Supplementary Information

1. Solid Phase Synthesis of Carbonyl-CILAT Reagent

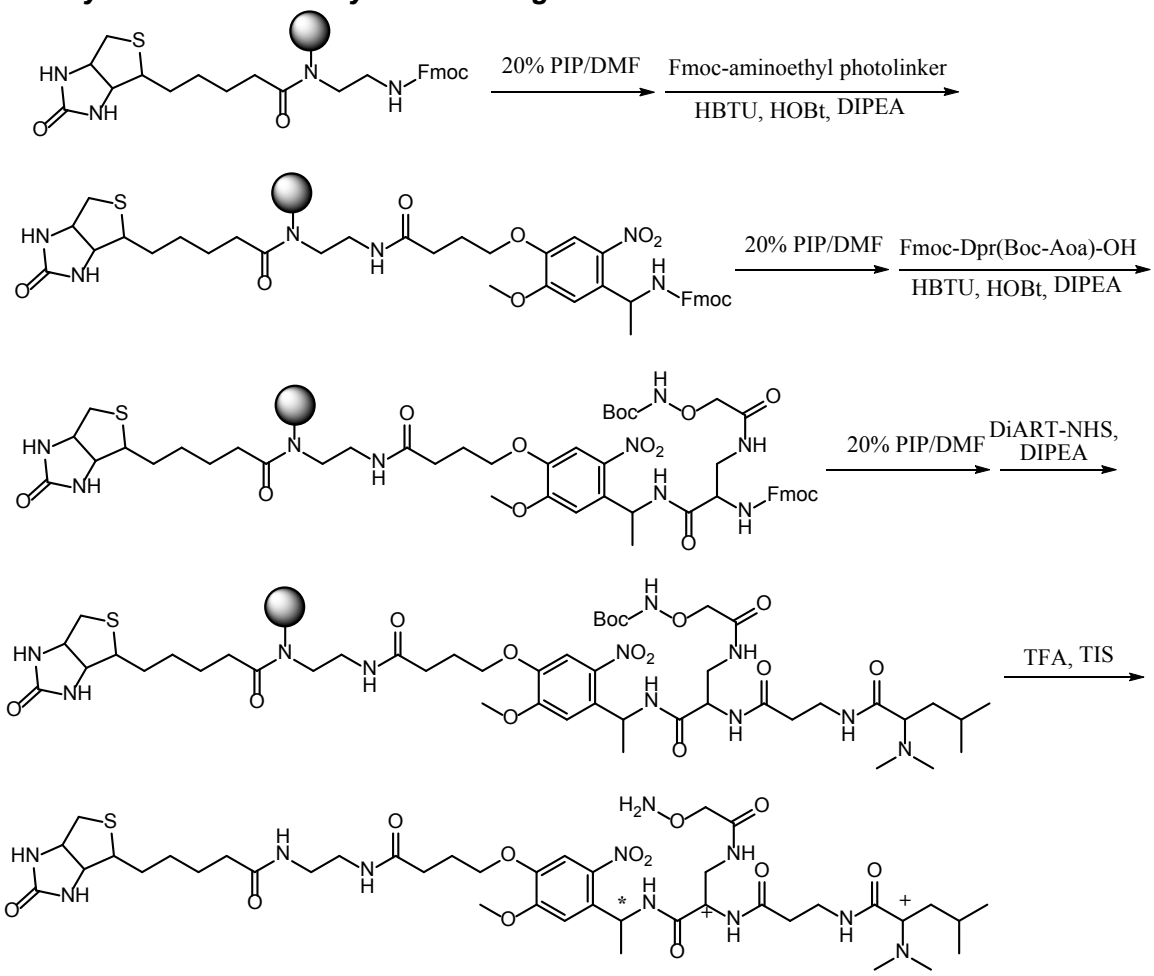

Figure S1. Solid phase synthesis of carbonyl-CILAT reagent. Asterisk (*) in the final product indicates a racemic center. Plus (+) indicates chiral centers. **PIP**: piperidine; **DMF**: N,N'-dimethylformamide; **HBTU**: 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; **HOBt**: 1-Hydroxybenzotriazole; **DIPEA**: N,N-Diisopropylethylamine; **TFA**: trifluoroacetic acid; **TIS**: triisopropylsilane; **Fmoc**: Fluorenylmethyloxycarbonyl; **Boc**: butyloxycarbonyl; **NHS**: N-Hydroxysuccinimide ester.

Biotin NovaTag resin (Novabiochem, Cat No. 855051, load capacity 0.51 mmol/g, 200 mg) was added into a reaction vessel and shaken with 5 mL 20% PIP in DMF for 30 min. After washing, Fmoc-aminoethyl photolinker (Novabiochem, Cat No. 01-60-0042, discontinued) 110 mg, HBTU 76 mg, HoBt 27 mg, and 250 µL DIPEA (2M in DMF) were dissolved in 1.5 mL DMF, added into the resin, and shaken for 2 h. The resin was deprotected with 20% PIP in DMF for 30 min. Fmoc-Dpr(Boc-Aoa)-OH (Novabiochem, Cat No. 852216) 250 mg, HBTU 190 mg, HOBt 80mg, and 625 µL DIPEA (2M in DMF) were dissolved in 3 mL DMF, added into the resin, and shaken for 2 h. The resin was again deprotected with 20% PIP in DMF for 30 min. DIART-NHS 48 mg and 840 µL DIPEA (2M in DMF) were mixed with 800 µL DMF, added into the resin, and shaken for 1 h. After the resin was washed, the final product, carbonyl-DiART reagent, was cleaved from the resin by the cocktail solution (88% TFA, 5% H_2O, 5% phenol, 2% TIS), purified by C18 reverse phase HPLC to at least 95% purity (solvent A: 0.1%TFA in 100% water; solvent B: 0.1%TFA in 100% acetonitrile; Gradient: 0-5 min 10% B; 5-45 min from 10% to 90% B) and lyophilized to offer light yellow fluffy solid (32 µmol, recovery yield 32%), which was quantified by using biotin quantitation kit (Pierce Cat # 28005). The correct product was confirmed by MALDI-MS. Carbonyl-DiART114: cal. $[M+H]^+$ = 939.47, Obs. $[M+H]^+$ = 939.53 ; carbonyl-DiART115: cal. $[M+H]^+$ = 939.48, Obs. $[M+H]^+$ = 939.47.

Note: The carbonyl-DiART reagent showed two well-resolved peaks with almost equal intensity in HPLC chromatogram. The reagent was a pair of diastereoisomers because the compound contains a racemic carbon center (labeled by an asterisk *) and two L-configured chiral centers (labeled by an plus +). Both peaks were collected in one fraction. However, each labeled aldehyde or ketone only showed a single peak in LC-MS/MS analysis because the racemic center was removed by photo-cleavage and was not attached to the labeled carbonyls for LC-MS/MS analysis.

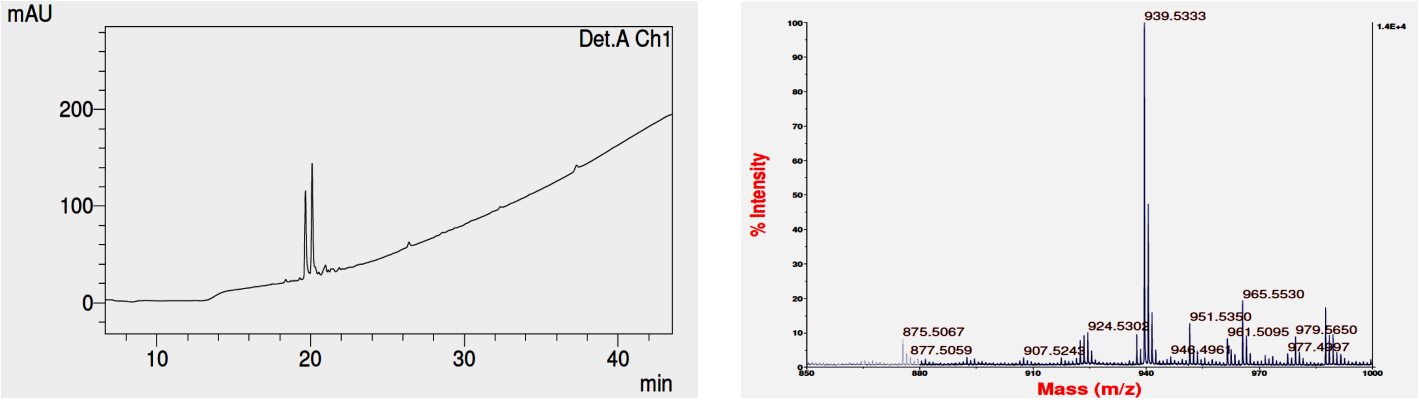

Figure S2. (left) HPLC chromatogram of crude carbonyl-DiART reagents after acid cleavage from beads. (right) MALDI-MS of carbonyl-DiART[114].

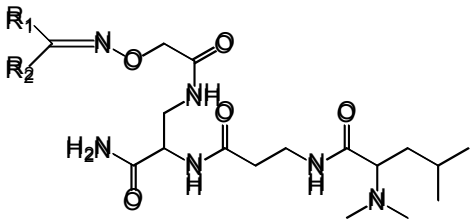

Figure S3. General structure of labeled carbonyls after UV release from streptavidin beads and ready for LC-MS/MS analysis. For aldehyde, R_1 = H.

2. Cell Lysate Extraction and Carbonyl Labeling

Human aortic endothelial cells (HAECs) obtained from Lonza (Walkersville, MD) were maintained in endothelial growth medium (EGM) containing 2% fetal bovine serum (FBS), growth factors and 5 mM (LG) or 30 mM glucose (HG) in 3.5 cm tissue culture dishes for consecutive 7 days with daily media change. At the end of the culture, metabolites were extracted as described in our previous report[1,2]. In brief, cells were rapidly rinsed by warm PBS twice and quenched with 300 µl of ice cold 70:30 acetonitrile-water (v/v) containing 2 µM formyl-benzoic acid and oxoheptanedioic acid as internal standards followed by freezing in a dry ice/ethanol bath (-70°C). Cells were then scraped on ice and refrozen. Subsequently, lysate was sonicated on ice using 10 1-second bursts at low power of a sonic dismembrator followed by centrifugation at 14,000 rpm for 10 min at 4°C. Supernatants were collected and one fourth of the collected cell lysate was used for CILAT labeling. Briefly, 20 µl of 20 mM CILAT[114] or CILAT[115] was added to the LG or HG lysate respectively. Aniline was added to the reaction as the catalyst to reach the final concentration of 100 mM. Then triethylammonium bicarbonate (40 mM, pH 4.5) in 70% acetonitrile was added to reach the final volume of reaction at 160 µL. Labeling reaction was conducted at 65ºC for 24 h on a PCR machine to avoid the solvent evaporation. At the end of labeling, LG lysate labeled with CILAT[114] and HG lysate labeled with CILAT[115] were mixed and 4-[3-(perfluorooctyl)-propyl-1-oxy]benzaldehyde was added to quench the unreacted CILAT. The reaction was continued for another 3 h at 65ºC. At the end of the reaction, 500 µL H_2O was added to the reaction mixture. Excess CILAT, now conjugated with 4-[3-(perfluorooctyl)-propyl-1-oxy]benzaldehyde, was removed by being extracted with 500 µL of ethyl acetate three times. The collected bottom phase containing labeled carbonyls was incubated with streptavidin beads at room temperature for 45 min in a spin column (streptavidin beads were preconditioned with triethylammonium bicarbonate (15 mM, pH 4.5) containing 10% acetonitrile twice followed by H_2O once). After incubation, the streptavidin beads were washed by triethylammonium bicarbonate

(15 mM, pH 4.5) containing 10% acetonitrile twice followed by H_2O once. 200 µL H_2O was then added to the washed beads and the mixture was transferred to a UV transparent glass vial. The labeled carbonyls were cleaved from streptavidin beads by being exposed to UV 365 nm for 45 min. The collected labeled carbonyls were dried by speedvac.

3. Liquid Chromatography Coupled Mass Spectrometry (LC-MS) Conditions

Analysis of CILAT labeled carbonyls were performed in positive ion mode on a Finnigan LTQ Orbitrap Discovery ion trap mass spectrometer (San Jose, CA) equipped with nanospray ionization (NSI) interface coupled with an Agilent 1200 HPLC (Palo Alto, CA). Separations were achieved on in-house packed 50 µm I.D. silica capillary (Polymicro Technology, Phoenix, AZ) columns with 3 µm Atlantis T3 C18 aqueous reversed phase particles (Waters, Milford, MA). All columns had 20 cm packed bed length. The flow rate directed to capillary column was reduced to 20-30 nl/min by using a micro cross (Upchurch, Oak Arbor, WA) and a 75 µm I.D. silica capillary as the flow splitter. Mobile phase A was 10 mM formic acid in H_2O and mobile phase B was 10 mM formic acid in methanol. A 45 min gradient was used to elute analytes: 30 min from 0 to 70% solvent B and 10 min from 70 to 95% solvent B. The temperature of the heated capillary was 200ºC. The spray voltage was 5kV. Fragmentation was activated by higher-energy collision induced dissociation (HCD). The collision energy for MS/MS was 45%. All labeled carbonyls were analyzed using data-dependent analysis at a resolving power of 15,000. The chromatographic function was activated during data-dependent analysis. The instrument control, data acquisition, and data analysis were performed by Xcalibur software (Thermo Electron Corporation, version 2.0.7 SP1). Statistics for technical replicates are reported in standard deviation (±SD).

4. Signal Enhancement as a Result of Labeling

Carbonyl standards were analyzed by capillary RPLC-MS as described above. Each analyte was analyzed in both the labeled and unlabeled form with a concentration of 5 µM. Many carbonyls of interest have no acidic or basic functional groups which generally prohibit ionization by ESI in positive or negative mode. Methylglyoxal and glucose gave poor detection signal when unlabeled. Comparison of the signal to noise ratio indicates dramatic increase in sensitivity from labeling carbonyls vs. unlabeled carbonyls. Overlapping chromatograms show graphically the increase in signal due to tagging of carbonyls. Signal to noise ratio was determined using the automated function on the Qual Browser Xcalibur software (Thermo). To render an equivalent comparison, both labeled and unlabeled were analyzed in full scan MS mode. Quantitative experiments (e.g. Figure 2) of labeled carbonyls are performed using MS/MS mode which generally yields S/N increases of 5-10 fold over full scan MS mode.

Table S1*. S/N Comparison of Tagged vs. Non-Tagged Carbonyl Standards

Carbonyl	S/N of Unlabeled Carbonyl	S/N of Labeled Carbonyl
Pyruvate	16667	25430
Oxobutyric	5671	53624
Glucose	2778	37100
Methylglyoxal	ND	630129
Glucose-6-phosphate	162839	24296
Glyceraldehyde-3-phosphate	42356	94532
4-oxo-heptanedioic acid	19400	137706
Formylbenzoic acid	32817	311141

*The S/N of unlabeled carbonyls were determined in negative mode as most of them did not yield measurable signal in positive mode. The S/N of labeled carbonyls were determined in positive modes.

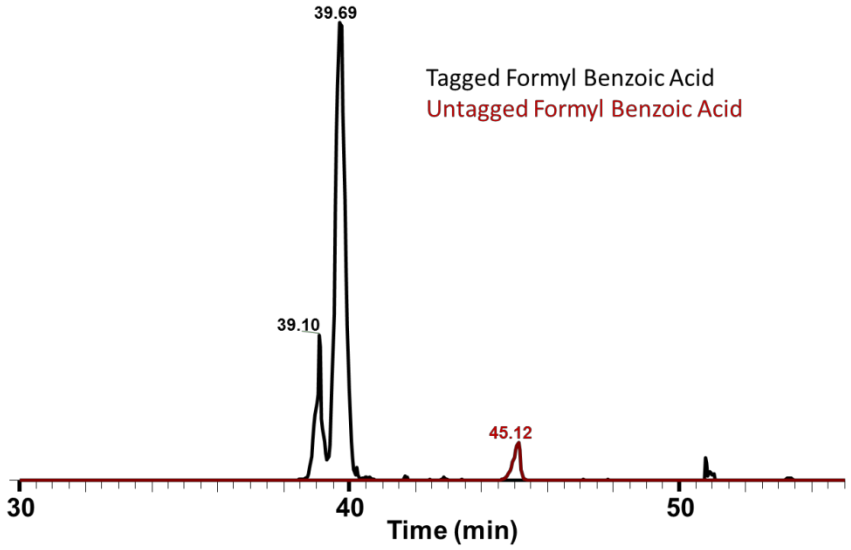

Figure S4. Sensitivity Comparison of CILAT-Labeled and Unlabeled Formyl Benzoic Acid

5. Linearity of Ratiometric Analysis and Technical Replicates of Endothelial Cells.

Three mixtures of eight carbonyl standards at different concentrations (0.5, 1 or 5 μM) were prepared, in each of which the concentrations of all carbonyls were the same. Each mixture was then reacted with both CILAT^{114} and CILAT^{115} respectively. After labeling, carbonyls with different concentration and different labels were further mixed, such as 5μM/0.5μM, 0.5/5μM, 5μM/1μM, 1μM/5μM, 1μM/0.5μM, 1μM/1μM, to get various ratios. Then the mixtures were analyzed by following all the rest of steps including clean up, streptavidin beads binding and UV cleavage.

The expected ratios and the ratio response were plotted and proved linear fits with correlation coefficients (R^2) greater than 0.95 for ratios ranging between 1:10-10:1 and greater than 0.97 for ratios between 1:5-5:1.

The endothelial cells were lysed and the resulting supernatant was split into two groups. The first group was labeled with carbonyl-CILAT^{114} and the second group labeled with carbonyl-CILAT^{115}. Both groups were mixed at 1:1 and analyzed by targeted MS^2 analysis. Resulting ratios of 114 to 115 are shown below.

Table S2. Linearity of Response and Technical Replicates

	Mass	R^2 for 10:1 to 1:10 ratios	R^2 for 5:1 to 1:5 ratios	Ratio of Technical Replicates (115/114)
Pyruvate	460.2538	0.975	0.960	1.08
Oxobutyric	474.2694	0.999	0.993	1.01
Glucose	552.3011	0.939	0.950	1.10
Methylglyoxal	408.2483	0.991	0.983	0.99
Glucose-6-phosphate	632.2674	0.959	0.918	1.00
Glyceraldehyde-3-phosphate	542.2357	0.996	0.999	1.06
4-oxo-heptanedioic acid	546.2905	0.901	0.974	1.09
Formylbenzoic acid	522.2694	0.846	0.999	1.07
Average		**0.951**	**0.972**	**1.05**
St. Dev.		**0.054**	**0.028**	**0.05**

6. Non-targeted Carbonyl Changes from Endothelial Cells in Response to High Glucose Stimulation.

Endothelial cells were treated with low or high glucose levels, lysed and reacted with carbonyl-CILAT as described above. Samples were analyzed by capillary LC-MS/MS in data dependent mode. Experiments were performed in triplicate (n=3). Ratios of 114:115 were determined by a PERL script written in house. Fold changes greater than 2.0 or less than 0.5 were used as the threshold for significant change. The number of components picked for MS/MS in data dependent mode was 1784±6. On average, 16% or 280±60 carbonyls increased by a factor of two in HG vs. LG and 2% or 35±2 decreased by a factor of two. Of the 280 which increased, 95 carbonyls were found in at least two of the three analyses based on exact mass (within 10 ppm) and retention time (within 3%). These 95 are found in Table S3. Of the 35 which decreased by 2 fold, 22 were found in two of the three analyses. These 22 are found in Table S4. The masses of known analytes based on mass and retention time (pyruvate, oxobutyrate etc.) showed mass accuracy of ±10ppm. Masses in Table S3 and S4 were matched to the metabolite database Metlin using 10 ppm as the mass accuracy and the presence of aldehyde and ketone group. Mass accuracy was determined by adding 372.23771 (multiplied by the number of Tags, generally 1) to the exact mass of the proposed METLIN ID metabolite and using that mass as the exact mass to compare against the detected mass. Both Tables tentatively identified 27% of the masses, which is consistent with our previous experiments using the database. Fold change is the average of the values of the ratio given by the data analysis.

Table S3. Carbonyl Increases in Endothelial Cells from High Glucose Stimulation

Retention Time (min)	Detected Mass	# of Tags	Proposed ID	Mass Accuracy to Proposed ID Mass (ppm)	Fold Change	Pathway
45.88	392.7303	2			2.7	
51.71	395.2346	1			2.0	
28.94	401.2189	1			2.3	
51.16	407.7502	2			2.3	
28.64	414.2529	1			4.8	
32.26	423.2537	2			3.6	
35.09	424.2638	2			2.7	
29.12	426.2539	2			9.3	
29.74	432.2643	1			2.3	
33.39	433.2450	1			2.1	
30.48	434.2399	1			2.4	
30.61	435.4200	1			3.0	
32.99	439.2492	2			3.8	
28.63	444.2137	1			2.3	
52.77	446.2619	1			2.5	
27.89	446.2784	1			4.8	
43.14	446.3173	1			2.0	
34.02	453.8715	1			2.2	

31.12	461.4432	1			3.1	
28.65	462.2702	1	Glyceraldehyde	-1.71	3.6	Pentose Phosphate Pathway
29.18	473.2853	1	Oxobutanamide	0.23	4.2	
35.53	474.2734	1	Methylmalonic acid semialdehyde	-8.43	2.1	Propanoate Pathway
28.75	476.2852	1			4.9	
50.30	479.2817	1			2.7	
49.40	486.4301	1			2.4	
28.65	487.2661	1			7.8	
29.98	487.2679	1			4.6	
35.02	487.3031	1	Acetamidopropanal	-4.29	4.4	Arg/Pro/Glu synthesis
30.40	490.2666	1	Hydroxy-2-oxobutanoic acid	-4.67	2.4	
28.52	492.2818	1	Erythrulose	-3.64	2.2	Pentose Phosphate Pathway
40.25	501.3167	1	Acetylaminobutanal	0.02	4.6	Arg/Pro Metabolism
38.01	501.3182	1			3.2	
57.13	502.3026	1	Adipate semialdehyde	-3.76	2.2	Lysine Degradation
36.45	504.2813	1	Acetolactate	-2.56	2.3	Valine Bio-synthesis
30.60	505.2774	1			6.2	
45.48	509.2874	1			2.1	
33.49	510.3736	1			3.3	
43.63	513.3194	1			3.1	
28.72	517.3127	1	Aminoadipate 6-semialdehyde	-2.11	4.5	Lysine Degradation
40.49	518.2965	1	Aceto-Hydroxybutanal	-1.70	5.3	Branched Chain Amino acid Biosynthesis
46.02	519.2801	1	Hydroxyglutamate semialdehyde	20.82	2.9	Proline Biosynthesis

34.06	519.2909	1			25.0	
28.04	522.2923	1	Pentose(s)	-3.43	4.9	
27.91	525.2903	1			13.7	
57.99	528.2955	1	Naphthaldehyde	-0.55	10.2	
29.15	532.2894	1	Formyl-1-indanone	1.33	11.1	
32.39	532.3232	1	Phenyl-4-pentenal	6.22	2.8	
33.83	542.2365	1	Glyceraldehyde-3-phosphate	-1.46	3.7	Glycolysis
36.69	544.3496	1	Oxo-nonanoic acid	-3.58	2.4	
32.78	546.9479	1			3.0	
44.87	549.2484	1			2.3	
27.92	550.2864	1	Dehydro-3-deoxy-D-gluconate	-8.34	11.6	Pentose Phosphate Pathway
26.98	552.3029	1	Hexose	-3.24	31.0	Glycolysis
51.70	552.3284	1	Hydroxykynurenamine	-1.47	2.0	Trp Metabolism
30.07	553.2850	1			21.6	
30.61	557.2826	1			2.2	
47.07	557.3452	1			11.0	
30.97	558.3033	1			6.3	
34.63	558.3389	1			3.8	
29.10	559.2999	1			2.9	
32.37	561.3026	1	Amino-6-oxoheptanedioate	2.12	2.0	
48.86	561.3416	1			2.1	
29.30	566.2828	1	Keto-D-Gluconic acid	-4.22	2.5	Pentose Phosphate Pathway
47.56	568.3597	1			2.1	
44.40	568.4793	1			4.0	
60.71	572.2161	1			2.6	
41.84	572.3527	1			2.3	
48.63	573.3365	1			3.8	

45.19	574.3723	1			2.0	
32.05	583.2974	1			5.6	
51.12	588.3846	1	Hexyl-3-phenyl-2-propenal	7.69	4.3	
50.14	591.2914	1	Hydroxy-indolepyruvate	-0.83	2.6	
48.04	593.3077	1			4.1	
29.98	594.3170	1			23.6	
56.70	602.1264	1			2.2	
40.72	609.3052	1			2.2	
27.54	610.3083	1	Deoxy-D-manno-octulosonate	-2.77	15.6	
48.67	614.4001	1			2.4	
32.57	615.3603	1			2.7	
38.89	617.3284	1			4.7	
59.69	625.2409	1			3.7	
29.91	628.3211	1			7.2	
33.35	629.2945	1			2.1	
45.89	636.3194	1			2.0	
50.49	637.3333	1			2.2	
51.49	642.3976	1			2.4	
44.84	645.4068	1			5.1	
30.20	646.3315	1			4.9	
39.01	648.3611	1			4.7	
45.10	651.3590	1			5.9	
47.91	702.3937	1			2.3	
44.76	715.4246	1			3.8	
32.17	717.3688	1			2.3	
37.36	727.3876	1			3.7	
51.91	746.4208	1			2.0	

Table S4. Carbonyl Decreases in Endothelial Cells from High Glucose Stimulation

Retention Time (min)	Mass Detected	# of Tags	Proposed ID	Mass Accuracy to Proposed ID Mass (ppm)	Fold Change	Pathway
48.54	387.2202	2			0.44	
42.24	397.2458	2			0.43	
40.80	433.7537	2			0.43	
44.19	467.2686	1			0.39	
44.66	494.2809	1	Hydroxybenzaldehyde	9.73	0.43	Aromatic Degradation
51.07	495.2634	1			0.33	
59.95	500.3599	1	Octanone	3.6E-5*	0.31	
29.85	503.3041	1	4-Amino-5-oxopentanoate	16.1	0.29	Arg Metabolism
48.79	510.2742	1	Dihydroxybenzaldehyde	-8.99	0.33	Aromatic Degradation
47.47	536.2899	1	Oxo-3-phenylpropanoate	-9.12	0.34	Aromatic Degradation
54.25	580.3156	1	Formyl-5-hydroxykynurenamine	11.9	0.37	Trp Metabolism
48.70	632.3801	1			0.35	
54.12	633.3632	1			0.34	
46.04	697.3328	1			0.40	
48.38	773.4335	1			0.45	
48.34	419.1753	2			0.49	
51.98	436.7547	2			0.45	
46.37	536.2175	1			0.38	
30.88	644.3543	1			0.28	
28.60	696.3487	1			0.35	
47.72	776.4329	1			0.45	
51.99	872.5026	1			0.41	

*The mass accuracy was calculated to be 3.6×10^{-5} ppm.

References:

1. Yuan, W.; Edwards, J. L. J Chromatogr A 2011, 1218, 2561-2568.
2. Yuan, W.; Zhang, J.; Li, S.; Edwards, J. L. J Proteome Res 2011, 10, 5242-5250.