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Application of Continuous Flow in Tazobactam Synthesis

Shuhao Zhou, Yunting Xin, Jiasheng Wang, Chengjun Wu,* and Tiemin Sun*

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ABSTRACT: Tazobactam is a β-lactamase inhibitor. In this work, a combination of continuous flow and batch experiments for the synthesis of tazobactam has been developed. The first three steps and the preparation of the peroxyacetic acid are continuously carried out in the microreactors, which improves the procedure safety and efficiency. There is also a final step of the deprotection reaction in the microreactor, which can increase the yield and reduce the formation of impurities. Under optimized process conditions, the total yield of the target product reached 37.09% (30.93% in batch). The continuous flow method not only greatly reduces the reaction time but also significantly improves procedure safety and increases the yield.

KEYWORDS: *continuous flow, tazobactam, synthesis, industrial process*

1. INTRODUCTION

Tazobactam is a β-lactamase inhibitor. It was first marketed in the United States in 1992 in combination with piperacillin. Although it has little antibacterial activity itself, but it can inhibit the bacterial β-lactamase activity to prevent bacteria from hydrolyzing β-lactam antibiotics.[1] Due to its low toxicity and strong activity, a variety of combinations of tazobactam and β-lactam antibiotics have been developed and applied in clinics widely.[2]

In our previous work, an industrialized route of tazobactam reported in the literature has been optimized. As shown in Scheme 1, key intermediate **5** was originally synthesized using a three-step process from 6-aminopenicilanic acid (6-APA) as a starting material. Potassium bromide and sulfuric acid were used to take the place of the corrosive and toxic hydrobromic acid or bromine. Next, peroxyacetic acid and benzophenone hydrazine were used for esterification in most literature studies,[3] but an overoxidation product was produced and a higher concentration of peroxyacetic acid (40%) was needed to increase the yield, which posed extreme safety hazards for industrial production (Scheme 2). Thus, diphenylmethanol was used for esterification and hydrogen peroxide for the oxidation reaction in our scheme, which increased the yield and improved production safety. Next, **9** was produced by **5** through three steps that were similar to the literature studies. Then, 1,2,3-triazole was introduced by an anion resin that can be recycled; it can avoid using azide and acetylene and has greater value in terms of economy and environmental protection. Finally, the target product tazobactam was obtained by traditional methods.

Each step has been further optimized in the batch experiments with our efforts, and the total yield has been increased from about 20% reported in the literature to 30.93%; the production process can be carried out stably including the yield, purity, safety, economy, and environmental protection, and other issues have been optimized to the maximum. We have scaled up the production process to industrial production in factories.

In recent years, the application of flow chemistry technology to the field of drug synthesis and production has become the focus of much attention.[4] The flow reactors have unique characteristics that can improve the mixing effect, provide precise temperature control, and greatly shorten the cycle of process screening and process amplification. Compared with traditional batch methods, the flow reactors can not only improve the reaction performance but also improve safety.[5−7] Due to the extremely fine pore size and high heat transfer efficiency of the microchannels, some dangerous reactions in batch experiments can be carried out safely, such as the nitration reaction, fluorination reaction, azide reaction, oxidation and reduction reaction, etc.[8−12]

If flow chemistry could be used, current tazobactam synthesis procedures will still have room for improvement. For example, the first three reactions use the same solvent; if the solids in the reaction procedure can be avoided, the continuous flow could be easily realized and production efficiency can be improved. In the process of producing **5** from **4** in the original literature,[13] 40% peroxyacetic acid was the oxidant that had a high yield close to 95% and overoxidized product **5′** was hardly tested, but the use of peroxyacetic acid was very dangerous in the industry. Therefore, a variety of alternative and safer oxidation systems have been developed.[14−16] However, they have not achieved such a high reaction yield as 40% peroxyacetic acid was used. Meanwhile, the content of overoxidized products **5′** was much high so that the purification was difficult; it would increase the corresponding cost and the postprocedure would be more complicated. As we all know, the safety of using hazardous reagents in microreactors can be greatly improved, so peroxyacetic acid

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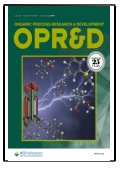

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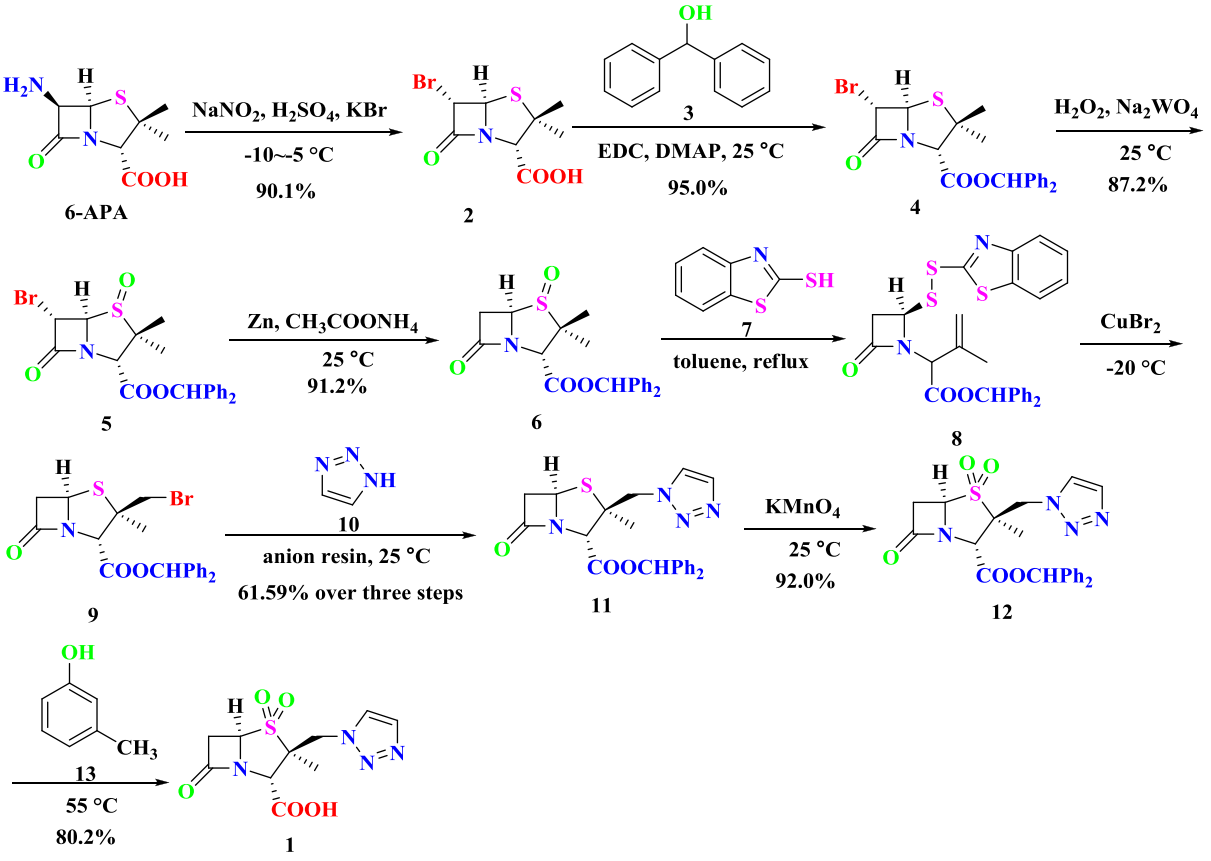
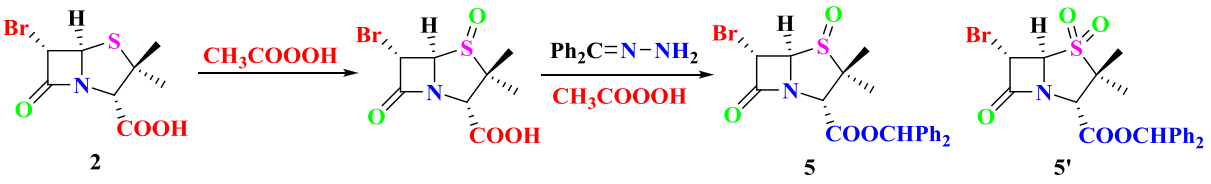
Scheme 1. Initial Synthesis Route to Tazobactam



Scheme 2. Synthesis of Intermediate 5 and Generation of Impurity



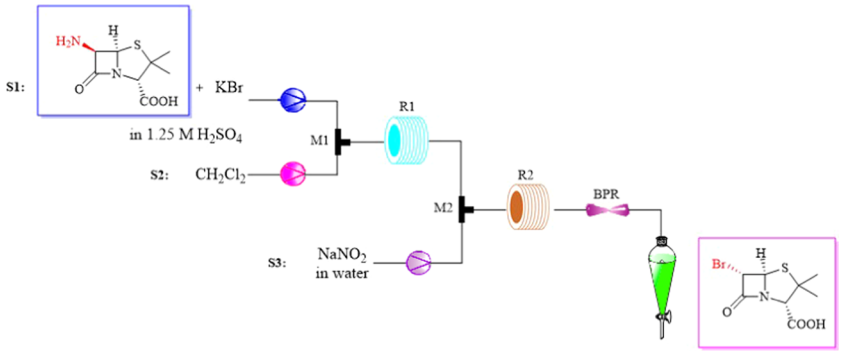


Figure 1. Semicontinuous flow process for the synthesis of intermediate 2.

was chosen in our scheme using continuous flow. Here, we report the synthesis method of tazobactam by combining continuous flow and batch experiments on the route used in our industrial production.

2. RESULTS AND DISCUSSION

2.1. Semicontinuous Method of Step 1. The first step of the reaction uses ethanol to solubilize intermediate **2** in the batch experiment, but it will cause the precipitation of potassium bromide, which will block the pipeline in the microreactor. When ethanol was not added, there was no potassium bromide solid precipitated, but intermediate **2** was not soluble in this aqueous solution. It adheres to the pipe wall as oil and results in low yield. When adhesion products in the pipeline gradually increase, they eventually result in blockage of the pipeline. Therefore, it is necessary to add a solvent to

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Table 1. Investigation into Reaction Conditions of Step 1

entry	total flow rate (mL/min)	residence time (min)	increased volume of water (mL)	volume of dichloromethane (mL)	temperature (°C)	yield (%)
1	6	5	0	0	0	30.2
2	5	6	100	50	0	46.2
3	4	7.5	100	50	0	40.8
4	3	10	100	50	0	39.2
5	8	3.75	100	50	0	62.1
6	10	3	100	50	0	68.7
7	12	2.5	100	50	0	71.0
8	14	2.14	100	50	0	75.3
9	16	1.875	100	50	0	73.0
10	18	1.67	100	50	0	68.6
11	15	2	100	50	0	77.2
12	15	2	80	50	0	70.2
13	15	2	100	80	0	80.1
14	15	2	100	100	0	85.5
15	15	2	100	120	0	82.8
16	15	2	100	100	−5	73.5
17	15	2	100	100	5	88.2
18	15	2	100	100	10	68.0

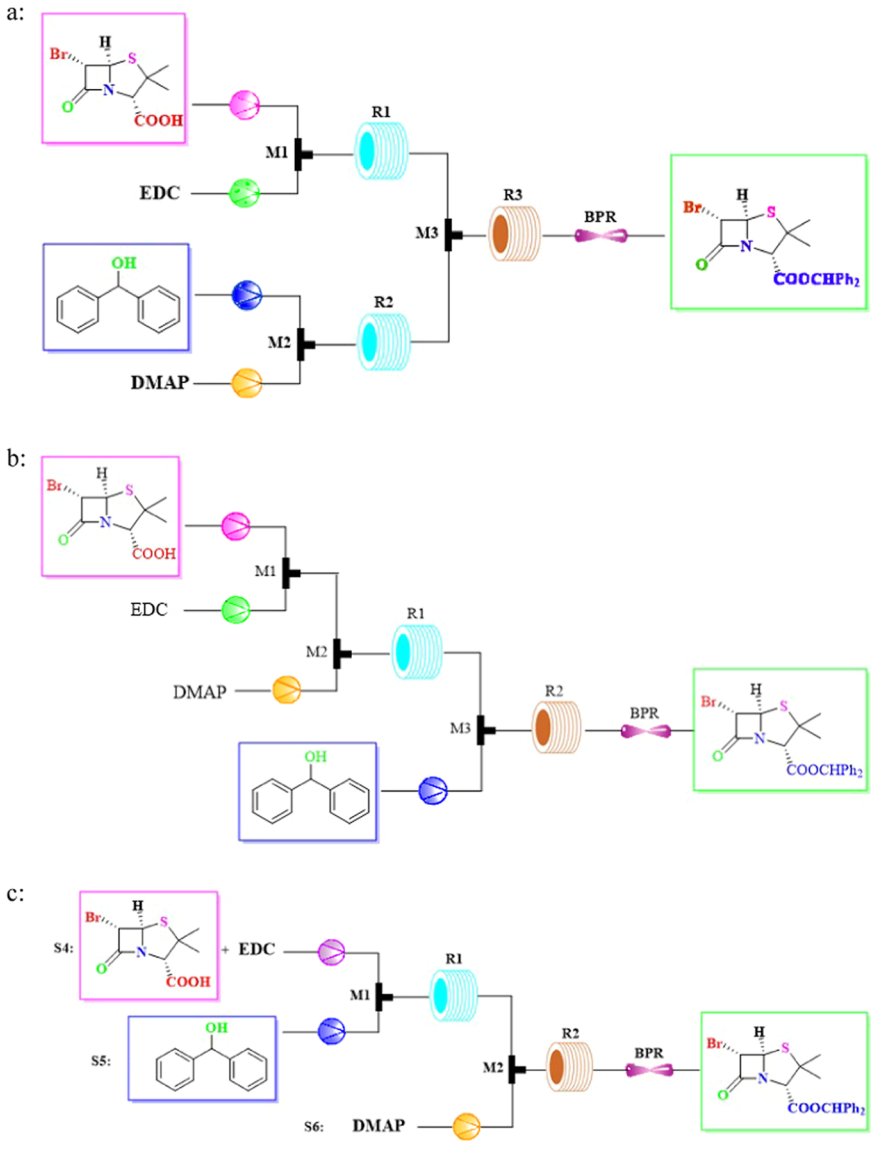

Figure 2. Semicontinuous flow process for the synthesis of **4**.

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dissolve intermediate **2** without causing the precipitation of potassium bromide. Dichloromethane was chosen as the solvent in our scheme to perform the two-phase reaction in sulfuric acid so that intermediate **2** was soluble in the organic phase. More importantly, dichloromethane can be used as a solvent for the next reaction. It can be used continuously without changing the solvent. The device was designed as shown in Figure 1, including a feed system (three plunger metering pumps), two T-shaped mixers (M1 and M2), two microreactors (R1 and R2), a backpressure regulator (BPR), and an external high- and low-temperature-integrated machine connected to control different reaction temperatures. The role of R1 was to mix and precool the reaction solution.

As shown in Figure 1, there were three streams in total. Two plunger pumps were used to separately introduce solution S1 [6-APA (21.6 g, 0.1 mol) and potassium bromide (60.1 g, 0.5 mol) in 1.25 M H_2SO_4 (250 mL)] and solution S2 (CH_2Cl_2, 100 mL) into M1 for mixing and precooling in microreactor R1 at 0 °C. The solution flow rates were 3.9 mL/min and 1.38 mL/min, respectively. The combined solution was mixed with solution S3 (sodium nitrite aqueous solution, 3 mol/L), which was introduced into M2 with a flow rate of 0.75 mL/min. The resulting solution passed through microreactor R2 (the residence time is 5 min; the temperature is 0 °C). After running for 6 min, the system reached a steady state. The output of the reactor was continuously collected in a flask for 10 min, and then, the liquid was separated. Finally, intermediate **2** was obtained after the organic layer was dried and the solvent was evaporated in vacuo. The purity and yield were tested by high-performance liquid chromatography (HPLC). The preliminary experiment yield was only 30%, and then, the specific parameters of the reaction were optimized in detail.

To find suitable reaction conditions, the effects of reaction parameters were systematically studied (Table 1). Considering that the total volume of the aqueous phase was reduced due to the replacement of ethanol with dichloromethane, this would inevitably lead to pH values of the aqueous phase being changed, which in turn would result in the lower yield of the reaction. Therefore, the appropriate amount of water was needed for the reaction solution to maintain a proper pH value. Water was added to the sodium nitrite solution, which can increase the volume of the sodium nitrite solution and improve the accuracy of the flow rate. It was found that adding 100 mL of water was more beneficial to the reaction after experiments. The amount of dichloromethane was also investigated. Although dichloromethane did not participate in the reaction, but its amount would affect the total flow rate and the mixing effect in the microreactors. We found that it was more appropriate when dichloromethane is 100 mL. In the investigation of residence time, when the total flow rate was 15 mL/min and the residence time was 2 min, the reaction yield can be greatly improved. Finally, the reaction temperature was investigated and 5 °C was selected as the optimum reaction temperature.

After a series of optimizations of the reaction conditions, the reaction yield in this step reached 88.2%, but the yield in conventional batch experiments could reach 90%. So, no better results were achieved in the microreactors than batch experiments. Although the yield was slightly reduced, it was still very valuable for research. More importantly, the reaction can continuously flow with the subsequent reactions, saving time, improving efficiency, and reducing the consumption of solvents.

2.2. Semicontinuous Method of Step 2. The initial plan was to combine intermediate **2** with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), diphenylmethanol, and 4-dimethylaminopyridine (DMAP) separately. They were fed into the microreactors using four plunger metering pumps directly, as shown in Figure 2a. After experiments, intermediate **2** was not completely transformed regardless of changes in the concentration, residence time, reaction temperature, or parameters such as the flow rate and pressure. Afterward, the order of feeding was changed; intermediate **2** was mixed with EDC and DMAP first and then the confluent reaction occurred with diphenylmethanol, but the results were still not as expected, as shown in Figure 2b. Finally, we designed the process illustrated in Figure 2c. After changing the order of reactants again, the reaction results were much improved. Then, this model as the basis for further experimental optimization was used.

The device designed includes a feeder system (three plunger metering pumps), two T-shaped mixers (M1 and M2), two microreactors (R1 and R2), and a backpressure regulator (BPR), as shown in Figure 2c.

There were three streams in total too. Two plunger pumps were used to separately introduce solution S4 [intermediate **2** (19.58 g, 0.07 mol) and EDC (13.4 g, 0.07 mol) in CH_2Cl_2 (300 mL)] and solution S5 [diphenylmethanol (12.88 g, 0.07 mol) in CH_2Cl_2 (100 mL)] into M1 and R1 for mixing. The solution flow rates were 6 and 2 mL/min, respectively. The combined solution was mixed with solution S5 [DMAP (0.4 g, 0.0032 mol) in CH_2Cl_2 (100 mL)], which was introduced into M2 at a flow rate of 2 mL/min. Also, the resulting solution passed through microreactor R2 (the residence time is 2 min; the temperature is 25 °C). After running for 6 min, the system reached a steady state. The output of the reactor was continuously collected in a flask for 10 min, and then, the collected mixture was added in 100 mL of water and separated. Finally, intermediate **4** was obtained after the organic layer was dried and the solvent was evaporated in vacuo. The purity and yield were tested by HPLC. It was exciting to obtain excellent results with a yield of 92.4% by the semicontinuous method.

To find suitable reaction conditions, the effects of residence time and reaction temperature were systematically studied (Table 2). When the residence time was shortened to 1 min,

Table 2. Investigation into Reaction Conditions of Step 2

entry	residence time (min)	temperature (°C)	pressure (MPa)	yield (%)
1	3	25	0	88.5
2	2	25	0	94.0
3	1	25	0	98.2
4	0.5	25	0	90.7
5	1	40	0	74.2
6	1	40	0.2	72.6
7	1	50	0.4	61.0
8	1	60	0.4	42.3
9	1	10	0	90.5
10	1	0	0	84.1
11	1	−10	0	80.4
12	1	−20	0	65.0
13	2	−20	0	74.2
14	3	−20	0	76.6

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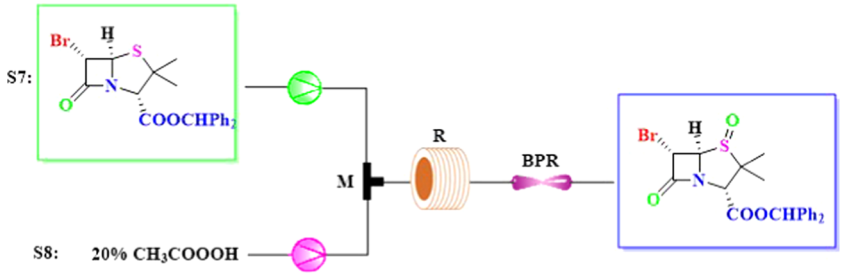

Figure 3. Semicontinuous flow process for the synthesis of **5**.

the yield increased to 98%. The shortened time was more favorable to improving production efficiency. In the investigation of the reaction temperature, when the reaction temperature increased up to 60 °C, the yield decreased sharply because higher temperatures may have some impacts on the stability of the reactants. The decrease of yield was found when the temperature decreased to −20 °C, which indicated that the lower temperature was not conducive to the rapid progress of the reaction so that the residence time was extended in an attempt to increase the yield at this temperature but the result did not reach our expectations. When the temperature was between 10 and 30 °C, the reaction yield was relatively constant. Room temperature was chosen as the reaction temperature without heating or cooling, which is green and environmentally friendly.

2.3. Semicontinuous Method of Step 3. Peroxyacetic acid was initially selected as the oxidant in the synthesis of tazobactam reported in the literature. There were numerous advantages such as high yield, few impurities, and high efficiency. However, because of its accidents in the industry, the use of 40% peroxyacetic acid was strictly restricted and a series of safeguards were required. After that, a variety of safer oxidation systems have been developed but none of them was better than peroxyacetic acid in efficiency and yield. Because of the small inner diameter of the pipe, larger contact area, high heat transfer efficiency, and continuous flow characteristics, the safety of peroxyacetic acid can be greatly improved. Here, peroxyacetic acid was chosen as the oxidant in the microreactor.

As shown in Figure 3, two plunger pumps were used to separately introduce solution S7 [intermediate **4** (28.2 g) in CH_2Cl_2 (300 mL)] and solution S8 (20% peroxyacetic acid) into a T-shaped mixer (M) at the flow rates of 18.3 mL/ min and 1.7 mL/min, respectively. Then, the solution passed through microreactor R (the residence time was 1 min; the reaction temperature was 25 °C). After running for 6 min, the system reached a steady state. The output of the reactor was continuously collected in a flask for 10 min, and then, the collected mixture was washed with saturated sodium bisulfite and water. Finally, intermediate **5** was obtained after the organic layer was dried and the solvent was evaporated in vacuo. The purity and yield were tested by HPLC. It was exciting to get better yields than in batch experiments. Then, some reaction conditions were investigated including the reaction temperature and residence time, as shown in Table 3. The results showed that the reaction temperature hardly affected the yield so that the same temperature as the previous step was chosen, which was 25 °C too; no additional temperature adjustment was required, which was convenient for continuous operation and saving energy. The residence time selected was 1 min, and 1.2 equiv of peroxyacetic acid would be better.

Table 3. Investigation into Reaction Conditions of Step 3

entry	residence time (min)	temperature (°C)	peroxyacetic acid equivalent	yield (%)
1	3	2	1.1	87.0
2	2	2	1.1	90.2
3	1	2	1.1	93.1
4	0.5	2	1.1	85.4
5	1	10	1.1	90.5
6	1	25	1.1	94.5
7	1	40	1.1	74.8
8	1	25	1.0	84.6
9	1	25	1.2	96.8
10	1	25	1.3	96.0

2.4. Semicontinuous Method of Preparing Peroxyacetic Acid. The danger of peroxyacetic acid was reduced in the microreactor, but its preparation, storage, and transportation had the same danger. If peroxyacetic acid could be prepared and used in situ in the microreactor, the above-mentioned dangerous problems could be greatly reduced, which was similar to the previous device, as shown in Figure 4.

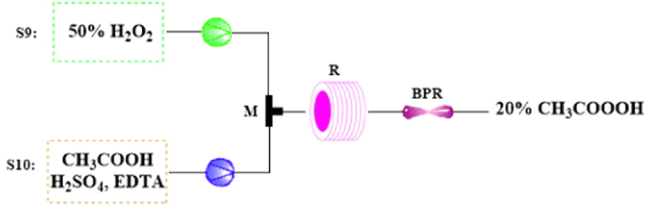

Figure 4. Semicontinuous flow process for the synthesis of peroxyacetic acid.

Two plunger pumps were used to introduce solution S9 (50% H_2O_2) and solution S10 (acetic acid with catalytic amounts of H_2SO_4 and EDTA) into a T-shaped mixer (M) and then passed through microreactor R. After investigation of reaction conditions, 20% content of peroxyacetic acid was obtained when the residence time was 10 min and reaction temperature was 55 °C, as shown in Table 4 (peroxyacetic acid concentration measured by the titration method). Now, we developed a continuous process of preparation of 20% peroxyacetic acid, which can be carried out simultaneously with the oxidation reaction and continuously flow into the microreactors. The preparation and use of 20% peroxyacetic acid all took place in microreactors, which can avoid the explosion risk that may occur in the preparation, storage, and transportation in batch experiments. What is more, this

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Table 4. Investigation into Reaction Conditions for Preparing Peroxyacetic Acid

entry	temperature (°C)	residence time (min)	EDTA	peroxyacetic acid content (%)
1	25	5	−	8.4
2	25	8	−	10.8
3	25	10	−	16.5
4	25	13.3	−	15.0
5	35	10	−	18.7
6	45	10	−	19
7	55	10	−	18.5
8	55	10	+	20.6
9	65	10	+	20.0

method can not only reduce the production risk but also improve efficiency greatly.

2.5. Fully Continuous Flow Process of These Four Reactions. We tried to develop a fully continuous flow process to perform these four steps. As shown in Figure 5, the four-step reactions were connected in sequence according to the semicontinuous method obtained in the previous experiments. First, three plunger pumps were separately used to introduce solutions S1, S2, and S3 through two T-shaped mixers (M1 and M2) and flowed into the microreactor (R2) for reaction. The reaction temperature was 5 °C, and the residence time was 2 min. It flowed into the separatory funnel through the pressure regulator (BPR). Now, the solution of intermediate **2** was obtained after separating the dichloromethane layer and washing it with saturated sodium chloride. Next, three plunger pumps were utilized to introduce solutions S4, S5, and S6 separately. They all flowed into microreactor R3. The reaction temperature was 25 °C, and the residence time was 1 min. Thus, the solution of intermediate **3** was obtained. The intermediate **3** solution was directly flowed into microreactor R6 without being separated. At the same time, two plunger pumps were used to introduce solutions S7 and S8 into microreactor R4 to prepare peroxyacetic acid in situ. Peroxyacetic acid later directly flowed into mixer M6, and it was mixed with the reaction solution flowing out of microreactor R3 to undergo the oxidation reaction in microreactor R6. Finally, it was derived through the back-pressure regulator (BPR). Then, the reaction solution was in turn washed with the sodium bisulfite solution, sodium bicarbonate solution, and saturated sodium chloride solution. Finally, intermediate **5** was obtained after drying and distilling off the solvent.

Since the reaction conditions of the relevant steps have been investigated separately in early experiments, here, we only optimized the adaptation conditions when they are jointly performed. The conditions for the first step were the same as the optimal conditions in the semicontinuous process. It should be noted that the concentration of intermediate **2** was 0.84 mol/L after the first step, while the required concentration of intermediate **2** was 0.23 mol/L in the second step. To achieve synergy, we tried to decrease the concentration of intermediate **2** in the first step or increase the concentration of other reactants in the second and third steps to match the high concentration of intermediate **2**, but no better results were obtained after many attempts. So, we decided to add some CH_2Cl_2 into the solution of intermediate **2** after the first step to maintain the concentration at 0.23 mol/L, which can be better carried out in the subsequent reactions. About 265 mL of CH_2Cl_2 was added per 100 mL of solution of intermediate **2** from the first step after calculations. It should increase the flow rates in the second and third steps because of the larger volume of the intermediate **2** solution so that it can avoid the accumulation of intermediate **2**. Thus, we increased the related flow rates in the second and third steps and made them suitable and sustainable. Considering that the reaction temperature was 55 °C during the preparation of 20% peroxyacetic acid in R4, the oxidation reaction temperature of step 3 was 25 °C in R6. We set a reactor device behind the microreactor R4 to precool the solution to prevent the

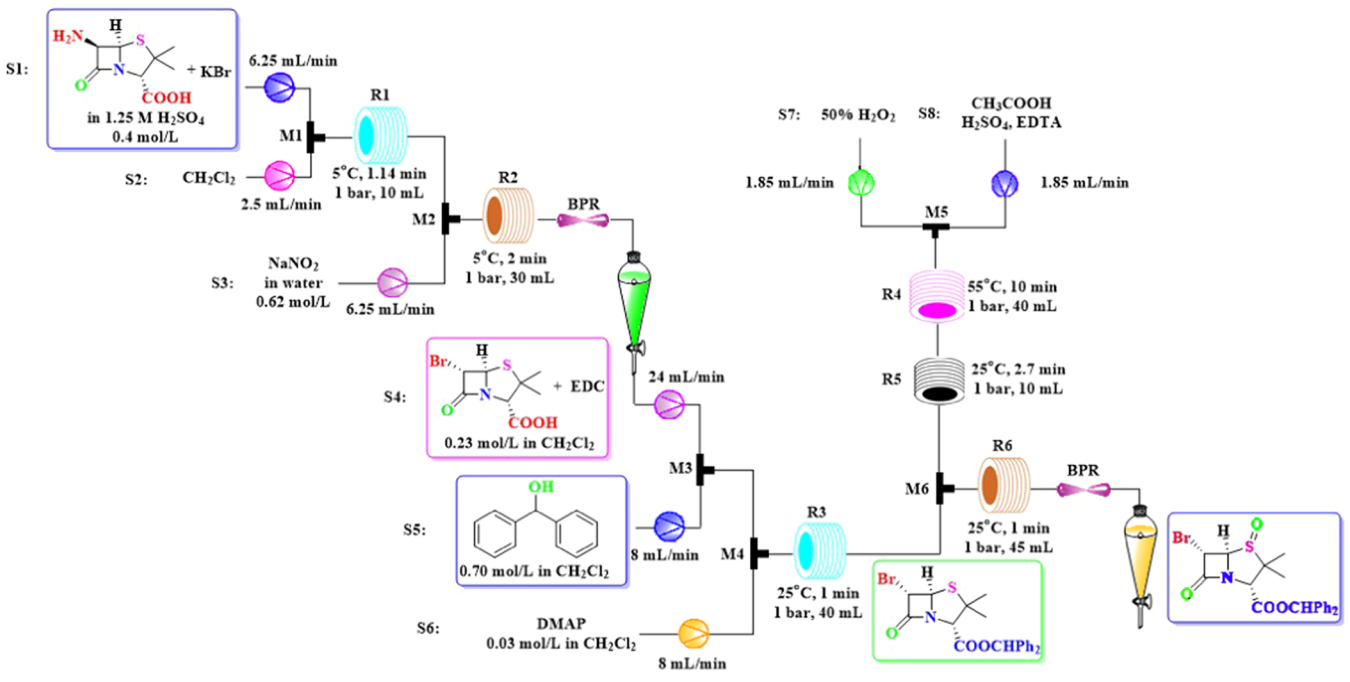

Figure 5. Fully continuous flow process of these four reactions.

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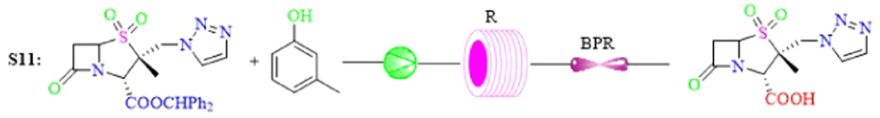

Figure 6. Semicontinuous Flow Process for the Synthesis of 1.

peroxyacetic acid from bringing more heat into microreactor R6. Now, all reaction conditions and reactant concentrations have been specified, as shown in Figure 5. Excitingly, we got intermediate **5** with higher yield and purity than batch experiments. What is more, it was continuously suitable and efficient for industrial production without the amplification effect.

2.6. Semicontinuous Method of Step 9. The reaction temperature was 55 °C and the reaction time was 4 h in batch experiments in this step. However, the temperature and reaction time were detrimental to the stability of the β-lactam ring. High temperatures and long reaction times may cause the β-lactam ring to be destroyed. As a result, the yield and purity were lower, which will affect the final product quality. Therefore, we hope to be able to reduce the adverse effects and increase the yield and purity by flow chemistry. As illustrated in Figure 6, a plunger pump is used to introduce the *m*-cresol solution of intermediate **12** into the microreactor (we have experimentally verified that intermediate **12** hardly reacts in m-cresol solution at room temperature). The conditions such as the flow rate, residence time, and reaction temperature were systematically studied, as provided in Table 5. When the flow rate was 20 mL/min, the residence time was 1 min, and the reaction temperature was 75 °C, better yield and purity were obtained.

Table 5. Investigation into Reaction Conditions of Step 9

entry	flow rate (mL/min)	residence time (min)	temperature (°C)	yield (%)
1	12	1.54	75	82.6
2	14	1.43	75	84.6
3	16	1.25	75	83.8
4	18	1.10	75	84.0
5	20	1.00	75	85.6
6	22	0.90	75	81.0
7	20	1.00	85	78.3

An improved synthetic route to tazobactam is shown in Scheme 3, and a comparison of process mass intensity (PMI) breakdown for initial and improved synthetic routes is shown in Figure 7. Compared to the initial synthetic route, application of the continual flow method is safer and efficient; it proceeds with a higher overall yield (36% increase) and a lower PMI (7% reduction).

However, because only four steps in the whole route were converted to continuous flow, the advantages of continuous flow cannot be fully reflected in the PMI and total yield. Therefore, we have compared the continuous flow method and batch method of the first three steps in productivity and space−time yield (STY) (Table 6). Thanks to these improvements, the productivity and STY of the whole route were estimated to increase by 20% and 80%, respectively, on a manufacturing scale.

3. CONCLUSIONS

A stable and effective synthetic method for the preparation of tazobactam in batch experiments combined with a continuous flow method has been developed. Compared with all traditional batch experiments, it has improved procedure safety and efficiency and cut back side reactions. The total yield was 37.09%, and the purity was 99.85%. The use of the continuous flow strategy provides a good solution for the synthesis of tazobactam, and it has been applied to industrial production.

4. EXPERIMENTAL SECTION

4.1. General Information. In this paper, ^{1}H NMR and ^{13}C NMR spectra were measured using a Bruker 600 MHz AVIII HD nuclear magnetic resonance spectrometer. TMS was the internal standard. The mass spectra of the compounds were all measured with an Agilent 1000 four-link liquid-mass spectrometer. High-performance liquid chromatography was measured with an Agilent 1100 high-performance liquid chromatograph. The infrared spectrum was measured with a Bruker IFS 55. The optical rotation was measured with an Agilent fully automatic polarimeter. Continuous flow instruments include microreactors (Himile Mechanical Science and Technology (Shandong) Co., Ltd.), pumps (Shanghai Sanotac Scientific Instruments Co., Ltd.), and a high- and low-temperature circulating device (Gongyi Yuhua Instrument Co., Ltd.). Other reaction instruments include an FC204 analytical balance (Shanghai Precision Balance Instrument Factory), a DF-101S collector-type constant temperature heating magnetic stirrer (Gongyi Yuhua Instrument Co., Ltd.), and an EYELA N-1001 rotary evaporator (EYELA, Germany). The reagents used in the experiments were all commercially available chemicals or of analytical grade.

4.2. 6α-Bromopenicillin-3-α-carboxylic acid (2). 6-APA (21.6 g) and potassium bromide (60.1 g) were added to 1.25 mol/L sulfuric acid (250 mL) to dissolve them at 0 °C, as solution S1. Dichloromethane (100 mL) was S2. Sodium nitrite (10.6 g) was dissolved in 250 mL of water as solution S3. Three plunger pumps were used to introduce the three solutions, respectively, into microreactor R2 after passing through two T-shaped mixers M1 and M2 and a microreactor R1 to precool; the flow rates were 6.25, 2.5, and 6.25 mL/min, respectively. The reaction temperature was 5 °C, and the residence time was 2 min. After the reaction, the solution was collected at the discharge port (placed in an ice bath to control temperature), the liquid was separated, the organic phase was washed with saturated brine, and then dichloromethane was added to the solution to prepare for the next reaction; 265 mL of dichloromethane was added per 100 mL of solution. ESI-MS: 279.4 ($M - H^+$).

4.3. 6α-Bromopenicillin-3α-carboxylic Acid Sulfoxide Diphenylmethyl Ester (5). EDC (13.4 g) was added to the total volume of 300 mL solution of the previous step, and it was introduced into microreactor R3 by a pump; the flow rate was 24 mL/min. Diphenylmethanol (12.90 g) was dissolved in dichloromethane (100 mL) and introduced into microreactor

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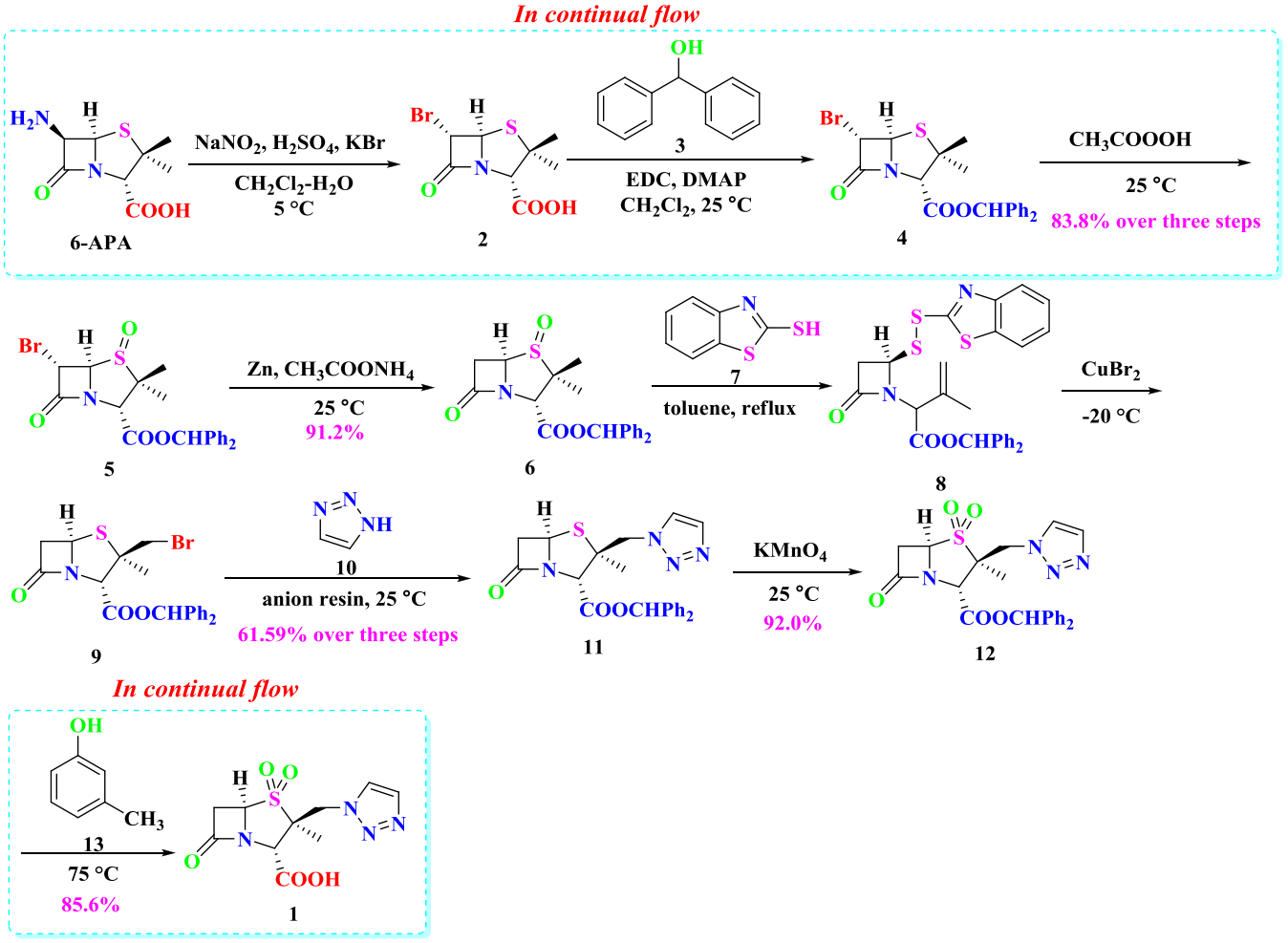
Scheme 3. Improved Synthetic Route to Tazobactam



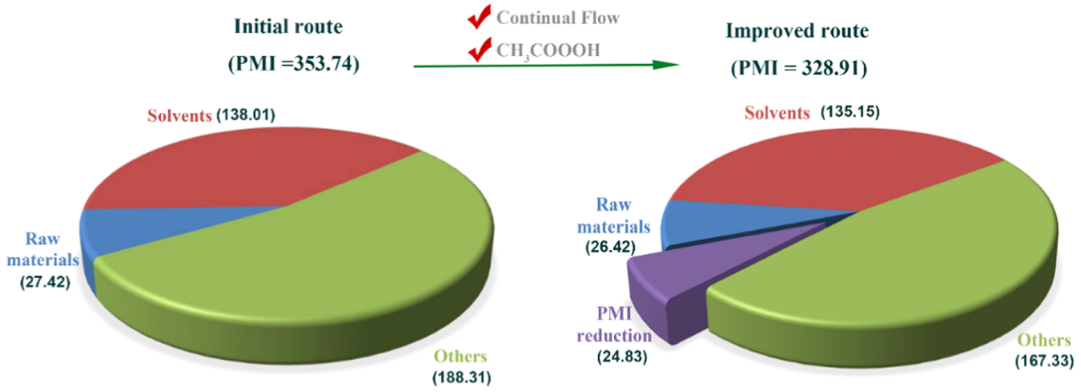


Figure 7. Comparison of PMI breakdown for initial and improved synthetic routes.

Table 6. Comparison of the First Three Steps in Batch and Continuous Flow Experiments

attribute yield (%)	batch experiment 74.6%	continual flow experiment 83.8%
PMI	96.64	77.1
productivity (mol/day)	4	8
STY (kg/(dm^3 d ay)	0.07	19

R3 by a pump at a flow rate of 8 mL/min. DMAP (0.4 g) was dissolved in dichloromethane (100 mL) and introduced into microreactor R3 by a pump at a flow rate of 8 mL/min. The residence time was 1 min, and the reaction temperature was 25 °C. After the reaction, the solution was directly inputted into microreactor R6 without separation. At the same time, 50% hydrogen peroxide was introduced into microreactor R4 by a pump at a flow rate of 1.85 mL/min. Acetic acid that was added with sulfuric acid and EDTA was introduced into microreactor R4 by a pump at a flow rate of 1.85 mL/min. The residence time was 10 min. The reaction temperature was 55

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°C. Finally, the reaction produced 20% peroxyacetic acid and then it flowed into microreactor R6 and underwent an oxidation reaction with intermediate **3** inputted by the previous pump. The residence time was 1 min, and the reaction temperature was 25 °C. The reaction solution was received at the discharge port and washed with saturated sodium bisulfite, saturated sodium bicarbonate, water, and saturated sodium chloride. A yellow oil (30.3 g) with a yield of 83.8% (based on 6-APA) was obtained after the organic layer was dried and evaporated in vacuo. ESI-MS: 485.3 ($M + Na^+$). HPLC analysis: 98.63%.

4.4. 6,6-Dihydropenicillin-3α-carboxylic Acid Sulfoxide Diphenylmethyl Ester (6). Intermediate **5** (32 g) and ammonium acetate solution (NH_4OAc, 21.696 g; H_2O, 173.44 mL) were added to tetrahydrofuran (170 mL). Zinc powder (14.72 g) was added in batches, and the raw materials completely disappeared after 1 h of reaction at room temperature. Tetrahydrofuran was distilled off, and ethyl acetate (200 mL) was added for extraction. The extract was washed with water to near-neutral and then with saturated sodium chloride solution (100 mL) and dried over anhydrous sodium sulfate. After filtration, the solvent was distilled off from the filtrate, cyclohexane−ethyl acetate (8: 1, 90 mL) was added at room temperature, and the mixture was stirred for 1.5 h. The white solid was filtered to obtain the product (24.2 g) with a yield of 91.2%. ESI-MS: 382.18 ($M - H^+$). ^1H NMR (600 MHz, $CDCl_3$): δ 7.38−7.30 (m, 10H), 6.99 (s, 1H), 4.92 (dd, J = 3.8, 2.8 Hz, 1H), 4.64 (s, 1H), 3.35 (dd, J = 3.3, 1.4 Hz, 2H), 1.69 (s, 3H), 0.94 (s, 3H). HPLC analysis: 98.99%.

4.5. 3-Methyl-[2-oxo-4-(2-benzothiazole dithio)-1-azacyclobutyl]-3-butenoic acid (8). Intermediate **6** (27 g) and 2-mercaptobenzothiazole (11.78 g) were added to toluene (450 mL) and refluxed for 80 min. Water was removed with a water separator. The solvent was distilled in vacuo at 65−70 °C, and the next reaction was directly performed. ESI-MS: 555.4 ($M + Na^+$).

4.6. 2β-Bromomethyl-2α-methylpenicillin-3α-carboxylic Acid Diphenylmethyl Ester (9). All of the products from the previous step were added to dichloromethane (360 mL) and cooled to −15 to −20 °C; then, copper bromide (18 g) was added and the reaction was maintained at −15 to −20 °C for 8 h. Diatomaceous earth (4 g) was added, stirred for 15 min, and filtered. The filtrate was washed twice with 1% sodium bicarbonate solution (200 mL) and then washed twice with water (200 mL) and saturated sodium chloride solution (100 mL). Then, it was dried over anhydrous sodium sulfate. After filtration, the solvent was distilled off and the next reaction was directly carried out.

4.7. 2β-(1,2,3-Triazol-1-yl) methyl-2α-methyl-6,6-dihydropenicillin-3α-carboxylic Acid Diphenylmethyl Ester (11). Acetone (300 mL) was added to the product in the previous step, and then, 1,2,3-triazole (112.5 g) and water (40 g) were added. The temperature was kept at −15 °C, resin sulfonic acid (47.5 g) was added, and then the reaction was maintained at −15 °C for 20 h. When the reaction completed, the organic phase was filtered and evaporated in vacuo at 40−50 °C. Then, dichloromethane (200 mL) was added and separated, and the organic layer was washed with water (200 mL) and saturated sodium chloride (50 mL). Intermediate **11** was obtained after the organic layer was dried and evaporated in vacuo. Then, the crude product was recrystallized from an ethyl acetate−*n*-hexane solution to give pure intermediate **11**. Yield: 61.59% (based on intermediate **6**), HPLC analysis: 98.25%.

4.8. 2β-(1,2,3-triazol-1-yl) Methyl-2α-methyl-6,6-dihydropenicillin-3α-carboxylic Acid Diphenylmethyl 1,1-Dioxide (12). Intermediate **11** (36 g), dichloromethane (216 mL), and acetic acid (63 g) were added to a flask and cooled to 10 °C; then, potassium permanganate (28.8 g) was added, and the reaction was kept at 25 °C for 6 h. After the reaction completed, water (80 g) was added and the temperature was kept between 5 and 10 °C. Hydrogen peroxide (50%) was added dropwise at a controlled temperature of 5−10 °C until the reaction solution turned white and stirred for 1 h. Dichloromethane was evaporated in vacuo, then ethyl acetate (50 mL) was added and stirred for 1 h at 0−5 °C, and intermediate **12** was obtained after filtration. Yield: 92%, ESI-MS: 465.22 ($M - H^+$), 467.23 ($M + H^+$)

4.9. Tazobactam (1). Intermediate **12** (30 g) was dissolved in *m*-cresol (200 mL) at 25 °C. It was pumped into the microreactor at a flow rate of 20 mL/min, the reaction temperature was 75 °C, and the retention time was 1 min. The reaction solution was received at the discharge port; ethyl acetate (660 mL) was added and extracted twice with saturated sodium bicarbonate solution (200 mL); and washed the water layer using methyl isobutyl ketone (30 mL), ethyl acetate (30 mL), dichloromethane (30 mL). Activated carbon (3 g) was added to the water layer to decolorize and then filtered. After filtration, the filtrate was cooled to 0−5 °C and 15% hydrochloric acid was added to adjust the pH value to 1.0−1.5 slowly, it was allowed to stand for 3 h and then filtered. Finally, white solid tazobactam (16.53 g) was obtained after drying. Yield: 85.6%. m.p. 197−200 °C. ESI-MS: 301.12 ($M + H^+$). IR (KBr), v/cm^{-1}: 3431.7 (−OH), 1799.7 (−C═O), 1713.5 (−C═O), 1328.5 (as, $-SO_2$), 1140.9 (as, $-SO_2$), 790.6 (−C═O). ^1H NMR (600 MHz, DMSO-d_6) δ = 8.09 (s, 1H), 7.79 (s, 1H), 5.24 (d, J = 15.3, 1H), 5.18 (d, J = 4.1, 1H), 4.93 (d, J = 15.3 Hz, 1H), 4.79 (s, 1H), 3.71 (dd, J = 16.5, 4.4 Hz, 1H), 3.33 (d, J = 16.4, 1H), 1.35 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ = 171.3, 167.8, 133.3, 126.8, 64.5, 61.9, 59.8, 49.8, 37.8, 15.7. HPLC analysis: 99.85%.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.oprd.1c00127.

Mass spectrum of 6α-bromopenicillin-3-α-carboxylic acid (**2**); mass spectrum and HPLC spectrum of 6α-bromopenicillin-3α-carboxylic acid sulfoxide diphenylmethyl ester (**5**); mass spectrum, ^1H NMR spectrum (600 MHz, $CDCl_3$), and HPLC spectrum of 6,6-dihydropenicillin-3α-carboxylic acid sulfoxide diphenylmethyl ester (**6**); mass spectrum of 3-methyl-[2-oxo-4-(2-benzothiazole dithio)-1-azacyclobutyl]-3-butenoic acid (**8**); HPLC spectrum of 2β-(1,2,3-triazol-1-yl) methyl-2α-methyl-6,6-dihydropenicillin-3α-carboxylic acid diphenylmethyl ester (**11**); mass spectrum of 2β-(1,2,3-triazol-1-yl) methyl-2α-methyl-6,6-dihydropenicillin-3α-carboxylic acid diphenylmethyl 1,1-dioxide (**12**); mass spectrum, ^1H NMR spectrum (600 MHz, DMSO-d_6), ^{13}C NMR spectrum (151 MHz, DMSO-d_6), HPLC spectrum, and infrared spectrum of tazobactam (**1**); and calculations of productivity, space−time yield (STY), and process mass intensity (PMI) (PDF)

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AUTHOR INFORMATION

Corresponding Authors

Chengjun Wu − *Key Laboratory of Structure-Based Drug Design and Discovery, Shenyang Pharmaceutical University, Ministry of Education, Shenyang 110016, P. R. China;* orcid.org/0000-0001-5785-8065; Email: chengjunspu@163.com

Tiemin Sun − *Key Laboratory of Structure-Based Drug Design and Discovery, Shenyang Pharmaceutical University, Ministry of Education, Shenyang 110016, P. R. China;* orcid.org/0000-0002-9981-0243; Email: suntiemin@126.com

Authors

Shuhao Zhou − *Key Laboratory of Structure-Based Drug Design and Discovery, Shenyang Pharmaceutical University, Ministry of Education, Shenyang 110016, P. R. China*

Yunting Xin − *Key Laboratory of Structure-Based Drug Design and Discovery, Shenyang Pharmaceutical University, Ministry of Education, Shenyang 110016, P. R. China*

Jiasheng Wang − *Key Laboratory of Structure-Based Drug Design and Discovery, Shenyang Pharmaceutical University, Ministry of Education, Shenyang 110016, P. R. China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.oprd.1c00127

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Kadima, T. A.; Weiner, J. H. Mechanism of suppression of piperacillin resistance in enterobacteria by tazobactam. *Antimicrob. Agents Chemother.* **1997**, *41*, 2177−2183.

(2) Perry, C. M.; Markham, A. Piperacillin/Tazobactam an updated review of its use in the treatment of bacterial infections. *Drugs* **1999**, *57*, 805−809.

(3) Zhou, Y.; Wu, C.; Ma, H.; Chen, J.; Sun, T. Precise Preparation of a High-Purity Key Intermediate of Tazobactam. *Org. Process Res. Dev.* **2020**, *24*, 2898−2905.

(4) Mason, B. P.; Price, K. E.; Steinbacher, J. L.; Bogdan Andrew, R.; Mcquade, D. T. Greener Approaches to Organic Synthesis Using Microreactor Technology. *Chem. Rev.,* **2007**, *107*, 2300−2318.

(5) Malet-Sanz, L.; Susanne, F. Continuous Flow Synthesis. A Pharma Perspective. *J. Med. Chem.* **2012**, *55*, 4062−4098.

(6) Porta, R.; Benaglia, M.; Puglisi, A. Flow Chemistry: Recent Developments in the Synthesis of Pharmaceutical Products. *Org. Process Res. Dev.* **2016**, *20*, 2−25.

(7) Pastre, J. C.; Browne, D. L.; Ley, S. V. Flow chemistry syntheses of natural products. *Chem. Soc. Rev.* **2013**, *42*, 8849−8869.

(8) Baumann, M.; Moody, T. S.; Smyth, M.; Wharry, S. A Perspective on Continuous Flow Chemistry in the Pharmaceutical Industry. *Org. Process Res. Dev.* **2020**, *24*, 1802−1813.

(9) Plutschack, M. B.; Pieber, B.; Gilmore, K.; Seeberger, P. H. The Hitchhiker's Guide to Flow Chemistry. *Chem. Rev.* **2017**, *117*, 11796−11893.

(10) Kawaguchi, T.; Miyata, H.; Ataka, K.; Mae, K.; Yoshida, J.-i. Room Temperature Swern Oxidations by Using a Microscale Flow System. *Angew. Chem. Int. Ed.* **2005**, *44*, 2413−2416.

(11) Meenen, E. V.; Moonen, K.; Acke, D.; Stevens, C. V. Straightforward continuous synthesis of α-aminophosphonates under microreactor conditions. *Arkivoc* **2006**, *2006*, 31−45.

(12) Movsisyan, M.; Delbeke, E.; Berton, J.; Battilocchio, C.; Ley, S. V.; Stevens, C. V. Taming hazardous chemistry by continuous flow technology. *Chem. Soc. Rev.* **2016**, *45*, 4892−4928.

(13) Micetich, R. G.; Maiti, S. N.; Spevak, P.; Tanaka, M.; Yamazaki, T.; Ogawa, K. Synthesis of 2β-Azidomethylpenicillin-1, 1-Dioxides and 3β-Azido-3α-methylcepham-1, 1-Dioxides. *Synthesis* **1986**, *1986*, 292−296.

(14) Deshpande, P. B.; Udayampalayam, P. S.; Karale, S. N.; Kannappan, J.; Gnanaprakasam, A. A Process for the Preparation of Benzyl-2-oxo-4-(heteroaryl) dithio-alpha-isoprenyl-1-azetidineazetate Derivatives. WO2004039776A22004.

(15) Xu, W.; Li, Y.; Zhang, Q.; Zhu, H. A new approach to the synthesis of tazobactam using an organosilver compound. *Synthesis* **2005**, *2005*, 442−446.

(16) Buynak, J.; Chen, H. Inhibitors of Serine and Metallo-β-Lactamases. WO2003087105A12003.