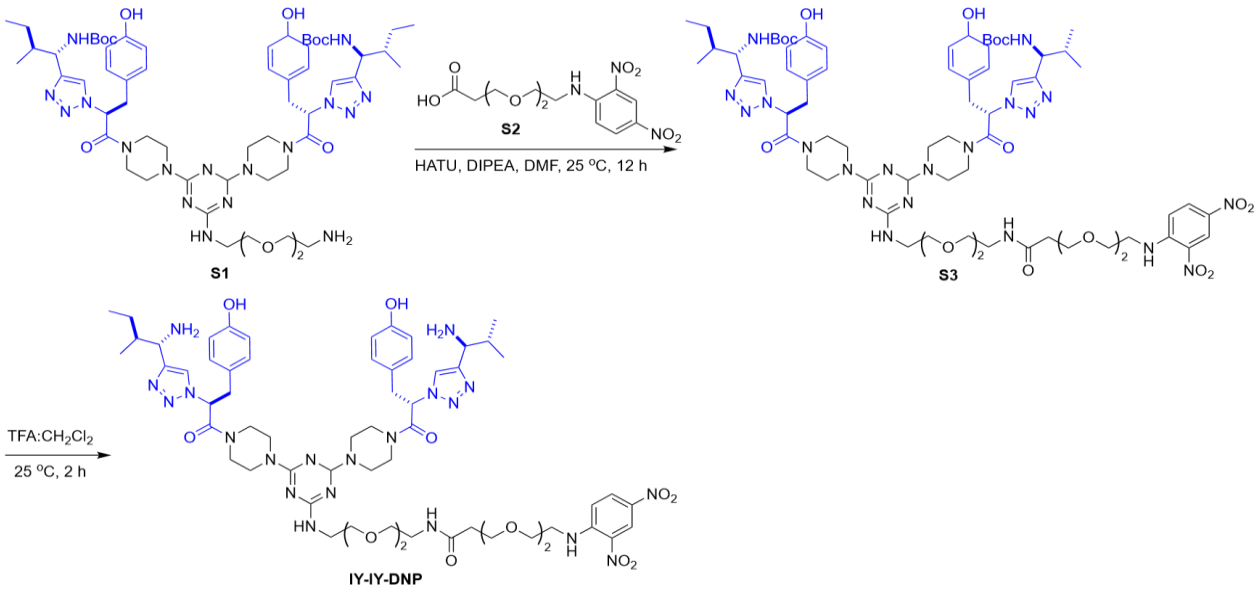
Antibody-Dependent Cellular Phagocytosis of Tropomyosin Receptor Kinase C (TrkC) Expressing Cancer Cells For Targeted Immunotherapy

Cancer Immunology, Immunotherapy (submitted in 2021) – Phei San, Lai et al.

Supporting Information

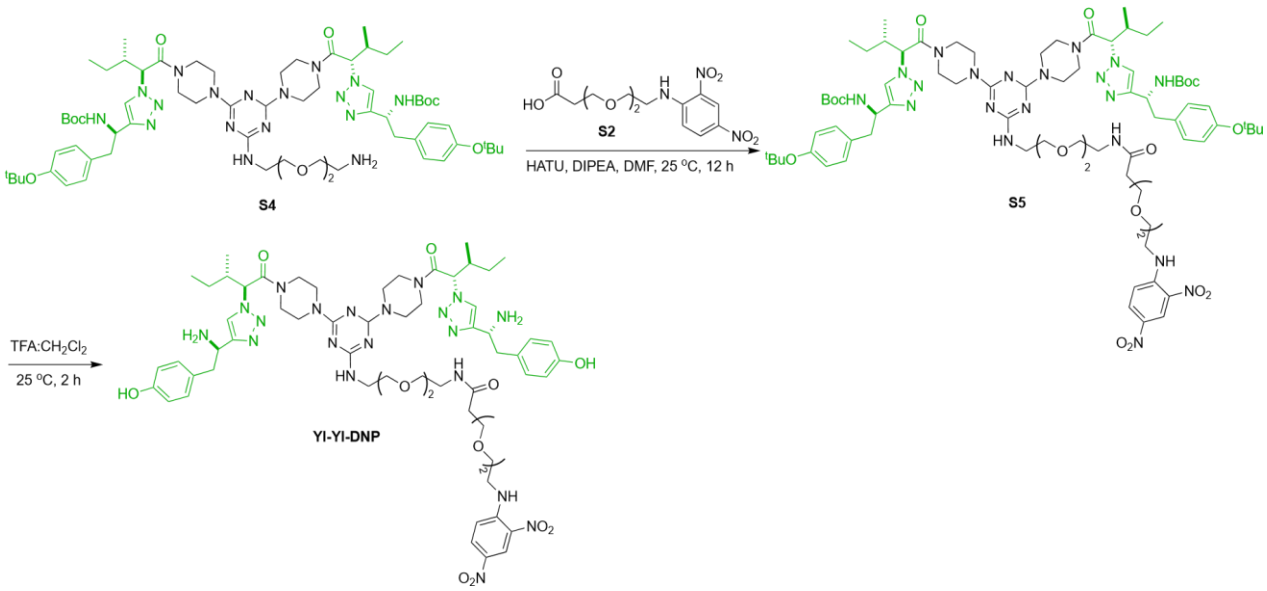


Scheme S1 Synthesis of IY-IY-DNP from **S1** (IY-IY-NH2) and **S2** (DNP-PEG2-acid).

Compound S3. Compound **S1** was synthesized according to reported literature.[1] To a 100 mL round bottom flask **S1** (200 mg, 0.17 mmol) and HATU (64.6 mg, 0.17 mmol) were added in dry DMF and stirred for 15 min. Afterwards, **S2** (57.4 mg, 0.15 mmol) and DIPEA (52.25 uL, 0.30 mmol) were added dropwise in the mixture. The reaction was stirred for 12 h under Argon atmosphere. Solvent was removed and the crude was purified by reversed phase column on prepHPLC {50% MeCN/50%H_2O – 90%MeCN/10%H_2O (containing 0.1% TFA) in 20 min} to get the desired product as amorphous yellow solid (198 mg, 77%). ^{1}H NMR (500 MHz, MeOD) δ 8.95 (d, J = 2.8 Hz, 1H), 8.26 (dd, J = 9.6, 2.8 Hz, 1H), 8.00 (s, 2H), 7.19 (dd, J = 10.6, 3.7 Hz, 1H), 7.02 (dd, J = 8.6, 2.7 Hz, 4H), 6.71 – 6.66 (m, 4H), 6.07 (t, J = 7.9 Hz, 2H), 3.80 (q, J = 5.1, 4.7 Hz, 3H), 3.74 (t, J = 6.2 Hz, 6H), 3.65 (tdt, J = 17.5, 9.5, 4.0 Hz, 26H), 3.54 (q, J = 6.0 Hz, 6H), 3.43 (dd, J = 13.7, 7.8 Hz, 3H), 3.38 – 3.31 (m, 7H), 2.47 (t, J = 6.1 Hz, 2H), 1.90 – 1.85 (m, 1H), 1.44 (d, J = 5.5 Hz, 18H), 1.13 (dt, J = 14.1, 7.8 Hz, 2H), 0.93 (t, J = 7.4 Hz, 6H), 0.81 (d, J = 6.7 Hz, 6H). ^{13}C NMR (125 MHz, MeOD) δ 173.91, 172.89, 167.03, 161.32, 160.17, 159.87, 157.41, 156.71, 156.58, 156.55, 156.38, 156.08, 154.58, 150.78, 148.52, 148.47, 141.79, 139.66, 135.64, 135.60, 130.25, 130.22, 130.20, 130.09, 130.06, 129.70, 129.60, 128.53, 125.65, 125.34, 123.30, 123.29, 121.71, 120.77, 117.19, 115.78, 115.25, 115.16, 115.14, 114.88, 114.80, 114.72, 113.52, 78.94, 70.12, 70.08, 70.04, 69.95, 69.88, 69.22, 68.54, 68.40, 68.36, 68.08, 66.92, 66.47, 60.54, 51.88, 51.61, 43.32, 42.76, 42.72, 41.62, 40.42, 39.27, 39.13, 39.08, 37.72, 37.66, 36.30, 34.40, 29.75, 27.39, 26.35, 25.74, 25.28, 24.87, 14.58, 13.74, 13.07, 10.44, 10.27, 10.07.

IY-IY-DNP. To a 20 mL scintillation vial bottom flask **S3** (170 mg, 0.11 mmol) was added and cooled at ice for 10 min. TFA:DCM (1:1; 4 mL) was added to solution and stirred for 2 h. The solvent was removed and purified by reversed phase column on prep-HPLC {50% MeCN/50%H_2O – 90%MeCN/10%H_2O (containing 0.1% TFA) in 20 min} to get the desired

product as amorphous yellow solid (140 mg, 95%). ^{1}H NMR (500 MHz, MeOD) δ 8.95 (d, J = 2.7 Hz, 1H), 8.31 – 8.19 (m, 3H), 7.19 (d, J = 9.5 Hz, 1H), 7.08 (d, J = 8.1 Hz, 4H), 6.71 (d, J = 8.3 Hz, 4H), 6.18 (t, J = 8.2 Hz, 2H), 4.47 (d, J = 6.2 Hz, 2H), 3.81 (t, J = 5.2 Hz, 3H), 3.75 (d, J = 6.1 Hz, 4H), 3.70 – 3.67 (m, 5H), 3.64 (dt, J = 9.3, 5.9 Hz, 15H), 3.60 (q, J = 5.4, 4.3 Hz, 6H), 3.54 (t, J = 5.6 Hz, 3H), 3.47 (dd, J = 13.7, 7.9 Hz, 3H), 3.39 – 3.34 (m, 5H), 3.33 (q, J = 1.9 Hz, 2H), 2.46 (d, J = 6.1 Hz, 1H), 2.06 (dd, J = 13.4, 6.8 Hz, 2H), 1.50 (td, J = 7.8, 3.8 Hz, 2H), 1.34 – 1.27 (m, 1H), 1.19 – 1.11 (m, 2H), 1.06 (s, 1H), 1.00 (t, J = 7.4 Hz, 6H), 0.89 (d, J = 6.8 Hz, 6H). ^{13}C NMR (125 MHz, MeOD) δ 173.91, 172.81, 166.99, 161.53, 161.01, 156.69, 156.67, 156.54, 154.97, 150.77, 148.52, 148.48, 142.71, 141.80, 135.62, 135.58, 130.25, 130.22, 130.17, 130.06, 129.68, 129.61, 128.49, 125.39, 125.34, 123.36, 123.29, 120.79, 115.24, 115.19, 70.11, 70.07, 70.03, 69.98, 69.89, 69.19, 68.52, 68.42, 68.40, 66.91, 66.47, 60.61, 60.58, 52.13, 51.60, 44.93, 44.36, 43.21, 42.77, 42.73, 41.60, 40.41, 39.07, 37.86, 37.70, 37.64, 37.52, 36.27, 34.41, 29.33, 25.29, 24.54, 22.31, 13.86, 13.06, 10.07, 9.87. HRMS (ESI) calculated for $C_{62}H_{89}N_{20}O_{13}$ $[M+H]^{+}$ 1321.6906; observed: 1321.6912.



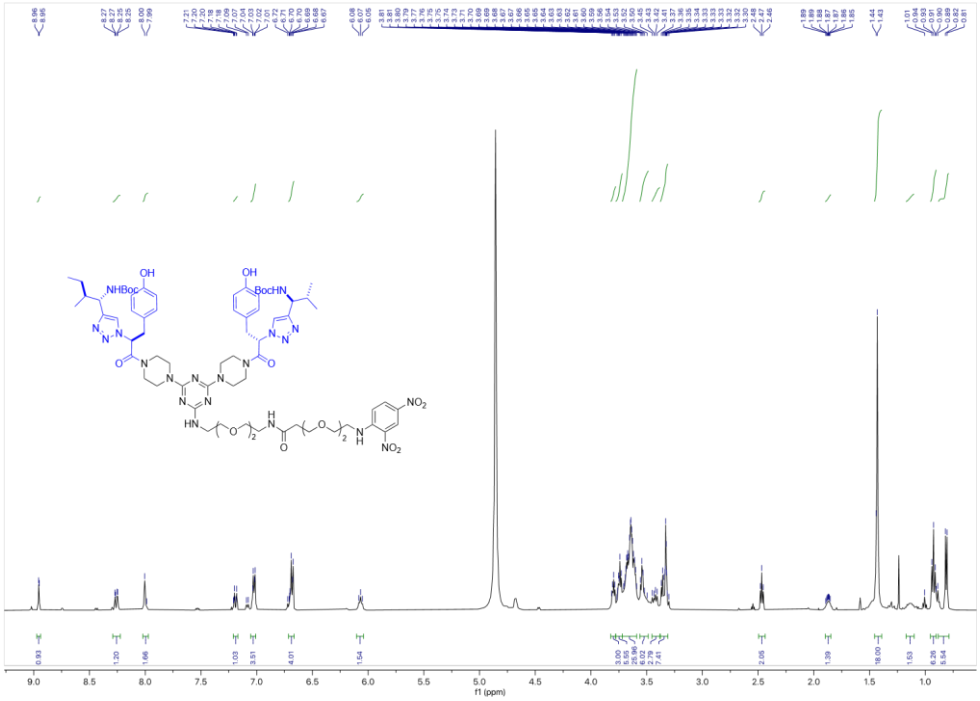
Scheme S2 Synthesis of YI-YI-DNP from **S4** (YI-YI-NH2) and **S2** (DNP-PEG2-acid).

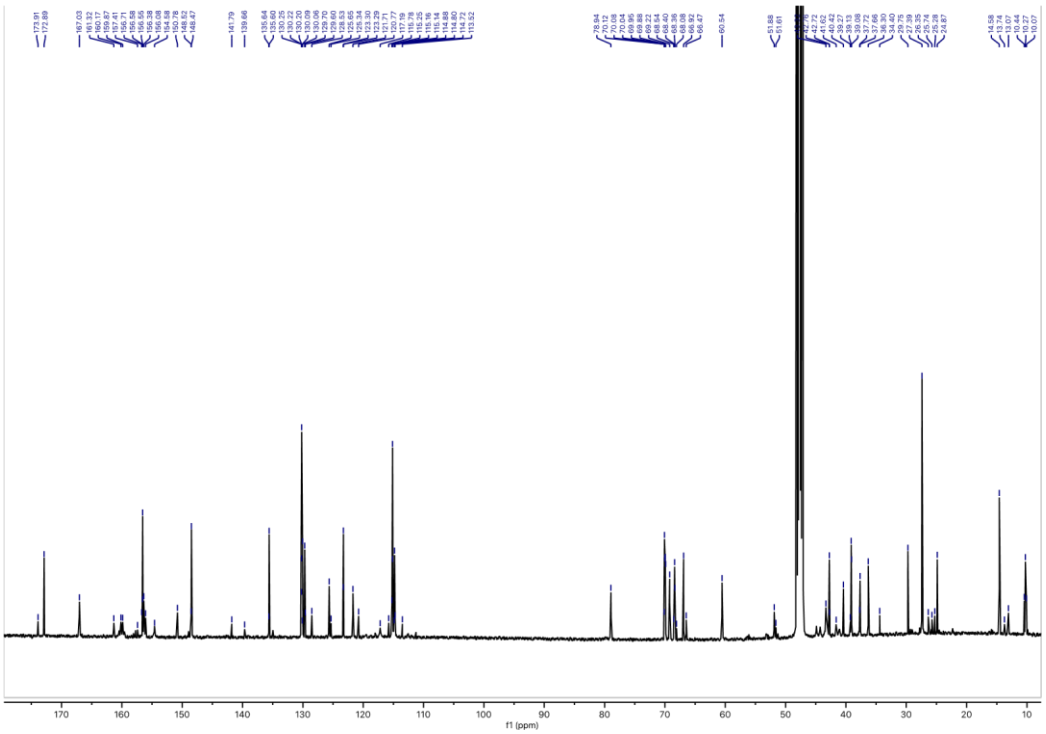
Compound S5. Compound S4 was synthesized according to literature procedure.[1] To a 100 mL round bottom flask **S4** (200 mg, 0.15 mmol) and HATU (57.94 mg, 0.15 mmol) were added in dry DMF and stirred for 15 min. Afterwards, **S2** (52.5 mg, 0.15 mmol) and DIPEA (52.25 uL, 0.30 mmol) were added dropwise in the mixture. The reaction was stirred for 12 h under Argon atmosphere. Solvent was removed and the crude was purified by reversed phase column on prepHPLC {50% MeCN/50%H_2O – 90%MeCN/10%H_2O (containing 0.1% TFA) in 20 min} to get the desired product as amorphous yellow solid (180 mg, 73%). ^{1}H NMR (500 MHz, MeOD) δ 8.97 (d, J = 2.7 Hz, 1H), 8.26 (dd, J = 9.5, 2.7 Hz, 1H), 7.88 (s, 1H), 7.19 (d, J = 9.6 Hz, 1H), 7.09 – 7.04 (m, 4H), 6.84 (d, J = 8.1 Hz, 4H), 5.62 (d, J = 10.4 Hz, 2H), 5.01 (t, J = 7.5 Hz, 1H), 4.04 – 3.81 (m, 8H), 3.80 (t, J = 5.2 Hz, 4H), 3.75 (t, J = 6.1 Hz, 3H), 3.70 – 3.59 (m, 16H), 3.55 (t, J = 5.6 Hz, 4H), 3.36 (t, J = 5.6 Hz, 2H), 3.33 (p, J = 1.6 Hz, 5H), 3.17 (dd, J = 13.7, 6.9 Hz, 2H), 3.05 (dd, J = 13.7, 8.0 Hz, 2H), 2.47 (t, J = 6.1 Hz, 2H), 2.43 – 2.37 (m, 2H), 1.37 (s, 17H), 1.29 (s,

18H), 1.04 (d, J = 6.5 Hz, 11H), 0.87 (t, J = 7.2 Hz, 7H). ^{13}C NMR (125 MHz, MeOD) δ 172.87, 166.87, 166.71, 156.06, 154.60, 153.74, 149.33, 148.47, 143.18, 135.62, 132.75, 130.09, 130.04, 129.87, 129.68, 129.66, 129.58, 128.19, 124.00, 123.66, 123.28, 120.91, 119.88, 117.56, 114.80, 78.94, 78.02, 78.01, 70.08, 70.05, 69.97, 69.89, 69.24, 68.57, 68.41, 66.92, 63.47, 43.71, 43.08, 42.77, 40.38, 40.08, 39.08, 37.50, 36.31, 27.82, 27.34, 24.16, 14.43, 14.29, 9.57.

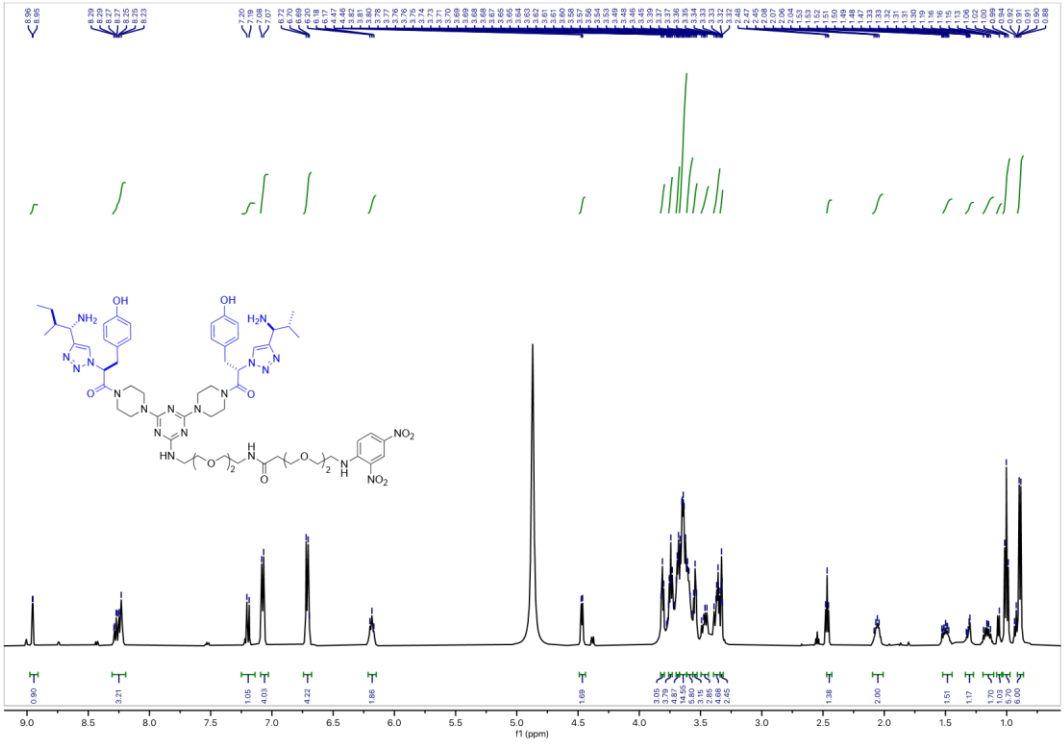
YI-YI-DNP. To a 20 mL scintillation vial bottom flask **S3** (140 mg, 0.91 mmol) was added and cooled at ice for 10 min. TFA:DCM (1:1; 4 mL) was added to solution and stirred for 2 h. The solvent was removed and purified by reversed phase column on prep-HPLC {50% MeCN/50%H_2O – 90%MeCN/10%H_2O (containing 0.1% TFA) in 20 min} to get the desired product as amorphous yellow solid (130 mg, 90%). ^{1}H NMR (500 MHz, MeOD) δ 8.94 (s, 1H), 8.24 (d, J = 9.5 Hz, 1H), 8.01 (s, 2H), 7.18 (d, J = 9.6 Hz, 1H), 6.94 (d, J = 8.2 Hz, 4H), 6.63 (d, J = 8.0 Hz, 4H), 5.66 (d, J = 10.0 Hz, 2H), 4.78 (dd, J = 9.7, 6.0 Hz, 2H), 4.06 – 3.94 (m, 2H), 3.94 – 3.72 (m, 12H), 3.66 (dt, J = 22.5, 7.0 Hz, 17H), 3.54 (t, J = 5.7 Hz, 4H), 3.42 – 3.28 (m, 7H), 3.28 – 3.08 (m, 2H), 2.47 (t, J = 6.2 Hz, 2H), 2.38 (q, J = 9.2 Hz, 2H), 1.32 (d, J = 20.2 Hz, 1H), 1.07 – 0.91 (m, 11H), 0.86 (t, J = 7.4 Hz, 6H). ^{13}C NMR (125 MHz, MeOD) δ 172.80, 166.64, 157.76, 157.42, 156.41, 156.37, 156.13, 154.54, 148.60, 148.48, 146.61, 143.33, 143.14, 141.70, 135.55, 130.13, 130.08, 130.03, 129.70, 127.33, 125.63, 125.57, 123.30, 122.90, 117.55, 115.78, 115.15, 115.05, 114.83, 113.52, 112.94, 70.07, 70.04, 69.97, 69.89, 69.19, 68.40, 68.37, 66.92, 63.60, 45.14, 44.54, 43.81, 43.21, 42.77, 41.76, 41.25, 40.48, 39.07, 38.01, 37.60, 36.26, 24.08, 14.38, 9.66, 9.58. HRMS (ESI) calculated for $C_{62}H_{89}N_{20}O_{13}$ $[M+H]^+$ 1321.6902; observed: 1321.6912.

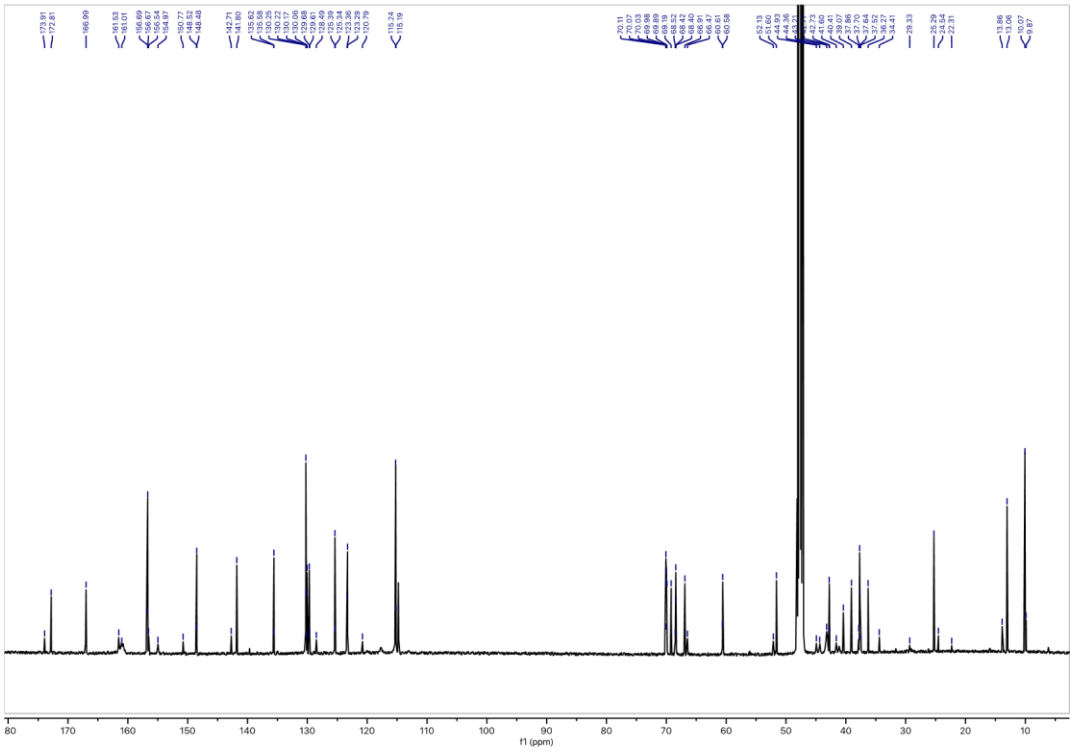
NMRs and HRMS spectra's Compound S3

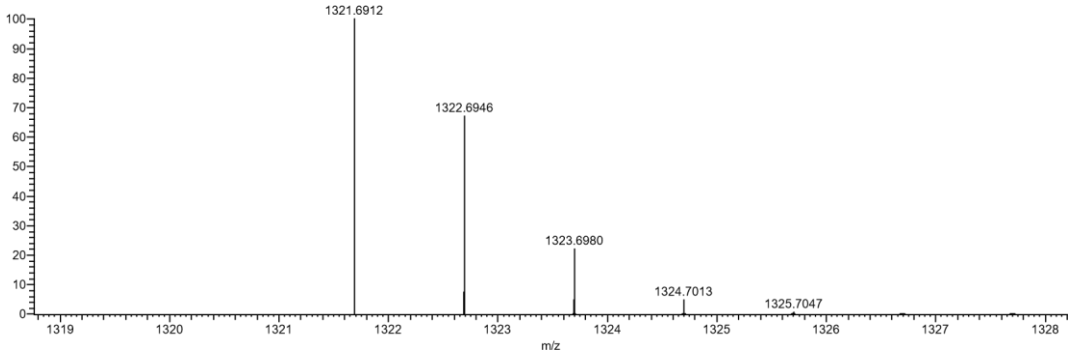




IY-IY-DNP



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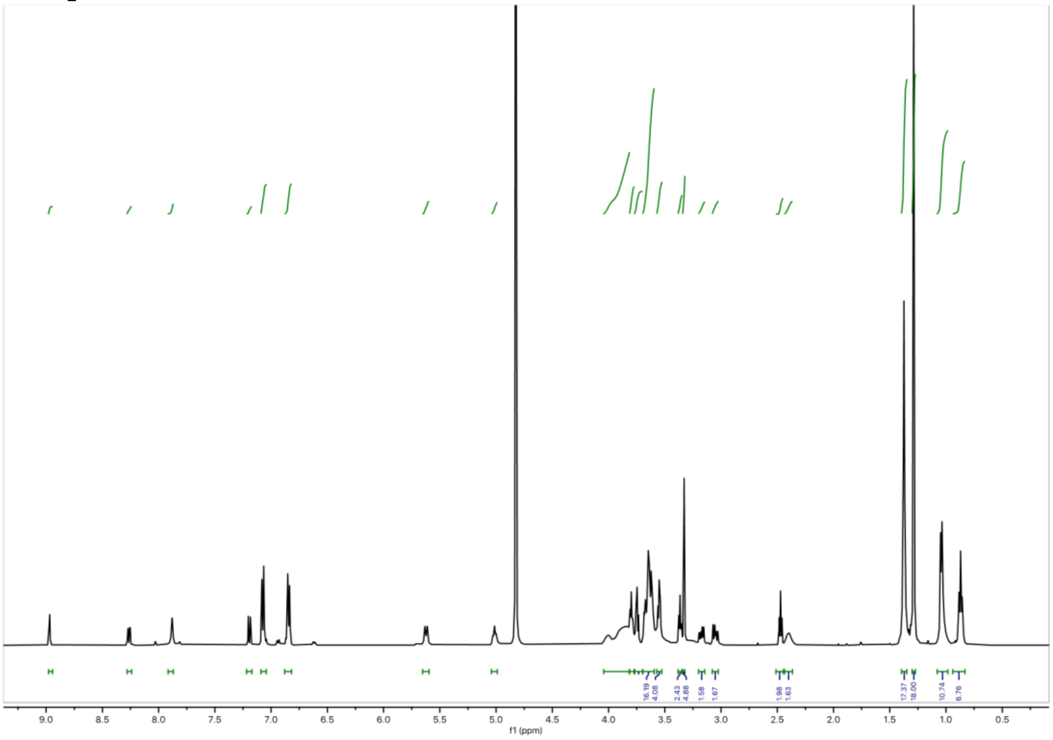
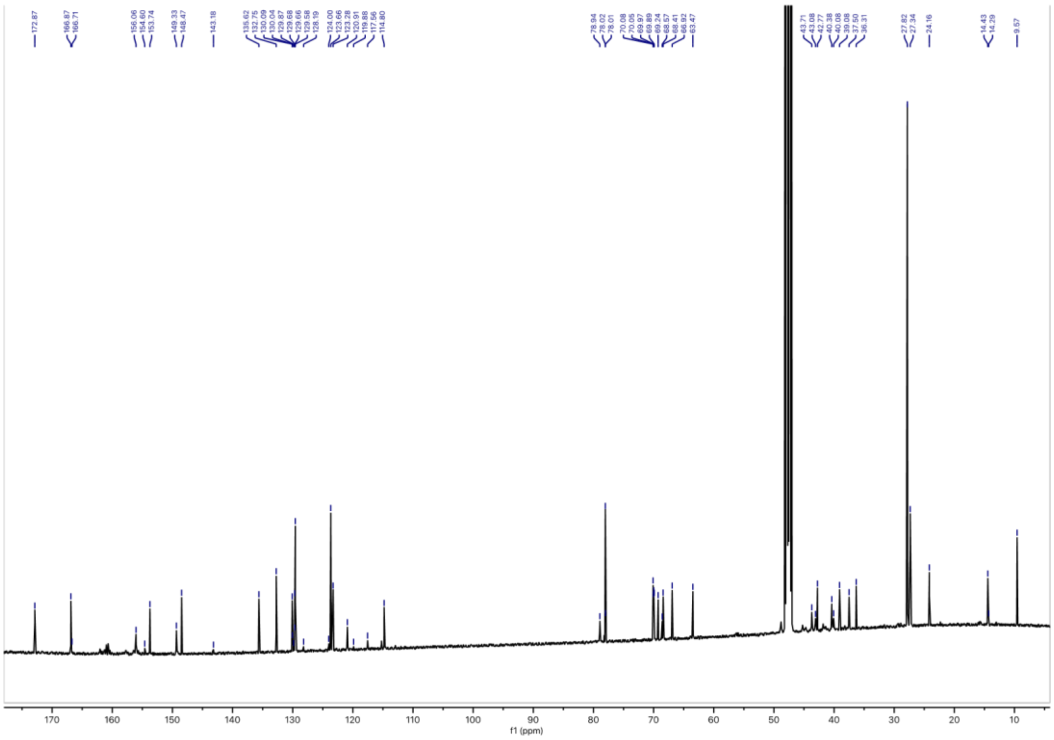
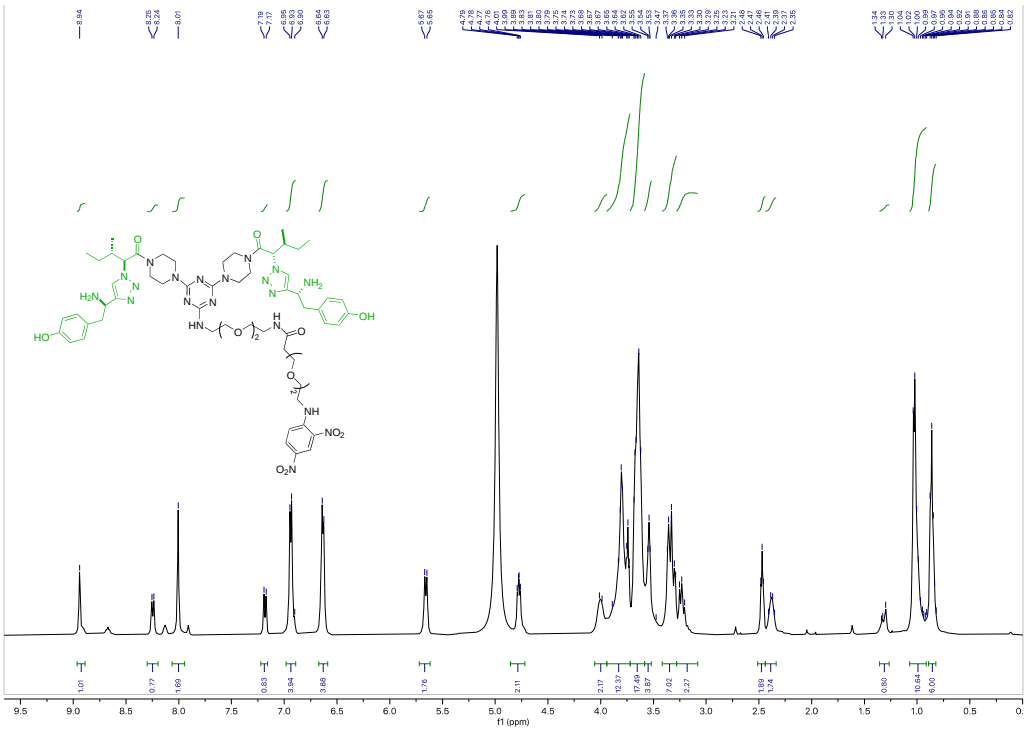
m/z

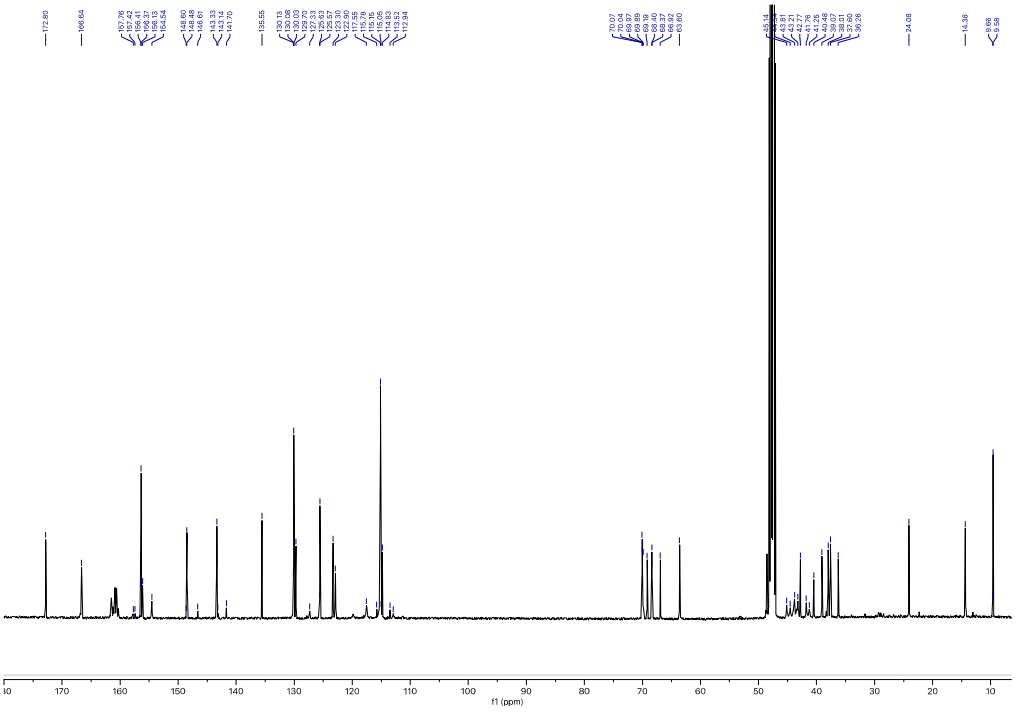
Compound S5





YI-YI-DNP





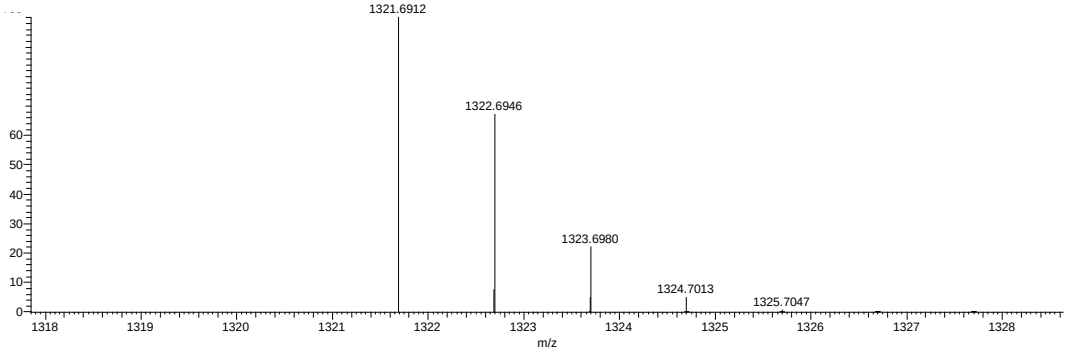


Fig S1 The synthesis and characterization of IYIY-DNP and YIYI-DNP.

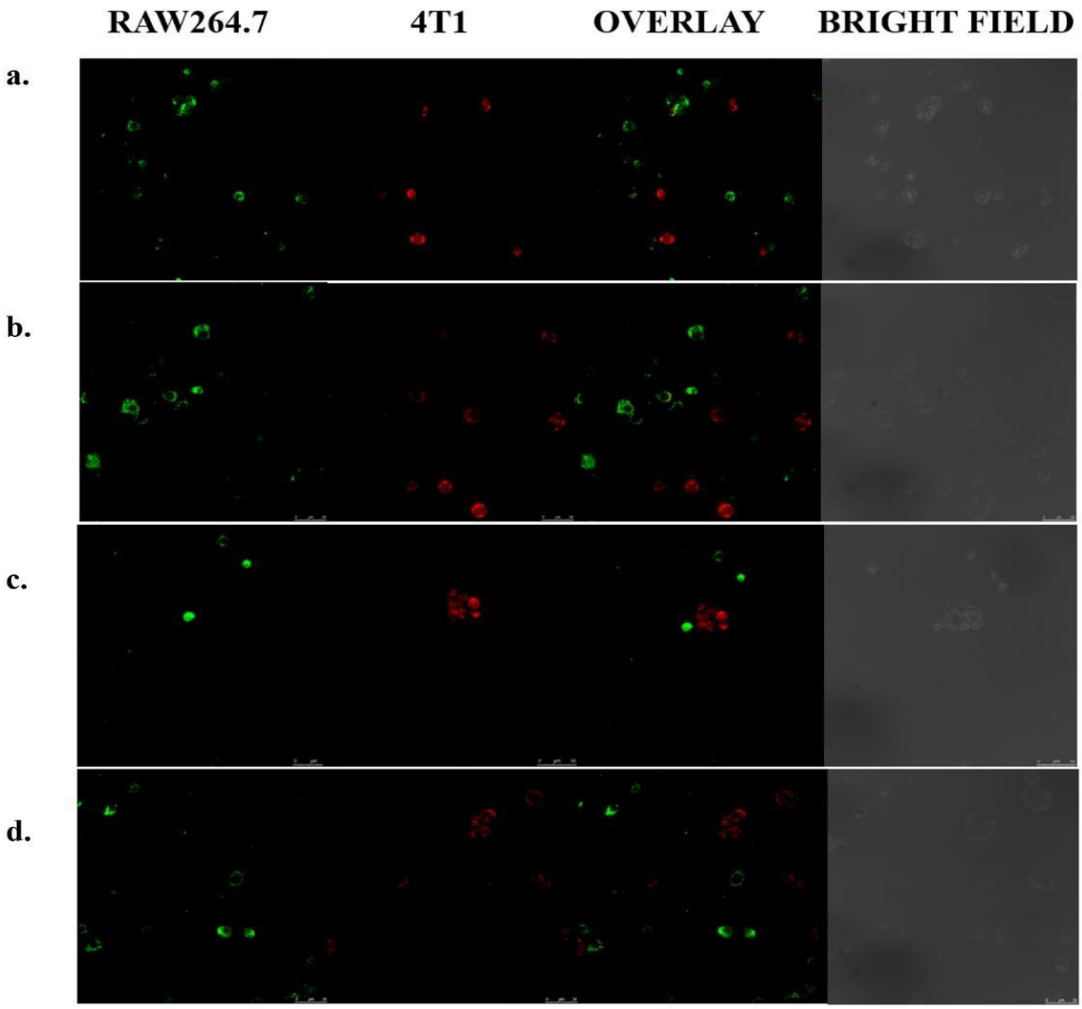


Fig S2 *In vitro* antibody-dependent cellular phagocytosis assay by using TrkC+ 4T1 cells (red) and RAW264.7 cells (green) (a) without anti-DNP antibodies; (b) with 0.1% anti-DNP antibodies; (c) with 10 μM IYIY-DNP without anti-DNP antibodies; and (d) with 10 μM YIYI-DNP without anti-DNP antibodies. The data shown are the representative of the three independent experiments with similar results.

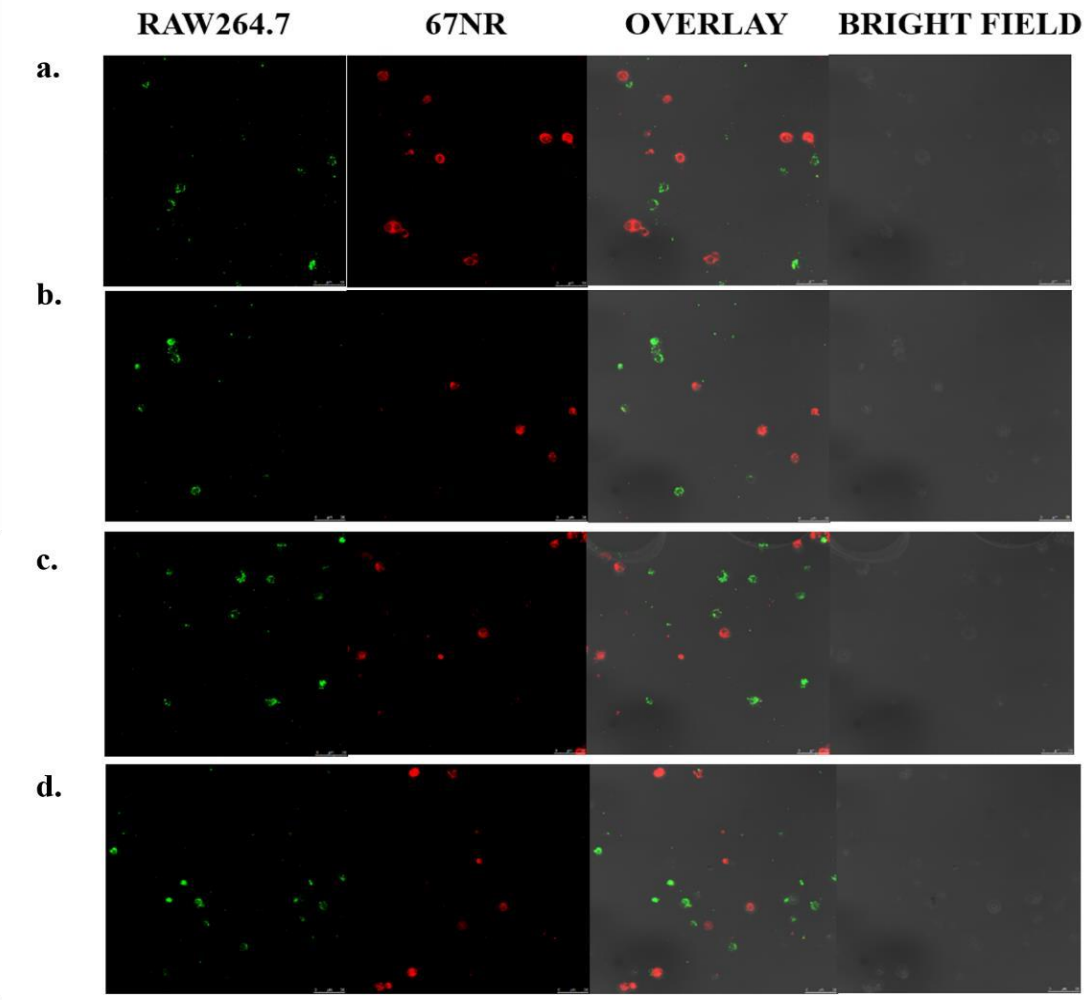


Fig S3 *In vitro* antibody-dependent cellular phagocytosis assay by using TrkC- 67NR cells (red) with RAW264.7 (green) (a) without anti-DNP antibodies; (b) with 0.1% anti-DNP antibodies; (c) with 10 μM IYIY-DNP in absence of anti-DNP antibodies, and (d) with 10 μM YIYI-DNP in absence of antiDNP antibodies. The data shown are the representative of the three independent experiments with similar results.

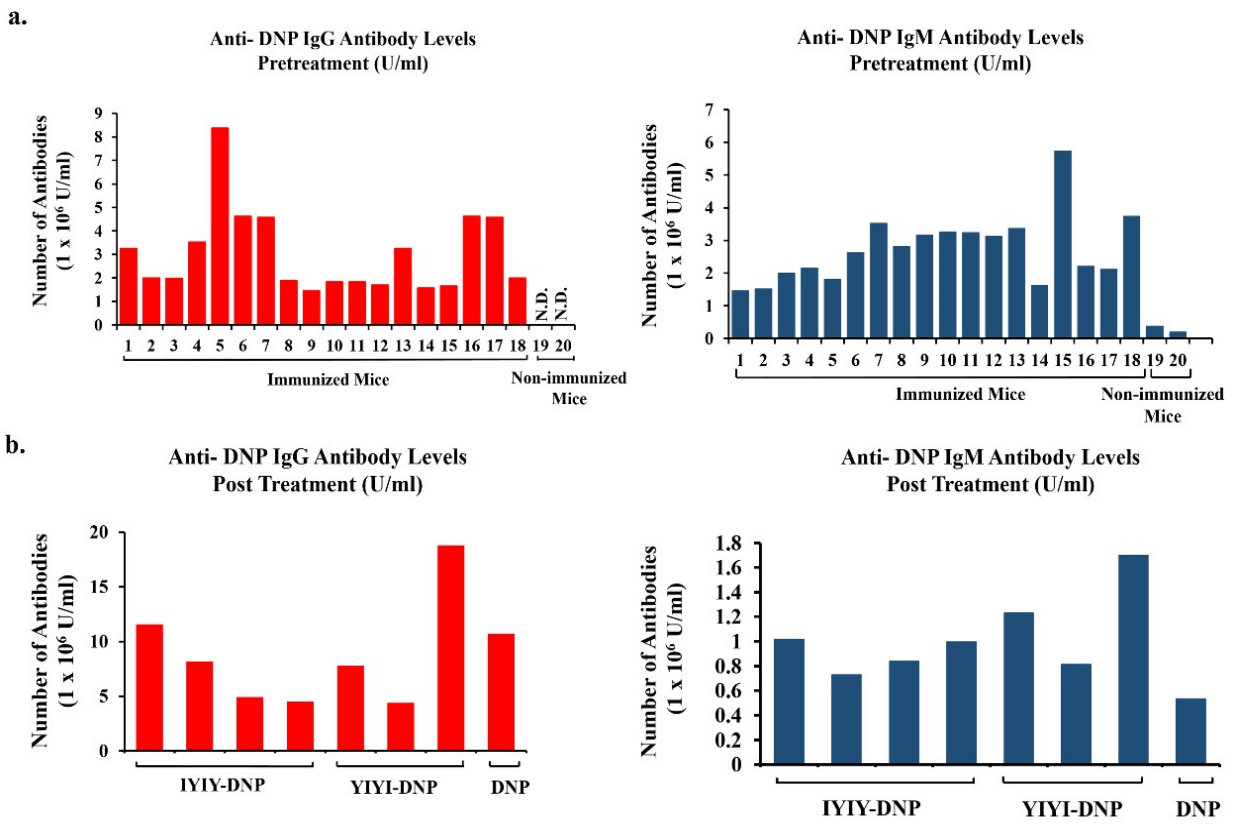


Fig S4 The immunized mice were randomly selected and blood was withdrawn for anti-DNP antibodies IgG and IgM quantification at (a) the week after booster immunization and (b) the next day after five cycle of treatment (day-10).