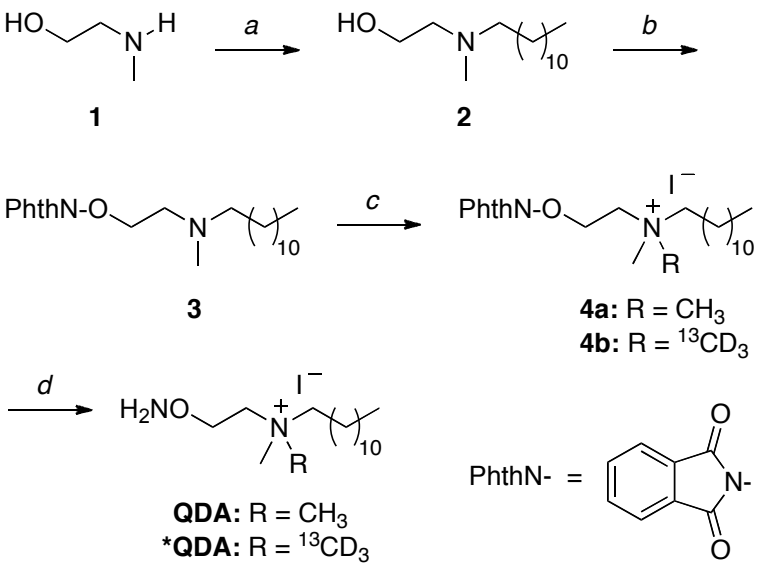
Supplementary Information

Experimental procedures for the synthesis of QDA and *QDA



Scheme S1. Synthetic scheme for QDA and *QDA. Reagents, conditions, and yields are as follows (a) 1-bromododecane (1.0 eq), anhydrous sodium carbonate (1.0 eq), ethanol, reflux, 20 h; 70%; (b) *N*-hydroxyphthalimide (1.1 eq), PPh_3 (1.1 eq), DIAD (1.1 eq), THF, rt, 12 h; 98%; (c) CH_3I or $^{13}CD_3I$ (2.5 eq), CH_2Cl_2, 50 °C, 12 h; (d) hydrazine monohydrate (5.0 eq), anhydrous ethanol, rt (for QDA) or 50 °C (for *QDA), 12 h; 65-75% (2 steps), respectively.

Materials and Methods

N, *N*-dimethylethanolamine (95.5%), 1-bromododecane (97%), *N*-hydroxyphthalimide (97%), triphenylphosphine (reagent plus, 99%), diisopropyl azodicarboxylate (DIAD, 95%), 1-iodooctane (98%), hydrazine hydrate, iodomethane (99%), sodium α-ketoglutarate, oxaloacetic acid, sodium α-ketobutaric acid, sodium pyruvate, *n*-butanol (99.9%) and anhydrous ethanol (200 proof, 99.5%) were purchased from Aldrich Chemical Company (Milwaukee, WI). Reagent grade ACS dichloromethane (DCM), tetrahydrofuran (THF), acetone, hexanes, and ethyl acetate were purchased from Pharmco-AAPER (Shelbyville, Ky). α-D(+)-glucose was purchased from Acros Organics. THF and DCM were dried using a solvent purification system by LC Technology Solutions, Inc. (Salisbury, MA). $CDCl_3$ (99.8%) was purchased from Cambridge Isotope Laboratories (Andover, Ma.). NMR spectra were acquired on a Varian *Inova* 500 MHz spectrometer. FTIR spectra were acquired on a Mattson galaxy series 5000 DRIFT

spectrometer using 64 scans, at a scan speed of 2.1 kHz and a resolution of 4v. DRIFT samples were prepared as a loose powder in KBr. FTMS spectra were acquired on a Finnigan LTQ- FT spectrometer (Thermo Electron Corp.). Heating of samples was done on a Barnstead/Thermolyne Type 17600 Dri-bath. Centrifugation was performed on an Eppendorf Centrifuge type 5415R; vacuum centrifugation was performed on an Eppendorf Vacufuge. Melting points were acquired on an SRS Digimelt.

Synthesis of N-(2-hydroxyethyl)-N-methyl-1-dodecanylamine (2). A solution of 2-(methylamino)ethanol (1.37 mL, 17.0 mmol), 1-bromododecane (4.08 mL, 17.0 mmol), and anhydrous sodium carbonate (1.93 g, 17.0 mmol) in anhydrous ethanol (34 mL) was heated to reflux. After 20h, the reaction suspension was cooled to room temperature and the precipitated solids were removed by filtration. The filtrate was concentrated to one-third volume by rotary evaporation and then transferred to a centrifuge vial and centrifuged to pellet precipitated solids. The supernatant was collected and evaporated by rotary evaporation. Bulb-to-bulb distillation (Kugelrohr, 190 °C, *ca.* -0.986 atm) of the crude residue afforded **2** (2.91 g, 70%) as a clear liquid. Spectral characterization of **2** gave values identical to those reported;[1] ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.0 Hz, 3H, C(12)H_3); 1.25 (m, 18H, C(3-11)H_2); 1.45 (m, 2H, C(2)H_2), 2.22 (s, 3H, N(CH_3); 2.38 (t, *J* = 8.50 Hz, 2H, C(1)H_2); 2.50 (t, *J* = 4.25 Hz, C(1')H_2); 3.57 (t, J = 5.5 Hz, 2H, C(2')H_2).

Synthesis of 2-(2-(dodecyl(methyl)amino)ethoxy)isoindoline-1,3-dione (3). To a solution of **2** (2.91 g, 11.9 mmol) in THF (50 mL) at 0 °C were added *N*-hydroxyphthalamide (2.14 g, 13.1 mmol), PPh_3 (3.44 g, 13.1 mmol) and DIAD (2.6 mL, 13.1 mmol). The reaction mixture was warmed to room temperature and stirred 12h before removing the solvents by rotary evaporation. To the yellow residue was added hexane (60 mL) and the resultant suspension was triturated and sonicated (room temperature bath, 30 sec intervals). The resultant fine solids were removed by filtration, and the filtrate was removed by rotary evaporation to afford crude **3** as an orange solid. The crude product was dissolved in ethyl acetate (50 mL) and the solution was washed with saturated $NaHCO_3$ (3X). The organic layer was dried (Na_2SO_4) and then evaporated by rotary evaporation to yield **3** (4.53 g, 98%) as a yellow, low melting semi-solid (38.7-40.1 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, *J* = 7.0 Hz, 3H, C(12)H_3); 1.24 (m, 18H, C(3-11)H_2); 1.41 (m, 2H, C(2)H_2); 2.29 (s, 3H, NCH_3); 2.39 (t, *J* = 7.8 Hz, 2H, C(1)H_2); 2.83 (t, *J* = 5.5 Hz, 2H, C(1')H_2); 4.32 (t, *J* = 5.5 Hz, 2H, C(2')H_2); 7.74 (m, 2H); 7.83 (m, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) 19C δ 14.1; 21.9; 22.6; 26.9; 27.4; 29.3; 29.5-29.6 (m); 31.9; 42.1; 55.4; 57.8; 75.6; 123.4; 128.9; 134.4; 163.4.

Synthesis of N-[2-(Aminooxy)ethyl]-N, N-dimethyl-1-dodecylammonium iodide (QDA). A glass pressure tube was charged with **3** (0.10 g, 1.79 mmol), dry CH_2Cl_2 (5 mL) and iodomethane (1.53 g 10.8 mmol). The tube was sealed and then immersed in an oil bath heated to 50 °C. After 12h, the reaction was cooled to room temperature, the tube was opened and the solvent was evaporated in a fume hood using a steady nitrogen stream. The resulting off-white solid was used directly in the next step. **4a:** ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, *J* = 7.0 Hz, 3H, C(12)H_3); 1.24 (m, 16H, C(4-11)H_2); 1.36 (m, 2H, C(3)H_2); 1.84 (m, 2H, C(2)H_2); 3.56 (s, 6H, NCH_3); 3.77 (m, 2H, C(1)H_2); 4.37 (t, *J* = 4.3 Hz, 2H, C(1')H_2); 4.75 (t, *J* = 4.0 Hz, 2H, C(2')H_2); 7.80 (m, 2H); 7.85 (m, 2H).

To a solution of **4a** (0.90 g, 1.70 mmol) in dry ethanol (9 mL) hydrazine monohydrate (0.43 g 8.49 mmol) was added. The reaction mixture was stirred at room temperature 12h whereupon the solvent was removed by rotary evaporation. To the residue was added CH_2Cl_2 (20 mL) and the resultant suspension was filtered. The filtrate was concentrated by rotary evaporation and toluene (15 mL) was added. The mixture was triturated with sonication (room temperature bath, 30 sec intervals) and the solids were collected and dried (vacuum) to afford **QDA** (0.45 g, 65%); mp 85.9-88.9 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.87 (t, *J* = 7.0 Hz, 3H, C(12)H_3); 1.20-1.43 (m, 18H, C(3-11)H_2); 1.75 (m, 2H, C(2)H_2); 3.42 (s, 6H, NCH_3); 3.58 (m, 2H, C(1)H_2); 3.96 (t, *J* = 4.5 Hz, 2H, C(1')H_2); 4.16 (m, 2H, C(2')H_2); 6.85 (br. s, 2H, NH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1; 22.6; 22.9; 26.2; 29.2; 29.3; 29.35; 29.4; 29.6 (2 signals); 31.9; 52.1; 62.4; 66.0; 68.9; IR (DRIFT, KBr powder) 3270, 2917, 2848, 1468, 1172, 956, 900 cm^{-1}; HRMS *m/z* calcd $[C_{16}H_{37}N_2O]^+$ 273.2906, observed 273.2903.

*Synthesis of N-[2-(Aminooxy)ethyl]-N-methyl-N-[($^{13}C^2H_3$)-methyl]-1-dodecylammonium iodide (*QDA).* Using the procedure described above for the synthesis of **QDA**, amine **3** (0.50 g, 1.29 mmol) was reacted with $^{13}CD_3I$ (0.47 g, 3.21 mmol) and hydrazine monohydrate (0.302g, 6.02mmol) to give ***QDA** (0.37 g, 75%) as a white powder; mp 86.6-87.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (t, *J* = 7.0 Hz, 3H, C(12)H_3); 1.14-1.43 (m, 16H, C(3-11)H_2); 1.74 (m, 2H, C(2)H_2); 3.41 (d, *J* = 3.5 Hz, 3H, NCH_3); 3.58 (m, 2H, C(1)H_2); 3.92 (m, 2H, C(1')H_2); 4.16 (m, 2H, C(2')H_2); 5.91 (br. s, NH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3; 22.8; 23.1; 26.4; 29.4; 29.5; 29.6; 29.7; 29.8 (2 signals); 32.1; 51.6 (m); 52.2; 62.6; 66.0; 69.2; IR (DRIFT, KBr powder) 3278, 2913, 2850, 1468, 1173, 1048, 947 ν(O-N), 720, 482, 418 cm^{-1}; HRMS *m/z* calcd $[C_{15}{}^{13}C^2H_3H_{34}N_2O]^+$ 277.3119, observed 277.3124.

Table S1. Fraction of QDA adducts partitioned into *n*-butanol per extraction over five consecutive extractions

Extraction	QDA-Pyruvate	QDA-α-Ketoglutarate	QDA-α-Ketobutyrate	QDA-Glucose
1	0.8334	0.8652	0.8028	0.9886
2	0.1550	0.1180	0.1957	0.0109
3	0.0022	0.0165	0.0015	0.0004
4	0.0094	0.0003	0.0000	0.0000
5	0.0000	0.0000	0.0000	0.0000
Aqueous Layer (post extraction)	0.0000	0.0000	0.0000	0.0001

Table S1: QDA-standard adducts were partitioned into *n*-butanol from 5 mM, pH 6 ammonium acetate buffer in 5 consecutive extraction. Ion counts used to calculate the partitioning fraction were normalized to external standard *N*,*N*-dimethyl-*N*-(2-((D_6-propan-2-ylideneamino)oxy)ethyl)octan-1-aminium iodide.

Table S2: Reactive carbonyl metabolites assigned based on a comparison of the molecular formulae derived from QDA adducts with those from our custom database.

Mass isomer assignments	**Molecular formula**	**Theoretical m/z for QDA adducts**	**Observed m/z**	**Mass difference**	**Ion count**
Pyruvate 3-Oxopropanoate	C3H4O3	343.295519	343.295769	0.00025	905472.8
Glyceraldehyde 1,3-Dihydroxyacetone	C3H6O3	345.311169	345.311429	0.00026	100706.3
1-Amino-2-propanone L-2-aminopropionaldehyde 3-Aminopropanal	C3H7NO	328.332238	328.331509	-0.000729	6147.6
Dihydroxyacetone phosphate Glyceraldehyde 3-phosphate	C3H7O6P	425.277501	425.277999	0.000498	36810.4
Oxaloacetate	C4H4O5	387.285349	387.285619	0.00027	2621.8
3-buten-2-one (cis)-2-butenal	C4H6O	325.321339	325.321899	0.00056	3126.2
isobutyraldehyde 2-butanone 1-Butanal	C4H8O	327.336989	327.339649	0.00266	18593.2

D-Erythrose D-Threose Erythrulose	C4H8O4	375.321734	375.322089	0.000355	22845.9
2-amino-4-oxo-pentoate Glutamate-1-semialdehyde L-glutamate γ-semialdehyde Amino-oxo-pentanoate	C5H9NO3	386.337718	386.338069	0.000351	33337.8
Deoxyribose 1-deoxy-xylulose 2-Deoxy-alpha-D-ribopyranose 2-Deoxy-L-arabinose	C5H10O4	389.337384	389.337689	0.000305	8659.2
α-Ketoglutarate Oxaloacetate 4-methyl ester Methyloxaloacetate acetonedicarboxylate 5-Hydroxy-2,4-dioxopentanoate Dehydro-D-arabinono-1,4-lactone D-erythro-ascorbate	C5H6O5	401.300999	401.301469	0.00047	193346.3
Dehydrodeoxypentonate D-xylono-1,5-lactone D-arabinono-1,4-lactone L-arabinono-1,4-lactone	C5H8O5	403.316649	403.317199	0.00055	2301.5
Indole-5,6-quinone Isatin	C8H5NO2	402.311503	402.311999	0.000496	7508.5
(2E)-4-hydroxy-5-methyl-2-propylidene-3(2H)-furanone	C8H10O3	409.342469	409.341009	-0.00146	1746.4
1,2-Dihydroxy-5-(methylthio)pent-1-en-3-one 2-oxo-5-methylthiopentanoate	C6H10O3S	417.314539	417.315089	0.00055	1541.8
L-fuculose Rhamnose L-rhamnofuranose fucose 1-O-methyl-β-D-xylopyranose Deoxy-D-glucose Deoxy-L-galactose 1,5-Anhydroglucitol	C6H12O5	419.347949	419.348329	0.00038	10340.3

D-galacto-Hexodialdose 5-Dehydro-D-fructose 2-keto-myo-inositol L-sorbosone 2,6-lactone D-galacto-hexodialdose 2-Deoxy-5-keto-D-gluconic acid L-sorbosone Dehydrodeoxygluconate L-sorbosone 1,4-lactone Gulonolactone Galactonolactone Dehydrodeoxygalactonate Dehydroglucose Gluconolactone Dehydrogalactose 5-Deoxy glucuronic acid 2-Inosose	C6H10O6	433.327214	433.327769	0.000555	69951.5
5-Methylthio-D-ribose	C6H12O4S	435.325104	435.325029	-7.5E-05	2982.9
9-Fluorenone	C13H8O	435.336989	435.337029	4E-05	18490.2
Hexose (ketose and aldose)	C6H12O6	435.342864	435.343289	0.000425	3235226.3
Hydroxykynurenamine	C9H12N2O2	435.369352	435.370609	0.001257	1106.1
oxo capric acid methyl 9-oxononanoate 1,3S-dihydroxy-8E-decen-5-one Oxodecanoate	C10H18O3	441.405069	441.405559	0.00049	16598.7
Sedoheptulose Mannoheptulopyranose	C7H14O7	465.353429	465.353969	0.00054	10502.8
oxo-dodecanoic acid	C12H22O3	469.436369	469.437049	0.00068	33371.2
S-(3-oxo-3-carboxy-n-propyl)cysteine	C7H11NO5S	476.315268	476.316249	0.000981	1017.3
N-Acetylhexosamine	C8H15NO6	476.369413	476.370119	0.000706	291404.4
5-isobutylthioribose	C9H18O4S	477.372054	477.373469	0.001415	77115.6
Pyridoxal 5-phosphate	C8H10NO6P	502.30405	502.304809	0.000759	69620.1
Muramic acid	C9H17NO7	506.379978	506.380799	0.000821	3817.1
Octadecenal	C18H34O	521.540439	521.541639	0.0012	1657.1
Eicosanal	C20H40O	551.587389	551.588249	0.00086	26901.3

Table S3: Slopes, intercepts, and R^2 values for the linear regressions of Figure S1 (below)

	*QDA-Pyr	*QDA-αKg	*QDA-αKb	*QDA-Glc
Slope	536847	579435	470851	265815
Intercept	813724	1523225	781273	488369
R^2	0.974	0.924	0.969	0.960

Figure S1: Log-log scatter plot of concentration versus ion count (non-normalized) for four *QDA standard adducts. αKg = α-ketoglutarate; Pyr = pyruvate ; αKb = ketobutyrate; Glc = glucose.

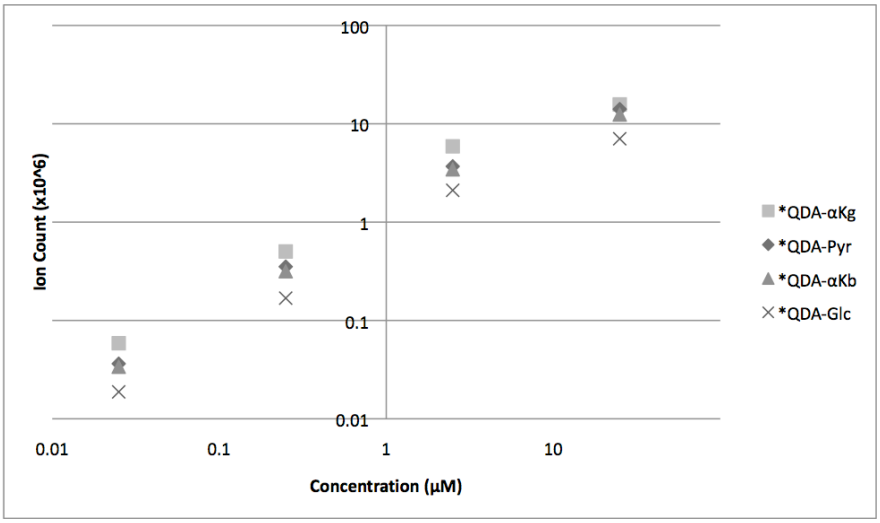


Figure S2: Scatter plot with linear regression of the normalized ion counts of QDA-$^{13}C_3$-pyruvate from buffer sample (**A**) and cell extract sample (**B**) at three concentrations, averaged over three injections.

Figure S2A

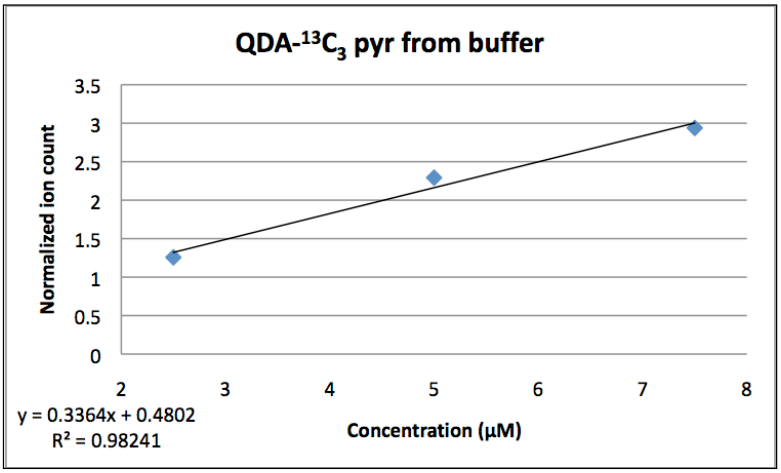
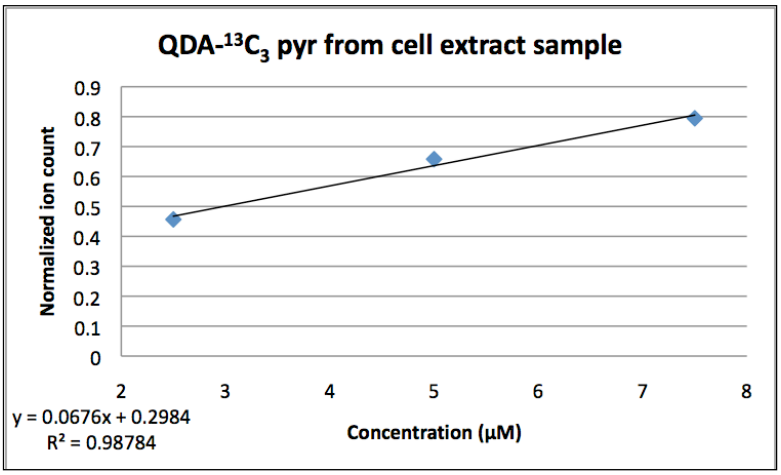
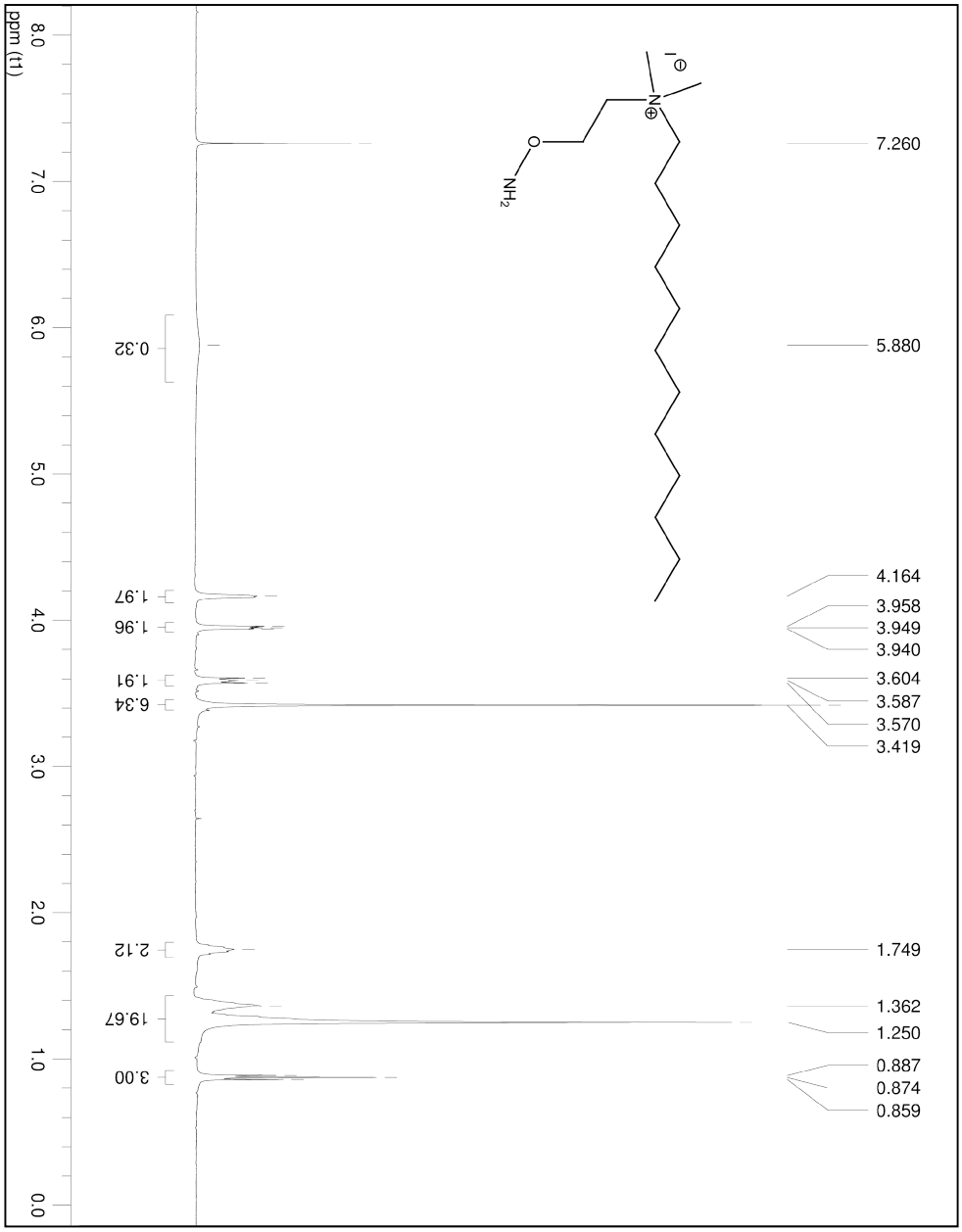


Figure S2B

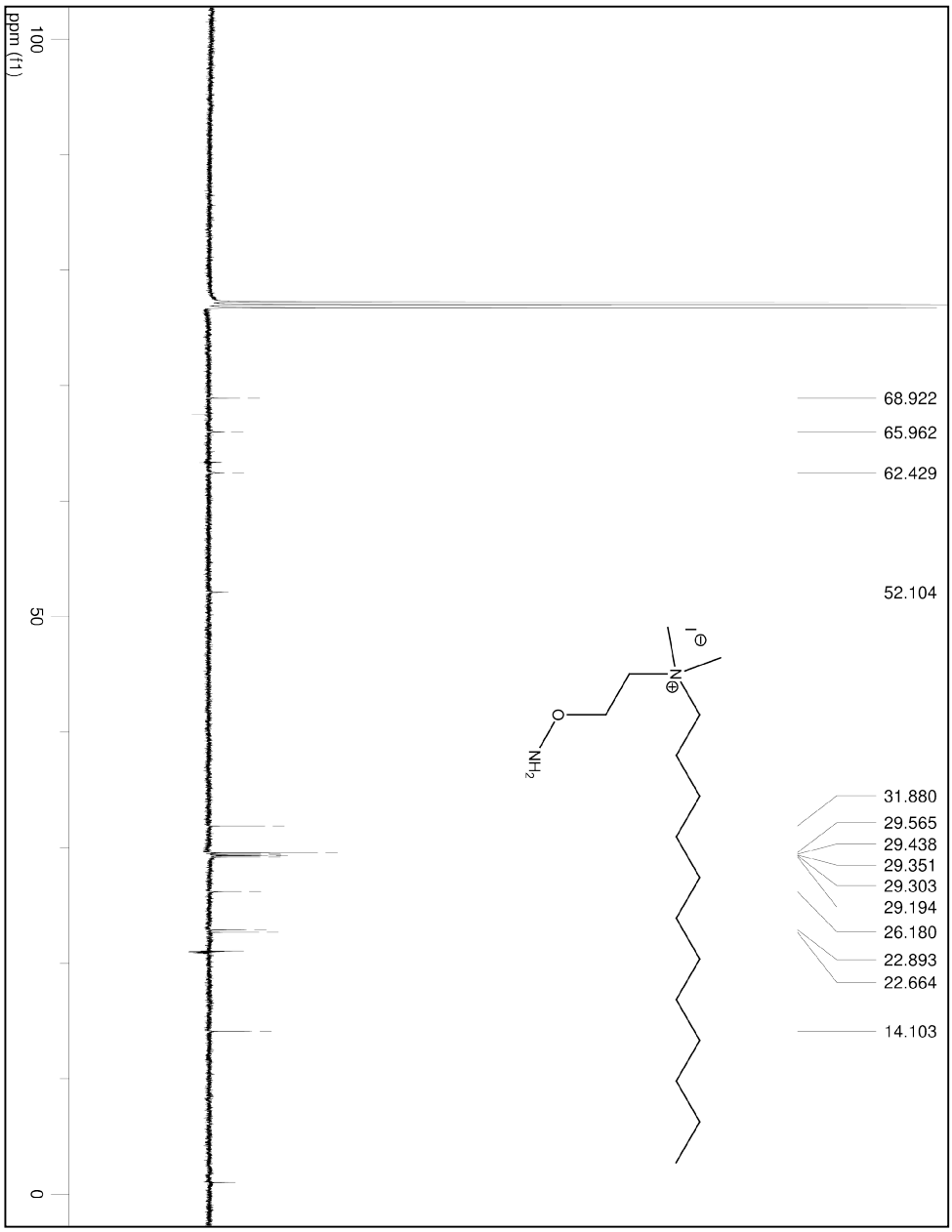

QDA-$^{13}C_3$ pyr from cell extract sample
Normalized ion count
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
3
4
5
6
7
8
y = 0.0676x + 0.2984
R² = 0.98784
Concentration (µM)

NMR Spectra of QDA and *QDA

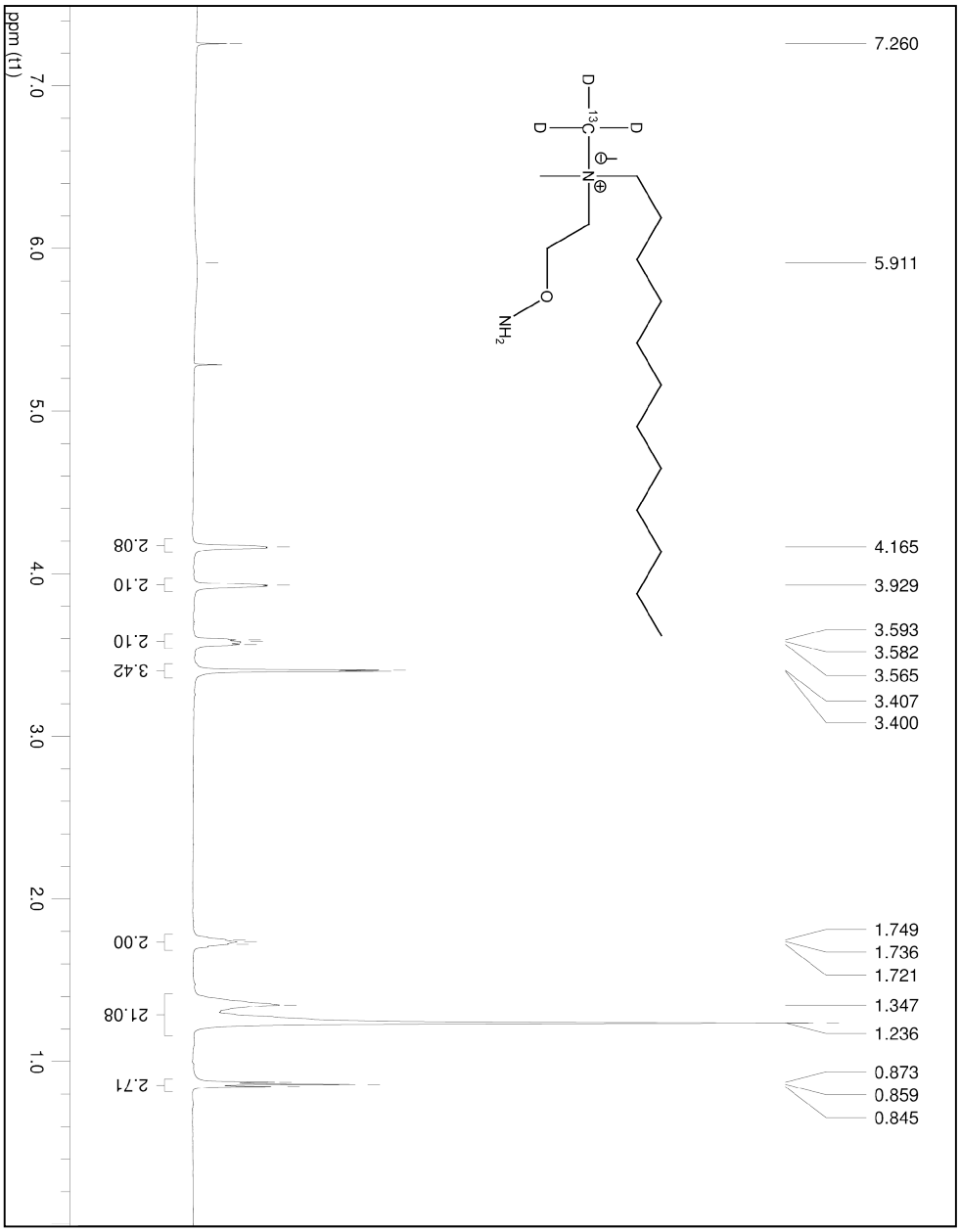
Spectrum 1: ^{1}H NMR spectrum QDA·I in $CDCl_3$



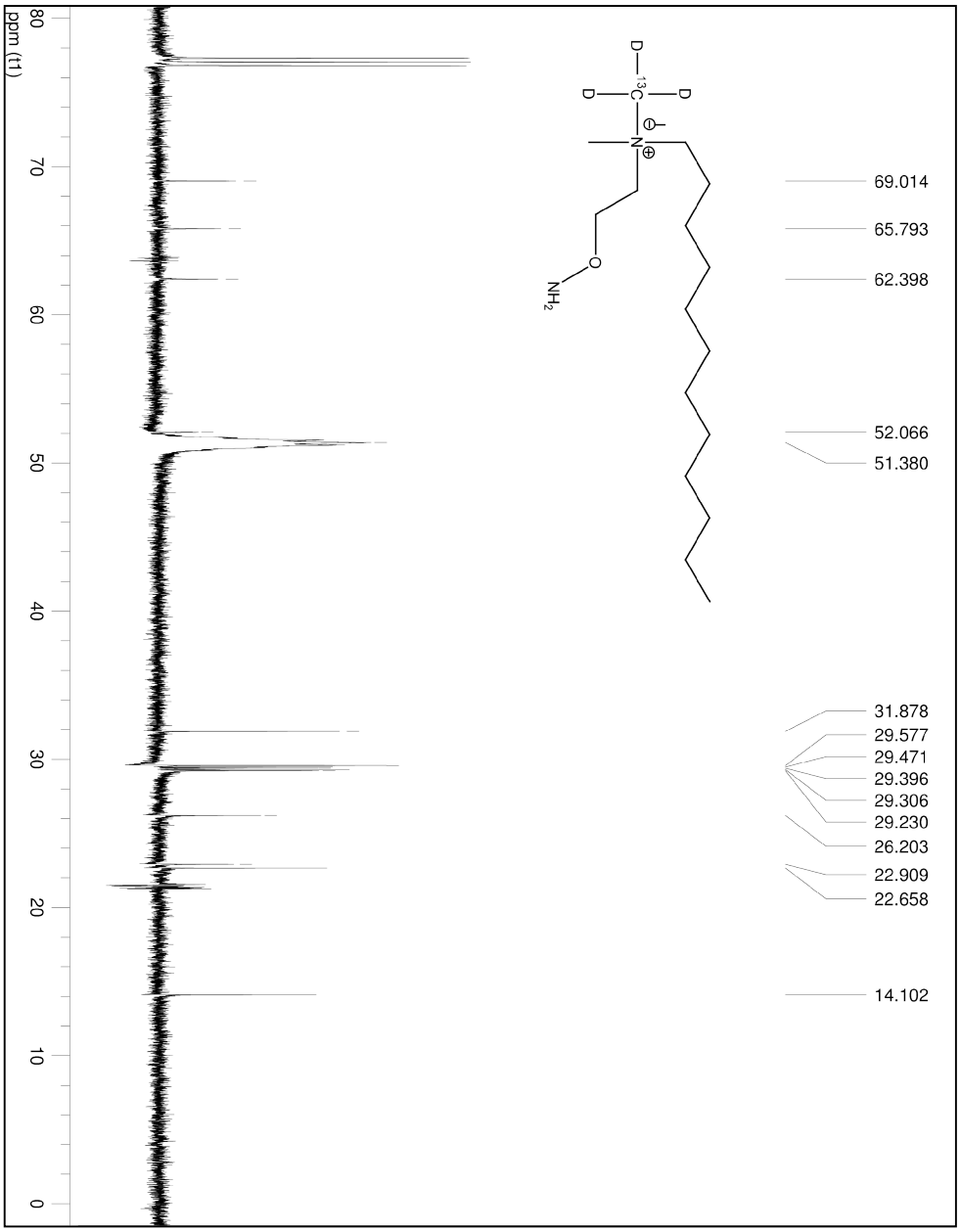
Spectrum 2: ^{13}C NMR spectrum QDA·I in $CDCl_3$



Spectrum 3: ^{1}H NMR spectrum *QDA·I in $CDCl_3$



Spectrum 4: ^{13}C NMR spectrum *QDA·I in $CDCl_3$



References

1. Tsubone, K.; Uchida, N.; Niwase, H.; Honda, K., Syntheses of Sodium 2-(N-alkyl-N-methylamino)ethanephosphates and Their Physicochemical Properties. *Journal of the American Oil Chemists' Society* **1989,** *66* (6), 829-833.
2. Lane, A. N.; Fan, T. W.-M.; Xie, Z.; Moseley, H. N. B.; Higashi, R. M., Isotopomer analysis of lipid biosynthesis by high resolution mass spectrometry and NMR. *Analytica Chimica Acta* **2009,** *651*, 201-208.