

TABLE 3.3 Methods of Covalent Binding of Enzymes to Supports (*Continued*)

	Supports with —COOH
(a) Via azide derivative	
1) $\left -\text{O}-\text{CH}_2-\text{COOH}\right \xrightarrow[\text{H}^+]{\text{CH}_3\text{OH}} \left -\text{O}-\text{CH}_2-\text{COOCH}_3\right \xrightarrow{\text{H}_2\text{NNH}_2} \left -\text{O}-\text{CH}_2-\text{CO}-\text{NH}-\text{NH}_2\right $	
2) $\left -\text{O}-\text{CH}_2-\text{CO}-\text{NH}-\text{NH}_2\right \xrightarrow[\text{HCl}]{\text{NaNO}_2} \left -\text{O}-\text{CH}_2-\text{CON}_3\right \xrightarrow{+\text{protein}-\text{NH}_2} \left -\text{O}-\text{CH}_2-\text{CO}-\text{NH}-\text{PROTEIN}\right $	
(b) Using a carbodiimide	
$\left -\text{COOH}\right + \begin{array}{c} \text{N}-\text{R}_1 \\ \parallel \\ \text{C} \\ \text{N}-\text{R} \end{array} \rightarrow \begin{array}{c} \text{O} \\ \\ \text{C}-\text{O}-\text{C} \\ \\ \text{N}-\text{R} \end{array} \xrightarrow{+\text{protein}-\text{NH}_2} \begin{array}{c} \text{O} \\ \\ \text{C}-\text{NH}-\text{protein} + \begin{array}{c} \text{HNR}_1 \\ \\ \text{O} \\ \\ \text{HNR} \end{array} \end{array}$	Supports containing anhydrides
	$\text{HOOC}-\text{CH}-\text{CH}_2-$
$-\text{CH}_2-\begin{array}{c} \text{CH} \\ \\ \text{O}=\text{C} \end{array}-\text{CH}-\text{CH}_2-$ + Protein—NH ₂ → $-\text{CH}_2-\begin{array}{c} \text{CH} \\ \diagup \\ \text{O}=\text{C}-\text{NH}-\text{protein} \end{array}$	

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