Easy assembly of ligands for glycosidase affinity chromatography

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An improved, high-yield synthesis of the corresponding *N*-carboxypentyl derivatives of three iminoalditol glycosidase inhibitors has been developed for affinity chromatography enzyme purification. Reductive amination of 1-deoxynojirimycin (or its D-manno or D-galacto analogues) with methyl 5-formylvalerate and NaBH₃CN at neutral pH afforded an aminoester which upon hydrolysis with aqueous 5% HCl gave the desired aminoacid in 97% overall yield. These amino acids could then be covalently attached using water-soluble carbodi-imide to 6-aminohexyl Sepharose 4B.

INTRODUCTION

One of the best methods for fractionating enzymes involves affinity chromatography, which takes advantage of biologically important binding interactions that occur on protein surfaces. Recent use of 1,5-iminoalditols like 1,5-dideoxy-1,5-imino-D-glucitol (1-deoxynojirimycin, 1-dNM) (1) (see Scheme 1) and 1,5-dideoxy-1,5-imino-D-mannitol (2) as affinity chromatography ligands has led to the isolation and purification of key glycosidases involved in the processing of asparagine-linked oligosaccharides in the late stages of mammalian glycoprotein biosynthesis (see [1] for review). For instance, Hettkamp *et al.* [2] have capitalized on the high specificity of 1-dNM for glucosidase I to separate it from glucosidase II. Similarly Paulsen & Matzke [3] linked 1,5-dideoxy-1,5-imino-L-fucitol to Sepharose through a six-carbon spacer arm for purification of α -L-fucosidase. The trimming α -mannosidases of glycoprotein biosynthesis have also been purified by Schweden *et al.* [4,5] using 2 that had been attached to Sepharose according to the procedure of Hettkamp *et al.* [2]. As the discovery of new iminoalditols continues to uncover potent competitive inhibitors of glycosidases (see [6] for review), we have developed a mild, general, and high-yield synthesis of the *N*-carboxypentyl derivatives like 4 which are suitable for covalent attachment to nucleophilic resins like 6-aminohexyl Sepharose 4B (see 6).

Scheme 1. Preparation of *N*-carboxypentyl derivatives of glycosidase inhibitors

MATERIALS AND METHODS

1-Deoxynojirimycin (1) was purchased from the Genzyme Corporation, Boston, MA 02111, U.S.A. Methyl 5-formylvalerate was prepared as described previously [7].

Synthesis of *N*-(5'-carboxypentyl)-1-deoxynojirimycin (4)

To a solution of 1-deoxynojirimycin (1) (hydrochloride form, 75 mg, 0.38 mmol) in anhydrous methanol (2 ml) was added methyl 5-formylvalerate (3) (300 mg, 2.1 mmol) and NaBH₃CN (34 mg, 0.54 mmol). After stirring for 44 h at room temperature, the reaction mixture was treated with 15% NaOH (0.15 ml, 0.56 mmol) and transferred to a strongly acidic ion exchange resin (Dowex 1, H⁺ form). Nonbasic material was eluted with ethanol. The desired intermediate ester 5 was then eluted with 5% HCl and the eluent concentrated *in vacuo*. The resulting oily solid was dissolved in 5% HCl (5 ml) and heated at 65 °C for 3.5 h. After concentrating the reaction mixture *in vacuo* the residue was dissolved in ethanol (2 ml) and filtered through a plug of glass wool to remove insoluble NaCl. The filtrate was concentrated to give 4 (101 mg, 97%) as an oil. Recrystallization from ethanol afforded pure 4 (m.p. 166–169 °C) identical with an authentic sample, lit. m.p. 168–170 °C [2]: ¹H-n.m.r. (300 MHz, ²H₂O) δ 4.27 (br. s, 1 H), 4.10, 4.02 (AB q, 2 H, *J* 13 Hz), 4.00 (t, 1 H, *J* 10 Hz), 3.72 (dd, 1 H, *J* 3, 9 Hz), 3.53 (dd, 1 H, *J* 4, 13 Hz), 3.16 (d, 1 H, *J* 10 Hz), 3.01 (t, 2 H, *J* 7.5 Hz), 2.42 (t, 2 H, *J* 7 Hz), 1.83 (m, 2 H), 1.67 (m, 2 H).

RESULTS AND DISCUSSION

Hettkamp *et al.* [2] linked a carboxypentyl group to the nitrogen of 1-dNM by heating with an excess of 6-bromohexanoic acid (50 h, 55 °C) to furnish 4 in unspecified yield. We found that the 6-carbon tether could be attached to 1-dNM in virtually quantitative yield using methyl 5-formylvalerate (3), which was readily prepared by the ozonolysis of cyclohexene in methanol according to the method of Schreiber *et al.* [7]. For instance, reductive amination of 1 (75 mg) with 3 (300 mg) using NaBH₃CN (34 mg) in methanol (2 ml, neutral pH, 44 h) afforded aminoester 5, which could be obtained pure after chromatography on a strongly acidic ion exchange resin (Dowex 1, H⁺ form). Treatment of 5 with aqueous 5% HCl gave the desired aminoacid 4 in 97% yield from 1-dNM. In exactly the same fashion the D-manno analogue 2 and the corresponding

Abbreviation used: 1-dNM, 1-deoxynojirimycin.

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D-galacto-derived iminoalditol (not shown) were joined to a hexanoic acid tether. All three ligands could then be attached to 6-aminohexyl Sepharose 4B according to the procedure of Hettkamp *et al.* [2] using a water-soluble carbodi-imide, and should find wide use in the purification of biologically interesting carbohydrate-processing enzymes.

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