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Protease-mediated preparation of valganciclovir intermediate

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Protease 6L
Alcalase 2.4L

ABSTRACT

An efficient and promising enzymatic process towards the synthesis of Valganciclovir intermediate, 2[(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)methoxy]-3-hydropropyl-N-[(benzyloxy)carbonyl]-L-valinate has been developed. Protex 6L, a bacterial alkaline protease derived from a selected strain of *Bacillus licheniformis* and Alcalase 2.4L, a subtilisin protease has been found as a excellent biocatalyst towards the desymmetrization of 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]-1,3-propanediyl bis(L-valinate), an intermediate of Valgancyclovir hydrochloride. The access to mono-ester synthesis of high purity has been possible by hydrolysis reaction where the reaction parameters have been optimized in terms of various hydrolases, source and amount of Protex-6L, temperature, solvent and reaction time.

1. Introduction

During the past recent years, tremendous research have been made to establish desymmetrization of symmetric compounds by eliminating one or more elements of symmetry of the substrate. Synthesis of Valganciclovir hydrochloride is one of them, an anti-viral active pharmaceutical ingredient used to treat cytomegalovirus infection, associated with AIDS disease [1–7]. Valganciclovir hydrochloride is a mono-L-valyl ester prodrug of ganciclovir that exists as a mixture of diasteromers [8]. Numerous routes were proposed by early researchers towards the synthesis of Valganciclovir. The process includes protection, deprotection as well as reductive halogenation of both amino moiety and hydroxyl moiety of valganciclovir intermediate [9–21]. WO199727197 describes a process which involves preparation of cyclic ortho ester of ganciclovir followed by hydrolysis, ganciclovir mono carboxylate, esterification of ganciclovir mono carboxylate and selective hydrolysis to yield mono valinate ester of ganciclovir [22]. EP18373361 describes a process with triacetyl ganciclovir as a starting material, comprising steps like selective hydrolysis, reacting with a coupling agent, followed by hydrolysis under basic condition and hydrogenolysis in presence of 10% palladium carbon catalyst [23]. A enzymatic process for the preparation of valganciclovir was been published out by preparing regioselective amino acylation of Ganciclovir [24]. Many research papers are also available on enzymatic desymmetrization of symmetric compounds using lipases and esterases which elimates one or more elements of symmetry of the substrate through either transesterification [25–38] or hydrolysis [39–50].

During our course of work, desymmetrization of 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]-1,3-propanediyl bis(L-valinate) has been studied using lipases, esterases and proteases (Scheme 1). It has been found that Protex 6L (a bacterial alkaline protease derived from a selected strain of *Bacillus licheniformis*) and Alcalase 2.4L (a Subtilisin protease) were effective.

2. Experimental

2.1. Analytical methods

2.1.1. HPLC

High performance liquid chromatography analysis was performed on Waters alliance 2645 High performance liquid chromatography instrument connected with UV detector at 254 nm using Zorbax, SB-C18 column (3.5 μm particle size, 150 mm × 4.6 mm length) eluted with gradient mobile phase

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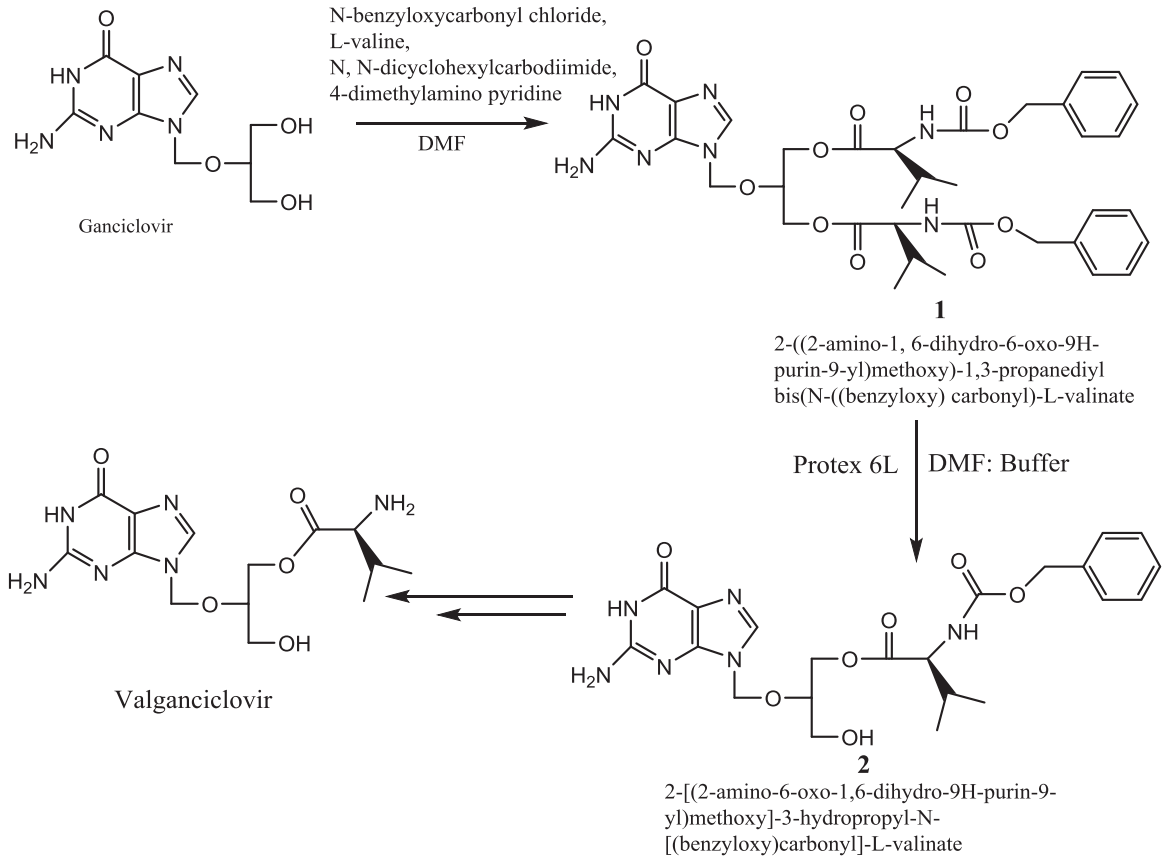

Scheme 1. Enzymatic desymmetrization of 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]-1,3-propanediyl bis.

system containing ammonium dihydrogen phosphate buffer pH 2.8 and methanol at a flow rate of 1 mL/min. The gradient mobile program is as follows: 0–5 min (92% ammonium dihydrogen phosphate buffer pH 2.8: 8% methanol), 15–30 min (92–80% ammonium dihydrogen phosphate buffer pH 2.8: 8–20% methanol), 15–30 min (80–30% ammonium dihydrogen phosphate buffer pH 2.8: 20–70% methanol), 30–40 min (30% ammonium dihydrogen phosphate buffer pH 2.8: 70% methanol), 40–41 min (30–92% ammonium dihydrogen phosphate buffer pH 2.8: 70–8% methanol) and 41–55 min (92% ammonium dihydrogen phosphate buffer pH 2.8: 8% methanol).

The retention time were found to be 1.8 min, 3.2 min, 28.2 min and 36.0 min for guanine, ganciclovir, 2[(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)methoxy]-3-hydropropyl-N-[(benzyloxy) carbonyl]-L-valinate and 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]-1,3-propanediyl bis(L-valinate) respectively.

2.1.2. NMR spectroscopy

The ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker Avance 300 spectrometer. The chemical shifts are reported in δ ppm relative to TMS (δ 0.00) and DMSO-d_6 as internal standards respectively.

2.1.3. Mass spectrometry

Electron Spray Ionization-Mass spectra (ESI-MS) of isolated compounds were measured using Agilent 1100 LC/MSD Trap SL instrument.

2.2. Enzymes

Novozyme 435, CALAL, CALBL, Savinase 16L, Savinase 12T, Esperase 8L, Resinase HT, Novocor ADL, Lipex 100L, lipozyme RM IM, Alacalase 2.4L, Neutrase 0.8L, Lecitase ultra, florozyme 1000L, Palatase 20000L and Protamex plus from Novozyme A/S, Denmark, Protex 6L, Protex 14L and Protex 16L from Genecor International, The Netherlands, Esterase EL01 from Fermenta Biotech Ltd., India, Acid protease, Pectin methyl esterase and Alkaline protease from Aumgene BioSciences Ltd., India

2.3. Chemicals

N-Benzyloxycarbonyl chloride from Sigma Chemical Co, L-valine, N,N-dicyclohexylcarbodiimide, 4-dimethylamino pyridine, 9-(1,3-dihydroxy propoxymethyl) guanine Methanol, methylene dichloride, liquid ammonia, conc. HCl, dimethyl formamide, ethanol, 1,4-dioxane, tetrahydrofuran, 2-methoxy ethanol, toluene, isopropyl alcohol, acetone, acetonitrile, ethyl acetate.

2.4. General procedure for preparation of 2-((2-amino-1, 6-dihydro-6-oxo-9H-purin-9-yl)methoxy)-1,3-propanediyl bis(N-((benzyloxy) carbonyl)-L-valinate **(1)**

Preparation of 2-((2-amino-1, 6-dihydro-6-oxo-9H-purin-9-yl) methoxy)-1,3-propanediyl bis(N-((benzyloxy) carbonyl)-L-valinate has done as per procedure given in US patent no.5043339 [51]. A 22.5 g (0.09 M) of N-benzyloxycarbonyl chloride (Sigma Chemical Co.) L-Valine, 18.6 g (0.09 mol) of N,N-dicyclohexylcarbodiimide and 1.2 g (0.01 mol) of 4-dimethylamino pyridine were added in 100 mL of N,N-dimethylformamide and stirred under nitrogen for 10 min. A 7.65 g (0.03 mol) of 9-(1, 3-dihydroxy propoxymethyl) guanine, the mixture was stirred for 18–24 h at ambient temperature. The progress of reaction was monitored by HPLC. The suspension was filtered, washed the precipitate with dichloromethane. The filtrate were combined