Synthesis and antioxidant evaluation of (*S*,*S*)- and (*R*,*R*)-secoisolariciresinol diglucosides (SDGs)

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Supporting information

Experimental Data for Biological Assays

Assay Kits. HORAC Assay kit (#TA30) was purchased from Oxford Biomedical Research. ORAC Assay kit (#STA345) was obtained from Cell Biolab, San Diego, CA. SDG standard was purchased from Chromadex Inc., San Diego, CA.

Reducing Power Activity Assay. The determination of reducing power was performed as described by Yen and Der.[1] Reducing power assay determines the reducing potential of the test compound which reacts with potassium ferricyanide (Fe^{+3}) to form potassium ferrocyanide (Fe^{+2}), which subsequently reacts with ferric chloride to produce a ferric-ferrous complex that has maximum absorption at 700 nm. Various concentrations (1–500 μM) of test compounds were taken in sodium phosphate buffer (0.1 M, pH 6.6) in 96-well microplates and mixed with potassium ferricyanide (1%). Samples were incubated at 50 °C and equal volume of 10% trichloroacetic acid was added. The upper layer was mixed with deionized water (1:1:2) and ferric chloride (0.1%). The absorbance was read at 700 nm on a Bio-Rad microplate reader (Bio-Rad, Hercules, CA). The increase in absorbance indicates increase in reducing power.

Hydroxyl Radical Scavenging Potential (HORAC Assay). The ability of SDGs to scavenge hydroxyl radicals in a chemical system was evaluated using HORAC Assay kit (#TA30) obtained from Oxford Biomedical Research. Hydroxyl radicals were generated from hydrogen peroxide by Fenton reaction. Oxidation of fluorescein was measured on a fluorescence microplate reader. Antioxidants inhibit

fluorescein oxidation. Gallic acid was used as a standard for calibration curve. Calculations used SDG concentration that fit the linear part of the calibration curve. SDG concentrations were used in the range of 8 µM– 1 mM. Antioxidant capacity against hydroxyl radicals was expressed as gallic acid equivalent (GAE).

Peroxyl Radical Scavenging Potential (ORAC ASSAY). The ability of SDGs to scavenge peroxyl radicals in a chemical system was evaluated using a ORAC assay kit (#STA345) obtained from Cell Biolab (San Diego, CA). Peroxyl radicals were generated by AAPH (2,2′-azobis(2-amidinopropane dihydrochloride). Oxidation of fluorescein was measured using a fluorescence microplate reader. Antioxidants inhibit fluorescein oxidation. Trolox was used as a standard for calibration curve. Calculations used SDG concentrations that fit the linear part of the calibration curve. SDG concentrations were used in the range of 8 µM – 1 mM. Antioxidant capacity against peroxyl radicals was expressed as Trolox equivalent (TE).

DPPH Radical Scavenging Assay. The ability of the SDGs to scavenge DPPH radicals was assessed as described by Moree et al with minor modification for use in microplates.[2] Briefly, different concentrations of SDG isomers and other test compounds were incubated with 200 µL medium in 96-well microplates containing 0.1 M Tris buffer (pH 7.4) and 250 µM DPPH solution, and kept in the dark for 20 min. The absorbance was read at 517 nm in a Bio-Rad microplate reader. Ascorbic acid and α-tocopherol were used as known antioxidants for comparison. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and calculated using the following equation: Percentage inhibition = [O.D. $_{control}$ − O.D. $_{treated}$ / O.D. $_{control}$] × 100.

Experimental Data for Syntheses of Compounds

General Procedures for Chemical Reactions. All reactions were carried out under an argon atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Dry tetrahydrofuran (THF), dimethylformamide (DMF), and methylene chloride (CH_2Cl_2) were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as visualizing agent and an ethanolic solution of phosphomolybdic acid and cerium sulfate, and heat as developing agents. E. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography. Preparative thin-layer chromatography (PTLC) separations were carried out on 2 mm E. Merck silica gel plates (60F-254) unless otherwise noted. Infrared (IR) spectra were recorded on a Perkin-Elmer 100 FT-IR spectrometer. NMR spectra were recorded on a Bruker DRX-600 instrument and calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet. IR spectra were recorded on a Perkin–Elmer Spectrum 100 FT-IR spectrometer. High-resolution mass spectra (HR-MS) were recorded on an Agilent ESI-TOF (time of flight) mass spectrometer using ESI (electrospray ionization). Optical rotations were recorded on a Perkin–Elmer Model 343 polarimeter at 589 nm, and are reported in units of 10^{-1} (deg cm^2 g^{-1}).

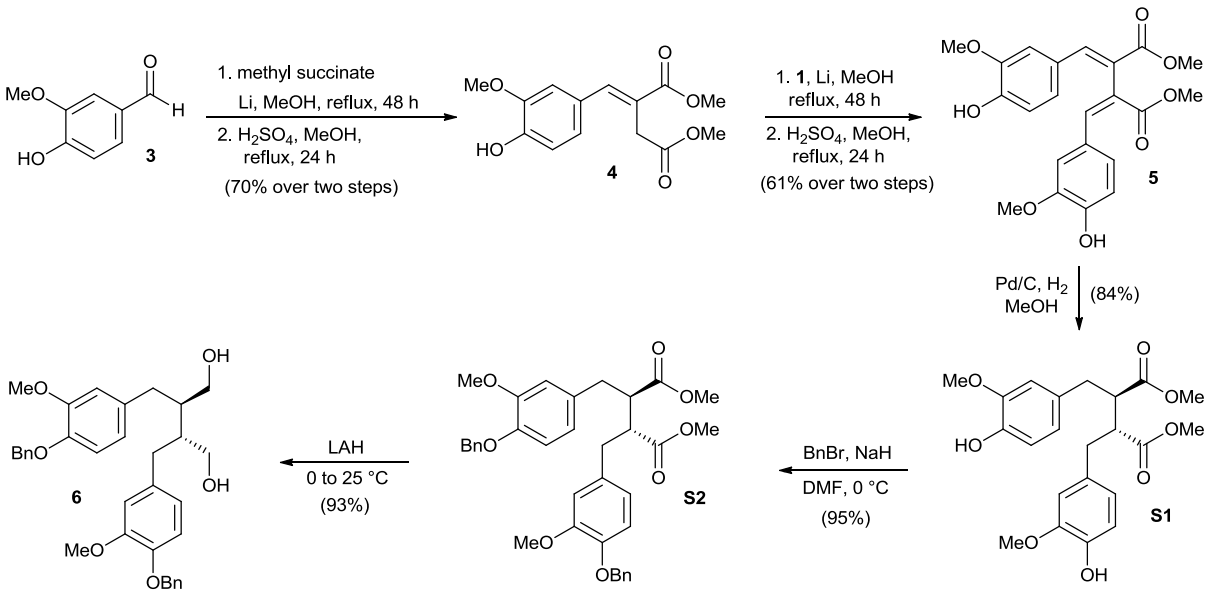
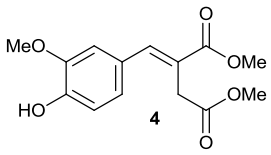
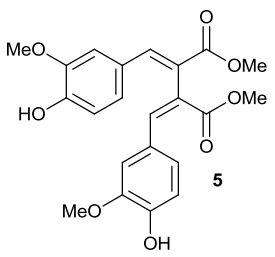


Figure S1. Synthesis of the secoisolariciresinol core.

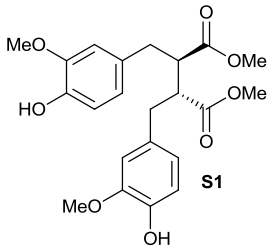
Phenol 4. To a solution of vanillin **3** (14.00 g, 92.1 mmol, 1 equiv) and dimethyl succinate (12.1 mL, 92.1 mmol, 1 equiv) in MeOH (450 mL), lithium wire (1.79 g, 257.9 mmol, 2.8 equiv) was added slowly piecewise with an ice bath to control the exotherm. After the initial lithium had fully dissolved, more lithium (1.98 g, 285.5 mmol, 3.1 equiv) was added slowly piecewise and stirred until fully dissolved. The reaction mixture was then heated at reflux for 48 h. After cooling the solution to room temperature, most of the methanol was removed by concentration on rotavap. EtOAc (1000 mL) was added and the solution was washed with a 2 M aq. HCl solution (700 mL), H_2O (3 × 1000 mL), then brine (200 mL). The organics layer was then dried ($MgSO_4$), filtered and concentrated. The crude was dissolved in MeOH (230 ml), H_2SO_4 (1 mL) was added, and the solution was heated at reflux overnight. Upon cooling the next morning, $NaHCO_3$ (3.00 g) was added to quench H_2SO_4 and the solution was mostly concentrated by rotavap. EtOAc (500 ml) was added and the solution was washed with H_2O (2 × 200 mL), then brine (100 mL). The organics layer was then dried ($MgSO_4$), filtered and concentrated to a brown oil. Dissolution in a small amount of CH_2Cl_2 and then flash column chromatography (silica, 1:9 → 2:8 → 3:7 → 4:6 → 5:5 ether:hexane) provided **4** (18.0 g, 64.2 mmol, 70% yield, unassigned olefin geometry) as an off-white solid. **4**: R_f = 0.17 (silica, ether:hexanes 1:1); IR (film): ν_{max} = 3422, 1702, 1514, 1434, 1258, 1195 1159, 1093, 1030, 924, 821 770, 729 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ = 7.79 (s, 1 H), 6.90–6.85 (m, 3 H), 6.19 (s, 1 H), 3.82 (s, 3 H), 3.78 (s, 3 H), 3.69 (s, 3 H), 3.57 (s, 2 H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ = 171.94, 168.13, 146.80, 146.61, 142.38, 126.96, 123.39, 123.31, 114.76, 111.80, 55.85, 52.24, 52.20, 33.61 ppm; HRMS (ESI-TOF): calcd for $C_{14}H_{16}O_6$ [M + H^+]: 280.102, found 281.1022.



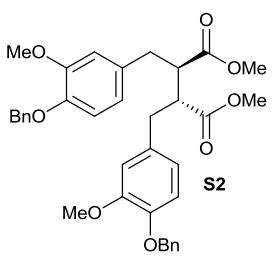


Diester 5. To a solution of diester **4** (15.00 g, 53.52 mmol, 1 equiv) and vanillin **3** (8.14 g, 53.52 mmol, 1 equiv) in MeOH (200 mL), lithium wire (2.60 g, 374.6 mmol, 7 equiv) was added slowly piecewise with an ice bath to control the exotherm and stirred until fully dissolved. The reaction mixture was then heated at reflux for 48 h. After cooling the solution to room temperature, most of the methanol was removed by concentration on rotavap. EtOAc (200 mL) was added, the solution was acidified with 2 M aq. HCl solution (500 mL), and then extracted with EtOAc (2 × 200 mL). The combined organics were washed with H_2O (2 × 200 mL), then brine (200 mL), and dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in MeOH (300 mL), conc. H_2SO_4 (1 mL) was added, and the solution was heated at reflux overnight. Upon cooling the next morning, $NaHCO_3$ (3.0 g) was added to quench H_2SO_4 and the solution was mostly concentrated by rotavap. EtOAc (400 ml) was added and the solution was washed with H_2O (2 × 200 mL), then brine (100 mL). The organics layer was then dried ($MgSO_4$), filtered and concentrated. Dissolution in a small amount of CH_2Cl_2 and then flash column chromatography (silica, 2:1:7 → 3:1:6 → 4:1:5 EtOAc:CH_2Cl_2:hexanes) provided **5** (13.60 g, 32.8 mmol, 61% yield, unassigned olefin geometry) as a yellow-orange solid. **5**: R_f = 0.28 (silica, EtOAc:hexanes 1:1); IR (film): ν_{max} = 3388, 1696, 1588, 1509, 1431, 1209, 1157, 1029, 817, 733 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ = 7.86 (s, 2 H), 7.11 (d, *J* = 1.64 Hz, 2 H), 7.04 (dd, *J* = 8.48, 1.72 Hz, 2 H), 6.82 (d, *J* = 8.38 Hz, 2 H), 6.08 (s, 2 H), 3.7 (s, 6 H), 3.69 (s, 6 H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ = 168.00, 147.59, 146.54, 142.64, 127.10, 125.38, 124.06, 114.71, 111.52, 55.77, 52.51 ppm; HRMS (ESI-TOF): calcd for $C_{22}H_{22}O_8$ [M + H^+]: 415.1387, found 415.1378.

Diester S1. A solution of **5** (5.00 g, 12.07 mmol, 1 equiv) in MeOH (120 mL) was saturated with an argon atmosphere by briefly exposing to vacuum and backfilling with argon several times. Palladium on carbon (10% Pd by weight, 0.500 g) was added and the solution was saturated with H_2 atmosphere by vacuum/backfill H_2. The solution was stirred overnight. The next morning the solution was put under an argon atmosphere, CH_2Cl_2 (400 mL) was added, and the solution was allowed to stir for 1 h. The mixture was then filtered through a pad of Celite (1.5 in, washing with MeOH and CH_2Cl_2), and the filtrate was concentrated. The resulting solid was dissolved in a small amount of CH_2Cl_2 and purified by flash column chromatography (2:8:1 → 3:7:1 → 4:6:1 EtOAc:hexanes:CH_2Cl_2) to give **S1** (4.24 g, 10.1 mmol, 84% yield) as an off-white solid. **S1**: R_f = 0.24 (silica, EtOAc:hexanes:CH_2Cl_2 4:6:1); IR (film): ν_{max} = 3440, 2951, 1726, 1514, 1432, 1267, 1198, 1151, 1121, 1029, 817, 797 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ = 6.78 (d, *J* = 8.09 Hz, 2 H), 6.58 (d, *J* = 8.09 Hz, 2 H), 6.45 (s, 2 H), 5.65 (s, 2 H), 3.75 (s, 6 H), 3.64 (s, 6 H), 3.00–2.94 (m, 2 H), 2.92–2.82 (m, 4 H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ = 174.03, 146.49, 144.21, 130.41, 121.88, 114.18, 111.26, 55.73, 51.86, 47.67, 35.38 ppm; HRMS (ESI-TOF): calcd for $C_{22}H_{26}O_8$ [M + H^+]: 419.17, found 419.1700.

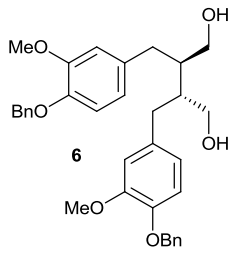


Benzyl Ether S2. To a solution of **S1** (1.00 g, 2.39 mmol, 1 equiv) in DMF (24.0 mL) cooled to 0 °C with an ice bath, NaH (0.201 g, 5.02 mmol, 60% dispersion, 2.1 equiv) was added slowly and the solution was stirred at 0 °C for 1 h. BnBr (910 μL, 7.65 mmol, 3.2 equiv) was added over 1 min, and the solution was stirred at 0 °C for 4 h. The reaction mixture was then poured into H_2O (300 mL) and EtOAc (100



mL). The organic layer was washed with H_2O (3 × 200 mL) and brine (1 × 50 mL), dried ($MgSO_4$), filtered and concentrated. The resulting solid was dissolved in a small amount of CH_2Cl_2 and purified by flash column chromatography (silica, 9:1:1 → 8:2:1 hexanes:EtOAc:CH_2Cl_2) to give **S2** (1.36 g, 2.27, 95% yield) as a white solid. **S2**: R_f = 0.23 (silica, EtOAc:hexanes 3:7); IR (film): ν_{max} = 2949, 1730, 1512, 1453, 1253, 1225, 1138, 1023, 733 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ = 7.45–7.40 (m, 4 H), 7.38–7.33 (m, 4 H), 7.31–7.27 (m, 2 H), 6.74 (d, *J* = 8.12 Hz, 2 H), 6.59 (d, *J* = 1.69 Hz, 2 H), 6.53 (dd, *J* = 8.12, 1.69 Hz, 2 H), 5.12 (s, 4 H), 3.80 (s, 6 H), 3.61 (s, 6 H), 3.03–2.85 (m, 6 H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ = 174.00, 149.58, 146.87, 137.37, 131.74, 128.65, 127.93, 127.37, 121.09, 114.05, 112.73, 71.14, 56.00, 51.91, 47.91, 35.32 ppm; HRMS (ESI-TOF): calcd for $C_{36}H_{38}O_8$ [M + H^+]: 599.2639, found 599.2651.

Diol 6. To a solution of **S2** (0.341 g, 0.570 mmol, 1 equiv) in THF (6 mL) at 0 °C, lithium aluminum hydride (1 M soln in THF, 1.1 mL, 1.14 mmol, 2 equiv) was added dropwise. The solution was allowed to come to 25 °C overnight. The resulting mixture was poured into a flask containing H_2O (200 mL) and EtOAc (100 mL). Then 50 mL of an aq. sat. soln of Rochelle's salt was added and the mixture was stirred until the layers were readily separable. The organic layer was then washed with H_2O (2 × 100 mL) and brine (1 × 50 mL), and dried ($MgSO_4$), filtered and concentrated. The resulting solid was dissolved in a small amount of CH_2Cl_2 and purified by flash column chromatography (silica, 5:5:1 → 7:3:1 → 8:2:1 EtOAc:hexanes:CH_2Cl_2) to give diol **6** (0.286 g, 0.527 mmol, 93% yield) as a white solid. **6**: R_f = 0.2 (silica, 6:4 EtOAc:hexanes); IR (film): ν_{max} = 3287, 2933, 1510, 1453, 1259, 1223, 1137, 1009, 733, 695 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ = 7.47–7.42 (m, 4 H), 7.40–7.34 (m, 4 H), 7.33–7.28 (m, 2 H), 6.78 (d, *J* = 8.15 Hz, 2 H), 6.69 (s, 2 H), 6.62 (d, *J* = 8.15 Hz, 2 H), 5.11 (s, 4 H), 4.09 (s, 2 H), 3.82 (s, 6 H), 3.78 (d, *J* = 11.07 Hz, 2 H), 3.49 (d, *J* = 11.07 Hz, 2 H), 2.80–2.72 (m, 2 H), 2.69–2.62 (m, 2 H), 1.86 (s, 2 H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.47, 146.38, 137.29, 133.87, 128.51, 127.80, 127.28, 121.02, 114.02, 112.79, 71.12, 60.19, 55.96, 43.79, 35.81 ppm; HRMS (ESI-TOF): calcd for $C_{34}H_{38}O_6$ [M + H^+]: 543.2741, found 543.2741.



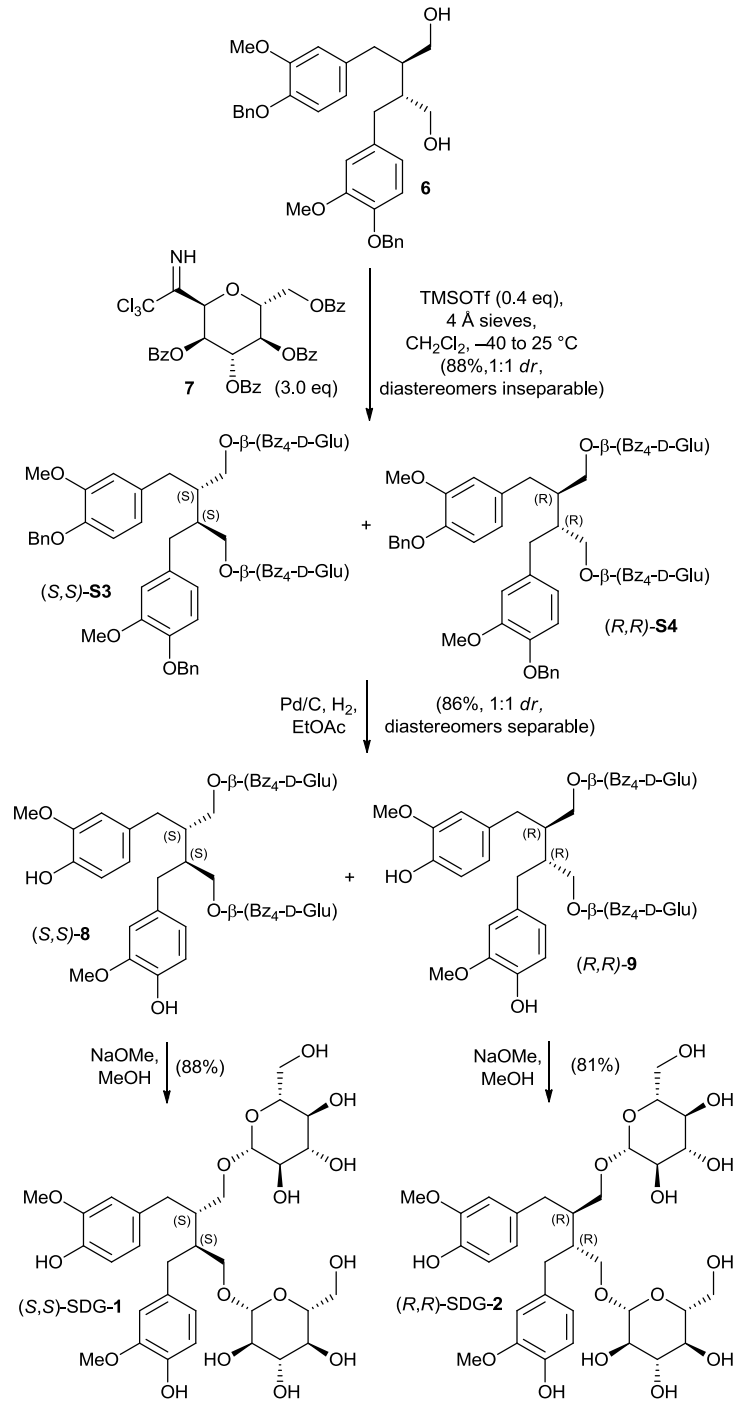
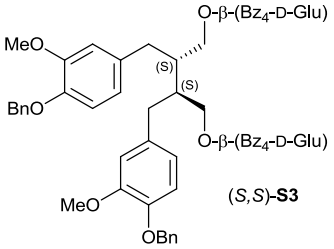
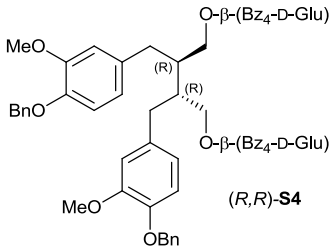


Figure S2. Glycosidation of secoisolariciresinol.

Glycosidated Isomers (*S,S*)-S3 and (*R,R*)-S4. A flask was charged with diol **6** (0.408 g, 0.751 mmol, 1 equiv) and trichloroacetimidate **7**[3] (1.67 g, 2.25 mmol, 3 equiv) and dried by benzene azeotrope (3 × 10 mL). Activated 4 Å molecular sieves (0.800 g) and CH_2Cl_2 (7.5 mL) were added and the solution was stirred for 1 h. After cooling to –40 °C, TMSOTf (54 μL, 0.300 mmol, 0.4 equiv) was added dropwise and the reaction mixture was allowed to warm to 25 °C overnight. The next morning, NEt_3 (200 μL) was



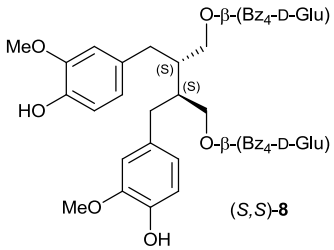
(*S*,*S*)-**S3**



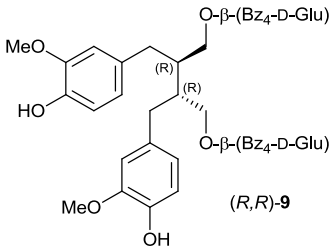
(*R*,*R*)-**S4**

added and the mixture was filtered through a silica pad (1 in, washing with EtOAc) and concentrated. The resulting crude was purified by flash column chromatography (silica, 9:1:1 → 8:2:1 → 7:2:2 hexanes:EtOAc:CH_2Cl_2) to give a 1:1 inseparable mixture of diastereomers (*S*,*S*)-**S3** and (*R*,*R*)-**S4** (1.128 g, 0.664 mmol, 88% yield). (*S*,*S*)-**S3**/(*R*,*R*)-**S4**: R_f = 0.22 (silica, 7:2:1 hexanes:EtOAc:CH_2Cl_2); IR (film): ν_{max} = 2941, 1727, 1601, 1511, 1451, 1262, 1092, 1026, 708 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ = 8.04 (d, *J* = 7.70 Hz, 4 H), 8.01 (d, *J* = 7.62 Hz, 4 H), 7.98–7.91 (m, 12 H), 7.87–7.81 (m, 12 H), 7.57–7.26 (m, 68 H), 6.61 (d, *J* = 8.26 Hz, 2 H), 6.49 (s, 2 H), 6.44 (d, *J* = 8.0 Hz, 2 H), 6.42–6.38 (m, 4 H), 6.23 (d, *J* = 8.14 Hz, 2 H), 5.93 (t, *J* = 10.07 Hz, 2 H), 5.81 (t, *J* = 9.68 Hz, 2 H), 5.72 (t, *J* = 10.07 Hz, 2 H), 5.67 (t, *J* = 9.68 Hz, 2 H), 5.56 (t, *J* = 7.72 Hz, 2 H), 5.45 (t, *J* = 8.24 Hz, 2 H), 5.08 (s, 8 H), 4.72 (dd, *J* = 12.19, 2.70 Hz, 2 H), 4.66 (d, *J* = 8.22 Hz, 2 H), 4.61 (dd, *J* = 12.19, 3.08 Hz, 2 H), 4.49–4.42 (m, 4 H), 4.39 (d, *J* = 7.70 Hz, 2 H), 4.14–4.08 (m, 2 H), 3.98–3.93 (m, 2 H), 3.92–3.87 (m, 2 H), 3.74 (s, 6 H), 3.70 (s, 6 H), 3.65–3.61 (m, 2 H), 3.40–3.34 (m, 2 H), 3.24–3.18 (m, 2 H), 2.56–2.45 (m, 6 H), 2.41–2.34 (m, 2 H), 1.85 (s, 2 H), 1.73 (s, 2 H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ = 166.20, 166.19, 165.93, 165.90, 165.29, 165.16, 164.98, 149.38, 149.35, 146.32, 146.20, 137.56, 133.73, 133.66, 133.56, 133.53, 133.45, 133.37, 133.34, 133.28, 129.93, 129.91, 129.86, 129.84, 129.76, 129.66, 129.64, 129.38, 129.30, 128.95, 128.92, 128.91, 128.60, 128.58, 128.55, 128.53, 128.42, 127.83, 127.81, 127.39, 127.32, 121.38, 121.19, 113.61, 112.70, 112.51, 101.31, 101.23, 73.06, 72.93, 72.12, 72.10, 72.06, 71.99, 71.05, 71.00, 69.89, 69.81, 69.61, 69.32, 63.07, 62.98, 55.94, 55.93, 41.00, 40.78, 35.35, 35.14 ppm; HRMS (ESI-TOF): calcd for $C_{102}H_{90}O_{24}$ [M + H^+]: 1699.5895, found 1699.5917.

Phenols (*S*,*S*)-8 and (*R*,*R*)-9. The 1:1 mixture of (*S*,*S*)-**S3** and (*R*,*R*)-**S4** (0.612 g, 0.360 mmol, 1 equiv) in EtOAc (3.6 mL) was saturated with an argon atmosphere by briefly exposing to vacuum and backfilling with argon several times. Palladium on carbon (10% Pd by weight, 0.120 g) was added and the solution was saturated with H_2 by vacuum/backfill H_2. After stirring at 25 °C for 36 h, the solution was put under an argon atmosphere, filtered through a pad of Celite (1.5 in, washing with EtOAc and CH_2Cl_2), and the filtrate concentrated. The resulting solid was dissolved in a small amount of CH_2Cl_2 and purified by flash column chromatography (silica, 3:7 → 4:6 → 5:5 EtOAc:hexanes) to give the debenzylated glycoside (0.470 g, 0.309 mmol, 86% yield) as a 1:1 mixture of diastereomers. The diastereomers could be separated by preparative thin-layer chromatography (silica, 2 mm, multiple plates, 7:20 EtOAc:hexanes, >10 elution runs) to give (*S*,*S*)-**8** and (*R*,*R*)-**9** as off-white solids.



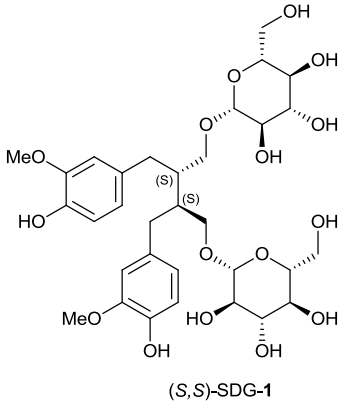
(*S*,*S*)-**8**



(*R*,*R*)-**9**

(*S*,*S*)-**8**: R_f = 0.10 (4:6 EtOAc:hexanes); $[\alpha]_D^{32}$ = +1.2 (EtOAc, *c* = 4.3); IR (film): ν_{max} = 3460, 2938, 1729, 1514, 1451, 1265, 1023, 1061, 1027, 709 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ = 8.03 (d, *J* = 8.42 Hz, 4 H), 7.92 (d, *J* = 8.22 Hz, 4 H), 7.83 (d, *J* = 7.48 Hz, 8 H), 7.58–7.46 (m, 6 H), 7.45–7.27 (m, 18 H), 6.62 (d, *J* = 7.90 Hz, 2 H), 6.43 (dd, *J* = 7.90, 1.16 Hz, 2 H), 6.38 (d, *J* = 1.16 Hz, 2 H), 5.80 (t, *J* = 9.57 Hz, 2 H), 5.64 (t, *J* = 9.57 Hz, 2 H), 5.44 (t, *J* = 9.74 Hz, 2 H), 5.40 (s, 2 H), 4.62 (dd, *J* = 12.09, 2.98 Hz, 2 H), 4.44 (dd, *J* = 12.09, 5.17 Hz, 2 H), 4.41 (d, *J* = 8.03 Hz, 2 H), 3.96 (m,

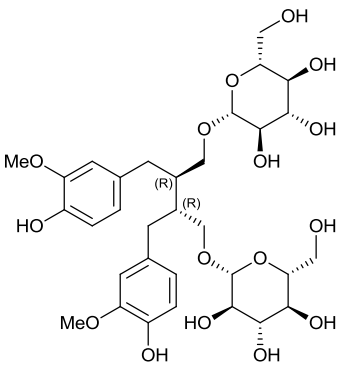
2 H), 3.69 (s, 6 H), 3.64 (dd, *J* = 9.38, 2.82 Hz, 2 H), 3.21 (dd, *J* = 9.43, 4.13 Hz, 2 H), 2.48 (d, *J* = 6.8 Hz, 4 H), 1.71 (s, 2 H) ppm; ^{13}C NMR (150 MHz, CD_3OD) δ = 166.26, 165.92, 165.35, 165.05, 146.27, 143.64, 133.60, 133.49, 133.38, 133.37, 132.41, 129.95, 129.88, 129.85, 129.78, 129.67, 129.30, 128.94, 128.60, 128.57, 128.45, 122.01, 113.83, 111.49, 101.37, 72.96, 72.15, 72.00, 69.89, 69.54, 63.16, 55.82, 40.87, 35.26 ppm; HRMS (ESI-TOF): calcd for $C_{88}H_{78}O_{24}$ [M + H$^+$]: 1519.4956, found 1519.4937. (*R*,*R*)-**9**: R_f = 0.10 (4:6 EtOAc:hexanes); $[\alpha]_D^{32}$ = +4.8 (EtOAc, *c* = 4.1); IR (film): ν_{max} = 3457, 2942, 1726, 1262, 1026, 707 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ = 8.00 (d, *J* = 7.62 Hz, 4 H), 7.97 (d, *J* = 7.71 Hz, 4 H), 7.93 (d, *J* = 7.98 Hz, 4 H), 7.62 (d, *J* = 7.71 Hz, 4 H), 7.56–7.27 (m, 24 H), 6.45 (d, *J* = 7.95 Hz, 2 H), 6.29 (s, 2 H), 6.24 (d, *J* = 7.95 Hz, 2 H), 5.91 (t, *J* = 9.82 Hz, 2 H), 5.70 (t, *J* = 9.67 Hz, 2 H), 5.54 (t, *J* = 9.23 Hz, 2 H), 5.40 (s, 2 H), 4.71 (dd, *J* = 11.87, 3.29 Hz, 2 H), 4.62 (d, *J* = 7.92 Hz, 2 H), 4.42 (dd, *J* = 12.14, 4.72 Hz, 2 H), 4.11–4.06 (m, 2 H), 3.85 (dd, *J* = 9.53, 3.46 Hz, 2 H), 3.65 (s, 6 H), 3.34 (dd, *J* = 9.70, 4.68 Hz, 2 H), 2.47–2.40 (m, 2 H), 2.37–2.30 (m, 2 H), 1.82 (s, 2 H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ = 166.23, 165.95, 165.34, 165.21, 146.33, 143.54, 133.56, 133.50, 133.36, 133.30, 132.44, 129.96, 129.92, 129.87, 129.65, 129.42, 128.98, 128.66, 128.55, 128.44, 121.98, 113.66, 111.12, 101.25, 73.07, 72.15, 72.08, 69.88, 69.78, 62.97, 55.78, 40.67, 35.50 ppm; HRMS (ESI-TOF): calcd for $C_{36}H_{38}O_8$ [M + H$^+$]: 1519.4956, found 1519.4947.



(*S*,*S*)-SDG-**1**

Secoisolariciresinol Diglucoside (*S*,*S*)-SDG-1. To a flask containing dry (*S*,*S*)-**8** (0.043 g, 0.028 mmol, 1 equiv), a freshly prepared solution of NaOMe in MeOH (0.4 M, 2 mL, 28 equiv) was added and the solution was stirred for 60 h at 25 °C. The solution was then filtered through a pad of silica (0.5 in, washing with MeOH) and the filtrate was concentrated. The resulting solid was purified by preparative thin-layer chromatography (silica, 2 mm, 9:1 → 7:3 CH_2Cl_2:MeOH, then 5:5 CH_2Cl_2:MeOH half of plate length) and then passed through a small plug of reversed phase silica (100 Å, C_{18}) to provide (*S*,*S*)-SDG-**1** (0.017 g, 0.025 mmol, 88% yield) as an off-white solid. (*S*,*S*)-SDG-**1**: R_f = 0.57 (silica, 1:1 CH_2Cl_2:MeOH); $[\alpha]_D^{32}$ = −0.3 (MeOH, *c* = 1.2); IR (film): ν_{max} = 3340, 2950, 1601, 1515, 1372, 1270, 1070, 1015, 798 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ = 6.65 (d, *J* = 8.05 Hz, 2 H), 6.59 (d, *J* = 1.31 Hz, 2 H), 6.56 (dd, *J* = 8.05, 1.31 Hz, 2 H), 4.24 (d, *J* = 7.42 Hz, 2 H), 4.08 (dd, *J* = 10.09, 5.58 Hz, 2 H), 3.85 (dd, *J* = 12.00, 2.43 Hz, 2 H), 3.73 (s, 6 H), 3.69 (dd, *J* = 11.85, 5.55 Hz, 2 H), 3.50–3.45 (m, 2 H), 3.38–3.28 (m, 4 H), 3.27–3.19 (m, 4 H), 2.69 (dd, *J* = 13.82, 6.72 Hz, 2 H), 2.61 (dd, *J* = 13.82, 7.98 Hz, 2 H), 2.12 (m, 2 H) ppm; ^{13}C NMR (150 MHz, CD_3OD) δ = 148.69, 145.35, 133.93, 122.89, 115.63, 113.51, 104.77, 78.16, 77.89, 75.25, 71.69, 71.18, 62.79, 56.25, 41.20, 35.60 ppm; HRMS (ESI-TOF): calcd for $C_{32}H_{46}O_{16}$ [M + H$^+$]: 687.2858, found 687.2856.

Secoisolariciresinol Diglucoside (*R*,*R*)-SDG-2. To a flask containing dry (*R*,*R*)-**9** (0.041 g, 0.027 mmol, 1 equiv), a freshly prepared solution of NaOMe in MeOH (0.4 M, 2 mL, 28 equiv) was added and the solution stirred for 60 h at 25 °C. The solution was then filtered through a pad of silica (0.5 in, washing with MeOH) and the filtrate concentrated. The resulting solid was purified by preparative thin-layer chromatography (silica, 2 mm, 9:1 → 7:3 CH_2Cl_2:MeOH, then 5:5 CH_2Cl_2:MeOH half of plate length) and then passed through a small plug of reversed phase silica (100 Å $C_{18,}$ washing with MeOH) to provide (*R*,*R*)-SDG-**2** (0.015 g, 0.022 mmol, 81% yield) as an off-white solid. (*R*,*R*)-SDG-**2**: R_f = 0.50 (silica, 1:1 CH_2Cl_2:MeOH); $[\alpha]_D^{32}$ = −22.2 (MeOH, *c* = 1.0); IR (film): ν_{max} = 3336, 2949, 1651, 1409,



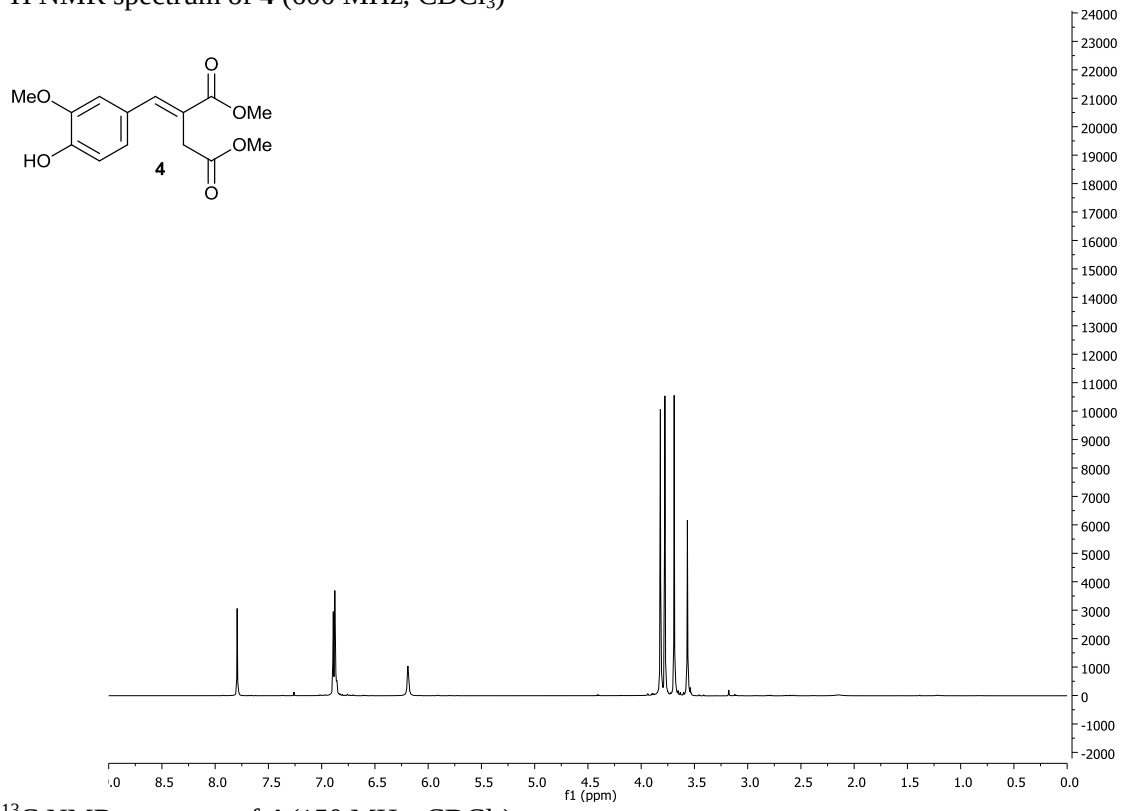
(*R*,*R*)-SDG-**2**

1014 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ = 6.66 (d, *J* = 8.06 Hz, 2 H), 6.64 (d, *J* = 1.63 Hz, 2 H), 6.59 (dd, *J* = 8.06, 1.63 Hz, 2 H), 4.21 (d, *J* = 7.82 Hz, 2 H), 3.91 (dd, *J* = 10.11, 5.69 Hz, 2 H), 3.87 (dd, *J* = 12.01, 2.01 Hz, 2 H), 3.75 (s, 6 H), 3.67 (dd, *J* = 12.02, 5.47 Hz, 2 H), 3.58 (dd, *J* = 9.90, 5.36 Hz, 2 H), 3.38–3.18 (m, 8 H), 2.76–2.62 (m, 4 H), 2.14–2.07 (m, 2 H) ppm; ^{13}C NMR (150 MHz, CD_3OD) δ = 148.77, 145.37, 134.05, 122.83, 115.70, 113.56, 104.59, 78.19, 77.93, 75.19, 71.70, 70.62, 62.79, 56.32, 41.63, 35.62 ppm; HRMS (ESI-TOF): calcd for $C_{32}H_{46}O_{16}$ [M + H^+]: 687.2858, found 687.2856.

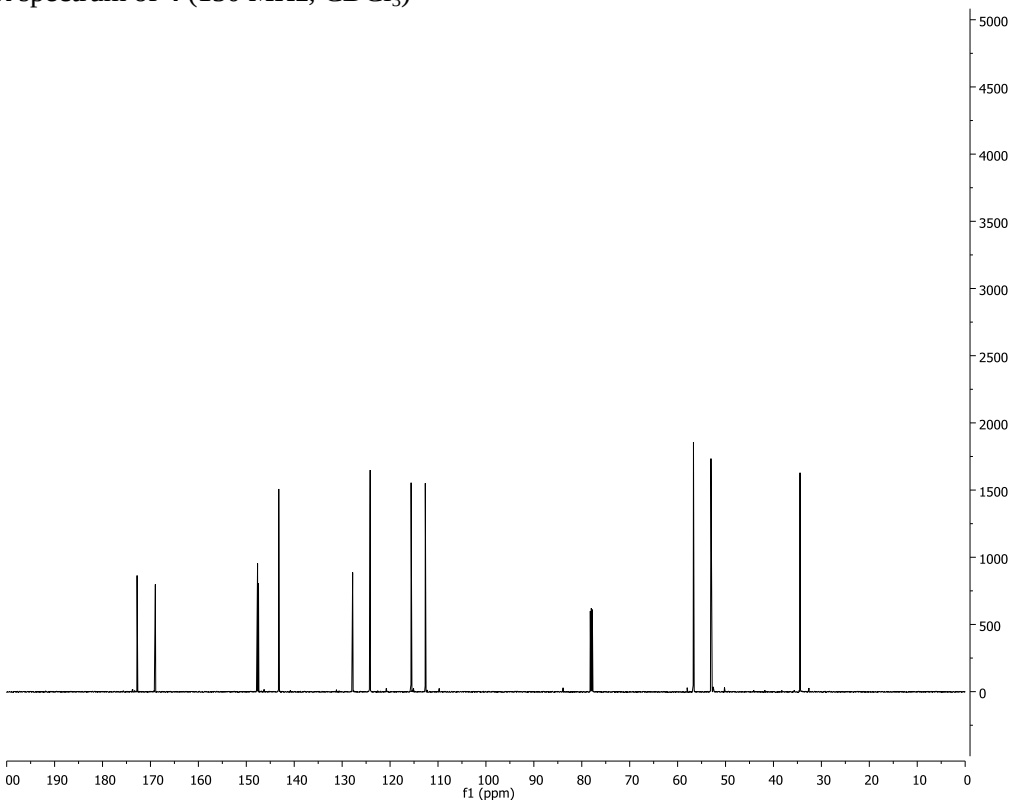
References

1. Yen, G. C.; Pin-Der, D. *J. Am. Oil Chem. Soc.* **1993**, *70*, 383.
2. Moree, S.; Khanum, S.A.; Rajesha, J. *Free Rad. Antiox.* **2011**, *1*, 31.
3. Mühlhausen, U.; Schirrmacher, R.; Piel, M.; Lecher, B.; Briegert, M.; Piee-Staffa, A.; Kaina, B.; Rösch, F. *J. Med. Chem.* **2006**, *49*, 263.

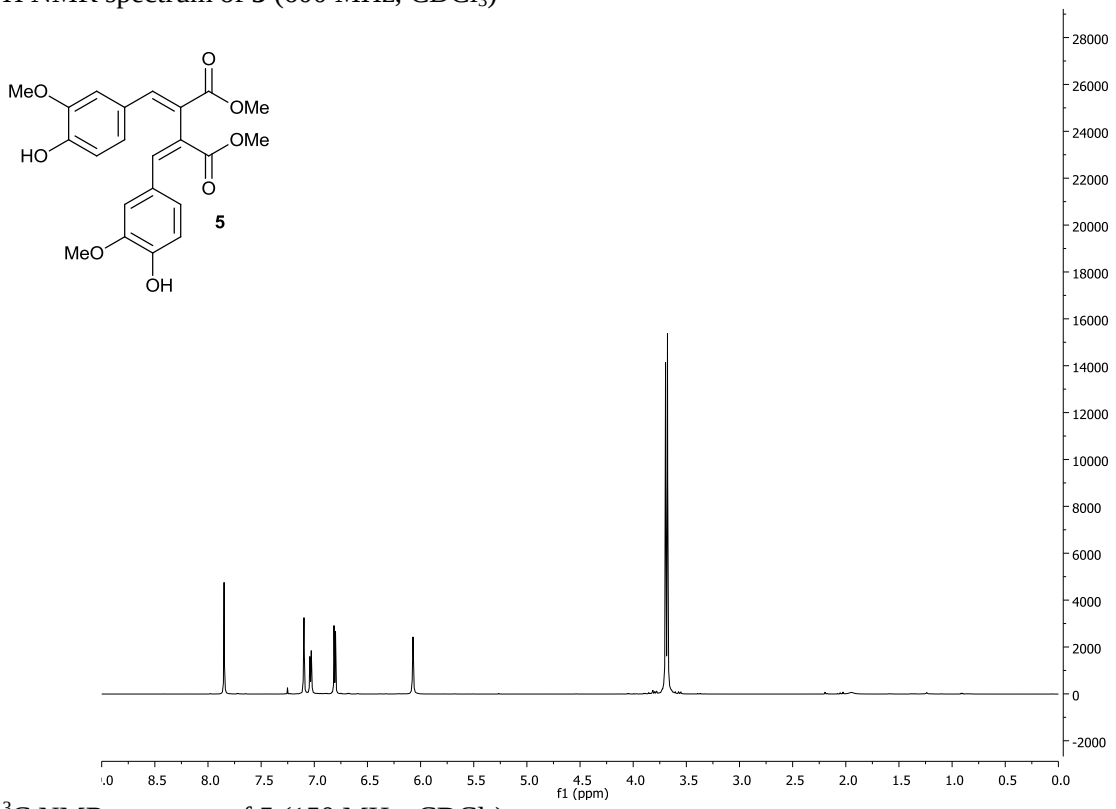
^{1}H NMR spectrum of **4** (600 MHz, $CDCl_3$)



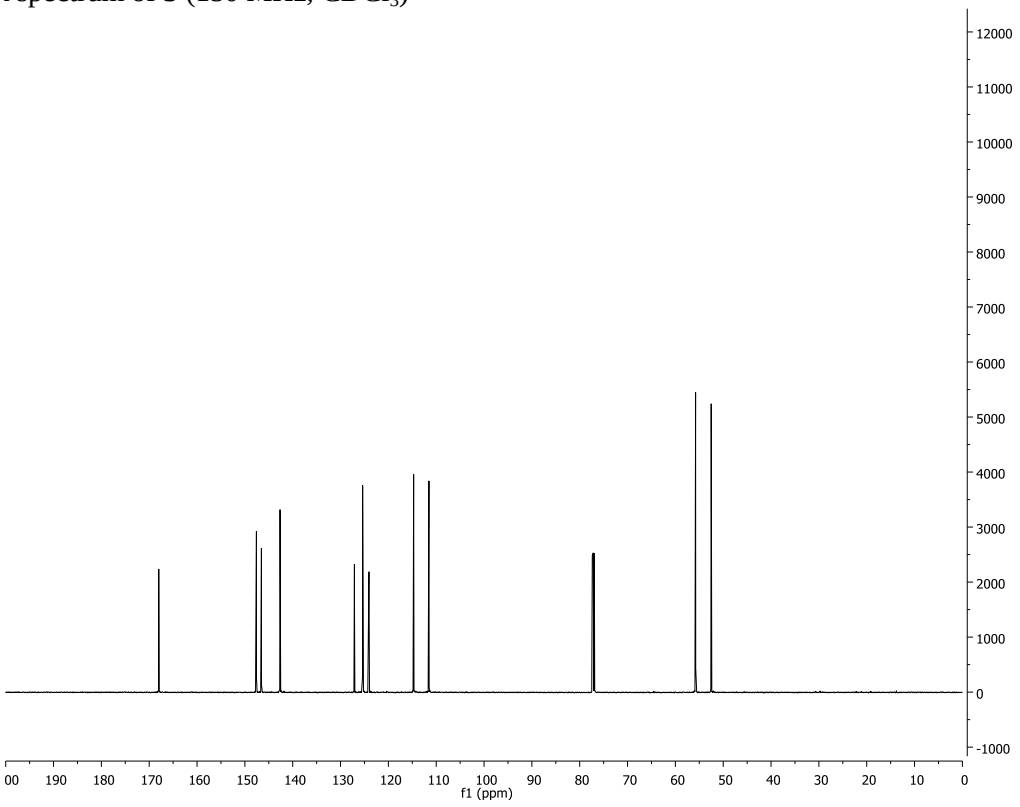
^{13}C NMR spectrum of **4** (150 MHz, $CDCl_3$)



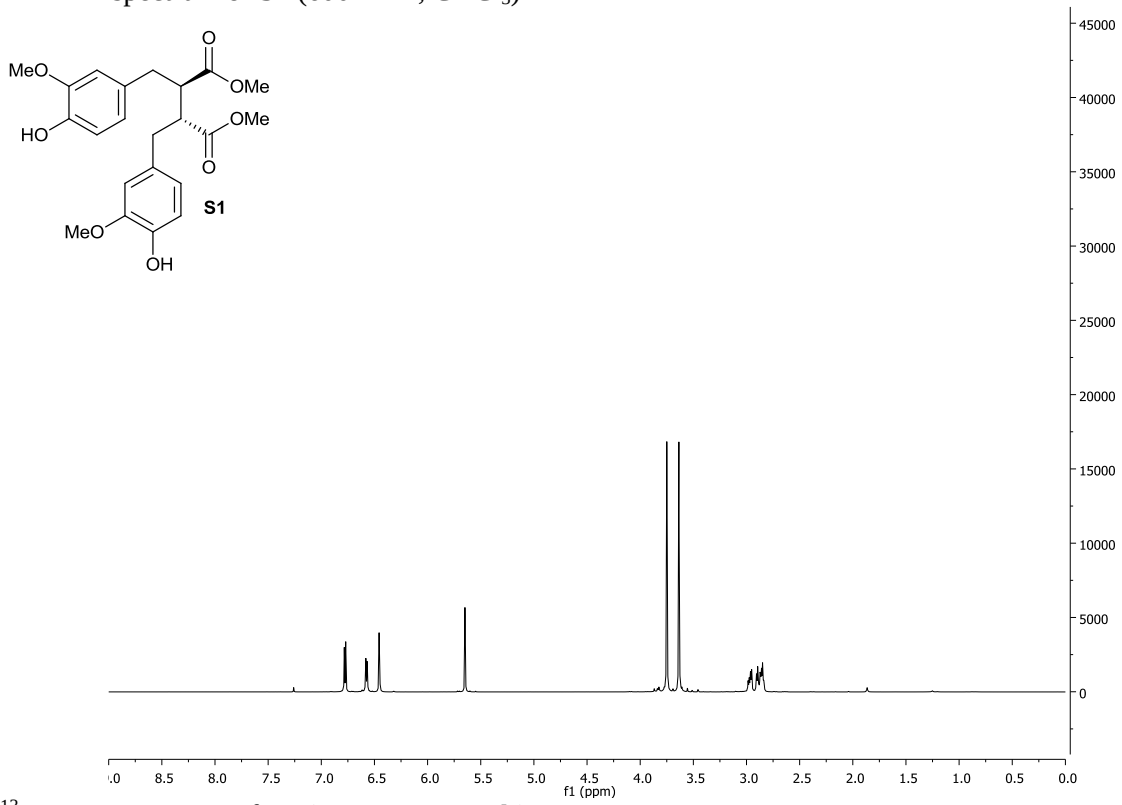
^{1}H NMR spectrum of **5** (600 MHz, $CDCl_3$)



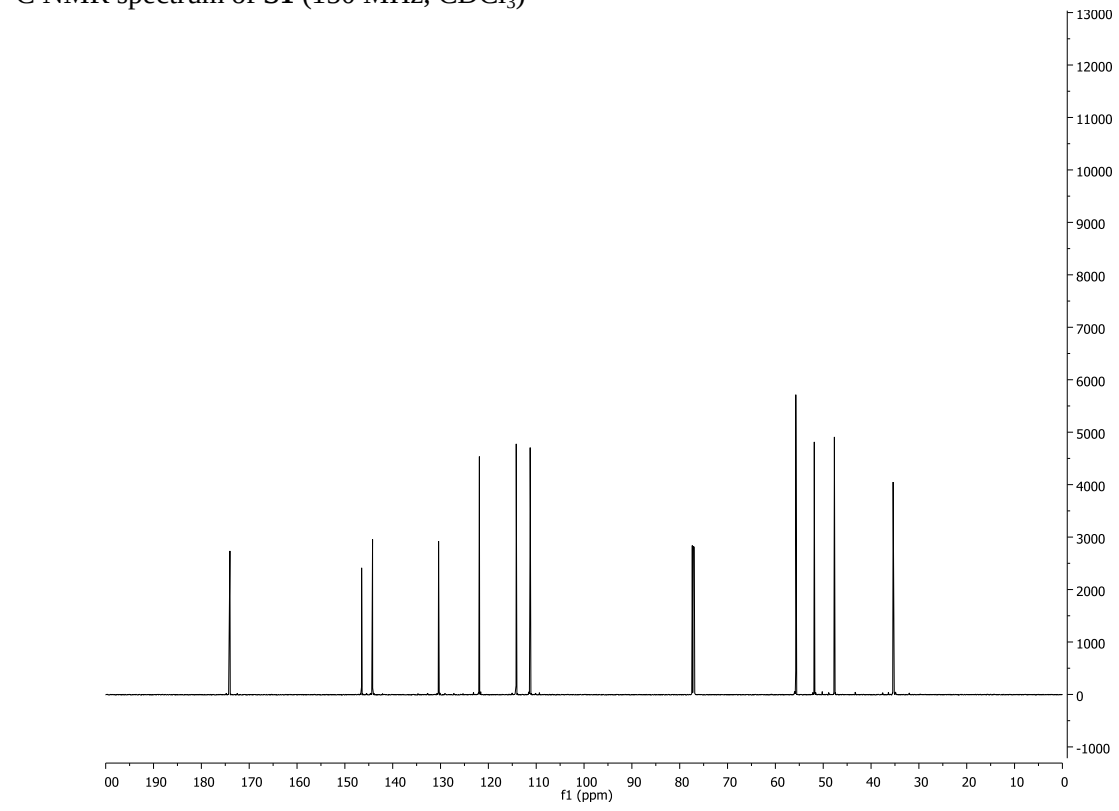
^{13}C NMR spectrum of **5** (150 MHz, $CDCl_3$)



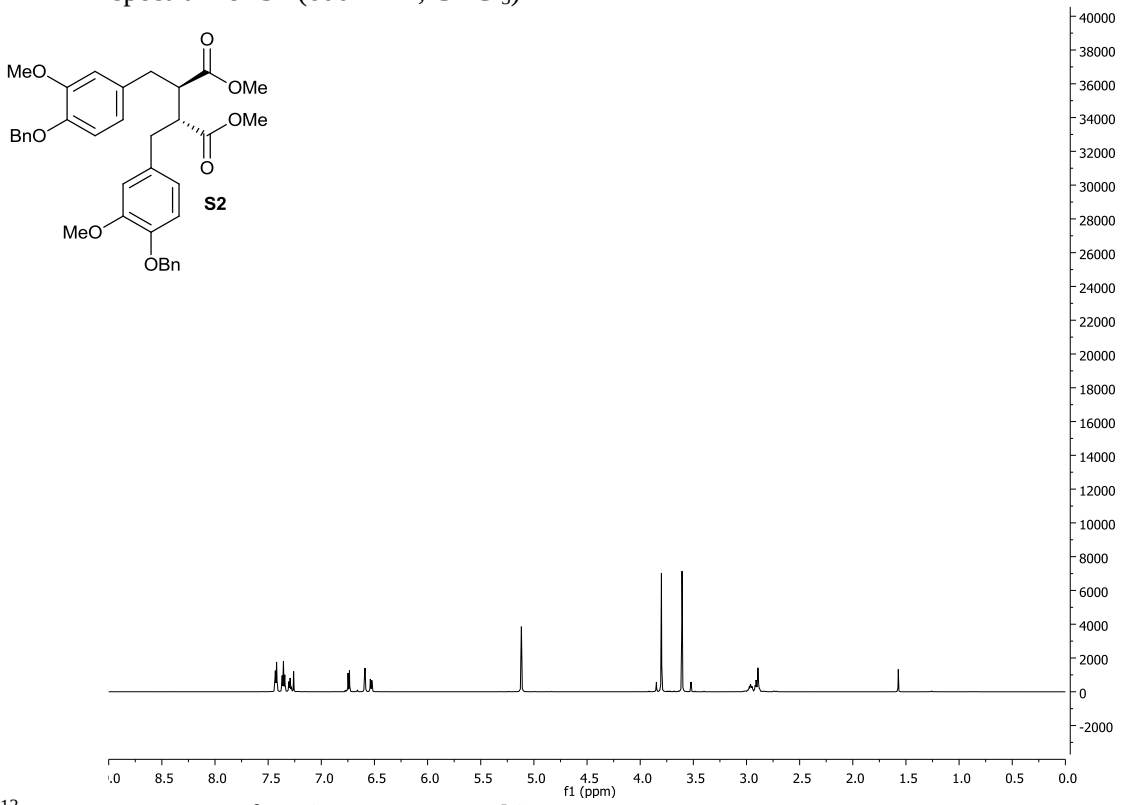
^{1}H NMR spectrum of **S1** (600 MHz, $CDCl_3$)



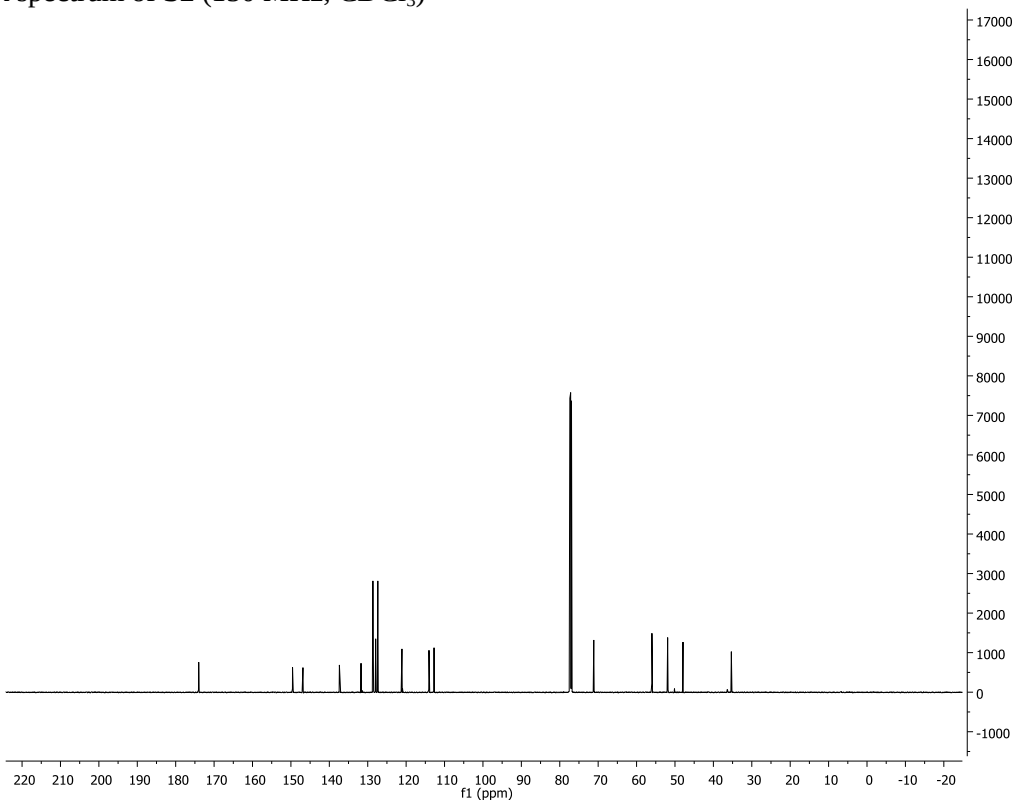
^{13}C NMR spectrum of **S1** (150 MHz, $CDCl_3$)



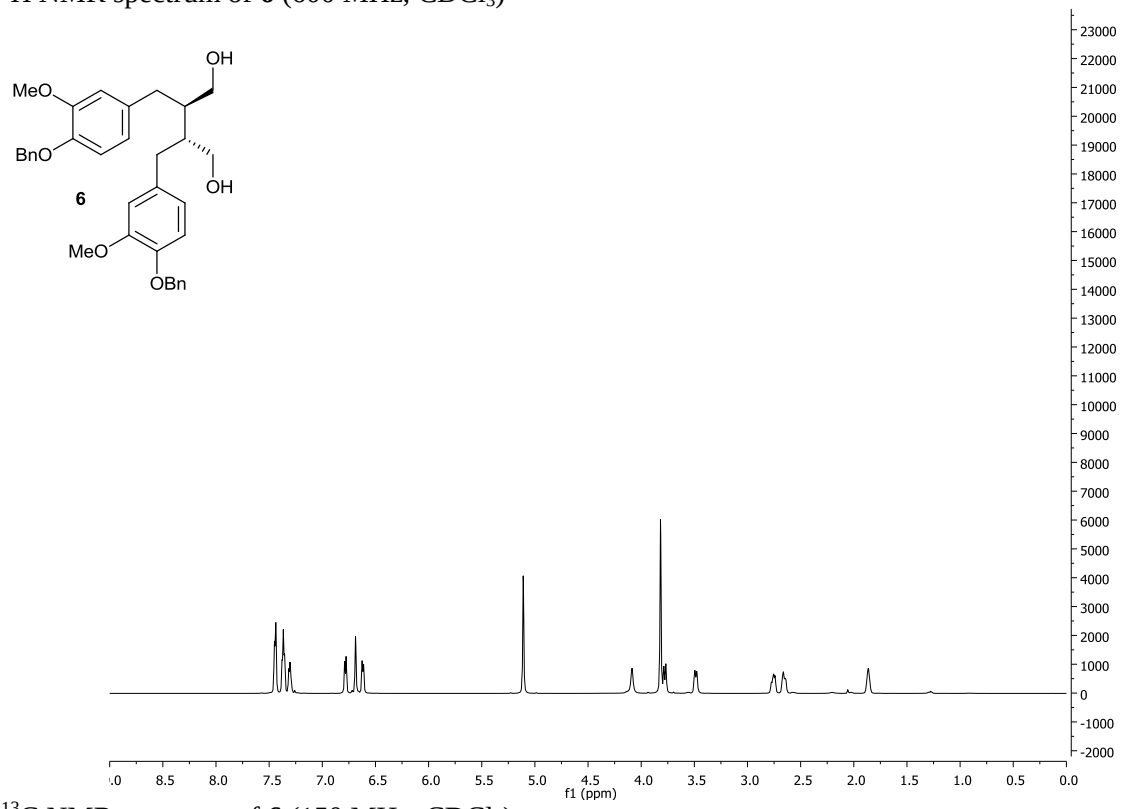
^{1}H NMR spectrum of **S2** (600 MHz, $CDCl_3$)



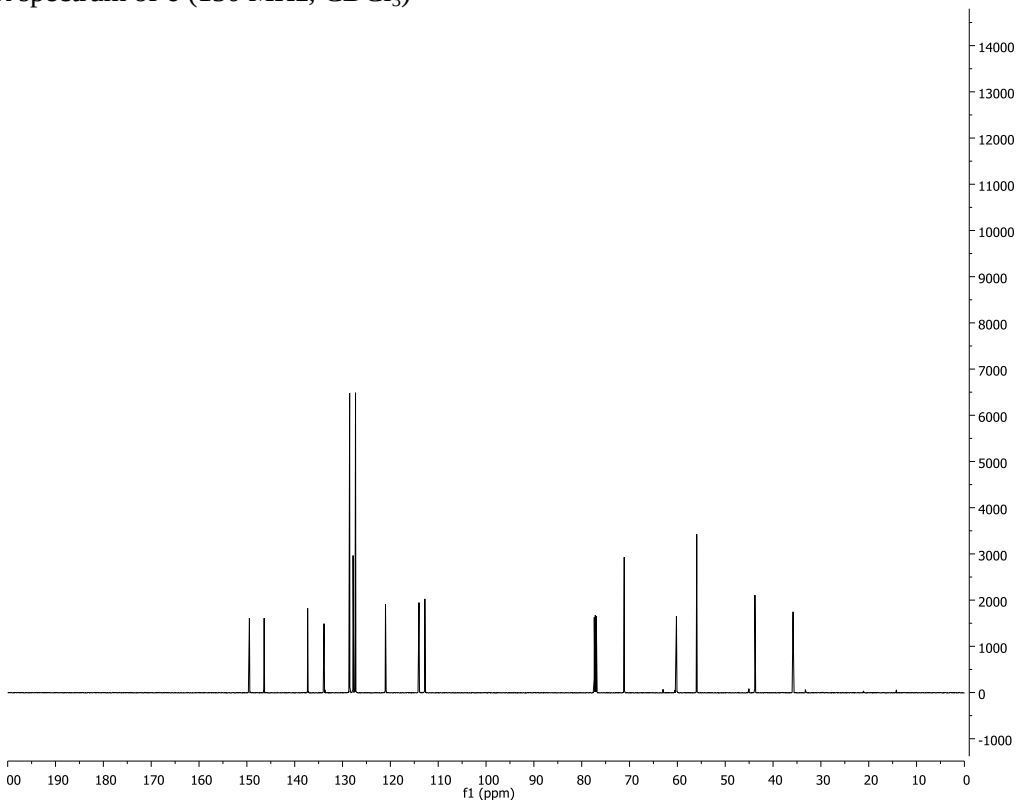
^{13}C NMR spectrum of **S2** (150 MHz, $CDCl_3$)



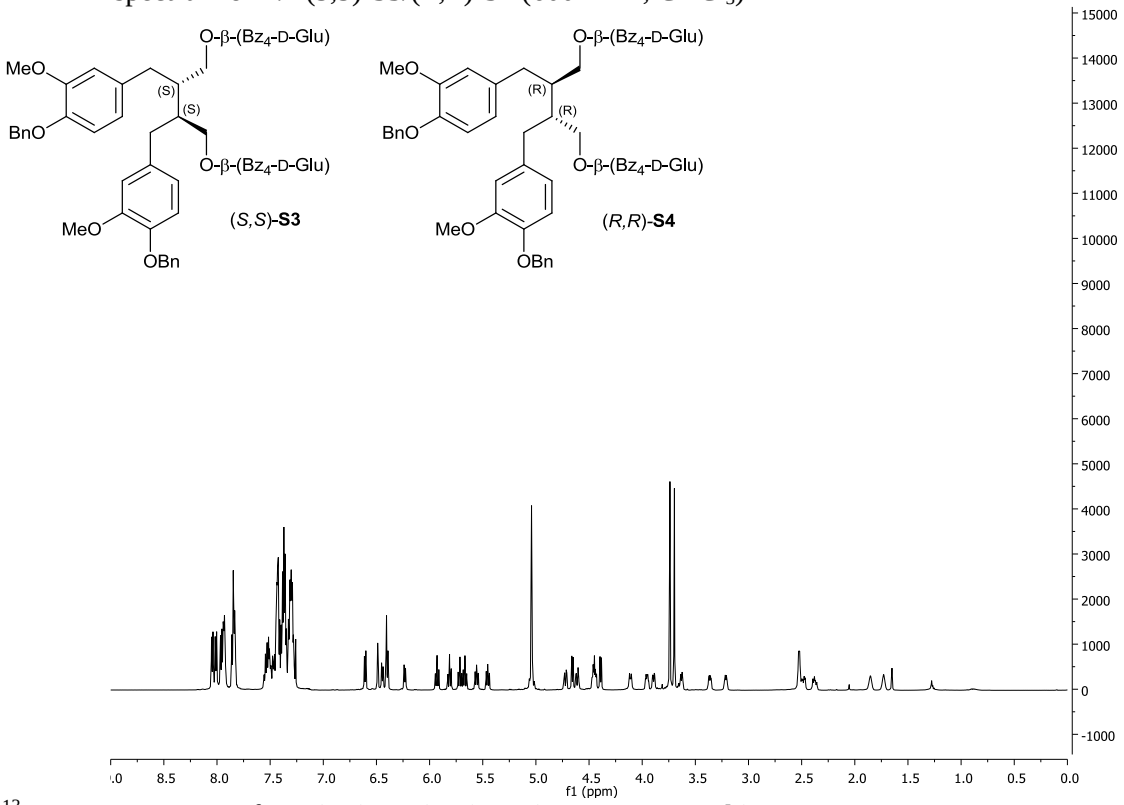
^{1}H NMR spectrum of **6** (600 MHz, $CDCl_3$)



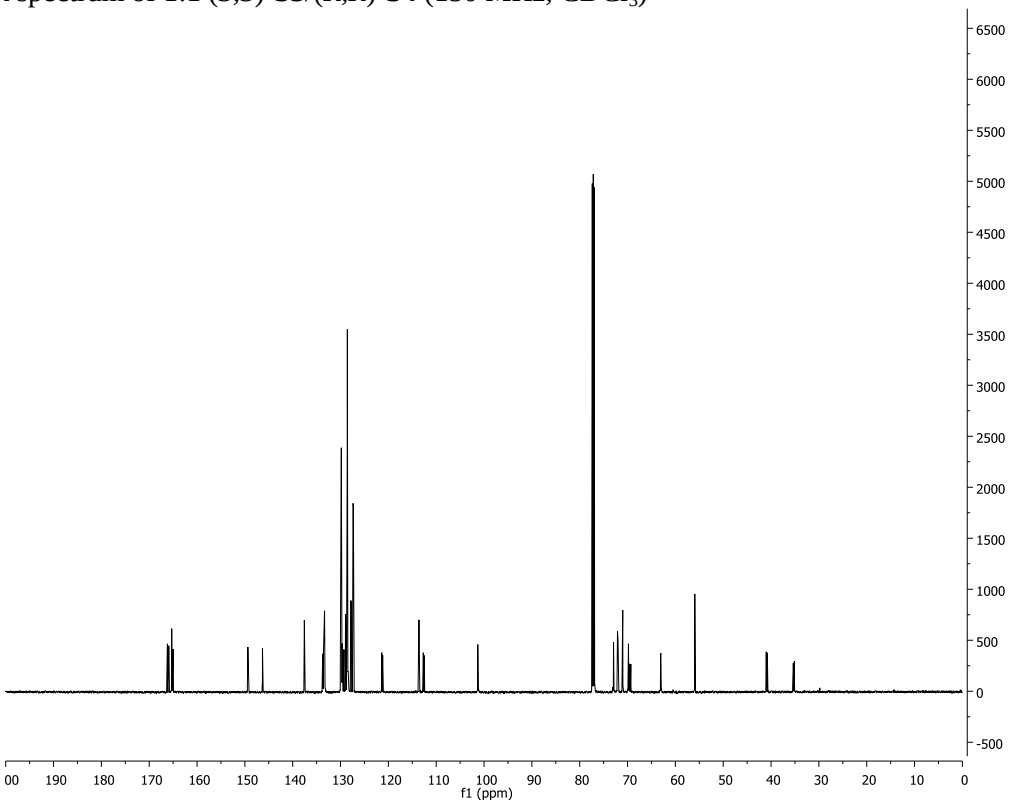
^{13}C NMR spectrum of **6** (150 MHz, $CDCl_3$)



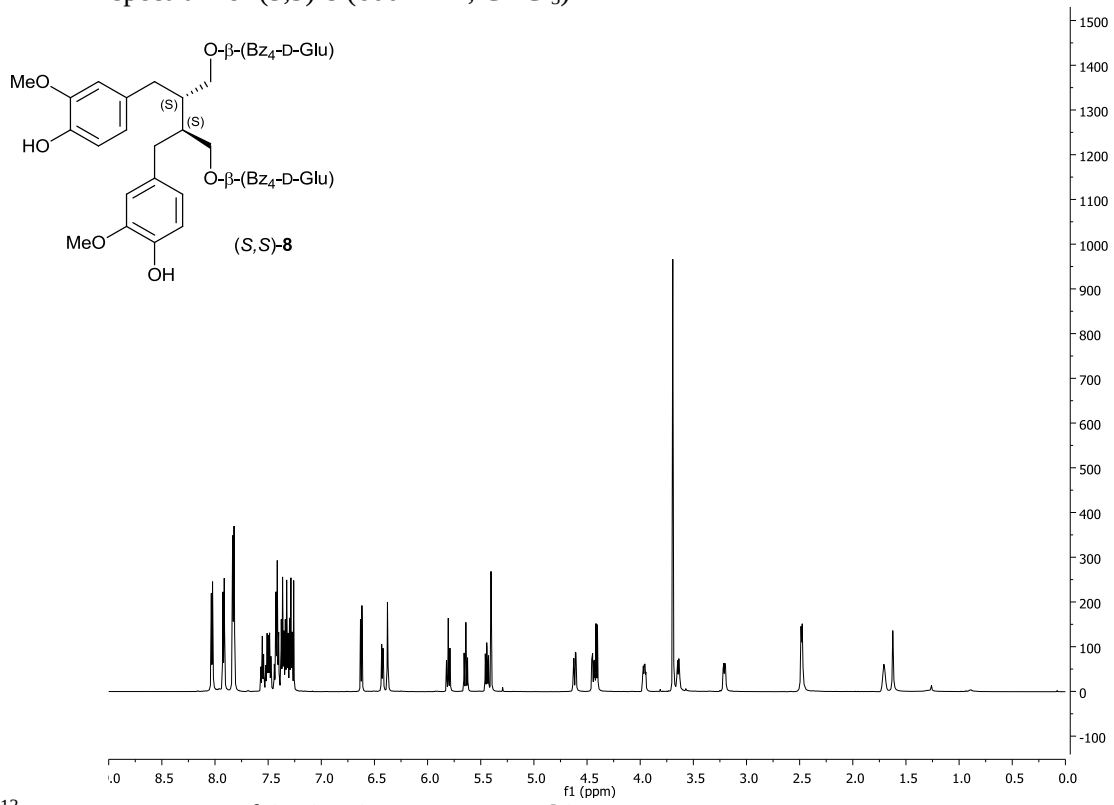
^{1}H NMR spectrum of 1:1 (*S*,*S*)-**S3**/(*R*,*R*)-**S4** (600 MHz, $CDCl_3$)



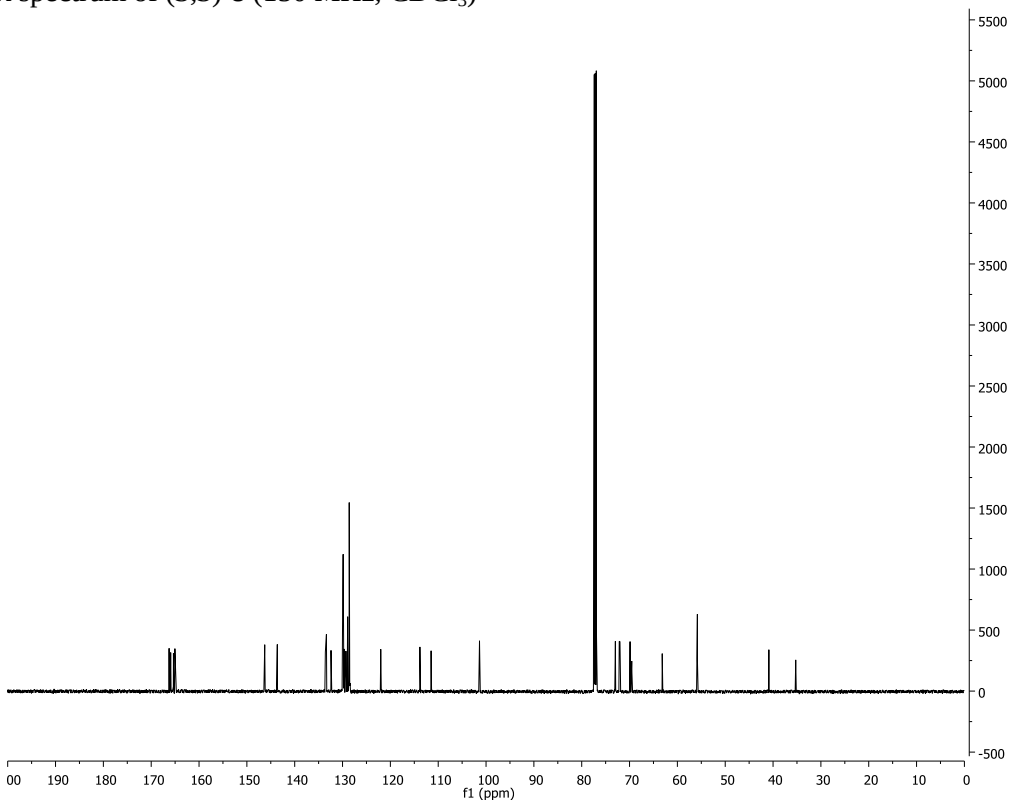
^{13}C NMR spectrum of 1:1 (*S*,*S*)-**S3**/(*R*,*R*)-**S4** (150 MHz, $CDCl_3$)



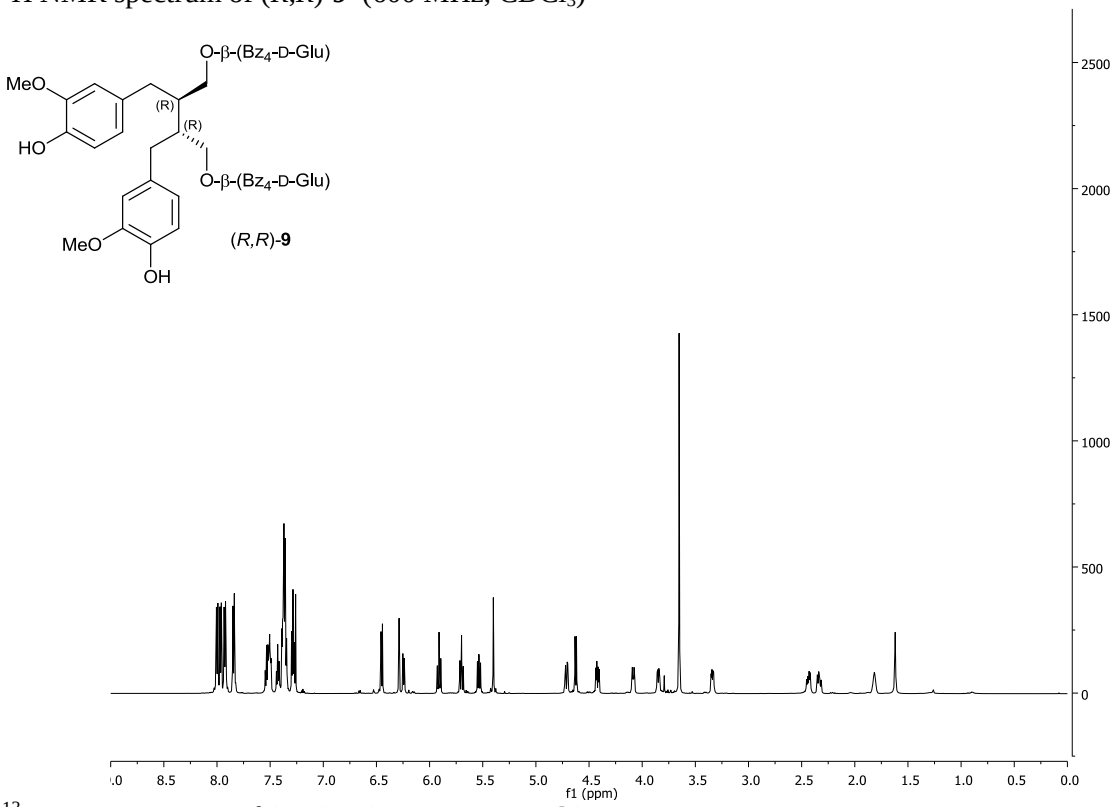
^{1}H NMR spectrum of (*S*,*S*)-**8** (600 MHz, $CDCl_3$)



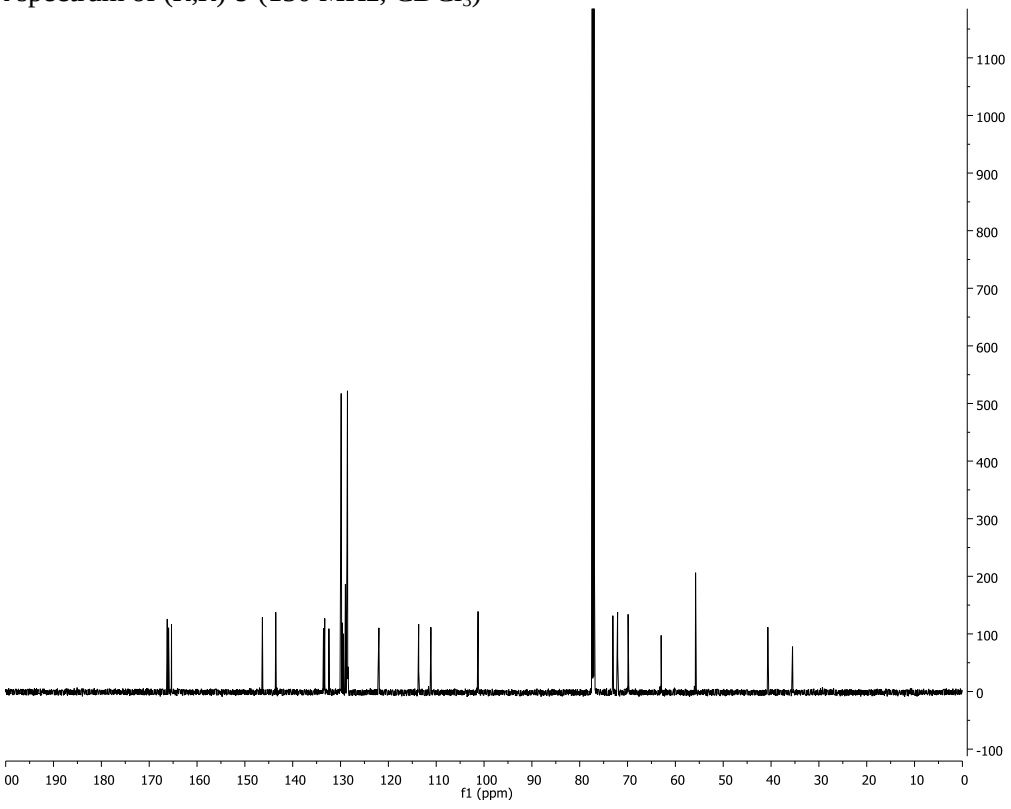
^{13}C NMR spectrum of (*S*,*S*)-**8** (150 MHz, $CDCl_3$)



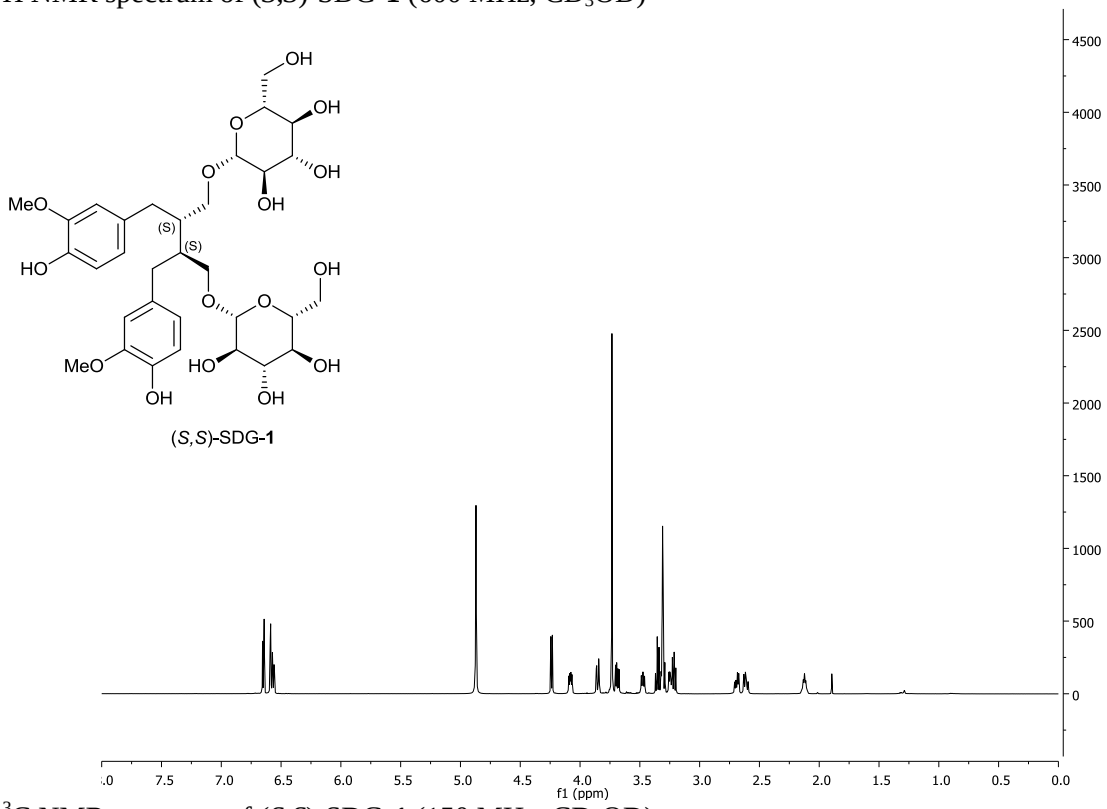
^{1}H NMR spectrum of (*R*,*R*)-**9** (600 MHz, $CDCl_3$)



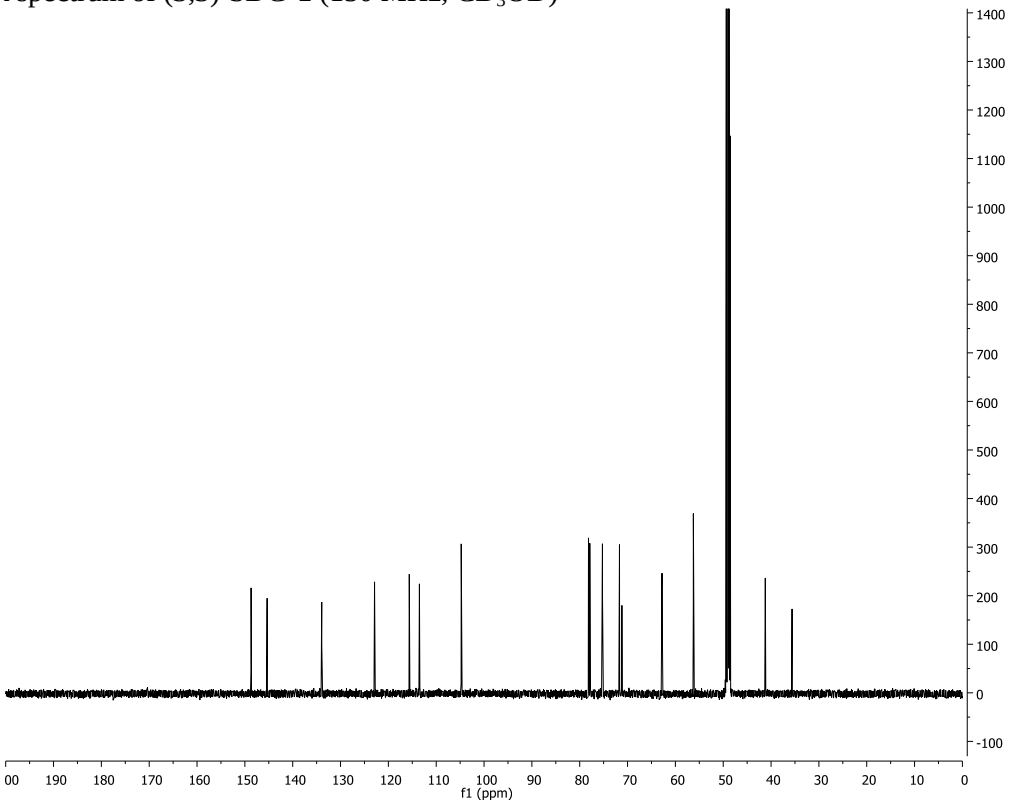
^{13}C NMR spectrum of (*R*,*R*)-**9** (150 MHz, $CDCl_3$)



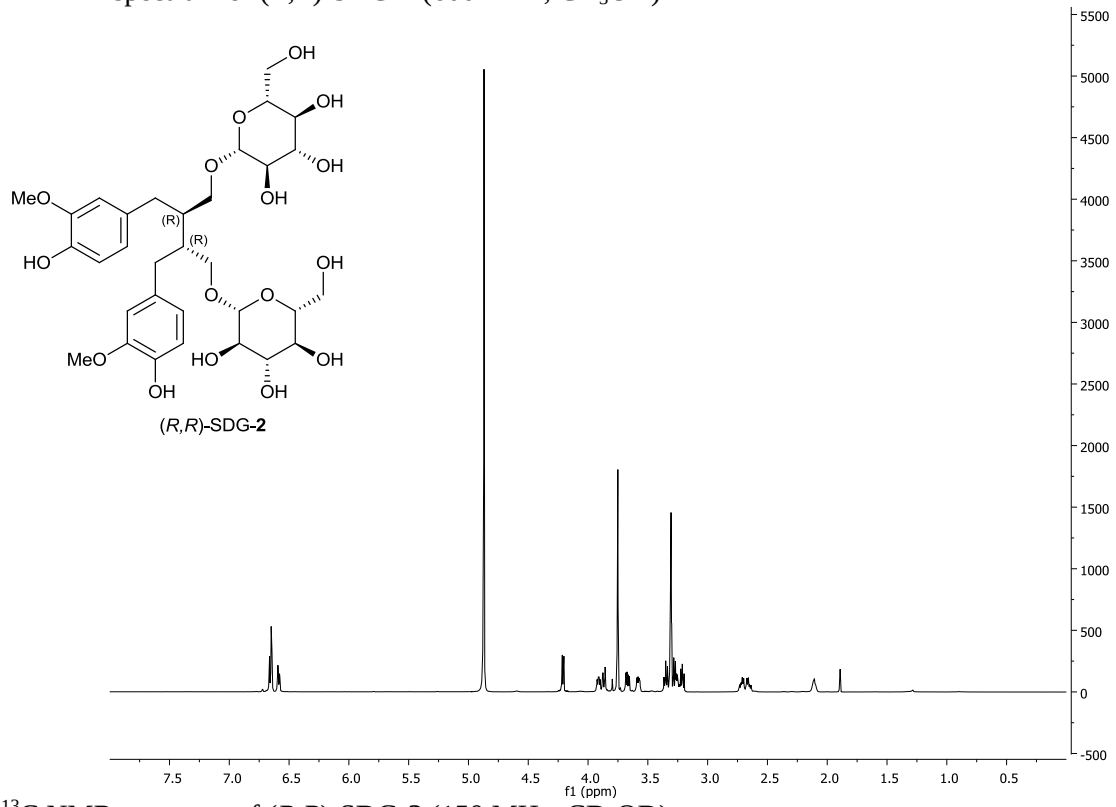
^{1}H NMR spectrum of (*S*,*S*)-SDG-**1** (600 MHz, CD_3OD)



^{13}C NMR spectrum of (*S*,*S*)-SDG-**1** (150 MHz, CD_3OD)



^{1}H NMR spectrum of (*R*,*R*)-SDG-**2** (600 MHz, CD_3OD)



^{13}C NMR spectrum of (*R*,*R*)-SDG-**2** (150 MHz, CD_3OD)