

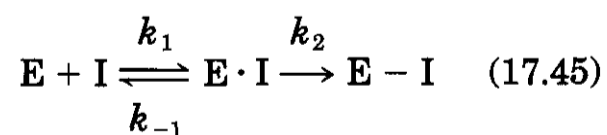
**Table 17.7 Commonly Used Reagents for Chemical Modification**

Residue Targeted	Reagent	Other Residues Labeled
Lysine	Acetic anhydride Isothiocyanates Trinitrobenzenesulfonate (TNBS) Cyanate	These reagents <b>can</b> also react with the N-terminal amino group
Histidine	Diethylpyrocarbonate (DEPC)	DEPC should be used at neutral pH to minimize reaction with lysines, <b>cysteines</b> , and tyrosines
Cysteine	Iodoacetamide, iodoacetate p-Hydroxymercuribenzoate Methyl methanethiosulfonate Ellman's reagent (DTNB) N-Ethylmaleimide	Iodoacetamide has the potential to modify histidines and <b>lysines</b>
Arginine	Phenylglyoxal Butanedione	Phenylglyoxal <b>can</b> react with lysine Butanedione should be used in the dark to prevent reaction with <b>tryptophans</b> , histidines, and tyrosines
Tyrosine	Tetranitromethane Chloramine T	Chloramine T also modifies <b>histidines</b> and methionines
Tryptophan	N-Bromosuccinimide 2-Hydroxy-5-nitrobenzyl bromide	
Serine	Diisopropylfluorophosphate Halomethyl ketones	
Aspartic Acid Glutamic Acid	Carbodiimides Trimethyl oxonium fluoroborate Isoxazolium salts	

listed in Table 17.7. These compounds are chemically reactive and may lead to the modification of both catalytic and nonessential residues. As a consequence, experimental design (such as choice of reagent and reaction conditions, use of substrate protection, etc.) is of utmost importance in carrying out and interpreting chemical modification studies. Although inhibitors of this type are not the prime focus of this chapter (and are not discussed further), it should be noted that most of the kinetic equations that apply to affinity labels also apply to chemical modifiers, and there are a number of texts available that cover this topic (40, 157, 158).

Although the organic modifiers are usually not specific for a given enzyme, the second group, the affinity labels, have a degree of specificity built in. Sometimes described as active-site directed, irreversible inhibitors, affinity labels are usually substrate or product analogs that contain an additional chemically reactive moiety. They **first** bind to the en-

zyme's active site in a noncovalent fashion, like rapid reversible inhibitors. However, upon formation of the enzyme-inhibitor complex ( $E \cdot I$ ), they react by various mechanisms with one or more amino acid residues in close proximity in the enzyme's active site. This results in covalent bond formation between the enzyme and the inhibitor ( $E-I$ ) (Equation 17.45).



Usually the inhibitor contains an electrophilic moiety that labels amino acids containing nucleophilic groups. However, in some cases, a nucleophilic species may be formed, which can react either with **arginine** or with any tightly bound organic or inorganic low molecular weight cofactors possessing electrophilic sites. Unlike the mechanism-based in-