Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.
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Supporting information

Advances in 1-phenanthryl-tetrahydroisoquinoline series of PAK4 inhibitors: Potent agent restrains tumor cell growth and invasion.

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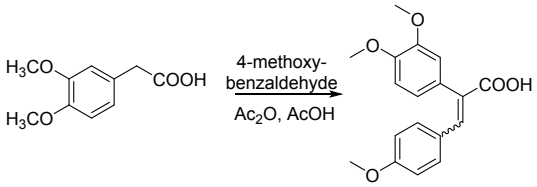
1. Synthesis of key intermediates(**5-10**).

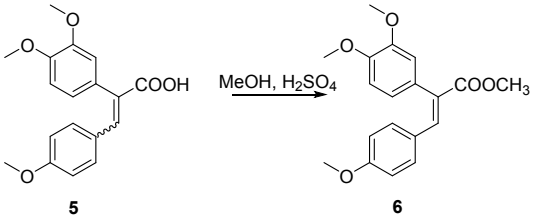
(*Z*/*E*)-2-(3,4-dimethoxyphenyl)-3-(4-methoxyphenyl)acrylic acid



4 **5** (*E*/*Z*)=9:1

A solution of (3,4-dimethoxyphenyl)acetic acid **4** (9.8 g, 50 mmol), 4-methoxybenzaldehyde (6.0mL, 50 mmol), acetic anhydride (19.5 mL), and triethylamine (9.5 mL) was refluxed with stirring under argon protection for 10 h. The resulting solution was cooled to room temperature, 10% NaOH solution (150 mL) was added, and then the resulting mixture was stirred for 2 h at 90°C. The reaction mixture was cooled to room temperature and acidified with concentrated hydrochloric acid (pH 4). The yellow solid obtained was collected by filtration and recrystallized from MeOH to yield pure **5** as yellow needles (12.5 g, 79%). (*E*)-isomer: mp 217.1–218.2 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ 3.66 (s, 3H), 3.71 (s, 3H), 3.78 (s, 3H), 6.67 (dd, J = 8.12, 1.88Hz, 1H), 6.74 (d, J = 1.88 Hz, 1H), 6.79 (d, J = 8.88 Hz, 2H), 6.97 (d, J = 8.24 Hz, 1H), 7.05 (d, J = 8.84 Hz, 2H), 7.67(s, 1H), 12.43(s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ 55.6, 55.9, 56.0, 112.4, 113.6, 114.3 (2C), 122.2, 127.5, 129.4, 131.0, 132.4 (2C), 139.0, 148.7, 149.3, 160.3, 169.2; *m/z* (ES+) $(M+H)^+$ =315.2, $(M+Na)^+$=337.2, $(M+K)^+$=353.1. (*Z*)-isomer: ^{1}H NMR (400MHz, DMSO-d_6) δ 3.78 (s, 6H), 3.81 (s, 6H), 6.97-6.93 (m, 5H), 7.12 (d, J = 1.48 Hz, 1H), 7.46 (d, J = 8.68Hz, 2H), 13.26 (s, 1H); *m/z* (ES-) 312.9 $(M-H)^-$.

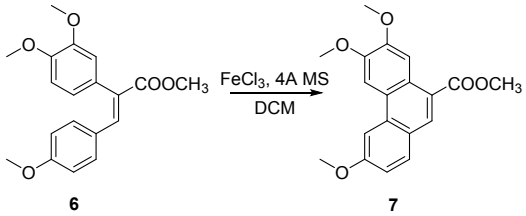
E-2-(3,4-Dimethoxyphenyl)-3-(4-methoxyphenyl)-acrylic acid methyl ester



5 **6**

To a mixture of **5** (7.9 g, 25 mmol) in MeOH (75 mL) was slowly added concentrated H_2SO_4 (0.5 mL). The mixture was refluxed for 6 h and then cooled to room temperature overnight. The crude solid was filtered and washed twice with ice-cold MeOH, dried in vacuo to give **6**. The filtrate was evaporated under reduced pressure and diluted with water. The mixture was extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (25% ethyl acetate in n-hexane) to afford an addition of **6** as an off-yellow solid (6.1 g, 74%): mp 110.6–111.7 °C; ^{1}H NMR (400MHz, $CDCl_3$) δ 3.76 (s, 1H), 3.79 (s, 1H), 3.80 (s, 1H), 3.93 (s, 1H), 6.70 (d, J = 8.88 Hz, 2H), 6.74 (d, J = 1.88 Hz,1H), 6.79 (dd, J = 8.16, 1.88 Hz, 1H), 6.90 (d, J = 8.20 Hz, 1H), 7.02 (d, J = 8.88 Hz, 1H), 7.78 (s, 1H); ^{13}C NMR (100MHz, $CDCl_3$) δ 52.3, 55.2, 55.8, 55.9, 111.5, 112.9, 113.7 (2C), 122.1, 127.3, 128.7, 129.6, 132.4 (2C), 140.2, 148.6, 149.1, 160.3, 168.7; *m/z* (ES+) $(M+H)^+$ =329.2, $(M+Na)^+$ =351.2, $(M+Na)^+$ =367.1.

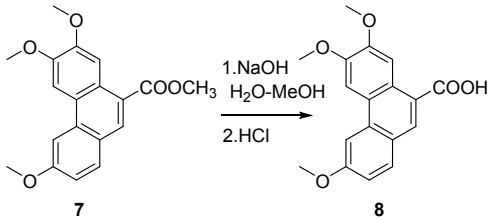
3, 6, 7-trimethoxyphenanthrene-9-carboxylic acid methyl ester



6 **7**

To a mixture of **6** (8.2 g, 25 mmol), 4Å molecular sieves (24.6 g) in anhydrous CH_2Cl_2 (250 mL) was added anhydrous $FeCl_3$ (14.2 g, 87.5 mmol) portion-wise at 0 °C. The mixture was stirred at 0 °C for a further 8h. The dark brown mixture was poured into saturated aqueous $NaHCO_3$ solution (250 mL).The mixture was subsequently filtered and the aqueous phase was extracted with CH_2Cl_2. The combined organic phase was dried over Na_2SO_4, and evaporated in vacuo to yield a dark brown solid. The residue was purified by silica gel column chromatography (n-hexane: ethyl acetate =3:1 as eluent) to afford **7** as a light yellow solid (5.3 g, 65%): mp 151.2–152.0 °C; ^{1}H NMR (400MHz, $CDCl_3$) δ 4.01(s, 1H), 4.03(s, 1H), 4.09(s, 1H), 4.11(s, 1H), 7.22 (dd, J = 8.8, 2.40 Hz, 1H), 7.81 (d, J = 2.2 Hz, 1H), 7.85 (d, J = 8.8 Hz, 1H), 7.87(s, 1H), 8.44 (s, 1H), 8.65 (s, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ 52.0, 55.6, 55.8, 55.9, 103.3, 103.8, 107.0, 116.0, 121.7, 124.3, 125.0, 125.2, 131.3, 131.9, 133.4, 148.9, 149.9, 160.2, 168.2; *m/z* (ES+) $(M+H)^+$ =327.2, $(M+Na)^+$=349.2.

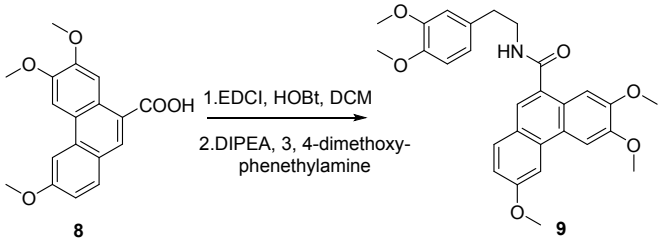
3,6,7-trimethoxyphenanthrene-9-carboxylic acid



7 **8**

To a suspension of **7** (0.99 g, 3 mmol) in MeOH (9 mL) and water (9 mL), NaOH (0.72 g, 18 mmol) was added, and the resulting mixture was refluxed for 4 h. After cooling, the solution was acidified with concentrated HCl (pH 2). The formed precipitate was filtered, washed with water, and dried under vacuum for 12 h to give **8** as a white solid (0.92 g, 98%): mp 213.0–214.6 °C; ^{1}H NMR (400MHz, $CDCl_3$) δ 3.92 (s, 3H), 4.04 (s, 3H), 4.06 (s, 3H), 7.29 (dd, J = 8.8, 2.3 Hz, 1H), 8.04 (d, J = 8.8 Hz, 1H), 8.11 (d, J = 2.2 Hz, 1H), 8.14 (s,1H), 8.45 (s, 1H), 8.58 (s, 1H), 12.94 (s, 1H); ^{13}C NMR (100MHz, $CDCl_3$) δ 55.7, 56.2, 56.3, 104.6, 104.7, 107.3, 117.0, 124.3, 125.0, 125.2, 130.8, 132.2, 133.3, 149.3, 150.0, 160.4, 169.4; *m/z* (ES-) m/z 310.9 $[M-H]^-$.

N-(3,4-dimethoxyphenethyl)-3,6,7-trimethoxyphenanthrene-9-carboxamide

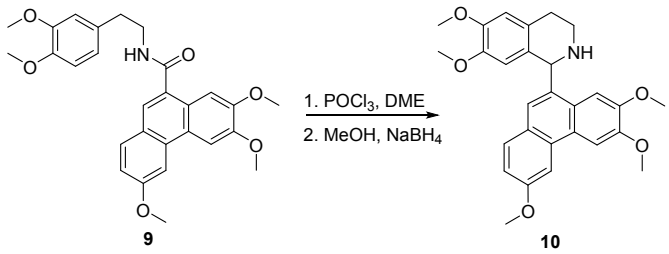


8 **9**

A solution of **8** (0.62 g, 2 mmol), EDCI (0.57g, 3 mmol) and HOBt (0.41 g, 3 mmol) in anhydrous CH_2Cl_2 (12 mL) were stirred at 25°C for 3.5 h. Then 3,4-dimethoxyphenethylamine (0.33 g, 2 mmol) and DIPEA (0.38mL, 4 mmol) were added and the reaction mixture was stirred at the same temperature for 1.5 h. The organic layer was washed with water and dried over Na_2SO_4. Removal of the solvent gave a residue that was purified by column chromatography (silica gel, CH_2Cl_2: acetone 20:1 as eluent)

to furnish **9** as a white solid (0.78 g, 82%): mp 149.8–151.5 °C; ^{1}H NMR (400MHz, $CDCl_3$) δ 2.98 (t, J = 6.8 Hz, 2H), 3.81 (q, J = 13.2, 6.9 Hz, 2H), 3.85(s, 3H), 3.87(s, 3H), 4.02(s, 3H), 4.03(s, 3H), 4.11(s, 3H), 6.16 (t, J = 5.7 Hz, 1H), 6,82(s, 1H), 6.83(s, 2H), 7.20 (dd, J = 8.8, 2.3 Hz, 1H), 7.60(s, 1H), 7.72(d, J = 8.8 Hz, 1H), 7.81 (d, J = 2.3 Hz, 1H), 7.86 (s, 1H), 7.89(s, 1H); ^{13}C NMR (100MHz, $CDCl_3$) δ 35.3, 41.2, 55.5, 55.9, 55.9 (2C), 56.0, 103.3, 103.8, 106.5, 111.5, 112.1, 116.0, 120.8, 124.3, 124.4, 124.5, 124.9, 129.8, 130.7, 131.4, 132.0, 149.1, 149.3, 149.7, 159.3, 170.1; *m/z* (ES+) $(M+H)^+$ =476.2.

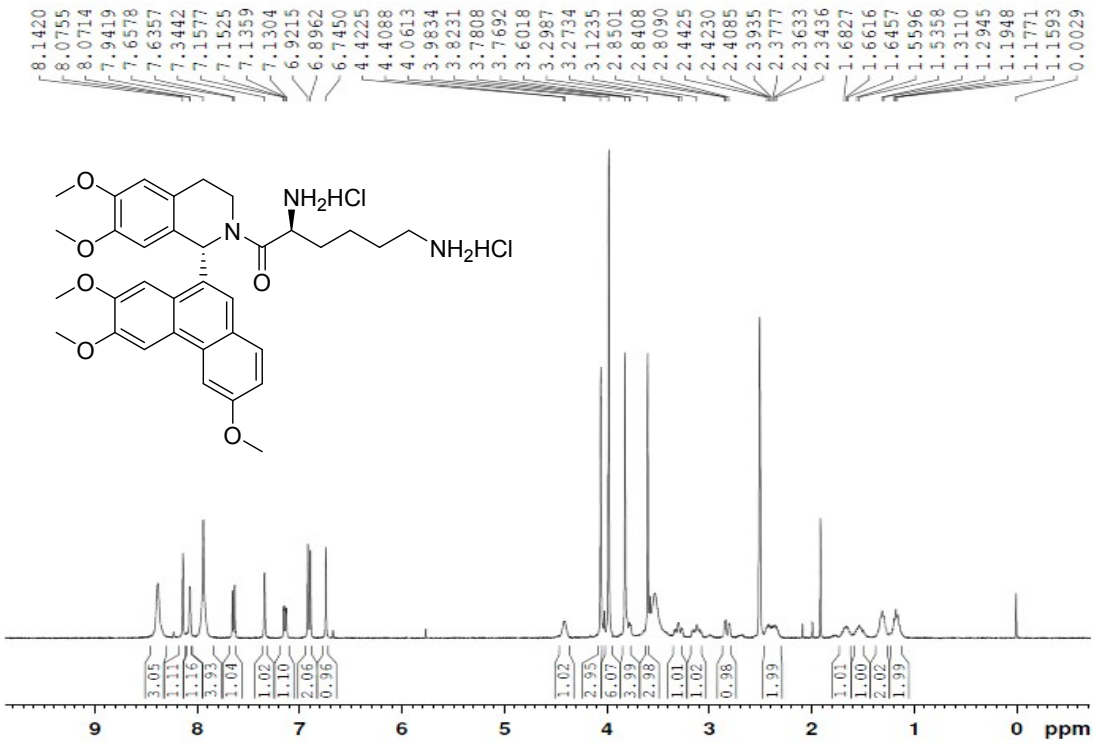
6, 7-dimethoxy-1-(3, 6, 7-trimethoxyphenanthren-9-yl)-1, 2, 3, 4-tetrahydroisoquinoline



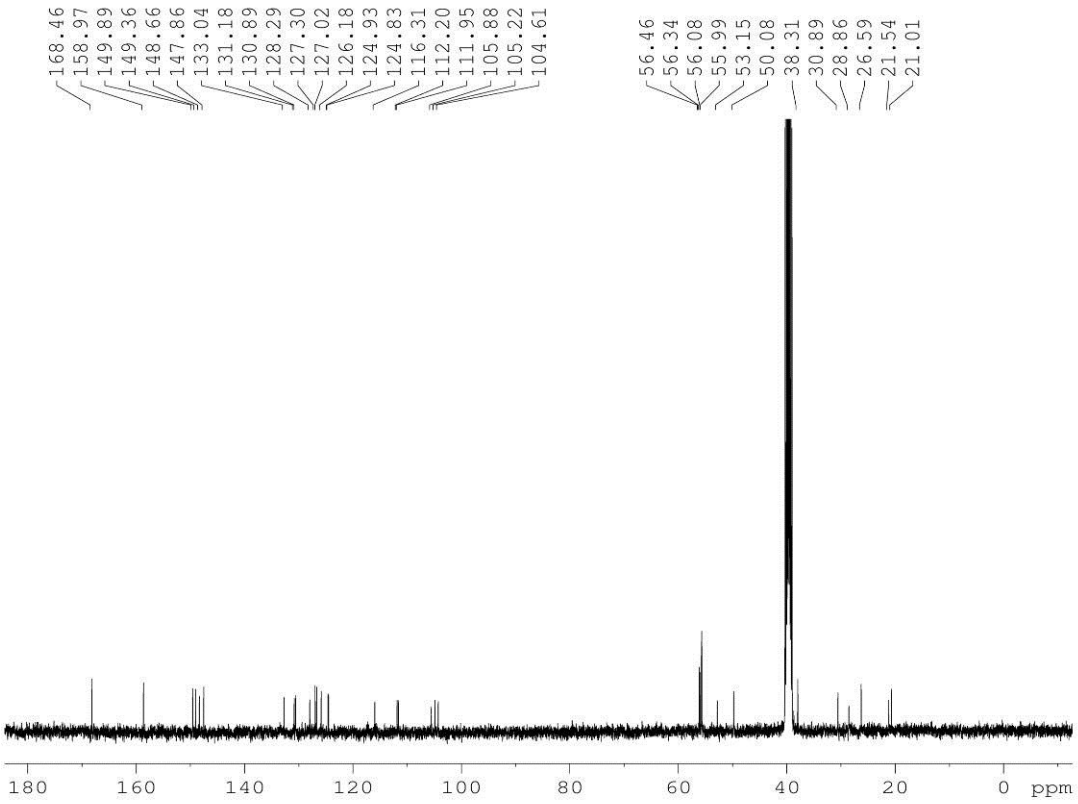
9 **10**

To a solution of **9** (0.48 g, 1 mmol) in dimethoxyethane (5 mL), phosphorus oxychloride (2 mL, 4.0 mmol) was added, and the resulting mixture was refluxed for 3.5 h. The reaction mixture was cooled to 0°C for 1h and formed precipitate was filtered to afford the dihydroisoquinoline intermediate without further purification. To a solution of the dihydroisoquinoline (0.35 g, 0.76 mmol) in MeOH (5 mL) at 0 °C, sodium borohydride (37.8 mg, 1 mmol) was added slowly. The resulting mixture was stirred for 1.5 h at 0 °C and diluted with water. The mixture was extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, DCM: MeOH 20:1 as eluent) to furnish **10** as a white solid (0.29 g, 63% for 2 steps) : mp 270.5–272.8 °C; ^{1}H NMR (400MHz, $CDCl_3$) δ 2.75-2.79(m, 1H), 2.97-3.00(m, 1H), 3.15-3.17(m, 4H), 3.68(s, 3H), 3.74(s, 3H), 3.99(m, 3H), 4.00(s, 3H), 4.11(q, 1H), 4.13(s, 3H), 5.58(s, 1H), 6.20(s, 1H), 6,80(s, 1H), 7.19 (dd, J = 8.7, 2.2 Hz, 1H), 7.44(s, 1H), 7.71(s, 1H), 7.78 (d, J = 8.7 Hz, 1H), 8.06(d, J = 2.2 Hz, 1H), 8.06(s, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ 29.4, 49.1, 55.4, 55.8, 55.9, 55.99(2C), 56.3, 104.5, 104.7, 111.0, 112.5, 116.26(2C), 125.37(2C), 125.5, 126.0, 126.8, 128.0, 130.5(2C), 131.1, 147.4, 147.8, 148.6, 148.8, 158.5; m/z (ES^+) $(M+H)^+$ =460.3, $(M+Na)^+$ =482.3.

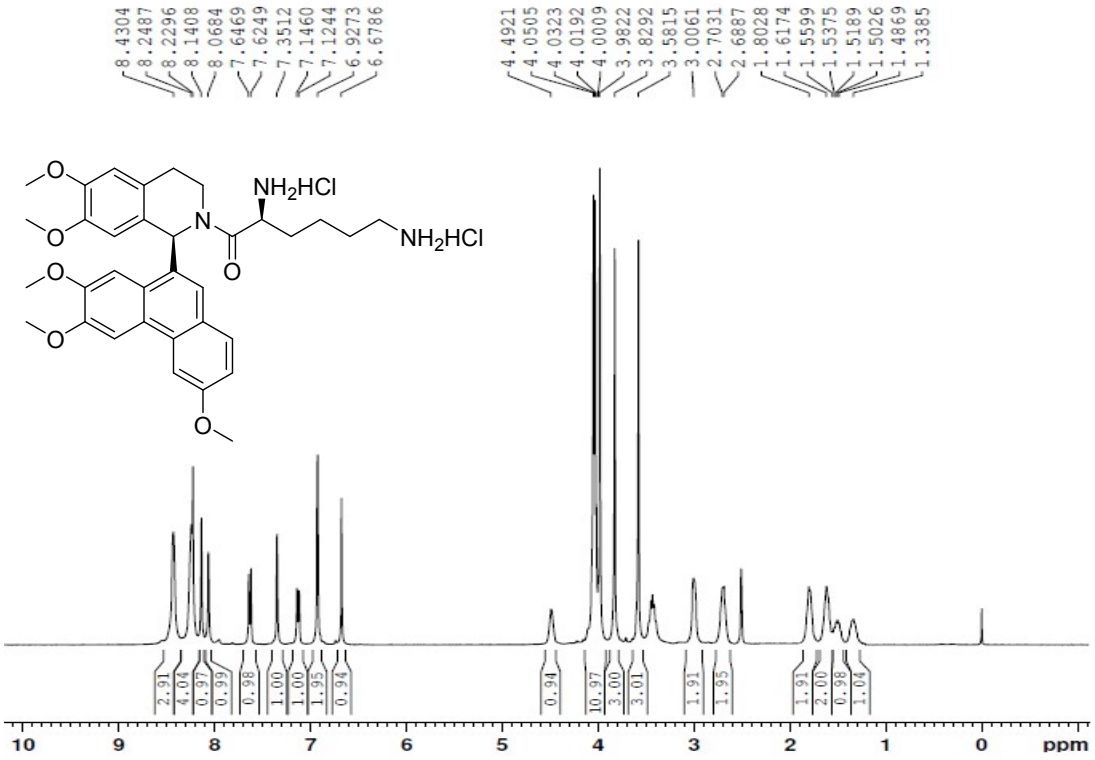
2. The ^{1}H-NMR and ^{13}C-NMR spectrums of compounds **(11h, 12a-k)**.



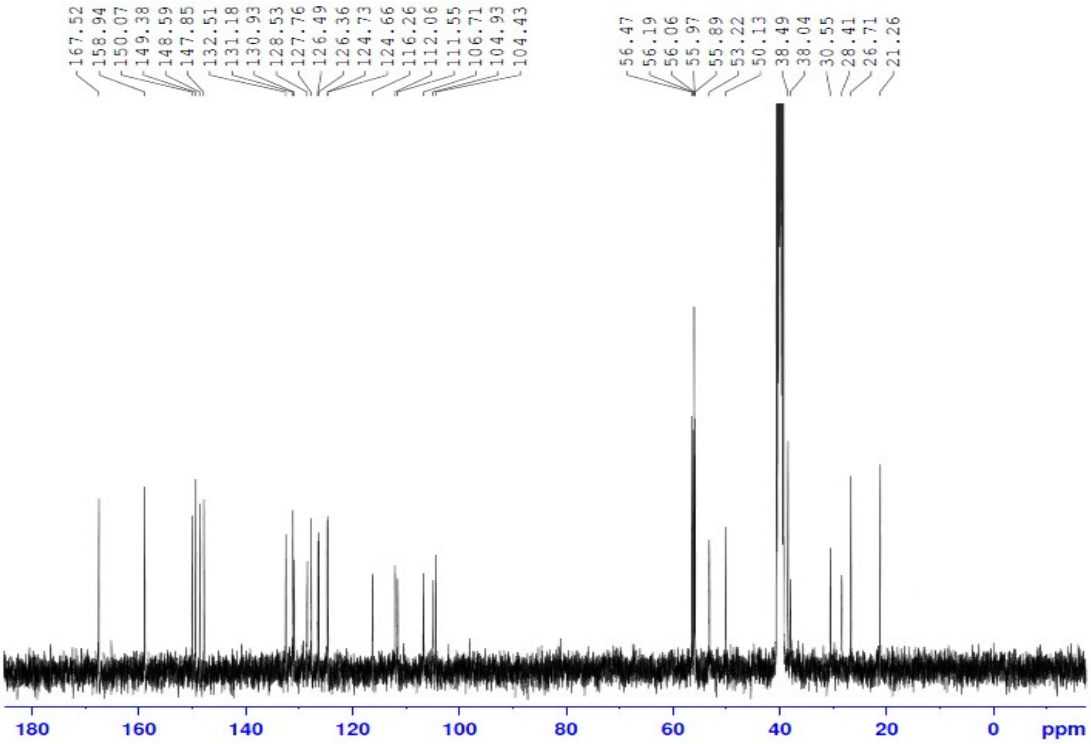
The ^{1}H-NMR spectrum of Compd. **12a**.



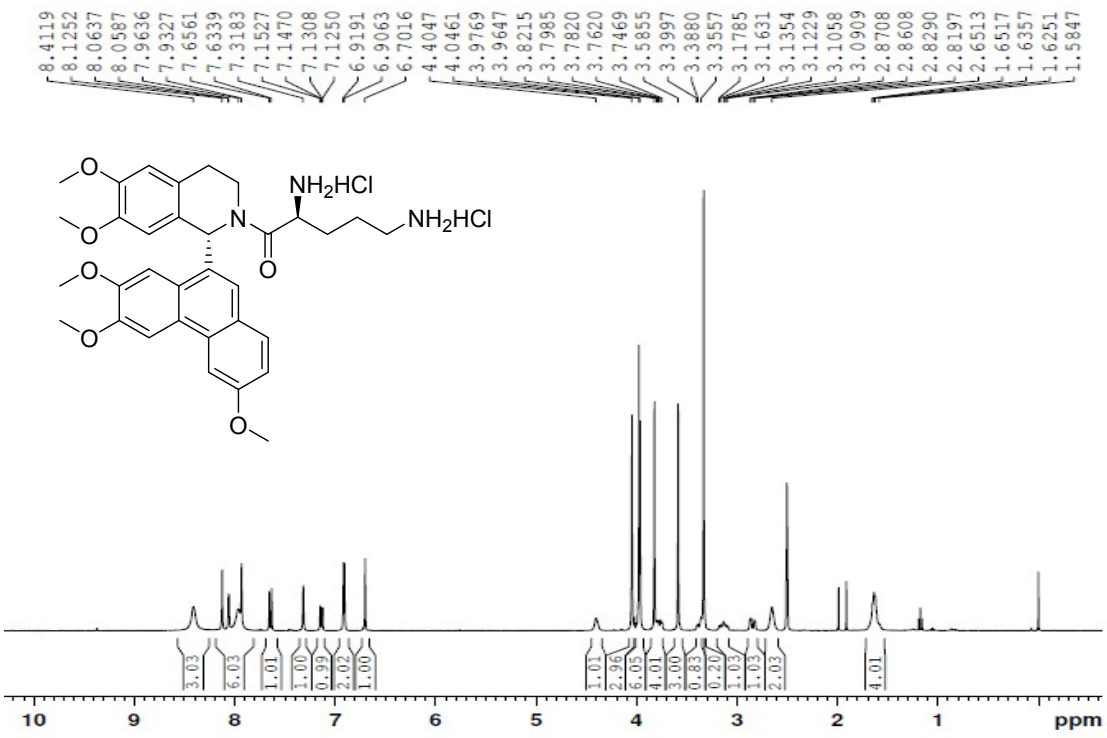
The ^{13}C-NMR spectrum of Compd. **12a**.



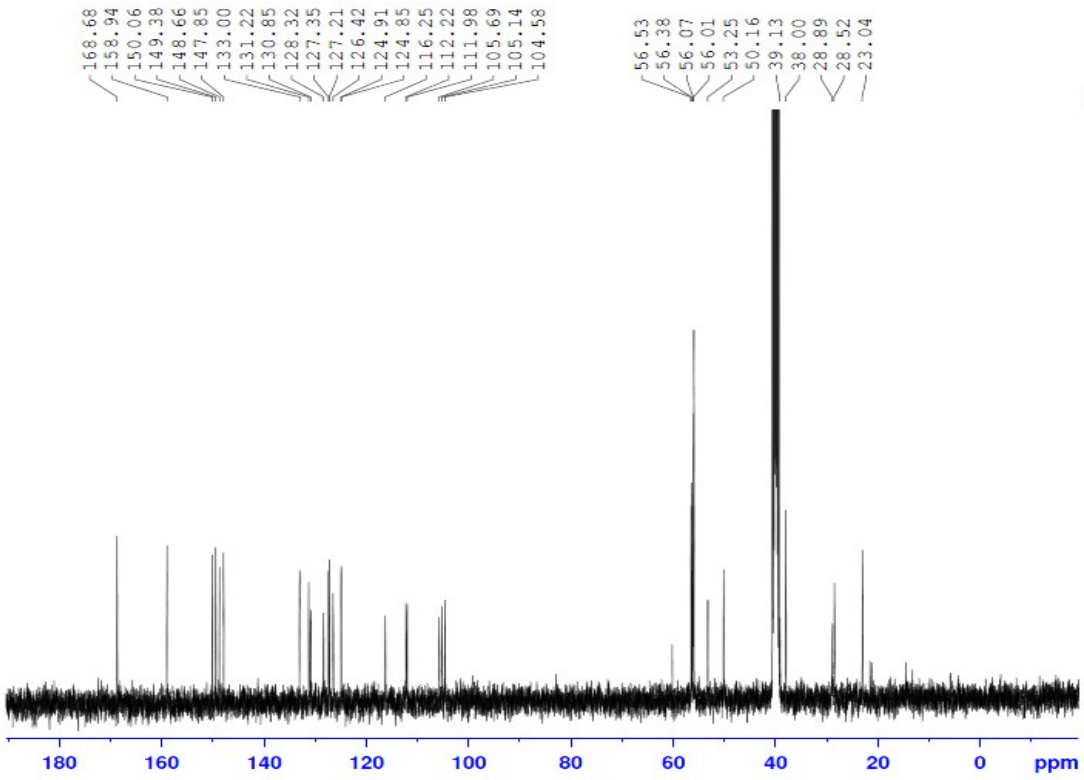
The ^{1}H-NMR spectrum of Compd. **12b**.



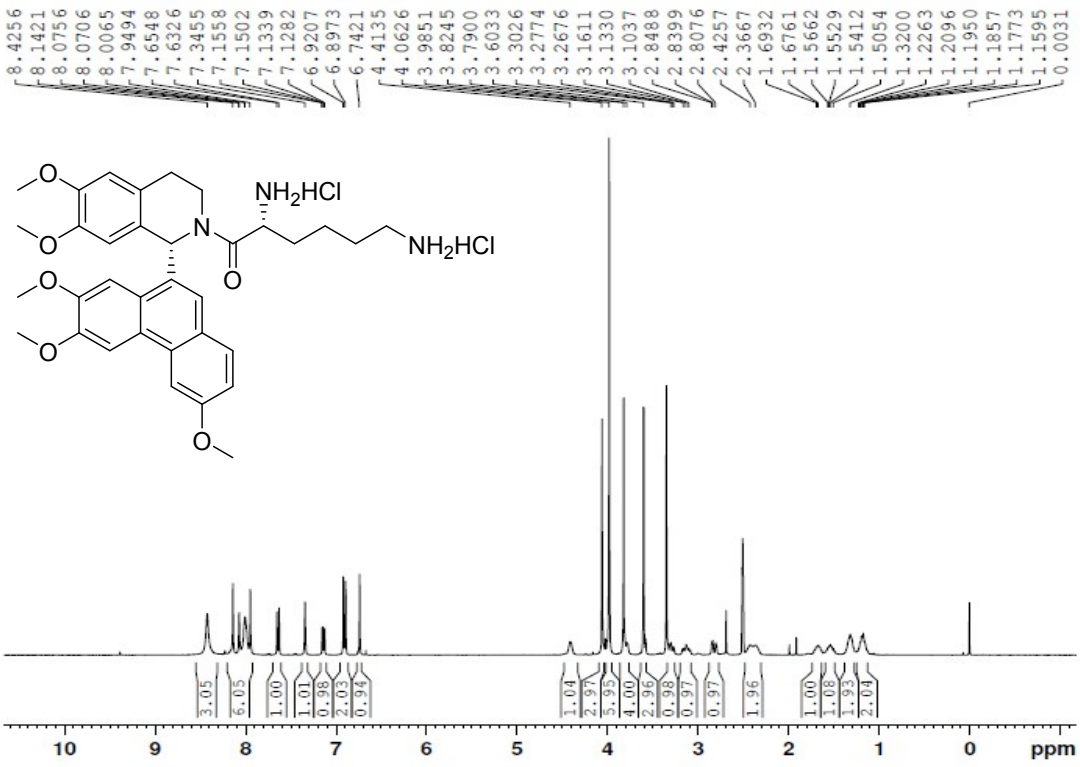
The ^{13}C-NMR spectrum of Compd. **12b**.



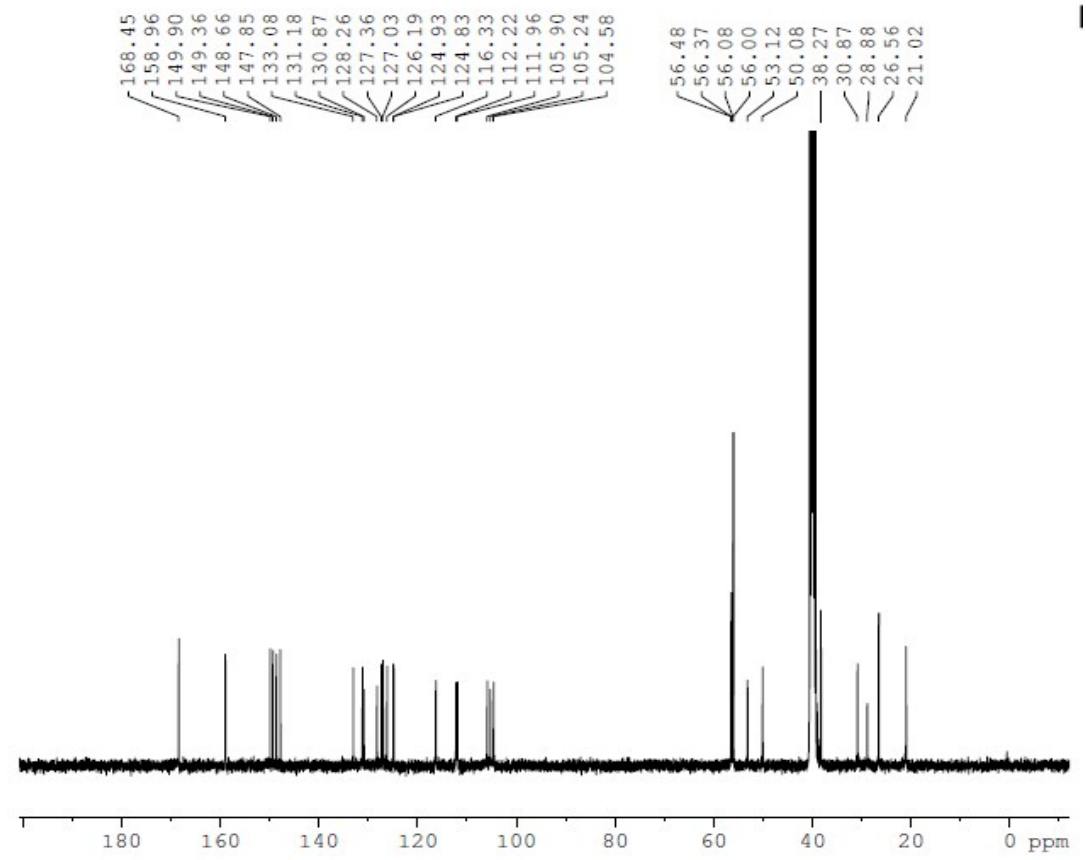
The [1]H-NMR spectrum of Compd. **12c**.



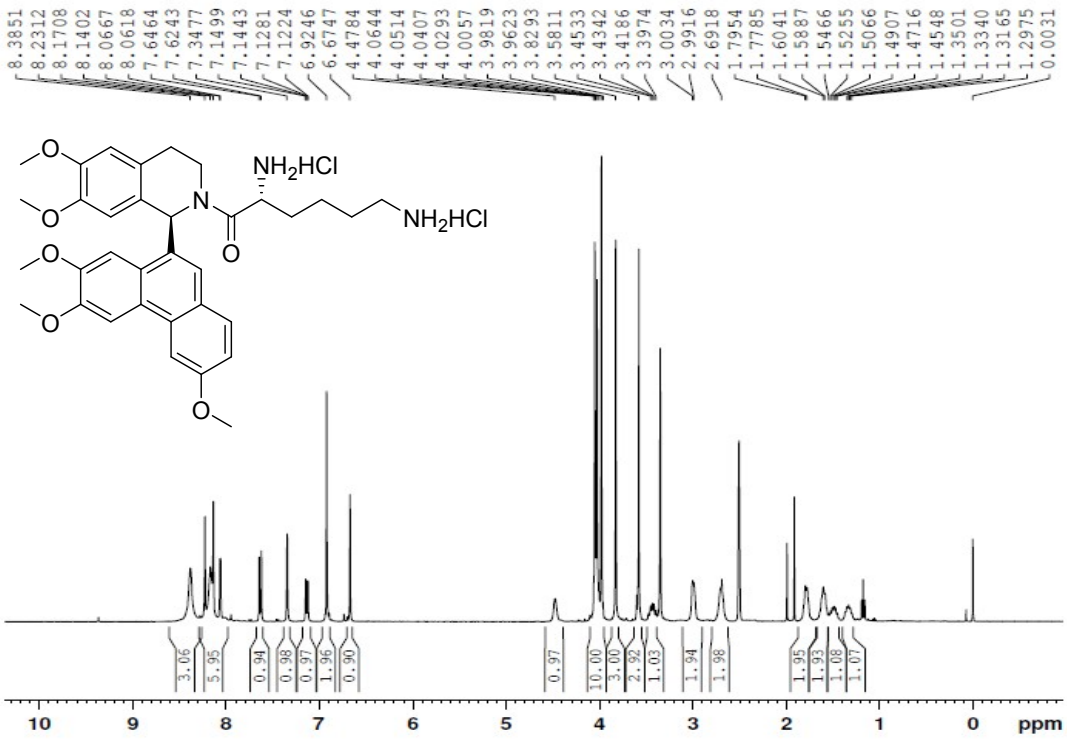
The [13]C-NMR spectrum of Compd. **12c**.



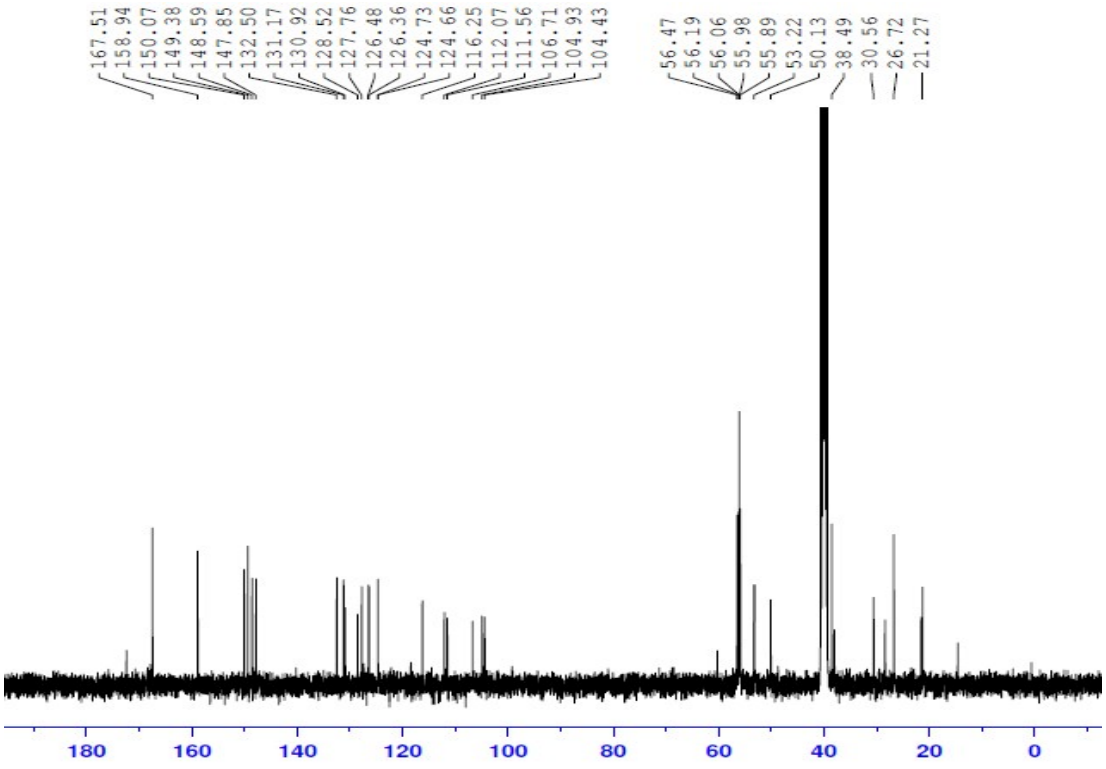
The ^{1}H-NMR spectrum of Compd. **12d**.



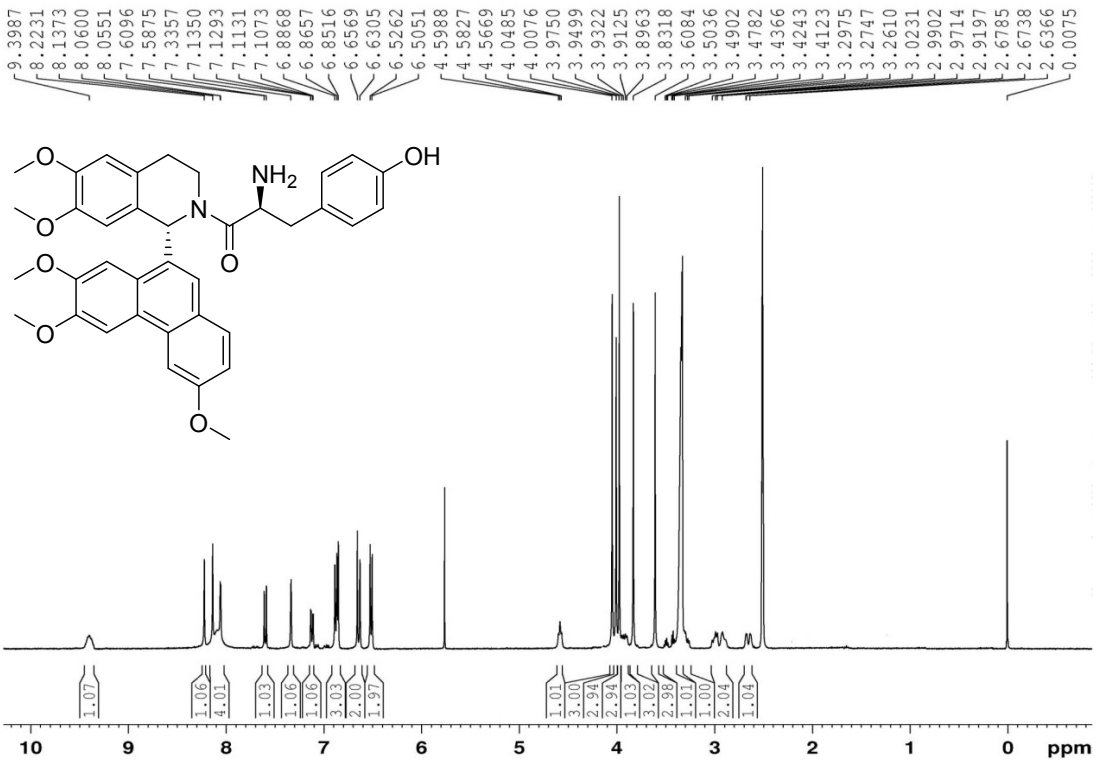
The ^{13}C-NMR spectrum of Compd. **12d**.



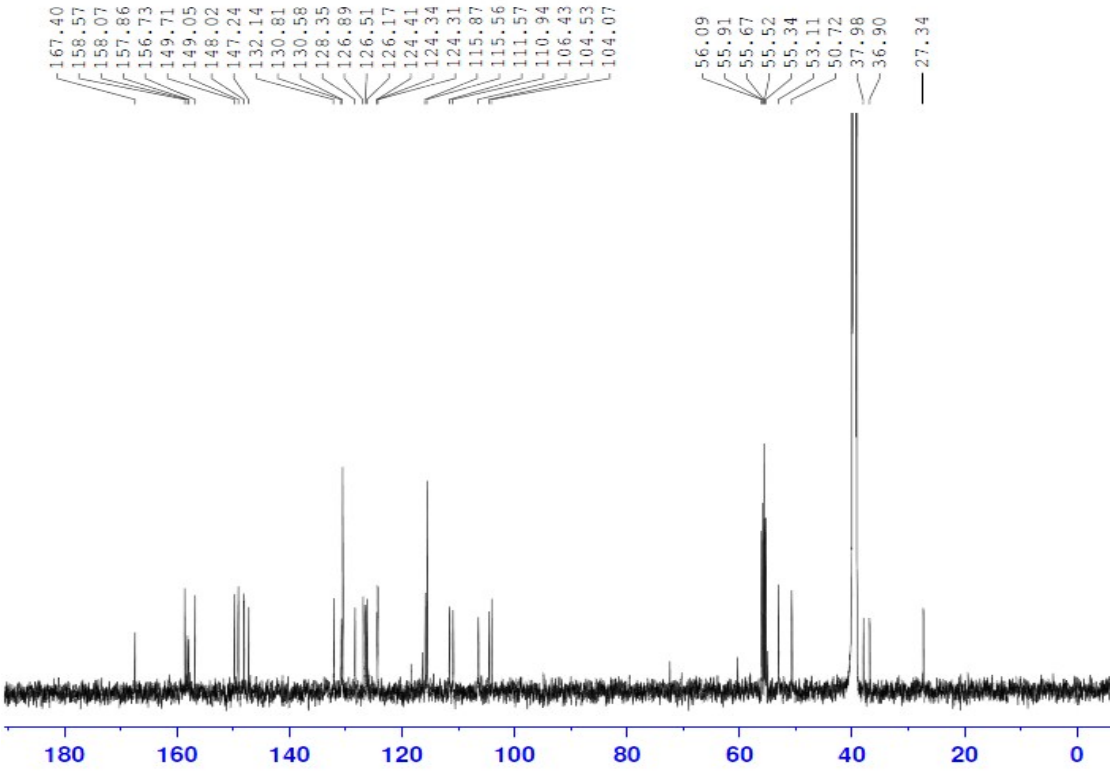
The ^{1}H-NMR spectrum of Compd. **12e**.



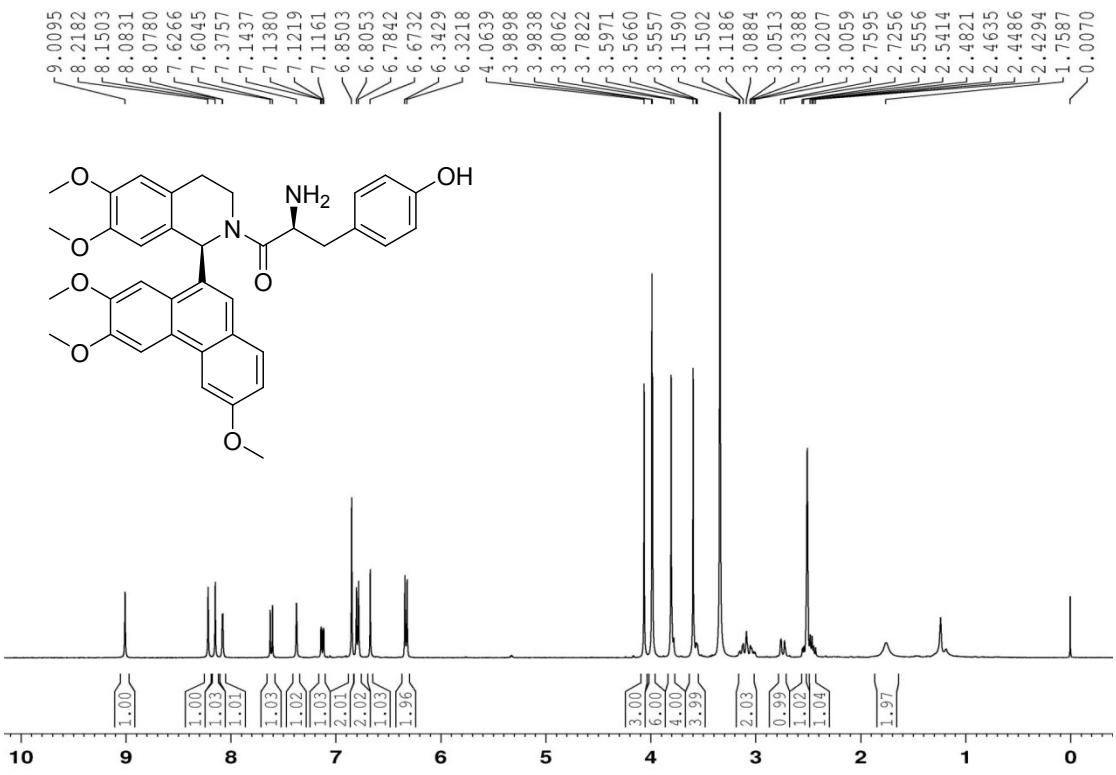
The ^{13}C-NMR spectrum of Compd. **12e**.



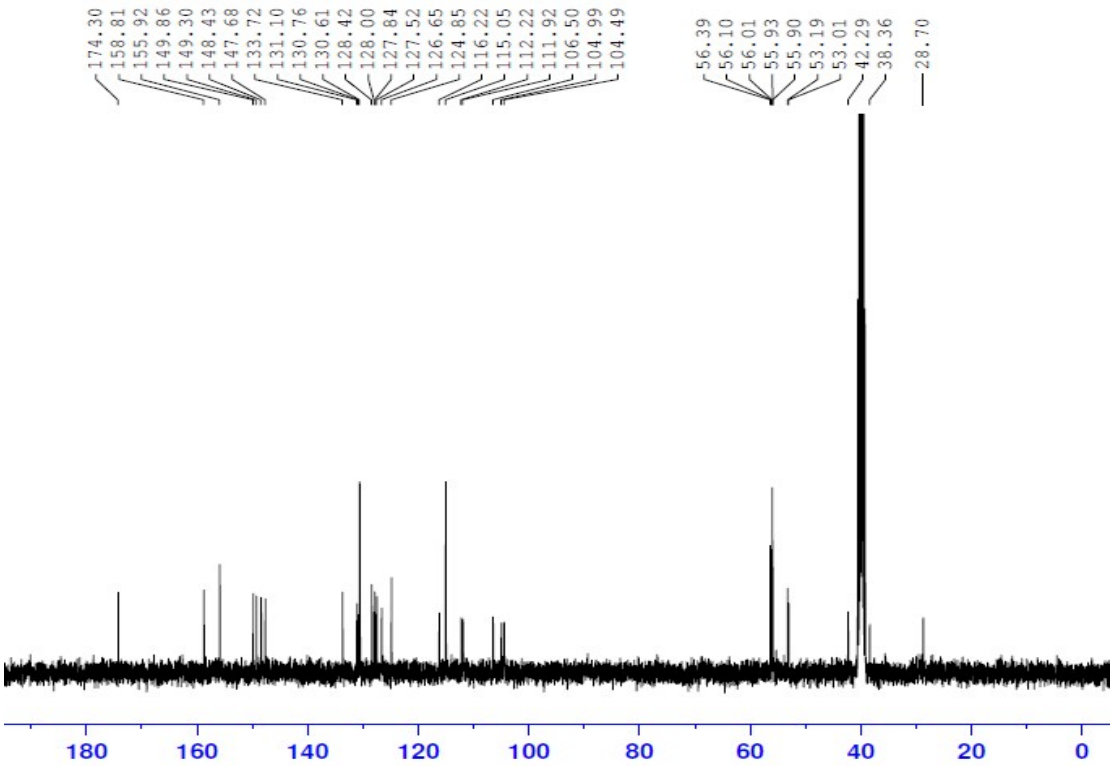
The [1]H-NMR spectrum of Compd. **12f**.



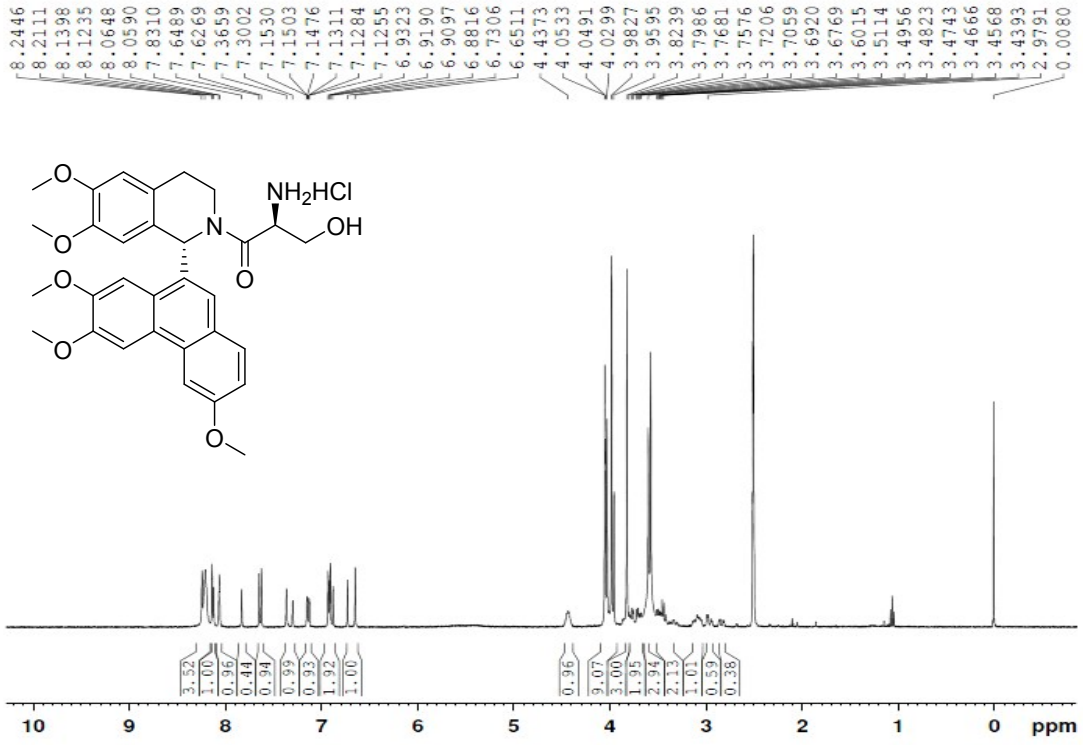
The [13]C-NMR spectrum of Compd. **12f**.



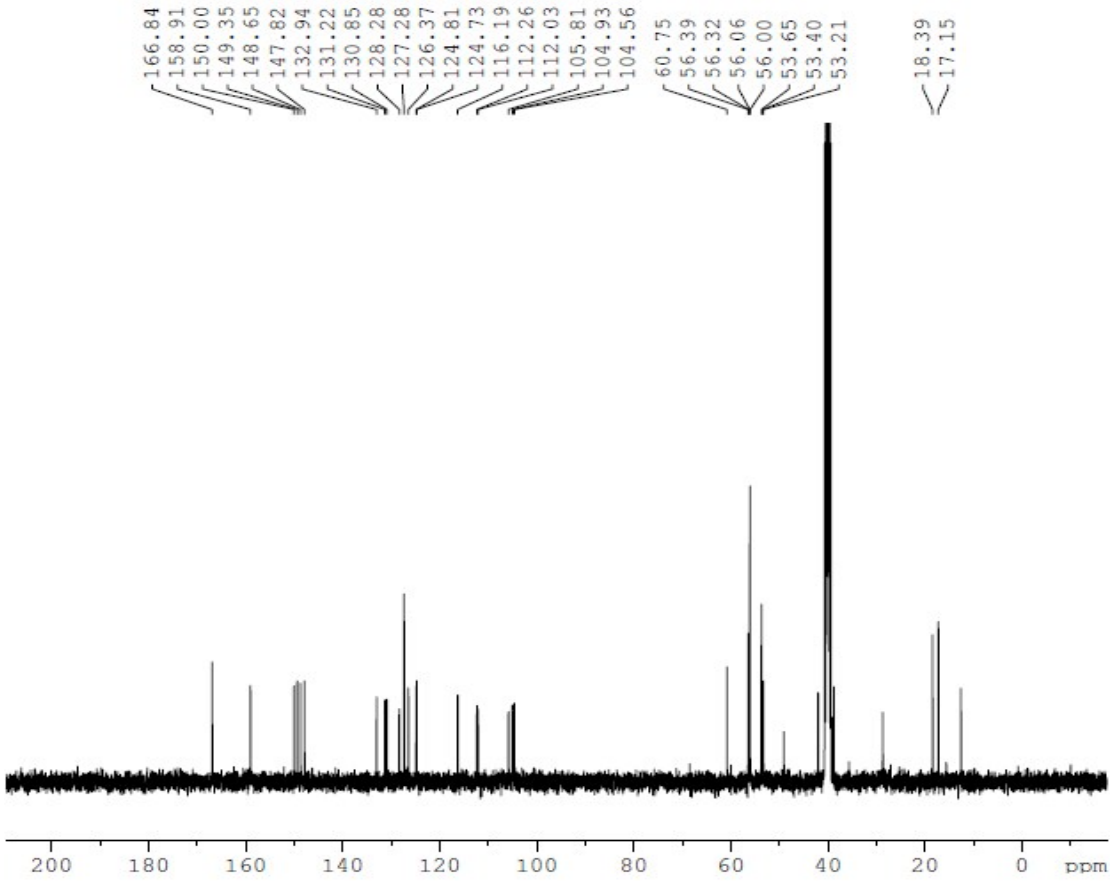
The ^{1}H-NMR spectrum of Compd. **12g**.



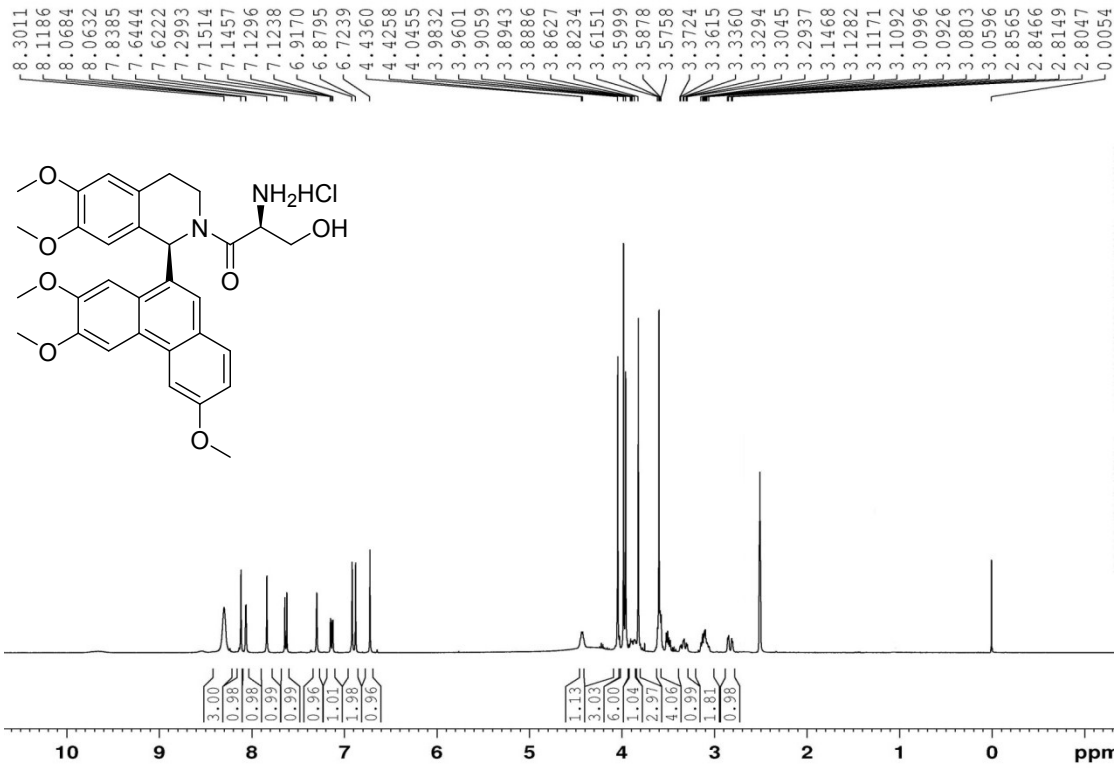
The ^{13}C-NMR spectrum of Compd. **12g**.



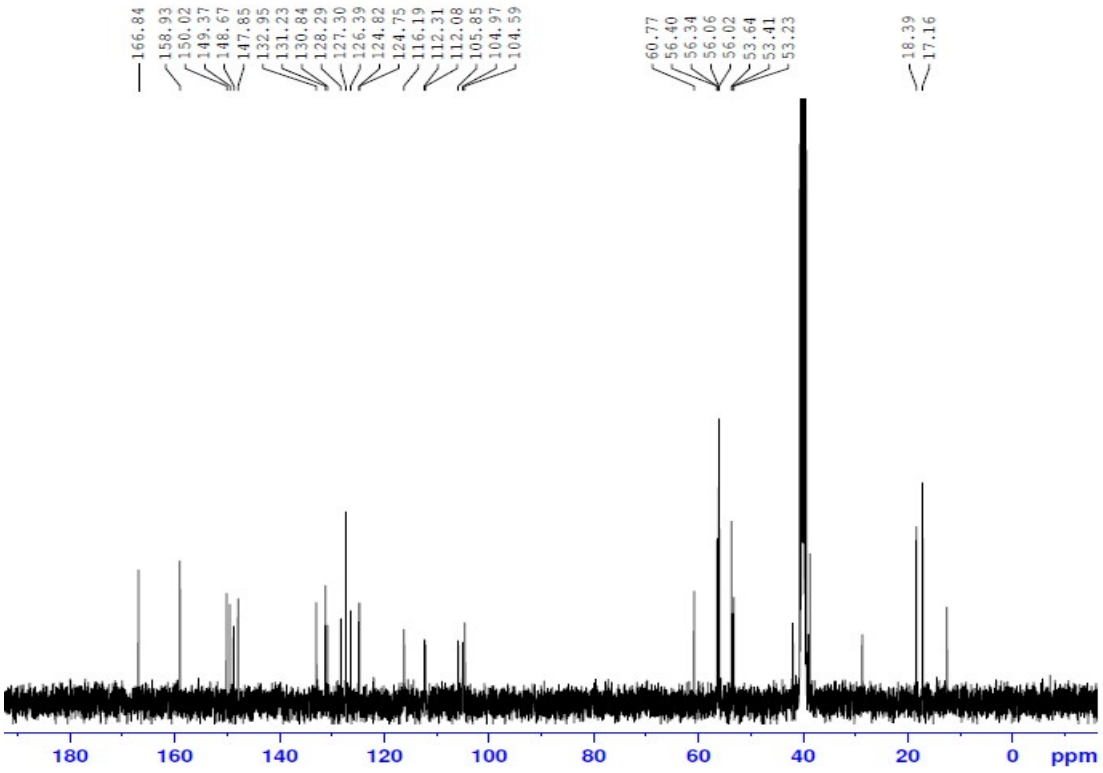
The ^{1}H-NMR spectrum of Compd. **12h**.



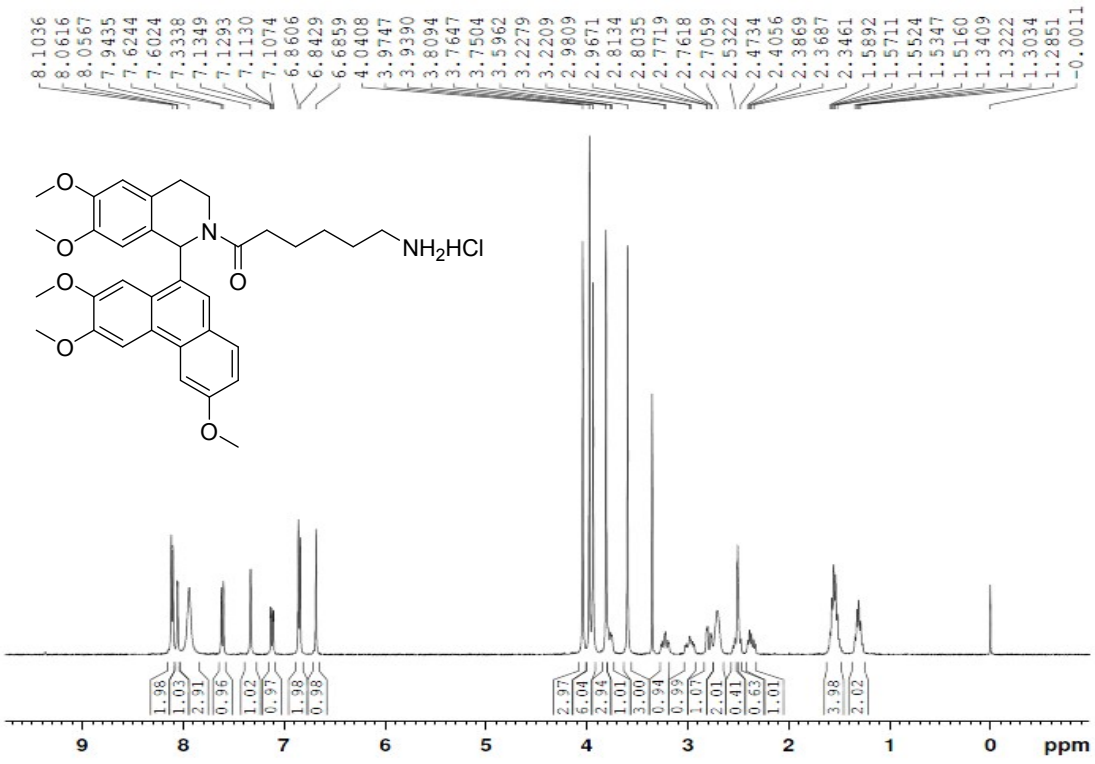
The ^{13}C-NMR spectrum of Compd.**12h**.



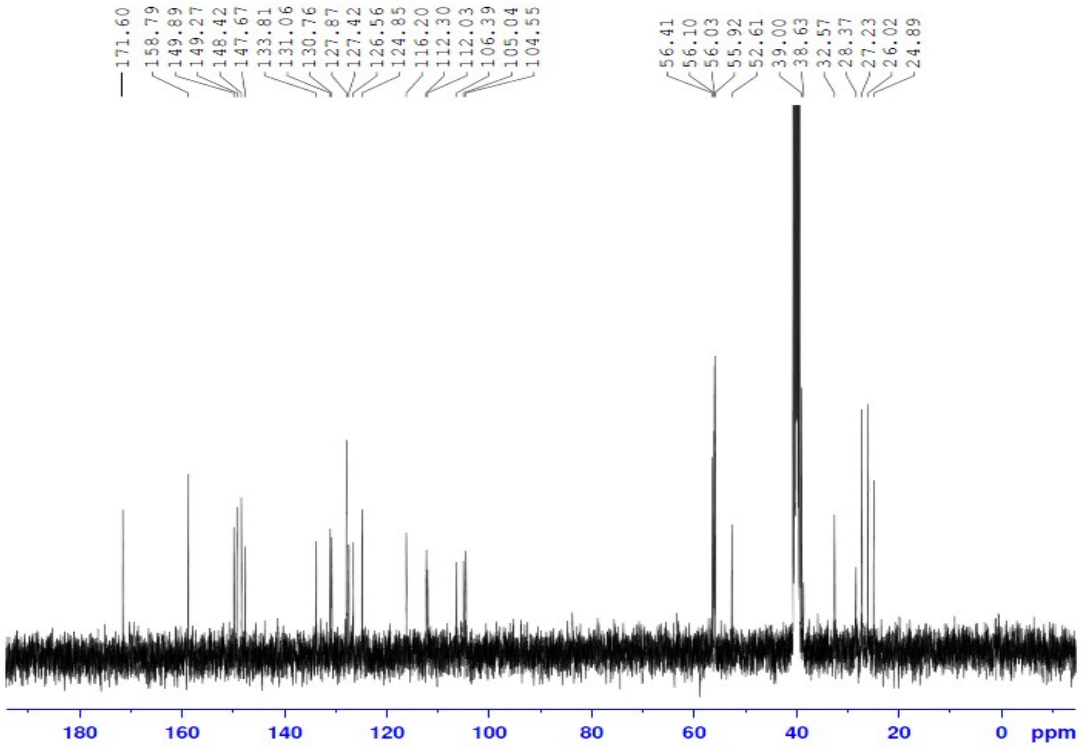
The ^{1}H-NMR spectrum of Compd.**12i**.



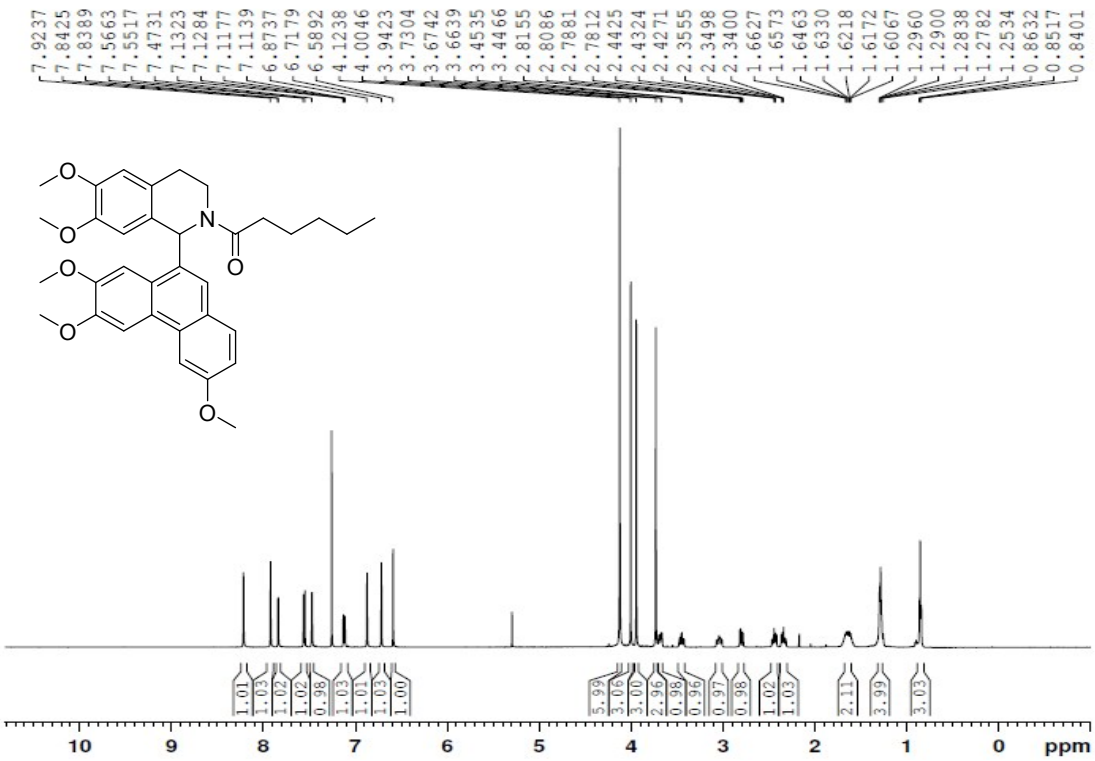
The ^{13}C-NMR spectrum of Compd.**12i**.



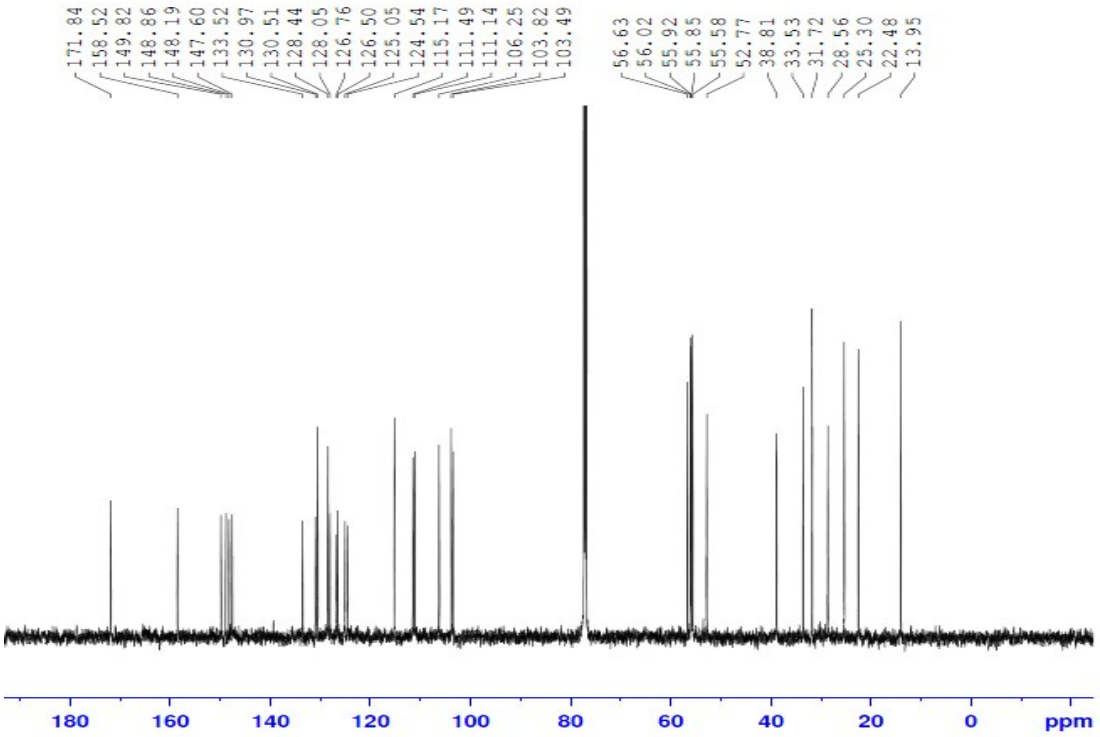
The ^{1}H-NMR spectrum of Compd.**12j**.



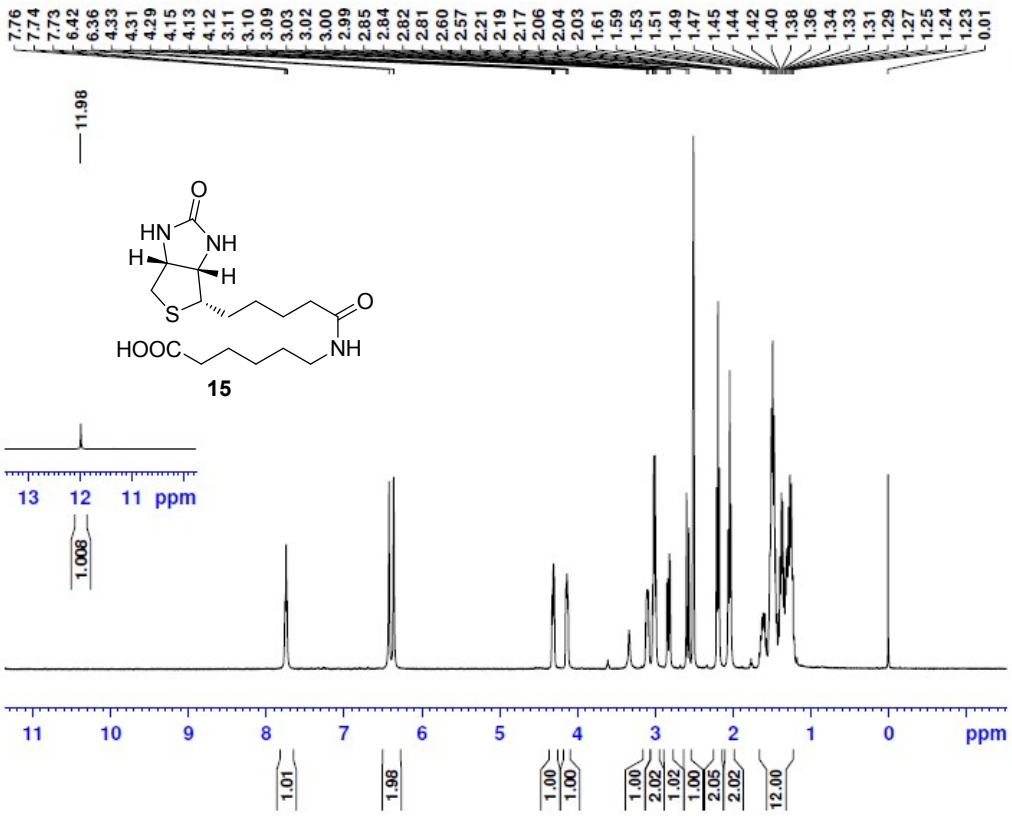
The ^{13}C-NMR spectrum of Compd.**12j**.



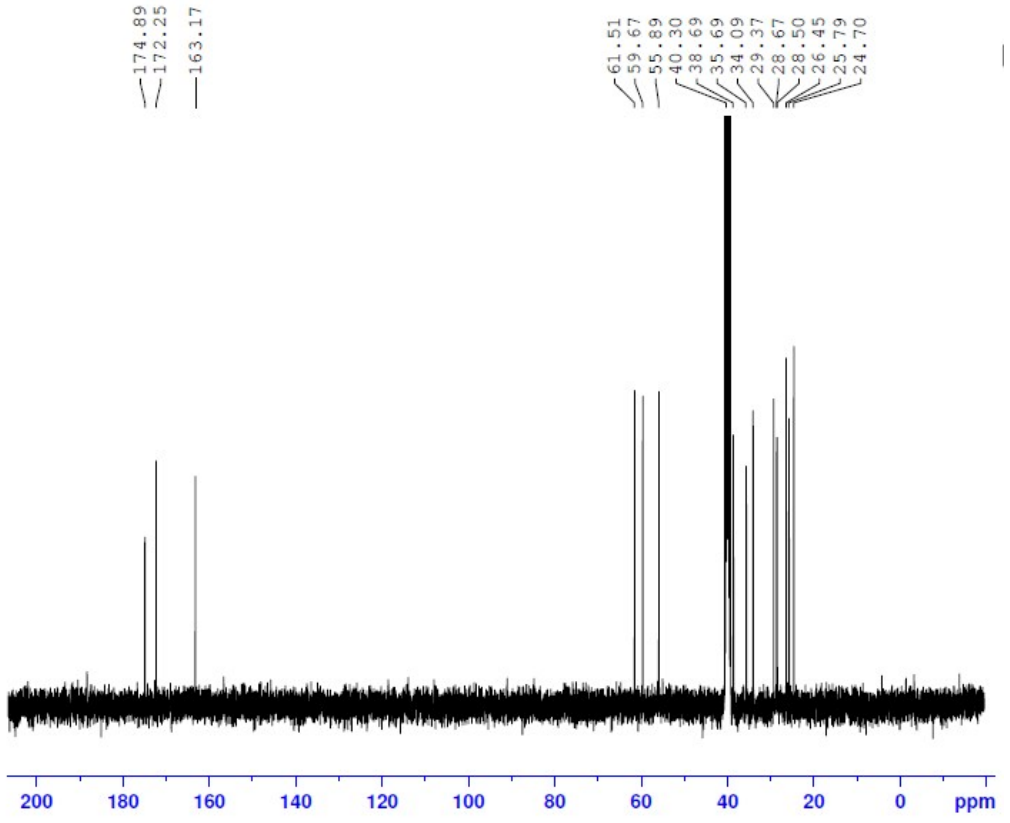
The [1]H-NMR spectrum of Compd.**11h**.



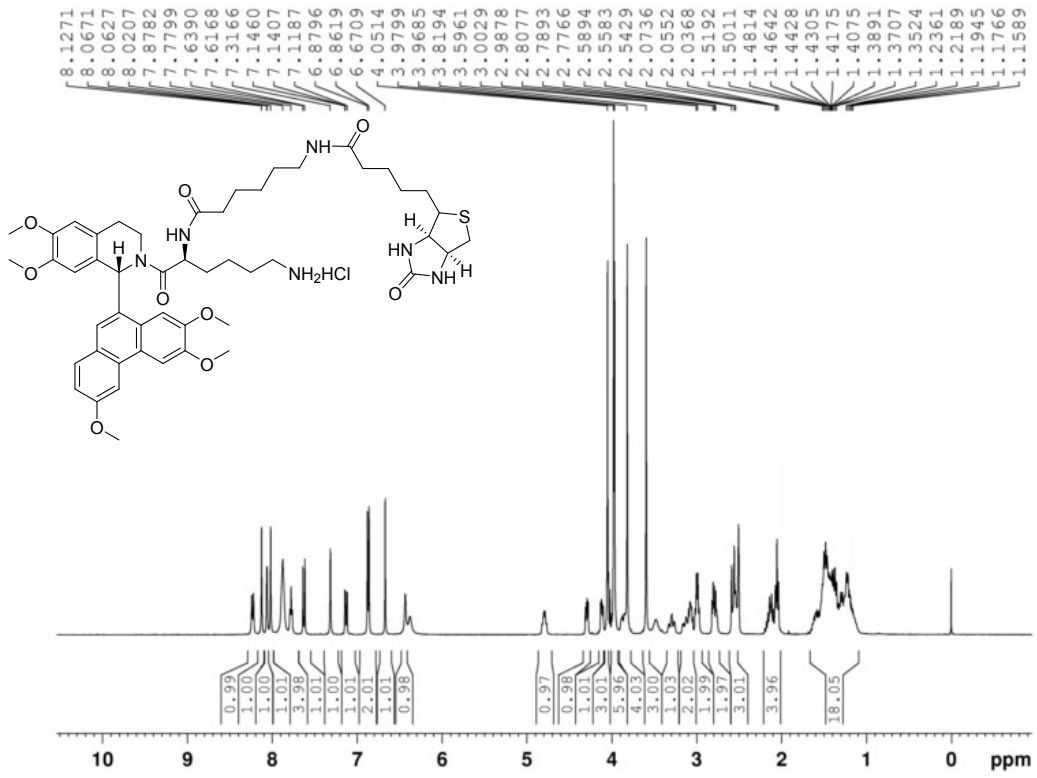
The [13]C-NMR spectrum of Compd. **11h**.



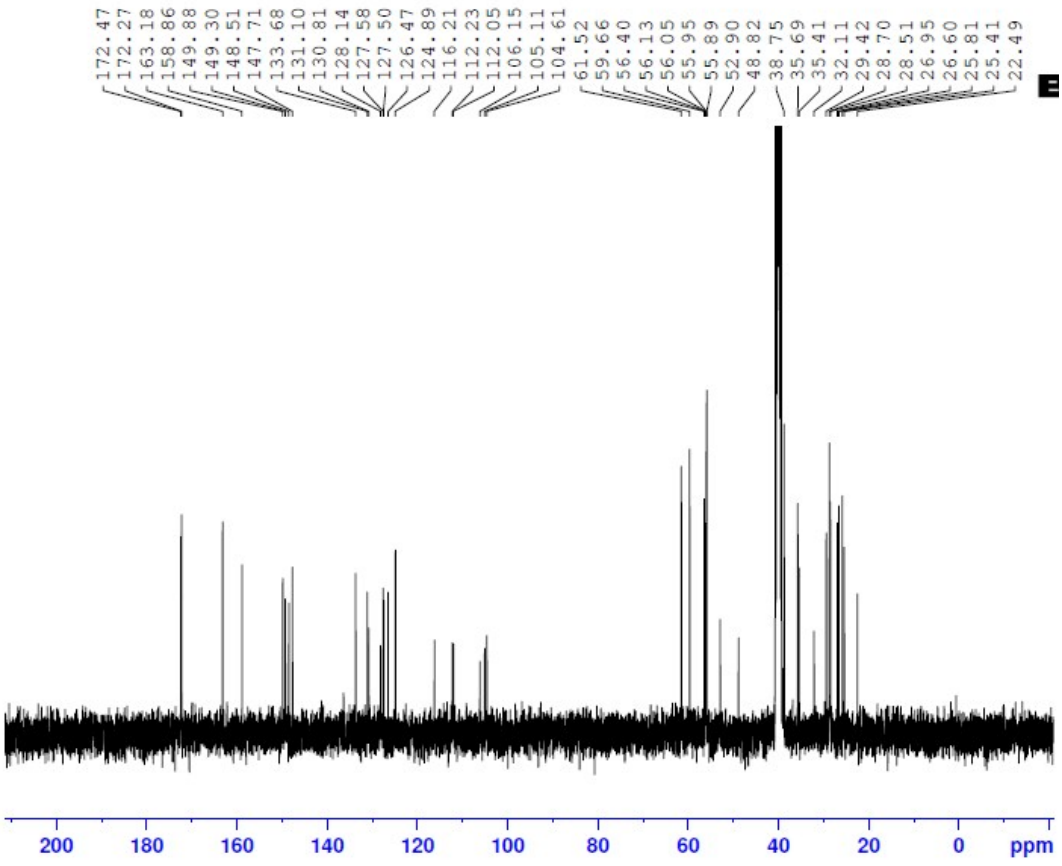
The ^{1}H-NMR spectrum of Compd.**15**.



The ^{13}C-NMR spectrum of Compd.**15**.



The [1]H-NMR spectrum of Compd.**17**(biotinconjugated **12a**).



The [13]C-NMR spectrum of Compd. **17**(biotinconjugated **12a**).

3. The ECD spectra of compounds (**12a-i, 12k**)

The absolute stereochemistry of compound **12a** and **12b** (diastereomers) was postulated via a comparison between the experimental and theoretical electronic circular dichroism (ECD) spectra. ECD spectra for each diastereomer (compound **12a** and **12b**) were acquired on Bio-logic MOS 450 spectropolarimeter, and the corresponding theoretical ECD spectra were determined quantum mechanically. The results are illustrated in Fig.1. Alignment of the experimental and theoretical ECD spectra allowed for the major absorption transitions to be mapped to their corresponding peaks in the ECD spectra. Comparison of these unambiguous regions of the corresponding experimental ECD traces of **12a** and **12b** to those computed for the (*S,S*)- and (*R,S*)- forms, led to the assignments of compounds **12a** and **12b** as the (*S,S*)- and (*R,S*)- diastereomers, respectively. The absolute configurations of the rest of analogs were assigned by comparing ECD spectra and specific rotations with **12a** and **12b**.

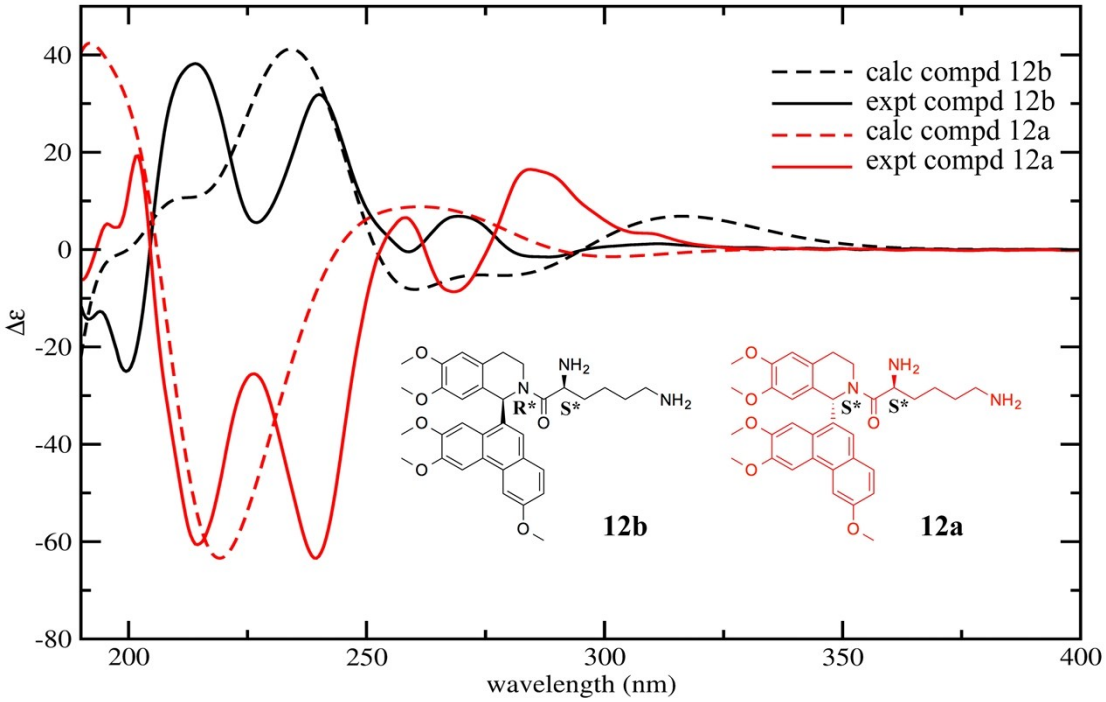
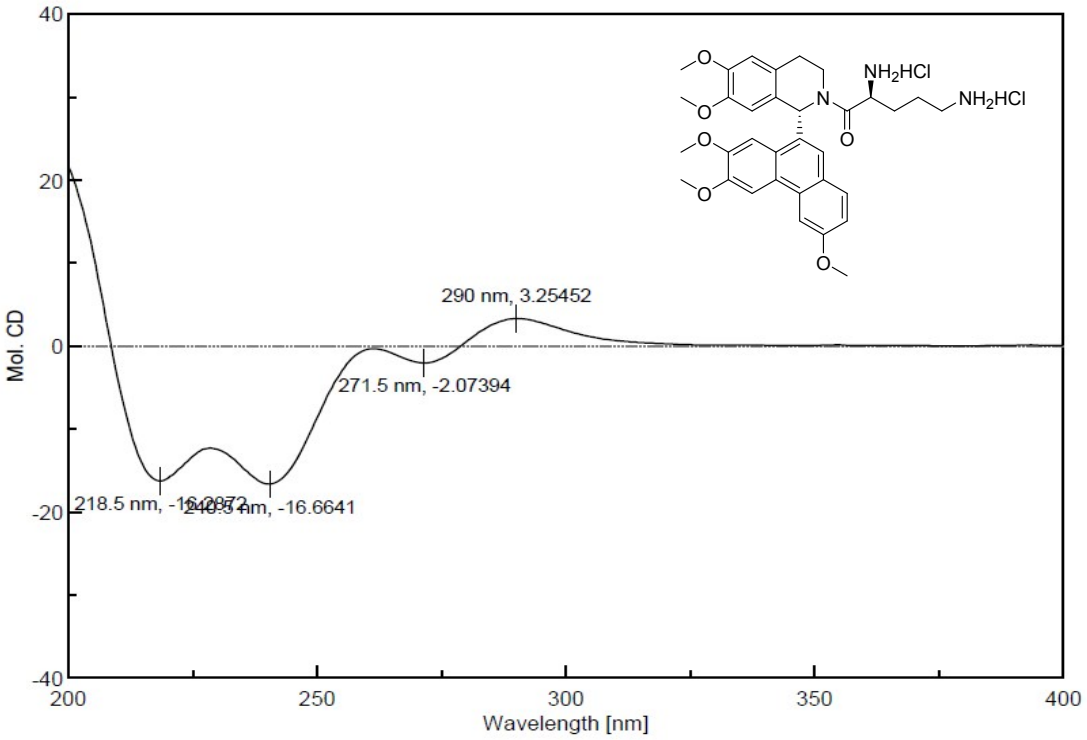
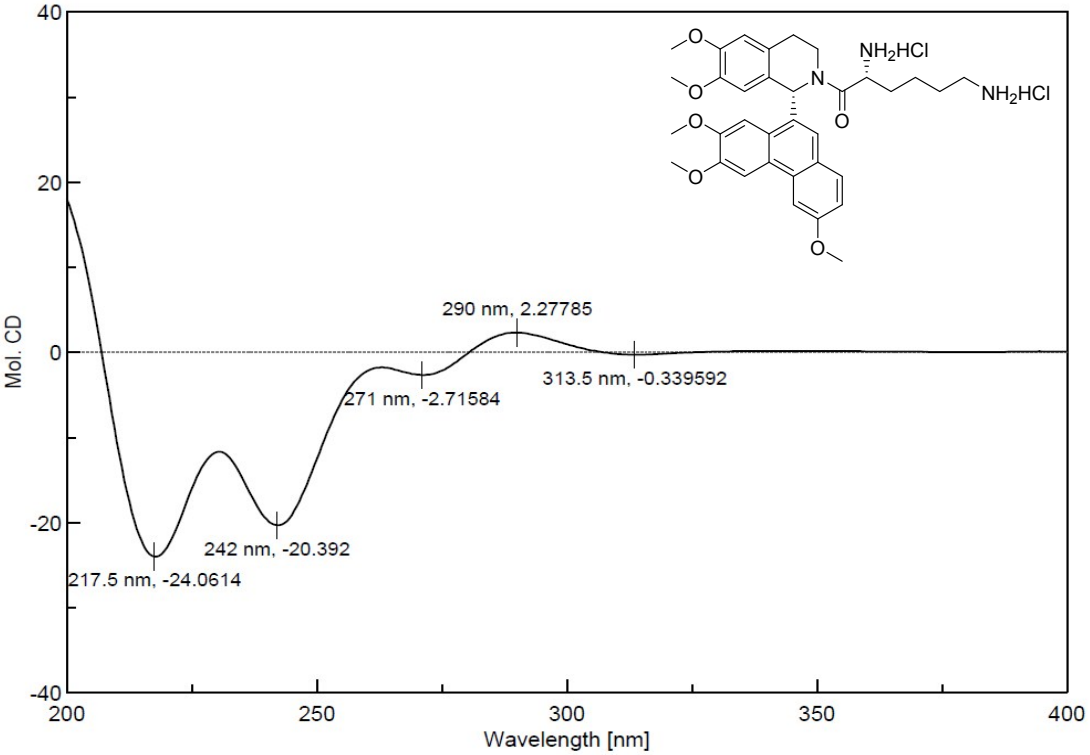


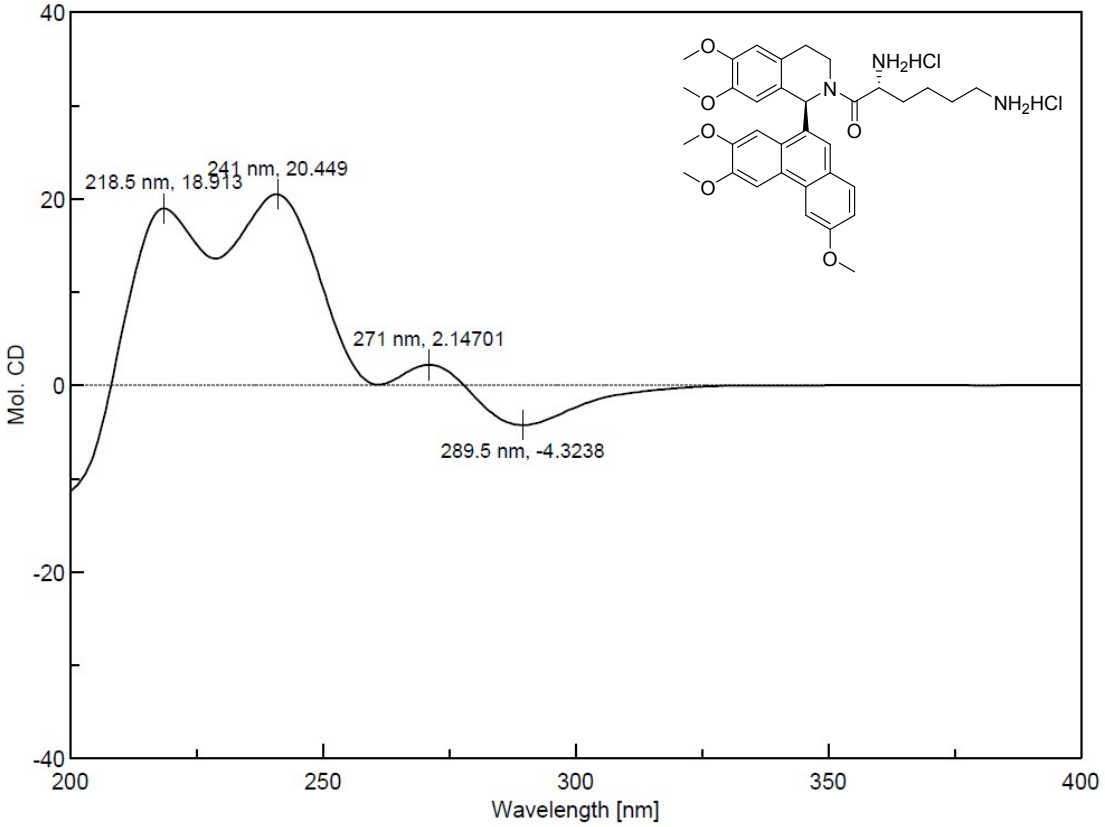
Fig. 1. Comparison of the experimental and calculated ECD spectra of compound **12a** and compound **12b**.



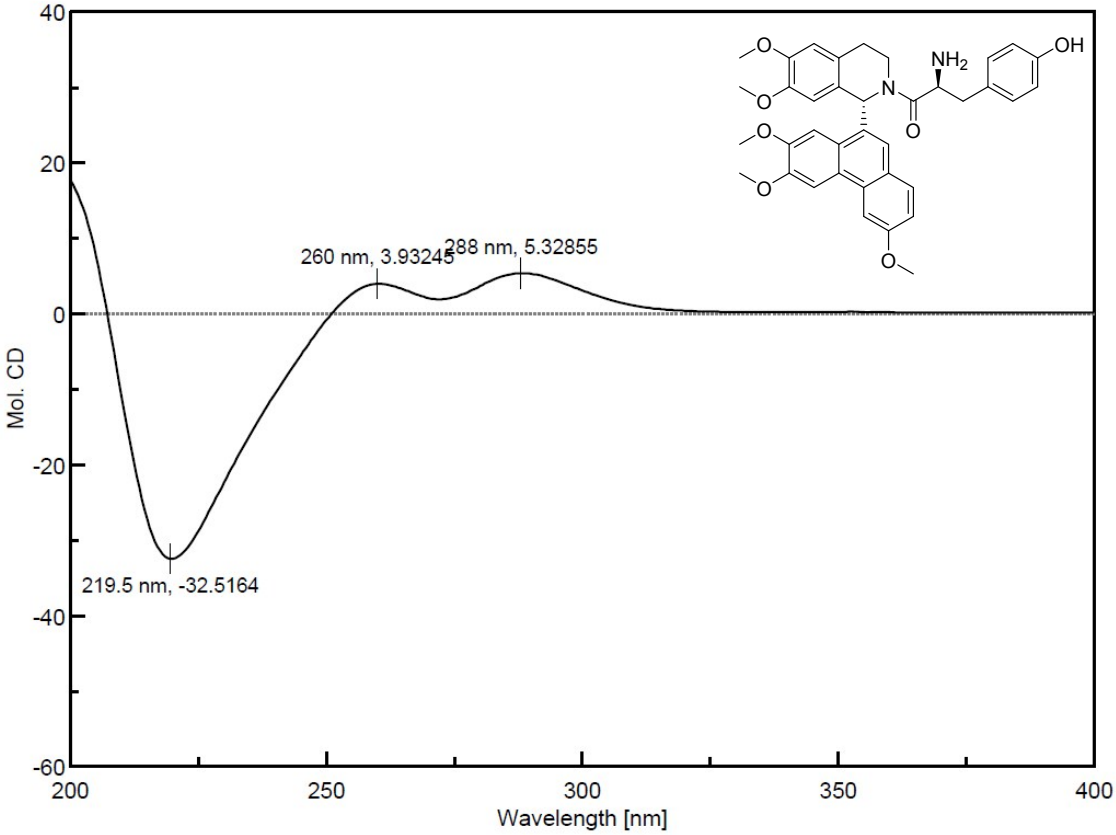
The ECD spectra of compound **12c.**



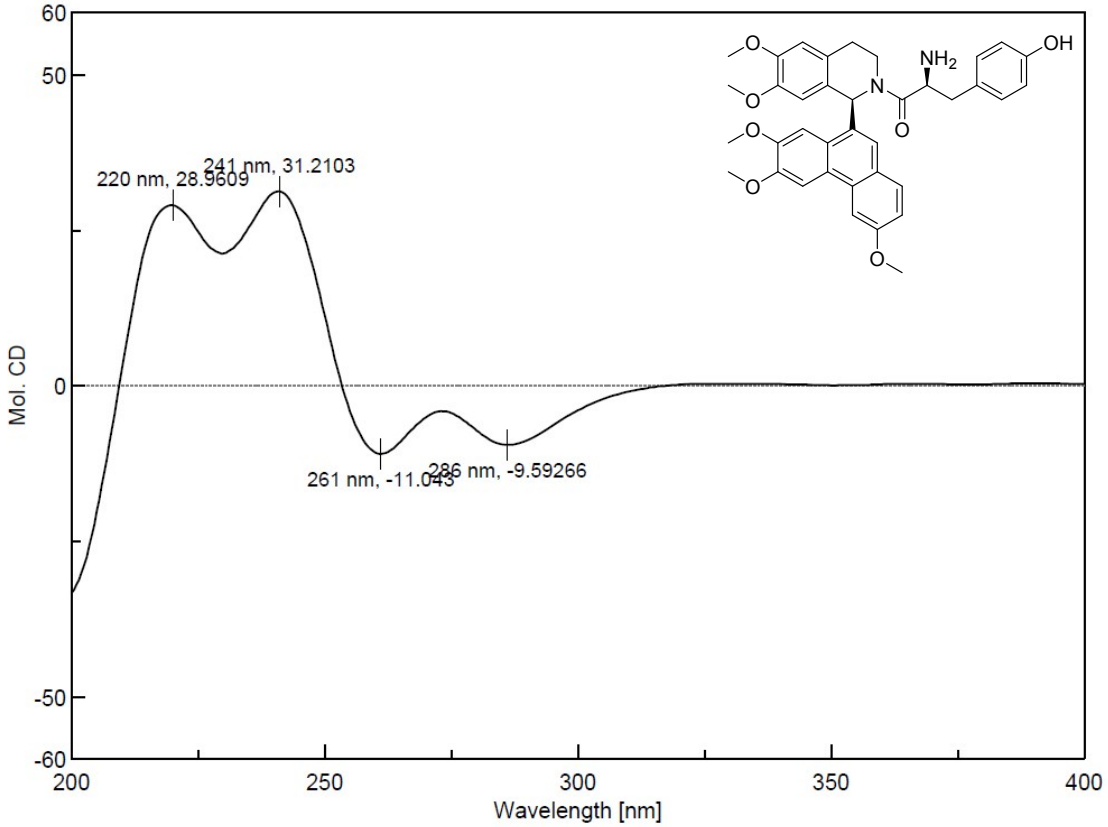
The ECD spectra of compound **12d.**



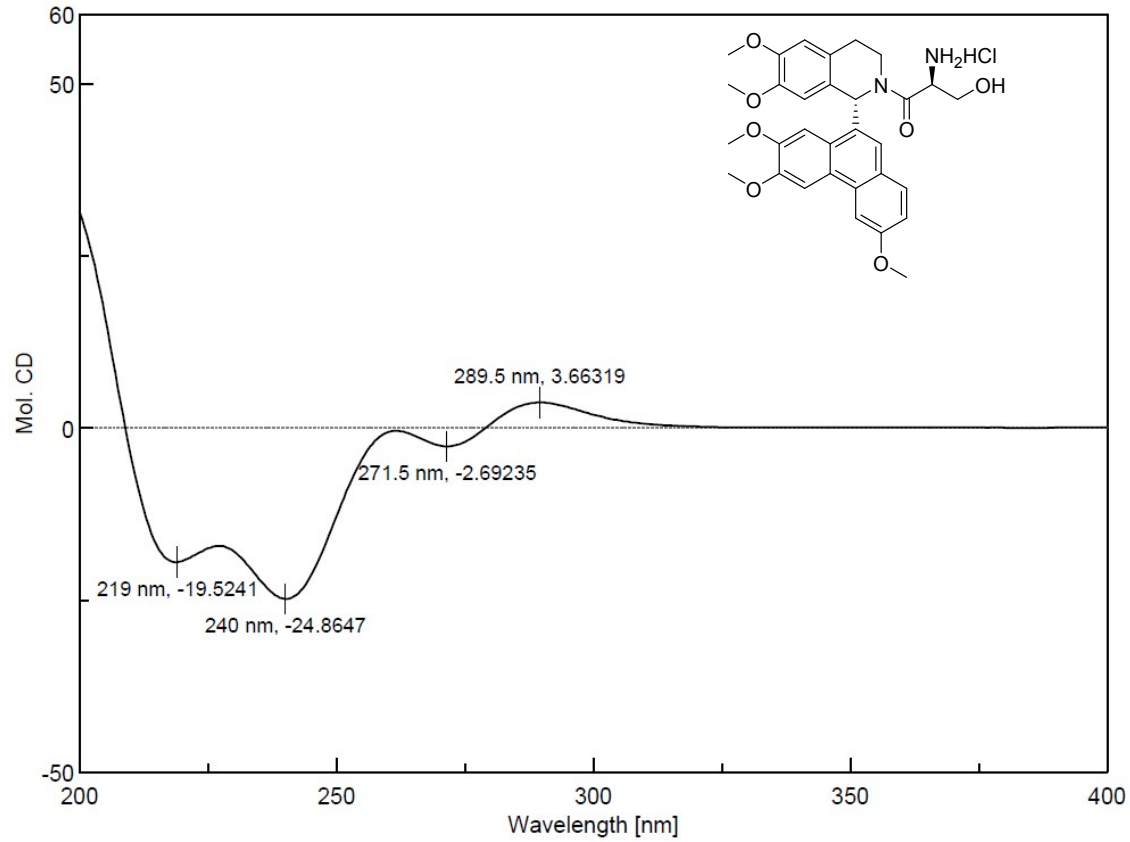
The ECD spectra of compound **12e.**



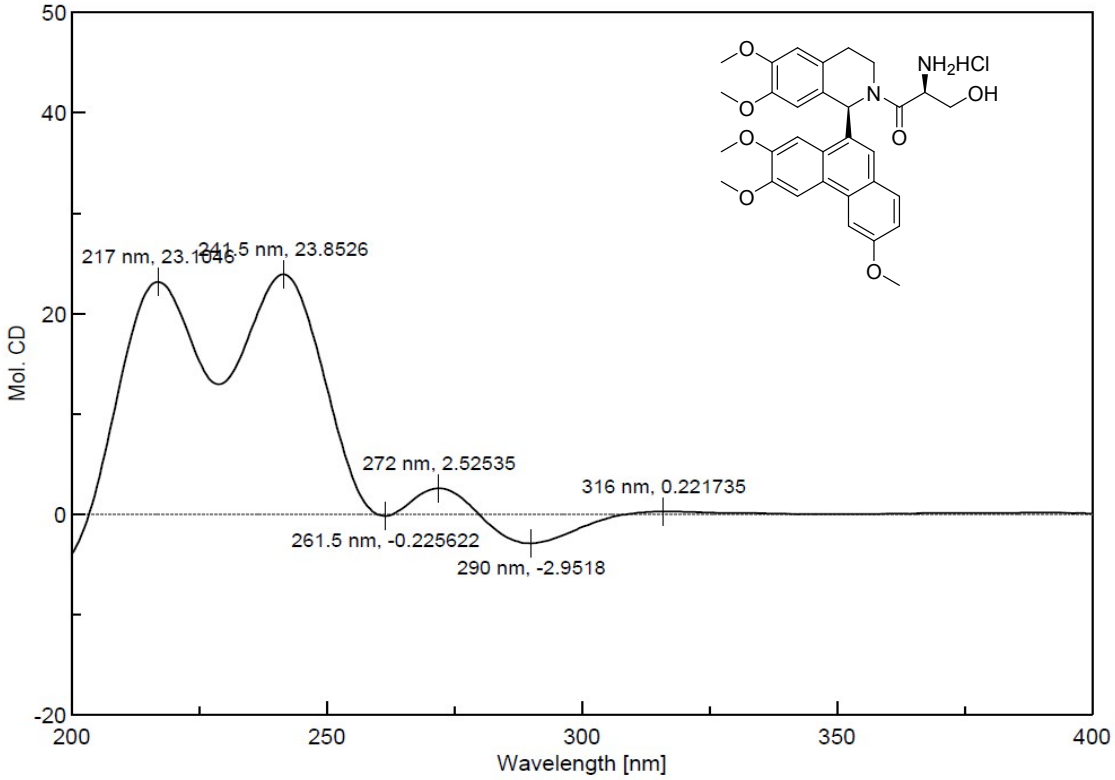
The ECD spectra of compound **12f.**



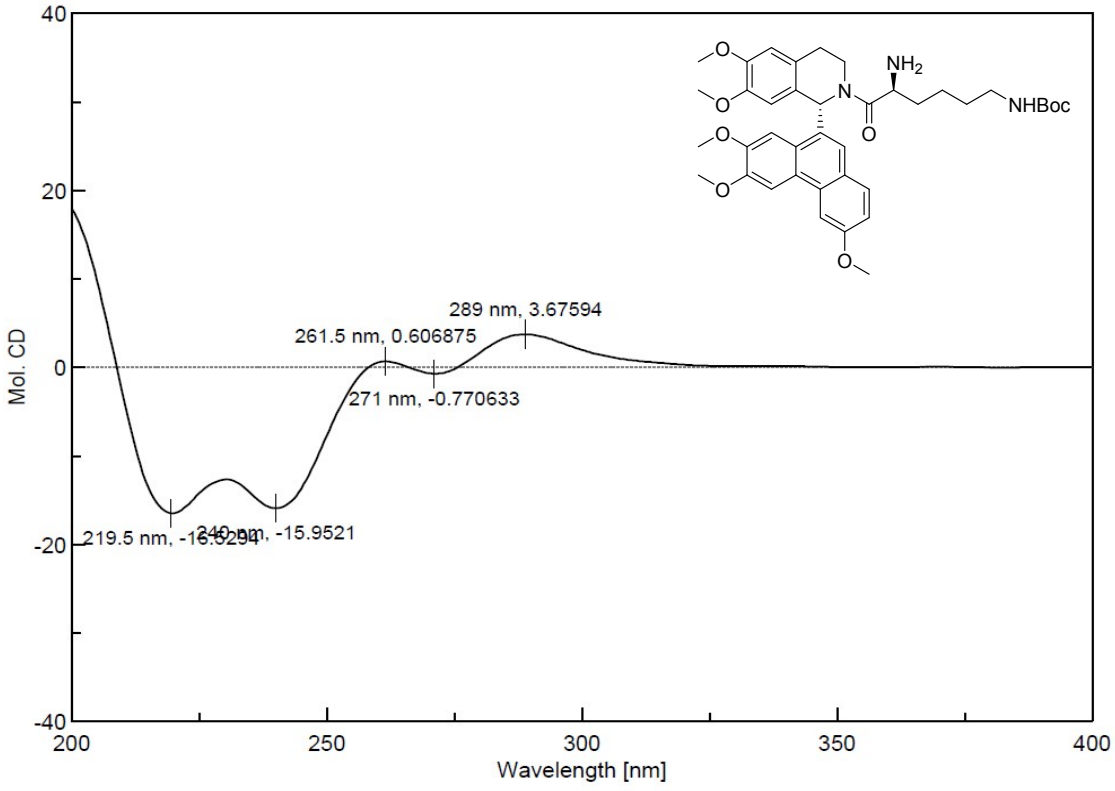
The ECD spectra of compound **12g.**



The ECD spectra of compound **12h.**



The ECD spectra of compound **12i.**



The ECD spectra of compound **12k.**