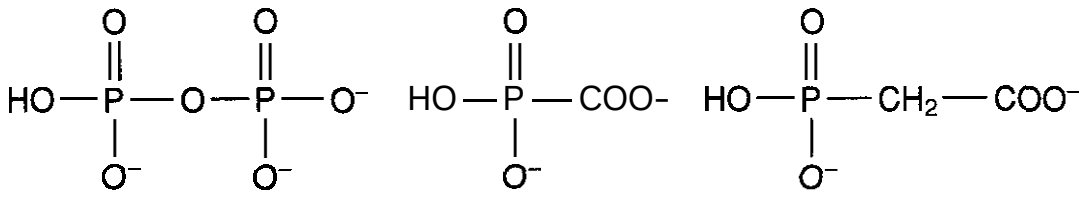


Pyrophosphate (PP_i) (22) (23)

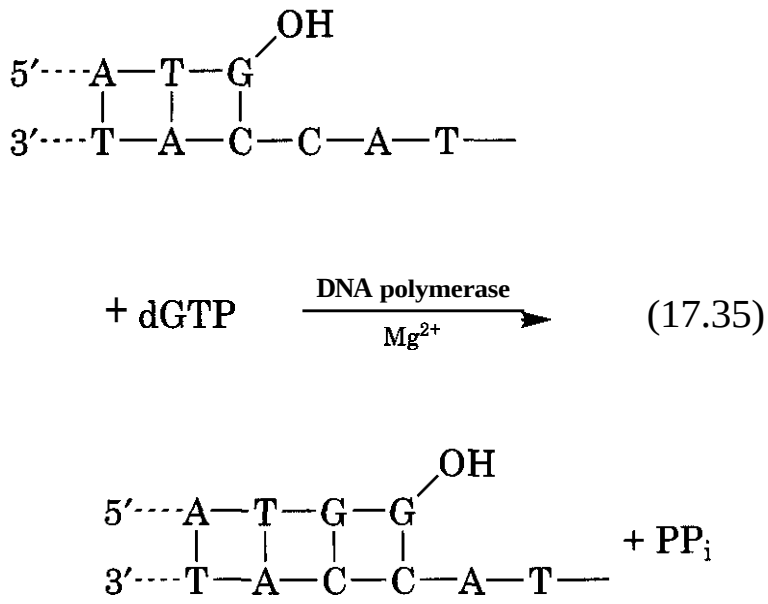
Figure 17.17. *Pyrophosphate* analogs used to inhibit DNA **polymerase.**

2.5 Inhibitors Classified on the Basis of Structure/Mechanism

As with any reaction, an enzyme-catalyzedreaction must proceed from the ground state through a transition state before products are formed. In addition, there are often some high-energy intermediates along the pathway. Knowledge and understanding of an enzyme's mechanism permits the identification of the high-energy intermediates and the prediction of the structures of the transition states. Armed with that knowledge, it is possible to design enzyme inhibitors based on the structures of the various intermediates along the reaction pathway. Inhibitors designed in this manner are occasionally referred to as mechanism-based inhibitors. However, for purposes of this chapter, we will reserve that term for the covalently binding inhibitors described in Section 3.

2.5.1 Ground-State Analogs. The ground state of an enzymatic reaction consists of the substrates and the products. Compounds that mimic the substrate of an enzymatic reaction have been examined earlier (Section 2.3) and are not discussed again here. There are many examples of enzymatic reactions that are inhibited by some or all of the reaction products. Both epinephrine and S-adenosyl-L-homocysteine, for example, are inhibitors of phenylethanolamine N-methyltransferase (Equation 17.35). In much the same way as described earlier for substrate analogs, product analogs can also be used to obtain information about the binding mechanism of enzymes (90).

Phosphonoformate (22) (Fig. 17.17) is an antiviral agent that is used clinically in the treatment of herpes simplex virus (HSV) and human cytomegalovirus (HCMV) (91). It acts as a product analog, blocking the pyrophosphate-binding site, in the reaction catalyzed by DNA polymerase (Equation 17.35). It is also effective, using the same mechanism, against HIV reverse transcriptase (91).



(17.35)

DNA polymerase catalyzes the transfer of a complementary deoxynucleoside monophosphate moiety from its triphosphate (dNTP) to the 3' hydroxyl of the primer terminus, with subsequent release of pyrophosphate (PP_i, eq. 17.35). Initially, phosphonoformate (22) and phosphonoacetate (23) were identified as inhibitors of HSV DNA synthesis (92). Detailed kinetic studies (93), using DNA polymerase induced by avian herpes viruses, showed that phosphonoacetate (23) was a noncompetitive inhibitor of the four dNTPs. At low levels of dNTPs it was a noncompetitive inhibitor of the substrate DNA, becoming uncompetitive at saturating dNTP levels. It was also found that (23) was a competitive inhibitor of pyrophosphate, with a K_i value in the low micromolar range, in the dNTP-PP_i exchange reaction catalyzed by a turkey virus DNA polymerase (93). The inhibition patterns were identical to those observed using pyrophosphate as an inhibitor. Therefore it was concluded that (23) acted as an analog of pyrophosphate and competed for the same binding site (93). Later, both (22) and (23) were confirmed as acting as pyrophosphate (i.e., product) analog inhibitors of isolated HSV DNA polymerase (94).