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Asymmetric Synthesis of Florfenicol via Dynamic Reductive Kinetic Resolution with Ketoreductases

Jie Zou[+],[a] Guowei Ni[+],[a,b] Jiawei Tang,[a] Jun Yu,[a] Luobin Jiang,[a] Dianwen Ju,[b] Fuli Zhang*[a], and Shaoxin Chen*[a]

Dedication ((optional))

Abstract: A novel chemo-enzymatic synthesis of the veterinary antibiotic florfenicol is described. The key step was the dynamic reductive kinetic resolution (DYRKR) of a keto ester with a ketoreductase (KRED-02) to afford two contiguous stereocenters of (2S,3R)-cis-1,2-amino alcohol in >99% ee and 99% dr in one step. This biocatalysis is green and environmental friendly with high enantioselectivity and productivity. Subsequently two new methods of nucleophilic fluorination via aziridines and cyclic sulfates were developed to prepare the fluoro amine in high regioselectivity, greatly reducing the safety risk. Additional studies have indicated that KRED-02 can also be used to afford chiral alcohol ***S*-21** with a good yield and stereoselectivity. By integrating biocatalysis into organic synthesis, it has an opportunity to be useful in industrial applications for florfenicol.

Introduction

Florfenicol (**1**) was discovered as a 3'-fluoro derivative of thiamphenicol (**2**), which had been widely used as a veterinary antibiotic for its significant superiority in antibacterial spectrum and activity (Figure 1).[1] Previous investigations, which focused on the deoxyfluorination, had identified the Ishakawa reagent, which severely corroded the equipment and was relatively high in cost, as the chief successful reagent for completing this transformation.[2] Construction of the two stereocenters was based upon the aldol reactions involving classical chemical resolution, which caused waste water pollution.[3] Since the large productivity (4000 ton per year) and environmentally inefficient industrial process, there has been considerable attention on using different strategies for the fluorination[4] and two adjacent stereocenters of *cis*-1,2-amino alcohol[5].

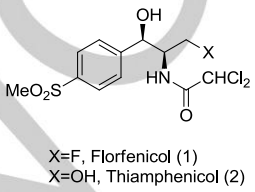
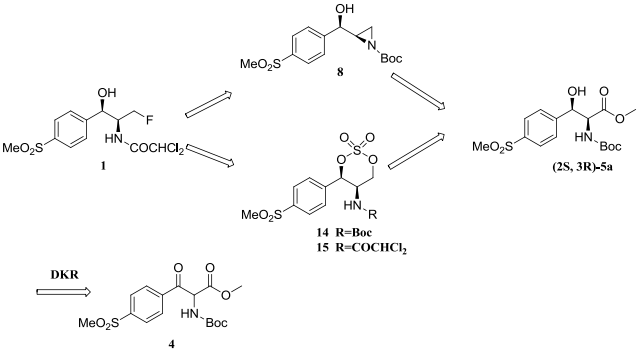


Figure 1. Structures of florfenicol (**1**) and thiamphenicol (**2**)

Recently, Chen developed two asymmetric methods of florfenicol based on sharpless epoxidation[5c,f] and catalytic aziridination[5d]. Lin performed a new chemo-enzymatic route with hydroxynitrile lyase to establish the first chiral carbon and generated the second by DIBAL reduction and hydrocyanation.[5e] However lengthy steps and expensive cost of catalysts limited their industrial application. Later on, Chen disclosed a new route of synthesizing florfenicol, involving DKR process with asymmetric hydrogenation catalysted by Ru and ligands, which afforded (*2S, 3S*)-1,2-amino alcohol with low stereoselectivity in 92% de and 78%ee, therefore its hydroxyl still needed to be inversed for the florfenicol.[6] Asymmetric hydrogenation combined with dynamic kinetic resolution (DKR) is particularly attractive for constructing two adjacent stereocenters in one step.[7] Ketoreductases have been widely applied as an efficient biocatalyst for pharmaceutical industry becausese of its high stereoselectivity and bioreductive activity.[8] However few literaures reported DKR with ketoreductases （KREDs） in organic synthesis.[9] Herein we report a novel chemo-enzymatic approach via DYRKR with ketoreductases and two new methods of introducing fluorine are designed for the synthesis of florfenicol.



Scheme 1. Retrosynthetic Analysis of Florfenicol **(1)**

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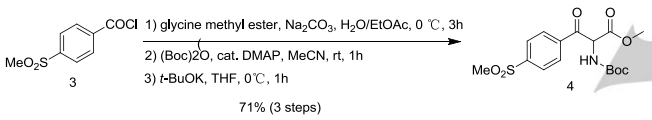
We envisioned that florfenicol (**1**) could be accomplished through nucleophilic fluorination of the corresponding aziridine (**8**) and cyclic sulfate (**14, 15**) intermediates with the two stereocenters established already. Further, the aziridines and cyclic sulfates might be synthesized from **(*2S, 3R*)-5a**. The two adjacent stereocenters could be elaborated via asymmetric bioreduction of the corresponding amino ketone (**4**) through enzymatic DYRKR (Scheme 1).

Results and Discussion

For the above synthetic strategy, an approach was developed to prepare the desired α-amino β-keto ester **4** for our investigation on ketoreductases (Scheme 2). Treatment of glycine methyl ester with benzoyl chloride **3** at 0 ℃, in the presence of Na_2CO_3, gave the corresponding benzamide. The subsequent protection of the benzamide with $(Boc)_2O$ under DMAP catalysis in acetonitrile at room temperature afforded the N-Boc amide. The N-Boc amide was then subjected to a base-promoted intramolecular N→C benzoyl migration reaction under *t*-BuOK in THF at 0 ℃. Thus β-keto ester (**4**) was prepared in 71% yield via three steps (Scheme 1).[10]



Scheme 2. Synthesis of β-keto ester **4**

Before our screening of KREDs, adequate chiral HPLC analyses were developed for **(*rac*)-5** to achieve a reliable method to measure the enantiomeric excesses of the final product from the ketoreductase-catalyzed reaction. Accordingly, four stereoisomers **(*2S, 3R*)-5a**, **(*2R, 3S*)-5b**, **(*2S, 3S*)-5c** and **(*2R, 3R*)-5d** were prepared to comfirm the corresponding peak in the chiral HPLC.[11]

Next, we chose to evaluate the putative DYRKR of **4** to prepare **(*2S, 3R*)-5a**. It is accepted that DKR, which combines asymmetric bioreduction and *in situ* racemization of the unreacted enantiomer, is a promising process to overcome the limitations in chemical resolution of 50% yield.[12] Fortunately **4** was found to be easily racemized with the ranging from pH 6-8 which is suitable for bioreduction. Using keto esters **4** as starting materials, KREDs were screened to carry out reductions that were stereoselective.[13] The selected data for the conversion and stereoselectivity of ketoreductases are presented in Table 1, where two out of the four stereoisomers were formed in optically pure form using different enzymes. KRED-02 was identified to perform the desired DKR bioreduction to afford ***cis*-(*2S, 3R*)-5a** in 91% yield with >99% ee and 99% dr, as judged by chiral HPLC. ***Trans*-(*2R, 3R*)-5c** could also be prepared with KRED-10 in high selectively >99% ee and 96.8% dr, which exhibited the diversity of reductases. The attainment of the catalytic cycle for the enzymatic DKR reduction, by consuming glucose to gluconic acid catalyzed by glucose dehydrogenase (GDH) or using *i*-propanol as co-substrate to acetone, delivered the hydride source to selectively reduce **(*S*)-4**. The enzymatic DKR reduction with the selected KRED-02 was carried out by treating in an aqueous phosphate buffer in the presence of glucose at 25℃. NaOH (1M) was added to neutralize the gluconic acid formed during the reaction and maintain the reaction pH at 7.5 to accomplish a fast epimerization of 6, thereby achieving 98% conversion at 24 h and 4-(methylsulfonyl)benzoic acid was the main byproduct as the unstability of the compound **4** in the basic condition.

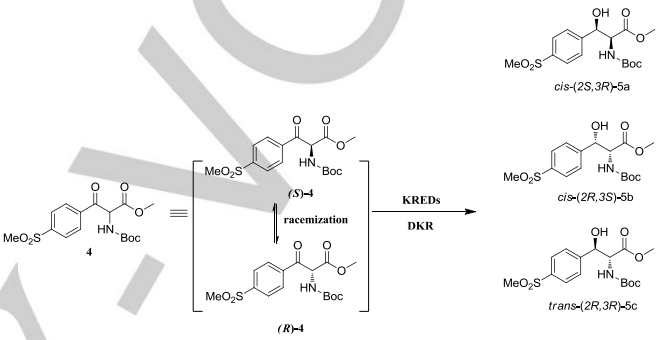


Table 1. Stereoselectivity of ketoreductase-catalyzed of keto ester **4**.

	KRED	Conversion[e] (%)	Diastereomeric ratio (%)[f] **5a**	5b	5c	5d
1	774[a]	95	43.8	6.7	49.5	0
2	0902[b]	85	28.2	7.8	64.0	0
3	740[c]	80	65.6	0	34.4	0
4	05[a]	95	0	86.9	0	13.1
5	10[d]	90	1.6	0	98.4	0
6	02[a]	98	99.5	0	0.5	0

[a] 0.05g ketone 4, 0.2g wet cells, 0.2g glucose, 1mg $NADP^+$ and PBS buffer (100 mM , pH 7.0) in 10mL reaction system for 24h at 25 °C and 200 rpm. [b] 0.05g ketone 4, 0.2g wet cells, 1mg $NADP^+$, 2mL IPA and PBS buffer (100 mM , pH 7.0) in 10mL reaction system for 24h at 25 °C and 200 rpm. [c] 0.05g ketone 4, 0.2g wet cells, 0.2g glucose, 1mg NAD^+ and PBS buffer (100 mM , pH 7.0) in 10mL reaction system for 24h at 25 °C and 200 rpm. [d] 0.05g ketone 4, 0.2g wet cells, 1mg NAD^+, 2mL IPA and PBS buffer (100 mM , pH 7.0) in 10mL reaction system for 24h at 25 °C and 200 rpm. [e] Measured by HPLC. [f] Measured by chiral HPLC using a chiral IB-3 column.

The poor solubility of **4** in buffer solutuin restricted the scale-up of the bioreduction by using KRED-02. We therefore tried to append organic co-solvents into the aqueous medium, DMSO

and toluene were chose to represent miscible and immiscible solvents with water. 50% DMSO or toluene relative to the buffer solution was well tolerated by KRED-02, which was also desired for improving the solubility of **4** and the concentration of the compound 6 was elevated to 50g/L from 5 g/L which showed the prospectivity for industrial application (Table 2).

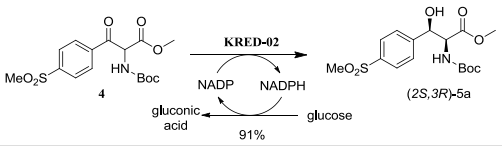


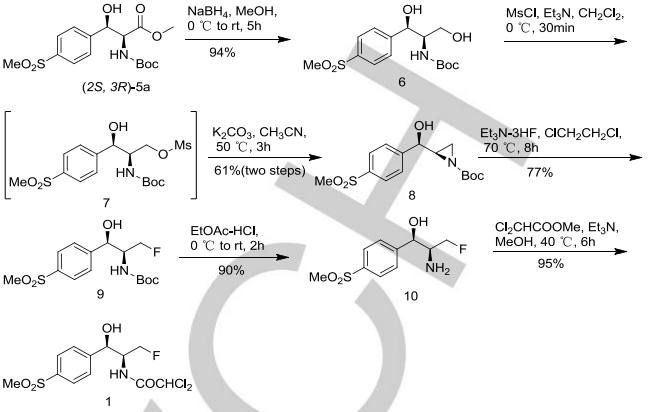
Table 2. DYRKR of keto ester **4** in phosphate buffer/ organic co-solvent (v/v).

	Co-solvent(%)	Conversion(%)[c]	ee (%)[d]	dr (%)[d]
1[a]	-	20%	>99	99
2[a]	Octadecane(50%)	54%	>99	99
3[a]	Toluene(50%)	95%	>99	99
4[a]	DMSO(50%)	98%	>99	99
5[b]	DMSO(50%)	98%	>99	99

[a] 0.2g ketone 4, 0.2g KERD-02, 0.5g glucose, 2mg $NADP^+$ and PBS buffer (100 mM , pH 7.0) in 10mL reaction system for 24h at 25 °C and 200 rpm. [b] 0.5g ketone 4, 0.5g KERD-02, 0.8g glucose, 5mg $NADP^+$ and PBS buffer (100 mM , pH 7.0) in 10mL reaction system for 24h at 25 °C and 200 rpm. [c] Measured by HPLC. [d] Measured by chiral HPLC using a chiral IB-3 column.

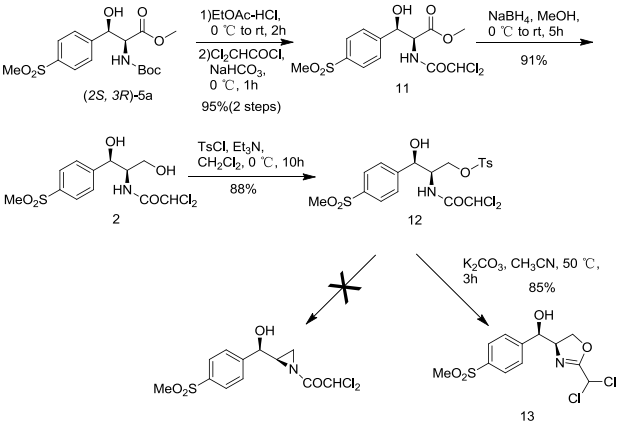
The high stereoselectivity of **(*2S, 3R*)-5a**, which is generally not readily prepared through chemical reduction, was prepared through a powerful DKR bioreduction of racemic substrate **4** in one step. The application of biotechnology into classical organic synthesis is a practical and efficient tool to deal with synthetic problems.[10]

With **(*2S, 3R*)-5a** in hand, we attempted to develop new methods of introducing fluorine atom. Many of these strategies rely on the hydroxyl group replaced by fluorine with (diethylamino)sulfur trifluoride (DAST) or Ishakawa reagent.[2] In contrast, aziridine ring-opening by fluoride offers a direct route to fluoamines. The studies of Doyle demonstrated that the hydrofluorination of aziridines to provide fluoroamines using amine-HF reagents was presented with regioselectivity.[14] Xu and Hammond reported conveniently achieving regioselectivity in the fluorination of the aziridine substrate under acidic conditions.[15] Additionally, studies on the ring-opening of aziridines are highly sought after.



Scheme 3. Synthesis of Florfenicol **1** via N-Boc aziridines

Our first approach was to obtain the corresponding aziridine and react with nucleophilic fluorinating reagents for fluorination (Scheme 3).[16] The reduction with $NaBH_4$ led to aminodiol **6**, followed by the selective masking of the primary hydroxy group with mesyl chloride to afford **7** without purification for the next step. The step of cyclization to the aziridine ring was carried out by potassium carbonate and aziridine **8** was obtained in 61% yield over two steps. By treating **8** with triethylamine trihydrofluoride in 1,2-dichloroethane (DCE) under 70℃, **8** was regioselectively converted into the desired **9** in 77% yield. The fluorination of β-hydroxyl aziridine **8** took place at the less hindered side leaving the hydroxyl group untouched; ^{19}F NMR confirmed the regioselectivity of fluorination. Other common HF-based reagents, DMPU-HF (65%HF in DMPU) and Olah's reagent (70% HF in pyridine), gave no reaction at all due to higher acidity. After deprotection of **9** with EtOAc-HCl at ambient temperature, the fluoro amine **10** HCl salt was directly isolated from EtOAc in 90% yield. Finally, florfenicol (1) was obtained in 95% yield by acylation of **10** with methyl dichloroacetate.

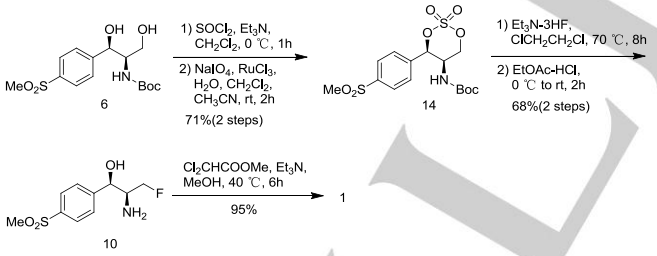


Scheme 4. Synthesis of the Intermediate **13**

Taking advantage of the efficient ring-opening reaction, the other synthesis of florfenicol was developed as outlined in Scheme 4. The *N*-Boc group of **(*2S, 3R*)-5a** was cleaved by using HCl-EtOAc, and the resulting amine was then acetylized by dichloroacetic chloride in the presence of saturated sodium bicarbonate solution to afford **11**. Reduction of ester **11** successfully gave the aminodiol 2 in good yield. The subsequent protection of the primary hydroxy group afforded O-tosyl derivative **12**. In constract , the intramolecular cyclization of the O-tosyl derivative **12** with potassium carbonate to prepare aziridine was attempted. After being stirred for 2h at 60℃, the starting material disappeared and a new product was observed by HPLC. To our surprise, the cyclic compound **13**, which is a vital intermediate for the synthesis of florfenicol (**1**), was the main product and the same result was obtained when the tosylation product was treated with KOH or other strong alkali in acetonitrile.

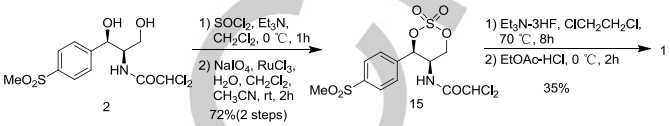
The foregoing method utilizing aziridines to obtain florfenicol **1**, could avoid waste water pollution caused by the present process. In addition, triethylamine hydrofluoride as a fluorinated ring-opening reagent, might improve safety compared to anhydrous HF and reduce corrosion of equipment.[17]

Although these routes were an improvement over fluorinations, there are still multiple chromatographic separations required. We searched for alternative fluorination methods through six-membered cyclic sulfates.[18] Gao and Sharpless reported efficiently opening cyclic sulfates with nucleophiles including fluoride.[19] Furthermore, Marc observed that a cyclic sulfate was opened by the nucleophile at the primary position while no secondary substitution product could be detected.[20] Opening cyclic sulfates from diols resulted in a high selectivity for the primary position. These results led us to try this approach on 1,3- diols.



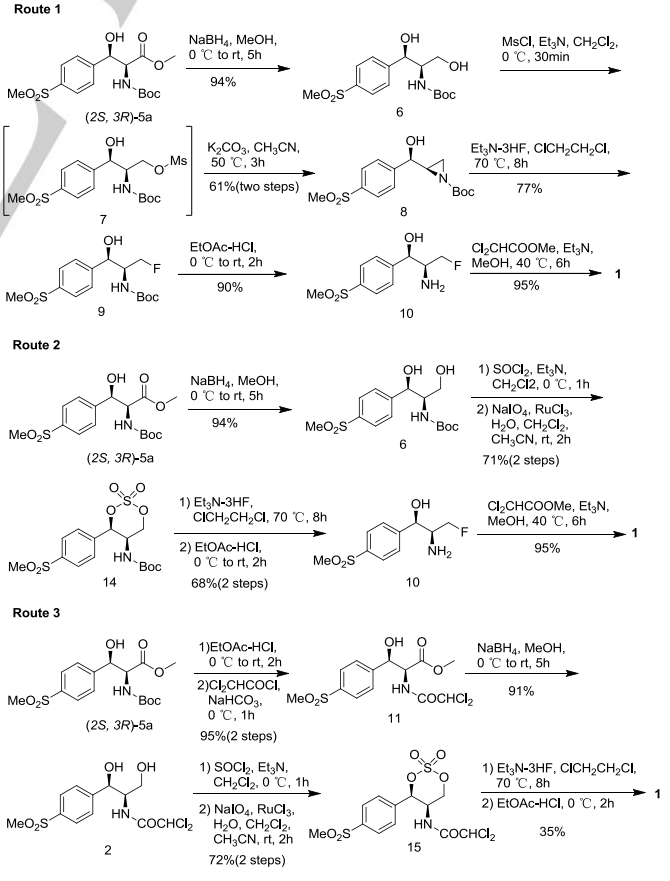
Scheme 5. Synthesis of Florfenicol **1** via N-Boc cyclic sulfates **14**

According to our strategy shown in Scheme 5, the cyclic sulfate **14** was obtained in 71% yield by reaction of diol **6** with $SOCl_2$/TEA followed by *in situ* oxidation with $NaIO_4$/$RuCl_3$. Attempts to perform direct sulfation of **6** using SO_2Cl_2/TEA also afford **14** but in lower yields.[21] Regioselective ring-opened reactions of the key precursor **14** using nucleophilic fluorinating reagents were studied. Et_3N-3HF in DCE worked best. We found that the phenyl group at the C4 position of **14** would sterically hinder approaching of the fluoride nucleophile to C4, directly attacking to the C6 position (cyclic sulfate numbering).[22] Hydrolysis of a sulfate ester was reported by Kim and Sharpless using catalytic concentrated sulfuric acid and 0.5 to 1.0 equiv of water in THF.[23] In our case, hydrolysis of the sulfate could be accomplished while deprotecting by treating the crude reaction mixture with EtOAc-HCl to gave the fluoro amine **10** in 74% yield. Finally, florfenicol (**1**) was obtained in 95% yield by acylation of **10** with methyl dichloroacetate.



Scheme 6. Synthesis of Florfenicol **1** via N-$COCHCl_2$ cyclic sulfates **15**

We envisaged that cyclic sulfates would be good substrates for substitution. The straightforward purification and facile separation encouraged us to look for a shorter route to synthesize florfenicol **1** (Scheme 6). Treatment of **2** with thionyl chloride gave a mixture of sulfites that was oxidized without further purification with $RuCl_3$/$NaIO_4$ to the corresponding cyclic sulfate **15**, which was obtained in 72% yield. Treatment of the key precursor **15** with Et_3N-3HF as the nuclephile, followed by acidic hydrolysis, gave florfenicol **1**.



Scheme 7. Synthetic routes towards florfenicol **1**

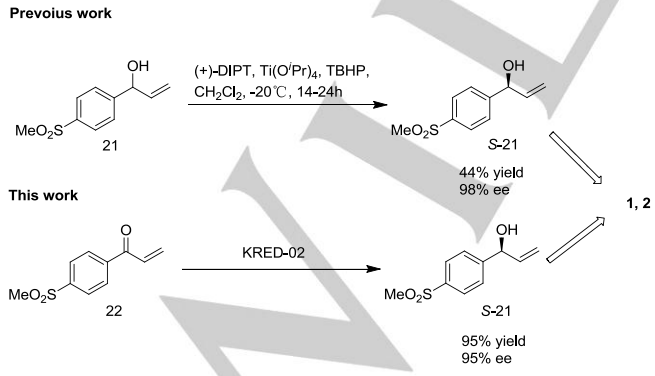
The synthetic routes towards florfenicol **1** (Scheme 7) were evaluated via using decision analysis as a tool based on a weighing of the desired characteristics for the synthesis and an evaluation of each route against these characteristics. [4c, 24]

Table 3. Evaluation of routes to florfenicol **1**.

Criteria	Weight[a]	Route 1[b]	Route 2[b]	Route 3[b]
number of steps	2	2	4	4
health and safety	5	2	3	3
environment	5	2	3	3
yield	3	3	4	2
flexibility	2	4	4	3
purity	4	3	3	2
total[c]		53	70	58

[a] Scores for each weighting were given from 1 = least important to 5 = most important. [b] Scores for each category were given from 1 = meets the criteria least successfully to 5 = meets the criteria most successfully. [c] The total score was given by the sum of the products of the weighting and the scoring for each category.

The evaluation shows clearly route 2 performs the best in this analysis (Table 3), primarily due to the nucleophilic fluorination via cyclic sulfates. The development of a new fluorination strategy, using Et_3N-3HF as a fluorinating reagent, improved the safety for the synthesis of florfenicol **1**. The application of enzymatic DYRKR significantly reduced the pollution of the process by chemical resolution and achieved an excellent yield. In developing a process we have therefore made the process operationally green and safer.



Scheme 8. Application of KRED-02 to intermediate **22**

Once the process was validated, direct application of our ketoreductases to an interesting intermediate **22** (Table 4),[13] which is an useful intermediate for the synthesis of florfenicol (**1**) and thiamphenicol (**2**), was tested. Compared with the previous chemical synthetic method through sharpless asymmetric epoxidation[25], the bioreduction with KRED-02 worked well in buffered aqueous solution, the yields are excellent and enantiomeric ratios almost in favor of allylic alcohol **(*S*)-21** (Scheme 8). This finding further demonstrated that recuction with ketoreductases showed the potential for industrial application.

Table 4. Bioreduction of ketone **22**.

	KRED	Conversion(%)[f]	ee (%)[g]	Configuration
1	774[a]	95	25.1	*S*
2	0902[b]	92	88.6	*S*
3	740[c]	92	30.9	*S*
4	05[a]	90	22.2	*S*
5	10[d]	95	37.3	*S*
6	1306[b]	90	74.6	*S*
7	1500[b]	95	>99	*R*
8	1184[b]	98	>99	*R*
9	787[d]	75	>99	*R*
10	1348[d]	50	94.5	*S*
11	02[a]	98	95.3	*S*
12	02[e]	98	95.3	*S*

[a] 0.05g ketone 22, 0.2g wet cells, 0.2g glucose, 1mg $NADP^+$ and PBS buffer (100 mM , pH 7.0) in 10mL reaction system for 24h at 25 °C and 200 rpm. [b] 0.05g ketone 22, 0.2g wet cells, 1mg $NADP^+$, 2mL IPA and PBS buffer (100 mM , pH 7.0) in 10mL reaction system for 24h at 25 °C and 200 rpm. [c] 0.05g ketone 22, 0.2g wet cells, 0.2g glucose, 1mg NAD^+ and PBS buffer (100 mM , pH 7.0) in 10mL reaction system for 24h at 25 °C and 200 rpm. [d] 0.05g ketone 22, 0.2g wet cells, 1mg NAD^+, 2mL IPA and PBS buffer (100 mM , pH 7.0) in 10mL reaction system for 24h at 25 °C and 200 rpm. [e] 0.5g ketone 22, 0.5g wet cells, 0.8g glucose, 5mg $NADP^+$ and PBS buffer (100 mM , pH 7.0) in 10mL reaction system for 24h at 25 °C and 200 rpm. [f] Measured by HPLC. [g] Measured by chiral HPLC using a chiral IC-3 column.

Conclusions

In summary, novel chemo-enzymatic routes has been developed for the synthesis of florfenicol, which features the application of enzymatic DYRKR to establish the two stereo centers of *cis*-1,2-amino alcohol **(*2S, 3R*)-5a** with >99% ee and 99% dr in one step. For the introduction of key fluorine atom, two new methods were developed through aziridines and cyclic sulfates with nucleophilic fluoride reagent Et_3N-3HF in a highly regioselective

manner. Additionally we found that KRED-02 can be used to afford **(*S*)-21** with a good yield and stereoselectivity. The integration of asymmetric dynamic bioreduction for the synthesis of other analogues and further studies involved with the evolution of KRED-02 are in progress.

Experimental Section

Materials and methods

Materials, reagents and solvents were obtained commercially and used without additional purification. All the KRED genes (the codon-optimized open reading frames encoding proteins) were synthesized by Bioligo (Shanghai). ClonExpress® Entry One Step Cloning Kit used for molecular cloning was from Vazyme Biotech (Nanjin, China) and other enzymes from Takara (Dalian, China). BCA Protein Assay Kit was purchased from cwbiotech (Beijin, China). All the KREDs were used in the form of wet cells. Column chromatography was performed on silica gel (GENERAL-REAGENT®, 200-300 mesh). After chromatography the fractions were pooled, evaporated to dryness and dried in vacuo. Melting points were determined on a WRS-3 digital melting-point apparatus and are uncorrected. NMR spectras were recorded on Bruker Advance 600 MHz instrument. Chemical shifts are reported in ppm, relative to TMS (1H; internal standard, δ_H = 0.00 ppm) or solvent peaks ($CDCl_3$: ^1H, δ_H = 7.26 ppm and ^{13}C, δ_C = 77.0 ppm; DMSO-d_6: ^1H, δ_H = 2.49 ppm and ^{13}C, δ_C = 39.5 ppm; MeOD: ^1H, δ_H = 3.31 ppm and ^{13}C, δ_C = 49.0 ppm). ^{19}F NMR spectra were recorded on a Agilent spectrometer (400 MHz) using CCl_3F (^{19}F, δ = 0.0 ppm) as an externalstandard. High resolution mass spectra (HRMS) were recorded on a Bruker MicroTOF mass spectrometer. HPLC analyses were carried out on a Dionex UltiMate 3000 HPLC instrument.

Methyl-2-((tert-butoxycarbonyl)amino)-3-(4-(methylsulfonyl)phenyl)-3- oxopropanoate (4)

To a suspension of Na_2CO_3 (7.6 g, 71.7 mmol) in water (80 mL) and EtOAc (120 mL) at 0 ℃ was added glycine methyl ester hydrochloride (8.3 g, 66.1 mmol) in portions over 30 min. The mixture was stirred for additional 30min, 3 (12.0 g, 54.9 mmol) was then added in portions over 30min at 0 ℃. The reaction mixture was warmed to 25 ℃ and formed a homogenous biphasic solution. The separated organic phase was evaporated in vacuo and dissloved in MeCN (150 mL). DMAP (0.04 g, 0.3 mmol) and a solution of $(Boc)_2O$ (18.0 g, 82.5 mmol) in MeCN was added at ambient temperature dropwise over 1 h. The reaction mixture was stirred at ambient temperature for 3 h and the solvent was evaporated under reduced pressure. THF (200 mL) was added. Then, a solution of *t*-BuOK (13.5 g, 120.3 mol) in THF was added at 0 ℃ dropwise over 1 h. After aging at 0 ℃ for 1h, 10 % citric acid was added to adjust to pH = 7 at 0 ℃. The organic phase was washed with brine (50 mL × 3) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether / ethyl acetate 1:1, V/V) to give 4 (14.5 g, 71 %) as a white solid. Mp: 135.7-136.6 ℃. ^1H NMR (600MHz, $CDCl_3$): δ=8.28(d, J=8.22Hz, 2H), 8.09(d, J=8.34Hz, 2H), 5.96(d, J=7.92Hz, 1H), 5.87(d, J=7.62Hz, 1H), 3.75(s, 3H), 3.10(s, 3H), 3.27(s, 1H), 1.45(s, 9H); ^{13}C NMR (150MHz, $CDCl_3$): δ=191.53, 166.90, 154.92, 145.04, 138.44, 130.23, 127.89, 81.15, 59.37, 53.46, 44.27, 28.21; HR-MS calcd for $C_{16}H_{21}NNaO_7S$ $[M+Na]^+$ m/z 394.0931, found m/z 394.0938.

(2*S*,3*R*)-Methyl-2-((tert-butoxycarbonyl)amino)-3-hydroxy-3-(4-(methylsulfonyl)phenyl)propanoate ((*2S, 3R*)-5a)

To a solution of phosphate buffer (pH 7.0, 10mL) was added dextrose (0.8g) followed by KRED-02 wet cells (0.5g), 5mg $NADP^+$, ketone 4 (0.5g), DMSO (5mL). The resulting solution was pH adjusted to a minimum of 7.5 with 1M NaOH prior to use. The reaction was carried out at 30 ℃ and shaken at 200 rpm shaker for 24 h. The reaction mixture was then extracted with DCM (10 mL×3), and the DCM layers were combined. The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure, obtaining (*2S, 3R*)-5a (0.46 g, 91 %) as a white solid. Mp: 143.7-144.5 ℃. ^1H NMR (600MHz, $CDCl_3$): δ=7.85(d, J=8.16Hz, 2H), 7.57(d, J=8.28Hz, 2H), 5.37(s, 1H), 5.34(d, J=9.06Hz, 1H), 4.55(d, J=8.52Hz, 1H), 3.80(s, 3H), 3.27(s, 1H), 3.00(s, 3H), 1.29(s, 9H); ^{13}C NMR (150MHz, $CDCl_3$): δ=170.75, 155.53, 146.39, 139.74, 127.28, 127.16, 80.33, 73.19, 59.09, 52.83, 44.49, 28.08; HR-MS calcd for $C_{16}H_{27}N_2O_7S$ $[M+NH_4]^+$ m/z 391.1533, found m/z 391.1532. The chiral HPLC was performed using a CHIRALPAK IB-3 (4.6×250 mm, 3μm, DAICEL, Shanghai) column with a mobile phase of 95% 0.2%(DEA+TFA) Hex-5% EtOH. The product was detected at 220 nm, and the retention times of diastereoisomers were 34.4 min, 36.1 min, 39.5 min ((*2S, 3R*)-5a), and 43.2 min, respectively.

***tert*-Butyl ((*1R,2R*)-1,3-dihydroxy-1-(4-methylsulfonyl)phenyl) propan-2-yl carbamate (6)**

$NaBH_4$ (4.0 g, 105.7 mmol) was added to a solution of (2*S*, 3*R*)-5a (13.5 g, 34.8 mmol) in MeOH (60 mL) at 0℃. The mixture was stirred for 5h and H_2O (15 mL) was added and the mixture was stirred further for 1h. The mixture was then filtered and the filtrate was concentrated. The residue was diluted with CH_2Cl_2 (80 mL), washed with brine (30 mL), dried (Na_2SO_4), filtered, and concentrated under vacuum. The residue was purified by flash column chromatography [silica gel petroleum ether-EtOAc (2:1)] to give a white solid (11.3 g, 94 %). Mp: 144.3-145.9 ℃. ^1H NMR (600MHz, DMSO-d_6): δ=7.85(d, J=8.16Hz, 2H), 7.55(d, J=8.16Hz, 2H), 6.11(d, J=9.3Hz, 1H), 5.51(d, J=5.52Hz, 1H), 4.92-4.91(m, 1H), 4.75(t, J=5.46Hz, 1H), 3.68-3.64(m, 1H), 3.54-3.50(m, 1H), 3.32-3.28(m, 1H), 3.14(s, 3H) , 1.23(s, 9H); ^{13}C NMR (150MHz, DMSO-d_6): δ=155.62, 150.27, 139.44, 127.52, 126.82, 78.12, 70.42, 61.14, 58.02, 44.11, 28.51; HR-MS calcd for $C_{15}H_{23}NNaO_6S$ $[M+Na]^+$ m/z 368.1138, found m/z 368.1138.

(*1R,2R*)-2-((*tert*-Butoxycarbonyl)amino)-1-hydroxy-1-(4-(methylsulfonyl)phenyl)propyl methanesulfonate (7)

Compound 6 (7.0 g, 20.3 mmol) was dissolved in dry dichloromethane (70 mL), triethylamine (7.2 g, 71.2 mmol) and methanesulfonyl chloride (7.0 g, 61.1 mmol) were added at 0℃, and the reaction mixture was stirred for 1 h. The reaction mixture was quenched with ice-cold water and extracted with DCM (3 × 20 mL) , the solvent was removed under vacuum. The crude compound was used in the next step without further purification.

(*S*)-[4-(methylsulfonyl)phenyl][(*R*)-1-(*tert*-Butoxycarbonyl)amino)aziridine] methanol (8)

To a solution of 7 (obtained in the previous step) in acetonitrile (80 mL) was added K_2CO_3 (8.0 g, 57.9 mmol), the reaction mixture was stirred at 50℃ for 5h. The solvent was evaporated in vacuo and the residue was diluted with CH_2Cl_2 (80 mL), washed with brine (30 mL), dried with Na_2SO_4, filtered, and concentrated under vacuum. the residue was purified by flash chromatography (petroleum ether / ethyl acetate 1:1, V/V) to afford 8 (4.0 g, 61%, over two steps) as a colorless oil. ^1H NMR (600MHz, $CDCl_3$): δ=7.94(d, J=8.4Hz, 2H), 7.68(d, J=8.22Hz, 2H), 4.52(t, J=5.25Hz, 1H), 3.26(d, J=5.04Hz, 1H), 3.04(s, 3H), 2.71-2.69(m, 1H), 2.39(d, J=5.94Hz, 1H), 2.29(d, J=3.66Hz, 1H), 1.44(s, 9H); ^{13}C NMR

(150MHz, $CDCl_3$): δ=161.66, 146.93, 140.11, 127.66, 127.28, 82.36, 73.00, 44.53, 42.35, 29.29, 27.85; HR-MS calcd for $C_{15}H_{21}NNaO_5S$ $[M+Na]^+$ m/z 350.1033, found m/z 350.1032.

tert-butyl((1_R_,2_S_)-3-fluoro-1-hydroxy-1-(4-(methylsulfonyl)phenyl)propan-2-yl) carbamate(9)

The aziridine 8 (3.0 g, 9.2 mmol) was dissolved in DCE (30 mL). Triethylamine trihydrofluoride (12.0 g, 74.4 mmol) was added and the reaction mixture was stirred at 70℃ for 10h. The reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (20 mL × 3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (petroleum ether / ethyl acetate 1:1, V/V) to afford 9 (3.1 g, 77 %) as a white solid. Mp: 175.2-175.7 ℃. ^{1}H NMR (600MHz, DMSO-d_6): δ=7.86(d, J=8.88Hz, 2H), 7.58(d, J=8.22Hz, 2H), 6.63(d, J=9.24Hz, 1H), 5.74(d, J=5.16Hz, 1H), 4.87(t, J=4.02Hz, 1H), 4.61-4.50(m, 1H), 4.35-4.24(m, 1H), 4.01-3.94(m, 1H), 3.15(s, 3H), 1.23(s, 9H); ^{13}C NMR (150MHz, DMSO-d_6): δ=155.62, 148.98, 139.77, 127.70, 126.87, 82.95(d, J=167.85Hz), 78.38, 70.61(d, J=6.03Hz), 55.95(d, J=19.67Hz), 44.08, 28.48; HR-MS calcd for $C_{15}H_{22}FNNaO_5S$ $[M+Na]^+$ m/z 370.1095, found m/z 370.1096.

(_1R,2S_)-2-amino-3-fluoro-1-[4-(methylsulfonyl)phenyl]-1-propanol(10)

To a solution of 9 (3.0 g, 8.6 mmol) in EtOAc (10 mL) was added 2N HCl in EtOAc (10mL) at 0℃. The reaction mixture was allowed to warm to room temperature and stirred for 2h. The resulting slurry was filtered, and the cake was washed with EtOAc. The wet cake was dried at 45℃ in vacuum to give HCl salt 10 (2.1 g, 90 %). Mp: 155.9-157.3 ℃. ^{1}H NMR (600MHz, DMSO-d_6): δ=8.54(s, 3H), 7.95(d, J=8.46Hz, 2H), 7.68(d, J=8.34Hz, 2H), 6.67(d, J=4.5Hz, 1H), 4.91-4.89(m, 1H), 4.68-4.58(m, 1H), 4.35-4.25(m, 1H), 3.64-3.57(m, 1H), 3.22(s, 3H); ^{13}C NMR (150MHz, DMSO-d_6): δ=146.64, 141.00, 128.42, 127.65, 81.31(d, J=167.96Hz), 69.61(d, J=5.43Hz), 56.15(d, J=18.15Hz), 43.97; HR-MS calcd for $C_{10}H_{15}FNO_3S$ $[M+H]^+$ m/z 248.0751, found m/z 248.0752.

2,2-dichloro-N-((1_R_,2_S_)-3-fluoro-1-hydroxy-1-(4-(methylsulfonyl)phenyl)propan-2-yl)acetamide(1)

A solution of 10 (5.0 g, 17.6 mmol) in dry MeOH (25 mL), Et_3N (6.1 g, 60.3 mmol) and methyl dichloroacetate (7.2 g, 50.4 mmol) was stirred at 50℃ for 10h. The solvent was evaporated in vacuo and the residue was purified by flash chromatography (petroleum ether / ethyl acetate 1:1, V/V) to afford 1 (7.1 g, 95 %) as a white solid. Mp: 155.7-156.2 ℃. ^{1}H NMR (600MHz, DMSO-d_6): δ=8.62(d, J=9.06Hz, 1H), 7.86(d, J=8.4Hz, 2H), 7.62(d, J=8.28Hz, 2H), 6.46(s, 1H), 6.16(d, J=4.38Hz, 1H), 4.99(t, J=3.6Hz, 1H), 4.71-4.60(m, 1H), 4.44-4.33(m, 1H), 4.31-4.25(m, 1H), 3.16(s, 3H); ^{13}C NMR (150MHz, DMSO-d_6): δ=164.19, 148.39, 140.02, 127.62, 126.97, 82.83(d, J=168.96Hz), 69.8 (d, J=6.0Hz), 66.72, 55.09(d, J=19.71Hz), 44.04; HR-MS calcd for $C_{12}H_{14}Cl_2FNNaO_4S$ $[M+Na]^+$ m/z 379.9897, found m/z 379.9900. The chiral HPLC was performed using a CHIRALPAK IC-3 (4.6×250 mm, 3μm, DAICEL, Shanghai) column with a mobile phase of 70% Hex-30% IPA. The product was detected at 224nm, and the retention times of diastereoisomers were 45.6 min, 48.2min (1), 51.1min and 57.4 min, respectively.

(2S,3R)-methyl2-(2,2-dichloroacetamido)-3-hydroxy-3-(4-(methylsulfonyl) phenyl)propanoate (11)

To a solution of (2_S_, 3_R_)-5a (5 g, 13.4 mmol) in EtOAc (20 mL) was added 2N HCl in EtOAc (20 mL) at 0℃. The reaction mixture was allowed to warm to room temperature and stirred for 2h. The resulting slurry was filtered, and the cake was washed with EtOAc. The cake was suspended in dichloromethane (40 mL). Saturated sodium bicarbonate solution (20 mL) and a solution of dichloroacetic chloride (2.0 g, 13.6 mmol) was added and the mixture was stirred for 1h at 0 ℃. The reaction mixture was quenched with water and extracted with DCM (3 × 20 mL) , the DCM layer was removed under vacuum. to leave a residue. The crude product was recrystallized with petroleum ether / ethyl acetate (1:1, V/V) to give white solid (4.9 g, 95 %). Mp: 164.3-166.2 ℃. ^{1}H NMR (600MHz, DMSO-d_6): δ=8.93(d, J=9.12Hz, 1H), 7.85(d, J=8.34Hz, 2H), 7.66(d, J=8.4Hz, 2H), 6.56(s, 1H), 6.37(d, J=4.38Hz, 1H), 5.35(t, J=3.63Hz, 1H), 4.72(dd, J=9.12Hz, 2.76Hz, 1H), 3.72(s, 3H), 3.16(s, 3H); ^{13}C NMR (150MHz, DMSO-d_6): δ=169.93, 164.24, 147.42, 140.19, 127.64, 126.98, 71.79, 66.27, 58.66, 52.93, 44.00; HR-MS calcd for $C_{13}H_{15}Cl_2NNaO_6S$ $[M+Na]^+$ m/z 405.9889, found m/z 405.9889.

2,2-dichloro-N-((1_R_,2_R_)-1,3-dihydroxy-1-(4-(methylsulfonyl)phenyl)propan-2-yl)acetamide (2)

$NaBH_4$ (1 g, 26.4 mmol) was added to a solution of 11 (3.5 g, 8.8 mmol) in MeOH (20 mL) at 0℃. The mixture was stirred for 5h and H_2O (5 mL) was added and the mixture was stirred for a further 1h. The mixture was then filtered and the filtrate was concentrated. The residue was diluted with CH_2Cl_2 (40 mL), washed with brine (20 mL), dried (Na_2SO_4), filtered, and concentrated under vacuum. The residue was purified by flash column chromatography [silica gel petroleum ether-EtOAc (1:1)] to give a white solid (2.8 g, 91 %). Mp: 164.4-164.9 ℃. ^{1}H NMR (600MHz, DMSO-d_6): δ=8.30(d, J=9.06Hz, 1H), 7.84(d, J=8.4Hz, 2H), 7.58(d, J=8.28Hz, 2H), 6.49(s, 1H), 5.97(d, J=4.38Hz, 1H), 5.03-5.01(m, 1H), 4.98-4.96(m, 1H), 3.94-3.90(m, 1H), 3.60-3.56(m, 1H), 3.36-3.32(m, 1H), 3.16(s, 3H); ^{13}C NMR (150MHz, DMSO-d_6): δ=163.89, 149.65, 139.72, 127.49, 126.93, 69.50, 66.96, 60.65, 57.35, 44.06; HR-MS calcd for $C_{12}H_{16}Cl_2NO_5S$ $[M+H]^+$ m/z 356.0121, found m/z 356.0121.

(2_R_,3_R_)-2-(2,2-dichloroacetamido)-3-hydroxy-3-(4-(methylsulfonyl)phenyl) propyl 4-methylbenzenesulfonate (12)

Compound 2 (3 g, 8.4 mmol) was dissolved in dry dichloromethane (30 mL), pyridine (10 mL) and 4-methylbenzenesulfonyl chloride (1.9 g, 10.0 mmol) were added at 0℃, and the reaction mixture was stirred for 8 h. The reaction mixture was quenched with ice-cold water and extracted with DCM (3 × 20 mL) , the solvent was removed under vacuum. The residue was purified by flash column chromatography [silica gel petroleum ether-EtOAc (1:1)] to give a white solid (3.8 g, 88 %); ^{1}H NMR (600MHz, $CDCl_3$): δ=7.81-7.77(m, 4H), 7.50(d, J=8.28Hz, 2H), 7.38(d, J=8.04Hz, 2H), 6.95(d, J=8.46Hz, 1H), 5.74(s, 1H), 5.19(s, 1H), 4.32-4.25(m, 2H), 4.06-4.04(m, 1H), 3.60(d, J=4.02Hz, 1H), 3.01(s, 3H), 2.45(s, 3H); ^{13}C NMR (150MHz, $CDCl_3$): δ=164.31, 146.39, 145.68, 139.84, 132.05, 130.20, 128.01, 127.46, 126.94, 69.95, 67.25, 65.94, 54.09, 44.50, 21.73; HR-MS calcd for $C_{19}H_{21}Cl_2NNaO_7S_2$ $[M+Na]^+$ m/z 532.0029, found m/z 532.0029.

(_R_)-((_R_)-2-(dichloromethyl)-4,5-dihydrooxazol-4-yl)(4-(methylsulfonyl)phenyl) methanol (13)

To a solution of 12 (4 g, 7.8 mmol) in acetonitrile (40 mL) was added K_2CO_3 (4.3 g, 31.1 mmol), the reaction mixture was stirred at 50℃ for 2h. The reaction mixture was quenched with water and extracted with DCM (20 × 3 mL), the solvent was removed under vacuum, the residue was purified by flash chromatography (petroleum ether / ethyl acetate 1:1, V/V) to afford 13 (2.3 g, 85 %) as a white solid. Mp: 129.7-130.4 ℃. ^{1}H NMR (600MHz, $CDCl_3$): δ=7.93(d, J=8.4Hz, 2H), 7.61(d, J=8.16Hz, 2H), 6.22(s, 1H), 4.82-4.80(m, 1H), 4.59-4.55(m, 1H), 4.43(t, J=9.39Hz, 1H),

4.36(t, J=8.4Hz, 1H), 3.04(s, 3H), 2.92(d, J=3.78Hz, 1H); ^{13}C NMR (150MHz, $CDCl_3$): δ=164.13, 145.73, 140.38, 127.81, 127.68, 74.71, 71.83, 70.84, 60.95, 44.49; HR-MS calcd for $C_{12}H_{14}Cl_2NO_4S$ $[M+H]^+$ m/z 338.0015, found m/z 338.0015.

tert-butyl((4*R*,5*R*)-4-(4-(methylsulfonyl)phenyl)-2,2-dioxido-1,3,2-dioxathian-5-yl)carbamate (14)

To a stirred solution of diol 6 (5.0 g, 14.48 mmol) in anhydrous dichloromethane (50 mL) and triethylamine (40 mL) at 0℃ was slowly added thionyl chloride (13.8 g, 116.00 mmol). After 30min, the reaction was diluted with dichloromethane (40 mL) and washed with dilute hydrochloric acid (25 mL × 2) and brine (50 mL). The organic layer was concentrated under reduced pressure. the crude product was dissolved in a mixture of water (10 mL), MeCN (10 mL), and CH_2Cl_2 (15 mL). $NaIO_4$ (4.0 g, 18.70 mmol) and $RuCl_3$ hydrate (3 mg, 0.01 mmol) were added and the solution was vigorously stirred for 7h at ambient temperature. EtOAc (50 mL) was added to the cooled mixture. The organic layer was removed, dried (Na_2SO_4), and concentrated. The crude product was chromatographed to give the corresponding compound 14 (4.2g, 71 %) as a white solid. Mp: 232.8-234.8 ℃. ^{1}H NMR (600MHz, $CDCl_3$): δ=7.99(d, J=8.4Hz, 2H), 7.57(d, J=8.22Hz, 2H), 6.19(s, 1H), 5.31(d, J=9.9Hz, 1H), 5.15(dd, J=11.79Hz, 1.77Hz, 1H), 4.66(dd, J=11.76Hz, 1.2Hz, 1H), 4.38(dd, J=9.93Hz, 1.53Hz, 1H), 3.03(s, 3H), 1.23(s, 9H); ^{13}C NMR (150MHz, $CDCl_3$): δ=154.49, 141.34, 138.82, 127.75, 126.73, 86.33, 81.13, 47.06, 44.39, 27.96; HR-MS calcd for $C_{15}H_{21}NNaO_8S_2$ $[M+H]^+$ m/z 430.0601, found m/z 430.0606.

2,2-dichloro-N-((4R,5R)-4-(4-(methylsulfonyl)phenyl)-2,2-dioxido-1,3,2- dioxathian-5-yl)acetamide (15)

To a stirred solution of diol 2 (5.0 g, 14.0 mmol) in anhydrous dichloromethane (50 mL) and triethylamine (40 mL) at 0℃ was slowly added thionyl chloride (13.4 g, 112.6 mmol). After 30min, the reaction was diluted with dichloromethane (40 mL) and washed with dilute hydrochloric acid (2 ×25 mL) and brine (50 mL). The organic layer was concentrated under reduced pressure. the crude product was dissolved in a mixture of water (10 mL), MeCN (10 mL) and CH_2Cl_2 (15 mL). $NaIO_4$ (4.0 g, 18.70 mmol) and $RuCl_3$ hydrate (3 mg, 0.01 mmol) were added and the solution was vigorously stirred for 7h at ambient temperature. EtOAc (50 mL) was added to the cooled mixture. The organic layer was removed, dried (Na_2SO_4), and concentrated. The crude product was chromatographed to give compound 15 (4.3g, 72 %) as a white solid. Mp: 187.0-189.0 ℃. ^{1}H NMR (600MHz, DMSO-d_6): δ=9.48(d, J=9.6Hz, 1H), 7.96(d, J=8.52Hz, 2H), 7.61(d, J=8.28Hz, 2H), 6.54(d, J=1.92Hz, 1H), 6.36(s, 1H), 5.17(dd, J=12.09Hz, 2.01Hz, 1H), 4.85(dd, J=12.09Hz, 1.41Hz, 1H), 4.71(dd, J=9.63Hz, 1.77Hz, 1H), 3.17(s, 3H); ^{13}C NMR (150MHz, DMSO-d_6): δ=163.98, 141.52, 139.08, 127.50, 127.19, 86.95, 77.98, 66.11, 46.04, 43.89; HR-MS calcd for $C_{12}H_{13}Cl_2NNaO_7S_2$ $[M+Na]^+$ m/z 439.9403, found m/z 439.9402.

(*S*)-1-(4-(methylsulfonyl)phenyl)prop-2-en-1-ol (*S*-21)

To a solution of phosphate buffer (pH 7.0, 10mL) was added dextrose (0.8g) followed by KRED-02 wet cells (0.5g), 5mg $NADP^+$, ketone 22 (0.5g). The resulting solution was pH adjusted to a minimum of 7.5 with 2M NaOH prior to use. The reaction was carried out at 30 ℃ and shaken at 200 rpm shaker for 24 h. The reaction mixture was then extracted with DCM (10 mL×3), and the DCM layers were combined. The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure, obtaining 22 (0.48 g, 95 %) as a white solid. Mp: 54.8-55.8 ℃. ^{1}H NMR (600MHz, $CDCl_3$): δ=7.89(d, J=8.34Hz, 2H), 7.57(d, J=8.28Hz, 2H), 6.01-5.93(m, 1H), 5.38(d, J=17.1Hz, 1H), 5.29(d, J=5.7Hz, 1H), 5.25(d, J=10.26Hz, 1H), 3.03(s, 3H), 2.41(s, 1H); ^{13}C NMR (150MHz, $CDCl_3$): δ=148.76, 139.45, 139.32, 127.57, 127.12, 116.57, 74.63, 44.55; HR-MS calcd for $C_{10}H_{12}N_aO_3S$ $[M+Na]^+$ m/z 235.0399, found m/z 235.0403. The chiral HPLC was performed using a CHIRALPAK IC-3 (4.6×250 mm, 3μm, DAICEL, Shanghai) column with a mobile phase of 90% Hex-10% EtOH. The product was detected at 220 nm, and the retention times of diastereoisomers were 47.2 min, and 49.7 min (*S*-21), respectively.

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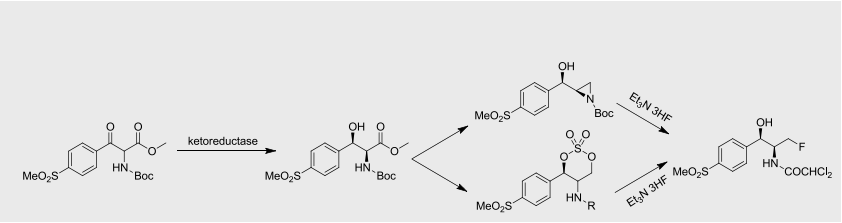

Chemo-enzymatic synthesis: The new synthesis of florfenicol features the application of enzymatic DYRKR to establish the two stereo centers of cis-1,2-amino alcohol with >99% ee and 99% dr in one step. Two new methods via aziridines and cyclic sulfates with Et_3N-3HF in high regioselectivuty to introduce fluorine atom were developed.

Jie Zou, Guowei Ni, Jiawei Tang, Jun Yu, Luobin Jiang, Dianwen Ju, Fuli Zhang, and Shaoxin Chen**

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Asymmetric Synthesis of Florfenicol via Dynamic Reductive Kinetic Resolution with Ketoreductases