A Novel and Practical Synthesis of Isavuconazonium Sulfate via Anion Exchange Resin

Lei Huang[1,2] Yi Zhang[1,2] Hua Chen[1,2] Wei-Wei Wang[1,2] Jian-Qi Li[1,2,*] Yu Liu[1,2,*]

[1] Novel Technology Center of Pharmaceutical Chemistry, Shanghai Institute of Pharmaceutical Industry Co., Ltd., China State Institute of Pharmaceutical Industry, Shanghai, People's Republic of China
[2] Shanghai Engineering Research Center of Pharmaceutical Process, Shanghai Institute of Pharmaceutical Industry Co., Ltd., China State Institute of Pharmaceutical Industry, Shanghai, People's Republic of China

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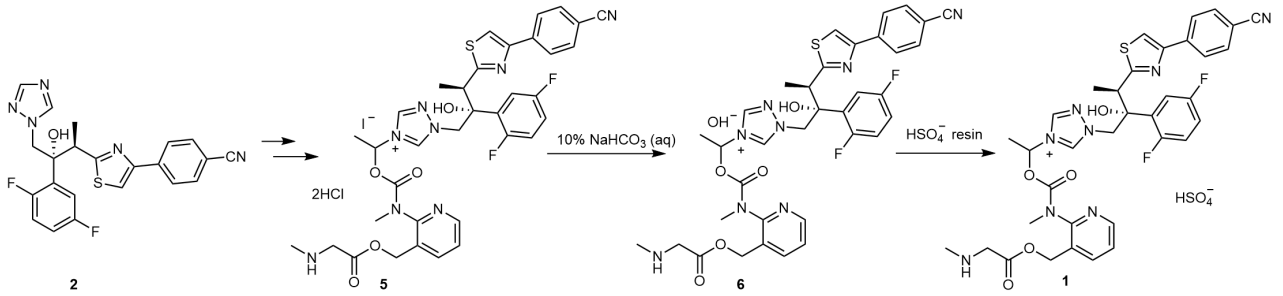
Address for correspondence Jian-Qi Li, PhD, Novel Technology Center of Pharmaceutical Chemistry, Shanghai Institute of Pharmaceutical Industry, 285 Gebaini Road, Shanghai 201203, People's Republic of China (e-mail: lijianqb@126.com).

Yu Liu, PhD, Novel Technology Center of Pharmaceutical Chemistry, Shanghai Institute of Pharmaceutical Industry, 285 Gebaini Road, Shanghai 201203, People's Republic of China (e-mail: liuyu_tianjin@126.com).



Overall yield: 57.0%, HPLC purity of **1** was 97.0%

Abstract

In this study, an efficient and practical process for the synthesis of isavuconazonium sulfate (compound **1**), an antifungal agent, was described. Highlights in the synthesis route are the usage of the ion exchange resin instead of H_2SO_4 to introduce the HSO_4^- anion in the formulation of quaternary ammonium salt (**1**), and the reaction condition was further optimized to facilitate the scale-up. The overall yield of the process was 57.0% and the high-performance liquid chromatography purity of product was 97.25%, which was higher than that of the reference-listed drug.

Keywords
- isavuconazonium sulfate
- synthesis
- anion exchange resin

Introduction

Isavuconazonium sulfate (**1**) is a prodrug of isavuconazole (**2**), which is a broad-spectrum triazole antifungal agent and widely used for the treatment of invasive fungal infections.[1–7] The water-soluble compound **1** includes a triazolium salt tethered to isavuconazole via an ester moiety (►**Fig. 1**). Evidence suggested that many antifungal drugs, such as itraconazole and voriconazole, often used cyclodextrin vehicle, to facilitate the solubility in their intravenous formulation, yet had been speculated to be nephrotoxic in humans.[8] Inspiringly, compound **1** does not require a cyclodextrin vehicle due to its natural water-soluble nature, and thereby can represent a better option in the therapy of fungal infections in comparison to other triazole antifungal agents.[8–10]

The current two reported approaches for the synthesis of compound **1** are described in **Scheme 1**. The original

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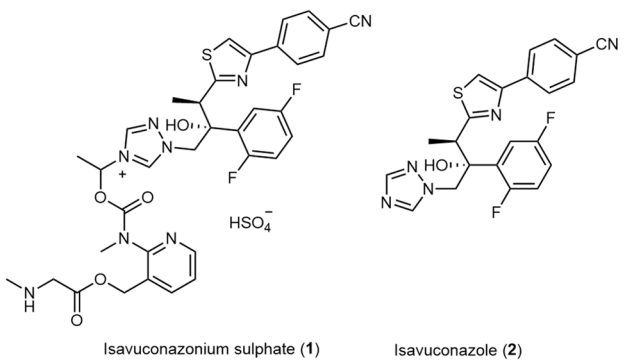


Fig. 1 Structures of isavuconazonium sulfate (**1**) and isavuconazole (**2**).

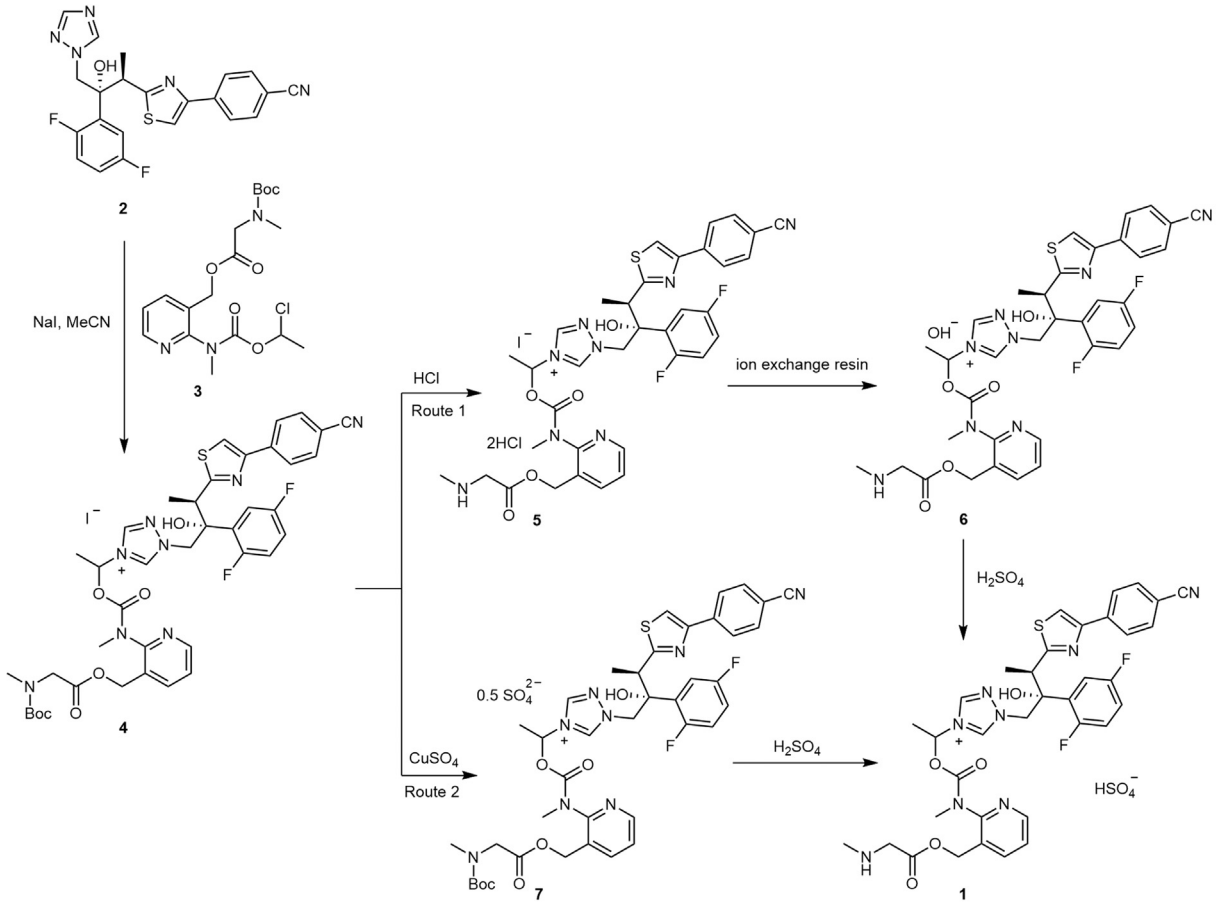
preparation of compound **1** was based on Fukuda et al's method using isavuconazonium chloride hydrochloride rather than bisulfate as the key intermediate.[11] Later, Zhou et al reported a new synthetic route in 2017, directly using $CuSO_4$ to introduce SO_4^{2-} to the triazolium salt.[12] The generated key intermediate (**7**) was acidified by H_2SO_4 to obtain the target compound. However, these routes had three shortcomings: first, compound **5** degraded a lot owing to basic ion exchange resin in the preparation of compound **6**. Second, due to multiple alkaline sites in isavuconazonium, it is difficult to form the HSO_4^- salt of isavuconazonium in a 1:1 ratio through directly adding H_2SO_4. Finally, both the reported two routes suffered from purification problems due to the thermal instability of the target product. Thus, developing a method for preparing compound **1** with a high yield and purity is necessary.

In this study, we first report a novel synthetic route (**Scheme 2**), wherein compound **5** was obtained according to Fukuda et al's method (Route 1 in **Scheme 1**), then neutralized in basic solution to give compound **6**. The OH^- anion of compound **6** was ion exchanged by HSO_4^- from resin loaded with HSO_4^- to form the target compound **1**.

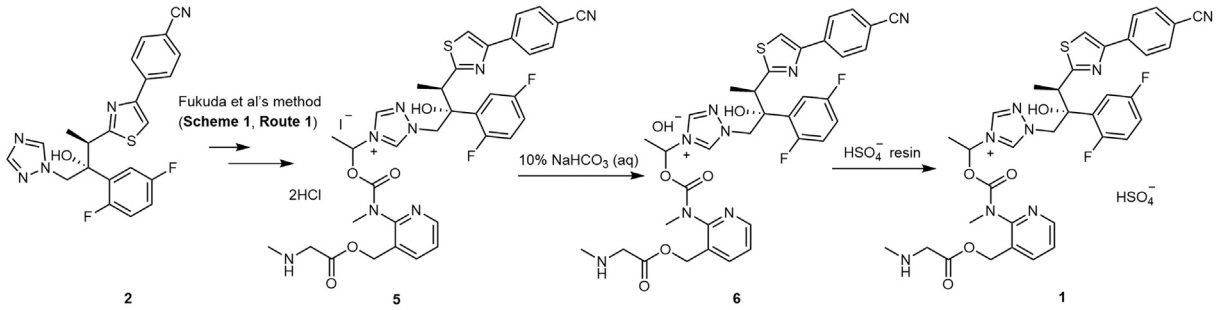
Results and Discussion

Improved Synthesis of Key Intermediate 4 and the underlying Mechanism

Fukuda et al used a catalytic amount of NaI to prepare key intermediate **4** (Route 1 in **Scheme 1**).[1] However, the yield was too low to be acceptable when the ratio of NaI was below 30% (►**Table 1**, entries 1–9). Interestingly, this work found that higher equivalents of NaI afforded higher yields of **4** (►**Table 1**, entries 7–12), and when 1.1 equivalent of NaI was added (►**Table 1**, entries 12 and 13), compound **2** was reacted completely, indicating that NaI may take a part in the reaction rather than acting as a catalyst. A possible reaction mechanism is proposed in **Scheme 3**.



Scheme 1 Reported synthetic routes of isavuconazonium sulfate (**1**) by Fukuda et al's method (Route 1) and Zhou et al's method (Route 2).



Scheme 2 New synthetic route via ion exchange resin reported by this work.

Preparation of Compound 6

Removing the Boc of compound **4** in HCl (aq) solution obtained compound **5**, and the I^- of which was replaced with OH^- to obtain compound **6**. Fukuda et al used an ion-exchange resin to introduce OH^-. However, under basic conditions, the ester groups in compound **5** were easy to hydrolyze, and the hydrolyzed products (**2**, **8**, **9**) are shown in **Scheme 4**. Even if compound **5** was suspended in 5% $NaHCO_3$ solution and extracted with dichloromethane (DCM), the hydrolysis of compound **5** was still unstoppable. In this work, the process of obtaining compound **6** was improved. Compound **5** was added into a two-phase DCM/water solvent system at a low temperature, followed by HCl neutralization and the replacement of I^- with OH^- under basic conditions. The generated compound **6** was immediately transferred into an organic phase, thus avoiding degradation. This solution was used for the next step without further work-up.

Preparation of Target Compound 1 (Isavuconazonium Sulfate)

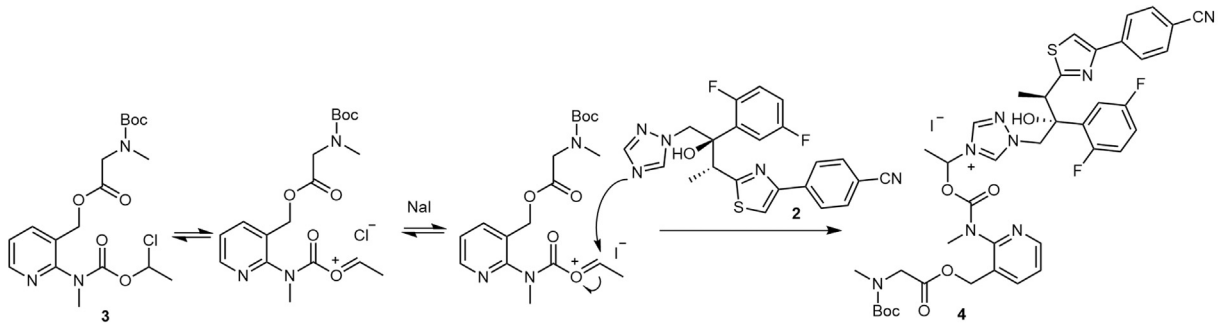
To obtain the target compound **1**, Fukuda et al replaced the OH^- group of compound **6** with HSO_4^- by addition of H_2SO_4. However, it was found that when H_2SO_4 was added to the reaction mixture, compound **6** was degraded into impurity **8** and isavuconazole (**2**), and the product precipitated as a syrup, which could not be purified because of its poor solid form and thermal instability. Given above, the introduction of HSO_4^- anion via a medium that would provide a pure convenient ion pair is significantly important.

Evidence suggested that some I^--containing imidazolium ionic liquids can be anion-exchanged with anion–exchange resin loaded with different anions.[13] Considering that isavuconazonium has a similar quaternary ammonium moiety to these imidazolium ionic liquids, we used the anion-exchange resin loading with HSO_4^- as a medium for the introduction of HSO_4^- into isavuconazonium, and the ion-exchange reaction is described in **Scheme 5**. Our explored method thereby achieved better outcomes compared with the use of H_2SO_4 (aq) solution.

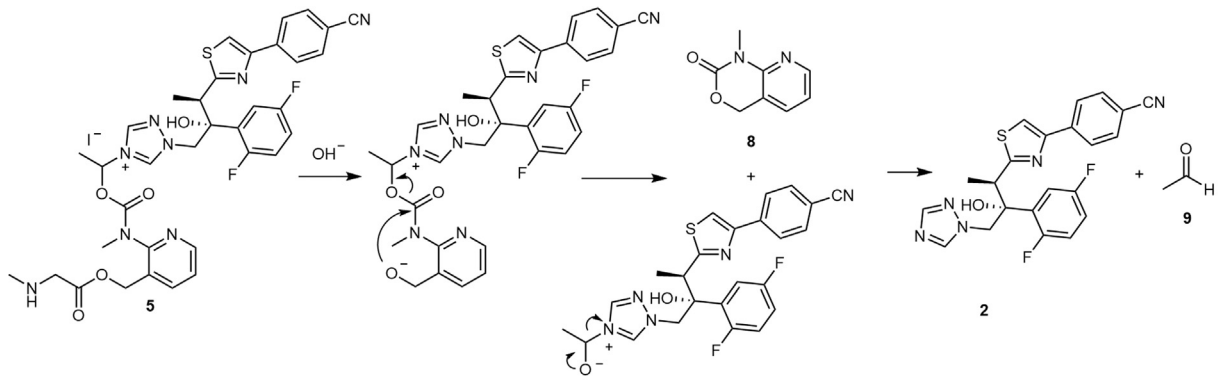
The conditions for the ion-exchange reaction were also explored. Initially, a strongly basic anion-exchange resin Amberlyst A-26 (OH^- form) was used. The resin was treated with ammonium hydrogen sulfate (NH_4HSO_4) solution to retain HSO_4^- in the resin. The solution of compound **6** in DCM was concentrated and the residue was re-dissolved in methanol and stirred with A-26 resin (HSO_4^- form) according to a reported study.[13] However, the

Table 1 Optimization of conditions for the forming of intermediate 4

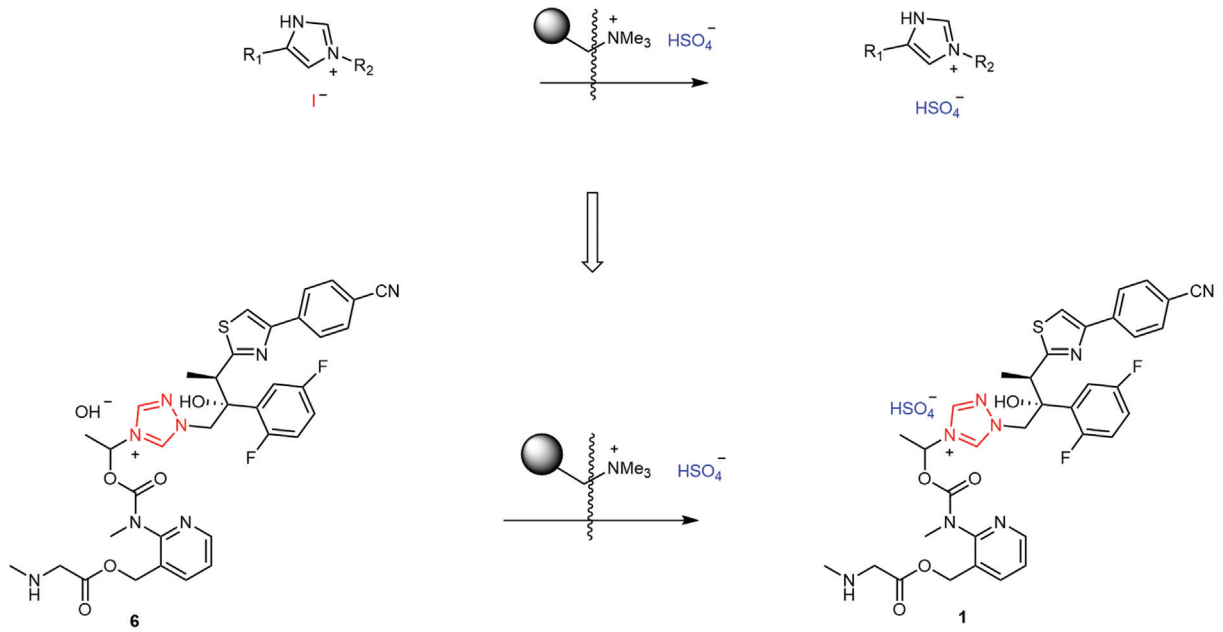
Entry	Solvent	Temp (°C)	Time (h)	NaI (mol%)	Yield (%)
1	THF	70	3	0.1	10.0
2	Acetone	70	3	0.1	9.7
3	CH_3CN	70	3	0.1	9.9
4	CH_3CN	50	3	0.1	9.9
5	CH_3CN	90	3	0.1	9.8
6	CH_3CN	70	5	0.1	9.9
7	CH_3CN	70	7	0.1	9.7
8	CH_3CN	70	3	20	19.4
9	CH_3CN	70	3	30	31.0
10	CH_3CN	70	3	50	44.2
11	CH_3CN	70	3	90	80.4
12	CH_3CN	70	3	110	95.6
13	CH_3CN	70	3	130	95.2



Scheme 3 Proposed mechanism for the synthesis of isavuconazonium iodide **4**.



Scheme 4 Degradation mechanism of compound **5** under basic conditions.



Scheme 5 Ion exchange between ionic compound and the specific resin.

reaction outcome was disappointing, with a formation of a complex product mixture. We assumed that the concentration process may contribute to the degradation of isavuconazonium, because compound **6** was thermally sensitive in solution. Besides, an additional stability experiment also indicated that compound **1** was unstable in methanol. Therefore, DCM solution of compound **6** was moved to the next procedure directly instead of using after concentration. Ion exchange was performed directly using DCM solution of compound **6**, unfortunately, the residue was found again to contain a complex mixture, which was hard to be purified.

Table 2 Effect of the type of different resins on the reaction outcome

Entry	Resin type	Yield	HPLC purity
1	D301	24.0%	97.65%
2	IRA402	34.0%	96.70%
3	IRA410	60.0%	98.03%

Abbreviation: HPLC, high-performance liquid chromatography.

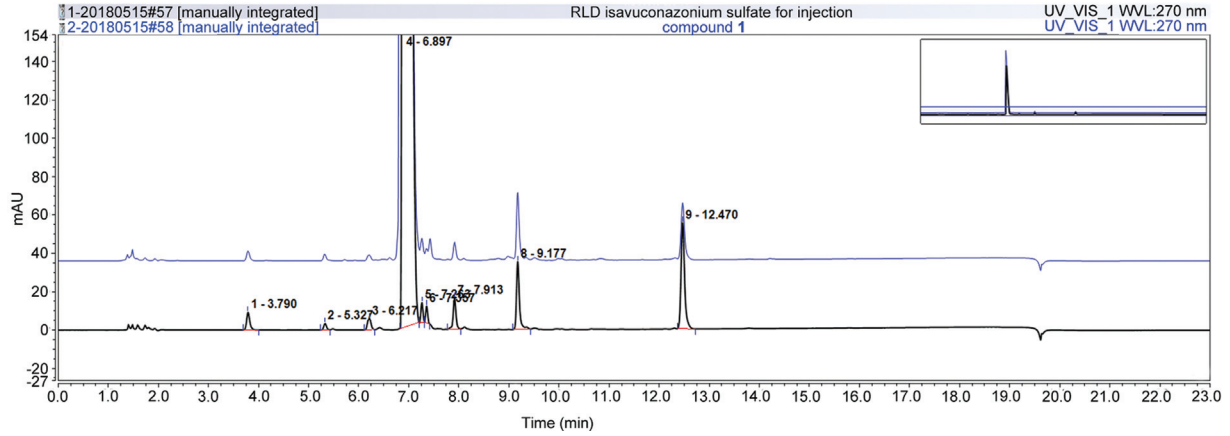


Fig. 2 Overlay map of RLD isavuconazonium sulfate for injection and compound **1** from HPLC analysis. HPLC, high-performance liquid chromatography; RLD, reference listed drug.

Then, a biphasic system was trialed in this step. The approach was based on the excellent water solubility of the expected product, with the expectation that performing anion exchange in a biphasic system would partition the targeted water-soluble products (mostly generated from the coordination of isavuconazonium with HSO_4^- in the aqueous phase), and the lipophilic impurities were reserved in the organic phase. Excitingly, after the reaction, the resulting aqueous solution was washed with DCM and lyophilized to give the target product in 97.0% purity, without requiring additional purification.

Due to the importance of the anion-exchange resin in this process, three types of commercially available strongly basic anion-exchange resins were screened to compare the resulting product purity and yield. The results indicated that resin Amberlite IRA410 gave the optimal results with a good yield (60.0%) and high-performance liquid chromatography (HPLC) purity (98.03%), as shown in ►**Table 2**.

Based on this novel and improved process, the batch was scaled up to 100 g and the results were reproduced in three validation productions. Furthermore, results from HPLC analysis showed that the peak position of compound **1** was consistent with that of RLD isavuconazonium sulfate for injection, of which the inactive ingredient was mannitol (►**Fig. 2**); however, the purity by measuring area percentage of the main peak according to the area normalization method showed that the purity of compound **1** was much higher (97.25%) in comparison to that of RLD isavuconazonium sulfate for injection (94.10%) (►**Figs. S6** and **S7 [online only]).**

Conclusion

In this study, an improved method for the synthesis of isavuconazonium sulfate (**1**) was reported. An anion-exchange resin was first used to introduce HSO_4^- to the target compound with the major advantages as the following: (1) only dissociation and ion exchange were mentioned in the new synthetic route, indicating the mild and facile reaction conditions in the whole preparation of the target compound; (2) high HPLC purity (97.25%) of compound **1** was obtained without the need for techniques such as recrystallization and column chromatography; (3) the process met the requirements of "green chemistry" based on the conversion of the starting material isavuconazole, and the overall yield of the synthetic route was 57%; and (4) the used resin could be recycled via acid–base neutralization reactions.

In summary, the novel and practical synthesis of isavuconazonium sulfate (**1**) was explored in this study, and this may also provide guidance for the synthesis other HSO_4^- salt analogues in the future. It is a promising reference of application of ion-exchange resins in organic synthesis.

Experimental Section

General

Unless otherwise noted, reagents were commercially available (obtained from Titan, Energy Chemical, Meryer, etc.) and used without purification. NMR data were obtained using a Bruker V-400 instrument (Bruker BioSpin AG, Industriestrasse 26, CH-8117, Fallanden) at 400 MHz for ^{1}H and

100 Hz for ^{13}C in either $CDCl_3$ or DMSO-d_6. The chemical shifts are reported in δ ppm relative to tetramethylsilane. HPLC analysis was performed on Agilent 1200 (Agilent Technologies, California, United States) using a Dionex U3000, a Symmetry Shield RP C18 HPLC column (4.6 mm × 250 mm, particle size 5 μm) under the following conditions: mobile A (0.05% TFA aqueous solution) and mobile B (acetonitrile) with gradient condition of 0–17 minutes: mobile A 75%; 17–23 minutes, mobile A 25%, and detection wavelength was 289 nm.

General Procedure to Load HSO_4^- Anion in Resin IRA410

NH_4HSO_4 aqueous solution (1 mol/L) was passed through a glass column (Shanghai Heqi Glassware Co., Ltd., Shanghai, China) packed with commercial resin IRA410 (OH^- form) (Titan) until the pH of the eluent reached the same value as that of the original solution. The process was performed at room temperature using gravity as the driving force.

Preparation of 4-(1-(((3-(((*N*-(*tert*-butoxycarbonyl)-*N*-methylglycyl)oxy)methyl)pyridin-2-yl)(methyl)carbamoyl)oxy)ethyl)-1-((2*R*,3*R*)-3-(4-(4-cyanophenyl)thiazol-2-yl)-2-(2,5-difluorophenyl)-2-hydroxybutyl)-1*H*-1,2,4-triazol-4-ium Iodide (4)

A solution of compound **2** (100 g, 228.83 mmol), sodium iodide (51.5 g, 343.25 mmol), and compound **3** (142.4 g, 343.25 mmol) was stirred in acetonitrile (1,000 mL) for 3 hours at 70°C under a nitrogen atmosphere. The reaction mixture was concentrated under reduced pressure, diluted with ethyl acetate (3 L), and then washed with HCl solution (2%, 1.5 L × 2) and H_2O (1.5 L). The organic layer was dried over $MgSO_4$, filtered, and the filtrate was evaporated under vacuum to give the crude product **4** as a light-yellow oil (211.7 g, 98% yield). b.p. 86°C (decomposed). ^1H NMR (400 MHz, DMSO-d_6) δ 10.16 (brd, J = 4.9, 1H), 9.01 (brd, J = 3.6, 1H), 8.49 (s, 1H), 8.24–8.20 (m, 2H), 7.96–7.91 (m, 2H), 7.44–7.26 (m, 6H), 7.15–7.05 (m, 1H), 6.66–6.61 (m, 1H), 6.17–5.90 (m, 2H), 5.76 (s, 2H), 5.09 (d, J = 14.8, 2H), 4.91–4.79 (m, 3H), 4.15 (q, J = 7.3, 1H), 3.15 (s, 1H), 3.12 (s, 2H), 1.99 (s, 3H), 1.42 (s, 9H), 1.20 (d, J = 6.9, 3H). ESI-MS (m/z): calcd. for $C_{40}H_{43}F_2IIN_8O_7S$ $[M-I]^+$ 817.2938; found 817.70.

Preparation of 1-((2*R*,3*R*)-3-(4-(4-cyanophenyl)thiazol-2-yl)-2-(2,5-difluorophenyl)-2-hydroxybutyl)-4-(1-((methyl(3-(((methylglycyl)oxy)methyl)pyridin-2-yl)carbamoyl)oxy)ethyl)-1*H*-1,2,4-triazol-4-ium Iodide Hydrochloride (5)

To a solution of compound **4** (211.7 g, 224.25 mmol) in ethyl acetate (1 L) was added hydrogen chloride ethyl acetate solution (2 N, 1121.3 mL) at room temperature. After stirring for 2 hours, the precipitate was filtered and washed with ethyl acetate. The precipitate was dried to afford the crude product **5** as a yellow solid (199.3 g, 97%). b.p. 95°C (decomposed). ^1H NMR (DMSO-d_6) δ 10.62–10.35 (m, 1H), 9.39–9.08 (m, 3H), 8.48 (s, 1H), 8.20 (d, J = 7.92 Hz, 2H), 7.99 (d, J = 8.25 Hz, 2H), 7.49–7.02 (m, 5H), 6.84–6.59 (m, 1H), 5.22–4.56 (m, 5H), 4.28–3.79 (m, 2H), 3.30–3.10 (m, 3H), 1.64–1.10 (m, 12H). ESI-MS (m/z): calcd. For $C_{35}H_{35}F_2IN_8O_5S$ $[M-I]^+$ 717.2414; found 717.60.

Preparation of 1-((2*R*,3*R*)-3-(4-(4-cyanophenyl)thiazol-2-yl)-2-(2,5-difluorophenyl)-2-hydroxybutyl)-4-(1-((methyl(3-(((methylglycyl)oxy)methyl)pyridin-2-yl)carbamoyl)oxy)ethyl)-1*H*-1,2,4-triazol-4-ium OH^- (6)

Compound **5** (199.3 g, 217.5 mmol) was dissolved in a mixture of DCM (1.5 L) and H_2O (500 mL). Then, $NaHCO_3$ solution (aq, 5%) was slowly added at 0°C until the pH of the mixture was 7.8 to 8.0. The organic layer was separated and maintained at 0°C for the next procedure without further work-up. The product was not isolated and structure conformed.

Preparation of the Target Product (1)

To a solution of the organic layer above (1.5 L) and deionized water (100 mL) was added resin IRA410 (HSO_4^- form, 1.36 L). The mixture was stirred for 1.5 hours. Water layer was separated, washed with DCM (200 mL × 2), and then lyophilized to give the target compound **1** (106.3 g, 60%) as a white solid. HPLC purity: 97.25%. b.p. 100°C (decomposed); m.p. 143.6–146.2°C. ^1H NMR (400 MHz, D_2O) δ 8.85 (d, J = 30.5 Hz, 1H), 8.66 (s, 1H), 8.44–8.29 (m, 1H), 7.99 (t, J = 7.7 Hz, 1H), 7.89–7.70 (m, 3H), 7.51 (ddd, J = 21.7, 18.0, 6.9 Hz, 3H), 6.87 (ddd, J = 47.0, 19.8, 14.0 Hz, 3H), 5.37–4.99 (m, 3H), 4.99–4.74 (m, 3H), 4.67–4.50 (m, 2H), 4.29–4.10 (m, 1H), 4.01 (d, J = 16.0 Hz, 2H), 3.29–3.06 (m, 3H), 2.80–2.62 (m, 3H), 1.58–1.42 (m, 1H), 1.84 (s, 1H), 1.12 (dd, J = 23.0, 6.9 Hz, 3H). $[\alpha]_D^{24}$ +3.505° (c 1.0, H_2O). ^{13}C NMR (151 MHz, D_2O) δ 171.76, 166.98, 159.40, 157.79, 155.35, 153.78, 152.95, 151.86, 151.23, 150.70, 149.13, 142.45, 140.19, 137.80, 132.64 (×2), 128.12, 126.45, 125.01, 124.69, 119.15, 117.62, 115.38, 110.06, 79.36, 76.41, 63.04, 59.08, 50.53, 48.63, 43.90, 35.98, 33.00, 19.09, 16.69. ESI-MS (m/z): calcd. For $C_{35}H_{36}F_2N_8O_9S_2$ $[M-HSO_4]^+$ 717.2414; found 717.60.

Supporting Information

Spectroscopic characterization processes (NMR and ESI-MS) for **4** and **1**, as well as HPLC results for the purities of compound **1** following the improved synthesis route, are included in the Supporting Information (►**Figs. S1–S7** [online only]).

Conflict of Interest
None.

Acknowledgments
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