CHAPTER-4

STUDIES TOWARDS THE NEW SYNTHESIS OF

GANCICLOVIR
4.1 INTRODUCTION

Cytomegalovirus (CMV) retinitis is the infection of the retina of the eye caused by cytomegalovirus, another class of viruses and causes blindness if it is untreated [139-142]. CMV can also be responsible for HIV progression [143]. Ganciclovir (73), a nucleoside analogue (Chapter-III, Table-3.2) used to treat or prevent the cytomegalovirus (CMV) infections [144] and herpes simplex virus (HSV) in human clinical studies [145-148]. Ganciclovir, 73 shows some side effects, especially bone-marrow toxicity, therefore it usually cannot be combined with AZT (Zidovudine or azidothymidine, an antiretroviral drug and an analog of thymidine) which has similar toxicity. Generally, 73 is used for; i) Sight-threatening CMV retinitis in severely immunocompromised & AIDS confirmed people ii) Prevention and cure of CMV pneumonitis in bone marrow transplant recipients [143].

AIDS patients may require indefinite ganciclovir, 73 maintenance therapy to prevent disease progression, as ganciclovir (like other antivirals) does not eradicate latent viral infection. For the treatment of immunocompromised patients, 73 therapy is found to be a key move [149].

Mechanism of action [150-153]: Ganciclovir, 73 acts by phosphorylating first to the monophosphate form by a CMV-encoded (UL97 gene) protein kinase homologue, then to the di- and triphosphate forms by cellular kinases [150]. Ganciclovir triphosphate concentrations
may be 100-fold greater in CMV-infected than in uninfected cells, indicating preferential phosphorylation in infected cells. Ganciclovir triphosphate, once formed, persists for days in the CMV-infected cell and is believed to inhibit viral DNA synthesis by; (1) competitive inhibition of viral DNA polymerases; and (2) incorporation into viral DNA, resulting in eventual termination of viral DNA elongation as mentioned in Figure-4.1.

**Figure 4.1: Mechanism of action**

Absorption of the oral form is very limited about 5% fasting, and about 8% with food [152]. Concentration of ganciclovir, 73 in the central nervous system, is found at the level of about 50% of the plasma level. About 90% of plasma ganciclovir, 73 is eliminated unchanged in the urine with a half-life of 2-6 h depending on renal function (elimination takes over 24 hours in end-stage renal disease).
4.2 REVIEW OF LITERATURE

4.2.1 Development History: The first synthesis of ganciclovir, 73 was reported by Julien Verheyden and John Martin at Syntex Research in California in 1980 and is marketed as sodium salt (Ganciclovir sodium) under the trade names Cytovene® and Cymevene® [154b]. For ocular use, ganciclovir is marketed under the trade name Vitrascert® (Bausch & Lomb) [154c]. A prodrug of ganciclovir, valganciclovir, 74 with improved oral bioavailability under the trade name Valcyte® has also been approved in 2001 and is marketed [154d].

4.2.2 Chemical structure and properties: Ganciclovir (73) is a synthetic analogue of 2'-deoxyguanosine and its chemical name is 2-amino-9-[[1,3-dihydroxypropan-2-yl]oxy]methyl]-6,9-dihydro-3H-purin-6-one (Figure 4.2). It is a white to off-white crystalline solid powder and soluble in water (0.6 mg/mL in water at 25 °C).

![Figure 4.2: Structures of Ganciclovir(73) and Valganciclovir(74)](image)

Ganciclovir, 73 is also available in slow-release formulations for insertion into the vitreous humour of the eye, as treatment for CMV retinitis (associated with HIV infection). Valganciclovir [154a], 74 an L-valyl ester of ganciclovir, 73
is rapidly converted to 73 after oral administration, by intestinal and hepatic esterases [154e].

4.2.3 Previously reported synthetic approaches

Ganciclovir, 73 is a derivative of acyclovir, 45 having a Purine moiety and a side chain with glycerol derivative. Purine and diacetylguanine are the building blocks for the synthesis of Ganciclovir (73). Therefore, several approaches reported are utilizing them as key starting materials for the synthesis of ganciclovir (73). Upon extensive review, all the reported approaches can be categorized based on the starting material used for its synthesis;

4.2.3.1 Synthetic Approaches from 6-Oxopurines

4.2.3.2 Synthetic Approaches from 6-Halopurines

4.2.3.3 Synthetic Approach from 6-Aminopurines and

4.2.3.4 Synthetic Approach from Imidazoles

The synthetic routes falling under each category were discussed briefly in the following sections.

4.2.3.1 Synthetic approaches to 73 from 6-oxopurines:

Martin et al. in 1983 [155] reported the synthesis of 73 starting from epichlorohydrin (76). Treatment of 76 with benzyl alcohol in the presence of 50% aqueous NaOH furnished 1,3-di-O-benzylglycerol (77a) in 63% yield after distillation. (Scheme-4.1, Path A). Alternativley, Beachamp et al., reported an alternative method for the synthesis of this requisite acyclic chain (77a), using 1, 3-dichloropropan-2-ol (75) (Scheme-4.1,
Path B) [156]. Chloromethylation of 77a with HCl and paraformaldehyde gave the chloromethyl ether 77b, which was treated with potassium acetate to yield glycerol derivative 77c. Condensation of crude 77c with 78b in the presence of a catalytic amount of p-toluenesulfonic acid in sulfolane produced 3:2 mixture of $N^2$-acetyl-9-[[1,3-bis(benzyloxy)-2-propoxy]methyl]guanine (80) and its corresponding $N^7$ isomer 79, from which the desired isomer 80 was crystallized from toluene in 31% yield.

Scheme 4.1: Martin's approach
Debenzylation of 80 over 20% palladium hydroxide on carbon with cyclohexene in refluxing ethanol gave intermediate 81, which, without isolation, was deacetylated with 1:1 concentrated NH₄OH/ methanol to furnish ganciclovir (73).

Ogilvie et al. investigated the condensation of N²-acetylguanine (78c) with 77b using tetrabutylammonium iodide (TBAI), molecular sieves, stannic chloride, and mercuric cyanide as condensation catalysts and found that TBAI gave the best results with 28% yields each for the N⁹-isomer 80 and the N⁷-isomer 79 [157]. Direct condensation of guanine (78a) with 77b using TBAI as catalyst furnished largely a 7:3 mixture of 83 and 82 which were separated by fractional crystallization from ethanol. The initial silylation of guanine (78a) was the slow step in this route which required 4 days. Catalytic transfer reduction of 83 was carried out using palladium black and cyclohexene in refluxing ethanol provided 73 after 18h in 84% yield (Scheme-4.2).

Scheme 4.2: Ogilvie's approach
An alternate Birch reduction [158] of 83 produced 73 in similar yields with an advantage of being faster (15 min) compared to catalytic reduction. Similarly, in 1989, Hakimelahi and Khalafi-Nezhad [159] also reported the preparation of 73 by condensation of guanine (78a) with hexamethyldisilazane (HMDS) to give tris(trimethylsilyl) derivative, 84 which on further condensation with chloromethyl ether 77b in the presence of Bu₄NF as a catalyst to afford 83 (98%), which upon deprotection by catalytic transfer reduction provided 73 (Scheme-4.3).

\[ \text{Scheme 4.3: Hakimelahi and Khalafi-Nezhad's approach} \]

Similar efforts were devoted by various research groups for the synthesis of 73 with some modifications in the above reported procedures starting from 78a and 78b [160-162]. In another report, the synthesis of 73 was described under PTC conditions using afforded 1,3-di-O-benzylglycerol derivative (85) as side chain [163].(Scheme-4.4).
Scheme 4.4: Reported synthesis for 73 under PTC conditions

Tolman et al.[164,165] prepared ganciclovir (73) by a fusion method in which diacetylguanine, 78b was reacted with 89 at 155-160 °C in the presence of ethanesulfonic acid to furnish 90 in 33% yield followed by deprotection employing aqueous methylamine (Scheme-4.5)

Scheme 4.5: Tolman's approach

The key intermediate 89 was prepared either from 4-chloromethyl-1,3-dioxolane (87) or glycerol formal, a mixture of 1,3-dioxan-5-ol (91) and 1,3-dioxolane-4-methanol (92). In both the cases, the synthesis resulted in the formation of a mixture of isomers from which the desired
product (89) (Scheme-4.6) was isolated with great difficulty using HPLC. A patent application filed by Chu et al. [166] in 1994, discloses an improved process for the preparation of this key intermediate 89 that results in the exclusive formation of the desired product by chloromethylation of 1,3-dichloropropan-2-ol (75) with HCl and paraformaldehyde followed by reaction with potassium acetate.

Scheme 4.6: Chu’s approach

A thioether route to 73 with 10.7% overall yield from glycerol (94) was described by McGee et al. in 1988 [167]. The starting material, 1,3-di-O-pivaloylglycerol (95a), was prepared by acylation of glycerol 94 with pivaloyl chloride (Scheme-4.7)
In this reaction, some of the isomeric dipivalate 95b was initially formed; however 95b reacted faster than 95a with excess pivaloyl chloride to form the tripivalate 95c. Treatment of a mixture of 95a, 95b and 95c with acetic anhydride in DMSO yielded methylthiomethyl ether 96. Reaction of 96 with m-CPBA generated sulfoxide 97 which was suitably activated for use in acid catalysed condensation reactions. The condensation of 97 with diacetylguanine 78b in DMF resulted in the desired product 99 and the $N^\prime$-isomer 98. $N^\prime$- Isomer (98) was found to be recyclable to 99 upon heating in DMSO with $p$-toluenesulfonic acid at 105 °C for 4 hours to generate 36% of 99. Deprotection of 99 with
sodium methoxide in methanol followed by crystallization in water containing NH₄Cl (to adjust the pH) gave of 73 with 72% yield.

4.2.3.2 Synthetic approach to Ganciclovir (73) from 6-halopurines:

Ogilvie et al.[168] explored the synthesis of 73 starting from 6-chloropurine (100). Condensation of 100 with 1,3-dibenzylxoxy-2-chloromethoxypropane (77b) yielded 6-chloroguanine derivative 101, which was then converted into the desired guanine derivative 102 by reacting with sodium methoxide, mercaptoethanol, and a trace of water in methanol at reflux for 2 to 3 hours. After workup, the product 102 was obtained in 70% yield. Debenzylation of 102 under palladium catalyzed reduction in refluxing ethanol gave 73 in 84% yield (Scheme-4.8).

![Scheme 4.8: Ogilvie et al. approach](image)

In 1994, Kim et al.[169] introduced a fluoro atom at the 6 position of ganciclovir (73) to prepare their potential prodrugs (Scheme-4.9). The 6-chloropurine acyclonucleoside 103 was treated with anhydrous trimethylamine in DMF to afford the corresponding quaternary
trimethylammonium salt 104 in 70% yield. Reaction of trimethylammonium salt 104 with KF and DMF afforded 6-fluoropurine acyclonucleoside 105 in 56% yield. Enzymatic defluorination of 105 with an excess of calf intestinal mucosa adenosine deaminase in phosphate buffer solution at pH 7.5 resulted in complete conversion of this prodrug into ganciclovir (73). It was found that the 6-fluoropurine acyclonucleoside 105 was more efficiently metabolized to 73 by adenosine deaminase than the corresponding 6-aminopurine acyclonucleoside.

![Chemical structure](image)

**Scheme 4.9: Kim et al. approach**

### 4.2.3.3 Synthetic approach to 73 from 6-aminopurines:

In 1984, Ogilvie et al.[168] described an alternative synthetic route starting from $N^2,N^6$-diacetyl-2,6-diaminopurine (106). Direct coupling of 2,6-diaminopurine with 77b was not successful under the usual conditions (Scheme-4.10). However, coupling of diacetyl purine (106) with 77b gave 107 in 55% yield which was then subjected for
deacetylation and debenzylation in Palladium catalyzed reduction in ethanol and cyclohexane to furnish the corresponding compound 109. 2,6-diaminopurine derivative (109) was then treated with an excess of adenosinedeaminase to obtain ganciclovir (73) in quantitative yields.

Scheme 4.10: Ogilvie et al. approach

4.2.3.4 Synthetic approach to Ganciclovir (73) from imidazoles:

In 1991, Alhede et al.[170] reported the synthesis of ganciclovir (73) starting from imidazoles (Scheme-4.11). By preparing the potassium salt of 110 in DMF using KOH powder followed by addition of the alkylating agent, 111 was isolated from the reaction mixture in 75% yield. The benzyl groups of 111 were deprotected by catalytic hydrogenation. Treatment of this compound with benzoyl isothiocyanate in acetone afforded 113 which was hydrolyzed in situ to the corresponding thiourea 114. The key step, ring closure of the thiourea, was carried out in the
presence of one equivalent of heavy metal ion (preferably Cu$^{2+}$) in excess aqueous NaOH.

Scheme 4.11: Alhede et al. approach
Summary of literature:

Figure 4.3: Schematic representation of all previous approaches (in a single view)

Reagents and Conditions: (i) (a) (NH₄)₂SO₄/ HMDS, reflux, 4 d, then 77b/ Bu₄NI, reflux, 15 h, 41% (b) Pd/C/Cyclohexene/ EtOH, reflux, 18 h, 73: 84% (ii) (a) p-TsOH/ sulfolane, 95 °C, 31% (b) 20% Pd(OH)₂/C/Cyclohexene/EtOH, reflux, 32 h, 92% (c) NH₄OH/MeOH, 25 °C, 16 h, 94% (iii) (a) EtSO₃H, 165-170 °C, 0.5 h, 33% (b) 40% aq MeNH₂, 75 °C, 1.5 h, 95%; (iv) (a) p-TsOH/ DMSO/ DMF, 105 °C, 42 h, 45% (b) NH₄OH/ MeOH, reflux, 40 h, 72%; (v) (a) (NH₄)₂SO₄/ HMDS, reflux, 2 h, then 77b/ Hg(CN)₂/ benzene, reflux, 3 h (b) Na/ HSCH₂CH₂SH/ MeOH, reflux, 2.5 h (c) Cyclohexene/ Pd/ EtOH, reflux, 18 h; (vi) (a) Et₃N/ DMF, 60 h, 55% (b) MeONa/MeOH, reflux, 2 h, 85% (c) Cyclohexene/ Pd/ EtOH, reflux, 3 h, 80% (d) Adenosine deaminase, pH 7.5, r.t., 18 h.
In summary (Figure-4.3), of the numerous methods explored in the synthesis of ganciclovir, 73 uses silylated guanine, \(N^2\)-acetylguanine, \(N_2,9\)-diacetylguanine and 6-chloroguanine as the source of guanine moiety. Martin et al. synthesis of 73 pose a difficulty in deprotection of benzyl groups and requires costly palladium reagents and specialized equipment like pressure hydrogenator thereby preventing the process from being easily scaled up to industrial size, whereas Ogilvie synthesis [168] 6-chloroguanine is not a preferred substrate for industrial scale synthesis of 73 because it is relatively expensive. Alternatively, Tolman approach [164,165] involved reaction of \(N^2\), 9-diacetylguanine (78b) with 1,3-diacetoxy-2-acetoxyethoxy propane (89), in which the preparation of 89 is tedious and time consuming HPLC separations thereby making the route a non-commercial one for the preparation of ganciclovir. The McGee approach [167] involved multiple stages for the preparation of protected glycerol derivative (97). Though this process eliminates the costly and time consuming hydrogenation step for removal of the benzyl protection and also avoids the chloromethylation step, which yields a by-product bischloromethylether, which is carcinogen. However, the process does not seem to be an improvement in terms of reaction stages and cost effectiveness. Nevertheless, Use of expensive reagents, involvement of more no. of steps and low yields made these approaches inferior for industrial scale; hence, we felt the need of a simple and production friendly process for ganciclovir, 73.
4.3. OBJECTIVE OF THE PRESENT WORK:

The present work was aimed towards overcoming the limitations of the prior art procedures, such as;

1. Usage of high-pressure reactions involving expensive metal catalysts.
2. Usage of expensive reactants/starting material like Chloroguanine.
3. Usage of non-commercial processes like chromatographic separations.
4. More number of steps and lengthy procedures due to protection-deprotection strategies.

The objective of present work is to develop an alternative synthesis of ganciclovir (73) from commercially available and inexpensive raw materials, also which is easier to produce at industrial scale at lower cost when compared with existing routes. Another objective of this work is to find a new route that is not encumbered with patent protection.

Considering all above factors, for the industrial scale up and the commercial synthesis of 73, Process research and development activity has been taken up as illustrated in here.
4.4 RESULTS & DISCUSSION

We started our efforts [171] towards Retro synthetic analysis of ganciclovir, 73 as outlined below in Figure-4.4.

Figure 4.4: Retro synthetic analysis of Ganciclovir (73).

Based on retro synthetic analysis and the literature precedence, we considered N2,9-diacetylguanine (78b) as aromatic key building block due to its ready availability and reactivity. Next target was the selection of the appropriate side-chain, a glycerol derivative to achieve the formation of final molecule. We had derived a simple approach for the synthesis of the desired glycerol derivative, which would avoid the challenges involved in the reported approaches, and the investigations are summarized herein.

4.4.1 Investigations on side chain synthesis

In order to achieve desired side chain 117, we explored the reported approaches to understand the challenges involved in the process. As a
first attempt, we followed the key reactions involved in the prior art approaches [172,173] as depicted broadly in Scheme-4.12.

**Scheme 4.12: Reported approaches for side chain synthesis**

Chloromethylation of protected 115 using paraformaldehyde and HCl gas at 0 °C for about 16 hrs provided the 116 containing lot of impurities. Further, acetoxy substitution on 116 using potassium acetate to get the desired 117 failed to provide the desired quality and yield. Chloromethylation of Benzyl protected 115 was found superior compared to other groups as we observed the desired yield, but at the end they require costly palladium reagents with specialized equipment for deprotection, which render complications in industrial scale up. Other options using acetyl and ether protections though feasible, faced difficulty in the isolation of intermediates due to lack of chemo and regio selectivity and gave a mixture of products which led to the usage of tedious and time consuming chromatographic separations. Using glycerol as the starting material for side chain synthesis always led to selectivity problems over primary and secondary hydroxyl functions. In
addition, there is always possibility to form mixture of products that led to lower yields during the synthesis, which make the process inferior.

Overall chloromethylation conducted as per Scheme-4.12 posed various challenges; (i) improper conversion of reactants due to reversible nature of formed aliphatic ether to alcohol before its conversion to corresponding desired alkyl halide under acidic conditions, (ii) deprotection of acetyl and benzyl groups on primary alcohol under the reaction conditions, and (iii) Usage of dry HCl gas employed for halogenation. These drawbacks of chloromethylation step forced us to devise an alternative, which circumvents these issues.

Based on our experimental findings on the above approaches, we identified a new glycerol derivative (121) having an aldehyde group instead of one hydroxyl group in the glycerol, which allows easy conversion of aldehyde to hydroxyl when desired as shown in Scheme-4.13.
Scheme 4.13: Proposed pathway for Ganciclovir synthesis

In order to achieve 121, without chloromethylation, chloromethyl acetate (CMA, 124) was found to be an effective reagent for acetoxylation of hydroxyl group due to its ready availability in bulk quantities and its stability under normal reactions. Synthesis of the desired glycerol derivative (121) was attempted initially using two methodologies starting from glycerol but the results were unsuccessful as outlined in Scheme-4.14.
Scheme-4.14: Explored synthesis of aliphatic key building block 121

Tritylation of 94 gave mixture of products containing ditrityl (desired primary hydroxyl protected) and tritrityl (undesired protection of secondary hydroxyl) derivatives which led to only 50% yield. Even though, the second stage, acetoxy methylation gave 60% yield, poor selectivity was observed at third stage (partial hydrolysis) and resulted in only 20% yield (Scheme 4.14, Path A). This process demands chromatographic purifications due to poor stage wise yields and inferior quality of the intermediates and hence hindered us to pursue the scheme. On the other hand, lower yields (30%) were observed during selective tritylation of one primary hydroxyl (stage-1) and oxidation of primary alcohol in presence of secondary (stage-2) due to poor reaction quality, only 20% yield was obtained (Scheme 4.14, Path B). Because of the formation of undesired and multiple products during oxidation and acetoxy methylation, we dropped this approach.
Our further exploration aimed to make protected aldehyde 129, starts with acrolein, 125 (Scheme-4.15) gave overall yield of about 20% due to poor reaction quality during cyclic acetal (126) formation and epoxidation (Scheme-4.15, step: 2). This approach was also not preferred due to the usage of chromatographic purifications and unstable intermediate like epoxide.

Scheme 4.15: Explored synthesis of aliphatic key building block 129

With the above failures, we have identified alternate glycerol derivative 133 and strategy for the synthesis of the same starts from 125 (Scheme 4.16). It involves protection of aldehyde (125) as acyclic acetal (130) using triethyl orthoformate and ammonium nitrate to give acrolein diethyl acetal, 130 which is further subjected to oxidation using potassium permanganate to give glyceraldehyde diethyl acetal, 131. The other terminal hydroxy group is protected by trityl group[174] to obtain 132. Finally, incorporation of the desired acetoxyethyl functionality in
132 is achieved using chloromethyl acetate, 124 to give 133 as captured in Scheme-4.16, which hitherto is unknown structural unit for 73.

Scheme 4.16: Selected approach for aliphatic side chain

Reaction of 132 with 124 in the presence of sodium hydride in DMF provided the desired intermediate in good yields post optimization studies. The plausible mechanism of acetoxymethylation using CMA is shown in Figure-4.5.

Figure 4.5: Mechanism of acetoxymethylation using chloromethylacetate

In order to derive parameters for optimum reaction conditions, various bases and solvents were screened as depicted in Table-4.1 and NaH as base and DMF as solvent were found to be suitable for the transformation of 132 to 133.
Table 4.1: Screening of bases and solvents for preparation of 133

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Solvent</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>K$_2$CO$_3$</td>
<td>Acetone</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>K$_2$CO$_3$</td>
<td>DCM</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>K$_2$CO$_3$, NaI</td>
<td>Acetone</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>DMAP</td>
<td>DCM</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>KOH</td>
<td>DMF</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>NaOMe</td>
<td>DMF</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>K'tBuo</td>
<td>DMF</td>
<td>31</td>
</tr>
<tr>
<td>8</td>
<td>LiOH</td>
<td>DMF</td>
<td>15</td>
</tr>
<tr>
<td>9</td>
<td>NaH</td>
<td>DMF</td>
<td>63</td>
</tr>
<tr>
<td>10</td>
<td>NaH</td>
<td>Toluene</td>
<td>49</td>
</tr>
<tr>
<td>11</td>
<td>NaH</td>
<td>THF</td>
<td>52</td>
</tr>
<tr>
<td>12</td>
<td>NaH</td>
<td>DMSO</td>
<td>45</td>
</tr>
</tbody>
</table>

There was no reaction initiation when relatively weak bases like potassium carbonate and DMAP were used in presence of Acetone and dichloromethane even after prolong duration at elevated temperatures. When a halo exchange reaction was attempted using sodium iodide in presence of potassium carbonate, reaction progress was very poor. Even attempts using sodium methoxide, potassium tert-butoxide and lithium hydroxide were discouraging. Finally, a strong base, sodium hydride served the purpose of achieving satisfactory yield.

The mode of addition of reactants 132 and 124 also had a tremendous effect on reaction yield. Impurity formation was more upon successive addition of sodium hydride, 132 and 124 in DMF medium.
thereby resulting in low yield of 133. Addition of 124 to an activated 132 (using NaH) was also resulted in low yields with increased amount of impurities. In contrast, drastic improvement in yield was observed upon activation of 132 using NaH followed by its drop wise addition to 124 in DMF.

Upon thorough investigations, it was reasoned that, the major impurity which resulted during acetoxymethylation was the acetyl derivative of 132 (134, Scheme-4.16). Impurity 134 is likely to be formed due to the attack of activated 132 on carbonyl carbon of chloromethyl acetate in highly basic medium. Thus, drop wise addition of activated 132 to chloromethylacetate (124) was preferred. The optimum reaction temperature was found to be 15-20 °C. The final optimized procedure is described in experimental section. The compound 133 was confirmed by proton NMR spectroscopy (Figure-4.8b) in which 4.29 ppm corresponds to methylene group attached to the acetoxy group and 2.01 ppm shows the presence of acetoxy group and it was further confirmed by mass spectral data, which exhibited a mass value corresponds to m/z 496 (Figure-4.8a).

4.4.2 Investigations on ganciclovir, 73 synthesis

Next step to achieve ganciclovir, 73 was the alkylation reaction of diacetyl guanine (78b) with the masked glycerol derivative 133 (Scheme-4.17). The N-9 alkylation of purines remains an important synthetic
route to pharmaceutically important acyclic nucleoside analogues, however these reactions are rarely regiospecific, giving rise to mixtures of N-9 and N-7 alkylated products. In particular, base-catalysed alkylations of guanine and protected guanines exhibit very poor regioselectivity, resulting in approximately equal amounts of N-9 and N-7 isomers [175a,b]. Conversely, acid-mediated alkylations of guanines using alpha-halo or alpha acetoxy ether alkylating agents give N-9 isomer predominantly under thermal conditions [175b,c].

**Scheme 4.17: Synthesis of Ganciclovir**

The intermediate \textbf{135} was obtained by the N-alkylation of \textbf{78b} using \textbf{133} as alkylating agent under acidic conditions using methane sulfonic acid (Figure-4.6), and the solvent used was dimethyl acetamide. The alkylation reaction resulted in predominantly N-9 isomer over N-7 isomer. The fusion reaction of \textbf{78b} with triacetoxy derivative, similar to \textbf{133}, reported earlier required high temperature and reduced pressure.
The condensation of 78b and 133 was easily achieved at 75-80 °C at normal atmospheric pressure to give 135 [176] with 44% yield.

**Figure 4.6: Plausible Mechanism of condensation of 78b and 133**

During N-alkylation of diacetyl guanine, 78b with the glycerol derivative, 133 reaction profiles were optimized using various acid catalysts like paratoluene sulphonic acid, ethane sulphonic acid, and methane sulphonic acid and some of them were reported as mentioned in Table-4.2.
Table 4.2: Experimental details on condensation using acid catalyst

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acid</th>
<th>Solvent</th>
<th>Temp (°C)</th>
<th>Time (h)</th>
<th>Yield (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>PTSA</td>
<td>DMF, DMSO</td>
<td>100-105</td>
<td>42</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>PTSA</td>
<td>DMSO</td>
<td>100-105</td>
<td>30</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>PTSA</td>
<td>DMA</td>
<td>100-105</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>PTSA</td>
<td>Toluene</td>
<td>100-105</td>
<td>20</td>
<td>29</td>
</tr>
<tr>
<td>5</td>
<td>PTSA</td>
<td>DMF</td>
<td>95-100</td>
<td>42</td>
<td>22</td>
</tr>
<tr>
<td>6</td>
<td>PTSA</td>
<td>Xylene</td>
<td>100-110</td>
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</tr>
<tr>
<td>7</td>
<td>PTSA</td>
<td>Dioxane</td>
<td>100-110</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>ESA</td>
<td>DMA</td>
<td>95-100</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
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<td>ESA</td>
<td>DMSO</td>
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<tr>
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<td>MSA</td>
<td>DMA</td>
<td>75-80</td>
<td>22</td>
<td>44</td>
</tr>
</tbody>
</table>

As presented in Table-4.2, a combination of methane sulphonic acid and dimethylacetamide at a reaction temperature of 75-80 °C is found to be an ideal reaction condition to get optimum yield and quality of 135.

The structure of 135 was further confirmed by proton NMR (Figure-4.9b) which showed that 8.46 ppm corresponds to H-8 proton of compound 135. The compound was further confirmed by HRMs which exhibited a mass value corresponds to m/z: 612 (Figure-4.9a).

In the next step, acetyl group 135 was deprotected under basic conditions using sodium hydroxide and methanol to afford the deacetylated compound 136 [176] in 79% yield. The proton NMR of the
compound 136 showed the absence of acetyl group (Figure-4.10b) and was further confirmed by mass spectroscopy, which exhibited a mass value corresponds to m/z 568.2 (Figure-4.10a). The compound 136 was subjected to hydrolysis under acidic conditions to remove acetal and trityl group using the trifluoroacetic acid to furnish the aldehyde 137 in situ and the presence of aldehyde was confirmed by its IR spectrum having 1720 peak. The aldehyde 137 upon reduction with sodium borohydride in a tandem manner in methanol gave the final compound ganciclovir (73) in 73% yield (from 136 to 73). The structure of the ganciclovir (73) was confirmed by spectral data (Figure-4.7a –c) including proton NMR data, which is similar to the values reported in literature [177].
4.5 CONCLUSIONS

The approach used to prepare ganciclovir, 73 provided the following advantages compared to that reported in literature. Firstly, the key intermediate 133 was synthesized utilizing acetoxymethylation to avoid cumbersome chloromethylation reaction. The new glycerol derivative, 133 allowed easy conversion to 135. Secondly, the requisite acyclic side chain in ganciclovir was conveniently achieved by simple chemical transformations after the alkylation step. Thus, an alternative, non-infringing synthesis of ganciclovir, 73 from commercially available and inexpensive raw materials is accomplished.

4.6 EXPERIMENTAL SECTION

General procedures: IR spectra were recorded in the solid state as a KBr dispersion using a Perkin-Elmer FT-IR spectrophotometer. 1H NMR spectra were scanned in DMSO-d₆ on a Mercury Plus spectrometer with TMS as an internal standard. HR-MS spectra were obtained on Waters LCT Premier XE (Micro mass Oa-TOF) instrument. The solvents and reagents were used without any purification.

4.6.1 Preparation of 2-acetoxymethoxy-1,1-diethoxy-3-trityloxypropane (133): To a solution of NaH (144 mg, 6 mmol) in anhydrous DMF (25 mL) was added 1,1-diethoxy-3-trityloxy-propan-2-ol 132 (4.06 g, 10 mmol) at room temperature and reaction mixture was
stirred for 10 h under N\textsubscript{2}. This solution was added dropwise at regular intervals to a solution of chloromethyl acetate, \textbf{124} (1.0 mL, 10 mmol) in DMF (25 mL) at 15-20 °C. The reaction mixture was stirred for 5-6 h, quenched with chilled water and extracted with diisopropyl ether. The ether layer was dried over sodium sulfate and concentrated under vacuum to give crude compound \textbf{133} as a residue, which was purified by column chromatography eluting with 10\% MeOH in EtOAc to get the product \textbf{133} in 63\% yield (3 g). IR (KBr): 3444, 3086, 3059, 2974, 2874, 1747, 1649, 1490, 1448, 1227, 1154, 1066, 1012, 947, 704 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (400 MHz, Me\textsubscript{2}SO-\textit{d}_6): \textdelta = 7.46-7.42 (m, 6H), 7.30-7.20 (m, 9H), 5.35-5.55 (dd, 2H), 4.53 (d, \textit{J} = 6.3 Hz, 1H), 4.29 (s, 2H), 3.81-3.77 (m, 1H), 3.60-3.54 (m, 2H), 3.33-3.29 (m, 2H), 3.23-3.18 (m, 2H), 2.01 (s, 3H), 1.00 (t, 3H), 0.86 (t, 3H); HRMS for C\textsubscript{29}H\textsubscript{34}O\textsubscript{6} [M+H]\textsuperscript{+}: m/z Calcd: 479.2699; Found: 479.2702.

\textbf{4.6.2 Preparation of N\textsuperscript{2}-acetyl-9-[(1,1-diethoxy-3-trityloxy-2-propoxy)methyl] guanine (135):} To a solution of diacetylguanine \textbf{78b} (1.41 g, 6 mmol) and 2-acetoxymethoxy-1,1-diethoxy-3-trityloxypropane \textbf{133} (2.9 g, 6 mmol) in dimethylacetamide (30 mL) was added methanesulfonic acid (0.1 mL, 1.2 mmol) at room temperature. The reaction mixture was heated at 75-80 °C for 20-21 h, cooled to room temperature and extracted with DCM. The DCM layer was dried over sodium sulfate and concentrated under vacuum to give crude compound \textbf{135}, which was
purified by column chromatography eluting with 10% MeOH in CHCl₃ to get the product 135 in 44% yield (1.6 g). IR (KBr): 3238, 3167, 3054, 2927, 2855, 1692, 1617, 1547, 1449, 1373, 1265, 1083, 1062, 896, 738 cm⁻¹; ¹H NMR (400 MHz, Me₂SO-d₆): δ = 12.15 (s, 1H), 11.63 (s, 1H), 8.46 (s, 1H), 7.28-7.19 (m, 15H), 5.8 (s, 2H), 4.38 (d, J = 6.1 Hz, 1H), 4.03-3.99 (m, 1H), 3.57-3.37 (m, 4H), 2.93-2.89 (m, 2H), 2.16 (s, 3H), 0.86 (t, 3H), 0.83 (t, 3H); HRMS for C₃₄H₃₇N₅O₆ [M+H]^+: m/z Calcd: 612.2822; Found: 612.2835.

4.6.3 Preparation of 9-[(1,1-diethoxy-3-trityloxy-2-propoxy)methyl]guanine (136): A solution of compound 135 (1.83 g, 3 mmol) and NaOH (0.12 g, 3 mmol) in MeOH (15 mL) was stirred at 40-50 °C for 3-4 h. The reaction mixture was cooled to room temperature and extracted with EtOAc. The organic extract was dried over sodium sulfate and concentrated under vacuum to give compound 136 in 79% yield (1.35 g). IR (KBr): 3426, 3321, 3167, 3059, 3033, 2973, 2928, 1685, 1560, 1475, 1448, 1377, 1213, 1089 cm⁻¹; ¹H NMR (400 MHz, Me₂SO-d₆): δ = 10.82 (s, 1H), 8.17 (s, 1H), 7.43-7.20 (m, 15H), 6.15 (s, 2H), 5.75 (s, 2H), 4.36 (d, J = 5.9 Hz, 1H), 4.01-3.98 (m, 1H), 3.54-3.43 (m, 4H), 2.94-2.88 (m, 2H), 1.01 (t, 3H), 0.86 (t, 3H); HRMS for C₃₂H₃₅N₅O₅ [M+H]^+: m/z Calcd: 568.2560; Found: 568.2542.
4.6.4 Preparation of Ganciclovir (73): A mixture of compound 136 (569 mg, 1 mmol) and TFA (0.3 mL, 4 mmol) in DCM (10 mL) was stirred at room temperature for 4 h. The solvent was stripped off under reduced pressure and MeOH (20 mL) was added to the residue and the solution was stirred at 10-15 °C. Sodium borohydride (45.6 mg, 1.2 mmol) was added in portions and the mixture was stirred for 2 h at 25 °C. The reaction mixture was poured into cold water, the solid obtained was filtered and recrystallized from MeOH to give ganciclovir (73) as a white solid in 73% yield (186 mg). mp 248-250 °C; IR (KBr): 3420, 3320, 3159, 3101, 2942, 2892, 1687, 1658, 1491, 1475, 1367, 1064, 1045 cm\(^{-1}\); \(^1\)H NMR (400 MHz, Me\(_2\)SO-\(d_6\)): 10.6 (s, 1 H), 7.79 (s, 1 H), 6.47 (s, 2 H), 5.43 (s, 2 H), 4.62 (m, 1 H), 3.30-3.52 (m, 4 H).
Spectral data of active pharmaceutical ingredient, novel intermediates and impurities are mentioned as follows.

Figure 4.7a: Mass spectrum of Compound 73

Figure 4.7b: $^1$HNMR Spectrum (DMSO-$d_6$, 400 MHz) of Compound 73
Figure 4.7c: $^{13}$C NMR Spectrum (DMSO-$d_6$, 200 MHz) of Compound 73

Figure 4.8a: HRMS Spectrum of Compound 133
Figure 4.8b: $^1$H NMR Spectrum of Compound 133

Figure 4.8c: $^{13}$C Spectrum of Compound 133
**Figure 4.9a: HRMS of Compound 135**

**Figure 4.9b: $^1$H NMR Spectrum (DMSO-d$_6$, 400 MHz) of Compound 135**
Figure 4.9c: $^{13}$C Spectrum (DMSO-d$_6$, 200 MHz) of Compound 135

Figure 4.10a: HRMS of Compound 136
Figure 4.10b: $^1$H NMR Spectrum (DMSO-$d_6$, 400 MHz) of Compound 136

Figure 4.10c: $^{13}$C Spectrum (DMSO-$d_6$, 200 MHz) of Compound 136