Synthetic and Immunological Studies on Trimeric MUC1 Immunodominant Motif Antigens-based Anti-cancer Vaccine Candidates

Mingjing Li,[a] Fan Yu,[a, b] Chao Yao,[a] Peng George Wang,[a] Yonghui Liu,[a,] Wei Zhao[a,]**

[a]State Key Laboratory of Medicinal Chemical Biology, College of Pharmacy and Tianjin Key Laboratory of Molecular Drug Research, Nankai University, Haihe Education Park, 38 Tongyan Road, Tianjin 300353, P.R.China

[b]College of Life Sciences, Nankai University, Tianjin 300071, P.R.China

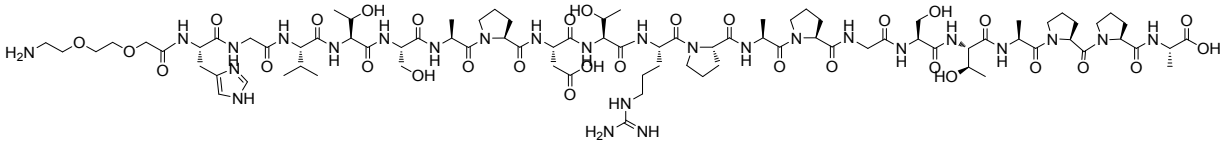
Corresponding Authors:

Wei Zhao
*Email: wzhao@nankai.edu.cn

Yonghui Liu
*Email: yhliu@mail.nankai.edu.cn

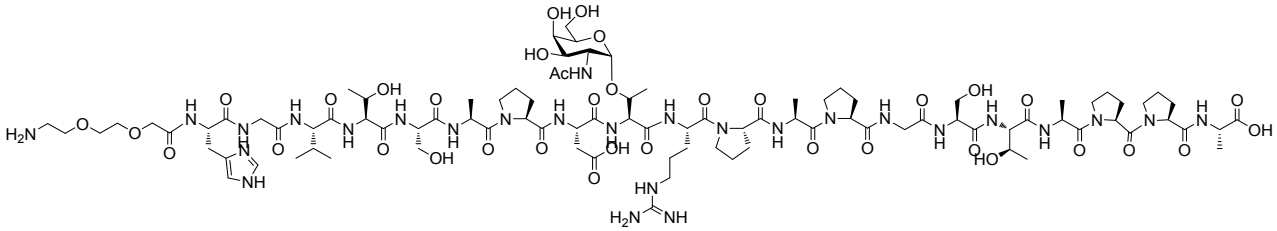
Peptides used for ELISA plate coating

1) $NH_2(CH_2CH_2O)_2CH_2CO$-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala-OH (**12**)



The compound was characterized in our previous report.[1]

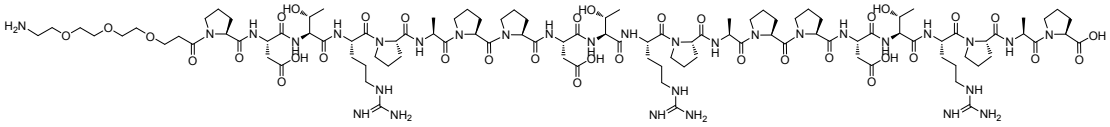
2) $NH_2(CH_2CH_2O)_2CH_2CO$-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr(α-D-GalNAc)-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala-OH (**13**)



The compound was characterized in our previous report.[1]

Figure S1-10. Analytical data of (glyco)peptides and their conjugates with BSA

1) NH_2-PEG_4-PDTRPAPPDTRPAPPDTRPAP (**8**)



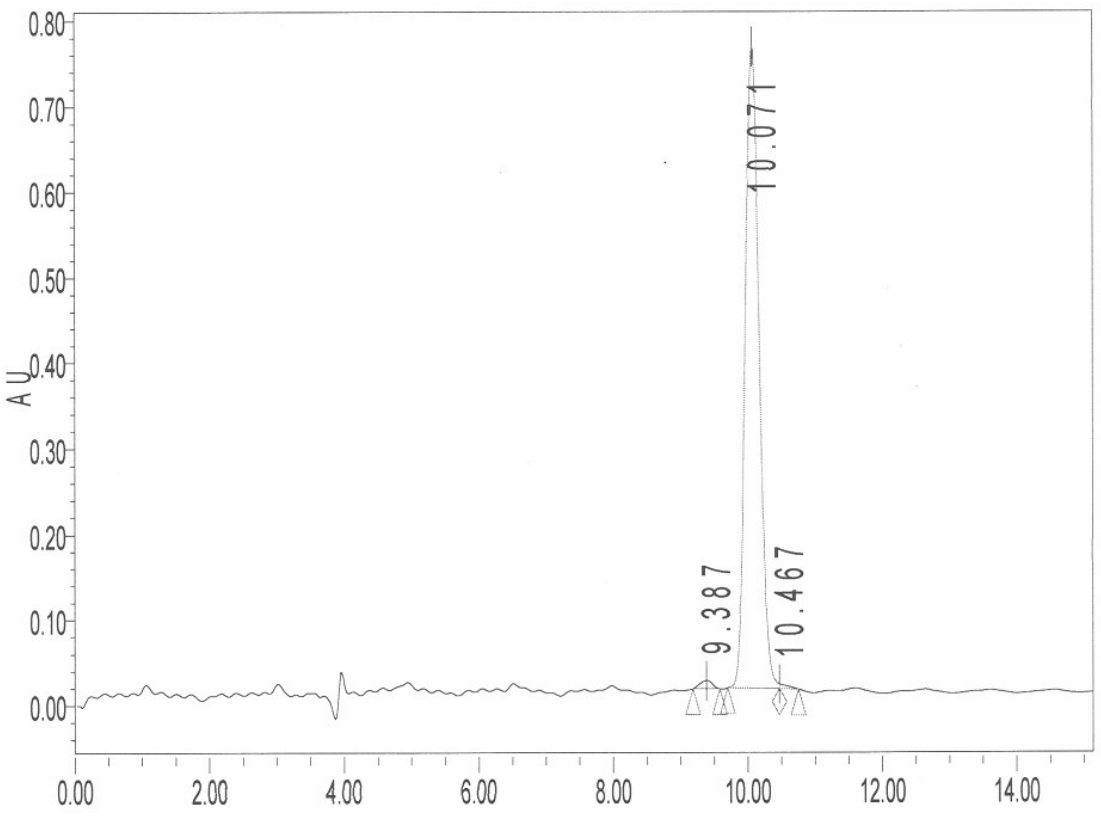


Figure S1. Analytical HPLC of **8**: R_t(retention time) = 10.1 min (10-25% of acetonitrile and 0.05% trifluoroacetic acid over 15 min on a C-18 column, λ=220 nm)

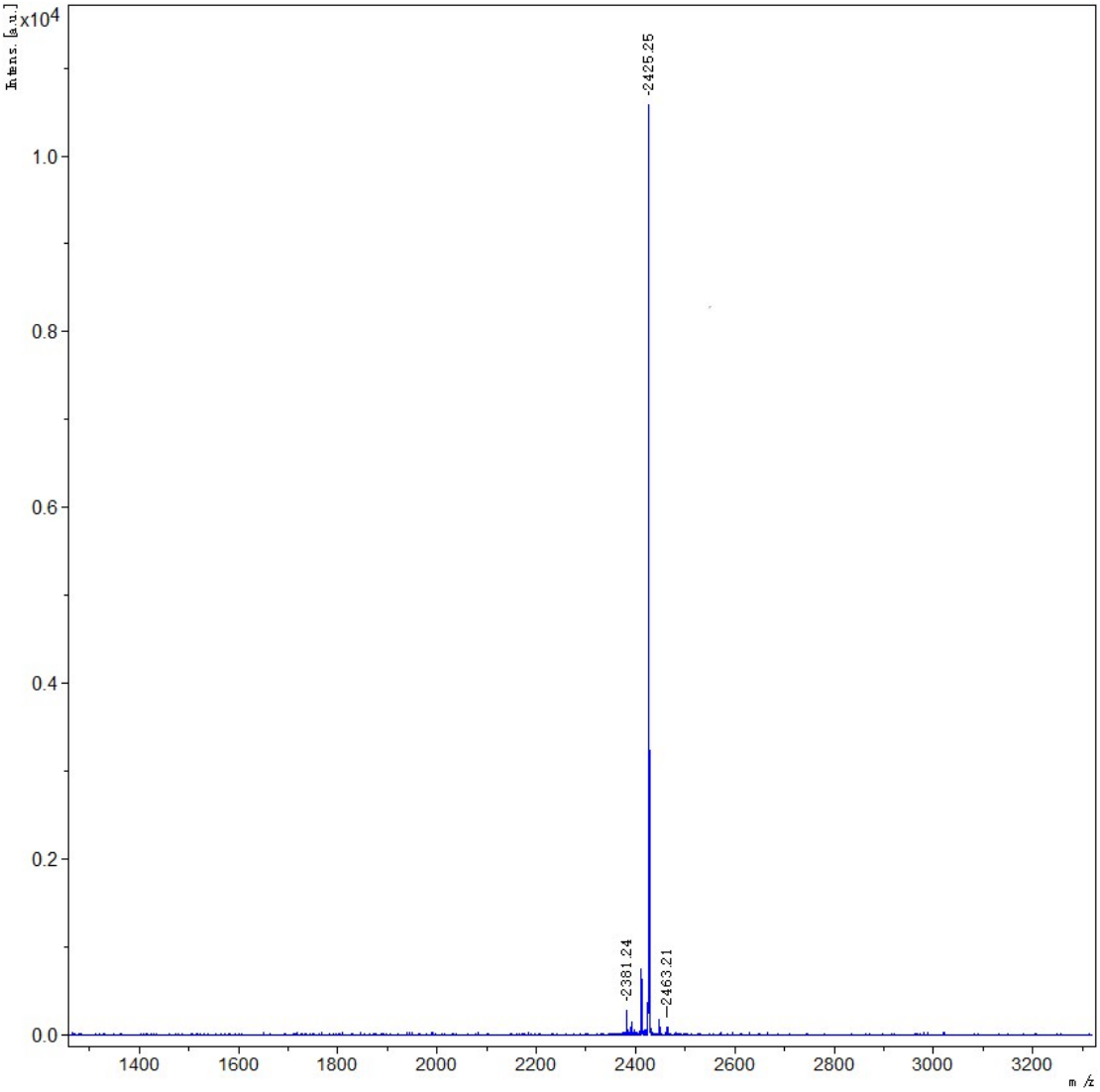


Figure S2. MALDI-MS of **8**:*m/z*for $C_{105}H_{169}N_{31}O_{35}$ $[M+H]^+$calcd 2425.24, found $[M+H]^+$2425.25.

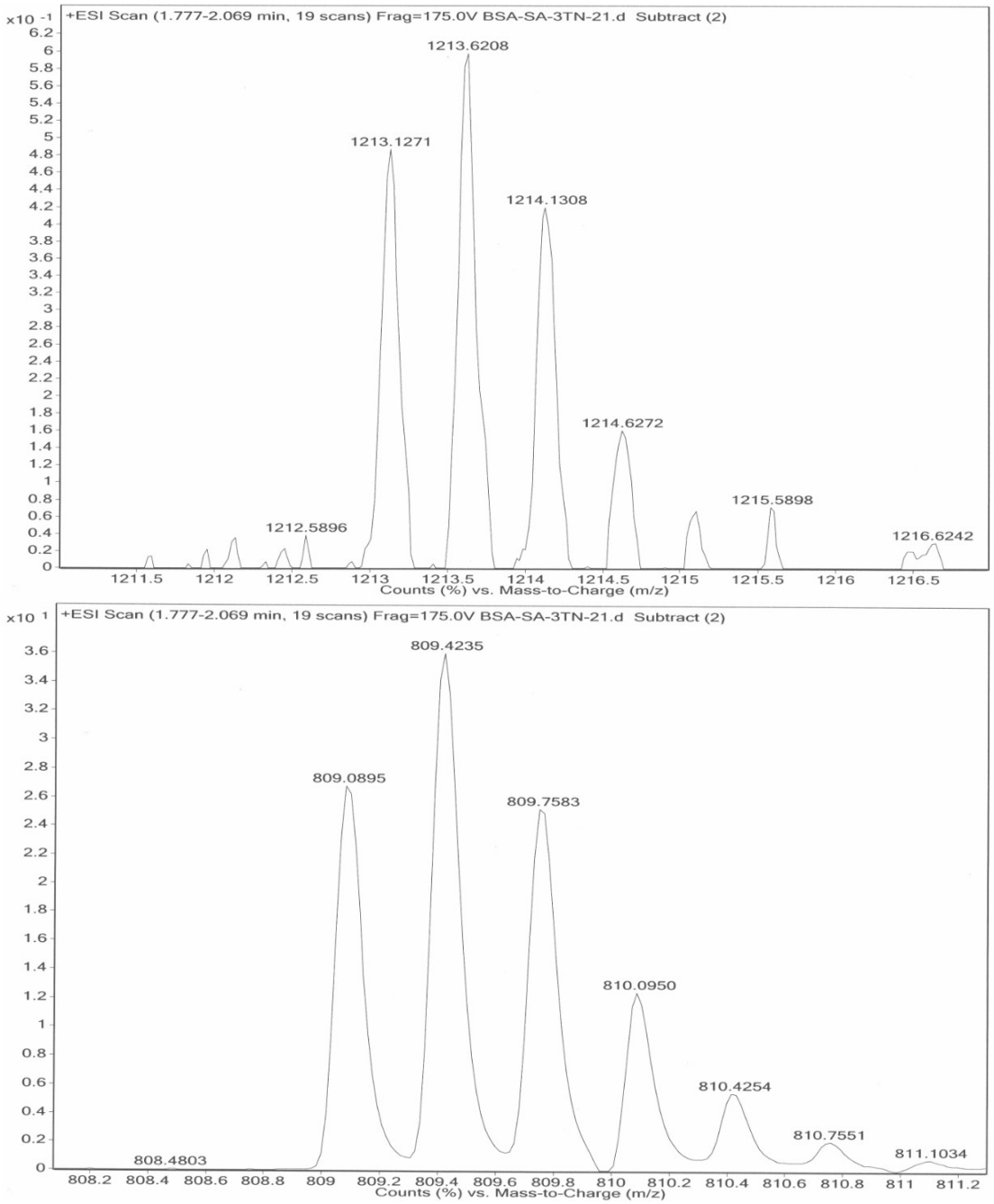


Figure S3. HR-ESI-MS of **8**:$C_{105}H_{169}N_{31}O_{35}$ $[M+2H]^{2+}$calcd 1213.1277, found 1213.1277;$[M+3H]^{3+}$calcd809.0877, found 809.0895.

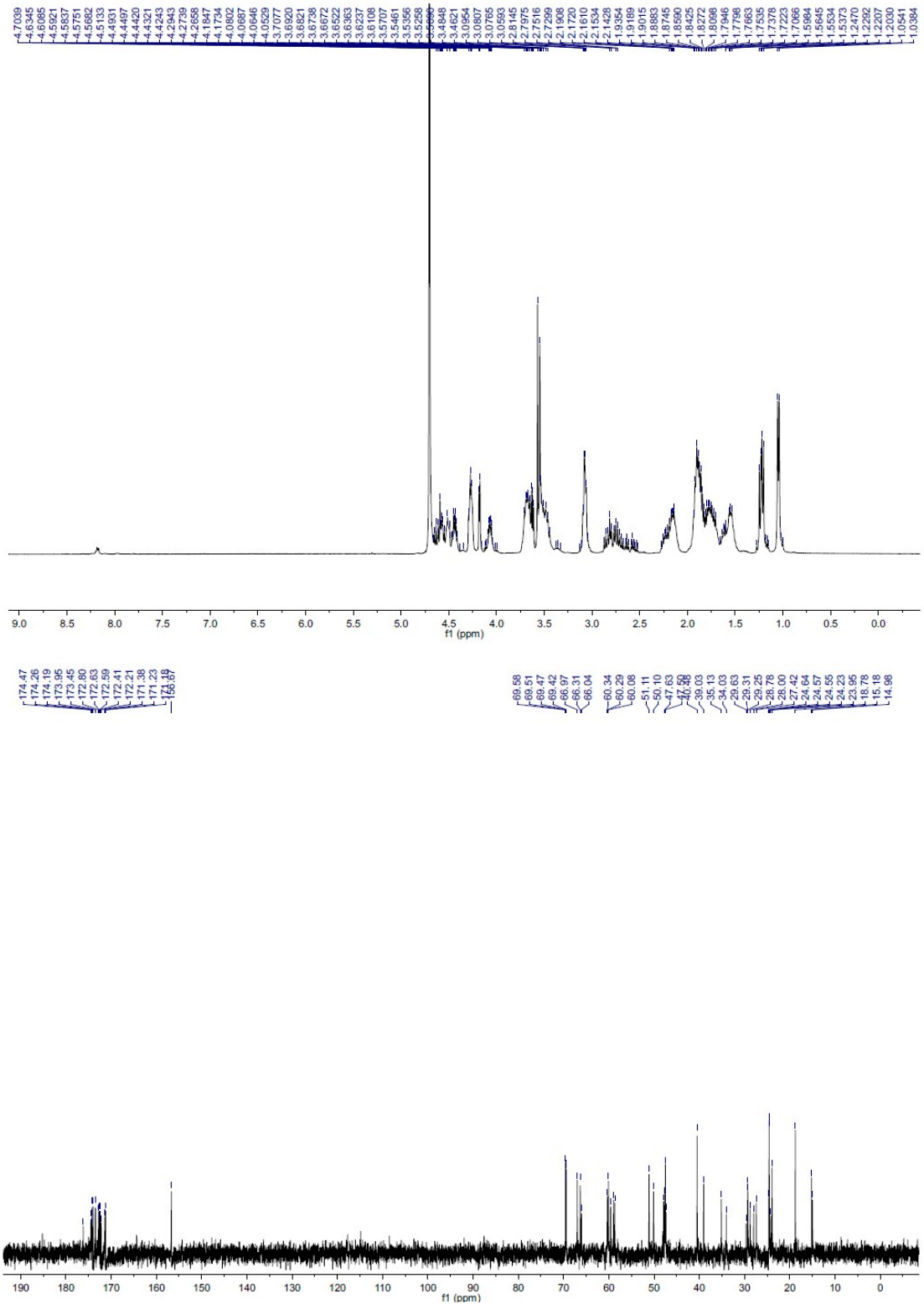
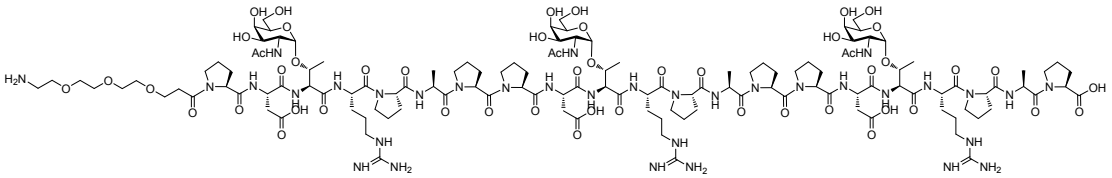


Figure S4. NMR data of **8**

2) NH_2-PEG_4-PDT(α-D-GalNAc)RPAPPDT(α-D-GalNAc)RPAPPDT(α-D-GalNAc)RPAP (**9**)



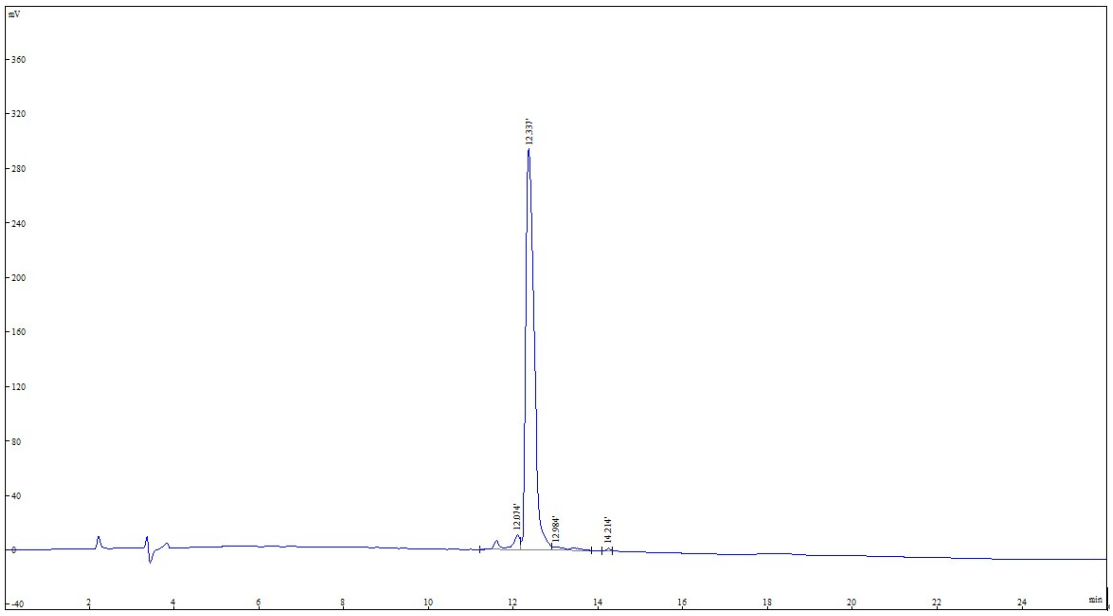


Figure S5. Analytical HPLC of **9**: R_t(retention time) = 12.3 min (10-100% of acetonitrile and 0.1% trifluoroacetic acid over 30 min on a C-18 column, λ=220 nm)

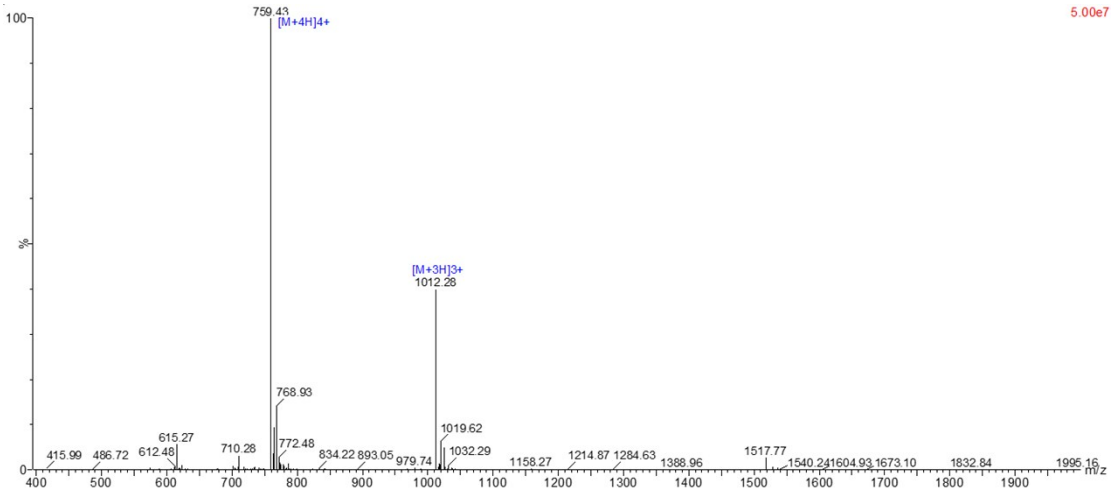


Figure S6. ESI-MS of **9**: *m/z* for $C_{129}H_{208}N_{34}O_{50}$ $[M+3H]^{3+}$calcd 1012.50, found1012.28, $[M+4H]^{4+}$calcd 759.62, found 759.43.

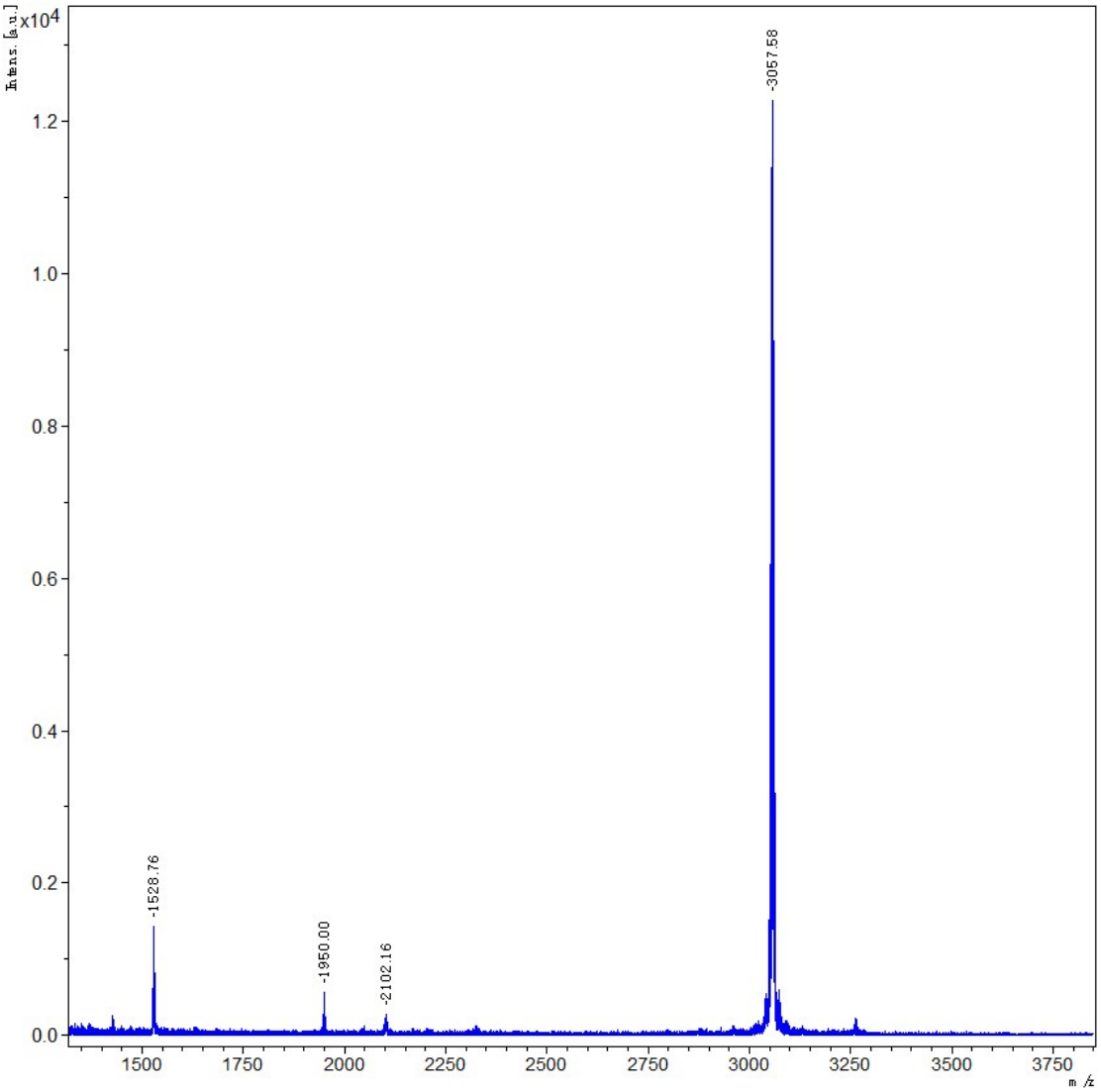


Figure S7. MALDI-MS of **9**: *m/z* for $C_{129}H_{208}N_{34}O_{50}$ $[M+Na]^+$calcd 3057.47, found3057.58.

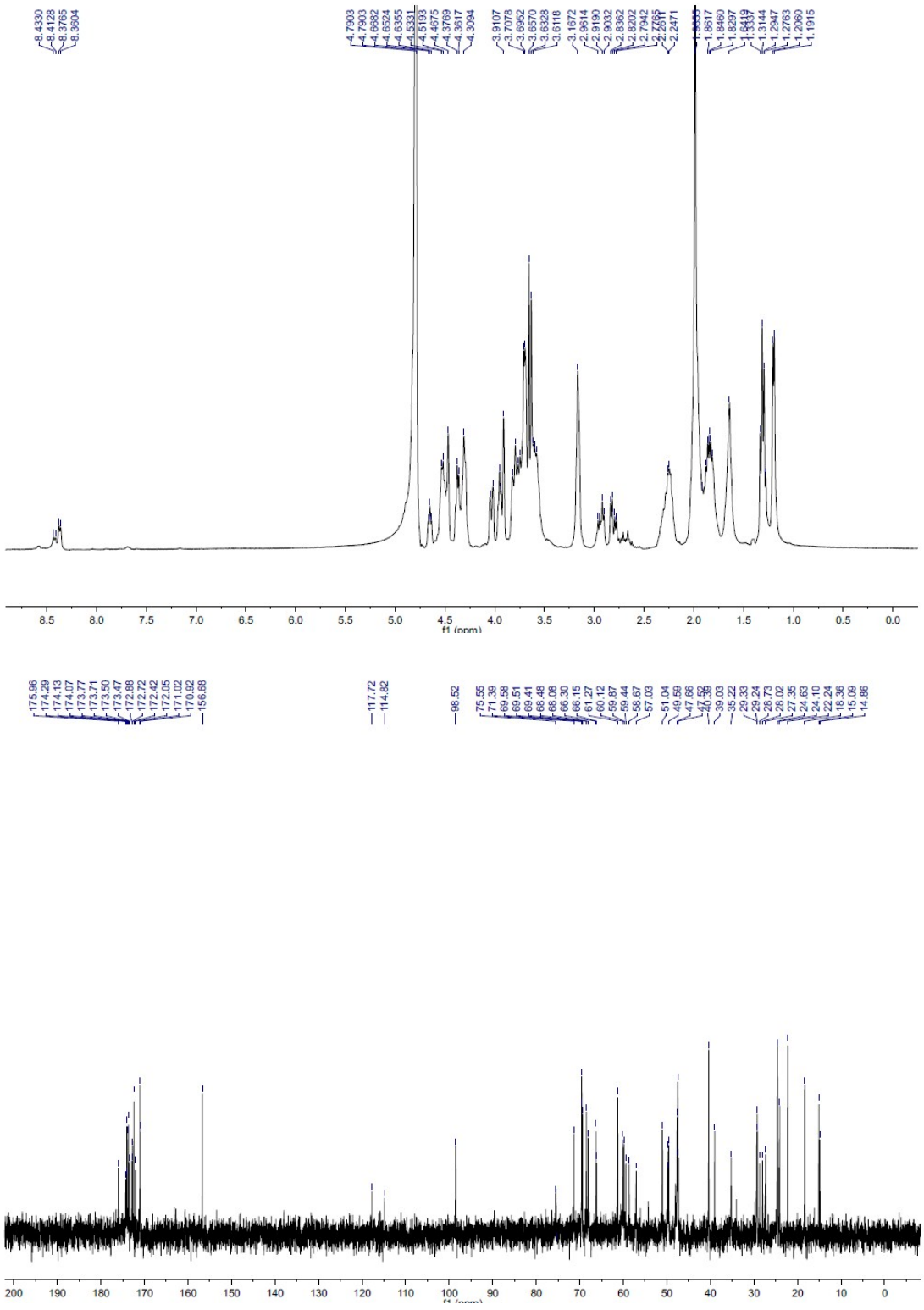


Figure S8. NMR data of **9**

3) BSA-NH_2-PEG_4-PDTRPAPPDTRPAPPDTRPAP (**10**)

The peptide **8** (10.3 mg, 4.24 μmol) was dissolved in the solution of EtOH/H_2O (1 mL, 1:1), then 3, 4-Diethoxy-3-cyclobutene-1, 2-dione (0.63 μL, 4.24 μmol) was added slowly. (Note: 0.63 μL 3, 4-Diethoxy-3-cyclobutene-1, 2-dione was very

difficult to get exactly, we usually diluted 6.3 μL 3, 4-Diethoxy-3-cyclobutene-1, 2-dione to 63 μL with EtOH/H_2O (1:1), and drain 6.3 μL of the diluent) Then sat. aq. $NaHCO_3$ was added until pH value of 8 was reached. After stirring for 2 h at room temperature, the reaction mixture was neutralized by adding HOAc. Followed by lyophilisation. The product was then dissolved in 0.07M $Na_2B_4O_7$/0.035 M $NaHCO_3$ buffer solution (1 mL), and BSA (11.2 mg, 0.17 μmol) was added. The mixture was stirred for 3 days at room temperature. The peptide-BSA conjugate was ultra-filtered with ultrafiltration (MWCO, 30 kDa) and washed with ultrapure water three times. The residue was redissolved in deionized water followed by lyophilization to obtain whilte solid (12.1mg).

BSA conjugated vaccine. The antigen loading level of **8** was determined by MALDI-TOF mass spectrometry and indicated on average three molecules of peptide per molecule of BSA.

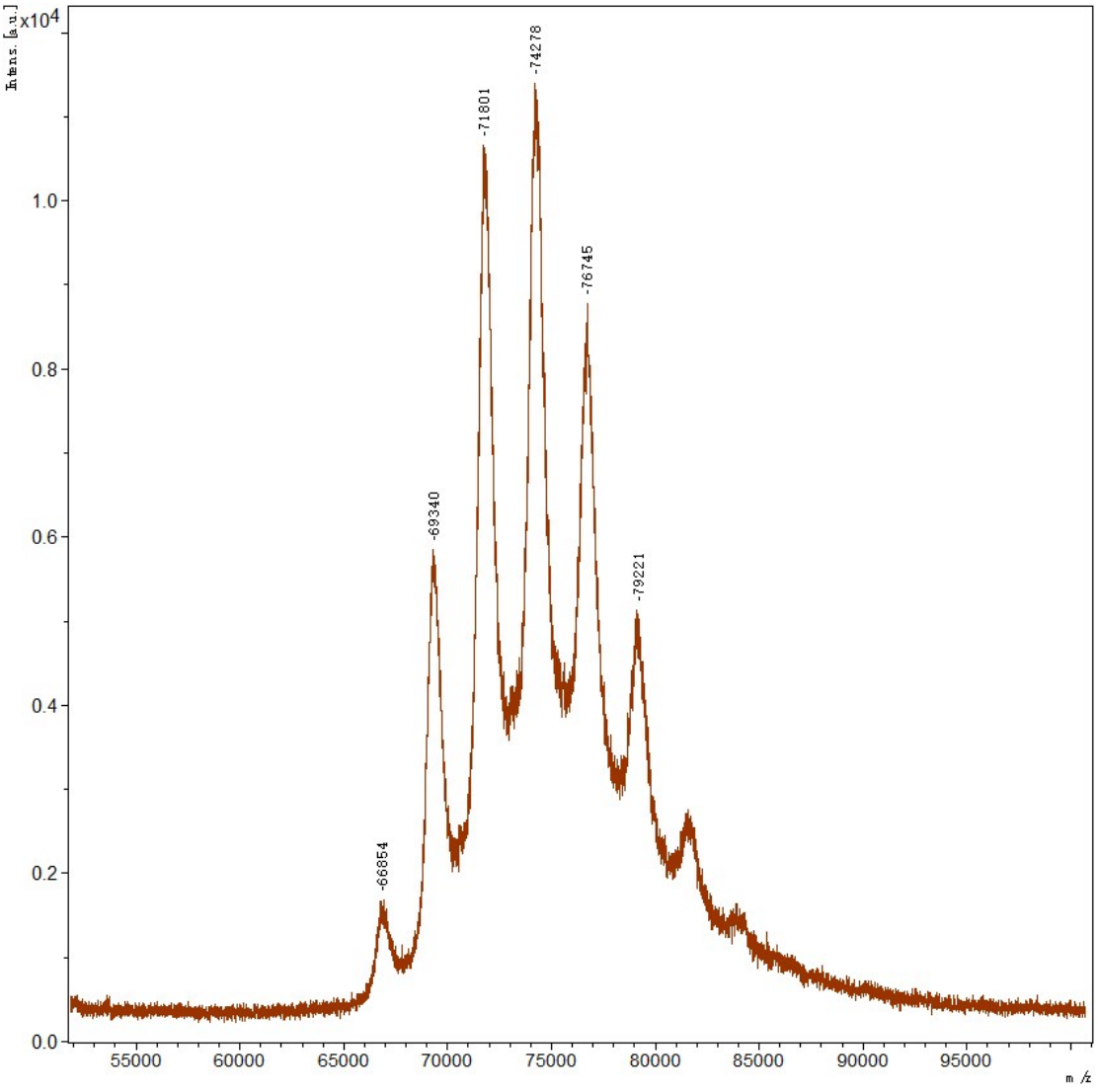


Figure S9. MALDI-TOF mass spectrometry of **10**

4) BSA-NH_2-PEG_4-PDT(α-D-GalNAc)RPAPPDT(α-D-GalNAc)RPAPPDT(α-D-GalNAc)RPAP (**11**)

The peptide **9** (5.6 mg, 1.84 μmol) was dissolved in the solution of EtOH/H_2O (0.6 mL, 1:1), then 3, 4-Diethoxy-3-cyclobutene-1, 2-dione (0.27 μL, 1.84 μmol) was added slowly. (Note: the 3, 4-Diethoxy-3-cyclobutene-1, 2-dione was diluted 10 times as above) Then sat. aq. $NaHCO_3$ was added until pH value of 8 was reached. After stirring for 2 h at room temperature, the reaction mixture was neutralized by adding HOAc. Followed by lyophilisation. The product was then dissolved in 0.07M $Na_2B_4O_7$/0.035 M $NaHCO_3$ buffer solution (0.6 mL), and BSA (4.7 mg, 0.073 μmol) was added. The mixture was stirred for 3 days at room temperature. The peptide-BSA conjugate was ultra-filtered with ultrafiltration (MWCO, 30 kDa) and washed with ultrapure water three times. The residue was redissolved in deionized water followed by lyophilization to obtain whilte solid (5.2 mg).

BSA conjugated vaccine. The antigen loading level of **9** was determined by MALDI-TOF mass spectrometry and indicated on average two molecules of glycopeptide per molecule of BSA.

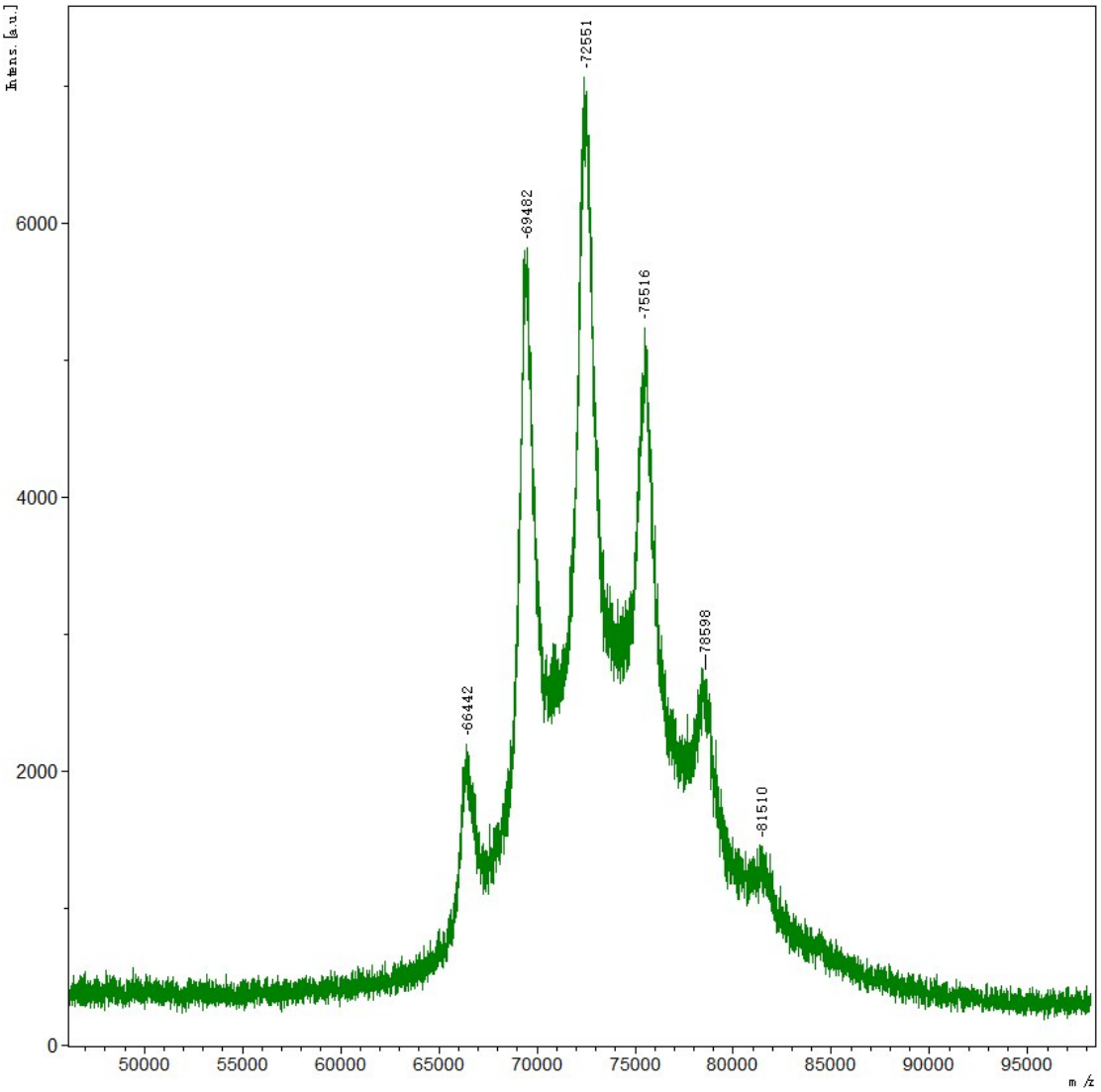


Figure S10. MALDI-TOF mass spectrometry of **11**

1. Y. Liu, Y. Wang, F. Yu, Z. Zhang, Z. Yang, W. Zhang, P. G. Wang and W. Zhao, *Chem. Commun.*, 2017, **53**, 9486-9489.