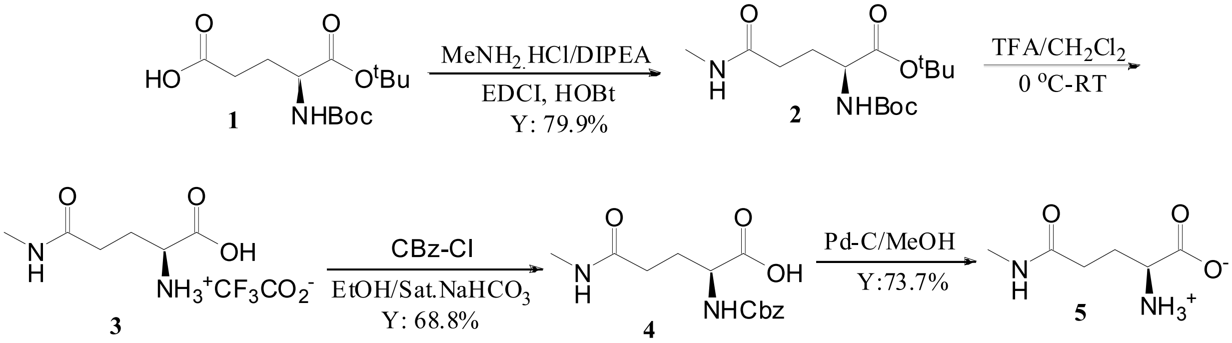
Fig. S1. Gamma-glutamylmethylamide (GMA) chemical synthesis



(S)-tert-butyl 2-((tert-butoxycarbonyl)amino)-5-(methylamino)-5-oxopentanoate (**2**): To a stirred solution of *(S)-5-(tert-butoxy)-4-((tert-butoxycarbonyl)amino)-5-oxopentanoic acid* **1** (2 g, 6.598 mmol) in CH_2Cl_2 (20 mL) at 0°C, 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide (EDCI, 1.517 g, 7.918 mmol) and 1-hydroxybenzotriazole (HOBt monohydrate, 1.211 g, 7.918 mmol) were added. After 10 minutes of stirring, methylamine hydrochloride ($MeNH_2$.HCl, 0.294 g, 9.897 mmol) and diisopropylethylamine (DIPEA, 2.30 mL, 13.196 mmol) were added, and stirring was maintained overnight. The reaction mixture was then diluted with CH_2Cl_2 (20 mL), subsequently washed with saturated aqueous $NaHCO_3$ solution (2 × 10 mL), 1N aqueous $KHSO_4$ solution (2 × 10 mL), water (10 mL), and brine (10 mL), dried over $MgSO_4$, and the solvent concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with 30 to 60% EtOAc in cyclohexane to afford **2** (1.66 g, 79.9%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.24 (brs, 1H), 5.27 (d, *J* = 7.6 Hz, 1H), 4.25-4.08 (m, 1H), 2.85 (d, *J* = 4.8 Hz, 3H), 2.35-2.11 (m, 1H), 2.01-1.79 (m, 1H), 1.49 (s, 9H), 1.48 (s, 9H); ^{13}C NMR (75 MHz $CDCl_3$): δ 172.8, 171.4, 155.9, 82.0, 79.8, 53.6, 32.5, 29.2, 28.2, 27.9, 26.2; ESI-MS: *m/z* 654.9 $[2M+Na]^+$, 339.0 $[M+Na]^+$, 316.9 $[M+H]^+$.

(S)-2-(((benzyloxy)carbonyl)amino)-5-(methylamino)-5-oxopentanoic acid (**4**):

Compound **2** (1.50 g, 4.743 mmol) was treated with trifluoroacetic acid (TFA, 15 mL) at 0 °C and allowed to stir at RT until completion of the reaction. TFA was concentrated under reduced pressure, co-evaporated with cyclohexane, and the residue was dried under high vacuum. The resulting TFA salt **3** was dissolved in ethanol (EtOH, 10 mL) cooled to 0°C, saturated aqueous $NaHCO_3$ (10 mL) followed by benzyl chloroformate (CbZ-Cl, 1.01 mL, 7.114 mmol) were added (reaction mixture P^H = 8-9) and the resulting mixture stirred for 24 h. The aqueous layer was washed with diethyl ether (2x 10 mL), acidified with $KHSO_4$, extracted with ethyl acetate (EtOAc, 4 x 20 mL), dried over $MgSO_4$ and the solvent concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with 80 to 100% EtOAc in cyclohexane to afford **4** (0.96 g, 68.8%) as a solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.49-7.33 (m, 5H), 6.94 (brs, 1H), 6.32 (s, 1H), 6.00 (d, *J* = 6.9 Hz, 1H), 5.13 (s, 2H), 4.36 (dd, *J* = 12.7, 6.8 Hz, 1H), 2.83 (d, *J* = 4.7 Hz, 3H), 2.54-2.32 (m, 2H), 2.31-2.15 (m, 1H), 2.14-1.99 (m, 1H); ^{13}C NMR (75 MHz $CDCl_3$): δ 174.6, 174.3, 156.7, 136.1, 128.5, 128.1, 127.9, 67.0, 32.0, 28.3, 26.4; ESI-MS: *m/z* 610.8 $[2M+Na]^+$, 317.1 $[M+Na]^+$, 295.0 $[M+H]^+$.

(S)-2-ammonio-5-(methylamino)-5-oxopentanoate (**5**): To a stirred solution of compound **4** (0.96 g, 3.264 mmol) in methanol (MeOH, 30 ml) at RT, 10% Pd-C (0.15 g) catalyst was added and the reaction was stirred overnight under a H_2 gas atmosphere. The reaction mixture was filtered over Millipore filter paper, the filter washed subsequently with MeOH (20 mL) and water (30 mL), and the MeOH and water solutions collected separately. The MeOH solution was evaporated, the resulting solid washed in EtOAc (10 mL) under stirring, filtered, dissolved in water (20 mL), combined with the water washing, and lyophilized to afford compound **5** (0.385 g, 73.7%) as a white solid. ^{1}H NMR (300 MHz, D_2O): δ 3.68 (t, *J* = 6.1 Hz, 1H), 2.65 (s, 3H), 2.37-2.28 (m, 2H), 2.10-2.00 (m, 2H); ^{13}C NMR (75 MHz D_2O): δ 175.1, 173.9, 54.1, 31.4, 26.4, 25.9; ESI-MS: *m/z* 161.0 $[M+H]^+$.

Table S1. List of primer sets and PCR specifications

Use and target	Sequence (5' -> 3') [a]		Product size (bp)	Tm (°C)
	Forward primer (localization)	Reverse primer (localization)		
Cloning in pCM80 [b]				
METDI2327	cgattctagaTTTTTCTTTTGTGGCGatgg (2224946)	tgcatctaGATCGAGCAACCGACCTCAT (2226415)	1488	63
METDI4690	agattctagaAATGGGCCACGCGAACGAC (4574476)	tcagtctagaCTAGAACAGGTGGCTGTAGC (4573139)	1394	65
Site directed-mutagenesis [c]				
ΔMETDI2324-2327, left	tgcactgcaGGTTCCTGCACAACCAGATT(2221393)	gatcgcttaCGGCGCCGCCCCGTCCGTT (2221922-2221940)	556	62
ΔMETDI2324-2327, right	gcgccgTGAGCGATCCTCCGGCATCC (2226294)	attagagctCACCGGCGTACCAGAATG (2226775-2226792)	504	67
ΔMETDI2319-2322, left	aactgcaGGGGATCGGCTGTCTCTGG (2214937)	ATGCCGGCCCGACCtacgacat (2215370-2215391)	455	64
ΔMETDI2319-2322, right	ggtcgggccgGCATGGGGTCAGCCGCTCTTG (2220564)	ttggatccGACGGCGACGTCGGTGAG (2221011-2221028)	465	64
ΔMETDI4690, left	atgctcagaAATGGGGTTTCGATGTCCT (4572648)	agcctgaaTAGGCGGAGGCTCCCATGAA (4573103-4573122)	491	64
ΔMETDI4690, right	tccgcctaTTCAGGCTCCTGGCATGGG (4574494)	tgcatctagaAAGCCAAGCCTACCATTCGG (4574968-4574987)	511	64
RT-PCR				
METDI2317/METDI2319	CGAGATCATCAACAAGCCCTA (2214507)	CCGAGTTCGGCTATGAGGTC (2215313)	807	54
METDI2318/METDI2319	GGGATGCTGGCTGGTCTCAC (2214719)	CGACTTACAAACGGCTCTCG (2215732)	1014	61
METDI2319/METDI2320	GCGAGAGCCGTTTGTAAGTC (2215712)	GAAATCAACGGCCAGACCAC (2216293)	582	62
METDI2320/METDI2322	AAGCGTGGTCGTATGTGTCG (2218366)	TTCTGCTTCGCTCACCTGAT (2219445)	1080	60
METDI2322/METDI2323	GCGTCCGAGTGGTACAGGTT (2220194)	GTGCCACCAGCGATAGAGTT (2220962)	769	61
METDI2323/METDI2324	TGCCGGACTTTCCGCTGATG (2220868)	CGGTTCAAGCTTCGGATTCT (2221988)	1141	61
METDI2324/METDI2325	ATGTGCGGTATTGTCGGACT (2221941)	ACGGTCCAGTGGGTCTCGTT (2222970)	1030	61
METDI2325/METDI2326	CTGCGTCGAGAAAGAGATGC (2223406)	CGAGGGCAGGTACTGGTAGA (2224011)	606	61
METDI2326/METDI2327	CACCTGCACAACCTCGAAC (2224795)	GTGCCGAACAGATCGGTGTA (2225077)	283	57
METDI2327/METDI2328	CATGTACACCGACGGACACA (2226089)	AAGAGGATTACGGCCATCAC (2226694)	606	57
METDI2318	GGGATGCTGGCTGGTCTCAC (2214719)	GTGTGTTGAACGGGAAGGCC (2215124)	406	60
METDI2320	CATGACGTAATGGGTCTCG (2216713)	CCGGTCGTTCACCTCGATTC (2217616)	904	59
METDI2323	TGCCGGACTTTCCGCTGATG (2220868)	GTGCAGGTAATGCCGGTGGA (2221625)	758	59
METDI2324	ATGTGCGGTATTGTCGGACT (2221941)	CTCCCACGCGTAGACCTTG (2222837)	897	59
METDI2327	CGCGGCATCAAGTATTTCCT (2225031)	TGTTGCCCGTATAGGTCACG (2225931)	901	60
METDI1560	GCCCTGATAGATCACGAAGG (1425068)	GAGGAGTGGATCTCCGACAA (1425904)	837	56
METDI1773	GCATCACCTCGAACATCACG (1646466)	GTAATCCTCGGCCGGTCCTTC (1647696)	331	60
METDI3639	GCCGACAAGATCATCCAAGA (3582273)	GGTTGTAGAAGCCACGGACT (3582417)	145	55
METDI4690	TCGCCCTCAAGAATGTGCTG (4574140)	TGGTGCGGATGAACTCTTCG (4573213)	966	60

[a], Sequence written in capital case hybrize with *M. extorquens* DM4 chromosome (nucleotide position as defined in MaGe). Sequence in lower case and underligned correspond to restriction sites: tctaga (XbaI); ctgcag (PstI); gagctc (SacI); gacgtc (AatII)

[b], For plasmids pME8280 (*gmaS,* chromosome nucleotide positions 2224946-2226415) and pME8285 (METDI4690, 4574476-4573139)

[c], For construction of plasmids pME8282 (nucleotide positions 4573123-4574493 deletion in the chromosome of strain DM4, strain DM4Δmetdi4690), pME8283 (position 2221941-2226293, strain DM4Δ*mgs-gmaS*) and pME8284 (position 2215392-2220563, mutant DM4Δ*mgd*)

Table S2. Protein comparison of *gmaS* homologs and representatives of the three known types of glutamine synthetase (GS)

Strain	**Homolog affiliation**	**aa identity (%)**		**Length (aa)**	**Accession number**
		METDI2327 [a]	**METDI4690** [b]		
Acidithiobacillus ferrivorans SS3	METDI4690	38	66	453	YP_004784771
Acidithiobacillus ferroxidans	GS type I	31	34	468	P07804
Bacteroides fragilis	GS type III	29	27	729	AAA62314
Clostridium acetobutylicum DSM 1731	GS type III	29	27	696	AEI33778
Coraliomargarita akajimensis DSM 45221	METDI4690	39	67	472	YP_003549260
Cyanobium sp. PCC7001	METDI4690	42	55	461	WP_006911610
Dechloromonas aromatica RCB	METDI4690	40	79	453	YP_283306
Escherichia coli K12	GS type I	29	34	469	AAC76867
Frankia alni	GS type II	22	23	352	AAA62803
Geobacter bemidjiensis	GS type III	26	29	695	YP_002139877
Halothiobacillus neapolitanus c2	METDI4690	40	69	461	YP_003262690
Homo sapiens	GS type II	23	35	373	AAS57904
Hyphomicrobium sp. MC1	METDI4690	58	80	459	YP_004676841
	GMAS	68	39	435	YP_004678139
Methylobacillus flagellatus KT	GMAS	41	42	459	YP_544567
	METDI4690	39	77	453	YP_545964
Methylobacterium extorquens DM4	GMAS	100	39	432	YP_003067875
	GS type I	30	27	481	YP_003068149
	GS type II	25	27	348	YP_003068665
	GS type I	30	26	469	YP_003068666
	METDI4690	39	100	457	YP_003070133
Methylobacterium radiotolerans JCM2831	GMAS	85	40	432	YP_001757450
Methylocella silvestris BL2	GS type I	36	27	469	ACK49401
	GMAS	68	38	435	ACK51558
Methylococcus capsulatus str. Bath	GS type I	29	28	469	P15124
Methyloversatilis universalis FAM5	GMAS	42	42	442	ADH10360
Methylovorus glucosetrophus SIP3-4	GMAS	41	43	454	YP_003052188
	METDI4690	39	78	444	YP_003050724
Methylovorus mays n°9	GMAS	41	43	444	BAF99006
Mycobacterium sp. MCS	METDI4690	41	44	453	YP_638042
	GMAS	44	42	436	YP_642052
	METDI4690	40	51	451	YP_642501
Parvibaculum lavamentivorans DS-1	METDI4690	35	44	458	YP_001412913
Prevotella bryantii B14	GS type III	31	24	729	AAL87245
Pseudomonas stutzeri A1501	METDI4690	42	53	454	YP_001172508
Ralstonia eutropha H16	GMAS	44	43	464	YP_841703
Saccharomyces cerevisiae	GS type II	28	23	346	AAA34644
Sideroxydans lithotrophicus ES-1	METDI4690	40	77	453	YP_003522723
Streptomyces viridochromogenes	GS type II	29	26	343	P19432
Synechococcus sp. CB205	METDI4690	42	53	452	WP_010317406
Synechococcus sp. WH5701	METDI4690	41	54	461	WP_006172961
Synechocystis sp. PCC 6803	GS type III	24	28	724	NP441832
	GS type I	37	36	473	P77961
Thioalkalivibrio sulphidophilus HL-EbGr7	GMAS	42	43	444	YP_002513768
	METDI4690	40	77	455	YP_002515141
Thiomicrospira crunogena XCL-2	GMAS	42	41	444	YP_391616
	METDI4690	41	72	451	YP_392064

[a], *M. extorquens* DM4 (accession number YP_003067875)

[b], *M. extorquens* DM4 (accession number YP_003070133)