The role of substrate-induced conformational changes in enzyme specificity has been controversial since the first suggestion of “induced-fit” nearly 60 years ago. In studies on HIV reverse transcriptase, the problem takes on important medical relevance regarding the effectiveness versus toxicity of nucleoside analogs used to treat viral infections. In our studies we use transient state kinetics methods that allow direct measurement of the rates of enzyme closing and opening relative to rates of incorporation of nucleotides during DNA polymerization by HIV reverse transcriptase. This analysis demonstrates that the nucleotide-induced enzyme isomerization is the major determinant of specificity governing both fidelity and selection against nucleotide analogs. In the case of lamivudine (3TC), the two-step binding reaction helps to make the drug more effective by affording a lower Km to compensate for the slower rate of incorporation. Similarly, evolution of resistance to zidovudine (AZT) is attenuated by the two-step binding in that slower chemistry results in a lower Km for incorporation. Molecular dynamics simulations reveal the molecular details underlying the large conformational changes to provide a comprehensive understanding of the role of substrate-induced changes in enzyme structure that are responsible for nucleotide selectivity.