

Application Data: The Utility of QuadraPure™ C For Peptide Conjugate Purification

Introduction

The following example is a typical procedure utilised for conjugation of a thiol containing peptide with a macromolecule. The procedure utilises the activated disulphide Aldrithiol-2 to form an 'activated disulphide' intermediate. The intermediate must be isolated as a pure species and all trace thiol removed from the reaction to ensure directed conjugation and appropriate stoichiometry is maintained.

QuadraPure™ C is used to fully remove all low molecular weight contaminants from the crude peptide following activation with the Aldrithiol-2. In this example the peptide has side chain functionalities including free thiol, hydroxyl, carboxylic acid, guanidino and primary amine groups. Despite the functionality on the peptide the QuadraPure™ C efficiently removes EDT, 2-mercaptopyridine and excess Aldrithiol-2 impurities with no detectable loss of peptide to the resin.

Activated Peptide Synthesis: A Typical Experimental Procedure

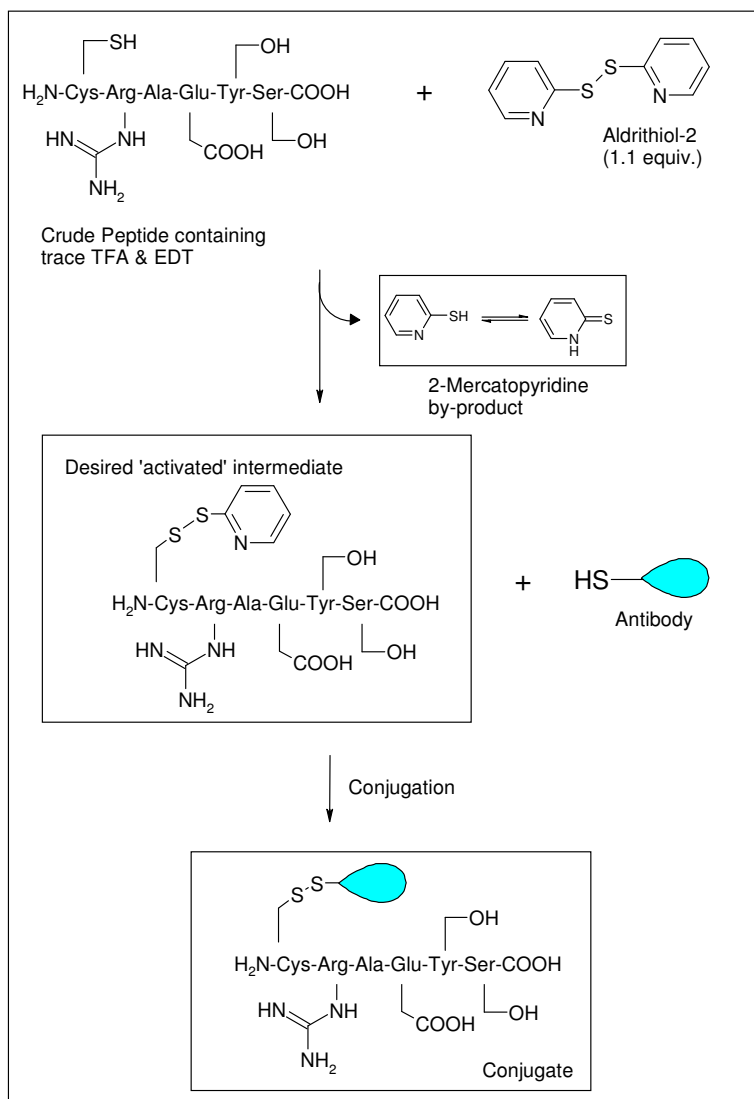


Figure 1: Typical Conjugation Procedure Employing Aldrithiol-2

Experimental

The crude peptide (0.050g, 0.069mmoles, MW 727.80, synthesised in-house) was dissolved in 0.1% v/v TFA in MeCN/H₂O (1:1, pH 1) at 5mg/ml⁻¹ concentration and activated by treatment with an excess of Aldrithiol-2 (17mg, 0.076mmoles, 1.1 equiv., MW 220.31, Sigma-Aldrich). The activated disulphide reacts specifically with the free sulphhydryl group of cysteine to liberate 2-mercaptopyridine (highly chromophoric by-product). The reaction is accompanied by a visual colour change. After 1 hour incubation at RT the crude peptidic solution was sampled then analysed by RP-HPLC. The elution profile clearly indicated the reaction had reached completion by the disappearance of the peptide starting material and the appearance of a new, more hydrophobic peak; representative of the activated peptide. The elution profile also shows the presence of a molar equivalent of 2-mercaptopyridine (0.069mmoles) and excess Aldrithiol-2.

Treatment of the above crude peptide solution (approx 50mg peptide in 10ml MeCN/H₂O) with QuadraPure™ C (0.5g) reduced the Aldrithiol-2 and 2-mercaptopyridine contaminants to ppb levels (accurately quantified) with no peptide lost to the resin. The negligible peptide loss was substantiated by quantitative RP-HPLC. In addition, all trace EDT was removed from the peptide solution affording a more manageable crude.




		
Crude Peptide solution in 0.1% v/v TFA in MeCN / H ₂ O (1:1, 5mg/ml ⁻¹)	Crude Reaction after 1 hour incubation with Aldrithiol-2 at RT	Crude reaction mixture after treatment with 0.500g QuadraPure™ C for 1 hour at RT

Figure 2: Visual representation of sulphydryl activation with Aldrithiol-2 and subsequent scavenging of 2-mercaptopyridine by-product & excess Aldrithiol-2 starting material with QuadraPure™ C

This is an industrially significant example because the experiment is a typical preparative CONJUGATION reaction practiced at scale. In practice, it is essential that all traces of thiol contaminate such as Aldrithiol-2, EDT and/or 2-mercaptopyridine are removed from the crude intermediate prior to conjugation with a thiol containing macromolecule such as an antibody. Obviously the presence of thiol containing impurities would be deleterious to the outcome of the conjugation reaction between the activated peptide and the thiol functional antibody. In the past this purification has been typically achieved by preparative reverse phase chromatography. This method demonstrates a cost effective, efficient process for peptide purification.