SI APPENDIX

A quorum-sensing inhibitor blocks *Pseudomonas aeruginosa* virulence and biofilm formation

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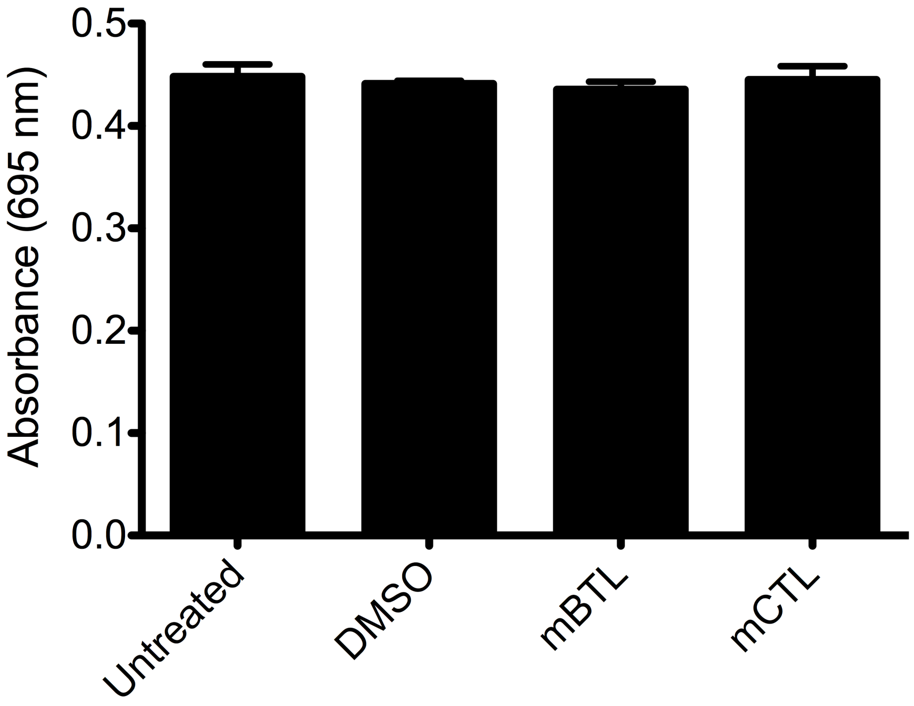


Fig. S1: Treatment of cell-free culture fluids with mBTL or mCTL does not alter pyocyanin levels

Cell-free culture fluids from WT *P. aeruginosa* PA14 following 17 hr growth were left untreated or supplied with 100 μM mCTL, mBTL, or an equivalent amount of DMSO. Subsequently, the fluids were incubated shaking at 37°C for 17 hr. Pyocyanin levels were assessed by absorption. Error bars indicate S.D. of three replicates.

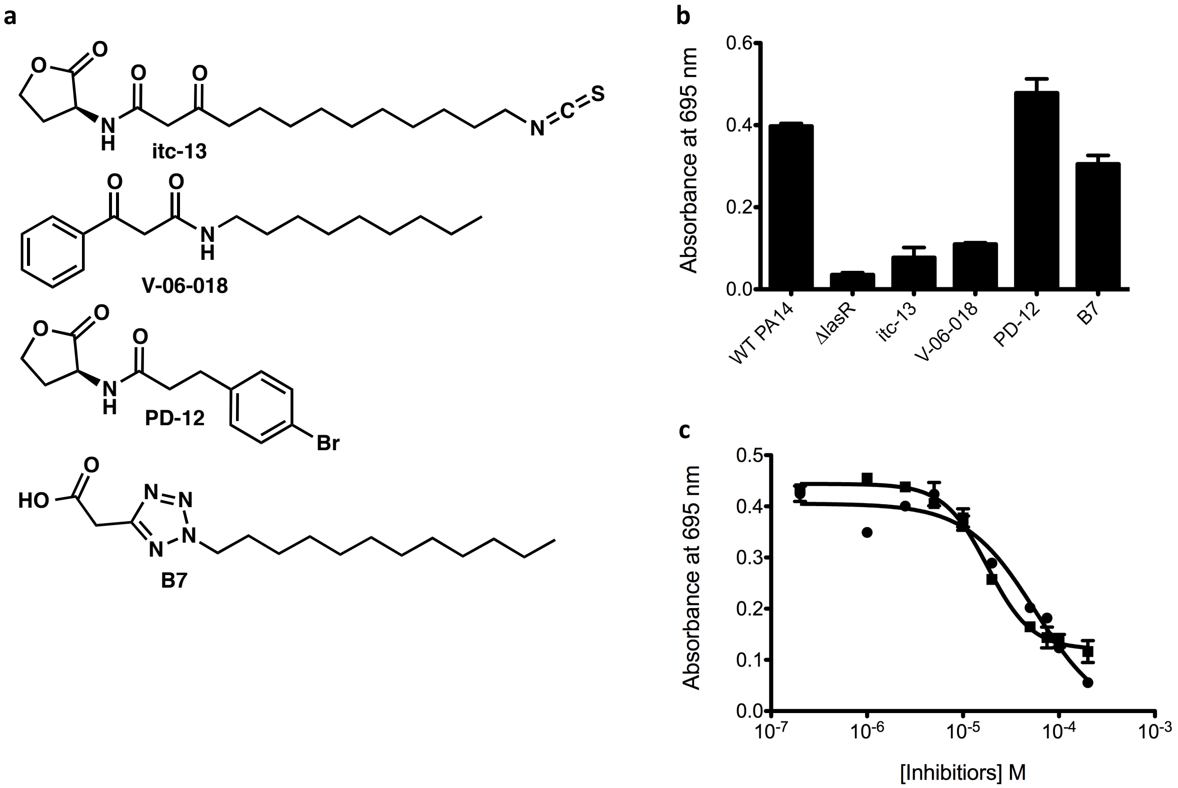


Fig. S2: Inhibition of pyocyanin production in *P. aeruginosa* PA14 by previously reported LasR inhibitors

a. Structures of four previously published LasR inhibitors (1-3). **b.** Pyocyanin inhibition assays were performed with 100 μM of each of the four molecules in panel a. on WT *P. aeruginosa* PA14. **c.** Pyocyanin inhibition titration assays were performed with itc-13 (circles) and V-06-018 (squares) at the concentrations shown using WT *P. aeruginosa* PA14. Error bars represent S.D. of three replicates.

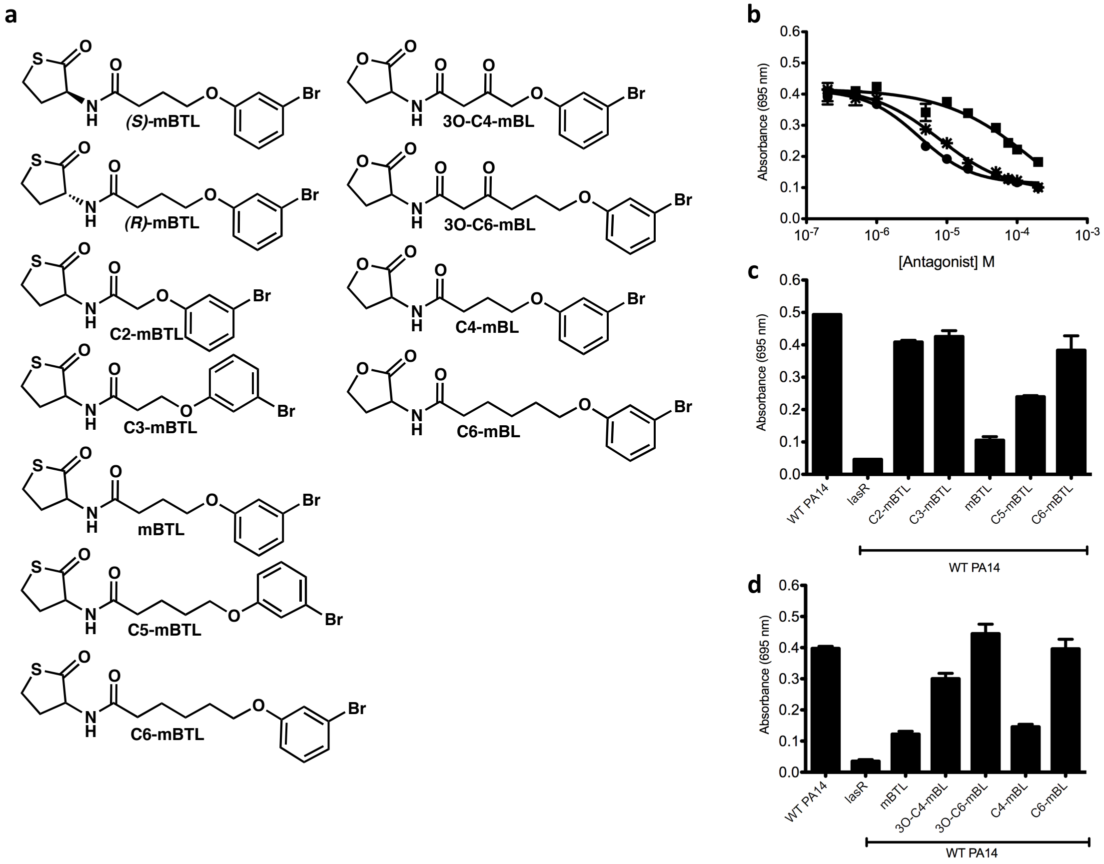


Fig. S3: Structure-function analysis of mBTL and closely related derivatives

a. Structures of mBTL and derivatives tested for inhibition of pyocyanin production. **b.** Pyocyanin inhibition titrations were performed with the (*R*) (squares) and (*S*) (circles) enantiomers of mBTL and the racemic mixture (asterisks). **c.** mBTL derivatives containing 2-6 carbon chains were tested at 100 μM . **d.** Lactone variants were examined at 100 μM . In all panels, error bars indicate S.D. of three replicates.

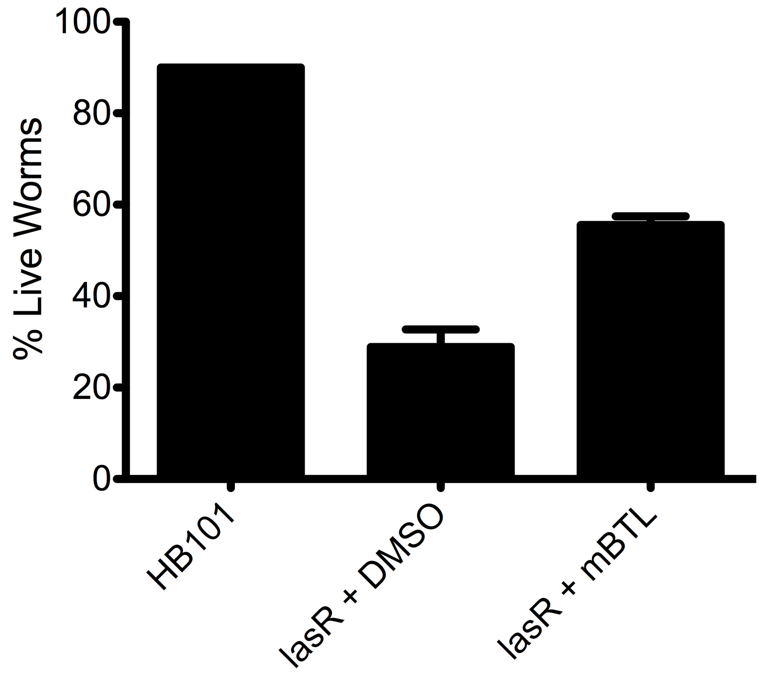


Fig. S4: mBTL rescues *C. elegans* from killing by the *P. aeruginosa* PA14 *lasR* strain

The % living worms was scored on plates containing the *lasR P. aeruginosa* mutant in the presence of 100 μM mBTL and compared to plates containing DMSO after 5 hr. HB101 denotes the *E. coli* control strain. Error bars represent S.E.M. of three replicates.

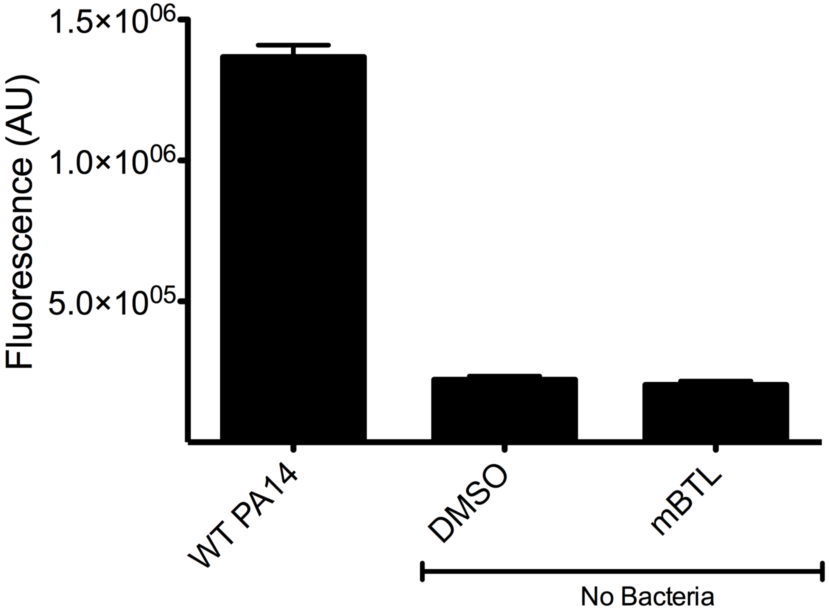


Fig. S5: mBTL is not cytotoxic to A549 human lung cells
A549 human lung cells were treated with 100 μM mBTL or an equivalent amount of DMSO in the absence of bacteria. Fluorescence from propidium iodide uptake was measured after 8 hr. Error bars represent S.E.M. of six replicates. Lung cells treated with WT *P. aeruginosa* PA14 show the maximum level of propidium iodide uptake.

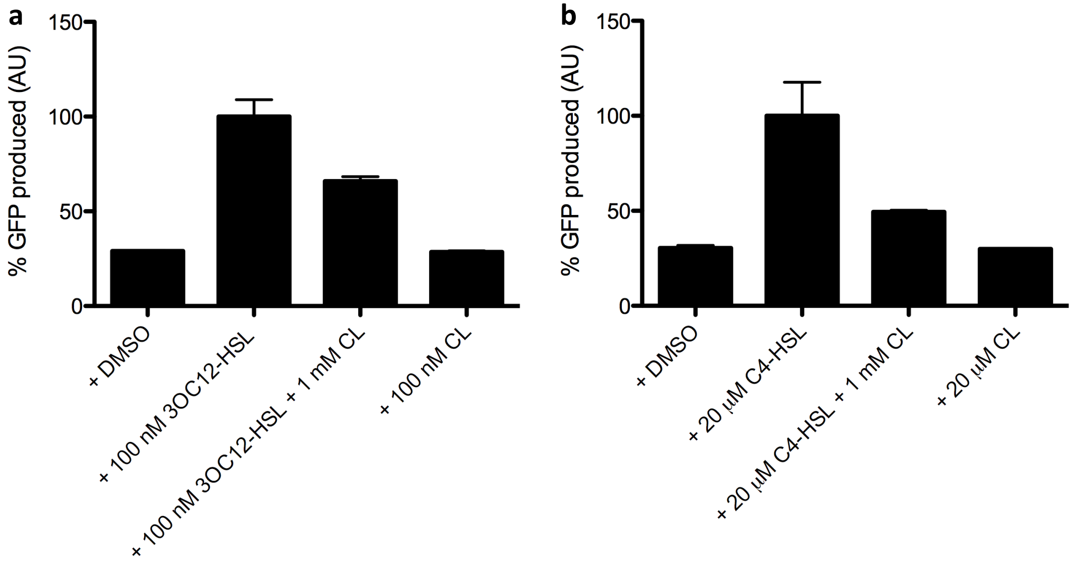


Fig. S6: CL inhibits LasR- and RhlR-controlled transcription in *E. coli*

a. LasR activation of expression of *rsaL-gfp* in *E. coli*. **b.** RhlR activation of expression of *rhlA-gfp* in *E. coli*. In each panel, *gfp* expression in the presence of the cognate autoinducer (100 nM 3OC12-HSL or 20 μM C4-HSL) is set to 100%. CL was tested for inhibition at 1 mM and agonism at 100 nM for LasR and 20 μM for RhlR. Error bars represent S.D. of three replicates.

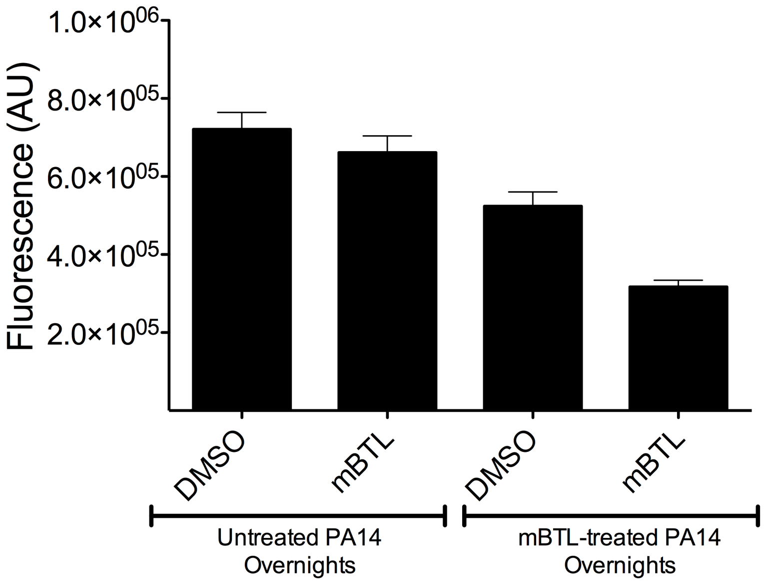


Fig. S7: mBTL functions as a prophylactic

Pelleted *P. aeruginosa* PA14 cells that had been grown for 17 hr in the presence of 100 μM mBTL or an equivalent amount of DMSO were washed, resuspended in PBS, and added to human lung A549 cells. An additional 100 μM mBTL or an equivalent amount of DMSO was added at the start of infection. Cell death was measured using fluorescence from propidium iodide uptake. Error bars indicate S.E.M. of six replicates.

Table S1. Genes down-regulated by mBTL in the wild-type strain determined through microarray analysis. The ratio of gene expression in untreated cells to that of mBTL treated cells (fold down-regulation) is displayed for genes that exhibited changes greater than 2-fold. Additional columns indicate the genes that are also downregulated in the lasR and rhlR null strains. Ratios are the average of three independent experiments.

Gene locus	Gene name	Description	Ratio	LasR-regulated	RhlR-regulated
PA14_20020	*hasAp*	heme acquisition protein HasAp	13.6	*	*
PA14_21530		ankyrin domain-containing protein	9.2	*	*
PA14_54840		tRNA-Gly	7.6	*	*
PA14_53300		alkyl hydroperoxide reductase	7.5	*	*
PA14_30720		tRNA-Cys	7.0	*	*
PA14_39420		hypothetical protein	6.6	*	*
PA14_07470		tRNA-Met	6.5	*	*
PA14_03090		hypothetical protein	6.5	*	*
PA14_39960	*phzB2*	phenazine biosynthesis protein	6.2		*
PA14_26990		hypothetical protein	5.9	*	*
PA14_14570		tRNA-Leu	5.7	*	*
PA14_58710		tRNA-Thr	5.7	*	*
PA14_27190		tRNA-Ser	5.6	*	*
PA14_39970	*phzA2*	phenazine biosynthesis protein	5.6		*
PA14_30420		tRNA-Ser	5.5	*	*
PA14_62790		tRNA-Met	5.5	*	*
PA14_51830		DNA-binding stress protein	5.5	*	*
PA14_52320		tRNA-Met	5.5	*	*
PA14_24870		tRNA-Pro	5.4	*	*
PA14_51230		tRNA-Ser	5.4	*	*
PA14_09150	*katA*	catalase	5.3	*	*
PA14_68150		tRNA-Thr	5.0	*	*
PA14_06810	*norC*	nitric-oxide reductase subunit C	4.9	*	*
PA14_61830		tRNA-Met	4.9	*	*
PA14_60160		tRNA-Pro	4.8	*	*
PA14_22320		hypothetical protein	4.8	*	*
PA14_18590		hypothetical protein	4.7	*	*
PA14_51420	*pqsB*	PqsB	4.6		
PA14_41330		tRNA-His	4.5	*	*
PA14_27330		phospho-2-dehydro-3-deoxyheptonate aldolase	4.3	*	*
PA14_28190		tRNA-Val	4.1	*	*
PA14_05860		hypothetical protein	4.0	*	*
PA14_18800		hypothetical protein	4.0	*	*
PA14_06830	*norB*	nitric-oxide reductase subunit B	3.9	*	*
PA14_23570		tRNA-Ala	3.8	*	*
PA14_06875		rsmYregulatoryRNA	3.7	*	*
PA14_01490		hemolysin	3.7		*
PA14_48060	*aprA*	alkaline metalloproteinase	3.6	*	*
PA14_28660	*infC*	translation initiation factor IF-3	3.5		
PA14_01710	*ahpC*	alkyl hydroperoxide reductase	3.4	*	*
PA14_36330	*hcnA*	hydrogen cyanide synthase HcnA	3.4		*

PA14_20500		tRNA-Arg	3.4	*	*
PA14_51410	*pqsC*	PqsC	3.3		
PA14_28740		tRNA-Pro	3.3		*
PA14_13950		hypothetical protein	3.2		
PA14_50290	*fliC*	flagellin type B	3.2		
PA14_08900	*rplV*	50S ribosomal protein L22	3.2		
PA14_08220		hypothetical protein	3.2		
PA14_58040		hypothetical protein	3.1	*	*
PA14_15990	*trmD*	tRNA (guanine-N(1)-)-methyltransferase	3.1		
PA14_01720	*ahpF*	alkyl hydroperoxide reductase	3.1	*	*
PA14_51610		hypothetical protein	3.1		
PA14_53290	*trxB2*	thioredoxin reductase 2	3.1	*	*
PA14_09470	*phzB1*	phenazine biosynthesis protein	3.1		*
PA14_26980		hypothetical protein	3.0	*	*
PA14_63460		tRNA-Sec	3.0		*
PA14_41340		tRNA-Arg	3.0	*	*
PA14_28720	*ihfA*	integration host factor subunit alpha	3.0		
PA14_08930	*rpmC*	50S ribosomal protein L29	3.0		
PA14_59230		colicin immunity protein	3.0		
PA14_39470		hypothetical protein	3.0		
PA14_69200	*trxA*	thioredoxin	3.0	*	*
PA14_61040	*katB*	catalase	2.9	*	*
PA14_60480		hypothetical protein	2.9		
PA14_08880	*rplB*	50S ribosomal protein L2	2.9		
PA14_14560		hypothetical protein	2.9		
PA14_08660		tRNA-Gly	2.9	*	*
PA14_36320	*hcnB*	hydrogen cyanide synthase HcnB	2.8		*
PA14_03410		tRNA-Arg	2.8	*	*
PA14_28670	*rpmI*	50S ribosomal protein L35	2.8		
PA14_53610		hypothetical protein	2.8		
PA14_09130	*rplQ*	50S ribosomal protein L17	2.7		
PA14_07680		hypothetical protein	2.7		
PA14_41320		tRNA-Leu	2.7	*	
PA14_68030		tRNA-Phe	2.7	*	*
PA14_62680		hypothetical protein	2.7		
PA14_38180		hypothetical protein	2.7		
PA14_10230	*adh*	2,3-butanediol dehydrogenase	2.6		
PA14_58010		hypothetical protein	2.6		
PA14_20290		DNA binding-protein	2.6		
PA14_09080	*rpsM*	30S ribosomal protein S13	2.6		
PA14_49520	*pyoS3A*	pyocin killing protein	2.6		
PA14_55940		hypothetical protein	2.6	*	*
PA14_70190	*rpmB*	50S ribosomal protein L28	2.6		
PA14_09540	*mexG*	hypothetical protein	2.6		*
PA14_58000	*sodM*	superoxide dismutase	2.5		
PA14_51380	*pqsE*	quinolone signal response protein	2.5		
PA14_35840		hypothetical protein	2.5		
PA14_18690		peroxidase	2.5		

PA14_49030		hypothetical protein	2.5		
PA14_28680	*rplT*	50S ribosomal protein L20	2.5		
PA14_48140		hypothetical protein	2.5	*	*
PA14_23410	*orfJ*	glycosyl transferase family protein	2.5		
PA14_58030	*fumC*	fumarate hydratase	2.5		
PA14_18070		periplasmic metal-binding protein	2.5	*	*
PA14_51390	*pqsD*	3-oxoacyl-ACP synthase	2.5		
PA14_23330	*rpsA*	30S ribosomal protein S1	2.5		
PA14_24650	*rmf*	ribosome modulation factor	2.5		
PA14_14300		zinc-binding oxidoreductase	2.5		
PA14_49470	*nrdB*	ribonucleotide-diphosphate reductase subunit beta	2.5		
PA14_57460		cell division protein MraZ	2.5		
PA14_08820	*fusA1*	elongation factor G	2.4		
PA14_17480	*rpoS*	RNA polymerase sigma factor RpoS	2.4	*	
PA14_09090	*rpsK*	30S ribosomal protein S11	2.4		
PA14_35160		hypothetical protein	2.4		*
PA14_62690		hypothetical protein	2.4	*	
PA14_19590		molybdopterin-binding protein	2.4		*
PA14_57950		hypothetical protein	2.4		
PA14_23360	*wzz*	O-antigen chain length regulator	2.4		
PA14_20050		outer membrane protein	2.4	*	
PA14_41230	*clpX*	ATP-dependent protease ATP-binding subunit ClpX	2.4		
PA14_59430		hypothetical protein	2.4		
PA14_50880		hypothetical protein	2.4		
PA14_06750	*nirS*	nitrite reductase	2.4	*	*
PA14_49510	*pyoS3I*	immunity protein S3I structureal gene	2.4		
PA14_64500		transcriptional regulator	2.4	*	*
PA14_28780		hypothetical protein	2.3		
PA14_30210	*clpS*	ATP-dependent Clp protease adaptor protein ClpS	2.3		
PA14_69370	*algP*	alginate regulatory protein AlgP	2.3		
PA14_41570	*oprF*	major porin and structural outer membrane porin OprF precursor	2.3		
PA14_09270	*pchE*	dihydroaeruginoic acid synthetase	2.3		*
PA14_08790	*rpsL*	30S ribosomal protein S12	2.3		
PA14_35760		hypothetical protein	2.3		
PA14_53590		hypothetical protein	2.3		
PA14_19110	*rhlB*	rhamnosyltransferase chain B	2.3		*
PA14_13940		S-type pyocin protein	2.3		
PA14_35850		hypothetical protein	2.3		
PA14_08810	*rpsG*	30S ribosomal protein S7	2.3		
PA14_09340	*fptA*	Fe(III)-pyochelin outer membrane receptor	2.3		
PA14_65180	*rpsF*	30S ribosomal protein S6	2.2		
PA14_48040	*aprI*	alkaline proteinase inhibitor AprI	2.2	*	*
PA14_68940		hypothetical protein	2.2		*
PA14_51430	*pqsA*	coenzyme A ligase	2.2		
PA14_29570		hypothetical protein	2.2		
PA14_08720	*rplK*	50S ribosomal protein L11	2.2		
PA14_08910	*rpsC*	30S ribosomal protein S3	2.2		

PA14_59400		hypothetical protein	2.2		
PA14_09210	*pchA*	salicylate biosynthesis isochorismate synthase	2.2		
PA14_59610		hypothetical protein	2.2		
PA14_59190		hypothetical protein	2.2		
PA14_14750		iron-binding protein IscA	2.2		*
PA14_56070	*mvaT*	transcriptional regulator MvaT, P16 subunit	2.2		
PA14_08690		tRNA-Trp	2.2		*
PA14_19100	*rhlA*	rhamnosyltransferase chain A	2.2		*
PA14_33750		outer membrane protein	2.2	*	*
PA14_08840	*rpsJ*	30S ribosomal protein S10	2.2		
PA14_45960	*lasR*	transcriptional regulator LasR	2.2	*	
PA14_60960		hypothetical protein	2.2		
PA14_68340	*arcB*	ornithine carbamoyltransferase	2.2		
PA14_19490		antioxidant protein	2.2		*
PA14_61760		tRNA-Gln	2.2	*	*
PA14_25630	*rpmF*	50S ribosomal protein L32	2.2		
PA14_23400		hypothetical protein	2.2		
PA14_70180	*rpmG*	50S ribosomal protein L33	2.2		
PA14_51810		hypothetical protein	2.2		
PA14_08730	*rplA*	50S ribosomal protein L1	2.2		
PA14_23390	*orfE*	polysaccharide biosynthesis protein	2.2		
PA14_30190	*icd*	isocitrate dehydrogenase	2.2		
PA14_15850		hypothetical protein	2.2		
PA14_10560		hypothetical protein	2.2		*
PA14_23340	*ihfB*	integration host factor subunit beta	2.2		
PA14_53070	*hpd*	4-hydroxyphenylpyruvate dioxygenase	2.2		
PA14_20010	*hasR*	heme uptake outer membrane receptor HasR	2.2		
PA14_41250	*tig*	trigger factor	2.2		
PA14_08850	*rplC*	50S ribosomal protein L3	2.2		
PA14_08960	*rplX*	50S ribosomal protein L24	2.2		
PA14_51350	*phnB*	anthranilate synthase component II	2.2		
PA14_09115	*rpoA*	DNA-directed RNA polymerase subunit alpha	2.2		
PA14_59390		hypothetical protein	2.1		*
PA14_67190		hypothetical protein	2.1		
PA14_37520		hypothetical protein	2.1		
PA14_61380		hypothetical protein	2.1	*	
PA14_17440	*truD*	tRNA pseudouridine synthase D	2.1		
PA14_20530		hypothetical protein	2.1		
PA14_08890	*rpsS*	30S ribosomal protein S19	2.1		
PA14_14730	*iscS*	cysteine desulfurase	2.1		
PA14_73300	*atpE*	F0F1 ATP synthase subunit C	2.1		
PA14_54490		hypothetical protein	2.1	*	
PA14_09480	*phzA1*	phenazine biosynthesis protein	2.1		
PA14_12260		hypothetical protein	2.1		
PA14_59620		hypothetical protein	2.1		
PA14_28710	*pheT*	phenylalanyl-tRNA synthetase subunit beta	2.1		
PA14_20610	*lecB*	fucose-binding lectin PA-IIL	2.1		*
PA14_08970	*rplE*	50S ribosomal protein L5	2.1		

PA14_56780	*sodB*	superoxide dismutase	2.1		
PA14_31350		hypothetical protein	2.1		*
PA14_16830		hypothetical protein	2.1		
PA14_52990	*phhA*	phenylalanine 4-monooxygenase	2.1		
PA14_49200	*oprH*	PhoP/Q and low Mg2+ inducible outer membrane prote	2.1		
PA14_53340		hypothetical protein	2.1		
PA14_49860		hypothetical protein	2.1		
PA14_08750	*rplL*	50S ribosomal protein L7/L12	2.1		
PA14_13460		RNA polymerase sigma factor	2.1	*	*
PA14_07480		reverse transcriptase	2.0		
PA14_08870	*rplW*	50S ribosomal protein L23	2.0		
PA14_09220	*pchB*	isochorismate-pyruvate lyase	2.0		
PA14_59590		hypothetical protein	2.0		
PA14_62720	*rpsO*	30S ribosomal protein S15	2.0	*	*
PA14_66875	*phaF*	polyhydroxyalkanoate synthesis protein PhaF	2.0		
PA14_04300		hypothetical protein	2.0		
PA14_37420		transmembrane sensor protein	2.0		
PA14_14740		scaffold protein	2.0		*
PA14_55810		two-component response regulator	2.0		
PA14_41210	*hupB*	DNA-binding protein HU	2.0		*
PA14_10490		hypothetical protein	2.0		*
PA14_16910		hypothetical protein	2.0		
PA14_57010	*groEL*	chaperonin GroEL	2.0		
PA14_02260		two-component response regulator	2.0	*	
PA14_50280		hypothetical protein	2.0		
PA14_09000	*rplF*	50S ribosomal protein L6	2.0		
PA14_66620	*pilQ*	type 4 fimbrial biogenesis outer membrane protein PilQ precursor	2.0		
PA14_59380		hypothetical protein	2.0		
PA14_09020	*rpsE*	30S ribosomal protein S5	2.0		

Supplemental Table 2. Genes down-regulated by disruption of the *lasR* gene determined through microarray analysis. The ratio of gene expression of the wild-type strain to that of the *lasR* null strain (fold down-regulation) is displayed for genes that exhibited changes greater than 2-fold. Ratios are the average of three independent experiments.

Gene locus	Gene name	Description	Ratio
PA14_45960	*lasR*	transcriptional regulator LasR	81.7
PA14_06810	*norC*	nitric-oxide reductase subunit C	74.5
PA14_22320		hypothetical protein	52.0
PA14_20020	*hasAp*	heme acquisition protein HasAp	34.9
PA14_21530		ankyrin domain-containing protein	33.1
PA14_33290		hypothetical protein	28.1
PA14_45950	*rsaL*	regulatory protein RsaL	23.4
PA14_01720	*ahpF*	alkyl hydroperoxide reductase	23.3
PA14_26990		hypothetical protein	21.5
PA14_53290	*trxB2*	thioredoxin reductase 2	20.9
PA14_09150	*katA*	catalase	16.7
PA14_03090		hypothetical protein	15.8
PA14_53300		alkyl hydroperoxide reductase	15.3
PA14_06830	*norB*	nitric-oxide reductase subunit B	15.3
PA14_40260		hypothetical protein	12.8
PA14_01710	*ahpC*	alkyl hydroperoxide reductase	12.8
PA14_52320		tRNA-Met	12.4
PA14_62790		tRNA-Met	12.3
PA14_10380		hypothetical protein	11.8
PA14_58710		tRNA-Thr	11.2
PA14_39420		hypothetical protein	11.1
PA14_06875		rsmYregulatoryRNA	11.1
PA14_68150		tRNA-Thr	11.0
PA14_18590		hypothetical protein	9.7
PA14_51230		tRNA-Ser	8.7
PA14_30420		tRNA-Ser	8.7
PA14_05860		hypothetical protein	8.6
PA14_72370		hypothetical protein	7.9
PA14_27330		phospho-2-dehydro-3-deoxyheptonate aldolase	7.5
PA14_55940		hypothetical protein	7.3
PA14_20500		tRNA-Arg	7.3
PA14_07470		tRNA-Met	7.2
PA14_39780		hypothetical protein	6.8
PA14_48060	*aprA*	alkaline metalloproteinase	6.4
PA14_54840		tRNA-Gly	6.3
PA14_28190		tRNA-Val	6.2
PA14_41340		tRNA-Arg	5.9
PA14_24870		tRNA-Pro	5.7
PA14_51830		DNA-binding stress protein	5.7
PA14_18800		hypothetical protein	5.6
PA14_61040	*katB*	catalase	5.4
PA14_14570		tRNA-Leu	5.2

PA14_09900	*prpL*	Pvds-regulated endoprotease, lysyl class	5.1
PA14_26980		hypothetical protein	5.0
PA14_03410		tRNA-Arg	4.8
PA14_52560		tRNA-Ser	4.7
PA14_60160		tRNA-Pro	4.4
PA14_23570		tRNA-Ala	4.1
PA14_64500		transcriptional regulator	4.0
PA14_36820		hypothetical protein	4.0
PA14_08670		tRNA-Thr	4.0
PA14_41330		tRNA-His	4.0
PA14_40240		ABC transporter ATP-binding protein/permease	4.0
PA14_36560		hypothetical protein	3.9
PA14_01290	*coxB*	cytochrome c oxidase subunit II	3.9
PA14_27190		tRNA-Ser	3.8
PA14_48040	*aprI*	alkaline proteinase inhibitor AprI	3.8
PA14_40230		secretion protein	3.8
PA14_16720		hypothetical protein	3.7
PA14_35340		2-ketogluconate kinase	3.7
PA14_61380		hypothetical protein	3.7
PA14_28600		hypothetical protein	3.6
PA14_48610		sparagine synthase	3.6
PA14_72360		hypothetical protein	3.6
PA14_61760		tRNA-Gln	3.5
PA14_13390		hypothetical protein	3.5
PA14_17980	*glpF*	glycerol uptake facilitator protein	3.5
PA14_22100		hypothetical protein	3.5
PA14_01780		nucleoside 2-deoxyribosyltransferase	3.5
PA14_49130	*dctA*	C4-dicarboxylate transporter DctA	3.4
PA14_72060		hypothetical protein	3.3
PA14_24860	*snr1*	cytochrome c Snr1	3.3
PA14_46750		hypothetical protein	3.3
PA14_62720	*rpsO*	30S ribosomal protein S15	3.2
PA14_01300	*coxA*	cytochrome c oxidase subunit I	3.2
PA14_28620		hypothetical protein	3.2
PA14_56910		hypothetical protein	3.1
PA14_66880		hypothetical protein	3.1
PA14_55790		hypothetical protein	3.0
PA14_06750	*nirS*	nitrite reductase	3.0
PA14_61830		tRNA-Met	3.0
PA14_01320	*coIII*	cytochrome c oxidase subunit III	2.9
PA14_01350		hypothetical protein	2.9
PA14_00300	*plcB*	phospholipase C, PlcB	2.8
PA14_62810	*secG*	preprotein translocase subunit SecG	2.7
PA14_22880		Fe-S protein	2.7
PA14_13350		hypothetical protein	2.7
PA14_13460		RNA polymerase sigma factor	2.7
PA14_62870	*rrmJ*	cell division protein FtsJ	2.7
PA14_68030		tRNA-Phe	2.7

PA14_69200	*trxA*	thioredoxin	2.7
PA14_53250	*cpbD*	chitin-binding protein CbpD	2.7
PA14_58040		hypothetical protein	2.6
PA14_57030	*fxsA*	FxsA protein	2.6
PA14_08660		tRNA-Gly	2.6
PA14_53630		hypothetical protein	2.6
PA14_17480	*rpoS*	RNA polymerase sigma factor RpoS	2.6
PA14_33050		hypothetical protein	2.6
PA14_54490		hypothetical protein	2.5
PA14_02500	*exbB1*	transport protein ExbB	2.5
PA14_20050		outer membrane protein	2.5
PA14_62860	*ftsH*	cell division protein FtsH	2.5
PA14_06770	*nirQ*	regulatory protein NirQ	2.5
PA14_53840		hypothetical protein	2.5
PA14_38550	*maiA*	maleylacetoacetate isomerase	2.5
PA14_00790	*prlC*	oligopeptidase A	2.5
PA14_18070		periplasmic metal-binding protein	2.5
PA14_07890		ABC transporter permease	2.4
PA14_38825	*pqqA*	coenzyme PQQ synthesis protein PqqA	2.4
PA14_55920		type II secretion system protein	2.4
PA14_13380		hypothetical protein	2.4
PA14_20770		hypothetical protein	2.4
PA14_22310		hypothetical protein	2.4
PA14_63170		transcriptional regulator	2.4
PA14_47190	*cyoB*	cytochrome o ubiquinol oxidase subunit I	2.3
PA14_02510	*exbD1*	transport protein ExbD	2.3
PA14_67770	*pgm*	phosphoglyceromutase	2.3
PA14_62690		hypothetical protein	2.3
PA14_26020		aminopeptidase	2.3
PA14_61060		oxidoreductase	2.3
PA14_30720		tRNA-Cys	2.3
PA14_48140		hypothetical protein	2.3
PA14_36920		hypothetical protein	2.3
PA14_25180	*psrA*	transcriptional regulator PsrA	2.3
PA14_58070		hypothetical protein	2.3
PA14_53160		hypothetical protein	2.3
PA14_42200		hypothetical protein	2.3
PA14_35980		acyl-CoA dehydrogenase	2.3
PA14_49050		hypothetical protein	2.3
PA14_12130	*lis*	lipoyl synthase	2.3
PA14_40250		outer membrane protein	2.3
PA14_60750	*pra*	protein activator	2.3
PA14_05020		hypothetical protein	2.2
PA14_13370		hypothetical protein	2.2
PA14_23680	*ibpA*	heat-shock protein IbpA	2.2
PA14_20480		hypothetical protein	2.2
PA14_11510	*ribA*	GTP cyclohydrolase II	2.2
PA14_53210		hypothetical protein	2.2

PA14_41320		tRNA-Leu	2.2
PA14_35360		hypothetical protein	2.1
PA14_36980		hypothetical protein	2.1
PA14_72870		aminotransferase	2.1
PA14_20780		two-component response regulator	2.1
PA14_63480		amino acid permease	2.1
PA14_54430	*algU*	RNA polymerase sigma factor AlgU	2.1
PA14_33060		hypothetical protein	2.1
PA14_28140		hypothetical protein	2.1
PA14_72170		citrate transporter	2.1
PA14_53040		lysozyme inhibitor	2.1
PA14_11610		ABC transporter permease	2.1
PA14_02260		two-component response regulator	2.1
PA14_61020		hypothetical protein	2.1
PA14_19870	*ldh*	leucine dehydrogenase	2.1
PA14_72970	*tonB*	TonB protein	2.1
PA14_33750		outer membrane protein	2.1
PA14_34330		hypothetical protein	2.1
PA14_00480		hypothetical protein	2.1
PA14_60500	*fklB*	peptidyl-prolyl cis-trans isomerase FklB	2.1
PA14_09700		monooxygenase	2.1
PA14_16250	*lasB*	elastase LasB	2.0
PA14_49460	*nrdA*	ribonucleotide-diphosphate reductase subunit alpha	2.0
PA14_72140		hypothetical protein	2.0
PA14_35370	*ptxS*	transcriptional regulator PtxS	2.0
PA14_66550	*hemE*	uroporphyrinogen decarboxylase	2.0
PA14_12030		hypothetical protein	2.0
PA14_70490		lipoprotein	2.0
PA14_38060		hypothetical protein	2.0
PA14_73230	*atpC*	F0F1 ATP synthase subunit epsilon	2.0
PA14_72770		hypothetical protein	2.0
PA14_47530		hypothetical protein	2.0
PA14_66400		potassium efflux protein KefA	2.0
PA14_37410		hypothetical protein	2.0
PA14_44700	*alkB2*	alkane-1 monooxygenase	2.0

Supplemental Table 3. Genes down-regulated by disruption of the *rhlR* gene determined through microarray analysis. The ratio of gene expression of the wild-type strain to that of the *rhlR* null strain (fold down-regulation) is displayed for genes that exhibited changes greater than 2-fold. Ratios are the average of three independent experiments.

Gene locus	Gene name	Description	Ratio
PA14_10360		hypothetical protein	54.5
PA14_22320		hypothetical protein	47.2
PA14_34870	*chiC*	chitinase	44.6
PA14_15350		integrase	43.8
PA14_18800		hypothetical protein	42.7
PA14_15600		hypothetical protein	35.7
PA14_01720	*ahpF*	alkyl hydroperoxide reductase	34.7
PA14_53250	*cpbD*	chitin-binding protein CbpD	33.7
PA14_21530		ankyrin domain-containing protein	32.7
PA14_20610	*lecB*	fucose-binding lectin PA-IIL	29.6
PA14_53290	*trxB2*	thioredoxin reductase 2	26.8
PA14_19100	*rhlA*	rhamnosyltransferase chain A	24.4
PA14_06810	*norC*	nitric-oxide reductase subunit C	23.6
PA14_01490		hemolysin	23.2
PA14_15480	*merR*	transcriptional regulator MerR	23.1
PA14_15610		hypothetical protein	22.7
PA14_26990		hypothetical protein	22.6
PA14_53300		alkyl hydroperoxide reductase	19.6
PA14_11140		nonribosomal peptide synthetase	18.4
PA14_09540	*mexG*	hypothetical protein	16.3
PA14_01710	*ahpC*	alkyl hydroperoxide reductase	16.0
PA14_40310		acyl carrier protein	15.9
PA14_03090		hypothetical protein	15.6
PA14_28360		hypothetical protein	15.2
PA14_38270		hypothetical protein	15.1
PA14_37745		carbamoyl transferase	14.3
PA14_16250	*lasB*	elastase LasB	14.2
PA14_06830	*norB*	nitric-oxide reductase subunit B	13.9
PA14_09150	*katA*	catalase	13.6
PA14_15560		hypothetical protein	11.1
PA14_51230		tRNA-Ser	10.5
PA14_09530	*mexH*	RND efflux membrane fusion protein	10.3
PA14_05860		hypothetical protein	9.9
PA14_54840		tRNA-Gly	9.8
PA14_03510		hypothetical protein	9.7
PA14_68940		hypothetical protein	9.7
PA14_39420		hypothetical protein	9.2
PA14_31290	*pa1L*	PA-I galactophilic lectin	9.0
PA14_19590		molybdopterin-binding protein	8.7
PA14_16100		hypothetical protein	8.4
PA14_68150		tRNA-Thr	8.2
PA14_52320		tRNA-Met	8.1
PA14_62790		tRNA-Met	7.7
PA14_15520	*trbJ*	conjugal transfer protein TrbJ	7.3
PA14_40290	*lasA*	LasA protease	7.2
PA14_30720		tRNA-Cys	7.1
PA14_58710		tRNA-Thr	6.6
PA14_03520		hypothetical protein	6.4
PA14_31360		hypothetical protein	6.4

PA14_24870		tRNA-Pro	6.2
PA14_15580		Type II restriction enzyme, methylase subunit	6.2
PA14_32950		hypothetical protein	6.1
PA14_51830		DNA-binding stress protein	6.1
PA14_53840		hypothetical protein	6.0
PA14_18590		hypothetical protein	5.8
PA14_34880		GntR family transcriptional regulator	5.6
PA14_28190		tRNA-Val	5.5
PA14_49760	*rhlC*	rhamnosyltransferase 2	5.5
PA14_10490		hypothetical protein	5.4
PA14_68210	*rmlC*	dTDP-4-dehydrorhamnose 3,5-epimerase	5.4
PA14_59390		hypothetical protein	5.3
PA14_30420		tRNA-Ser	5.3
PA14_21030		ATP-dependent Clp protease proteolytic subunit	5.3
PA14_09520	*mexI*	RND efflux transporter	5.2
PA14_41330		tRNA-His	5.2
PA14_55940		hypothetical protein	5.1
PA14_63210		two-component response regulator	5.0
PA14_20500		tRNA-Arg	4.9
PA14_27330		phospho-2-dehydro-3-deoxyheptonate aldolase	4.9
PA14_16720		hypothetical protein	4.9
PA14_34750		taurine catabolism dioxygenase	4.9
PA14_15400		replication protein, RepC	4.9
PA14_23570		tRNA-Ala	4.9
PA14_49130	*dctA*	C4-dicarboxylate transporter DctA	4.8
PA14_20020	*hasAp*	heme acquisition protein HasAp	4.7
PA14_15360		hypothetical protein	4.7
PA14_56590		hypothetical protein	4.6
PA14_15460	*merA*	mercuric reductase	4.6
PA14_48040	*aprI*	alkaline proteinase inhibitor AprI	4.5
PA14_27190		tRNA-Ser	4.5
PA14_61040	*katB*	catalase	4.4
PA14_68190	*rmlD*	dTDP-4-dehydrorhamnose reductase	4.3
PA14_11130		short chain dehydrogenase	4.3
PA14_36330	*hcnA*	hydrogen cyanide synthase HcnA	4.3
PA14_31350		hypothetical protein	4.2
PA14_41340		tRNA-Arg	4.2
PA14_36320	*hcnB*	hydrogen cyanide synthase HcnB	4.2
PA14_52560		tRNA-Ser	4.1
PA14_09500	*opmD*	outer membrane protein	4.0
PA14_19110	*rhlB*	rhamnosyltransferase chain B	4.0
PA14_61830		tRNA-Met	4.0
PA14_26980		hypothetical protein	3.9
PA14_34780		ABC transporter ATP-binding protein	3.9
PA14_09470	*phzB1*	phenazine biosynthesis protein	3.9
PA14_60160		tRNA-Pro	3.9
PA14_06875		rsmYregulatoryRNA	3.9
PA14_18680		hypothetical protein	3.9
PA14_03410		tRNA-Arg	3.8
PA14_15590		hypothetical protein	3.8
PA14_13460		RNA polymerase sigma factor	3.8
PA14_39960	*phzB2*	phenazine biosynthesis protein	3.8
PA14_43040		hypothetical protein	3.8
PA14_02500	*exbB1*	transport protein ExbB	3.8
PA14_61060		oxidoreductase	3.6
PA14_62390		hypothetical protein	3.6

PA14_15510	*traJ*	conjugal transfer relaxosome component TraJ	3.6
PA14_17980	*glpF*	glycerol uptake facilitator protein	3.6
PA14_15500		oriT-binding protein, TraK	3.6
PA14_50740		hypothetical protein	3.6
PA14_45950	*rsaL*	regulatory protein RsaL	3.5
PA14_15450	*merD*	transcriptional regulator MerD	3.5
PA14_19540		hypothetical protein	3.5
PA14_48140		hypothetical protein	3.5
PA14_40860		hypothetical protein	3.4
PA14_03490		hypothetical protein	3.4
PA14_33290		hypothetical protein	3.3
PA14_15490		hypothetical protein	3.3
PA14_68170	*rmlB*	dTDP-D-glucose 4,6-dehydratase	3.3
PA14_00640	*phzH*	potential phenazine-modifying enzyme	3.3
PA14_62860	*ftsH*	cell division protein FtsH	3.3
PA14_55580	*nemO*	heme oxygenase	3.3
PA14_69200	*trxA*	thioredoxin	3.3
PA14_35160		hypothetical protein	3.3
PA14_31170		hypothetical protein	3.3
PA14_72060		hypothetical protein	3.3
PA14_66460		hypothetical protein	3.2
PA14_14570		tRNA-Leu	3.2
PA14_07470		tRNA-Met	3.1
PA14_30620		AraC family transcriptional regulator	3.1
PA14_38825	*pqqA*	coenzyme PQQ synthesis protein PqqA	3.1
PA14_64930		hypothetical protein	3.1
PA14_61760		tRNA-Gln	3.1
PA14_10350		secretion protein	3.1
PA14_53040		lysozyme inhibitor	3.1
PA14_02510	*exbD1*	transport protein ExbD	3.0
PA14_19530		NAD(P)H-dependent FMN reductase	3.0
PA14_28050		chemotaxis transducer	3.0
PA14_72970	*tonB*	TonB protein	3.0
PA14_62810	*secG*	preprotein translocase subunit SecG	2.9
PA14_25180	*psrA*	transcriptional regulator PsrA	2.9
PA14_35170		redox-sensing activator of soxS	2.9
PA14_08670		tRNA-Thr	2.9
PA14_66550	*hemE*	uroporphyrinogen decarboxylase	2.9
PA14_58040		hypothetical protein	2.9
PA14_06750	*nirS*	nitrite reductase	2.9
PA14_41210	*hupB*	DNA-binding protein HU	2.9
PA14_00790	*prlC*	oligopeptidase A	2.8
PA14_68930		permease	2.8
PA14_62720	*rpsO*	30S ribosomal protein S15	2.8
PA14_34730		XRE family transcriptional regulator	2.8
PA14_57030	*fxsA*	FxsA protein	2.8
PA14_64500		transcriptional regulator	2.8
PA14_09490	*phzM*	phenazine-specific methyltransferase	2.8
PA14_53160		hypothetical protein	2.8
PA14_32520		hypothetical protein	2.8
PA14_14710		Rrf2 family protein	2.8
PA14_06770	*nirQ*	regulatory protein NirQ	2.7
PA14_12970	*tauD*	taurine dioxygenase	2.7
PA14_23980	*xcpP*	secretion protein XcpP	2.7
PA14_24860	*snr1*	cytochrome c Snr1	2.7
PA14_43050		hypothetical protein	2.7

PA14_38550	*maiA*	maleylacetoacetate isomerase	2.7
PA14_34770		ABC transporter substrate-binding protein	2.7
PA14_30410		hypothetical protein	2.7
PA14_19490		antioxidant protein	2.6
PA14_26780		hypothetical protein	2.6
PA14_39800		ECF subfamily RNA polymerase sigma-70 factor	2.6
PA14_30670	*pgsA*	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase	2.6
PA14_10330		outer membrane protein	2.6
PA14_06180		RNA polymerase sigma factor	2.6
PA14_12920		taurine ABC transporter periplasmic protein	2.6
PA14_28620		hypothetical protein	2.6
PA14_60030		hypothetical protein	2.6
PA14_40260		hypothetical protein	2.6
PA14_39780		hypothetical protein	2.6
PA14_36820		hypothetical protein	2.6
PA14_71900		hypothetical protein	2.5
PA14_34460		hypothetical protein	2.5
PA14_19520		ABC transporter ATP-binding protein	2.5
PA14_13210		hypothetical protein	2.5
PA14_09270	*pchE*	dihydroaeruginoic acid synthetase	2.5
PA14_08660		tRNA-Gly	2.5
PA14_06130		hypothetical protein	2.5
PA14_05020		hypothetical protein	2.5
PA14_62800		tRNA-Leu	2.5
PA14_31370		hypothetical protein	2.5
PA14_15370		hypothetical protein	2.5
PA14_60750	*pra*	protein activator	2.5
PA14_37470		flavin-dependent oxidoreductase	2.5
PA14_03080		acetyltransferase	2.4
PA14_06580		hypothetical protein	2.4
PA14_37990		RNA polymerase sigma factor	2.4
PA14_68200	*rmlA*	glucose-1-phosphate thymidylyltransferase	2.4
PA14_30550		periplasmic aliphatic sulfonate-binding protein	2.4
PA14_12130	*lis*	lipoyl synthase	2.4
PA14_58580		hydroxylase	2.4
PA14_15110		hypothetical protein	2.4
PA14_11010		hypothetical protein	2.4
PA14_00800		hypothetical protein	2.4
PA14_18070		periplasmic metal-binding protein	2.3
PA14_41930		hypothetical protein	2.3
PA14_28250		secreted acid phosphatase	2.3
PA14_09370		hypothetical protein	2.3
PA14_10560		hypothetical protein	2.3
PA14_10500		cbb3-type cytochrome c oxidase subunit I	2.3
PA14_31700		CDP-alcohol phosphatidyltransferase	2.3
PA14_72870		aminotransferase	2.3
PA14_21260		hypothetical protein	2.3
PA14_22880		Fe-S protein	2.3
PA14_26190		hypothetical protein	2.3
PA14_23090		keto-hydroxyglutarate-aldolase/keto-deoxy-phosphogluconate aldolase	2.3
PA14_37440		MFS transporter	2.3
PA14_52250		two-component response regulator	2.3
PA14_08690		tRNA-Trp	2.2
PA14_13140		hypothetical protein	2.2

PA14_16110		hypothetical protein	2.2
PA14_63460		tRNA-Sec	2.2
PA14_20770		hypothetical protein	2.2
PA14_28740		tRNA-Pro	2.2
PA14_40510	*ccoN-2*	cbb3-type cytochrome c oxidase subunit I	2.2
PA14_60540		hypothetical protein	2.2
PA14_38060		hypothetical protein	2.2
PA14_35340		2-ketogluconate kinase	2.2
PA14_39970	*phzA2*	phenazine biosynthesis protein	2.2
PA14_72550		adhesin	2.2
PA14_42200		hypothetical protein	2.2
PA14_68030		tRNA-Phe	2.2
PA14_33750		outer membrane protein	2.2
PA14_56910		hypothetical protein	2.2
PA14_41450		hypothetical protein	2.2
PA14_52130		hypothetical protein	2.2
PA14_62870	*rrmJ*	cell division protein FtsJ	2.2
PA14_52690	*aruG*	arginine/ornithine succinyltransferase AII subunit	2.2
PA14_36310	*hcnC*	hydrogen cyanide synthase HcnC	2.2
PA14_49800		oxidoreductase	2.2
PA14_34820		regulatory protein	2.1
PA14_43520		hypothetical protein	2.1
PA14_38220		hypothetical protein	2.1
PA14_15570		hypothetical protein	2.1
PA14_40240		ABC transporter ATP-binding protein/permease	2.1
PA14_31050		hypothetical protein	2.1
PA14_47190	*cyoB*	cytochrome o ubiquinol oxidase subunit I	2.1
PA14_34830		regulatory protein	2.1
PA14_14750		iron-binding protein IscA	2.1
PA14_58690		hypothetical protein	2.1
PA14_12940		taurine ABC transporter ATP-binding protein	2.1
PA14_68070		periplasmic binding protein	2.1
PA14_19120	*rhlR*	transcriptional regulator RhlR	2.1
PA14_40630		hypothetical protein	2.1
PA14_37770		hydrolase	2.1
PA14_02530		hypothetical protein	2.1
PA14_64940		hypothetical protein	2.1
PA14_09290	*pchG*	pyochelin biosynthetic protein PchG	2.1
PA14_06170		transmembrane sensor	2.0
PA14_48060	*aprA*	alkaline metalloproteinase	2.0
PA14_15540		mating pair formation protein TrbL	2.0
PA14_34510		hypothetical protein	2.0
PA14_65320	*miaA*	tRNA delta(2)-isopentenylpyrophosphate transferase	2.0
PA14_52700	*aruF*	arginine/ornithine succinyltransferase AI subunit	2.0
PA14_13000		transcriptional regulator	2.0
PA14_19370		asparagine synthetase	2.0
PA14_14740		scaffold protein	2.0
PA14_30630	*pqsH*	FAD-dependent monooxygenase	2.0

Supplemental Table 4. Genes up-regulated by mBTL in the wild-type strain determined through microarray analysis. The ratio of gene expression in mBTL treated cells to that of untreated cells (fold up-regulation) is displayed for genes that exhibited changes greater than 2-fold. Ratios are the average of three independent experiments.

Gene locus	Gene name	Description	Ratio
PA14_10380		hypothetical protein	2.6
PA14_31290	*pa1L*	PA-I galactophilic lectin	2.4
PA14_10370		hypothetical protein	2.3
PA14_61200		hypothetical protein	2.3
PA14_49300		lipoxygenase	2.3
PA14_10350		secretion protein	2.2
PA14_31840		hypothetical protein	2.2
PA14_52000		hypothetical protein	2.2
PA14_45310	*ccmF*	cytochrome C-type biogenesis protein CcmF	2.1
PA14_37070		hypothetical protein	2.1
PA14_31280		integrase	2.1
PA14_31890		RND efflux transporter	2.0
PA14_20640		hypothetical protein	2.0

Supplemental Table 5. Genes down-regulated by mBTL in the *lasR* mutant determined through microarray analysis. The ratio of gene expression in untreated cells to that of mBTL treated cells (fold down-regulation) is displayed for genes that exhibited changes greater than 2-fold. Ratios are the average of three independent experiments.

Gene locus	Gene name	Description	Ratio
PA14_33810	*pvdA*	L-ornithine N5-oxygenase	18.4
PA14_09490	*phzM*	phenazine-specific methyltransferase	12.8
PA14_33510		hypothetical protein	12.6
PA14_33820	*pvdQ*	penicillin acylase-related protein	11.0
PA14_33610		peptide synthase	10.9
PA14_51380	*pqsE*	quinolone signal response protein	7.8
PA14_09480	*phzA1*	phenazine biosynthesis protein	7.7
PA14_51420	*pqsB*	PqsB	6.7
PA14_09470	*phzB1*	phenazine biosynthesis protein	6.6
PA14_49720		hypothetical protein	5.7
PA14_33730		dipeptidase	5.4
PA14_51410	*pqsC*	PqsC	5.4
PA14_33280	*pvdL*	peptide synthase	5.1
PA14_33650	*pvdD*	pyoverdine synthetase D	4.6
PA14_51430	*pqsA*	coenzyme A ligase	4.5
PA14_36320	*hcnB*	hydrogen cyanide synthase HcnB	4.4
PA14_28470		hypothetical protein	4.4
PA14_51390	*pqsD*	3-oxoacyl-ACP synthase	4.2
PA14_36330	*hcnA*	hydrogen cyanide synthase HcnA	4.1
PA14_51350	*phnB*	anthranilate synthase component II	4.0
PA14_39970	*phzA2*	phenazine biosynthesis protein	3.9
PA14_39960	*phzB2*	phenazine biosynthesis protein	3.9
PA14_33690	*pvdE*	pyoverdine biosynthesis protein PvdE	3.8
PA14_09400	*phzS*	hypothetical protein	3.7
PA14_10960		ferredoxin oxidoreductase subunit	3.7
PA14_54910		thioesterase	3.6
PA14_03370		hypothetical protein	3.5
PA14_25640	*plsX*	glycerol-3-phosphate acyltransferase PlsX	3.5
PA14_21020		non-ribosomal peptide synthetase	3.5
PA14_34490		hypothetical protein	3.5
PA14_29560		hypothetical protein	3.4
PA14_33760		ABC transporter ATP-binding protein/permease	3.3
PA14_52800	*acsA*	acetyl-CoA synthetase	3.3
PA14_19710		alpha/beta hydrolase	3.3
PA14_53230		oxidoreductase	3.3
PA14_67190		hypothetical protein	3.3
PA14_02970		hypothetical protein	3.2
PA14_33630	*pvdJ*	protein PvdJ	3.1
PA14_30720		tRNA-Cys	3.1
PA14_08220		hypothetical protein	3.1
PA14_54870		hypothetical protein	3.0
PA14_55110		hypothetical protein	3.0
PA14_14560		hypothetical protein	3.0
PA14_59410		hypothetical protein	3.0
PA14_29250		hypothetical protein	2.9
PA14_55550		ECF subfamily RNA polymerase sigma-70 factor	2.9
PA14_68040		short-chain dehydrogenase	2.9
PA14_41800		transcriptional regulator	2.9
PA14_51610		hypothetical protein	2.9

PA14_55360	*exbB2*	transport protein ExbB2	2.9
PA14_51920		acylphosphatase	2.8
PA14_33270	*pvdG*	protein PvdG	2.8
PA14_34420		maltose/mannitol ABC transporter substrate-binding prot	2.8
PA14_69550		hypothetical protein	2.8
PA14_50050		MFS family transporter	2.8
PA14_63070		GntR family transcriptional regulator	2.7
PA14_48950		hypothetical protein	2.7
PA14_18350		bifunctional UDP-glucuronic acid decarboxylase/UDP-4-	2.7
PA14_32790		hypothetical protein	2.7
PA14_20330	*phnE*	phosphonate ABC transporter permease	2.7
PA14_35880		gamma-aminobutyraldehyde dehydrogenase	2.7
PA14_33700	*pvdF*	pyoverdine synthetase F	2.7
PA14_57460		cell division protein MraZ	2.7
PA14_18150		acetyl-coa synthetase	2.7
PA14_23080	*pgl*	6-phosphogluconolactonase	2.7
PA14_33250		hypothetical protein	2.7
PA14_68380	*nudE*	ADP-ribose diphosphatase NudE	2.6
PA14_01600		aldehyde dehydrogenase	2.6
PA14_41740		hypothetical protein	2.6
PA14_49010		hypothetical protein	2.6
PA14_38170		hypothetical protein	2.6
PA14_09660		acyl-CoA synthetase	2.6
PA14_19960		hypothetical protein	2.6
PA14_35950		dehydrogenase	2.6
PA14_50310		sugar nucleotidyltransferase	2.5
PA14_19750		hypothetical protein	2.5
PA14_23670		hypothetical protein	2.5
PA14_63330		glycerolphosphodiesterase	2.5
PA14_42910		hypothetical protein	2.5
PA14_51850		hypothetical protein	2.5
PA14_69430		hypothetical protein	2.5
PA14_59590		hypothetical protein	2.5
PA14_35390	*pvcD*	pyoverdine biosynthesis protein PvcD	2.5
PA14_38420		hypothetical protein	2.5
PA14_58900		large exoprotein	2.5
PA14_57770	*hisC1*	histidinol-phosphate aminotransferase	2.4
PA14_33560		adhesion protein	2.4
PA14_22550		LysR family transcriptional regulator	2.4
PA14_55160	*toxR*	transcriptional regulator ToxR	2.4
PA14_67350	*hutU*	urocanate hydratase	2.4
PA14_21680		hypothetical protein	2.4
PA14_47650	*cobS*	cobalamin synthase	2.4
PA14_33740	*pvdP*	protein PvdP	2.4
PA14_51050		aldehyde dehydrogenase	2.4
PA14_52720	*argD*	bifunctional N-succinyldiaminopimelate-aminotransferas	2.4
PA14_01250		sulfate transporter	2.4
PA14_72980		G3E family GTPase	2.4
PA14_10130		short chain dehydrogenase	2.4
PA14_29390		hypothetical protein	2.4
PA14_61620		MerR family transcriptional regulator	2.4
PA14_20950	*fabH2*	3-oxoacyl-ACP synthase	2.4
PA14_05890		stomatin-like protein	2.3
PA14_54740		hypothetical protein	2.3
PA14_72960		MFS dicarboxylate transporter	2.3
PA14_36310	*hcnC*	hydrogen cyanide synthase HcnC	2.3

PA14_04030		hypothetical protein	2.3
PA14_61170		transmembrane protein	2.3
PA14_33550		ABC transporter ATP-binding protein	2.3
PA14_46170		LysR family transcriptional regulator	2.3
PA14_46000		hypothetical protein	2.3
PA14_63605	*fdnG*	formate dehydrogenase-O, major subunit	2.3
PA14_72230		hypothetical protein	2.3
PA14_35940		acyl-CoA synthetase	2.3
PA14_28630		hydrolase	2.3
PA14_05430		chemotaxis protein	2.3
PA14_22600		glycosyl transferase family protein	2.3
PA14_34320		DszC family monooxygenase	2.3
PA14_51020		hypothetical protein	2.3
PA14_29500		type II secretion system protein	2.3
PA14_18880	*nth*	endonuclease III	2.3
PA14_29050		molybdopterin oxidoreductase	2.3
PA14_54110		transporter	2.3
PA14_52900		acyl-CoA dehydrogenase	2.3
PA14_42100		hypothetical protein	2.3
PA14_33720	*pvdN*	protein PvdN	2.3
PA14_20130		LysR family transcriptional regulator	2.2
PA14_03265		hypothetical protein	2.2
PA14_33240		hypothetical protein	2.2
PA14_73000		hypothetical protein	2.2
PA14_27410		outer membrane protein	2.2
PA14_41020		Orn/Arg/Lys decarboxylase	2.2
PA14_58110		Maf-like protein	2.2
PA14_32650		glutathione S-transferase	2.2
PA14_03250		hypothetical protein	2.2
PA14_27700		transcriptional regulator	2.2
PA14_26810		two-component sensor	2.2
PA14_67540		hypothetical protein	2.2
PA14_31740		hypothetical protein	2.2
PA14_33770		hypothetical protein	2.2
PA14_33570		hypothetical protein	2.2
PA14_03320		hypothetical protein	2.2
PA14_22060		hypothetical protein	2.2
PA14_17260	*dnaE*	DNA polymerase III subunit alpha	2.2
PA14_03360		hypothetical protein	2.2
PA14_14630	*secD*	preprotein translocase subunit SecD	2.2
PA14_34250		glycerophosphoryl diester phosphodiesterase	2.2
PA14_66700		nuclease	2.2
PA14_53740		hypothetical protein	2.2
PA14_20480		hypothetical protein	2.1
PA14_55390		hypothetical protein	2.1
PA14_34460		hypothetical protein	2.1
PA14_69890		multidrug efflux protein NorA	2.1
PA14_43510		hypothetical protein	2.1
PA14_61340		hypothetical protein	2.1
PA14_00560	*exoT*	exoenzyme T	2.1
PA14_33710	*pvdO*	protein PvdO	2.1
PA14_13330		hypothetical protein	2.1
PA14_27450		hypothetical protein	2.1
PA14_43100		Rhs family protein	2.1
PA14_72750		hypothetical protein	2.1
PA14_54890		hypothetical protein	2.1

PA14_40900		short-chain dehydrogenase	2.1
PA14_41780		hypothetical protein	2.1
PA14_31770		oxidoreductase	2.1
PA14_50570		hypothetical protein	2.1
PA14_33580		hypothetical protein	2.1
PA14_45830		hypothetical protein	2.1
PA14_34510		hypothetical protein	2.1
PA14_10600		MFS transporter	2.1
PA14_08630		pantothenate kinase	2.1
PA14_26890	*pyrF*	orotidine 5'-phosphate decarboxylase	2.1
PA14_33540		ABC transporter permease	2.1
PA14_37380		flavin-binding monooxygenase	2.1
PA14_06650	*nirN*	c-type cytochrome	2.1
PA14_28020		hypothetical protein	2.1
PA14_17650		hypothetical protein	2.1
PA14_23640		hypothetical protein	2.1
PA14_46290		TetR family transcriptional regulator	2.1
PA14_65390		hypothetical protein	2.1
PA14_68740	*argA*	N-acetylglutamate synthase	2.1
PA14_55000		ABC transporter periplasmic protein	2.1
PA14_28660	*infC*	translation initiation factor IF-3	2.1
PA14_33530		hypothetical protein	2.1
PA14_51540		transposase	2.1
PA14_29000		hypothetical protein	2.1
PA14_58980		hypothetical protein	2.1
PA14_55040		ferric enterobactin transporter ATP-binding protein	2.1
PA14_33500	*pvdH*	diaminobutyrate--2-oxoglutarate aminotransferase	2.1
PA14_59940		hypothetical protein	2.1
PA14_26610		hypothetical protein	2.1
PA14_59540		hypothetical protein	2.1
PA14_08620	*birA*	biotin--protein ligase	2.1
PA14_38020		ntibiotic biosynthesis monooxygenase	2.1
PA14_06030		acyltransferase	2.0
PA14_40790		transcriptional regulator	2.0
PA14_28170		formate/nitrate transporter	2.0
PA14_36090		porin	2.0
PA14_36400		hypothetical protein	2.0
PA14_22350	*actP*	acetate permease	2.0
PA14_63380		hypothetical protein	2.0
PA14_46010		ABC transporter ATP-binding protein	2.0
PA14_44480		hypothetical protein	2.0
PA14_52080		hypothetical protein	2.0
PA14_51360	*phnA*	anthranilate synthase component I	2.0
PA14_73100		hypothetical protein	2.0
PA14_26390		hypothetical protein	2.0
PA14_64580		two-component sensor	2.0
PA14_41563	*cobA*	uroporphyrin-III C-methyltransferase	2.0
PA14_20940		acyl carrier protein	2.0
PA14_26070		hypothetical protein	2.0
PA14_67260		histidine/phenylalanine ammonia-lyase	2.0

Supplemental Table 6. Genes down-regulated by mBTL in the *rhlR* mutant determined through microarray analysis. The ratio of gene expression in untreated cells to that of mBTL treated cells (fold down-regulation) is displayed for genes that exhibited changes greater than 2-fold. Ratios are the average of three independent experiments.

Gene locus	Gene name	Description	Ratio
PA14_58970		hypothetical protein	3.0
PA14_33360		hypothetical protein	2.2
PA14_52320		tRNA-Met	2.0
PA14_62790		tRNA-Met	2.0

Supplemental Table 7. Genes up-regulated by mBTL in a *rhlI* null strain determined through microarray analysis. The ratio of gene expression in mBTL treated cells to that of untreated cells (fold up-regulation) is displayed for genes that exhibited changes greater than 2-fold. Additional columns indicate the genes that are down-regulated in the *lasR* and *rhlR* null strains. Ratios are the average of three independent experiments.

Gene locus	Gene name	Description	Ratio	LasR-regulated	RhlR-regulated
PA14_10360		hypothetical protein	39.5		*
PA14_53250	*cpbD*	chitin-binding protein CbpD	10.1	*	*
PA14_01710	*ahpC*	alkyl hydroperoxide reductase	9.4	*	*
PA14_06810	*norC*	nitric-oxide reductase subunit C	5.8	*	*
PA14_10380		hypothetical protein	4.9	*	
PA14_11140		nonribosomal peptide synthetase	4.7		*
PA14_28360		hypothetical protein	4.2		*
PA14_66720	*priA*	primosome assembly protein PriA	3.9		
PA14_03520		hypothetical protein	3.8		*
PA14_58040		hypothetical protein	3.7	*	*
PA14_14710		Rrf2 family protein	3.5		*
PA14_17690		hypothetical protein	3.4		
PA14_46450	*aceK*	bifunctional isocitrate dehydrogenase kinase/phosphatase	3.3		
PA14_35730		hypothetical protein	3.1		
PA14_22320		hypothetical protein	3.0	*	*
PA14_38270		hypothetical protein	3.0		*
PA14_16250	*lasB*	elastase LasB	2.9	*	*
PA14_18800		hypothetical protein	2.9	*	*
PA14_53290	*trxB2*	thioredoxin reductase 2	2.9	*	*
PA14_19640		short chain dehydrogenase	2.8		
PA14_52380		cytochrome b561	2.7		
PA14_33360		hypothetical protein	2.5		
PA14_26990		hypothetical protein	2.5	*	*
PA14_40290	*lasA*	LasA protease	2.5		*
PA14_03490		hypothetical protein	2.5		*
PA14_03510		hypothetical protein	2.4		*
PA14_03770	*speB1*	agmatinase	2.4		
PA14_06830	*norB*	nitric-oxide reductase subunit B	2.3	*	*
PA14_44140		hypothetical protein	2.3		
PA14_56110		hypothetical protein	2.3		
PA14_05860		hypothetical protein	2.2	*	*
PA14_06990		hypothetical protein	2.2		
PA14_17510		hypothetical protein	2.2		
PA14_64390	*ureC*	urease subunit alpha	2.2		
PA14_01720	*ahpF*	alkyl hydroperoxide reductase	2.1	*	*
PA14_51830		DNA-binding stress protein	2.1	*	*
PA14_71840		hypothetical protein	2.1		
PA14_05660		transcriptional regulator	2.1		
PA14_60480		hypothetical protein	2.1		

PA14_18860	hypothetical protein	2.0	
PA14_19210	hypothetical protein	2.0	
PA14_46420	short chain dehydrogenase	2.0	
PA14_24440	lipoprotein	2.0	
PA14_14740	scaffold protein	2.0	*

Supplemental Experimental Procedures:

Strain Construction

The region spanning approximately 600 bp upstream of *lasR* and including the start codon (called *lasR*') and the sequence encoding the C-terminal 6 amino acids of LasR and approximately 600 bp downstream (called '*lasR*) were amplified by PCR. The FRT-*aacC1*-FRT region in pAS03 (33) was amplified using primers that span sequences in *lasR'* or *'lasR*. The *lasR'*, FRT-*aacC1*-FRT and *'lasR* PCR products were combined through overlap extension PCR and amplified. The resulting *lasR'*-FRT-*aacC1*-FRT-*'lasR* product was transformed into *P. aeruginosa* PA14 harboring pUCP18-RedS (34). Gentamicin resistance was selected to yield *lasR*::*aacC1* in the chromosome. Following excision of the gentamicin resistance gene (35), the *lasR, rhlR* double mutant strain was constructed by inserting *rhlR*::*MAR2xT7* into the *lasR* strain background followed by selection for gentamicin resistance (32). This strategy yielded the *lasR*::FRT, *rhlR*::*MAR2xT7* strain.

LasR and RhlR Overexpression

Overnight cultures of *E. coli* BL21-Gold (DE3) carrying the LasR and RhlR overexpression constructs were diluted 1:100 into fresh LB supplemented with antibiotics and grown shaking at 37°C to an OD_{600} of 0.4. Autoinducer or antagonist molecules were added at 100 μM and incubated an additional 30 min at 20°C, after which protein production was induced by the addition of 1 mM IPTG for 6 hr at 20°C. Cells were harvested by centrifugation and resuspended in 1 mL of 20 mM Tris (pH 7.5), 0.5 mM EDTA, 300 mM NaCl, 1 mM DTT, and 5% glycerol and 100 μM of the appropriate ligand. Resuspended pellets were sonicated twice for 15 seconds to produce lysates containing all of the cell contents. We refer to this preparation as the whole cell (WC) fraction. The WC fraction was subjected to centrifugation at 4°C at

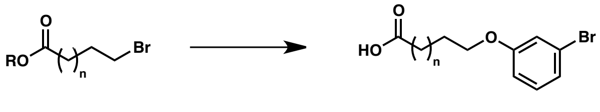
13,300 RPM for 15 min to remove insoluble material and the membrane fraction (22). The supernatant from this pellet is referred to as the soluble (S) fraction. SDS-PAGE gels (4% stacking and 12% resolving) were used followed by Coomassie blue (BioRad) staining to visualize protein. Contrast was uniformly adjusted for both gels.

Biofilm-Induced Clogging of Microfluidic Flow Cells

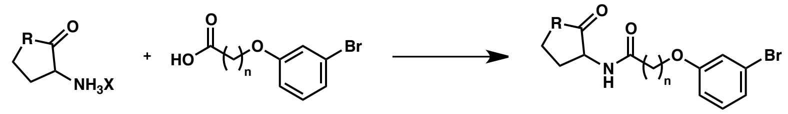
Overnight *P. aeruginosa* PA14 cultures were back-diluted 1:1000 into 800 mL of tryptone broth (1% tryptone in H_2O) with or without 100 μM mBTL and grown to mid-logarithmic phase (OD_{600} = 0.5). These cultures were used to fill 100 mL reservoirs that fed into microfluidic flow channels via Tygon tubing with an inner diameter of 2.4 mm. Similar tubing connected the outlet of the microfluidic channel to a collection dish on an analytical balance controlled via LabVIEW. The elevation of the culture reservoir above the collection dish on the balance set the constant pressure difference that drove the flow through the microfluidic channel. The microfluidic channel is 200 μm wide, 90 μm high, and contains a sequence of 37 bends that mimic corners in porous materials. The weight of the effluent culture was measured as a function of time t with measurement intervals of 4 s, and the data were converted into a flow rate $Q(t)$ via the equation $Q(t) = \frac{w(t+\Delta t) - w(t-\Delta t)}{1\text{min}} \frac{1}{\text{density}}$, where $\Delta t = 30$ s and the density is assumed to be that of water, 1 kg/L. To the resulting flow rate time series $Q(t)$, the function $Q_0 \Big/ \left[1 + \exp\left(\frac{t+T}{\tau/2}\right)\right]$ was fitted which yields the measurement of the time until clogging (corresponding to the time at which the flow rate declined to 50% of its baseline value Q_0).

Chemistry Materials and Methods

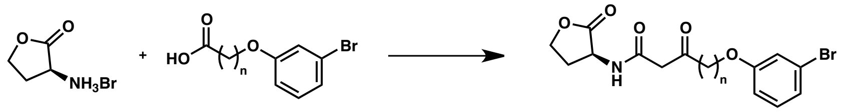
Unless otherwise stated, reactions were performed in flame-dried glassware fitted with rubber septa under a nitrogen atmosphere and were stirred with Teflon-coated magnetic stirring bars. Liquid reagents and solvents were transferred via syringe using standard Schlenk techniques. Reaction solvents were dried by passage over a column of activated alumina. All other solvents and reagents were used as received unless otherwise noted. Reaction temperatures above 23°C refer to the oil bath temperature, which was controlled by an OptiCHEM temperature modulator. Thin layer chromatography was performed using SiliCycle silica gel 60 F-254 precoated plates (0.25 mm) and visualized by UV irradiation and anisaldehyde, ceric ammonium molybdate, or potassium permanganate stain. Sorbent standard silica gel (particle size 40-63 μm) was used for flash chromatography. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance II (500 MHz for 1H; 125 MHz for ^{13}C) spectrometer fitted with either a ^{1}H-optimized TCI (H/C/N) cryoprobe or a ^{13}C-optimized dual C/H cryoprobe. Chemical shifts (δ) are reported in ppm relative to the residual solvent signal (δ = 7.26 for ^{1}H NMR and δ = 77.0 for ^{13}C NMR). Data for ^{1}H NMR spectra are reported as follows: chemical shift (multiplicity, coupling constants, number of hydrogens). Abbreviations are as follows: s (singlet), d (doublet), t (triplet), m (multiplet). High-resolution mass spectral analysis was performed using an Agilent 1200-series electrospray ionization – time-of-flight (ESI-TOF) mass spectrometer in the positive ESI mode. The following compounds were synthesized as previously described: CL, CTL, mBTL, mCTL (4), itc-13 (3), PD-12 (5).



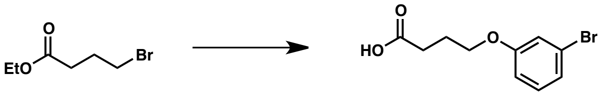
General Procedure A. Synthesis of acids: To a flame-dried flask was added the 3-bromophenol (1.0 equiv), the appropriate bromo-ester (1.0 equiv), potassium carbonate (1.2 equiv), and DMF (0.50 M). The reaction was stirred for 3 d or until complete by TLC. After completion, H_2O was added, and the aqueous layer was extracted 3x with Et_2O. The combined organic layer was washed 3x with H_2O and 1x with 1 M NaOH. The solution was dried over Na_2SO_4, filtered, and concentrated. The product was purified by column chromatography to remove excess 3-bromophenol if necessary. The resulting ester (1.0 equiv) was added to a solution of sodium hydroxide (5.0 equiv) in 3:1 THF/H_2O (0.30 M). The reaction was heated to 65°C for 12 hr, or until complete by TLC. The reaction was cooled and acidified with 1 M HCl. The aqueous layer was extracted 3x with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The product was carried forward crude.



General Procedure B. Synthesis of amides: To a flame-dried flask were added the acid (1.0 equiv), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (1.1 equiv), 1-hydroxybenzotriazole (0.25 equiv), triethylamine (2.2 equiv), the appropriate (thio)lactone (1.0 equiv), and CH_2Cl_2 (0.10 M). After the mixture was stirred at room temperature for 24 hr, H_2O was added, and the aqueous layer was extracted 3x with EtOAc. The combined organic layer was washed sequentially with 1 M $NaHSO_4$, saturated aqueous $NaHCO_3$, and brine. The solution was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography with a hexanes/EtOAc gradient.

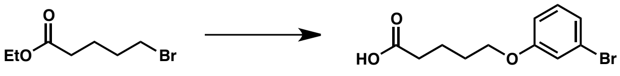


General Procedure C. Synthesis of β-keto amides: The acid (1.0 equiv) was combined with CH_2Cl_2 (0.5 M) and cooled to 0°C. *N*,*N′*-dicyclohexylcarbodiimide (1.0 equiv) was added, and the reaction was stirred at 0°C for 30 min. Meldrum's acid (1.0 equiv) and 4-(dimethylamino)pyridine (1.0 equiv) were added, and the reaction mixture was stirred at room temperature overnight. The solution was filtered through a Celite plug and concentrated. The residue was dissolved in CH_3CN (0.10 M). After L-homoserine lactone hydrobromide (1.0 equiv) and trifluoroacetic acid (1.0 equiv) were added, the reaction was heated to 65°C for 4 hr. The reaction mixture was cooled, diluted with EtOAc, and washed sequentially with 1 M $NaHSO_4$, saturated aqueous $NaHCO_3$, and brine. The solution was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography with a hexanes/EtOAc gradient.



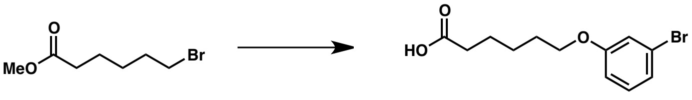
S1

C4 acid (S1): Prepared from ethyl 4-bromobutyrate using general procedure A to give **S1** in 90% yield over two steps. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.16-7.01 (m, 3H), 6.84-6.79 (m, 1H), 4.00 (t, *J* = 6.0 Hz, 2H), 2.59 (t, *J* = 7.2 Hz, 2H), 2.16-2.08 (m, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 179.1, 159.4, 130.5, 123.9, 122.8, 117.6, 113.4, 66.6, 30.4, 24.2; **HRMS** (ESI-TOF) calculated for $C_{10}H_{12}BrO_3$ $[M+H]^+$: *m/z* 258.9971, found 258.9967.



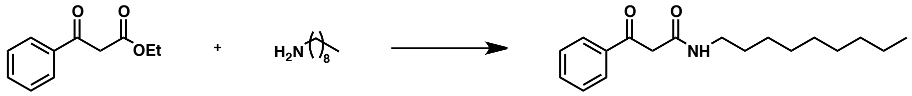
S2

C5 acid (S2): Prepared from ethyl 5-bromovalerate using general procedure A to give **S2** in 93% yield over two steps. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.18-7.01 (m, 3H), 6.85-6.77 (m, 1H), 3.96 (t, *J* = 5.6 Hz, 2H), 2.50-2.41 (m, 2H), 1.89-1.80 (m, 4H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 178.6, 159.6, 130.5, 123.7, 122.8, 117.6, 113.5, 67.5, 33.4, 28.4, 21.3; **HRMS** (ESI-TOF) calculated for $C_{11}H_{14}BrO_3$ $[M+H]^+$: *m/z* 273.0127, found 273.0135.



S3

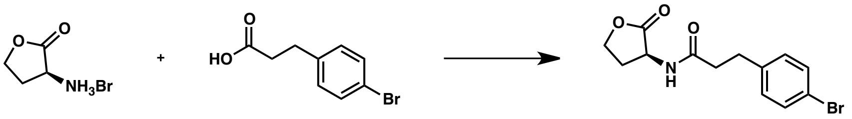
C6 acid (S3): Prepared from methyl 6-bromohexanoate using general procedure A to give **S3** in 80% yield over two steps. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.16-7.01 (m, 3H), 6.85-6.78 (m, 1H), 3.94 (t, *J* = 6.4 Hz, 2H), 2.40 (t, *J* = 7.4 Hz, 2H), 1.84-1.76 (m, 2H), 1.76-1.67 (m, 2H), 1.60-1.49 (m, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 179.0, 159.7, 130.5, 123.6, 122.8, 117.6, 113.5, 67.8, 33.7, 28.8, 25.5, 24.3; **HRMS** (ESI-TOF) calculated for $C_{12}H_{16}BrO_3$ $[M+H]^+$: *m/z* 287.0283, found 287.0277.



V-06-018

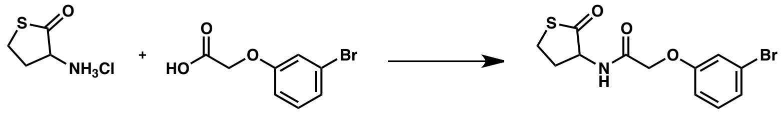
V-06-018: Ethyl benzylacetate (0.10 mL, 0.58 mmol, 1.0 equiv) was combined with ethanol (5.8 mL, 10 M). Nonylamine (0.11 mL, 0.58 mmol, 1.0 equiv) was added dropwise, and the mixture was heated to reflux for 6 hr. The reaction mixture was concentrated, and the residue was dissolved in EtOAc. The solution was washed sequentially 2x with 1 M HCl, 1x with brine, then dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by column chromatography (hexanes/EtOAc gradient) to afford 8.3 mg of **V-06-018** in a 5.0% yield. **^{1}H NMR** (500

MHz, $CDCl_3$) δ 8.00 (d, *J* = 7.5 Hz, 2H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.50 (t, *J* = 7.8 Hz, 2H), 7.15 (s, 1H), 3.95 (s, 2H), 3.31-3.27 (m, 2H), 1.55-1.50 (m, 2H), 1.38-1.17 (m, 12H), 0.87 (t, *J* = 6.9 Hz, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 196.4, 165.5, 136.1, 134.1, 128.9, 128.6, 45.2, 39.7, 31.8, 29.5, 29.4, 29.2, 29.2, 26.9, 22.7, 14.1; **HRMS** (ESI-TOF) calculated for $C_{18}H_{28}NO_2$ $[M+H]^+$: *m/z* 290.2120, found 290.2120.



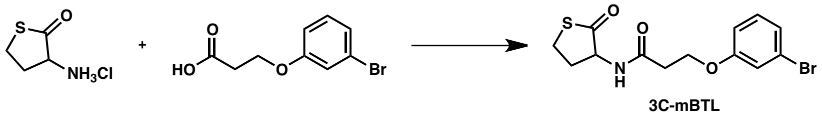
B7

B7: Prepared with L-homoserine lactone and 3-(4-bromophenyl)propionic acid using general procedure B to give **B7** in a 52% yield. The spectral data agreed with that reported for **B7** (*17*). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.3 Hz, 2H), 7.07 (t, *J* = 9.1 Hz, 2H), 5.86 (s, 1H), 4.57-4.41 (m, 2H), 4.30-4.25 (m, 1H), 2.93 (t, *J* = 7.5 Hz, 2H), 2.89-2.78 (m, 1H), 2.61-2.43 (m, 2H), 2.09-2.00 (m, 1H).; **^{13}C NMR** (125 MHz, $CDCl_3$) δ 175.2, 172.2, 139.3, 131.6, 130.1, 120.2, 66.1, 49.3, 37.5, 30.6, 30.6; **HRMS** (ESI-TOF) calculated for $C_{13}H_{15}BrNO_3$ $[M+H]^+$: *m/z* 312.0236, found 312.0239.

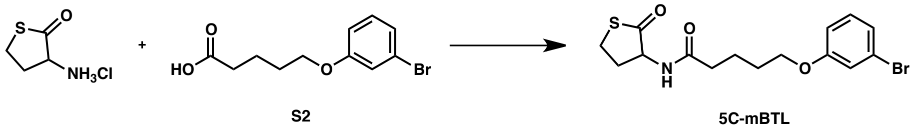


2C-mBTL

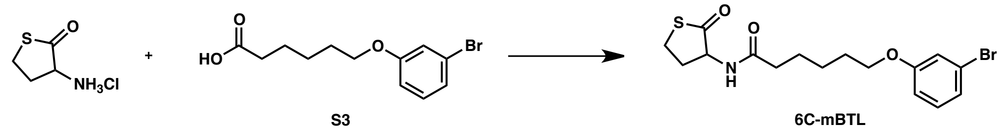
2C-mBTL: Prepared with homocysteine thiolactone hydrochloride and (3-bromophenoxy)acetic acid using general procedure B to give **2C-mBTL** in a 50% yield. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.22-7.09 (m, 3H), 6.95 (s, 1H), 6.89-6.84 (m, 1H), 4.68-4.58 (m, 1H), 4.57-4.46 (m, 2H), 3.44-3.35 (m, 1H), 3.34-3.26 (m, 1H), 3.00-2.91 (m, 1H), 2.08-1.95 (m, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 204.6, 168.1, 157.5, 130.9, 125.5, 123.0, 118.3, 113.4, 67.2, 58.9, 31.6, 27.5; **HRMS** (ESI-TOF) calculated for $C_{12}H_{13}BrNO_3S$ $[M+H]^+$: *m/z* 329.9800, found 329.9830.



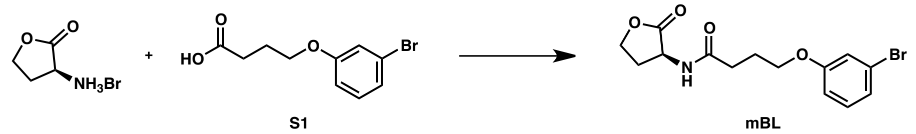
3C-mBTL: Prepared with homocysteine thiolactone hydrochloride and 3-(3-bromo-phenoxy)-propionic acid using general procedure B to give **3C-mBTL** in a 42% yield. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.21-7.07 (m, 3H), 6.90-6.84 (m, 1H), 6.32 (s, 1H), 4.57-4.49 (m, 1H), 4.30-4.21 (m, 2H), 3.43-3.33 (m, 1H), 3.32-3.24 (m, 1H), 3.04-2.96 (m, 1H), 2.80-2.67 (m, 2H), 2.01-1.87 (m, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 205.3, 170.7, 158.9, 130.6, 124.4, 122.8, 117.9, 113.6, 64.0, 59.6, 36.2, 32.0, 27.7; **HRMS** (ESI-TOF) calculated for $C_{13}H_{15}BrNO_3S$ $[M+H]^+$: *m/z* 343.9956, found 343.9984.



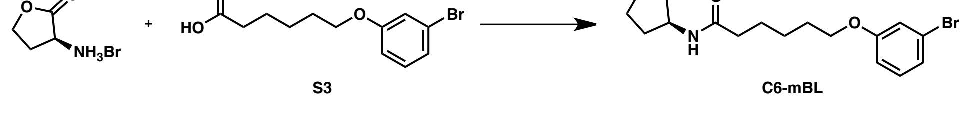
5C-mBTL: Prepared with homocysteine thiolactone hydrochloride and **S2** using general procedure B to give **5C-mBTL** in a 68% yield. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.16-6.99 (m, 3H), 6.85-6.77 (m, 1H), 5.91 (s, 1H), 4.56-4.46 (m, 1H), 3.95 (t, *J* = 5.5 Hz, 2H), 3.42-3.31 (m, 1H), 3.30-3.22 (m, 1H), 3.01-2.90 (m, 1H), 2.37-2.29 (m, 2H), 1.97-1.78 (m, 5H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 205.6, 173.0, 159.6, 130.5, 123.7, 122.8, 117.6, 113.4, 67.6, 59.5, 35.8, 32.1, 28.5, 27.6, 22.1; **HRMS** (ESI-TOF) calculated for $C_{15}H_{19}BrNO_3S$ $[M+H]^+$: *m/z* 372.0269, found 372.0300.



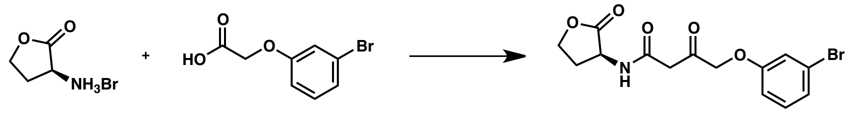
6C-mBTL: Prepared with homocysteine thiolactone hydrochloride and **S3** using general procedure B to give **6C-mBTL** in a 74% yield. **^{1}H NMR** (500 MHz, CDCl$_3$) δ 7.16-7.00 (m, 3H), 6.85-6.76 (m, 1H), 5.89 (s, 1H), 4.57-4.45 (m, 1H), 3.93 (t, *J* = 6.4 Hz, 2H), 3.41-3.31 (m, 1H), 3.30-3.20 (m, 1H), 3.03-2.91 (m, 1H), 2.35-2.21 (m, 2H), 1.98-1.83 (m, 1H), 1.83-1.66 (m, 4H), 1.54-1.44 (m, 2H); **^{13}C NMR** (125 MHz, CDCl$_3$) δ 205.7, 173.3, 159.7, 130.5, 123.6, 122.7, 117.6, 113.4, 67.8, 59.5, 36.2, 32.1, 28.8, 27.6, 25.6, 25.1; **HRMS** (ESI-TOF) calculated for $C_{16}H_{21}BrNO_3S$ $[M+H]^+$: *m/z* 386.0426, found 386.0427.



mBL: Prepared with L-homoserine lactone hydrobromide and **S1** using general procedure B to give **mBL** in a 62% yield. **^{1}H NMR** (500 MHz, CDCl$_3$) δ 7.14-6.98 (m, 3H), 6.83-6.76 (m, 1H), 5.98 (s, 1H), 4.56-4.47 (m, 1H), 4.44 (t, *J* = 9.0 Hz, 1H), 4.30-4.21 (m, 1H), 3.97 (t, *J* = 5.9 Hz, 2H), 2.87-2.78 (m, 1H), 2.44 (t, *J* = 6.8 Hz, 2H), 2.17-2.02 (m, 3H); **^{13}C NMR** (125 MHz, CDCl$_3$) δ 175.3, 172.7, 159.5, 130.6, 123.9, 122.8, 117.7, 113.4, 66.9, 66.1, 49.3, 32.2, 30.6, 24.7; **HRMS** (ESI-TOF) calculated for $C_{14}H_{17}BrNO_4$ $[M+H]^+$: *m/z* 342.0341, found 342.0345.

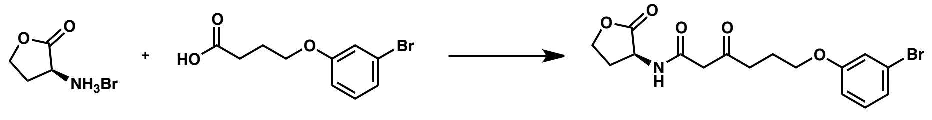


C6-mBL: Prepared with L-homoserine lactone hydrobromide and **S3** using general procedure B to give **C6-mBL** in a 61% yield. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.17-7.01 (m, 3H), 6.85-6.77 (m, 1H), 5.95 (s, 1H), 4.59-4.50 (m, 1H), 4.48 (t, *J* = 9.0 Hz, 1H), 4.34-4.25 (m, 1H), 3.93 (t, *J* = 6.3 Hz, 2H), 2.93-2.82 (m, 1H), 2.35-2.23 (m, 2H), 2.19-2.06 (m, 1H), 1.86-1.68 (m, 4H), 1.55-1.46 (m, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 175.4, 173.3, 159.7, 130.5, 123.6, 122.8, 117.6, 113.4, 67.7, 66.1, 49.3, 36.0, 30.7, 28.8, 25.6, 25.0; **HRMS** (ESI-TOF) calculated for $C_{16}H_{21}BrNO_4$ $[M+H]^+$: *m/z* 370.0654, found 370.0666.



3O-mBL

3O-mBL: Prepared with L-homoserine lactone hydrobromide and (3-bromophenoxy)acetic acid using general procedure C to give **3O-mBL** in a 46% yield. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.20-7.03 (m, 3H), 6.87-6.80 (m, 1H), 4.68 (s, 2H), 4.64-4.56 (m, 1H), 4.48 (t, *J* = 8.9 Hz, 1H), 4.35-4.24 (m, 1H), 3.66 (s, 2H), 2.93-2.88 (m, 1H), 2.81-2.75 (m, 1H), 2.29-2.15 (m, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 201.7, 174.8, 165.6, 157.9, 130.9, 125.3, 123.0, 118.0, 113.3, 72.6, 66.0, 49.3, 45.5, 29.9; **HRMS** (ESI-TOF) calculated for $C_{14}H_{15}BrNO_5$ $[M+H]^+$: *m/z* 356.0134, found 356.0127.



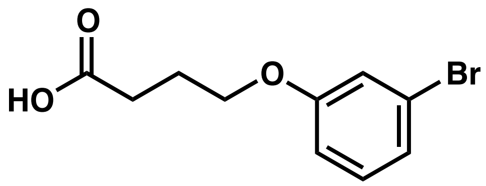
S1 **3O-C6-mBL**

3O-C6-mBL: Prepared with L-homoserine lactone hydrobromide and **S1** using general procedure C to give **3O-C6-mBL** in a 34% yield. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.18-6.99 (m, 3H), 6.85-6.76 (m, 1H), 4.63-4.55 (m, 1H), 4.48 (t, *J* = 8.6 Hz, 1H), 4.32-4.23 (m, 1H), 3.96 (t, *J* = 5.9 Hz, 2H), 3.51 (s, 2H), 2.83-2.70 (m, 3H), 2.23-2.15 (m, 1H), 2.14-2.02 (m, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 205.6, 174.7, 166.1, 159.3, 130.6, 124.0, 122.8, 117.6, 113.4, 66.6, 65.9, 49.1, 48.2, 40.1, 29.9, 22.9; **HRMS** (ESI-TOF) calculated for $C_{16}H_{19}BrNO_5$ $[M+H]^+$: *m/z* 384.0447, found 384.0455.

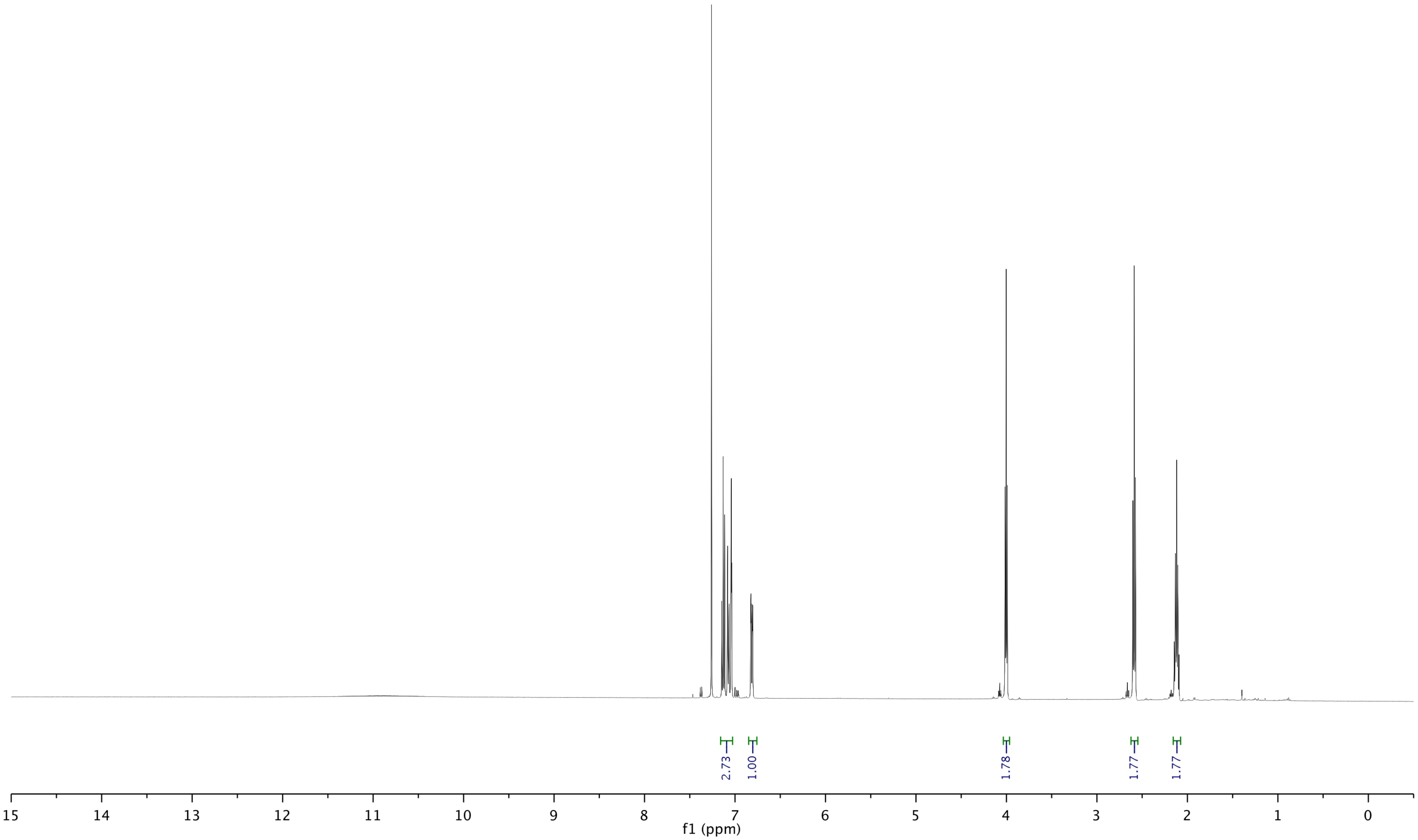
Chiral resolution of mBTL: mBTL enantiomers were resolved using a Berger Multigram II SFC system equipped with two Varian SD-1 pumps, a Knauer K-2501 multi-wavelength detector set at 220 nm, a Knauer K-1900 pump, a Vatran SGP-50-100 condenser, and using a Chiralpak IC (2 x 15 cm) column. An isocratic method using a mixture of 30% MeOH/CO_2 (100 bar) at 60 mL/min was employed. The two peaks eluted at 1.66 min and 2.13 min. The identity of the enantiomers was determined through comparison of the HPLC trace with that of authentic (*S*)-mBTL synthesized from L-homocysteine thiolactone hydrochloride. Based on this analysis, peak 1 (>99:1 *er*) is (*S*)-mBTL and peak 2 (>99:1 *er*) is (*R*)-mBTL.

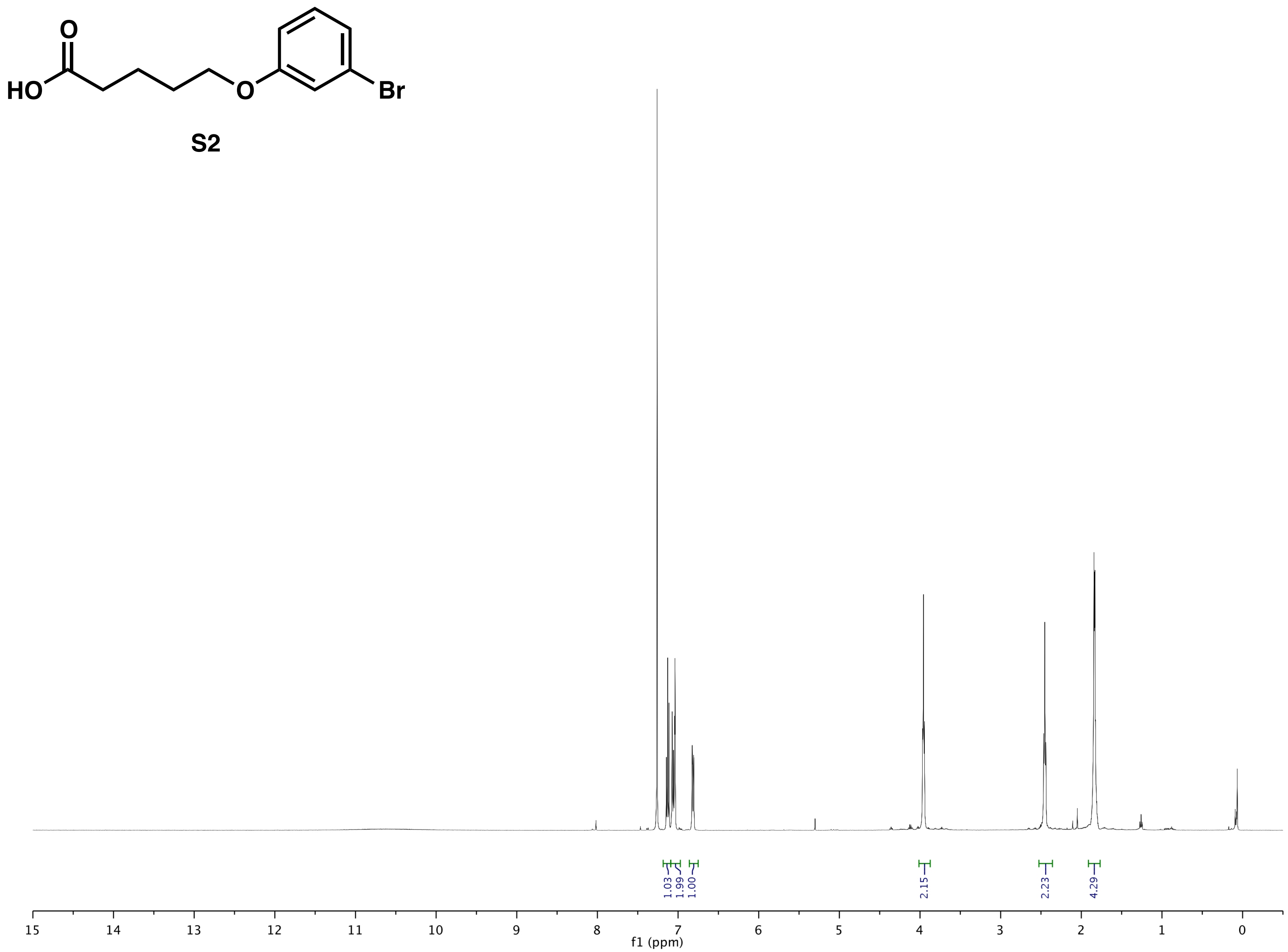
References:

1. Müh U, Schuster, M., Heim, R., Singh, A., Olson, E. R., Greenberg, E. P. (2006) Novel Pseudomonas aeruginosa Quorum-Sensing Inhibitors Identified in an Ultra-High-Throughput Screen. *Antimicrobial Agents and Chemotherapy* 50(11):3674-3679.
2. Geske GD, O'Neill, Jennifer C., Miller, David M., Mattmann, Margrith E., Blackwell, Helen E. (2007) Modulation of Bacterial Quorum Sensing with Synthetic Ligands: Systematic Evaluation of N-Acylated Homoserine Lactones in Multiple Species and New Insights into Their Mechanisms of Action. *Journal of the American Chemical Society* 129(44):13613-13625.
3. Amara N, et.al. (2009) Covalent Inhibition of Bacterial Quorum Sensing. *Journal of the American Chemical Society* 131(30):10610-10619.
4. Swem LR, Swem, D. L., O'Loughlin, C. T., Gatmaitan, R., Zhao, B., Ulrich, S. M., et. al. (2009) A Quorum-Sensing Antagonist Targets Both Membrane-Bound and Cytoplasmic Receptors and Controls Bacterial Pathogenicity. *Molecular Cell* 35(2):143-153.
5. Morkunas B, Galloway, Warren R. J. D., Wright, Megan, Ibbeson, Brett M., Hodgkinson, James T., O'Connell, Kieron M. G., et. al. (2012) Inhibition of the production of the Pseudomonas aeruginosa virulence factor pyocyanin in wild-type cells by quorum sensing autoinducer-mimics. *Organic & Biomolecular Chemistry* 10(42):8452-8464.



S1




S2

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O
Br
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1.00
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2.23
4.29
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13
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11
10
9
8
7
6
5
4
3
2
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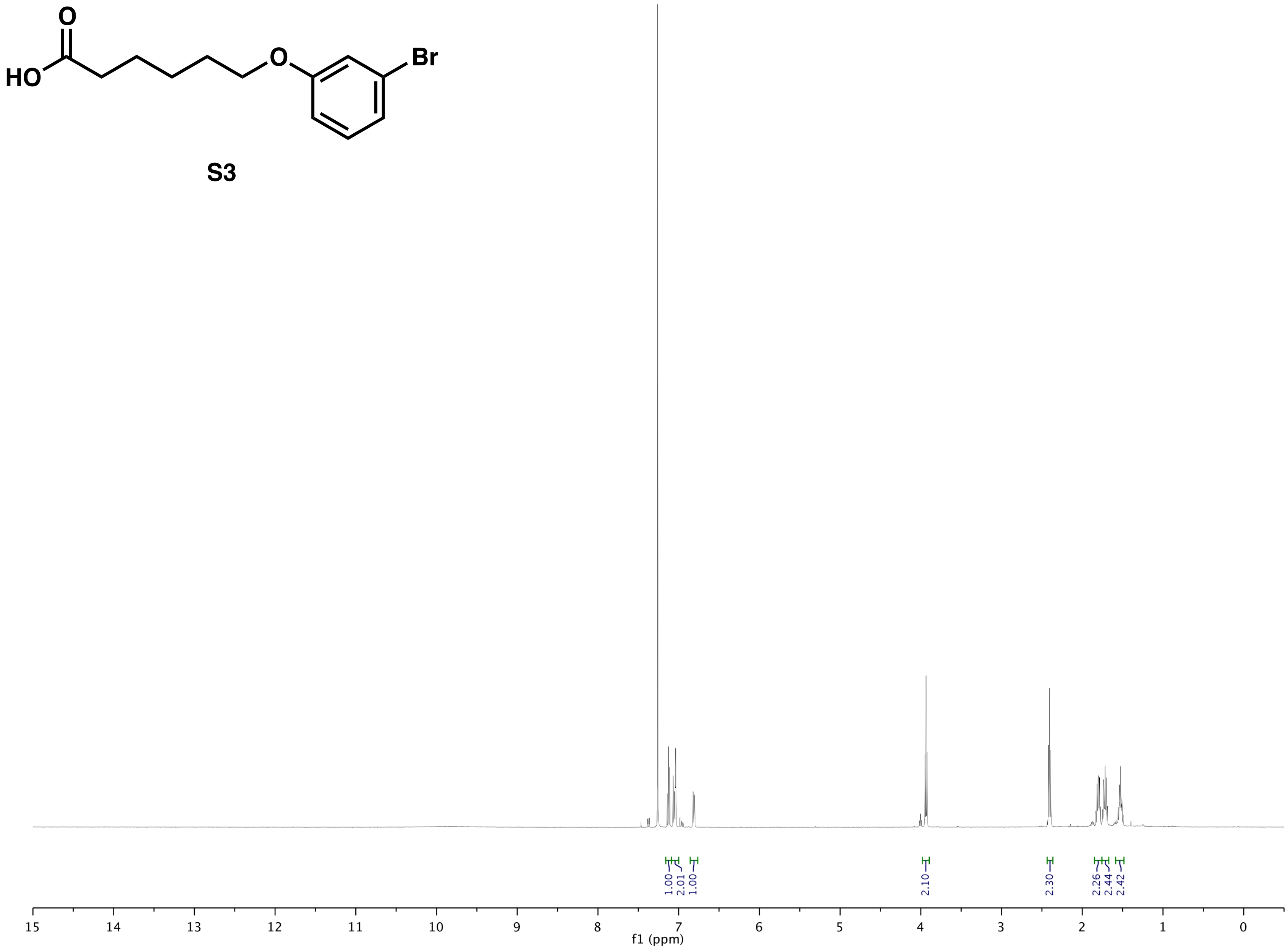
f1 (ppm)



S3

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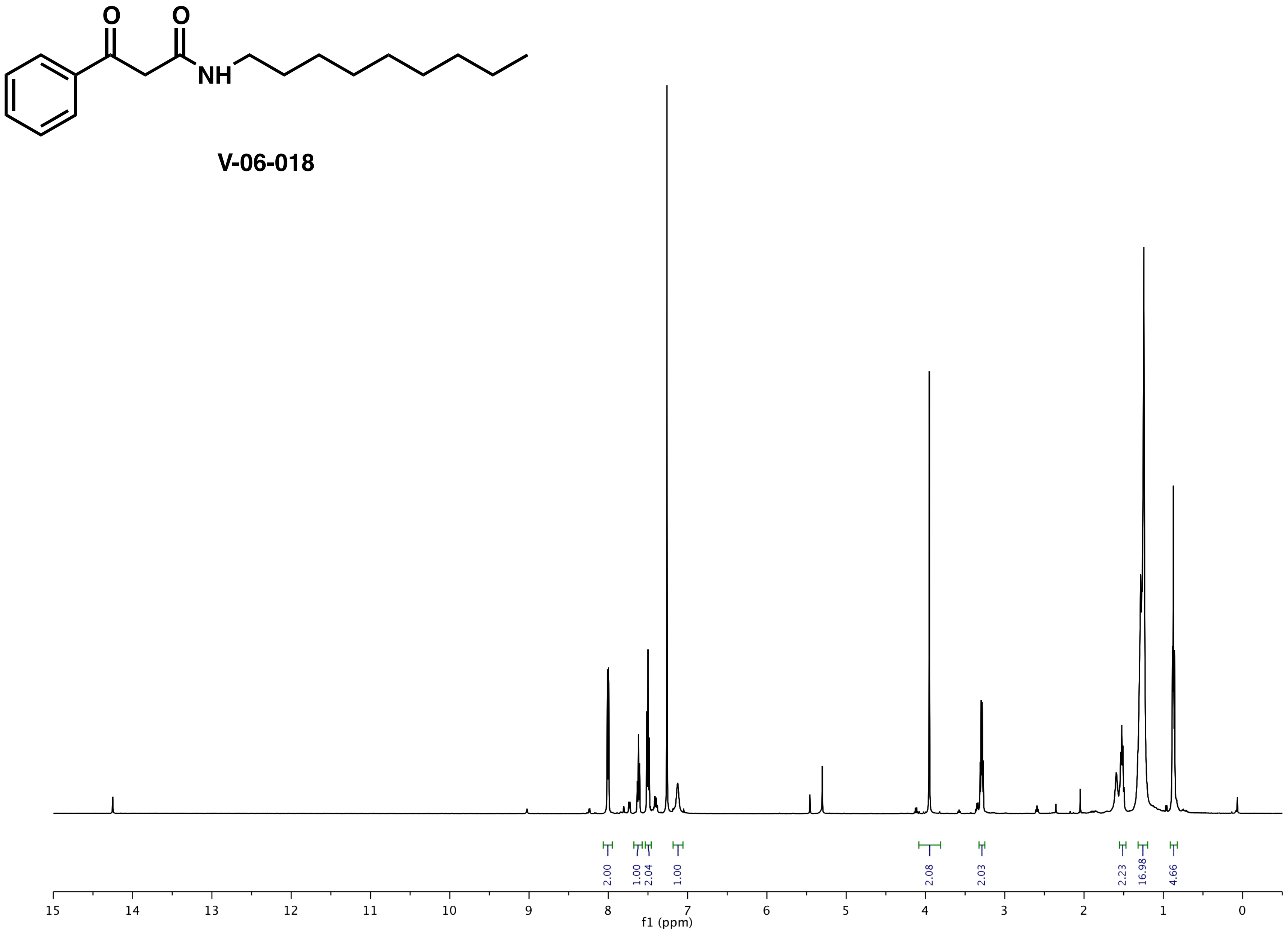
f1 (ppm)



V-06-018

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13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

B7

Br
O
N
H
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1.00
2.07
1.05
2.09
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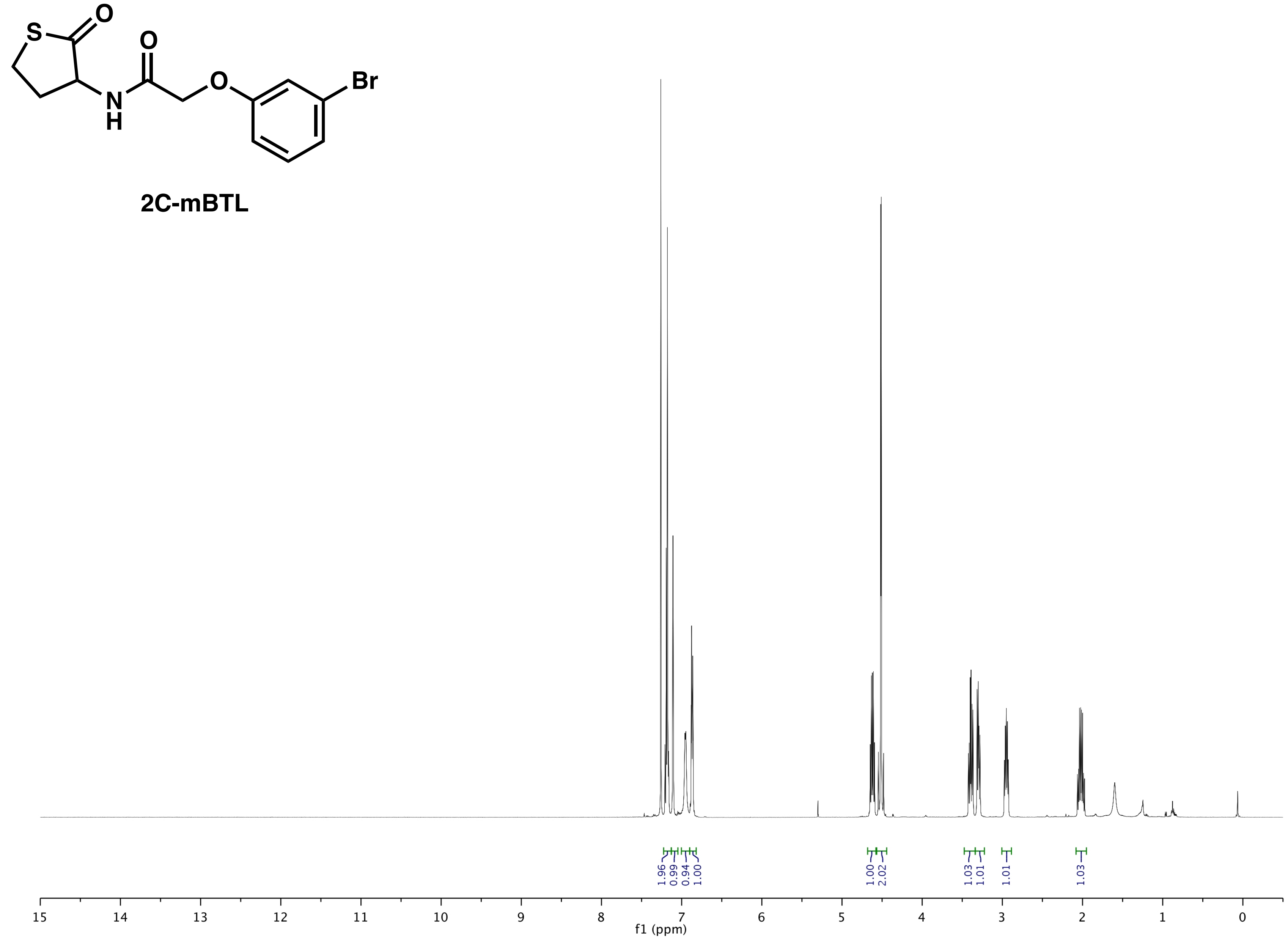

2C-mBTL

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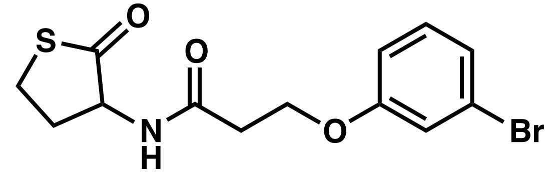
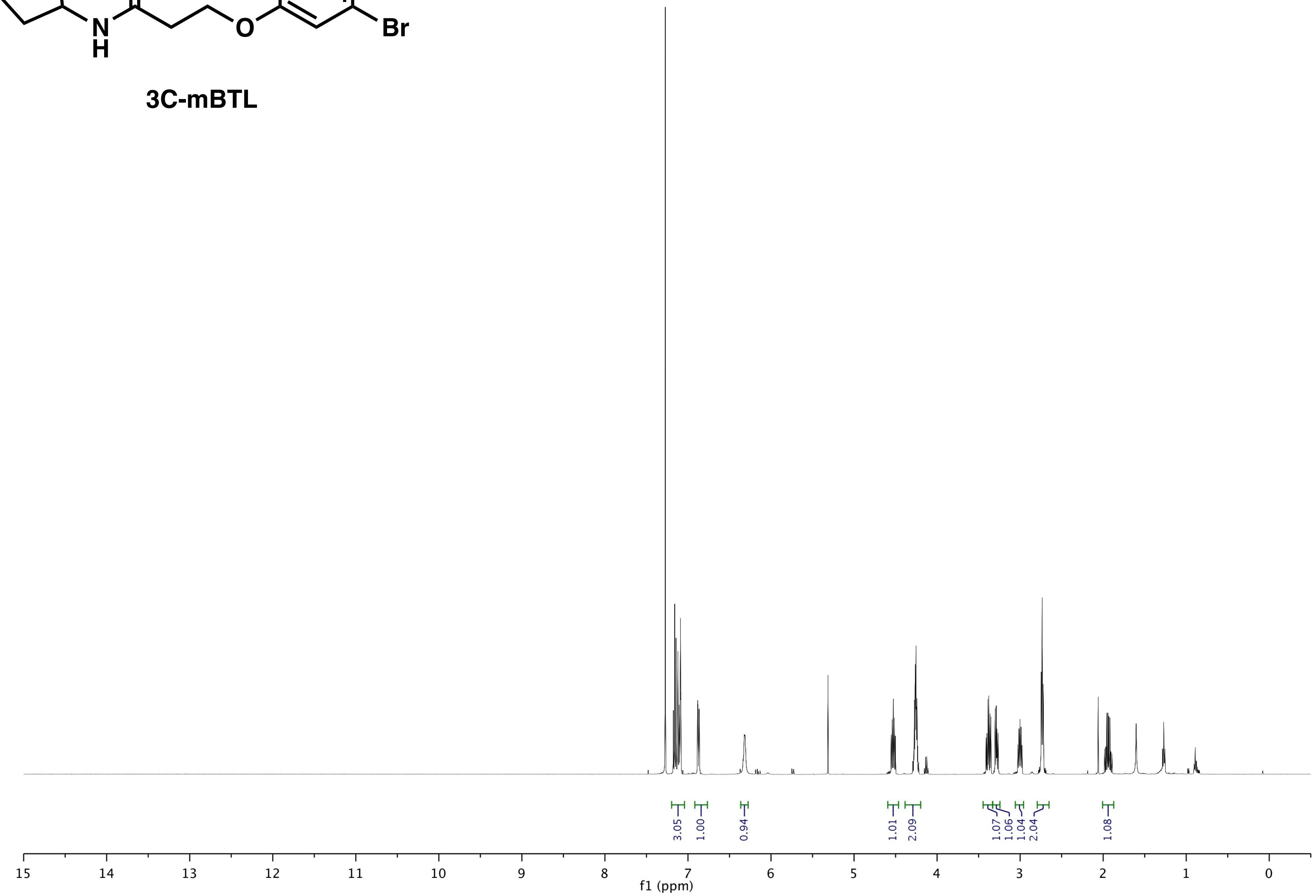
f1 (ppm)



3C-mBTL



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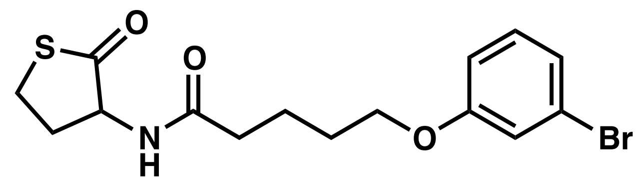
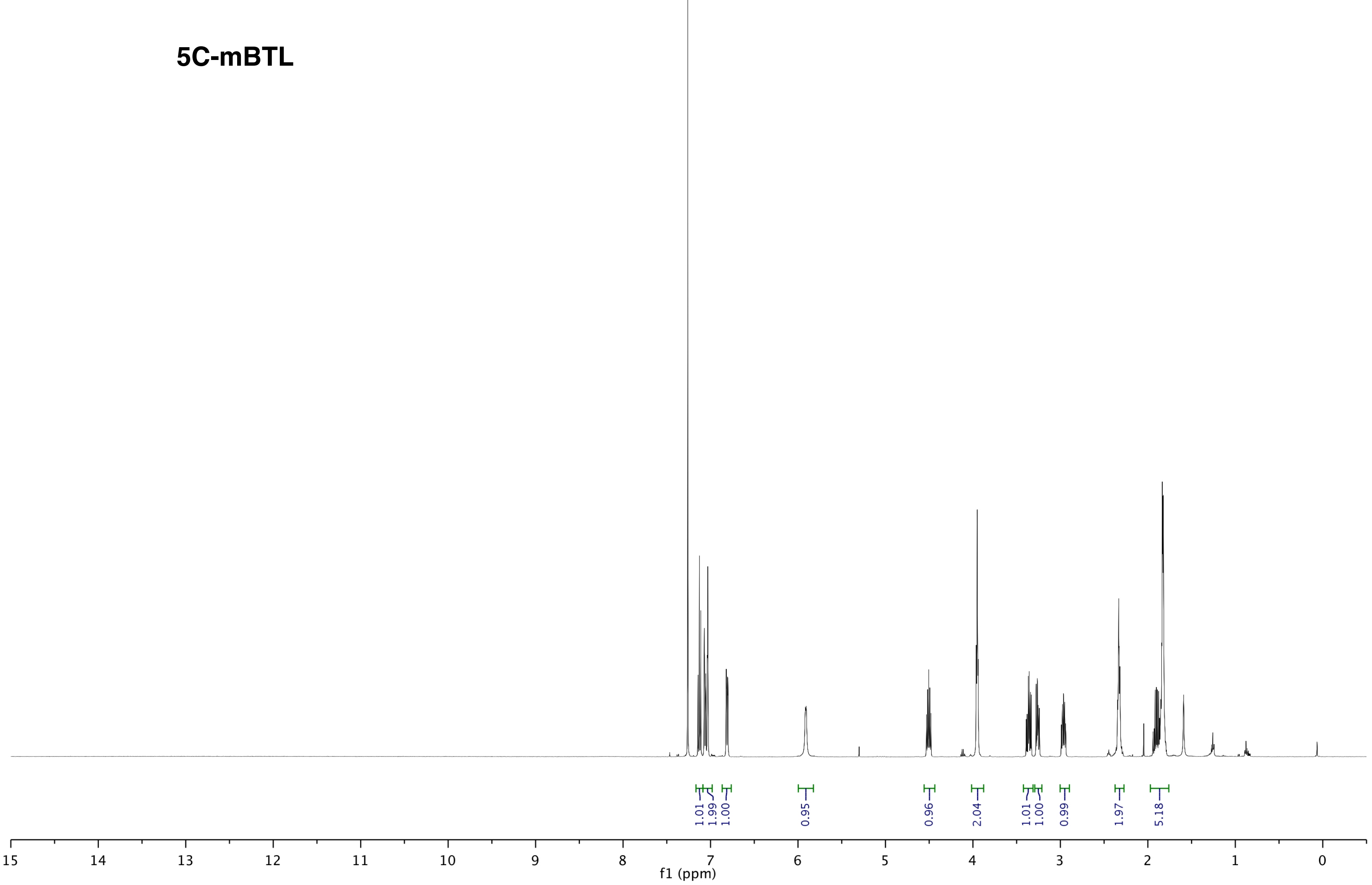
f1 (ppm)



5C-mBTL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0.99
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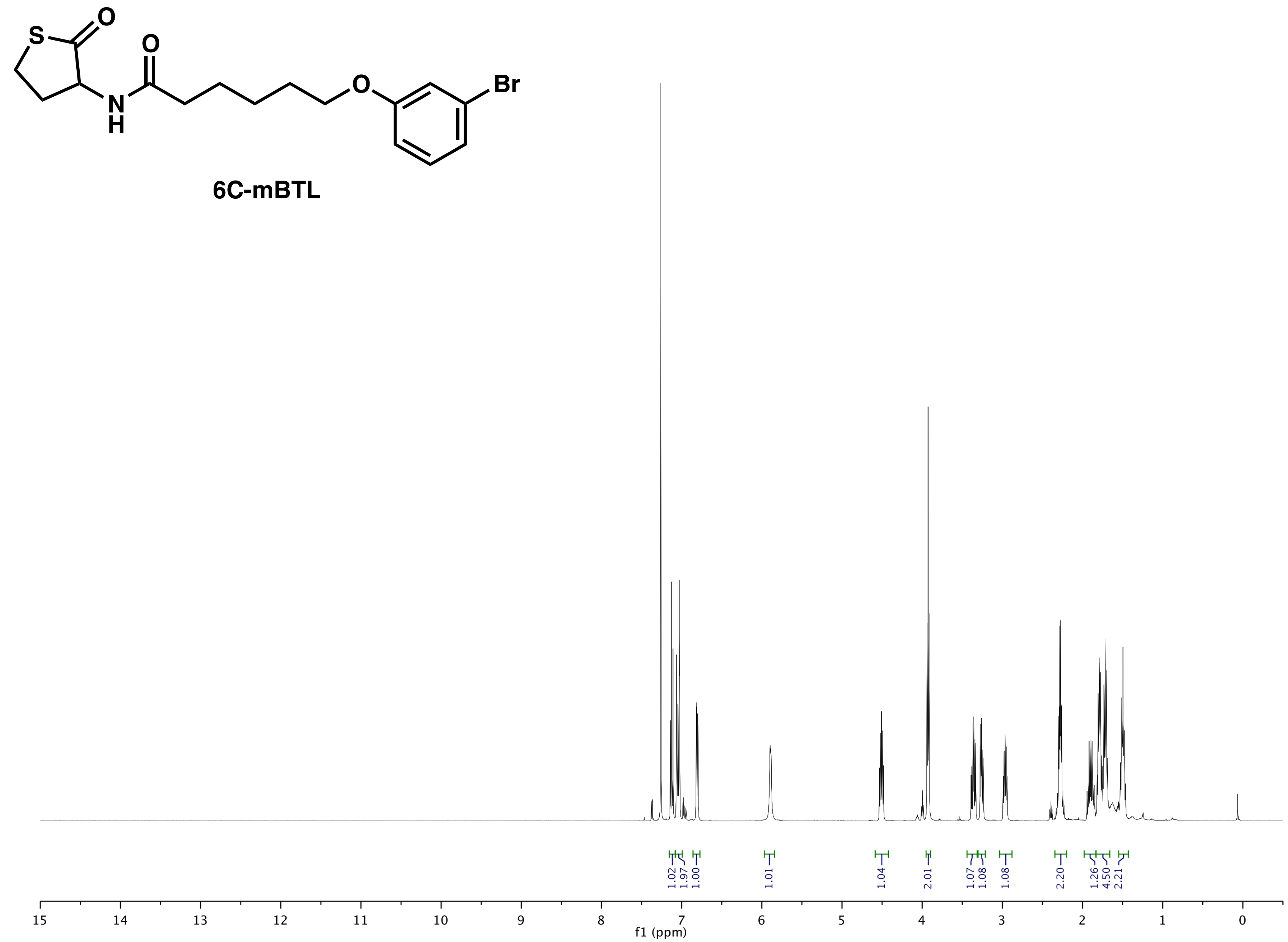
f1 (ppm)



6C-mBTL

S
O
O
N
H
O
Br
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1.26
4.50
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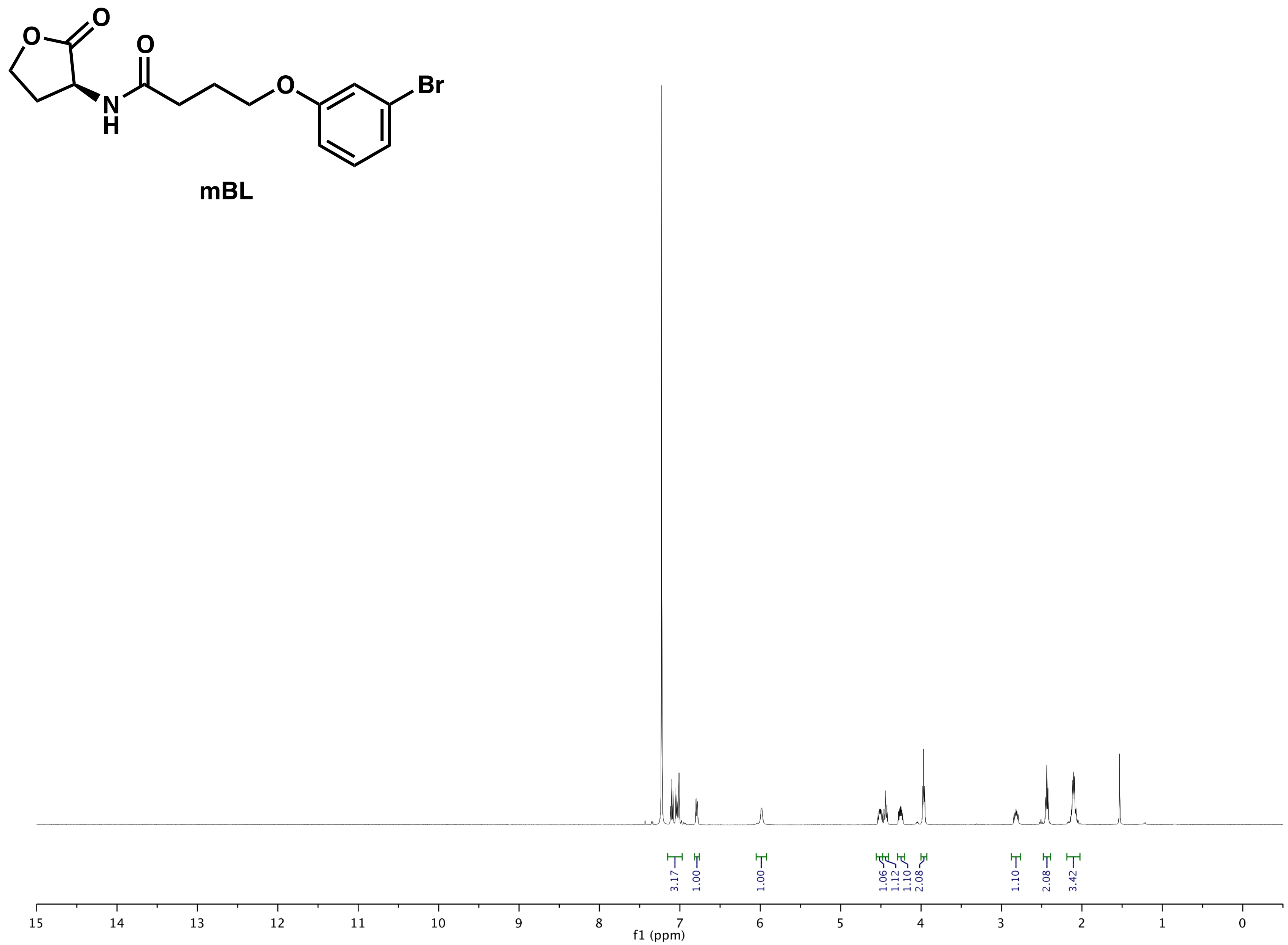
0
f1 (ppm)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2.08
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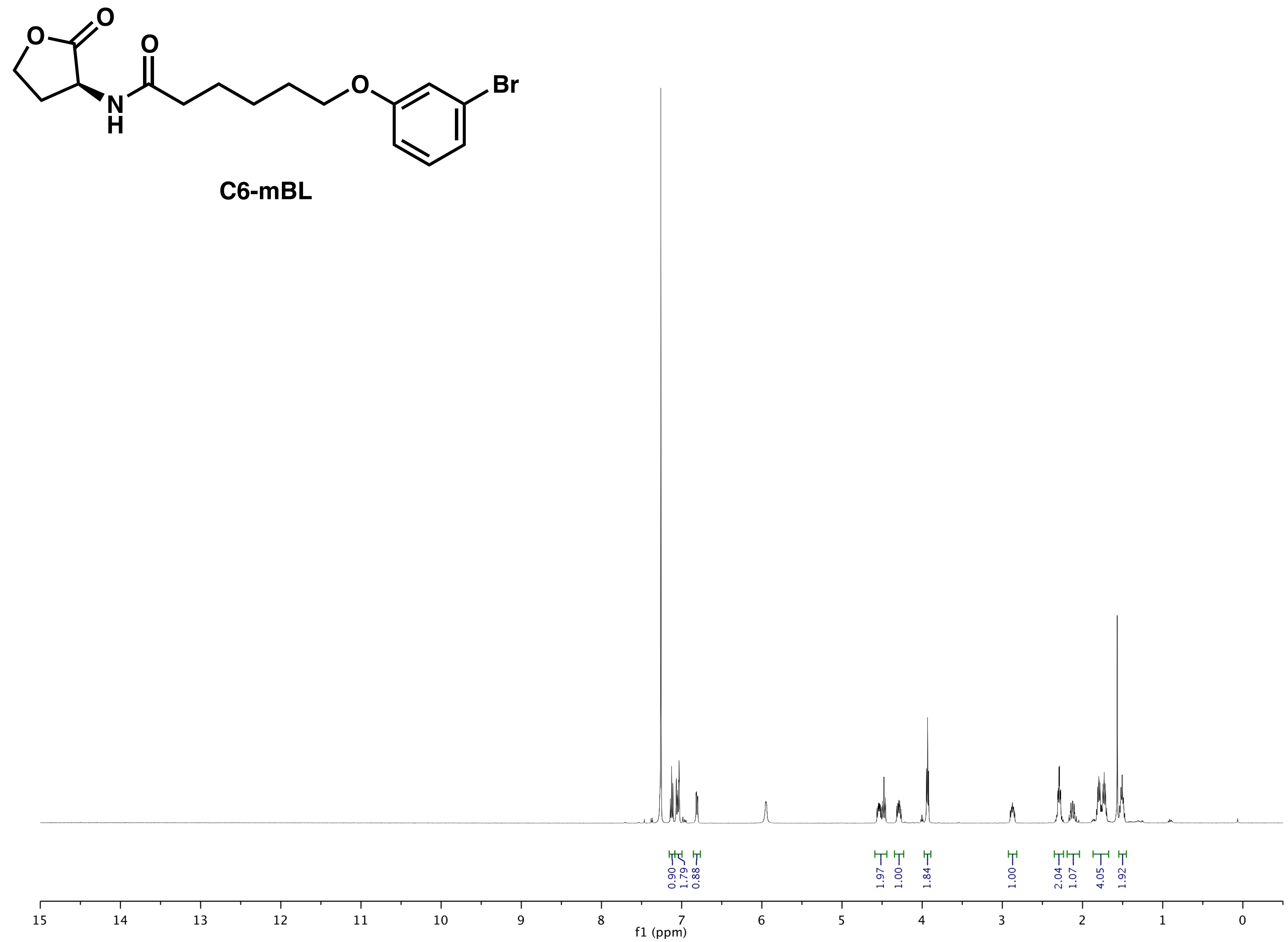
f1 (ppm)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H
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Br
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1.00
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2.04
1.07
4.05
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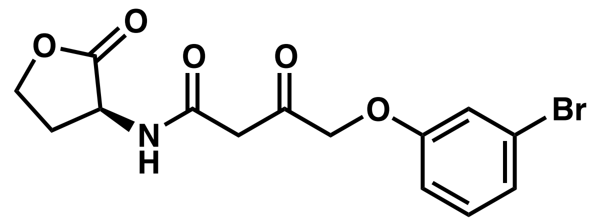
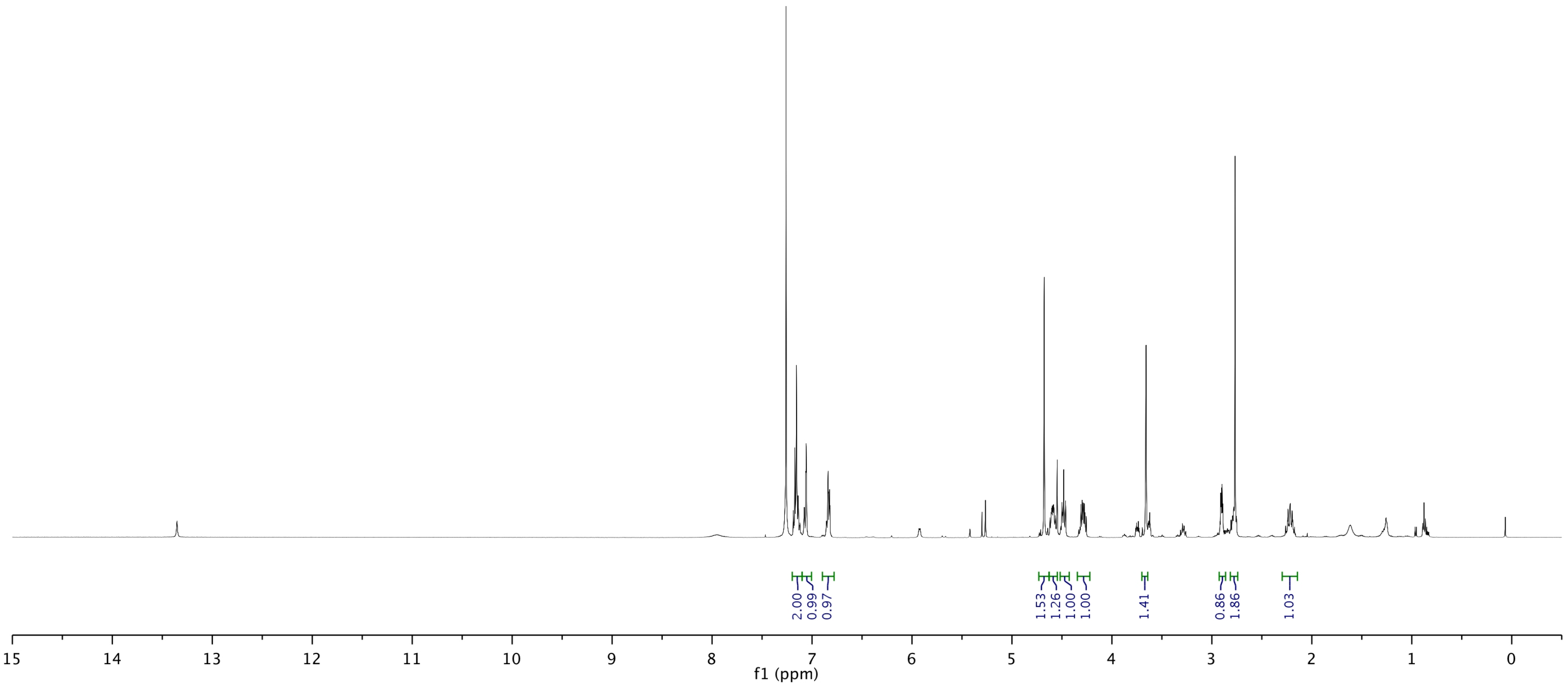
f1 (ppm)



3O-mBL



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1.41
0.86
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2
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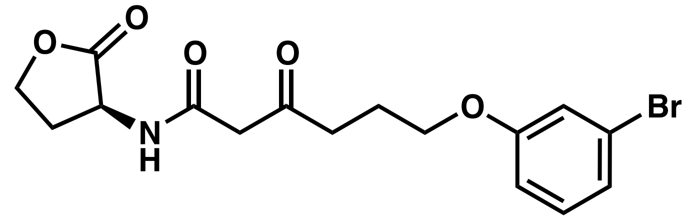
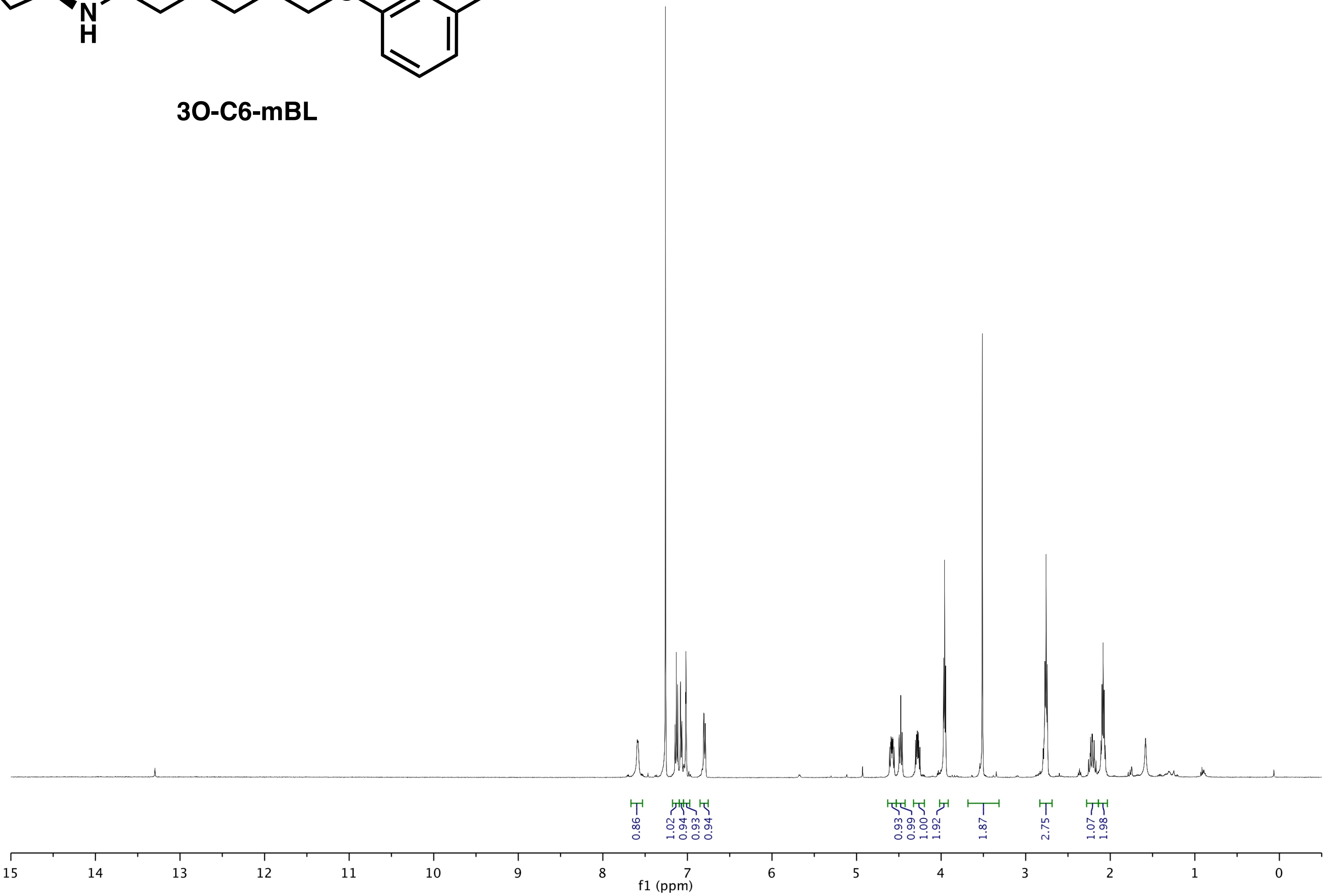
0
f1 (ppm)



3O-C6-mBL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0.94
0.93
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1.00
1.92
1.87
2.75
1.07
1.98
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)