Supporting Information for

Supramolecular Assembly of Fluorogenic Glyco-Dots from Perylenediimide-based Glycoclusters for Targeted Imaging of Cancer Cells

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[d] *Equal contribution*

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S1. Additional figures

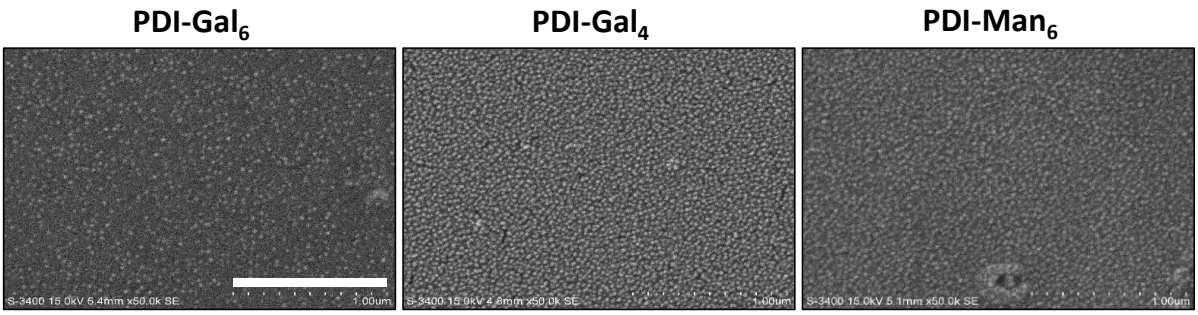


Fig. S1. Morphology of **PDI-Gal$_6$**, **PDI-Gal$_4$** and **PDI-Man$_6$** glyco-dots determined by scanning electron microscopy (Scale bar: 1 μm).

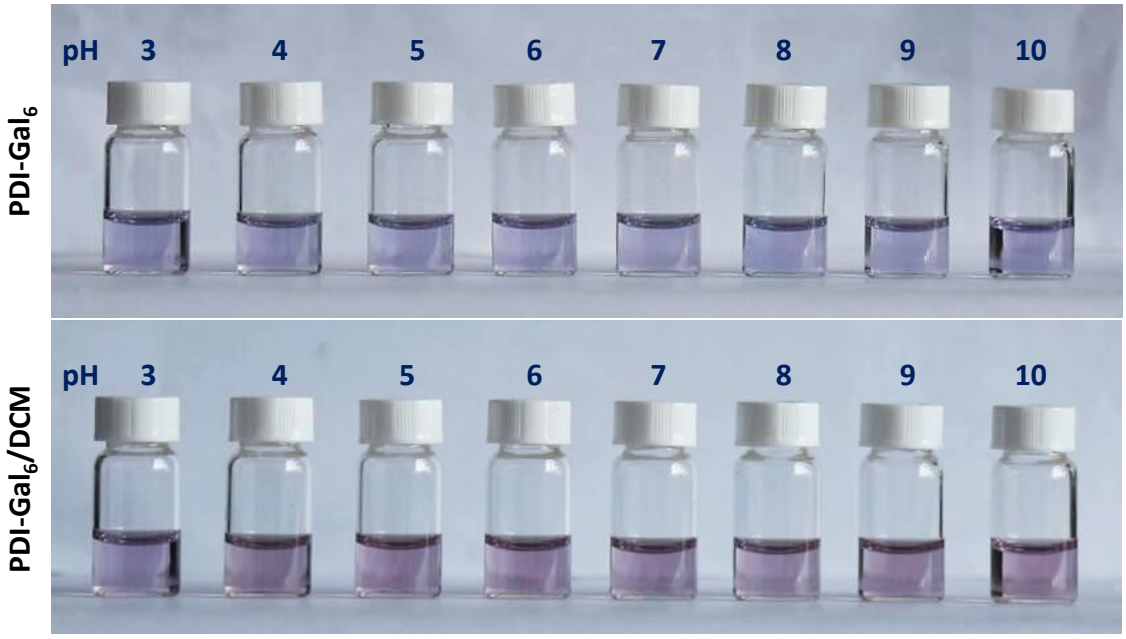


Fig. S2. Stability of **PDI-Gal$_6$** (30 μM) and **PDI-Gal$_6$/DCM** (30/10 μM) in Tris-HCl solution with different pH after storage for 3 months.

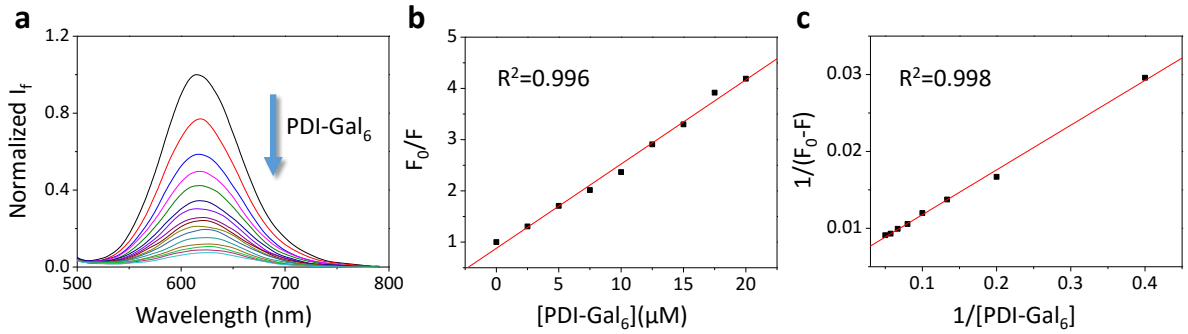


Fig. S3 (a) Fluorescence titration of **DCM** (5 μM) in the presence of increasing **PDI-Gal$_6$** (0-30 μM). (b) Stern-Volmer plots and (c) Lineweaver–Burk plots for **DCM** (5 μM) with increasing **PDI-Gal$_6$**, where F_0 and F are the fluorescence intensity of **DCM** in the absence and presence of **PDI-Gal$_6$**, respectively. The data for **DCM** were recoded with an excitation and emission wavelength of 460 and 620 nm, respectively. The dynamic and static quenching can be described by the Stern–Volmer's equation (eq 1) and Lineweaver–Burk equation (eq 2), respectively.

$$F_0/F = 1 + K_{SV}c_q \quad (1)$$

$$1/(F_0-F) = 1/F_0 + K_{LB}/(F_0c_q) \quad (2)$$

where F_0 and F are the fluorescence intensities of the fluorophores in the absence and the presence of a quencher (**PDI-Gal$_6$**), respectively, c_q is the concentration of the quencher, K_{SV} is the dynamic quenching constant, and K_{LB} is the static quenching constant.

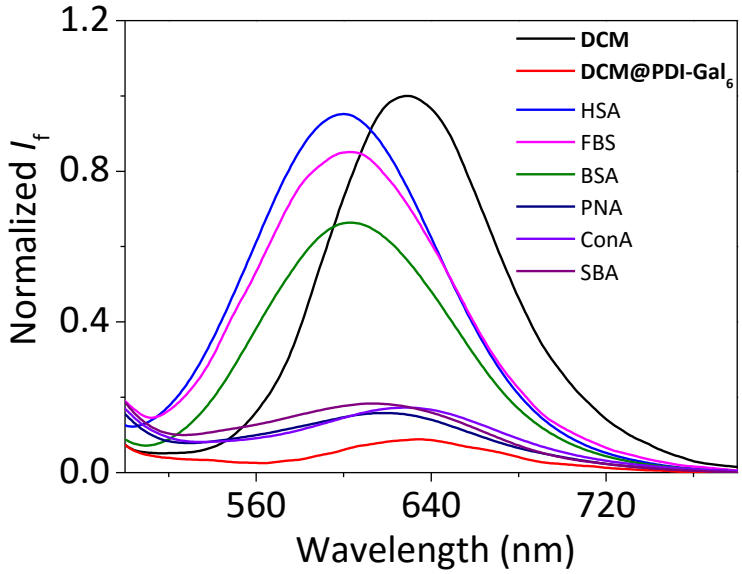


Fig. S4. Fluorescence spectra of **PDI-Gal$_6$/DCM** (5/30 μM) in the absence and presence of different serum proteins including human serum albumin (HSA) (7.5 μM), fetal bovine serum (FBS) (14 μL, from cesarean section fetal bovine) and bovine serum albumin (BSA) (7.5 μM), and different lectins including soybean agglutinin (SBA, 7.5 μM), concanavalin A (Con A, 7.5 μM) and peanut agglutinin (PNA, 7.5 μM). All fluorescence measurements were tested in Tris-HCl buffer (0.02 M, pH 7.4) with an excitation wavelength of 460 nm.

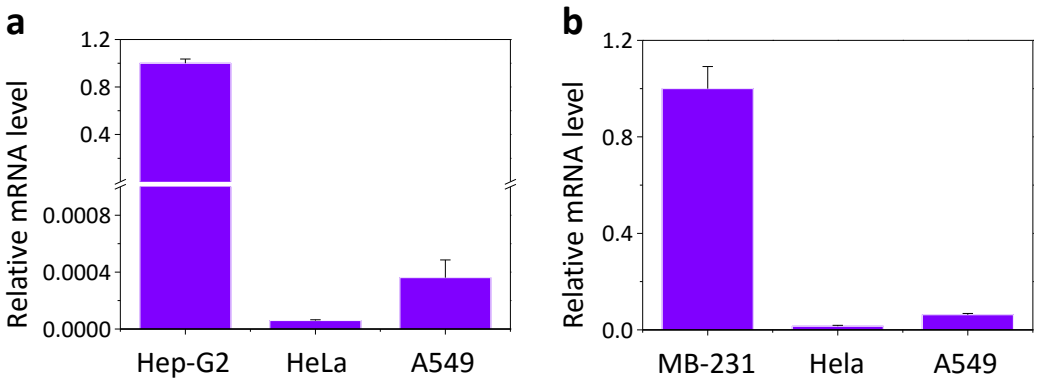


Fig. S5. Relative mRNA level of (a) asialoglycoprotein receptor (ASGPr) in Hep-G2, HeLa and A549 cells, and (b) mannose receptor (MR) in MDA-MB-231, HeLa and A549 cells determined by real-time quantitative polymerase chain reaction.

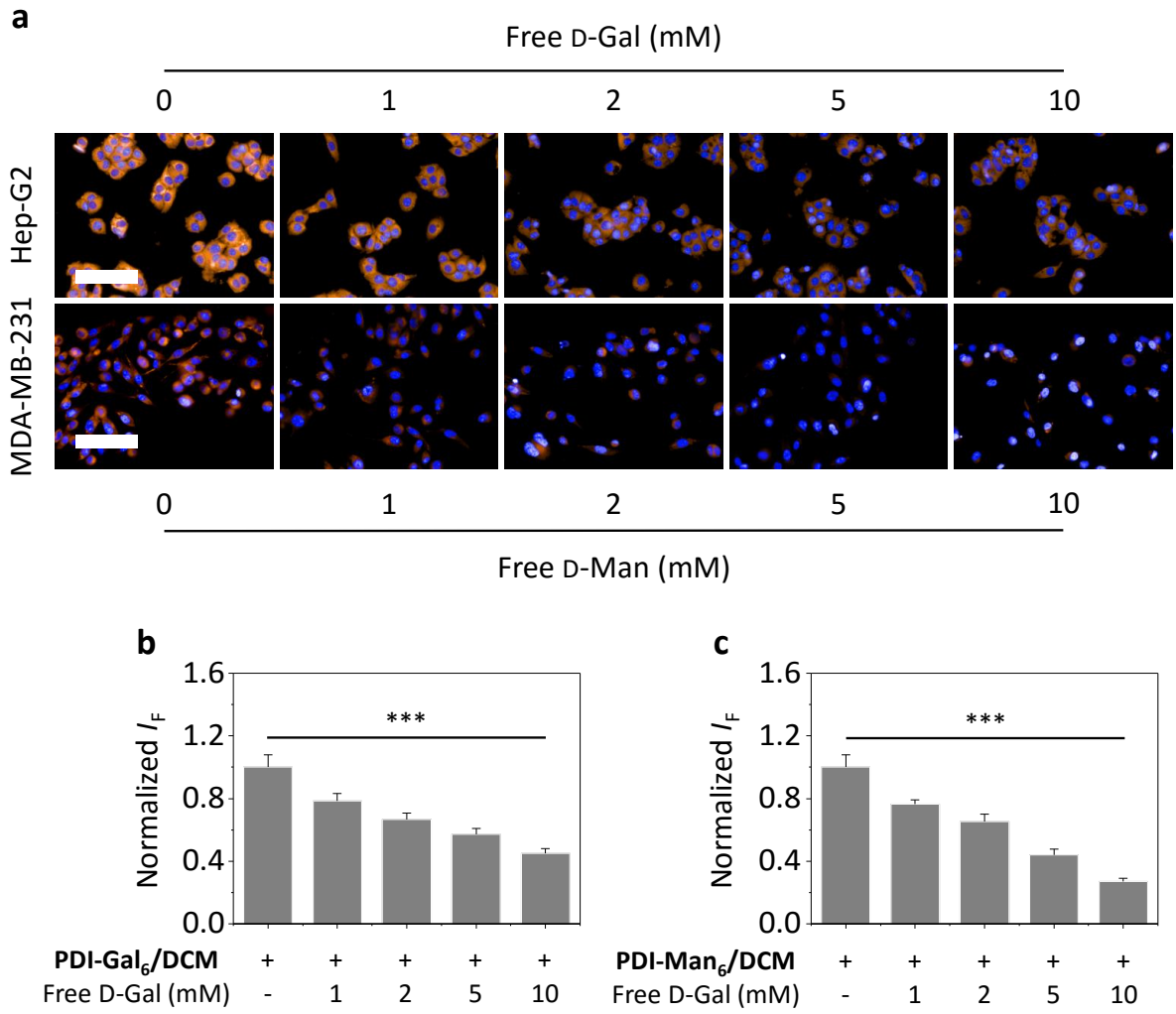


Fig. S6. Fluorescence imaging (a) and quantification (b,c) of different cancer cells with **PDI-Gal$_6$/DCM** or **PDI-Man$_6$/DCM** in the presence of increasing free D-galactose or D-mannose (***P < 0.001). Excitation channel: 460–490 nm, emission: 580–650 nm; the cell nucleus was stained by Hoechst 33342; scale bar: 100 μm.

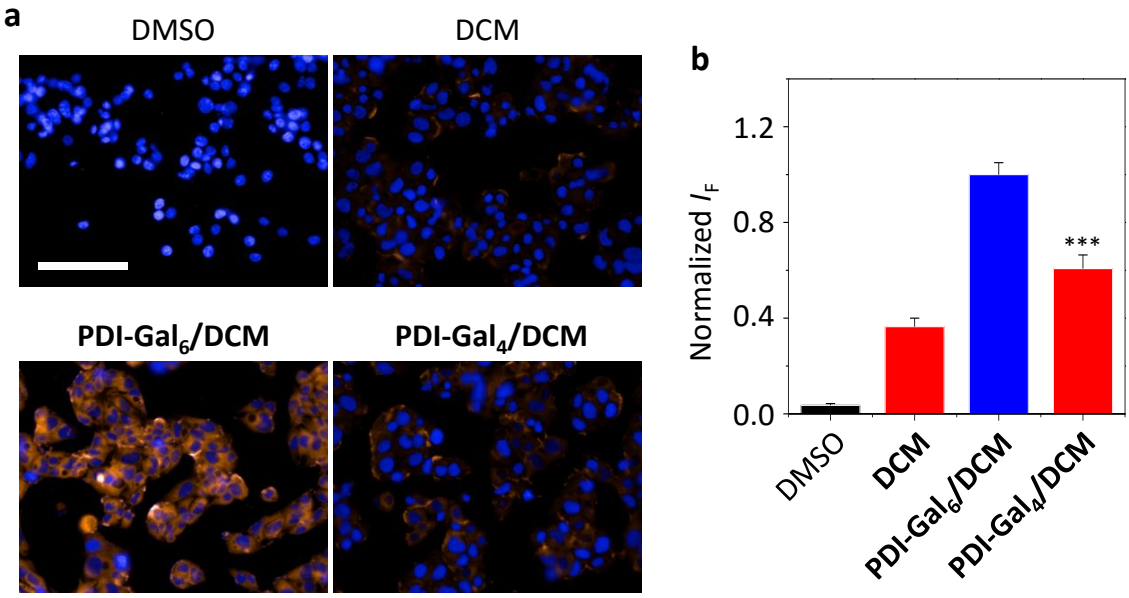


Fig. S7. Fluorescence imaging (a) and quantification (b) of Hep-G2 cells with **DCM** (10 μM), **PDI-Gal$_6$/DCM** (30/10 μM) and **PDI-Gal$_4$/DCM** (30/10 μM). Excitation channel: 460-490 nm, emission: 580-650 nm; the cell nucleus was stained by Hoechst 33342; scale bar: 100 μm.

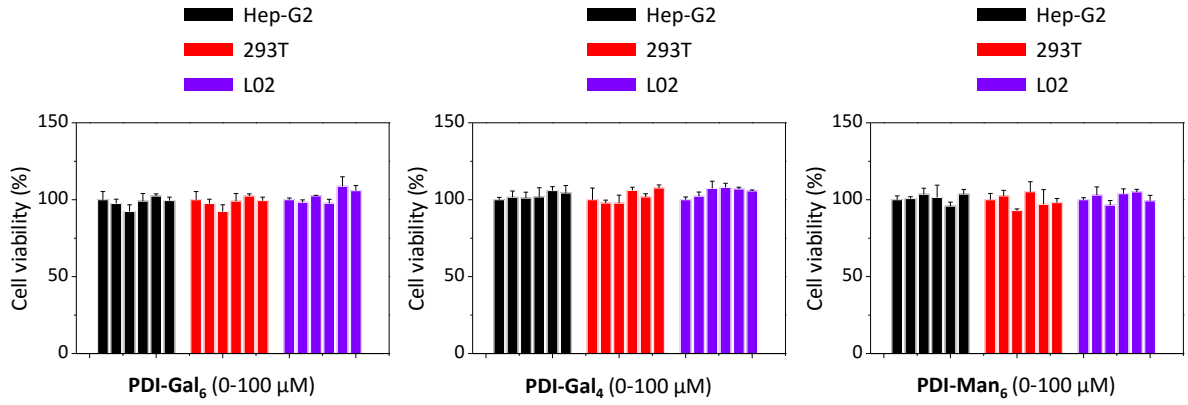


Fig. S8. Cell viability of Hep-G2 (human liver cancer) cells, 293T (human embryonic kidney) cells and L02 (human normal liver) cells with increasing **PDI-Gal$_6$**, **PDI-Gal$_4$** or **PDI-Man$_6$** determined by MTS cell proliferation assay.

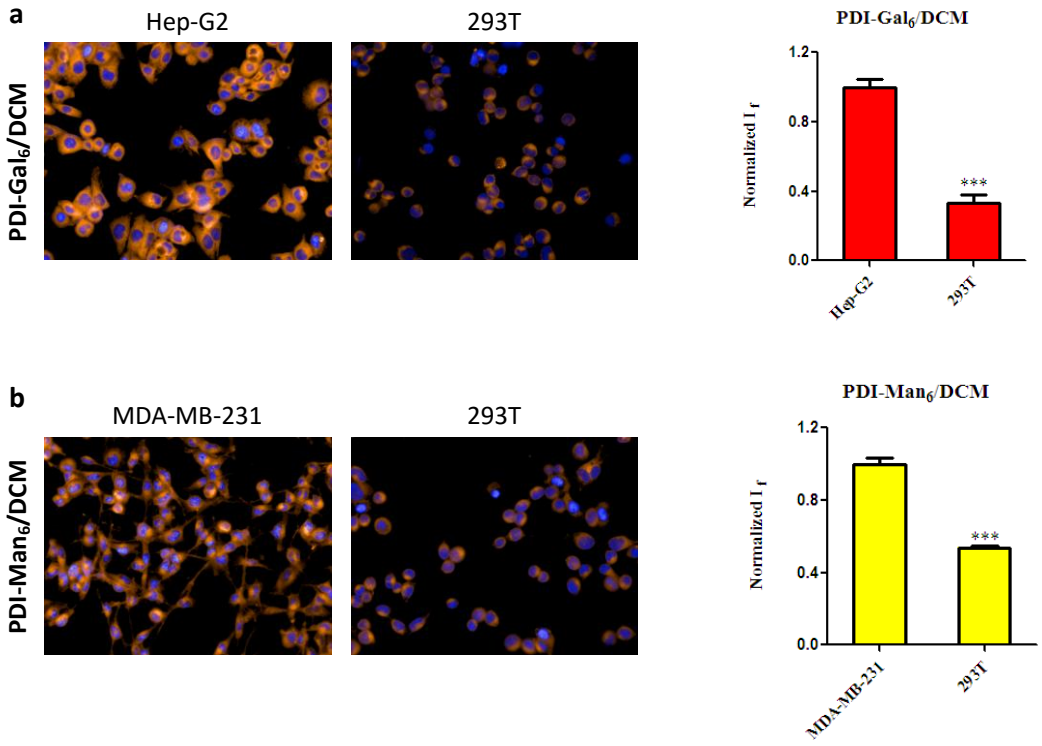


Figure S9. (a) Fluorescence imaging and quantification of Hep-G2 and 293T with **PDI-Gal$_6$/DCM** (30/10 μM). (a) Fluorescence imaging and quantification of MDA-MB-231 and 293T with **PDI-Man$_6$/DCM** (30/10 μM). Excitation channel: 460–490 nm, emission: 580–650 nm; the cell nucleus was stained by Hoechst 33342 (***P<0.001).

S2. Experimental section

General. UV-absorbance spectra were carried out on a Varian Cary 500 spectrophotometer. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer. Zeta potential was determined by a Horiba LB-550 DLS Nano-Analyzer. Ultrapure water was obtained from a Milli-Q integral Pure/Ultrapure Water Production unit.

Supramolecular self-assembly of glyco-dots. DCM (1 mM, DMSO, 100 μL) was added to a solution of **glyco-PDIs** (3 mM, Tris-HCl, 0.02 M, pH 7.4, 100 μL). The resulting mixture was sonicated for 10 min, and then stirred at room temperature for 12 h in the dark to produce the supramolecular glyco-dots, which can be used as is.

Scanning electron microscopy (SEM). A droplet of glycocluster (60 μM), which had been sonicated for 15 min in ultrapure water, was cast onto a freshly cleaved mica surface, followed by drying at room temperature. Then, SEM images of the materials were obtained by S-3400N (HITACHI, Japan).

High-resolution transmission electron microscope (HRTEM). A droplet of glycocluster (60 μM) with or without **DCM** (20 μM) was dropped onto carbon copper grids for HRTEM characterizations. JEOL 2100 equipped with a Gatan Orius charged-coupled device camera and Tridiem energy filter operating at 200 kV was used for TEM images, and the data were processed using Image J software.

Cell culture. Hep-G2 Hela and 293T cells were cultured in Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco, Gland Island, NY, USA). A549 cells were cultured in F12 supplemented with 10% FBS in a humidified atmosphere of 5% CO_2 and 95% air at 37 ˚C and split when the cells reached 85% confluency. L02 cells were cultured in Roswell Park Memorial Institute (1640) supplemented with 10% FBS. MDA-MB-231 cells were cultured in 1640 supplemented with 5% FBS in a humidified atmosphere of 5% CO_2 and 95% air at 37˚C and split when the cells reached 90% confluency.

Generation of shASGPR1 and control shRNA-HepG2 stable cell lines infected with lentivirus. pCAGVSVG (a plasmid encoding envelope protein) and PAX2 (packaging plasmid) were kind gifts from Dr. J. Wong (East China Normal University, China). The shRNA plasmids encoding ASGPR1-specific shRNA or scramble shRNA were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Lentiviral particles were generated according to the manufacturer's instructions. Briefly, 293T cells were seeded in a six-well tissue culture plate and were grown to a 80-90% confluency in antibiotic-free normal growth medium supplemented with FBS. Then, shRNA plasmid (shRNA of ASGPR1 or control, 3 μg) was cotransfected with pCAG-VSVG (1.8 μg) and PAX2 (2.7 μg) into 293T cells using 15 μL of lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After 6 h, the medium was changed to

fresh DMEM with 10% FBS. After 72 h, the lentivirus-cotaining supernatant were collected, filtered, and then employed for analysis.

Hep-G2 cells were plated in a 12-well plate 24 h prior to viral infection. The cells at approximately 50% confluency were infected with the lentiviral particles prepared as described above. The plates were incubated overnight and the medium was then changed to fresh complete medium. Two days after infection, the cells were splited at 1:5 and incubated for another 24 h in complete medium. Then puromycin (4 μg mL^{-1}) was added to select the stable clones expressing the shRNA. Medium was replaced with fresh puromycin-containing medium every 3-4 days until resistant colonies can be identified. Several colonies were picked, expanded, and then assayed for stable shRNA expression by evaluating level of ASGPR1 mRNA via real-time quantitative polymerase chain reaction (qPCR).

Generation of shMR and control shRNA-MDA-MB-231 stable cell lines infected with lentivirus. MDA-MB-231 cells were plated in a 6-well plate 24 h prior to viral infection. The cells at approximately 50% confluency were infected with the lentiviral particles containing shRNA targeting human MR or control, which were purchased from Genomeditech (Genomeditech, Shanghai, China). The plates were incubated for 6-8 h according to the manufacturer's instructions and the medium was then changed to fresh complete medium. Two days after infection, the cells were splited at 1:3 and incubated for another 24 h in complete medium. Then puromycin (4 μg mL^{-1}) was added to select the stable clones expressing the shRNA. Medium was replaced with fresh puromycin-containing medium every 3-4 days until resistant colonies can be identified. Several colonies were picked, expanded, and then assayed for stable shRNA expression by evaluating level of MR mRNA via real-time qPCR.

Fluorescence imaging of cells. Cells (Hep-G2/MDA-MB-231, 25000/well; HeLa, 12000/well; A549, 15000/well) were seeded on a black 96-well microplate with optically clear bottom (Greiner bio-one, Germany) overnight. Then, the cells were incubated with **DCM** in the absence and presence of glyco-dots for 15 min. For the competition assay, Hep-G2 and MDA-MB-231 cells were preincubated with free D-galactose and D-mannose for 2 h, followed by incubation with **PDI-Gal_6/DCM** and **PDI-Man_6/DCM** for 15 min, respectively. Then, the cells were gently washed with PBS (phosphate buffered saline) three times, and stained with Hoechst 33342 (5 μg mL^{-1}) at 37 °C in a humidified atmosphere of 5% CO_2 in air for 5 min. Then, cells were washed with PBS three times. The fluorescence images were recorded using an Operetta high content imaging system and quantified by the Columbus image data analysis system (Perkinelmer, US).

Real-time quantitative PCR. Total RNA was isolated from cells using TRIzol Reagent (Invitrogen) according to the manufacturer's protocol. Complementary DNA generated using a PrimeScript® RT reagent kit (TaKaRa, Dalian, China) was analyzed by quantitative PCR using SYBR® Premix Ex TaqTM. Real-time PCR was performed using a 7300 Real-Time

PCR system (Applied Biosystems, CA, USA). GAPDH was detected as the housekeeping gene. Primers for qPCR were as follows:

GAPDH forward, 5'-ATCACTGCCACCCAGAAGAC-3'
and reverse, 5'-ATGAGGTCCACCACCCTGTT-3'
ASGPR1 forward, 5'-CTGGACAATGAGGAGAGTGAC-3'
and reverse, 5'-TTGAAGCCCGTCTCGTAGTC-3'
Mannose Receptor forward,5'-GCAGCTCTGGGAACTTGGAT-3'
and reverse, 5'-TTGCCTGGTGTCCAGTAGGA-3'

Cell viability assay. Cells were plated overnight on 96-well plates at 8000 cells per well in growth medium. After seeding, cells were treated with glycoclusters at different concentrations for 48 h. After 48 h exposure, a MTS/PMS (20:1, Promega Corp) solution (10 µL per well) was added to each well, followed by a gentle shake. After 2-4 h incubation at 37 °C under 5% CO_2, the absorbance of the mixture solutions was measured at 490 nm as a reference with an M5 microplate reader (Molecular Device, USA). The optical density of the result in MTS assay was directly proportional to the number of viable cells.

S3. Syntheses of the PDI-based glycoclusters

General procedures

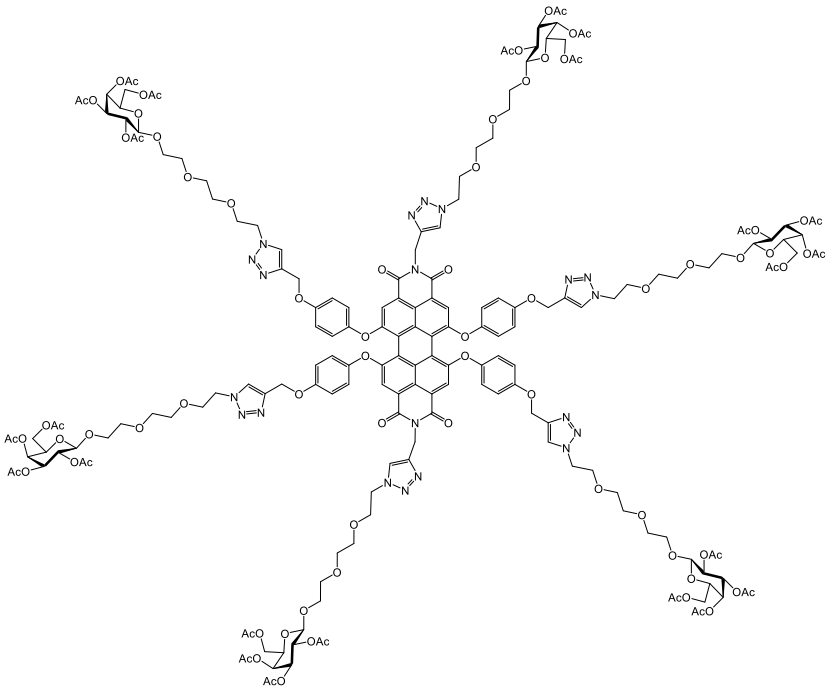
All reagents for synthesis commercially available (highest purity available for reagent grade compounds) were used without further purification. Solvents were distilled over CaH_2 (CH_2Cl_2), Mg/I_2 (MeOH), Na/benzophenone (THF) or purchased dry. Reactions under microwave activation were performed on a Biotage Initiator system. Thin-layer chromatography (TLC) was carried out on aluminum sheets coated with silica gel 60 F_{254} (Merck). TLC plates were inspected by UV light (λ = 254 nm, 365 nm) and developed by treatment with a mixture of 10% H_2SO_4 in EtOH/H_2O (95:5 v/v) followed by heating. Silica gel column chromatography was performed with silica gel Si 60 (40–63 μm). Optical rotation was measured using a Perkin Elmer polarimeter. NMR spectra were recorded at 293 K, unless stated otherwise. Chemical shifts are referenced relative to deuterated solvent residual peaks. The following abbreviations are used to explain the observed multiplicities: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; p, pseudo and b, broad. Complete signal assignments were based on 1D and 2D NMR correlations COSY and HSQC. High resolution (HR-ESI-QToF) mass spectra were recorded using a Bruker MicroToF-Q II XL spectrometer. The glycoclusters tested in bioassays were purified using automated purification systems with medium pressure chromatography on reverse C_{18} silica gel. Their purity was verified by ^{1}H and ^{13}C NMR techniques, indicating ca. 95% purity.

General procedure for 1,3-dipolar cycloadditions (Method A)

The alkyne-functionalized perylenediimide **5** or **6** (1 eq.), CuI (0.5 eq,), DIPEA (1.5 eq. per alkyne function) and azido-derivative **7** (1.5 eq. per alkyne function) in DMF were introduced into a Biotage Initiator 2-5 mL vial. The vial was sealed with a septum cap and heated at 110°C for 15 min under microwave irradiation (solvent absorption level : High). The crude mixture was concentrated and co-evaporated with toluene 6 times then purified by flash silica gel column chromatography to afford the desired cycloadducts.

General procedure for the Zemplén deacetylation (Method B)

To a suspension of acetylated glycocluster (1 eq.) in distilled MeOH was added MeONa (0.2 eq.). The mixture was stirred at r.t. for 16 hours, neutralized with Amberlite IR-120 resin (H^+ form), filtrated and concentrated *in vacuo* to afford the corresponding hydroxylated glycoclusters.



N*,*N*'-Bis-{1-[1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethyl}-1,6,7,12-tetra-(4-{1-[1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethyloxy}phenoxy)perylene-3,4,9,10-tetracarboxylic diimide (2-Gal):** Obtained as a purple foam following ***Method A: **1** (10 mg, 0.010 mmol, 1 eq.), compound **Ac_4Gal-TEG-N_3** (45 mg, 0.089 mmol, 9.4 eq.), CuI (1 mg, 0.005 mmol, 0.5 eq.) and DIPEA (12 µL, 0.066 mmol, 7 eq.). Purified by silica gel flash chromatography (CH_2Cl_2/MeOH 99/1 to 95/5).

Yield = 77% (30 mg), $\mathbf{R_f}$ = 0.45 (CH_2Cl_2/MeOH 95/5).

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.09 (s, 4H, perylene-H), 7.98 (s, 4H, H-triaz), 7.75 (s, 2H, H-triaz), 6.91 (d, *J* = 9.9 Hz, 16H, H-ar), 5.43 (bs, 4H, NCH_2-triaz), 5.38 (d, *J* = 3.1 Hz, 4H, H-4), 5.36 (d, *J* = 3.1 Hz, 2H, H-4'), 5.23 (bs, 8H, OCH_2-triaz), 5.21-5.13 (M, 6H, H-2, H-2'), 5.02 (dd, *J* = 10.4, 3.2 Hz, 4H, H-3), 5.00 (dd, *J* = 10.4, 3.3 Hz, 2H, H-3'), 4.64 (s, 8H, NCH_2CH_2), 4.55 (d, *J* = 8.0 Hz, 4H, H-1), 4.53 (d, *J* = 8.1 Hz, 2H, H-1'), 4.47 (bs, 4H, NCH_2CH_2), 4.21-4.06 (m, 12H, H-6, H-6'), 4.02-3.87, 3.87-3.79 (2m, 18H, OCH_2, H-5, H-5'), 3.79-3.39 (m, 48H, 4×OCH_2), 2.13, 2.12, 2.04, 2.02, 2.01, 2.01, 1.97, 1.96 (8s, 72H, 24×$COCH_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 170.5, 170.4, 170.3, 169.58, 169.55 (4×*C*OCH_3), 163.1 (Ar*C*ON), 156.63, 155.58, 149.0 (3×C^{IV}-ar-O), 143.8, 143.3 (2×C^{IV}-triaz), 133.0 (C^{IV}-ar), 124.3 (6C, CH-triaz), 122.4 (C^{IV}-ar), 121.6 (8C, CH-ar), 120.2, 119.4 (2×C^{IV}-ar), 119.3 (CH-perylene), 116.3 (8C, CH-ar), 101.51 (C-1), 101.47 (C-1'), 71.0 (C-3, C-3'), 70.82 (C-5), 70.80, 70.78, 70.74, 70.72, 70.66, 70.38, 70.34, 69.61, 69.56 (C-5', 4×OCH_2), 69.32, 69.29 (GalO*C*H_2), 69.96 (C-2), 68.95 (C-2'), 67.2 (C-4, C-4'), 62.7 (OCH_2-triaz), 61.4 (C-6), 60.5 (C-6'), 50.5, 50.2 (NCH_2CH_2), 35.4 (NCH_2-triaz), 20.93, 20.91, 20.82, 20.81, 20.7 (4×$COCH_3$).

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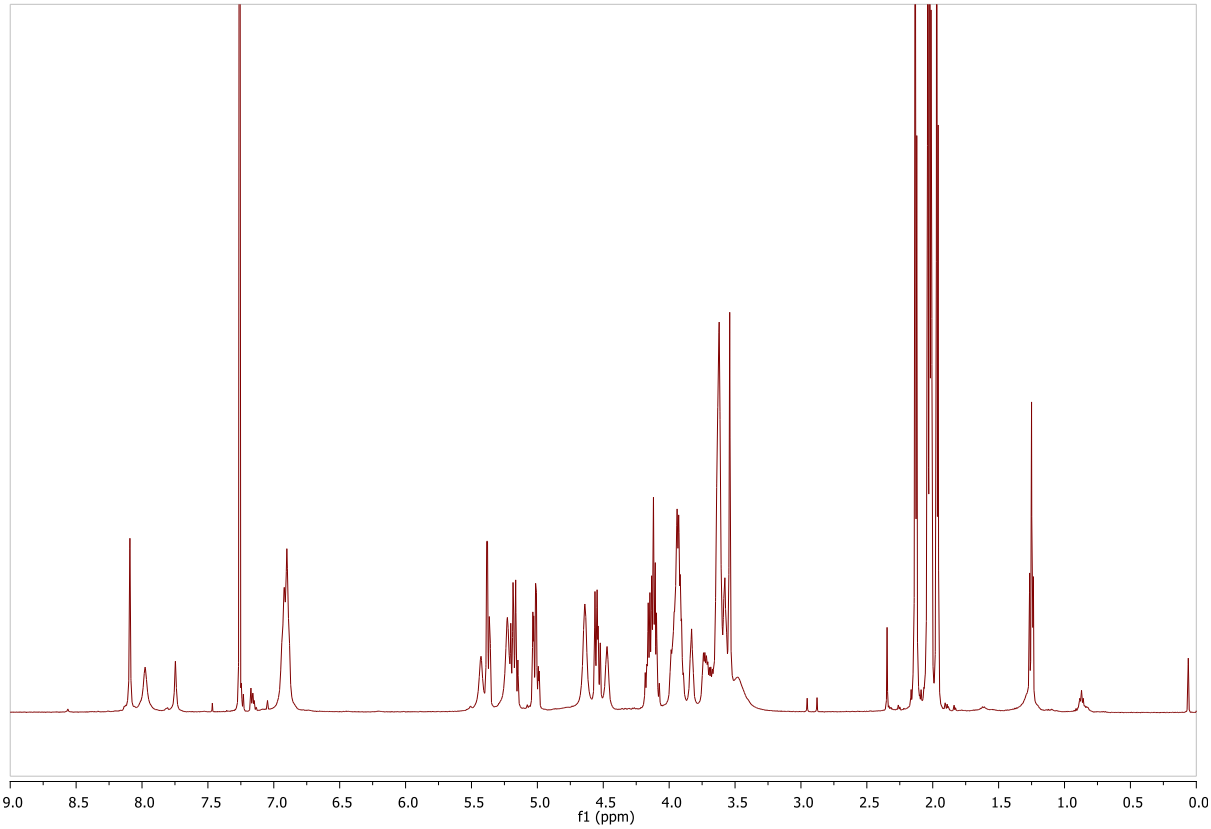
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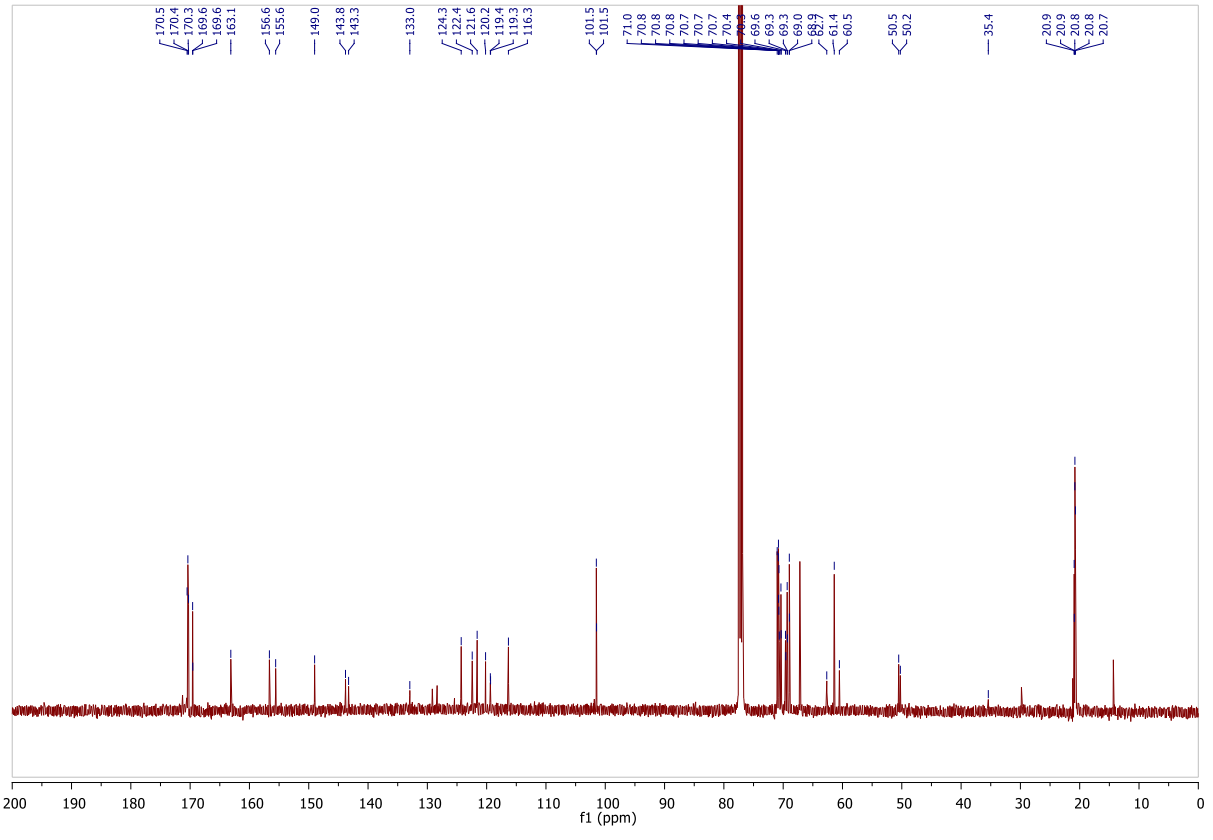
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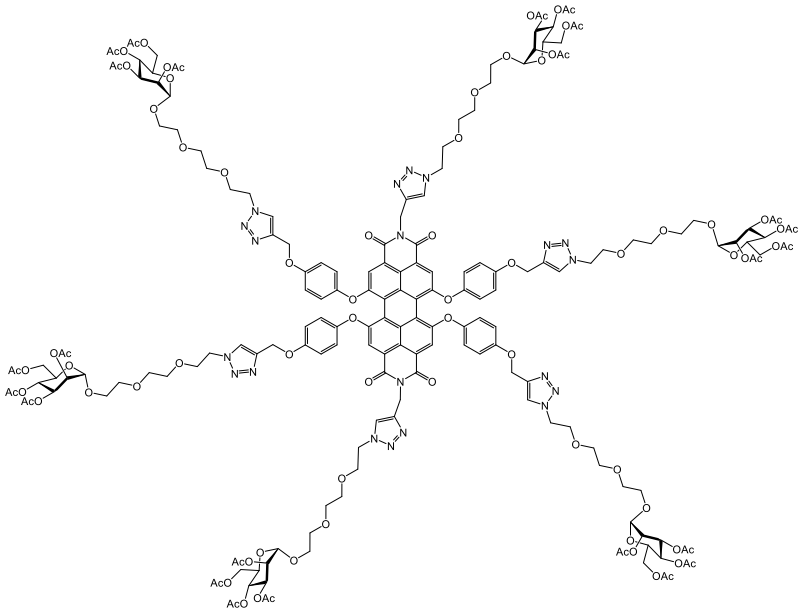


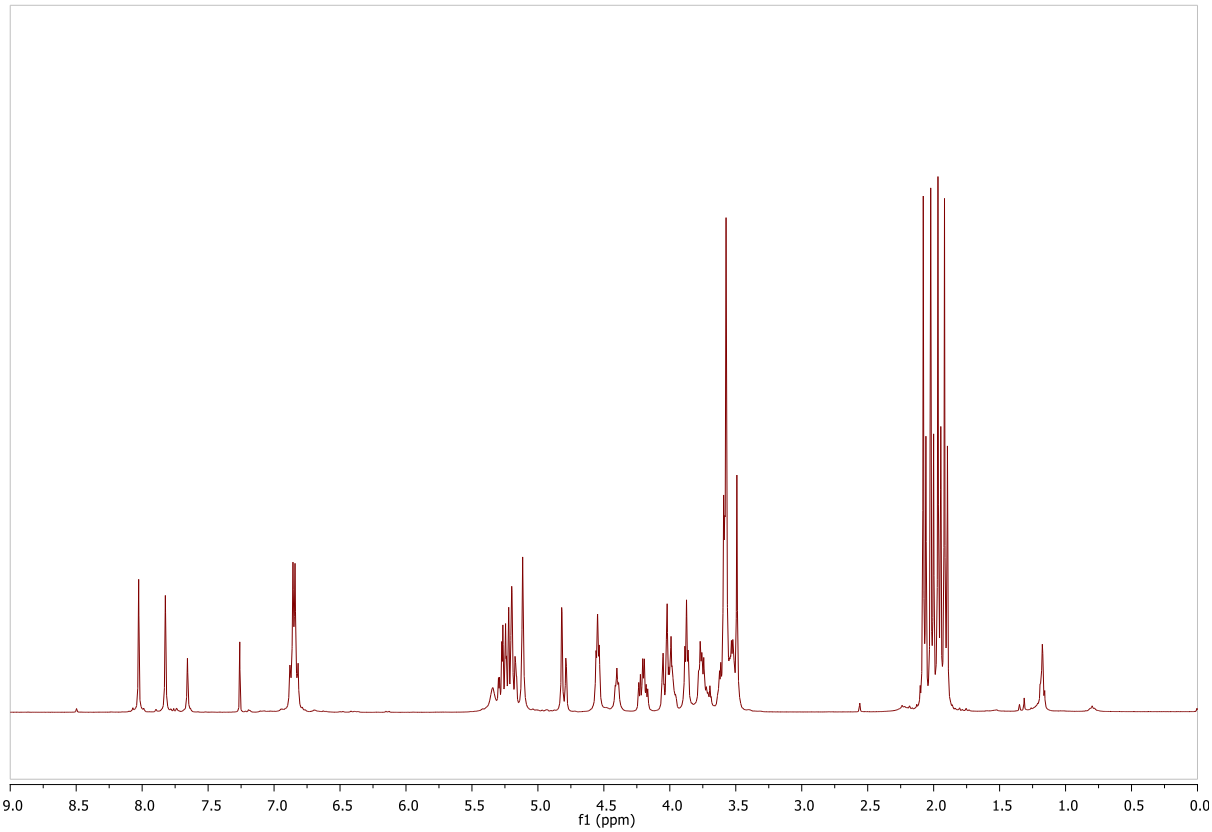
N*,*N*'-Bis-{1-[1-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethyl}-1,6,7,12-tetra-(4-{1-[1-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethyloxy}phenoxy)perylene-3,4,9,10-tetracarboxylic diimide (2-Man):** Obtained as a purple foam following ***Method A: **1** (50 mg, 0.048 mmol, 1 eq.), compound **Ac_4Man-TEG-N_3** (216 mg, 0.428 mmol, 9 eq.), CuI (4.5 mg, 0.024 mmol, 0.5 eq.) and DIPEA (74 µL, 0.428 mmol, 9 eq.). Purified by silica gel flash chromatography (CH_2Cl_2/MeOH 99/1 to 95/5).

Yield = 67% (130 mg), $\mathbf{R_f}$ = 0.45 (CH_2Cl_2/MeOH 95/5).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.03 (s, 4H, perylene-H), 7.82 (s, 4H, H-triaz), 7.66 (s, 2H, H-triaz), 6.85 (d, *J* = 6.3 Hz, 16H, H-ar), 5.34 (bs, 4H, NCH_2-triaz), 5.31-5.15 (m, 18H, H-2, H-2', H-3, H-3', H-4, H-4'), 5.12 (bs, 8H, OCH_2-triaz), 4.82 (d, *J* = 1.0 Hz, 4H, H-1), 4.79 (2d, *J* = 1.1 Hz, 2H, H-1'), 4.55 (t, *J* = 4.9 Hz, 8H, NCH_2CH_2), 4.40 (t, *J* = 4.9 Hz, 4H, NCH_2CH_2), 4.25-4.15 (m, 6H, H-6a, H-6a'), 4.08-3.95 (m, 12H, H-5, H-5', H-6b, H-6b'), 3.87 (t, *J* = 5.0 Hz, 8H, OCH_2), 3.80-3.47 (m, 52H, OCH_2), 2.08, 2.06, 2.02, 2.00, 1.97, 1.95, 1.92, 1.90 (8s, 72H, 24×$COCH_3$).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 170.56, 170.54, 170.0, 169.92, 169.86, 169.81, 169.6 (7s, $COCH_3$), 162.9 (ArCON), 156.4, 155.4, 148.8 (3×C^{IV}-ar-O), 143.6, 143.1 (2×C^{IV}-triaz), 132.7 (C^{IV}-ar), 124.1 (6C, CH-triaz), 122.2 (C^{IV}-ar), 121.4 (8C, CH-ar), 119.9 (C^{IV}-ar), 119.1 (CH-perylene, C^{IV}-ar), 116.1 (8C, CH-ar), 97.59, 97.55 (2s, C-1, C-1'), 70.60, 70.54, 70.45, 70.40, 69.9 (3×OCH_2), 69.45, 69.42, 69.37 (OCH_2, C-2, C-2'), 69.0 (C-3, C-3'), 68.4, 68.3 (C-5, C-5'), 67.3, 67.2 (OCH_2), 66.0 (C-4, C-4'), 62.4 (OCH_2-triaz), 62.32, 62.29 (C-6, C-6'), 50.3, 50.0 (NCH_2CH_2), 35.2 (NCH_2-triaz), 20.9-20.8, 20.7-20.6 (2m, 4×$COCH_3$).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{186}H_{224}N_{20}Na_2O_{84}$ $[M+2Na]^{2+}$ 2063.6828, found 2063.6744.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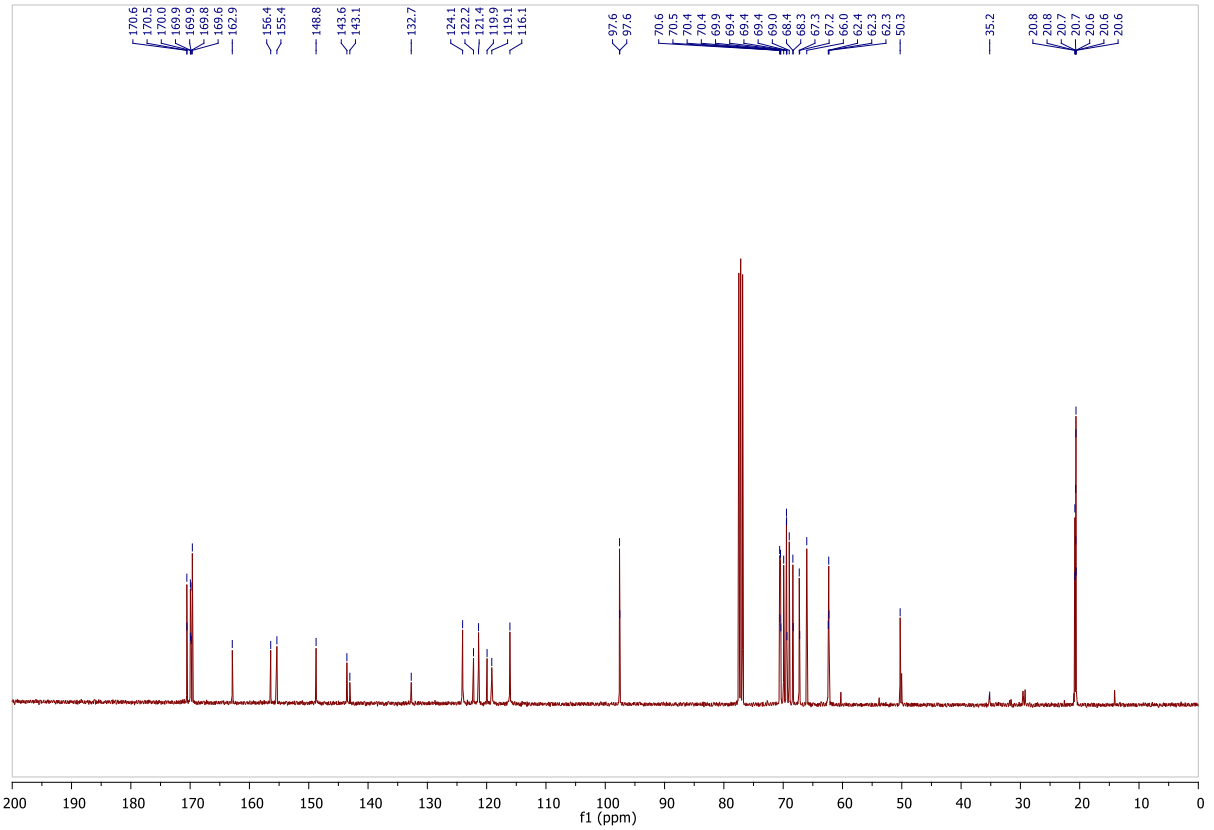
f1 (ppm)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170.5
170.0
169.9
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169.6
162.9
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155.4
148.8
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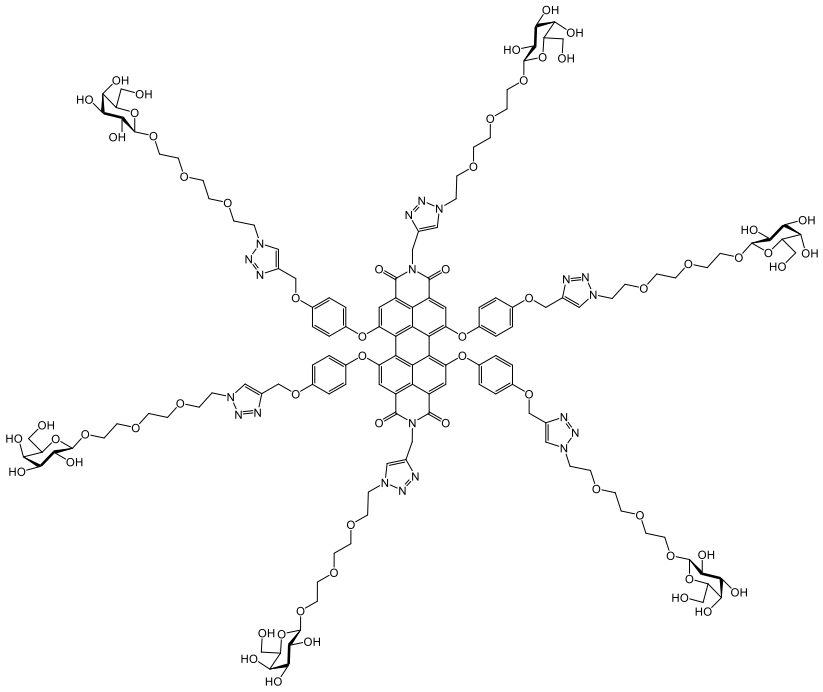
132.7
124.1
122.2
121.4
119.9
119.1
116.1
97.6
97.6
70.6
70.5
70.4
70.4
69.9
69.4
69.4
69.4
69.0
68.4
68.3
67.3
67.2
66.0
62.4
62.3
62.3
50.3
35.2
20.8
20.8
20.7
20.7
20.6
20.6
20.6
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



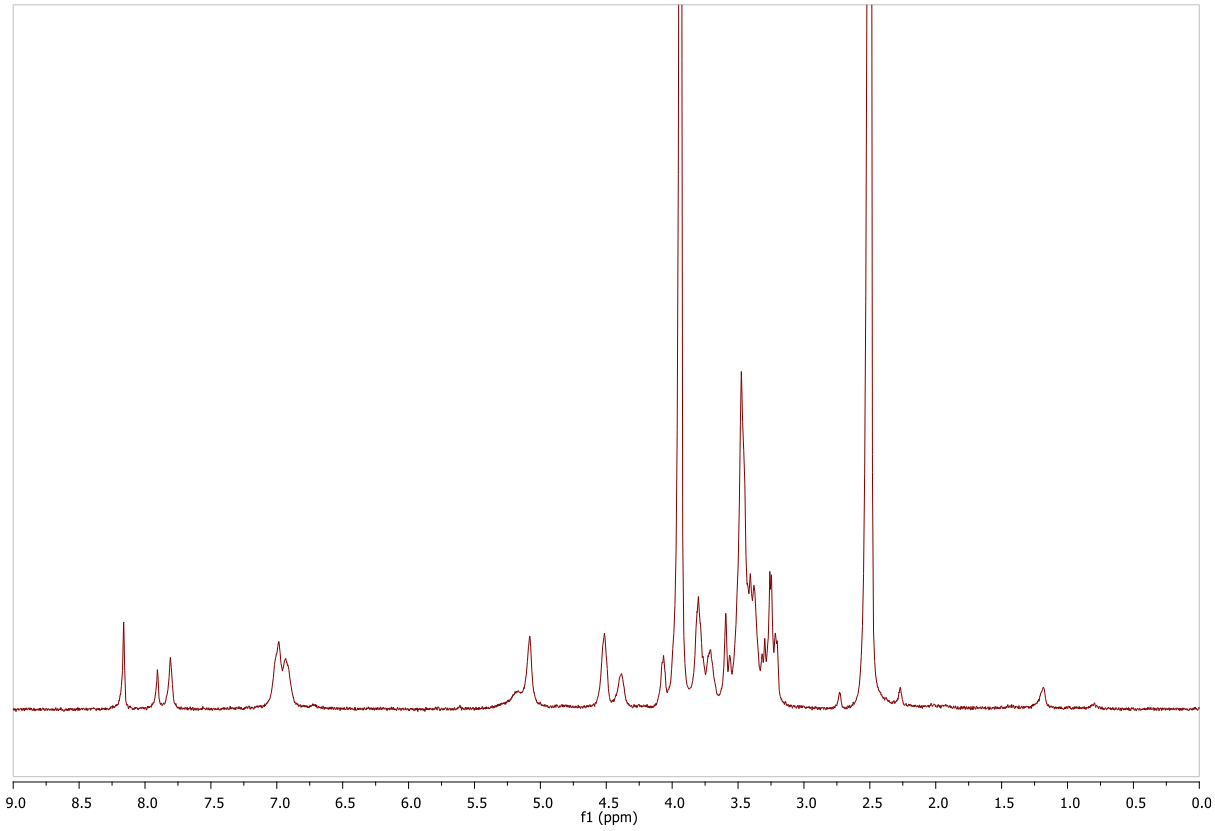
N*,*N*'-Bis-{1-[1-(β-D-galactopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethyl}-1,6,7, 12-tetra-(4-{1-[1-(β-D-galactopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethyloxy} phenoxy)perylene-3,4,9,10-tetracarboxylic diimide (PDI-Gal$_6$):** Obtained from **2-Gal** (112 mg, 0.027 mmol) as a deep purple foam following ***Method B.

Yield = 88% (75 mg).

^{1}H NMR (300 MHz, DMSO-d$_6$ + βD$_2$O) δ (ppm): 8.16 (s, 4H, H-triaz), 7.91 (s, 2H, H-triaz), 7.81 (s, 4H, perylene-H), 6.96 (d, *J* = 15.9 Hz, 16H, CH-ar), 5.08 (s, 12H, OC*H*$_2$-triaz, NC*H*$_2$-triaz), 4.51, 4.38 (2s, 12H, NC*H*$_2$CH$_2$), 4.11-4.04 (m, 6H, H-1), 3.88-3.17 (m, 96H, H-2, H-3, H-4, H-5, H-6, 5×OCH$_2$).

^{13}C NMR (100 MHz, DMSO-d$_6$ + βD$_2$O) δ (ppm): 162.5 (Ar*C*ON), 156.2, 155.5, 148.4 (3×C^{IV}-ar-O), 142.8, 142.7 (2×C^{IV}-triaz), 132.5 (C^{IV}-ar), 125.4, 124.0 (2×CH-triaz), 122.4 (C^{IV}-ar), 121.6 (CH-ar), 119.2, 118.8 (2×C^{IV}-ar), 118.1 (CH-perylene), 116.4 (CH-ar), 103.7 (C-1), 75.4 (C-3), 75.3 (C-2), 73.5 (C-5), 70.7, 70.0, 69.9, 69.8, 69.7, 69.0, 68.8 (OCH$_2$), 68.5-68.2 (m, C-4, OCH$_2$), 68.1 (OCH$_2$), 61.7 (OCH$_2$-triaz), 60.6 (C-6), 49.8, 49.7 (N*C*H$_2$CH$_2$), 35.7 (NCH$_2$-triaz).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{138}H_{176}N_{20}Na_2O_{60}$ $[M+2Na]^{2+}$ 1559.5580, found 1559.5608.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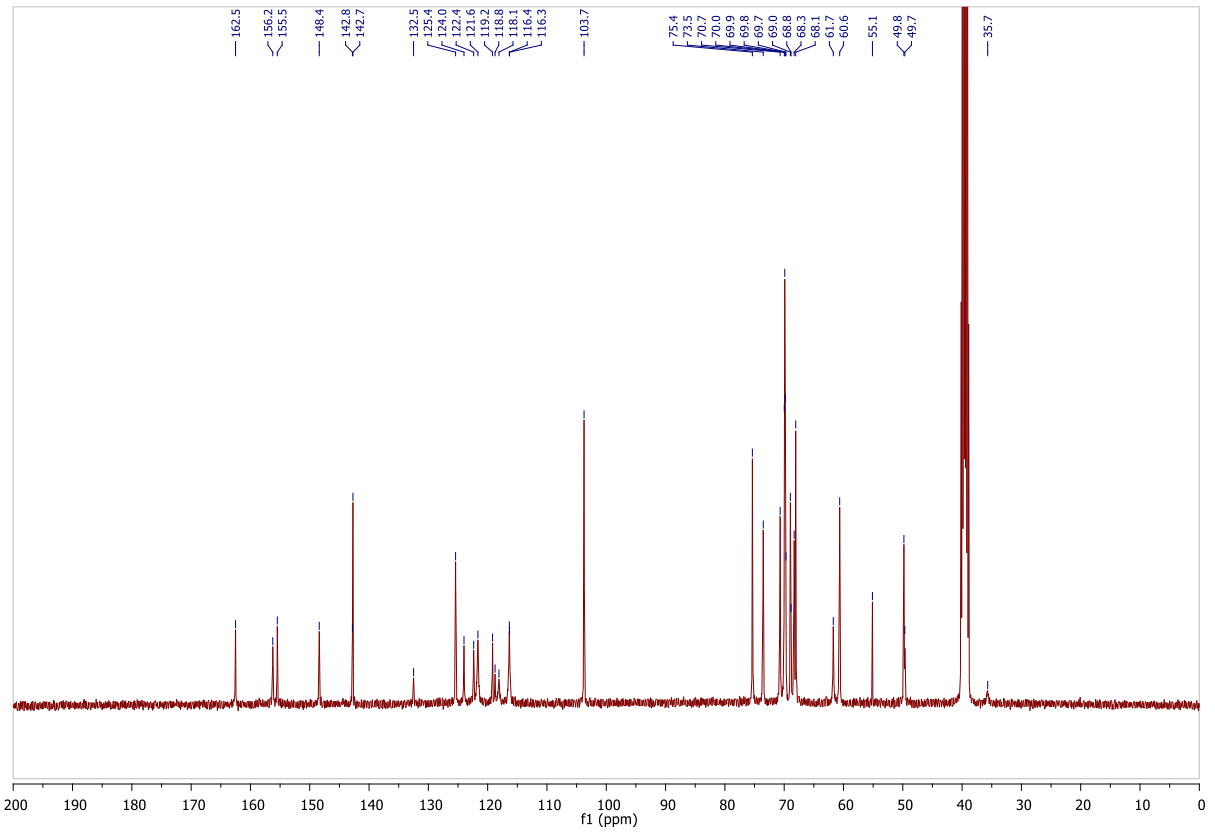
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


162.5
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155.5
148.4
142.8
142.7
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125.4
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121.6
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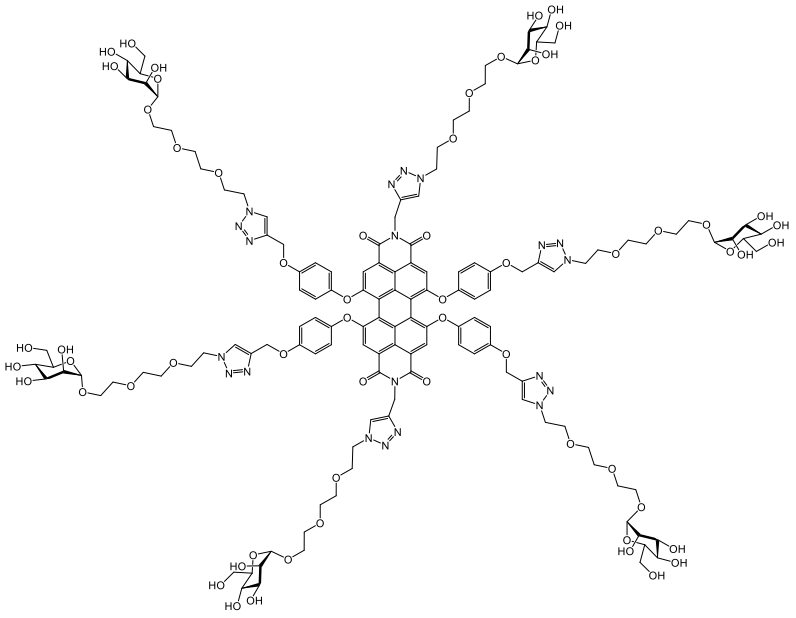
118.1
116.4
116.3
103.7
75.4
73.5
70.7
70.0
69.9
69.8
69.7
69.0
68.8
68.3
68.1
61.7
60.6
55.1
49.8
49.7
35.7
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



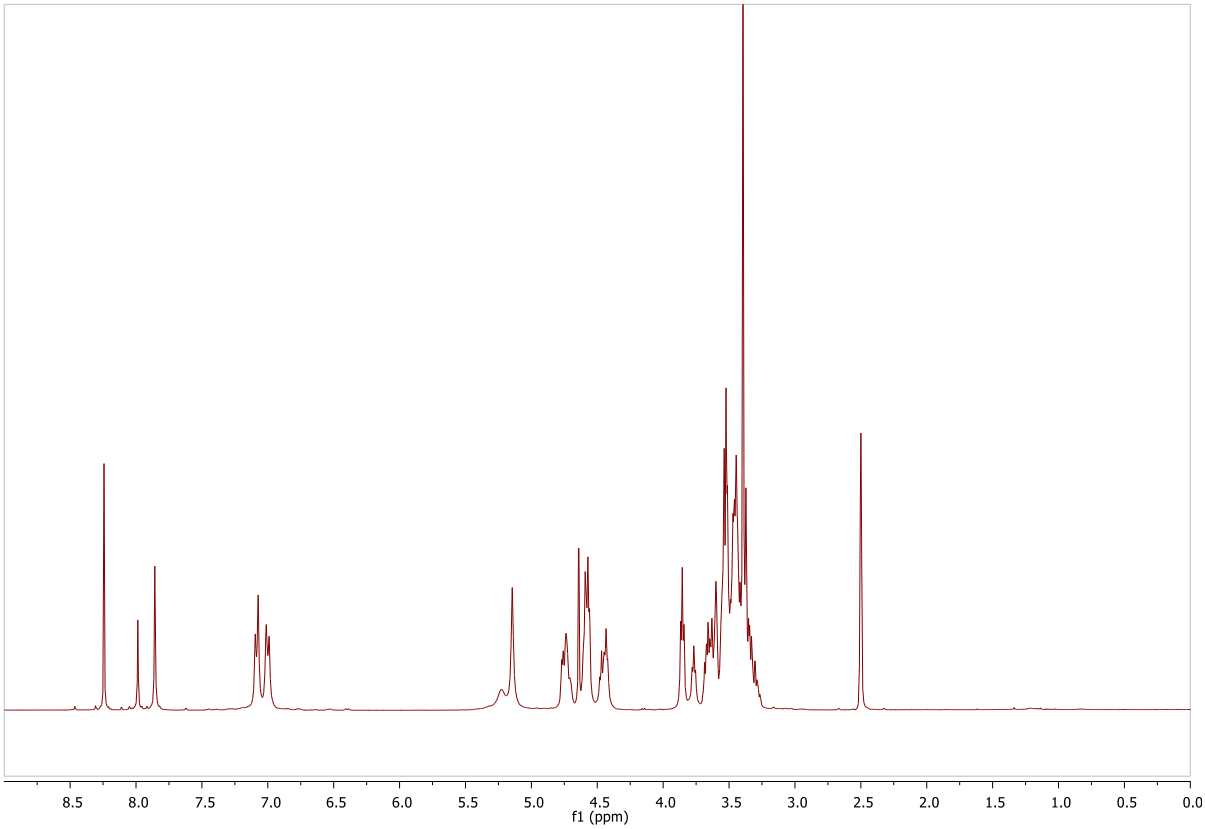
N*,*N*'-Bis-{1-[1-(α-D-mannopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethyl}-1,6,7, 12-tetra-(4-{1-[1-(α-D-mannopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethyloxy} phenoxy)perylene-3,4,9,10-tetracarboxylic diimide (PDI-Man$_6$):** Obtained from **2-Man** (119 mg, 0.029 mmol) as a deep purple foam following ***Method B.

Yield = 87% (78 mg).

^{1}H NMR (400 MHz, DMSO-d$_6$ + αD$_2$O) δ (ppm): 8.24 (s, 4H, H-triaz), 7.99 (s, 2H, H-triaz), 7.86 (s, 4H, perylene-H), 7.04 (dd, *J* = 33.3, 8.7 Hz, 16H, CH-ar), 5.14 (bs, 12H, OC*H*$_2$-triaz, NC*H*$_2$-triaz), 4.64 (d, *J* = 1.2 Hz, 4H, H-1), 4.62-4.54 (m, 10H, H-1', NC*H*$_2$CH$_2$), 4.50-4.39 (m, 4H, NC*H*$_2$CH$_2$), 3.85 (t, *J* = 5.1 Hz, 8H, OCH$_2$), 3.77 (t, *J* = 5.1 Hz, 4H, OCH$_2$), 3.71-3.26 (m, 84H, H-2, H-2', H-3, H-3', H-4, H-4', H-5, H-5', H-6, H-6', 4×OCH$_2$).

^{13}C NMR (100 MHz, DMSO-d$_6$ + αD$_2$O) δ (ppm): 162.3 (Ar*C*ON), 156.1, 155.4, 148.2 (3×C^{IV}-ar-O), 142.6, 142.5 (2×C^{IV}-triaz), 132.3 (C^{IV}-ar), 125.1, 123.8 (2s, 2×CH-triaz), 122.3 (C^{IV}-ar), 121.5 (CH-ar), 118.9, 118.6 (2s, 2×C^{IV}-ar), 117.9 (CH-perylene), 116.2 (CH-ar), 100.0 (C-1, C-1'), 74.0 (C-4, C-4'), 71.0 (C-3, C-3'), 70.3 (C-2, C-2'), 69.74, 69.72, 69.60, 69.56, 69.50, 68.8, 68.7 (4×OCH$_2$), 67.0 (C-5, C-5'), 65.74, 65.70 (OCH$_2$), 61.6 (O*C*H$_2$-triaz), 61.3 (C-6, C-6'), 49.6, 49.4 (2s, N*C*H$_2$CH$_2$) 35.5 (NCH$_2$-triaz).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{138}H_{178}N_{20}O_{60}$ $[M+2H]^{2+}$ 1537.5741, found 1537.5681.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6.0
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5.0
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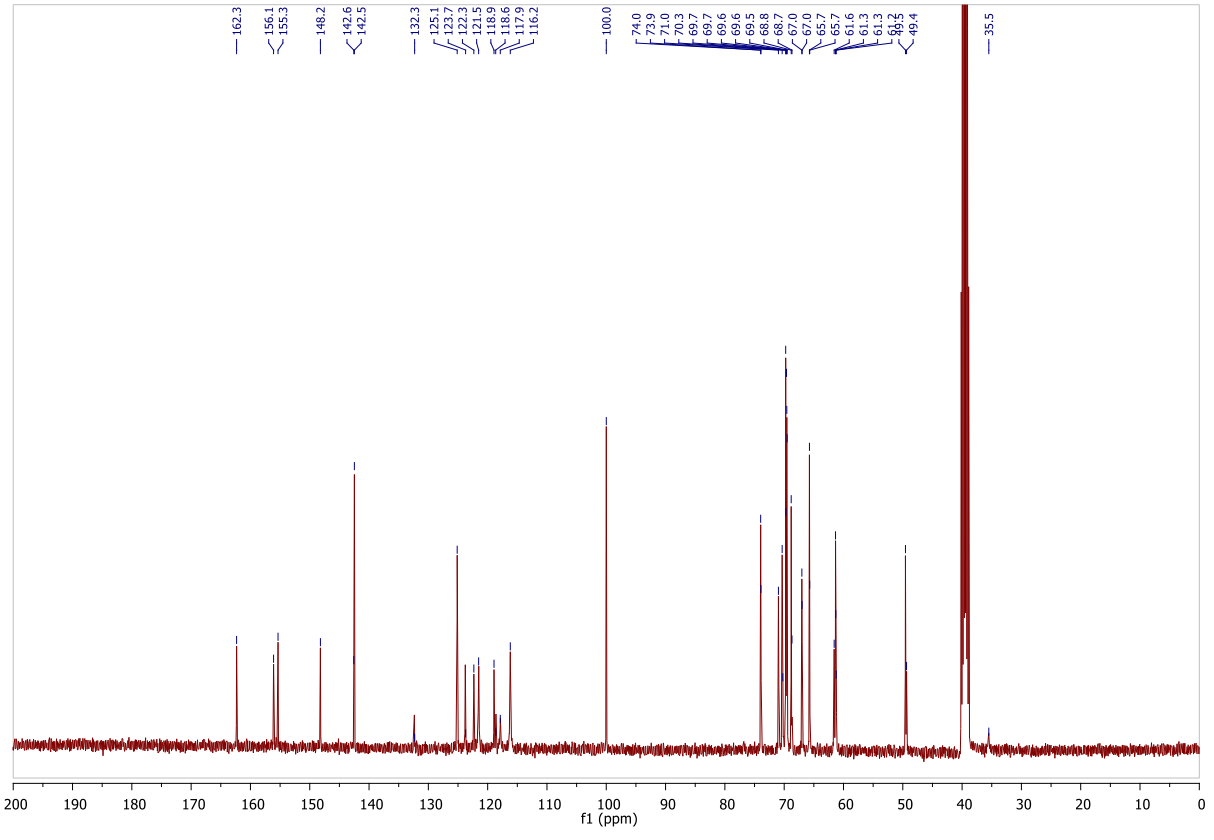
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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125.1
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121.5
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117.9
116.2
100.0
74.0
73.9
71.0
70.3
69.7
69.7
69.6
69.6
69.5
68.8
68.7
67.0
67.0
65.7
65.7
61.6
61.3
61.3
49.5
49.4
35.5
200
190
180
170
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150
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130
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110
100
90
80
70
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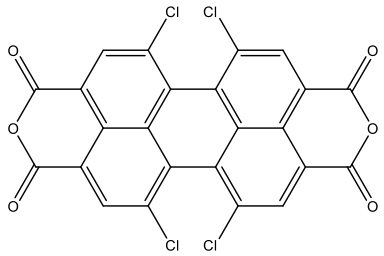
50
40
30
20
10
0
f1 (ppm)



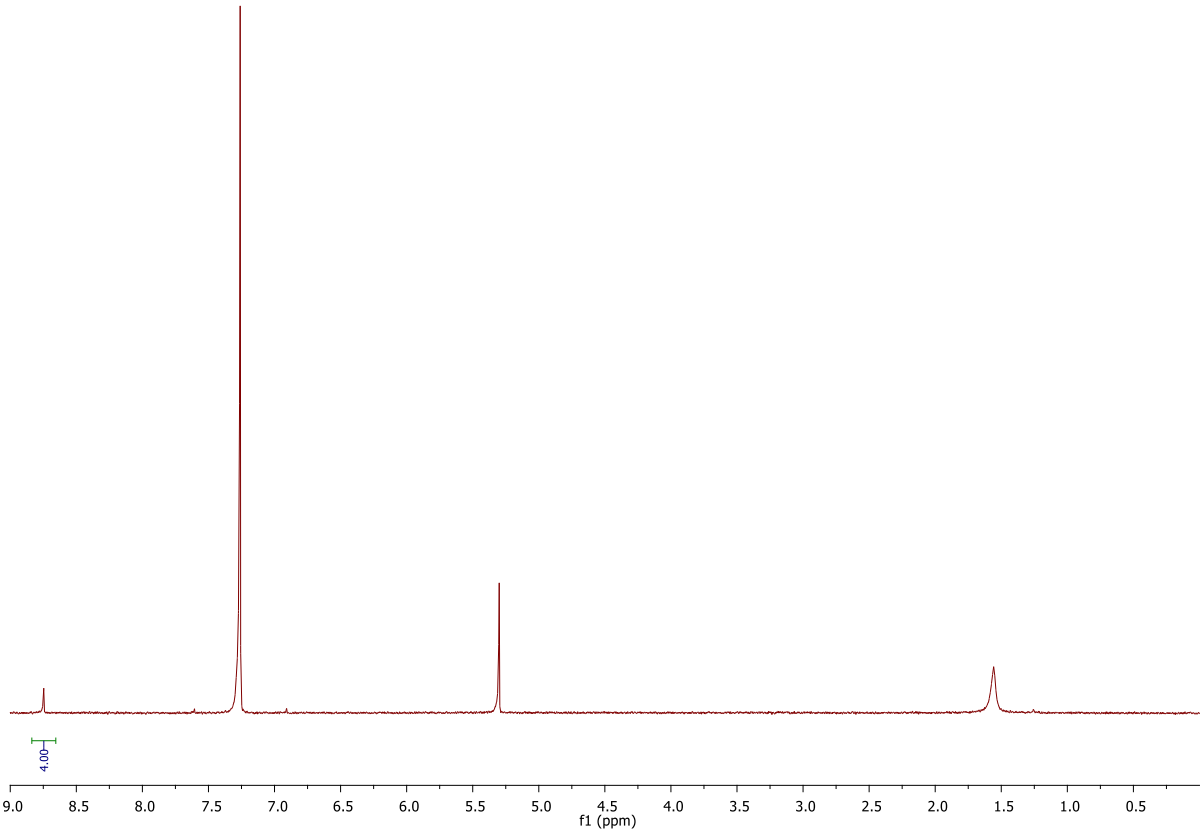
Nia, A. S. *et al. Tetrahedron* **2012**, *68*, 722-729.

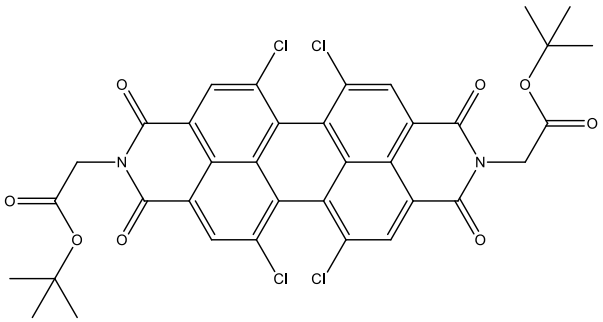
1,6,7,12-Tetrachloroperylene-3,4,9,10-tetracarboxylic dianhydride (4)

Iodine (1.62 g, 6.4 mmol, 0.25 eq.) was added to a solution of 3,4,9,10-perylene tetracarboxylic dianhydride **3** (10 g, 25.5 mmol, 1 eq.) in chlorosulfonic acid (100 mL) at room temperature. The reacting mixture was heated to 70°C for 5 h. Afterwards, the mixture was poured slowly into an ice-water mixture (1 L). The insoluble product was collected by filtration and washed with water until pH reached 7. The crude product was dried at 100°C in oven for 3 days and purified by soxhlet extraction using dichloromethane as solvent. The solvent was evaporated off to afford **4** as a red solid.

Yield = 51% (6.96 g).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.75 (s, 4H, perylene-H).





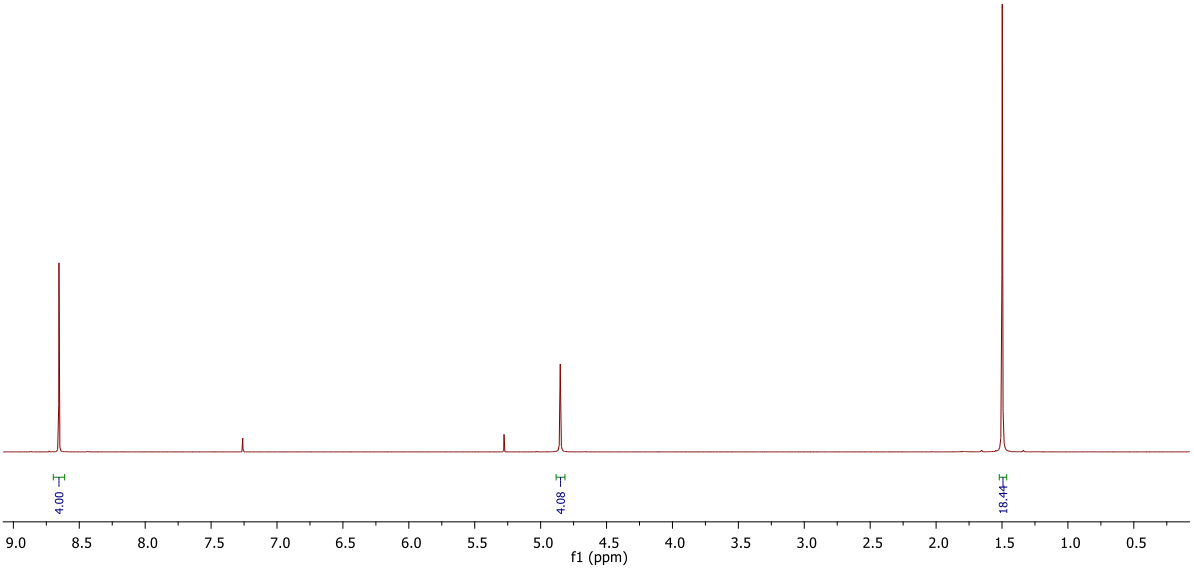
***N*,*N*'-Bis-(*t*-butoxycarbonylmethyl)-1,6,7,12-tetrachloroperylene-3,4,9,10-tetracarboxylic diimide (5)**: Triethylamine (188 µL, 1.36 mmol, 3.6 eq.) was added to a suspension of **4** (200 mg, 0.377 mmol, 1 eq.) and glycine *t*-butyl ester hydrochloride (190 mg, 1.13 mmol, 3 eq.) in isopropanol (10 mL). The mixture was stirred at 90°C for 22 h. The mixture was then poured into water (100 mL) and the red solid was filtered off then washed with water (2×10mL) until neutral pH. The red residue was collected with CH_2Cl_2 (50 mL) and the solvent was evaporated off. The crude solid was purified by silica gel flash chromatography (CH_2Cl_2/EtOAc 97/3) to afford **5** as a red solid.

Yield = 87% (247 mg); $\mathbf{R_f}$ = 0.30 (CH_2Cl_2/EtOAc 95/5).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.65 (s, 4H, perylene-H), 4.85 (s, 4H, NCH_2), 1.50 (s, 18H, CMe_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.6 (COO), 162.0 (NCO), 135.5 (CCl), 133.3 (CH-ar), 131.5 (C^{IV}-ar), 128.9 (C^{IV}-ar), 123.5 (C^{IV}-ar), 122.9 (C^{IV}-ar), 82.8 (*C*Me_3), 42.4 (NCH_2), 28.1 (C*Me*$_3$).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{36}H_{26}Cl_4N_2NaO_8$ $[M+Na]^+$ 777.0335, found 777.0320.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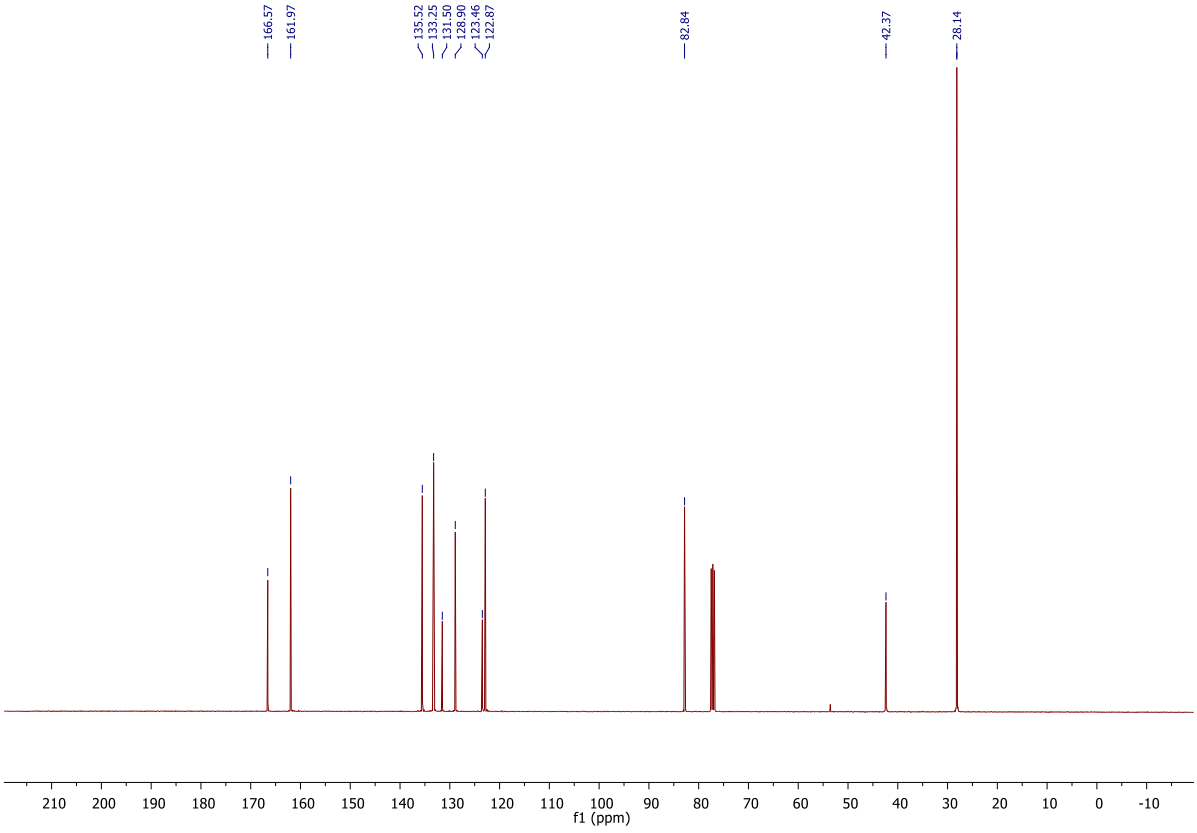
f1 (ppm)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128.90
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122.87
82.84
42.37
28.14
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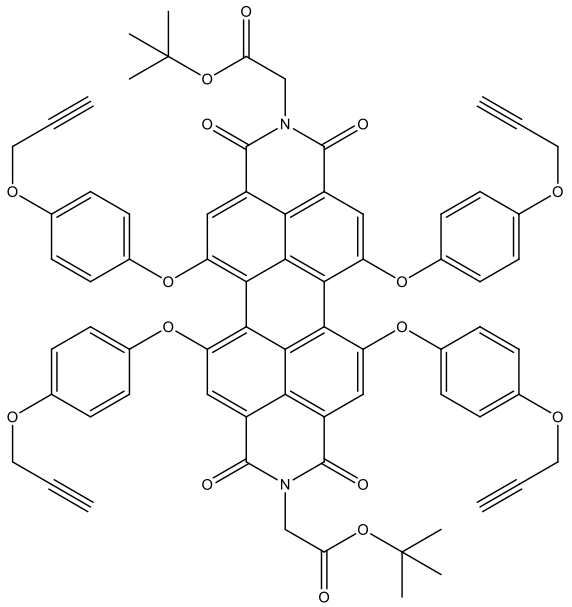
f1 (ppm)



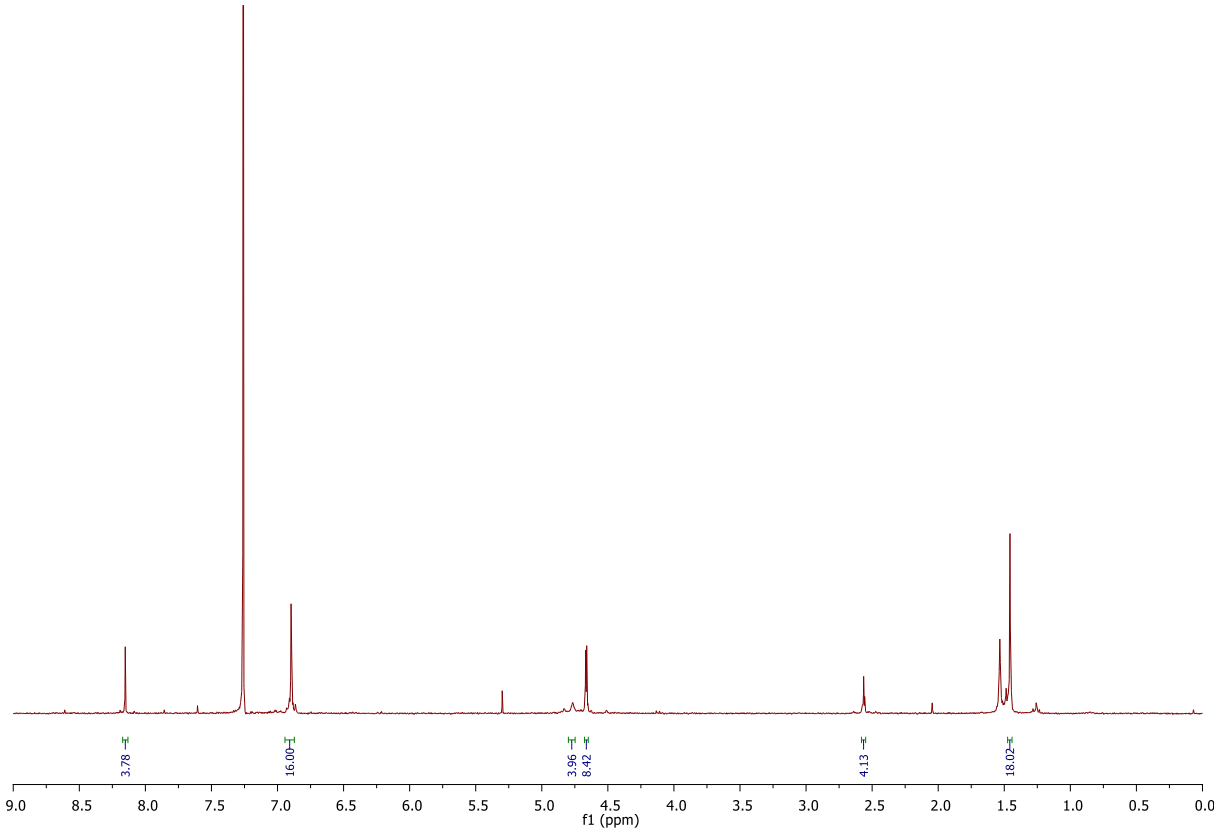
***N*,*N*'-Bis-(*t*-butoxycarbonylmethyl)-1,6,7,12-tetra-(4-propargyloxyphenoxy)perylene-3,4,9, 10-tetracarboxylic diimide (6)**: Potassium carbonate (239 mg, 1.73 mmol, 8 eq.) was added to a suspension of **5** (163 mg, 0.216 mmol, 1 eq.) and *p*-propargyloxyphenol (192 mg, 1.30 mmol, 6 eq.) in *N*-methyl-2-pyrrolidone (15 mL). The reaction was stirred at r.t. and turned immediately from dark red to black. After 30 min, the reaction mixture was stirred at 90°C for 15 h. The mixture was then poured into 1N HCl (150 mL) and the deep purple solid was filtered off then washed with water (2×20mL) until neutral pH. The deep purple residue was collected with CH_2Cl_2 (50 mL) then evaporated and purified by silica gel flash chromatography (CH_2Cl_2/EtOAc 97/3) to afford **6** as a deep purple solid.

Yield = 23% (61 mg); $\mathbf{R_f}$ = 0.70 (CH_2Cl_2/EtOAc 99/1).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.13 (s, 4H, perylene-H), 6.88 (s, 16H, CH-ar), 4.75 (bs, 4H, NCH_2), 4.66 (d, J = 2.4 Hz, 8H, OCH_2), 2.57 (t, J = 2.4 Hz, 4H, C≡CH), 1.46 (s, 18H, CMe_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 167.1 (COO), 163.1 (NCO), 156.4, 154.6, 149.4 (3×C^{IV}-ar-O), 133.0, 122.1 (2×C^{IV}-ar), 121.3 (CH-ar), 120.4, 119.6 (2×C^{IV}-ar), 119.5 (CH-perylene), 116.5 (CH-ar), 82.5 (*C*Me_3), 78.5 (*C*≡CH), 75.9 (C≡*C*H), 56.5 (OCH_2), 42.2 (NCH_2), 28.2 (C*Me*$_3$).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{72}H_{54}N_2NaO_{16}$ $[M+Na]^+$ 1225.3366, found 1225.3354.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16.00
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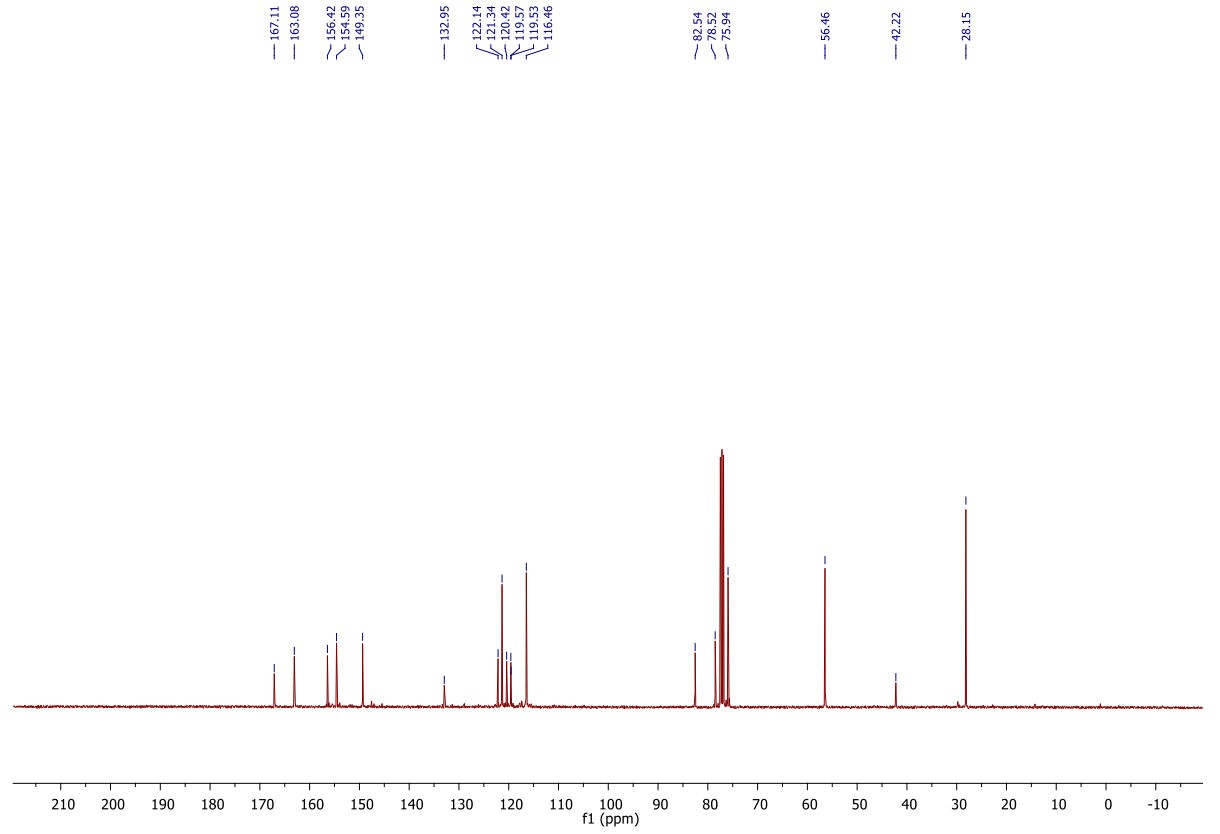
8.42
4.13
18.02
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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121.34
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119.57
119.53
116.46
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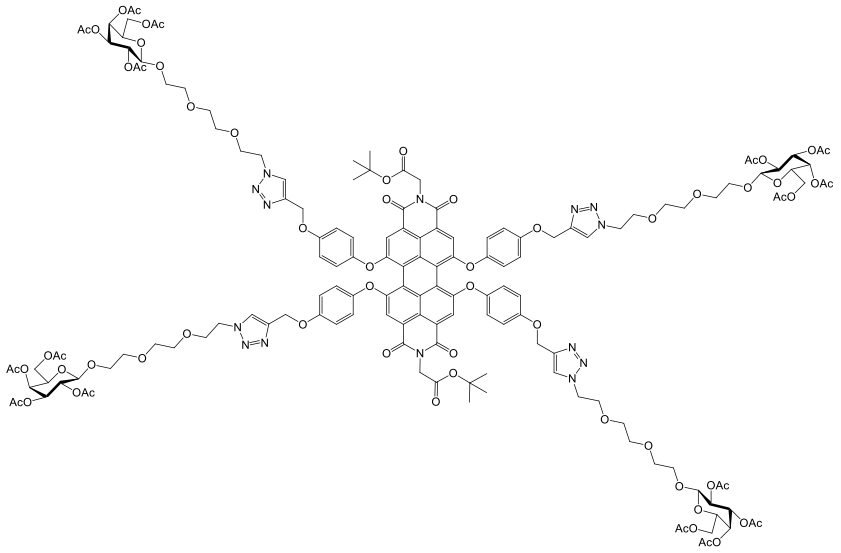
f1 (ppm)



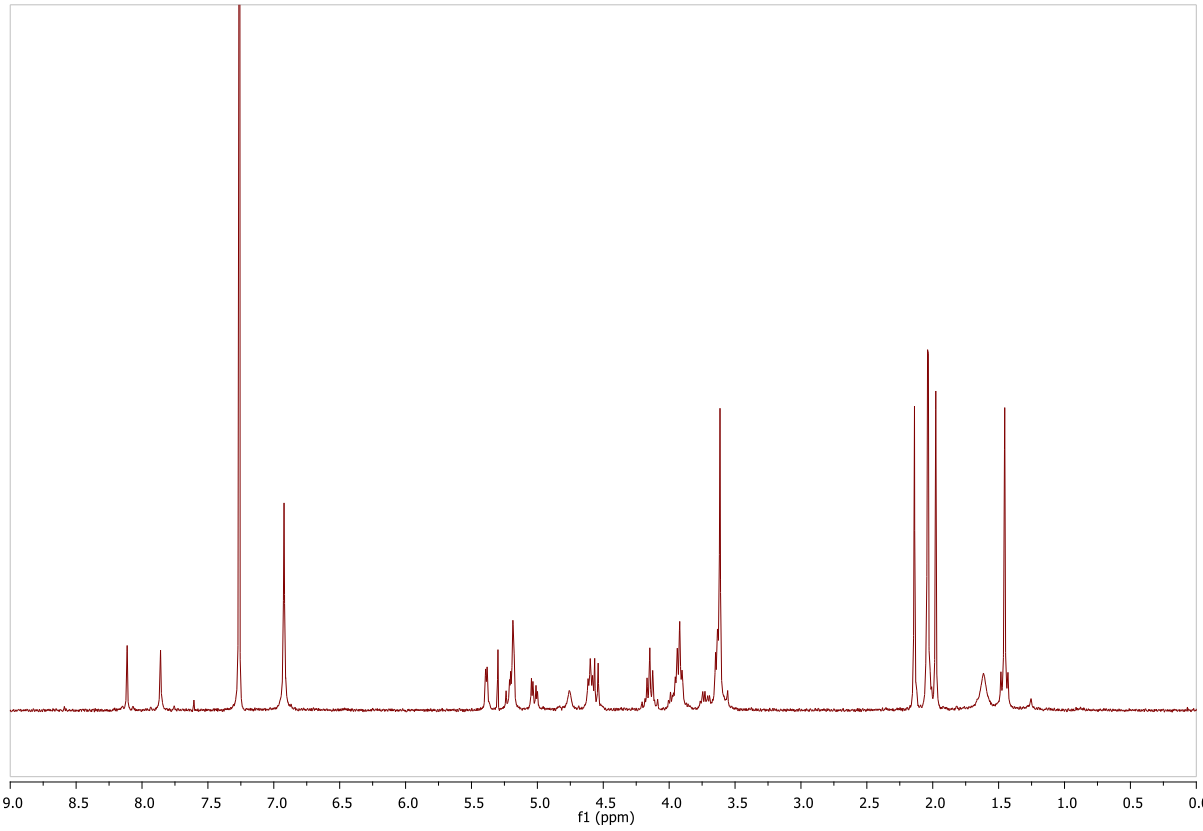
***N*,*N*'-Bis-(*t*-butoxycarbonylmethyl)-1,6,7,12-tetra-(4-{1-[1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethyloxy}phenoxy)perylene-3,4,9,10-tetracarboxylic diimide (7-Gal) :** Obtained as a purple foam following Method A: **6** (47 mg, 0.039 mmol, 1 eq.), **Ac_4Gal-TEG-N_3** (99 mg, 0.196 mmol, 5 eq.), CuI (4 mg, 0.020 mmol, 0.5 eq.) and DIPEA (34 µL, 0.196 mmol, 5 eq.). Purified by silica gel flash chromatography (CH_2Cl_2/MeOH 99/1 to 96/4).

Yield = 52% (66 mg), $\mathbf{R_f}$ = 0.45 (CH_2Cl_2/MeOH 95/5).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.11 (s, 4H, H-triaz), 7.86 (s, 4H, perylene-H), 6.92 (s, 16H, CH-ar), 5.39 (d, *J* = 3.4 Hz, 4H, H-4), 5.25-5.15 (m, 12H, H-2, OC*H*$_2$-triaz), 5.02 (dd, *J* = 10.4, 3.4 Hz, 4H, H-3), 4.76 (bs, 4H, NCH_2CO), 4.60 (t, *J* = 5.2 Hz, 8H, NCH_2CH_2), 4.55 (d, *J* = 7.9 Hz, 4H, H-1), 4.13 (m, 8H, H-6), 4.02-3.88 (m, 12H, H-5, OCH_2), 3.78-3.54 (m, 32H, 4×OCH_2), 2.14, 2.04, 2.03, 1.98 (4s, 48H, $COCH_3$), 1.46 (s, 18H, CMe_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 170.5, 170.3, 170.2, 169.5 (4×*C*OCH_3), 167.0 (COO), 163.1 (Ar*C*ON), 156.5, 155.5, 149.0 (3×C^{IV}-ar-O), 132.9 (C^{IV}-ar), 124.2 (C^{IV}-triaz), 122.1 (C^{IV}-ar), 121.5 (CH-ar), 120.3, 119.4 (2×C^{IV}-ar), 119.3 (CH-perylene), 116.2 (CH-ar), 101.4 (C-1), 82.4 (*C*Me_3), 70.9 (C-3), 70.7 (C-5, OCH_2), 70.6, 70.3, 69.5, 69.2 (4×OCH_2), 68.9 (C-2), 67.1 (C-4), 62.5 (O*C*H_2-triaz), 61.3 (C-6), 50.4 (N*C*H_2CH_2), 42.2 (N*C*H_2CO), 28.1 (C*Me*$_3$), 20.9, 20.8, 20.7 (4×CO*C*H_3).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{152}H_{180}N_{14}O_{64}$ $[M+2H]^{2+}$ 1612.5625, found 1612.5634.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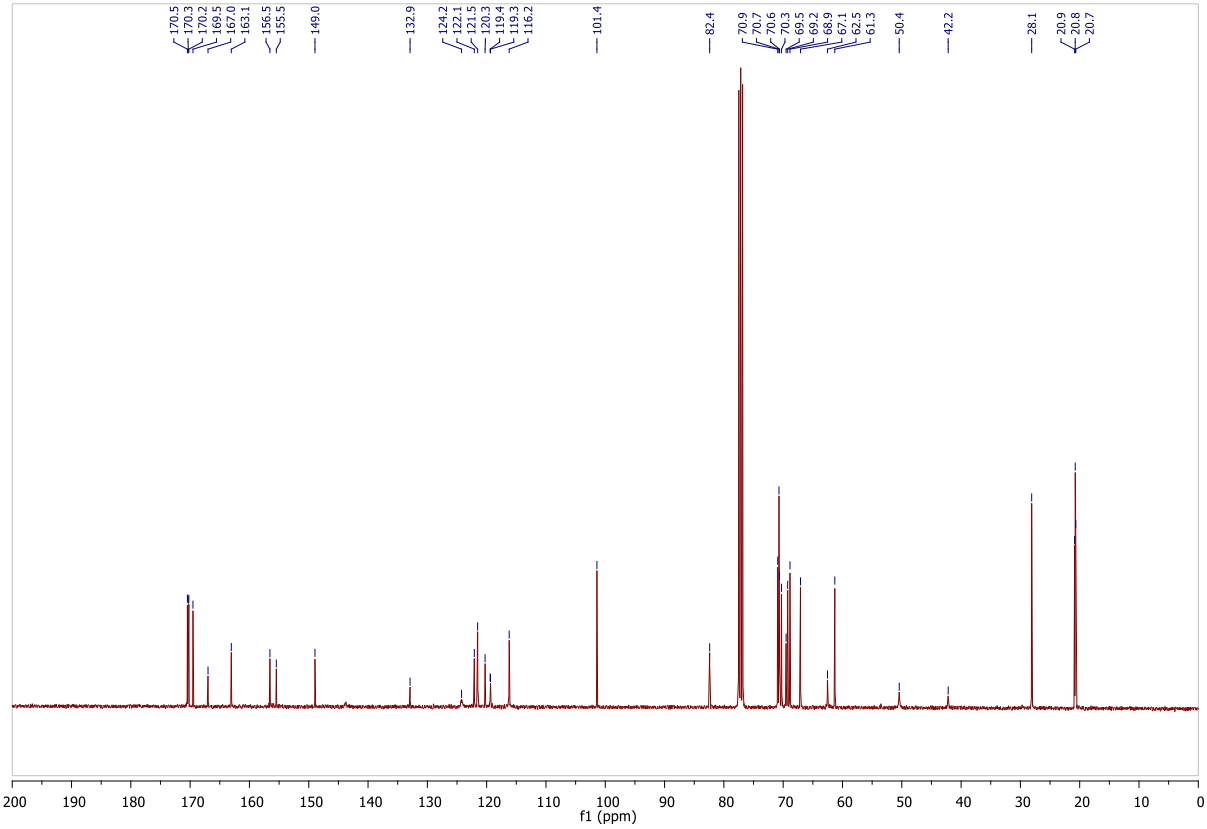
0.0
f1 (ppm)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120.3
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119.3
116.2
101.4
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70.9
70.7
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200
190
180
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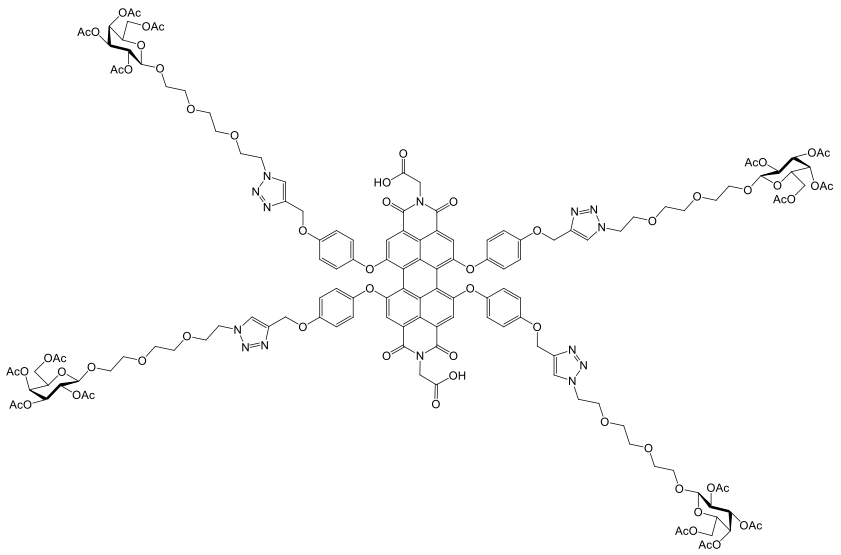
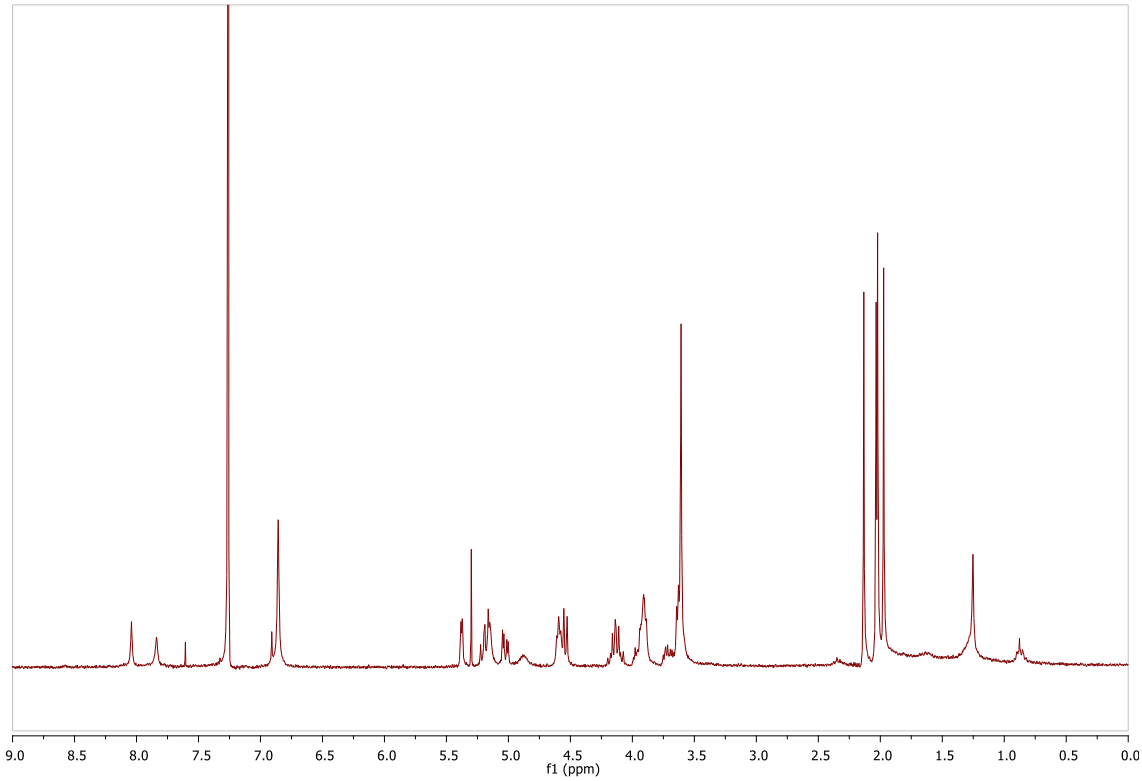


N*,*N*'-Bis-(carboxymethyl)-1,6,7,12-tetra-(4-{1-[1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethyloxy}phenoxy)perylene-3,4,9,10-tetracarboxylic diimide (7-Gal-CO_2H):** Obtained from **7-Gal** (47 mg, 0.039 mmol, 1 eq.) as a purple foam following ***Method C.

$\mathbf{R_f}$ = 0.30 (CH_2Cl_2/MeOH 95/5).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.04 (s, 4H, H-triaz), 7.84 (s, 4H, perylene-H), 6.86 (s, 16H, CH-ar), 5.38 (d, *J* = 3.2 Hz, 4H, H-4), 5.19 (dd, *J* = 10.5, 7.9 Hz, 4H, H-2), 5.15 (bs, 8H, OCH_2-triaz), 5.02 (dd, *J* = 10.4, 3.2 Hz, 4H, H-3), 4.93-4.83 (bs, 4H, NCH_2CO), 4.63-4.57 (m, 8H, NCH_2CH_2), 4.54 (d, *J* = 7.9 Hz, 4H, H-1), 4.14 (m, 8H, H-6), 4.00-3.84 (m, 12H, H-5, OCH_2), 3.77-3.54 (m, 32H, 4×OCH_2), 2.13, 2.03, 2.02, 1.97 (4s, 48H, $COCH_3$).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{144}H_{164}N_{14}O_{64}$ $[M+2H]^{2+}$ 1556.4999, found 1556.4916.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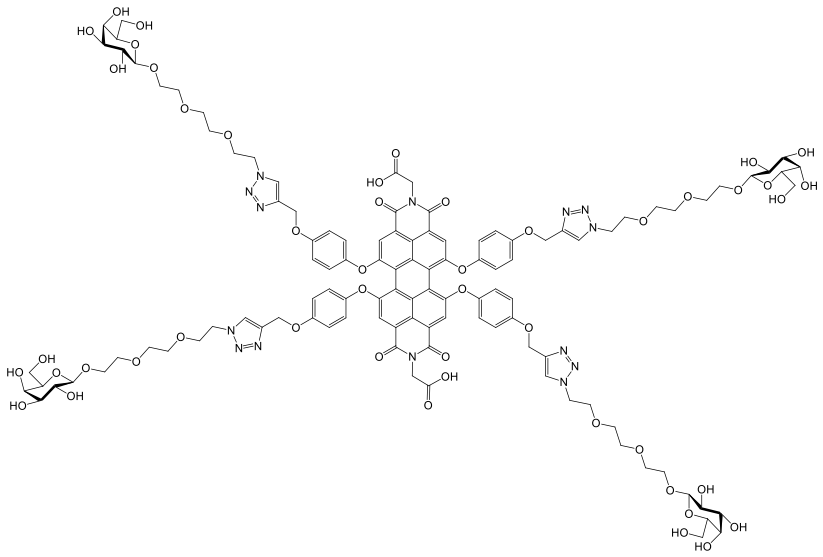
f1 (ppm)



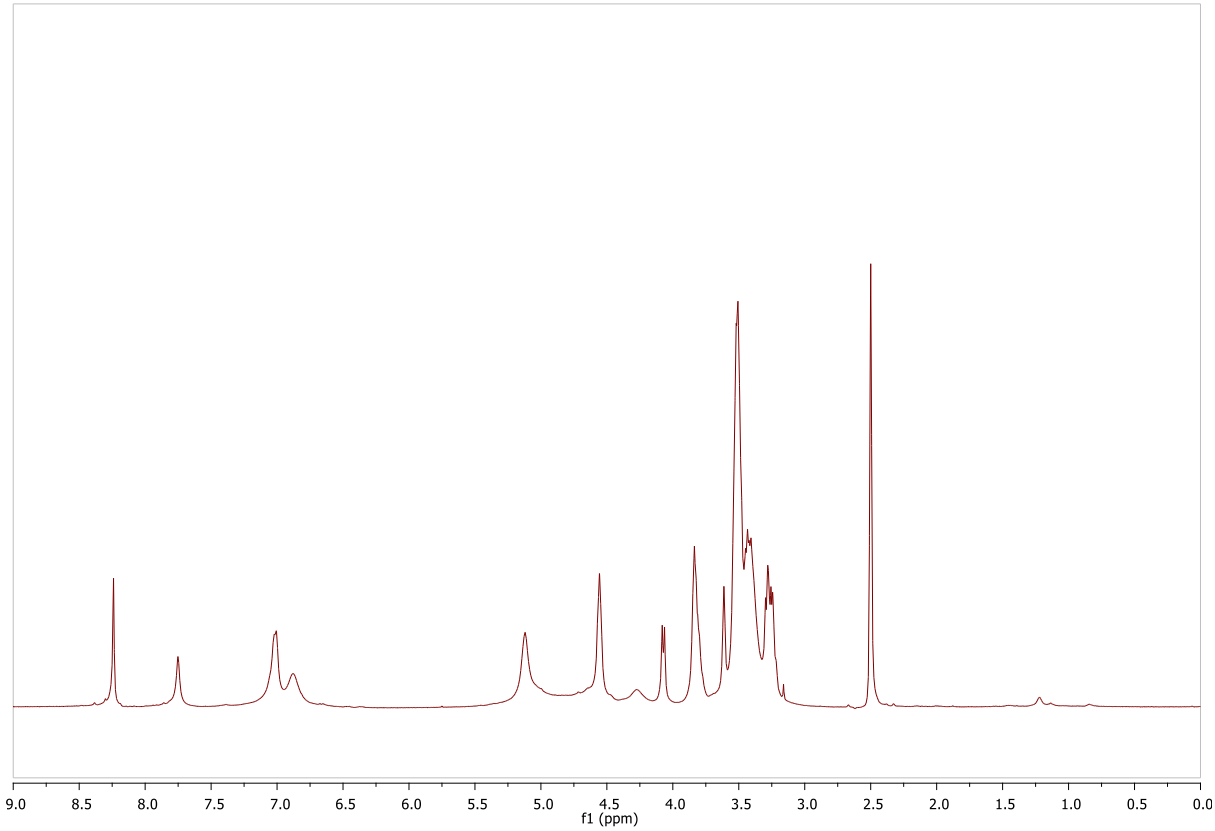
N*,*N*'-Bis-(carboxymethyl)-1,6,7,12-tetra-(4-{1-[1-(β-D-galactopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethyloxy}phenoxy)perylene-3,4,9,10-tetracarboxylic diimide (PDI-Gal$_4$):** Obtained from **7-Gal-CO$_2$H** (121 mg, 0.038 mmol) as a deep purple foam following ***Method B.

Yield = 90% (82 mg). After two steps

^{1}H NMR (400 MHz, DMSO-d$_6$ + βD$_2$O) δ (ppm): 8.24 (s, 4H, H-triaz), 7.75 (s, 4H, perylene-H), 7.01 (bs, 8H, CH-ar), 6.88 (bs, 8H, CH-ar), 5.12 (s, 12H, OC*H*$_2$-triaz, NC*H*$_2$-triaz), 4.56 (s, 12H, NC*H*$_2$CH$_2$), 4.07 (d, *J* = 7.0 Hz, 4H, H-1), 3.84 (bs, 12H, OCH$_2$), 3.61(s, 4H, H-4), 3.70-3.34 (m, 52H, OCH$_2$), 3.34-3.20 (m, 12H, H-2, H-3, H-5).

^{13}C NMR (100 MHz, DMSO-d$_6$ + βD$_2$O) δ (ppm): 170.0 (COO), 162.2 (Ar*C*ON), 155.9, 155.0, 148.5 (3×C^{IV}-ar-O), 142.5 (C^{IV}-triaz), 132.2 (C^{IV}-ar), 125.16 (CH-triaz), 122.29 (C^{IV}-ar), 121.3 (CH-ar), 118.9, 118.4 (2×C^{IV}-ar), 117.9 (CH-perylene), 116.1 (CH-ar), 103.6 (C-1), 75.2 (C-3), 73.49 (C-2), 70.53 (C-5), 69.8, 69.7, 69.6, 68.7 (4×OCH$_2$), 68.0 (C-4), 67.74 (OCH$_2$), 61.5 (O*C*H$_2$-triaz), 60.3 (C-6), 49.5 (N*C*H$_2$CH$_2$), 43.9 (N*C*H$_2$CO).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for C$_{112}$H$_{132}$N$_{14}$O$_{48}$ [M+2H]$^{2+}$ 1220.4154, found 1220.4117.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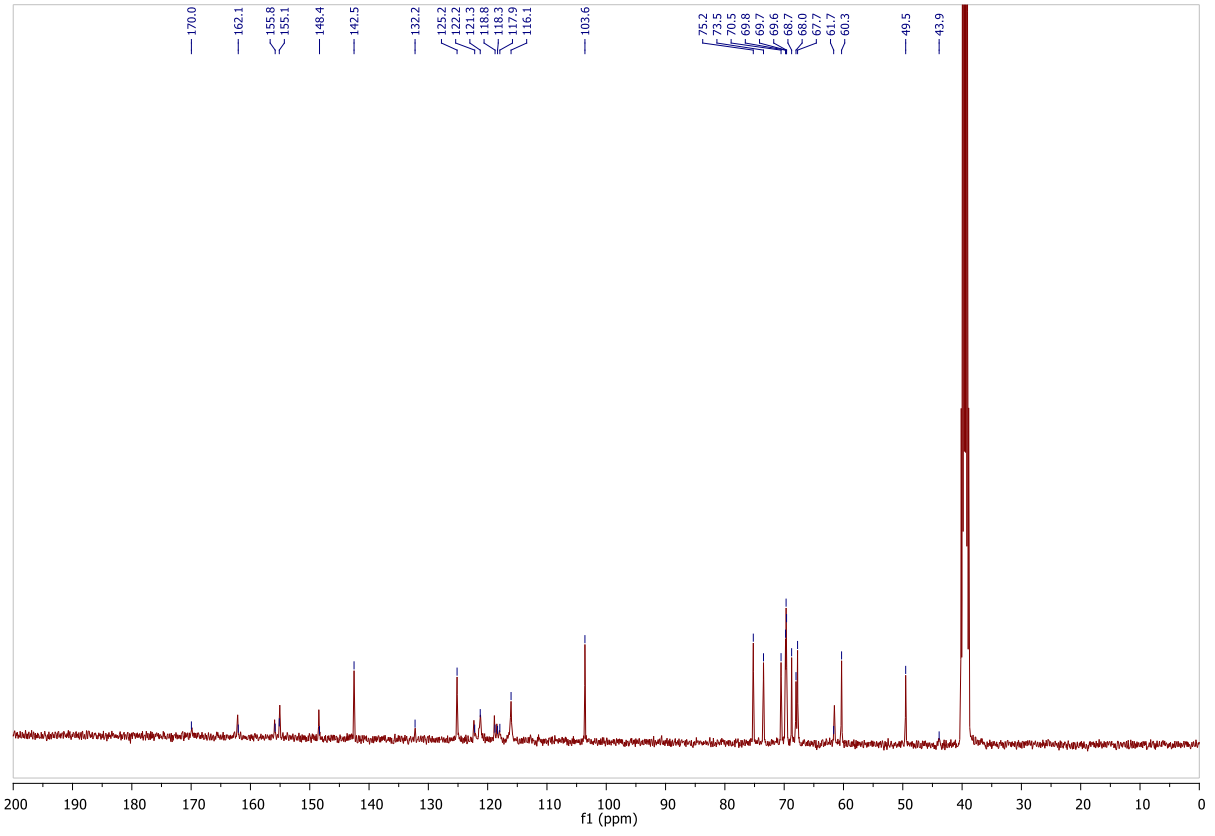
f1 (ppm)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116.1
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75.2
73.5
70.5
69.8
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69.6
68.7
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61.7
60.3
49.5
43.9
200
190
180
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160
150
140
130
120
110
100
90
80
70
60
50
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30
20
10
0
f1 (ppm)