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Note

Origin of the carbamate functional groups in cyanogen bromide-activated, alkylamine-substituted Sepharose

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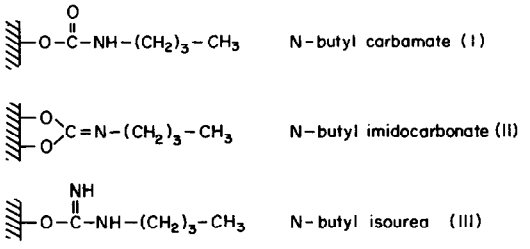
In a recent study[1], it was shown by using a combination of ion-exchange reactions and NMR spectroscopy that one of the major products obtained on coupling butylamine to cyanogen-bromide-activated Sepharose is an uncharged carbamate derivative together with N-butyl imidocarbonate and N-butylisourea (Scheme 1).



Scheme 1.

These results appeared to imply that the mechanism for cyanogen bromide activation of polysaccharides, which we have recently described[2–4], is not completely correct (Scheme 2).

The reason for the discrepancy between the two studies is that in each instance a different type of activated resin was used. In our study, we used a freshly activated Sepharose, which therefore contained only cyanate esters and linear imidocarbonates, both of which give N-substituted isourea as the main product (Scheme 1, III). On the other hand, in the reported study[1] commercially available Sepharose was used, which is known to be treated with acid in order to achieve better stabilization of the activated resin. Such treatment results in a preparation containing cyanate esters and linear carbonates, as acid treatment of imidocarbonate results in the respective carbonate derivative (Scheme 3).

The carbonate can react further with amino-containing ligands to give the corresponding carbamate (Scheme 4).

In order to show that this is the case, we activated Sepharose with cyanogen bromide under basic conditions[4]. The reaction was carried out until no more cyanate ester could be detected[3]. The resulting activated resin (which contained only imido-

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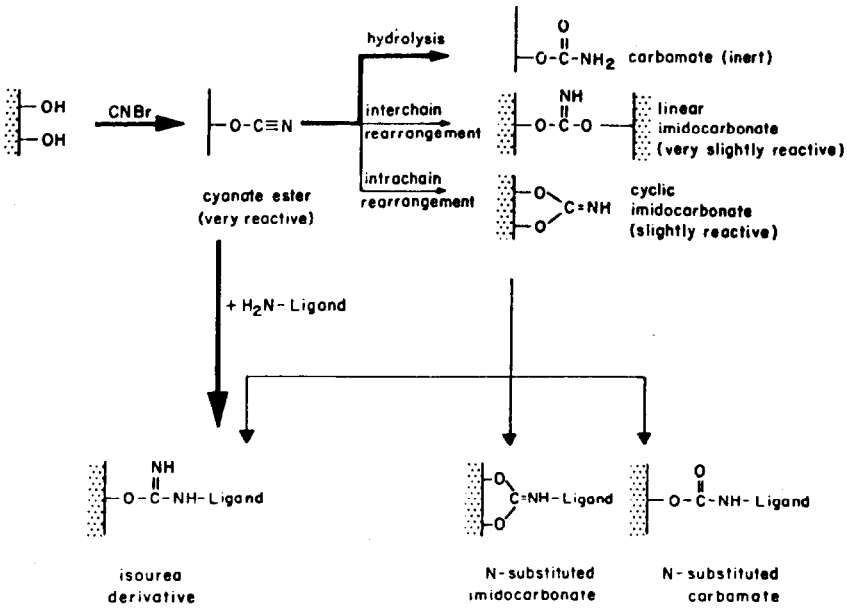
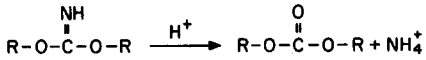
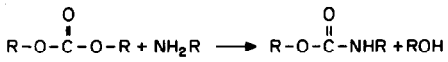


Scheme 2.



Scheme 3.



Scheme 4.

carbonate) was treated with 1 *N* hydrochloric acid for 1 h in order to form the carbonate. When this Sepharose derivative was treated with butylamine or 1,4-diaminobutane at pH 9.5 for 24 h, derivatives containing up to 20 μmole of butylamine per millilitre of Sepharose were obtained. The butylamine-substituted Sepharose was devoid of charge, as it contained only carbamates (see Scheme 1, I).

There is therefore no discrepancy between our results and those of Johansson and Drevin[1]; simply two different derivatives of cyanogen bromide-activated Sepharose were used.

Finally, the bonus from this short study is the introduction of a new activated form of Sepharose containing only carbonate groups. This new type of activated Sepharose can be used to couple amino-containing ligands to the resin, yielding columns that consist of stable and uncharged carbamate groups. These activated resins will mainly be useful for coupling low-molecular-weight ligands such as diaminohexane or aminocaproic acid, as the coupling to the resin has to be performed at high pH (*ca.* 9.5), owing to the low activity of the carbonate formed. The derivatized Sepharose can be further used to couple proteins under mild conditions and in high

yields. When coupling of protein directly to the carbonate was attempted only low levels of coupling were obtained (0.5 mg of trypsin per gram wet weight of Sepharose or 15 mg per gram dry weight of Sepharose), even when the coupling was carried out at pH 9.

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