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# Total Synthesis of Quinolactacin-H from Marine-derived *Penicillium* sp. ENP701 and Biological Activities

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## General experimental Information – Chemistry

All solvents were purified and dried according to standard methods prior to use. All other reagents were used without purification as commercially available. All reactions were carried out in oven-dried glassware with magnetic stirring under air unless stated otherwise. All reactions were monitored by thin layer chromatography (TLC), column chromatography purification was carried out using silica gel. 4Å molecular sieves were activated at 400 °C for 5 h. $^{1}$H NMR spectra were recorded on Bruker Avance Ⅲ 500 MHz or 600 MHz spectrometer and $^{13}$C NMR spectra were recorded on 125 MHz or 150 MHz spectrometer in $CDCl_3$, $CD_3OD$ or in DMSO-d6 using tetramethylsilane (TMS) as internal standard. HR-ESI-MS were performed on a Thermo Scientific ITQ 1100 TM spectrometer, equipped with an electrospray ionization interface (ESI). Analytical HPLC was recorded on a HPLC machine equipped with Agilent 1200 HPLC system with a UV diode array detector or Shimadzu LC-20AT with a UV/VIS detector. The chiral stationary phase was Daicel Chiralcel AD-H and OD-H column. Optical rotations were determined using a Rudolph Autopol V polarimeter at 20°C or 25 °C. IR spectra were measured on a Nicolet 5DX-FT-IR spectrometer using a KBr piece.

## Separation of natural products and characterization data

Fungal material and culture conditions: *Penicillium* sp. ENP701 isolated from Seawater sample which was collected from the East China Sea north latitude 28°26', east longitude 121°60'. Large-scale cultures of *Penicillium* sp. ENP701 were performed in conical flask using GYT medium consisting of glucose (1%), peptone (0.5%), yeast extract (0.1%), and seawater (60%). Cultures (40 L) were incubated for 8 days at 28 °C on a rotary shaker at 200 rpm and then static for 22 days.

Extraction and isolation: The mycelium and culture fluid were separated by filtration. Freeze-dried mycelium was prepared for later treatment of MeOH extract and the filtrate was concentrated below 50 °C. 20 g of crude extract was obtained from 40 L of culture. The extract was analyzed by HPLC (solvents: MeCN/$H_2O$ =90/10; flow: 0.8 mL/min; 30 ℃). According to the result above, a crude organic extract was subjected to chromatography on RP-18 silica-gel column using gradient mixtures of MeOH-$H_2O$ (10:90 to 90:10) to obtain 9 fractions. The fractions of 7 were further chromatographically separated by HPLC and eluted with MeCN-$H_2O$ (90/10) to yield the Quinolactacin-H (20 mg).

Quinolactacin-H: $C_{34}H_{40}N_4O_4Mg$ (OH) Cl·$H_2O$, white acerate crystal. IRmax (KBr): 3440.96, 2964.49, 1684.89, 1606.58, 1526.53, 1456.00, 1420.99, 1384.33, 1265.75, 1225.87, 1177.16, 1119.96, 816.70, 768.99, 640.94, 531.56, 468.75cm$^{-1}$. UV max (MeOH): 245nm. HR-ESI-MS: $C_{17}H_{20}N_2O_2$, calcd for $[M+H]^+$ 285.1598; Found: 285.1586. The X-ray diffraction data were collected at 296 K on a Rigaku RAXIS-RAPID diffractometer with Mo Kα radiation (λ = 0.71073 Å). The structure was solved by direct methods using the SHELXS-97 program. $C_{34}H_{43}ClMgN_4O_6$, space group *P*-1, $a$ = 11.1374(6) Å, $b$ = 11.8740(6) Å, $c$ = 13.7441(9) Å, $\alpha$ = 84.977°, $\beta$ = 82.117°, $\gamma$ = 72.4380°, $V$ = 1714.45(17) Å$^3$, $Z$ = 2, ρCalcd = 1.352 g/cm$^3$, $\mu$ = 0.176 mm$^{-1}$, and $F$ (000) = 668. Crystal size: 0.32 × 0.22 × 0.18 mm$^3$. 13318 reflections in *h (−13/11), k (−14/13), l (−16/16)*, measured in the range 3.00° ≤ $\vartheta$ ≤ 25.01°, 5938 independent reflections, of which 4103 unique reflection with $I > 2\sigma(I)$ were collected for the analysis, $R_{int}$ = 0.0265. The final $R$ indices: $R_1$ = 0.1216, $wR_2$ = 0.2572 [I > 2σ(I)], R indices (all data): $R_1$ = 0.1498, $wR_2$ = 0.2686, and largest difference peak and hole: 1.052 and −0.414 e Å$^{-3}$. Crystallographic data of compound have been deposited at the Cambridge Crystallographic Data Centre (CCDC) under the reference number CCDC 1517245.

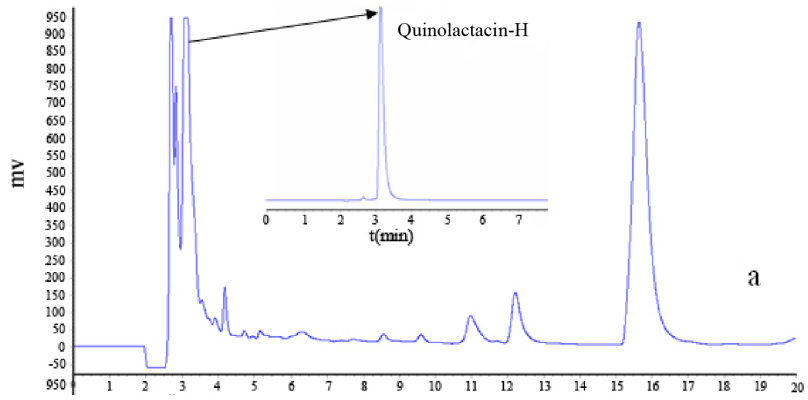



**Figure 1**. HPLC–DAD (210 nm) of MeOH extract of marine-derived fungus *Penicillium* sp.701

**Table 1.** Physico-chemical properties of compound

| Compound | |
|---|---|
| Appearance | White acerate crystal |
| MP | 208-210℃ (dec.) |
| Molecular formula | $C_{34}H_{40}N_4O_4Mg(OH)Cl{\cdot}H_2O$ |
| Molecular weight | 634.5 |
| HR- ESI-MS (*m/z*) | |
| Found: | 285.1586 $[C_{17}H_{21}N_2O_2]^+$ |
| Calcd.: | 285.1598 for $[C_{17}H_{21}N_2O_2]^+$ |
| UV $\lambda_{max}^{MeOH}$ nm | 245 |
| IR $\gamma_{max}^{KBr}$ ($cm^{-1}$) | 3440.96, 2964.49, 1684.89, 1606.58, 1526.53, 1456.00, 1420.99, 1384.33, 1265.75, 1225.87, 1177.16, 1119.96, 816.70, 768.99, 640.94, 531.56, 468.75 |

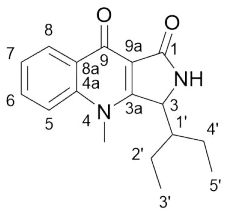


**Table 2**. $^{1}H$ and $^{13}C$ NMR chemical shift assignments of compound in $CD_3OD$

| | $^{13}C(\delta)$ | $^{1}H(\delta)$ |
|---|---|---|
| 1 | 172.01 | |
| 3 | 37.84 | 2.33(1H, d,J=2.5Hz) |
| 3a | 166.39 | |
| 4a | 143.08 | |
| 5 | 126.15 | 7.57(1H, d,J=2Hz) |
| 6 | 117.93 | 7.87(1H, dd,J=2Hz,1.5Hz) |
| 7 | 134.46 | 7.88(1H, dd,J=4Hz,1.5Hz) |
| 8 | 127.39 | 8.43(1H, d,J=4Hz) |
| 8a | 129.21 | |
| 9 | 175.16 | |
| 9a | 111.59 | |
| 4-Me | 36.99 | 3.94(3H, s) |
| 1’ | 18.10 | 1.26(1H, m) |
| 2’ | 29.05 | 1.53(1H, m), 1.69(1H, m) |
| 3’ | 12.00 | 0.76(3H, t, 7.5Hz) |
| 4’ | 12.33 | 1.14(2H, m) |
| 5’ | 11.94 | 0.57(3H, t, 6.5Hz) |

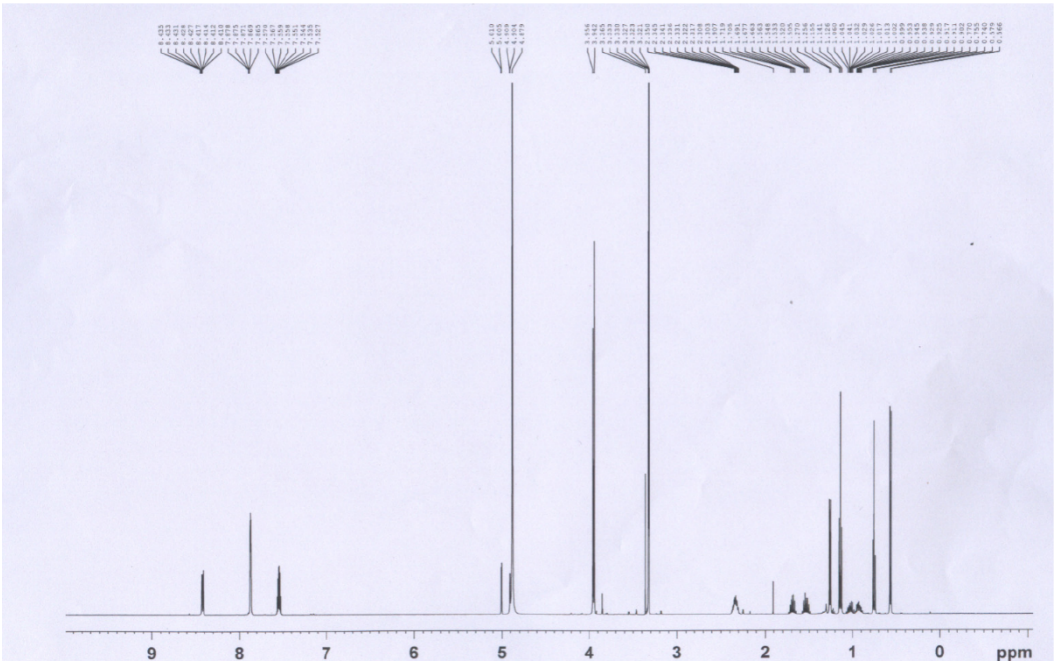



**Figure 2**. $^{1}H$ NMR Spectrum of compound recorded at 500 MHz in $CD_3OD$

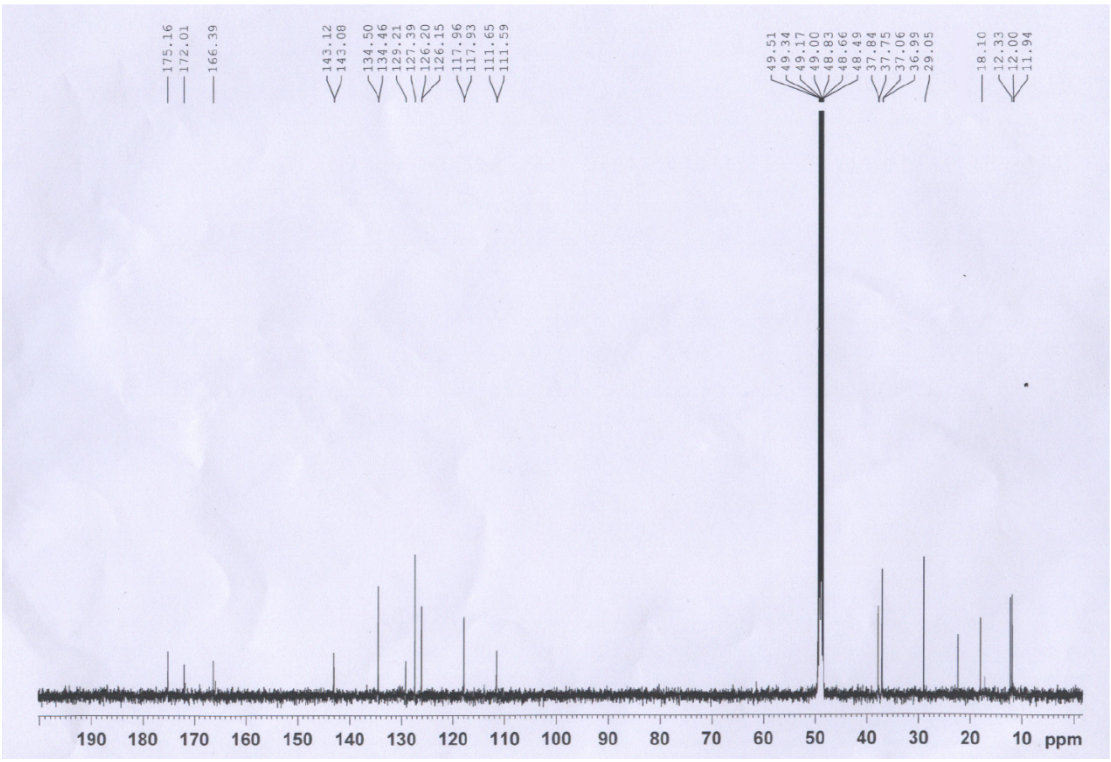



**Figure 3**. $^{13}C$ NMR Spectrum of compound recorded at 500 MHz in $CD_3OD$

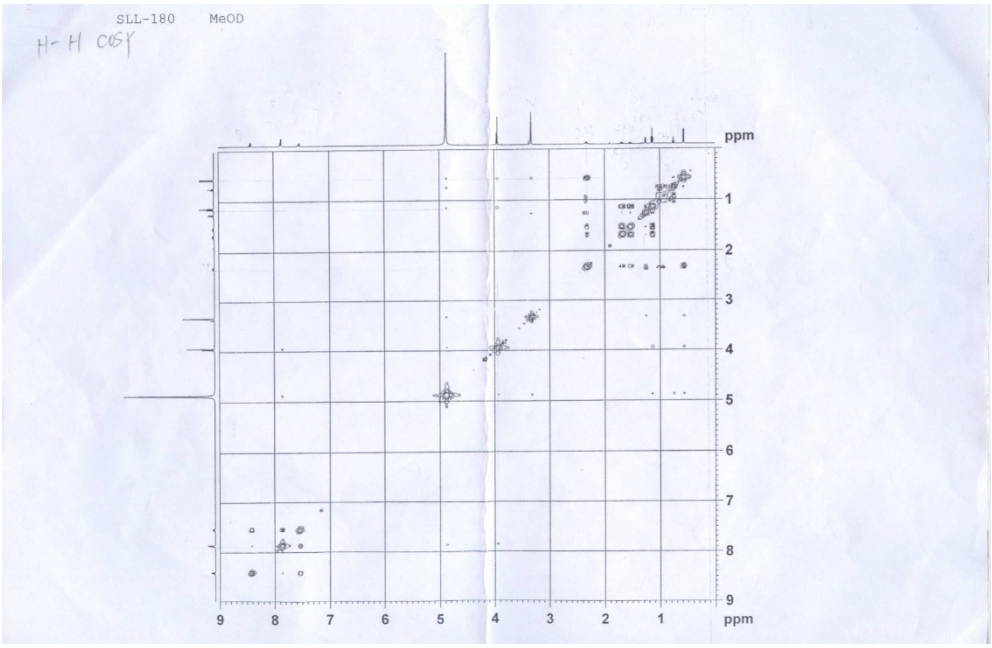



**Figure 4**. $^{1}H$-$^{1}H$ COSY NMR Spectrum of compound recorded at 500 MHz in $CD_3OD$

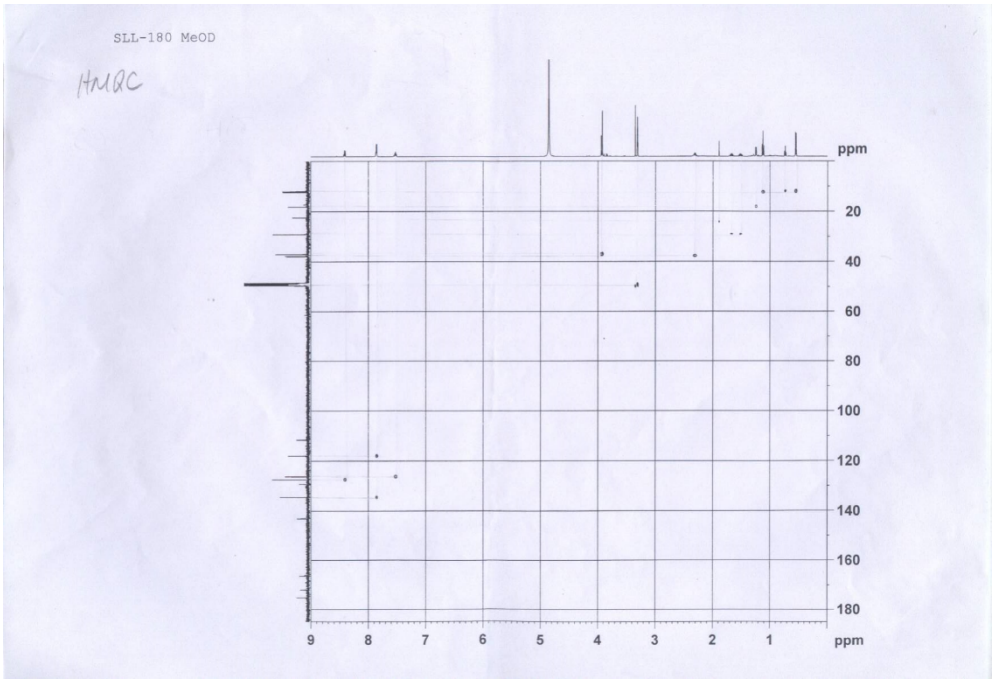



**Figure 5**. HMQC NMR Spectrum of compound recorded at 500 MHz in $CD_3OD$

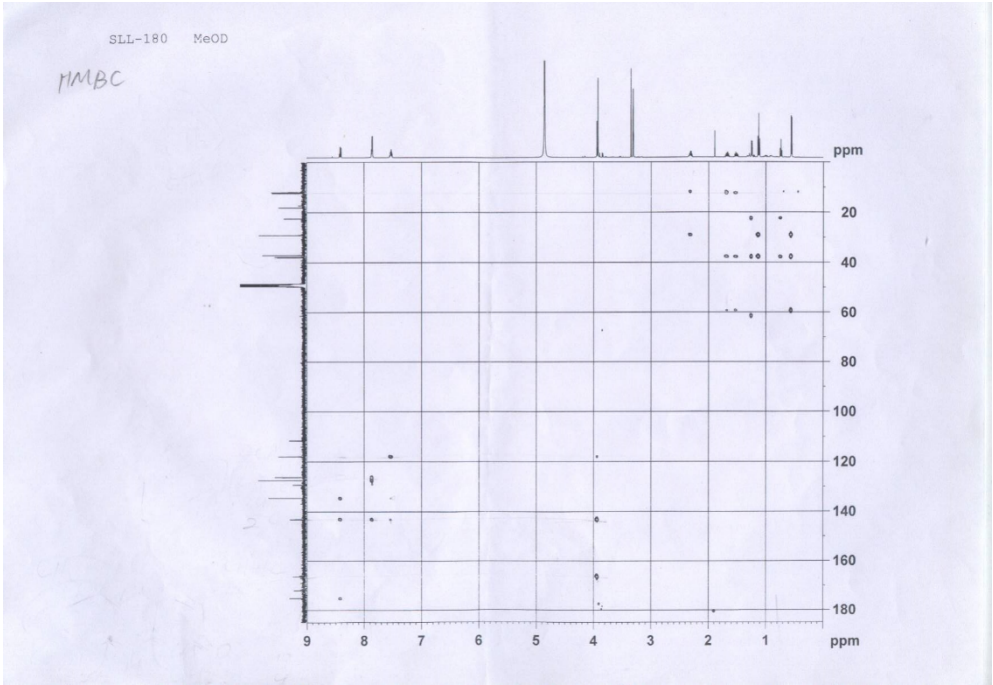



**Figure** 6. HMBC NMR Spectrum of compound recorded at 500 MHz in $CD_3OD$

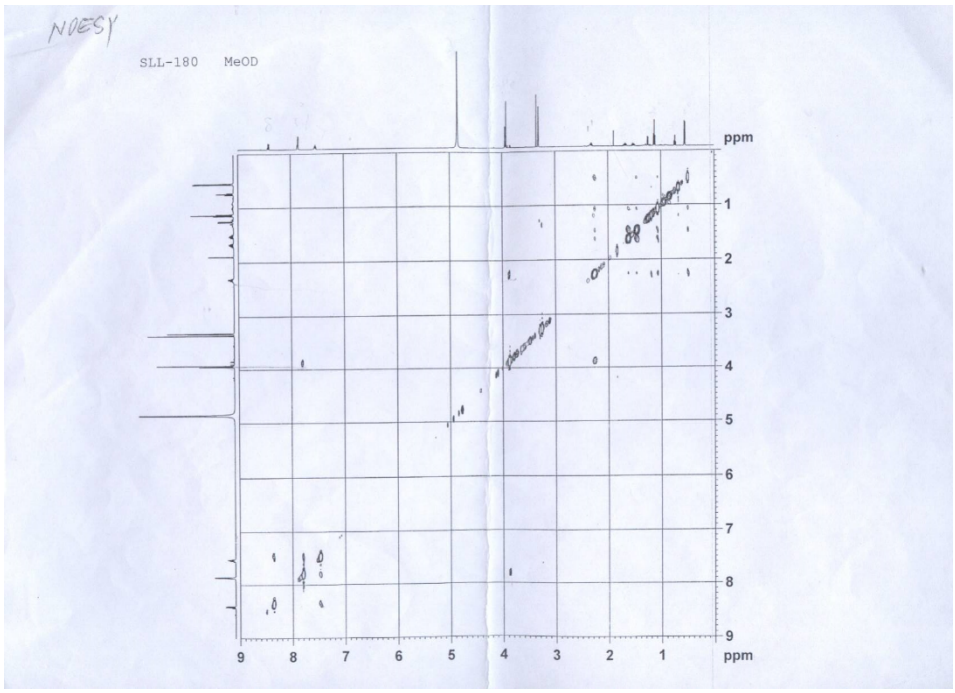



**Figure 7**. NOESY Spectrum of compound recorded at 500 MHz in $CD_3OD$

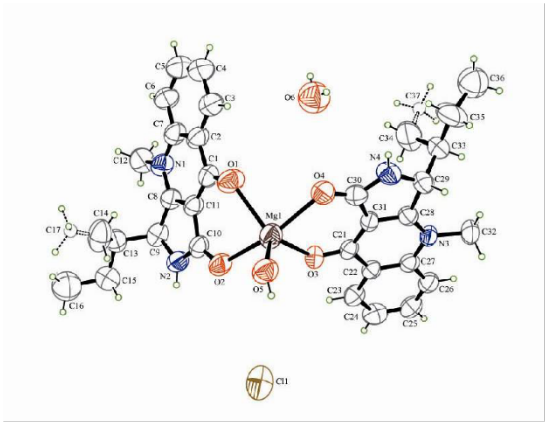
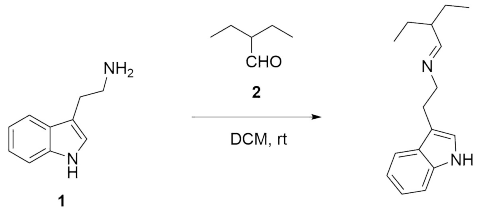



**Figure 8.** X-ray crystal structure of compound

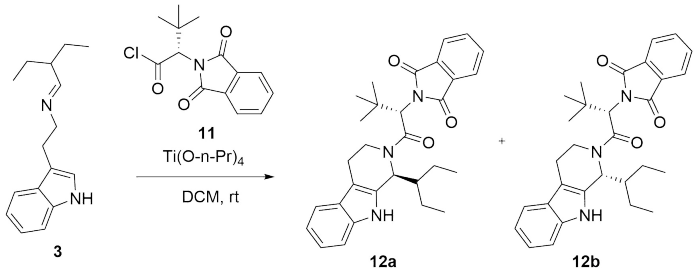
## Total synthesis of (*R*)-Quinolactacin-H and (*S*)-Quinolactacin-H[1].

**(*E*)-*N*-(2-(1*H*-indol-3-yl) ethyl)-2-ethylbutan-1-imine (3)**



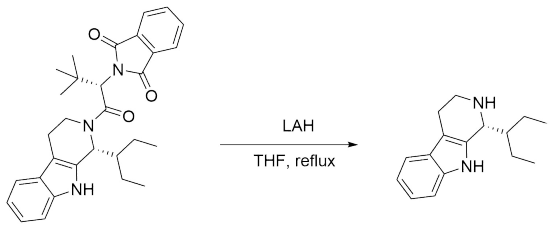

To a solution of tryptamine (8 g, 50 mmol) in dichloromethane (200 mL) was added dropwise 2-ethylbutyraldehyde (7.5 mL, 75 mmol) via syringe followed by $MgSO_4$ (10 g) at room temperature. The mixture was stirred for 2 hours. The resulting solution was filtered, then the filtrate was concentrated to give the product as a brown oil. The Schiff base was recrystallized ($Et_2O$/n-hexane 5:1) as a bright yellow solid for further use.

**2-((*S*)-3,3-dimethyl-1-oxo-1-((*R*)-1-(pentan-3-yl)-1,3,4,9-tetrahydro-2*H*-pyrido[3,4-*b*] indol-2-yl) butan-2-yl) isoindoline-1,3-dione (12b)**




To a solution of **3** (6 g, 24.8 mmol) in dry dichloromethane (100 mL), then d-amino acid chloride (17.2 g, 61.5 mmol) was added dropwise Ti(O-n-Pr)$_4$ at room temperature for 10 minutes. A solution of the Schiff base in dichloromethane was added into the reaction and the mixture was stirred at room temperature for 4 hours. The solvent was removed and the residue was purified by silica gel column chromatography (ethyl acetate / petroleum ether = 1/12, with 1%TEA) to give pure product as a yellow solid **12b** (6.6 g, 55%). $[\alpha]_D^{25}$=-148 (c=1.0, MeOH); **$^1$H NMR** (500 MHz, CDCl3) δ 7.97 (d, J = 2.8 Hz, 1H), 7.80 (d, J = 2.5 Hz, 2H), 7.76 – 7.68 (m, 2H), 7.29 (d, J = 8.1 Hz, 1H), 7.14 (d, J = 7.8 Hz, 1H), 7.10 (dd, J = 11.1, 4.0 Hz, 1H), 6.96 (t, J = 7.4 Hz, 1H), 5.78 (d, J = 8.5 Hz, 1H), 4.94 (s, 1H), 3.94 (dd, J = 14.4, 5.7 Hz, 1H), 3.59 – 3.46 (m, 1H), 2.53 (dd, J = 15.4, 4.0 Hz, 1H), 2.20 – 2.06 (m, 1H), 1.78 (dd, J = 7.2, 3.7 Hz, 1H), 1.62 (s, 3H), 1.59 – 1.54 (m, 1H), 1.54 – 1.45 (m, 2H), 1.45 – 1.35 (m, 1H), 1.20 (s, 8H), 0.95 (t, J = 7.4 Hz, 3H), 0.91 (t, J = 7.4 Hz, 3H). **$^{13}$C NMR** (126 MHz, CDCl3) δ 167.77, 165.90, 135.78, 134.37, 134.33, 131.31, 126.51, 123.69, 121.59, 119.17, 117.65, 110.86, 107.52, 77.29, 77.03, 76.78, 57.36, 51.53, 45.02, 40.53, 37.15, 31.60, 27.90, 22.66, 21.93, 21.88, 14.12, 11.06, 10.83. **HRMS** (ESI-TOF): calcd for $C_{30}H_{35}N_3O_3$ $[M+H]^+$: 486.2751; Found: 486.2737.

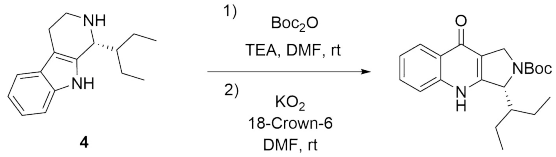
**(*R*)-1-(pentan-3-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*] indole ((*R*)-4)**




12b

To a solution of **12b** (1 g, 2.06 mmol) in dry THF (20 mL) was added LAH (155 mg, 4.12 mmol) at room temperature. The reaction mixture was heated to reflux for 30 minutes. Then the reaction was diluted with ether and cooled to 0℃, slowly added water (155 μL), added 15% aqueous sodium hydroxide (155 μL), and then added water (465 μL), warmed to room temperature stirred. After 15 minutes, dried over anhydrous magnesium sulfate, filtered and concentrated to give the crude product. Purified by silica gel column chromatography (methanol/dichloromethane =1/20) to give the desired product as a yellow oil (101.6mg, 42%). $[\alpha]_D^{20}$=-33 (c=0.9, MeOH); **$^1$H NMR** (500 MHz, $CDCl_3$) δ 8.21 (s, 1H), 4.57 (s, 1H), 3.62 (dd, J = 7.9, 3.3 Hz, 1H), 3.21 – 3.09 (m, 1H), 3.08 – 2.94 (m, 1H), 2.85 (d, J = 15.7 Hz, 1H), 2.00 (s, 1H), 1.80 (d, J = 3.2 Hz, 1H), 1.71 – 1.55 (m, 2H), 1.50 – 1.40 (m, 1H), 1.40 – 1.31 (m, 1H), 1.07 (t, J = 7.4 Hz, 3H), 0.93 (t, J = 7.4 Hz, 3H). **$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 135.80, 135.63, 127.76, 121.36, 119.32, 117.88, 110.65, 110.51, 54.60, 45.98, 43.65, 23.22, 22.89, 22.70, 12.98, 12.62. HRMS (ESI-TOF): calcd for $C_{16}H_{22}N_2$ $[M+H]^+$: 243.1856; Found: 243.1858.

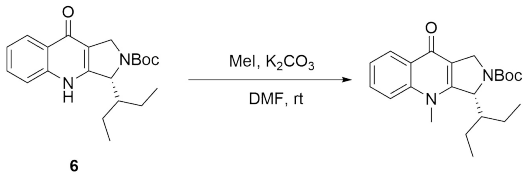
***Tert*-butyl (*R*)-9-oxo-3-(pentan-3-yl)-1,3,4,9-tetrahydro-2*H*-pyrrolo[3,4-*b*] quinoline-2-carboxylate (6)**




4

To a solution of **4** (1 g, 4.1 mmol) in DMF (15 mL) was added $Boc_2O$ (1.07 mg, 4.92 mmol) and TEA (0.85 mL, 6.15 mmol) at room temperature. The reaction mixture was stirred at 40 ℃ for 2 hours. Then reaction mixture was partitioned between dichloromethane (50 mL) and water (50 mL). The aqueous phase was separated, and the aqueous extracted twice with dichloromethane (50 mL). The combined organic phase was washed with 1N HCl, water, and brine, dried over anhydrous sodium sulfate and concentrated. The product as light yellow solid (**5**) for further use. To a solution of **5** and 18-crown-6 (1.08 g, 4.1 mmol) in DMF (15 mL) was added potassium superoxide (1.15 g, 16.4 mmol) at room temperature. Then the reaction mixture turned red. After 18 hours, the red color disappeared and the reaction mixture was stirred for another 2 hours. The reaction was quenched with saturated ammonium chloride, ether acetate was added into the reaction. The aqueous phase was separated, and the aqueous extracted three time with ether acetate. The combined organic phase was washed with brine, dried over anhydrous sodium sulfate and concentrated. The crude product was purified by chromatography on silica (1% triethylamine in ether acetate), yielding the product as a light yellow solid (901 mg, 62%). $[\alpha]_D^{20}$=-84 (c=0.76, MeOH); **$^1$H NMR** (500 MHz, DMSO-$d_6$) δ 11.93 (br, s, 1H), 8.12 (d, J = 8.1 Hz, 1H), 7.66 (d, J = 4.8 Hz, 2H), 7.38 – 7.21 (m, 1H), 5.21 (d, J = 34.1 Hz, 1H), 4.55 (d, J = 13.8 Hz, 1H), 4.22 (t, J = 15.0 Hz, 1H), 1.99 (s, 1H), 1.78 (s, 1H), 1.46 (s, 8H), 1.42-1.33 (m, 2H), 1.17 (t, J = 7.1 Hz, 1H), 0.97 (d, J = 20.9 Hz, 3H), 0.89-0.78 (m, 3H). **$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 173.99, 154.86, 151.49, 140.70, 131.79, 125.39, 125.34, 123.93, 118.82, 116.35, 80.08, 64.85, 50.96, 47.10, 28.40, 23.16, 22.43, 12.44, 12.34. HRMS (ESI-TOF): calcd for $C_{21}H_{28}N_2O_3$ $[M+H]^+$: 357.2173; Found: 357.2176.

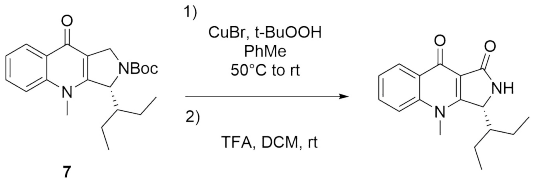
***Tert*-butyl (*R*)-4-methyl-9-oxo-3-(pentan-3-yl)-1,3,4,9-tetrahydro-2*H*-pyrrolo[3,4-*b*] quinoline-2-carboxylate (7)**




**6**

To a solution of **6** (500 mg, 1.4 mmol) in DMF (8 mL) was added $K_2CO_3$ (387 mg, 2.8 mmol) and MeI (0.173 mL, 2.8 mmol) at room temperature. The reaction was stirred 2 hours, then was partitioned with ether acetate and water. The aqueous phase was separated, and the aqueous extracted three time with ether acetate. The combined organic phase was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was purified by chromatography on silica (hexanes/ethyl acetate = 2/1), yielding the product as a white solid (395 mg, 77%). $[\alpha]_D^{20}$=-78 (c=0.54, MeOH). $^1$H NMR (600 MHz, $CDCl_3$) δ 8.50 (d, J = 7.6 Hz, 1H), 7.69 (t, J = 7.5 Hz, 1H), 7.50 (d, J = 8.6 Hz, 1H), 7.42 (t, J = 7.5 Hz, 1H), 5.59 (d, J = 2.5 Hz, 1H), 4.86 (d, J = 14.3 Hz, 1H), 4.40 (d, J = 14.2 Hz, 1H), 3.76 (s, 3H), 1.67 – 1.54 (m, 4H), 1.49 (s, 9H), 1.26 – 1.23 (m, 1H), 1.19 (t, J = 6.7 Hz, 3H), 0.75 (t, J = 7.4 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 173.32, 155.79, 152.48, 141.75, 131.96, 126.93, 126.68, 123.68, 117.93, 115.12, 80.25, 77.26, 77.04, 76.83, 63.98, 51.82, 48.66, 36.20, 28.40, 23.80, 20.88, 12.62, 12.26. HRMS (ESI-TOF): calcd for $C_{22}H_{30}N_2O_3$ $[M+H]^+$: 371.2329; Found: 371.2322.

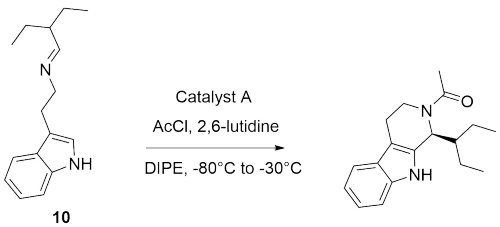
**(*R*)-4-methyl-3-(pentan-3-yl)-2,3-dihydro-1*H*-pyrrolo[3,4-*b*] quinoline-1,9(4H)-dione (9)**




**7**

To a solution of **7** (184 mg, 0.5 mmol) and Cuprous Bromide (28 mg, 0.2 mmol) in methylbenzene (5 mL) was added t-BuOOH (5.5 M in decane, 0.182 mL, 1 mmol) ar room temperature. The reaction mixture was heated at 50 ℃ for 2 hours and stirred at room temperature for another 20 hours. Then the reaction was filtered through a pad of Celite.. The solution was concentrated to give a light green oil (**8**). To a solution of **8** and trifluoroacetic acid (150 μL) in DCM (4 mL), then the reaction mixture stirred at room temperature for 2 h. The solvent was removed. Then the residue was adjusted pH to 8-9 with saturated $NaHCO_3$. The mixture was extracted three times with dichloromethane. The combined organic layer was concentrated and purified by column chromatography on silica (ethyl acetate, then methanol/dichloromethane =1/10) to afford product as a light yellow solid (74 mg, 54%). $[\alpha]_D^{20}$=-64 (c=0.43, MeOH). HRMS (ESI-TOF): calcd for $C_{22}H_{28}N_2O_4$ $[M+H]^+$: 385.2122; Found: 385.2124.

**(*S*)-1-(1-(pentan-3-yl)-1,3,4,9-tetrahydro-2*H*-pyrido[3,4-*b*] indol-2-yl) ethan-1-one (13)**[2]

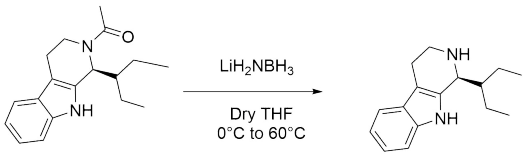



**10**

To a solution of **10** (500 mg, 2.07mmol) in isopropyl ether (40 mL) was added catalyst A (52 mg, 0.1 mmol) at room temperature. The reaction mixture cooled to -80 ℃. 2,6-lutidine (0.244 mL, 2.1 mmol), then acetyl chloride (0.148 mL, 2.1 mmol) were added dropwise by syringe. The reaction was stirred at -80 ℃ for 10 minutes, then

warmed to -30 ℃ and stirred for 28 hours. The solvent was removed. The residue was purified by chromatography on silica (ethyl acetate/hexane=1/10, then ethyl acetate) to afford product as a white solid (357 mg, 63%). The enantiomeric excess was determined to be 99.5 % by chiral HPLC (Daicel Chiralpak OD-H, n-hexane/i-propanol= 90:10, 1.0 mL/min, λ=254 nm, t (major)=16.4 min, t (minor)=28.8 min). $[\alpha]_D^{20}$=+57 (c=0.3, MeOH). **$^1$H NMR** (500 MHz,$CDCl_3$) δ 8.15 (S, 1H), 7.44 (dd, J = 7.8, 1.5 Hz, 1H), 7.33 – 7.30 (m, 1H), 7.15 (m, 1H), 7.12 – 7.07 (m, 1H), 5.67 (dd, J = 16.9, 8.4 Hz, 1H), 4.07 – 3.89 (m, 1H), 3.59 (ddt, J = 14.2, 11.4, 5.7 Hz, 1H), 2.84 – 2.75 (m, 2H), 2.22 (s, 3H), 1.74 (m,, 1H), 1.60 – 1.45 (m, 3H), 1.40 – 1.32 (m, 1H), 0.98 – 0.95 (t, 3H), 0.92 – 0.88 (t, 3H). **$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 171.14, 135.95, 134.37, 126.75, 121.80, 119.47, 117.91, 110.99, 108.05, 51.22, 45.26, 41.47, 22.07, 22.05, 22.00, 21.93, 11.24, 10.92. HRMS (ESI-TOF): calcd for $C_{18}H_{24}N_2O$ $[M+H]^+$: 285.1961; Found: 285.1953.

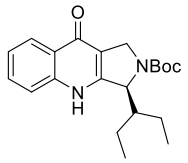
**(*S*)-1-(pentan-3-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*] indole ((*S*)-4)**



13

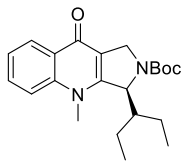
(*S*)-1-(pentan-3-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-*b*] indole (**(*S*)-4**) was isolated as a white solid (46% yield). $[\alpha]_D^{20}$=+39 (c=0.47, MeOH). **$^1$H NMR** (500 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.49 (d, J = 7.7 Hz, 1H), 7.33 (d, J = 7.8 Hz, 1H), 7.15 (t, J = 6.9 Hz, 1H), 7.10 (t, J = 6.8 Hz, 1H), 4.26 (d, J = 2.3 Hz, 1H), 3.42 (m, 1H), 2.96 (m, 1H), 2.75 (m, 2H), 1.57 (m, 3H), 1.34 – 1.20 (m, 3H), 1.05 (t, J = 7.1 Hz, 3H), 0.88 (t, J = 7.4 Hz, 3H). **$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 135.70, 135.59, 121.33, 119.28, 117.85, 110.64, 110.44, 54.52, 45.90, 43.60, 23.17, 22.82, 22.65, 12.96, 12.61. HRMS (ESI-TOF): calcd for $C_{16}H_{22}N_2$ $[M+H]^+$: 243.1856; Found: 243.1847.

***Tert*-butyl (*S*)-9-oxo-3-(pentan-3-yl)-1,3,4,9-tetrahydro-2*H*-pyrrolo[3,4-*b*] quinoline-2-carboxylate**




*Tert*-butyl (*S*)-9-oxo-3-(pentan-3-yl)-1,3,4,9-tetrahydro-2*H*-pyrrolo[3,4-*b*] quinoline-2-carboxylate was isolated as a light white solid (59% yield for two steps). $[\alpha]_D^{20}$=+71 (c=1.4, MeOH). **$^1$H NMR** (600 MHz, $CDCl_3$) δ 11.59 (br, s, 1H), 8.37 (d, J = 8.2 Hz, 1H), 7.76 (d, J = 8.5 Hz, 1H), 7.60 (t, J = 7.5 Hz, 1H), 7.35 (t, J = 7.6 Hz, 1H), 5.31 (d, J = 39.7 Hz, 1H), 4.81 (dd, J = 77.5, 14.0 Hz, 1H), 4.54 – 4.43 (m, 1H), 1.52 – 1.46 (m, 4H), 1.43 (s, 9H), 1.29 – 1.21 (m, 1H), 0.90 (t, J = 7.4 Hz, 3H), 0.87 (t, J = 7.2 Hz, 3H). **$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 173.99, 154.87, 151.56, 140.72, 131.78, 125.39, 125.31, 123.93, 118.86, 116.32, 80.07, 64.83, 50.97, 47.15, 28.40, 23.18, 22.37, 14.13, 12.44. HRMS (ESI-TOF): calcd for $C_{21}H_{28}N_2O_3$ $[M+H]^+$: 357.2173; Found: 357.2177.

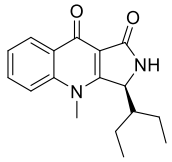
***Tert*-butyl (*S*)-4-methyl-9-oxo-3-(pentan-3-yl)-1,3,4,9-tetrahydro-2*H*-pyrrolo[3,4-*b*] quinoline-2-carboxylate**




*Tert*-butyl (*S*)-4-methyl-9-oxo-3-(pentan-3-yl)-1,3,4,9-tetrahydro-2*H*-pyrrolo[3,4-*b*] quinoline-2-carboxylate was isolated as a white solid (75% yield). $[\alpha]_D^{20}$=+69 (c=0.91, MeOH). **$^1$H NMR** (600 MHz,$CDCl_3$) δ 8.51 (d, J = 7.1 Hz,

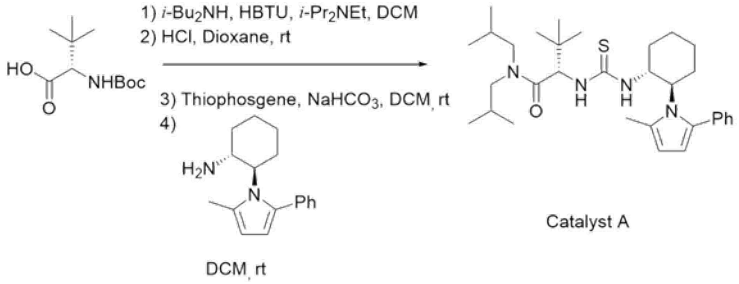
1H), 7.70 (t, J = 7.4 Hz, 1H), 7.51 (d, J = 8.5 Hz, 1H), 7.43 (t, J = 7.5 Hz, 1H), 5.60 (d, J = 2.4 Hz, 1H), 4.87 (d, J = 14.3 Hz, 1H), 4.40 (d, J = 14.5 Hz, 1H), 3.76 (s, 3H), 1.64 – 1.61 (m, 3H), 1.59 – 1.56 (m, 2H), 1.50 (s, 9H), 1.26 – 1.25 (m, 1H), 1.19 (t, J = 6.6 Hz, 3H), 0.76 (t, J = 7.4 Hz, 3H). **$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 173.37, 155.80, 152.47, 141.77, 131.96, 126.96, 126.71, 123.69, 117.96, 115.09, 80.27, 64.00, 51.82, 48.68, 36.19, 28.41, 23.82, 20.90, 12.62, 12.26. HRMS (ESI-TOF): calcd for $C_{22}H_{30}N_2O_3$ $[M+H]^+$: 371.2329; Found: 371.233.

**(*S*)-4-methyl-3-(pentan-3-yl)-2,3-dihydro-1*H*-pyrrolo[3,4-*b*] quinoline-1,9(4H)-dione**



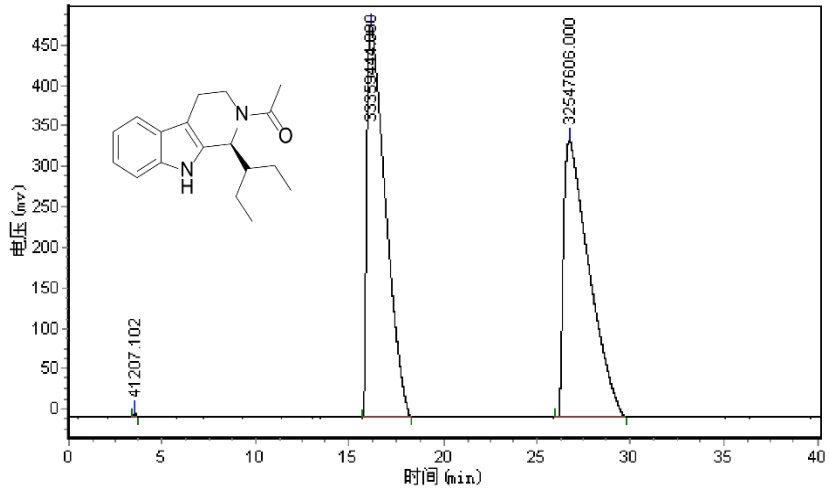
(*S*)-4-methyl-3-(pentan-3-yl)-2,3-dihydro-1*H*-pyrrolo[3,4-*b*] quinoline-1,9(4H)-dione was isolated as a white solid (51% yield for two steps). $[\alpha]_D^{20}$=+53.3 (c=0.53, MeOH). HRMS (ESI-TOF): calcd for $C_{17}H_{20}N_2O_2$ $[M+H]^+$: 285.1598; Found: 285.1589.

**(*S, R, R*)-*N, N*-Diisobutyl-3,3-dimethyl-2-{3-[2-(2-methyl-5-phenyl-pyrrol-1-yl)-cyclohexyl]-thioureido}-butyramide (Catalyst A)**




*(S, R, R) -N, N* -Diisobutyl-3, 3-dimethyl-2- {3- [2- (2-methyl-5-phenyl-pyrrol-1-yl) -cyclohexyl] -thioureido}-butyramide was synthesized by using the method Jacobsen reported in 2002[2]. **$^{1}$H NMR** (600 MHz, $CDCl_3$) δ 7.42 (t, J = 7.6 Hz, 2H), 7.37 – 7.34 (m, 2H), 7.32 (d, J = 7.2 Hz, 1H), 6.15 (s, 1H), 6.02 (s, 1H), 5.86 (s, 1H), 5.48 (s, 1H), 5.11 – 5.00 (m, 1H), 4.38 (s, 1H), 3.96 (d, J = 12.7 Hz, 1H), 3.78 (dd, J = 13.4, 6.6 Hz, 1H), 3.51 – 3.38 (m, 1H), 3.02 (dd, J = 14.5, 6.7 Hz, 1H), 2.59 (dd, J = 13.4, 7.9 Hz, 1H), 2.46 (s, 1H), 2.29 – 2.14 (m, 3H), 2.07 – 1.93 (m, 2H), 1.88 – 1.82 (m, 1H), 1.75 – 1.66 (m, 1H), 1.44 – 1.36 (m, 0H), 1.32 – 1.21 (m, 2H), 0.97 (s, 9H), 0.92 (d, J = 6.8 Hz, 6H), 0.89 (d, J = 6.9 Hz, 6H), 0.86 – 0.83 (m, 1H). **$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 181.24, 171.96, 134.26, 129.60, 128.74, 127.02, 108.68, 59.82, 56.41, 55.86, 53.73, 37.16, 33.73, 32.38, 28.00, 26.95, 26.75, 25.69, 24.62, 20.79, 20.56, 20.42, 19.52, 15.28, 14.12.

## HPLC Spectra




分析结果表

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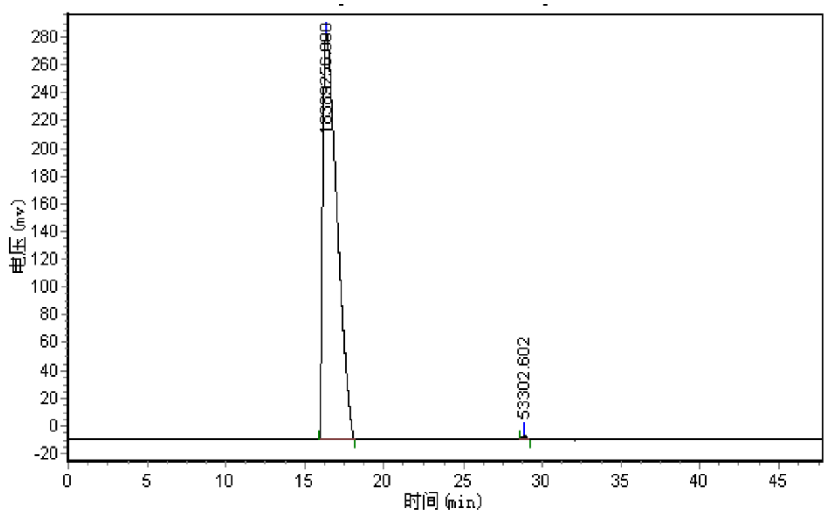
| 2 | | 16.190 | 483679.719 | 33359444.000 | 50.5843 |
| 3 | | 26.732 | 341959.625 | 32547606.000 | 49.3532 |
| 总计 | | | 830670.870 | 65948257.102 | 100.0000 |




分析结果表

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|---|---|---|---|---|---|
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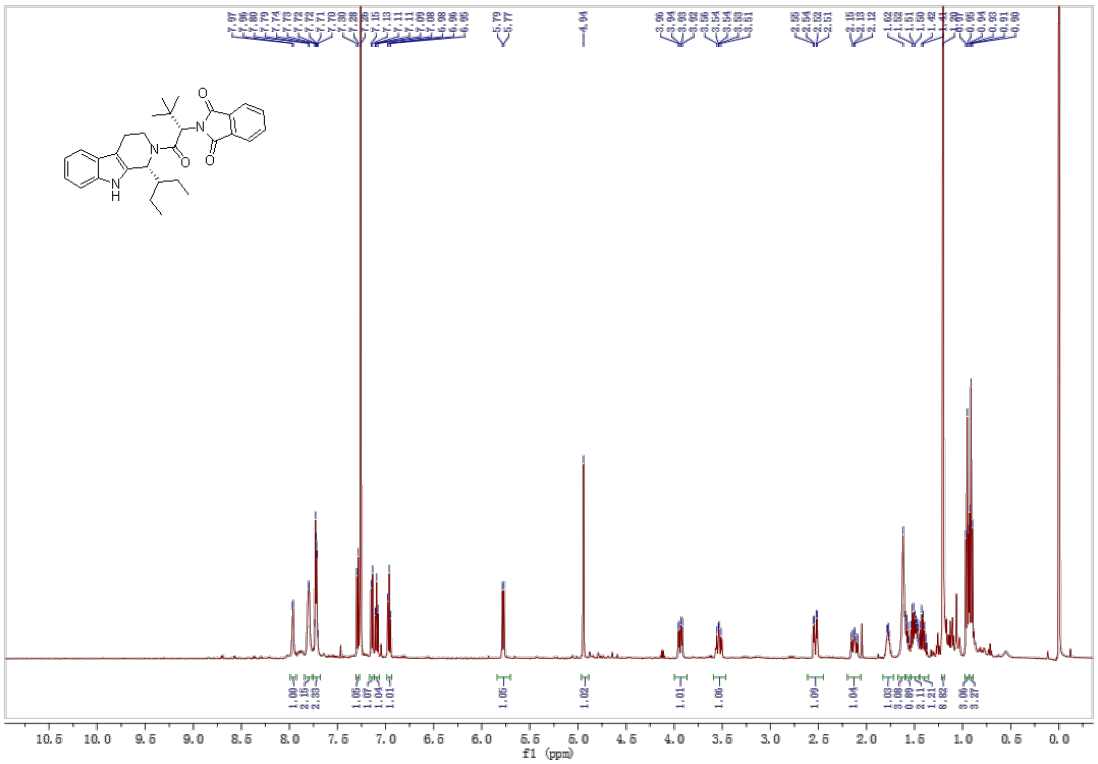
| 总计 | | | 294304.851 | 18442558.602 | 100.0000 |

**NMR spectra for all compounds**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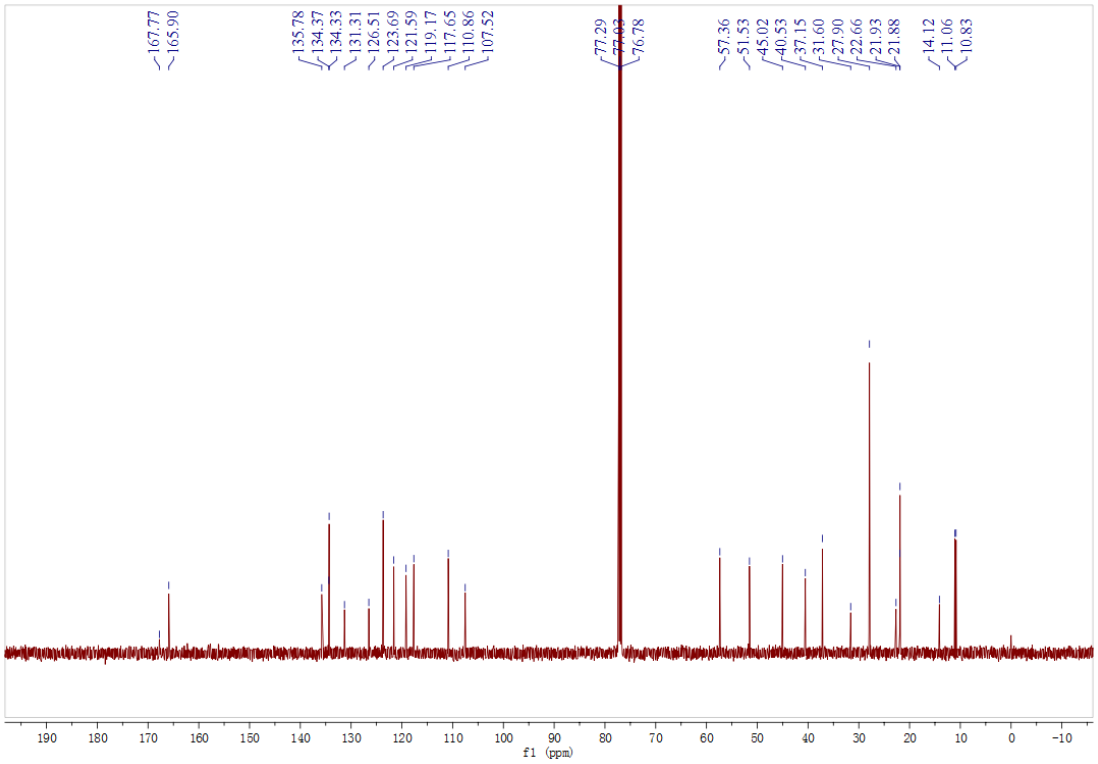
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77.03
76.78
57.36
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22.66
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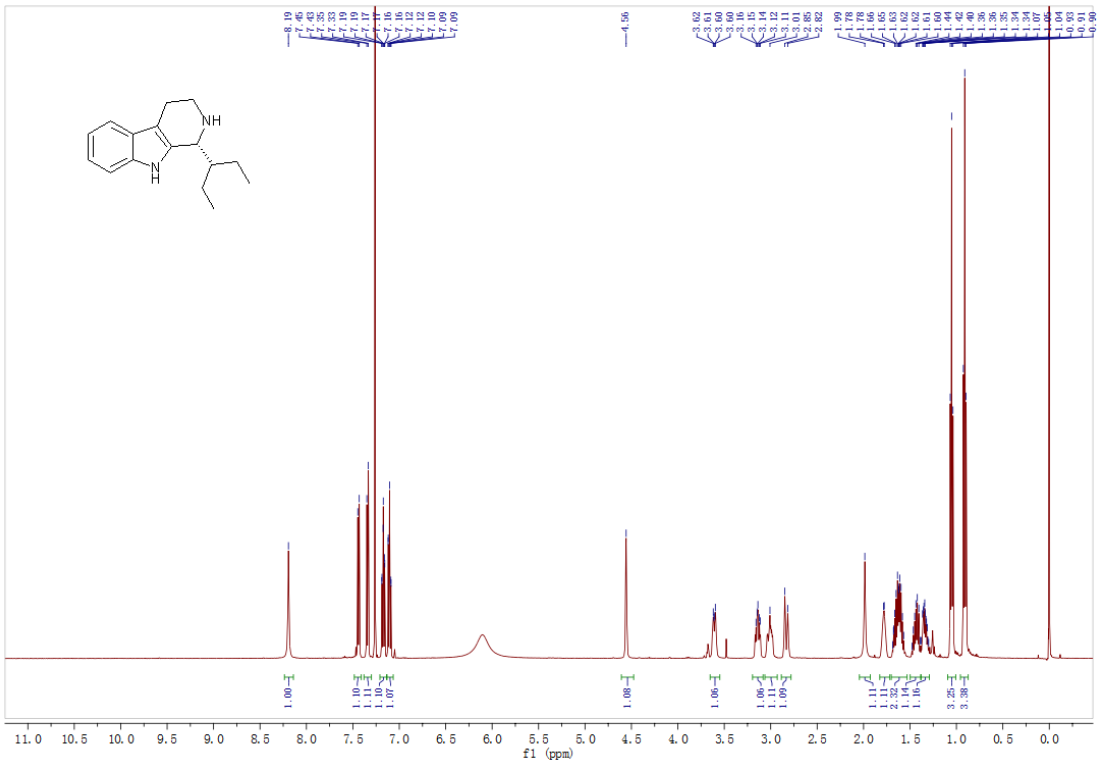
21.88
14.12
11.06
10.83
f1 (ppm)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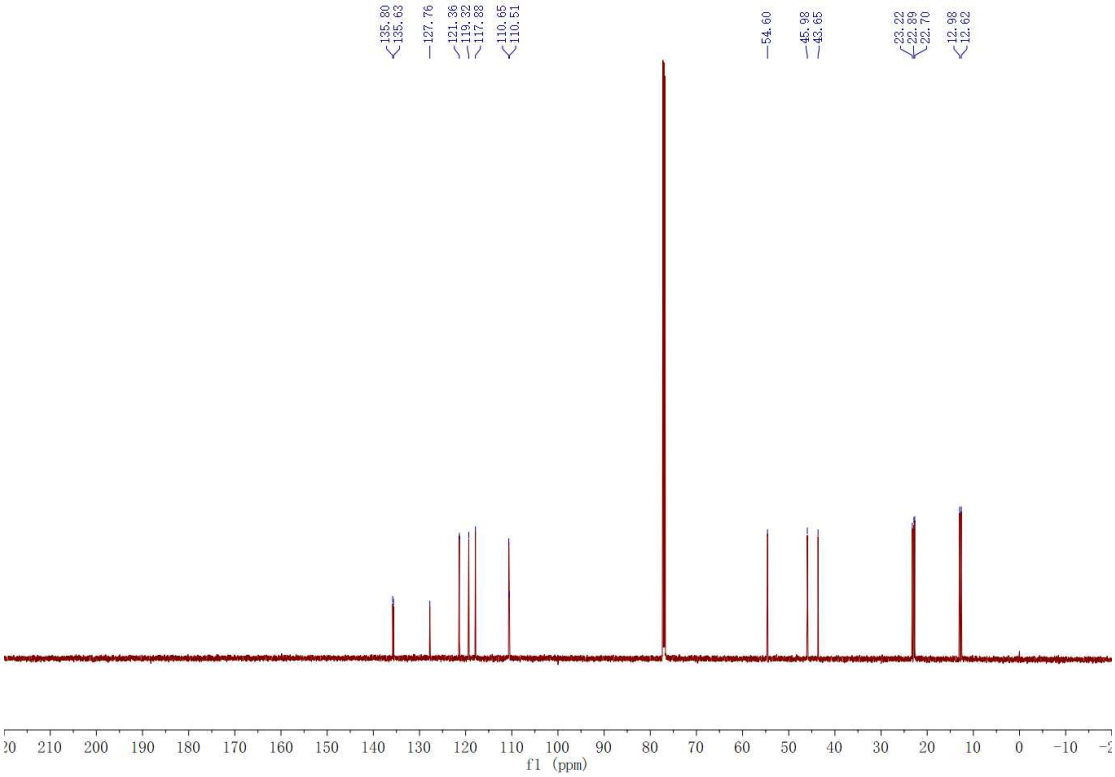
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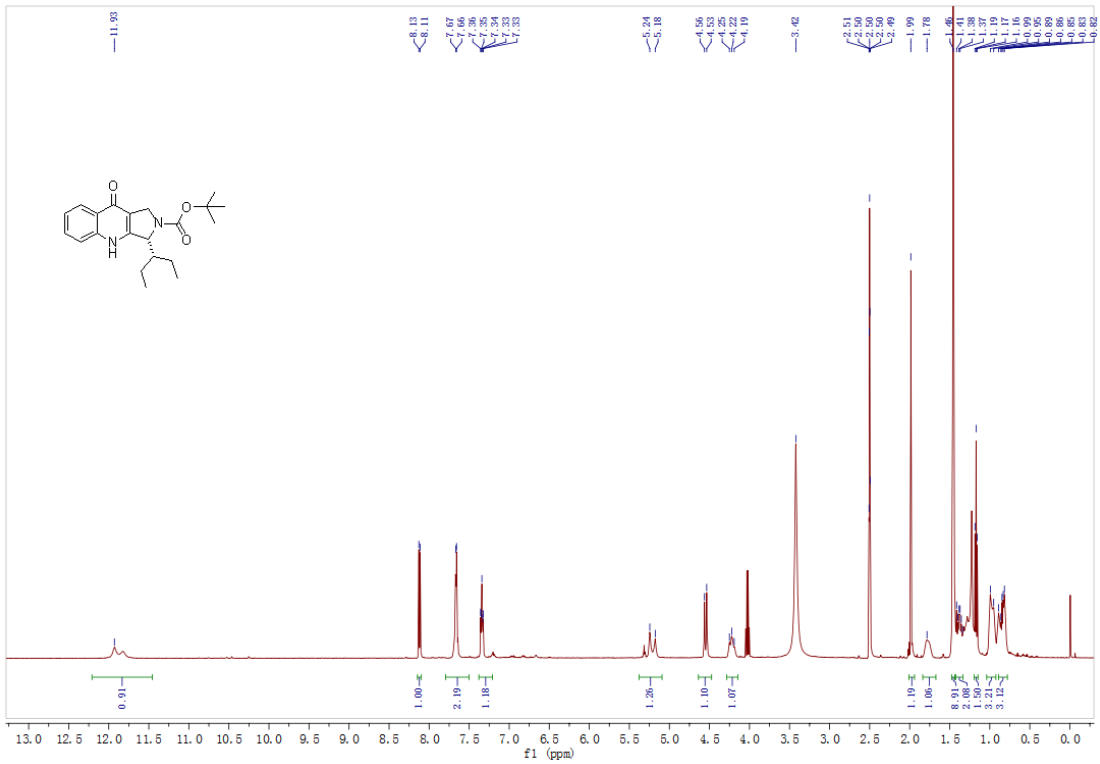
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4.25
4.22
4.19
3.42
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2.50
2.50
2.50
2.49
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1.78
1.46
1.41
1.38
1.37
1.19
1.17
1.16
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0.85
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0.82
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O
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O
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1.20
1.10
1.07
1.19
1.06
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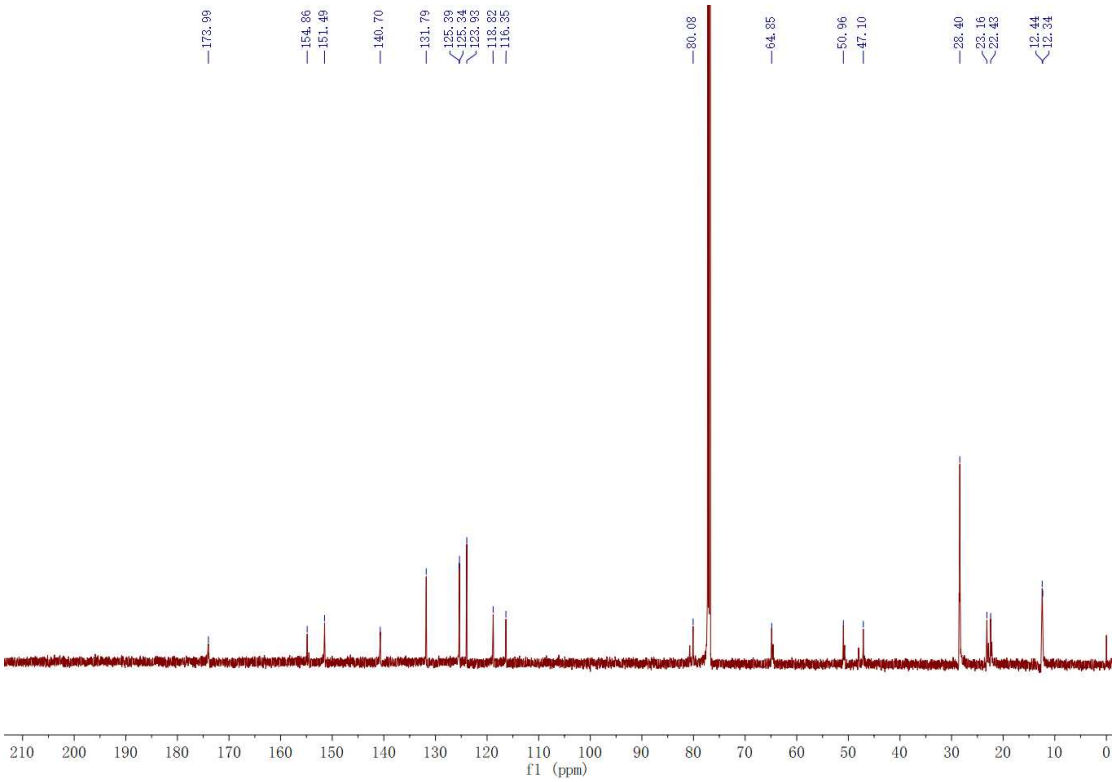
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50.96
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12.34
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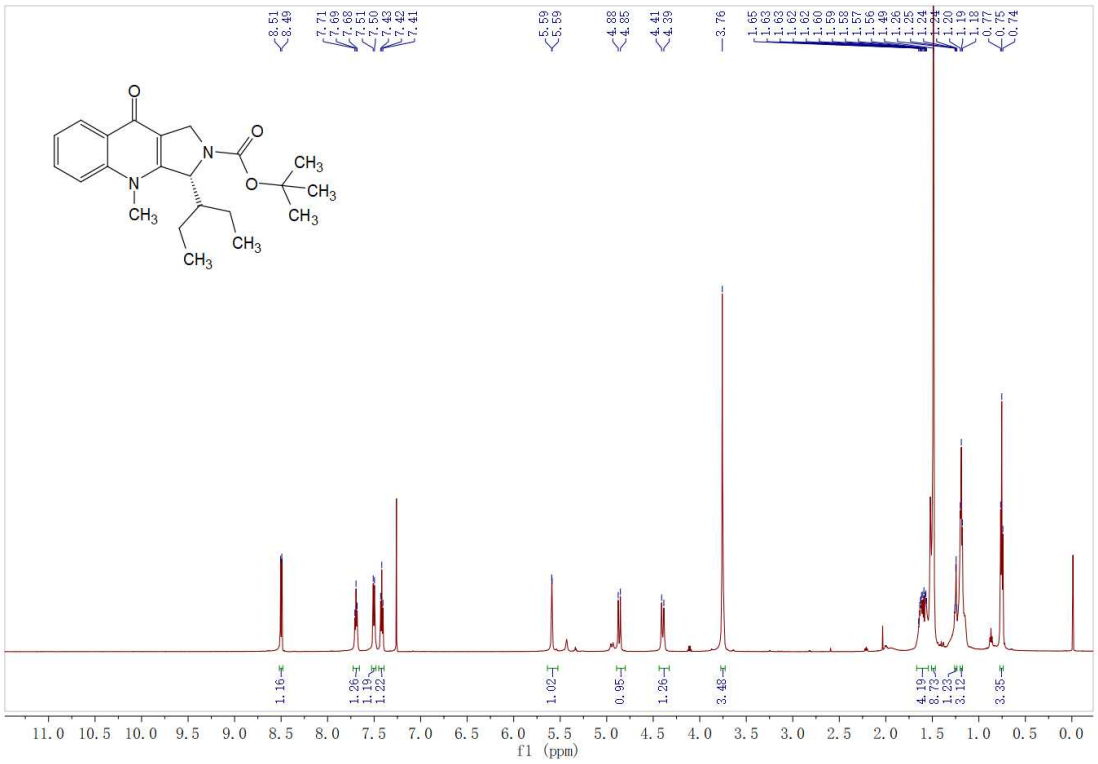
f1 (ppm)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1.59
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1.57
1.56
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CH3
CH3
N
CH3
CH3
CH3
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1.26
1.19
1.22
1.02
0.95
1.26
3.48
4.19
8.73
1.23
3.12
3.35
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
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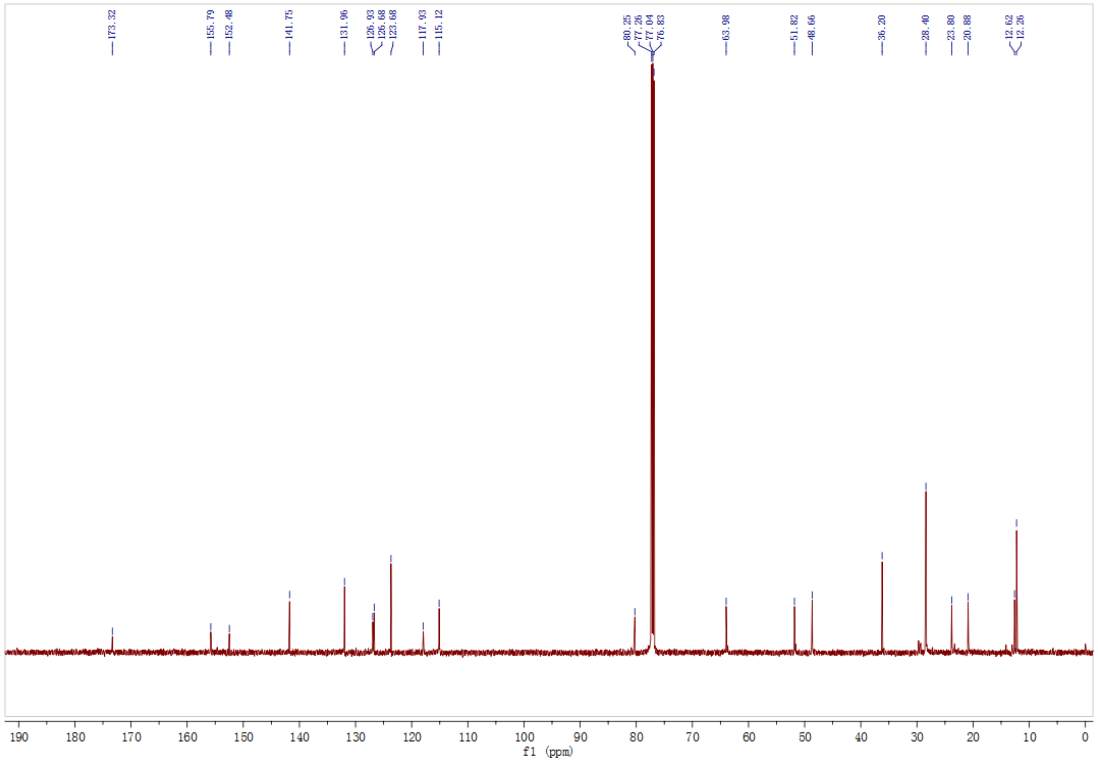
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51.82
48.66
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12.62
12.26
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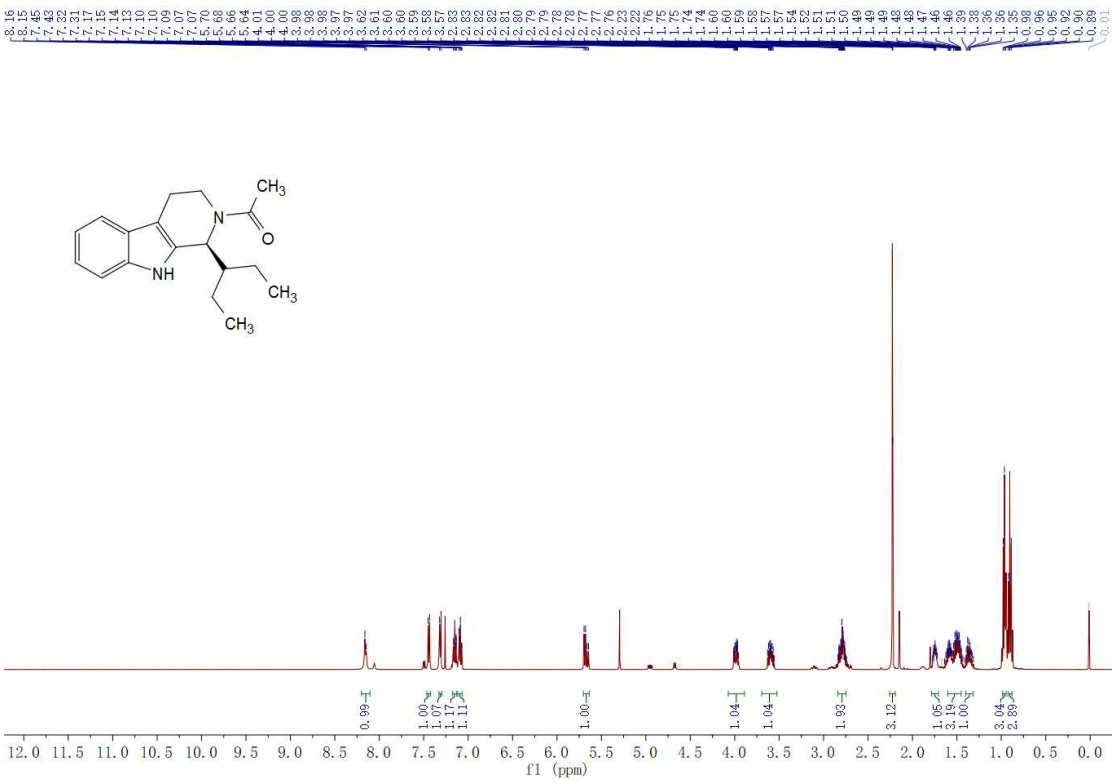
f1 (ppm)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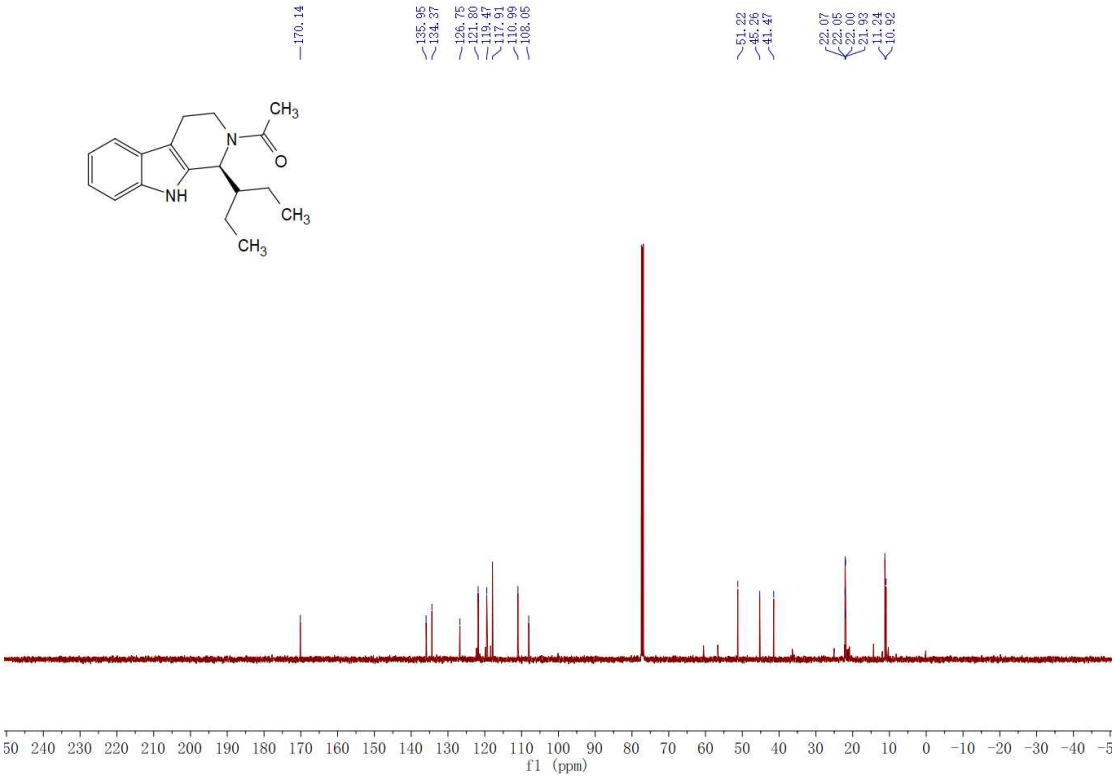
2.89
f1 (ppm)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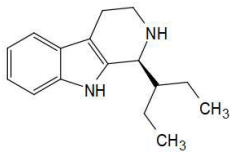
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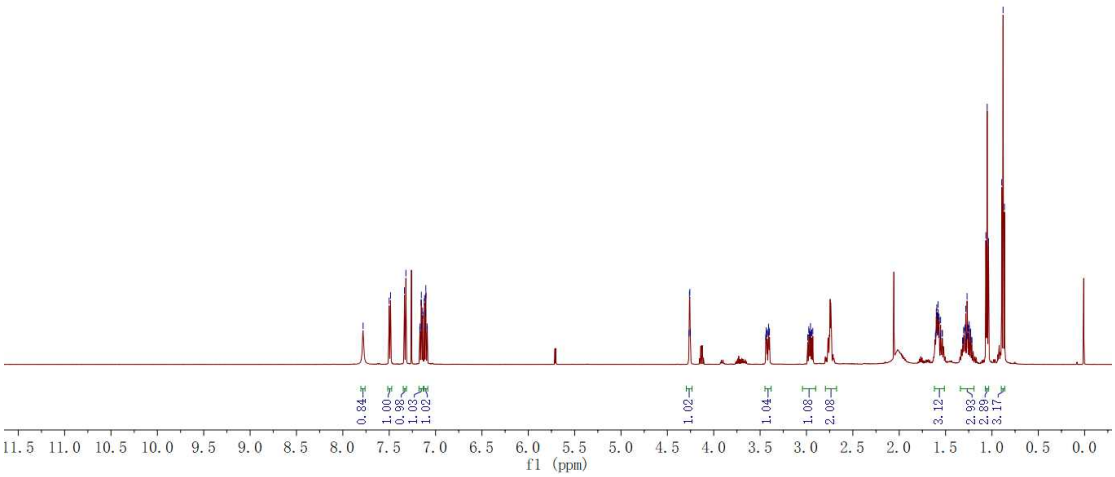
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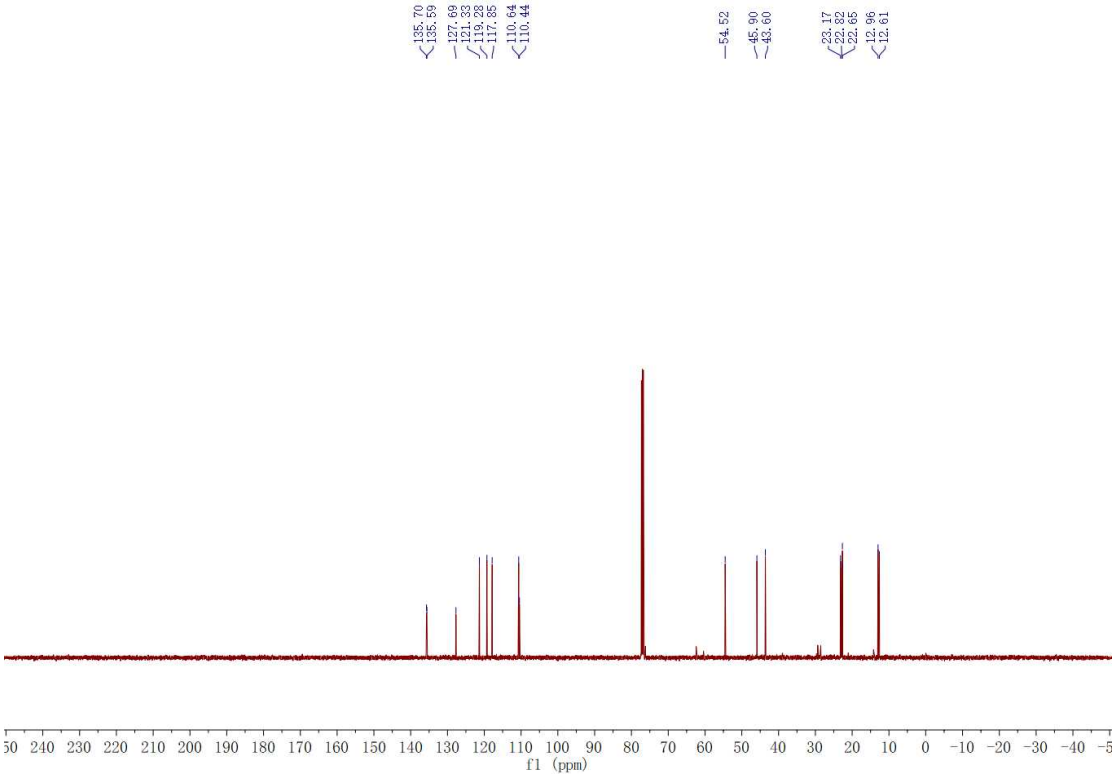
f1 (ppm)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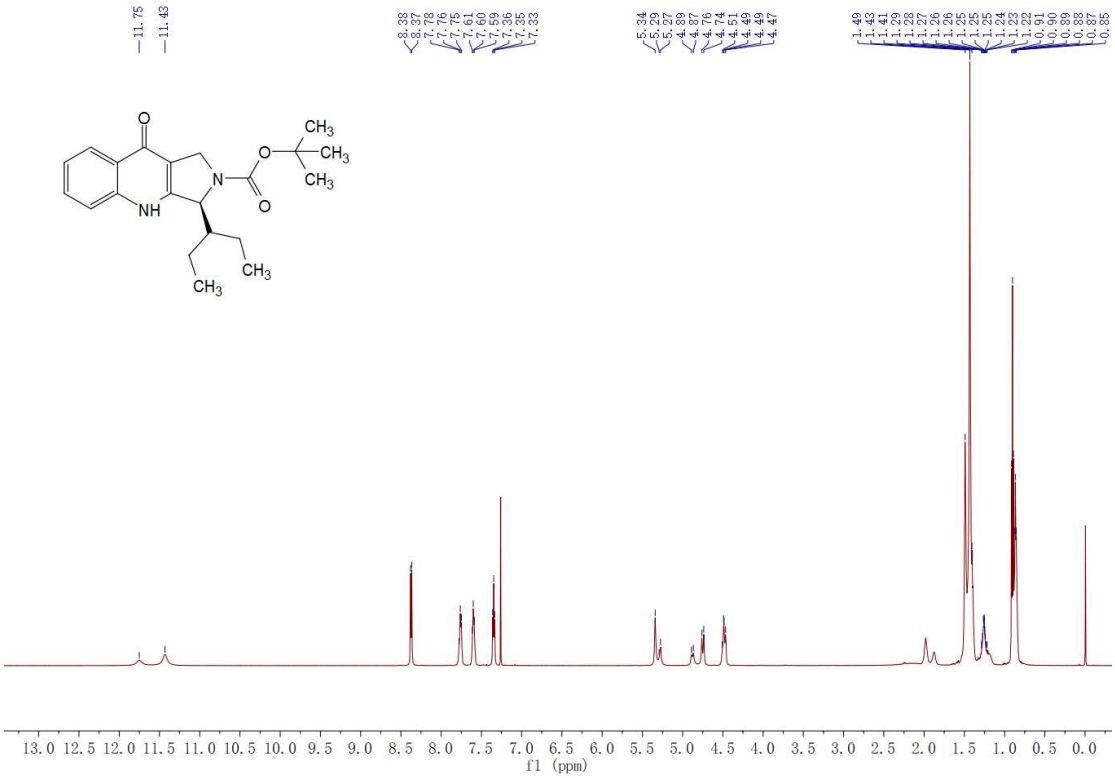
f1 (ppm)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0.85
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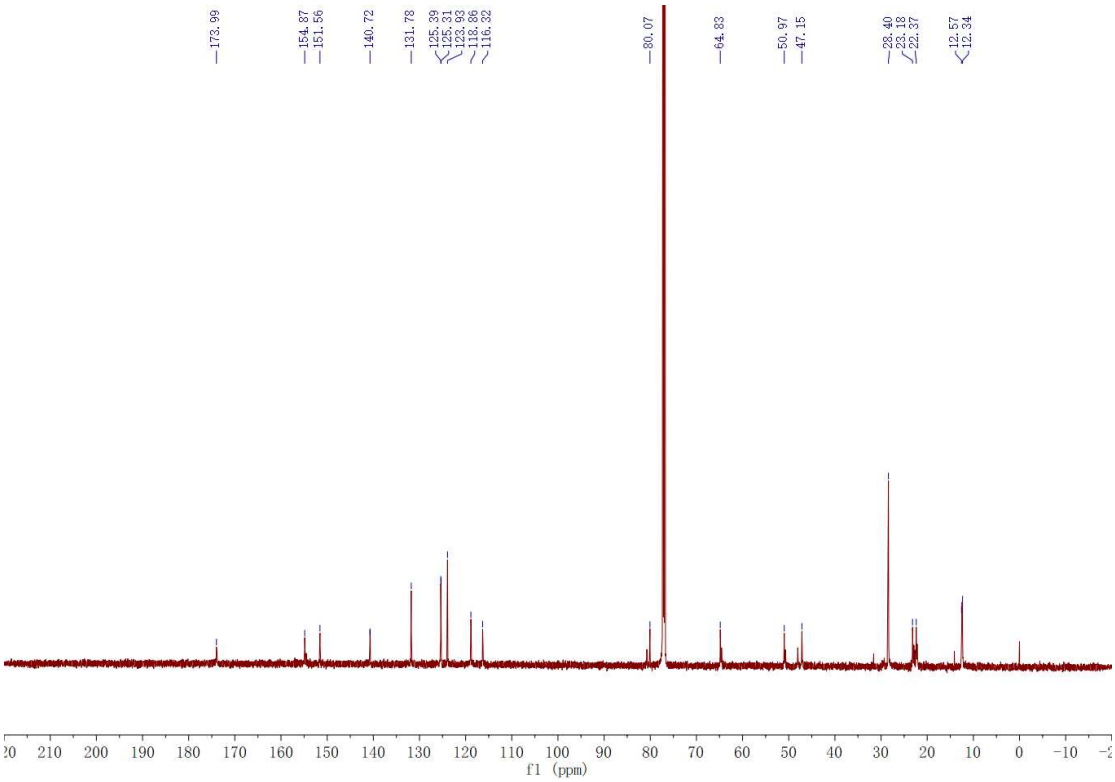
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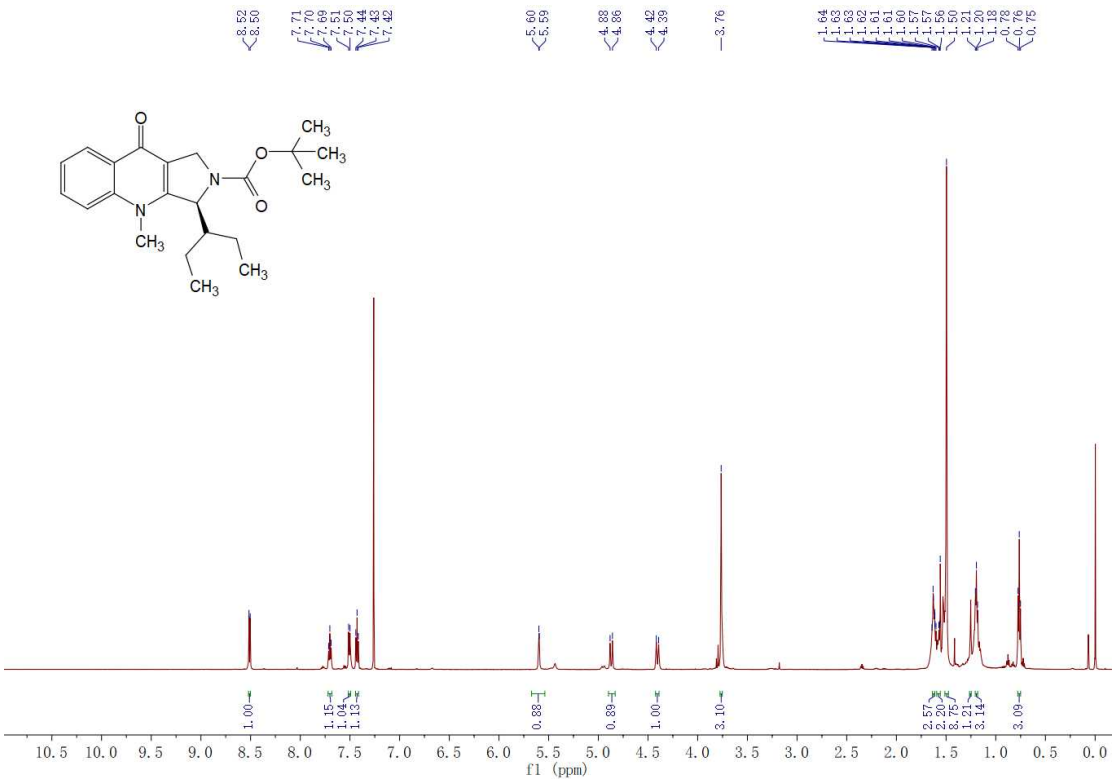
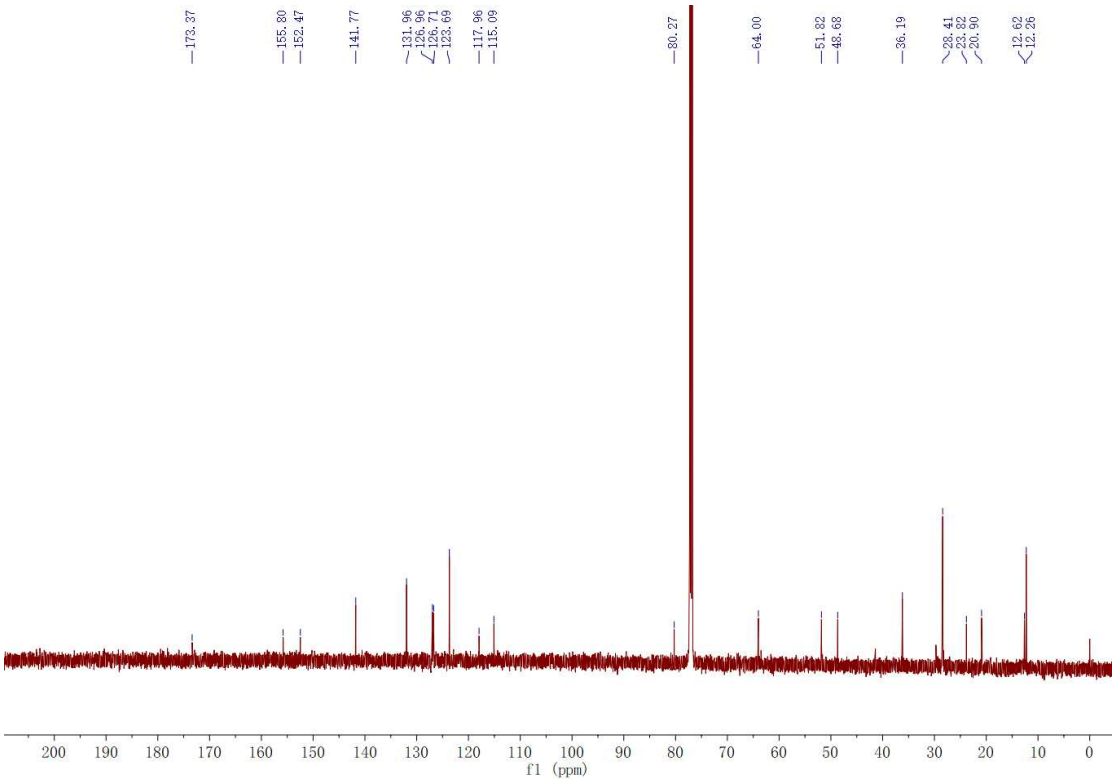
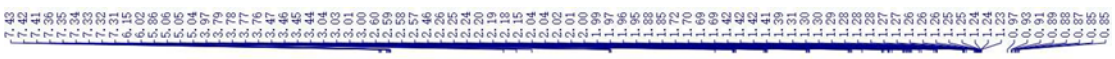
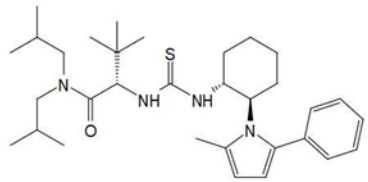
125.39
125.31
123.93
118.86
116.32
80.07
64.83
50.97
47.15
28.40
23.18
22.37
12.57
12.34
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

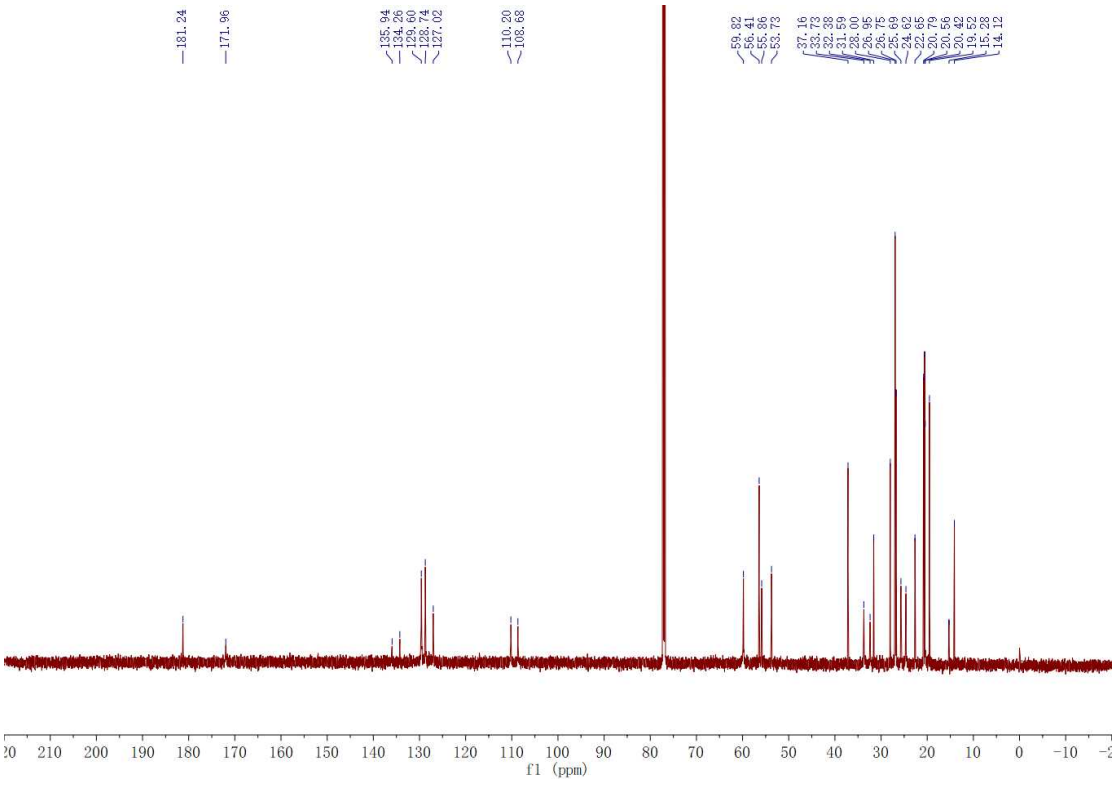

8.52
8.50
7.71
7.70
7.69
7.51
7.50
7.44
7.43
7.42
5.60
5.59
4.88
4.86
4.42
4.39
3.76
1.64
1.63
1.63
1.62
1.61
1.61
1.60
1.57
1.57
1.56
1.50
1.21
1.20
1.18
0.78
0.76
0.75
O
N
O
O
N
CH3
CH3
CH3
CH3
CH3
1.00
1.15
1.04
1.13
0.88
0.89
1.00
3.10
2.57
2.20
3.75
1.27
3.14
3.09
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


173.37
155.80
152.47
141.77
131.96
126.96
126.71
123.69
117.96
115.09
80.27
64.00
51.82
48.68
36.19
28.41
23.82
20.90
12.62
12.26
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)




S
N
NH
NH
O
N


1.97
2.13
1.15
1.00
1.06
1.03
0.93
0.93
0.82
1.04
1.21
1.15
1.11
1.20
3.17
3.06
2.13
1.96
1.03
3.53
9.06
5.85
5.94
1.28
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


181.24
171.96
135.94
134.26
129.60
128.74
127.02
110.20
108.68
59.82
56.41
55.86
53.73
37.16
35.73
32.38
31.59
31.00
26.95
26.75
25.69
24.62
22.65
20.79
20.56
20.42
19.52
15.28
14.12
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

## General experimental Information – Biology[3]

Standard solvents, salts, and media were purchased from commercial sources and used as received. All biological assays were performed in untreated, round-bottomed polystyrene 96-well microtiter plates (Costor 3799). Absorbance measurements were obtained using a SPECTRA MAX 190 plate reader running SoftMax Pro 7 software. Spectroscopic measurements were made in 200 µL of media in 96-well microtiter plates unless otherwise noted. Bacterial growth was assessed by measuring the culture cell density according to absorbance at 600 nm ($OD_{600}$). Biofilm was quantified by crystal violet (CV) staining according to absorbance at 590 nm ($A_{590}$). All reported CV measurements represent the amount of surface-attached (SA) biofilm at the bottom of the microtiter plate wells; the amount of air-liquid interface (Int) biofilm and total (TtI) biofilm was also measured, but are not reported here. Assay data were analyzed using Microsoft Office Excel 2017 and GraphPad Prism version 7 for Windows (GraphPad Software, Inc.). Data are shown as percent of the "Benzimidazole" positive control and represent the average and standard error (STE) of 3 replicate wells from a single trial; we note that replicate trials gave similar results as the single trials reported here. Half maximal inhibitory concentration ($IC_{50}$) values and half maximal dispersal concentration ($DC_{50}$) values were calculated using a sigmoidal curve fit (three parameters) in GraphPad Prism and are reported with a 95% confidence interval (95% CI).

## Crystal violet static biofilm assay protocol

A widely used Crystal violet (CV) microtiter plate biofilm assay was used in this study. Bacterial samples for biofilm assessment were prepared by first streaking a freezer stock of *Pseudomonas aeruginosa* PAO1 on an LB/agar plate and incubating at 37 °C for 16 hours. Single colonies were selected and used to inoculate Luria-Bertani (LB) medium (10 mL) in a 25 mL Erlenmeyer flask. The culture was grown on an incubator-shaker (200 rpm) at 37 °C for 16 hours, then diluted with fresh LB medium to $OD_{600}$ ~0.1. Using a multi-channel pipette, the culture was added to plates in 200 µL aliquots. Plates were covered, and incubated statically at 37 °C for 24 hours (the maximal biofilm formation is obtained at 24 h). Bacterial suspension was removed by inverting and shaking the plate, then glue with paper towels. Non-biofilm associated biomaterial was removed by washing with 200 µL Phosphate buffered saline (PBS) and then repeating this process twice. Biofilm was fixed to 96-well microtiter plates by uncovering the plate and thermally dehydrating at 37 °C. Dried biofilm was stained for 15 minutes at room temperature with a 200 µL aliquot of the CV staining solution. The stain was removed, then residual stain was removed by washing with 200 µL PBS. The amount of CV retained by the biofilm was quantified by using with 30% AcOH to re-solubilizing the CV and measuring the absorbance at 590 nm. This number is directly related to the amount of biofilm in the well.

There are four experiment groups of compounds, Quinolactacin-H, (*R*)-Quinolactacin, (*S*)-Quinolactacin and Benzimidazole as positive control. Compound stock solutions in DMSO were prepared at 10 mM. The final well concentrations upon inoculum addition were as follows: $10^x$ µM where x = 3, 2.5, 2, 1.5, 1, 0, -1, or -2. All compounds were tested in replicates of 3 wells and were assessed after 24 hours incubation. All groups were tested in replicates of 3 wells and were assessed after 24 h incubation. $IC_{50}$ values were calculated using a sigmoidal curve fit (three parameters) and are reported with a 95% CI (Figure 9).

For biofilm dispersion assessment, biofilms allowed to form in 96-well microtiter plates in the absence of compound for 24 h, then non-biofilm material was removed by dumping and washing as normal with fresh LB media. These Stocks DMSO with compound were diluted in series to obtain final concentrations as follows: $10^x$ µM where x = 3, 2.5, 2, 1.5, 1, 0, -1, or -2. Then, fresh LB media was added to let each of the wells with 200 µL solution. The 96-well microtiter plates were incubated at 37 °C for an additional 24 hours. After the first 24 h culture, to assess the amount of biofilm, one set of wells were given a fresh LB medium to assess the amount of biofilm for 48

h, while another set of wells was given DMSO without compound to assess the effect of DMSO to dispersion biofilm. After 48 hours incubation, non-biofilm material was removed by dumping and washing 3× with fresh PBS. Biofilm was fixed and quantified with CV as described above. The amount of dispersed biofilm was determined via comparison of the amount of biofilm at 48 hours in the presence of compound versus the amount of biofilm in the "no compound" blank control at 48 hours. All compounds were tested in replicates of 5 wells. $DC_{50}$ values for biofilm dispersion were calculated using a sigmoidal curve fit (three parameters) and are reported with a 95% CI (Figure 10).

### *P. aeruginosa* biofilm assay data for (*R*)-Quinolactacin-H and (*S*)-Quinolactacin-H

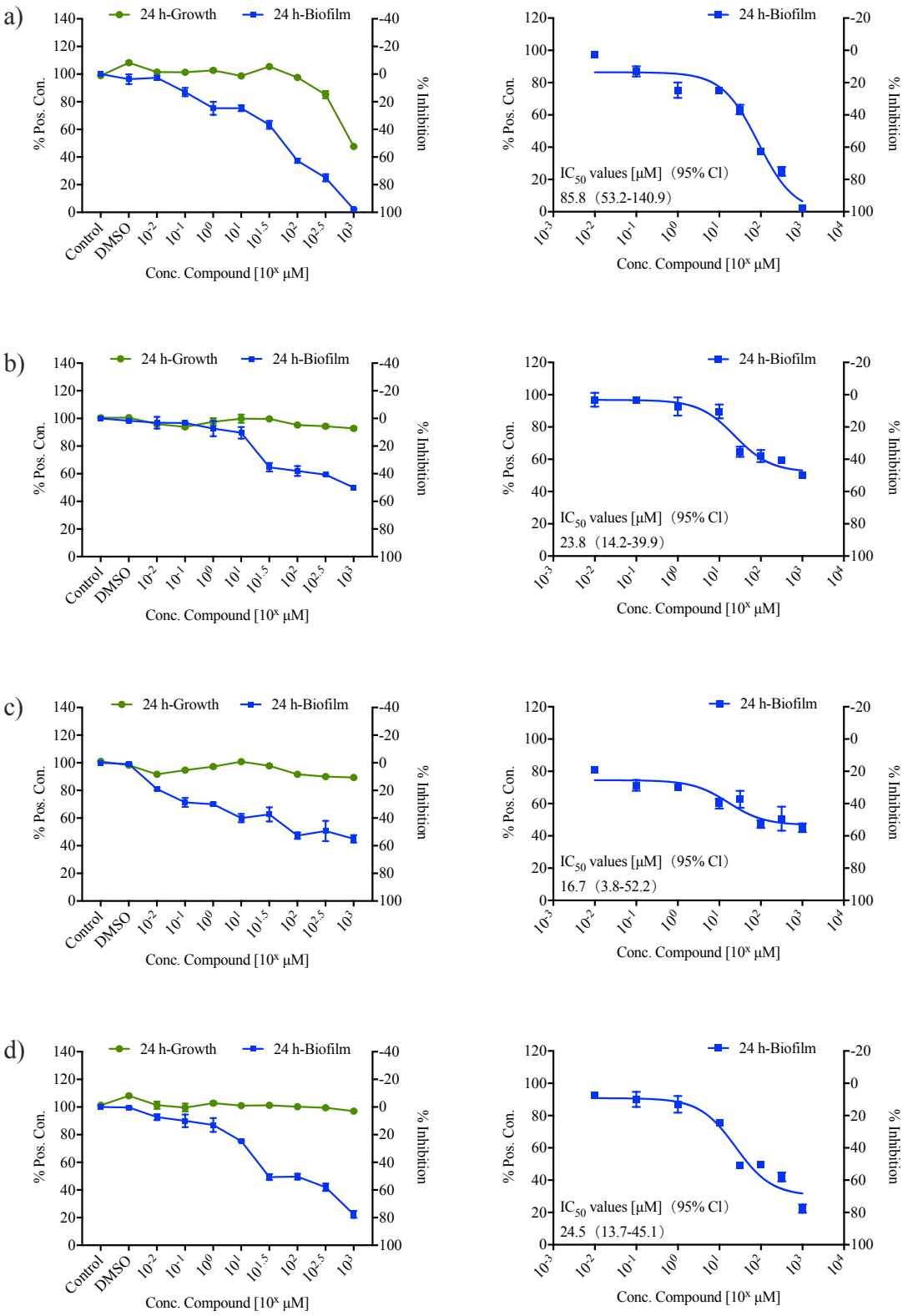



**Figure 9**. *P. aeruginosa* PAO1 biofilm formation and bacterial growth dose-response curves (left) and sigmoidal curve fits with $IC_{50}$ values (right) for biofilm inhibition.

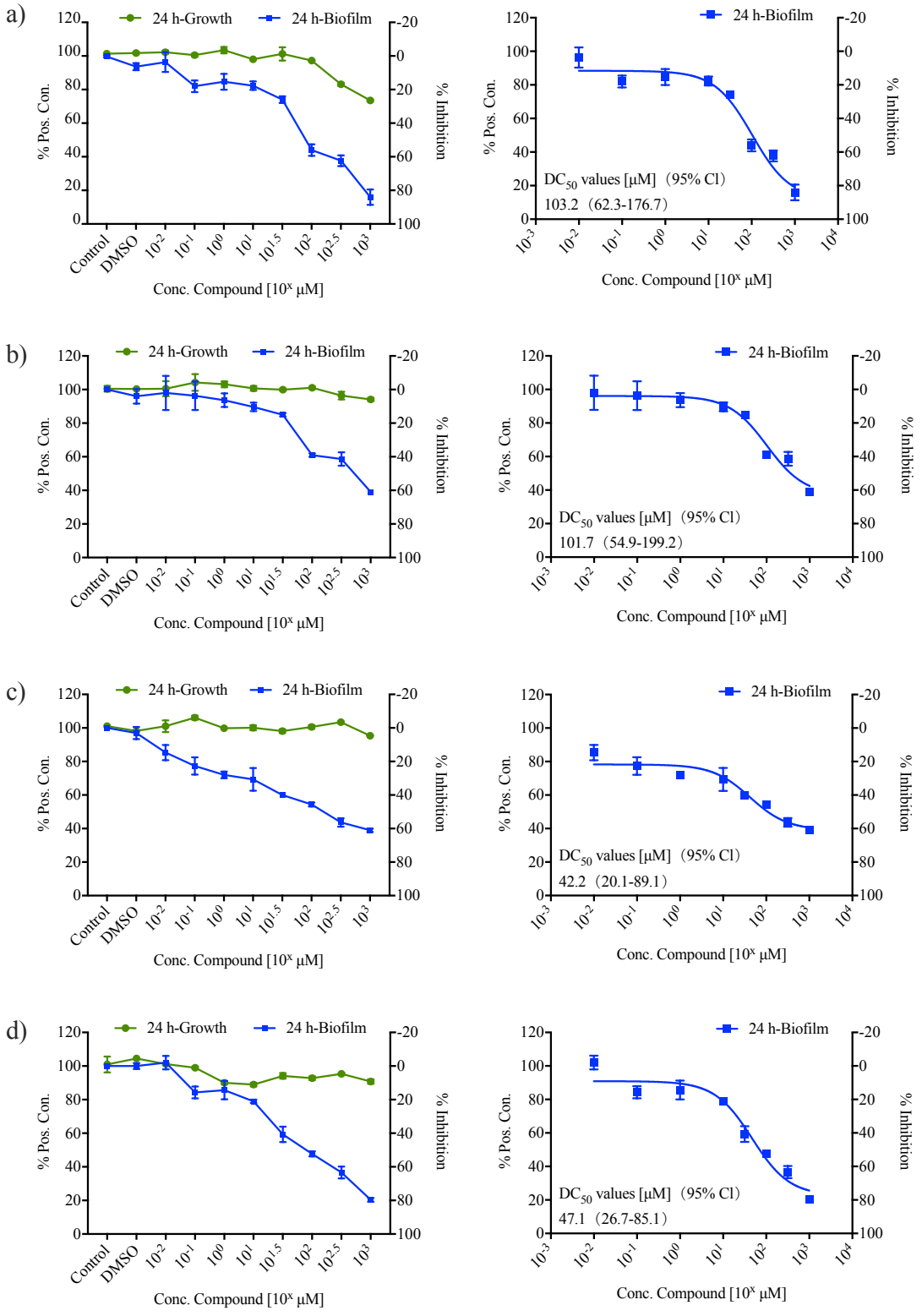



**Figure 10.** *P. aeruginosa* PAO1 biofilm dispersion and bacterial growth dose-response curves (left) and sigmoidal curve fits with $DC_{50}$ values (right) for biofilm dispersion.

## References and Notes


1. X. Q. Zhang, W.Q. Jiang and Z. H. Sui, *J. Org. Chem.,* 2003, **68**, 4523.
2. M. S. Taylor and E. N. Jacobsen, *J. Am. Chem. Soc.,* 2004, **126**, 10558.
3. Reto Frei, Anthony S. Breitbach and Helen E. Blackwell, *Angew. Chem. Int. Ed.*, 2012, **51**, 5226.