Research Article

Ultrasonic-Assisted Synthesis of Two \( t \)-Butoxycarbonylamino Cephalosporin Intermediates on SiO\(_2\)

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Herein, we describe a facile and high efficient strategy for the synthesis of two forms of the \( 7\beta-t \)-butoxycarbonylamino-3-chloromethyl-3-cephem-4-carboxylates using ultrasonic irradiation. By SiO\(_2\) as weak Lewis acid catalyst, 4-methoxybenzyl \( 7\beta-t \)-butoxycarbonylamino-3-chloromethyl-3-cephem-carboxylate (Boc-ACLE) and benzhydryl \( 7\beta-t \)-butoxycarbonylamino-3-chloromethyl-3-cephem-4-carboxylate (Boc-ACLH) were successfully synthesized through the efficient protection of the \( N-t \)-butoxycarbonyl (N-Boc), and the reactions occurred at low temperature requiring short reaction times and exhibiting excellent isolated yields (96% and 96.2%, resp.). The advantages of this reaction route including the usage of economical reagents and mild reaction conditions and high isolated yield make the two significant \( t \)-butoxycarbonylamino cephalosporin intermediates possible in large-scale production.

1. Introduction

\( 7\beta-t \)-Butoxycarbonylamino-3-chloromethyl-3-cephem-4-carboxylates serving as the extremely important intermediates are employed to synthesize the commonly used antibiotics, cephalosporin. As shown in Figure 1, the chemical structure of cephalosporin intermediate contains a chemically active 3-chloromethyl-group at 3-side chain, which can be easily coupled with drug-active functional groups forming cephalosporin derivatives, especially the fourth-generation cephalosporins Cefoselis sulfate [1, 2]. However, owing to their presence in various biological environments, the amine functionality at 7 positions needs to be protected which is one of the most challenging issues in this synthetic chemistry. Among the current amine protection methods, the \( t \)-butoxycarbonyl (Boc) protection has been considered as the useful one because of its excellent stability regarding catalytic hydrogenation and extreme resistance to underlying basic or nucleophilic reactions [3].

In the past decade, multiple processes have been available to introduce the Boc protecting group using Boc\(_2\)O (di-\( t \)-butyl dicarbonate) to synthesize 4-methoxybenzyl \( 7\beta-t \)-butoxycarbonylamino-3-chloromethyl-3-cephem-carboxylate (Boc-ACLE). The synthesis of Boc-ACLE has been reported by Lee et al. [4] that they dissolved 4-methoxybenzyl \( 7\beta\)-amino-3-chloromethyl-3-cephem-carboxylate (ACLE: HCl) into CH\(_2\)Cl\(_2\) solvent first. Next, the mixture was reacted with di-\( t \)-butyldicarbonate (Boc\(_2\)O) in the presence of a catalyst of \( N-(t \)-trimethylsilyl) acetamide (NSA). However, the catalyst of NSA has numerous drawbacks for the practical reaction such as its high costs, sensitivity to moisture, the low yield (51%), and other further troubles from processing (number 1, Table 1). Recently, Du [5] used tetrabutylammonium bromide (TEBA) as phase transfer catalyst to synthesize Boc-ACLE compound, where ACLE.HCl was reacted with Boc\(_2\)O in weak basic aqueous solution at room temperature. However, Boc\(_2\)O was easily decomposed under basic aqueous solution environment in which Boc\(_2\)O was largely lost, and the reaction required long reaction times (10 h) giving a yield of 80% (number 2, Table 1). In 2010, Lin [6] reported that the Boc-ACLE could be synthesized via a catalyst-free method starting from the reaction of ACLE.HCl and Boc\(_2\)O in tetrahydrofuran (THF). Using triethylamine as acid binding agent, they finally got a high yield of 87.21%.

Unfortunately,
Another $t$-butoxy carbonyl amino cephalosporin intermediate, benzhydryl $7\beta$-$t$-butoxy carbonyl amino-3-chloromethyl-3-cephem-4-carboxylate (Boc-ACLH), was typically synthesized by Daï’s group using 7-aminocephalosporanic acid (7-ACA) as starting material following the approach outlined in Scheme 1 [7]. Key intermediate 1 was obtained when 7-ACA was hydrolyzed in basic solution at $\text{-30}^\circ\text{C}$. Sequentially, compound 1 reacted with Boc$_2$O to protect amine group catalyzed by TEBA in NaCO$_3$-H$_2$O-acetone solution to produce compound 2, which was then treated with diphenyl-diazomethane in $n$-hexane forming compound 3. In the last step, the hydroxyl group of compound 3 was substituted by chlorine in presence of phosphorus pentachloride and pyridine in dichloromethane at $-45^\circ\text{C}$ to get the desired product 4b (Boc-ACLH). It is apparent that this synthetic route includes tedious steps, complex experimental operations, and harsh conditions. Alternative synthetic strategies with advantages such as short steps, mild conditions, and high efficiency are keen to be developed.

It can be deduced from the above-mentioned cases that the choice of raw materials, catalyst, solvent, and the reaction conditions play significant roles in synthesizing the two $t$-butoxy carbonyl amino cephalosporin intermediates. Generally, in terms of producing these N-Boc derivatives, there are two kinds of catalytic methods (i.e., using base catalyst and Lewis acid catalyst). There have been plenty of base-catalyzed B-protection studies reported by using Boc$_2$O/DMAP system; however, the high toxicity of DMAP was not negligible [8]. What is worse, the base-catalyzed reactions reported often resulted in the generation of byproducts like isocyanate, urea, and $NN$-$di$-Boc derivatives [9, 10]. Other catalytic approaches involving Lewis acids, such as La(NO$_3$)$_3$-6H$_2$O, ZrCl$_4$, LiClO$_4$, Cu(BF$_4$)$_2$-6H$_2$O, Zn(ClO$_4$)$_2$-6H$_2$O, Yttria-Zirconia, HClO$_4$/SiO$_2$, montmorillonite K-10, amberlyst-15, H$_3$PW$_12$O$_{40}$, sulfamic acid, I$_2$, and hexafluoroisopropanol (HFIP), have been attempted [11–15]. As the increasing demand of mild reaction conditions encourages the development of greener route to achieve these significant synthetic works in recent years, the sonochemistry has offered a solution that a large variety of organic/medical transformations proceeded using this more efficient and facile method [16]. For instance, Amira et al. [17] have applied ultrasonic irradiation technology for the N-Boc protection which not only eliminated the harsh reaction conditions but also assisted in impressive yield. Dighe and Jadhav [18] found microwave assisted chemoselective method could shorten the reaction time for the generation of N-Boc products with excellent isolated yield. It is worth mentioning that solvent-free synthetic strategies using ionic liquid catalyst have emerged which show great potential for N-Boc formation [19, 20].

In this paper, we aim at proposing an optimum route for the synthesis of the two $t$-butoxy carbonyl amino cephalosporin intermediates based upon the synergetic effect of ultrasonic assistance and SiO$_2$ catalyst. The efficiency of our route for N-Boc formation of our targeted product was analyzed and the mechanism of the proposed ultrasonic-assisted strategy for N-Boc protection of amines was investigated.
Table 2: The effect of SiO$_2$ and ultrasound on the yields of the Boc-ACLE.

<table>
<thead>
<tr>
<th>Number</th>
<th>SiO$_2$</th>
<th>Ultrasound</th>
<th>Reaction time</th>
<th>Yield%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>×</td>
<td>×</td>
<td>6 h</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>✓</td>
<td>×</td>
<td>6 h</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>×</td>
<td>✓</td>
<td>6 h</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>✓</td>
<td>✓</td>
<td>30 min</td>
<td>96</td>
</tr>
</tbody>
</table>

Note: ×: no; ✓: yes.

In addition, the products were confirmed by spectrometric methods. In comparison to conventional methods, our route may offer a highly efficient and methodologically simple catalytic process to introduce N-Boc protecting group into our desired products.

2. Experimental Section

2.1. General Information. ACLE-HCl and ACLH-HCl were purchased from Shanghai Arbor Chemical Co., Ltd. The other reagents and solvents were obtained from Sigma-Aldrich and used as received without any further purification. All reactions were monitored by thin-layer chromatography (TLC) using commercial silica gel plates. IL-120DTH ultrasonic bath was purchased from Shanghai Jnlsh Testmart Co., Ltd. The purity was measured by high performance liquid chromatography (HPLC) on Agilent 1,100 series. The liquid chromatographic system was equipped with an ODS-3 C18 column (GL Science Co. Ltd., 180 × 4.6 mm i.d., 5.0 μm). Melting points were observed on YRT-3 Melting Point Tester and were uncorrected. NMR spectra were determined on Bruker AV300 in DMSO-$_d_6$ with TMS as internal standard for $^1$H NMR (300 MHz) and $^{13}$C NMR (75 MHz), respectively. HRMS were carried out on an Agilent 6230-TE/TOF MS mass spectrometer.

2.2. General Procedure for t-Butoxycarbonylamino Cephalosporin Intermediates. ACLE-HCl (5a) or ACLH-HCl (5b) as starting reagent was added to CH$_2$Cl$_2$ (200 mL) firstly; triethylamine was then introduced into the mixture to neutralize the pH at 7-8 at 0–5°C. Then, appropriate amounts of SiO$_2$ and Boc$_2$O were added to the mixture for the appropriate time of bath ultrasonication at 0–5°C (Table 2). After completion of the reaction indicated by thin-layer chromatography (TLC), the mixture was poured into water and the organic phase was separated followed by washing with brine water and drying with anhydrous sodium sulfate under vacuum evaporation. The crude product was purified by column chromatography over silica gel to yield the desired compounds.

4-Methoxyphenyl 7β-t-Butyl-carbonylamino-3-chloromethyl-3-cephem-4-carboxylate (4a). 4a: Solid, m.p. 63–65°C. The yield was 96.0%. HPLC assay confirmed the purity of 4a was 98.5%. The column oven temperature was set at 295 K. The mobile phase consisted of methanol and deionized water (75:25, v/v) and flowed through the column in 15 min with the flow rate of 1.0 mL/min. Photodiode array detection was used to detect the Boc-ACLE at the wavelength of 254 nm. $^1$H and $^{13}$C NMR refer to Tables 3 and 4. ESI–HRMS: calcd. for C$_{26}$H$_{33}$ClN$_2$O$_5$S ([M–H]$^-$) 467.1049, found 467.1045.

Benzhydryl 7β-t-Butoxycarbonylamino-3-chloromethyl-3-cephem-4-carboxylate (4b). (4b): Solid, m.p. 140–142°C (decomposed). The yield was 96.2%. (HPLC assay of 4b was 98.1%. The HPLC method of 4b was similar to the 4a.) $^1$H NMR refer to Table 5. $^{13}$C NMR (75 MHz, DMSO-$d_6$): δ 170.86, 165.01, 161.06, 159.34, 135.70, 130.25, 28.93, 128.16, 126.84, 126.43, 125.29, 124.83, 113.77, 67.28, 59.18, 57.96, 57.86, 55.06, 43.72, 41.55, 40.33, 26.09. ESI–HRMS: calcd. for C$_{26}$H$_{33}$ClN$_2$O$_5$NaS ([M+Na]$^+$) 537.1221, found 537.1243.

Table 3: Comparison of $^1$H NMR data (4a).

<table>
<thead>
<tr>
<th>Lee et al. reported (500 MHz, CDCl$_3$)$^a$</th>
<th>Synthetic 4a (300 MHz, DMSO-$d_6$)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.45 (s, 9H)</td>
<td>1.37 (s, 9H)</td>
</tr>
<tr>
<td>3.47 (d, 1H, J = 18.2 Hz),</td>
<td>3.48 (d, 1H, J = 17.91 Hz)</td>
</tr>
<tr>
<td>3.66 (d, 1H, J = 18.2 Hz)</td>
<td>3.64 (d, 1H, J = 9.65 Hz)</td>
</tr>
<tr>
<td>3.73 (s, 3H)</td>
<td></td>
</tr>
<tr>
<td>4.55 (d, 1H, J = 12.1 Hz)</td>
<td>4.41 (d, 1H, J = 11.31 Hz)</td>
</tr>
<tr>
<td>4.95 (d, 1H, J = 4.6 Hz)</td>
<td>4.49 (d, 1H, J = 11.07 Hz)</td>
</tr>
<tr>
<td>5.23 (s, 2H)</td>
<td>5.07 (d, 1H, J = 3.93 Hz)</td>
</tr>
<tr>
<td>5.23 (s, 2H)</td>
<td>5.12 (d, 1H, J = 12.09 Hz)</td>
</tr>
<tr>
<td>5.61 (dd, 1H, J = 5.0, 9.5 Hz)</td>
<td>5.19 (d, 1H, J = 11.73 Hz)</td>
</tr>
<tr>
<td>5.68–6.91 (dt$_{app}$, 2H)</td>
<td>5.48 (s, 2H)</td>
</tr>
<tr>
<td>6.89–6.92 (dt$_{app}$, 2H)</td>
<td></td>
</tr>
<tr>
<td>7.33–7.36 (dt$_{app}$, 2H)</td>
<td>7.32–7.34 (dt$_{app}$, 2H)</td>
</tr>
</tbody>
</table>

$^a$Data taken from [4]; $^b$this work.

3. Results and Discussion

In present work, as described in Scheme 2, we selected inexpensive ACLE-HCl (5a) or benzhydryl 7β-amino-3-chloromethyl-3-cephem-carboxylate (ACLE-HCl, 5b) as starting materials. They were neutralized by triethylamine in CH$_2$Cl$_2$ solvent at 0–5°C for 30 min, and then the free base (ACLE or ACLH) was reacted with Boc$_2$O by N-acylation reaction at presence of SiO$_2$ under ultrasonic irradiation for 30 min to form 7β-t-butoxycarbonylamino-3-chloromethyl-3-cephem-4-carboxylates (4a, Boc-ACLE, or 4b, Boc-ACLE).

To overcome the exothermicity of the reaction while carrying out the reaction on a large-scale operation, the reaction temperature should be controlled and determining an appropriate solvent which depends on substrate reactivity and solubility seems very important [14]. As the exothermic phenomenon was found in our N-acylation reaction, therefore, to maintain the stability of thermos-sensitive cephalosporin compounds, we chose dichloromethane as solvent and kept the reaction temperature below 10°C.
The effect of SiO$_2$ and ultrasound on the yields of the formation of Boc-ACLE was recorded in Table 2. The proposed route without both catalyst and ultrasonic irradiation took 6 hours to achieve the reaction but the resulting isolated yield was only 15%. When Lewis acid catalyst SiO$_2$ was added to the route, the yield of the reaction was improved up to 35%. To find the synergic effect of ultrasound and SiO$_2$, another control reaction using ultrasound alone was performed under the same reaction time without SiO$_2$, that a low yield of 40% was found. Notably, when the Lewis acid catalyst of SiO$_2$ and ultrasonic power were utilized together, an overwhelming yield of 96% was discovered after 30 mins. The similar impressive result also happened to synthesize Boc-ACLH (the yield is 96.2%) using both ultrasound and SiO$_2$ which implied these results were attributed to the synergy effect of ultrasonic irradiation and SiO$_2$.

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To explain this mechanism, we believe that, as a result of the electronic and steric molecular structure of 7β-amino-3-chloromethyl-3-cephem-4-carboxylates, the amine group is low nucleophilic which could be activated by the weak Lewis acid support body of SiO$_2$ under ultrasonic irradiation. Figure 2 describes the structure of SiO$_2$ surface. Tran et al. [21] believed that the adjacent hydroxyl and double hydroxyl groups of SiO$_2$ surface showing weak acidity could activate the carbonyl compounds. We hypothesize that the surface of SiO$_2$ support body formed the polyhydroxyl matrix and the (Boc)$_2$O was attacked by the free base (ACLE or ACLH) on the matrix which was consistent with the conclusion of Sunseri et al. [22]. Ultrasonic vibrations enable the formation of intermolecular extrusions and confusions which has a significant influence on carbon dioxide generated from mono-t-butyl carbonate (compound 6). We believe there are two reasonable mechanisms of facilitating the nucleophilic attack of amine group on the carbonyl group involved during the process; the former is that with the bubble formation and breakdown; the cavitation contributes to the efficient generation of the N-Boc amide (Boc-ACLE or BOC-ACLH); the latter is based on the reaction equilibrium that the continuous escape of CO$_2$ results in the reaction preferring a forward tendency (Scheme 3).
In summary, the ultrasonic-assisted approach catalyzed by SiO$_2$ for the synthesis of two $t$-butoxycarbonylamino compounds at low temperature was investigated. By means of the synergetic effect of the ultrasound and SiO$_2$, the designed reaction system showed a high efficiency on the desired products without any harsh conditions. Additionally, compared with existing production processing of the two $t$-butoxycarbonylamino cephalosporin intermediates, the work described in this study allows for stabilization of the real-time reaction temperature and low cost for the reaction. Importantly, this study greatly advances not only the practical cephalosporin production field but also the field of other synthetic drugs and their derivatives requiring Boc protection of amines.

### Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

### Acknowledgments

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