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Supplementary Materials for

A modular approach toward producing nanotherapeutics targeting the innate immune system

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Supplementary Methods

Synthetic procedures and characterization

BODIPY-aliphatic. BODIPY FL carboxylic acid (9.3 mg, 0.032 mmol) was dissolved in $CHCl_3$ (3 mL) with EDC•HCl (9.8 mg, 0.051 mmol) and 4-(dimethylamino)pyridine (5.1 mg, 0.042 mmol) were added and the mixture was stirred for 10 min. 1-octadecanol (25.0 mg, 0.092 mmol) was added to the reaction flask. The mixture was stirred overnight in a sealed vial at room temperature. The reaction mixture was transferred to a centrifuge tube (15 mL). Water (3 mL) was added to the tube and the $CHCl_3/H_2O$ solution was centrifuged at 4500 rpm for 5 minutes. The water was removed and appropriately discarded. This was repeated 3 times. After the third wash, the organic layer was dried over Na_2SO_4, isolated by vacuum filtration, and concentrated under vacuum. The crude product was purified by preparative TLC using 6% MeOH in $CHCl_3$ to yield the product as an orange film. Yield = 13.06 mg, 0.024 mmol. η = 75 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.10 (s, 1H), 6.90, (d, *J*=2.68 Hz, 1H), 6.29 (d, *J*= 4.68 Hz, 1H), 6.13 (s, 1H), 4.11 (t, *J*= 6.53 Hz, 2H), 3.32 (t, *J*=7.84 Hz, 2H), 2.78 (t, *J*=7.84 Hz, 2H), 2.59 (s, 3H), 2.28 (s, 3H), 1.70-1.51 (m, 4H), 1.38-1.20 (m, 28H), 0.90 (t, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ = 172.88, 160.36, 157.27, 143.72, 133.36, 127.83, 123.93, 120.35, 116.77, 67.98, 64.77, 33.52, 32.99, 29.72, 29.62, 29.49, 29.35, 28.72, 25.98, 25.66, 23.98, 22.66, 15.07, 14.12, 11.28. SFC-MS for [$C_{32}H_{51}BF_2N_2O_2$] (m/z): Calculated: 544.40 [M], Found: 567.39 [M+Na^+].

BODIPY-cholesterol. BODIPY FL carboxylic acid (10.0 mg, 0.034 mmol) was dissolved in $CHCl_3$ (3 mL) with EDC•HCl (7.9 mg, 0.041 mmol) and 4-(dimethylamino)pyridine (5.1 mg, 0.007 mmol) were added and the mixture was stirred for 10 min. Cholesterol (14.0 mg, 0.052 mmol) was added to the reaction flask. The mixture was stirred overnight in a sealed vial at room temperature. The reaction mixture was transferred to a centrifuge tube (15 mL). Water (3 mL) was added to the tube and the $CHCl_3/H_2O$ solution was centrifuged at 4500 rpm for 5 minutes. The water was removed and appropriately discarded. This was repeated 3 times. After the third wash, the organic layer was dried over Na_2SO_4, isolated by vacuum filtration, and concentrated under vacuum. The crude product was purified by preparative TLC using 6% MeOH in $CHCl_3$ to yield the product as an orange film. Yield = 13.06 mg, 0.024 mmol. η = 75 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.11 (s, 1H), 6.90 (d, 1H), 6.30 (d, 1H), 6.13 (s, 1H), 5.39 (m, 2H), 4.66 (m, 1H), 4.08 (t, 2H), 3.11 (t, 2H), 2.58 (t, 2H), 2.59 (s, 3H), 2.34

(t, 4H), 2.27 (s, 3H), 2.08-0.71 (m, 36H). ^{13}C NMR (150 MHz, $CDCl_3$) δ = 160.27, 157.60, 143.80, 139.85, 135.25, 133.45, 128.15, 123.94, 122.72, 120.39, 116.84, 74.27, 64.33, 56.75, 56.15, 50.08, 42.35, 39.76, 39.52, 38.12, 36.99, 36.64, 36.22, 35.84, 33.89, 33.73, 32.77, 31.86, 30.098, 29.42, 28.57, 28.27, 28.02, 27.74, 27.11, 25.55, 24.42, 24.9, 24.04, 23.77, 22.86, 22.67, 21.00, 19.76, 19.37, 18.77, 14.87, 14.13, 11.86, 11.31. SFC-MS for [$C_{41}H_{59}BF_2N_2O_2$] (m/z): Calculated: 660.46 [M], Found: 683.45 [M+Na^+].

Malonate-aliphatic - Ethyl octadecyl malonate. 1-octadecanol (250 mg, 1.08 mmol) was dissolved in dry chloroform (30 mL) at 40 °C, trimethylamine (165 μL, 119 mmol) was added followed by ethyl 3-chloro-3-oxopropanoate (140 μL, 1.30 mmol). The mixture was stirred for 2 hours, allowed to cool to room temperature and washed with water (3 x 30 mL). The organic phase was dried using $MgSO_4$ and under vacuum, the crude product was purified by column chromatography (3 % methanol in chloroform) to yield the product as a yellowish wax. Yield = 314 mg, 0.82 mmol. η = 76 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ = 4.14 (q, *J* = 7.2 Hz, 1H), 4.07 (t, *J* = 6.7 Hz, 1H), 3.30 (s, 2H), 1.61-1.44 (m, 4H), 1.36-1.01 (m, 30H), 1.21 (t, *J* = 7.2 Hz, 6H), 0.81 (t, *J* = 6.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ = 166.77, 65.84, 61.65, 41.85, 32.10, 29.87, 29.74, 29.68, 29.54, 29.38, 28.63, 25.96, 22.86, 14.28. SFC-MS for [$C_{23}H_{44}O_4$] (m/z): Calculated: 384.32 [M], Found: 386 [M+H^+], 408 [M+Na^+].

Malonate-cholesterol - (3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ethyl malonate. Cholesterol (194 mg, 0.50 mmol) was dissolved in DCM (30 mL), pyridine (60 μL, 0.75 mmol) was added and the mixture was cooled to 0 °C. Ethyl 3-chloro-3-oxopropanoate (80 μL, 0.75 mmol) was dropwise added and the mixture was stirred for 2 hours at 0 °C, allowed to warm to room temperature and stirred for an additional 16 hours. Water (60 mL) was added, the layers separated, and the aqueous phase was washed twice with DCM (50 mL). The combined organic fractions were dried using $MgSO_4$ and under vacuum. The crude product was purified using column chromatography (hexane:ethyl acetate 1:1) to yield the product as a yellowish solid. Yield: 243 mg, 49 mmol. η = 97 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ = 5.41 (br, 1H), 4.69 (m, 1H), 4.22 (q, *J* = 7.1 Hz, 2H), 3.37 (s, 2H), 2.37 (m, 2H), 2.1–1.1 (m, 26H), 1.30 (t, *J* = 7.2Hz, 3H), 1.03 (s, 3H), 0.92 (d, *J* = 6.5 Hz, 3H), 0.87 (dd, *J* = 6.5, 2.6 Hz, 6H), 0.69 (s, 3 H). ^{13}C NMR (150 MHz, $CDCl_3$) δ = 166.88, 166.20, 139.52, 123.07, 75.40,

61.61, 56.85, 56.30, 50.17, 42.48, 42.16, 39.89, 39.70, 38.05, 37.09, 36.74, 36.36, 35.97, 32.07, 32.02, 28.41, 28.19, 27.76, 24.46, 24.01, 23.01, 22.75, 21.21, 19.48, 18.90, 14.28, 12.04. SFC-MS for [$C_{32}H_{52}O_4$] (m/z): Calculated: 500.39 [M], Found: 501.67 [$M+H^+$], 369.63 [fragment where the malonate-cholesterol bond is split].

(+)-JQ1 carboxylic acid - (S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid. (+)-JQ1 (90 mg, 0.20 mmol) was dissolved in 5 % TFA in chloroform (5 mL) and stirred for 16 hours at 40 °C after which the solvent was evaporated. Chloroform (5 mL) was added and evaporated under vacuum, this was repeated twice to yield the product which was used without further characterization. Yield = 78 mg, 0.20 mmol. η = >99 %.

(+)-JQ1-aliphatic - Octadecyl (S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate. (S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid (78 mg, 0.20 mmol) was dissolved in dry chloroform (5 mL), EDC•HCl (45 mg, 0.23 mmol) and 4-(dimethylamino)pyridine (37 mg, 0.30 mmol) were added and the mixture was stirred for 30 minutes. 1-octadecanol (63 mg, 0.23 mmol) was added and the mixture was stirred for 12 hours at room temperature. The mixture was washed with water (3 x 5 mL) and dried using $MgSO_4$ and under vacuum. The crude product was purified using preparative TLC (6 % methanol in chloroform) to yield the product as a white wax. Yield = 40 mg, 61 μmol. η = 31 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.40 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 8.6 Hz, 2H), 4.60 (m, 1H), 4.16 (t, *J* = 6.7 Hz, 2H), 3.65 – 3.59 (m, 2H), 2.67 (s, 3H), 2.41 (s, 3H), 1.74 (s, 3H), 1.73-1.62 (m, 2H), 1.39-1.32 (m, 2H), 1.32-1.17 (m, 28H), 0.87 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ = 171.87, 163.91, 155.57, 150.05, 136.92, 136.79, 132.45, 131.04, 130.87, 130.54, 130.01, 128.85, 65.15, 53.99, 37.08, 32.11, 29.89, 29.81, 29.75, 29.55, 29.49, 28.85, 26.13, 22.88, 14.60, 14.32, 13.29, 12.06. LC-MS for [$C_{37}H_{53}ClN_4O_2S$] (m/z): Calculated: 652.36 [M], Found: 653.6 [$M+H^+$].

(+)-JQ1-cholesterol - (3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate). (S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic

acid (75 mg, 0.19 mmol) was dissolved in dry chloroform (5 mL), EDC•HCl (50 mg, 0.26 mmol) and 4-(dimethylamino)pyridine (40 mg, 0.33 mmol) were added and the mixture was stirred for 30 minutes. Cholesterol (92 mg, 0.23 mmol) was added and the mixture was stirred for 12 hours at room temperature. The mixture was washed with water (3 x 5 mL) and dried using $MgSO_4$ and under vacuum. The crude product was purified using preparative TLC (6 % methanol in chloroform) to yield the product (R_f = 0.52) as a white powder. Yield = 49 mg, 64 μmol. η = 34 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.40 (d, *J* = 8.3Hz, 2H), 7.32 (d, *J* = 8.6Hz 2H), 5.36 (d, *J* = 4.1Hz, 1H), 4.69 (m, 1H), 4.60 (t, 1H), 3.59 (t, *J* = 6.5Hz, 2H), 2.67 (s, 3H), 2.41 (s, 3H), 2.36 (d, *J* = 6.9Hz, 2H), 2.1-0.9 (m, 19H), 1.68 (s, 3H), 1.03 (s, 3H), 0.91 (d, *J* = 6.5Hz, 3H), 0.87 (m, 3H), 0.68 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ = 171.21, 163.87, 155.58, 150.03, 139.81, 136.91, 136.80, 132.47, 131.02, 130.87, 130.54, 130.00, 128.87, 122.84, 74.70, 56.89, 56.32, 54.08, 50.23, 42.50, 39.93, 39.70, 38.28, 37.29, 37.22, 36.81, 36.37, 35.97, 32.10, 32.03, 29.89, 28.03, 24.47, 24.01, 23.01, 22.75, 21.23, 19.52, 18.91, 14.58, 13.30, 12.05. LC-MS for [$C_{46}H_{61}ClN_4O_2S$] (m/z): Calculated: 768.42 [M], Found: 769.82 [$M+H^+$].

GSK-aliphatic - Octadecyl 3-((2-(pyridin-2-yl)-6-(1,2,4,5-tetrahydro-3H-benzo[d]azepin-3-yl)pyrimidin-4-yl)amino)propanoate. GSK-J1 (20 mg, 51.4 μmol) was dissolved in dry chloroform (3 mL), EDC•HCl (12.8 mg, 66.6 μmol) and 4-(dimethylamino)pyridine (1.8 mg, 14.8 μmol) were added and the mixture was stirred for 30 min. 1-octadecanol (15.4 mg, 66.6 μmol) was added and the mixture was stirred overnight at room temperature. The mixture was washed with water (3 x 5 mL) and dried using $MgSO_4$ and under vacuum. The crude product was purified using preparative TLC (6 % methanol in chloroform) to yield the product (R_f = 0.38) as a white solid. Yield = 25.4 mg, 39.5 μmol. η = 77 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ =8.75 (s, 1H), 8.45 (d, *J* = 7.7 Hz, 1H), 7.81 (t, *J* = 7.1 Hz, 1H), 7.35 (b, 1H), 7.15 (s, 4H), 5.55 (s, 1H), 5.42 (b, 1H), 4.10 (t, *J* = 6.8 Hz, 2H), 3.95 (s, 4H), 3.63 (q, *J* = 6.4 Hz, 2H), 3.05 - 3.00 (m, 4H), 2.66 (t, *J* = 6.6 Hz, 2H), 1.62 (dt, *J* = 14.7, 6.8 Hz, 4H), 1.37-1.13 (m, 28H), 0.88 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ = 172.13, 163.74, 162.54, 156.41, 149.39, 141.03, 136.80, 130.17, 126.64, 124.48, 123.60, 120.07, 79.65, 65.29, 47.64, 37.74, 37.09, 34.36, 32.11, 29.89, 29.79, 29.71, 29.55, 29.46, 28.77, 26.11, 22.88, 14.32. LC-MS for [$C_{40}H_{59}N_5O_2$] (m/z): Calculated: 641.47 [M], Found: 642.73 [$M+H^+$].

GSK-cholesterol - (3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 3-((2-(pyridin-2-yl)-6-(1,2,4,5-tetrahydro-3H-benzo[d]azepin-3-yl)pyrimidin-4-yl)amino)propanoate.

GSK-J1 (25 mg, 64.2 μmol) was dissolved in dry chloroform (3 mL), EDC•HCl (16.0 mg, 83.3 μmol) and 4-(dimethylamino)pyridine (2.3 mg, 18.8 μmol) were added and the mixture was stirred for 30 min. Cholesterol (27 mg, 69.8 μmol) was added and the mixture was stirred overnight at room temperature. The mixture was washed with water (3 x 5 mL) and dried using $MgSO_4$ and under vacuum. The crude product was purified using preparative TLC (6 % methanol in chloroform) to yield the product as a white solid. Yield = 17.2 mg, 22.7 μmol. η = 35 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ = 8.75 (br, 1H), 8.45 (d, *J* = 7.3, 1H), 7.83 (b, 1H), 7.36 (b, 1H), 7.15 (s, 4H), 5.57 (s, 1H), 5.36 (b, 1H), 4.64 (m, 1H), 3.95 (br, 4H), 3.63 (q, *J* = 6.2Hz, 2H), 3.03 (m, 4H), 2.65 (t, *J* = 6.4, 2H), 2.33 (d, *J* = 7.5 Hz, 2H), 2.1–1.0 (m, 26H), 1.01 (s, 3H), 0.92 (d, *J* = 6.5 Hz, 3H), 0.86 (dd, *J* = 6.6, 2.7 Hz, 6H), 0.67 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ = 171.45, 163.60, 162.45, 161.40, 155.17, 149.88, 140.95, 139.68, 137.02, 130.19, 126.67, 124.83, 123.74, 122.96, 79.68, 74.77, 56.86, 56.31, 50.18, 47.68, 42.49, 39.90, 39.70, 38.29, 37.80, 37.14, 37.07, 36.76, 36.37, 35.97, 34.63, 32.08, 29.90, 28.41, 28.20, 27.96, 24.47, 24.01, 23.02, 22.76, 21.21, 19.48, 18.90, 12.04. LC-MS for [$C_{49}H_{67}N_5O_2$] (m/z): Calculated: 757.53 [M], Found: 758.77 [$M+H^+$], 1516.27 [$2M+H^+$].

Rapamycin-aliphatic Rapamycin (100 mg, 110 μmol) and vinyl stearate (170 mg, 548 μmol) were dissolved in dry toluene (40 mL) and Novozyme 435 (50 mg) was added. The mixture was stirred on a rotavapor at 60 °C for 4 days under mild vacuum. The Novozyme beads were filtered off, the solvent evaporated, and the crude product purified using column chromatography (0 – 6 % MeOH in chloroform), to yield the product as a white solid. Yield = 108 mg, 89.4 μmol. η = 84 %. Conversion was monitored by ^{1}H NMR (600 MHz, $CDCl_3$) through monitoring of the signal corresponding to the proton adjacent to the alcohol group being esterified, which is present at 2.73 ppm and 4.67 ppm in the unfunctionalized and functionalized Rapamycin, respectively. LC-MS for [$C_{69}H_{113}NO_{14}$] (m/z): Calculated: 1179.82 [M], Found: 1131.0 [M -OCH_3 -H_2O], 1149.0 [M-OCH_3], 1203.0 [$M+Na^+$] (A similar fragmentation pattern was observed for unfunctionalized Rapamycin). Elemental analysis for [$C_{69}H_{113}NO_{14}$] Found (Calc.): C, 70.04 (70.19); H, 9.40 (9.65); N, 1.10 (1.19).

Formulating the 20 nm nanobiologics

For the synthesis of the 20 nm sized nanoparticles a similar microfluidic procedure as for the 35 nm sized particles was used. Here, the acetonitrile mixture contained (again from 10 mg/ml stock solutions): POPC (250 μL), PHPC (15 μL), Cholesterol (13 μL). The acetonitrile solution was injected with a rate of 0.75 mL/min. The APOA1 solution (0.1 mg/mL in PBS) was injected with 3 mL/min. To obtain nanotherapeutics for FACS measurements, DiO-C_{18} (0.25 mg) was added to the acetonitrile solution.

Formulating the 65 nm nanobiologics

For the synthesis of the 65 nm sized nanoparticles a similar microfluidic procedure as for the 35 nm sized particles was used. Here, the acetonitrile mixture contained (again from 10 mg/ml stock solutions): POPC (250 μl), Cholesterol (12 μL), Tricaprylin (1400 μL). The acetonitrile solution was injected with a rate of 0.75 mL/min. The APOA1 solution (0.1 mg/ml in PBS) was injected with 4 mL/min. To obtain nanotherapeutics for FACS measurements, DIO-C_{18} (0.25 mg) of was added to the acetonitrile solution.

Formulating the 120 nm nanobiologics

For the synthesis of the 120 nm sized nanoparticles a similar microfluidic procedure as for the 35 nm sized particles was used. Here, the acetonitrile mixture contained (again from 10 mg/ml stock solutions): POPC (100 μl), Cholesterol (10 μL), Tricaprylin (4000 μL). The acetonitrile solution was injected with a rate of 0.75 mL/min. The APOA1 solution (0.10 mg/ml in PBS) was injected with 1.50 mL/min. To obtain nanotherapeutics for FACS measurements, DIO-C_{18} (0.25 mg) of was added to the acetonitrile solution.

Determining drug recovery and hydrolysis

(Pro-)drug recovery was evaluated by taking a small aliquot of nanobiologic solution (20 μL or less) and diluting in acetonitrile to 200 μL. The suspension was evaluated by HPLC using the parameters outlined in Supplementary Table 3. Hydrolysis was determined by drying an aliquot (typically 200 μL) of a nanobiologic solution, adding acetonitrile (typically 600 μL), and sonication the suspension for 20 minutes. The suspension was centrifuged to precipitate any solids and analyzed by HPLC using water and acetonitrile as eluents (each containing 0.1%TFA); except for both malonate derivatives which were analyzed using SFC-MS, and diethylmalonate which was analyzed by GC-MS.

Supplementary Figures

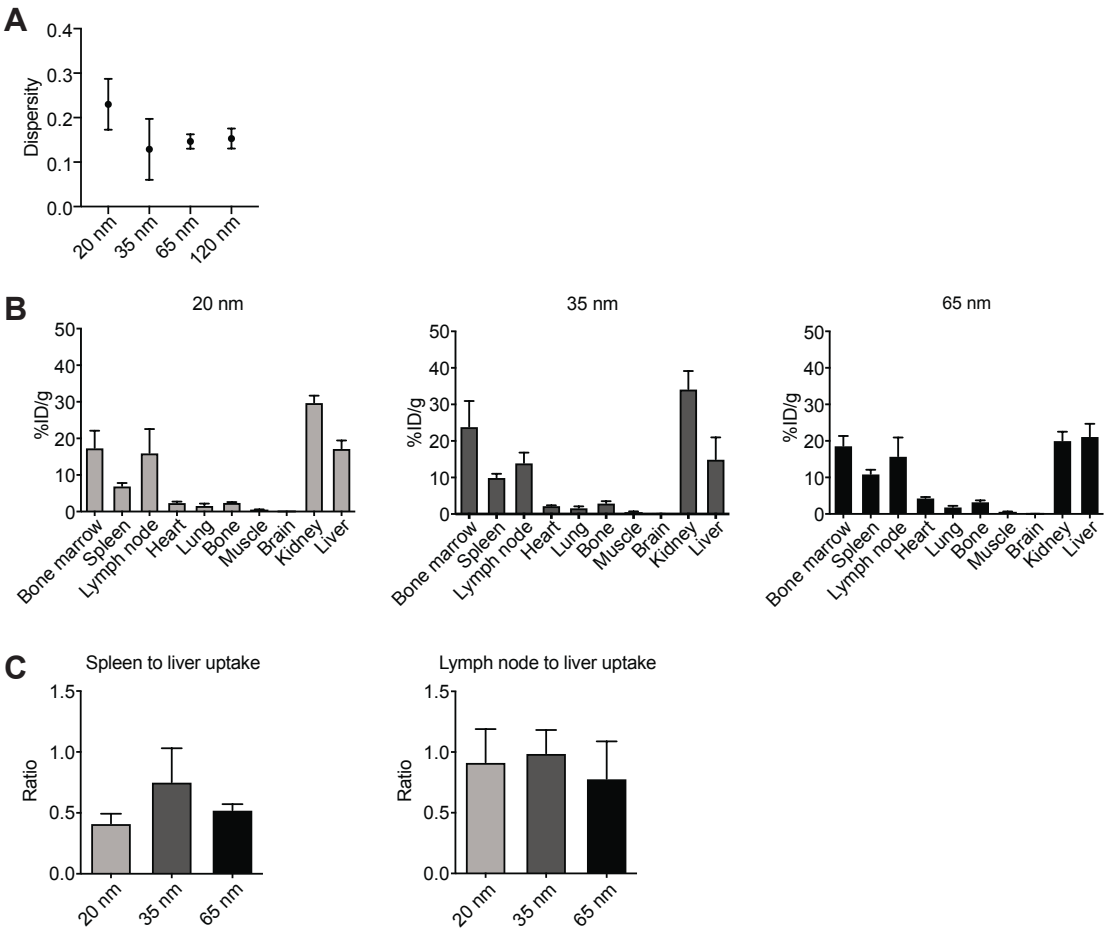

Figure S1. Dispersity and biodistribution of the nanobiologics. (**A**) Dispersity of the nanobiologics as measured by dynamic light scattering. (**B**) Biodistribution of ^{89}Zr-labeled nanobiologics in C57BL/6 mice at 24 hours post injection. (**C**) Nanobiologic uptake in the spleen (left) or iliac lymph nodes (right) divided by nanobiologic uptake in the liver, measured at 24 hours post-injection.

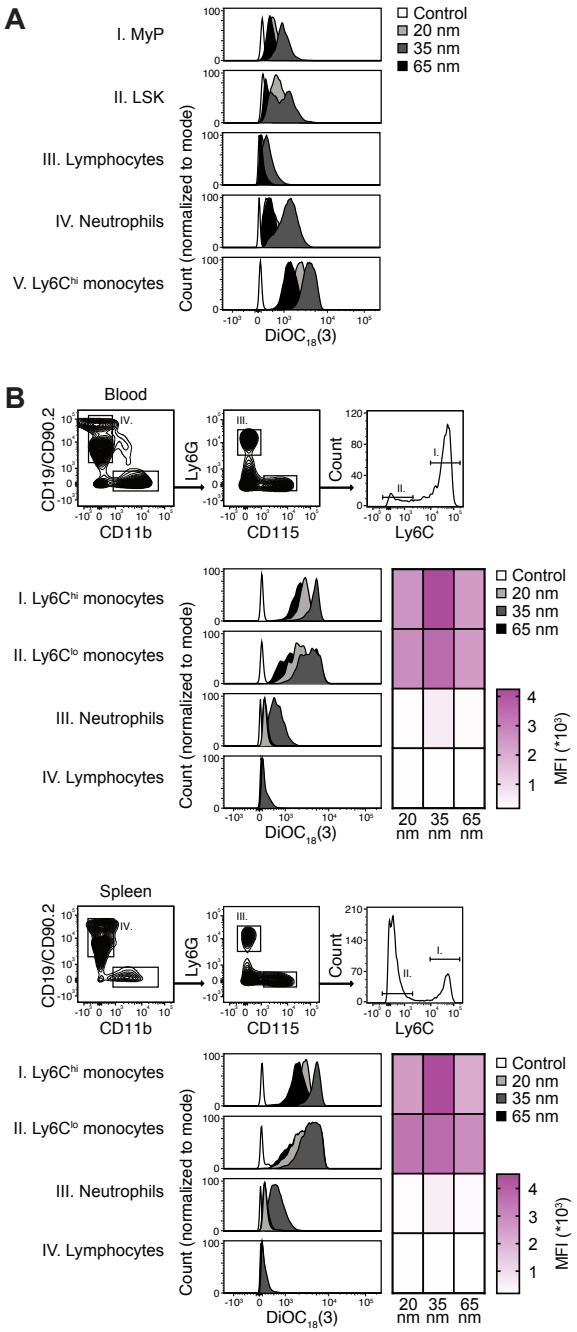

Figure S2. Cellular specificity of the nanobiologics. C57BL/6 mice were injected with $DiOC_{18}(3)$-labeled nanobiologics. Twenty-four hours post injection, $DiOC_{18}(3)$ uptake was measured by flow cytometry in the immune cells of the bone marrow, blood and spleen. (**A**) Representative histograms showing $DiOC_{18}(3)$ signal in different immune cell subsets in the bone marrow. (**B**) Gating strategy, representative histograms and heatmap depicting average mean fluorescence intensity (MFI) values for the blood (top) and spleen (bottom), n=4. For all graphs, data are represented as mean +/- standard deviation. MyP = Myeloid progenitors, LSK = Lin^- $Sca1^+$ and $c\text{-}Kit^+$.

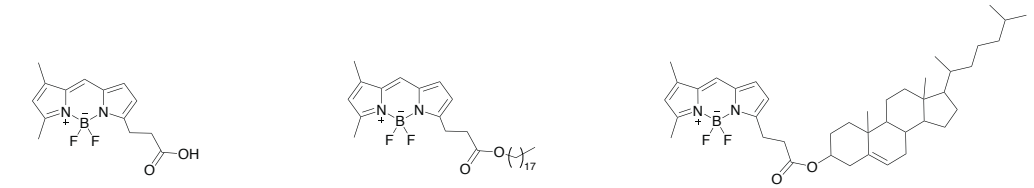

Figure S3. Molecular structures of the fluorescent model drug BODIPY FL carboxylic acid and its functionalized derivatives.

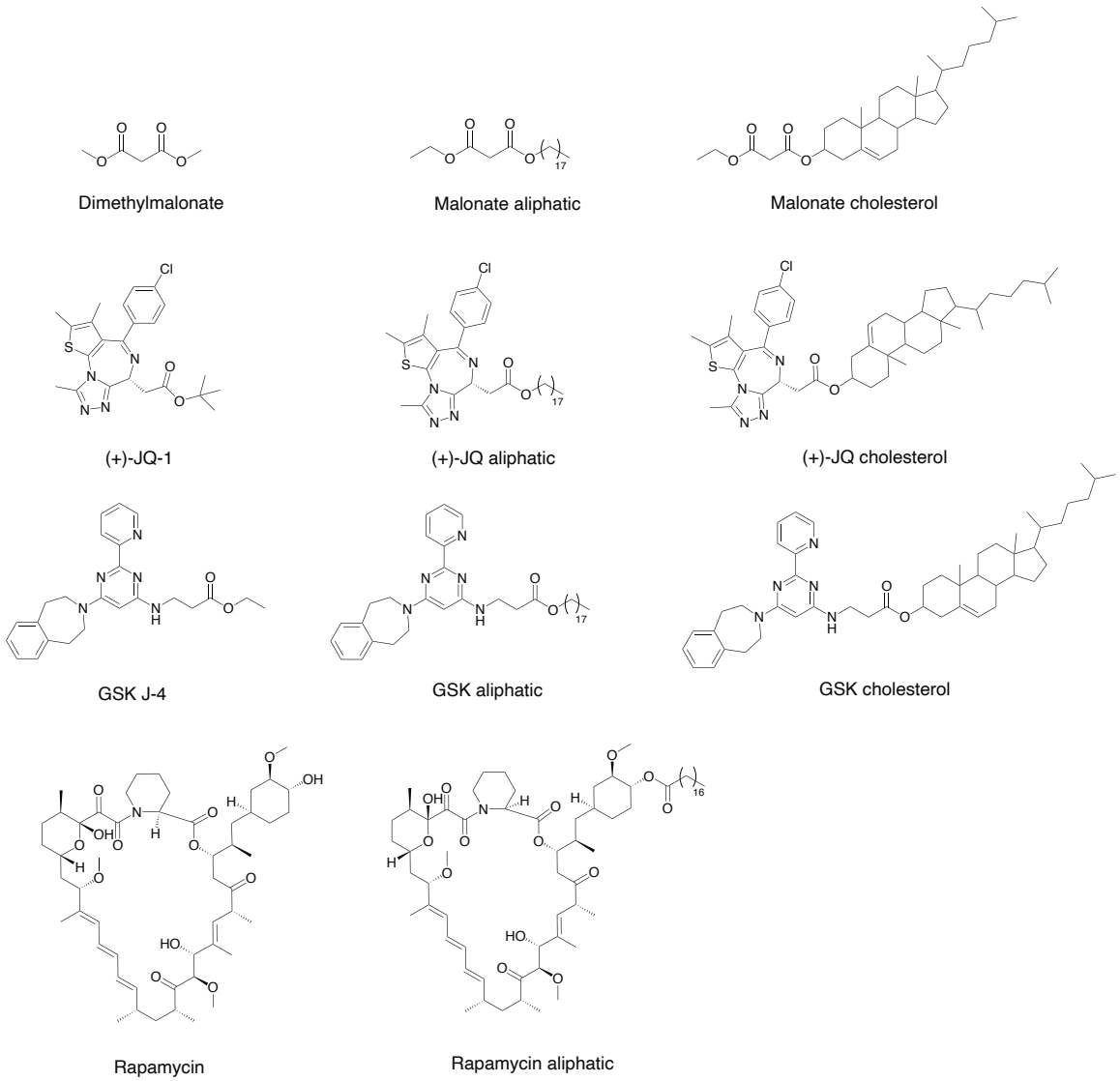

Figure S4. Chemical structures of malonate, (+)-JQ-1, GSK J-4 and rapamycin, as well as their functionalized derivatives.

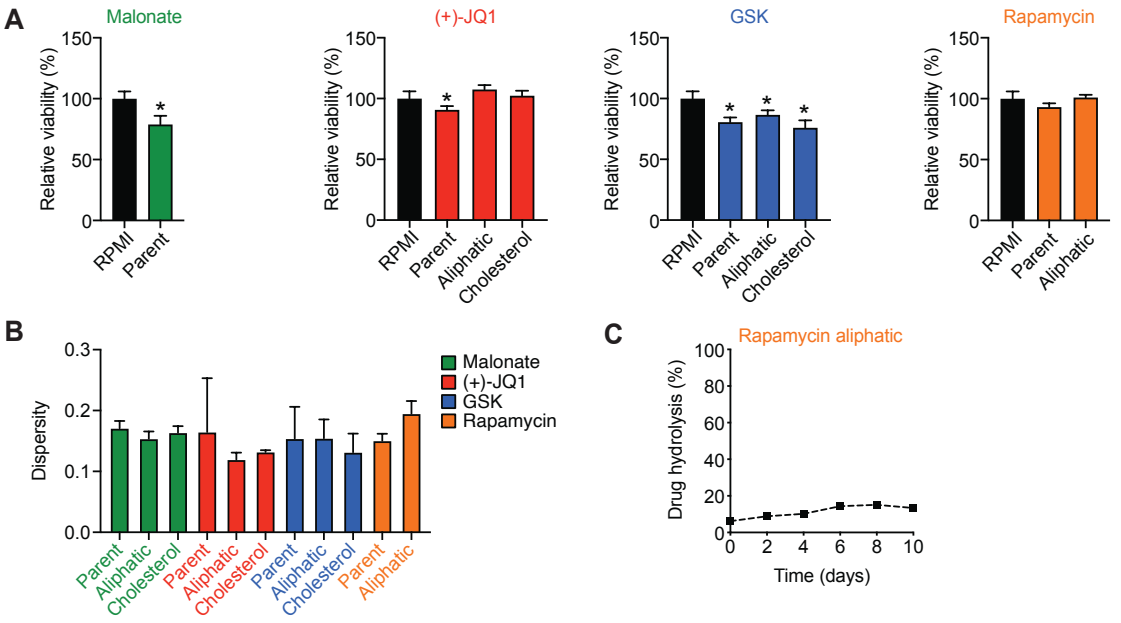

Figure S5. Establishing a library of nanotherapeutics (**A**) Murine bone marrow cells were incubated with (pro)drugs during 24 hours, after which cell viability was assessed, n=3. (**B**) Dispersity of 35 nm the various nanobiologics as measured by dynamic light scattering, n=3. (**C**) Hydrolysis kinetics of rapamycin aliphatic when incorporated in the nanobiologics and stored at 4 °C in PBS. No hydrolysis was observed for the other (pro)drugs. Hydrolysis rates were measured using HPLC, line is to guide the eye, n=2. Data are represented as mean +/- standard deviation.

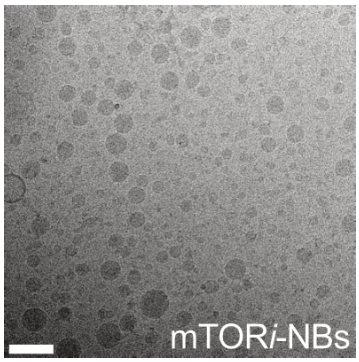

Figure S6. Cryo-TEM image of mTORi-NBs. Scale bar = 100 nm.

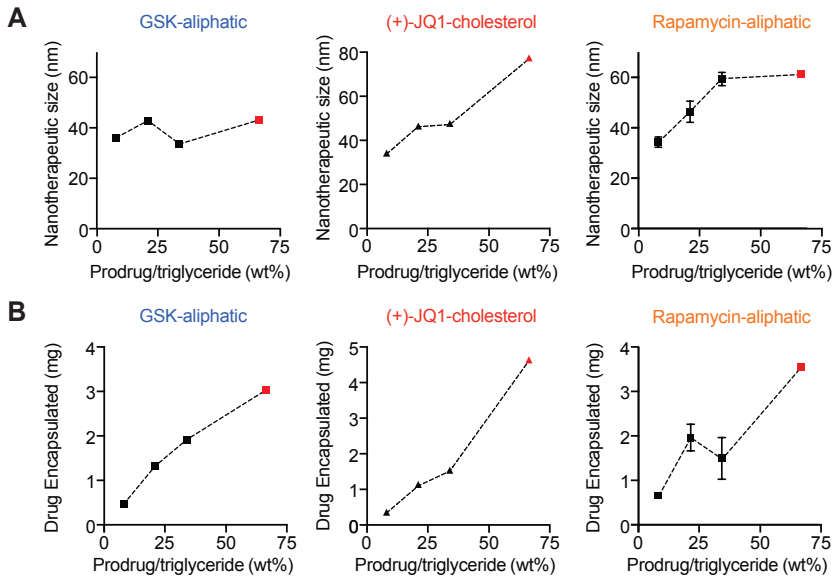

Figure S7. Optimizing GSK-aliphatic, (+)-JQ1-cholesterol, and rapamycin-aliphatic loading of the nanobiologics. Nanobiologics were formulated using varying amounts of prodrugs and their (**A**) size and (**B**) the amount of prodrug incorporated determined. Nanobiologic suspension that appeared cloudy are indicated by red symbols. N=1 for GSK-aliphatic and (+)-JQ1-cholesterol, n=2 for rapamycin-aliphatic.

Supplementary Tables

	20 nm	35 nm	65 nm
Spleen	0.45 ± 0.02	0.64 ± 0.05	0.69 ± 0.04
Heart	0.25 ± 0.03	0.23 ± 0.02	0.41 ± 0.03
Lung	0.25 ± 0.03	0.22 ± 0.01	0.25 ± 0.02
Kidney	7.49 ± 0.23	8.27 ± 0.94	4.84 ± 0.21
Liver	13.94 ± 1.28	13.31 ± 5.42	16.63 ± 0.93

Table S1. Percentage of injected ^{89}Zr-labeled nanobiologics in organs of C57BL/6 mice 24 hours after injection.

	20 nm	35 nm	65 nm
% fast	35.20	45.37	52.04
$t_{1/2}$ fast (minutes)	5.154	1.930	2.051
% slow	64.80	54.63	47.96
$t_{1/2}$ slow (minutes)	204.1	132.7	159.6
Weighted $t_{1/2}$ (minutes)	134.1	73.37	77.61

Table S2. Half-lives of the nanobiologics in C57BL/6 mice.

(Pro-)drug	**Column**	**Gradient** (acetonitrile %)	**Injection Vol** (µL)	λ_{max} (nm)	**Retention time** (min)
BODIPY aliphatic	CN	20% to 80% (6 min) 80% (10 min) 80% to 20% (3min)	5	503	4.9
BODIPY cholesterol	CN	20% to 80% (6 min) 80% (10 min) 80% to 20% (3min)	5	503	7.9
(+)-JQ-1*	CN	30% to 99% (8 min) 99% (2 min) 99% to 30% (2min)	20	258	4.6
(+)-JQ aliphatic	CN	30% to 99% (8 min) 99% (7 min) 99 to 30% (2min)	20	258	6.9
(+)-JQ cholesterol	CN	50% to 99% (7 min)	20	258	2.2
GSK J-4	CN	40% to 70% (4 min) 70% (4 min) 70 to 40% (1 min)	20	278	3.7
GSK aliphatic	CN	Isocratic 50% (5 min)	20	278	2.1
GSK cholesterol	CN	30% to 99% (8min) 99% (2min) 99% to 30% (2min)	20	278	6.8
Rapamycin	C_{18}	Isocratic 65% (5min)	10	278	3.0
Rapamycin aliphatic	C_{18}	60% to 99% (5 min), 99% (7 min), 99% to 60% (3 min)	20	278	13.3

Table S3. HPLC parameters used to quantify (pro-)drug concentrations in nanobiologics. *) solvents without TFA were used.

	mTORi-NB
% fast	74.35
$t_{1/2}$ fast (minutes)	7.158
% slow	25.65
$t_{1/2}$ slow (minutes)	212.3
Weighted $t_{1/2}$ (minutes)	59.77

Table S4. Half-life of mTORi-NB in non-human primates.