Sugar Vinyl Sulfoxide Glycoconjugation of Peptides and Lysozyme: Abrogation of Proteolysis at the Lysine Sites

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1. General Information

Solvents were dried and distilled according to literature procedures. All the chemicals and lysozyme protein were purchased from commercial sources and used without further purification. Silica gel (100−200 mesh) was used for column chromatography and thin layer chromatography (TLC) analysis was performed on commercial plates coated with silica gel 60 F254. Visualization of the spots on the TLC plates was achieved by UV radiation or spraying 5% sulfuric acid in ethanol or ninhydrin in ethanol solution. Mass spectral characterizations were performed on ESI-QTOF (Waters Xevo® G2-XS QTOF), operating in the positive ion mode, on samples in either MeCN/water or MeOH/water solution. ^{1}H and ^{13}C NMR spectral analyses were performed on a spectrometer operating at 400 and 100 MHz, respectively. Processing of the FID data was performed on Bruker TopSpin™ and Mnova softwares, with default settings. Chemical shifts are reported with respect to tetramethylsilane for ^{1}H NMR and the central line (77.0 ppm) of $CDCl_3$ for ^{13}C NMR spectra. Coupling constants (*J*) are reported in Hertz. Standard abbreviations s, d, t, dd, br s, m, and app refer to singlet, doublet, triplet, doublet of doublet, broad singlet, multiplet, and apparent, respectively.

2. Experimental Procedures

5: A mixture of benzyl 4,6-di-*O*-acetyl-2,3-dideoxy-3-(*p*-tolylsulfinyl)-α-D-*erythro*-hex-3-enopyranoside (**1**) (0.10 g, 0.22 mmol) and ethanolamine (8 μL, 0.13 mmol) was dissolved in aq. MeOH (40% v/v) (10 mL), stirred at 40 °C for 12 h, solvents evaporated *in vacuo* and purified (SiO_2) (EtOAc) to afford **5** (0.035 g, 64%), as a foamy solid. R_f = 0.25 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (d, *J* = 7.6 Hz, 2 H, aromatic), 7.31-7.21 (band, 7 H, aromatic), 6.59 (app.

s, 1 H, H-4), 5.03 (d, J = 3.8 Hz, 1 H, H-1), 4.76 (d, J = 12 Hz, 1 H, PhCH_{2a}), 4.52 (d, J = 12 Hz, 1 H, PhCH_2), 4.45 (d, J = 3.8 Hz, 1 H, H-2), 3.81 (dd, J = 3.2Hz, 11.6 Hz, 1 H, H-6a), 3.68 (dd, J = 2.8 Hz, 11.6 Hz, 1 H, H-6_b), 3.31 (t, J = 4.8 Hz, 2 H, CH_2OH), 2.93 (app.d, J = 2.8 Hz, 1 H, H-5), 2.46-2.41 (band, 1 H, CH_{2a}NH), 2.31 (s, 3 H), 2.08-2.017 (m, 1 H, CH$_{2b}$NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 143.2, 142.3, 139.2, 137.0, 130.1, 128.6, 128.6, 128.2, 128.2 (aromatic), 126.8 (*C*-4), 126.7 (aromatic), 94.1 (*C*-1), 71.0 (PhCH_2), 69.9 (*C*-5), 64.5 (*C*-6), 61.6 (*C*-2), 54.5 (CH_2OH), 48.4 (CH_2NH), 21.5 (CH_3); ESI-MS m/z: $[M+Na]^+$ calcd. For $C_{22}H_{27}NO_5SNa$, 440.1508; found: 440.1507.

7: A mixture of benzyl 4,6-di-*O*-acetyl-2,3-dideoxy-3-(*p*-tolylsulfinyl)-α-D-*erythro*-hex-3-enopyranoside (**1**) (103 mg, 0.23 mmol), serine methyl ester hydrochloride (**6**) (24 mg, 0.15 mmol) and $NaHCO_3$ (5 mg) was dissolved in aq. MeOH (40% v/v) (5 mL, pH 7.5-8) and stirred at 40 °C for 16 h, solvents evaporated *in vacuo* and purified (SiO_2) (pet. ether/EtOAc, followed by $CHCl_3$/MeOH) to afford **7** (58 mg, 74 %), as a diastereomeric mixture (ratio 5:1). R_f = 0.2 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (d, J = 7.6 Hz, 2.7 H), 7.32-7.26 (m, 8.9 H), 5.60 (s, 1 H), 5.30 (s, 0.36 H), 5.09 (d, J= 2.8 Hz, 1.02 H), 5.04 (s, 0.3 H), 4.77 (d, J= 11.6 Hz, 1.4 H), 4.60 (d, J= 12 Hz, 1.3 H), 4.43 (s, 1.1 H), 4.16 (s, 0.5 H), 4.10 (d, J=3.6 Hz, 2.2 H), 3.89-3.82 (m, 2.8 H), 3.71 (s, 3.2 H), 3.67-3.65 (m, 2.6 H), 3.37 (app. s, 1.1 H), 2.43 (s, 4.2 H), 2.06 (s, 1.7 H), 1.25 (s, 0.8 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.1, 169.5, 169.4, 145.1, 144.8, 141.7, 137.5, 137.0, 136.8, 136.6, 135.4, 130.0, 129.8, 128.6, 128.3, 128.2, 128.1, 127.8, 92.8, 91.4, 70.8, 70.3, 68.3, 62.4, 61.2, 60.6, 21.6, 20.7, 20.6, 20.5; ESI-MS m/z: $[M+Na]^+$ calcd. For $C_{26}H_{31}NO_8SNa$, 540.1668; found: 540.1666.

9: A mixture of benzyl 4,6-di-*O*-acetyl-2,3-dideoxy-3-(*p*-tolylsulfinyl)-α-D-*erythro*-hex-3-enopyranoside (**1**) (89 mg, 0.20 mmol), lysine hydrochloride (**8**) (23.8 mg, 0.13 mmol) and $NaHCO_3$ (5 mg) was dissolved in aq. MeOH (40%) (5 mL, pH 7.5-8) and stirred at 40 °C for 16 h. solvents were evaporated *in vacuo* and purified (SiO_2) (pet ether/EtOAc) to afford **9** (55 mg, 81%), as a diastereomeric mixture (ratio = 1:0.35). R_f = 0.18 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (br s, 1.3 H), 7.67 (d, *J*= 6.8 Hz, 1.5 H), 7.55-7.53 (m, 0.5 H), 7.49 (d, *J*= 7.6 Hz, 2.1 H), 7.35-7.29 (m, 1.6 H), 7.19-7.11 (m, 8.4 H), 7.00 (d, *J*= 7.2 Hz, 1.8 H), 6.74 (br s, 1.0 H), 6.60 (s, 0.2 H), 4.81 (app s, 1.4 H), 4.71-4.63 (m, 2.5 H), 4.56 (d, *J*= 12 Hz, 0.5 H), 4.49 (d, *J*= 12 Hz, 1.3 H), 4.24 (t, *J*= 4.4 Hz, 0.7 H), 4.12 (app s, 2.7 H), 3.84 (br s, 1.8 H), 3.65 (s, 0.6 H), 3.47 (s, 0.9 H), 3.09 (s, 1.6 H), 2.40-2.33 (m, 1.5 H), 2.27 (s, 3 H), 2.20 (s, 2.1 H), 2.08-2.03 (m, 1.0 H), 1.88 (app s, 3.7 H), 1.46 (br s, 4.0 H), 1.25 (s, 1.0 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.45, 174.23, 170.36, 170.29, 142.72, 141.49, 140.42, 138.97, 136.41, 136.39, 130.18, 128.91, 128.49, 128.20, 126.46, 126.00, 93.66, 93.37, 70.11, 68.36, 66.63, 65.21, 57.71, 54.63, 48.78, 46.85, 46.72, 35.18, 30.37, 30.18, 29.68, 22.60, 22.56, 21.43, 21.24, 20.67. ESI-MS m/z: $[M+H]^+$ calcd. For $C_{28}H_{37}N_2O_7S$, 545.2327; found 545.2327.

11: A mixture of benzyl 4,6-di-*O*-acetyl-2,3-dideoxy-3-(*p*-tolylsulfinyl)-α-D-*erythro*-hex-3-enopyranoside (**1**) (0.124 g, 0.27 mmol), arginine (**10**) (31.4 mg, 0.18 mmol) and $NaHCO_3$ (3 mg) was dissolved in aq. MeOH (2:1) (5 mL, pH 7.5-8) and heated at 40 °C for 16 h, solvents were evaporated *in vacuo* and purified (SiO_2) (MeOH/$CHCl_3$, linear gradient) to afford **11** (70 mg, 70%), as a diastereomeric mixture (ratio 9:1). R_f = 0.6 (20% MeOH/$CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 7.64-7.50 (m, 5.1 H), 7.36-7.32 (m, 4.1 H), 7.01-6.9 (br., 4 H), 6.6 (app. s, 1.2 H), 5.04 (app. s, 1 H), 4.86-4.76 (m, 0.4 H), 4.69-4.42 (m, 3.2 H), 4.25-4.17 (m, 3 H), 3.04 (br s, 3.6

H), 2.73 (br s, 1.3 H), 2.41-2.31 (m, 4.4 H), 2.06-2.01 (m, 4.3 H), 1.40-1.36 (m, 2.7 H), 1.28 (s, 1.9 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.22, 170.62, 157.28, 157.22, 143.09, 142.43, 140.84, 137.91, 137.10, 136.49, 130.18, 128.64, 128.45, 128.17, 127.87, 127.78, 127.64, 127.28, 126.97, 95.21, 95.09, 70.68, 68.57, 65.34, 65.10, 65.01, 54.06, 41.16, 29.63, 24.68, 21.52, 21.46, 20.86. ESI-MS m/z: $[M+H]^+$ calcd. for $C_{28}H_{37}N_4O_7S$, 573.2383; found 573.2393.

14: A mixture of benzyl 4,6-di-*O*-acetyl-2,3-dideoxy-3-(*p*-tolylsulfinyl)-α-D-*erythro*-hex-3-enopyranoside (**1**) (0.21 g, 0.45 mmol), Boc-Lys-Lys-OMe (**12**) (58 mg, 0.15 mmol) and $NaHCO_3$ (2 mg) was dissolved in aq. MeOH (40%) (10 mL, pH 7.5-8) and stirred at 40 ^oC for 16 h, solvents evaporated *in vacuo* and purified (SiO_2) ($CHCl_3$/MeOH) to afford **14** (0.12 g, 72%), as a diastereomeric mixture (ratio = 1:0.46). $\underline{R}_f$ = 0.6 (20% MeOH/$CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 7.58-7.50 (m, 6.4 H), 7.38-7.27 (m, 17.8 H), 7.24 (s, 1.6 H), 7.09-6.99 (m, 1.2 H), 6.78-6.76 (m, 0.6 H), 6.59 (br s, 1.4 H), 6.16 (br s, 0.5 H), 5.37-5.36 (m, 0.5 H), 5.05-4.94 (m, 2.8 H), 5.05-4.94 (m, 1.2 H), 4.85-4.79 (m, 4.3 H), 4.70-4.56 (m, 3.5 H), 4.54-4.51 (br. m, 3.9 H), 4.14-4.08 (m, 1.3 H), 3.80 (app. s, 4.9 H), 3.73-3.71 (m, 6.9 H), 3.52 (app d, *J*=2.4 Hz, 0.6 H), 3.23-3.14 (m, 4.0 H), 2.87 (br s, 1.5 H), 2.37 (app s, 10.3 H), 2.01-1.97 (m, 2.5 H), 1.85-1.71 (m, 12.0 H), 1.50 (br s, 3.7 H), 1.44 (s, 9 H), 1.27-1.23 (m, 9.8 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.5, 171.0, 155.3, 151.3, 143.8, 142.1, 139.1, 136.7, 130.6, 130.1, 130.0, 128.5, 128.4, 128.2, 128.0, 127.8, 126.8, 126.7, 126.4, 126.0, 95.4, 94.8, 93.9, 79.7, 77.32, 70.9, 70.4, 69.9, 64.5, 64.2, 60.1, 54.3, 54.0, 52.2, 46.5, 42.0, 32.8, 32.8, 32.1, 31.3, 28.8, 28.3, 27.9, 22.7, 21.5, 21.4. ESI-MS *m*/*z*: $[M+H]^+$ calcd. for $C_{58}H_{77}N_4O_{13}S_2$, 1101.4929; found 1101.4978.

15: A mixture of benzyl 4,6-di-*O*-acetyl-2,3-dideoxy-3-(*p*-tolylsulfinyl)-α-D-*erythro*-hex-3-enopyranoside (**1**) (38.5 mg, 0.084 mmol), Boc-Lys-Ala-Lys-OMe (**13**) (13 mg, 0.028 mmol) and $NaHCO_3$ (2 mg) was dissolved in aq. MeOH (40%) (5 mL, pH 7.5-8) and stirred at 40 ^oC for 16 h, solvents evaporated *in vacuo* and re-suspended in EtOAc/MeOH (1:1). Characterization was carried out using LCMS technique and the product was eluting at between 10.7 and 11.1 min. under method A (*vide infra*).

3. Mass, ^{1}H and ^{13}C NMR Spectra

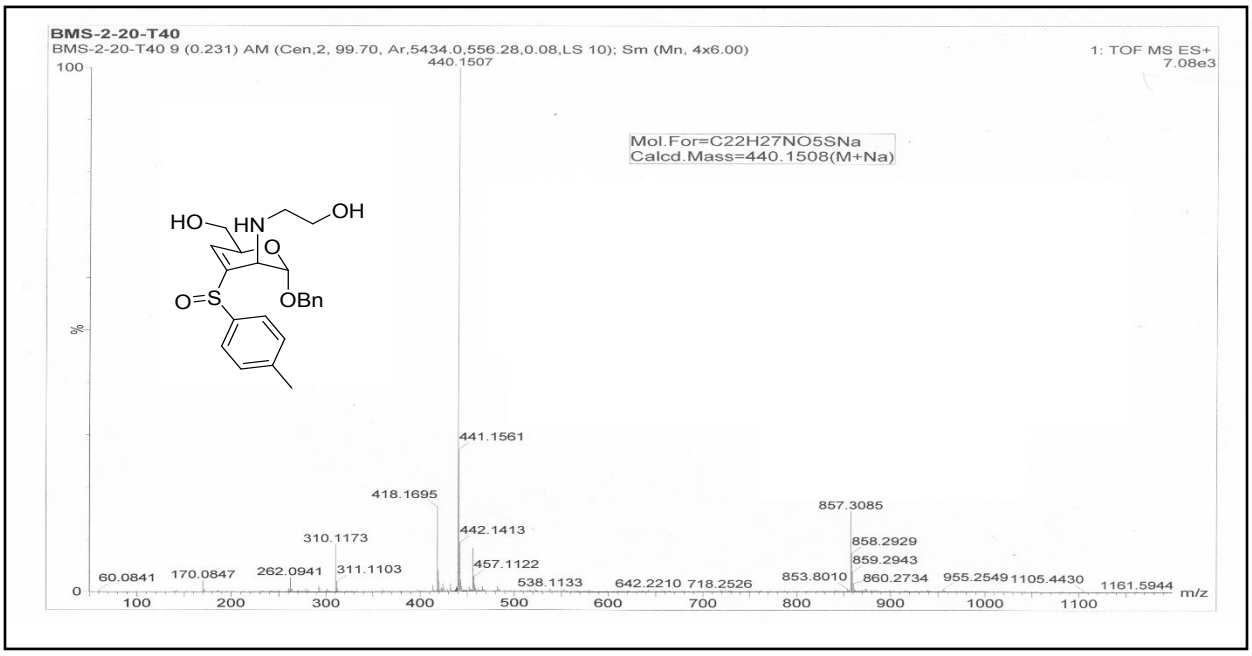

Figure S1. ESI-MS spectrum of **5**.

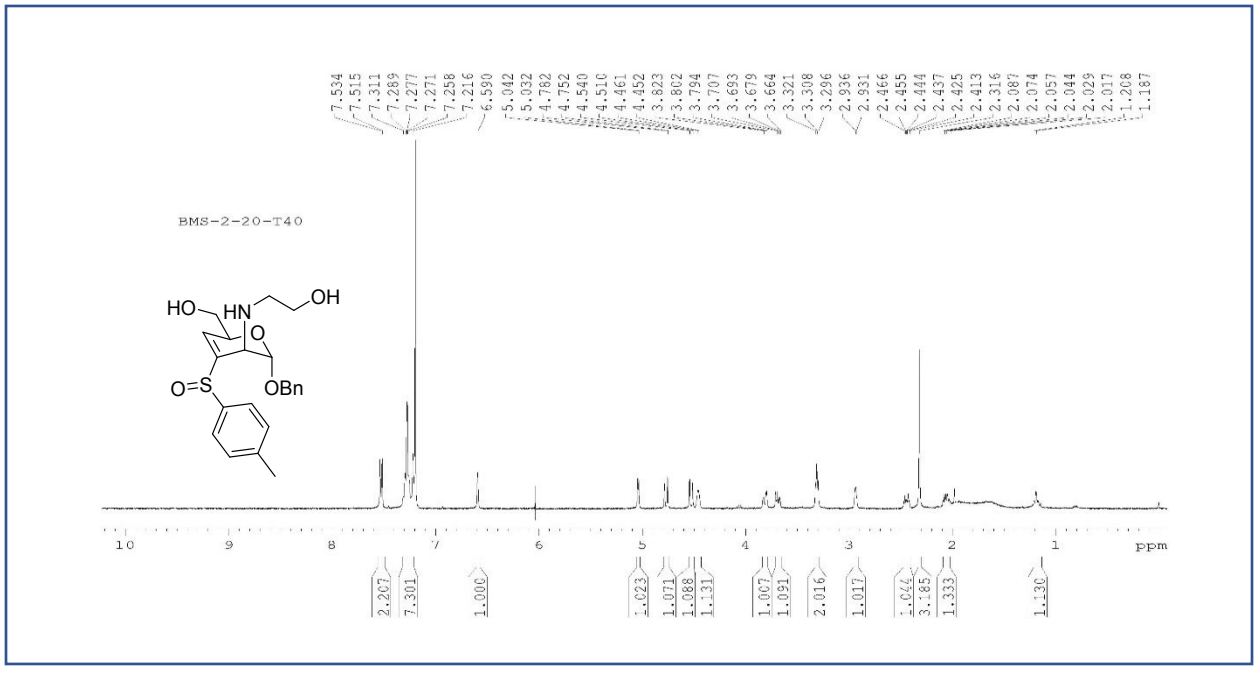

Figure S2. ^{1}H NMR spectrum of **5** ($CDCl_3$, 400 MHz).

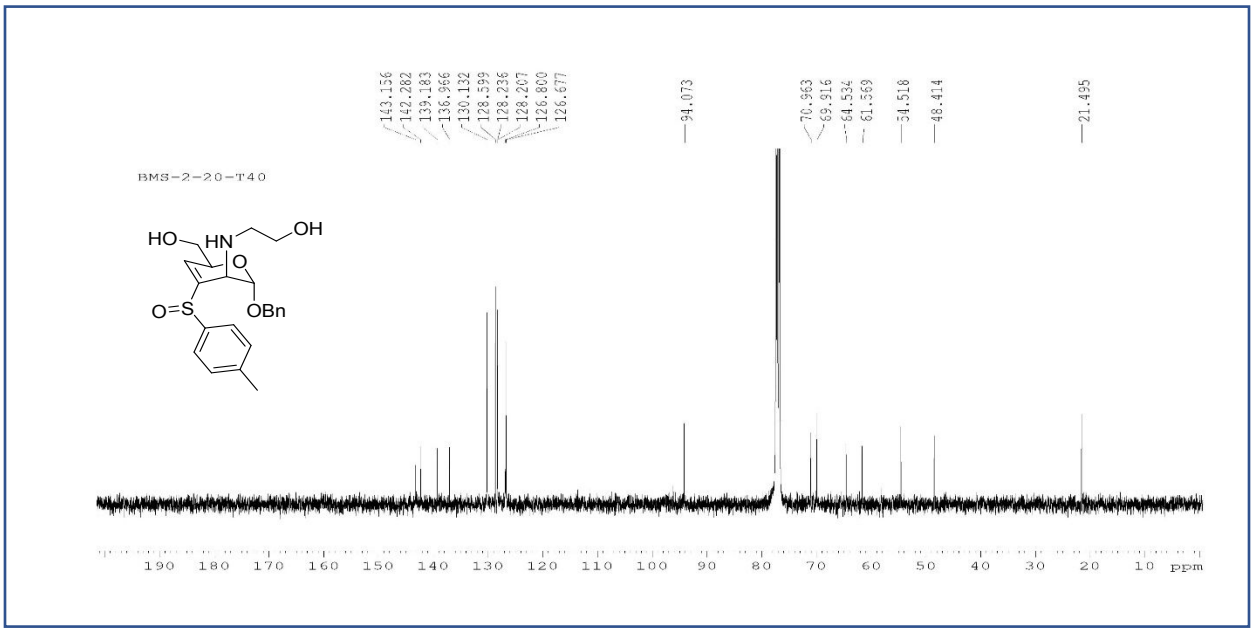

Figure S3. ^{13}C NMR spectrum of **5** ($CDCl_3$, 100 MHz).

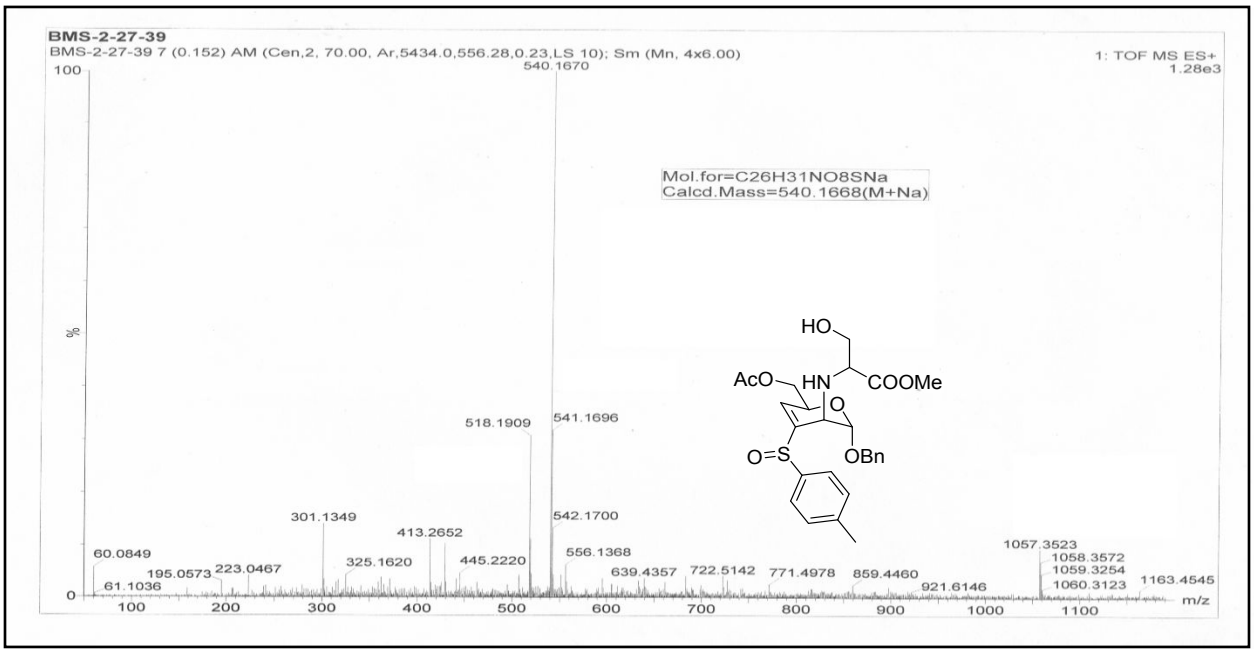

Figure S4. ESI-MS spectrum of **7**.

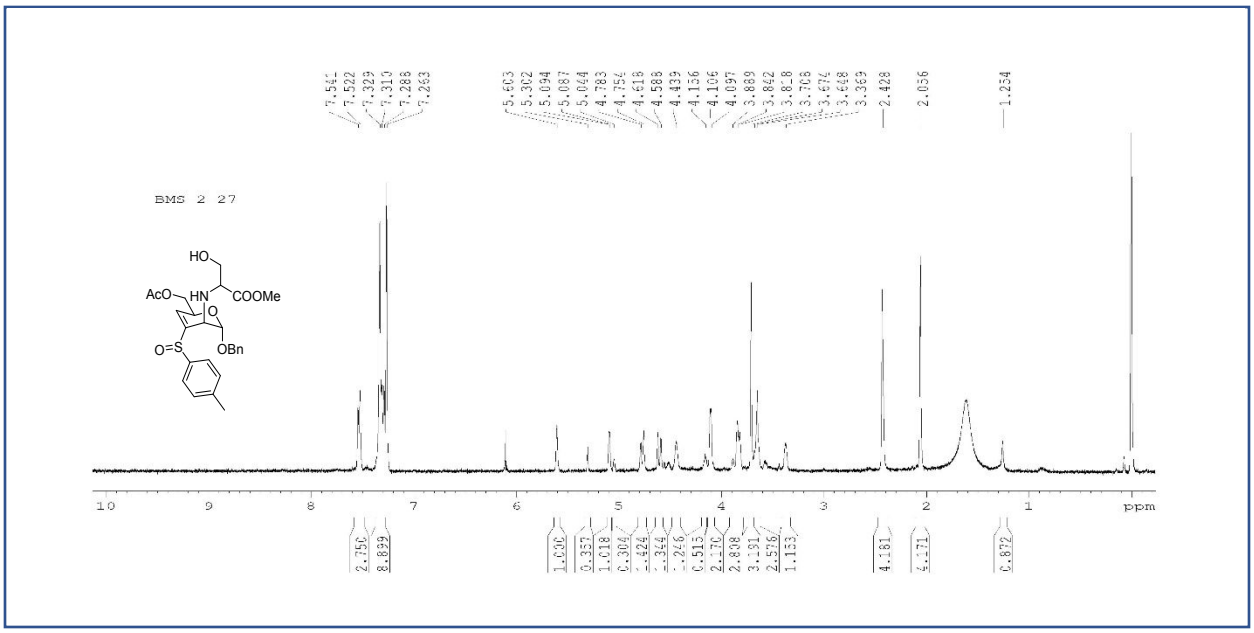

Figure S5. ^{1}H NMR spectrum of **7** ($CDCl_3$, 400 MHz).

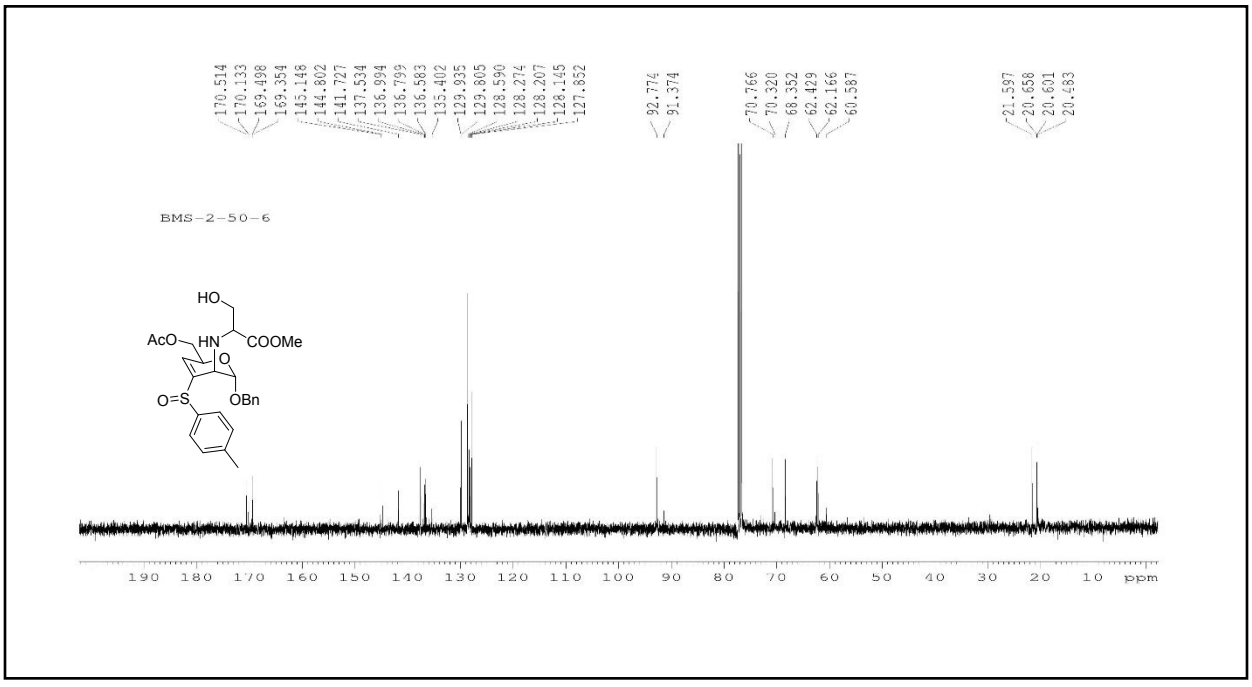

Figure S6. ^{13}C NMR spectrum of **7** ($CDCl_3$, 100 MHz).

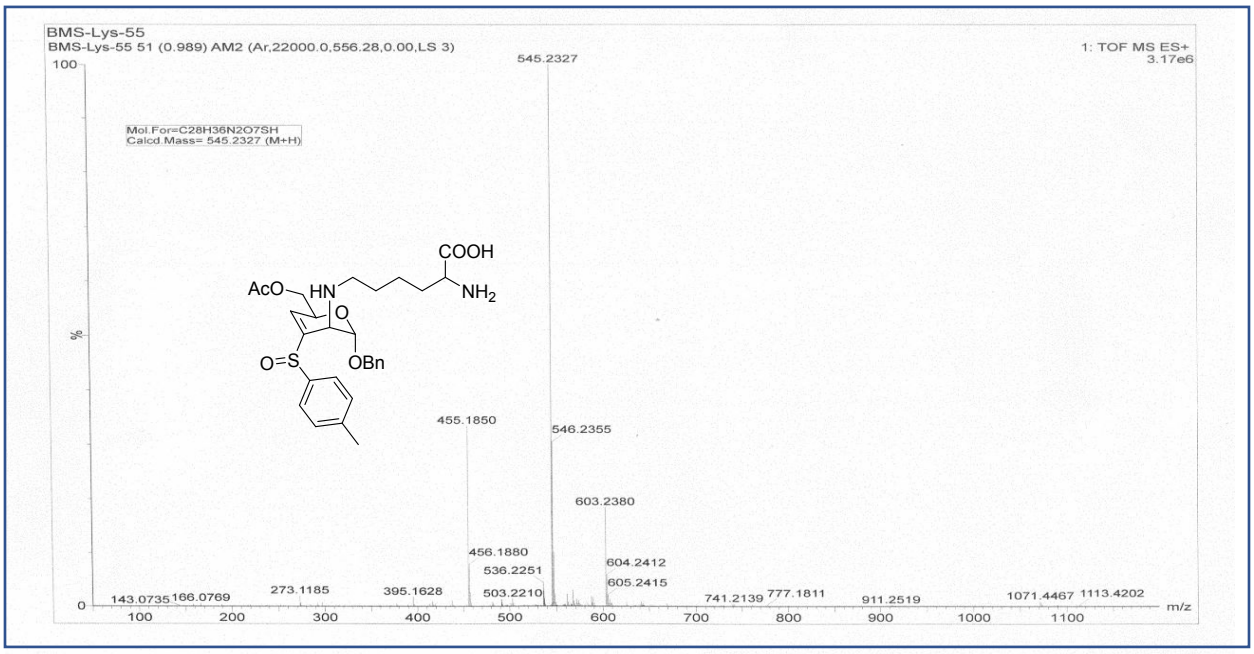

Figure S7. ESI-MS spectrum of **9**.

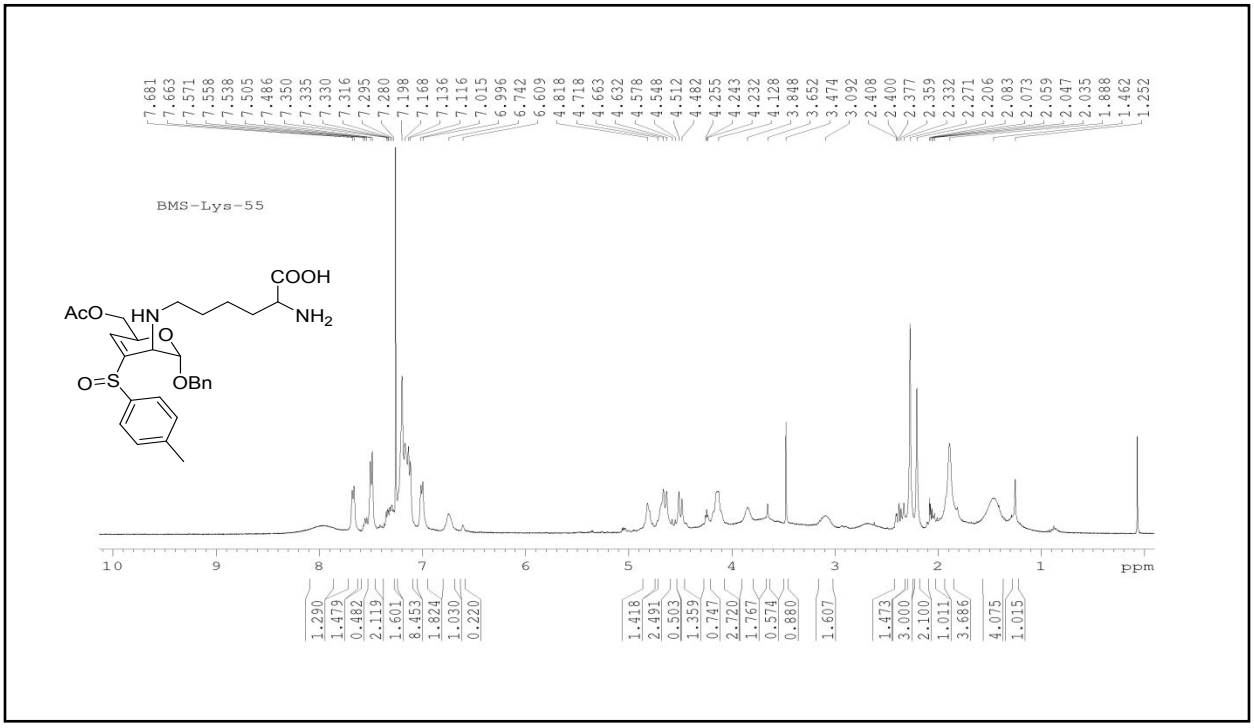

Figure S8. ^{1}H NMR spectrum of **9** ($CDCl_3$, 400 MHz).

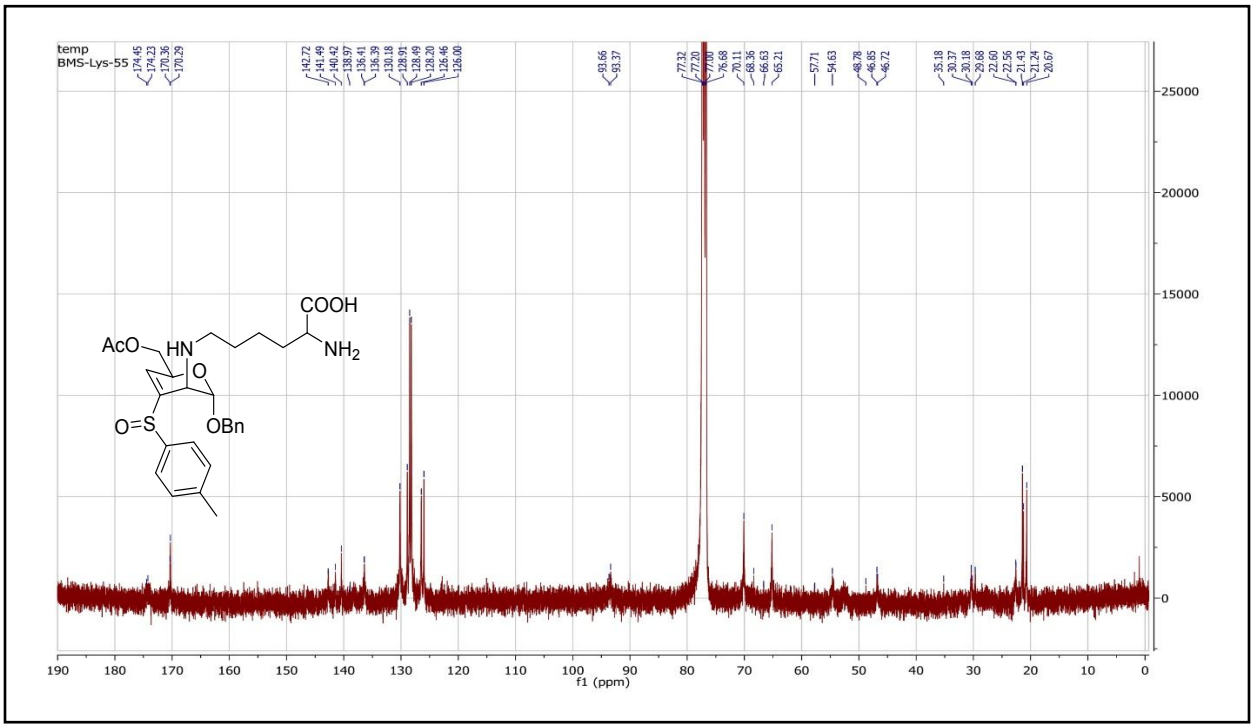

Figure S9. ^{13}C NMR spectrum of **9** ($CDCl_3$, 100 MHz).

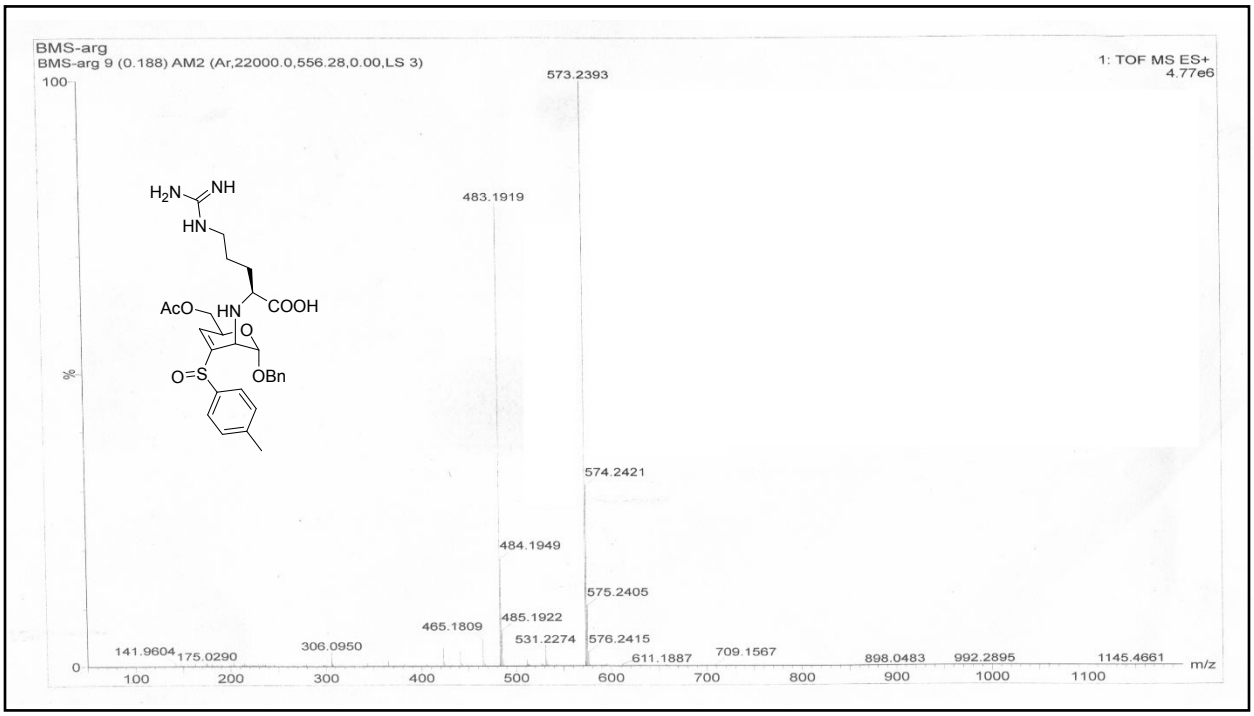

Figure S10. ESI-MS spectrum of **11**.

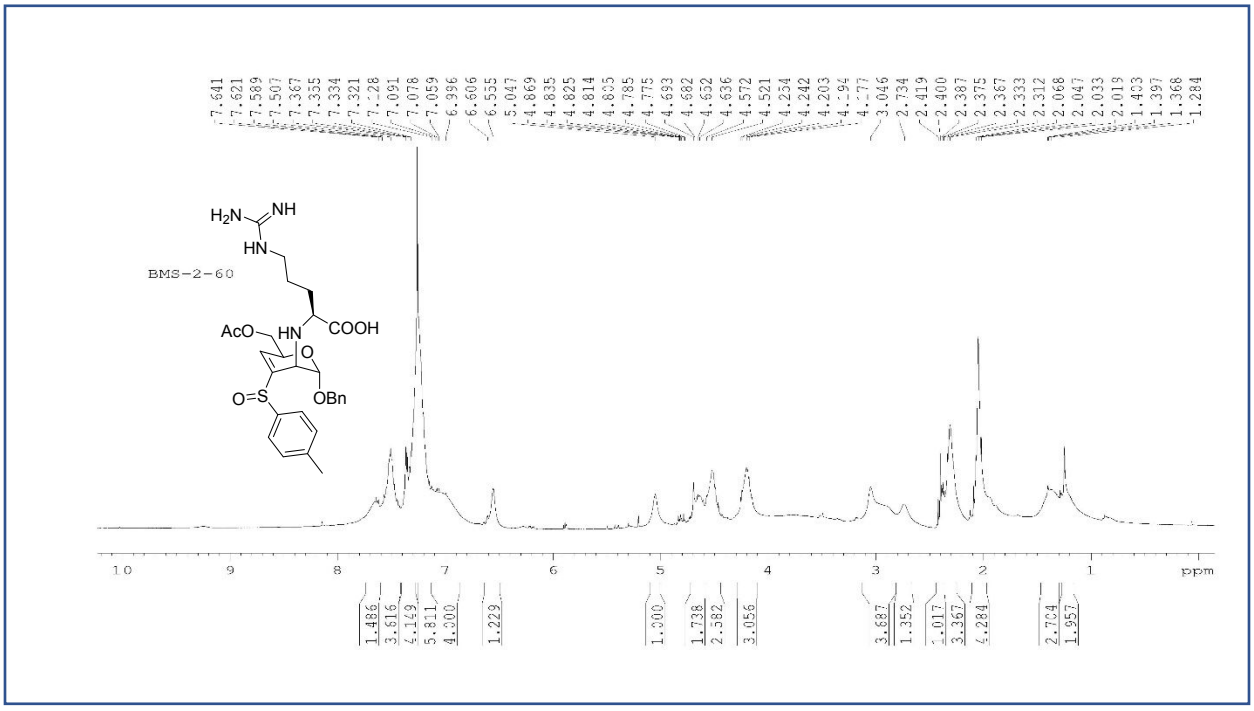

Figure S11. ^{1}H NMR spectrum of **11** ($CDCl_3$, 400 MHz).

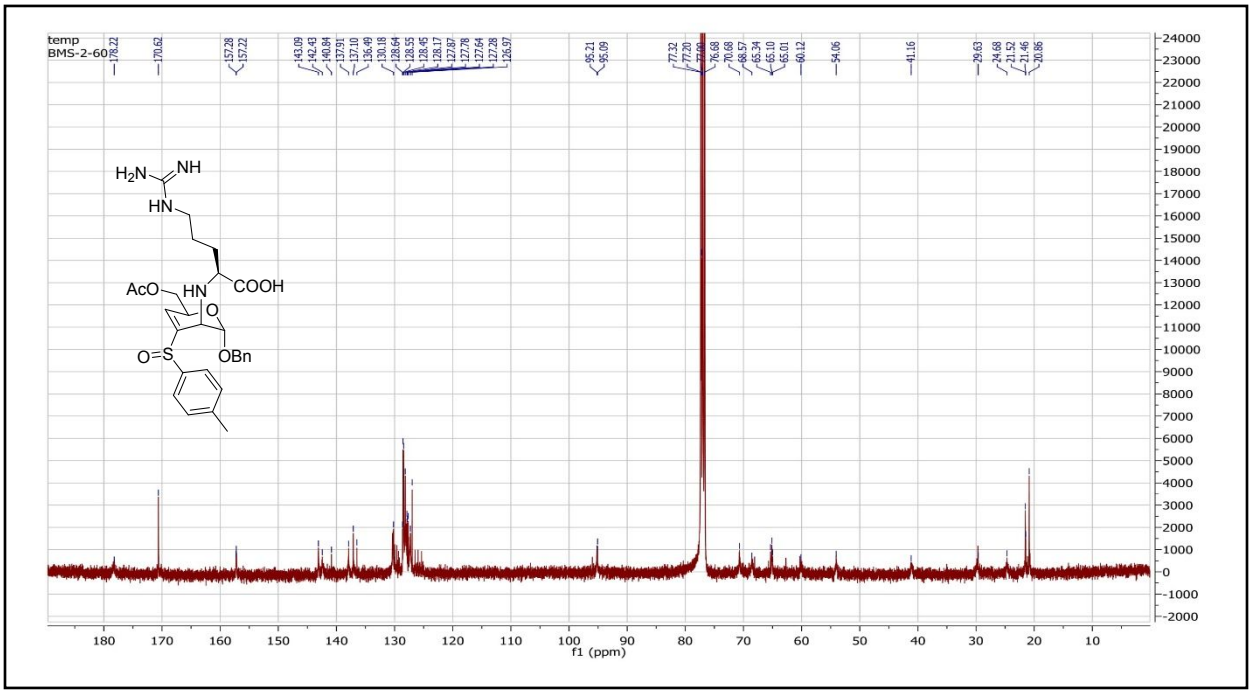

Figure S12. ^{13}C NMR spectrum of **11** ($CDCl_3$, 100 MHz).

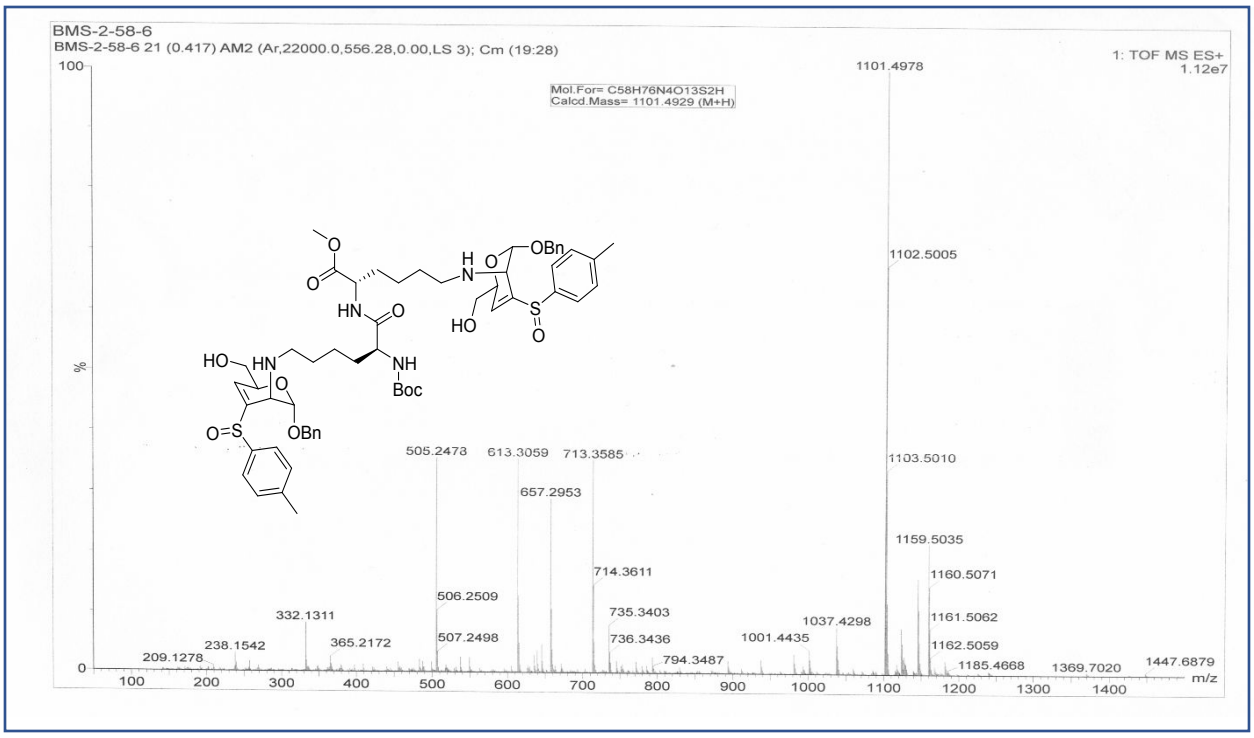

Figure S13. ESI-MS spectrum of **14**.

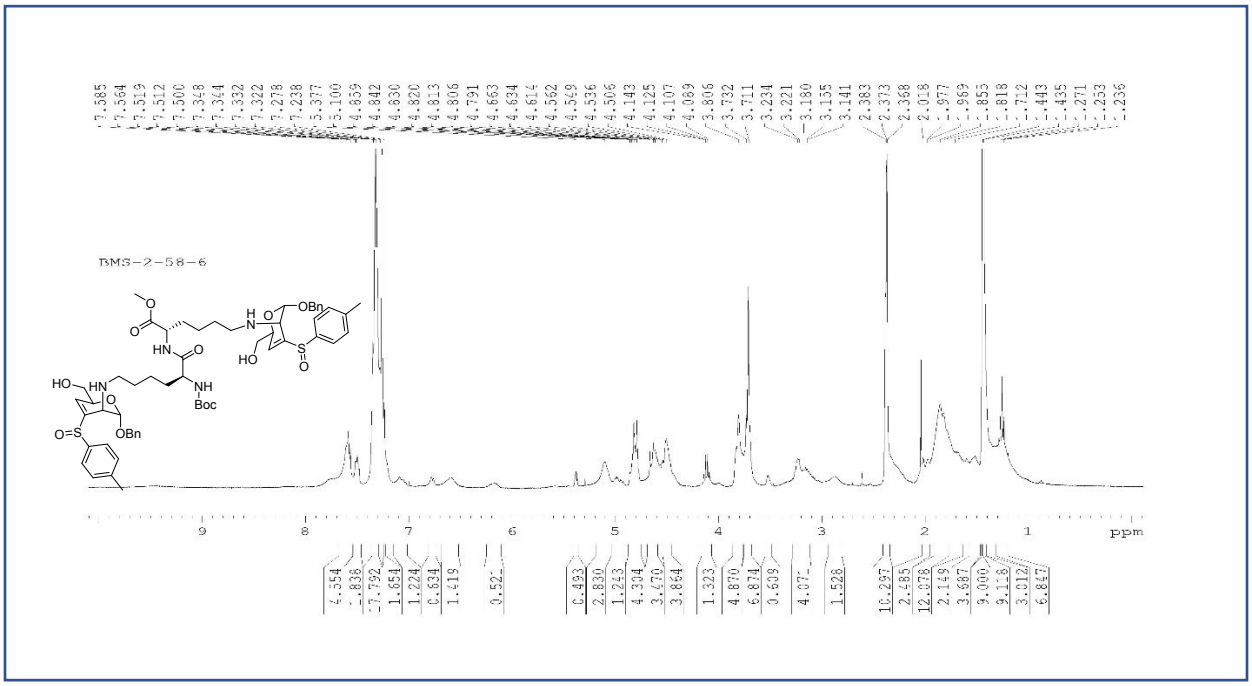

Figure S14. ^{1}H NMR spectrum of **14** ($CDCl_3$, 400 MHz).

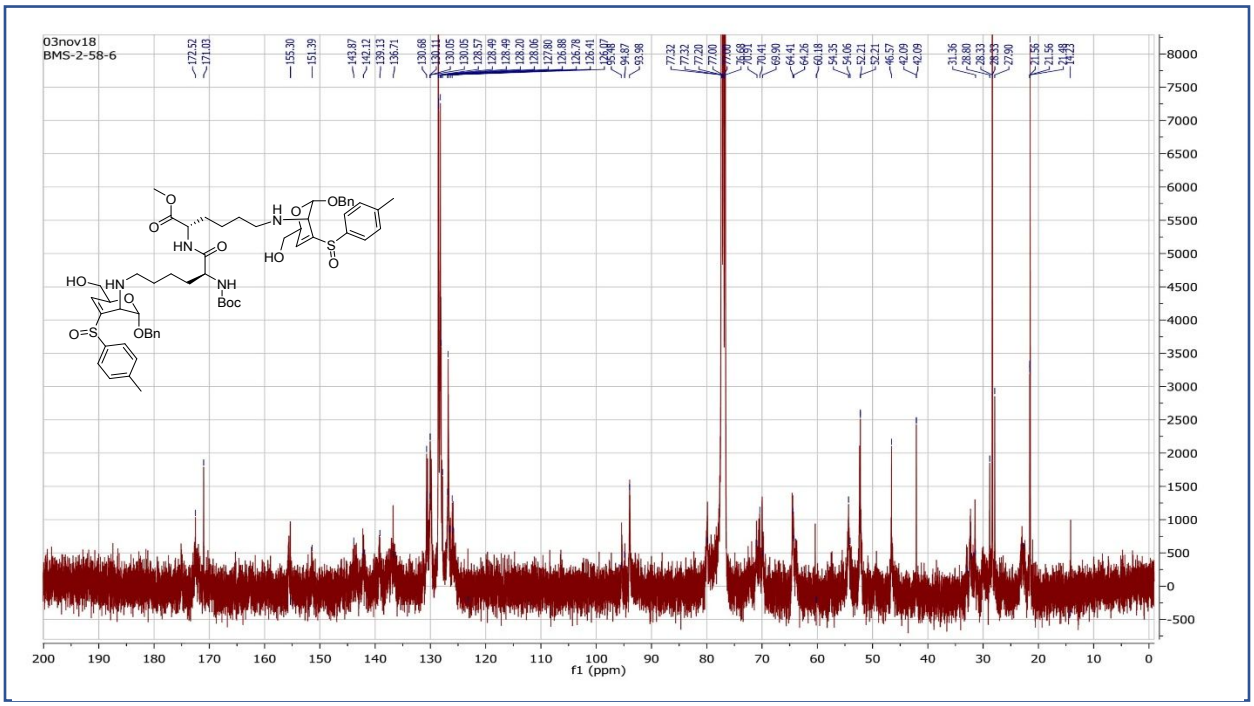

Figure S15. ^{13}C NMR spectrum of **14** ($CDCl_3$, 100 MHz).

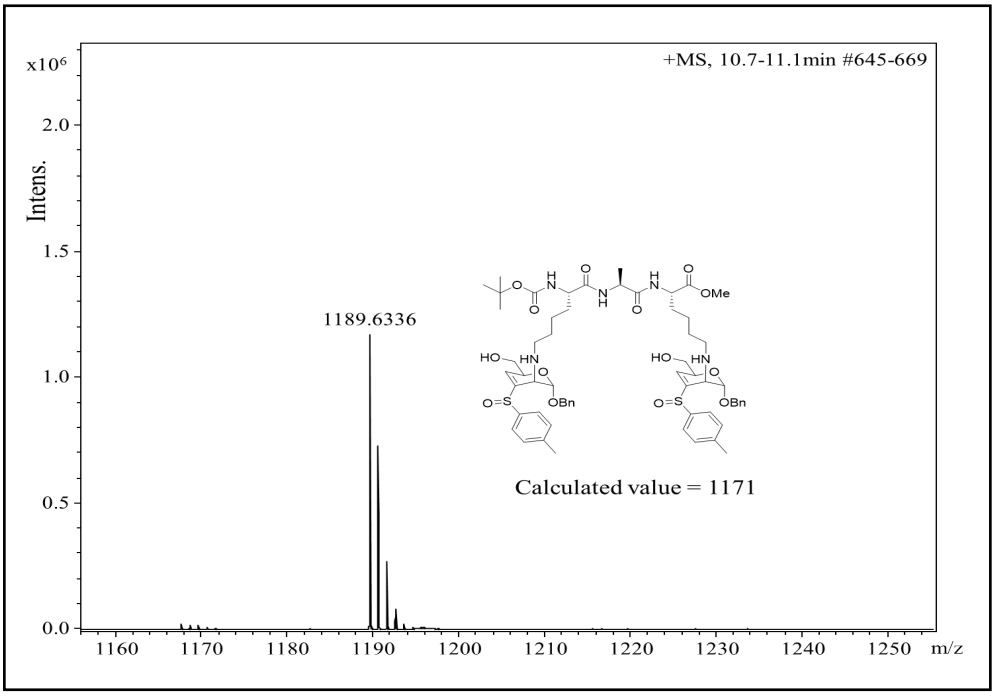

Figure S16. LCMS spectrum of **15**.

4. Protein Mass Spectrometry

The protein analyses were performed on HCT Ultra PTM Discovery LCMS System (ETD II-Bruker Daltonics), connected with 1100 series HPLC (Agilent). Agilent Poroshell 120 SB-C_{18} (4.6 x 250 mm), paticle size 2.7 μm column was used for LCMS. HRMS data were recorded on Bruker Daltonics MicroTOF-Q-II with electrospray ionization (ESI). MALDI-TOF/TOF mass spectrometry was performed with Bruker Daltonics UltrafleXtreme. Peptide mass and fragment were analyzed using Bruker Daltonics Sequence Editor 3.2 for peptide mapping and sequencing.

5. Methods and Solvents for LCMS

A mixture of acetonitrile and H_2O containing formic acid (0.01%) was used as the mobile phase in LCMS analysis. Following two methods of solvent gradient system were used in the analysis.

Method A

Column: Agilent Poroshell 120 SB-C_{18} (4.6 x 250 mm), particle size 2.7 μm, flow rate 0.2 ml/min.

Table S1. Method A for LCMS analysis.

Time	H_2O (%)	Acetonitrile (%)
0	95	5
5	95	5
20	5	95
25	5	95
30	95	5

Method B

Column: Agilent Poroshell 120 SB-C_{18} (4.6 x 250 mm), particle size 2.7 μm, flow rate 0.2 ml/min.

Table S2. Method B for LCMS analysis.

Time	H_2O (%)	Acetonitrile (%)
0	90	10
10	70	30
30	10	90
35	10	90
40	90	10

6. Modification of Lysozyme

A mixture of benzyl 4,6-di-*O*-acetyl-2,3-dideoxy-3-(*p*-tolylsulfinyl)-α-D-*erythro*-hex-3-enopyranoside (4 mg, 8.7 μmol), lysozyme (4.7 mg, 0.3 μmol) (PDB ID: 1DPX_A) and $NaHCO_3$ (1 mg) was dissolved in aq. MeOH (40%) (5 mL) and incubated at 37 ^oC for 24 h. The reaction mixture was taken for further analysis by MALDI-TOFMS, LCMS, SDS-PAGE and trypsin digestion analysis. In LCMS analysis, the modified lysozyme monomer eluted between 22.9 and 23.8 min. and dimer eluted between 19.2 and 19.9 minutes, under method B. Native lysozyme eluted between 5.2-5.6 minutes, under method B.

7. In-Gel Tryptic Digestion Protocol

In-gel tryptic digestions for lysozyme and modified lysozyme were carried out by the following protocol, with modifications.[1] Samples containing gel pieces were minced into small pieces and transferred into a sterile microcentrifuge tube, washed with wash solution aq. acetonitrile containing aq. ammonium bicarbonate (50 mM), incubated at room temperature for 15 min. with gentle agitation and dehydrated in acetonitrile for 5 min. Gel pieces were then rehydrated in reduction solution containing dithiothreitol (10 mM) in aq. ammonium bicarbonate (50 mM) for 30 min. at 56 ^{o}C. After discarding reduction solution, alkylation solution (iodoacetamide (50 mM) in aq. ammonium bicarbonate (50 mM)) was added to gel pieces, incubated for 30 min. in dark at room temperature, alkylation solution discarded, washed with wash solution and incubated again for 15 min. at room temperature with gentle agitation. The gel pieces was again dehydrated gel in acetonitrile for 5 min., dried and samples allowed for tryptic digestion (20 μg mL^{-1}) for 15 h at 37 ^{o}C. After digestion, samples were centrifuged at a speed of 12,000 rpm for 30 sec. Supernatant was collected, the gel pieces were dipped into the extraction solution (trifluoroacetic acid (0.1%) and acetonitrile (50%)), sonicated for 10 min., centrifuged, supernatants combined and evaporated by centrifugal evaporation. A re-suspension solution (5 μL) was added to each tube, sonicated and/or agitated gently on a vortex. The samples were subjected for MALDI-TOF analysis (alpha-cyano-4-hydroxycinnamic acid matrix). The unknown samples were calibrated using internal tryptic peaks of 842.5 and 2211.1 Da.

8. LCMS Data of Native Lysozyme

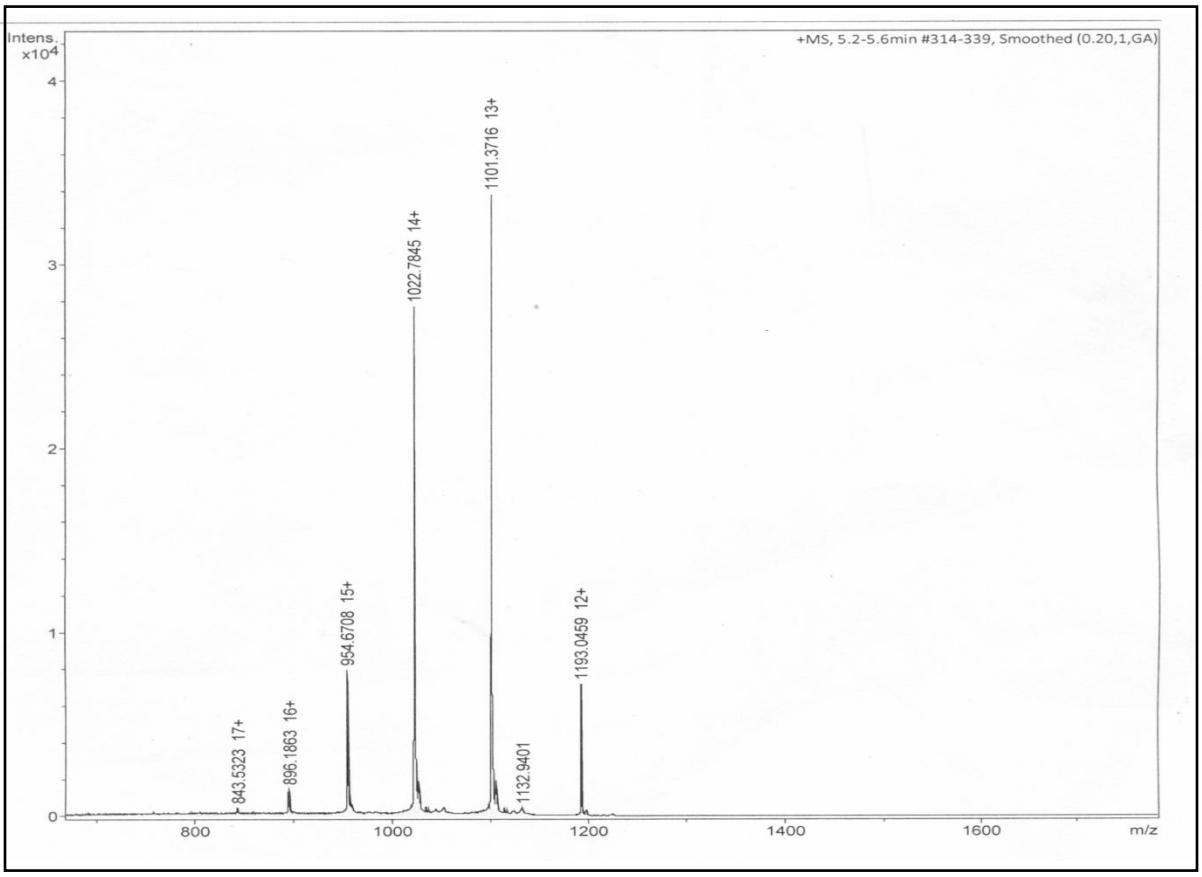

Figure S17. LCMS spectrum of native lysozyme.

Table S3. LCMS data of native lysozyme.

Component	Mass	Molecule	Abund. [%]
A	14304.7943	Mr	100.00
B	14322.1509	Mr	24.02
C	14362.4472	Mr	7.73

Abbreviation: Mr = molecular ion

9. LCMS Spectrum and Data of Modified Lysozyme

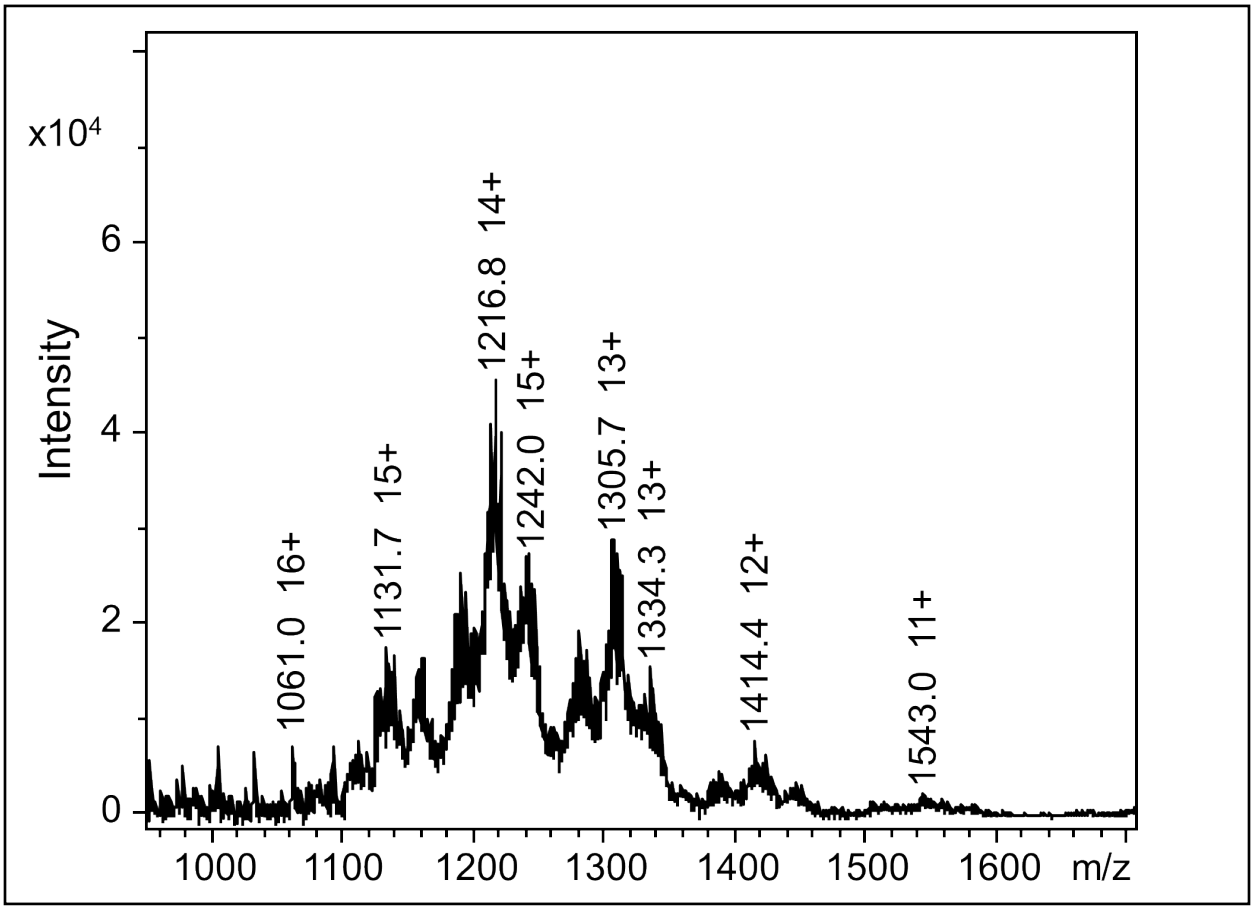

Figure S18. LCMS spectrum of modified Lysozyme.

Table S4. LCMS data of modified lysozyme.

Component	Mass	Molecule	Abund. [%]
A	17021.6	Mr	99
B	15788.8	Mr	69
C	16960.9	Mr	100
D	17063.1	Mr	88
E	15827.8	Mr	62
F	15733.1	Mr	71
G	16918.0	Mr	68
H	18615.7	Mr	51
I	15693.2	Mr	61
J	17333.2	Mr	60

Abbreviation: Mr = Molecular ion

10. LCMS Spectrum and Data of Native Lysozyme after Trypsin Digestion

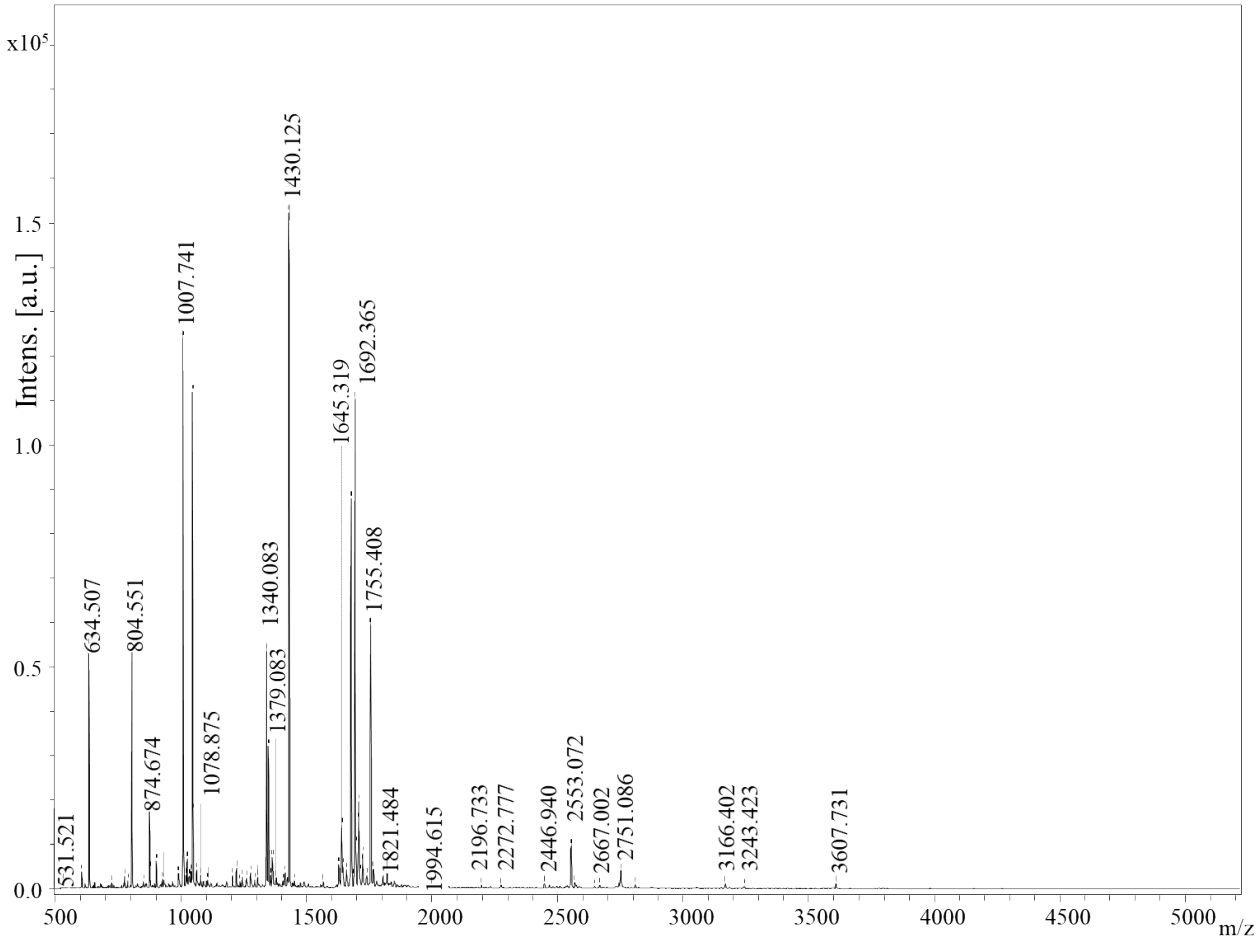

Figure S19. LCMS spectrum of native lysozyme after trypsin digestion.

Table S5. LCMS data of native lysozyme after trypsin digestion.

m/z	S/N	Quality Fac.	Res.	Intens.	Area
531.521	12	3254	2479	1678	546
550.813	10	8402	3316	1408	380
606.477	28	31071	2873	3996	1447
634.507	371	340589	3395	54389	17116
636.504	12	3911	2933	1798	685
724.571	9	826	3954	1434	457
776.509	18	9000	3991	2775	980
788.658	11	3211	2652	1792	936

804.551	354	80737	3791	56173	21857
806.561	41	45047	3749	6473	2574
851.587	9	2438	4162	1414	538
874.674	117	46092	4311	19547	7695
879.615	10	3696	4149	1587	660
902.72	38	26166	3484	6356	3263
904.669	9	1770	2824	1518	1005
925.659	11	7462	1695	1849	2025
932.708	12	963	4772	2044	793
988.829	11	878	5074	1969	763
989.778	14	2759	3599	2482	1341
990.689	14	172	5703	2395	795
992.699	10	488	4385	1695	759
1005.717	16	138	4057	2794	1499
1007.741	648	464201	3583	113843	68121
1008.755	361	185935	3742	63415	33583
1009.737	65	36236	3931	11344	6244
1011.746	22	4669	3885	3923	2143
1019.753	17	2996	4444	2974	1399
1023.755	29	37225	4304	5224	2603
1024.747	21	3609	4354	3759	1800
1030.793	13	1849	3379	2363	1487
1033.82	12	1677	3285	2124	1480
1039.713	15	3274	4305	2732	1490
1040.716	8	307	4489	1476	754
1045.9	542	114293	3235	97031	69667
1046.94	333	5097	3715	59671	32408
1049.892	21	3192	2403	3805	3667
1061.912	15	6225	4402	2743	1382
1062.909	14	1225	4368	2483	1228
1077.887	10	625	4803	1799	843
1078.875	10	2023	4010	1889	1113
1102.784	7	1593	3439	1379	1058
1106.884	9	1715	3570	1623	1169
1204.97	15	1744	4772	3007	1801
1220.966	22	6620	4568	4275	2709
1235.005	7	1007	4419	1460	1006
1244.055	11	2432	3436	2246	2031
1262.012	11	945	4775	2226	1432
1278.023	17	5273	4833	3333	2164
1294.044	7	2547	3835	1494	1275
1305.59	10	1999	2120	2109	3512
1340.083	189	60410	4799	38421	27690

1341.102	133	2909	5915	27068	14103
1342.087	25	1913	4923	4978	3350
1348.145	87	8128	6429	17594	9288
1349.138	111	34437	4947	22565	15658
1356.096	13	2831	4878	2579	1868
1362.059	15	1198	4221	3084	2644
1364.12	27	6471	4144	5515	4807
1366.157	14	244	7528	2844	1240
1367.126	12	2757	4656	2392	1852
1379.083	10	1865	4516	2094	1666
1406.14	7	3863	4037	1375	1359
1412.078	12	940	5181	2415	1771
1414.069	13	943	3130	2574	3142
1428.082	210	407	4011	42016	45209
1430.125	750	122053	3809	149836	153712
1452.084	8	7215	4906	1521	1244
1566.17	7	7148	4525	1362	1425
1628.315	23	14742	4813	4324	4700
1630.321	9	554	5384	1696	1417
1639.239	56	4126	3331	10175	16733
1640.334	34	460	13297	6284	2286
1643.315	11	752	7535	1956	1294
1645.319	23	60155	5419	4291	3996
1659.302	22	19461	4983	3945	4244
1675.353	16	9.26	5821	2813	2310
1676.329	407	33251	4658	73673	87294
1678.352	134	24345	5045	24325	23574
1688.314	16	16.7	5109	2866	3272
1692.365	505	23013	4190	90691	122576
1694.4	198	2915	5413	35522	31468
1698.346	11	5786	4062	1910	2663
1704.399	16	440	5397	2811	2748
1706.378	27	1394	5292	4870	4872
1708.378	92	28378	4924	16280	18233
1710.404	30	448	10439	5245	2548
1714.344	8	2601	5455	1341	1326
1720.393	9	431	6042	1660	1476
1722.384	10	318	5722	1691	1568
1724.38	38	13733	5322	6785	7057
1739.335	9	586	2353	1340	3455
1753.398	118	766	9555	20625	12923
1755.408	290	366028	4432	50763	65538
1766.374	20	11368	4740	3412	4472

1805.499	10	7179	4229	1628	2346
1821.484	23	185489	4991	3811	4870
1984.571	1		4790	324	129
2063.618	2		4775	456	196
2196.733	3		6072	766	317
2279.772	1		4965	272	139
2446.94	4		4645	892	494
2553.072	95	2748	3778	6141	14236
2567.925	17	3244	5050	978	2103
2662.051	2		7353	246	88.2
2751.086	55	8351	4868	2484	6035
3166.402	9		7243	808	471
3238.415	1		10940	97.5	48.3
3607.731	17	9448	909	604	

Abbreviation: m/z = molecular ion / charge; S/N = signal / noise; Quality Fac. = quality factor; Res. = resolution; Intens. = intensity

11. LCMS Spectrum and Data of Modified Lysozyme after Trypsin Digestion

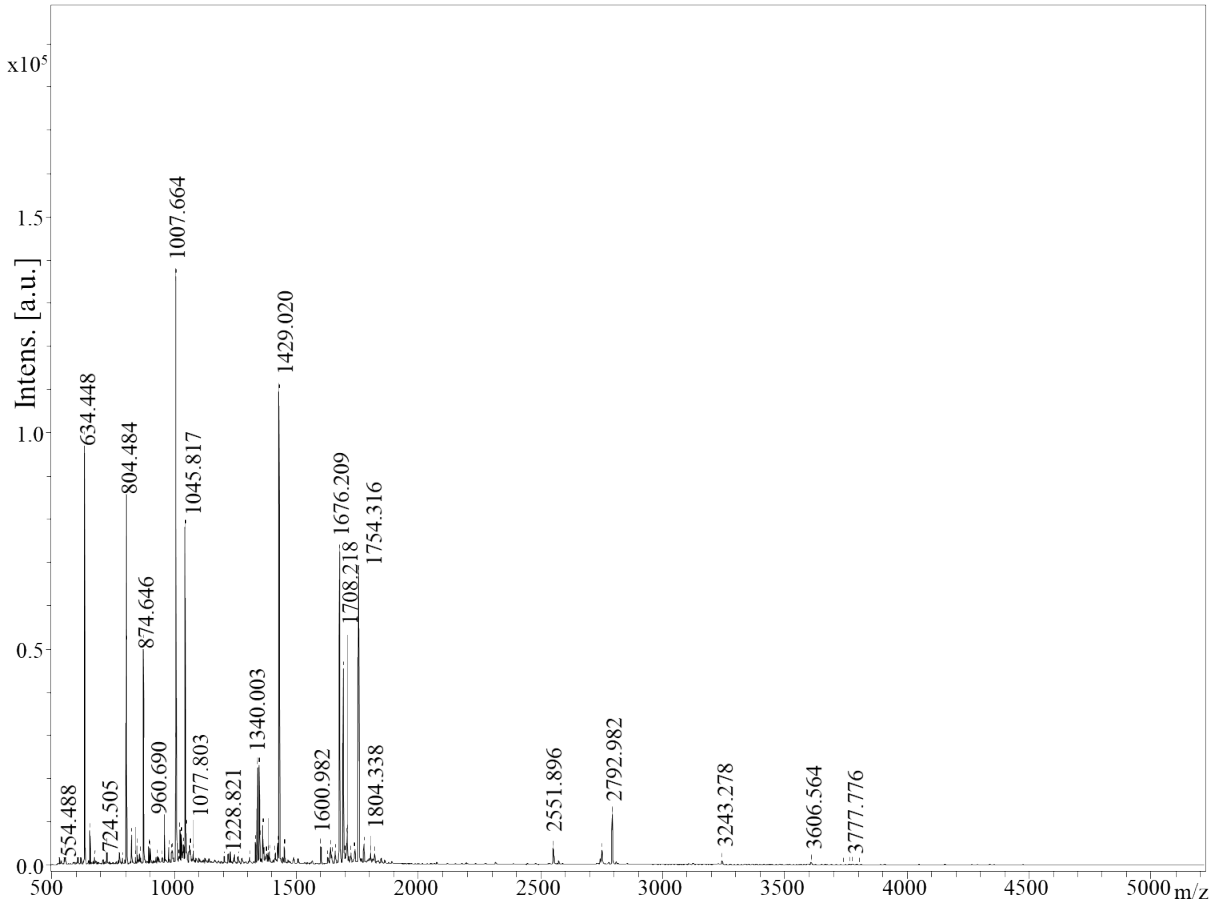

Figure S20. LCMS spectrum of modified lysozyme after trypsin digestion.

Table S6. LCMS data of modified lysozyme after trypsin digestion.

m/z	S/N	Quality Fac.	Res.	Intens.	Area
531.471	10	2961	3513	1759	401
550.793	17	6037	5121	3051	500
554.488	18	9559	4319	3247	636
556.466	14	4865	4621	2396	430
606.506	48	10975	2409	8677	3276
618.433	14	11709	4098	2486	611
634.448	539	139177	4349	98860	23777
635.47	108	14711	5338	19757	3591
636.458	11	1977	3565	1948	596

656.426	41	16325	4463	7651	1915
672.403	9	5613	4846	1695	408
724.505	23	14164	5279	4401	1012
726.484	15	7317	5424	2856	639
776.445	22	10966	5391	4203	1071
788.513	9	6544	3223	1758	821
804.484	447	80199	5005	88712	25730
806.496	39	4142	5377	7660	2072
826.47	36	6078	5322	7203	2074
842.423	8	2119	4980	1705	581
848.445	23	6785	5806	4604	1245
859.619	13	1536	4793	2598	844
874.646	250	232425	4378	51728	19958
879.575	8	1945	4779	1562	534
896.641	21	27437	5909	4436	1214
902.645	20	3250	4278	4108	1747
932.654	9	777	5942	1992	580
951.59	8	992	5624	1739	625
960.69	56	19423	6096	12024	3770
978.724	17	41105	5699	3758	1282
989.66	11	5129	5108	2361	948
990.635	10	314	6754	2118	580
992.647	10	1469	6015	2260	725
1005.64	10	74.9	5194	2071	878
1007.66	619	319866	4764	134428	60600
1008.68	248	69056	4975	54010	22437
1009.71	55	15342	6450	12014	3789
1011.68	18	7595	4798	3861	1696
1019.69	9	277	5340	1979	724
1023.69	36	47904	5943	7831	2718
1029.68	32	6652	5071	7004	2995
1030.81	19	964	6024	4078	1348
1033.75	8	405	5276	1799	688
1039.68	21	5700	5749	4520	1690
1045.82	351	195642	5030	77087	34402
1046.81	128	51622	4340	28098	14764
1051.72	12	1307	5234	2680	1112
1061.83	13	1094	5514	2956	1146
1063.86	20	1930	5043	4326	1807
1067.79	16	2853	5858	3439	1234
1077.8	14	4049	5071	3104	1387

1079.79	9	4949	5291	2099	936
1204.87	9	1905	5924	2052	971
1220.8	11	8980	5076	2525	1491
1228.82	22	22090	5525	4837	2646
1246.86	10	22092	5380	2197	1197
1261.91	9	1482	5930	1965	984
1308.82	9	3270	5412	1903	1159
1332.87	25	17087	6426	5407	2834
1340	105	16599	6165	22729	12255
1341.99	24	11084	6238	5226	2747
1348.04	83	46126	5795	17954	10688
1349.03	47	1590	7131	10235	4465
1350.03	10	1633	5647	2187	1329
1352.02	8	942	6317	1654	817
1361.99	42	11227	6764	9114	4484
1364	18	7936	5573	3984	2521
1367.06	18	5128	5873	3880	2258
1374.89	8	4159	5619	1788	1184
1377.97	12	5187	6049	2577	1480
1384.97	13	11013	5967	2774	1689
1390.05	13	3273	5408	2695	1881
1413.98	10	2021	4459	2166	1920
1424.09	22	6616	5893	4662	3052
1429.02	468	18475	4920	97919	80929
1431.04	195	40367	5349	40704	28829
1451	12	4913	9025	2550	1094
1451.99	13	12437	6124	2646	1715
1600.98	25	45691	6412	4427	3360
1628.19	9	2842	5269	1623	1612
1640.21	18	19783	3174	3010	4668
1645.19	11	8962	7649	1809	1220
1659.18	17	27549	6628	2886	2233
1676.21	399	41994	6230	65671	56140
1678.19	103	27361	6089	16927	14042
1692.21	246	37279	6210	39900	34831
1694.21	66	30057	6180	10690	8991
1698.17	12	6840	6132	1987	1790
1706.2	21	3921	6865	3333	2598
1708.22	41	27289	6246	6499	5656
1724.21	15	16229	6713	2338	1915
1736.24	11	2768	5955	1753	1731

1739.24	14	11217	4324	2202	3012
1754.32	379	45855	5130	58977	69274
1756.33	139	27603	5382	21537	21172
1776.29	26	13286	6673	4032	3479
1804.34	11	3224	4682	1675	2208
1821.31	11	16381	5831	1626	1808
2551.9	57	154556	6503	2731	4119
2750.96	53	7723	4980	2015	3972
2792.98	212	274893	5398	7394	15480
3243.28	16		7944	1003	579
3606.56	8		7344	395	251
3738.33	1		14461	48.4	18.8
3767.42	1		10545	56.6	19.5
3777.78	4		9229	195	119
3805.63	1		12460	48.7	15.2

Abbreviation: m/z = molecular ion / charge; S/N = signal / noise; Quality Fac. = quality factor; Res. = resolution; Intens. = intensity

12. Tryptic Digestion Pattern of Native and Modified Lysozymes

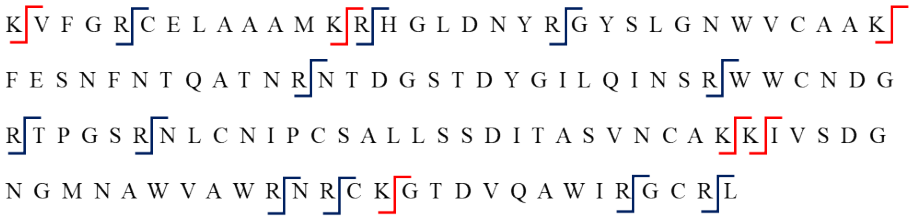

Figure S21. Summary of the peptide bond cleavages as a result of trypsin digestion on lysozyme. Red and blue brackets correspond to the lysine and arginine cleavage sites, respectively.

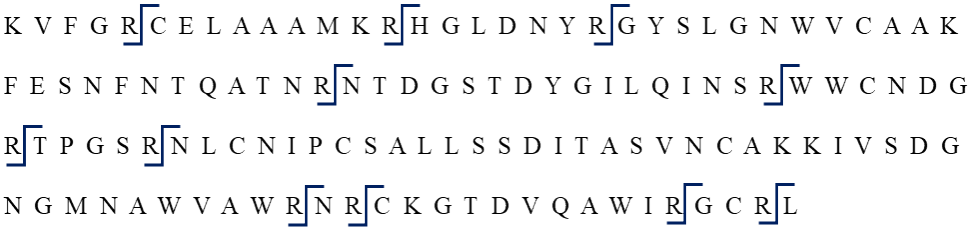

Figure S22. Summary of the trypsin digestion on lysozyme after glycoconjugation. Brackets refer to the cleavage sites.

13. Assay of Lysozyme Lytic Activity

The lytic activity of lysozyme was determined according to the turbidometric method by using *E. coli* cells. Bacterial cells were harvested (7000 rpm for 10 min.) and re-suspended in Tris-Cl buffer (pH 7.5) at the OD_{600} of 0.8. Lysozyme and modified lysozyme solution were added to the bacterial cell at the concentration of 60 μg mL^{-1}. Change in the OD was monitored at 37 °C and recorded using a spectrophotometer up to 6 hours.

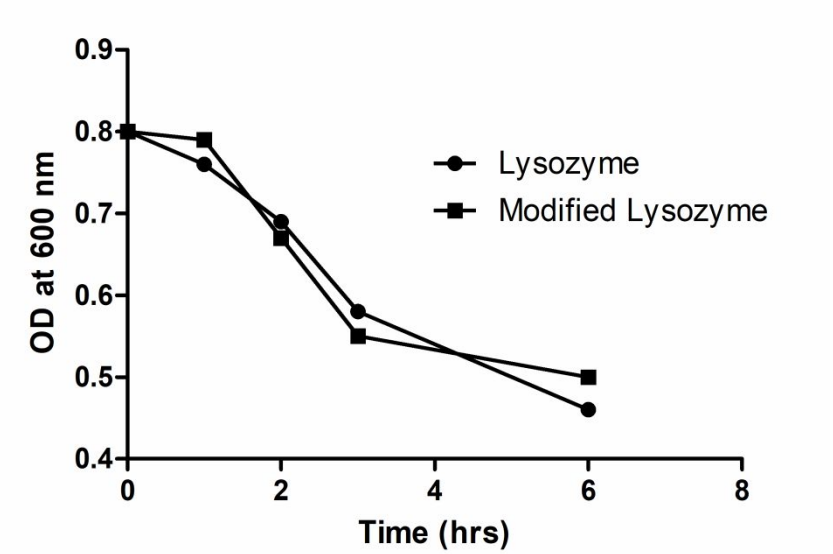

Figure S23. Plot of the lytic activity of native and modified lysozymes on *E. coli*.

Reference

1. Sambrook, J.; Fritsch, E. F.; Maniatis, T. (2001) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press.