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CHOLINE CHLORIDE-UREA: AN EFFICIENT, GREEN MEDIA FOR PARTIAL HYDROLYSIS OF *DI*- AND *TRI*-PROTECTED GANCICLOVIR AND SELECTIVE *O*-ACYLATION IN THE SYNTHESIS OF ANTIVIRAL DRUG VALGANCICLOVIR

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ABSTRACT

A simple, mild, and environmentally benign protocol for the synthesis of pharmaceutically important Valganciclovir hydrochloride was developed by using Choline Chloride-Urea DES as solvent. The intermediate steps (steps 1 to 3) viz. partial hydrolysis of di, tri protected ganciclovir and the selective *o*-acylation of mono protected ganciclovir were efficiently proceeded in Choline Chloride-Urea DES. The use of DES as solvent not only minimizing the use of volatile organic solvents but also offer good selectivity with excellent yields of desired products. The simple operating process, easy isolation of product, gram scale study and recyclability of DES have made the process more efficient than the conventional processes and feasible on larger scale.

KEYWORDS: Valganciclovir, cytomegalovirus, CMV-retinitis, L-Valine ester, hepatic esterases, orthoesters.

1. INTRODUCTION

The Cytomegalovirus CMV retinitis is a sight threatening disease commonly associated with AIDS and organ transplant. In the majority of cases the virus lies dormant in the body throughout the life, but may reactivated at time when the immune system is weakened (in the case of AIDS patients). The CMV[1,2,3] infection is also observed in the organ transplant patients during first few months of transplant causes complications in the lung, liver, gastrointestinal tract. Ganciclovir, is an efficient

antiviral drug on CMV. Which inhibits the replication of human CMV both in vitro and in vivo. But has very low rate of absorption when administered orally. Therefore, it is compulsory to take either higher oral dosage or to administrate it intravenously, which is very problematic to patients. So, it is highly desirable to provide ganciclovir[4] with an improved oral absorption profile. Valganciclovir is an L-valine ester of ganciclovir, (monoester/prodrug of ganciclovir) which belongs to a group of antiviral was proven to be effective to treat such infections. Valganciclovir after administrating orally it is well absorbed from gastrointestinal tract and rapidly and comprehensively metabolized to ganciclovir by intestinal wall and liver by hepatic esterases.

The synthesis procedure of Valganciclovir involves synthesis of monoprotectedganciclovir which on further condensation with CBZ-L-Valine, followed by hydrolysis and hydrogenationgives desired final product. The first step to synthesize the monoprotectedganciclovir is very important and critical step asboth the –OH groups of ganciclovir are chemically equivalent. Therefore various protection strategies are need to apply to get the desired product. The previous protocols reported for the synthesis of monoprotectedganciclovir involves reaction of ganciclovir with the orthoformate to give orthoester which subsequently on hydrolysis produces monoprotected ganciclovir[5] or it was treated with aldehyde to give protected diol in DMSO which on further reduction with $NaBH_4$ or treatment with $AlCl_3$ in THF gives monoprotected ganciclovir.[6,7] The another report comprises multi step process for the synthesis of monoprotectedganciclovir which involves use of reagents such as trityl chloride, TEA, mesyl chloride and solvents such as DMF, DCM, and acetic acid.[8]

Rao et al.[9] have reported new protocol for the synthesis of valganciclovir which comprises use of tri-protected ganciclovir such as tri-acylatedganciclovir. Although the triacylatedganciclovir is inexpensive than the ganciclovir, the protocol involves excess use of various organic solvents such as dichloromethane, *N*,*N*-dimethyl acetamide, *N*,*N*-formamide, piperidine, hexane, acetone etc., required longer reaction time, tedious isolation process, and give moderate yields of the products (**Fig.1**). Thus from the previous reports it was clear that all the prior processes involves use of costly reagents, excess use of hazardous organic solvents, and required longer reaction time which not only make the process tedious and time consuming but also increase the

cost of the final product i.e. valganciclovir. Thus, the scope to develop an efficient, environmentally benign, and selective protocol for the synthesis of valganciclovir is still in demand.

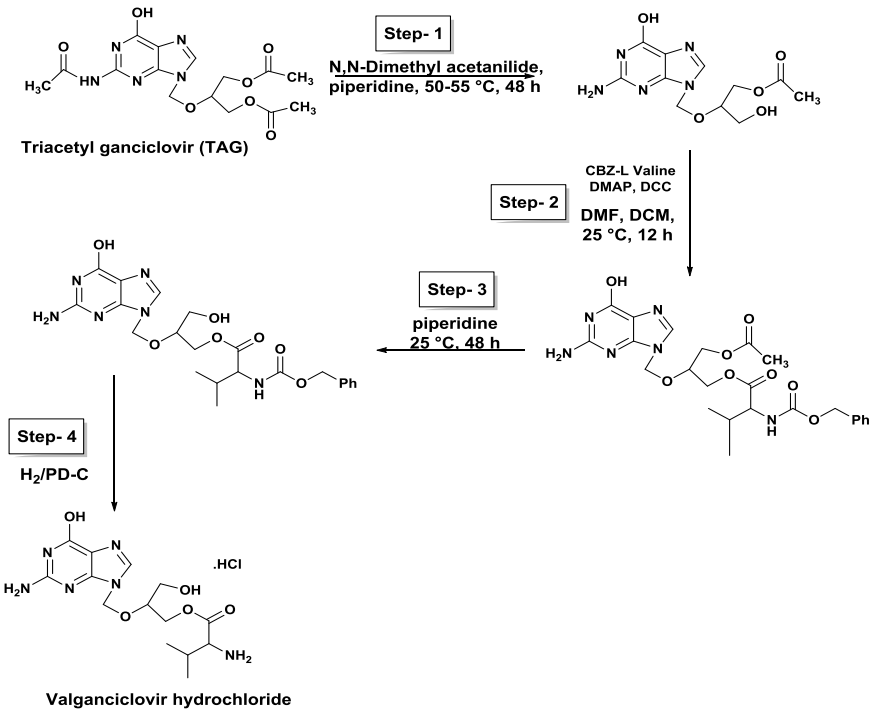


Fig 1: Previous report for the synthesis of Valganciclovir by using TAG.

ChCl-Urea DES is well known and broadly explored in various transformations as an efficient alternative to volatile organic solvent. The unique features of this environmentally benign solvent such as low vapour pressure, bio-degradable nature, readily availability, and inexpensive starting materials have attracted much attention in research laboratory as well as in Industries. In addition to this, DES also provide excellent selectivity over conventional toxic and hazardous solvents.[10,11]

Thus, here in we report a mild efficient strategy employing environment friendly ChCl-Urea DES as solvent for partial hydrolysis of di- and tri-protected ganciclovir and selective *o*-acylation of mono-protected ganciclovir in the synthesis of valganciclovir. The ChCl-Urea DES offer good selectivity as well as excellent yield of desired product. Additionally, the comparatively lower cost of tri-acylatedganciclovir than ganciclovir and use of inexpensive and readily available choline chloride and urea have made the present protocol cost effective over previously reported protocols.

2. MATERIALS AND METHODS

All the reagents and reactants were purchased from commercial suppliers and were used without further purification. In all the experiments 40% Monomethyl amine solution was used. All products were confirmed by melting point, FTIR spectroscopy, ^{1}H NMR spectroscopy and mass spectrometry. ^{1}H NMR spectrums were recorded by using 300 MHz spectrometers and chemical shifts are expressed in δ ppm. Mass spectral data were obtained with a micromass-Q-Tof (YA105) spectrometers. For hydrogenation H_2-gas pressure reactor was used.

2.1. Experimental

Step 1: Preparation of 2-((2-amino-6-hydroxy-9*H*-purin-9-yl)methoxy)-3-hydroxypropyl acetate (2) from triacetylganciclovir (1)

In 500 mL 3 neck round bottom flask fitted with overhead stirrer, 50 g of 1 (0.13 mol) was added in 100 ml of Choline Chloride-Urea DES at room temperature. The reaction mass was cooled to 15-20 °C and 12.6 g of monomethylamine (0.40 mol) was added drop wise in 30 min. The progress of the reaction was monitored on TLC. The solid product was isolated by adding 200 mL of chilled water (5-10 °C) in to the reaction mass and stirred for 15 min. The solid obtained was filtered and washed with chilled water (5 mL X 2) and dried under vacuum at 50-55 °C to give crude compound **2** as white solid.

White solid; HPLC conversion for compound **2** is 60%; IR: λmax/cm^{-1}: 1713 (-CO-), 3478 (-NH), 3113 (-CH=C-H), 3198 (-OH), 1650 (-CO), 1052 (-CO), 1200 (-C-O-C); EI-MS m/z: 298 (M+H).

Step 2: Preparation of 2-(2-amino-1,6-dihydro-6-oxo-purine-9-yl)-methoxy-3-acetoxy-1-propyl-*N*-(benzyloxycarbonyl)-L-valinate (5) from 2-((2-amino-6-hydroxy-9*H*-purin-9-yl)methoxy)-3-hydroxypropyl acetate (2).

In 500 mL 3 neck round bottom flask fitted with overhead stirrer, 50 g of compound **2** (0.17 mol), 46.5 g of CBZ-L-Valine (0.18 mol), and 2.5 g of *N*,*N*-dimethylaminopyridine (0.02 mol) were taken in 150 mL of Choline Chloride –Urea DES. The reaction mass was cooled to 10-15 °C and 41.68 g of *N*,*N*-dicyclohexylcarbodimide (0.20 mol) was added with maintaining the temperature to 10-15 °C. After addition of *N*,*N*-dicyclohexylcarbodimide the reaction temperature was allowed to increase to room temperature (30-32 °C) and maintained for 20 h. The

progress of the reaction was monitored on TLC. After completion of reaction, the reaction mass was cooled to 10-15 °C and 50 mL of water was added and the reaction mass was filtered. The aqueous layer was extracted with dichloromethane (2 X 100ml). All the organic layers were dried over anhydrous Na_2SO_4 and the solvent was evaporated under vacuum to give oily product. The oil obtained was stirred in 100 mL of isopropyl alcohol for 30 min at 15-20 °C to afford compound **5** as white solid.

Yield 92%, 82 g; M. P.:140 °C; IR: λmax/cm^{-1}: 1600-1700 (C=O broad peak), 3324 (N-H), 3033 (O-H), 1276 (C-O-C), 1031 (C-O of ester); ^{1}H NMR (300 MHz, DMSO) δ 10.66 (s, 1H), 7.80 (d, *J* = 1.8 Hz, 1H), 7.66 (dd, *J* = 8.0, 5.5 Hz, 1H), 7.42 – 7.24 (m, 5H), 6.48 (s, 2H), 5.40 (s, 2H), 5.02 (s, 2H), 4.36 – 3.78 (m, 6H), 1.92 (d, *J* = 3.4 Hz, 1H), 1.86 (d, *J* = 3.2 Hz, 3H), 0.82 (dd, *J* = 6.7, 2.2 Hz, 6H). EI-MS m/z: 529 (M-H).

Step 3a: Preparation of 2-(2-amino-1,6-dihydro-6-oxo-purine-9-yl)-methoxy-3-hydroxy-1-propyl-*N*-(benzyloxycarbonyl)-L-valinate (6) from 2-(2-amino-1,6-dihydro-6-oxo-purine-9-yl)-methoxy-3-acetoxy-1-propyl-*N*-(benzyloxycarbonyl)-L-valinate (5)

In 500 ml 3 neck round bottom flask fitted with overhead stirrer, 100 ml of Choline Chloride-Urea DES and 50 g of compound **5** (0.09 mol) was taken. The reaction mass was cooled to 15-20 °C. To the cooled solution, 9 g of mono methylamine solution (0.28 mol) was added drop wise maintaining the temperature to 15-20 °C. After addition of monomethyl amine solution reaction temperature was allowed to increase to room temperature (30-32 °C) and maintained at room temperature for 18 h. The progress of the reaction was monitored on TLC. After completion of reaction, the reaction mass was cooled to 5-10 °C and 200 ml of water was added slowly. The reaction mass was acidified to pH 2 by 0.5 N HCl to isolate the solid product. The solid obtained was washed with chilled water and dried under vacuum to afford compound **6** as white solid.

Yield 90%, 41 g; M. P.: 165 °C; IR: λmax/cm^{-1}: 1630 (C=O), 1725 (C=O ester), 3325 (N-H), 3033 (O-H), 3194 (O-H), 1077 (CH_2-OH), 1227 (C-O-C), 1394 (C-N of C-NH_2); ^{1}H NMR (300 MHz, DMSO) δ 10.68 (s, 1H), 7.80 (d, *J* = 2.5 Hz, 1H), 7.60 (dd, *J* = 8.1, 6.0 Hz, 1H), 7.35 – 7.12 (m, 5H), 6.48 (s, 2H), 5.41 (s, 2H), 5.02 (s, 2H), 4.90

(s, 1H), 4.18 (ddd, *J* = 28.4, 11.7, 3.2 Hz, 2H), 4.02 – 3.73 (m, 4H), 1.89 (dd, *J* = 13.1, 6.5 Hz, 1H), 0.80 (dd, *J* = 6.6, 4.7 Hz, 6H). EI-MS m/z: 486 (M-2).

Step 3b: Preparation of 2-(2-amino-1,6-dihydro-6-oxo-purine-9-yl)-methoxy-3-hydroxy-1-propyl-*N*-(benzyloxycarbonyl)-L-valinate (6) **from 2-((2-amino-6-hydroxy-9*H*-purin-9-yl)methoxy)-3-((((benzyloxy)carbonyl)valyl)oxy)propyl ((benzyloxy)carbonyl)-L-valinate (8)**

In 500 ml 3 neck round bottom flask fitted with overhead stirrer, 100 ml of Choline Chloride-Urea DES and 50 g of compound **8** (0.07 mol) was taken. The reaction mass was cooled to 15-20 °C. To the cooled solution, 12.6 g of monomethyl amine solution (0.40 mol) was added drop wise maintaining the temperature to 15-20 °C. After addition of monomethyl amine solution reaction temperature was allowed to increase to room temperature (30-32 °C) and maintained at room temperature for 18 h. The progress of the reaction was monitored on TLC. After completion of reaction, the reaction mass was cooled to 5-10 °C and 200 mL of water was added slowly. The reaction mass was acidified to pH 2 by 0.5 N HCl to isolate the solid product. The solid obtained was washed with chilled water and dried under vacuum to give compoun(8) **6** as white solid.

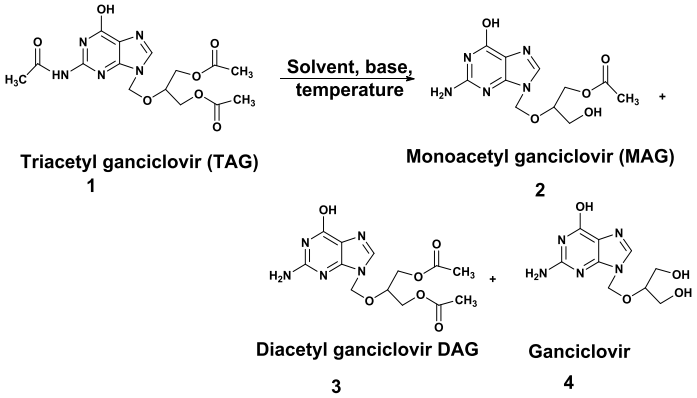
Yield 85%, 29 g; M. P.: 167 °C; IR: λmax/cm^{-1}:1640 (C=O), 1735 (C=O ester), 3400 (N-H), 3100 (O-H), 3035 (O-H), 1200 (C-O-C).

Step 4: Preparation of 2-((2-amino-6-hydroxy-9*H*-purin-9-yl)methoxy)-3-hydroxypropyl valinate hydrochloride (7) from 2-(2-amino-1,6-dihydro-6-oxo-purine-9-yl)-methoxy-3-hydroxy-1-propyl-*N*-(benzyloxycarbonyl)-L-valinate.(6)[12]

To the H_2 gas pressure reactor, 50 g of compound **6** (0.10 mol), 100 ml of ethanol, 2.5 ml of conc. HCl and 12 g of 50% wet Pd on carbon were added and stirred under 3 kg pressure of H_2 gas. The progress of reaction was monitored on TLC. After 3 h the reaction mass was filtered through inert inorganic bed, and the filtrate was concentrated under vacuum at 40-45°C. The crude product obtained after distillation was treated with isopropyl alcohol to give solid which after filtration and drying at 50-55 °C afford final compound as white solid.

Yield 80%, 32 g; M. P.: 161-162 °C; IR: λmax/cm^{-1}: 1633 (C=O), 1728 (C=O ester), 3317 (N-H), 3191 (O-H). ^{1}H NMR: (300 MHz, DMSO) δ (ppm): 10.89 (s,1H), 8.40-8.50 (s, 2H), 7.78-7.86 (d, 1H), 6.60-6.71 (s, 2H), 5.37-5.48 (m, 2H), 4.90-5.00 (s, 1H), 4.20-4.30 (dd, 1H), 4.00-4.10 (dd, 1H), 3.80-3.90 (dd, 1H), 3.63-3.75 (dd, 1H), 3.40-3.50 (m, 2H), 2.00-2.10 (m, 1H), 0.80-0.85 (d, 6H). EI-MS m/z: 388(M-2).

3. RESULTS AND DISCUSSION



The optimization of partial hydrolysis was started by taking tri-acylatedganciclovir (TAG) **1** (13 mmol), Monomethyl amine (40 mmol) as base at room temperature (**Table 1**).

Table 1: Optimization of reaction parameters.[a]

Entry	Solvent	Base	Conversion[b]		
			2	3	4
Solvent study					
1	Toluene	Monomethyl amine	-	-	-
2	Water	Monomethyl amine	21	29	50
3	Methanol	Monomethyl amine	25	40	35
4	IPA	Monomethyl amine	30	55	15
5	Acetone	Monomethyl amine	26	61	13
6	Hexamethylphosphoramide	Monomethyl amine	47	43	10
7	DMF	Monomethyl amine	45	34	21
8	DMA	Monomethyl amine	48	32	20
9	DMSO	Monomethyl amine	46	32	22
10	NMP	Monomethyl amine	40	36	24
11[c]	ChCl-Urea	Monomethyl amine	60	27	13
12	ChCl-Dextrose	Monomethyl amine	0	10	0
13	ChCl-Malonic acid	Monomethyl amine	0	8	0
14	ChCl-Oxalic acid	Monomethyl amine	0	20	15
Base study					
15	ChCl-Urea	Liq. NH_3	11	20	69
16		NaOH	26	41	33

17		CH_3ONa	22	48	34
18		$NH(Et)_2$	35	45	20
19		$N(Et)_3$	32	51	17
20		*N*,*N*-Diisopropylethylamine	49	34	17
21		Piperidine	43	32	25
22		Pyridine	30	56	14
Temperature study					
23[d]	ChCl-Urea	Monomethyl amine	57	35	8
24[e]		Monomethyl amine	51	23	26
Base equivalent study					
25[f]	ChCl-Urea	Monomethyl amine	50	42	8
26[g]		Monomethyl amine	60	25	15

[a]*Reaction conditions:* ***1****: 5 g (13 mmol), Solvent: 10 ml, Base: 40 mmol added owly at 15-20°C in 15-mins, Time: 24 h, Temperature: 30-32 °C;* [b]*Conversion determined by HPLC;* [c]*Time: 18 h;* [d]*Temperature: 20-22 °C;* [e]*Time:10 h, Temperature: 40-42 °C;* [f]*Base: 30 mmol;,* [g]*Base: 50 mmol.*

Various solvents were tested to investigate suitable candidate to perform partial hydrolysis of **1** (**Table 1**, entries 1-6). The hydrolysis of **1** was failed in non-polar solvent toluene as starting materialremains as it is even after 24 h at room temperature (Table 1, entry 1). Although the polar solvents facilitated the hydrolysis of **1** giving 100% conversion but failed to give higher selectivity for **2** (Table 1, entries 2-10). In the case of water, higher selectivity was obtained for completely hydrolysed product **4** (Table 1, entry 2), whereas acetone gives higher selectivity for **3** in 24 h (Table 1, entry 5). Noticeably when Choline chloride-Urea DES was used, 100% conversion along with higher selectivity of 60% for desired monoacetylganciclovir**2** was obtained in 18 h at room temperature (Table 1, entry 11). The other DES investigated under present conditions were failed to give such selectivity as well as conversion (Table 1, entries 12-14). The Choline Chloride-Dexrose and Choline Chloride-Malonic acid DES although give poor conversion but gives selectively 3 as solo product in 24 h (Table 1, entries 12, 13). Thus the solvent study demonstrated that the Choline Chloride-Urea DES served as best solvent to carry out this transformation.

After shortlisting the desired solvent, effect of base on the rate of hydrolysis of 1 was investigated. All the bases studied here also gives good conversion but lack the selectivity for **2** (Table 1, entries 15-22). When liq. NH_3 was used higher selectivity was obtained for **4** in 24 h (Table 1, entry 15), whereas the triethyl amine and pyridine showed higher selectivity for **3** (table 1, entries 19, 22). The effect of temperature on

hydrolysis of **1** was investigated by lowering temperature below room temperature to 20-22 °C as well as by increasing the temperature to 40-42 ° (Table 1, entries 23, 24). Although lowering the temperature to 20-22 °C does not showed much effect on the selectivity of **2** but reduces the reaction rate as reaction took 24 h for completion (Table 1, entry 23). Whereas on increasing the reaction temperature from room temperature to 40-42 °C the selectivity for **2** reduced from 60% to 51% (Table 1, entry 24). The effect of equivalents on Monomethyl amine on hydrolysis of **1** was also investigated. When 30 mmols of Monomethyl amine were used the rate of hydrolysis of **1** as well as the selectivity of **2** was reduced (Table 1, entry 25). Whereas, on increasing the base equivalents from 40 mmol to 50 mmol does not show much influence on the rate of reaction as well as the selectivity of **2** (Table 1, entry 26).

The synthesis of Valganciclovir from triacylatedganciclovir involves following steps as shown in Fig **1**. The first step involves partial hydrolysis of triacylatedganciclovir to give monoprotectedganciclovir derivative. The next step is selective *o*-acylation of monoprotected derivative with CBZ-L Valine to give di-protected ganciclovir. In the third step, the di-protected ganciclovir on further selective partial hydrolysis gives mono-protected ganciclovir derivative of CBZ-L Valine which in forth step on reduction with Pd/C give desired valganciclovir.

The optimized parameters for the partial hydrolysis reaction of triacylatedganciclovir applied on larger scale by using Choline Chloride-Urea DES as solvent. The triacylatedganciclovir on reaction with monomethylamine in Choline Chloride: Urea DES as solvent give the crude product which is mixture of **2**, **3**, and **4**. The selectivity of these three product in the crude was determined by HPLC analysis which showed 60% selectivity for 2. Since all the attempts to isolate these three products from the crude mass was failed we proceeded for the next step on the basis of HPLC conversion. During addition of monomethyl amine temperature must keep to 15-20 °C as selectivity for **2** was greatly affected by the temperature at this stage. We found that if addition of monomethyl amine was performed at room temperature or at elevated temperature, rate of complete hydrolysis was increased over partial hydrolysis to give **4** as major product. In the next step, the crude mass of step 1 was treated with CBZ-L-Valine in the presence of *N*,*N*-dimethylaminopyridine and *N*,*N*-dicyclohexylcarbodimide with Choline:Urea DES as solvent. The desired product was

isolated by adding water in to the reaction mass and then extracted in dichloromethane. The oily mass obtained after evaporation of dichloromethane was further treated with isopropyl alcohol to give desired product **5** as white solid. The compound **5** on treatment with monomethylamine in Choline:Urea DES underwent selective hydrolysis of acetyl group to afford L-valinemonoprotectedganciclovir. The desired product **6** was isolated as white solid by addition of water to the reaction mass followed by acidification by 0.5 M HCl at 5-10 °C. It was necessary to maintain lower temperature during this step as increase in temperature resulted in loss of selectivity for **6**.

Similar to partial hydrolysis of **5** (in which one hydroxyl group was protected by acyl group and second hydroxyl group was protected by L-Valine group), the conditions used in step 3 were applied to ganciclovir in which both the hydroxyl groups were protected with L-Valine group. The partial hydrolysis of this L-valine protected ganciclovir efficiently proceeded in Choline Chloride:Urea DES to afford **6** in excellent yield. This confirm the efficiency of DES for partial hydrolysis of diprotectedganciclovir irrespective of the protecting group. The last step of this reaction, i. e. reduction of amine to amine was performed as per the conventional process by using Pd-C.

Recyclability studies of DES

The recyclability study was performed on 10 g scale. The progress of the reaction was monitored on HPLC. After completion of reaction water was added to separate the product from DES. The aqueous layer with DES was filtered from the reaction mass and water was evaporated under vacuum at 70-75 °C. The recovered DES was successfully recycled up to 3 consecutive cycles without much loss in its activity.

Entry	No. of cycle	Conversion of 2 (%)
1	Fresh	60
2	1St	58
3	2nd	55
4	3rd	55

4. CONCLUSIONS

In summary, Choline Chloride: Urea DES efficiently replaced various solvents in the partial hydrolysis and selective *o*-acylation steps in the synthesis of valganciclovir.

The reaction proceeds under mild conditions and give desired products in the yields comparable to the conventional process. The Choline Chloride: Urea DES provides an environmentally benign media for the synthesis of valganciclovir. The use of inexpensive starting material, recyclable reaction media and easy to operate conditions which minimizes the excess use of solvents are some important features of present protocol.

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