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ORIGINAL ARTICLE

Synthesis and characterisation of novel glycoclusters based on cell penetrating heptakis(6-aminoethylamino-6-deoxy)-β-cyclodextrin

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Abstract The modification of a polyamino βCD, heptakis(6-aminoethylamino-6-deoxy)-βCD (bpen) with several monosaccharides (Man, GlcNAc, Gal, Glc), specific for bacterial lectin targeting is described. The first synthetic approach, based on disuccinimidyl carbonate-substituted monosaccharides had moderate success, whereas the second approach, based on thiopropanoic acid-linked monosaccharides, was more efficient. Each method gave the best result with a different monosaccharide. Given that bpen is known to penetrate cells, the new products are expected to possess both lectin recognition ability and membrane crossing properties.

Keywords Monosaccharides · Amino cyclodextrin · Glycoclusters · Cell penetrating

Introduction

Biological recognition phenomena involving cells and proteins are based on specific sugar–protein binding interactions. Multivalent scaffolds bearing multiple recognition sugars (glycoclusters) interact strongly with special proteins, the lectins [1]. Therefore, a specific recognition sugar can play the role of a targeting moiety, able to attach to a specific cell via its lectins (for example, the lectins of bacterial cells). Cyclodextrins (CDs) offer unique features towards the above goals since they are biocompatible and can be easily modified in their primary side with carbohydrates. Per-carbohydrate modified CDs [2] have been reported previously to display much higher affinity towards blood proteins in hemagglutination experiments or related assays [3, 4] than the mono-substituted CDs, especially when there is a 2–5 carbon atom linker between the CD and the sugar of interest [5, 6]. Our group has shown recently [7] that per-6-guanidinoalkylamino- and per-6-aminoalkylamino-CDs are able to penetrate cell membranes and to form inclusion complexes with anionic guests, such as nucleotides [8]. The present work is focused on the synthesis and characterisation of novel CD glycoclusters based on a cell penetrating CD, heptakis (6-aminoethylamino-6-deoxy)-βCD (bpen) modified with D(+)-mannose (Man), *N*-acetyl-D-(+)-glucosamine (GlcNAc), D(+)-galactose (Gal) and D(+)-glucose (Glc), identified as monosaccharides that recognize bacterial lectins [1] using: (i) disuccinimidyl carbonate-substituted monosaccharides, or (ii) thiopropanoic acid-substituted monosaccharides. Each pathway was successful with different monosaccharides, yielding finally four different bpen-based glycoclusters. Pathway (i) was the most efficient.

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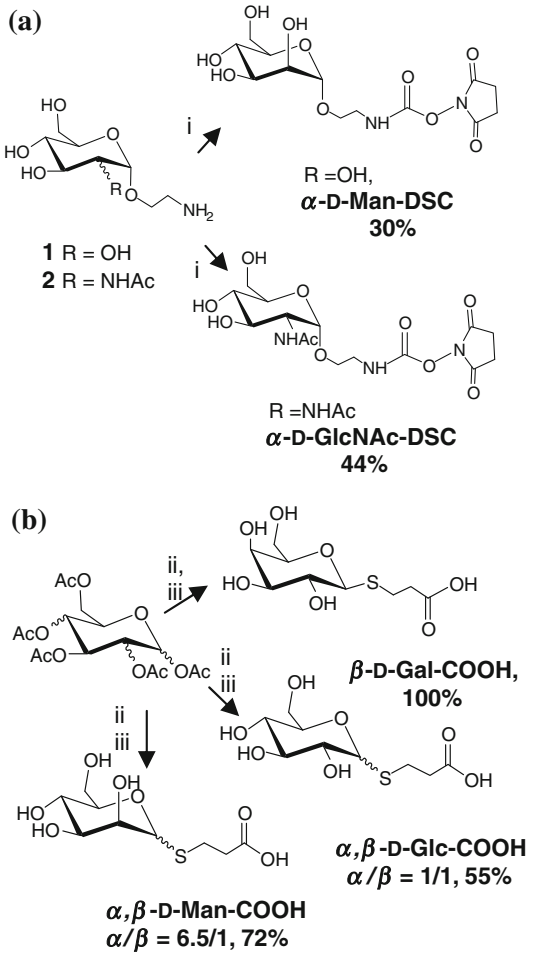
Results and discussion

Modification of monosaccharides

The synthesis of novel glycoclusters based on bpen required modification of the targeting monosaccharides, i.e. Man, GlcNAc, Gal, Glc. Appendage of an aminoethyl group on C1 (OH) of Man and of GlcNAc to obtain 2′-aminoethyl mannopyranoside (**1**) and 2′-aminoethyl-*N*-acetyl glucosaminopyranoside (**2**), respectively (Scheme 1a) required several steps. Thus compound **1** was

prepared according to published procedures [9–12] in five steps and 59% total yield, as follows: acetylation of the starting D-(+)-mannose (mixture of anomers, [α]/[β] = 1:1) gave α,β-D-mannose pentaacetate enriched in the α-anomer ([α]/[β] = 3:1), which reacted with bromoethanol to produce only the α anomer of 2′-bromoethyl-1-manno-pyranoside. The latter was transformed to the 2′-azido derivative, which was deacetylated and subsequently reduced to α-**1**. Compound **2** was prepared in three steps and 69% total yield by chloroethylation of α-GlcNAc followed by introduction of the azido group [13, 14] and subsequent reduction to α-**2**. Finally, reaction of α-**1** and of α-**2** with *N,N′*-disuccinimidyl carbonate (DSC) yielded the monosuccinimidyl carbonate monosaccharides, α-Man-DSC [15] and α-GlcNAc-DSC (Scheme 1a).

The carboxy-terminated β-D-Gal-COOH and α,β-D-Glc-COOH (Scheme 1b) were prepared in high yields from the commercially available β-D-(+)-galactose pentaacetate and α-D-(+)-glucose pentaacetate, respectively. α,β-D-Man-COOH was obtained from the synthesised α,β-D-(+)-mannose pentaacetate ([α]/[β] = 3:1). Use of the acetylated starting materials enabled purification of all the products in the various steps using column chromatography, not feasible with natural Gal, Glc or Man. Following literature procedures [16–20] anomerically pure β-Gal-COOH was obtained in two steps quantitatively. Glc-COOH was prepared as a mixture of anomers ([α]/[β] = 1:1) in two steps and 55% yield. The anomeric ratio was depended on the reaction time during introduction of the thiopropanoic acid tail: after 12 h, the product was isolated as [α]/[β] = 1:1 in 55% yield, whereas after 4 h the product was isolated as [α]/[β] = 9:1 in 24% yield. Attachment of thiopropanoic acid in Man enriched the contents of the α anomer in Man-COOH ([α]/[β] = 6.5:1) and the overall yield was 72%.



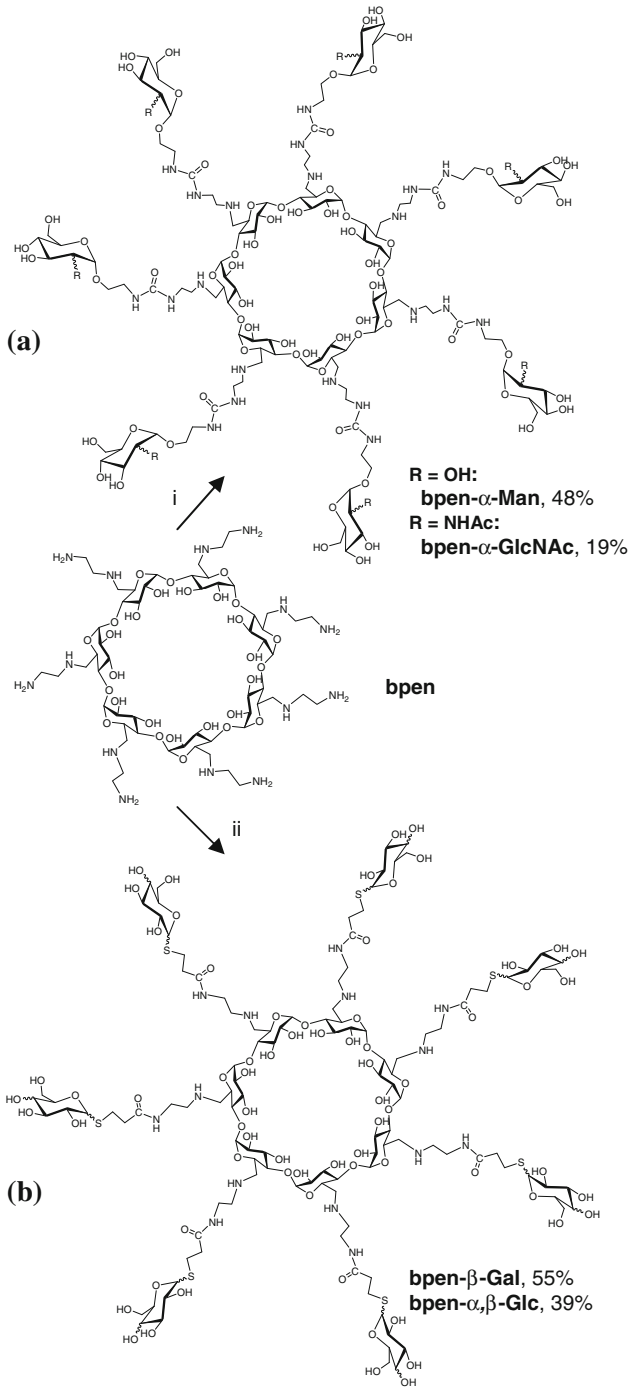
Scheme 1 **a** (*i*) Et_3N, DMF, DSC. **b** (*ii*) 3-Mercaptopropanoic acid, $BF_3{\cdot}OEt_2$, CH_2Cl_2, r.t. (*iii*) MeONa, MeOH, r.t., 100%

Coupling of modified monosaccharides with bpen

The DSC modified monosaccharides of Scheme 1a reacted with bpen to afford glycoclusters bpen-α-Man and bpen-α-GlcNAc (Scheme 2a) where the CD core is connected to the monosaccharide substituents via urea-type linkers, spanning a total length of eight-bonds.

The coupling reaction was satisfactory in terms of yields and degree of substitution only for bpen-α-Man (per-6-substitution, 48% yield) whereas bpen-α-GlcNAc proceeded poorly (only 50% substitution, 19% yield). A DSC-modified galactose derivative, β-D-Gal-DSC was also prepared in a way similar to the one described for α-D-Man-DSC but its coupling with bpen was unsuccessful (no reaction). The glycoclusters obtained (Scheme 2a) were characterised by ^{1}H NMR spectra, where the ratio between H1-CD/H1-Monosaccharide was calculated by integration (1:1 for bpen-α-Man, Fig. 1a). The NMR spectra showed broad peaks for bpen, but sharp peaks for the monosaccharides in D_2O, especially the ^{13}C NMR spectra (Fig. 1b). Such behaviour has been reported previously for β-CD based branched glycoclusters [21]. The assignment was aided by 2D NMR spectra. Given the incomplete substitution with GlcNAc and the low isolated yields, an alternative approach was pursued, based on the coupling reaction between bpen and the carboxy-terminated monosaccharides of Scheme 1b using HATU as the coupling agent (Scheme 2b).

Coupling of bpen with β-D-Gal-COOH and α,β-D-Glc-COOH gave the corresponding per-6-substituted products in moderate to good yields, whereas with α,β-D-Man-COOH no reaction took place. The ^{1}H NMR spectra of the glycoclusters bpen-β-Gal and bpen-α,β-Glc in DMSO-d_6 showed relatively broadened peaks for the cyclodextrin moiety. The presence of an amide peak at 7.85 ppm (not visible in aqueous solution) certified the successful coupling. Integration suggested full substitution on the primary side of bpen. Further support was obtained from MS and analytical data.



Scheme 2 **a** (*i*) α-D-Man-DSC or α-D-GlcNAc-DSC, Et_3N, DMF, 30 °C. **b** (*ii*) β-D-Gal-COOH or α,β-D-Glc-COOH, HATU, DIPEA, DMF, 35 °C

Contrary to the ^{1}H NMR spectra, the monosaccharide peaks in the ^{13}C NMR spectra were sharp, indicating symmetrically substituted molecules.

Assignment of the signals was carried out based on 2D spectra (Fig. 2a, b). The 2D ROESY spectrum showed dipolar coupling between the terminal NH of bpen and the

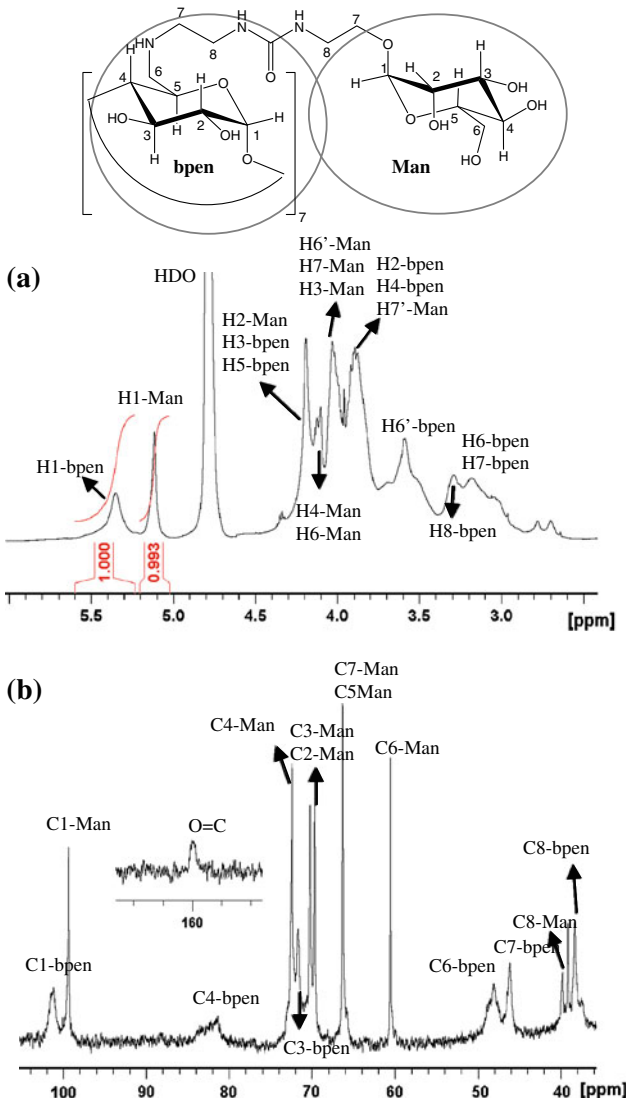


Fig. 1 Bpen-α-Man. **a** ^{1}H NMR spectrum (500 MHz, D_2O, 318 K). **b** ^{13}C NMR spectrum (125 MHz, D_2O, 298 K)

methylene protons H8 of the sugar (Fig. 2b), providing concrete proof for the coupling.

In summary, using two different approaches and based on a cell penetrating polyamino CD, bpen, glycoclusters bearing four different monosaccharides (Man, GlcNAc, Gal, Glc) were obtained in moderate yields. The products are expected to retain their cell penetrating ability combined with lectin targeting ability.

Experimental

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. βCD was a product of CycloLab. βCD and derivatives were dried by heating at 70 °C under vacuum for 20 h before reaction. 1,2-diaminoethane was distilled from a mixture of CaO-KOH and stored over molecular sieves type 4 Å. The dialysis membrane (cellulose tubing, benzoylated, MWCO 1.2 kD), β-D-(+)-galactose pentaacetate, α-D-(+)-glucose pentaacetate, α,β-D-(+)-mannose and *N*-acetyl-D-(+)-glucosamine, *O*-(7-azabenzotriazol-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. The following starting materials were prepared according to the literature: **1** [7–10], bpen [5].

2′-Aminoethyl-2-acetamide-2-deoxy-α-D-glucopyranoside (**2**)

A solution of 2′-azidoethyl-2-acetamide-2-deoxy-α-D-glucopyranoside [11, 12] (150 mg, 0.517 mmol) in MeOH (4.5 mL), containing Pd/C 10% (15 mg) was stirred at room temperature under a hydrogen atmosphere for 15 h until the starting material had been consumed (TLC). The mixture was filtered through a Celite pad which was also washed with MeOH (3 × 5 mL). The combined filtrates were evaporated under reduced pressure to give product **2** quantitavely. ^{1}H NMR (D_2O, 298 K, 500 MHz) δ 4.90 (s, 1H, H1), 3.97–3.95 (m, 1H, H2), 3.91–3.88 (m, 1H, H6), 3.82–3.74 (m, 4H, H3, H5, H6, CH_2O), 3.53–3.49 (m, 2H, H4, CH_2O), 2.90–2.88 (m, 2H, CH_2NH_2), 1.07 (s, 3H) ppm. ^{13}C NMR (D_2O, 125 MHz) δ 174.0 (O=C), 96.7 (C1), 71.5 (C5), 70.6 (C3), 69.6 (C4), 68.5 (CH_2O), 60.2 (C6), 53.2 (C2), 39.6 (CH_2NH_2), 21.5 (CH_3) ppm. ESI-MS *m/z*: 265.3 ($[M + H]^+$), calcd. for $C_{10}H_{20}N_2O_6H$: 265.13.

1-(2′-*N*-Succinimidyloxycarbonylaminoethyl)-2-acetamide-2-deoxy-α-D-glucopyranoside (α-GlcNAc-DSC)

A solution of **2** (41.26 mg, 0.156 mmol) and triethylamine (10.87 μL, 0.078 mmol) in DMF (4 mL) was added dropwise at 0 °C to a solution of *N,N′*-disuccinimidyl carbonate (20 mg, 0.078 mmol) in DMF (4 mL). The reaction mixture was left to stir for 48 h at room temperature under a nitrogen atmosphere. The solvent was then evaporated under reduced pressure, the crude was washed with $CHCl_3$ and purified by flash column chromatography (10% MeOH in CH_2Cl_2) to obtain α-GlcNAc-DSC, as a sticky solid in 44% yield. $R_f = 0.26$ (CH_2Cl_2:MeOH, 9:1, v/v). ^{1}H NMR (CD_3OD, 298 K, 500 MHz) δ 4.79 (s, 1H, H1), 4.57 (brs, 1H, NH), 3.95–3.89 (m, 1H), 3.85–3.81 (m, 2H), 3.71–3.60 (m, 4H), 3.52–3.33 (m, 3H), 2.68 (brs, 2H), 2.0 (s, 3H, CH_3) ppm. ^{13}C NMR (CD_3OD, 125 MHz) δ 173.9 (O–C=O) 172.8 (O=*C*–CH_3), 157.5 (N–C=O), 99.2 (C1), 74.1 (C5), 73.1 (C3), 72.5 (C4), 67.7 (C7), 62.8 (C6), 55.41 (C2), 42.7 (C8), 26.4 (CH-DSC) ppm. ESI-MS *m/z*: 460.4 ($[M + MeOH + Na]^+$), calcd. for $C_{15}H_{23}N_3O_{10}$·MeOH: 460.39.

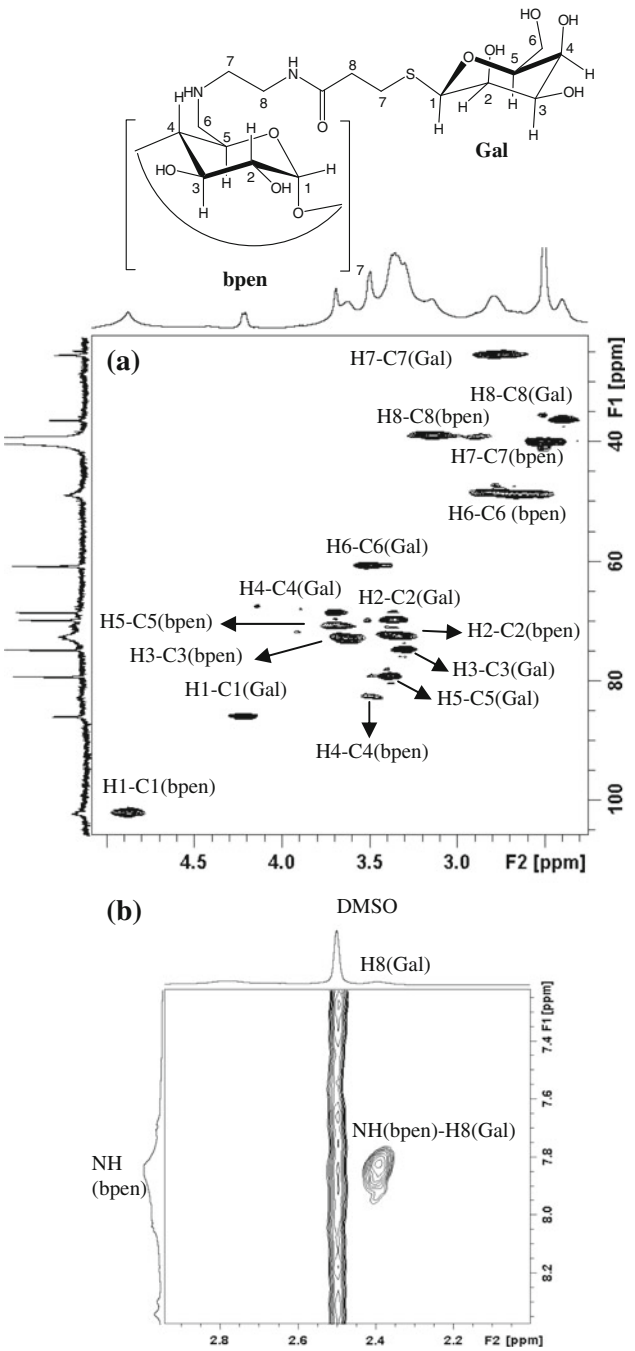


Fig. 2 **a** HSQC spectrum of bpen-β-Gal (500 MHz, DMSO-d_6, 298 K). **b** Partial ROESY spectrum of bpen-β Gal (500 MHz, DMSO-d_6, 298 K)

2′-*N*-Succinimidyloxycarbonylethyl-α-D-mannopyranoside (α-D-Man-DSC)

It was prepared from α,β-D-(+)-mannose ([α]/[β] = 1:1) following literature procedures in six steps [13]. Overall yield: 18%. $R_f = 0.25$. ^{1}H NMR (CD_3OD, 298 K, 500 MHz) δ 5.35 (OH), 5.22 (OH), 5.16 (OH), 4.77 (s, 1H, H1), 4.55 (brs, 1H, NH), 3.84–3.76 (m, 3H), 3.72–3.66 (m, 3H), 3.61–3.53 (m, 3H), 2.79 (s, 4H) ppm. ^{13}C NMR (CD_3OD, 125 MHz) δ 172.6 (C=O), 101.6 (C1), 74.7 (C4), 72.5 (C3), 72.0 (C2), 68.6 (C7), 66.9 (C5), 62.9 (C6), 42.5 (C8), 26.4 (CH-DSC) ppm.

1-(2′-Carboxylethylthio)-1-deoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside

It was prepared from β-D-(+)-galactose pentaacetate in one step following literature procedures [14–18]. Yield: 100%. $R_f = 0.48$ in CH_2Cl_2:MeOH (95:5). ^{1}H NMR ($CDCl_3$, 298 K, 500 MHz) δ 10.33 (brs, OH), 5.31 (brs, 1H, H4), 5.09 (t, $J = 9.6$ Hz, 1H, H2), 4.97–4.95 (m, 1H, H3), 4.47 (d, $J = 9.7$ Hz, 1H, H1), 4.06–3.97 (m, 2H, H6), 3.88–3.85 (m, 1H, H5), 2.89–2.76 (m, 2H, S-CH_2), 2.64 (m, 2H, CH_2COOH), 2.05 (s, CH_3), 1.95 (s, CH_3x2), 1.87 (s, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.67 (O=*C*–OH), 170.57 (O=*C*–CH_3), 170.29 (O=*C*–CH_3), 170.06 (O=*C*–CH_3), 169.6 (O=*C*–CH_3), 82.43 (C1), 74.34 (C5), 71.76 (C3), 67.34 (C4), 67.06 (C2), 61.58 (C6), 35.22 (*C*H_2COOH), 25.24 (S-*C*H_2), 20.66 (CH_3), 20.54 (CH_3x2), 20.47 (CH_3) ppm.

1-(2′-Carboxyethylthio)-1-deoxy-β-D-galactopyranoside (β-D-Gal-COOH)

It was prepared from 2′-carboxylethylthio-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside by deacetylation following literature procedures [14–18]. Yield: 100%. ^{1}H NMR (D_2O, 298 K, 500 MHz) δ 4.66 (d, $J = 10.2$ Hz, 1H, H1), 4.14 (d, $J = 2.8$ Hz, 1H, H4), 3.87 (brs, 3H, H5, H6), 3.84–3.82 (m, 1H, H3), 3.69 (t, $J = 9.7$ Hz, 1H, H2), 3.15–3.03 (m, 2H, S-CH_2), 2.83 (t, $J = 6.9$ Hz, 2H, CH_2COOH) ppm. ^{13}C NMR (D_2O, 125 MHz) δ 178.68 (O=*C*–OH), 86.25 (C1), 79.19 (C5), 74.14 (C3), 69.9 (C2), 69.3 (C4), 61.56 (C6), 36.64 (*C*H_2COOH), 26.16 (S-*C*H_2) ppm.

1-(2′-Carboxylethylthio)1-deoxy-2,3,4,6-tetra-*O*-acetyl-α,β-D-mannopyranoside

3-Mercaptopropanoic acid (893 μL, 10.24 mmol) and BF_3·OEt_2 (485 mL, 3.84 mmol) were added to a solution of α,β-D-(+)-mannose pentaacetate ([α]/[β] = 3:1) (1 g, 2.56 mmol) in CH_2Cl_2 (30 mL). The reaction mixture was stirred for 10 h at room temperature under a nitrogen atmosphere. More CH_2Cl_2 (60 mL) and HCl 0.1 M (20 mL) were added in the reaction mixture and the whole was washed with distilled water (3 × 40 mL). The organic layer was separated and dried over $MgSO_4$. The solvent was then evaporated to dryness under reduced pressure and the crude product was purified by flash column chromatography (Hex:AcOEt 7:5) to obtain the desired product in 72% yield ([α]/[β] = 6.5:1). $R_f = 0.23$ (CH_2Cl_2:MeOH, 9:1, v/v). ^{1}H NMR ($CDCl_3$, 298 K, 500 MHz) δ 9.65 (brs, OH), 5.18 (m, 2H, H2, H4), 5.13–5.10 (m, 1H, H3), 5.06 (m, 1H, H1), 4.24 (1H, H6), 4.15–4.12 (m, 1H, H6), 3.97 (m, 1H, H5), 2.83–2.70 (m, 2H, H7), 2.59 (m, 2H, H8), 2.02 (s, CH_3), 1.95 (s, CH_3), 1.91 (s, CH_3), 1.83 (s, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.8 (O=C–OH), 170.7 (O=C–CH_3), 169.9 (O=C), 169.7 (O=C), 82.8 (C1), 70.7 (C5), 69.24 (C3), 68.9 (C4), 66.1 (C2), 62.3 (C6), 34.3 (CH_2O), 26.2 (SCH_2), 20.4 (CH_3) ppm. ESI-MS *m/z*: 459.3 ($[M + Na]^+$), calcd. for $C_{17}H_{24}O_{11}SNa$: 459.1.

1-(2′-Carboxyethylthio)-1-deoxy-α,β-D-mannopyranoside (α,β-D-Man-COOH)

Solid MeONa (450 mg, 8.33 mmol) was added to a solution of 2′-carboxyethylthio-2,3,4,6-tetra-*O*-acetyl-α,β-D-mannopyranoside ([α]/[β] = 6.5:1) (442 mg, 1.012 mmol) in MeOH (8.3 mL). The reaction mixture was left to stir for 3 h at room temperature until the acetylation was complete (TLC). The pH was adjusted to 4.5 with DOWEX 50WX2 (H^+) resin, filtered and the solvent was removed under reduced pressure to obtain the desired product, α,β-D-Man-COOH ([α]/[β] = 6.5:1), quantitatively as a sticky solid. ^{1}H NMR (D_2O, 298 K, 500 MHz) δ 5.35 (brs, 1H, H1-α), 4.88 (brs, 1H, H1-β), 4.07–4.01 (m, 2H, H4-α,β), 3.91–3.88 (m, 2H, H6-α,β), 3.82–3.78 (m, 2H, H6-α,β), 3.73–3.68 (m, 2H, H3-α,β), 2.96–2.85 (m, 4H, S-CH_2-α,β), 2.62 (brs, 4H, CH_2COOH-α,β) ppm. ^{13}C NMR (D_2O, 125 MHz) δ 179.8 (O=C–α,β), 85.1 (C1-α), 84.7 (C1-β), 74.01 (C5-β), 73.3 (C5-α), 72.4 (C3-β), 71.9 (C3-α), 71.2 (C2-α,β), 67.3 (C4-α), 66.8 (C4-β), 61.3 (C6-β), 61.0 (C6-α), 37.4 (*C*H_2COOH-β), 36.8 (*C*H_2COOH-α), 27.4 (S-*C*H_2-β), 27.2 (S-*C*H_2-α) ppm. MS *m/z*: 305.3 ($[M + HCl + H]^+$), calcd. for $C_9H_{17}O_7S$·HCl: 305.3.

1-(2′-Carboxylethylthio)-1-deoxy-2,3,4,6-tetra-*O*-acetyl-α,β-D-glucopyranoside

3-Mercaptopropanoic acid (893 μL, 10.24 mmol) and BF_3·OEt_2 (485 mL, 3.84 mmol) were added to a solution of α-D-(+)-glucose pentaacetate (1 g, 2.56 mmol) in CH_2Cl_2 (30 mL). The reaction mixture was stirred for 1 day at room temperature under a nitrogen atmosphere. More CH_2Cl_2 (30 mL) and HCl 0.1 M (40 mL) were then

added and the mixture was washed with distilled water (2 × 20 mL). The organic layer was separated and dried over $MgSO_4$. The solvent was then evaporated to dryness under reduced pressure and the crude product was purified by flash column chromatography (1% MeOH in CH_2Cl_2) to obtain the desired product in 55% yield ([α]/[β] = 1:1). $R_f = 0.64$ (CH_2Cl_2:MeOH, 9:1, v/v). ^{1}H NMR ($CDCl_3$, 298 K, 500 MHz) δ 8.98 (brs, OH), 5.53 (brs, 1H, H1-α), 5.14–5.04 (m, 2H), 4.91–4.84 (m, 3H), 4.45–4.43 (d, $J = 10.3$ Hz, 1H, H1-β), 4.26 (brs, 1H), 4.12–3.93 (m, 3H), 3.62 (brs, 1H) 2.96–2.53 (m, 10H), 1.91–1.82 (brs, 24H, CH_3x8) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.9 (O=*C*–OH), 175.7 (O=*C*–OH), 170.7 (O=*C*–CH_3), 170.6 (O=*C*–CH_3), 170.1 (O=*C*–CH_3), 169.8 (O=*C*–CH_3), 169.7 (O=*C*–CH_3), 169.6 (O=*C*–CH_3), 169.4 (O=*C*–CH_3), 169.3 (O=*C*–CH_3), 83.4 (C1-α), 82.3 (C1-β), 75.4 (C5-β), 73.5 (C5-α), 70.3 (C3-α), 70.1 (C3-β), 69.5 (C4-α), 68.3 (C4-β), 67.6 (C2-β), 62.0 (C6-α), 61.8 (C6-β), 35.0 (*C*H_2COOH-α), 34.3 (*C*H_2COOH-β), 25.1 (S-*C*H_2-β), 24.8 (S-*C*H_2-α), 23.7, 20.4–20.3 (CH_3) ppm. ESI-MS *m/z*: 459.3 ([*M* + Na]$^+$), calcd. for $C_{17}H_{24}O_{11}SNa$: 459.1.

1-(2′-Carboxyethylthio)-1-deoxy-α,β-D-glucopyranoside (α,β-D-Glc-COOH)

Solid MeONa (542 mg, 10.03 mmol) was added to a solution of 2′-carboxyethylthio-2,3,4,6-tetra-*O*-acetyl-α,β-D-glucopyranose ([α]/[β] = 1:1) (532 mg, 1.218 mmol) in MeOH (10 mL). The reaction mixture was left to stir for 3 h at room temperature until the deprotection reaction was complete (TLC). It was then neutralized with 1 M HCl and filtered. The solvent was removed under reduced pressure to obtain the desired product, α,β-D-Glc-COOH, quantitatively, as a sticky solid ([α]/[β] = 1:1). ^{1}H NMR (D_2O, 298 K, 500 MHz) 5.64–5.63 (d, $J = 4.9$ Hz, 1H, H1-α), 4.14 (d, $J = 8.2$ Hz, 1H, H1-β), 4.04–3.94 (m, 4H), 3.88–3.85 (m, 1H), 3.74–3.59 (m, 6H), 3.52–3.48 (t, $J = 9.3$ Hz, 1H), 3.11–2.97 (m, 4H, S-CH_2), 2.89–2.83 (m, 4H, CH_2COOH) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ 178.4 (O=*C*–OH), 86.1 (C1-β), 85.7 (C1-α), 80.04 (C5-α), 77.4 (C5-β), 73.9 (C3-β), 72.9 (C3-α), 72.5 (C4-α), 71.3 (C4-β), 69.9 (C2-α), 69.8 (C2-β), 61.3 (C6-α), 60.9 (C6-β), 36.6 (*C*H_2COOH-α), 36.2 (*C*H_2COOH-β), 26.1 (S-*C*H_2-β), 26.0 (S-*C*H_2-α) ppm. ESI-MS *m/z*: 305.3 ([*M* + HCl + H]$^+$), calcd. for $C_9H_{17}O_7S$·HCl: 305.3.

Heptakis{6-deoxy-6-aza-[7-(α-D-mannopyranos-1′-yl)-3-aza-4-oxo-5-aza-hept-1-yl]-6-amino}-β-cyclodextrin (bpen-α-Man)

A solution of α-D-Man-DSC (36 mg, 0.095 mmol) in DMF (2 mL) was added dropwise into a solution of heptakis [6-aminoethyl-6-deoxy]-β-cyclodextrin (bpen) (17.6 mg, 0.0123 mmol) and triethylamine (150 μL, 1.076 mmol) in DMF (0.6 mL). The mixture was stirred for 168 h at 30 °C under a nitrogen atmosphere. The solvent was then evaporated to dryness, the resulting mixture was dissolved in doubly distilled water (3 mL), and the pH was adjusted to 7 with 1 N HCl. The resulting solution was dialysed for 72 h. Lyophilization afforded the product as an off-white solid (48%). ^{1}H NMR (D_2O, 298 K, 500 MHz) δ 5.12 (brs, 1H, H1-bpen), 4.88 (brs, 1H, H1-man), 3.96 (brs, 3H, H3-bpen, H5-bpen, H2-man), 3.88 (m, 2H, H4-man, H6-man), 3.78 (brs, 3H, H6′-man, H7-man, H3-man), 3.65 (brs, 3H, H7′-man, H2-bpen, H4-bpen), 3.36 (brs, 1H, H6-bpen), 3.10 (brs, 2H, H8-bpen), 3.01-2.88 (m, 3H, H6′-bpen, H7-bpen) ppm. ^{13}C NMR (D_2O, 125 MHz) δ 160.1 (O=C), 101.5 (C1-bpen), 99.4 (C1-man), 82.3 (C4-bpen), 72.4 (C4-man), 71.7 (C3-bpen), 70.2 (C3-man, C2-bpen), 69.7 (C2-man, C5-bpen), 66.4 (C7-man, C5-man), 60.5 (C6-man), 48.1 (C6-bpen), 46.1 (C7-bpen), 39.8 (C8-man), 38.3 (C8-bpen) ppm. MALDI-TOF MS *m/z*: 1678.79 ([*M* + 4HCl + H + K]$^{2+}$), calcd. for $C_{119}H_{217}O_{77}N_{21}$·4HCl·H$^+$·K$^+$: 3356.25. Elemental analysis calcd. for $C_{119}H_{217}O_{77}N_{21}$·8HCl·9$H_2O$ (%): C 39.39, H 6.75, N 8.11; found C 38.87, H 7.06, N 7.80.

Heptakis{6-deoxy-6-aza-[7-(2-acetamide-2-deoxy-α-D-glucopyranose-1′-yl)-3-aza-4-oxo-5-aza-hept-1-yl]-6-amino}-β-cyclodextrin (bpen-α-GlcNAc)

Solid bpen (6.4 mg, 0.00448 mmol) was added in three equal portions during 48 h to a solution of α-D-GlcNAc-DSC (14 mg, 0.0123 mmol) and triethylamine (97.5 μL, 0.699 mmol) in DMF (1.5 mL). The mixture was stirred for 78 h at 30 °C under a nitrogen atmosphere. The solvent was then evaporated to dryness, the resulting mixture was dissolved in doubly distilled water (3 mL) and the pH was adjusted to 7 with 1 N HCl. The solution was dialysed for 72 h. Lyophilization afforded the product as an off-white solid (19%). ^{1}H NMR (D_2O, 298 K, 500 MHz) δ 5.12 (brs, 1H, H1-bpen), 4.87 (brs, 1H, H1-GlcNAc), 3.96 (brs, 2H, H3-bpen, H4-GlcNAc), 3.85 (brs, 1H, H6-GlcNAc), 3.80 (brs, 1H, H2-GlcNAc), 3.77 (brs, 1H, H6′-GlcNAc), 3.73 (brs, 1H, H7-GlcNAc), 3.67 (brs, 2H, H2-bpen, H3-GlcNAc), 3.52 (brs, 1H, H4-bpen), 3.38 (brs, 1H, H7′-GlcNAc), 3.27 (brs, 1H, H8-bpen), 3.06-2.82 (m, 5H, H6-bpen, H7-bpen, H8′-bpen) ppm. ^{13}C NMR (D_2O, 125 MHz) δ 160.1 (O=C), 100.7 (C1-bpen), 96.2 (C1-GlcNAc), 82.0 (C4-bpen), 72.2 (C4-GlcNAc), 71.5 (C3-GlcNAc), 70.7 (C2-GlcNAc), 70.0 (C3-bpen), 66.6 (C7-GlcNAc), 60.0 (C6-GlcNAc), 48.1 (C6-bpen), 46.3 (C7-bpen), 38.0 (C8-bpen) ppm. Degree of substitution based on NMR integration: 3.5/7.

Heptakis{6-deoxy-6-aza-[6-(β-D-galactopyranos-1′-ylthio)-3-aza-4-oxohex-1-yl]-6-amino}-β-cyclodextrin (bpen-β-Gal)

HATU (181 mg, 0.212 mmol) was added to a stirred solution of β-D-Gal-COOH (57 mg, 0.212 mmol) in DMF (7 mL) at 0 °C. After 1 h diisopropylethylamine (DIPEA) (85 μL, 0.336 mmol) and bpen (30.2 mg, 0.0178 mmol) were added during 48 h. The mixture was left to stir for 120 h. The solvent was then evaporated to dryness, the resulting mixture was dissolved in doubly distilled water (2 mL) and the pH was adjusted to 7 with 1 N HCl. The solution was dialysed for 48 h to remove the low-molecular-weight impurities. Lyophilization afforded an off-white solid (55%). ^{1}H NMR (DMSO-d_6, 298 K, 500 MHz) δ 7.85 (s, 1H, –N*H*–, amide), 5.79 (s, OH), 4.87 (brs, 1H, H1-bpen), 4.21 (d, $J = 8.8$ Hz, 1H, H1-gal), 3.68 (s, 2H, H4-gal, H5-bpen), 3.62 (brs, 1H, H3-bpen), 3.49 (s, 3H, H6-gal, H4-bpen), 3.64–3.28 (m, 4H, H5-gal, H2-gal, H2-bpen, H3-gal), 3.14 (brs, 2H, H8-bpen), 2.78 (brs, 2H, H7-gal, SCH_2), 2.66 (1H, H6-bpen), 2.53 (1H, H6′-bpen), 2.39 (s, 2H, H8-gal, CH_2C=O) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz) δ 171.0 (O=*C*–NH–), 102.2 (C1-bpen), 85.9 (C1-gal), 82.7 (C4-bpen), 79.1 (C5-gal), 74.6 (C3-gal), 72.8–72.3 (C3-bpen, C2-bpen), 70.9 (C5-bpen), 69.7 (C2-gal), 68.4 (C4-gal), 60.6 (C6-gal), 48.7 (C6-bpen, C7-bpen), 36.2 (C8-gal-*C*H_2C=O), 25.3 (C7-gal-S*C*H_2) ppm. The compound contained azabenzotriazole ($C_5H_4N_4O$). ESI-TOF MS *m/z*: 1590.62 ($[M + 2H]^{2+}$), calcd. for $C_{119}H_{210}N_{14}O_{70}S_7$: 3179.13. Elemental analysis calcd. for $C_{119}H_{210}N_{14}O_{70}S_7 \cdot 0.5C_5H_4N_4O$ (%): C 44.9, H 6.57, N 6.89; found C 45.05, H 7.08, N 7.45.

Heptakis{6-deoxy-6-aza-[6-(α,β-D-glucopyranos-1′-ylthio)-3-aza-4-oxohex-1-yl]-6-amino}-β-cyclodextrin (bpen-α,β-Glc)

The title compound was obtained using α,β-D-Glc-COOH (76 mg, 0.283 mmol) and bpen (40 mg, 0.0238 mmol) as described above for β-D-Gal-COOH. The reagents used were HATU (242 mg, 0.283 mmol), DMF (8 mL) and DIPEA (114 μL, 0.449 mmol). Yield 39% ([α]/[β] = 1:1). ^{1}H NMR (DMSO-d_6, 298 K, 500 MHz) δ 7.89 (s, 1H, –N*H*–, amide), 5.86 (s, OH), 5.22 (brs, 1H, H1-glc-α), 4.87 (brs, 1H, H1-bpen), 4.25 (ap. d, 1H, H1-glc-β), 3.67 (s, 2H, H6-glc-α, H6-glc-β), 3.48 (brs, 1H, H2-glc-α), 3.41 (brs, 3H, H6′-glc-α, H6′-glc-β, H4-bpen), 3.32 (brs, 1H, H2-bpen), 3.12 (brs, 6H, H3-glc-α, H3-glc-β, H5-glc-α, H5-glc-β, H8-bpen), 2.99 (brs, 2H, H2-glc-β, H3-bpen), 2.77 (5H, H6-bpen, H7-bpen, H7-glc-β-SCH_2), 2.64 (3H, H6′-bpen, H7-glc-α-SCH_2), 2.37 (s, 4H, H8-glc-α, H8-glc-β-CH_2C=O) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz) δ 171.0 (O=*C*–NH–), 101.6 (C1-bpen), 84.9 (C1-glc-β), 84.8 (C1-glc-α), 82.6 (C4-bpen), 80.4 (C5-glc), 77.7 (C3-glc), 73.5 (C2-bpen), 72.4 (C3-bpen), 71.1 (C2-glc), 60.7 (C6-glc), 48.3 (C6-bpen), 38.7 (C7-bpen), 38.4 (C8-bpen), 35.8 (C8-glc-*C*H_2C=O), 24.9 (C7-glc-S*C*H_2) ppm. The product contained azabenzotriazole ($C_5H_4N_4O$). ESI-TOF MS *m/z*: 1090.47 ($[M + K + 2Na]^{3+}$), calcd. for $C_{119}H_{210}N_{14}O_{70}S_7 \cdot 2Na \cdot K$: 3266.55. Elemental analysis calcd. for $C_{119}H_{210}N_{14}O_{70}S_7 \cdot 2C_5H_4N_4O \cdot H_2O$ (%): C 44.63, H 6.38, N 8.87; found C 44.72, H 6.97, N 8.66.

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