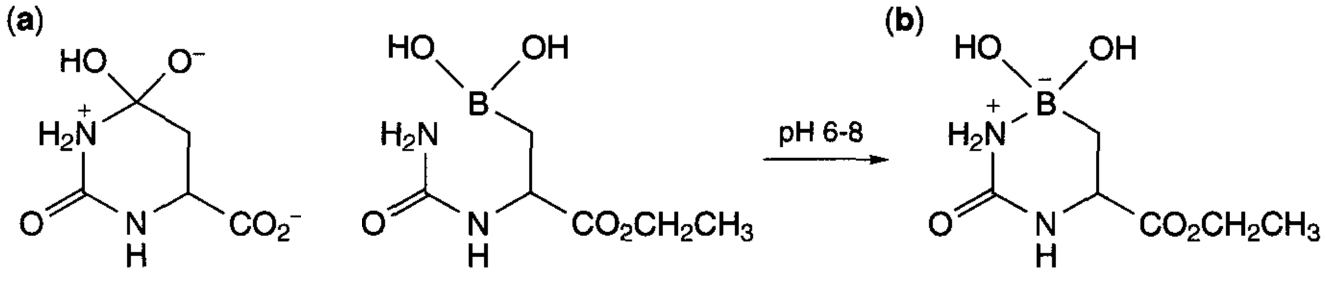


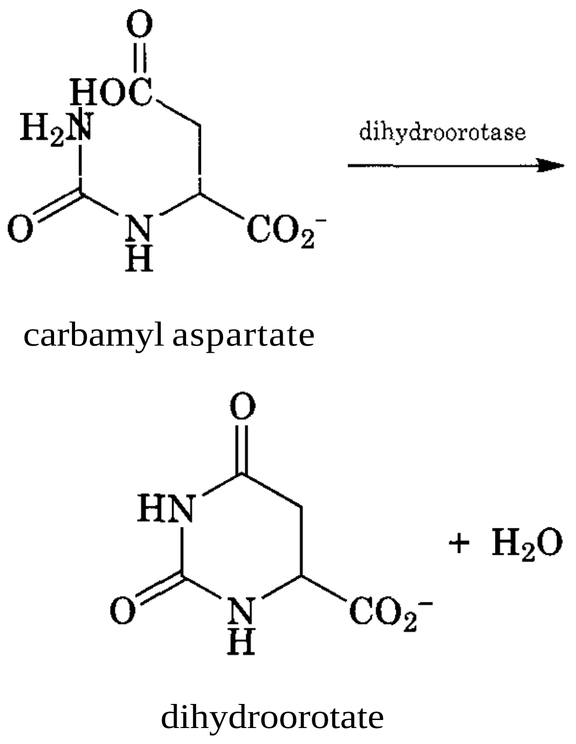
(56) **(57)** **(58)**

Figure 17.24. (a) Putative transition state for the dihydroorotase reaction, and (b) boronic acid transition-state analogs.

rapid onset of inhibition, it appears that purine riboside (53) itself initially binds and is then rapidly converted to (54) in the active site (141). This result, along with the high affinities of (*R*)-**coformycin** and (*R*)-**deoxycoformycin**, argues that the reaction proceeds by a **stereospecific**, direct attack of water rather than the double-displacement mechanism that also had been proposed (141). More recently, an X-ray study on adenosine deaminase, which had been crystallized in the presence of purine ribonucleoside, confirmed that it was the hydrated species of purine ribonucleoside that was present in the active site (143). Further, a triad of a zinc atom, a histidine residue, and an **aspartic** acid residue ensured that the binding was stereospecific, with the 6R isomer (55) being favored.

The adenosine deaminase story, in many ways, provides a perfect example of the general principles of enzymatic catalysis and the utility of enzyme inhibitors. ADA is an extremely efficient catalyst, producing a rate enhancement of 12 orders of magnitude. **6*R*-Hydroxy-1,6-dihydropurine** riboside (55) has an affinity for ADA about 8 orders of magnitude greater than that for substrates or products; that is, it expresses a substantial fraction of the free energy of binding that separates the transition state from the ground state in an enzymatic reaction. Evidence of the extraordinary ability of an enzyme to discriminate between stereoisomers is provided by the 10^7-fold difference in binding affinities of the 8*R*-OH (50) and 8*S*-OH (51) stereoisomers of 2'-deoxycoformycin. Inhibitors were used to differentiate among several potential reaction mechanisms for ADA and, finally, an ADA inhibitor (pentostatin) has proved to be of therapeutic benefit.

Inhibitors of pyrimidine and purine **biosynthesis** are used as antineoplastic agents. As a consequence, dihydroorotase, which catalyzes the third step of ***de novo*** pyrimidine **biosynthesis**, the conversion of carbamyl aspartate to dihydroorotate (Equation **17.43**), is a target for therapeutic intervention.



(17.43)

The reaction is thought to proceed through the tetrahedral-activated complex (56) (Fig. **17.24**), which is a highly charged, unstable *sp*^{3} carbon species (144, 145). At around neutral pH, compound (**57**), a boron-containing analog of carbamyl aspartate, rearranges to the stable, tetrahedral boronic acid derivative (58). The affinity of (58) for **dihydroorotase** (K_i = 5 μM) was found to be 10-fold greater than that of the carbamyl aspartate