Supporting Information

Direct and Regioselective Di-\(\alpha\)-fucosylation on the Secondary Rim of \(\beta\)-Cyclodextrin

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Figure S2. $^1$H NMR (400 MHz, CDCl$_3$) of the crude mixture of fucosylated $\alpha$-CDs

$J = 3.5$ to $4$ Hz

H-$\text{1}_{\text{Fuc}}$
Figure S3. Full MS spectrum of nonasaccharide 10, showing the parent ions

\[ [\text{M-H}]^{-} \]

\[ [\text{M-2H}]^{2-} \]
Figure S4. Dihedral angles between two neighboring sugars within the β-CD rings of nonasaccharide 10 as a function of time for the combined trajectory. 0-4000 ns: GROMOS force field; 4000-8000 ns: Q4MD force field. The dashed vertical bar at 4000 ns separates the runs with the two force fields. Coloring is based on the naming of the β-CD ring sugars. For example, blue represents the dihedral between rings G and A (for naming of the rings, see Scheme 3 in the main text), with the dihedral angle defined by the O5-C1-C4-C5 atoms as shown in Figure 4A in the main text.
Figure S5. Representative snapshots of major conformational clusters. Representative snapshots viewed from the bottom (left) and side (right) from the first six conformational clusters obtained from 16,000 conformations from the combined trajectories. The numbers indicate the rank of the cluster (bold yellow), the percentage of conformations in this cluster contributed to the cluster from the combined (yellow), GROMOS (orange), and Q4MD (magenta) trajectories, respectively, (see also Table S5). Protein Data Bank (pdb) coordinates of all representative conformations of the clusters are provided in the archive MD-SUPPINFO.zip, and can be visualized with appropriate software, for example VMD.\textsuperscript{1}
Figure S6. Distribution of distances characterizing the interaction of the fucose rings with respect to each other in product 10.
Black: fucose center to center distance; Orange: fucose C6 to C6 distance. Solid lines: Q4MD force field, dashed lines: GROMOS force field.
Figure S7. Non-trivial NOE candidates for characterization of conformational diversity of β-CD-FUC(3A,3D) (compound 10), specifically looking at the fucose on ring A. The figure shows snapshots of the eight most populated clusters of the simulation in the Q4MD model, highlighting the atoms involved in the close approaches. Magenta: H3 on residue G, yellow: H5 and H6s on the fucose attached to residue A (downstream of G); orange: H1 on the fucose attached to residue A.

Figure S7 shows the most prominent candidates for close contacts with the fucose on ring A (for definition, see Scheme 3 in the main text). The H-atoms on the fucose C5 and C6 (H5,6FA) are relatively close to the H-atom on C3 of the upstream barrel sugar (H3G) in many of the most populated clusters. The closest distance between H5FA and H3G is 1.9 Å in cluster 1; the closest approach distances between the H6FA and H3G are about 2 Å. The most common conformational variation of the fucose is the turning with respect to the cyclodextrin barrel, cf. clusters 1 and 2-4 in Figure S7. In this conformational variation of the fucose, the H-atom on C1 of fucose (H1FA) approaches H3G; the closest distance is 2.28 Å in cluster 2.
Figure S8. Non-trivial NOE candidates for characterization of conformational diversity of β-CD-FUC(3A,3D) (compound 10), specifically looking at the fucose on ring D. The figure shows snapshots of the eight most populated clusters of the simulation in the Q4MD model, highlighting the atoms involved in the close approaches. Magenta: H3 on residue C; yellow: H5 and H6s on the fucose attached to residue D (downstream of C); orange: H1 on the fucose attached to residue D; green: H3 on the fucose attached to residue D.

Figure S8 shows the most prominent candidates for close contacts with the fucose on ring D (for definition, see Scheme 3 in the main text). Similar to the fucose on ring A, the H-atoms on the fucose C5 and C6 (H5,6FD) are relatively close to the H-atom on C3 of the upstream barrel sugar (H3C) in many of the most populated clusters. The closest distance between H5FA and H3G is 2.06 Å in cluster 4; the closest approach distances between the H6FD and H3C are about 2.5 Å. The most common conformational variation of the fucose is the turning with respect to the cyclodextrin barrel, cf. clusters 1 and 2 in Figure S8. In this conformational variation of the fucose, the H-atom on C1 of fucose (H1FD) approaches H3C; the closest distance is 2.44 Å in cluster 1. In addition, there is a close approach to H3C by the H-atom on C3 of the fucose (H3FD); the closest distance is 2.43 Å in cluster 5.
| Entry | Reaction Conditions | Bnβ-CD (4) | BnFuc₁-β-CD | BnFuc₂-β-CD (9) | BnFuc₃-β-CD | BnFuc₄-β-CD | BnFuc₅-β-CD |
|-------|---------------------|------------|--------------|-----------------|-------------|-------------|-------------|
| 1     | -80°C (o/n)         | 2          | 5            | **46**          | 27          | 15          | 5           |
| 2     | -70°C (3h)          | 5          | 9            | **33**          | 21          | 20          | 12          |
| 3     | quenched at -70°C   | 19         | 24           | **35**          | 15          | 7           | nd          |
| 4     | quenched at -60°C   | 5          | 12           | **57**          | 18          | 9           | nd          |
| 5     | quenched at -50°C   | 2          | 5            | **35**          | 29          | 20          | 9           |
| 6₁    | quenched at -60°C   | 6          | 13           | **61**          | 20          | 6           | nd          |

₁ Performed on large scale
Detailed explanation of the NMR assignment

To identify the connections within a fucosylated glucoside ring, HH-COSY (Figure S9) was used: first the $H_1^{\text{Glc}} \rightarrow H_2^{\text{Glc}}$ correlations were identified, but further $H_2^{\text{Glc}} \rightarrow H_3^{\text{Glc}}$ correlations were overlapping. Thus, a TOCSY (Figure S10) experiment was used to identify the $H_1^{\text{Glc}} \rightarrow H_3^{\text{Glc}}$ correlations, and two overlapping H-3 signals were identified at 4.15 ppm, and a cross peak resulting from $H_1^{\text{Fuc}} \rightarrow C_3^{\text{Glc}}$ was observed in a HMBC experiment (Figure S11, linkage A), providing confirmation that the fucoside unit was attached through an $\alpha$-linkage to the C-3 of the glucoside. These $C_3^{\text{Glc}}$ signals have a $^{13}$C signal at 77.5 ppm and a H-3 signal at 4.15 ppm (based on HSQC, Figure S12). From HMBC experiments (Figure S11, linkage B) the connection from $H_3^{\text{Glc}} \rightarrow C_4^{\text{Glc}}$ was found, with the corresponding $C_4^{\text{Glc}}$ signals at 78.5 ppm. Using the HMBC experiment (Figure S11, linkage C), the C4 signals appeared to have a cross-peak at 5.08 ppm, which connected them to $H_1^{\text{Glc'}}$ (a so-called upstream glucoside unit). The peak at 5.08 ppm appears as an app. triplet with integral = 2, so it was identified as two $H_1^{\text{Glc'}}$ signals. TOCSY correlations (Figure S10) revealed that these signals coupled to $H_3^{\text{Glc'}}$ signals that were not linked to a fucoside, leading to the conclusion that each of the (Fuc)Glc motifs has a neighbouring non-modified residue, eliminating the 3A,3B modification pattern.

Scheme S1. Fucosylated region of CD. NMR experiments used to identify certain linkages are presented in two colors: green – TOCSY, red – HMBC.
Figure S9. COSY NMR (600 MHz, D$_2$O), showing the region with H$_1$Glc→H$_2$Glc and H$_1$Fuc→H$_2$Fuc correlations. Further on all figures in the section: H-peaks of fucoses are highlighted with green, fucosylated glucoses with red and non-fucosylated with blue.

Figure S10. TOCSY (600 MHz, D$_2$O) mixing time 80 ms. The H$_1$Glc→H$_3$Glc and H$_1$Fuc→H$_3$Fuc correlations are framed.
Figure S11. HMBC (600 MHz, D₂O) the following connections are shown in frames: A) 2xH₁_Fuc → 2xC₃_Glc (green bonds) B) 2xH₃_Glc → C₄_Glc (red bonds) C) 2xC₄_Glc → 2xH₁_Glc (blue bonds)

Figure S12. HSQC (600 MHz, D₂O) with the cross-peak H₃_Glc-C₃_Glc framed.
Detailed explanation of the MS/MS experiments

Figure S13. Full MS/MS spectrum

Upon fragmentation of the CD, opening of the ring occurs, forming a parent-ion precursor (Scheme S2). The parent ion can undergo different types of cleavages leading to 2 types of adducts: cross-ring fragments (\(2,4^A/2,4^X\) and \(0,2^A/0,2^X\) series) and fragments originating from glycosidic bond cleavages (B, Y, C and Z). In the MS/MS spectrum (Figure S13) three major fragments were identified (Scheme S3): two were originating from cleavage of one fucose unit, leading to Z- and Y-ions (1262 and 639) and a cross-ring \(2,4^A\)-fragment resulting from cleavage of the reducing end glucoside (652).

For the modified β-CDs, seven different possibilities of opening the CD ring are possible, with many similar fragments originating from these options. For the 3A,3C isomer only the linear parent ion depicted in Scheme S4 leads to characteristic fragments (i.e. fragments not possible with the 3A,3D isomer), that have \(m/z\) values of 717, 707, 657 and 767 (Table S2). These fragments are not observed in the MS/MS spectrum (Figure S13). For the 3A,3D regioisomer three linear parent ions were considered (Scheme S5) and for linear parent ion II several ions corresponding to glycosidic bond fragments were observed (Table S3, values found are highlighted in red). Fragments originating from cross-ring cleavages were dominating in the MS/MS spectrum and are given in Table S4, where all the \(m/z\) values that were found in MS/MS spectrum are shown in red. Because
the pattern of found m/z values only confirm the presence of the 3A,3D isomer, and characteristic fragments for 3A,3C isomer were not found, it was concluded that nonasaccharide 10 is substituted in a 3A,3D fashion.

Scheme S2. Possible initial fragmentation to form the linear parent ion. Typical oligosaccharide fragmentations are shown with arrows.

Scheme S3. Major fragments found in the MS/MS spectrum.
Scheme S4. Linear parent ion leading to characteristic 3A,3C fragmentations

Table S2. Cross-ring fragmentations for the 3A,3C nonasaccharide isomer. Characteristic m/z values are shown in blue.

| Parent molecule | AC  |
|-----------------|-----|
| Ring            | 0.2A| 0.2X| 2.4A| 2.4X|
| A               | 248 | 1177| -   | -   |
| B               | 409 | 1015| 349 | 1075|
| C               | 717 | 707 | 657 | 767 |
| D               | 879 | 545 | 819 | 605 |
| E               | 1041| 383 | 981 | 443 |
| F               | 1203| 221 | 1143| 281 |

Scheme S5. Three linear parent ions of the 3A, 3D isomer
Table S3. M/z values of the fragments originating from glycosidic bond cleavages. M/z values found in the MS/MS spectrum are shown in red.

| AD from parent II |   |   |   |
|------------------|--|--|--|
| B    | Y  | C  | Z  |
| 145  | 1280 | 160 | 1264 |
| 452  | 972  | 468 | 956  |
| 614  | 810  | 630 | 794  |
| 776  | 648  | 792 | 632  |
| 1084 | 340  | 1100 | 324 |
| 1246 | 178  | 1262 | 162 |
Table S4. M/z values of the fragments from different linear parent ions of the 3A,3D isomer. M/z values found in the MS/MS spectrum are shown in red.

|     | I     | II    | III   |
|-----|-------|-------|-------|
| 0.2A| 0.2X  | 2.4A  | 2.4X  |
| 409 | 1015  | 349   | 1075  |
| 571 | 853   | 511   | 913   |
| 733 | 691   | 673   | 751   |
| 1041| 383   | 981   | 443   |
| 1203| 221   | 1143  | 281   |
| 1365| 59    | 1305  | 119   |

|     | 0.2A  | 0.2X  | 2.4A  | 2.4X  |
|-----|-------|-------|-------|-------|
| 409 | 1015  | 349   | 1075  |       |
| 571 | 853   | 511   | 913   |       |
| 733 | 691   | 673   | 751   |       |
| 1041| 383   | 981   | 443   |       |
| 1203| 221   | 1143  | 281   |       |
| 1365| 59    | 1305  | 119   |       |

|     | 0.2A | 0.2X | 2.4A | 2.4X |
|-----|------|------|------|------|
| 264 | 1161 | 203  | 1221 |
| 571 | 853  | 511  | 913  |
| 733 | 691  | 673  | 751  |
| 895 | 529  | 835  | 589  |
| 1057| 367  | 997  | 427  |
| 1365| 59   | 1305 | 119  |
General Experimental Procedures

All solvents used were of commercial grade and used without further purification. Dry DCM, toluene and THF were generated by an MBraun SPS 800 solvent purification system. Solvents used for workup and column chromatography were of technical or HPLC grade from Boom, Biosolve or Honeywell and used as purchased. Solvents were removed by rotary evaporation under reduced pressure at 45°C. Reagents were purchased from Sigma-Aldrich, Acros, TCI Europe or CarboSynth and used without further purification. Reaction temperature refers to temperature of the cooling bath equipped with stirring bar, unless stated otherwise. Reactions were monitored by TLC analysis on Merck silica gel 60/Kieselguhr F254 and spots were visualized by UV light, or spraying with orcinol stain (a mixture of 180 mg orcinol, 10 mL 85% H₃PO₄, 5 mL EtOH and 85 mL H₂O) or with Seebach’s stain (2.5 g phorphomolybdic acid, 1 g Ce(SO₄)₂, 6 mL H₂SO₄ and 94 mL H₂O) followed by heating with a heat gun. Column chromatography was performed using silica (Standard Silica 60M, 0.04 – 0.063 mm, 230-400 mesh, Macherey-Nagel GmbH, Germany) as the stationary phase. Size-exclusion chromatography was performed on Sephadex LH-20 using DCM/MeOH (1/1, v/v) as eluent. Molecular sieves 4Å (Merck, Germany) were activated by heating with a heat gun in vacuo.

¹H and ¹³C NMR spectra were recorded on a Varian 400-MR (400/100 MHz) or a Bruker Avance NEO (600/150 MHz). Chemical shifts are given in ppm with the solvent resonance as internal standard (CDCl₃: δ7.26 for ¹H, δ 77 for ¹³C; D₂O: δ 4.79 for ¹H). All individual signals were assigned using 2D NMR spectroscopy: HH-CONJ, gHSQC or NOE. Data are reported as follows: chemical shifts (δ), multiplicity (s = singlet, d = doublet, dd = double doublet, ddd = double double doublet, t = triplet, q = quartet, p = quintet, m = multiplet, apparent quartet = app q), coupling constants J (Hz), and integration. High resolution mass measurements were performed on a LTQ Orbitrap XL spectrometer (Thermo Fisher Scientific) with an ESI ionization source.

UPLC-UV/MS measurements were performed on a Vanquish UHPLC system coupled to a LCQ Fleet mass spectrometer (Thermo Fisher Scientific) using an Acquity UPLC BEH C4 column (Waters, 2.1×150 mm, 1.7 µm) in combination with eluents A (10 mM ammonia acetate in H₂O) and B (acetonitrile), or using a Cortecs UPLC HILIC column (Waters, 2.1×150 mm, 1.6 µm) in combination with eluents A (H₂O + 0.025% NH₄OH) and B (acetonitrile + 0.025% NH₄OH). The UV traces were measured at 208 and 254 nm simultaneously and the ionization was performed with an ESI-source. In positive mode the [M+NH₄]⁺ or [M+2NH₄]²⁺ were most prominent. In negative mode the [M-H]⁻ or [M-2H]²⁻ were most prominent. For chromatography on the C4 a 25 min run (flow rate 0.3 mL/min) was used, and the gradient used in the method was as follows: from 70% to 95% of B (from 0 to 20 min) and 70% of B (from 21 to 25 min). For chromatography on the Cortects HILIC a 25 min run (flow rate 0.3 mL/min) was used, and the gradient was as follows: 70% of B (from 0 to 2 min), from 70% to 50% of B (from 2 to 15 min) and 70% of B (from 16 to 25 min).

For preparative HPLC purification (Shimadzu), an Xbridge BEH C4 column (Waters, 10×150 mm, 5 µm) was used in combination with eluents A (H₂O) and B (acetonitrile) with UV-detection at
208 nm. For purification, a sample was dissolved in acetonitrile in a concentration of 2 mg/mL and up to 800 µL was injected without affecting the separation. A 15 min column flush with 70% of B was performed before each run. A method of 40 min (flow rate 5 mL/min) was used and the gradient was as follows: from 70% to 95% (from 0 to 30 min) and 95% of B (from 30 to 40 min).

For fragmentation experiments separation on Cortects HILIC was performed. Samples were prepared in 70% acetonitrile in water in a concentration of 1 mg/mL, and 10 µL was used per injection. Upon chromatography an eluent was introduced into ESI ion source where the following conditions were set: heater temperature 75°C, spray voltage 2 kV, capillary temperature 200°C, capillary voltage -2 V. Collision induced dissociation was set at 25% normalized collision energy and an isotopic width of 3 m/z. The MS/MS spectra were obtained in negative ion mode in a mass range from 195 to 1440 m/z.
Synthesis of fucoside-donor 5

Scheme S6. Synthesis of fucosyl donor 5. Reagents and conditions: i – 1. Ac₂O, pyridine, 0°C to RT 2. 33% HBr/AcOH 3. HSTol, TBAB (aq), KOH (aq), CHCl₃; ii – Na in MeOH; iii – BnBr, NaH, DMF, 0°C to RT

p-Methylphenyl 2,3,4-tri-O-acetyl-1-thio-β-L-fucopyranoside (F1).

L-(–)-fucose (5.03 g, 31 mmol) was portionwise added to a stirred solution of pyridine (54 mL, 52.8 g, 0.668 mol) and acetic anhydride (42 mL, 45.4 g, 0.444 mol) under nitrogen at 0°C over the course of 15 min. The reaction was left to stir overnight at +4 °C after which time it was poured on a mixture of crushed ice and water. The aqueous layer was extracted with DCM (3x). The combined organic layers were washed with H₂O (2x), brine, dried over MgSO₄ and concentrated in vacuo. The crude compound was flushed through a pad of silica gel (pentane/EtOAc = 2/1) and concentrated in vacuo. Next, a mixture of peracetylated fucose (~31 mmol) in DCM (120 mL, 0.25 M) was cooled to 0°C under nitrogen, and HBr (33 wt. % in acetic acid, 40 mL, 56.0 g, 0.692 mol) was added dropwise in 20 min. When TLC analysis indicated complete consumption of the starting material (2.5h) the reaction mixture was carefully poured on crushed ice and stirred. The resulting aqueous layer was extracted with DCM (2x). The combined organic layers were washed with NaHCO₃ (3x) until neutral pH, then washed with H₂O (1x), brine (1x), dried over MgSO₄ and concentrated in vacuo. Without further purification the resulting anomeric bromide was dissolved in CHCl₃ (300 mL) and p-thiocresol (5.5 g, 44 mmol) and TBABₐq (dissolved in 47 mL H₂O, 1.88 g, 5.8 mmol) were added. To the reaction mixture KOHₐq (dissolved in 45 mL H₂O, 3.4 g, 61 mmol) was added dropwise in 10 min. The two layered reaction mixture was stirred vigorously overnight at ambient temperature. TLC analysis (DCM/MeOH/H₂O, 10/5/1, v/v/v) indicated complete consumption of the anomeric bromide. The organic layer was washed with H₂O (1x), brine (1x), dried over MgSO₄ and concentrated in vacuo. Purification by flash column chromatography (silica gel, gradient from pentane/EtOAc, 6/1 to 2/1) afforded the title compound as a white solid (Yield: 10.62 g, 26.8 mmol, 89% over 3 steps). The analytical data were in accordance with those reported previously.² TLC: Rₜ 0.21 (pentane/EtOAc, 6/1, v/v).
**p-Methylphenyl 1-thio-β-L-fucopyranoside (F2).**

To a stirred solution of monosaccharide F1 (7.52 g, 19.0 mmol) in anhydrous MeOH (40 mL) a piece of sodium (approx. 300 mg) was added. When TLC analysis (DCM/MeOH, 5/1, v/v) indicated complete consumption of the starting material (25 min) the reaction mixture was neutralized by the addition of Amberlite IR-120H+ until neutral pH, filtered and concentrated in vacuo. The title compound was obtained as a white solid (Yield: 5.01 g, 18.5 mmol, 98%). The analytical data were in accordance with those reported previously.³ TLC: Rf = 0.33 (EtOAc).

**p-Methylphenyl 2,3,4-tri-O-benzyl-1-thio-β-L-fucopyranoside (5).**

To a stirred solution of p-methylphenyl 1-thio-β-L-fucopyranoside (8.72 g, 32 mmol) in DMF (108 mL) under nitrogen atmosphere, NaH (60% dispersion in mineral oil, 6.6 g, 0.165 mol) was added. The reaction was stirred for 20 minutes, after which time benzyl bromide (20 mL, 0.165 mol) was added dropwise in 1 h. The reaction mixture was stirred overnight at ambient temperature (18h) and then diluted by Et₂O and quenched by the slow addition of an ice-water mixture. The aqueous layer was extracted with Et₂O (3x). The organic layer was washed with brine, dried over MgSO₄ and concentrated in vacuo. Recrystallisation from EtOH afforded the title compound as a white fluffy solid (Yield: 13.8 g, 25 mmol, 79%). The analytical data were in accordance with those reported previously.⁴⁵ Rf = 0.33 (pentane/Et₂O, 6/1, v/v).

¹H NMR (400 MHz, CDCl₃) δ 7.50 – 7.47 (m, 2H, CHarom STol), 7.42 – 7.27 (m, 13H, CHarom Bn), 7.01 (d, 2H, J = 7.8 Hz, CHarom STol), 5.00 (d, 1H, J = 11.6 Hz, CHH Bn), 4.80 (d, 1H, J = 10.2 Hz, CHH Bn), 4.75 – 4.70 (m, 3H, CHH Bn, CH₂ Bn), 4.66 (d, 1H, J = 11.6 Hz, CHH Bn), 4.54 (d, 1H, J = 9.6 Hz, H-1), 3.89 (t, 1H, J = 9.4 Hz, H-2), 3.63 (d, 1H, J = 2.8 Hz, H-4), 3.58 (dd, 1H, J = 9.2, 2.8 Hz, H-3), 3.51 (q, 1H, J = 6.4 Hz, H-5), 2.30 (s, 3H, CH₃ STol), 1.26 (d, 3H, J = 6.3 Hz, H-6).

¹³C NMR (100 MHz, CDCl₃) δ 138.8, 138.5, 138.4, 137.1 (C₄), 132.2, 130.5, 129.5, 128.4, 128.3, 128.1, 128.0, 127.7, 127.6, 127.4 (CHarom), 87.9 (C-1), 84.6 (C-3), 77.2 (C-2), 76.6 (C-4), 75.5 (CH₂ Bn), 74.6 (C-5), 72.8 (CH₂ Bn), 21.1 (CH₃ STol), 17.3 (C-6).
Synthesis of the cyclodextrin acceptors

Scheme S7. Synthesis of CD acceptors. Reagents and conditions: i – for α-CD: BnBr, NaH, DMF; for β-CD: BnCl, NaH, DMSO; ii – I₂, Et₃SiH, DCM, -60° to -35°C.

Perbenzylated α-CD (1).
To a stirred solution of α-cyclodextrin (1.05 g, 1.08 mmol) in DMF (13.5 mL) under nitrogen at 0°C, NaH (60% dispersion in mineral oil, 4.7 g, 117.5 mmol) was added portionwise. After stirring the reaction mixture for 15 min, benzyl bromide (14 mL, 118 mmol) was added to the solution dropwise in 30 min. After 4.5h when TLC control indicated complete conversion of the starting material into a single spot, the reaction mixture was diluted with Et₂O and quenched by the slow addition of ice-water at 0°C. The water layer was extracted with Et₂O (3x), the combined organic layers were washed with H₂O, brine, dried over MgSO₄ and concentrated. Purification by flash column chromatography (silica gel, pentane/Et₂O, 6/1 to 2/1) afforded the title compound as a white foam (Yield: 2.43 g, 0.94 mmol, 87%). The analytical data were in accordance with those reported previously.⁶ Rᶠ = 0.41 (pentane/Et₂O, 2/1, v/v).

2⁴-F, 6⁴-F-dodeca-O-benzyl-α-CD (3).
Perbenzylated α-cyclodextrin 1 (580 mg, 0.23 mmol) and iodine (379 mg, 1.5 mmol) were suspended in DCM (83 mL, 18 mM I₂) under nitrogen. After complete dissolution of iodine, the purple reaction mixture was cooled down to -45°C (temperature measured inside the flask), followed by the addition of triethylsilane (0.24 mL, 1.5 mmol). The reaction mixture was allowed to warm up to -35°С (1.5h) and was then neutralized by the addition of solid K₂CO₃ and then the reaction mixture was washed with a saturated solution of Na₂S₂O₃. The water layer was extracted with DCM (2x) and the combined organic layers were washed with brine, dried over MgSO₄ and concentrated. Purification using flash column chromatography (silica gel, toluene/EtOAc, from 9/1 to 5/1, v/v) yielded the title compound as a white foam (Yield: 279 mg, 0.14 mmol, 60%). The analytical data were in accordance with those reported previously.⁷ TLC: Rᶠ = 0.44 (toluene/EtOAc, 5/1, v/v).
**β-CD**

^1H NMR (400 MHz, Deuterium Oxide) δ 5.03 (d, J = 3.7 Hz, 1H, H-1), 3.92 (t, J = 9.5 Hz, 1H, H-3), 3.82 (m, 3H, H-5, H-6a,b), 3.61 (dd, J = 10.0, 3.6 Hz, 1H, H-2), 3.55 (t, J = 9.1 Hz, 1H, H-4).

**Perbenzylated β-CD (2).**

To a stirred solution of β-cyclodextrin (12.1 g, 10.7 mmol) in DMSO (213 mL) at 0°C under nitrogen, NaH (60% dispersion in mineral oil, 17.9 g, 0.45 mol) was added portionwise in 15 min. Benzyl chloride (51 mL, 0.45 mol) was added dropwise in 1 h. The reaction mixture was left to proceed overnight (19 h) at ambient temperature and then neutralized by the slow addition of MeOH (55 mL) at 0°C. Then the resulting mixture was diluted with H2O and extracted with Et2O (3 x 500 mL). The combined organic layers were separated, dried over MgSO4 and concentrated. Purification by flash column chromatography (silica gel, pentane/Et2O = from 4/1 to 1/1) afforded the title compound as a white foam (Yield: 28.4 g, 9.4 mmol, 88%). The analytical data were in accordance with those reported previously.6 Rf = 0.21 (pentane/Et2O, 2/1, v/v).

**2A-G,6A-G-tetradeca-O-benzyl-β-CD (4).**

Perbenzylated β-cyclodextrin 2 (2.80 g, 0.9 mmol) and iodine (1.81g, 7.1 mmol) were suspended in DCM (396 mL, 18 mM I2) under nitrogen. After complete dissolution of iodine, the purple reaction mixture was cooled down to -60°C (temperature measured inside the flask), followed by the addition of triethylsilane (1.14 mL, 7.1 mmol). The reaction mixture was allowed to warm up to -35°C (1.5h) and was then neutralized by the addition of K2CO3. Then the reaction mixture was washed with a saturated solution of Na2S2O3. The water layer was extracted with DCM (2x) and the combined organic layers were washed with brine, dried over MgSO4 and concentrated. Purification using flash column chromatography (toluene/EtOAc, 8/1 to 3/1, v/v) yielded the title compound as a white foam (Yield: 570 mg, 0.24 mmol, 27%). TLC: Rf = 0.24 (toluene/EtOAc, 5/1, v/v).

^1H NMR (400 MHz, CDCl3) δ 7.46 – 7.32 (m, 7×H, CHarom), 7.22 – 7.12 (m, 7×H, CHarom), 5.11 (s, 7×H, 7×OH), 5.00 (d, 7H, J = 11.9 Hz, 7×CHH Bn), 4.84 – 4.73 (m, 14H, 7×H-1, 7×CHH Bn), 4.48 (d, 7H, J = 12.2 Hz, 7×CHH Bn), 4.25 (d, 7H, J = 12.2 Hz, 7×CHH Bn), 4.05 (t, 7H, J = 9.2 Hz, 7×H-3), 3.73 (m, 7H, 7×H-5), 3.63 – 3.55 (m, 7H, 7×H-6a), 3.46 (m, 21H, 7×H-2, 7×H-4, 7×H-6b).
$^{13}$C-APT NMR (100 MHz, CDCl$_3$) $\delta$ 138.1 (7×C$_q$), 137.6 (7×C$_q$), 128.9, 128.4, 128.08, 127.6, 127.5 (CH$_{arom}$), 102.1 (7×C-1), 83.4 (7×C-4), 78.3 (7×C-2), 74.0 (7×CH$_2$ Bn), 73.8 (7×C-3), 73.1 (7×CH$_2$ Bn), 70.3 (7×C-5), 68.5 (7×C-6).

ESI-HRMS: [M-H]$^-$ calcd for C$_{140}$H$_{153}$O$_{35}$ 2395.02205 found 2395.02620.
Fucosylations of cyclodextrins

**3A-O-(2,3,4-tri-O-α-L-benzylfucopyranosyl)-2A-G,6A-G-tetradeca-O-benzyl-β-CD (Bn-Fuc-β-CD)**

A mixture of donor 5 (87 mg, 0.161 mmol), Ph$_2$SO (42 mg, 0.21 mmol) and TTBP (100 mg, 0.40 mmol) was co-evaporated 3 times with dry toluene. The residue was dissolved in DCM (0.4 mL) under nitrogen and activated molecular sieves (4Å) were added. The resulting mixture was stirred at room temperature for 20 min and then it was cooled down to -80°C (acetone cooling bath equipped with a stirring bar). Tf$_2$O (35 µL, 0.21 mmol) was added and the process of donor activation was monitored by TLC analysis. After 40 minutes, when TLC control indicated complete activation of donor, the reaction mixture was cooled down to -85°C and a solution of acceptor 4 (48 mg, 0.020 mmol) in DCM (0.3 mL) was added in portions of 0.15 mL slowly (in 2 minutes) via the wall of the flask. Upon completion of the addition the temperature reached -80°C and was allowed to warm up to -65°C (12 min). The reaction mixture was then diluted with DCM, and neutralized by addition of H$_2$O and Et$_3$N. The mixture was concentrated in vacuo and purified on size-exclusion Sephadex LH-20 (DCM/MeOH, 1/1, v/v). Fraction of fucosylated cyclodextrins (45 mg, 70% yield) contained: 27% of octasaccharide and 37% of nonasaccharide 9, the rest contained acceptor and overfucosylated products. R$_f$ = 0.14 (Toluene/EtOAc, 8/1, v/v). The title compound was purified by preparative HPLC on BEH C4 column (5 mg) (RT 22.5) and purity was checked by UPLC-UV/MS (RT 14.1 min).

**1H NMR (600 MHz, CDCl$_3$)** δ 7.44 – 6.96 (m, 85H, CH$_{arom}$), 5.81 (d, 1H, $J$ = 4.0 Hz, H-1Fuc), 4.96 – 4.81 (m, 10H, 9×CHH Bn, H-1Glc), 4.78 (d, 1H, $J$ = 3.6 Hz, H-1Glc), 4.76 – 4.64 (m, 13H, 4×H-1Glc, H-5Fuc, CH$_2$ Bn, 6×CHH Bn), 4.54 – 4.48 (m, 3H, 3×CHH Bn), 4.47 (d, $J$ = 3.4 Hz, 1H, H-1Glc), 4.44 – 4.20 (m, 13H, CHH Bn, 5×CH$_2$ Bn, H-3Glc, H-3Fuc), 4.18 – 4.05 (m, 4H, 3×CHH Bn, H-2Fuc), 3.98 (t, $J$ = 9.3 Hz, 4H, 4×H-3Glc), 3.90 – 3.85 (m, 2H, H-3Glc, H-4Fuc), 3.80 (dt, 1H, $J$ = 3.6, 7.3 Hz, H-5Glc), 3.76 – 3.71 (m, 2H, H-4Glc, H-5Glc), 3.71 – 3.67 (m, 3H, 3×H-5Glc), 3.63 – 3.49 (m, 8H, H-3Glc, H-5Glc, 3×H-6Glc), 3.49 – 3.46 (m, 1H, H-2Glc), 3.45 – 3.30 (m, 17H, 5×H-2Glc, 4×H-4Glc, 8×H-6Glc), 3.29 – 3.17 (m, 6H, H-2Glc, 2×H-4Glc, 3×H-6Glc), 1.24 (d, $J$ = 6.5 Hz, 3H, CH$_3$ Fuc).

**13C NMR (151 MHz, CDCl$_3$)** δ 139.4, 139.2, 139.0, 139.0, 138.3, 138.1, 138.1, 138.0, 137.7, 137.6, 137.4, 136.8 (C$_c$); 129.1, 129.1, 128.9, 128.9, 128.8, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.5, 127.4, 127.3, 127.2, 127.2, 126.9 (C$_{arom}$); 102.4, 102.2, 102.0, 102.0, 101.8, 101.7 (6×C-1glic); 99.4 (C-1glic), 97.5 (C-1Fuc); 84.3, 83.9, 83.7, 83.3, 83.3, 83.0 (6×C-4glic); 82.5 (C-2glic), 79.7 (C-3glic), 79.4 (C-4glic), 79.0 (C-2glic), 78.7(C-3glic), 78.5, 78.4, 78.3, 77.9, 77.8 (5×C-2glic), 76.2 (C-2Fuc), 75.1, 74.2, 74.2, 74.1, 74.1 (CH$_2$ Bn); 73.9, 73.8, 73.8, 73.6 (4×C-3glic), 73.3, 73.2, 73.2, 73.2, 73.1, 73.0 (CH$_2$ Bn), 72.7 (C-5glic), 72.2 (C-3glic), 71.8 (C-3Fuc) 71.5 (CH$_2$ S26
Bn), 70.4, 70.3, 70.3, 70.0, 69.9, (7xC-5Glc), 69.8, 68.9, 68.8, 68.4, 68.4, 68.3, 66.1 (7xC-6Glc), 16.5 (C-6Fuc).

ESI-HRMS: [M+2×NH₄]²⁺ calcd for C₁₆₇H₁₉₂O₃₉N₂ 1424.14842 found 1424.14862.

**3⁴⁺,3⁶⁺-Di-O-(2,3,4-tri-O-α-L-benzylfucopyranosyl)-2⁴-G,6⁶-G-tetradeca-O-benzyl-β-CD (9)**

A mixture of donor 5 (1.81 g, 3.35 mmol), Ph₂SO (0.881 g, 4.36 mmol) and TTBP (2.08 g, 8.39 mmol) was co-evaporated 3 times with dry toluene. The residue dissolved in dry DCM (8 mL) under nitrogen and activated molecular sieves (4Å) were added. The resulting mixture was stirred at room temperature for 20 min and then cooled down to -80°C (acetone cooling bath equipped with a stirring bar). Tf₂O (0.73 mL, 4.36 mmol) was added dropwise and the process of donor activation was monitored by TLC analysis (pentane/Et₂O, 6/1, v/v, Rₚ of donor = 0.33). After 40 minutes when TLC control indicated complete activation of the donor, the reaction mixture was cooled down to -85°C and a solution of acceptor 4 (1.0 g, 0.42 mmol, 1 eq) in dry DCM (6 mL) was added in portions of 2 mL slowly (in 10 minutes) via the wall of the flask. Upon complete addition the temperature reached -80°C and the reaction mixture was allowed to warm up to -60°C (15 min). The mixture was diluted with DCM and neutralized by addition of H₂O and Et₃N. Then the crude mixture was concentrated and purified on size-exclusion Sephadex LH-20 (DCM/MeOH, 1/1, v/v). Additional purification by column chromatography provided nonasaccharide 9 as a white foam (Yield: 898 mg, 0.28 mmol, 67%, 69% purity based on UPLC UV absorbance 208 nm) TLC: Rₚ = 0.19 (toluene/EtOAc, 8/1, v/v). For detailed analysis the title compound was purified by preparative HPLC on BEH C4 (RT 27.1 min) and purity was checked by UPLC-UV/MS (RT 17.0 min).

¹H NMR (600 MHz, CDCl₃) δ 7.62 – 6.95 (m, 100H, CHₐrom), 5.97 (d, 1H, J = 4.1 Hz, H-1Fuc), 5.93 (d, 1H, J = 3.9 Hz, H-5Fuc), 5.09 – 4.72 (m, 29H, 8xCH₂ Bn, 5xH-1Glc, 2xH-5Fuc), 4.71 – 4.50 (m, 16H, 2xH-1Glc, 7xCH₂ Bn), 4.50 – 4.12 (m, 38H, 5xCH₂ Bn, 2xH-3Glc, 2xH-2Fuc, 2xH-3Fuc), 4.11 – 3.95 (m, 6H, 4xH-3Glc, 2xH-4Fuc), 3.94 – 3.83 (m, 4H, 2xH-4Glc, 2xH-5Glc), 3.79 – 3.63 (m, 7H, H-3Glc, 5xH-5Glc), 3.63 – 3.22 (m, 26H, 7xH-2Glc, 5xH-4Glc, 14xH-6Glc), 1.38 (d, 3H, J = 6.4 Hz, H-6Fuc), 1.34 (d, 3H, J = 6.5 Hz, H-6Fuc).

¹³C NMR (150 MHz, CDCl₃) δ 139.4, 139.2, 139.2, 139.1, 139.1, 138.9, 138.3, 138.3, 138.3, 138.1, 138.1, 137.9, 137.9, 137.7, 137.6, 137.5, 137.4, 137.3, 136.8, 136.8, (Cₐ), 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.1, 128.1, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.5, 127.5 (Cₐrom), 102.6, 102.2, 102.1, 101.9, 101.6, 99.5, 99.4 (7xC-1Glc), 97.5, 97.3 (2xC-1Fuc), 84.5, 84.1, 83.9, 83.8, 83.6, 83.0, 82.5 (2xC-2Glc, 5xC-4Glc), 79.8, 79.7, 79.4, 79.3, 79.1, 79.1, 78.8, 78.7, 78.6, 78.3, 78.1, 78.0, 77.4 (5xC-2Glc, 3xC-3Glc, 3xC-5Glc, 4xC-6Glc).
2×C-4\text{Glc}), 76.5, 76.1 (2×C-2\text{Fuc}), 75.1, 75.0, 74.3, 74.2, 74.1, 74.0, 73.9, 73.3, 73.1, 73.1, 73.0, 72.9, 72.8, 72.6, 72.5, 72.1, 71.7, 71.4, 71.4 (5×C-3\text{Glc}, 20×\text{CH}_2\text{Bn}); 70.4, 70.3, 70.1, 70.0, 69.9, 69.8 (7×C-5\text{Glc}), 69.4, 69.0, 68.9, 68.5, 68.4, 68.2, 68.1 (7×C-6\text{Glc}); 66.2, 66.2 (2×C-5\text{Fuc}), 16.7, 16.6 (2×C-6\text{Fuc}).

ESI-HRMS: [M+\text{NH}_4]^+ \text{calcd for } C_{194}H_{214}O_{43}N_1 3247.46512 \text{ found 3227.45595.}

\textbf{3A-O-(α-L-fucopyranosyl)-β-CD (Fuc1-β-CD)}

Octasaccharide BnFuc1-β-CD, as isolated from preparative HPLC, was dissolved in a THF/H$_2$O mixture (2.5 ml/0.2 mL) and excess of Pd/C was added. The mixture was purged with nitrogen, followed by purging with hydrogen, and the mixture was left to react overnight under a blanket of hydrogen gas. The mixture was filtered over a pad of celite, concentrated and lyophilized (1 mg).

$^1$H NMR (600 MHz, D$_2$O) $\delta$ 5.54 (d, 1H, $J = 3.8$ Hz, H-1\text{Fuc}), 5.16 (d, 1H, $J = 3.8$ Hz, H-1\text{Glc}), 5.12 – 5.04 (m, 6H, 6×H-1\text{Glc}), 4.46 (q, 1H, $J = 6.7$ Hz, H-5\text{Fuc}), 4.21 – 4.15 (m, 1H, H-6\text{Glc}), 4.15 – 4.10 (m, 1H, H-3\text{Glc}), 4.00 – 3.93 (m, 8H, 6×H-3\text{Glc}, H-6\text{Glc}, H-4\text{Fuc}), 3.92 – 3.80 (m, 22H, H-4\text{Glc}, 7×H-5\text{Glc}, 12×H-6\text{Glc}, H-2\text{Fuc}, H-3\text{Fuc}), 3.71 – 3.55 (m, 13H, 7×H-2\text{Glc}, 6×H-4\text{Glc}), 1.26 (d, 3H, $J = 6.5$ Hz, H-6\text{Fuc}).

$^{13}$C NMR (150 MHz, D$_2$O) $\delta$ 102.0, 101.9, 101.8, 101.7, 101.6 (6×C-1\text{Glc}); 99.9 (C-1\text{Glc}), 98.8 (C-1\text{Fuc}), 81.1, 81.0, 80.9, 80.8 (6×C-4\text{Glc}), 78.7 (C-4\text{Glc}), 77.3 (C-3\text{Glc}); 73.2, 73.1, 73.0, 73.0, (6×C-3\text{Glc}), 72.6, 72.1, 71.9, 71.9, 71.8, 71.6, 71.6, 71.4, 71.2 (7×C-2\text{Glc}, 7×C-5\text{Glc}, C-3\text{Fuc}), 69.2 (C-4\text{Fuc}), 68.9 (C-2\text{Fuc}), 67.8 (C-5\text{Fuc}), 60.5, 60.2, 60.1 (7×C-6\text{Glc}); 15.3 (C-6\text{Fuc}).

ESI-HRMS: [M+\text{NH}_4]^+ \text{calcd for } C_{48}H_{84}O_{39}N_1 1298.46150 \text{ found 1298.46375.}

\textbf{3A,3\text{D}-Di-O-(α-L-fucopyranosyl)-β-CD (10)}

Nonasaccharide 9 (830 mg, 0.26 mmol) was dissolved in THF (10 mL) and H$_2$O (1 mL) and Pd/C (6 g) was added. The reaction mixture was purged with nitrogen followed by purging with hydrogen. The reaction was left to stir over 3 days under a blanket of hydrogen gas, after which the mixture was filtered over celite, concentrated and lyophilized to yield the title compound as a white solid (Yield: 275 mg, 0.19 mmol, 75%, 69% purity). RT on Cortecs HILIC 3.5 min.
\(^1\)H NMR (600 MHz, D\(_2\)O) \(\delta\) 5.56 (d, 1H, \(J = 3.8\) Hz, H-1_Fuc), 5.54 (d, 1H, \(J = 3.9\) Hz, H-1_Fuc), 5.19 (d, 1H, \(J = 4.1\) Hz, H-1_Glc), 5.18 (d, 1H, \(J = 3.9\) Hz, H-1_Glc), 5.15 (app t, 2H, \(J = 3.5\) Hz, 2xH-1_Glc), 5.12 (d, 1H, \(J = 3.7\) Hz, H-1_Glc), 5.08 (app t, 2H, \(J = 3.1\) Hz, 2xH-1_Glc), 4.47 (q, \(J = 6.2, 5.5\) Hz, 2H, 2xH-5_Fuc), 4.21 (dt, 2H, \(J = 4.1, 12.6\) Hz, 2xH-6_Glc), 4.16 (t, 2H, \(J = 8.3\) Hz, 2xH-3_Glc), 4.06 – 3.98 (m, 6H, 3xH-3_Glc, 2xH-6_Glc, 2xH-4_Fuc), 3.96 – 3.80 (m, 35H, 2xH-3_Glc, 2xH-4_Glc, 7xH-5_Glc, 10xH-6_Glc, 2xH-2_Fuc, 2xH-3_Fuc), 3.75 – 3.67 (m, 5H, 5xH-2_Glc), 3.65 – 3.54 (m, 7H, 2xH-2_Glc, 5xH-4_Glc), 1.28 (d, 6H, \(J = 6.6\) Hz, 2xH-6_Fuc).

\(^{13}\)C NMR (150 MHz, D\(_2\)O) \(\delta\) 101.9, 101.8, 101.7, 101.5, 101.2 (5xC-1_Glc), 99.5, 99.3 (2xC-1_Glc), 99.0, 98.9 (2xC-1_Fuc), 81.3, 81.1, 81.0, 80.4 (5xC-4_Glc), 78.5, 78.4 (2xC-4_Glc), 77.5, 77.2 (2xC-3_Glc); 73.2, 73.2, 73.1, 72.8, 72.6 (5xC-3_Glc), 72.1, 72.0, 72.0, 71.9, 71.7, 71.6, 71.4, 71.3, 71.2 (7xC-5_Glc, 7xC-2_Glc, 2xC-3_Fuc), 69.3 (2xC-4_Fuc), 69.0, 68.9 (2xC-2_Fuc), 67.9, 67.8 (2xC-5_Fuc), 60.7, 60.4, 60.3, 60.2, 60.2 (7xC-6_Glc), 15.3 (2xC-6_Fuc).

HRMS: [M-H]\(^-\) calcd for C\(_{54}\)H\(_{89}\)O\(_{43}\) 1425.47721 found 1425.48104.

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Computational details

Molecular Dynamics (MD) simulations were performed for beta-cyclodextrin (β-CD) and the doubly fucosylated β-CD molecule 10 (β-CD-FUC(3A,3D)) using two different force fields, GROMOS 53a6 and Q4MD, using the GROMACS simulation package, version 2016.3. GROMOS is a so-called united atom model, in which aliphatic H-atoms are not treated explicitly, but modeled effectively together with the C-atom they are attached to. Gebhardt et al. studied multiple flavors of the GROMOS force field as they constitute several stages in the refinement of the force field to model carbohydrates. Q4MD is an AMBER-based all-atom force field, optimized for carbohydrates, with a version specific for cyclodextrins (Q4MD-CD) which is used here. These force fields were recently part of an extensive comparative study of cyclodextrins by Gebhardt et al., which highlighted the structural flexibility of cyclodextrins, which was shown to be captured to a different extent in different force fields. The study identifies Q4MD-CD as the force field that is the most consistent with the known experimental data, although a number of other force fields are nearly as good.

Topology
Topologies were built by extending the libraries (.rtp files) that contain building blocks (residues or fragments) that are employed by the GROMACS program pdb2gmx to build topologies for molecules consisting of multiple connected residues. Atom types dictating the non-bonded interactions, partial charges, and the parameters for bond stretching, angle bending, and dihedral (torsional) motions have been described within each of the force fields and were implemented according to the literature. The GROMOS model used in this study is 53a6, with the important modification that dihedral restraints were employed to all sugar rings ensuring the maintenance of the predominant chair conformation $4C_1$ (see also the discussion on the tilting of the β–CD rings with respect to the barrel wall). After completion, the topologies were checked by hand against published parameter sets for the two force fields. The topologies are available in the archive containing the simulation data. Water as solvent is described by the SPC water model in combination with the GROMOS force field; Q4MD is combined with the TIP3P model for water. Note that (a) in contrast to the study by Gebhardt et al., the GROMACS engine was used exclusively for the simulations; (b) in standard GROMOS 53a6, dihedral restraints to fix the predominant chair conformation $4C_1$ are not imposed; and (c) the force field referred to as Q4MD in this manuscript is designated Q4MD-CD in the study by Gebhardt et al.

Starting structures
The coordinates of β-CD were available from an earlier project, and were used for the β-CD simulations described here. Fucosylated β-CD coordinates were generated by placing fucose moieties close to their target position on the β-CD ring (substitution is on O3). Structures were then optimized first in vacuo, and subsequently solvated in water (SPC molecules for GROMOS,
TIP3P molecules for Q4MD) in a cubic simulation box of approx. 5.7×5.7×5.7 nm³, followed by a round of energy minimization runs of the combined system. This system was then equilibrated in a series of short (1-10 ps) runs, increasing the time-step from 0.1 to 2.5 fs, switching on constraints on all bonds in the later stages. All bond lengths were constrained to their reference value using the LINCS algorithm. The geometry of the SPC and TIP3P water models was kept rigid using the SETTLE algorithm.

Production runs
The production system contains a single β-CD or β-CD-FUC(3A,3D) molecule, and 5661 or 5680 water molecules, respectively. Production MD simulations of 4 μs (microseconds) were performed for both force fields for the β-CD -FUC(3A,3D) molecule. The simulations were done with a time-step of 2.5 fs under periodic boundary conditions. The Verlet buffered neighbor list update scheme was used with a buffer tolerance of 0.005, based on a cut-off distance of 1.4 nm for the van der Waals interactions (modeled by a Lennard-Jones potential). GROMOS was run with the reaction-field (RF) modified coulombic potential due to Tironi, with a cut-off of 1.4 nm, and a value of 62 for the RF dielectric constant (parameter epsilon_rf in GROMACS). For the Q4MD model, the Particle Mesh Ewald (PME) scheme was used to calculate the long-range coulombic interactions, with a cut-off for switching between the direct and reciprocal space terms at 1.4 nm. For both LJ and coulomb potentials, a modifier was used to smoothen the potential energy term around the cut-off distance.

The temperature was maintained using the Berendsen algorithm, coupling the β-CD (or β-CD FUC(3A,3D)) and water separately to a temperature bath at 298 K, with a coupling constant (GROMACS parameter tau_t) of 0.1 ps. Pressure coupling was isotropic at 1 bar, using the Berendsen algorithm, with a coupling constant (GROMACS parameter tau_p) of 0.5 ps, and a compressibility 4.6 × 10⁻⁵ bar⁻¹. The GROMACS file run.mdp containing these (and other) settings is part of the archive MD-SUPPINFO.zip. The data provided for each system (β−CD and β−CD-FUC(3A,3D), each with the GROMOS and Q4MD model) is briefly described in a README file contained in the archive.

Analysis
Coordinates were saved every 10 ps during the trajectories, but only a subset of the snapshots were used for analysis. The dynamics and structure of the β-CDs was characterised primarily by monitoring dihedral (torsional) angles between the glucose rings that make up the cyclodextrin, calculating the angles every 200 ps, using the GROMACS tool gmx angle. We chose to monitor dihedral angles between successive CD ring sugars as defined by the quartet of O5-C1-(+C4)-(+C5) atoms, where the + indicates the atom on the next residue along the β-CD ring, i.e. the direction of the sugars is defined as C1→O1(=O4)→C4. These dihedral angles provide similar information as the dihedrals defined by Gebhart et al. in their recent β-CD force field comparison. The dihedral angles inform about the tilt of the sugars with respect to each other. In the canonical β-CD structure, these angles are between -30 and 30 degrees, depending on the dihedral of interest.
The tilting of the glucose rings with respect to each other best characterizes the larger scale motion and flexibility of the β-CDs. The orientation of the fucose rings with respect to the β-CD scaffold was monitored by the dihedral angles C2-C3-O3-FC1 and C3-O3-FC1-FO5, where the prefix F denotes the Fucose. Figure S14 provides a visual guide to the definition of these angles (see also Figure 4A in the main text). Molecules were visualized using the VMD software.¹

Figure S14. Structure of β–CD-FUC(3A,3D) (compound 10), and annotation of sugar residues in β–CD and definition of dihedral angles used to quantify the tilting of the sugars with respect to each other. Left: side view. Right: bottom view, where the fucose rings point to the front; the CD-O6 alcohols point to the back. The structures are represented by a stick model, with the atoms of the fucose rings drawn with thicker bonds. Aliphatic hydrogens are not shown. The β–CD ring letters are indicated next to the rings, except for rings B and C in the side view for which the letters are inside the ring. The fucoses are substituted on rings A and D. The C1 of each β–CD-glucose residue is shown as a colored sphere: A: purple, B: green, C: orange, D: magenta, E: cyan, F: maroon, G: blue. These colors are used in a number of graphs showing the values and distributions of these angles. The magenta lines denote one of the dihedral angles used to quantify the tilt; in this case the dihedral connecting the sugar rings B and C, with the dihedral defined by the sequence O5(B)-C1(B)-C4(C)-C5(C).

In addition to monitoring the dihedral angles, the conformational space of the molecules was investigated by preparing an overlay of conformations visited during the simulations. To this end, all structures are fit on the canonical starting structure by means of minimizing the root mean squared deviation (RMSD) between each conformation and the starting conformation. The RMSD was used to perform a clustering analysis, grouping similar conformations into a conformational state, using the GROMACS tool gmx cluster. The clustering was done using the GROMOS algorithm,²² which counts the number of neighbors using the given cut-off, and takes the conformation with the largest number of neighbors and all its neighbors as the first state. Then all
conformations of this state are eliminated from the pool. The procedure is repeated with the remaining conformations to find the next state, until all conformations are assigned to a state. Each conformational state or cluster was then represented by the conformation that forms the center of the cluster, i.e. the conformation that is most similar to all conformations in the cluster or conformational state. The criterion for grouping conformations into a state used in this study is that the RMSD is below 0.16 nm. The criterion was set such that the individual runs (i.e. performing the analysis on the 4 μs of each of the force fields separately) yielded less than 100 conformational states. We used 8,000 snapshots, spaced by 500 ps from 4 μs total time for each force field. In order to compare the united-atom GROMOS force field and the all-atom Q4MD force field, all H-atoms were removed from both trajectories, yielding mapped trajectories for both force fields. Trajectories were then combined (with a total of 16,000 snapshots, a total of 8 μs, the first 4 μs taken from the GROMOS force field run, the second 4 μs taken from the Q4MD force field run), and the similarity between the force fields investigated. Note that in Figure S5, which shows the representative conformations, the alcoholic H-atoms have been added for more natural presentation of sugars, although the positions of these H-atoms were not used in defining the conformational states.

Discussion of the simulations

Tilting of β-CD ring sugars with respect to barrel wall and connection to fucose substituents

Figure S4 shows the dihedral angles characterizing the tilting of the sugars with respect to the barrel of the β-CD ring of compound 10 as a function of time for the GROMOS and Q4MD simulations. The dihedral angles are indicated in Figure S14. It can be observed in Figure S4 that in both force fields, sugars within the β-CD ring tilt out of the barrel-shape; these tilts are mostly seen as concerted changes in two neighboring dihedrals connecting consecutive sugars (one dihedral angle becomes positive, while the other becomes negative). Interestingly, the dynamics of the two force fields differ in this tilting, with the GROMOS force field being the less dynamic one. The study of Gebhardt et al. for a native (i.e. unsubstituted) β-CD shows that force fields differ in this aspect both concerning thermodynamics and kinetics, but generally do show (partial) tilting of the sugars with respect to the barrel wall.\(^\footnote{12}\) Most GROMOS force field variants optimized for carbohydrates show limited tilting dynamics and a generally less flexible structure than Q4MD. GROMOS 53a6, however, was seen by Gebhardt et al. to yield different dihedral distributions concerning width and average position, a behavior that is not reproduced in our simulations. Possible causes are (1) the fact that we use dihedral restraints on the sugar rings to maintain the predominant \(^4\)C\(_1\) chair conformation, where Gebhardt et al. found that the G53a6 force fields favors the \(^1\)C\(_4\) conformation, and (2) the fact that we use the GROMACS simulation engine and implementation of this force field instead of GROMOS. By removing the dihedral restraints on the sugar rings, we confirmed that the cause of the apparent discrepancy is the difference in preference for the \(^1\)C\(_4\) conformation in the standard G53a6 force field (data not shown), for which we find similar behavior as Gebhardt et al.
Our results for the Q4MD force field are consistent with those reported by Gebhardt et al. It can be seen that for 10, both inward and outward tilts occur. Although the tilting events are not frequent, the tilted states can persist over several nanoseconds and constitute important alternative conformations.

Quantification of the thermodynamics can be achieved by determining the occurrence of the different tilt angles. Figure 4 (main text) shows the distributions of these angles over the entire simulation, dashed lines. Angles between -30 and +30 degrees are within the normal range for a canonical β–CD structure, and the distributions show that these regions are indeed predominant, but there is considerable probability for tilt angles around -90 and +90 degrees indicating sugars being tilted perpendicular to the barrel wall. These tilts can be inward as well as outward, i.e. the hydroxyl groups and/or fucose on the C2 and C3 can be pointing toward or away from the center of the βCD ring. Figure 4 shows that tilting is more frequently observed for the CD-glucose residues bearing the fucose and the ones next to the fucosylated sugars also display more frequent tilting. It can also be seen that their outward tilting is considerably more favorable than their inward tilting. The results remain qualitative, however, because the tilting events are rare and the 4 μs simulations do not offer enough statistics to determine rates or free energy differences.

It is interesting to compare the tilting of the ring sugars in the doubly fucosylated product 10 and native β–CD. The direct comparison between the distributions is shown in Figure 4, drawn lines for the native β–CD, dashed lines for the doubly fucosylated product 10. Apart from the general conclusion that the Q4MD force field is more flexible than the GROMOS force field also for βCD, two effects can be seen for both force fields: (1) the tilt angle distributions for the fucosylated sugars shift, the upstream ones to more negative angles, the downstream ones to more positive angles, indicative of a slight out-of-barrel-wall tilting of the substituted sugars; and (2) the substitution of the sugars increases the probability of substantial tilting with respect to the barrel wall, apparent from the higher shoulders at dihedral angles at values around 60-90 degrees larger or smaller than those of the main peaks. In summary, the substitution of the β–CD sugars increases the tilting propensity with respect to the barrel wall as shown in Figure 4. The distributions from the (3A,3D)-difucosylated βCD show much more pronounced shoulders than the distributions from the native βCD, indicating the tilted conformations.

Also, a comparison of the fucose dihedral angles reinforces the observation that the Q4MD model is more flexible: distributions are wider and transitions more frequent. Figure S15 shows the distributions of the dihedral angles that connect the β–CD ring sugars to the fucose substituents. It can be seen in Figure S15 that the distributions of the dihedral angles connecting a fucose to its βCD ring sugar are unimodal and relatively narrow in the GROMOS model, indicating a relatively well-defined relative orientation of the fucose group with respect to its β–CD ring sugar. In contrast, the Q4MD model shows a wider and multimodal distribution, indicating more flexibility of the fucoses with respect to their β–CD ring sugars, but also more diverse conformational states.
Conformational clustering and visualization of the most predominant conformations

For each simulation of (3A,3D)-difucosylated β–CD 10, 8,000 conformations were taken from 4 μs of simulation and subjected to conformational clustering based on the RMSDs between all pairs of conformations, providing insight into the possible conformational states and their relative occurrence. In order to be able to compare both force fields, only the non-H atoms were considered in calculating the RMSD between conformations. The 16,000 pooled conformations were also clustered; this provides insight in the overlap between the conformational space of the two force fields, as far as sampled in the simulations. Table S5 contains an overview of the numerical results of the cluster analysis of the combined trajectory, showing the top 8 states, and Figure S5 shows representative conformations of the top 6 conformational states. The output of the clustering and representative conformations of all 81 clusters found in the analysis are available in the archive MD-SUPPINFO.zip.

The cluster analysis (most important data presented in Table S5 and Figure S5) shows that there is clearly a most predominant conformational state, making up 67% of all conformations in the combined trajectories. Within GROMOS, this is the overwhelmingly dominant conformational state, with 93%. In the Q4MD force field it is also clearly the most abundant state, but contains only 41% of all conformations. The most abundant state is a canonical βCD barrel with the two fucose moieties slightly tilted inward from the barrel wall, but not residing within the barrel, see Figure S5, top left. The cluster analysis bears witness to the difference in structural flexibility of the two force fields. Of the 81 total clusters, many are populated by structures contributed only by the Q4MD trajectory; this trajectory also leads to more equally sized clusters in terms of number of structures, whereas the number of conformations in the lower-ranked clusters of the GROMOS force field rapidly drops.
Table S5. Top 8 clusters found in the combined cluster analysis.

| Combined cluster % | GROMOS | | Q4MD |
|--------------------|--------|---------------------|---------------------|
| rank within GROMOS | % within combined | rank within Q4MD | % within combined |
| a                  | b      | c                   | b                   | c                   |
| 66.9               | 1      | 93                  | 69                  | 1                   | 41                  | 31                  |
| 8.0                | >6     | <0.1                | 0.2                 | 2                   | 16                  | 99.8                |
| 3.9                | ---    | ---                 | 0                   | 3                   | 8                   | 100                 |
| 3.8                | 5      | 0.7                 | 9                   | 4                   | 7                   | 91                  |
| 2.0                | >6     | 0.3                 | 7                   | 5                   | 4                   | 93                  |
| 1.8                | 2      | 3                   | 88                  | >10                 | 0.4                 | 12                  |
| 1.6                | >6     | <0.1                | 0.4                 | 6                   | 3                   | 99.6                |
| 1.4                | 3      | 2                   | 69                  | >10                 | 0.9                 | 31                  |

a Ranking of the combined cluster within this force field. b Contribution of this cluster to total within this force field. c Contribution of each force field to combined cluster.

The most predominant non-canonical structures are the ones in which the fucosylated residues tilt out of the barrel by about 90 degrees; in the second most populated cluster (8% of the total, 16% of the Q4MD snapshots, representative conformation in Figure S5, top right), this is one of the fucosylated sugars, viz. sugar D, in the third most populated cluster (3.9% of the total, 8% of the Q4MD snapshots, representative conformation in Figure S5, middle left) both fucosylated sugars are tilted outward. The contribution of these conformations from the GROMOS force field is very low. In contrast, the sixth cluster (representative conformation in Figure S5, bottom right) is predominantly found in the GROMOS run and hardly in the Q4MD run. In this cluster, the fucosylated sugar A is tilted out of the plane. This analysis shows that similar structures can be found in the two force fields, but also points to the need for more extensive sampling (either by performing longer runs or by employing enhanced sampling methods) to obtain proper statistics for extracting free energy differences between the conformational states.

Further insight into the conformational space of the doubly fucosylated βCD is obtained by superposing representative structures from the trajectories and observing the 'brush' of conformations, as shown in Figure S16. (Note that the superposition is not of the representative structures of the clusters, but the conformations are taken from the 4 μs of simulation at equally spaced time intervals.) The superpositions clearly show that the fucose rings sample a range of orientations with respect to the β–CD barrel, being more inward and more outward oriented. The observation that the flexibility of the Q4MD model is clearly higher is nicely borne out by displaying the conformations in this manner.
The different states may play a role in recognition by presenting alternative binding modes to possible targets. The orientation of the fucose rings with respect to the barrel and/or with respect to each other may be relevant. We therefore calculated the distribution of the distance between the centers of the two fucose rings and the distance between the C6 atoms of the two fucose rings. The center of a fucose ring is defined as the center of mass of the six atoms that make up the sugar ring (C1, C2, C3, C4, C5, and O5). These distributions are shown in Figure S6. The distributions bear witness to the larger flexibility of the Q4MD force field, but are otherwise comparable in the values found. The distance between the fucose ring centers is peaked at around 0.7 nm in both force fields; the C6 methyl group distance peaks at around 0.4 nm, reflecting the predominant conformational state of the most populated cluster in which the fucose rings are parallel to the barrel wall (Figure S5 cluster 1). However, there is a population in which the fucose-fucose center-to-center distance is considerably smaller. In this case, the fucose rings are both tilted toward the β-CD center.

**Detailed explanation of the calculated NOE intensities and 3J couplings**

The Q4MD model simulation was used to specifically investigate NOEs between the H-atoms on the modified glucose residues. The chemical shifts of the hydrogens H1-H4 are sufficiently resolved to distinguish NOE signals. Ideally, NOE signals between these hydrogens should inform about the conformational equilibria observed in the simulations and be reflected in the experimental NOE signals. It should be stressed that the simulations reported here are not long enough to be quantitatively reliable, i.e. the conformations sampled do not constitute a converged ensemble. There are not enough transitions between the conformational states to extract reliable equilibrium statistics. The reported intensities should be interpreted qualitatively.

The GROMACS tool gmx rmsdist calculates quantities that are reflected in NOEs, most reliably the averages over the inverse cube of the distance ($<r^3>$) and the inverse sixth power of the distance ($<r^6>$) - the latter is the appropriate one for orientation-averaged dipole-dipole interactions. In an all-atom model such as Q4MD, the distances between the H-atoms are trivially...
generated in a simulation. Here, the averages are taken over ca. 23,000 frames over a simulation time of 4.6 microseconds. In Table S6, the first and second columns give the hydrogens involved, the third and fourth columns give the distances calculated from the average inverse cube and sixth power of the distance, respectively, and the final column the relative intensity as calculated by the GROMACS tool gmx rmsdist.

The highest NOE intensities calculated are between the hydrogens in the glucose-glucose bridge, viz. H1 and H4 on neighboring residues (see Table S6), which are a factor 2.5-8 higher than the intraresidue values (Table S7). Hydrogens H1 and H2 have the highest calculated intensity (lowest "distance") because they are in a gauche relation to each other; the other intensities are lower, because the hydrogens are predominantly in a trans relation to each other. Monitoring the distances as a function of time, it can be seen that there are conformational changes: distances jump between different values (data not shown).
Table S6. Calculated NOE metrics for interresidue H-H contacts of the modified glucose residues. In bold are the NOE signals that are described in the manuscript.

| Hydrogen 1 | Hydrogen 2 | $<r^{3}>^{-1/3}$ (nm) | $<r^{6}>^{-1/6}$ (nm) | Intensity |
|------------|------------|-----------------------|-----------------------|-----------|
| H1 D       | H4 E       | 0.211                 | 0.209                 | 12057     |
| H1 A       | H4 B       | 0.215                 | 0.213                 | 10793     |
| H4 D       | H1 C       | 0.225                 | 0.222                 | 8401      |
| H4 A       | H1 G       | 0.221                 | 0.219                 | 9031      |
| H3 D       | H1 FD      | 0.229                 | 0.223                 | 8041      |
| H3 A       | H1 FA      | 0.229                 | 0.224                 | 8011      |
| H61 D      | H1 C       | 0.273                 | 0.256                 | 3517      |
| H62 D      | H1 C       | 0.298                 | 0.267                 | 2768      |
| H61 A      | H1 G       | 0.281                 | 0.260                 | 3205      |
| H62 A      | H1 G       | 0.309                 | 0.276                 | 2267      |
| H1 D       | H61 D      | 0.367                 | 0.297                 | 1446      |
| H1 A       | H61 A      | 0.315                 | 0.266                 | 2818      |
| H3 C       | H5 FD      | 0.305                 | 0.267                 | 2753      |
| H3 G       | H5 FA      | 0.312                 | 0.267                 | 2748      |
| H3 C       | H6 FD      | 0.377                 | 0.324                 | 2592      |
| H3 G       | H6 FA      | 0.407                 | 0.342                 | 1863      |
| H3 B       | H6 FD      | 0.421                 | 0.343                 | 1835      |
| H3 F       | H6 FA      | 0.450                 | 0.357                 | 1439      |
| H4 D       | H61 D      | 0.288                 | 0.281                 | 2023      |
| H4 D       | H62 D      | 0.305                 | 0.289                 | 1729      |
| H4 A       | H61 A      | 0.293                 | 0.287                 | 1807      |
| H4 A       | H62 A      | 0.291                 | 0.275                 | 2293      |
| H3 C       | H1 FD      | 0.341                 | 0.295                 | 1516      |
| H3 G       | H1 FA      | 0.360                 | 0.305                 | 1243      |
| H1 C       | H5 D       | 0.327                 | 0.301                 | 1348      |
| H1 G       | H5 A       | 0.327                 | 0.307                 | 1197      |
| H2 D       | H1 FD      | 0.341                 | 0.313                 | 1061      |
| H2 A       | H1 FA      | 0.330                 | 0.301                 | 1349      |
| H4 A       | H5 FA      | 0.349                 | 0.312                 | 1085      |

Table S7: Calculated NOE metrics for intraresidue H-H contacts.

| Hydrogen 1 | Hydrogen 2 | $<r^{3}>^{-1/3}$ (nm) | $<r^{6}>^{-1/6}$ (nm) | Intensity |
|------------|------------|-----------------------|-----------------------|-----------|
| H1 D       | H2 D       | 0.241                 | 0.240                 | 5251      |
| H1 A       | H2 A       | 0.240                 | 0.239                 | 5376      |
| H2 D       | H4 D       | 0.281                 | 0.274                 | 2360      |
The H-H distance changes are a reflection of dihedral flips along the glucose ring. Analysis of all H-C-C-H dihedral angles in the glucose ring reveals these dihedral flips in the fucosylated glucose rings. The dihedral flips are a reflection of the conformational transitions between different chair and boat conformations. Interestingly, such conformational flexibility is almost absent in the five non-modified glucoses along the cyclodextrin ring. As an example, the angles of the H2-C2-C3-H3 dihedral are shown as a function of time in Figure S17.

![Figure S17](image)

*Figure S17*. Conformational diversity in the glucoses of β–CD-FUC(3A,3D) (compound 10). The figure shows instantaneous values of the H2-C2-C3-H3 dihedral angles as a function of time for all 7 glucoses. The dihedral angles on modified glucoses A (purple) and D (magenta) can be seen to show multiple trans-gauche transitions.
Of the other 5 glucoses, only ring B (green) shows a few short-lived transitions. Similar graphs are obtained for the other 3-bond H-H couplings in the glucose ring (not shown).

The dihedral changes should be reflected in $^3J$ coupling constants, at least for the H2-H3, H3-H4, and H4-H5 pairs, because they change between trans and gauche states. The H1-H2 pair switches between gauche+ and gauche- that have the same $^3J$ coupling. The Karplus relation for analysis of cyclohexane rings (given in eq. 1, taken from https://www.chem.wisc.edu/areas/reich/nmr/05-hmr-05-3j.htm) was used to calculate the $^3J$ couplings between all H-H pairs over the entire duration of the Q4MD simulation.

$$^3J(Hz)=\begin{cases} 
14 \times \cos^2 \theta ; & 90^\circ < |\theta| \leq 180 \\
10 \times \cos^2 \theta ; & |\theta| \leq 90
\end{cases}
$$

(Eq. 1)

The analysis showed that significantly different $^3J$ couplings are to be expected for the H2-H3, H3-H4, and H4-H5 pairs of the modified glucoses compared to the non-modified ones, see Table S8.

Table S8: Calculated $^3J$ couplings in Hz (variance in parentheses) between hydrogens in the glucose rings of 10.

| Glucose ring | H1-H2   | H2-H3  | H3-H4  | H4-H5  |
|-------------|--------|--------|--------|--------|
| A           | 3.4 (1.4) | 11.4 (4.6) | 10.7 (4.6) | 11.0 (4.5) |
| D           | 3.6 (1.5) | 9.8 (5.8)  | 9.2 (5.6)  | 9.6 (5.5) |
| B           | 3.2 (1.3) | 13.3 (0.7) | 12.7 (1.3) | 12.9 (1.6) |
| C           | 3.3 (1.2) | 13.5 (0.6) | 12.8 (1.3) | 13.0 (1.3) |
| E           | 3.1 (1.1) | 13.5 (0.6) | 12.8 (1.1) | 13.1 (1.0) |
| F           | 3.3 (1.2) | 13.5 (0.6) | 13.0 (1.0) | 13.2 (0.9) |
| G           | 3.4 (1.2) | 13.5 (0.6) | 13.0 (1.0) | 13.3 (1.0) |

The calculated $J$ couplings reflect the difference in conformational flexibility observed for H2-H3, H3-H4, and H4-H5 by a different (lower) average value and a much higher variance for the modified than for the unmodified glucoses. For the H1-H2 pair, both metrics are similar when compared, because the modified glucoses have a gauche-gauche transition, which does not change the instantaneous $J$ coupling.

The experimentally observed $J$ couplings in compound 10 are reported in the SI (see below). The somewhat higher couplings (4.1 and 3.9 Hz compared to 3.5 (2x), 3.7, and 3.1 (2x)) for two H1
hydrogens could be ascribed to the modified glucoses, but the evidence from the simulations is not that strong.

The low value of 8.3 Hz reported as a triplet for two H3 hydrogens (at 4.16 ppm) is consistent with two $^3J$ couplings to hydrogen H-3 of similar magnitude (11.4 and 10.7 for ring A and 9.8 and 9.2 for ring D). The triplet is a double doublet with very similar coupling strengths. In the glucose rings that do not show the conformational diversity, the $^3J$ couplings of hydrogen H-3 are all close to the maximum value of 14 Hz in the Karplus parameterization used here, indicating that both H2 and H3 and H3 and H4 are all essentially permanently in a trans relation to each other.
NMR data

$^1$H NMR (400 MHz, CDCl$_3$) of compound 5.
$\text{H}^1$-APT NMR (100 MHz, CDCl$_3$) of monosaccharide 5.

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$^1$H NMR (400 MHz, CDCl$_3$) of α-CD acceptor 3.
$^{13}$C NMR (100 MHz, CDCl$_3$) of α-CD acceptor 3.
$^{1}$H NMR (400 MHz, CDCl$_3$) of β-CD acceptor 4.
$^{13}$C-APT NMR (100 MHz CDCl$_3$) of β-CD acceptor 4.
$^1$H NMR (600 MHz, CDCl$_3$) of octasaccharide BnFuc$_1$-β-CD after preparative HPLC.
\textsuperscript{1}H NMR (600 MHz, CDCl\textsubscript{3}) of octasaccharide BnFuc\textsubscript{1}-\textbeta-CD after preparative HPLC, zoomed in on the region from 6.0 to 1.0 ppm.
$^{13}$C-DEPT-135 (150 MHz, CDCl$_3$) of octasaccharide BnFuc$_1$-\(\beta\)-CD after preparative HPLC.
$^1$C-DEPT-135 (150 MHz, CDCl$_3$) of octasaccharide BnFuc$_1$-β-CD after preparative HPLC, zoomed in on the region from 105 to 95 ppm.
COSY (600 MHz, CDCl₃) of octasaccharide BnFuc₁-β-CD.
Multiplicity-edited HSQC (600 MHz, CDCl₃) of octasaccharide BnFuc₁-β-CD.
$^1$H NMR (400 MHz, CDCl$_3$) of the mixture obtained after size-exclusion chromatography containing nonasaccharide 9.
$^1$H NMR (600 MHz, CDCl$_3$) of nonasaccharide 9 (purified by preparative HPLC).
$^1$H NMR (600 MHz, CDCl$_3$) of a nonasaccharide 9 (purified by preparative HPLC), zoomed in on the region from 6.0 to 1.0 ppm.
Multiplicity-edited HSQC (600 MHz, CDCl$_3$) of nonasaccharide 9 (purified by preparative HPLC).

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\(^1\)H NMR (600 MHz, CDCl₃) of nonasaccharide 9 (purified by column chromatography on silica gel), obtained from the large scale reaction.
\(^1\)H NMR (600 MHz, CDCl\(_3\)) of nonasaccharide 9 (purified by column chromatography on silica gel) obtained from the large scale reaction, zoomed in on the region from 6.0 to 1.0 ppm.
$^{13}$C-APT NMR (150 MHz, CDCl$_3$) of nonasaccharide 9 (purified by column chromatography on silica gel) obtained from the large scale reaction.
\( ^{13}C\)APT NMR (150 MHz, CDCl\(_3\)) of nonasaccharide 9 (purified by column chromatography on silica gel) obtained from the large scale reaction, zoomed in on the region from 105 to 60 ppm.
COSY NMR (600 MHz, CDCl₃) of nonasaccharide 9 (purified by column chromatography on silica gel) obtained from the large scale reaction.
Multiplicity-edited HSQC (600 MHz, CDCl₃) of nonasaccharide 9 (purified by column chromatography on silica gel) obtained in the large scale reaction.
$^1$H NMR (600 MHz, D$_2$O) of unprotected octasaccharide Fuc$_1$-$\beta$-CD.
$^1$H NMR (600 MHz, D$_2$O) of unprotected octasaccharide Fuc-$\beta$-CD, zoomed in on the region from 6.0 to 1.0 ppm.
$^{13}$C-DEPT 135 (150 MHz, D$_2$O) of unprotected octasaccharide Fuc$_3$-β-CD.
$^{13}$C-DEPT 135 (150 MHz, D$_2$O) of unprotected octasaccharide Fuc$_1$-β-CD, zoomed in on the region from 105 to 95 ppm.
COSY (600 MHz, D$_2$O) of unprotected octasaccharide Fuc$_1$-β-CD.
TOCSY mixing time 30 ms (600 MHz, D₂O) of unprotected octasaccharide Fuc₁-β-CD.
Multiplicity-edited HSQC (150 MHz, D$_2$O) of unprotected octasaccharide Fuc$_1$-$\beta$-CD.
HSQC (150 MHz, D₂O) unprotected octasaccharide Fuc₁-β-CD (zoom-in).
$^1$H NMR (600 MHz, D$_2$O) of nonasaccharide 10 (purity >90%).
$^{13}$C NMR (150 MHz, D$_2$O) of nonasaccharide 10.
$^{13}$C NMR (150 MHz, D$_2$O) of nonasaccharide 10, zoomed in on the region from 110 to 90 ppm.
DQF-COSY (600 MHz, D$_2$O) of nonasaccharide 10.
TOCSY (600 MHz, D₂O) of nonasaccharide 10 mixing time 80 ms.
ROESY (600 MHz, D$_2$O) of nonasaccharide 10.
HMBC (600 MHz, D$_2$O) of nonasaccharide 10.
HSQC (600 MHz, D$_2$O) of nonasaccharide 10.
NOESY (600 MHz, D₂O) of nonasaccharide 10.
Zoom-in of NOESY (600 MHz, D$_2$O) of nonasaccharide 10.
Zoom-in of NOESY (600 MHz, D$_2$O) of nonasaccharide 10, highlighting the cross-peak between H5-Fuc(A,D) and H3-Glc(G,C)
Zoom-in of NOESY (600 MHz, D$_2$O) of nonasaccharide 10, highlighting the cross-peak between H$_6$-Fuc(A,D) and H$_3$-Glc(G,C)
$^1$H NMR (600 MHz, D$_2$O) of unprotected nonasaccharide 10 (obtained in the large scale reaction), purity 69%. 
$^{13}$C-APT (150 MHz, D$_2$O) of unprotected nonasaccharide 10 (obtained in the large scale reaction).
$^{13}$C-APT (150 MHz, D$_2$O) of unprotected nonasaccharide 10 (obtained in the large scale reaction) (zoom-in from 110 to 90 ppm).
$\text{HSQC (600 MHz, } D_2O\text{) of unprotected nonasaccharide 10 (obtained in the large scale reaction).}$
UPLC-UV/MS traces

UPLC-UV/MS on Acquity C4 column (Waters) trace of crude mixture of fucosylated β-cyclodextrins (optimized conditions). UV absorbance 208

UPLC-UV/MS on Acquity C4 column (Waters) trace of octasaccharide BnFuc1-β-CD purified by preparative HPLC (C4 column).

UPLC-UV/MS on Acquity C4 column (Waters) trace of nonasaccharide 9 purified by preparative HPLC (C4 column).
UPLC-UV/MS on Acquity C4 column (Waters) trace of the crude mixture of fucosylated β-CDs obtained from the large scale reaction, containing 60% of nonasaccharide 9.

UPLC-UV/MS on Acquity C4 column (Waters) of nonasaccharide 9 (large scale reaction) after purification by column chromatography on silica gel.

UPLC-MS trace on Cortecs HILIC (Waters) of nonasaccharide 10 used for analysis.
UPLC-MS trace Cortecs HILIC (Waters) of nonasaccharide 10 (obtained from large scale reaction).
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