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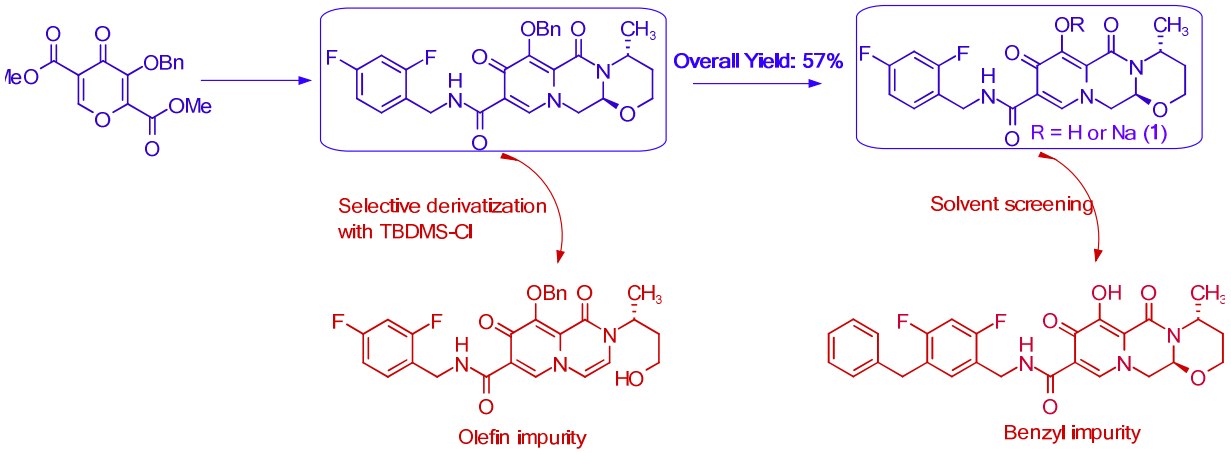


Identification and control of critical process impurities: An improved process for the preparation of dolutegravir

ABSTRACT

A four-stage manufacturing route for the preparation of dolutegravir sodium **(1)** was assessed and optimized leading to a higher yielding, simpler and scalable process. Key improvements in the process include the development of mild work-up procedure by selective derivatization of difficult to remove process impurity using *tert*-butyldimethylsilyl chloride. Metal based hydrogenation free *O*-debenzylation is optimized and the critical isomeric impurity formed was identified and eliminated from the process by establishment of proper control strategy.

KEY WORDS: Dolutegravir sodium, *O*-debenzylation, Silylation, Process impurities.

INTRODUCTION

Inhibition of HIV integrase, the enzyme that mediates the integration of the viral DNA into the host cell genome, can halt the further spread of HIV and represents a viable treatment for HIV infection.[1] In this context, integrase strand transfer inhibitors (INSTi's) have become an attractive drug target over the past few years.[2] Dolutegravir **1** alongside raltegravir and elvitegravir (Figure 1) are metal-binding INSTi's approved for the treatment of antiretroviral therapy.[3] Dolutegravir is particularly advantageous due to low 50 mg dosing given once daily without pharmacokinetic boosting and it's display of antiviral activity in multiple cell-types and cell-based assay formats.[4] Synthesis of dolutegravir is well documented in the literature starting from routes employed during initial structure-activity relationship findings of a series of carbamoyl pyridone heterocycles to improved process for scale-up.[5] The synthetic route based on a densely functionalized pyridinone core as starting material for constructing dolutegravir is by far the most impressive synthetic route available compared to tedious route based on inexpensive maltol.[6] Though numerous methods of constructing substituted pyranone and further converting to dolutegravir is reported, an improved process with detailed impurity profiling and subsequent control strategy is not yet reported. Herein we report identification of critical process impurities observed during the laboratory optimization of dolutegravir sodium. An improved process with appropriate control strategies to reduce the level of these impurities in the final API is described.

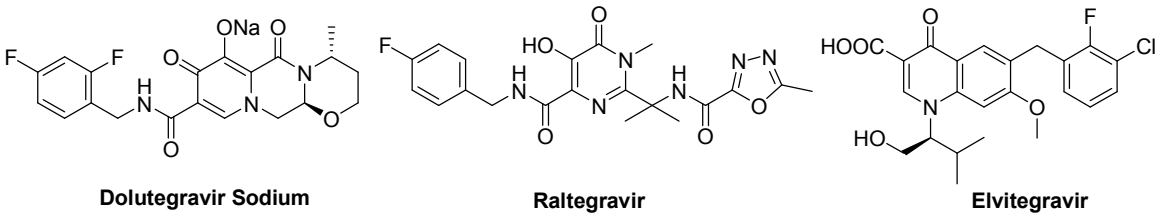
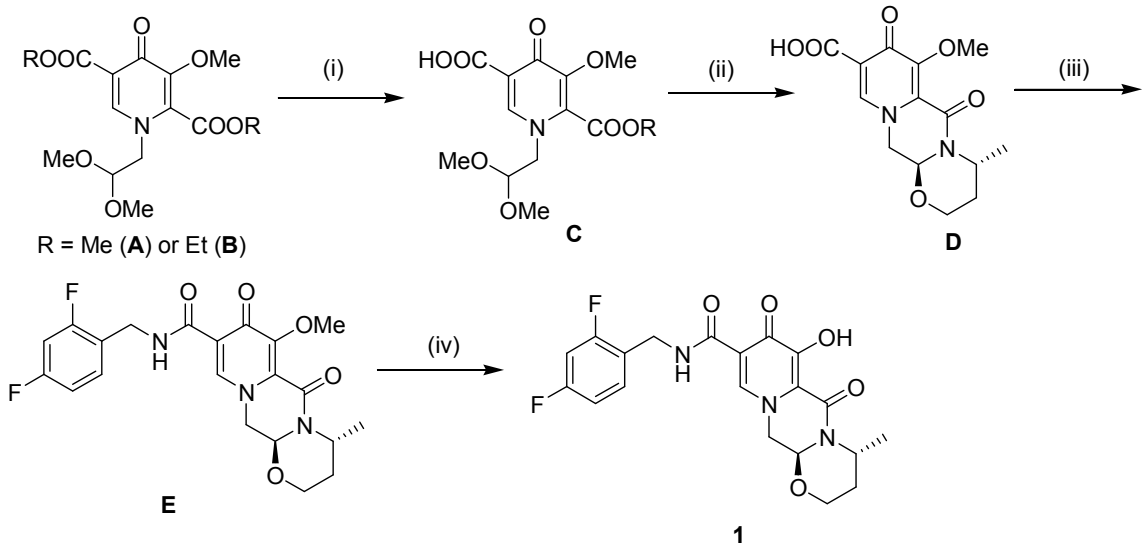


Figure 1 Metal-binding integrase strand transfer inhibitors for antiretroviral therapy

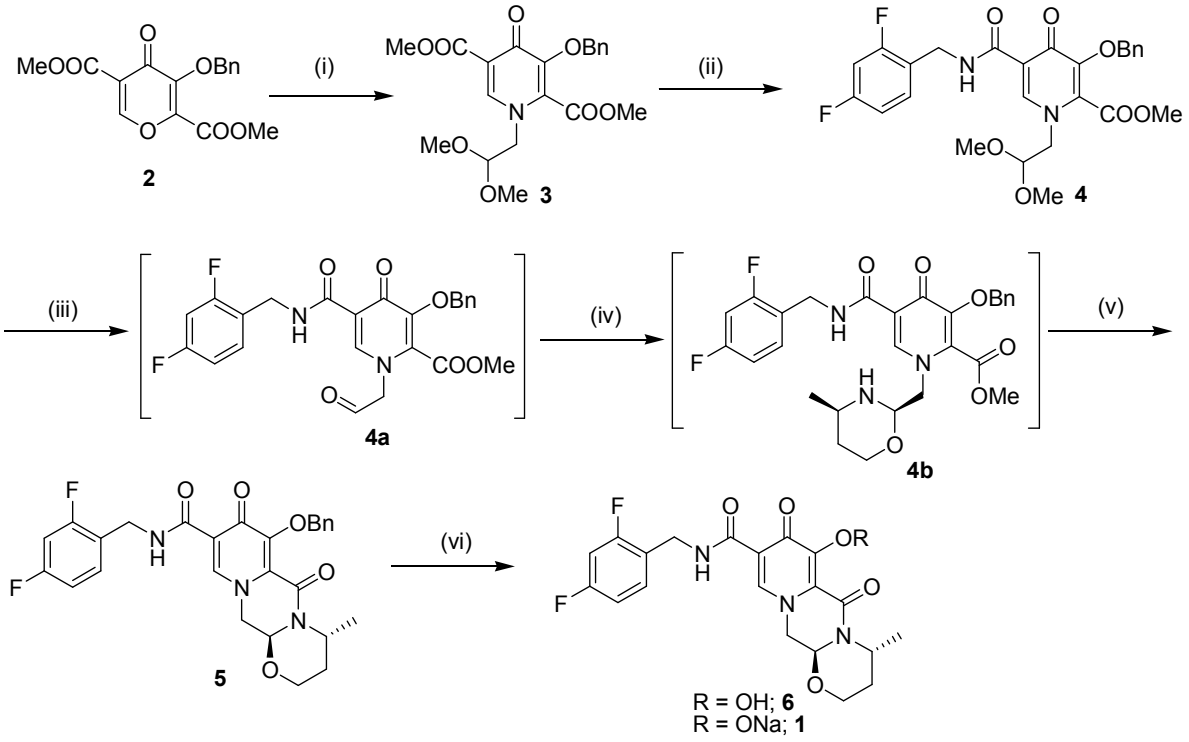
RESULTS AND DISCUSSION

The synthetic route taken up for lab optimization is adopted from innovator's patent application where different schemes are disclosed and demonstrated on milligram scale.[7] In a subsequent prior-art, one of these routes is demonstrated at higher scales (Scheme 1) and appears to be scalable.[8] The key feature of this route was selective hydrolysis of diester raw material **B** to a half-ester **C** which allows for easier isolation and purification by salt formation with the carboxylic group in the molecule. The phenolic moiety of the intermediates (**B**, **C** and **D**) was masked as methyl ether which was liberated to the free phenol at the last stage by the action of Lewis acids. The alternate easily removable benzyl protection of phenolic group is not opted due to a possible defluorination side-reaction during deprotection under catalytic hydrogenation. We observed that this selective hydrolysis route did however did not appear to be reproducible and high-yielding in our hands (Table-S1, supporting information). Therefore alternatively reported mono-amidation of diester using 2,4-difluorobenzylamine was explored and identified to be comparatively easier, reproducible and scalable. The finalized route of synthesis starting from benzyl protected pyranone, dimethyl 3-(benzyloxy)-4-oxo-4*H*-pyran-2,5-dicarboxylate **2** and based on regioselective mono amidation is shown in scheme 2.



Scheme 1 Reported route for the synthesis of Dolutegravir sodium: (i) LiOH; (ii) (a) MsOH, AcOH; (b) 3*R*-aminobutan-1-ol; (iii) CDI, 2,4-difluorobenzylamine; (iv) LiCl or $MgCl_2$

Accordingly, the starting material pyran **2** was ring-opened with aminoacetaldehyde dimethyl acetal and then cyclized back forming pyridinone **3** which reacted selectively with 2,4-difluorobenzylamine to form amidoester **4**. Acetal hydrolysis followed by reaction with 3*R*-aminobutan-1-ol in the presence of acid led to chiral hemiaminal **4b** that undergoes critical substrate controlled diastereoselective cyclization[9] to form benzyl protected dolutegravir intermediate **5**. *O*-Debenzylation of **5** under acidic conditions followed by salification using aqueous sodium hydroxide resulted in dolutegravir sodium.



Scheme 2 Optimized route of synthesis of Dolutegravir sodium: (i) Aminoacetaldehyde dimethyl acetal, DIPEA, MeOH, 25°C, 85%; (ii) Toluene, AcOH, 2,4-difluorobenzylamine, 90°C, 85%; (iii) MsOH, AcOH, ACN, 65°C; (iv) 3*R*-aminobutan-1-ol; 25°C; (v) (a) 65°C; (b) MeOH, 25°C, 78%; (vi) (a) TFA, DCM, 35°C; (b) Toluene, 80°C; 90% (c) aq. NaOH, MeOH, 65°C, 95%.

The yields of all the stages were low to moderate during the initial optimization either due to lower conversion or due to formation of more impurities. The first stage namely ring opening of **2** with aminoacetaldehyde dimethyl acetal proceeded instantaneously to form a mixture of acyclic intermediates,[10] which cyclised to **3** in varying yields and purity depending upon the conditions employed. A preliminary screening of solvents (ester, chlorinated, hydrocarbon and protic solvents) pointed out that the reaction was efficient in alcoholic solvent, especially methanol (Table-S2, supporting information). When performed in methanol at elevated temperatures the reaction was faster but generated more impurities and those performed at room

temperature did not go to completion. The use of sodium sulfate (for trapping water) and acid catalysts showed marginal improvement in yield and purity. Surprisingly addition of base to the reaction mass conveniently improved the yield and minimized the formation of impurities during the cyclization of acyclic intermediates. Due to the homogeneous nature of reaction mass and better control of water content of bases employed, organic bases, preferably diisopropylethylamine was employed instead of inorganic bases (sodium bicarbonate) in the final optimized process. **Imp-1** and **imp-2** (Figure 2) are major impurities formed in stage-1 at varying levels under different conditions screened (Table-S3, supporting information). Under the optimized conditions, these non-critical impurities are well-controlled and intermediate **3** was formed in very good yield. Additionally **3** could be conveniently crystallized from the reaction mass after the regular work-up using toluene, xylene, methanol or isopropanol.

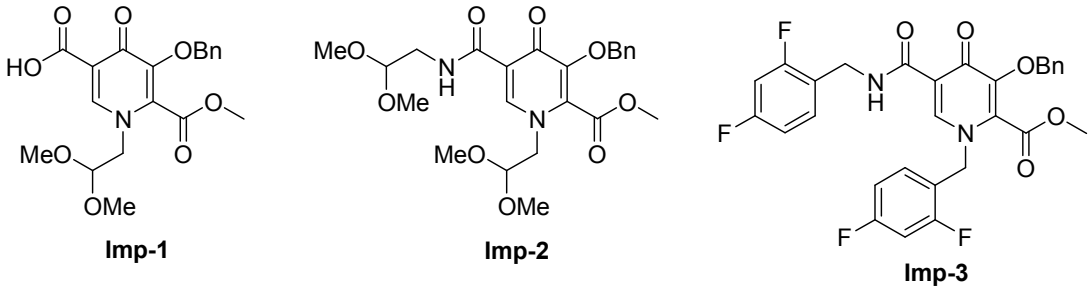


Figure 2 Structures of process impurities formed in stage-1 of the process

However in view of a higher and clean conversion of **3** (typically around 90-93% area by HPLC) and to evade material loss due to isolation, an *in situ* conversion of **3** to **4** was investigated. Accordingly the reaction mass after work-up was distilled off, exchanged with fresh solvent and reacted with 2,4-difluorobenzylamine in presence of acetic acid. A preliminary screening of solvents pointed out the necessity of high temperature for reaction completion and toluene was identified as the solvent of choice for the transformation. The reaction completion was however

observed to be always be inconsistent and required higher mole equivalents of 2,4-difluorobenzylamine which in turn generated more of **imp-3**. Attempts including the use of higher equivalents of acetic acid and portion-wise addition of acetic acid were not beneficial in driving the reaction to completion. This bottle-neck was overcome by portion-wise dosing of 2,4-difluorobenzylamine towards the termination of the reaction. This modified process terminated the reaction consistently at various scales employed. The next attempt was to improve the purity of intermediate **4** by effective crystallization (Table-S4, supporting information). The crude reaction mass was highly soluble in most of the solvents and did not crystallize out easily. After several attempts, effective and practical crystallization of **4** was achieved in isopropyl alcohol that enabled the removal of many impurities along with **imp-3**.

The next stage involving the construction of the tricyclic ring system was investigated in detail due to the presence of many in situ intermediates (**4a** and **4b**) and formation of a second chiral center. Therefore hydrolysis of acetal **4** to aldehyde **4a**, its further reaction with 3*R*-aminobutan-1-ol to form hemiaminal **4b** and its diasteroselective cyclisation to benzyl dolutegravir **5** were studied individually to afford control on chiral and process impurities.

Acid hydrolysis of **4** to aldehyde **4a** was faster in aqueous acids and slower with anhydrous acidic conditions (Table-S5, supporting information). The yield in most of the cases was limited by competitive side reactions.[5d] In the presence of formic acid and sulphuric acid, hydrolysis was faster but generated more impurities upon hold-up of reaction mass which may turn troublesome during scale-up. The use of formic acid-methanesulfonic acid in place of sulphuric acid also produced lower yields. Incomplete reaction and lower yield was observed when using excess of methanesulphonic acid under anhydrous conditions. Hydrolysis in the presence of sulphonic

acids like pyridinium *p*-toluenesulfonate (PPTS) and tosylic acid did not provide encouraging results. Heteropoly acid like phosphomolybdic acid turned the reaction mass highly colored without any yield improvement and impurity reduction. The use of a catalytic amount of methanesulfonic acid in acetonitrile gave a comparatively cleaner reaction at lower temperature (50-70^{o}C) but did not go to completion. Raising the reaction temperature to reflux resulted in completion of the reaction but generated more impurities with lower product yield. In most of the cases, the major side reaction was identified to be formation of *O*-debenzylated hemiacetal or lactol impurities (**imp-4** and **imp-5**) caused by the internal cyclisation of the hemiacetal or aldehyde hydrate moiety with the ester functionality. Fine tuning of the reaction parameters (temperature of reaction mass and mole equivalents of methanesulfonic acid; Refer to the experimental section) alongside portion-wise dosing of methanesulfonic acid afforded clean conversion of **4** to **4a** with minimum of **imp-4** and **imp-5** (typically less than 1.0%).

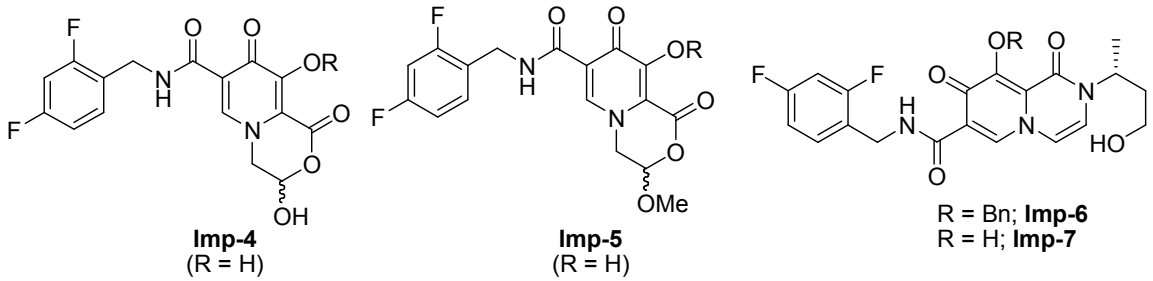
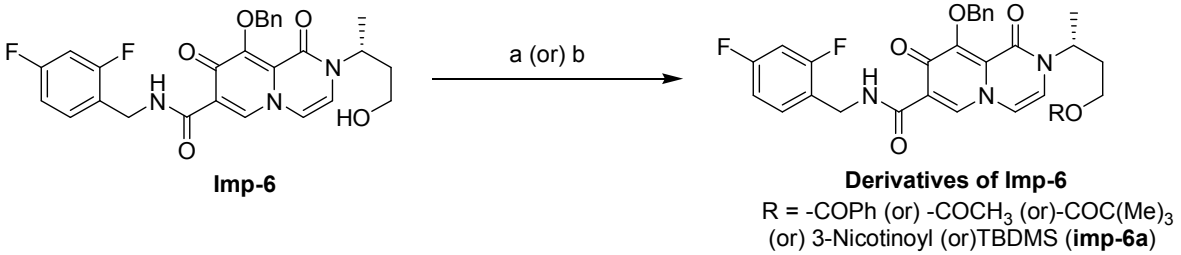


Figure 3 Structures of process impurities formed in stage-2 of the process

Isolation of intermediate **4a** was not attempted due to unstable nature of the aldehyde functionality and the accomplishment of a cleaner hydrolysis prompted for in situ reaction with 3*R*-aminobutan-1-ol at ambient temperature. Slow addition of 3*R*-aminobutan-1-ol formed an imine which converted to hemiaminal **4b** instantaneously. Further cyclisation of hemiaminal **4b** with the ester functionality to generate tricyclic core bearing second chiral center of benzyl dolutegravir **5** was studied by varying the temperature of cyclisation, the nature of reaction

conditions (by externally adding acid or base), the water content of reaction mass and the addition of catalyst (Table-S6, supporting information). Optimized condition (refer to experimental section) afforded **5** containing around 0.8-1.0% of the unwanted diastreomer. Further optimization to control diastereomer during the reaction was not explored since this impurity was removed easily below the desired ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) limit in the downstream process. The main process impurity of concern that formed in all of the attempted experiments was **imp-6**. For a structurally similar congener, competitive dealkylation of the ether moiety in ring under acidic condition was reported as main source of this impurity. Assuming that an acidic nature and temperature of the reaction mass to trigger this side-reaction, different experimental parameters were explored like neutralizing reaction mass (containing methanesulfonic acid) with various bases before further reaction with 3*R*-aminobutan-1-ol, reverse mode addition *viz* reaction mass added dropwise to 3*R*-aminobutan-1-ol, isolation of *in situ* intermediates (**4a** and **4b**) before further reaction and modifying the temperature of reaction mass. Unfortunately none of the trials controlled the formation of **imp-6**. The second obvious strategy opted was purification of the isolated intermediate **5** in various solvent medium (single and binary combination). Only a marginal reduction of impurity was observed irrespective of purifications employed. Most importantly, the removal of this impurity was identified as being critical as it reacted in subsequent stages and the corresponding debenzylated impurity (**imp-7**) was carried forward to active pharmaceutical ingredient (API) at same levels without any further purging. Similarly purification attempts at the API and intermediate stages did not assist in removing **imp-7**. After failing with regular methods of removing **imp-6**, control strategy based on chemoselective derivatization of the **imp-6** was investigated. **Imp-6** has a distinct primary

alcohol group which can selectively be functionalised without disturbing the main molecule. The main challenge was to identify a derivatization method that should be simple enough to implement on production scale and most importantly the resulting derivative of **imp-6** should be effectively removed from the system by the existing downstream process. Commercial acid chlorides like benzoyl chloride, acetyl chloride and pivaloyl chloride readily formed the corresponding ester derivative of **imp-6**; but further removal did not happen as expected. Therefore nicotinyl chloride was attempted as a substitute on the basis of assumption that the nicotinyl ester of **imp-6** can be effectively removed by simple acid base work-up. Unfortunately the derivatization reaction was not successful and complete derivatization of the impurity was found to be highly inconsistent. Finally the more facile silylation of the primary alcohol was identified as a derivatization strategy. Due to lesser stability of trimethylsilyl chloride, commercially available *tert*-butyldimethylsilyl chloride (TBDMSCl) was selected as a appropriate reagent for selective reaction. The reagent system as expected, demonstrated successful derivatization followed by complete removal of the impurity formed even at higher levels.



Imp-6

Derivatives of Imp-6

R = -COPh (or) -$COCH_3$ (or)-$COC(Me)_3$ (or) 3-Nicotinoyl (or)TBDMS (**imp-6a**)

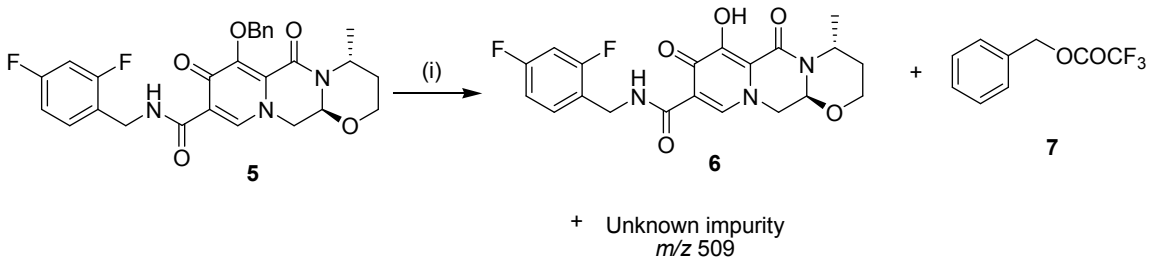
Scheme 3 Attempted derivatization of **imp-6** in stage-2: (a) BzCl or AcCl or PivCl or 3-Nicotinoyl chloride, TEA; (b) Imidazole, TBDMSCl

The derivatization step was implemented as a part of the workup procedure and integrated with the main process. Accordingly, the reaction mass was distilled off after the completion of the

reaction, taken up in dichloromethane-water to remove reagents and by-products and treated with imidazole and TBDMSCl at ambient temperature for the selective derivatization of **imp-6** to **imp-6a**. The derivatization was found to be instantaneous and after a second aqueous work-up, the crude reaction mass was crystallized in methanol to afford pure **5** that is free of **imp-6a**. The use of other common bases (triethylamine, diisopropylethylamine and potassium carbonate) was not as effective as imidazole for the derivatization reaction. The identified strategy permitted for an easy scalable process demonstrating strong impurity control and overall yield of 70% (conversion of **4** to **5**).

The final chemical stage of the process is the *O*-debenzylation of **5** to form the free acid of dolutegravir. Most of the literature methods use the conventional catalytic hydrogenation in the presence of Pd/C. Two main drawbacks in implementing catalytic hydrogenation was the poor solubility of the starting material and product in most of the common solvents employed for hydrogenation and the potential aromatic defluorination side-reactions generating isomeric desfluoro impurities that are extremely difficult to remove by purification. Different acidic and basic reagents were alternatively screened for establishing simple and clean conversion (Table-S7, supporting information). Brønsted acids like methanesulphonic acid, tosylic acid and trifluoroacetic acid (neat) completed the debenzylation reaction but generated more of **imp-7** which was difficult to remove by purification. No conversion was observed with mild acid like PPTS. Lewis acids like LiBr gave quick reaction but formed bromide impurities (by LCMS) as by-products. LiCl and $MgCl_2$ reactions were slower and did not go to completion. The reaction completed in the presence of sodium hydride as reagent and formed dolutegravir sodium directly but generated more of desfluoro impurity and **imp-7**. After screening the above-said reagents at different concentrations, it was found that 5.0 mole equivalent of trifluoroacetic acid (TFA) in

dichloromethane gave clean conversion (Scheme 4). Attempts to reduce mole equivalents of TFA again gave incomplete conversion. Reactions at various scales were consistent in terms of conversion, yield and impurity-profile using 5.0 mole equivalents of TFA (Table-S8, supporting information). Similarly no rise in olefin impurity (**imp-7**) was observed under these conditions as against observed using neat TFA. After the regular acid base workup, the by-product of the reaction, benzyl trifluoroacetate (**7**) was removed by recrystallization in toluene and the pure dolutegravir was isolated in 86% yield.



Scheme 4 O-Debenzylation of 5 to 6: (i) (a) TFA, DCM; (b) Toluene.

Though all the known impurities are controlled during the debenzylation stage, one unknown impurity with m/z 509 (**imp-8**) matching the molecular weight of benzyl dolutegravir **5** was observed at a level of 0.06-0.08% during the purity analysis by HPLC. The impurity formed a sodium salt and was carried forward to the final API at the same level. The identification of this unknown impurity therefore became critical to understand the origin and its appropriate control. The formation of the sodium salt by the impurity indicated a structure similar to API, however a mass similar to benzyl dolutegravir with a different HPLC retention time indicate that an additional benzyl group is attached at some part of the API molecule. Initially a rearrangement of **5** was assumed involving a [3,3]-sigmatropic rearrangement of *O*-benzyl group to form **8** in the presence of Bronsted acid, TFA. Though the rearrangement product has identical mass, it does not have any phenolic group to form a sodium salt. Also the impurity was not formed

significantly under conditions like Lewis acid ($AlCl_3$, $BF_3.OEt_2$) and thermal reaction (250^oC) that are favorable to [3,3]-sigmatropic rearrangement. A pure sample of the impurity isolated using preparatory HPLC followed by structural characterization revealed that no sigmatropic rearrangement occurred and indeed the benzyl group was attached to 2,4-difluorobenzyl moiety (Figure 4).

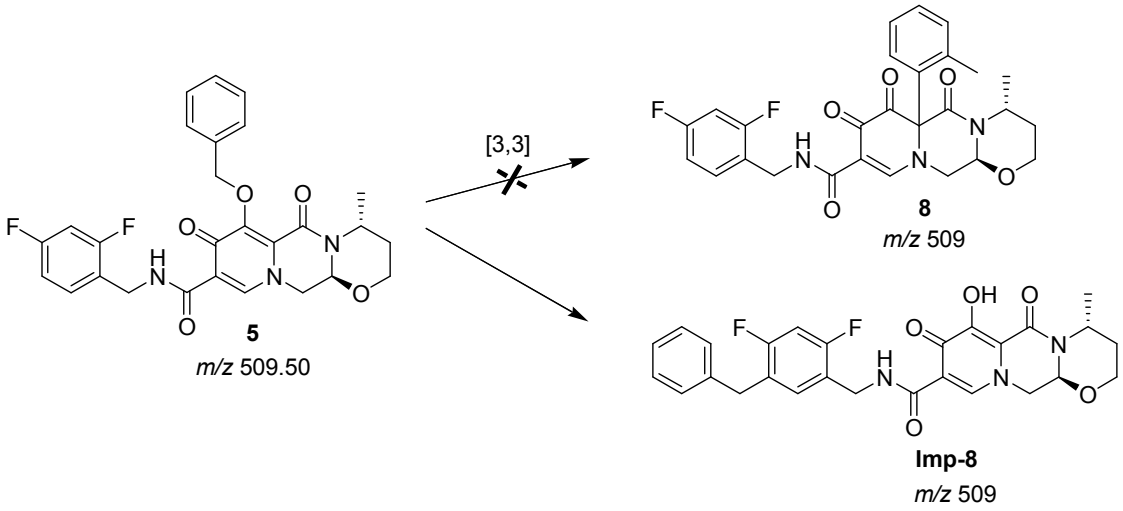


Figure 4 Most probable structure of process impurity **imp-8**

The presence of an additional benzyl group at the aromatic region is a suggestive of mechanism of **imp-8** formation involving electrophilic substitution (Friedel-Crafts like alkylation) at the aromatic part of dolutegravir **6** with by-product, benzyltrifluoroacetate **7** in the presence of trifluoroacetic acid. The regioselectivity of electrophilic substitution during the impurity formation is supported by facile acylation reported for 2,4-difluorotoluene in the presence of Lewis acid.[11] If the proposed mechanism of impurity formation is valid, suitable process conditions can be identified to control the formation of **imp-8**. Since the debenzylation reaction was performed in dichloromethane, the use of a sacrificial component having a higher affinity to electrophilic substitution can suppress this side reaction. Accordingly, addition of 10% v/v of

toluene to the original debenzylation reaction conditions, as expected reduced the level of imp-8 was from 0.08% to 0.02% and a separate reaction performed exclusively in toluene did not form any **imp-8** supporting the mechanism of origin of the impurity. The identified solvent system provided excellent reaction condition for an impurity-free debenzylation. Treatment of **6** with aqueous sodium hydroxide at reflux temperature afforded high-pure dolutegravir sodium **1**. The overall yield of the optimized process was 57%.

CONCLUSION

A robust and scalable process for the synthesis of dolutegravir sodium was developed. All unknown impurities detected during the development of dolutegravir sodium was either synthesized or isolated via preparative HPLC and identified by NMR and MS. The structural knowledge of these impurities led to the identification of their root cause of formation and allowed for establishing a suitable control strategy that can produce high purity API in a consistent way. The improved process demonstrates consistent control of chiral impurities (diastereomeric impurity and enantiomeric impurity) to a level of not more than 0.15% w/w in the final API.

The optimized process was successfully demonstrated and validated at pilot plant scale matching the proposed lab yield and quality at all the stages (Table-S9, supporting information).

EXPERIMENTAL SECTION

All materials were purchased from commercial suppliers. Unless specified otherwise, all reagents and solvents were used as supplied by manufacturers. Melting points were determined by open air capillary with Buchi M-565 and are uncorrected. IR spectra of samples were

recorded on Shimadzu IR Affinity-I FT-IR spectrophotometer. ^{1}H NMR spectra (400 MHz) and ^{13}CNMR spectra (100 MHz) were recorded in $CDCl_3$, DMSO-d6, on a Bruker 400 MHz NMR (ASCEND, 5 mm PABBO) instrument and mass spectra were determined on Velos Pro Ion Trap Mass Spectrophotometer (Thermo Scientific)

Methyl 5-(2,4-difluorobenzylcarbamoyl)-3-(benzyloxy)-1,4-dihydro-1-(2,2-dimethoxyethyl)-4-oxopyridine-2-carboxylate (4) Dimethyl 3-(benzyloxy)-4-oxo-4*H*-pyran-2,5-dicarboxylate **2** (150 g, 0.471 mol) was suspended in methanol (1500 mL) in a 3-neck RB flask at room temperature. Aminoacetaldehyde dimethyl acetal (54.5 g, 0.518 mol) was added drop-wise followed by addition of diisopropylethylamine (60.9 g, 0.471 mol) and the resulting clear solution was stirred untill the consumption of starting material was observed. After the completion of the reaction (16-18 h), the pH of reaction mass was adjusted to around 6.0-7.0 using aqueous citric acid (50% solution, 300 mL) and solvent was distilled off completely under vacuum. The residue was taken up in dichloromethane (1200 mL) and water (300 mL), separated organic layer and distilled off the dichloromethane layer to afford product as crude solid. Toluene (1350 mL) and 2,4-difluorobenzylamine (67.45 g, 0.471) was added to the crude reaction mass at room temperature. Acetic acid (11.32 g, 0.189 mol) was then added drop-wise and the reaction mass was heated to 90-95^{o}C, two more portions of 2,4-difluorobenzylamine (16.86 g, 0.118 mol) after every 3.0 h. After the consumption of the starting material, the reaction mass was cooled to room temperature and washed the toluene layer sequentially with aqueous citric acid (25% solution, 300 mL) and water (300 mL). The toluene layer was separated and distilled under reduced pressure to give product **4** as a transparent viscous oil. Isopropyl alcohol (300 mL) was added and heated the reaction mass to 55-60^{o}C. Cooled the reaction mass was cooled to 0-5^{o}C gradually and maintained for 2.0 h, filtered and dried under vacuum to give

210 g (86%) of **4** as an off-white solid. Characterization data of **3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.1(s, 1H), 7.4 (m, 2H), 7.3 (m, 3H), 5.3 (s, 2H), 4.4-4.5 (t, *J*=4.8Hz, 1H), 3.9 (m, 5H), 3.8 (s, 3H), 3.3 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.0, 165.5, 162.2, 149.2, 146.1,136.9, 133.9, 128.7, 128.2, 128.1, 118.1, 102.7, 74.1, 56.7, 55.7, 53.0, 52.3; IR (KBr, cm^{-1}): 3041, 2999, 2954, 2835, 1722, 1616, 1602. ESI-MS *m/z* 406.17; Characterization data of **4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.43-10.45 (t, *J*=5.6Hz, 1H), 8.44(s, 1H), 7.33-7.42 (m, 6H), 6.83-6.87 (m, 2H), 4.66-4.67 (d, *J*=6.0Hz, 2H), 4.47-4.50 (t, *J*=4.8Hz, 1H), 4.03-4.04 (d, *J*=4.8Hz, 2H), 3.81 (s, 1H), 3.38 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.2, 164.1, 162.1, 160.9, 161.0, 163-3, 163-5, 159.5, 159.6, 162.0, 162.1, 148.3, 144.5, 136.7, 135.0, 130.6, 130.8, 128.5, 128.3, 128.2, 121.4-121.6, 119.3, 111.0, 111.2, 103.5, 104.0, 102.6, 74.4, 56.9, 55.6, 53.1, 36.4, 36.5; IR (KBr, cm^{-1}): 3172, 3039, 2956, 2875, 2841, 1734, 1658, 1608, 1546, 1132; ESI-MS *m/z* 517.34.

(4*R*,12a*S*)-*N*-(2,4-Difluorobenzyl)-7-benzyloxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1',2':4,5]pyrazino[2,1-*b*][1,3]oxazine-9-carboxamide (5) A solution of **4** (210 g, 0.406 mol) in acetonitrile (1470 mL) was treated with acetic acid (146.5 g, 2.440 mol) under nitrogen atmosphere in a 3-necked RB flask. A solution of methanesulphonic acid (7.81 g, 0.081 mol) in acetonitrile (210 mL) was added slowly at ambient temperature and the reaction mass heated to 60-65^{o}C. A second lot of methanesulphonic acid (3.90 g, 0.041 mol) was added and continued heating until the completion of the reaction. The reaction was cooled to room temperature and treated with a solution of 3*R*-aminobutan-1-ol (50.74 g, 0.569 mol) in acetonitrile (210 mL). Maintained the reaction mass for 2 h at same temperature and again heated to 60-65^{o}C until the consumption of **4b**. The reaction mass was completely distilled under vacuum and the residue was taken-up in dichloromethane (1680 mL). The organic layer was washed sequentially with 10% sodium carbonate solution (1050 mL) and water (840 mL). The

dichloromethane layer was separated, dried over sodium sulphate and treated with TBDMS-Cl (12.26 g, 0.081 mol) and imidazole (11.07 g, 0.163 mol) in a fresh RB flask under nitrogen atmosphere. After stirring for 2 h, 15% sodium chloride solution (840 mL) was added to the reaction mass and the layers are separated. The organic layer was taken in a fresh RB flask and distilled out completely under reduced pressure. Methanol (420 mL) was added to the concentrated residue and heated the reaction mass to 55-60^{o}C for 1.0 h. The solid formed was cooled gradually to 5-10^{o}C, filtered and dried under vacuum to afford 162.0 g (78%) of **5** as pale-yellow solid. Characterization data of **5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.4 (t, *J*=6Hz, 1H), 8.3 (s, 1H), 7.6 (d, *J*=7.2Hz, 2H), 7.2-7.4 (m, 4H), 6.8 (m, 2H), 5.2-5.3 (dd, *J*=16.4Hz and 10Hz, 2H), 5.1 (m, 1H), 4.9-5.0 (m, 1H), 4.6 (d, *J*=6Hz, 2H), 4.2 (dd, *J*=13.2Hz and 3.2Hz, 1H), 4.0-4.1 (dd, *J*=13.2Hz and J=6Hz, 1H), 3.9 (m, 2H), 2.1-2.2 (m, 1H), 1.4-1.5 (dd, *J*=14Hz and *J*=2Hz, 1H), 1.3 (d, *J*=6.8Hz, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 174.5, 164.0, 160.9, 161.0, 163.4, 163.5, 159.4, 159.5, 161.9, 162.0, 155.5, 153.2, 142.0, 136.6, 130.5, 130.6, 129.4, 128.9, 128.2, 128.1, 121.3, 121.5, 118.7, 111.0, 111.2, 103.5, 104.4, 76.0, 74.4, 62.5, 53.5, 44.5, 36.4, 36.5, 29.3, 15.9; IR (KBr, cm^{-1}): 3180, 3061, 2974, 2941, 2870, 1656, 1606, 1546, 1093; ESI-MS *m/z* 510.17

(4*R*,12a*S*)-*N*-(2,4-Difluorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1',2':4,5]pyrazino[2,1-*b*][1,3]oxazine-9-carboxamide (6) A solution of **5** (135 g, 0.265 mol) in dichloromethane (2430 mL) was heated to reflux in a 3-necked RB flask. A solution of trifluoroacetic acid (151 g, 1.325 mol) in dichloromethane (270 mL) was slowly added to the reaction mass at reflux over a period of 1.0-2.0 h and continued stirring until the completion of the reaction. The reaction mass was cooled to 10-15^{o}C, carefully treated with water (540 mL) and pH (~7.0-9.0) was adjusted using aq. ammonia (114 mL). The reaction mass

was warmed to room temperature, the layers were separated and washed sequentially with water (270 mL), 10% citric acid solution (270 mL) and brine (540 mL). The dichloromethane layer was taken in a fresh RB flask and distilled out completely under reduced pressure. Toluene (405 mL) was added to the concentrated residue and heated the reaction mass to 60-65^{o}C for 1.0 h. The solid formed was cooled gradually to 10-15^{o}C, filtered, washed with toluene (67.5 mL) and dried under vacuum to afford 100 g (90%) of **6** as off-white to pale-yellow solid. Characterization data of **6**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.4 (s, 1H), 10.3 (t, *J*=5.6Hz), 8.3 (s, 1H), 7.3 (m, 1H), 6.7-6.8 (m, 2H), 5.2 (m, 1H), 4.9-5.0 (m, 1H), 4.6 (d, *J*=6.0Hz, 2H), 4.2-4.3 (dd, *J*=13.2Hz and 4Hz, 1H), 4.1 (dd, *J*=13.2Hz and 6Hz), 4.0 (m, 2H), 2.1-2.2 (m, 1H), 1.5 (dd, *J*=14Hz and 2.0Hz, 1H), 1.3-1.4 (d, *J*=7.2Hz, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.3, 164.1, 162.5, 160.9-161.0, 163.3-163.4, 159.4, 159.5, 161.9, 162.0, 156.0, 140.1, 130.2, 130.4, 121.4, 121.6, 116.6, 115.8, 111.0, 111.2, 103.4, 103.9, 76.3, 62.7, 52.4, 44.7, 36.5, 36.5, 29.2, 15.5; IR (KBr, cm^{-1}): 3442, 3190, 3068, 2974, 2949, 2885, 1660,1629, 1544, 1087; ESI-MS *m/z* 420.17

Sodium(4*R*,12a*S*)-9-{[(2,4-difluorophenyl)methyl]carbamoyl}-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1`,2`:4,5]pyrazino[2,1-*b*][1,3]oxazol-7-olate (1) A slurry of **6** (85 g, 0.203 mol) in methanol (765 mL) was heated to reflux in a 3-necked RB flask. A solution of sodium hydroxide (8.5 g, 0.213 mol) in water (25.5 mL) was slowly added to the reaction mass at reflux over a period of 30-45 min and further continued stirring for a period of 2.0 h. The solid formed was cooled gradually to 25-30^{o}C, filtered, washed with methanol (170 mL) and dried under vacuum to afford 85.0 g (95%) of **1** as off-white to pale-yellow solid. Characterization data of **1**: ^{1}H NMR (400 MHz, DMSO-d6) δ 10.6-10.7 (t, *J*=6.0Hz, 1H), 7.8 (s, 1H), 7.3 (dd, *J*=8.4Hz and 7.2Hz, 1H), 7.1-7.2 (m, 1H), 7.0 (t, *J*=8.0Hz, 1H), 5.1 (bs, 1H), 4.7-4.8 (m, 1H), 4.5 (d, *J*=5.6Hz, 2H), 4.2-4.3 (d, *J*=11.2Hz, 1H), 4.1 (m, 1H), 3.9 (m, 1H), 3.7-

3.8 (m, 1H), 1.8 (m, 1H), 1.3 (d, *J*=13.2Hz, 1H), 1.2 (d, *J*=6.8Hz, 3H); ^{13}C NMR (400 MHz, DMSO-d6) δ 177.9, 167.0, 166.0, 161.0, 159.9, 160.0, 162.4, 162.5, 158.6, 158.8, 161.1, 161.2, 134.2, 130.4, 130.5, 122-8, 123.0, 114.8, 111.0, 111.3, 108.6, 103.3, 103.8, 75.5, 61.8, 53.1, 42.9, 35.3, 29.1, 15.3; IR (KBr, cm^{-1}): 3165, 3072, 2974, 2941, 2873, 1643, 1539, 1504, 1101; ESI-MS *m/z*: 418.17

1-(2,2-Dimethoxyethyl)-5-(benzyloxy)-6-(methoxycarbonyl)-4-oxo-1,4-dihydropyridine-3-carboxylic acid (imp-1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.0 (s, 1H), 8.4 (s, 1H), 7.3-7.4 (m, 5H), 5.3 (s, 2H), 4.4 (t, *J*=4.8Hz, 1H), 4.0 (d, *J*=4.4Hz, 2H), 3.8 (s, 3H), 3.4 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 174.8, 165.8, 161.4, 147.4, 145.1, 136.4, 136.1, 128.7, 128.5, 128.4, 116.5, 102.2, 74.6, 57.4, 55.8, 53.4; IR (KBr, cm^{-1}): 3076, 3043, 2999, 2954, 2835, 1724, 1620; ESI-MS *m/z* 392.13.

Methyl 5-(2,2-dimethoxyethylcarbamoyl)-3-(benzyloxy)-1,4-dihydro-1-(2,2-dimethoxyethyl)-4-oxopyridine-2-carboxylate (imp-2): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.1 (t, *J*=5.2Hz, 1H), 8.4 (s, 1H), 7.3-7.4 (m, 5H), 5.2 (s, 2H), 4.5(t, *J*=5.2Hz, 1H), 4.4 (t, *J*=4.4Hz, 1H), 4.0 (d, *J*=4.4hz, 2H), 3.7 (s, 3H), 3.6 (t, *J*=6.0Hz, 2H), 3.4 (s, 6H), 3.3 (s, 6H) ; ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.2, 164.2, 162.1, 148.2, 144.4, 136.7, 134.8, 128.5, 128.3, 128.1, 119.3, 102.7, 102.4, 74.3, 56.8, 55.6, 53.9, 53.1, 40.8; IR (KBr, cm^{-1}): 3201, 3045, 2949, 2835, 1734, 1662, 1610, 1548; ESI-MS *m/z* 479.26

***N*-(2,4-Difluorobenzyl)-2,8-dihydro-9-benzyloxy-2-(4-hydroxybutan-2-yl)-1,8-dioxo-1*H*-pyrido[1,2-*a*]pyrazine-7-carboxamide (imp-6):** ^{1}H NMR (400 MHz, DMSO-d6) δ 10.6 (t, *J*=5.6Hz, 1H), 8.9 (s, 1H), 7.6 (d, *J*=7.2Hz, 2H), 7.3-7.4 (m, 5H), 7.2 (m, 1H), 7.0-7.1 (m, 1H), 7.0 (d, J=6.4Hz, 1H), 5.0-5.1 (dd, *J*=12.8Hz and 10.4Hz), 4.9 (m, 1H), 4.57-4.59 (d, *J*=5.6Hz, 2H), 4.51-4.5 (t, *J*=4.8Hz,1H), 3.3-3.4 (dd, *J*=11.2Hz and 1.5Hz, 2H), 1.7-1.8 (m, 2H), 1.2 (d,

J=6.8Hz, 3H); ^{13}C NMR (400 MHz, DMSO-d6) δ 171.8, 163.1, 160.2-160.3, 162.6, 162.7, 158.8, 158.9, 161.3, 161.4, 153.5,151.7, 138.2, 137.4, 130.9, 131.0, 129.8, 128.1, 127.9, 127.6, 122.0, 122.2, 119.7,115.3,111.6, 111.2, 111.5, 103.5, 104.0, 73.2, 57.6, 47.5, 36.8, 35.8, 35.8, 18.5; IR (KBr, cm^{-1}): 3419, 3192, 3059, 2933, 2881, 1660, 1604, 1543, 1095; ESI-MS *m/z* 510.31

***N*-(2,4-Difluorobenzyl)-2,8-dihydro-9-benzyloxy-2-(4-(tert-butyldimethylsilyloxy)butan-2-yl)-1,8-dioxo-1H-pyrido[1,2-*a*]pyrazine-7-carboxamide (imp-6a):** ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.6 (t, *J*=6.0Hz, 1H), 8.6 (s, 1H), 7.6 (d, *J*=7.2Hz, 2H), 7.2-7.3 (m, 4H), 6.7-6.8 (m, 2H), 6.7 (d, *J*=6.0Hz, 1H), 6.3 (d, *J*=6.4Hz, 1H), 5.2 (dd, *J*=15.6Hz and 10Hz), 5.0 (m, 1H), 4.6 (d, *J*=6.0Hz, 2H), 3.6 (t, *J*=6.0Hz, 2H), 1.8-1.9 (m, 2H), 1.3 (d, *J*=6.8Hz, 3H), 0.0 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 172.8, 163.8, 160.9-161.0, 163.3, 163.4, 159.3-159.5, 161.8, 161.9, 153.7, 152.1, 137.6, 136.7, 130.4, 130.6, 129.5, 128.9, 128.2, 128.0, 121.2, 121.4, 120.5, 115.2, 111.0, 111.2, 110.8, 103.4, 10.3-9, 74.3, 59.8, 48.6, 37.4, 36.5, 25.9, 19.3, 18.1, -5.5; IR (KBr, cm^{-1}): 3192, 3059, 2953, 2856, 1664, 1604, 1543, 1095, 839; ESI-MS *m/z* 624.42

***N*-(2,4-Difluorobenzyl)-2,8-dihydro-9-hydroxy-2-(4-hydroxybutan-2-yl)-1,8-dioxo-1*H*-pyrido[1,2-a]pyrazine-7-carboxamide (imp-7):** ^{1}H NMR (400 MHz, DMSO-d6) 12.2 (s, 1H), 10.5-10.6 (t, *J*=6.0Hz, 1H), 8.8 (s, 1H), 7.5 (d, *J*=6.4Hz, 1H), 7.3-7.4 (dd, *J*=15.6Hz and 6.8Hz, 1H), 7.2 (m, 1H), 7.05-7.09 (m, 1H), 7.01(d, *J*=6.4Hz, 1H), 4.8-4.9 (m, 1H), 4.5 (m, 3H), 3.3-3.4 (m, 2H), 1.7-1.9 (m, 2H), 1.2 (d, *J*=6.8Hz, 3H); ^{13}C NMR (400 MHz, DMSO-d6) δ 168.1, 163.4, 160.5, 160.2-160.3, 162.6, 162.7, 158.8, 158.9, 161.3, 161.4, 153.0, 134.8, 130.7, 130.9, 122.0, 122.2, 117.6, 117.2, 114.7, 113.5, 111.2, 111.4, 103.5, 104.0, 57.4, 47.4, 36.5, 35.8, 18.3; IR

(KBr, cm^{-1}): 3450, 3236, 3101, 3049, 2941, 2885, 1658, 1624, 1558, 1504, 1099; ESI-MS *m/z* 420.16

(4*R*,12a*S*)-*N*-(5-Benzyl-2,4-difluorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1',2':4,5]pyrazino[2,1-*b*][1,3]oxazine-9-carboxamide (imp-8): ^{1}H NMR (400 MHz, DMSO-d6) δ 12.3 (m, 1H), 10.2 (s, 1H), 8.2 (s, 1H), 7.0-7.3 (m, 6H), 6.6-6.7 (t, J=9.6Hz, 1H), 5.1 (bs, 1H), 4.8-4.9 (m, 1H), 4.5 (m, 2H), 3.5-4.0 (m, 6H), 2.0-2.1 (m, 1H), 1.4 (m, 1H), 1.3 (m, 1H); ^{13}C NMR (400 MHz, DMSO-d6) δ 171.2, 164.0, 162.4, 158.7, 158.6, 161.2, 161.1, 160.6, 160.5, 158-1, 158.0, 155.9, 140.2, 139.5, 131.4, 128.5, 128.4, 126.1, 123.7, 123-8, 121.2, 121.3, 116.4, 115.7, 103.3, 103.8, 75.7, 62.6, 52.3, 44.6, 36.6, 34.4, 29.2, 15.4; IR (KBr, cm^{-1}): 3464, 3242, 3059, 2972, 2926, 1658, 1633, 1541, 1504, 1091; ESI-MS *m/z* 508.16

ASSOCIATED CONTENT

SUPPORTING INFORMATION. Copies of IR, ESI-MS, ^{1}H-NMR and ^{13}C-NMR of compounds **3**, **4**, **5**, **6**, **1** and impurities **imp-1**, **imp-2**, **imp-6**, **imp-6a**, **imp-7** and **imp-8**. LCMS of **imp-8**, HPLC chromatogram of isolated intermediates **3** and **4** along with reaction monitoring for the conversion of **2** to **3** in the presence and absence of water is provided. Results of optimization studies for preparation of intermediates and API are also provided.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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