

Engineering ribonucleoside triphosphate specificity in a thymidyltransferase

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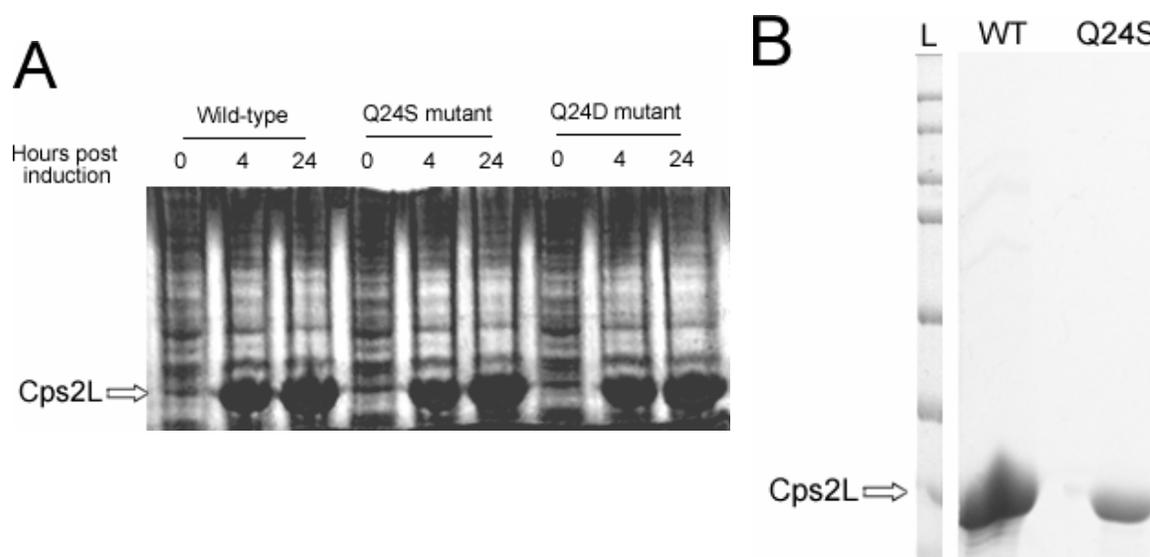
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SDS-page gel of wild-type and mutant Cps2L catalysts



Panel A shows over-expression of the wild-type and mutant catalysts and panel B show a representative purification of the Cps2L Q24S mutant.

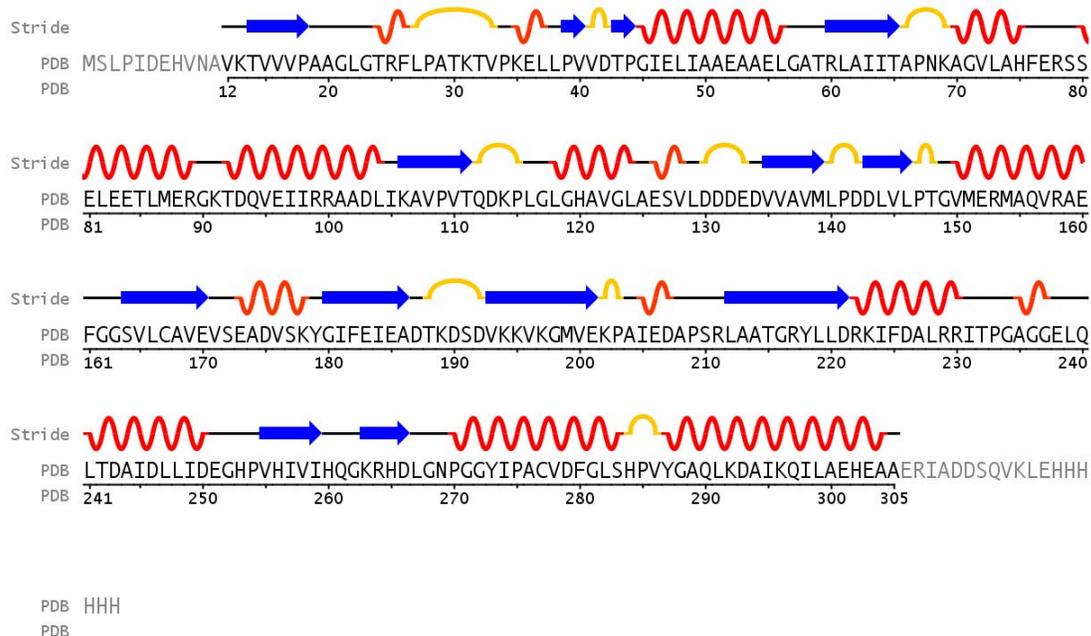
Retention times for sugar nucleotide products

Sugar-1-Phosphate	NTP	sugar nucleotide retention time (min)	Conversion (%)	Time
α -D-Glucose-1-Phosphate	ATP	5.4	6	72 h, 0.2 EU
α -D-Glucose-1-Phosphate	CTP	4.5	27	73 h, 0.2 EU
α -D-Glucose-1-Phosphate	GTP	5.3	51	74 h, 0.2 EU
3-O-(2Ethylbutyl)- α -D-Glucose-1-Phosphate	dTTP	9.6	96	48 h
3-O-(2-MethylPropyl)- α -D-Glucose-1-Phosphate	dTTP	7.4	98	48 h
3-O-Butyl- α -D-Glucose-1-Phosphate	dTTP	7.5	99	48 h
3-O-Dodecyl- α -D-Glucose-1-Phosphate	dTTP	12.8	97	48 h
3-O-Hexadecyl- α -D-Glucose-1-Phosphate	dTTP	12.4	33	48 h
3-O-Hexyl- α -D-Glucose-1-Phosphate	dTTP	*		
3-O-Methyl- α -D-Glucose-1-Phosphate	dTTP	5.6	98	48 h
3-O-Octyl- α -D-Glucose-1-Phosphate	dTTP	8.2	99	48 h
3-O-(2Ethylbutyl)- α -D-Glucose-1-Phosphate	UTP	9.2	39	48 h
3-O-(2-MethylPropyl)- α -D-Glucose-1-Phosphate	UTP	7.0	51	48 h

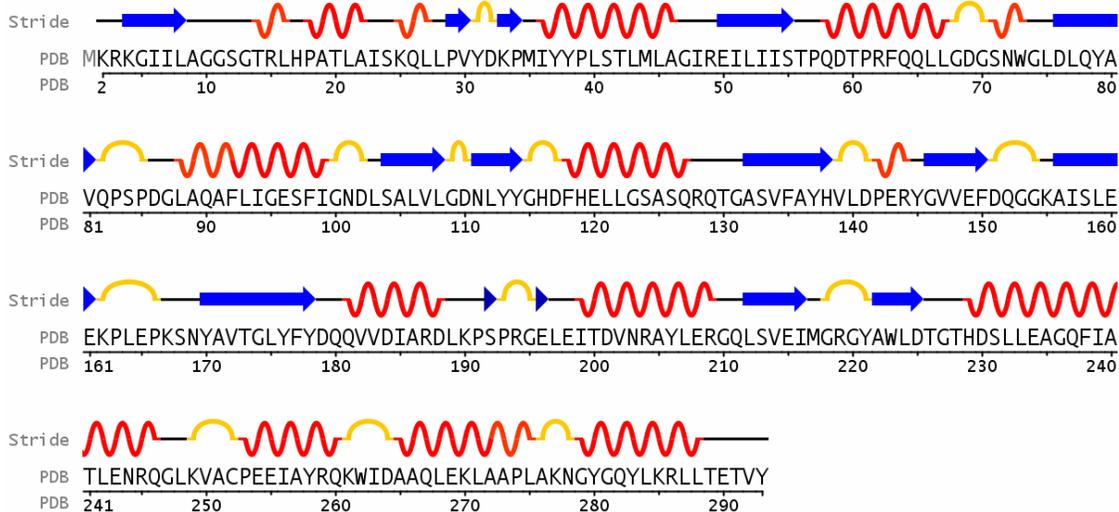
3-O-Butyl- α -D-Glucose-1-Phosphate	UTP	7.1	50	48 h
3-O-Dodecyl- α -D-Glucose-1-Phosphate	UTP	12.2	73	48 h
3-O-Hexadecyl- α -D-Glucose-1-Phosphate	UTP	9.5	64	48 h
3-O-Hexyl- α -D-Glucose-1-Phosphate	UTP	*		
3-O-Methyl- α -D-Glucose-1-Phosphate	UTP	5.3	97	48 h
3-O-Octyl- α -D-Glucose-1-Phosphate	UTP	8.1	27	48 h
α/β -D-6Fluoro-Galactofuranose-1-Phosphate	dTTP	5.9	51	24 h
α/β -D-Fucofuranose-1-Phosphate	dTTP	5.6	23	24 h
α/β -D-Galactofuranose-1-Phosphate	dTTP	5.7	98	24 h
α/β -D-Glucofuranose-1-Phosphate	dTTP	-		
α/β -L-Arabinofuranose-1-Phosphate	dTTP	5.7	51	24 h
α/β -D-6Fluoro-Galactofuranose-1-Phosphate	UTP	-		
α/β -D-Fucofuranose-1-Phosphate	UTP	4.5	4	24 h
α/β -D-Galactofuranose-1-Phosphate	UTP	5.5	9	24 h
α/β -D-Glucofuranose-1-Phosphate	UTP	-		
α/β -L-Arabinofuranose-1-Phosphate	UTP	5.6	50	24 h
α -D-Galactose-1-Phosphate	dTTP	5.6	100	24 h
α -D-Glucose-1-Phosphate	dTTP	5.7	100	24 h
α -D-Mannose-1-Phosphate	dTTP	5.6	100	24 h
α -D-Glucosamine-1-Phosphate	dTTP	2.7	100	24 h
N-Acetyl- α -D-Glucosamine-1-Phosphate	dTTP	5.6	94	24 h
α -D-Galactose-1-Phosphate	UTP	5.5	27	24 h
α -D-Glucose-1-Phosphate	UTP	5.5	96	24 h
α -D-Mannose-1-Phosphate	UTP	5.4	57	24 h
α -D-Glucosamine-1-Phosphate	UTP	2.4	98	24 h
N-Acetyl- α -D-Glucosamine-1-Phosphate	UTP	5.5	37	24 h

* co-eluted with NDP
- no observed
conversion

Sequence analysis of *Corynebacteria Glutamicum* uridylyltransferase (PDB: 2pa4)



Sequence analysis of *P. aeruginosa* thymidyltransferase (PDB: 1g1l)



Sequence alignment of representative prokaryotic uridylyltransferases and thymidylyltransferases

CLUSTAL 2.0.5 multiple sequence alignment

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Paenibacillus sp.          -----MTKVKKAIIPAAAGLGRFLPATKAMPKEMLPIVDKPTIQYIVEEVAAGIED 52
Lactobacillus johnsonii   -----MKVVRKAVIPAAAGLGRFLPATKAMPKEMVPIVDKPTIQFIVEEAKKSGIED 51
C. glutamicum             MSLPIDEHVNAVKTVVVPAAGLGRFLPATKTVPKELLPVVDTPGIELIAAAEAEELGATR 60
S. pneumoniae (Cps2L)    -----MKGIILAGGSGTRLYPLTRATSKQLMPVYDKPMIYYPLSTLMLAGIKD 48
S. mutans (RmlA)         -----MKGIILAGGSGTRLYPLTRAASKQLMPVYDKPMIYYPLSTLMLAGIKD 48
P_Aeruginosa (RmlA)      -----MKRKGIIILAGGSGTRLHPATLAISKQLLPVYDKPMIYYPLSTLMLAGIRE 50
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Paenibacillus sp.        I I I V T G K G K R A I E D H F D S A F E L E H N L M E K G K L T L L D E V Q K S S -- R V D I H Y I R Q K E A K G L G 110
Lactobacillus johnsonii  I L I V T G K N K R S I E D H F D A N F E L E Q D L E E K G K T E L L H L T Q S I T N L G V N L Y Y T R Q P H P A G L G 111
C. glutamicum            L A I I T A P N K A G V L A H F E R S S E L E E T L M E R G K T D Q V E I I R R A A D - L I K A V P V T Q D K P L G L G 119
S. pneumoniae (Cps2L)   I L I I S T P ----- Q D L P R F K D L L L D G S E F G I R L S Y A E Q P S P D G L A 87
S. mutans (RmlA)        I L I I S T P ----- Q D L P R F K E L L Q D G S E F G I K L S Y A E Q P S P D G L A 87
P_Aeruginosa (RmlA)     I L I I S T P ----- Q D T P R F Q Q L L G D G S N W G L D L Q Y A V Q P S P D G L A 89
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Paenibacillus sp.        H A V W C A R N F I G D E P -- F A V L L G D D I V D S E V P C T K Q L M D Q F E Q T G R S V I G V K P V P T N E T E R 168
Lactobacillus johnsonii  D A I L R A R S F V G D E P -- F A V M L G D D L M E D K V P L T K Q L I D R Y D K T H A S T I A V M P V P H E E V S K 169
C. glutamicum            H A V G L A E S V L D D D E V V A V M L P D D L V L P T G - V M E R M A Q V R A E F G G S V L C A V E V S E A D V S K 178
S. pneumoniae (Cps2L)   Q A F L I G E D F I G D D S -- V A L I L G D N I Y H G P G L S K M L Q K T V S K E K G A T V F G Y Q V K --- D P E R 142
S. mutans (RmlA)        Q A F I G E E F I G D D H -- V A L I L G D N I Y G P G L S R M L Q K A A S K E S G A T V F G Y Q V K --- D P E R 142
P_Aeruginosa (RmlA)     Q A F L I G E S F I G N D L -- S A L V L G D N L Y Y G H D F H E L L G S A S Q R Q T G A S V F A Y H V L --- D P E R 144
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Paenibacillus sp.        Y G I V E Y - S E K N G - L L S L V D R F I E K P T P G T T E S N L A I M G R Y V L T P D I F K F L S K Q E K G A G G E 226
Lactobacillus johnsonii  Y G V I D P D S E I E P - G L I N V K A F V E K P D V D K A P S D Y A I I G R Y L L T P E I F D I L A H Q K P G R G G E 228
C. glutamicum            Y G I F E I E A D T K D S V K K V K G M V E K P A I E D A P S R L A A T G R Y L L D R K I F D A L R R I T P G A G G E 238
S. pneumoniae (Cps2L)   F G V V E F D E N M N A ----- I S I E E K P -- E C P R S N Y A V T G L Y F Y D N D V V E I A K S I K P S A R G E 194
S. mutans (RmlA)        F G V V E F D N D R N A ----- I S I E E K P -- E H P K S H Y A V T G L Y F Y D N S V V D I A K N I K P S P R G E 194
P_Aeruginosa (RmlA)     Y G V V E F D Q G G K A ----- I S L E E K P -- L E P K S N Y A V T G L Y F Y D Q Q V V D I A R D L K P S P R G E 196
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Paenibacillus sp.        I Q L T D A I Q K L N E S ----- Q G V Y A Y E F E G R R F D V G E K L G F I T T T L D Y A L R N ---- E S L 274
Lactobacillus johnsonii  I Q L T D A I D T M N K T ----- Q R V F A H V F N G E R H D V G N K E G Y L E T S I E Y G L S H ---- P Q I 276
C. glutamicum            L Q L T D A I D L L I D E G ----- H P V H I V I H Q G K R H D L G N P G G Y I P A C V D F G L S H P V Y G A Q L 291
S. pneumoniae (Cps2L)   L E I T D V N K A Y L D R G N L S V E V M G R G F A W L D T G T H E S L L E A S Q Y I E T V Q R M Q N V Q V A N L E E I 254
S. mutans (RmlA)        L E I T D V N K A Y L D R G D L S V E V M R G F A W L D T G T H E S L L E A A Q Y I E T V Q R M Q N L Q V A N L E E I 254
P_Aeruginosa (RmlA)     L E I T D V N R A Y L E R G Q L S V E I M G R Y A W L D T G T H D S L L E A G Q F I A T L E N R Q G L K V A C P E E I 256
                          : : * * . . . . * : : : : : : : : :

Paenibacillus sp.        K G P L L E ----- A M K E I L E R E L I H K F K ----- 295
Lactobacillus johnsonii  K D D L R E Y ---- I I N M A Q K L Q K E D K N S K K K ----- 301
C. glutamicum            K D A I K Q ---- I L A E H E A A E R I A D D S Q V K ----- 315
S. pneumoniae (Cps2L)   A Y R M G Y I S R E D V L E L A Q P L K K N E Y G Q Y L L R L I G E V -- 289
S. mutans (RmlA)        A Y R M G Y I T A D Q V R E L A Q P L K K N E Y G Q Y L L R L I G E V -- 289
P_Aeruginosa (RmlA)     A Y R Q K W I D A A Q L E K L A A P L A K N G Y G Q Y L K R L L T E T V Y 293

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ESI-MS/MS EPI Scan Data of Enzyme-Catalyzed Production of UDP-furanoses

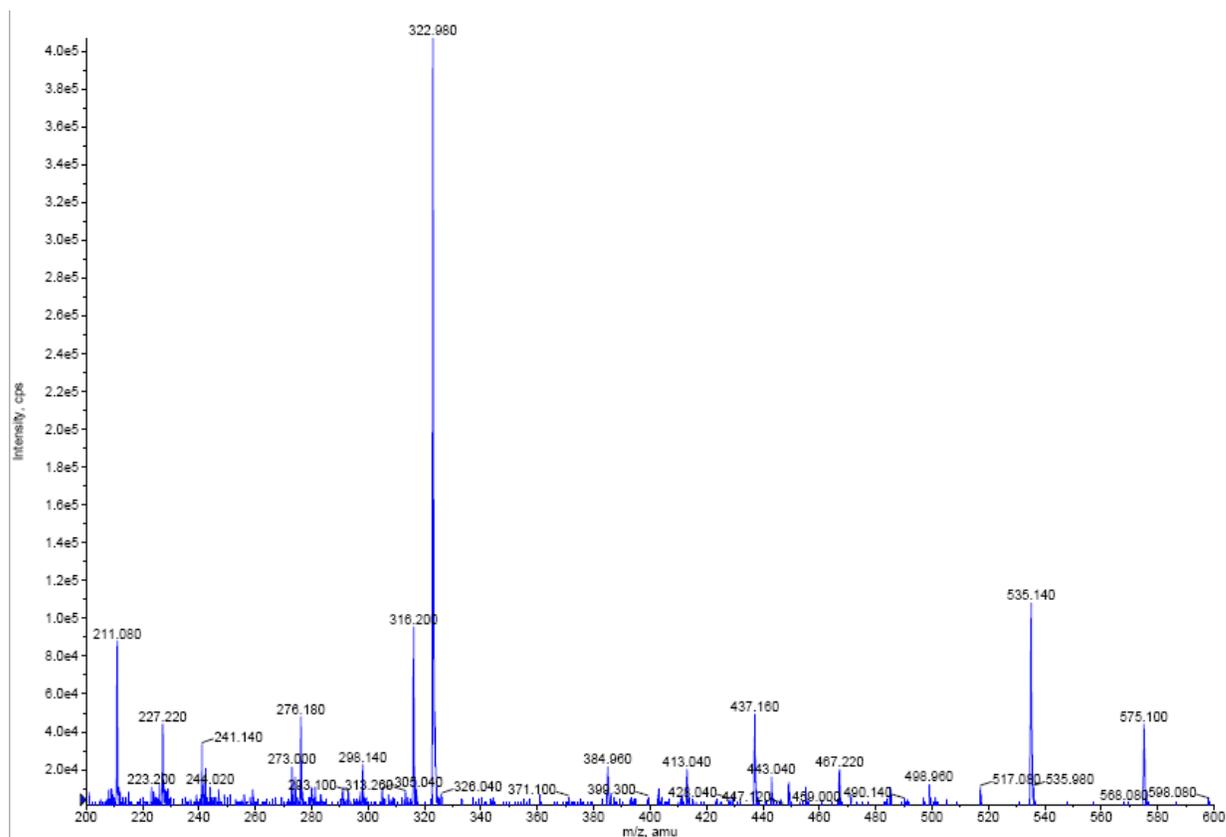


Figure S1: Cps2L Q24S + α/β -L-arabinofuranose-1-phosphate + UTP

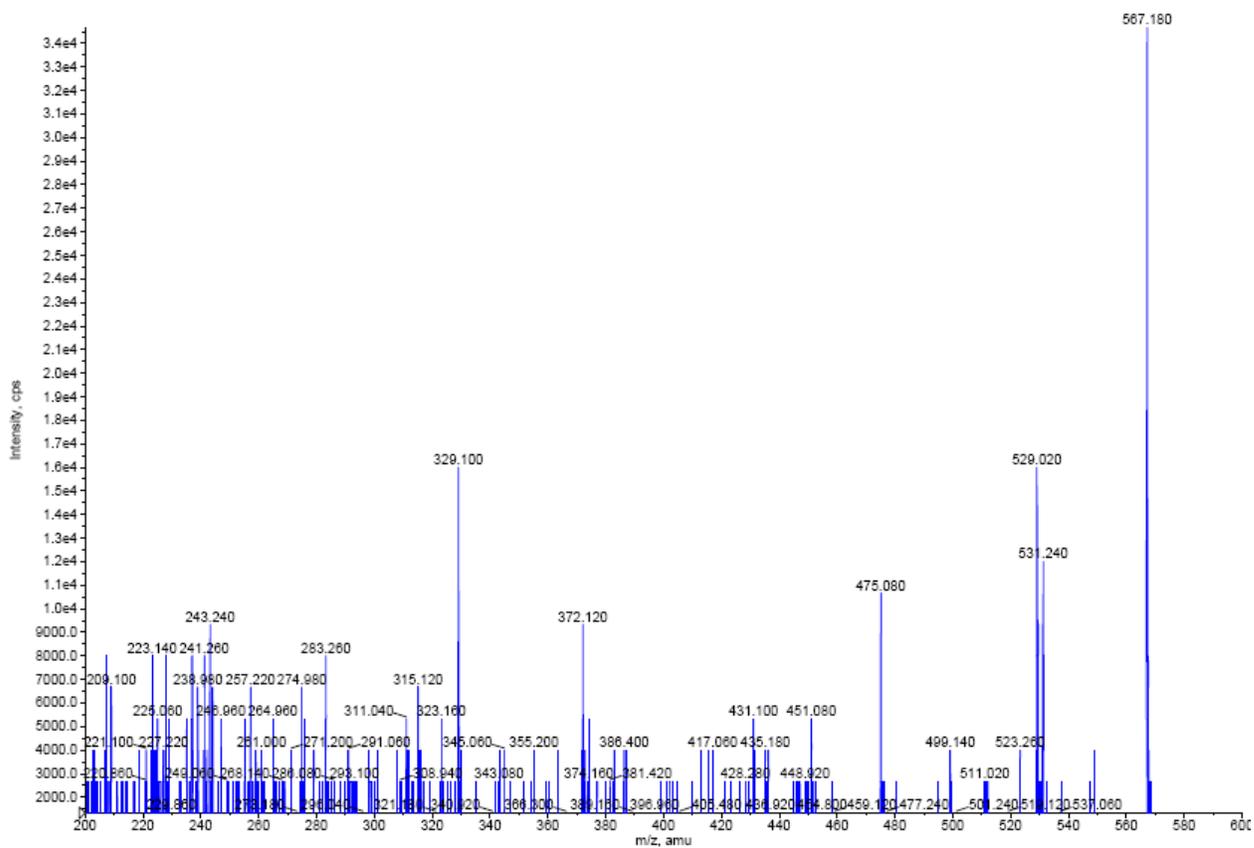


Figure S2: Cps2L Q24S + α/β -D-6-deoxy-6-fluorogalactofuranose-1-phosphate + UTP

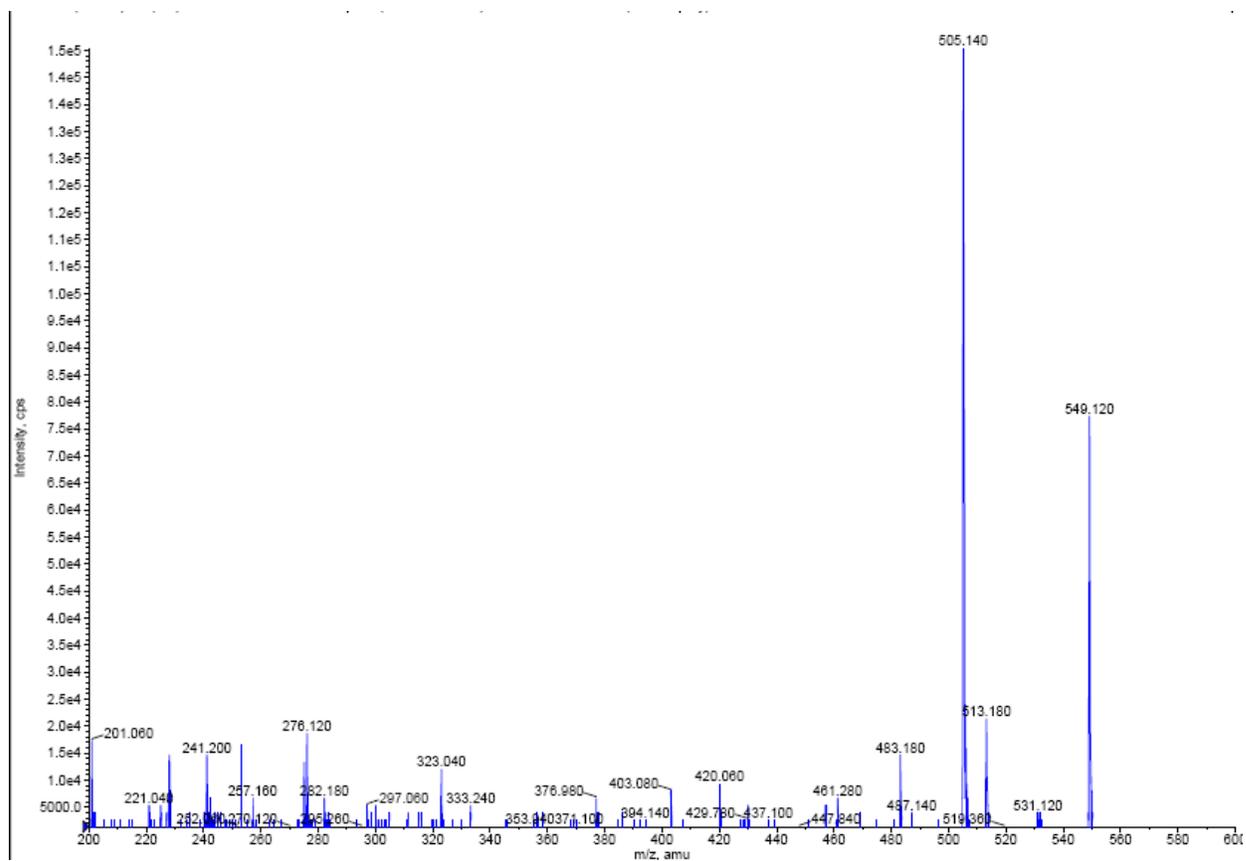


Figure S3: Cps2L Q24S + α/β -D-6-deoxygalactofuranose-1-phosphate + UTP

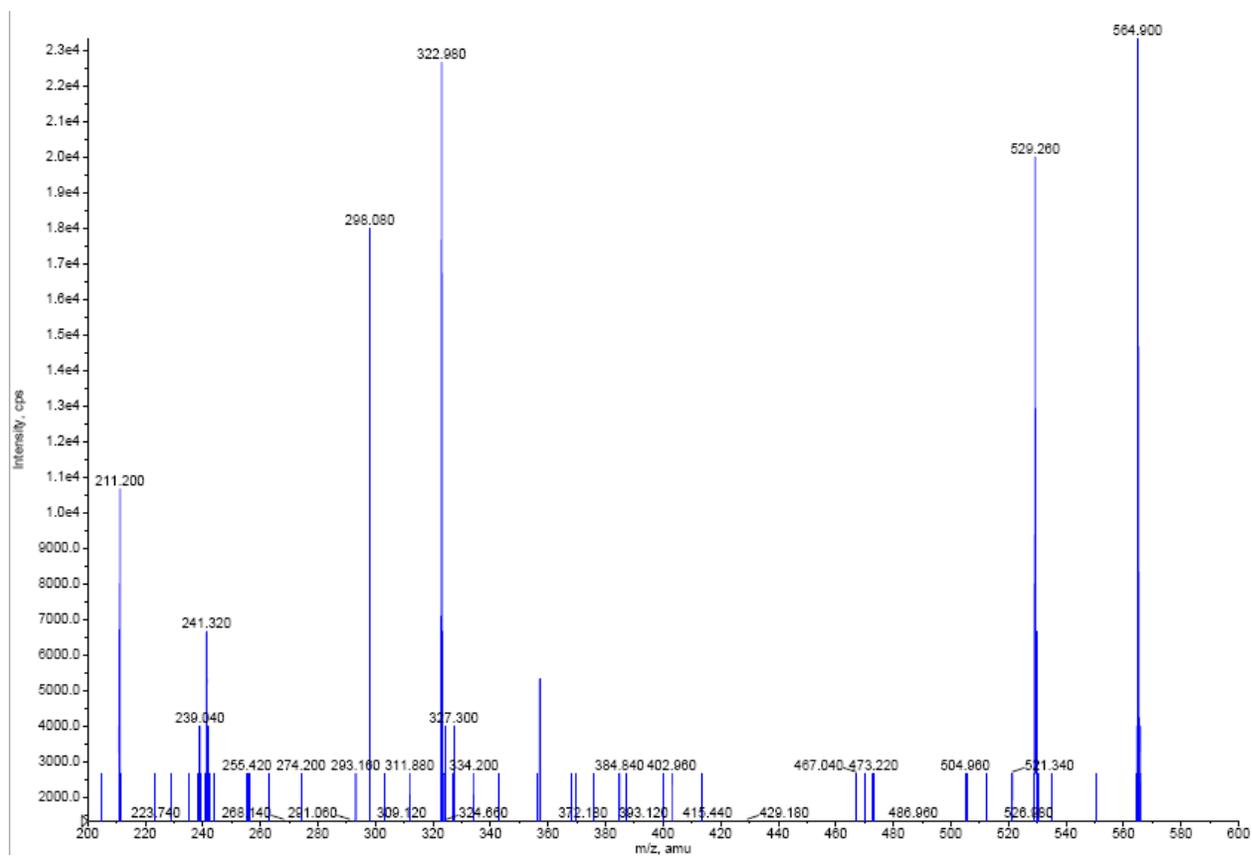


Figure S4: Cps2L Q24S + α/β -D-galactofuranose-1-phosphate + UTP

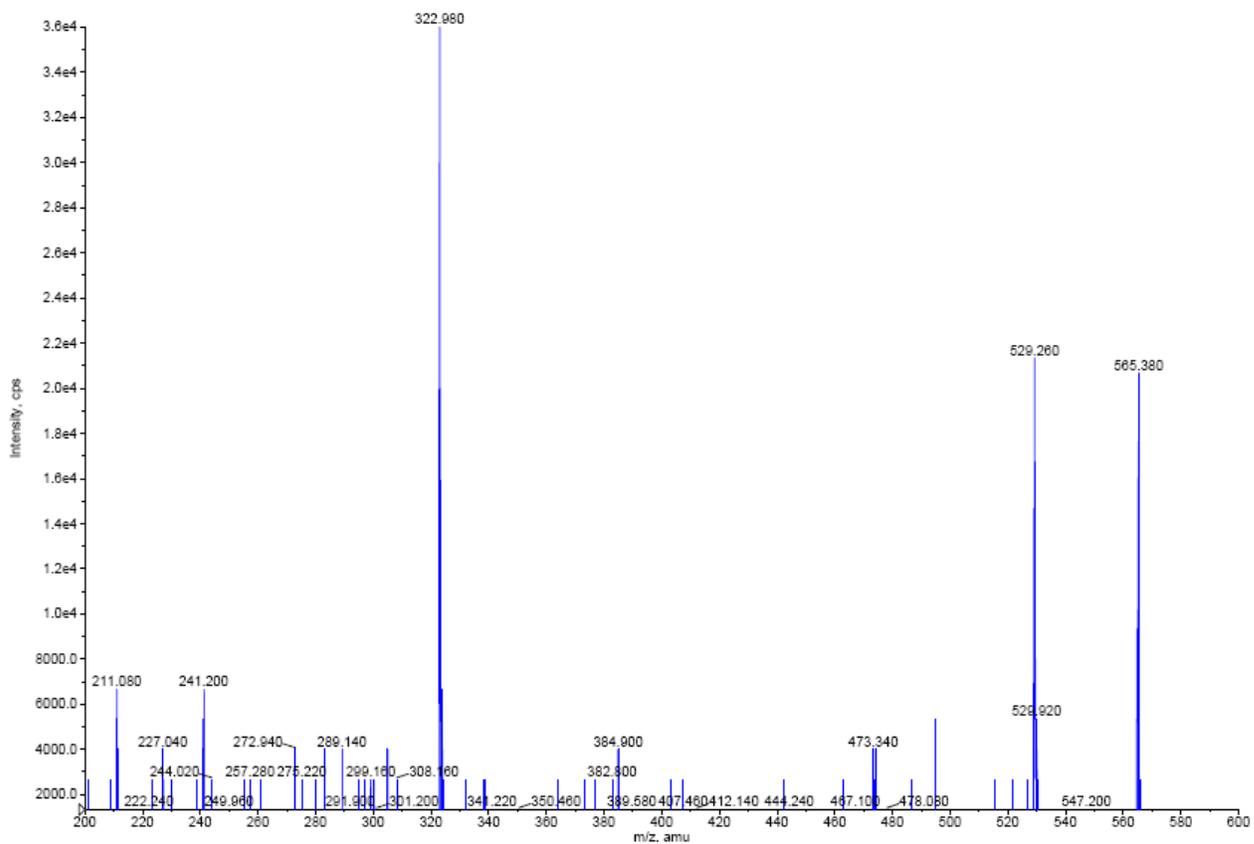


Figure S5: Cps2L Q24S + α/β -D-glucofuranose-1-phosphate + UTP