SUPPORTING INFORMATION

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Supplementary Methods

Media preparation

Fecal culture medium (FCM) was prepared according to a protocol by McDonald *et al.*[1] autoclaving for 20 min at 121 °C. The media was brought into an anaerobic chamber and left overnight with the lid slightly ajar to remove any remaining oxygen from the media. Then, an aqueous solution of L-cysteine was pushed through a 0.2uM syringe filter into the media solution for a concentration of 0.5% L-Cysteine (w/v) to maintain anoxic solution.

Data analysis of HMP culture extracts

MS1 feature detection and MS/MS pairing was performed using MZmine 2.37corr17.7_kai_merge2[2,3]. An intensity threshold of 1E3 and 50 were set for MS1 and MS2 detection, respectively, with centroid data. MS1 chromatogram construction was performed using the ADAP chromatogram builder, where the minimum group size was set to 3, group intensity threshold was 1E3, minimum highest intensity was 3E3, and mass tolerance was 0.01 *m/z* or 20ppm. Chromatogram deconvolution was then performed using a local minimum search algorithm with a chromatographic threshold of 90%, a search minimum in retention time (RT) range of 0.2 min, minimum relative height of 1%, minimum absolute threshold height of 3E3, minimum ratio for top/edge of 1, and a peak duration of 0.01-2 min. Pairing between MS1 and MS2 was performed with a mass tolerance of 0.01 *m/z* or 20ppm and RT range of 0.3 min. Isotope peaks were grouped, then features from different samples were aligned using the same mass and RT tolerances; alignment was performed by placing a weight of 75 on *m/z* and 25 on RT. A peak area feature table was exported as a .csv file and consensus MS/MS spectral data were exported in .mgf format.

Synthesis of pure conjugated bile acids

Materials: Organic solutions were concentrated under reduced pressure on a Büchi rotary evaporator using a water bath. Chromatographic purification of products was accomplished by flash chromatography on Silicycle F60 silica gel. All reactions were carried out in well ventilated fume hoods. Thin-layer chromatography (TLC) was performed on Silicycle 250 μm silica gel plates. Visualization of the developed chromatogram was performed by irradiation with 254 nm UV light or treatment with a solution of ceric ammonium molybdate stain followed by heating. Yields refer to purified compounds unless otherwise noted.

Instrumentation: ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 600 (600 and 151 MHz for ^{1}H and ^{13}C, respectively) instrument, and are internally referenced to residual protiosolvent signals of CD_3OD at δ 3.31 and 49.0 ppm and $(CD_3)_2SO$ at δ 2.50 and 39.51 ppm. Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), integration, multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), and coupling constant (Hz). Data for ^{13}C NMR are reported in terms of chemical shift and no special nomenclature is used for equivalent carbons.

Glutamate conjugated cholic acid (Glu-CA): $NaHCO_3$ was used as the inorganic base. Product was purified using 6-18% CH_3OH in CH_2Cl_2 with 1% acetic acid to obtain a 21% isolated yield as an off-white amorphous solid. ^{1}H NMR (600 MHz, CD_3OD) δ 4.37 (dd, J = 8.8, 5.0 Hz, 1H), 3.96

(t, *J* = 3.0 Hz, 1H), 3.80 (q, *J* = 3.0 Hz, 1H), 3.37 (tt, *J* = 11.2, 4.4 Hz, 1H), 2.38 (t, *J* = 7.7 Hz, 2H), 2.37 – 2.22 (m, 3H), 2.18 (ddd, *J* = 13.4, 9.3, 6.6 Hz, 2H), 2.05 – 1.78 (m, 6H), 1.79 – 1.70 (m, 1H), 1.69 – 1.62 (m, 1H), 1.63 – 1.50 (m, 5H), 1.49 – 1.29 (m, 6H), 1.12 (qd, *J* = 11.8, 5.7 Hz, 1H), 1.04 (d, *J* = 6.5 Hz, 3H), 0.98 (td, *J* = 14.2, 3.5 Hz, 1H), 0.92 (s, 3H), 0.72 (s, 3H); ^{13}C NMR (151 MHz, $(CD_3)_2SO$) δ 174.41, 172.68, 71.17, 70.57, 66.38, 51.74, 46.31, 45.86, 41.61, 41.46, 35.40, 35.29, 34.97, 34.49, 32.55, 31.82, 31.04, 31.00, 30.47, 28.63, 27.43, 27.24, 26.30, 22.93, 22.72, 17.23, 12.45; HRMS (ESI) exact mass calculated for [M+H]+ ($C_{29}H_{48}NO_8$) requires m/z 538.3375, found 538.3376 with a difference of 0.19 ppm.

Glutamate conjugated chenodeoxycholic acid (Glu-CDCA): $NaHCO_3$ was used as the inorganic base. Product was purified using 6-12% CH_3OH in CH_2Cl_2 with 1% acetic acid to obtain a 50% yield as an off-white amorphous solid. ^{1}H NMR (600 MHz, CD_3OD) δ 4.37 (t, *J* = 6.7 Hz, 1H), 3.79 (t, *J* = 3.3 Hz, 1H), 3.37 (tt, *J* = 10.5, 4.2 Hz, 1H), 2.38 (t, *J* = 7.5 Hz, 2H), 2.35 – 2.22 (m, 2H), 2.21 – 2.12 (m, 2H), 2.03 – 1.99 (m, 1H), 1.97 – 1.80 (m, 5H), 1.74 (dt, *J* = 13.3, 8.0 Hz, 1H), 1.69 – 1.58 (m, 2H), 1.54 – 1.45 (m, 5H), 1.39 – 1.27 (m, 7H), 1.23 – 1.15 (m, 2H), 1.15 – 1.06 (m, 1H), 0.99 (d, *J* = 6.1 Hz, 3H), 0.93 (s, 3H), 0.70 (s, 3H); ^{13}C NMR (151 MHz, $(CD_3)_2SO$) δ 174.34, 172.57, 70.43, 66.27, 55.71, 51.68, 50.08, 42.00, 41.49, 40.82, 35.37, 35.11, 34.88, 34.81, 32.37, 32.32, 31.67, 30.87, 30.59, 27.89, 27.13, 23.25, 22.79, 20.33, 18.43, 11.73; HRMS (ESI) exact mass calculated for [M+H]+ ($C_{29}H_{48}NO_7$) requires m/z 522.3426, found 522.3423 with a difference of 0.57 ppm.

Glutamate conjugated deoxycholic acid (Glu-DCA): $NaHCO_3$ was used as the inorganic base. Product was purified using 6-12% CH_3OH in CH_2Cl_2 with 1% acetic acid to obtain a 55% yield as an off-white amorphous solid. ^{1}H NMR (600 MHz, CD_3OD) δ 4.37 (dd, *J* = 8.4, 4.8 Hz, 1H), 3.96 (t, *J* = 3.1 Hz, 1H), 3.57 – 3.48 (m, 1H), 2.38 (t, *J* = 7.6 Hz, 2H), 2.35 – 2.29 (m, 1H), 2.21 – 2.12 (m, 2H), 1.96 – 1.75 (m, 8H), 1.66 – 1.57 (m, 3H), 1.55 – 1.50 (m, 2H), 1.48 – 1.23 (m, 9H), 1.17 (qd, *J* = 13.0, 3.9 Hz, 1H), 1.09 (dd, *J* = 12.1, 5.8 Hz, 1H), 1.03 (d, *J* = 6.5 Hz, 3H), 0.98 (td, *J* = 14.1, 3.4 Hz, 1H), 0.93 (s, 3H), 0.71 (s, 3H); ^{13}C NMR (151 MHz, $(CD_3)_2SO$) δ 174.36, 174.08, 172.70, 71.16, 70.07, 51.71, 47.55, 46.35, 46.08, 41.70, 36.34, 35.75, 35.22, 35.18, 33.91, 33.02, 32.48, 31.76, 30.84, 30.28, 28.68, 27.32, 27.08, 27.08, 26.20, 23.61, 23.18, 17.18, 12.54; HRMS (ESI) exact mass calculated for [M+H]+ ($C_{29}H_{48}NO_7$) requires m/z 522.3426, found 522.3427 with a difference of 0.19 ppm.

Isoleucine conjugated cholic acid (Ile-CA): Purified according to literature procedure[4] and characterization data is consistent with reported data.

Leucine conjugated cholic acid (Leu-CA): Purified according to literature procedure[4] and characterization data is consistent with reported data.

Methionine conjugated chenodeoxycholic acid (Met-CDCA): $NaHCO_3$ was used as the inorganic base. Product was purified using 3-6% CH_3OH in CH_2Cl_2 with 1% acetic acid to obtain a 79% yield as a white amorphous solid. ^{1}H NMR 600 MHz, CD_3OD) δ 4.53 (dd, *J* = 9.3, 4.6 Hz, 1H), 3.79 (q, *J* = 2.9 Hz, 1H), 3.37 (tt, *J* = 10.9, 4.2 Hz, 1H), 2.62 – 2.47 (m, 2H), 2.36 – 2.23 (m, 2H), 2.21 – 2.10 (m, 2H), 2.09 (s, 3H), 2.05 – 1.79 (m, 7H), 1.78 – 1.70 (m, 1H), 1.68 – 1.59 (m, 2H), 1.56 – 1.42 (m, 5H), 1.40 – 1.27 (m, 5H), 1.24 – 1.15 (m, 2H), 1.14-1.06 (m, 1H), 1.03 – 0.94 (m, 4H), 0.93 (s, 3H), 0.69 (s, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 176.93, 175.25, 72.83, 69.05, 57.34, 52.54, 51.50, 43.66, 43.12, 41.02, 40.73, 40.41, 36.80, 36.54, 36.19, 35.86, 34.01, 33.81, 33.21, 32.10, 31.32, 31.29, 29.28, 24.62, 23.42, 21.78, 18.94, 15.24, 12.23; HRMS (ESI) exact mass calculated for [M+H]+ ($C_{29}H_{50}NO_5S$) requires m/z 524.3404, found 524.3406 with a difference of 0.38 ppm.

<u>Methionine conjugated deoxycholic acid (Met-DCA):</u> $NaHCO_3$ was used as the inorganic base. Product was purified using 3-6% CH_3OH in CH_2Cl_2 with 1% acetic acid to obtain a 76% yield as a white amorphous solid. ^{1}H NMR (600 MHz, CD_3OD) δ 4.53 (dd, *J* = 9.3, 4.6 Hz, 1H), 3.96 (t, *J* = 3.0 Hz, 1H), 3.53 (tt, *J* = 11.2, 4.6 Hz, 1H), 2.62 – 2.55 (m, 1H), 2.55 – 2.48 (m, 1H), 2.37 – 2.28 (m, 1H), 2.24 – 2.10 (m, 2H), 2.09 (s, 3H), 1.99 – 1.93 (m, 1H), 1.93 – 1.73 (m, 7H), 1.65 – 1.57 (m, 3H), 1.57 – 1.49 (m, 2H), 1.49 – 1.37 (m, 6H), 1.36 – 1.30 (m, 1H), 1.30 – 1.25 (m, 2H), 1.22 – 1.06 (m, 2H), 1.03 (d, *J* = 6.5 Hz, 3H), 0.98 (td, *J* = 14.2, 3.2 Hz, 1H), 0.93 (s, 3H), 0.71 (s, 3H). ^{13}C NMR (151 MHz, CD_3OD) δ 177.0099, 175.27, 74.06, 72.52, 52.54, 49.24, 48.11, 47.54, 43.58, 37.42, 37.15, 36.72, 36.41, 35.27, 34.78, 33.80, 33.17, 32.11, 31.27, 31.01, 29.86, 28.67, 28.38, 27.43, 24.86, 23.72, 17.68, 15.22, 13.24; HRMS (ESI) exact mass calculated for [M+H]+ ($C_{29}H_{50}NO_5S$) requires m/z 524.3404, found 524.3405 with a difference of 0.19 ppm.

<u>Phenylalanine conjugated cholic acid (Phe-CA):</u> Purified according to literature procedure[4] and characterization data is consistent with reported data.

<u>Phenylalanine conjugated chenodeoxycholic acid (Phe-CDCA):</u> $NaHCO_3$ was used as the inorganic base. Product was purified using 3-6% CH_3OH in CH_2Cl_2 with 1% acetic acid to obtain a 94% yield as a white amorphous solid. ^{1}H NMR (600 MHz, CD_3OD) δ 7.29 – 7.17 (m, 5H), 4.64 (dd, *J* = 9.5, 4.9 Hz, 1H), 3.79 (q, *J* = 2.9 Hz, 1H), 3.41 – 3.33 (m, 1H), 3.22 (dd, *J* = 14.0, 4.8 Hz, 1H), 2.93 (dd, *J* = 13.9, 9.5 Hz, 1H), 2.27 (q, *J* = 11.7 Hz, 1H), 2.23 – 2.17 (m, 1H), 2.10 – 2.03 (m, 1H), 1.98 – 1.94 (m, 1H), 1.90 – 1.81 (m, 3H), 1.76 – 1.59 (m, 4H), 1.55 – 1.43 (m, 4H), 1.40 – 1.27 (m, 5H), 1.26 – 1.04 (m, 5H), 1.02 – 0.94 (m, 1H), 0.94 – 0.91 (m, 6H), 0.66 (s, 3H); ^{13}C NMR (151 MHz, $(CD_3)_2SO$) δ 173.41, 172.64, 137.99, 129.15, 128.10, 126.32, 70.41, 66.23, 55.60, 53.59, 50.05, 41.94, 41.47, 36.87, 35.36, 35.01, 34.87, 34.78, 32.34, 32.15, 31.54, 30.59, 27.79, 23.22, 22.77, 20.31, 18.35, 11.70; HRMS (ESI) exact mass calculated for [M+H]+ ($C_{33}H_{50}NO_5$) requires m/z 540.3684, found 540.3683 with a difference of 0.19 ppm.

<u>Phenylalanine conjugated deoxycholic acid (Phe-DCA):</u> $NaHCO_3$ was used as the inorganic base. Product was purified using 3-6% CH_3OH in CH_2Cl_2 with 1% acetic acid to obtain a 99% yield as a white amorphous solid. ^{1}H NMR (600 MHz, CD_3OD) δ 7.30 – 7.17 (m, 5H), 4.65 (dd, *J* = 9.3, 4.9 Hz, 1H), 3.94 (t, *J* = 3.0 Hz, 1H), 3.57 – 3.48 (m, 1H), 3.21 (dd, *J* = 13.9, 4.9 Hz, 1H), 2.94 (dd, *J* = 13.9, 9.3 Hz, 1H), 2.25 – 2.17 (m, 1H), 2.11 – 2.03 (m, 1H), 1.95 – 1.84 (m, 2H), 1.85 – 1.74 (m, 4H), 1.71 – 1.62 (m, 1H), 1.64 – 1.55 (m, 3H), 1.54 – 1.48 (m, 2H), 1.50 – 1.32 (m, 6H), 1.31 – 1.24 (m, 1H), 1.24 – 1.11 (m, 3H), 1.12 – 1.01 (m, 1H), 1.02 – 0.93 (m, 4H), 0.93 (s, 3H), 0.67 (s, 3H); ^{13}C NMR (151 MHz, $(CD_3)_2SO$) δ 173.42, 172.74, 137.97, 129.15, 128.11, 126.33, 71.11, 70.04, 53.56, 48.66, 47.52, 46.22, 46.01, 41.68, 36.86, 36.33, 35.72, 35.21, 35.08, 33.87, 32.98, 32.27, 31.63, 30.27, 28.66, 27.21, 27.05, 26.18, 23.57, 23.14, 21.14, 17.10, 12.49; HRMS

(ESI) exact mass calculated for [M+H]+ ($C_{33}H_{50}NO_5$) requires m/z 540.3684, found 540.3684 with a difference of 0.00 ppm.

Threonine conjugated chollc acid (Thr-CA): $NaHCO_3$ was used as the inorganic base. Product was purified using 6-12% CH_3OH in CH_2Cl_2 with 1% acetic acid to obtain a 97% yield as a white amorphous solid. ^1H NMR (600 MHz, CD_3OD) δ 4.37 (s, 1H), 4.28 (s, 1H), 3.96 (t, *J* = 3.0 Hz, 1H), 3.80 (q, *J* = 3.0 Hz, 1H), 3.37 (tt, *J* = 11.4, 4.5 Hz, 1H), 2.39 (ddd, *J* = 14.7, 10.1, 5.1 Hz, 1H), 2.33 – 2.19 (m, 3H), 2.04 – 1.96 (m, 2H), 1.96 – 1.79 (m, 4H), 1.78 – 1.71 (m, 1H), 1.68 – 1.63 (m, 1H), 1.63 – 1.51 (m, 5H), 1.48 – 1.28 (m, 5H), 1.18 (d, *J* = 6.3 Hz, 3H), 1.16 – 1.07 (m, 1H), 1.05 (d, *J* = 6.5 Hz, 3H), 0.98 (td, *J* = 14.2, 3.5 Hz, 1H), 0.92 (s, 3H), 0.72 (s, 3H). ^{13}C NMR (151 MHz, CD_3OD) δ 176.98, 74.04, 72.86, 69.08, 68.75, 48.08, 47.48, 43.15, 42.96, 40.97, 40.42, 36.91, 36.46, 35.87, 35.82, 34.08, 33.21, 31.14, 29.52, 28.68, 27.84, 24.22, 23.14, 20.26, 17.77, 13.00; ^{13}C NMR (151 MHz, $(CD_3)_2SO$) δ 173.13, 71.28, 70.66, 66.73, 66.47, 46.41, 45.93, 41.67, 41.52, 35.46, 35.43, 35.02, 34.55, 32.72, 31.97, 30.50, 28.67, 27.51, 26.36, 23.01, 22.77, 19.92, 17.29, 12.52; HRMS (ESI) exact mass calculated for [M+H]+ ($C_{28}H_{48}NO_7$) requires m/z 510.3426, found 510.3424 with a difference of 0.39 ppm.

Tyrosine conjugated cholic acid (Tyr-CA): Purified according to literature procedure[4] and characterization data is consistent with reported data.

Tyrosine conjugated chenodeoxycholic acid (Tyr-CDCA): NaOH was used as the inorganic base. Product was purified using 3-12% CH_3OH in CH_2Cl_2 with 1% acetic acid to obtain a 74% yield as a white amorphous solid. ^1H NMR (600 MHz, CD_3OD) δ 7.03 (d, *J* = 8.5 Hz, 2H), 6.69 (d, *J* = 8.5 Hz, 2H), 4.58 (dd, *J* = 9.3, 4.9 Hz, 1H), 3.80 (q, *J* = 3.0 Hz, 1H), 3.41 – 3.33 (m, 1H), 3.11 (dd, *J* = 14.0, 4.9 Hz, 1H), 2.84 (dd, *J* = 14.0, 9.3 Hz, 1H), 2.31 – 2.17 (m, 2H), 2.11 – 2.02 (m, 1H), 2.02 – 1.93 (m, 2H), 1.91 – 1.81 (m, 3H), 1.77 – 1.58 (m, 4H), 1.55 – 1.43 (m, 4H), 1.43 – 1.04 (m, 9H), 0.98 (td, *J* = 14.2, 3.4 Hz, 1H), 0.93 (t, *J* = 3.3 Hz, 6H), 0.67 (s, 3H). ^{13}C NMR (151 MHz, CD_3OD) δ 176.67, 175.21, 157.21, 131.22, 129.19, 116.15, 72.85, 69.10, 57.31, 55.23, 51.49, 43.64, 43.13, 41.01, 40.72, 40.43, 37.62, 36.80, 36.52, 36.19, 35.85, 34.02, 33.84, 33.23, 31.32, 29.20, 24.61, 23.38, 21.76, 18.85, 12.17; HRMS (ESI) exact mass calculated for [M+H]+ ($C_{33}H_{50}NO_6$) requires m/z 556.3633, found 556.3634 with a difference of 0.18 ppm.

Supplementary Tables

Supplementary Table 1: Number of unique spectral matches per compound for Q-ToF data in positive ionization mode.

	aMCA	bMCA	CA	CDCA	DCA	gMCA	HDCA	UDCA
Ala	241	4	342	418	420	74	264	40
Arg	180	101	409	38	38	103	44	72
Asn	152	159	55	2	35	159	0	2
Asp	39	36	39	0	8	39	2	0
Cit	33	33	64	224	226	33	224	224
Cys	0	N/A	4	0	0	0	0	0
DOPA	0	0	0	0	0	0	0	0
Gln	13	8	20	4	4	11	4	4
Glu	77	48	181	164	170	76	137	N/A
His	720	673	605	242	239	724	244	244
Ile/Leu	982	601	757	1563	1590	920	1376	1420
Lys	1181	963	1250	551	579	672	548	626
Met	115	115	10	204	214	115	217	207
Orn	305	199	215	80	83	203	73	84
Phe	1658	1453	1507	2629	2629	1544	2517	2624
Pro	0	0	0	0	0	0	0	0
Ser	1	3	44	30	32	24	30	30
Thr	349	328	139	7	22	340	0	0
Trp	446	420	518	1286	1286	502	1283	1283
Tyr	568	465	563	431	431	534	412	436
Val	0	0	48	8	4	0	4	4

Supplementary Table 2: Number of unique spectral matches per compound for QE data in positive ionization mode.

	aMCA	bMCA	CA	CDCA	DCA	gMCA	HDCA	UDCA
Ala	27	27	68	139	301	27	165	45
Arg	217	235	366	46	51	217	46	48
Asn	152	152	28	6	4	152	4	4
Asp	36	36	3	2	8	36	2	2
Cit	2	2	34	188	187	2	188	188
Cys	N/A	N/A	N/A	N/A	0	N/A	N/A	N/A
DOPA	0	0	0	0	0	0	0	0
Gln	8	2	15	2	2	8	2	2
Glu	51	36	113	149	178	111	136	N/A
His	595	591	595	244	244	595	244	225
Ile/Leu	595	574	712	1476	1548	584	1434	1414
Lys	517	464	856	548	570	141	542	548
Met	115	97	10	199	198	115	198	198
Orn	203	196	422	80	83	23	36	83
Phe	1509	639	1407	2513	2513	1274	2450	2526
Pro	0	0	0	0	0	0	0	0
Ser	8	4	37	36	32	11	28	30
Thr	231	235	108	5	35	229	5	10
Trp	499	471	506	1283	1284	495	1268	1283
Tyr	486	361	563	333	324	355	310	333
Val	0	15	16	26	42	0	4	4

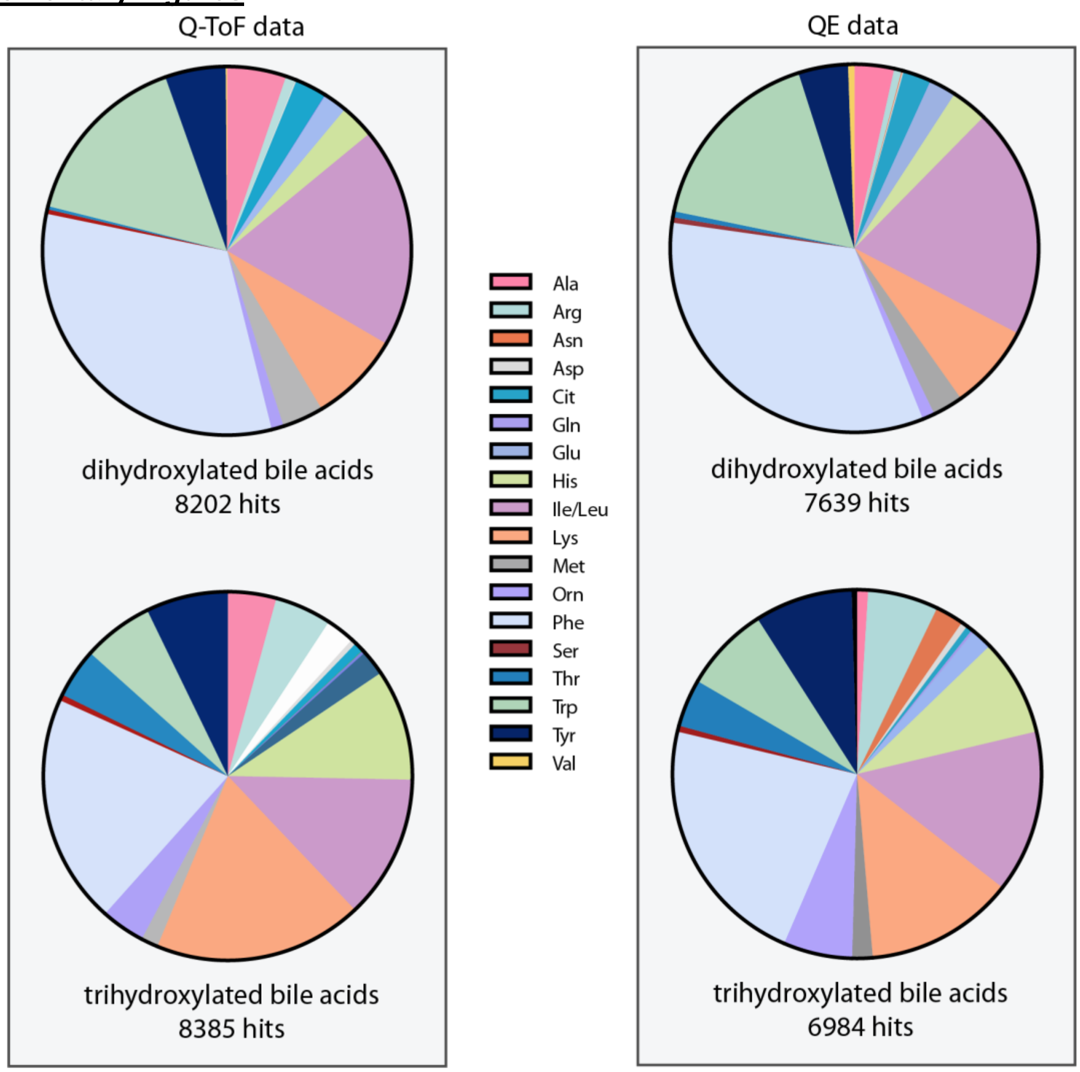
Supplementary Table 3: Number of unique spectral matches per compound for Q-ToF data in negative ionization mode. There is very little negative mode data in the public domain resulting in very few matches.

	aMCA	bMCA	CA	CDCA	DCA	gMCA	HDCA	UDCA
Ala	0	0	0	0	0	0	0	0
Arg	0	0	0	0	0	0	0	0
Asn	0	0	0	0	2	0	0	0
Asp	0	0	0	0	0	0	0	0
Cit	0	0	0	0	0	0	0	0
Cys	0	0	0	0	0	0	0	0
DOPA	0	0	0	0	0	0	0	0
Gln	0	0	0	0	0	0	0	0
Glu	0	0	0	0	0	0	0	0
His	0	0	0	0	0	0	0	0
Ile/Leu	0	0	0	0	0	0	0	0
Lys	0	0	0	0	0	0	0	0
Met	0	0	0	0	0	0	0	0
Orn	0	0	0	0	0	0	0	0
Phe	0	0	0	0	0	0	0	4
Pro	0	0	0	0	0	0	0	0
Ser	0	0	0	0	0	0	0	0
Thr	0	0	0	0	0	0	0	0
Trp	0	0	0	0	0	0	0	0
Tyr	0	0	0	0	0	0	0	2
Val	0	0	0	0	0	0	0	0

Supplementary Table 4: Organisms, bile acids and intensities. Provided in separate extended tables.

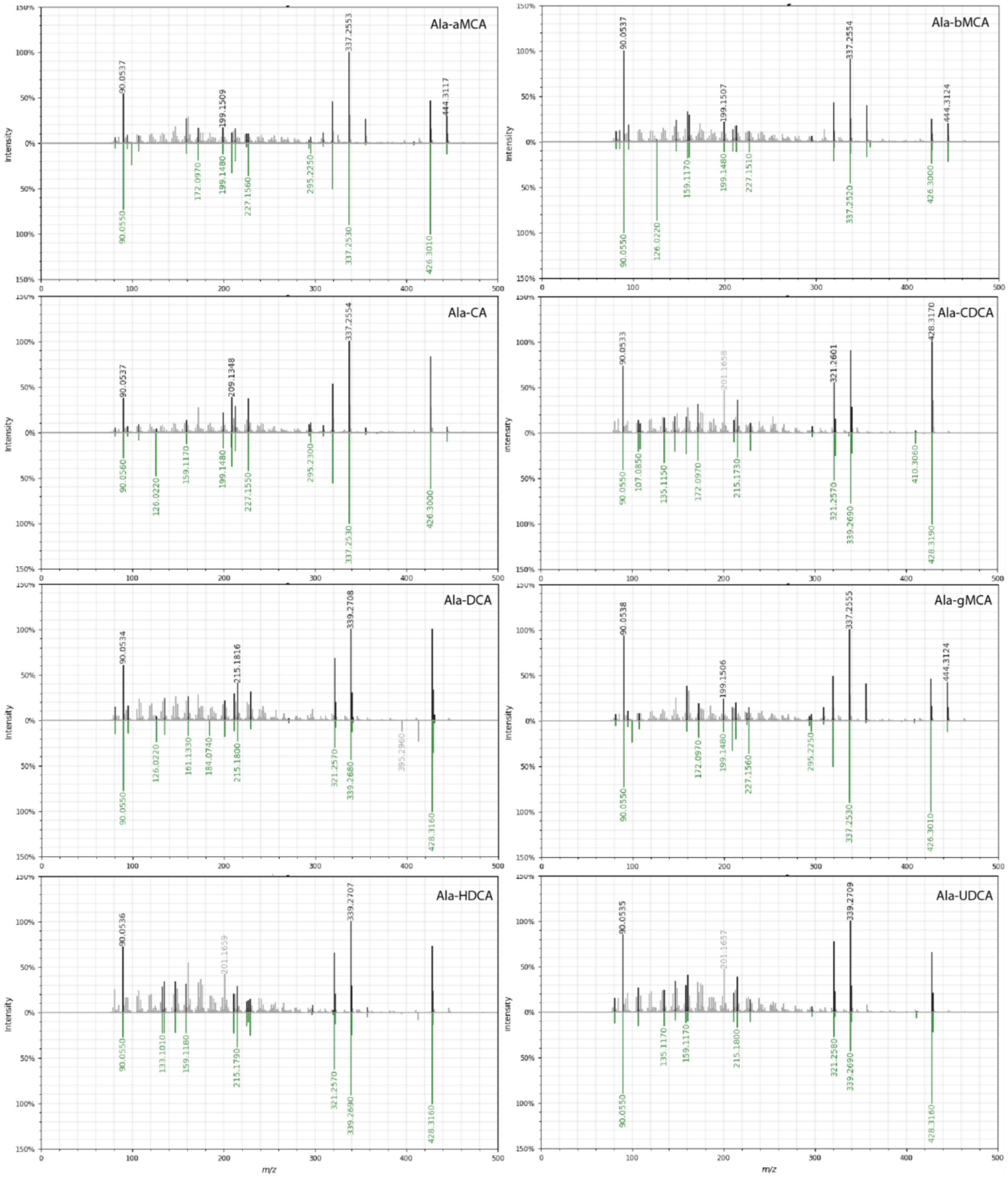
Supplementary Table 5: Universal spectrum identifiers and MASST job links for all conjugated BAs in synthetic mixtures. Provided in separate extended tables.

Supplementary Figures

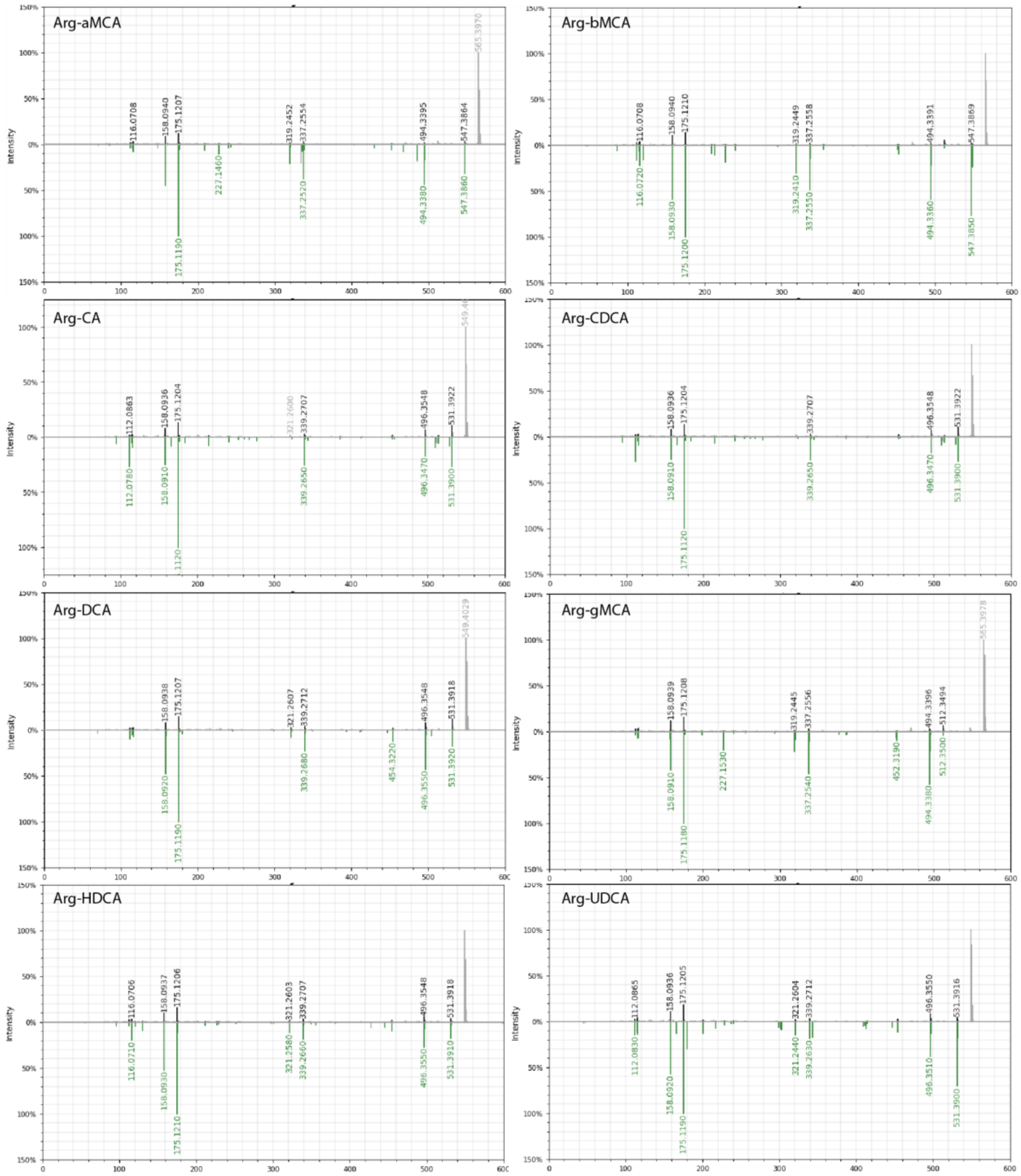


Supplementary Fig. 1: Comparison of amino acid distributions for MASST searches of Orbitrap (Thermo QE) vs. Q-ToF (Bruker MaXis) data in positive ionization mode.

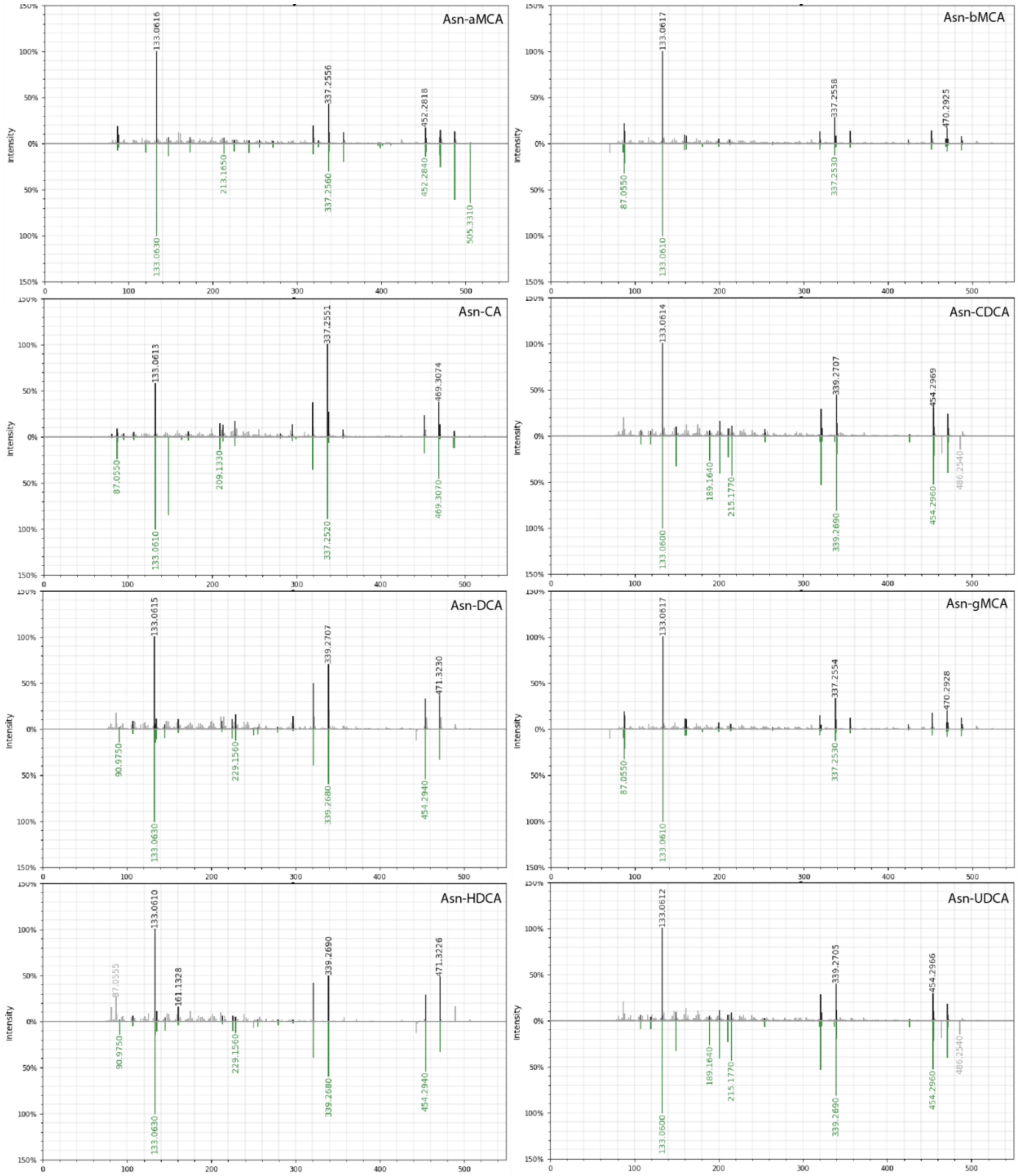
All mirror plots were produced with Metabolomics Spectral Resolver available at https://metabolomics-usi.ucsd.edu/[5].



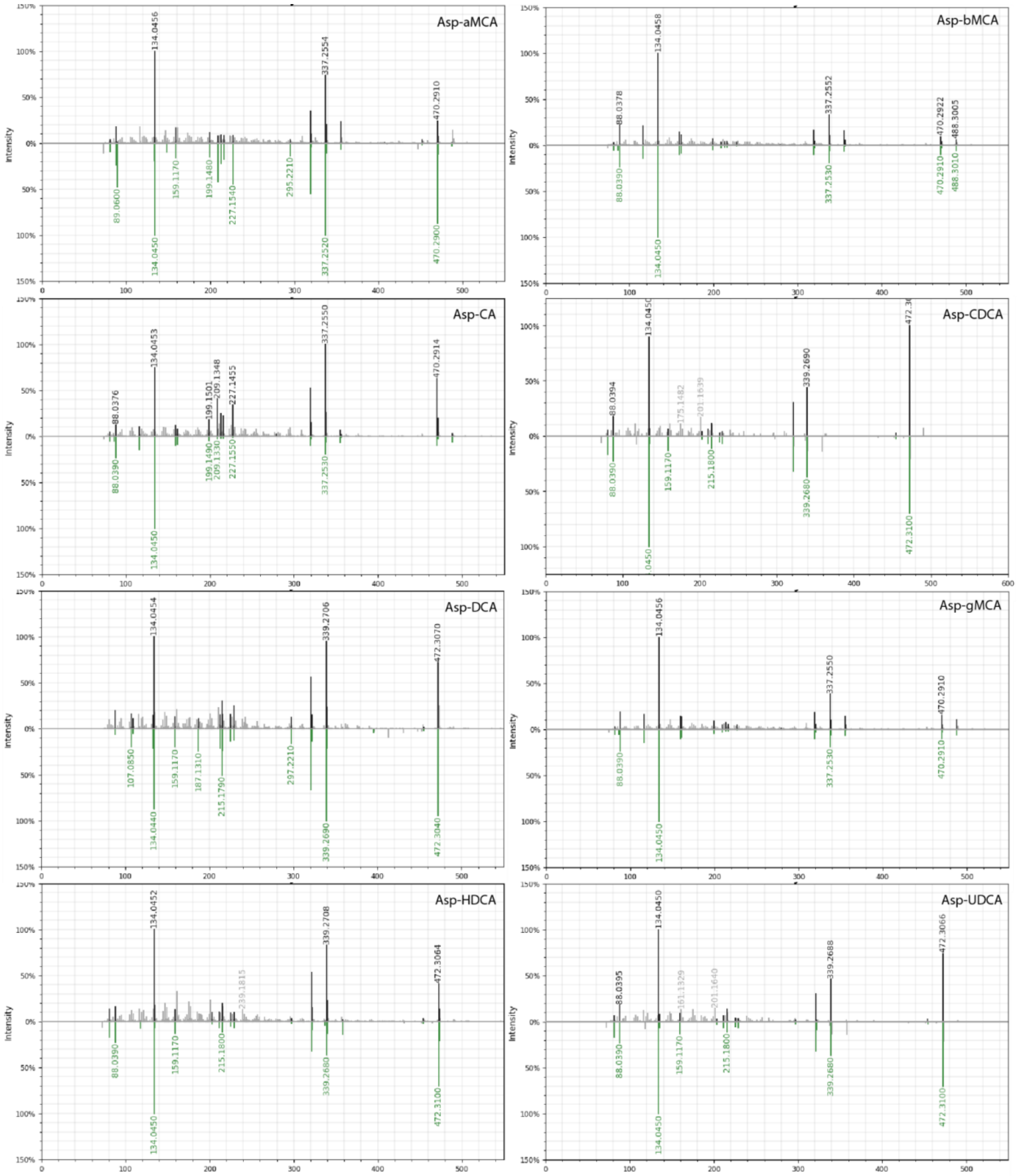
Supplementary Fig. 2: Mirror plots for MS/MS matches to Ala conjugated bile acids.



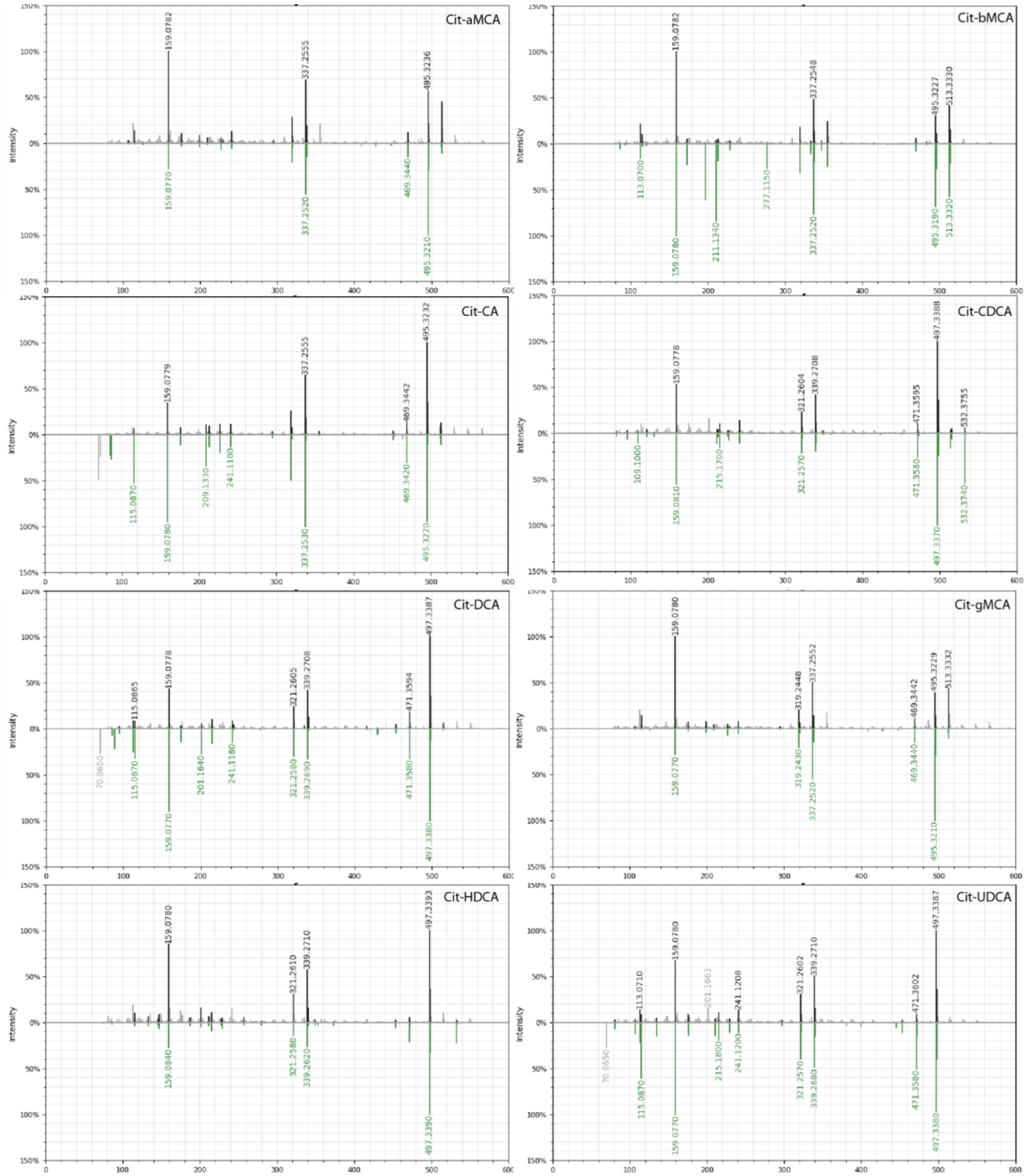
Supplementary Fig. 3: Mirror plots for MS/MS matches to Arg conjugated bile acids.



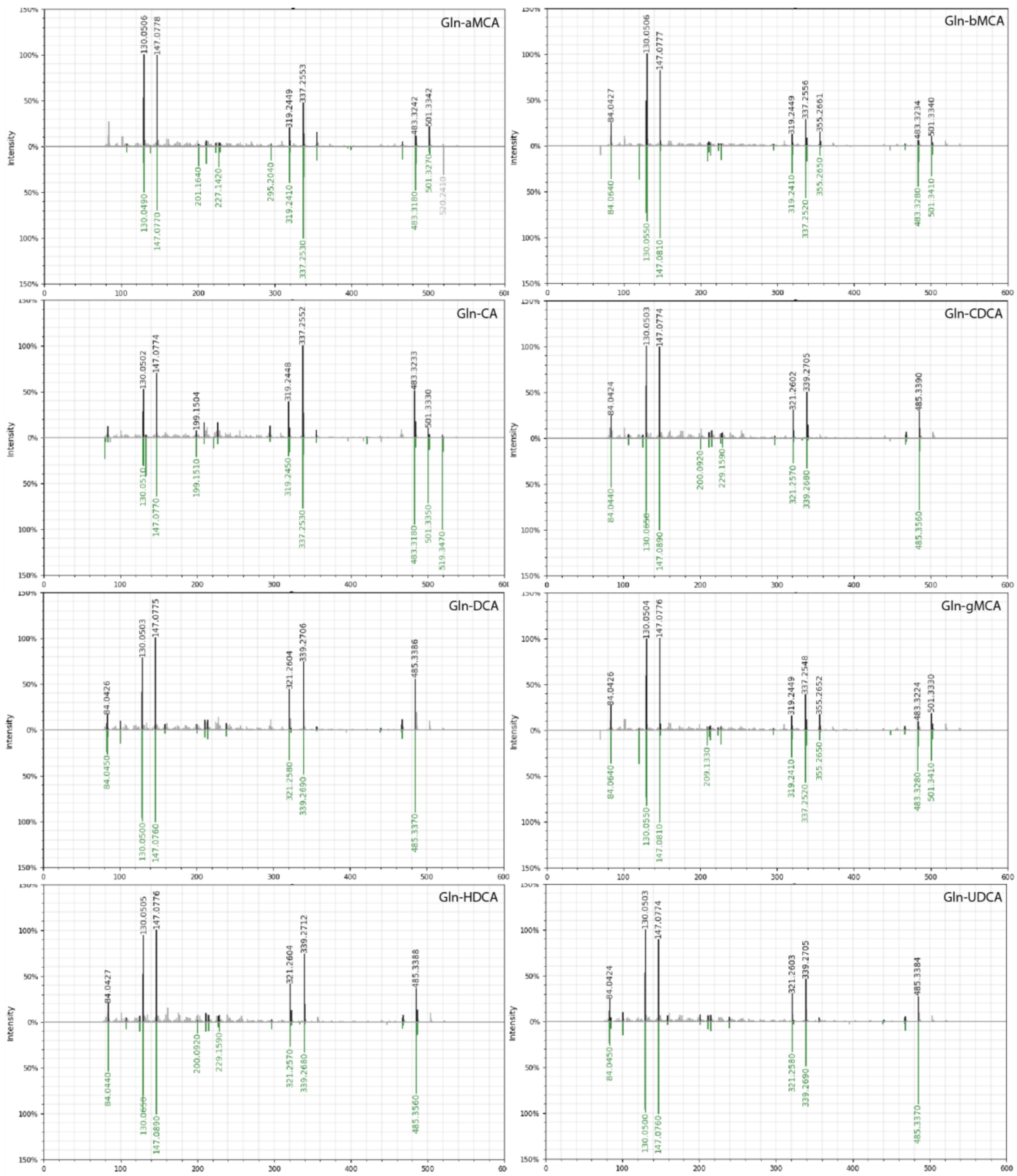
Supplementary Fig. 4: Mirror plots for MS/MS matches to Asn conjugated bile acids.



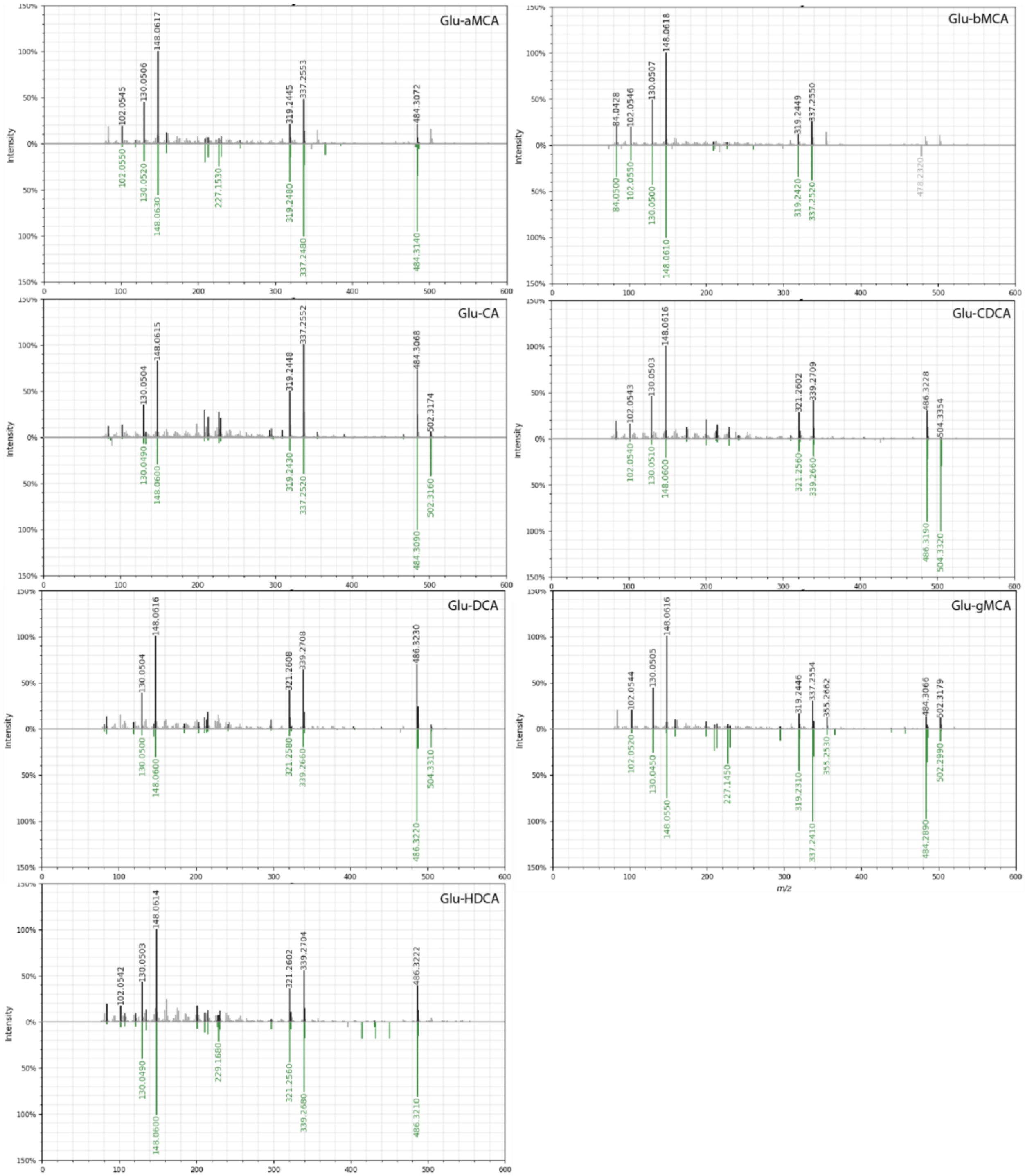
Supplementary Fig. 5: Mirror plots for MS/MS matches to Asp conjugated bile acids.



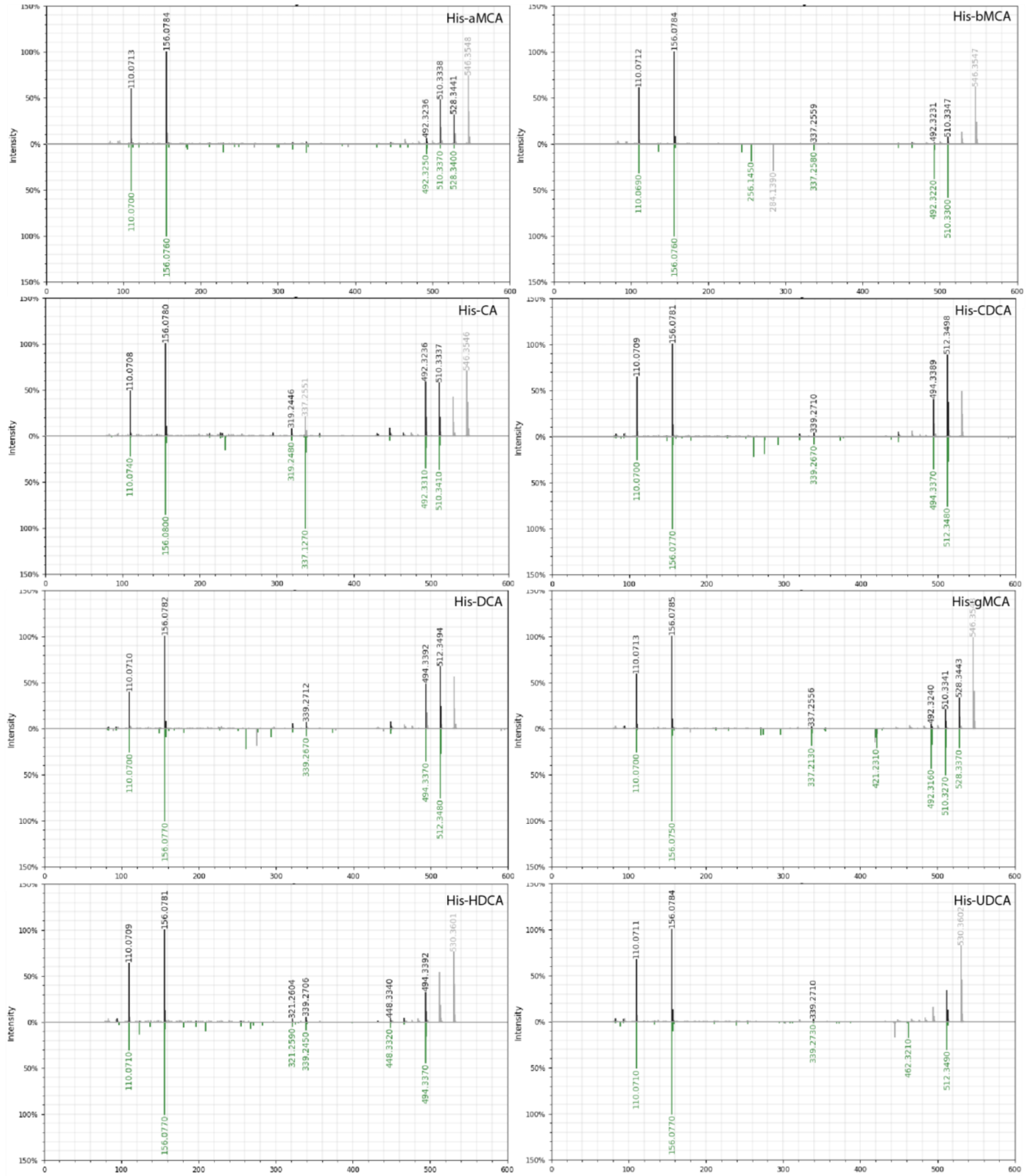
Supplementary Fig. 6: Mirror plots for MS/MS matches to Cit conjugated bile acids.



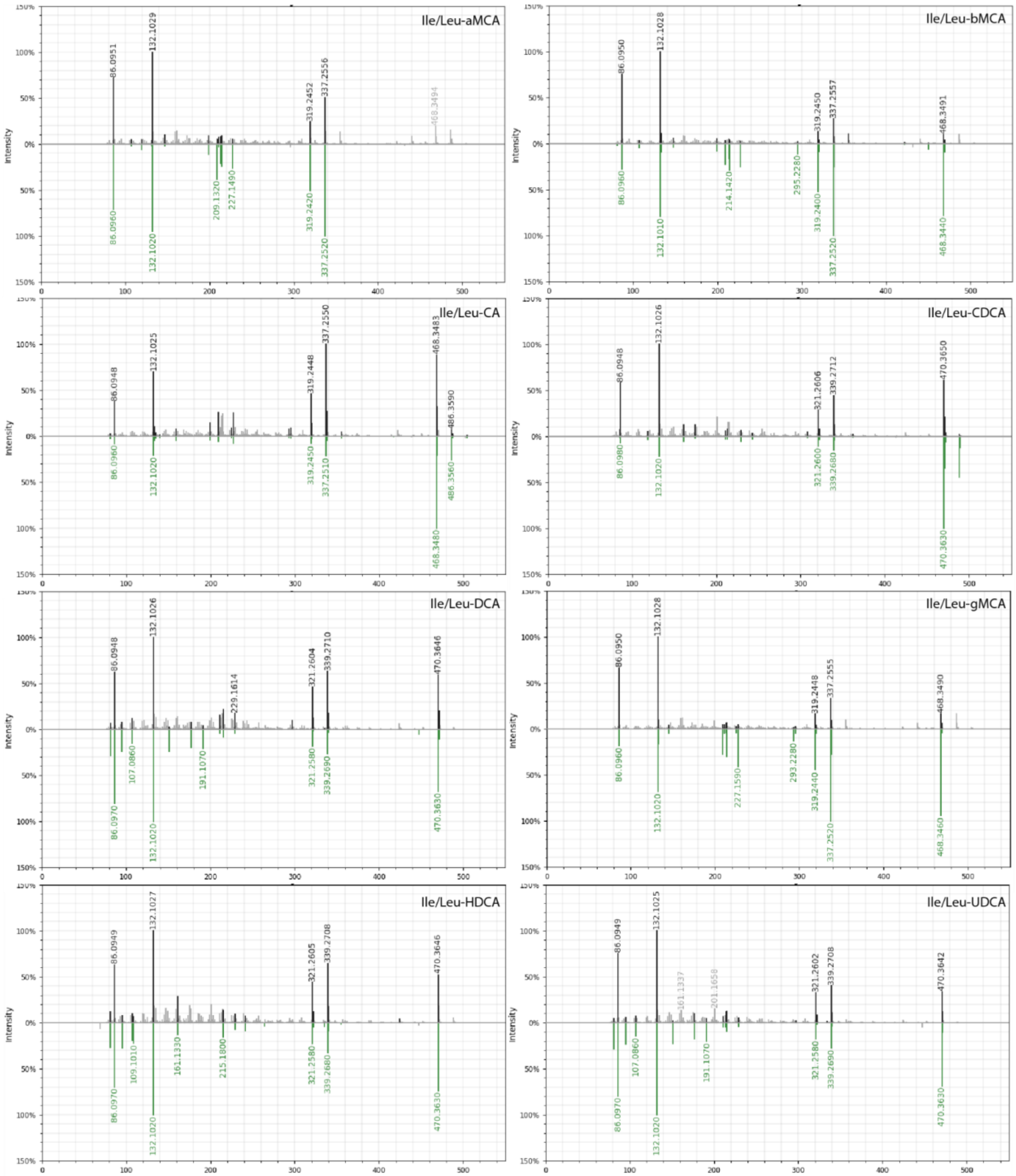
Supplementary Fig. 7: Mirror plots for MS/MS matches to Gln conjugated bile acids.



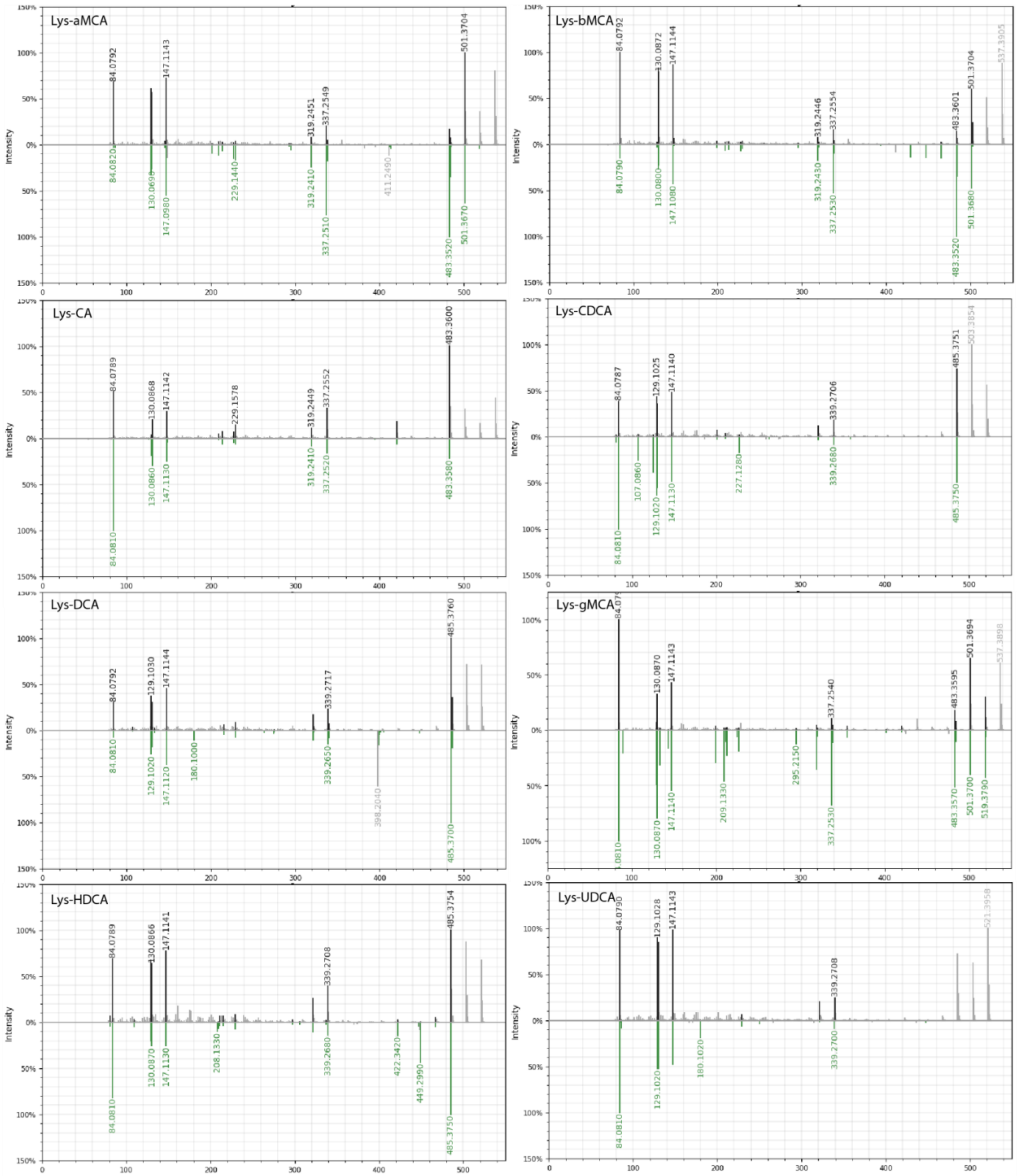
Supplementary Fig. 8: Mirror plots for MS/MS matches to Glu conjugated bile acids.



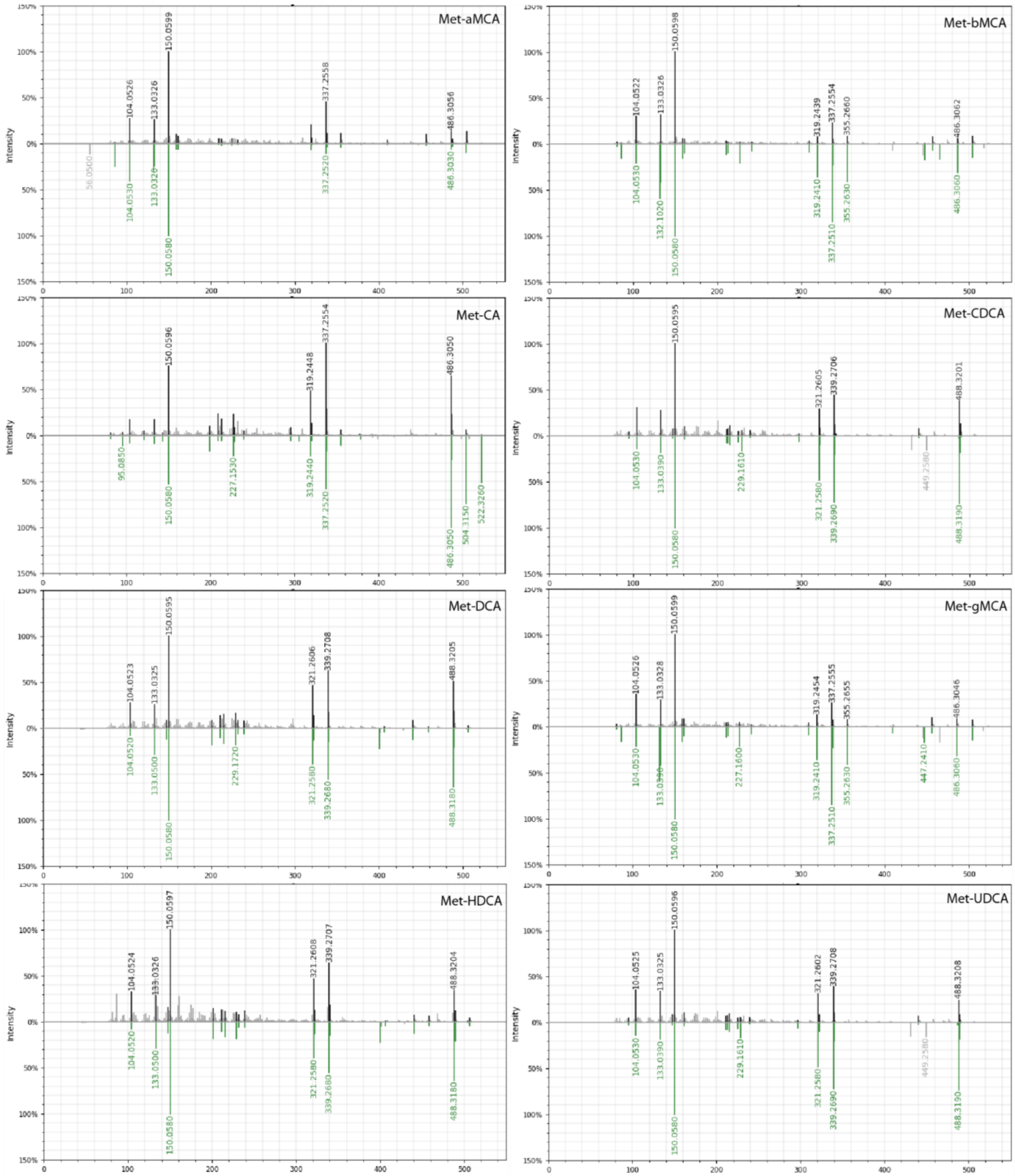
Supplementary Fig. 9: Mirror plots for MS/MS matches to His conjugated bile acids.



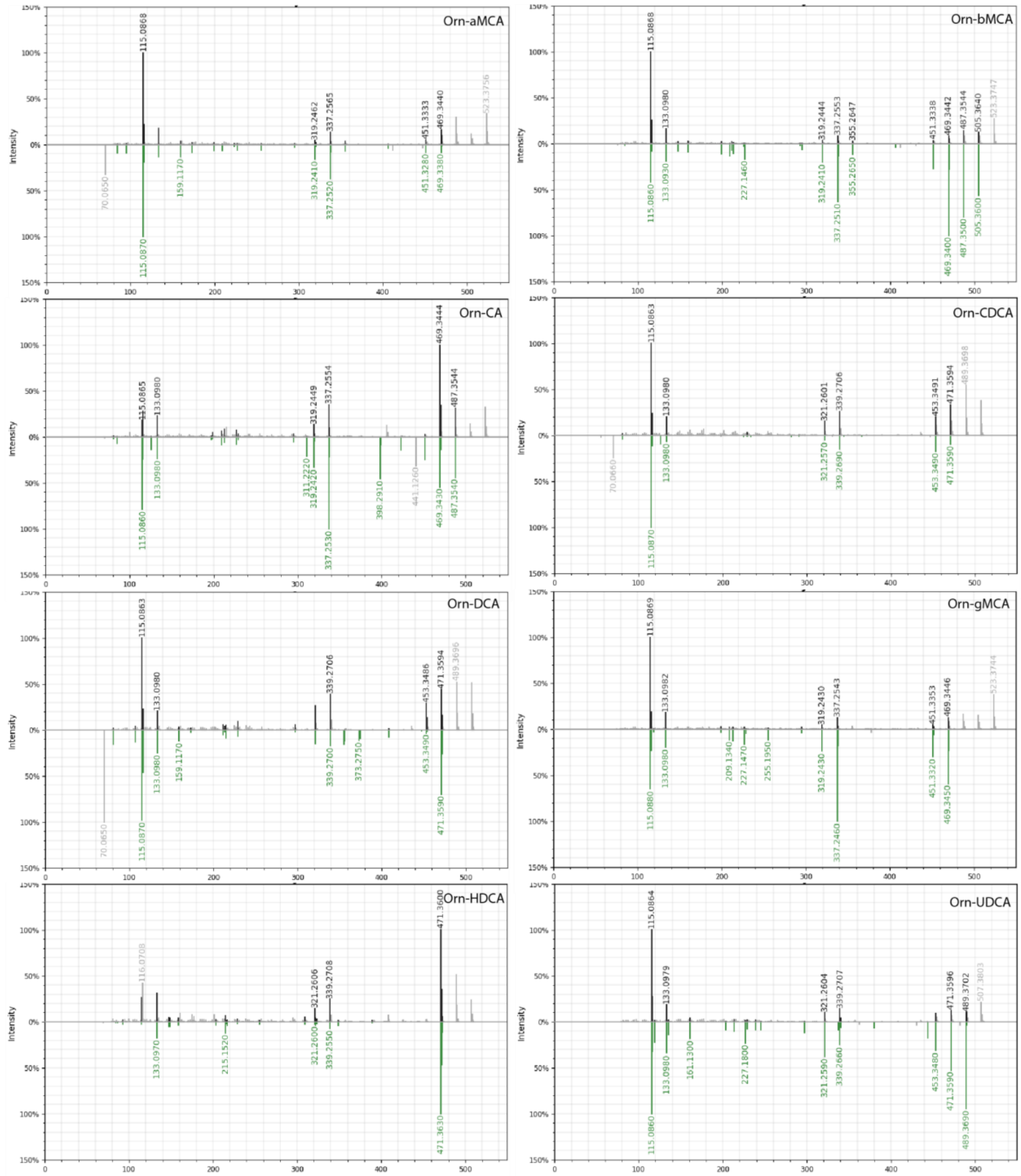
Supplementary Fig. 10: Mirror plots for MS/MS matches to Ile/Leu conjugated bile acids.



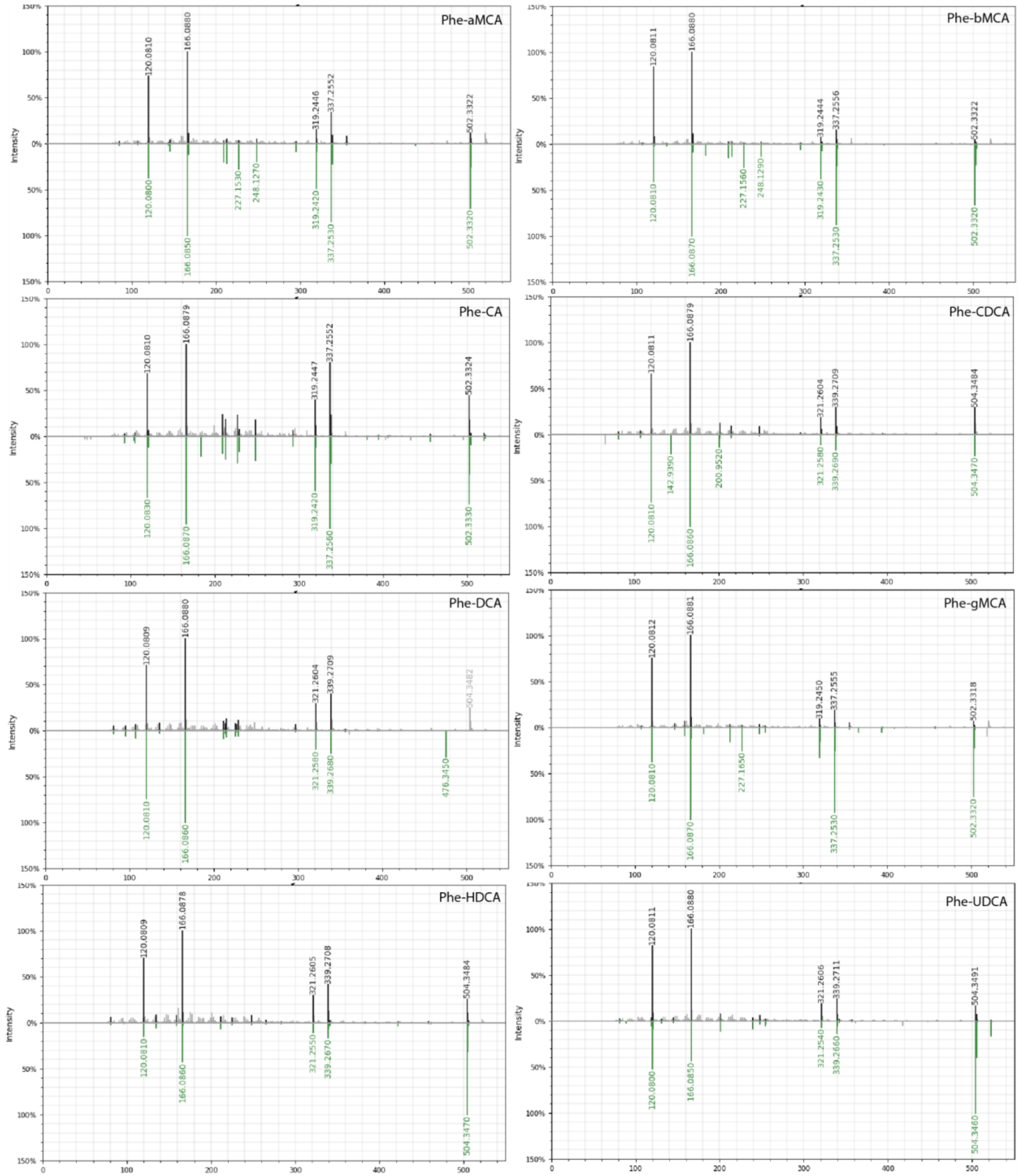
Supplementary Fig. 11: Mirror plots for MS/MS matches to Lys conjugated bile acids.



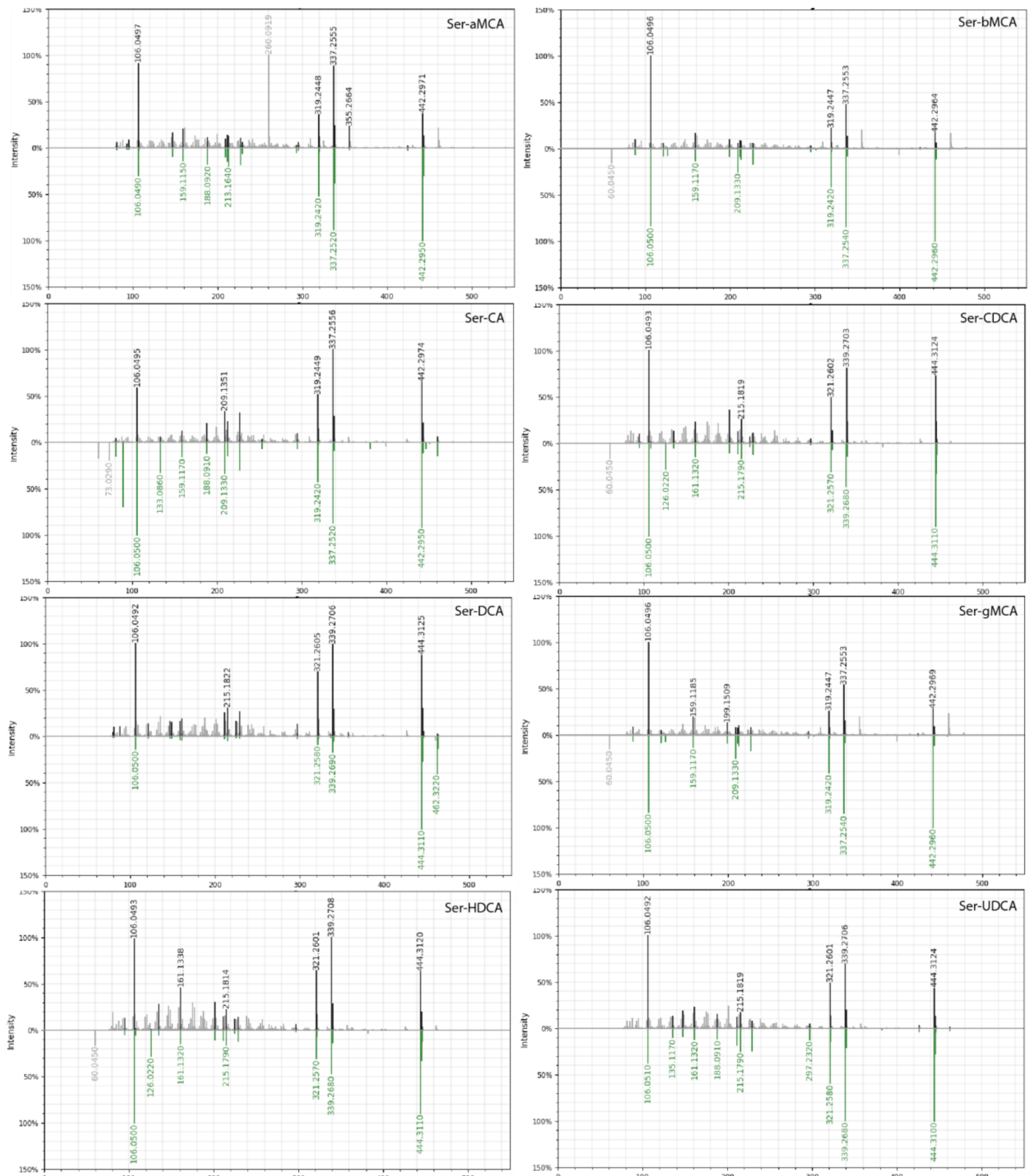
Supplementary Fig. 12: Mirror plots for MS/MS matches to Met conjugated bile acids.



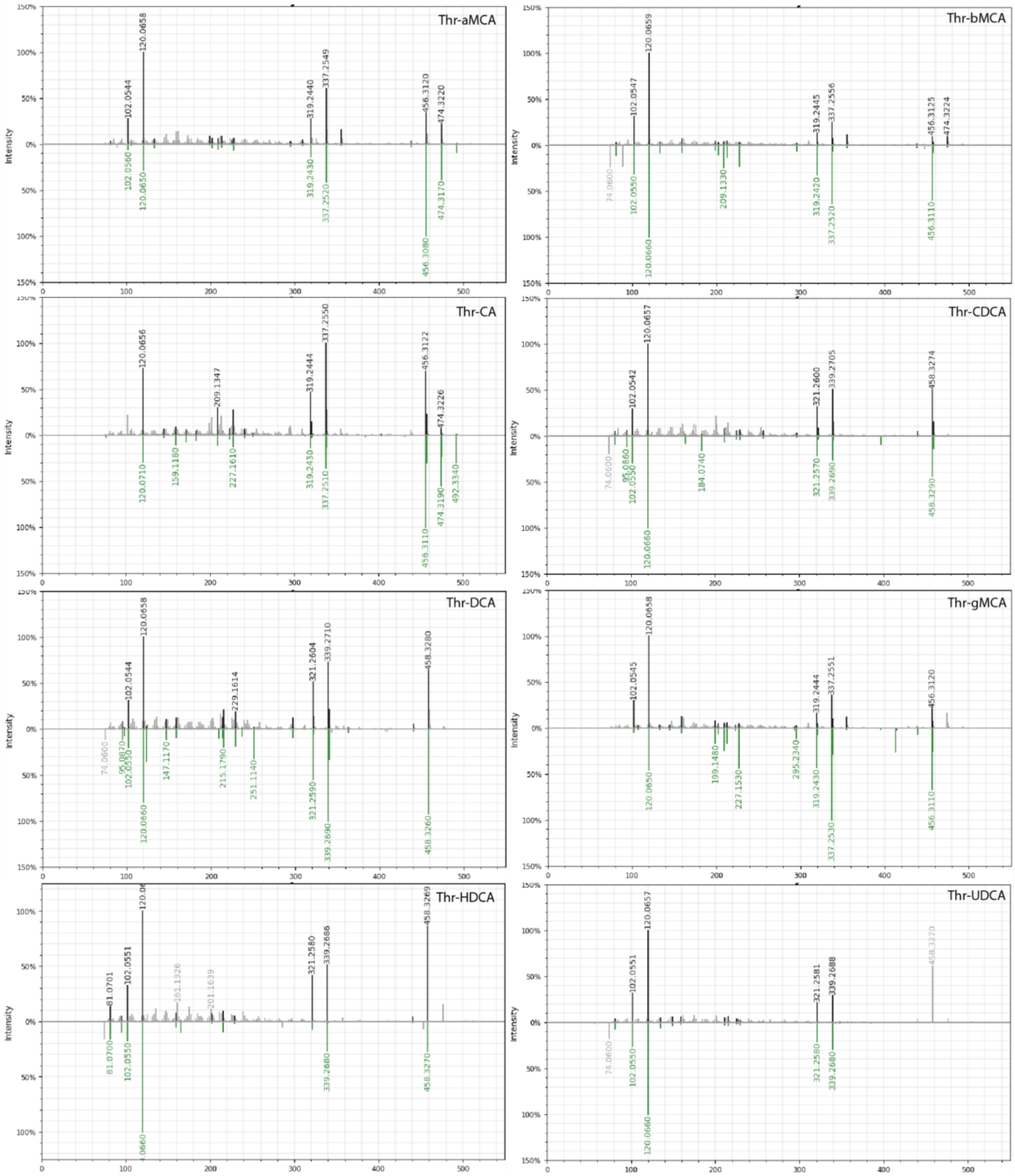
Supplementary Fig. 13: Mirror plots for MS/MS matches to Orn conjugated bile acids.



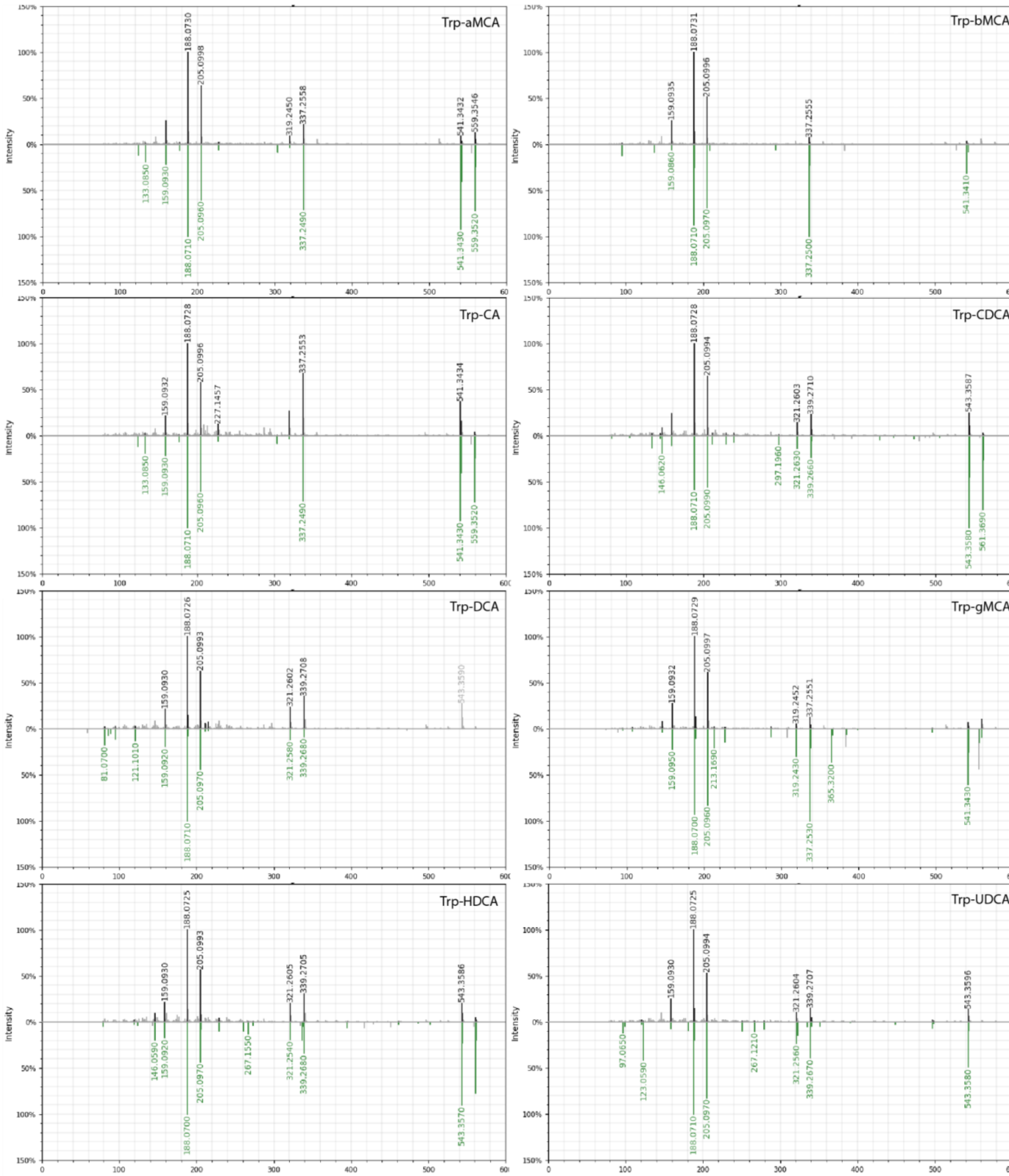
Supplementary Fig. 14: Mirror plots for MS/MS matches to Phe conjugated bile acids.



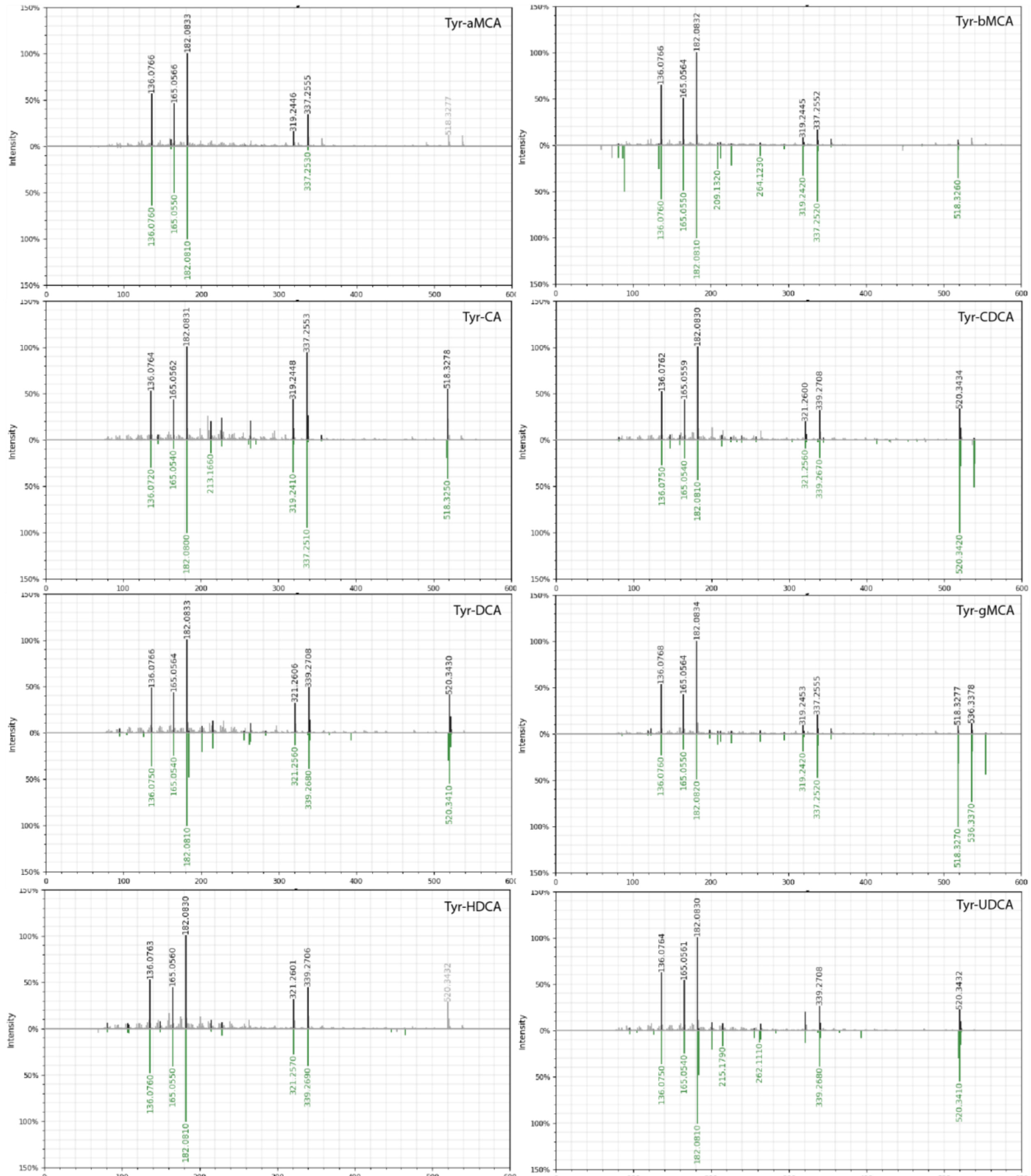
Supplementary Fig. 15: Mirror plots for MS/MS matches to Ser conjugated bile acids.



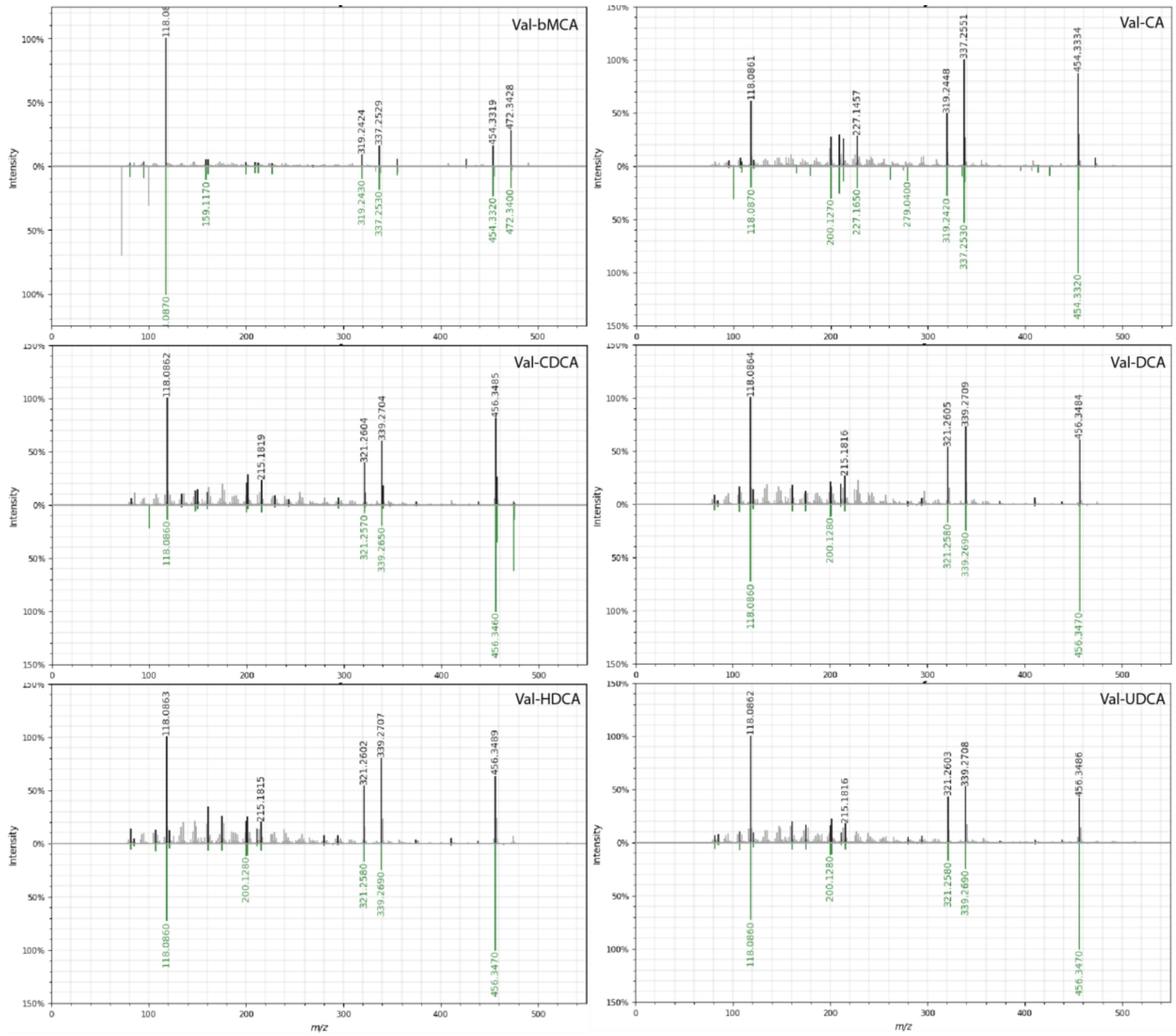
Supplementary Fig. 16: Mirror plots for MS/MS matches to Thr conjugated bile acids.



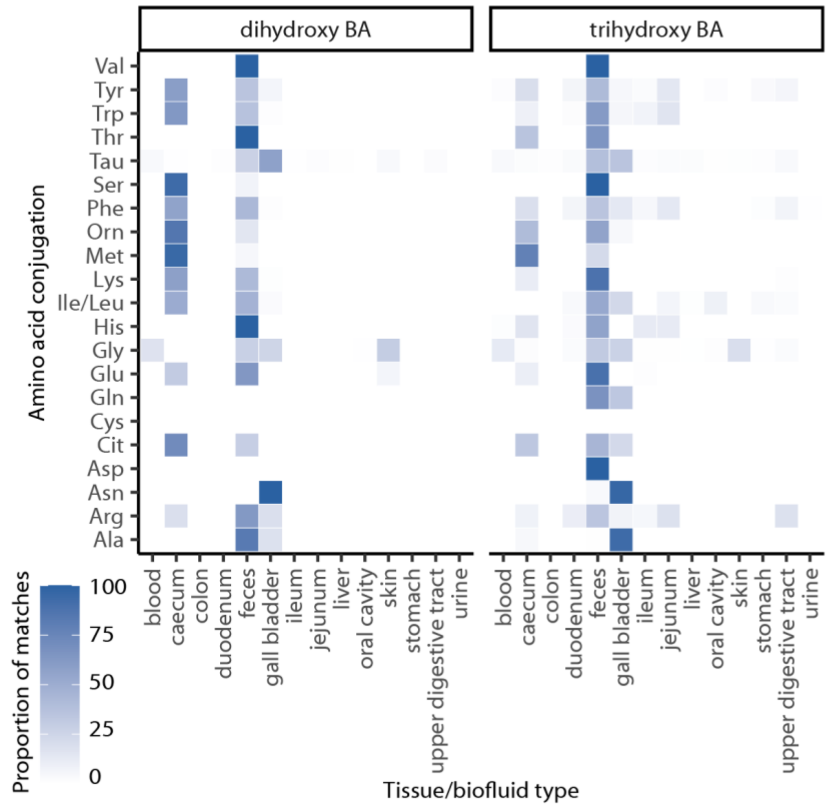
Supplementary Fig. 17: Mirror plots for MS/MS matches to Trp conjugated bile acids.



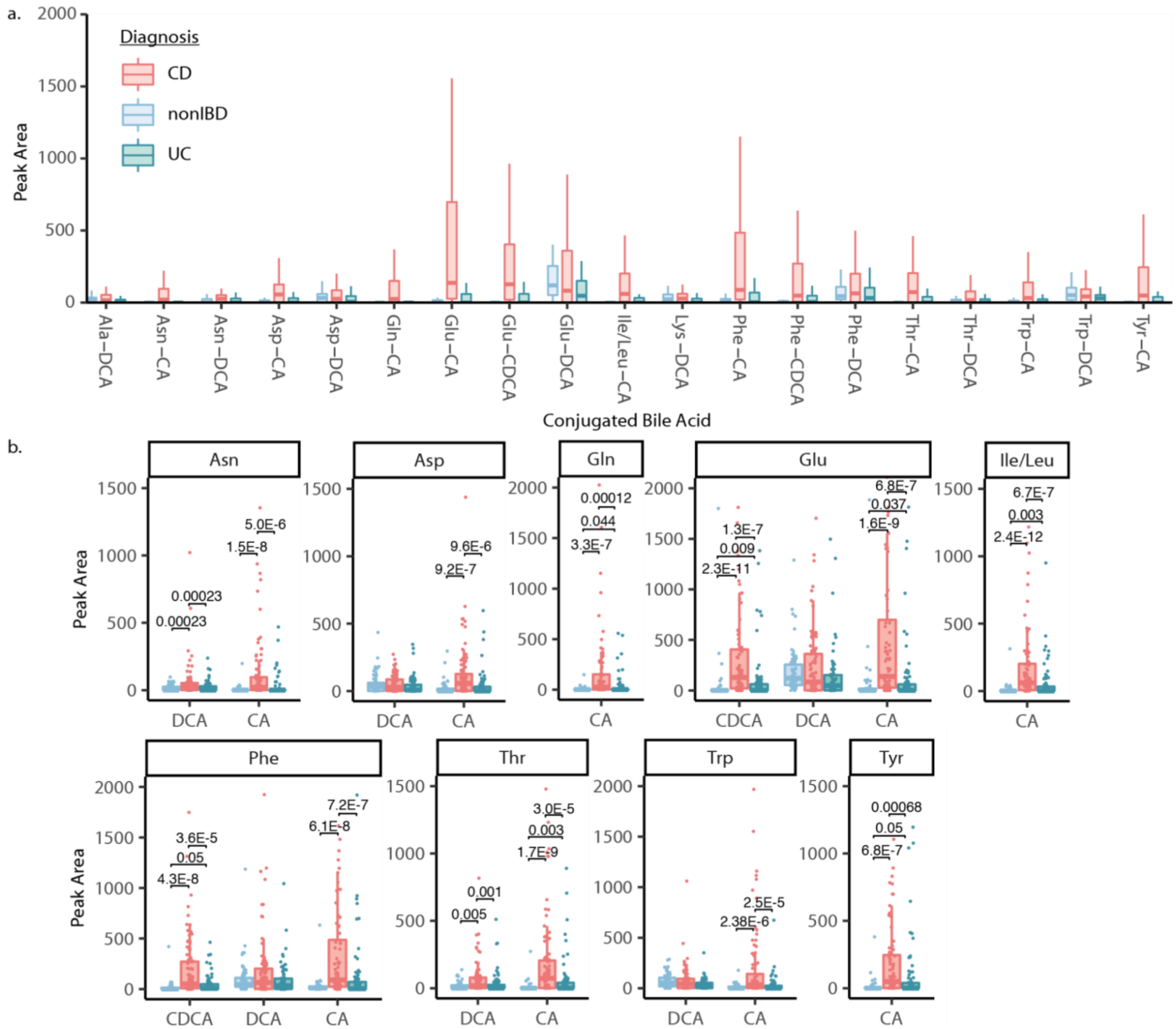
Supplementary Fig. 18: Mirror plots for MS/MS matches to Tyr conjugated bile acids.



Supplementary Fig. 19: Mirror plots for MS/MS matches to Val conjugated bile acids.



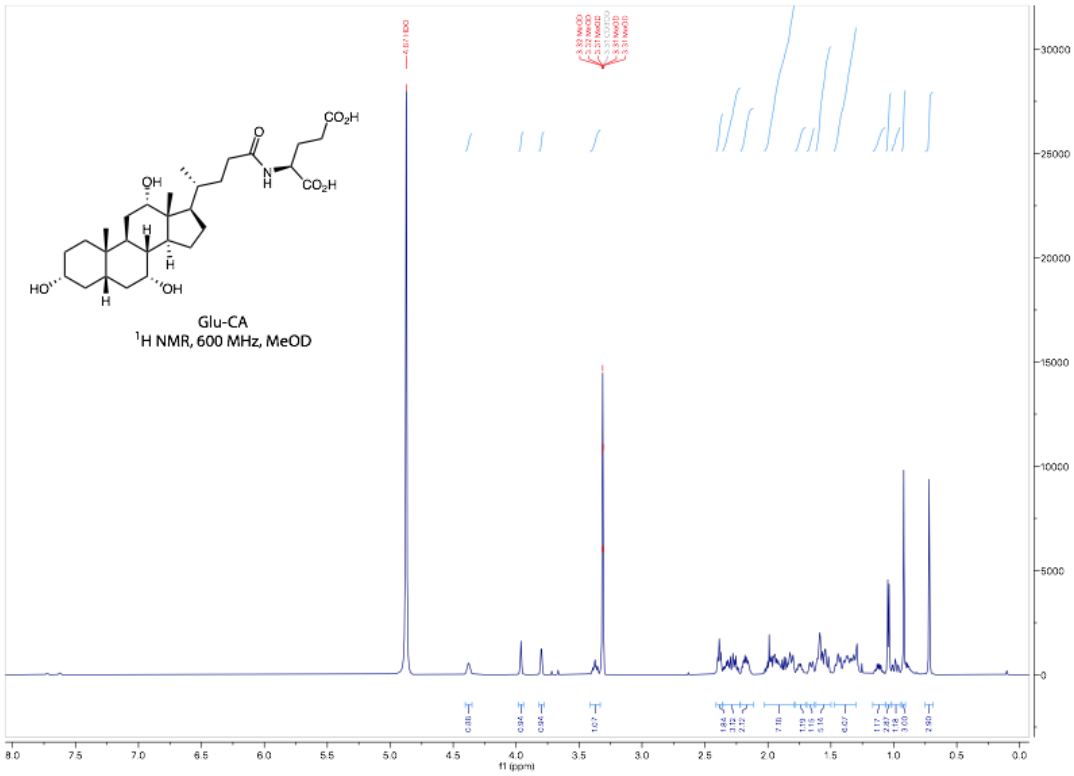
Supplementary Fig. 20: Proportion of spectral matches per bile acid across tissue types and biofluids, faceted by number of hydroxyl groups. Each row or amino acid conjugation was binned and summed to one.

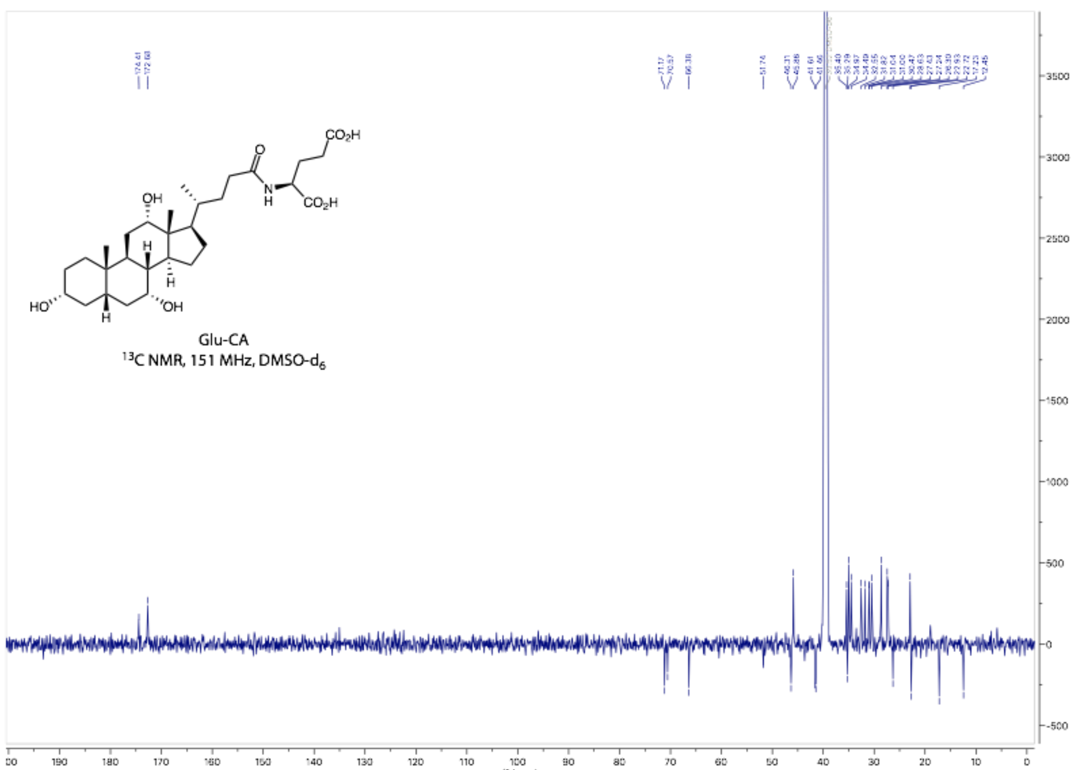


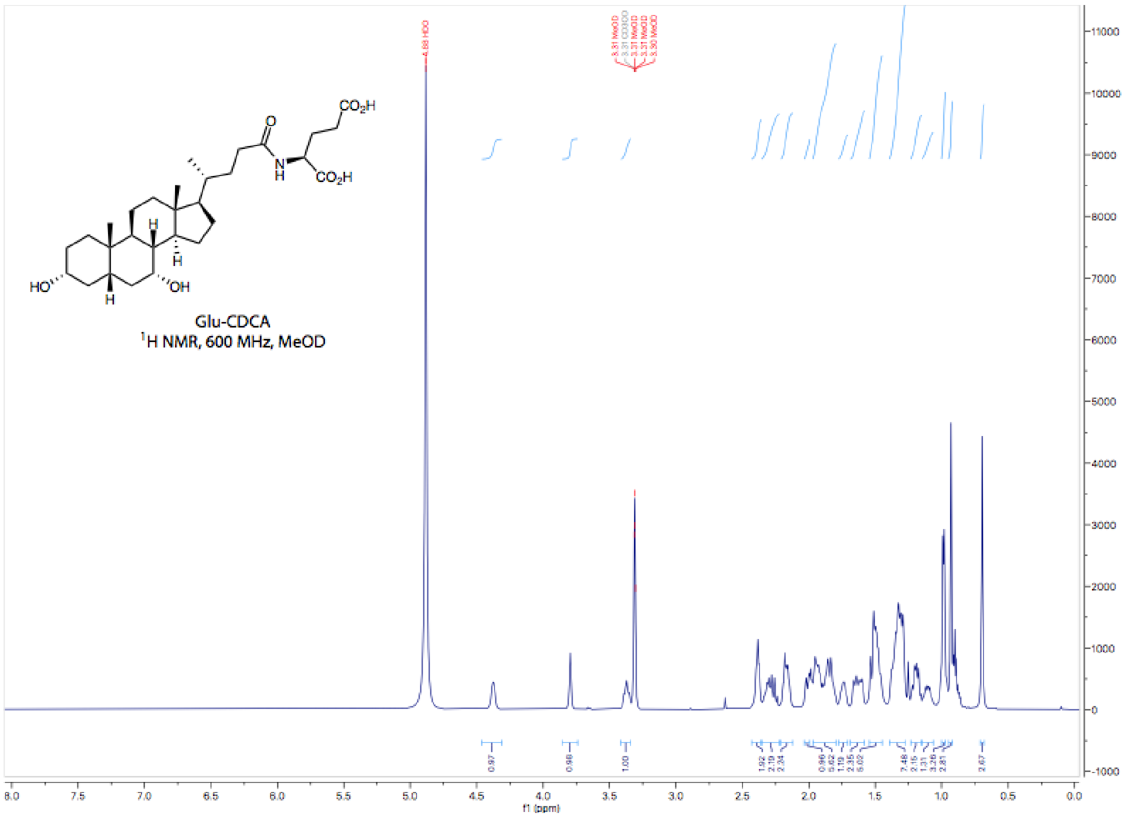
Supplementary Fig. 21: Independent validation of new conjugated bile acids in PRISM human IBD cohort. a) Peak area abundances of conjugated bile acids detected in longitudinal PRISM dataset.[6] Boxes represent the interquartile range, center line is the median and whiskers are 1.5 times the interquartile range. b) Peak area abundances of selected bile acids that were higher in Crohn's and/or Ulcerative Colitis patients relative to non-IBD individuals, as determined by pairwise two-sided Wilcoxon tests. Significance is shown using Benjamini-Hochberg corrected p-values (n for CD = 68, n for non-IBD = 34 and n for UC = 53).

<u>Supplementary Data</u>

^{1}H and ^{13}C NMR spectra of pure synthesized conjugated bile acids.

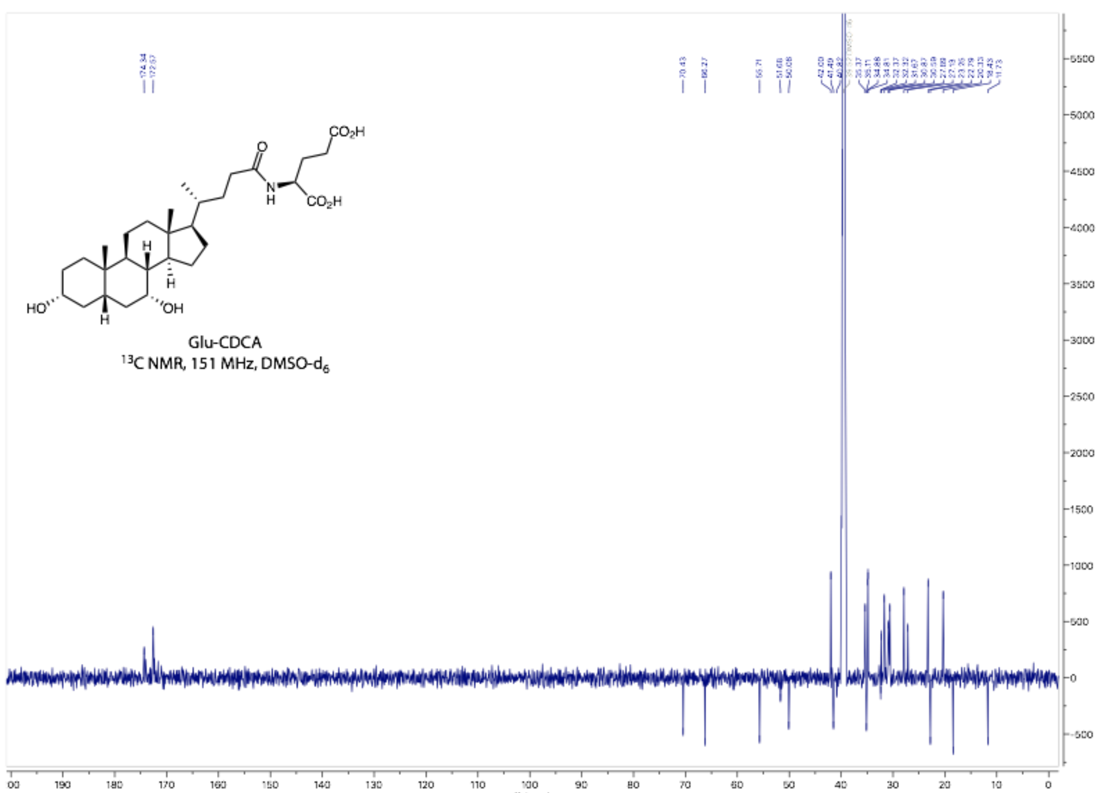






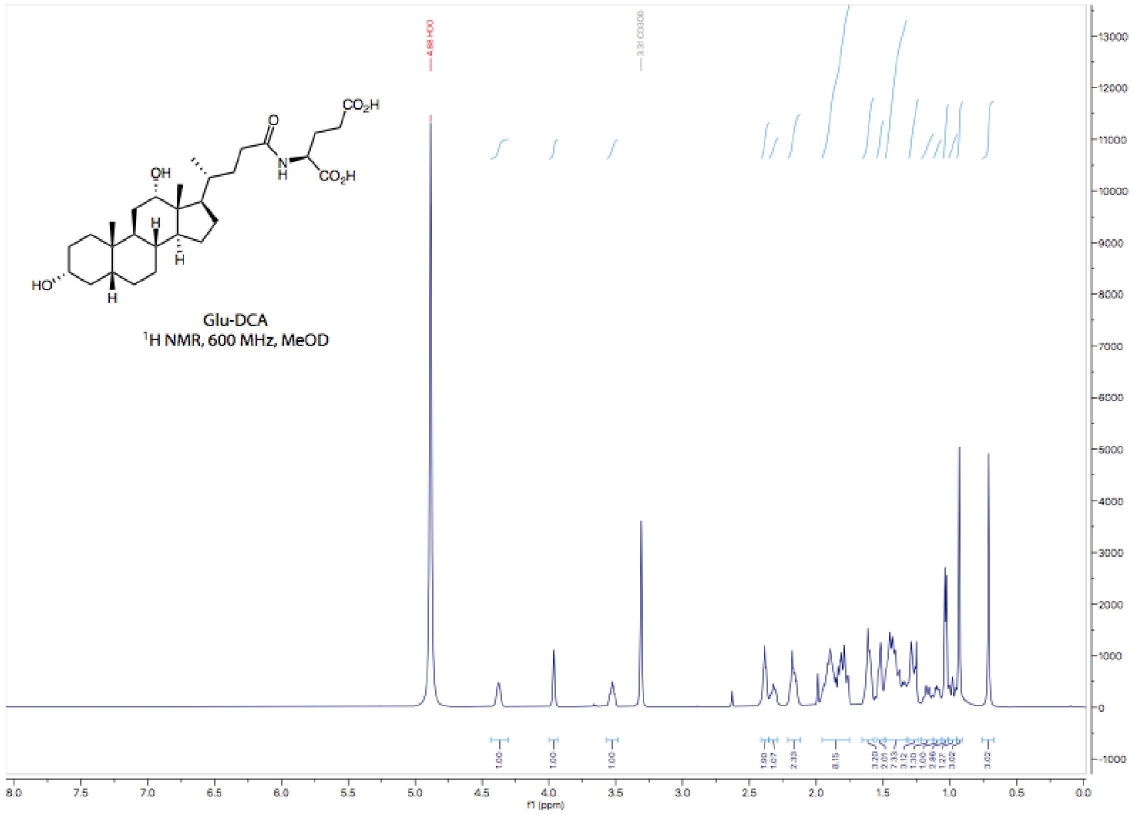
Glu-CDCA
^{1}H NMR, 600 MHz, MeOD
f1 (ppm)

342



Glu-CDCA
^{13}C NMR, 151 MHz, DMSO-d_6
f1 (ppm)

343



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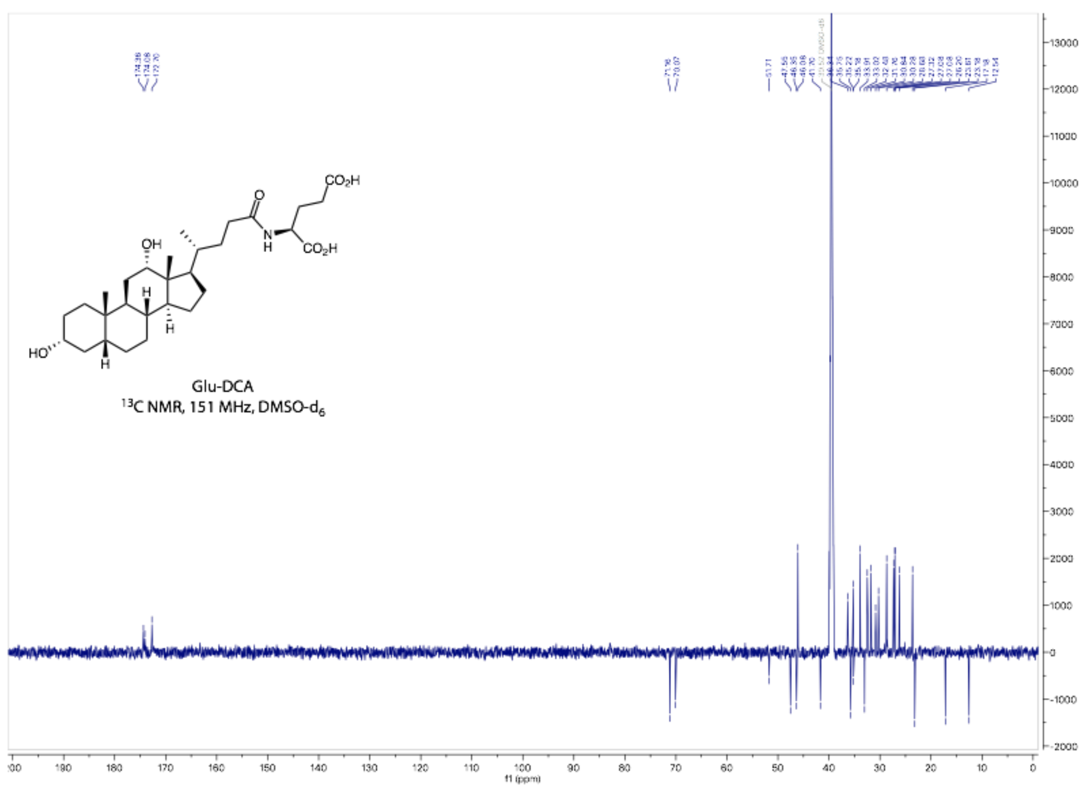

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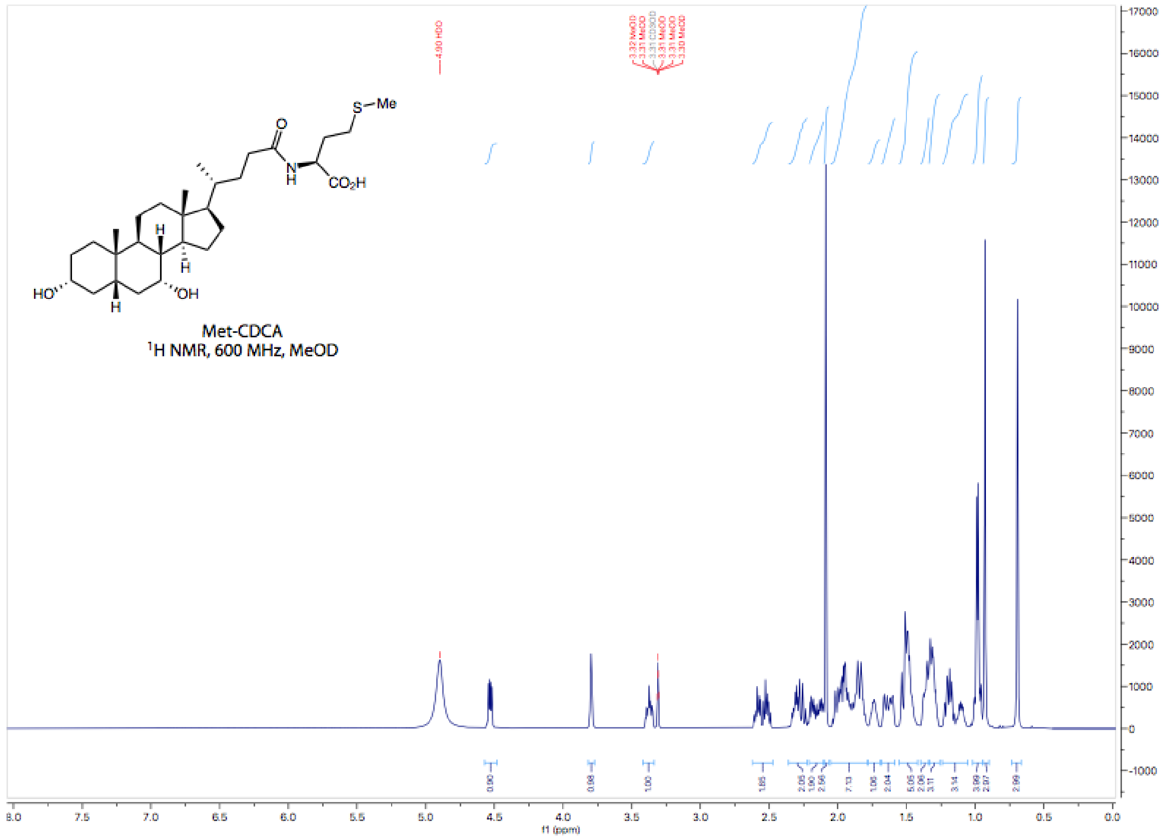
S–Me
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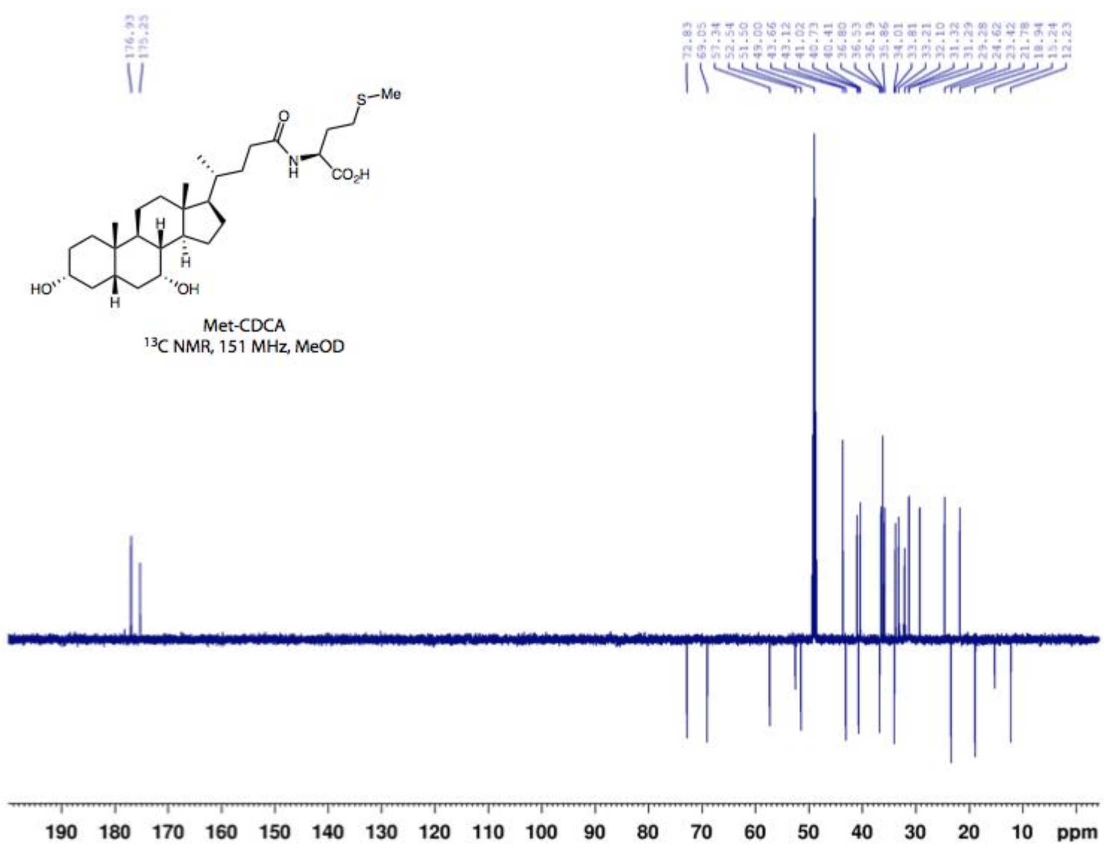
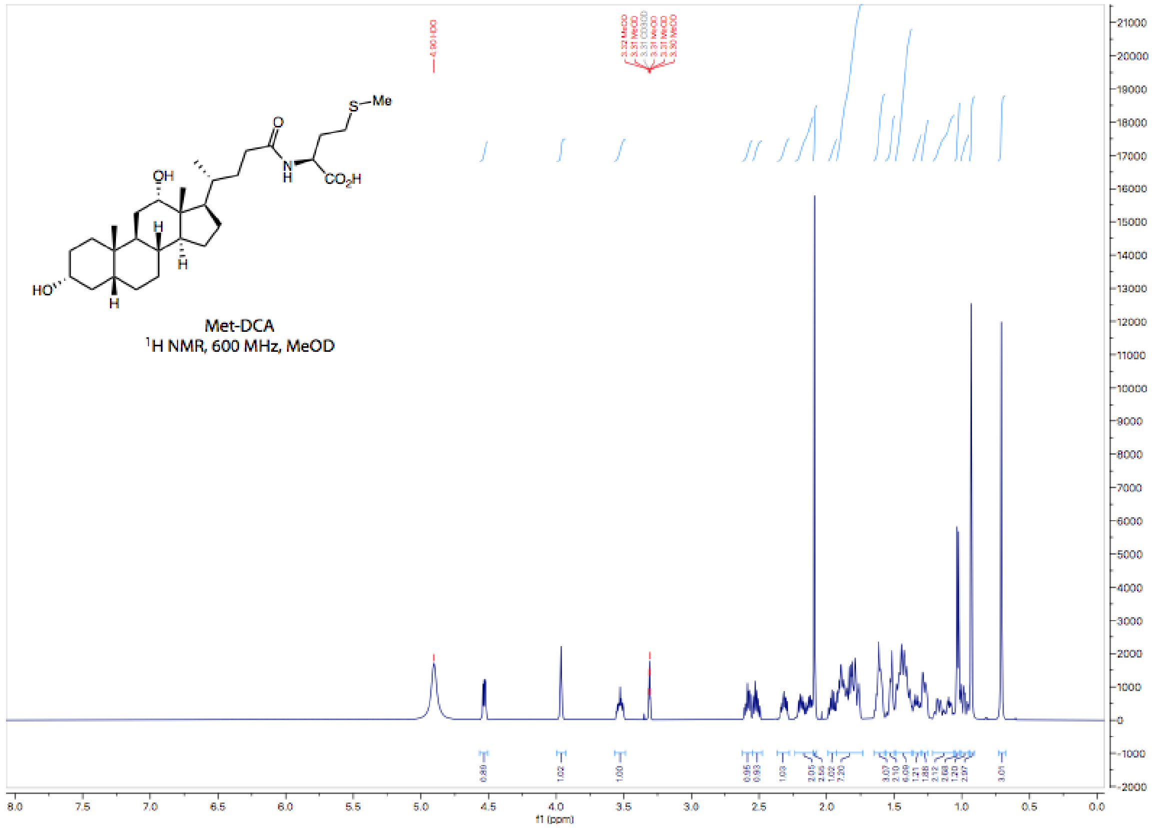
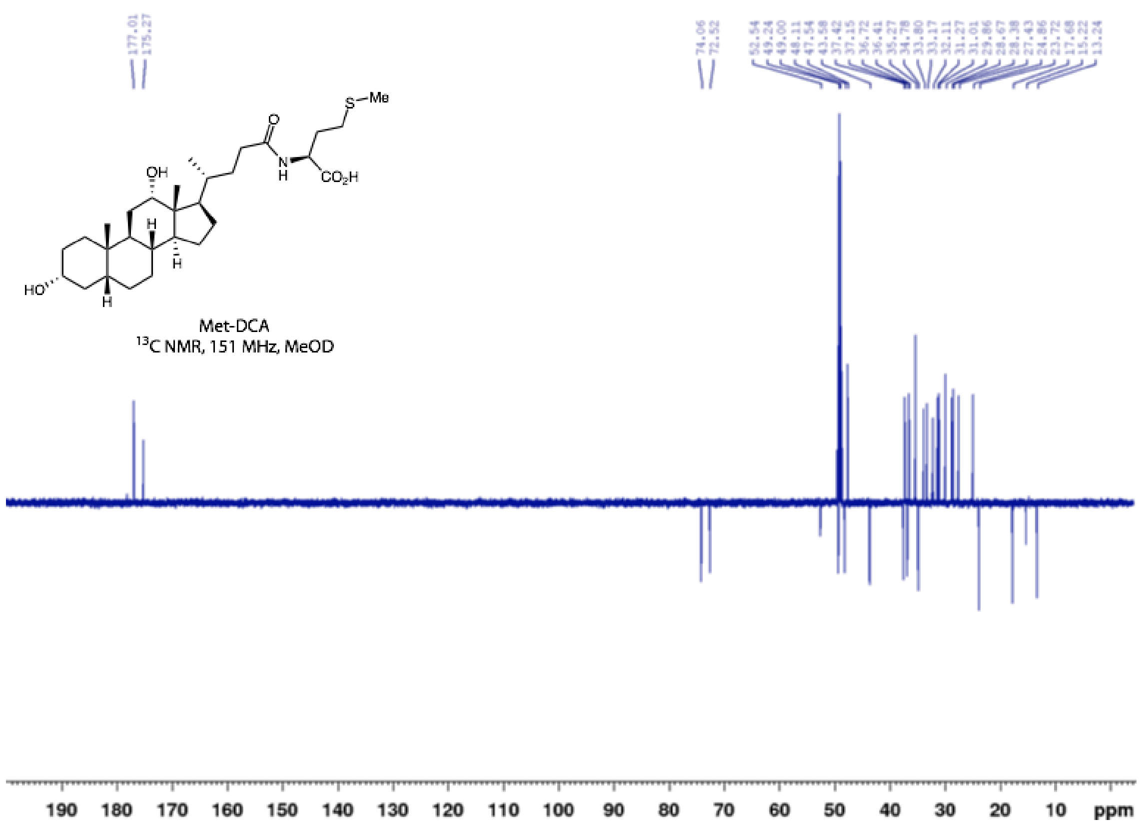
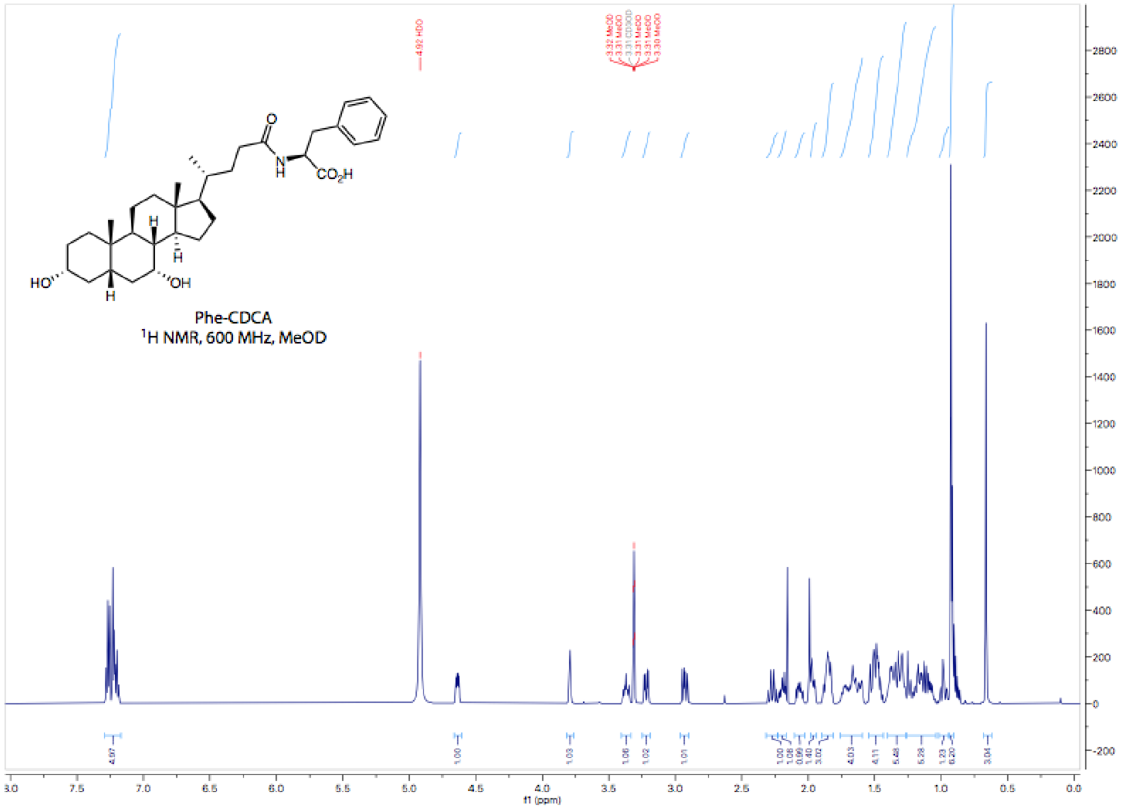
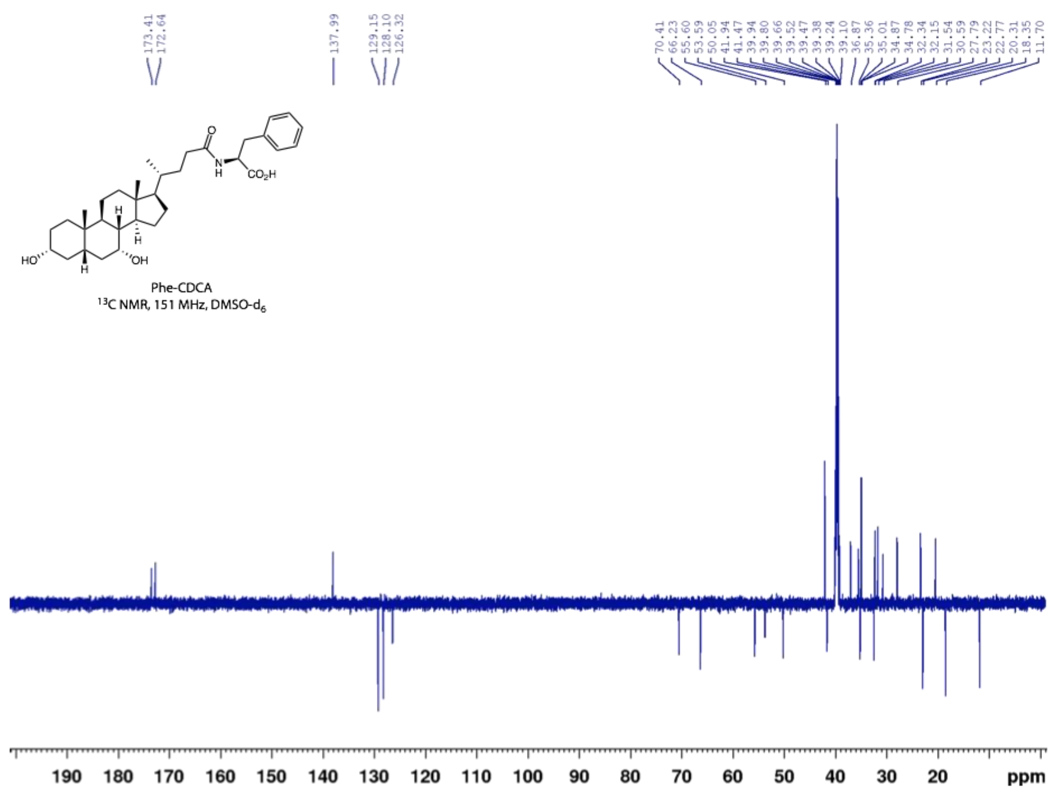
N
H
CO2H
H
H
HO
H
OH
Met-CDCA
1H NMR, 600 MHz, MeOD
f1 (ppm)


S–Me
O
N
H
CO2H
H
H
HO
H
OH
Met-CDCA
13C NMR, 151 MHz, MeOD
ppm

348 
Met-DCA
^{1}H NMR, 600 MHz, MeOD
f1 (ppm)

349 
Met-DCA
^{13}C NMR, 151 MHz, MeOD
ppm


Phe-CDCA
^{1}H NMR, 600 MHz, MeOD
f1 (ppm)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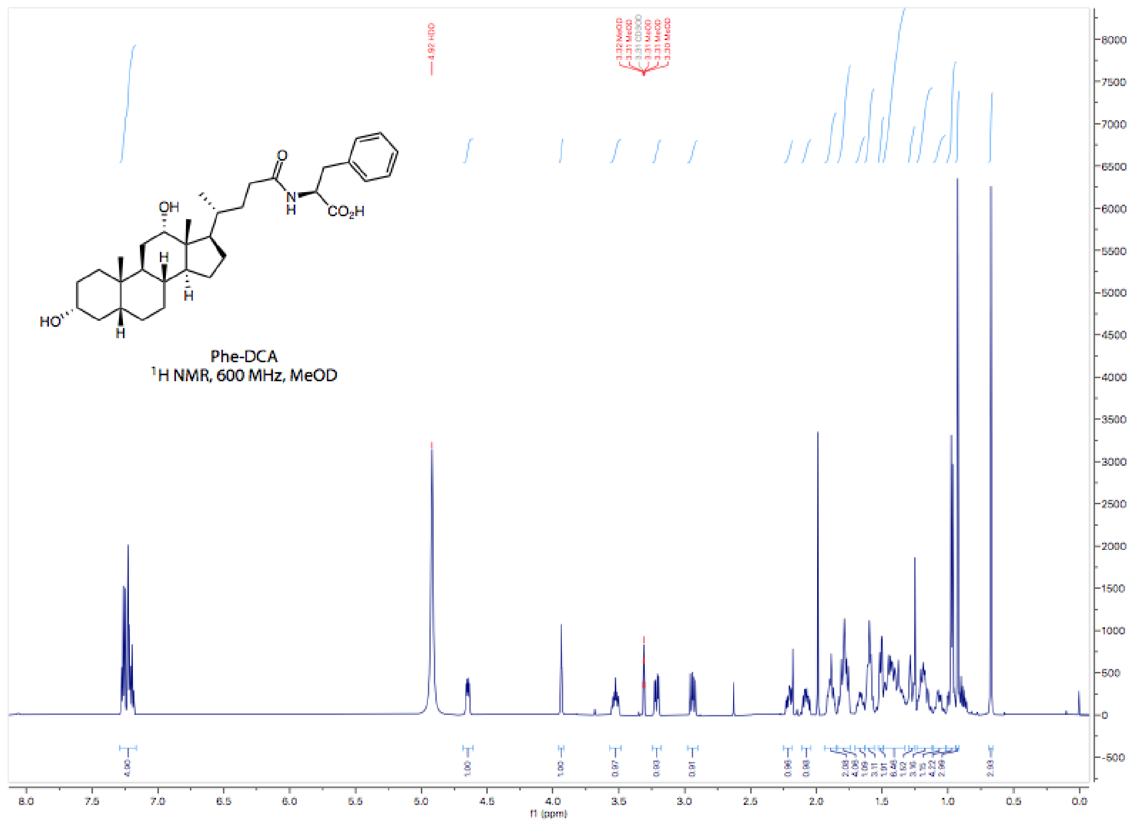
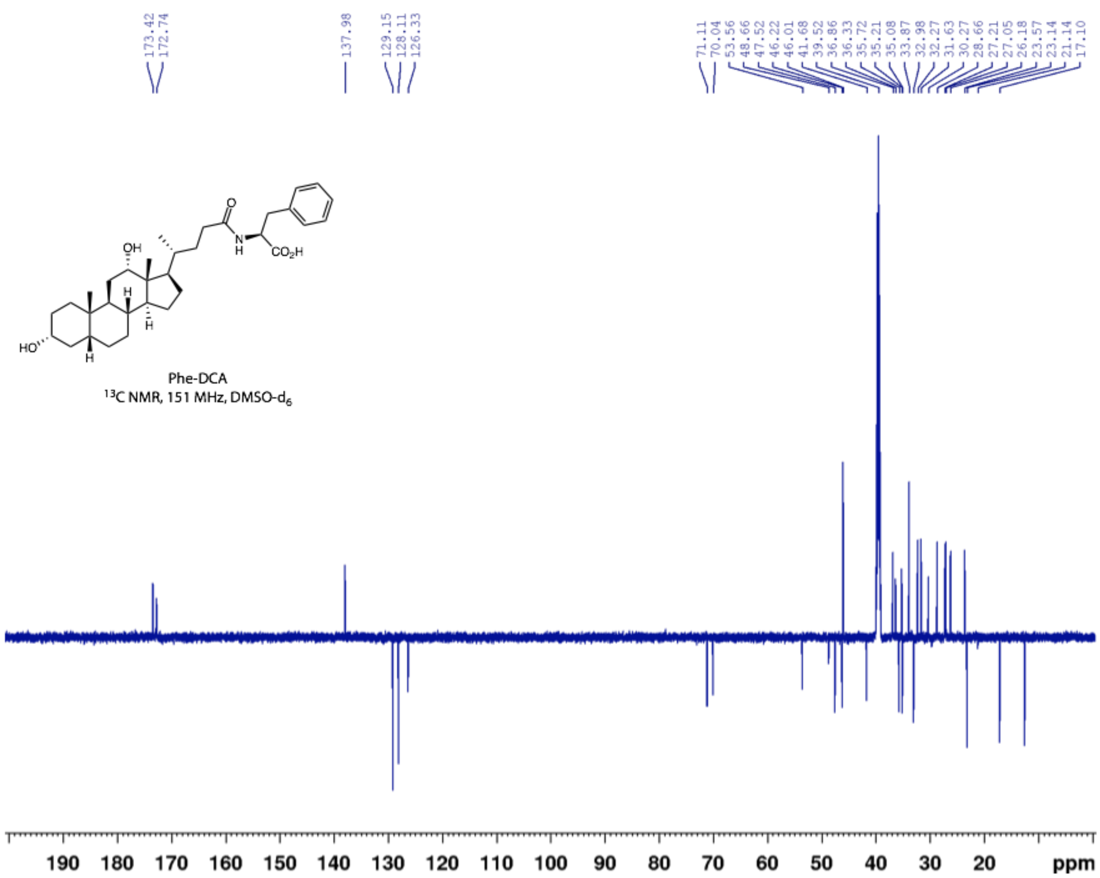
137.99
129.15
128.10
126.32
70.41
66.23
55.60
53.59
50.05
41.94
41.47
39.94
39.80
39.66
39.52
39.47
39.38
39.24
39.10
36.87
35.36
35.01
34.87
34.78
32.34
32.15
31.54
30.59
27.79
23.22
22.77
20.31
18.35
11.70
Phe-CDCA
^{13}C NMR, 151 MHz, DMSO-d_6
ppm


Phe-DCA
1H NMR, 600 MHz, MeOD
f1 (ppm)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128.11
126.33
71.11
70.04
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48.66
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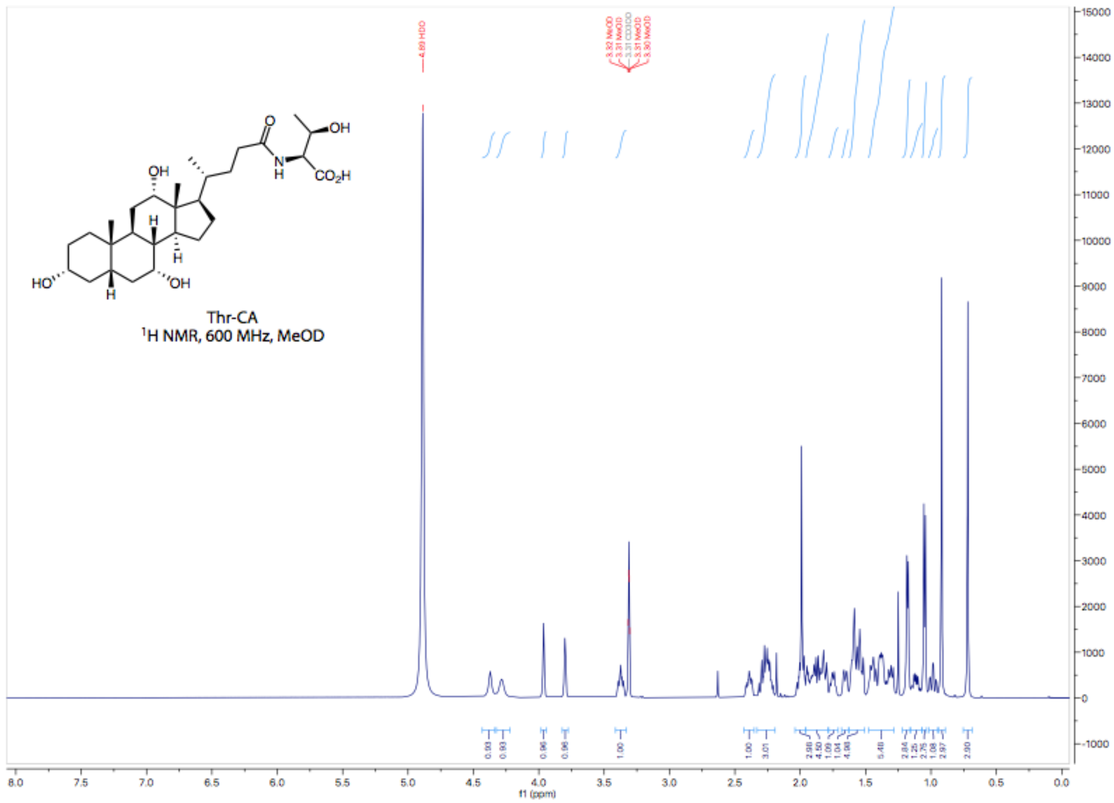
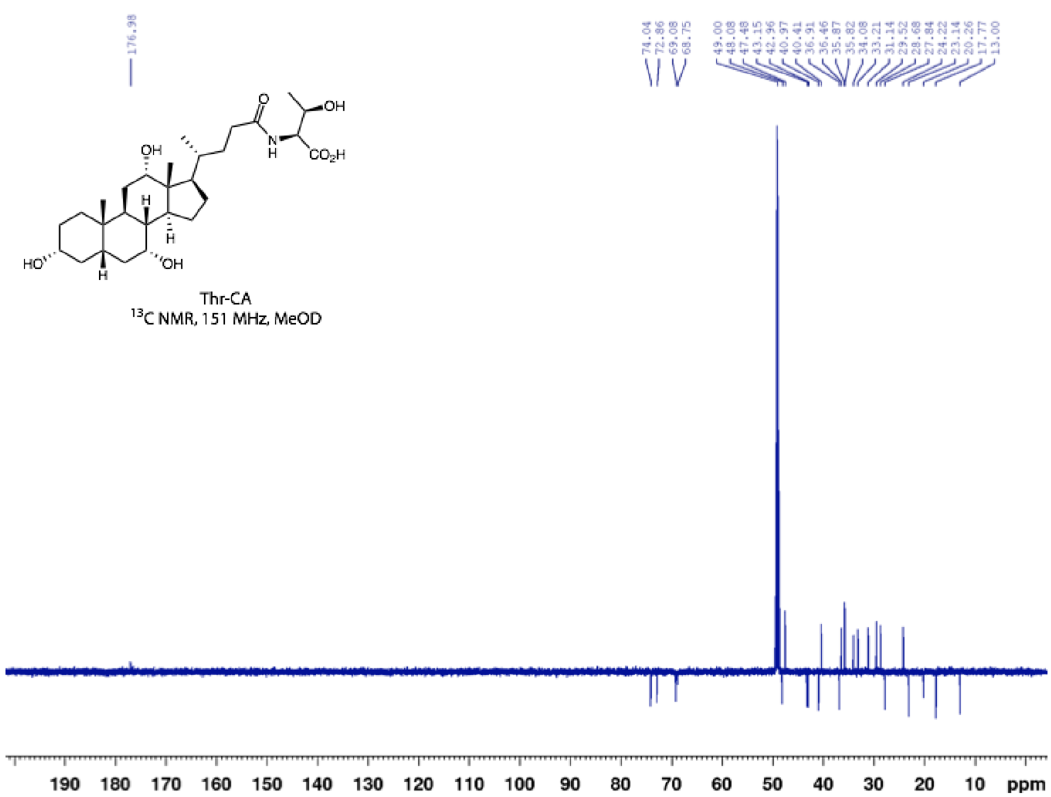
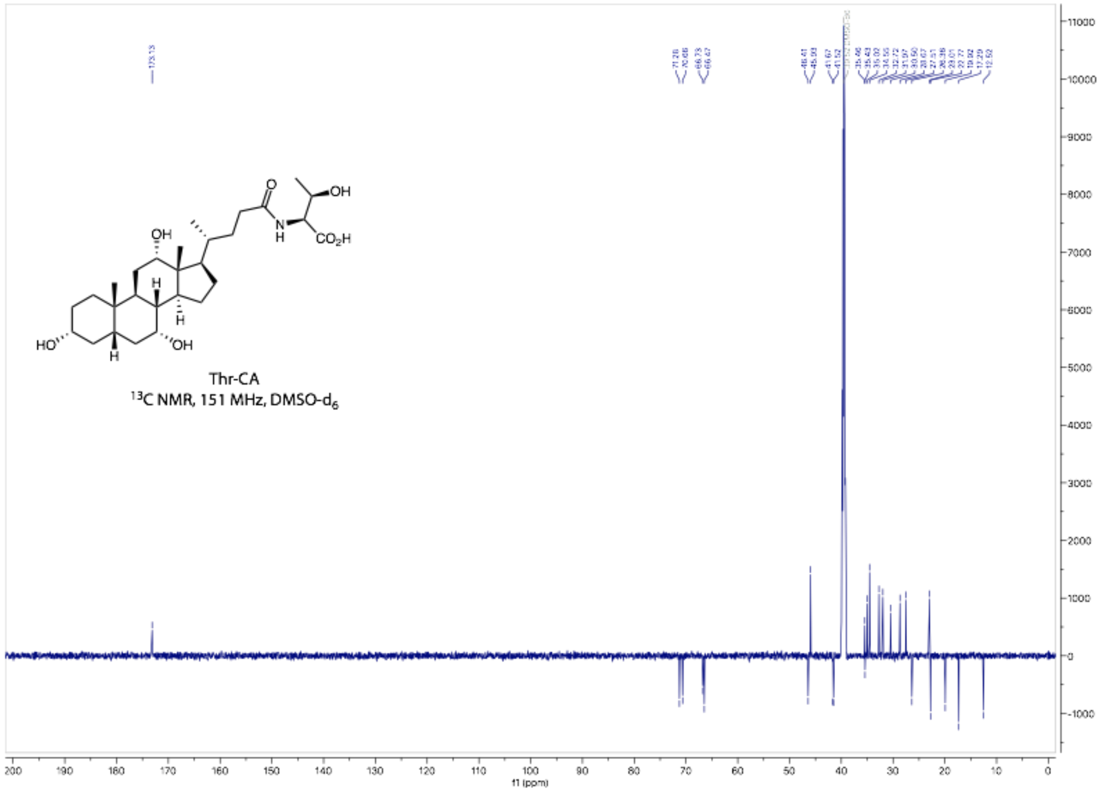
41.68
39.52
36.86
36.33
35.72
35.21
35.08
33.87
32.98
32.27
31.63
30.27
28.66
27.21
27.05
26.18
23.57
23.14
21.14
17.10
Phe-DCA
13C NMR, 151 MHz, DMSO-d6
ppm

356 

357 



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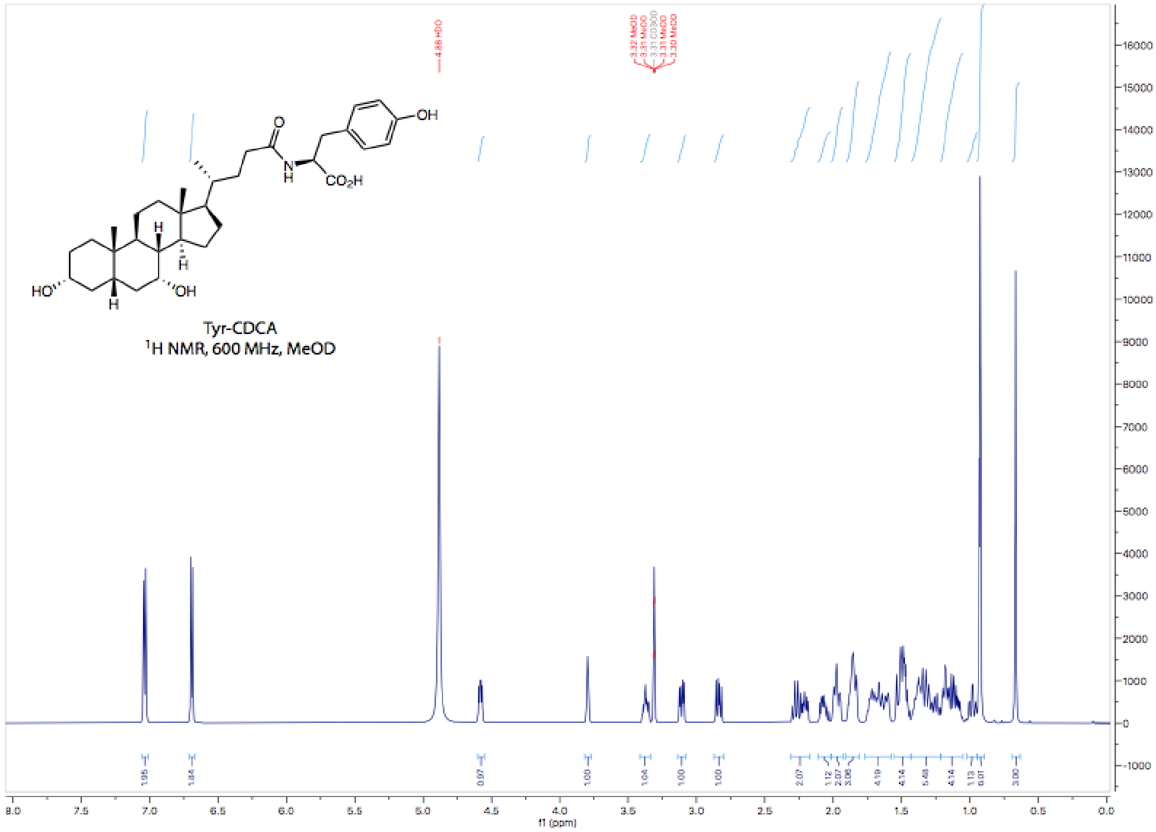

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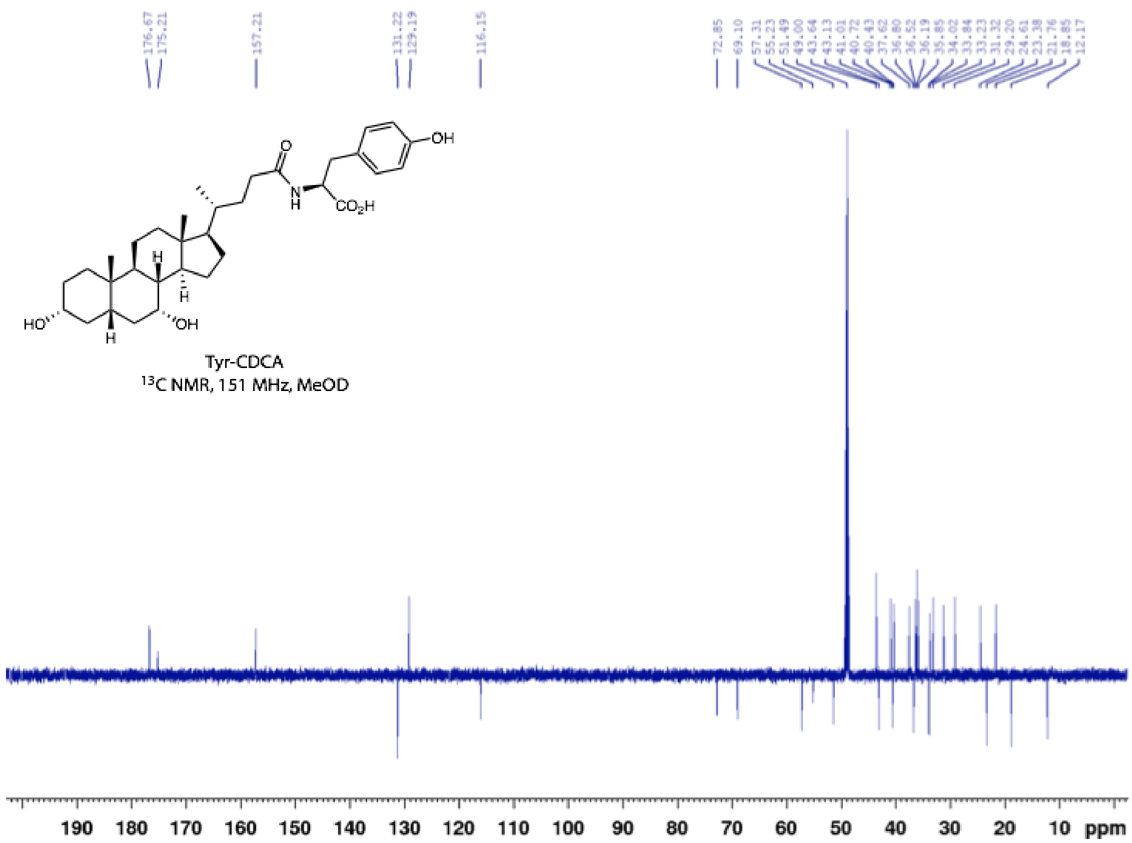

Supplementary References

1. McDonald, J. A. K. et al. Evaluation of microbial community reproducibility, stability and composition in a human distal gut chemostat model. J. Microbiol. Methods 95, 167–174 (2013).
2. Katajamaa, M., Miettinen, J. & Oresic, M. MZmine: toolbox for processing and visualization of mass spectrometry based molecular profile data. Bioinforma. Oxf. Engl. 22, 634–636 (2006).
3. Pluskal, T., Castillo, S., Villar-Briones, A. & Orešič, M. MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinformatics 11, 395 (2010).
4. Quinn, R. A. et al. Global chemical effects of the microbiome include new bile-acid conjugations. Nature 579, 123–129 (2020).
5. Wang, M. et al. Interactive MS/MS Visualization with the Metabolomics Spectrum Resolver Web Service. bioRxiv 2020.05.09.086066 (2020) doi:10.1101/2020.05.09.086066.
6. Franzosa, E. A. et al. Gut microbiome structure and metabolic activity in inflammatory bowel disease. Nat. Microbiol. 4, 293–305 (2019).