Preparation of Dolutegravir Intermediate Diastereomer

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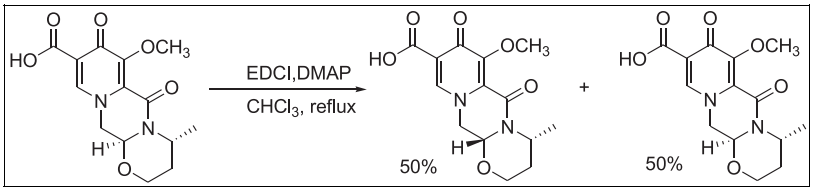

A convenient method was developed to prepare the diastereomer of dolutegravir tricyclic intermediate in the catalysis of EDCI/DMAP in up to 87% yield. Different solvents, temperature, and times were optimized. The synthesized diastereomer **6′** could be used as a standard for the industrial manufacture requirement of dolutegravir active pharmaceutical ingredient.

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INTRODUCTION

AIDS became a serious global public health and social problem for a long time [1]. It has been found that integrase plays an important role in the replication of HIV-1 virus because there is no functional analogue of integrase in human body, and this enzyme has gradually become an ideal target for anti-HIV drugs. In recently, a number of integrase inhibitors including raltegravir, elvitegravir, dolutegravir, cabotegravir, and bictegravir have been launched or approved to enter into clinic phases [2–5].

Dolutegravir (trade name Tivicay), (4R,12aS)-*N*-(2,4-dif luorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12 a-hexahydro-2*H*-pyrido[1′,2′:4,5]pyrazino[2,1-*b*][1,3]oxaz ine-9-carboxamide, was developed by Viiv Healthcare and Glaxo SmithKline as a second generation of integrase inhibitor [6], which was first approved by Food and Drug Administration (FDA) in August 2013. Compared with the first generation of integrase inhibitors, raltegravir and elvitegravir, dolutegravir possesses a higher barrier for developing resistance, as well as that the clinical efficacy for AIDS treatment is better than raltegravir [7,8]. Recently, there is a great need for process research [9–15] for the forthcoming industrial manufacture. Hence, an efficient synthetic route of dolutegravir is urgently needed. In this course, it was found that the existence of other enantimers of dolutegravir contaminated the quality of final product. Therefore, in order to meet the requirement of API manufacture, it is necessary to prepare sufficient amounts of dolutegravir enantimers for impurity profiling. Among of these enantiomeric impurities, dolutegravir diastereomer could be easily prepared from diastereomer of intermediate **6** (Fig. 1). Herein, we described a convenient preparation method for this compound.

RESULTS AND DISCUSSION

A facile and direct process to prepare dolutegravir was developed by Glaxo SmithKline, which was started from 4-methoxyacetoacetic acid methyl ester [12]. In this modified process, the compound **4** was synthesized in a total of 36% yield for a continuous four steps. Similarly, without additional separation and purification of aldehyde **5**, the key intermediate **6**, (4S,12aR)-4-methyl-7-(meth yloxy)-6,8 -dioxo-3,4,6,8,12,12a-hexahydro[1,3]oxazolo [3,2-*a*]pyrido[1,2-*d*]pyrazine-9-carboxylic acid was smoothly obtained in a good yield upon treatment with R-2-aminobutanol; and after the coupling with 2, 4-difluorobenzylamine and de-methylation, the target compound dolutegravir was obtained in a medium yield (Scheme 1).

As an important scaffold of dolutegravir, compound **6** controls the optical purity of the drug. Therefore, on the one hand, how to improve and optimize stereo-selectivity of compound **6** is becoming a critical problem for the whole industrial production (Scheme 1); on the other hand, it seems that we can also easily obtain dolutegravir intermediate diastereomer **6′** in the same reaction.

In order to prepare diastereomer **6′**, the first thought occurring to our mind is that diastereomer **6′** could be isolated from the cyclization reaction of compound **5** with (R)-3-amino-1-butanol, which theoretically can produce two chiral centers and one pair of diastereomers (Scheme 2). However, result shows that this reaction produces enantiomer **6** stereo-selectively with a

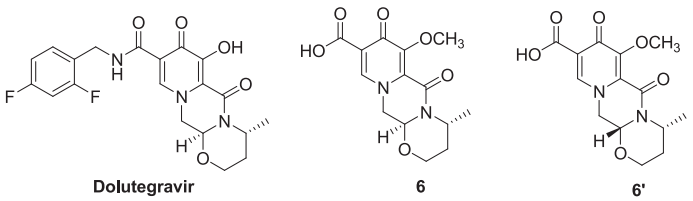
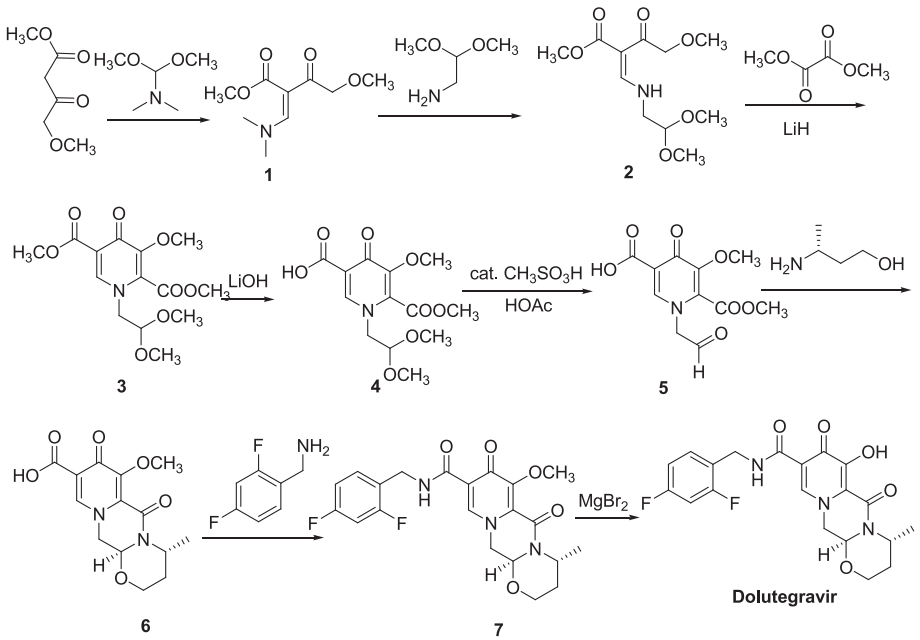
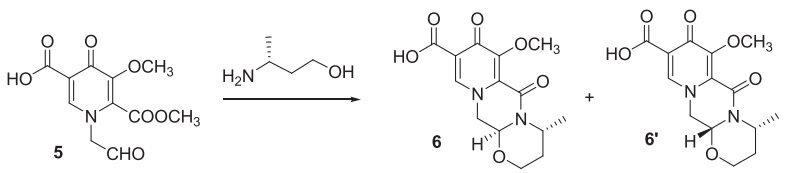


Figure 1. Structure of dolutegravir, intermediate **6** and **6′**.

Scheme 1. Synthetic route for dolutegravir [12].



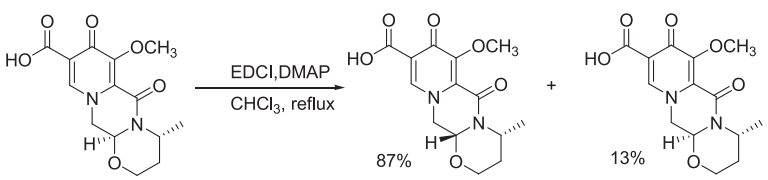
Scheme 2. Preparation of intermediate **6** and diastereomer **6′**.



diastereoismer ratio >99:1. Only traces of diastereomer **6′** can be detected by thin-layer chromatography (TLC).

More attention was then paid on the conversion of intermediate **6**. It was expected that the carboxylic acid **6** could undergo the ring-opening and ring-cycling reaction in the proton acid condition, which will finally lead to the formation for its diastereomer **6′** (Scheme 3).

Scheme 3. Proposed synthetic route for diastereomer **6′**



Different coupling reagents, solvents, and reaction temperature were then tried to optimize this reaction. And the best result shows that the diastereomer **6′** can be prepared in up to 50% yield which is promoted by EDCI/DMAP in a sealed tube reaction (Table 1, entry 7).

A mechanism was then proposed to explain this reaction (Scheme 4). The acetal **6** was hydrolyzed in the acid condition to form carbon cation **9**. Consequently, the carbon cation **9** will be attacked by OH-group from two sides (**a** and **b**) to form configuration keeping and conversing compound correspondingly.

MATERIALS AND METHODS

Melting points were taken on a Mettler Toledo FP62 melting point apparatus, uncorrected and reported in degrees. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian Unity INOVA 400 MHz spectrometer (400 and 100 MHz, respectively) using $CDCl_3$, or DMSO-d_6 as solvents with tetramethylsilane as an internal standard. Chemical shifts were reported as δ (ppm) and spin–spin coupling constants as *J* (Hz) values. The mass spectra (MS) were recorded on a Finnigan MAT-95 mass spectrometer. TLC plates (GF 254) were bought from Branch Qingdao Haiyang Chemical Plant. IR spectra were measured on Fourier infrared spectrometer (Nicolet, USA, KBr tablet).

EXPERIMENTAL

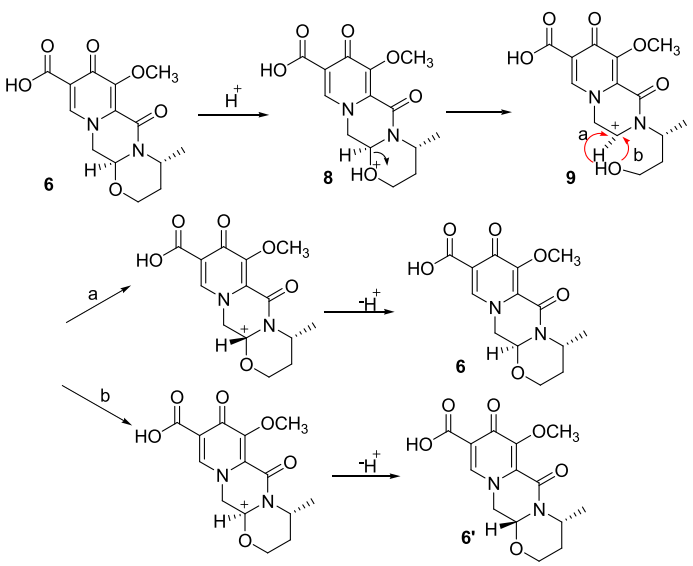
Synthesis of 1-[2,2-bis(methoxy)ethyl]-5-(methoxy)-6-[(methoxy)-carbonyl]-4-oxo-1,4-dihydro-3-pyridinecarboxylic acid 4. Methyl 4-methoxyacetoacetate (8 m, 61.80 mmol) and *N*,*N*-dimethylformamide dimethylacetal (DMF-DMA, 9.6 mL, 72.26 mmol) were stirred at room temperature for 1.5 h to form a brown mixture containing

Table 1

Optimization for the preparation of diastereomer **6′**.

Entry		Base	Solvent	Temp/Time	Yield (**6′**)	Yield (**6**)
1	CH_3SO_3H	-	DCM	Reflux/3 days	0	100%
2	ECDI	-	DCM	Reflux/3 days	0	100%
3	ECDI	DMAP	DCM	Reflux/3 days	5%	95%
4	ECDI	-	Chloroform	Reflux/3 days	0	100%
5	-	DMAP	Chloroform	Reflux/3 days	0	100%
6	ECDI	DMAP	Chloroform	Reflux/3 days	20%	80%
7	ECDI	DMAP	Chloroform	70°C (sealed tube)/3 days	87%	13%
8	CDI	DMAP	Chloroform	70°C (sealed tube)/3 days	0	100%

Scheme 4. Proposed reaction mechanism for the formation of diastereomer **6′**. [Color figure can be viewed at wileyonlinelibrary.com]



the target compound **1** (R_f = 0.14, ethyl acetate/petroleum ether = 5/1). The resulted mixture was added with methanol (20 mL) and aminoacetaldehyde dimethyl acetal (6.68 mL, 61.31 mmol). After stirring at room temperature for 1 h, the solution turned reddish and TLC showed a new point. Compound **2** (R_f = 0.56, ethyl acetate). The reaction was stopped and evaporated to give a red-brown oily liquid containing the crude product of compound **2**; 45.2 mL of methanol and 18.344 g (155.34 mmol) of dimethyl oxalate were dissolved. After the dimethyl oxalate was completely dissolved, the temperature was controlled at 25 under an ice-water bath. After adding lithium hydride LiH 0.864 g (108.67 mmol) in batches, the solution turns into a brownish-red suspension. After addition, the reaction solution is placed in an oil bath at 40°C for 14 h, and the solution changes from a suspension to a brick red solution. A new spot occurred in TLC (compound **3**, R_f = 0.23, ethyl acetate). The reaction solution was cooled to −5°C and anhydrous lithium hydroxide (5.94 g, 123.8 mmol) was added while the temperature was controlled at 3–5°C. The reaction solution changed from brick red to orange-yellow suspension and reacted for 2 h. The reaction was quenched by the addition of 2N hydrochloric acid (146.8 mL), and the reaction temperature was controlled below 5°C in an ice-water bath. Add 180 mL of ethyl acetate to the extraction, raise the temperature to 20°C, filter the lower solid by filtration, and discard it; collect the liquid phase and separate the liquid; add 90 mL of water to the organic phase, concentrate under reduced pressure, filter by suction, and the filter cake was collected and dried *in vacuo* at 50°C to obtain compound **4** (6.93 g, 35.87%) as a colorless solid. R_f = 0.441 (dichloromethane/methanol = 40/1, containing 0.5% glacial acetic acid). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 8.40–8.42 (m, 1H), 4.49–4.53 (m, 1H), 4.10–4.14 (m, 2H), 3.98 (s, 3H), 3.97 (s, 3H), 3.38 (s, 3H), 3.37 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 174.86, 165.99, 161.60, 148.65, 145.47, 136.59, 116.53, 102.31, 60.97, 57.26, 55.97, 53.77.

Synthesis of (4S,12aR)-4-methyl-7-(methyloxy)-6,8-dioxo-3,4,6,8,12,12a-hexahydro[1,3]oxazolo[3,2-*a*]pyrido[1,2-*d*] pyrazine-9-carboxylic acid (6). Compound **4** (3.38 g, 107.24 mmol) was dissolved in 33 mL of acetonitrile at room temperature. To the solution was added acetic acid (3 mL) and methanesulfonic acid (0.21 mL, 3.24 mmol). The solution was heated to 60°C for 20 h. A solution of R-3-amino-1-butanol (1.44 mL, 1.34 g) in acetonitrile (2.25 mL) was slowly added to the previous reaction solution. The reaction solution gradually changed from light yellow to brownish yellow, and the solution was white after the addition was completed. The suspension was heated to 64°C and continued to stir for 18.5 h. TLC showed a new spot. Compound 5, R_f = 0.42 (dichloromethane/methanol = 20/1 with 0.5% glacial acetic acid). The reaction solution was concentrated under reduced pressure and a pale yellow solid precipitated; 25.5 mL of DCM was added and redissolved. After adding 25.5 mL of 1N hydrochloric acid, the liquid was separated and traced by TLC. The organic phase was collected, and the aqueous layer was extracted twice with 25.5 mL of DCM and organic. Combine under reduced pressure and concentrate. Add 7 mL of methanol and evaporate to a light yellow solid. After adding 13 mL of methanol and heating to reflux for 4 h, the reaction was stopped, and the mixture was slowly cooled to 20°C and allowed to stand for 15 h. After suction filtration, the cake was collected and vacuum dried at 50°C to give compound **6** (1.72 g, 52.12%) as a pale yellow solid. R_f = 0.42 (dichloromethane/methanol = 20/1 with 0.5% glacial acetic acid). $[\alpha]^{25}$ = −36.57 (c = 5.0, CH_3OH). IR (KBr) ν (cm^{-1}) = 3410, 2972, 2876, 1630, 1449, 1275, 1093, 775. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 15.02 (d, *J* = 16.7 Hz, 1H), 8.42 (d, *J* = 11.8 Hz, 1H), 5.29 (dd, *J* = 5.4, 3.9 Hz, 1H), 4.97 (q, *J* = 6.4 Hz, 1H), 4.52 (t, *J* = 4.5 Hz, 1H), 4.43 (dd, *J* = 13.6, 3.5 Hz, 1H), 4.26 (dd, *J* = 13.6, 5.8 Hz, 1H), 4.13 (d, *J* = 4.5 Hz, 1H), 4.05 (d, *J* = 13.0 Hz, 2H), 3.98 (d, *J* = 2.2 Hz, 3H), 3.96 (d, *J* = 2.9 Hz, 1H), 3.39 (s, 3H), 2.22–2.09 (m, 1H), 1.52 (dd, *J* = 13.9, 1.6 Hz, 1H), 1.35 (d, *J* = 7.0 Hz, 2H)^{13}C-NMR (100 MHz, $CDCl_3$) δ = 173.23, 164.17, 162.24, 149.45, 144.64, 135.03, 130.77, 130.73, 130.67, 119.35, 111.34, 111.30, 111.13, 111.09, 103.81, 102.73, 60.78, 56.81, 55.72, 53.47, 36.60, 36.56. HRMS (ESI-TOF): 309.1166 [M + 1].

Synthesis of (4S,12aS)-4-methyl-7-(methyloxy)-6,8-dioxo-3,4,6,8,12,12a-hexahydro[1,3]oxazolo [3,2-*a*]pyrido [1,2-*d*] pyrazine-9-carboxylic acid (6′). A solution of compound **6** (67.6 mg, 0.23 mmol), EDCI (155.5 mg, 0.81 mmol), and DMAP (11.9 mg, 0.1 mmol) in chloroform (15 mL) was stirred at 70°C in a sealed tube for 3 days. After being cooled to the room temperature, the reaction mixture was quenched with the addition of water (5 mL). The separated aqueous phase was extracted with DCM (2 *20 mL) and the combined organic phases were dried over $MgSO_4$. The solvent was evaporated completely under vacuo, and the residue was purified by chromatography through a silical gel column using DCM: CH_3OH = 97: 3 as eluent to give the title product (**6′**, 58.8 mg, 87%) as a pale yellow solid. R_f = 0.23 (DCM: CH_3OH = 97: 3). m.p. 86.2°C. $[\alpha]^{25}$ = −28.38 (c = 4.96, CH_3OH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.97 (s, 1H), 5.20 (s, 1H), 4.97–4.81 (m, 1H), 4.33–4.10 (m, 3H), 3.99 (dd, *J* = 13.3, 6.1 Hz, 1H), 3.92 (s, 4H), 2.30 (d, *J* = 9.5 Hz, 1H), 2.08 (dt, *J* = 11.6, 6.8 Hz, 1H), 1.27 (dt, *J* = 9.3, 4.6 Hz, 5H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 172.29, 164.55, 155.84, 143.33, 128.06, 117.71, 76.10, 62.44, 60.88, 53.35, 44.35, 29.66, 15.98, 14.29.

CONCLUSIONS

A convenient method was developed to prepare the diastereomer of dolutegravir tricyclic intermediate catalyzed by EDCI/DMAP in up to 50% yield. The synthesized diastereomer **6′** could be used not only as a standard for the industrial manufacture requirement of dolutegravir but also as a key building block for the preparation of dolutegravir diastereomer derivatives.

AUTHOR CONTRIBUTIONS

Dr. Xianheng Wang designed the study and Dr. Changkuo Zhao was responsible for the synthetic route design. Mr. Song Chen validated the procedure and prepared the sample. Ms. Liangye Long collected and analyzed the data. Prof. Yuhe did some administration on this project. All authors gave the approval for the final submission.

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