Supporting Information

Accurate programmed multifunctional nano-missiles for self-promoted deep delivery and synergistic cascade tumor therapy: Tactfully collaborating chemosynthesis with tumor microenvironment remodeling

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1. The synthesis of the ligands Bio$_3$-PEG-BIm, Bio$_3$-PEG-AIm, Bio$_3$-PEG-PIm, Bio$_3$-PEG-Hz and Bio$_3$-PEG-BSa

1.1 Chemicals and materials

All reactions were carried out under usual atmosphere unless otherwise stated. All liquid reagents were distilled before use. All unspecified reagents were from commercial resources. TLC was performed using precoated silica gel GF254 (0.2 mm), while column chromatography was performed using silica gel (100-200 mesh). $^1$H NMR spectra was taken on a Varian INOVA 400 (Varian, Palo Alto, CA, USA) using CDCl$_3$ or DMSO-$d_6$ as solvents. Chemical shifts are expressed in $\delta$ (ppm), with tetramethylsilane (TMS) functioning as the internal reference, and coupling constants ($J$) were expressed in Hz.

1.2 Synthesis of ligand Bio$_3$-PEG-BIm

![Scheme S1. The synthetic route of the ligand Bio$_3$-PEG-BIm. Reagents and conditions: (a) Biotin, EDCI, DMAP, DIPEA, DCM, DMF, r.t., 18h, 74.1%; (b) CF$_3$COOH, DCM, r.t., 3 h, 81.2%; (c) Succinic anhydride, Et$_3$N, DCM, r.t., 6 h, 77.1%; (d) Triethylene glycol, EDCI, DMAP, DIPEA, DCM, r.t., 5 h, 76.3%; (e) 3-Butyn-1-ol, DCC, DMAP, DCM, r.t., 2 h, 84.6%; (f) DBU, DCM, r.t., 30 min, 58.1%; (g) Compound 4, HATU, DIPEA, DCM/DMF, r.t., 2 h, 84.7%; (h) CF$_3$COOH, DCM, r.t., 2 h, 70.3%; (i) Biotin, HATU, DIPEA, DCM/DMF, r.t., 2 h, 70.3%; (j) Biotin, HATU, DIPEA, DCM/DMF, r.t., 2 h, 70.3%; (k) Biotin, HATU, DIPEA, DCM/DMF, r.t., 2 h, 70.3%; (l) Biotin, HATU, DIPEA, DCM/DMF, r.t., 2 h, 70.3%; (m) Biotin, HATU, DIPEA, DCM/DMF, r.t., 2 h, 70.3%]
2-Azidoethanol, EDCI, DMAP, DIPEA, DCM, r.t., 12 h, 83.5%; (k) CF₃COOH, Et₃SiH, DCM, r.t., 3 h, 71.9%; (l) PEG3350, EDCI, DMAP, DIPEA, DCM, r.t., 16 h, 90.1%; (m) 4-Formylbenzoic acid, DCC, DMAP, DCM, r.t., 24 h, 75.2%; (n) Compound 12, sodium ascorbate, CuI, DMF/H₂O, r.t., 16 h, 55.2%; (o) 4-(tert-Butoxycarbonyl)benzoic acid, EDCI, DMAP, DIPEA, DCM, r.t., 5 h, 84.7%; (p) CF₃COOH, Et₃SiH, DCM, r.t., 2 h, 76.3%; (q) Compound 6, DCC, DMAP, DCM, r.t., 2 h, 70.3%; (r) CF₃COOH, DCM, r.t., 2 h, 76.2%; (s) Compound 18, AcOH, molecular sieves, DMSO, 40°C, 4 h, 77.4%.

Ligand Bio₃-PEG-BIm was prepared using the methodologies depicted in Scheme S1. In the initial step, the esterification reaction of compound 1 with excess biotin resulted in the intermediate 2, which was then subjected to the Boc-deprotection reaction and condensation reaction to yield intermediate 4. Both intermediates 6 and 8 could be successfully obtained via esterification reaction using commercially sourced raw materials. Fmoc-deprotection with DBU in dichloromethane afforded the primary amine 9, which was followed by amidation with compound 4, Boc-deprotection with TFA and amidation with biotin to produce the intermediate alkyne 12. By reacting with commercially available 2-azidoethanol, compound 13 was converted to intermediate 14. After removing the tert-butyl group, it was successively esterified with PEG3350 and 4-formylbenzoic acid to yield intermediate 17. Subsequently, the click reaction of compound 17 with alkyne 12 in the presence of CuI generated the key intermediate 18. Similarly, another key intermediate, benzylamine 23, could be easily prepared in four steps starting from the known compound 19. Finally, benzaldehyde 18 reacted with benzylamine 23 to reach the target compound (Bio₃-PEG-BIm) by using anhydrous acetic acid as the catalyst.

1.2.1 Synthesis of compound 2.

To a solution of biotin (2.38 g, 9.76 mmol) in dichloromethane (100 mL) and DMF (100 mL) was added EDCI (2.81 g, 14.64 mmol), DMAP (1.79 g, 14.64 mmol) and DIPEA (2.84 g, 21.96 mmol), and the reaction was stirred at -5 °C for 30 min. Then, compound 1 (500 mg, 2.44 mmol) in dichloromethane (10 mL) was added slowly. After stirring for another 18 h at room temperature, the mixture was washed with 1 mol/L HCl (100 mL x 2) and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography to afford compound 2 as a white solid (1.19 g, 74.1%). M.p. > 200°C. ¹H-NMR (600 MHz, CDCl₃, ppm) δ 6.56 (s, 1H), 6.48 (s, 1H), 5.86 (brs, 1H), 5.77 (brs, 1H), 4.50 (t, J = 6.0 Hz, 2H), 4.31-4.33 (m, 2H), 4.17-4.18 (m, 4H), 3.50 (s, 2H), 3.42-3.48 (m, 2H), 3.13-3.15 (m, 2H), 2.90 (dd, J₁ = 12.6 Hz, J₂ = 4.2 Hz, 2H), 2.73 (d, J = 12.6 Hz, 2H), 2.36 (s, 4H), 1.63-1.74 (m, 8H), 1.39-1.45 (m, 13H).

¹³C-NMR (150 MHz, CDCl₃, ppm) δ 173.7, 164.1, 155.2, 80.4, 62.6, 62.4, 61.9, 61.8, 60.2, 55.4, 47.5, 47.4, 40.7, 33.9, 28.4, 28.2, 28.1, 25.0, 24.9. LR-MS: (ESI) calcd. for C₂₂H₄₅N₃O₅S₂ [M+H]+ 658.3, found 658.2.

1.2.2 Synthesis of compound 3.

Trifluoroacetic acid (2.5 mL, 10% v/v) was added to the solution of compound 2 (1.3 g) in dichloromethane (25 mL). After stirred at room temperature for 3h, the mixture was adjusted to pH 9 with saturated sodium bicarbonate
solution and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography to afford compound 3 as a white solid (896.6 mg, 81.2%). M.p. > 200°C. 1H-NMR (400 MHz, DMSO-d6, ppm) δ 8.81 (brs, 1H), 6.41 (s, 2H), 6.37 (s, 2H), 4.30-4.33 (m, 2H), 4.27 (t, J = 4.8 Hz, 4H), 4.12-4.15 (m, 2H), 3.28 (s, 4H), 3.08-3.13 (m, 2H), 2.82 (dd, J1 = 12.4 Hz, J2 = 5.2 Hz, 2H), 2.58 (d, J = 12.4 Hz, 2H), 2.36 (t, J = 7.2 Hz, 4H), 1.43-1.67 (m, 8H), 1.32-1.41 (m, 4H). 13C-NMR (100 MHz, CDCl3, ppm) δ 173.7, 164.0, 63.3, 61.9, 60.2, 55.5, 47.8, 40.6, 33.8, 28.4, 28.2, 24.9.

1.2.3 Synthesis of compound 4.

The solution of succinic anhydride (63 mg, 0.62 mmol) in dichloromethane (3 mL) was stirred at 0°C for 5 min, then a mixture of compound 3 (210 mg, 0.31 mmol) and trethylamine (174 μL, 1.24 mmol) in dichloromethane (5 mL) was added dropwise. After stirring for another 6 h at room temperature, the reaction mixture was concentrated and purified by silica gel column chromatography to afford compound 4 as a white solid (157.2 mg, 77.1%). M.p. 194.6-196.2°C. 1H-NMR (400 MHz, DMSO-d6, ppm) δ 6.47 (s, 1H), 6.45 (s, 1H), 6.41 (s, 1H), 6.38 (s, 1H), 4.31 (s, 2H), 4.14-4.18 (m, 4H), 4.07 (s, 2H), 3.61 (s, 2H), 3.50 (s, 2H), 3.10 (s, 2H), 2.83 (dd, J1 = 12.4 Hz, J2 = 4.8 Hz, 2H), 2.56-2.63 (m, 4H), 2.42 (s, 2H), 2.26-2.35 (m, 4H), 1.42-1.60 (m, 8H), 1.31-1.33 (m, 4H). LR-MS: (ESI) calcd. for C23H46N2O3S2 [M + H]+ 658.3, found 658.2.

1.2.4 Synthesis of compound 6.

To a solution of compound 5 (500 mg, 1.99 mmol) in dichloromethane (8 mL) was added EDCI (573 mg, 2.98 mmol), DMAP (365 mg, 2.98 mmol) and DIPEA (515 mg, 3.98 mmol), and the reaction was stirred at -5°C for 30 min. Then, triethylene glycol (747 mg, 4.97 mmol) in dichloromethane (1 mL) was added slowly. After stirring for another 5 h at room temperature, the mixture was washed with 1 mol/L HCl and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography to afford compound 2 as a yellowish oily (582.1 mg, 76.3%). 1H-NMR (400 MHz, DMSO-d6, ppm) δ 7.92 (d, J = 8.0 Hz, 2H), 7.48 (t, J = 6.0 Hz, 1H), 7.37 (d, J = 8.0 Hz, 2H), 4.57 (t, J = 5.2 Hz, 1H), 4.37 (t, J = 4.8 Hz, 2H), 4.19 (d, J = 6.0 Hz, 2H), 3.74 (t, J = 4.8 Hz, 2H), 3.58-3.60 (m, 2H), 3.52-3.54 (m, 2H), 3.45-3.49 (m, 2H), 3.41 (t, J = 4.8 Hz, 2H), 1.39 (s, 9H).

1.2.5 Synthesis of compound 8.

To a solution of compound 7 (10 g, 21.3 mmol) in dichloromethane (80 mL) was added DCC (8.8 g, 42.6 mmol) and DMAP (522 mg, 4.26 mmol), and the reaction was stirred at -5°C for 30 min. Then, 3-Butyn-1-ol (2.24 g, 32 mmol) in dichloromethane (5 mL) was added slowly. After stirring for another 2 h at room temperature, the mixture was cooled at -20°C for 5 h and filtered through a Buchner funnel. The filtrate was washed with 1 mol/L HCl and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous
sodium sulfate, filtered, concentrated and purified by silica gel column chromatography to afford compound 8 as a yellowish oily (9.38 g, 84.6%). 1H-NMR (400 MHz, CDCl3, ppm) δ 7.77 (d, J = 7.6 Hz, 2H), 7.61 (d, J = 7.6 Hz, 2H), 7.40 (t, J = 7.6 Hz, 2H), 7.32 (t, J = 7.6 Hz, 2H), 5.41 (brs, 1H), 4.57 (brs, 1H), 4.18-4.45 (m, 6H), 3.12 (d, J = 6.0 Hz, 2H), 2.58 (td, J1 = 6.4 Hz, J2 = 2.8 Hz, 2H), 2.02 (t, J = 2.8 Hz, 1H), 1.84-1.93 (m, 1H), 1.65-1.78 (m, 3H), 1.36-1.51 (m, 11H). 13C-NMR (100 MHz, CDCl3, ppm) δ 172.2, 156.1, 155.9, 143.9, 143.7, 141.3, 127.7, 127.1, 125.1, 120.0, 79.6, 79.2, 70.3, 67.0, 63.0, 53.7, 47.2, 40.1, 32.2, 29.6, 28.4, 22.3, 18.9. LR-MS: (ESI) calcd. for C23H28N2O4 [M-Boc+H]+ 421.2, found 421.1.

1.2.6 Synthesis of compound 9.

DBU (6.37 g, 41.9 mmol) was added to the solution of compound 8 (7.27 g, 13.96 mmol) in dichloromethane (60 mL). After stirred at room temperature for 30 min, the mixture was washed with water and brine. Then, the combined organic layers were dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography to afford compound 9 as a colorless transparent oil (2.42 g, 58.1%). 1H-NMR (400 MHz, DMSO-d6, ppm) δ 6.75 (brs, 1H), 4.03-4.16 (m, 2H), 3.26-3.29 (m, 1H), 2.85-2.90 (m, 3H), 2.49-2.53 (m, 2H), 1.72 (brs, 2H), 1.51-1.60 (m, 1H), 1.42-1.47 (m, 1H), 1.24-1.37 (m, 13H). 13C-NMR (100 MHz, CDCl3, ppm) δ 175.7, 156.0, 79.9, 79.1, 70.1, 62.5, 54.3, 40.4, 34.4, 29.8, 28.4, 22.8, 19.0. LR-MS: (ESI) calcd. for C13H27N2O4 [M+H]+ 299.2, found 299.2.

1.2.7 Synthesis of compound 10.

To a solution of compound 4 (3.98 g, 6.05 mmol) in dichloromethane (60 mL) and DMF (6 mL) was added HATU (2.63 g, 6.91 mmol) and DIPEA (1.49 g, 11.52 mmol), and the reaction was stirred at -5 °C for 30 min. Then, compound 9 (1.72 g, 5.76 mmol) in dichloromethane (5 mL) was added slowly. After stirring for another 2h at room temperature, the mixture was washed with 1 mol/L HCl and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography to afford compound 10 as a yellowish oily (4.58 g, 84.7%). 1H-NMR (400 MHz, DMSO-d6, ppm) δ 8.19 (d, J = 7.2 Hz, 1H), 6.74 (brs, 1H), 6.40 (s, 2H), 6.35 (s, 2H), 4.29-4.32 (m, 2H), 4.11-4.17 (m, 6H), 4.03-4.08 (m, 2H), 3.60 (t, J = 4.8 Hz, 2H), 3.48-3.51 (m, 2H), 3.38 (q, J = 6.8 Hz, 1H), 3.07-3.12 (m, 2H), 2.80-2.90 (m, 5H), 2.54-2.59 (m, 6H), 2.36-2.43 (m, 2H), 2.26-2.34 (m, 4H), 1.43-1.68 (m, 10H), 1.37 (s, 9H), 1.24-1.33 (m, 8H). LR-MS: (ESI) calcd. for C34H46N2O8S2 [M+H]+ 938.4, found 938.3.

1.2.8 Synthesis of compound 11.

Trifluoroacetic acid (7.5 mL, 30% V/V) was added to the solution of compound 10 (5.7 g, 6.08mmol) in dichloromethane (25 mL). After stirred at room temperature for 2h, the mixture was adjusted to pH 9 with saturated sodium bicarbonate solution and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column
chromatography to afford compound **11** as a yellowish oily (3.58 g, 70.3%). $^1$H-NMR (600 MHz, DMSO-$d_6$, ppm) δ 8.22 (brs, 1H), 7.71 (brs, 1H), 7.67 (brs, 1H), 6.42 (s, 2H), 6.37 (s, 2H), 4.30-4.31 (m, 2H), 4.14-4.23 (m, 6H), 4.05-4.07 (m, 2H), 3.60 (s, 2H), 3.49-3.50 (m, 3H), 3.09 (s, 2H), 2.87 (s, 1H), 2.82 (dd, $J_1 = 12.0$ Hz, $J_2 = 4.8$ Hz, 2H), 2.68 (t, $J = 6.0$ Hz, 2H), 2.55-2.61 (m, 4H), 2.37-2.43 (m, 2H), 2.27-2.33 (m, 4H), 1.97-2.02 (m, 2H), 1.59-1.65 (m, 2H), 1.52-1.53 (m, 2H), 1.45-1.46 (m, 2H), 1.28-1.38 (m, 6H), 1.23 (s, 6H). LR-MS: (ESI) calcd. for C$_{38}$H$_{60}$N$_2$O$_{10}$S$_2$ [M+H]$^+$ 838.4, found 838.4.

1.2.9 Synthesis of compound **12**.

To a solution of biotin (268 mg, 1.09 mmol) in dichloromethane (10 mL) and DMF (10 mL) was added HATU (501 mg, 1.32 mmol) and DIPEA (284 mg, 2.19 mmol), and the reaction was stirred at -5 °C for 30 min. Then, compound **11** (613 mg, 0.73 mmol) in dichloromethane (5 mL) was added slowly. After stirring for another 2h at room temperature, the mixture was washed with 1 mol/L HCl and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography to afford compound **12** as a yellowish oily (546.2 mg, 70.3%). $^1$H-NMR (400 MHz, DMSO-$d_6$, ppm) δ 8.22 (d, $J = 7.2$ Hz, 1H), 7.75 (t, $J = 5.6$ Hz, 1H), 6.44 (s, 3H), 6.38 (s, 3H), 4.29-4.32 (m, 3H), 4.10-4.19 (m, 7H), 4.02-4.09 (m, 3H), 3.59-3.61 (m, 2H), 3.50 (t, $J = 5.6$ Hz, 2H), 3.17 (d, $J = 5.2$ Hz, 3H), 3.09-3.10 (m, 3H), 2.98-3.02 (m, 2H), 2.87 (t, $J = 2.8$ Hz, 1H), 2.82 (dd, $J_1 = 12.4$ Hz, $J_2 = 4.8$ Hz, 3H), 2.54-2.62 (m, 4H), 2.47-2.48 (m, 2H), 2.34-2.45 (m, 2H), 2.26-2.34 (m, 4H), 2.04 (t, $J = 7.2$ Hz, 2H), 1.41-1.69 (m, 14H), 1.30-1.37 (m, 8H). LR-MS: (ESI) calcd. for C$_{42}$H$_{47}$N$_2$O$_2$S$_2$ [M+H]$^+$ 1064.5, found 1064.5.

1.2.10 Synthesis of compound **14**.

To a solution of compound **13** (1.0 g, 4.50 mmol) in dichloromethane (36 mL) was added EDCI (1.3 g, 6.75 mmol), DMAP (830 mg, 6.75 mmol) and DIPEA (1.16 g, 9.0 mmol), and the reaction was stirred at -5 °C for 30 min. Then, 2-azidoethanol (587 mg, 6.75 mmol) in dichloromethane (3 mL) was added slowly. After stirring for another 12h at room temperature, the mixture was washed with 1 mol/L HCl and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography to afford compound **14** as a white solid (1.09 g, 83.5%). M.p. > 200°C. $^1$H-NMR (400 MHz, DMSO-$d_6$, ppm) δ 8.08 (d, $J = 8.4$ Hz, 2H), 8.03 (d, $J = 8.4$ Hz, 2H), 4.48 (t, $J = 4.8$ Hz, 2H), 3.69 (t, $J = 4.8$ Hz, 2H), 1.55 (s, 9H).

1.2.11 Synthesis of compound **15**.

The solution of compound **14** (1.2 g, 4.12 mmol) in dichloromethane (2 mL) was stirred at 0°C for 5min. Then, triethylsilane (1.2 g, 10.3 mmol) in dichloromethane (1.5 mL) and trifluoroacetic acid (8 mL) was added slowly. After stirring for another 3h at room temperature, the mixture was concentrated and purified by recrystallization to afford
compound 15 as a white solid (696.7 mg, 71.9%). M.p. > 200°C. 1H-NMR (400 MHz, DMSO-d6, ppm) δ 8.08 (s, 4H), 4.48 (t, J = 4.8 Hz, 2H), 3.70 (t, J = 4.8 Hz, 2H). 13C-NMR (100 MHz, DMSO-d6, ppm) δ 167.3, 165.7, 135.8, 133.6, 130.4, 130.2, 64.9, 50.1.

1.2.12 Synthesis of compound 16.

To a solution of compound 15 (500 mg, 2.13 mmol) in dichloromethane (30 mL) was added EDCI (612 mg, 3.20 mmol), DMAP (390 mg, 3.20 mmol) and DIPEA (550 mg, 4.26 mmol), and the reaction was stirred at -5 °C for 30 min. Then, PEG3350 (7.13 g, 2.13 mmol) in dichloromethane (5 mL) was added slowly. After stirring for another 16h at room temperature, the mixture was washed with 1 mol/L HCl and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by recrystallization (DCM/Et2O) to afford compound 16 as a white solid (5.85 g, 90.1%). M.p. 72.6-74.2°C. 1H-NMR (400 MHz, DMSO-d6, ppm) δ 8.11 (s, 4H), 4.56 (t, J = 5.2 Hz, 2H), 4.49 (t, J = 4.8 Hz, 2H), 4.43 (t, J = 4.8 Hz, 2H), 3.76 (t, J = 4.8 Hz, 2H), 3.67-3.72 (m, 4H), 3.40-3.59 (m, 248H).

1.2.13 Synthesis of compound 17.

To a solution of 4-formylbenzoic acid (407 mg, 2.7 mmol) in dichloromethane (20 mL) was added DCC (741.6 mg, 3.6 mmol) and DMAP (44.0 mg, 0.36 mmol), and the reaction was stirred at -5 °C for 30 min. Then, compound 16 (6.45 g, 1.8 mmol) in dichloromethane (5 mL) was added slowly. After stirring for another 24h at room temperature, the mixture was cooled at -20 °C for 5h and filtered through a Buchner funnel. The filtrate was washed with 1 mol/L HCl and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by recrystallization (DCM/Et2O) to afford compound 17 as a white solid (4.3 g, 75.2%). M.p. 88.8-90.2°C. 1H-NMR (400 MHz, DMSO-d6, ppm) δ 10.12 (s, 1H), 8.16 (d, J = 8.4 Hz, 2H), 8.11 (s, 4H), 8.06 (d, J = 8.4 Hz, 2H), 4.42-4.43 (m, 4H), 3.77 (s, 4H), 3.67-3.72 (m, 4H), 3.59-3.60 (m, 6H), 3.51 (s, 242H).

1.2.14 Synthesis of compound 18.

To a solution of compound 12 (100 mg, 0.094 mmol) and compound 17 (448.8 mg, 0.141 mmol) in the mixed solvent of DMF (3 mL) and water (0.3 mL) was added CuI (5.3 mg, 0.028 mmol) and sodium ascorbate (11.1 mg, 0.056 mmol). After stirring for another 16h at room temperature under the protection of argon, the mixture was concentrated and purified by silica gel column chromatography to afford compound 18 as a yellowish oily (220.4 mg, 55.2%). 1H-NMR (600 MHz, DMSO-d6, ppm) δ 10.11 (s, 1H), 8.21 (d, J = 6.6 Hz, 1H), 8.15-8.18 (m, 2H), 8.08 (s, 1H), 8.05 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 8.4 Hz, 2H), 7.73-7.75 (m, 3H), 6.42 (s, 3H), 6.36 (s, 3H), 4.76-4.77 (m, 2H), 4.67-4.68 (m, 2H), 4.41-4.45 (m, 3H), 4.21-4.36 (m, 6H), 4.12-4.16 (m, 6H), 4.03-4.06 (m, 2H), 3.75-3.76 (m, 2H), 3.58 (s, 4H), 3.47-3.50 (m, 250H), 3.08 (s, 3H), 2.94-3.00 (m, 5H), 2.80-2.85 (m, 3H), 2.58 (s, 2H), 2.56 (s, 2H), 2.38-2.44 (m, 2H), 2.34-2.37 (m, 1H), 2.26-2.29 (m, 1H), 2.12-2.15 (m, 1H), 1.99-2.02 (m, 1H), 1.78-1.81 (m, 1H), 1.70-1.73 (m, 1H), 1.58-1.60 (m, 1H).
2.25-2.37 (m, 6H), 2.02-2.04 (m, 2H), 1.43-1.59 (m, 14H), 1.22-1.30 (m, 8H).

1.2.15 Synthesis of compound 20.

To a solution of compound 13 (1.0 g, 4.50 mmol) in dichloromethane (36 mL) was added EDCI (1.3 g, 6.75 mmol), DMAP (830 mg, 6.75 mmol) and DIPEA (1.16 g, 9.0 mmol), and the reaction was stirred at -5 °C for 30 min. Then, compound 19 (1.56 g, 3.0 mmol) in dichloromethane (3 mL) was added slowly. After stirring for another 5h at room temperature, the mixture was washed with 1 mol/L HCl and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography to afford compound 20 as a yellowish oily (1.84 g, 84.7%). 1H-NMR (400 MHz, CDCl3, ppm) δ 8.09 (d, J = 8.4 Hz, 2H), 8.03 (d, J = 8.4 Hz, 2H), 5.32-5.33 (m, 1H), 4.49 (t, J = 4.8 Hz, 2H), 3.85 (t, J = 4.8 Hz, 2H), 3.70 (d, J = 4.8 Hz, 4H), 3.63 (s, 4H), 3.13-3.20 (m, 1H), 1.00-2.37 (28H, remaining cholesterol protons), 1.61 (s, 9H), 0.98 (s, 3H), 0.91 (d, J = 6.4 Hz, 3H), 0.86 (d, J = 6.8 Hz, 6H), 0.67 (s, 3H).

1.2.16 Synthesis of compound 21.

The solution of compound 20 (1.15 g, 1.59 mmol) in dichloromethane (2 mL) was stirred at 0°C for 5 min, Then, triethylsilane (462.4 mg, 3.98 mmol) in dichloromethane (1 mL) and trifluoroacetic acid (7 mL) was added slowly. After stirring for another 2 h at room temperature, the mixture was concentrated and purified by silica gel column chromatography to afford compound 21 as a colorless transparent oil (808.9 mg, 76.3%). 1H-NMR (400 MHz, CDCl3, ppm) δ 8.13 (s, 4H), 5.31-5.33 (m, 1H), 4.51 (t, J = 4.8 Hz, 2H), 3.86 (t, J = 4.8 Hz, 2H), 3.71 (d, J = 4.8 Hz, 4H), 3.63 (s, 4H), 3.13-3.20 (m, 1H), 1.00-2.37 (28H, remaining cholesterol protons), 0.98 (s, 3H), 0.91 (d, J = 6.4 Hz, 3H), 0.86 (d, J = 6.8 Hz, 6H), 0.67 (s, 3H).

1.2.17 Synthesis of compound 22.

To a solution of compound 21 (250 mg, 0.36 mmol) in dichloromethane (3 mL) was added DCC (155 mg, 0.72 mmol) and DMAP (9.5 mg, 0.07 mmol), and the reaction was stirred at -5 °C for 20 min. Then, compound 6 (216 mg, 0.54 mmol) in dichloromethane (0.5 mL) was added slowly. After stirring for another 2 h at room temperature, the mixture was washed with 1 mol/L HCl and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography to afford compound 22 as a yellowish oily (261.3 mg, 70.3%). 1H-NMR (400 MHz, DMSO-d6, ppm) δ 8.07 (s, 4H), 7.89 (d, J = 8.0 Hz, 2H), 7.46 (t, J = 5.6 Hz, 1H), 7.35 (d, J = 8.0 Hz, 2H), 5.25 (s, 1H), 4.40 (t, J = 8.8 Hz, 4H), 4.31 (t, J = 4.4 Hz, 2H), 4.18 (d, J = 5.6 Hz, 2H), 3.73-3.76 (m, 6H), 3.58-3.61 (m, 6H), 3.52-3.54 (m, 2H), 3.47 (s, 4H), 3.03-3.09 (m, 1H), 0.93-2.28 (28H, remaining cholesterol protons), 1.39 (s, 9H), 0.89 (s, 3H), 0.87 (s, 3H), 0.84 (d, J = 6.4 Hz, 6H), 0.62 (s, 3H).

1.2.18 Synthesis of compound 23.
Trifluoroacetic acid (1.5 mL, 30% V/V) was added to the solution of compound 22 (200 mg, 0.194 mmol) in dichloromethane (5 mL). After stirred at room temperature for 2h, the mixture was adjusted to pH 9 with saturated sodium bicarbonate solution and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography to afford compound 23 as a yellowish oily (137.8 mg, 76.2%). 1H-NMR (400 MHz, DMSO-d6, ppm) δ 8.07 (s, 4H), 7.87 (d, J = 8.0 Hz, 2H), 7.45 (d, J = 8.0 Hz, 2H), 5.24(s, 1H), 4.40 (t, J = 8.8 Hz, 4H), 4.34 (t, J = 4.4 Hz, 2H), 3.78 (s, 2H), 3.73-3.77 (m, 6H), 3.58-3.61 (m, 6H), 3.51-3.54 (m, 2H), 3.47 (s, 4H), 3.23 (brs, 2H), 3.03-3.09 (m, 1H), 0.93-2.27 (28H, remaining cholesterol protons), 0.89 (s, 3H), 0.87 (s, 3H), 0.84 (d, J = 6.4 Hz, 6H), 0.62 (s, 3H).

1.2.19 Synthesis of ligand Bio3-PEG-BIm.

Compound 18 (42.5 mg, 0.01 mmol) and Compound 23 (14.0 mg, 0.015 mmol) were dissolved in anhydrous DMSO (1 mL). Then, molecular sieves (20 mg) and anhydrous AcOH (2 drops) were added under the protection of argon. After stirred at 40°C for 4h, the mixture was diluted with saturated sodium bicarbonate solution, filtered, and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by dialysis and lyophilization to afford Bio3-PEG-BIm as a white solid (40.0 mg, 77.4%). 1H-NMR (400 MHz, DMSO-d6, ppm) δ 8.62 (s, 1H), 8.16 (d, J = 7.2 Hz, 1H), 8.07-8.09 (m, 5H), 8.04 (d, J = 8.4 Hz, 2H), 8.02 (d, J = 8.4 Hz, 2H), 7.93 (d, J = 8.0 Hz, 2H), 7.88 (d, J = 8.0 Hz, 2H), 7.69-7.71 (m, 2H), 7.48 (d, J = 8.4 Hz, 2H), 7.37 (t, J = 5.6 Hz, 1H), 6.40 (s, 3H), 6.34 (s, 3H), 5.24 (s, 1H), 4.77 (d, J = 4.4 Hz, 2H), 4.68 (t, J = 4.4 Hz, 2H), 4.35-4.42 (m, 7H), 4.24-4.32 (m, 8H), 4.12-4.16 (m, 6H), 4.06 (t, J = 4.8 Hz, 2H), 3.76 (s, 10H), 3.68 (t, J = 4.8 Hz, 2H), 3.58-3.61 (m, 12H), 3.40-3.51 (m, 252H), 3.09 (s, 4H), 2.94-2.98 (m, 5H), 2.79-2.83 (m, 3H), 2.54-2.59 (m, 4H), 2.37-2.42 (m, 2H), 2.25-2.32 (m, 7H), 2.04 (t, J = 7.2 Hz, 3H), 1.03-1.97 (48H, including 28H remaining cholesterol protons), 0.89 (s, 3H), 0.88 (s, 3H), 0.84 (d, J = 6.4 Hz, 6H), 0.63 (s, 3H). HRMS calculated for C240H222N13O92S3 [M+2H]2⁺ 2582.9020, found 2579.7344.

1.3 Synthesis of ligand Bio3-PEG-Alm
Scheme S2. The synthetic route of the ligand Bio₃-PEG-Alm. Reagents and conditions: (a) Fmoc-6-aminohexanoic acid, HATU, DIPEA, DCM, r.t., 2 h, 74.2%; (b) DBU, DCM, r.t., 30 min, 55.3%; (c) Compound 18, AcOH, molecular sieves, DMSO, 40°C, 4 h, 75.6%.

1.3.1 Synthesis of compound 24.

The synthesis of compound 24 had been reported in our previous work [1].

1.3.2 Synthesis of compound 25.

To a solution of Fmoc-6-aminohexanoic acid (420.5 mg, 1.19 mmol) in dichloromethane (10 mL) was added HATU (539.7 mg, 1.42 mmol) and DIPEA (307.3 mg, 2.37 mmol), and the reaction was stirred at -5 °C for 30 min. Then, compound 24 (500 mg, 0.79 mmol) in dichloromethane (2 mL) was added slowly. After stirring for another 2 h at room temperature, the mixture was washed with 1 mol/L HCl and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography to afford compound 25 as a yellowish oily (567.1 mg, 74.2%). ¹H-NMR (400 MHz, CDCl₃, ppm) δ 7.76 (d, J = 7.2 Hz, 2H), 7.59 (d, J = 7.2 Hz, 2H), 7.40 (t, J = 7.2 Hz, 2H), 7.31 (t, J = 7.2 Hz, 2H), 5.33-5.34 (m, 1H), 4.87 (brs, 1H), 4.78 (brs, 1H), 4.40 (s, 2H), 4.22 (s, 3H), 3.49-3.69 (m, 10H), 3.20-3.27 (m, 5H), 1.06-2.36 (44H, including 28H remaining cholesterol protons), 0.99 (s, 3H), 0.91 (d, J = 6.4 Hz, 3H), 0.86 (d, J = 6.8 Hz, 6H), 0.67 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃, ppm) δ 173.5, 156.4, 144.0, 141.3, 141.0, 127.6, 127.0, 125.0, 121.5, 120.0, 79.5, 70.9, 70.6, 70.4, 69.2, 67.3, 66.4, 56.8, 56.2, 50.2, 47.4, 42.3, 40.8, 39.8, 39.5, 39.1, 39.0, 37.3, 36.9, 36.2, 35.8, 34.0, 32.0, 31.9, 29.7, 29.6, 28.4, 28.3, 28.2, 28.0, 26.1, 24.5, 23.4, 22.8, 22.2, 21.1, 19.4, 18.7, 11.9.

1.3.3 Synthesis of compound 26.

DBU (2.36 g, 15.5 mmol) was added to the solution of compound 25 (5 g, 5.17 mmol) in dichloromethane (50 mL). After stirred at room temperature for 30 min, the mixture was washed with 1 mol/L HCl, water and brine. Then, the combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated to afford compound 26 as a colorless oil (2.13 g, 55.3%), which was used for next step directly without further purification.

1.3.4 Synthesis of ligand Bio₃-PEG-Alm.

Compound 18 (42.5 mg, 0.01 mmol) and Compound 26 (11.2 mg, 0.015 mmol) were dissolved in anhydrous DMSO (1 mL). Then, molecular sieves (20 mg) and anhydrous AcOH (0.5 drop) were added under the protection of argon. After stirred at 40°C for 4 h, the mixture was diluted with saturated sodium bicarbonate solution, filtered, and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by dialysis and lyophilization to afford Bio₃-PEG-Alm as a white solid (37.6 mg, 75.6%). ¹H-NMR (400 MHz, DMSO-d₆, ppm) δ 8.42 (s, 1H), 8.16 (d, J = 7.6 Hz, 1H), 8.09 (s, 1H), 8.06 (d, J = 6.0 Hz, 2H), 8.02 (d, J = 8.0 Hz, 2H), 8.01 (d, J = 6.0 Hz, 2H), 7.95 (d, J = 8.0 Hz, 2H), 7.87 (s, 1H), 7.85 (s, 1H), 6.41 (s, 3H), 6.35 (s, 3H), 5.30 (s, 1H), 4.77 (t, J = 4.8 Hz, 2H), 4.68 (t, J = 4.8 Hz, 2H), 4.39-4.45 (m, 3H),...
4.21-4.31 (m, 8H), 4.11-4.17 (m, 6H), 4.06 (t, J = 4.8 Hz, 2H), 3.76 (s, 4H), 3.68 (t, J = 4.8 Hz, 4H), 3.40-3.59 (m, 258H), 3.08-3.12 (m, 5H), 2.94-2.99 (m, 8H), 2.79-2.85 (m, 3H), 2.54-2.59 (m, 4H), 2.36-2.42 (m, 6H), 2.25-2.33 (m, 8H), 0.97-2.08 (62H, including 28H remaining cholesterol protons), 0.94 (s, 3H), 0.89 (s, 3H), 0.84 (d, J = 6.4 Hz, 6H), 0.65 (s, 3H).

1.4 Synthesis of ligand Bio\textsubscript{3}-PEG-Plm

\textbf{Scheme S3}. The synthetic route of the ligand Bio\textsubscript{3}-PEG-Plm. Reagents and conditions: (a) (Boc)\textsubscript{2}O, Et\textsubscript{3}N, 1,4-dioxane, H\textsubscript{2}O, r.t., 24 h, 92.3%; (b) Triethylene glycol, EDCI, DMAP, DIPEA, DCM, r.t., 5 h, 78.5%; (c) Compound 21, DCC, DMAP, DCM, r.t., 2 h, 74.4%; (d) CF\textsubscript{3}COOH, DCM, r.t., 2 h, 72.8%; (e) Compound 18, AcOH, molecular sieves, DMSO, 40°C, 4 h, 79.9%.

1.4.1 Synthesis of compound 28.

Triethylamine (10 mL, 0.73 mol) was added to the solution of compound 27 (5.0 g, 0.36 mmol) in 1,4-dioxane (75 mL) and water (35 mL), and the reaction was stirred at room temperature for 5 min. Then, Di-tert-butyl dicarbonate (16 g, 0.73 mol) was added to the above solution. After stirring for another 24 h at room temperature, the mixture was concentrated and 3N HCl was added to produce precipitation. After filtration, the filter cake was recrystallized with methanol to afford compound 28 as a white solid (7.95 g, 92.3%). M.p. 192-194°C. \textsuperscript{1}H-NMR (400 MHz, DMSO-d\textsubscript{6}, ppm) \(\delta 12.61 (s, 1H), 9.73 (s, 1H), 7.83 (d, J = 8.8 Hz, 2H), 7.55 (d, J = 8.8 Hz, 2H), 1.48 (s, 9H).

1.4.2 Synthesis of compound 29.

To a solution of compound 28 (250 mg, 1.05 mmol) in dichloromethane (5 mL) was added EDCI (303 mg, 1.58 mmol), DMAP (193 mg, 1.58 mmol) and DIPEA (272 mg, 2.1 mmol), and the reaction was stirred at -5 °C for 30 min. Then, triethylene glycol (396 mg, 2.63 mmol) in dichloromethane (1 mL) was added slowly. After stirring for another 5 h at room temperature, the mixture was washed with 1 mol/L HCl and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography to afford compound 29 as a yellowish oily (304.5 mg, 78.5%). \textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}, ppm) \(\delta 7.98 (d, J = 8.8 Hz, 2H), 7.42 (d, J = 8.8 Hz, 2H), 6.77 (s, 1H), 4.46 (t, J = 4.8 Hz, 2H), 3.83 (t, J = 4.8 Hz, 2H), 3.67-3.72 (m, 6H), 3.60 (t, J = 4.8 Hz, 2H), 1.52 (s, 9H).
1.4.3 Synthesis of compound 30.

To a solution of compound 21 (250 mg, 0.36 mmol) in dichloromethane (3 mL) was added DCC (155 mg, 0.72 mmol) and DMAP (9.5 mg, 0.07 mmol), and the reaction was stirred at -5 °C for 20 min. Then, compound 29 (199.5 mg, 0.54 mmol) in dichloromethane (0.5 mL) was added slowly. After stirring for another 2h at room temperature, the mixture was washed with 1 mol/L HCl and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography to afford compound 30 as a yellowish oily (272.7 mg, 74.4%). 1H-NMR (400 MHz, CDCl₃, ppm) δ 8.08 (s, 4H), 7.96 (d, J = 8.8 Hz, 2H), 7.41 (d, J = 8.8 Hz, 2H), 6.83 (s, 1H), 5.31-5.32 (m, 1H), 4.47-4.51 (m, 4H), 4.44 (t, J = 4.8 Hz, 2H), 3.81-3.87 (m, 6H), 3.69-3.72 (m, 8H), 3.62 (s, 4H), 3.12-3.20 (m, 1H), 1.01-2.36 (28H, remaining cholesterol protons), 1.52 (s, 9H), 0.97 (s, 3H), 0.91 (d, J = 6.4 Hz, 3H), 0.86 (d, J = 6.4 Hz, 6H), 0.67 (s, 3H).

1.4.4 Synthesis of compound 31.

Trifluoroacetic acid (1.5 mL, 30% V/V) was added to the solution of compound 30 (200 mg, 0.196 mmol) in dichloromethane (5 mL). After stirred at room temperature for 2h, the mixture was adjusted to pH 9 with saturated sodium bicarbonate solution and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography to afford compound 31 as a yellowish oily (131 mg, 72.8%). 1H-NMR (400 MHz, CDCl₃, ppm) δ 8.09 (s, 4H), 7.84 (d, J = 8.4 Hz, 2H), 6.64 (d, J = 8.4 Hz, 2H), 5.30-5.32 (m, 1H), 4.47-4.51 (m, 4H), 4.41 (t, J = 4.8 Hz, 2H), 3.80-3.86 (m, 6H), 3.69-3.72 (m, 8H), 3.63 (s, 4H), 3.13-3.20 (m, 1H), 1.01-2.37 (28H, remaining cholesterol protons), 0.97 (s, 3H), 0.91 (d, J = 6.4 Hz, 3H), 0.86 (d, J = 6.4 Hz, 6H), 0.67 (s, 3H).

1.4.5 Synthesis of ligand Bio₃-PEG-Plm.

Compound 18 (42.5 mg, 0.01 mmol) and Compound 31 (13.8 mg, 0.015 mmol) were dissolved in anhydrous DMSO (1 mL). Then, molecular sieves (20 mg) and anhydrous AcOH (2 drops) were added under the protection of argon. After stirred at 40°C for 4h, the mixture was diluted with saturated sodium bicarbonate solution, filtered, and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by dialysis and lyophilization to afford Bio₃-PEG-Plm as a white solid (41.1mg, 79.9%).

1.5 Synthesis of ligand Bio₃-PEG-Hz
1.5.1 Synthesis of compound 32.

To a solution of 4-acetylbenzoic acid (74.42 mg, 0.45 mmol) in dichloromethane (5 mL) was added DCC (123.6 mg, 0.60 mmol) and DMAP (7.33 mg, 0.06 mmol), and the reaction was stirred at -5 °C for 30 min. Then, compound 16 (915.4 mg, 0.3 mmol) in dichloromethane (3 mL) was added slowly. After stirring for another 24 h at room temperature, the mixture was cooled at -20 °C for 5 h and filtered through a Buchner funnel. The filtrate was washed with 1 mol/L HCl and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by recrystallization (DCM/Et2O) to afford compound 32 as a white solid (801.9 mg, 83.6%). M.p. 95.8-97.2°C. 1H-NMR (400 MHz, DMSO-d6, ppm) δ 8.11 (s, 4H), 8.08 (s, 4H), 4.49 (t, J = 4.8 Hz, 2H), 4.43 (t, J = 4.0 Hz, 4H), 3.76 (t, J = 4.8 Hz, 4H), 3.67-3.72 (m, 4H), 3.48-3.61 (m, 246H), 2.63 (s, 3H).

1.5.2 Synthesis of compound 33.

To a solution of compound 12 (100 mg, 0.094 mmol) and compound 32 (450.9 mg, 0.141 mmol) in the mixed solvent of DMF (3 mL) and water (0.3 mL) was added CuI (5.3 mg, 0.028 mmol) and sodium ascorbate (11.1 mg, 0.056 mmol). After stirring for another 16 h at room temperature under the protection of argon, the mixture was concentrated and purified by silica gel column chromatography to afford compound 33 as a yellowish oily (229.1 mg, 57.2%). 1H-NMR (400 MHz, DMSO-d6, ppm) δ 8.15-8.17 (m, 1H), 8.11 (s, 1H), 8.05-8.08 (m, 6H), 8.02 (d, J = 8.4 Hz, 2H), 7.71 (t, J = 5.6 Hz, 1H), 6.41 (s, 3H), 6.35 (s, 3H), 4.76-4.78 (m, 2H), 4.67-4.69 (m, 2H), 4.41-4.42 (m, 3H), 4.22-4.36 (m, 6H), 4.13-4.17 (m, 6H), 4.06-4.08 (m, 2H), 3.76 (s, 2H), 3.68 (t, J = 4.8 Hz, 2H), 3.37-3.59 (m, 252H), 3.08-3.10 (m, 3H), 2.94-3.01 (m, 5H), 2.80-2.86 (m, 3H), 2.63 (s, 3H), 2.59 (s, 2H), 2.56 (s, 2H), 2.36-2.42 (m, 2H), 2.25-2.32 (m, 6H), 2.03 (t, J = 6.8 Hz, 2H), 1.48-1.59 (m, 14H), 1.23-1.31 (m, 8H).

1.5.3 Synthesis of compound 34.
The synthesis of compound 34 had been reported in our previous work.  

1.5.4 Synthesis of ligand Bio3-PEG-Hz

Compound 33 (42.6 mg, 0.01 mmol) was dissolved in a mixture of anhydrous dichloromethane (1 mL) and tetrahydrofuran (0.9 mL). Then, compound 34 (45 mg, 0.06 mmol) in anhydrous dichloromethane (1 mL) was added dropwise into the above solution under the protection of argon. After that, anhydrous formic acid (1 drop) was added and the mixture was stirred at 20 °C for 72h. At last, the mixture was concentrated and purified by flash column chromatography to afford ligand Bio3-PEG-Hz as a faint yellow oily (29.6 mg, 60.3%). 1H-NMR (400 MHz, DMSO-d6, ppm) δ 10.56 (s, 1H), 8.17 (d, J = 7.2 Hz, 1H), 8.11 (s, 1H), 8.07 (d, J = 7.6 Hz, 2H), 8.02 (d, J = 8.4 Hz, 2H), 7.98 (d, J = 8.4 Hz, 2H), 7.91 (d, J = 7.6 Hz, 2H), 7.80 (s, 1H), 7.73 (t, J = 5.6 Hz, 1H), 6.41 (s, 3H), 6.35 (s, 3H), 5.30 (s, 1H), 4.77 (t, J = 4.8 Hz, 2H), 4.68 (t, J = 4.8 Hz, 2H), 4.38-4.42 (m, 3H), 4.21-4.30 (m, 6H), 4.11-4.17 (m, 8H), 4.06 (t, J = 4.8 Hz, 2H), 3.76 (s, 2H), 3.68 (t, J = 4.8 Hz, 2H), 3.51-3.59 (m, 26H), 3.07-3.10 (m, 4H), 2.93-3.02 (m, 7H), 2.79-2.85 (m, 3H), 2.54-2.59 (m, 4H), 2.37-2.41 (m, 5H), 2.26-2.32 (m, 12H), 0.97-2.08 (58H, including 28H remaining cholesterol protons), 0.94 (s, 3H), 0.89 (d, J = 6.0 Hz, 3H), 0.84 (d, J = 6.4 Hz, 6H), 0.65 (s, 3H).

1.6 Synthesis of ligand Bio3-PEG-BSa

NaBH₄ (5.7 mg, 0.15 mmol) was slowly added to the solution of Bio3-PEG-BIm (154.8 mg, 0.03 mmol) in anhydrous DMF (1 mL) at 0°C under the protection of argon. After stirring for 24h at room temperature, the mixture was diluted with saturated sodium bicarbonate solution and extracted three times with dichloromethane. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by flash column chromatography to afford Bio3-PEG-BSa as a faint yellow oily (125.8 mg, 81.2%). 1H-NMR
(400 MHz, DMSO-\textit{d}_6, ppm) δ 8.18 (d, \( J = 7.2 \) Hz, 1H), 8.07 (s, 5H), 8.02 (d, \( J = 7.6 \) Hz, 2H), 7.92 (d, \( J = 8.0 \) Hz, 2H), 7.89 (d, \( J = 7.6 \) Hz, 2H), 7.73 (t, \( J = 5.6 \) Hz, 1H), 7.53 (d, \( J = 8.0 \) Hz, 1H), 7.51 (d, \( J = 7.6 \) Hz, 1H), 7.46 (d, \( J = 8.0 \) Hz, 2H), 7.38 (d, \( J = 7.6 \) Hz, 1H), 7.36 (d, \( J = 8.0 \) Hz, 1H), 6.42 (s, 3H), 6.36 (s, 3H), 5.25 (s, 1H), 4.89 (brs, 1H), 4.77 (d, \( J = 4.4 \) Hz, 2H), 4.68 (t, \( J = 4.4 \) Hz, 2H), 4.57 (t, \( J = 5.6 \) Hz, 4H), 4.35-4.41 (m, 10H), 4.28-4.32 (m, 3H), 4.12-4.26 (m, 4H), 4.06 (t, \( J = 4.8 \) Hz, 2H), 3.75 (s, 12H), 3.68 (t, \( J = 4.8 \) Hz, 2H), 3.58-3.61 (m, 12H), 3.41-3.50 (m, 252H), 3.08 (s, 4H), 2.94-2.98 (m, 5H), 2.81 (dd, \( J_1 = 12.8 \) Hz, \( J_2 = 4.4 \) Hz, 3H), 2.55-2.59 (m, 4H), 2.38-2.42 (m, 2H), 2.25-2.32 (m, 7H), 2.01-2.05 (m, 3H), 1.05-1.94 (48H, including 28H remaining cholesterol protons), 0.89 (s, 6H), 0.84 (d, \( J = 6.40 \) Hz, 6H), 0.63 (s, 3H).

2. The hydrolysis of ligands Bio3-PEG-BIm, Bio3-PEG-Alm, Bio3-PEG-Plm and Bio3-PEG-Hz

To test the responsiveness of different acid-sensitive bonds to the tumor microenvironment, ligands Bio3-PEG-BIm, Bio3-PEG-Hz, Bio3-PEG-Alm and Bio3-PEG-Plm was treated with the follow conditions: (A) the PBS (pH 7.4) solution of the ligand Bio3-PEG-BIm or Bio3-PEG-Hz or Bio3-PEG-Plm (10 mg/mL) was shaken at 37 °C for 24h; (B) the PBS (pH 7.4) solution of the ligand Bio3-PEG-Alm (10 mg/mL) was shaken at 37 °C for 2h; (C) the PBS (pH 6.5) solution of the ligand Bio3-PEG-Hz or Bio3-PEG-Plm (10 mg/mL) was shaken at 37 °C for 2h; (D) the PBS (pH 6.5) solution of the ligand Bio3-PEG-BIm (10 mg/mL) was shaken at 37 °C for 1h; (E) the ligand Bio3-PEG-BIm (10 mg) was added into the PBS (pH 6.5, 1 mL) solution of GOx (100 \( \mu \)g/mL) and glucose (1 mg/mL), then shaken at 37 °C for 1h. Subsequently, using the same method for reprocessing: (1) Each solution was diluted with saturated sodium bicarbonate solution and extracted three times with dichloromethane. (2) The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, concentrated, and then identified by \(^1\)H NMR.

![Figure S1. \(^1\)H NMR spectra of Bio3-PEG-Alm after treatment by the follow condition: PBS (pH 7.4), 37 °C, 2h.](image-url)
As shown in Figure S1, after shaking at pH 7.4 for 2h, about 36.4% \((0.39/(0.39+0.68)\times 100\%)\) of Bio3-PEG-Alm was hydrolyzed, indicating that the alkyl-imine bond (Alm) was not stable under normal physiological conditions of blood circulation and could not be used as acid-sensitive bond in guidance unit.

After Bio3-PEG-PIm was oscillated at pH 7.4 for 24 hours, there was still no new signal peak in the corresponding hydrogen spectrum (Figure S2A), indicating that this ligand remained stable for a long time under normal physiological conditions of blood circulation. In contrast, about 18.7% \((0.20/(0.20+0.87)\times 100\%)\) of Bio3-PEG-PIm was hydrolyzed at pH 6.5 for 2 h (Figure S2B), indicating that phenyl-imine bond (PIm) was slowly broken under weakly acidic conditions in the tumor microenvironment.

![Figure S2. ¹H NMR spectra of Bio3-PEG-PIm after treatment by different conditions: (A) PBS (pH 7.4), 37 °C, 24h; (B) PBS (pH 6.5), 37 °C, 2h.](image)

Figure S3. \(^1\)H NMR spectra of Bio\(_3\)-PEG-Hz after treatment by different conditions: (A) PBS (pH 7.4), 37 °C, 24h; (B) PBS (pH 6.5), 37 °C, 2h.

Similarly, no new signal peak appeared in the hydrogen spectrum of Bio\(_3\)-PEG-Hz after shock at pH 7.4 for 24h (Figure S3A). In contrast, about 14.3% \((0.15/(0.15+0.90)\times100\%)\) of Bio\(_3\)-PEG-Hz was hydrolyzed at pH 6.5 for 2h (Figure S3B), indicating that the hydrazone bond (Hz) fractured slowly under weakly acidic conditions in tumor microenvironment, and the rate was slower than that of Bio\(_3\)-PEG-PIm.

Table S1. The hydrolysis rate of ligands Bio\(_3\)-PEG-Hz, Bio\(_3\)-PEG-Alm, Bio\(_3\)-PEG-PIm and Bio\(_3\)-PEG-BIm in different conditions.

| Ligand          | Hydrolysis rate at pH 7.4 \(^a\) | Hydrolysis rate at pH 6.5 \(^b\) |
|-----------------|----------------------------------|----------------------------------|
| Bio\(_3\)-PEG-Hz| 0                                | 14.3%                            |
| Bio\(_3\)-PEG-Alm| 36.4% \(^c\)                     | N.T. \(^d\)                      |
| Bio\(_3\)-PEG-PIm| 0                                | 18.7%                            |
| Bio\(_3\)-PEG-BIm| 0                                | 47.7% \(^e\)                     |

\(^a\) pH 7.4, 24h; \(^b\) pH 6.5, 2h; \(^c\) pH 7.4, 2h; \(^d\) Not tested; \(^e\) pH 6.5, 1h.

3. Cell culture

4T1 cells (Murine TNBC cell line) were maintained in RPMI 1640, whereas MDA-MB-231 cells (Human TNBC cancer cell line), MCF7 ((Human breast cancer cell line) and L02 cells (Human hepatocytes with normally-expressed SMVT) were cultivated in Dulbecco’s Modified Eagles Medium (DMEM), all supplemented with 10% FBS, 100 U/mL
streptomycin, and 100 U/mL penicillin at 37 °C under a humidified atmosphere containing 5% CO₂.

4. Animals

Female BALB/c mice (6–8 weeks old, SPF) were bought from Dashuo Biotechnology Co., Ltd (Chengdu, China). All animal procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Sichuan University, and approved by the Animal Ethics Committee of Sichuan University.

5. Other experimental results

Table S2. Characterization of different nano-rockets. (means ± SD, n = 3).

| Nano-rockets (NR) | pH | Size (nm) | PDI  | Zeta potential (mV) |
|-------------------|----|-----------|------|---------------------|
| LF-NR             | 7.4| 113.4±1.3 | 0.190±0.016 | -1.24±0.50 |
|                   | 6.5| 114.1±1.5 | 0.173±0.022 | -0.57±0.39 |
| NH₂-NR            | 7.4| 119.7±2.1 | 0.198±0.034 | 28.8±0.62  |
|                   | 6.5| 120.8±1.6 | 0.193±0.030 | 39.7±0.41  |
| Bio₃-BSa-NR       | 7.4| 151.6±1.8 | 0.185±0.019 | -15.9±0.55 |
|                   | 6.5| 151.0±2.3 | 0.153±0.028 | -14.7±0.37 |
| Bio₃-BIm-NR       | 7.4| 152.2±1.6 | 0.162±0.025 | -16.7±0.26 |
|                   | 6.5| 126.9±2.3 | 0.177±0.029 | 30.8±0.55  |

Table S3. Characterization of GOx+L-Arg-NM and PTX-NM. (means ± SD, n = 3).

| Nano-missiles (NM) | Size(nm) | PDI  | EE (%)       | Zeta potential (mV) |
|--------------------|----------|------|--------------|---------------------|
| GOx+L-Arg-NM      | 173.6±0.9| 0.111±0.019 | 11.06±1.09 (GOx) | -18.22±0.46 |
|                   |          |      | 53.74±1.83 (L-Arg) |                     |
| PTX-NM            | 155.3±1.5| 0.134±0.025 | 92.79±2.22 | -15.02±0.34 |

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Table S4. IC$_{50}$ values of PTX to 4T1, MCF7 and MDA-MB-231 cells (means ± SD, n = 6).

| Cell Lines   | IC$_{50}$ (μg/mL) | GOx+L-Arg-NM | PTX-NM& GOx+L-Arg-NM |
|--------------|-------------------|--------------|---------------------|
| 4T1          | 0.89              | /            | 0.08                |
| MCF7         | 1.13              | /            | 0.10                |
| MDA-MB-231   | 3.27              | /            | 0.09                |

Figure S4. (A) The quantitative cellular internalization of MDA-MB-231 cells after incubation with nano-rockets at pH 7.4 (left) or pH 6.5 (right), respectively ((a) CFPE-LF-NR, (b) CFPE-Bio-BSa-NR, (c) CFPE-Bio-Blm-NR, (d) CFPE-Bio-Blm-NR&GOx+L-Arg-NM) (means ± SD, n = 3, * indicates p < 0.05, ** indicates p < 0.01, *** indicates p <0.001). (B) was the semi-quantitative intensity of these nano-rockets in MDA-MB-231 tumor spheroids ((a) CFPE-LF-NR, (b) CFPE-Bio-BSa-NR, (c) CFPE-Bio-BSa-NR&GOx+L-Arg-NM, (d)
CFPE-Bio3-BIm-NR, (c) CFPE-Bio3-BIm-NR&GOx+L-Arg-NM) (means ± SD, n = 3, ** indicates p < 0.01, **** indicates p < 0.0001 versus CFPE-LF-NR). Confocal laser scanning microscopy (CLSM) images on (C) 4T1 cells and (D) MDA-MB-231 cells at pH 6.5, showing FITC channel (green), LysoTracker-stained lysosome channel (red), and DAPI-stained nucleus channel (blue), the scale bar = 20 µm ((a) CFPE-LF-NR, (b) CFPE-Bio3-BSa-NR, (c) CFPE-Bio3-Blm-NR, (d) CFPE-Bio3-BIm-NR&GOx+L-Arg-NM) (n = 3). (E) and (F) were the fluorescence distribution of 4T1 and MDA-MB-231 tumor spheroids under pH 6.5 conditions, respectively ((a) CFPE-LF-NR, (b) CFPE-Bio3-BSa-NR, (c) CFPE-Bio3-BSa-NR&GOx+L-Arg-NM, (d) CFPE-Bio3-Blm-NR, (e) CFPE-Bio3-Blm-NR&GOx+L-Arg-NM) (n = 3). The scale bar = 100 µm.

Figure S5. (A) In vivo fluorescence imaging of the 4T1 tumor xenografts bearing mice after intravenous injection with DiD-LF-NR (a), DiD-Bio3-BSa-NR (b), DiD-Bio3-Blm-NR (c), and DiD-Bio3-BSa-NR&GOx+L-Arg-NM (d). (B) Ex vivo imaging of the major organs and tumor ex vivo 24 h post-injection. (C) Radiant efficiency of the organs measured by ex vivo imaging (means ± SD, *** indicates p < 0.001).
Figure S6. Confocal fluorescence and bright field images (A), NO generation (D) of MCF7 cells after incubation with different nano-missiles for 6 h ((a) control, (b) PTX-NM, (c) GOx+L-Arg-NM, (d) GOx+L-Arg-NM&PTX-NM) (means ± SD, n = 3, *** indicates p < 0.001, N.S. indicates no significant difference). (E) Time-dependent changes in the pH values of MCF7 cells at pH 6.5 ((a) control, (b) GOx+L-Arg-NM (GOx: 10 μg/mL, glucose: 0 mg/mL), (c) GOx+L-Arg-NM (GOx: 10 μg/mL, glucose: 1 mg/mL)) (means ± SD, n = 3). (B) Confocal laser scanning microscopy (CLSM) images on MCF7 cells at pH 6.5, showing FITC channel (green), LysoTracker-stained lysosome channel (red), and DAPI-stained nucleus channel (blue), the scale bar = 20 μm (n = 3). (F) The quantitative cellular internalization of MCF7 cells after incubation with nano-rockets at pH 7.4 or pH 6.5, respectively ((a) CFPE-LF-NR, (b) CFPE-Bio3-BSa-NR, (c) CFPE-Bio3-BIm-NR, (d) CFPE-Bio3-BIm-NR&GOx+L-Arg-NM) (means ± SD, n = 3, ** indicates p < 0.01, *** indicates p <0.001). (C) was the fluorescence distribution and (H) was the semi-quantitative intensity of these nano-rockets in MCF7 tumor spheroids under pH 6.5 conditions ((a) CFPE-LF-NR, (b) CFPE-Bio3-BSa-NR, (c) CFPE-Bio3-BSa-NR&GOx+L-Arg-NM, (d) CFPE-Bio3-BIm-NR, (e) CFPE-Bio3-BIm-NR&GOx+L-Arg-NM) (means ± SD, n = 3, * indicates p < 0.05, ** indicates p < 0.01, *** indicates p <0.001, **** indicates p < 0.0001 versus CFPE-LF-NR). (G) The cytotoxicity study of different nano-missiles on MCF7 cells ((a) PTX-NM, (b) GOx+L-Arg-NM, (c) GOx+L-Arg-NM&PTX-NM) (means ± SD, n = 6). (I) Flow cytometry analysis and the apoptotic proportion of MCF7 cells after incubation with PTX-NM, GOx+L-Arg-NM, GOx+L-Arg-NM&PTX-NM (means ± SD, n = 3, *** indicates p < 0.001).
Figure S7. The cytotoxicity study of nano-rockets without ammunitions on (A) 4T1, (B) MCF7, (C) MDA-MB-231, (D) L02 cells (means ± SD, n = 6). (E) Confocal laser scanning microscopy (CLSM) images on L02 cells after incubation with different nano-rockets for 1 h, the scale bar = 20 µm (n = 3). (F) the quantitative cellular internalization of L02 cells after incubation with nano-rockets at pH 7.4 (means ± SD, n = 3, ** indicates p < 0.01 versus CFPE-LF-NR).
Figure S8. H&E analyses of the major organs (heart, liver, spleen, lung and kidney) after treatments of PBS (a), Free PTX (b), Free GOx+L-Arg (c), PTX-NM (d), GOx+L-Arg-NM (e), GOx+L-Arg-NM&PTX-NM (PTX = 3 mg/kg) (f), GOx+L-Arg-NM&PTX-NM (PTX = 1 mg/kg) (g). The white circle and arrow represent the blood cells in the intercellular space, and the scale bar = 200 µm.

6. Reference

1. Lu RX, Zhou L, Liu QJ, Wang SQ, Yang CY, Hai L, et al. Skillfully collaborating chemosynthesis with GOx-enabled tumor survival microenvironment deteriorating strategy for amplified chemotherapy and enhanced tumor ablation. Biomater Sci. 2021; 9: 1855-1871.
Figure S9. $^1$H-NMR of compound 2

Figure S10. $^{13}$C-NMR of compound 2
Figure S11. LR-MS of compound 2

![LR-MS of compound 2](image)

| m/z  | z | Abund       |
|------|---|-------------|
| 279.7| 1 | 100000.58   |
| 301.6| 1 | 65931.89    |
| 558.2| 1 | 15933.78    |
| 559.1| 1 | 46905.06    |
| 658.2| 1 | 22050.96    |
| 659.2| 1 | 75879.75    |
| 660.2| 1 | 33451.38    |
| 661.2| 1 | 88130.98    |
| 680.2| 1 | 182944.84   |
| 681.2| 1 | 64316.82    |

Figure S12. ¹H-NMR of compound 3

![¹H-NMR of compound 3](image)
Figure S13. $^{13}$C-NMR of compound 3

Figure S14. $^1$H-NMR of compound 4
Figure S15. LR-MS of compound 4

![LR-MS Graph]

| m/z | z  | Abund |
|-----|----|-------|
| 151.1 | 14190.49 |
| 152.1 | 99583.64 |
| 329.6 | 65823.64 |
| 658.2 | 512577.19 |
| 659.2 | 181955.58 |
| 660.2 | 84332.59 |
| 661.2 | 20563.41 |
| 680.2 | 116413.38 |
| 681.1 | 37163.34 |
| 682.2 | 18638.59 |

Figure S16. $^1$H-NMR of compound 6

![^1H-NMR Graph]
Figure S17. $^1$H-NMR of compound 8

Figure S18. $^{13}$C-NMR of compound 8
**Figure S19. LR-MS of compound 8**

![LR-MS spectrum of compound 8](image)

**Peak List**

| m/z  | z | Abund       |
|------|---|-------------|
| 421.1| 1 | 2225940.75  |
| 422.1| 1 | 670074.25   |
| 423.1| 1 | 105492.27   |
| 431.3| 1 | 262617.22   |
| 432.3| 1 | 78380.63    |
| 543.1| 1 | 338587.53   |
| 544.2| 1 | 113391.56   |
| 545.1| 1 | 22344.17    |

**Figure S20. ^1^H-NMR of compound 9**

![^1^H-NMR spectrum of compound 9](image)
Figure S21. $^{13}$C-NMR of compound 9

Figure S22. LR-MS of compound 9
Figure S23. $^1$H-NMR of compound 10

Figure S24. LR-MS of compound 10

| m/z  | z  | Abund  |
|------|----|--------|
| 419.7| 1  | 682705.75 |
| 554.3| 1  | 35297.46  |
| 658.2| 1  | 482966.85 |
| 938.3| 1  | 1044226.06|
| 939.3| 1  | 512137.16  |
| 940.3| 1  | 241601.31  |
| 941.3| 1  | 78373.3   |
| 960.3| 1  | 218829.7  |
| 961.3| 1  | 111051.05 |
| 962.3| 1  | 53541.01  |
Figure S25. $^1$H-NMR of compound 11

Figure S26. LR-MS of compound 11
Figure S27. $^1$H-NMR of compound 12

![H-NMR spectrum of compound 12](image)

Figure S28. LR-MS of compound 12

![LR-MS spectrum of compound 12](image)

**Peak List**

| m/z | Intensity (x10^5) |
|-----|-------------------|
| 169 | 0.0253            |
| 209 | 0.0451            |
| 532.8 | 0.08478855      |
| 838.4 | 0.02307939      |
| 1026.5 | 0.02171668      |
| 1064.5 | 0.015718033     |
| 1065.5 | 0.009146     |
| 1066.5 | 0.005018281    |
| 1086.5 | 0.005111581    |
| 1087.5 | 0.003113376    |
Figure S29. $^1$H-NMR of compound 14

Figure S30. $^1$H-NMR of compound 15
Figure S31. $^{13}$C-NMR of compound 15

Figure S32. $^1$H-NMR of compound 16
Figure S33. $^1$H-NMR of compound 17

![Figure S33. $^1$H-NMR of compound 17](image1)

Figure S34. $^1$H-NMR of compound 18

![Figure S34. $^1$H-NMR of compound 18](image2)
Figure S35. $^1$H-NMR of compound 20

Figure S36. $^1$H-NMR of compound 21
Figure S37. $^1$H-NMR of compound 22

Figure S38. $^1$H-NMR of compound 23
Figure S39. $^1$H-NMR of ligand Bio$_3$-PEG-BIm

Figure S40. HR-MS of ligand Bio$_3$-PEG-BIm
Figure S41. $^1$H-NMR of compound 25

Figure S42. $^{13}$C-NMR of compound 25
Figure S43. $^1$H-NMR of ligand Bio$_2$-PEG-Alm

Figure S44. $^1$H-NMR of compound 28
Figure S45. $^1$H-NMR of compound 29

![NMR Spectrum of Compound 29](image)

Figure S46. $^1$H-NMR of compound 30

![NMR Spectrum of Compound 30](image)
Figure S47. $^1$H-NMR of compound 31

![H-NMR of compound 31](image1.png)

Figure S48. $^1$H-NMR of ligand Bio$_2$-PEG-EIm

![H-NMR of Bio$_2$-PEG-EIm](image2.png)
Figure S49. $^1$H-NMR of compound 32

Figure S50. $^1$H-NMR of compound 33
Figure S51. $^1$H-NMR of ligand Bio$_2$-PEG-Hz

Figure S52. $^1$H-NMR of ligand Bio$_2$-PEG-BSa