Supplementary Files

**Functionalized asymmetric bola-type amphiphiles for efficient gene and drug delivery**

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![Fluorescent quenching assay of EB/DNA with the addition of bolasomes. The molar ratio of bolaamphiphile/DOPE was 1:1.](image1)

**Figure S1.** Fluorescent quenching assay of EB/DNA with the addition of bolasomes. The molar ratio of bolaamphiphile/DOPE was 1:1.

![Mean particle sizes (columns) and zeta potentials (dots) of the five bolasomes with the DOPE/bolaamphiphile ratio of 1:1 obtained by DLS at room temperature. Data represent mean ± SD (n = 3).](image2)

**Figure S2.** Mean particle sizes (columns) and zeta potentials (dots) of the five bolasomes with the DOPE/bolaamphiphile ratio of 1:1 obtained by DLS at room temperature. Data represent mean ± SD (n = 3).
Figure S3. CLSM images of HeLa cells transfected with Cy5-labelled DNA by the bolaplexes at the optimal transfection N/P ratio. For each row, left: cell nuclei stained by Hoechst 33342 (blue); middle: Cy5-labelled pGL-3 DNA (red); right: merged image. Scale bar: 10 μm.

Figure S4. Relative cellular uptake of Lys-His/DNA bolaplexes at optimal transfection N/P ratio in HeLa cells in the presence of various endocytic inhibitors quantified by flow cytometry analysis. (***P < 0.001)
**Figure S5.** Acid–base titration profiles of Lys-14-10, Lys-His, 25 kDa PEI and 150 mM NaCl solutions. Bolaamphiphiles or PEI (0.050 mmol of amino groups) was first treated with 1 N HCl to adjust pH to 2.0, and then the solution pH was measured after each addition of 20 μL of 0.1 N NaOH.

**Figure S6.** In vitro cytotoxicity of the bolaplexes at various N/P ratios in HepG-2 (A) and A549 cells (B) for a 24 h incubation. Data represent mean ± SD (n= 3).

**Figure S7.** TEM images of the bolaamphiphile aggregates without DOPE
Figure S8. Plots of the intensity ratio $I_{383}/I_{373}$ from the pyrene emission spectra versus the logarithm of the concentration for self-assembling aggregates in aqueous media from Lys-His, Lys-TPP, Lys-Gal and Lys-Biotin, respectively.

Figure S9. Aqueous drug-loaded bolaamphiphiles solutions after storage under 4°C for 5 months.
Figure S10. Mean particle sizes (A) and zeta-potentials (B) of drug-free bolaamphiphiles nanoparticles. Mean particle sizes (C) and zeta-potentials (D) of drug-loaded bolaamphiphiles nanoparticles measured by DLS at room temperature at a fixed angle (90°). Data represent mean ± SD (n = 3).

Figure S11. *In vitro* cytotoxicity of DOX-free bolaamphiphiles nanoparticles in HepG-2 (A) and HeLa (B) cells treated with various concentrations blank materials for 48 h. Data represent mean ± SD (n = 3).
Analysis data of Compound 2

Diol-14-10-OTBS: Colourless liquid, yield: 65.2%. 1H NMR (CDCl3, 400 MHz): δ (ppm) = 4.02 (t, 4H, J=8.0 Hz, COOH2), 3.62-3.55 (m, 4H, HO-CH2, Si-OC2H5), 2.26 (t, 4H, J=8.0 Hz, CH2-OCOC), 1.58-1.23 (m, 52H, (CH2)3), 0.86 (s, 9H, -(CH3)3), 0.02 (s, 6H, Si-CH3). 13C NMR (CDCl3, 100 MHz): δ (ppm) = 174.02, 64.39, 64.35, 63.30, 63.29, 34.37, 32.84, 32.75, 29.55, 29.51, 29.48, 29.44, 29.41, 29.37, 29.26, 29.22, 29.19, 29.13, 28.60, 25.96, 25.90, 25.88, 25.75, 25.70, 25.62, 24.99, -5.28. HR-MS (ESI): CaH58O3SiNa [M+Na]+, 707.5622, found: 707.5623.

Analysis data of Compound 3

2Boc-Lys-14-10-OH: White powder, yield: 53.8%. 1H NMR (CDCl3, 400 MHz): δ (ppm) = 4.63 (s, 1H, CONH-CH2), 4.06 (t, 2H, J=8.0 Hz, COO-CH2), 4.00 (t, 4H, J=8.0 Hz, CH2-OCOC), 3.58 (t, 2H, J=8.0 Hz, HO-CH2), 3.09-2.98 (m, 2H, CONH-CH2), 2.24 (t, 4H, J=8.0 Hz, OOC-CH2), 1.83-1.48 (m, 16H, CONH-CH2), CONH-CH2), CONH-CH2), COO-CH2), COO-CH2), OOC-CH2), HO-CH2), 1.39 (s, 18H, Boc-H), 1.34-1.15 (m, 40H, (CH2)3), 0.31-0.02 (s, 6H, Si-C2H5). 13C NMR (CDCl3, 100 MHz): δ (ppm) = 173.99, 172.84, 171.12, 156.01, 155.44, 79.71, 79.02, 65.37, 64.32, 62.87, 60.35, 53.24, 40.05, 34.34, 32.73, 32.36, 29.51, 29.46, 29.41, 29.38, 29.35, 29.22, 29.17, 29.12, 29.10, 28.58, 28.48, 28.37, 28.28, 25.86, 25.76, 25.69, 24.96. HR-MS (ESI): CaH58O3SiNa [M+Na]+, 707.655, found: 707.657.

Analysis data of target products

Lys-His: Flavescent ropy liquid, yield: 90.9%. 1H NMR (CD3OD, 400 MHz): δ (ppm) = 8.86 (s, 1H, imidazole-H), 7.45 (s, 1H, imidazole-H), 4.27-4.18 (m, 4H, Lys-COO-CH2), His-COO-CH2), 4.04 (t, 5H, J=8.0 Hz, CH2-OCOC), 3.44-3.33 (m, 2H, imidazole-CH2), 3.31-3.27 (m, 1H, NH2-(CH2)3-CH2), 2.92 (t, 2H, J=8.0 Hz, NH2-CH2), 2.28 (t, 4H, J=8.0 Hz, OOC-CH2), 1.99-1.86 (m, 2H, NH2-(CH2)3-CH2), 1.75-1.20 (m, 56H, (CH2)3), 13C NMR (CD3OD, 100 MHz): δ (ppm) = 174.24, 169.00, 167.71, 134.52, 127.29, 118.09, 66.68, 66.23, 64.04, 52.25, 51.53, 38.76, 33.69, 29.62, 29.25, 29.18, 29.17, 29.12, 28.96, 28.90, 28.75, 28.32, 28.13, 28.03, 26.59, 25.61, 25.44, 25.36, 25.23, 24.68, 21.67. HR-MS (ESI): CaH58O3SiNa [M+Na]+, 858.6296, found: 858.6238.

Lys-TPP: Flavescent ropy liquid, yield: 93.4%. 1H NMR (CD3OD, 400 MHz): δ (ppm) = 7.91-7.70 (m, 15H, benzene-H), 4.27-4.19 (m, 2H, TPP-COO-CH2), 4.07-3.97 (m, 6H, Lys-COO-CH2), CH2-OCOC), 3.45-3.41 (m, 1H, NH2-(CH2)3-CH2), 2.94 (t, 2H, J=8.0 Hz, NH2-CH2), 2.38 (t, 2H, J=8.0 Hz, (Ph)-P-CH2), 2.28 (t, 4H, J=8.0 Hz, OOC-CH2), 2.00-1.90 (m, 2H, NH2-(CH2)3-CH2), 1.83 (t, 2H, J=8.0 Hz, (Ph)-P-(CH2)3-CH2), 1.76-1.20 (m, 58H, (CH2)3), 13C NMR (CD3OD, 100 MHz): δ (ppm) = 174.20, 173.24, 169.01, 134.90, 134.87, 133.43, 133.33, 130.18, 130.05, 118.84, 117.98, 114.92, 66.23, 64.23, 64.04, 61.56, 52.26, 38.78, 33.72, 32.48, 29.62, 29.26, 29.18, 29.13, 28.97, 28.91, 28.75, 28.34, 28.29, 28.14, 26.58, 25.63, 25.57, 25.45, 25.43, 25.25, 24.70, 21.69. HR-MS (ESI): CaH108N3O3P [M+H]+, 1044.7296, found: 522.3609(2).

Lys-Biotin: Flavescent ropy liquid, yield: 92.5%. 1H NMR (CD3OD, 400 MHz): δ (ppm) = 4.51-4.46 (m, 1H, CONH-CH2), 4.32-4.27 (m, 1H, CONH-CH2), 4.26-4.20 (m, 2H, Lys-COO-CH2), 4.07-4.00 (m, 6H, CH2-OCOC),, Biotin-COO-CH2), 3.31-3.21 (m, 1H, NH-CH2), 3.22-3.16 (m, 1H, S-CH2), 2.96-2.66 (m, 4H, NH2-CH2, S-CH2), 2.35-2.25 (m, 6H, OOC-CH2), 1.99-1.88 (m, 2H, NH2-(CH2)3-CH2), 1.78-1.20 (m, 62H, (CH2)3), 13C NMR (CD3OD, 100 MHz): δ (ppm) = 174.19, 173.99, 169.02, 164.66, 66.24, 64.15, 64.06, 61.97, 60.20, 55.58, 52.26, 39.66, 38.77, 33.74, 33.72, 33.47, 29.62, 29.27, 29.21, 29.19, 29.18, 28.98, 28.93, 28.91, 28.77, 28.35, 28.14, 28.09, 25.58, 25.60, 25.64, 25.45, 24.72, 24.71, 24.61, 21.67. HR-MS (ESI): CaH108N3O3S [M+H]+, 925.6663, found: 925.6654.
**Lys-Gal:** Flavescent ropy liquid, yield: 91.5%. $^1$H NMR (CD$_3$OD, 400 MHz): $\delta$ (ppm) = 4.36-4.31 (m, 1H, Galactose-$H$), 4.28-4.00 (m, 10H, -COO-$CH_2$), 3.82-3.40 (m, 5H, NH-$CH$, Galactose-$H$), 2.94 (t, 2H, $J$=8.0 Hz, NH$_2$-$CH_2$), 2.66-2.57 (m, 4H, Galactose-$CH_2$-OOC-$CH_2$), 2.28 (t, 4H, $J$=8.0 Hz, OOC-$CH_2$), 1.95-1.87 (m, 2H, NH$_2$-(CH$_2$)$_3$-$CH_2$), 1.77-1.22 (m, 56H, (CH$_2$)$_2$). $^{13}$C NMR (CD$_3$OD, 100 MHz): $\delta$ (ppm) = 174.15, 172.74, 172.48, 169.06, 97.25, 92.79, 73.30, 72.44, 72.22, 69.56, 66.44, 66.23, 64.50, 64.09, 63.76, 63.56, 52.31, 38.82, 33.77, 33.59, 31.69, 29.60, 29.30, 29.22, 29.01, 28.96, 28.79, 28.54, 28.47, 28.37, 28.32, 28.14, 27.29, 26.55, 25.69, 25.62, 25.46, 24.74, 22.94, 21.67. HR-MS (ESI): C$_{50}$H$_{93}$N$_2$O$_{15}$ [M+H]$^+$; 961.6576, found: 961.6559.