Supporting Information

Synthesis and Structure-Activity-Relationship Study of 5a-Carbasugar Analogues of SL0101

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Section A: General Information

Section B: Experimental Procedure

Section C: ^{1}H, ^{13}C Spetra

General chemistry methods and materials

^{1}H and ^{13}C spectra were recorded on 400 MHz and 500 MHz spectrometers. Chemical shifts were reported relative to $CDCl_3$ (δ 7.26 ppm), CD_3OD (δ 3.31 ppm), acetone-d_6 (δ 2.05 ppm) for ^{1}H, and $CDCl_3$ (δ 77.0 ppm), CD_3OD (δ 49.15 ppm), acetone-d_6 (δ 29.92 ppm) for ^{13}C. Optical rotations were measured with a digital polarimeter at sodium D line (589 nm) and were reported in concentration of g/100 mL at 25 ºC in the solvent specified. Infrared (IR) spectra were obtained on a FT-IR spectrometer. Flash chromatography was performed using the indicated solvent system on silica gel standard grade 60 (230-400 mesh). R_f values are reported for analytical TLC using the specified solvents and 0.25 mm silica gel 60 F254 plates that were visualized by UV irradiation (254 nm and 365 nm) or by staining with $KMnO_4$ stain or *p*-anisaldehyde stain. Ethyl ether, tetrahydrofuran, methylene chloride, toluene, and triethylamine were dried by passing through activated alumina (8 x 14 mesh) column with argon gas pressure. Commercial reagents were used without purification unless otherwise noted. Air and/or moisture-sensitive reactions were carried out under an atmosphere of argon/nitrogen using oven/flamed-dried glassware and standard syringe/septum techniques. Melting points are uncorrected. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were obtained using α-cyano-4-hydroxycinnamic acid (CCA) as the matrix on a MALDI-TOF mass spectrometer.

Biologic Methods

Purified recombinant RSK2

Baculovirus encoding His-tagged RSK2 cDNA was generated using the Bac-to-Bac Baculovirus Expression System (Invitrogen, Carlsbad, CA). Recombinant RSK2 was expressed in Sf9 cells and activated by a 20 min treatment with phorbol 12-myristate 13-acetate (PMA). Protein was purified using the Ni-NTA Spin Kit (Qiagen, Valencia, CA).

In vitro kinase assays

The assays were performed as previously described[1]. Briefly, a fusion protein consisting of glutathione S-transferase and the amino acid sequence RRRLAS**T**TNDKG (1 μg/well) was adsorbed to MaxiSorp-treated LumiNunc 96-well white polystyrene plates (Thermo Scientific, Roskilde, Denmark). The wells were blocked with 3% tryptone in phosphate-buffered saline. Kinase (5 nM) in kinase buffer (25 mM HEPES pH 7.4, 150 mM NaCl, 5 mM β-glycerophosphate, 1.5 mM DTT, 30 mM $MgCl_2$, 1% BSA) was added. Reactions were incubated with or without inhibitor. Reactions were initiated by the addition of ATP (10 μM) for 20 min, which is in the linear range of the assay. The reactions were terminated by addition of EDTA (500 mM, pH 8.0). The plates were washed and phosphorylation was measured using rabbit polyclonal anti- LApSTND[1] and horseradish peroxidase (HRP)-conjugated donkey anti-rabbit (Jackson ImmunoResearch Laboratories, West Grove, PA) antibodies. Western Lightning Enhanced Chemiluminescent Reagent Plus (PerkinElmer Life Sciences, Waltham, MA) was used to measure HRP activity. To determine IC_{50} values, non-linear regression analysis was performed using GraphPad Prism version 6.0a (La Jolla, CA).

Proliferation assays

The assays were performed as previously described[1]. Briefly, 200,000 MCF-7 or MCF-10A cells were seeded in 24-well tissue culture treated plates in media recommended by ATCC. Inhibitor or vehicle was added and proliferation was measured after 48 h using CellTiterGlo reagent (Promega, Madison, WI) according to the manufacturer's protocol. To determine IC_{50} values, non-linear regression analysis was performed using GraphPad Prism version 6.0a (La Jolla, CA).

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1 Smith, J. A.; Poteet-Smith, C. E.; Xu, Y.; Errington, T. M.; Hecht, S. M.; Lannigan, D. A. *Cancer Res.* **2005,** *65*, 1027-34.

Immunoblot analysis

The assays were performed as previously described[2]. Briefly, 250,000 MCF-7 cells were seeded onto a 35-mm tissue culture dish. The next day the medium was replaced with serum-free medium. After 16 hours, the medium was changed for serum-free medium with DMSO, serum-containing medium with DMSO, or serum-containing medium with inhibitor. Cells were treated for 2 hours before stimulation with PMA (500 nM) for 20 minutes. Cell lysis was performed as previously described[3]. Lysates were normalized for total protein, electrophoresed, and immunoblotted. Antibodies used for immunoblotting include: anti-(K/R)x(K/R)xx(pS/pT) motif (9611), anti-(K/R)(K/R)x(pS/pT) motif (9621), anti-(K/R)x(pS/pT)Φ(K/R) motif (2261), monoclonal anti-phospho-Y (9411), anti-eEF2 (2332), anti-phospho-eEF2 (2331), and anti-cyclin D1 (2926), from Cell Signaling Technology (Danvers, MA). Secondary antibodies used were HRP-conjugated donkey anti-rabbit and goat anti-mouse (Jackson ImmunoResearch Laboratories, West Grove, PA).

2 Joel, P.B.; Traish, A.M.; Lannigan, D.A. *J. Biol. Chem.* **1998,** *273,* 13317-23.

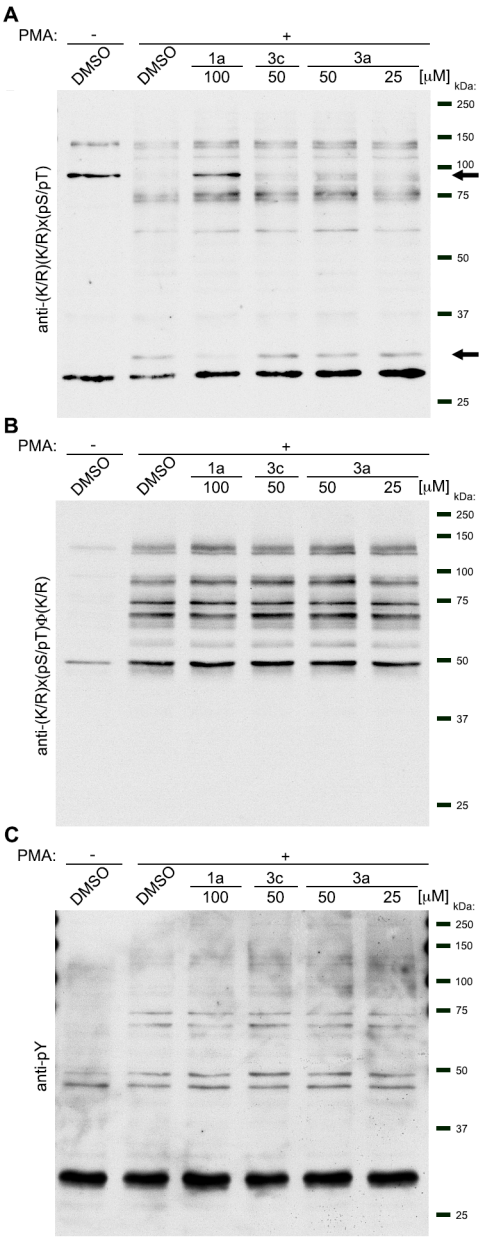
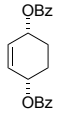


Figure S1. Characterization of selectivity of inhibitors **3a** and **3c** compared to SL0101 (**1a**) in MCF-7 cells. MCF-7 cells were treated with PMA after pre-treatment with the indicated inhibitors. Lysates were electrophoresed and immunoblotted. The arrows indicate bands whose intensity changes upon treatment with SL0101 (**1a**). Antibodies used detect the PKA phosphorylation motif (A), the PKC phosphorylation motif (B) or the tyrosine kinase motif (C).

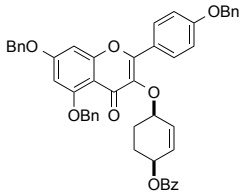
Compounds characterization:

(1*R*,4*S*)-cyclohex-2-ene-1,4-diyl dibenzoate (10): Prepared from literature procedure[3]



To a stirred solution of $Pd(OAc)_2$, *p*-Benzoquinone and benzoic acid in 200 mL acetone was added a solution of 1,3-cyclohexadiene in 50 mL acetone with addition funnel over 4 hours. Then the reaction mixture was stirred at rt overnight. The solvent was removed under reduced pressure with rotovap. The residue was then diluted with ether, washed with a 2M NaOH aq. solution (200 mL x 2, 100 mL x 1). The combined aqueous layers were extracted back with ether. The combined organic layers were then dried over Na_2SO_4, concentrated. The crude product was then purified by recrystallization with petroleum ether and dichloromethane (9: 1) to give cis-bisbenzoate **10** (7.6 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 – 8.04 (m, 4H), 7.63 – 7.53 (m, 2H), 7.45 (t, *J* = 7.7 Hz, 4H), 6.10 (s, 2H), 5.54 (s, 2H), 2.17 – 2.03 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 133.1, 130.6, 130.4, 129.8, 128.5, 68.0, 25.2. NMR spectra match with the reported data.

(1*S*,4*R*)-4-((5,7-bis(benzyloxy)-2-(4-(benzyloxy)phenyl)-4-oxo-4*H*-chromen-3-yl)oxy)cyclohe x-2-en-1-yl benzoate (11):

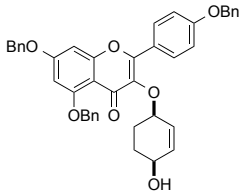


To a solution of perbenzylated flavonol **7** (112 mg, 0.2 mmol), 1,8-Diazabicycloundec-7-ene (DBU, 30 μL, 0.2 mmol) and bis-benzoate **10** (130 mg, 0.4 mmol) in 2 mL CH_2Cl_2 was added a solution of η^3-$(C_3H_5PdCl)_2$ (3.6 mg, 0.01 mmol) and (*S*,*S*)-phenyl-DACH-Trost ligand (21 mg, 0.03 mmol) in 0.5 mL CH_2Cl_2 at 0 °C. The reaction mixture was stirred and allowed to warmed up to rt overnight then loaded onto chromatography column on silica gel, eluting with hexane-EtOAc (5: 1) gave glycosylated product **11** (85 mg, 56%). Light yellow oil. R_f = 0.25 (5: 1 (v/v) hexane/EtOAc). $[\alpha]_D^{20}$ = –21.7 (*c* = 1.46, CH_2Cl_2). IR (thin film, cm^{-1}) 3062, 3032, 2933, 2874, 1712, 1625, 1603, 1508, 1451, 1353, 1267, 1255, 1143, 1105, 1014, 917, 834. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* = 9.0 Hz, 2H), 8.03 (d, *J* = 7.2 Hz, 2H), 7.59 (d, *J* = 7.3 Hz, 2H), 7.53 (dd, *J* = 7.3, 7.4 Hz, 1H), 7.48 – 7.23 (m, 15H), 7.05 (d, *J* = 9.0 Hz, 2H), 6.59 (d, *J* = 2.2 Hz, 1H), 6.46 (d, *J* = 2.2 Hz, 1H), 6.20 (dd, *J* = 10.1, 2.4 Hz, 1H), 5.89 (dd, *J* = 10.1, 2.2 Hz, 1H), 5.43 – 5.36 (m, 1H), 5.28 (s, 2H), 5.09 (s, 2H), 5.05 (s, 2H), 5.02 – 4.96 (m, 1H), 1.99 – 1.74 (m,

3 Bäckvall, J. E.; Granberg, K. L.; Hopkins, R. B. *Acta. Chem. Scand.* **1990***, 44,* 492-9

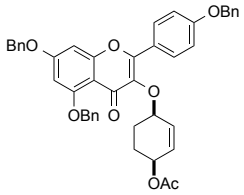
4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 166.2, 162.9, 160.4, 160.0, 158.9, 153.8, 139.0, 136.6, 136.5, 135.9, 133.1, 132.7, 130.7, 129.8, 128.9, 128.8, 128.75 128.6, 128.5, 128.3, 127.9, 127.8, 127.7, 126.8, 123.9, 114.6, 110.1, 98.3, 94.1, 74.5, 71.0, 70.6, 70.2, 68.4, 25.5, 25.3. HRMS (MALDI-TOF/CCA): Calcd. for $[C_{49}H_{40}O_8 + H]^+$: 757.2796 , Found: 757.2764.

5,7-bis(benzyloxy)-2-(4-(benzyloxy)phenyl)-3-(((1*R*,4*S*)-4-hydroxycyclohex-2-en-1-yl)oxy)-4*H*-chromen-4-one (12):



To a solution of benzoate **11** (80 mg, 0.1 mmol) in 4 mL MeOH-THF (1: 1) was added K_2CO_3 (20 mg, 0.15 mmol) at 0 °C. The reaction mixture was stirred overnight and then diluted with EtOAc, washed with water (25 mL × 3), saturated aqueous NaCl, dried over Na_2SO_4 and concentrated under reduced pressure to give crude product. The crude product was then purified with chromatography on silica gel, eluting with Hexane-EtOAc (4: 1) gave allylic alcohol **12** (56 mg, 82%) as light yellow oil. R_f = 0.25 (7: 5 (v/v) hexane/EtOAc). $[\alpha]_D^{20}$ = +15.4 (*c* = 1.14, CH_2Cl_2). IR (thin film, cm^{-1}) 3583, 3450, 3442, 2967, 2925, 2869, 2684, 2583, 2478, 2376, 2278, 2180, 2092, 1726, 1624, 1603, 1509, 1452, 1375, 1355, 1295, 1253, 1178, 973, 913, 834. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 9.0 Hz, 2H), 7.57 (d, *J* = 7.6 Hz, 2H), 7.51 – 7.21 (m, 13H), 7.05 (d, *J* = 9.0 Hz, 2H), 6.57 (d, *J* = 2.2 Hz, 1H), 6.44 (d, *J* = 2.2 Hz, 1H), 6.02 (dd, *J* = 10.1, 3.0 Hz, 1H), 5.80 (dd, *J* = 10.1, 2.2 Hz, 1H), 5.27 (s, 2H), 5.13 (s, 2H), 5.08 (s, 2H), 4.87 – 4.79 (m, 1H), 4.09 – 4.01 (m, 1H), 1.88 – 1.76 (m, 1H), 1.75 – 1.56 (m, 3H), 1.45 – 1.37 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 162.8, 160.4, 159.9, 158.9, 153.9, 139.2, 136.6, 135.9, 133.3, 130.7, 130.2, 128.9, 128.9, 128.8, 128.6, 128.3, 127.8, 127.8, 127.7, 126.8, 123.9, 114.6, 110.2, 98.3, 94.0, 74.4, 71.0, 70.6, 70.2, 65.9, 28.5, 25.5. HRMS (MALDI-TOF/CCA): Calcd. for $[C_{42}H_{36}O_7 + H]^+$: 653.2534, Found: 653.2522.

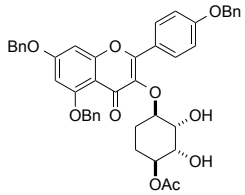
(1*S*,4*R*)-4-((5,7-bis(benzyloxy)-2-(4-(benzyloxy)phenyl)-4-oxo-4*H*-chromen-3-yl)oxy)cyclohex-2-en-1-yl acetate (13):



To the solution of allylic alcohol (58 mg, 0.089 mmol) in 3 mL CH_2Cl_2 at 0 °C was added pyridine (100 µL, 1.2 mmol), DMAP (2 mg, 0.016 mmol), Ac_2O (85 µL, 0.88 mmol) sequentially. The reaction mixture was stirred at 0 °C for 4 hours. The mixture was then diluted with EtOAc, washed with sat. $NaHCO_3$, saturated aqueous NaCl, dried over Na_2SO_4 and concentrated under reduced pressure to give crude product. The crude product was then purified with

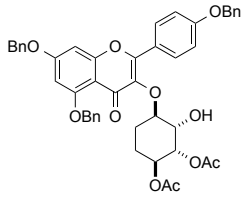
chromatography on silica gel, eluting with Hexane-EtOAc (9: 1), gave allylic acetate **13** (53 mg, 86%) as yellow oil. R_f = 0.42 (7: 3 (v/v) hexane/EtOAc). $[\alpha]_D^{20}$ = –3.2 (*c* = 2.23, CH_2Cl_2). IR (thin film, cm^{-1}) 3417, 2923, 2864, 2830, 2669, 2534, 2371, 1721, 1711, 1620, 1600, 1493, 1439, 1401, 1370, 1354, 1246, 1173, 1119, 1032, 832, 822. ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 8.9 Hz, 2H), 7.58 (d, *J* = 7.4 Hz, 2H), 7.49 – 7.23 (m, 13H), 7.06 (d, *J* = 8.9 Hz, 2H), 6.58 (d, *J* = 2.0 Hz, 1H), 6.44 (d, *J* = 2.0 Hz, 1H), 6.15 (dd, *J* = 10.1, 2.0 Hz, 1H), 5.75 (dd, *J* = 10.1, 2.0 Hz, 1H), 5.27 (s, 2H), 5.18 – 5.10 (m, 3H), 5.08 (s, 2H), 4.86 (s, 1H), 2.05 (s, 3H), 1.84 – 1.76 (m, 2H), 1.76 – 1.62 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 170.9, 162.9, 160.4, 159.9, 158.9, 153.8, 139.3, 136.6, 135.9, 132.6, 130.7, 128.9, 128.9, 128.8, 128.7, 128.6, 128.4, 127.9, 127.8, 127.7, 126.8, 123.9, 114.7, 110.2, 98.3, 94.0, 74.7, 71.0, 70.6, 70.2, 68.0, 25.5, 25.1, 21.5. Mass (MALDI-TOF/CCA): Calcd. for $[C_{44}H_{38}O_8 + H]^+$: 695.2639, Found: 695.2643.

(1*S*,2*S*,3*R*,4*R*)-4-((5,7-bis(benzyloxy)-2-(4-(benzyloxy)phenyl)-4-oxo-4*H*-chromen-3-yl)oxy)-2,3-dihydroxycyclohexyl acetate (14):



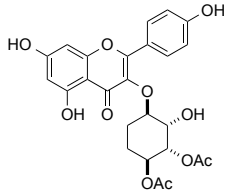
A solution of allylic acetate **13** (44 mg, 0.063 mmol) in 2 mL acetone-*t*-BuOH (1: 1) was cooled down to 0 °C. To the solution was added 26 μL NMO-H_2O (w/w 1:1), and then catalytic amount of crystalline OsO_4 was added into the mixture when stirring. The reaction mixture was kept stirring at 0 °C for 3 hours then warmed up to rt. The reaction mixture was then quenched by 1 mL saturated aqueous $Na_2S_2O_3$. After stirring for another 0.5 hours, all the staff was passed through a small pad of celite and silica gel, washed by EtOAc-MeOH (1:1) (10 mL×3). The organic effluent was dried by Na_2SO_4, and concentrated under reduced pressure to give crude prodcut. The crude product was then purified with chromatography on silica gel eluting with EtOAc-Hexane (1: 1), gave acetate diol **14** (44 mg, 92%) as light yellow solid. m.p. 88-90 ^oC (recrystallized from EtOAc and Hexane), R_f = 0.29 (1: 1(v/v) hexane/EtOAc). $[\alpha]_D^{20}$ = – 64.7 (*c* = 1.45, CH_2Cl_2). IR (thin film, cm^{-1}) 3406, 3005, 2988, 2927, 2870, 2706, 2692, 1735, 1604, 1509, 1454, 1373, 1275, 1260, 1178, 1102, 1015, 909, 834. ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 8.9 Hz, 2H), 7.57 (d, *J* = 7.5 Hz, 2H), 7.52 – 7.16 (m, 13H), 7.08 (d, *J* = 8.9 Hz, 2H), 6.61 (d, *J* = 2.1 Hz, 1H), 6.50 (d, *J* = 2.1 Hz, 1H), 5.26 (s, 2H), 5.16 (s, 2H), 5.10 (s, 2H), 5.04 – 4.98 (m, 1H), 4.07 – 3.96 (m, 2H), 3.96 – 3.80 (m, 1H), 3.03 (brs, 1H), 2.08 (s, 3H), 1.80 – 1.51 (m, 3H), 1.48 – 1.38 (m, 1H), 1.34 – 1.18 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 170.1, 163.5, 160.8, 160.1, 159.1, 155.3, 139.4, 136.5, 136.3, 135.7, 130.9, 129.0, 128.9, 128.8, 128.7, 128.5, 128.0, 127.8, 127.8, 126.8, 123.4, 114.7, 109.6, 98.7, 94.2, 85.0, 72.5, 71.2, 70.8, 70.8, 70.3, 26.2, 23.8, 21.5. HRMS (MALDI-TOF/CCA): Calcd. for $[C_{44}H_{40}O_{10} + Na]^+$: 751.2514, Found: 751.2485.

(1*S*,2*S*,3*S*,4*R*)-4-((5,7-bis(benzyloxy)-2-(4-(benzyloxy)phenyl)-4-oxo-4*H*-chromen-3-yl)oxy)-3-hydroxycyclohexane-1,2-diyl diacetate (15):



A solution of acetate diol **14** (35 mg, 0.048 mmol) in 1 mL acetonitrile was cooled down to 0 °C, then sequentially N,N-Diisopropylethylamine (DIPEA) (17 µL, 0.096 mmol), 10 mol% catalytic amount of Taylor catalyst and AcCl (5 µL, 0.072 mmol) were added. The reaction mixture was stirred at 0 °C overnight. The reaction mixture was diluted by 15 mL EtOAc and washed by 5 mL saturated aqueous $NaHCO_3$, 5 mL saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure to give a residue which was subjected to chromatography on silica gel. Elution with hexane-EtOAc (2: 1) afforded C3'', C4''-diacetate **15** as major product (22 mg, 59%, 5: 1 ratio). Light yellow solid. m.p. 98-100 ^oC (recrystallized from EtOAc and Hexane). R_f = 0.54 (1: 1 (v/v) hexane/EtOAc). $[\alpha]_D^{20}$ = –55.55 (*c* = 1.6, EtOAc). IR (thin film, cm^{-1}) 3405, 3063, 3030, 3005, 2966, 2926, 2870, 1740, 1604, 1509, 1499, 1453, 1438, 1371, 1275, 1259, 1225, 1178, 1104, 1060, 1020, 888, 834. ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 8.9 Hz, 2H), 7.60 (d, *J* = 7.4 Hz, 2H), 7.52 – 7.27 (m, 13H), 7.09 (d, *J* = 8.9 Hz, 2H), 6.60 (d, *J* = 1.7 Hz, 1H), 6.50 (d, *J* = 1.7 Hz, 1H), 6.02 (brs, 1H), 5.36 – 5.28 (m, 1H), 5.25 (d, *J* = 12.8 Hz, 1H), 5.21 (d, *J* = 12.8 Hz, 1H), 5.15 (s, 2H), 5.11 (s, 2H), 4.95 – 4.88 (m, 1H), 4.21 – 4.14 (m, 1H), 4.13 – 4.01 (m, 1H), 2.08 (s, 3H), 2.02 (s, 3H), 1.75 – 1.38 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 169.9, 169.8, 163.4, 160.7, 160.0, 159.1, 154.6, 139.5, 136.5, 136.4, 135.7, 130.8, 129.0, 128.9, 128.8, 128.7, 128.5, 127.9, 127.8, 127.7, 126.8, 123.5, 114.8, 109.8, 98.5, 94.1, 83.3, 72.4, 71.0, 70.9, 70.8, 70.3, 69.5, 26.1, 24.5, 21.4, 21.3. HRMS (MALDI-TOF/CCA): Calcd. for $[C_{46}H_{42}O_{11} + Na]^+$: 793.2619, Found: 793.2598.

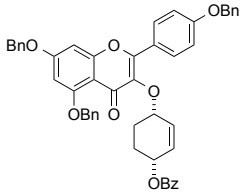
(1*S*,2*S*,3*S*,4*R*)-4-((5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H*-chromen-3-yl)oxy)-3-hydroxycyclohexane-1,2-diyl diacetate (4):



A solution of **15** (14 mg, 0.018 mmol) in 2 mL THF-EtOH (1: 1) was added Pearlman's catalyst (Pd-C, 10%, 2 mg). The solution was degassed using vacuum at room temperature and refilling with H_2. This procedure was repeated three times. The reaction was stirred under a H_2 atmosphere overnight. The reaction mixture was loaded onto silica gel and elution with Et_2O-MeOH (20: 1) gave **4** (8 mg, 96%): Pale yellow solid. m.p. 185-187 ^oC (recrystallized from EtOAc and Hexane). R_f = 0.32 (1: 2 (v/v) hexane/EtOAc). $[\alpha]_D^{20}$ = –54.1 (*c* = 0.74, EtOAc). IR (thin film, cm^{-1}) 3587, 3421, 3397, 3385, 3288, 3253, 3229, 2966, 2935, 2376, 1734, 1701, 1654, 1608, 1569, 1507, 1448, 1363, 1260, 1177, 1089, 1061, 1032, 975, 840. ^1H NMR (400 MHz, CD_3OD) δ 7.86 (d, *J* = 8.7 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 2H), 6.37 (d, *J* = 1.8 Hz, 1H), 6.19 (d, *J* = 1.8 Hz, 1H), 5.10 (dd,

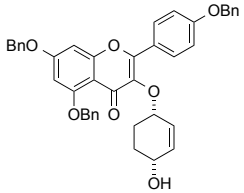
J = 8.2, 2.9 Hz, 1H), 4.97 (td, *J* = 8.2, 4.0 Hz, 1H), 4.51 – 4.43 (m, 1H), 4.20 – 4.14 (m, 1H), 2.05 (s, 3H), 1.98 (s, 3H), 1.77 – 1.65 (m, 1H), 1.65 – 1.52 (m, 2H), 1.52 – 1.30 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 178.7, 170.7, 164.8, 161.9, 160.4, 157.9, 157.3, 135.6, 130.9, 121.5, 115.2, 104.6, 98.6, 93.6, 80.5, 72.9, 69.8, 24.1, 23.2, 19.7, 19.6. HRMS (MALDI-TOF/CCA): Calcd. for $[C_{25}H_{24}O_{11} + Na]^+$: 523.1211, Found: 523.1185.

(1*R*,4*S*)-4-((5,7-bis(benzyloxy)-2-(4-(benzyloxy)phenyl)-4-oxo-4*H*-chromen-3-yl)oxy)cyclohex-2-en-1-yl benzoate ((*ent*)-11):



To a solution of perbenzylated flavonol **7** (168 mg, 0.3 mmol), DBU (45 μL, 0.3 mmol) and bis-benzoate **10** (193 mg, 0.6 mmol) in 3 mL CH_2Cl_2 was added a solution of η^3-$(C_3H_5PdCl)_2$ (5.5mg, 0.015 mmol) and (*R*,*R*)-phenyl-DACH-Trost ligand (31 mg, 0.045 mmol) in 0.5 mL CH_2Cl_2 at 0 °C. The reaction mixture was stirred and allowed to warmed up to rt overnight then loaded onto chromatography column on silica gel, eluting with hexane-EtOAc (5: 1) gave glycosylated product **(*ent*)-11** (138 mg, 61%). m.p. 76-78 °C. R_f = 0.25 (5: 1 (v/v) hexane/EtOAc). $[\alpha]_D^{20}$ = + 9.74 (*c* = 0.12, CH_2Cl_2). IR (thin film, cm^{-1}) 3062, 3032, 2932, 2870, 1712, 1625, 1603, 1508, 1451, 1353, 1268, 1255, 1143, 1105, 1015, 917, 834, 820. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 8.9 Hz, 2H), 8.04 (d, *J* = 7.3 Hz, 2H), 7.60 (d, *J* = 7.4 Hz, 2H), 7.53 (dd, *J* = 7.3, 7.4 Hz, 1H), 7.49 – 7.28 (m, 15H), 7.05 (d, *J* = 8.9 Hz, 2H), 6.59 (d, *J* = 1.9 Hz, 1H), 6.46 (d, *J* = 1.9 Hz, 1H), 6.21 (dd, *J* = 10.1, 2.4 Hz, 1H), 5.90 (dd, *J* = 10.1, 2.2 Hz, 1H), 5.43 – 5.36 (m, 1H), 5.28 (s, 2H), 5.09 (s, 2H), 5.05 (s, 2H), 5.02 – 4.96 (m, 1H), 1.99 – 1.76 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 166.2, 162.9, 160.5, 160.0, 158.9, 153.8, 139.1, 136.6, 136.6, 135.9, 133.1, 132.8, 130.7, 129.8, 129.0, 128.8, 128.7, 128.7, 128.6, 128.4, 127.9, 127.8, 127.7, 126.9, 123.9, 114.7, 110.2, 98.4, 94.1, 74.5, 71.0, 70.7, 70.2, 68.5, 25.6, 25.3. HRMS (MALDI-TOF/CCA): Calcd. for $[C_{49}H_{40}O_8 + H]^+$: 757.2796, Found: 757.2776.

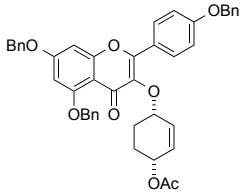
5,7-bis(benzyloxy)-2-(4-(benzyloxy)phenyl)-3-(((1*S*,4*R*)-4-hydroxycyclohex-2-en-1-yl)oxy)-4*H*-chromen-4-one ((*ent*)-12):



To a solution of benzoate **(*ent*)-11** (130 mg, 0.17 mmol) in 4 mL MeOH-THF (1: 1) was added K_2CO_3 (38 mg, 0.28 mmol) at 0 °C. The reaction mixture was stirred overnight and then diluted with EtOAc, washed with water (25 mL × 3), saturated aqueous NaCl, dried over Na_2SO_4 and concentrated under reduced pressure to give crude product. The crude product was then purified

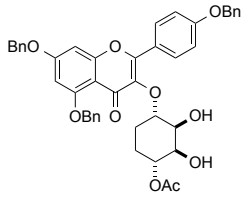
with chromatography on silica gel, eluting with Hexane-EtOAc (4: 1), gave allylic alcohol **(*ent*)-12** (94 mg, 85%) as light yellow solid. m.p. 87-89 ^{o}C (recrystallized from EtOAc and Hexane), R_f = 0.25 (7: 5(v/v) hexane/EtOAc). $[\alpha]_D^{20}$ = –17.0 (*c* = 1.35, CH_2Cl_2). IR (thin film, cm^{-1}) 3399, 3369, 3350, 3063, 3031, 3005, 2929, 2868, 2050, 1734, 1600, 1573, 1507, 1451, 1436, 1374, 1352, 1275, 1259, 1172, 1099, 1013, 910, 833, 819. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 8.9 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.52 – 7.24 (m, 13H), 7.05 (d, *J* = 8.9 Hz, 2H), 6.57 (d, *J* = 1.9 Hz, 1H), 6.44 (d, *J* = 1.9 Hz, 1H), 6.03 (dd, *J* = 10.0, 2.3 Hz, 1H), 5.82 (dd, *J* = 10.0, 2.2 Hz, 1H), 5.25 (s, 2H), 5.12 (s, 2H), 5.07 (s, 2H), 4.87 – 4.80 (m, 1H), 4.08 – 4.00 (m, 1H), 1.88 – 1.76 (m, 1H), 1.76 – 1.58 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 162.9, 160.4, 159.9, 158.9, 153.9, 139.3, 136.7, 135.9, 133.6, 130.7, 130.1, 128.9, 128.9, 128.8, 128.6, 128.4, 127.9, 127.9, 127.8, 126.9, 123.9, 114.7, 110.2, 98.3, 94.1, 74.5, 70.9, 70.7, 70.3, 65.9, 28.5, 25.6. HRMS (MALDI-TOF/CCA): Calcd. for $[C_{42}H_{36}O_7 + H]^+$: 653.2534, Found: 653.2556.

(1*R*,4*S*)-4-((5,7-bis(benzyloxy)-2-(4-(benzyloxy)phenyl)-4-oxo-4*H*-chromen-3-yl)oxy)cyclohex-2-en-1-yl acetate ((*ent*)-13):



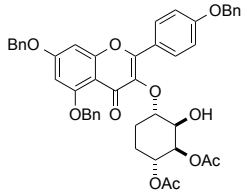
To the solution of 75 mg allylic alcohol **(*ent*)-12** (0.115 mmol) in 4 mL CH_2Cl_2 at 0 ^{o}C was added pyridine (100 μL, 1.2 mmol), DMAP (2 mg, 0.016 mmol), Ac_2O (43 μL, 0.46 mmol) sequentially. The reaction mixture was stirred at 0 °C for 4 hours. The mixture was then diluted with EtOAc, washed with sat. $NaHCO_3$, saturated aqueous NaCl, dried over Na_2SO_4 and concentrated under reduced pressure to give crude product. The crude product was then purified with chromatography on silica gel, eluting with Hexane-EtOAc (9: 1) gave allylic acetate **(*ent*)-13** (70 mg, 88%) as light yellow solid. m.p. 70-72 ^{o}C (recrystallized from EtOAc and Hexane). R_f = 0.42 (7: 3 (v/v) hexane/EtOAc). $[\alpha]_D^{20}$ = +1.47 (*c* = 1.4, CH_2Cl_2). IR (thin film, cm^{-1}) 3417, 2923, 2864, 2830, 2669, 2534, 2371, 1721, 1711, 1620, 1600, 1493, 1439, 1401, 1370, 1354, 1246, 1173, 1119, 1032, 832, 822. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 8.8 Hz, 2H), 7.58 (d, *J* = 7.5 Hz, 2H), 7.51 – 7.27 (m, 13H), 7.06 (d, *J* = 8.8 Hz, 2H), 6.58 (d, *J* = 1.7 Hz, 1H), 6.44 (d, *J* = 1.7 Hz, 1H), 6.16 (dd, *J* = 10.1, 2.3 Hz, 1H), 5.76 (dd, *J* = 10.1, 2.3 Hz, 1H), 5.26 (s, 2H), 5.18 – 5.10 (m, 3H), 5.08 (s, 2H), 4.87 (s, 1H), 2.05 (s, 3H), 1.85 – 1.62 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 170.9, 162.9, 160.4, 159.9, 158.9, 153.7, 139.3, 136.6, 135.9, 132.6, 130.7, 128.9, 128.9, 128.8, 128.8, 128.6, 128.4, 127.9, 127.8, 127.7, 126.8, 123.9, 114.7, 110.2, 98.4, 94.1, 74.7, 70.9, 70.7, 70.3, 68.1, 25.6, 25.2, 21.6. HRMS (MALDI-TOF/CCA): Calcd. for $[C_{44}H_{38}O_8 + H]^+$: 695.2639, Found: 695.2626.

(1*R*,2*R*,3*S*,4*S*)-4-((5,7-bis(benzyloxy)-2-(4-(benzyloxy)phenyl)-4-oxo-4*H*-chromen-3-yl)oxy)-2,3-dihydroxycyclohexyl acetate ((*ent*)-14):



A solution of allylic acetate **(*ent*)-13** (65 mg, 0.094 mmol) in 3 mL acetone-*t*-BuOH (1: 1) was cooled down to 0 °C. To the solution was added 40 mL NMO-H_2O (w/w 1: 1), and then catalytic amount of crystalline OsO_4 was added into the mixture when stirring. The reaction mixture was kept stirring at 0 °C for 3 hours then warmed up to rt. The reaction mixture was then quenched by 1 mL saturated aqueous $Na_2S_2O_3$. After stirring for another 0.5 hours, all the staff was passed through a small pad of celite and silica gel, washed by EtOAc-MeOH (1: 1) (10 mL×3). The organic effluent was dried by Na_2SO_4, and concentrated under reduced pressure to give crude prodcut. The crude product was then purified with chromatography on silica gel eluting with EtOAc-Hexane (1: 1), gave acetate diol **(*ent*)-14** (62 mg, 91%). m.p. 94-96^oC. R_f = 0.29 (1: 1 (v/v) hexane/EtOAc). $[\alpha]_D^{20}$ = +44.87 (c = 0.93, CH_2Cl_2) IR (thin film, cm^{-1}) 3490, 3090, 3066, 2938, 2292, 2252, 1963, 1891, 1735, 1604, 1509, 1453, 1438, 1406, 1297, 1251, 1178, 1121, 1015, 918, 891, 836. ^1H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, J = 8.6 Hz, 2H), 7.59 (d, J = 7.5 Hz, 2H), 7.53 – 7.29 (m, 13H), 7.10 (d, J = 8.6 Hz, 2H), 6.62 (d, J = 2.0 Hz, 1H), 6.51 (d, J = 2.0 Hz, 1H), 5.26 (s, 2H), 5.17 (s, 2H), 5.12 (s, 2H), 5.06 – 5.00 (m, 1H), 4.08 – 3.98 (m, 2H), 3.97 – 3.82 (m, 1H), 3.13 (brs, 1H), 2.10 (s, 3H), 1.79 – 1.55 (m, 3H), 1.49 – 1.40 (m, 1H), 1.36 – 1.19 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.8, 169.9, 163.3, 160.6, 159.8, 158.9, 155.1, 139.2, 136.3, 136.2, 135.5, 130.8, 128.8, 128.7, 128.7, 128.6, 128.3, 127.8, 127.6, 127.6, 126.6, 123.2, 114.6, 109.4, 98.5, 94.0, 84.7, 72.3, 70.9, 70.7, 70.6, 70.1, 26.1, 23.6, 21.3. HRMS (MALDI-TOF/CCA): Calcd. for $[C_{44}H_{40}O_{10} + H]^+$: 729.2694, Found: 729.2717.

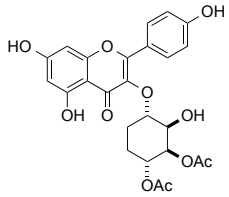
(1*R*,2*R*,3*R*,4*S*)-4-((5,7-bis(benzyloxy)-2-(4-(benzyloxy)phenyl)-4-oxo-4*H*-chromen-3-yl)oxy)-3-hydroxycyclohexane-1,2-diyl diacetate ((*ent*)-15):



A solution of acetate diol **(*ent*)-14** (50 mg, 0.069 mmol) in 1.5 mL Acetonitrile was cooled down to 0 °C, then sequentially DIPEA (25 μL, 0.138 mmol), 10 mol% catalytic amount of Taylor catalyst and AcCl (7.3 μL, 0.105 mmol) were added. The reaction mixture was stirred at 0 °C overnight. The reaction mixture was diluted by 15 mL EtOAc and washed by 5 mL saturated aqueous $NaHCO_3$, 5 mL saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure to give a residue which was subjected to chromatography on silica gel. Elution with hexane-EtOAc (2: 1) afforded C3'',C4''-diacetate **(*ent*)-15** as major product (29 mg, 55%, 5: 1 ratio). Light yellow solid. m.p. 78-80 ^oC (recrystallized from EtOAc and Hexane). R_f = 0.32 (1: 2 (v/v) hexane/EtOAc). $[\alpha]_D^{20}$ = +21.70 (c =0.25, CH_2Cl_2), IR (thin film, cm^{-1}) 3271, 3089, 3063,

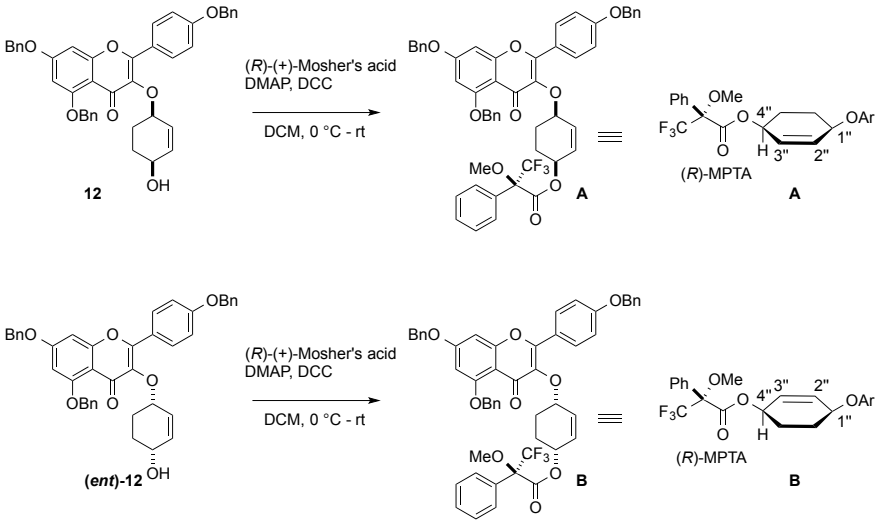
2936, 2873, 1743, 1605, 1508, 1498, 1487, 1454, 1436, 1370, 1297, 1253, 1225, 1179, 1104, 1021, 939, 908, 835. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 8.9 Hz, 2H), 7.59 (d, *J* = 7.3 Hz, 2H), 7.55 – 7.28 (m, 13H), 7.09 (d, *J* = 8.9 Hz, 2H), 6.60 (d, *J* = 2.2 Hz, 1H), 6.50 (d, *J* = 2.2 Hz, 1H), 6.01 (brs, 1H), 5.36 – 5.28 (m, 1H), 5.25 (d, *J* = 12.6 Hz, 1H), 5.21 (d, *J* = 12.6 Hz, 1H), 5.15 (s, 2H), 5.11 (s, 2H), 4.94 – 4.87 (m, 1H), 4.20 – 4.15 (m, 1H), 4.13 – 4.04 (m, 1H), 2.08 (s, 3H), 2.02 (s, 3H), 1.75 – 1.38 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 169.9, 169.8, 163.3, 160.7, 160.0, 159.1, 154.6, 139.4, 136.5, 136.4, 135.7, 130.8, 129.0, 128.9, 128.8, 128.7, 128.4, 127.9, 127.8, 127.7, 126.8, 123.4, 114.8, 109.8, 98.5, 94.1, 83.4, 72.4, 71.1, 70.9, 70.7, 70.3, 69.5, 26.4, 24.5, 21.4, 21.3. HRMS (MALDI-TOF/CCA): Calcd. for $[C_{46}H_{42}O_{11} + Na]^+$: 793.2619, Found: 793.2627.

(1*R*,2*R*,3*R*,4*S*)-4-((5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H*-chromen-3-yl)oxy)-3-hydroxycyclohexane-1,2-diyl diacetate ((*ent*)-4):



A solution of **(*ent*)-15** (20 mg, 0.026 mmol) in 2 mL THF-EtOH (1: 1) was added Pd-C (10%, 3 mg). The solution was degassed using vacuum at room temperature and refilling with H_2. This procedure was repeated three times. The reaction was stirred under a H_2 atmosphere overnight. The reaction mixture was loaded onto silica gel and elution with Et_2O-MeOH (20:1) gave **(*ent*)-4** (11 mg, 91%): Pale yellow solid. m.p. 160-162 ^{o}C (recrystallized from EtOAc and Hexane). R_f = 0.32 (1: 2 (v/v) hexane/EtOAc). $[\alpha]_D^{20}$ = +42.09 (*c* = 0.2, MeOH). IR (thin film, cm^{-1}) 3448, 3405, 3356, 3334, 3251, 3228, 3200, 2956, 2934, 2926, 1725, 1655, 1606, 1570, 1498, 1439, 1364, 1309, 1258, 1210, 1177, 1088, 1062, 1022, 975, 841. ^{1}H NMR (400 MHz, CD_3OD) δ 7.86 (d, *J* = 8.7 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 2H), 6.37 (d, *J* = 1.8 Hz, 1H), 6.18 (d, *J* = 1.7 Hz, 1H), 5.09 (dd, *J* = 8.2, 2.8 Hz, 1H), 4.97 (td, *J* = 8.5, 3.9 Hz, 1H), 4.51 – 4.43 (m, 1H), 4.20 – 4.14 (m, 1H), 2.04 (s, 3), 1.98 (s, 3H), 1.77 – 1.65 (m, 1H), 1.65 – 1.52 (m, 2H), 1.52 – 1.30 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 178.7, 170.7, 164.8, 161.9, 160.4, 157.9, 157.3, 135.6, 130.9, 121.5, 115.2, 104.6, 98.6, 93.6, 80.5, 72.9, 69.8, 24.2, 23.3, 19.7, 19.7. HRMS (MALDI-TOF/CCA): Calcd. for $[C_{25}H_{24}O_{11} + Na]^+$:523.1211 , Found: 523.1182.

Mosher Ester Method to determine the enantiomeric excesses of **12** and (***ent***)-**12**:



To an ice-bath cooled solution of allylic alcohol **12** (3 mg, 4.6 μmol), DMAP (0.1 mg, 0.8 μmol) and DCC (4 mg, 19 μmol) in DCM was added (*R*)-(+) Mosher's acid (2 mg, 8.5 μmol). The mixture was then allowed to warm to rt and stirred for 4 hours. Then the solvent was removed with reduced pressure and the crude mixture was examined by proton NMR to determine the enantiomeric excess as > 96% (see the spectra copies). After purification with flash chromatography on silica gel, the pure product was obtained, 3.6 mg, 92%.

Similar procedure was applied to (***ent***)-**12** to determine the % ee as > 96%.

Assignment of absolute configuration for **12** and (***ent***)-**12**:

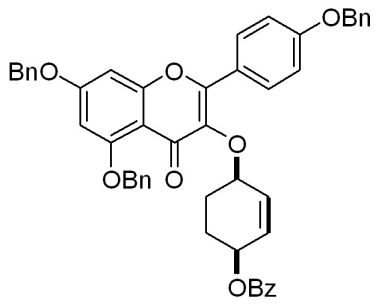
Mosher ester analysis was used to assign the absolute configuration of compounds **12** and (***ent***)-**12** with proton NMR.[4,5]

4 Kusumi, T.; Ohtani, I. Determination of the Absolute Configuration of Biologically Active Compounds by the Modified Mosher's Method. In The Biology-Chemistry Interface; Cooper, R., Snyder, J. K., Eds.; Marcel Dekker: New York, 1999; pp 103.

5 Seco, J. M.; Quinoa, E.; Riguera, R. The Assignment of Absolute Configuration by NMR. *Chem. Rev.* **2004**, *104*, 17−117

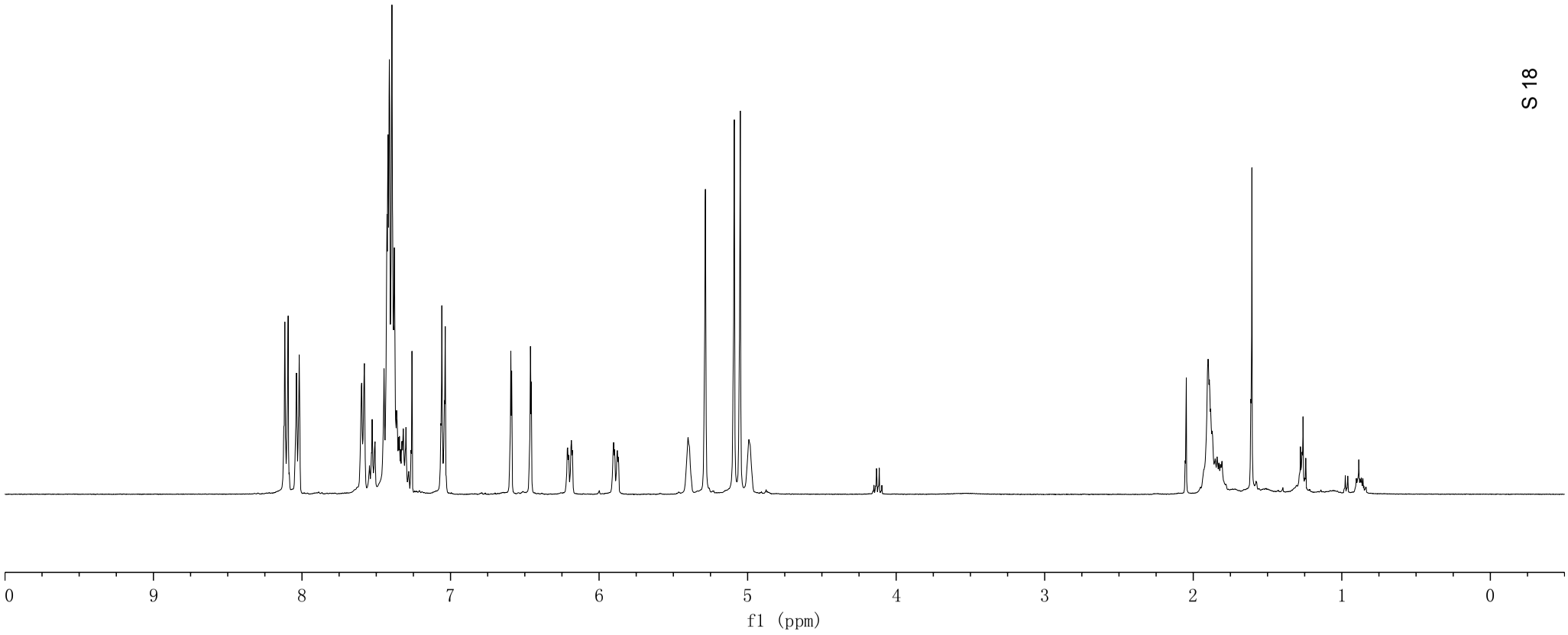
Proton	δ_A (ppm)	δ_B (ppm)	$\Delta\delta = \delta_B - \delta_A$ (ppm)
2″	5.7704	5.8545	0.0841
3″	6.1626	6.2079	0.0453

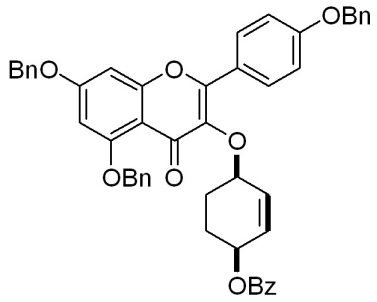
This Mosher ester assignment was also consistent with the stereochemistry one would predict following the Trost mnemonic and from the relative biological activity of the enantiomeric products **4** and (***ent***)-**4**.



11

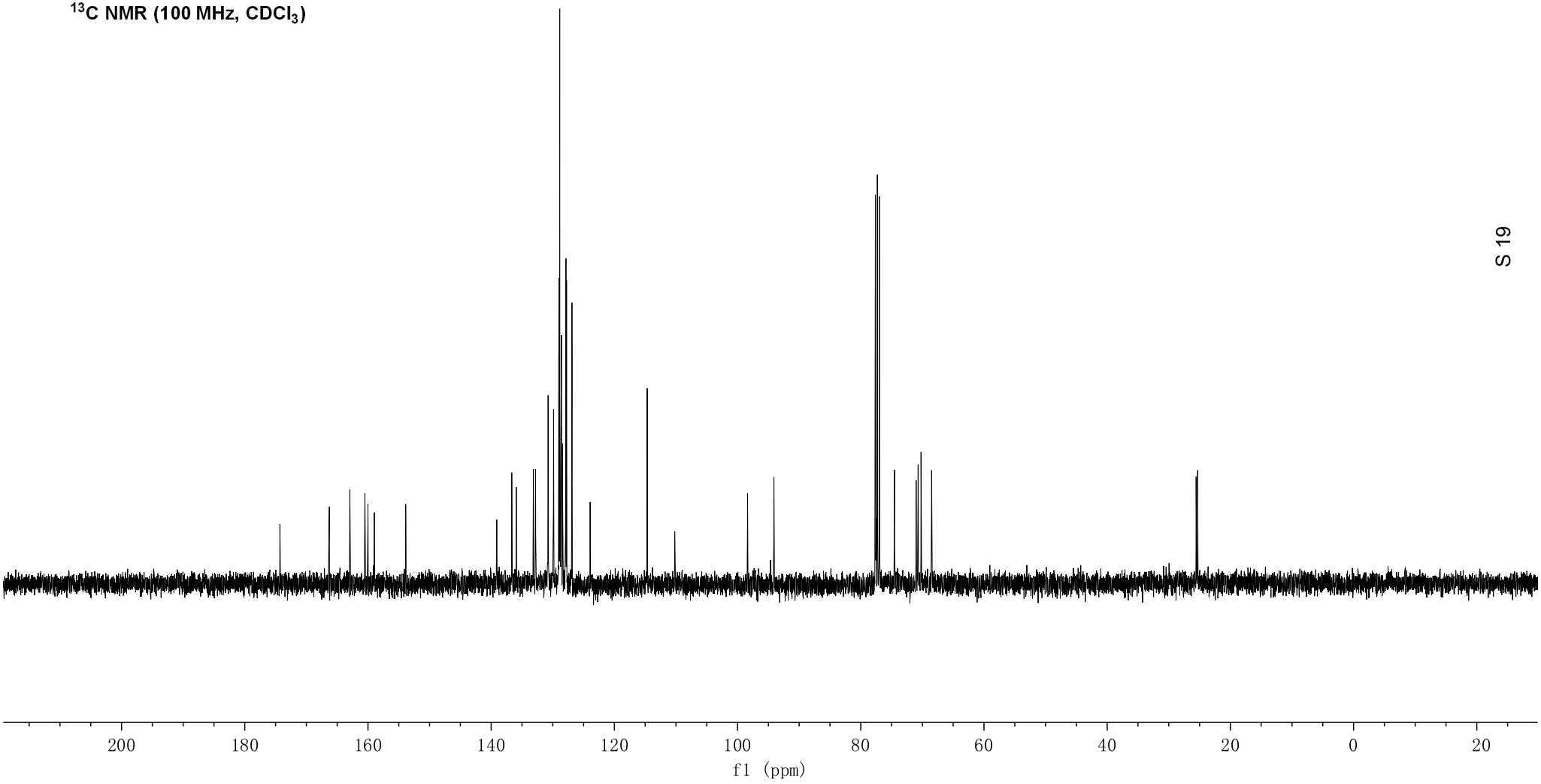
^{1}H NMR (400 MHz, CDCl3)

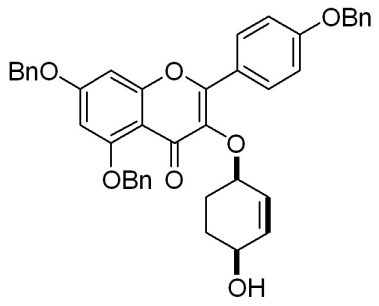




11

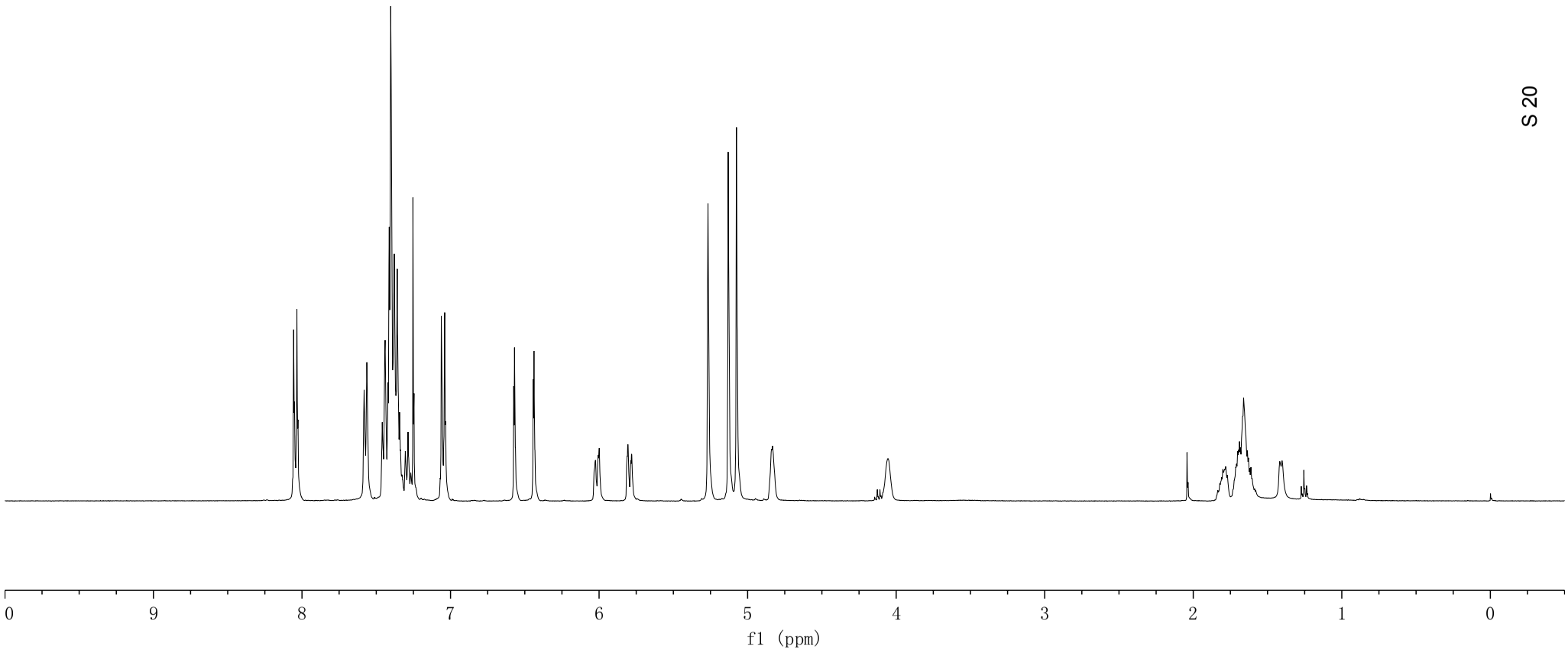
^{13}C NMR (100 MHz, $CDCl_3$)

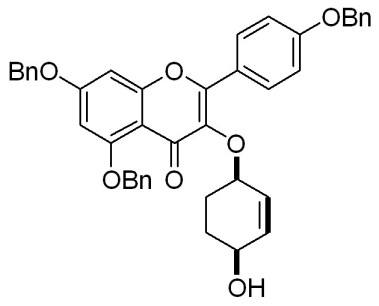




12

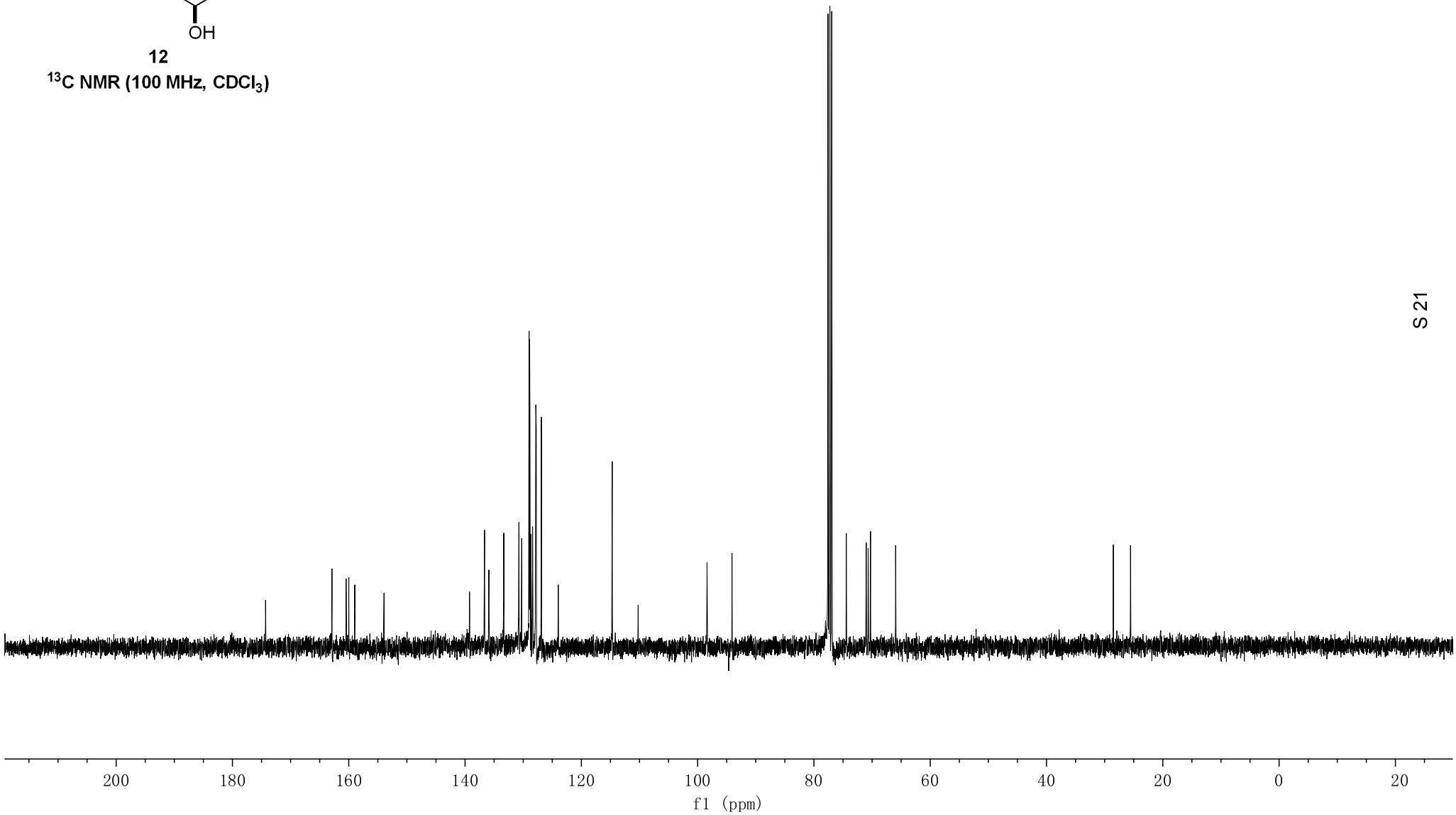
^{1}H NMR (400 MHz, $CDCl_3$)

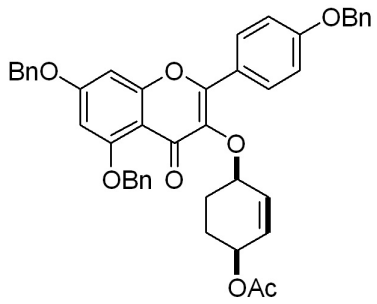




12

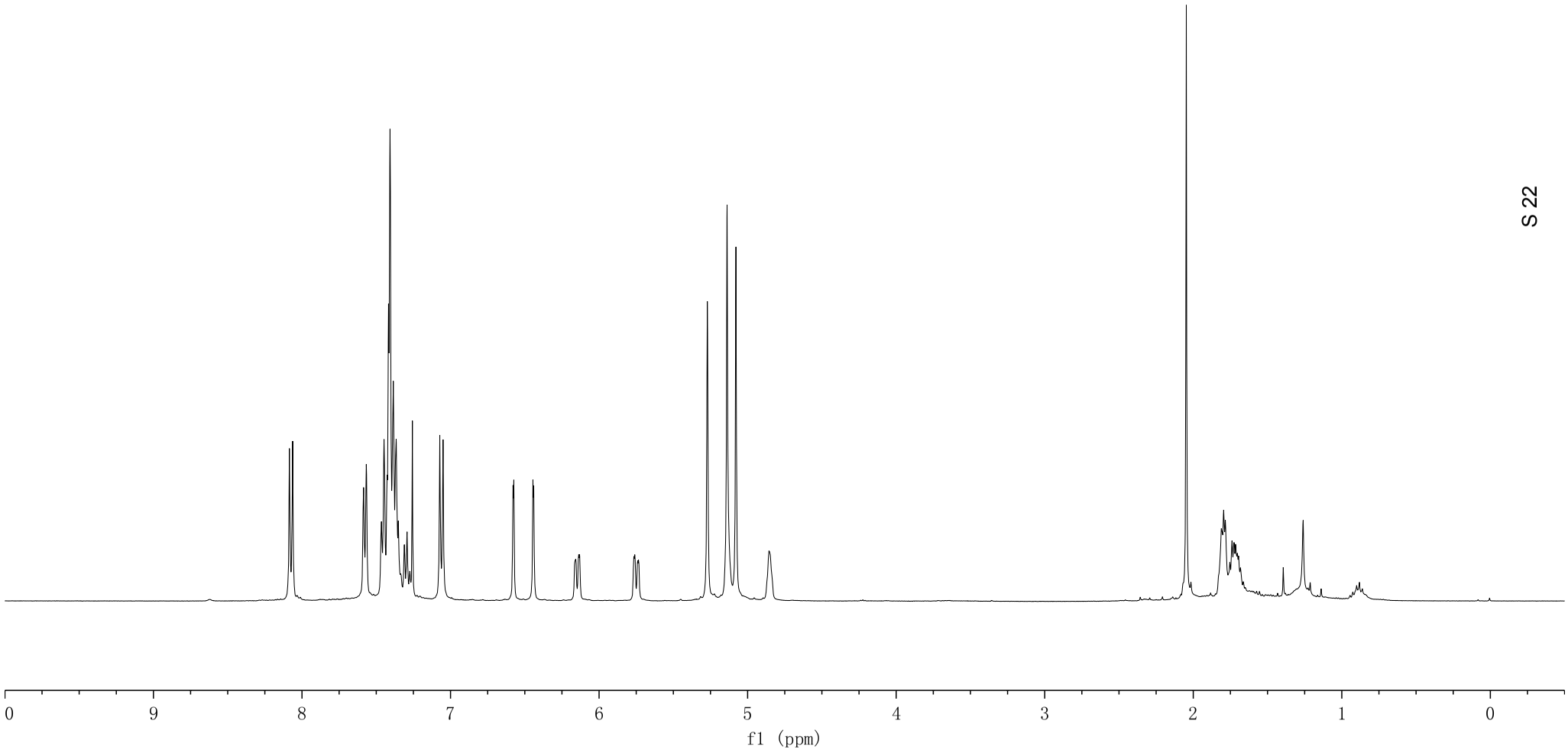
^{13}C NMR (100 MHz, $CDCl_3$)

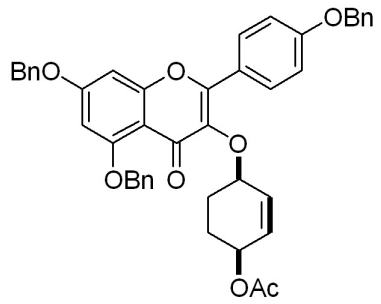




13

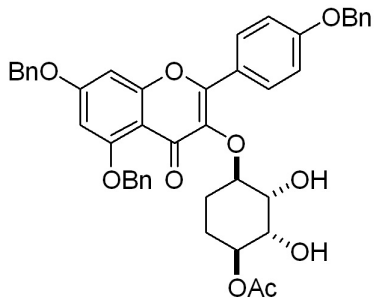
^{1}H NMR (400 MHz, $CDCl_3$)





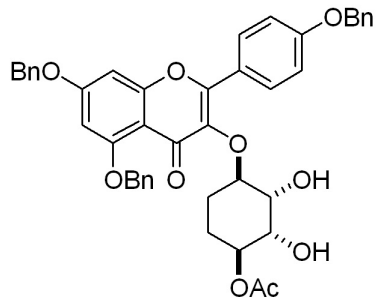
13

^{13}C NMR (100 MHz, $CDCl_3$)



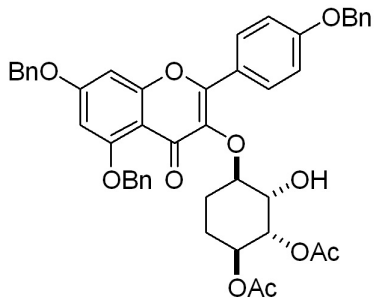
14

^{1}H NMR (400 MHz, $CDCl_3$)



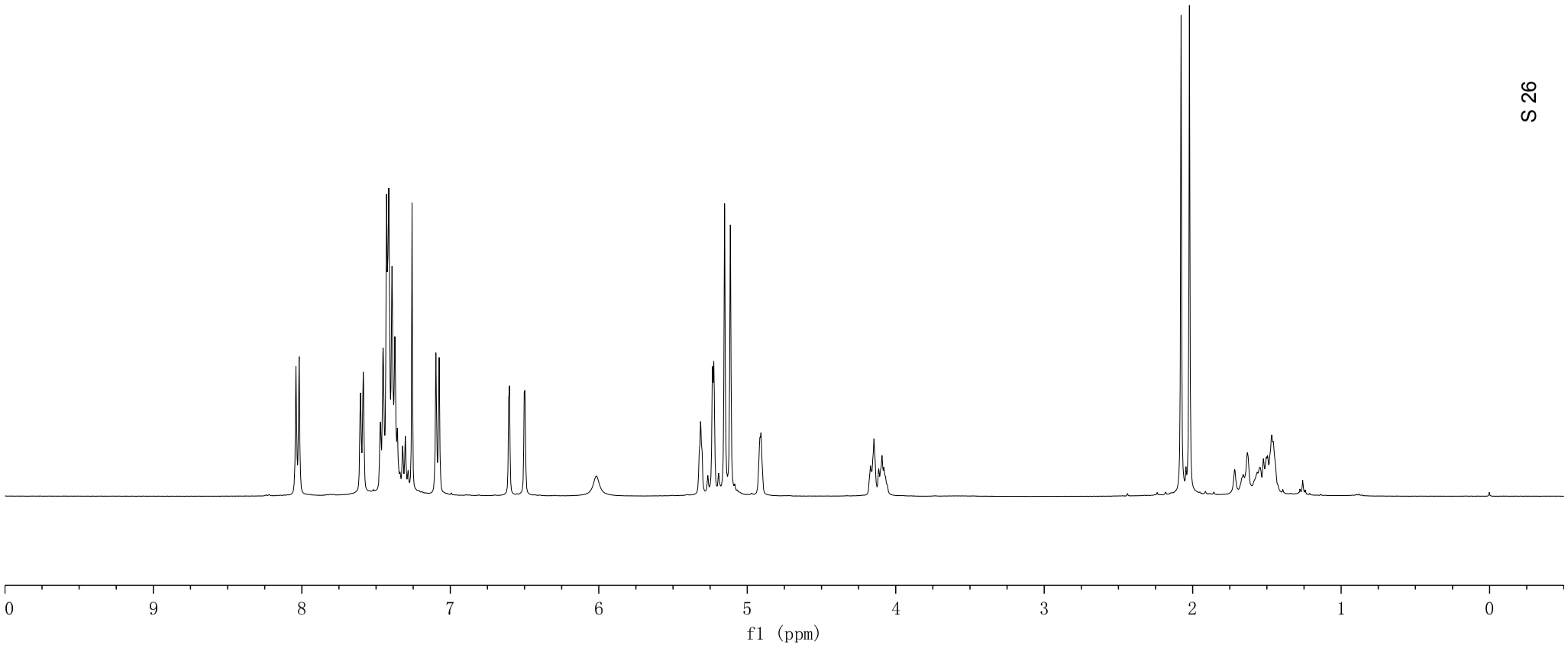
14

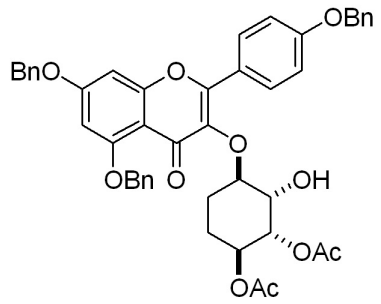
^{13}C NMR (100 MHz, $CDCl_3$)



15

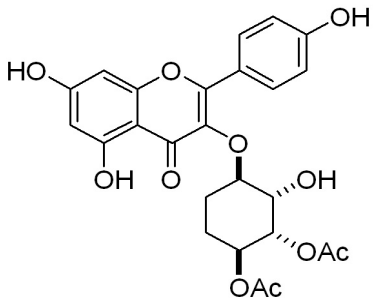
^{1}H NMR (400 MHz, $CDCl_3$)





15

^{13}C NMR (100 MHz, $CDCl_3$)



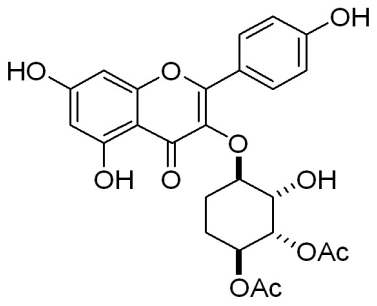
4

^{1}H NMR (400 MHz, CD_3OD)

S 28

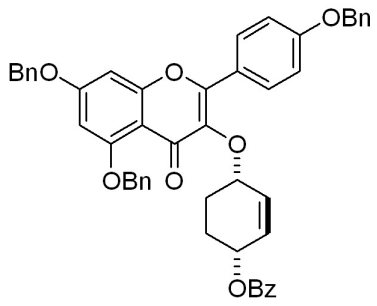
0 9 8 7 6 5 4 3 2 1 0

f1 (ppm)



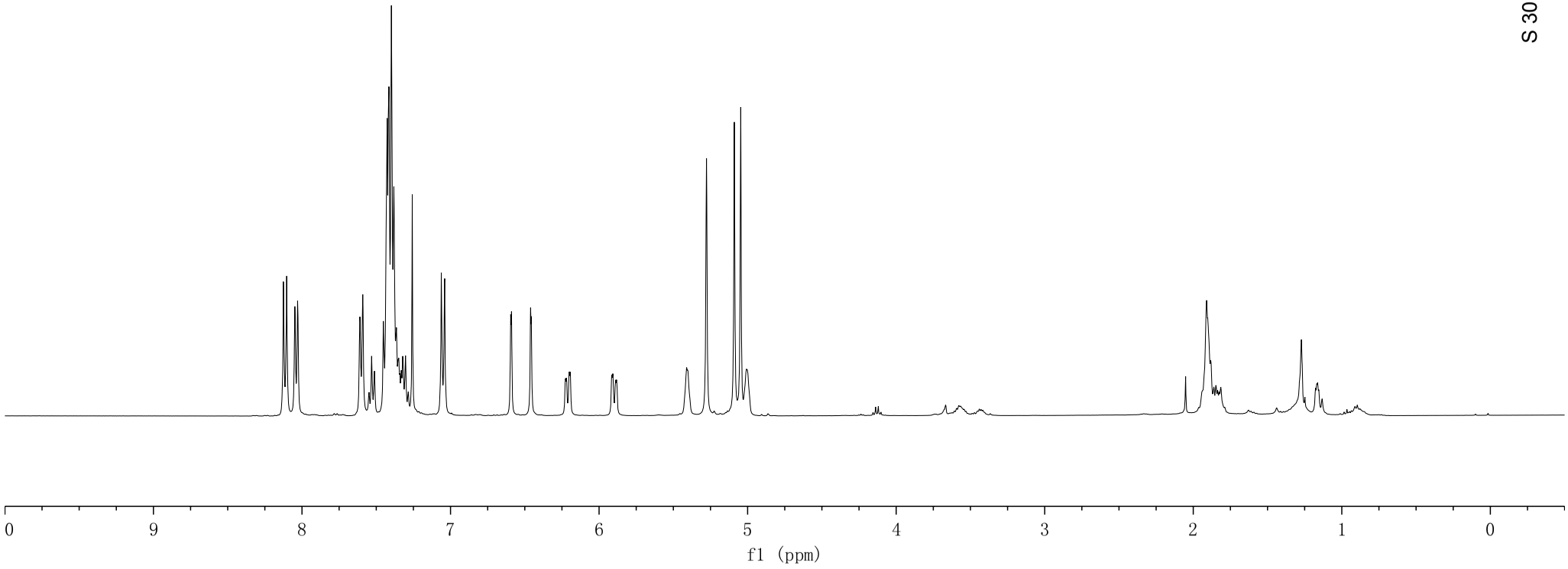
4

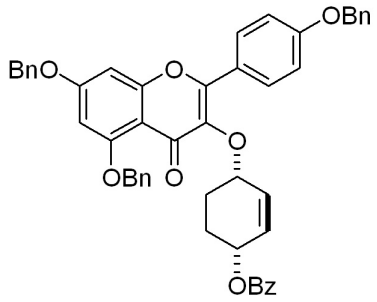
^{13}C NMR (100 MHz, CD_3OD)



(*ent*)-11

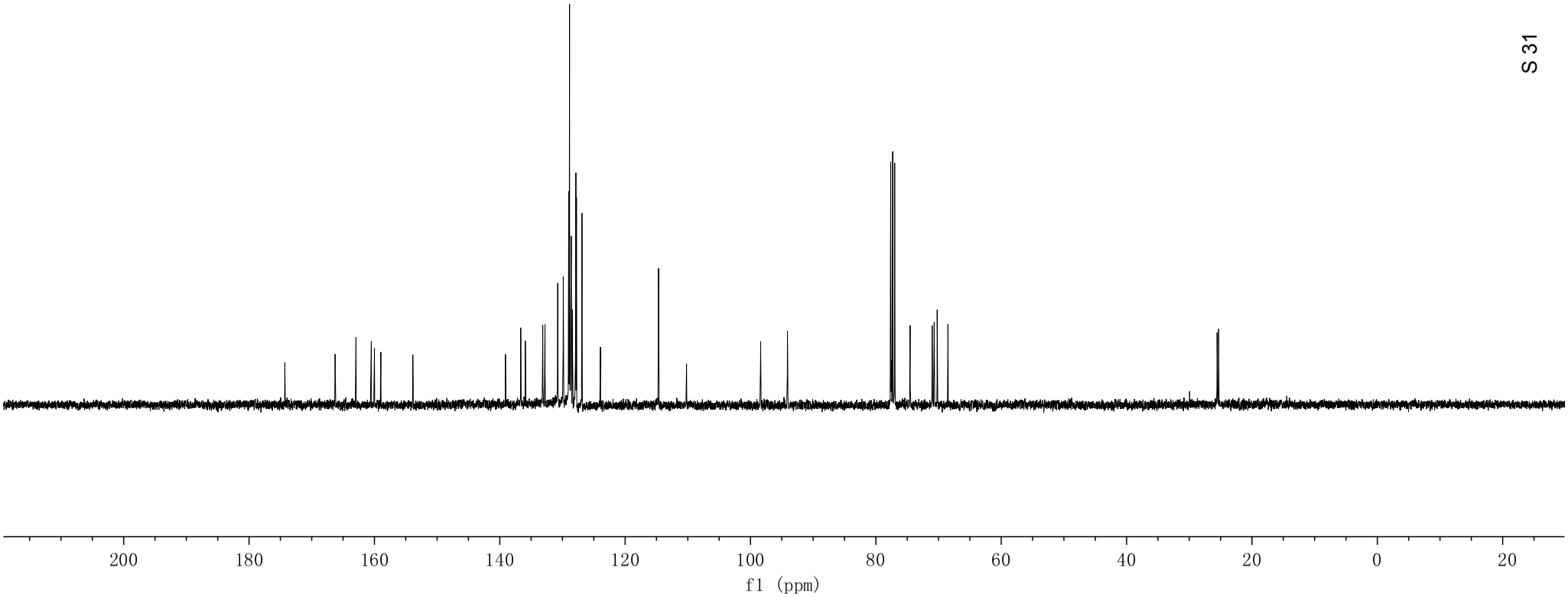
^{1}H NMR (400 MHz, $CDCl_3$)

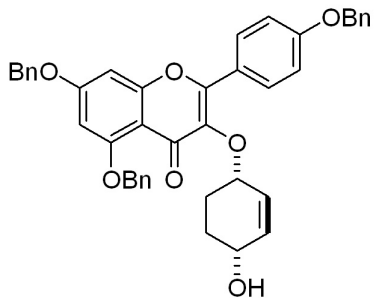




(*ent*)-11

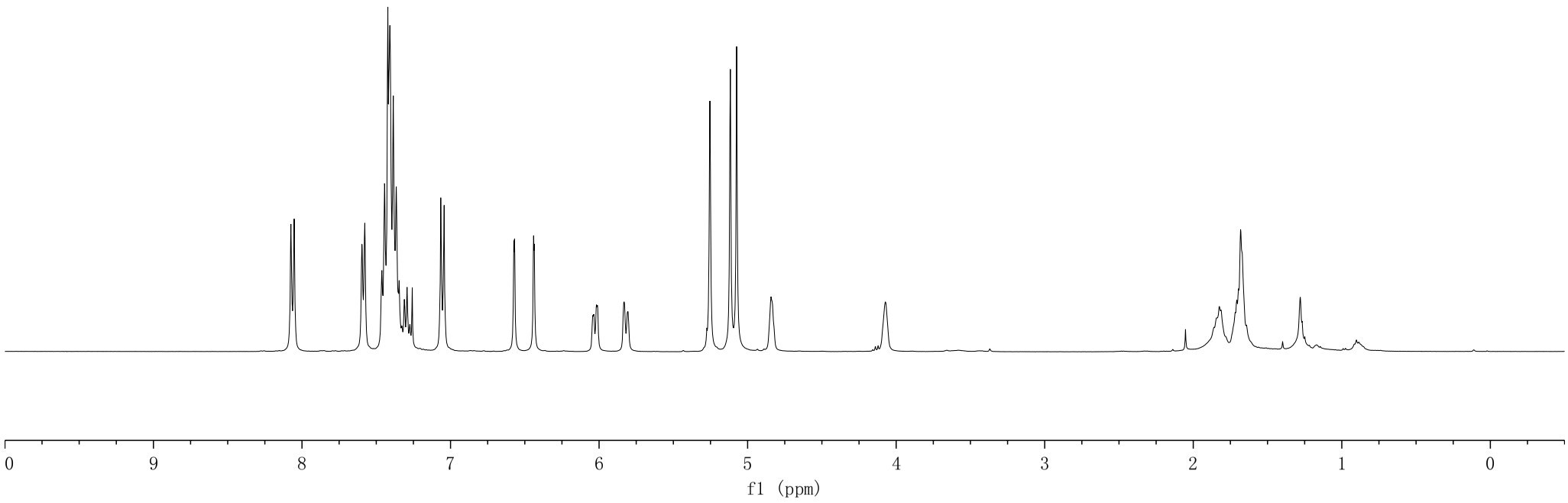
^{13}C NMR (100 MHz, $CDCl_3$)

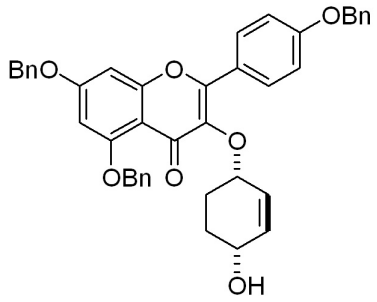




(*ent*)-12

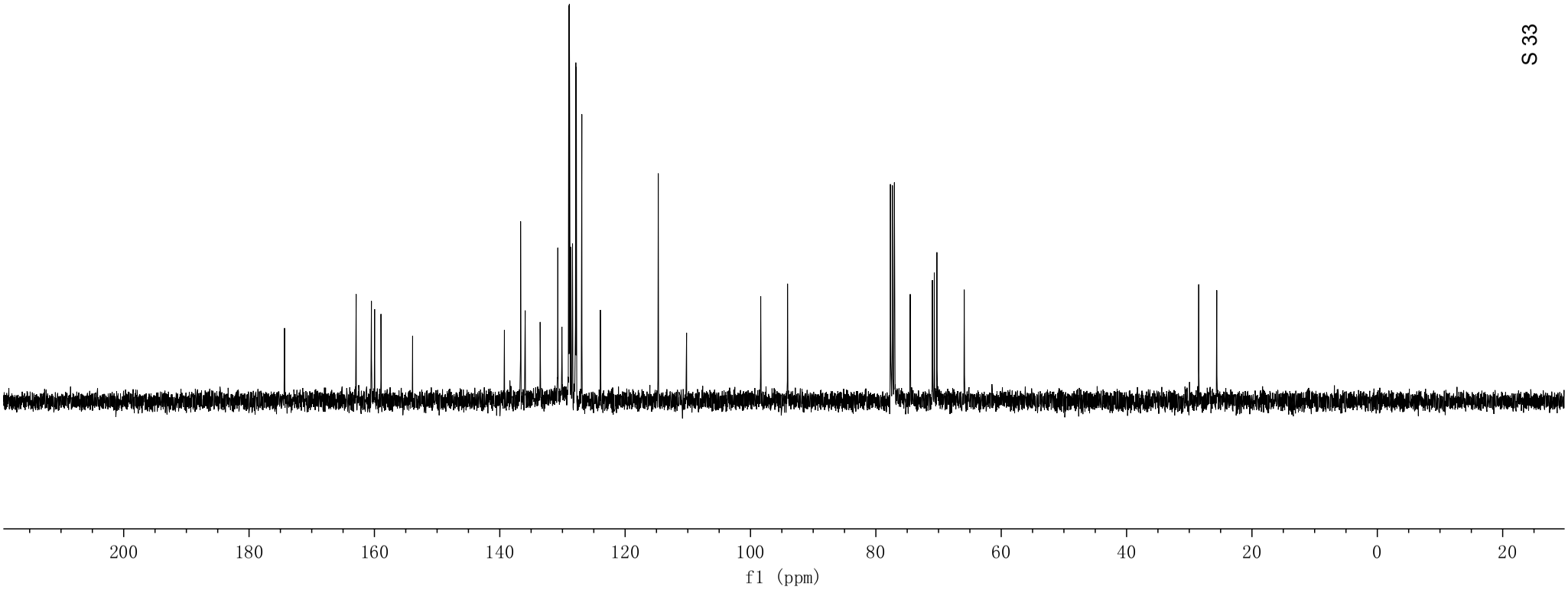
^{1}H NMR (400 MHz, $CDCl_3$)

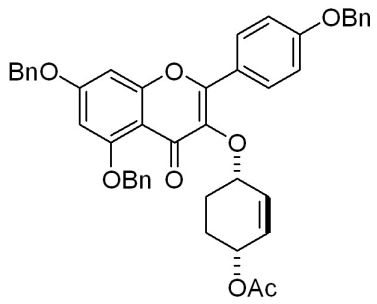




(*ent*)-12

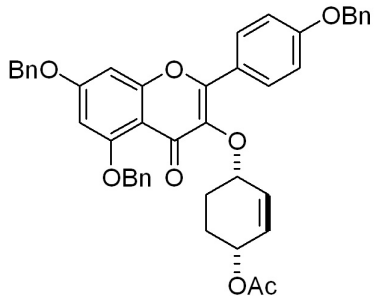
^{13}C NMR (100 MHz, $CDCl_3$)





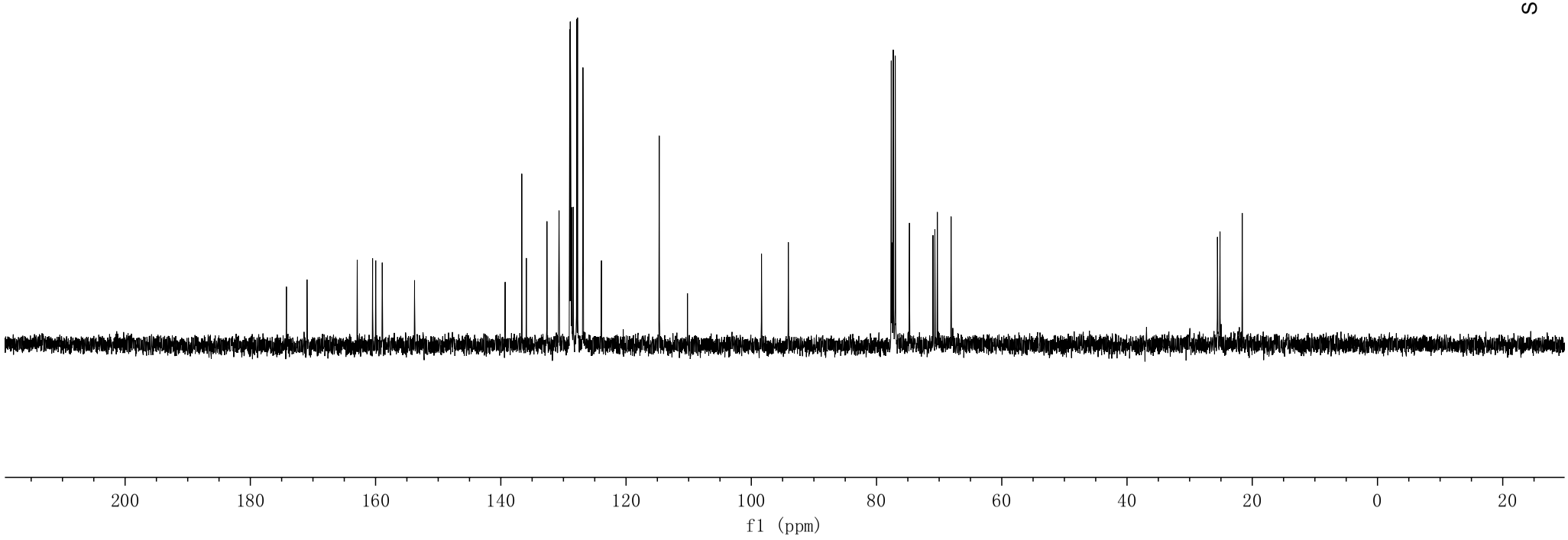
(*ent*)-13

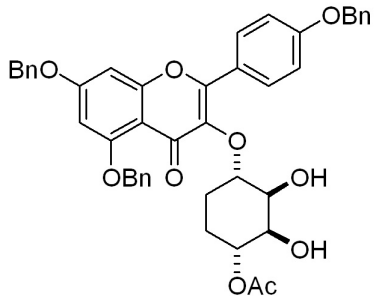
^{1}H NMR (400 MHz, $CDCl_3$)



(*ent*)-13

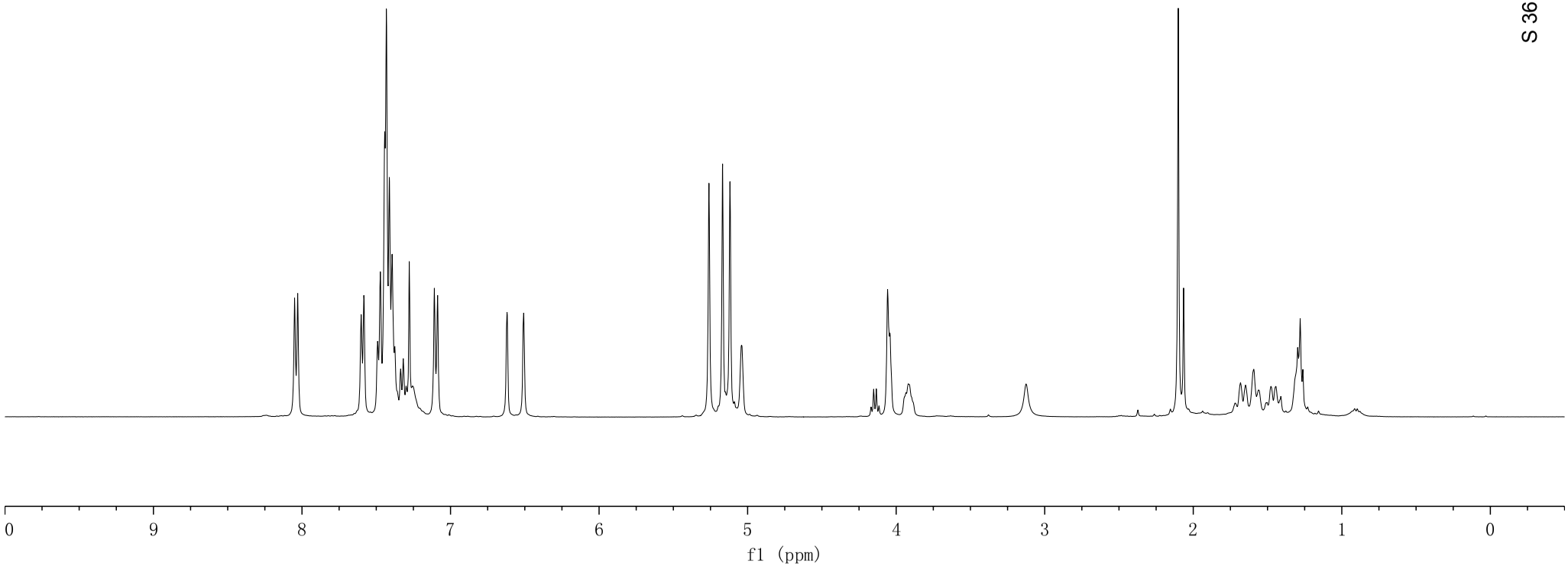
^{13}C NMR (100 MHz, $CDCl_3$)

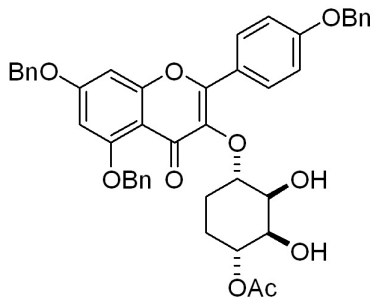




(*ent*)-14

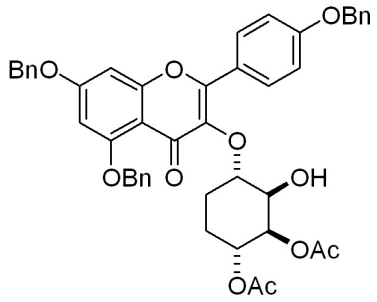
^{1}H NMR (400 MHz, $CDCl_3$)





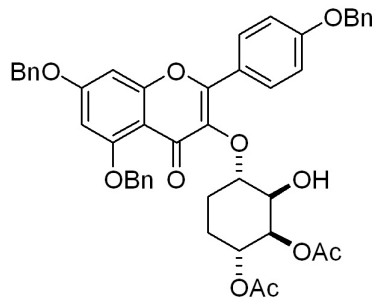
(*ent*)-14

^{13}C NMR (100 MHz, $CDCl_3$)



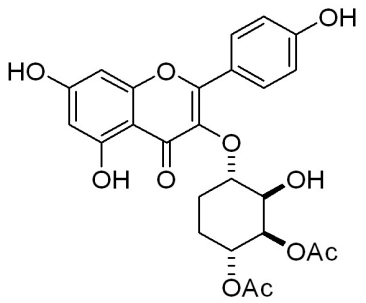
(*ent*)-15

^{1}H NMR (400 MHz, $CDCl_3$)



(*ent*)-15

^{13}C NMR (100 MHz, $CDCl_3$)



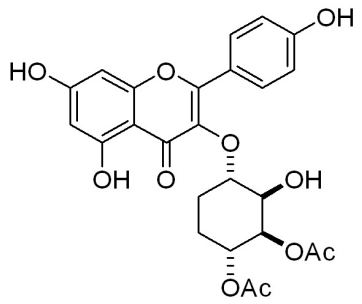
(*ent*)-4

^{1}H NMR (400 MHz, CD_3OD)

S 40

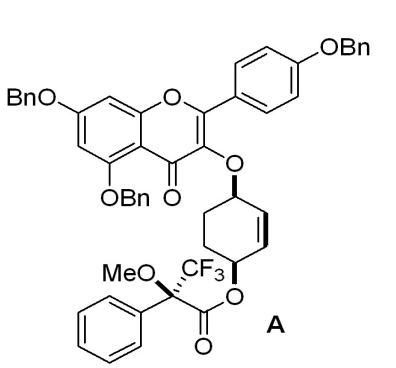
0 9 8 7 6 5 4 3 2 1 0

f1 (ppm)

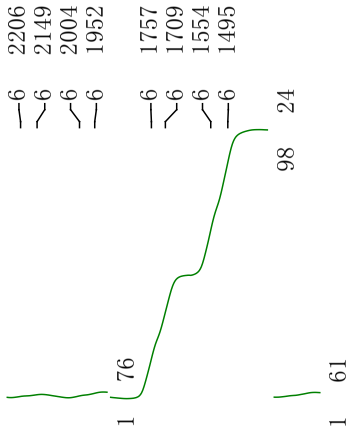


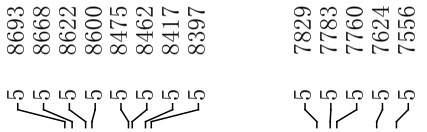
(*ent*)-4

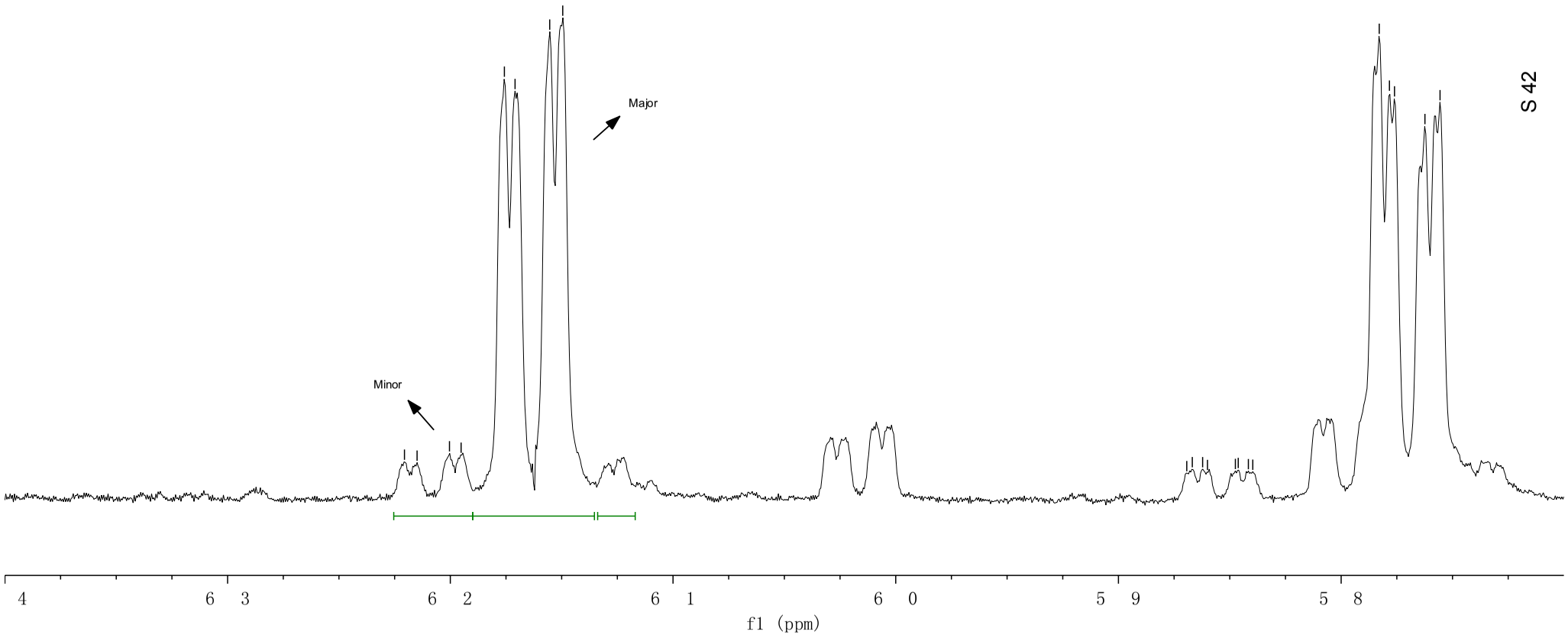
^{13}C NMR (100 MHz, CD_3OD)

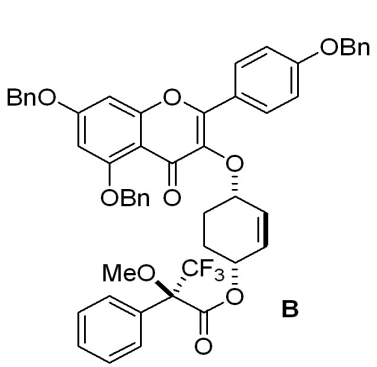


Crude H^1 NMR 500 MHz in $CDCl_3$
% ee: > 96% based on integration



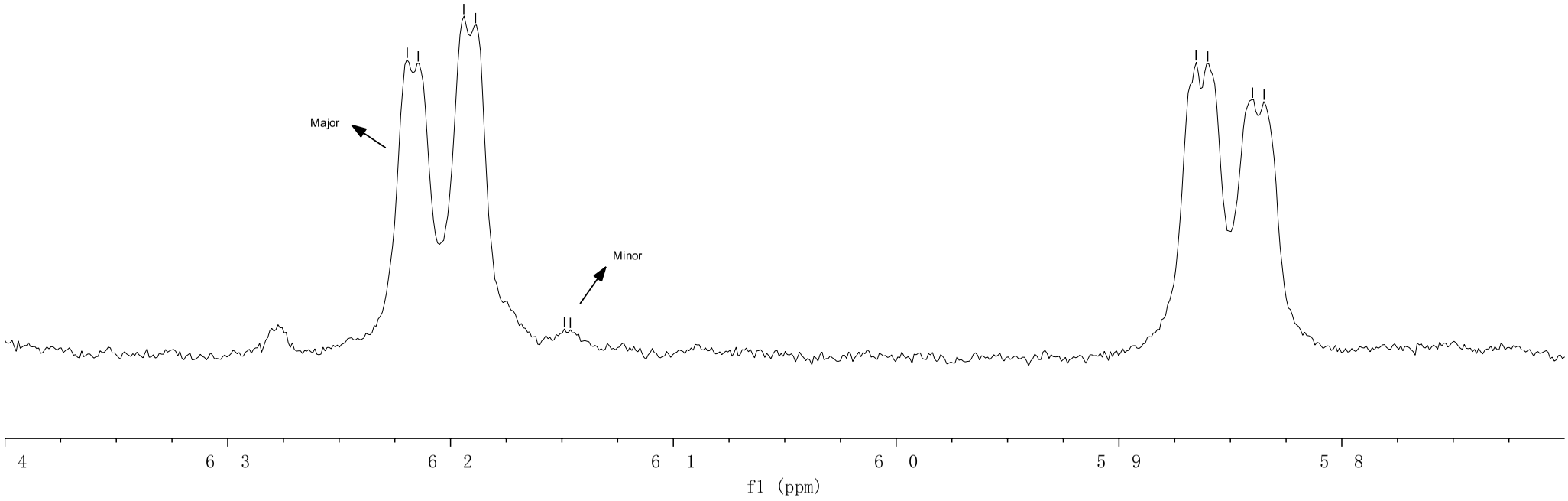


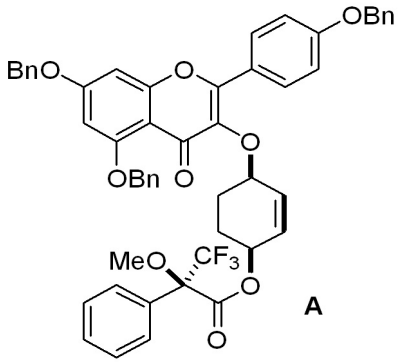




2194 2145 1940 1888 1489 1462 8654 8602 8400 8349

Crude H^1 NMR 400 MHz in $CDCl_3$
% ee: > 96% based on integration





A

H^1 NMR 400 MHz in $CDCl_3$

1833 6
1775 6
1571 6
1533 6
7895 5
7827 5
7641 5
7588 5