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An Easy Preparation of Hapten Active Esters via Solid Supported EDAC

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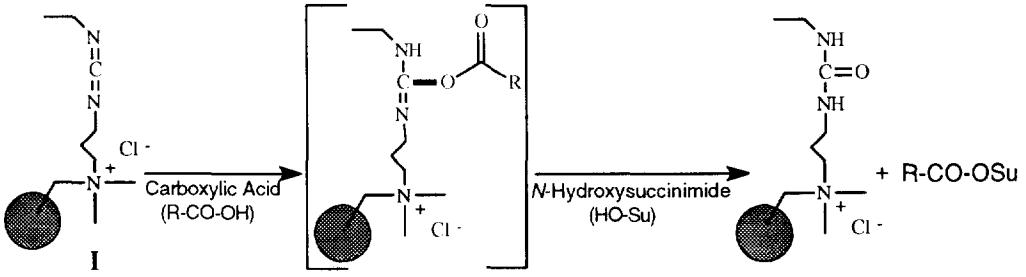
Abstract: Bioconjugates are preferably prepared by reacting an active ester of the hapten of interest to the protein. Preparation of active esters with solid supported EDAC and *N*-hydroxysuccinimide or pentafluorophenol affords active esters in excellent yield and purity.

Almost every immunoassay incorporates at least one reagent that is derived from the synthesis of a bioconjugate.[1,2] Bioconjugates, such as immunogens[1,2] and enzyme conjugates[3], are usually prepared by direct coupling of carboxylic acid haptens to proteins using EDAC[4] (ethyl dimethylaminopropylcarbodiimide) or DCC[5] (dicyclohexylcarbodiimide). This process, however, leads to undesired side reactions such as cross-linking of the protein, *N*-acylurea formation on the protein, etc.[6] Furthermore, removal of urea by-products is not trivial[7] and complicates bioconjugate preparations. These disadvantages are not avoided by the use of *in situ* generated active esters, however, the use of pure active esters overcomes these disadvantages to a large extent. Thus, there is a need for an easy preparation of active esters for use in bioconjugation.

Activation of carboxylic acids utilizing pentafluorophenol is an attractive choice since many pentafluorophenyl esters can be purified by crystallization.[8] Various chromatographic techniques can be applied to the purification of active esters, however, these methods are laborious and not always effective. Very polar active esters, which require aqueous solutions and pH adjustment for their elution, frequently undergo degradation during the process of chromatographic purification, especially on preparative scale.

In this context, we describe herein an easy and practical method for the synthesis of active esters for use in bioconjugation. Polymer supported EDAC[9] was applied to activate carboxylic acids which were reacted with either *N*-hydroxysuccinimide (HOSu) or pentafluorophenol (PFP-OH) as the limiting reagent as shown below.[10]

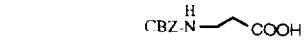
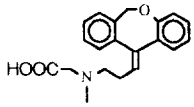
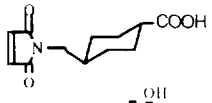
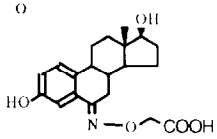
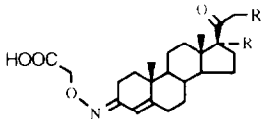
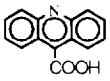
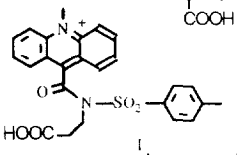
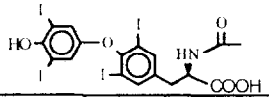
Scheme of Active Ester Synthesis using Solid Supported EDAC (I)



A variety of carboxylic acid haptens were selected in order to exemplify the use of this method, see Table 1. Of special importance is entry 8 which exemplifies the extension of this method to extremely water soluble active esters which can not be purified by conventional extraction methods. The product is not stable in aqueous/ organic mixtures and previous attempts at purification by HPLC were not successful.

In conclusion, we demonstrated the use of solid supported EDAC to produce *N*-hydroxysuccinimidyl and pentafluorophenyl active esters. Both classes of activated esters were obtained in good to excellent yield in pure form. This method can be applied to polar, water soluble haptens whose active esters could not otherwise

Table 1. *N*-Hydroxysuccimidyl and Pentafluorophenyl Active Esters Obtained via Solid Supported EDAC

Entry No.	Carboxylic Acid	HOSu or PFP-OH / DMF:$CHCl_3$ (1:2)	OSu Active Ester (% yield)	or OPFP Active Ester (% yield)
1			98	95
2			95	63
3			81	73
4			60	53
5		R = H	93	78
6		R = OH	85	80
7			92	72
8			83	86
9			71	56

be prepared. Furthermore, this method can be expanded to other nucleophiles which activate carboxylic acids for future conjugation.

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10. In general, to a 10 mL round bottom flask under nitrogen was placed freshly crushed polymer supported EDAC (350 mg) followed by the addition of chloroform (3.6 mL). Subsequently, a solution of carboxylic acid (0.11 mmol) in dimethylformamide (0.9 mL) was added via syringe followed by the addition of a solution of *N*-hydroxysuccinimide or pentafluorophenol (0.10 mmol) in 0.9 mL dimethylformamide. Typically, the reaction mixture was stirred for 14 hours under nitrogen followed by workup which consisted of filtering the crude reaction mixture through a pad of Celite and removing all solvents *in vacuo* to afford the desired active ester. Satisfactory analysis of each active ester product was obtained [^{1}H NMR, Mass spec and HPLC (analytical C_{18} column eluted with acetonitrile/ water/acetic acid)].

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