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RELATED SUBSTANCES DERIVATION IN SELECTIVE ANTI HEPATITIS C VIRUS AGENT AND THEIR SYNTHESIS

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ABSTRACT

This manuscript aims to illustrate the impurities stimulated during preparation of Propan-2-yl (2S) -2-{[(S)-(pentafluorophenoxy) (phenoxy) phosphoryl] amino} propanoate (compound 2) and their impact on active pharmaceutical ingredient (1).

KEYWORDS

Benzoyl (Bz), Cinnamoyl (Cin), Hepatitis C virus (HCV), HCV NS5B polymerase, Nucleoside Phosphoramidate and Propan-2-yl (2S) -2-{[(S)- (pentafluoro-phenoxy) - (phenoxy) phosphoryl] amino} propanoate.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a major health concern[1] that leads to chronic liver disease[2] in a substantial number of patients. This viral disease is transmitted sexually and parenterally by contaminated blood, blood products, and contaminated needles. Over the last several years, ribonucleotide analogues with a 20-C-methyl substituent have been identified as potent and selective inhibitors[3]. Clark *et al*[4] disclosed the synthesis and biological activity of a fluorinated cytidine analogue, 20-deoxy-20-fluoro-20-C-methylcytidine. As nucleoside phosphoramidates are useful as inhibitors of HCV NS5B polymerase, as inhibitors of HCV replication, active pharmaceutical ingredient (1) chemically known as

(S) -Isopropyl 2- ((S) - (((2R, 3R, 4R, 5R) -5-(2, 4-dioxo3, 4-dihydro-pyrimidin-1 (2H) - yl) -4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2yl) - meth-oxy) - (phenoxy) -phosphoryl-amino) propanoate, was developed by Pharmasset.

It is currently marketed in a tablet form as SOVALDI®, in combination with ledipasvir in HARVONI®, and in combination with Velpatasvir in EPCLUSA®, all by Gilead Sciences, Inc[5]. It is used for the treatment of chronic hepatitis C (CHC) infection, often as a component of a combination anti-viral treatment regimen.

Active pharmaceutical ingredient and a process for its preparation are disclosed in U.S. patent No.7, 964, 580 B2 and PCT Publication No. WO 2008/121634 A2, which are hereby incorporated by reference.

Present study provides the origin and synthesis of isomeric and analogues impurities probable to be present in (S) -Isopropyl 2- ((S) - (((2R, 3R, 4R, 5R) -5- (2, 4-dioxo3, 4-dihydro-pyrimidin-1(2H)-yl) -4- fluoro -3- hydroxy -4- methyltetrahydrofuran-2yl) methoxy) (phenoxy) -phosphorylamino) propanoate.

It is well known that presence of its related compounds in a drug substance can have a significant impact on the quality and safety of the drug product. Therefore, we have studied the impurity profile/control of active pharmaceutical ingredient (API) during its manufacturing and found that the product meets the requirement of general guidelines comfortably as recommended by ICH [4] to qualify the drug substance for acceptable level for known and unknown related compounds respectively.

As described above, compound 3 on reaction with compound 2 yield compound 1. At this juncture, existence of possible isomers in compound 2 may also result in the formation of corresponding isomer of active pharmaceutical ingredient i.e. compound 1.

Compound 2 preparation consists preparation of L-Alanine Isopropyl ester using L-Alanine and isopropyl alcohol, which on further reaction with phenyl dichlorophosphate in presence of triethylamine yields corresponding mono chloro intermediate (in situ), which on reaction with pentafluorophenol produces a mixture of compound 2 and ScRp isomer. Which were isolated by chromatography. Similarly, usage of D-Alanine Isopropyl ester will produce a mixture of compound RcSp and Rc Rp isomer**.** Schematic presentation of the preparation of all possible isomers has been presented below.

In addition of above isomers, presence of analogs in compound 2 inclines to afford a like API analog impurities to contaminate it.

Presence of any alcohol in isopropyl alcohol, a reactant, to prepare L-alanine isopropyl ester tends to give corresponding ester, which can carry through the synthesis to afford equivalent analog of compound 2.

Synthesis of compound 2 involves reaction of phenyl dichloro phosphate with isopropyl ester of L-alanine and subsequently with pentafluorophenol. Any contamination of alcohols in isopropyl alcohol, used in isopropyl ester of L-alanine preparation lead to corresponding L-alanine esters and carry through the synthesis to afford equivalent analogs of compound 2. Analog impurities of compound 2 can originate/ be prepared, by following the procedure of compound 2. However, schematic presentation of analog impurities in compound 2 is presented below.

Analog impurities of compound 2 present in it, can carry through the synthesis to give corresponding analogs of compound-1, shown below

Experimental Section

Unless stated, all reagents and solvents used in this study were commercially available. During development, reactions were monitored by TLC using commercial silica gel plates/HPLC. IR spectra were obtained on *Perkin Elmer Spectrum One FT-IR Spectrometer*. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO d_6/$CDCl_3$ at 300 MHz and 75 MHz on *Bruker 300 MHz Avance NMR spectrometer* with Tetramethylsilane as an internal reference. Mass spectra were recorded on Waters Xevo G2-XS Q-TOF LC/MS/MS system. Or *Agilent 1100 Series LC-MSD-TRAP-SL system.*

General process to prepare compound 2

Stage-I: Preparation of L-Alanine isopropyl ester

N,N-dimethylformamide (10ml) and thionyl chloride (267 g) were added sequentially and slowly to L-Alanine (100g) in isopropyl alochol (100ml) and reaction mass stirred at 67-70°C. After completion, reaction mass was concentrated and obtained residue was taken in methylene dichloride (500ml) and water (500ml) to adjust its pH 9.5-10.5 with ~10% w/v aqueous sodium hydroxide solution. Thereafter, organic layer was separated, washed with water and concentrated to afford L-Alanine isopropyl ester (130g)

Stage-II: Preparation of Compound 2

Under nitrogen atmosphere, to a solution of Phenyl dichloro phosphate (100g) in methylene dichloride (500ml), L-Alanine isopropyl ester (65.3g) was added at -30±3°C. Thereafter, triethylamine (57.5g), 87.2 g of pentafluorophenol in 300 ml of methylene dichloride and again triethylamine (57.5g) were added sequentially and slowly at the same temperature. Stirring was continued at the same temperature to complete the reaction. After accomplishment, temperature was raised to 0 to10°C, diluted with water (500ml) and adjusted the pH to 1.5-2.5 with dilute hydrochloric acid. Thereafter, organic layer was separated, washed with water and concentrated. Obtained concentrated mass was crystallized with Isopropyl acetate / n-heptane mixture to afford 130g of desired compound 2 i.e. (ScSp), by parting undesired ScRp-2 isomer in filtrate, which was isolated from preparative HPLC.

Compound 2 (Sc, Sp)

^{1}HNMR ($CDCl_3$, 300MHz) δ (ppm):1.23-1.27 (6H,m), 5.00-5.08(1H,m), 4.08-4.20 (1H,m), 1.46 (3H,d), 7.19-7.39 (5H,m), 3.94-4.01 (1H,m); ^{13}CNMR ($CDCl_3$, 75MHz), δ (ppm): 21.53, 21.51, 69.49, 172.46(d), 20.81(d), 150.10(d), 120.46(d), 129.75, 125.55, 125.71-125.98(m), 129.99-136.41(m), 136.90-140.63(m), 139.45-143.11(m), IR: (cm^{-1}, KBr): 3228, 3066, 2985, 2941, 1740, 1593, 1527, 1493, 1378, 1266,1207, 1111, 1068, 1024, 1011, 888, 868, 820, 775; m/z 454(M+1).

Compound 2 (Sc, Rp isomer)

^{1}HNMR ($CDCl_3$, 300MHz) δ (ppm): 1.25-1.29(6H,m), 5.01-5.11(1H,m), 4.09-4.22(1H,m), 1.44(3H,d), 7.20-7.40(5H,m), 3.97-4.04(1H,m); ^{13}CNMR ($CDCl_3$, 75MHz) δ (ppm), 21.61, 69.69, 172.33(d), 50.59, 21.04(d), 150.24(d), 120.04(d), 129.86, 125.63, 125.49-125.95(m), 129.52-136.46(m), 137.09-140.86(m), 139.60-143.21(m), IR:(cm^{-1}, KBr): 3201, 3059, 2987, 2941, 1736, 1592, 1524, 1491, 1475, 1440, 1377, 1266,1235, 1153, 1141,1107, 1066, 1026, 1012, 890, 862, 819, 774, m/z 454(M+1).

Process to prepare Compound 2 Rc, Rp and Rc, Spisomers

Compound 2 Rc, Rp isomer was prepared by using D-Alanine instead of L-Alanine and by following the general process of compound 2, whereas Rc, Sp isomer, parting in filtrate, was isolated from preparative HPLC.

Rc, Rp isomer of compound 2

^{1}HNMR ($CDCl_3$, 300MHz) δ (ppm), 1.14-1.28 (6H,m), 4.90-5.13(1H,m), 3.87-4.22(2H,m), 1.43-1.47(3H,m), 7.01-7.40(5H,m), ^{13}CNMR ($CDCl_3$, 75MHz) δ (ppm): 21.52,21.55(m), 69.62, 172.31(d), 50.55(d), 20.89(d), 150.12(d), 119.97(d), 129.79, 125.58, 128.97-129.50(m), 132.44-136.20(m), 137.18-140.54(m), 139.57-143.14(m), IR(cm^{-1}, KBr), 3423, 3218, 2986, 2942, 1737, 1594, 1520, 1493, 1377, 1263, 1219, 1154, 1108, 1069, 866, 819, 774, 758, m/z 454(M+1).

Rc, Sp isomer of compound 2

^{1}HNMR (DMSO, 300MHz) δ (ppm), 1.17-1.32(6H,m), 5.02-5.10(2H,m), 4.12-4.20(1H,m), 1.47(3H,d), 7.14-7.41(5H,m), 3.96-4.03(1H,m), ^{13}CNMR (DMSO 75MHz) δ (ppm), 21.54, 21.56, 69.54, 172.46(d), 50.57, 20.85(d), 150.08(d), 120.00(d), 129.79(s), 125.59(s), 125.93-126.77(m), 130.21-136.46(m), 137.25-143.09(m), 139.52-144.67(m), IR:(cm^{-1}, KBr), 3434, 3232, 3067, 2985, 2941, 1740, 1593, 1521, 1493, 1478, 1438, 1378, 1266, 1207, 1154, 1111, 1068, 1024, 1011, 888, 868, 820, 775, m/z: 454(M+1).

Methyl (2*S*)-2-{[(*S*) - (pentafluorophenoxy) (phenoxy) phosphoryl] amino} propanoate (Methyl analog of Compound 2)

^{1}HNMR ($CDCl_3$, 300MHz) δ (ppm), 3.75(3H,s), 4.16-4.24(1H,q), 1.41-1.49(3H,m), 7.02-7.39(2H,m), 7.02-7.39(2H,m), 7.02-7.39(1H,m), 3.94-4.02(1H,m),^{13}CNMR ($CDCl_3$, 300MHz) δ(ppm): 52.66(s), 173.39(d), 50.41(d), 20.80(d), 150.05(d), 119.97(d), 129.81, 125.65, 120.32-120.60(m), 122.96-132.74(m), 135.94-140.55(m), 136.79-143.11(m); IR:(cm^{-1}, KBr), 3439, 3229, 1735, 1633, 1593, 1522, 1493, 1458, 1437, 1376, 1263, 1223, 1153, 1137, 1071, 1025, 997, 822, 774, m/z: 426(M+1).

Ethyl (2*S*) -2- {[(*S*)- (pentafluorophenoxy) (phenoxy) phosphoryl] amino}propanoate (Ethyl analog of Compound 2)

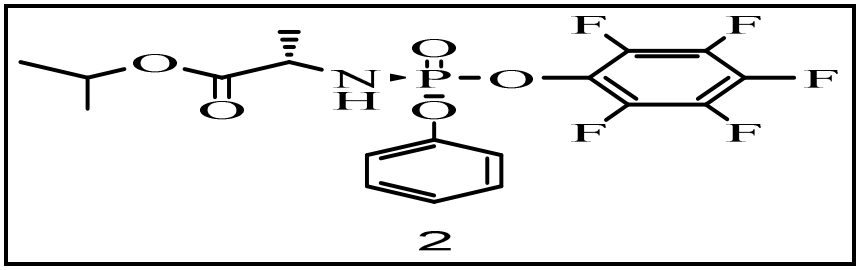
^{1}HNMR ($CDCl_3$, 300MHz) δ (ppm): 1.27(3H,t), 4.14-4.23(3H,m), 1.47(3H,d), 7.19-7.39(5H,m), 3.91-3.98(1H,m), ^{13}CNMR ($CDCl_3$, 75MHz) δ (ppm): 14.01, 61.78, 172.93(d), 50.47(d), 20.85 (d), 150.10(d), 120.00(d), 129.79, 125.59, 128.99-129.40(m), 136.01-139.74(m), 136.99-140.73(m), 139.52-143.08(m), IR:(cm^{-1}, KBr): 3215, 2991, 1737, 1593, 1522, 1492, 1375, 1316, 1266, 1204, 1153, 1135, 1069, 1026, 1014, 909, 858, 821, 774, m/z: 440(M+1).

Propyl (2*S*) -2- {[(*S*) - (pentafluorophenoxy) (phenoxy) phosphoryl] amino} propanoate (n-Propylanalog of Compound 2)

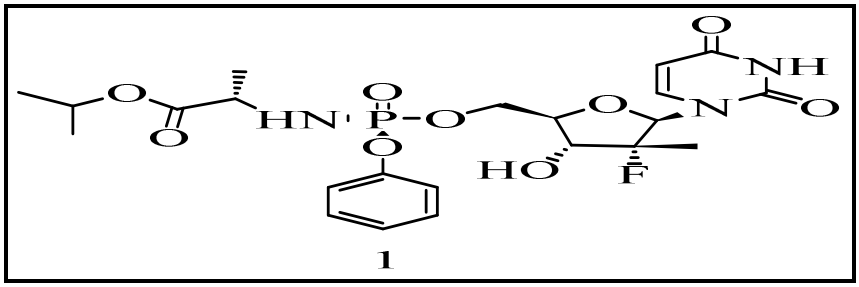
^{1}HNMR ($CDCl_3$, 300MHz) δ (ppm): 0.94(3H,t), 1.58-1.73(2H,m), 4.10(2H,t), 4.16-4.24(1H,m), 1.48(3H,d), 7.14-7.39(5H,m), 3.96-4.04(1H,m), ^{13}CNMR ($CDCl_3$, 75MHz) δ (ppm), 10.21, 21.83, 67.40, 173.03(d), 50.49(d), 20.02(d), 150.07(d), 120.01(d), 129.84, 125.66, 120.04-120.44(m), 129.02-136.21 (m), 129.49-139.73(m), IR:(cm^{-1}, KBr): 2969, 1747, 1596, 1522, 1494, 1384, 1330, 1265, 1199, 1155, 1070, 1026, 997, 908, 823, 774, m/z: 454(M+1).

2-Methylpropyl (2*S*) -2- {[(*S*)- (pentafluorophenoxy) (phenoxy) phosphoryl] amino} propanoate (Isobutyl analog of Compound 2)

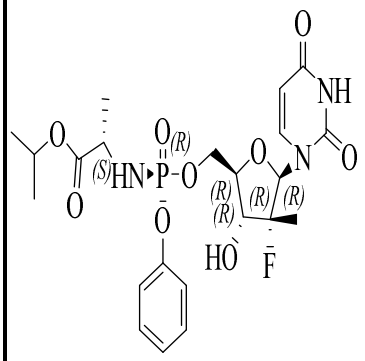
^{1}HNMR ($CDCl_3$, 300MHz) δ (ppm): 0.93 (3H,d), 1.90-2.01(2H,m), 3.88-4.02(2H,m), 4.14-4.25(1H,m), 1.49(3H,d), 7.19-7.39(5H,m), 3.88-4.02(1H,m), ^{13}CNMR ($CDCl_3$, 75MHz) δ (ppm): 18.89, 27.66, 71.81, 173.00(d), 50.50(d), 21.08(d), 150.10(d), 120.02(d), 129.84, 125.65, 129.54-129.97(m), 135.97-139.87(m), 136.94-140.54(m), 139.35-143.14(m), IR:(cm^{-1}, KBr): 3222, 2963, 1749, 1593, 1559, 1521, 1494, 1383, 1329, 1266, 1195, 1154, 1137, 1068, 1026, 1013, 909, 820, 775, m/z:468(M+1).



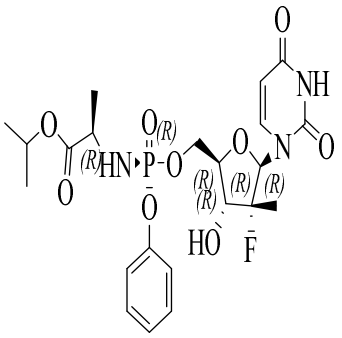
2



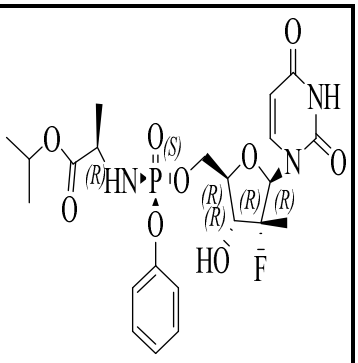
1



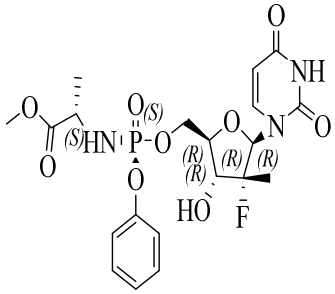
Sc,Rp-isomer of **1**



Rc,Rp-isomer of **1**

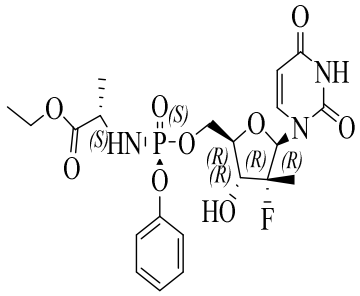


Rc,Sp-isomer of **1**



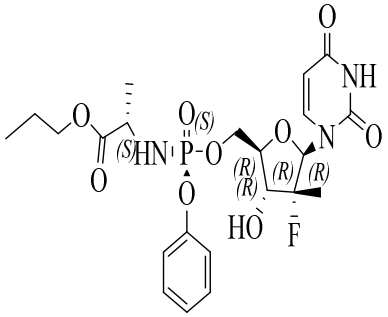
(2S)-Methyl 2-((S)-(((2R,3R,4R,5R)-5-(2,4-dioxo3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2yl)-methoxy)-(phenoxy)-phosphorylamino) propanoate

Compound I



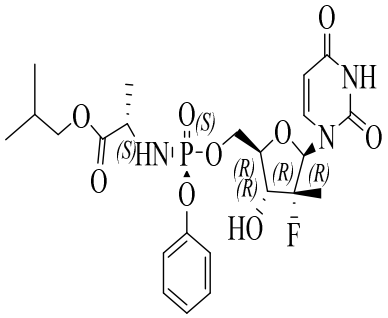
(2S)-Ethyl 2-((S)-(((2R,3R,4R,5R)-5-(2,4-dioxo3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2yl)-methoxy)-(phenoxy)-phosphorylamino) propanoate

Compound J



(2S)-n-Propyl 2-((S)-(((2R,3R,4R,5R)-5-(2,4-dioxo3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2yl)-methoxy)-(phenoxy)-phosphorylamino) propanoate

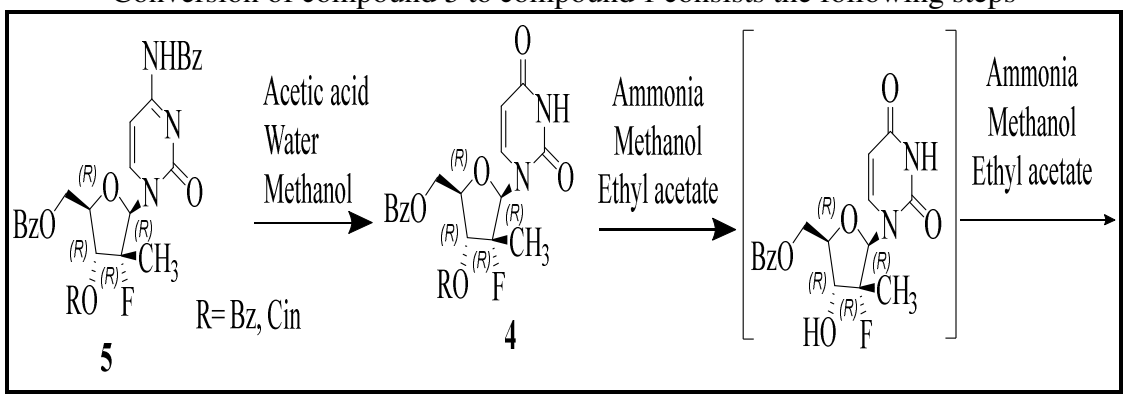
Compound K

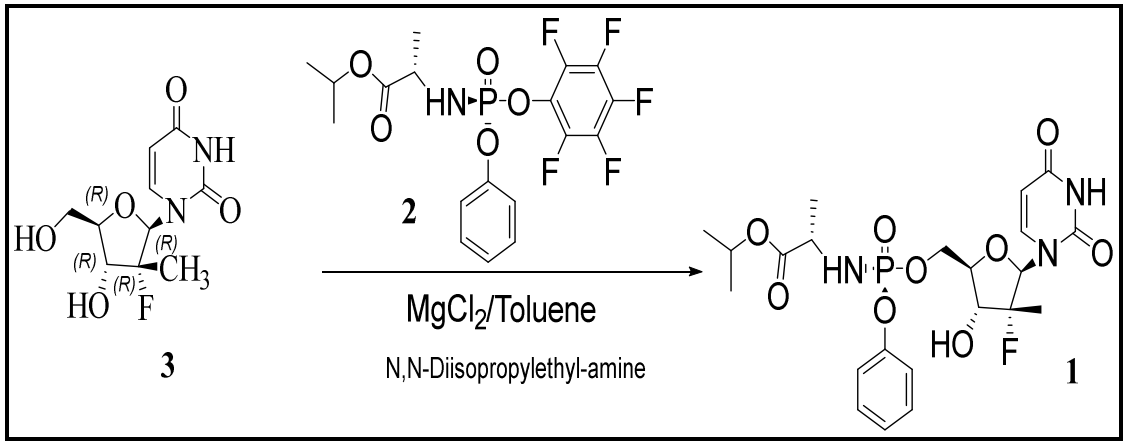


(2S)-Isobutyl 2-((S)-(((2R,3R,4R,5R)-5-(2,4-dioxo3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2yl)-methoxy)-(phenoxy)-phosphorylamino) propanoate

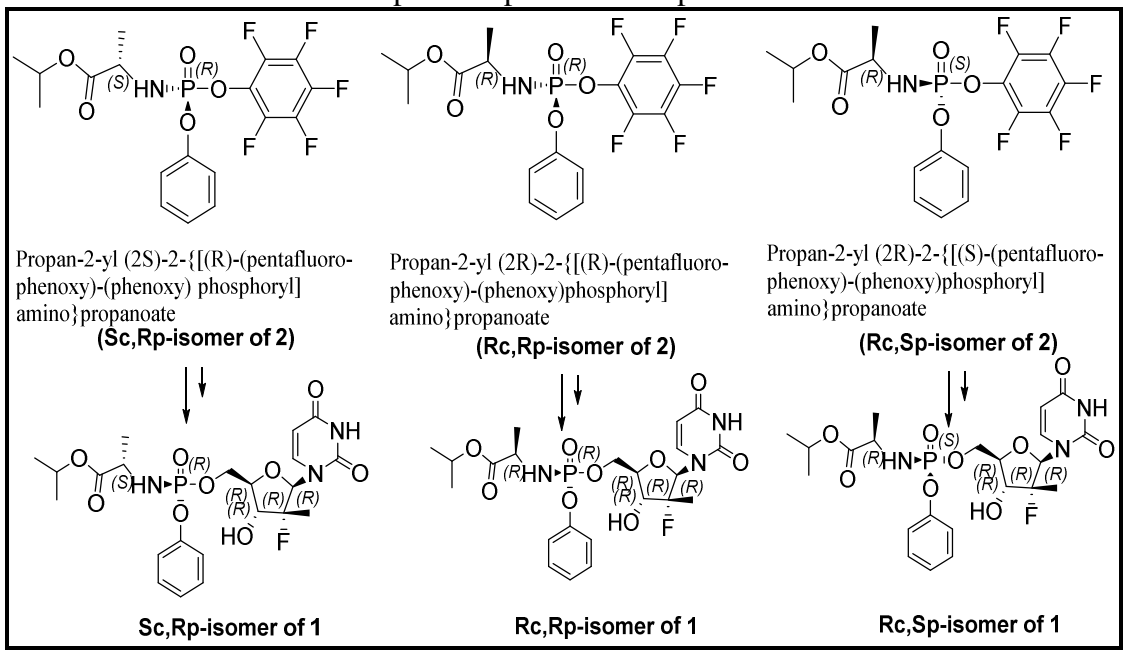
Compound L

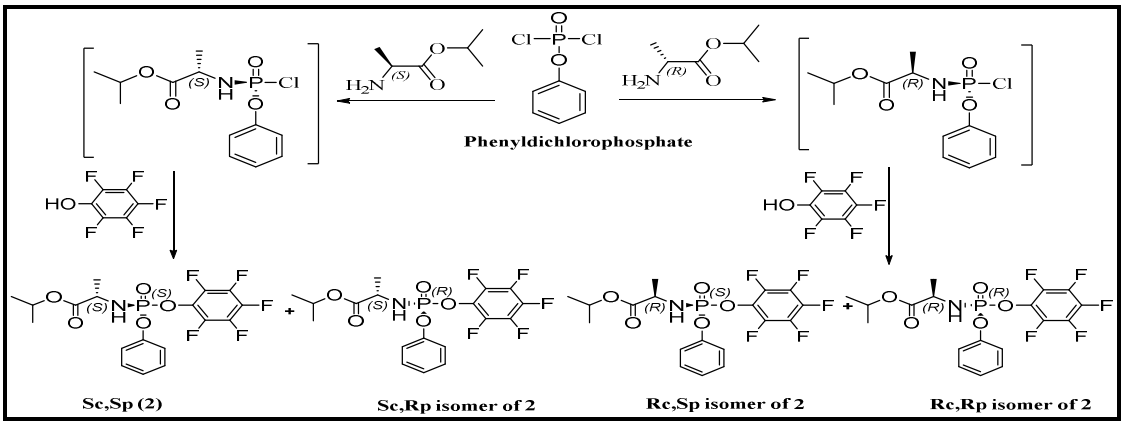
Conversion of compound 5 to compound 1 consists the following steps

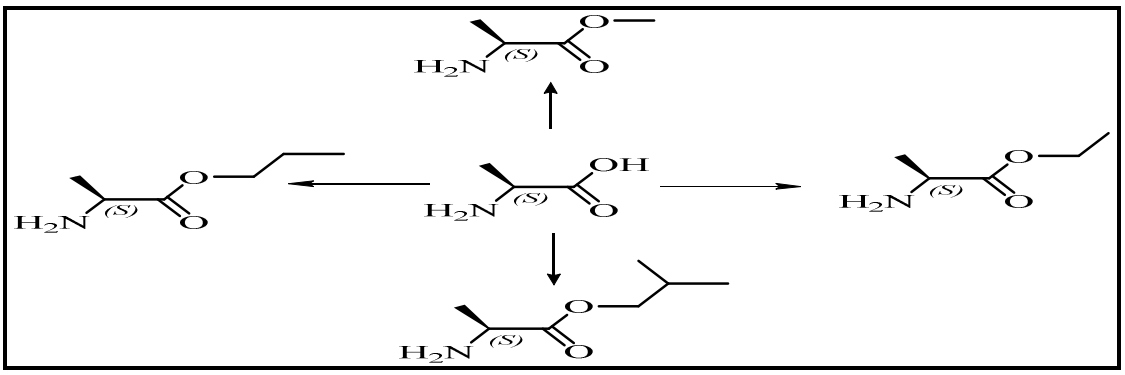


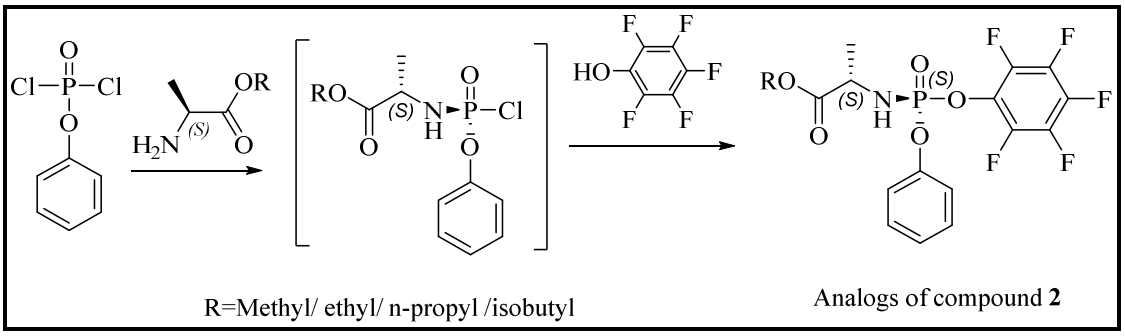


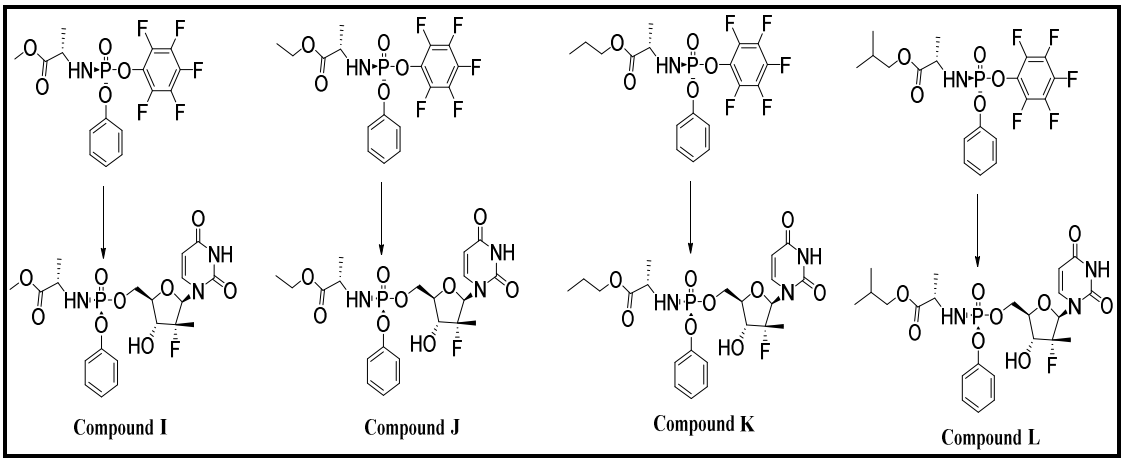
Compound 5 is hydrolyzed by heating, in presence of acetic acid to afford compound 4, which on basic hydrolysis with ammonia in methanol yields compound 3. Finally, compound 3, on condensation with compound 2 provides compound 1.




Phenyldichlorophosphate
Sc,Sp (2)
Sc,Rp isomer of 2
Rc,Sp isomer of 2
Rc,Rp isomer of 2




R=Methyl/ ethyl/ n-propyl /isobutyl
Analogs of compound **2**


Compound I
Compound J
Compound K
Compound L

CONCLUSION

It is clear from above study that analogous impurities and isomeric impurities should be circumscribed in compound 2 to evade the presence of their corresponding related substances in Compound 1 specifications.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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