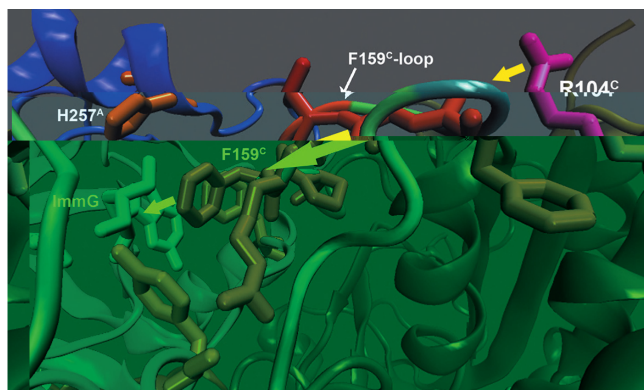

Figure 15.4. Dynamic perturbation of the purine nucleoside phosphorylase reaction coordinate, simulated by molecular dynamics calculations by Saen-Oon et al.[16] The motion of Arg104 (magenta) is coupled to the movement of Phe159 (red), located in a catalytic loop at the interface of two subunits (A, blue and C, tan) in the trimeric protein. Phe159 in turn impinges on the ligands (green) ImmG and phosphate (partially obscured) and surrounding active-site residues (orange) in the adjacent subunit. The vector of dynamic perturbation is shown as yellow arrows. Modified from Saen-Oon et al.[16]

Transition-state mimicry

The various transition-state theories share an essential feature in their description of an enzyme's structure and function: enzymes have evolved to interact most optimally to generate the transition state. This is achieved by strategic placement of appropriate functional groups, which operate collectively to convert the stable substrate molecule into an unstable structure within a relatively short (typically ms) time scale. It has been recognized for many decades that if a chemically stable version of the transition state could be engineered and introduced into the active site, very strong associations with the enzyme would be expected.[2,3,17]

The tight binding of transition-state mimics is best explained by the thermodynamic model of transition-state stabilization (see "Enzymatic transition-state formation"). In the hypothetical binding equilibrium between the enzyme and its transition state (Figure 15.1), the transition state is held more tightly than the substrate by a factor equal to the rate acceleration, k_{enz}/k_{non}, and the energy of this "association" is given by $\Delta\Delta G^{\ddagger}$ (Figure 15.2). Although a *virtual* thermodynamic equilibrium cannot exist because of the subbond vibrational lifetime of the transition state, it is instructive to imagine capturing the system at the moment the transition state is formed. This energetic interaction is approximated by the *real* binding equilibrium with a transition-state analog, which, if it were a perfect mimic, would completely convert the transition-state stabilization energy, $\Delta\Delta G^{\ddagger}$, into binding energy.[18,19]

The dynamic view of transition-state theory explains the tight-binding property of transition state analogs by a conformational collapse of the enzyme around the chemically stable mimic.[20] The chemically inert transition-state analog converts the dynamic excursions found at the transition state to a stable convergence of the enzyme conformation,

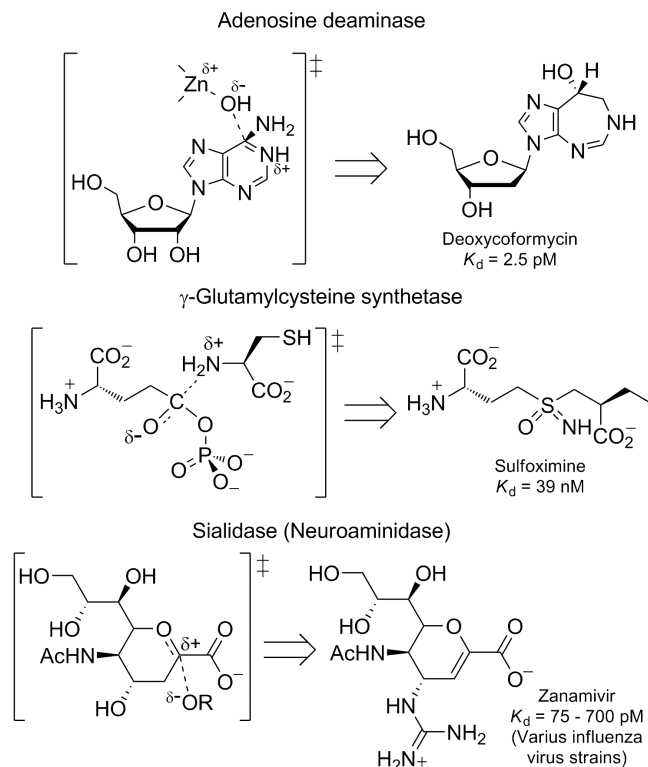

Figure 15.5. Enzymatic transition states and their chemically stable analogs. Dissociation constants for inhibitors of adenosine deaminase,[135] γ-glutamylcysteine synthetase,[136] and sialidase (neuraminidase)[137] are given.

resulting in the conversion of catalytic dynamics into static binding energy.

Regardless of the precise mechanism by which enzymatic transition states are formed, the incredibly potent inhibition exhibited by their analogs – in contrast to substrate and product analogs, for instance – underscores the importance of their development. Figure 15.5 illustrates examples of transition-state analogs from among the hundreds of known enzyme targets.[21] To design transition-state analogs, we must first have knowledge of the structure of the transition state.

Determination of transition-state structure from kinetic isotope effects

Unfortunately, the structure of the transition state cannot be determined by the analytical methods used for stable compounds, including crystallography, nuclear magnetic resonance (NMR), infrared/Raman, ultraviolet/visible spectroscopy, and mass spectrometry. With a lifetime less than a single bond vibration, methods probing the ground state are clearly insufficient. Indirect kinetic methods provide some structural information. For instance, variations in the reactivity of functional groups (e.g., varying pK_a or electron-withdrawing properties, analyzed by Hammett plots) in the substrate have been introduced to examine