Fig S1

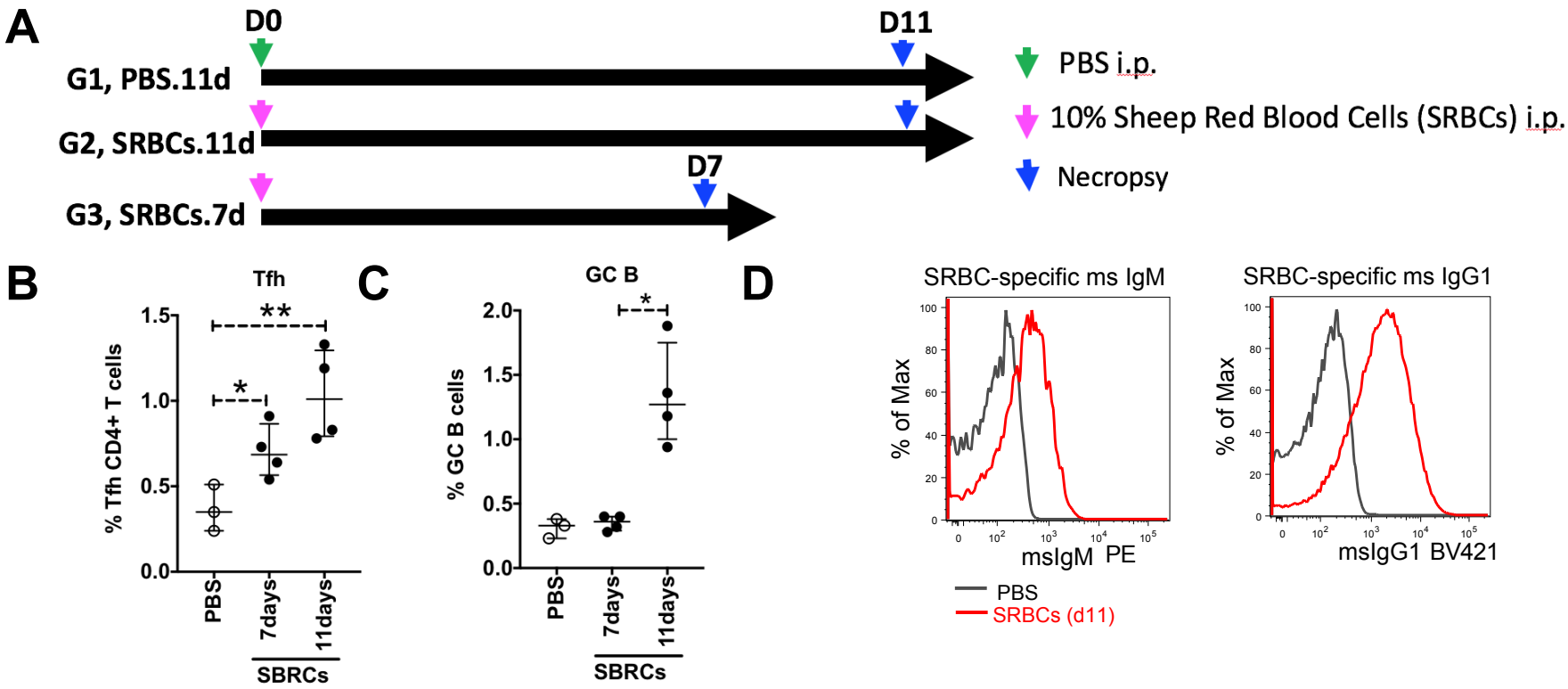


Figure S1. Sheep red blood cell vaccinated T cell-dependent immune response model. **(A)** Study design analyze germinal center reaction and antibody response against SRBC vaccination in mice. Control mice received 100uL PBS i.p. and proceeded with necropsy at 11 days later; experimental mice were vaccinated with 100uL 10% SRBC and necropsied at 11 days or 7 days later. Spleen samples were harvested for single cell isolation and analyzing Tfh and GC B cells with flow cytometry method; blood specimen were collected for analyzing the titer of antibody (IgG1 and IgM) against SRBC. **(B-C)** Data obtained with flow cytometry analysis showed the frequency of $PD1^{hi}CXCR5^{+}$ Tfh $CD4^{+}$ T cells **(B)** and $B220^{+}GL7^{+}CD95^{+}$ activated B cell **(C)** were highest in the spleen of mice vaccinated with SRBC for 11days. Welch-test was performed to compare the results between two groups. * $p<0.05$; ** $p<0.01$. **(D)** Representative histogram showing the mean fluorescent intensity (MFI) of SRBC incubated with plasma from PBS-treated and SRBC-vaccinated mice (at 11 days later) following stained with PE-conjugated anti-mouse IgM or BV421-conjugated anti-mouse IgG1.

Fig S2

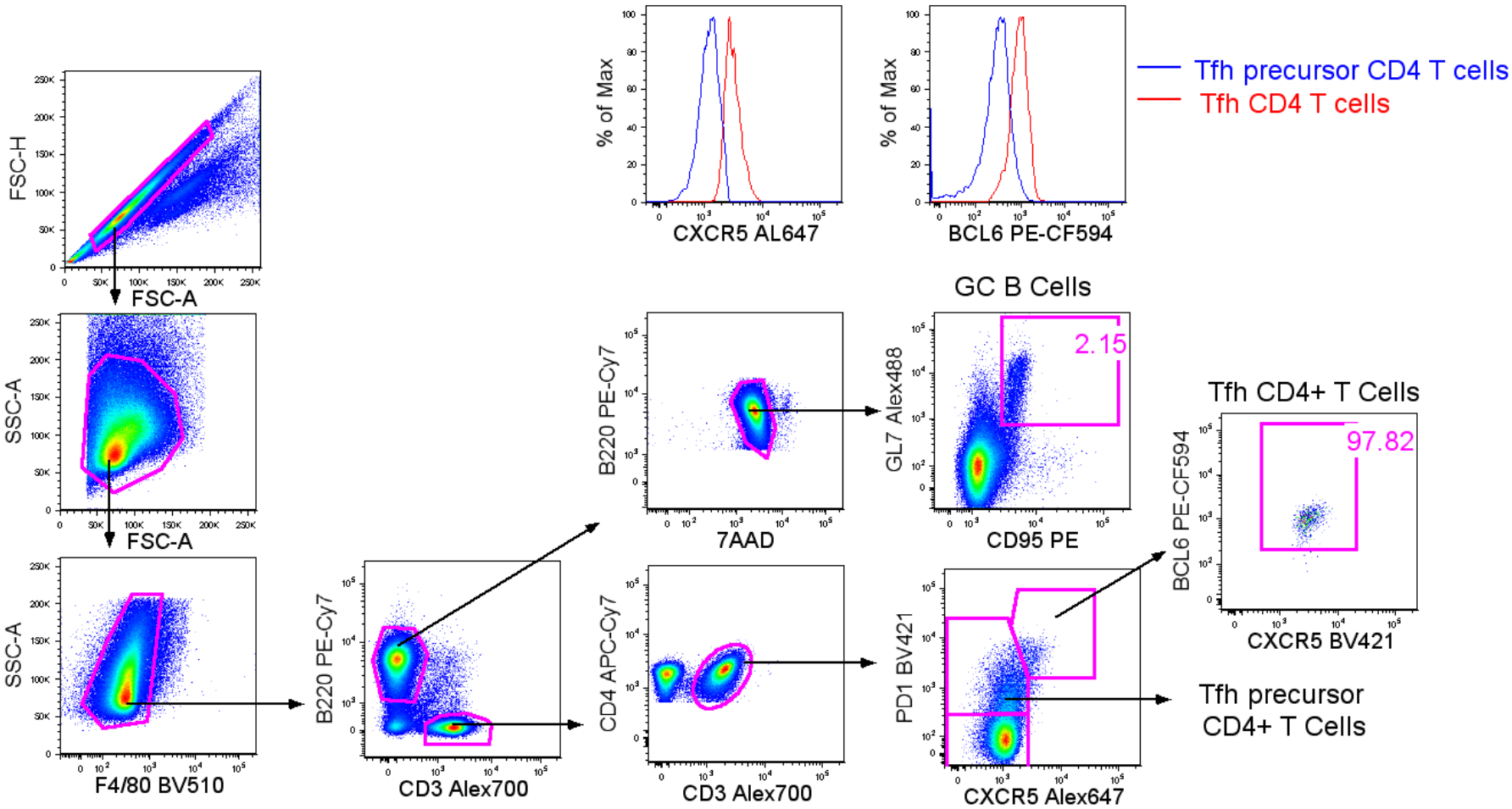


Figure S2. Flow gating strategy for analyzing CD4$^+$ Tfh, Tfh precursor & GC B cells.

Fig. S3

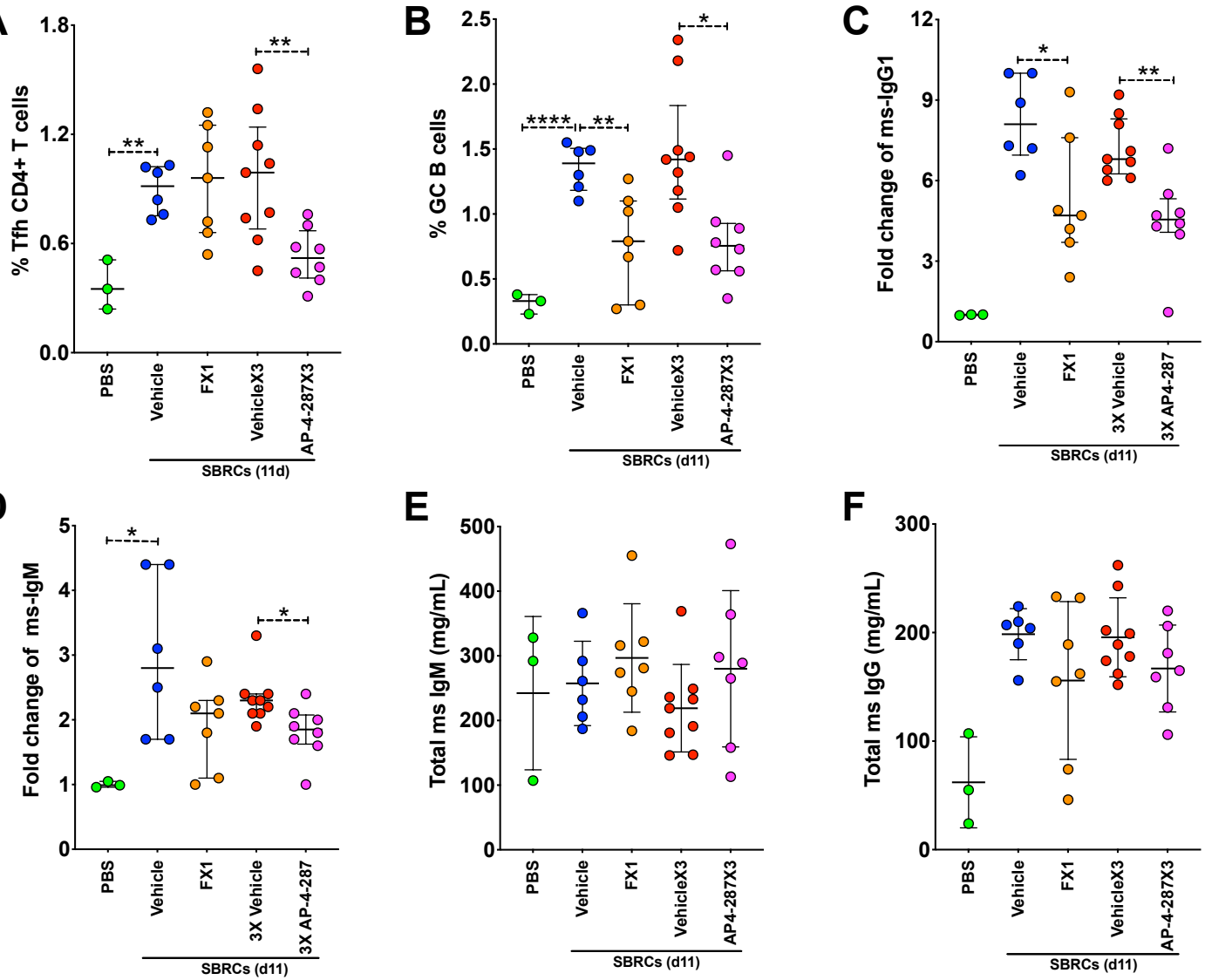


Figure S3. Comparison GC reaction and antibody responses in SRBC-vaccinated mice treated with FX1 and AP-4-287. The vaccinated mice in the study received 100uL 10% SRBC vaccination, 3-day resting and an 8-day course treatment with either vehicle (100uL once or three times daily), or FX1 (80mg/kg in 100uL vehile, once daily) or AP-4-287(25mg/kg in 100uL vehicle, three times daily) prior necropsy. Control mice received 100uL PBS and resting for 11 days prior necropsy. Spleen and blood were collected at the necropsy to analyze germinal center reaction and antibody responses. Data obtained from cytometry analysis showed that the frequency of splenic Tfh $CD4^+$ T cells **(A),** splenic activated GC B **(C)** and the level of SRBC-specific IgG1 **(D)** from PBS-treated, SRBC-vaccinated mice treated with vehicle, or FX1, or AP-4-287. The level of pan IgM **(E)** and IgG **(F)** were measured by ELISA method as well. Welch-test was performed to compare the results between two groups. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

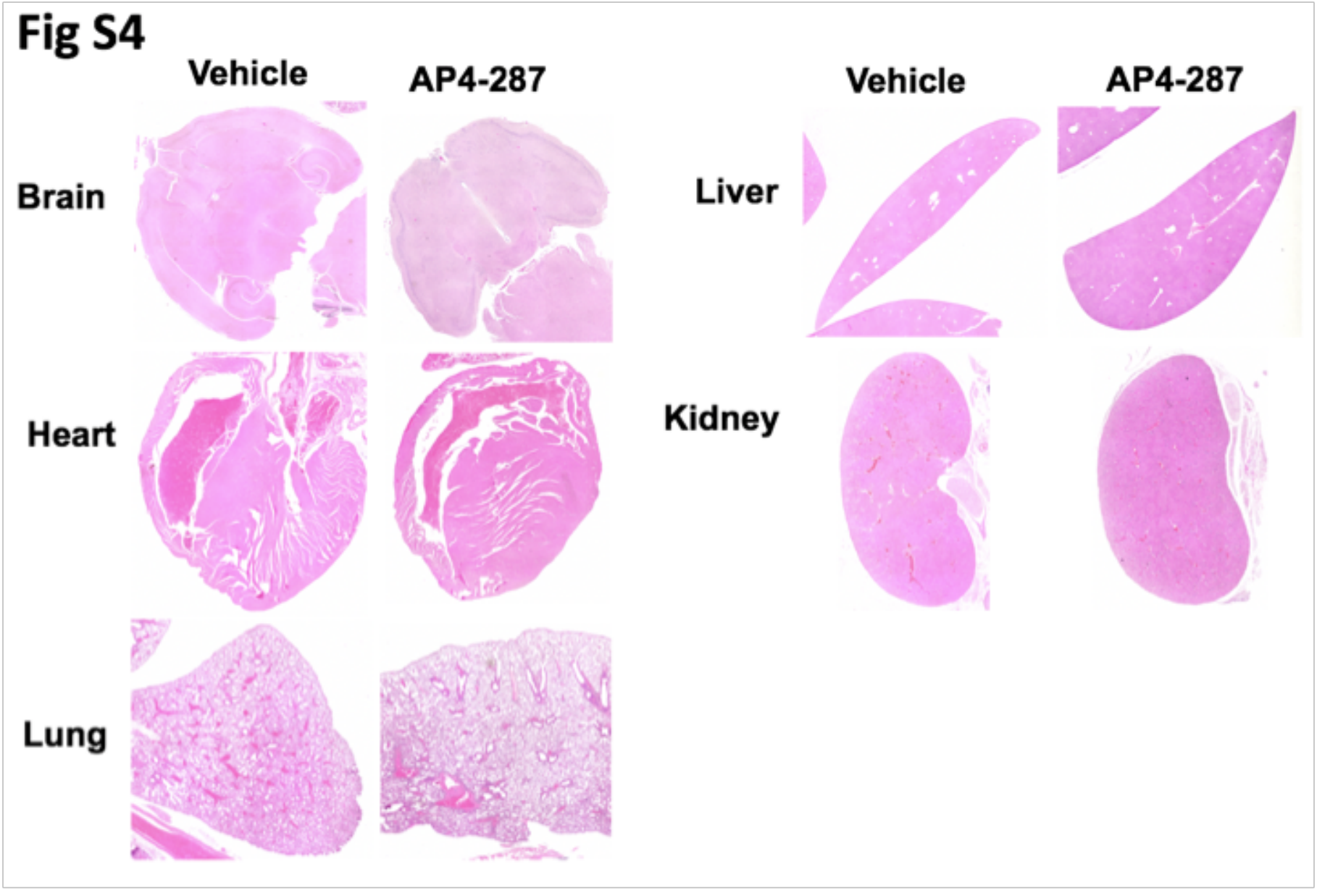


Figure S4. H&E stained tissues sections from brain, heart, lung, liver and kidney of mice receiving Vehicle or AP-4-287.

Fig S5

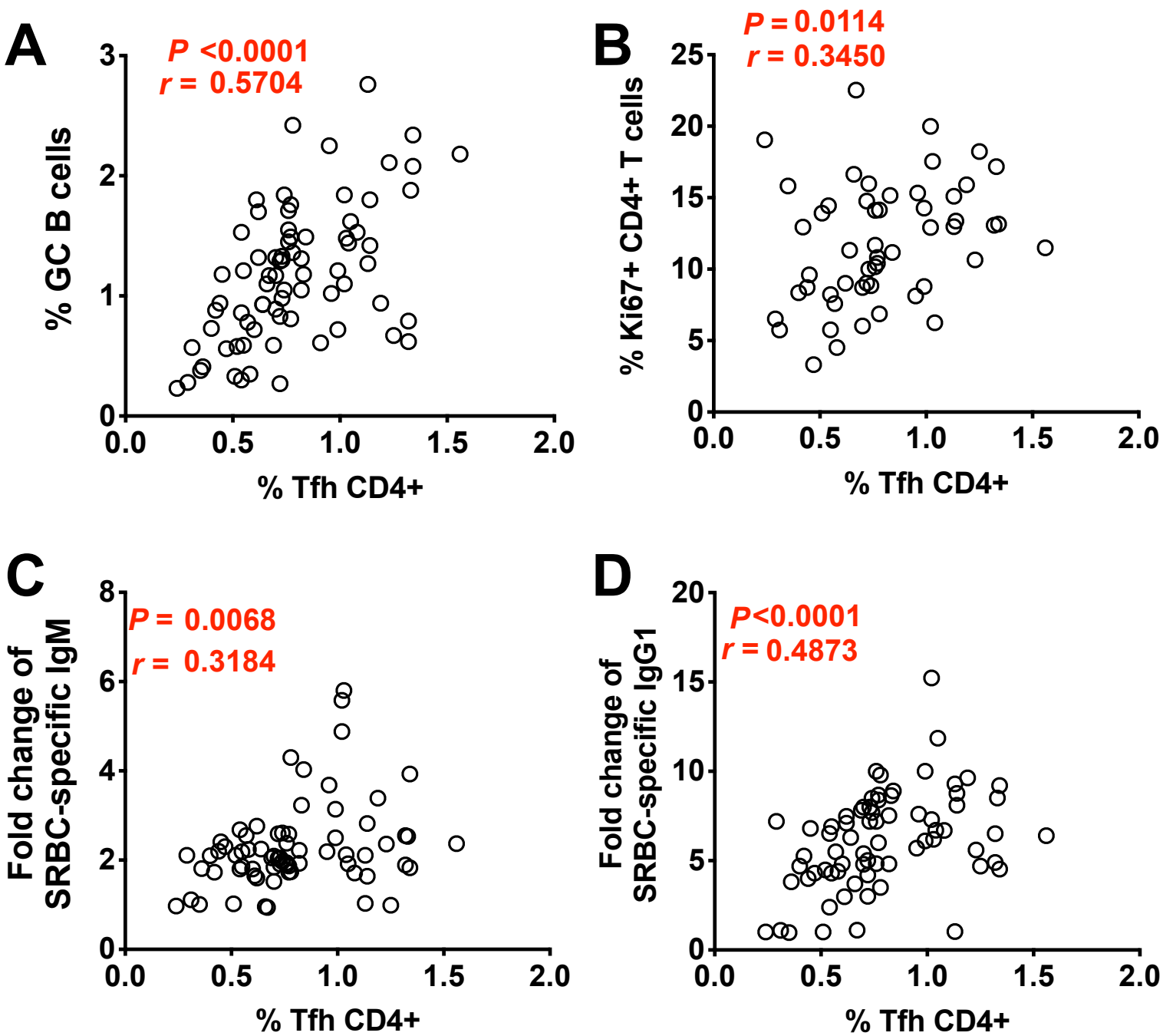
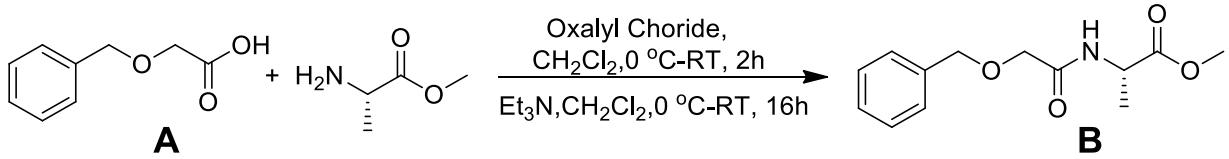


Figure S5. Tfh CD4$^+$ T cells positively correlated with activated GC B, Ki67$^+$CD4$^+$ T cells, and the production of SRBC-specific IgM and SRBC-specific IgG1 in vaccinated mice.

Spearman correlation analysis was performed to relate the frequency of splenic Tfh CD4$^+$ T cells and the frequency of activated splenic GC B, Tfh and Ki67$^+$CD4$^+$ T cells, and the titer of SRBC-specific IgM and IgG1.

Experimental procedures for synthesis of AP-4-287, ^{1}H NMR, and LCMS Spectra

(S)-Methyl 2-(2-(benzyloxy)acetamido)propanoate (B):



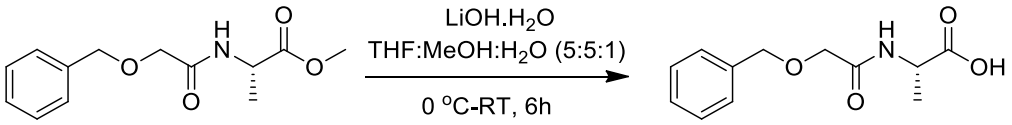
A **B**

To a stirred solution of 2-(benzyloxy)acetic acid, **A** (14 g, 0.0842 mol) in 200 mL of dry CH_2Cl_2 was added oxalyl chloride (8.8 mL, 0.101 mol) at 0 ^oC dropwise followed by 0.2 mL of DMF. The reaction mixture was then slowly brought to room temperature and stirred for 2h. Volatiles were evaporated under reduced pressure and the crude product was co-distilled with dry toluene to provide a thick liquid that was dissolved in 75 mL of dry CH_2Cl_2 and added dropwise to a premixed solution of (S)-methyl 2-aminopropanoate hydrochloride (11.76 g, 0.0842 mol) and triethylamine (35.4 mL, 2.53 mol) in 200 mL of dry CH_2Cl_2 at 0 ^oC. The reaction mixture was slowly brought to room temperature and stirred for 16h. Completion of the reaction was confirmed by LC-MS. Then the reaction mixture was diluted with ice cold water and the product was extracted with CH_2Cl_2. The organic layer was washed with *aq* 1N HCl, *aq* sat $NaHCO_3$, brine solution and then dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and dried under high vacuum to afford **B** as a thick liquid (18.3 g, 0.072 mmol, 86%), product confirmed by ^{1}H NMR and LC-MS.

δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.30 (m, 5H), 7.10 (d, *J* = 5.8 Hz, 1H), 4.71 – 4.53 (m, 3H), 4.06 – 3.92 (m, 2H), 3.76 (s, 3H), 1.43 (d, *J* = 7.2 Hz, 3H).

Mass: m/z $[M+H]^+$ 252

(S)-2-(2-(Benzyloxy)acetamido) propanoic acid (C):



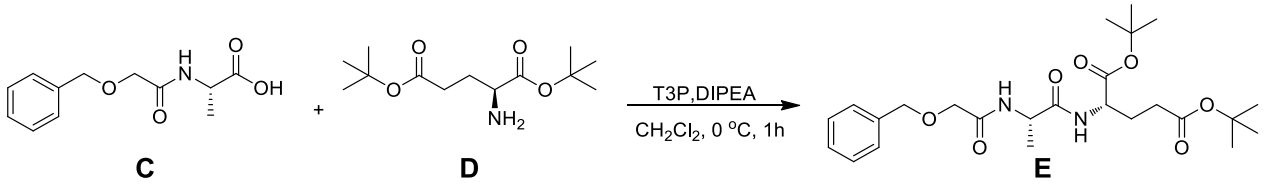
B **C**

To a stirred solution (S)-methyl 2-(2-(benzyloxy)acetamido)propanoate, **B** (18 g, 0.072 mol) in 200 mL of (THF:MeOH:H_2O) (5:5:1) at 0 ^oC was added $LiOH{\cdot}H_2O$ (9.02 g, 0.215 mol) and the reaction mixture was brought to room temperature and stirred for 6h. Completion of the reaction was confirmed by LC-MS. The volatiles were evaporated under reduced pressure to provide crude product, which was cooled to 0 ^oC and acidified with 1N HCl (aq) to pH 5. The reaction product was extracted with CH_2Cl_2 and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and dried under high vacuum to afford **C** (16.16g, 0.067 mmol, 94%) title compound was confirmed by ^{1}H NMR and LC-MS.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (s, 1H), 7.42 – 7.28 (m, 5H), 7.14 (d, *J* = 7.4 Hz, 1H), 4.70 – 4.49 (m, 3H), 4.08 – 3.95 (m, 2H), 1.48 (d, *J* = 7.2 Hz, 3H).

Mass: m/z $[M-H]^+$ 236

(**S)-Di-tert-butyl 2-((S)-2-(2-(benzyloxy)acetamido)propanamido)pentanedioate (E):**



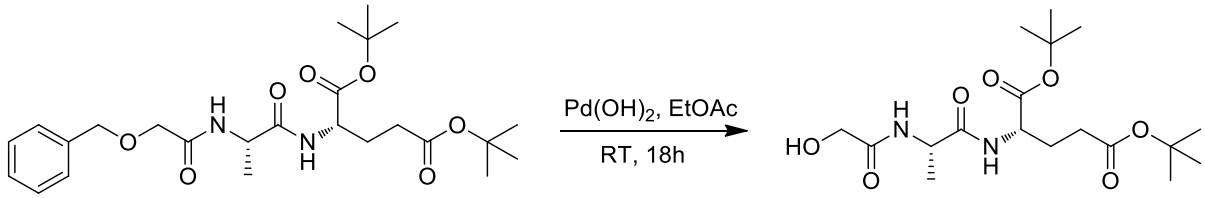
C **D** **E**

To a stirred solution of (S)-2-(2-(benzyloxy)acetamido)propanoic acid, **C** (16 g, 0.067 mol) and (S)-di-tert-butyl 2-aminopentanedioate, **D** (17.49 g, 0.067 mol) in 250 mL dry CH_2Cl_2 at 0 °C, was added diisopropyl ethylamine (47 mL, 0.27 mmol) and T3P (50% solution in CH_2Cl_2 by weight) (55.8 g, 0.087mol) simultaneously dropwise. Then the reaction mixture was stirred for 30 mins at 0 °C. Completion of the reaction was confirmed by LC-MS. The reaction mixture was diluted with ice cold water and the product was extracted with CH_2Cl_2. The organic layer was washed with *aq* 1N HCl, aq sat $NaHCO_3$, brine solution, and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and dried under high vacuum to afforded title compound as thick liquid (29.04 g, 0.060 mol, 90%). The product **E** was confirmed by ^{1}H NMR and LC-MS.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.29 (m, 5H), 7.15 (d, *J* = 7.6 Hz, 1H), 6.73 (d, *J* = 7.6 Hz, 1H), 4.66 – 4.47 (m, 3H), 4.44 (td, *J* = 7.8, 4.9 Hz, 1H), 4.00 (d, *J* = 5.6 Hz, 2H), 2.39 – 2.18 (m, 2H), 2.18 – 2.05 (m, 1H), 1.93 (dtd, *J* = 14.3, 8.3, 6.2 Hz, 1H), 1.46 (d, *J* = 4.8 Hz, 9H), 1.44 – 1.37 (m, 12H).

Mass: m/z $[M+Na]^+$ 501; m/z $[M-H]^+$ 479

(S)-Di-tert-butyl 2-((S)-2-(2-hydroxyacetamido)propanamido)pentanedioate (F):



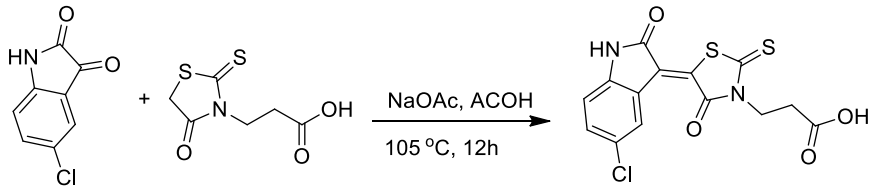
E **F**

To a stirred solution of (S)-di-tert-butyl 2-((S)-2-(2-(benzyloxy)acetamido) propanamido) pentanedioate, **E** (29 g, 0.061 mol) in 300 mL of ethyl acetate at room temperature was added 10% $Pd(OH)_2$ (3g) stirred for 16h for under a balloon pressure hydrogen atmosphere. Completion of reaction was confirmed by LC-MS. The reaction mixture was filtered through a small pad of celite, the solvent was evaporated under reduced pressure, and dried under high vacuum to afford **F** as thick liquid (22.36 g, 0.057, 94%), product confirmed by ^{1}H NMR and LC-MS.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (d, *J* = 7.5 Hz, 1H), 6.87 (d, *J* = 7.6 Hz, 1H), 4.57 (p, *J* = 7.1 Hz, 1H), 4.43 (td, *J* = 7.8, 4.7 Hz, 1H), 4.19 – 4.02 (m, 2H), 3.13 (s, 1H), 2.43 – 2.18 (m, 2H), 2.17 – 2.05 (m, 1H), 1.94 (tt, *J* = 14.4, 7.2 Hz, 1H), 1.44 (t, *J* = 9.8 Hz, 21H).

Mass: m/z $[M+H]^+$ 389; m/z $[M+Na]^+$ 411.

(Z)-3-(5-(5-Chloro-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)propanoic acid (FX-1):



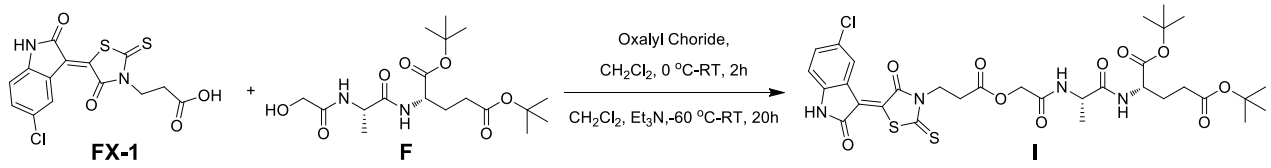
G **H** **FX-1**

To stirred solution of 5-chloroindoline-2,3-dione, **G** (8.85 g, 0.049 mol) and 3-(4-oxo-2-thioxothiazolidin-3-yl)propanoic acid, **H** (10 g, 0.049 mol) in 350 ml of acetic acid at room temperature was added sodium acetate (40 g, 0.49 mol). The reaction temperature was slowly raised to 105 °C and stirred for 12h. Completion of the reaction was confirmed by LC-MS. The Reaction mixture was cooled to room temperature and diluted with 350 mL of water, and the resulting precipitate was filtered and washed with H_2O, then dried under high vacuum to afford **FX-1** as a dark red colored solid (16.2 g, 0.044, 90%). Product confirmed by ^{1}H NMR and LC-MS.

^{1}H NMR (400 MHz, DMSO) δ 12.51 (s, 1H), 11.40 (s, 1H), 8.79 (d, *J* = 2.0 Hz, 1H), 7.68 – 7.28 (m, 1H), 6.97 (d, *J* = 8.4 Hz, 1H), 4.37 – 4.15 (m, 2H), 2.79 – 2.56 (m, 2H).

Mass: m/z $[M-H]^+$ 367.

(S)-Di-tert-butyl 2-((S)-2-(2-(3-((Z)-5-(5-chloro-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl) propanoyloxy) acetamido) propanamido)pentanedioate (I):



FX-1 **F** **I**

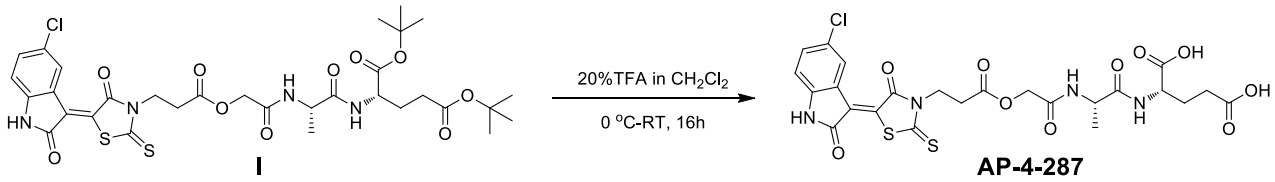
To a stirred solution of (Z)-3-(5-(5-chloro-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)propanoic acid, **FX-1** (10 g, 0.027 mol) in 300 mL of dry CH_2Cl_2 at 0 °C was added oxalyl chloride (3.02 mL, 0.035 mol) dropwise followed by 0.3 mL of DMF. The reaction mixture was slowly brought to room temperature and stirred for 12h. The volatiles were evaporated under reduced pressure and the crude product was co-distilled with dry toluene. The resulting red colored solid was suspended in 400 mL of dry CH_2Cl_2 and cooled to -78 °C in a dry ice acetone bath. Then Et_3N (15.2 mL, 0.108 mol) and (S)-di-tert-butyl 2-((S)-2-(2-hydroxyacetamido)

propanamido)pentanedioate, **F** (10.53 g, 0.027 mol) dissolved in 50 mL of dry CH_2Cl_2 was added dropwise to the reaction mixture. The reaction mixture was then slowly brought to room temperature and stirred for an additional 16 h. Completion of the reaction was confirmed by LC-MS. The reaction mixture was diluted with ice cold water, and the volatiles were evaporated under reduced pressure to provide the crude product. This was washed with water and diethyl ether and dried under high vacuum. Crude product was stirred in MeOH at room temperature for 3h, and any insoluble material was filtered off through a small pad of celite. The MeOH filtrate was concentrated under reduced pressure to obtain the solid product, which was dried under high vacuum to afford **I** as a red colored solid (14 g, 0.019, 70%). The product was confirmed by ^{1}H NMR and LC-MS.

^{1}H NMR (400 MHz, DMSO) δ 11.42 (s, 1H), 8.84 (d, *J* = 2.1 Hz, 1H), 8.19 (dd, *J* = 17.3, 7.6 Hz, 2H), 7.49 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 4.60 – 4.45 (m, 2H), 4.39 – 4.25 (m, 3H), 4.10 (dd, *J* = 13.8, 8.1 Hz, 1H), 2.89 – 2.79 (m, 2H), 2.26 (td, *J* = 8.1, 3.5 Hz, 2H), 1.91 (dt, *J* = 13.8, 7.8 Hz, 1H), 1.75 (dt, *J* = 14.9, 8.6 Hz, 1H), 1.38 (d, *J* = 2.1 Hz, 16H), 1.21 (d, *J* = 7.1 Hz, 3H).

Mass: m/z $[M+1]^+$ 739; m/z $[M+Na]^+$ 761.

(S)-2-((S)-2-(2-(3-((Z)-5-(5-Chloro-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)propanoyloxy)acetamido)propanamido)pentanedioic acid (AP-4-287):



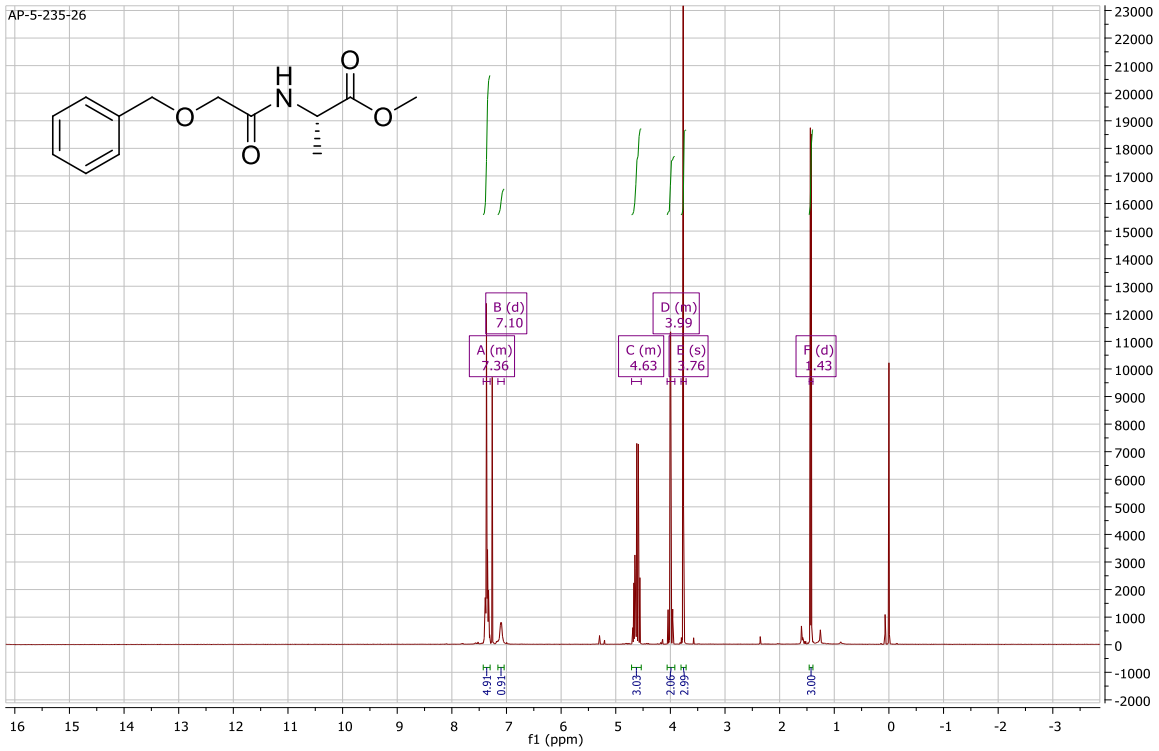
I **AP-4-287**

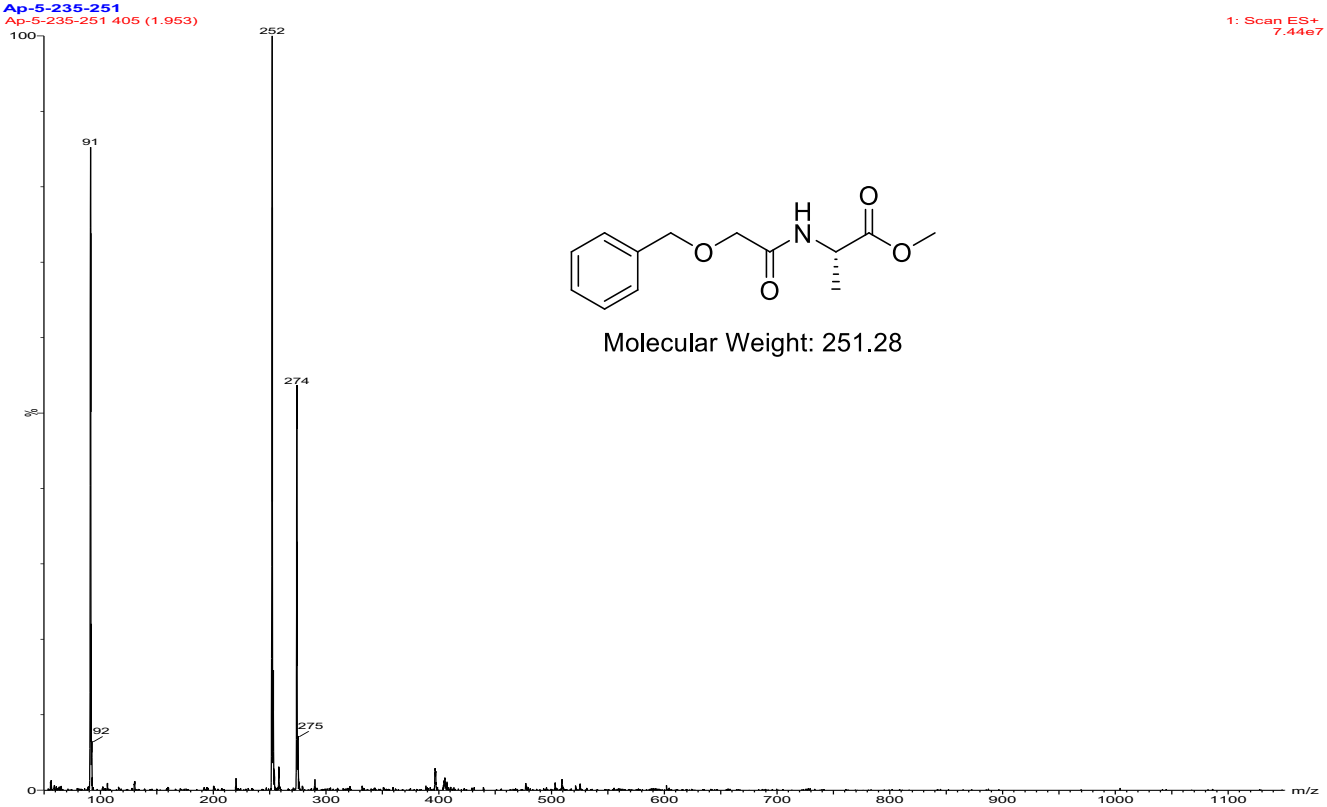
To a stirred solution of (S)-di-tert-butyl 2-((S)-2-(2-(3-((Z)-5-(5-chloro-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)propanoyloxy)acetamido)propanamido)pentanedioate, **I** (13.9 g, 0.019 mol) in 320 mL of CH_2Cl_2 at 0 °C was slowly added 80 mL of TFA. Then the reaction mixture was slowly brought to room temperature and stirred for 16h. Completion of the reaction was confirmed by LC-MS. Volatiles were evaporated under reduced pressure, and the product was co-distilled with toluene. The product was suspended in diethyl ether and sonicated for 30 min to remove traces of the remaining TFA, and then filtered to collect the red colored solid which was dried under high vacuum to afford **AP-4-287** as red color solid (10.61 g, 0.017 mol, 89%), product confirmed by ^{1}H NMR and LC-MS.

1H NMR (400 MHz, DMSO) δ 12.42 (s, 2H), 11.41 (s, 1H), 8.80 (t, *J* = 23.8 Hz, 1H), 8.36 – 8.01 (m, 2H), 7.49 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 4.52 (q, *J* = 14.8 Hz, 2H), 4.40 – 4.27 (m, 3H), 4.19 (td, *J* = 8.9, 5.1 Hz, 1H), 2.91 – 2.76 (m, 2H), 2.35 – 2.17 (m, 2H), 1.97 (td, J = 13.0, 7.7 Hz, 1H), 1.78 (dt, *J* = 14.3, 8.2 Hz, 1H), 1.21 (d, *J* = 7.1 Hz, 3H).

Mass: m/z $[M-H]^+$ 627.

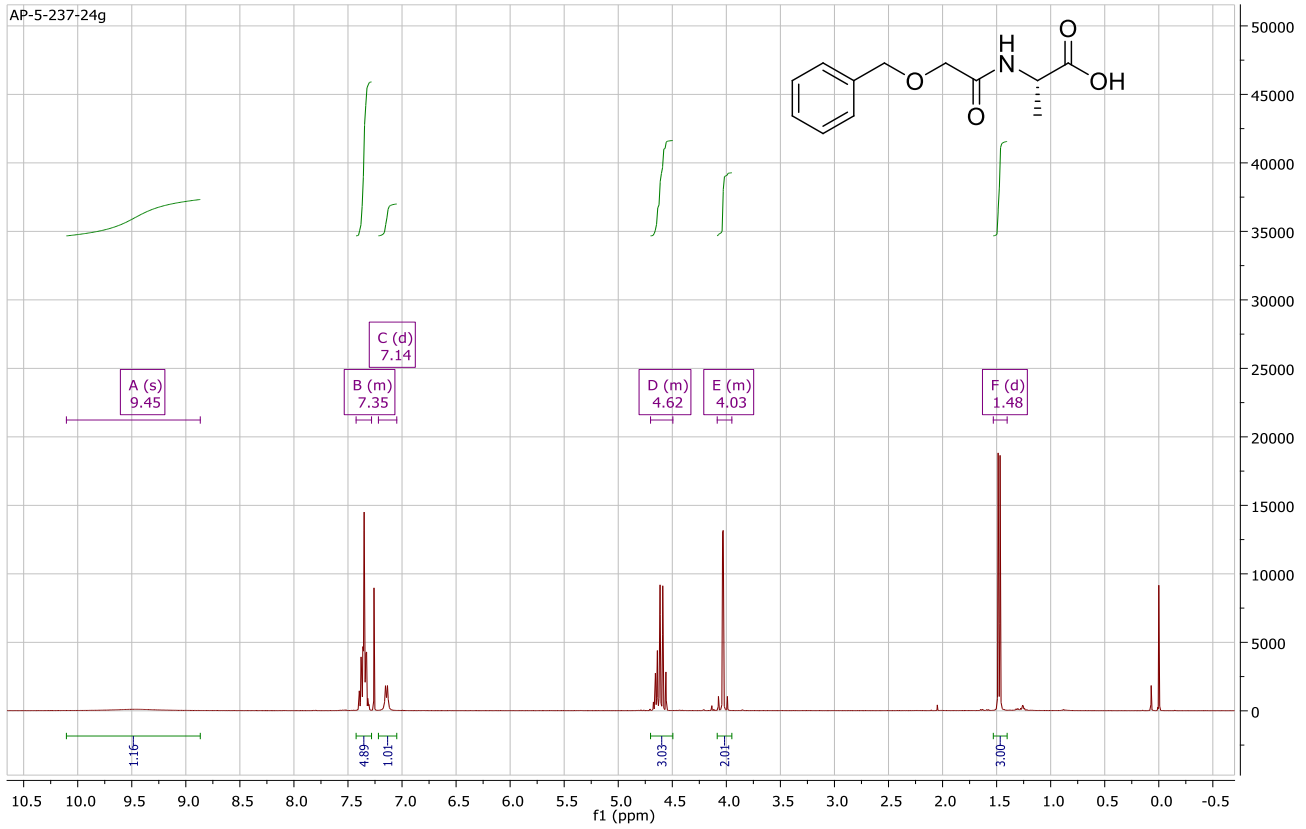
(S)-Methyl 2-(2-(benzyloxy)acetamido)propanoate (B):

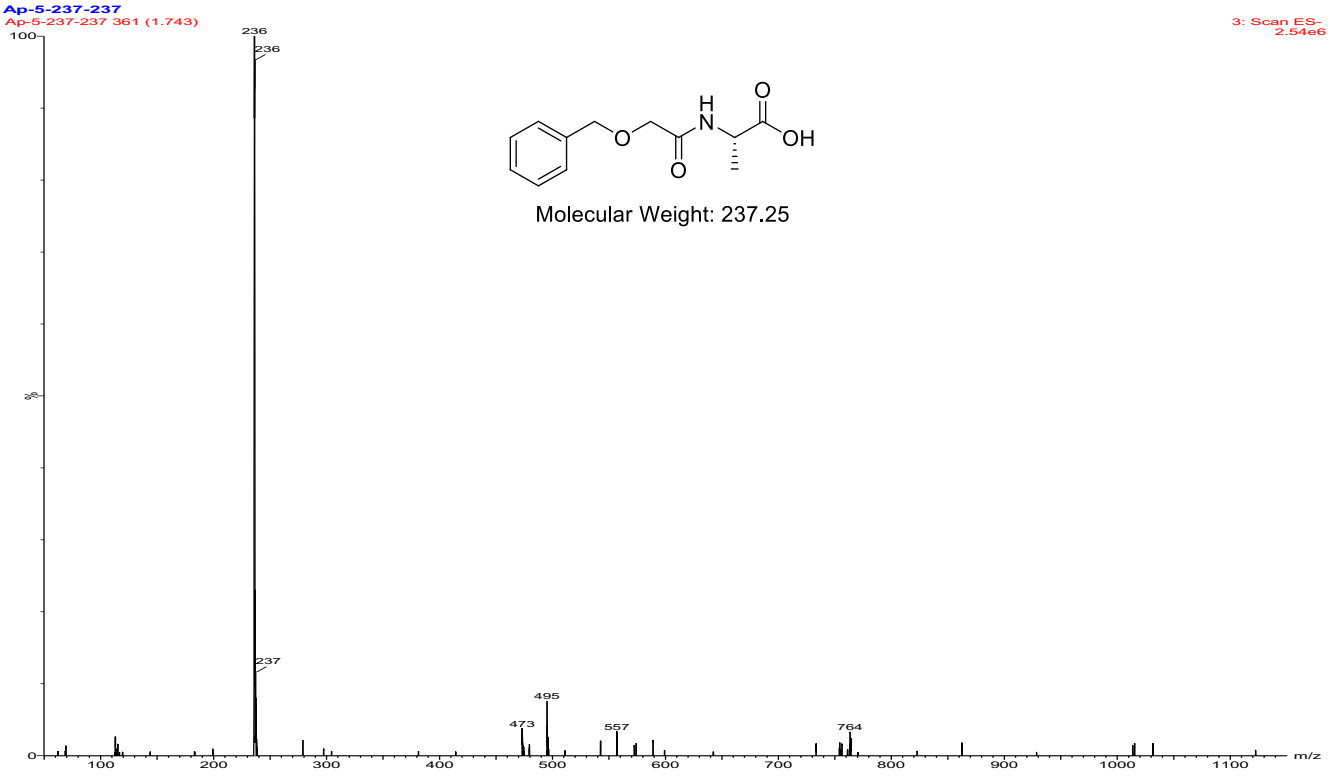




Molecular Weight: 251.28

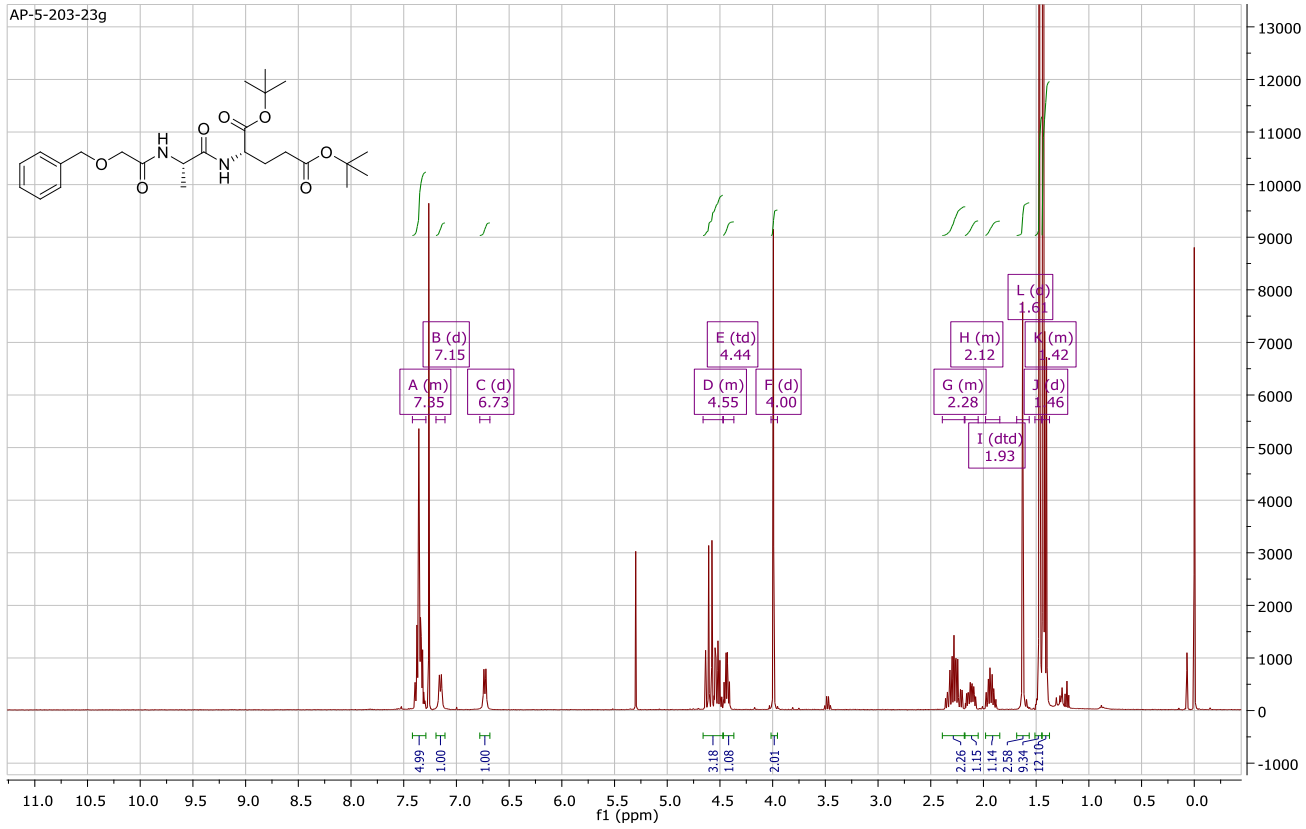
(S)-2-(2-(Benzyloxy)acetamido) propanoic acid (C):

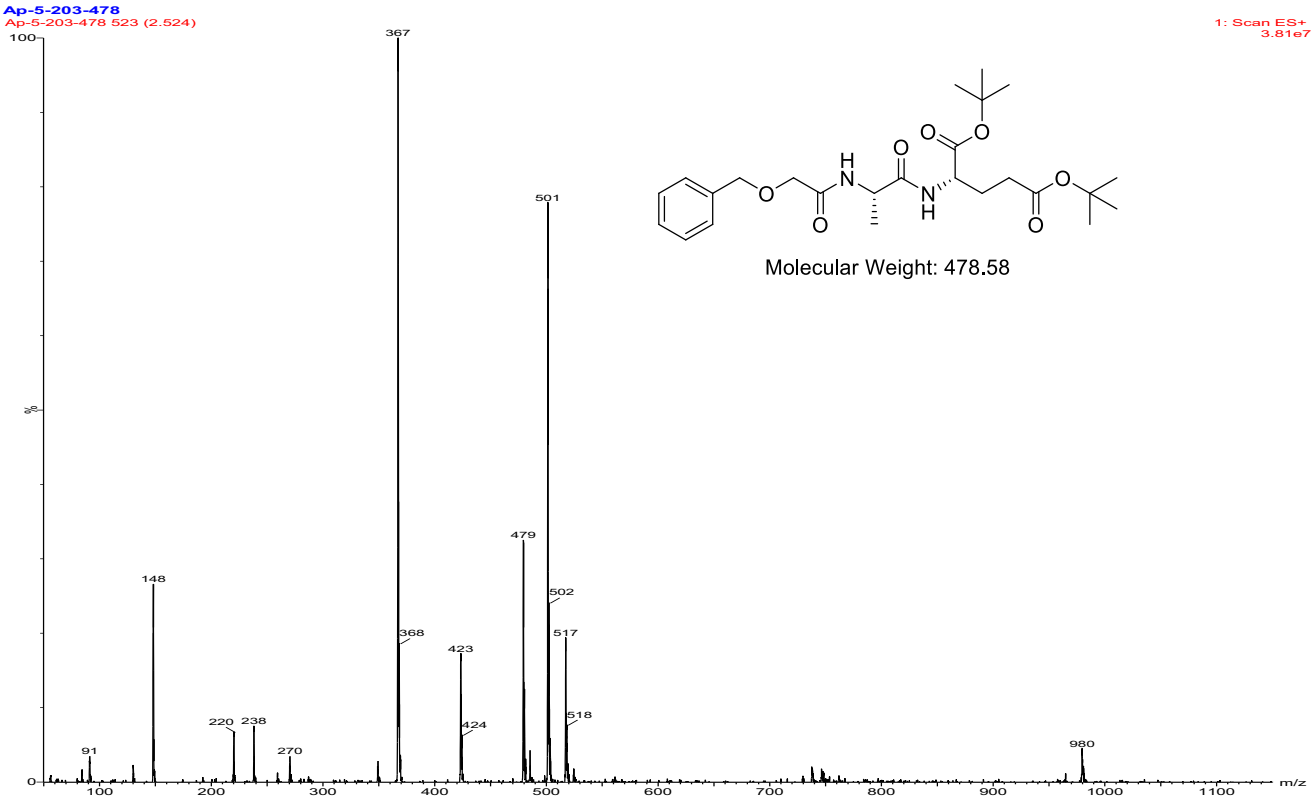




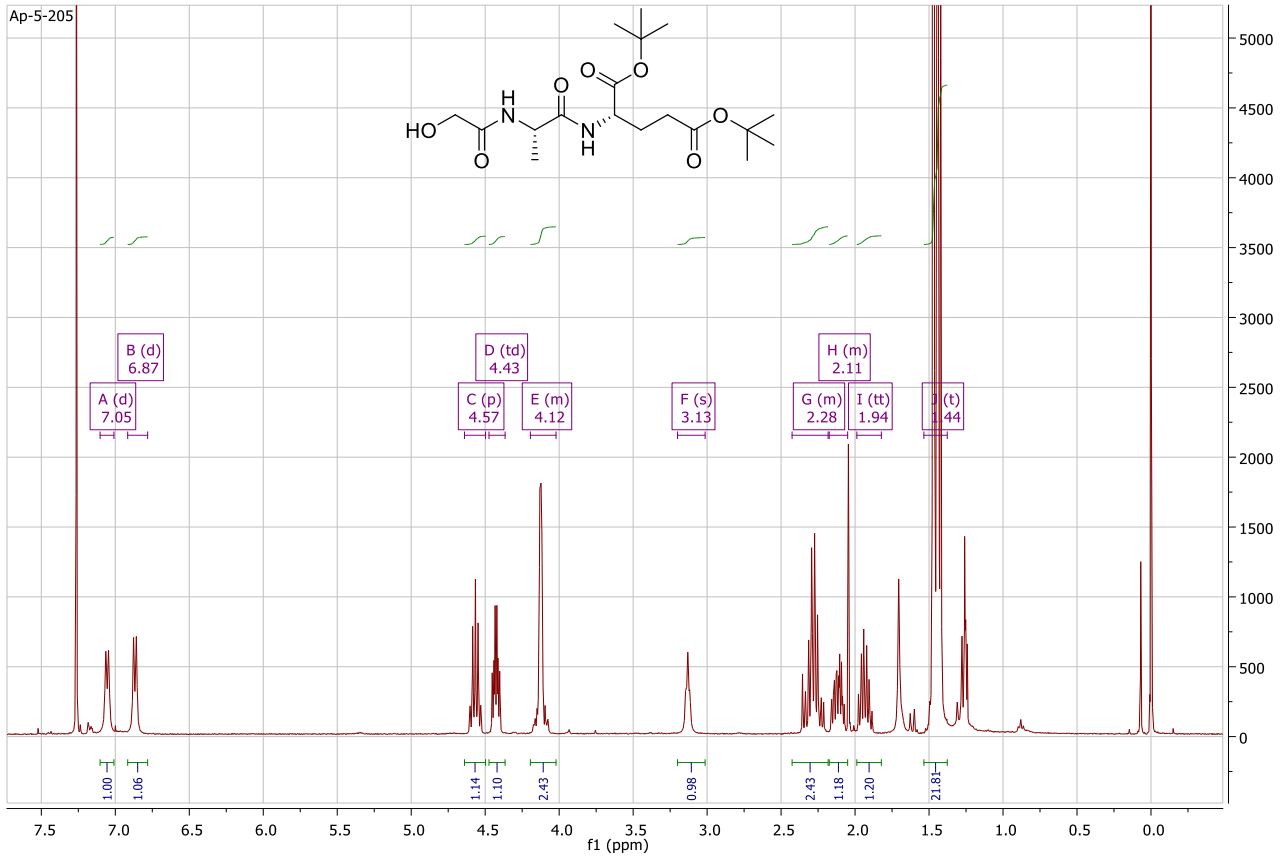
Molecular Weight: 237.25

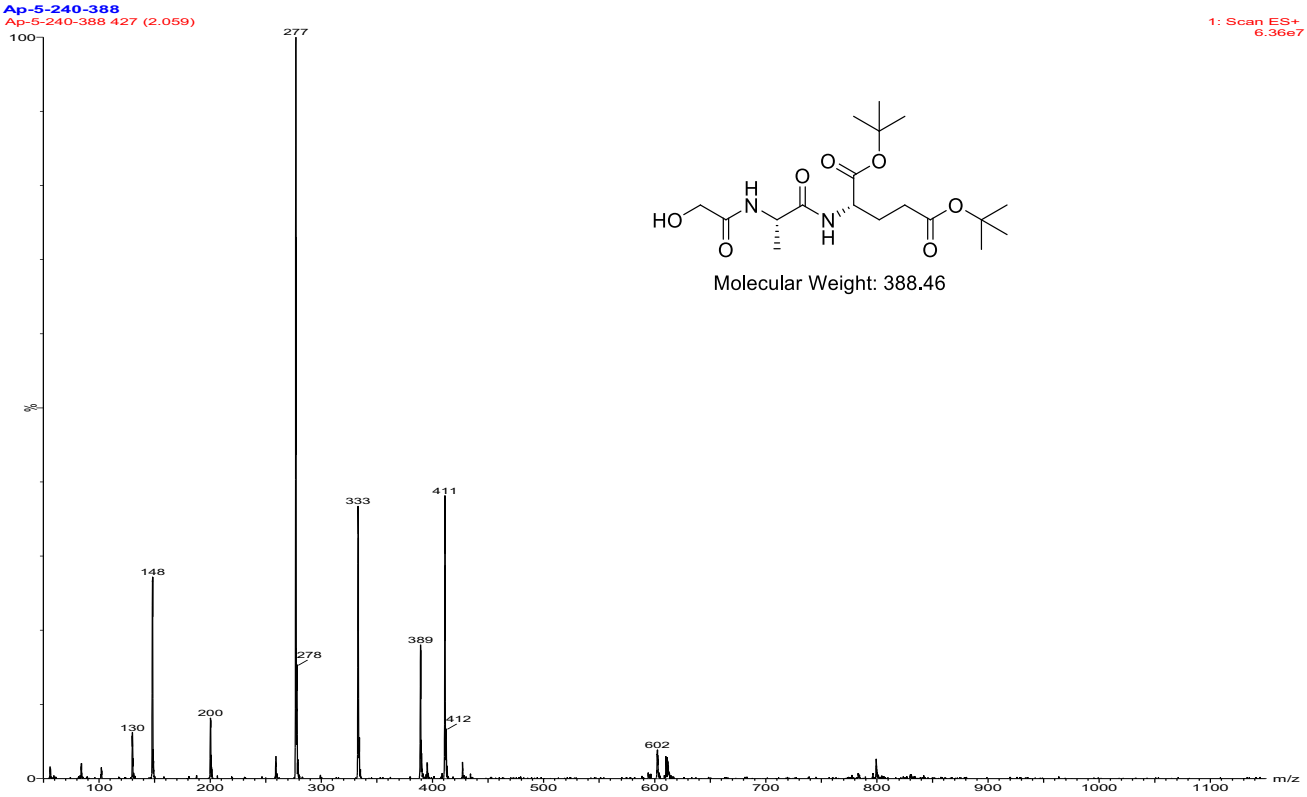
(S)-Di-tert-butyl 2-((S)-2-(2-(benzyloxy)acetamido)propanamido)pentanedioate (E):





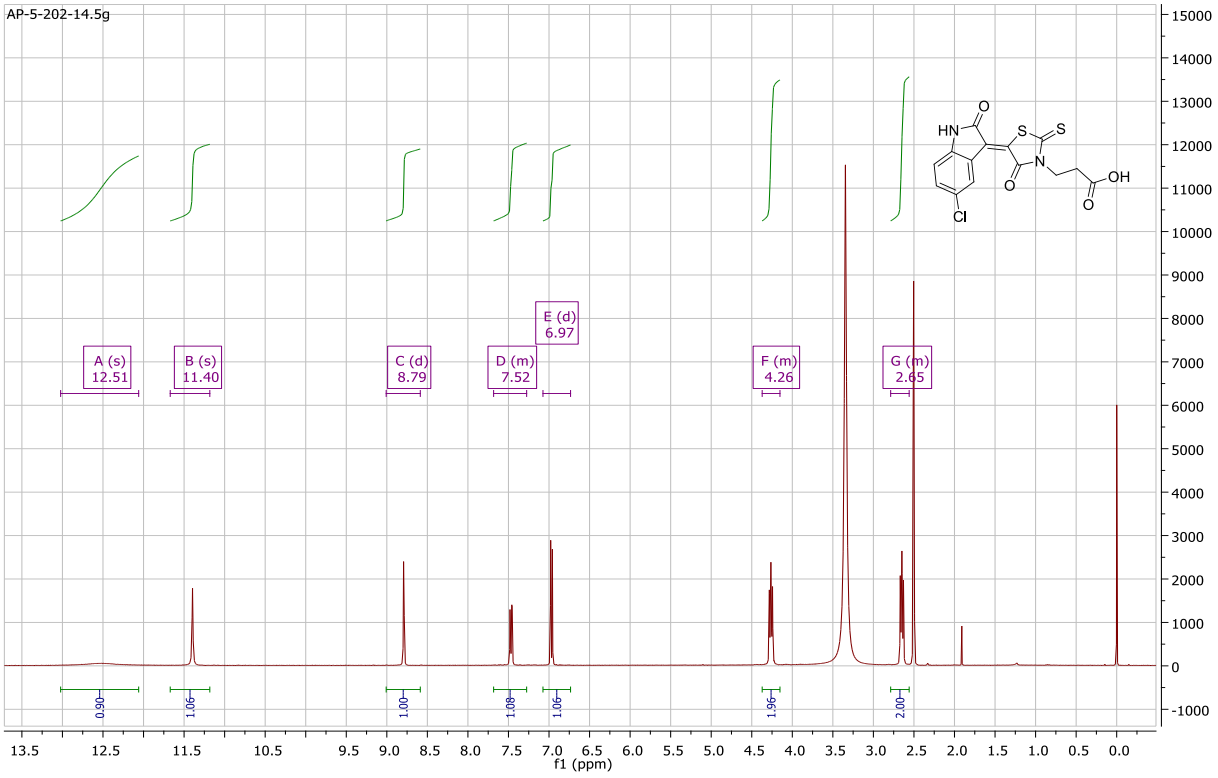
(S)-Di-tert-butyl 2-((S)-2-(2-hydroxyacetamido)propanamido)pentanedioate (F):

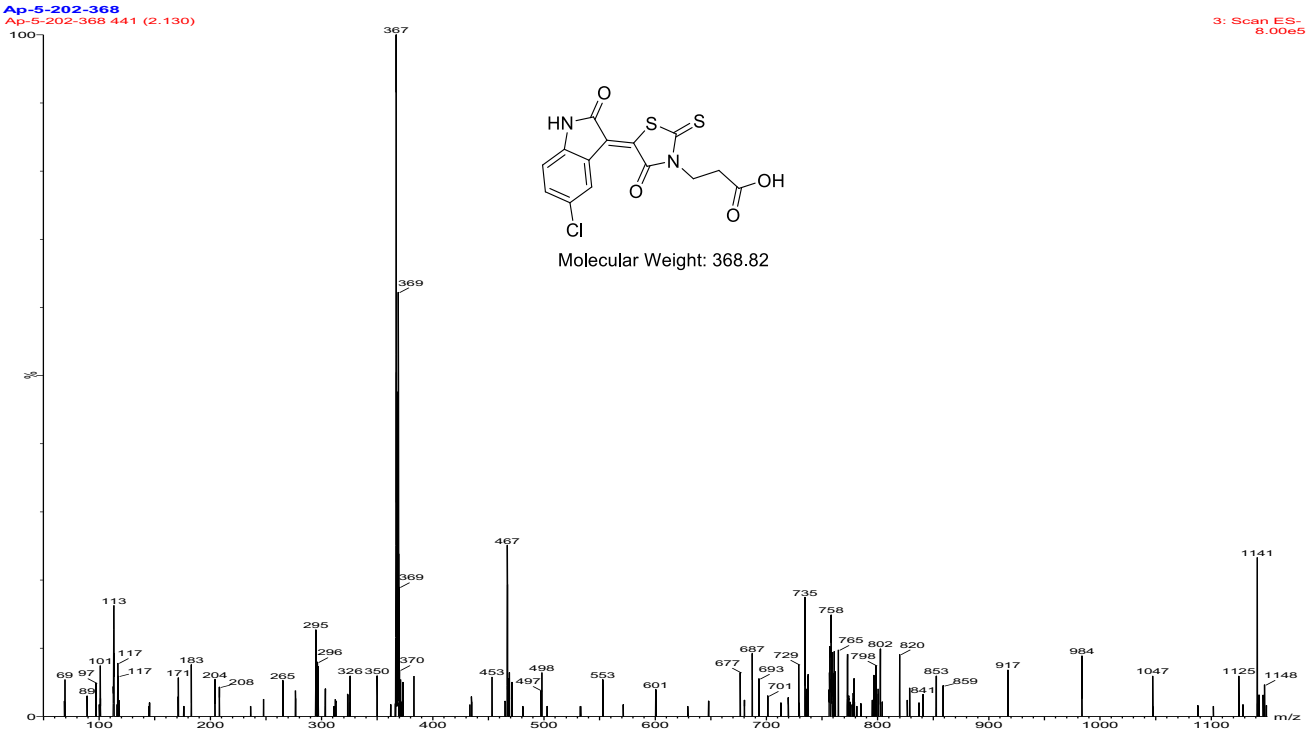




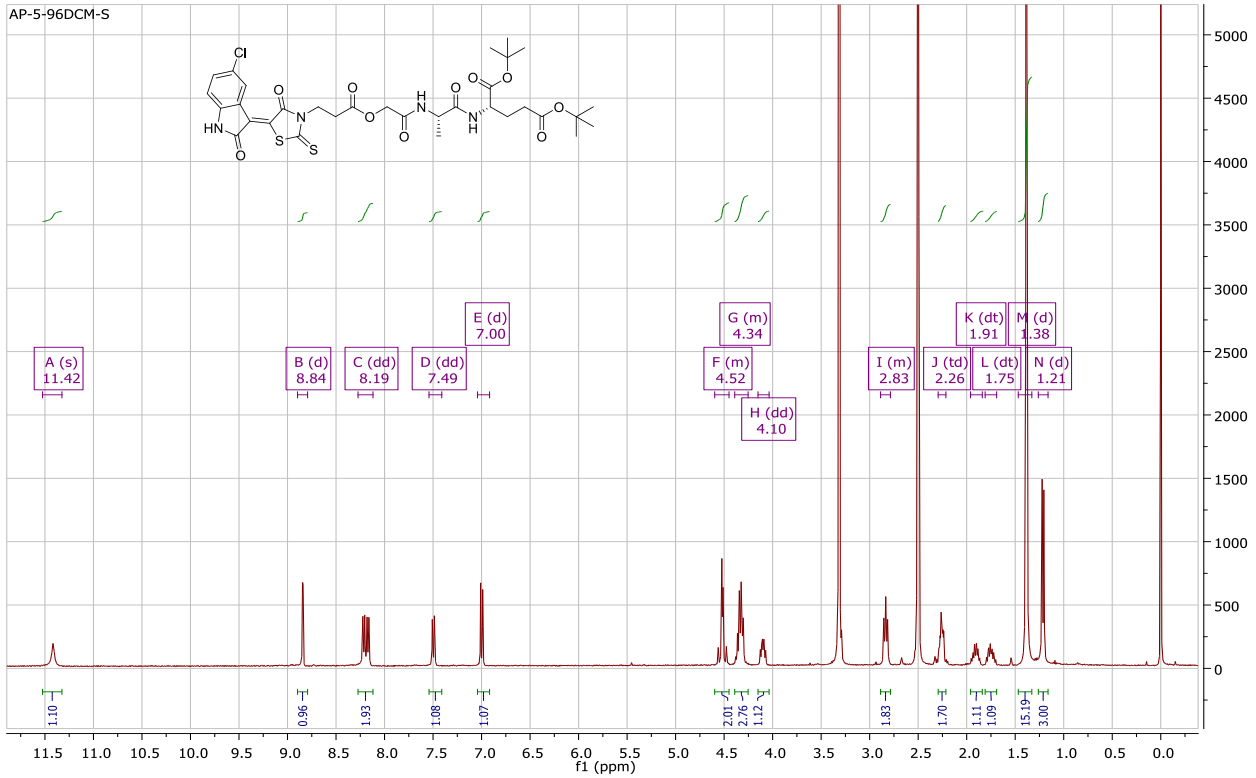
Molecular Weight: 388.46

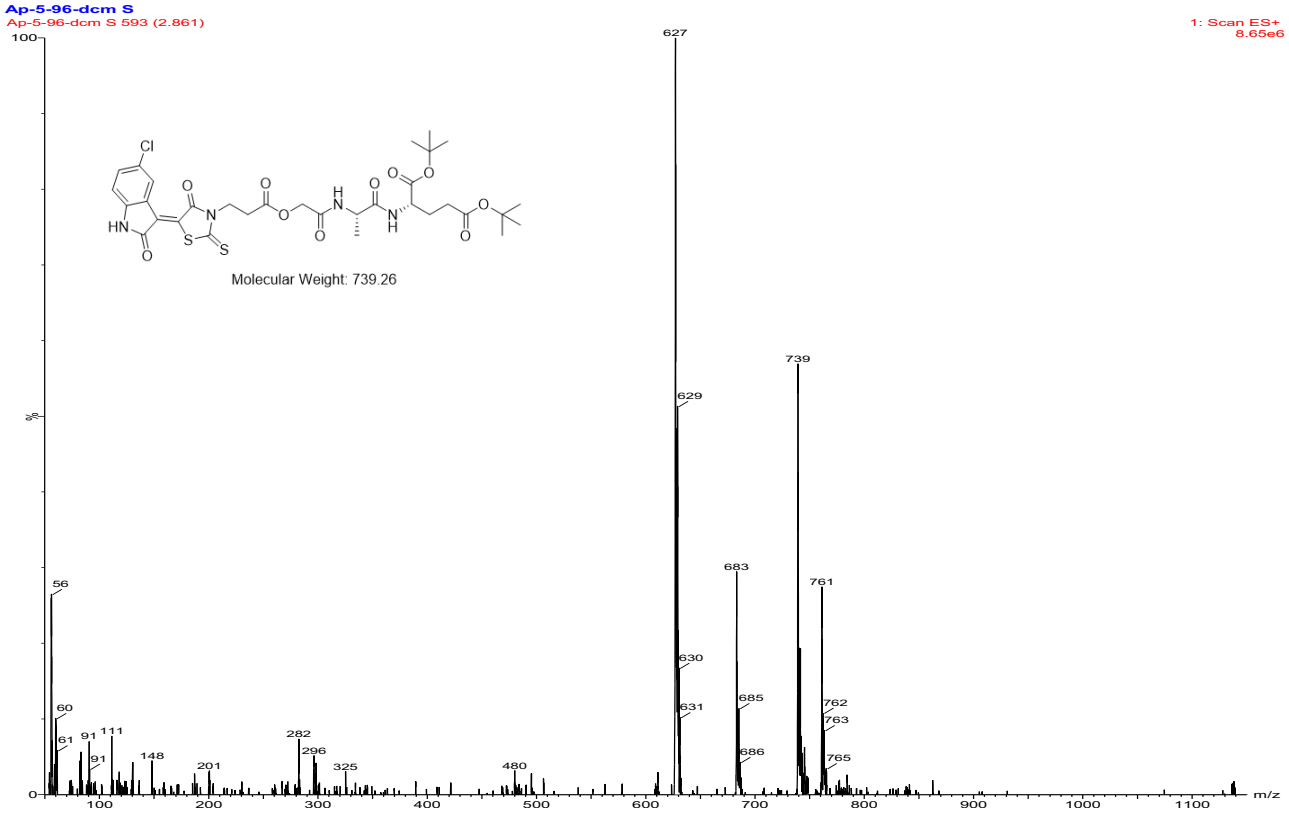
(Z)-3-(5-(5-Chloro-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)propanoic acid (FX-1) :



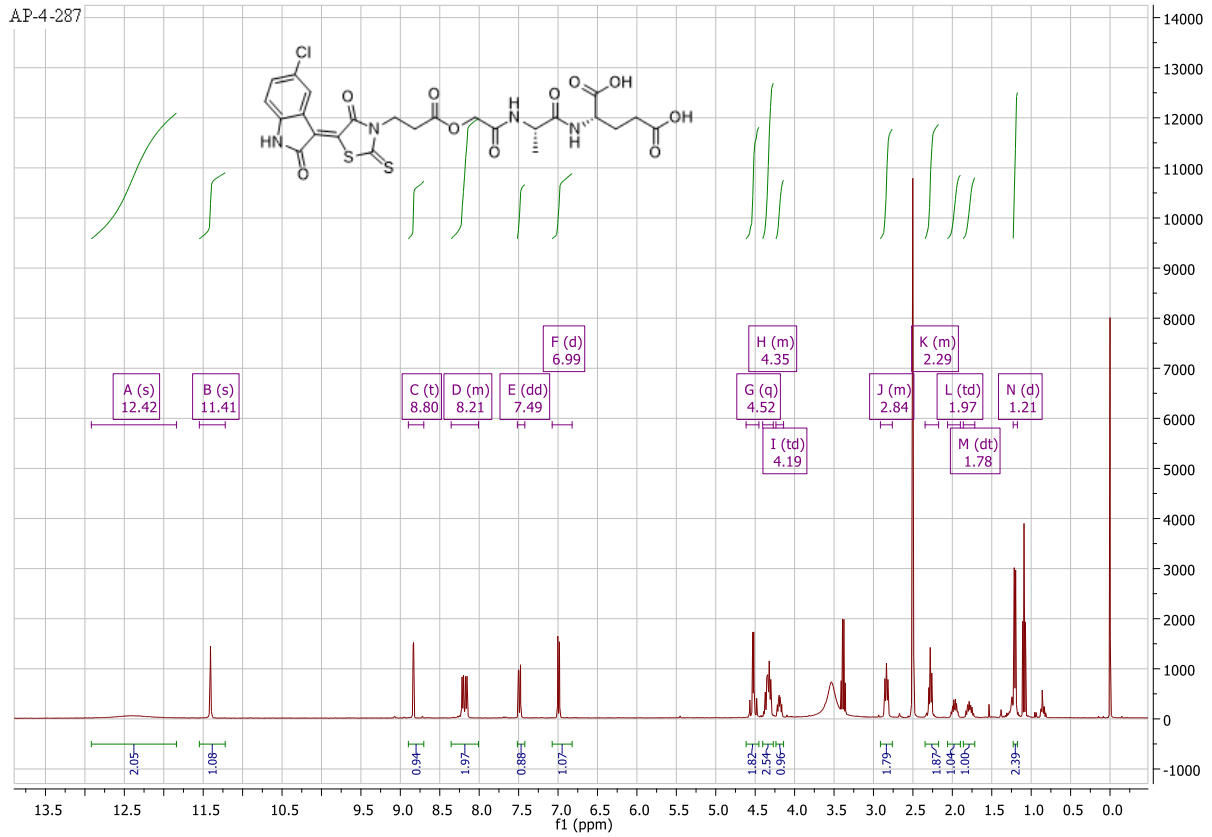


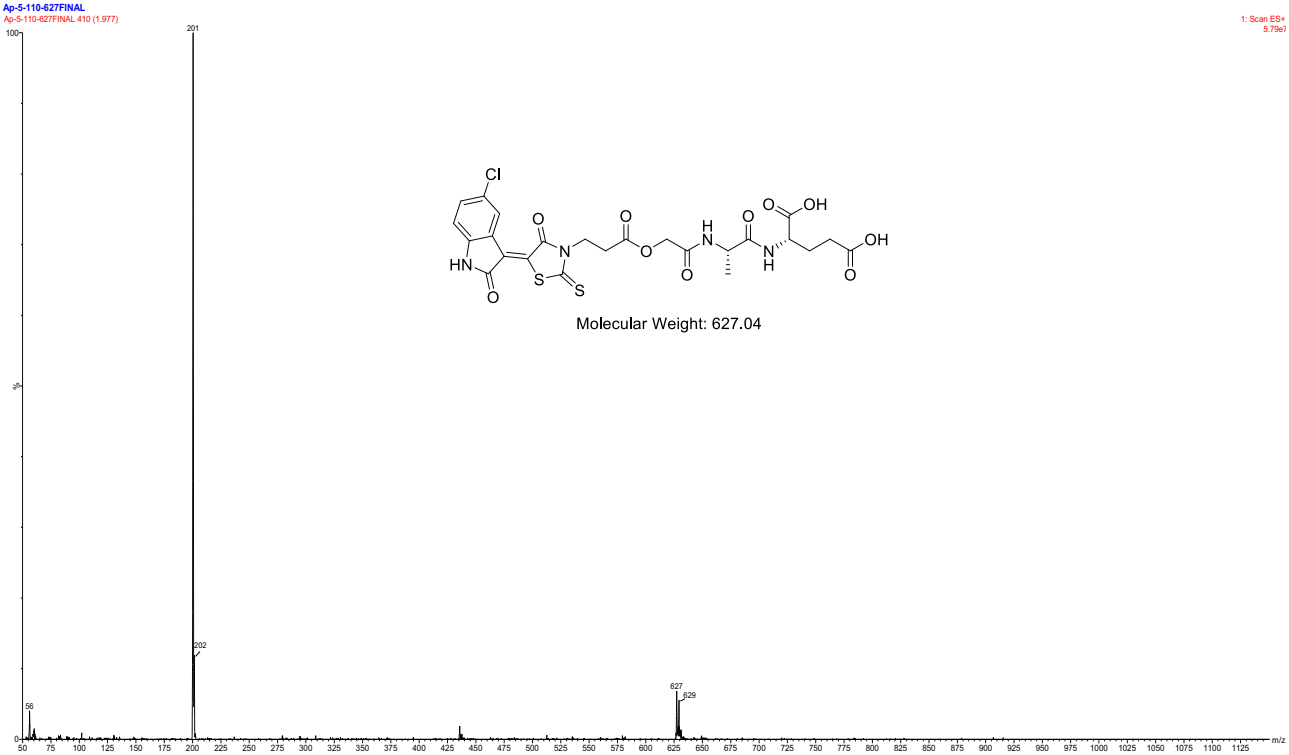
(S)-Di-tert-butyl 2-((S)-2-(2-(3-((Z)-5-(5-chloro-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl) propanoyloxy) acetamido) propanamido)pentanedioate (I):





(S)-Di-tert-butyl 2-((S)-2-(2-(3-((Z)-5-(5-chloro-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)propanoyloxy)acetamido)propanamido)pentanedioate (AP-4-287)





Molecular Weight: 627.04