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Synthesis and antibacterial activity of 6(*R*)- and 6(*S*)-fluoropenibruguieramine As: Fluorine as a probe for testing the powerfulness of memory of chirality (MOC)

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ABSTRACT

The synthesis of 6(*R*)- and 6(*S*)- fluoropenibruguieramine As has been achieved, employing the elegant strategy developed by Kim and co-workers. Single diastereomers were formed via the key intramolecular aldol reaction, and both of the products were unambiguously confirmed by X-ray diffraction crystallography. This reaction shows that the fluorine amide effect could not compete with the memory of chirality (MOC) effect, thus further demonstrating the powerfulness of MOC effect in asymmetric synthesis. The biological testing carried out in this work indicates that the principal of antibacterial activity of the natural extract is probably not penibruguieramine A.

1. Introduction

'Memory of chirality' (MOC) refers to the effect that a sole chiral center in a molecule directs the stereochemical course of a reaction even though it is destroyed during the reaction [1–3]. MOC is an important strategy in asymmetric synthesis [1–3], which has been elegantly applied for the synthesis of several natural products [4–6]. In this context, Kim's work on the synthesis of penibruguieramine A (PA) stands out as a notable example [6]. PA is a natural marine alkaloid which was isolated from the fungal strain *Penicillium sp.GD6* by Guo and co-workers [7]. As presented in Scheme 1, PA is featured by an unprecedented 1-hydroxy-2-methyl pyrrolizidin-3-one skeleton with an alkenyl chain and a hydroxymethyl group attached to the C1 position and the C8 position, respectively. In 2015, Kim and co-workers achieved the first total synthesis of PA from proline employing a biomimetic intramolecular Aldol reaction [6], wherein memory of chirality (MOC) were elegantly used for the control of the very challenged quaternary C8 stereochemistry.

The incorporation of fluorine into a given molecule can alter its conformation because the highly polarized C-F bond engages in a variety of stereoelectronic interactions with neighboring functional groups such as ammonium, amine, and amide [8–10]. In this context, 4-fluoroproline [11] is undoubtedly a meritorious scaffold for both molecular design and organic synthesis, owing to the so-called fluorine gauche effect [8–10,12]. For example, Raines and co-workers showed that incorporating 4-fluoroproline into a collagen significantly stabilize its secondary structure [13,14]. Florison and co-workers showed 4-fluoroprolines as viable substrates for highly diastereoselective allylation reaction [15]. List and co-workers demonstrated that 4-fluoroprolines as organocatalysts for asymmetric transannular aldolizations of 1,4-cyclooctanediones [16].

Based on the previous reports and as the continuous interests of one of the authors in the synthesis of stereoselectively fluorinated biomolecules [17–20]. We became interested in the synthesis of 6(*R*)- and 6(*S*)-fluoropenibruguieramines **2** and **3** from 4-fluorproline. We expected: sterically, the fluorine atom is as small as a hydrogen, so the key intramolecular Aldol reaction would be directed by the MOC effect to give the product **7** and **8**, as observed by Kim and co-workers [6]. Electronically, however, the aforementioned fluorine amide gauche effect may compete with the MOC effect in **5I**, and possibly afford the wrong diastereomer **9** (Scheme 1) [15]. Therefore, fluorine was expected to serve as a probe to test the powerfulness of MOC effect in this synthesis. In addition, although the crude extract of the natural product manifests promising antibacterial activity, the anti-bacterial study had not been conducted for PA due to the scarcity of the natural compound [7]. Therefore, We also aimed to test the anti-bacterial activity the natural PA and its fluorinated analogues to see whether the introduction of fluorine atom could bring some positive impact to the parent molecule [21].

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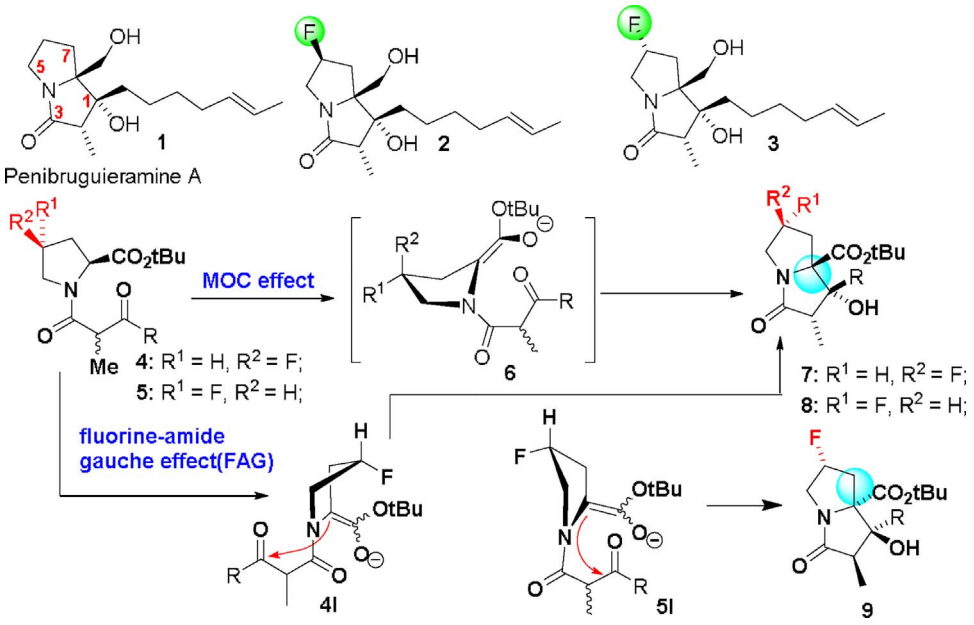


Scheme 1. Major product prediction based on MOC effect and FAG effect.

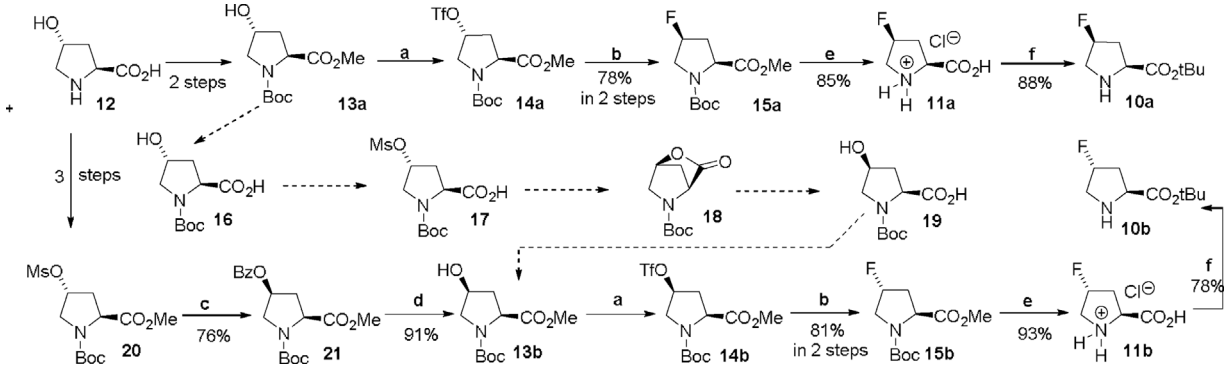
2. Results and discussion

As outlined in Scheme 2, the synthesis of 6(*R*)- and 6(*S*)-fluoropenibruguieramines **2** and **3** commenced with the preparation of *tert*-Butyl esters of 4-fluoroproline, **10a** and **10b**. Their precursors, fluorinated prolines **11a** and **11b,** were synthesized from 4-hydroxyproline with modifications of a procedure reported by Raines and co-workers [22,23]. The major improvements include: (1) the addition of activated 4 Å molecular sieve in the crucial fluorinating step increased the yield significantly, from 40% in the original report to 78% in this work [22]. (2) a more practical strategy for the inversion of the hydroxyl group in **13** to yield **21** was used [24], which avoided the handling of three very polar compounds **16, 17** and **18** and two unstable intermediates **17–18** (dashed arrow, Scheme 2). With this improved approach, both **11a** and **11b** could be obtained readily on gram scale. Global deprotection of **15a** and **15b** followed by selective protection of the resulting free amino acids with *tert*-butyl acetate affords the desired **10a** and **10b** in 84% and 74% yields, respectively [25].

With ample quantity of **10a** and **10b** in hand, the next task was to couple them with the side chain fragment **9** prepared from alcohol **7**. Initially, we attempted to use the DCC as the coupling agent [6]. To our disappointment, no desired product could be obtained and the failure was largely attributed to possible decarboxylation of **24** to yield ketone **25**. After some optimization, the desired products **4** and **5** were obtained in moderate yields using HATU as the coupling reagent.

With **4** and **5** secured, attention was next turned to the key intramolecular aldol reaction. Under the identical cyclization condition (EtONa in EtOH) as reported by Kim and co-workers [6], only one product was observed by TLC for both **4** and **5**. Although well resolved NOE spectra were obtained (Supporting information), it turned out to be impossible to assign the relative chemistry of the products, partly because no hydrogen attached to two of the newly generated stereogenetic centers(C1 and C8). Finally, the problem was solved by obtaining material of **7** and **26** (free acid of **8**) suitable for X-ray diffraction [26]. As depicted in Fig. 1, the absolute configuration at C1, C2 and C8 of compounds **7** and **26** are identical to those of natural **1**. We could therefore conclude safely that the stereochemistry of **7** and **8** were controlled by MOC effect during the intramolecular Aldol reaction.

Though the rational of the stereocontrol was mentioned previously in paragraph 3, it is worth further discussion based on previous experimental and theoretical work. The MOC effect of proline-derived enolate **6** (Scheme 1) could be quite large, as a literature survey showed that a related phenylalaline-derived enolate's MOC effect, measured by Kawabata and co-workers [27], is 16.8 kcal/mol. In contrast, the fluorine amide effect is relatively subtle, usually about 2.0 kal/mol [8–10]. Thus, it is reasonable that fluorine amide effect could not compete with the MOC effect in this 4-fluoroproline system, which is supported experimentally by our work.



Scheme 2. Synthesis of fluorinated proline **10a** and **10b** based on an improved route of 4-fluoroproline. Conditions: a) Tf_2O (1.1 equiv), Pyridine (1.2 equiv), 4 Å MS, CH_2Cl_2, 0 °C,1 h; b) TBAF·$3H_2O$ (1.2 equiv), 4 Å MS, CH_2Cl_2, rt, 1.5 h; c) BzONa (2 equiv), DMSO, rt, 4.5 h; d) K_2CO_3 (1 equiv), MeOH, rt, 1 h; e) 2N HCl, reflux 24 h; f) ^tBuOAc (15 equiv), $HClO_4$ (1.4 equiv), rt, 18 h.

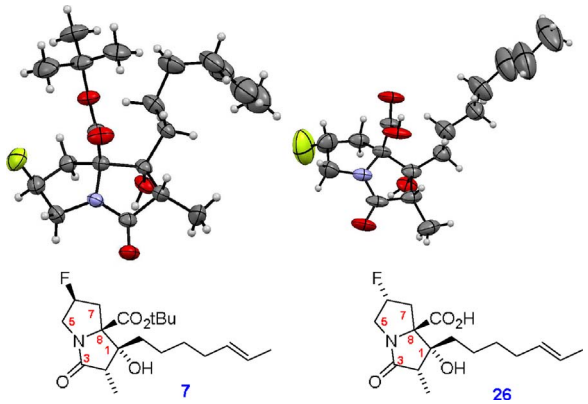


Fig. 1. X-ray crystal structure of **7** and **26**.

In order to obtain material for antibacterial testing, **11a** and **11b** was then treated with trifluoromethyl acetic acid to yield their corresponding carboxylic acids **27** and **26**, which were then transformed to activated ester and reduced in situ to afford the fluorinated fluoropenibruguieramine **2** and **3** (Scheme 3) [6].

With penibruguieramine A **1** [6] and its fluorinated analogues **2** and **3** in hand, We performed an antibacterial experiments according to a reported microplate bioassay method [28]. Although we can see some difference among the three compounds, unfortunately, the IC_{50} of each compound is quite high, which indicates that the principal of antibacterial of the natural extracts is probably not penibruguieramine A (Table 1) [7].

3. Conclusion

In summary, we have synthesized two fluorinated analogues of penibruguieramine A, **2** and **3,** employing the elegant strategy developed by Kim and co-workers [6]. Single diastereomers were formed from **4** and **5** via the intramolecular aldol reaction, and the structures of **7** and **8** were unambiguously confirmed by X-ray diffraction crystallography. This reaction shows that the fluorine amide effect could not compete with the MOC effect, thus further demonstrating the powerfulness of MOC effect in asymmetric synthesis. The biological testing carried out in this work indicates that the principal of antibacterial activity of the natural extract is probably not penibruguieramine A. During the synthesis, the reported synthetic route [22] of 4-Fluroprolines was improved and both **11a** and **11b** could be obtained readily on gram scale, which should be useful for researchers who want to employ these important fluorinated building blocks.

Table 1
IC_{50} of penibruguieramine A **1** and its fluorinated analogues **2** and **3**.[a]

Compound	*E.coli* (mg/mL)	*Staphylococcus aureus* (mg/mL)
1	1.35 (0.05)[b]	0.86 (0.02)[b]
2	1.16 (0.14)[b]	1.00 (0.05)[b]
3	1.49 (0.09)[b]	0.75 (0.07)[b]

[a] Three repetitions were made for each experiment.
[b] Numbers in parentheses are standard deviations.

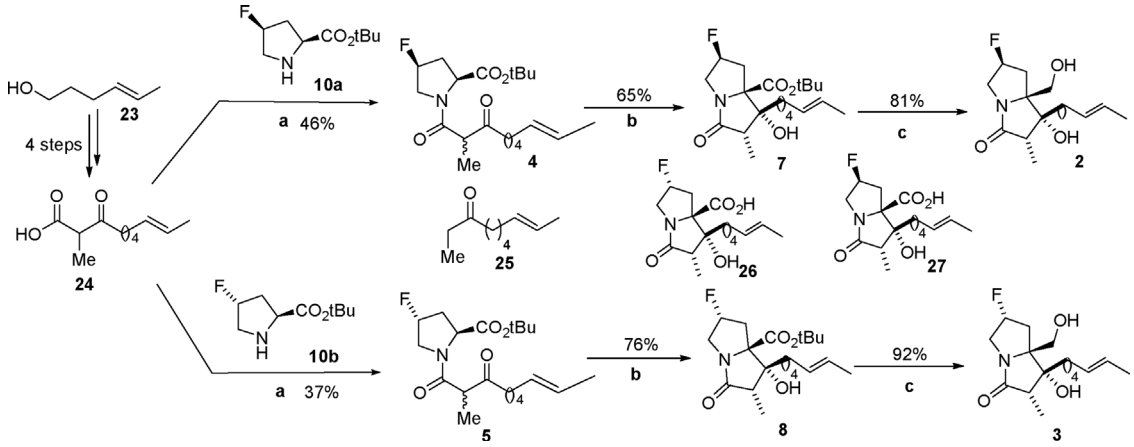
4. Experimental

4.1. N-Boc-(2S,4S)-4-fluoroproline methylester (15a)

To a solution of compound **13a** (1.44 g, 5.8 mmol) in dry methylene chloride (100 mL) was added activated 4 Å molecular sieve powder. This solution was cooled to 0 °C, and dry pyridine (0.65 mL, 6.96.mmol) was added, followed by trifluoromethanesulfonic anhydride (1.0 mL, 6.38 mmol). The solution was allowed to stir for 1 h and then quenched with 1N HCl solution. The aqueous phase was extracted with methylene chloride (2 × 50 mL), and the combined organic extracts were dried over $MgSO_4$(s) and then filtered (the work-up should be as fast as possible to avoid the decomposition of the unstable intermediate). To this filtrate was added activated 4 Å molecular sieve powder and TBAF·$3H_2O$ (2.2 g, 1.2 equiv, in one portion) successively. The resulting mixture was stirred at room temperature overnight, and then concentrated under reduced pressure. Chromatography (silica gel, 1:1 ethyl acetate/petroleum ether) furnished **15a** (1.1 g, 78%) as colorless oil; mixture of rotamers. $[\alpha]_D^{25}$ −58.7 (c = 1 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.20 (dm, J_{HF} = 53.3 Hz, 1H), 4.56 and 4.42 (total 1H, each d, each J = 9.4 Hz, amide isomers), 3.89–3.58 (m, 5H), 2.53–2.44 (m, 2H), 1.49 and 1.41 (total 9H, s, each amide isomers); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 171.9, 153.9, 153.6, 92.2 (d, $^1J_{CF}$ = 177.8 Hz), 91.1, (d, $^1J_{CF}$ = 177.8 Hz), 80.4, 80.3, 57.6, 57.2, 53.2 (d, $^2J_{CF}$ = 24.5 Hz), 52.8 (d, $^2J_{CF}$ = 24.2 Hz), 52.3, 52.2, 37.4 (d, $^2J_{CF}$ = 22.0 Hz), 36.6 (d, $^2J_{CF}$ = 22.0 Hz), 29.7, 28.3, 28.2. {NMR data are consistent with those reported [29]}.

4.2. 1-tert-Butyl 2-methyl (2S,4S)-4-hydroxy-1,2-pyrrolidinedicarboxylate (15b)

Compound **15b** was synthesized from **13b** [24] according to procedure for **15a** (4.1 g, 84%) as colourless oil; $[\alpha]_D^{25}$–77.2 (c = 1 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.21 (dm, J_{HF} = 52.3 Hz, 1H), 4.46–4.38 (m, 1H), 3.95–3.56 (m, 5H), 2.61–2.58 (m, H), 2.16–2.04



Scheme 3. Synthesis of 6(*R*)- and 6(*S*)-fluoropenibruguieramines. Conditions: a: **10a**/**10b** (1.0 equiv), HATU (1.1equiv), DIPEA (2.9 equiv), DMF, rt, 18 h; b: EtONa (5equiv), EtOH, rt, 24 h; c: i) TFA (45 equiv), CH_2Cl_2; ii) BOP (1.1equiv), DIPEA (1.5equiv), THF, 16 h; then $NaBH_4$, 0 °C to rt, 1 h. HATU = [Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium 3-oxid hexafluorophosphate; BOP = benzotriazolyloxy-tris(dimethylamino)].

(m,1H), 1.47 and 1.42 (total 9H, s, each amide isomers); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.1, 172.9, 154.1, 153.5, 91.8 (d, $^1J_{CF}$ = 178.6 Hz), 91.0 (d, $^1J_{CF}$ = 178.6 Hz), 80.5, 77.4, 77.1, 76.8, 57.6, 57.3, 53.2 (d, $^2J_{CF}$ = 23.0 Hz), 52.9 (d, $^2J_{CF}$ = 23.3 Hz), 52.3, 52.1, 37.5 (d, $^2J_{CF}$ = 22.8 Hz), 36.6 (d, $^2J_{CF}$ = 22.8 Hz), 28.3, 28.2. {NMR data are consistent with those reported [30]}.

4.3. tert-butyl (2S,4S)-4-fluoropyrrolidine-2-carboxylate (10a)

Compound **15a** (1.89 g, 7.64 mmol) was dissolved in 10 mL of 2N HCl. The resulting solution was heated under reflux for 2–4 h. After the reaction was complete (as monitored by TLC), the solution was decolorized with charcoal while still hot, and filtered through a pad of celite. Water was removed by rotary evaporation and further dried with azeotropic distillation with dry toluene under reduced pressure. Recrystallization (1:1 ethyl acetate/petroleum ether) afforded the hydrochloride salt of **11a** (1.1 g, 85%) as a white solid. To a suspension of compound **11a** (0.945 g, 5.57 mmol) and ^{t}BuOAc (0.5 mL) was added $HClO_4$ (70%, 0.17 mL, 0.35 equiv) and the solution was stirred for 24 h at room temperature. It was poured into a saturated aqueous $NaHCO_3$ solution (25 mL). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure to afford the title compound **10a** (885 mg, 88%). colourless oil; $[\alpha]_D^{25}$ 8.9 (c = 0.45, $CHCl_3$); IR (cm^{-1}): 525, 660, 898, 1095, 1238, 1662, 1739, 2854, 3074, 3195; ^{1}H NMR (400 MHz, CD_3OD): δ 5.22 (dm, J_{HF} = 52.9 Hz, 1H), 4.26–4.23 (m, 1H), 3.53 (ddd, J = 20.3 Hz, 13.6 Hz, 2.0 Hz, 1H), 3.29 (ddd, J = 36.1 Hz, 13.6 Hz, 3.6 Hz, 1H), 2.57–2.35 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD): δ 169.8, 92.2 (d, $^1J_{CF}$ = 179.4 Hz), 84.5, 59.4, 53.5 (d, $^2J_{CF}$ = 23.4 Hz), 36.9 (d, $^2J_{CF}$ = 20.9 Hz), 28.0, 27.9, 27.8; ^{19}F NMR (376 MHz, $CDCl_3$): δ (−173.7) – (−174.1) (m, 1F); ^{19}F {^{1}H} NMR (376 MHz, $CDCl_3$): δ −173.9 (s, 1F); HRMS (ESI): m/z calcd for $C_9H_{17}FNO_2^+$ $[M+H]^+$ 190.1238, found 190.1236.

4.4. tert-butyl (2S,4R)-4-fluoropyrrolidine-2-carboxylate (10b)

Compound **10b** was synthesized according to the procedure for **10a** (1.6 g, 78%). colourless oil; $[\alpha]_D^{25}$3.8 (c = 0.175, $CHCl_3$); **IR** (cm^{-1}): 668, 968, 1076, 1262, 1668, 1733, 728, 3195; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.24 (dm, J_{HF} = 54.1 Hz, 1H), 3.97 (t, J = 8.1 Hz, 1H), 3.33–3.16 (m, 2H), 2.50–2.38 (m, 1H), 2.05–2.01 (m, 2H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.9, 94.1 (d, $^1J_{CF}$ = 179.4 Hz), 81.9, 58.7, 52.9 (d, $^2J_{CF}$ = 23.3 Hz), 37.7 (d, $^2J_{CF}$ = 22.5 Hz), 28.0; ^{19}F NMR (376 MHz, $CDCl_3$): δ (−175.4) – (−175.7) (m, 1F); ^{19}F {^{1}H} NMR (376 MHz, $CDCl_3$): δ −175.4 (s, 1F); HRMS (ESI): m/z calcd for $C_9H_{17}FNO_2^+$ $[M+H]^+$ 190.1238, found 190.1236.

4.5. tert-butyl (2S,4S)-4-fluoro-1-((E)-2-methyl-3-oxodec-8-enoyl) pyrrolidine-2-carboxylate (4)

To a solution of acid **24** [6] (120 mg, 0.60 mmol) in dry DMF (20 mL) was added HATU (250 mg, 0.66 mmol). After stirring for 10 min, **10a** (113 mg, 0.6 mmol) was added and the mixture was stirred at room temperature for 20 min. To this mixture was added DIPEA (0.28 mL, 1.74 mmol) and stirring was continued for 24 h. The solution was washed with brine and distilled water, extracted with ethyl acetate. The combined organic extracts were dried over magnesium sulphate and evaporated in vacuo to give a 1.2:1 diastereomers (101 mg, 46%) as a white solid. m.p.: 73–75 °C; $[\alpha]_D^{25}$-95.8 (c = 0.19, CH_3OH); IR (cm^{-1}): 962, 1014, 1057, 1153, 1222, 1632; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 5.39–5.36 (m, 3.3H), 5.37 (dm, J_{HF} = 51.7 Hz, 1.7H), 4.47 (d, J = 9.9 Hz, 1H), 3.97–3.84 (m, 2H), 3.82–3.65 (m, 2H), 3.62–3.48 (m, 1H), 2.64–2.53 (m, 1H), 2.53–2.50 (m, 4H), 2.48–2.43 (m, 3H), 2.39 (dd, J = 9.3 Hz, 5.2 Hz, 1H), 2.23 (ddd, J = 19.8 Hz, 14.6 Hz, 5.4 Hz, 1H), 1.94–1.89 (m, 3H), 1.60–1.59 (m, 5H), 1.47–1.43 (m, 1H), 1.42–1.41 (m, 7H), 1.38–1.36 (m, 11H), 1.30–1.20 (m, 5H), 1.21–1.14 (m, 3H), 1.12–1.09 (m, 3H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ 206.8, 206.5, 206.2, 170.6, 170.0, 169.9, 169.6, 169.6, 131.6, 131.5, 131.5, 125.0, 124.9, 93.1 (d, $^1J_{CF}$ = 174.7 Hz), 91.8 (d, $^1J_{CF}$ = 174.7 Hz), 82.1, 82.0, 81.1, 80.9, 58.8, 58.1, 58.0, 54.2 (d, $^2J_{CF}$ = 23.4 Hz), 54.0 (d, $^2J_{CF}$ = 23.2 Hz), 51.3, 51.2, 36.1 (d, $^2J_{CF}$ = 21.3 Hz), 32.3, 32.3, 32.1, 28.9, 28.8, 28.8, 28.0, 28.0, 27.9, 23.2, 23.0, 22.9, 22.8, 18.2, 13.8, 13.2, 13.0, 12.6; ^{19}F NMR (376 MHz, $(CD_3)_2SO$): δ (−171.0)–(−171.4) (m, 1F); ^{19}F {^{1}H} NMR (376 MHz, $(CD_3)_2SO$): δ −171.20 (s, 1F), −171.26 (s, 1F), −171.54 (s, 1F), −171.92 (s, 1F); HRMS (ESI): m/z calcd for $C_{20}H_{32}NFO_4Na^+$ $[M+Na]^+$ 392.2208, found 392.2206.

4.6. (2R,4R)-tert-butyl 4-fluoro-1-((E)-2-methyl-3-oxodec-8-enoyl) pyrrolidine-2-carboxylate (5)

To a solution of acid **24** [6] (438 mg, 2.2 mmol) in dry DMF (30 mL) was added HATU (912 mg, 2.4 mmol). After stirring for 10 min, **10b** (416 mg, 2.2 mmol) was added and the mixture was stirred at room temperature for 20 min. To this mixture was added DIPEA (0.99 mL, 6.6 mmol), and stirred for 24 h. The solution was washed with brine and distilled water, extracted with ethyl acetate. The combined organic extracts were dried over magnesium sulphate and evaporated in vacuo to give a 1.7:1 diastereomers (270 mg, 37%) as a colourless oil. $[\alpha]_D^{25}$ −44.4 (c = 0.34, $CHCl_3$); IR (cm^{-1}): 771, 846, 964, 1081, 1189, 1209, 1367, 1457, 1653, 2340, 2853, 2923; ^{1}H NMR (400 MHz, CD_3OD): δ 5.45–5.41 (m, 2.9H), 5.38 (dm, J_{HF} = 55.1 Hz, 1.5H), 4.46–4.39 (m, 1H), 4.15–4.01 (m, 2H), 3.93–3.88 (m, 1H), 3.86–3.82 (m, 1H), 3.81–3.76 (m, 1H), 2.70–2.60 (m, 2H), 2.58–2.48 (m, 2H), 2.24–2.16 (m, 1H), 2.14–2.05 (m, 1H), 2.14–2.05 (m, 1H), 2.04–1.94 (m, 3H), 1.65–1.63 (m, 4H), 1.61–1.54 (m, 3H), 1.53–1.52 (m, 2H), 1.50–1.48 (m, 11H), 1.39–1.34 (m, 3H), 1.31 (d, J = 6.9 Hz, 3H), 1.28 (d, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ 206.5, 205.9, 170.8, 170.8, 170.7, 170.5, 130.9, 130.8, 130.7, 124.6, 124.5, 92.0 (d, $^1J_{CF}$ = 178.1 Hz), 92.0 (d, $^1J_{CF}$ = 178.1 Hz), 81.7, 81.7, 58.6, 58.6, 54.0 (d, $^2J_{CF}$ = 22.5 Hz), 53.8 (d, $^2J_{CF}$ = 22.6 Hz), 51.8, 51.6, 40.0, 39.9, 35.4 (d, $^2J_{CF}$ = 22.4 Hz), 31.9, 31.9, 28.7, 28.6, 26.8, 26.8, 26.8, 22.7, 22.6, 16.7, 11.7, 11.4; ^{19}F NMR (376 MHz, CD_3OD): δ (−178.8)–(−179.5) (m, 1F) ^{19}F {^{1}H} NMR (376 MHz, CD_3OD): δ −179.1 (s, 1F), −179.1 (s, 1F), −179.3 (s, 1F), −179.4 (s, 1F); HRMS (ESI): m/z calcd for $C_{20}H_{32}NFO_4Na^+$, $[M+Na]^+$ 392.2208, found 392.2207.

4.7. tert-butyl (1R,2S,6S,7aR)-6-fluoro-1-((E)-hept-5-en-1-yl)-1-hydroxy-2-methyl-3-oxotetrahydro-1H-pyrrolizine-7a(5H)-carboxylate (7)

To a solution of **4** (257 mg, 0.69 mmol) in EtOH (3 mL) was added NaOEt (234 mg, 3.5 mmol), and the mixture was stirred for 9 h at room temperature. The reaction was quenched by the addition of a saturated NH_4Cl aqueous solution and extracted twice with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (hexane/EtOAc, 2:1) to afford **7** (163 mg, 65%) as a white crystalline solid; m.p.: 142–143 °C; $[\alpha]_D^{25}$ −48.9 (c = 0.5, CH_3OH); IR (cm^{-1}): 668, 759, 843, 964, 1016, 1153, 1284, 1368, 1430, 1669, 2361, 2854, 2931; ^{1}H NMR (400 MHz, CD_3OD): δ 5.47–5.45 (m, 2H), 5.42 (dm, J_{HF} = 52,8 Hz, 1H), 3.68 (ddd, J = 34.7 Hz, 13.6 Hz, 4.4 Hz, 1H), 3.33 (qd, J = 3.4 Hz, 2.2 Hz, 1.6 Hz, 1H), 3.31–3.24 (m, 1H), 2.91 (q, J = 7.1 Hz, 1H), 2.62 (dd, J = 18.3 Hz, 14.4 Hz, 1H), 2.46 (ddd, J = 42.5 Hz, 14.4 Hz, 4.1 Hz, 1H), 2.06 (td, J = 7.5 Hz, 4.7 Hz, 2H), 1.75–1.69 (m, 1H), 1.67–1.65 (m, 3H), 1.51 (s, 9H), 1.38–1.36 (m, 2H), 1.34–1.31 (m, 2H), 1.03 (d, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ 176.6, 170.8, 131.0, 124.5, 95.6 (d, $^1J_{CF}$ = 174.7 Hz), 82.5, 81.3, 79.9, 49.6, 48.5 (d, $^2J_{CF}$ = 25.6 Hz), 35.2 (d, $^2J_{CF}$ = 20.8 Hz), 32.3, 30.1, 26.7, 22.7, 16.7, 6.2; ^{19}F NMR (376 MHz, CD_3OD): δ −169.93 (ddddd, J = 52.8 Hz, 42.3 Hz, 34.7 Hz, 27.6 Hz, 18.3 Hz, 1F); ^{19}F {^{1}H} NMR (376 MHz,

CD_3OD): δ −169.93 (s, 1F); HRMS (ESI): *m/z* calcd for $C_{20}H_{32}NFO_4Na^+$, $[M+Na]^+$ 392.2208, found 392.2207.

4.8. (1R,2S,6S,7aS)-6-fluoro-1-((E)-hept-5-en-1-yl)-1-hydroxy-7a-(hydroxymethyl)-2-methylhexahydro-3H-pyrrolizin-3-one **(2)**

To a solution of 7 (180 mg, 0.49 mmol) in CH_2Cl_2 (5.0 mL) was added trifluoroacetic acid (1.6 mL) at 0 °C, and the mixture was stirred for 16 h at room temperature. The reaction mixture was concentrated in vacuo to afford crude free acid **27** as a white solid, which was used in the subsequent step. m.p.: 213–215 °C; $[\alpha]_D^{25}$ −27.4 (*c* = 0.32, CH_3OH); IR (cm^{-1}): 668, 763, 896, 993, 1039, 1143, 1332, 1405, 1700, 2342, 2521, 2856; ^1H NMR (400 MHz, CD_3OD): δ 5.46–5.43 (m, 2H, H15, H16), 5.34 (dm, J_{HF} = 54.8 Hz, 1H, H1), 3.85 (dd, *J* = 21.9 Hz, 13.4 Hz, 1H), 3.18 (ddd, *J* = 28.9 Hz, 13.3 Hz, 4.6 Hz, 1H), 2.96 (q, *J* = 7.2 Hz, 1H), 2.80–2.75 (m, 1H), 2.71 (dd, *J* = 18.8 Hz, 4.5 Hz, 1H), 2.02–1.97 (m, 2H), 1.78–1.70 (m, 2H), 1.65 (d, *J* = 3.7 Hz, 3H), 1.59–1.46 (m, 3H), 1.40–1.29 (m, 3H), 1.11 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ 176.6, 173.8, 131.0, 124.3, 94.0 (d, $^1J_{CF}$ = 182.2 Hz), 81.1, 79.2, 48.5 (d, $^2J_{CF}$ = 32.5 Hz), 35.4, 34.7 (d, $^2J_{CF}$ = 23.4 Hz), 32.1, 29.9, 22.7, 16.7, 6.5; ^{19}F NMR (376 MHz, CD_3OD): δ −174.5 (m, 1F); ^{19}F {^1H} NMR (376 MHz, CD_3OD): δ −174.5 (s, 1F); HRMS (ESI): *m/z* calcd for $C_{16}H_{25}NFO_4^+$, $[M+H]^+$ 314.1762, found 314.1762. To a solution of crude acid in THF (2.0 mL) was added benzotriazol-1-yloxytris (dimethylamino) phosphonium hexafluorophosphate (BOP, 150 mg, 0.35 mmol) and diisopropylethyl amine (30.0 μL, 0.48 mmol) at room temperature. The resulting solution was stirred for 10 min, and then $NaBH_4$ (60.0 mg, 1.60 mmol) was added in portions at 0 °C. After 1 h of stirring at room temperature, the reaction was quenched by the addition of a saturated NH_4Cl aqueous solution and was extracted four times with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 20:1) to yield **2** (93.0 mg, 81%) as a white solid. m.p.: 189–190 °C; $[\alpha]_D^{25}$-8.3 (*c* = 0.5, CH_3OH); IR(cm^{-1}): 860, 964, 1049, 1299, 1635, 2199, 3422; ^1H NMR (400 MHz, CD_3OD): δ 5.48–5.45 (m, 2H), 5.32 (dm, J_{HF} = 55.5 Hz, 1H), 3.94 (ddd, *J* = 23.9 Hz, 13.4 Hz, 6.0 Hz, 1H), 3.80 (d, *J* = 12.2 Hz, 1H), 3.68 (d, *J* = 12.2 Hz, 1H), 3.37–3.31 (m, 1H), 3.25 (ddd, *J* = 24.4 Hz, 12.1 Hz, 2.3 Hz, 1H), 3.11 (q, *J* = 7.3 Hz, 1H), 2.75–2.62 (m, 2H), 2.06–2.01 (m, 1H), 1.85–1.80 (m, 1H), 1.78–1.76 (m, 1H), 1.71 (dt, *J* = 8.1 Hz, 3.4 Hz, 1H), 1.66 (dq, *J* = 2.5 Hz, 1.2 Hz, 3H), 1.63–1.56 (m, 1H), 1.42 (q, *J* = 7.4 Hz, 1H), 1.33–1.24 (m, 2H), 1.02 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ 178.2, 131.1, 124.5, 94.5 (d, $^1J_{CF}$ = 177.6 Hz), 81.1, 76.2, 63.6, 49.4, 48.6 (d, $^2J_{CF}$ = 27.5 Hz), 33.6 (d, $^2J_{CF}$ = 20.0 Hz), 33.4, 32.1, 29.9, 23.0, 16.7, 6.2; ^{19}F NMR (376 MHz, CD_3OD): δ −167.1 (tt, *J* = 31.6 Hz, 24.6 Hz, 1F); ^{19}F {^1H} NMR (376 MHz, CD_3OD): δ −167.1 (s, 1F); HRMS (ESI): *m/z* calcd for $C_{16}H_{26}NFO_3Na^+$, $[M+Na]^+$ 322.1789, found 322.1788.

4.9. tert-butyl (1R,2S,6R,7aR)-6-fluoro-1-((E)-hept-5-en-1-yl)-1-hydroxy-2-methyl-3-oxotetrahydro-1H-pyrrolizine-7a(5H)-carboxylate **(8)**

Compound **8** was synthesized according to the procedure for **7** from compound **5** (150 mg, 88%) as colourless oil; $[\alpha]_D^{25}$ −52.5 (*c* = 0.3, CH_3OH); IR (cm^{-1}): 758, 842, 965, 984, 1078, 1156, 1255, 1287, 2855, 2934, 3420; ^1H NMR (400 MHz, CD_3OD): δ 5.50–5.44 (m, 2H), 5.31 (dm, J_{HF} = 53.2 Hz, 1H), 3.83 (dd, *J* = 21.9 Hz, 13.4 Hz, 1H), 3.14 (dddd, *J* = 28.4 Hz, 13.5 Hz, 4.9 Hz, 1.5 Hz, 1H), 2.90 (qd, *J* = 7.3 Hz, 1.3 Hz, 1H), 2.80–2.72 (m, 1H), 2.70–2.62 (m, 1H), 2.03 (td, *J* = 7.2 Hz, 4.1 Hz, 2H), 1.76–1.69 (m, 1H), 1.67–1.65 (m, 1H), 1.51 (s, 9H), 1.40–1.38 (m, 2H), 1.36–1.31 (m, 2H), 1.10 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ 176.4, 171.0, 130.9, 124.5, 93.9 (d,$^1J_{CF}$ = 182.5 Hz), 82.9, 81.0, 79.7, 48.8, 35.4, 34.7 (d, $^2J_{CF}$ = 23.4 Hz), 32.3, 301, 26.7, 22.6, 16.7, 6.5; ^{19}F NMR (376 MHz, CD_3OD): δ −174.7 (ddd, *J* = 60.4 Hz, 29.0 Hz, 23.1 Hz, 1F); ^{19}F {^1H} NMR (376 MHz, CD_3OD): δ −174.7 (s, 1F); HRMS (ESI): *m/z* calcd for $C_{20}H_{32}NFO_4Na^+$, $[M+Na]^+$ 392.2208, found 392.2204.

4.10. (1R,2S,6R,7aS)-6-Fluoro-1-((E)-hept-5-en-1-yl)-1-hydroxy-7a-(hydroxymethyl)-2-methylhexahydro-3H-pyrrolizin-3-one **(3):**

Compound **3** was synthesized from **26** according to the procedure for **2**. Data of **26:** 83% yield, white solid; m.p.: 158–160 °C; $[\alpha]_D^{25}$ −37.4 (*c* = 0.21, CH_3OH); IR (cm^{-1}): 643, 792, 897, 990, 1190, 1208, 1284, 1336, 1463, 1718, 2604, 2853; ^1H NMR (400 MHz, CD_3OD): δ 5.38–5.32 (m, 2H), 5.29 (dm, J_{HF} = 53.0 Hz, 1H), 3.60 (ddd, *J* = 33.9 Hz, 13.5 Hz, 4.5 Hz, 1H), 3.24–3.14 (m, 2H), 2.86 (q, *J* = 7.2 Hz, 1H), 2.54 (dd, *J* = 18.9 Hz, 14.3 Hz, 1H), 2.38 (dd, *J* = 41.4 Hz, 14.3 Hz, 1H), 1.64–1.59 (m, 1H), 1.54 (d, *J* = 3.3 Hz, 3H), 1.45–1.37 (m, 3H), 1.24–1.20 (m, 2H), 0.94 (d, *J* = 7.3 Hz, 3H); ^{13}CNMR (100 MHz, CD_3OD): δ 179.1, 175.9, 133.3, 126.5, 97.6 (d, $^1J_{CF}$ = 174.5 Hz,), 83.7, 81.5, 51.5, 50.8 (d, $^2J_{CF}$ = 25.6 Hz), 37.4, 39.2 (d, $^2J_{CF}$ = 20.6 Hz), 34.3, 32.1, 24.9, 18.9, 8.5; ^{19}F NMR (376 MHz, CD_3OD): δ −170.1 (dtdd, *J* = 52.1 Hz, 34.2 Hz, 15.1 Hz, 8.7 Hz, 1F); ^{19}F {^1H} NMR (376 MHz, CD_3OD),: δ −170.1 (s, 1F); HRMS (ESI): *m/z* calcd for $C_{16}H_{25}NFO_4^+$, $[M+H]^+$ 314.1762, found 314.1762. Data of **3**: (43 mg, 92%) as a white solid; m.p.: 162–163 °C; $[\alpha]_D^{25}$ −12.4 (*c* = 0.6, CH_3OH); IR (cm^{-1}): 559, 601, 651, 724, 837, 893, 966, 997, 1055, 1101, 1136, 1168, 1250, 2851, 2985; ^1H NMR (400 MHz, CD_3OD): δ 5.51–5.45 (m, 2H), 5.42 (dtt, *J* = 55.9 Hz, 5.3 Hz, 5.3 Hz, 1H), 3.82 (ddd, *J* = 23.7 Hz, 13.2 Hz, 1.5 Hz, 1H), 3.71 (d, *J* = 11.5 Hz, 1H), 3.48 (d, *J* = 11.6 Hz, 1H), 3.40 (ddd, *J* = 13.4 Hz, 5.0 Hz, 1.5 Hz, 1H), 3.34–3.32 (m, 1H), 2.94 (q, *J* = 7.24 Hz, 1H), 2.69–2.57 (m, 1H), 2.08–1.97 (m, 3H), 1.71–1.68 (m, 1H), 1.67–1.64 (m, 3H), 1.63–1.59 (m, 1H), 1.42–1.36 (m, 2H), 1.34–1.19 (m, 2H), 1.08 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ 176.8, 130.9, 124.6, 95.2 (d, *J* = 179.5 Hz), 80.3, 76.9, 64.6, 49.1 (d, *J* = 4.1 Hz), 48.9, 34.2 (d, *J* = 23.0 Hz), 33.4, 32.0, 29.8, 22.9, 16.9, 6.3; ^{19}F NMR (376 MHz, CD_3OD): δ −170.9 (dtt, *J* = 55.1 Hz, 30.5 Hz, 24.2 Hz, 1F); ^{19}F{^1H} NMR (376 MHz, CD_3OD): δ −170.9 (s, 1F); HRMS (ESI): *m/z* calcd for $C_{16}H_{26}NFO_3Na^+$, $[M+Na]^+$ 322.1789, found 322.1789.

4.11. Bioassay procedures [28]

Escherichia coli and Staphylococcus aureus were purchased from China Center for Type Culture Collection. The microplate bioassay (microdilution) was used to study the antimicrobial activities of PA and its fluorinated analogues towards *Escherichia coli*(1×10^8 cfu/mL) *and Staphylococcus aureus*(1×10^8 cfu/mL). Antibiotic solution of streptomycin was used in the presence of microorganisms for negative control. The microplate was aseptically sealed and incubated at 37 °C for 16 h. After agitation, microorganism growth was estimated by reading the absorbance in microplate wells at 410 nm with a microplate spectrophotometer (SynergyH1, Bio Tek). According to inhibition rate of different samples at various concentrations, the inhibition curve was drawn and half-inhibitory concentration value IC_{50} of the sample was calculated. Three repetitions were made for each experiment.

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