1-Cyano-4-dimethylamino pyridinium tetrafluoroborate as a cyanylating agent for the covalent attachment of ligand to polysaccharide resins

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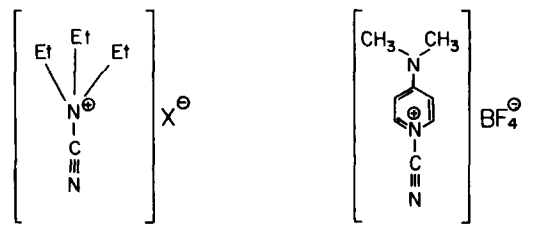
1. INTRODUCTION

Cyanogen bromide (CNBr) is widely used as an activating agent for polysaccharide resins [1–3]. Activation by CNBr, which can be performed in an aqueous medium within a few minutes, is a very convenient technique except for the extremely hazardous nature of CNBr. Therefore, we searched for alternative, non-hazardous activating agents which would yield activate resins, identical, for all practical purposes, with the original CNBr-activated resins.

The reaction of triethylamine (TEA) with CNBr results in the formation of the highly unstable *N*-cyano-triethylammonium bromide (fig. 1, complex 1a), which was found (when prepared in situ) to be an extremely efficient activating agent for polysaccharide resins [4]. Unfortunately *N*-cyano-triethylammonium bromide cannot be isolated, since it decays at above −10°C [5]. However, when Br^- is replaced by non-nucleophilic anions such as perchlorate (ClO_4^-), or tetrafluoroborate (BF_4^-), the respective *N*-cyano-ammonium salts could be obtained as stable, crystalline compounds [5,6]. Based on the same considerations, the particularly stable 1-cyano-4-dimethylamino pyridinium tetrafluoroborate (CDAP, complex 2) could be synthesized [7]. These *N*-cyano derivatives are good cyanylating agents [5,6], and CDAP has been used as a selective reagent for the modification of protein sulfhydryl groups [7]. However, despite their remarkable chemical properties, neither CTEA nor CDAP has been used since to any extent.

Here we report that CTEA and CDAP have been found to be highly efficient activating agents for polysaccharide resins. The activation yields (i.e., mol ligand coupled/mol activating agent employed) of CTEA and CDAP were 15% and 50%, respectively. (The activation yield for conventional CNBr-activation is 1–2% only.) Treatment of polysaccharides with CTEA or CDAP resulted in the formation of cyanate ester derivatives (Resin-O-C≡N) on the activated resins. These resins are therefore identical, in terms of coupling mechanism, to conventional CNBr-activated resins [8,9]. Activations with CTEA or

Abbreviations: CNBr, cyanogen bromide; CDAP, 1-cyano-4-dimethyl-amino pyridinium tetrafluoroborate; CTEA, *N*-cyano triethylammonium tetrafluoroborate; TEA, triethylamine



complex 1
N-cyanotriethylammonium
Ia) $X^{\ominus}=Br^{\ominus}$
Ib) $X^{\ominus}=BF_4^{\ominus}$

complex 2
1-cyano-4-dimethylamino
pyridinium tetrafluoroborate

Fig. 1. Molecular structures of CTEA and CDAP

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Table 1
Amounts of activating agent required for activation of 10 g drained Sepharose 4B

Degree of activation	Approx. coupling capacity (μmol ligand/g resin)	CTEA-Activation[a]		CDAP-Activation[a]	
		CTEA (mg)	TEA[b] (ml)	CDAP[c] (mg)	TEA[b] (ml)
Weak	5	60	0.6	25	0.2
Moderate	15	180	1.8	75	0.6
Strong	30	360	3.6	150	1.2

[a] The illustrative procedure was used as described
[b] 0.2 M aqueous solution of TEA
[c] For convenient use a stock solution of CDAP (0.1 g/ml) was prepared; this stock solution could be stored at 4°C for over 1 month

CDAP could be performed safely without a hood, making it possible to obtain 'cyanogen bromide'-activated resins without significant health hazard.

Preliminary data on the composition on CTEA-activated agarose indicated that cyanate esters account for >75% of the total nitrogen content of the resin; the remaining 25% being mostly inert carbamates and traces of imidocarbonates. On CDAP-activated agarose a significant amount of pyridine derivatives was found, in addition to cyanate esters and traces of carbamates and imidocarbonates. During the coupling reaction, the pyridine derivatives were released from the CDAP-activated resin in the form of the 4-dimethylamino pyridinium salt, which was identified by its strong UV absorption at $\lambda_{max} = 280$ nm (in 0.1 N HCl $\epsilon = 19000$ liter.mol^{-1}). After coupling of the ligand, <0.1 μmol/g resin of pyridine derivatives remained on the resin. Their exact nature is under investigation.

2. ILLUSTRATIVE PROCEDURE FOR THE ACTIVATION OF SEPHAROSE 4B

CTEA was synthesized as in [6], and CDAP was obtained from Makor Chemicals, PO Box 6570, Jerusalem. Drained Sepharose 4B (10 g) was washed sequentially with water, 30% acetone, 60% acetone, and resuspended in 10 ml 60% acetone. The suspension was cooled to 0°C. According to the desired degree of activation, the required amount of activating agent was added, followed by a corresponding amount of TEA (table 1). The TEA solution (0.2 M in water) was added dropwise with vigorous stirring. After 2 min, the entire reaction mixture was rapidly transferred into 100 ml ice-cold washing medium (acetone:0.1 N HCl = 1:1). The resin could be stored in this manner for over 1 h without loss of activation. For coupling, the resin was washed with plenty of cold water followed by one quick wash with the coupling medium. Coupling of ligand to CTEA- or CDAP-activated resins was performed for CNBr-activated resins [2].

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