Electronic Supplementary Material (ESI) for MedChemComm.

Supporting information

Design of a MUC1-based tricomponent vaccine adjuvanted with FSL-1 for cancer immunotherapy

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Table of Contents

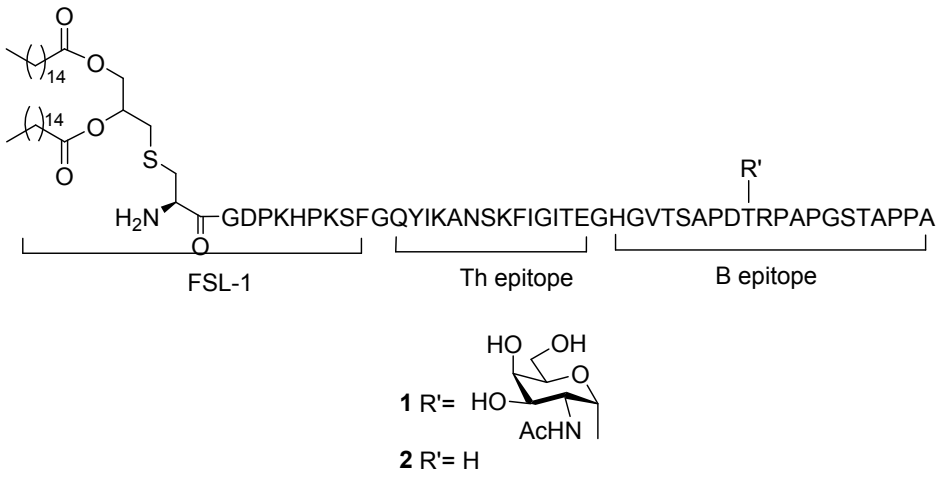
Materials and instruments

Matrix-assisted laser desorption/ionization time of flight mass spectra (MALDI-TOF MS) were performed using sinapinic acid (SA) or α-cyano-4-hydroxycinnamic acid (CHCA) as matrix on UltrafleXtreme MALDI-TOF/TOF (Brucker, Germany). Reversed-phase HPLC separations were performed on a Waters system 2487 using solution A (0.1% trifluoroacetic acid in acetonitrile) and solution B (0.1% trifluoroacetic acid in water) for elutions. UV absorption signals were detected with an UV detector at a wavelength of 220nm. Semi-preparative HPLC was used for separation and purification of the peptides on a C-18 column (10 μm, 10×250 mm, 100 μL per injection) at a flow rate of 2 mL/min. Mouse IgG antibody and Monoclonal Antibody Isotyping kit were purchased from Sigma-Aldrich (USA). ELISA kits detecting IL-6 and IL-12 were purchased from Biolegend (San Diego, CA, USA). MCF-7 and B16-MUC1 cell lines were purchased from China Infrastructure of Cell Line Resources.

General Procedure for peptides synthesis

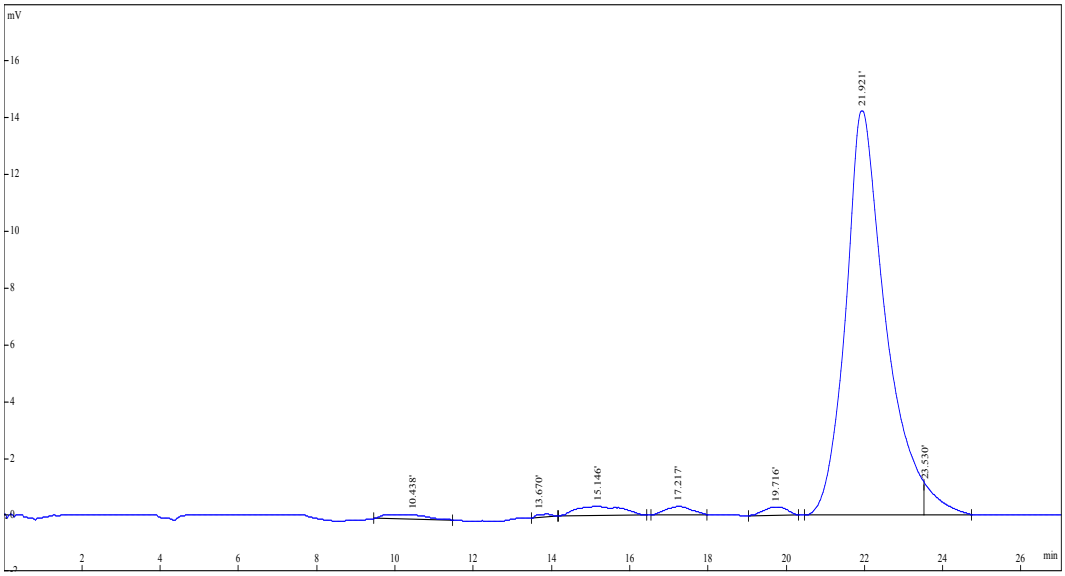
(Glycolipo)peptides were synthesized as we previously reported[1]. The solid-phase peptide synthesis (SPPS) for the preparation of vaccine candidate **1** was conducted using 2-chlorotrityl resin preloaded with Fmoc-Alanine (loading: 0.13 mmol g^{-1}, 3.85 g, 0.5 mmol). Fmoc amino acids were introduced with 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 4.0 equiv), 1-hydroxybenzotriazole (HOBt, 4.0 equiv), N,N-diisopropylethylamine (DIPEA, 8.0 equiv). The introduction of glycosylated amino acid was conducted using more reactive 1-[dis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium 3-oxid hexafluorophosphate (HATU, 2.0 equiv), 1-hydroxy-7-azabenzotriazole (HOAt, 2.0equiv), DIPEA (4.0 equiv) and its acetyl moieties were removed by MeONa/MeOH (pH = 10–11). Fmoc-Pam_2Cys-OH was coupled to the sequence with HATU/HOAt (2.0 equiv) and DIPEA (4.0 equiv). The protecting groups of side chain were removed and the glycopeptide was detached from the resin by adding 90% TFA, 5% TIPS, and 5% H_2O. The glycolipopeptide was purified by semi-preparative HPLC on a C18 column. Compound **2**, **3**, **4** and **5** were prepared in a similar way. The yield of HPLC is defined as the purification yield.

Analytical data for MUC1-(lipo)peptide

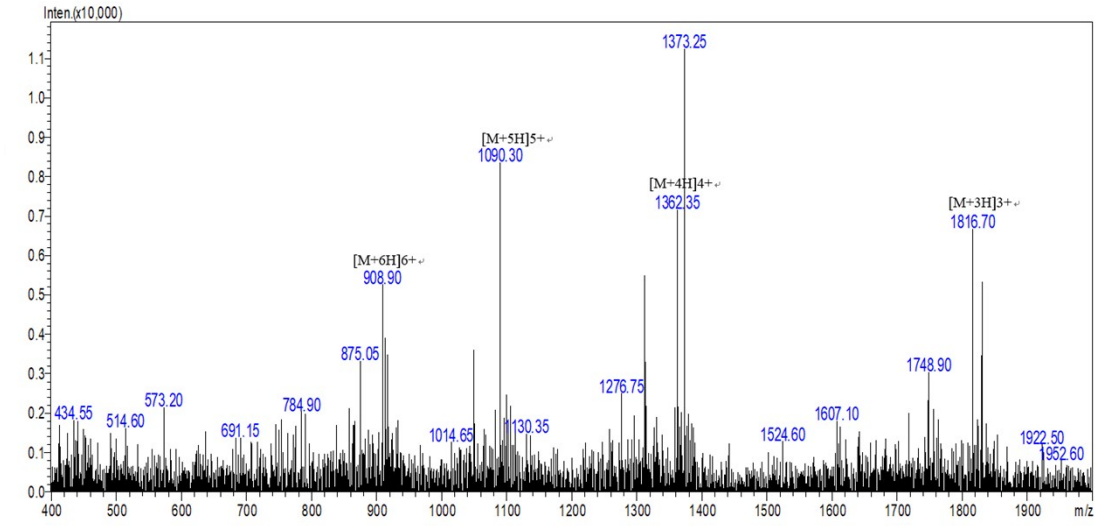


Compound 1

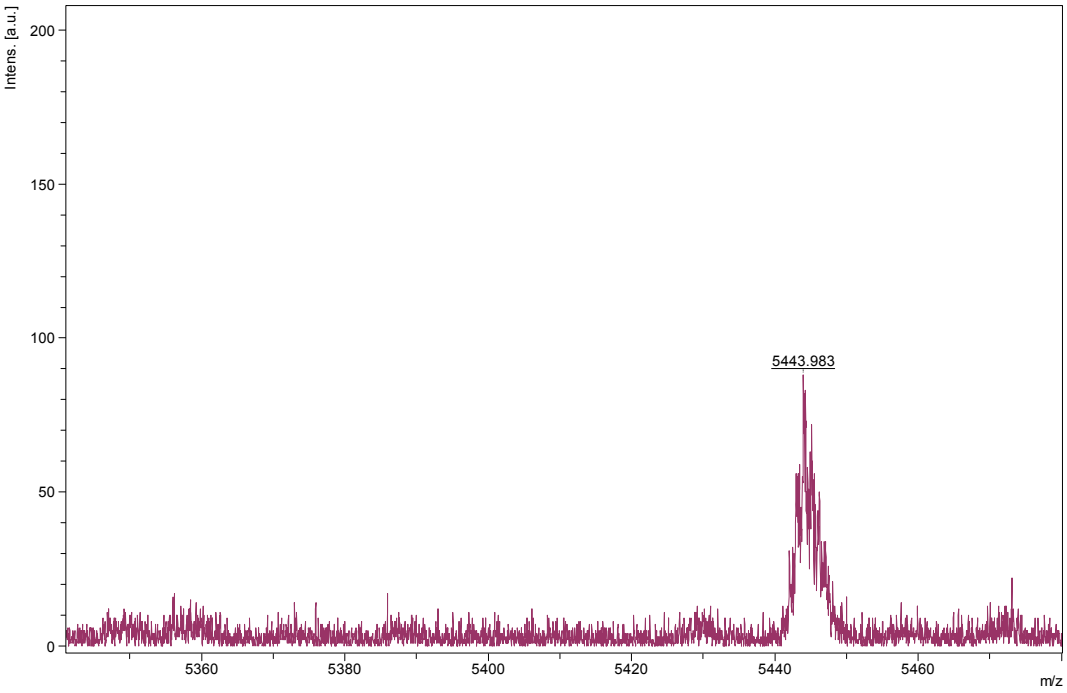
H-S-[2,3-Bis(palmitoyloxy)-(2RS)-propyl]-(R)-cysteine-Gly-Asp-Pro-Lys-His-Pro-Lys-Ser-Phe-Gly-Gln-Tyr-Ile-Lys-Ala-Asn-Ser-Lys-Phe-Ile-Gly-Ile-Thr-Glu-Gly-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr(α-GalNAc)-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala-OH.



Analytical HPLC: Rt (retention time) = 21.9 min (50-75% of acetonitrile and 0.1% trifluoroacetic acid over 30 min on a C-18 column, λ=220 nm). Yield 25% (10.8 mg).



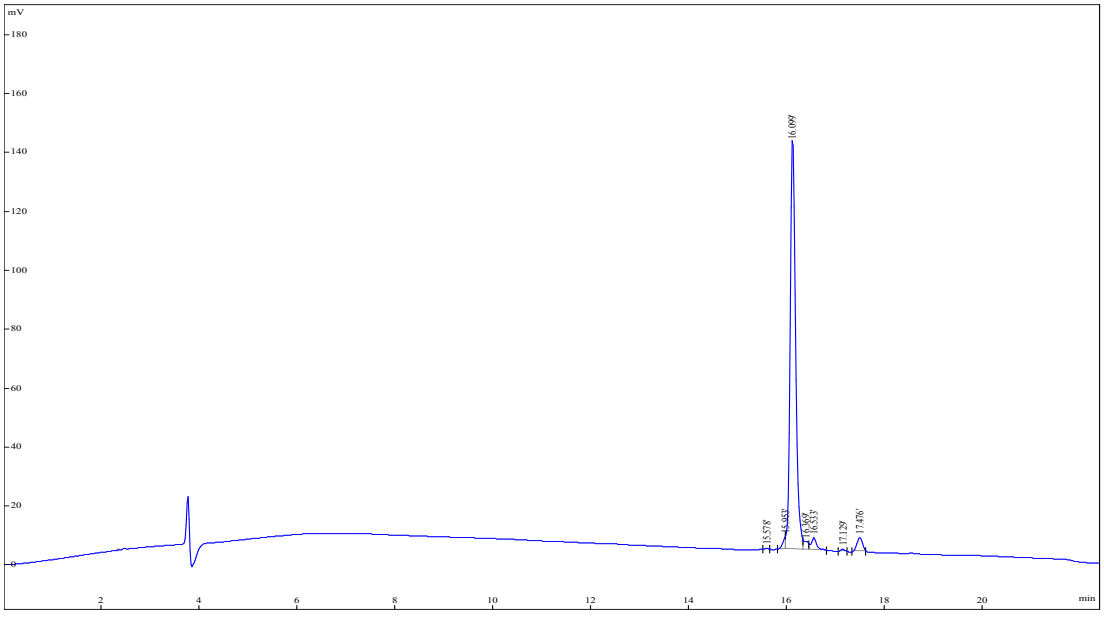
ESI-MS: m/z for $C_{250}H_{400}N_{60}O_{73}S$ $[M+3H]^{3+}$ calcd 1815.31, found 1816.70; $[M+4H]^{4+}$ calcd 1361.73, found 1362.35; $[M+5H]^{5+}$ calcd 1089.59, found 1090.30 ; $[M+6H]^{6+}$ calcd 908.16, found 908.90.



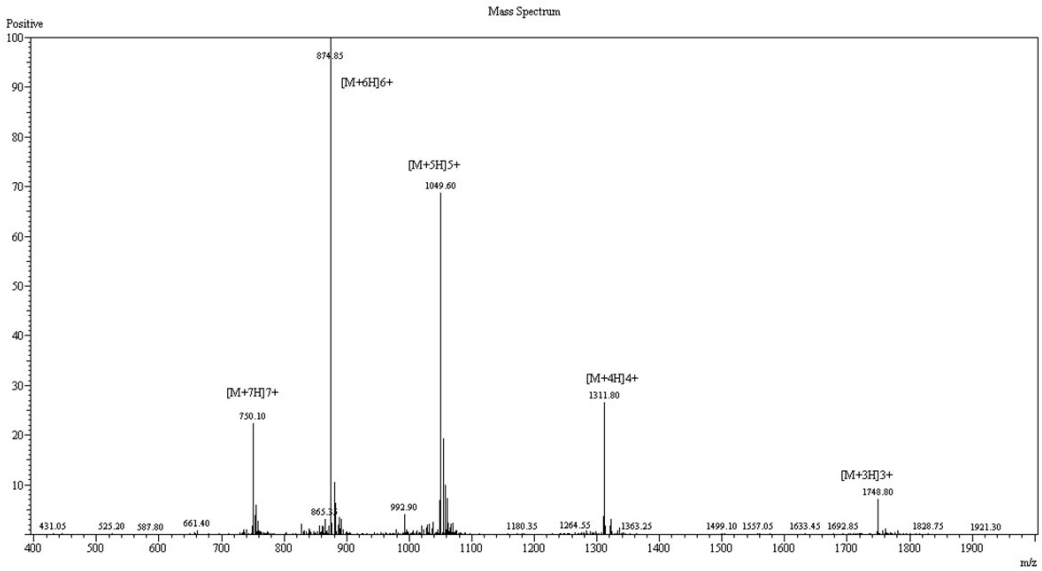
MALDI-TOF-MS：$C_{250}H_{400}N_{60}O_{73}S$ $[M+H]^+$ calcd 5443.9226, found 5443.983.

Compound 2

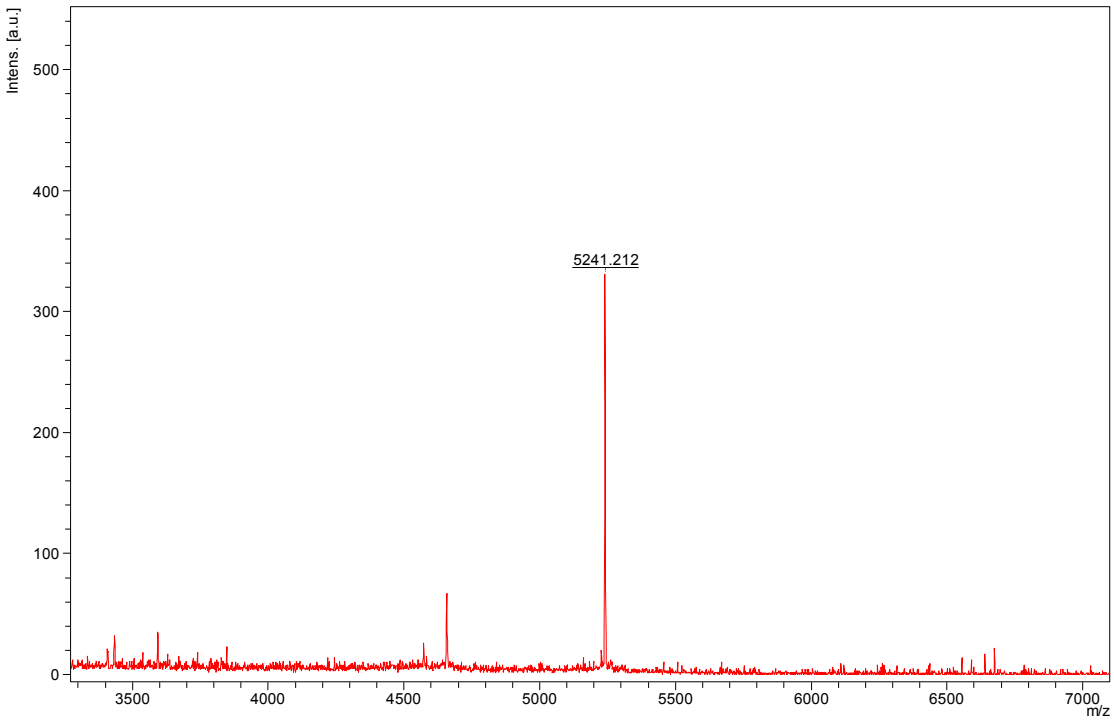
H-S-[2,3-Bis(palmitoyloxy)-(2RS)-propyl]-(R)-cysteine-Gly-Asp-Pro-Lys-His-Pro-Lys-Ser-Phe-Gly-Gln-Tyr-Ile-Lys-Ala-Asn-Ser-Lys-Phe-Ile-Gly-Ile-Thr-Glu-Gly-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala-OH.



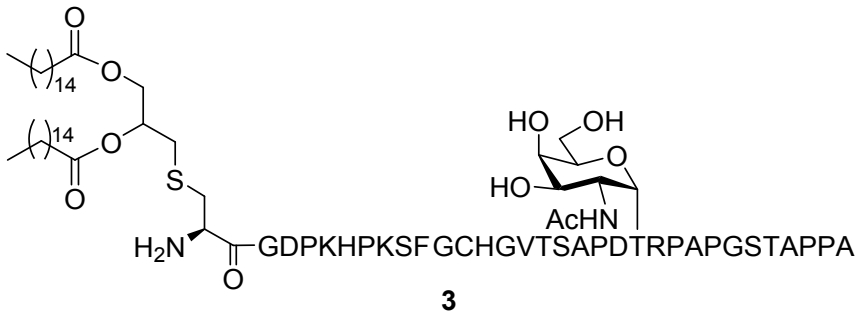
Analytical HPLC: Rt (retention time) = 16.1 min (50-75% of acetonitrile and 0.1% trifluoroacetic acid over 30 min on a C-18 column, λ=220 nm). Yield 29% (12.0 mg).



ESI-MS: m/z for $C_{242}H_{387}N_{59}O_{68}S$ $[M+3H]^{3+}$ calcd 1747.62, found 1748.8; $[M+4H]^{4+}$ calcd 1310.97, found 1311.80; $[M+5H]^{5+}$ calcd 1048.97, found 1049.60 ; $[M+6H]^{6+}$ calcd 874.31, found 874.85; $[M+7H]^{7+}$ calcd 749.56, found 750.10.



MALDI-TOF-MS：$C_{242}H_{387}N_{59}O_{68}S$ $[M+H]^{+}$ calcd 5240.8432, found 5241.212.

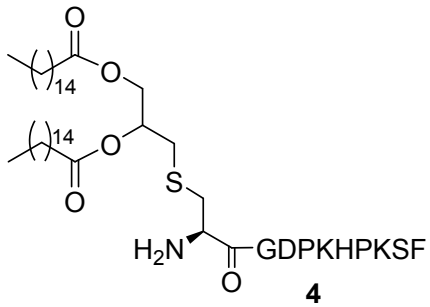


3

Compound 3

H-S-[2,3-Bis(palmitoyloxy)-(2RS)-propyl]-(R)-cysteine-Gly-Asp-Pro-Lys-His-Pro-Lys-Ser-Phe-Gly-Cys-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala-OH.

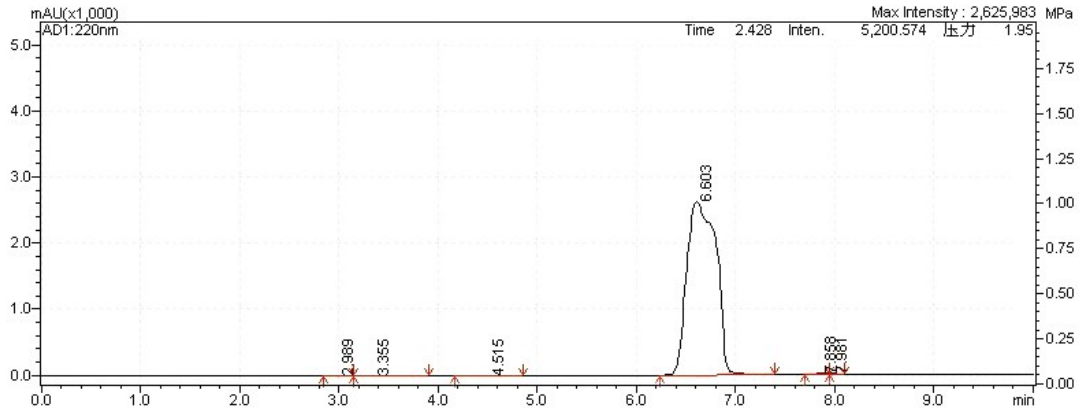
The compound was characterized in our previous report[1].



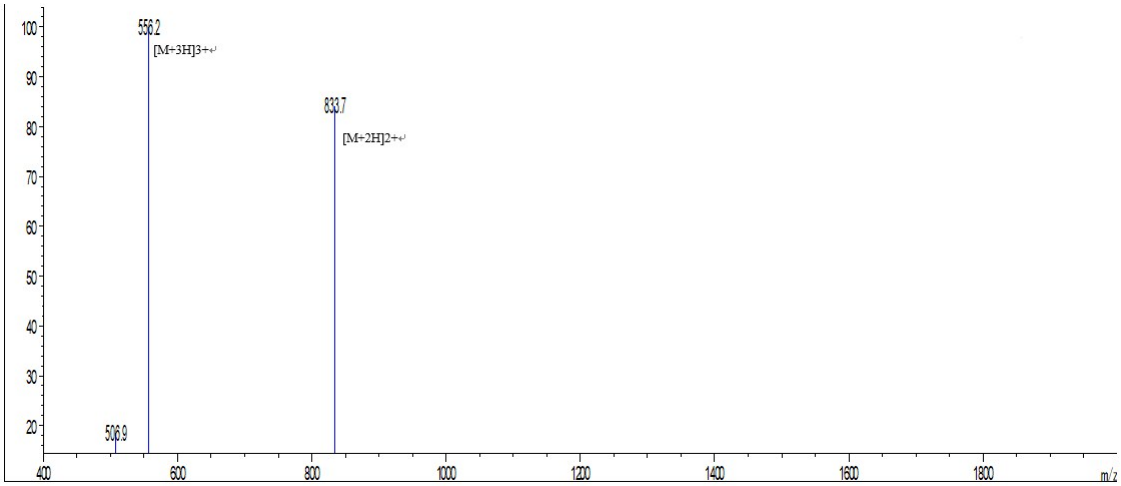
4

Compound 4

H-S-[2,3-Bis(palmitoyloxy)-(2RS)-propyl]-(R)-cysteine-Gly-Asp-Pro-Lys-His-Pro-Lys-Ser-Phe

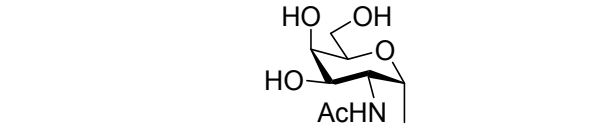


Analytical HPLC: Rt (retention time) = 6.603 min (10-100% of acetonitrile and 0.1% trifluoroacetic acid over 10 min on a C-18 column, λ=220 nm). Yield 49% (9.6 mg).



ESI-MS: m/z for $C_{84}H_{141}N_{15}O_{17}S$ $[M+2H]^{2+}$ calcd 833.0, found 833.7; $[M+3H]^{3+}$ calcd 555.7, found 556.2.

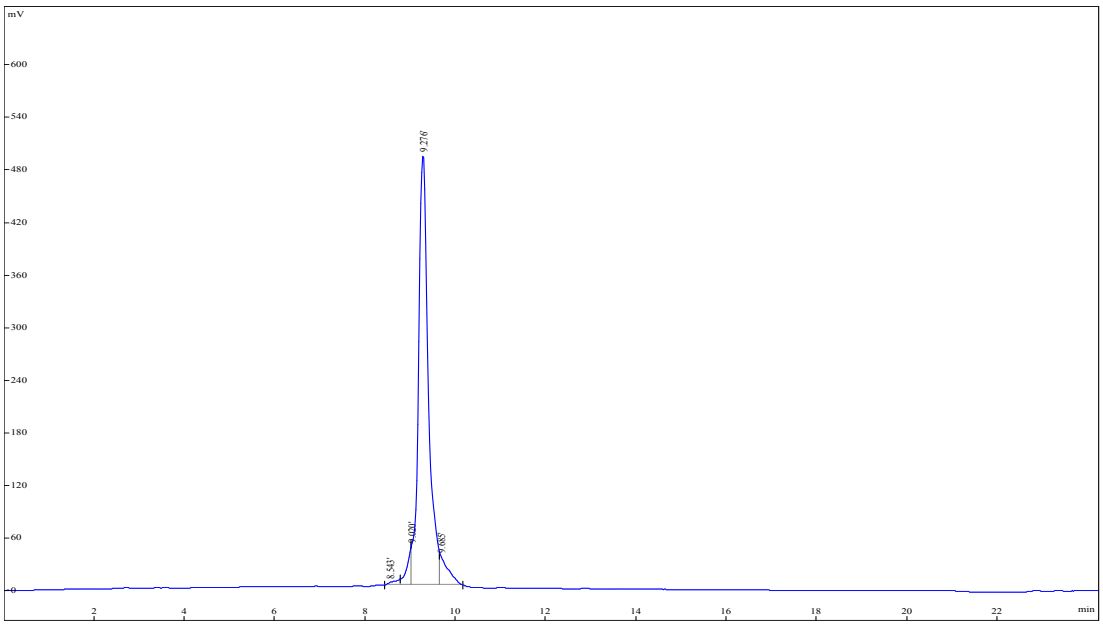
The compound was characterized in previous report[2].



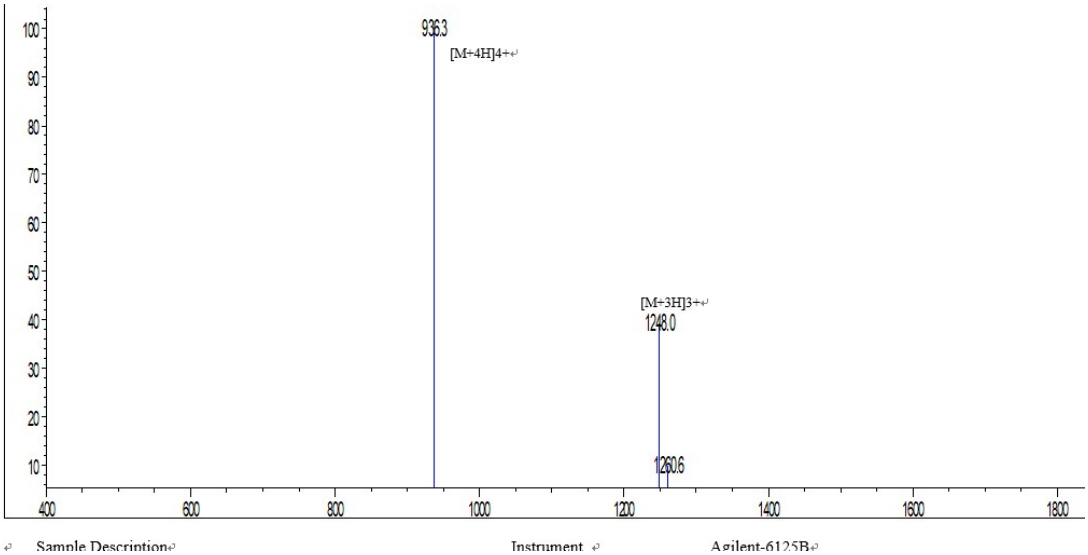
5

Compound 5

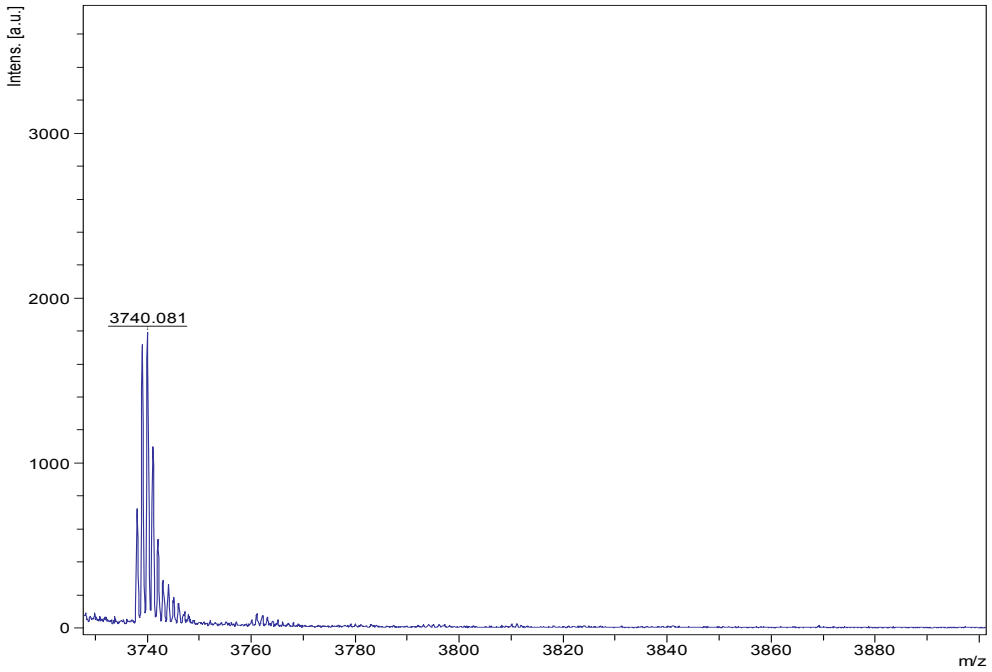
H-Gln-Tyr-Ile-Lys-Ala-Asn-Ser-Lys-Phe-Ile-Gly-Ile-Thr-Glu-Gly-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr(α-GalNAc)-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala-OH.



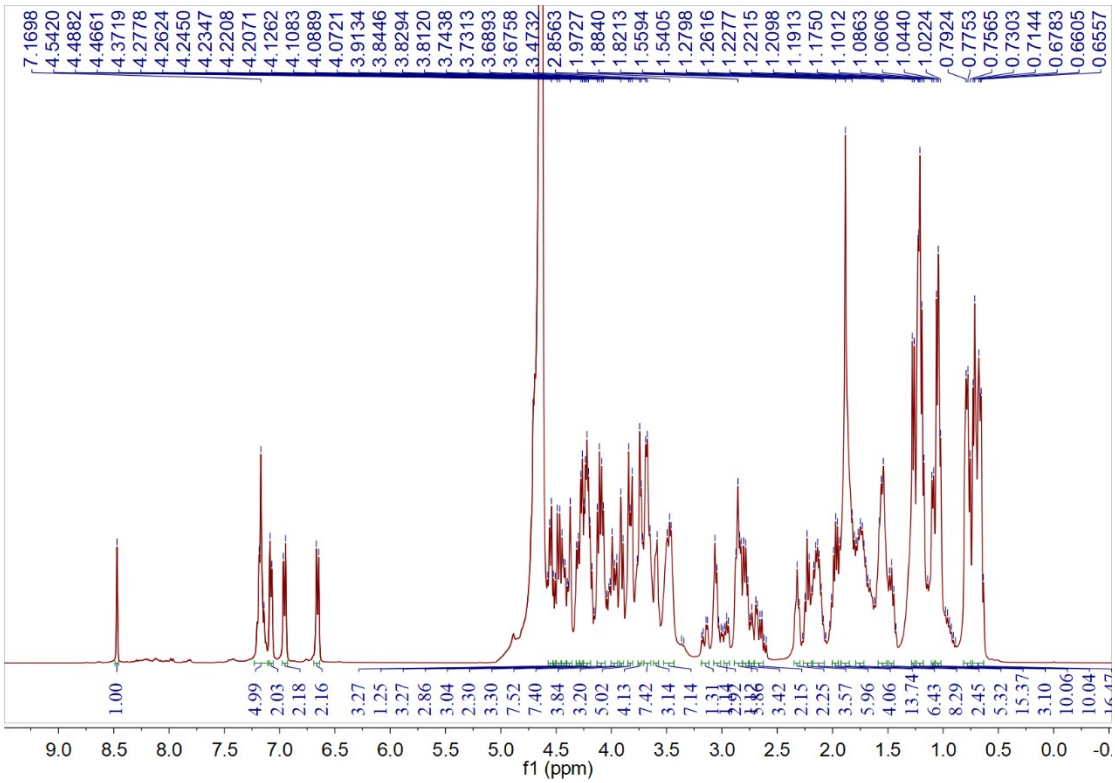
Analytical HPLC: Rt (retention time) = 9.3 min (16-40% of acetonitrile and 0.1% trifluoroacetic acid over 30 min on a C-18 column, λ=220 nm). Yield 38% (10.1 mg).



ESI-MS: m/z for $C_{164}H_{259}N_{45}O_{55}$ $[M+3H]^{3+}$ calcd 1247.3, found 1248.0; $[M+4H]^{4+}$ calcd 935.7, found 936.3.

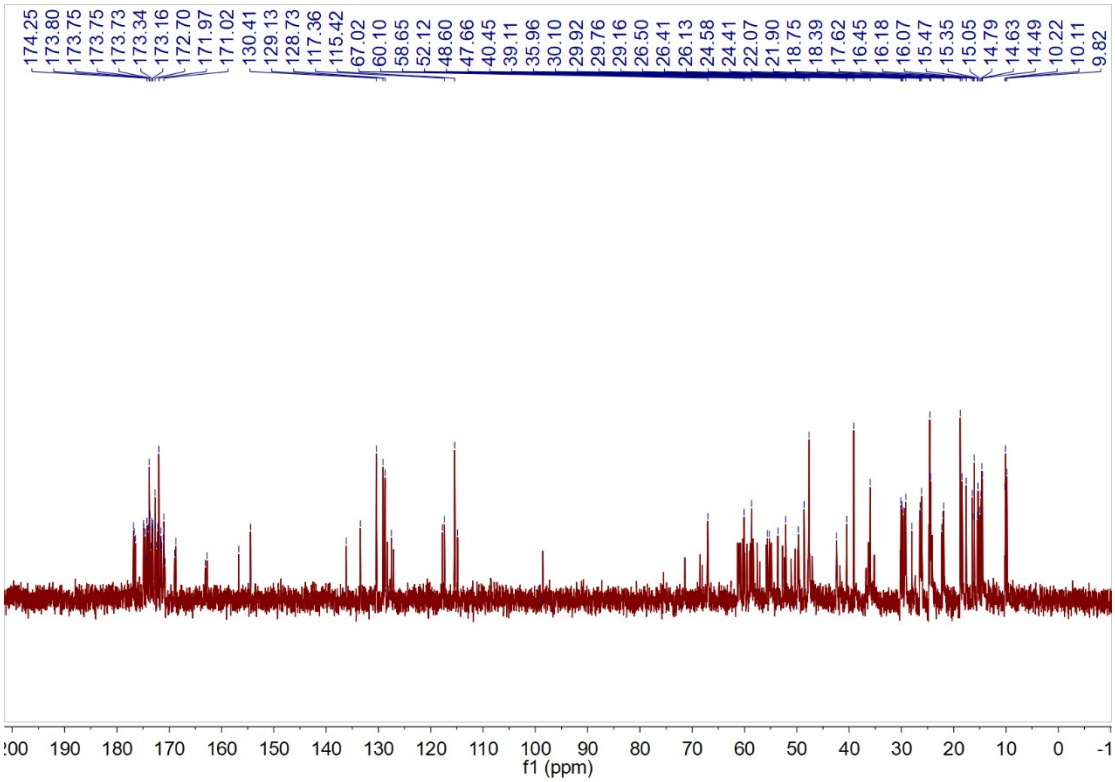


MALDI-TOF-MS：$C_{164}H_{259}N_{45}O_{55}$ $[M+H]^{+}$ calcd 3739.8926, found 3740.081



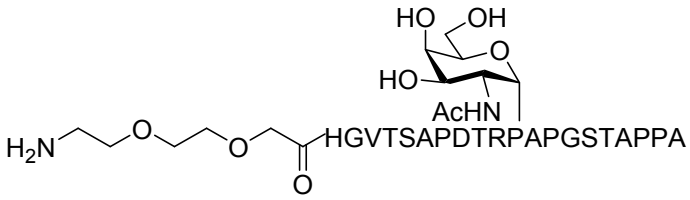
^{1}H NMR (400 MHz, D_2O) δ 8.47 (s, 1H), 7.23 – 7.11 (m, 5H), 7.08 (d, *J* = 6.9 Hz, 2H), 6.96 (d, *J* = 8.0 Hz, 2H), 6.66 (d, *J* = 8.2 Hz, 2H), 4.57 – 4.53 (m, 3H), 4.52 (d, *J* = 6.9 Hz, 2H), 4.48 (d, *J* = 8.8 Hz, 2H), 4.45 – 4.41 (m, 4H), 4.40 – 4.36 (m, 3H), 4.32 – 4.29 (m, 2H), 4.27 (s, 3H), 4.25 – 4.19 (m, 8H), 4.16 – 4.08 (m, 7H), 4.00 – 3.94 (m, 4H), 3.92 – 3.86 (m, 3H), 3.85 – 3.81 (m, 5H), 3.74 (d, *J* = 5.0 Hz, 4H), 3.71 – 3.64 (m, 7H), 3.59 (s, 3H), 3.53 – 3.43 (m, 8H), 3.16 (s, 1H), 3.08 – 3.02 (m, 3H), 2.98 – 2.93 (m, 1H), 2.89 – 2.81 (m, 6H), 2.81 – 2.76 (m, 3H), 2.74 (d, *J* = 6.3 Hz, 1H), 2.67 (dd, *J* = 15.6, 6.5 Hz, 2H), 2.31 (d, *J* = 7.0 Hz, 2H), 2.26 – 2.20 (m, 4H), 2.18 – 2.07 (m, 6H), 2.00 – 1.95 (m, 4H), 1.92 – 1.84 (m, 14H), 1.79 – 1.72 (m, 6H), 1.59 – 1.51 (m, 8H), 1.49 – 1.45 (m, 2H), 1.31 – 1.24 (m, 5H), 1.24 – 1.18 (m, 15H), 1.11 – 1.07 (m, 3H), 1.07 – 1.02 (m, 10H), 0.82 – 0.75 (m, 10H), 0.72 – 0.66 (m, 16H).

Selected signals δ 8.47 (1H, Arg); 7.23 – 6.66 (11 H, Tyr, Phe, His-aryl group).



^{13}C NMR (101 MHz, D_2O, selected signals for carbonyl groups, Arg, Tyr and C-1) δ176.84, 176.69, 176.46, 174.90, 174.88, 174.58, 174.29, 174.25, 174.19, 174.02, 173.80, 173.75, 173.75, 173.73, 173.51, 173.34, 173.32, 173.27, 173.18, 173.16, 172.70, 172.64, 172.42, 172.20, 172.07, 171.97, 171.87, 171.64, 171.57, 171.52, 171.52, 171.46, 171.13, 171.09, 171.02, 171.00, 170.92, 170.80, 168.94, 168.72, 163.10, 162.74, 156.70 (Arg), 154.46 (Tyr), 98.48 (C-1).

Antigen for ELISA plate coating



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

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

FACS analysis of the antisera binding with B16-MUC1 cells

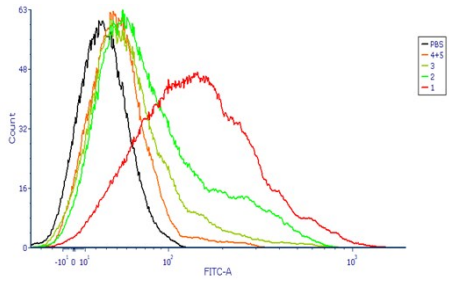


Fig. S1 FACS analysis of the antisera binding with B16-MUC1 cells. The sera from mice injected with PBS was used as control (black)

References

1. Y. Liu, W. Zhang, Q. He, F. Yu, T. Song, T. Liu, Z. Zhang, J. Zhou, P. G. Wang and W. Zhao, *Chem. Commun.*, 2016, **52**, 10886-10889.
2. L. Israel, Y. Wang, K. Bulek, E. Della Mina, Z. Zhang, V. Pedergnana, M. Chrabieh, N. A. Lemmens, V. Sancho-Shimizu, M. Descatoire, T. Lasseau, E. Israelsson, L. Lorenzo, L. Yun, A. Belkadi, A. Moran, L. E. Weisman, F. Vandenesch, F. Batteux, S. Weller, M. Levin, J. Herberg, A. Abhyankar, C. Prando, Y. Itan, W. J. B. van Wamel, C. Picard, L. Abel, D. Chaussabel, X. Li, B. Beutler, P. D. Arkwright, J. L. Casanova and A. Puel, *Cell*, 2017, **168**, 789-800.