Synthesis of cyclodextrin derivatives with monosacharides and their binding with ampicillin and selected lectins

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Dedicated to Professor Michael Orfanopoulos on the occasion of his 67th birthday

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Abstract

β- and *γ*-Cyclodextrins connected on their narrow side *via* 6 bond-linkers with monosaccharides (Man, GlcNAc) suitable for bacterial lectin recognition, along with reference mono-analogues were prepared. The resulting compounds: (a) posses a functional cavity forming inclusion complexes with a model guest molecule and with the antibiotic ampicillin; (b) inhibit by more than 30% the hydrolysis of ampicillin in the presence of the enzyme *β*-lactamase; (c) interact stronger with surface immobilized lectins than the reference mono-analogues. The present work suggests that combining the lectin recognition property of the compounds with their ability to encapsulate antibiotics could be used to target and kill bacteria.

Keywords: Cyclodextrin, glycoclusters, monosaccharides, bacteria, antibiotics, ampicillin

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Introduction

Pathogenic bacteria frequently colonize and infect host tissues by adhesion of lectins present on their surface to complementary carbohydrates of the host cells.[1,2] Among hundreds of monosaccharides found in nature lectins recognize just a few, primarily D-glucose (Glc), D-galactose (Gal), D-mannose (Man), D-*N*-acetylglucosamine (GlcNAc), D-*N*-acetylgalactosamine (GalNAc), L-fucose (Fuc) and D-*N*-acetylneuraminic acid.[3] Therefore, a monosaccharide can be a targeting moiety recognized by a bacterial lectin. However, since the binding affinities of monovalent carbohydrates to lectins are weak to moderate[1] nature utilizes multivalency to create cooperative mechanisms to increase lectin-carbohydrate affinities. The multivalency of ligands[4,5] as well as the multiple carbohydrate binding modules on the lectins provide strengthened binding with amplified specificity of the recognition.[1]

Cyclodextrins (CDs) offer unique features towards the formation of glycoclusters for biological applications, since they are biocompatible and can be easily modified in their primary side with carbohydrate moieties. Per-carbohydrate modified CD derivatives have been reported[6-15] demonstrating the advantages of multivalency and clustering effect in homo- and heterogeneously decorated cyclodextrin scaffolds. Herein, the synthesis of a series of new cyclodextrin-based glycoclusters with seven and eight monosaccharide units connected to the CD core *via* optimally sized linkers, as well as the corresponding monosubstituted derivatives, is reported. The compounds also form complexes with a common *β*-lactam antibiotic, ampicillin, and partially inhibit its hydrolysis rate in the presence of a *β*-lactamase enzyme, in buffer conditions and at the same time they bind strongly with specific lectins, which is exactly the objective of the present work: to show the potential of the synthesized compounds to act as stabilizing agents for antibiotics which can be carried to the bacterial surface.

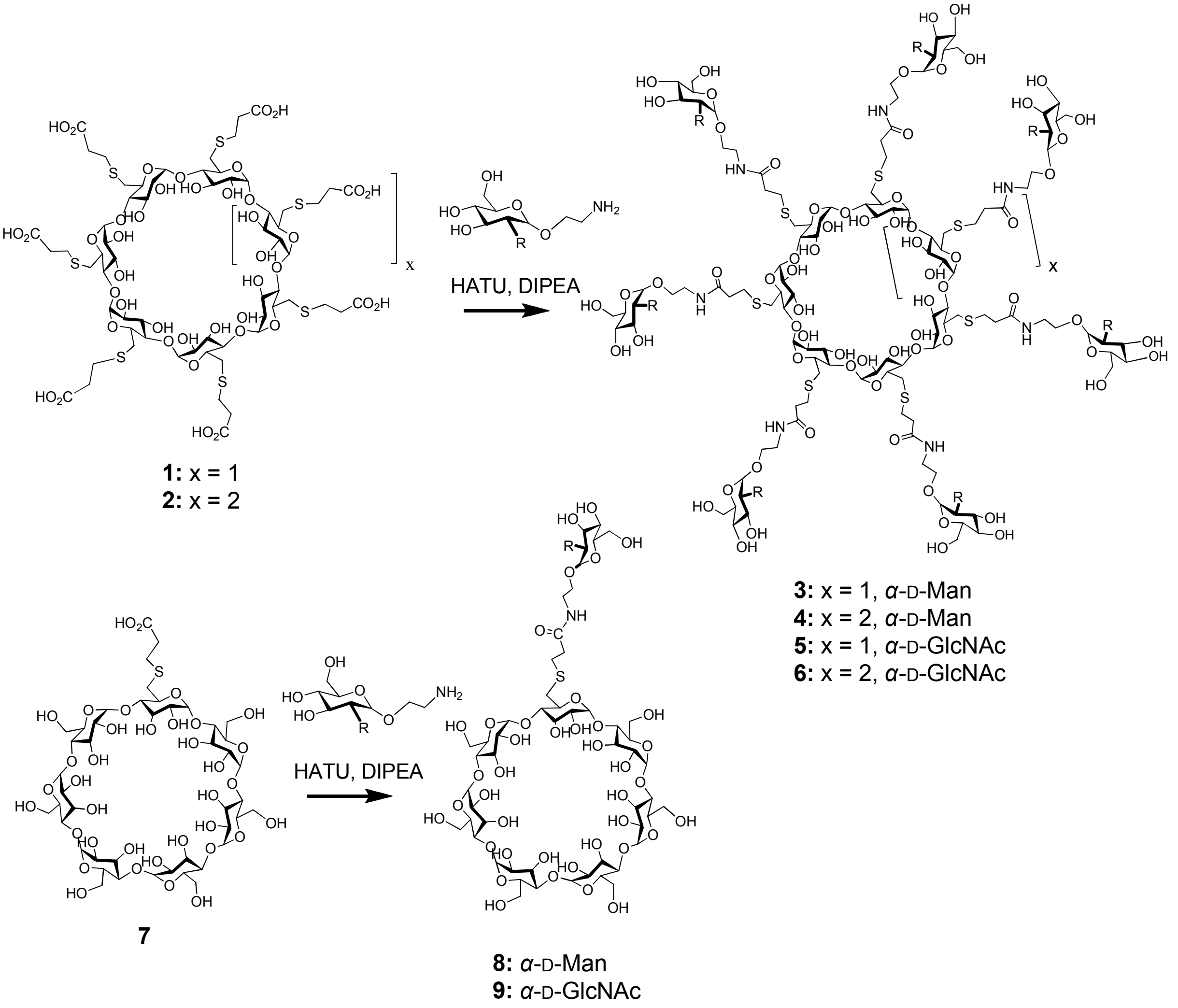
Results and Discussion

HATU-mediated amide bond formation between 2'-aminoethyl–appended monosaccharides and the per-carboxyethylthio-terminated cyclodextrin derivatives **1** and **2**[16] and the novel mono-carboxy-derivative **7** (Scheme 1) gave the monodisperse products **3-6** and **8-9**, respectively, whereas using HOBT/DCC coupling under numerous conditions (ratio, temperature, duration) products of inferior quality were obtained.

The monosaccharides with 2'-aminoethyl linkers were synthesized according to literature methods from the parent monosaccharides: 2'-aminoethyl-*α*-D-mannopyranose was prepared in five steps and 59% overall yield[17] and 2'-aminoethyl-2-acetamide-2-deoxy-*α*-D-glucopyranose was prepared in three steps and 69% overall yield from the known azido precursor.[18,19] The linker chain between the amino-end and the monosaccharides was limited to two carbon atoms so that after connection to the CD core the total carbon atoms would be 6. Literature suggests[14] that CD-based glycoclusters with connectors 2-5 carbon atoms apart from the core have much higher affinity towards blood glycoproteins, compared with glycoclusters having longer linkers.

In all compounds (Scheme 1), connection of the monosaccharide part with the CD core was verified by detecting either the N$\underline{H}$---$\underline{C}$=O scalar coupling through bonds (2D HMBC NMR spectra) or the dipolar through space coupling (ROESY/NOESY NMR spectra) between the two components. In addition, two derivatives with only one monosaccharide unit on the CD were synthesised from the mono-carboxyl precursor **7** and characterised in a similar manner.

Guest binding and partial inhibition of enzymatic degradation of ampicillin by *β*-lactamase. Although per-substitution of the primary hydroxyl groups of a cyclodextrin with carbohydrate moieties is expected to produce glycoclusters that bind stronger to lectins than the mono-analogs[14] the cavity inclusion ability may be decreased or increased, compared with the unsubstituted CDs, while hyperbranched derivatives may totally loose their cavity binding capacity.[14,20] Therefore, the inclusion ability of the cavity of the current glycoclusters was initially tested with a typical guest molecule, *p*-*tert*-butylbenzoic acid, which is known to form strong inclusion complexes with β-CD in solution[21] ($K_{\text{assoc}} \geq 10^4$ M^{-1}) and in the crystalline state.[22] In addition, this guest molecule lacks NMR signals in the carbohydrate region (5-2.5 ppm). 2D ROESY NMR spectra revealed clear dipolar interactions between the AA'XX' aromatic proton doublets of the guest at 7.75 and 7.45 ppm and the signals attributed to the cyclodextrin cavity protons of **4** at ~3.9 ppm. Therefore the cavity is indeed capable of hosting a model molecule while the linkers with the monosaccharide appendages seem to neither block the cavity nor interfere with the guest inclusion.

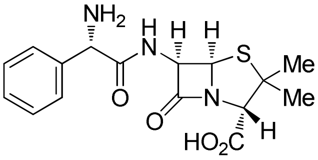


Scheme 1. Preparation of glycoclusters and monosaccharide-CD derivatives.

Ampicillin, a very frequently used broad spectrum β-lactam antibiotic, was tested next. We have previously shown by NMR spectroscopy and mass spectrometry[23] that ampicillin complexes with **2** *via* both cavity inclusion and external interactions,[23,24] thus demonstrating the contribution and importance of non-cavity effects.[24] Ampicillin solutions in the presence of **4** and **6** were examined presently by 2D ROESY NMR experiments. The spectra showed clear intermolecular interactions of the drug's phenyl group with the cavity protons of **4** and **6** indicating that the drug is encapsulated in the cavity of the compounds.

The rate of enzymatic hydrolysis of ampicillin in the presence of β-lactamase (EC 3.5.2.6) from *Enterobacter cloacae* was studied in buffered aqueous solutions (phosphate, pH 7) of **2**, **4** and **6** and of the linear analogue of β-CD, maltoheptaose**,** at constant temperature (25 ºC). Pseudo first-order conditions were used, *i.e.*, 5- to 10-fold excess of host compounds and maltoheptaose. Degradation was quantified by monitoring the area decrease corresponding to the signals of the two methyl groups of ampicillin by ^{1}H NMR spectroscopy; therefore the rate of degradation was defined as the ratio of the integral of the methyl peaks over the total integral of the phenyl peaks, *vs* time. The error of the method was assessed as ±10% by running the hydrolysis experiments of ampicillin alone in triplicate. The degradation profiles were fitted satisfactorily into a mono-exponential decay equation and consequently linear plots of $\ln([A_0]/[A])$ *vs* time were obtained (where $[A_0]$ and $[A]$ are the ampicillin concentrations initially and at time t). The rates thus calculated (Table 1) show that **2** inhibits the degradation of ampicillin by ~50%, as shown previously by this group,[23] while **4** and **6** inhibit hydrolysis by ~30 and ~40%, respectively, statistically different from linear maltoheptaose (~20%). The derivatives thus offer protection to ampicillin presumably *via* cavity inclusion. However, external interactions (non-cavity) effects, as shown before[23] cannot be ruled out. The combined effects result in partial inhibition of the ampicillin degradation rates which are, in descending order, **2** > **6** ≥ **4** > maltoheptaose. The results show that the prepared glycoclusters can bind and partially protect ampicillin from degradation.

Table 1. Rates of hydrolysis of ampicillin in the presence of β-lactamase (25 ºC)



k (× 10^{-4} h^{-1})	Enzymatic Hydrolysis of	R^2
108 (±4.0)	ampicillin	0.996
44.2 (±2.6)	ampicillin/**2**	0.988
74.7 (±2.5)	ampicillin/**4**	0.995
63.3 (±4.3)	ampicillin/**6**	0.986
90.3 (±1.6)	ampicillin/maltoheptaose	0.999

Interaction of glycoclusters with selected lectins: real-time monitoring of binding by White Light Reflectance Spectroscopy. Two selected commercial lectins were tested *in vitro*: (a) wheat germ agglutinin (WGA) that shows binding affinity for GlcNAc, has four sugar binding pockets per monomer and in the crystal forms tetramers;[25] (b) galanthus nivalis (GNL),[26] a mannose-binding lectin that possesses three sugar binding pockets per monomer. White Light Reflectance Spectroscopy (WLRS) was used, as was described previously,[27,28] to monitor and quantify GNL interaction with **3** and WGA interaction with **5**. The lectins were deposited on a surface in a fluidic cell (see Experimental) and formed a stable layer upon which solutions of **3** (or **5**) were introduced. The binding events were detected in real time as red shifts of the resonant wavelength, $\lambda_{\max}$, where resonance is meant as a local extremum in the reflectance spectrum. An initial $\Delta\lambda_{\max}$ increase was followed by a plateau signifying the end of the binding event; subsequent washing resulted in a drop of the $\Delta\lambda_{\max}$ change (*off* process). From the experimental data ($\Delta\lambda_{\max}$ *vs* time) and the slopes of the *on* and *off* curves which are proportional to the *on* and *off* binding reaction rates (k_{on}, k_{off}, respectively, see Supplementary Material) combined with the compound solution concentrations the binding as well as dissociation rate constants and the corresponding equilibrium constants, $K = k_{on}/k_{off}$ (M^{-1}) were determined (Table 2).

Table 2. Binding, k_{on}, dissociation, k_{off}, rate constants and equilibrium constants, K, obtained from the WLRS curves

ASSOCIATION				DISSOCIATION		
Comp.	Conc. (M)	R^2	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	R^2	$K = k_{on}/k_{off}$ (M^{-1})
			GNL			
3	10^{-4}	0.986	10	3.3×10^{-6}	0.902	3.0×10^{6}
			WGA			
5	10^{-5}	0.973	49	1.0×10^{-3}	0.933	4.9×10^{4}

The binding to the corresponding underlying lectin layer was strong for both **3** and **5** whereas no binding was observed for the mono-substituted derivatives (*e.g.*, solution of **8** applied onto WGA layer) or the monosaccharide alone. The binding constant for **5** with WGA as determined falls within the range of association constants (0.6×10^4 to 6.6×10^4 M^{-1}) determined by SPR measurements of surface immobilized WGA with di- and trivalent GlcNAc glycosides.[29] A high association constant was determined between **3** and GNL that forms three binding pockets per lectin molecule, although the goodness of fit is not perfect. Fitting of the binding curves to a bi-exponential equation with two time constants was successful as well ($R^2 > 0.990$), indicating that the binding between the above lectins and **3** or **5** is not a straightforward process, thus the data in Table 2 represent trends and orders of magnitude rather than strict values. However, these experiments indicate that under the conditions described the lectins were immobilized with retention of their functionality and displayed strong binding with the compounds bearing multiple copies of complementary recognition monosaccharides (multivalency effect) but not with the singly substituted compounds.

The synthesized compounds are capable for binding with the antibiotic ampicillin, the enzymatic hydrolysis of which they inhibit by up to 30% while they are also recognized and bound by complementary lectins. Thus they may be useful in targeting bacteria expressing complementary lectins and in combining this property with inclusion and transfer of antibiotics.

Experimental Section

General. Deuterium oxide (D_2O), deuterated dimethylsulfoxide (DMSO-d_6) and deuterated chloroform ($CDCl_3$) were purchased from Deutero GmbH. Deuterated methanol (CD_3OD) was a product of Merck. *β*-CD was a product of CycloLab. *β*-CD and derivatives were dried by heating at 70 ^oC under vacuum for 20 h before reaction. 1,2-Diaminoethane was distilled from a mixture of CaO-KOH and stored over 4 Å molecular sieves. The dialysis membranes (cellulose tubing, benzoylated, MW_{CO} 2 kD), *α,β*-D-(+)-mannose and *N*-acetyl-D-(+)-glucosamine, *O*-(7-aza-benzotriazol-1-yl)-*N*,*N*,*N′*,*N′*-tetramethyluronium hexafluorophosphate (HATU) and dry DMF were purchased from Sigma-Aldrich. Flash column chromatography silica gel 60 (Mesh 0.040-0.063 mm) was purchased from Merck. *N*,*N*-diisopropylethylamine (DIPEA) was used as received. The known compounds (**1**, **2** and 2′-aminoethyl-monosaccharides), were prepared according to literature methods, as indicated in the introduction. *β*-Lactamase (EC 3.5.2.6) from *Enterobactercloacae* ≥ 97% pure powder was obtained from Sigma-Aldrich. Lectins WGA (wheat germ agglutinin from Triticum vulgaris, lyophylized powder, < 20 μg/mL agglutination activity) and GNL (galanthus nivalis, lyophylized powder, < 20 μg/mL agglutination activity) were purchased from Sigma. 1D and 2D NMR spectra were run on a Bruker Avance 500 MHz spectrometer. MALDI-TOF MS spectra were obtained using Autoflex (Bruker Daltonics) at the "G. Ronzoni Institute, Milan, Italy; the samples were solutions in H_2O (~3 mg/mL); matrix solution: 10 mg/mL of 2,5-dihydroxybenzoic acid (DHB) in EtOH 80%; 1 μL of the sample solution was added to 5 μL of the matrix solution and then 1 μL of the mixture was spotted on the target and left to air-dry. The spectra were recorded both in Linear and Reflectron modes, in positive polarity, both calibrated in the expected mass ranges. Elemental analyses were performed in house on a Perkin Elmer 2400 instrument. Melting points were determined on an Electrothermal IA9100 digital apparatus (25-400 ºC range).

Mono[6-(carboxyethylthio)-6-deoxy]-*β*-cyclodextrin (7). To a solution of 3-mercapto-propanoic acid (14.6 μL, 0.168 mmol) in DMF (2 mL) sodium hydride (15 mg, 0.620 mmol) was added in 10 equal portions and the mixture was stirred for 1 h. Then 6-mono-*p*-toluenesulfonyl-*β*-cyclodextrin[30] (100 mg, 0.077 mmol) was added and the whole was stirred at 100 ^oC for 2 h. The mixture was allowed to cool to room temperature and then water was added (2 mL). The solvent was driven off under vacuum, the compound was dissolved in minimum volume of water, the pH was adjusted to 7 and the solution was dialysed for 1 day. The desired product was obtained after drying as a colorless solid (94 mg, 98% yield). Mp >222 °C (decomp.); ^{1}H NMR (D_2O, 298 K, 500 MHz) δ_H 5.15 (brs, 1H, H1'), 5.09 (brs, 6H, H1), 3.96-3.88 (m, 22H), 3.69-3.59 (m, 12H), 3.17 (m, 1H), 2.94-2.85 (m, 2H), 2.63 (m, 2H) ppm. ^{13}C NMR (D_2O, 125 MHz) δ_C 180.8 (C=O), 102.0 (C1), 101.8 (C1'), 81.3 (C4), 73.3 (C3), 72.3 (C2), 72.1 (C5), 60.4 (C6'), 37.8 (C7), 33.2 (C6), 29.4 (C8) ppm. MS (MALDI-TOF) *m/z*: 1245.67 ([M+Na]$^+$, calcd.: 1245.37).

Coupling reactions

Octakis-{6-deoxy-6-*S*-[6-(*α*-D-mannopyranose-1'-yl)-4-aza-3-oxohex-1-yl]-6-thio}-*γ*-cyclodextrin (4). HATU (266 mg, 0.701 mmol) was added to a stirred solution of octakis[6-(oxycarbonylethylthio)-6-deoxy]-*γ*-cyclodextrin **2** (50 mg, 0.023 mmol) in DMF (16 mL) at 0 °C. After 1 h at ambient temperature DIPEA (85 μL, 0.499 mmol) and 2'-aminoethyl-*α*-D-mannopyranose (69 mg, 0.312 mmol) were added and the mixture was left to stir for 48 h. The solvent was then evaporated to dryness, the resulting solid was dissolved in doubly distilled water (3 mL) the pH was adjusted to 7 with 1 N HCl and the solution was dialysed for 48 h to remove the low-molecular-weight impurities. Lyophilization afforded an off-white solid (84 mg, 74%). Mp >188 °C (decomp.); ^{1}H NMR (D_2O, 298 K, 500 MHz) δ_H 5.15 (brs, 1H, CD-H1), 4.88 (s, 1H, Man-H1), 3.96 (s, 1H, Man-H2), 3.91-3.86 (m, 3H, CD-H3, Man-H4, Man-H6), 3.80 (brs, 3H, Man-H3, Man-H7, Man-H6'), 3.70 (s, 1H, CD-H5), 3.68 (s, 1H, Man-H5), 3.66 (s, 1H, CD-H2), 3.63 (brs, 2H, Man-H7', CD-H4), 3.49-3.43 (m, 2H, Man-H8), 3.28-3.26 (m, 1H, CD-H6), 2.96 (s, 3H, CD-H6', CD-H8), 2.62 (s, CD-H7) ppm. ^{13}C NMR (D_2O, 125 MHz) δ_C 173.6 (C=O), 101.4 (CD C1), 99.4 (Man-C1), 83.7 (CD-C4), 72.4 (Man-C4, CD-C2), 71.8 (CD-C3, -C5), 70.3 (Man-C3), 69.8 (Man-C2), 66.3 (Man-C5), 65.6 (Man-C7), 60.5 (Man-C6), 38.7 (Man-C8), 35.6 (CD-C7), 32.8 (CD-C6), 28.3 (CD-C8) ppm. MS (MALDI-TOF) *m/z*: 3664.24 ([M+Na]$^+$), calcd. for $C_{136}H_{232}O_{88}S_8N_8Na$: 3664.17. Elemental analysis calcd. for $C_{136}H_{232}N_8O_{88}S_8 \cdot 12H_2O$ (%): C 42.32, H 6.68, N 2.90; found C 42.12, H 6.62, N 2.84.

Heptakis{6-deoxy-6-*S*-[6-(*α*-D-mannopyranose-1'-yl)-4-aza-3-oxohex-1-yl]-6-thio}-*β*-cyclodextrin (3). The title compound was obtained using heptakis[6-(oxycarbonylethylthio)-6-deoxy]-*β*-cyclodextrin (**1**) (85 mg, 0.045 mmol) and 2'-aminoethyl-*α*-D-mannopyranose (118 mg, 0.529 mmol). The same ratio of equivalents of reagents as for **4** were used: HATU (454 mg, 1.195 mmol), DMF (13 mL), DIPEA (143 μL, 0.541 mmol). Off-white solid, (142 mg, 81%). Mp >158 °C (decomp.); ^{1}H NMR (D_2O, 298 K, 500 MHz) δ_H 5.13 (brs, 1H, CD-H1), 4.88 (s, 1H, Man-H1), 3.96 (s, 2H, Man-H2, CD-H3), 3.89 (brs, 1H, Man-H4), 3.87 (brs, 1H, Man-H6), 3.80 (brs, 3H, Man-H3, Man-H7, Man-H6'), 3.49-3.42 (m, 4H, Man-H5), 3.25-3.20 (m, 1H, CD-H6), 2.95 (brs, 3H, CD-H8, CD-H6'), 2.60 (s, 2H, CD-H7) ppm. ^{13}C NMR (D_2O, 125 MHz) δ_C 173.6 (C=O), 101.4 (CD-C1), 99.4 (Man-C1), 83.9 (CD-C4), 72.5 (Man-C4, CD-C2), 71.5 (CD-C3, CD-C5), 70.2 (Man-C3), 69.7 (Man-C2), 66.3 (Man-C5), 65.5 (Man-C7), 60.5 (Man-C6), 38.6 (Man-C8), 35.6 (CD-C7), 32.8 (CD-C6), 28.4 (CD-C8) ppm. MS (MALDI-TOF) *m/z*:

3209.15 ([*M*+Na]$^+$), calcd for $C_{119}H_{203}O_{77}S_7N_7Na$: 3209.02. Elemental analysis calcd. for $C_{119}H_{203}N_7O_{77}S_7 \cdot 9H_2O$ (%): C 42.66, H 6.65, N 2.93; found C 42.19, H 6.56, N 3.30.

Heptakis{6-deoxy-6-*S*-[6-(2-acetamide-2-deoxy-*α*-D-glucopyranose-1'-yl)-4-aza-3-oxohex-1-yl]-6-thio}-*β*-cyclodextrin (5). The title compound was obtained using **1** (100 mg, 0.052 mmol) and 2'-aminoethyl-2-acetamide-2-deoxy-*α*-D-glucopyranose (165 mg, 0.624 mmol). The same ratio of equivalents of reagents as described above for **4** were used: HATU (535 mg, 1.407 mmol), DMF (23 mL), DIPEA (168 μL, 0.991 mmol). Colorless solid (182 mg, 83%). Mp >180 °C (decomp.); ^{1}H NMR (DMSO-d_6, 298 K, 500 MHz) δ_H 7.83 (s, 1H, -<u>NH</u>-, amide), 7.62 (d, *J* 7.8 Hz, 1H, -<u>NH</u>-Ac), 4.99 (s, OH), 4.86 (brs, 1H, CD-H1), 4.77 (s, OH), 4.60 (s, 1H, GlcNAc-H1), 4.53 (s, OH), 3.77 (brs, 2H, GlcNAc-H2, GlcNAc-H3), 3.68 (brs, 2H, GlcNAc-H4, GlcNAc-H6), 3.62 (brs, 1H, GlcNAc-H7), 3.56 (brs, 3H, GlcNAc-H6', CD-H3, CD-H2), 3.47 (brs, 1H, GlcNAc-H7'), 3.39 (brs, 1H, GlcNAc-H8), 3.34 (overlapping with H_2O, 1H, GlcNAc-H5), 3.47 (brs, 1H, GlcNAc-H8'), 3.02-2.95 (m, 1H, CD-H6), 2.83-2.76 (m, 3H, CD-H6', CD-H8), 2.36 (brs, 2H, CD-H7), 1.86 (s, 3H, GlcNAc, CH_3) ppm. ^{13}C NMR (D_2O, 125 MHz) δ_C 173.8 (O=<u>C</u>-Ac, O=<u>C</u>-NH-), 101.4 (CD-C1), 96.8 (GlcNAc-C1), 83.9 (CD-C4), 72.2 (CD-C2), 71.7 (GlcNAc-C3), 70.9 (GlcNAc-C4), 70.3 (CD-C5, CD-C3), 66.1 (GlcNAc-C7), 60.2 (GlcNAc-C6), 53.2 (GlcNAc-C2), 38.7 (GlcNAc-C8), 35.6 (CD-C7), 32.8 (CD-C6), 28.5 (CD-C8), 21.7 (GlcNAc-CH_3) ppm. MS (MALDI-TOF) *m/z*: 3496.50 ([M+Na]$^+$), calcd for $C_{133}H_{224}O_{77}S_7N_{14}Na$: 3496.21. Elemental analysis calcd. for $C_{133}H_{224}N_{14}O_{77}S_7 \cdot 0.5C_8H_{19}N$ (%): C 46.47, H 6.64, N 5.73; found C 46.08, H 7.17, N 5.98.

Octakis{6-deoxy-6-*S*-[6-(2-acetamide-2-deoxy-*α*-D-glucopyranose-1'-yl)-4-aza-3-oxohex-1-yl]-6-thio}-*γ*-cyclodextrin (6). The title compound was obtained from octakis[6-(oxycarbonylethylthio)-6-deoxy]-*γ*-cyclodextrin (**2**) (100 mg, 0.046 mmol) and 2'-aminoethyl-2-acetamide-2-deoxy-*α*-D-glucopyranose (165 mg, 0.624 mmol). The same ratio of equivalents of reagents as for **4** were used: HATU (535 mg, 1.407 mmol), DMF (22 mL), DIPEA (168 μL, 0.991 mmol). Off-white solid (182 mg 83%). Mp >175 °C (decomp.); ^{1}H NMR (DMSO-d_6, 298 K, 500 MHz) δ_H 7.84 (s, 1H, -<u>NH</u>-, amide), 7.63 (d, *J* 6.5 Hz, 1H, -<u>NH</u>-Ac), 4.99 (s, OH), 4.90 (brs, 1H, CD-H1), 4.78 (s, OH), 4.66 (s, 1H, GlcNAc-H1), 4.54 (s, OH), 3.68 (s, 1H, GlcNAc-H2), 3.64-3.63 (brs, 2H, GlcNAc-H6, GlcNAc-H7), 3.49-3.45 (brs, 2H, GlcNAc-H6', GlcNAc-H7'), 3.39 (1H, GlcNAc-H8), 3.33 (overlapping with H_2O, 1H, GlcNAc-H3), 3.15 (brs, GlcNAc-H7', GlcNAc-H8'), 2.98 (m, 1H, CD-H6), 2.77 (brs, 3H, CD-H6', CD-H8), 2.36 (brs, 2H, CD-H7), 1.86 (s, 3H, GlcNAc, CH_3) ppm. ^{13}C NMR (D_2O, 125 MHz) δ_C 173.8 (O=<u>C</u>-Ac), 173.6 (O=<u>C</u>-NH-), 101.4 (CD-C1), 96.8 (GlcNAc-C1), 83.4 (CD-C4), 72.3 (GlcNAc-C4), 71.7 (CD-C3, CD-C2), 70.8 (GlcNAc-C3), 69.4 (GlcNAc-C7), 66.2 (CD-C5), 60.2 (GlcNAc-C6), 53.1 (GlcNAc-C2), 38.7 (GlcNAc-C8), 35.6 (CD-C7), 32.7 (CD-C6), 28.4 (CD-C8), 21.7 (GlcNAc-CH_3) ppm. MS (MALDI-TOF) *m/z*: 3992.94 ([M+Na]$^+$), calcd for $C_{152}H_{256}O_{88}S_8N_{16}Na$: 3992.38. Elemental analysis calcd. for $C_{152}H_{256}N_7O_{88}S_8 \cdot 2H_2O$ (%): C 45.54, H 6.53, N 5.59; found C 45.72, H 6.57, N 5.76.

Mono{6-deoxy-6-*S*-[6-(*α*-D-mannopyranose-1'-yl)-4-aza-3-oxohex-1-yl]-6-thio}-*β*-cyclodextrin (8). The title compound was obtained using mono[6-(oxycarbonylethylthio)-6-deoxy]-*β*-

cyclodextrin (**7**) (52 mg, 0.0417 mmol) and 2'-aminoethyl-*α*-D-mannopyranose (16 mg, 0.071 mmol). The same ratio of equivalents of reagents as described for **9** below were used: HATU (61 mg, 0.159 mmol), DMF (9 mL), DIPEA (19 μL, 0.113 mmol). Off-white solid (60 mg, 52%). Mp >225 °C (decomp.); ^{1}H NMR (DMSO-d_6, 298 K, 500 MHz) δ_H 5.75 (m, OH), 4.82 (brs, 1H, CD-H1), 4.59 (s, 1H, Man-H1), 4.45 (s, OH), 3.37 (ap.d, 1H, Man-H2), 3.66 (m, 4H, CD-H3, CD-$\underline{H6}$-OH, Man-H7), 3.53 (m, 1H, Man-H7'), 3.36 (overlapping with H_2O, 6H, Man-H6, CD-H4, CD-H2, Man-H8), 3.13 (m, 2H, CD-$\underline{H6, H6'}$-SCH_2), 3.02 (m, 2H, CD-H8), 2.34 (m, 2H, CD-H7) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz) δ_C 170.7 (O=$\underline{C}$-Ac), 169.5 (O=$\underline{C}$-NH-), 101.7 (CD-C1), 99.5 (Man-C1), 81.0 (CD-C4), 72.4 (CD-C3), 71.8 (CD-C2), 69.8 (Man-C2), 65.2 (Man-C7), 60.8 (Man-C6), 60.2 (CD-$\underline{C6}$-OH), 38.3 (CD-$\underline{C6}$-SCH_2), 35.2 (CD-C7), 32.9 (CD-C8) ppm. MS (ESI) *m/z*: 1450.9 ($[M+Na]^+$), calcd for $C_{53}H_{89}NO_{41}SNa$: 1450.46. Elemental analysis calcd. for $C_{53}H_{89}NO_{41}S.0.2C_5H_4N_4O\cdot 6H_2O$ (%): C 41.48, H 6.56, N 1.61; found C 41.21, H 6.55, N 2.02.

Mono{6-deoxy-6-*S*-[6-(2-acetamide-2-deoxy-*α*-D-glucopyranose-1'-yl)-4-aza-3-oxohex-1-yl]-6-thio}-*β*-cyclodextrin (9). HATU (121 mg, 0.320 mmol) was added to a stirred solution of mono[6-(oxycarbonylethylthio)-6-deoxy]-*β*-cyclodextrin (**7**) (104 mg, 0.084 mmol) in DMF (22 mL) at 0 °C. After 1 h DIPEA (38 μL, 0.225 mmol) and 2'-aminoethyl-2-acetamido-2-deoxy-*α*-D-glucopyranose (37.5 mg, 0.142 mmol) in DMF (0.3 mL) were added and the mixture was left to stir for 24 h. The solvent was then evaporated to dryness, the resulting mixture was dissolved in doubly distilled water (2 mL), the pH was adjusted to 7 with 1 N HCl and the solution was dialysed for 72 h to remove the low-molecular-weight impurities. Lyophilization afforded an off-white solid (123 mg, 76%). Mp >218 °C (decomp.); ^{1}H NMR (DMSO-d_6, 298 K, 500 MHz) δ_H 5.78-5.70 (m, OH), 4.82 (brs, 1H, CD-H1), 4.59 (s, 1H, GlcNAc-H1), 4.54 (s, OH), 4.45 (s, OH), 3.65-3.60 (m, 4H, GlcNAc-H2, GlcNAc-H8, CD-$\underline{H6, H6'}$-OH), 3.56 (brs, 1H, GlcNAc-H7), 3.46 (m, 2H, GlcNAc-H6), 3.33 (overlapping with H_2O, 4H, GlcNAc-H7, CD-H4, CD-H2, CD-H3), 3.15-3.11 (m, 1H, GlcNAc-H8'), 3.01 (m, 2H, CD-$\underline{H6, H6'}$-SCH_2), 2.75 (m, 2H, CD-H8), 2.35 (m, 2H, CD-H7), 1.85 (s, 3H, GlcNAc, CH_3) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz) δ_C 170.7 (O=$\underline{C}$-Ac), 169.5 (O=$\underline{C}$-NH-), 101.9 (CD-C1), 97.4 (GlcNAc-C1), 81.5 (CD-C4), 73.0 (CD-C2), 66.4 (GlcNAc-C7), 60.8 (GlcNAc-C6), 58.9 (CD-$\underline{C6}$-OH), 35.9 (GlcNAc-C8), 35.6 (CD-C7), 33.3 (CD-$\underline{C6}$-SCH_2), 28.5 (CD-C8), 22.7 (GlcNAc-CH_3) ppm. MS (MALDI-TOF) *m/z*: 1491.7 ($[M+Na]^+$), calcd for $C_{55}H_{92}N_2O_{41}SNa$: 1491.49. Elemental analysis calcd. for $C_{55}H_{92}N_2O_{41}S\cdot 8H_2O$ (%): C 40.94, H 6.74, N 1.73; found C 41.27, H 6.16, N 2.22.

Hydrolysis of ampicillin in the presence of the enzyme *β*-lactamase. *β*-Lactamase (EC 3.5.2.6) from *Enterobacter cloacae* ≥ 97% pure powder was obtained from Sigma-Aldrich. Measurements were carried out in a phosphate buffer solution (Na_2HPO_4/NaH_2PO_4) in D_2O (250 μL) at pH 7. To a solution of ampicillin (2.69 mM, 0.5 mg ampicillin/0.5 mL buffer) a solution of *β*-lactamase (Sigma) in D_2O (100 μL, 0.5 mg *β*-lactamase/1 mL) was added. The mixture was placed in a 5 mm NMR tube and kept at 25 °C in a thermostated bath near the NMR instrument. ^{1}H NMR spectra were recorded at various time intervals, and the growth of the methyl peaks at

1.15 ppm, owing to emergence of hydrolysis products relative to the total phenyl group signal, were monitored with time. The solutions **2**, **4**, **6** and maltoheptaose added were 13.45 mM. The points of each curve were normalised. The rate constants (*k*) were obtained from linear fitting to the equation $\ln([A_0]/[A]) = kt$, where $[A_0]$ is the initial ampicillin concentration and [A] is the concentration at time t.

Binding studies with White Light Reflectance Spectroscopy (WLRS). Protein solutions were prepared: WGA (0.8 μM; GNL, 0.2 μM) in PBS, pH 7.4. The solutions were left to stand for 12 h at 4 °C before deposition on the interferometric WLRS setup, previously described in detail.[27,28] Briefly, four-inch silicon wafers with a thermally grown 300 nm thick SiO_2 film as the interferometric layer were used, upon which a thin novolac resin film (AZ-5214, AZ-EM materials) was deposited by spin coating and post-baked at a high temperature (180 °C, 1 h) to improve coating efficiency (50 to 100 Å). The coated wafer was placed in the incubation–fluidic cell and the protein solutions were channeled in at 60 μL/min and $\Delta\lambda_{max}$ was monitored until a plateau was reached. The protein solutions were allowed to stand without flow in the setup for 12 h at room temperature (21-25 ºC). The effective thickness of the formed layer was inferred by fitting the experimental spectrum with the reflectance equation in the region around the extremum (590-680 nm). The films were subsequently washed with PBS with a flow of 60 μL/min followed by the introduction of each of the compounds dissolved in PBS with the same flow rate at the following concentrations: **5** (10 μM), **9** (100 μM), D-GlcNAc (1 mM) and **3** (100 μM). with WGA; **3** (100 μM) and **8** (100 μM) with GNL.

Supporting information. Reaction schemes and conditions for the monosaccharides with 2'-aminoethyl linkers; 2D spectra and assignments of key compounds showing the connectivity of the monosaccharide appendages to the cyclodextrin core; 2D ROESY NMR of the cavity bound complexes; hydrolysis kinetics; analysis of WLRS results.

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References

1. Lis, H.; Sharon, N. *Chem. Rev*. **1998**, *98*, 637.
   http://dx.doi.org/10.1021/cr940413g
2. Sharon, N. *Biochim. Biophys. Acta* **2006**, *1760*, 527.

3. Sharon, N.; Lis, H.: *Lectins*, 2nd Ed. Springer:Dordrecht,, 2007. http://dx.doi.org/10.1007/978-1-4020-6953-6
4. Badjica, J. D., Nelson, A., Cantrill, S. J., Turnbull, W. B., Stoddart, J. F. *Acc. Chem. Res*. **2005**, *38*, 723. http://dx.doi.org/10.1021/ar040223k
5. Mammen, M.; Choi, S.-K.; Whitesides, G. M.: *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2754. http://dx.doi.org/10.1002/(SICI)1521-3773(19981102)37:20<2754::AID-ANIE2754>3.0.CO;2-3
6. Attioui, F.; Al-Omar, A.; Leray, E.; Parrot-Lopez, H.; Finance, C. R. B. *Biol. Cell.* **1994**, *82*, 161. http://dx.doi.org/10.1016/S0248-4900(94)80018-9
7. Ortiz Mellet, C.; Benito, J. M.; García Fernández, J. M.; Law, H.; Chmurski, K.; Defaye, J.; O'Sullivan, M. L.; Caro, H. N. *Chem. -Eur. J.* **1998**, *4*, 2523. http://dx.doi.org/10.1002/(SICI)1521-3765(19981204)4:12<2523::AID-CHEM2523>3.0.CO;2-2
8. García-López, J. J.; Santoyo-González, F.; Vargas-Berenguel, A.; Giménez-Martínez, J. J. *Chem. -Eur. J.* **1999**, *5*, 1775. http://dx.doi.org/10.1002/(SICI)1521-3765(19990604)5:6<1775::AID-CHEM1775>3.0.CO;2-2
9. Baussane, I.; Benito, J. M.; Ortiz Mellet, C.; García Fernández, J. M.; Defaye, J. *ChemBiochem*. **2001**, *10*, 777. http://dx.doi.org/10.1002/1439-7633(20011001)2:10<777::AID-CBIC777>3.0.CO;2-C
10. Furuike, T.; Sadamoto, R.; Niikura, K.; Monde, K. N. S.; Nishimura, S. I. *Tetrahedron* **2005**, *61*, 1737. http://dx.doi.org/10.1016/j.tet.2004.12.035
11. Carpenter, C.; Nepogodiev, S.A. *Eur. J. Org. Chem.* **2005**, 3286. http://dx.doi.org/10.1002/ejoc.200500146
12. Gómez-García, M.; Benito, J. M.; Butera, A. P.; Ortiz Mellet, C.; García Fernández, J. M.; Jiménez Blanco, J. L. *J. Org. Chem*. **2012**, *77*, 7620.
13. Grunstein, D.; Maglinao, M.; Kikkeri, R.; Collot, M.; Barylyuk, K.; Lepenies, B.; Kamena, F.; Zenobi, R.; Seeberger, P. H. *J. Am. Chem. Soc.* **2011**, *33*, 13957. http://dx.doi.org/10.1021/ja2036767
14. Vargas-Berenguel, A.; Ortega-Caballero, F.; Casas-Solvas, J. M. *Mini-Rev. Org. Chem.* **2007**, *4*, 1. http://dx.doi.org/10.2174/157019307779815901
15. Furuike, T.; Aiba, S.; Nishimura, S. I. *Tetrahedron* **2000**, *56*, 9909. http://dx.doi.org/10.1016/S0040-4020(00)00962-5
16. Adam, M. J.; Bennett, D. J.; Bom, A.; Clark, J. K.; Feilden, H.; Hutchinson, E. J.; Palin, R.; Prosser, A.; Rees, D. C.; Rosair, G. M.; Stevenson, D.; Tarver, G. J.; Zhang, M.-Q. *J. Med. Chem*. **2002**, *45*, 1806.

http://dx.doi.org/10.1021/jm011107f

17. Izumi, M.; Okumura, S.; Yuasa, H.; Hashimoto, H. *J. Carbohydr. Chem.* **2003**, *22*, 317.
http://dx.doi.org/10.1081/CAR-120023475
18. Roy, R.; Kim, J. M. *Tetrahedron* **2003**, *59*, 3881.
http://dx.doi.org/10.1016/S0040-4020(03)00438-1
19. Disney, M. D.; Seeberger, P. H. *Chem. Biol.* **2004**, *11*, 1701.
http://dx.doi.org/10.1016/j.chembiol.2004.10.011
20. Vargas-Berenguel, A.; Ortega-Caballero, F.; Santoyo-González, F.; Giménez-Martínez, J. J.; García-Fuentes, L.; Ortiz-Salmerón, E. *Chem. -Eur. J.* **2002**, *8*, 812.
http://dx.doi.org/10.1002/1521-3765(20020215)8:4<812::AID-CHEM812>3.0.CO;2-P
21. Hoefler, T.; Wenz, G. *J. Inclusion Phenom. Mol. Recognit. Chem.* **1996**, *25*, 81.
http://dx.doi.org/10.1007/BF01041541
22. Rontoyianni, A.; Mavridis, I. M.; Hadjoudis, E.; Duisenberg, A. J. M. *Carbohydr. Res.* **1994**, *252*, 19.
http://dx.doi.org/10.1016/0008-6215(94)90003-5
23. Maffeo, D.; Leondiadis, L.; Mavridis, I.; Yannakopoulou, K. *Org. Biomol. Chem.* **2006**, *4*, 1297.
http://dx.doi.org/10.1039/b517275f
24. Messner, M.; Kurkov, S. V.; Flavià-Piera, R.; Brewster, M. E.; Loftsson, T. *Int. J. Pharm.* **2011**, *408*, 235.
http://dx.doi.org/10.1016/j.ijpharm.2011.02.008
25. Schwefel, D.; Maierhofer, C.; Beck, J. G.; Seeberger, S.; Diederichs, K.; Moeller, H. M.; Welte, W.; Wittmann, V. *J. Am. Chem. Soc.* **2010**, *132*, 8704.
http://dx.doi.org/10.1021/ja101646k
26. Hester, G.; Kaku, H.; Goldstein, I. J.; Wright, C. S. *Nat. Struct. Biol.* **1995**, *2*, 472.
http://dx.doi.org/10.1038/nsb0695-472
27. Zavali, M.; Petrou, P. S.; Kakabakos, S. E.; Kitsara, M.; Raptis, I.; Beltsios, K.; Misiakos, K. *Micro Nano Lett.* **2006**, *1*, 94.
http://dx.doi.org/10.1038/nsb0695-472
28. Maffeo, D.; Velkov, Z.; Misiakos, K.; Mergia, K.; Paulidou, A.; Zavali, M.; Mavridis, I. M.; Yannakopoulou, K. *J. Colloid Interface Sci.* **2011**, *358*, 369.
http://dx.doi.org/10.1016/j.jcis.2011.03.024
29. Masaka, R.; Ogata, M. Y. M.; Yano, M.; Hashimoto, C.; Murata, T.; Kawagishi, H.; Usui, T. *Bioorg. Med. Chem.* **2010**, *18*, 621.
http://dx.doi.org/10.1016/j.bmc.2009.12.006
30. Brady, B.; Lynam, N.; O'Sullivan, T.; Ahern, C.; Darcy, R. *Org. Synth.* **2000**, *77*, 220.