Three-step synthetic procedure to prepare dolutegravir, cabotegravir, and bictegravir

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ABSTRACT

Dolutegravir, cabotegravir, and bictegravir are three integrase inhibitors, which were used for HIV-1 in the clinic. In this paper, a continuous three-step synthetic strategy was developed to prepare dolutegravir, cabotegravir, and bictegravir in a better yield, compared to the initial step-by-step procedure. Different solvents, temperature, and times were optimized. In the course of industrial production of active pharmaceutical ingredient (API), this three-step synthetic protocol has several advantages, including low cost, time-saving, and better yield. This process was successfully scaled up to tens of grams.

Introduction

AIDS remains a serious global public health threat to humans even in modern society. With the development of science and technology, it has been found that integrase plays an important role in the replication of HIV-1 virus, and this enzyme has become an attractive drug target over the past few years (1). So far, a lot of integrase inhibitors including raltegravir, elvitegravir, dolutegravir, cabotegravir, and bictegravir have been launched globally or approved to enter into phase III clinic trials (Figure 1) (2–4).

Dolutegravir, cabotegravir, and bictegravir are three closely related molecules designed and developed by GSK, Viiv Healthcare, and Gilead. These three molecules are structurally very similar, with the only difference being the chiral five-membered (cabotegravir), the six-membered (dolutegravir), or bicyclic (bictegravir) hemiaminal (5–7). Due to the forthcoming patent expiration, many pharmaceutical companies have paid much attention to their process research (8–12). Considering the potential market demand, an economical, efficient, and convenient synthetic route of dolutegravir and its analogues is urgently needed. Herein, we describe a convenient preparation method for these compounds.

Results and discussion

A straightforward process to prepare dolutegravir was developed by Glaxo SmithKline, which was started from 4-methoxyacetoacetic acid methyl ester (12). In this modified process, building block 4 was synthesized in a total 36% yield for a multi-component reaction. In the following, scaffold 4 was treated with CH3SO3H in acetonitrile to give aldehyde 5 smoothly. Without additional separation and purification, the aldehyde 5 was cyclized with (R)-2-aminobutanol to afford the key intermediate 6 (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 in a good yield. Finally, intermediate 6 was coupled with 2,4-difluorobenzylamine and followed by de-methylation, the target compound dolutegravir was obtained in a medium yield (Scheme 1).
The initial route to cabotegravir and bictegravir developed by ViiV Healthcare and Gilead is outlined in Scheme 2 and employs the same building block acid acetal 4 used for the preparation of dolutegravir. In a condition of HOAc/MeSO₃H in CH₃CN, the acetal 4 was hydrolyzed to aldehyde 5, which was further treated with (S)-2-aminopropan-1-ol or (1R, 3S)-3-aminocyclopentanol to give key intermediates 6a and 6b. The further coupling with 2, 4-difluorobenzyl amine and 2, 4, 6-trifluorobenzyl amine provided penultimate intermediate 7a or 7b, followed by demethylation with MgBr₂ to furnish cabotegravir or bictegravir respectively.

However, the initial synthetic procedure results in more synthetic steps and is less atom-economical, which was not suitable for the manufacture in the pharmaceutical industry. Many patents or articles have been published for their process optimization. After perusal of the lit., we envisioned that a convergent synthetic protocol can be turned into reality by means of a continuous ‘three-step’ synthetic strategy (Scheme 3).

To this protocol, the acetal 4 is a key intermediate that has been prepared in a modified multi-component reaction (Scheme 4) starting from 4-methoxyβ-ketoester, wherein the methyl ether serves as a hydroxyl protecting group.
group until the final step of the synthesis (13–15). Therefore, this convenient procedure was employed in our process research and the optimized yield can be increased to 65% (lit., 61%).

Encouraged by these findings (16, 17), we envisioned that a more convenient protocol could be further developed for the following several steps. Considering the similar acid condition for the following two steps, we attempted to carry out these two reactions in one batch in one step manner. Employing the same strategy, the desired products 6, 6a, and 6b were fortunately obtained in a moderate yield respectively (Scheme 5).

In this step, the reaction yields of the three compounds 6, 6a, and 6b were slightly optimized. However, the diastereomer ratio (dr) was significantly increased above 100:1. Especially for compound 6b, in the initial Gilead’s scheme, two diastereomers were formed at the same time, but in our one-step method, compound 6b was obtained selectively as a single diastereomer.

Scheme 2. Initial synthetic route for cabotegravir and Bictegravir.

Scheme 3. Envisioned synthetic pathway for dolutegravir and analogues in ‘three-step’ manner.

The first step

Scheme 4. A modified multi-component reaction to synthesize intermediate 4.
The same strategy was also employed for the last two steps. Thus different solvents and temperature, reaction time and molar ratios were then tried to optimize this reaction. And the results were summarized in Table 1. It was found that the final product dolutegravir, cabotegravir and bictegravir can be also prepared in ‘one step’ manner in up to 90%, 87%, and 56% yield respectively. Meanwhile, it was also found that LiBr is better than MgBr₂ to remove the methyl group, probably because MgBr₂ is more hygroscopic than LiBr.

UPLC-MS was used to monitor the reaction progress. As an example for dolutegravir, the result was recorded as shown in Figure 2. The content of intermediate 7 reached the maximum concentration after 2 h. The raw material of carboxylic acid 6 can be completely consumed. After 4 h of the addition of LiBr, the content of dolutegravir can reach 65%. After the simple workup, the yield of dolutegravir is 56%, higher than the original route.

Materials

¹H and ¹³C nuclear magnetic resonance (NMR) spectra were recorded on a Varian Unity INOVA 400 MHz spectrometer (400 and 100 MHz, respectively) using CDCl₃ or DMSO-d₆ as solvents with TMS as an internal standard. Chemical shifts were reported as δ (ppm) and spin–spin coupling constants as J (Hz) values. Melting points were taken on a Mettler Toledo FP62 melting point apparatus, uncorrected and reported in degrees. TLC plates (GF 254) were bought from Branch Qingdao Haiyang Chemical Plant.

Experimental

**The first step procedure for 1-[2,2-bis(methoxy)ethyl]-5-(methoxy)-6-[(methoxy)-carbonyl]-4-oxo-1,4-dihydro-3-pyridinecarboxylic acid 4**

Methyl 4-methoxyacetoacetate (16 mL, 123.6 mmol), N,N-dimethylformamide dimethylacetal (DMF-DMA, 19.2 mL, 144.52 mmol) were stirred at room temperature for 1.5 h to form a brown mixture (Rᶠ = 0.14, ethyl acetate/petroleum ether = 5/1). The resulted mixture was added with methanol (40 mL) and aminoacetaldehyde dimethyl acetal (13.36 mL, 122.62 mmol). After stirring at room temperature for 1 h, the solution turned reddish and TLC showed a new point (Rᶠ = 0.56, ethyl acetate). The reaction was stopped and evaporated to give a red-brown oily liquid. 90 mL of methanol was then added to dissolve the mixture, followed by the
addition of dimethyl oxalate (36.68 g, 310.68 mmol). After the dimethyl oxalate was completely dissolved, the temperature was controlled at 25°C under an ice-water bath. After adding lithium hydride (1.72 g, 217.4 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 (R_f = 0.23, ethyl acetate). The reaction solution was cooled to −5°C and anhydrous lithium hydroxide (11.88 g, 247.6 mmol) was added while the temperature was controlled at 3–5°C. The reaction solution changes from brick red to orange-yellow suspension and reacted for 2 h. The reaction was quenched by the addition of 300 mL of 2N hydrochloric acid, and the reaction temperature was controlled below 5°C in an ice-water bath. Add 360 mL of ethyl acetate to the extraction, and raise the temperature to 20°C, filter the lower solid by filtration and discard it; collect the liquid phase and separate the liquid; add 180 mL of water to the organic phase, concentrate under reduced pressure, filter by suction, add a small amount of water was washed, and the filter cake was collected and dried in vacuo at 50°C to obtain 25.02 g of compound 4 as a colorless solid, R_f = 0.44 (dichloromethane/methanol = 40/1, containing 0.5% glacial acetic acid); The total reaction yield was 65%. 1H 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); 13C NMR (100 MHz, CDCl_3) δ = 174.86,

| Entry | EDCI | Solvent | Temp | Time (h) | Product (dolutegravir) | Product (cabotegravir) | Product (bictegravir) |
|-------|------|---------|------|----------|-----------------------|-----------------------|----------------------|
| 1b    | 7 equ. | DCM     | 50°C | 4        | –                     | –                     | –                    |
| 2     | 3 equ. | DCM     | 50°C | 4        | –                     | –                     | –                    |
| 3     | 3 equ. | CH_3CN  | 80°C | 6        | 56%                   | 90% (88%)             | 87%                 |
| 4     | 3 equ. | DCM/CH_3CN | 50°C | 14       | 28%                   | 24%                   | 27%                 |
| 5     | 3 equ. | DCM/CH_3CN | 50°C | 14       | 33%                   | 31%                   | 35%                 |
| 6     | 3 equ. | DME     | 80°C | 13       | 10%                   | –                     | –                    |
| 7     | 3 equ. | CHCl_3  | 70°C | 17       | trace                 | trace                 | trace               |
| 8     | 3 equ. | THF     | 65°C | 6        | trace                 | trace                 | trace               |

*aFor entries 2–8, the molar ratio of amine: DMAP: carboxylic acid = 1:2:0.4:2.2:1. 
*bFor entry 1, the molar ratio of amine: DMAP: carboxylic acid = 1:2:0:2.1, and no LiBr was added.
The second step procedure for dolutegravir intermediate 6

(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

A mixture of compound 4 (prepared according to lit. (14) procedure, 20 g, 63 mmol), methanesulfonic acid (1.2 mL, 18.5 mmol), acetic acid (100 mL) and (R)-3-amino-1-butanol (8 mL, 188 mmol) in 100 mL of CH3CN was refluxed for 15 h. The reaction solution was concentrated under reduced pressure. The residue was diluted with 200 mL of DCM and 100 mL of 1N hydrochloric acid. The organic phase was separated and the aqueous phase was extracted with DCM (100 mL×2). The combined organic phases were collected, and the aqueous layer was extracted twice with 100 mL of DCM. The combined organic layers were concentrated completely under reduced pressure. 50 mL of methanol was then added and the mixture was refluxed for 2 h. A yellow solid was precipitated from the solution. After being cooled to room temperature, the solid was collected by filtration and vacuum dried at 50°C to give 12.6 g of compound 6 as a pale yellow solid with a yield of 65%.

1H NMR (400 MHz, CDCl3) 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); 13C NMR (100 MHz, CDCl3) 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].

The second step procedure for cabotegravir intermediate 6a

Acetal (4, 1 g, 3.2 mmol) was dissolved in 50 mL of CH3CN. HOAc (1 mL) and CH3SO3H (0.3 mL, 5.7 mmol) and L-alaninol (0.4 mL, 9.4 mmol) in CH3CN (50 mL) were added at rt and the mixture was refluxed for 30 h. The mixture was concentrated, and the residue was re-dissolved in CH2Cl2 (100 mL). HCl (1 N, 50 mL) was added and the layers were separated. The aqueous layer was extracted with CH2Cl2 (150 mL×2) and the organic layers were combined and concentrated. MeOH (80 mL) was added and the resultant mixture was concentrated again. MeOH (50 mL) was added and the resultant mixture was heated at reflux for 2 h, gradually cooled to 20°C and held at 20°C for 15 h. The product was collected by filtration and dried under vacuum to give the title compound (6a, 746 mg, 80%) as a white solid. RF = 0.69 (ethyl acetate). 1H NMR (400 MHz, Chloroform-d) δ = 8.43 (s, 1H), 5.39 (dd, J = 9.9, 3.4 Hz, 1H), 4.56 (dd, J = 12.4, 3.4 Hz, 1H), 4.47–4.33 (m, 2H), 4.07 (s, 3H), 3.96 (dd, J = 12.1, 10.2 Hz, 1H), 3.71 (t, J = 7.5 Hz, 1H), 1.42 (d, J = 6.0 Hz, 3H). 13C NMR (101 MHz, CDCl3) δ = 176.16, 165.78, 153.85, 152.48, 143.04, 131.51, 116.04, 82.38, 73.15, 61.60, 55.74, 52.20, 17.02.

The second step procedure for bictegravir intermediate 6b

The bictegravir intermediate 6b was obtained in 62% yield using the above procedure. RF = 0.69 (DCM: CH3OH = 97:3). 1H NMR (400 MHz, DMSO-d6) δ = 8.70 (s, 1H), 5.40...
The third step procedure for dolutegravir

To a 1-L round flask was added intermediate (6, 10 g, 34 mmol), CH3CN (300 mL), EDCI (19.5 g, 100 mmol), DMAP (1.67 g, 13.65 mmol) and 2, 4-Difluorobenzylamine (5 mL, 40.9 mmol) in sequence. The resultant suspension was heated to 80°C in the oil bath and become clear after 2 h. Then LiBr (5.5 g, 60 mmol) was added. After 4 h, the solid was collected by filtration and dried under vacuum to give the title compound (dolutegravir, 7.9 g, 56%) as a white solid. 1H NMR (400 MHz, DMSO-

The third step procedure for cabotegravir

Compound (2 g, 6.8 mmol) was dissolved in 100 mL of CH3CN. EDCI (3.87 g, 20.2 mmol), DMAP (334 mg, 2.7 mmol), and 2, 4-difluorobenzylamine (1 mL, 8.2 mmol) were added to form a suspension solution. The mixture was heated to 80°C in an oil bath and dissolved completely after 2 h. Then LiBr (1 g, 12 mmol) was added. After 4 h, the product cabotegravir was collected by filtration and dried under vacuum to give the title compound (2.5 g, 90%) as a white solid. Rf = 0.31 (DCM: CH3OH = 97:3). 1H NMR (400 MHz, DMSO-

The third step procedure for bictegravir

The compound bictegravir can also be prepared in a similar procedure, using 2, 4, 6-trifluorobenzylamine to replace 2, 4-difluorobenzylamine. Yield: 87% as a white solid. Rf = 0.38 (DCM: CH3OH = 97:3). 1H NMR (400 MHz, CDCl3) δ = 12.32 (s, 1H), 10.25 (s, 1H), 8.25 (s, 1H), 6.61 (t, J = 8.0 Hz, 2H), 5.36 (dd, J = 9.4, 4.0 Hz, 1H), 5.25 (d, J = 9.5 Hz, 1H), 4.65–4.58 (m, 2H), 4.24 (dd, J = 13.1, 4.1 Hz, 1H), 3.95 (dd, J = 12.8, 9.3 Hz, 1H), 3.43 (d, J = 11.0 Hz, 1H), 2.12–1.89 (m, 6H). 13C NMR (101 MHz, CDCl3) δ = 171.13, 163.70, 160.45, 155.68, 140.21, 116.27, 115.78, 100.48, 100.23, 100.20, 100.18, 99.95, 99.93, 77.28, 74.24, 53.36, 51.27, 37.89, 30.59, 28.98, 27.85.

Summary

In this paper, we have developed a continuous three-step synthetic route to prepare dolutegravir, cabotegravir, and bictegravir. Compared to the original step-by-step procedure, this three-steps synthetic protocol is more practical and scalable, which has several advantages, including low cost, no chromatography, time-saving and better yield, and can be applied to the industrial manufacture of active pharmaceutical ingredient (API).

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Supporting information

1HNMR and 13CNMR spectrum for all of the synthesized compounds are available in the supporting information file.

Data availability

Data are available as part of the electronic supplementary material.
Research ethics
No requirement for completing an ethical assessment prior to conducting this research.

Animal ethics
No need for the ethics permission from local agent. Since no animal was used in this project.

Permission to carry out fieldwork
No fieldwork was conducted in this project.

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