



FIGURE 11.5

Use of D-2-deoxyribose-5-phosphate aldolase (DERA) in the synthesis of precursors to cholesterol-lowering drugs (statins). **(A)** DERA-catalyzed aldol reaction between the natural donor, acetaldehyde, and the acceptor, D-glyceraldehyde-3-phosphate, to form D-2-deoxyribose-5-phosphate. **(B)** DERA-catalyzed tandem aldol reaction in which two moles of acetaldehyde react consecutively with chloroacetaldehyde to form a lactol product. This product is converted by mild oxidation to (3R,5S)-6-chloro-2,4,6-trideoxy-erythro-hexonolactone with an enantiomeric excess of greater than 99.9% and a diastereoisomeric excess of 96.6%. The latter compound is the precursor to the portions enclosed by dotted lines of the statin drugs Lipitor **(C)** and Crestor **(D)**.

genes on the insert was under the control of *cis*-acting vector-based promoters. To generate the library, a standard laboratory strain of *E. coli* was transformed with these constructs. An appropriately diluted culture was then incubated in a microtiter plate format at 37°C in a growth medium in which each clone would replicate to at least 10^4 clones in 24 hours. The clone array was then subjected to high-throughput expression screening, and DERA-expressing clones were detected using a fluorogenic substrate analog designed to release a fluorescent molecule, 4-methylumbelliferone, upon DERA-catalyzed hydrolysis.

Screening of the environmental DNA library yielded a DERA (from an unknown source organism) with properties much improved over those of the *E. coli* enzyme. The inhibition by chloroacetaldehyde was avoided by a process modification – slow feeding of substrates at a constant ratio of 2:1 acetaldehyde to chloroacetaldehyde. The catalyzed formation of the two carbon–carbon bonds giving rise to the two stereogenic centers led to an enantiomeric excess of greater than 99.9% and a diastereomeric excess of