An improved Synthetic Process of Two Key Intermediates and Their Application in the Synthesis of Lifitegrast

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Pharmaceut Fronts

Abstract

Benzofuran-6-carboxylic acid 2 and 2-(tert-butoxycarbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxylic acid 21 are two key intermediates for the synthesis of lifitegrast (1). The present study aimed to obtain lifitegrast from the key intermediates of 2 and 5,7-dichloro-2-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroisoquinoline-6-carboxylic acid (31), which had the same core structure as 21. In this study, the synthetic routes of 2 and 31 were explored. 2 and 31 were synthesized from 4-bromo-2-hydroxybenzaldehyde (25) and 2-(2,4-dichlorophenyl)ethan-1-amine (28), with the yields of 78 and 80%, respectively. The route avoided the harsh reaction conditions of generating 2 in a previous study and could more efficiently achieve the core structure of 5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxylic acid. Besides, the hydrolysis reaction conditions of preparing lifitegrast were also optimized. In this work, lifitegrast was obtained from 2 and 31 with high purity (>99.9%) and an overall yield of 79%, which was higher than the reported yield of 66%.
**Introduction**

Dry eye disease (DED), also known as keratoconjunctivitis sicca, is a multifactor disease (e.g., low blink rate) characterized by decreased tear secretion or increased tear evaporation while affecting the optical tables and tear glands.\(^1\) DED is classified into aqueous-deficient DED and evaporative dry eye as the major two subtypes. Studies have shown that DED affects approximately 5 to 50% of the global population.\(^2\) With the increase in age, the prevalence of DED is gradually increased, and the incidence of DED in Asian people is higher than that of other people.\(^3,4\)

Lifitegrast is a novel small-molecule integrin antagonist that can competitively block the binding of lymphocyte function-associated antigen-1 and intercellular adhesion molecule-1,\(^5\)–\(^11\) thus affecting the activation of T cells and the release of cytokines (proteins). It is a new chemical entity developed by Shire Development LLC and approved by the Food and Drug Administration in July 2016 for the treatment of the symptoms and signs of DED. It provides a new option for patients with DED.\(^12\) With the acceleration of the pace of life, a large number of people have the symptom of DED in China, shown in recent survey reports. So, it would be of great interest to develop a more economical route for process development researchers.

The retrosynthetic analysis for lifitegrast (1) is depicted in **Scheme 1**, and there were three intermediates, including benzofuran-6-carboxylic acid (2), benzyl (S)-2-amino-3-(3-(methylsulfonyl)phenyl propanoate (3), and 5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxylic acid (4).

A reported method for the synthesis of lifitegrast is illustrated in **Scheme 2**, which starts from the amidation reaction of compounds 3 and 21 (Boc-4).\(^13\) After removing the Boc group in a HCl solution, the resulting compound 23 is subjected to amidation with 2 to provide 24, which undergoes ester hydrolyzation to give the target compound 1. However, there are some challenges in the reported method: (1) the reaction conditions for the preparation of intermediate 2 were harsh and difficult to operate; (2) the yields of intermediate 4 were low and the process is difficult to scale up; and (3) the degradation of lifitegrast occurred in hydrolyzation, so the conditions should be controlled strictly. In this manuscript, we are dedicated to solving the problems mentioned above.

Intermediate 2 can be obtained in two ways (**Scheme 3**).\(^13,14\) One starts from commercial 2-(2-bromoethyl)-1,3-dioxolane 5, through a substitution reaction, Wittig olefination reaction, cyclization reaction, and amide hydrolysis reaction to give intermediate 2 (route A, **Scheme 3**). However, the reaction time of the route A was too long, and the operation of the reaction was complex. The other way employed the inaccessible and expensive 6-hydroxy-2(3H)-benzofuranone 9 as the key material, which was converted to intermediate 2 through five steps, including a substitution reaction, a reduction reaction, dehydration, carbonyl insertion, and an ester

![Scheme 1](image1.png)

**Scheme 1** Retrosynthetic analysis of lifitegrast (1).

![Scheme 2](image2.png)

**Scheme 2** Original approach to synthesizing lifitegrast.
hydrolysis reaction (route B, Scheme 3). Unfortunately, the operation is complicated, and the reaction time is long, while the introduction of carboxyl groups requires palladium acetate in the fourth step, which may have heavy metal palladium residual problem.

Compound 18 was a derivative of intermediate 4. Zeller et al. achieved 18 (Ph3C-4) from two routes (routes C and D, Scheme 4) using 3,5-dichlorobenzaldehyde (14) as a starting material. As for route C, the second step involves cyclization to construct the tetrahydroisoquinoline ring at high temperature in a sealed tube without solvent. This step required a high reaction vessel and had a risk of explosion. In addition, the yield of the first step was only 35%, which severely limited the practicability of the route. Route D also had some insufficiency, including complex operations that are not conducive to actual production. Besides, it would be dangerous to reduce the isoquinoline ring of compound 20 under high-pressure hydrogen (the third step). Route D not only had a low yield but also used expensive catalysts. Thus, it is crucial to explore a method to construct the tetrahydroisoquinoline ring more reasonably and economically.

As for the preparation of lifitegrast, intermediate 3 coupled with compound 21 (Boc-4) under HATU and triethylamine in N,N-dimethylformamide. After removing the Boc group to get compound 23, it was coupled with compound 2 to get compound 24, followed by hydrolysis to get the final product compound 1. During the last step, there were three impurities (Fig. 1; impurity 1, 2, and 3) produced when 2 mol/L sodium hydroxide was used for hydrolysis or hydrogenation with Pd/C to remove benzyl.

In this article, we will focus our effort on solving the synthetic problems given bellow: (1) avoid the harsh reaction conditions of compound 2; (2) design a more efficient route to synthesize compound 4; (3) optimization of the hydrolysis condition of compound 1 to improve the final product’s purity.

Results and Discussion

Synthesis of Intermediate 2

We designed a novel synthetic route to obtain 2. First (Scheme 5, route E), compound 25 and bromoacetic acid are coupled to afford 26 through Williamson ether synthesis.
Compound 26 underwent a ring-closure reaction to give 6-bromobenzofuran 27, which was reacted with CO2 in the presence of n-butyllithium to give the target product. However, 26 was obtained with an unacceptable yield of 30%, despite several attempts to optimize the reaction conditions, and 25, as a starting material, remained even though the reaction time lasted for 24 hours at 75°C. To solve this problem, 26 was prepared through etherification of 25 with methyl 2-chloroacetate as the initial step to get 25-1 followed by its ester hydrolysis (Scheme 5, route F,). Fortunately, all the efforts were effective, as the yield of 26 was improved, reaching 90%.

**Synthesis of Intermediate 31**

We aimed to obtain 4 by getting rid of the use of platinum catalysis and acquiring an improved yield. The synthesis route of 31 (CF3CO-4) was designed in this work (Scheme 6). In this route, N-(2,4-dichlorophenethyl)-2,2,2-trifluoroacetamide 29 was obtained via a substitution amidation reaction of 2-(2,4-dichlorophenethyl)ethan-1-amine 28 and trifluoroacetic anhydride, and converted to 1-(5,7-dichloro-3,4-dihydroisoquinolin-2(1H)-yl)-2,2,2-trifluoroethan-1-one 30 via a Pictet–Spengler reaction with paraformaldehyde in a mixture of sulfuric acid and acetic acid. Then 31 (Tfa-4) was synthesized by a reaction of compound 30 with carbon dioxide under n-butyllithium.

The substrates of the Pictet–Spengler reaction were screened (Table 1). When R = H (compound 28), no satisfactory results were achieved (Table 1, entry 1). When R = t-butoxycarbonyl, (a Boc-protecting group), there was also no reaction. Then, some strong electron-withdrawing groups were tried, with acceptable yields (Table 1, entries 3–5). Considering the convenience of removing the protective groups, we finally chose trifluoromethyl as the protective group for the subsequent reaction.

**Table 1** Substituents screening of the Pictet–Spengler reaction

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>No reaction</td>
</tr>
<tr>
<td>2</td>
<td>t-Butoxycarbonyl</td>
<td>No reaction</td>
</tr>
<tr>
<td>3</td>
<td>Trifluoroacetyl</td>
<td>98.54%</td>
</tr>
<tr>
<td>4</td>
<td>Tosyl</td>
<td>96.24%</td>
</tr>
<tr>
<td>5</td>
<td>4-Nitrobenzenesulfonyl</td>
<td>96.90%</td>
</tr>
</tbody>
</table>

Note: Reagents and conditions: reaction substrate (2.67 mmol), paraformaldehyde (10.68 mmol), concentrated sulfuric acid: acetic acid = 3:2 (V:V), 10 mL, 8 hours, r.t.
The condition of the Pictet–Spengler reaction was further optimized. The ratios of concentrated sulfuric acid to acetic acid (V:V), as well as the reaction time, were screened (►Table 2). We found that when the proportion of concentrated sulfuric acid was too low, the reaction time would be prolonged or it would not react (►Table 2, entries 4 and 5). When the ratio is too high, the reaction time would be shortened, but the raw material may be decomposed, affecting the yield (►Table 2, entry 1). Finally, the volume ratio of 3:2 was selected as the optimal condition (►Table 2, entry 2).

**Synthesis of Lifitegrast (1)**

We noticed that the original synthesis process has a problem to be resolved: compound 32 (►Fig. 2), which is a byproduct of the reaction between compound 4 and HATU, is difficult to be converted to amide ester 22 and the yield was only 70%.

![Structure of intermediate 32](image)

**Table 2** Effect of different ratios of sulfuric acid to acetic acid on the synthesis of compound 30

<table>
<thead>
<tr>
<th>Entry</th>
<th>$V_{\text{sulfuric acid}}:V_{\text{acetic acid}}$</th>
<th>Time (h)</th>
<th>Yield of 30 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2:1</td>
<td>4.00</td>
<td>81.58</td>
</tr>
<tr>
<td>2</td>
<td>3:2</td>
<td>4.00</td>
<td>98.79</td>
</tr>
<tr>
<td>3</td>
<td>1:1</td>
<td>9.00</td>
<td>83.49</td>
</tr>
<tr>
<td>4</td>
<td>1:2</td>
<td>48.00</td>
<td>No reaction</td>
</tr>
<tr>
<td>5</td>
<td>1:4</td>
<td>48.00</td>
<td>No reaction</td>
</tr>
</tbody>
</table>

**Table 3** Reaction condition optimization of intermediate 22

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base (equiv.)</th>
<th>Catalyst (equiv.)</th>
<th>Time (h)</th>
<th>Yield of 22 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Et3N (2)</td>
<td>HATU (1)</td>
<td>28.00</td>
<td>45.52</td>
</tr>
<tr>
<td>2</td>
<td>Et3N (3)</td>
<td>HATU (1)</td>
<td>20.00</td>
<td>94.19</td>
</tr>
<tr>
<td>3</td>
<td>Et3N (4)</td>
<td>HATU (1)</td>
<td>4.75</td>
<td>97.33</td>
</tr>
<tr>
<td>4</td>
<td>Et3N (5)</td>
<td>HATU (1)</td>
<td>5.00</td>
<td>98.38</td>
</tr>
<tr>
<td>5</td>
<td>Et3N (6)</td>
<td>HATU (1)</td>
<td>4.75</td>
<td>96.42</td>
</tr>
<tr>
<td>6</td>
<td>Et3N (5)</td>
<td>HATU (0.5)</td>
<td>6.00</td>
<td>81.63</td>
</tr>
<tr>
<td>7</td>
<td>Et3N (5)</td>
<td>HATU (0.75)</td>
<td>6.00</td>
<td>94.13</td>
</tr>
<tr>
<td>8</td>
<td>Et3N (5)</td>
<td>HATU (2)</td>
<td>30.00</td>
<td>50.60</td>
</tr>
<tr>
<td>9</td>
<td>Et3N (5)</td>
<td>HATU (3)</td>
<td>30.00</td>
<td>40.81</td>
</tr>
<tr>
<td>10</td>
<td>DMAP (1.5)</td>
<td>EDC·HCl (1.1)</td>
<td>10.00</td>
<td>No reaction</td>
</tr>
</tbody>
</table>

So, we contributed to increasing the yield of 22 and reducing the reaction time.

Condensing reagents, the equiv. amounts of HATU and triethylamine of the reaction were screened. We found the yield of 22 was still lower and the reaction time was longer when the amount of catalyst was increased (►Table 3, entries 8 and 9). Using a base is necessary for the reaction to achieve a better yield of 22. While we tried to change the equiv. of the base, a high yield of 22 (98%) could be provided in a short reaction time with the 5 equiv. base and 1.0 equiv. HATU (►Table 3, entry 4). Finally, under the improved reaction conditions, the yield of 22 was improved from 75% to 98%. Meanwhile, the reaction time was reduced to 5 hours.

As for the synthesis of the target product (1), the conditions for hydrolysis of benzyl ester derivative 24 were screened (►Table 4). In the beginning, Pd/C (►Table 4, entry 1) or sodium hydroxide solution (►Table 4, entry 2) was tried. Unfortunately, the desired products had a low purity and were contaminated with impurities 1–3, which could not be easily removed by recrystallization. Suffering from the above issues of the economy of Pd/C and the residue of heavy metals, a hydrolysis reaction was chosen to obtain the target product. Subsequently, sodium carbonate, potassium carbonate, cesium carbonate, sodium hydroxide, and lithium hydroxide at a concentration of 1 mol/L were screened. Our
data showed that the use of K₂CO₃ solution (1 mol/L) was
effective and should be selected as the optimal condition for
debenzylation. Crude product 1 was purified by recrystalli-
zation of isopropanol and n-heptane (HPLC: >99.9%) to
achieve lifitegrast.

Conclusion
In this work, we have developed a novel and practical method
for the synthesis of 2 starting from 4-bromo-2-hydroxyben-
zaldehyde 25. The synthesis was accomplished in three steps
with a total yield of 78%. In addition, we used 2-(2,4-
dichlorophenyl)ethylamine as a raw material to obtain the
key intermediate 31 in three steps with a yield of 80%. With
an optimized synthetic route in hand, lifitegrast was
obtained a total yield of 79% (calculated by compound
21). The total synthesis route of lifitegrast including the two key
intermediates is shown in Scheme 7.

Experimental
General Procedures
Unless specified otherwise, all starting materials, reagents,
and solvents are commercially available (Shanghai Haoyuan
Chemexpress Co., Ltd., Shanghai, China; Bidepharmatech
Ltd., Shanghai, China). Thin layer chromatography (TLC) on
silica gel GF254 was used to monitor the progress of all
reactions. Melting points were obtained on a melting point
apparatus (WRS-2A, Shanghai INESA Scientific Instruments
Co., Ltd., Shanghai, China) and were uncorrected. The electro-
spray ionization (ESI) mass spectra were collected on a
Waters ZQ2000 spectrometer (Waters, United States). Com-
pound purity was determined by high-performance liquid
chromatography (HPLC) (Waters e2695 HPLC, 2998 PAD,
Waters Corp., Milford, MA, United States). Nuclear magnetic
resonance (NMR) spectra were recorded in chloroform-
D₆ or dimethyl sulfoxide-D₆ on a 400 MHz or 600 MHz Bruker NMR
spectrometer (Bruker Bio-Spin, Rheinstetten, Germany) with
tetramethylsilane as the internal reference, and all chemical
shifts were reported in parts per million (ppm).
The purity of the products was measured by a HPLC-peak
area normalization method using a Welch Ultimate XB
C18 column (4.6 mm × 250 mm, 5 µm) under the following
conditions: mobile phase A (ACN) and phase B (0.05% TFA [v/v]
water solution) with gradient elution of 0 to 50 minutes: 80 to
10% B; 50 to 51 minutes: 10 to 80% B; 51 to 60 minutes: 80% B.
The flow rate was 1 mL/min; the column temperature was 30°
C; and the detection wavelength was 220 nm.

Benzofuran-6-carboxylic Acid (2)
Compound 27 (1.77 g, 8.98 mmol) was suspended in
anhydrous tetrahydrofuran (20 mL) and was slowly added
2.5 mol/L n-butyl lithium in tetrahydrofuran (7.2 mL, 17.96 mmol) at –68°C under a nitrogen atmosphere. After stirring for 1 hour, benzyl chloroformate (3.06 g, 19.79 mmol) was slowly added at the same temperature, and the reaction mixture was stirred for 2 hours. After completion of the reaction, 40 mL of 2 mol/L NaOH solution was added and stirred at 70°C overnight. Then, the mixture was adjusted with 1 mol/L HCl solution, extracted with ethyl acetate (100 mL), washed with brine (50 mL), and concentrated under reduced pressure. The residue was purified by silica column chromatography (100% petroleum ether) to give compound 2 (1.2 g, yield 82%). mp: 186.8–188.2°C. 1H NMR (400 MHz, DMSO-d6) δ 7.63 (d, J = 7.6 Hz, 1H), 7.54 (d, J = 8.1 Hz, 1H), 2.32 (dd, J = 11.3, 4.1 Hz, 1H), 7.21 (t, J = 7.5 Hz, 1H), 7.10 (s, 1H). HPLC: tR = 10.46 minutes, 96.30% purity. ESI-MS (m/z) calcd for [M – H]− 161.0317; found 161.03. HR-MS (ESI) calcd. for [M – H]− 161.0317, found: 161.0232.

5,7-Dichloro-2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinoline-6-carboxylic acid (31)

Compound 30 (11.0 g, 36.90 mmol) and N,N,N′,N′-tetramethylthelylenediamine ([TMEDA], 8.58 g, 73.8 mmol) were suspended in anhydrous tetrahydrofuran (200 mL) and slowly added 2.5 mol/L n-butyl lithium in tetrahydrofuran (29.52 mL, 73.80 mmol) at –68°C under a nitrogen atmosphere. After stirring for 2 hours, the excess carbon dioxide gas was slowly bubbled into the reaction solution over 1 hour, and a well-dispersed suspension was formed. The mixture was stirred for an additional 4 hours, then warmed to 0°C over 1 hour after monitoring the completion of the reaction using TLC. Water was added and the mixture was added 2 mol/L HCl solution (100 mL), stirred at room temperature for 2 hours, extracted with ethyl acetate (200 mL), washed with brine, and concentrated under reduced pressure to give 31 as a yellowish solid (10.36 g, yield 82%). mp: 174.6–175.5°C. 1H NMR (400 MHz, DMSO-d6) δ 7.59 (d, J = 7.9 Hz, 1H), 7.71 (s, 1H), 7.67 (d, J = 6.7 Hz, 1H), 7.57 (t, J = 7.7 Hz, 1H), 7.38 (d, J = 6.2 Hz, 2H), 7.05 (d, J = 1.5 Hz, 1H), 4.87–4.76 (m, 2H), 4.73–4.59 (m, 1H), 3.93–3.56 (m, 2H), 3.30 (dd, J = 14.2, 4.5 Hz, 1H), 3.15 (s, 3H), 3.03 (dd, J = 13.9, 10.6 Hz, 1H), 2.75 (d, J = 16.6 Hz, 2H). 13C NMR (DMSO-d6) δ 172.5, 169.9, 164.0, 154.1, 148.2, 141.1, 139.5, 137.5, 134.9, 132.1, 131.6, 129.7, 129.3, 129.1, 128.8, 126.8, 128.2, 125.7, 125.5, 122.4, 121.9, 110.8, 107.3, 53.3, 59.1, 53.5, 44.1, 36.8, 21.5. HPLC: tR = 18.80 minutes, 100% purity. ESI-MS (m/z): calcd. for [M + H]+ 615.0681; found 614.90. HRMS (ESI) calcd. for [M + H]+ 615.0681; found: 615.0750.

Supporting Information

Full experimental detail, as well as 1H, 13C NMR, HRMS spectra of compound 26, 27, 2, 29, 30, 31, 22, 23, 24, and 1 can be found via the ‘Supporting Information’ section of this article’s webpage.

Conflict of Interest

None declared.

Reference

11. Keating GM. Lifitegrast ophthalmic solution 5%; a review in dry eye disease. Drugs 2017;77(02):201–208
15. Xu W, Gong X, Odidov A, et al. Scalable process for making 5,7-dichlorotetrahydroisoquinoline-6-carboxylic acid using...
