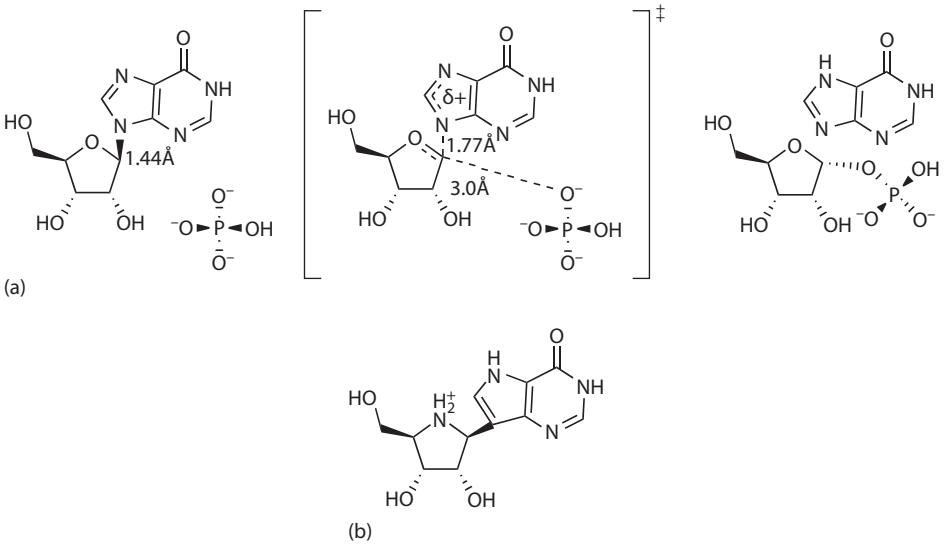

FIGURE 11.6 The structures of PNP reaction substrate inosine, transition state and reaction product (a) and transition state-based inhibitor Immucillin H/BCX-1777 (b).

and genetic deficiency in this enzyme leads to profound T-cell-mediated immunosuppression. Inhibition of PNP has applications in treating aberrant T lymphocyte activity which is implicated in T-cell leukemia and autoimmune diseases. The challenge to inhibitor design for PNP arises from the abundance of the enzyme in human tissues. It has been shown that near complete inhibition of PNP (>95%) is required for significant reduction in T-cell function. Structure-based inhibitor design produced some inhibitors with K_d values in the nanomolar range. However, clinical evaluations showed that these inhibitors did not produce sufficient inhibition of PNP to be effective anti-T-cell therapies. Much more potent PNP inhibitors were later designed with the aid of transition state analysis. In theory, a perfect transition state inhibitor of PNP should bind with a K_d value of approximately 10^{-17} M (10 attomolar). The structure of the transition state for human PNP was determined by Schramm and coworkers in 1995 by measuring kinetic isotope effects. Their studies revealed a transition state with significant ribooxycarbenium character (Figure 11.6). On the basis of the features of this transition state, compounds with picomolar affinity to PNP were synthesized. Among them, Immucillin H/BCX-1777 was a 56 pM inhibitor of human PNP with good potency against cultured human T-cell lines in the presence of deoxyguanosine. Early-stage clinical trials of Immucillin H/BCX-1777 showed moderate efficacy in peripheral and cutaneous T-cell lymphomas and the molecule is currently under investigation for recurrent refractory peripheral T-cell lymphoma.

11.5 BIOSTRUCTURE-BASED DESIGN

In the preceding section of this chapter, we established the fundamental importance to drug discovery of a deep, mechanistic understanding of the reaction mechanism of an enzyme target. While this can be accomplished by the application of mechanistic enzymology, it can be facilitated greatly by the knowledge of the three-dimensional structure of the protein, ideally obtained via experimental techniques such as X-ray crystallography and NMR spectroscopy or by computational methods such as homology modeling. Visualization of the detailed architecture of an enzyme's active site, in complex with a small-molecule inhibitor, can be an important driver in the optimization of a medicinal chemistry effort. The structural insights obtained allow for improvements in target potency, selectivity, and inhibitor physicochemical properties, all of which are paramount in establishing inhibitor SAR.