Supporting Information for

Mutagenesis and immunological evaluation of group A streptococcal C5a peptidase as an antigen for vaccine development and as a carrier protein for glycoconjugate vaccine design

Hui Li,[a] Subo Wang,[a] Yisheng Zhao,[a] Zonggang Chen,[a] Guofeng Gu,[a,*] and Zhongwu Guo[a,b,*]

[a] National Glycoengineering Research Center, School of Life Science, Shandong University, 27 Shanda Nan Lu, Jinan 250100, China

[b] Department of Chemistry, University of Florida, 214 Leigh Hall, Gainesville, Florida 32611, United States

*Corresponding authors. Tel: +86 (531) 88363612, +1 (352) 392 9133; E-mail: guofenggu@sdu.edu.cn, zguo@chem.ufl.edu

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I. The primers designed for site-directed mutagenesis

Table S1. The primers designed for site-directed mutagenesis

Entry	Mutational site	primers	
1	D130A	forward	5’TGTTGTTGCAGTGATTGCTGCTGGTTTTGA3’
		reverse	3’CTCGACCCTGACAACAACGTCACTAACGAC5’
2	S512A	forward	5’CAAACTTTCTGGAACTGCTATGTCTGCGCC3’
		reverse	3’TGTTCATACGGTTTGAAAGACCTTGACGAT5’
3	H193A	forward	5’ GCTGTCGATCAAGAGGCCGGCACACACG 3’
		reverse	3’TACCATTTTGGCGACAGCTAGTTCTCCGGC 5’
4	N295A	forward	5’ AATATGAGCTTTGGTGCTGCTGCACTAGCTT3’
		reverse	3’TCGATTCCACTAATTATACTCGAAACCACGACG5’

II. MALDI-TOF MS results for the hydrolysis of human C5a peptide by various ΔScpA

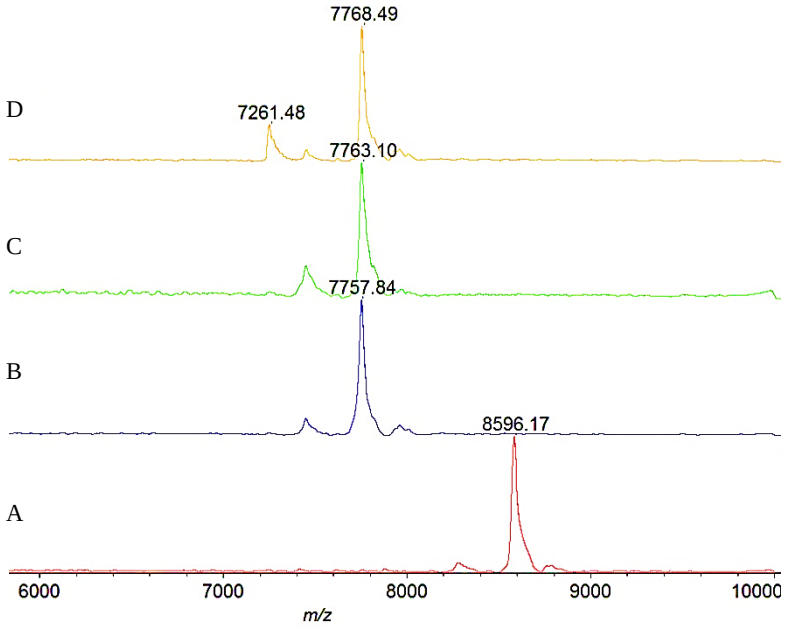


Figure S1. MALDI-TOF MS results for (A) human C5a peptide and for the hydrolysis of human C5a peptide (30 μg/mL) in the presence of truncated wild-type ΔScpA (B) 1 μg/mL, (C) 3 μg/mL and (D) 30 μg/mL at 20 °C for 30 min.

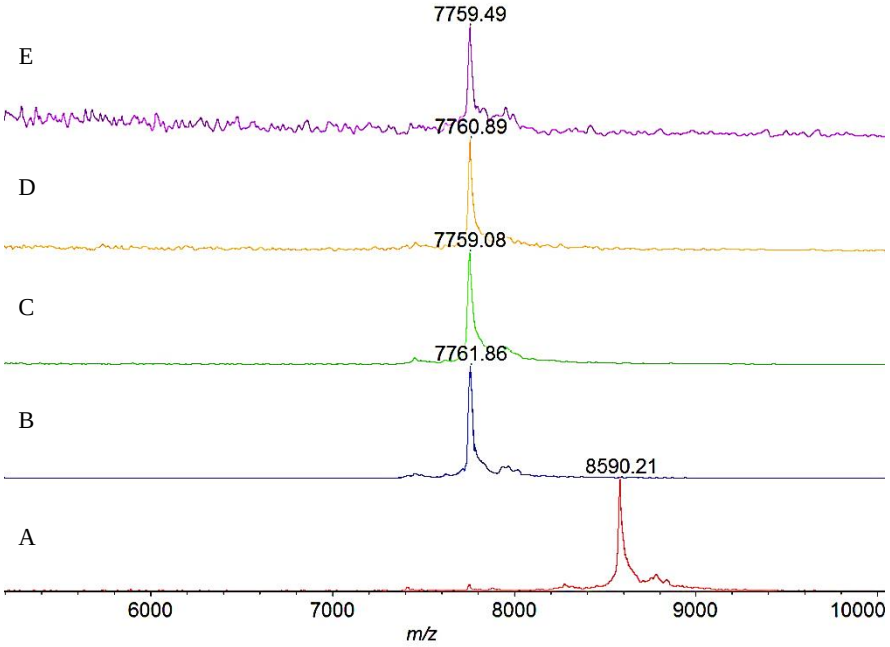


Figure S2. MALDI-TOF MS results for (A) human C5a peptide and for the hydrolysis of human C5a peptide (30 μg/mL) in the presence of $\Delta ScpA^{D130A}$ (B) 1 μg/mL, (C) 3 μg/mL, (D) 30 μg/mL and (E) 300 μg/mL at 20 °C for 30 min.

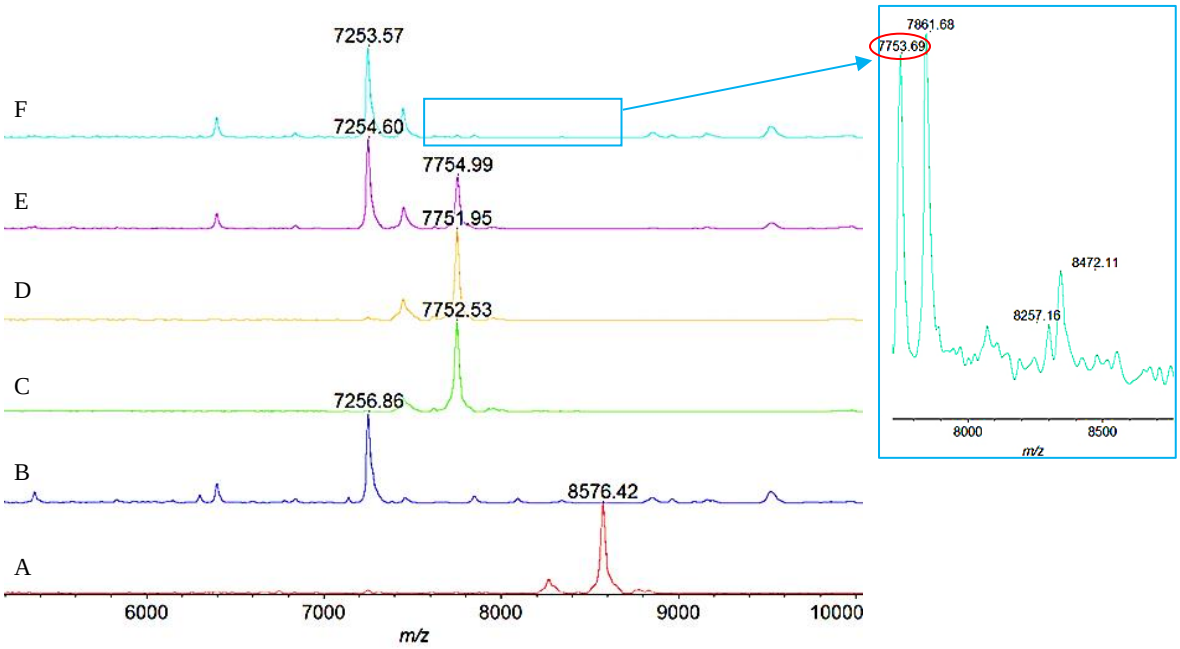


Figure S3. MALDI-TOF MS results for (A) human C5a peptide, (B) ΔScpA^{N295A}, and for the hydrolysis of human C5a peptide (30 μg/mL) in the presence of ΔScpA^{N295A} (C) 1 μg/mL, (D) 3 μg/mL, (E) 30 μg/mL and (F) 300 μg/mL at 20 °C for 30 min.

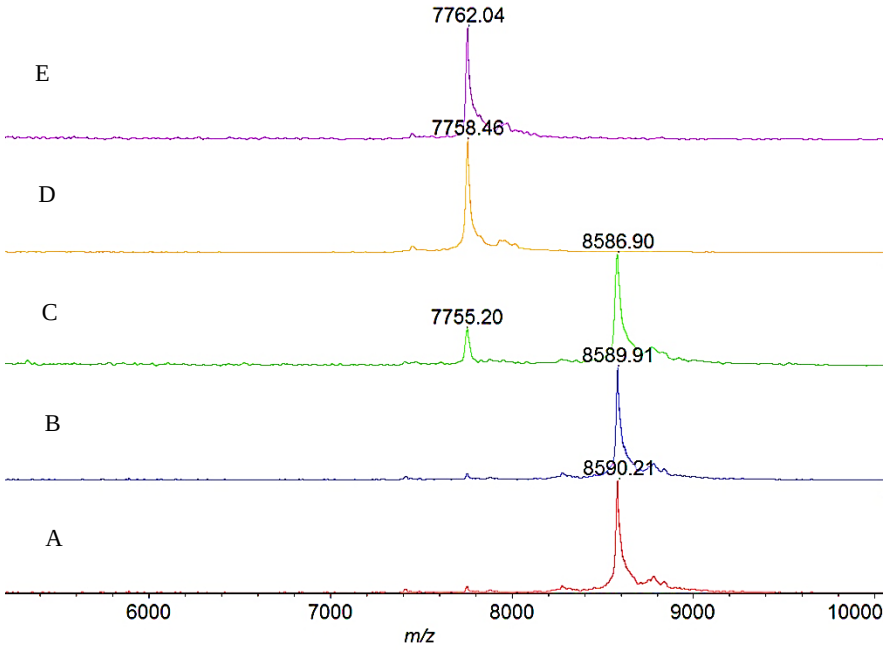


Figure S4. MALDI-TOF MS results for (A) human C5a peptide and for the hydrolysis of human C5a peptide (30 μg/mL) in the presence of ΔScpA^{S512A} (B) 1 μg/mL, (C) 3 μg/mL, (D) 30 μg/mL and (E) 300 μg/mL at 20 °C for 30 min.

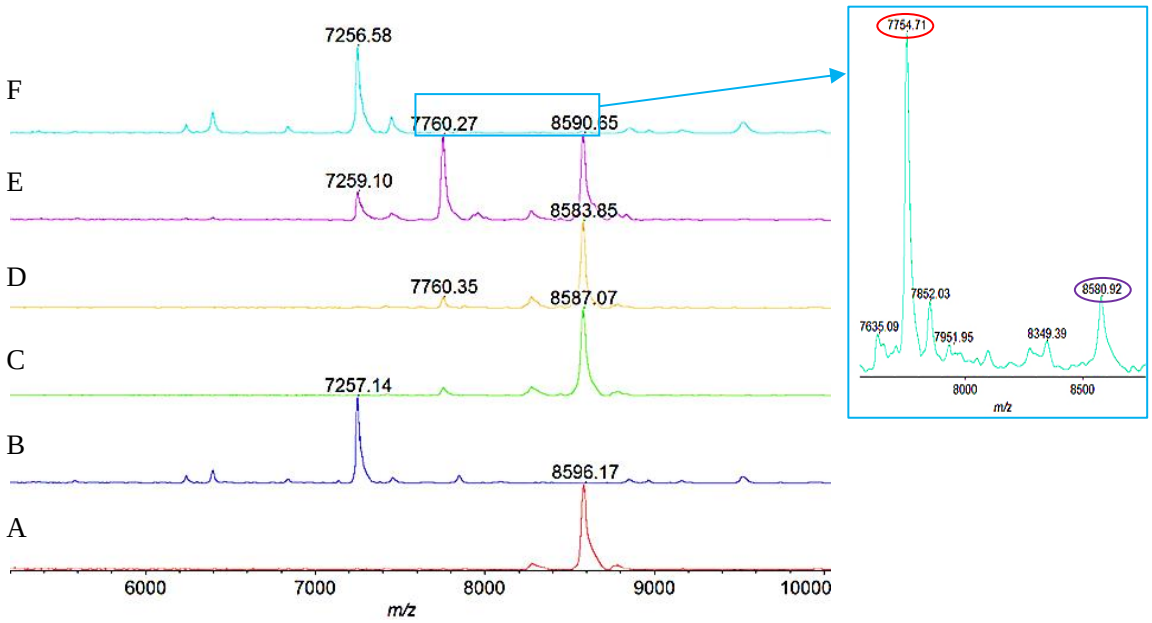


Figure S5. MALDI-TOF MS results for (A) human C5a peptide, (B) ΔScpA$^{D130S, S512A}$, and for the hydrolysis of human C5a peptide (30 μg/mL) in the presence of ΔScpA$^{D130S, S512A}$ (C) 1 μg/mL, (D) 3 μg/mL, (E) 30 μg/mL and (F) 300 μg/mL at 20 °C for 30 min.

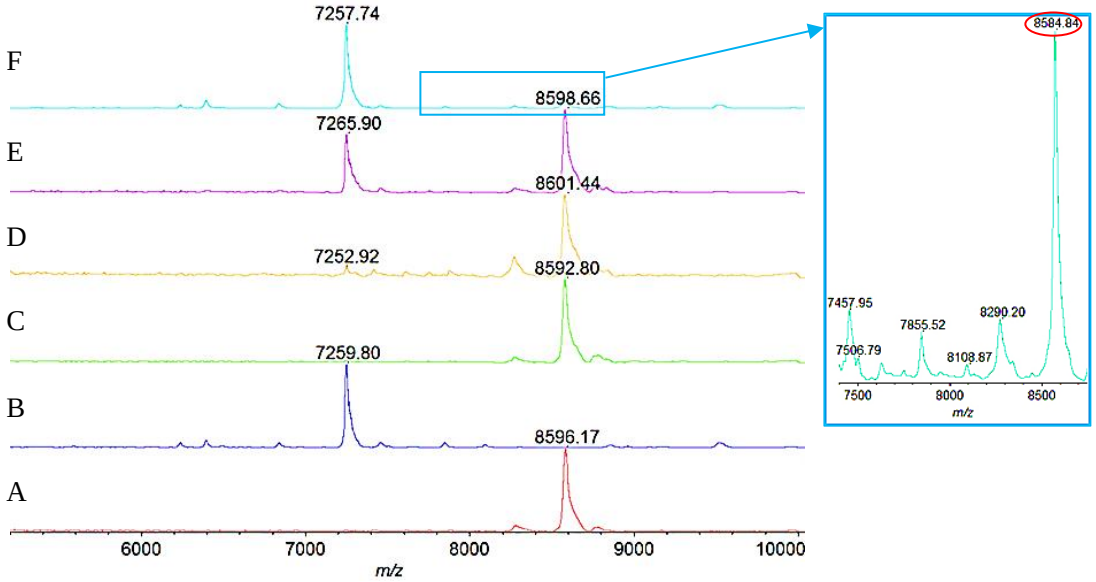
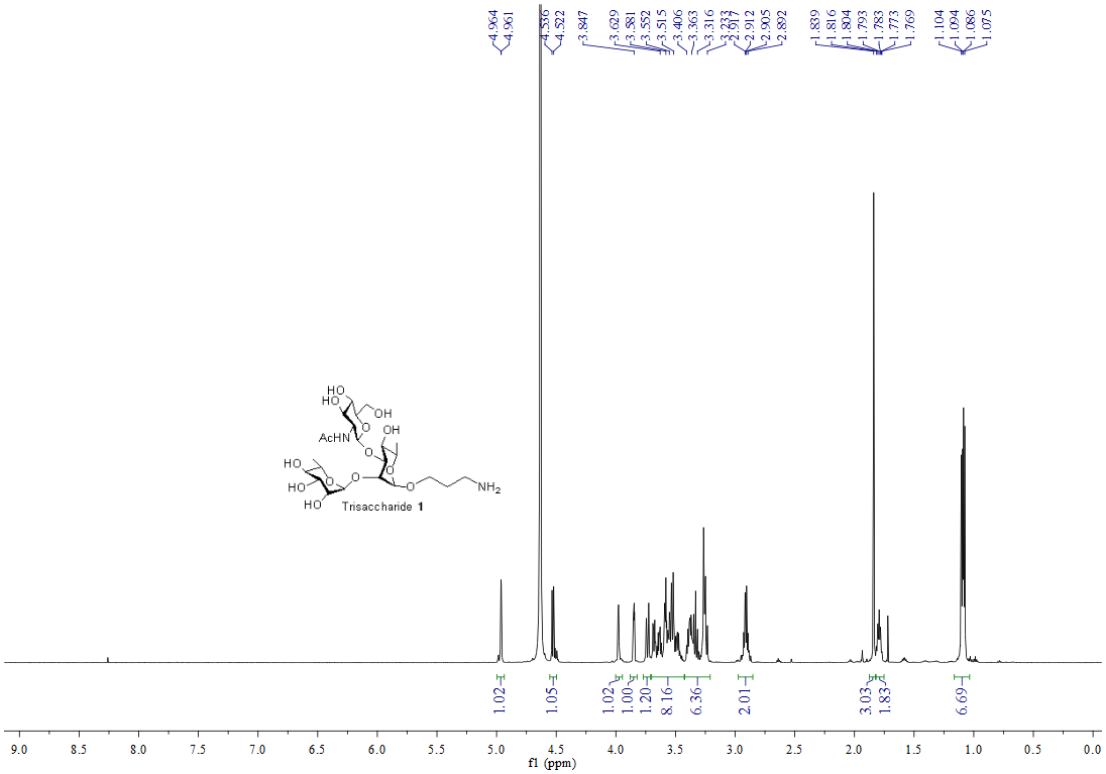


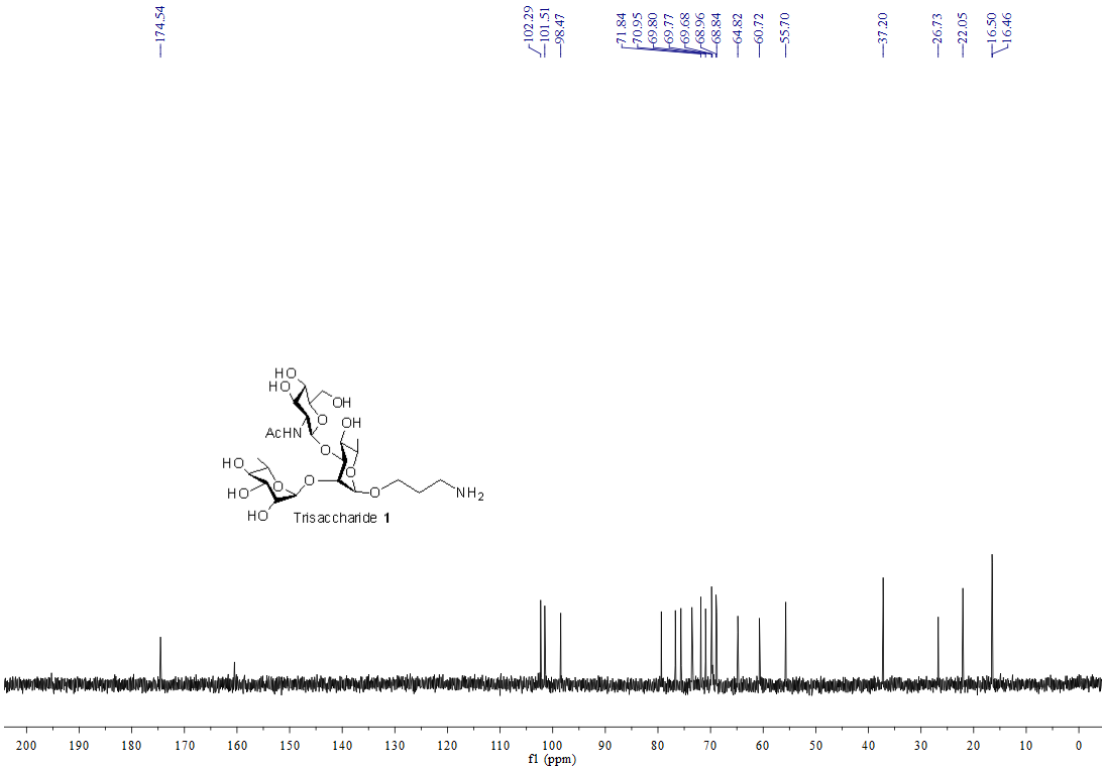
Figure S6. MALDI-TOF MS results for (A) human C5a peptide, (B) ΔScpA^{H193A}, and for the hydrolysis of human C5a peptide (30 μg/mL) in the presence of ΔScpA^{H193A} (C) 1 μg/mL, (D) 3 μg/mL, (E) 30 μg/mL and (F) 300 μg/mL at 20 °C for 30 min.

III. Characterization of GAS trisaccharide hapten 1

A



B



C

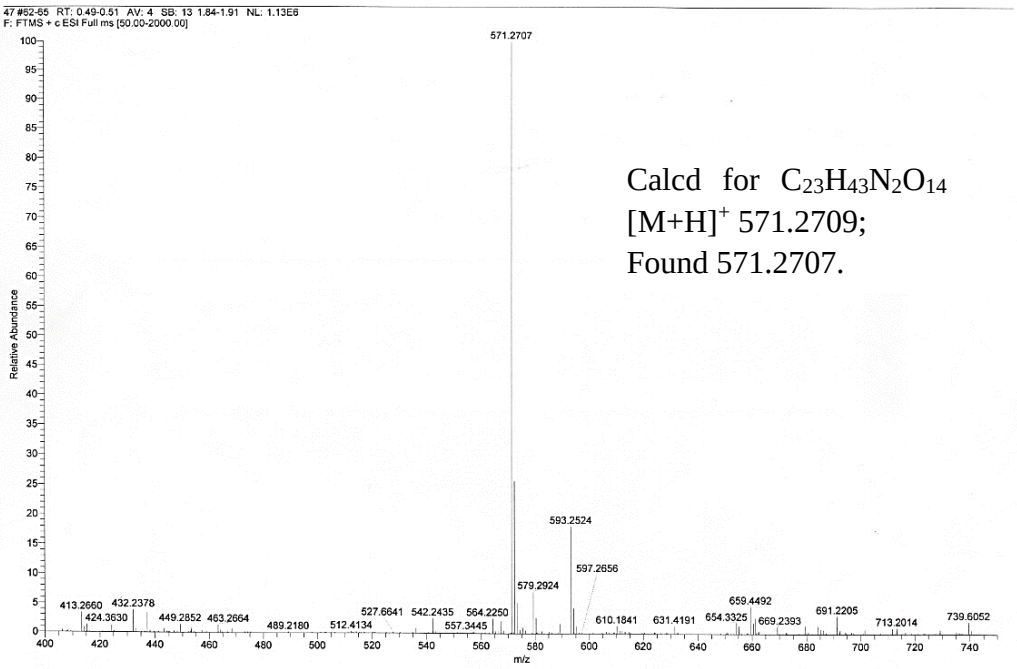


Figure S7. NMR and MS spectra of GAS trisaccharide **1**: (A) ^{1}H-NMR (600 MHz, D_2O), (B) ^{13}C-NMR (150 MHz, D_2O), and (C) ESI-TOF HRMS (positive).

IV. Preparation and characterization of the GAS trisaccharide-protein conjugates

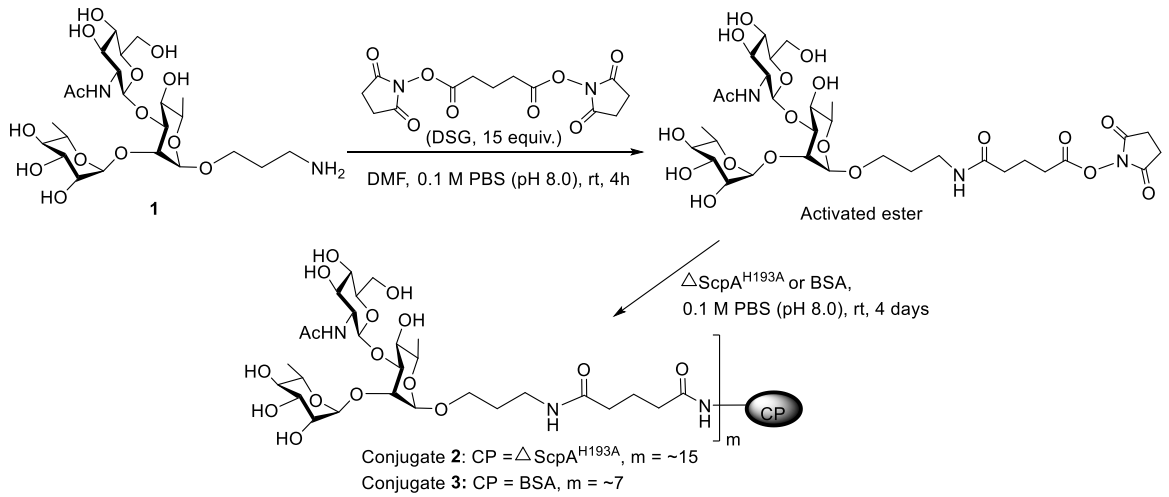


Figure S8. Synthetic scheme for GAS trisaccharide-protein conjugates.

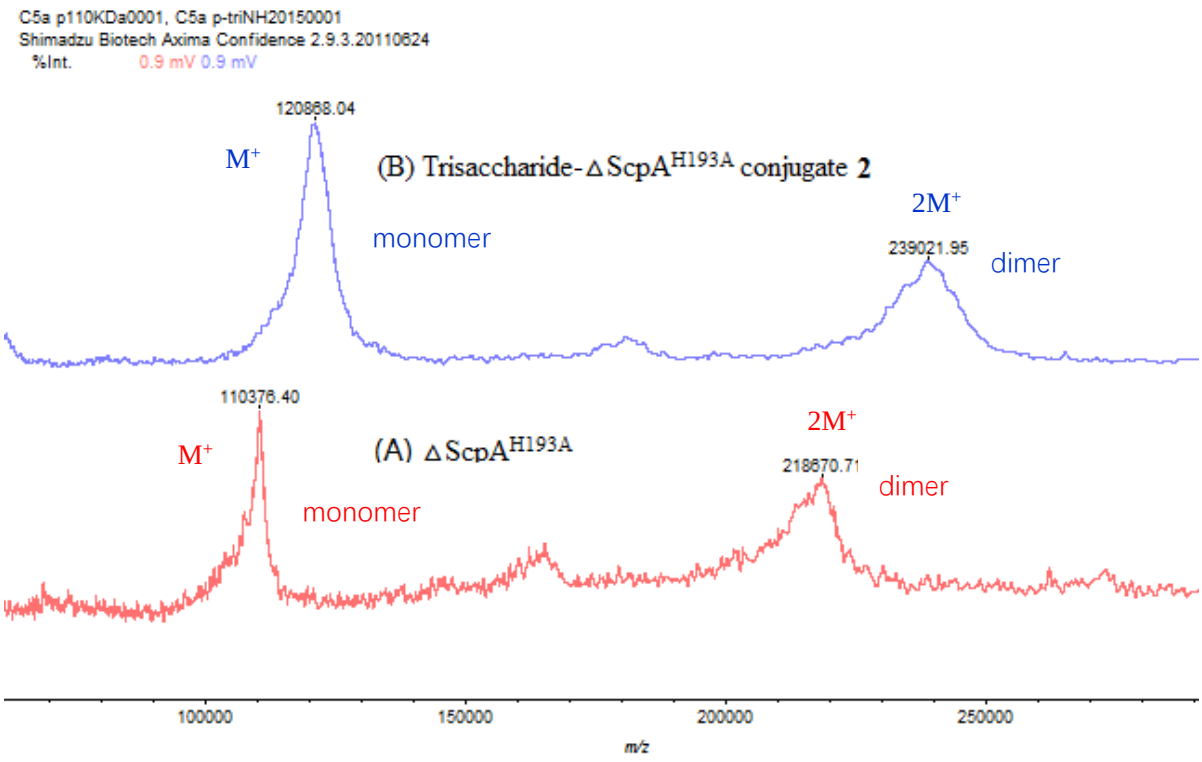


Figure S9. MALDI-TOF MS results for (A) ΔScpA^{H193A} and (B) trisaccharide-ΔScpA^{H193A} conjugate **2**

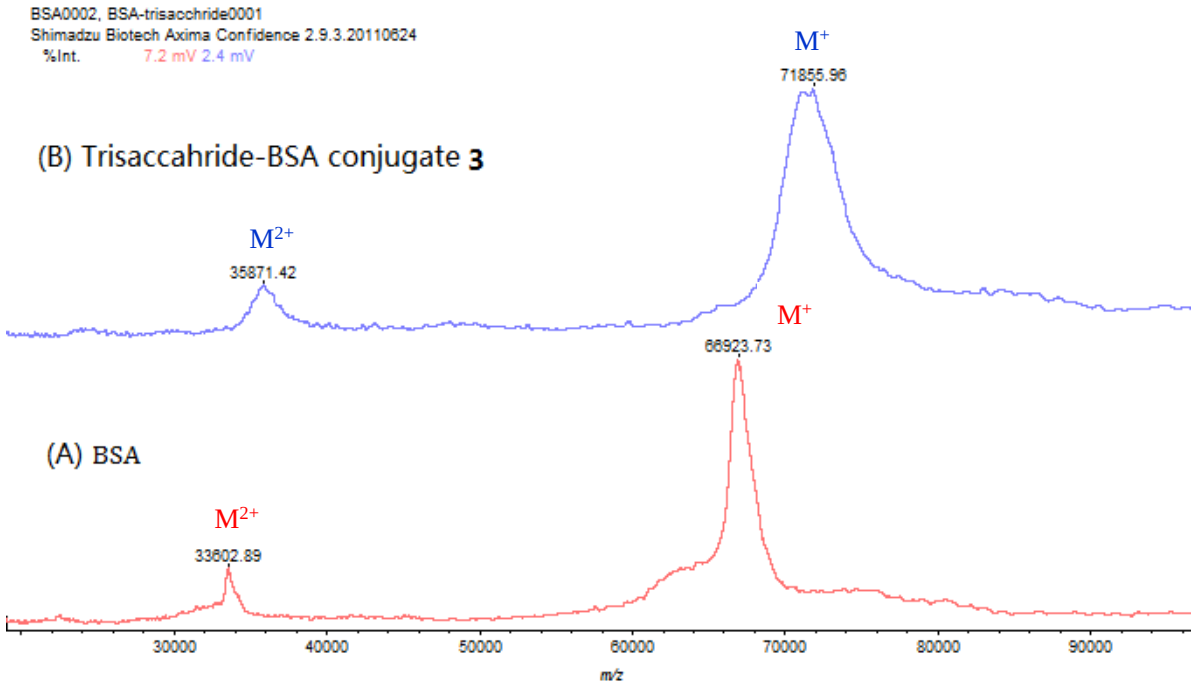
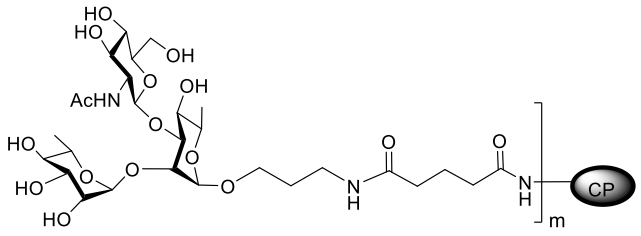


Figure S10. MALDI-TOF MS results for (A) BSA and (B) and trisaccharide-BSA conjugate **3**

Table S2. The carbohydrate loading of GAS trisaccharide-protein conjugates



Neoglycoprotein	Carrier Protein (CP)	Hapten residues/ protein molecule (m)	Carbohydrate loading (%)
2	△ScpA^{H193A}	15	8.7
3	BSA	7	6.8