Supporting Information

Identification of Fumarate Hydratase Inhibitors with Nutrient-Dependent Cytotoxicity

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1. Supporting Information Figures and Tables

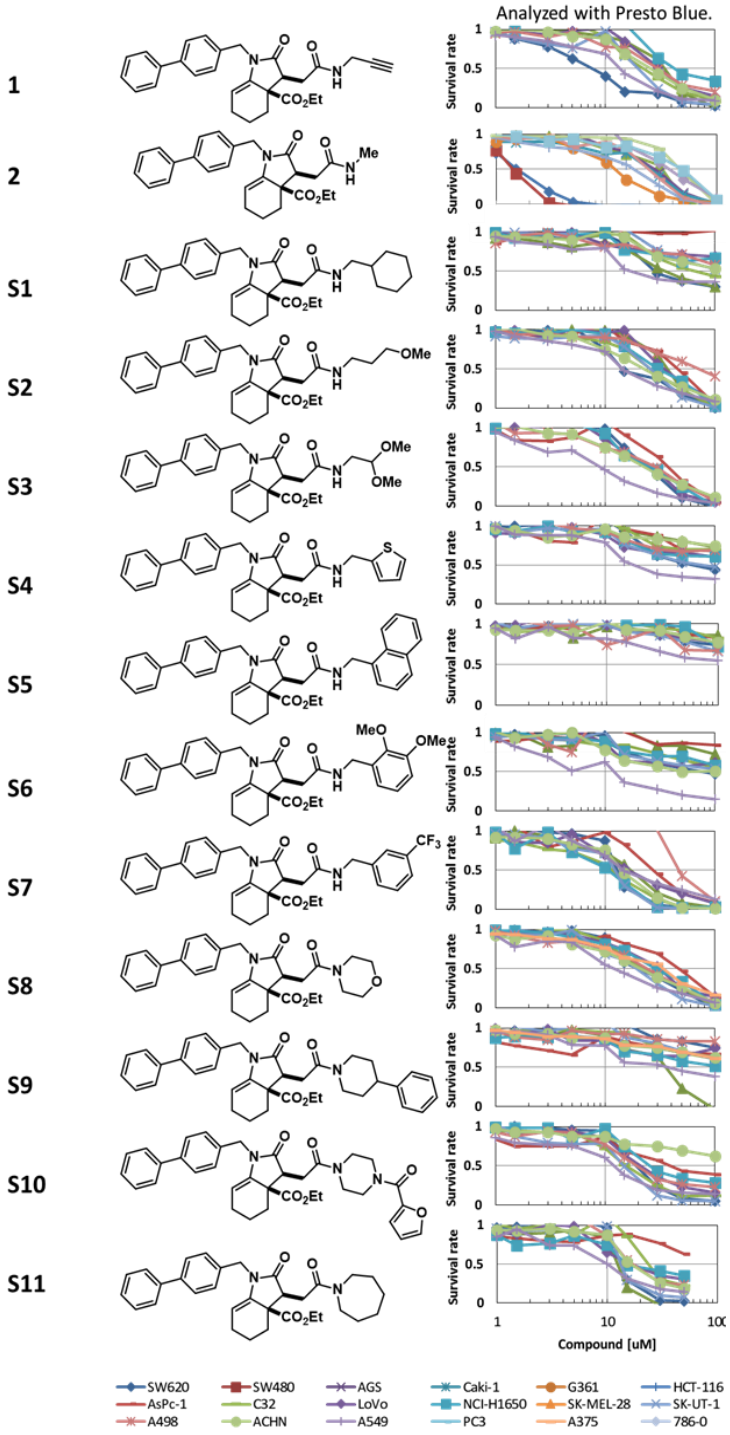


Figure S1. Structure activity relationship studies. Dose-dependent effect of each of the compounds[1] shown on cell growth was analyzed. Each cell line was grown in recommended medium (as described in the Experimental protocols section) and was treated with each compound for 72 h.

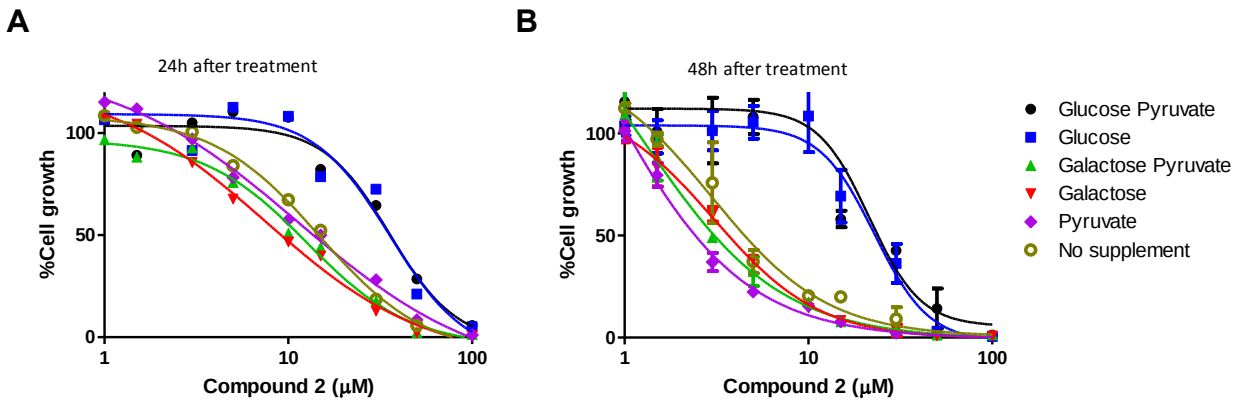


Figure S2. Nutrient dependence on activity of compound **2**. Each nutrient, including glucose (2 mM), galactose (2 mM) or sodium pyruvate (2 mM), was added to glucose-free DME medium supplemented with dialyzed FBS. Cell growth rate of SW620 cell line was analyzed after 24 h (A) or 48 h (B) of treatment with compound **2**.

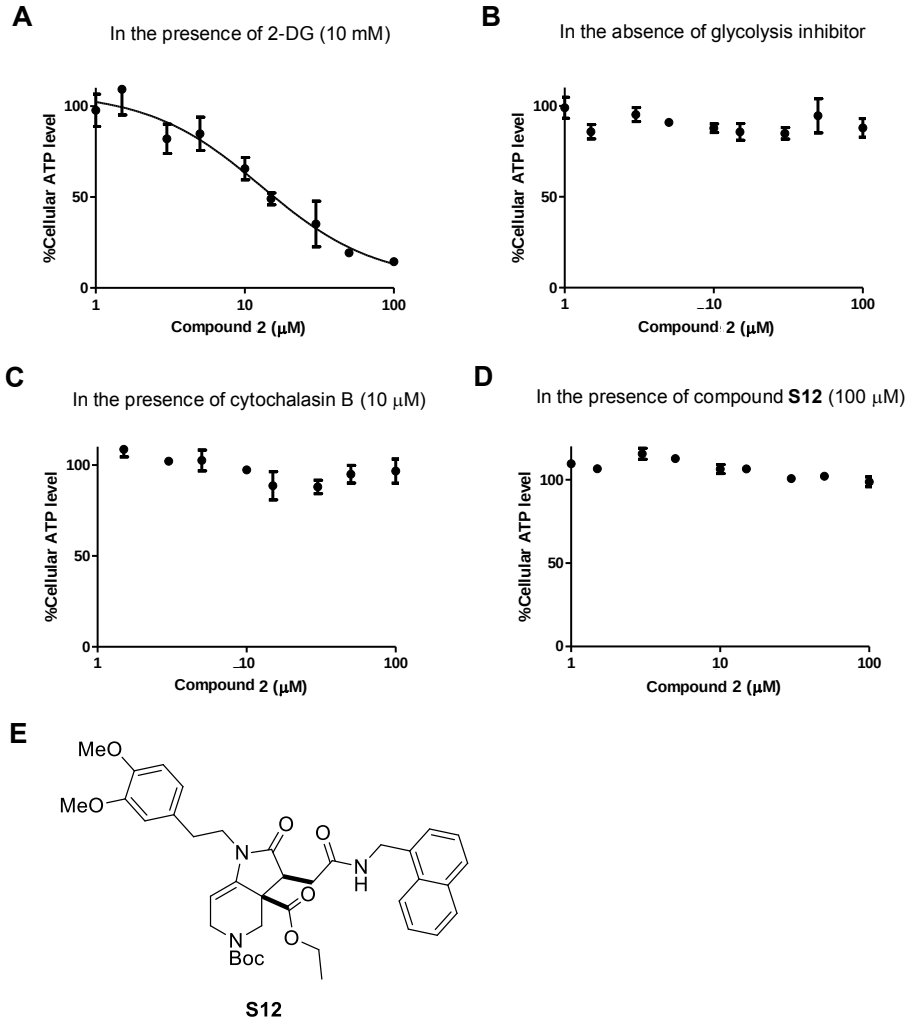


Figure S3. Cellular ATP level of SW620 cells treated with compound **2** along with glycolysis inhibitors. (A-D) Inhibition of ATP production in SW620 cells in response to treatment with compound **2**, in the presence or absence of a glycolysis inhibitor, such as 2-deoxy-glucose (2-DG, 10 mM) (A), DMSO (B), cyochalasin B (10 μM) (C) or **S12** (100 μM) (D) . All values are presented as percentage of DMSO-treated sample. Each value is mean ± s.e.m. of triplicate values from a representative experiment. (A) ATP depletion upon treatment with a combination of compound **2** and 2-DG (10 mM). (B) Constant level of ATP after treatment of cells with compound **2** in the absence of a glycolysis inhibitor. (C) Treatment of cells with a combination of compound **2** and cytochalasin B (10 μM) does not affect ATP levels significantly within 30 min of incubation. (D) Treatment of cells with a combination of compound **2** and compound **S12** does not affect ATP levels significantly within 30 min of incubation. (E) Structure of previously reported glycolysis inhibitor **S12** by our group.[1]

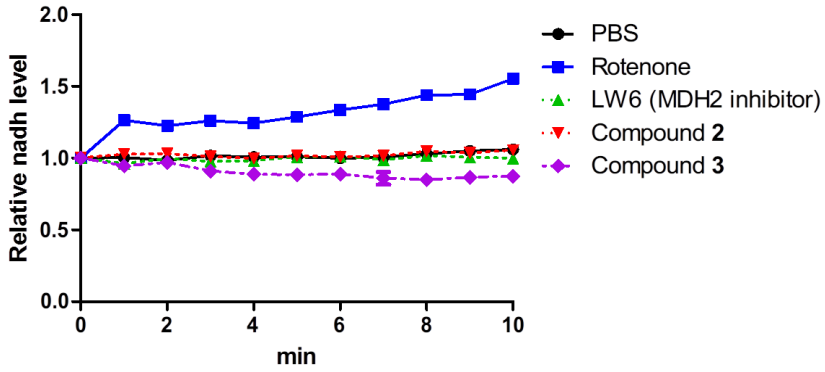


Figure S4. Levels of cellular NADH concentration were estimated using autofluorescence of cells by confocal microscopy (350 nm excitation and 457 nm emission). SW620 cells were treated with rotenone (1 μM), LW6[2] (10 μM), compound **2** (10 μM) or compound **3** (10 μM). As soon as compound was added, fluorescence was measured every 1 min for 10 min.

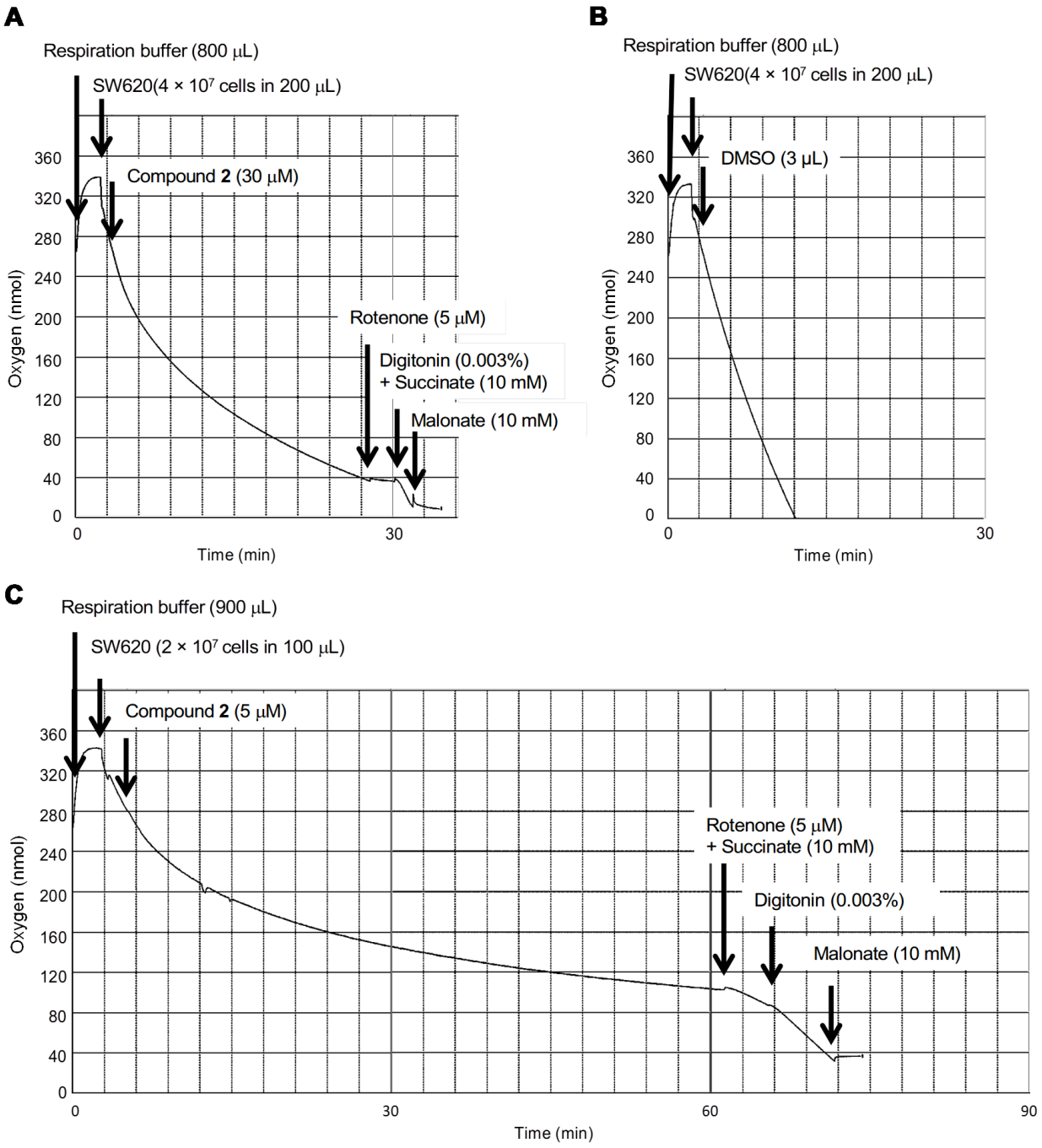


Figure S5. Real-time measurement of oxygen consumption by Clark-type oxygen electrode. Respiration of SW620 treated with compound **2** (30 μM) (A), DMSO (3 μL) (B) or compound **2** (5 μM) (C) was monitored. ETC complex activities were measured by adding complex-specific substrates after inhibiting prior complexes in the chain. Complex II-IV activities were measured by adding succinate, complex II-specific substrate, after inhibition of complex I with rotenone (5 μM). Then complex II-IV activities were inhibited by malonate (10 mM), a complex II inhibitor. Digitonin was added to permeabilize the cells.

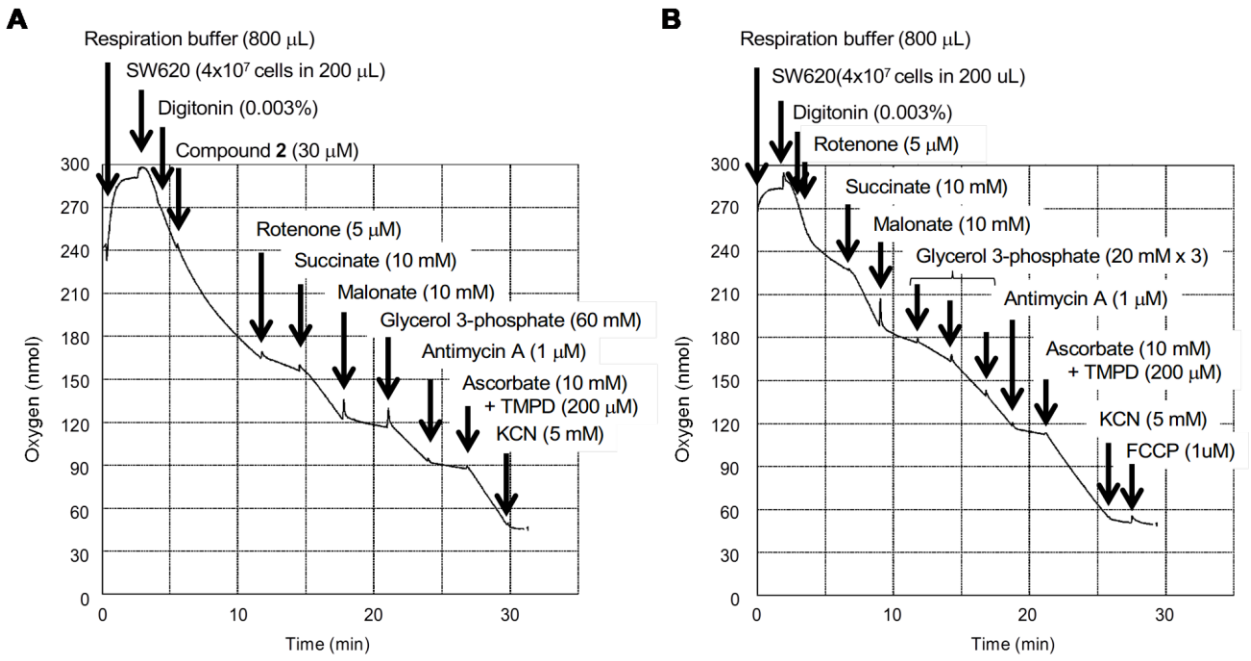


Figure S6. Measurement of electron transport chain activity by Clark-type oxygen electrode. Respiration of SW620 treated with compound **2** (5 μM) was monitored. ETC complex activities were measured by adding complex-specific substrates after inhibiting prior complexes in the chain. Complex II-IV activities were measured by adding succinate, complex II-specific substrate, after inhibition of complex I with rotenone (5 μM). Complex III-IV activities were measured by adding glycerol-3-phosphate after inhibition of complex II with malonate (10 mM). Complex IV activity was measured by adding ascorbate (10 mM) and TMPD (200 μM) after inhibition of complex III with antimycin A (1 μM). KCN, complex IV inhibitior, was added to stop oxygen consumption. Digitonin was added to permeabilize the cells. TMPD: tetramethyl-*p*-phenylenediamine.

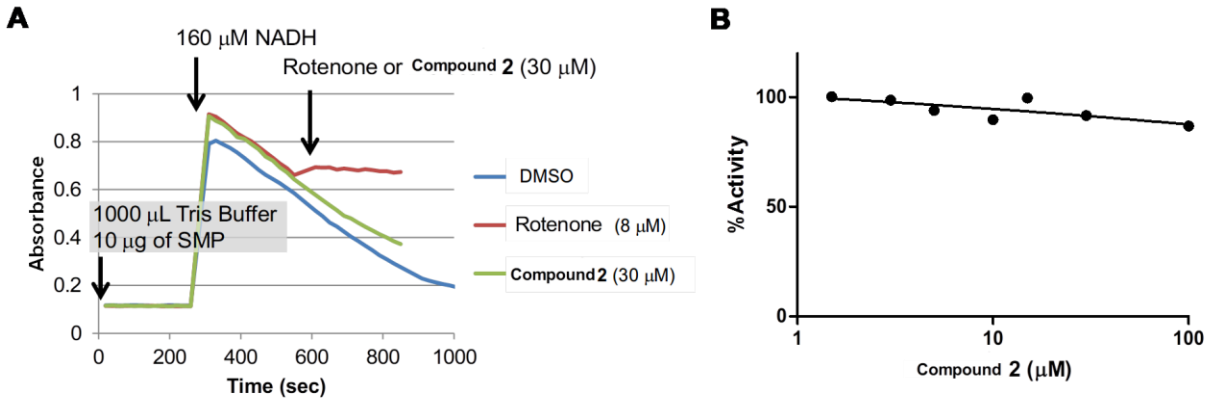


Figure S7. Effect of compound **2** on mitochondrial complex I activity. (A) Real-time consumption of NADH by SMP was monitored. SMP from SW620 cells was incubated in tris buffer. After addition of NADH, the consumption was monitored using absorbance change of 340 nm. (B) Dose-dependent effect of compound **2** on mitochondrial complex I activity. Absorbance change of SMP from SW620 cells treated with several concentration of compound **2** was analyzed, after addition of NADH.

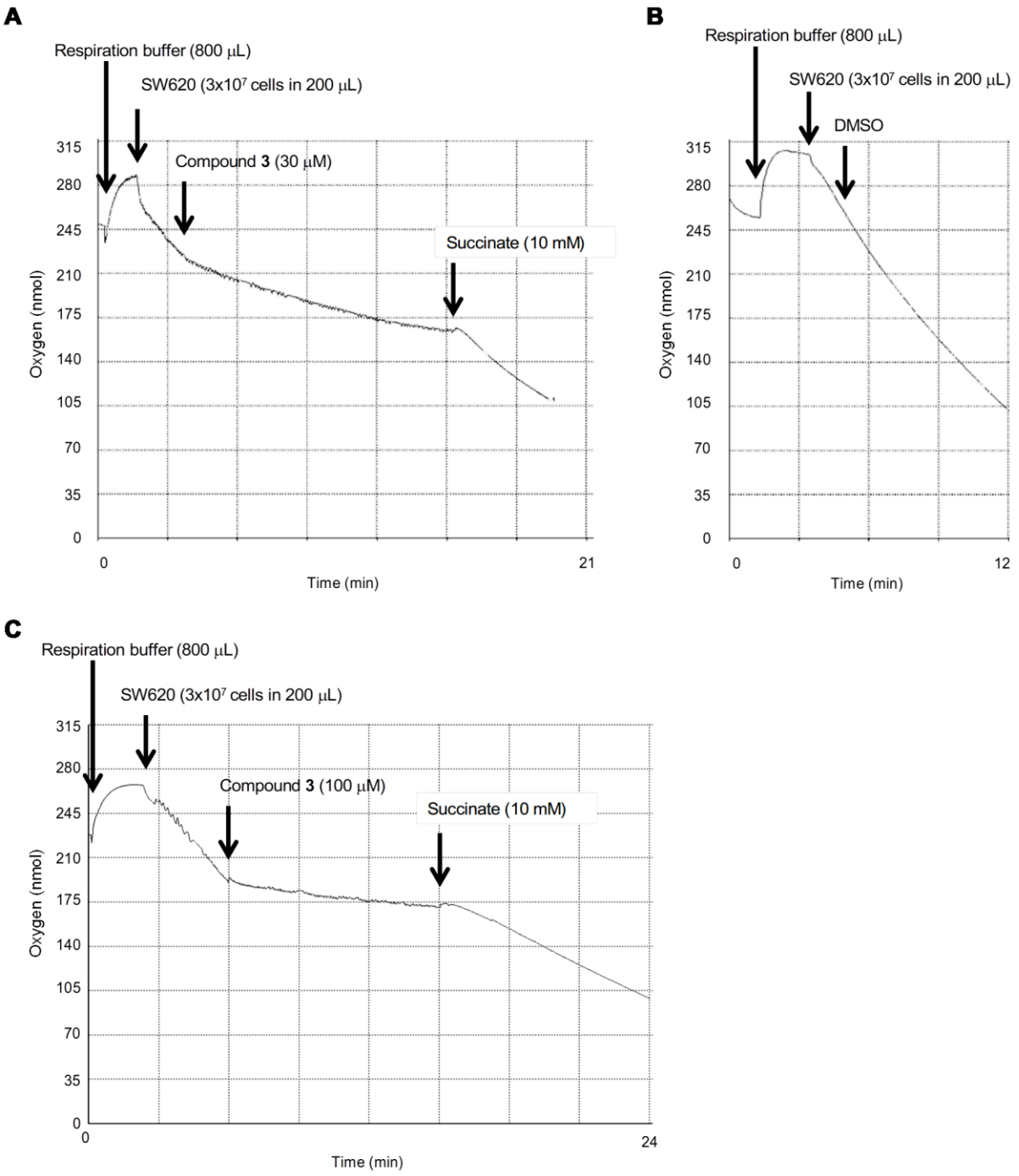


Figure S8. Measurement of electron transport chain activity by Clark-type oxygen electrode. Respiration of SW620 treated with compound **3** (30 or 100 μM) was monitored. Respiration was reinitiated by adding succinate (10 mM), substrate of complex II.

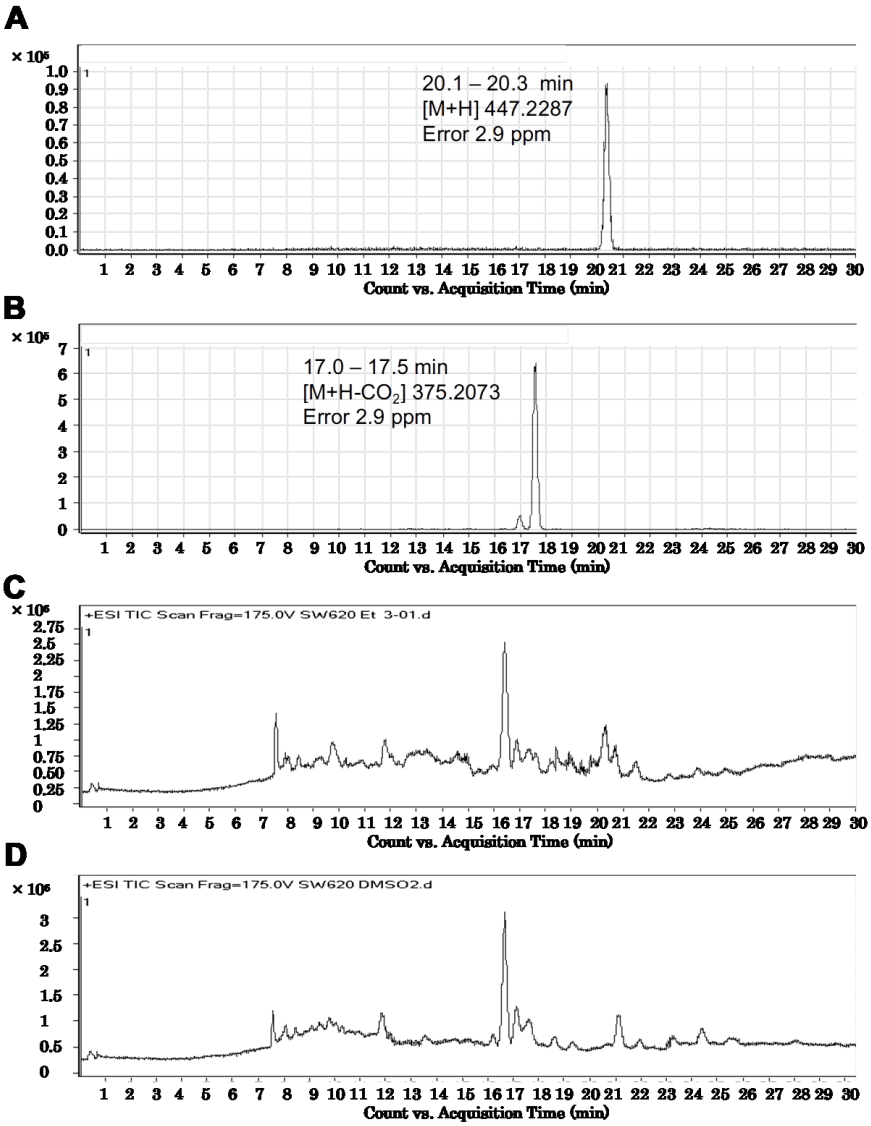


Figure S9. LCMS analysis of compound **2** alone (1 pmol) (A), compound **3** alone (1 pmol) (B), metabolite-extract from compound **2**-treated SW620 cells (C), and metabolite-extract from DMSO-treated SW620 cells (D). SW620 cells (1×10^6) treated with DMSO or compound **2** (10 μM) for 2 h were extracted with MeOH/H_2O (80:20) and the metabolite-extract (4 μL of 1 mL) was analyzed by LCMS.

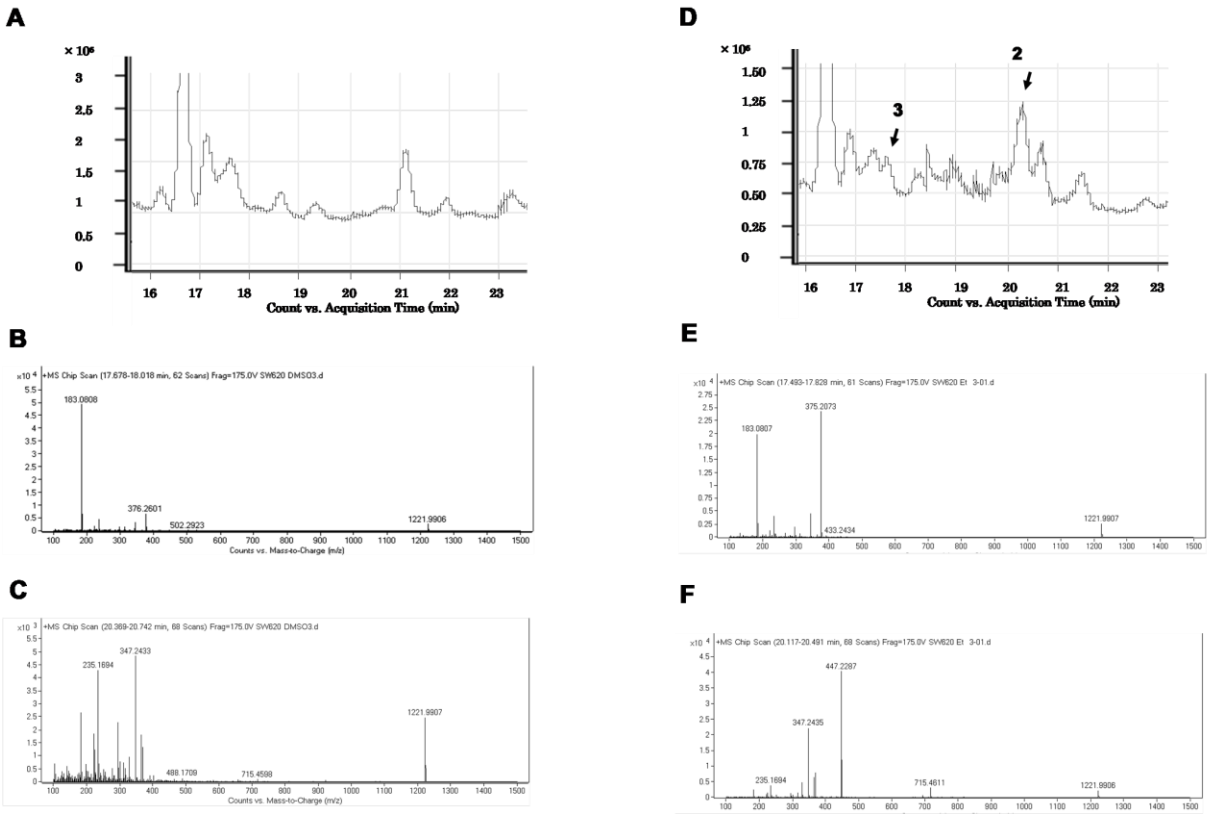


Figure S10. LCMS analysis of metabolite-extract from metabolite-extract from DMSO- (A) and compound **2**-treated SW620 cells (D). (AD) Magnified spectrum of Figure S9CD. (BC) Mass spectrum of metabolite-extract from DMSO-treated SW620 cells at 17.7 (B) and 20.3 min (C). (EF) Mass spectrum of metabolite-extract from compound **2**-treated SW620 cells at 17.7 (E) and 20.3 min (F).

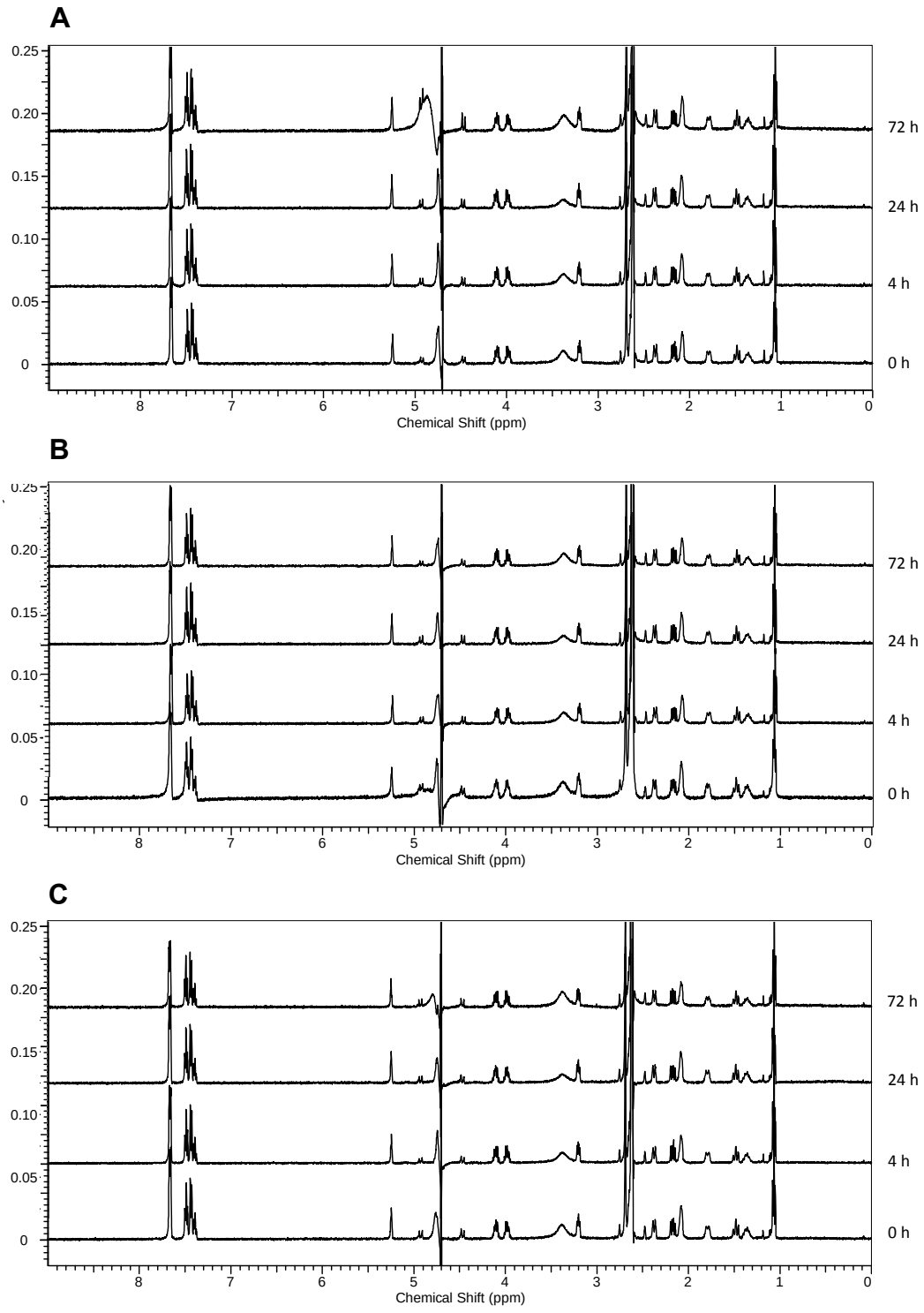


Figure S11. Stability of compound **2** under biologically acidic, neutral or basic condition. Compound **2** (250 μM) incubated in phosphate buffer (25 mM sodium phosphate in D_2O containing 25%(v/v) d_6-DMSO [pH 6 (A), 7 (B) or 8 (C)]) for 0, 4, 24 and 72 h at 37 °C was analyzed by ^{1}H NMR employing water suppression method.

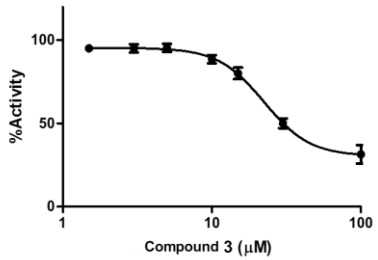


Figure S12. Dose-dependent effect of compound **3** on mitochondrial complex I activity. Absorbance change of SMP from SW620 cells treated with several concentration of compound **3** was analyzed, after addition of NADH.

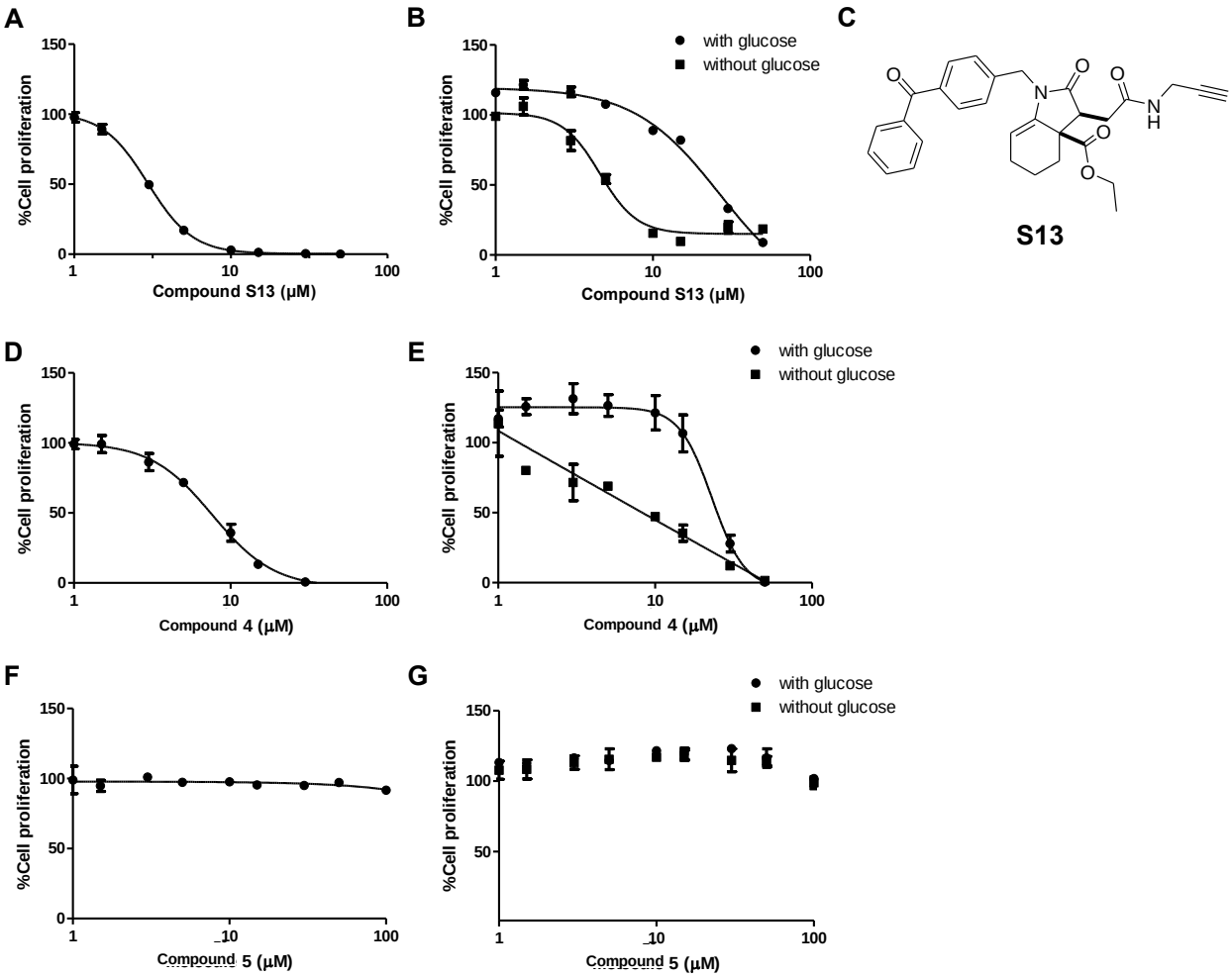


Figure S13. (ABDEFG) Effect of compound **S13**, **4** and **5** on antiproliferative activity of SW620 cultured in L-15 medium (A, D and F, respectively) or in DME medium with or without glucose (B, E and G, respectively). Each value is mean ± SEM of triplicate values from three independent experiments. (C) Structure of compound **S13**.

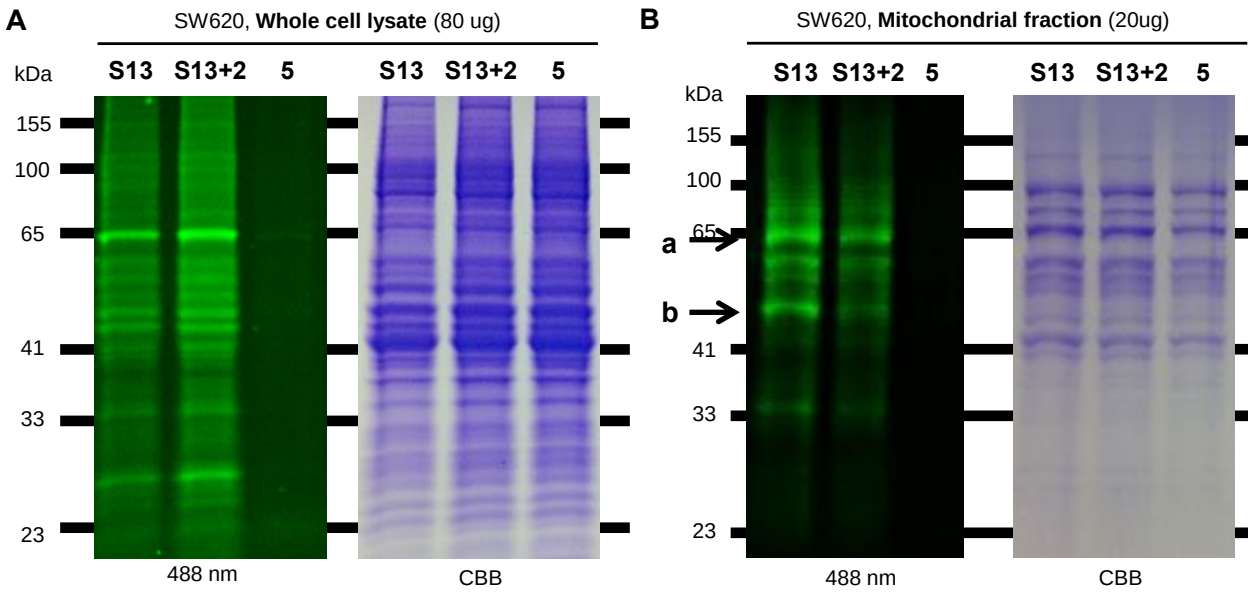


Figure S14. Target identification by photoaffinity labeling. The cells were treated with either of the probe **S13** (5 μM) or **5** (5 μM) in the absence or presence of **2** (10 μM), irradiated at 365 nm and lysed. Photocrossslinked proteins were next labeled with azide-conjugated Alexa Fluor 488 and analyzed by SDS-PAGE. (A) Fluorescence imaging and coomassie brilliant blue (CBB) staining of SDS-PAGE separated proteins from whole SW620 cell lysate following treatment with photoaffinity probes, UV irradiation and fluorescent dye labeling. (B) Fluorescence imaging and CBB staining of SDS-PAGE separated proteins from mitochondrial fraction of SW620 cells following treatment with photoaffinity probes, UV irradiation and fluorescent dye labeling. Arrowed bands a and b were identified as Hsp70 and fumarate hydratase, respectively (see Table S3).

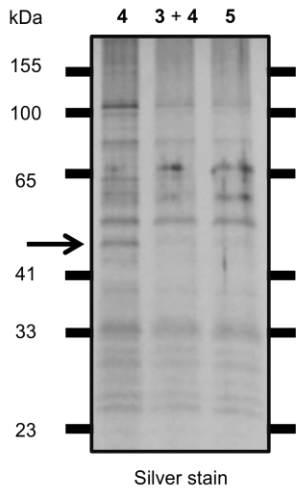


Figure S15. Pull-down assay using chemical probe **4**. SW620 cells treated with chemical probe **4**, both **4** and **3** as a competitor, or **5** were irradiated by UV. Proteins conjugated to probe **4** or **5** in lysate of isolated mitochondria was labeled with azide-conjugated biotin using click chemistry, which were recovered using avidin-agarose beads. Recovered proteins were analyzed using SDS-PAGE and visualized by silver stain.

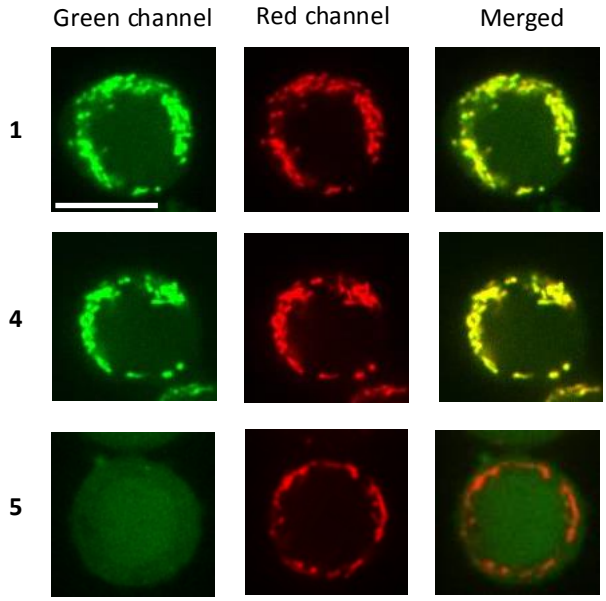


Figure S16. Subcellular localization of compounds **1**, **4** and **5**. SW620 cells were treated with chemical probe **3**, **4** or **5**, followed by click reaction with azide-conjugated Alexa Fluor 488 to visualize subcellular localization of compounds (green channel). Mitochondria were selectively stained with Mito Tracker Red (red channel). Scale bars = 10 μm.

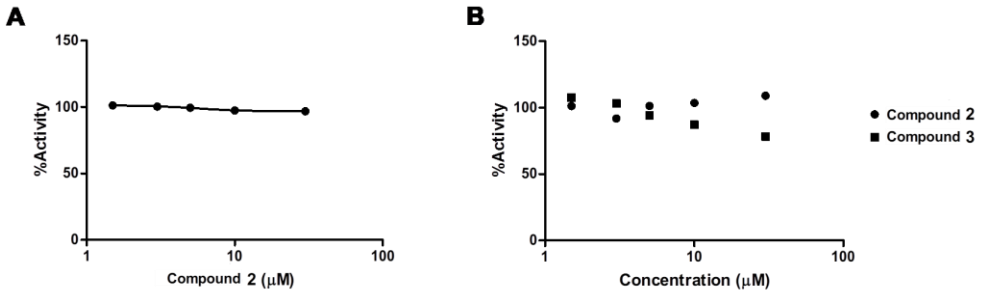


Figure S17. Effect of compounds **2** and **3** on fumarate hydratase and malate dehydrogenase activity. (A) Effect of compound **2** on isolated fumarate hydratase from SW620 cells. (B) Effect of compounds **2** and **3** on malate dehydrogenase from porcine heart.

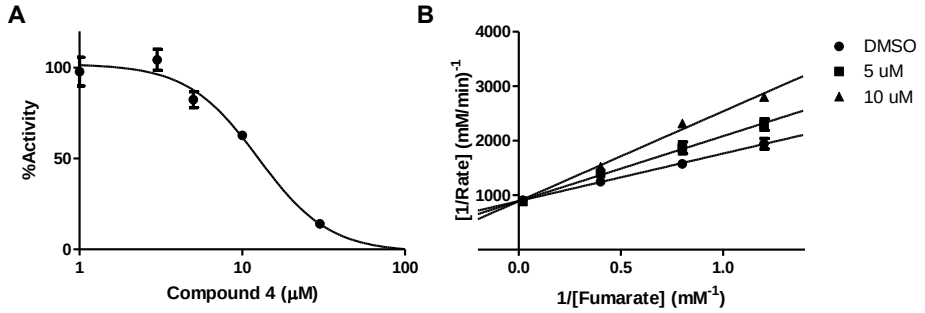


Figure S18. Inhibition of fumarate hydratese with compound **4** in vitro. (A) Dose-dependent inhibition of fumarate hydratase, which was iso-lated from SW620 cells, by compound **4**. (B) Lineweaver-Burk plot of the inhibition of fumarate hydratase by **4**. Kinetic parameters: Ki = 6.8 μM (Competitive inhibition), Km = 1.2 mM, Vmax = 1.2 μM/min.

Scheme S1. Fumarate hydratase activity assay.

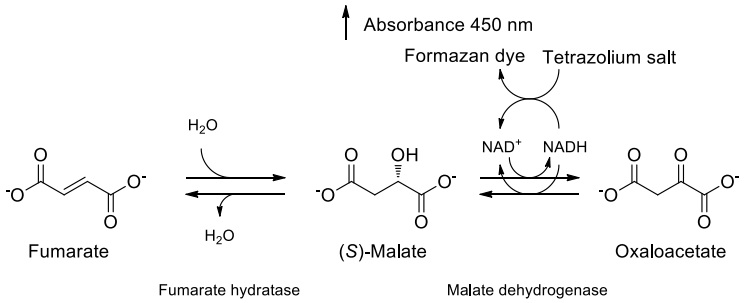


Table S1. Proteins identified in arrowed-50 kDa protein band in supplementary Fig. S15. Sliced protein band was processed by in situ digestion using trypsin. Digested peptides were analyzed using LCMS to identify proteins listed.

	Score	MW	Protein
1	336	54815	Fumarate hydratase, mitochondrial OS=Homo sapiens GN=FH PE=1 SV=3
2	252	48029	Keratin, type I cytoskeletal 18 OS=Homo sapiens GN=KRT18 PE=1 SV=2
3	215	61229	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2
4	134	42136	Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1
5	87	48930	Ornithine aminotransferase, mitochondrial OS=Homo sapiens GN=OAT PE=1 SV=1
6	69	46664	Adipocyte plasma membrane-associated protein OS=Homo sapiens GN=APMAP PE=1 SV=2
7	67	49936	Elongation factor Tu, mitochondrial OS=Homo sapiens GN=TUFM PE=1 SV=2
8	63	51964	Citrate synthase, mitochondrial OS=Homo sapiens GN=CS PE=1 SV=2
9	55	48451	Thioredoxin domain-containing protein 5 OS=Homo sapiens GN=TXNDC5 PE=1 SV=2

Table S2. Results of mass spectrometric analysis.

1) Identification of 100 kDa protein.

Rank	Score	Mass	Description
1	637	31840	Complement component 1 Q subcomponent-binding protein, mitochondrial OS=Homo sapiens GN=C1QBP PE=1 SV=1
2	559	92767	Endoplasmin OS=Homo sapiens GN=HSP90B1 PE=1 SV=1
3	232	107375	Neutral alpha-glucosidase AB OS=Homo sapiens GN=GANAB PE=1 SV=3
4	204	35484	Electron transfer flavoprotein subunit alpha, mitochondrial OS=Homo sapiens GN=ETFA PE=1 SV=1
5	180	45493	Lysosome-associated membrane glycoprotein 1 OS=Homo sapiens GN=LAMP1 PE=1 SV=3
6	149	48930	Ornithine aminotransferase, mitochondrial OS=Homo sapiens GN=OAT PE=1 SV=1
7	112	42136	Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1
8	109	68236	4F2 cell-surface antigen heavy chain OS=Homo sapiens GN=SLC3A2 PE=1 SV=3
9	102	114927	Isoleucine--tRNA ligase, mitochondrial OS=Homo sapiens GN=IARS2 PE=1 SV=2
10	97	105357	Alpha-actinin-4 OS=Homo sapiens GN=ACTN4 PE=1 SV=2
11	80	92477	Integrin beta-1 OS=Homo sapiens GN=ITGB1 PE=1 SV=2
12	76	33046	ATP synthase subunit gamma, mitochondrial OS=Homo sapiens GN=ATP5C1 PE=1 SV=1
13	67	114458	Sodium/potassium-transporting ATPase subunit alpha-1 OS=Homo sapiens GN=ATP1A1 PE=1 SV=1
14	64	106860	Monofunctional C1-tetrahydrofolate synthase, mitochondrial OS=Homo sapiens GN=MTHFD1L PE=1 SV=1
15	60	61229	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2
16	51	62311	Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3
17	50	107062	Lon protease homolog, mitochondrial OS=Homo sapiens GN=LONP1 PE=1 SV=2

2) Identification of 90 kDa protein.

Rank	Score	Mass	Description
1	2562	92767	Endoplasmin OS=Homo sapiens GN=HSP90B1 PE=1 SV=1
1	722	83638	Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4
1	398	85104	Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1 PE=1 SV=5
1	157	80415	Heat shock protein 75 kDa, mitochondrial OS=Homo sapiens GN=TRAP1 PE=1 SV=3
2	1223	105357	Alpha-actinin-4 OS=Homo sapiens GN=ACTN4 PE=1 SV=2
3	907	68094	Calnexin OS=Homo sapiens GN=CANX PE=1 SV=2
4	727	68236	4F2 cell-surface antigen heavy chain OS=Homo sapiens GN=SLC3A2 PE=1 SV=3
5	428	107375	Neutral alpha-glucosidase AB OS=Homo sapiens GN=GANAB PE=1 SV=3
6	412	61229	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2
7	358	107062	Lon protease homolog, mitochondrial OS=Homo sapiens GN=LONP1 PE=1 SV=2
8	209	66212	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6
9	188	106860	Monofunctional C1-tetrahydrofolate synthase, mitochondrial OS=Homo sapiens GN=MTHFD1L PE=1 SV=1
10	184	71954	Drebrin OS=Homo sapiens GN=DBN1 PE=1 SV=4
11	183	57131	Neutral amino acid transporter B(0) OS=Homo sapiens GN=SLC1A5 PE=1 SV=2
12	169	114458	Sodium/potassium-transporting ATPase subunit alpha-1 OS=Homo sapiens GN=ATP1A1 PE=1 SV=1
13	165	114927	Isoleucine--tRNA ligase, mitochondrial OS=Homo sapiens GN=IARS2 PE=1 SV=2
14	142	73990	Stress-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2
15	127	45643	Lysosome-associated membrane glycoprotein 2 OS=Homo sapiens GN=LAMP2 PE=1 SV=2
16	111	45493	Lysosome-associated membrane glycoprotein 1 OS=Homo sapiens GN=LAMP1 PE=1 SV=3
17	110	88171	Delta-1-pyrroline-5-carboxylate synthase OS=Homo sapiens GN=ALDH18A1 PE=1 SV=2
18	102	90118	Transitional endoplasmic reticulum ATPase OS=Homo sapiens GN=VCP PE=1 SV=4
19	88	31840	Complement component 1 Q subcomponent-binding protein, mitochondrial OS=Homo sapiens GN=C1QBP PE=1 SV=1
20	83	93205	Villin-1 OS=Homo sapiens GN=VIL1 PE=1 SV=4
21	61	27251	Trypsin-1 OS=Homo sapiens GN=PRSS1 PE=1 SV=1
22	57	96485	Elongation factor 2 OS=Homo sapiens GN=EEF2 PE=1 SV=4
23	54	117353	2-oxoglutarate dehydrogenase, mitochondrial OS=Homo sapiens GN=OGDH PE=1 SV=3

3) Identification of 65 kDa protein.

Rank	Score	Mass	Description
1	2799	73990	Stress-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2
2	2591	72431	78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 SV=2
2	373	71138	Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1
2	344	70829	Heat shock 70 kDa protein 1-like OS=Homo sapiens GN=HSPA1L PE=1 SV=2
2	287	70364	Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A PE=1 SV=5
3	2126	73313	Protein disulfide-isomerase A4 OS=Homo sapiens GN=PDIA4 PE=1 SV=2
4	1085	80415	Heat shock protein 75 kDa, mitochondrial OS=Homo sapiens GN=TRAP1 PE=1 SV=3
5	268	61229	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2
6	94	68094	Calnexin OS=Homo sapiens GN=CANX PE=1 SV=2
7	87	92767	Endoplasmin OS=Homo sapiens GN=HSP90B1 PE=1 SV=1
8	72	63612	Peptidyl-prolyl cis-trans isomerase FKBP9 OS=Homo sapiens GN=FKBP9 PE=1 SV=2
9	69	57131	Neutral amino acid transporter B(0) OS=Homo sapiens GN=SLC1A5 PE=1 SV=2
10	54	68236	4F2 cell-surface antigen heavy chain OS=Homo sapiens GN=SLC3A2 PE=1 SV=3

4) Identification of 60 kDa protein

Rank	Score	Mass	Description
1	3702	61229	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2
2	2384	57578	Protein disulfide-isomerase OS=Homo sapiens GN=P4HB PE=1 SV=3
3	2159	73990	Stress-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2
4	1863	57244	Protein disulfide-isomerase A3 OS=Homo sapiens GN=PDIA3 PE=1 SV=4
5	1620	72431	78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 SV=2
5	342	70333	Heat shock-related 70 kDa protein 2 OS=Homo sapiens GN=HSPA2 PE=1 SV=1
6	819	73313	Protein disulfide-isomerase A4 OS=Homo sapiens GN=PDIA4 PE=1 SV=2
7	801	59856	ATP synthase subunit alpha, mitochondrial OS=Homo sapiens GN=ATP5A1 PE=1 SV=1
8	648	65748	Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2
8	296	66212	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6
8	216	53671	Keratin, type II cytoskeletal 8 OS=Homo sapiens GN=KRT8 PE=1 SV=7
8	93	58155	Keratin, type II cytoskeletal 79 OS=Homo sapiens GN=KRT79 PE=1 SV=2
9	417	53690	Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4
10	375	59076	Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6
10	127	51956	Keratin, type I cytoskeletal 14 OS=Homo sapiens GN=KRT14 PE=1 SV=4
11	316	42136	Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1
12	302	54853	Dihydrolipoyl dehydrogenase, mitochondrial OS=Homo sapiens GN=DLD PE=1 SV=2
13	257	56985	Aldehyde dehydrogenase, mitochondrial OS=Homo sapiens GN=ALDH2 PE=1 SV=2
14	246	56525	ATP synthase subunit beta, mitochondrial OS=Homo sapiens GN=ATP5B PE=1 SV=3
15	216	56526	Serine hydroxymethyltransferase, mitochondrial OS=Homo sapiens GN=SHMT2 PE=1 SV=3
16	210	74479	Glutaminase kidney isoform, mitochondrial OS=Homo sapiens GN=GLS PE=1 SV=1
17	154	57131	Neutral amino acid transporter B(0) OS=Homo sapiens GN=SLC1A5 PE=1 SV=2
18	118	56690	Succinyl-CoA:3-ketoacid-coenzyme A transferase 1, mitochondrial OS=Homo sapiens GN=OXCT1 PE=1 SV=1
19	116	60293	T-complex protein 1 subunit theta OS=Homo sapiens GN=CCT8 PE=1 SV=4
20	116	60003	Catalase OS=Homo sapiens GN=CAT PE=1 SV=3
21	103	68094	Calnexin OS=Homo sapiens GN=CANX PE=1 SV=2
22	86	42671	Basigin OS=Homo sapiens GN=BSG PE=1 SV=2
23	83	80415	Heat shock protein 75 kDa, mitochondrial OS=Homo sapiens GN=TRAP1 PE=1 SV=3
24	68	58527	T-complex protein 1 subunit delta OS=Homo sapiens GN=CCT4 PE=1 SV=4
25	63	54475	Solute carrier family 2, facilitated glucose transporter member 1 OS=Homo sapiens GN=SLC2A1 PE=1 SV=2
26	61	61934	Methylcrotonoyl-CoA carboxylase beta chain, mitochondrial OS=Homo sapiens GN=MCCC2 PE=1 SV=1
27	58	61366	Prolyl 4-hydroxylase subunit alpha-1 OS=Homo sapiens GN=P4HA1 PE=1 SV=2
28	56	67214	Apoptosis-inducing factor 1, mitochondrial OS=Homo sapiens GN=AIFM1 PE=1 SV=1
29	53	59968	T-complex protein 1 subunit eta OS=Homo sapiens GN=CCT7 PE=1 SV=2
30	51	55424	ERO1-like protein alpha OS=Homo sapiens GN=ERO1L PE=1 SV=2

5) Identification of 55 kDa protein.

Rank	Score	Mass	Description
1	1222	59856	ATP synthase subunit alpha, mitochondrial OS=Homo sapiens GN=ATP5A1 PE=1 SV=1
2	679	53671	Keratin, type II cytoskeletal 8 OS=Homo sapiens GN=KRT8 PE=1 SV=7
2	472	60363	Keratin, type II cytoskeletal 6A OS=Homo sapiens GN=KRT6A PE=1 SV=3
2	296	58155	Keratin, type II cytoskeletal 79 OS=Homo sapiens GN=KRT79 PE=1 SV=2
3	450	56526	Serine hydroxymethyltransferase, mitochondrial OS=Homo sapiens GN=SHMT2 PE=1 SV=3
4	412	57244	Protein disulfide-isomerase A3 OS=Homo sapiens GN=PDIA3 PE=1 SV=4
5	384	66212	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6
6	345	61229	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2
7	225	56525	ATP synthase subunit beta, mitochondrial OS=Homo sapiens GN=ATP5B PE=1 SV=3
8	147	62311	Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3
9	121	50956	Tubulin alpha-1A chain OS=Homo sapiens GN=TUBA1A PE=1 SV=1
10	116	42136	Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1
11	107	48325	Calreticulin OS=Homo sapiens GN=CALR PE=1 SV=1
12	81	53690	Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4
13	81	51662	Keratin, type I cytoskeletal 16 OS=Homo sapiens GN=KRT16 PE=1 SV=4
14	52	42671	Basigin OS=Homo sapiens GN=BSG PE=1 SV=2
15	51	50207	Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2

6) Identification of 40 kDa protein.

Rank	Score	Mass	Description
1	724	42136	Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1
1	606	42479	Actin, aortic smooth muscle OS=Homo sapiens GN=ACTA2 PE=1 SV=1
2	329	48029	Keratin, type I cytoskeletal 18 OS=Homo sapiens GN=KRT18 PE=1 SV=2
3	285	51964	Citrate synthase, mitochondrial OS=Homo sapiens GN=CS PE=1 SV=2
4	250	30896	Voltage-dependent anion-selective channel protein 1 OS=Homo sapiens GN=VDAC1 PE=1 SV=2
5	224	49936	Elongation factor Tu, mitochondrial OS=Homo sapiens GN=TUFM PE=1 SV=2
6	211	32268	Thioredoxin-related transmembrane protein 1 OS=Homo sapiens GN=TMX1 PE=1 SV=1
7	175	48930	Ornithine aminotransferase, mitochondrial OS=Homo sapiens GN=OAT PE=1 SV=1
8	131	35484	Electron transfer flavoprotein subunit alpha, mitochondrial OS=Homo sapiens GN=ETFA PE=1 SV=1
9	101	61229	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2
10	88	32186	Voltage-dependent anion-selective channel protein 2 OS=Homo sapiens GN=VDAC2 PE=1 SV=2
11	84	33046	ATP synthase subunit gamma, mitochondrial OS=Homo sapiens GN=ATP5C1 PE=1 SV=1
12	67	34343	Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial OS=Homo sapiens GN=HADH PE=1 SV=3
13	65	66212	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6
14	58	37021	L-lactate dehydrogenase A chain OS=Homo sapiens GN=LDHA PE=1 SV=2
15	54	27251	Trypsin-1 OS=Homo sapiens GN=PRSS1 PE=1 SV=1
16	53	33692	Thiosulfate sulfurtransferase OS=Homo sapiens GN=TST PE=1 SV=4
17	50	54815	Fumarate hydratase, mitochondrial OS=Homo sapiens GN=FH PE=1 SV=3
18	50	33624	Pyrroline-5-carboxylate reductase 1, mitochondrial OS=Homo sapiens GN=PYCR1 PE=1 SV=2

7) Identification of 35 kDa protein.

Rank	Score	Mass	Description
1	924	42136	Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1
1	830	42402	Beta-actin-like protein 2 OS=Homo sapiens GN=ACTBL2 PE=1 SV=2
2	398	53671	Keratin, type II cytoskeletal 8 OS=Homo sapiens GN=KRT8 PE=1 SV=7
3	146	36049	Malate dehydrogenase, mitochondrial OS=Homo sapiens GN=MDH2 PE=1 SV=3
4	139	51964	Citrate synthase, mitochondrial OS=Homo sapiens GN=CS PE=1 SV=2
5	130	66212	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6
6	72	53009	NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial OS=Homo sapiens GN=NDUFS2 PE=1 SV=2
7	67	33276	Prohibitin-2 OS=Homo sapiens GN=PHB2 PE=1 SV=2
8	57	48930	Ornithine aminotransferase, mitochondrial OS=Homo sapiens GN=OAT PE=1 SV=1
9	56	35952	Sideroflexin-1 OS=Homo sapiens GN=SFXN1 PE=1 SV=4

Table S3. Proteins identified in arrowed protein bands a (1) and b (2) in Figure S14B. Sliced protein band was processed by in situ digestion using trypsin. Digested peptides were analyzed using LCMS to identify proteins listed.

1) Identification of protein band a.

	Score	MW	Protein
1	2359	73990	Stress-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2
2	2120	72431	78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 SV=2
2	304	71138	Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1
2	275	70829	Heat shock 70 kDa protein 1-like OS=Homo sapiens GN=HSPA1L PE=1 SV=2
3	1556	73313	Protein disulfide-isomerase A4 OS=Homo sapiens GN=PDIA4 PE=1 SV=2
4	740	80415	Heat shock protein 75 kDa, mitochondrial OS=Homo sapiens GN=TRAP1 PE=1 SV=3
5	171	61229	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2
6	108	92767	Endoplasmin OS=Homo sapiens GN=HSP90B1 PE=1 SV=1
7	99	68094	Calnexin OS=Homo sapiens GN=CANX PE=1 SV=2
8	57	57131	Neutral amino acid transporter B(0) OS=Homo sapiens GN=SLC1A5 PE=1 SV=2
9	54	68236	4F2 cell-surface antigen heavy chain OS=Homo sapiens GN=SLC3A2 PE=1 SV=3

2) Identification of protein band b.

	Score	MW	Protein
1	382	54815	Fumarate hydratase, mitochondrial OS=Homo sapiens GN=FH PE=1 SV=3
2	367	48029	Keratin, type I cytoskeletal 18 OS=Homo sapiens GN=KRT18 PE=1 SV=2
3	231	61229	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2
4	160	42136	Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1
5	93	48930	Ornithine aminotransferase, mitochondrial OS=Homo sapiens GN=OAT PE=1 SV=1
6	84	49936	Elongation factor Tu, mitochondrial OS=Homo sapiens GN=TUFM PE=1 SV=2
7	83	46664	Adipocyte plasma membrane-associated protein OS=Homo sapiens GN=APMAP PE=1 SV=2
8	78	51964	Citrate synthase, mitochondrial OS=Homo sapiens GN=CS PE=1 SV=2
9	67	48451	Thioredoxin domain-containing protein 5 OS=Homo sapiens GN=TXNDC5 PE=1 SV=2
10	66	130348	Reticulon-4 OS=Homo sapiens GN=RTN4 PE=1 SV=2
11	62	50513	Elongation factor 1-gamma OS=Homo sapiens GN=EEF1G PE=1 SV=3
12	55	44185	Proliferation-associated protein 2G4 OS=Homo sapiens GN=PA2G4 PE=1 SV=3

2. Experimental Protocols

Cell Culture. SW620, LoVo, AGS, HCT-116, A549, AsPC-1, SK-MEL-28, C32, A375, G-361, A498, ACHN, Caki-1, NCI-H1650, 786-0, SK-UT-1, PC-3, C2BBe1, SW48, SW480, NCI-H23, NCI-H2087 and SK-MES-1 cell lines were obtained from ATCC. SW620, SW480, SW48, 786-0, NCI-H1650, AsPc-1 cell lines were grown in L-15 or RPMI supplemented with 10% FBS, 2 mM L-glutamin, penicillin (100 U/ml) and streptomycin (100 µg/ml). PC-3, LoVo and AGS cell lines were grown in L-15, RPMI or F12K supplemented with 10% FBS, 2 mM L-glutamin, penicillin and streptomycin. ACHN, A498, SK-UT-1, C32 and SK-MEL-28 cell lines were grown in L-15, RPMI or EMEM supplemented with 10% FBS, 2mM L-glutamin, penicillin and streptomycin. Caki-1, HCT-116 and G361 cell lines were grown in L-15, RPMI or McCoy's supplemented with 10% FBS, 2 mM L-glutamin, penicillin and streptomycin. A375 cell line was grown in L-15, RPMI or DMEM supplemented with 10% FBS, 2 mM L-glutamin, penicillin and streptomycin. C2Bbe1 was grown in DMEM supplemented with transferrine (0.01 mg/ml), 10% FBS, 2 mM L-glutamin, penicillin and streptomycin. Neonatal normal human fibroblast was obtained from ATCC and was cultured in fibroblast basal medium (ATCC) supplemented with fibroblast growth kit-serum-free (ATCC), penicillin-streptomycin-amphotericin B and Phenol Red. The medium was routinely changed every 3 days, and cells were passaged by trypsinization before confluence.

High Throughput Screening. SW620, LoVo, AGS, HCT-116, A549, AsPC-1, SK-MEL-28, C32, A375, G-361, A498, ACHN, Caki-1, NCI-H1650, 786-0, SK-UT-1, PC-3, C2BBe1, SW48 cell lines and neonatal normal human fibroblast were plated on 384-well plate (Corning Inc., Corning, NY) at 1,000 cells/well and treated after 24 h with individual chemical library members at a concentration of ca. 16 µM using Automated Workstation JANUS (PerkinElmer, Waltham, MA). Growth inhibition was determined 72 h following compound addition using ATPLite (1:3 dilution with PBS)(PerkinElmer, Waltham, MA). Luminescence intensity was measured using Perkin Elmer Victor3 (Waltham, MA). Cellular growth scores were normalized to robust Z score using cellHTS2[3] and analyzed using ROKU method,[4] which was calculated using a custom program written in R software (The R Foundation).

Cell Growth Inhibition Assay. Cells seeded one day before treatment in 96-well tissue culture plates at 10,000 cells/well were treated with several compounds for indicated time (Nunc; Thermo Fisher Scientific Inc., Waltham, MA). Cell growth was measured by PrestoBlue cell viability assay (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Fluorescence intensity was measured using Perkin Elmer Victor3 (530 mm excitation, 615 nm emission).

Measurement of Cellular ATP Levels. Cells were plated in 96-well format at 10,000 cells/well. The following day, cells were treated with several concentrations of small molecules in presence or absence of glycolysis inhibitors (10 mM 2-DG or 10 μM cytochalasin B) for 30 min at 37 °C. ATP level was analyzed using ATPLite. Luminescence intensity was measured using Perkin Elmer Victor3.

Measurment of Normalized ATP Levels. Cells were plated in 96-well format at 10,000 cells/well. The following day, cells were treated with several concentrations of compound **2**. After incubation for indicated time, ATP level and protein level of each well were analyzed by ATPLite and sulforhodamine B assay, respectively. Normalized ATP levels were calculated by dividing ATP level with protein levels.

Cell Growth Inhibition Assay under Several Different Nutrient Conditions. Cells were seeded in 96-well plate at 5,000 cells/well in normal DMEM. The following day, medium was replaced to glucose-free DMEM with dialyzed FBS (10%) supplemented with or without glucose (2 mM), galactose (2 mM) or sodium pyruvate (1 mM). Then cells were treated with several concentration of compound **2** for indicated time. Cell growth was measured by PrestoBlue according to the manufacturer's instructions. Fluorescence intensity was measured using Perkin Elmer Victor3.

Visualization of Subcellular Localization of Probe Compounds. SW620 cells on 35-mm glass bottom dishes (Greiner) were incubated with either a probe compound armed with alkyne

or MitoTracker Red (Invitrogen, Carlsbad, CA) for 2 h. After wash of cells with PBS, cells were fixed with 4% paraformaldehyde in PBS for 15 min. Then cells were washed with PBS, permeabilized with 0.2% Triton X-100 in PBS for 5 min, and blocked with 1% bovine serum albumin in PBS for 10 min. Click chemistry reaction was carried out using Click-iT Cell Reaction Buffer Kit (Invitrogen, Carlsbad, CA) with Alexa 488 azide (Invitrogen) according to the manufacturer's instructions. After wash of cells with PBS, resulting cells were analyzed using confocal microscope (Marianas advanced spinning disk confocal microscope, Intelligent Imaging Innovations Inc., Denver, CO).

Measurement of Oxygen Consumption Rate. The XF96 extracellular flux analyzer (Seahorse Bioscience, North Billerica, MA) was used to analyze realtime changes of cellular respiration and glycolysis rate. SW620 cells were plated on XF96 cell culture microplate at 10,000 cells/well in DME media and were incubated for 24 h at 37 °C. After replacement of media with XF assay media (Seahorse Biosciences) (175 μL/well), cells were further incubated for 1 h at 37 °C in 100% air. After measurement of basal oxygen consumption rate (OCR), several concentrations of small molecule, oligomycin (1 μM), carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (1 μM), and rotenone (1 μM) + antimycina A (1 μM) were sequentially added at time points of 60, 150, 168 and 186 min, respectively, and OCR was measured every 6 min.

Measurement of Electron Transport Chain Activity. The Hansatech Oxygraph (Hansatech Instruments, King's Lynn, Norfolk, UK) was used to analyze electron transport chain (ETC) activity. Respiration buffer (0.25 M sucrose, 20 mM tris (pH 7.2), 40 mM KCL, 2 mM ethylene glycol tetra acetic acid (EGTA), 1mg/ml fatty acid free bovine serum albumin (BSA)) and suspended SW620 cells in respiration buffer (total volume of 1 ml) were added in cuvette at 37 °C. Digition (0.003% (w/v)) was added to permeabilize cellular plasma membrane. ETC complex activities were measured at 37 °C by adding complex-specific substrates after addition of prior complex inhibitor. After addition of several concentration of small molecule, complex II-IV activity was measured by addition of sodium succinate (10 mM) after addition of rotenone (3.3 μM). Complex III and IV activity was measured by adding grycerol-3-phosphate (60 mM) after addition of malonate (10 mM). Complex IV

activity was measured by adding sodium ascorbate (10 mM) + *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (200 μM) after addition of antimycin A. Then KCN (5 mM) was added to inhibit complex IV.

Measurement of complex I activity. Submitochondrial particles (SMP) were prepared from mitochondria-enriched fraction of SW620 cells as described. SMP in assay buffer (10 mM Tris, 1 mg/ml BSA [pH 8.0]) (100 μL, 10 μg/mL) was plated on clear-bottom 96 well plate. Complex I activity was analyzed by monitoring consumption of NADH using absorbance change of 340 nm. This assay was initiated by adding NADH (160 μM) after addition of several concentration of small molecule. Rotenone (5 μM) was used as positive control.

Measurement of Amount of Small Molecule in Cells. SW620 cells on 6-well plate in DMEM were treated with small molecule for 2 h at 37 °C. After wash of cells, small molecule in cells was extracted with 1 ml of ice cold MeOH/H_2O (80:20) using scraper. Wells were washed with 0.5 ml of MeOH/H_2O two times. Combined extract was centrifuged at 5,000 × g for 5 min at 4 °C to remove insoluble material. Resulting extract was evaporated for 2 h at room temperature, and analyzed using Agilent 6540 Q-Tof MS-MS equipped with 1260 nano LC-Chip with SmMol-Chip-43 (II) (Agilent Technologies, Inc., Santa Clara, CA).

Measurement of Stability of Small Molecule under acidic, neutral or basic condition. Compound **2** (0.35 mg, 0.75 μmol) incubated in phosphate buffer (3 ml, 25 mM sodium phosphate in D_2O containing 25% d_6-DMSO [pH 6, 7 or 8]) at 37 °C for 0, 4, 24 or 72 h was analyzed by ^1H NMR employing water suppression method.

Target Identification using photo-affinity labeling. SW620 cells on 15-cm dish in DMEM media were treated with probe compound (5 μM), competitor (10 μM) or negative control probe (5 μM) for 2 h at 37 °C. After wash of cells, cells were suspended on 6-cm tissue culture-untreated dish in PBS, and were irradiated with 365 nm UV light for 30 min on ice using high-intensity UV inspection lamp (B100AP; UVP LLC, Upland, CA) at distance of 10

cm. For mitochondrial fraction analysis, mitochondria-enriched fraction was obtained from irradiated cells using mitochondria isolation kit for cultured cells (Thermo Fisher Scientific Inc.) and Dounce homogenizer (20 strokes). Resulting cells or mitochondrial fraction was lysed in lysis buffer (50 mM Tris HCl, 0.1 mM PMSF, 1% Protease Inhibitor Cocktail (Sigma-Aldrich, St. Louis, MO), 1% Phosphatase Inhibitor Cocktail (Sigma-Aldrich, St. Louis, MO) [pH 8.0] containing 1% triton X-100 or 2% CHAPS, respectively) on ice for 30 min. Lysates were centrifuged at 15,000 × g for 15 min at 4 °C to remove insoluble material. Protein concentration was measured using DC protein assay kit (Bio-Rad, Hercules, CA). To label alkyne tag-labeled protein in supernatants (50 µl, 2 µg/µl) with Alexa 488 azide (Invitrogen), click chemistry reaction was carried out using Click-iT Protein Reaction Buffer Kit (Invitrogen) according to the manufacturer's instructions. Methanol/chloroform precipitation removed residual reaction components. The precipitates were re-dissolved in 1 × SDS sample buffer (200 µL) (Boston BioProducts Inc., Ashland, MA) by incubating for 10 min at 70 °C. The resultant mixtures were electrophoresed on 12% tris-glycine gels (Invitrogen, Carlsbad, CA) and scanned with PharosFX imaging systems (Bio-Rad, Hercules, CA). After scan, the gels were stained with coomassie brilliant blue (CBB) and scanned again.

Pull-Down Analysis of Fumarate Hydratase using photo-affinity labeling. SW620 cells on 15-cm dish in DMEM media were treated with probe compound, competitor or negative control probe for 2 h at 37 °C. After wash of cells, cells were suspended on 6-cm tissue culture-untreated dish in PBS, and were irradiated with 365 nm UV light for 30 min on ice at distance of 10 cm. Mitochondria-enriched fraction was obtained using Mitochondria isolation kit for cultured cells and Dounce homogenizer (20 strokes) from the irradiated cells. Resulting mitochondrial fraction was lysed in lysis buffer containing 2% CHAPS on ice for 30 min. Lysates were centrifuged at 15,000 × g for 15 min at 4 °C and protein concentration was measured. To label alkyne tag-labeled protein in supernatants (50 µl, 2 µg/µl) with PEG4 carboxamide-6-azidohexanyl biotin (Invitrogen), click chemistry reaction was carried out. Methanol/chloroform precipitation removed residual reaction components. The precipitates were re-dissolved in 1% SDS-containing PBS (50 µL) by incubating for 5 min at 80 °C. The protein solution was diluted with PBS (200 µL) to 0.2% SDS solution and was added to avidin-agarose beads (50 µL, wet volume) (Sigma-Aldrich, St. Louis, MO). The

suspension was rotated at room temperature for 3 h. After wash of the beads with 0.2% SDS-containing PBS for 3 times and with PBS for 3 times, the beads was incubated in 2 × SDS sample buffer (50 μL) for 5 min at 95 °C. The resulting proteins were electrophoresed on 12% tris-glycine gels (Invitrogen, Carlsbad, CA) and visualized by silver stain (SilverQuest; Invitrogen, Carlsbad, CA).

Proteomics Analysis. Protein bands were manually excised from the gel. Diced gels were destained, if needed, and washed with water for 3 min 3 times using sonication in ice-cold water. Gel pieces were incubated in equilibrium buffer (100 mM NH_4HCO_3) for 10 min, and were dehydrated with MeCN for 10 min. Then gel pieces were incubated in reduction buffer (100 mM NH_4HCO_3, 10 mM DTT) for 60 min at 55 °C, treated with alkylation buffer (100 mM NH_4HCO_3, 50 mM acrylamide) was added and incubated for additional 45 min at 20 °C. The sample was washed with 50% MeOH for 10 min 4 times, dehydrated with MeCN for 10 min, equilibrated with equilibrium buffer (50 mM NH_4HCO_3) for 10 min and dehydrated with MeCN for 10 min. A few drops of sequencing grade modified trypsin (20 μg/ 50 mM NH_4HCO_3) (Promega, Madison, WI) was added and the sample was incubated 37 °C for 16 h. Digested peptides were extracted with 50% MeCN with 0.1% TFA. The peptides were analyzed with an Ultraflex Extreme MALDI tandem TOF mass spectrometer (Bruker, Billerica, MA) or Agilent 6540 Q-Tof MS-MS equipped with 1260 nano LC-Chip with ProtID-Chip-150 (II) (Agilent Technologies, Inc., Santa Clara, CA).

Measurement of Fumarate Hydratase Activity. Fumarase Specific Activity Microplate Assay Kit (abcam) was used to measure fumarate hydaratase activity according to the manufacturer's instructions. Briefly, lysate of SW620 cells (64 μg/well) in assay plate were incubated for 3 h at room temperature. After wash of the plate, assay buffer (100 μL) with small molecule was added, and the plate was incubated for 3 h at room temperature. Then assay buffer (100 μL) containing malate dehydrogenase, small molecule and substrates was added. Reaction was monitored using absorbance change of 450 nm by UV spectrometer (Infinite M200 PRO; Tecan, Seestrasse, Männedorf, Switzerland).

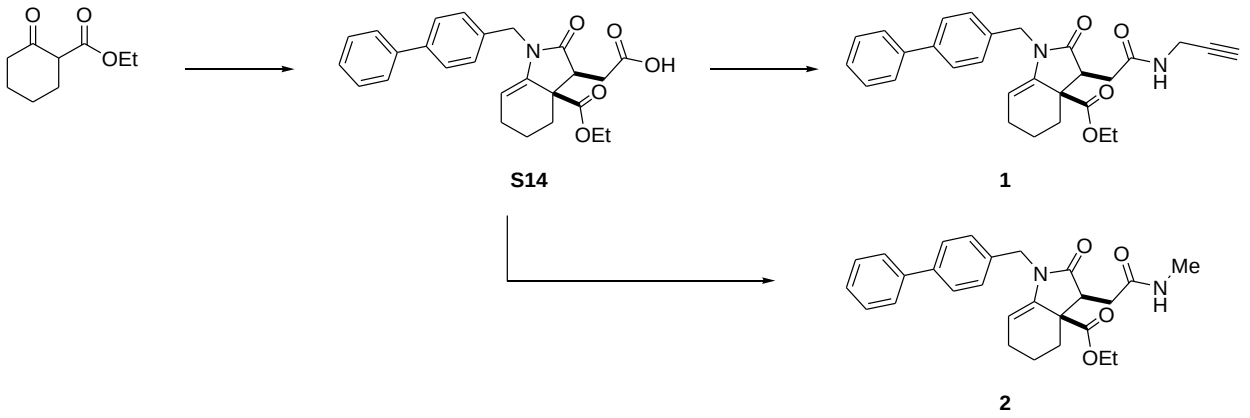
Measurement of Cellular NADH Levels. Cellular NADH level was estimated by measuring autofluorescence of cells using confocal microscope (DSU spinning disk confocal microscope (Olympus, Center Valley, PA)) at an excitation wavelength of 350 ± 25 nm and emission wavelength of 457 ± 25 nm. SW620 cells were seeded on 35-mm glass bottom dish (Greiner) at 200,000 cells/dish. The following day, the fluorescence was measured every 1 min for 10 min after addition of small molecule. Relative NADH level was calculated by dividing initial average autofluorescence by that after treatment.

1. Synthetic Procedures and Compound Characterization

General Information

^{1}H- and ^{13}C- NMR were recorded on a BRUKER DRX500 (Billerica, MA). Chemical shifts were reported in δ, parts per million (ppm), calibrated using residual solvent protons as an internal reference. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and br, broad. Coupling constants are recorded in Hertz (Hz). Mass spectra were obtained on Agilent 6540 Ultra High Definition Accurate-Mass Q-TOF LC/MS system. Column chromatography was carried out on SiliaFlash (Silicycle Inc., Quebec City, CANADA). Analytical thin-layer chromatography (TLC) was performed on precoated Whatman Silica Gel 60 A plates (Fairfield, CT), and compounds were visualized by UV illumination (254 nm) or heating 150 °C after spraying phosphomolybdic acid in ethanol. THF was distilled from sodium/benzophenone. All other solvent and reagents were obtained from commercial sources and used without further purification.

Scheme S2. Synthesis of conpound **1** and **2**.



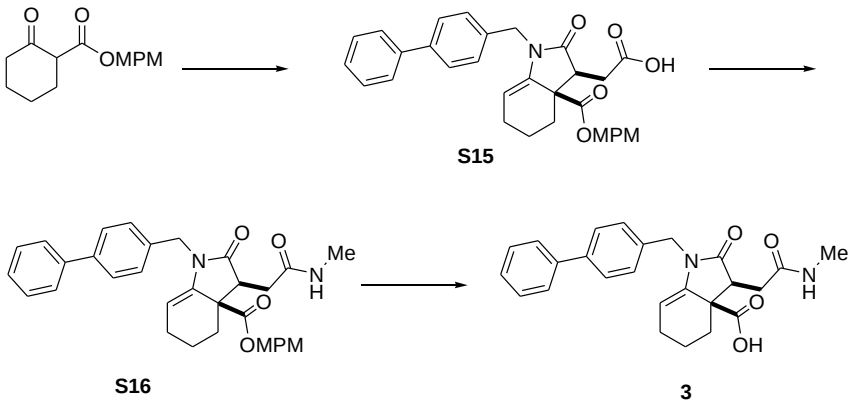
Synthesis of compound S14[1]. To a stirred solution of 4-phenylbenzylamine (1.1 g, 6.3 mmol) in $CHCl_3$ (20 ml) was added ethyl 2-oxocycloheanecarboxylate (1 mL, 6.3 mmol), and the mixture was stirred at 60 °C for 16 h. Maleic anhydride (0.74 g, 7.5 mmol) was added, and the resulting mixture was stirred at rt for 2 h. After the addition of saturated $NaHCO_3$ solution, the reaction mixture was extracted with EtOAc. The combined organic layer was washed with H_2O, dried over brine and $MgSO_4$, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography to give carboxylic acid as an amorphous solid (1.8 g, 67%). TLC (hexane:EtOAc=1:2): R_f = 0.2; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.57 (dd, J = 8.0, 1.4 Hz, 2H), 7.55 (d, J = 8.2 Hz, 2H), 7.43 (t, J = 8.0 Hz, 2H), 7.39 (d, J = 8.2 Hz, 2H), 7.34 (tt, J = 8.0, 1.4 Hz, 1H), 6.90 (br s, 1H), 5.06 (t, J = 3.7 Hz, 1H), 4.91 (d, J = 15.5 Hz, 1H), 4.56 (d, J = 15.5 Hz, 1H), 4.13 (dq, J = 10.7, 7.2, 1H), 4.06 (dq, J = 10.7, 7.2, 1H), 3.10 (dd, J = 7.3, 7.0 Hz, 1H), 2.91 (dd, J = 17.0, 7.0 Hz, 1H), 2.60 – 2.65 (m, 1H), 2.44 (dd, J = 17.0, 7.3 Hz, 1H), 2.05 – 2.21 (m, 2H), 1.81 – 1.87 (m, 1H), 1.46 – 1.59 (m, 2H), 1.16 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.1, 174.6 171.1, 140.7, 140.5, 138.2, 134.8, 128.8 (2C), 128.2 (2C), 127.4, 127.2 (2C), 127.1 (2C), 102.3, 61.5, 42.0, 47.5, 44.1, 31.5, 30.6, 22.7, 19.5, 14.1; HRMS (m/z): $[M+H]^+$ calcd. for $C_{26}H_{27}NO_5$, 433.1889; found, 433.188.

Synthesis of compound 1. To a stirred solution of **S14** (119 mg, 0.27 mmol) in CH_2Cl_2 (1 ml) was added EDCI (62 mg, 0.32 mmol), and the mixture was stirred at rt for 2 h. Propargylamine (26 μL, 0.41 mmol) was added, and the reaction mixture was stirred at rt for 2 h. After the addition of saturated $NaHCO_3$ solution, the reaction mixture was extracted with EtOAc. The combined organic layer was washed with H_2O, dried over brine

and $MgSO_4$, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography to give **1** as a white amorphous solid (66 mg, 52%). TLC (hexane:EtOAc=1:2): R_f = 0.5; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.58 (d, J = 7.9 Hz, 2H), 7.56 (d, J = 8.2 Hz, 2H), 7.45 (t, J = 7.9 Hz, 2H), 7.40 (d, J = 7.9 Hz, 2H), 7.36 (t, J = 7.9 Hz, 1H), 7.23 (brs, NH, 1H), 5.06 (t, J =3.4 Hz, 1H), 4.90 (d, J = 15.3 Hz, 1H), 4.57 (d, J =15.3 Hz, 1H), 4.03 – 4.18 (m, 4H), 3.08 (dd, J = 7.5, 6.0 Hz, 1H), 2.72 (dd, J = 15.1, 7.5 Hz, 1H), 2.62 (dt, J = 12.5, 2.8 Hz, 1H), 2.28 (dd, J = 15.1, 6.0 Hz, 1H), 2.25 (t, J = 2.4 Hz, 1H), 2.10 – 2.23 (m, 2H), 1.81 – 1.87 (m, 1H), 1.45 – 1.60 (m, 2H), 1.16 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.4, 171.3, 170.4, 140.7, 140.4, 138.1, 134.9, 128.7 (2C), 128.2 (2C), 127.3, 127.1 (2C), 127.0 (2C), 101.9, 79.5, 71.4, 61.3, 52.2, 48.3, 44.0, 33.2, 30.2, 29.3, 22.7, 19.4, 14.1; HRMS (m/z): $[M+H]^+$ calcd. for $C_{29}H_{30}N_2O_4$, 470.2206; found, 470.2209.

Synthesis of compound 2. Under the conditions analogous to those used for the preparation of **1**, **S14** (1.8 g, 4.2 mmol) and methylamine (3.1 ml, 2 M in THF, 6.2 mmol) gave **2** as an amorphous solid (1.4 g, 75%). TLC (hexane:EtOAc=1:2): R_f = 0.2; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.56 (dd, J = 7.9, 1.2 Hz, 2H), 7.54 (d, J = 8.2 Hz, 2H), 7.43 (dd, J = 7.9, 7.6 Hz, 2H), 7.38 (d, J = 8.2 Hz, 2H), 7.34 (tt, J = 7.6, 1.2 Hz, 1H), 6.90 (brs, NH, 1H), 5.04 (t, J = 3.4 Hz, 1H), 4.85 (d, J = 15.5 Hz, 1H), 4.56 (d, J = 15.5 Hz, 1H), 4.10 (dq, J = 10.7, 7.1 Hz, 1H), 4.03 (dq, J = 10.7, 7.1 Hz, 1H), 3.08 (dd, J = 7.1, 5.8 Hz, 1H), 2.83 (d, J = 4.6 Hz, 3H), 2.67 (dd, J = 15.0, 7.1 Hz, 1H), 2.59 (dt, J = 11.9, 2.4 Hz, 1H), 2.24 (dd, J =15.0, 5.8 Hz, 1H), 2.05 – 2.20 (m, 2H), 1.79 – 1.86 (m, 1H), 1.43 – 1.59 (m, 2H), 1.14 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.5, 171.3, 171.3, 140.7, 140.4, 138.2, 134.9, 128.7 (2C), 128.1 (2C), 127.3, 127.1 (2C), 126.7 (2C), 101.8, 61.2, 62.2, 48.5, 43.9, 33.1, 30.1 26.4, 22.7, 19.4, 14.0; HRMS (m/z): $[M+H]^+$ calcd. for $C_{27}H_{30}N_2O_4$, 446.2206; found, 446.2202.

Scheme S3. Synthesis of carboxylic acid **3**.



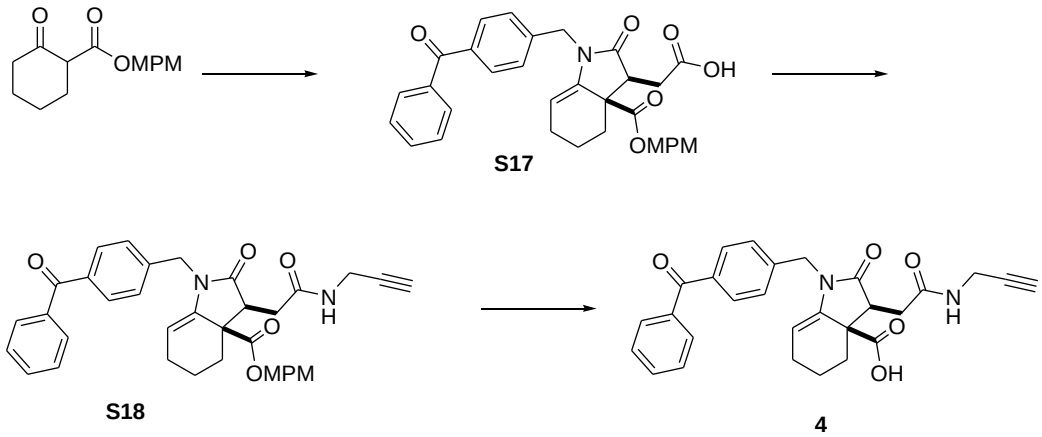
Synthesis of compound S15. Under the conditions analogous to those used for the preparation of **S14**, 4-methoxybenzyl 2-oxocyclohexanecarboxylate[2] (1.1 g, 4.4 mmol), 4-phenylbenzylamine (800 mg, 4.4 mmol) and maleic anhydride (510 mg, 5.2 mmol) gave **S15** as a colorless oil (2.1g, 92%). TLC (hexane:EtOAc=1:2): R_f = 0.2; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.55 (dt, J = 7.9 Hz, 2H), 7.49 (d, J = 8.2 Hz, 2H), 7.43 (t, J = 7.9 Hz, 2H), 7.35 (d, J = 8.2 Hz, 2H), 7.35 (t, J = 7.9 Hz, 1H), 7.18 (dt, J = 8.8, 1.8 Hz, 2H), 6.85 (dt, J = 8.8, 1.8 Hz, 2H), 5.08 (d, J = 11.9 Hz, 1H), 5.05 (t, J = 3.7, 1H), 4.92 (d, J = 11.9 Hz, 1H), 4.88 (d, J = 15.6 Hz, 1H), 4.55 (d, J = 15.6 Hz, 1H), 3.17 (s, 3H), 3.06 (t, J = 7.0 Hz, 1H), 2.69 (dd, J = 16.8, 7.6 Hz, 1H), 2.61 (dd, J = 9.2, 3.7 Hz, 1H), 2.32 (dd, J = 16.8, 6.7 Hz, 1H), 2.06 – 2.15 (m, 2H), 1.76 – 1.85 (m, 1H), 1.45 – 1.50 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2, 170.9, 159.8, 140.6, 138.0, 134.6, 130.2 (2C), 128.8 (2C), 128.1 (2C), 127.3, 127.2 (2C), 127.0 (2C), 114.0 (2C), 102.5, 66.9, 55.2 (2C), 52.0, 47.5, 44.2, 31.3, 30.5, 22.7, 19.4, 14.2; HRMS (m/z): $[M+H]^+$ calcd. for $C_{32}H_{31}NO_6$, 525.2151; found, 525.2151.

Synthesis of compound S16. Under the conditions analogous to those used for the preparation of **1**, **S15** (100 mg, 0.19 mmol) and methylamine (143 μL, 2M in THF, 0.29 mmol) gave **S16** as a clear oil (64 mg, 63%). TLC (hexane:EtOAc=1:2): R_f = 0.05; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.55 (d, J = 7.3 Hz, 2H), 7.50 (d, J = 7.9 Hz, 2H), 7.43 (t, J = 7.3 Hz, 2H), 7.36 (d, J =7.9 Hz, 2H), 7.34 (t, J = 7.3 Hz, 1H), 7.22 (d, J = 8.2 Hz, 2H), 6.87 (d, J = 8.2 Hz, 2H), 6.20 (brs, 1H), 5.06 (d, J = 11.7 Hz, 1H), 5.01 (t, J = 3.4 Hz, 1H), 4.90 (d, J = 11.7 Hz, 1H), 4.85 (d, J = 15.6 Hz, 1H), 4.54 (d, J = 15.6 Hz, 1H), 3.79 (s, 3H), 3.08 (t, J =7.0 Hz, 1H), 2.76 (d, J = 4.6 Hz, 3H), 2.52 – 2.57 (m, 1H), 2.41 (dd, J = 15.0, 7.0 Hz, 1H), 2.05 – 2.18 (m, 2H), 1.97 (dd, J = 15.0, 7.0 Hz, 1H), 1.77 – 1.83 (m, 1H), 1.40 – 1.55 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 171.3, 171.2, 159.8, 140.7, 140.3, 138.1, 134.9, 130.5 (2C), 128.7

(2C), 128.1 (2C), 127.5, 127.3, 127.1 (2C), 127.0 (2C), 113.9 (2C), 101.8, 66.7, 55.2, 52.1, 48.5, 44.0, 32.8, 30.2, 26.4, 22.7, 19.4; HRMS (m/z): $[M+H]^+$ calcd. for $C_{33}H_{34}N_2O_5$, 538.2468; found, 538.2468.

Synthesis of compound 3. To a stirred solution of **S16** (70 mg, 0.13 mmol) in CH_2Cl_2 (2 ml) and PBS (800 μL) was added 2,3-dichloro-5,6-dicyano-p-benzoquinone (59 mg, 0.26 mmol), and the mixture was stirred at rt for 2 h. After the addition of Et_3N (300 μL), the reaction mixture was concentrated. The resultant residue was purified by silica gel column chromatography to give **3** as a colorless oil (15.6 mg, 29%). TLC (EtOAc:MeOH=4:1): R_f = 0.3; ^{1}H NMR (500 MHz, MeOD): δ 7.59 (d, J = 8.2 Hz, 2H), 7.55 (d, J = 8.2 Hz, 2H), 7.37 – 7.44 (m, 4H), 7.31 (t, J = 7.3 Hz), 4.92 (d, J = 16.2 Hz, 1H), 4.78 (t, J = 3.7 Hz, 1H), 4.57 (d, J = 16.2 Hz, 1H), 2.95 (dd, J = 8.2, 5.7 Hz, 1H), 2.76 (s, 3H), 2.66 (dd, J = 14.9, 5.57 Hz, 1H), 2.47 (dt, J = 12.2, 3.4 Hz,1H), 2.41 (dd, J = 14.9, 8.2 Hz, 1H), 2.03 – 2.15 (m, 2H), 1.73 – 1.80 (m, 2H), 1.34 (dt, J = 12.2, 4.6 Hz, 1H); ^{13}C NMR (125 MHz, MeOD) δ 178.1, 177.5, 175.5, 144.0, 142.4, 141.4, 137.6, 129.9 (2C), 128.7 (2C), 128.4, 128.2 (2C), 128.1 (2C), 100.5, 55.8, 50.5, 45.2, 34.7, 32.8, 26.6, 24.2, 21.5; HRMS (m/z): $[M+H\text{-}CO_2]^+$ calcd. for $C_{24}H_{26}N_2O_2$, 375.2067; found, 375.2073.

Scheme S4. Synthesis of carboxylic acid **4**.



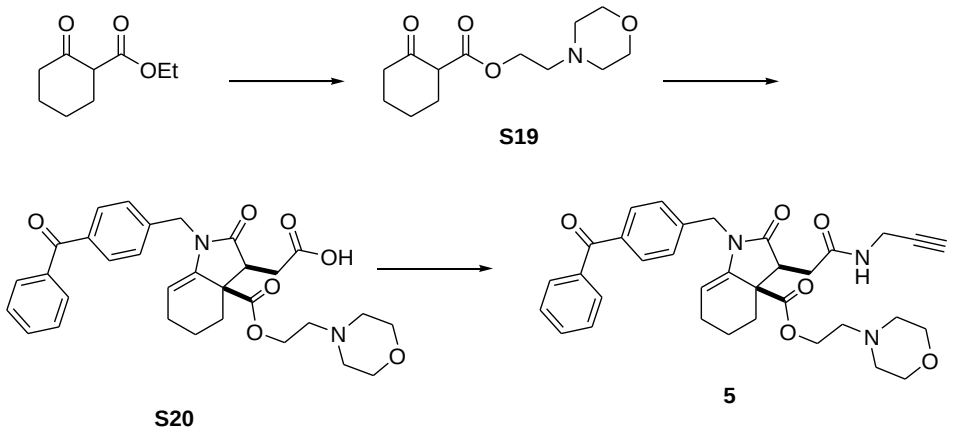
Synthesis of compound S17. Under the conditions analogous to those used for the preparation of **S14**, 4-methoxybenzyl 2-oxocyclohexanecarboxylate[5] (105 mg, 0.40 mmol), 4-benzoylbenzylamine (84 mg, 0.40 mmol) and maleic anhydride (47 mg, 0.48 mmol) gave carboxylic acid **S17** as an amorphous solid (129 mg, 58%). TLC (hexane:EtOAc=1:2): R_f = 0.2; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.78 (d, J = 8.2 Hz, 2H), 7.71 (d, J = 7.6 Hz, 2H), 7.61 (t, J = 8.2 Hz, 1H), 7.50 (t, J = 8.2 Hz, 2H), 7.39 (d, J = 7.6 Hz, 2H), 7.19 (d, J = 7.3 Hz, 2H), 6.85 (d, J = 7.3 Hz, 2H), 5.11 (d, J = 11.9 Hz, 1H), 4.92 – 5.03 (m, 3H), 4.57 (d, J = 15.7 Hz, 1H), 3.78 (s, 3H), 3.10 (t, J = 6.7 Hz, 1H), 2.77 (dd, J = 17.4, 6.5 Hz, 1H), 2.62 – 266 (m, 1H), 2.35 (dd, J = 17.4, 6.5 Hz, 1H), 2.05 – 2.15 (m, 2H), 1.77 – 1.85 (m, 1H), 1.45 – 1.55 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 196.2, 174.0, 174.9, 171.0, 159.8, 140.3, 140.3, 137.9, 137.5, 136.8, 132.4, 130.4 (2C), 130.2 (2C), 130.0 (2C), 128.2 (2C), 127.4 (2C), 127.1, 114.0 (2C), 102.4, 67.1, 55.2, 55.2, 52.0, 47.5, 44.1, 31.1, 30.6, 22.6, 19.4; HRMS (m/z): $[M+H]^+$ calcd. for $C_{33}H_{31}NO_7$, 533.2101; found, 533.2089.

Synthesis of compound S18. Under the conditions analogous to those used for the preparation of **1**, **S17** (129 mg, 0.23 mmol) and propargylamine (22 μL, 0.35 mmol) gave **S18** as an amorphous solid (80 mg, 59%). TLC (hexane:EtOAc=1:2): R_f = 0.3; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.77 (d, J = 7.4 Hz, 2H), 7.72 (d, J = 8.2 Hz, 2H), 7.60 (t, J = 7.4 Hz), 7.48 (t, J = 7.4 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.24 (d, J = 8.5 Hz, 2H), 6.87 (d, J = 8.5 Hz, 2H), 6.22 (br s, 1H), 5.09 (d, J = 11.9 Hz, 1H), 4.94 (t, J = 3.7 Hz, 1H), 4.93 (d, J = 11.9 Hz, 1H), 4.93 (d, J = 15.8 Hz, 1H), 4.56 (d, J = 15.8 Hz, 1H), 3.97 – 4.03 (m, 1H), 3.80 (s, 3H), 3.09 (t, J = 6.7 Hz, 1H), 2.56 (dd, J = 9.8, 3.4 Hz, 1H), 2.43 (dd, J = 15.3, 6.7 Hz, 1H), 2.25 (t, J = 2.4 Hz, 1H), 2.05 – 2.17 (m, 2H), 1.91 (dd, J = 15.3, 6.7 Hz, 1H), 1.70 – 1.85 (m, 2H), 1.42 – 1.55 (m,

2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 196.2, 174.1, 171.2, 170.1, 159.9, 140.6, 137.9, 137.5, 136.7, 132.4, 130.6 (2C), 130.3 (2C), 129.9 (2C), 128.3 (2C), 127.4 (2C), 127.3, 114.0 (2C), 101.9, 79.4, 71.5, 66.8, 55.3, 52.0, 48.3, 44.0, 32.7, 30.2, 29.3, 22.7, 19.4; HRMS (m/z): $[M+H]^+$ calcd. for $C_{36}H_{34}N_2O_6$, 590.2417; found, 590.2404.

Synthesis compound of 4. Under the conditions analogous to those used for the preparation of **3**, **S18** (12.5 mg, 0.021 mmol) gave **4** as an colorless oil (9.2 mg, 93%). TLC (EtOAc): R_f = 0.2; ^{1}H NMR (500 MHz, MeOD): δ 7.76 (dd, J = 7.4, 1.5 Hz, 2H), 7.73 (d, J = 8.2 Hz, 2H), 7.64 (t, J = 7.4, 1.5 Hz, 1H), 7.50 – 7.55 (m, 4H), 4.95 (t, J = 3.7 Hz, 1H), 4.86 (d, J = 16.2 Hz, 1H), 4.72 (d, J = 4.72 Hz, 1H), 3.97 – 4.01 (m, 2H), 3.14 (dd, J = 7.9, 5.8 Hz, 1H), 2.75 (dd, J = 15.7, 5.8 Hz, 1H), 2.58 (t, J = 2.4 Hz, 1H), 2.50 – 2.56 (m, 1H), 2.36 (dd, J = 15.7, 7.9 Hz, 1H), 2.06 – 2.18 (m, 2H), 1.76 – 1.85 (m, 1H), 1.64 – 1.76 (m, 1H), 1.41 – 1.50 (m, 1H); ^{13}C NMR (125 MHz, MeOD) δ 198.4, 176.7, 175.8, 173.4, 143.2, 141.1, 139.1, 137.9, 133.9, 131.4 (2C), 131.1 (2C), 129.7 (2C), 128.6 (2C), 102.3, 80.6, 72.4, 54.0, 50.0, 44.9 33.8, 31.8, 29.8, 23.9, 21.0; HRMS (m/z): $[M+H\text{-}CO_2]^+$ calcd. for $C_{27}H_{26}N_2O_3$, 427.2016; found, 427.2009.

Scheme S5. Synthesis of carboxylic acid **6**.



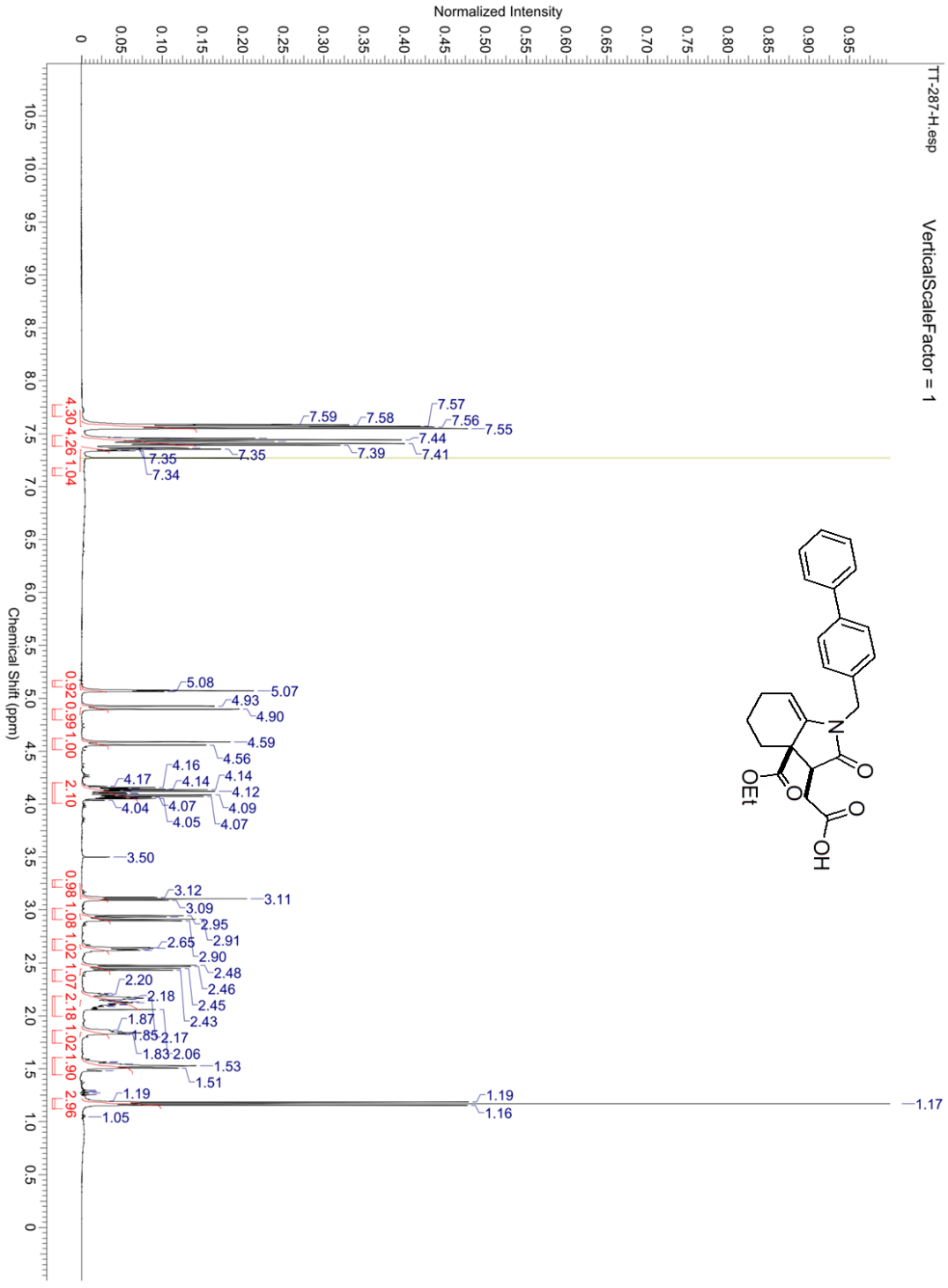
Synthesis of compound S19. To a stirred solution of 2-oxocycloheanecarboxylate (2.4 ml, 15 mmol) in toluene (10 ml) was added 2-morpholinoethanol (2.7 ml, 23 mmol), and the mixture was refluxed for 5 days.[6] Resulting reaction mixture was concentrated and the residue was purified by silica gel column chromatography to give **S19** as a colorless oil (3.7 g , 98%). TLC (EtOAc): R_f = 0.2; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.20 – 4.48 (m, 2H), 3.65 – 3.79 (m, 4H), 3.33 – 3.45 (m, 1H), 2.62 – 2.76 (m, 2H), 2.45 – 2.76 (m, 5H), 2.30 – 2.40 (m, 1H), 2.03 – 2.20 (m, 2H), 1.75 – 2.02 (m, 3H), 1.60 – 1.74 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.1, 169.8, 66.6, 61.6, 57.1, 56.9, 53.6, 41.5, 29.9, 27.1, 23.2; HRMS (m/z): $[M+H]^+$ calcd. for $C_{13}H_{21}NO_4$, 225.1471; found, 255.1475.

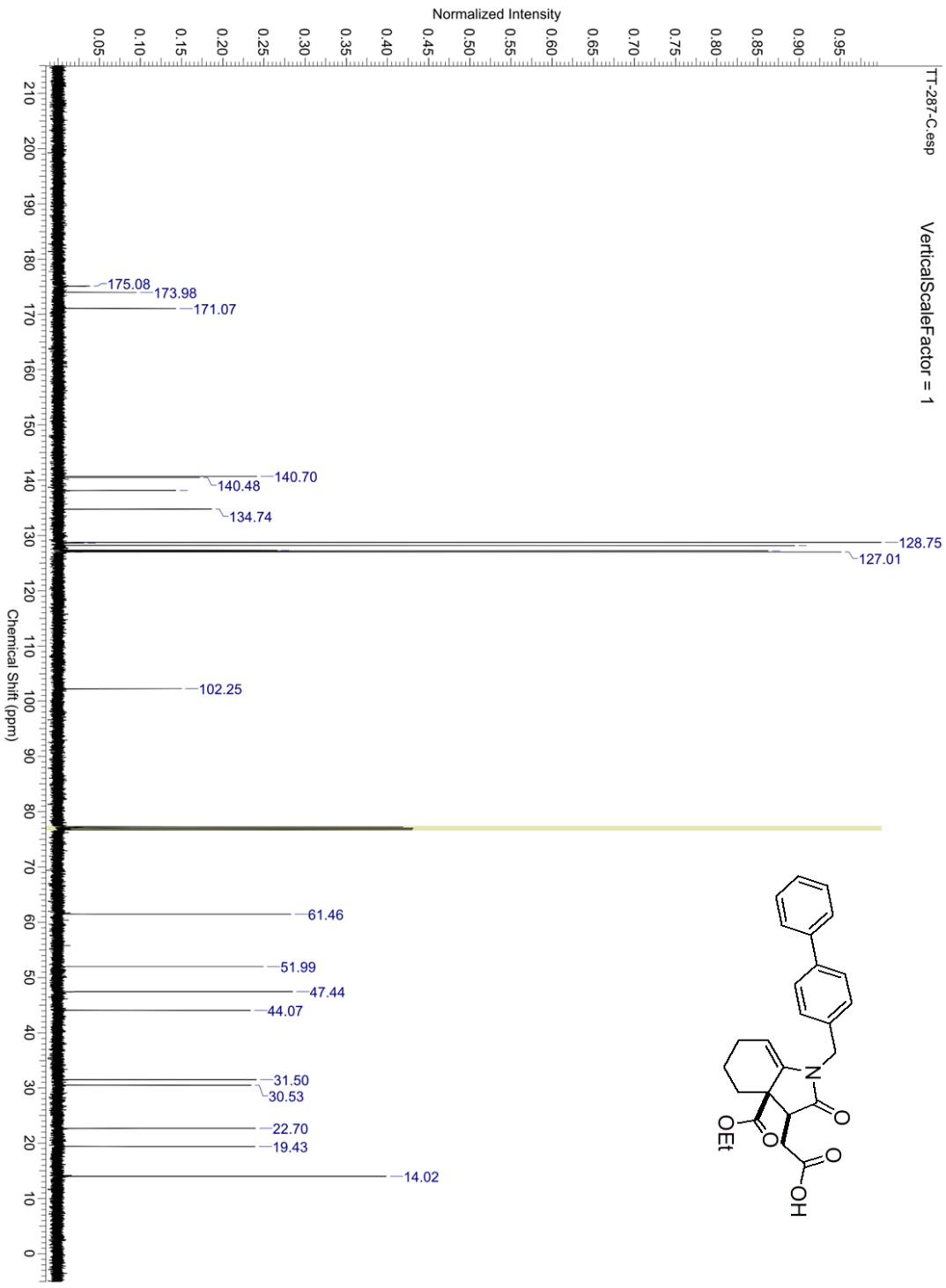
Synthesis of compound S20. Under the conditions analogous to those used for the preparation of **S14**, **S19** (102 mg, 0.40 mmol), 4-benzoylbenzylamine (84 mg, 0.40 mmol) and maleic anhydride (47 mg, 0.48 mmol) gave **S20** as a colorless oil (112 mg, 51%). TLC (hexane:EtOAc=1:2): R_f = 0.2; ^{1}H NMR (500 MHz, MeOD): δ 7.74 – 7.83 (m, 4H), 7.64 – 7.70 (m, 1H), 7.52 – 7.59 (m, 4H), 5.13 – 5.17 (m, 1H), 4.92 – 4.96 (m, 1H), 4.68 – 4.75 (m, 1H), 4.51 (ddd, J = 13.2, 7.0, 3.4 Hz, 1H), 4.41 (ddd, J = 13.2, 7.0, 3.4 Hz, 1H), 3.70 – 4.05 (m, 5H), 3.05 – 3.50 (m, 7H), 2.96 (ddd, J = 17.4, 7.0, 4.6 Hz, 1H), 2.60 – 2.66 (m, 1H), 2.48 (dd, J = 17.4, 8.2 Hz, 1H), 2.14 – 2.20 (m, 2H), 1.60 – 1.70 (m, 1H), 1.45 – 1.57 (m, 1H); ^{13}C NMR (125 MHz, MeOD) δ 198.3, 175.4, 175.2, 172.8, 142.9, 139.4, 138.8, 138.3, 134.1, 131.5 (2C), 131.1 (2C), 130.0, 129.7 (2C), 129.0, 103.3, 65.1 (2C), 60.9, 56.7, 53.9 (2C), 53.4, 49.1, 44.8, 32.4, 32.0, 23.8, 20.8; HRMS (m/z): $[M+H]^+$ calcd. for $C_{31}H_{34}N_2O_7$, 546.2366; found, 546.2355.

Synthesis of compound 5. Under the conditions analogous to those used for the preparation of **1**, **S20** (80 mg, 0.15 mmol) and propargylamine (19 μL, 0.18 mmol) gave **5** as an

amorphous solid (29 mg, 33%). TLC (EtOAc:MeOH=4:1): R_f = 0.2; 7.75 – 7.78 (m, 4H), 7.65 (t, J = 7.3 Hz, 1H), 7.52 – 7.56 (m, 4H), 5.13 (t, J = 3.4 Hz, 1H), 4.91 (d, J = 15.9 Hz, 1H), 4.70 (d, J = 15.9 Hz, 1H), 4.57 (ddd, J = 13.5, 6.4, 2.8 Hz, 1H), 4.41 (ddd, J = 13.5, 6.4, 2.8 Hz), 3.97 – 4.02 (m, 2H), 3.70 – 4.05 (m, 4H), 3.40 – 3.52 (m, 2H), 3.00 – 3.50 (m, 4H), 3.16 (dd, J = 9.5, 4.3 Hz, 1H), 2.93 (dd, J = 15.5, 4.3 Hz, 1H), 2.66 (t, J =2.8 Hz, 1H), 2.55 – 2.60 (m, 1H), 2.32 (dd, J = 15.5, 9.5 Hz, 1H), 2.11 – 2.18 (m, 2H), 1.80 – 1.89 (m, 1H), 1.54 – 1.60 (m, 2H); ^{13}C NMR (125 MHz, MeOD) δ 198.3, 175.3, 173.4, 172.6, 142.8, 139.3, 138.9, 138.3, 134.1, 131.5 (2C), 131.1 (2C), 129.7 (2C), 128.9 (2C), 103.7, 80.6, 72.7, 65.1 (2C), 60.3, 57.0, 53.7 (2C), 53.4, 50.1, 44.8, 33.7, 32.0, 29.9, 23.7, 20.7; HRMS (m/z): $[M+H]^+$ calcd. for $C_{31}H_{34}N_2O_7$, 546.2366; found, 546.2355.

4. ^{1}H NMR and ^{13}C NMR Spectral

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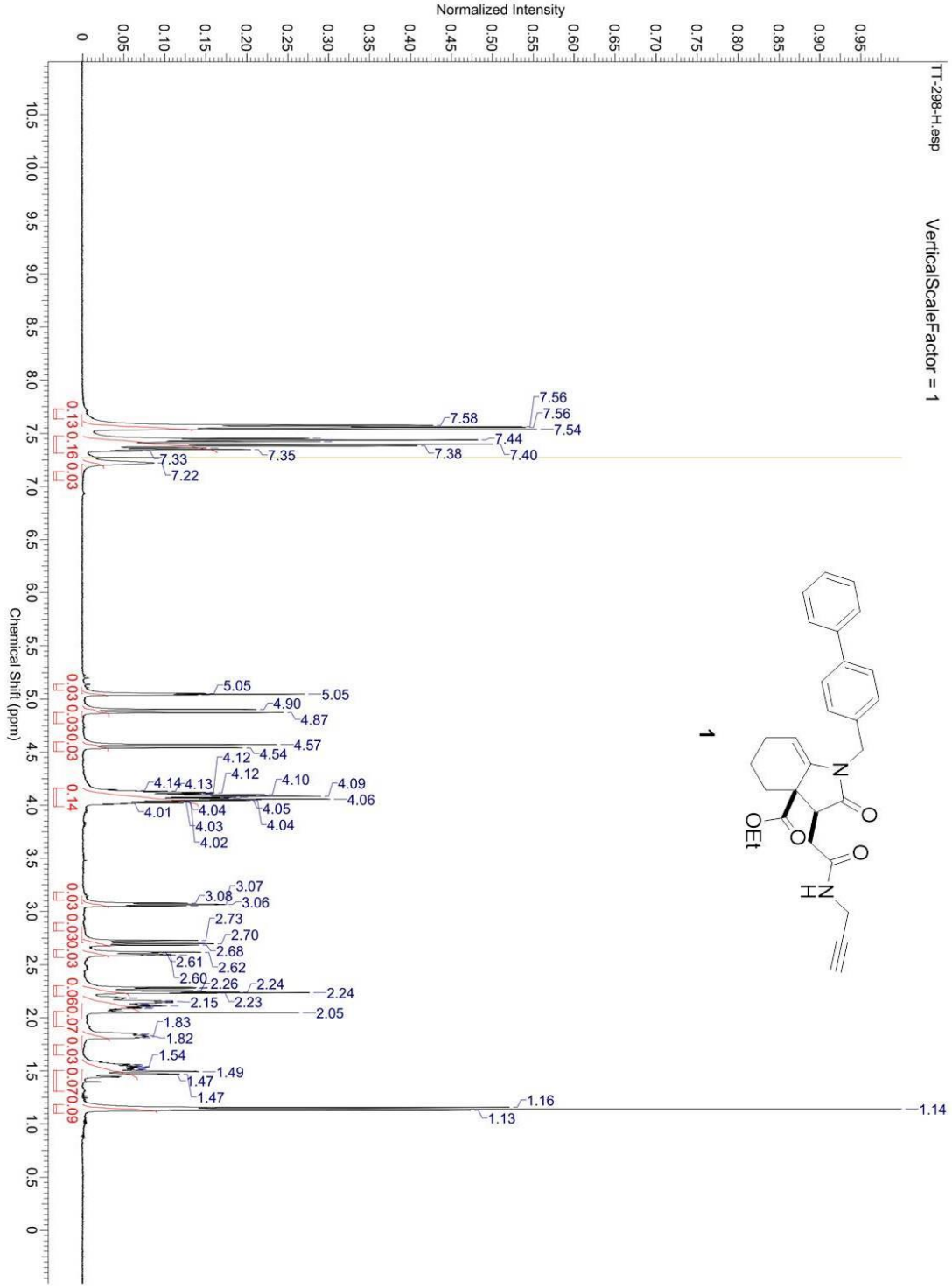
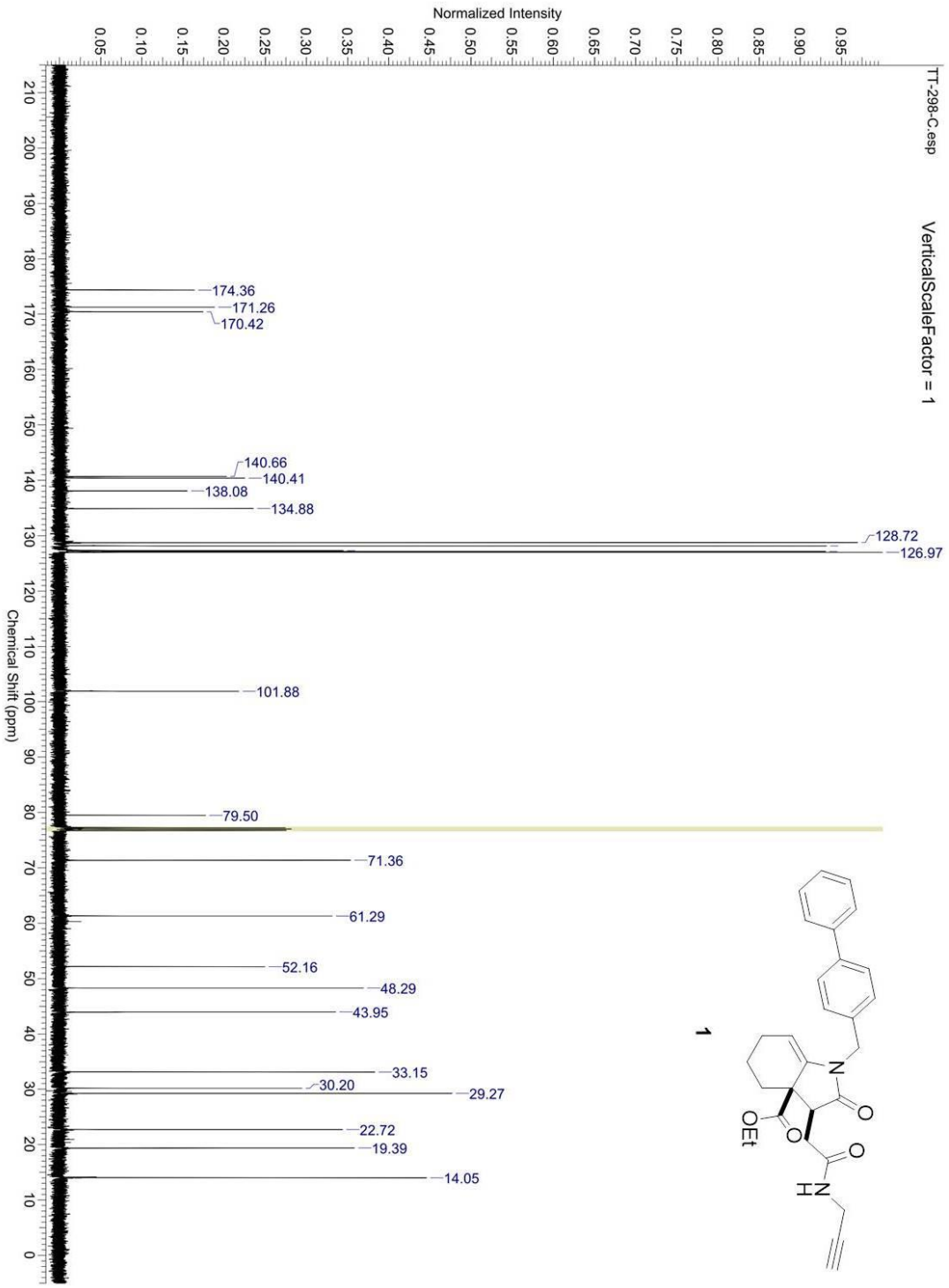
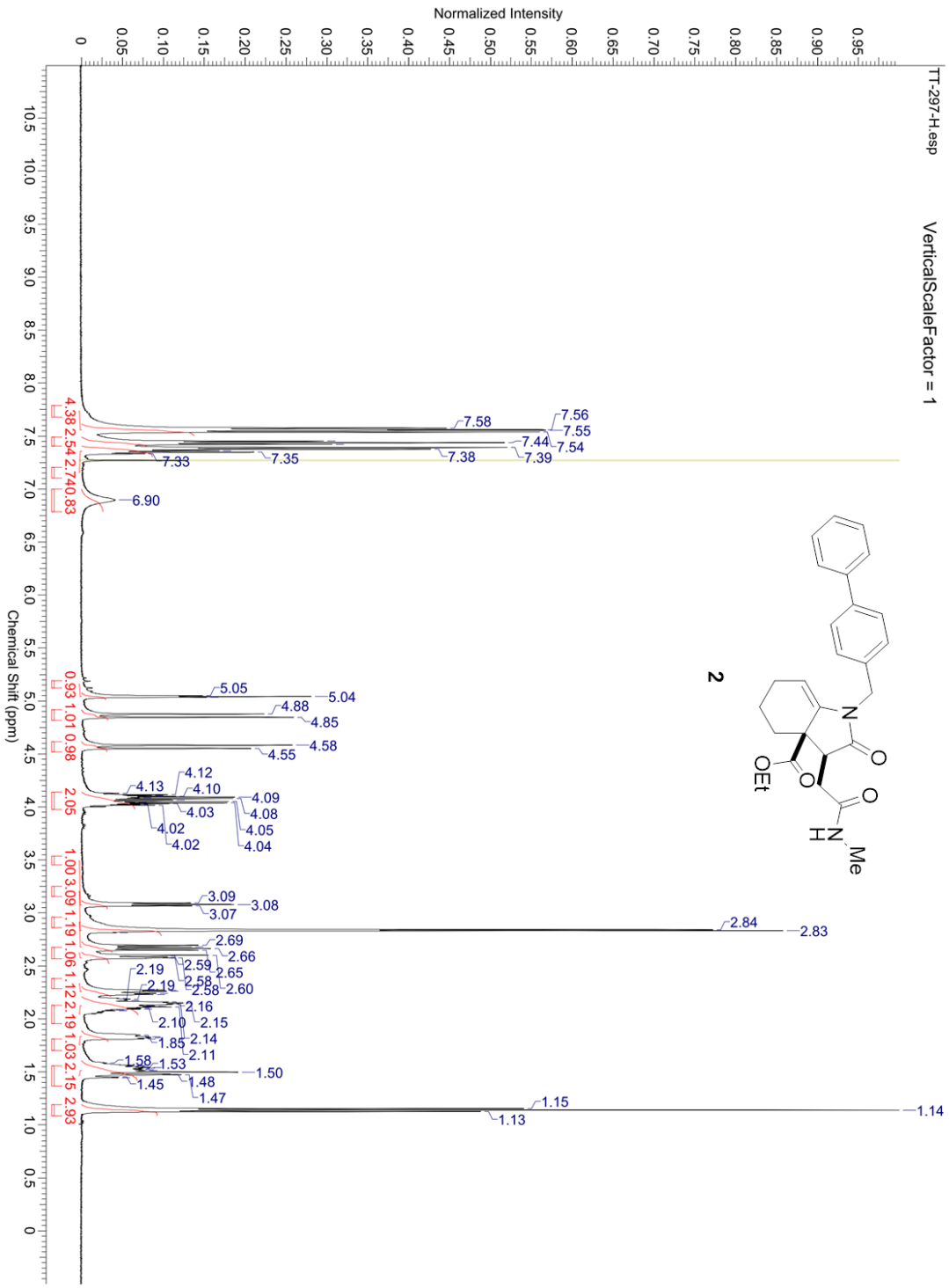
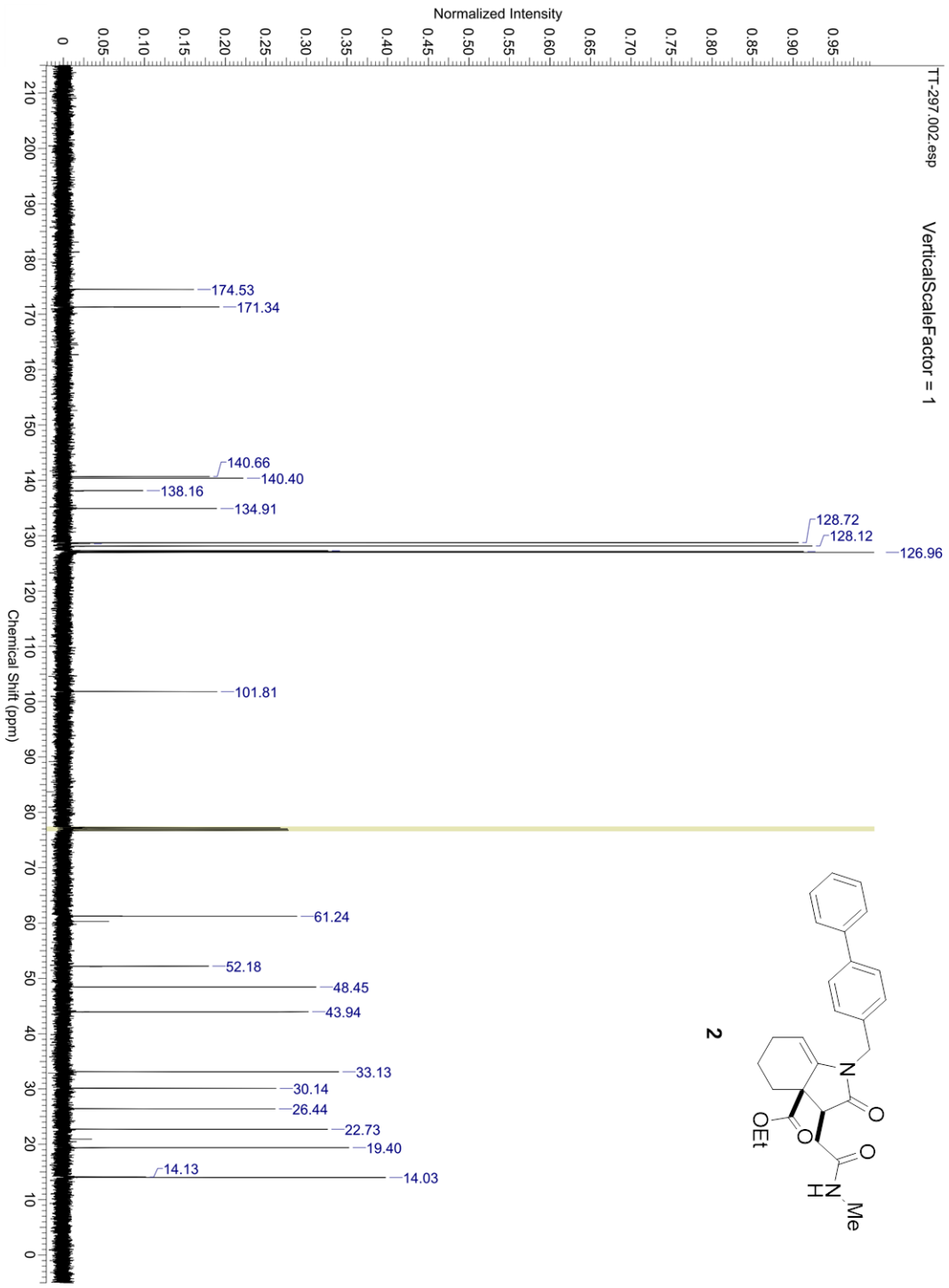
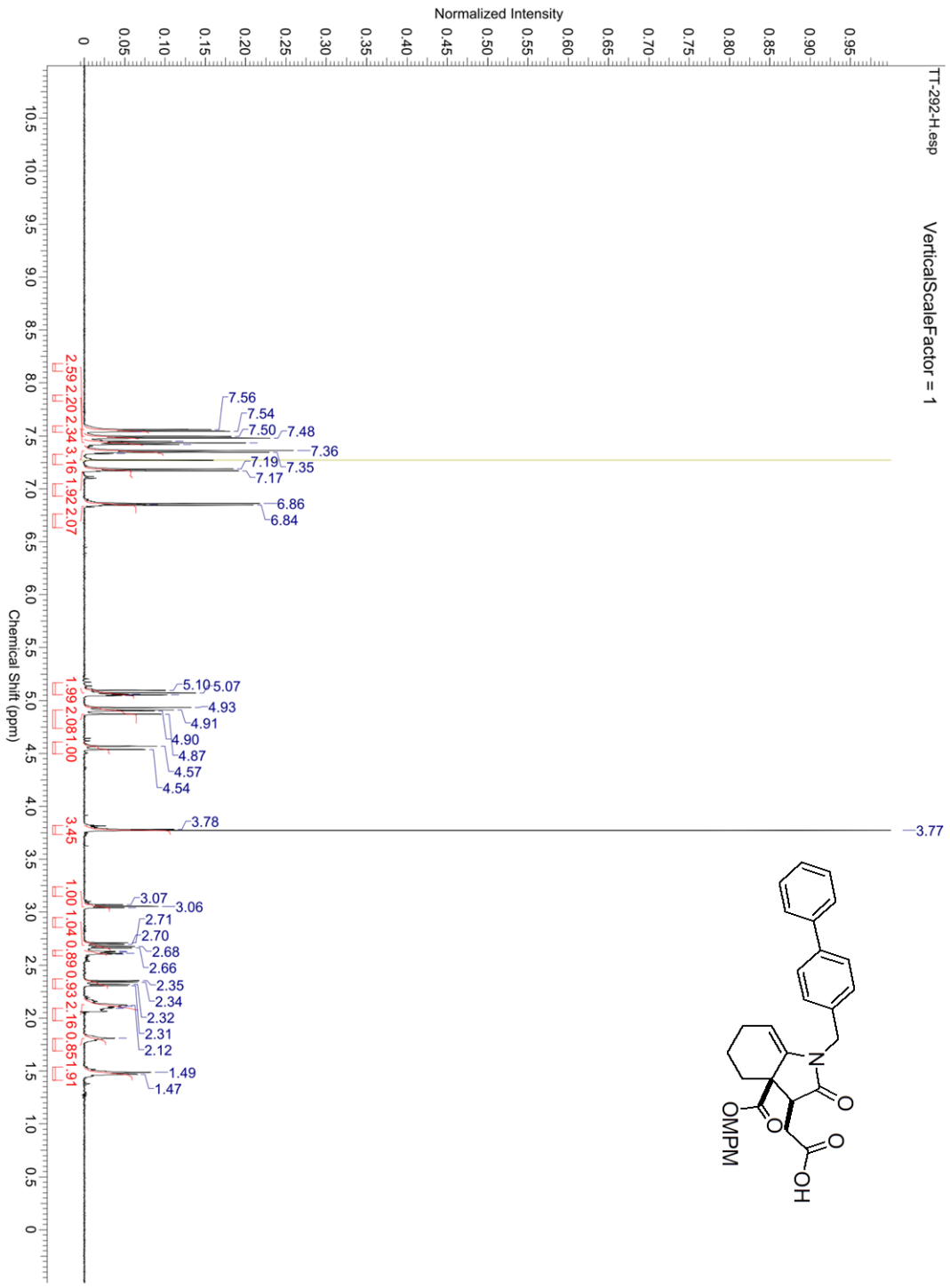
O
OH

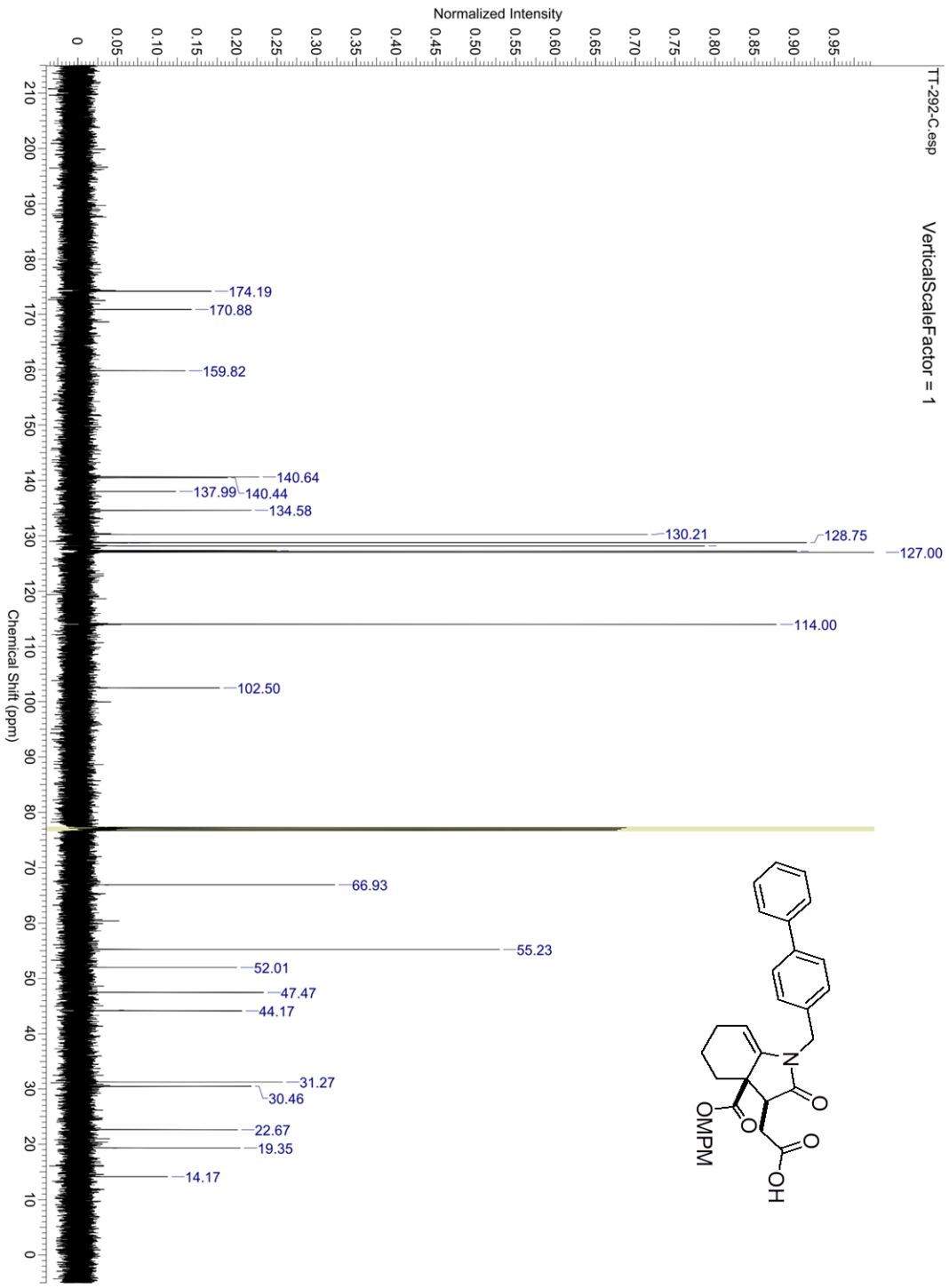

Normalized Intensity
TT-298-H.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
1
OEt
N
O
O
O
O
HN

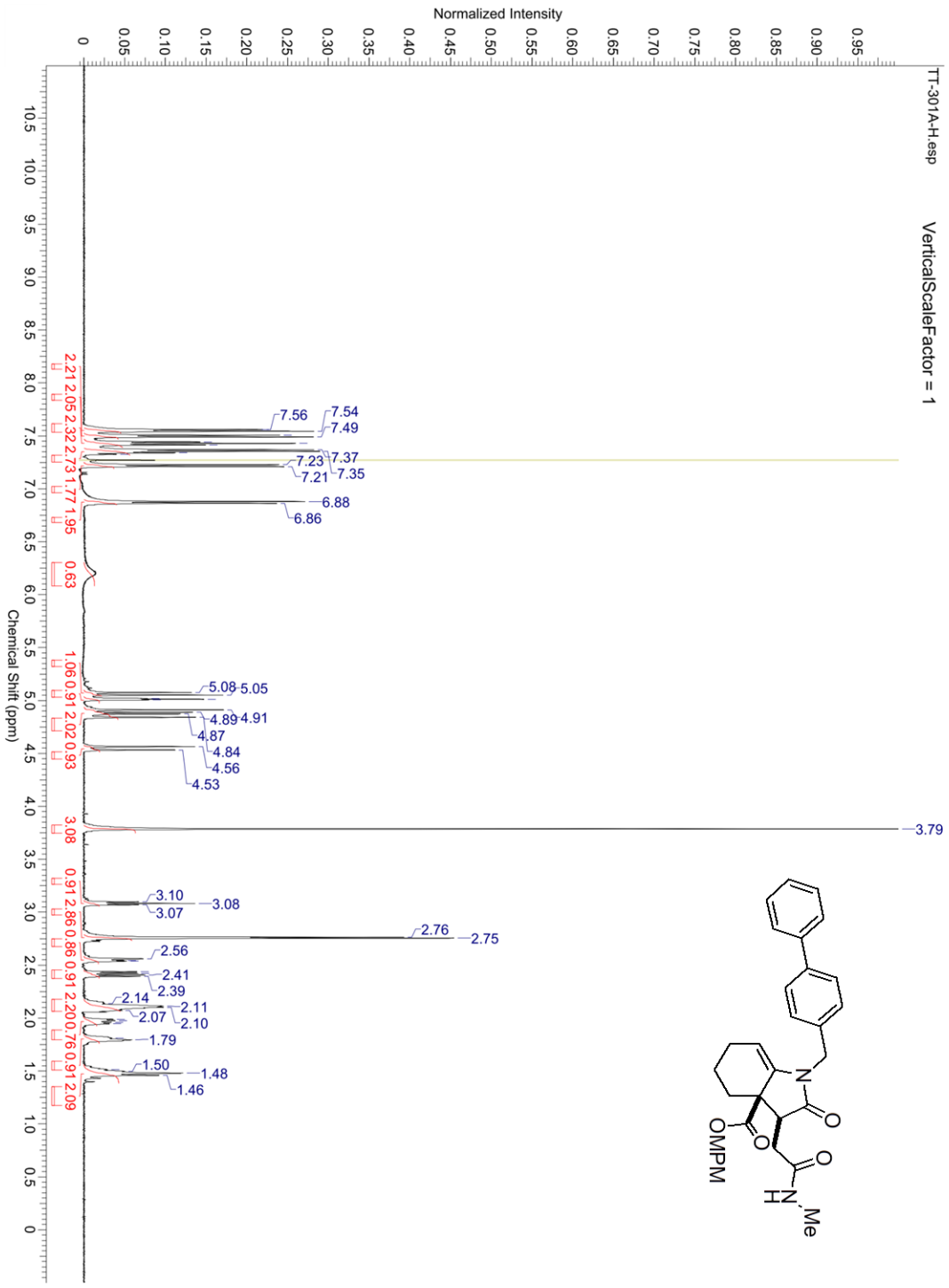

Normalized Intensity
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95
TT-298-C.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
174.36
171.26
170.42
140.66
140.41
138.08
134.88
128.72
126.97
101.88
79.50
71.36
61.29
52.16
48.29
43.95
33.15
30.20
29.27
22.72
19.39
14.05
1
N
O
O
OEt
O
H
N

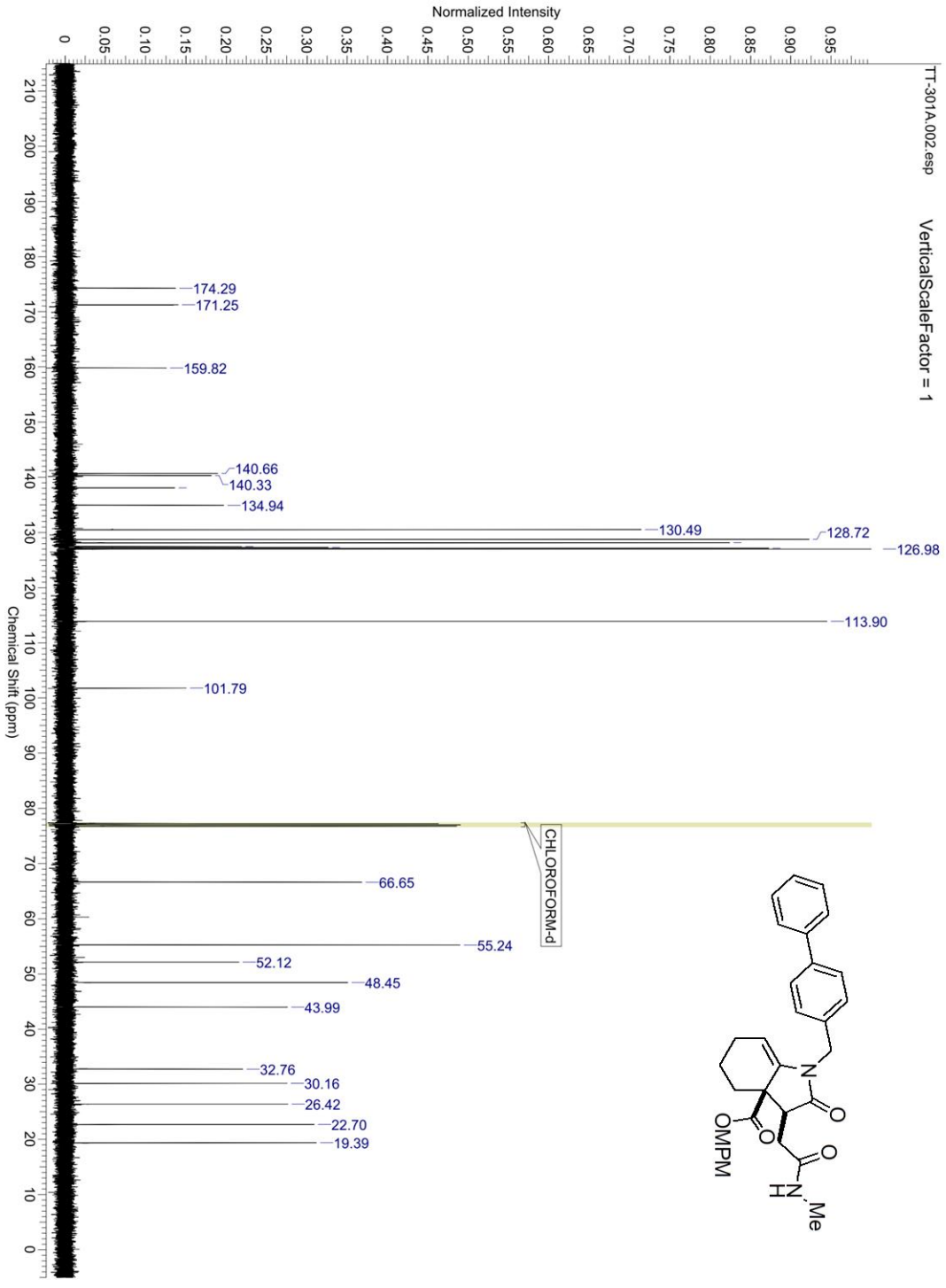

Normalized Intensity
Chemical Shift (ppm)
TT-297-H.esp
VerticalScaleFactor = 1
2
OEt
N
O
O
O
N
H
Me

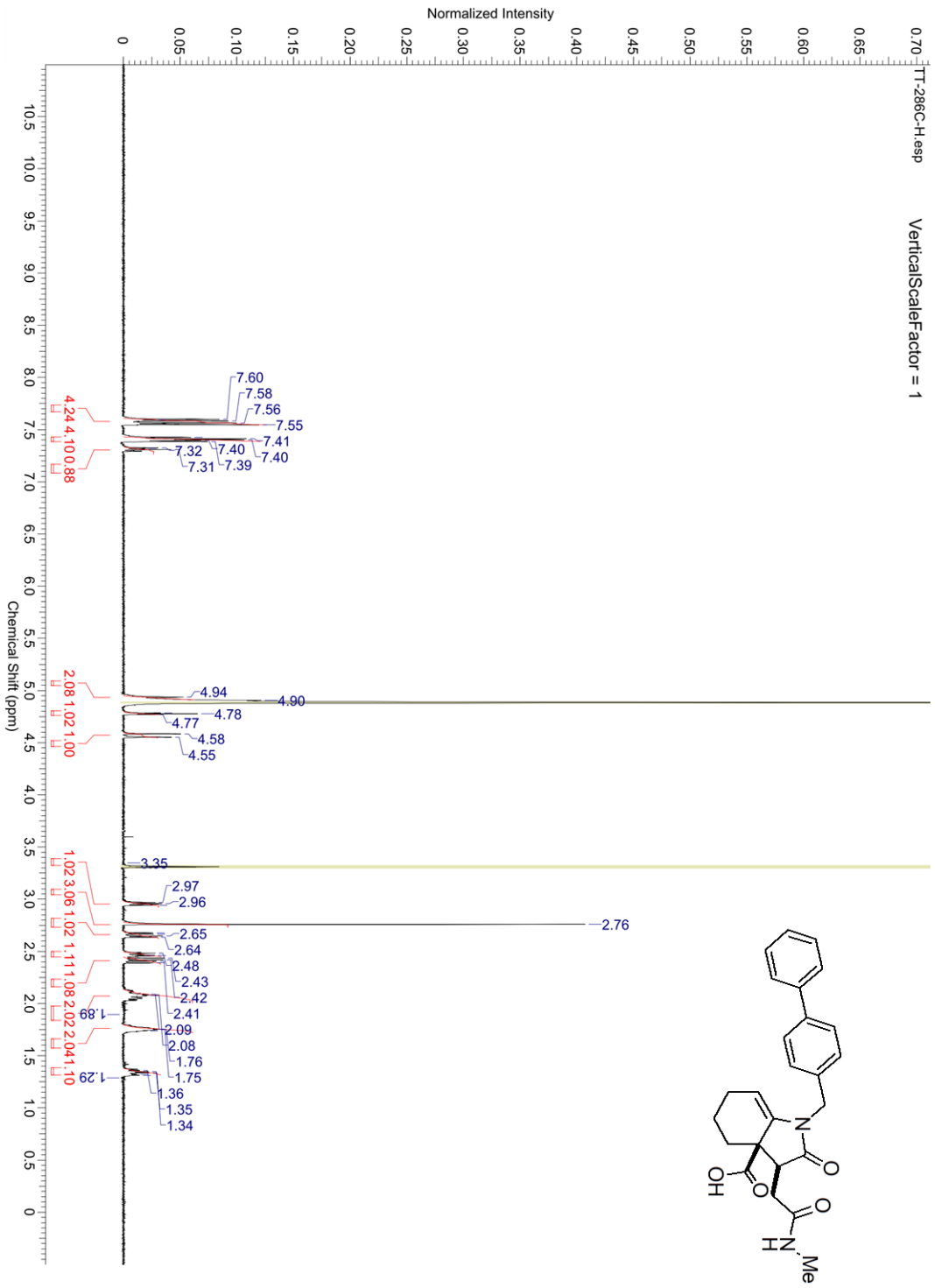

Normalized Intensity
0
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95
TT-297.002.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
174.53
171.34
140.66
140.40
138.16
134.91
128.72
128.12
126.96
101.81
61.24
52.18
48.45
43.94
33.13
30.14
26.44
22.73
19.40
14.13
14.03
2
OEt
N
O
O
O
HN
Me

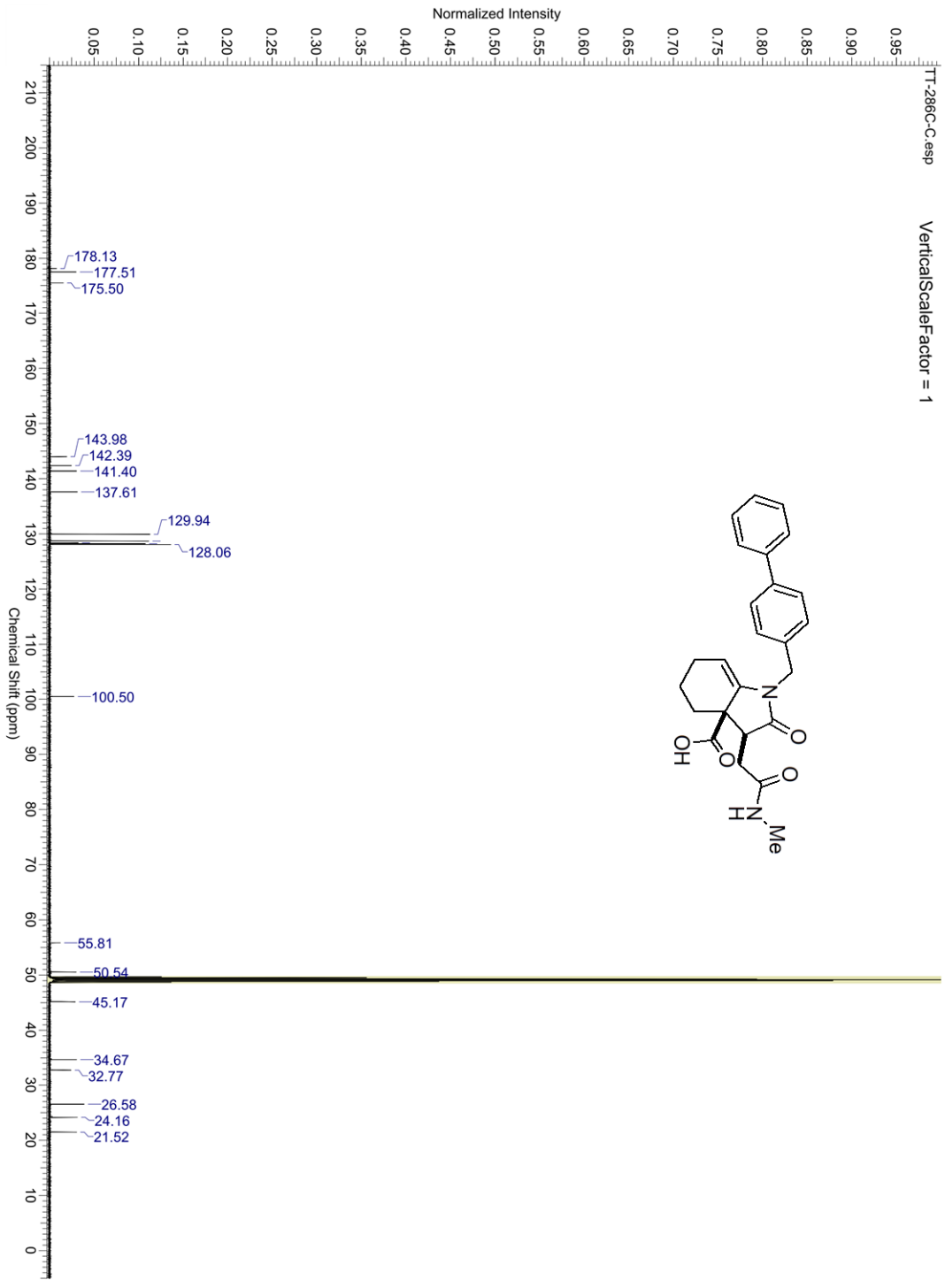

Normalized Intensity
TT-292-H.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
7.56
7.54
7.50
7.48
7.36
7.19
7.35
7.17
6.86
6.84
5.10
5.07
4.93
4.91
4.90
4.87
4.57
4.54
3.78
3.77
3.07
3.06
2.71
2.70
2.68
2.66
2.35
2.34
2.32
2.31
2.12
1.49
1.47
2.59 2.20 2.34 3.16 1.92 2.07
1.99 2.08 1.00
3.45
1.00 1.04 0.89 0.93 2.16 0.85 1.91
OMPM
OH

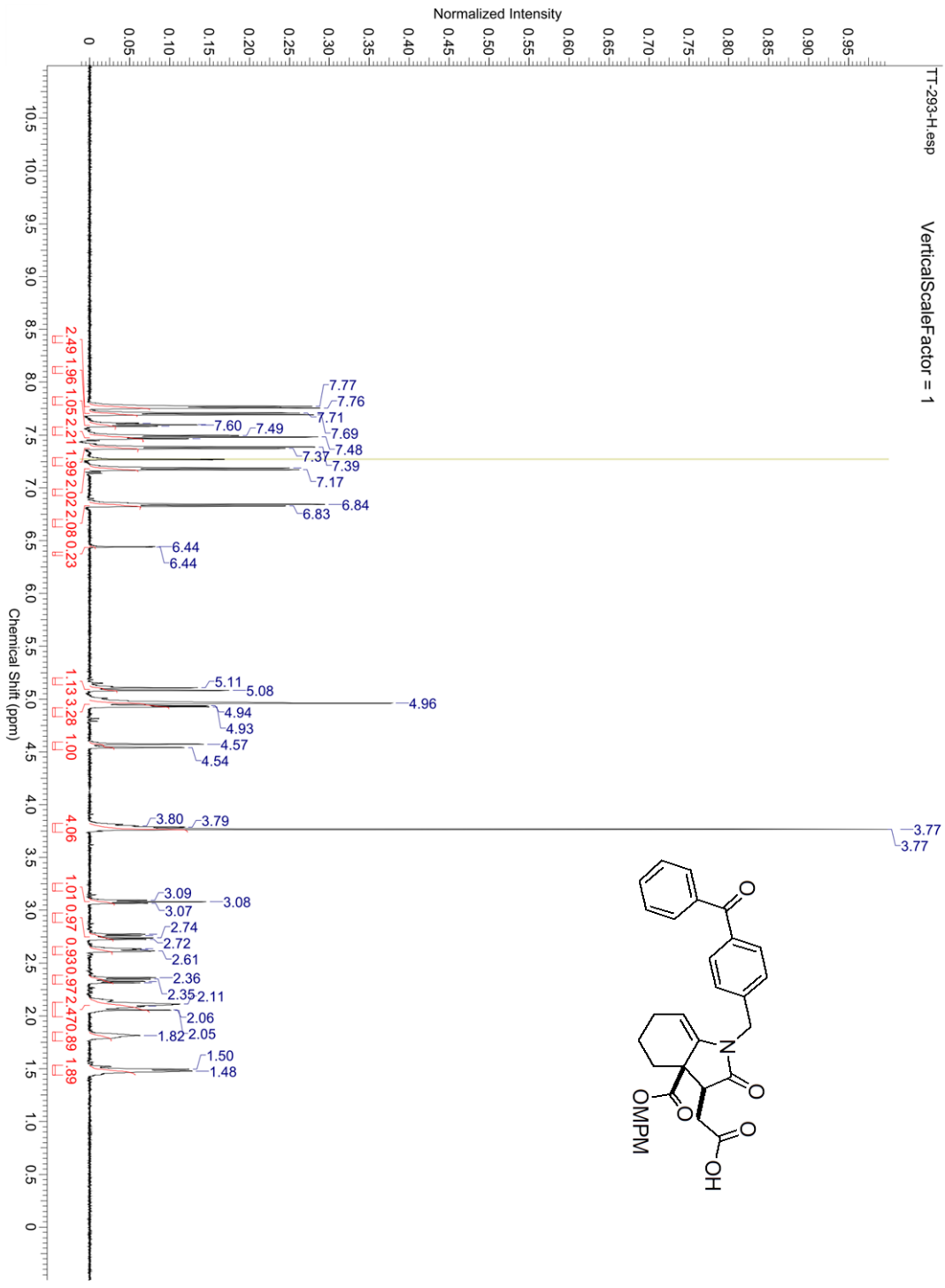

Normalized Intensity
TT-292-C.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
174.19
170.88
159.82
140.64
137.99
140.44
134.58
130.21
128.75
127.00
114.00
102.50
66.93
55.23
52.01
47.47
44.17
31.27
30.46
22.67
19.35
14.17
OMPM
OH

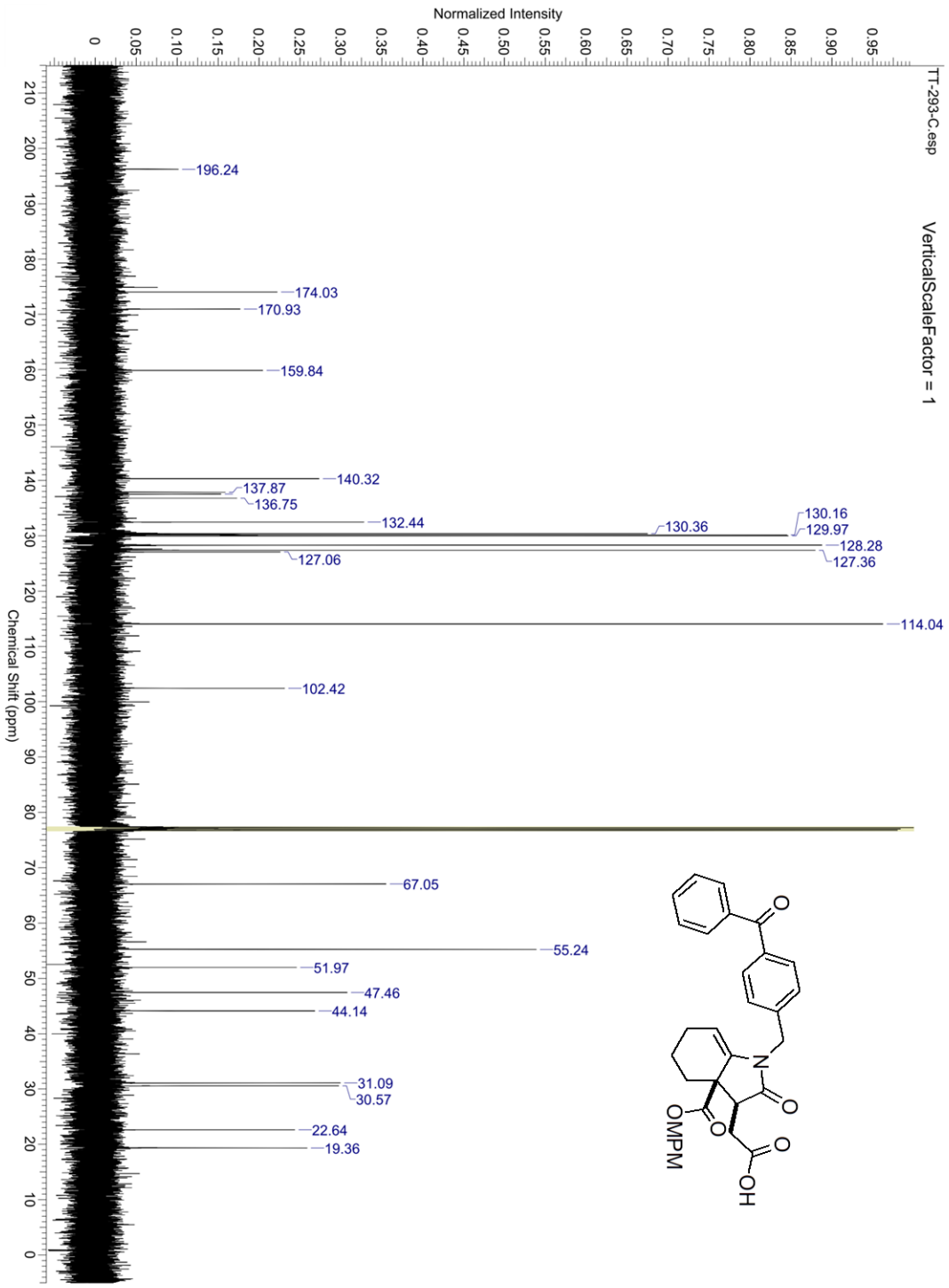

Normalized Intensity
TT-301A-H.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
7.56
7.54
7.49
7.37
7.35
7.23
7.21
6.88
6.86
5.08
5.05
4.91
4.89
4.87
4.84
4.56
4.53
3.79
3.10
3.08
3.07
2.76
2.75
2.56
2.41
2.39
2.14
2.11
2.10
2.07
1.79
1.50
1.48
1.46
2.21
2.05
2.32
2.73
1.77
1.95
0.63
1.06
0.91
2.02
0.93
3.08
0.91
2.86
0.86
0.91
2.20
0.76
0.91
2.09
OMPM
N
O
O
O
N
H
Me

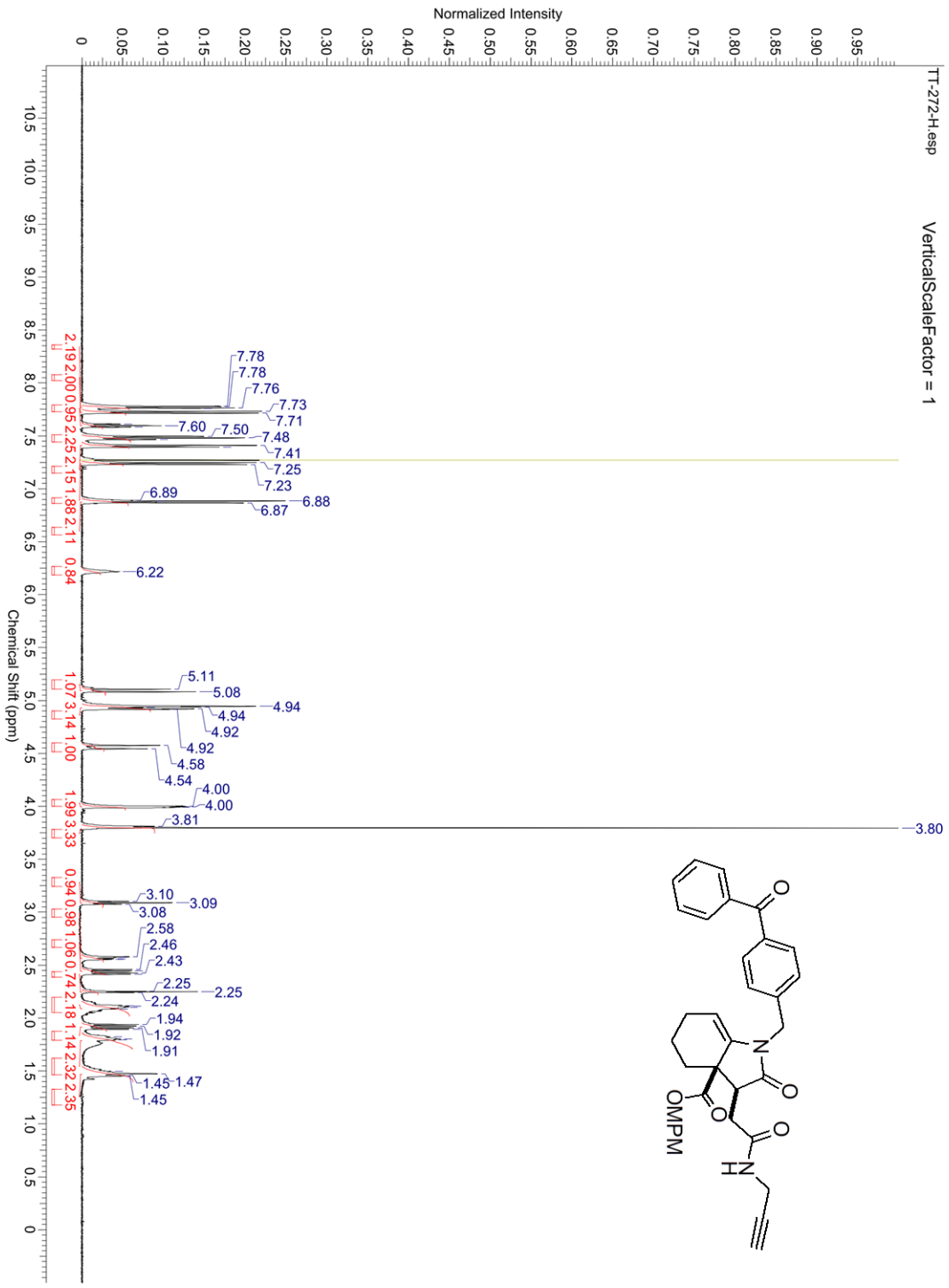

Normalized Intensity
TT-301A.002.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
174.29
171.25
159.82
140.66
140.33
134.94
130.49
128.72
126.98
113.90
101.79
CHLOROFORM-d
66.65
55.24
52.12
48.45
43.99
32.76
30.16
26.42
22.70
19.39
OMPM
N
O
O
O
O
H
N
Me

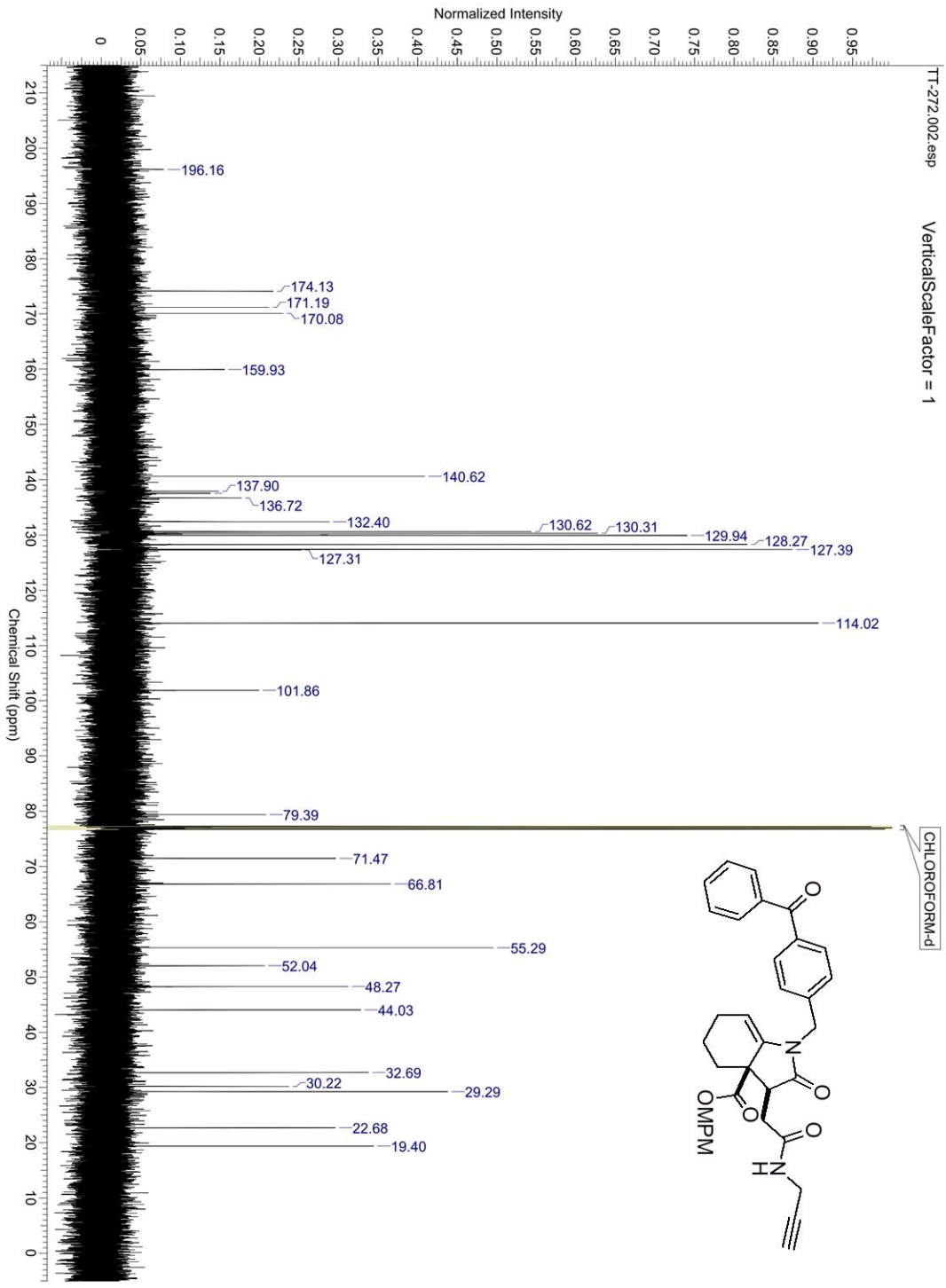

Normalized Intensity
TT-286C-H.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
7.60
7.58
7.56
7.55
7.41
7.40
7.40
7.32
7.40
7.31
7.39
4.24
4.10
0.88
4.94
4.90
4.78
4.77
4.58
4.55
2.08
1.02
1.00
3.35
2.97
2.96
2.76
2.65
2.64
2.48
2.43
2.42
2.41
2.09
2.08
1.76
1.75
1.36
1.35
1.34
1.89
1.29
1.02
3.06
1.02
1.11
1.08
2.02
2.04
1.10
OH
O
O
N
O
N
H
Me

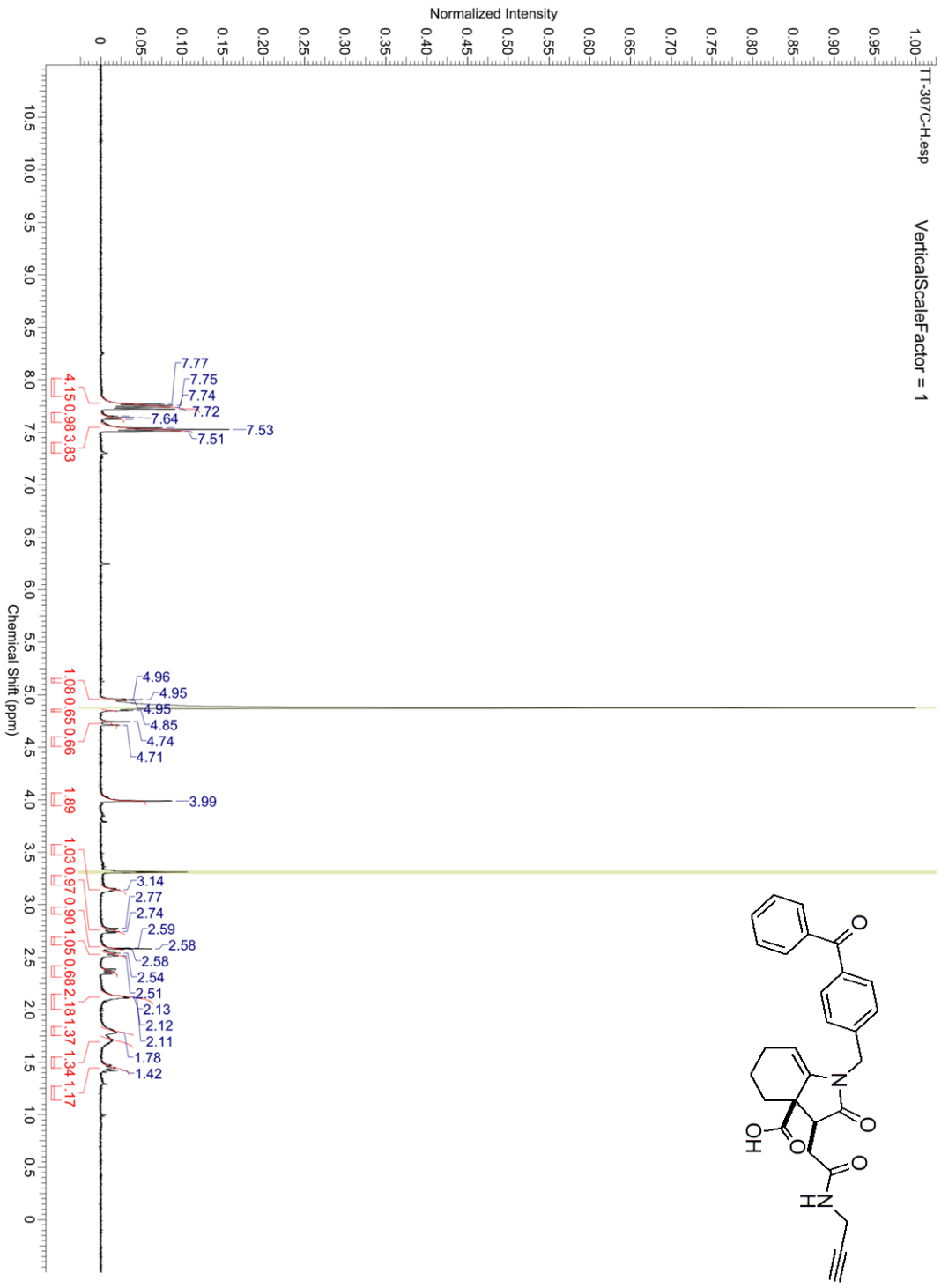

Normalized Intensity
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95
TT-286C-C.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
178.13
177.51
175.50
143.98
142.39
141.40
137.61
129.94
128.06
100.50
55.81
50.54
45.17
34.67
32.77
26.58
24.16
21.52
N
O
OH
O
O
HN
Me

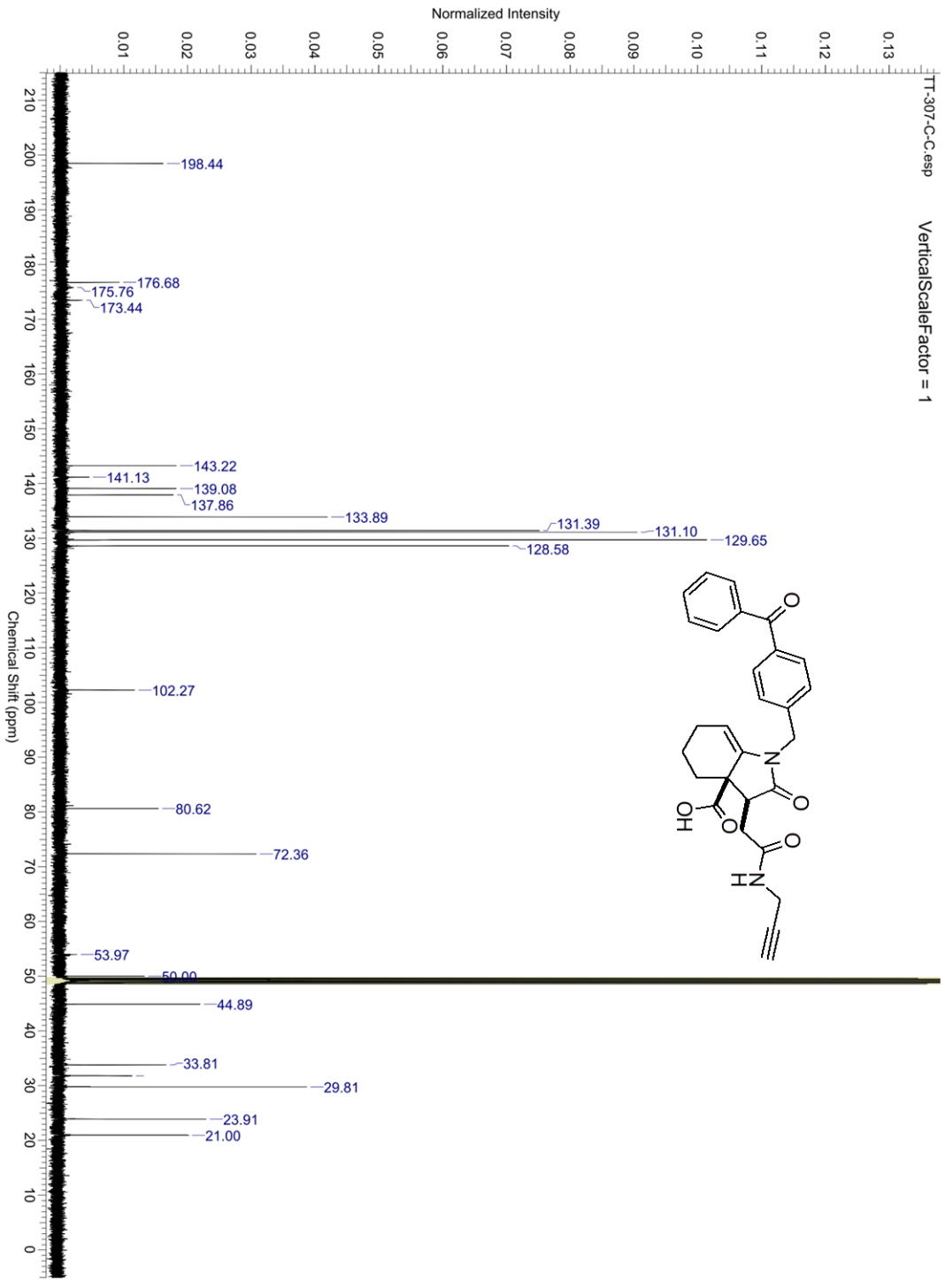

Normalized Intensity
TT-293-H.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
7.77
7.76
7.71
7.60
7.49
7.69
7.48
7.37
7.39
7.17
6.84
6.83
6.44
6.44
5.11
5.08
4.96
4.94
4.93
4.57
4.54
3.80
3.79
3.77
3.77
3.09
3.07
3.08
2.74
2.72
2.61
2.36
2.35
2.11
2.06
1.82
2.05
1.50
1.48
2.49
1.96
1.05
2.21
1.99
2.02
2.08
0.23
1.13
3.28
1.00
4.06
1.01
0.97
0.93
0.97
2.47
0.89
1.89
OMPM
OH

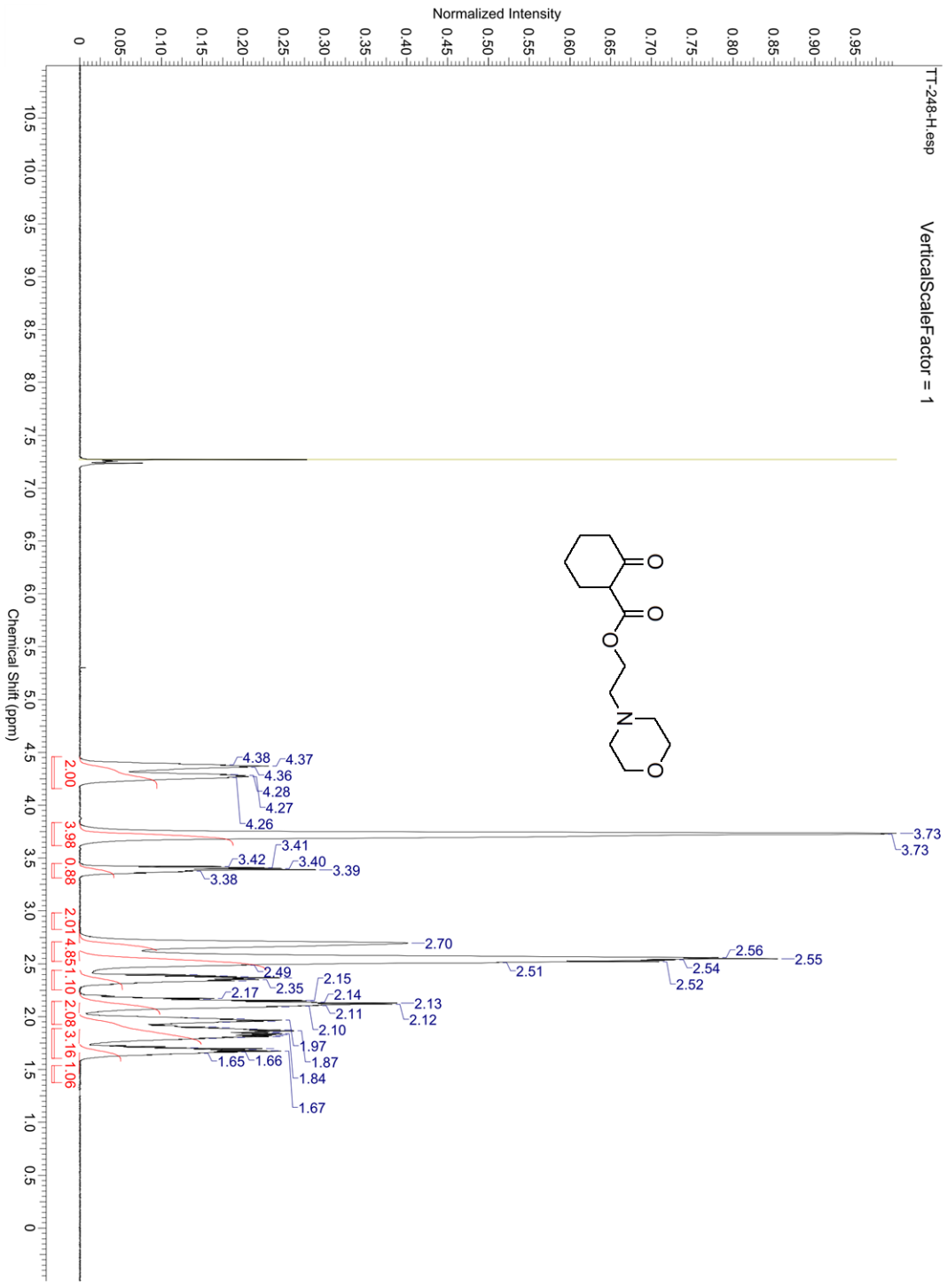

Normalized Intensity
Chemical Shift (ppm)
TT-293-C.esp
VerticalScaleFactor = 1
196.24
174.03
170.93
159.84
140.32
137.87
136.75
132.44
130.36
130.16
129.97
128.28
127.36
127.06
114.04
102.42
67.05
55.24
51.97
47.46
44.14
31.09
30.57
22.64
19.36
OMPM
OH

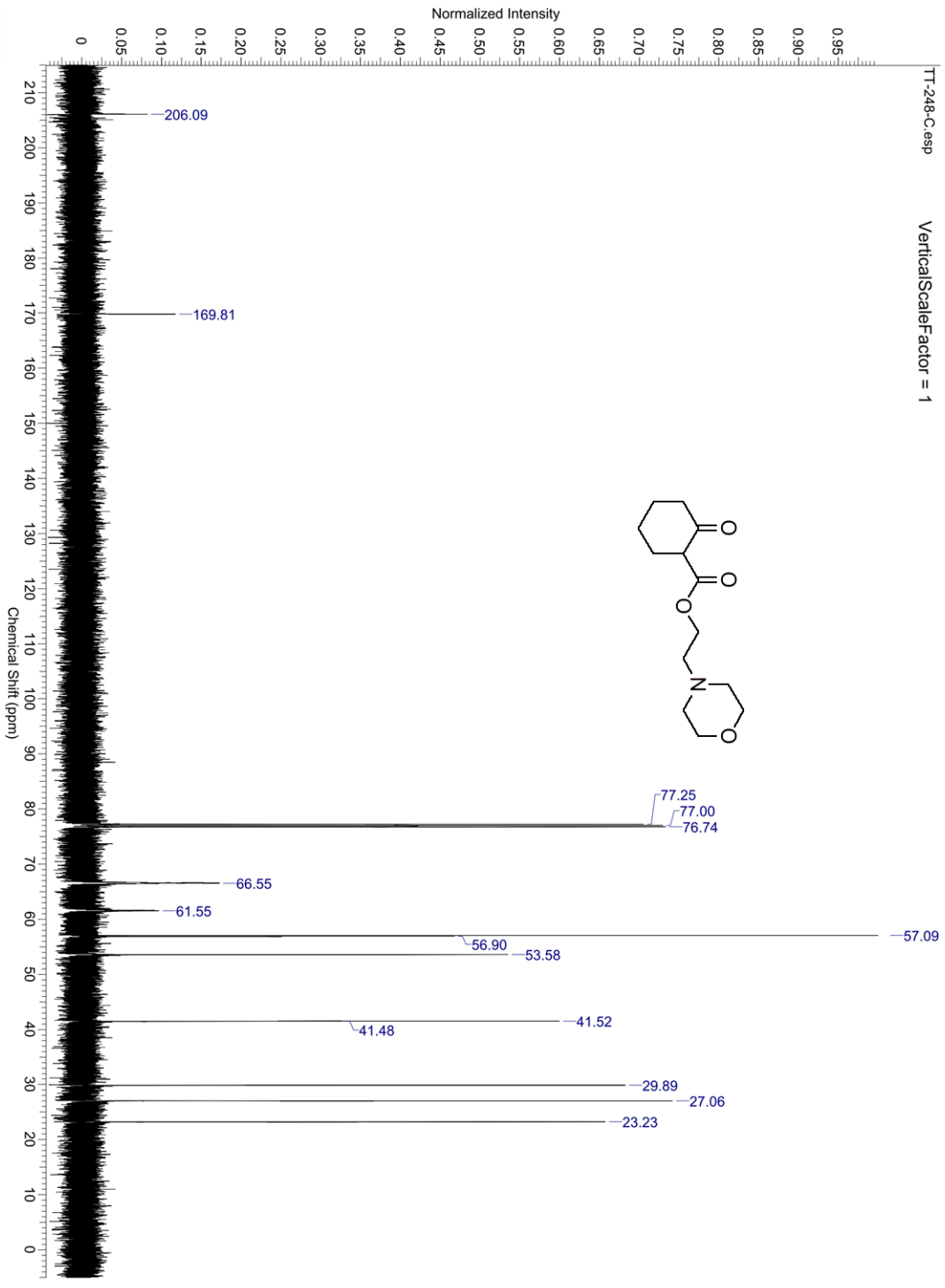

Normalized Intensity
TT-272-H.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
7.78
7.78
7.76
7.73
7.71
7.60
7.50
7.48
7.41
7.25
7.23
6.89
6.88
6.87
6.22
5.11
5.08
4.94
4.94
4.92
4.92
4.58
4.54
4.00
4.00
3.81
3.80
3.10
3.09
3.08
2.58
2.46
2.43
2.25
2.25
2.24
1.94
1.92
1.91
1.47
1.45
1.45
2.19 2.00 0.95 2.25 2.15 1.88 2.11 0.84
1.07 3.14 1.00 1.99 3.33 0.94 0.98 1.06 0.74 2.18 1.14 2.32 2.35
OMPM

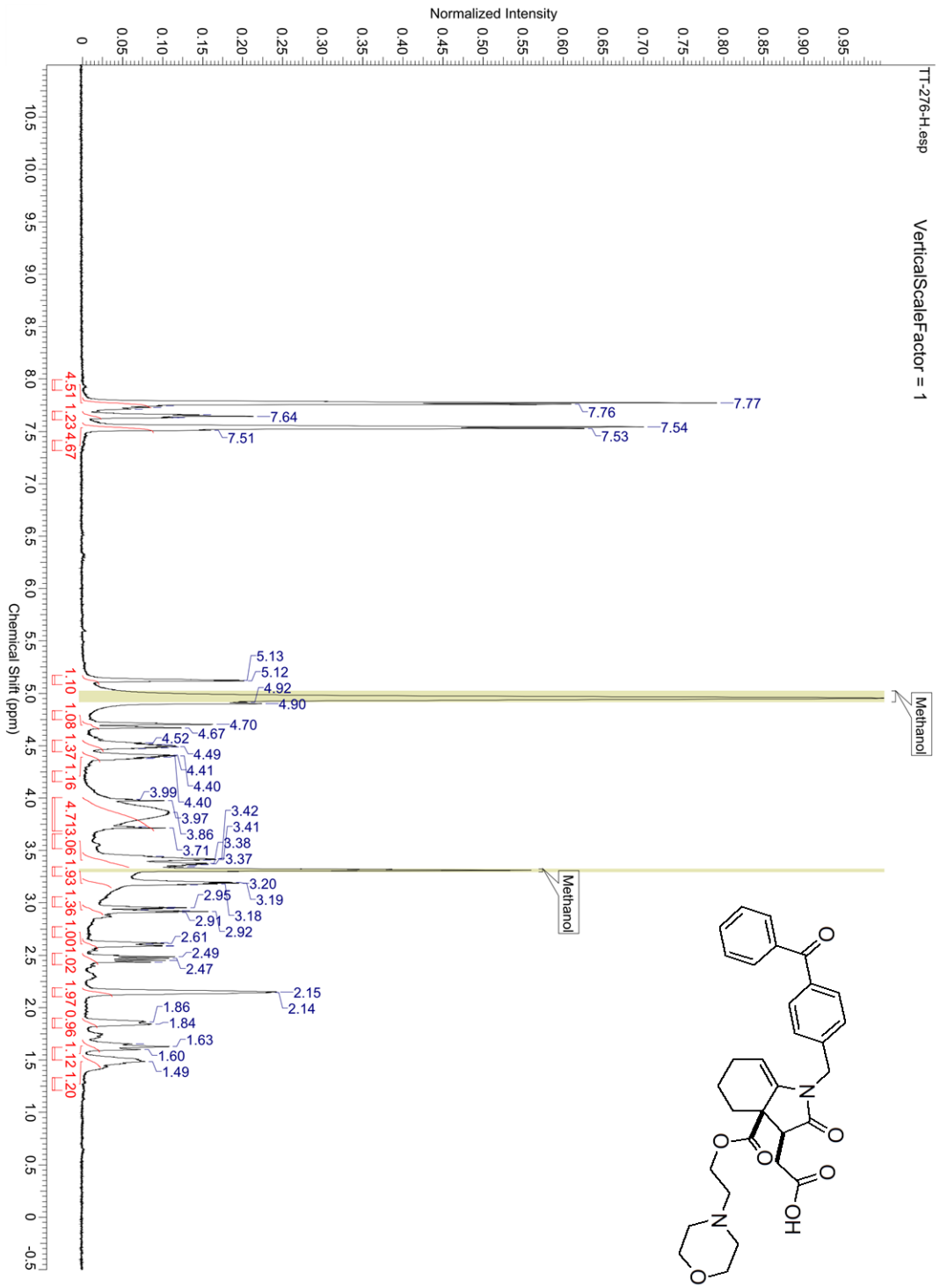

Normalized Intensity
Chemical Shift (ppm)
TT-272.002.esp
VerticalScaleFactor = 1
CHLOROFORM-d
196.16
174.13
171.19
170.08
159.93
140.62
137.90
136.72
132.40
130.62
130.31
129.94
128.27
127.39
127.31
114.02
101.86
79.39
71.47
66.81
55.29
52.04
48.27
44.03
32.69
30.22
29.29
22.68
19.40
OMPM

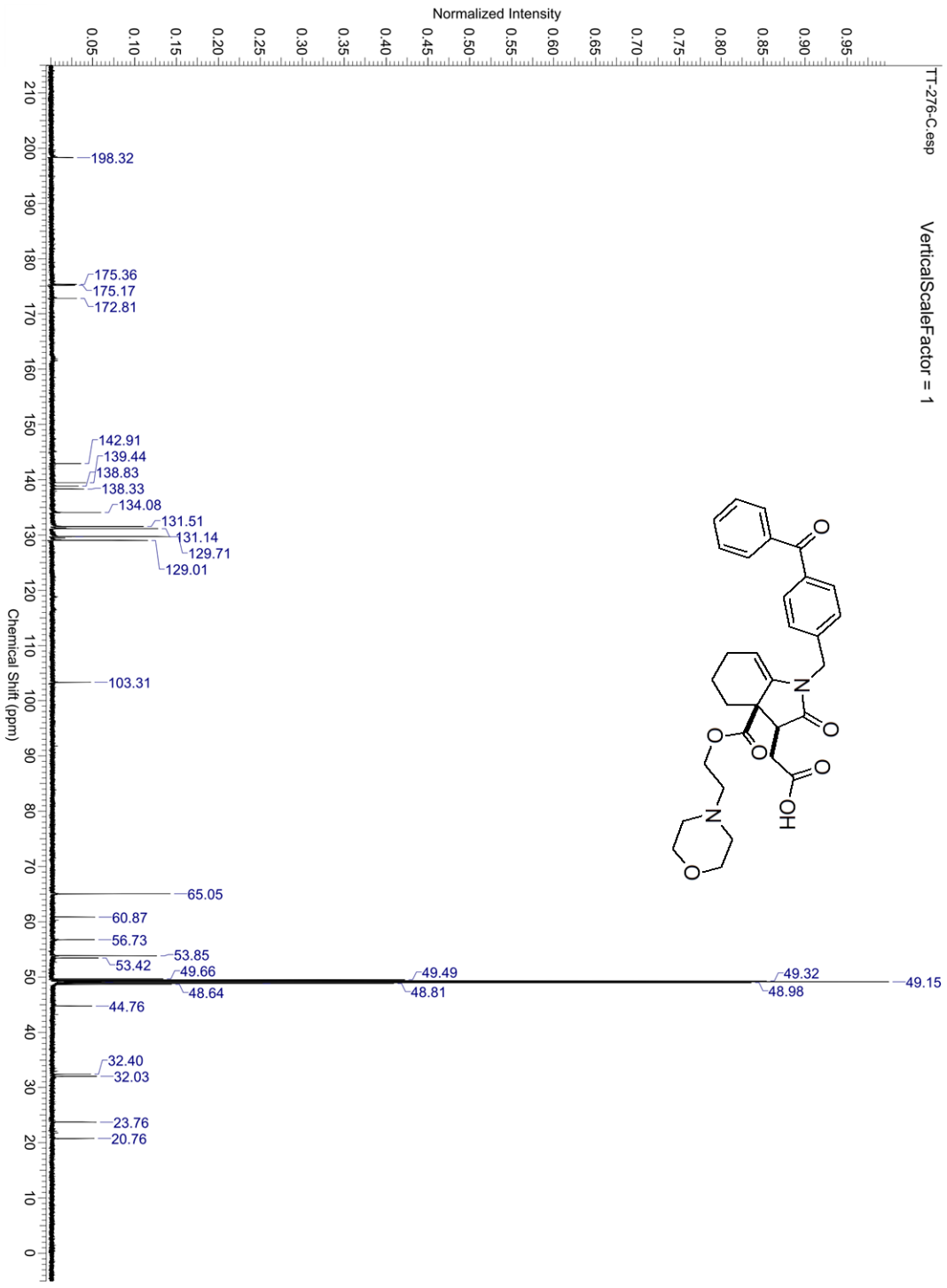

Normalized Intensity
TT-307C-H.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
7.77
7.75
7.74
7.72
7.64
7.53
7.51
4.96
4.95
4.95
4.85
4.74
4.71
3.99
3.14
2.77
2.74
2.59
2.58
2.58
2.54
2.51
2.13
2.12
2.11
1.78
1.42
4.15
0.98
3.83
1.08
0.65
0.66
1.89
1.03
0.97
0.90
1.05
0.68
2.18
1.37
1.34
1.17
O
N
O
OH
O
O
HN

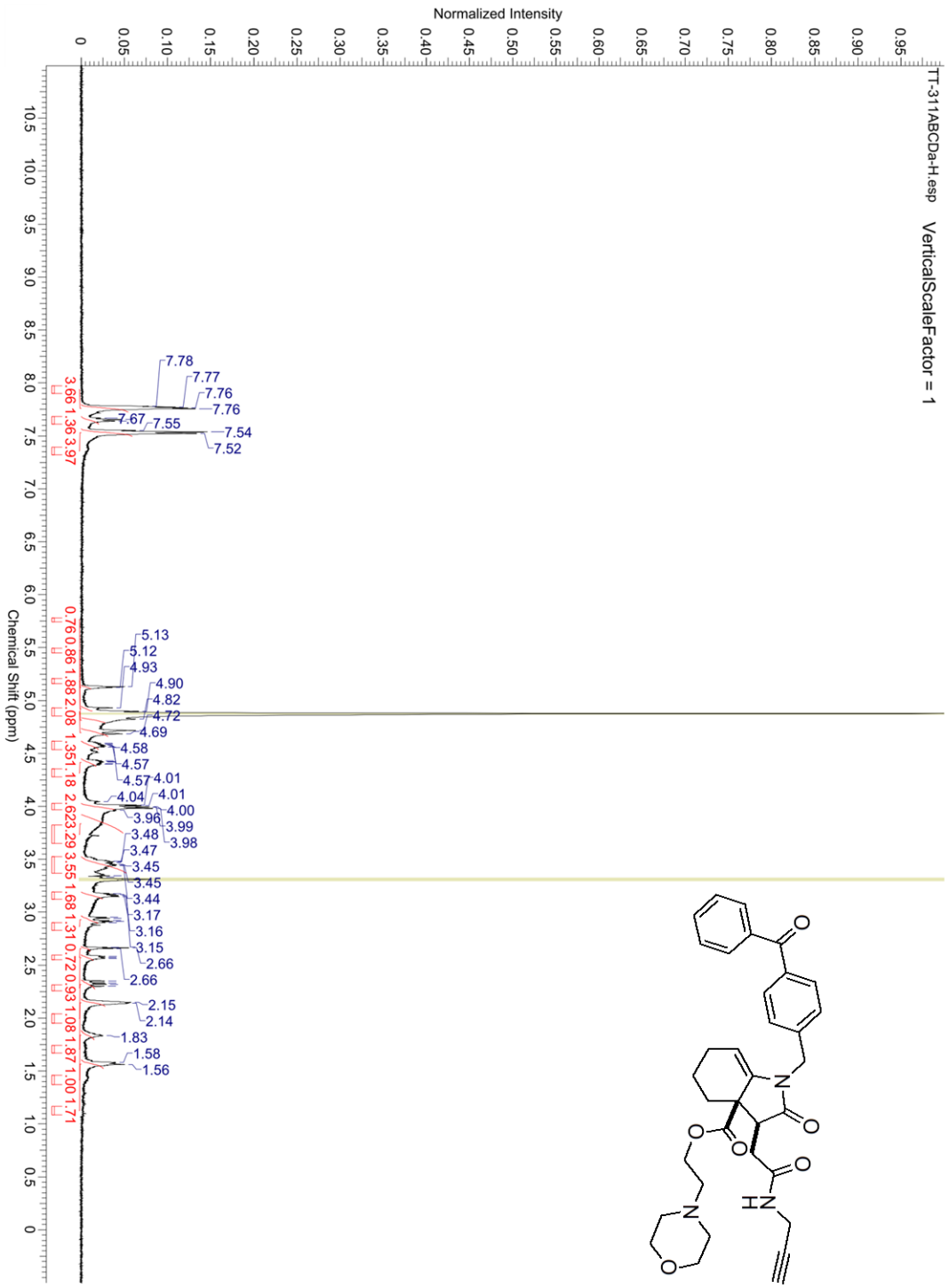

Normalized Intensity
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.10
0.11
0.12
0.13
TT-307-C-C.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
198.44
176.68
175.76
173.44
143.22
141.13
139.08
137.86
133.89
131.39
131.10
129.65
128.58
102.27
80.62
72.36
53.97
50.00
44.89
33.81
29.81
23.91
21.00
O
N
O
OH
O
O
HN

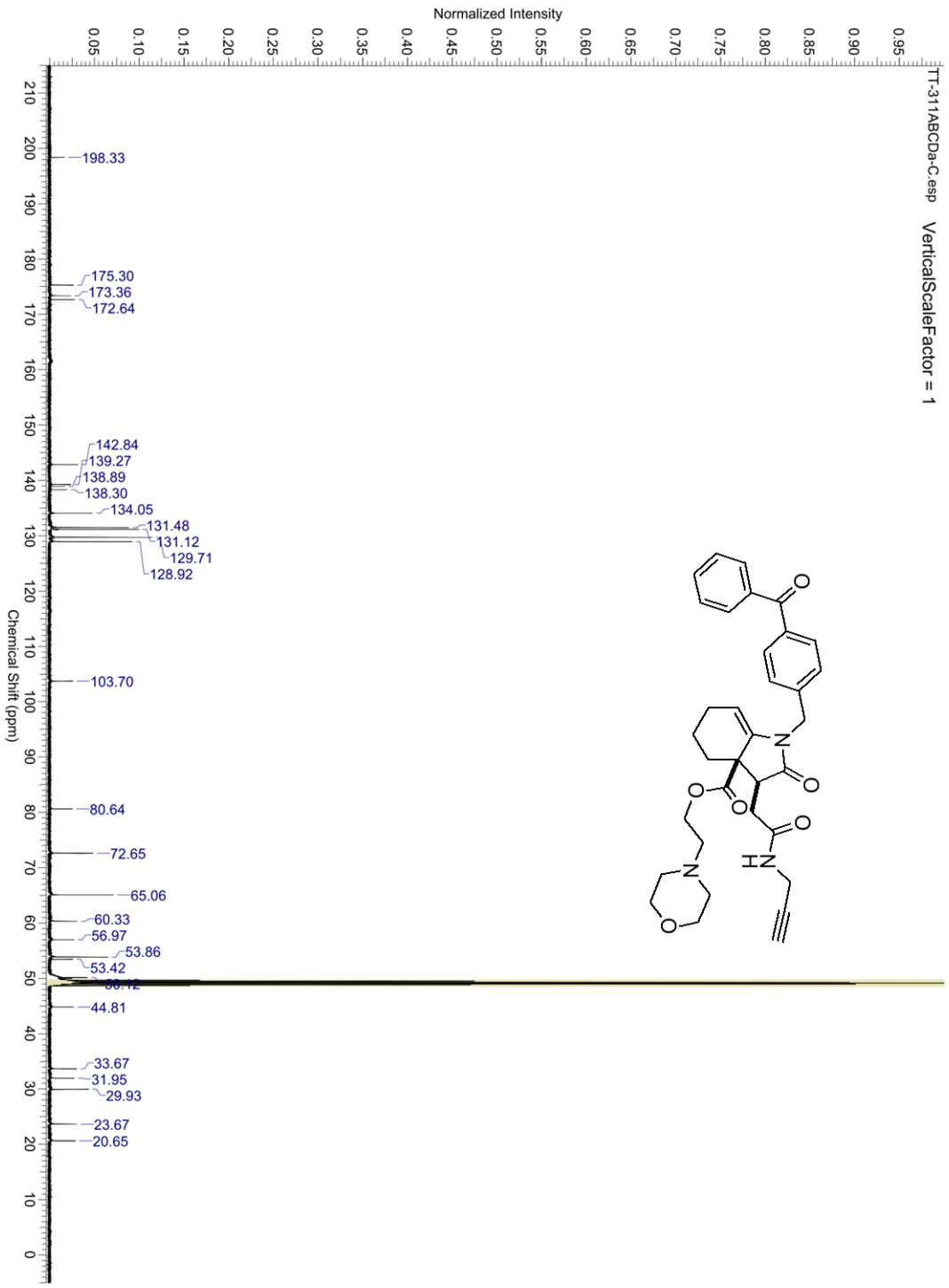

Normalized Intensity
TT-248-H.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
4.38
4.37
4.36
4.28
4.27
4.26
3.73
3.73
3.41
3.42
3.40
3.39
3.38
2.70
2.56
2.55
2.51
2.54
2.52
2.49
2.15
2.35
2.17
2.14
2.13
2.11
2.12
2.10
1.97
1.87
1.65
1.66
1.84
1.67
2.00
3.98
0.88
2.01
4.85
1.10
2.08
3.16
1.06
O
O
O
N
O


Normalized Intensity
0
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95
TT-248-C.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
206.09
169.81
77.25
77.00
76.74
66.55
61.55
57.09
56.90
53.58
41.52
41.48
29.89
27.06
23.23
O
O
O
N
O


Normalized Intensity
TT-276-H.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
Methanol
Methanol
7.77
7.76
7.64
7.54
7.53
7.51
5.13
5.12
4.92
4.90
4.70
4.67
4.52
4.49
4.41
4.40
3.99
4.40
3.97
3.86
3.71
3.42
3.41
3.38
3.37
3.20
3.19
2.95
3.18
2.91
2.92
2.61
2.49
2.47
2.15
2.14
1.86
1.84
1.63
1.60
1.49
4.51 1.23 4.67
1.10 1.08 1.37 1.16 4.71 3.06 1.93 1.36 1.00 1.02 1.97 0.96 1.12 1.20
O
O
N
O
O
O
O
N
O
OH


Normalized Intensity
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95
TT-276-C.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
198.32
175.36
175.17
172.81
142.91
139.44
138.83
138.33
134.08
131.51
131.14
129.71
129.01
103.31
65.05
60.87
56.73
53.85
53.42
49.66
49.49
49.32
49.15
48.81
48.98
48.64
44.76
32.40
32.03
23.76
20.76
O
N
O
O
O
O
OH
N
O


Normalized Intensity
TT-311ABCDa-H.esp VerticalScaleFactor = 1
Chemical Shift (ppm)
7.78
7.77
7.76
7.76
7.67
7.55
7.54
7.52
5.13
5.12
4.93
4.90
4.82
4.72
4.69
4.58
4.57
4.57
4.04
4.01
4.01
4.00
3.99
3.98
3.96
3.48
3.47
3.45
3.45
3.44
3.17
3.16
3.15
2.66
2.66
2.15
2.14
1.83
1.58
1.56
3.66 1.36 3.97
0.76 0.86 1.88 2.08 1.35 1.18 2.62 3.29 3.55 1.68 1.31 0.72 0.93 1.08 1.87 1.00 1.71


Normalized Intensity
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95
TT-311ABCDa-C.esp
VerticalScaleFactor = 1
Chemical Shift (ppm)
198.33
175.30
173.36
172.64
142.84
139.27
138.89
138.30
134.05
131.48
131.12
129.71
128.92
103.70
80.64
72.65
65.06
60.33
56.97
53.86
53.42
44.81
33.67
31.95
29.93
23.67
20.65
O
N
O
O
O
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6. Supporting Information References

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