Electronic Supplementary Information

Synthesis and biological evaluation of bergenin derivatives as new immunosuppressants

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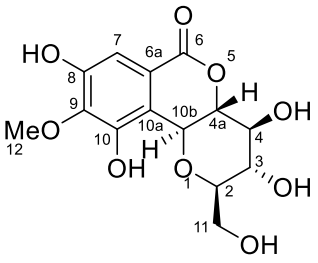
CONTENTS

Synthetic Protocols

General: All chemicals purchased were reagent grade and used without further purification unless otherwise noted. Dichloromethane (CH_2Cl_2) were distilled over calcium hydride (CaH_2). Methanol was distilled from magnesium and iodine. Toluene and tetrahydrofuran (THF) were distilled over sodium/benzophenone. Reactions were monitored by analytical thin-layer chromatography (TLC) on silica gel 60 F_{254} precoated on aluminum plates (E. Merck). Spots were detected under UV (254 nm) light and/or by staining with acidic ceric ammonium molybdate. Solvents were evaporated under reduced pressure and below 45 °C (water bath). ^{1}H NMR, ^{13}C NMR, ^{19}F NMR and 2D NMR spectra were recorded on an Avance III Bruker-600 or an Avance III Bruker-400 spectrometer. Chemical shifts were referenced to an internal $SiMe_4$ standard or to residual solvent protons. Mass spectra were recorded by a Waters Xevo G2 Q-TOF spectrometer. All the final compounds have a purity of at least 95%, as determined by analytical HPLC which was performed on an Agilent 1260 Infinity system equipped with VWD detector and the data were collected at 280 nm.

General procedure A for the preparation of compounds 2a, 2d, 2f: To a solution of bergenin (100.0 mg, 0.30 mmol) in *N*,*N*-dimethylformamide (5.0 mL) were added potassium carbonate (62.2 mg, 0.45 mmol), KI (5.1 mg, 10% mmol) and the respective alkyl bromide (1.5 equiv., 0.45 mmol). The reaction mixture was heated to 80 °C and stirred overnight, then the solvent was evaporated under reduced pressure and water was added. The mixture was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate. After filtration, the filtrate was concentrated and the residue was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH = 25:1) to give **2a, 2d, 2f** (22-26% yield) as white solids.[1]

Scheme S1. The structure of bergenin:



Bergenin

(2R,3S,4S,4aR,10bS)-8,10-Dibutoxy-3,4-dihydroxy-2-(hydroxymethyl)-9-methoxy-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (2a)

Following the general procedure A, 1-bromobutane (49 μL, 0.45 mmol) was used to afford **2a** (33.6 mg, 25% yield) as white solids. $[\alpha]_D^{25}$ +25.2 (c 0.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.35 (s, 1H), 4.72 (d, *J* = 10.2 Hz, 1H), 4.12 (t, *J* = 9.8 Hz, 1H), 4.08 – 3.90 (m, 7H), 3.88 (s, 3H), 3.78 (t, *J* = 9.0 Hz, 1H), 3.60 – 3.58 (m, 1H), 1.86 – 1.64 (m, 4H), 1.55 – 1.40 (m, 4H), 0.99 – 0.94 (m, 6H). The spectroscopic data coincide with the previous report.[1]

(2R,3S,4S,4aR,10bS)-3,4-Dihydroxy-2-(hydroxymethyl)-9-methoxy-8,10-bis(pentyloxy)-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (2b): To a solution of bergenin (100.0 mg, 0.30 mmol) in *N*,*N*-dimethylformamide (5.0 mL) were added potassium carbonate (62.2 mg, 0.45 mmol), KI (5.1 mg, 10% mmol) and 1-bromopentane (93 μL, 0.75 mmol). The reaction was heated to 60 °C and stirred overnight, then the solvent was evaporated under reduced pressure and water was added. The mixture was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate. After filtration, the filtrate was concentrated and then the residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 25:1) to give **2b** (128.4 mg, 90% yield) as white solid. $[\alpha]_D^{25}$ -25.2 (c 0.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.31 (s, 1H), 5.44 (s, 1H), 5.01 (d, *J* = 3.1 Hz, 1H), 4.70 (d, *J* = 10.1 Hz, 1H), 4.13 (t, *J* = 9.8 Hz, 1H), 4.04 (t, *J* = 8.9 Hz, 1H), 4.01 – 3.89 (m, 6H), 3.87 (s, 3H), 3.79 (t, *J* = 9.0 Hz, 1H), 3.62 – 3.49 (m, 1H), 1.88 – 1.65 (m, 4H), 1.49 – 1.28 (m, 8H), 0.97 – 0.85 (m, 6H). The spectroscopic data coincide with the previous report.[1]

(2R,3S,4S,4aR,10bS)-8,10-Bis(hexyloxy)-3,4-dihydroxy-2-(hydroxymethyl)-9-methoxy-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (2c): This compound was prepared in the same manner as described in the preparation of **2b**. 1-Bromohexane (105 μL, 0.75 mmol) instead of 1-bromopentane was used to afford **2c** (130.1 mg, 86% yield) as white solid. $[\alpha]_D^{25}$ -22.2 (c 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 1H), 5.28 (s, 1H), 4.84 (s, 1H), 4.71 (d, *J* = 10.1 Hz, 1H), 4.12 (t, *J* = 9.8 Hz, 1H), 4.08 – 4.00 (m, 1H), 4.00 – 3.88 (m, 6H), 3.87 (s, 3H), 3.79 (t, *J* = 9.0 Hz, 1H), 3.62 – 3.53 (m, 1H), 3.42 (s, 1H), 1.85 – 1.67 (m, 4H), 1.50 – 1.39 (m, 4H), 1.39 – 1.27 (m, 8H), 0.96 – 0.83 (m, 6H); ^{13}C NMR (101 MHz, CDCl3) δ 164.70, 153.25, 150.63, 148.85, 125.84, 118.87, 110.58, 80.32, 79.95, 74.70, 72.24, 70.43, 69.17, 62.15, 61.10, 31.75, 31.63, 30.28, 29.17, 25.79, 25.75, 22.78, 22.67, 14.18, 14.11. The spectroscopic data coincide with the previous report.[2]

(2R,3S,4S,4aR,10bS)-8,10-Bis(heptyloxy)-3,4-dihydroxy-2-(hydroxymethyl)-9-methoxy-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (2d): Following the general procedure A, 1-bromoheptane (71 μL, 0.45 mmol) was used to afford **2d** (41.6 mg, 26% yield) as white solid. $[\alpha]_D^{25}$ +17.2 (c 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 1H), 5.28 (s, 1H), 4.85 (s, 1H), 4.71 (d, *J* = 10.1 Hz, 1H), 4.12 (t, *J* = 9.8 Hz, 1H), 4.07 – 3.89 (m, 7H), 3.87 (s, 3H), 3.80 (t, *J* = 7.4 Hz, 1H), 3.59 – 3.56 (m, 1H), 3.41 (s, 1H), 1.86 – 1.65 (m, 4H), 1.52 – 1.38 (m, 4H), 1.39 – 1.21 (m, 12H), 0.95 – 0.81 (m, 6H). The spectroscopic data coincide with the previous report.[1]

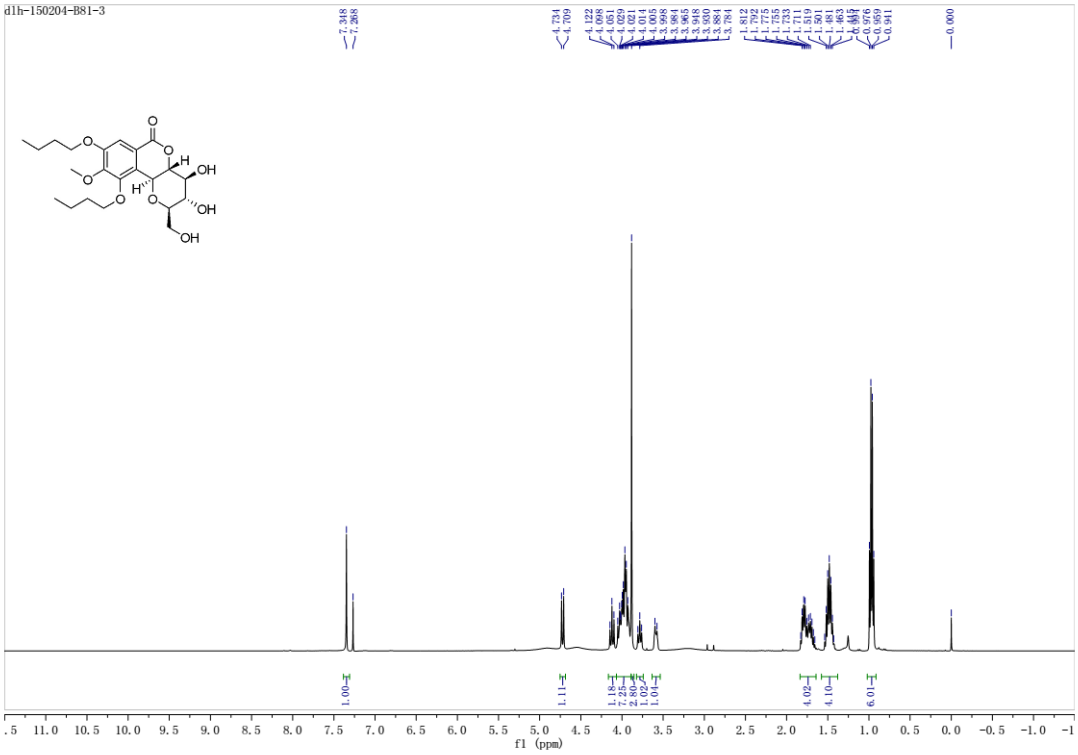
(2R,3S,4S,4aR,10bS)-3,4-Dihydroxy-2-(hydroxymethyl)-9-methoxy-8,10-bis(octyloxy)-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (2e): This compound was prepared in the same manner as described in the preparation of **2b**. Using 1-bromooctane (130 μL, 0.75 mmol) instead of 1-bromopentane to afford **2e** (149.8 mg, 89% yield) as white solid. $[\alpha]_D^{25}$ -18.7 (c 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (s, 1H), 5.30 (brs, 1H), 4.85 (brs, 1H), 4.70 (d, *J* = 10.1 Hz, 1H), 4.12 (t, *J* = 9.8 Hz, 1H), 4.04 (t, *J* = 9.0 Hz, 1H), 4.00 – 3.88 (m, 6H), 3.87 (s, 3H), 3.80 (t, *J* = 9.1 Hz, 1H), 3.62 – 3.52 (m, 1H), 3.40 (brs, 1H), 1.87 – 1.67 (m, 4H), 1.50 – 1.39 (m, 4H), 1.38 – 1.23 (m, 16H), 0.94 – 0.84 (m, 6H). The spectroscopic data coincide with the previous report.[1]

(2R,3S,4S,4aR,10bS)-8,10-Bis(decyloxy)-3,4-dihydroxy-2-(hydroxymethyl)-9-methoxy-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (2f): Following the general procedure, 1-bromodecane (94 μL, 0.45 mmol) was used to afford **2f** (40.9 mg, 22% yield). $[\alpha]_D^{25}$ -3.9 (c 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (s, 1H), 4.95 (s, 1H), 4.72 (d, *J* = 10.1 Hz, 1H), 4.52 (brs, 1H), 4.12 (t, *J* = 9.8 Hz, 1H), 4.06 – 3.90 (m, 7H), 3.88 (s, 4H), 3.79 (t, *J* = 9.0 Hz, 1H), 3.59 – 3.58 (m, 1H), 3.05 (s, 1H), 1.90 – 1.63 (m, 4H), 1.53 – 1.39 (m, 4H), 1.39 – 1.16 (m, 24H), 0.87 (dt, *J* = 6.9, 3.3 Hz, 6H); ^{13}C NMR (100 MHz, CDCl3) δ 164.63, 153.33, 150.69, 148.92, 125.76, 118.84, 110.63, 80.26, 79.99, 74.80, 74.74, 72.29, 70.54, 69.23, 62.29, 61.14, 32.04, 32.03, 30.38, 29.83, 29.76, 29.70, 29.63, 29.51, 29.48, 29.45, 29.25, 26.17, 26.13, 22.81, 14.24. The spectroscopic data coincide with the previous report.[2]

(2R,3S,4S,4aR,10bS)-8,10-Bis(benzyloxy)-3,4-dihydroxy-2-(hydroxymethyl)-9-methoxy-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (2g): To a solution of bergenin (1.00 g, 3.0 mmol) in *N*,*N*-dimethylformamide (20.0 mL) were added potassium carbonate (3.30 g, 24.0 mmol) and benzyl bromide (1.25 g, 7.5 mmol). The reaction was allowed to stir at room temperature for 12 h, then the solvent was evaporated under reduced pressure and water was added. The mixture was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate. After filtration, the filtrate was concentrated and then the residue was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH = 25:1) to give **2g** (1.39 g, 90% yield) as white solid. $[\alpha]_D^{25}$ +8.4 (c 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.49 – 7.31 (m, 10H), 5.10 – 4.96 (m, 4H), 4.50 (d, *J* = 10.3 Hz, 1H), 4.08 (t, *J* = 9.9 Hz, 1H), 3.98 – 3.90 (m, 1H), 3.86 (s, 3H), 3.80 – 3.66 (m, 3H), 3.47 – 3.39 (m, 1H). The spectroscopic data coincide with the previous report.[3]

NMR Spectra

^{1}H NMR of compound **2a**



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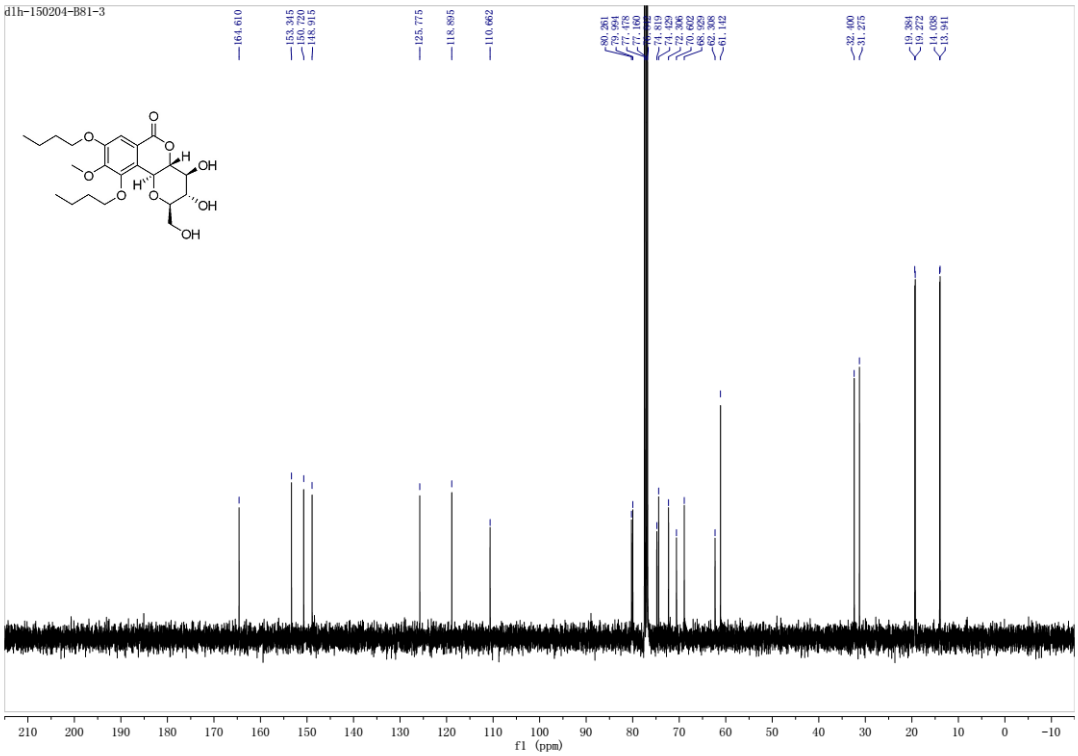

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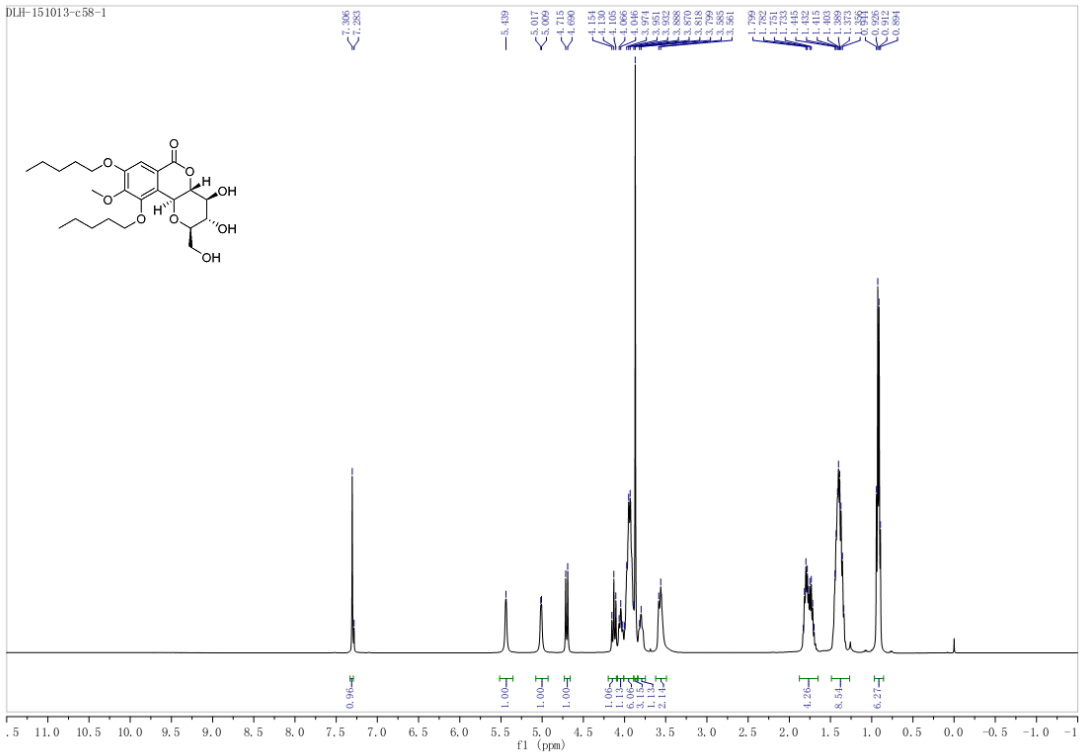

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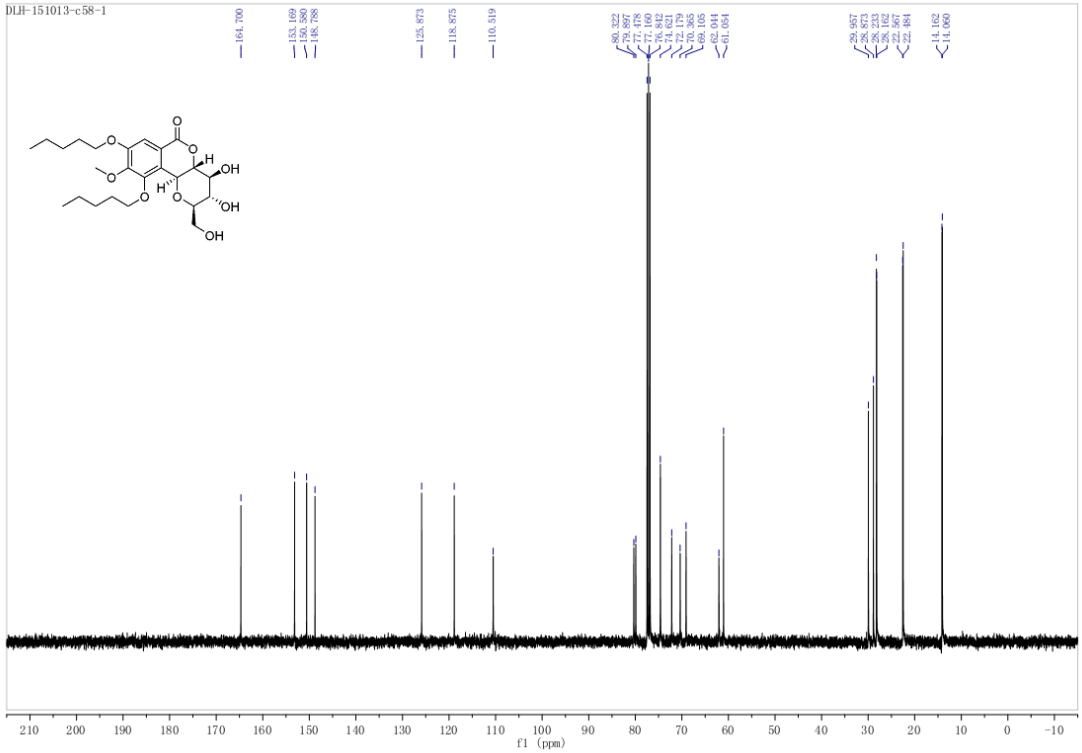

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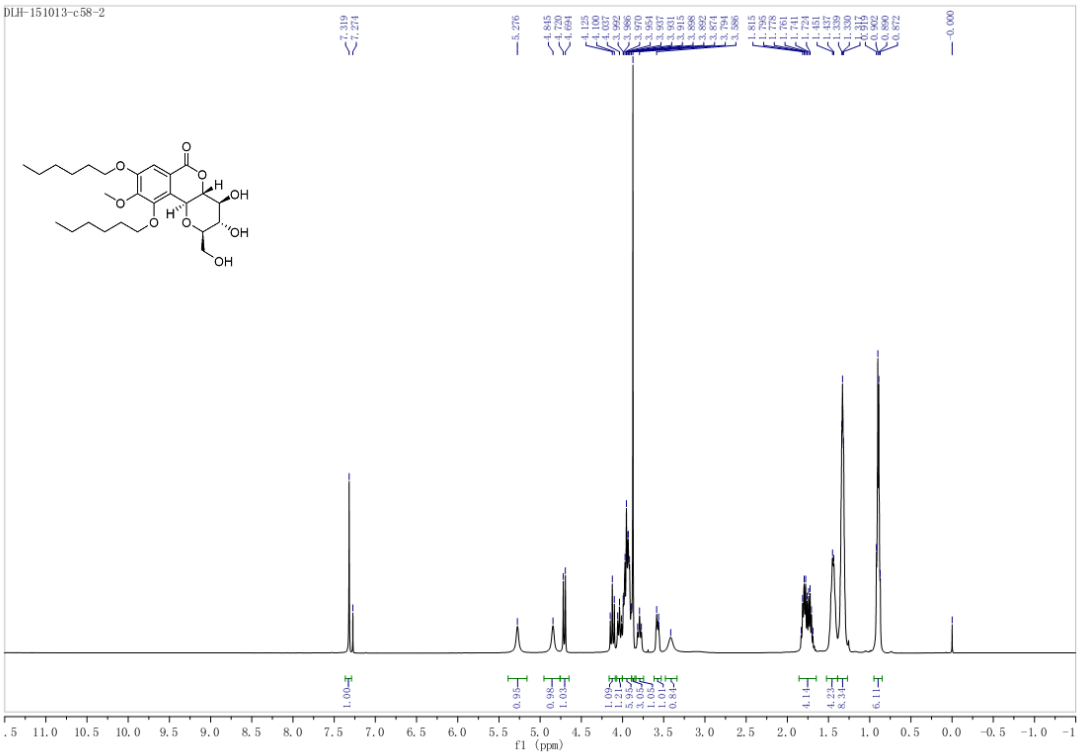

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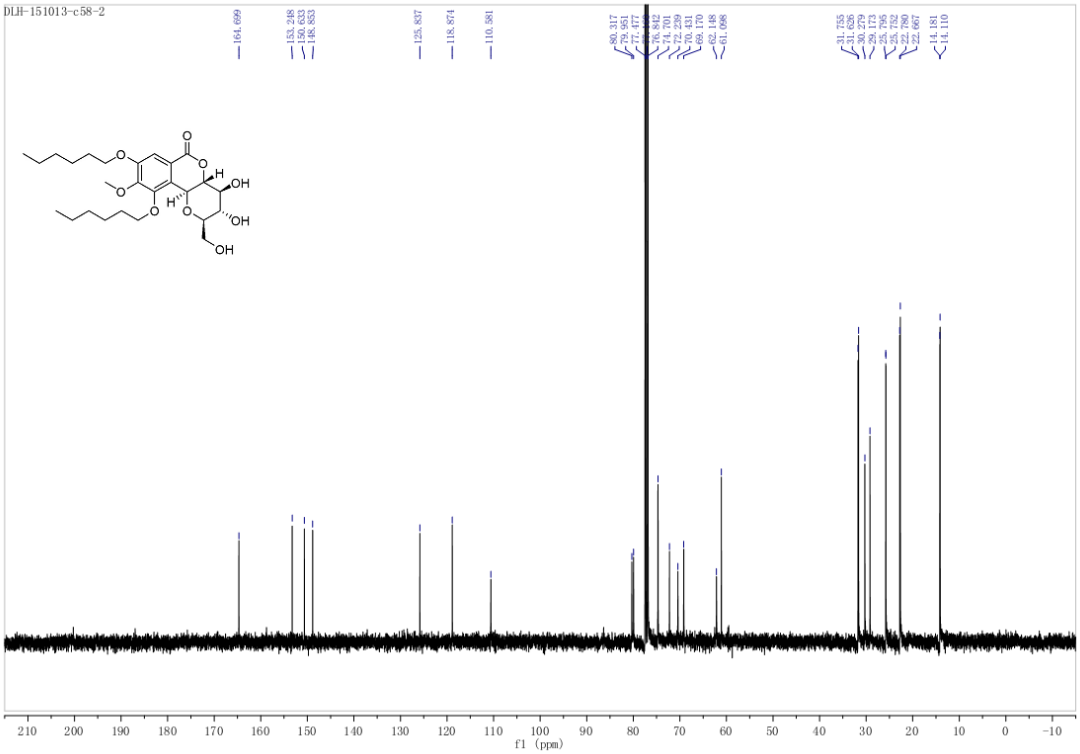

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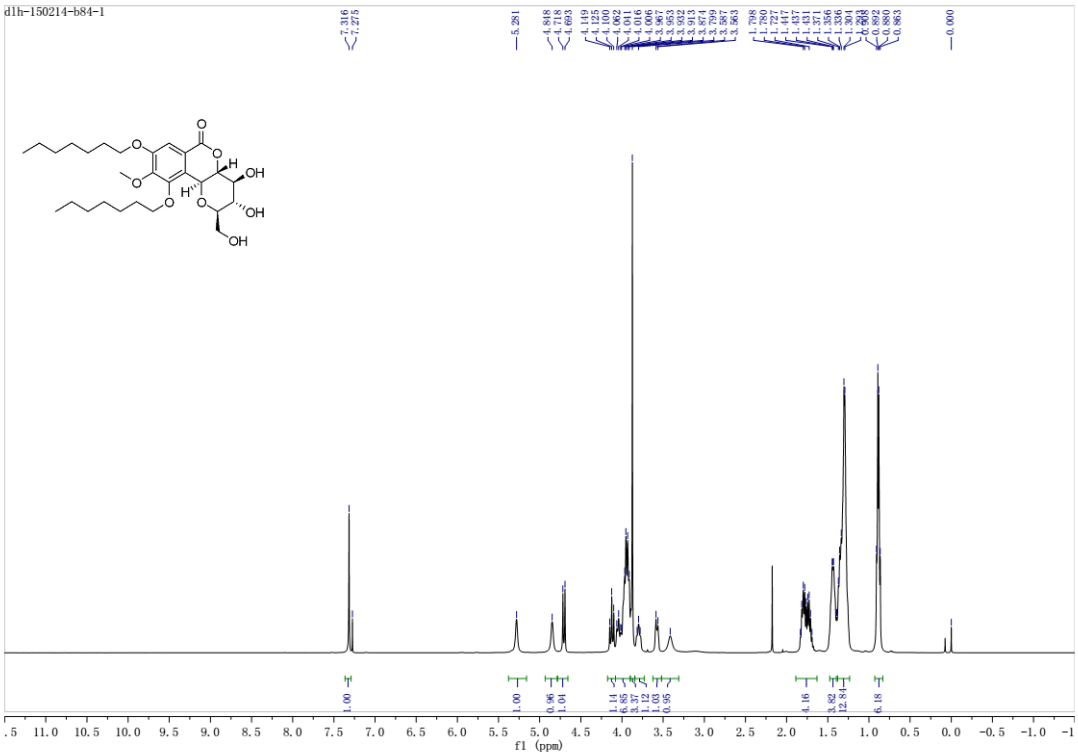

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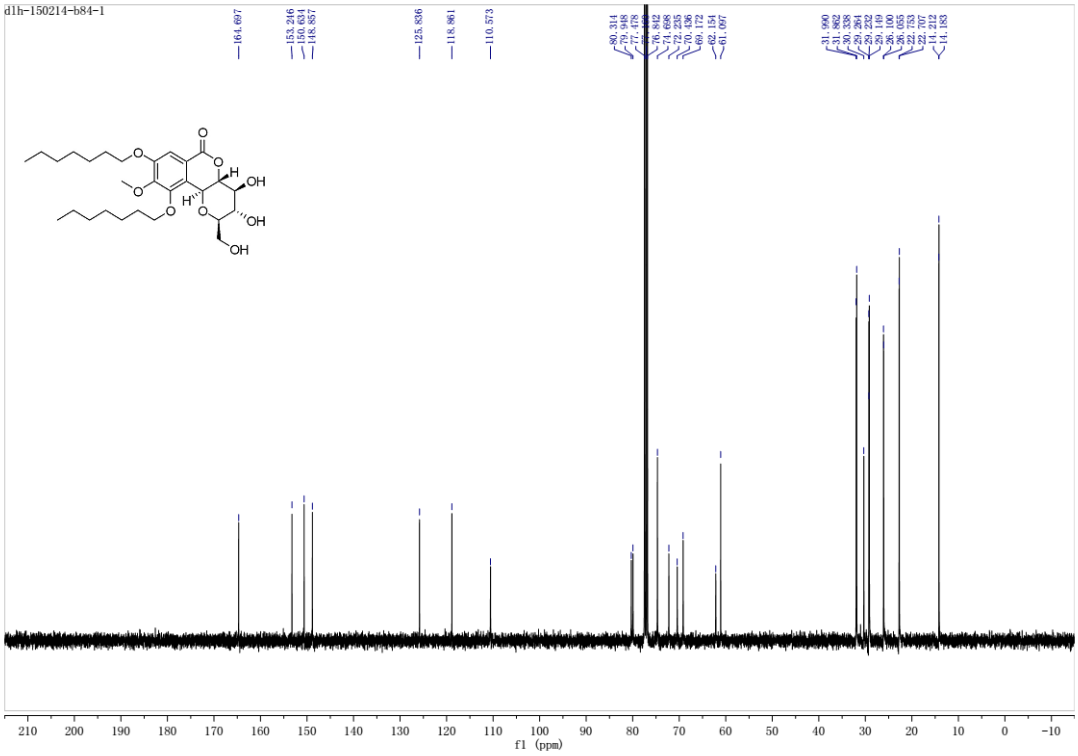

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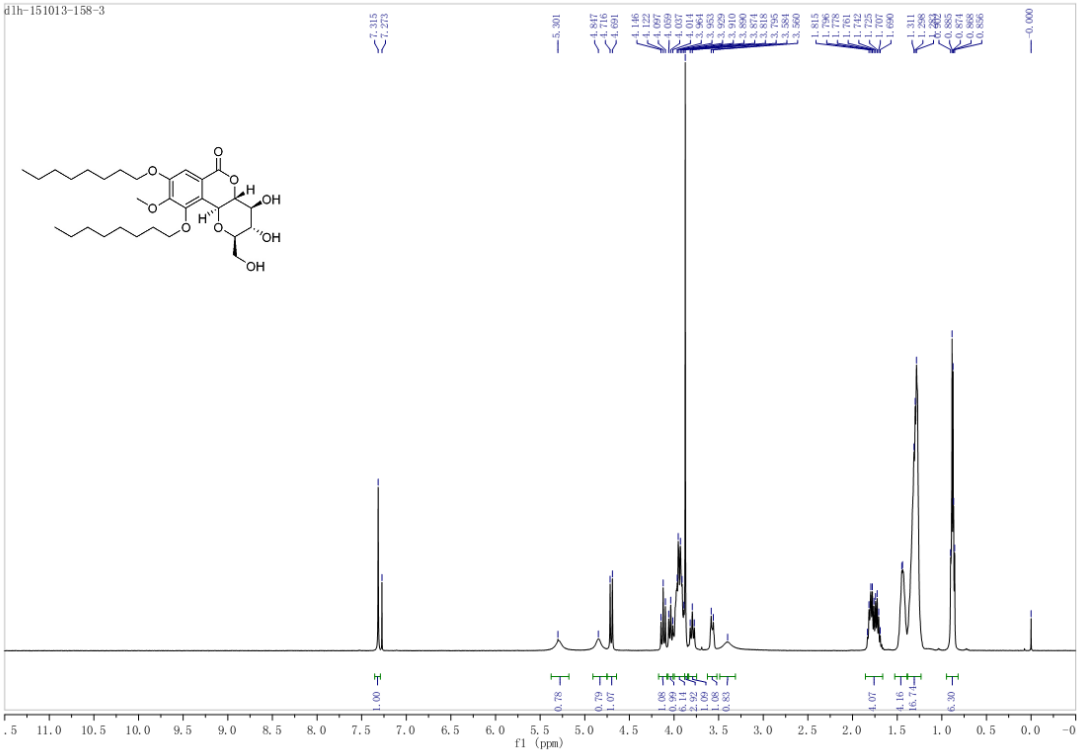

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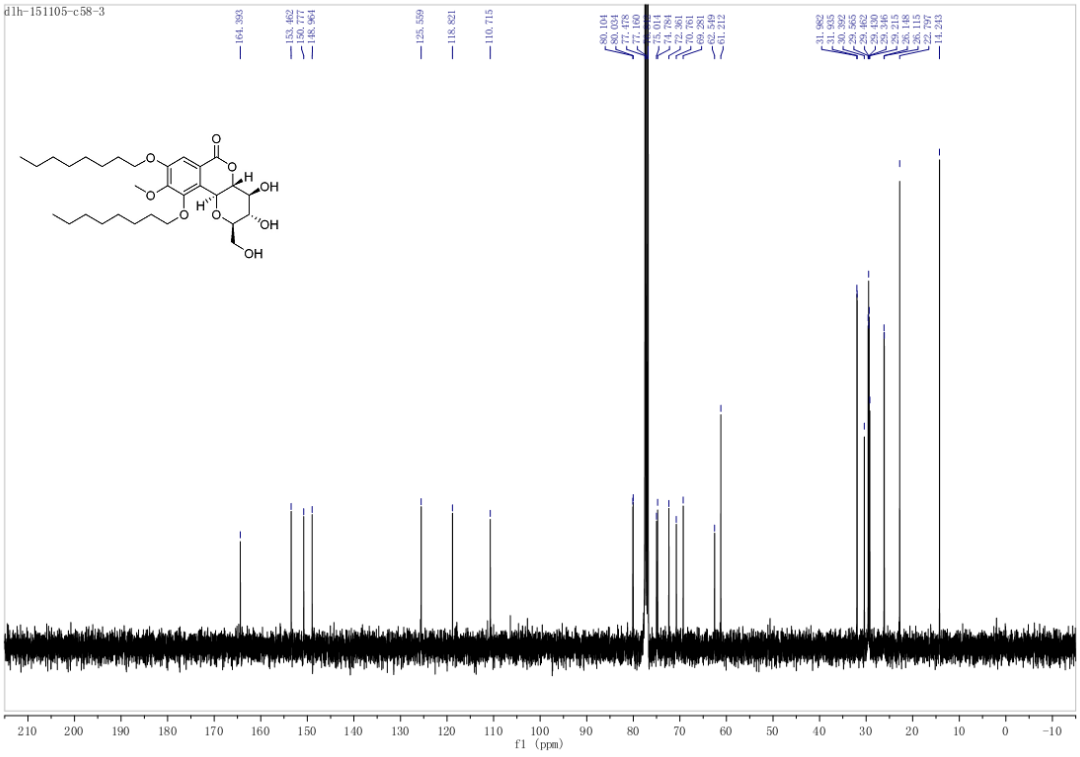

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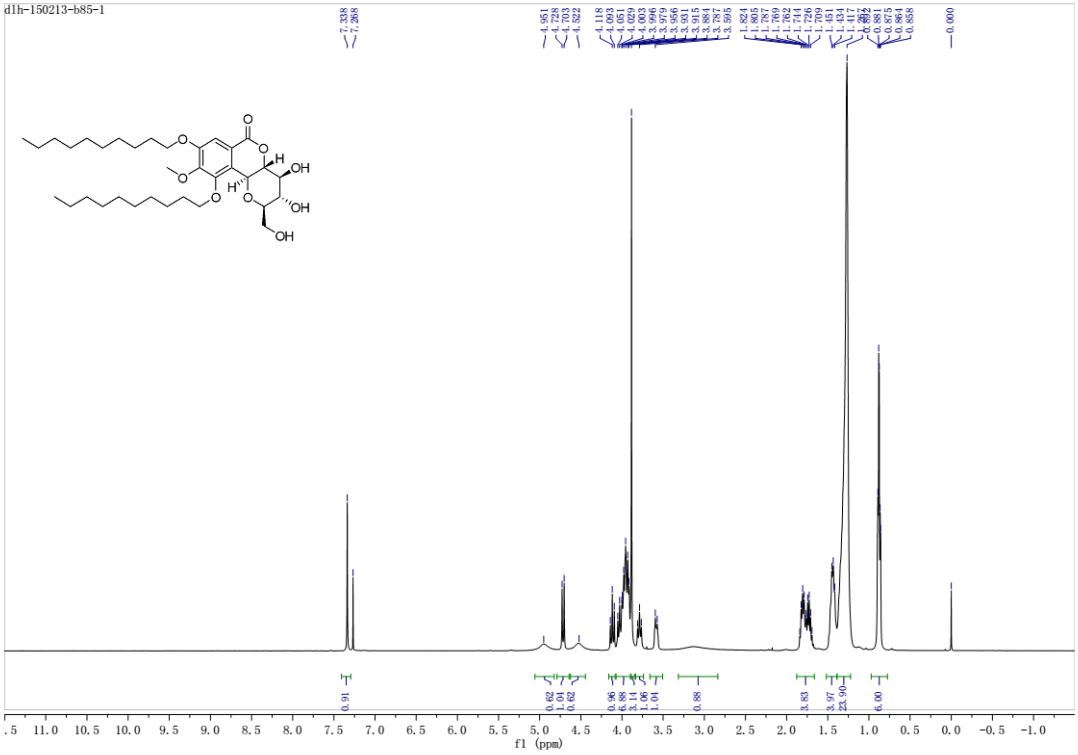

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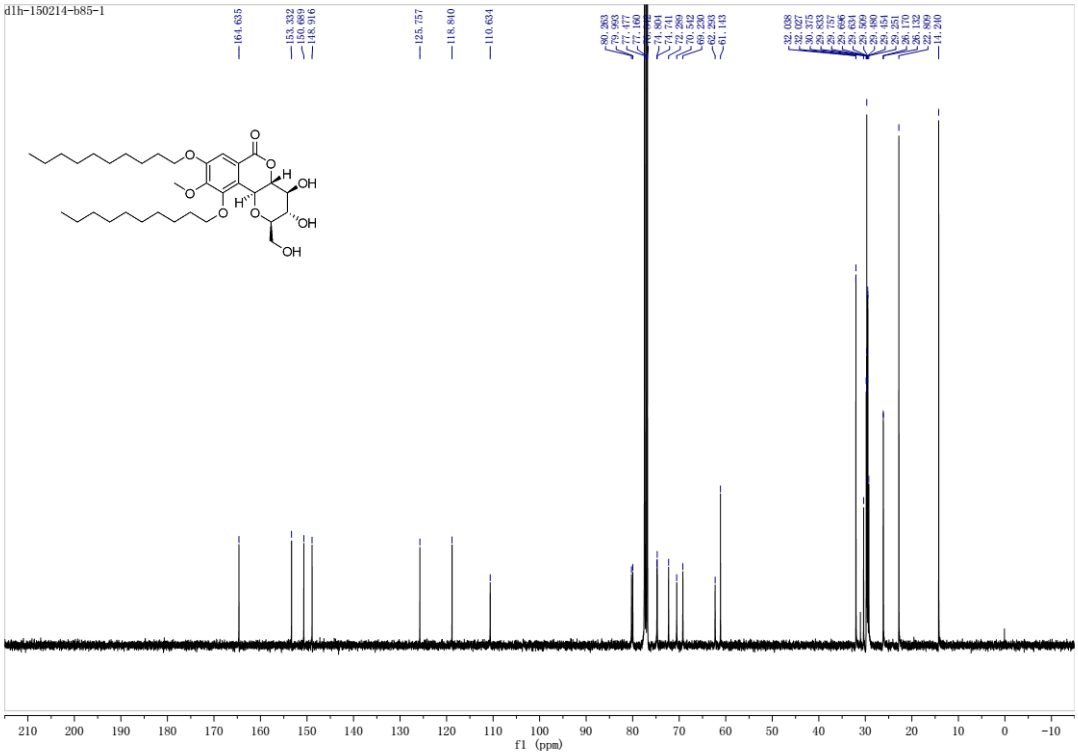

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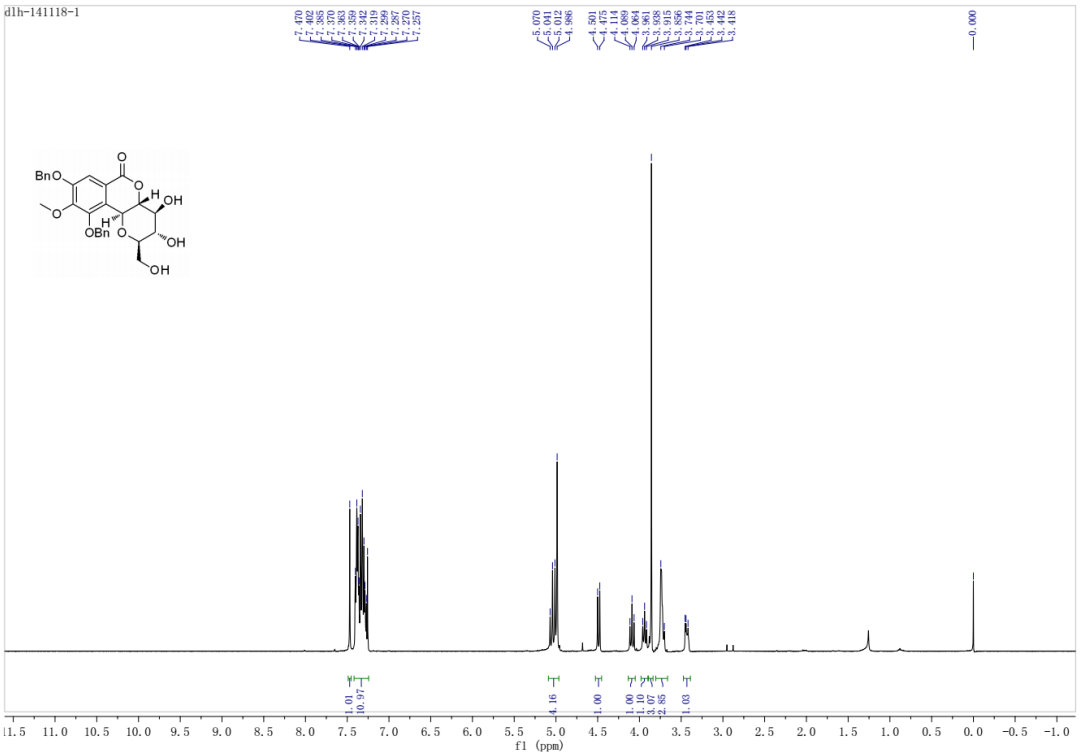

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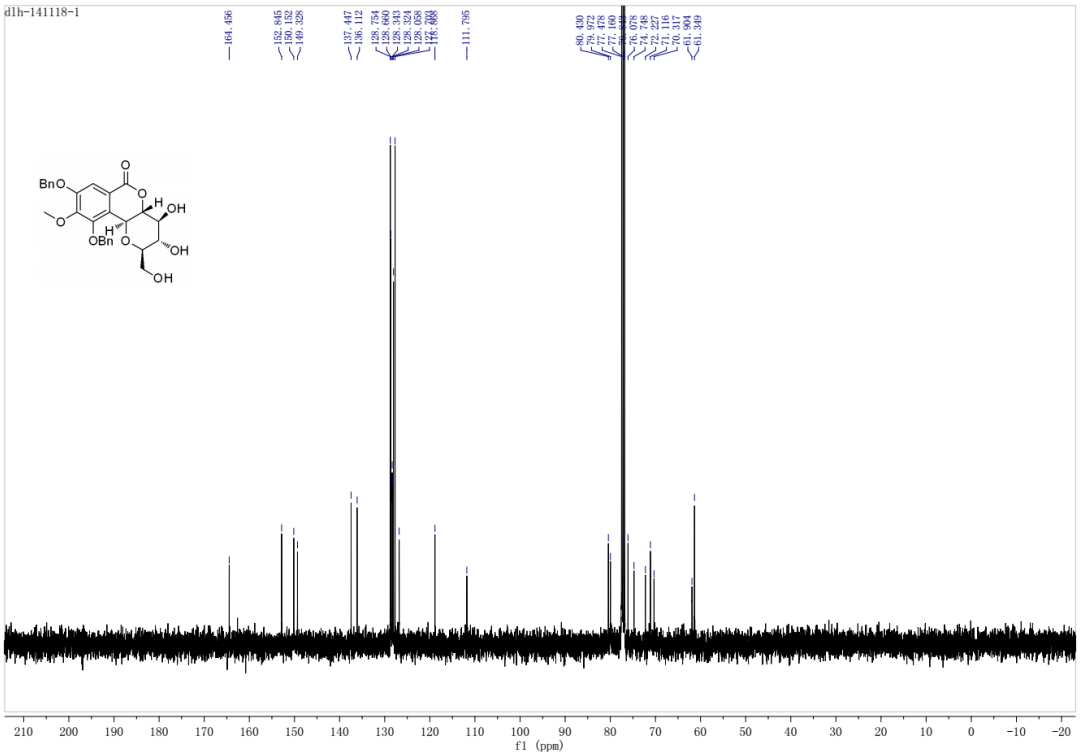

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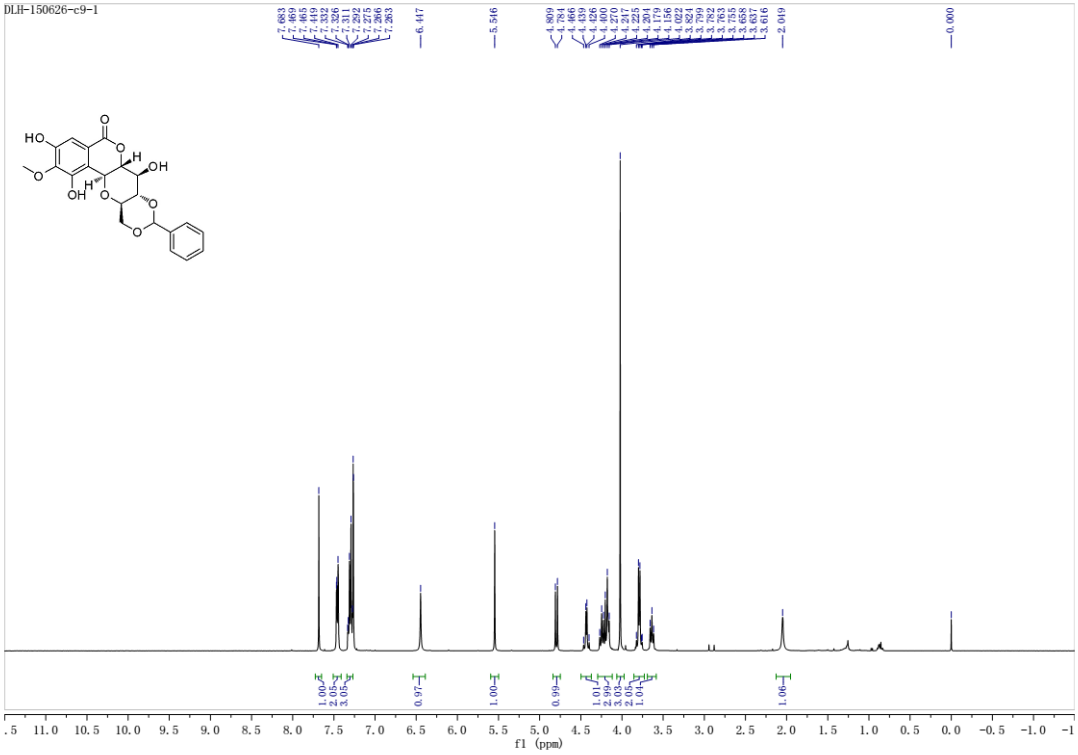

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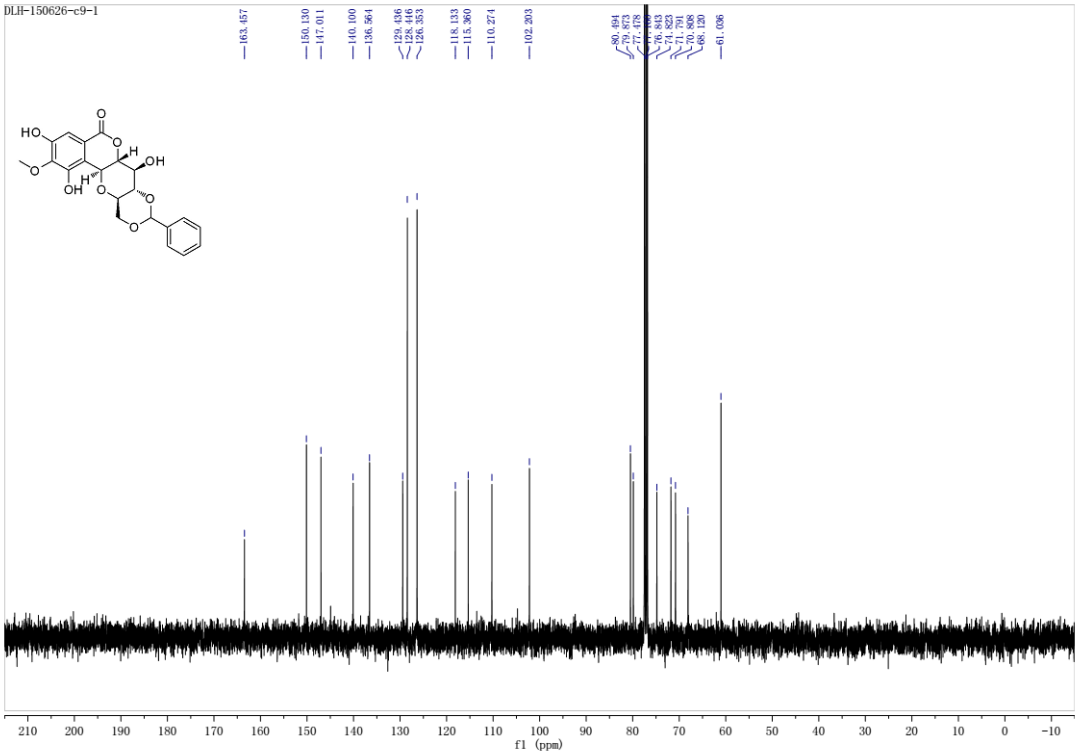

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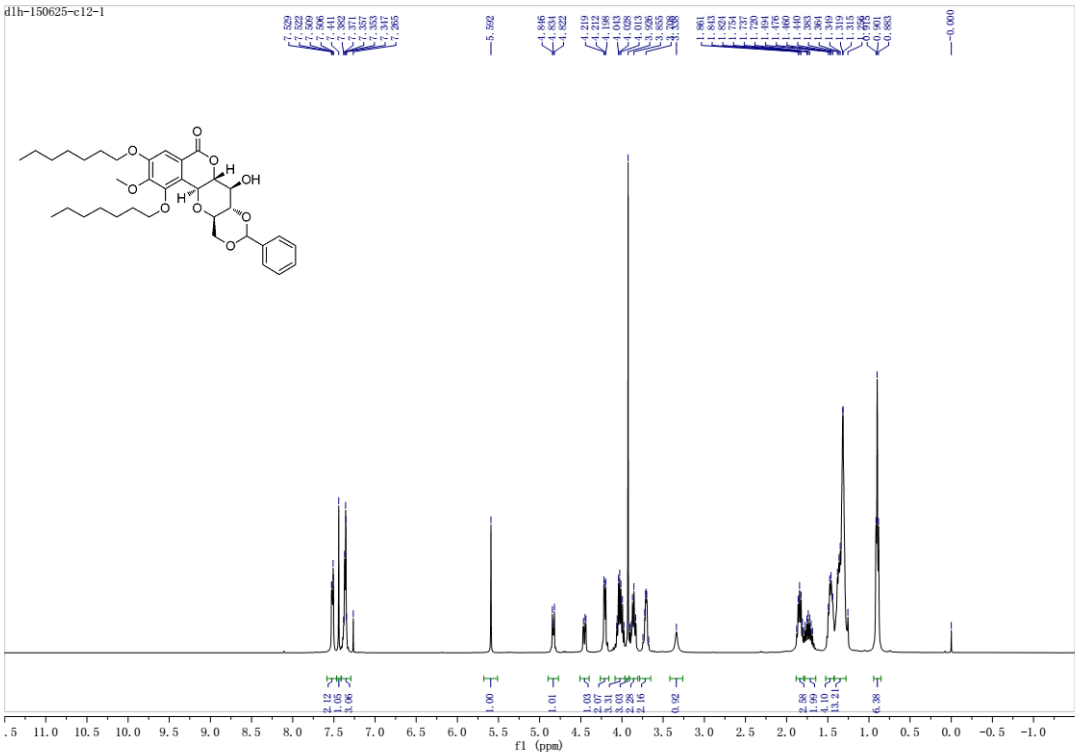

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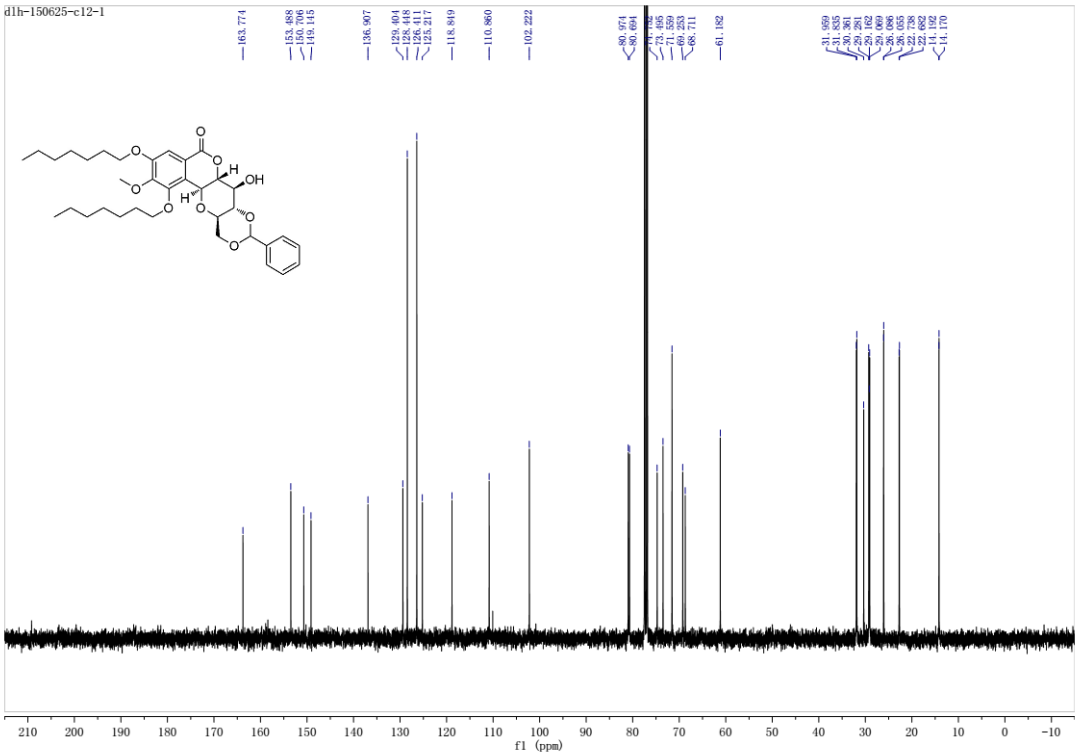

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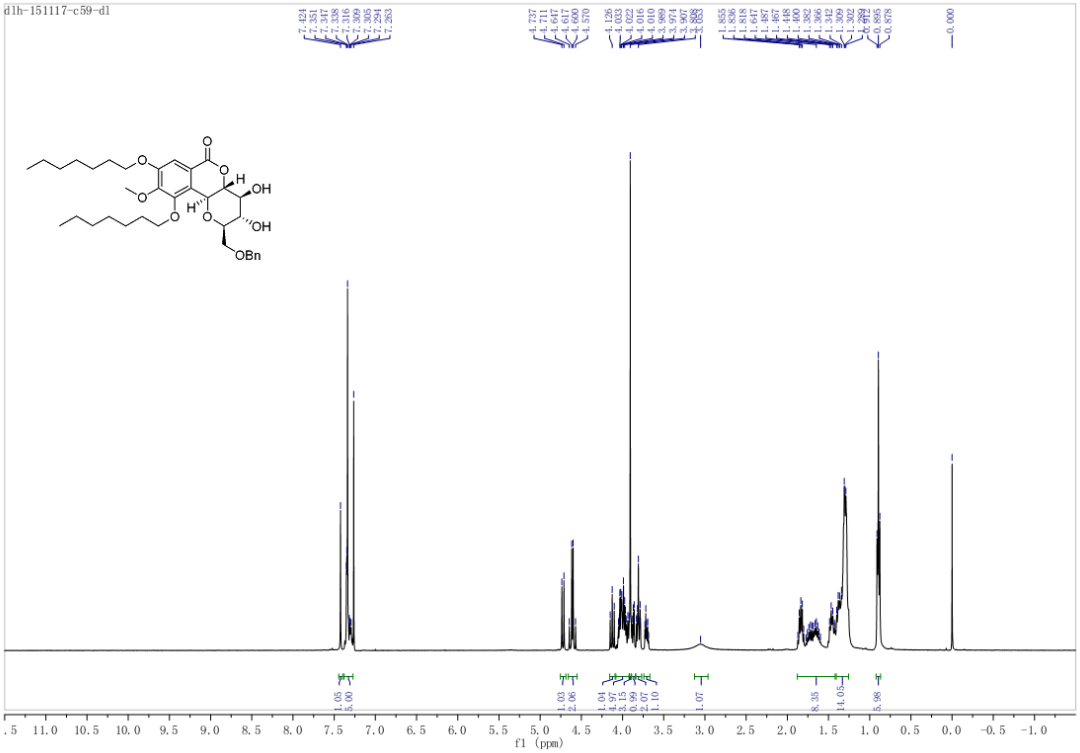

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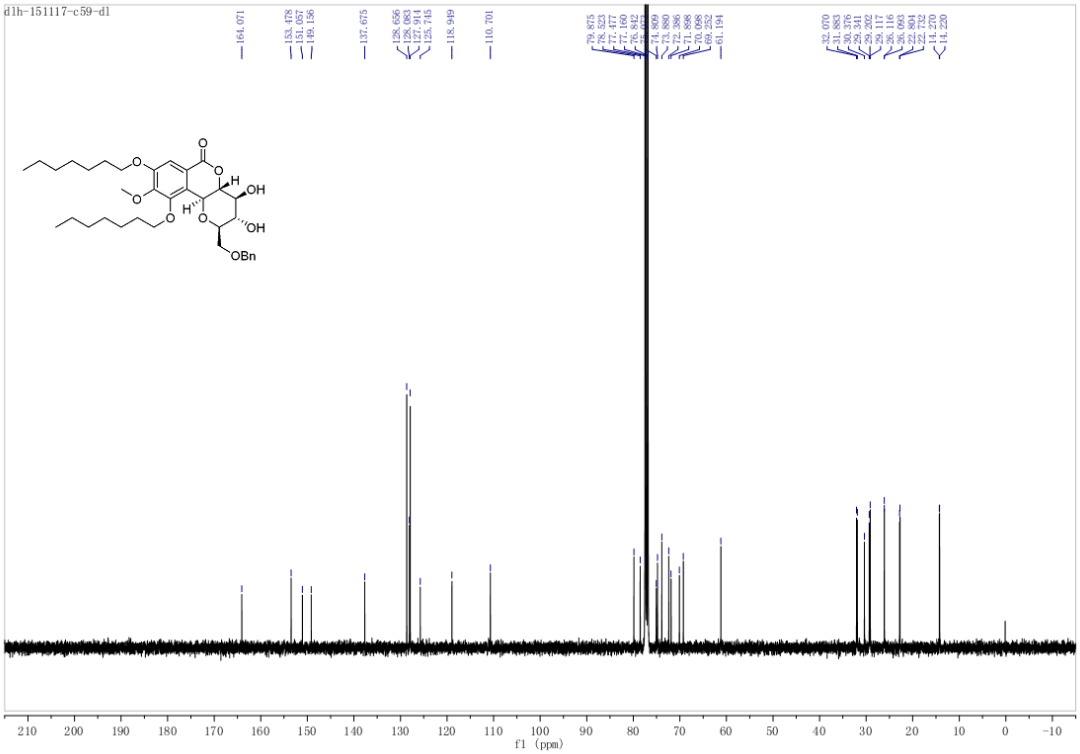

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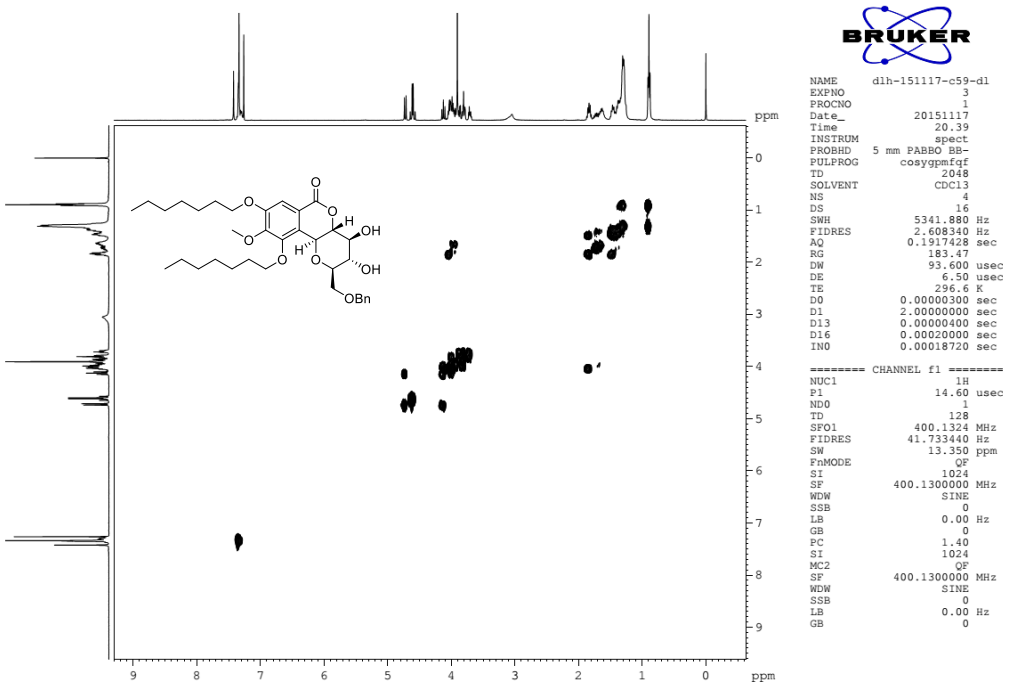

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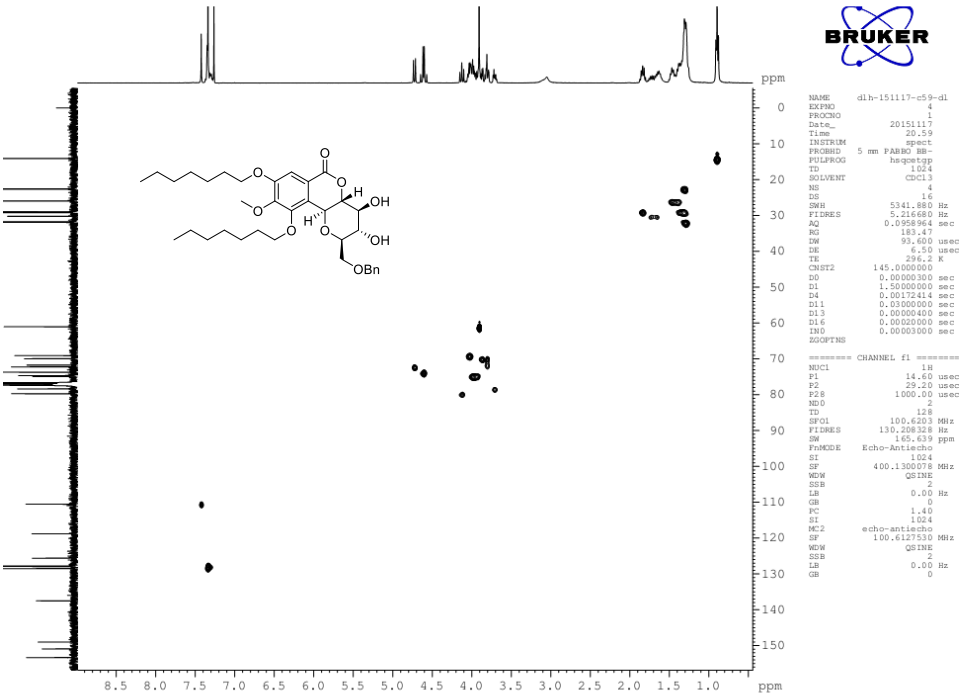

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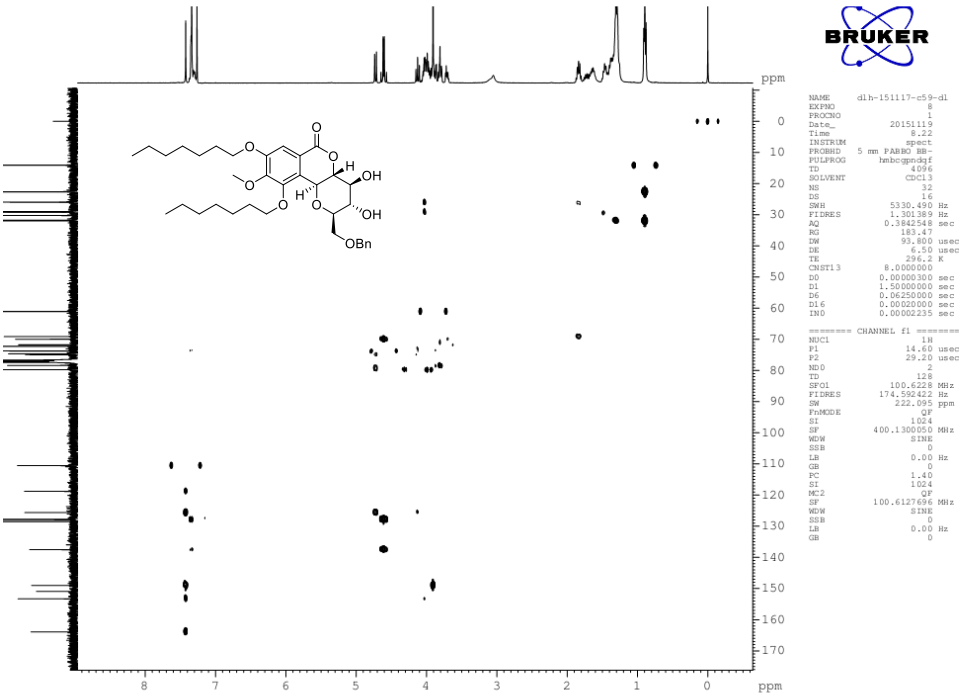

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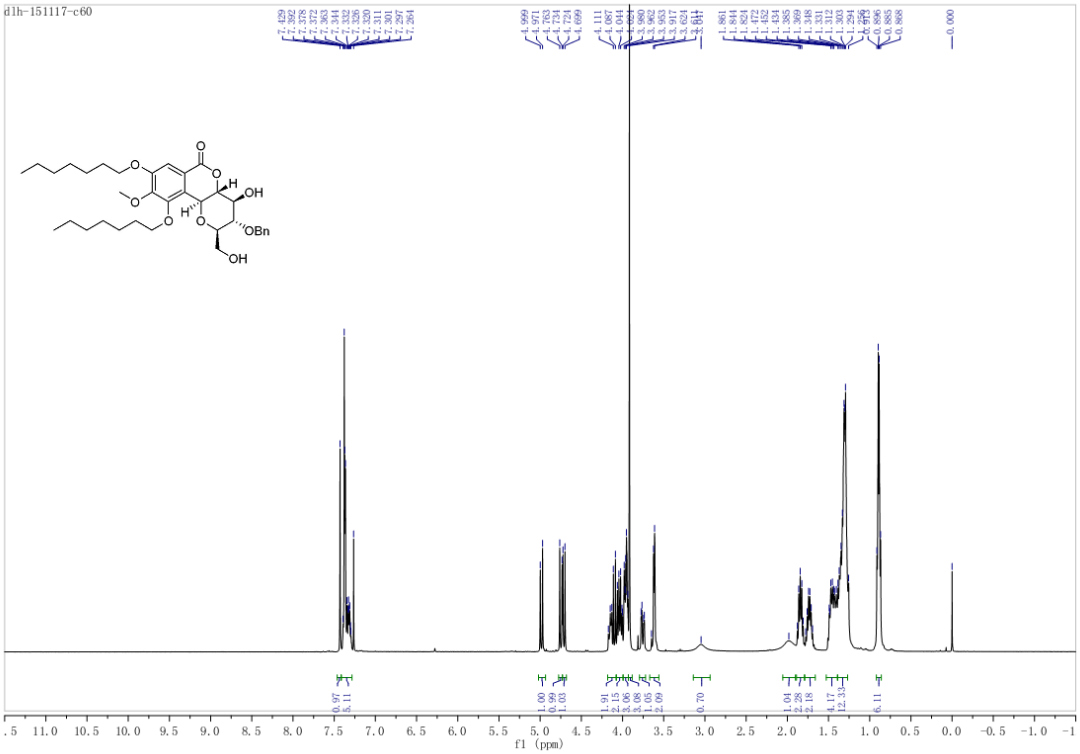

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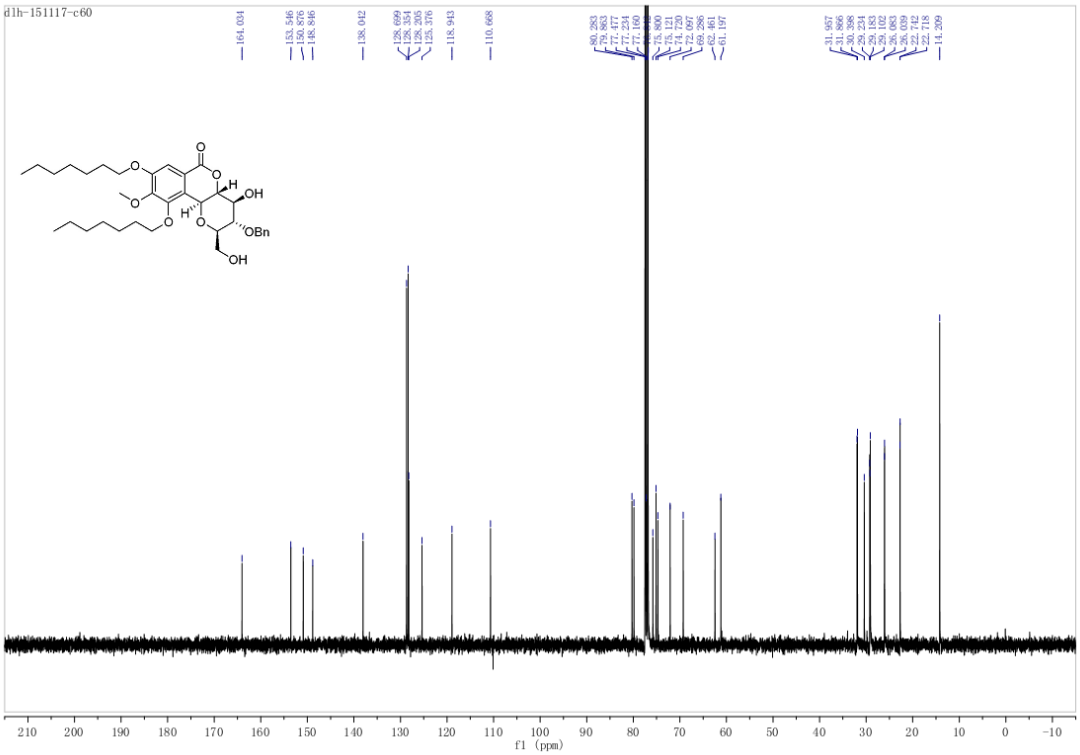
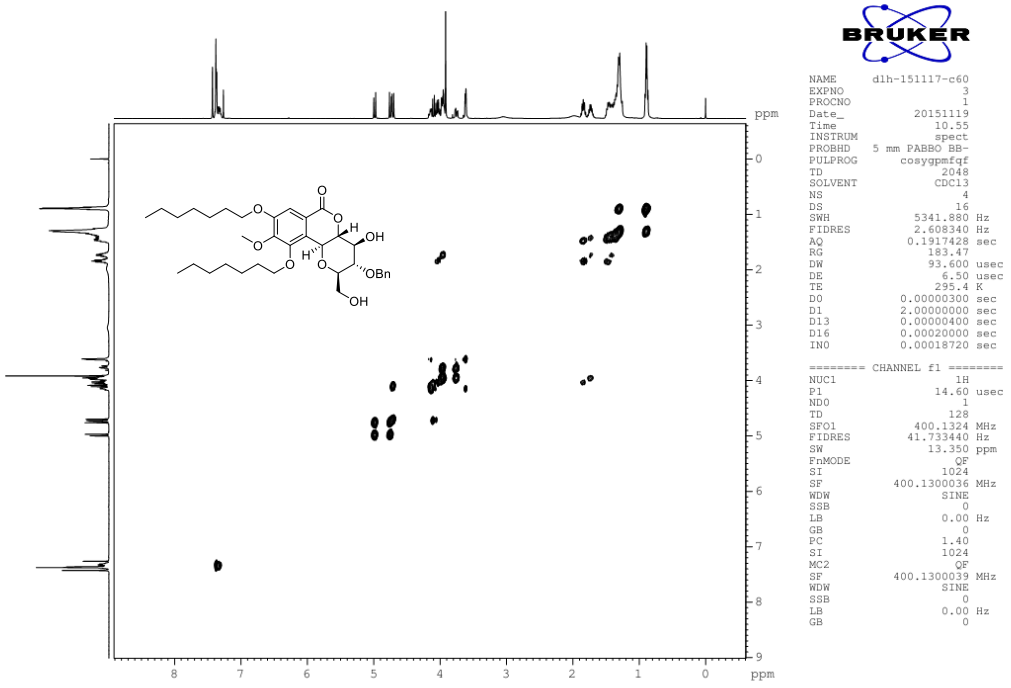


^{1}H-^{1}H COSY of compound **6**



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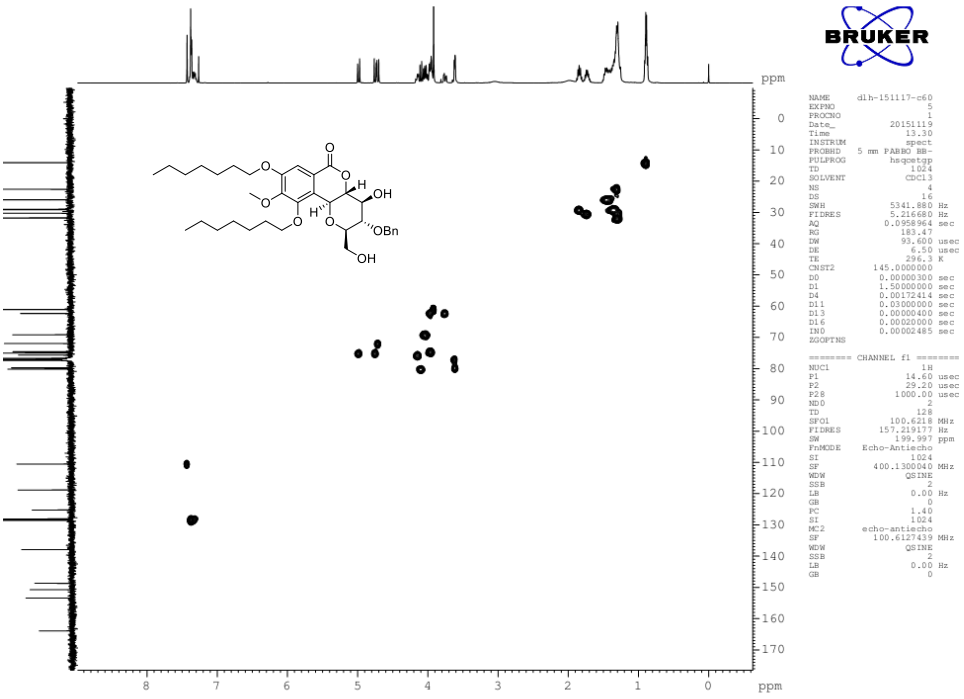
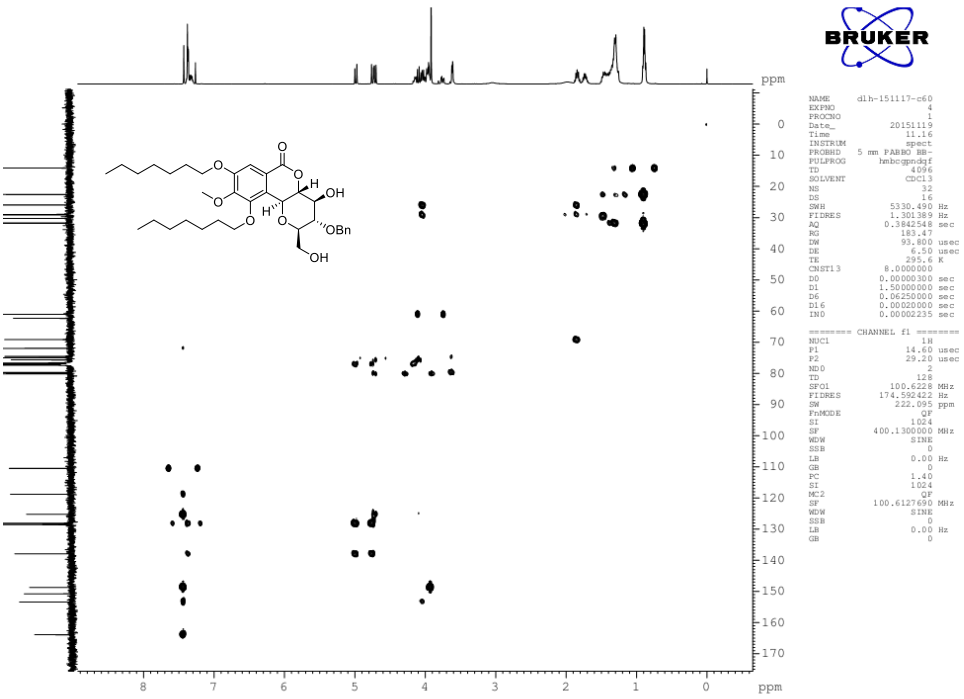
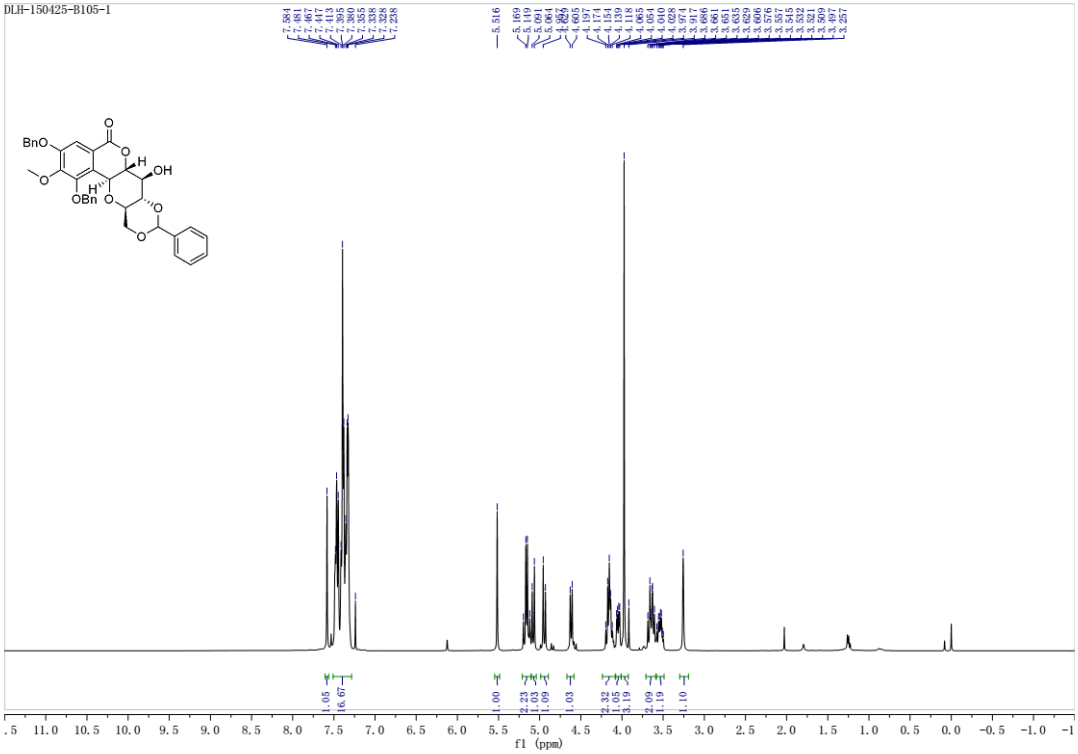
HSQC of compound **6**



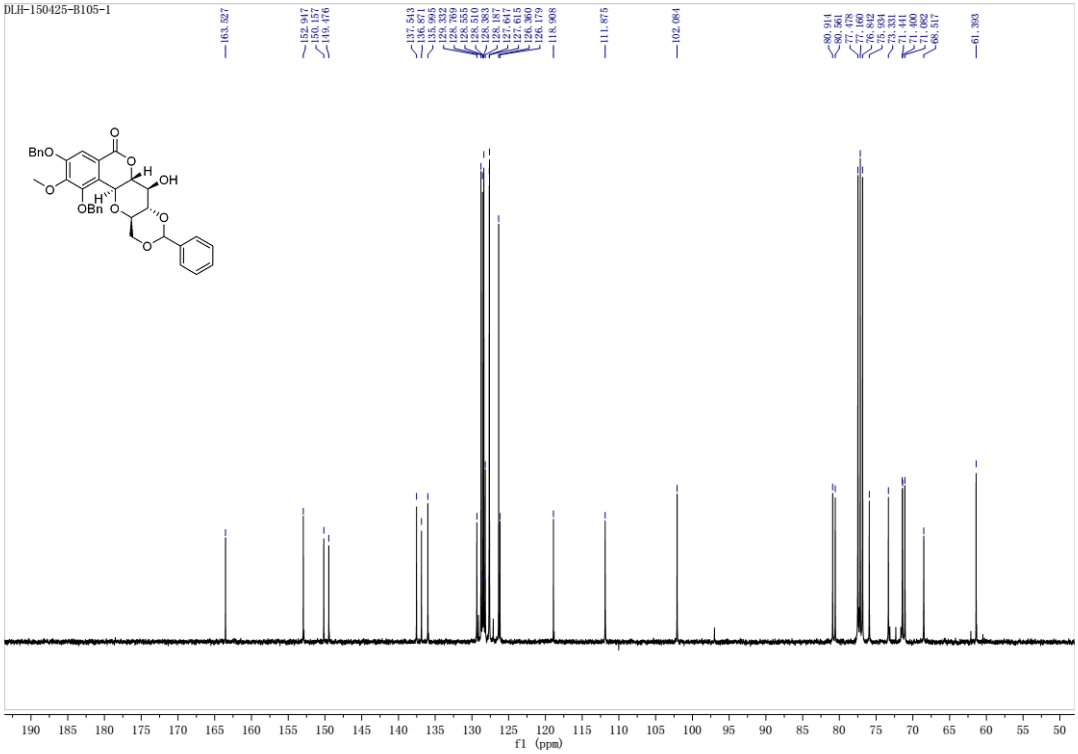
HMBC of compound **6**



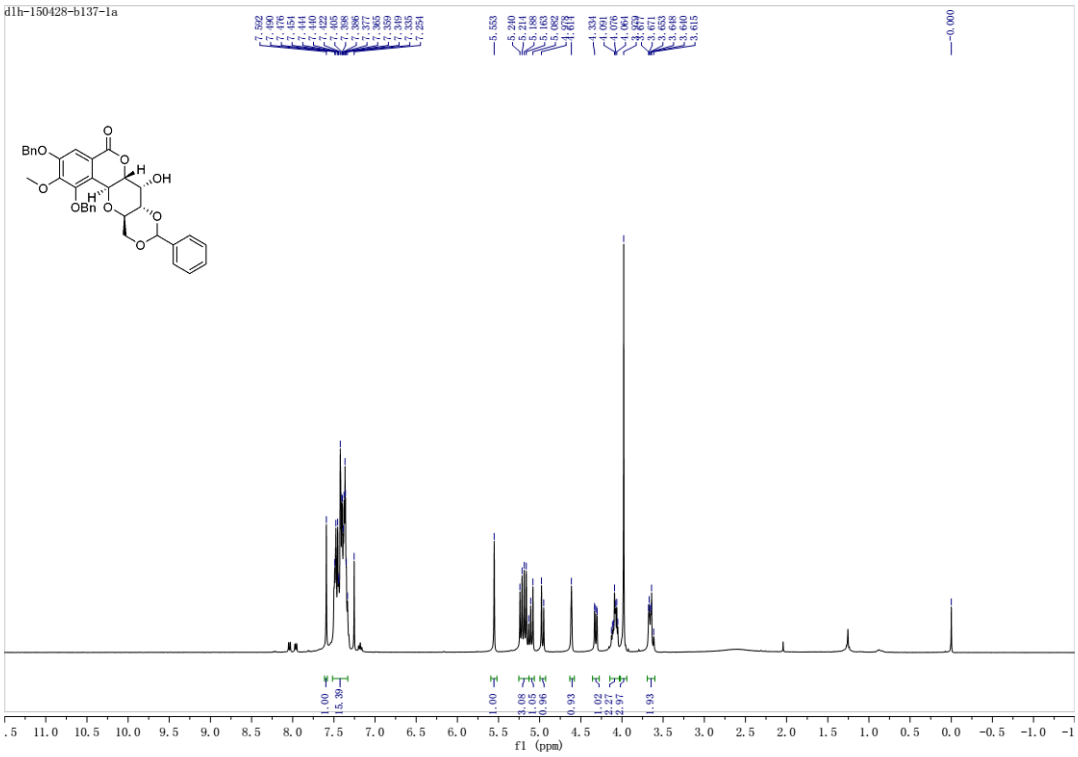
^{1}H NMR of compound **7**



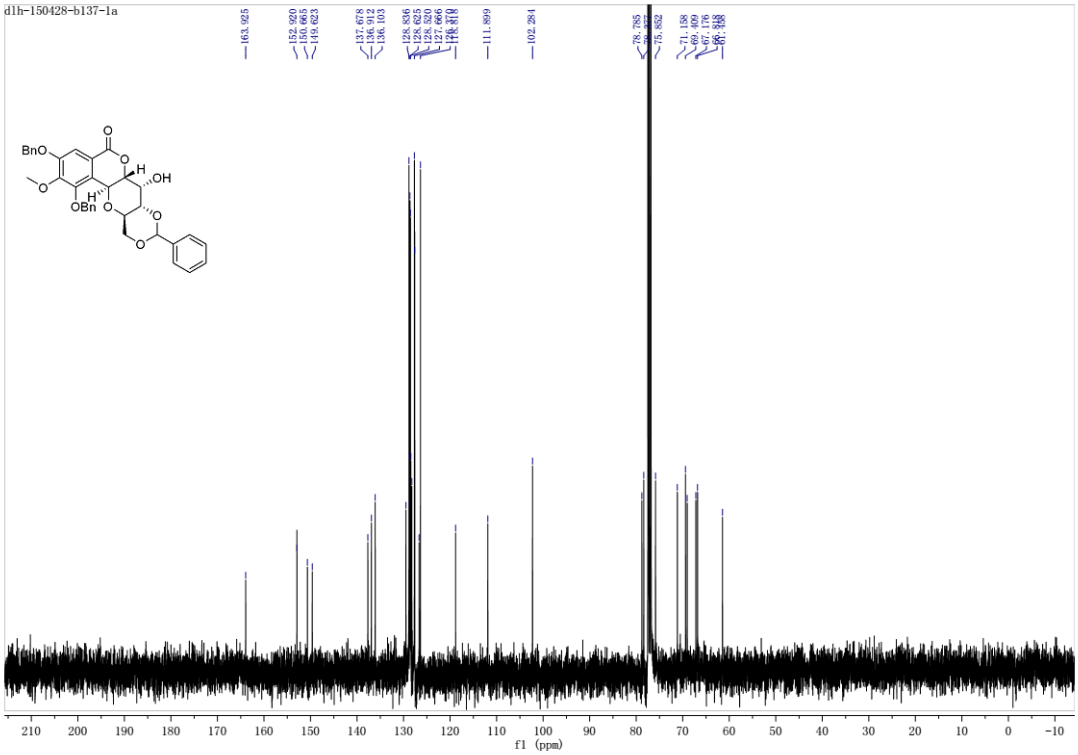
^{13}C NMR of compound **7**



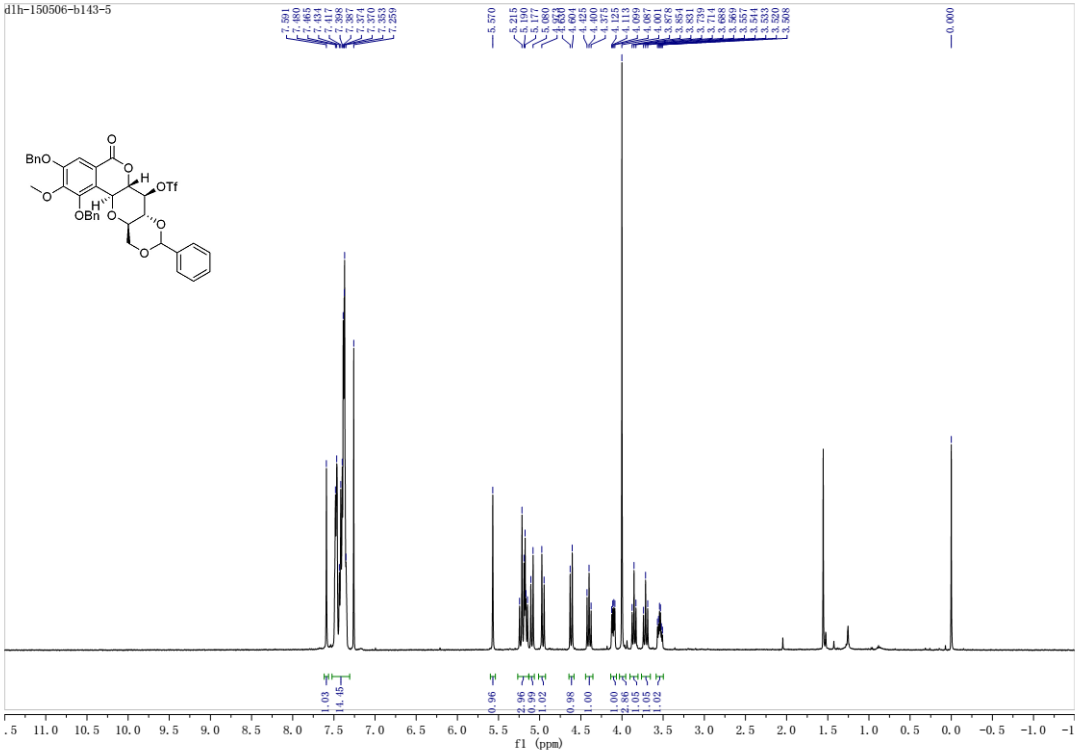
^{1}H NMR of compound **8**



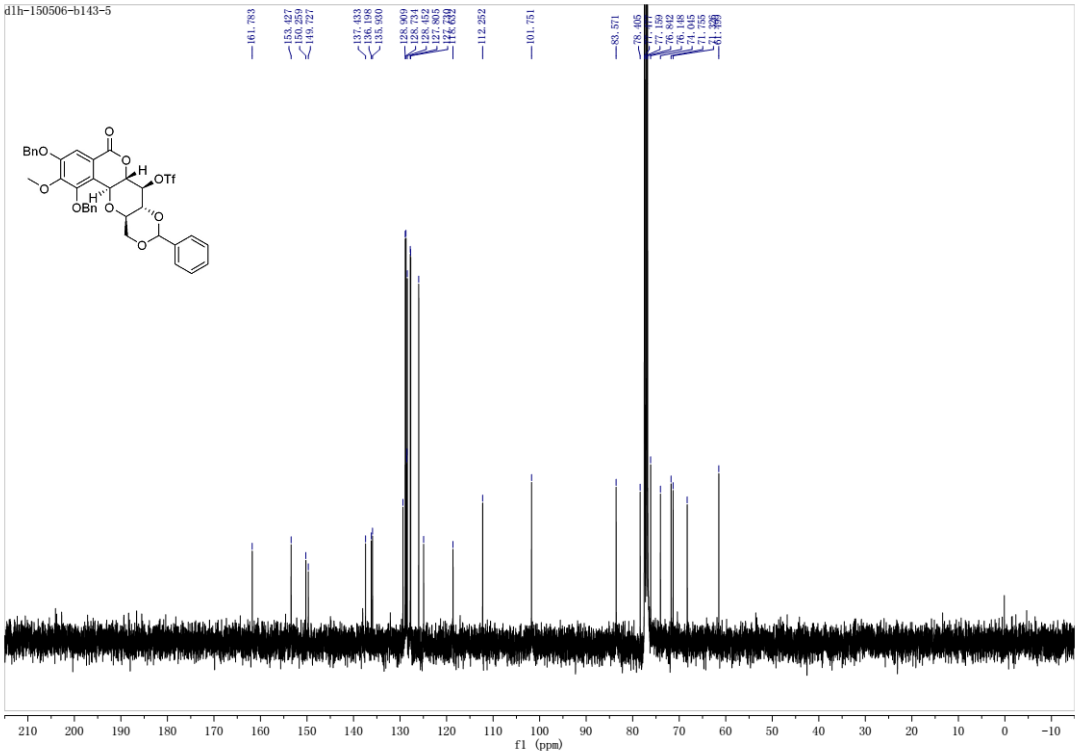
^{13}C NMR of compound **8**



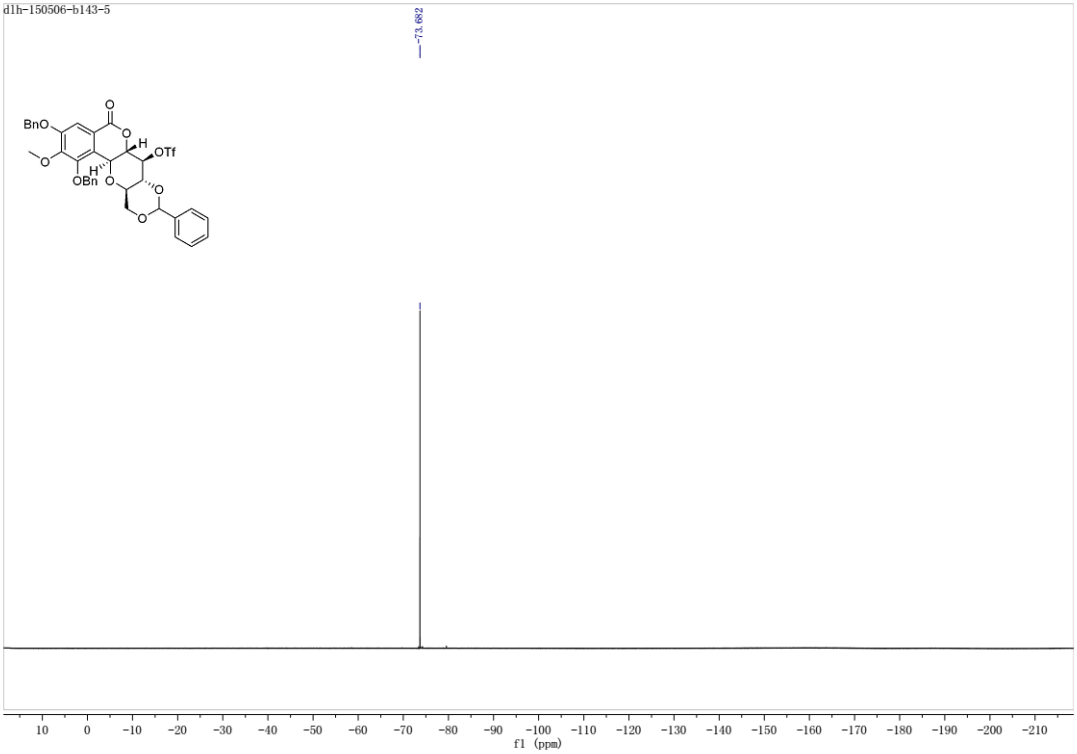
^{1}H NMR of compound **9**



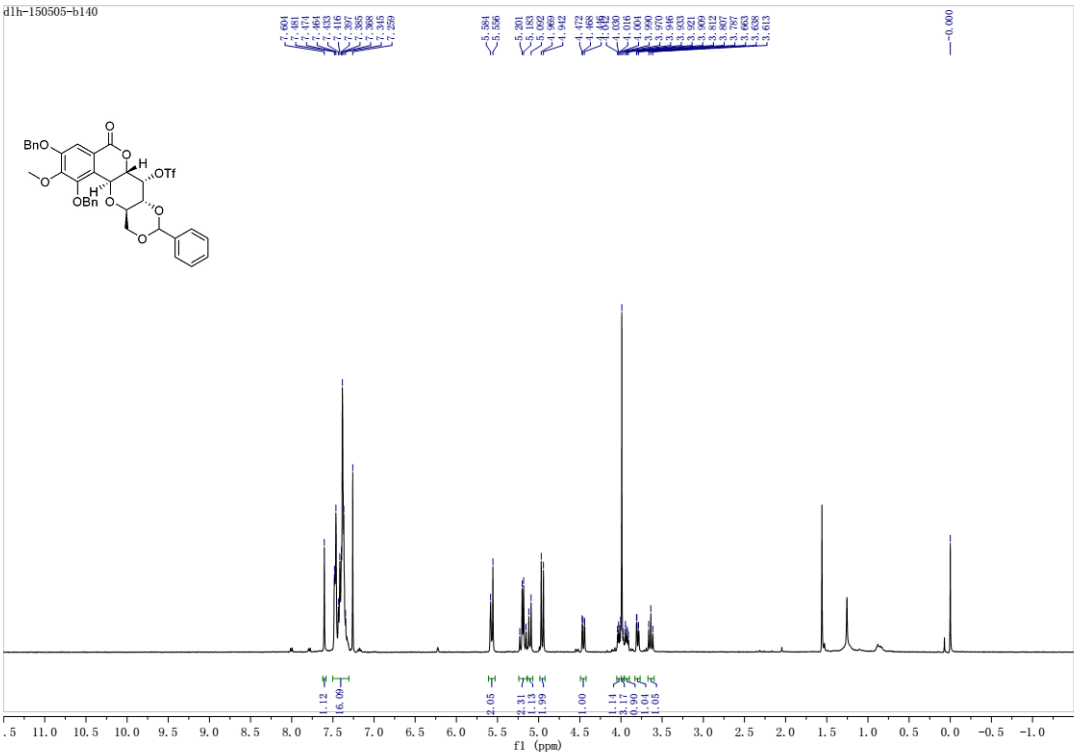
^{13}C NMR of compound **9**



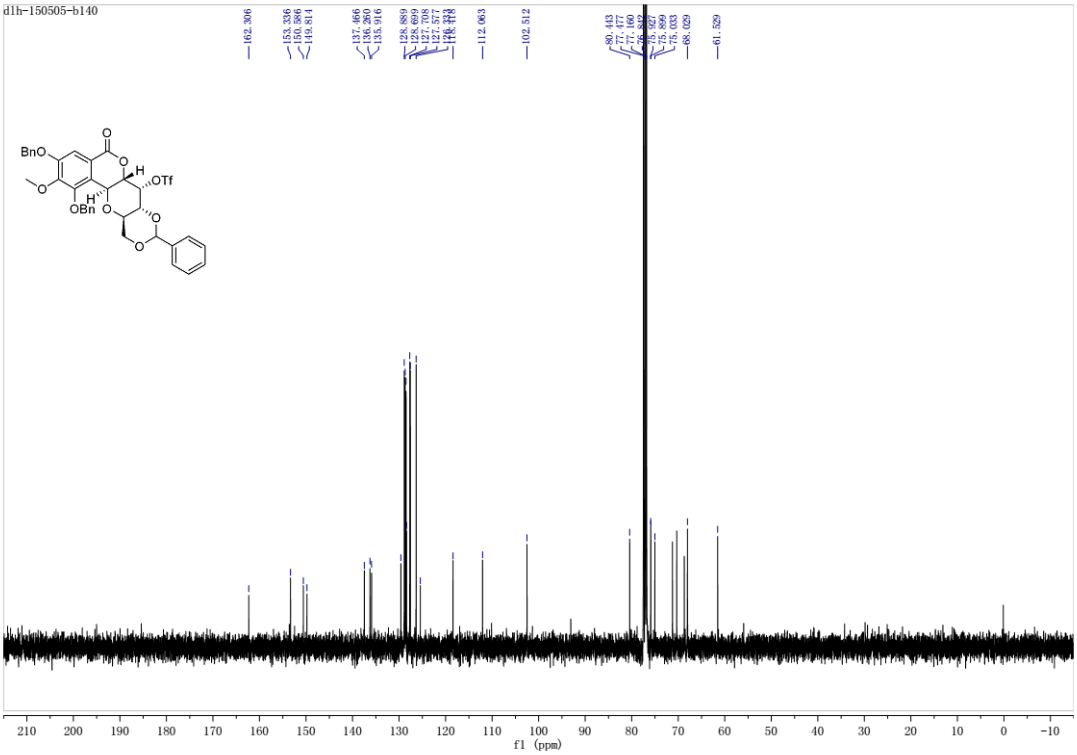
^{19}F NMR of compound **9**



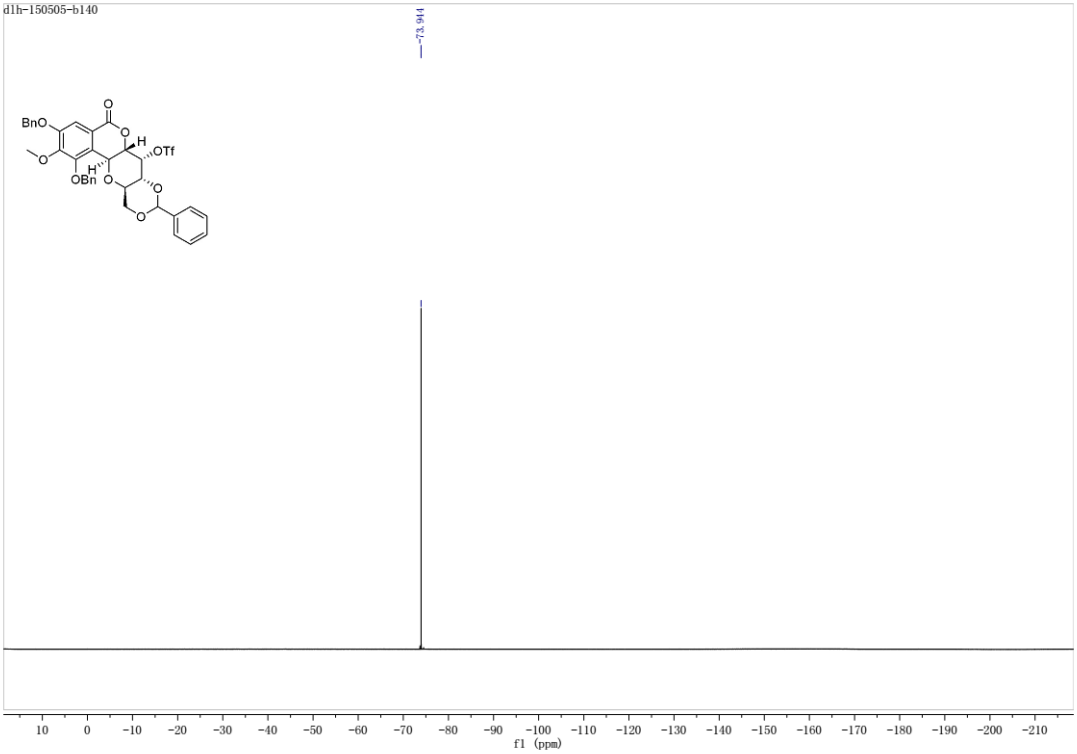
^{1}H NMR of compound **10**



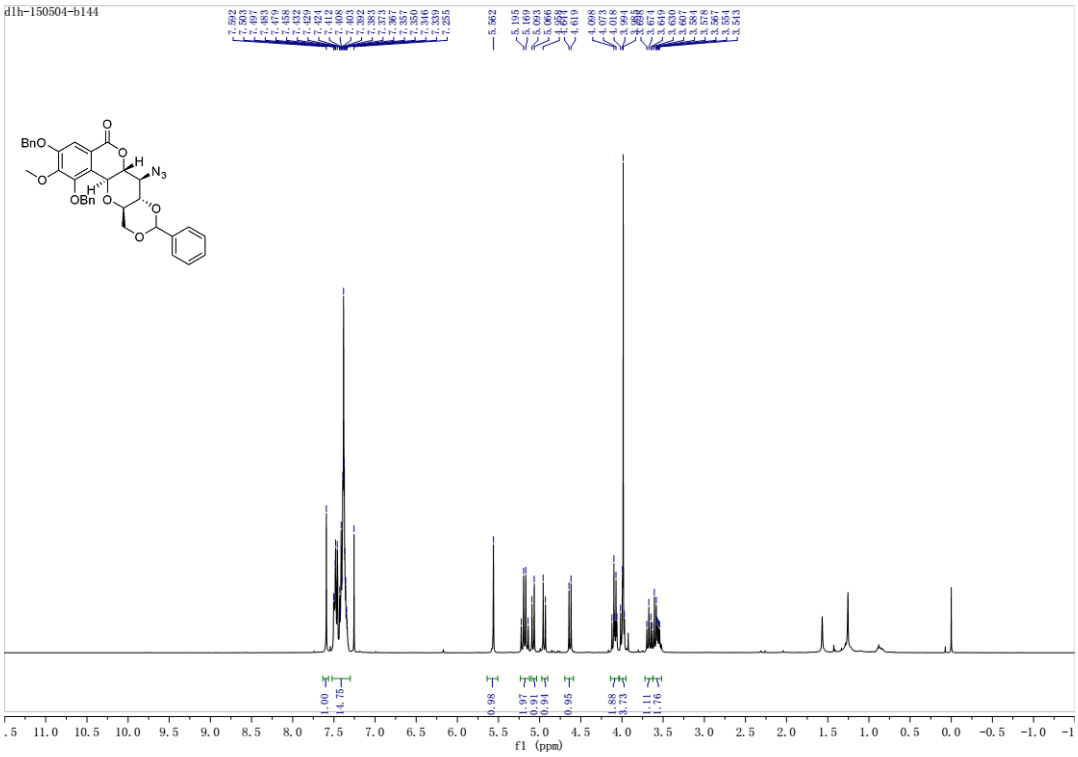
^{13}C NMR of compound **10**



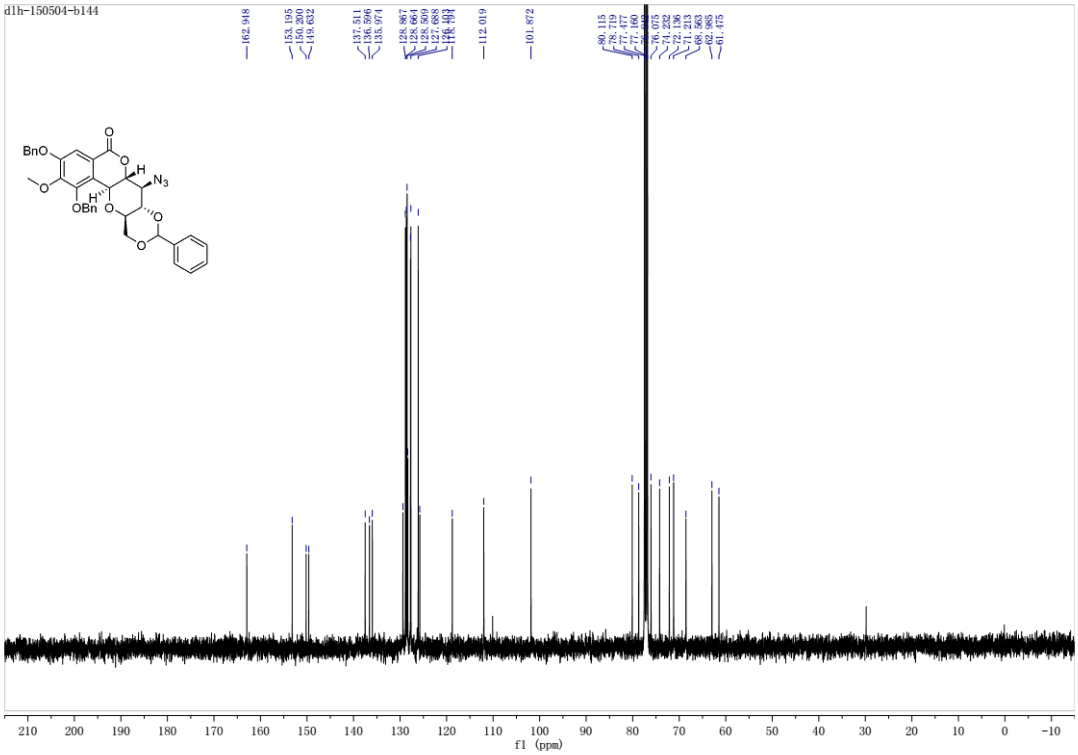
^{19}F NMR of compound **10**



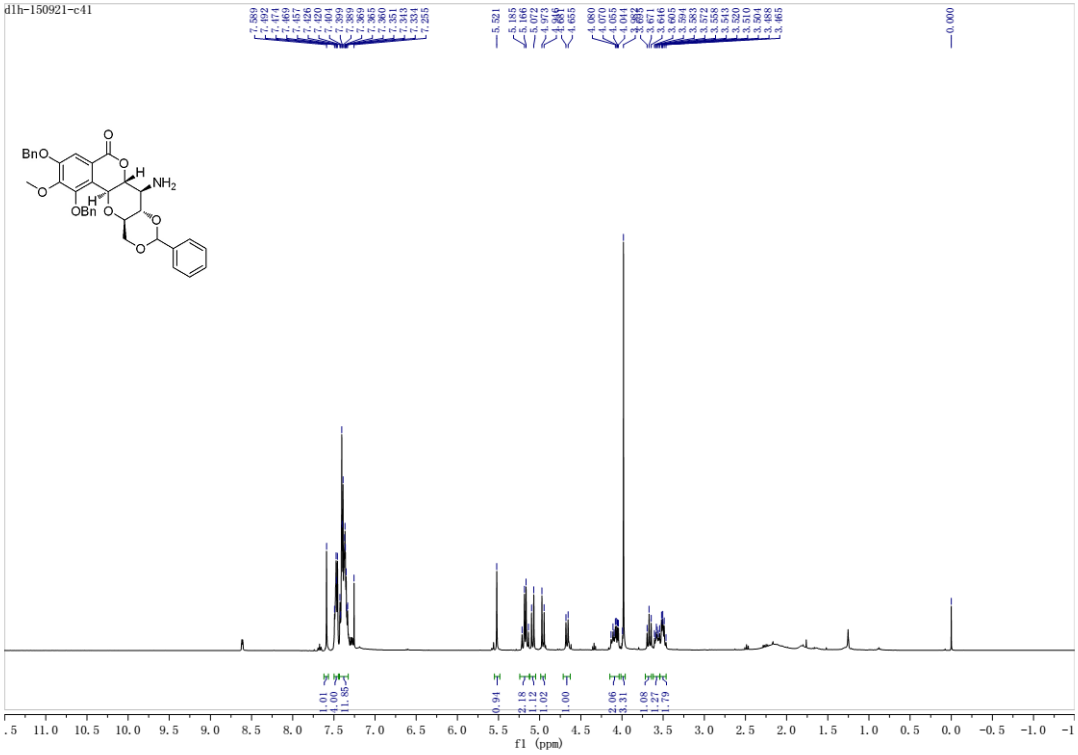
^{1}H NMR of compound **11**



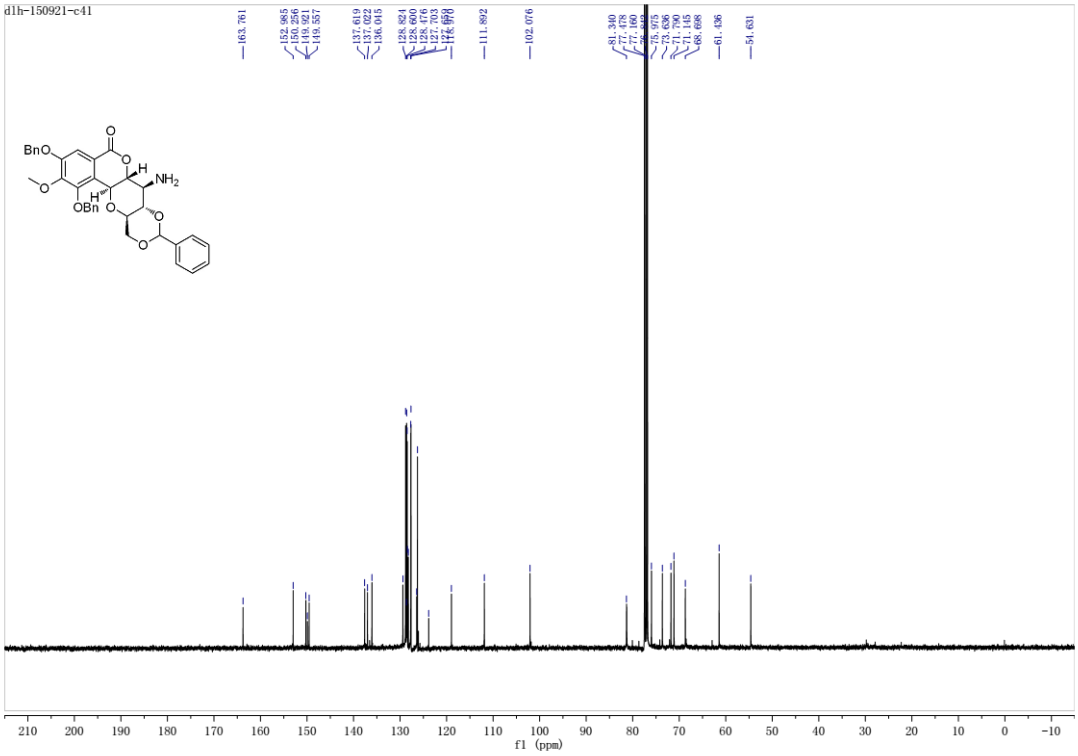
^{13}C NMR of compound **11**



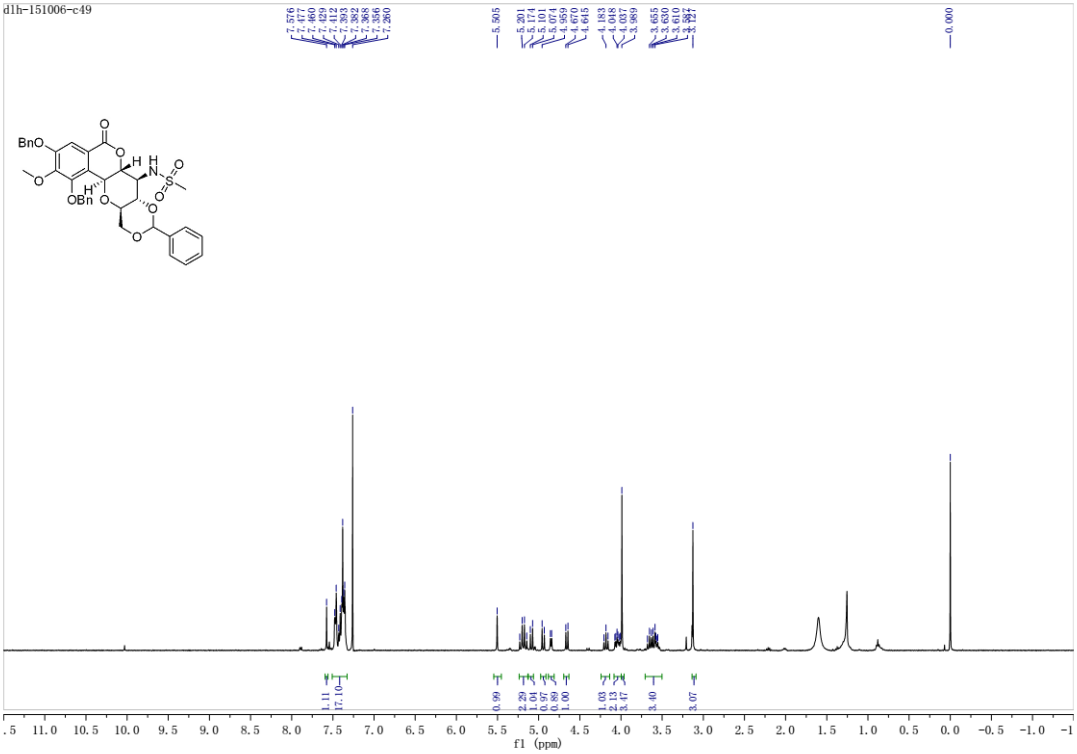
^{1}H NMR of compound **12**



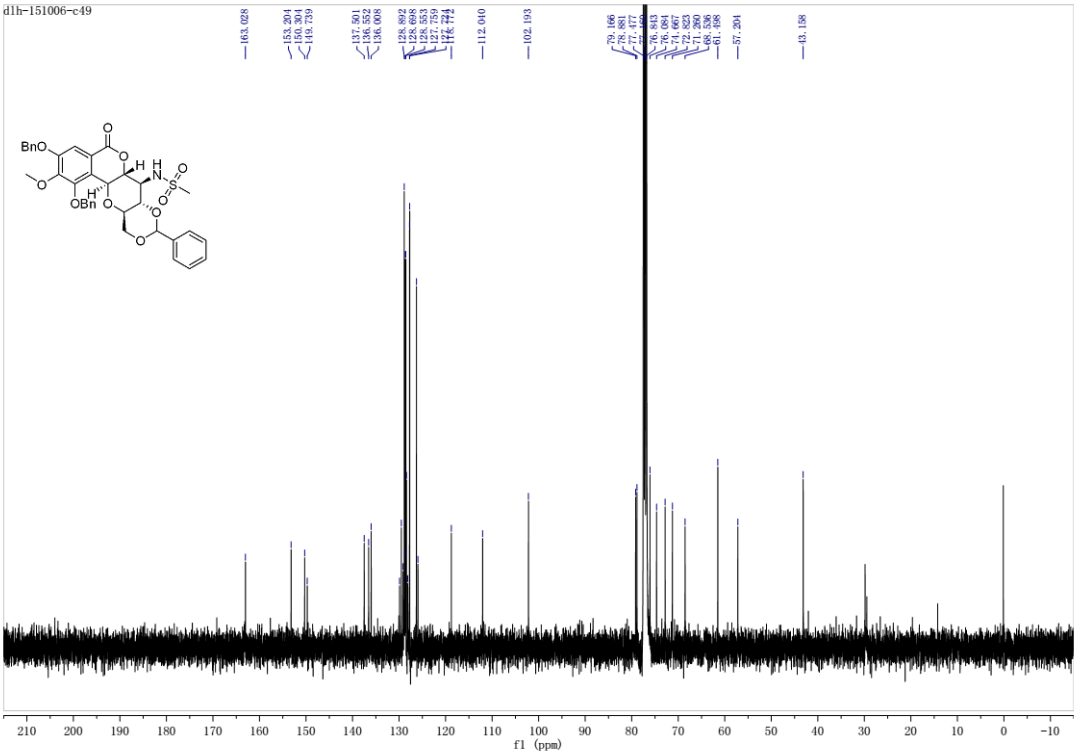
^{13}C NMR of compound **12**



^{1}H NMR of compound **13**



^{13}C NMR of compound **13**



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