ELECTRONIC SUPPLEMENTARY INFORMATION

Colloid-based multiplexed screening for plant biomass-degrading glycoside hydrolase activities in microbial communities

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SUPPLEMENTARY FIGURES

Fig. S1 Structures of amphiphilic substrates. All substrates were composed of a sugar head group coupled to a perfluorinated heptadecafluoro-1,1,2,2-tetrahydrodecyl (F17) tag. The linker, containing a dimethyl-arginine, is separated from the sugar moiety by a spacer of 5 methylene groups. The linker ensures accessibility of the sugar head groups, while the dimethyl-arginine greatly enhances ionization[1]. The sugar oligomers used in this study were (a) cellobiose (CB), (b) cellotetraose (CT), and (c) xylobiose (XB).

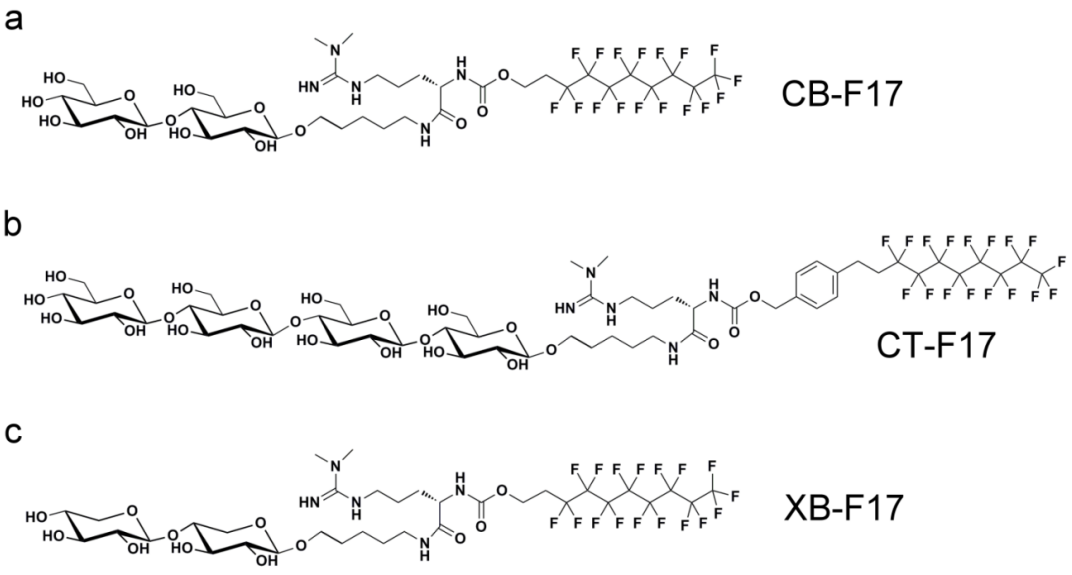


Fig. S2 Comparison of ionization efficiencies. Analysis of an equimolar mixture of perfluorinated glucose (G-F17; *m/z* 940), cellobiose (CB-F17; *m/z* 1102), and cellotetraose (CT-F17; *m/z* 1516) (0.1 mM per substrate in 20 μL 50% H_2O / 50% methanol) shows comparable ionization efficiencies, a prerequisite for the measurement of product-to-substrate ratios.

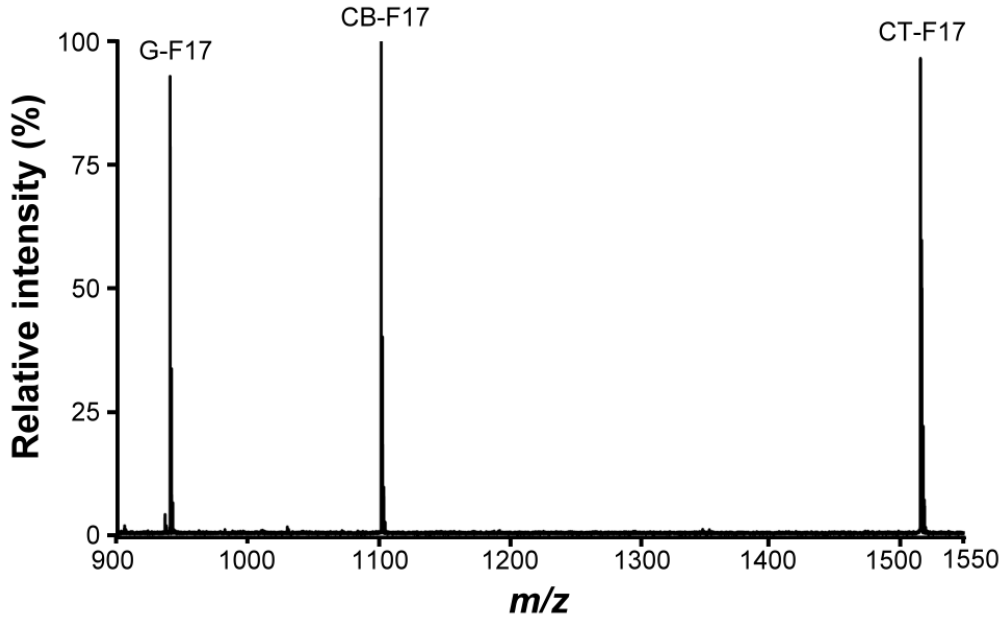


Fig. S3 Influence of the perfluorinated tag on enzyme activity. To test if the perfluorinated tags of the used substrates might interfere with enzyme activity, the time course using pure β-glucosidase (An_BG) and 0.1 mM perfluorinated cellobiose (CB-F17) (Fig. 3) was repeated with a combination of 0.02 mM CB-F17 and 0.08 mM untagged natural cellobiose. As the overall cellobiose concentrations remains constant at 0.1 mM and only the ratios between CB-F17 and formed glucose (G-F17) are determined, the time curve should not be altered in the case of no interference. If natural cellobiose has a higher affinity to the enzyme than CB-F17, the conversion of CB-F17 would be slowed down. The competition experiment yielded virtually the identical curve showing that the perfluorinated tags do negatively affect the activity of the studied enzymes.

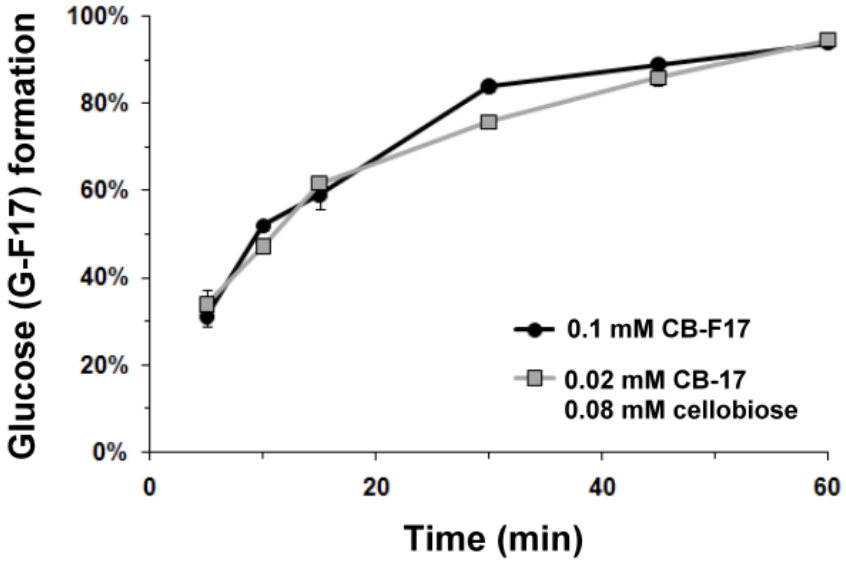


Fig. S4 Assessment of activities of control enzymes. For an analysis of the oligosaccharide degrading capabilities of cellulases, pure β-glucosidases (An_BG, UBG), exoglucanases (Cs_GH5-1, Cs_GH5-2), and endoglucanases (Pr_GH5, Tm_Cel5A, Ac_Cel9A) were incubated with perfluorinated cellotetraose (CT-F17) as substrate. All activities were corrected by the values of negative control samples without enzyme. Error bars represent standard deviation of three independent experiments.

Bars 1-2: Both β-glucosidases were able to stepwise degrade CT-F17 from the tetraose to cellotriose, cellobiose, and glucose. The preferred reaction of β-glucosidases is the conversion of cellobiose to glucose, but the production of sugar monomers from longer cellodextrins is also catalyzed,[2] presumably at a slower reaction speed.

Bars 3-7: The main reaction for exo- and endoglucanases with cellotetraose is hydrolysis into cellobiose units.[2,3] Accordingly, both groups of enzymes showed cellobiose as the most significant signal present. However, in some cases significant signals for glucose were detectable, showing that these enzymes are also capable of cleaving off cellotriose units directly. This effect was more significant for the endoglucanases. Exo- and endoglucanases showed basically no triose signal, indicating that in general these two groups of enzymes cannot cleave off sugar monomers.

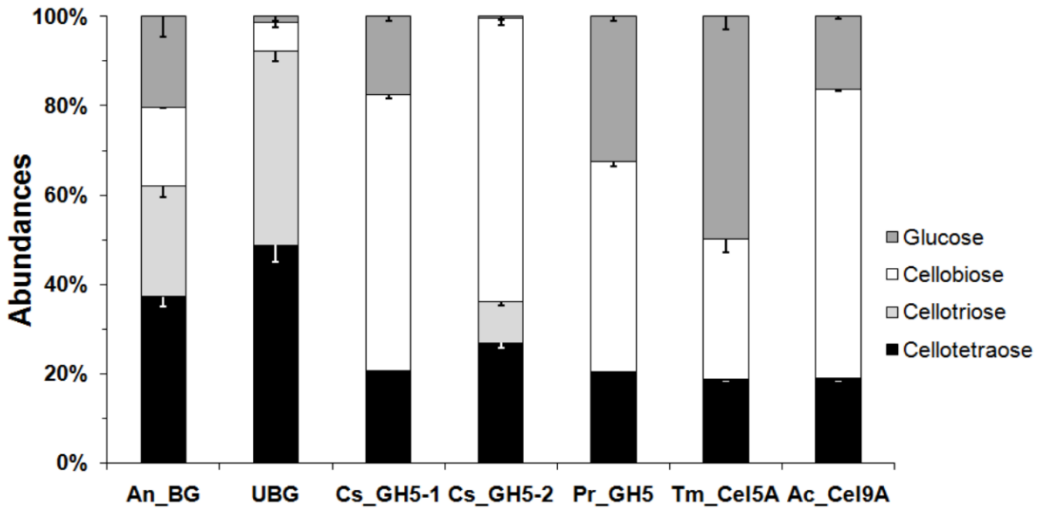


Fig. S5 Synthesis of perfluorinated tags. Perfluorinated tags (**Compound 3** and **Compound 5**) were obtained by coupling dimethyl-arginine (**Compound 1**) to perfluorinated (F17) molecules of varying length by amide bond formation (**Compound 2** and **Compound 4**).

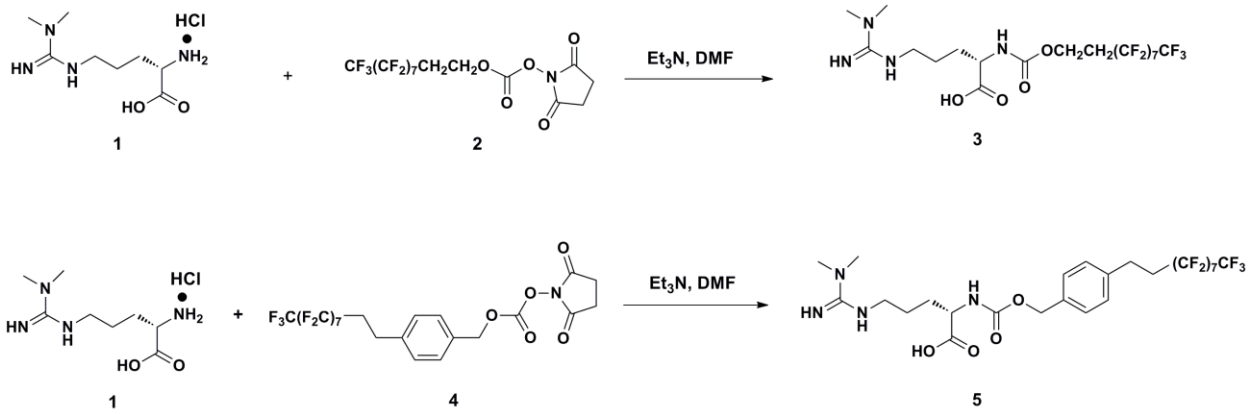


Fig. S6 Synthesis of xylobiose-F17. A $(CH_2)_5$-linker (**Compound 7**) was coupled to the reducing end of a xylobiose molecule (**Compound 6**) using Schmidt imidate chemistry to yield **Compound 8**. After removing the acetyl protection groups from the hydroxyl groups of the sugar molecule (**Compound 9**), hydrogenation by Pd/C removed the Carbobenzyloxy (Cbz) protection group to give a primary amine (**Compound 10**). A peptide coupling reaction with a fluorous tag (**Compound 3**) yielded the desired perfluorinated enzyme substrate (**Compound 11**).

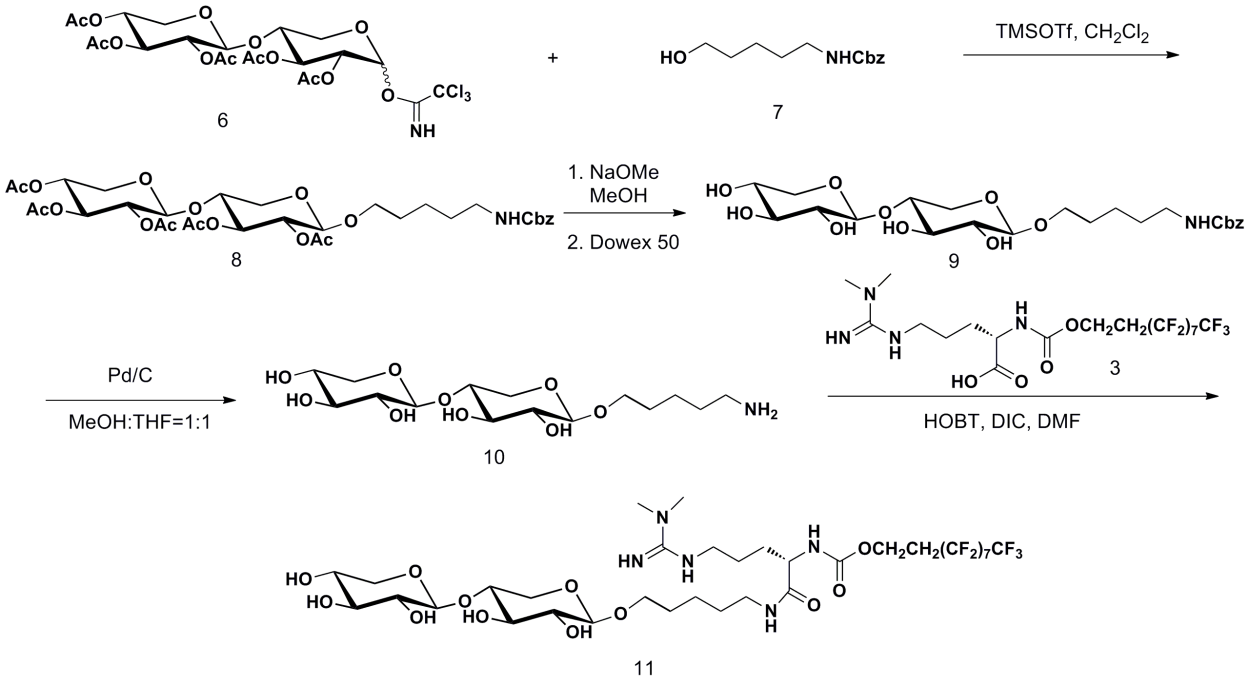


Fig. S7 Synthesis of cellobiose-F17. A $(CH_2)_5$-linker (**Compound 7**) was coupled to the reducing end of a cellobiose molecule (**Compound 12**) using Schmidt imidate chemistry to yield **Compound 13**. After removing the acetyl protection groups from the hydroxyl groups of the sugar molecule (**Compound 14**), hydrogenation by Pd/C removed the carbobenzyloxy (Cbz) protection group to give a primary amine (**Compound 15**). A peptide coupling reaction with a fluorous tag (**Compound 3**) yielded the desired perfluorinated enzyme substrate (**Compound 16**).

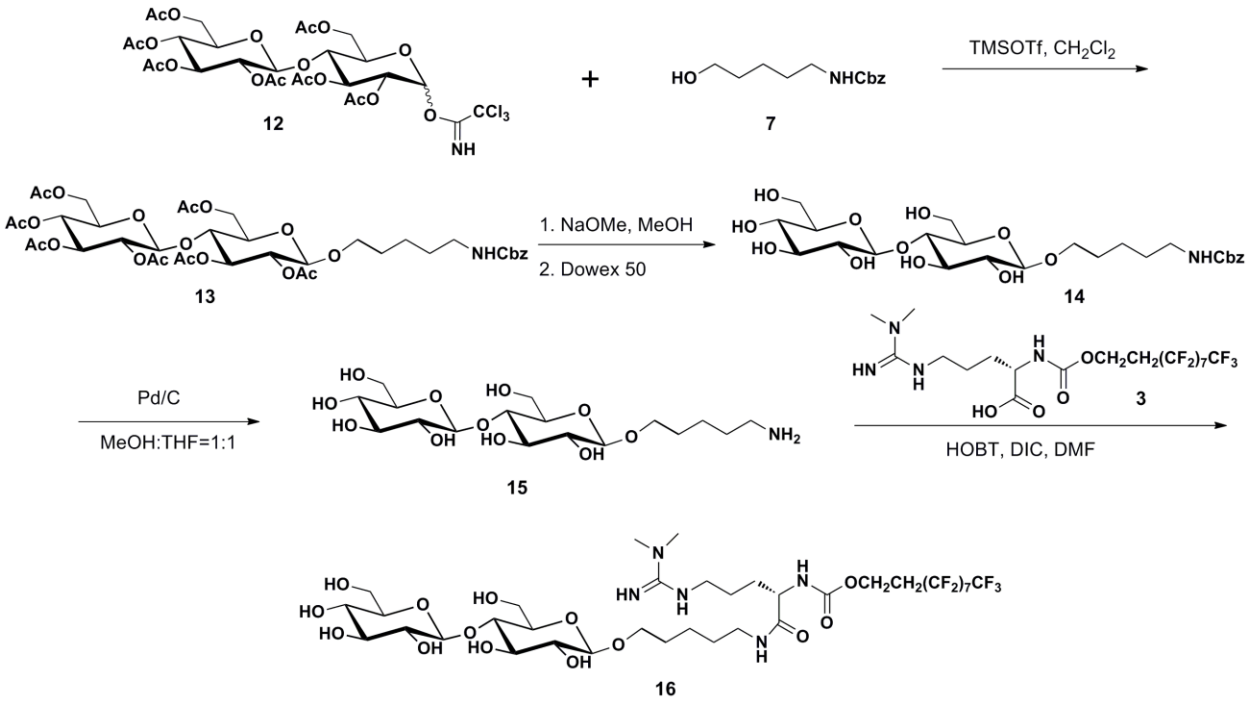


Fig. S8 Synthesis of cellotetraose-F17. A fully acetylated cellotetraose molecule (**Compound 17**) was first converted to a trichloro-imidate (**Compound 18**), and a $(CH_2)_5$-linker (**Compound 7**) was subsequently coupled to the reducing end of the cellotetraose molecule (**Compound 18**) using Schmidt imidate chemistry to yield **Compound 19**. After removing the acetyl protection groups from the hydroxyl groups of the sugar molecule (**Compound 20**), hydrogenation by Pd/C removed the Carbobenzyloxy (Cbz) protection group to give a primary amine (**Compound 21**). A peptide coupling reaction with a fluorous tag (**Compound 5**) yielded the desired perfluorinated enzyme substrate (**Compound 22**).

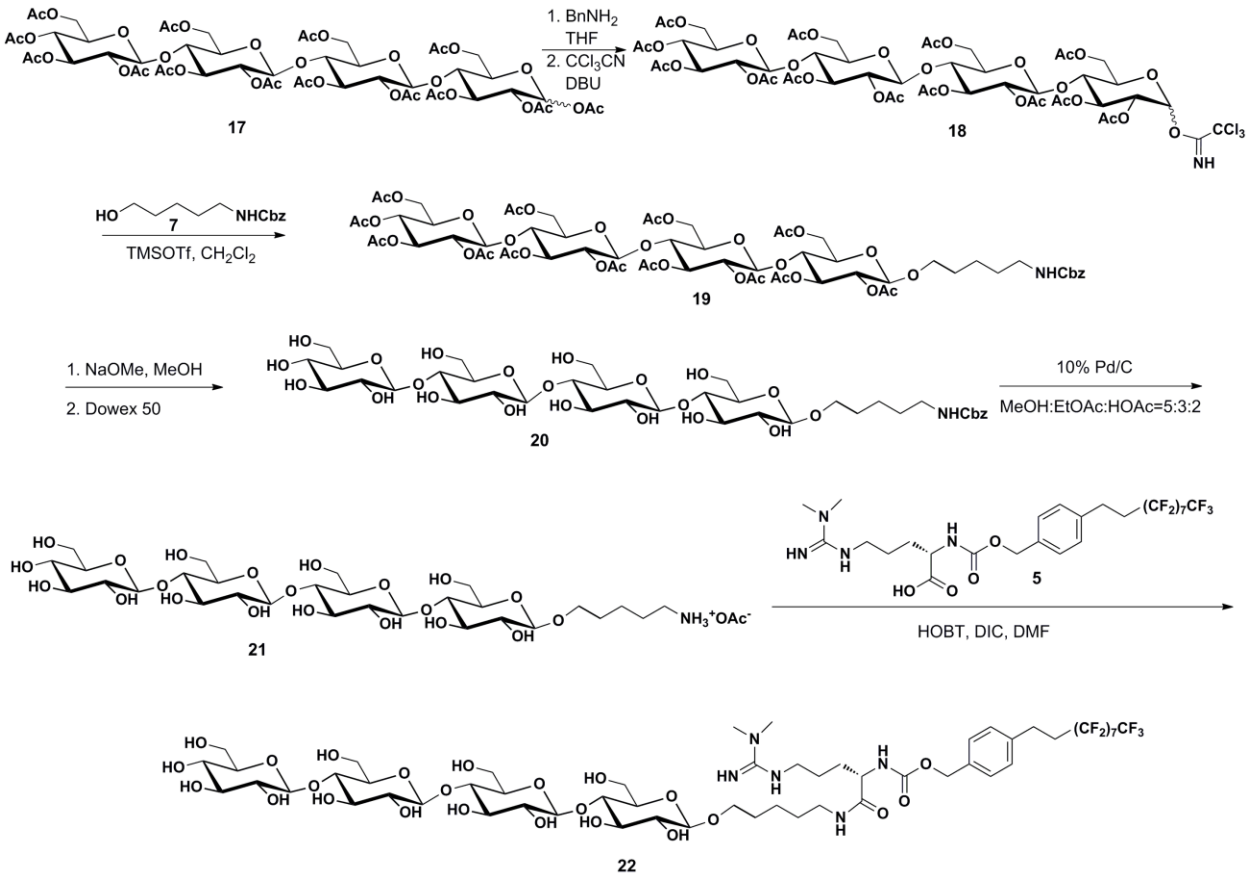
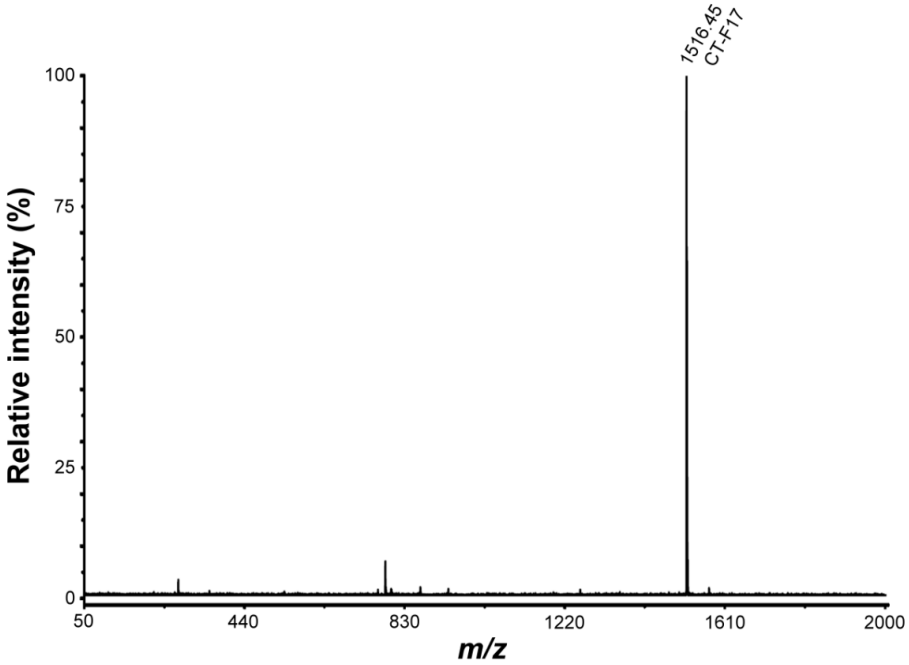


Fig. S9 Mass spectrum of cellotetraose-F17. After synthesis, cellotetraose-F17 (CT-F17) was purified by flash column chromatography. Purity was controlled by nanostructure-initiator mass spectrometry.



SUPPLEMENTARY TABLE

Table S1 Overview of enzymes and reaction conditions. Table shows the enzymes used to assess the spectrum of potentially catalyzed reactions by the three different groups of cellulose degrading enzymes (β-glucosidases, exoglucanases, and endoglucanases), amounts of enzyme applied, and reactions conditions. All enzymes were used at or close to their optimal pH and temperatures.

Enzyme name	Enzyme type	Enzyme amount	Buffer	Temp
An_BG	β-glucosidase	0.05 μg	50 mM sodium acetate (pH 4.0)	50°C
UBG	β-glucosidase	2 μg	McIlvaine's citrate/phosphate buffer (pH 7.0)[4]	50°C
Cs_GH5-1	exoglucanase	2.9 μg	McIlvaine's citrate/phosphate buffer (pH 6.0)[4]	80°C
Cs_GH5-2	exoglucanase	3.2 μg	McIlvaine's citrate/phosphate buffer (pH 6.0)[4]	80°C
Pr_GH5	endoglucanase	3.8 μg	McIlvaine's citrate/phosphate buffer (pH 6.0)[4]	37°C
Tm_Cel5A	endoglucanase	1.9 μg	McIlvaine's citrate/phosphate buffer (pH 6.0)[4]	80°C
Ac_Cel9A	endoglucanase	12.5 μg	McIlvaine's citrate/phosphate buffer (pH 6.0)[4]	70°C

SUBSTRATE SYNTHESIS

General information

All chemicals were purchased as reagent grade and used without further purification. Flash column chromatography steps were performed on a CombiFlash Rf chromatography system from Teledyne ISCO (Lincoln, NE). Reactions were monitored using analytical thin-layer chromatography (TLC) in EM silica gel 60 F254 plates and/or by staining with acidic ceric ammonium molybdate or ninhydrin. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVB-400 or AV-600. Chemical shifts (in ppm) were assigned according to the internal standard signal of $CDCl_3$ (δ = 7.26 ppm), CD_3OD (δ = 3.31 ppm), or D_2O (δ = 4.79 ppm) for ^{1}H NMR and $CDCl_3$ (δ = 77.16 ppm) or CD_3OD (δ = 49.00 ppm) for ^{13}C NMR. Coupling constants (*J*) are reported in Hertz, and the splitting patterns are described by using the following abbreviations: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiple; AB, AB spin system.

Synthesis of perfluorinated tags (Fig. S5)

Compound 3. Triethylamine (0.18 mL, 1.31 mmol) was added to a stirred solution of asymmetric dimethyl-arginine HCl salt (**Compound 1**; 120 mg, 0.436 mmol) and **Compound 2**[5] (308 mg, 0.523 mmol) in DMF (11.0 mL) under nitrogen at 0^{o}C. Subsequently, the ice-bath was removed and the reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue was purified by column chromatography (MeOH/EtOAc/H_2O = 5/3/0.1 to 5/3/1) to provide the desired **Compound 3** (112 mg, 37% yield) as a white viscous solid substance. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm) 4.36 (t, *J* = 6.0 Hz, 2H), 4.08 (t, *J* = 7.2 Hz, 1H), 3.32-3.27 (m, 2H), 3.05 (s, 6H), 2.65-2.52 (m, 2H), 1.92-1.82 (m, 1H), 1.76-1.65 (m, 3H).

Compound 5. Triethylamine (0.19 mL, 1.35 mmol) was added to a stirred solution of asymmetric dimethyl-arginine HCl salt (**Compound 1**; 125 mg, 0.450 mmol) and **Compound 4** (2,5-dioxopyrrolidin-1-yl-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl)benzyl carbonate; 380 mg, 0.550 mmol) in DMF (11.0 mL) under nitrogen at 0^{o}C. Subsequently, the ice-bath was removed and the reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue was purified by column chromatography (MeOH/EtOAc/H_2O = 5/3/0.1 to 5/3/1) to provide the desired **Compound 5** (120 mg, 34% yield) as a white solid substance. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm) 7.33 (d, *J*

= 7.8 Hz, 2H), 7.27 (d, *J* = 7.8 Hz, 2H), 5.06 (AB, *J* = 12.6 Hz, 2H), 4.07 (t, *J* = 6.0 Hz, 1H), 3.32-3.27 (m, 2H), 3.04 (s, 6H), 2.95-2.89 (m, 2H), 2.52-2.40 (m, 2H), 1.91-1.84 (m, 1H), 1.75-1.63 (m, 3H).

Synthesis of xylobiose-F17 (Fig. S6)

Compound 8. Compound 6[6] (114 mg, 0.18 mmol) and **Compound 7** (51 mg, 0.21 mmol) were mixed in 4.0 mL of anhydrous methylene chloride under nitrogen. Then 3 Å molecular sieves were added. After the resulting mixture was stirred at room temperature for 0.5 h, it was cooled to -20ºC, followed by injection of TMSOTf (5 μL, 0.027 mmol). After the reaction mixture was stirred at this temperature for 1h, triethylamine was added to quench the reaction. The solvent was removed under reduced pressure and the substance was purified by flash column chromatography to give 49 mg of **Compound 8** with 37% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.36-7.27 (m, 5H), 5.14-5.03 (m, 4H), 4.91-4.75 (m, 4H), 4.52 (d, *J* = 6.0 Hz, 1H), 4.37 (d, *J* = 7.6 Hz, 1H), 4.07 (dd, *J* = 12.0, 4.8 Hz, 1H), 3.96 (dd, *J* = 11.6, 5.2 Hz, 1H), 3.85-3.72 (m, 2H), 3.49-3.40 (m, 1H), 3.37 (dd, *J* = 12.0, 7.6 Hz, 1H), 3.27 (dd, *J* = 11.6, 10.0 Hz, 1H), 3.16 (q, *J* = 6.8 Hz, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.62-1.51 (m, 2H), 1.51-1.42 (m, 2H), 1.38-1.28 (m, 2H).

Compound 9. NaOMe in MeOH (3.4 μL; 25% w/w) were added to **Compound 8** (43 mg, 0.06 mmol) in 2 mL of methanol. After the resulting mixture was stirred at room temperature for 4 h, prewashed DOWEX 50WX2-200 (H^+) was added. The mixture was stirred for 0.5 h and filtered. The solvent was evaporated under reduced pressure and the remaining residue was purified by flash column chromatography using 30% MeOH in chloroform to give **Compound 9** (30 mg) in quantitative yield. ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 7.40-7.28 (m, 5H), 5.07 (s, 2H), 4.31 (d, *J* = 5.6 Hz, 1H), 4.22 (d, *J* = 7.8 Hz, 1H), 4.01 (dd, *J* = 11.6, 5.2 Hz, 1H), 3.91 (dd, *J* = 11.2, 5.2 Hz, 1H), 3.82 (m, 1H), 3.64 (m, 1H), 3.58-3.48 (m, 2H), 3.45 (t, *J* = 8.8 Hz, 1H), 3.38-3.27 (m, 2H), 3.27-3.18 (m, 3H), 3.13 (t, *J* = 6.8 Hz, 2H), 1.67-1.59 (m, 2H), 1.58-1.48 (m, 2H), 1.47-1.36 (m, 2H).

Compound 10. Compound 9 (163 mg, 0.325 mmol) was dissolved in a mixture of methanol (2.0 mL) and THF (2.0 mL). Then 10% Pd/C (34 mg, 0.016 mmol) was added and the reaction mixture was stirred under H_2 (g; 1atm) at room temperature for 14 h. The mixture was filtered and the filtrate was concentrated by solvent evaporization under reduced pressure. The

resulting residue containing **Compound 10** (117 mg, 98% yield) was used in the next step without further purification. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm) 4.32 (d, *J* = 7.8 Hz, 1H), 4.22 (d, *J* = 7.8 Hz, 1H), 4.00 (dd, *J* = 11.4, 5.2 Hz, 1H), 3.91 (dd, *J* = 11.4, 5.2 Hz, 1H), 3.82 (m, 1H), 3.64 (m, 1H), 3.58-3.48 (m, 2H), 3.45 (t, *J* = 8.8 Hz, 1H), 3.38-3.27 (m, 2H), 3.27-3.18 (m, 3H), 3.13 (t, *J* = 6.8 Hz, 2H), 1.67-1.59 (m, 2H), 1.58-1.48 (m, 2H), 1.47-1.36 (m, 2H). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm) 104.85, 103.98, 78.23, 77.61, 75.93, 74.72, 74.27, 71.03, 70.54, 67.07, 64.51, 42.00, 31.85, 30.36, 24.24.

Compound 11. N,N'-Diisopropylcarbodiimide (11 μL, 0.071 mmol) and 1-hydroxybenzotriazole (11 mg, 0.071 mmol) were added to a stirred solution of **Compound 10** (26 mg, 0.071 mmol) and **Compound 3** (36 mg, 0.052 mmol) in DMF (2.0 mL) at room temperature, and the reaction was kept stirring overnight. The mixture was concentrated under reduced pressure, and the residue was purified by flash column chromatography to provide **Compound 11** (40 mg, 74% yield). ^{1}H NMR (600 MHz, CD_3OD) δ (ppm) 4.43-4.34 (m, 2H), 4.32 (d, *J* = 7.8 Hz, 1H), 4.21 (d, *J* = 7.8 Hz, 1H), 4.11-4.05 (m, 1H), 4.02-3.96 (m, 1H), 3.89 (dd, *J* = 10.8, 4.8 Hz, 1H), 3.87-3.74 (m, 2H), 3.65 (m, 1H), 3.66-3.59 (m, 1H), 3.58-3.46 (m, 2H), 3.46 (t, *J* = 8.4 Hz, 1H), 3.36-3.14 (m, 7H), 3.04 (s, 6H), 2.93 (t, *J* = 6.0 Hz, 2H), 2.69-2.53 (m, 2H), 1.85 (m, 1H), 1.78-1.60 (m, 5H), 1.60-1.47 (m, 2H), 1.47-1.36 (m, 2H).

Synthesis of cellobiose-F17 (Fig. S7)

Compound 13. Compound 12[7] (330 mg, 0.42 mmol) and **Compound 7** (150 mg, 0.55 mmol) were mixed in 8 mL of anhydrous methylene chloride under nitrogen. Then 3 Å molecular sieves were added. After the resulting mixture was stirred at room temperature for 0.5 h, it was cooled down to -20ºC, followed by injection of TMSOTf (14 μL, 0.077 mmol). After the reaction mixture was stirred at this temperature for 1h, triethylamine was added to quench the reaction. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography to give 140 mg of **Compound 13** in 39% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.40-7.28 (m, 5H), 5.19-5.08 (m, 5H), 4.95-4.79 (m, 3H), 4.60-4.50 (m, *2*H), 4.45 (d, *J* = 8.0 Hz, 1H), 4.39 (dd, *J* = 12.8, 4.4 Hz, 1H), 4.18-4.00 (m, 2H), 3.88-3.75 (m, 2H), 3.69 (m, 1H), 3.61 (m, 1H), 3.48 (m, 1H), 3.20 (q, *J* = 6.8 Hz, 2H), 2.10 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H), 2.00 (s, 6H), 1.99 (s, 3H), 1.96 (s, 3H), 1.67-1.56 (m, 2H), 1.56-1.47 (m, 2H), 1.47-1.32 (m, 2H).

Compound 14. NaOMe in MeOH (20 μL; 25% w/w) were added to **Compound 13** (217 mg, 0.25 mmol) in 2.5 mL of methanol. After the resulting mixture was stirred at room temperature for 4 h, prewashed DOWEX 50WX2-200 (H^+) was added. The mixture was stirred for 0.5 h and filtered. The solvent was evaporated under reduced pressure and the remaining residue was purified by flash column chromatography using 30% MeOH in chloroform to give **Compound 14** (128 mg, 90% yield). ^{1}H NMR (600 MHz, CD_3OD) δ (ppm) 7.40-7.28 (m, 5H), 5.06 (s, 2H), 4.40 (d, *J* = 7.8 Hz, 1H), 4.27 (d, *J* = 7.8 Hz, 1H), 3.90-3.82 (m, 4H), 3.66 (dd, *J* = 11.4, 5.4 Hz, 1H), 3.58-3.48 (m, 3H), 3.41-3.28 (m, 4H), 3.26-3.20 (m, 2H), 3.11 (t, *J* = 6.8 Hz, 2H), 1.67-1.58 (m, 2H), 1.55-1.46 (m, 2H), 1.45-1.35 (m, 2H).

Compound 15. Compound 14 (89 mg, 0.16 mmol) was dissolved in a mixture of methanol (1.5 mL) and THF (1.5 mL). Then 10% Pd/C (16.8 mg, 0.008 mmol) was added and the reaction mixture was stirred under H_2 (g; 1atm) at room temperature for 14 h. The mixture was filtered and the filtrate was concentrated by solvent evaporization under reduced pressure. The resulting residue containing **Compound 15** (65 mg, 96% yield) was used in the next step without further purification. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm) 4.41 (d, *J* = 7.8 Hz, 1H), 4.28 (d, *J* = 7.8 Hz, 1H), 3.94-3.83 (m, 4H), 3.65 (dd, *J* = 12.0, 5.4 Hz, 1H), 3.59-3.53 (m, 2H), 3.51 (t, *J* = 9.0 Hz, 1H), 3.42-3.32 (m, 4H), 3.32-3.29 (m, 2H), 3.27-3.20 (m, 2H), 2.72 (t, *J* = 7.2 Hz, 2H), 1.68-1.60 (m, 2H), 1.60-1.51 (m, 2H), 1.50-1.41 (m, 2H). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm) 104.9, 104.5, 81.1, 78.4, 78.2, 76.8, 76.7, 75.2, 75.1, 71.7, 70.9, 62.7, 62.2, 42.2, 32.2, 30.6, 24.5.

Compound 16. N,N'-Diisopropylcarbodiimide (8.1 μL, 0.052 mmol) and 1-hydroxybenzotriazole (8.0 mg, 0.052 mmol) were added to a stirred solution of **Compound 15** (22 mg, 0.052 mmol) and **Compound 3** (30 mg, 0.043 mmol) in DMF (1.5 mL) at room temperature, and the reaction was kept stirring overnight. The mixture was concentrated under reduced pressure, and the residue was purified by flash column chromatography to provide **Compound 16** (35 mg, 74% yield). ^{1}H NMR (600 MHz, CD_3OD) δ (ppm) 4.41 (d, *J* = 7.8 Hz, 1H), 4.40-4.32 (m, 2H), 4.28 (d, *J* = 7.8 Hz, 1H), 4.10-4.04 (m, 1H), 3.94-3.83 (m, 4H), 3.66 (dd, *J* = 12.0, 5.4 Hz, 1H), 3.61-3.47 (m, 3H), 3.44-3.25 (m, 4H), 3.25-3.16 (m, 4H), 3.04 (s, 6H), 2.96-2.91 (t, *J* = 7.2 Hz, 2H), 2.65-2.53 (m, 2H), 1.88-1.79 (m, 1H), 1.73-1.59 (m, 5H), 1.58-1.48 (m, 2H), 1.47-1.39 (m, 2H)

Synthesis of cellotetraose-F17 (Fig. S8)

Compound 18. Benzylamine (52 μL, 0.47 mmol) was added to a stirred solution of D-(+)-cellotetraose tetradecaacetate (**Compound 17**; 391 mg, 0.31 mmol) in THF (13 mL) at room temperature every 12 h for 60 h. Subsequently, the solvent was removed and the resulting residue was purified by flash column chromatography to give Compound 17 (intermediate) (365 mg, 97% yield). CCl_3CN (0.30 mL, 2.97 mmol) was added to a stirred solution of Compound 17 (intermediate) (360 mg, 0.30 mmol) in methylene chloride (10.0 mL) at 0ºC, followed by the addition of DBU (9 μL, 0.06 mmol). The resulting mixture was stirred for 2 h and the solvent was evaporated under reduced pressure. The residue was subjected to flash column chromatography purification to give **Compound 18** (341 mg, 84% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.65 (s, 1H), 6.47 (s, 1H), 5.69 (br, 1H), 5.50 (t, *J* = 9.6 Hz, 1H), 5.16-5.00 (m, 6H), 4.90 (t, *J* = 8.0 Hz, 1H), 4.89 (q, *J* = 8.8 Hz, 2H), 4.55-4.38 (m, 7H), 4.34 (dd, J = 12.8, 4.4 Hz, 1H), 4.15-4.05 (m, 3H), 4.34 (dd, J = 12.4, 2.0 Hz, 1H), 3.85-3.68 (m, 3H), 3.66-3.51 (m, 3H), 2.14 (s, 6H), 2.11 (s, 3H), 2.08 (s, 3H), 2.034 (s, 3H), 2.031 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.998 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H).

Compound 19. Compound 18 (112 mg, 0.0825 mmol) and **Compound 7** (25 mg, 0.107 mmol) were mixed in 5 mL of anhydrous methylene chloride under nitrogen. Then 3 Å molecular sieves were added. After the resulting mixture was stirred at room temperature for 0.5 h, it was cooled down to -20ºC, followed by injection of TMSOTf (14 μL, 0.077 mmol). After the reaction mixture was stirred at this temperature for 2h, triethylamine was added to quench this reaction. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography to give **Compound 19** (35 mg, 30% yield). ^{1}H NMR (600 MHz, CD_3OD) δ (ppm) 7.37-7.27 (m, 5H), 5.16-5.00 (m, 7H), 4.92-4.78 (m, 5H), 4.51 (dd, *J* = 12.0, 1.8 Hz, 1H), 4.47-4.36 (m, 5H), 4.34 (dd, J = 12.6, 4.2 Hz, 1H), 4.13-4.02 (m, 3H), 4.01 (dd, J = 12.6, 2.4 Hz, 1H), 3.83-3.67 (m, 4H), 3.65-3.59 (m, 1H), 3.57-3.50 (m, 3H), 3.47-3.39 (m, 1H), 3.15 (q, *J* = 6.8 Hz, 2H), 2.13 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.999 (s, 3H), 1.996 (s, 3H), 1.992 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.60-1.51 (m, 2H), 1.51-1.42 (m, 2H), 1.40-1.27 (m, 2H).

Compound 20. NaOMe in MeOH (30 μL; 25% w/w) was added to **Compound 19** (32 mg, 0.022 mmol) in a mixture of methanol (1.5 mL) and methylene chloride (1.5 mL). After the resulting mixture was stirred at room temperature for 14 h, prewashed DOWEX 50WX2-200 (H^+) was added. The mixture was stirred for 0.5 h and filtered. The solvent was evaporated under

reduced pressure to give a white solid substance (18 mg; **Compound 20**), which was used in the next step without further purification. ^{1}H NMR (600 MHz, D_2O) δ (ppm) 7.41-7.28 (m, 5H), 5.06 (s, 2H), 4.49 (d, *J* = 7.8 Hz, 1H), 4.48 (d, *J* = 7.8 Hz, 1H), 4.47 (d, *J* = 7.8 Hz, 1H), 4.41 (d, *J* = 7.8 Hz, 1H), 3.97-3.90 (m, 3H), 3.90-3.82 (m, 2H), 3.81-3.72 (m, 3H), 3.69 (dd, *J* = 12.6, 6.0 Hz, 1H), 3.68-3.55 (m, 9H), 3.53 (m, 1H), 3.49-3.42 (m, 2H), 3.39-3.22 (m, 5H), 3.08 (t, *J* = 6.0 Hz, 2H), 1.61-1.53 (m, 2H), 1.50-1.43 (m, 2H), 1.35-1.28 (m, 2H).

Compound 21. Compound 20 (18 mg, 0.020 mmol) was dissolved in a mixture of methanol (1.5 mL), ethyl acetate (1.5 mL), and acetic acid (1.0 mL). Then 10% Pd/C (18 mg, 0.008 mmol) was added and the reaction mixture was stirred under H_2 (g; 1atm) at room temperature for 14 h. The mixture was filtered and the filtrate was concentrated by solvent evaporation under reduced pressure. The resulting residue (14 mg; **Compound 21**) was used in the next step without further purification. ^{1}H NMR (600 MHz, D_2O) δ (ppm) 4.51-4.47 (m, 2H), 4.46 (d, *J* = 7.8 Hz, 1H), 4.43 (d, *J* = 8.4 Hz, 1H), 3.94-3.81 (m, 5H), 3.80-3.71 (m, 3H), 3.70-3.49 (m, 9H), 3.48-3.39 (m, 2H), 3.40-3.33 (m, 1H), 3.31 (t, *J* = 8.4 Hz, 2H), 3.28-3.21 (m, 2H), 2.95 (t, *J* = 7.2 Hz, 2H), 1.67-1.56 (m, 4H), 1.43-1.35 (m, 2H).

Compound 22. N,N'-Diisopropylcarbodiimide (1.0 μL, 0.0063 mmol) and 1-hydroxybenzo-triazole (1.0 mg, 0.0063 mmol) were added to a stirred solution of **Compound 21** (4.0 mg, 0.0053 mmol) and **Compound 5** (4.1 mg, 0.0053 mmol) in DMF (0.4 mL) at room temperature, and the reaction was kept stirring overnight. The resulting mixture containing **Compound 22** was purified by flash column chromatography as before, however, in this case it was not possible to purify sufficiently enough for NMR. Nanostructure-initiator mass spectrometry was used to confirm the success of the coupling reaction. The calculated exact mass for $C_{55}H_{78}F_{17}N_5O_{24}$ is 1515.48, which was correctly identified at 1516.45 $[M+H]^+$ (Fig. S9).

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