylamine)imine (**15**)[3a] with di-*tert*-butyl dicarbonate to provide the *N*-Boc derivative **16** in 99% yield (Scheme 2). Hydrogenolysis of the benzyloxycarbonyl groups of **16**, followed by treatment with chloroacetic anhydride and triethylamine, yielded the *N*-chloroacetylated derivative **17**. Coupling of the galactopyranoside, lactose and melibiose residues was performed by reaction of **17** with the isothiouronium salt **18**,[11c] and thiols **1**[1a] and **19**, respectively. The divalent saccharides **20**–**22** were obtained in 83%, 91% and 67% yield, respectively, when Cs_2CO_3 was employed in dimethylformamide (DMF) at room temperature. Trifluoroacetolysis of **20**–**22**, followed by *N*-chloroacetylation afforded **23**–**25** in 92–94% yield. Subsequent treatment of the *N*-chloroacetyl derivatives **23**–**25** with thiourea and aqueous Na_2SO_3 gave the thiols **26**–**28** (56%, 55% and 75% yield, respectively).

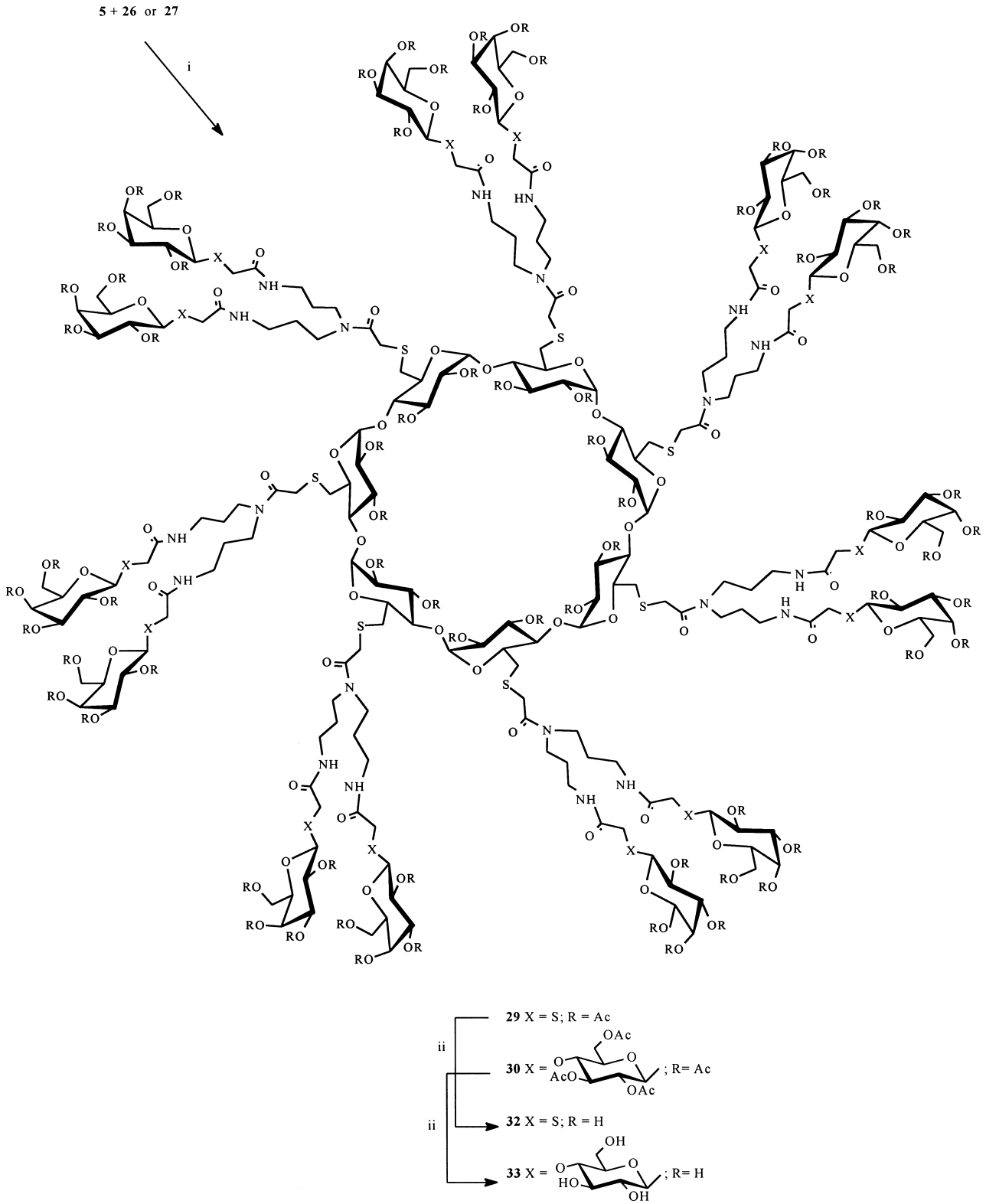
Reaction of compounds **26**–**28** with per-6-deoxy-6-iodo-β-CD (**5**) was carried out at 60 °C in dry DMF under Ar atmosphere using Cs_2CO_3 in order to generate in situ the cesium thiolate derivatives (Schemes 3 and 4). After seven days, acetic anhydride, pyridine and DMAP were added. Peracetylation reaction was kept at 40 °C for 48 h and then the per-O-acetylated hyperbranched glyco-CDs **29**–**31** were isolated in high yields (79%, 80% and 92% yield, respectively). Removal of the acetyl groups of **29**–**31** under Zemplén conditions furnished the tetradecavalent glycodendrimers **32**–**34** based on a β-CD core in 84–98% yields.

Branched and hyperbranched β-CDs **7**–**14** and **29**–**34** were characterised by NMR spectroscopic techniques with COSY, HMQC and selective TOCSY experiments and MALDI-TOF mass spectrometry. Measurements of the NMR data were performed at 80–100 °C to avoid broadening of the signals and to improve the resolution of the spectra. The NMR spectra show a single signal pattern for all saccharide residues. The ^{13}C NMR spectra show two anomeric carbon signals at $\delta = 96.3$ and 102.5 (C-1), and 82.0 and 85.4 (C-1′) for compounds **29** and **32**, respectively, and three anomeric signals at $\delta = 96.1$–102.1 (C-1), 76.9–79.4 (C-1′) and 99.3–102.8 (C-1″) for compounds **9**, **10**, **13** and **14** and at $\delta = 95.9$–102.5 (C-1), 81.3–85.7 (C-1′) and 95.4–103.8 (C-1″) for compounds **7**, **8**, **11**, **12**, **30**, **31**, **33** and **34**. The ratios of the integrals for the signals of the saccharide residue protons and for the signals of those belonging to the CD core are in accordance with the structures of the products. A useful fact for the assignment of the ^{13}C NMR signals is the expected lower intensity of the signals corresponding to the CD moiety probably due in part to the lack of flexibility of the cyclodextrin torus.[14]

Guest binding ability: The inclusion complexation behaviour of these branched and hyperbranched CDs **11**–**14** and **32**–**34** as well as the reported[4] branched CDs **35**–**38** (Scheme 1) with the guests sodium 8-anilino-1-naphthalenesulfonate (ANS) (**39**) and sodium 2-naphthalenesulfonate (**40**) was studied by using isothermal titration calorimetry (ITC) (Table 1). The thermodynamic parameters for the complexation of compounds **39** and **40** with β-CD are reported[15] and were used as reference for comparison purpose with those ones obtained from the branched and hyperbranched CDs. ITC measurements provide direct determination of n, the stoichiometry, ΔH°, the enthalpy change of binding, and K, the affinity constant. From measurements of K, the free energy of binding, ΔG°, can be calculated and hence the



Scheme 2. Synthesis of divalent glycoside building blocks **26**–**28**: i) $Et_3N/[(CH_3)_3OCO]_2O/CH_3CN$, 12 h, RT: **16** (99%); ii) a) Pd/C/MeOH, H_2 (2.5 atm), 1 h; b) $Et_3N/(ClCH_2CO)_2O/CH_3CN$, 24 h, RT: **17** (81%); iii) Cs_2CO_3/DMF, 24 h, RT: **20** (83%), **21** (91%), **22** (67%); iv) a) TFA/CH_2Cl_2, 3–6 h, 0 °C; DIPEA/$(ClCH_2CO)_2O/CH_3CN$, 7–8 h, RT: **23** (92%), **24** (99%), **25** (92%); v) a) $(NH_2)_2CS/(CH_3)_2CO$, 12 h, RT; b) Na_2SO_3/H_2O, 30 min, RT: **26** (56%), **27** (55%), **28** (75%).



Scheme 3. Synthesis of hyperbranched β-cyclodextrin derivatives **32** and **33**: i) a) Cs_2CO_3/DMF, 7 d, 60 °C; b) Ac_2O/Py, 48 h, 40 °C: **29** (79 %), **30** (80 %); ii) NaOMe/MeOH, 12 h, RT: **32** (84 %), **33** (86 %).

entropy of binding, ΔS°, determined from $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = -RT\ln K$ (standard state = 1 mol L^{-1}). The calculation of thermodynamic functions implies the usual approximation of setting standard enthalpies equal to the observed values. Stepwise addition of aliquots of guest-containing solution to a solution of branched CDs led to a decrease in the extent of released heat as shown by a typical illustration of the thermogram and isotherm (Figure 1).

In all cases, the best fit for the three variables, n, ΔH° and K was found for 1:1 stoichiometry in accordance with the most

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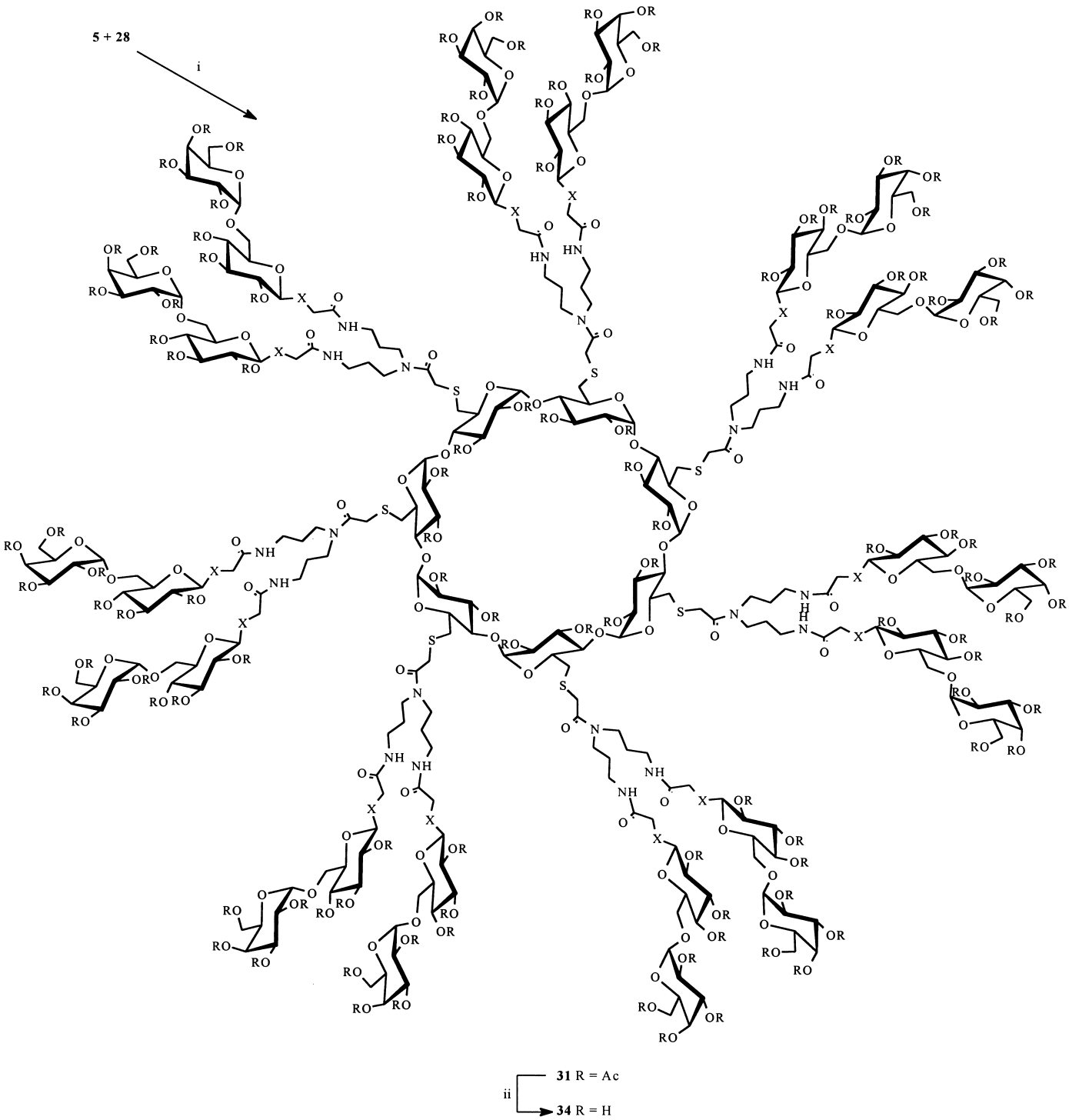

Scheme 4. Synthesis of melibiose β-cyclodextrin derivative **34**: i) a) Cs_2CO_3/DMF, 7 d, 60 °C; b) Ac_2O/Py, 48 h, 40 °C: **31** (92 %); ii) NaOMe/MeOH, 12 h, RT: **34** (98 %).

commonly claimed stoichiometry ratio for CD complexes.[16] The forces responsible for the formation of the CD complexes involve a number of different contributions such as steric fit, release of high-energy water, hydrophobic effects, dispersive forces, hydrogen bonds, and van der Waals, dipole–dipole, charge-transfer and electrostatic interactions, the importance of which is still a matter of some debate.[16] Previous thermodynamic studies of molecular recognition by mono-modified CDs have shown the influence of the relative size between the cavity of the CD and the guest molecule, as well as the shape, dipole, charge and functional group of the branch attached to the primary face edge of the CD in determing how the guest molecule fits into the host cavity.[15] In our case, the structure of the CD derivatives **11**–**14** and **32**–**38** is the result of the substitution the OH-6 of every single glucose unit by bulky groups separated from the CD cavity edge by spacer arms of variable length. Most likely, this β-CD persubstitution may affect the conformation of the oligosac-

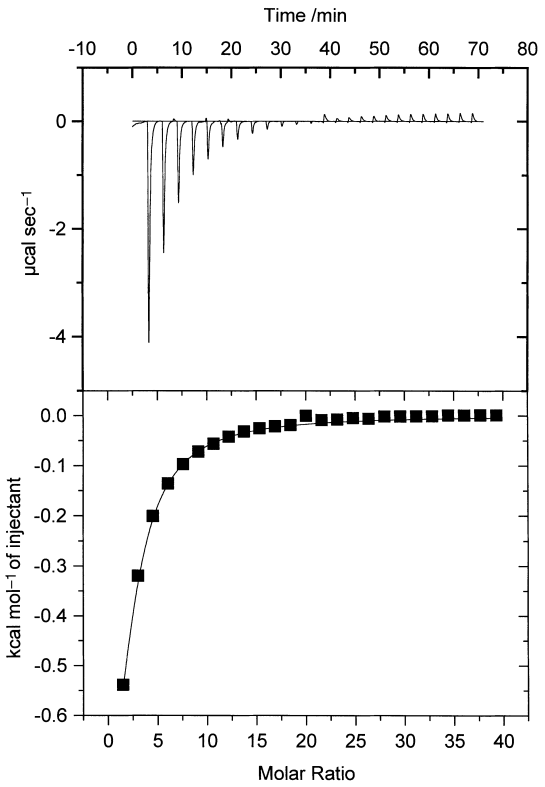

Figure 1. Titration of 0.105 mM of branched CD **13** with 25 aliquots (5 µL each) of sodium 2-naphthalenesulfonate (**40**) (stock concentration of 43.78 mM) in 10 mM phosphate buffer at pH 7.4 and 25 °C. The top panel shows the raw data, denoting the amount of generated heat (negative exothermic peaks) following each injection of guest. The area under each peak represents the amount of heat released upon the binding of sodium 2-naphthalenesulfonate to CD **13**. Note that, as the titration progresses, the area under the peaks progressively becomes smaller due to an increased occupancy of the CD by guest. The area under each peak was integrated and plotted against the molar ratio of guest to CD **13**. The smooth solid line represents the best fit of the experimental data to a model with 1:1 stoichiometry.

charide ring and therefore modify the overall shape of the CD cavity. In addition, the spacer arms can modify the CD primary face microenviromental hydrophobicity depending on their lipophilic pattern. Thus, it is expected that the presence of the amide group located just above the cavity may increase the host hydration when compared with presence of the sulfide function directly attached at C-6. As can be seen from Table 1 and Figure 2, when **39** was used as a guest, CDs **13** and **37** gave strongest inclusion complexes ($K = 408.5$ and

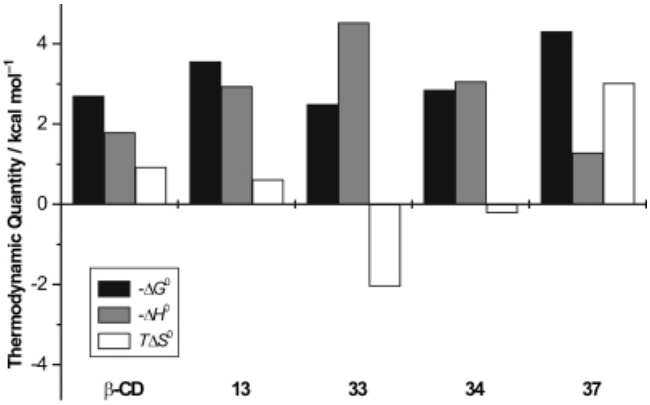

Figure 2. Free energy ($-\Delta G°$), enthalpy ($-\Delta H°$), and entropy changes ($T\Delta S°$) for the inclusion complexation of sodium 8-anilino-1-naphthalenesulfonate (**39**) with β-cyclodextrins and derivatives **13**, **33**, **34** and **37** in a buffered aqueous solution (pH 7.4) at 25 °C.

1321.0 M^{-1}, respectively) than the β-CD ($K \approx 115\,M^{-1}$), although the complexation with the former is enthalpy-driven and the complexation of the latter is entropy-driven. The only apparent differences between both host Lac-NHCOCH$_2$S-CD **13** and Gal-NHCOCH$_2$S-CD **37** are due to the respective lactosyl and galactosyl substituents which could contribute to differentiate both cavity shapes either because conforma-

Table 1. Thermodynamics of binding of guests **39** and **40** to CDs **11**–**14** and **34**, **36**–**38** in H_2O at 25 °C.[a]

Host	Guest	K [M^{-1}]	$-\Delta G°$ [kcal mol^{-1}]	$-\Delta H°$ [kcal mol^{-1}]	$T\Delta S°$ [kcal mol^{-1}]
***β*-CD**	**39**[b]	114.81	2.70	1.79	0.93
11	**39**	N.B.[c]	–	–	–
12	**39**	N.B.	–	–	–
13	**39**	408.50 ± 46.98	3.56 ± 0.07	2.94 ± 0.22	0.62 ± 0.23
14	**39**	N.B.	–	–	–
32	**39**	N.B.	–	–	–
33	**39**	66.69 ± 14.93	2.49 ± 0.13	4.53 ± 0.85	− 2.04 ± 0.86
34	**39**	122.50 ± 97.24	2.85 ± 0.47	3.06 ± 1.91	− 0.21 ± 1.97
37	**39**	1321.00 ± 166.10	4.31 ± 0.08	1.29 ± 0.09	3.02 ± 0.12
38	**39**	N.B.	–	–	–
***β*-CD**	**40**[d]	2.3x10^5	7.33	7.00	0.31
11	**40**	652.50 ± 18.99	3.85 ± 0.02	2.20 ± 0.04	1.65 ± 0.04
12	**40**	700.00 ± 33.00	3.88 ± 0.03	3.02 ± 0.08	0.84 ± 0.09
13	**40**	3274.00 ± 97.43	4.80 ± 0.02	2.78 ± 0.04	2.02 ± 0.04
14	**40**	380.70 ± 16.18	3.52 ± 0.03	2.63 ± 0.07	0.89 ± 0.07
32	**40**	N.B.	–	–	–
33	**40**	N.B.	–	–	–
34	**40**	N.B.	–	–	–
35	**40**	N.B.	–	–	–
36	**40**	534.30 ± 65.17	3.72 ± 0.07	3.73 ± 0.27	− 0.01 ± 0.28
37	**40**	3116.00 ± 135.2	4.77 ± 0.03	3.17 ± 0.05	1.60 ± 0.05
38	**40**	401.90 ± 20.75	3.55 ± 0.03	3.42 ± 0.11	0.13 ± 0.11

[a] Determined in buffered aqueous solution at pH 7.4 (10 mM sodium phosphate). [b] Ref. [15a]. [c] N.B. = no binding. [d] Ref. [15b].

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tional or ring flexibility changes or both. The enthalpic gain for Gal-NHCOCH$_2$S-CD **37** is of the same magnitude than for β-CD but smaller than for Lac-NHCOCH$_2$S-CD **13**. However, the entropic gain for **37** is higher than for **13** which is slightly smaller than for β-CD. The small negative value for ΔH° and the positive value for $T\Delta S^\circ$ in the case of Gal-NHCOCH$_2$S-CD **37** could be caused by a weak inclusion interaction and an extensive host and guest desolvation. By contrast, host Lac-NHCOCH$_2$S-CD **13** affords more negative ΔH° but much smaller value for $T\Delta S^\circ$, suggesting a stronger inclusion interaction but resulting in a less flexible structure as a result of a more rigid oligosaccharide ring of the host **13** as the observation of its ^{13}C NMR spectra suggests. In this respect, the steric congestion on the primary face of CD due to the attachment of fourteen branches at the CD lower rim led to almost negligible binding constants for **33** and **34**. Similarly, we did not detect the formation of inclusion complexes between compound **40** and CDs **32**–**34**. These steric factors could lead to a conformational distortion of the CD ring due to the rotation of one or more glucose units around the glycosidic linkages[17] and might reduce the secondary face average diameter of the cavity, thus preventing guest penetration. In addition, despite the lack of accuracy of the thermodynamic data for inclusion complex **33** and **34** with guest **39**, the negative values ΔH° and $T\Delta S^\circ$ jointly with what is observed from the ^{13}C NMR spectra for those compounds supports that the reduced flexibility of the macrocycle could contribute against the complex formation. Previous reports[15c–e] have shown that mono-modified β-CDs with functional groups attached to the edge of the cavity can undergo self-inclusion processes preventing the guest inclusion. In our case, NMR NOESY experiments gave spectra with not enough resolution for a reliable evaluation of a possible self-inclusion complex. However, one-dimensional ^{1}H NMR spectra of these compounds showed in all cases the seven-fold symmetry of the compounds and it did not show the expected peak splitting arising from symmetry breakdown due to self-inclusion.

The inclusion complexation of 2-naphthalenesulfonate (**40**) with β-CD is very strong. It is an enthalpy-driven complex with minimal entropic gain.[15b] As previously reported for mono modified CDs,[15c] Lac-CDs **11**–**14** and Gal-CDs **36**–**38** afforded substantially less stable complexes with the guest **40** than the native β-CD. As can be seen from Table 1 and Figure 3, all the complexes formed by host **11**–**14**, and **36**–**38** and guest **40** are exothermic and enthalpy-driven. When compared with the complexation of **40** and native β-CD, the persubstitution on the primary face of the CD with the galactosyl residues led to the reduction of the enthalpic gain up to 3.3–4.8 kcal mol^{-1} for **11**–**14**, and **36**–**38**, suggesting a less penetration of the naphthalene part of **40** into the CD cavity. However, the chemical nature of the spacer arm of the branched CDs seems to influence the binding inducing different variations of the $T\Delta S^\circ$ value. Thus, for Lac-S-CD **11**, Lac-NHCOCH$_2$S-CD **13** and Gal-NHCOCH$_2$S-CD **37**, having sulfur bound at C-6, the $T\Delta S^\circ$ value increases up to 1.3–1.7 kcal mol^{-1}, while for Lac-SCH$_2$CONH-CD **12**, Lac-NHCOCH$_2$SCH$_2$CONH-CD **14**, Gal-SCH$_2$NH-CD **36** and Gal-NHCOCH$_2$SCH$_2$CONH-CD **38**, having and amide nitrogen bound at C-6, the variation of the $T\Delta S^\circ$ is almost negligible (from -0.3 to 0.6 kcal mol^{-1}). This different entropic gain may be attributable to the more extensive desolvation of the host when with a more lipophilic environment just on the lower rim of the CD. The different length of the spacer arms also influences the binding affinity between host and guest. The heptakis-Lac-NHCOCH$_2$S-CD **13** forms a more stable inclusion complex with **40** than the heptakis-Lac-S-CD **11**, in which the glycosidic moiety is directly attached onto the CD core through sulfur. We did not detect inclusion complexation between CD Gal-S-CD **35**, the galactosyl analogue of **11** and **40**. However, by increasing the spacer arm length in compounds Lac-SCH$_2$CONH-CD **12** and Gal-SCH$_2$CONH-CD **36**, to give rise to branched Lac-NHCOCH$_2$SCH$_2$CONH-CD **14** and Gal-NHCOCH$_2$SCH$_2$-CONH-CD **38**, resulted in a lower binding with the guest **40**. The inclusion complex formation between host **13** and guest **40** was also confirmed by NMR experiments. Thus, upfield shifts of the host inner protons H-5 and H-3 from $\delta = 3.90$ and 3.80, respectively, to the spectral overlapping region of $\delta =$ 3.64–3.32 are observed, when the ^{1}H NMR spectrum of **13** is compared with that for **13** in the presence of a equimolar amount of **40**. A two-dimensional T-ROESY experiment showed cross peaks between the aromatic protons of **40** and CD protons from the above-mentioned spectral region of $\delta =$ 3.64–3.32, presumably due to dipolar contacts with protons H-3,5 of the cavity.

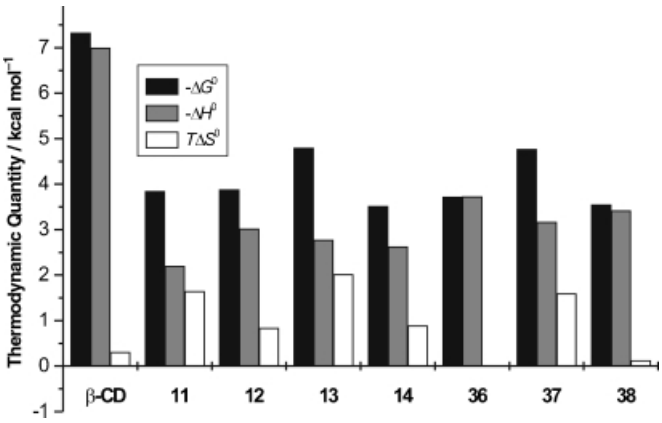

Figure 3. Free energy ($-\Delta G^\circ$), enthalpy ($-\Delta H^\circ$), and entropy changes ($T\Delta S^\circ$) for the inclusion complexation of sodium 2-naphthalenesulfonate (**40**) with β-cyclodextrins and derivatives **11**–**14**, **36**–**38** in a buffered aqueous solution (pH 7.4) at 25 °C.

PNA binding affinity: The binding affinity of branched and hyperbranched CDs **11**–**14** and **32**–**38** with PNA was also studied by using ITC. In these experiments soluble CD derivative is titrated into a solution containing binding lectin and the heat released or absorbed during binding is measured as a function of the [CD]/[PNA] molar ratio (Figure 4). ITC experiments showed that the binding interaction between PNA and CDs **11**–**14**, with seven 4-*O*-β-D-galactopyranosyl (or lactosyl) ligands, **36**, with seven *S*-D-galactopyranosyl ligands, **32**, with fourteen *S*-β-D-galactopyranosyl ligands, and **33**, with fourteen 4-*O*-β-D-galactopyranosyl (or lactosyl) ligands, was exothermic (Figure 4, Table 2). By contrast,

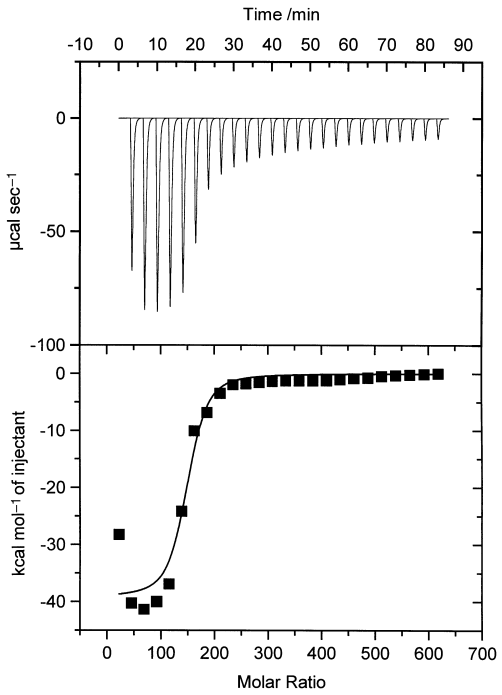

Figure 4. Titration of 2.5 µM of peanut lectin (PNA) with 25 aliquots (10 µL each) of **13** (7.83 mM) in 10 mM phosphate buffer at pH 7.4 and 25 °C. The top panel shows the raw calorimetric data, denoting the amount of heat released (negative exothermic peaks) following each injection of guest. The area under each peak represents the amount of heat released upon the binding of CD **13** to PNA. Note that, as the titration progresses, the area under the peaks progressively becomes smaller due to an increase in saturation fraction. The bottom panel shows the plot of amount of heat generated per injection in kcal mol^{-1} of guest injected as a function of the molar ratio of CD **13** to PNA.

Table 2. Thermodynamics of the binding of CDs **11**–**14**, **32**–**38** and **13** **14** to PNA in H_2O at 25 °C.[a]

	n	$K \cdot 10^{-5}$ [M^{-1}]	$-\Delta G^\circ$ [kcal mol^{-1}]	$-\Delta H^\circ$ [kcal mol^{-1}]	$T\Delta S^\circ$ [kcal mol^{-1}]
lactose[b]		0.0199			
11	61.4 ± 0.6	2.8 ± 0.5	7.3 ± 0.1	10.8 ± 0.2	−3.5 ± 0.2
12	164.1 ± 2.8	1.8 ± 0.7	7.2 ± 2.3	6.9 ± 0.2	0.2 ± 2.3
13	79.8 ± 2.6	2.3 ± 1.3	7.3 ± 0.3	39.3 ± 1.9	±31.9 ± 1.9
14	138.3 ± 0.6	1.9 ± 0.1	7.2 ± 0.1	8.2 ± 0.1	−1.0 ± 0.1
33	98.8 ± 0.7	0.7 ± 0.0	6.6 ± 0.1	5.5 ± 0.1	1.1 ± 0.1
34	–	N.B.[c]	–	–	–
Me-β-Gal[d]		0.0187			
35	–	N.B.	–	–	–
36	275.8 ± 2.4	0.9 ± 0.1	6.8 ± 0.1	3.7 ± 0.1	3.1 ± 0.1
37	–	N.B.	–	–	–
38	–	N.B.	–	–	–
13·**40**	190.4 ± 1.5	5.5 ± 1.1	7.9 ± 0.1	15.9 ± 0.2	−8.1 ± 0.2

[a] Determined in a buffered aqueous solution at pH 7.4 (10 mM sodium phosphate). [b] Ref. [10d]. [c] N.B. = no binding. [d] Methyl-β-D-galactopyranoside, ref. [10d].

binding interaction was not detected between the lectin and CDs **34** and **37**, bearing seven D-galacopyranosylamine residues, and **38**, with 6-*O*-α-D-galactopyranosyl (or melibiosyl) residues. ITC data can be fit using a nonlinear least-square algorithm with three independent variables (such as n, ΔH°, and K) for the simplest model based on equal and independent binding sites. When we tried to fit our results using that model we obtained n values of 61–276 for the heptavalent CDs **11**–**14** and **36** and 95 for the tetradecavalent CD **36**. Previous ITC experiments[10d] have shown that lactose and methyl β-D-galactopyranoside bind to native PNA with affinity constants of 1990 and 1870.6 M^{-1}, respectively, and with a stoichiometry (n) close to 1. These n values are in concordance with the four saccharide-binding sites, one per monomer, revealed by X-ray diffraction data of the crystal structure of the complex of the tetrameric PNA with lactose and methyl β-D-galactopyranoside.[10b, c] The n values give the [ligand]/[receptor] ratio when the lectin binding sites are fully saturated. In previous ITC studies on interaction between multivalent carbohydrates and lectins the n values have been related with the multivalent or glycoside cluster effect.[18, 19] Thus, n values below one are associated with binding of a multivalent carbohydrate to a lectin due to the formation of a soluble one-dimensional cross-linked complex between the monovalent lectin and the multivalent carbohydrate.[19] In our case, it is expected to obtain high n values, since the cross-linked complexes would be formed by combination of a C_7-symmetry multiligand system with a tetravalent receptor. Thus, occupation of all lectin binding sites would give rise to a complex surrounded by saccharide residues not involved in binding.

As can be seen from Table 2, all the CD/PNA complexes formation are enthalpy-driven with a substantial increase in affinity when compared with the binding of lactose and methyl β-D-galactopyranoside to native PNA. Thus, heptavalent lacto-CDs **11**–**14** and the tetradecavalent lacto-CD **33** have 90–141 and 35-fold, respectively, higher K values for native PNA relative to lactose, and the heptavalent Gal-SCH_2NH-CD **36** has 48-fold higher K value for native PNA relative to methyl β-D-galactopyranoside. Consequently, although the increase in valency from a heptavalent to a tetradecavalent CD, as occurs for **33**, improved the binding affinity of the CD to the PNA relative to lactose, it did not yield an improvement of the affinity of the CD to the lectin relative to the heptavalent analogues **11**–**14**. A similar phenomenon has been previously observed with other reported glycodendrimers.[3a] Both, Gal-SCH_2CONH-CD **36** and Lac-SCH_2CONH-CD **12**, with the same spacer-arm but with galactosyl and lactosyl residues, respectively, improved substantially the CD/PNA cross-linked complex stability with respect to methyl β-D-galactopyranoside and lactose, respectively. However, the latter one **12** afforded a two-fold more stable complex with PNA ($K = 1.8 \times 10^5$ M^{-1}) than **36** ($K = 0.9 \times 10^5$ M^{-1}), in spite of the close K values for lactose and methyl β-D-galactopyranoside (Table 2). This indicates that the affinity enhancement relative to the monovalent analog by using heptavalent CDs is more efficient when the saccharide residue grafted on the CD ring is lactose.

When compared, the heptavalent CDs **11**–**14**, in which the lactosyl residues are bound to the CD core through different spacer-arms, the typical energetics of protein–carbohydrate association is observed,[2b] that is, ΔH° more negative or equal to the ΔG° and a strong linear enthalpy–entropy compensation (slope of 1.00, with a correlation coefficient of 1, Figure 5). Lac-S-CD **11** and Lac-$NHCOCH_2S$-CD **13**, with sulfur at C-6 of the CD afforded the most stable complexes

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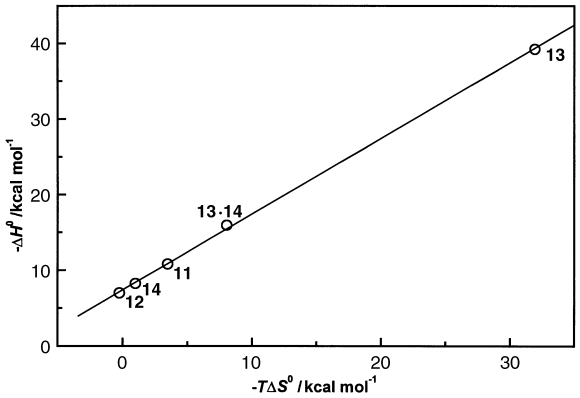

Figure 5. Enthalpy–entropy compensation for the binding of CDs **11**–**14** to PNA at 25 °C. The linear correlation coefficient for the fit was 1.

with PNA, independently on the length of the spacer-arm. In this respect, it is noteworthy that the least flexible heptavalent analogue **11** yields a similar K value to that for **13** and higher than that for Lac-SCH_2CONH-CD **12** and Lac-$NHCOCH_2SCH_2CONH$-CD **14**, with longer spacer arms.

Within the best two PNA ligands **11** and **13**, the first does not form an inclusion complex with 2-naphthalenesulfonate (**40**); however, the latter **13** is the best host for guest **40** of the series. When an aqueous solution containing a mixture of branched Lac-$NHCOCH_2S$-CD **13** ($[\mathbf{13}] = 4$ mM) and an excess of guest **40** ($[\mathbf{40}] = 140$ mM), such that only complex **13**·**40** and compound **40** is present in the solution, is titrated into a solution containing PNA, a release of heat is observed (Figure 6, Table 2). Previous experiments did not show binding interaction between 2-naphthalenesulfonate (**40**) and PNA. The obtained K value was the best of the series ($5.5 \times 10^5\,M^{-1}$) with $n = 190$. The thermodynamic profile corresponds to that of a protein–carbohydrate interaction. Remarkably, the obtained enthalpy–entropy compensation fits perfectly with the lineal correlation obtained for the lactosyl CDs **11**–**13** (Figure 5). These results seems to suggest that a binding interaction takes place between the complex **13**·**40** and the protein PNA giving rise to a three components cross-linked complex and therefore, the cluster lactoside host **13** could be used as a molecular carrier for guest **40** towards the biological target PNA.

Conclusion

We report the synthesis of heptavalent and tetradecavalent cluster galactosides based on a β-CD core. The heptavalent structures consist in a perbranched β-CD with lactose residues bound to C-6 through several spacer arms. The tetradecavalent structures are constructed by coupling of glycodendrons to the β-CD by nucleophilic displacement of the iodide in per-6-iodo-β-CD by the thiolate anion located at the focal point of the glycodendrimer. The persubstitution of the primary face of the CD with saccharides does not necessarily diminish the binding constant value for the inclusion complexation with a guest compared with the native β-CD (for example for guest **39**, the K value increases). However, the increase of the steric congestion on the narrow rim of the CD can cause a

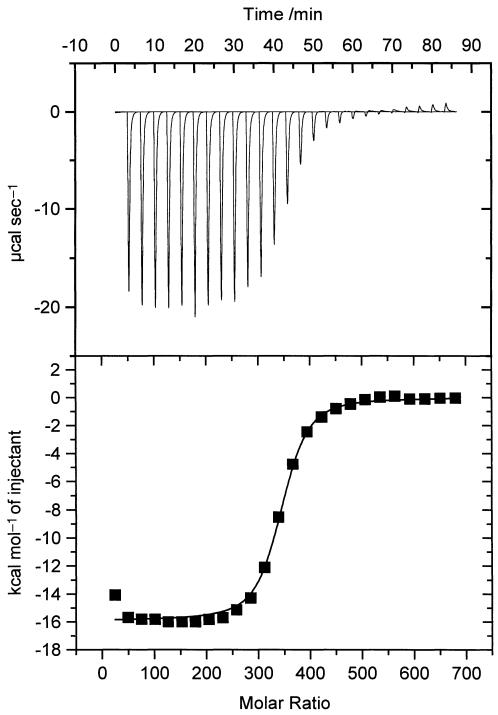

Figure 6. Titration of 1.25 µM of peanut lectin (PNA) with 25 aliquots (10 µL each) of complex **13**·**40** (4.13 mM, 140 mM, respectively) in 10 mM phosphate buffer at pH 7.4 and 25 °C. The top panel shows the raw calorimetric data, denoting the amount of heat released (negative exothermic peaks) following each injection of guest. The area under each peak represents the amount of heat produced upon the binding of complex **13**·**40** to PNA. Note that, as the titration progresses, the area under the peaks progressively becomes smaller due to an increase in saturation fraction. The bottom panel shows the plot of amount of heat generated per injection as a function of the molar ratio of complex **13**·**40** to PNA.

substantial decrease of the binding ability as shown for guest 2-naphthalenesulfonate (**40**). The synthesised hyperbranched CDs lose the host ability or do not bind well the guests ANS **39** and 2-naphthalenesulfonate (**40**). The spacer arms are not passive elements and influence the CD binding ability according to their chemical nature. PNA forms soluble cross-linked complexes with cluster galactosides and lactosides scaffolded on β-CD but not with cluster galactopyranosylamines or melibiose. Both, perbranched and hyperbranched CDs, form stronger complexes with PNA than the monomeric analogues. However, the use of hyperbranched CDs does not contribute to the improvement of the complex stability relative to heptakis-CDs. PNA lectin recognises a cluster lactoside host with a guest molecule inside its cavity. These results allow us to conclude that a cluster lactoside based on a β-CD core could play the role of a molecular carrier for transporting a guest towards a specific biological lactoside receptor such as PNA.

Experimental Section

General methods: TLC was performed on Merck silica gel 60 F_{254} aluminium sheets with detection by charring with sulfuric acid, and by UV light when applicable. Flash column chromatography was performed on silica gel Scharlau (230–400 mesh, ASTM). Melting points were measured on a Büchi melting point apparatus and are uncorrected. Optical

rotations were recorded on a Perkin–Elmer 141 polarimeter at room temperature. IR spectra were recorded on a Mattson Genesis II FTIR. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance DPX 300 (300 MHz) and AM 400 (400 MHz) spectrometers. Chemical shifts are given in ppm and referenced to internal $SiMe_4$ (δ_H, δ_C 0.00). J values are given in Hz. Mass spectra were recorded on a Micromass Autospec-Q spectometer. MALDI-TOF Mass spectra were recorded on a Voyager-DE-RP Perspective Biosystems using 2,5-dihydroxybenzoic acid matrix. Sodium 8-anilino-1-naphthalenesulfonate (ANS) (**39**) and 2-naphthalenesulfonate (**40**) were purchased from Aldrich and Fluka, respectively. The lectin from *Arachis hypogaea* (PNA) was purchased from Sigma. The lectin solutions were prepared in 0.1M sodium phosphate buffer (pH 7.4), dialysed against a large volume of the same buffer, and centrifugated to remove any insoluble material. The protein concentrations were determined using the lectin specific absorbance $A^{1\%}_{280\,nm} = 7.7$.[10a]

Isothermal titration calorimetry experiments were performed using an MCS isothermal titration calorimeter (ITC) from Microcal, Inc. (Northampton, MA). A complete description of its predecessor, OMEGA-ITC, experimental strategies, and data analyses are given Wiseman et al.[20] The calorimeter was calibrated by known heat pulses as recommended by the manufacturer. During titration, the reference cell was filled with Milli Q water. Prior to the titration experiments, guest, CD derivatives and lectin were degassed for 10 min with gentle stirring under vacuum. The sample cell was filled either with 1.8 mL (effective volume: 1.38 mL) of buffer (for control experiment) or with an appropriately receptor solution (glycosyl β-CD or PNA). During the titration, the reaction mixture was continuously stirred at 400 rpm. The background titration profiles, under identical experimental conditions, were obtained by injecting the guest or glycosyl β-CD (when used glycosyl β-CD or PNA as receptors, respectively) into appropriate buffer solutions. The observed heat effects were concentration-independent and were identical to the heat signals detected after the saturation is reached. The raw experimental data were presented as the amount of heat produced per second following each injection of guest or ligand into the CD derivative or PNA solution (corrected for the ligand heats of dilution) as a function of time. The amount of heat produced per injection was calculated by integration of the area under individual peaks by the Origin software provided with the instrument. The errors are provided by software from the best fit of the experimental data to the model of equal and independent sites, and correspond to the standard deviation in the fitting of the curves.

2,3,6-Tri-*O*-acetyl-4-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-chloroacetyl-β-D-glucopyranosylamine (3)

With nBu_3P (*procedure A*): nBu$_3$P (1.75 mL, 7.02 mmol) was added dropwise to a solution of the glycosyl azide **2**[11b] (2.33 g, 3.51 mmol) in anhydrous CH_2Cl_2 (30 mL) at room temperature under Ar. Gas evolution was observed to have ceased after 1 h; thereupon, the reaction mixture was cooled to $-80\,^\circ$C, and a solution of chloroacetic anhydride (1.20 g, 7.02 mmol) in anhydrous CH_2Cl_2 (15 mL) was added. The reaction mixture was allowed to warm to room temperature and kept overnight. CH_2Cl_2 (150 mL) was added and the organic solution was washed with saturated aqueous $NaHCO_3$ (2×100 mL) and H_2O (150 mL). The organic layer was dried (Na_2SO_4), filtered, evaporated, and the crude product purified by chromatography on silica gel (EtOAc/hexane 1:1), to give **3** (2.21 g, 88%) as a solid.

With 1,3-propanedithiol (*procedure B*): 1,3-Propanedithiol (0.40 mL, 4.0 mmol) and triethylamine (0.42 mL, 3.0 mmol) were added to a solution of compound **2** (0.66 g, 1.0 mmol) in anhydrous MeOH (15 mL) under Ar. The solution was stirred at room temperature for 3 h and then, chloroacetic anhydride (1.37 g, 8.0 mmol) was added. After 6 h at room temperature, the solvent was evaporated and the crude product purified by chromatography on silica gel (EtOAc/hexane 1:1 → 2:1) to give **3** (0.42 g, 58%) as a solid. M.p. 96–98 °C; $[\alpha]_D^{25} = -14$ ($c = 1$ in chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.23$ (d, ^3J(NH,H1) = 9.2 Hz, 1H; NH), 5.36 (br d, ^3J(H3′,H4′) = 2.8 Hz, 1H; H-4′), 5.32 (t, $^3J = 9.2$ Hz, 1H; H-3), 5.16 (t, $^3J = 9.2$ Hz, 1H; H-1), 5.11 (dd, ^3J(H2′,H3′) = 10.2, ^3J(H1′,H2′) = 7.8 Hz, 1H; H-2′), 4.95 (dd, ^3J(H2′,H3′) = 10.2, ^3J(H3′,H4′) = 3.5 Hz, 1H; H-3′), 4.92 (t, $^3J = 9.2$ Hz, 1H; H-2), 4.47 (d, ^3J(H1′,H2′) = 7.8 Hz, 1H; H-1′), 4.45 (m, 1H; H-6), 4.13 (m, 3H; H-6,6′,6′), 4.06 (d, $^2J = 15.5$ Hz, 1H; CHCl), 4.00 (d, $^2J = 15.5$ Hz, 1H; CHCl), 3.88 (m, 1H; H-5′), 3.78 (m, 2H; H-4,5), 2.16, 2.13, 2.07, 2.06, 2.05, 2.04, 1.97 (7s, 21H; 7Ac); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 171.0$–166.0 (CO), 100.9 (C-1′), 78.4 (C-1), 75.9 (C-4), 74.7 (C-5), 72.2 (C-3), 71.0, 70.8, 70.6 (C-3′,5′,2), 69.0 (C-2′), 66.7 (C-4′), 61.9 (C-6), 60.9 (C-6′), 42.3 (CH_2Cl), 20.9–20.5 (CH_3CO); IR (KBr): $\tilde{\nu} = 3350$, 1749, 1705, 1532, 1369, 1229 cm^{-1}; elemental analysis calcd (%) for $C_{28}H_{38}ClNO_{18}$ (712): C 47.23, H 5.38, N 1.96; found: C 47.50, H 5.50, N 1.86.

2,3,6-Tri-*O*-acetyl-4-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-mercaptoacetyl-β-D-glucopyranosylamine (4): Thiourea (2.14 g, 28.1 mmol) was added to a solution of **3** (2.00 g, 2.81 mmol) in anhydrous acetone (30 mL). The reaction mixture was stirred at room temperature for 12 h. The solution was concentrated approximately 10 mL under reduced pressure without heating. Then a solution of Na_2SO_3 (1.06 g, 8.43 mmol) in H_2O was added and the reaction mixture was stirred for 30 min. Aqueous HCl (5%, 8 mL), H_2O (100 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (2×150 mL). The combined organic phases were washed with H_2O (100 mL). The organic layer was dried (Na_2SO_4), filtered, evaporated, and the crude product purified by chromatography on silica gel (EtOAc/hexane 1:1) to give **4** (1.59 g, 80%) as a solid. M.p. 90–91 °C; $[\alpha]_D^{25} = +10$ ($c = 1$ in chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.25$ (d, ^3J(NH,H1) = 9.4 Hz, 1H; NH), 5.32 (br d, ^3J(H3′,H4′) = 3.4 Hz, 1H; H-4′), 5.28 (t, $^3J = 9.4$ Hz, 1H; H-3), 5.15 (t, $^3J = 9.4$ Hz, 1H; H-1), 5.08 (dd, ^3J(H2′,H3′) = 10.3, ^3J(H1′,H2′) = 7.9 Hz, 1H; H-2′), 4.92 (dd, ^3J(H2′,H3′) = 10.3, ^3J(H3′,H4′) = 3.4 Hz, 1H; H-3′), 4.87 (t, $^3J = 9.4$ Hz, 1H; H-2), 4.44 (d, ^3J(H1′,H2′) = 7.9 Hz, 1H; H-1′), 4.41 (br d, $^2J = 11.5$ Hz, 1H; H-6), 4.08 (m, 3H; H-6,6′,6′), 3.85 (br t, $^3J = 6.8$ Hz, 1H; H-5′), 3.76 (t, $^3J = 9.4$ Hz, 1H; H-4), 3.74 (m, 1H; H-5), 3.21 (dd, $^2J = 16.2$, ^3J(CH,SH) = 8.8 Hz, 1H; CHS), 3.15 (dd, $^2J = 16.2$, ^3J(CH,SH) = 9.2 Hz, 1H; CHS), 2.13, 2.09, 2.04, 2.03, 2.02, 2.01, 1.93 (7s, 21H; 7Ac), 1.87 (dd, ^3J(CH,SH) = 9.2, ^3J(CH,SH) = 8.8 Hz, 1H; SH); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 171.1$–168.9 (CO), 100.8 (C-1′), 78.2 (C-1), 75.8 (C-4), 74.5(C-5), 72.1 (C-3), 70.8 (C-2), 70.6 (C-5′,3′), 68.8 (C-2′), 66.5 (C-4′), 61.8 (C-6), 60.8 (C-6′), 28.8 (CH_2SH), 20.8–20.4 (CH_3CO); IR (KBr): $\tilde{\nu} = 3505$, 2961, 1748, 1694, 1537, 1369, 1229, 1044 cm^{-1}; HRMS (FAB): m/z: calcd for $C_{28}H_{39}NO_{18}SNa$: 732.1785; found: 732.1784 $[M+Na]^+$.

General procedure for the synthesis of lactose-CD 7–10: A mixture of **5**[13] (0.12 mmol for reaction with **1**,[11a] 0.70 mmol for reaction with **4**) or **6**[4a] (0.13 mmol for reaction with **1**, 0.70 mmol for reaction with **4**), Cs_2CO_3 (2.5 equiv) and compound **1** (2.5 equiv) and **4** (2 equiv) in anhydrous DMF (8 mL) was kept under Ar for 7 d at 60 °C. After this time, Ac_2O (14 mL), pyridine (8 mL), and DMAP (cat.) were added and the reaction mixture was stirred for 48 h at 40 °C. Then the precipitated material was filtered and the filtrate was poured over ice/H_2O. Aqueous HCl (5%, 100 mL) was added and the aqueous layer extracted with CH_2Cl_2 (2×100 mL). The combined organic phases were washed successively with aqueous HCl (5%, 2×100 mL), H_2O (100 mL), saturated $NaHCO_3$ (2×100 mL) and H_2O (100 mL). The organic solution was dried (Na_2SO_4), filtered, evaporated and gave a residue that was subjected to column chromatography.

Heptakis{2,3-di-*O*-acetyl-6-*S*-[2′,3′,6′-tri-*O*-acetyl-4′-*O*-(2″,3″,4″,6″-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-6-thio}cyclomaltoheptaose (7): Column chromatography (EtOAc/MeOH 1:0 → 40:1) gave **7** (606 mg, 82%) as a solid. The isolated solid was dissolved in CH_2Cl_2 and diethyl ether was added. The resulting precipitate was filtered and compound **7** was obtained (586 mg, 79%). M.p. 171 °C (decomp); $[\alpha]_D^{25} = +25$ ($c = 0.5$ in chloroform); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 80 °C): $\delta =$ 5.37 (d, $^3J = 3.7$ Hz, 7H; H-4″), 5.30 (t, $^3J = 7.8$ Hz, 7H; H-3), 5.24 (m, 21H; H-1,3′,3″), 4.99 (dd, ^3J(H2″,H3″) = 9.9 Hz, ^3J(H1″,H2″) = 10.1 Hz, 7H; H-2″), 4.85 (m, 28H; H-1′,1″,2,2′), 4.55 (br d, $^2J = 10.8$ Hz, 7H; H-6$_a$′), 4.28 (br t, $^3J = 5.8$ Hz, 7H; H-5″), 4.18 (m, 28H; H-5,6$_b$′,6$_a$″,6$_b$″), 4.08 (t, $^3J =$ 7.8 Hz, 7H; H-4), 3.95 (t, $^3J = 9.3$ Hz, 7H; H-4′), 3.82 (m, 7H; H-5′), 3.28 (br d, $^2J = 12.5$ Hz, 7H; H-6$_a$), 3.18 (dd, ^2J(H6a,H6b) = 14.4 Hz, ^3J(H5,H6b) = 4.3 Hz, 7H; H-6$_b$), 2.19, 2.14, 2.13, 2.11, 2.10, 2.00 (6s, 189H; 63Ac); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO, 80 °C): $\delta = 169.2$–168.2 (CO), 99.4 (C-1″), 96.7 (C-1), 82.5 (C-1′), 77.9 (C-4), 75.7, 74.9 (C-4′,5′), 73.2 (C-3′ or C-3″), 70.6, 70.1, 69.6, 69.4 (C-2,3,5,2′,3′ or 3″,5″), 68.9 (C-2″), 66.8 (C-4″), 61.6 (C-6′), 60.3 (C-6″), 31.5 (C-6), 19.8–19.4 (CH_3CO); IR (KBr): $\tilde{\nu} = 2957$, 2923, 1750, 1371, 1232, 1046 cm^{-1}; MS (MALDI-TOF): m/z: calcd for $C_{252}H_{336}O_{161}S_7$: 6165.73; found: 6188.20 $[M+Na]^+$.

Heptakis{2,3-di-*O*-acetyl-6-amino-6-deoxy-6-*N*-[2′,3′,6′-tri-*O*-acetyl-4-*O*-(2″,3″,4″,6″-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl-1′-thiomethylcarbonyl]}cyclomaltoheptaose (8): Column chromatography (EtOAc/MeOH 1:0 → 40:1) gave **7** (689 mg, 84%) as a solid. The isolated solid was dissolved in CH_2Cl_2 and diethyl ether was added. The resulting precipitate was filtered and compound **8** was obtained (662 mg, 81%). M.p.

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163 °C (decomp); $[\alpha]_D^{25} = +31$ ($c = 0.5$ in chloroform); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 80 °C): $\delta = 7.56$ (brs, 7H; NH), 5.37 (d, $^3J = 3.2$ Hz, 7H; H-4″), 5.30 (t, $^3J = 8.7$ Hz, 7H; H-3), 5.17 (m, 21H; H-1,3″,3′), 4.98 (dd, ^3J(H2″,H3″) = 10.0, ^3J(H1″,H2″) = 7.8 Hz, 7H; H-2″), 4.97 (d, ^3J(H1′,H2′) = 9.7 Hz, 7H; H-1′), 4.89 (dd, ^3J(H2,H3) = 8.7, ^3J(H1,H2) = 2.7 Hz, 7H; H-2), 4.88 (t, $^3J = 9.8$ Hz, 7H; H-2′), 4.87 (d, ^3J(H1″,H2″) = 7.8 Hz, 7H; H-1″), 4.49 (brd, $^2J = 11.1$ Hz, 7H; H-6$_a$′), 4.28 (brt, $^3J = 6.5$ Hz, 7H; H-5″), 4.21 (m, 7H; H-5′), 4.15 (m, 21H; H-5,6$_a$″,6$_b$″), 3.94 (t, $^3J = 9.7$ Hz, 7H; H-4′), 3.87 (m, 14H; H-6′,6), 3.80 (t, $^3J = 8.7$ Hz, 7H; H-4), 3.58 (brs, 7H; H-6), 3.52 (d, $^2J = 15.4$ Hz, 7H; CHS), 3.47 (d, $^2J = 15.4$ Hz, 7H; CHS), 2.28, 2.18, 2.13, 2.11, 2.10, 2.09, 2.00 (7s, 189H; 63Ac); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO, 80 °C): $\delta = 169.4-168.2$ (CO), 99.4 (C-1″), 95.9 (C-1), 81.3 (C-1′), 76.6 (C-4), 75.7, 75.3, 73.1, 70.1, 69.6, 68.9 (C-2,3,5,2′,3′,4′,5′,2″,3″,5″), 66.9 (C-4″), 61.7 (C-6′), 60.5 (C-6″), 39.0 (C-6), 32.4 (CH$_2$S), 19.9–19.5 (*C*H$_3$CO); IR (KBr): $\tilde{\nu} = 3502$, 2940, 1751, 1661, 1539, 1435, 1371, 1230, 1050 cm^{-1}; MS (MALDI-TOF): *m*/*z*: calcd for $C_{266}H_{357}N_7O_{168}S_7$: 6565.09; found: 6588.35 for $[M+Na]^+$.

Heptakis{2,3-di-*O*-acetyl-6-*S*-[*N*-((2′,3′,6′-tri-*O*-acetyl-4′-*O*-(2″,3″,4″,6″-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl)aminocarbonylmethyl]-6-thio}cyclomaltoheptaose (9): Column chromatography (EtOAc/MeOH 1:0 → 40:1) gave **9** (604 mg, 92%) as a solid. The isolated solid was dissolved in CH_2Cl_2 (2 mL) and diethyl ether (30 mL) was added. The resulting precipitate was filtered and compound **9** was obtained (570 mg, 86%). M.p. 162 °C (decomp); $[\alpha]_D^{25} = +28$ ($c = 0.5$ in chloroform); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 80 °C): $\delta = 8.42$ (d, ^3J(NH,H1′) = 9.3 Hz, 7H; NHC-1′), 5.36 (brd, ^3J(H3″,H4″) = 3.7 Hz, 7H; H-4″), 5.34 (t, ^3J(H1′,H2′) = 9.3 Hz, 7H; H-1′), 5.32 (dd, ^3J(H2,H3) = 10.0, ^3J(H3,H4) = 8.9 Hz, 7H; H-3), 5.28 (t, $^3J = 9.3$ Hz, 7H; H-3′), 5.24 (dd, ^3J(H2″,H3″) = 10.0, ^3J(H3″,H4″) = 3.7 Hz, 7H; H-3″), 5.15 (d, ^3J(H1,H2) = 3.3 Hz, 7H; H-1), 4.98 (dd, ^3J(H2″,H3″) = 10.0, ^3J(H1″,H2″) = 7.9 Hz, 7H; H-2″), 4.91 (t, $^3J = 7.9$ Hz, 7H; H-2′), 4.86 (dd, ^3J(H2,H3) = 10.0, ^3J(H1,H2) = 3.3 Hz, 7H; H-2), 4.84 (d, ^3J(H1″,H2″) = 7.9 Hz, 7H; H-1″), 4.44 (brd, $^2J = 11.7$ Hz, 7H; H-6′), 4.30 (brt, $^3J = 6.7$ Hz, 7H; H-5″), 4.19 (m, 7H; H-5), 4.15 (m, 21H; H-6″,6″,6′), 4.02 (brt, $^3J = 8.9$ Hz, 7H; H-4), 3.93 (m, 7H; H-5′), 3.91 (t, $^3J = 9.3$ Hz, 7H; H-4′), 3.47 (d, $^2J = 14.4$ Hz, 7H; CHS), 3.32 (d, $^2J = 14.4$ Hz, 7H; CHS), 3.22 (brd, $^2J = 13.5$ Hz, 7H; H-6), 3.12 (m, 7H; H-6), 2.20, 2.17, 2.13, 2.11, 2.10, 2.08, 2.06, 2.00 (8s, 189H; 63Ac); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO, 80 °C): $\delta = 169.5-168.3$ (CO), 99.4 (C-1″), 96.2 (C-1), 77.9 (C-4), 77.0 (C-1′), 75.3 (C-4′), 73.5 (C-5′), 72.8 (C-3′), 71.3 (C-5), 70.6 (C-2′), 70.2 (C-3″), 70.0, (C-3), 69.8 (C-2), 69.6 (C-5″), 68.9 (C-2″), 66.9 (C-4″), 61.9 (C-6′), 60.5 (C-6″), 36.2 (CH$_2$S), 33.6 (C-6), 20.0–19.6 (*C*H$_3$CO); IR (KBr): $\tilde{\nu} = 3617$, 3643, 2940, 1751, 1531, 1434, 1232, 1047 cm^{-1}; MS (MALDI-TOF): *m*/*z*: calcd for $C_{266}H_{357}N_7O_{168}S_7$: 6565.09; found: 6588.30 $[M+Na]^+$.

Heptakis{2,3-di-*O*-acetyl-6-amino-*N*-[*S*-(*N*-(2′,3′,6′-tri-*O*-acetyl-4′-*O*-(2″,3″,4″,6″-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl)aminocarbonylmethyl)-mercaptoacetyl]-6-deoxy}cyclomaltoheptaose (10): Column chromatography (EtOAc/MeOH 1:0 → 40:1) gave **10** (558 mg, 80%) as a solid. The isolated solid was dissolved in CH_2Cl_2 (2 mL) and diethyl ether (30 mL) was added. The resulting precipitate was filtered and compound **10** was obtained (530 mg, 76%). M.p. 165 °C (decomp); $[\alpha]_D^{25} = +14$ ($c = 0.25$ in chloroform); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 80 °C): $\delta = 8.55$ (d, ^3J(NH,H1′) = 8.8 Hz, 7H; NHC-1′), 7.76 (brs, 7H; NHC-6), 5.36 (d, ^3J(H3″,H4″) = 3.6 Hz, 7H; H-4″), 5.28 (m, 21H; H-1′,3,3′), 5.25 (d, ^3J(H1,H2) = 4.1 Hz, 7H; H-1), 5.24 (dd, ^3J(H2″,H3″) = 10.1, ^3J(H3″,H4″) = 3.6 Hz, 7H; H-3″), 4.98 (dd, ^3J(H2″,H3″) = 10.1, ^3J(H1″,H2″) = 8.0 Hz, 7H; H-2″), 4.90 (t, $^3J = 9.3$ Hz, 7H; H-2′), 4.90 (m, 7H; H-2), 4.85 (d, ^3J(H1″,H2″) = 8.0 Hz, 7H; H-1″), 4.42 (brd, $^2J = 11.4$ Hz, 7H; H-6′), 4.30 (brt, $^3J = 6.5$ Hz, 7H; H-5″), 4.14 (m, 28H; H-5,6′,6″,6″), 3.90 (m, 28H; H-4,4′,5′,6), 3.58 (brd, $^2J = 11.7$ Hz, 7H; H-6), 3.45 (d, $^2J = 14.2$ Hz, 7H; CHS), 3.37 (d, $^2J = 14.2$ Hz, 7H; CHS), 3.35 (d, $^2J = 14.2$ Hz, 7H; CHS), 3.31 (d, $^2J = 14.2$ Hz, 7H; CHS), 2.19, 2.15, 2.11, 2.09, 2.08, 2.04, 2.00 (7s, 189H; 63Ac); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO, 80 °C): $\delta = 169.6-168.3$ (CO), 99.3 (C-1″), 96.1 (C-1), 76.9 (C-1′), 76.6 (C-4), 75.4 (C-4′), 73.5 (C-5′), 72.9 (C-3′), 70.7 (C-2′), 70.2 (C-3″,3), 69.8 (C-5), 69.6, (C-2,5″), 68.9 (C-2″), 66.9 (C-4″), 61.9 (C-6′), 60.6 (C-6″), 39.1 (C-6), 34.8, 34.6 (CH$_2$S), 20.0–19.6 (*C*H$_3$CO); IR (KBr): $\tilde{\nu} = 3471$, 2941, 1750, 1654, 1540, 1434, 1371, 1232, 1049 cm^{-1}; MS (MALDI-TOF): *m*/*z*: calcd for $C_{280}H_{378}N_{14}O_{175}S_7$: 6964.41; found: 6987.20 $[M+Na]^+$.

General procedure for the Zemplén de-O-acetylation of lacto-CD 7–10: A solution of compound **7** (460 mg, 0.08 mmol), **8** (230 mg, 0.04 mmol), **9** (300 mg, 0.05 mmol) or **10** (200 mg, 0.03 mmol) in dry MeOH (5–8 mL) was made alkaline to pH 9 (indicator paper) with a methanolic solution of NaOMe (1M). The reaction mixture was stirred overnight at room temperature and the precipitated material was filtered, washed with MeOH and dissolved in H_2O (6–8 mL). The solution was concentrated by lyophilization and gave a solid.

Heptakis{6-*S*-[4′-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-6-thio}-cyclomaltoheptaose (11): Yield: 251 mg, 95%; m.p. 213 °C (decomp); $[\alpha]_D^{25} = +42$ ($c = 0.25$ in H_2O); ^{1}H NMR (300 MHz, D_2O): $\delta = 5.06$ (brs, 7H; H-1), 4.59 (d, ^3J(H1′,H2′) = 9.3 Hz, 7H; H-1′), 4.40 (d, ^3J(H1″,H2″) = 7.4 Hz, 7H; H-1″), 4.07 (brs, 7H; H-5), 3.96–3.43 (m, 98H; H-2,3,2′,3′,5′,6′,6′,2″,3″,4″,5″,6″,6″,4 or 4′), 3.33 (m, 14H; H-4 or 4′,6), 3.11 (brd, $^2J = 10.4$ Hz, 7H; H-6); ^{13}C NMR (75.5 MHz, D_2O): $\delta = 102.8$ (C-1″), 101.9 (C-1), 85.7 (C-1′), 83.0 (C-4), 78.5, 78.1, 75.7, 75.2, 72.7, 72.4, 72.2, 70.8, 70.3, 68.4 (C-2,3,5,2′,3′,4′,5′,2″,3″,4″,5″), 60.9, 60.3 (C-6′,6″), 31.4 (C-6); IR (KBr): $\tilde{\nu} = 3397$, 2914, 1651, 1435, 1374, 1155, 1068, 1040 cm^{-1}; MS (MALDI-TOF): *m*/*z*: calcd for $C_{126}H_{210}O_{98}S_7$ 3517.41; found: 3540.54 $[M+Na]^+$.

Heptakis{6-amino-6-deoxy-6-*N*-[4′-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl-1′-thiomethylcarbonil]}cyclomaltoheptaose (12): Yield: 132 mg, 96%; m.p. 204 °C (decomp); $[\alpha]_D^{25} = +14$ ($c = 0.25$ in H_2O); ^{1}H NMR (300 MHz, D_2O): $\delta = 4.87$ (brs, 7H; H-1), 4.45 (d, ^3J(H1′,H2′) = 9.3 Hz, 7H; H-1′), 4.29 (d, ^3J(H1″,H2″) = 7.1 Hz, 7H; H-1″), 3.89–3.18 (m, 140H; H-2,2′,2″,3,3′,3″,4,4′,4″,5,5′,5″,6,6,6′,6′,6″,6″,CH$_2$S); ^{13}C NMR (75.5 MHz, D_2O): $\delta = 172.3$ (CO), 102.7 (C-1″), 102.0 (C-1), 85.1 (C-1′), 82.8 (C-4), 78.5, 77.8, 75.6, 75.2, 72.8, 72.4, 71.9, 70.7, 69.9, 68.4 (C-2,3,5,2′,3′,4′,5′,2″,3″,4″,5″), 60.8, 60.0 (C-6′,6″), 40.0 (C-6), 32.7 (CH$_2$); IR (KBr): $\tilde{\nu} = 3395$, 2918, 1652, 1557, 1539, 1417, 1159, 1042 cm^{-1}; MS (MALDI-TOF): *m*/*z*: calcd for $C_{140}H_{231}N_7O_{105}S_7$ 3916.77; found: 3940.47 $[M+Na]^+$.

Heptakis{6-*S*-[*N*-((4′-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl)amino-carbonylmethyl]-6-thio}cyclomaltoheptaose (13): Yield: 175 mg, 98%; m.p. 201 °C (decomp); $[\alpha]_D^{25} = +30$ ($c = 0.25$ in H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta = 4.98$ (brs, 7H; H-1), 4.91 (d, ^3J(H1′,H2′) = 9.0 Hz, 7H; H-1′), 4.40 (d, ^3J(H1″,H2″) = 8.0 Hz, 7H; H-1″), 3.90–3.35 (m, 84H; H-2,2′,3′,4′,5′,5″,6′,6′,6″,6″,CH$_2$S), 3.90 (m, 7H; H-5), 3.81 (d, ^3J(H3″,H4″) = 3.1 Hz, 7H; H-4″), 3.80 (m, 7H; H-3), 3.74 (m, 7H; H-4), 3.58 (dd, ^3J(H2″,H3″) = 9.0 Hz, ^3J(H3″,H4″) = 3.1 Hz, 7H; H-3″), 3.45 (t, $^3J = 9.0$ Hz, 7H; H-2″), 3.19 (brd, $^2J = 13.3$ Hz, 7H; H-6), 2.92 (brs, 7H; H-6); ^{13}C NMR (75.5 MHz, D_2O): $\delta = 173.6$ (CO), 102.8 (C-1″), 102.1 (C-1), 84.2 (C-4), 79.6 (C-1′), 77.6, 76.3, 75.3, 73.1, 72.5, 71.6, 70.8, 68.5 (C-2,2′,2″,3,3′,3″,4,4′,4″,5,5′,5″), 60.9, 59.8 (C-6′,6″), 36.2 (CH$_2$S), 33.7 (C-6); IR (KBr): $\tilde{\nu} = 3411$, 2918, 1662, 1543, 1411, 1375, 1156, 1041 cm^{-1}; MS (MALDI-TOF): *m*/*z*: calcd for $C_{140}H_{231}N_7O_{105}S_7$: 3916.77; found: 3939.00 $[M+Na]^+$.

Heptakis{6-amino-*N*-[*S*-(*N*-(4′-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl)aminocarbonylmethyl]-mercaptoacetyl]-6-deoxy}cyclomaltoheptaose (14): Yield: 122 mg, 98%; m.p. 195 °C (decomp); $[\alpha]_D^{25} = +46$ ($c = 0.25$ in H_2O); ^{1}H NMR (300 MHz, D_2O): $\delta = 4.99$ (brs, 7H; H-1), 4.94 (d, ^3J(H1′,H2′) = 9.2 Hz, 7H; H-1′), 4.40 (d, ^3J(H1″,H2″) = 7.7 Hz, 7H; H-1″), 4.05–3.25 (m, 140H; H-2,2′,2″,3,3′,3″,4,4′,4″,5,5′,5″,6,6′,6″,6,6′,6″,CH$_2$S); ^{13}C NMR (75.5 MHz, D_2O): $\delta = 172.6$, 171.5 (CO), 102.8 (C-1″), 102.0 (C-1), 82.4 (C-4), 79.4 (C-1′), 72.8, 72.2, 69.9 (C-2,3,5), 77.7, 76.3, 75.0, 71.4 (C-2′,3′,4′,5′), 75.2, 72.4, 70.8, 68.4 (C-2″,3″,4″,5″), 60.9, 59.8 (C-6′,6″), 40.1 (C-6), 34.6 (CH$_2$S); IR (KBr): $\tilde{\nu} = 3415$, 2912, 1651, 1555, 1416, 1375, 1159, 1081, 1041 cm^{-1}; MS (MALDI-TOF): *m*/*z*: calcd for $C_{154}H_{252}N_{14}O_{112}S_7$: 4316.13; found: 4340.03 $[M+Na]^+$.

Bis(3-benzyloxycarbonylaminopropyl)-*N*-*tert*-butoxycarbonylamine (16): Boc_2O (1.24 g, 5.67 mmol) and triethylamine (498 mg, 4.93 mmol) were added to a solution of compound **15** (1.97 g, 4.93 mmol) in CH_3CN (20 mL). The reaction mixture was stirred overnight, then the solvent was evaporated and the crude product was purified by chromatography on silica gel (EtOAc/hexane 1:1 → 2:1) to give **16** (2.45 g, 99%) as a syrup. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.40-7.30$ (m, 10H; Ph), 5.80 (brs, 2H; NH), 5.10 (s, 4H; CH_2O), 3.18 (brs, 8H; CH_2NH, CH_2N), 1.66 (brs, 4H; $CH_2CH_2CH_2$), 1.45 (s, 9H; CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 156.4-155.9$ (CO), 136.6–127.9 (Ph), 80.0 (*C*H$_3$), 66.4 (*C*H$_2$CO), 44.0–43.2 (CH$_2$N), 38.2–37.6 (CH$_2$NH), 28.7–27.8 (CH$_2$*C*H$_2$CH$_2$), 28.2 (CH$_3$); IR (KBr): $\tilde{\nu} = 3333$, 2983, 1694, 1524, 1250, 1149, 1026 cm^{-1}; HRMS (FAB): *m*/*z*: calcd for $C_{27}H_{37}N_3O_6Na$: 522.2580; found: 522.2589 $[M+Na]^+$.

Bis(3-chloroacetamidopropyl)-*N*-*tert*-butoxycarbonylamine (17): A suspension of compound **16** (8.05 g, 16.11 mmol) and Pd/C (822 mg) in MeOH (100 mL) was stirred under H_2 atmosphere (2.5 atm) for 1 h at room temperature. The mixture was filtered and the Pd/C washed twice with MeOH. The combined filtrates were dried and the solvent evaporated. The obtained crude product was dissolved in CH_3CN (50 mL) and chloroacetic anhydride (6.61 g, 38.67 mmol) and triethylamine (3.26 g, 32.22 mmol) were added to the solution. The reaction mixture was stirred for 24 h at room temperature. Then the solvent was evaporated, and the crude product purified by chromatography on silica gel (EtOAc/hexane 5:1) to give **17** (5.03 g, 81 %) as a solid. M.p 96.6 – 98.8 °C; ^{1}H NMR (300 MHz, ($CDCl_3$): $\delta = 7.61$ (brs, 1H; NH), 6.71 (brs, 1H; NH), 4.04 (s, 4H; CH_2Cl), 3.28 (brs, 8H; CH_2NH,CH_2N), 1.71 (brs, 4H; $CH_2CH_2CH_2$), 1.46 (s, 9H; CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 166.2 – 156.2$ (CO), 80.3 (CH_3C), 44.5 – 43.3 (CH_2N), 42.6 (CH_2Cl), 37.5 – 36.2 (CH_2NH), 28.6 – 27.5 ($CH_2CH_2CH_2$), 28.4 (CH_3); IR (KBr): $\tilde{\nu} = 3327$, 3260, 2970, 2948, 1690, 1664, 1419, 1173, 1142 cm^{-1}; HRMS (FAB): *m*/*z*: calcd for $C_{15}H_{27}N_3O_4Cl_2Na$: 406.1276; found: 406.1279 $[M+Na]^+$.

2,3,4-Tri-*O*-acetyl-6-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-galactopyranosyl)-1-thio-β-D-glucopyranose (19): Thiourea (1.23 g, 16.15 mmol) was added to a solution of 2,3,4-tri-*O*-acetyl-6-*O*-(2′,3′,4′,6′-*O*-acetyl-α-D-galactopyranosyl)-α-D-glucopyranosyl bromide (2.26 g, 3.23 mmol) in anhydrous acetone (30 mL). The reaction mixture was stirred under reflux for 4 h. The solution was concentrated approximately 15 mL. Then a solution of Na_2SO_3 (1.22 g, 9.69 mmol) in H_2O (25 mL) was added and the reaction mixture was stirred for 30 min. Aqueous HCl (5 %, 8 mL), H_2O (100 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (2 × 150 mL). The combined organic phases were washed with H_2O (100 mL). The organic layer was dried (Na_2SO_4), filtered, evaporated, and the crude product purified by chromatography on silica gel (EtOAc/hexane 1:3 → 1:2) to give **19** (1.54 g, 73 %) as a solid. M.p. 202.5 – 204.8 °C; $[\alpha]_D^{25} = +100$ ($c = 0.25$ in chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.45$ (brd, ^3J(H3′,H4′) = 3.2 Hz, 1H; H-4′), 5.35 (dd, ^3J(H2′,H3′) = 10.8, ^3J(H3′,H4′) = 3.2 Hz, 1H; H-3′), 5.21 (d, ^3J(H1′,H2′) = 4.0 Hz, 1H; H-1′), 5.17 (t, $^3J = 9.4$ Hz, 1H; H-3), 5.07 (dd, $^3J = 9.4$ Hz, 1H; H-4), 5.07 (dd, ^3J(H2′,H3′) = 10.8, ^3J(H1′,H2′) = 4.0 Hz, 1H; H-2′), 4.89 (t, $^3J = 9.4$ Hz, 1H; H-2), 4.50 (t, $^3J = 9.4$ Hz, 1H; H-1), 4.25 (brt, $^3J = 6.5$ Hz, 1H; H-5′), 4.14 – 4.05 (m, 2H; H-6′,6′), 3.72 – 3.60 (m, 3H; H-5,6,6), 2.27 (d, $^3J = 9.4$ Hz, 1H; SH), 2.14 – 1.98 (6s, 21H; Ac); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 170.6 – 169.3$ (CO), 96.0 (C-1′), 78.4 (C-1), 77.0 (C-5), 73.5 (C-2,3), 68.6, 68.1 (C-2′,4), 67.4 (C-4′), 66.2 (C-3′), 66.0 (C-6), 61.5 (C-6′), 20.7 – 20.6 (CH_3CO); IR (KBr): $\tilde{\nu} = 2954$, 1754, 1375, 1224, 1049 cm^{-1}; HRMS (FAB): *m*/*z*: calcd for $C_{26}H_{36}O_{17}S$: 652.1674; found: 652.1672 $[M]^+$.

General procedure for the synthesis of thioglycoside 20 – 22: A mixture of **17** (2.72 mmol for reaction with **18**,[11c] 0.42 mmol for reaction with **1**,[11a] 1.32 mmol for reaction with **19**), Cs_2CO_3 (5 equiv) and the compound **1**, **18** or **19** (3 equiv) in anhydrous DMF (10 – 15 mL) was kept under Ar for 24 h at room temperature. After this time, the precipitated material was filtered. Aqueous HCl (5 %, 100 mL) was added and the aqueous layer extracted with CH_2Cl_2 (2 × 150 mL). The combined organic phases were washed successively with aqueous HCl (5 %, 100 mL), H_2O (100 mL), saturated aqueous $NaHCO_3$ (2 × 100 mL), and H_2O (2 × 100 mL). The organic solution was dried (Na_2SO_4), filtered and evaporated, giving a residue that was subjected to column chromatography.

Bis[3-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-1-thiomethylcarbonylamino)propyl]-*N*-*tert*-butoxycarbonylamine (20): Column chromatography (EtOAc/MeOH 1:0 → 30:1) gave **20** (2.33 g, 83 %) as a solid. M.p. 63.6 – 65.2 °C; $[\alpha]_D^{25} = -12$ ($c = 0.5$ in chloroform); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 80 °C): $\delta = 7.73$ (brt, $^3J = 5.2$ Hz, 2H; NH), 5.43 (dd, ^3J(H3,H4) = 3.6, ^3J(H4,H5) = 1.1 Hz, 2H; H-4), 5.29 (dd, ^3J(H2,H3) = 9.8, ^3J(H3,H4) = 3.5 Hz, 2H; H-3), 5.10 (t, $^3J = 9.8$ Hz, 2H; H-2), 5.00 (d, ^3J(H1,H2) = 9.8 Hz, 2H; H-1), 4.29 (ddd, ^3J(H5,H6) = 6.4, ^3J(H5,H6′) = 6.1, ^3J(H4,H5) = 1.1 Hz, 2H; H-5), 4.17 (dd, ^2J(H6,H6′) = 11.2, ^3J(H5,H6) = 6.0 Hz, 2H; H-6), 4.13 (dd, ^2J(H6,H6′) = 11.3, ^3J(H5,H6′) = 6.5 Hz, 2H; H-6′), 3.44 (d, $^2J = 14.3$ Hz, 2H; CHS), 3.37 (d, $^2J = 14.3$ Hz, 2H; CHS), 3.26 (t, $^3J = 7.2$ Hz, 4H; CH_2N), 3.16 (dd, $^2J = 12.9$, $^3J = 7.0$ Hz, 4H; CH_2NH), 2.21 – 2.02 (4s, 24H; 8Ac), 1.75 (m, 4H, $CH_2CH_2CH_2$), 1.51 (s, 9H; $(CH_3)_3$C); ^{13}C NMR (75 MHz, [D$_6$]DMSO, 80 °C): $\delta = 169.6$, 168.6, 167.4, 154.3 (CO), 82.0 (C-1), 78.0 ($(CH_3)_3C$), 73.4 (C-5), 70.8 (C-3), 67.3(C-2,4), 60.9 (C-6), 44.2 (CH_2NH), 36.5 (CH_2N), 32.7 (CH_2S), 27.8 ($CH_2CH_2CH_2$), 27.7 ($(CH_3)_3$C), 19.9, 19.8, 19.7, 19.6 (CH_3CO); IR (KBr): $\tilde{\nu} = 3470$, 2938, 1752, 1668, 1541, 1478, 1423, 1370, 1224, 1149, 1082, 1053, 950, 919, 775, 713, 648 cm^{-1}; HRMS (FAB): *m*/*z*: calcd for $C_{43}H_{65}N_3O_{22}S_2$Na: 1062.3399; found: 1062.3408 $[M+Na]^+$.

Bis{3-[2,3,6-tri-*O*-acetyl-4-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl-1-thiomethylcarbonylamino]propyl}-*N*-*tert*-butoxycarbonylamine (21): Column chromatography (EtOAc/MeOH 1:0 → 30:1) gave **21** (621 mg, 91 %) as a solid. M.p. 99.5 – 101.3 °C; $[\alpha]_D^{25} = -15$ ($c = 0.5$ in chloroform); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 80 °C): $\delta = 7.71$ (t, $^3J = 5.6$ Hz, 2H; NH), 5.36 (dd, ^3J(H3′,H4′) = 3.6 Hz, ^3J(H4′,H5′) = 1.1 Hz, 2H; H-4′), 5.25 (dd, ^3J(H2′,H3′) = 10.2, ^3J(H3′,H4′) = 3.7 Hz, 2H; H-3′), 5.24 (t, $^3J = 9.0$ Hz, 2H; H-3), 4.99 (d, ^3J(H1,H2) = 10.0 Hz, 2H; H-1), 4.97 (dd, ^3J(H2′,H3′) = 10.1, ^3J(H1′,H2′) = 8.0 Hz, 2H; H-2′), 4.88 (dd, $^3J = 10.0$, $^3J = 9.0$ Hz, 2H; H-2), 4.85 (d, ^3J(H1′,H2′) = 7.9 Hz, 2H; H-1′), 4.45 (brd, 2H, $^2J = 12.8$ Hz, 2H; H-6), 4.30 (brt, $^3J = 6.9$ Hz, 2H; H-5′), 4.15 (m, 6H; H-6,6′,6′), 3.93 (t, $^3J = 9.7$ Hz, 2H; H-4), 3.91 (m, 2H; H-5), 3.40 (d, $^2J = 14.2$ Hz, 2H; CHS), 3.32 (d, $^2J = 14.2$ Hz, 2H; CHS), 3.25 (t, $^3J = 7.1$ Hz, 4H; CH_2N), 3.15 (dd, $^2J = 13.7$, $^3J = 6.0$ Hz, 4H; CH_2NH), 2.19 – 2.00 (6s, 42H; 14Ac), 1.83 (m, 4H; $CH_2CH_2CH_2$), 1.51 (s, 9H; $(CH_3)_3$C); ^{13}C NMR (75 MHz, [D$_6$]DMSO, 80 °C): $\delta = 169.5$, 169.2, 168.8, 168.6, 168.4, 167.4, 161.1 (CO), 99.4 (C-1′), 81.2 (C-1), 78.1 ($(CH_3)_3C$), 75.6, 75.5 (C-4,5), 73.1 (C-3), 70.1 (C-2,3′), 69.1 (C-5′), 68.9 (C-2′), 66.9 (C-4′), 62.0 (C-6), 60.6 (C-6′), 44.2 (CH_2NH), 36.5 (CH_2N), 32.6 (CH_2S), 27.8 ($CH_2CH_2CH_2$), 27.7 ($(CH_3)_3$C), 20.0, 19.8, 19.7 (CH_3CO); IR (KBr): $\tilde{\nu}$ 3449, 2940, 1752, 1540, 1455, 1371, 1231, 1169, 1048, 953, 912, 771, 647, 604 cm^{-1}; HRMS (FAB): *m*/*z*: calcd for $C_{67}H_{97}N_3O_{38}S_2$Na: 1638.5091; found: 1638.5092 $[M+Na]^+$.

Bis{3-[2,3,4-tri-*O*-acetyl-6-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-glucopyranosyl-1-thiomethylcarbonylamino]propyl}-*N*-*tert*-butoxycarbonylamine (22): Column chromatography (EtOAc/MeOH 1:0 → 30:1) gave **22** (1.43 g, 67 %), as a solid. M.p. 98.0 – 101.2 °C; $[\alpha]_D^{25} = +43$ ($c =$ 0.5 in chloroform); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 80 °C): $\delta = 7.70$ (brs, 2H; NH), 5.47 (dd, ^3J(H3′,H4′) = 3.1, ^3J(H4′,H5′) = 1.0 Hz, 2H; H-4′), 5.35 (t, $^3J = 9.4$ Hz, 2H; H-3), 5.33 (dd, ^3J(H2′,H3′) = 10.6, ^3J(H3′,H4′) = 3.1 Hz, 2H; H-3′), 5.25 (d, ^3J(H1′,H2′) = 3.6 Hz, 2H; H-1′), 5.12 (t, $^3J = 9.4$ Hz, 2H; H-4), 5.10 (dd, ^3J(H2′,H3′) = 10.6, ^3J(H1′,H2′) = 3.6 Hz, 2H; H-2′), 5.05 (d, ^3J(H1,H2) = 10.0 Hz, 2H; H-1), 4.92 (t, $^3J = 10.0$ Hz, 2H; H-2), 4.30 (brt, $^3J = 5.9$ Hz, 2H; H-5′), 4.13 (m, 4H; H-6′,6′), 4.02 (m, 2H; H-5), 3.80 (dd, ^2J(H6,H6) = 12.0, ^3J(H5,H6) = 4.3 Hz, 2H; H-6), 3.74 (dd, ^2J(H6,H6) = 12.0, ^3J(H5,H6) = 2.7 Hz, 2H; H-6), 3.43 (d, $^2J = 13.9$ Hz, 2H; CHS), 3.36 (d, $^2J = 14.2$ Hz, 2H; CHS), 3.26 (t, $^3J = 7.2$ Hz, 4H; CH_2N), 3.18 (dd, $^2J =$ 13.5, $^3J = 6.2$ Hz, 4H; CH_2NH), 2.21 – 2.04 (5s, 42H; 14Ac), 1.75 (m, 4H; $CH_2CH_2CH_2$), 1.52 (s, 9H; $(CH_3)_3$C); ^{13}C NMR (75 MHz, [D$_6$]DMSO, 80 °C): $\delta = 169.3$, 169.2, 168.8, 168.5, 168.4, 167.3 (CO), 95.3 (C-1′), 81.3 (C-1), 78.1 ($(CH_3)_3C$), 75.3 (C-5), 73.1 (C-3), 69.8 (C-2), 68.1 (C-4), 67.6 (C-4′), 67.2 (C-2′), 66.8 (C-3′), 65.9 (C-5′), 65.4 (C-6), 61.0 (C-6′), 44.2 (CH_2NH), 36.5 (CH_2N), 32.4 (CH_2S), 27.9 ($CH_2CH_2CH_2$), 27.7 ($(CH_3)_3$C), 19.9, 19.8, 19.7, 19.6, 19.5 (CH_3CO); IR (KBr): $\tilde{\nu} = 3459$, 2943, 1753, 1664, 1374, 1226, 1039 cm^{-1}; HRMS (FAB): *m*/*z*: calcd for $C_{67}H_{97}N_3O_{38}S_2$: 1615.5193; found: 1615.5124 $[M]^+$.

General procedure for the synthesis of *N*-chloroacetyl compounds 23 – 25: A solution of **20** (2.11 mmol), **21** (1.15 mmol) or **22** (0.29 mmol) in 22 % trifluoroacetic acid in CH_2Cl_2 (40 mL) was stirred for 3 – 6 h at 0 °C (ice bath). The solution was concentrated and dried under vacuum. The obtained residue was suspended in CH_3CN (30 mL). Diisopropylethylamine was added until basic moist pH, and then was added chloroacetic anhydride (2 equiv). The reaction mixture was stirred at room temperature for 7 – 8 h. The solution was concentrated at 30 °C, aqueous HCl (5 %, 100 mL) was added and the aqueous layer extract with CH_2Cl_2 (2 × 150 mL). The combined organic phases were washed successively with aqueous HCl (5 %, 100 mL), H_2O (100 mL), saturated aqueous $NaHCO_3$ (2 × 100 mL), and H_2O (2 × 100 mL). The organic solution was dried (Na_2SO_4), filtered and evaporated, and the crude product purified by chromatography on silica gel.

Bis[3-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-1-thiomethylcarbonylamino)-propyl]-*N*-chloroacetylamine (23): Column chromatography (EtOAc/MeOH 30:1) to give **23** (1.99 g, 92 %) as a solid. M.p. 57.5 – 59.3 °C; $[\alpha]_D^{25} = -10$ ($c = 0.5$ in chloroform); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 80 °C): $\delta = 7.68$ (brs, 2H; NH), 5.44 (dd, ^3J(H3,H4) = 3.4, ^3J(H4,H5) = 1.2 Hz, 2H; H-4), 5.29 (dd, ^3J(H2,H3) = 9.7, ^3J(H3,H4) = 3.5 Hz, 2H; H-3), 5.12 (t, $^3J = 9.7$ Hz, 2H; H-2), 5.01 (d, ^3J(H1,H2) = 9.9 Hz, 2H; H-1), 4.35 (s, 2H; CH_2Cl), 4.29 (ddd, ^3J(H5,H6′) = 6.4, ^3J(H5,H6) = 6.1, ^3J(H4,H5) = 1.0 Hz, 2H; H-5), 4.17 (dd, $^2J = 11.2$, $^3J =$

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5.9 Hz, 2H; H-6), 4.13 (dd, ^2J = 11.3, ^3J = 6.6 Hz, 2H; H-6′), 3.47 (d, ^2J = 14.3 Hz, 2H; CHS), 3.44 (t, ^3J = 3.6 Hz, 4H; CH_2N), 3.38 (d, ^2J = 14.1 Hz, 2H; CHS), 3.22 (dd, ^2J = 12.6, ^3J = 6.6 Hz, 4H; CH_2NH), 2.21–2.02 (4s, 24H; 8Ac), 1.83 (m, 4H; $CH_2CH_2CH_2$); ^{13}C NMR (75 MHz, [D$_6$]DMSO, 80 °C): δ = 169.0, 168.5, 167.4, 165.3 (CO), 82.0 (C-1), 73.4 (C-5), 70.8 (C-3), 67.4, 67.3 (C-2,4), 60.8 (C-6), 41.0 (CH_2Cl), 36.2 (CH_2N), 32.7 (CH_2S), 27.4 ($CH_2CH_2CH_2$), 19.7, 19.6, 19.5, 19.4 (CH_3CO); IR (KBr): $\tilde{\nu}$ = 3419, 2937, 1749, 1650, 1541, 1435, 1371, 1225, 1149, 1082, 1053, 950, 919, 599 cm^{-1}; HRMS (FAB): *m*/*z*: calcd for $C_{40}H_{58}N_3O_{21}S_2ClNa$: 1038.2590; found: 1038.2588 $[M+Na]^+$.

Bis{3-[2,3,6-tri-*O*-acetyl-4-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl-1-thiomethylcarbonylamino]propyl}-*N*-chloroacetylamine (24): Column chromatography ($CHCl_3$/MeOH 40:1) to give **24** (1.83 g, 99%) as a solid. M.p. 78.3–80.2 °C; $[\alpha]_D^{25} = -14$ (*c* = 0.5 in chloroform); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 80 °C): δ = 7.61 (brs, 2H, NH), 5.37 (dd, ^3J(H3′,H4′) = 3.6, ^3J(H4′,H5′) = 1.0 Hz, 2H; H-4′), 5.25 (dd, ^3J(H2′,H3′) = 10.1, ^3J(H3′,H4′) = 3.6 Hz, 2H; H-3′), 5.24 (t, ^3J = 8.7 Hz, 2H; H-3), 4.99 (d, ^3J(H1,H2) = 10.0 Hz, 2H; H-1), 4.98 (dd, ^3J = 7.8 Hz, 2H; H-2′), 4.89 (dd, ^3J = 9.0 Hz, 2H; H-2), 4.85 (d, ^3J(H1′,H2′) = 8.0 Hz, 2H; H-1′), 4.48 (dd, ^2J = 12.0, ^3J = 1.7 Hz, 2H; H-6), 4.34 (s, 2H; CH_2Cl), 4.28 (m, 2H; H-5′), 4.16 (m, 6H; H-6,6′,6′), 3.95 (t, ^3J = 9.8 Hz, 2H; H-4), 3.89 (m, 2H; H-5), 3.42 (d, ^2J = 14.3 Hz, 2H; CHS), 3.41 (t, ^3J = 7.6 Hz, 4H; CH_2N), 3.34 (d, ^2J = 14.3 Hz, 2H; CHS), 3.21 (dd, ^2J = 12.6, ^3J = 6.7 Hz, 4H; CH_2NH), 2.19–2.00 (6s, 42H; 14Ac), 1.82 (m, 4H; $CH_2CH_2CH_2$); ^{13}C NMR (75 MHz, [D$_6$]DMSO, 80 °C): δ = 169.8, 169.4, 168.8, 168.6, 167.8 (CO), 99.6 (C-1′), 81.5 (C-1), 75.8, 75.6 (C-4,5), 73.3 (C-3), 70.3 (C-2,3′), 69.8 (C-5′), 69.1 (C-2′), 67.1 (C-4′), 62.1 (C-6), 60.8 (C-6′), 41.5 (CH_2Cl), 36.5 (CH_2N), 32.8 (CH_2S), 20.2, 20.0, 19.9, 19.8 (CH_3CO); IR (KBr): $\tilde{\nu}$ = 3457, 2982, 1752, 1653, 1541, 1435, 1372, 1230, 1170, 1137, 1049, 911, 604 cm^{-1}; HRMS (FAB): *m*/*z*: calcd for $C_{64}H_{90}N_3O_{37}S_2ClNa$: 1614.4281; found: 1614.4277 $[M+Na]^+$.

Bis{3-[2,3,4-tri-*O*-acetyl-6-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-glucopyranosyl-1-thiomethylcarbonylamino]propyl}-*N*-chlororacetylamine (25): Column chromatography (EtOAc/MeOH 1:0 → 9:1) to give **25** (432 mg, 92%) as a solid. M.p. 103.0–105.2 °C; $[\alpha]_D^{25} = +58$ (*c* = 0.5 in chloroform); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 80 °C): δ = 7.76 (brs, 2H; NH), 5.47 (brd, ^3J(H3′,H4′) = 3.1 Hz, 2H; H-4′), 5.35 (t, ^3J = 9.5 Hz, 2H; H-3), 5.33 (dd, J(H2′,H3′) = 10.7, ^3J(H3′,H4′) = 3.2 Hz, 2H; H-3′), 5.25 (d, ^3J(H1′,H2′) = 3.6 Hz, 2H; H-1′), 5.12 (t, ^3J = 9.5 Hz, 2H; H-4), 5.10 (dd, ^3J(H2′,H3′) = 10.8, ^3J(H1′,H2′) = 3.4 Hz, 2H; H-2′), 5.05 (d, ^3J(H1,H2) = 9.5 Hz, 2H; H-1), 4.92 (t, ^3J = 9.5 Hz, 2H; H-2), 4.36 (s, 2H; CH_2Cl), 4.30 (brt, ^3J = 6.4 Hz, 2H; H-5′), 4.16 (m, 4H; H-6′,6′), 4.02 (m, 2H; H-5), 3.80 (dd, ^2J(H6,H6) = 11.9, ^3J(H5,H6) = 4.4 Hz, 2H; H-6), 3.74 (dd, ^2J(H6,H6) = 11.9, ^3J(H5,H6) = 2.3 Hz, 2H; H-6), 3.44 (d, ^2J = 14.2 Hz, 2H; CHS), 3.41 (t, ^3J = 6.7 Hz, 4H; CH_2N), 3.37 (d, ^2J = 13.9 Hz, 2H; CHS), 3.20 (dd, ^2J = 12.6, ^3J = 6.5 Hz, 4H; CH_2NH), 2.21–2.04 (5s, 42H; 14Ac), 1.81 (m, 4H; $CH_2CH_2CH_2$); ^{13}C NMR (75 MHz, [D$_6$]DMSO, 80 °C): δ = 168.8, 168.5, 168.4, 167.5, 165.4 (CO), 95.3 (C-1′), 81.4 (C-1), 75.3 (C-5), 73.1 (C-3), 69.9 (C-2), 68.1 (C-4), 67.6 (C-4′), 67.2 (C-2′), 66.8 (C-3′), 65.9 (C-5′), 65.4 (C-6), 61.0 (C-6′), 41.2 (CH_2Cl), 36.3 (CH_2N), 32.4 (CH_2S), 29.1 ($CH_2CH_2CH_2$), 19.9, 19.8, 19.7, 19.6 (CH_3CO); IR (KBr): $\tilde{\nu}$ = 3475, 2943, 1753, 1375, 1226, 1039 cm^{-1}; HRMS (FAB): *m*/*z*: calcd for $C_{64}H_{90}N_3O_{37}S_2Cl$: 1591.4385; found: 1591.4392 $[M]^+$.

General procedure for synthesis of thiol 26–28: A solution of **23** (1.83 mmol), **24** (0.75 mmol) or **25** (0.27 mmol) in anhydrous acetone (15–30 mL) was added thiourea (10 equiv). The reaction mixture was stirred at room temperature for 24 h. The solution was concentrated approximately up to half volume without heating. Then a solution of Na_2SO_3 (3 equiv) in H_2O (10–30 mL) was added and the reaction was stirred for 30 min. Aqueous HCl (5%, 5 mL), H_2O (100 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (2 × 150 mL). The combined organic phases was washed with H_2O (100 mL). The organic layer was dried (Na_2SO_4), filtered, evaporated, and the crude product purified by chromatography on silica gel.

Bis[3-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-1-thiomethylcarbonylamino)propyl]-*N*-mercaptoacetylamine (26): Column chromatography (EtOAc/MeOH 30:1) to give **26** (1.05 g, 56%) as a solid. M.p. 61.6–63.0 °C; $[\alpha]_D^{25} = -12$ (*c* = 0.5 in chloroform); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 80 °C): δ = 7.80 (brs, 2H; NH), 5.43 (brd, ^3J = 3.2 Hz, 2H; H-4), 5.29 (dd, ^3J(H2,H3) = 9.5, ^3J(H3,H4) = 3.6 Hz, 2H; H-3), 5.09 (t, ^3J = 9.8 Hz, 2H; H-2), 5.01 (d, ^3J(H1,H2) = 10.1 Hz, 2H; H-1), 4.29 (brt, ^3J = 6.1 Hz, 2H; H-5), 4.15 (m, 4H; H-6,6′), 3.47 (d, ^3J = 6.8 Hz, 2H; CH_2SH), 3.43–3.35 (m, 8H; CH_2S, CH_2N), 3.20 (brs, 4H; CH_2NH), 2.66(t, ^3J = 7.1 Hz, 1H; SH), 2.21–2.02 (4s, 24H; 8Ac), 1.80 (brs, 4H; $CH_2CH_2CH_2$); ^{13}C NMR (75 MHz, [D$_6$]DMSO, 80 °C): δ = 169.3, 168.9, 168.8, 167.6 (CO), 82.1 (C-1), 73.4 (C-5), 70.9 (C-3), 67.4 (C-2,4), 61.0 (C-6), 36.3 (CH_2N), 32.8 (CH_2S), 25.1 (CH_2SH), 20.0, 19.9, 19.8, 19.7 (CH_3CO); IR (KBr): $\tilde{\nu}$ = 3457, 2939, 1750, 1646, 1372, 1227, 1053 cm^{-1}; HRMS (FAB): *m*/*z*: calcd for $C_{40}H_{59}N_3O_{21}S_3Na$: 1036.2701; found: 1036.2698 $[M+Na]^+$.

Bis{3-[2,3,6-tri-*O*-acetyl-4-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl-1-thiomethylcarbonylamino]propyl}-*N*-mercaptoacetylamine (27): Column chromatography ($CHCl_3$/MeOH 30:1) to give **27** (660 mg, 55%) as a solid. M.p. 98.8–100.7 °C; $[\alpha]_D^{25} = -24$ (*c* = 0.5 in chloroform); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 80 °C): δ = 7.70 (brs, 2H; NH), 5.36 (brd, ^3J = 3.5 Hz, 2H; H-4′), 5.25 (dd, ^3J(H2′,H3′) = 10.2 Hz, ^3J(H3′,H4′) = 3.7 Hz, 2H; H-3′), 5.25 (t, ^3J = 8.7 Hz, 2H; H-3), 5.00 (d, ^3J(H1,H2) = 10.0 Hz, 2H; H-1), 4.97 (dd, ^3J(H2′,H3′) = 10.0, ^3J(H1′,H2′) = 7.9 Hz, 2H; H-2′), 4.88 (t, ^3J = 9.2 Hz, 2H; H-2), 4.85 (d, ^3J(H1′,H2′) = 7.9 Hz, 2H; H-1′), 4.47 (brd, ^2J = 12.2 Hz, 2H; H-6), 4.29 (brt, ^3J = 6.5 Hz, 2H; H-5′), 4.15 (m, 6H; H-6,6′,6′), 3.94 (t, ^3J = 9.9 Hz, 2H; H-4), 3.91 (m, 2H; H-5), 3.47 (d, ^3J = 7.0 Hz, 2H; CH_2SH), 3.42 (d, ^2J = 14.5 Hz, 2H; CHS), 3.39 (t, ^3J = 7.5 Hz, 4H; CH_2N), 3.33 (d, ^2J = 14.5 Hz, 2H; CHS), 3.19 (dd, ^2J = 12.4, ^3J = 6.2 Hz, 4H; CH_2NH), 2.64 (t, ^3J = 6.8 Hz, 1H; SH), 2.19–2.00 (7s, 42H; 14Ac), 1.79 (brs, 4H; $CH_2CH_2CH_2$); ^{13}C NMR (75 MHz, [D$_6$]DMSO, 80 °C): δ = 169.3, 169.2, 168.8, 168.7, 168.6, 168.3, 167.6 (CO), 99.4 (C-1′), 81.3 (C-1), 75.7, 75.4 (C-4,5), 73.1 (C-3), 70.2 (C-2,3′), 69.7 (C-5′), 68.9 (C-2′), 67.0 (C-4′), 61.9 (C-6), 60.6 (C-6′), 36.3 (CH_2N), 32.6 (CH_2S), 29.9 ($CH_2CH_2CH_2$), 25.1 (CH_2SH), 19.9, 19.8, 19.7, 19.6, 19.5 (CH_3CO); IR (KBr): $\tilde{\nu}$ = 3482, 2939, 1753, 1374, 1225, 1038 cm^{-1}; HRMS (FAB): *m*/*z*: calcd for $C_{64}H_{91}N_3O_{37}S_3Na$: 1612.4391; found: 1612.4385 $[M+Na]^+$.

Bis{3-[2,3,4-tri-*O*-acetyl-6-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-glucopyranosyl-1-thiomethylcarbonylamino]propyl}-*N*-mercaptoacetylamine (28): Column chromatography (EtOAc/MeOH 30:1) to give **28** (325 mg, 75%) as a solid. M.p. 98.0–100.8 °C; $[\alpha]_D^{25} = +62$ (*c* = 0.5 in chloroform); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 80 °C): δ = 7.75 (brs, 2H; NH), 5.47 (brd, ^3J(H3′,H4′) = 2.7 Hz, 2H; H-4′), 5.36 (t, ^3J = 9.6 Hz, 2H; H-3), 5.33 (dd, ^3J(H2′,H3′) = 10.3, ^3J(H3′,H4′) = 3.6 Hz, 2H; H-3′), 5.25 (d, ^3J(H1′,H2′) = 3.6 Hz, 2H; H-1′), 5.12 (t, ^3J = 9.6 Hz, 2H; H-4), 5.10 (dd, ^3J(H2′,H3′) = 10.3, ^3J(H1′,H2′) = 3.6 Hz, 2H; H-2′), 5.05 (d, ^3J(H1,H2) = 9.6 Hz, 2H; H-1), 4.92 (t, ^3J = 9.6 Hz, 2H; H-2), 4.30 (brt, ^3J = 5.9 Hz, 2H; H-5′), 4.16 (m, 4H; H-6′,6′), 4.02 (m, 2H; H-5), 3.80 (dd, ^2J(H6,H6) = 12.0, ^3J(H5,H6) = 4.5 Hz, 2H; H-6), 3.74 (dd, ^2J(H6,H6) = 12.1, ^3J(H5,H6) = 3.2 Hz, 2H; H-6), 3.47 (d, ^3J = 7.0 Hz, 2H; CH_2SH), 3.44 (d, ^2J = 14.1 Hz, 2H; CHS), 3.40 (t, ^3J = 7.3 Hz, 4H; CH_2N), 3.37 (d, ^2J = 14.3 Hz, 2H; CHS), 3.20 (m, dd, ^2J = 12.4, ^3J = 6.3 Hz, 4H; CH_2NH), 2.66 (t, ^3J = 6.9 Hz, 1H; SH), 2.21–2.04 (5s, 42H; 14Ac), 1.80 (m, 4H; $CH_2CH_2CH_2$); ^{13}C NMR (75 MHz, [D$_6$]DMSO, 80 °C): δ = 169.3, 169.2, 168.9, 168.5, 168.4 (CO), 95.3 (C-1′), 81.4 (C-1), 75.3 (C-5), 73.1 (C-3), 69.9 (C-2), 68.1 (C-4), 67.6 (C-4′), 67.2 (C-2′), 66.8 (C-3′), 65.9 (C-5′), 65.4 (C-6), 61.0 (C-6′), 36.3 (CH_2N), 32.4 (CH_2S), 25.0 (CH_2SH), 19.9, 19.8, 19.6, 19.5, 19.4 (CH_3CO); IR (KBr): $\tilde{\nu}$ = 3494, 2942, 1753, 1374, 1225, 1038 cm^{-1}; HRMS (FAB): *m*/*z*: calcd for $C_{64}H_{91}N_3O_{37}S_3$: 1589.4493; found: 1589.4514 $[M]^+$.

General procedure for the synthesis of CDs 29–31: A mixture of **5**[13] (0.02 mmol for reaction with **26**, 0.03 mmol for reaction with **27** and 0.03 mmol for reaction with **28**), Cs_2CO_3 (2.5 equiv) and the thiol **26, 27** or **28** (2 equiv) in anhydrous DMF (8–20 mL) was kept under Ar for 7 d at 60 °C. After this time, Ac_2O (4 mL), pyridine (2 mL), and DMAP (cat.) were added and the reaction mixture was stirred for 48 h at 40 °C. Then the precipitated material was filtered and the filtrate was poured over ice/H_2O. Aqueous HCl (5%, 100 mL) was added and the aqueous layer extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were washed successively with aqueous HCl (5%, 100 mL), H_2O (100 mL), saturated $NaHCO_3$ (2 × 150 mL) and H_2O (2 × 100 mL). The organic solution was dried (Na_2SO_4), filtered, evaporated to obtain a residue that was subjected to column chromatography.

Glyco-CD (29): The product was purified by column chromatography (EtOAc/MeOH 1:0 → 10:1). The isolated solid was dissolved in CH_2Cl_2 and diethyl ether was added until precipitation. The resulting precipitated was filtered, and gave **29** (120 mg, 79%) as a solid. M.p. 93.4 °C (decomp); $[\alpha]_D^{25} = +2$ (*c* = 0.5 in chloroform); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 80°C): δ = 7.69 (brs, 14H; NH), 5.44 (brd, ^3J(H3′,H4′) = 3.5 Hz, 14H; H-4′), 5.35

(brt, 7H; H-3), 5.29 (dd, ^3J(H2′,H3′) = 9.7, ^3J(H3′,H4′) = 3.6 Hz, 14H; H-3′), 5.20 (brd, ^3J(H1,H2) = 3.2 Hz, 7H; H-1), 5.11 (t, ^3J(H1′,H2′) = 9.8 Hz, 14H; H-2′), 5.00 (d, ^3J(H1′,H2′) = 9.8 Hz, 14H; H-1′), 4.84 (brdd, ^3J(H2,H3) = 9.8, ^3J(H1,H2) = 3.4 Hz, 7H; H-2), 4.28 (brt, ^3J(H5′,H6′) = 6.2 Hz, 14H; H-5′), 4.20 (m, 7H; H-4), 4.19–4.15 (m, ^3J(H5′,H6′) = 6.5 Hz, 35H; H-5,6′,6′), 3.76 (brd, ^2J = 14.4 Hz, 7H; H-6), 3.58 (brd, ^2J = 14.4 Hz, 7H; H-6), 3.50–3.38 (m, 28H; CH_2N), 3.47 (d, ^2J = 14.1 Hz, 21H; CHS), 3.40 (d, ^2J = 14.1 Hz, 21H; CHS), 3.23 (m, 28H; CH_2NH), 2.22, 2.14, 2.12, 2.11, 2.02 (5s, 210H; 70Ac), 1.82 (brs, 28H; $CH_2CH_2CH_2$); ^{13}C NMR (75 MHz, [D$_6$]DMSO, 80 °C): δ = 167.6–169.4 (CO), 96.3 (C-1), 82.0 (C-1′), 77.6 (C-4), 73.4 (C-5′), 71.3 (C-5), 70.8 (C-3′), 69.9, 69.8 (C-2,3), 67.4, 67.3 (C-2′,4′), 60.8 (C-6′), 45.3, 43.2 (CH_2N), 36.5, 36.2 (CH_2NH), 35.1 (C-6), 32.7 (CH_2S), 28.0, 27.1 ($CH_2CH_2CH_2$), 19.5–19.9 (CH_3CO); IR (KBr): $\tilde{\nu}$ = 3471, 2939, 1750, 1650, 1371, 1227, 1054 cm^{-1}; MS (MALDI-TOF): *m*/*z*: calcd for $C_{350}H_{497}N_{21}O_{189}S_{21}$: 8696.12; found: 8718.60 [*M*+Na]$^+$.

Glyco-CD (30): The product was purified by column chromatography (EtOAc/MeOH 1:0 → 20:1). The isolated solid was dissolved in CH_2Cl_2 and diethyl ether was added until precipitation. The resulting precipitated was filtered, and gave **30** (329 mg, 80%) as a solid. M.p. 117.9 °C (decomp); $[\alpha]_D^{25} = -3$ (*c* = 0.5 in chloroform); ^{1}H NMR (400 MHz, [D$_6$]DMSO, 100 °C): δ = 7.51 (brs, 14H; NH), 5.25 (brd, ^3J(H3″,H4″) = 3.6 Hz, 14H; H-4″), 5.25 (brd, ^3J = 8.7 Hz, 7H; H-3), 5.13 (dd, ^3J(H2″,H3″) = 10.3, ^3J(H3″,H4″) = 3.7 Hz, 14H; H-3″), 5.12 (t, ^3J = 8.4 Hz, 14H; H-3′), 5.07 (brd, ^3J(H1,H2) = 2.6 Hz, 7H; H-1), 4.86 (dd, ^3J(H2″,H3″) = 10.2, ^3J(H1″,H2″) = 7.9 Hz, 14H; H-2″), 4.86 (d, ^3J(H1′,H2′) = 10.0 Hz, 14H; H-1′), 4.76 (t, ^3J = 9.5 Hz, 14H; H-2′), 4.73 (d, ^3J(H1″,H2″) = 7.9 Hz, 14H; H-1″), 4.36 (brd, ^2J = 12.0 Hz, 14H; H-6′), 4.17–3.97 (m, 56H; H-4,5,6′,6″,6″), 4.17 (brt, ^3J = 6.9 Hz, 14H; H-5″), 3.82 (t, ^3J = 8.6 Hz, 14H; H-4′), 3.79–3.74 (m, 14H; H-5′), 3.62 (brd, ^2J = 14.0 Hz, 7H; H-6), 3.44 (brd, ^2J = 13.7 Hz, 7H; H-6), 3.31 (d, ^2J = 14.2 Hz, 21H; CHS), 3.25 (d, ^2J = 14.0 Hz, 21H; CHS), 3.32–3.24 (m, 28H; CH_2N), 3.09 (m, 28H; CH_2NH), 2.08, 2.06, 1.99, 1.98, 1.97, 1.88 (6s, 336H; 112Ac), 1.68 (brs, 28H; $CH_2CH_2CH_2$); ^{13}C NMR (75 MHz, [D$_6$]DMSO, 80 °C): δ = 169.6–161.8 (CO), 99.5 (C-1″), 96.3 (C-1), 81.3 (C-1′), 77.6 (C-4), 75.6, 75.4 (C-4′,5′), 73.1 (C-3′,3″), 71.3 (C-5), 70.1 (C-2′,3′,3″), 69.9 (C-2,3), 69.6 (C-5″), 68.9 (C-2″), 66.9 (C-4″), 61.9 (C-6′), 60.5 (C-6″), 45.3, 43.2 (CH_2N), 36.5 (CH_2NH), 35.2 (C-6), 32.6 (CH_2S), 28.3, 27.1 ($CH_2CH_2CH_2$), 19.7–20.0 (CH_3CO); IR (KBr): $\tilde{\nu}$ = 3511, 2940, 1751, 1434, 1232, 1049 cm^{-1}; MS (MALDI-TOF): *m*/*z*: calcd for $C_{518}H_{721}N_{21}O_{301}S_{21}$: 12731.63; found: 12756.70 [*M*+Na]$^+$.

Glyco-CD (31): The product was purified by column chromatography (EtOAc/MeOH 1:0 → 20:1.) The isolated solid was dissolved in CH_2Cl_2 and diethyl ether was added until precipitation. The resulting precipitated was filtered, and gave **30** (345 mg, 92%) as a solid. M.p. 131.6 °C (decomp); $[\alpha]_D^{25} = +39$ (*c* = 0.5 in chloroform); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 80°C): δ = 7.63 (brs, 14H; NH), 5.47 (brd, ^3J(H3″,H4″) = 2.3 Hz, 14H; H-4″), 5.37 (m, 7H; H-3), 5.34 (t, ^3J = 8.4 Hz, 14H; H-3′), 5.32 (dd, ^3J(H2″,H3″) = 10.8, ^3J(H3″,H4″) = 2.6 Hz, 14H; H-3″), 5.25 (d, ^3J(H1″,H2″) = 3.6 Hz, 14H; H-1″), 5.21 (brs, 7H; H-1), 5.16 (t, ^3J = 9.2 Hz, 14H; H-4′), 5.10 (dd, ^3J(H2″,H3″) = 10.8, ^3J(H1″,H2″) = 3.6 Hz, 14H; H-2″), 5.03 (d, ^3J(H1′,H2′) = 9.9 Hz, 14H; H-1′), 4.92 (t, ^3J(H1′,H2′) = 9.5 Hz, 14H; H-2′), 4.84 (brd, ^3J = 9.6 Hz, 7H; H-2), 4.29–4.17 (m, 42H; H-4,5,6′,6′), 4.29 (brt, ^3J = 6.0 Hz, 14H; H-5″), 4.00 (m, 14H; H-5′), 3.85–3.73 (m, 35H; H-6,6′,6′), 3.57 (brd, ^2J = 13.0 Hz, 7H; H-6), 3.42 (brs, 70H; CH_2S, CH_2N), 3.22 (brs, 28H; CH_2NH), 2.21, 2.18, 2.14, 2.11, 2.05, 2.04 (6s, 336H; 112Ac),1.82 (brs, 28H; $CH_2CH_2CH_2$); ^{13}C NMR (75 MHz, [D$_6$]DMSO, 80 °C): δ = 169.3–167.4 (CO), 96.3 (C-1), 95.4 (C-1″), 81.5 (C-1′), 77.5 (C-4), 75.3 (C-5′), 73.2 (C-3′,3″), 71.3 (C-5), 69.9 (C-2′), 68.3 (C-2,3), 68.1 (C-4′), 67.6 (C-4″), 67.2 (C-2″), 66.8 (C-3′,3″), 65.9 (C-5″), 65.4 (C-6′), 61.0 (C-6″), 45.1, 43.3 (CH_2N), 36.5 (CH_2NH), 35.1 (C-6), 32.4 (CH_2S), 28.1, 27.3 ($CH_2CH_2CH_2$), 19.5–19.9 (CH_3CO); IR (KBr): $\tilde{\nu}$ = 3447, 2940, 1751, 1654, 1374, 1229, 1040 cm^{-1}; MS (MALDI-TOF): *m*/*z*: calcd for $C_{518}H_{721}N_{21}O_{301}S_{21}$: 12731.63; found: 12757.90 [*M*+Na]$^+$.

General procedure for the Zemplén de-O-acetylation of *S*-CDs 29–31: A solution of compound **29** (250 mg, 0.03 mmol), **30** (380 mg, 0.03 mmol) and **31** (307 mg, 0.02 mmol) in dry MeOH (6–8 mL) was made alkaline to pH 9 (indicator paper) with a methanolic solution of NaOMe (1M). The reaction mixture was stirred overnight at room temperature and the precipitated material was filtered, washed with MeOH and dissolved in H_2O (6 mL). The solution was concentrated by lyophilization and gave a solid.

Glyco-CD (32): Yield: 138 mg, 84%; m.p. 156.3 °C (decomp); $[\alpha]_D^{25} = -22$ (*c* = 0.25 in H_2O); ^{13}C NMR (75 MHz, D_2O): δ = 172.2–177.1 (CO), 102.5 (C-1), 85.4 (C-1′), 79.0, 73.1, 72.4, 72.3, 69.4, 68.6 (C-2,2′,3,3′,4,4′,5,5′), 61.0 (C-6′), 46.1, 43.9, 37.2, 32.7, 27.9, 26.5 (CH_2); IR (KBr): $\tilde{\nu}$ = 3421, 2924, 1636, 1043 cm^{-1}; MS (MALDI-TOF): *m*/*z*: calcd for $C_{210}H_{357}N_{21}O_{119}S_{21}$: 5753.54; found: 5778.90 [*M*+Na]$^+$.

Glyco-CD (33): Yield: 206 mg, 86%; m.p. 182.8 °C (decomp); $[\alpha]_D^{25} = -6$ (*c* = 0.25 in H_2O); ^{13}C NMR (75 MHz, D_2O, 80 °C): δ = 172.5–171.7 (CO), 103.8 (C-1″), 102.9 (C-1), 85.7 (C-1′), 85.4 (C-4), 79.7, 79.6, 76.6, 76.1, 73.7, 69.5 (C-3′,3″,4′,4″,5′,5″), 73.1 (C-2′), 76.5, 73.0 (C-2,3,5), 71.9 (C-2″), 61.6 (C-6′,6″), 47.1, 44.9, 38.2, 34.0, 29.0, 27.7, (CH_2); IR (KBr): $\tilde{\nu}$ = 3405, 2935, 1637, 1071, 1042 cm^{-1}; MS (MALDI-TOF): *m*/*z*: calcd for $C_{294}H_{497}N_{21}O_{189}S_{21}$: 8023.50; found: 8044.60 [*M*+Na]$^+$.

Glyco-CD (33): Yield: 189 mg, 98%; m.p. 152 °C (decomp); $[\alpha]_D^{25} = +186$ (*c* = 0.25 in H_2O); ^{13}C NMR (75 MHz, D_2O): δ = 171.9–168.2 (CO), 102.6 (C-1), 98.0 (C-1″), 85.4 (C-1′), 78.3, 77.4, 75.1, 72.7, 72.3, 70.9, 69.5, 69.1, 69.0, 68.4 (C-2,2′,2″,3,3′,3″,4,4′,4″,5,5′,5″), 65.3, 61.0 (C-6′,6″), 46.2, 44.0, 37.3, 32.7, 27.9, 26.6 (CH_2); IR (KBr): $\tilde{\nu}$ = 3412, 2928, 1646, 1030 cm^{-1}; MS (MALDI-TOF): *m*/*z*: calcd for $C_{294}H_{497}N_{21}O_{189}S_{21}$: 8023.50; found: 8043.40 [*M*+Na]$^+$.

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