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Novel synthesis of 4-amino-2-hydroxymethyl-1-butanol and its purine analogues

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ABSTRACT

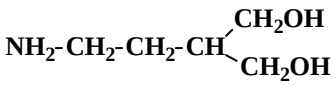
Novel and improved synthesis of Famciclovir drug substance through N-(2-amino-4-chloro-6-[[4-hydroxy-3-(hydroxymethyl)butyl]amino]pyrimidin-5-yl)formamide from 4-amino-2-hydroxymethyl-1-butanol and N-(2-Amino-4,6-dichloro-5-pyrimidinyl)formamide. Further this synthetic method involves the 2-acetoxymethyl-4-azido-1-butyl acetate and 4-azido-2-hydroxymethyl-1-butanol as novel intermediates in the preparation of 4-amino-2-hydroxymethyl-1-butanol.

Keywords: Purine analogue, famciclovir, dechlorination, cyclization and acetylation.

INTRODUCTION

Apart from Acyclovir, Penciclovir (**3)** and Famciclovir (**2)** (**Figure 1**) are found to be potent and highly selective anti-viral agents active against a number of the human herpes virus such as simplex virus type-1 and 2 (HSV-1 and HSV-2), Varicella zoster virus (VZV), hepatitis B virus (HBV) etc. Penciclovir and Famciclovir are analogues of Acyclovir, which have alkyl side chain at the N-9 position of the guanine / purine moiety. Famciclovir has been licensed as **Famvir**™ and Penciclovir has been licensed as **Vectavir**™ in United Kingdom and **Denavir**™ in United States.[1]

A new synthetic method for the preparation of 4-amino-2-hydroxymethyl-1-butanol (**1**) starting from 2-acetoxymethyl-4-methanesulfonyl-1-butyl acetate (**5**) is described via these new intermediates 2-acetoxymethyl-4-azido-1-butyl acetate (**6**) and 4-azido-2-hydroxymethyl-1-butanol (**7**). Compound **1** is a key intermediate in the preparation of 9-[4-Acetoxy-3-(acetoxymethyl)butyl]-2-aminopurine (Famciclovir drug substance, **2**) and Penciclovir drug substance (**3**). Another new synthetic method for the preparation of *N*-(2-amino-4-chloro-6-[[4-hydroxy-3-(hydroxymethyl)butyl]amino]pyrimidin-5-yl)formamide (**4**), an important and novel intermediate for the preparation of famciclovir is also described, which involves the condensation of 4-amino-2-hydroxymethyl-1-butanol **1** with *N*-(2-amino-4,6-dichloro-5-pyrimidinyl)formamide (**19**) to result *N*-(2-amino-4-chloro-6-[[4-hydroxy-3-(hydroxymethyl)-butyl]amino]pyrimidin-5-yl)formamide **4**. This compound **4** on further sequential reactions results Famciclovir **2**.



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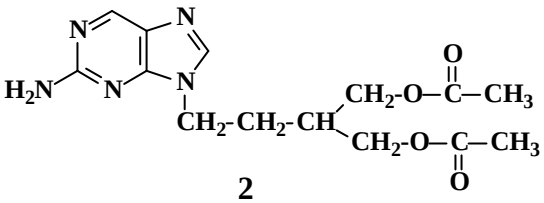

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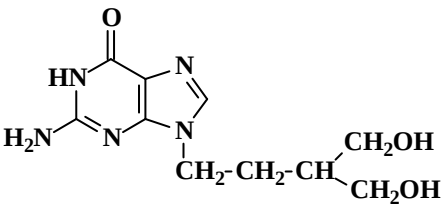

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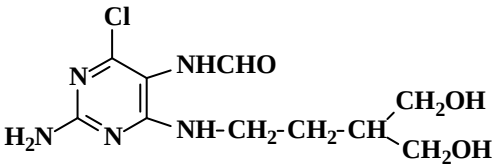


4

Figure 1

MATERIALS AND METHODS

In general, solvents & reagents were used as purchased without further purification. Melting points were determined by Polman melting point apparatus (Model No. mp - 96) and are uncorrected. The IR spectra (*v* max cm^{-1}) were recorded on Perkin-Elmer FT-IR spectrophotometer. The ^{1}H NMR, ^{13}C NMR spectra were recorded on 300 MHz, Bruker-Avance instrument using TMS as an internal standard. The mass spectra were scanned on Perkin-Elmer SCIEX API 2000 instrument. The compounds **6, 7** and **4** are novel compounds. The synthesis of all the compounds as described in this section.

Preparation of 2-Acetoxymethyl-4-azido-1-butyl acetate (6)

Sodium azide (6 g, 92 mmol) was added to the solution of 2-Acetoxymethyl-4-methanesulfonyl-1-butyl acetate **5** (20 g, 75 mmol) in DMF (60 mL) at 20-25°C and stirred for ~8 h. Thereafter, reaction mass was added slowly to DM water (180 mL).The product was extracted with ethyl acetate (2 x 50 mL), washed with water (20 mL), dried over anhydrous sodium sulphate and concentrated under reduced pressure to give 2-acetoxymethyl-4-azido-1-butyl acetate **6** as an oily residue (15.6 g, 90.5%); IR (Nujol mull): 2100.8 cm^{-1} (N≡N str.), 1743.6 cm^{-1} (C=O str.), 1462.9 cm^{-1} (aliphatic CH_2-bending vibration), 1199.4 cm^{-1} (C-N-Str.), 1040.8 cm^{-1} (C-O-C str.); ^{1}H NMR(300 MHz, $CDCl_3$): δ 1.62 (m, 2H, N_3-CH_2-**CH_2**), 2.03 (s, 6H, 2 x **CH_3**), 2.13 (m, 1H, **CH**), 3.39 (t, *J* = 9.0Hz, CH_2, N_3**CH_2**), 4.07 (d, *J* = 6Hz, 4H, 2 x **CH_2**-O); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.2, 28.0, 35.3, 49.3, 64.2, 171.2; Mass: *m/z* 228.3[$(M-H)^-$]; Anal. Calcd. for $C_9H_{15}N_3O_4$ (229.2);C, 47.15; H, 6.58; N, 18.33; Found: C, 47.20; H, 6.52; N, 18.25.

Preparation of 4-Azido-2-hydroxymethyl-1-butanol (7)

2-Acetoxymethyl-4-azido-1-butyl acetate **6** (20 g, 87 mmol) was added to 20% w/w aqueous sodium hydroxide solution (40 g) and continued stirring at 25-30°C for ~60 min. Product was extracted with ethyl acetate (2 x 80 mL), dried over anhydrous sodium sulphate and concentrated under reduced pressure to give 4-azido-2-hydroxymethyl-1-butanol **7** as an oily residue (11.6 g, 91.6%); IR (Nujol mull): 2100.8 cm^{-1} (N≡N str.), 3368.4 cm^{-1} (O-H str.), 1459.0 cm^{-1} (aliphatic CH_2-bending vibration), 1115.1 cm^{-1} (C-N-str.); ^{1}H NMR(300 MHz, $CDCl_3$): δ 1.67 (m, 2H, N_3-CH_2-**CH_2**), 1.84(m, 1H, **CH**), 3.40(t, *J* = 15Hz, 2H, -**CH_2**-N_3), 3.67, 3.80, 3.70, 3.82 (2ABq, 4H, 2 x **CH_2**OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.4, 39.2, 49.8, 64.7; Mass: *m/z* 144.1 [$(M-H)^-$]; Anal. Calcd. for $C_5H_{11}N_3O_2$ (145.1); C, 41.37; H, 7.63; N, 28.94; Found: C, 41.32; H, 7.60; N, 28.90.

Preparation of 4-Amino-2-hydroxymethyl-1-butanol (1)

10% w/w Palladium on charcoal (50% wet, 3 g) was added to the solution of 4-Azido-2-hydroxymethyl-1-butanol **7** (20 g, 138 mmol) in ethanol (140 mL) under nitrogen atmosphere. The mixture was hydrogenated under hydrogen pressure (3.0-4.0 Kg / cm^2) for 4 h at 25-30°C. Thereafter, the catalyst was filtered and concentrated the solvent under reduced pressure to obtain 4-amino-2-hydroxymethyl-1-butanol **1** as an oily residue (14 g, 85.3%); IR (Neat): 3368.7 cm^{-1} (N-H, O-H, str.), 1447.3 cm^{-1} (aliphatic CH_2 bending vibration), 1205.2 cm^{-1} (C-N str.); ^{1}H NMR(300 MHz, D_2O): δ 1.62 (m, 2H, **CH_2**-CH), 1.66 (m, 1H, **CH**), 2.98 (t, *J* = 6.0 Hz, 2H, **CH_2**NH_2), 3.44-3.55 (m, 4H, 2 x **CH_2**OH); ^{13}C NMR (75 MHz, D_2O): δ 17.3, 40.4, 57.7, 62.0; Mass: *m/z* 120.1 [$(M+H)^+$]; Anal. Calcd. for $C_5H_{13}NO_2$ (119.1); C, 50.40; H, 10.98; N, 11.75; Found: C, 50.50; H, 10.92; N, 11.70.

Preparation of *N*-(2-amino-4-chloro-6-[[4-hydroxy-3-(hydroxymethyl)butyl]amino]pyrimidin-5-yl)formamide (4)

Ethanol solution of 4-amino-2-hydroxymethyl-1-butanol **1** (12.9 g, 108 mmol in 60 mL) and water (6 mL) was slowly added to a mixture of sodium bicarbonate (16.25 g, 193 mmol) and *N*-(2-Amino-4,6-dichloro-5-pyrimidinyl)formamide **19** (20 g, 96.6 mmol) in ethanol (200 mL) at reflux temperature. The mixture was stirred at 75-80°C for ~90 min and cooled to 25°C. The salts were filtered and washed with ethanol. The filtrate is directly taken for the next step without further purification. However, small sample was subjected to cooling to crystallize the material for analytical characterization purpose. The isolated sample is confirmed the structure of *N*-(2-Amino-4-chloro-6-[[4-hydroxy-3-(hydroxymethyl)butyl]amino]pyrimidin-5-yl)formamide **4,** Melting point: 146-152°C; IR(KBr pellet): 3376.3 cm^{-1} (N-H str.), 1672.9 cm^{-1}, 1649.46 cm^{-1} (C=O str.), 1452.7 cm^{-1} (CH_2 bending vibration); ^{1}H NMR(300 MHz, DMSO-d_6): δ 1.48 (m, 2H, NH-CH_2-**CH_2**), 1.53 [m, 1H, **CH**-(CH_2-OH)$_2$], 3.30 (t, *J* = 6.0 Hz, 2H, **CH_2**-NH), 3.42 (m, 4H, 2 x **CH_2**OH), 4.36 (m, 2H, 2 x CH_2-**OH**, D_2O exchangeable proton), 6.38 (*brs*, 2H,NH_2, D_2O exchangeable proton), 6.73 (t, 1H, **NH**-CH_2, D_2O exchangeable proton), 8.11 (s, 1H, CHO), 9.00 (s, 1H, **NH**-CHO D_2O exchangeable proton); ^{13}C NMR (75 MHz, DMSO): δ 27.9, 40.0, 61.0, 101.5, 155.3, 156.4, 160.4, 161.1, 165.9; Mass: *m/z* 290.1 [(M + H)$^+$] (chloro pattern is observed); Anal. Calcd. for $C_{10}H_{16}ClN_5O_3$ (289.7); C, 41.46; H, 5.56; N, 24.17; Found: C, 41.50; H, 5.49; N, 24.13.

Preparation of 2-amino-9-[4-hydroxy-3-(hydroxymethyl)butyl]purine Hydrochloride (18)

The mixture of compound **4** and triethylamine (10.85 g, 107 mmol) was subjected to hydrogenation in the presence of 10% palladium on carbon (50% wet, 3.0 g) at 50°C under 5-6 Kg / cm^2 hydrogen pressure for ~18 h. Thereafter, the solution was cooled to room temperature, filtered the catalyst and filtrate was concentrated up to ~80 mL at < 60°C under reduced pressure. Added ethanolic hydrochloric acid (33 g, ~15% w/w) to the above solution of **17** and stirred at 25-30°C for 10 min. Thereafter, triethylorthoformate (65 g, 439 mmol) was added to the mixture and heated to 40-45°C and stirred at this temperature for ~3 h. Cooled the reaction mixture to 15-18°C and stirred for ~1 h to precipitate the product. The solid was filtered and dried at 40-45^{0}C under reduced pressure get compound **18** (14.0 g, 53%), Purity: 98.56% by HPLC; Melting point: 170-173°C; IR (KBr pellet): 3341.3 cm^{-1} (N-H str.), 2973.5 cm^{-1} (aliphatic C-H str.), 1444.4 cm^{-1} (aliphatic CH_2 bending vibration), 1242.3 cm^{-1} (C-N str.); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.47 (m, 1H, CH), 1.80 (m, 2H, N-CH_2-**CH_2**) 3.33-3.47 (m, 4H, 2 x **CH_2**OH), 4.18(t, *J* = 7.5 Hz, 2H, N-**CH_2**-CH_2), 4.65 (*brs*, 2H, 2 x OH), 8.08 (*brs*, 2H, NH_2), 8.64 (s, 1H, CH of Guanine), 8.98 (s, 1H, CH of Guanine); ^{13}C NMR (75 MHz, DMSO-D_6): δ 28.1, 40.9, 41.8, 61.3, 125.9, 138.6, 149.6, 154.4, 156.8; Mass: *m/z* 238.0 [(M + H)$^+$] (as free base); Anal. Calcd. for $C_{10}H_{15}N_5O_2$.HCl (273.6); C, 43.89; H, 5.88; N, 25.59; Found: C, 43.80; H, 5.84; N, 25.50.

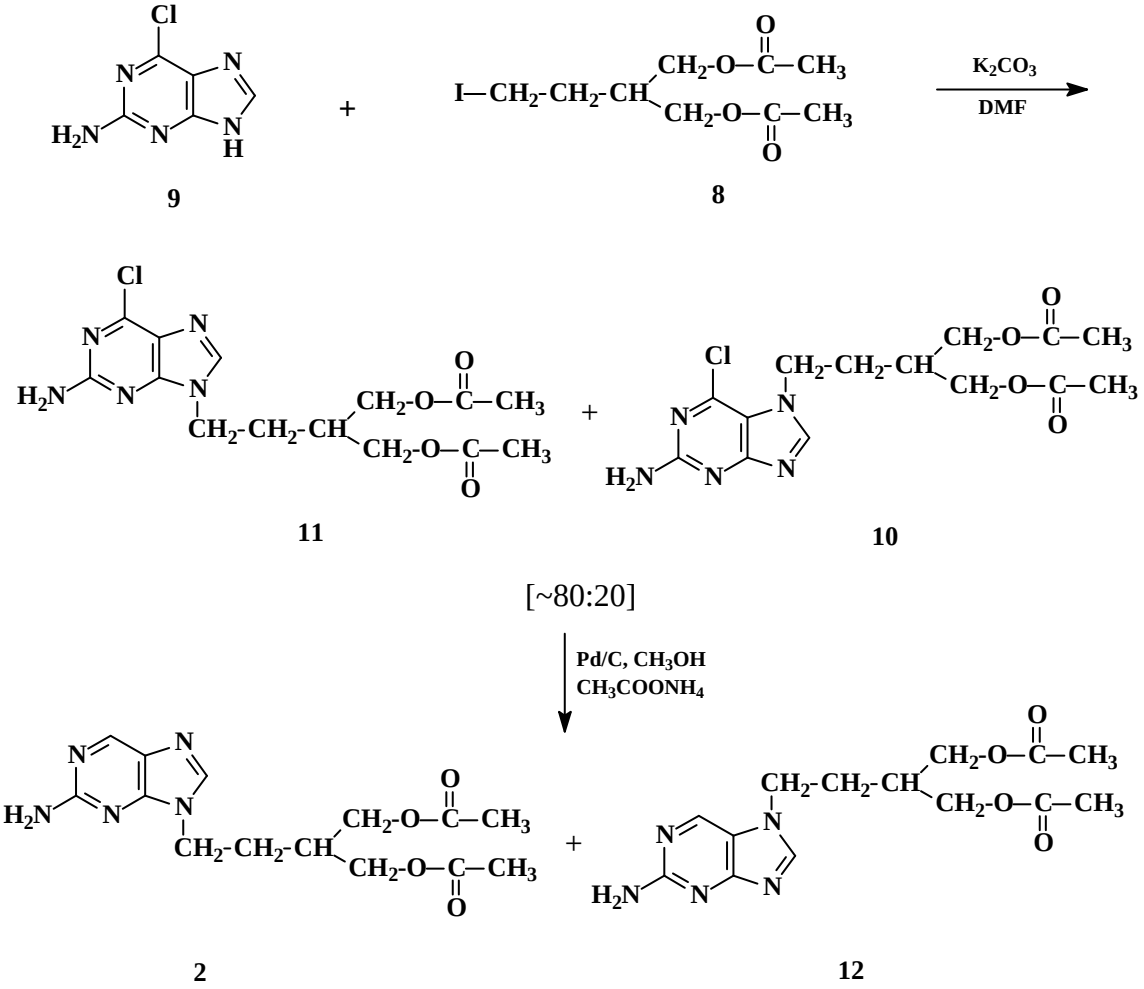
Preparation of 9-[4-Acetoxy-3-(acetoxymethyl) butyl]-2-aminopurine (Famciclovir, 2)

Triethylamine (23.90 g, 236 mmol) was added to the suspension of 2-Amino-9-[4-hydroxy-3-(hydroxymethyl) butyl]purine hydrochloride **18** (20 g, 73 mmol) in methylene chloride (160 mL) at 22-30°C and stirred for ~15 min. Thereafter, 4-(Dimethylamino)pyridine (DMAP, 0.4 g) is added to the mixture and cooled to 8-10°C. Acetic anhydride (15.60 g, 153 mmol, diluted with 40 mL methylene chloride) was slowly added to the reaction mixture during ~45 min at 8-11°C and continued stirring at 8-10°C for ~2 h. Methanol (5 mL) was added to the reaction mass and stirred for 20 min to quench the excess of acetic anhydride. Reaction mass was concentrated under reduced pressure at below 45°C and diluted with water (90 mL). The mixture was cooled to 15-18°C, stirred for 6 h to crystallize the product. The solid was filtered and washed with cold water (15 mL). The crude product was purified by re-crystallization from *n*-butanol to give pure **2** (18.0 g, 76.6%); purity: 99.69% (by HPLC); Melting point: 102-104°C; IR(KBr pellet): 1730.0 cm^{-1}, 1656.4 cm^{-1} (C=O, str.), 2982.0 cm^{-1}, 2952.4 cm^{-1} (aliphatic C-H, str.), 1445.5 cm^{-1} (aliphatic CH_2 bending vibration), 1216.4 cm^{-1} (C-N, str.), 1032.1 cm^{-1} (C-O-C str.), 3337.5 cm^{-1} (N-H str.); ^{1}H NMR(300 MHz, DMSO-d_6): δ 1.88 (m, 2H, CH_2-**CH_2**-CH), 1.94 [m, 1H, **CH**-(CH_2OH)$_2$], 1.99 (s, 6H, 2 x $COCH_3$), 4.02 (m, 4H, 2 x **CH_2**OH), 4.14 (t, *J* = 6 Hz, 2H, N-**CH_2**), 6.49 (*brs*, 2H, NH_2), 8.09 (s, 1H), 8.57 (s, 1H); ^{13}C NMR(75 MHz, DMSO-d_6): δ 20.9, 28.2, 34.9, 40.6, 63.8, 127.3, 143.0, 149.3, 153.3, 160.8, 170.8; Mass: *m/z* 322.1 [(M + H)$^+$]; Anal. Calcd. for $C_{14}H_{19}N_5O_4$ (321.3); C, 52.33; H, 5.96; N, 21.79, found C, 53.10; H, 5.80; N, 21.30.

RESULTS AND DISCUSSION

There are several synthesis methods reported [2-5] in literature for the preparation of Famciclovir. A general route [4] for the preparation of Famciclovir involves the alkylation of 2-amino-6-chloropurine (**9**) with 2-acetoxymethyl-4-iodo-1-butyl acetate (**8**) to get 2-[(acetoxy)methyl]-4-(2-amino-6-chloro-9*H*-purine-9-yl)butyl acetate (**11**), which is further dechlorinated to give Famciclovir **2**. Although, this process is simple and straightforward, it has a number of disadvantages. The main disadvantage of this process is the N-alkylation reaction, which is not selective and generates the undesired N-7 isomer (**10**) along with the desired compound **11** in the ratio of ~80:20 (**Scheme-1**). The dechlorination of **11** along with **10** results the required compound **2** and the major impurity 7-[4-acetoxy-3-

(acetoxymethyl)-butyl]-2-aminopurine (**12**). Further, removal of **12** requires conventional crystallization method and often requires chromatographic separation, which results in poor yield. In addition to this, the removal of the residual palladium from the final API is also a difficult task as the Pd/C is used in the final step of this process to prepare Famciclovir drug substance.

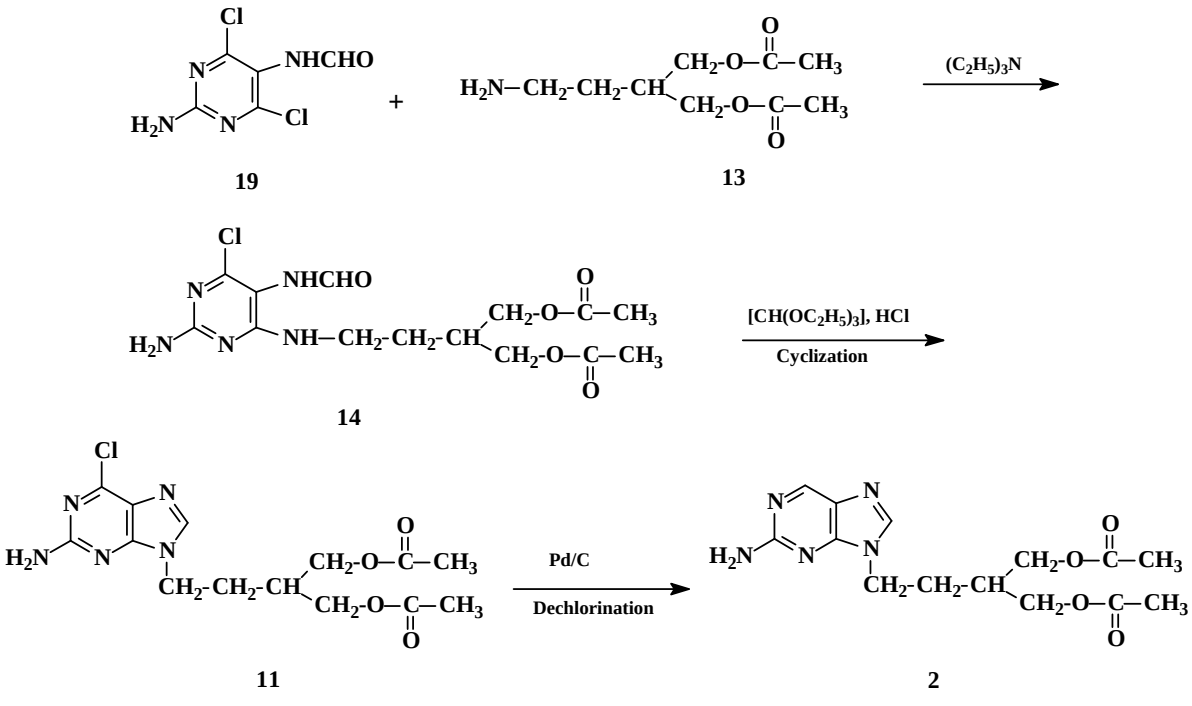


Scheme - 1

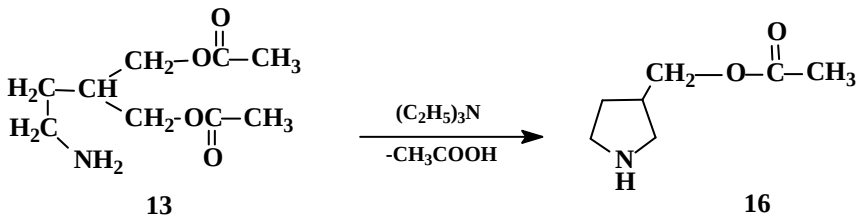
An alternate route [2,5] discloses the preparation of 2-aminopurine derivatives including Famciclovir **2**, wherein, *N*-(2-amino-4,6-dichloro-5-pyrimidinyl)formamide **19** is reacted with 2-acetoxymethyl-4-amino-1-butyl acetate (**13**) to give (**14**)**,** which is subsequently cyclised to yield **11**, which is dechlorinated to produce the required compound **2** as shown in **Scheme - 2**.

However, in this process, (Pyrrolidin-3-yl)methyl acetate (**16**) is forming as an impurity in the range of 25-30%, which is forming during the condensation reaction of **13** with compound **19** in the presence of base to prepare **14**. This impurity **16** may be forming due to the self-cyclisation of the ester **13** in the presence of base (**Scheme - 3**). The formation of this impurity requires the additional purification in this process to get pure compound **2**, which leads to the lower yield.

Another problem associates with the above method is the formation of 6-ethoxy derivative of purine (**15**) in the range of 10-12% during the preparation of **11** from **14** using triethyl- orthoformate and hydrochloric acid, thereby causing poor yields in the preparation of Famciclovir **2**. The 6-ethoxy derivative **15** (Figure 2) is forming by the substitution of chloro group by ethoxy group of triethylorthoformate and subsequent cyclisation.



Scheme - 2



Scheme - 3

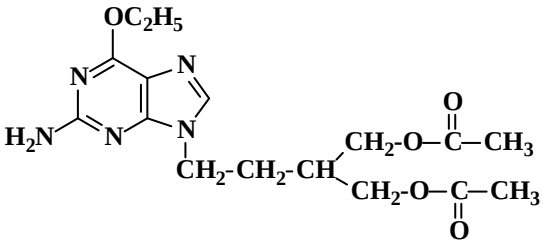
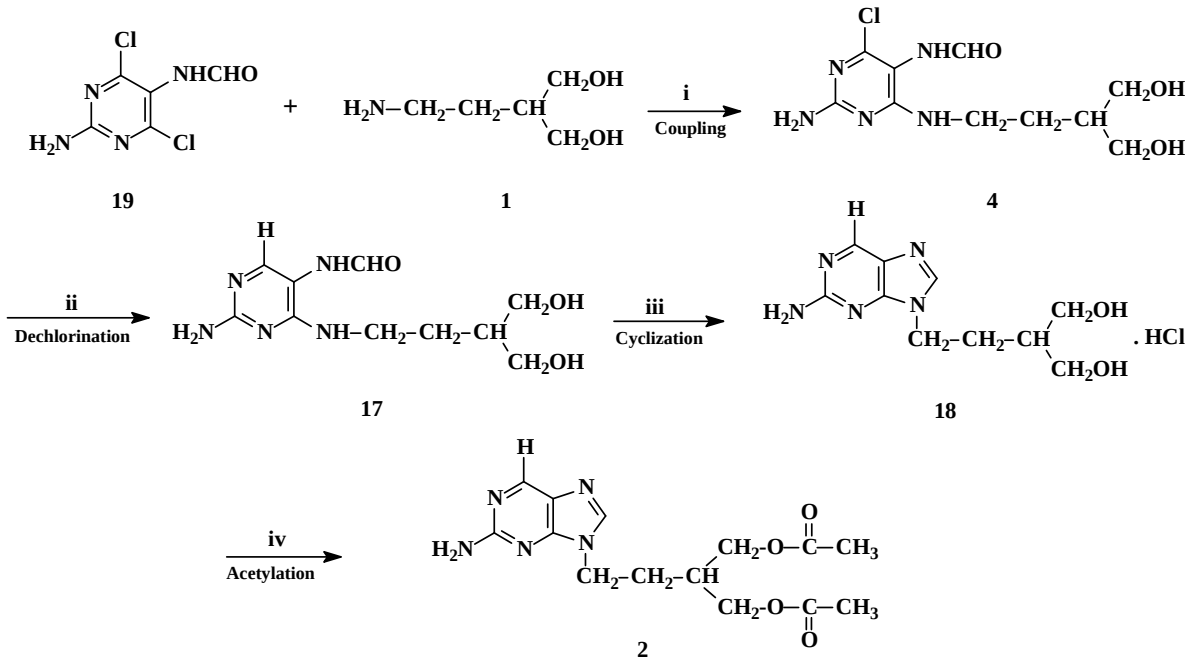


Figure 2

So, there is a need to develop a suitable process for the preparation of **2** to overcome the disadvantages of earlier processes as mentioned above. In this regard, herein we wish to report a novel synthetic method for the preparation of substantially pure Famciclovir **2** through a novel intermediate **4** (**Scheme - 4**) in good yield.

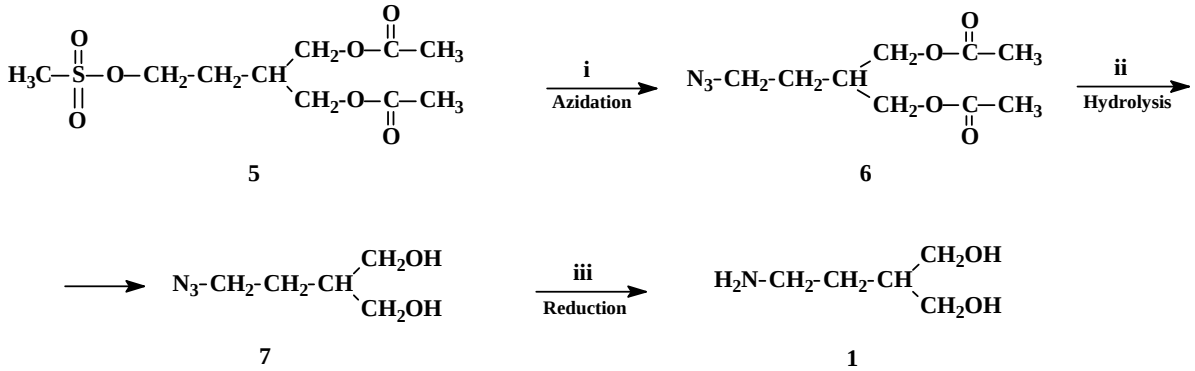


Scheme 4: Novel synthetic scheme of Famciclovir

Reagents and conditions: (i) $NaHCO_3$, EtOH, 75-80°C, 90 min. (ii) TEA, EtOH, 10% Pd/C (50% wet), 50°C, 18 h. (iii) $(C_2H_5O)_3CH$, EtOH.HCl, 40-45°C, 3 h. (iv) CH_2Cl_2, Ac_2O, TEA, 8-10°C, 2 h.

Condensation of 4-amino-2-hydroxymethyl-1-butanol **1** with N-(2-amino-4,6-dichloro-5- pyrimidinyl)formamide **19** in the presence of base gives the novel intermediate N-(2-amino-4-chloro-6-[[4-hydroxy-3-(hydroxymethyl)butyl]amino]pyrimidin-5-yl)formamide **4**, which is first subjected to dechlorination to avoid the formation of 6-ethoxyderivative of purine **15** and to produce exclusively compound (**17**) and then subjected to cyclisation using triethylorthoformate to yielded 2-amino-9-[4-hydroxy-3-(hydroxymethyl)butyl]purine hydrochloride (**18**). Triethylorthoformate is not causing the formation of compound **15** due to lack of chloro group at 6-position of compound **17**. Acetylation of **18** results Famciclovir **2** in good yield and also free from N-7 isomeric impurity **12**. There is no possibility of formation of **12** as one of the nitrogen is already formylated as in **19**. The use of diol **1** in the above synthetic method is preventing the formation of (Pyrrolidin-3-yl)methyl acetate **16,** which is forming when ester **13** is used in the condensation reaction, thereby; increasing the yield of Famciclovir **2**.

Further, we developed a novel synthetic process for the synthesis of 4-Amino-2-hydroxymethyl-1-butanol **1** through new intermediates **6** and **7** from **5,** which is depicted in the Scheme 5. 4-Amino-2-hydroxymethyl-1-butanol **1** is key intermediate in the above novel synthetic method for the preparation of Famciclovir and Penciclovir.



Scheme 5: Novel synthetic scheme of 4-Amino-2-hydroxymethyl-1-butanol 1

Reagent and conditions: (i) DMF, NaN_3, 20-25°C, 8 h. (ii) 20% w/w Aq. NaOH, 25-30°C, 1 h. (iii) 10% w/w Pd/c, H_2, EtOH, 25-30°C, 4 h.

The compound **5** was prepared by known literature procedure [3,6,7] and reacted with sodium azide in N,N-dimethylformamide to obtain azido compound **6**. The azido compound **6** was hydrolyzed by using sodium hydroxide in aqueous ethanol to give intermediate **7.** The intermediate **7** is hydrogenated in the presence of Pd/C in ethanol medium to yield 4-amino-2-hydroxymethyl-1-butanol **1** in excellent yield.

CONCLUSION

We have developed a new, scalable and convenient procedure for the synthesis of 4-amino-2-hydroxymethyl-1-butanol **1**, which is used in the preparation of Famciclovir **2**. The formation of undesired N-7 isomer **12** was eliminated by selecting the right intermediate **19**. The optimization was quite successful with much easier and more reliable operation. Therefore, this process is cost-effective and affords the product in better quality and also provides improved overall yield.

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