Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Material

Novel stable dendrimersome formulation for safe bioimaging applications

Miriam Filippi, Deyssy Patrucco, Jonathan Martinelli, Mauro Botta, Pablo Castro Hartmann, Lorenzo Tei, and Enzo Terreno**

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Synthesis of JDG0G1(3,5) and JDG0G1(3,4)

Materials and Instrumentation

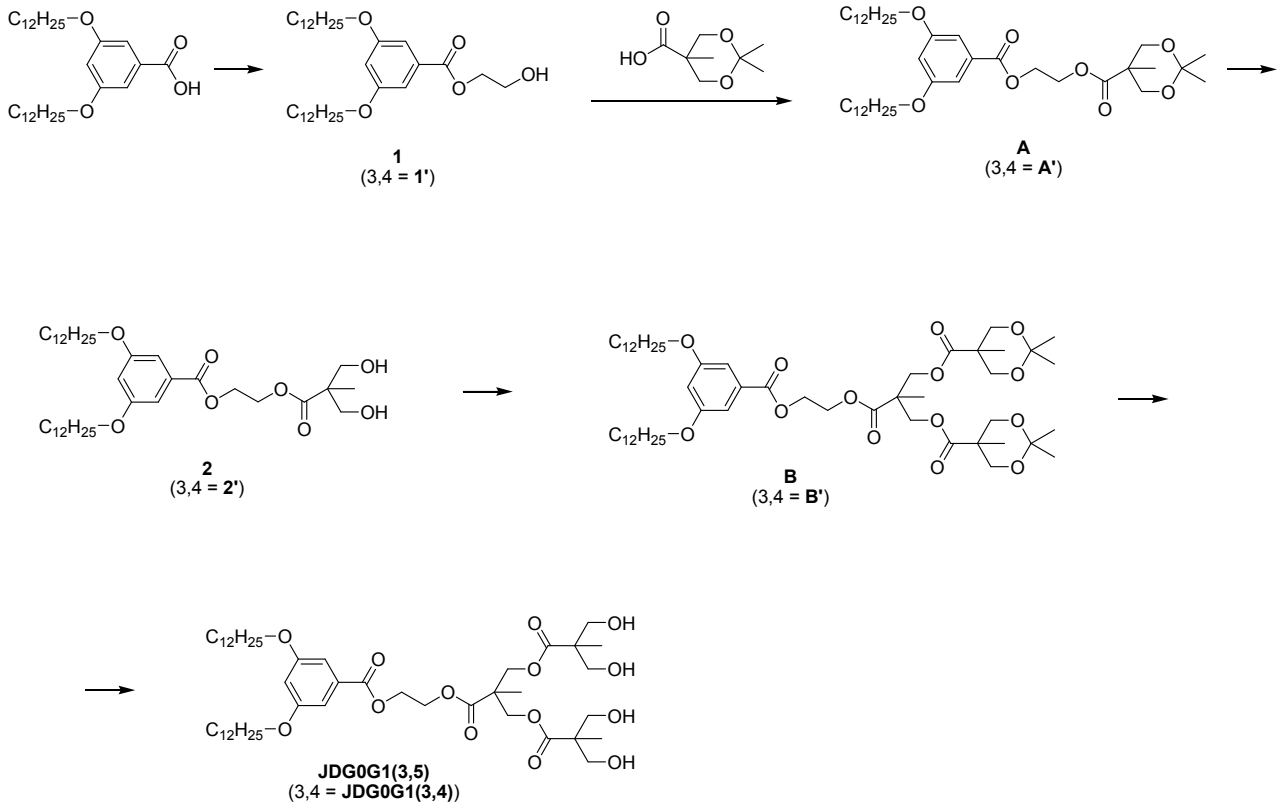
All chemicals were purchased from Sigma-Aldrich Co., Alfa Aesar Co. and Bachem Co. and were used without purification unless otherwise stated. "H_2O" refers to high purity water with conductivity of 0.04 μS cm-1, obtained from a "MILLI-Q" purification system. "Petroleum ether" (PetEt) refers to petroleum ether with boiling point in the range 40-60 °C.

DPTS,[1] isopropyliden-2,2-bis(methoxy)propionic acid,[2] 3,5-(didodecyloxy)benzoic acid[3] and 3,4-(didodecyloxy)benzoic acid[3] were synthesized according to literature procedures.

Thin-layer chromatography (TLC) was carried out on silica plates (silica gel 60 F254, Merck 5554) and visualized by UV lamp (254 nm) or stained in $KMnO_4$ solution. Preparative column chromatography was carried out using silica gel (Merck Silica Gel 60, 230 ± 400 mesh) pre-soaked in the starting eluent. Dialysis tubes (Spectra/Por 7, Spectrum Labs; regenerated cellulose) were soaked in water for 30 minutes and thoroughly rinsed before use. pH measurements were carried out by using a Hanna 211 pH-meter combined with an Aldrich Chemical Company micro-pH electrode, calibrated with buffer solutions at pH 4, 7 and 10. ^1H and ^{13}C NMR spectra were recorded on a JEOL Eclipse Plus 400 and on a Bruker AvanceIII spectrometers operating at 9.4 and 11.7 T, respectively. Chemical shifts are reported relative to TMS and were referenced using the residual proton solvent resonances. Chemical shifts are reported in ppm and coupling constants in Hz. Splitting patterns are described as singlet (s), broad singlet (bs), doublet (d), double doublet (dd), triplet (t), multiplet (m) or broad multiplet (bm). Electrospray ionization mass spectra (ESI MS) were recorded on a SQD 3100 Mass Detector (Waters), operating in positive or negative ion mode, with 1% v/v HCOOH in methanol as the carrier solvent. MALDI-TOF (Matrix Assisted Laser-Desorption/Ionization Time-Of-Flight) mass spectra were recorded on a Voyager DE-PRO MALDI-TOF (Applied Biosystems, Oggi AB-Sciex, Foster City, California) equipped with a nitrogen laser (337 nm); data acquisition and analysis were performed with a Data-Explorer software, version 4.0.0.0

(Applied Biosystems, Oggi AB-Sciex, Foster City, California); a 3,5-dimethoxycinnamic acid matrix, obtained from LaserBio Labs (Sophia Antipolis Cedex, France), was prepared as 10 mg/ml solution in acetonitrile / ultrapure H2O / TFA 50:50:0.1%. Infrared (IR) spectra were recorded in the range 4000–400 cm^{-1} at 4 cm^{-1} resolution using a Bruker Equinox 55 spectrometer.

Synthesis of JDG0G1(3,5) and JDG0G1(3,4)



Scheme S1. Synthetic procedure for JDG0G1(3,5) and JDG0G1(3,4).

2-hydroxyethyl 3,5-(didodecyloxy)benzoate (1)

3,5-(didodecyloxy)benzoic acid (1.00 g, 2.04 mmol) and DPTS (300 mg, 1.02 mmol) were suspended in DCM (5 ml) and ethylene glycol (5 ml). N_2 was bubbled for 10 minutes, then DCC (505 mg, 2.45 mmol) was added and the mixture was stirred at 50 °C for 15 hours. Then,

DCM (20 ml) was added to the suspension, that was washed with H_2O (3x10 ml). The organic phase was dried over anhydrous Na_2SO_4 and evaporated. The remaining oil was purified by flash chromatography (SiO_2, PetEt/EtOAc 9:1, R_f 0.18), and the product was obtained as a white solid (679 mg, 62% yield).

ESI^+ MS, m/z: 535 ($[M+H]^+$), 557 ($[M+Na]^+$). IR (KBr disk), cm^{-1}: 3445, 2921, 2852, 1721, 1594, 1447, 1374, 1346, 1322, 1298, 1229, 1166, 1056, 843, 766, 721, 676. ^1H NMR (500 MHz, 25 1721, 1_3), δ (ppm): 7.18 (d, $^4J_{HH}$ = 2.3 Hz, 2H, CH_{Ar}^o), 6.67 (t, $^4J_{HH}$ = 2.3 Hz, 1H, CH_{Ar}^p), 4.47 (m, 2H, $C\underline{H}_2CH_2OH$), 3.98 (m, 6H, $C\underline{H}_2OH$ + OCH_2^{chain}), 2.16 (bm, 1H, OH), 1.77 (m, 4H, $OCH_2C\underline{H}_2^{chain}$), 1.47 (m, 4H, $OCH_2CH_2C\underline{H}_2$), 1.3-1.2 (m, 32H, $[CH_2]_8$), 0.90 (t, $^3J_{HH}$ = 6.9 Hz, 6H, CH_3). ^{13}C NMR (125 MHz, 25 [$CHCDCl_3$), δ (ppm): 166.9 (CO), 160.2 (C_{Ar}^m), 131.5 (C_{Ar}^i), 107.8 (CH_{Ar}^o), 106.5 (CH_{Ar}^p), 68.4 (OCH_2^{chain}), 66.8 ($\underline{C}H_2CH_2OH$), 61.4 (CH_2OH), 32-22 ($[CH_2]_{10}$), 14.1 (CH_3).

2-hydroxyethyl 3,4-(didodecyloxy)benzoate (1')

3,4-(didodecyloxy)benzoic acid (2.57 g, 5.23 mmol) and DPTS (770 mg, 2.62 mmol) were dissolved in DCM (50 ml) and ethylene glycol (1.46 ml, 26.15 mmol). N_2 was bubbled for 10 minutes, then DCC (1.30 g, 6.28 mmol) was added and the mixture was stirred at room temperature for 15 hours. The resulting suspension was filtered under vacuum, and the filtrate was evaporated. The solid residue was purified by flash chromatography (SiO_2, PetEt/EtOAc 8:2, R_f 0.20), and the product was obtained as a white solid (1.90 g, 68% yield).

ESI^+ MS, m/z: 535 ($[M+H]^+$), 557 ($[M+Na]^+$). IR (KBr disk), cm^{-1}: 3441, 2917, 2849, 1690, 1598, 1518, 1467, 1432, 1307, 1278, 1222, 1148, 1069, 763. ^1H NMR (400 MHz, 25 1690, 1_3), δ (ppm): 7.64 (dd, $^4J_{HH}$ = 2.2 Hz, $^3J_{HH}$ = 8.4 Hz, 1H, CH_{Ar}^o), 7.53 (d, $^4J_{HH}$ = 2.2 Hz, 1H, CH_{Ar}^o), 6.85 (d, $^3J_{HH}$ = 8.4 Hz, 1H, CH_{Ar}^m), 4.43 (m, 2H, CH_2CH_2OH), 4.02 (m, 4H, OCH_2^{chain}), 3.93 (m, 2H, CH_2OH), 1.82 (m, 4H, $OCH_2CH_2^{chain}$), 1.46 (m, 4H, $OCH_2CH_2CH_2$), 1.4-1.2 (m, 32H, $[CH_2]_8$), 0.87 (t, $^3J_{HH}$ = 6.6 Hz, 6H, CH_3). ^{13}C NMR (100 MHz, 25 [CH0,

1_3), δ (ppm): 167.0 (CO), 153.6 (C_{Ar}^{p}), 148.6 (C_{Ar}^{m}), 123.8 ($CH_{Ar}^{o}CH_{Ar}$), 122.0 (C_{Ar}^{i}), 114.4 (CH_{Ar}^{m}), 111.9 ($CH_{Ar}^{o}C_{Ar}$), 69.4, 69.1 (OCH_2^{chain}), 66.6 (CH_2CH_2OH), 61.6 (CH_2OH), 32-22 ($[CH_2]_{10}$), 14.2 (CH_3).

JDG0G0(3,5)-acetonide (A)

1 (679 mg, 1.27 mmol), 2,2,5-trimethyl-1,3-dioxane-5-carboxylic acid (243 mg, 1.40 mmol) and DPTS (187 mg, 0.64 mmol) were dissolved in DCM (5 ml). N_2 was bubbled for 10 minutes, then DCC (314 mg, 1.52 mmol) was added and the mixture was stirred at room temperature for 15 hours. The resulting suspension was filtered and the filtrate was dried under vacuum. The solid residue was purified by flash chromatography (SiO_2, PetEt/EtOAc 9:1, R_f 0.31), and the product was obtained as a white solid (597 mg, 68% yield).

ESI^+ MS, m/z: 692 ($[M+H]^+$). IR (KBr disk), cm^{-1}: 2925, 2854, 1729, 1596, 1448, 1370, 1348, 1324, 1298, 1219, 1165, 1083, 1056, 833, 765. ^1H NMR (500 MHz, 25 °C, $CDCl_3$), δ (ppm): 7.17 (d, $^4J_{HH}$ = 2.3 Hz, 2H, CH_{Ar}^{o}), 6.66 (t, $^4J_{HH}$ = 2.3 Hz, 1H, CH_{Ar}^{p}), 4.52 (m, 4H, OCH_2CH_2O), 4.20 (d, $^2J_{HH}$ = 11.7 Hz, 2H, CCHHC, CDCl448, 13^3J_{HH} = 6.6 Hz, 4H, OCH_2^{chain}), 3.65 (d, $^2J_{HH}$ = 11.7 Hz, 2H, CCHHC, CDCl448, 1370, $OCH_2CH_2^{chain}$), 1.47 (m, 4H, $OCH_2CH_2CH_2$), 1.43 (s, 3H, $OCCH_3$), 1.38 (s, 3H, $OCCH_3$), 1.4-1.2 (m, 32H, $[CH_2]_8$), 1.23 (s, 3H, $CCCH_3$) 0.90 (t, $^3J_{HH}$ = 6.9 Hz, 6H, CH_3^{chain}). ^{13}C NMR (125 MHz, 25 H [$CHDCl_3$), δ (ppm): 174.0 (CCO), 166.2 (ArCO), 160.2 (C_{Ar}^{m}), 131.4 (C_{Ar}^{i}), 107.7 (CH_{Ar}^{o}), 106.7 (CH_{Ar}^{p}), 98.1 ($O\underline{C}CH_3$), 68.3 (OCH_2^{chain}), 65.9 ($C\underline{C}H_2O$), 62.5, 62.4 (OCH_2CH_2O), 41.9 ($\underline{C}CH_2O$), 42-22 ($[CH_2]_{10}$), 24.2, 23.1 ($OC\underline{C}H_3$), 18.7 ($CC\underline{C}H_3$) 14.1 (CH_3^{chain}).

JDG0G0(3,4)-acetonide (A')

1' (1.90 g, 3.55 mmol), 2,2,5-trimethyl-1,3-dioxane-5-carboxylic acid (741 mg, 4.26 mmol) and DPTS (522 mg, 1.78 mmol) were dissolved in DCM (14 ml). N_2 was bubbled for 10 minutes, then DCC (878 mg, 4.26 mmol) was added and the mixture was stirred at room temperature for 15 hours. The resulting suspension was filtered and the filtrate was dried

under vacuum. The solid residue was purified by flash chromatography (SiO_2, PetEt/EtOAc 9:1, R_f 0.11), and the product was obtained as a white solid (2.29 g, 93% yield).

ESI^+ MS, m/z: 692 ($[M+H]^+$). IR (KBr disk), cm^{-1}: 3496, 2920, 2850, 1716, 1601, 1518, 1468, 1433, 1392, 1344, 1277, 1217, 1136, 1045, 762. ^1H NMR (500 MHz, 25 °C, $CDCl_3$), δ (ppm): 7.62 (dd, $^3J_{HH}$ = 8.4 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, CH_{Ar}^o), 7.53 (d, $^4J_{HH}$ = 1.7 Hz, 1H, CH_{Ar}^o), 6.86 (d, $^3J_{HH}$ = 8.4 Hz, 1H, CH_{Ar}^m), 4.50 (m, 4H, OCH_2CH_2O), 4.19 (d, $^2J_{HH}$ = 11.9 Hz, 2H, CCHHC, CDCl601, 1518, 146_2^{chain}), 3.63 (d, $^2J_{HH}$ = 11.9 Hz, 2H, CCHHC, CDCl601, 1518, $1468_2CH_2^{chain}$), 1.48 (m, 4H, $OCH_2CH_2CH_2$), 1.41 (s, 3H, $OCCH_3$), 1.36 (s, 3H, $OCCH_3$), 1.4-1.2 (m, 32H, $[CH_2]_8$), 1.21 (s, 3H, $COCCH_3$), 0.89 (t, $^3J_{HH}$ = 6.8 Hz, 6H, CH_3^{chain}). ^{13}C NMR (125 MHz, 25 CH[$CHDCl_3$), δ (ppm): 174.0 (CCO), 166.1 (ArCO), 153.4 (C_{Ar}^p), 148.6 (C_{Ar}^m), 123.7 ($CH_{Ar}^oCH_{Ar}$), 121.9 (C_{Ar}^i), 114.3 (CH_{Ar}^m), 111.9 ($CH_{Ar}^oC_{Ar}$), 98.0 ($OCCH_3$), 69.2, 69.0 ($2xOCH_2^{chain}$), 65.9 (CCH_2O), 62.5, 62.2 (OCH_2CH_2O), 41.8 (CCH_2O), 42-22 ($[CH_2]_{10}$), 24.1, 23.1 ($2xOCCH_3$), 18.6 ($CCCH_3$) 14.1 (CH_3^{chain}).

JDG0G0(3,5) (2)

A (408 mg, 0.59 mmol) was dissolved in THF (6 ml). 6 M HCl (6 ml) was added, and the solution was stirred at room temperature for 15 hours. The resulting mixture was diluted with H_2O (30 ml) and extracted with DCM (3x20 ml). The organic phase was dried over anhydrous Na_2SO_4 and evaporated. The oil obtained was purified by flash chromatography (SiO_2, PetEt/EtOAc 7:3, R_f 0.23), and the product was obtained as a white solid (242 mg, 63% yield).

ESI^+ MS, m/z: 651 ($[M+H]^+$). IR (KBr disk), cm^{-1}: 3442, 2924, 2854, 1726, 1597, 1448, 1348, 1325, 1300, 1227, 1167, 1055, 766. ^1H NMR (500 MHz, 25 1726, 1_3), δ (ppm): 7.17 (d, $^4J_{HH}$ = 2.3 Hz, 2H, CH_{Ar}^o), 6.67 (t, $^4J_{HH}$ = 2.3 Hz, 1H, CH_{Ar}^p), 4.54 (m, 4H, OCH_2CH_2O), 3.98 (t, $^3J_{HH}$ = 6.6 Hz, 4H, OCH_2^{chain}), 3.90 (d, $^2J_{HH}$ = 11.7 Hz, 2H, CCHH= 11.7 Hz, 2H, C^2J_{HH} = 11.7 Hz, 2H, CCHH=O), 2.84 (bs, 2H, OH), 1.8-1-2 (m, 40H, $OCH_2CH_2^{chain}$), 1.09

(s, 3H, CC<u>C</u>H_3), 0.90 (t, $^3J_{HH}$ = 6.9 Hz, 6H, CH_3^{chain}). ^{13}C NMR (125 MHz, 25 HC, $CDCl_3$), δ (ppm): 175.6 (CCO), 166.5 (ArCO), 160.2 (C_{Ar}^m), 131.2 (C_{Ar}^i), 107.8 (CH_{Ar}^o), 106.8 (CH_{Ar}^p), 68.4 (OCH_2^{chain}), 68.3 (CH_2OH), 62.9, 62.5 (OCH_2CH_2O), 49.4 (<u>C</u>CH_2OH), 32-22 ($[CH_2]_{10}$), 17.3 (C<u>C</u>H_3) 14.1 (CH_3^{chain}).

JDG0G0(3,4) (2')

A' (2.02 g, 2.92 mmol) was dissolved in THF (20 ml). 6 M HCl (20 ml) was added, and the solution was stirred at room temperature for 15 hours. The resulting mixture was extracted with $CHCl_3$ (3x40 ml). The organic phase was dried over anhydrous Na_2SO_4 and evaporated. The oil obtained was purified by flash chromatography (SiO_2, PetEt/EtOAc 5:5, R_f 0.34), leading to the product as a white solid (1.35 g, 71% yield).

ESI$^+$ MS, m/z: 651 ($[M+H]^+$). IR (KBr disk), cm^{-1}: 3444, 2954, 2918, 2848, 1716, 1599, 1520, 1464, 1275, 1215, 1136, 1043, 762. ^{1}H NMR (500 MHz, 25 2848, 1_3), δ (ppm): 7.62 (dd, $^3J_{HH}$ = 8.4 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, CH_{Ar}^o), 7.53 (d, $^4J_{HH}$ = 1.7 Hz, 1H, CH_{Ar}^o), 6.86 (d, $^3J_{HH}$ = 8.4 Hz, 1H, CH_{Ar}^m), 4.54 (m, 4H, OCH_2CH_2O), 3.98 (t, $^3J_{HH}$ = 6.6 Hz, 4H, OCH_2^{chain}), 3.90 (d, $^2J_{HH}$ = 11.7 Hz, 2H, CC<u>H</u>H= 11.7 Hz, 2H, C$^2J_{HH}$ = 11.7 Hz, 2H, CCH<u>H</u>=O), 2.84 (bs, 2H, OH), 1.8-1-2 (m, 40H, $OCH_2CH_2^{chain}$), 1.09 (s, 3H, CC<u>C</u>H_3), 0.90 (t, $^3J_{HH}$ = 6.9 Hz, 6H, CH_3^{chain}). ^{13}C NMR (125 MHz, 25 °C, $CDCl_3$), δ (ppm): 174.0 (CCO), 166.1 (ArCO), 153.4 (C_{Ar}^p), 148.6 (C_{Ar}^m), 123.7 ($CH_{Ar}^oCH_{Ar}$), 121.9 (C_{Ar}^i), 114.3 (CH_{Ar}^m), 111.9 ($CH_{Ar}^oC_{Ar}$), 68.4 (OCH_2^{chain}), 68.3 (CH_2OH), 62.9, 62.5 (OCH_2CH_2O), 49.4 (<u>C</u>CH_2OH), 32-22 ($[CH_2]_{10}$), 17.3 (C<u>C</u>H_3) 14.1 (CH_3^{chain}).

JDG0G1(3,5)-acetonide (B)

2 (283 mg, 0.43 mmol), 2,2,5-trimethyl-1,3-dioxane-5-carboxylic acid (167 mg, 0.95 mmol) and DPTS (128 mg, 0.43 mmol) were dissolved in DCM (2 ml). N_2 was bubbled for 10 minutes, then DCC (215 mg, 1.03 mmol) was added and the mixture was stirred at room temperature for 15 hours. The resulting suspension was filtered under vacuum, and the filtrate

was evaporated, dissolved in EtOAc (10 ml) and washed with brine (3x5 ml). The organic phase was dried over anhydrous Na_2SO_4 and evaporated, and the white wax obtained was purified by flash chromatography (SiO_2, PetEt/EtOAc 8:2, R_f 0.33). The product was obtained as a colorless oil (325 mg, 79% yield).

ESI^+ MS, m/z: 985 ($[M+Na]^+$), 1001 ($[M+K]^+$). IR (KBr disk), cm^{-1}: 2925, 2854, 1734, 1595, 1457, 1221, 1165, 1083, 1043, 832, 766. ^1H NMR (500 MHz, 25 1595, 1_3), δ (ppm): 7.16 (d, $^4J_{HH}$ = 2.3 Hz, 2H, $CH_{Ar}{}^o$), 6.66 (t, $^4J_{HH}$ = 2.3 Hz, 1H, $CH_{Ar}{}^p$), 4.51 (m, 4H, OCH_2CH_2O), 4.36 (s, 4H, *G1*-$CC\underline{H}_2O$), 4.15 (d, $^2J_{HH}$ = 11.7 Hz, 2H, *G2*-$CC\underline{H}_2O$), 3.99 (t, $^3J_{HH}$ = 6.6 Hz, 4H, $OCH_2{}^{chain}$), 3.61 (d, $^2J_{HH}$ = 11.7 Hz, 2H, *G2*-$CC\underline{H}_2O$), 1.8-1-2 (m, 40H, $[CH_2]_{10}$), 1.59 (s, 3H, $CCCH_3$), 1.42, 1.36 (2x $OCCH_3$), 1.14 (s, 6H, $CC\underline{C}H_3$), 0.91 (t, $^3J_{HH}$ = 6.9 Hz, 6H, $CH_3{}^{chain}$). ^{13}C NMR (125 MHz, 25 CCHCH, 1_3), δ (ppm): 173.5, 172.4, 171.1 (3x CCO), 166.1 (ArCO), 131.3 ($C_{Ar}{}^i$), 107.8 ($CH_{Ar}{}^o$), 106.7 ($CH_{Ar}{}^p$), 98.1 ($O\underline{C}CH_3$), 68.4 ($OCH_2{}^{chain}$), 65.9 (*G2*-$C\underline{C}H_2O$), 65.2 (*G1*-$C\underline{C}H_2O$), 63.0, 62.5 (OCH_2CH_2O), 46.8 (*G1*-$\underline{C}CH_2O$), 42.0 (*G2*-$\underline{C}CH_2O$), 32-22 ($[CH_2]_{10}$), 25.1, 22.1 (2x $OC\underline{C}H_3$), 18.5 (*G2*-$CC\underline{C}H_3$), 17.3 (*G2*-$CC\underline{C}H_3$), 14.1 ($CH_3{}^{chain}$).

JDG0G1(3,4)-acetonide (B')

2' (1.35 g, 2.07 mmol), 2,2,5-trimethyl-1,3-dioxane-5-carboxylic acid (867 mg, 4.97 mmol) and DPTS (611 mg, 2.07 mmol) were dissolved in DCM (15 ml). N_2 was bubbled for 10 minutes, then DCC (1.03 g, 4.97 mmol) was added and the mixture was stirred at room temperature for 15 hours. The resulting suspension was filtered under vacuum and the filtrate was evaporated. The white wax obtained was purified by flash chromatography (SiO_2, PetEt/EtOAc 7:3, R_f 0.13). The product was obtained as a colorless oil (1.47 g, 74% yield).

ESI^+ MS, m/z: 985 ($[M+Na]^+$), 1001 ($[M+K]^+$). IR (KBr disk), cm^{-1}: 3400, 2919, 2850, 1721, 1599, 1514, 1467, 1273, 1215, 1134, 1041, 762. ^1H NMR (500 MHz, 25 1721, 1_3), δ (ppm): 7.62 (dd, $^3J_{HH}$ = 8.4 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, $CH_{Ar}{}^o$), 7.54 (d, $^4J_{HH}$ = 1.7 Hz, 1H, $CH_{Ar}{}^o$), 6.88

(d, $^{3}J_{HH}$ = 8.4 Hz, 1H, CH_{Ar}^{m}), 4.51 (m, 4H, OCH_2CH_2O), 4.36 (s, 4H, *G1*-CC$\underline{H}_2$O), 4.15 (d, $^{2}J_{HH}$ = 11.7 Hz, 2H, *G2*-CC$\underline{H}_2$O), 4.05 (t, $^{3}J_{HH}$ = 6.6 Hz, 4H, OCH_2^{chain}), 3.61 (d, $^{2}J_{HH}$ = 11.7 Hz, 2H, *G2*-CC$\underline{H}_2$O), 1.8-1-2 (m, 40H, $[CH_2]_{10}$), 1.59 (s, 3H, $CCCH_3$), 1.42, 1.36 (2x s, 3H, $OCCH_3$), 1.14 (s, 6H, CC$\underline{C}H_3$), 0.91 (t, $^{3}J_{HH}$ = 6.9 Hz, 6H, CH_3^{chain}). ^{13}C NMR (125 MHz, 25 , 3H, OC_3), δ (ppm): 173.5, 172.4 (2x C$\underline{C}$O), 166.1 (ArCO), 153.5 (C_{Ar}^{p}), 148.6 (C_{Ar}^{m}), 123.7 ($\underline{C}H_{Ar}{}^{o}CH_{Ar}$), 121.8 ($C_{Ar}^{i}$), 114.3 ($CH_{Ar}^{m}$), 111.9 ($\underline{C}H_{Ar}{}^{o}C_{Ar}$), 98.1 (O$\underline{C}CH_3$), 68.4 ($OCH_2^{chain}$), 65.9 (*G2*-C$\underline{C}H_2$O), 65.2 (*G1*-C$\underline{C}H_2$O), 63.0, 62.5 ($OCH_2CH_2O$), 46.8 (*G1*-$\underline{C}CH_2$O), 42.0 (*G2*-$\underline{C}CH_2$O), 32-22 ($[CH_2]_{10}$), 25.1, 22.1 (2x OC$\underline{C}H_3$), 18.5 (*G2*-CC$\underline{C}H_3$), 17.3 (*G2*-CC$\underline{C}H_3$), 14.1 ($CH_3^{chain}$).

JDG0G1(3,5)

B (1.38 g, 1.43 mmol) was dissolved in THF (5 ml). 6 M HCl (5 ml) was added, and the solution was stirred at room temperature for 15 hours. The resulting mixture was extracted with DCM (3x5 ml). The organic phase was dried over anhydrous Na_2SO_4 and evaporated. The oil obtained was purified by flash chromatography (SiO_2, PetEt/EtOAc 4:6, R_f 0.07), and the product was obtained as a colorless oil (934 mg, 74% yield).

ESI$^+$ MS, m/z: 884 ($[M+H]^+$), 906 ($[M+Na]^+$), 922 ($[M+K]^+$). IR (KBr disk), cm^{-1}: 3442, 2924, 2854, 1732, 1597, 1456, 1387, 1300, 1227, 1167, 1047, 766. ^{1}H NMR (500 MHz, 25 1732, 1_3), δ (ppm): 7.15 (d, $^{4}J_{HH}$ = 2.3 Hz, 2H, CH_{Ar}^{o}), 6.66 (t, $^{4}J_{HH}$ = 2.3 Hz, 1H, CH_{Ar}^{p}), 4.54 (m, 4H, OCH_2CH_2O), 4.44 (d, $^{2}J_{HH}$ = 11.7 Hz, 2H, *G1*-CCH_2O), 4.28 (d, $^{2}J_{HH}$ = 11.7 Hz, 2H, *G1*-CCH_2O), 3.98 (t, $^{3}J_{HH}$ = 6.6 Hz, 4H, OCH_2^{chain}), 3.81 (m, 4H, C$\underline{H}_2$OH), 3.69 (m, 4H, C$\underline{H}_2$OH), 3.40 (bs, 4H, OH), 1.8-1-2 (m, 40H, $[CH_2]_{10}$), 1.31 (s, 3H, *G1*-CCH_3), 1.05 (*G2*-CCH_3), 0.89 (t, $^{3}J_{HH}$ = 6.9 Hz, 6H, CH_3^{chain}). ^{13}C NMR (125 MHz, 25 °C, $CDCl_3$), δ (ppm): 175.0 (*G2*-CO), 172.8 (*G1*-CO), 166.2 (ArCO), 131.2 (C_{Ar}^{i}), 107.8 (CH_{Ar}^{o}), 106.7 (CH_{Ar}^{p}), 68.4 (OCH_2^{chain}), 67.7 (CH_2OH), 64.8 (*G1*-C$\underline{C}H_2$O), 63.1, 62.5 (OCH_2CH_2O), 49.7

($\underline{C}CH_2OH$), 46.4 (*G1*-$\underline{C}CH_2O$), 32-22 ($[CH_2]_{10}$), 18.5 (*G1*-CH_3), 17.3 (*G2*-CH_3), 14.1 (CH_3^{chain}).

JDG0G1(3,4)

B' (940 mg, 0.98 mmol) was dissolved in THF (10 ml). 6 M HCl (10 ml) was added, and the solution was stirred at room temperature for 15 hours. The resulting mixture was extracted with DCM (3x10 ml). The organic phase was dried over anhydrous Na_2SO_4 and evaporated. The oil obtained was purified by flash chromatography (SiO_2, PetEt/EtOAc 3:7, R_f 0.11), and the product was obtained as a colorless oil (560 mg, 65% yield).

ESI^+ MS, m/z: 884 ($[M+H]^+$), 906 ($[M+Na]^+$), 922 ($[M+K]^+$). IR (KBr disk), cm^{-1}: 3416, 2920, 2851, 1720, 1599, 1514, 1467, 1271, 1214, 1134, 1040, 762. ^1H NMR (500 MHz, 25 1720, 1_3), δ (ppm): 7.62 (dd, $^3J_{HH}$ = 8.4 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, CH_{Ar}^o), 7.53 (d, $^4J_{HH}$ = 1.7 Hz, 1H, CH_{Ar}^o), 6.86 (d, $^3J_{HH}$ = 8.4 Hz, 1H, CH_{Ar}^m), 4.54 (m, 4H, OCH_2CH_2O), 4.44 (d, $^2J_{HH}$ = 11.7 Hz, 2H, *G1*-CCH_2O), 4.28 (d, $^2J_{HH}$ = 11.7 Hz, 2H, *G1*-CCH_2O), 3.98 (t, $^3J_{HH}$ = 6.6 Hz, 4H, OCH_2^{chain}), 3.81 (m, 4H, $C\underline{H}_2OH$), 3.69 (m, 4H, $C\underline{H}_2OH$), 3.40 (bs, 4H, OH), 1.8-1-2 (m, 40H, $[CH_2]_{10}$), 1.31 (s, 3H, *G1*-CCH_3), 1.05 (*G2*-CCH_3), 0.89 (t, $^3J_{HH}$ = 6.9 Hz, 6H, CH_3^{chain}). ^{13}C NMR (125 MHz, 25 OH), 1.8_3), δ (ppm): 174.0 (CCO), 166.1 (ArCO), 153.4 (C_{Ar}^p), 148.6 (C_{Ar}^m), 123.7 ($CH_{Ar}^oCH_{Ar}$), 121.9 (C_{Ar}^i), 114.3 (CH_{Ar}^m), 111.9 ($CH_{Ar}^oC_{Ar}$), 68.4 (OCH_2^{chain}), 67.7 (CH_2OH), 64.8 (*G1*-$C\underline{C}H_2O$), 63.1, 62.5 (OCH_2CH_2O), 49.7 ($\underline{C}CH_2OH$), 46.4 (*G1*-$\underline{C}CH_2O$), 32-22 ($[CH_2]_{10}$), 18.5 (*G1*-CH_3), 17.3 (*G2*-CH_3), 14.1 (CH_3^{chain}).

2. Vesicle preparation protocol

The dendrimersomes herein reported were prepared by thin film hydration method, as previously described.[4] The total amount of required amphiphilic material was calculated depending on the experimental purpose, being the selected concentrations dedicated to stability and cellular tests 10 mg/ml and 40 mg/ml respectively. Briefly, appropriate amounts of Janus dendrimers and DSPE-PEG2000-COOH were weighted and dissolved into chloroform inside a round-bottomed flask in the molar ratio of 19:1 respectively. This solution underwent a drying process on a rotary evaporator (Rotavapor Heidolph, Schwabach, Germany), during which the organic solvent was slowly removed due to the reduced pressure, by letting the amphiphilic components distributed in a thin homogeneous film. The film remained at least two hours under vacuum, before being hydrated with a 300 mOsm hydration buffered solution (pH 7.4) containing 250 mM of Gadoteridol (Gd-HPDO3A, ProHance®), in addition to NaCl and 4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid (HEPES). The hydration process was performed at 50°C and accompanied by vigorous shaking to optimize the material detachment from the flask glass surface. During this process, a raw suspension of multilamellar vesicular structures of different dimensions was obtained, that required an additive extrusion procedure. The samples were therefore forced to pass through different extrusion polycarbonate filters (Lipex extruder, Northern Lipids Inc.) with pore diameters decreasing from 1 μm to 200 nm. This operation was repeated at least 4 times for each selected kind of filter. The Dynamic Light Scattering (DLS, Zetasizer Nano 90 ZS, Malvern, UK) was used to determine the mean hydrodynamic diameter of vesicles, which was found to be ranging from 105 to around 195 nm according to the molecular shape of the employed dendrimeric components. After the extrusion process was completed, suspensions with Polydispersity Index (PDI) value lower than 0.2 were obtained. The final suspensions were inserted inside dialysis cellulose tubing membranes (dialysis sacks of 25 mm flat width, Sigma-Aldrich) provided with 12000 Dalton-sized pores. Finally, exhaustive dialysis was

carried out at 4°C against isotonic dialysis buffer, in order to purify vesicular suspensions from the non-encapsulated probe molecules. Dialysis buffer consisted of a pH 7.4 solution 0.15 M of NaCl and 0.004 of M 4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid (HEPES). For the preparative protocol, dialysis was always performed in a total volume of 2 L and for a minimal duration of 16 hours, with a buffer renewal after the first 4 hours.

3. Dynamic Light Scattering

Dynamic Light Scattering measurements (DLS) were performed with a Malvern Instruments particle sizer (Zetasizer Nano 90 ZS, Malvern, UK). In order to calculate particle size, a scattering angle of 90 degrees was used. All measurements were performed in triplicate at 25°C, by diluting samples into isosmotic HEPES buffer. The Malvern instrument operates a cumulant analysis of the dynamic light scattering intensity autocorrelation function to estimate two values: a width parameter known as the Polydispersity Index (PDI) and a mean value for the size (often referred to as Z-average). On one hand, the information about polydispersity was needed to evaluate whether the employed preparation protocol was useful to produce dendrimersomes with dimensional homogeneity. On the other hand, vesicular diameter was carefully investigated to determine if sizes could be compared to other well established nanosystems, such as liposomes. In general terms, being exclusively calculated from the signal intensity, the hydrodynamic parameter Z-Average should not be intended as a mass or number mean. However, Z-Average can be considered a reliable term to describe the size of particles, when the sample is monomodal, monodisperse, and hallmarked by a distribution curve consisting of only one peak with a narrow width. Since dendrimersomes prepared by film hydration method presented these qualities, Z-Average was accepted as a reliable size indicator, also useful to compare to values measured with other techniques. The calculations for these parameters are defined in the ISO standard document 13321:1996.

Specifically, Z-Average is determined on the basis of the Z-Average diffusion coefficient (b value or second order cumulant), the viscosity index of the dispersant and some instrumental constants. PDI can be calculated as ratio of the squares of standard deviation and Z-average size, since it can be intended as a measure of relative variance on a Gaussian distribution describing a single population of particles characterized by a certain value of Z-Average. This Index is scaled such that it normally varies in a range of values included between 0.05 and 0.7: only extremely monodisperse and uniform standards provide values below the first number, while values higher than the second number denote broad size distributions not suitable for DLS analysis. For most dendrimersomes, the mean hydrodynamic size (expressed as Z-Average) was found to be ranging from 105 to 195 nm (depending on employed Janus Dendrimers). Unimodal size distributions and low values of PDI (ranging from 0.05 to 0.19) demonstrate the formation of monodisperse vesicle populations (Figure S1).

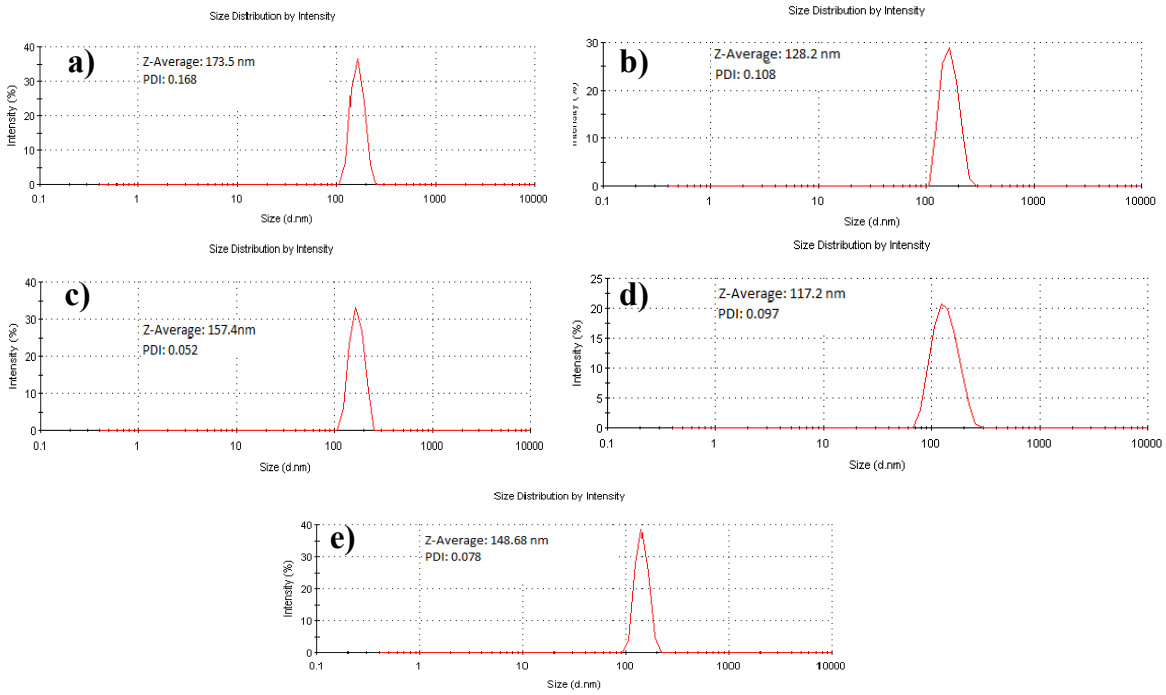


Figure S1. Polydispersity and size of dendrimersome suspensions. Representative plots of DLS intensity vs. diameter, collected from dendrimersomes assembled from a) JDG1G2(3,5), b) JDG1G2(3,4), c) JDG0G1(3,5) and d) JDG0G1(3,4) dendrimers and e) control liposomes composed of DPPC and DSPE-PEG (Avanti Polar Lipids Inc., Alabaster, AL, USA) (19:1 molar ratio).

4. Stability and content release

In order to test the stability within different biological environments, some incubation experiments were performed, where suspensions containing Gadoteridol-loaded vesicles were maintained for a certain time range into isosmotic solutions of plasmatic components at physiologic pH and temperature (7.4 and 37°C, respectively). The stability was then evaluated on the basis of the Gadoteridol release, by determining the residual observed water protons longitudinal relaxation rate (R_1^{obs}) and the Gadolinium ion (Gd^{3+}) content. The initial concentration of amphiphilic material in vesicular suspensions dedicated to incubation experiments was 10 mg/ml, which was further diluted to perform stability assays (dilution ratio 1:2) into isosmotic solutions of human serum (HS) proteins and human serum albumin (HSA) inside small plastic tubes. Freeze-dried aliquots of HS proteins were reconstituted following manufacturer's instructions (SERO, Billingstad, Norway), in order to obtain a final protein concentration in the incubation sample replicating the physiological one. Similarly, appropriate amounts of HSA (Sigma-Aldrich, St. Louis, USA) were dissolved into water in order to simulate the physiologic concentration value (50 mg/ml) after dilution with vesicular suspensions. In order to take into account the effects of physiologic temperature on the vesicle integrity, the incubation tubes were stored at 37°C. Correspondent control tests were carried out by incubating vesicles inside simple isosmotic buffer at 4°C or at 37°C. Gadoteridol molecules released during incubation (likely due to a loss of vesicular structural integrity) were removed by dialysis at 4°C (lasting at least 6 hours, with a minimal buffer volume of 1.5 L).

In a first set of experiments, the incubation was performed in the aforementioned conditions over the same time interval (1 h) for all tested samples and then a massively prolonged dialysis (30 h) was carried out at 4°C with recursive dialysis buffer renewals, in order to completely wash out all the non-encapsulated molecules. The Gadolinium content of samples

was relaxometrically assessed before and after dialysis process, while the R_1^{obs} was measured on the suspensions in time at each buffer renewal.

In a second set of experiments, the incubation was performed by testing previous incubation conditions over different time intervals. At each time point, one aliquot of the incubated sample was picked up and inserted into a cellulose membrane sack to undergo a 6 h dialysis at 4°C. For each data point, the Gadolinium content and the R_1^{obs} of the sample were measured and their variation over time (up to 6 or 48 hours) was graphically represented.

In order to evaluate structural changes, some aliquots of samples incubated with buffer, serum and albumin at 37°C were collected to be tested at DLS. Because the analysis was importantly affected by the presence of proteins inside the sample, serum components (especially albumin) had to be removed. Consequently, before performing DLS, samples were dialysed inside cellulose tubing membranes provided with a pore size of 100 kDa, which are expected to be permissive to the transit of albumin, but not of the much bigger supramolecular vesicles.

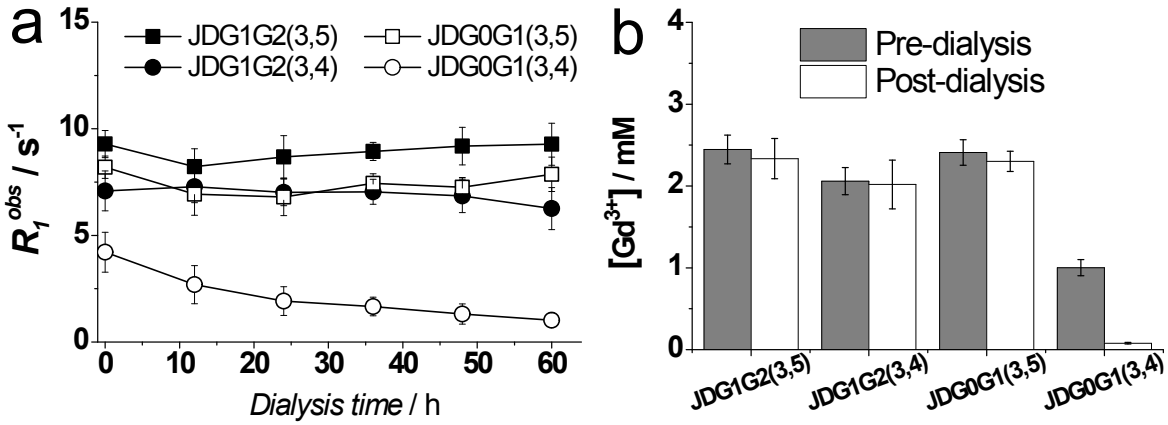


Figure S2. Stability and payload release. a) Water protons longitudinal relaxation rate (R_1^{obs}) values measured for suspensions of dendrimersomes composed of different JDs, incubated for 1 h in isotonic buffer at 4°C and successively dialyzed along consecutive time intervals (12 h each). b) Concentration of Gd^{3+} in the dendrimersomes suspension before and after dialysis (6 cycles).

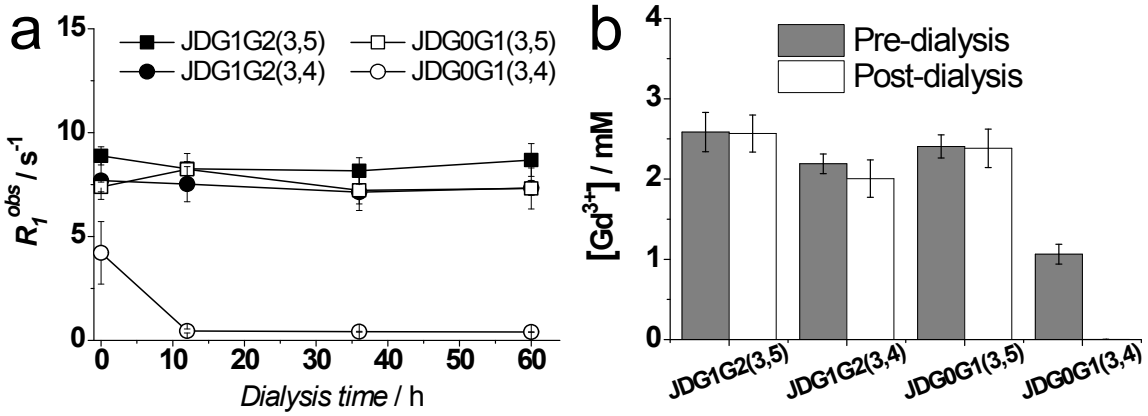


Figure S3. Stability and content release. a) Water protons longitudinal relaxation rate (R_1^{obs}) values measured for suspensions of dendrimersomes composed of different JDs, incubated for 1 h in isotonic buffer at 37°C and successively dialyzed along consecutive time intervals (12 h each). b) Concentration of Gd^{3+} in the dendrimersomes suspension before and after dialysis (6 cycles).

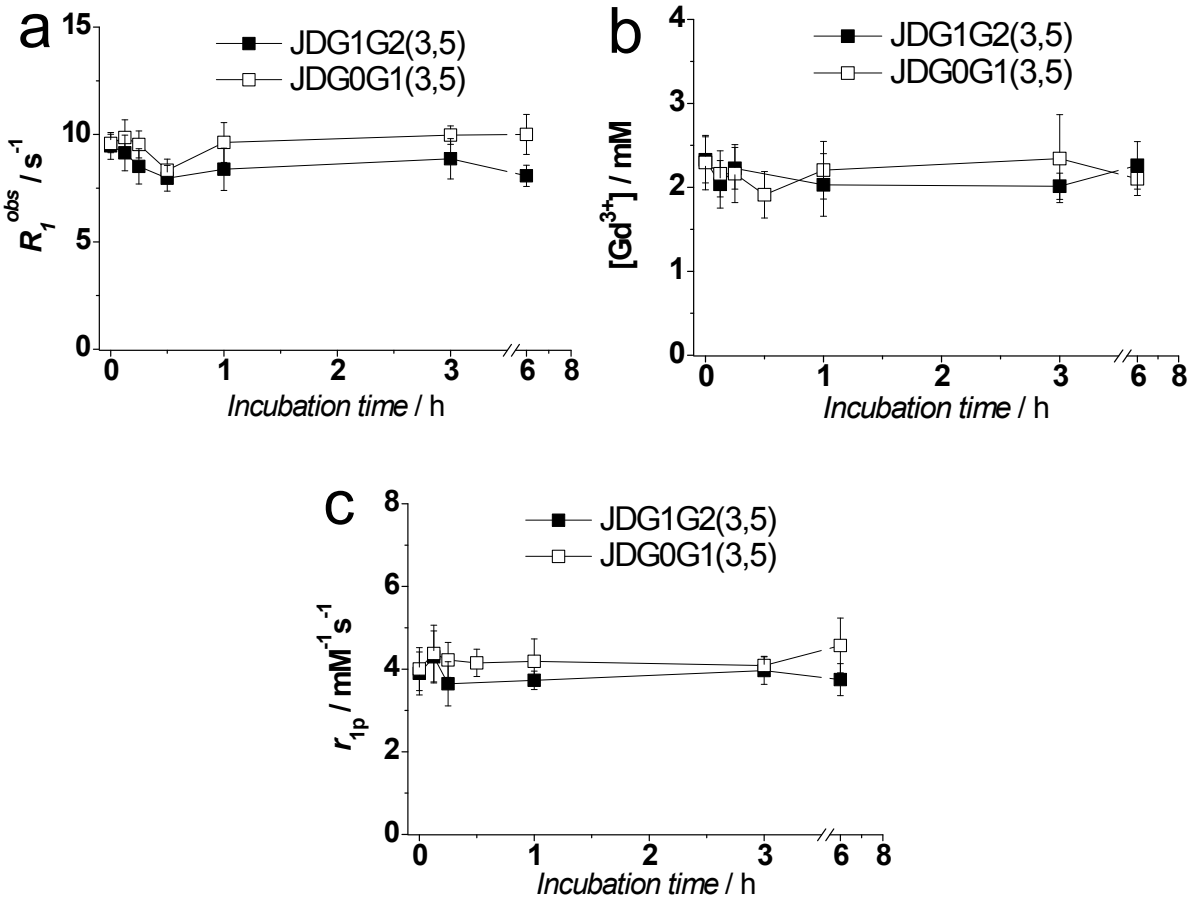


Figure S4. *In vitro* stability of (3,5)JD-based dendrimersomes at physiologic temperature. a) Water protons longitudinal relaxation rate (R_1^{obs}) of different suspensions of dendrimersomes in isosmotic buffer as a function of the incubation time at 37°C. R_1^{obs} was measured on the samples after dialysis. b) Residual concentration of Gd^{3+} in the sample, and c) normalized longitudinal relaxivity (r_1) at each time point.

5. Cell experiments

All cell lines were obtained from American Type Culture Collection (ATCC, Manassass, VA, USA) and resulted negative for mycoplasma test (MycoAlert™ Mycoplasma Detection Kit, Lonza Sales AG, Verviers, Belgium). Murine macrophages (J774A.1 and RAW 264.7 cell lines) and fibroblasts (NIH/3T3 cell lines) were cultured as monolayers at 37°C in a 5% CO_2-containing humidified atmosphere in Dulbecco's modified Eagle's medium (DMEM). The medium was supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum, 2 mM Glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. When cells reached confluence, the detachment was accomplished by mechanical removal by means of a scraper (macrophages) or by trypsin addition (fibroblasts). All cells employed for cellular tests were at passages included between 3 and 7. Culture medium, fetal bovine serum, penicillin-streptomycin mixture and trypsin were all purchased from Lonza (Lonza Sales AG, Verviers, Belgium).

5.1 Cell viability

In order to assess cellular viability after the interaction with investigated vesicles, 5 x 10^4 RAW 264.7, J774A.1 and NIH/3T3 cells were plated in culture dishes and after 24h they were treated with vesicular suspensions for variable time ranges (up to 2 days). To perform the incubation, vesicular suspensions added to cells were previously diluted into the appropriate culture medium to obtain three different dosages, among those that most likely could reproduce the biological dispersion after systemic injection in a common murine model. Being the total blood volume of a 25 g adult mouse around 2 ml (beyond individual variability), immediately after the injection a single dose of vesicular suspension (200 ul of 40 mg/ml) would be diluted to a final concentration of 3.6 mg/ml. The tested concentration range was therefore centered on this estimated value (from 0.1 mg/ml to 10 mg/ml). Viable cells were then counted by Trypan Blue exclusion assay before and after incubation procedure. A

0.4% solution of Trypan Blue in Phosphate Buffered Saline (pH 7.2-7.3; 1:1 v/v) was added to harvested cells, before counting. The Trypan Blue dye traverses the membrane only in dead cells and enables to perform a precise estimation on viable cells by excluding the stained ones from the count. Before being used for cellular incubation, vesicular suspensions were always filtered by sterile 200 um sized filters (Whatman™, GE Healthcare Life Sciences, Amersham Place Little Chalfont, Buckinghamshire HP7 9NA, UK) and diluted into culture medium. Incubation with neat buffered solution diluted into culture medium was used as control condition. Each incubation experiment was repeated in the same conditions at least eight times, and the reported cell viability percentage value represents the average ratio between the number of vital cells and the total number of cells counted in each experiment (N_v/N_t * 100).

Dendrimersome cytotoxicity was further investigated by means of the CellTiter-Blue® Cell Viability Assay (Promega, Madison, USA) in order to confirm results obtained by Trypan blue exclusion assay. The CellTiter-Blue® Cell Viability Assay uses the indicator dye Resazurin to measure the metabolic capacity of cells. While viable cells retain the ability to reduce Resazurin into the highly fluorescent Resorufin, nonviable cells rapidly lose metabolic capacity and do not generate a fluorescent signal. Briefly, J774A.1, RAW 264.7 and NIH/3T3 cells were plated in 96-well dishes at a concentration of 1.0×10^4, 1.5×10^4 and 1.0×10^4 cells per well respectively, placing them at a density for linear growth rate which is optimal for the assay. Cells were allowed to grow overnight at 37°C in a 5% CO_2-containing humidified atmosphere in Dulbecco's modified Eagle's medium (DMEM). The existing culture medium was then replaced with fresh media containing Gadoteridol-encapsulating dendrimersomes at different concentrations. After one hour, the incubation medium was removed and wells were washed three times with sterile PBS, before media containing the CellTiter-Blue® reagent was added following manufacturer's instructions (20 µl of reagent diluted 1:5 into culture medium for each well). Cells were exposed to the reagent for 4 hours, before supernatants

were collected and placed in a black 96-well multiwell plate for fluorescence detection. The fluorescent signal generated from the assay was monitored by using a 530-560 nm excitation wavelength and a 590 nm emission wavelength. Each fluorescence measurement was repeated three times. Each single incubation condition was investigated by six independent experiments all performed in triplicate, to provide statistical significance. Results are expressed as the mean ± SD of the percentage of viable cells, normalized with respect to control samples obtained by incubating cells with PBS containing medium.

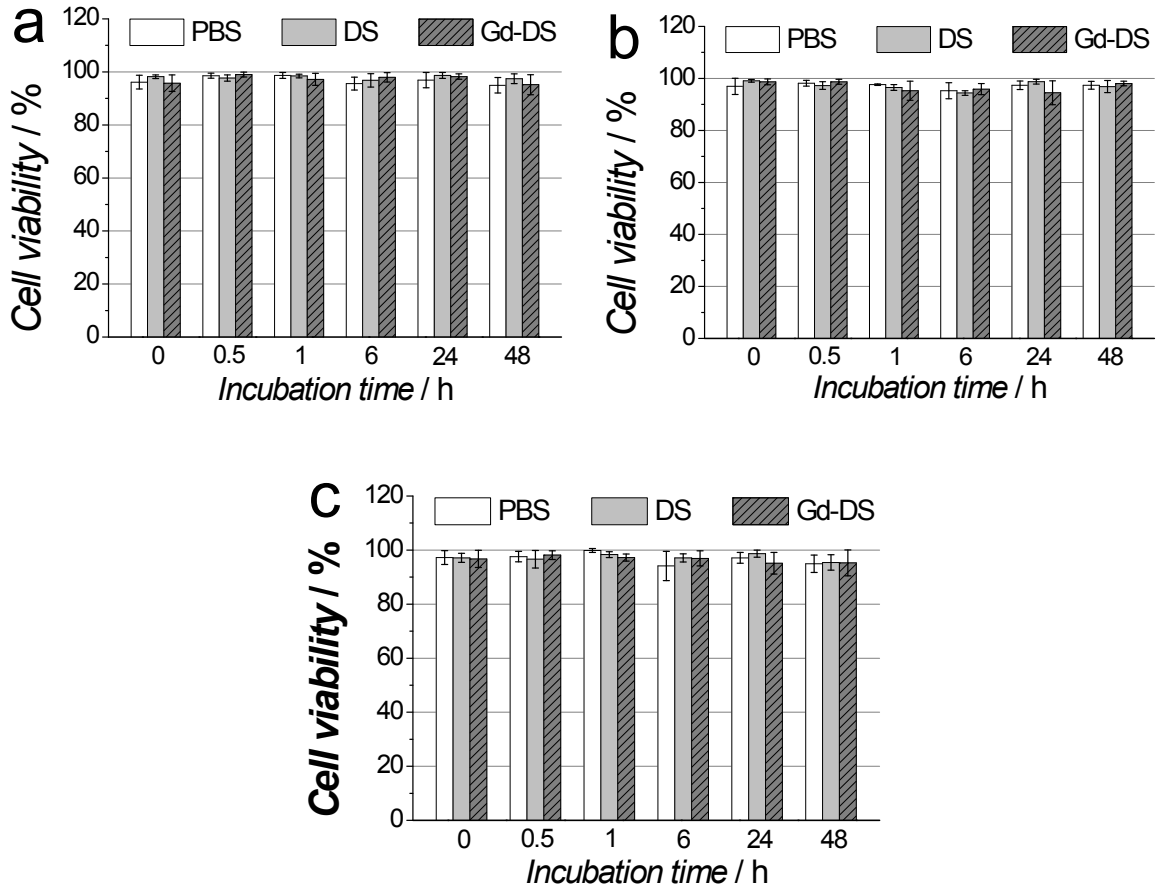


Figure S5. Effects of JDG0G1(3,5)-based dendrimersomes on cell viability (intermediate dose). Cell viability estimated by Trypan Blue assay on a) RAW 264.7 cells, b) J774A.1 cells and c) NIH/3T3 cells after incubation along different incubation times with PBS or JDG0G1(3,5)-based vesicles (1 mg/ml) encapsulating Gadoteridol or not (Gd-DS and DS respectively).

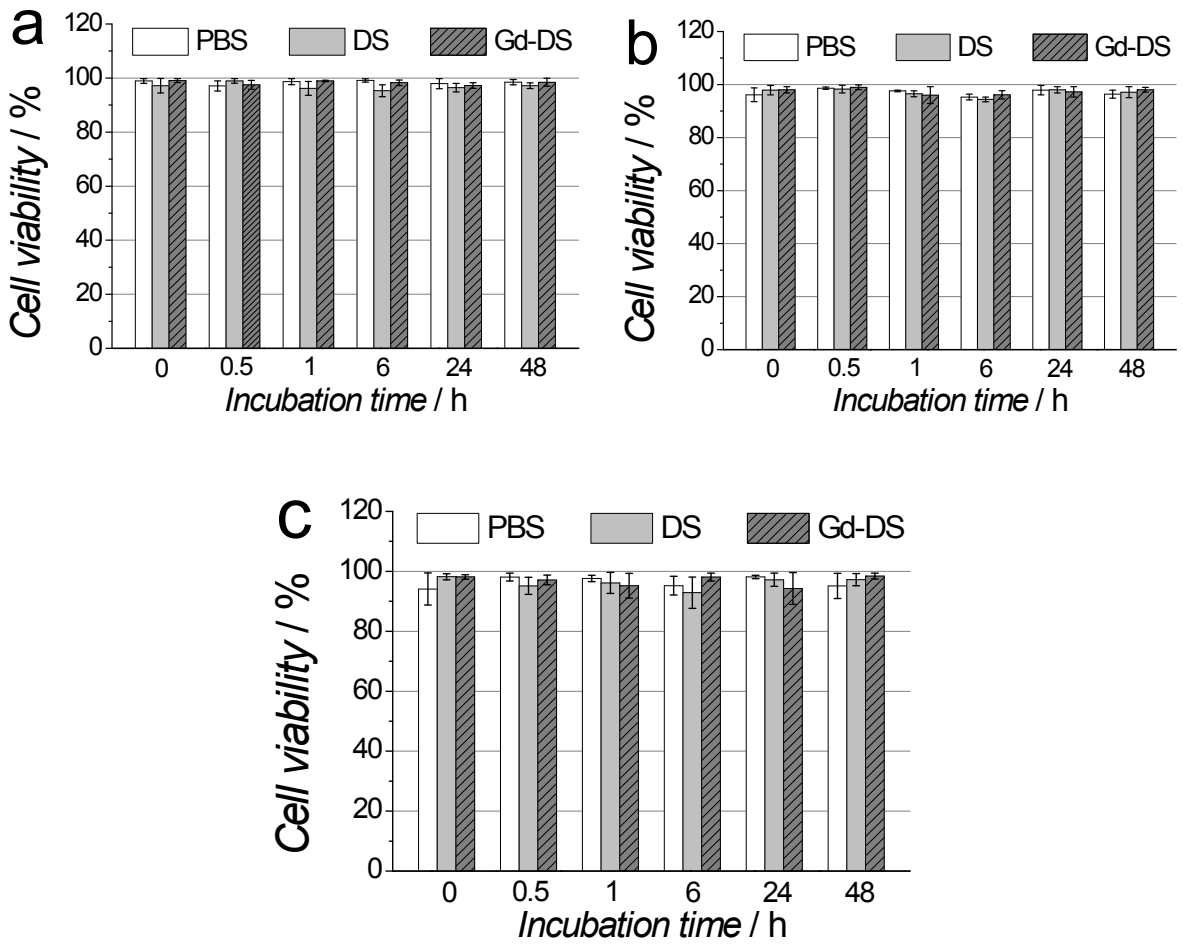
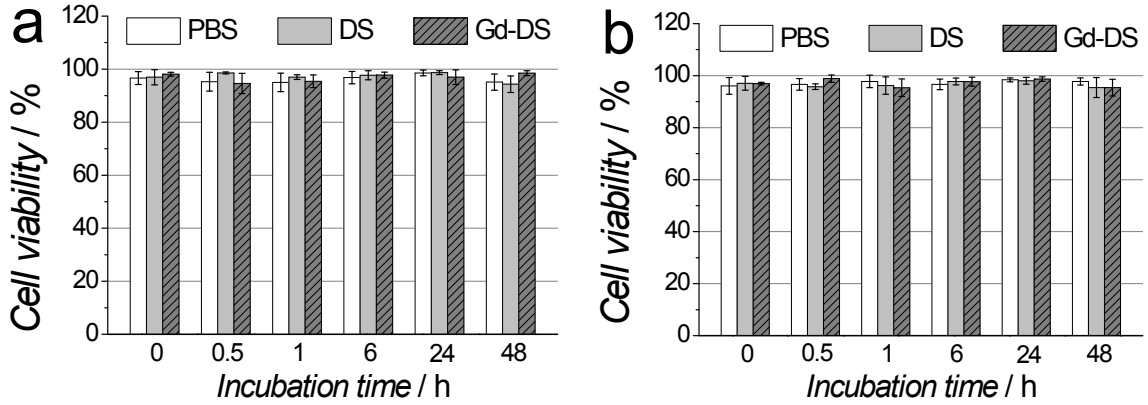


Figure S6. Effects of JDG0G1(3,5)-based dendrimersomes on cell viability (low dose). Cell viability estimated by Trypan Blue assay on a) RAW 264.7 cells, b) J774A.1 cells and c) NIH/3T3 cells after incubation along different incubation times with PBS or JDG0G1(3,5)-based vesicles (0.1 mg/ml) encapsulating Gadoteridol or not (Gd-DS and DS respectively).



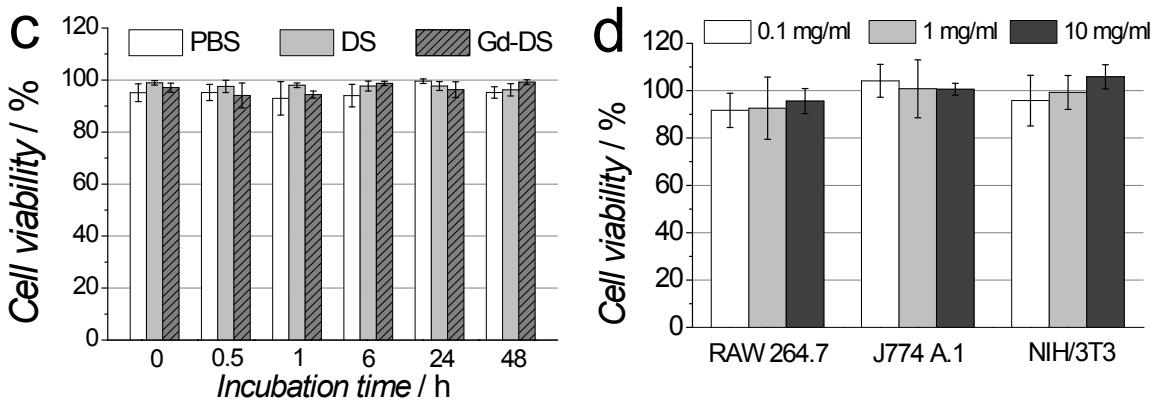


Figure S7. Effects of JDG1G2(3,5)-based dendrimersomes on cell viability. Cell viability estimated by Trypan Blue assay on a) RAW 264.7 cells, b) J774A.1 cells and c) NIH/3T3 cells after incubation along different incubation times with PBS or JDG1G2(3,5)-based vesicles at high concentration (10 mg/ml), encapsulating Gadoteridol or not (Gd-DS and DS respectively; d) cell viability estimated by Resazurin/Resofurin assay after 1 h incubation with JDG1G2(3,5)-based vesicles at different concentrations (0.1/1/10 mg/ml).

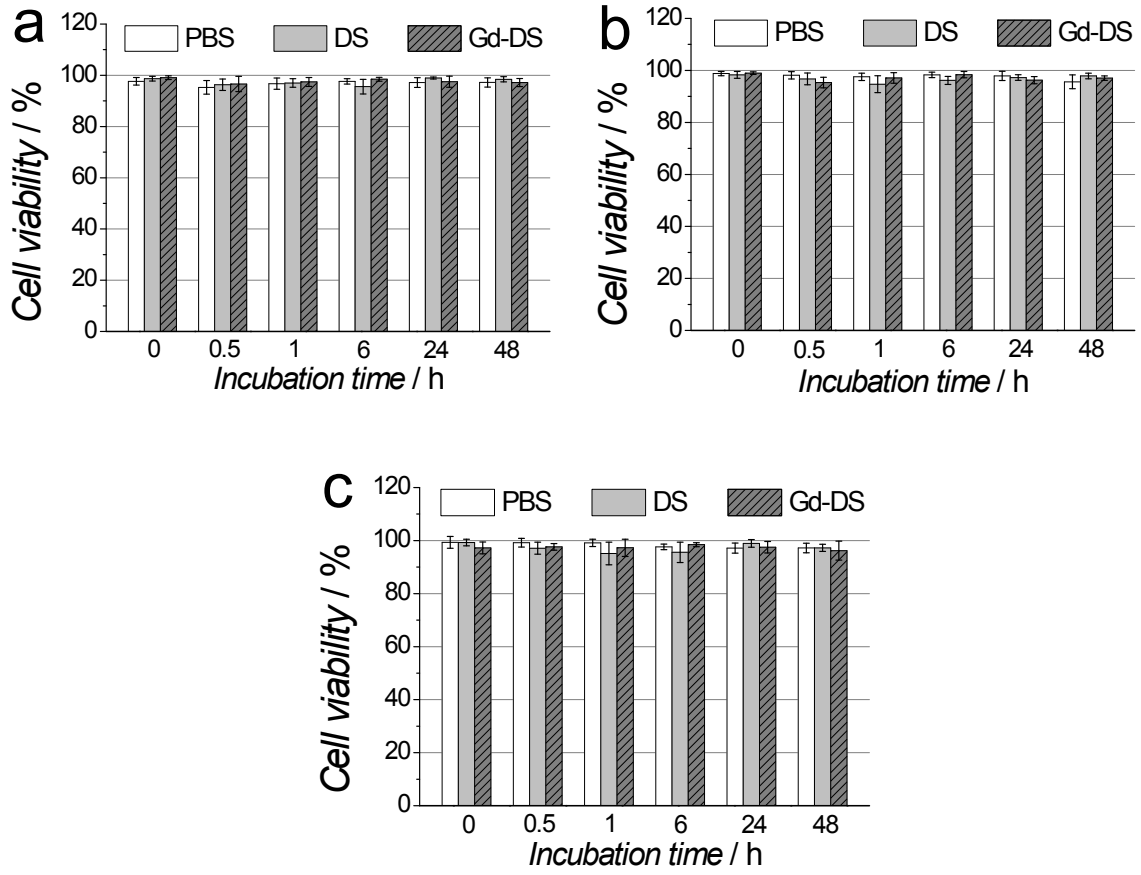


Figure S8. Effects of JDG1G2(3,5)-based dendrimersomes on cell viability (intermediate dose). Cell viability estimated by Trypan Blue assay on a) RAW 264.7 cells, b) J774A.1 cells and c) NIH/3T3 cells after incubation along different incubation times with PBS or JDG1G2(3,5)-based vesicles (1 mg/ml) encapsulating Gadoteridol or not (Gd-DS and DS respectively).

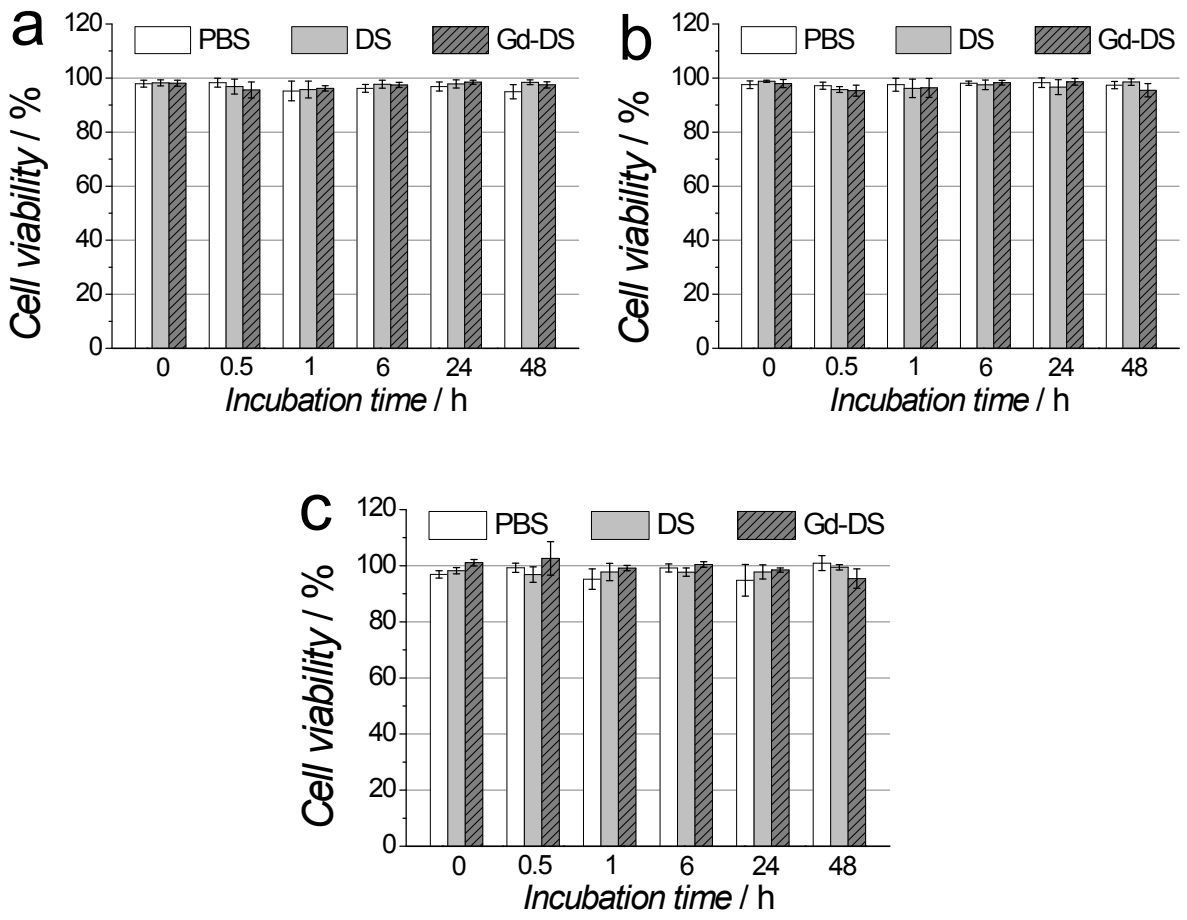


Figure S9. Effects of JDG1G2(3,5)-based dendrimersomes on cell viability (low dose). Cell viability estimated by Trypan Blue assay on a) RAW 264.7 cells, b) J774A.1 cells and c) NIH/3T3 cells after incubation along different incubation times with PBS or JDG1G2(3,5)-based vesicles (0.1 mg/ml) encapsulating Gadoteridol or not (Gd-DS and DS respectively).

5.2 Cell proliferation

For proliferation test, cells were first incubated for 1 h with vesicles at the highest investigated concentration (10 mg/ml in culture medium), before being seeded in round plastic dishes and maintained in fresh culture medium for different time ranges (up to 7 days). Cells were then detached, collected and counted by Trypan Blue assay at each time point. Each experiment was repeated with the same conditions at least five times. Finally, the proliferation ability was reported as the ratio between the number of cells at each time point and the number of cells present at the beginning of the experiment (N_t/N_0).

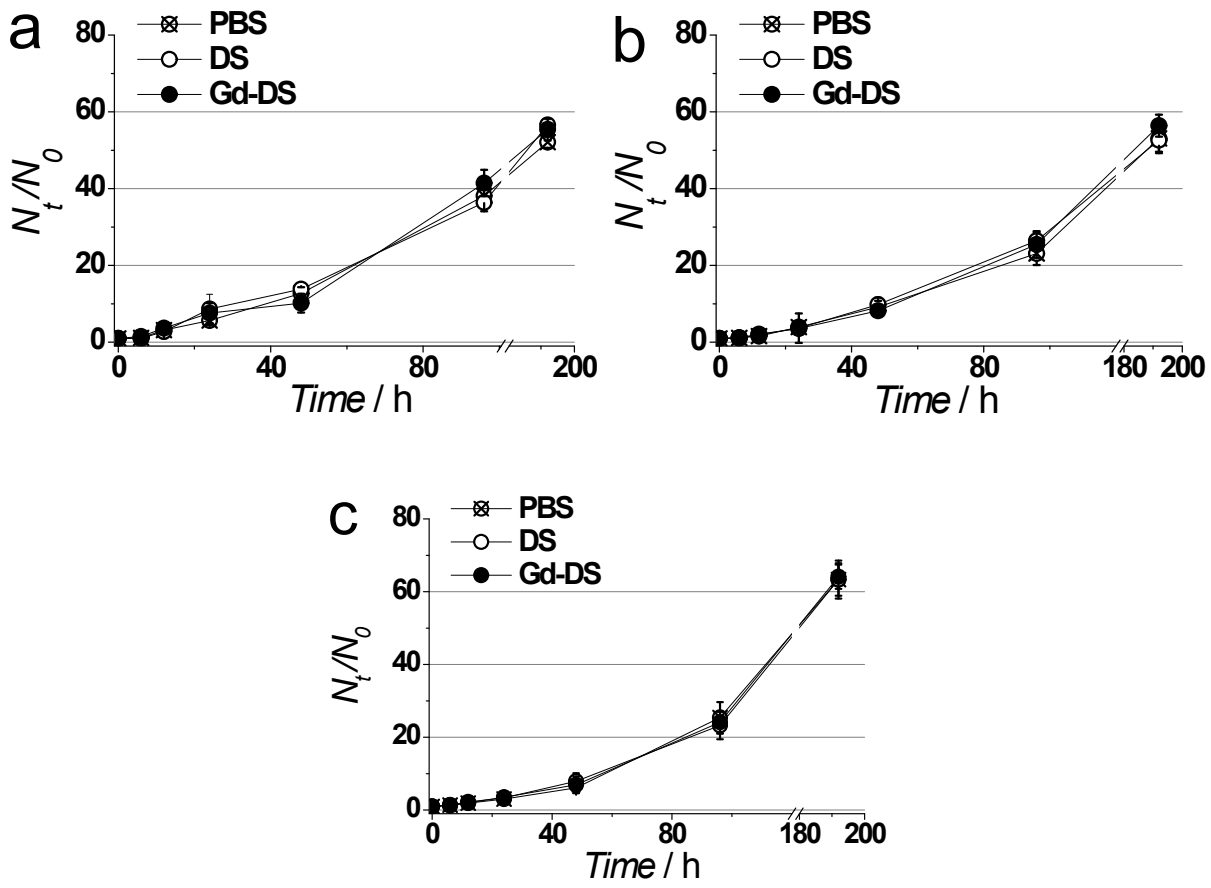


Figure S10. Effects of JDG1G2(3,5)-based dendrimersomes on cell proliferation. Proliferation rate of a) RAW 264.7 cells, b) J774A.1 cells and c) NIH/3T3 cells after 1 h incubation with PBS or JDG1G2(3,5)-based dendrimersomes (10 mg/ml of JD), loaded with Gadoteridol or not (Gd-DS and DS, respectively). N_t and N_0 represent the number of cells at each time point and the number of cells present at the beginning of the experiment respectively.

6. *In vivo* biodistribution

After last blood sampling (namely 24 h after being administered with Gadoteridol-loaded dendrimersomes or liposomes, or free Gadoteridol), animals were sacrificed to obtain the organs (liver, spleen, kidneys, muscle, heart, lung). The content of Gd(III) was quantified by ICP-MS after acidic tissue digestion. Briefly, the weights of all organs were registered before they were added with 70% concentrated HNO_3 (5 mL and 3 mL for liver and all other organs, respectively). After several days, the tissue dissolution was accomplished, and the samples were ready to be digested by applying microwave heating (Milestone MicroSYNTH, Microwave labstation equipped with an optical fiber temperature control and HPR-1000/6M six position high-pressure reactor, Bergamo, Italy). After digestion, the remaining material was diluted in ultrapure water (final volume of 3 mL) and further filtered with 0.2 μm filters. Finally, samples were analysed by ICP-MS (Thermo Scientific ELEMENT 2 ICP-MS - Finnigan, Rodano, Milano, Italy) to measure the Gd(III) content, which was then expressed as a function of the organ mass as moles/kg).

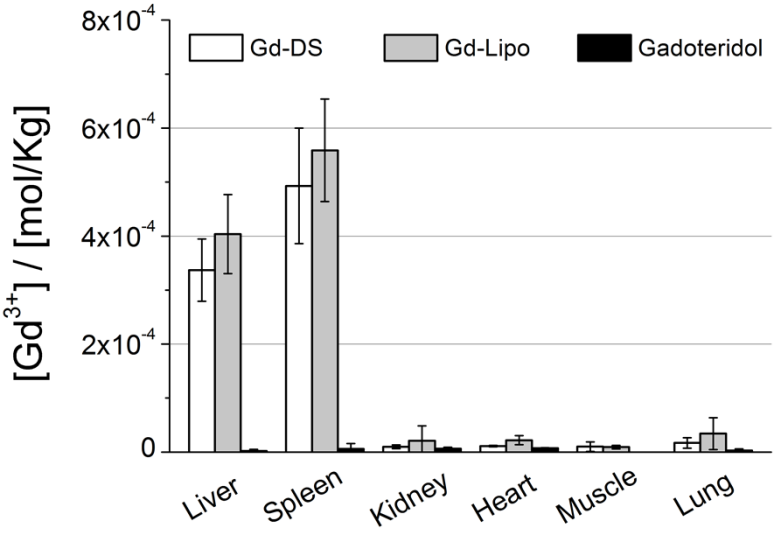


Figure S11. In vivo biodistribution of JDG0G1(3,5)-based dendrimersomes. Gd(III) concentration measured in different organs (liver, spleen, kidney, heart, muscle, lung) 24 h after the intravenous injection of Gadoteridol (0.16 mmol/kg), administered in a free form (Gadoteridol, black bars) or loaded onto dendrimersomes (Gd-DS, white bars) or liposomes (Gd-Lipo, grey bars).

While no traces of the metal were detected in any analysed organ after simple Gadoteridol administration, in animals injected with both vesicular systems, the expected accumulation sites for nanoparticles (liver and spleen) showed high Gd(III) uptake. The reported concentration values did not indicate any significant difference between dendrimersomes and control liposomes. As confirmed by the quantification of circulating Gd(III), free gadoteridol molecules followed a very rapid clearance kinetics with no residual metal content remaining in the analyzed organs after 24 h. On the other side, relatively high amounts of Gd(III) were detected in liver and spleen, when the animals received Gd-loaded particles. Moreover, in this case the particle were not taken up by the other control organs, with the only exception of lungs, where a very low concentration was measured, likely related to the phagocytic activity of alveolar macrophages.

7. References

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