Supporting Information

Modular Synthesis of Self-Assembling Janus-Dendrimers and Facile Preparation of Drug-Loaded Dendrimersomes

Sami Nummelin\textsuperscript{a}, Markus Selin\textsuperscript{b}, Sacha Legrand\textsuperscript{c}, Jarmo Ropponen\textsuperscript{c}, Jani Seitsonen\textsuperscript{d}, Antti Nykänen\textsuperscript{d}, Jari Koivisto\textsuperscript{e}, Jouni Hirvonen\textsuperscript{b}, Mauri A. Kostiainen\textsuperscript{a}, Luis M. Bimbo\textsuperscript{b,f}

\textsuperscript{a}Biohybrid Materials, Department of Bioproducts and Biosystems, Aalto University, FI-00076, Finland
\textsuperscript{b}Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, FI-00014, Finland
\textsuperscript{c}VTT-Technical Research Centre of Finland Ltd, P.O. Box 1000, FI-02044 VTT, Finland
\textsuperscript{d}Molecular Materials, Department of Applied Physics, Aalto University, FI-00076, Finland
\textsuperscript{e}Department of Chemistry and Materials Science, Aalto University, FI-00076, Finland
\textsuperscript{f}Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow, G4 0RE, United Kingdom
\textsuperscript{‡}Current address: Optitune Oy, Kaitoväylä 1, 90590 Oulu, Finland

Contents

S1. Instrumentation and Techniques ..................................................................................2
S2. Synthesis and Characterization of Janus-Dendrimers ...............................................4
  S.2.2. General procedure for the preparation of G1-azides................................................5
S3. \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectra ................................................................13
S4. Size Exclusion Chromatograms (SEC) .......................................................................19
S5. Dynamic Light Scattering (DLS) Measurements ..........................................................19
  S5.1 Z-average size as a function of concentration ..........................................................19
  S5.2. Z-average size after dilution with ultrapure water ..................................................37
  S5.3. Stability over time in ultrapure water at ambient temperature .................................38
S6. Zeta Potential of Assemblies Injected from Ethanol ......................................................39
S7. Additional cryo-TEM Images .....................................................................................39
S8. Preparation of Drug-Loaded Dendrimersomes .............................................................41
S9. References ..................................................................................................................41
S1. Instrumentation and Techniques

**Instrumentation**

**NMR:** $^1$H NMR (500 MHz) spectra and proton-decoupled $^{13}$C NMR (125 MHz) spectra were recorded on a 500 MHz Bruker UltraShield Plus spectrometer at 25°C. Additional measurements were recorded on a Bruker Avance DPX400 spectrometer equipped with a 5 mm BBFO probehead. Chemical shifts (δ) are reported in ppm. Residual protic solvent of CDCl$_3$ ($^1$H, δ 7.26 ppm; $^{13}$C, δ 77.0 ppm) was used as the internal reference. Coupling constants (J) are reported in Hertz (Hz). Hydrogen multiplicity (CH, CH$_2$, CH$_3$) was obtained from DEPT 135 experiments. Heteronuclear $^1$H-$^{13}$C connectivity was determined by using HSQC experiments.

**Melting points:** were measured on Stuart SMP30 melting point apparatus for compounds 5a-e and are uncorrected. Melting points for all Janus-dendrimers were taken from differential scanning calorimeter (DSC) measurements (TA Instruments Q100). The isotropization temperatures were determined as the maxima of their endothermic peak from the first heating cycle using 10 °C/min heating rate. Indium was used as calibration standard.

**Mass spectrometry:** HRMS spectra were recorded on Waters Micromass LCT Premier (ESI/TOF-MS) mass spectrometer.

**Size exclusion chromatography (SEC):** Molecular weights were determined with size exclusion chromatography (SEC) at 30 °C. The system was equipped with PSS GRAM 100 Å and PSS GRAM 3000 Å columns and Waters 2414 Refractive Index Detector. THF was used as an eluent and was delivered at a flow rate of 0.6 mL min$^{-1}$. SEC results were calibrated against polystyrene standards.

**Elemental Analysis (EA):** analyses were purchased from Microanalytisches Labor Pascher (Remagen-Bandorf, Germany; [http://www.mikrolabor.info](http://www.mikrolabor.info)).

**Techniques**

**Purity:** the purity of the products was determined by a thin-layer chromatography (TLC) on silica gel coated aluminium plates (Merck silica gel 60 F 254 (230-400 mesh, aluminium) using potassium permanganate (KMnO$_4$) stains to visualize the plates and size exclusion chromatography (SEC) in 1 mg mL$^{-1}$ concentration using THF as an eluent with flow rate 0.6 mL min$^{-1}$. Flash chromatography was performed on Merck Silica Gel 60 silica gel using CH$_2$Cl$_2$/MeOH mixtures as eluent. The residual copper was removed by dissolving the product in CH$_2$Cl$_2$ and washing with 1 M EDTA (3×).
**Preparation of dendrimersomes:** Dendrimersomes were fabricated via injection from organic solvents as reported previously.\(^1\) A 10 mg mL\(^{-1}\) stock solutions in absolute EtOH and 5 mg mL\(^{-1}\) stock solutions in THF and acetone of each Janus dendrimer was prepared, respectively. 100 µL of a stock solution was injected quickly into 1.9 mL of Milli-Q\(^\circledR\) followed by 5 sec. of vortex mixing to obtain a final concentration of 0.5 mg mL\(^{-1}\) (0.9 mL H\(_2\)O for THF or acetone). Different final concentration of dendrimersomes ranging from 0.1-4.0 mg mL\(^{-1}\) (ethanol injection) and 0.1-1.0 mg mL\(^{-1}\) (THF and acetone injection) in Milli-Q\(^\circledR\) was prepared. For dilution experiments stock solutions of 10, 5, 2.5 and 1.25 mg mL\(^{-1}\), in absolute EtOH were prepared, respectively. 100 µL of each stock solution was injected quickly into 0.9 mL of Milli-Q\(^\circledR\) followed by vortex mixing to obtain a final concentrations of 1.0, 0.5, 0.25 and 0.125 mg mL\(^{-1}\), respectively. Each sample were then diluted stepwise to the above mentioned concentrations to obtain 0.125 mg mL\(^{-1}\) plateau. No purifications or further manipulation was applied. The Foetal Bovine Serum (FBS) stability studies were done as follows: 10 µL of dendrimer solutions (c = 30 mg mL\(^{-1}\)) in ethanol were injected into 90 µL Milli-Q\(^\circledR\) in plastic cuvettes resulting in dendrimersomes that were diluted to a final concentration of 300 µg mL\(^{-1}\) by addition of 900 µL of FBS. Samples were capped and stored at 37 °C for seven days. For each dendrimer, a control was prepared as described above. All samples and controls were measured daily in triplicate by DLS. The Z-average size and changes in PDI were calculated from the analysis results and plotted using GraphPad Prism v. 6.07 (GraphPad Software Inc., USA).

**Cryo-TEM:** samples were freshly prepared from a 5 mg mL\(^{-1}\) stock solution in ethanol and injected into Milli-Q\(^\circledR\) in 0.5 mg mL\(^{-1}\) final concentration as described previously. Vitrification was done with Vitrobot in a saturated water vapor environment (FEI Vitrobot Mark IV, USA). TEM-grids were cleaned using Gatan Solarus Model 950 plasma cleaner (Gatan, Inc.; USA) prior use. Sample volumes of 3 µL were placed on Quantifoil R 3.5/grids and the excess sample was blotted away with filter paper. Blot time and drain time were both 0.5 s. Alternatively, Quantifoil C-flat 2/1 grids, blot time 1,5 s and drain time 5 s was used. After blotting the grids were plunged into liquid ethane/propane mixture which was cooled with liquid nitrogen surrounding the ethane/propane vessel.

**Confocal microscopy:** samples were prepared by mixing 60 µL of a 10 mg mL\(^{-1}\) solution of the Janus-dendrimers in absolute ethanol, with 2 µL of a Nile red (NR) solution (1 mg mL\(^{-1}\)) in absolute ethanol into a flat-bottom vial. Ethanol was subsequently evaporated under mild nitrogen flow and then overnight under vacuum to form a film. The film was hydrated by adding 1 mL of pre-heated (65 °C) Milli-Q\(^\circledR\) and then kept in an oven at 65 °C overnight. The
samples were then placed in the middle of a Vaseline circle on a glass slide and covered using a glass cover slip.

**Compound encapsulation and release:** Firstly, NR was dissolved in pure ethanol for a final concentration of 1 mg mL\(^{-1}\) (3.141 mM). Then 2 µL of the resulting solution was added to 98 µL of 3,4,5-G1 (11c) dendrimer solution in EtOH (c =10 mg mL\(^{-1}\)). After vortex the mixture was injected into 1900 µL of Milli-Q® (final c = 490 µg mL\(^{-1}\) (11c) and 3.141 µM NR in 5% (v/v) ethanol). For a control sample, NR was injected into Milli-Q® (alone or in 98 µL of pure ethanol) having the same ratio as with dendrimers. Identical preparation method was applied for 3,5-G2 sample (12b). The Z-average size of NR-loaded dendrimersomes was measured by DLS. The fluorescence was measured for NR-loaded dendrimersomes, Nile red in Milli-Q®, and pure dendrimersomes at an excitation wavelength of 552 nm and emission wavelengths between 552 nm and 700 nm (step size 2 nm). Spectra were smoothened across 5 data points. After the scan, 500 µL of 3% (w/w) Triton X-100 was added to the dendrimersome solution to disrupt the assemblies and the fluorescence scan was repeated. For the drug encapsulation, propranolol hydrochloride (5 g) was dissolved in 100 mL Milli-Q® and made alkaline by gradually adding 5 M sodium hydroxide. The resulting precipitate was filtered, washed with water (5×) on Whatman filter paper No.1 and dried in vacuum oven overnight. The purity of propranolol was verified by TLC, \(^{1}\)H-NMR and \(^{13}\)C-NMR (data not shown). For the encapsulation of propranolol, 49 µL of an ethanolic solution of 3,4,5-G1 (11c) or 3,5-G2 (12b) dendrimer (10 mg mL\(^{-1}\)) was mixed to 49 µL of neat ethanol (99.5%) and 2 µL of an ethanolic propranolol solution (12.5 mM) with vortex. The resulting 100 µL mixture was then injected into 900 µL of Milli-Q® (for a final concentration of 490 µg mL\(^{-1}\) of Janus-dendrimer and 25 µM of propranolol). The Z-average size of the assemblies obtained was measured by DLS and the propranolol fluorescence in the dendrimersomes and in an ethanol-Milli-Q® solution with the same ratio of solvents was measured at excitation wavelength of 296 nm and emission wavelength of 332 nm for 15 mins at 25 s intervals. Then, 50 µL of 3% aqueous Triton X-100 solution was added to the propranolol-loaded dendrimersome solution and the propranol in Milli-Q® solution. The size was measured once and fluorescence was measured for further 15 min at 25 s intervals.

**S2. Synthesis and Characterization of Janus-Dendrimers**

Propargyl modified bis-MPA dendrons 8 and 10 (G1 and G2 hydrophilic blocks),\(^2\) and Percec-type constitutionally isomeric AB\(_2\) 4a,b and AB\(_3\) benzyl chlorides 4c (G1 hydrophobic blocks)\(^3\) were prepared by using literature procedures. Corresponding azides 5a-c were prepared by using a modified literature procedure.\(^1\) Herein, one should be cautious when
working with azides and their derivatives, since they can form toxic gases and explosive compounds.\(^4\) Due to this, a rule which expresses the safety ratio between the total amount of carbon and oxygen divided by the total amount of nitrogen which should not be less than three \((N_C + N_O)/N_N \geq 3\) \((N=\text{number of atoms})\).\(^5\) In our work the azide-dendrons had \(N\) values of ca. 10 for 5a,b and 15 for 5c, which should make them safe to handle.

**Supporting Scheme S1. Synthesis of the First and Second Generation Janus-dendrimers**

![Supporting Scheme S1](image)

S.2.2. General procedure for the preparation of G1-azides

To a solution of benzyl chloride 4a-c (1.0 eq.) in DMF was added NaN\(_3\) (3.0 eq.). The mixture was stirred at 80 °C for 6-8 h after which TLC indicated complete conversion. The reaction mixture was cooled down while stirring and finally poured into cold water. The resulting precipitate was filtered, washed with water and dried in vacuum. Recrystallization from acetone gave the G1-azides 5a-c as white solids.

3,4-Bis(dodecyl-1-oxy)benzylazide (5a): Starting from the corresponding benzyl chloride (5.00 g, 10.10 mmol) and NaN\(_3\) (1.97 g, 33.32 mmol) in 70 mL DMF, the title compound 5a was isolated as a white solid. Yield 4.97 g (98%).
M.p.: 58.0–59.0 °C; TLC (5:1 hexane/EtOAc) $R_f = 0.83$.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta = 0.88$ (t, $J = 6.9$ Hz, 6H, CH$_3$), 1.26 (m, 32H, CH$_3$(CH$_2$)$_8$), 1.47 (m, 4H, CH$_3$(CH$_2$)$_2$OAr), 1.82 (m, 4H, CH$_2$CH$_2$OAr), 3.99 and 4.00 (2x t, $J = 6.6$ Hz, 4H, CH$_2$OAr, 3,4 positions), 4.24 (s, 2H, CH$_2$N$_3$), 6.85 (m, 3H, ArH).

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta = 14.10$ (CH$_3$), 22.68 (CH$_3$CH$_2$), 26.02 (CH$_2$(CH$_2$)$_2$OAr), 29.26, 29.36, 29.41, 29.62 (x2), 29.65, 29.69, (CH$_2$CH$_2$OAr and CH$_3$CH$_2$CH$_2$(CH$_2$)$_6$), 31.91 (CH$_3$CH$_2$CH$_2$), 54.78 (CH$_2$N$_3$), 69.28 and 69.30 (CH$_2$OAr, 3,4 positions), 113.66 (ArCH, 5 position), 113.91 (ArCH, 2 position), 120.93 (ArCH, 6 position), 127.80 (ArC, 1 position), 149.26 and 149.34 (ArC, 3,4 positions).

TOF-ESI-ES$^+$: m/z calcd for C$_{31}$H$_{55}$N$_3$O$_2$ [M+Na]$^+$ 524.4192, found 524.4194.

Anal. calcd for C$_{31}$H$_{55}$N$_3$O$_2$: C, 74.20; H, 11.05; N, 8.37. Found: C, 74.38; H, 11.23; N, 8.35.

3,5-Bis(dodecyl-1-oxy)benzylazide (5b): Starting from the corresponding benzyl chloride (5.00 g, 10.10 mmol) and NaN$_3$ (1.97 g, 33.32 mmol) in 70 mL DMF, compound 5b was isolated as an off-white solid (4.36 g, 86%).

M.p.: 36.0–37.0 °C; (lit.$^6$ 35.0–37.0); TLC (5:1 hexane/EtOAc) $R_f = 0.85$.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta = 0.88$ (t, $J = 6.8$ Hz, 6H, CH$_3$), 1.27 (m, 32H, CH$_3$(CH$_2$)$_8$), 1.45 (m, 4H, CH$_2$(CH$_2$)$_2$OAr), 1.77 (m, 4H, CH$_2$CH$_2$OAr), 3.93 (t, $J = 6.6$ Hz, 4H, CH$_2$OAr, 3,5 positions), 4.24 (s, 2H, CH$_2$N$_3$), 6.41 (t, $J = 2.1$ Hz, 1H, ArH, 4 position), 6.44 (d, $J = 2.1$ Hz, 2H, ArH, 2,6 positions).

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta = 14.11$ (CH$_3$), 22.69 (CH$_3$CH$_2$), 26.04 (CH$_2$(CH$_2$)$_2$OAr), 29.22, 29.35, 29.38, 29.57, 29.60, 29.63, 29.66 (CH$_2$CH$_2$OAr and CH$_3$CH$_2$CH$_2$(CH$_2$)$_6$), 31.92 (CH$_3$CH$_2$CH$_2$), 54.94 (CH$_2$N$_3$), 68.12 (CH$_2$OAr, 3,5 positions), 101.05 (ArCH, 4 position), 106.45 (ArCH, 2,6 positions), 137.37 (ArC, 1 position), 160.62 (ArC, 3,5 positions).

TOF-ESI-ES$^+$: m/z calcd for C$_{31}$H$_{56}$N$_3$O$_2$ [M+H]$^+$ 502.4386, found 502.4384.

Anal. calcd for C$_{31}$H$_{56}$N$_3$O$_2$: C, 74.20; H, 11.05; N, 8.37. Found: C, 74.33; H, 11.24; N, 8.47.
3,4,5-Tris(dodecyl-1-oxy)benzylazide (5c): Starting from the corresponding benzyl chloride\(^3\) (5.00 g, 7.36 mmol) and NaN\(_3\) (1.43 g, 22.07 mmol) in 70 mL DMF, the title compound 5c was isolated as a white solid. Yield 4.90 g (97%).

M.p.: 57.0–58.0 °C; (lit.\(^7\) 58.0); TLC (5:1 hexane/EtOAc) \(R_f\) = 0.85.

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) = 0.88 (t, \(J = 6.8\) Hz, 9H, \(CH\_3\_CH2\)), 1.26 (m, 48H, \(CH\_3\_CH2\)), 1.47 (m, 6H, \(CH\_2\_CH\_2\_OAr\)), 1.71-1.84 (m, 6H, \(CH\_2\_CH\_2\_OAr\)), 3.94 (t, \(J = 6.6\) Hz, 2H, \(CH\_2\_OAr, 4\) position), 3.97 (t, \(J = 6.5\) Hz, 4H, \(CH\_2\_OAr, 3,5\) positions), 4.24 (s, 2H, \(CH\_2\_N3\)), 6.49 (s, 2H, Ar\(H\), 2,6 positions).

\(^1^3\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) = 14.10 (\(CH\_3\)), 22.69 (\(CH\_3\_CH2\)), 26.09 (\(CH\_2\_CH\_2\_OAr, 3,5\) positions), 26.12 (\(CH\_2\_CH\_2\_OAr, 4\) position), 29.36, 29.39, 29.41, 29.61, 29.64, 29.65, 29.69, 29.73, 29.75, 30.33, 30.90 (\(CH\_2\_CH\_2\_OAr\) and \(CH\_3\_CH\_2\_CH\_2\_CH\_2\)), 31.92 (\(CH\_3\_CH\_2\_CH\_2\_3,5\) positions), 31.94 (\(CH\_3\_CH\_2\_CH\_2\_4\) positions), 55.18 (\(CH\_2\_N3\)), 69.16 (\(CH\_2\_OAr, 3,5\) positions), 73.41 (\(CH\_2\_OAr, 4\) position), 106.62 (Ar\(CH\), 2,6 positions), 130.33 (Ar\(C\), 1 position), 138.15 (Ar\(C\), 4 position), 153.35 (Ar\(C\), 3,5 positions).

TOF-ESI-ES\(^+\): \(m/z\) calcd for C\(_{43}\)H\(_{79}\)N\(_3\)O\(_3\)Na [M+Na]\(^+\) 708.6019, found 708.6035.

Anal. calcd for C\(_{43}\)H\(_{79}\)N\(_3\)O\(_3\): C, 75.27; H, 11.61; N, 6.12. Found: C, 75.43; H, 11.83; N, 6.10.

S2.3. General procedure for the copper catalyzed click reaction

The azide 5a-c (1.0 eq.) was dissolved in THF (5-8 mL). The bis-MPA-alkyne 8 or 10 (1.1 eq.) and Na-ascorbate (20 mol\%) were added to the reaction mixture. Cu(II)SO\(_4\)·5H\(_2\)O (10 mol\%) was dissolved in minimal H\(_2\)O (0.2-1 mL) and added to the reaction flask. The reaction mixture was stirred 5 minutes at rt before DMSO (0.2 mL) was added. Temperature was raised to 50 °C and stirred 16 h. The reaction mixture was cooled to rt. Evaporation of the solvents gave G1- and G2-dendrimers as a brown solids, respectively. Crude products were purified by flash chromatography on SiO\(_2\) (19:1 CH\(_2\)Cl\(_2\)/MeOH) and dried in vacuum affording the 11a-c and 12a-c as a white solids. The residual copper can be removed by dissolving the product in CH\(_2\)Cl\(_2\) and washing with 1 M EDTA (3x). However, due to the increased solubility of the G2-dendrimers into water, the yields can drop substantially.
3,4-G1 (11a): starting from the azide 5a (1.00 g, 1.99 mmol), bis-MPA-alkyne 8 (0.38 g, 2.20 mmol), Na-ascorbate (79 mg, 0.40 mmol), and Cu(II)SO₄·5H₂O (49 mg, 0.20 mmol), the title compound 11a was obtained as a white solid. Yield 1.25 g (94 %).
M.p.: 70.5–71.5 °C; TLC (19:1 CH₂Cl₂/MeOH) Rᵣ = 0.44.

1H NMR (500 MHz, CDCl₃) δ 0.87 (t, J = 6.9 Hz, 6H, C₃H₃CH₂), 1.06 (s, 3H, G1-C₃H₃), 1.25-1.33 (m, 32H, CH₃(CH₂)₈), 1.44 (m, 4H, CH₂(CH₂)₂OAr), 1.78 (m, 4H, CH₂CH₂OAr), 3.15 (bs, 2H, OH), 3.73 (d, J = 11.4 Hz, 2H, G1-C₃H₂OH), 3.86 (d, J = 11.4 Hz, 2H, G1-C₃H₂OH), 3.92 and 3.97 (2x t, J = 6.6 Hz, 4H, C₆H₂OAr, 3,4 positions), 5.29 (s, 2H, NCC原子), 5.41 (s, 2H, ArCH), 6.78-6.85 (m, 3H, ArH), 7.47 (s, 1H, NC原子).

13C NMR (125 MHz, CDCl₃) δ 14.10 (C₃H₃CH₂), 16.98 (G1-C₃H₃), 22.67 (CH₃CH₂), 25.99 (CH₂(CH₂)₂OAr), 29.18, 29.22, 29.35, 29.40, 29.61 (x3), 29.67, (CH₂CH₂OAr and CH₃CH₂CH₂(CH₂)₉), 31.90 (CH₃CH₂CH₂), 49.57 (G1-C), 54.23 (ArCH₂), 57.86 (1C, NCCH₂), 68.02 (G1-C₂OH), 69.22 and 69.36 (CH₂OAr, 3,4 positions), 113.61 and 113.66 (ArCH, 2,5 positions), 121.00 (ArCH, 6 position), 122.52 (NCHC), 126.45 (ArC, 1 position), 142.98 (CHC), 149.56 and 149.68 (ArC, 3,4 positions), 175.57 (G1-CO₂).

3,5-G1 (11b): starting from the azide 5b (1.00 g, 1.99 mmol), bis-MPA-alkyne 8 (0.38 g, 2.20 mmol), Na-ascorbate (79 mg, 0.40 mmol), and Cu(II)SO₄·5H₂O (49 mg, 0.20 mmol), the title compound 11b was obtained as a white solid. Yield 1.20 g (95 %).
M.p.: 64.5–65.5 °C; TLC (19:1 CH₂Cl₂/MeOH) Rᵣ = 0.36.
$^1$H NMR (500 MHz, CDCl$_3$) δ 0.88 (t, $J$ = 6.9 Hz, 6H, CH$_3$CH$_2$), 1.07 (s, 3H, G1-CH$_3$), 1.25-1.34 (m, 32H, CH$_3$(CH$_2$)$_3$), 1.42 (m, 4H, CH$_2$(CH$_2$)$_2$OAr), 1.74 (m, 4H, CH$_2$CH$_2$OAr), 3.09 (bs, 2H, OH), 3.73 (d, $J$ = 11.3 Hz, 2H, G1-CH$_2$OH), 3.85-3.89 (m, 6H, CH$_2$OAr, 3,5 positions and G1-CH$_2$OH), 5.31 (s, 2H, NCCH$_2$), 5.40 (s, 2H, ArCH$_2$), 6.35 (d, $J$ = 2.0 Hz, 2H, ArH, 2,6 position), 6.41 (t, $J$ = 1.9 Hz, 1H, ArH, 4 position), 7.53 (s, 1H, NCH).

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 14.11 (CH$_3$), 16.97 (G1-CH$_3$), 22.67 (CH$_3$CH$_2$), 26.00 (CH$_2$(CH$_2$)$_2$OAr), 29.16, 29.33, 29.37, 29.55, 29.58, 29.61, 29.64, (CH$_2$CH$_2$OAr and CH$_3$CH$_2$CH$_2$(CH$_2$)$_6$), 31.90 (CH$_3$CH$_2$CH$_2$), 49.57 (G1-C), 54.38 (ArCH$_2$), 57.87 (NCCH$_2$), 68.14 (G1-CH$_2$OH), 68.17 (CH$_2$OAr, 3,5 positions), 101.21 (ArCH, 4 position), 106.50 (ArCH, 2,6 positions), 122.79 (NCHC), 136.13 (ArC, 1 position), 143.07 (CHC), 160.83 (ArC, 3,5 positions), 175.59 (G1-CO$_2$).

TOF-ESI-ES$: m/z$ calcd for C$_{39}$H$_{67}$N$_3$O$_6$Na [M+Na]$^+$ 696.4928, found 696.4959.

Anal. calcd for C$_{39}$H$_{67}$N$_3$O$_6$: C, 69.50; H, 10.02; N, 6.23. Found: C, 69.70; H, 10.22; N, 6.30.

3,4,5-G1 (11c): starting from the azide 5e (1.00 g, 1.46 mmol), bis-MPA-alkyne 8 (0.28 g, 1.63 mmol), Na-ascorbate (57 mg, 0.29 mmol), and Cu(II)SO$_4$·5H$_2$O (36 mg, 0.14 mmol), the title compound 11c was obtained as a white solid. Yield 1.20 g (97 %).

M.p.: 73.0–74.0 °C; TLC (19:1 CH$_2$Cl$_2$/MeOH) $R_f$ = 0.44.

$^1$H NMR (500 MHz, CDCl$_3$) δ 0.89 (t, $J$ = 6.9 Hz, 9H, CH$_3$CH$_2$), 1.07 (s, 3H, G1-CH$_3$), 1.26-1.31 (m, 48H, CH$_3$(CH$_2$)$_3$), 1.45 (m, 6H, CH$_2$(CH$_2$)$_2$OAr), 1.69-1.80 (m, 6H, CH$_2$CH$_2$OAr), 3.05 (bs, 2H, OH), 3.74 (d, $J$ = 11.2 Hz, 2H, G1-CH$_2$OH), 3.87 (d, $J$ = 11.3 Hz, 2H, G1-CH$_2$OH), 3.91 (t, 4H, $J$ = 6.5 Hz, CH$_2$OAr, 3,5 positions), 3.93 (t, $J$ = 6.9 Hz, 2H, CH$_2$OAr, 4 position), 5.32 (s, 2H, NCCH$_2$), 5.39 (s, 2H, ArCH$_2$), 6.45 (s, 2H, ArH, 2,6 positions), 7.50 (s, 1H, NCH).

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 14.12 (CH$_3$CH$_2$), 16.97 (G1-CH$_3$), 22.69 (CH$_3$CH$_2$), 26.07 (CH$_2$(CH$_2$)$_2$OAr), 26.10 (CH$_2$(CH$_2$)$_2$OAr), 29.35, 29.36, 29.38, 29.41, 29.59, 29.63, 29.66, 29.69, 29.70, 29.73, 29.75, 30.31 (CH$_2$CH$_2$OAr and CH$_3$CH$_2$CH$_2$(CH$_2$)$_6$), 31.92 (CH$_3$CH$_2$CH$_2$, 3,5 positions), 31.93 (CH$_3$CH$_2$CH$_2$, 4 position), 49.56 (G1-C), 54.64 (ArCH$_2$), 57.91 (NCCH$_2$), 68.30 (G1-CH$_2$OH), 69.26 (CH$_2$OAr, 4 position), 73.47 (CH$_2$OAr, 3,5 positions).
positions), 106.71 (ArCH, 2,6 positions), 122.59 (NCH), 129.03 (ArC, 1 position), 138.56 (ArC, 4 position), 143.08 (CHC), 153.62 (ArC, 3,5 positions), 175.61 (G1-CO2).

TOF-ESI-ES^+: m/z calcd for C_{51}H_{91}N_{3}O_{7}Na [M+Na]^+ 880.6755, found 880.6750.
Anal. calcd for C_{51}H_{91}N_{3}O_{7}: C, 71.37; H, 10.69; N, 4.90. Found: C, 71.49; H, 10.73; N, 5.00.

3,4-G2 (12a): starting from the azide 5a (0.80 g, 1.59 mmol), bis-MPA-alkyne 10 (0.70 g, 1.73 mmol), Na-ascorbate (63 mg, 0.32 mmol), Cu(II)SO_4·5H_2O (40 mg, 0.16 mmol), the title compound 12a was obtained as a white solid. Yield 1.40 g (94%).
M.p.: 115.50–116.50 °C; TLC (19:1 CH_2Cl_2/MeOH) R_f = 0.53.

^1^H NMR (500 MHz, CDCl_3) δ 0.87 (t, J = 6.9 Hz, 6H, C-H_3CH_2), 1.00 (s, 6H, G2-CH_3), 1.25-1.35 (m, 32H, CH_3(C(H_2)_n)), 1.27 (s, 3H, G1-CH_3), 1.44 (m, 4H, CH_2(CH_2)_2OAr), 1.79 (m, 4H, CH_2CH_2OAr), 3.40 (bs, 4H, OH), 3.65-3.69 (dd, J = 11.3 Hz, J = 7.6 Hz, 4H, G2-CH_2OH), 3.75-3.78 (dd, J = 11.2 Hz, J = 4.7 Hz, 4H, G2-CH_2OH), 3.93 and 3.97 (2x t, J = 6.6 Hz, 2H, CH_2OAr, 3 and 4 positions), 4.26 (d, J = 11.1 Hz, 2H, G1-CH_2O), 4.35 (d, J = 11.2 Hz, 2H, G1-CH_2O), 5.21 (s, 2H, NCCH_2), 5.41 (s, 2H, ArCH_2), 6.80-6.85 (m, 3H, ArH, 2,5,6 positions), 7.53 (s, 1H, NCH).

^1^3^C NMR (125 MHz, CDCl_3) δ 14.10 (CH_3CH_2), 17.08 (G2-CH_3), 17.98 (G1-CH_3), 22.67 (CH_3CH_2), 26.00 (CH_2(CH_2)_2OAr), 29.18, 29.23, 29.34, 29.39, 29.41, 29.60, 29.63, 29.67 (CH_2CH_2OAr and CH_3CH_2CH_2(CH_2)_n), 31.90 (CH_3CH_2CH_2), 46.42 (G1-C), 49.70 (G2-C), 54.25 (ArCH_2), 58.18 (NCCH_2), 64.87 (G1-CH_2O), 67.70 and 67.82 (G2-CH_2OH), 69.21 and 69.40 (CH_2OAr, 3,4 positions), 113.60 and 113.80 (ArCH, 2,5 positions), 121.13 (ArCH, 6 position), 123.69 (NCHC), 126.33 (ArC, 1 position), 149.55 (CHC), 149.74 (ArC, 3,4 positions), 172.91 (G1-CO_2), 175.02 (G2-CO_2).

TOF-ESI-ES^+: m/z calcd for C_{49}H_{83}N_{3}O_{12}Na [M+Na]^+ 928.5874, found 928.5903.
Anal. calcd for C_{49}H_{83}N_{3}O_{12}: C, 64.94; H, 9.23; N, 4.64. Found: C, 65.07; H, 9.40; N, 4.57.
3,5-G2 (12b): starting from the azide 5b (0.50 g, 0.10 mmol), bis-MPA-alkyne 10 (0.45 g, 0.11 mmol), Na-ascorbate (43 mg, 0.22 mmol), and Cu(II)SO₄·5H₂O (25 mg, 0.11 mmol), the title compound 12b was obtained as a white solid. Yield 0.83 g (92 %).

M.p.: 90.0–91.0 °C; TLC (19:1 CH₂Cl₂/MeOH) Rₐ = 0.42.

1H NMR (500 MHz, CDCl₃) δ 0.88 (t, J = 6.9 Hz, 6H, C₆H₃CH₂), 1.00 (s, 6H, CH₂C₆H₃), 1.26 (m, 35H, CH₃(CH₂)₈ and G1-CH₂), 1.45 (m, 4H, CH₂(CH₂)OAr), 1.77 (m, 4H, CH₂CH₂OAr), 3.23 (bs, 4H, OH), 3.70 (dd, J = 11.3 Hz, J = 5.8 Hz, 4H, G2-CH₂OH), 3.80 (m, 4H, G2-CH₂OH), 3.90 (t, J = 6.5 Hz, 4H, CH₂OAr, 3,5 positions), 4.31 (d, J = 11.1, 2H, G1-CH₂O), 4.38 (d, J = 11.1 Hz, 2H, G1-CH₂O), 5.23 (s, 2H, NCCH₂), 5.41 (s, 2H, ArCH₂), 6.39 (d, J = 2.0 Hz, 2H, ArH, 2,6 positions), 6.42 (t, J = 2.1 Hz, 1H, ArH, 4 position), 7.57 (s, 1H, NCH).

13C NMR (125 MHz, CDCl₃) δ 14.12 (C₆H₃CH₂), 17.09 (G2-CH₃), 18.01 (G1-CH₃), 22.68 (CH₂CH₂), 26.01 (CH₂(CH₂)₂OAr), 29.17, 29.34, 29.38, 29.56, 29.59, 29.62, 29.65 (CH₂CH₂OAr and CH₂CH₂CH₂(CH₂)₆), 31.90 (CH₃CH₂CH₂), 46.47 (G1-C), 49.65 (G2-C), 54.42 (ArCH₂), 58.20 (NCCH₂), 64.92 (G1-CH₂O), 68.16 and 68.21 (G2-CH₂OH), 68.30 (CH₂OAr, 3,5 positions), 101.26 (ArCH, 4 position), 106.67 (ArCH, 2,6 positions), 123.84 (NCHC), 136.00 (ArC, 1 position), 142.25 (CHC), 160.86 (ArC, 3,5 positions), 172.92 (G1-CO₂), 175.08 (G2-CO₂).

TOF-ESI-ES⁺: m/z calcd for C₄₉H₸₃N₃O₁₂Na [M+Na]⁺ 928.5874, found 928.5901.

Anal. calcd for C₄₉H₸₃N₃O₁₂: C, 64.94; H, 9.23; N, 4.64. Found: C, 65.23; H, 9.16; N, 4.68.
3,4,5-G2 (12c): starting from the azide 5c (1.00 g, 1.45 mmol), bis-MPA-alkyne 10 (0.65 g, 1.60 mmol), Na-ascorbate (57 mg, 0.29 mmol), and Cu(II)SO₄·5H₂O (36 mg, 0.14 mmol), the title compound 12c was isolated as a white solid. Yield 1.47 g (93%).

M.p.: 88.0–89.0 °C; TLC (19:1 CH₂Cl₂/MeOH) Rᵣ = 0.43.

¹H NMR (500 MHz, CDCl₃) δ 0.87 (t, J = 6.9 Hz, 9H, C₆H₃CH₂), 1.01 (G2-C₆H₃), 1.25-1.32 (m, 48H, CH₃(CH₂)₈), 1.28 (s, 3H, G1-CH₃), 1.45 (m, 6H, CH₃(CH₂)₂OAr), 1.69-1.80 (m, 6H, CH₂CH₂OAr), 3.11 (bs, 4H, OH), 3.66-3.70 (dd, J = 11.2 Hz, J = 5.9 Hz, 4H, G2-CH₂OH), 3.77-3.80 (dd, J = 10.7 Hz, J = 3.5 Hz, 4H, G2-CH₂OH), 3.91 and 3.92 (t, overlapped, J = 6.3 Hz, 4H, CH₂OAr, 3,5 positions; t, J = 6.1 Hz, 2H, CH₂OAr, 4 position), 4.28 (d, J = 11.2 Hz, 2H, G1-CH₂O), 4.36 (d, J = 11.1 Hz, 2H, G1-CH₂O), 5.23 (s, 2H, NCCH₂), 5.39 (s, 2H, ArCH₂), 6.47 (s, 2H, ArH), 6.50 (s, 2H, 2,6 positions), 7.56 (s, 1H, NCCH₂).

¹³C NMR (125 MHz, CDCl₃) δ 14.06 (CH₃CH₂), 17.03 (G2-CH₂), 17.93 (G1-CH₃), 22.63 (CH₂CH₂), 26.04 (CH₂(CH₂)₂OAr), 29.30, 29.33, 29.37, 29.54, 29.58, 29.60, 29.63, 29.64, 29.67, 29.69, 30.26 (CH₂CH₂OAr and CH₃CH₂CH₂(CH₂)₂), 31.86 (CH₃CH₂CH₂, 3,5 positions), 31.87 (CH₃CH₂CH₂, 4 position), 46.37 (G1-C), 49.74 (G2-C), 54.59 (ArCH₂), 58.10 (NCCH₂), 64.80 (G1-CH₂O), 67.13 and 67.24 (G2-CH₂OH), 69.21 (CH₂OAr, 3,5 positions), 73.42 (CH₂OAr, 4 position), 106.74 (ArCH, 2,6 positions), 123.93 (NCHC), 128.92 (ArC, 1 position), 138.49 (ArC, 4 position), 142.22 (CHC), 153.56 (ArC, 3,5 positions), 172.89 (G1-CO₂), 174.93 (G2-CO₂).

S3. $^1$H NMR and $^{13}$C NMR spectra

Figure S1. $^1$H-NMR (top) and $^{13}$C-NMR (bottom) of 3,4-G1 in CDCl$_3$, $\delta = 7.26$ and 77.0 ppm, respectively.
Figure S2. $^1$H-NMR (top) and $^{13}$C-NMR (bottom) of 3,5-G1; in CDCl$_3$, $\delta = 7.26$ and 77.0 ppm, respectively.
Figure S3. $^1$H-NMR (top) and $^{13}$C-NMR (bottom) of 3,4,5-G1; in CDCl$_3$, $\delta = 7.26$ and 77.0 ppm, respectively.
Figure S4. $^1$H-NMR (top) and $^{13}$C-NMR (bottom) of 3,4-G2; in CDCl$_3$, $\delta = 7.26$ and 77.0 ppm, respectively.
Figure S5. $^1$H-NMR (top) and $^{13}$C-NMR (bottom) of 3,5-G2; in CDCl$_3$, $\delta$ = 7.26 and 77.0 ppm, respectively.
Figure S6. $^1$H-NMR (top) and $^{13}$C-NMR (bottom) of 3,4,5-G2; in CDCl$_3$, $\delta = 7.26$ and 77.0 ppm, respectively.
S4. Size Exclusion Chromatograms (SEC)

Figure S7. Normalized size exclusion chromatograms (SEC) of all Janus-dendrimers.

S5. Dynamic Light Scattering (DLS) Measurements

S5.1 Z-average size as a function of concentration

3,4-G1 in ethanol

Figure S8. DLS data of 3,4-G1 (11a). Injection from ethanol solution (5 mg mL\(^{-1}\)) into Milli-Q\(^®\). Final concentrations are from 0.1 to 0.5 mg mL\(^{-1}\).
3,5-G1 in ethanol

Figure S9. DLS data of 3,5-G1 (11b). Injection from ethanol solution (10 mg mL\(^{-1}\)) into Milli-Q\(^{®}\). Final concentrations are from 0.1 to 4.0 mg mL\(^{-1}\).
3,4,5-G1 in ethanol

\[ c = 0.1 \text{ mg mL}^{-1} \text{ in EtOH} \]

\[ c = 0.25 \text{ mg mL}^{-1} \text{ in EtOH} \]

\[ c = 0.5 \text{ mg mL}^{-1} \text{ in EtOH} \]

\[ c = 1.0 \text{ mg mL}^{-1} \text{ in EtOH} \]

\[ c = 2.0 \text{ mg mL}^{-1} \text{ in EtOH} \]

\[ c = 4.0 \text{ mg mL}^{-1} \text{ in EtOH} \]

**Figure S10.** DLS data of 3,4,5-G1 (11c). Injection from ethanol solution (10 mg mL\(^{-1}\)) into Milli-Q®. Final concentrations are from 0.1 to 4.0 mg mL\(^{-1}\).
3,4-G2 in ethanol

$c = 0.1 \text{ mg mL}^{-1}$ in EtOH

$c = 0.25 \text{ mg mL}^{-1}$ in EtOH

$c = 0.5 \text{ mg mL}^{-1}$ in EtOH

$c = 1.0 \text{ mg mL}^{-1}$ in EtOH

$c = 2.0 \text{ mg mL}^{-1}$ in EtOH

$c = 4.0 \text{ mg mL}^{-1}$ in EtOH

Gel is formed during the DLS measurement

Figure S11. DLS data of 3,4-G2 (12a). Injection from ethanol solution (10 mg mL$^{-1}$) into Milli-Q®. Final concentrations are from 0.1 to 2.0 mg mL$^{-1}$.
3,5-G2 in ethanol

Figure S12. DLS data of 3,5-G2 (12b). Injection from ethanol solution (10 mg mL\(^{-1}\)) into Milli-Q\(^{®}\). Final concentrations are from 0.1 to 4.0 mg mL\(^{-1}\).
3,4,5-G2 in ethanol

**$c = 0.1 \text{ mg mL}^{-1} \text{ in EtOH}$**

![Graph showing DLS data for $c = 0.1 \text{ mg mL}^{-1}$ in EtOH.](image)

**$c = 0.25 \text{ mg mL}^{-1} \text{ in EtOH}$**

![Graph showing DLS data for $c = 0.25 \text{ mg mL}^{-1}$ in EtOH.](image)

**$c = 0.5 \text{ mg mL}^{-1} \text{ in EtOH}$**

![Graph showing DLS data for $c = 0.5 \text{ mg mL}^{-1}$ in EtOH.](image)

**$c = 1.0 \text{ mg mL}^{-1} \text{ in EtOH}$**

![Graph showing DLS data for $c = 1.0 \text{ mg mL}^{-1}$ in EtOH.](image)

**$c = 2.0 \text{ mg mL}^{-1} \text{ in EtOH}$**

![Graph showing DLS data for $c = 2.0 \text{ mg mL}^{-1}$ in EtOH.](image)

**$c = 4.0 \text{ mg mL}^{-1} \text{ in EtOH}$**

![Graph showing DLS data for $c = 4.0 \text{ mg mL}^{-1}$ in EtOH.](image)

**Figure S13.** DLS data of 3,4,5-G2 (12c). Injection from ethanol solution (10 mg mL$^{-1}$) into Milli-Q®. Final concentrations are from 0.1 to 4.0 mg mL$^{-1}$.

---

24
3,4-G1 in THF

Figure S14. DLS data of 3,4-G1 (11a). Injection from THF solution (5 mg mL$^{-1}$) into Milli-Q®. Final concentrations are from 0.1 to 1.0 mg mL$^{-1}$. 

$\textit{c} = 0.1 \text{ mg mL}^{-1} \text{ in THF}$

\begin{align*}
\text{Z} &= 152.3 \pm 1.3 \text{ nm} \\
\text{PDI} &= 0.10 \pm 0.02
\end{align*}

$c = 0.25 \text{ mg mL}^{-1} \text{ in THF}$

\begin{align*}
\text{Z} &= 152.7 \pm 1.4 \text{ nm} \\
\text{PDI} &= 0.13 \pm 0.03
\end{align*}

$c = 0.5 \text{ mg mL}^{-1} \text{ in THF}$

\begin{align*}
\text{Z} &= 189.7 \pm 1.5 \text{ nm} \\
\text{PDI} &= 0.99 \pm 0.03
\end{align*}

$c = 1.0 \text{ mg mL}^{-1} \text{ in THF}$

\begin{align*}
\text{Z} &= 245.5 \pm 2.7 \text{ nm} \\
\text{PDI} &= 0.05 \pm 0.03
\end{align*}
3,5-G1 in THF

$c = 0.1 \text{ mg mL}^{-1}$ in THF

$c = 0.25 \text{ mg mL}^{-1}$ in THF

$c = 0.5 \text{ mg mL}^{-1}$ in THF

$c = 1.0 \text{ mg mL}^{-1}$ in THF

Figure S15. DLS data of 3,5-G1 (11b). Injection from THF solution (5 mg mL$^{-1}$) into Milli-Q®. Final concentrations are from 0.1 to 1.0 mg mL$^{-1}$.
3,4,5-G1 in THF

$c = 0.1 \text{ mg mL}^{-1}$ in THF

$c = 0.25 \text{ mg mL}^{-1}$ in THF

$c = 0.5 \text{ mg mL}^{-1}$ in THF

$c = 1.0 \text{ mg mL}^{-1}$ in THF

**Figure S16.** DLS data of 3,4,5-G1 (11c). Injection from THF solution (5 mg mL$^{-1}$) into Milli-Q®. Final concentrations are from 0.1 to 1.0 mg mL$^{-1}$.
3,4-G2 in THF

$c = 0.1 \text{ mg mL}^{-1}$ in THF

$c = 0.25 \text{ mg mL}^{-1}$ in THF

$c = 0.5 \text{ mg mL}^{-1}$ in THF

$c = 1.0 \text{ mg mL}^{-1}$ in THF

Figure S17. DLS data of 3,4.G2 (12a). Injection from THF solution (5 mg mL$^{-1}$) into Milli-Q®. Final concentrations are from 0.1 to 1.0 mg mL$^{-1}$.
**3,5-G2 in THF**

$c = 0.1 \text{ mg mL}^{-1}$ in THF

$c = 0.25 \text{ mg mL}^{-1}$ in THF

$c = 0.5 \text{ mg mL}^{-1}$ in THF

$c = 1.0 \text{ mg mL}^{-1}$ in THF

**Figure S18.** DLS data of 3,5-G2 (12b). Injection from THF solution (5 mg mL$^{-1}$) into Milli-Q®. Final concentrations are from 0.1 to 1.0 mg mL$^{-1}$.
3,4,5-G2 in THF

$c = 0.1 \text{ mg mL}^{-1}$ in THF

$c = 0.25 \text{ mg mL}^{-1}$ in THF

$c = 0.5 \text{ mg mL}^{-1}$ in THF

$c = 1.0 \text{ mg mL}^{-1}$ in THF

Figure S19. DLS data of 3,4,5-G2 (12c). Injection from THF solution ($5 \text{ mg mL}^{-1}$) into Milli-Q®. Final concentrations are from 0.1 to 1.0 mg mL$^{-1}$. 
3,4-G1 in acetone

**Figure S20.** DLS data of 3,4-G1 (11a). Injection from acetone solution (5 mg mL\(^{-1}\)) into Milli-Q\(^{®}\). Final concentrations are from 0.1 to 1.0 mg mL\(^{-1}\).
3,5-G1 in acetone

Figure S21. DLS data of 3,5-G1 (11b). Injection from acetone solution (5 mg mL\(^{-1}\)) into Milli-Q\textsuperscript{®}. Final concentrations are from 0.1 to 1.0 mg mL\(^{-1}\).
3,4,5-G1 in acetone

$c = 0.1$ mg mL$^{-1}$ in acetone

$c = 0.25$ mg mL$^{-1}$ in acetone

$c = 0.5$ mg mL$^{-1}$ in acetone

$c = 1.0$ mg mL$^{-1}$ in acetone

Figure S22. DLS data of 3,4,5-G1 (11c). Injection from acetone solution (5 mg mL$^{-1}$) into Milli-Q®. Final concentrations are from 0.1 to 1.0 mg mL$^{-1}$. 
3,4-G2 in acetone

$c = 0.1 \text{ mg mL}^{-1}$ in acetone

$c = 0.25 \text{ mg mL}^{-1}$ in acetone

$c = 0.5 \text{ mg mL}^{-1}$ in acetone

$c = 1.0 \text{ mg mL}^{-1}$ in acetone

Figure S23. DLS data of 3,4-G2 (12a). Injection from acetone solution (5 mg mL$^{-1}$) into Milli-Q®. Final concentrations are from 0.1 to 1.0 mg mL$^{-1}$. 
3,5-G2 in acetone

$c = 0.1 \text{ mg mL}^{-1}$ in acetone

$c = 0.25 \text{ mg mL}^{-1}$ in acetone

$c = 0.5 \text{ mg mL}^{-1}$ in acetone

$c = 1.0 \text{ mg mL}^{-1}$ in acetone

Figure S24. DLS data of 3,5-G2 (12b). Injection from acetone solution (5 mg mL$^{-1}$) into Milli-Q®. Final concentrations are from 0.1 to 1.0 mg mL$^{-1}$. 
Figure S25. DLS data of 3,4,5-G2 (12c). Injection from acetone solution (5 mg mL⁻¹) into Milli-Q®. Final concentrations are from 0.1 to 1.0 mg mL⁻¹.
S5.2. Z-average size after dilution with ultrapure water

Figure S26. DLS data comparing the size of dendrimersomes vs. dendrimer concentration when ethanolic stock solutions with different dendrimer concentrations were injected into Milli-Q®. Samples were measured in triplicate. Error bars represent ±SD.
S5.3. Stability over time in ultrapure water at ambient temperature

**Figure S27.** Injection from dilute THF solution (5 mg mL\(^{-1}\)) in Milli-Q\(^{®}\) (\(c = 0.5 \text{ mg mL}^{-1}\)). All time points were measured in triplicate. Error bars represent ±SD.

**Figure S28.** Injection from dilute acetone solution (5 mg mL\(^{-1}\)) in Milli-Q\(^{®}\) (\(c = 0.5 \text{ mg mL}^{-1}\)). All time points were measured in triplicate. Error bars represent ±SD.
S6. Zeta Potential of Assemblies Injected from Ethanol

Table S1. Zeta potential of assemblies injected from ethanol ($c = 0.5 \text{ mg mL}^{-1}$). The values are an average of two independent sets of measurements, each one measured in triplicate.

<table>
<thead>
<tr>
<th>Janus-dendrimer</th>
<th>ζ-Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,4-G1 (11a)</td>
<td>-11.5 ± 0.5</td>
</tr>
<tr>
<td>3,5-G1 (11b)</td>
<td>-10.4 ± 1.1</td>
</tr>
<tr>
<td>3,4,5-G1 (11c)</td>
<td>-9.8 ± 0.5</td>
</tr>
<tr>
<td>3,4-G2 (12a)</td>
<td>-14.2 ± 0.1</td>
</tr>
<tr>
<td>3,5-G2 (12b)</td>
<td>-13.3 ± 2.1</td>
</tr>
<tr>
<td>3,4,5-G2 (12c)</td>
<td>-18.7 ± 1.3</td>
</tr>
</tbody>
</table>

S7. Additional cryo-TEM Images

Figure S29. Representative cryo-TEM images of uniform dendrimersomes from 3,4,5-G2 (12c) injected from a 5 mg mL$^{-1}$ ethanolic stock solution. Final concentration in Milli-Q® is 0.5 mg mL$^{-1}$. Scale bar is 100 nm.
Figure S30. Representative cryo-TEM images of dendrimersomes self-assembled from 3,5-G2 (12b). Samples were injected from a 5 mg mL\(^{-1}\) ethanolic stock solution. Final concentration in Milli-Q\(^{®}\) is 0.5 mg mL\(^{-1}\). Scale bars are 100 nm.
S8. Preparation of Drug-Loaded Dendrimersomes

Figure S31. (a) Simple injection of Nile red into Milli-Q®, (b) NR-ethanol mixture injected into Milli-Q®, (c) Dendrimersomes 12b in Milli-Q®, (d) Nile red-loaded dendrimersomes in ethanol mixture in Milli-Q®. All Nile red and Janus-dendrimer 12b concentrations were the same in the final volume.

S9. References


