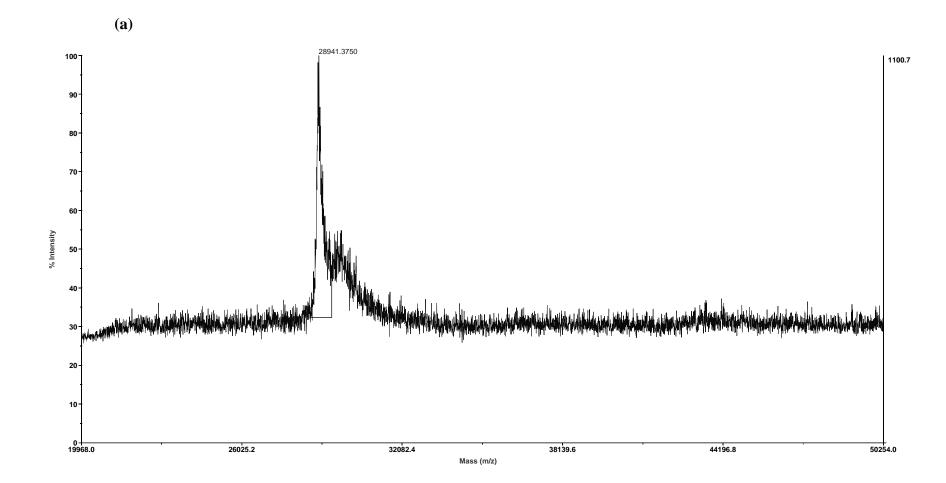


Figure S1. Purified Abp1 (a) and Abp2 (b) after SDS-polyacrylamide gel electrophoresis. Proteins were denatured at 100°C for 5 min in 0.1% SDS and 1% 2-mercaptoethanol before being loaded onto a 5% (w/v) stacking gel and separated in a 12% (w/v) separation gel. Gels were stained with Coomassie bright 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.



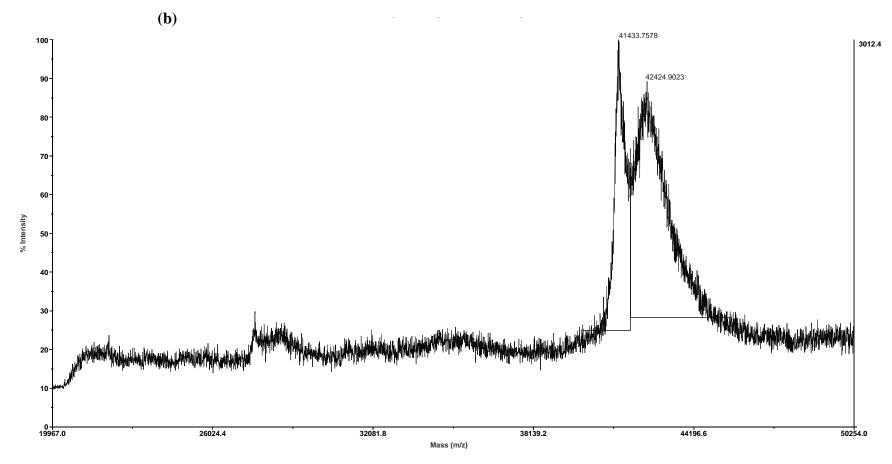
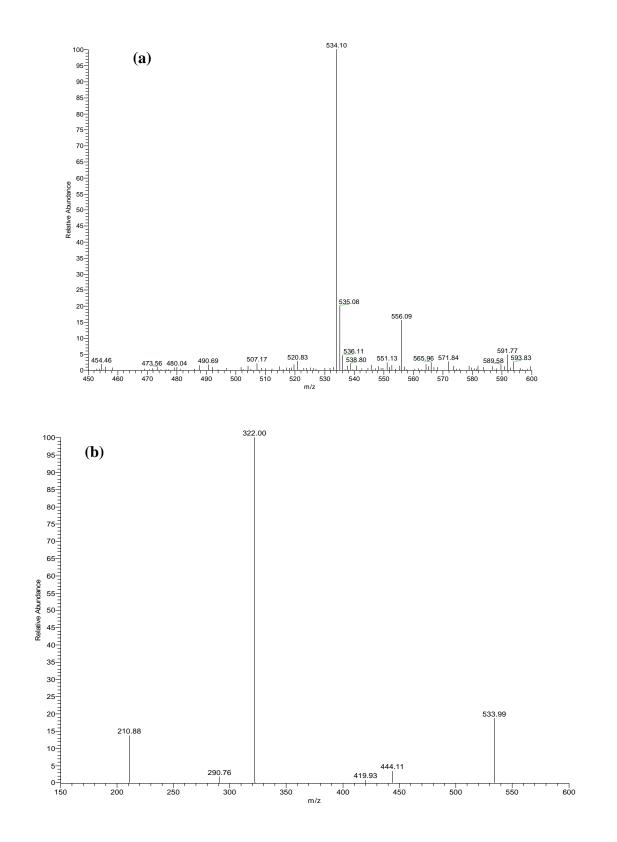


Figure S2. Molecular masses analysis of His₆-tagged Abp1 (a) and Abp2 (b).



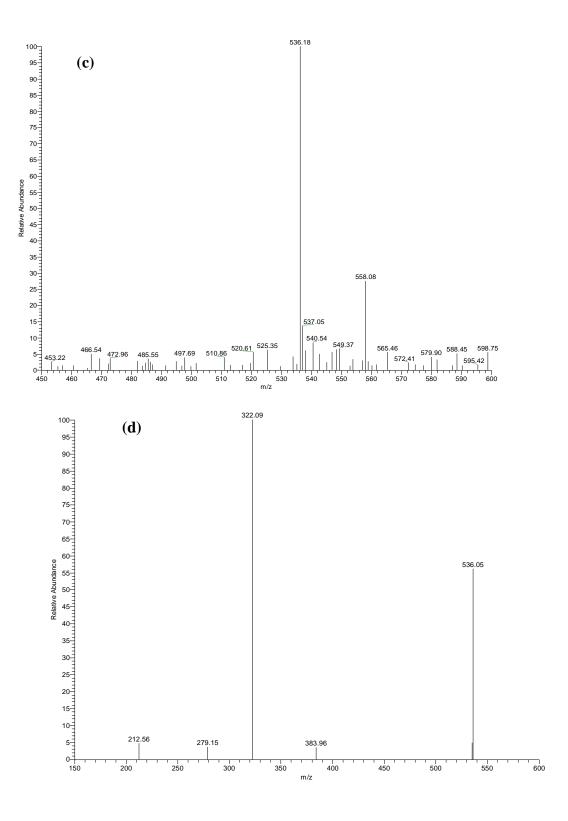


Figure S3. ESI MS of CDP-D-xylulose (a), MS2 analysis of the ion with m/z 534.10 (b), ESI MS of CDP-D-arabinitol (c), and MS2 analysis of the ion with m/z 536.18 (d).

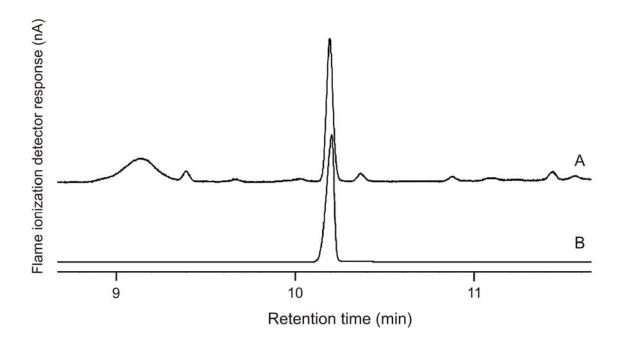
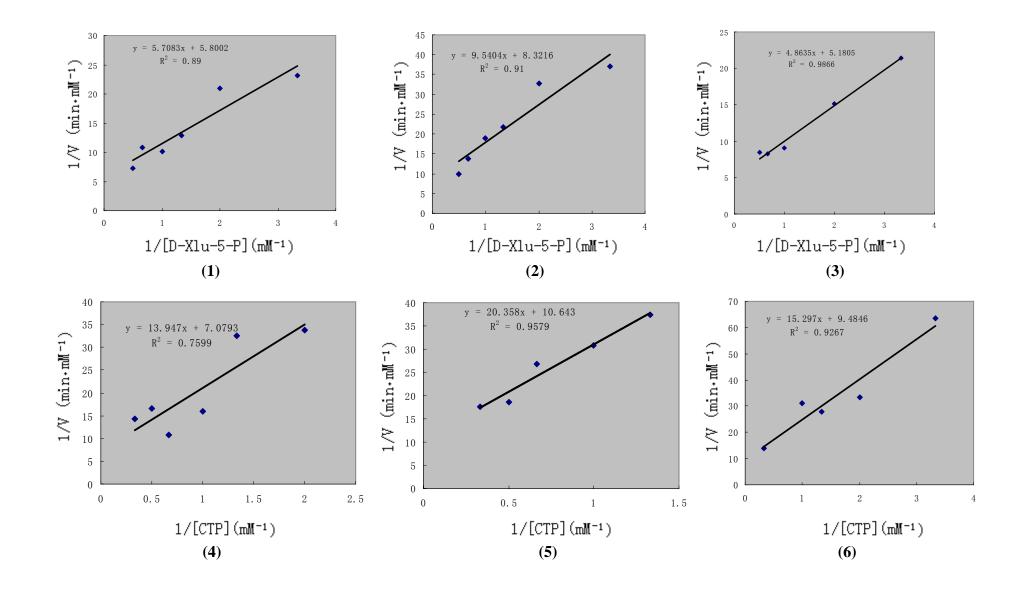
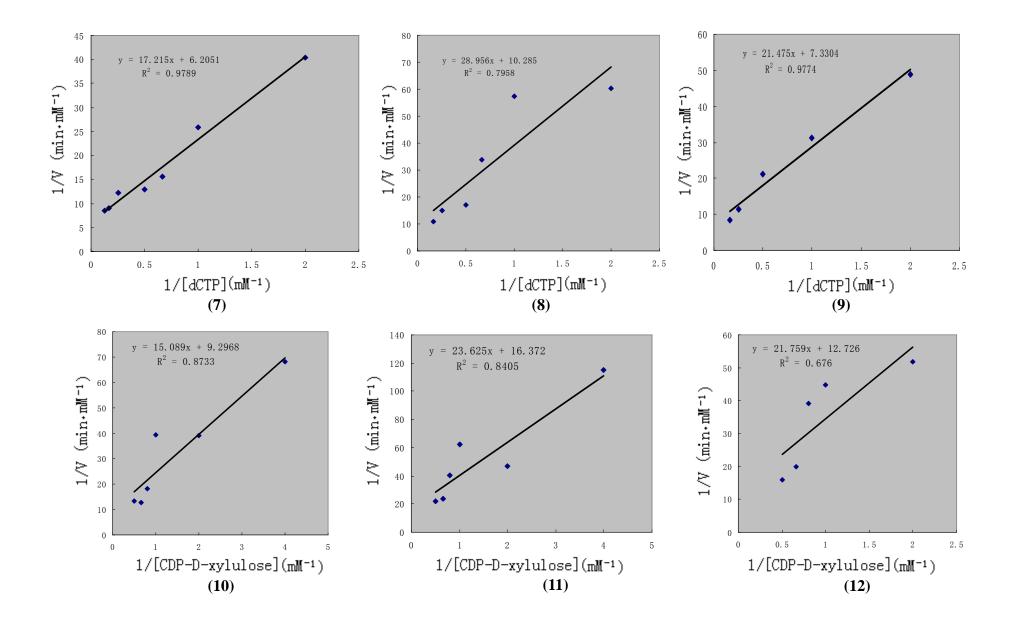


Figure S4. GLC analysis of the alditol acetates derived from the Abp2 product (A) and an authentic sample of arabinitol (B).





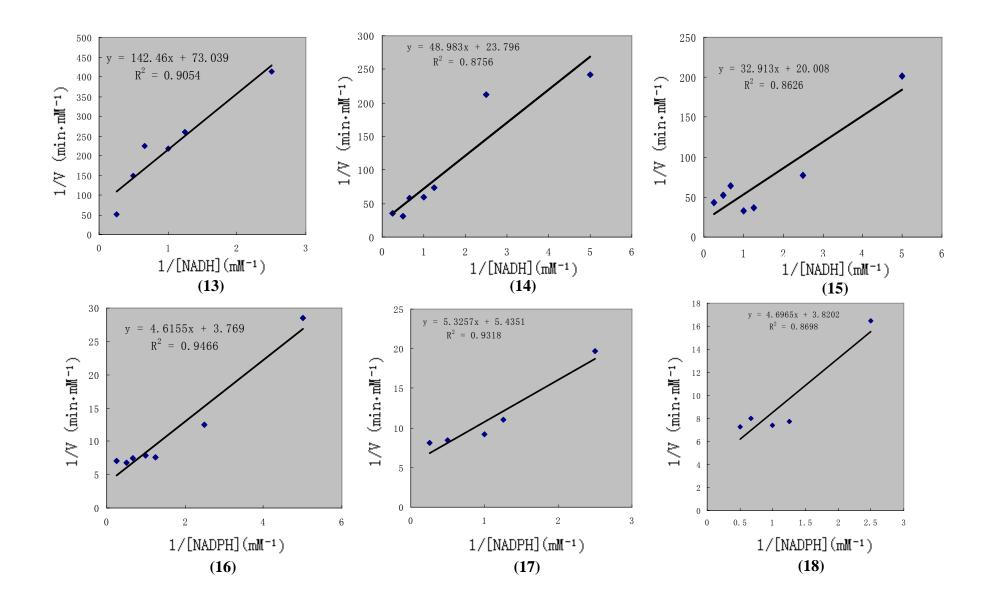


Figure S5. Kinetic analysis of Abp1 and Abp2. The double reciprocal plots are the initial velocities of reactions catalyzed by Abp1 and Abp2 using different substrates and cofactors, and three independent experiments were carried out for each substrate or cofactor. The kinetic parameters were calculated 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 NADH as the cofactor.

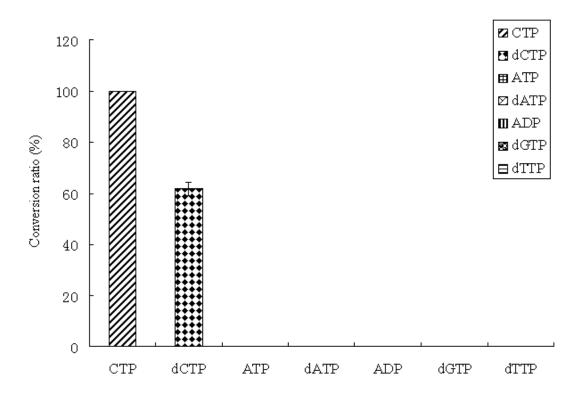


Figure S6. Activities of Abp2 in the reactions using different nucleotide phosphate donors.

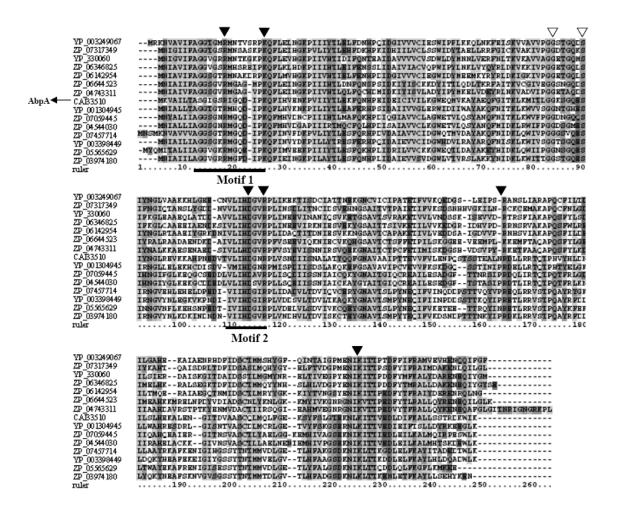


Figure S7. Alignment of AbpA and 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferases from different species. The GenBank accession numbers are shown. Two conserved motifs are marked. The residues involved in sequestering CTP and CDP-ME are indicated by white triangles, and those involved in binding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

AbpB◀	ZP_07381374 YP_001451264 CBL07771 CBK83759 ZP_06758642 ZP_03646123 YP_003249066 ZP_05427423 ZP_0102178 YP_001300213 ZP_003974181 ZP_03974181 YP_003398450 ZP_06252401 CAB3511 rular	
	ZP_07381374 YP_001451264 CBL07771 CBE03759 ZP_06758642 ZP_03646123 YP_003249066 ZP_0427423 ZP_06644524 ZP_01102178 YP_001300213 ZP_0374181 ZP_03576491 YP_003398450 ZP_06252401 CAI33511 rular	KAEKLF-KYAEK-DALEFLÜQUVTAPYN
	ZP_07381374 YP_001451264 CBL07771 CBK33759 ZP_06758642 ZP_03646123 YP_003249066 ZP_05427423 ZP_00644524 ZP_01102178 YP_001300213 ZP_0374181 ZP_03398450 ZP_005576491 YP_003398450 ZP_06252401 CAI33511 rular	ELAKAKQVEGMVYLSSLEIYGVDLSMESVDEKTFGSIDPMSVRSSYSEGKRIVETLCSSTAHEYGVPVKVARLCQS ELAKNKKVHSMVYLSSLEYYGTTNPEVSISEKDVGYLDPISVRSSYSESKMARSLCVGYCHQVQVPVKARLSQT KFATEKKINGMVYLSSLEYYGTPEDADLITENDYGYIEPLSVRSSYSESKMARSLCAGYCHAVSVPVKIARLSQT EFAKINECKSVVYVSSMEYGSFAEANKNVTTNEEGYIDPLAVRSSYSESKRACCCASVAHEYSVPVKIARLSQT ELARRQKVKKLYLSSMEYGGFAESQCVMTERLGYLDPLAVRSSYSESKRLCCYKSXAVEYGVPAVIARLSQT ELARRQKVKKUYLSSMEYGGFAESQCVMTERLGYLDPLAVRSSYSESKRLCCYKSXAVEYGVPAVIARLSQT ELARRQKVKKUYLSSMEYGGFAESQCVMTERLGYLDPLAVRSSYSESKRLCCYKSXAVEYGVPAVIARLSQT ELARRQKSSKIYSSMEIYGMKQGTEHLDFSAVGVIDPSKSSYSKALAETMCVSFKQQUCHWKIARLSQT ELARRQKSSGIFFISSEIYGGFTENQTEFFTDVGCULDPLSVRSSYPMAKRATENMCCLFAAEYQVVKVARLTQT KLAEKSNSSGIFFISSEIYGGFTENQTEFFTDVGCULDPLSVRSSYPMAKRATENMCSKAAETMCVSFKQQUCHWKIARLAHT SLAKKNARGTMFISSEIYGGPTEUKFFFTNTGUUPLDASCYPEGKRAGEALCQAFIAGSLCVIFFISSEIYGAAGGEVPLTASPOTLSALPRSCYPEGKRAGEALCQAFIAGGLUVFFIS NFVARHARGTMFISSEIYGGENGG IEKFFEKYCGVIDKINTARGYPESKRCGEALCQAFIAGGLUVFFISSEI NFVARHASKVLFLSSVEIYGGLKG VDRFSSDILGYINSNTLRGYPESKRCGEALCQAFIAGGLUVFFISSET EYGRTHKKENKLFLSSVEIYGGENGG IEKFFEKYCGVIDKINSNTLAGYPESKRCGEALCQAFIAGGLUVFFISSET EYGRTHKKENKLFLSSVEIYGGLKT EVVPDFKYCVIDYCITICNTLRGYPESKRCGEALCQAFIAGGLUVFNIPLISE EYGRTHKKENKLFLSSVEIYGGIKG UKFFSDILGYINSNTLRGYPESKRCGEALCQAFIAGGLUVFNIPLSEN EYGRTHKKENKLFLSSVEIYGGITSNNP-LESYIGTVDLSVRSYASKRATETLQASFSEGIKIMIVKPGHI
		Motif 2
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	ZP_07381374 YP_001451264 CBL07771 CBK33759 ZP_06758642 ZP_03646123 YP_003249066 ZP_05427423 ZP_06644524 ZP_01102178 YP_001300213 ZP_03974181 ZP_03598450 ZP_06252401 CAI33511 rular	IENYPDSGTKLVFNIVEDVTKLGTNPKVKMNLATDKAESLGWRAEIG-LKEMFDELITSMRYDKNK IELSGSNDTKLAFDLAEDVRKLGYNPTVKIRINTDKLESLGWQAHTD-LETMFDELIESMAADRKK CTTIGSGKIKTVIDIPEDLAESFGYNPENIRLDSKKLMQLGWKATIG-LEHMFPELIKGMKK CEKCGYGKINVVFDIPEDVQGFGYAPTVHMFLSSKKLMQLGKKPKVS-MAQAYVFLAFYIQEEGL ATHVSNNKSKVVFDIPEDVQGFGYAPTVHMFLSSKKLKSLGMKPKVS-MAQAYVFLAFYIQEEGL TREFGTGDARVIIDVDNAPYDPEHLFLDVSFLEALGWRPKVG-MAQAYVFLAFYIQEEGL VENDERNIKLVFSIFEDQALAGTAPFKRGILNSKKLKGLGWSPKNT-LAQGIRTVKYLES VENDERNIKLVFSIFEDQALAGTAPFKRGILNSKKLKGLGWSPKNT-LAQGIRTVKYLES VKINPEKHLKLCFDIFFD-ANTGCAPFYNGILSSKRLERLGWGPLNT-IEGFTFILKYLEFEKANGRL VEVAPSPGLKVTVSDEGYRPGYMPSKLQRLPSTAKLEDLGWRARVS-PREGFRFTIGAHL

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.