greatly reduced with knowledge of geometries permitted in the active site, which is often available from crystallographic data, especially from bound substrate and substrate analogs.

General approach to transition-state-based inhibitor design

In summary, solving the transition-state structure of an enzymatic reaction requires the following steps:

1. Chemical or biochemical synthesis of substrate molecules containing isotopic labels at specific positions.
2. Determination of experimental conditions wherein intrinsic isotope effects – that is, KIEs reflecting only the chemical step – can be measured.
3. Measurement of KIEs for each position labeled in step 1.
4. Iterative computation of theoretical KIEs from quantum mechanical calculations with model structures until the KIEs match those from experiment in step 3.

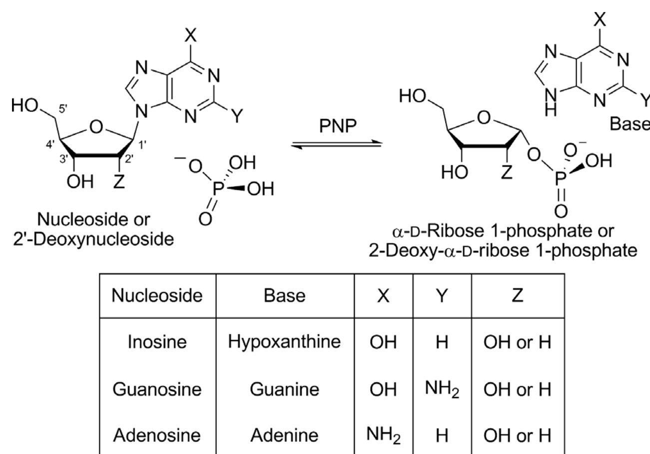
The demands of these conditions often limit the enzymatic systems that can be analyzed by this approach to inhibitor design. For instance, unstable or elaborate substrates may render isotopic label incorporation prohibitive. In other cases, the structure of substrates and their transition states may be too simple (e.g., in kinase reactions), such that structural information obtained from transition-state analysis is of no utility for inhibitor design. Additionally, intrinsic KIEs may be obscured by kinetically significant steps other than chemistry; however, alternative substrates, active site mutants, altered pH, and single-turnover analyses have been used to avoid these complications.[21,39] Finally, multiple steps in the enzymatic reaction coordinate, including substrate binding, and multiple chemical steps may contribute to the observed KIE, and these must be separated to establish the intrinsic KIE.[40–42]

Once the transition-state structure has been determined, aspects of its geometric and electrostatic properties can be incorporated into the design of appropriate chemically stable analogs. The remainder of this chapter demonstrates the methods of transition-state structure determination by the example of purine nucleoside phosphorylases and how this approach has led to the development of several generations of tight-binding inhibitors now in clinical trials for the treatment of various diseases.

PURINE NUCLEOSIDE PHOSPHORYLASE

Physiological role and basis for drug targeting

The cleavage of purine nucleosides (i.e., inosine and guanosine) and their 2′-deoxy counterparts is achieved by the phosphorolytic reaction catalyzed by PNP. This reaction forms a purine base (i.e., hypoxanthine or guanine) and (deoxy)ribose 1-phosphate (Figure 15.9) and is an essential process in human nucleoside metabolism and purine salvage. A rare genetic disorder of T-cell immunodeficiency has been attributed to a deficiency of PNP.[43,44] Without PNP, deoxyguanosine (dGuo) accumulates in the blood and is phosphorylated to deoxyguanosine monophosphate (dGMP) by deoxycytidine kinase most actively in rapidly dividing T cells (Figure 15.10).[43] Further phosphorylation to deoxyguanosine triphosphate (dGTP) causes inhibition of T-cell proliferation[45] and apoptosis.[46] This T-cell-specific effect has been exploited in the development of pharmaceuticals for the treatment of a variety of T-cell immunodisorders, including T-cell lymphoma, rheumatoid arthritis, lupus, psoriasis, and multiple sclerosis.[21]



Nucleoside	Base	X	Y	Z
Inosine	Hypoxanthine	OH	H	OH or H
Guanosine	Guanine	OH	NH_2	OH or H
Adenosine	Adenine	NH_2	H	OH or H

Figure 15.9. Phosphorolysis of the purine nucleosides inosine (Ino), guanosine (Guo), and adenosine (Ado) catalyzed by PNP.

Kinetic mechanism

The acid-catalyzed hydrolysis of purine nucleosides proceeds via N-7 protonation of the leaving group, followed by cleavage of the *N*-ribosidic bond to generate an oxacarbenium-ion intermediate, which is immediately

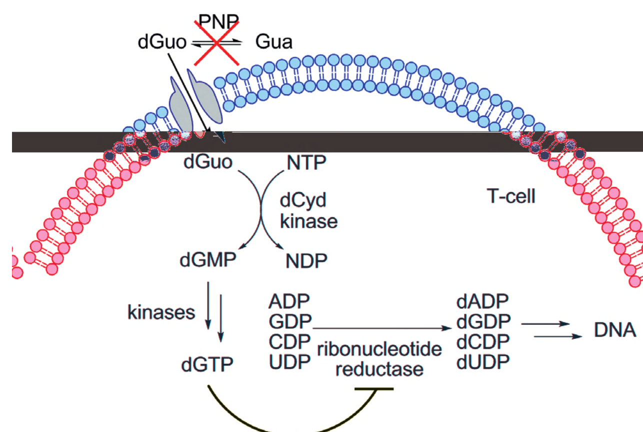


Figure 15.10. Simplified schematic of deoxyguanosine (dGuo) metabolism in human T cells. In the absence of PNP activity, high levels of dGuo are available for entry into T cells. Deoxycytidine (dCyd) kinase phosphorylates dGuo to dGMP, which, unlike the enzyme's normal product dCMP, does not cause product inhibition. Thus, dGMP is available for efficient conversion to dGTP, which allosterically inhibits ribonucleotide reductase-mediated production of deoxynucleosides and ultimately halts DNA synthesis and T-cell replication.