

A succinct synthesis of valganciclovir hydrochloride, a cytomegalovirus (CMV) retinitis inhibitor[#]

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Abstract

A concise and efficient synthesis of valganciclovir hydrochloride **1**, a CMV retinitis inhibitor, without involving protection-deprotection sequences, is described. The synthetic utility of (2S)-azido-3-methylbutyric acid, which acts as a masked L-valine equivalent, is demonstrated in the synthesis of **1**.

Keywords: Antiviral, CMV retinitis inhibitor, valganciclovir, selective ester hydrolysis

Introduction

Cytomegalovirus (CMV) is a common and opportunistic infection in adult population.¹ When the human immune defences are weak, CMV can attack several parts of the body and cause severe damage.² Patients with immunosuppression caused by various diseases including acquired immunodeficiency syndrome (AIDS) are at higher risk of being affected by CMV.³ The most common illness caused by CMV is retinitis *i.e.* the death of cells in retinas, which can lead to blindness unless treated.

Valganciclovir hydrochloride (Valcyte[®]) **1** has been used for the treatment of CMV retinitis in patients with weakened immune systems.⁴ Valganciclovir hydrochloride is the hydrochloride salt of an ester of L-valine with ganciclovir **2** that exists as a mixture of two diastereomers.⁵ After oral administration, intestinal and hepatic esterases hydrolyze both diastereomers to ganciclovir **2**, which inhibits replication of the human cytomegalovirus *in vitro* and *in vivo*. It is evident from

the literature that valganciclovir hydrochloride **1** is a recognized substrate of the intestinal peptide transporter PEPT1, which instigates the tenfold higher bioavailability of ganciclovir subsequent to valganciclovir compared to oral ganciclovir administration.⁶

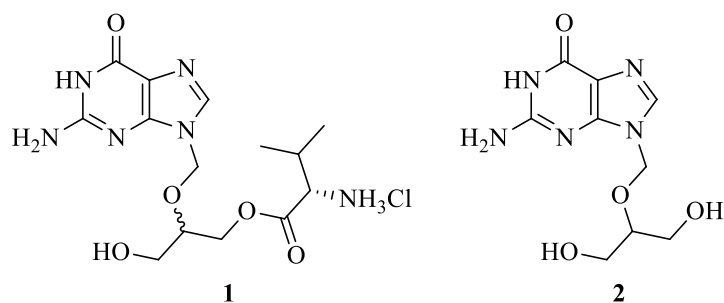
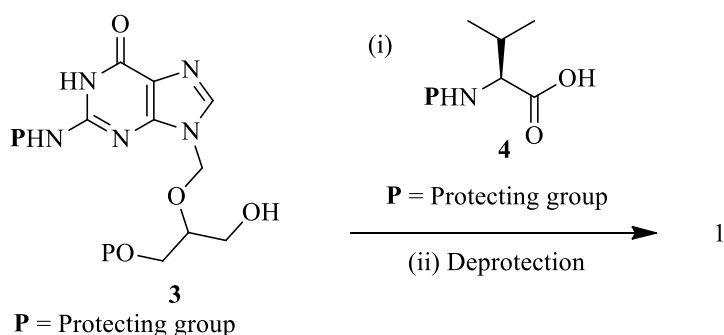


Figure 1. Structures of valganciclovir **1** and ganciclovir **2**.

In Dr Reddy's Laboratories, we have been dynamically involved in the synthesis and process development of various active pharmaceutical ingredients (APIs). While working on the synthesis of antiretroviral drugs, we became fascinated by valganciclovir hydrochloride. In this communication, we divulge our efforts toward the synthesis of valganciclovir hydrochloride **1**.

Results and Discussion

Since valganciclovir is a L-valyl ester of ganciclovir **2**, most of the hitherto known literature on the synthesis of **1** involves the coupling of the protected form of ganciclovir **3** with *N*-protected L-valine derivative **4** followed by deprotection (Scheme 1).



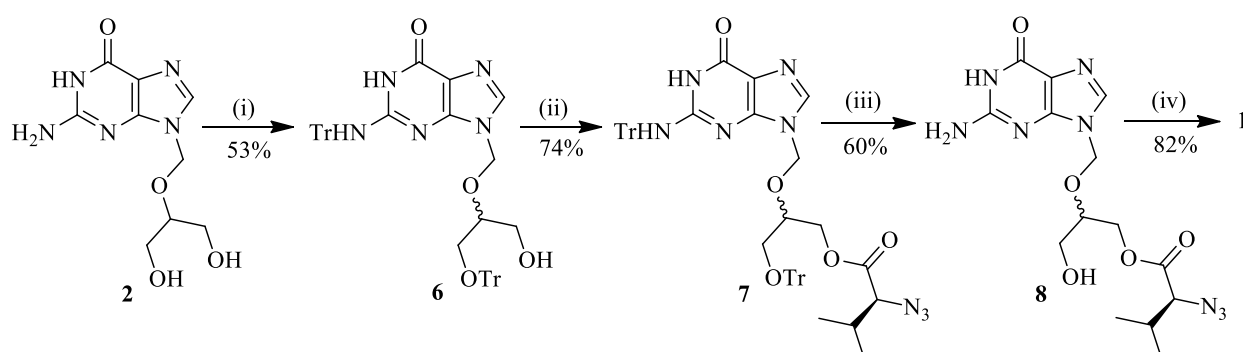
Scheme 1. Known synthesis of **1**.

Scientists from Syntex Inc. have published a number of patent applications, in which **1** was obtained through coupling of N and O protected ganciclovir with Boc or Cbz protected L-valine followed by deprotection.⁷ Nestor and co-workers have reported the synthesis of **1** from

guanine.⁸ Rao's team has described an alternative method for the preparation of **1** by coupling mono *O*-acetyl protected ganciclovir with Cbz-L-valine followed by hydrogenolysis.⁹ Recently, Sharma and co-workers have filed a patent application, in which the synthesis of **1** was achieved by partial hydrolysis of bis Cbz-L-valine ester of ganciclovir followed by deprotection.¹⁰

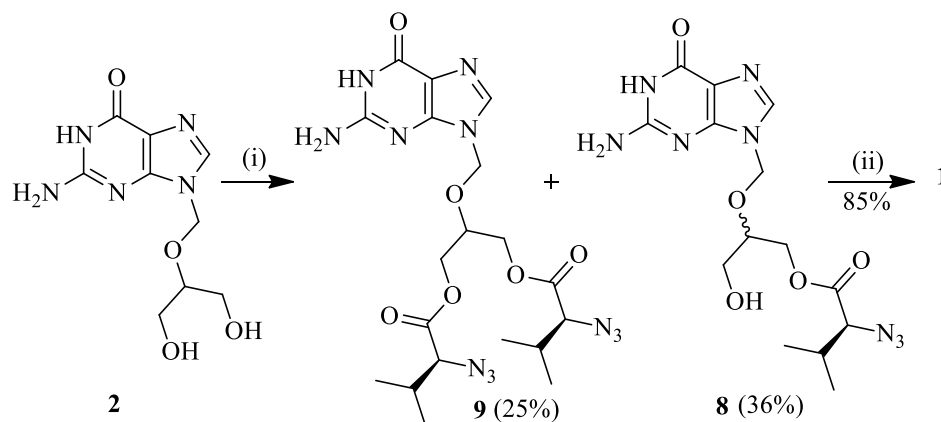
The precedented literature methods for the synthesis of **1** clearly show that the protection of amino group in L-valine is inescapable to coerce the coupling reaction. It was anticipated that bulky protecting group on L-valine might cause some sort of steric influence while coupling with ganciclovir **2** or its protected derivative, thereby reducing the reaction rate or yield of the coupled product. Moreover, the protection-deprotection strategy may further increase the risk of racemization at the amino acid chiral center. These problems could be avoided by the use of small size versatile precursor of amino group such as azide functionality. The azido group is relatively stable under acidic and basic conditions, and can be readily converted to amine by simple reduction. Since there are few potential advantages of replacing the protected L-valine by the azido equivalent; we decided to utilize this protocol for the synthesis of valganciclovir hydrochloride **1**.

The required α -azido acid, (2*S*)-azido-3-methylbutyric acid **5**, was prepared from L-valine through azido-transfer reaction with imidazole sulfonyl azide.¹¹ Initially, we were interested to learn the coupling reaction of **5** with *N,O*-ditrityl protected ganciclovir **6**.^{7a} The DCC mediated coupling reaction of **6** with **5** in DMF gave the coupled product **7** in 74% yield (Scheme 2). Deprotection of trityl groups in **7** was carried out using trifluoroacetic acid in DCM. Unfortunately, the yield of the deprotected compound **8** was disappointing (60%). Attempts to increase the yield of **8** by using different conditions and reagents failed to give satisfactory results. Then, the azido group in **8** was reduced with Zn and acetic acid to give valganciclovir acetate, which in turn was converted in to the hydrochloride salt **1** through anion exchange with aqueous HCl. Whilst the yield of the product in the final stage was moderate (82%), it was observed that the product was always contaminated with zinc acetate. Since valganciclovir hydrochloride is highly soluble in water, the removal of zinc salts from **1** became cumbersome, and we were not able to isolate **1** in pure form.¹²



Scheme 2. Synthesis of valganciclovir hydrochloride **1**.

In view of the fact that the yields of the intermediates in most of the stages in Scheme 2 were unacceptable, we have decided to amend the synthetic strategy. It is evident from the above scheme that the overall yield of the product **1** was significantly diminished by the integration of protection-deprotection sequence. So, it is obvious that the protection-deprotection strategy should be evaded in order to increase the overall yield of **1**. This triggered us to investigate the selective acylation of ganciclovir **2** with (2*S*)-azido-3-methylbutyric acid **5**. Thus, the coupling reaction of **2** with **5** was carried out using DCC conditions to provide the desired monoester derivative **8** (36%) along with bisester derivative **9** (25%) and unreacted ganciclovir **2** (20%) under the optimized conditions (Scheme 3). Although, the yield of the monoester derivative **8** was unimpressive, the overall yield of **8** from ganciclovir was better when compared to Scheme 2, *i.e.* the protection-deprotection strategy. Since the reduction of azido group of **8** with Zn and acetic acid was found to be problematic in Scheme 2, catalytic hydrogenation method was opted (Pd-C/H₂). In this case, the reduction of **8** proceeded smoothly and **1** was isolated in 85% yield with good purity (Scheme 3).¹²

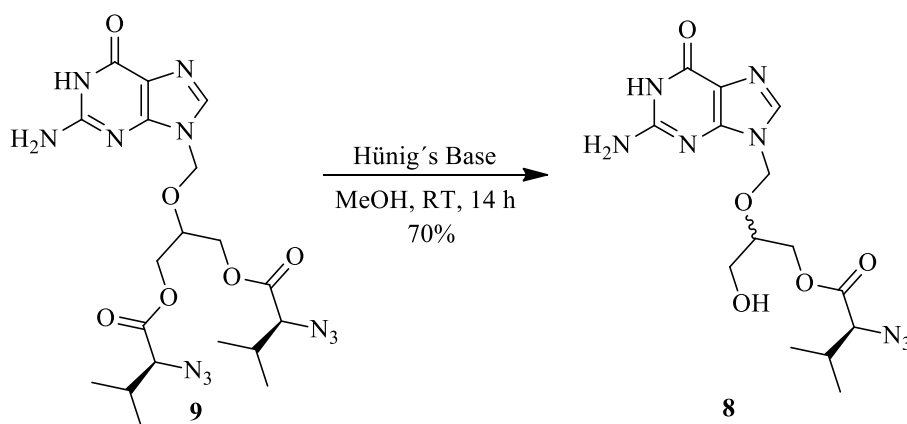


Conditions: (i) **5**, DCC, HOBt, Et₃N, DMF (ii) Pd-C, H₂, HCl, MeOH

Scheme 3. Direct coupling route to **1**.

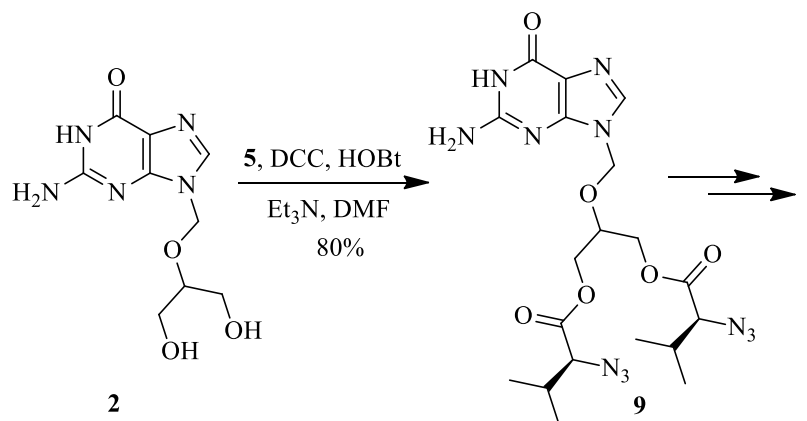
After completing the synthesis of **1** through direct coupling route (Scheme 3), we turned our attention towards optimization of the coupling stage to improve the yield of **8** under different conditions. Unfortunately, none of the reaction conditions facilitated in improving the yield of **8**. This observation provoked us to investigate the synthetic scheme 3 further in detail. In the direct coupling route (Scheme 3), the bisester derivative **9**, which was considered as a waste by-product, was always associated with a considerable quantity of monoester derivative **8**. To improve the efficiency of the reaction, it was decided to convert the by-product **9** to the useful monoester intermediate **8** by partial hydrolysis. A number of bases and reagents were tried for this transformation, and finally *N,N*-diisopropyl ethylamine (Hünig's base) was found to be the better option. The partial hydrolysis of **9** was optimized with equimolar quantity of Hünig's base

to give the required product **8** in 70% (in HPLC)¹³ along with over-hydrolyzed product **2** (20%) and unreacted **9** (7%) [Scheme 4].



Scheme 4. Partial hydrolysis of diester derivative **9**.

The yield and the quality of **8** were satisfactory and the over-hydrolyzed product **2**, which is nothing but the starting material ganciclovir, was recycled by crystallization. Since the yield of **8** through partial hydrolysis route (Scheme 4) was better than that of selective esterification method (Scheme 3), the strategy of synthesis was slightly modified. In the modified route, ganciclovir **2** was converted to the bisester derivative **9** using excess of **5** under DCC mediated coupling conditions (Scheme 5). The bisester derivative **9** was subsequently converted to the monoester derivative **8** (by partial hydrolysis method) followed by reduction with Pd-C/H₂ to provide **1** in good yield and purity.



Scheme 5. Modified route of synthesis to **1**.

Conclusions

An efficient synthesis of valganciclovir hydrochloride **1**, which is an inhibitor of CMV retinitis, has been developed without involving protection – deprotection strategy. The synthetic utility of (2*S*)-azido-3-methylbutyric acid **5**, which acts as a masked L-valine equivalent, has been demonstrated in the synthesis of **1**. We have also established a proficient method for the partial hydrolysis of **9** to **8**.

Experimental Section

General. ¹H NMR spectra were recorded at 400 MHz Varian FT-NMR Spectrometer. The chemical shifts are reported in δ ppm relative to TMS. The IR spectra were obtained using Perkin Elmer, Spectrum One FTIR spectrophotometer, with substances being pressed in a KBr pellet. The mass analyses have been performed on AB-4000 Q-trap LC-MS/MS mass spectrometer (MDS SCIEX, Applied Biosystems, California, USA). All the solvents and reagents were used without further purification.

Preparation of *N*-trityl-2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)methoxy-3-trityloxy-propan-1-ol (**6**).^{7a}

A solution of trityl chloride (178.5 g, 0.64 mol) in dimethylformamide (570 mL) was added to a suspension of ganciclovir (61.5 g, 0.241 mol), 4-dimethylaminopyridine (142.5 mg, 0.001 mol), and triethylamine (181.3 mL, 1.29 mol) in dimethylformamide (570 mL) was added at 50 °C over 25 minutes and the reaction mixture was stirred for about 9 hours at same temperature. The reaction mixture was cooled to 10 °C, filtered and washed with dimethylformamide (150 mL). The filtrate was then warmed to 20 °C and water was added in two portions (1350 mL and 900 mL) and decanted. Ethyl acetate (1500 mL) was added to the obtained gummy solid and stirred at room temperature for 12 h. The separated solid was filtered, washed with ethyl acetate (100 mL) and dried at 54 °C to afford the title compound as a white solid. Yield 76.0 g (43 %); FT IR (KBr) 3422, 3310, 1681, 1671 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 3.38 (d, *J* = 4.4 Hz, 2 H), 3.52 (m, 1 H), 3.87 (m, 3H), 5.41 (s, 2H), 6.46 (brs, 1H), 7.23-7.86 (m, 30 H), 7.79 (s, 1 H), 10.60 (s, 1H); MS *m/z* (DIP) 740.0 (M⁺ + 1).

Preparation of *N*-trityl-2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)methoxy-3-trityloxy-propyl-2'-(*S*)-azido-3'-methylbutanoate (**7**)

A solution of 2-(*S*)-azido-3-methylbutanoic acid **5**¹¹ (0.93 g, 0.006 mol) in dichloromethane (40 mL) was added to a solution of *N,N*-dicyclohexylcarbodiimide (0.7 g, 0.003 mol) in dichloromethane (80 mL) at 0 °C and stirred for about 30 minutes. It was filtered and the filtrate was added in a drop-wise to a solution of *N*-trityl-2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)methoxy-3-trityloxy-propan-1-ol **6** (2.0 g, 0.0027 mol), 4-dimethylaminopyridine (35.0 mg,

0.001 mol) and triethylamine (0.35 g, 0.003 mol) in dimethylformamide (40 mL) at 25 °C over a period of 17 hours. Water (50 mL) was added to the reaction mixture and the layers were separated. Solvent from the organic layer was evaporated and then ether (20 mL) was added to the residue and the resulting suspension was stirred for 2 hours. The solid was filtered and dried at 50 °C for an hour to afford the title compound as a white solid. Yield 1.7 g (74.0 %); mp 183 °C; FT IR (KBr) 3328, 2930, 2103, 1742, 1684 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) [1:1 mixture of diastereomers] δ 0.87 (d, $J = 7.2$ Hz, 3H), 0.88 (d, $J = 7.2$ Hz, 3H), 1.60 – 1.66 (m, 1H), 2.86 (d, $J = 6.8$ Hz, 0.5 H), 2.94 (d, $J = 6.8$ Hz, 0.5 H), 3.28 – 3.38 (m, 2H), 3.81 – 3.88 (m, 1H), 4.39 (d, $J = 5.2$ Hz, 2H), 5.00 (s, 2H), 5.98 (brs, 1H), 6.96-7.35 (m, 30 H), 7.99 (s, 1 H), 11.4 (s, 1H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 17.1, 18.9, 24.4, 25.3, 30.0, 33.1, 47.5, 66.8, 69.9, 85.7, 126.5, 127.0, 127.6, 127.9, 128.0, 128.3, 137.9, 143.3, 144.4, 149.5, 150.9, 156.4, 169.3; MS m/z (DIP) 865.4 ($\text{M}^+ + 1$).

Preparation of 2-[(2-amino-1,6-dihydro-6-oxopurin-9-yl)methyloxy]-3-hydroxypropyl 2'-(S)-azido-3'-methylbutanoate (8)

A solution of trifluoroacetic acid (8.0 mL, 0.104 mol) in dichloromethane (8 mL) was added to a solution of *N*-trityl-2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)methoxy-3-trityloxy-1'-propyl-2'-(S)-azido-3'-methylbutanoate **7** (2.0 g, 0.002 mol) in dichloromethane (4 mL) at 15 °C over a period of 10 minutes and stirred for 3 hours. Methyl *t*-butyl ether (25 mL) and *n*-hexane (100 mL) were added to it and the resulting mixture was stirred for 20 minutes. The solvent was decanted and dichloromethane (15 mL) was added to the resultant reaction mixture. The solvent was evaporated and the solid was dried under vacuum at 25 °C to afford **8** as a white crystalline powder. Yield 0.545 g (60 %); mp 163 °C; FT IR (KBr) 3444, 3198, 2110, 1737, 1707 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) [1:1 mixture of diastereomers] δ 0.77 (d, $J = 6.8$ Hz, 1.5 H), 0.78 (d, $J = 6.8$ Hz, 1.5 H), 0.87 (d, $J = 6.8$ Hz, 3H), 1.90 – 1.94 (m, 1H), 3.34 – 3.44 (m, 2H), 3.81 – 3.88 (m, 1H), 3.93 (d, $J = 5.2$ Hz, 0.5 H), 3.96 (d, $J = 5.0$ Hz, 0.5 H), 4.03 – 4.06 (m, 1H), 4.21 – 4.29 (m, 1H), 4.88 – 4.89 (m, 1H), 5.42 (d, $J = 4.0$ Hz, 2H), 6.47 (brs, 2H), 7.79 (s, 0.5 H), 7.80 (s, 0.5 H), 10.63 (s, 1H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 17.1, 18.9, 30.1, 60.1, 64.1, 64.5, 66.8, 71.0, 71.1, 76.5, 76.6, 116.4, 137.6, 151.3, 153.9, 156.7, 169.6; MS m/z (DIP) 403 ($\text{M}^+ + \text{Na}$).

Preparation of valganciclovir hydrochloride (1)

2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)methoxy-3-hydroxy-1'-propyl-2'-(S)-azido-3'-methylbutanoate **8** (10.0 g, 0.026 mol) and zinc dust (30.0 g, 0.459 mol) are charged into a round bottom flask and cooled to 5–10 °C. A solution of acetic acid (100 mL) in isopropanol (400 mL) was slowly added to the reaction mixture and the resulting suspension was stirred at 25–30 °C for about 45 minutes. It was filtered through celite and washed with a mixture of acetic acid (10 mL) and isopropanol (40 mL). The solvent was evaporated to dryness at 35 °C and the residue was suspended in acetone (200 mL) and stirred for 30 min. The solid obtained was filtered, washed with acetone (50 mL) and dried under reduced pressure. The solid is suspended in water

(50 mL) and stirred for 5 minutes at about 5 °C. Acetone (330 mL) is added to the suspension and stirred for about 5 minutes. The obtained white solid was filtered, washed with acetone (40 mL), and dried under reduced pressure to afford the valganciclovir acetate. It was suspended in isopropanol (140 mL) and acetic acid (7 mL) was added to it followed by trifluoroacetic acid (7 mL) and stirred for 5 minutes. The resulting mixture was filtered through celite and washed with isopropanol (10 mL). The filtrate was treated with 4% HCl in isopropanol (14 mL) for 30 minutes and filtered. The obtained solid was again washed with isopropanol (20 mL) and dried under reduced pressure to afford **1** as a white solid. Yield 8.3 g (82 %); mp 143 °C (lit. mp 142 °C);^{8b} FT IR (KBr) 3410, 1742, 1727, 1694 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) [1:1 mixture of diastereomers] δ 0.87 (d, *J* = 6.4 Hz, 6H), 2.01 – 2.05 (m, 1H), 3.44 – 3.48 (m, 1H), 3.71 (d, *J* = 4.4 Hz, 0.5 H), 3.77 (d, *J* = 4.4 Hz, 0.5 H), 3.83 – 3.90 (m, 1H), 4.02 – 4.12 (m, 1H), 4.21 (d, *J* = 2.8 Hz, 0.5 H), 4.24 (d, *J* = 3.0 Hz, 0.5 H), 4.30 (d, *J* = 3.2 Hz, 0.5 H), 4.32 (d, *J* = 3.2 Hz, 0.5 H), 4.94 (brs, 1H, OH), 5.42 – 5.47 (m, 2H), 6.6 (brs, 2H), 7.81 (s, 0.5 H), 7.83 (s, 0.5 H), 8.40 (brs, 2H), 10.7 (s, 1H); MS *m/z* (DIP) 355.1 (M⁺ + 1).

Synthesis of bisester derivative (9)

To a stirred solution of ganciclovir **2** (50 g, 0.196 mol) in DMSO (500 mL) was added 4-hydroxybenzotriazole (26.5, 0.196 mol) followed by triethylamine (20 g, 0.196 mol) and the resulting mixture was stirred at 25-30 °C for 10 min. A solution of (2S)-azido-3-methyl butyric acid **5** (98 g, 0.685 mol) in DMSO (20 mL) was added to the reaction mixture in a drop-wise manner. The reaction mass was maintained over 30-45 min at 25-30 °C. It was cooled to 20 °C and added a solution of dicyclohexylcarbodiimide (121 g, 0.587 mol) in DMSO (150 mL). The resultant mixture was stirred vigorously for 2 h at 25-30 °C. The solid was filtered and washed with DMSO (80.0 mL). To the filtrate was added water (4.5 L) for about 1 h at 25-30 °C and stirred further for another 1 h. The solid thus formed was filtered and washed with water (500 mL), and finally dried at 50-60 °C under vacuum to give **9** as a white solid. Yield 88.0 g (88.8 %); mp 138 °C; FT IR (KBr) 3423, 3320, 2108, 1745, 1682 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 0.80 (d, *J* = 6.4 Hz, 6H), 0.89 (d, *J* = 6.8 Hz, 6H), 1.95 – 2.09 (m, 2H), 4.02 (d, *J* = 5.2 Hz, 1H), 4.08 (d, *J* = 5.2 Hz, 1H), 4.16 – 4.18 (m, 3H), 4.32 – 4.35 (m, 2H), 5.4 (s, 2H), 6.4 (brs, 2H), 7.8 (s, 1H), 10.6 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 17.2, 19.0, 30.2, 63.5, 63.8, 66.7, 70.8, 73.3, 116.5, 137.5, 151.3, 153.8, 156.7, 169.4; MS *m/z* (DIP) 506.1 (M⁺ + 1).

Synthesis of monoester derivative (8) by partial hydrolysis

To a stirred solution of bisvaline ester of ganciclovir **9** (50 g, 0.099 mol) in methanol (750 mL) was added *N,N*-diisopropyl ethylamine (12.8 g, 0.099 mol) at 25-30 °C and the resulting reaction mass was maintained for 14 h at RT. It was quenched with acetic acid (5.94 g, 0.099 mol) and the solvent was removed up to 90%. To this solution, was added hexane (200 mL) and the resulting suspension was stirred for 30 min at RT. The solid thus formed was filtered and washed with hexane (50 mL). The wet material was dried under vacuum to provide a residue, which contains 70% of **8**, 20% of **2** and 7% of **9** (by HPLC). The crude material was then purified

repeatedly using a mixture of *n*-butanol and water, followed by methanol to give pure mono ester of ganciclovir **8** as a white solid. Yield 13.0 g (34.5 %).

Synthesis of valganciclovir hydrochloride (**1**)

To a stirred solution of mono ester derivative **8** (5g, 0.013 mol) in methanol (100 mL) was added 10% Pd/C (1 g) followed by 33% aqueous hydrochloric acid (1.6 mL, 0.014 mol). The resulting suspension was hydrogenated in the autoclave at 4 kg/cm² pressure for about 2 h. The reaction mixture was filtered through celite® and washed with methanol (25 mL). The solvent was removed under vacuum and the residue was recrystallized from water: isopropanol (1:10) mixture to provide **1** as a white solid. Yield 3.6 g (70.6 %).

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13. An in-house HPLC gradient method was developed for the separation of all possible related substances of valganciclovir hydrochloride. Waters make HPLC system equipped with 515 pump and 2998-photodiode-array (PDA) detector was used for better separation. The buffer solution used for the preparation of mobile phases A and B consists of 0.001M aqueous potassium dihydrogen phosphate monohydrate and 1.0 mL of TEA and its pH was adjusted to 2.8 with diluted phosphoric acid. Mobile phase A was buffer solution; mobile phase B was prepared in the ratio of 10:90 (v/v) of water and acetonitrile. Vertisep AQS C-18, 250 mm x 4.6 mm, 3 μ particle size column was used with a time gradient program of *T* (min)/%B (v/v). Initial gradient starts with 10 % of B and at 5 min it is 25%. The ratio being continued up to 35 min, at 40 min it is 60 %, which is continued up to 45 min, at 50 min it is 90 %, which is continued up to 55 min, at 60 min it is 10%, which is continued up to 65 min with a flow rate of 1.0ml/min, column oven temperature was 30 °C and column eluent was monitored by PDA detector at 254 nm.