Electronic Supplementary Information

Multifunctional squalene-based prodrug nanoparticles for targeted cancer therapy

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1. Materials

2. Analytical methods
   2.1 Nuclear magnetic resonance spectroscopy (NMR)  
   2.2 Transmission electron microscopy (TEM)  
   2.3 Dynamic light scattering (DLS) and zeta potential

3. Synthesis methods
   3.1 Synthesis of 2-(2-(2-(((4E,8E,12E,16E)-4,8,13,17,21-pentamethyldocosa-4,8,12,16,20-pentaen-1-yl)oxy)ethoxy)ethoxy)ethanol (3)  
   3.2 Synthesis of 2-(2-(2-(((4E,8E,12E,16E)-4,8,13,17,21-pentamethyldocosa-4,8,12,16,20-pentaen-1-yl)oxy)ethoxy)ethoxy)ethyl 5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate (Biotin-Sq, 4)  
   3.3 Synthesis of rhodamine B 4-(1,1’,2-trisnorsqualenoyl)piperazine (Rho-Sq, 7)  
   3.4 Nanoparticle preparation

4. Biological activity
   4.1 Cell lines and cell culture  
   4.2 Cell internalization  
   4.3 In vivo anticancer activity  
   4.4 Endocytosis in the presence of inhibitors

5. References
1. Materials

Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl. Dimethylformamide (DMF) and dichloromethane (DCM) were distilled from calcium hydride, under a nitrogen atmosphere. All reactions involving air- or water-sensitive compounds were routinely conducted with a flame-dried glassware under a positive pressure of nitrogen. Diisopropyl azodicarboxylate (94%) was purchased from Acros Organics. Sodium hydride (95%), Filipin III (>85%), chlorpromazine (98%), DMA (5-(N,N-dimethyl) amiloride hydrochloride, triethylene glycol (99%), methanol (99.8%) and 3-[4,5-dimethylthiazol-2-yl]-3,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich Chemical Co., France. Gemcitabine, squalene, biotin (98%), triphenylphosphine (99%), ethyl chloroformate (97%), 4-dimethylaminopyridine (DMAP, 99%) and imidazole (99%) were purchased from Alfa-Aesar (A Johnson Matthey Co., France). RPMI 1640 GlutaMAX I, DMEM GlutaMAX I and fetal bovine serum were purchased from Dulbecco (Invitrogen, France). Penicillin and streptomycin solution were purchased from Lonza (Verviers, Belgium). Chemicals obtained from commercial suppliers were used without further purification. Rhodamine-B piperazine and trisnorsqualenyl methanesulfonate were synthesized as published elsewhere.1,2

2. Analytical methods

2.1 Nuclear magnetic resonance spectroscopy (NMR). NMR spectroscopy was performed in 5 mm diameter tubes at 25 °C. 1H and 13C NMR spectroscopy were performed on a Bruker Avance 400 spectrometer at 400 MHz (1H) or 100 MHz (13C). The chemical shift scale was calibrated on the basis of the solvent peak. Recognition of methyl, methylene, methine, and quaternary carbon nuclei in 13C NMR spectra rested on the J-modulated spin-echo sequence. 19F NMR spectroscopy was performed on a Bruker 200 spectrometer at 188 MHz.
2.2 Transmission electron microscopy (TEM). The morphology of the different nanoassemblies was examined by cryogenic transmission electron microscopy (Cryo-TEM). Briefly, 5 μL of the nanoparticle suspension (5 mg.mL⁻¹) was deposited on a Lacey Formvar/carbon 300 mesh copper microscopy grid (Ted Pella). Most of the drop was removed with a blotting filter paper and the residual thin film remaining within the holes was vitrified by plunging into liquid ethane. Samples were then observed using a JEOL 2100HC microscope.

2.3 Dynamic light scattering (DLS) and zeta potential. Nanoparticle diameters (Dₚ) and zeta potentials (ζ) were measured by dynamic light scattering (DLS) with a Nano ZS from Malvern (173° scattering angle) at a temperature of 25 °C. The surface charge of the nanoparticles was investigated by ζ-potential (mV) measurement at 25 °C, after dilution with 1 mM NaCl, using the Smoluchowski equation. Measurements were performed in triplicate following dilution of the nanoparticle suspensions in water.

3. Synthesis methods

3.1 Synthesis of 2-(2-(2-(((4E,8E,12E,16E)-4,8,13,17,21-pentamethyldocosa-4,8,12,16,20-pentaen-1-yl)oxy)ethoxy)ethoxy)ethoxy)ethanol (3).

![Chemical structure](image)

To an ice-cooled suspension of sodium hydride (190 mg, 7.5 mmol) in anhydrous THF (10 mL) was added dropwise a solution of triethylene glycol (1.13 g, 7.5 mmol). After 30 min at 0 °C, the hydrogen evolution has totally ceased and a solution of trisnorsqualenyl methanesulfonate (2) (700 mg, 1.5 mmol)
in anhydrous THF (5 mL) was added dropwise. After being stirred at 20 °C for 1 day, the volatiles were removed under reduced pressure. The residue was taken up in water (10 mL) and extracted with ethyl acetate (4 × 20 mL). The combined organic layers were washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude product was then purified by flash chromatography (SiO₂, petroleum ether/AcOEt 1:1) to give trisnorsqualenyl triethylene-glycol ether (3) (300 mg, 40%) as a colorless viscous oil. ¹H NMR (400 MHz, CDCl₃, δ in ppm) 5.18−5.05 (5H, m, HC=C(CH₃)CH₂), 3.74−3.70 (2H, m, HOCH₂CH₂), 3.69−3.55 (10H, m, HOCH₂CH₂, OCH₂CH₂O), 3.42 (2H, t, J = 6.8 Hz, OCH₂CH₂CH₂), 2.11−1.93 (16H, m, =C(CH₃)CH₂CH₂CH=), 1.67 (2H, m, CH₂CH₂CH₂C(CH₃)), 1.67 (3H, s, HC=C(CH₃)₂), 1.59 (15H, s, HC=C(CH₃)); ¹³C NMR (100 MHz, CDCl₃, δ in ppm) 135.2 (C, HC=C(CH₃)CH₂), 135.2 (C, HC=C(CH₃)CH₂), 135.0 (C, HC=C(CH₃)CH₂), 134.4 (C, HC=C(CH₃)CH₂), 131.3 (C, C=C(CH₃)₂), 124.7 (CH, HC=C(CH₃)₂), 124.5 (CH, HC=C(CH₃)CH₂), 124.4 (3CH, HC=C(CH₃)CH₂), 72.7 (CH₂, HOCH₂CH₂O), 71.2 (CH₂, OCH₂CH₂CH₂), 70.7 (CH₂, OCH₂CH₂O), 70.7 (CH₂, OCH₂CH₂O), 70.5 (CH₂, OCH₂CH₂O), 70.2 (CH₂, OCH₂CH₂O), 61.9 (CH₂, HOCH₂CH₂), 39.8 (3CH₂, =C(CH₃)CH₂CH₂CH=), 36.0 (CH₂, CH₂CH₂CH₂C(CH₃), 28.4 (2CH₂, =CHCH₂CH₂CH=), 27.9 (CH₂, CH₂CH₂CH₂C(CH₃), 26.9 (CH₂, =C(CH₃)CH₂CH₂CH=), 26.8 (2CH₂, =C(CH₃)CH₂CH₂CH=), 25.8 (CH₃, CH₂C=C(CH₃)₂), 17.8 (CH₃, CH₂C=C(CH₃)₂), 16.2 (2CH₃, =C(CH₃)CH₂), 16.1 (CH₃, =C(CH₃)CH₂), 16.0 (CH₃, =C(CH₃)CH₂); MS (+APCI) m/z (%): 519.5 (100) [M + H]⁺.
3.2 Synthesis of 2-(2-((4E,8E,12E,16E)-4,8,13,17,21-pentamethyldocosa-4,8,12,16,20-pentaen-1-yl)oxy)ethoxy)ethoxy)ethyl 5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate (Biotin-Sq, 4).

To an ice-cooled solution of biotin (145 mg, 0.59 mmol) and triphenylphosphine (156 mg, 0.59 mmol) in anhydrous DMF (8 mL) was added dropwise diisopropyl azodicarboxylate (127 mg, 7.5 mmol). The reaction mixture was stirred for 1 h and a solution of trisnorqualenyl tri(ethylene glycol) ether (3) (200 mg, 0.39 mmol) in anhydrous DMF (2 mL) was added dropwise. After being stirred at 20 °C for 3 days, the volatiles were removed under reduced pressure. The residue was taken up in water (5 mL) and extracted with dichloromethane (3 × 10 mL). The combined organic layers were washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude was then purified by flash chromatography (SiO₂, CH₂Cl₂/methanol 95:5) to give trisnorqualenyl tri(ethylene glycol) biotin ester (Biotin-Sq, 4) (87 mg, 30%) as a colorless viscous oil. $^1$H NMR (400 MHz, CD₃OD, δ in ppm) 5.18–5.06 (5H, m, HC=C(CH₃)CH₂), 4.49 (1H, ddd, J = 7.9 Hz, J = 5.0 Hz, J = 0.8 Hz, HNCHCH₂S), 4.31 (1H, dd, J = 7.9 Hz, J = 4.5 Hz, Hz, HNCHCHS), 4.24–4.19 (2H, m, CO₂CH₂CH₂), 3.72–3.68 (2H, m, CO₂CH₂CH₂O), 3.65–3.60 (6H, m, OCH₂CH₂O), 3.59–3.54 (2H, m, OCH₂CH₂O), 3.44 (2H,
t, $J = 6.6$ Hz, OCH$_2$CH$_2$CH$_2$), 3.20 (1H, m, SCHCH$_2$), 2.93 (1H, dd, $J = 12.8$ Hz, $J = 5.0$ Hz, HNCHCH$_2$S), 2.70 (1H, d, $J = 12.8$ Hz, HNCHCH$_2$S), 2.37 (2H, t, $J = 7.3$ Hz, CH$_2$CH$_2$CO$_2$), 2.14–1.93 (18H, m, SCHCH$_2$CH$_2$, CH$_2$CH$_2$CO$_2$), 1.67 (3H, s, HC=C(CH$_3$)$_2$), 1.61 (15H, s, HC=C(CH$_3$)$_2$), 1.47 (2H, q, $J = 8.0$ Hz, CH$_2$CH$_2$CH$_2$CO$_2$); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 175.2 (C, CH$_2$CO$_2$CH$_2$), 166.1 (C, NHCONH)*, 136.0 (2C, HC=C(CH$_3$)CH$_2$), 135.8 (C, HC=C(CH$_3$)CH$_2$), 135.5 (C, HC=C(CH$_3$)CH$_2$), 132.0 (C, C=C(CH$_3$)$_2$), 125.7 (CH, HC=C(CH$_3$)$_2$), 125.6 (2CH, HC=C(CH$_3$)CH$_2$), 125.5 (2CH, HC=C(CH$_3$)CH$_2$), 71.9 (CH$_2$, OCH$_2$CH$_2$CH$_2$), 71.62 (2CH$_2$, OCH$_2$CH$_2$O), 71.57 (CH$_2$, OCH$_2$CH$_2$O), 71.2 (CH$_2$, OCH$_2$CH$_2$O), 70.2 (CH$_2$, CO$_2$CH$_2$CH$_2$O), 64.6 (CH$_2$, CO$_2$CH$_2$CH$_2$O), 63.4 (CH, NHCHCHCH$_2$), 61.6 (CH, NHCHCH$_2$S), 57.0 (CH, NHCHCHCH$_2$), 41.0 (CH$_2$, NHCHCH$_2$S), 40.9 (CH$_2$, =C(CH$_3$)CH$_2$CH$_2$CH=), 40.8 (2CH$_2$, =C(CH$_3$)CH$_2$CH$_2$CH=), 37.1 (CH$_2$, CH$_2$CH$_2$CH$_2$C(CH$_3$)$_3$), 34.7 (CH$_2$, CH$_2$CH$_2$CO$_2$), 29.7 (CH$_2$, CH$_2$CH$_2$CH$_2$CO$_2$), 29.5 (CH$_2$, SCHCH$_2$CH$_2$), 29.2 (2CH$_2$, =CHCH$_2$CH$_2$CH=), 29.1 (CH$_2$, CH$_2$CH$_2$CH$_2$C(CH$_3$)$_3$), 27.8 (CH$_2$, =C(CH$_3$)CH$_2$CH$_2$CH=), 27.6 (CH$_2$, =C(CH$_3$)CH$_2$CH$_2$CH=), 27.6 (CH$_2$, =C(CH$_3$)CH$_2$CH$_2$CH=), 25.9 (CH$_2$, CH$_2$CH$_2$CO$_2$), 25.9 (CH$_3$, CH$_2$C=C(CH$_3$)$_2$), 17.8 (CH$_3$, CH$_2$C=C(CH$_3$)$_2$), 16.2 (2CH$_3$, =C(CH$_3$)CH$_2$), 16.1 (CH$_3$, =C(CH$_3$)CH$_2$), 16.1 (CH$_3$, =C(CH$_3$)CH$_2$); MS (+APCI) $m$/z (%): 745.6 (100) [M + H]$^+$.  

*: HMBC detected.

3.3 Synthesis of rhodamine B 4-(1,1’,2-trisnorsqualenoyl)piperazine (Rho-Sq, 7).

![Chemical structure of Rho-Sq](image-url)
To a solution of trisnorsqualenic acid (5) (120 mg, 0.3 mmol) in anhydrous THF (2 mL) was added Et$_3$N (90 µL, 0.6 mmol). The mixture was cooled at 0 °C and a solution of ethyl chloroformate (30 µL, 0.33 mmol) in THF (1 mL) was added dropwise. The mixture was stirred for 30 min at 0 °C and a solution of rhodamine B piperazine (6) (181 mg, 0.33 mmol) in THF (1 mL) was added dropwise. After being stirred at 20 °C for 1 day, the volatiles were removed under reduced pressure. The residue was taken up in sat. NaHCO$_3$ aqueous solution (4 mL) and extracted with CH$_2$Cl$_2$ (3 × 15 mL). The combined organic layers were washed with brine, dried over MgSO$_4$ and concentrated under reduced pressure. The crude was then purified by flash chromatography (SiO$_2$, CH$_2$Cl$_2$/Methanol 90:10) to give rhodamine B 4-(1,1',2-trisnorsqualenoyl)piperazine (7) (231 mg, 90%) as a dark purple waxy solid. $^1$H NMR (400 MHz, CDCl$_3$, δ in ppm) 7.78 (2H, m, H-4’, H-5’), 7.70 (1H, dd, J = 6.6 Hz, J = 3.9 Hz, H-3’), 7.52 (1H, m, H-6’), 7.29 (2H, d, J = 9.5 Hz, H-1, H-8), 7.08 (2H, dd, J = 9.5 Hz, J = 2.4 Hz, H-2, H-7), 6.97 (2H, d, J = 2.4 Hz, H-4, H-5), 5.03–5.20 (5H, m, HC=C(CH$_3$)CH$_2$), 3.69 (8H, q, J = 7.1 Hz, H$_3$CC$_2$H$_2$N), 3.32–3.48 (8H, m, NC$_2$H$_2$C$_2$N), 2.48–2.39 (2H, m, NOCC$_2$CH$_2$), 2.24–2.16 (2H, m, NOCCH$_2$CH$_2$), 2.13–1.95 (16H, m, =C(CH$_3$)CH$_2$CH$_2$CH=), 1.66 (3H, s, HC=C(CH$_3$)$_2$), 1.60 (3H, s, HC=C(CH$_3$)$_2$), 1.59 (12H, s, HC=C(CH$_3$)$_2$), 1.31 (12H, t, J = 7.1 Hz, H$_3$CCH$_2$N); $^{13}$C NMR (100 MHz, CD$_3$OD, δ in ppm) 173.7 (C, NCOCH$_2$CH$_2$), 169.5 (C, ArCON), 159.3 (2C, C-4a, C-4b), 157.2 (2C, CNEt$_2$), 157.0 (C, C-9), 163.6 (2C, C-1’, C-2’), 136.1 (C, HC=C(CH$_3$)CH$_2$), 135.9 (2C, HC=C(CH$_3$)CH$_2$), 134.8 (C, HC=C(CH$_3$)CH$_2$), 133.2 (2CH, C-1, C-8), 132.2 (C, C=C(CH$_3$)$_2$), 131.8 (CH, C-6’), 131.4 (CH, C-4’ or C-5’), 131.3 (CH, C-4’ or C-5’), 128.9 (CH, C-3), 126.3 (CH, HC=C(CH$_3$)$_2$), 125.7 (CH, HC=C(CH$_3$)CH$_2$), 125.5 (CH, HC=C(CH$_3$)CH$_2$), 125.4 (2CH, HC=C(CH$_3$)CH$_2$), 115.4 (2CH, C-2, C-7), 114.9 (2C, C-8a, C-9a), 97.4 (2CH, C-4, C-5), 48.1 (CH$_2$, NCH$_2$CH$_2$N)*, 46.9 (4CH$_2$, H$_3$CCH$_2$N), 46.2 (CH$_2$, NCH$_2$CH$_2$N)*, 42.5 (2CH$_2$, NCH$_2$CH$_2$N)*, 40.9 (CH$_2$, =C(CH$_3$)CH$_2$CH$_2$CH=), 40.8 (CH$_2$, =C(CH$_3$)CH$_2$CH$_2$CH=), 40.7 (CH$_2$, =C(CH$_3$)CH$_2$CH$_2$CH=), 36.1 (CH$_2$, OCCH$_2$CH$_2$C(CH$_3$)=), 29.2 (2CH$_2$, =CHCH$_2$CH$_2$CH=), 27.8
(CH₂, =C(CH₃)CH₂CH₂CH=), 27.6 (2CH₂, =C(CH₃)CH₂CH₂CH=), 25.9 (CH₃, C=C(CH₃)₂), 17.8 (CH₃, CH₂C=C(CH₃)₂), 16.2 (3CH₃, =C(CH₃)CH₂), 12.8 (4CH₃, H₂CCH₂N); MS (+APCI) m/z (%): 909.6 (16) [M – Cl + H₂O]⁺, 893.9 (100) [M – Cl]⁺.

*: HMBC detected.

3.4 Nanoparticle preparation

Nanoparticles were prepared by the nanoprecipitation technique. Nanoparticles N1* at 1 mg.mL⁻¹ were prepared as follows. Stock solutions of Gem-Sq (1 mg) in 0.1 mL of THF, Biotin-Sq (1 mg) in 0.1 mL of methanol and Rho-Sq (1 mg) in 0.1 mL of methanol were prepared. A mixed solution of Gem-Sq/Biotin-Sq/Rho-Sq (86:9:5 wt.%) was then prepared and 0.1 mL of this solution was added dropwise under vigorous stirring (500 rpm) to 1 mL of MilliQ water. Formation of the nanoparticles occurred spontaneously and stirring was continued for 3 min. The suspension was then transferred into a weighted round bottom flask and the solvents were evaporated at ambient temperature using a Rotavapor. Other nanoparticles were identically prepared and weight ratios were adjusted accordingly. Colloidal stability of the nanoparticles was assessed for at least a week.

Table S1. Average Diameters and Particle Size Distributions (PSD) for the Different Nanoparticles Employed in this Study.

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Composition (x:y:z wt.%)</th>
<th>Dₜ</th>
<th>PSDᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1*</td>
<td>Gem-Sq/Biotin-Sq/Rho-Sq (86:9:5)</td>
<td>149</td>
<td>0.15</td>
</tr>
<tr>
<td>N1</td>
<td>Gem-Sq/Rho-Sq (95:5)</td>
<td>120</td>
<td>0.19</td>
</tr>
<tr>
<td>N2*</td>
<td>Gem-Sq/Biotin-Sq/Rho-Sq/Chol-BODIPY (85:9:5:1)</td>
<td>94</td>
<td>0.20</td>
</tr>
<tr>
<td>N2</td>
<td>Gem-Sq/Rho-Sq/Chol-BODIPY (94:5:1)</td>
<td>101</td>
<td>0.17</td>
</tr>
<tr>
<td>N3</td>
<td>Rho-Sq/Chol-BODIPY (80:20)</td>
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<td>0.17</td>
</tr>
<tr>
<td>N4</td>
<td>Gem-Sq</td>
<td>140</td>
<td>0.14</td>
</tr>
<tr>
<td>N5</td>
<td>Biotin-Sq/Rho-Sq (77:33)</td>
<td>261</td>
<td>0.18</td>
</tr>
</tbody>
</table>

ᵃParticle size distribution determined by the DLS apparatus.
4. Biological activity

4.1 Cell lines and cell culture

Human breast adenocarcinoma cells (MCF-7) and human cervix carcinoma cells (HeLa) were obtained from the American Type Culture Collection. Murin lung tumor cells (M109) were obtained from the University of Florida, USA. All cell lines were maintained as recommended. Briefly, M109 cells were cultured in RPMI 1640 medium. MCF-7 and Hela cells were grown in Dulbecco's minimal essential medium (DMEM). All media were supplemented with 10% heat-inactivated foetal bovine serum (FBS) (56°C, 30 min), penicillin (100 U.mL\(^{-1}\)) and streptomycin (100 μg.mL\(^{-1}\)). Cells were maintained in a humid atmosphere at 37 °C with 5% CO\(_2\).

4.2 Cell internalization

To quantitatively measure the cell internalization of the nanoparticles, Hela, M109 and MCF-7 cells were cultured on 12-well plates for 24 h to achieve 60-80 % confluence. The different nanoparticle samples were then added at the concentration of 1 μM to each well. After incubation, the cells were collected at different time intervals for measurement of rhodamine B fluorescence. Cells were incubated for 4 h at 4 °C or 37 °C. The fluorescence from individual cells was examined using a flow cytometer C6 (Accuri Cytometers Ltd., UK). For fluorescence detection of nanoparticles, excitation was carried out with the 488-nm line of an argon laser, and emission fluorescence was measured between 560 and 606 nm. 10000 cells were measured in each sample. All experiments were set up in triplicate to determine means and SDs. For the experiment with the dual fluorescently labelled nanoparticles, emission of fluorescence was performed at 560 nm (rhodamine B) and 515 nm (BODIPY).
Figure S1. Kinetics of cell capture of non-functionalized (Gem-Sq/Rho-Sq/Chol-BODIPY) N2 and biotin-functionalized (Gem-Sq/Biotin-Sq/Rho-Sq/Chol-BODIPY) N2* nanoparticles in HeLa cells at 37 °C monitored at 560 nm (a) and 515 nm (b).

For confocal microscopy experiment, Hela cells were cultured on a coverslip in a culture dish for 24 h to achieve approximately 40% confluence. Cells were then incubated with different kinds of nanoparticles at the concentration of 10 µM at 37 °C for different time periods. After incubation, the cells were washed with Dulbecco's PBS five times and imaged using a confocal laser scanning microscope LSM 510 META (Zeiss, Germany) equipped with a 1 mW Helium Neon laser and a Plan-Apochromat 63X objective lens (Numerical Aperture / 1.4, oil immersion). Excitation was carried out with the 488-nm line of an argon laser, and emission was performed at 560 nm (rhodamine B) and 515 nm (BODIPY).

4.3 *In vitro* anticancer activity

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] was used to test the cytotoxic activity of the nanoparticles. Briefly, cells (5 × 10^3/well) were seeded in 96-well plates. After overnight incubation, the cells were exposed to different kinds of nanoparticles at a concentration of 1 mg.mL⁻¹ for 72 h. The medium was then removed and 100 µL of MTT solution (0.5 mg.mL⁻¹ in DMEM containing
10% FBS) were added to each well. The plates were incubated for 2 h at 37 °C and 100 µL of 20% SDS solution were then added to each well for 24 h at 37 °C. Absorbance was measured at 570 nm using a plate reader (Metertech Σ 960, Fisher Bioblock, Illkirch, France). The percentage of surviving cells was calculated as the absorbance ratio of treated to untreated cells. The inhibitory concentration 50% (IC\textsubscript{50}) of the treatments was determined from the dose-response curve. All experiments were set up in quadruplicate to determine means and SDs.

### 4.4 Endocytosis in the presence of inhibitors

Hela cells were cultured in 24-well plates for 24 h to achieve 60-80 % confluence. The cells were then treated with endocytosis inhibitors (filipin III at 5 µg.mL\(^{-1}\), chlorpromazine at 11 µg.mL\(^{-1}\), and DMA (5-\((N,N\text{-dimethyl})amiloride\) hydrochloride) at 40 µM) for 30 min before their incubation with 1 µM of nanoparticles (either Sq-Gem/Chol-BODIPY at 99:1 wt.% or Sq-Gem/Biotin-Sq/Chol-BODIPY at 89:10:1 wt.%) for 6 h at 37°C. Cells were then washed with Dulbecco's PBS two times and treated with 0.25% trypsin for 10 min at 37°C. The fluorescence from individual cells was examined using a flow cytometer C6 (Accuri Cytometers Ltd., UK). For fluorescence detection of nanoparticles, excitation was carried out with the 488-nm line of an argon laser, and emission fluorescence was collected at 515 nm. 10000 cells were measured in each sample.
Figure S2. Internalization in Hela cells of Gem-Sq/Chol-BODIPY (99:1 wt.%) and (Gem-Sq/Biotin-Sq/Chol-BODIPY (89:10:1 wt.%) nanoparticles in the presence of endocytosis inhibitors by means of flow cytometry.

5. References