

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	
	$\left \text{COOH} \right + \begin{array}{c} \text{N}-\text{R}_1 \\ \parallel \\ \text{C} \\ \parallel \\ \text{N}-\text{R} \end{array} \rightarrow \left \text{C}-\text{O}-\text{C} \right \begin{array}{c} \text{O} \\ \parallel \\ \text{HN}-\text{R}_1 \end{array} \xrightarrow{+\text{protein}-\text{NH}_2} \left \text{C}-\text{NH}-\text{protein} \right + \begin{array}{c} \text{O} \\ \parallel \\ \text{HNR}_1 \\ \parallel \\ \text{O}=\text{C} \\ \parallel \\ \text{HNR} \end{array}$
Supports containing anhydrides	
	$\begin{array}{c} \text{HOOC}-\text{CH}-\text{CH}_2- \\ \diagup \\ -\text{CH}_2-\text{CH}-\text{CH}-\text{CH}_2- + \text{Protein}-\text{NH}_2 \longrightarrow -\text{CH}_2-\text{CH} \\ \diagdown \\ \text{O}=\text{C} \quad \text{C}=\text{O} \end{array}$ $\text{O}=\text{C}-\text{NH}-\text{protein}$

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