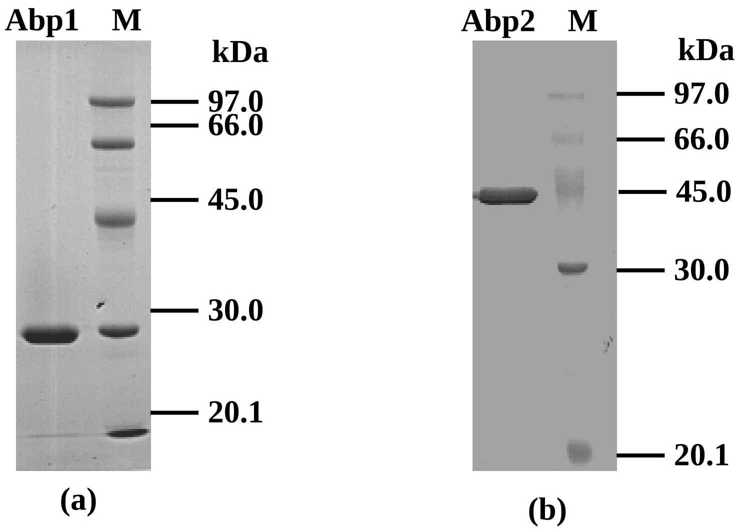
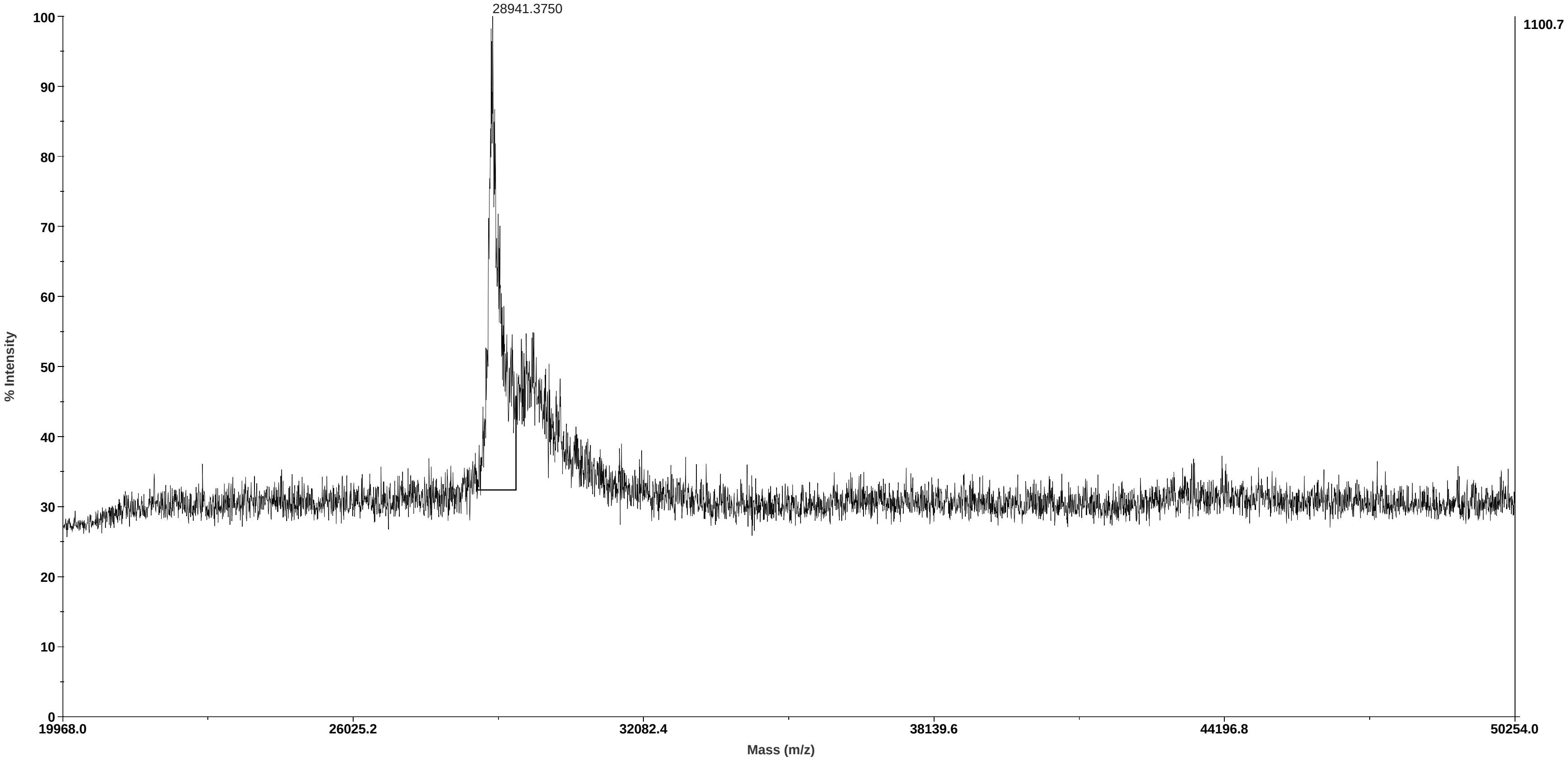


Figure S1. Purified Abp1 (a) and Abp2 (b) after SDS-polyacrylamide gel electrophoresis. Proteins were denatured at 100°C for 5 min in 0.1% SDS and 1% 2-mercaptoethanol before being loaded onto a 5% (w/v) stacking gel and separated in a 12% (w/v) separation gel. Gels were stained with Coomassie bright blue R250. The molecular weight markers from the LMW-SDS marker kit (lane M in (a) and (b), GE Healthcare) are indicated to the right of the panel.

(a)


28941.3750
1100.7
% Intensity
100
90
80
70
60
50
40
30
20
10
0
19968.0
26025.2
32082.4
38139.6
44196.8
50254.0
Mass (m/z)

(b)

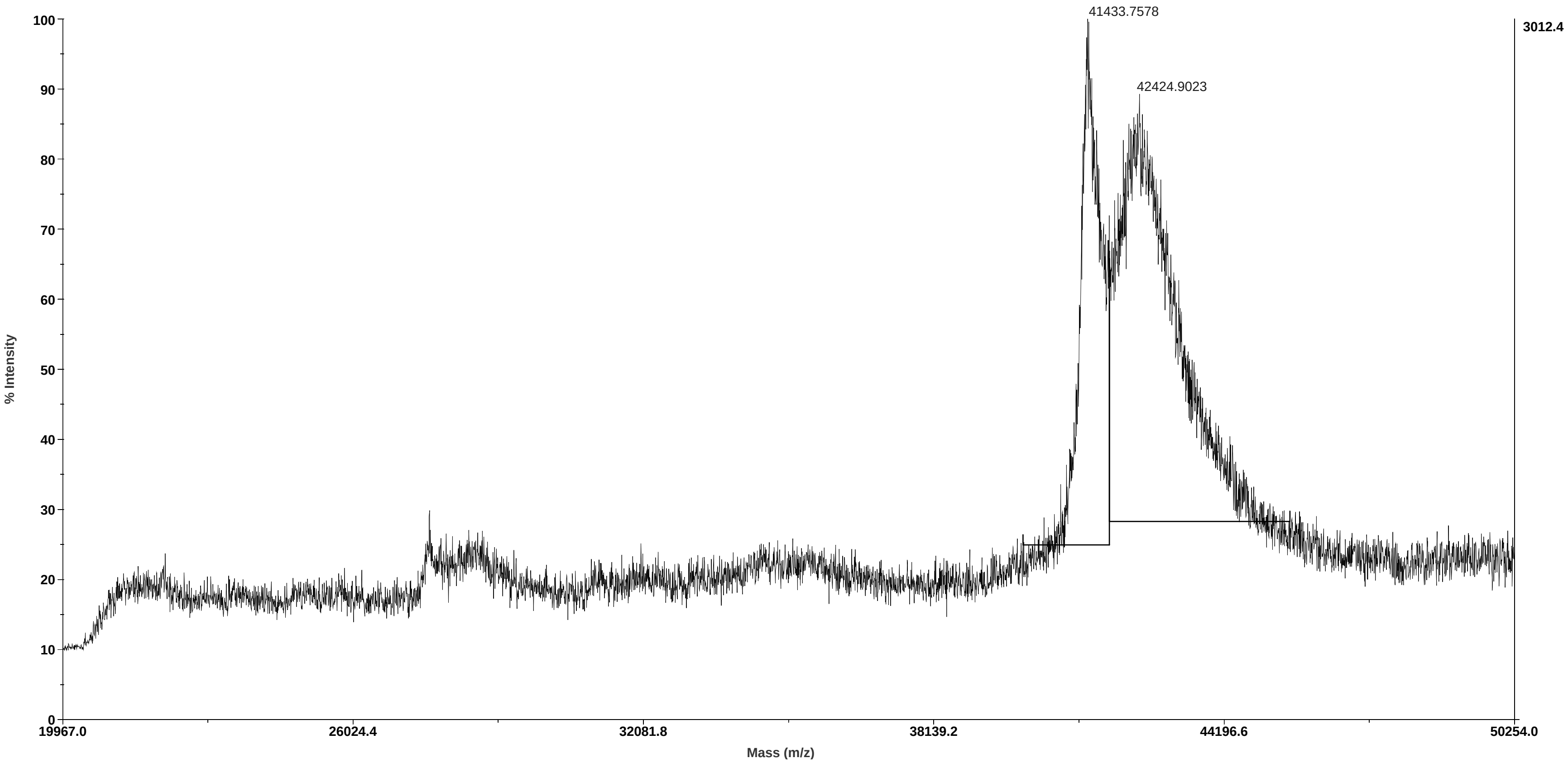
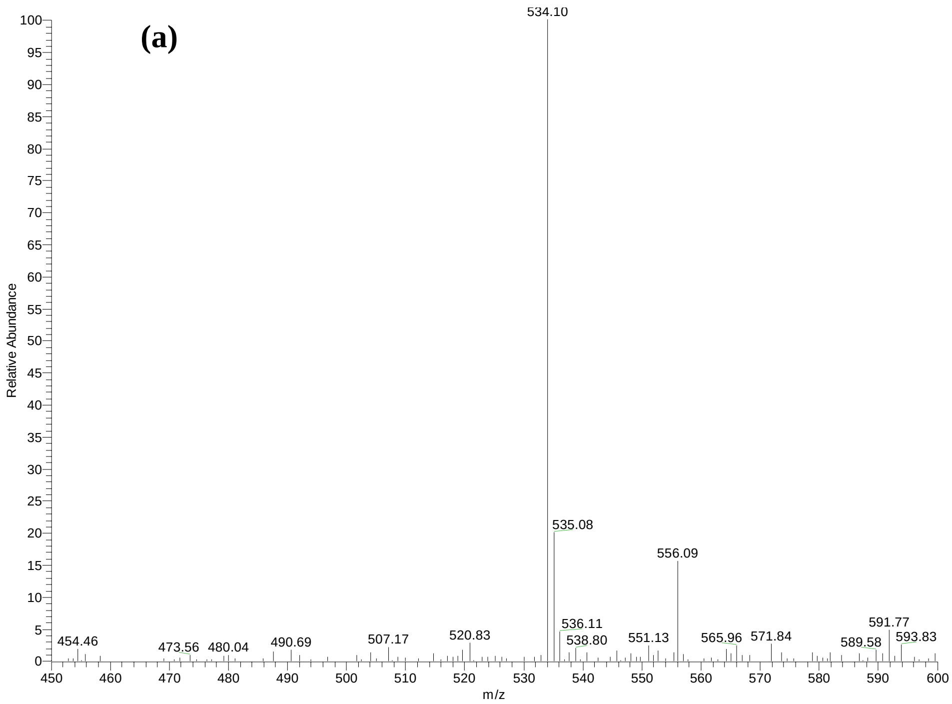
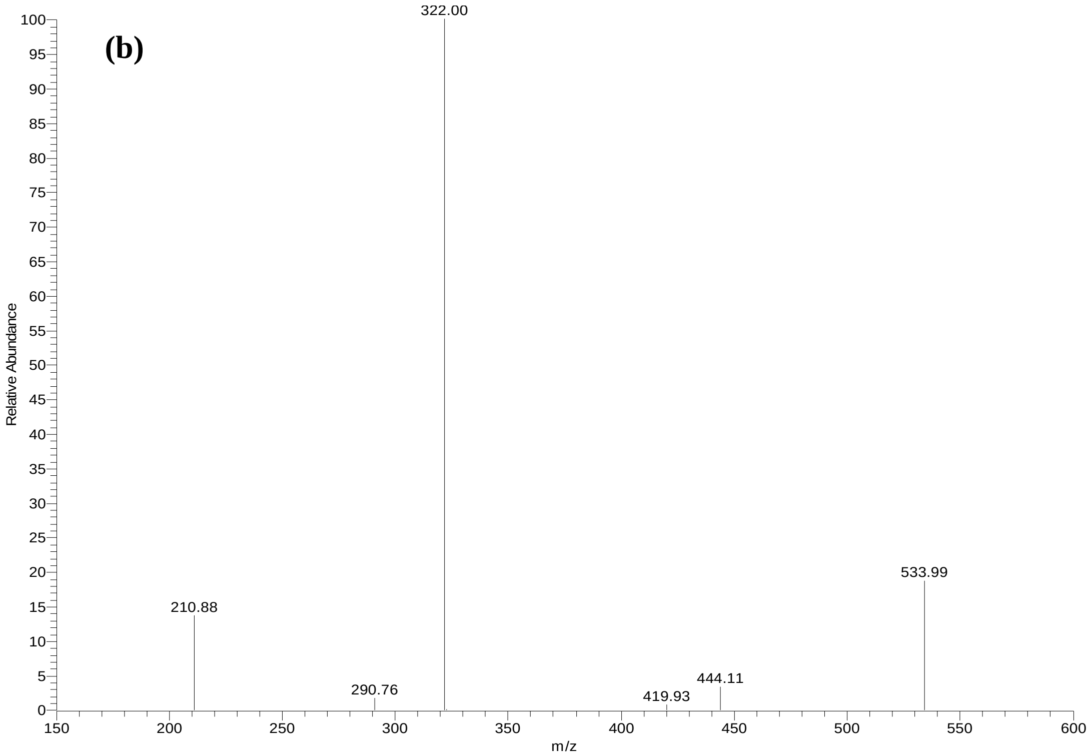


Figure S2. Molecular masses analysis of His_6-tagged Abp1 (a) and Abp2 (b).


(a)
Relative Abundance
m/z
534.10
535.08
556.09
536.11
538.80
551.13
565.96
571.84
591.77
593.83
589.58
454.46
473.56
480.04
490.69
507.17
520.83
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
450
460
470
480
490
500
510
520
530
540
550
560
570
580
590
600


(b)
Relative Abundance
m/z
322.00
533.99
210.88
290.76
419.93
444.11
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
150
200
250
300
350
400
450
500
550
600

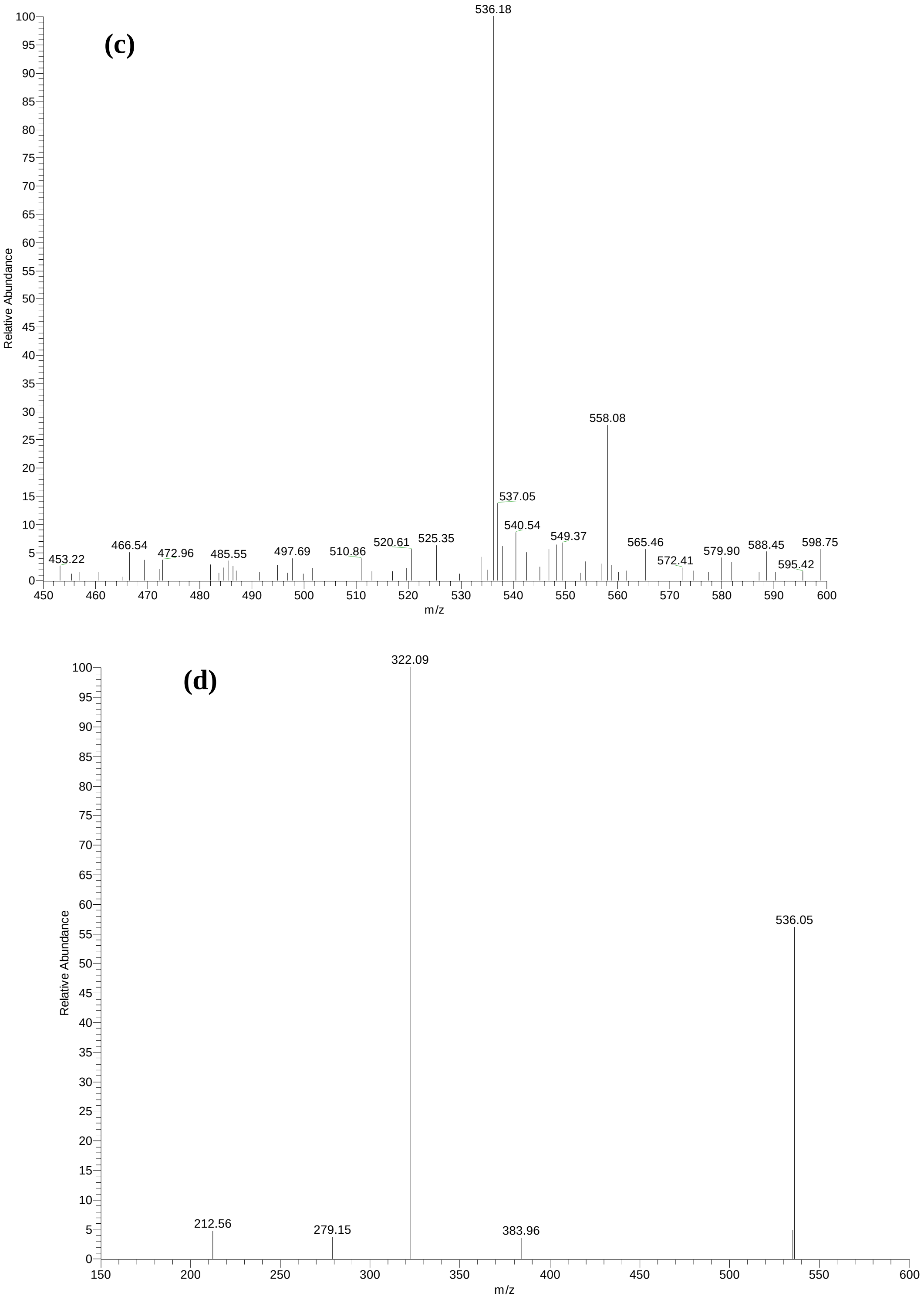


Figure S3. ESI MS of CDP-D-xylulose (a), MS2 analysis of the ion with *m/z* 534.10 (b), ESI MS of CDP-D-arabinitol (c), and MS2 analysis of the ion with *m/z* 536.18 (d).

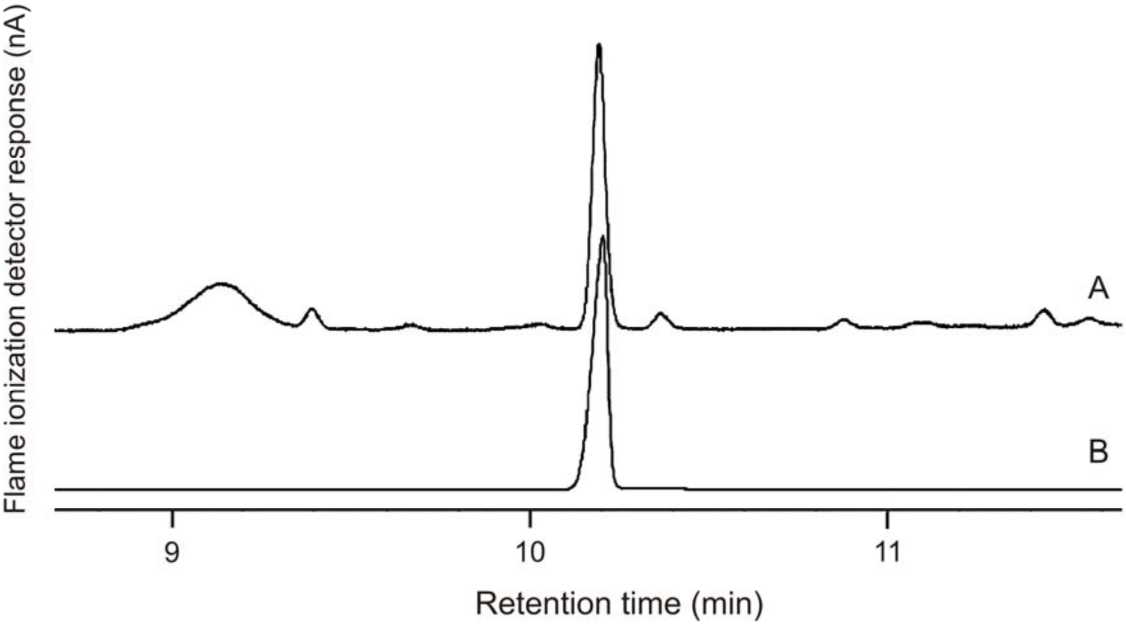
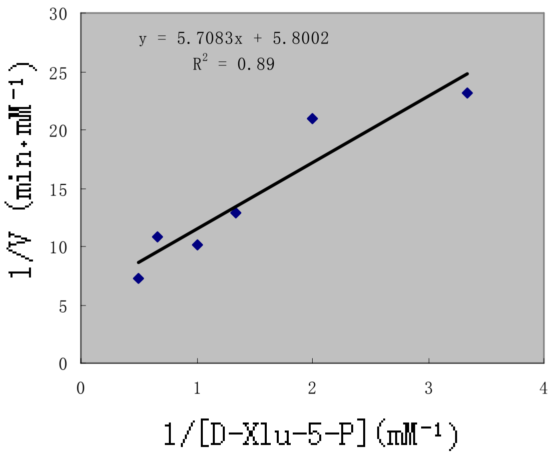
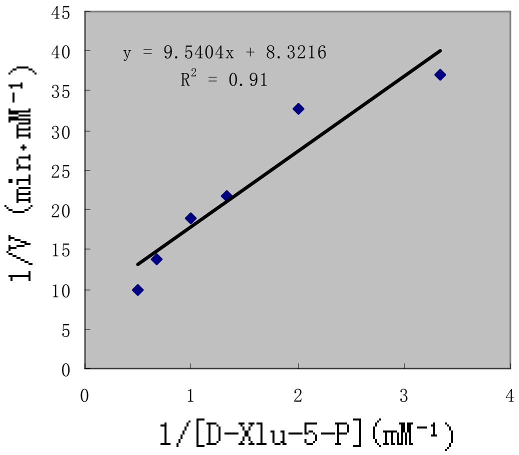


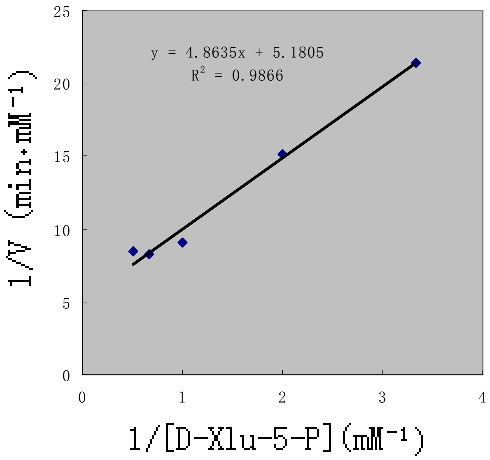
Figure S4. GLC analysis of the alditol acetates derived from the Abp2 product (A) and an authentic sample of arabinitol (B).


y = 5.7083x + 5.8002
R2 = 0.89
1/V (min·mM-1)
0
5
10
15
20
25
30
0
1
2
3
4
1/[D-Xlu-5-P](mM-1)

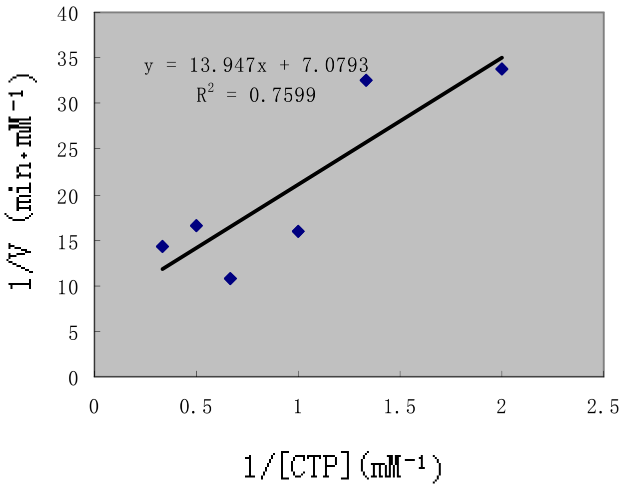
(1)


y = 9.5404x + 8.3216
R2 = 0.91
1/V (min·mM-1)
0
5
10
15
20
25
30
35
40
45
0
1
2
3
4
1/[D-Xlu-5-P](mM-1)

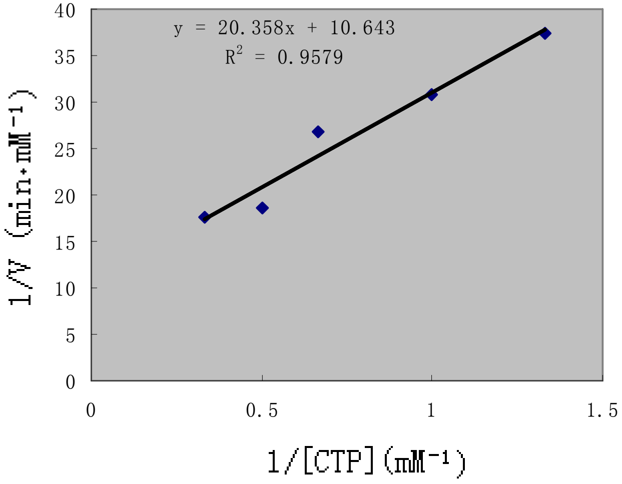
(2)


y = 4.8635x + 5.1805
R2 = 0.9866
1/V (min·mM-1)
0
5
10
15
20
25
0
1
2
3
4
1/[D-Xlu-5-P](mM-1)

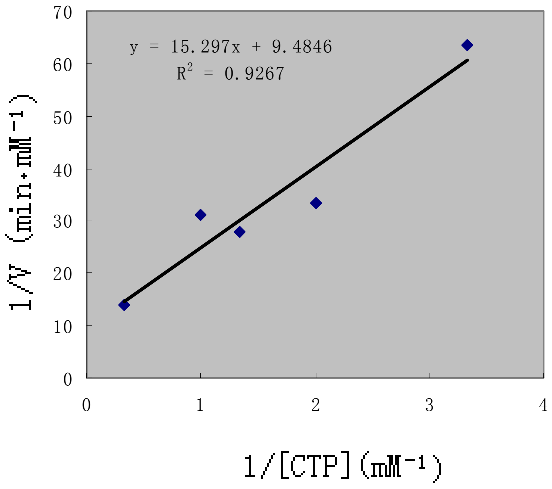
(3)


y = 13.947x + 7.0793
R2 = 0.7599
1/V (min·mM-1)
0
5
10
15
20
25
30
35
40
0
0.5
1
1.5
2
2.5
1/[CTP](mM-1)

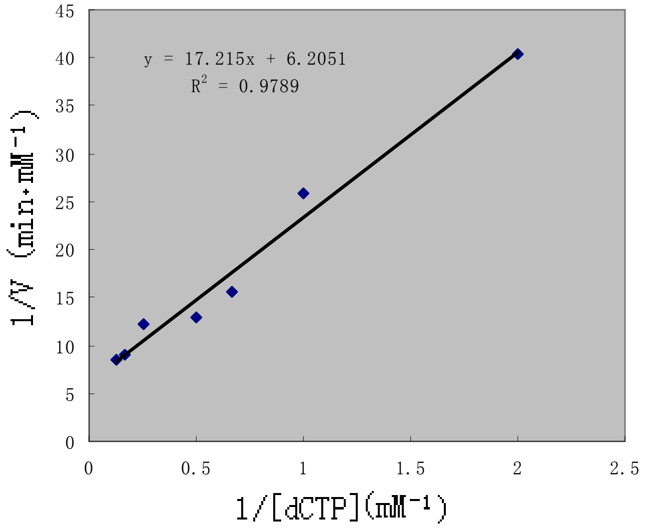
(4)


y = 20.358x + 10.643
R2 = 0.9579
1/V (min·mM-1)
0
5
10
15
20
25
30
35
40
0
0.5
1
1.5
1/[CTP](mM-1)

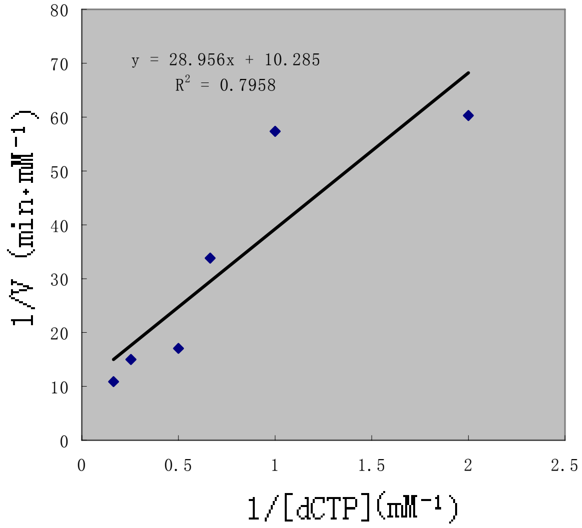
(5)


y = 15.297x + 9.4846
R2 = 0.9267
1/V (min·mM-1)
0
10
20
30
40
50
60
70
0
1
2
3
4
1/[CTP](mM-1)

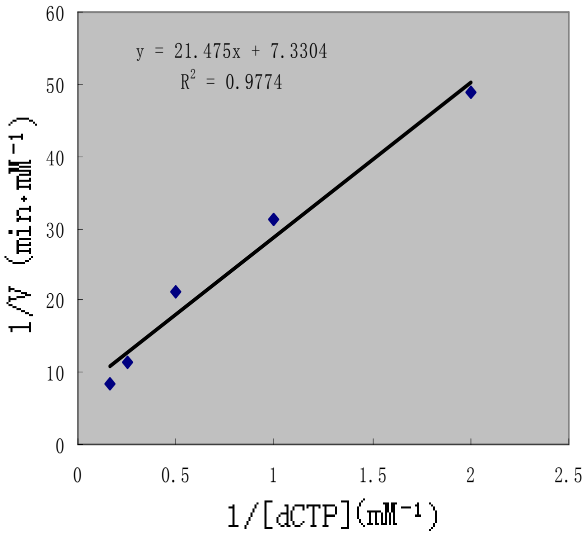
(6)


y = 17.215x + 6.2051
R² = 0.9789
1/V (min•mM⁻¹)
1/[dCTP](mM⁻¹)
0
5
10
15
20
25
30
35
40
45
0.5
1
1.5
2
2.5

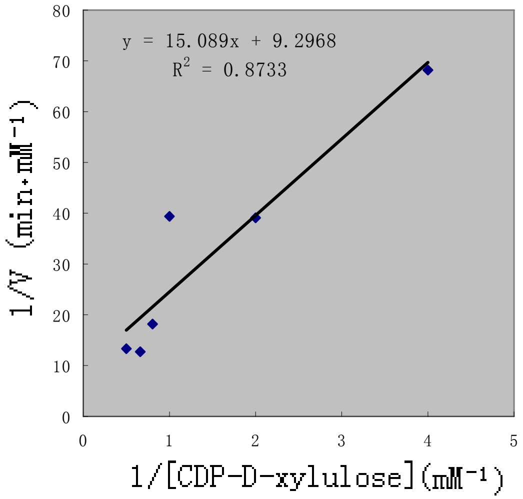
(7)


y = 28.956x + 10.285
R² = 0.7958
1/V (min•mM⁻¹)
1/[dCTP](mM⁻¹)
0
10
20
30
40
50
60
70
80
0.5
1
1.5
2
2.5

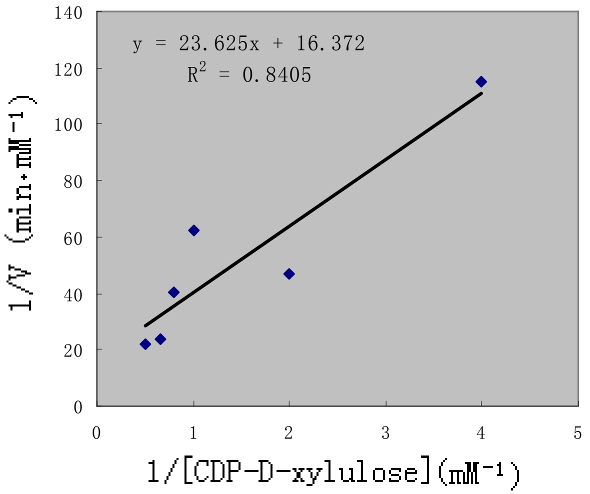
(8)


y = 21.475x + 7.3304
R² = 0.9774
1/V (min•mM⁻¹)
1/[dCTP](mM⁻¹)
0
10
20
30
40
50
60
0.5
1
1.5
2
2.5

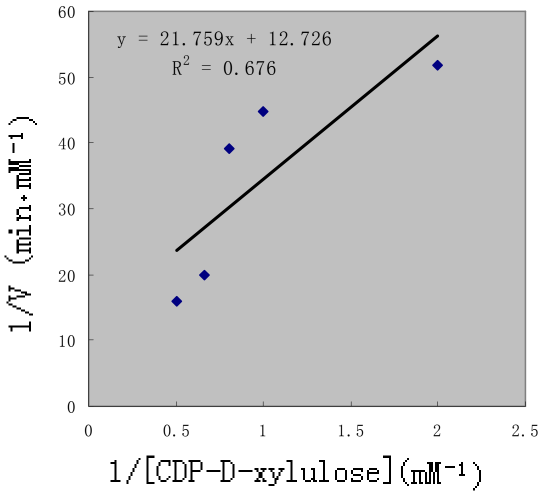
(9)


y = 15.089x + 9.2968
R² = 0.8733
1/V (min•mM⁻¹)
1/[CDP-D-xylulose](mM⁻¹)
0
10
20
30
40
50
60
70
80
1
2
3
4
5

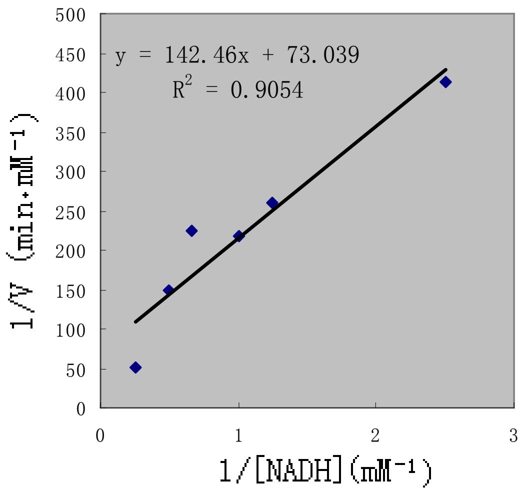
(10)


y = 23.625x + 16.372
R² = 0.8405
1/V (min•mM⁻¹)
1/[CDP-D-xylulose](mM⁻¹)
0
20
40
60
80
100
120
140
1
2
3
4
5

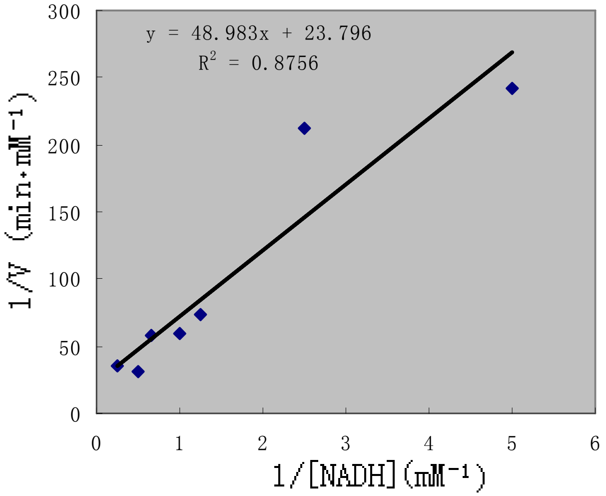
(11)


y = 21.759x + 12.726
R² = 0.676
1/V (min•mM⁻¹)
1/[CDP-D-xylulose](mM⁻¹)
0
10
20
30
40
50
60
0.5
1
1.5
2
2.5

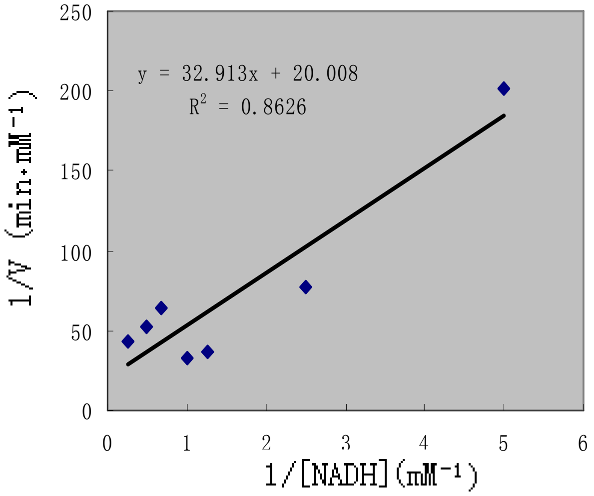
(12)



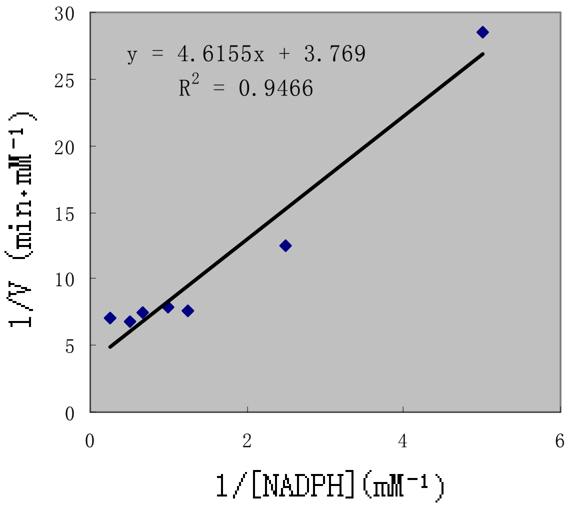
(13)



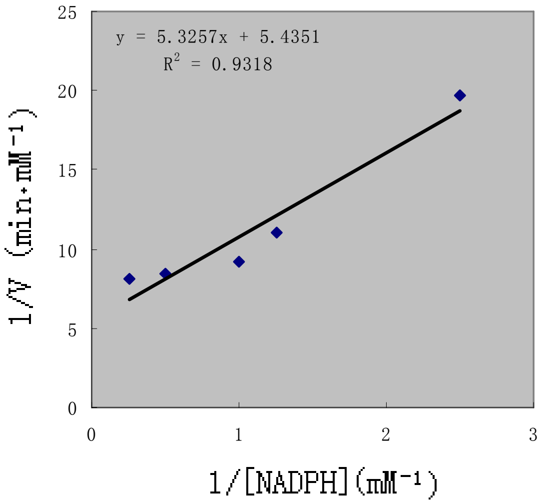
(14)



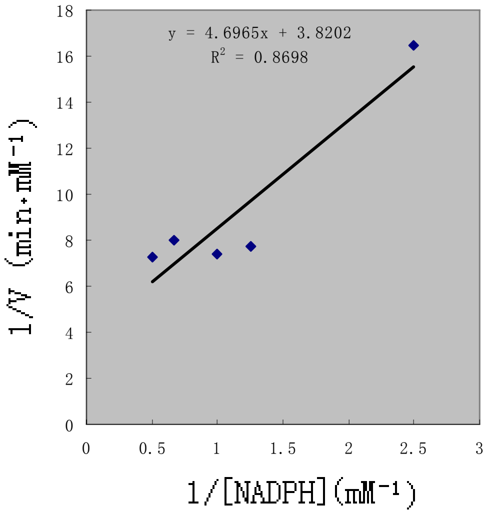
(15)



(16)



(17)



(18)

Figure S5. Kinetic analysis of Abp1 and Abp2. The double reciprocal plots are the initial velocities of reactions catalyzed by Abp1 and Abp2 using different substrates and cofactors, and three independent experiments were carried out for each substrate or cofactor. The kinetic parameters were calculated based on the averages of acceptable results (R2>0.8) and summarized in Table 4. (1)-(3): reactions catalyzed by Abp1 using d-Xlu-5-P as the substrate, (4)-(6): reactions catalyzed by Abp1 using CTP as the substrate, (7)-(9): reactions catalyzed by Abp1 using dCTP as the substrate, (10)-(12): reactions catalyzed by Abp2 using CDP-D-xylulose as the substrate, (13)-(15): reactions catalyzed by Abp2 using NADH as the cofactor, (16)-(18): reactions catalyzed by Abp2 using NADPH as the cofactor.

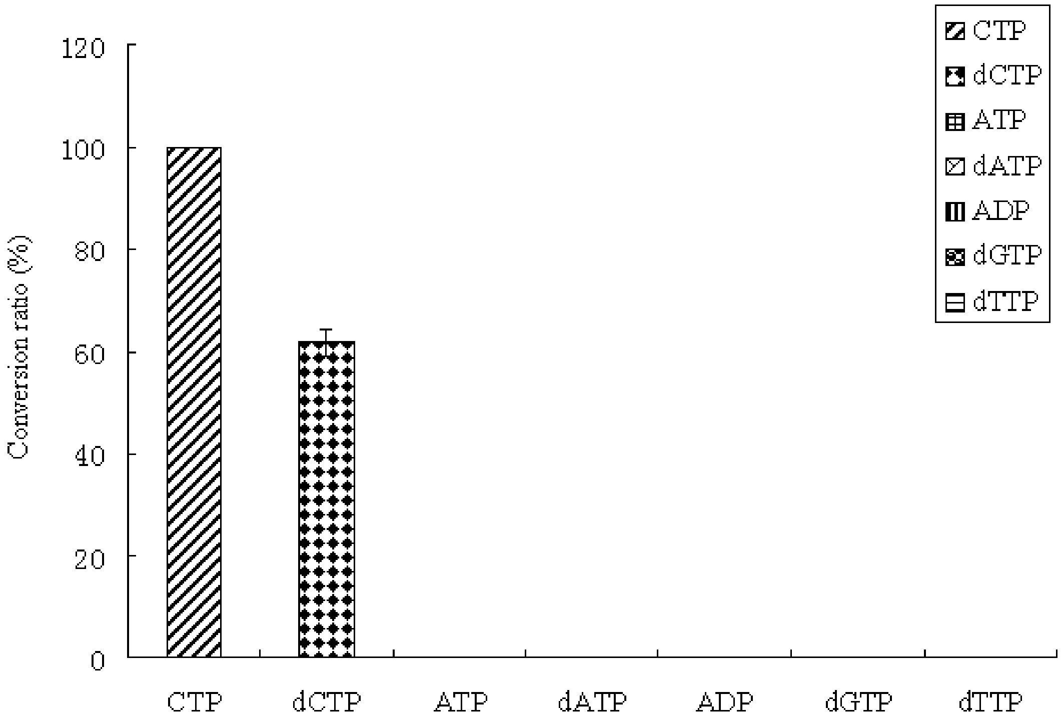


Figure S6. Activities of Abp2 in the reactions using different nucleotide phosphate donors.

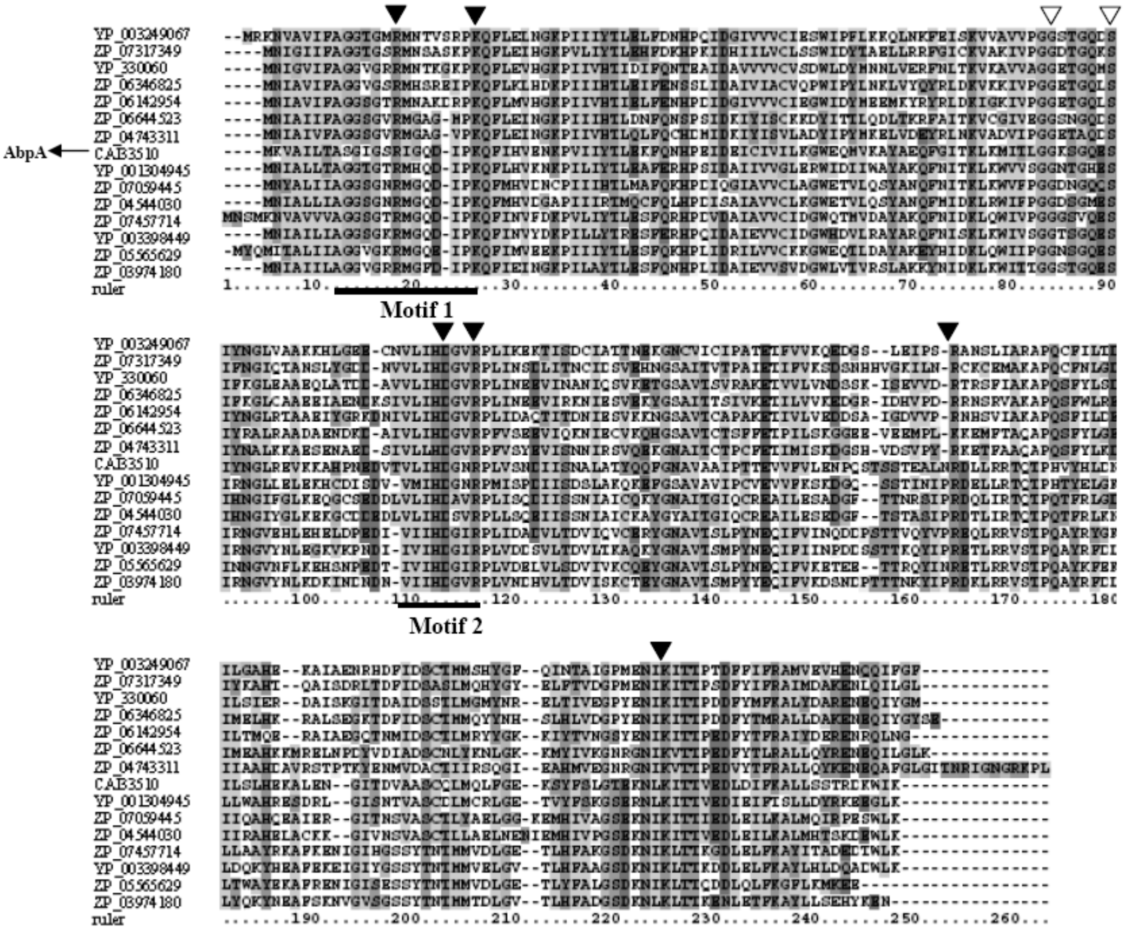


Figure S7. Alignment of AbpA and 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferases from different species. The GenBank accession numbers are shown. Two conserved motifs are marked. The residues involved in sequestering CTP and CDP-ME are indicated by white triangles, and those involved in binding and processing substrates are indicated by black triangles.

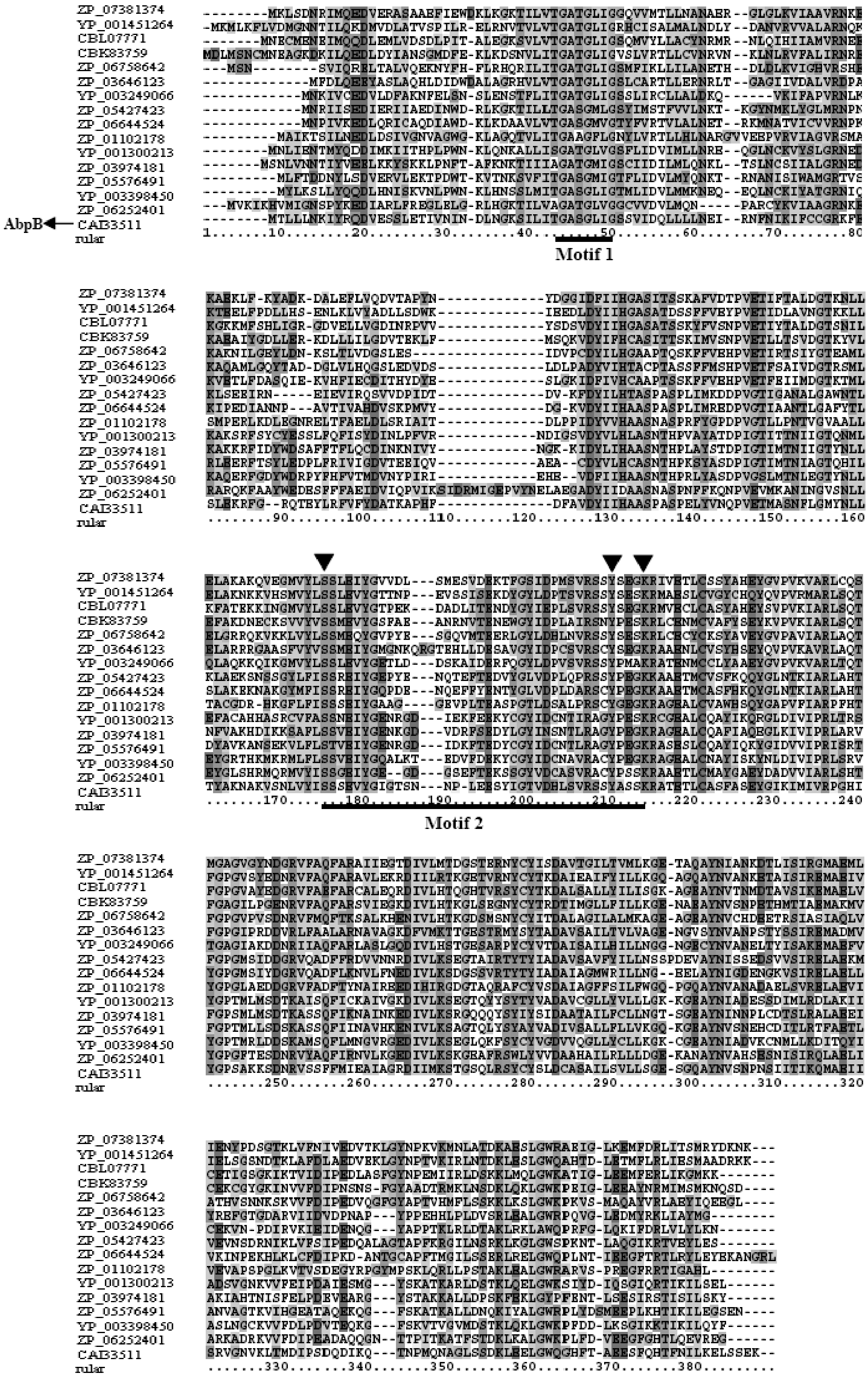


Figure S8. Alignment of AbpB and NAD-dependent epimerase/dehydratases from different species. The GenBank accession numbers are shown. Two conserved motifs are marked. The residues involved in catalysis are indicated by black triangles.