Supporting Information

Target-Selective Vesicle Fusion System with pH-Selectivity and Responsiveness

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Synthesis of Compound III as the raw material for Compound 1

2-Methylphenylboronic acid neopentylglycol ester (I)

2-Methylphenylboronic acid (2.72 g, 20.0 mmol) and 2,2-dimethyl-1,3-propanediol (2.50 g, 24.0 mmol) were dissolved in toluene (50 cm$^3$) in a 100 cm$^3$ oven dried round bottom flask. To this solution, $p$-toluenesulfonic acid (catalytic amount) was added. The round bottom flask was connected to a Dean Stark apparatus under reflux condition to remove the water. The progress of the reaction was monitored by TLC (silica gel 60, hexane-ethyl acetate = 8:2 (v/v)). After 6 h when the spot of 2-methylphenylboronic acid ($R_f = 0.16$) disappeared, the reaction was finished. After cooling the reaction mixture was washed rapidly with water (50 cm$^3$). The organic layer was separated, dried over anhydrous sodium sulfate, and concentrated to dryness. The residue being further purified through column chromatography (silica gel 60, hexane-ethyl acetate = 8:2 (v/v)) to obtain the pure title compound I as a pale yellow oil (4.03 g, 97% yield).

FAB-MS spectroscopy for MH$^+$: 205.18 (calcd. 205.08). $^1$H NMR (400 MHz, DMSO-d$_6$) : $\delta$ = 0.97 (s, 6H), 2.43 (s, 3H), 3.75 (s, 4H), 7.10-7.13 (br t+t, 2H), 7.26 (d, 1H), 7.60 (d, 1H).

2-Bromomethylphenylboronic acid neopentylglycol ester (II)

Compound I (4.00 g, 19.6 mmol) and N-bromosuccinimide (NBS) (3.69 g, 20.7 mmol) were dissolved in CCl$_4$ (50 cm$^3$) in a 100 cm$^3$ oven dried round bottom flask. To this solution,
α,α′-azobisisobutyronitrile (AIBN) (0.37 g, 10 wt% of NBS) was added. The solution was held at reflux for 3 h. The progress of the reaction was monitored by TLC (silica gel 60, hexane-ethyl acetate = 7:3 (v/v)). After cooling, insoluble materials were removed by filtration and then the filtrate was washed rapidly with water (50 cm³). The organic layer was separated, dried over anhydrous sodium sulfate, and concentrated to dryness. The residue being further purified through column chromatography (silica gel 60, hexane-ethyl acetate = 7:3 (v/v)) to obtain the pure title compound II as a brown oil (4.81 g, 86% yield from I).

FAB-MS spectroscopy for MH⁺: 286.12 (calcd. 283.98). ¹H NMR (400 MHz, DMSO-d₆): δ = 0.98 (s, 6H), 3.75 (s, 4H), 4.95 (s, 2H), 7.29 (d, 1H), 7.39-7.42 (br t+t, 2H), 7.69 (d, 1H). ²-

2-[N-Methyl-N-(p-carboxyphenyl)]aminomethylphenylboronic acid neopentylglycol ester (III)

Compound II (4.81 g, 16.9 mmol) and 4-(methylamino)benzoic acid (2.80 g, 18.5 mmol) were dissolved in N,N-dimethylformamide (20 cm³) in a 100 cm³ oven dried round bottom flask. The solution was held at reflux for 3 h in the presence of potassium carbonate (3.50 g, 25.4 mmol). After cooling, insoluble materials were removed by filtration and then the filtrate was concentrated to dryness. The residue was taken with a mixture of chloroform (20 cm³) and aqueous 5% sodium hydrogen carbonate solution (20 cm³). This heterogeneous mixture was stirred for 30 min at room temperature. The organic layer was separated, washed with water (50 cm³), dried over anhydrous sodium sulfate, and concentrated to dryness. The residue being further purified through column chromatography (silica gel 60, hexane-ethyl acetate = 7:3 (v/v)) to obtain the pure title compound III as a brown oil (2.95 g, 49% yield from II).

FAB-MS spectroscopy for MH⁺: 354.30 (calcd. 354.23). ¹H NMR (400 MHz, DMSO-d₆): δ = 0.75 (s, 6H), 3.11 (s, 3H), 3.76 (s, 4H), 5.25 (s, 2H), 6.57 (d, 2H), 7.30-7.40 (br d+t+t, 3H), 7.60 (d, 1H), 7.74 (d, 2H) ppm; ¹³C NMR (100 MHz, DMSO-d₆): δ = 22.1, 37.7, 65.6, 67.4, 110.8, 115.8, 127.2, 128.2, 128.9, 130.9, 131.6, 137.4, 138.1, 139.0, 166.3 ppm.
1H and 13C NMR charts of **Compound III**
Spectral data of Compound 1

HPLC trace of Compound 1 (after purification)

\[ \text{[mV]} \]

\[ 0 \quad 2 \quad 4 \quad 6 \quad 8 \quad 10 \quad 12 \quad 14 \quad 16 \quad 18 \quad 20 \quad 22 \quad 24 \quad 26 \quad 28 \]

\[ \text{Time (min)} \]

\[ 10.69 \]

\[ 0 \quad 5 \quad 10 \quad 15 \quad 20 \quad 25 \quad 30 \]

\[ \text{HPLC trace of Compound 1 (after purification)} \]

\[ \text{Solvent A: Water (containing 0.1% TFA)} \]

\[ \text{Solvent B: Acetonitrile (containing 0.1% TFA)} \]

\[ \text{Linear gradient system: B: 20% (0 min) → 30% (90 min)} \]

\[ \text{Flow: 1.0 mL/min} \]

\[ \text{Wavelength: 254 nm} \]

\[ \text{1H NMR chart of Compound 1} \]
$^{13}$C NMR chart of Compound 1
**Fig. S1** The lipid mixing experiment by the use of pre-incubated pilot vesicles (EggPC/DPGS/Compound 1) with 10 mM of free myo-inositol (open circle). The measurements were performed in 10 mM acetic acid/sodium acetate buffer (containing 100 mM NaCl, pH 5.0) at 30 °C.
Fig. S2  DLS size distribution profiles of target vesicles (EggPC/PI/NBD-PE/Rh-PE) before (A) and after (B) gel filtrations for inner leaflet mixing assays.

Fig. S3  Fluorescence spectra of gel filtrated target vesicles and further sodium dithionite added vesicles.