Improved synthesis of YT-14, a potent antibiotic to multidrug-resistant strains

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A new practical synthetic approach produced clinical drug candidate YT-14, improving the overall yield from 1.3% to 13.8%. Compared with the previous route, the new route is two steps shorter and all of the steps involve purifications without column chromatography. The advantages of this procedure include simple operating conditions and higher yields.

Keywords: drug candidate, YT-14, multidrug resistant, Gram-negative bacteria

The development and spread of bacterial resistance has evolved into a major public health crisis that endangers human health.[1] Especially for multidrug-resistant Gram-negative bacteria with high lethality, there is almost no completely effective therapeutic drug in the world.[2] Even worse, effective drugs against Gram-negative bacteria in phase II and III clinical trials are also rare. Hence, the development of a Gram-negative antibacterial drug is of great clinical significance and likely social value.[3]

Recently, a new siderophore conjugated antibiotic YT-14 (Fig. 1) has been reported.[4] With the Trojan strategy,[5–7] YT-14 shows excellent *in vitro* and *in vivo* activity against multidrug-resistant bacteria, including *Escherichia coli* (Eco), *Klebsiella pneumoniae* (Kpn), *Acinetobacter baumannii* (Aba) and *Pseudomonas aeruginosa* (Pae), which are resistant to meropenem and aztreonam (Table 1). Compared with the phase I clinical drug BAL30072 (Fig. 1),[8] YT-14 displays a potent *in vitro* activity against Kpn, whereas BAL30072 has very weak activity (Table 1). For the antibacterial activity against Eco, Aba, and Pae, YT-14 is significantly better than BAL30072. Meanwhile, compared with the current most promising phase III clinical drug S-649266 (Fig. 1),[9,10] the activity of YT-14 is as good as that of S-649266 against Kpn and Pae and is slightly better than S-649266 against Aba, which is recognised as a increasing class of aggressively pathogenic Gram-negative bacteria (Table 1).[11,12] Moreover, YT-14 exhibits a favourable pharmacokinetic profile and has no hERG inhibition. For a preclinical study, considerable material is needed. Considering this, we have developed a novel synthetic route for the potent antibiotic YT-14.

Table 1 Antibacterial activity of YT-14, BAL30072 and S-649266[a]

Compound	MIC (μg mL^{-1})			
	Eco[b]	Aba[c]	Kpn[d]	Pae[e]
YT-14	<0.03–0.5	1–4	0.125–4	0.5–4
BAL30072	0.125–1	1–>64	0.5–>64	1–8
S-649266	<0.03–0.125	1–8	0.125–4	0.5–4
Meropenem	0.25–8	64–>64	>64	32–64
Aztreonam	0.03–32	64–>64	>64	16–64

[a]Activity of YT-14, BAL30072 and S-649266 were tested under iron limitation.

[b]Eco, *E.* coli, five strains.

[c]Aba, multidrug-resistant *A. baumannii.*

[d]Kpn, multidrug-resistant *K. pneumoniae*, five strains.

[e]Pae, multidrug-resistant *P. aeruginosa*, five strains.

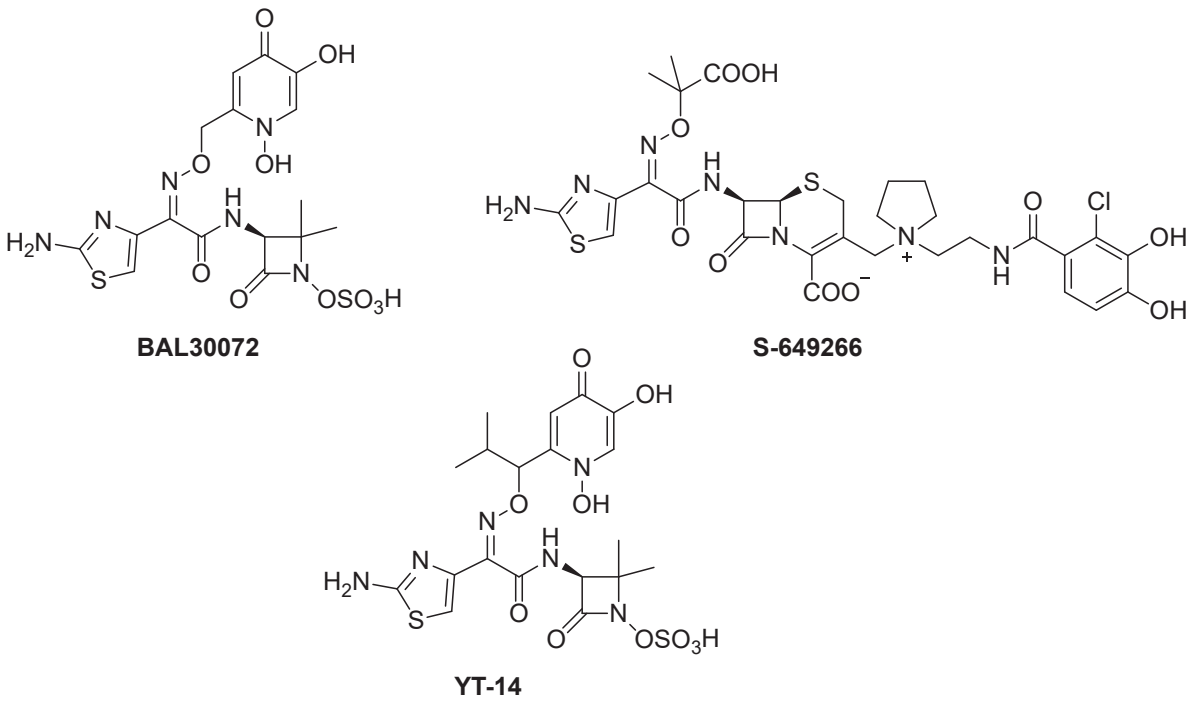

Fig. 1 Chemical structures of siderophore cephalosporin antibiotics.

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Results and discussion

We hoped to design a synthetic route for YT-14 based on the synthetic route for BAL30072. Mitsunobu reaction of compound **1** and *N*-hydroxyphthalimide gives key intermediate **2**, and BAL30072 is obtained through a series of reactions using compound **2** as a starting material. Therefore, compound **1** was oxidised first to provide aldehyde **3**, and alcohol **4** was obtained by Grignard reaction. Unfortunately, however, the Mitsunobu reaction from alcohol **4** to phthalimide **5** could not be carried out under any conditions (Fig. 2). Thus, we considered using a less-hindered benzyl group for a series of reactions first, followed by deprotection of alcohol **6** to give compound **7** and protection with a benzhydryl group to obtain the intermediate **8**. Finally, YT-14 was synthesised through a series of reactions using the intermediate **8** as a starting material. However, this route had three major problems that hampered its scale-up (Scheme 1): (1) the step of compound **6** to **8** was low yielding (total yield 38%), and the purification of compound **9** was difficult (purification by column chromatography) and hence not suitable for industrial amplification; (2) the synthetic route

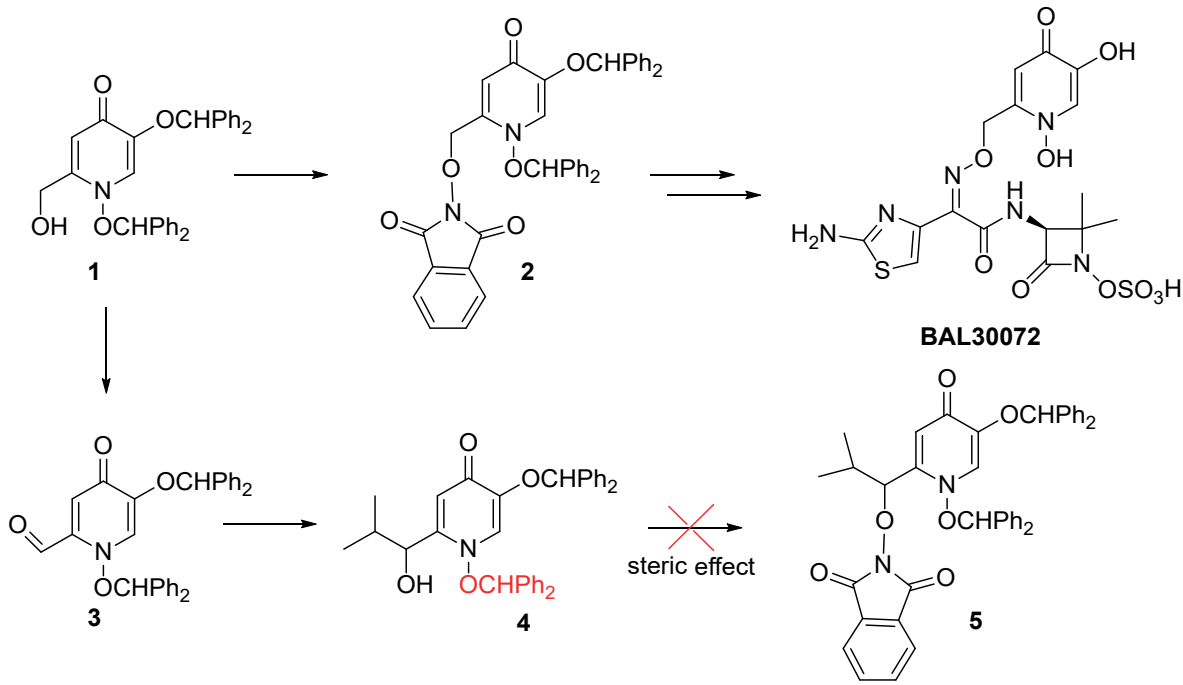
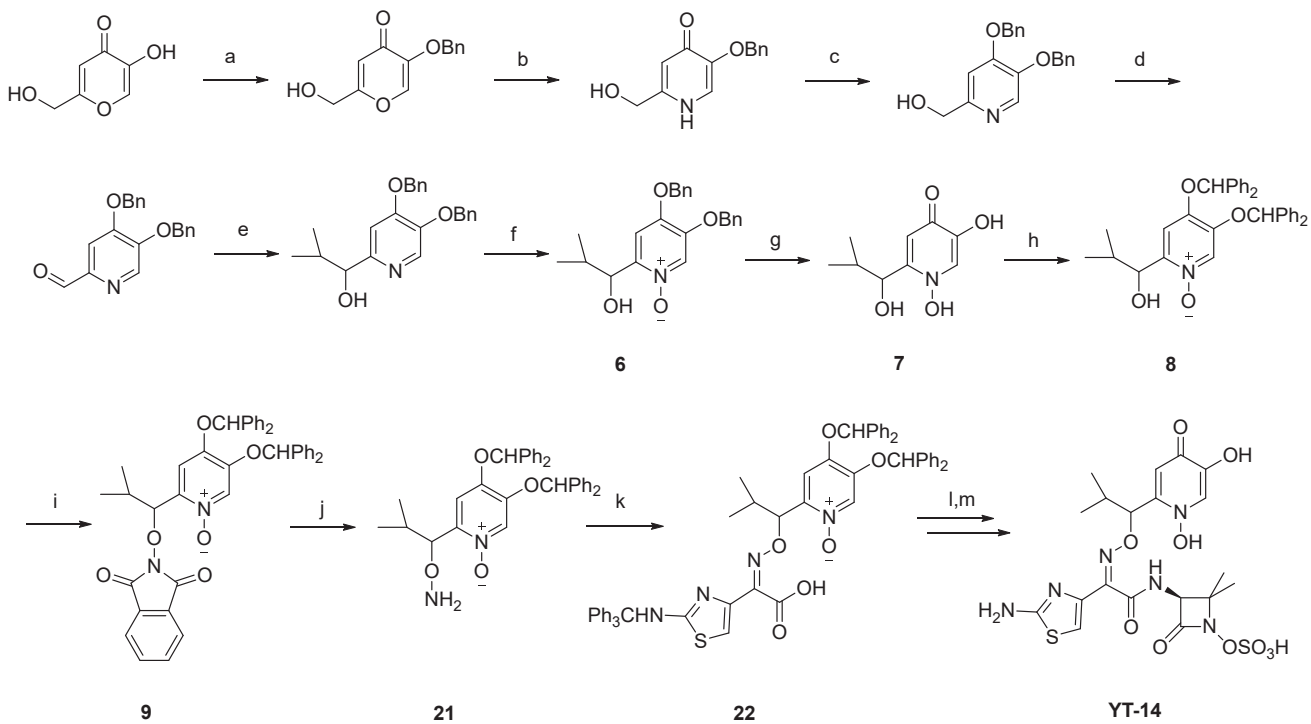


Fig. 2 Original proposed synthetic route of the key intermediate **5** towards YT-14.

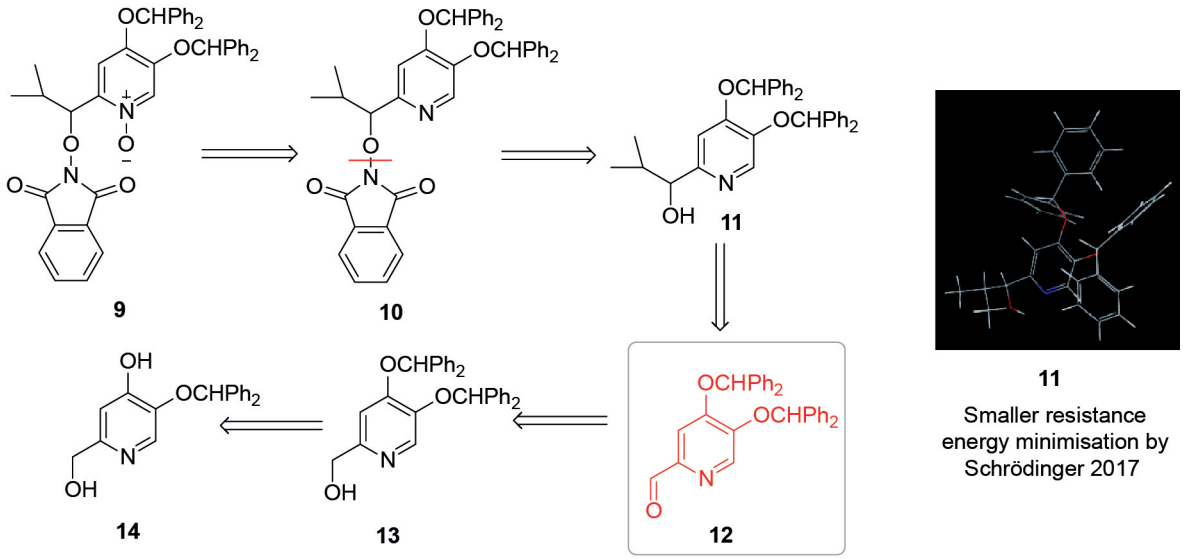


Scheme 1 Initial medicinal chemistry route to YT-14. Reagents and conditions: (a) BnCl, NaOH, MeOH, 60 °C, 91%; (b) NH_4OH, MeOH, 55 °C, 90%; (c) BnCl, K_2CO_3, DMSO, 50 °C, 43%; (d) pyridine-SO_3, DMSO, NEt_3, CH_2Cl_2, 95%; (e) *i*-PrMgBr, THF, 68%; (f) *m*-CPBA, CH_2Cl_2, r.t., 81%; (g) BCl_3, CH_2Cl_2, −10 °C; (h) diphenyldiazomethane, MeOH/CH_2Cl_2, r.t., 38% for two steps; (i) *N*-hydroxyphthalimide, PPh_3, DIAD, THF, 56%; (j) N_2H_4, MeOH, r.t., 79%; (k) MeOH/CH_2Cl_2, r.t., 65%; (l) HATU, $NaHCO_3$, DMSO, 94%; (m) TFA, Et_3SiH, CH_2Cl_2, −15 °C, 69%.

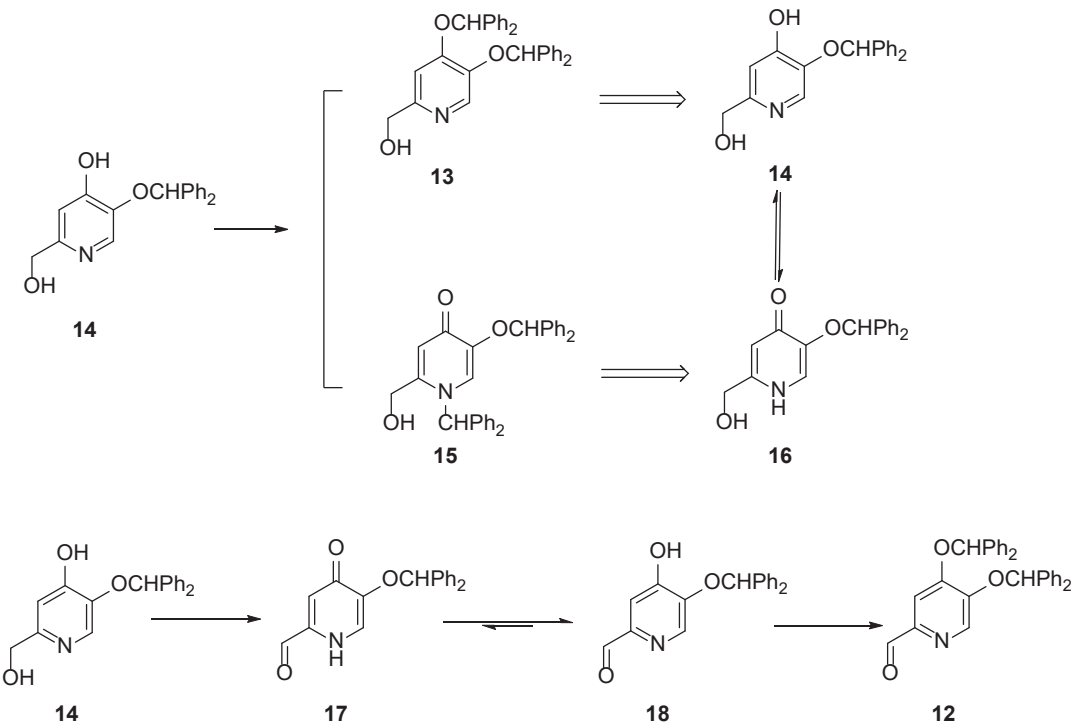
was long, while column chromatographic purification was required following most of the reactions; and (3) phthalimide **9** was difficult to obtain from the Mitsunobu reaction in the post-processing because of the polarity of triphenylphosphine oxide, and the reduction products DIAD and phthalimide **9** had very similar characteristics. These problems prompted us to seek a better synthesis of YT-14, which in turn meant that an efficient synthesis of the precursor **9** was needed.

As shown in Scheme 2, retrosynthetic analysis suggests that phthalimide **9** could be obtained by *m*-CPBA oxidation of compound **10**. Meanwhile, compound **10** is easily separated from triphenylphosphine and the reduction products of DEAD. With less steric hindrance, alcohol **11**, which is obtained by Grignard reaction of compound **12** and isopropylmagnesium bromide, can promote the Mitsunobu reaction. Furthermore, direct protection using the benzhydryl group can reduce the number of reaction steps. However, in the reaction of **14** to **13**, a large amount of the byproduct alcohol **15** was produced. This may be due to the equilibrium of **14** and **16** and steric effects in the reaction. Therefore, we considered whether it might be better to oxidise compound **14** first, giving compounds **17** and **18**. Owing to the conjugation effect of the aldehyde groups and aromatic rings, it might form the more stable intermediate **18** (Scheme 3). At the same time, the decrease in pKa of the phenolic hydroxyl group also facilitates the Sn_2 reaction of **18** to **12** (calculated by ACDLABS) (Scheme 4). Finally, aldehyde **12** was obtained in a very high yield (Scheme 3).

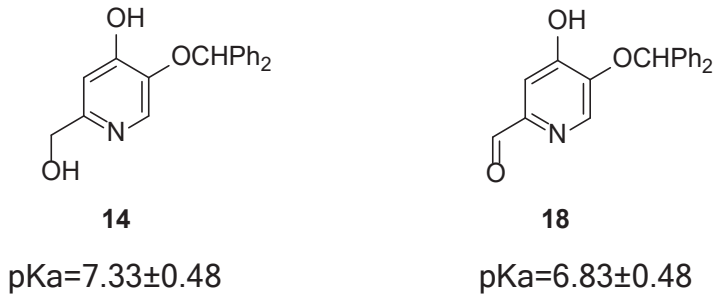
The final synthetic route to YT-14 is illustrated in Scheme 5. Selectively protecting the hydroxyl group of kojic acid **19** with diphenyldiazomethane provides intermediate **20**. By heating with ammonia, alcohol **14** was obtained, which was oxidised to give compound **18** by heating with manganese dioxide in



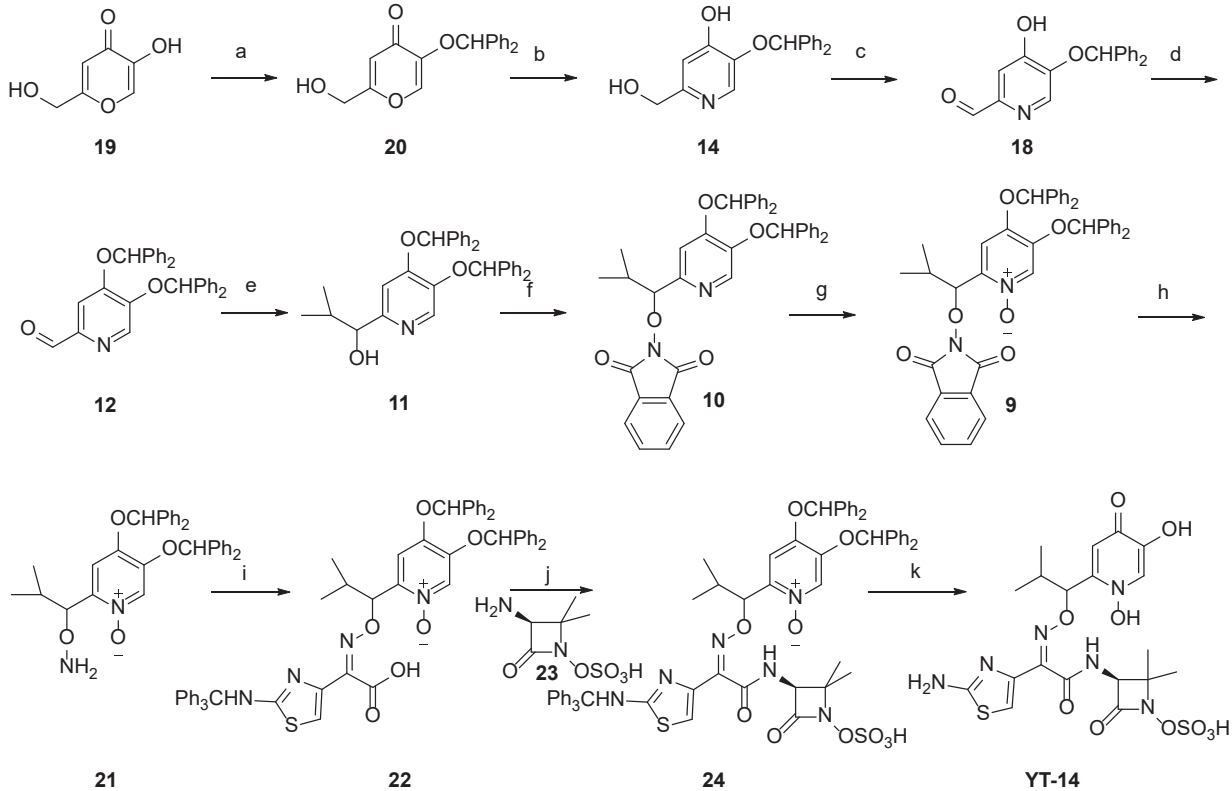
Scheme 2 Initial retrosynthetic analysis of compound **9**.



Scheme 3 Synthetic route changes of the key intermediate **13**.



Scheme 4 pKa prediction of phenolic hydroxyl group.



Scheme 5 Final synthetic route to YT-14. Reagents and conditions: (a) diphenyldiazomethane, MeOH/CH_2Cl_2, r.t., 82.7%; (b) NH_4OH, MeOH, 55 °C, 90.6%; (c) MnO_2, C_2H_5OH, 60 °C, 53.1%; (d) diphenylbromomethane, DMF, K_2CO_3, 78%; (e) *i*-PrMgBr, THF; (f) *N*-hydroxyphthalimide, PPh_3, DEAD, THF; (g) *m*-CPBA, CH_2Cl_2, r.t., 73.2% for three steps; (h) N_2H_4, MeOH, r.t.; (i) MeOH/CH_2Cl_2, r.t., 86% for two steps; (j) HATU, $NaHCO_3$, DMSO, 93%; (k) TFA, Et_3SiH, CH_2Cl_2, −15°C, 83%.

ethanol, and then reacting with bromodiphenylmethane, to give aldehyde **12**. Nucleophilic addition of isopropylmagnesium bromide afforded alcohol **11**. Mitsunobu reaction with *N*-hydroxyphthalimide provided compound **10**, which was converted to phthalimide **9** with *m*-CPBA. By reaction with hydrazine hydrate and removal of the phthaloyl groups, the alkoxyamine **21** was obtained. The key intermediate oxime acid **22** was prepared *via* condensation of 2-oxo-2-(2-(tritylamino)thiazol-4-yl) acetic acid and alkoxyamine **21**. With condensation of the oxime acid **22** with compound **23**, the acid **24** was provided. The final product YT-14 was obtained by deprotection of acid **24**.

Finally, the YT-14 that we obtained and the YT-14 obtained by the previous method were compared.[4] YT-14 has two isomers in the isopropyl position. In the previous route, YT-14 in the *S* and *R* configurations were synthesised by asymmetric synthesis. Furthermore, the crystal structure of compound **8** in the *S* configuration was resolved. However, the antibacterial activity of an isomeric mixture of YT-14 was better than either of the single *R* or *S* configurations of YT-14.[4] Therefore, a racemic form of YT-14 was chosen for further study. We synthesised YT-14 with a *R*/*S* ratio of 46:54 without column chromatography purification whereas the previous method obtained YT-14 with a *R*/*S* of 48:52 but column chromatography was required in many post-treatments.[4] Moreover, the relationship between the *R*/*S* ratio and the antibacterial activity of YT-14 needs further study in the future. However, it is undeniable that our new route greatly simplifies the synthesis of YT-14, and we were able to obtain key intermediate phthalimide **9** (*R*/*S* 50:50) in an overall yield of 20.8% without purification by column chromatography, while the yield obtained *via* the previous route was only 3.9% with complicated post-processing.

Conclusion

A novel synthetic route to drug candidate YT-14 has been developed. By changing the materials in the Mitsunobu reaction, the key alkoxyamine **10** could be easily separated from triphenylphosphine oxide and the reduction products of DEAD

by a silicone sand core funnel. Compared with the previous route, this new route is shorter by two steps. All of these reactions did not need to be purified by column chromatography and could be amplified. Using this new process, we were able to obtain key intermediate phthalimide **9** in an overall yield of 20.8% in seven steps while the previous route was only 3.9% in nine steps and the scale has been increased to more than 50 g. The final product YT-14 was obtained in an overall yield of 13.8%, which was better than the previous route with a yield of 1.3%. Furthermore, the key intermediate **9** was increased by more than 500 g by Xi'an Manareco through this route.

Experimental

Unless otherwise mentioned, all reagents were purchased from commercial suppliers and used without further purification. All reactions were monitored by TLC, using silica gel plates with fluorescence GF254 (Yu Cheng Chemical, Shanghai) and UV light visualisation. Analytical HPLC was conducted using UV detection and the following conditions: (A) Platisil ODS column (250 × 4.6 mm, 5 μm) using a gradient of 10% v/v MeCN in H_2O to 90% v/v MeCN in H_2O with detection at 254 nm; (B) Platisil ODS column (250 × 4.6 mm, 5 μm) using a gradient of 30% v/v MeCN in buffer solution (0.1% CF_3COOH and 0.1% NH_4OH in water, PH 3.5) to 70% v/v MeCN in H_2O with detection at 254 nm. Melting points were determined on an X-4 melting point apparatus without further correction. ^{1}H NMR and ^{13}C NMR spectra were provided on a Bruker ARX-400 or ARX-500 spectrometer using TMS as an internal standard. Chemical shifts (δ) are given in parts per million (ppm) relative to the internal residual solvent peak. Coupling constants (*J*) are recorded in Hertz (Hz). ESI-MS spectra were obtained on an Agilent G6520 Q-TOF mass spectrometer. All pure solid compounds, such as **10**, **11** and **21**, were obtained by column chromatography to test the melting point, ^{1}H NMR, ^{13}C NMR and ESI-MS spectra. Diphenyldiazomethane should be used and prepared carefully.

*Synthesis of 5-(benzhydryloxy)-2-(hydroxymethyl)-4*H*-pyran-4-one* (**20**)

A solution of benzophenone hydrazone (276 g, 1.406 mol) in petroleum ether (PE) (600 mL) was treated with MnO_2 (293.6 g, 3.377 mol). The reaction mixture was stirred at room temperature for 1 h. The resulting mixture was filtered and then the filtrate was concentrated *in vacuo* to give diphenyldiazomethane. Then diphenyldiazomethane was dissolved in dichloromethane (DCM) (300 mL), which was used in the next step without further purification. A suspension of kojic acid **19** (80 g, 562.9 mmol) in methanol (300 mL) was treated with the dichloromethane solution of diphenyldiazomethane. The mixture was stirred at room temperature for 20 h and concentrated *in vacuo* to remove methanol and DCM. The resulting residue was taken up in PE (500 mL) to give a precipitate. The solids were filtered off and the product was washed with a mixture 1:1 (v/v) of PE and water (25 °C, 300 mL) to obtain the desired product **20** without further purification. The product was dried at 40 °C under reduced pressure to provide compound **20** as a white solid; yield 143.6 g (82.7%); m.p. 126–128 °C; HPLC purity (condition A): 98%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (s, 1H), 7.41–7.28 (m, 10H), 6.48 (s, 1H), 6.34 (s, 1H), 4.39 (d, *J* = 6.5 Hz, 2H), 2.88 (t, *J* = 6.6 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 174.25, 168.51, 145.51, 145.07, 140.99 (2C), 129.02 (2C), 128.33 (4C), 127.18 (4C), 112.13, 81.37, 59.70; MS (ESI) *m/z*: 331 [M + Na]$^+$. HRMS (ESI) *m/z* calcd for $C_{19}H_{16}NaO_4$ [M + Na]$^+$: 331.0941; found: 331.0937.

*Synthesis of 5-(benzhydryloxy)-1-hydroxy-2-(hydroxymethyl)pyridin-4(1*H*)-one* (**14**)

A suspension of **20** (127.3 g, 412.9 mmol) in methanol (500 mL) was treated with NH_4OH (500 mL, 6.6 mol). The resulting mixture was heated to 55 °C and stirred for 20 h. The reaction mixture was cooled to room temperature and filtered off. The solids were washed with a 2:1 (v/v) mixture of water and dichloromethane (25 °C, 300 mL) and the desired product **14** was obtained without further purification. The product was dried at 40 °C under reduced pressure to provide compound **14** as a light brown solid; yield 115.0 g (90.6%); m.p. 191–193 °C; HPLC purity (condition A): 93%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.79 (s, 1H), 7.63–7.18 (m, 10H), 6.68 (s, 1H), 6.57 (s, 1H), 5.05 (s, 0H), 4.24 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 149.82, 145.36, 142.04 (3C), 128.88, 128.78 (4C), 127.91 (2C), 127.23 (4C), 112.63, 80.88, 60.39. MS (ESI) *m/z*: 306 [M – H]$^-$. HRMS (ESI) *m/z* calcd for $C_{19}H_{16}NO_3$ [M – H]$^-$: 306.1136; found: 306.1139.

Synthesis of 5-(benzhydryloxy)-4-hydroxypicolinaldehyde (**18**)

A solution of **14** (100 g, 325.2 mmol) in ethanol (800 mL) was treated with MnO_2 (428 g, 4.922 mol). The reaction mixture was heated to 65 °C and stirred 5 h. The resulting mixture was filtered and the filter residue was washed by ethanol. Then the filtrate was concentrated *in vacuo*. The resulting residue was taken up in ethyl acetate (EA) and filtered with a silicone core funnel. Then the filtrate was concentrated *in vacuo* to a final volume (150 to 250 mL). The solid was filtered off and the desired product **18** was obtained without further purification. The product was dried at 40 °C under reduced pressure to provide compound **18** as a white solid; yield 52.8 g (53.1%); m.p. 196–198 °C; HPLC purity (condition A): 93%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.14 (s, 1H), 9.70 (s, 1H), 8.34 (s, 1H), 7.64–7.19 (m, 11H), 6.86 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 193.00, 154.86, 147.99, 146.56, 141.19 (2C), 138.22, 129.09 (4C), 128.34 (2C), 127.09 (4C), 109.45, 81.24; MS (ESI) *m/z*: 304 [M – H]$^-$. HRMS (ESI) *m/z* calcd for $C_{19}H_{14}NO_3$ [M – H]$^-$: 304.0979; found: 304.0983.

Synthesis of 4,5-bis(benzhydryloxy)picolinaldehyde (**12**)

A solution of **18** (41.8 g, 136.9 mmol) in anhydrous *N*,*N*-dimethylformamide (DMF) (300 mL) was treated with $MgSO_4$ (41.8 g, 347.2 mmol), K_2CO_3 (37.8 g, 273.8 mmol) and bromodiphenylmethane (40.6 g, 164.3 mmol) under argon. The resulting mixture was heated to 55 °C and stirred for 12 h. The resulting mixture was extracted with EA and the organic phase was washed with water and brine, dried over anhydrous Na_2SO_4 and filtered, and the filtrate was concentrated *in vacuo*. Purification by recrystallisation from a 4:1 (v/v) mixture of PE and EA (50 °C, 750 mL) afforded compound **12**. The product was dried at 40 °C under reduced pressure to provide compound **12** as a white solid; yield 50.3 g (78%); m.p. 99–101 °C. HPLC purity (condition A): 99%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.76 (s, 1H), 8.23 (s, 1H), 7.52–7.26 (m, 21H), 6.43 (s, 1H), 6.42 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 192.22, 155.03, 148.23, 148.04, 140.37(2C), 139.98 (2C), 138.82, 128.87 (4C), 128.78 (4C), 128.28 (2C), 128.23 (2C), 126.76 (4C), 126.60 (4C), 107.75, 83.72, 82.43; MS (ESI) *m/z*: 472 [M + H]$^+$. HRMS (ESI) *m/z* calcd for $C_{32}H_{26}NO_3$ [M + H]$^+$: 472.1907; found: 472.1915.

Synthesis of 1-(4,5-bis(benzhydryloxy)pyridin-2-yl)-2-methylpropan-1-ol (**11**)

A solution of isopropylmagnesium bromide (3 M) in tetrahydrofuran (THF) (127.4 mL, 382.2 mmol) under argon atmosphere was cooled to 0 °C, and then a solution of **12** (60.1 g, 127.4 mmol) in dry THF (400 mL) was added dropwise to keep the reaction mixture below 10 °C. After addition, the resulting mixture was allowed to warm to room temperature and stirred for 5 h. After the reaction was completed, it was quenched by slow addition of saturated aqueous NH_4Cl. The resulting mixture was filtered and filter residue was washed with EA. Then the solution was extracted with EA, washed with brine, dried over anhydrous Na_2SO_4 and filtered, and the filtrate was concentrated *in vacuo* to obtain the crude racemic compound **11** as a light yellow oil (without further purification); yield 56.7 g. Pure racemic form compound **11** as a white waxy solid; m.p. 103–105 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (s, 1H), 7.51–7.18 (m, 20H), 6.63 (s, 1H), 6.33 (s, 1H), 6.24 (s, 1H), 4.23 (d, *J* = 4.6 Hz, 1H), 1.71 (pd, *J* = 6.8, 4.7 Hz, 1H), 0.76 (d, *J* = 6.8 Hz, 3H), 0.57 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.32, 155.37, 143.92, 141.08, 141.07, 140.26, 140.22, 138.51, 128.82 (4C), 128.60 (4C), 128.16, 128.12, 127.98, 127.96, 127.08 (2C), 127.01 (2C), 126.65 (2C), 126.63 (2C), 107.85,

84.45, 82.26,77.11, 34.98, 19.04, 16.34. MS (ESI) *m/z*: 516 $[M + H]^+$. HRMS (ESI) *m/z* calcd for $C_{35}H_{34}NO_3$ $[M + H]^+$: 516.2533; found: 516.2542.

Synthesis of 2-(1-(4,5-bis(benzhydryloxy)pyridin-2-yl)-2-methylpropoxy)isoindoline-1,3-dione (**10**)

A solution of **11** (56.7 g) in dry THF (300 mL) was treated with *N*-hydroxyphthalimide (24.6 g, 152.9 mmol) and PPh_3 (40.1 g, 152.9 mmol) under an argon atmosphere and the mixture was cooled to 0 °C. A solution of DEAD (24.0 mL, 152.9 mmol) in dry THF (80 mL) was added dropwise. After addition, the resulting mixture was allowed to warm to room temperature and stirred for 3 h. The solution was concentrated *in vacuo*. The resulting residue was taken up in PE (1.2 L) and EA (240.0 mL) and filtered with a silicone core funnel. Then the filtrate was concentrated *in vacuo* to afford the crude racemic form compound **10** as a colourless oil (without further purification); yield 93.5 g. Pure racemic form compound **10**: Colourless waxy solid; m.p. 128–130 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (s, 1H), 7.74–7.64 (m, 4H), 7.57–7.50 (m, 4H), 7.47–7.38 (m, 5H), 7.37–7.20 (m, 12H), 6.62 (s, 1H), 6.21 (s, 1H), 5.01 (d, *J* = 6.5 Hz, 1H), 2.00 (dq, *J* = 13.6, 7.4, 6.8 Hz, 1H), 0.88 (d, *J* = 6.7 Hz, 3H), 0.65 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 163.70 (2C), 154.92, 152.70, 144.43, 141.17, 141.14, 140.94, 140.50, 138.96, 134.28 (2C), 128.99, 128.85 (2C), 128.54 (4C), 128.49 (3C), 127.96, 127.89, 127.86, 127.80, 127.07 (2C), 126.88 (2C), 126.84 (2C), 126.67 (2C), 123.36 (2C), 109.68, 94.09, 84.21, 82.05, 32.72, 18.41, 17.82; MS (ESI) *m/z*: 661 $[M + H]^+$. HRMS (ESI) *m/z* calcd for $C_{43}H_{37}N_2O_5$ $[M + H]^+$: 661.2697; found: 661.2690.

Synthesis of 4,5-bis(benzhydryloxy)-2-(1-((1,3-dioxoisoindolin-2-yl)oxy)-2-methylpropyl)pyridine 1-oxide (**9**)

A solution of **10** (93.5 g) in DCM (450 mL) was treated with *m*-CPBA (65.9 g, 382.2 mmol). The resulting mixture was stirred for 1 h before being quenched by addition of saturated $Na_2S_2O_3$ solution. Then the resulting mixture was extracted with DCM, washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and filtered, and the filtrate was concentrated *in vacuo*. After purification by recrystallisation from a 3:1 (v/v) mixture of PE and EA (60 °C, 400 mL), the mixture was cooled to 15 °C for 12 h and filtered off. The product was dried at 40 °C under reduced pressure to provide compound **9** as a white solid; yield 58.0 g (67.2%) for three steps; m.p. 191–193 °C; HPLC purity (condition A): 98%, *R*/*S* 50:50; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (dd, *J* = 5.6, 3.1 Hz, 2H), 7.73 (s, 1H), 7.70 (dd, *J* = 5.6, 3.1Hz, 2H), 7.59 (s, 1H), 7.59–7.55 (m, 4H), 7.46–7.27 (m, 16H), 6.72 (s, 1H), 6.15 (s, 1H), 5.88 (d, *J* = 6.8 Hz, 1H), 2.11 (h, *J* = 6.9 Hz, 1H), 1.08 (d, *J* = 6.9 Hz, 3H), 0.64 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 163.64 (2C), 147.46, 145.49, 142.83, 140.66, 140.11 (2C), 140.04, 134.48 (2C), 130.34 (2C), 128.99 (2C), 128.94, 128.80 (2C), 128.72 (2C), 128.53 (2C), 128.29, 128.20, 128.17, 127.95, 126.79 (2C), 126.77 (2C), 126.73 (2C), 126.61 (2C), 123.49 (2C), 111.50, 85.90, 84.39, 82.83, 31.02, 18.95, 15.66; MS (ESI) *m/z*: 677 $[M + H]^+$. HRMS (ESI) *m/z* calcd for $C_{43}H_{37}N_2O_6$ $[M + H]^+$: 677.2646; found: 677.2662.

Synthesis of 4,5-bis(benzhydryloxy)-2-(1-((1,3-dioxoisoindolin-2-yl)oxy)-2-methylpropyl)pyridine 1-oxide (**21**)

A solution of **9** (10 g, 14.8 mmol) in methanol (150 mL) was treated with 85% hydrazine hydrate (1.69 ml, 29.6 mmol). The resulting mixture was stirred at room temperature for 60 min. Then the filtrate was concentrated *in vacuo*. Then EA (100 mL) and PE (200 mL) were added to the residue. The mixture was filtered and the filter residue was washed by a 2:1 (v/v) mixture of PE and EA (25 °C, 90 mL). The filtrate was concentrated in *vacuo* to afford crude compound **21** as: Colourless oil (without further purification); yield 9.3 g. Pure compound **21** as a colourless colloidal solid; m.p. 71–73 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.96 (s, 1H), 7.61–7.21 (m, 20H), 6.87 (s, 1H), 6.81 (s, 1H), 6.68 (s, 1H), 4.67 (d, *J* = 3.7 Hz, 1H), 1.82 (td, *J* = 7.0, 3.8 Hz, 1H), 0.81 (d, *J* = 6.9 Hz, 3H), 0.35 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 147.74, 145.70, 145.41, 140.04, 140.02, 139.96, 139.80, 130.87, 128.89 (2C), 128.81 (2C), 128.79 (2C), 128.77 (2C), 128.31 (2C), 128.25 (2C), 126.73 (4C), 126.68 (4C), 110.29, 84.58, 84.48, 83.14, 30.41, 19.00, 16.59; MS (ESI) *m/z*: 547 $[M + H]^+$. HRMS (ESI) *m/z* calcd for $C_{35}H_{35}N_2O_4$ $[M + H]^+$: 547.2591; found: 547.2600.

Synthesis of (Z)-4,5-bis(benzhydryloxy)-2-(1-(((carboxy(2-(tritylamino)thiazol-4-yl)methylene)amino) oxy)-2-methylpropyl)pyridine 1-oxide (**22**)

A solution of **21** (9.3 g) in anhydrous methanol and DCM (3:8, 110 mL) was treated with 2-oxo-2-(2-(tritylamino)thiazol-4-yl)acetic acid (5.8 g, 14.0 mmol) and the resulting mixture was stirred at room temperature for 4 h. 2-Oxo-2-(2-(tritylamino)thiazol-4-yl)acetic acid was synthesised according to a previous method.[13] When the reaction was complete, the mixture was filtered and the filter cake was washed with EA. After purification by recrystallisation from a 1:1 (v/v) mixture of PE and DCM (40 °C, 200 mL), the mixture was cooled to 15 °C for 12 h and filtered off. The product was dried at 40 °C under reduced pressure to provide compound **22** as a white solid; yield 12.0 g (86%) for two steps; m.p. 160–162 °C; HPLC purity (condition A): 98%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.88 (s, 1H), 7.99 (s, 1H), 7.61–7.14 (m, 35H), 6.96 (s, 1H), 6.90 (s, 1H), 6.69 (s, 1H), 6.55 (s, 1H), 5.29 (d, *J* = 3.2 Hz, 1H), 2.06 (pt, *J* = 6.7, 2.9 Hz, 1H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.44 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 167.84, 164.35, 145.66, 145.08, 144.14, 143.87, 141.10, 140.86, 140.62, 140.60, 140.52, 129.40, 129.25, 129.21, 129.17, 129.00, 128.48, 128.40, 128.33, 128.16, 127.28, 126.80, 126.74, 126.69, 111.61, 111.39, 83.21, 81.78, 81.62, 71.85, 29.15, 19.62, 16.05; MS (ESI) *m/z*: 941 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{59}H_{49}N_4O_6S$ $[M - H]^-$: 941.3378; found: 941.3379.

Synthesis of 4,5-bis(benzhydryloxy)-2-(1-((((Z)-2-(((S)-2,2-dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxo-1-(2-(tritylamino)thiazol-4-yl)ethylidene)amino)oxy)-2-methylpropyl) pyridine 1-oxide (**24**)

A solution of **22** (6.6 g, 7.0 mmol) in DMSO (100 mL) was treated with HATU (3.5g, 9.1 mmol), $NaHCO_3$ (1.8 g, 21.0mmol) and **23** (1.9 g, 9.1 mmol), and then the reaction mixture was stirred at room temperature for 5 h. After completion of the reaction, water (300 mL), EA (30 mL) and saturated ammonium chloride solution (100 mL) were added and the mixture was stirred for 10 min. The mixture was filtered and the filter cake was taken up in a 1:1 (v/v) mixture of PE and EA (25 °C, 30 mL) to obtain compound **24** without further purification. The product was dried at 40 °C under reduced pressure to provide compound **24** as a white solid: yield 7.4 g (93%); m.p. 186 °C decomp.; HPLC purity (condition B): 96%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.77 (d, *J* = 7.7 Hz, 1/2H), 9.68 (d, *J* = 7.0 Hz, 1/2H), 8.90 (s, 1H), 8.02 (s, 1/2H), 7.99 (s, 1/2H), 7.63–7.16 (m, 35H), 7.04 (s, 1/2H), 6.87 (s, 1/2H), 6.79 (s, 1/2H), 6.77 (s, 1/2H), 6.74 (s, 1/2H), 6.73 (s, 1/2H), 6.72 (s, 1/2H), 6.70 (s, 1/2H), 5.32 (d, *J* = 3.2 Hz, 1/2H), 5.26 (d, *J* = 3.7 Hz, 1/2H), 4.81 (d, *J* = 7.8 Hz, 1/2H), 4.65 (d, *J* = 7.1 Hz, 1/2H), 2.05–1.93 (m, 1H), 1.57 (s, 3/2H), 1.52 (s, 3/2H), 1.37 (s, 3/2H), 1.34 (s, 3/2H), 0.87 (d, *J* = 6.9 Hz, 3/2H), 0.82 (d, *J* = 7.0 Hz, 3/2H), 0.36 (d, *J* = 7.0 Hz, 3/2H), 0.32 (d, *J* = 7.1 Hz, 3/2H); ^{13}C NMR (126 MHz, DMSO-d_6) (most carbons show two peaks because of diastereomers): δ 168.23 and 168.17, 163.68 and 163.63, 162.07 and 161.88, 151.28 and 150.93, 145.72 and 145.67, 144.94 and 144.87, 144.30 and 144.10, 143.93, 141.75 and 141.71, 141.21, 141.10, 140.98, 140.80 and 140.54, 129.40, 129.26, 129.23, 129.05, 129.02, 128.48, 128.44, 128.26, 128.19, 127.44, 126.85, 126.75, 126.72, 126.64, 112.97 and 112.72, 111.75 and 111.65, 83.27 and 83.09, 81.60 and 81.57, 81.44 and 81.26, 71.62, 68.01 and 67.89, 61.58 and 61.22, 29.57 and 29.12, 24.02 and 23.97, 21.17 and 21.07, 19.46 and 19.37, 16.22 and 15.99. HRMS (ESI) *m/z* calcd for $C_{64}H_{57}N_6O_{10}S_2$ $[M - H]^-$: 1133.3583; found: 1133.3608.

Synthesis of (3S)-3-((Z)-2-(2-Aminothiazol-4-yl)-2-((1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-methylpropoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl hydrogen sulfate (YT-14)

A solution of **24** (2 g, 1.76 mmol) in anhydrous DCM (24 mL) was treated with triethylsilane (0.84 mL, 5.29 mmol) and cooled to −15 °C. Then trifluoroacetic acid (5.89 mL, 52.9 mmol) was added dropwise. The reaction mixture was stirred at −15 °C for 5 h. After completion

of the reaction, the reaction mixture was slowly warmed to 0 °C. Then, a mixture of 4:1 (v/v) EA and hexane (25 °C, 50 mL) was added dropwise, the resulting precipitate was further stirred for 20 min and collected by filtration, and then the cake was washed with EA without further purification. The product was dried at 25 °C under reduced pressure to provide compound YT-14 as a white solid; yield 820 mg (83%); m.p. 151 °C decomp. (lit.[4] 151 °C decomp.); HPLC purity (condition B): 97%, *R*/*S* in isopropyl position 46:54; IR (KBr) (υ cm^{-1}): 3390, 3306, 3235, 3103, 2976, 2939, 2879, 1776, 1672, 1639, 1541, 1456, 1390, 1263, 1200, 1142, 1051, 1016, 721, 600; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.69 (d, *J* = 8.0 Hz, 1/2H), 9.65 (d, *J* = 7.5 Hz, 1/2H), 8.24 (s, 1/2H), 8.23 (s, 1/2H), 7.02 (s, 1/2H), 7.00 (s, 1/2H), 6.83 (s, 1/2H), 6.81 (s, 1/2H), 5.44 (d, *J* = 4.8 Hz, 1/2H), 5.37 (d, *J* = 5.4 Hz, 1/2H), 4.71 (d, *J* = 7.9 Hz, 1/2H), 4.68 (d, *J* = 7.6 Hz, 1/2H), 2.23–2.09 (m, 1H), 1.48 (s, 3/2H), 1.47 (s, 3/2H), 1.35 (s, 3/2H), 1.34 (s, 3/2H), 1.00–0.96 (m, 3H), 0.92–0.85 (m, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) (most carbons show two peaks because of diastereomers): δ 169.35, 162.58, 162.02 and 161.98, 157.35 and 157.31, 150.93 and 150.90, 145.72 and 145.54, 145.13, 140.55 and 140.39, 127.97, 111.87 and 111.60, 110.97 and 110.88, 83.04 and 82.90, 68.17 and 68.05, 61.33 and 61.29, 31.90 and 31.67, 23.88 and 23.86, 21.13 and 21.06, 19.15 and 19.06, 17.32 and 17.08. HRMS (ESI) *m*/*z* calcd for $C_{19}H_{23}N_6O_{10}S_2$ $[M-H]^-$: 559.0917; found: 559.0923.

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