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An Efficient and Large Scale Process for Synthesis of Valacyclovir¶

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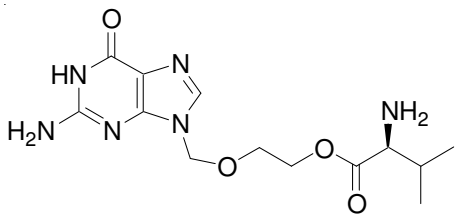
A facile, commercially viable and large scale process is developed for an antiviral drug substance, valacyclovir hydrochloride. Several process related critical factors including organic and heavy metal impurities are efficiently addressed in this process.

Key Words: Antiviral, Valacyclovir, Racemization, D-Isomer, Metal impurities, Resin.

INTRODUCTION

Valacyclovir[1,2], 2-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy]ethyl-L-valinate (**1**) is an antiviral drug used in the management of viral infections such as herpes simplex and varicella-zoster in humans. It is a prodrug, an esterifed version of acyclovir and having enhanced bioavailability (*ca.* 55 %) when compared to acyclovir (10-20 %) and is marketed by Glaxo Smith Kline under the trade name Valtrex[3] or Saltier. Valacyclovir is a high dosage drug with a daily dosage of 2 g per day. As per the regulatory (ICH) guidelines, for such a high dosage drug with maximum daily dosage of 2 g or > 2 g per day[4] unqualified related substance (impurity) in the drug substance should be within the limit of not more than 0.05 %[5].



Valacyclovir (**1**)

In development of the process for valacyclovir (**1**), we have successfully met this stringent quality requirement by efficiently addressing the process related organic and heavy metal impurities.

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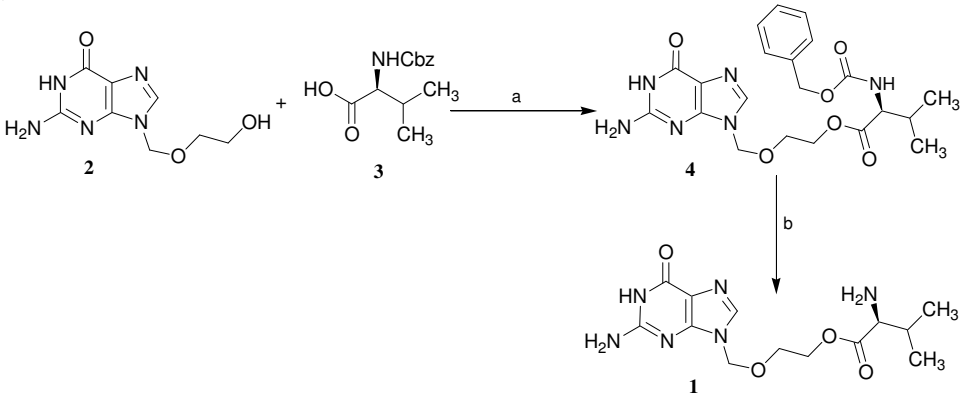
In the first synthesis of **1**, reported by Beauchamp and co-workers[1,2], acyclovir (**2**) and N-carbobenzyloxy-L-valine (**3**, Cbz-L-valine) were condensed to directly provide N-carbobenzyloxy protected valacyclovir (4), which was subjected to palladium catalyzed deprotection to furnish valacyclovir (**1**) in 55 % yield (**Scheme-I**).



Reagents and conditions: (a) DCC, DMAP, DMF, room temperature, 12 h
(b) H_2, 5% Pd/C, methanol, THF, aqueous HCl

Scheme-I: Synthetic scheme of valacyclovir

While developing our process for **1**, by practising this synthetic route and process as such, following criticalities were observed: (a) 3-4 % of enantiomer (D-isomer, **D-4**) was formed[6] along with N-Cbz valacyclovir (**4**); (b) metal impurities palladium and aluminium carried from palladium/alumina reagent and could not be removed to the desirable levels by the conventional purifications; (c) column chromatography and/or repeated crystallizations were required to meet the desired purity.

An efficient and scaleable process is developed by addressing the afore mentioned concerns and details are described in this work.

EXPERIMENTAL

General procedures: All starting materials were commercial products. The solvents and reagents were used without any purification. Melting points were recorded with Buchi melting point B-540 instrument and are uncorrected. IR spectra were recorded in the solid state as a KBr dispersion using a Perkin-Elmer FT-IR spectrophotometer and only diagnostic and/or intense peaks are reported. ^{1}H NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 with Varian Mercury Plus 400 MHz instrument. The chemical shifts are reported in δ ppm relative to TMS. Multiplicity is indicated by one or more of the following: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad); Analysis of metals was done by the use of inductively coupled plasma-optical emission spectroscopy (ICP-OES). ICP-OES analysis is accurate to parts per billion (ppb) to parts per trillion (ppt) range and applicable to most of the metals in pharmaceuticals[7].

Preparation of valacyclovir (1)

Preparation of Cbz-protected valacyclovir (4): Carbobenzyloxy-L-valine (**3**, 83.6 g, 0.332 mol) was dissolved in DMF (350 mL) and the solution was cooled to -5 °C. A solution of dicyclohexyl carbodiimide (68.6 g, 0.333 mol) in DMF (150 mL) was added below 0 °C. After aging for 20 min, acyclovir (**2**, 50 g, 0.222 mol) and 4-dimethylamino pyridine (4 g, 0.032 mol) were charged and reaction mixture was stirred at -5 to 0 ºC for *ca.* 6 h. Dicyclohexylurea was filtered, 80 % of the solvent was removed by distillation and the remaining solution was diluted with water (300 mL). Precipitated compound **4** was filtered at ambient temperature and recrystallized from methanol (88.5 g, 87 %). Purity by HPLC 99.3 %, m.p. 157-159 °C; IR (KBr, ν_{max}, cm^{-1}): 3311, 2931, 2855, 1726, 1630, 1606, 1536, 1390, 1183, 1128, 1101, 1041; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 10.8 (br, 1H), 7.9 (br, 1H), 7.7 (s, 1H), 7.22 (m, 5H), 5.38 (s, 2H), 5.1 (s, 2H), 4.25 (m, $1H_a$), 4.35 (m, $1H_b$), 3.75 (d, 1H), 3.7 (m, 2H), 2.15 (m, 1H), 0.91 (d, 3H), 0.88 (d, 3H).

Purification of Cbz-protected valacyclovir (4): Carbobenzyloxy protected valacyclovir (**4**, 25 g) having 3.1 % of D-isomer was dissolved in a mixture of acetone (300 mL) and water (75 mL) at reflux temperature and cooled to ambient temperature. The mixture was stirred for few hours after diluting with additional water (75 mL) and filtered to provide compound **4** (21.2 g, 84.8 %, 2.1 % **D-4** by HPLC).

Deprotection of Cbz group of 4: Carbobenzyloxy protected valacyclovir (**4**, 5 g, 0.011 mol) and dry 5 % Pd on alumina mixture (0.5 g) were taken in DMF (50 mL) in a hydrogenator vessel. Hydrogen pressure of 4 kg/cm^2 was applied at *ca.* 30 °C for the completion of the reaction. 70 % of the solvent was removed by distillation under vacuum below 80 °C and the resultant concentrated solution was cooled to 10 °C. pH was adjusted to 3.0-4.0 using aqueous HCl at 10 °C, diluted with water (12.5 mL) and catalyst was removed by filtration through celite at ambient temperature. Filtrate was saturated with acetone (225 mL), precipitated valacyclovir HCl (**1**) was filtered and dried under suction (4.7 g, 98.5 % pure by HPLC).

Purification of valacyclovir HCl (1) to remove D-1: Valacyclovir HCl (**1**, 25 g) having 3.5 % of D-1 was dissolved in 25 % aqueous acetonitrile (250 mL) at 70 °C. The mixture was cooled to 30 °C and diluted slowly with acetonitrile (75 mL) at 30 °C under stirring. The separated solid was filtered and dried under vacuum to yield compound **1** (19.5 g, 75 %, 2.6 % D-1 by HPLC).

Process for removing heavy metal impurities: A suspension of valacyclovir hydrochloride (**1**, 25 g) in water (50 mL) was heated to 65 °C with simultaneous stirring. To the obtained solution, T-63 resin (Thermax Ltd.) was added and the mixture was stirred for 20 min. The suspension was filtered through 0.45 μm filter paper and washed with water (25 mL). To the filtrate, acetone (500 mL) was added slowly. The obtained suspension was stirred for 1 h and the precipitate was filtered and dried to yield compound **1** (24 g, 96 %, both Pd and Al content less than 10 ppm).

Purification of valacyclovir HCl (1): Valacyclovir HCl (**1**, 50 g) wet material obtained from the above procedure was taken in DMF (230 mL) and maintained for 7 h at 30 °C. Isopropanol (115 mL) was added to the above mixture over a period of 1 h and isolated product was filtered at ambient temperature, washed with isopropanol and dried under vacuum at 60 °C. Dried material was subjected to same purification process to provide pure valacyclovir HCl which was further converted into dihydrate (**1**, 20.7 g, 99.66 % purity by HPLC) having individual impurity below 0.05 %, m.p. decomposed with foaming at 178 °C (lit. Valacyclovir HCl monohydrate m.p. 150 °C, decomposes with foaming at 195 °C); IR (KBr, ν_{max}, cm^{-1}): 3479, 2923, 2853, 1734, 1642, 1606, 1178, 1128, 1064, 1048, 926; ^{1}H NMR (DMSO-d_6, 200 MHz) δ: 10.9 (br, 1H), 7.83 (s, 1H), 5.38 (s, 2H), 4.40 (m, $1H_b$), 4.28 (m, $1H_a$), 3.82 (d, 1H), 3.74 (m, 2H), 2.13 (m, 1H), 0.91 (d, 3H), 0.88 (d, 3H).

RESULTS AND DISCUSSION

By following the disclosed route (**Scheme-I**), reaction of acyclovir (**2**) and Cbz-L-valine (**3**) was conducted in DMF medium using dicyclohexyl carbodiimide (DCC) in the presence of catalytic amount of 4-dimethylamino pyridine (DMAP) at *ca.* 60 °C temperature. This reaction yielded N-Cbz valacyclovir (**4**) along with 3-4 % of **D-4**. The extent of enhancement of D-isomer as a function of reaction temperature was derived from the control experiments (Table-1). Thus, reaction temperature was optimized to -5 to 0 °C, where **D-4** was controlled to a level of 1 %.

TABLE-1
EFFECT OF REACTION TEMPERATURE ON RACEMIZAITON

Entry	Reaction temperature (ºC)	**D-4** (%)	Entry	Reaction temperature (ºC)	**D-4** (%)
1	-10-5	1.0	5	10-15	1.5
2	-5-0	1.0	6	15-20	1.8
3	0-5	1.1	7	20-25	2.7
4	5-10	1.0	8	25-30	3.3

However, D-isomer content was increased significantly during the scale up of this process. For example, in 50 kg batch of N-Cbz valacyclovir (**4**), 2.6-3.0 % of D-isomer (**D-4**) was formed (Table-2).

TABLE-2
VARIATION IN **D-4** CONTENT AT DIFFERENT SCALES

Entry	Scale	**D-4** (%)
1	Lab scale (upto 100 g)	1.0-1.8
2	Kilo lab scale (upto 25 kg)	1.5-2.2
3	Plant scale (upto 50 kg)	2.6-3.0

Controlled experimental study revealed that compound **4** underwent racemization while distilling the DMF at 85 °C over a period of 10-12 h during the work-up (Table-3).

TABLE-3
STUDY ON THERMAL SENSITIVITY OF N-Cbz VALACYCLOVIR

Entry	Distillation condition	**D-4** (%)
1	Before distillation	1.1
2	Distillation at 85 ºC for 12 h	2.6
3	Distillation at 85 ºC for 30 h	3.2

Thus, appropriate solvent system was sought to purify **4** in order to reduce the level of **D-4**. Among the several solvents explored, acetone-water solvent combination had shown a better result in reducing the **D-4** content from 3.5 to 2.0 %. Thus, aqueous acetone (Table-4, entries 2 and 3) was finalized as the solvent system for the purification of N-Cbz valacyclovir (**4**).

TABLE-4
SOLVENT SCREENING FOR THE PURIFICATION OF N-Cbz VALACYCLOVIR (**4**)

Entry	Solvent (s) composition	Solvent ratio	Solvent quantity in volumes	**D-4** (%)
1	Acetone + water	2:1	18	2.0
2	Acetone + water	2:1	18	2.1
3	Acetone + water	4:1	15	2.3
4	Acetone + water	20:1	42	2.3
5	Methanol + water	2:1	30	2.3
6	Isopropanol	-	8	2.4
7	Methanol + water	2:1	30	2.5
8	1,4-Dioxane	-	25	2.5
9	Ethyl acetate + water	2.5:1	7	2.5
10	Acetone + water	2.5:1	7	2.6
11	Methanol	-	5	2.7
12	Methanol + water	3:1	20	2.7
13	DMF + water	0.2:1	24	3.0
14	Acetonitrile	-	50	3.0

In the reported process for Cbz group deprotection, N-Cbz valacyclovir (**4**) was subjected to catalytic hydrogenation in a mixture of methanol, THF and aqueous HCl. Distillation of solvent and subsequent purification of the crude product from aqueous ethanol provided valacyclovir (**1**).

In our optimized process for the deprotection, catalytic hydrogenation reaction was conducted in DMF using palladium supported on alumina. Filtration of the catalyst through celite/hyflow followed by usual work-up yielded crude valacyclovir (**1**) in 92 % yield with 98.5 % purity. However, this isolated product contains corresponding enantiomer (**D-1**) to an extent of 3.5-4.5 %. Various solvents were screened to remove D-isomer (**D-1**) from 1. Acetonitrile-water combination (Table-5, entries 13 and 14) was found to be ideal in reducing the **D-1** content from 4.2 % to 2.6 %.

After addressing the D-isomer issue in both the stages, we have focused on the content of heavy metals (palladium and aluminum) in valacyclovir (**1**). Despite hyflow bed filtration, these two metals were found in **1** at a level of 60-90 ppm.

TABLE-5
SOLVENT SCREENING FOR THE PURIFICATION OF VALACYCLOVIR (**1**)

Entry	Solvent (s) composition	Solvent ratio	Solvent quantity in volumes	**D-1** (%)
1	Methanol	–	10.0	4.57
2	Methanol	–	10.0	4.16
3	DMF	–	5.0	3.59
4	1,4-Dioxane	–	10.0	3.47
5	*tert*-Butanol	–	10.0	3.44
6	Dichloromethane	–	10.0	3.44
7	THF	–	10.0	3.44
8	Methanol + water	12:3	12.5	3.22
9	Methanol + water	11.25:3	19.0	3.00
10	Methanol + water	7.5:3	14.0	2.98
11	Ethanol + water	10:3	13.0	2.88
12	Acetonitrile + water	1.2:3	7.0	2.88
13	Acetonitrile + water	0.9:3	13.0	2.78
14	Acetonitrile + water	0.91:3	13.0	2.74

Furthermore, activated charcoal and activated clay were of little use in removing these metallic impurities. In this context, we have screened various resins and among them, T-63 resin was found[8] to be very efficient in reducing the heavy metal content to a level of less than 10 ppm using 10 % load (Table-6, entry 2).

TABLE-6
SCREENING STUDIES TO REMOVE METAL IMPURITIES

Entry	Solid support	Solvent	Resin load (% w/w)	Al content (ppm)	Pd content (ppm)
1	EDTA	Water	20	–	16.19
2	T-63† resin	Water	10	4.2	1.48
3	T -63† resin	Water	50	4.6	3.51
4	T-63† resin	Water	100	6.3	0.70
5	CH-97† resin	Water	20	12.6	95.20
6	CH-97† resin	Water	50	10.7	23.94
7	CH-97† resin	Water	100	8.9	18.21

†Manufactured by THERMAX Ltd., India.

Besides having control on D-isomer content at each stage and control of heavy metal impurities, it is also important to control individual impurity level below 0.05 % (by HPLC) in the API (**1**). Thus, substantially pure valacyclovir (**1**) was obtained from the technical grade material by following the crystallization procedures described in the experimental section. Purity details at each stage at different scales are provided in Table-7. Purity details of the representative samples of present optimized process are tabulated in Table-8.

Conclusion

A facile and efficient process is developed for valacyclovir hydrochloride by controlling the related substances including chiral impurity. Furthermore, process related heavy metal impurities were effectively controlled to acceptable limits using inexpensive and commercially available resin.

TABLE-7
STAGE WISE PURITY AND D-ISOMER DETAILS

	Compound **4**	Compound **4** after purification	Tech grade API (1)	Purified API (1)
Purity (%)	98.5-99.0	99.0-99.5	98.3-98.8	99.3-99.6
D-isomer (%)	1.5-3.5	1.0-2.1	1.5-3.8	1.0-2.5

TABLE-8
CONTENT OF IMPURITIES BY HPLC IN PURIFIED VALACYCLOVIR (**1**)

Impurity	Entry-1	Entry-2	Entry-3	Entry-4	Pharmeuropa[9] limit (%)	In-house limit (%)
Guanine†	0.003	0.003	0.003	0.003	2.00	0.15
Acyclovir†	0.23	0.25	0.26	0.46	2.00	1.00
Alanine†	ND	ND	0.01	0.01	0.10	0.10
O-Acetyl†	ND	ND	ND	0.002	0.20	0.15
N-Formyl†	0.02	0.02	0.03	0.04	1.50	0.15
Isoleucine†	ND	ND	ND	0.009	0.10	0.10
N-Cbz valacyclovir† (**4**)	ND	ND	0.008	ND	0.20	0.15
Cbz-L-Valine (**3**)	ND	ND	ND	ND	NA	0.05
Single maximum impurity	0.02	0.01	0.01	0.0008	0.10	0.05
Total content of impurities	0.28	0.29	0.33	0.53	5.00	2.00
D-isomer (**D-1**)	2.3	2.5	2.6	2.8	3.00	3.00

†PHARMEUROPA Vol. 18, No. 2, April (2006).

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