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Synthesis of bioactive fluoropyrrolidines via copper(i)-catalysed asymmetric 1,3-dipolar cycloaddition of azomethine ylides†

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Chiral pyrrolidinyl units are important building blocks in biologically active natural products and drugs, and the development of efficient methods for the synthesis of diverse structured pyrrolidine derivatives is of great importance. Meanwhile, incorporating fluorine containing groups into small molecules often changes their activities to a great extent due to the special physicochemical properties of fluorine atoms. Herein, we report an efficient route to obtain enantioenriched 3,3-difluoro- and 3,3,4-trifluoropyrrolidinyl derivatives by Cu(I)-catalysed enantioselective 1,3-dipolar cycloaddition of azomethine ylides with less active 1,1-difluoro- and 1,1,2-trifluorostyrenes. A series of new fluorinated pyrrolidines have been prepared in high yields (up to 96%) and with excellent stereoselectivities (up to >20 : 1 dr and 97% ee), and these unique structural blocks could be readily introduced into some natural compounds and pharmaceuticals. Additionally, antifungal activity investigation against four common plant fungi showed that some products possess general and high biological activities; comparison with the low antifungal activities of corresponding nonfluorinated compounds revealed that the fluorine atoms at the pyrrolidinyl rings play a crucial role in the antifungal activity.

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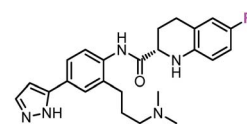
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Introduction

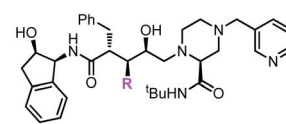
Fluoro-organic chemistry is one of the most fiery-hot areas in modern chemistry, and has currently become crucial in the evolution of many transdisciplinary research fields.^{1–4} The determinant impact of fluorine is undoubtedly associated with the pharmaceutical and agrochemical industries. Due to their special properties, the introduction of fluorine-containing groups into compounds often greatly affects their biological activity (Fig. 1) and thus has always been regarded as a powerful tactic in agrochemicals and creation of pharmaceuticals.^{5–14} According to a recent survey, approximately half of newly developed pesticides and 35% of new drug candidates in phase II–III clinical trials contain distinct kinds of fluorine-substituted moieties.^{15–17} Over the past few decades, the remarkable success of and great demand for fluorine compounds in various fields have provided infinite vitality for

organic chemists to explore methods for the construction of fluorinated molecules.^{18–22}

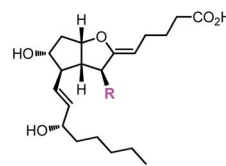
On the other hand, enantioenriched pyrrolidinyl groups, which are important building blocks in many biologically active natural alkaloids and pharmaceuticals, have attracted much attention in the last few decades.^{23–28} The transition-metal-catalysed asymmetric 1,3-dipolar cycloaddition of azomethine ylides with electron deficient alkenes represents one of the most powerful methods for the stereocontrolled synthesis of these molecules.^{29–34} Considering the crucial properties of fluorine



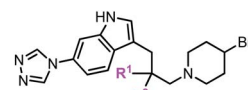
Inhibitors for Rho kinase
Analogue I (R = H): IC₅₀ = 590;
Analogue II (R = F): IC₅₀ = 7.



Inhibitors for HIV-1 protease
C17-*epi*-Indinavir (R = H): K_i = 160 nM;
Fluorinated analogue (R = F): K_i = 20 nM.



Half life in vivo (t_{1/2}, pH = 7.4)
PGI2 (R = H): 10 mins;
7-F-PGI2 (R = F): > 30 days.



Affinities at 5-HT_{1D} receptor
Analogue I: R¹ = R² = H: IC₅₀ = 0.3;
Analogue II: R¹ = H, R² = F: IC₅₀ = 0.9;
Analogue III: R¹ = R² = F: IC₅₀ = 78.

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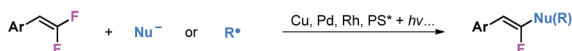
‡ These authors contributed equally.

Fig. 1 Fluorine effects on biological activity.

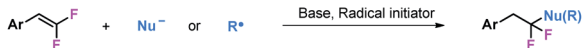


Previous work

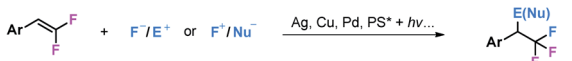
(a) Synthesis of monofluoro-alkenes by nucleophilic addition-elimination reactions



(b) Synthesis of difluoro-compounds via nucleophilic addition reactions



(c) Synthesis of trifluoro-compounds through F-addition induced cascade reactions



This work:

(d) Synthesis of bioactive fluoropyrrolidines by asymmetric [3+2] cycloaddition



- Regioselective & Stereoselective
- Broad substrate scope (55 cases)
- High antifungal activities

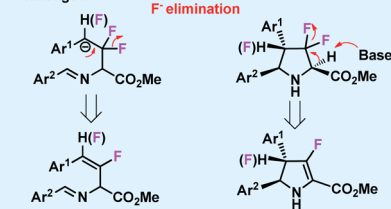
Enriched
3,3-Difluoropyrrolidines &
3,3,4-Trifluoropyrrolidines

Challenge I

Less reactive olefins
High activation barrier

$\text{H}(\text{F})$
 $\text{Ar}-\text{C}(\text{F})=\text{C}(\text{F})$
VS
 $\text{R}_1-\text{C}(\text{EWG})=\text{C}(\text{EWG})$
Reactive olefins
Low activation barrier

Challenge II



Scheme 1 Synthesis of fluorochemicals from fluorostyrenes.

atoms, incorporating fluorine atoms into heterocycle rings in chiral pyrrolidine derivatives would change their bioactivities to a great extent. In principle, the asymmetric 1,3-dipolar cycloaddition of azomethine ylides with fluorostyrenes could lead to multiple fluorinated pyrrolidines, however, few studies on the construction and bioactivity evaluation of such molecules have been reported to date.

Owing to their multiple reactivities, readily available *gem*-difluorostyrenes³⁵ can participate in various organic transformations to access structurally diversified fluorocompounds.^{36–43} Addition or addition–elimination of nucleophiles or free radicals to *gem*-difluoro-styrenes could lead to difluorocompounds^{44–46} and monofluoro-alkenes^{47–55} (Schemes 1a and b). Taking advantage of *gem*-difluorostyrenes as reliable trifluoromethyl (CF₃) precursors through F⁺/F[−] addition, several cascade reactions have been achieved to access trifluoromethyl compounds (Scheme 1c).^{56–60} Despite the comprehensive application of *gem*-difluorostyrenes in the synthesis of fluoro-compounds, the only example of the asymmetric transformation of *gem*-difluorostyrenes via fluoroarylation was reported by Zhang and coworkers,⁶¹ and asymmetric cycloaddition with these substrates still remains blank.

In view of the importance of fluoro-compounds and chiral N-heterocycles, we envisioned that enantioselective 1,3-dipolar cycloaddition of azomethine ylides with *gem*-1,1-difluorostyrenes and 1,1,2-trifluorostyrenes would lead to a broad scope of biologically active fluoropyrrolidines.^{62–68} To achieve this transformation, two challenges need to be considered (Scheme

1d). It is well known that the dipolarophiles of the 1,3-dipolar cycloaddition of azomethine ylide are generally limited to highly activated olefins bearing strong electron withdrawing substituents,^{29–34} and only a few cases are associated with less reactive olefins, however, the utilization of inactive alkenes as dipolarophiles remains a great challenge.^{69–72} In addition, the potential elimination of fluorine atoms in intermediates or products is another challenge for this reaction design.⁷³

Results and discussion

Reaction condition optimization

In the initial studies, imino ester **1a** was chosen as the azomethine ylide precursor and 4-Cl-*gem*-difluorostyrene **2a** was

Table 1 Investigation of optimal reaction conditions^a

Reaction scheme: **1a** + **2a** $\xrightarrow[\text{Solvent, 80 } ^\circ\text{C, 48 h}]{[\text{M}] (5 \text{ mol}\%), \text{L} (6 \text{ mol}\%), \text{Base} (20 \text{ mol}\%)}$ **3a** (dr > 20:1)^b

Entry	[M] ^c	L	Solvent	Base	T (°C)	Yield ^d (%)	Ee ^e (%)
1	CuPF ₆	L1	Toluene	KO ^t Bu	25	Trace	—
2	CuPF ₆	L1	Toluene	KO ^t Bu	80	60	60
3	CuPF ₆	L2	Toluene	KO ^t Bu	80	61	28
4	CuPF ₆	L3	Toluene	KO ^t Bu	25	48	96
5	CuPF ₆	L3	Toluene	KO ^t Bu	80	91	95
6	CuPF ₆	L4	Toluene	KO ^t Bu	80	28	28
7	CuPF ₆	L5	Toluene	KO ^t Bu	80	28	65
8	CuPF ₆	L6	Toluene	KO ^t Bu	80	34	94
9	CuBF ₄	L3	Toluene	KO ^t Bu	80	57	92
10	CuOAc	L3	Toluene	KO ^t Bu	80	Trace	—
11	Cu(OAc) ₂	L3	Toluene	KO ^t Bu	80	Trace	—
12	AgOAc	L3	Toluene	KO ^t Bu	80	85	77
13	CuPF ₆	L3	THF	KO ^t Bu	80	81	95
14	CuPF ₆	L3	AcOEt	KO ^t Bu	80	61	94
15	CuPF ₆	L3	1,4-Dioxane	KO ^t Bu	80	48	91
16	CuPF ₆	L3	Toluene	K ₂ CO ₃	80	42	95
17	CuPF ₆	L3	Toluene	DIPEA	80	90	95

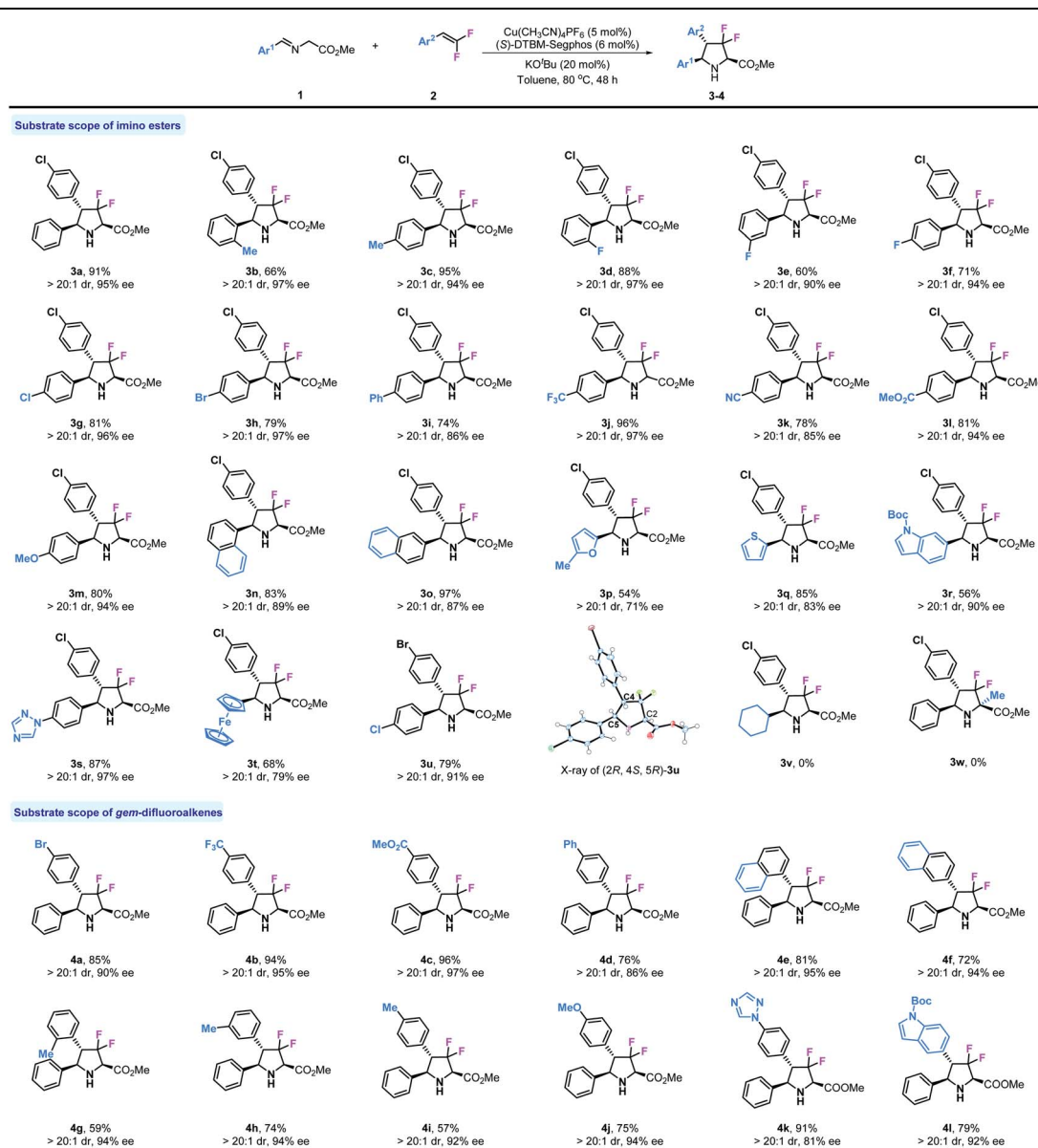
^a All reactions were carried out with 0.40 mmol of **1a** and 0.20 mmol of **2a** in 2 mL solvent. ^b Dr was determined by crude ¹H NMR. ^c CuPF₆ = Cu(CH₃CN)₄PF₆, CuBF₄ = Cu(CH₃CN)₄BF₄. ^d Isolated yield. ^e Ee was determined by chiral HPLC analysis.



chosen as the dipolarophile. In the presence of the $\text{CuPF}_6/(S)$ -BINAP complex (5 mol%) and KO^tBu (20 mol%) in toluene, only trace amounts of cycloadduct **3a** could be detected at room temperature (Table 1, entry 1); when the reaction temperature was increased to 80 °C, the desired product **3a** was obtained in 60% yield with >20 : 1 dr and 60% ee after 48 h (Table 1, entry 2). Encouraged by these results, we next tested other chiral diphosphine ligands, (*S*)-seghpos **L2** gave similar catalytic reactivity with (*S*)-BINAP (61% yield) but with poor enantioselectivity (28% ee) (Table 1, entry 3). To our delight, (*S*)-DTBM-seghpos **L3** exhibited high reactivity in this reaction, which made this reaction happen readily at room temperature and gave **3a** in 48% yield and 96% ee (Table 1, entry 4). When the reaction was carried out at 80 °C, the desired product **3a** was obtained in 91% yield with >20 : 1 dr and 95% ee (Table 1, entry

5). Chiral N-P ligands are not effective in this transformation; (*S*)-TF-biphamphos **L4**, which served as an efficient ligand in azomethine ylide-involved 1,3-dipolar cycloaddition, gave very poor results in this transformation (Table 1, entry 6), while chiral N-P ligands **L5** and **L6** also showed low reactivities (Table 1, entries 7 and 8). Besides ligands, metal salts also play an important role in this reaction; when CuBF_4 was used, the yield decreased from 91% to 57%, although stereoselectivities were maintained at the same level (>20 : 1 dr and 92% ee) (Table 1, entry 9), while neither CuOAc nor $\text{Cu}(\text{OAc})_2$ could cause this reaction to occur (Table 1, entries 10 and 11). Other metal salts, such as AgOAc , also worked in this transformation, however, they provided the corresponding product **3a** with moderate enantioselectivity (Table 1, entry 12). Next, we examined various common solvents such as tetrahydrofuran, ethyl acetate, and

Table 2 Substrate scope of imino esters **1** and *gem*-difluorostyrene **2**^a



1,4-dioxane, in which, catalytic reactivities decreased to various degrees while stereoselectivities remained at the same level. It seems that bases did not affect stereo-control at all; however, in the presence of K_2CO_3 , **3a** was obtained only in 42% yield, which may be due to the poor solubility of K_2CO_3 in toluene (Table 1, entry 16). Through the experiments above, we finally confirmed the optimal reaction conditions: 5 mol% $Cu(CH_3CN)_4PF_6$, 6 mol% (*S*)-DTBM-segphos, 20 mol% KO^tBu , and 2 mL of toluene, at 80 °C.

Substrate scope of azomethine ylides and *gem*-difluorostyrenes

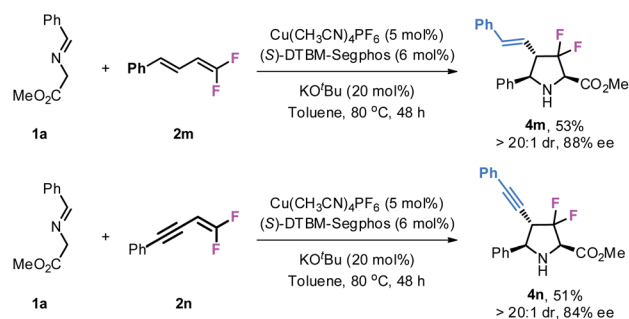
Then, we investigated the reaction of various substituted imino esters **1** with **2a** under optimal conditions, and the results are summarized in Table 2. In addition to product **3a**, various substituted chiral difluoropyrrolidines can be prepared as a single diastereomer (>20 : 1 dr) by this method. The steric effect of substituents did not influence the stereoselectivities, and *ortho*- and *para*-Me substituted products **3b** and **3c** were obtained with excellent enantioselectivities (97% and 94% ee respectively). Fluoro-substituted products **3d–3f** were obtained with moderate to good yields and with high stereoselectivities. Other halogen substituents, such as chloride or bromide, also tolerated these reaction conditions, providing corresponding products **3g** and **3h** in good yields (81%, 79%) and with high enantioselectivities (96%, 97% ee). Biphenyl derivative **3i** was produced in 74% yield and with high enantioselectivity (86% ee). As shown, substrates bearing strong electron withdrawing substituents such as $-CF_3$, $-CN$, and $-CO_2Me$ performed very well in this reaction, leading to desired products **3j–3l** with good reactivities (78–96% yields) and stereoselectivities (>20 : 1 dr, 85–97% ee). The strong electron donating group $-OMe$ also showed no influence on this reaction (**3m**). Both 1-naphthyl and 2-naphthyl substituted difluoropyrrolidines **3n** and **3o** were readily prepared with satisfactory results. Considering the important role of heterocycle units in biologically active compounds, we investigated four substrates containing furyl, thienyl, indolyl and triazolyl in this transformation; to our delight, all corresponding products **3p**, **3q**, **3r** and **3s** could be obtained in moderate to good yields (54–87%) and with good enantioselectivities (71–97% ee). We also tried an imino ester bearing a ferrocene group (**3t**), which worked but yielded the product in moderate yield (68%) and with only 79% ee. To confirm the absolute configuration of **3u**, X-ray analysis was carried out and the stereo-configuration of **3u** was determined to be (2*R*, 4*S*, 5*R*). Finally, more challenging substrates derived from cyclohexanal **1v** and alanine **1w** were tested, unfortunately, neither of them could participate in this reaction. Encouraged by the results of imino esters, we next examined a series of substituted *gem*-difluorostyrenes. As mentioned in the beginning, normal aromatic alkenes could not be used in the 1,3-dipolar cycloaddition of azomethine ylide, except those activated by strong electron withdrawing substituents on phenyl rings; however, due to the electron withdrawing inductive effect of fluorine atoms, all substrates with electron-rich, neutral and deficient substituents can be used in this reaction. Product **4a**

with a bromide substituent was prepared with similar result to **3a** (85% yield, >20 : 1 dr, 90% ee). As expected, substrates with electron withdrawing groups such as $-CF_3$ and $-CO_2Me$ performed very well in this reaction and provided corresponding products **4b** and **4c** in high yields (94%, 96%) and with excellent enantioselectivities (95%, 97% ee). Diphenyl alkene can be used in this transformation although the enantioselectivity is somewhat lower (86% ee). It seems that steric effect does not affect stereoselective control in this reaction; for example, 1-naphthyl and 2-naphthyl substituted *gem*-difluorostyrenes gave similar results (81%, 95% ee and 72%, 94% ee). Meanwhile, *ortho*-, *meta*- and *para*-methyl substituted products **4g–4i** could be created by this method with the same enantioselectivities (94%, 94%, 92% ee) but in different yields (59%, 74%, 57%). The substrate with a strong electron donating group $-OMe$ proceeded smoothly and gave product **4j** with >20 : 1 dr and 94% ee despite a moderate yield (75%). In addition, two *gem*-difluorostyrenes bearing 1-triazolyl and 6-indolyl substituents were synthesized and tested in this transformation, and both of the desired products **4k** and **4l** were prepared with satisfactory results.

Gem-difluorodiene **2m** derived from cinnamaldehyde also participated in this reaction, giving product **4m** bearing a styryl group at the 4-position with >20 : 1 dr and 88% ee although in moderate yield (53%). Similar results were also obtained with *gem*-difluoroenyne **2n** as the fluorinating precursor to access the corresponding product **4n** with an alkynyl group (Scheme 2).

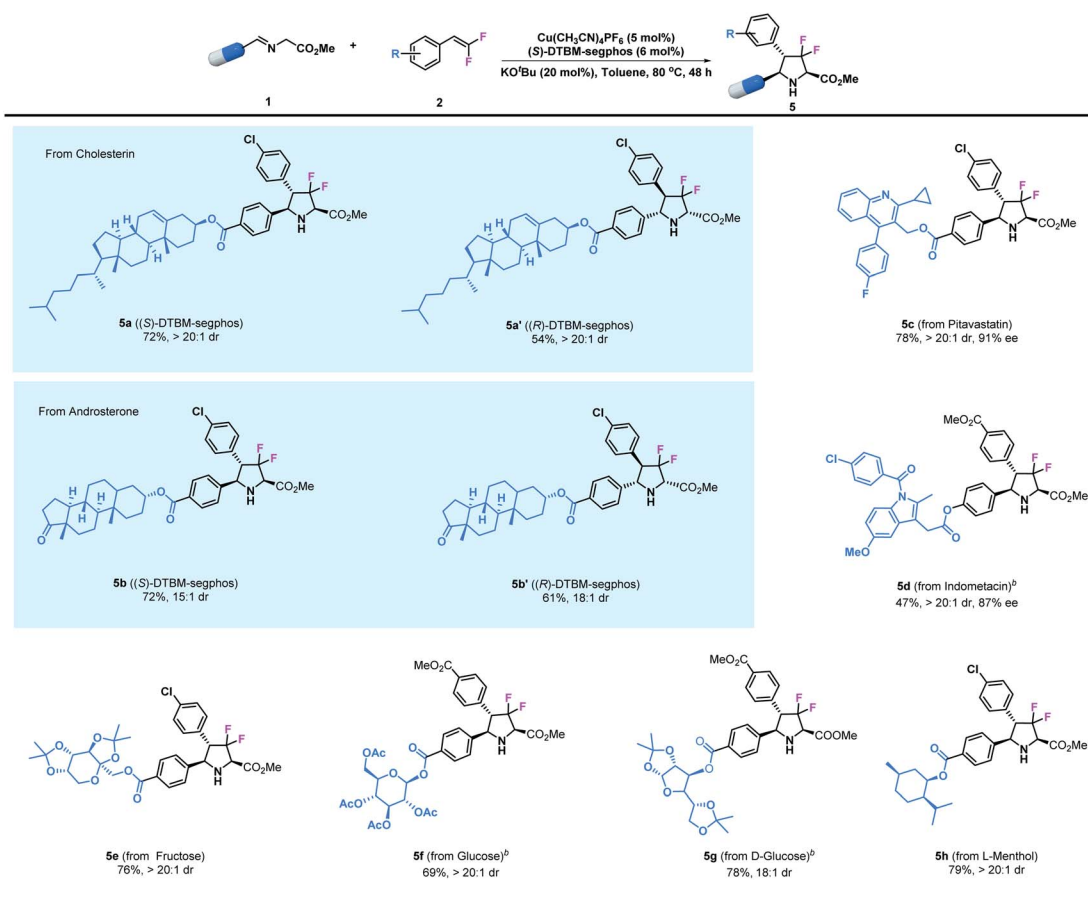
Modification of natural compounds and drugs

Additionally, since the modification of biologically active compounds is an important method for the development of agrochemicals and pharmaceuticals, the introduction of certain units into biologically active molecules is of great significance. Therefore, we further demonstrate the broad applicability of the asymmetric transformation protocol by examining imino esters derived from natural compounds and currently existing synthetic drugs. As shown in Table 3, the desired product **5a** bearing a cholesterol unit could be readily prepared in 72% yield and with >20 : 1 dr under the optimized reaction conditions; similarly, an androsterone derived imino ester could participate in this reaction to access adduct **5b**. Notably, the diastereomers **5a'** and **5b'** could also be prepared in high yield and stereoselectivity by using $Cu(I)/(R)$ -DTBM-segphos as the



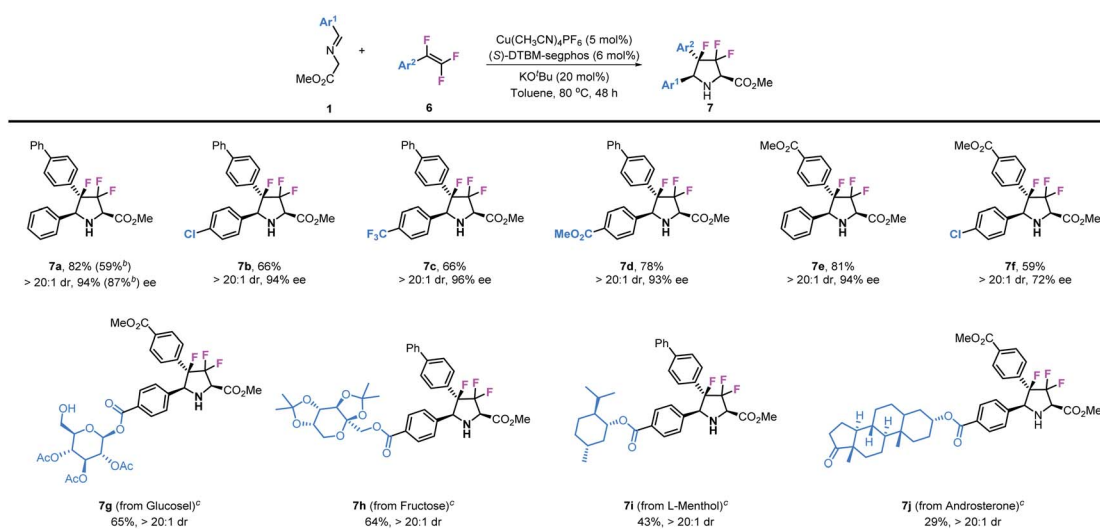
Scheme 2 Reactions of *gem*-difluorodiene **2m** and difluoroenyne **2n**.



Table 3 Modification of natural compounds and pharmaceuticals.^a

catalyst. Pitavastatin, which is an important building skeleton in drugs, could also be employed in this reaction to give **5c** in good yield (78%) and with excellent stereoselectivity (>20 : 1 dr and 91% ee). Meanwhile, the well-known commercial analgesic

indomethacin has been modified by this method, leading to the corresponding derivative **5d** in moderate yield (47%) together with excellent stereoselectivity (>20 : 1 dr and 87% ee). Encouraged by these results, we next tested saccharide

Table 4 Synthesis of trifluoropyrrolidine derivatives from trifluorostyrenes^a

derivatives which are important components in medicines that contain multiple sensitive functional groups. The asymmetric cycloaddition of imino esters derived from fructose and glucose was readily achieved, leading to the corresponding products **5e–5g** in high yields (69–78%) and with high stereoselectivities (18 : 1 → 20 : 1 dr). Finally, L-menthol is undoubtedly one of the most important chiral precursors for the synthesis of agrochemicals and pharmaceuticals. Herein, we easily incorporated this unit with a difluoropyrrolidiny motif to give product **5h** in 79% yield and with >20 : 1 dr.

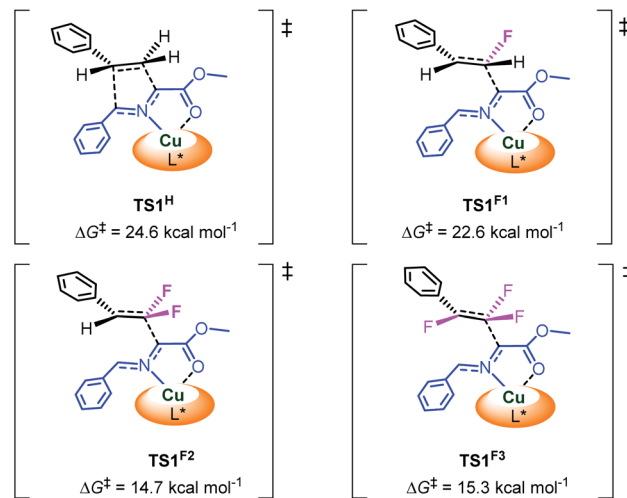
1,3-Dipolar cycloaddition of 1,1,2-trifluorostyrenes

Besides 3,3-difluoropyrrolidines, chiral 3,3,4-trifluoropyrrolidiny derivatives were also prepared by using 1,1,2-trifluorostyrenes as substrates in this transformation. As shown in Table 4, various substituted 1,1,2-trifluorostyrenes could readily participate in this reaction to access corresponding chiral 3,3,4-trifluoropyrrolidines. Imino esters and trifluorostyrenes with simple structures can be applied as compatible reactants, leading to the desired 3,3,4-trifluoro-pyrrolidines **7a–7f** in moderate to good yields (59–82%) and with excellent stereoselectivities (>20 : 1 dr and 72–96% ee). Similar to the synthesis of 3,3-difluoropyrrolidines, we introduced these unique structural motifs into some kinds of biologically active natural compounds such as glucose (**7g**), fructose (**7h**), L-menthol (**7i**) and androsterone (**7j**).

These reactions should be conducted at 60 °C to prevent decomposition of the obtained products.

Mechanism studies

To investigate the influence of fluorine atoms on the reactivity, the reactions of iminoester **1a** with styrene **2o** and monofluorostyrene **2p** were tested. As expected, neither of these reactions happened under the optimal conditions (Scheme 3), which proved that the strong electron withdrawing inductive effect plays a key role in the activation of double bonds. Next, density functional theory (DFT) calculations were performed on the reaction of iminoester **1a** with styrene, monofluorostyrene, difluorostyrene and trifluorostyrene. As shown in Scheme 4, in the case of styrene, the reaction tends to proceed *via* a concerted process, and the activation energy ΔG^\ddagger of **TS1^H** is approximately 24.6 kcal mol⁻¹. In contrast, the 1,3-dipolar cycloaddition of fluorostyrenes underwent stepwise routes that needed to

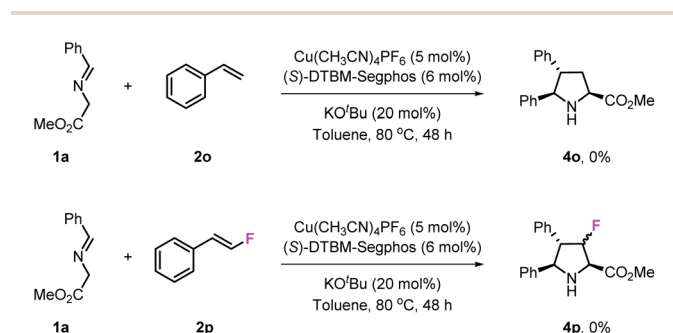


Scheme 4 Relative free energy barrier for cycloaddition of azomethine ylide with styrene and fluorostyrenes.

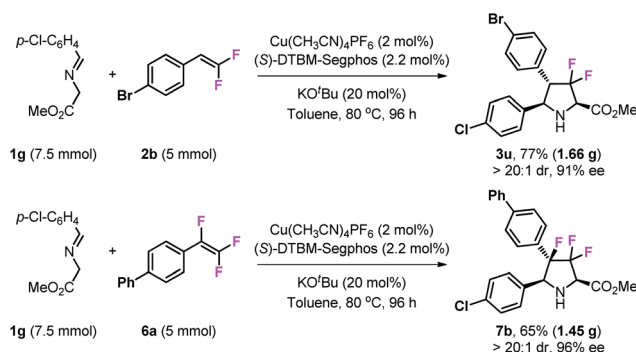
overcome two energy barriers **TS1** and **TS2** (see the ESI[†]). However, the activation energy ΔG^\ddagger values of key transition states **TS1** for the three reactions are quite different. In the case of monofluorostyrene, the activation energy ΔG^\ddagger of **TS1^{F1}** is calculated to be 22.6 kcal mol⁻¹ which is similar to that of styrene. However, with difluorostyrene and trifluorostyrene as substrates, the activation energy ΔG^\ddagger values are much lower (**TS1^{F2}** = 14.7 kcal mol⁻¹ and **TS1^{F3}** = 15.3 kcal mol⁻¹). These theoretical data are consistent with the experimental results perfectly and provide strong evidence for the crucial role of fluorine atoms in the activation of dipoles.

Scale-up reactions

To test the practicability of this methodology, gram-scale syntheses of **3u** and **7b** were examined under optimal conditions (Scheme 5). The reaction of 7.5 mmol of imino ester **1g** with 5 mmol of *gem*-difluorostyrene **2b** performed very well and gave 1.66 g of product **3u** in 77% yield, and with high stereoselectivity (>20 : 1 dr and 91% ee). In addition, 1.45 g of 3,3,4-trifluoropyrrolidine **7b** can be prepared by the reaction of 7.5 mmol of imino ester **1g** with 5 mmol of 1,1,2-trifluorostyrene **6a**.



Scheme 3 Reactions of styrene **2o** and monofluorostyrene **2p**.



Scheme 5 Gram scale reactions.



Antifungal activity evaluation

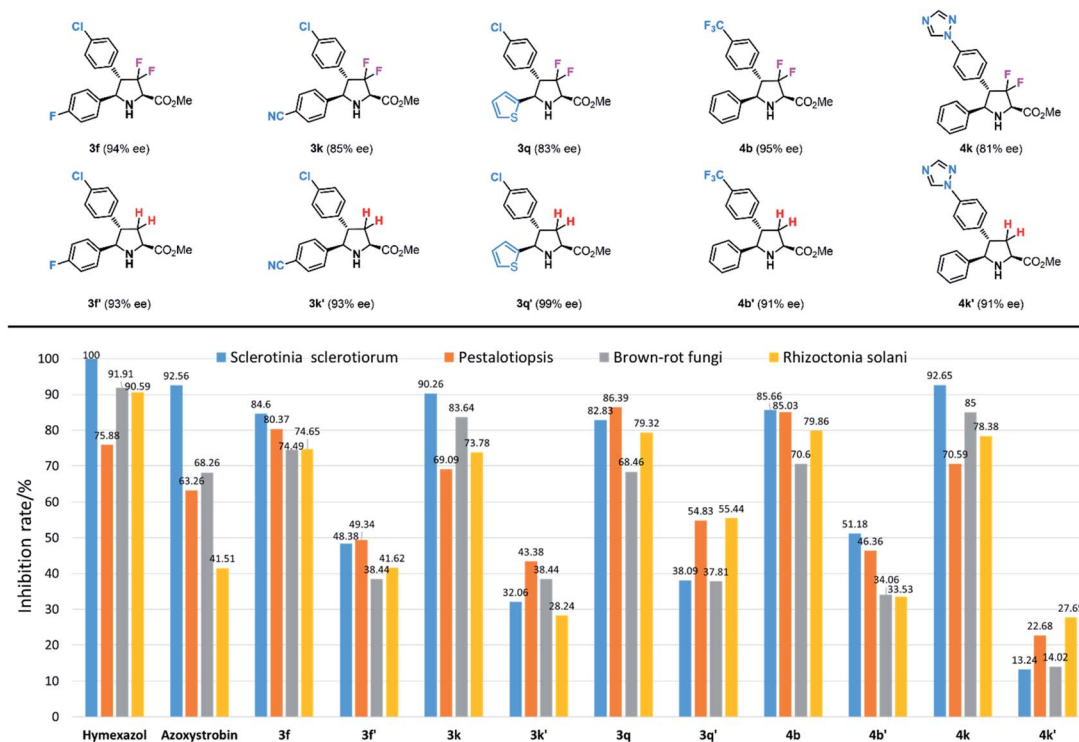
With these unique fluorinated compounds in hand, we next tested their antifungal activities. In this part, four common fungi, *Sclerotinia sclerotiorum*, *Pestalotiopsis*, brown-rot fungi and *Rhizoctonia solani* were chosen as experimental inhibitory subjects. Meanwhile, two popular and commercial antifungal insecticides hymexazol and azoxystrobin were used as contrapositives. As shown in Table S1,[†] most 3,3-difluorinated products showed general antifungal activities against the four fungi, however, in contrast, most 3,3,4-trifluoro-compounds gave poorer results. Among all biologically active difluoropyrrolidines, products **3f**, **3k**, **3q**, **4b** and **4k** provided similar and even better antifungal inhibition effects than hymexazol and azoxystrobin (Scheme 6). It seems that additional fluorine groups contribute greatly to antifungal inhibition. Product **3f** with an additional fluorine atom at the *para*-position of the phenyl group gave 84.60%, 80.37%, 74.49% and 74.65% inhibition rates against *Sclerotinia sclerotiorum*, *Pestalotiopsis*, brown-rot fungi and *Rhizoctonia solani* respectively, and similarly, compound **4b** which has a trifluoromethyl (CF₃) substituent also showed great results (85.66%, 85.03%, 70.62% and 79.86%). Meanwhile, product **3k** bearing a cyano-group (CN) also performed very well towards all fungi (90.26%, 69.09%, 83.64% and 73.78%). Heterocycles, which are important building blocks in agricultural compounds, often provide a key contribution to bioactivity; for example, thienyl-substituted product **3q** can inhibit these fungi effectively (82.83%, 86.39%, 68.46% and 79.32%), and the same results were obtained by compound **4k** with 1,2,4-triazolyl unit (92.65%, 70.59%, 85.05% and 78.38%). These experimental results reveal

Table 5 Inhibitory effect on *Sclerotinia sclerotiorum* at different concentrations

Concentration (mg L ⁻¹)	6.25	12.50	25.00	50.00	100.00	EC50
3f	40.00	44.56	64.41	83.24	84.60	23.8
3k	38.53	50.15	69.16	78.82	90.26	17.96
3q	0.00	28.68	62.35	71.62	82.83	13.60
4b	42.30	54.20	64.30	70.00	85.66	10.17
4k	0.00	13.18	47.95	70.62	92.65	25.78
Hymexazol	45.72	49.06	65.10	83.25	100.00	10.08
Azoxystrobin	45.32	55.30	92.55	95.38	92.56	15.14

that the combination of certain functional groups is necessary to enhance biological activities.

As mentioned in the beginning, fluorine factors play a crucial role in biological activity, therefore, to confirm the contribution of fluorine atoms in these structures to the antifungal inhibitory reactivity, corresponding chiral pyrrolidinyl derivatives **3f'**, **3k'**, **3q'**, **4b'** and **4k'** without fluorine atoms at the heterocyclic rings were prepared according to Carretero's methods.⁵⁵ The antifungal inhibition results of these contrapositive compounds are summarized in Tables 5 and S2,[†] and the antifungal activities between fluorinated compounds and their contrasts are quite different; as expected, all the contrapositive samples gave poor results in antifungal experiments. **3f'** showed only approximately half of the inhibition rate of **3f** against *Sclerotinia sclerotiorum*, *Pestalotiopsis* brown-rot fungi, and *Rhizoctonia solani* (48.38%, 49.34%, 38.44% and 41.62%). **3k'** and **3q'** led to even lower biological reactivities (32.06%, 43.38%, 38.44%, 28.24% and 38.09%, 54.83%, 37.81%, 43.38%, 38.44%, 28.24% and 38.09%, 54.83%, 37.81%,



Scheme 6 Investigation of "fluorine" influence on antifungal activities.



55.44%). Compound **4b'** bearing a trifluoromethyl (CF₃) group also provided a moderate inhibition rate towards the four fungi (51.18%, 46.36%, 34.06%, 33.53%). Furthermore, the non-fluorinated trizolyl-substituted contrast compound **4k'** showed almost no effect on the antifungal inhibition (13.24%, 22.68%, 14.02% and 27.65%). Through these control experiments, we can see that the two fluorine atoms at the pyrrolidine ring play a crucial role in biological activities.

Meanwhile, antifungal experiments were carried out with racemic samples under the same conditions. As shown in Table S2,† (±)-**3f**, (±)-**3q** and (±)-**4b** gave slightly poorer results than chiral samples, but (±)-**3k** and (±)-**4k** showed very poor antifungal inhibition. This revealed that stereoselectivity is also a key factor in biological activity. Considering the requirement for low pesticide doses in modern practical applications, we finally examined the antifungal inhibition effects of these active compounds at different concentrations (6.25, 12.50, 25.00, 50.00 and 100.00 mg L⁻¹) with *Sclerotinia sclerotiorum* as the research subject (Table 5). All inhibition rates decreased as the concentration decreased from 100.00 to 6.25 mg L⁻¹, but their rates of decline were different. The inhibitory effects of **3q** and **4k** dropped sharply, and no effects were observed at 6.25 mg L⁻¹ concentration, while those of **3f** and **3k** declined slower and still possessed similar inhibition rates to the commercial pesticides hymexazol and azoxystrobin at 6.25 mg L⁻¹. The best result was obtained for **4b**, which gave 42.3% inhibition rate even with 6.25 mg L⁻¹ concentration. And EC₅₀ (concentration for 50% of maximal effect) values of compounds **3q** (13.60 mg L⁻¹) and **4b** (10.17 mg L⁻¹) maintained at the same level of commercial pesticides hymexazol (10.08 mg L⁻¹) and azoxystrobin (15.14 mg L⁻¹). This result illustrates that this structure has some potential for applications in the creation of agricultural compounds.

Conclusion

In conclusion, we developed a Cu(I)-catalysed asymmetric 1,3-dipolar cycloaddition of azomethine ylides with less active 1,1-*gem*-difluorostyrenes and 1,1,2-trifluorostyrenes, leading to a series of novel chiral 3,3-difluoro- and 3,3,4-trifluoropyrrolidiny derivatives. This methodology features high yields (up to 96% yield), stereoselectivity (up to >20 : 1 dr and 97% ee) and broad substrate scope (55 cases). The biological activity of these newly prepared compounds was evaluated *via* antifungal experiments, and some of these fluorinated pyrrolidines performed very well in antifungal experiments against *Sclerotinia sclerotiorum*, *Pestalotiopsis*, brown-rot fungi and *Rhizoctonia solani*. Control experiments between fluorinated and nonfluorinated compounds proved that the fluorine atoms on heterocyclic rings are crucial to the biological activity.

Experimental section

General procedure for the synthesis of 3,3-difluoro- and 3,3,4-trifluoropyrrolidines

In a N₂ atmosphere, Cu(CH₃CN)₄PF₆ (3.7 mg, 0.01 mmol) and (*S*)-DTBM-segphos (14.2 mg, 0.012 mmol) were dissolved in

2.0 mL of toluene and stirred at room temperature for 1 h. Then, KO^tBu (0.04 mmol, 0.2 eq.), imino esters **1** (0.4 mmol, 2.0 eq.), 1,1-*gem*-difluorostyrenes **2** or 1,1,2-trifluorostyrenes **6** (0.2 mmol, 1.0 eq.) were added successively, the reaction was kept at 80 °C for 48 h. After completion, determined by TLC, the solvent was removed under vacuum and the diastereoselectivity was determined by crude ¹H NMR, then the reaction mixture was purified by flash chromatography on silica gel to give the corresponding fluorinated pyrrolidines, whose enantiomeric excess was analyzed directly by chiral HPLC.

Methods for antifungal activity investigation

All tested compounds (10 mg) were dissolved in 1.0 mL acetone, then the solution was added to 100 mL of Potato Dextrose Agar medium to prepare a drug-loaded PDA medium plate having a concentration of 100 mg L⁻¹, and 1.0 mL acetone was added to the PDA as a blank sample. Azoxystrobin and hymexazol were selected as control samples. *Sclerotinia sclerotiorum*, *Pestalotiopsis*, brown-rot fungi and *Rhizoctonia solani* were activated beforehand (prepared by a punch with $\phi = 0.50$ cm), and then they were cut from the edge of the colony and inoculated into the above PDA plate, each strain and blank control experiment were repeated three times. After sealing with a sealing film to prevent contamination of other bacteria, the poisonous PDA culture dishes were placed in a constant temperature incubator at the temperature of 24 ± 1 °C. After the blank control group culture was grown to about two-thirds of the culture dish, the colony diameter was measured by the cross method in millimeters (mm) with a cross and a vertical method, and the average value was taken, and the inhibition rate of each compound against the fungus was calculated.

Author contributions

X. X. developed the synthetic methods. L. B. performed the antifungal activity investigations. L. R. and Z. Y. participated in the synthetic work. D. Y. took charge of the characterization of compounds. C.-J. W. and H. T. wrote the manuscript with revisions provided by the other authors.

Conflicts of interest

There are no conflicts to declare.

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