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RESEARCH ARTICLE

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The preparation of optically active epineoclausenamide and enantiomeric separation of its racemate

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Abstract

We synthesized the optically active epineoclausenamide by utilizing chiral reagents, such as *R*-α-methylbenzylamine and *S*-α-methylbenzylamine, for the resolution of the intermediate (*trans*-3-phenyl-oxiranecarboxylic acid **12**), followed by amide exchange, cyclization, and reduction, unlike previously reported methods. The Meerwein–Ponndorf–Verley reduction was used to asymmetrically reduce neoclausenamidone. A plausible reduction mechanism of this method was elucidated. Thereafter, high-performance liquid chromatography (HPLC) was investigated for the resolution of the epineoclausenamide enantiomers. HPLC was also used to determine the optical purity of these isomers. Two chiral stationary phases (CSPs) for separating the enantiomers were compared. Different mobile phase compositions were tested at 298.15 K. The results showed that the best separation was obtained when the mobile phase was composed of *n*-hexane and isopropanol (IPA) (75/25, v/v), the racemate was separated on a Chiralcel OJ-H column, and the flow rate was 1.0 mL/min at a wavelength of 210 nm and a temperature of 25°C. The enantiomeric ratio (e.r.) values of both the synthetic (−)-epineoclausenamide and (+)-epineoclausenamide were 1.3(+):98.7(−) and 99.3(+):0.7(−), respectively. In this study, a new synthetic route was designed with a yield of 12.3–14.1%, and a quick (8 min) effective separation method was obtained. This provides basis for pharmacological research and quality control of clausenamide analogues.

KEYWORDS

(−)-epineoclausenamide, (+)-epineoclausenamide, asymmetric synthesis, chemical resolution, enantiomeric resolution, HPLC

1 | INTRODUCTION

Epineoclausenamide is the 6-epimer of neoclausenamide. The relative configuration of the former is (3*R**, 4*S**, 5*R**, 6*R**) and of the latter is (3*R**, 4*S**, 5*R**, 6*S**) (Figure 1). Both compounds are the reduction products of neoclausenamidone. However, different reduction methods produce neoclausenamide and epineoclausenamide at different ratios. Among the reduction methods, the Meerwein–Ponndorf–Verley method can be used to stereoselectively synthesize racemic epineoclausenamide. However, its mechanism is not clear.[1] Reports have revealed that epineoclausenamide and its enantiomers induced a significant increase in glutathione

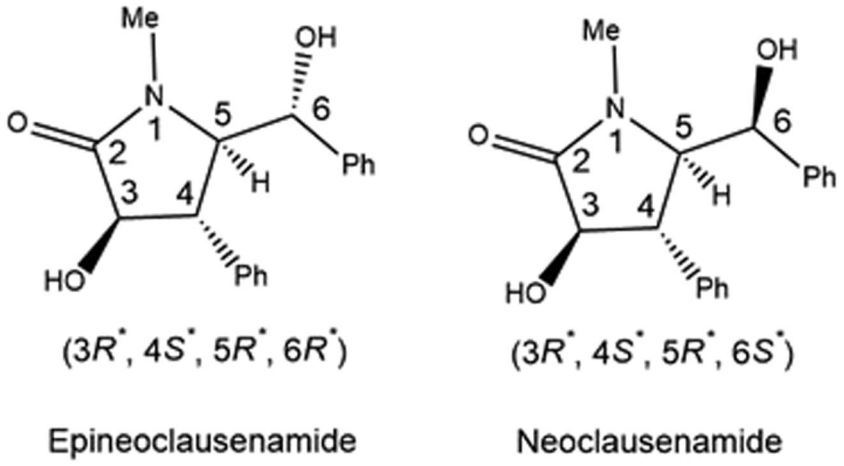

FIGURE 1 Structures of epineoclausenamide and neoclausenamide

transferase (GST) activity in mice, and (−)-epineoclausenamide showed the best activity.[2,3]

However, there have been few reports on the synthesis of optically active epineoclausenamide. One of the few published papers reports an interesting experiment by Luo and his coworkers[4] in which naturally occurring *N*-methyl-*N*-[(*Z*)-styryl]-3-phenyloxirane-2-carboxamide ((+)-SB204900)[5] was prepared by the CuI-catalyzed reaction of (2*S*,3*R*)-3-phenyloxirane-2-carboxamide with *Z*-styryl bromide, followed by *N*-methylation. (+)-SB204900 then underwent 5-endo cyclization to produce a mixture of neoclausenamide and its 6-epimer in sodium carbonate aqueous solution. Interestingly, the abovementioned mixture was oxidized by Jones' reagent to furnish (+)-neoclausenamidone **(+)-3** only. Hydrogenation of **(+)-3** afforded (−)-epineoclausenamide **(−)-4** in a reaction yield of 20%. In 2019, Zhouli et al.[6] asymmetrically synthesized (4*R*,5*S*)-5-benzoyl-1-methyl-4-phenylpyrrolidin-2-one via an *N*-heterocyclecarbene-catalyzed formal [3 + 2] annulation of bromoenals with α-amino ketones, followed by reduction and methylation. The 6-position carbonyl group of the thus-synthesized pyrrolidone was reduced by super hydride ($LiEt_3BH$), and the 3-position carbon was oxidized using a solution of hexamethyl phosphoric triamide and lithium diisopropylamide (LDA) in tetrahydrofuran (THF) at −70°C, adding trimethyl phosphite and oxygen after 1 h, to obtain **(−)-4**.[7]

The above examples illustrate that chiral catalysts need to be individually synthesized. The reaction conditions in each step are harsh, and the reaction will produce a single enantiomer, which is a disadvantage of these methods. Reported herein is a synthetic route towards the optically active epineoclausenamide, which enabled us to obtain (−)-epineoclausenamide and (+)-epineoclausenamide simultaneously. This route furnished pharmacological test samples for studying the three-dimensional structure–activity relationship of epineoclausenamide. Chiral reagents were employed to resolve cheap trans-cinnamic acid **11** as the starting material,[8] followed by cyclization by a phase transfer catalyst,[9] and the Meerwein–Ponndorf–Verley reduction.[1] The complete synthetic route is depicted in Figure 2.

Treatment of **11** with potassium peroxymonosulfate and $NaHCO_3$ in acetone in the presence of trace amounts of EDTA-2Na at 0–10°C afforded trans-3-phenyl oxiranecarboxylic acid sodium salt. The reaction liquid is acidified by HCl to pH 2–3 to obtain trans-3-phenyloxiranecarboxylic acid **12**.[8] Treatment of this compound with *R*-α-methylbenzylamine or *S*-α-methylbenzylamine in ethylacetate at 25°C afforded (2*S*,3*R*)-epoxycinnamate-(*R*)-α-methylbenzylamine salt **(+)-13** or (2*R*,3*S*)-epoxycinnamate-(*S*)-α-methylbenzylamine salt **(−)-13**.[8] The products, **(+)-13** and **(−)-13**, were acidified by HCl to pH 2–3 to obtain (2*S*,3*R*)-3-phenyloxirane-2-carboxylic acid **(+)-12** and (2*R*,3*S*)-3-phenyloxirane-2-carboxylic acid **(−)-12**. These products were then condensed with 2-(methylamino)-1-phenyl-1-ethanone hydrochloride to obtain chain amides **(+)-14** and **(−)-14** at 0–10°C.[8] The condensation products, **(+)-14** and **(−)-14**, were cyclized in a 3% aqueous solution of $N(CH_3)_4OH$ at 25°C to obtain (+)-neoclausenamide-3 and (−)-neoclausenamide-3. **(+)-3** and **(−)-3** were reduced with aluminum isopropoxide/IPA, and the produced acetone was distilled out at 85°C during the reaction to obtain (−)-epineoclausenamide-4 and (+)-epineoclausenamide-4.[1]

Due to the differences in the pharmacological activity of the two enantiomers, the HPLC resolution of (±)-epineoclausenamide was attempted to avoid the influence of invalid enantiomers on the pharmacological effects. The separation is essential for accurately determining the optical purity of the enantiomers according to various literature reports.[10,11] Many notation can expressing purity of enantiomeric mixtures. However, values of enantiomeric ratio (e.r.) has advantage for expressing selectivity in synthesis and is related to the

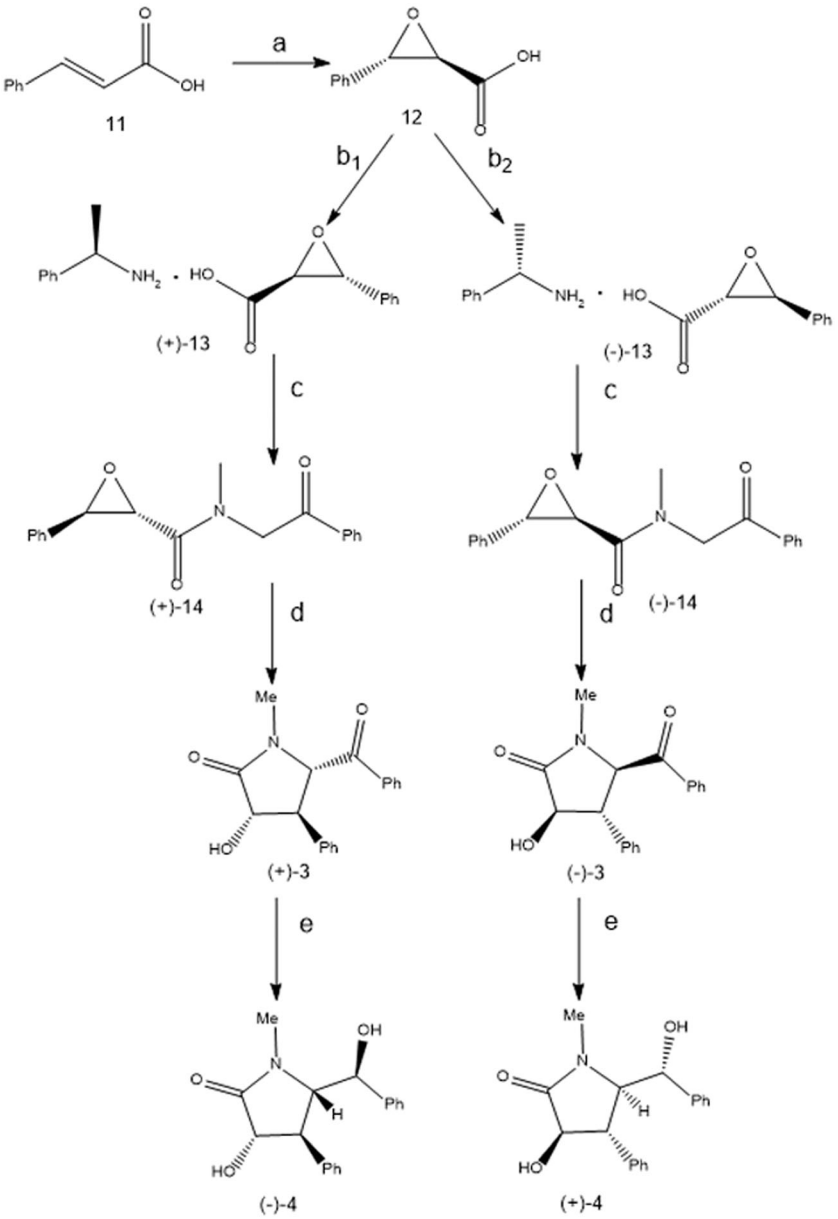

FIGURE 2 Synthesis of (−)-epineoclausenamide and (+)-epineoclausenamide. Reagents and conditions: (A) (i) Acetone, $NaHCO_3$, EDTA-2Na, $KHSO_5$, 0–10°C, 3 h; (ii) HCl, 0–10°C, 30 min; (B_1) ethylacetate, (*R*)-(+)-α-methylbenzylamine, 25°C, 3 h; (B_2) ethylacetate, (*S*)-(−)-α-methylbenzylamine, 25°C, 3 h; (C) (i) H_2O, DCM, HCl, 0°C; (ii) EDCI, HOBT, Et_3N, 2-methylamino-1-phenyl-1-ethanone hydrochloride, 0–10°C, 4 h. (D) DMC, 3% N $(CH_3)_4OH$, 25°C, 25 h; (E) Al(OPr-*i*)$_3$, anhydrous IPA, 85°C, 24 h

direct results of the chromatograms.[12] Therefore, we use e.r. to express the purity of enantiomeric purity.

In this study, two types of chiral stationary phases (CSPs), that is, amylose derivatized with *tris*-(3,-5-dimethylphenyl carbamate) and cellulose derivatized with *tris*-(4-methylbenzoate), were used to explore the optimal conditions for the separation of racemic epineoclausenamide, and both had a very good separation efficiency.[13]

2 | EXPERIMENTAL

2.1 | Materials and instruments

All the reagents were purchased from Aladdin and BCR and were used without further purification. Hexane, IPA, and isobutanol were of high-performance liquid chromatography (HPLC) grade, whereas all other reagents used were of AR grade. The racemic epineoclausenamide and 2-methylamino-1-phenyl-1-ethanone hydrochloride were prepared as previously reported.[14] Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 600-MHz spectrometer. The enantiomeric separations were conducted (and the e.r. values determined) on an HPLC system (model 1200 series, Agilent Technology) equipped with a quaternary pump, online degasser, autosampler, column oven, and diode array detector (DAD). Chiralpak AD-H (25 cm × 4.6 mm I. D., 5 μm) and Chiralcel OJ-H columns (25 cm × 4.6 mm I. D., 5 μm) were purchased from Daicel Chiral Technologies (China) Co. Ltd. Optical rotation was determined with a JASCO P1020 Polarimeter. High-resolution mass spectra (HRMS) were recorded on an AB SCIEX X500R mass spectrometer.

2.2 | Preparation of (−)-epineoclausenamide and (+)-epineoclausenamide

2.2.1 | General procedure for the synthesis of (2*S*,3*R*)-epoxycinnamate-(*R*)-α-methylbenzylamine salt and (2*R*,3*S*)-epoxycinnamate-(*S*)-α-methylbenzylamine salt ((+)-13 and (−)-13)

Compounds **(+)-13** and **(−)-13** were synthesized according to the method used in literature.[9] Figure 2 illustrates the reaction conditions and the reagents employed.

(+)-13: white solid (39.4% yield), m.p. 164–166°C. [α] 20 D = +125.8 (c = 1 in EtOH). ^{1}H NMR (600 MHz, DMSO-d_6), δ: 7.49–7.48 (m, 2H), 7.39 (t, J = 6.69 Hz, 2H), 7.35–7.26 (m, 6H), 4.31 (q, J = 6.69 Hz, 1H), 3.75 (d, J = 2.06 Hz, 1H), 3.16 (d, J = 2 Hz, 1H), 1.47 (d, J = 6.74 Hz, 3H). Enantiomeric ratio ((2*S*,3*R*)-3-phenyloxirane-2-carboxylic acid **(+)-12**): 96.5(+):3.5 (−), determined by HPLC (Daicel Chiralpak AD-H, *n*-hexane/IPA, 80:20 [v/v], flow rate 1.0 mL/min, λ = 254 nm): retention time: 4.990 min.

(−)-13: white solid (37.8% yield), m.p. 163–165°C. [α] D 20 = −124.7 (c = 1 in EtOH). ^{1}H NMR (600 MHz, DMSO-d_6), δ: 7.49–7.47 (m, 2H), 7.40–7.39 (t, J = 6.69 Hz, 2H), 7.35–7.26 (m, 6H), 4.30 (q, J = 6.69 Hz, 1H), 3.75 (d, J = 2.06 Hz, 1H), 3.15 (d, J = 2 Hz, 1H), 1.46 (d, J = 6.74, 3H). Enantiomeric ratio (((2*R*,3*S*)-3-phenyloxirane-2-carboxylic acid (−)-12): 0.1(+):99.9 (−), determined by HPLC (Daicel Chiralpak AD-H, *n*-hexane/IPA, 80:20 [v/v], flow rate 1.0 mL/min, λ = 254 nm): retention time: 8.141 min

2.2.2 | General procedure for the synthesis of (+)-*N*-methyl-*N*-benzoylmethyl-α,β-epoxy-β-phenylpropionamide and (−)-*N*-methyl-*N*-benzoylmethyl-α,β-epoxy-β-phenylpropionamide ((+)14 and (−)-14)

CH_2Cl_2 (250 mL) was added to a solution of (2*S*,3*R*)-epoxycinnamate-(*R*)-α-methylbenzylamine salt **(+)-13** (28.12 g, 98.6 mmol) or (2*R*,3*S*)-epoxycinnamate-(*S*)-α-methylbenzylamine salt **(−)-13** (27.4 g, 96.0 mmol) in deionized water (300 mL). The solution pH was adjusted to 3–4 using 1-M HCl at 0°C. The organic phase was isolated. The aqueous layer was then extracted with CH_2Cl_2 (3 × 150 mL). The combined organic phases were washed with saturated brine, dried over Na_2SO_4, and filtered. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (20.79 g, 108.5 mmol), 1-hydroxybenzotriazole (HOBT) (14.66 g, 108.5 mmol), and triethylamine (24.95 g, 246.5 mmol) were added in succession to the solution described above at 0°C. 2-(Methylamino)-1-phenyl-1-ethanone hydrochloride (18.25 g, 98.6 mmol) was added to the reaction solution in small quantities. The reaction mixture was stirred at 0–10°C and monitored by thin-layer chromatography. Upon completion of the reaction, water was added to the reaction mixture, and the mixture was stirred for 30 min. The organic phase was subjected to successive washes with saturated aqueous $NaHCO_3$ and brine. The organic phase was collected, dried over anhydrous Na_2SO_4, and concentrated by evaporation under reduced pressure. This was used in the next reaction. The concentrate was purified by column chromatography on silica gel using *n*-hexane/ethyl acetate (1:1, v/v) as an eluent to produce the desired amide compound **(+)14** or **(−)-14**.

(+)-14: yellow oil (79.6% yield), [α]D 20 = +161.2 (c = 1 in EtOH). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.97 (d, 2H), 7.63–7.60 (m, 1H), 7.50 (t, 2H), 7.41–7.36 (m, 2H), 5.04–4.78 (dd, J = 17.74, 2H), 4.13 (d, J = 2.07 Hz, 1H), 3.80 (d, J = 1.65 Hz, 1H), 3.21 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ: 193.55, 167.35, 135.58, 133.86, 128.98, 128.86, 128.72, 128.51, 128.00, 127.88, 125.81, 125.67, 57.84, 57.21, 54.40, 35.90. HRMS (ESI): *m*/*z* $[M + H]^+$, calculated: 296.1281, found: 296.1284. Enantiomeric ratio: 100.0(+):0(−), determined by HPLC (Daicel Chiralpak AD-H, *n*-hexane/isobutanol, 75:25 [v/v], flow rate 1.0 mL/min, λ = 254 nm): retention time: 16.658 min.

(−)-14: yellow oil (82.0% yield), [α]D 20 = −135.4 (c = 1 in EtOH). ^{1}H NMR (600 MHz, $CDCl_3$), δ: 7.95 (d, 2H), 7.60 (t, 3H), 7.48 (t, 3H), 7.37 (m, 5H), 4.89–4.77 (dd, J = 17.43 Hz, 2H), 4.12 (d, J = 2.06 Hz, 1H), 3.79 (d, J = 2.03 Hz, 1H), 3.21 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 193.54, 167.35, 134.96, 133.86, 128.99, 128.86, 128.71, 128.51, 128.00, 127.88, 125.81, 125.68, 57.85, 57.21, 54.38, 35.90. HRMS (ESI): *m*/*z* $[M + H]^+$, calculated: 296.1281, found: 296.1275. Enantiomeric ratio: 3.3 (+):96.7(−), determined by HPLC (Daicel Chiralpak AD-H, *n*-hexane/isobutanol, 75:25 [v/v], flow rate 1.0 mL/min, λ = 254 nm): retention time: 13.387 min.

2.2.3 | General procedure for the synthesis of (+)-neoclausenamidone and (−)-neoclausenamidone ((+)-3 and (−)-3)

A solution of the concentrate obtained above was prepared in CH_2Cl_2 (200 mL). To this solution, 3% aqueous $(CH_3)_4N^+OH^-$ (78 mmol, 100 mL) was added. The resulting solution was stirred at room temperature for 25 h. The organic phase was separated. The aqueous

layer was extracted with CH_2Cl_2(3 × 20 mL), and the combined CH_2Cl_2 phases were washed in succession with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (3:1, v/v) as an eluent to afford the desired product **(+)-3** or **(−)-3**. The e.r. values were determined in HPLC, and the configuration was assigned as **(+)-3** or **(−)-3** by using the same chromatography conditions as those reported in the literature.[15]

(+)-3: white solid (60.3% yield), m.p. 182.1–184.9°C. Enantiomeric ratio: 100.0(+):0(−), determined by HPLC (Daicel Chiralcel OJ-H, *n*-hexane/IPA 80:20 [v/v], flow rate 1.0 mL/min, λ = 254 nm): retention time: 20.275 min.

(−)-3: white solid (66.5% yield), m.p. 182.5–185.9°C. Enantiomeric ratio: 0(+):100.0(−), determined by HPLC (Daicel Chiralcel OJ-H, *n*-hexane/IPA 80:20 [v/v], flow rate 1.0 mL/min, λ = 254 nm): retention time: 14.488 min.

2.2.4 | General procedure for the synthesis of (−)-epineoclausenamide and (+)-epineoclausenamide ((−)-4 and (+)-4)

Aluminum isopropoxide (5.106 g, 25 mmol) and 0.5 mmol crude **(+)-3** or **(−)-3** were dissolved in anhydrous IPA (50 mL). The mixture was stirred at 85°C and monitored by thin-layer chromatography. During the reaction, IPA (about 20 mL) was added in appropriate intervals, and acetone was distilled out timely. The reaction was complete after 24 h. The solvent was removed at a reduced pressure. The residue was dissolved in water (50 mL) and then quenched by ammonia, thus adjusting the solution's pH to 11. This solution was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated under vacuum, obtaining the desired product.

(−)-4: white solid (81.5% yield), m.p. 220.5–221.3°C. [α]D 20 = −140 (c = 0.0125 in MeOH), ^{1}H NMR (600 MHz, DMSO-d_6), δ: 7.26–7.22 (m, 6H), 7.20–7.71 (m, 2H), 7.11–7.09 (d, 2H), 5.77 (d, J = 32.63 Hz, 2H), 4.59 (d, J = 4.39 Hz, 1H), 4.02 (d, J = 7.64 Hz, 1H), 3.86 (q, J = 4.52 Hz, 1H), 2.94 (t, J = 8.03 Hz, 1H), 2.70 (s, 3H); HRMS (ESI): *m/z* $[M + H]^+$, calculated: 298.1438, found: 298.1459.

(+)-4: white solid (86.3% yield), m.p. 220.2–220.9°C. [α]D 20 = +33.73 (c = 0.0075 in MeOH), ^{1}H NMR (600 MHz, DMSO-d_6), δ: 7.26–7.16 (m, 8H), 7.10–7.09 (m, 2H), 5.77 (d, J = 33.04 Hz, 2H), 4.59 (d, J = 4.44 Hz, 1H), 4.02 (d, 8.02 Hz, 1H), 3.86 (dd, J = 4.36 Hz, 1H), 2.94 (t, J = 7.54 Hz, 1H), 2.70 (s, 3H); HRMS (ESI): *m/z* $[M + H]^+$, calculated: 298.1438, found: 298.1438.

2.3 | Investigation of the enantioseparation of epineoclausenamide

2.3.1 | Sample preparation

A sample solution of 1.0 mg/mL^{-1} was prepared for the analysis. The samples were prepared by weighing 20 mg of (±)-epineoclausenamide, (−)-epineoclausenamide, and (−)-epineoclausenamide in a 20-mL volumetric flask; 10-mL IPA was added to the volumetric flask as a diluent. This solution was then sonicated for 5 min to completely dissolve the sample. Subsequently, the flask was filled with IPA to the 20-mL mark.

2.3.2 | Chromatography conditions

The mobile phase was a mixture of *n*-hexane and IPA. The detection wavelength was 210 nm, the flow rate was 1.0 mL/min, and the injection volume was 20 μL. Two CSPs, amylose derivatized with *tris*-(3,5-dimethylphenyl carbamate) (Chiralpak AD-H) and cellulose derivatized with *tris*-(4-methylbenzoate) (Chiralcel OJ-H), were evaluated. Different mobile phase compositions were tested for their separation efficiencies. Chromatography variables, such as the retention (*k*), separation (*α*), and resolution (*Rs*), were determined. 1,3,5-Tri (tert-butyl) benzene was used to calculate the dead time. The determination of the optical purities was performed by the best-performing separation method.

2.3.3 | Method validation

Specificity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and linearity were evaluated for validating based on a previous report.[16]

3 | RESULTS AND DISCUSSION

3.1 | Optimized synthetic route

Cinnamic acid **6** was oxidized and then combined with *R*-(+)-α-methylbenzylamine or *S*-(−)-α-methylbenzylamine to successfully isolate (2*S*,3*R*)-epoxycinnamic acid or (2*R*,3*S*)-epoxycinnamic acid. Using chiral α-methylbenzylamine was more effective in obtaining a single enantiomer. 2-(Methylamino)-1-phenyl-1-ethanone hydrochloride was involved in producing optically active *N*-methyl-*N*-benzoylmethyl-α,β-epoxy-β-phenylpropionamide **14**, and 14 was the key intermediate to obtaining pyrrolidone derivatives, as reported in the

literature.[17,18] The first three steps in our synthetic route gave high yields, with no need for purification through column chromatography. Tetramethylammonium hydroxide was used as a base for cyclization and phase transfer catalyst. Two isomers were produced, namely, (−)-clausenamidone and (+)-neoclausenamidone (+)-**3**, (or (+)-clausenamidone and (−)-neoclausenamidone (−)-**3**); the diastereoisomeric ratio was 1:3.5–5. Diastereoselective reduction of **(+)-3** or **(−)-3** by the Meerwein–Ponndorf–Verley method generated (−)-epineoclausenamide **(−)-4** (or **(+)-4**) in higher than 80% yield.

3.2 | Plausible mechanism of asymmetric reduction of Meerwein–Ponndorf–Verley method

The stereoselectivity of the aluminum alkoxide reduction is related to the structure of the substrate.[19] Considering (+)-neoclausenamidone as an example, we analyzed the process of the reduction reaction between (+)-neoclausenamidone-**3** and aluminum isopropoxide, as shown in Figure 3. The 3-position secondary hydroxyl group of **(+)-3** initially attacked aluminum isopropoxide to form a bridged cyclic transition state **15**, and subsequently, the 6-position carbonyl oxygen combined with the aluminum atom, forming a six-membered cyclic transition state **16** based on the bridged ring state, which limits the free rotation of the single bond between C_5 and C_6. The nonrotating carbon–carbon single bond is the key to stereoselectivity. Due to the uneven distribution of the electron cloud in the six-membered cyclic transition state and the electron migration under the influence of the electron-withdrawing center, the alcohol-aluminum derivative **17** was generated with the departure of acetone. The alcoholysis reaction of **17** produced (−)-epineoclausenamide **(−)-4**.

3.3 | Chromatographic data

The values of the separation parameters are shown in Table 1. The results suggested that the elution time, retention (k), and resolution decrease with the increase of the ratio of IPA to n-hexane in the mobile phase. It could be concluded that the interaction between the sample and the stationary phase weakened as the IPA content was increased. In summary, we have obtained the best conditions for chromatographic separation on Chiralcel OJ-H with 75/25 (v/v) n-hexane/IPA at 298.15 K.

3.4 | Validation of the HPLC method

3.4.1 | System suitability and specificity

HPLC suitability testing was performed using six replicate injections ($n = 6$) of the standard solution (1000 μg/mL). The tailing factor, relative standard deviation (RSD) of the peak area, and RSD of the retention time were deemed suitable, as shown in Table 2.

The specificity of the method was determined by searching for any interference from other peaks along with the main peaks. In order to determine the specificity, the eluent was injected onto the machine as a blank. No peak was observed from the blank solution, which indicated the specificity of the system.

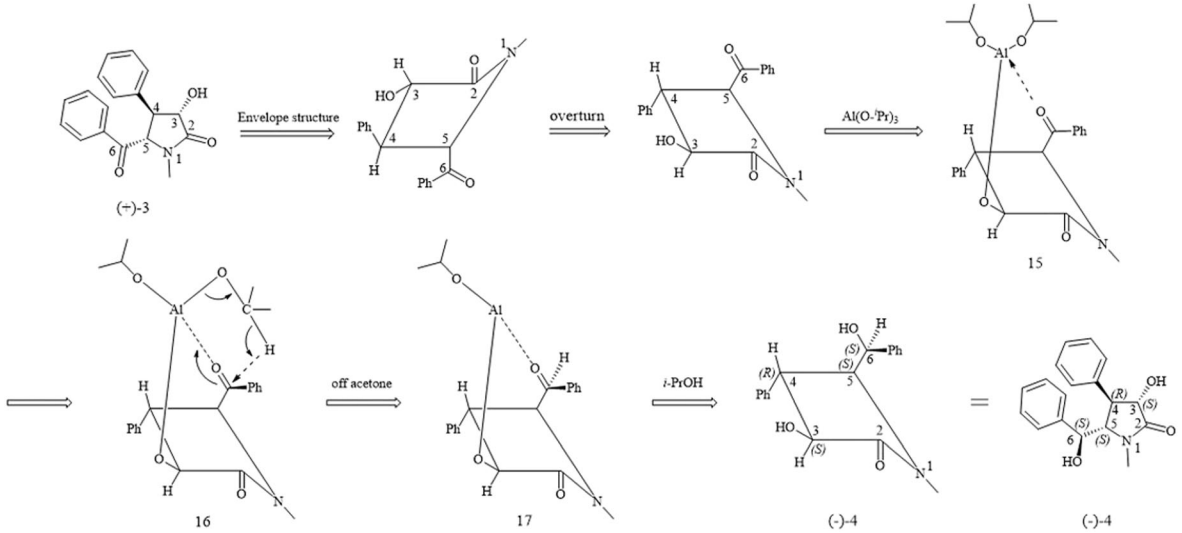

FIGURE 3 Plausible mechanism of the asymmetric reduction of **(+)-3**

TABLE 1 Results of the separation of racemic epineoclausenamide on Chiralcel OJ-H and Chiralpak AD-H

Compound	Column (CSP)	Mobile phase (v/v)	K_1	K_2	A	Rs
Racemic epineoclausenamide	Chiralcel OJ-H	*n*-hexane/IPA 90/10	3.20	4.51	1.41	4.11
		n-hexane/IPA 80/20	1.18	1.73	1.46	4.15
		n-hexane/IPA 75/25	0.85	1.27	1.50	3.45
	Chiralpak AD-H	*n*-hexane/IPA 90/10	5.36	6.86	1.28	6.72
		n-hexane/IPA 80/20	2.05	2.57	1.25	4.97
		n-hexane/IPA 75/25	1.24	1.59	1.27	3.90

Note: The temperature of the column was 298.15 K.
Abbreviations: CSP, chiral stationary phase; IPA, isopropanol.

TABLE 2 The results of the method validation

Method validation			*S*-epineoclausenamide	*R*-epineoclausenamide
System suitability	Tailing factor		0.86	0.73
	Resolution		-	2.06
	% RSD peak area		0.093	0.068
	% RSD retention time		0.139	0.195
Linearity	Slope		28,939	28,894
	Y-intercept		1145.6	799.94
	Regression coeff (r^2)		0.9997	0.9996
Limit of detection and quantification	LOD (μg/mL)		0.35	0.33
	LOQ (μg/mL)		1.1	0.97
Accuracy (percentage recovery data)	300 μg/mL		98.7%	99.1%
			98.5%	98.6%
			98.7%	98.7%
	600 μg/mL		101.5%	100.4%
			101.1%	100.0%
			101.6%	100.6%
	1000 μg/mL		100.4%	100.9%
			100.4%	100.9%
			100.5%	101.0%
Precision (%RSD)	200 μg/mL	A[a]	0.248	0.092
		T[a]	0.050	0.033
	400 μg/mL	A	0.138	0.304
		T	0.156	0.290
	800 (μg/mL)	A	0.120	0.099
		T	0.271	0.301

Abbreviation: RSD, relative standard deviation.
[a]A represents the peak area; T represents the retention time.

3.4.2 | Linearity, LOD, and LOQ

The linearity of the method was evaluated by regression analysis of the curve. The linearity values of the standardized peak areas versus concentrations for the two enantiomers were tested in the concentration range of 200–1000 μg/mL. The slopes, *y*-intercepts, and regression coefficients R^2 were calculated. These values are given in Table 2. The regression coefficients R^2 were 0.9996 and 0.9997 for the two enantiomers ($n = 6$), respectively.

The LOD and LOQ values were calculated at signal-to-noise ratios of 3 and 10, respectively. The LODs of (−)-epineoclausenamide and (+)-epineoclausenamide were 0.33 and 0.35 μg/mL, respectively. The LOQs of (−)-epineoclausenamide and (+)-epineoclausenamide were 0.97 and 1.1 μg/mL, respectively.

3.4.3 | Accuracy and precision

The accuracy was determined by calculating the recovery from the spike amounts. The percent recoveries were found to be in the range of 98.5–101.6% for the two enantiomers at three different spiking concentrations (300, 600, and 1000 μg/mL), as shown in Table 2.

The precision was determined by three concentrations of the enantiomers (200, 400, and 800 μg/mL) ($n = 3$). The RSDs of the retention times and peak area were 0.033–0.304, respectively, which indicated a suitably precise HPLC method.

3.5 | Determination of e.r.

It could be concluded that the best separation of racemic epineoclausenamide was achieved using *n*-hexane/IPA (75/25 v/v) at 1 mL/min^{-1} at 298.15 K using Chiralcel OJ-H according to Sections 3.3 and 3.4. Based on these conditions, racemic epineoclausenamide was used as the reference to determine the optical purities of (−)-epineoclausenamide and (+)-epineoclausenamide. The chromatograms are shown in Figure 4 and indicate that the e.r. values of optically active epineoclausenamide were 1.3(+):98.7(−) and 99.3(+):0.7(−) respectively.

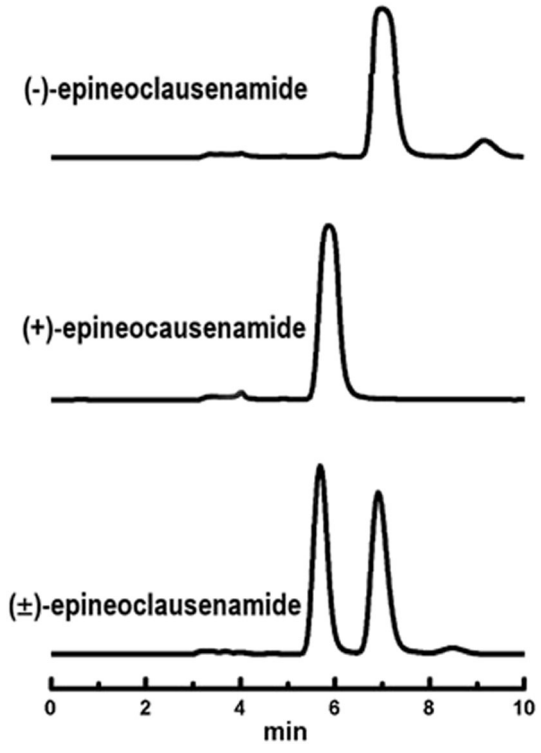

FIGURE 4 Chromatographic separation of epineoclausenamide on Chiralcel OJ-H

4 | CONCLUSION

The optically active epineoclausenamide was synthesized efficiently and concisely in acceptable-to-good yields (12.3–14.1%). The HPLC method of enantioseparations was performed on Chiralcel OJ-H with *n*-hexane/IPA (75/25 v/v) at 298.15 K. Under these chromatography conditions, the racemic epineoclausenamide was separated rapidly within 8 min, and e.r. values of (+)-epineoclausenamide or (−)-epineoclausenamide were higher than 97%. All method validation parameters showed acceptable results. Essentially, a new synthetic route has efficiently afforded optically active epineoclausenamide, and the best separation method has been achieved for its quality control. Our research still has some shortcomings. Two of the five-step reactions still require column chromatography for purification. The stereoselectivity of the cyclization reaction needs to be improved, and other base reagents need to be tried. There are still some deficiencies in the reduction method: this is a reversible reaction, and the operation is complicated, although it has good stereoselectivity. We will explore more stereoselective reduction methods of carbonyl in our subsequent research.

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DATA AVAILABILITY STATEMENT

Data that support the findings of this study are openly available in the supporting information of this article or are available from the corresponding author upon reasonable request.

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REFERENCES

1. Daofei H. *Synthesis of Neoclausenamide, Dehydroclausenamide and Their Optical Isomers and Derivatives[D]*. Peking Union Medical College; 1990.
2. Yuqun WU, Lide L, Hualing W, Gengtao L. Different effects of nine clausenamide enantiomers on liver glutathione biosynthesis and glutathione *S*-transferase activity in mice. *Acta Pharmacol Sin*. 2010;27(008):1024-1028.
3. Yuqun WU, Gengtao L. Protective effects of optically active clausenamide analogues on injured unprogrammed DNA synthesis in rat hepatocytes by aflatoxin B1. *J Toxicol Pharmacol*. 2006;20(5):393-398.

4. Luo Y, Dexian W, Qiyu Z, Jie P, Zhitang H, Meixiang W. Highly efficient and concise synthesis of both antipodes of SB204900, clausenamide, neoclausenamide, homoclausenamide and zeta-clausenamide. Implication of biosynthetic pathways of Clausena alkaloids. *Org Biomol Chem*. 2009;7(12):2628-2634.
5. Li SH, Wu SL, Li WS. Amides and coumarin from the leaves of Clausena lansium. *Zhongguo Yao Xue (Ying Wen Ban)*. 1996;48 (5):367-373.
6. Zhouli H, Ying Z, Zhenqian F, Wei H. Asymmetric synthesis of enantioenriched 6-hydroxyl butyrolactams promoted by *N*-heterocyclic carbene. *J Org Chem*. 2019;84(16):10328-10337.
7. Yingming P, Jinling L, Hengshan W. A process for preparing clausenamide: CN105017124 [P], 2015.
8. Hansen L, Junru M, Jingz X, Xuna L, Chengqiao F. Preparation method of optically active clausenamidone and their derivatives from trans-cinnamic acid: CN2018-11423524, 2018;11–26.
9. Hansen L. The synthesis of 3-hydroxy-4-phenyl-5-benzoyl-N-methyl-lactam. *Huaxi Yaoxue Zazhi*. 1999;04:260-262.
10. Abdul KKK, Jahfar N, Tajudheen KK, Zubair P, Binoy M, Michael S. Development and validation of a chiral LC-MS method for the enantiomeric resolution of (+) and (−)-medetomidine in equine plasma by using polysaccharide-based chiral stationary phases. *Chirality*. 2020;32(3):314-323.
11. Jiaxi L, Ruixia L, Liyang W, Hongjie G. Enantioseparation of chiral pharmaceuticals by vancomycin-bonded stationary phase and analysis of chiral recognition mechanism. *Chirality*. 2019;31(3):236-247.
12. Tiritan ME, Fernandes C, Maia AS, Pinto M, Cass QB. Enantiomeric ratios: why so many notations? *J Chromatogr A*. 2018; 1569:1-7. https://doi.org/10.1016/j.chroma.2018.07.039
13. Ianni F, Scorzoni F, Gentili PL, et al. Chiral separation of helical chromenes with chloromethyl phenylcarbamate polysaccharide-based stationary phases. *J Sep Sci*. 2018;41(6): 1266-1273.
14. Yu R, Jinlong S, Guantao H, Yixiao Y, Senmei Z, Hansen L. Synthesis of 2-methylamino-1-phenyl-ethanone hydrochloride. *Huaxue Yanjiu Yu Yingyong*. 2020;32(10):1911-1914.
15. Xuna L, Chengqiao F, Mi J, Jingzi X, Hansen L. Enantiomeric resolution, thermodynamic parameters, and modeling of clausenamidone and neoclausenamidone on polysaccharide-based chiral stationary phases. *Chirality*. 2019;31(6):423-433.
16. Zhiyan L, Kangzi R, Ye F, Tommy U, Scott K, Hong Y. Rapid and economic determination of 13 steviol glycosides in market-available food, dietary supplements, and ingredients: single-laboratory validation of an HPLC method. *J Agric Food Chem*. 2020;68:10142-10148.
17. Xingzhou L, Chuangjiang Z, Changhui L, Kemei W, Daofei H, Liang H. Synthesis of *N*-substituted Clausenamide analogues. *Eur J Med Chem*. 2010;45(11):5531-5538.
18. Erchang R, Hao H, Jiachong C, Guangzhong Y, Hansen L, Liang H. Total synthesis of clausenamide. *Chin Chem Lett*. 1994;5(4):267-268.
19. Ren W. *Organic Reactions for Drug Synthesis*. Beijing: Chemical Industry Press; 2017:304-305.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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