Supplementary Information for

Engineering Boronic Acid-Integrated Lipid Nanoparticles for Precision Glycan-Mediated Gene Transfer

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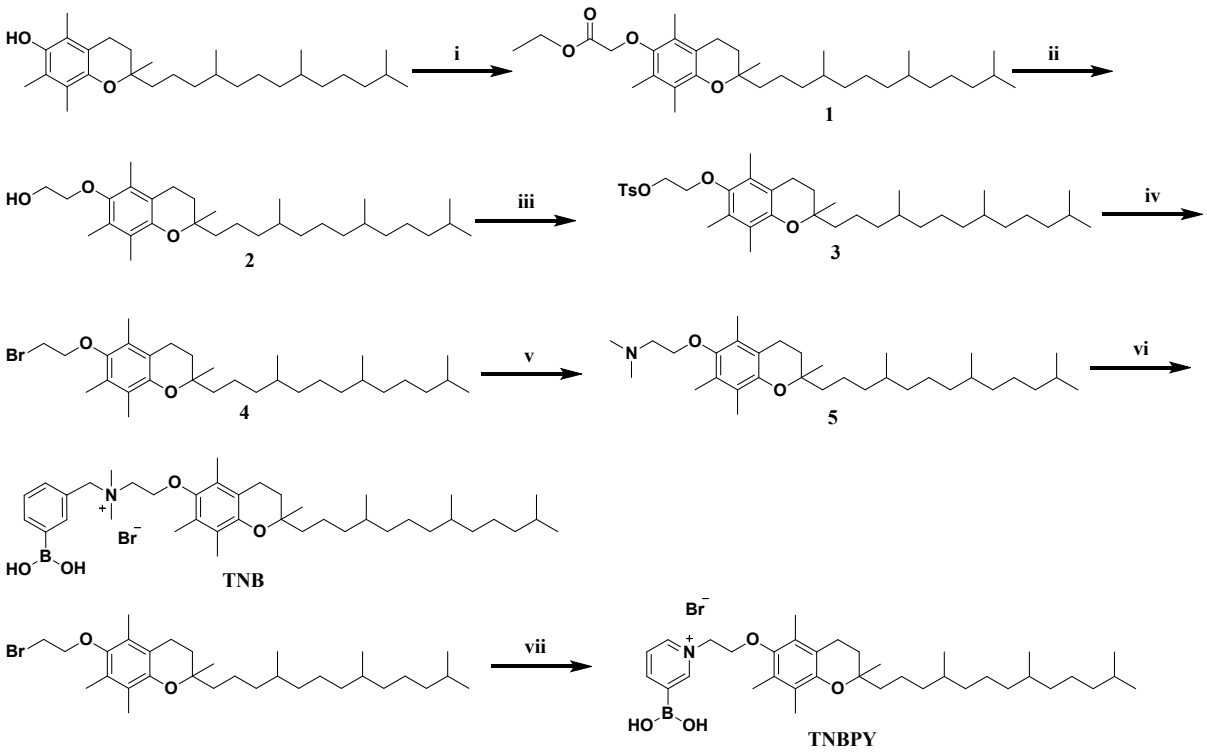
Scheme S1[a]



[a]**Reagents, conditions, and yield:** (i) NaH, ethyl bromoacetate, dry DMF, 0 °C–rt, 16 h, N_2 atm, 60%; (ii) $LiAlH_4$, THF, 0 °C–rt, 6 h, N_2 atm, 90%; (iii) p-TsCl, Py, CH_2Cl_2, DMAP (cat), 6 h, rt, 85%; (iv) LiBr, acetone, reflux, 12 h, 75%; v) aq. Dimethyl amine (40%), CH_3CN, EtOH, 80 °C, pressure tube, 24 h, 60%; (vi) 3-(Bromomethyl)phenylboronic acid, DMF, rt, 24h, 60%; (vii) 3-pyridinyl boronic acid, DMF, reflux, 72 h, 40%.

Synthesis of Tocopheryloxyethyl N,N-dimethylamine (5)

Compound 4 (1 g, 1.862 mmol) was dissolved in dry acetonitrile (10 mL) and ethanol (2 mL), and dimethylamine was added in a pressure tube. The mixture was heated at 80 °C for 12 h. After completion, the solvent was removed under reduced pressure, and the residue was diluted with chloroform (500 mL). The organic layer was washed sequentially with water (2 × 100 mL) and brine (2 × 100 mL), then dried over anhydrous sodium sulfate. The filtrate was concentrated under vacuum to obtain a crude solid, which was purified by column chromatography using a chloroform/methanol mixture (100:6, v/v) as the eluent. The product was isolated as a solid (0.6 g, 60% yield).

Synthesis of Boronic acid-based Lipids TNB. Compound 5 (100 mg, 1.0 equiv) was dissolved in dry DMF (5 mL), followed by the addition of 3-(bromomethyl)phenylboronic acid (1.0 equiv). The reaction mixture was refluxed for 12 h. After completion, the solvent was removed under reduced pressure, and the crude residue was repeatedly washed with diethyl ether to afford the pure product.

TNB, ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.83-0.87 (*m*, 12H, -CH-CH_3, phytyl chain), 1.07-1.83 (m, 26H), 2.07 (s, 3H, -CH_3), 2.14 (s, 3H, -CH_3), 2.18 (s, 3H, -CH_3), 2.5 (t, 2H, J = 6.8 Hz), 3.34 (s, 6H), 3.95 (t, 2H), 4.04 (t, 2H), 4.92 (s, 2H), 7.30-7.32 (d, 1H), 7.58-7.60 (d, 1H), 7.87-7.89 (d, 1H), 8.02 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 150.77, 148.58, 147.37 (oxygenated aromatic carbons of the tocopherol chromanol ring and deshielded aromatic carbon in the boronic acid-substituted benzyl headgroup), 137.01, 134.88, 128.26, 126.88, 125.96, 124.98, 123.41, 117.95 (remaining aromatic carbons of the phenylboronic acid benzyl group and chromanol aromatic carbons of the tocopherol moiety), 75.06 (oxygen-bearing aliphatic carbon adjacent to the chromanol ether linkage), 69.98 (ether-linked carbons), 66.19, 63.64, 63.44 (methylene carbons adjacent to the quaternary ammonium nitrogen including benzyl N–CH_2 and linker N–CH_2 groups), 50.96 (methyl carbons attached to the ammonium center), 40.08, 39.38, 37.61, 37.51, 37.29, 34.92, 32.79, 31.22, 29.71, 27.98 (aliphatic methylene and methine carbons of the tocopherol side chain and chromanol ring junction), 24.81, 24.46, 23.66, 22.64, 21.05, 20.68, 19.58 (branched alkyl carbons and methyl-substituted aliphatic carbons), 13.33, 12.50, 11.84 (terminal and branched methyl carbons of the tocopherol framework). ESI-MS calculated for m/z [$C_{40}H_{67}BNO_4^+$] = 636.5158, found 636.2063.

Synthesis of Boronic acid-based Lipids TNBPY. A solution of compound 4 (150 mg, 1.0 equiv) in dry DMF (7 mL) was treated with 3-pyridinylboronic acid (1.05 equiv), and the

mixture was refluxed for 24 h. Upon completion of the reaction, the solvent was removed under reduced pressure, and the resulting residue was thoroughly washed with diethyl ether to yield the desired pure product.

TNBPY, ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.82-0.86 (m, 12H, -CH-CH_3, phytyl chain), 1.06-1.54 (m, 26H), 1.75 (s, 3H, -CH_3), 1.79 (s, 3H, -CH_3), 1.9 (s, 3H, -CH_3), 2.5 (t, 2H, J = 6.8 Hz), 4.12 (t, 2H), 5.36 (t, 2H), 8.08 (s, 2H), 8.5 (s, 1H), 8.5 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 148.54, 146.14, 145.38 (deshielded aromatic carbons of the pyridinium–boronic acid headgroup and oxygenated aromatic carbon of the tocopherol ring), 128.69, 127.83, 126.93, 125.87, 125.13, 123.37, 117.90 (remaining aromatic carbons of the pyridinium ring and chromanol aromatic carbons of the tocopherol moiety), 75.02 (oxygen-bearing aliphatic carbon adjacent to the chromanol ether linkage), 70.08, 62.33 (ether-linked carbons), 39.96, 39.37, 37.57, 37.29, 32.83, 31.77, 31.15, 29.94, 29.37, 27.82 (aliphatic methylene and methine carbons of the tocopherol side chain and chromanol ring junction), 24.81, 24.45, 23.74, 22.73, 21.06, 20.57, 19.57 (branched alkyl carbons and ring methyl substituents), 14.10, 12.30, 11.73, 11.44 (terminal and branched methyl carbons of the tocopherol framework). ESI-MS calculated for m/z [$C_{36}H_{59}BNO_4^+$] = 580.6684, found 536.4362. The characteristic fragment peak at m/z = 536.4362 was detected, which can be attributed to the loss of a $B(OH)_2$ unit.

Quantification of Cell-Surface Sialylation by SNA-FITC Assay. Cell-surface sialic acid expression was analyzed using SNA-FITC, a lectin specific for α 2,6-linked sialic acid residues. Cells (4T1, MCF-7, MCF-10A, and McCoy) were cultured, washed with PBS, and incubated with SNA-FITC (5 μg mL^{-1}) at 4 °C for 30 min in the dark. After washing to remove excess lectin, fluorescence was measured by flow cytometry in the FITC channel. The mean fluorescence intensity (MFI) was used to quantify surface sialylation. All experiments were performed in triplicate (n = 3).

Figure S1. ^{1}H-NMR spectra of TNB in $CDCl_3$.

Figure S2. ^{1}H-NMR spectra of TNBPY in $CDCl_3$.

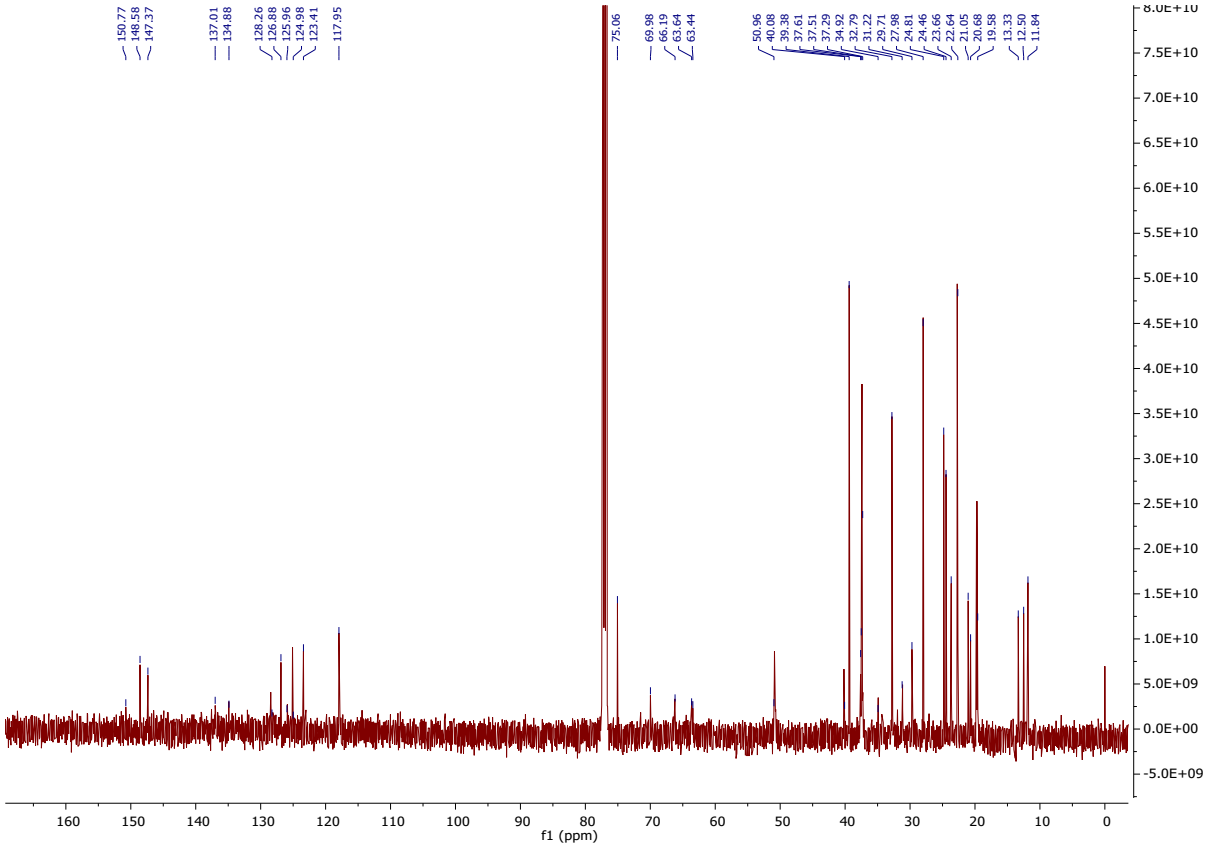


Figure S3. ^{13}C-NMR spectra of TNB in $CDCl_3$.

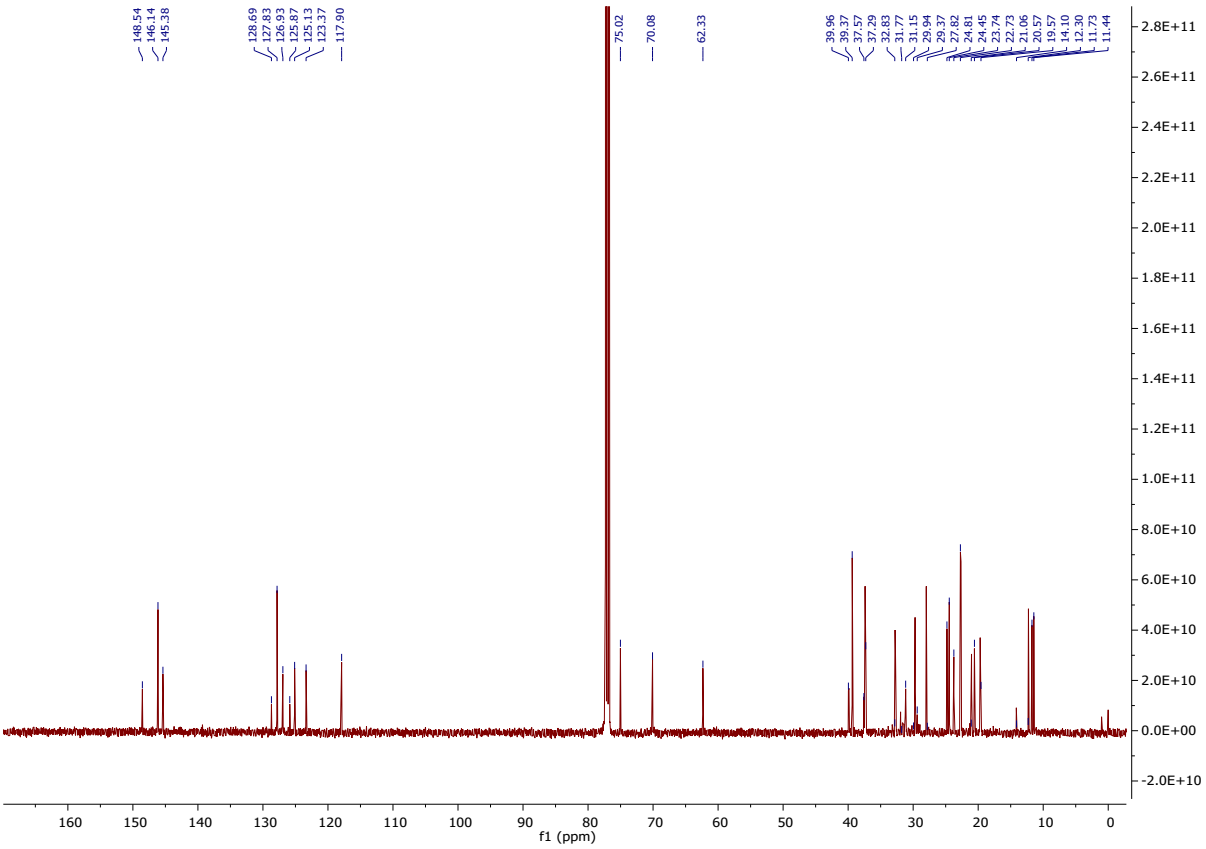


Figure S4. ^{13}C-NMR spectra of TNBPY in $CDCl_3$.

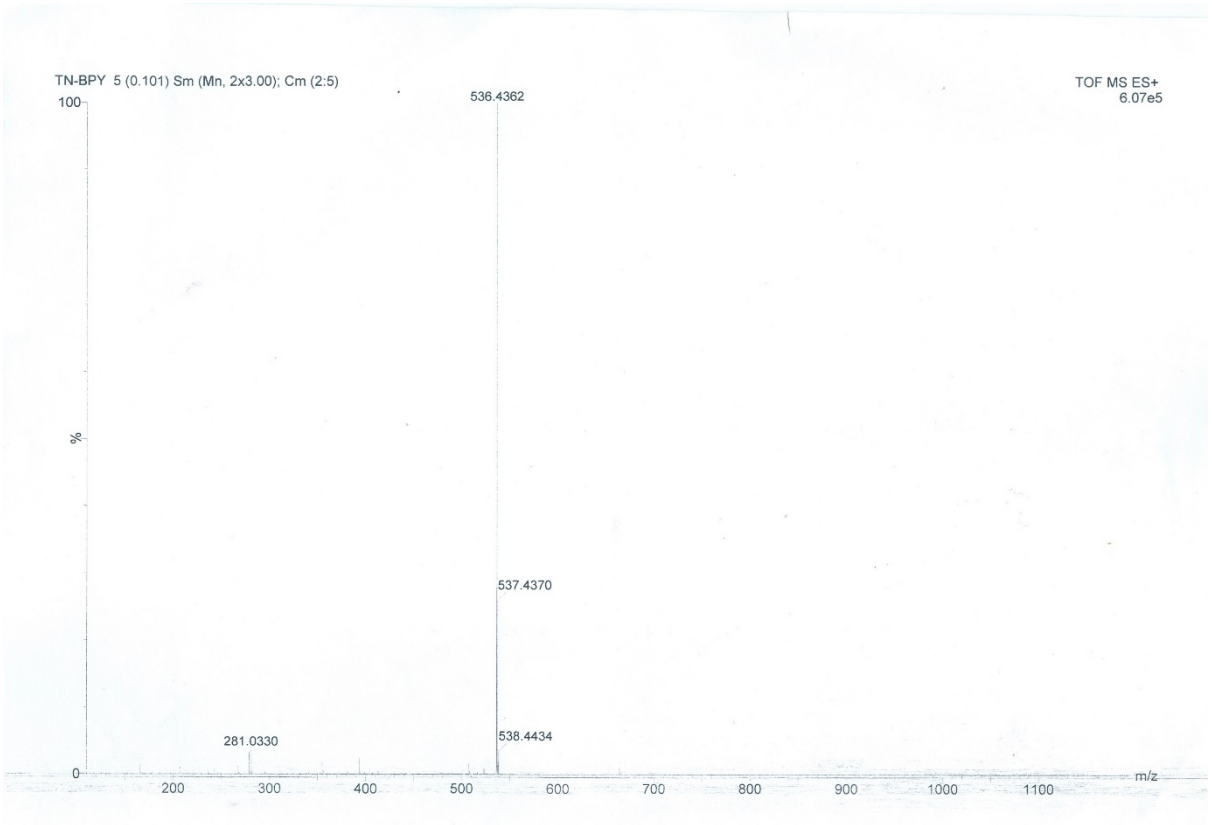


Figure S5. Mass spectra of TNBPY.

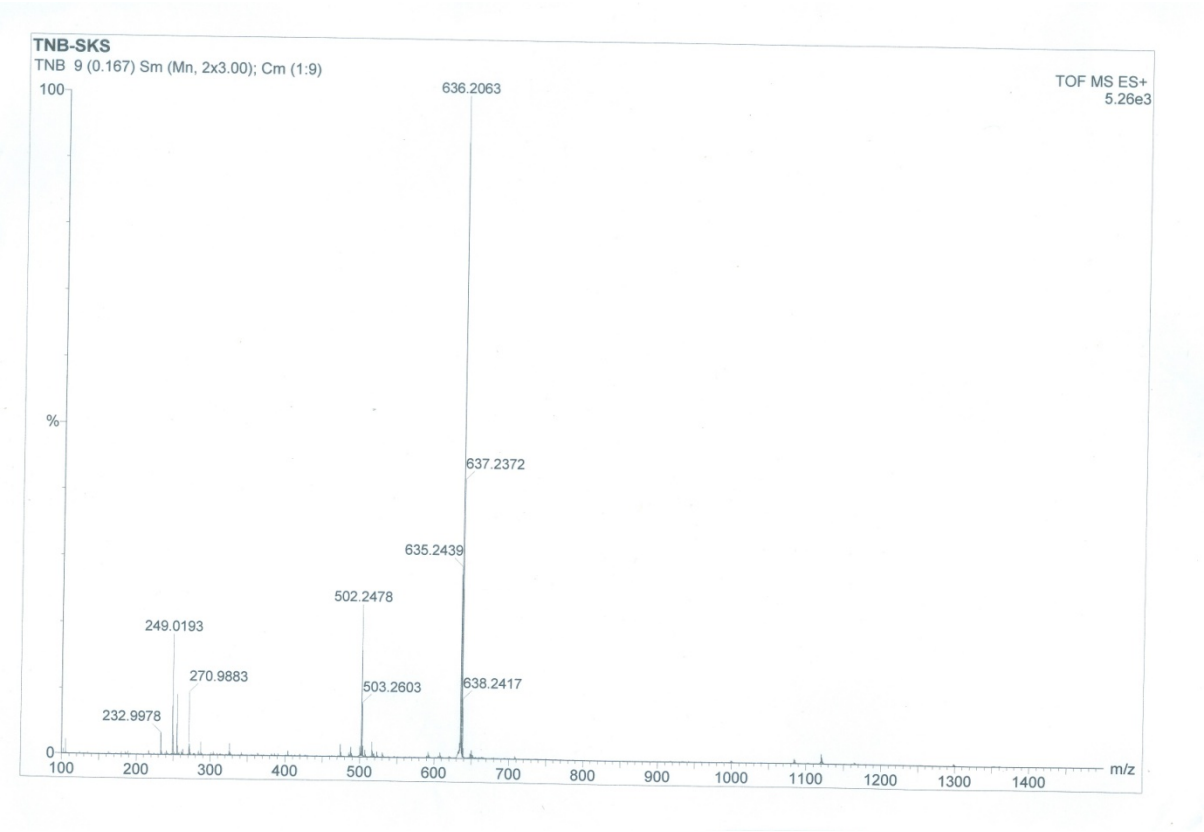


Figure S6. Mass spectra of TNB.

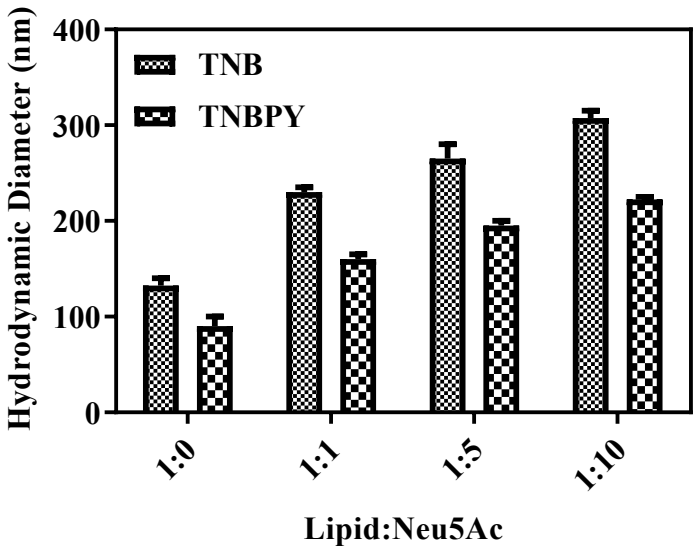
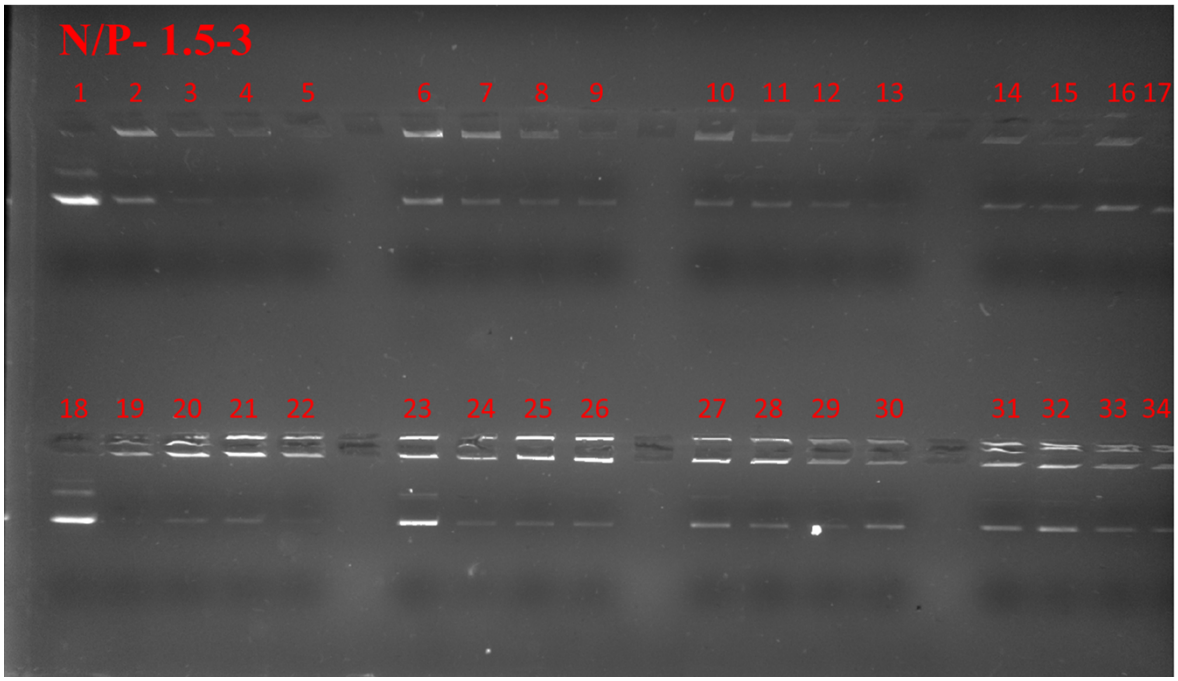


Figure S7. Changes in hydrodynamic diameter of lipids upon gradual addition of Neu5Ac. Data are presented as mean ± SD from three independent experiments (n = 3).



Lane 1 & 18 : DNA alone

Lane 2-5 : DOPE:TH8S:TNBPY (2:1:0.5) **Lane 19-22 : DOPE:TH8S:TNB (2:1:0.5)**

Lane 6-9 : DOPE:TH8S:TNBPY (2:1:1) **Lane 23-26 : DOPE:TH8S:TNB (2:1:1)**

Lane 10-13 : DOPE:TH8S:TNBPY (2:1:1.5) **Lane 27-30 : DOPE:TH8S:TNB (2:1:1.5)**

Lane 14-17 : DOPE:TH8S:TNBPY (2:1:2) **Lane 31-34 : DOPE:TH8S:TNB (2:1:2)**

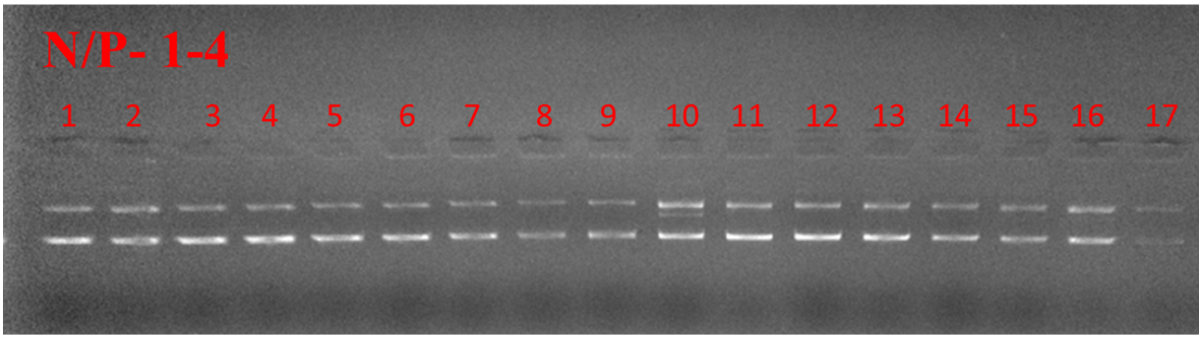
Lane 2, 6, 10, 14, 19, 23, 27, 31 : N/P = 1.5

Lane 3, 7, 11, 15, 20, 24, 28, 32 : N/P = 2.0

Lane 4, 8, 12, 16, 21, 25, 29, 33 : N/P = 2.5

Lane 5, 9, 13, 17, 22, 26, 30, 34 : N/P = 3.0

Figure S8. Agarose gel electrophoresis for the lipoplexes of DOPE:TH8S:TNB and DOPE:TH8S:TNBPY in different molar ratios from N/P = 1.5 to 3. The co-liposomes were complexed with *p*DNA (0.2 µg) and run on 1% agarose gel for ~30 min at 80V.



Lane 1 – only plasmid
Lane 2-5 – TNBPY:DOPE; 1:1
Lane 6-9- TNBPY:DOPE; 1:2
Lane 10-13- TNB:DOPE; 1:1
Lane 14-17- TNB:DOPE; 1:2

Figure S9. Agarose gel electrophoresis for the lipoplexes of DOPE:TNB and DOPE:TNBPY in different molar ratios from N/P = 1 to 4. The co-liposomes were complexed with *p*DNA (0.2 µg) and run on 1% agarose gel for ~30 min at 80V.

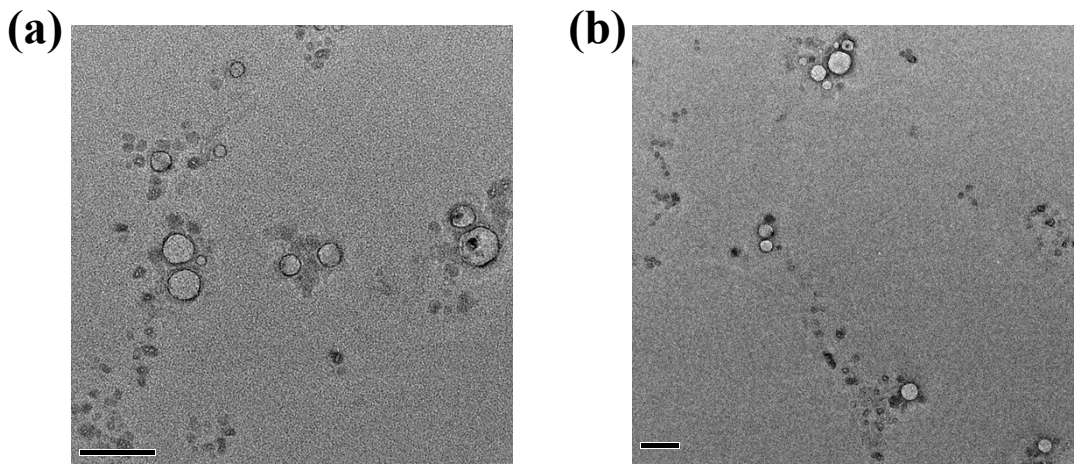


Figure S10. Additional TEM images of **(a).** DOPE:TH8S:TNB (2:1:1), **(b).** DOPE:TH8S:TNBPY (2:1:1).

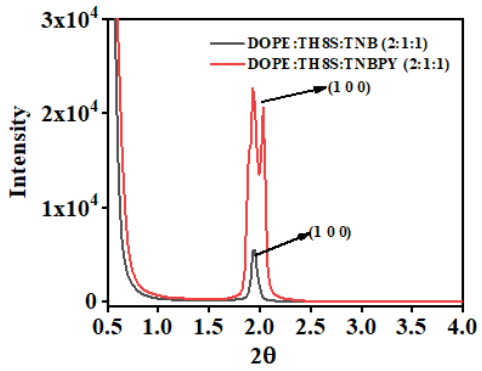


Figure S11. Small angle x-ray diffraction plot of mixed co-liposomal formulations.

Table S1. Calculation of bilayer widths of the co-liposomal formulations.

Co-liposomal formulation	2θ (degree)	$d_{(100)}$ (nm)
DOPE:TH8S:TNB (2:1:1)	1.94	4.55
DOPE:TH8S:TNBPY (2:1:1)	1.92, 2.03 (two peak)	4.6, 4.35

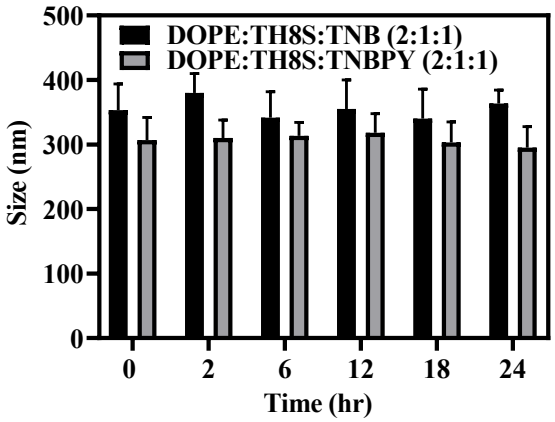


Figure S12. DLS studies to check the stability of the lipoplexes with time. Data are presented as mean ± SD from three independent experiments (n = 3).

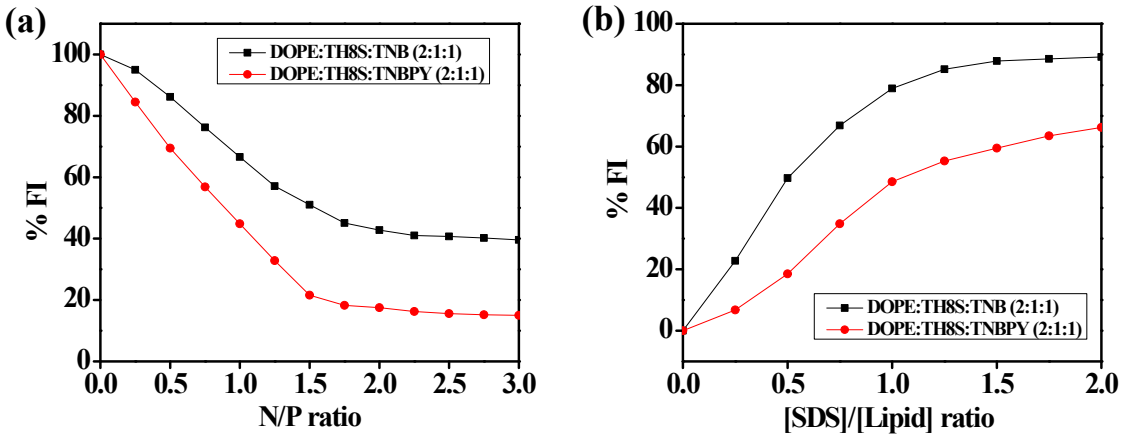


Figure S13. (a) Ethidium bromide exclusion assay showing the effect of the addition of transfection optimized co-liposomal formulations (DOPE:TH8S:TNB and DOPE:TH8S:TNBPY) at different N/P ratios into the EB-*p*DNA complex, **(b)** Ethidium bromide re-intercalation assay depicting the effect of addition of SDS micelles into the lipoplexes of co-liposomal formulations (DOPE:TH8S:TNB and DOPE:TH8S:TNBPY).

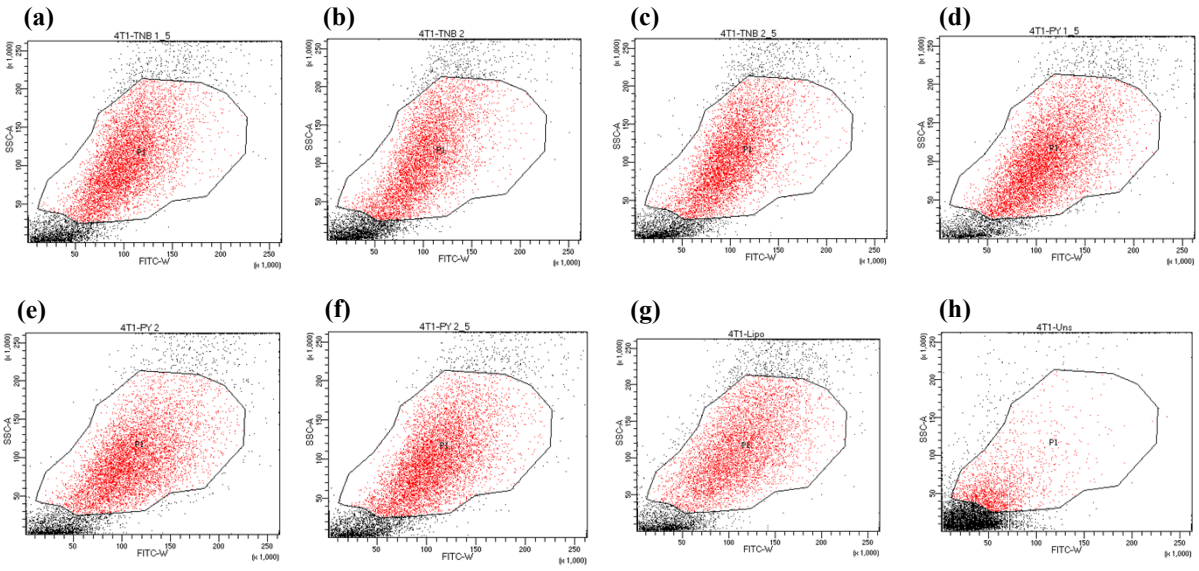


Figure S14. FACS data **(a-h)** of the co-liposomal formulations DOPE:TH8S:TNB (2:1:1) and DOPE:TH8S:TNBPY (2:1:1) from N/P=1.5 to 2.5 showing the EGFP expression in 4T1 cells.

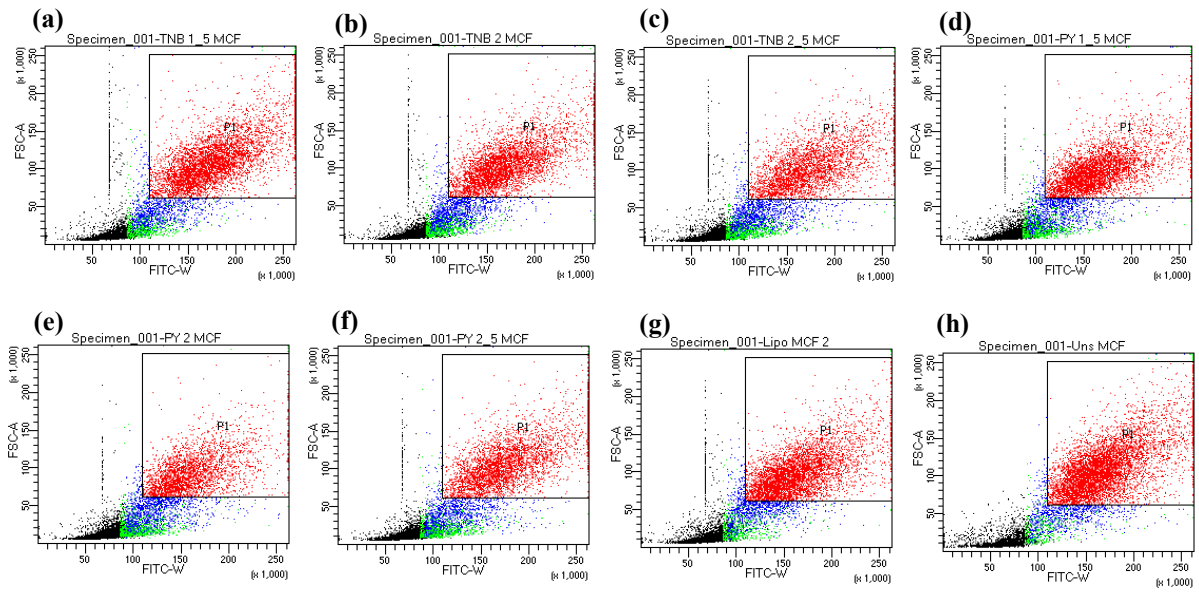


Figure S15. FACS data **(a-h**) of the co-liposomal formulations DOPE:TH8S:TNB (2:1:1) and DOPE:TH8S:TNBPY (2:1:1) from N/P=1.5 to 2.5 showing the EGFP expression in MCF-7 cells.

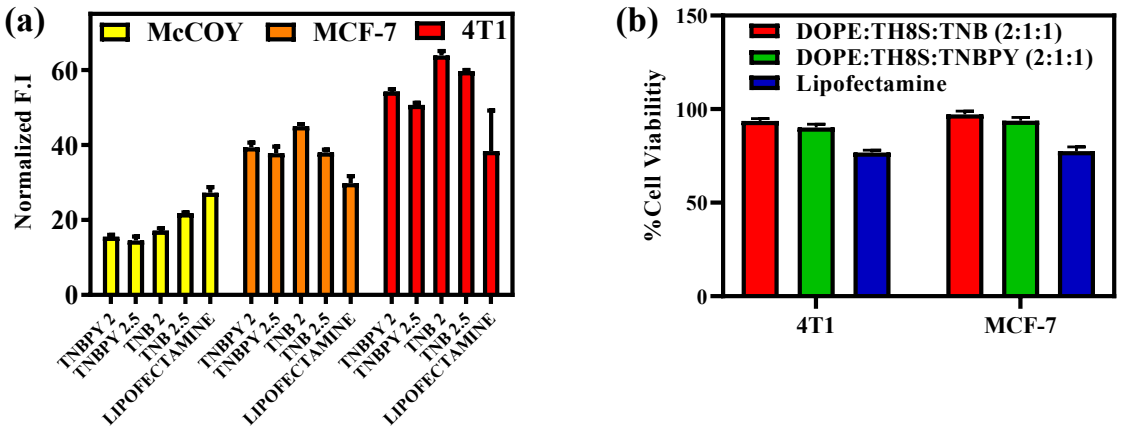


Figure S16. (a). FACS analysis of transfection optimized co-liposomal formulations of TNB, TNBPY (2:1:1) at different N/P ratios and lipofectamine showing the EGFP expression in different cell lines, **(b).** MTT assay showing the % cell viability upon treatment of transfection optimized co-liposomal formulations TNB, TNBPY (2:1:1) and lipofectamine. Data are presented as mean ± SD from three independent experiments (n = 3).

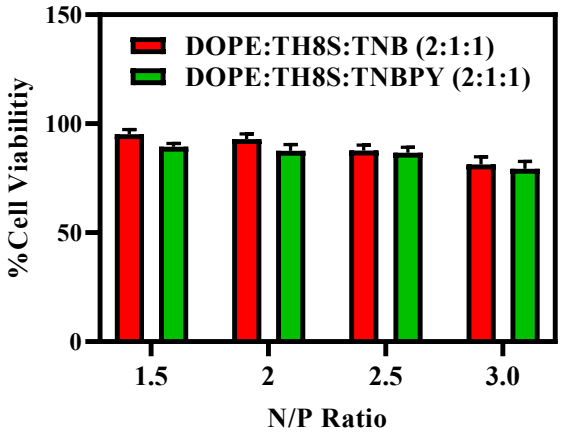


Figure S17. MTT assay showing the % cell viability of MCF-7 upon treatment of transfection optimized co-liposomal formulations TNB, TNBPY (2:1:1) across all N/P ratios. Data are presented as mean ± SD from three independent experiments (n = 3).

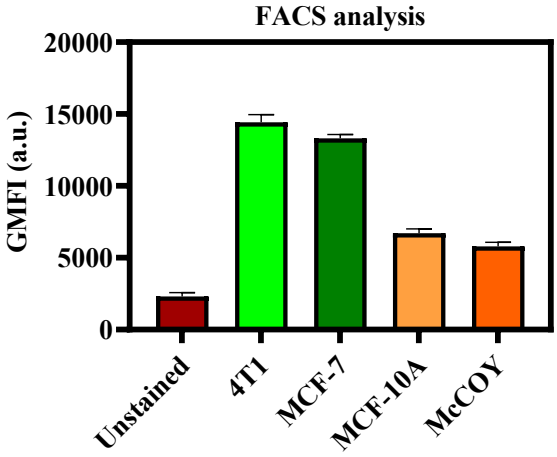


Figure S18. Quantification of cell-surface sialic acid expression using SNA-FITC lectin staining. Corresponding gross mean fluorescence intensity (GMFI) values showing higher

sialylation in cancer cells (MCF-7 and 4T1) compared to non-cancerous cells. Data are presented as mean ± SD (n = 3).

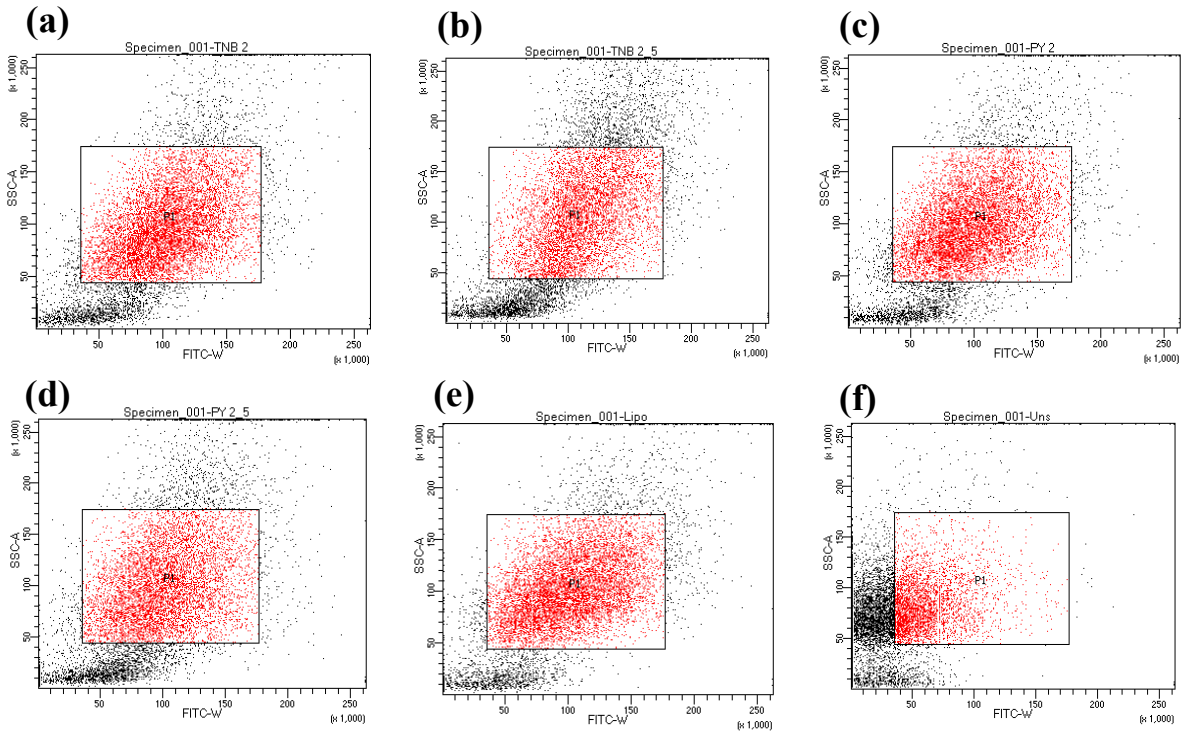


Figure S19. FACS data **(a-f)** of the co-liposomal formulations DOPE:TH8S:TNB (2:1:1) and DOPE:TH8S:TNBPY (2:1:1) from N/P=2 to 2.5 showing the EGFP expression in non-cancerous cells McCoy.

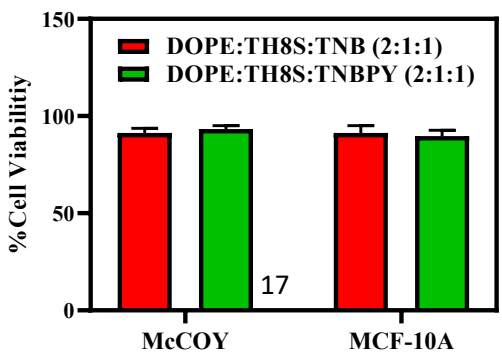


Figure S20. MTT assay showing the % cell viability of McCOY and MCF-10A upon treatment of transfection optimized co-liposomal formulations TNB, TNBPY (2:1:1) at N/P = 2. Data are presented as mean ± SD from three independent experiments (n = 3).

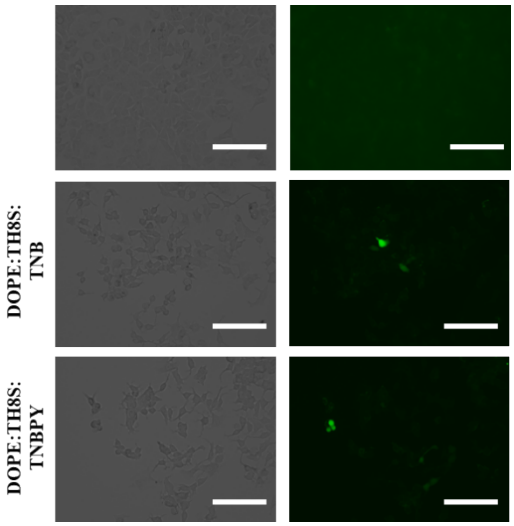


Figure S21. Cellular internalization of the lipoplexes showing the EGFP transfection efficiency of the co-liposomal formulations, DOPE:TH8S:TNB (2:1:1) and DOPE:TH8S:TNBPY (2:1:1) at N/P ratio 2 in non-cancerous cells MCF-10A, Scale bar: 150 µm.

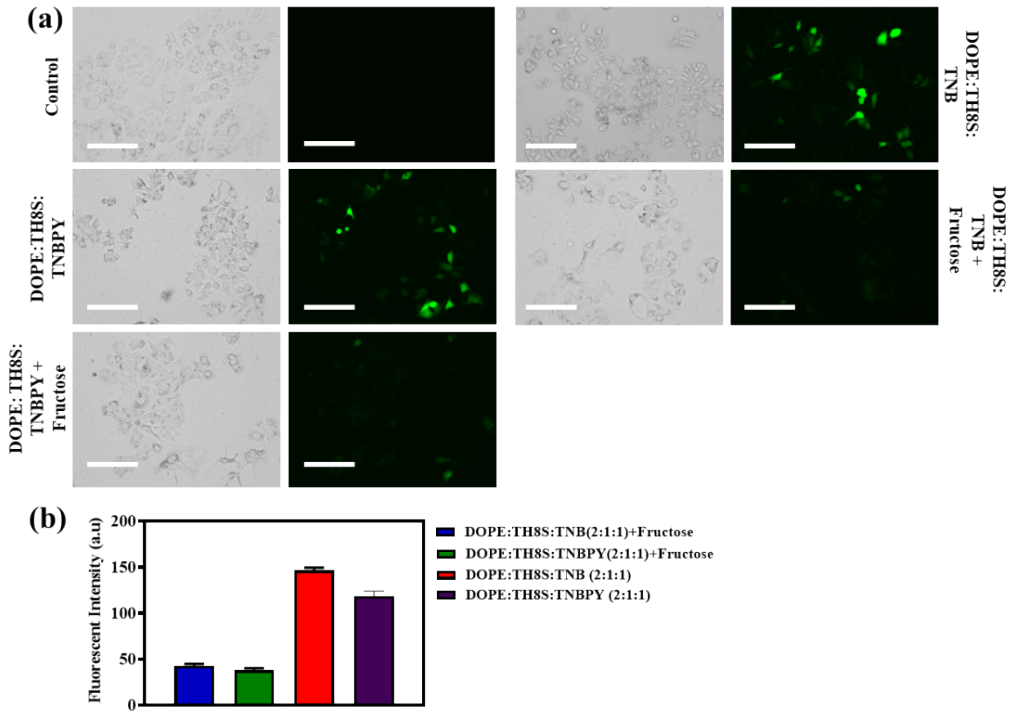


Figure S22. (a) Cellular internalization of the lipoplexes showing the EGFP expression DOPE:TH8S:TNBPY(2:1:1), DOPE:TH8S:TNB(2:1:1), and blocked co-liposomal

formulations (blocked by fructose) of TNBPY and TNB at N/P ratio 2.0 in MCF-7 cells, Scale bar: 150 µm. **(b)** image J quantification of the fluorescence microscopic images.

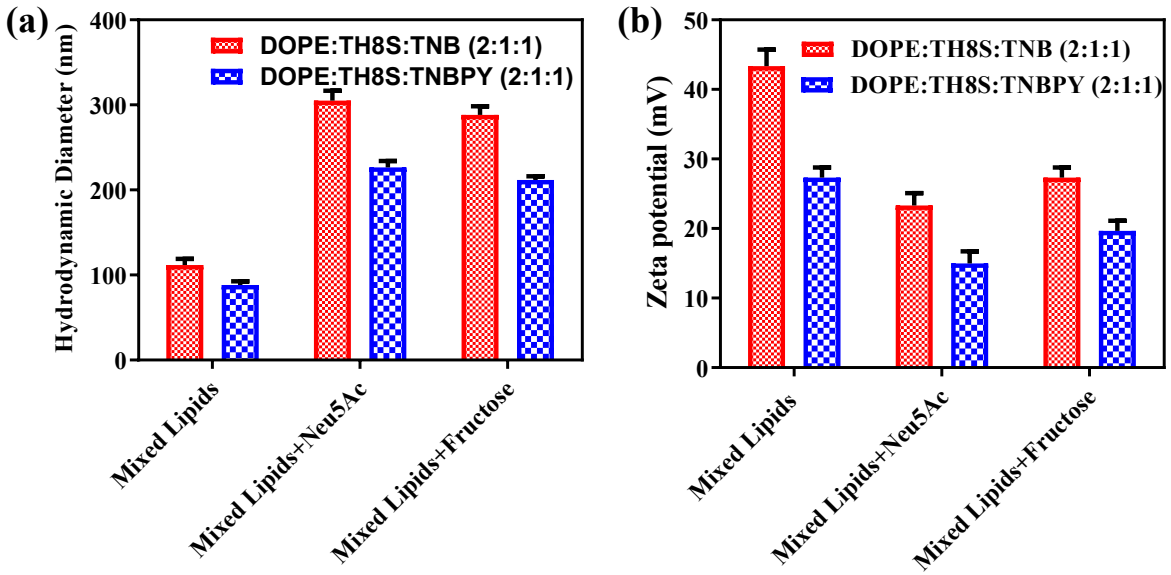


Figure S23. Physicochemical characterization of blocked liposomes upon interaction with competing diols. (a) Hydrodynamic diameter of co-liposomal formulations in the absence and presence of Neu5Ac and fructose, showing an increase in particle size upon blocking. (b) Corresponding zeta potential values indicating a decrease in surface charge after treatment with

Neu5Ac and fructose. These changes support the interaction of boronic acid headgroups with diol-containing molecules. Data are presented as mean ± SD (n = 4).

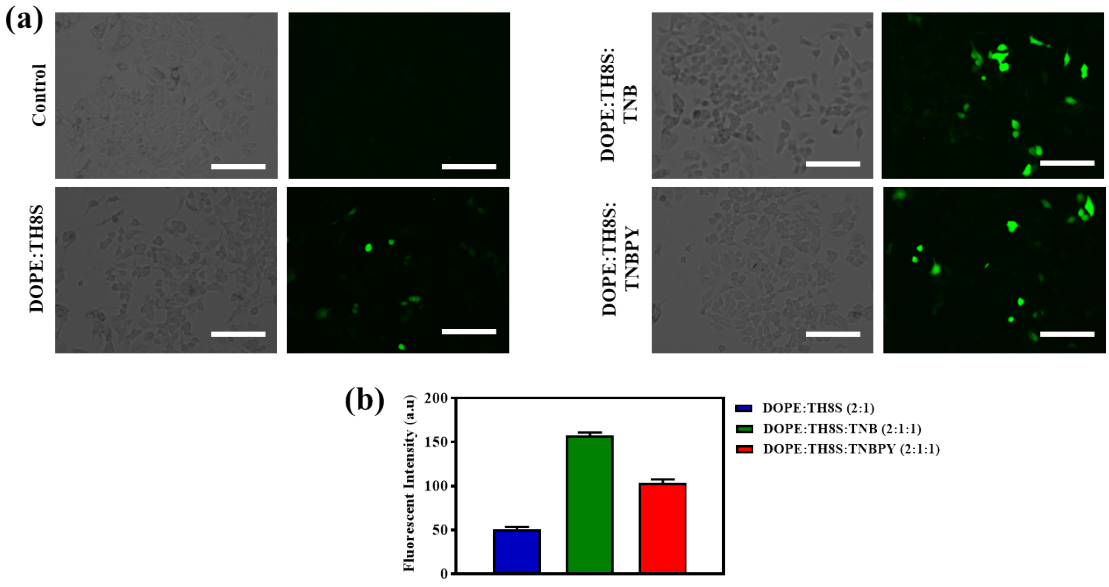


Figure S24. Transfection efficiency of control and co-liposomal formulations. (a) Representative fluorescence microscopy images showing GFP expression in MCF-7 cells treated with DOPE:TH8S(2:1) and DOPE:TH8S:TNB/TNBPY(2:1:1) co-liposomal

formulations at N/P=2. (b) Corresponding imageJ quantification analysis of the fluorescence microscopic images.