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

Supplementary Information

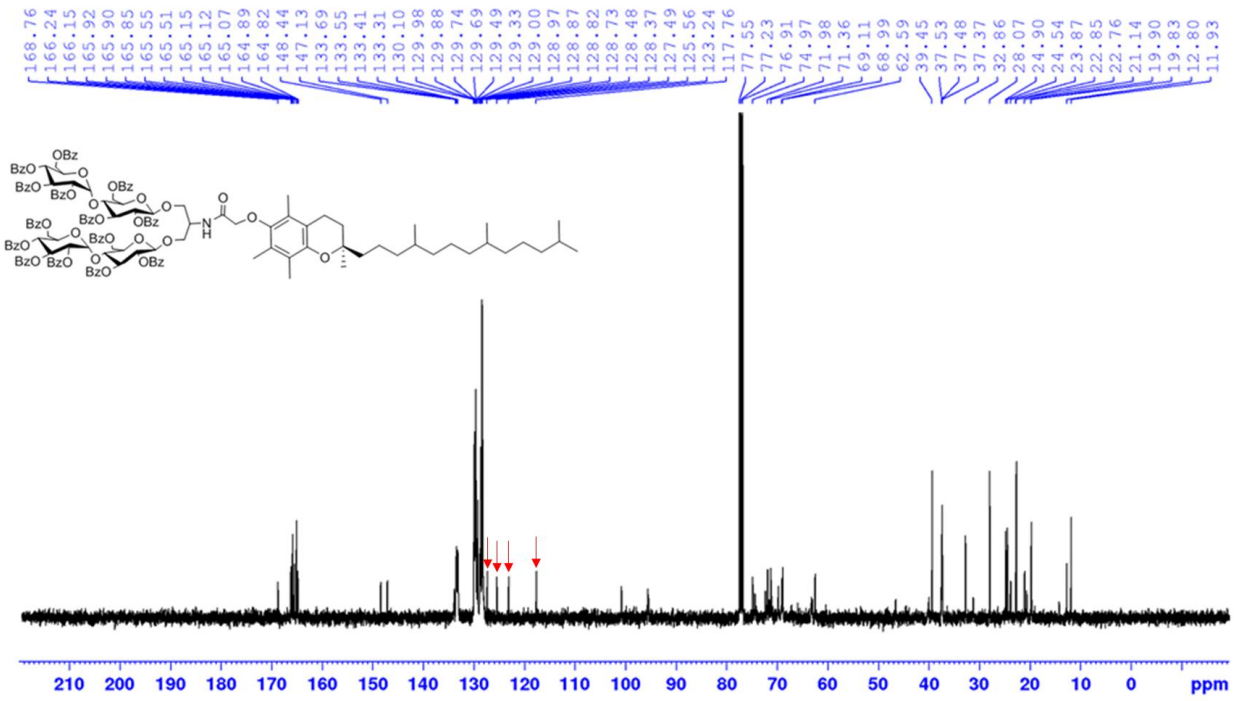


Fig. S1 ^{13}C NMR spectrum of the benzoyl protected form of VEG-3 (i.e., glycosylated product). The absence of an NMR peak at 121 ppm indicates that the orthoester isomer was not present in the isolated product. Note that the peaks at 117.8, 123.2, 125.6, and 127.5 ppm, indicated by the small arrows, correspond to benzene carbons in the vitamin E unit.

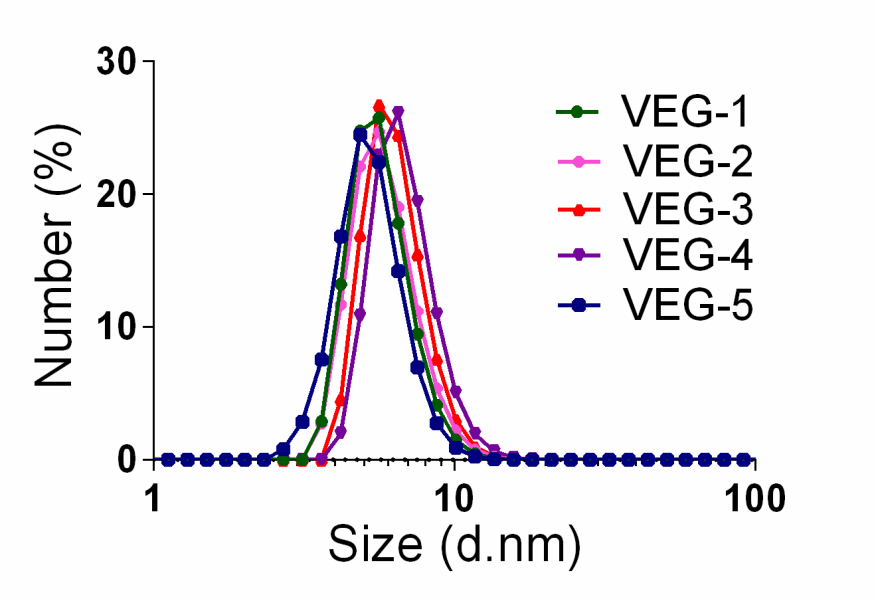


Fig. S2. Dynamic light scattering (DLS) profiles of individual novel detergents (VEGs). The detergents (VEG-1, VEG-2, VEG-3, VEG-4 and VEG-5) were tested at 1.0 wt% for this measurement. Based on scattered light intensity, the time scale of micelle diffusion was analyzed, which gives micellar distribution in term of the hydrodynamic diameter (R_h). All these agents tended to form monodisperse micelles with a set of single population.

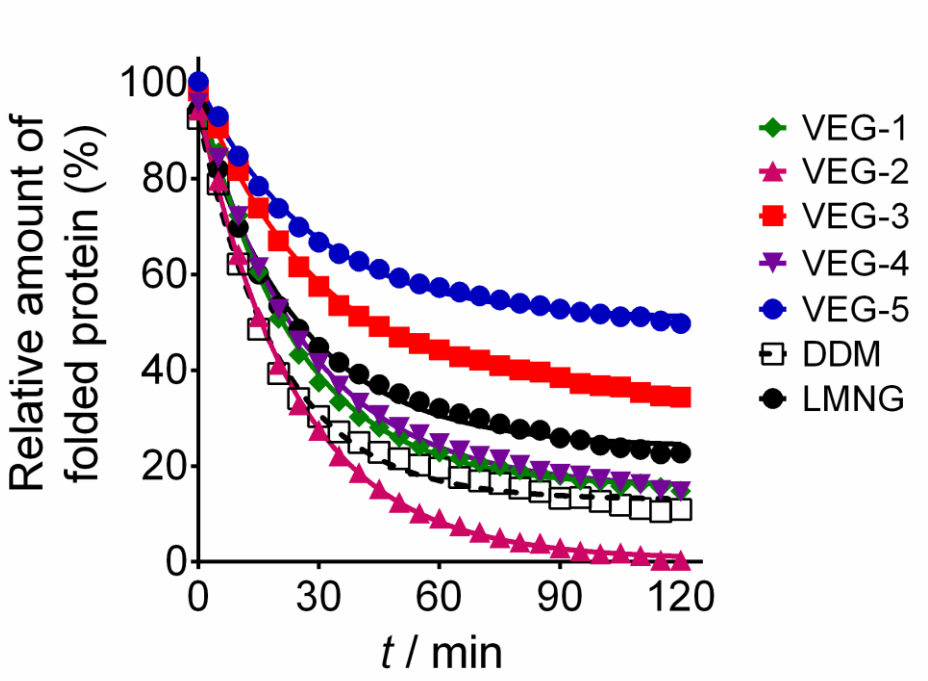


Fig. S3 Thermal denaturation profile of UapA solubilized in DDM, individual VEGs (VEG-1, VEG-2, VEG-3, VEPG-4, and VEG-5) or LMNG. The folded state of the transporter was monitored by thermal denaturation assay performed at 40 ℃ for 120 min, with a detergent concentration of CMC + 0.2 wt%. The relative amounts of folded protein were normalized using the most destabilizing condition in this experiment, that is, protein denaturation in VEG-2 after 120-min incubation as a reference.

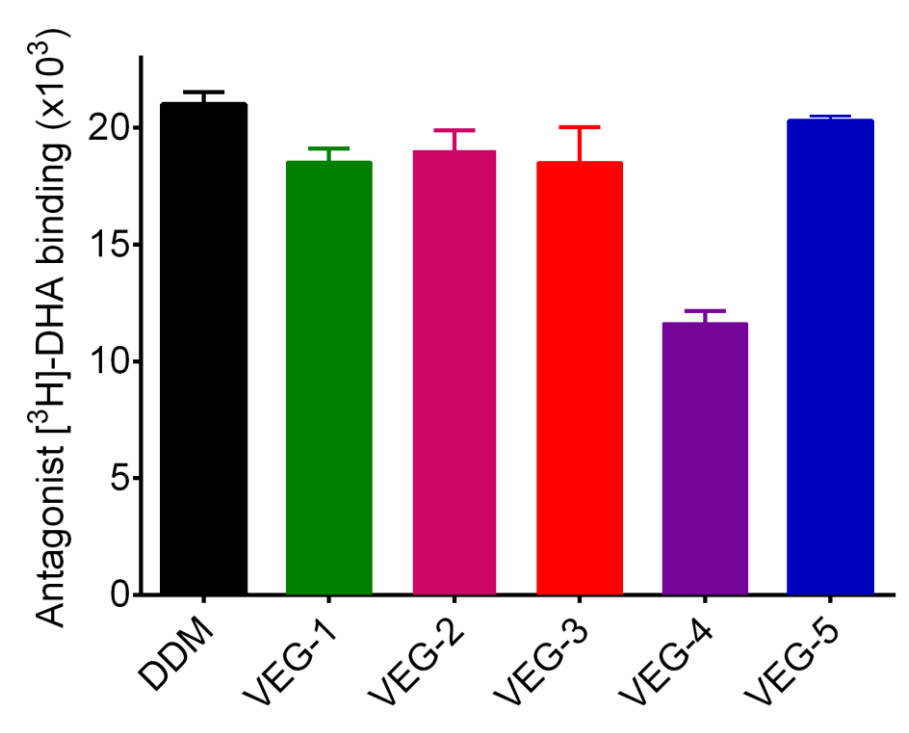


Fig. S4 Ligand binding ability of β_2AR solubilized in DDM, individual VEGs (VEG-1, VEG-2, VEG-3, VEG-4 and VEG-5). DDM-purified β_2AR was diluted into each detergent solution to give a final detergent concentration of 0.2 wt%. Receptor activity was measured using the radiolabeled antagonist ([^{3}H]-dihydroalprenolol (DHA)) *via* ligand-binding assay. Error bars, SEM, n = 3.

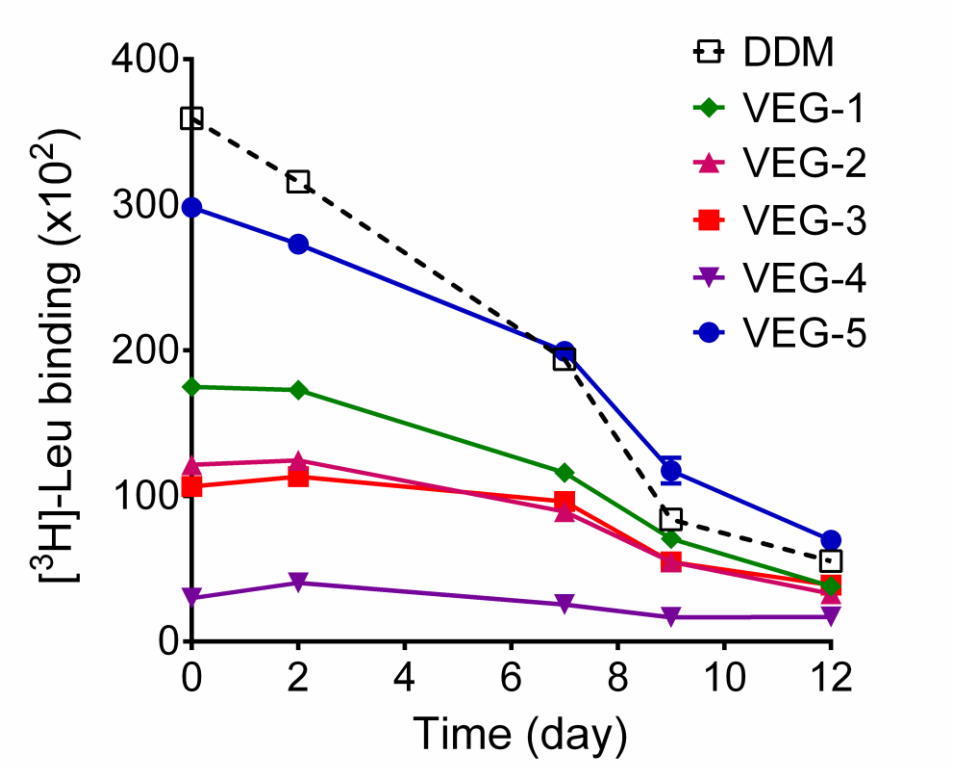


Fig. S5 Long-term stability of LeuT solubilized in DDM or a VEG (VEG-1, VEG-2, VEG-3, VEG-4, or VEG-5). The detergents were tested at CMC + 0.04 wt%. DDM-purified transporter was mixed with individual detergent-containing solutions and the resulting sample solutions were incubated for 12 days at room temperature. LeuT stability was monitored at regular intervals by measuring the ability of the transporter to bind the radiolabeled substrate ([^{3}H]-leucine (Leu)) *via* scintillation proximity assay (SPA). Error bars, SEM, n = 2.

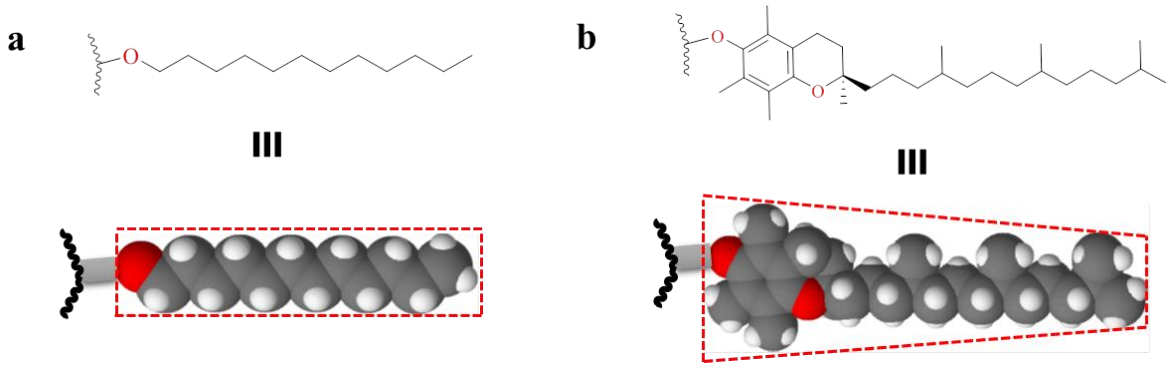


Fig. S6 Comparison of detergent hydrophobic group (dodecyl chain of DDM (a) *vs.* (b) vitamin E of the VEGs) in terms of molecular geometry. Each hydrophobic group was represented with the chemical structure (top) and space-filling model (bottom) while the hydrophilic group was omitted for clarity. The dodecyl chain of DDM has a cylindrical shape whereas the vitamin E unit is closer to a conical shape due to the presence of the bulky bicyclic ring at the interface between the hydrophilic and hydrophobic groups. Molecular geometry of detergent tail group was indicated by red dotted lines.

Detergent CMC determination by diphenylhexatriene (DPH) encapsulation

5.0 mM individual detergent stock solutions were prepared in deionized and distilled water. A series of detergent solutions were prepared to give a range of detergent concentrations using the stock solutions. 200 µL of each detergent sample was transferred to a 96-well plate in duplicate. A DPH stock solution was prepared by dissolving 3.0 mg DPH in 5.0 mL THF. 50 µL of the stock solution was added into 950 µL of distilled water to give a DPH working solution and then 2.0 µL of this DPH working solution was added into each well containing a detergent solution for dye encapsulation. After a 15 to 20 min incubation at room temperature, fluorescence intensity was measured at 430 nm upon excitation at 358 nm using a Synergy Mx Monochromator Based Multi-Mode Microplate reader. Detergent CMC values were determined by plotting florescence intensity as a function of detergent concentration.

Detergent micelle size measurement by dynamic light scattering (DLS)

The novel detergents (VEGs) were dissolved in distilled and deionized water to give a detergent concentration of 1.0 wt%. The solutions were filtered by a syringe filter with a pore size of 0.22 µm. Hydrodynamic diameters of micelles produced by these agents were measured at variable temperature using a Malvern Zeta Sizer Nano ZS90 particle analyzer. With a maximum power of 5 MW, a He-Ne laser set at 633 nm was used as the light source. The scattered light was collected at an angle of 90°. The translational diffusion coefficient and hydrodynamic diameter (D_h) of the detergent micelles was calculated by autocorrelation analysis on time-dependent scattered light intensity. Hydrodynamic diameter (D_h) for micelles formed by individual detergents were expressed as mean ± SD (n = 5).

Detergent evaluation with membrane proteins

***R. capsulatus* superassembly stability assay**

The superassembly was solubilized and purified according to the reported protocol.[1] Specialized photosynthetic membranes obtained from an engineered strain of *Rhodobacter capsulatus* were used for the experiment. A 10 ml aliquot of the frozen membranes was homogenized using a glass tissue homogenizer and incubated with mild agitation at 32 ℃ for 30 mins. The resulting homogenized membranes were treated with 1.0 wt% DDM for 30 min at 32 ℃. Following ultracentrifugation, the supernatant containing the solubilized LHI-RC complexes was collected and incubated with Ni^{2+}-NTA resin at 4 ℃ for one hour. The resin-containing solution was filtered onto 10 His-SpinTrap columns and the resin washed twice with 500 µl binding buffer (10 mM Tris (pH 7.8), 100 mM NaCl, 1 × CMC DDM). The elution buffer containing 1.0 M imidazole (2×300 µl) was used to collect DDM-purified LHI-RC complexes. 80 µl LHI-RC sample purified in DDM was diluted into 920 µL individual detergent solutions (a VEG (VEG-1, VEG-2, VEG-3, VEG-4, or VEG-5), DDM and OG). The final detergent concentration was CMC+0.04 wt% or CMC+0.2 wt%. The samples were incubated at 25 ℃ for the first 20-day and the incubation temperature was then increased to 32 ℃ for the next 7 days. Protein stability was measured at regular intervals during the incubation by taking UV-Visible spectra of the samples in the range of 650 to 950 nm. Protein integrity was assessed by monitoring absorbance at 875 nm (A_{875}).

UapA thermal denaturation assay

UapAG411V$_{\Delta 1\text{-}11}$ was expressed as a GFP fusion in *Saccharomyces cerevisiae* strain FGY217 and isolated as described previously in sample buffer (20 mM Tris (pH 7.5), 150 mM NaCl, 0.03% DDM, 1 mM xanthine).[2] The protein was concentrated to approximately 10 mg/ml using a 100 kDa molecular weight cut off filter (Millipore). The protein was diluted 1:150 into buffer containing either DDM, a VEG (VEG-1, VEG-2, VEG-3, VEG-4, or VEG-5) or LMNG at concentrations of CMC + 0.2 wt% in Greiner 96-well plates. The CPM dye (Invitrogen), stored in DMSO (Sigma), was diluted in dye buffer (20 mM Tris (pH 7.5), 150 mM NaCl, 0.03% DDM, 5 mM EDTA) and 3 µl of the diluted dye was added to individual protein samples. The reaction mixture was incubated for 120 min at 40 °C. The fluorescence emission intensity was monitored using a microplate spectrofluorometer set at excitation and emission wavelengths of 387 and 463 nm, respectively. The maximum fluorescence value was used to calculate the percentage of relative folded protein during this incubation period. The relative amounts of folded proteins were plotted against time using GraphPad Prism.

Fluorescence size exclusion chromatography (FSEC): Membranes containing UapA were resuspended in pH 8.0 PBS buffer containing 10 mM Imidazole, 150 mM NaCl, 10% glycerol and the protein concentration was measured. The membranes were adjusted to a concentration of 1 mg/ml and 1 ml aliquots were incubated individually with 1.0 wt% DDM, LMNG, VEG-3 and VEG-5 for 60 min on ice with mild agitation. 100 µl aliquots were taken from each tube, and fluorescent SEC (FSEC) was measured for each sample after ultracentrifugation at 150,000 g for 10 min. The remaining soluble fractions were thermally treated by incubating for 10 min at 45 °C. The thermally treated samples were subjected to FSEC to monitor integrity of the transporter. The FSEC was carried out using a Superose 6 column (GE Healthcare) equilibrated with buffer containing the appropriate agent (DDM, LMNG, VEG-3, or VEG-5).

MelB$_{St}$ solubilization and thermal stability assay

A published protocol[3] was modified to evaluate stability of MelB from *Salmonella typhimurium* (MelB$_{St}$) with DDM and representative VEGs. The plasmid pK95ΔAHB/WT MelB$_{St}$/CH10 encoding the wild-type MelB$_{St}$ with a C-terminal 10-His tag was expressed in DW2 cells (*ΔmelB* and *ΔlacZY*). Cell growth and membrane preparation were carried out as reported.[4] Protein assays were carried out with a Micro BCA kit (Thermo Scientific). To determine the detergent extraction efficiency, membrane samples containing MelB$_{St}$ (a final protein concentration was 10 mg.mL^{-1}) were incubated with a solubilization buffer (50 mM sodium phosphate, pH 7.5, 200 mM NaCl, 10% glycerol, 20 mM melibiose) and 1.5 wt% of DDM or individual VEGs (VEG-1, VEG-2, VEG-3, VEG-4 and VEG-5) at two different temperatures (0 and 23 °C) for 90 min. Following ultracentrifugation at 355,590 g in a Beckman Optima™ MAX Ultracentrifuge using a TLA-100 rotor for 30 min at 4 °C, 20 µg of each protein sample was separated by SDS-15% PAGE, followed by immunoblotting with a Penta-His-HRP antibody (Qiagen). To assess the thermostability of MelB$_{St}$ in various detergents, the membrane extracts at 23 °C by individual detergents was thermally treated at three different temperatures (45, 55 and 65 °C) for another 90 min, and analyzed by SDS-15% PAGE and Western blotting following ultracentrifugation. MelB$_{St}$ was detected using SuperSignal West Pico chemiluminescent substrate by

the ImageQuant LAS 4000 Biomolecular Imager (GE Healthcare Life Science).

β_2AR stability assay

Long-term stability measurement: β_2AR purified 0.1% DDM was diluted into buffer solutions containing DDM, or individual VEGs (VEG-1, VEG-2, VEG-3, and VEG-5) to reach a final detergent concentration of CMC + 0.2 wt%. β_2AR in each detergent was stored for 10 days at room temperature and its ligand binding capacity was measured at regular intervals by incubating with 10 nM of radioactive [^{3}H]-dihydroalprenolol (DHA) for 30 min at room temperature. The mixture was loaded onto a G-50 column and the flow-through collected with a certain amount of binding buffer (20 mM HEPES pH 7.5, 100 mM NaCl, supplemented with 0.5 mg/ml BSA), and 15 ml scintillation fluid added. Receptor-bound [^{3}H]-DHA was measured with a scintillation counter (Beckman). The binding capacity of each VEG-solubilized β_2AR for [^{3}H]-DHA was expressed as a column graph.

Purification and stability measurement on β_2AR-G_s complex in VEG-3: 100 μM β_2AR in 0.1% DDM was mixed with 120 μM G_s heterotrimer for 30 min at room temperature. 0.5 unit apyrase (NEB) and 2 mM $MgCl_2$ were added to facilitate complex formation and the solution was further incubated for another 1 hr. 1.0% VEG-3 was then added to give a final concentration of 0.2% and the resulting sample was incubated for 30 min to initiate detergent exchange from DDM to VEG-3. The protein solution was loaded onto an M1 Flag column, washed with a series of buffers with different molar ratios of 0.1% DDM buffer to 0.5% VEG-3 buffer to allow complete detergent exchange from DDM to VEG-3 and the receptor-G_s complex was finally eluted with 0.05% VEG-3 buffer. A preparative gel filtration was carried out to purify the β_2AR-G_s complex with running buffer (20mM HEPES pH 7.5, 100 mM NaCl, 0.005% VEG-3, 1 μM BI, 100 μM TCEP). To measure the stability of the β_2AR-G_s complex in VEG-3, analytical gel filtrations were performed following 3 and 10 day-incubations) in a detergent-free condition (i.e., with the same formulation of running buffer as above-mentioned, but with no detergent.

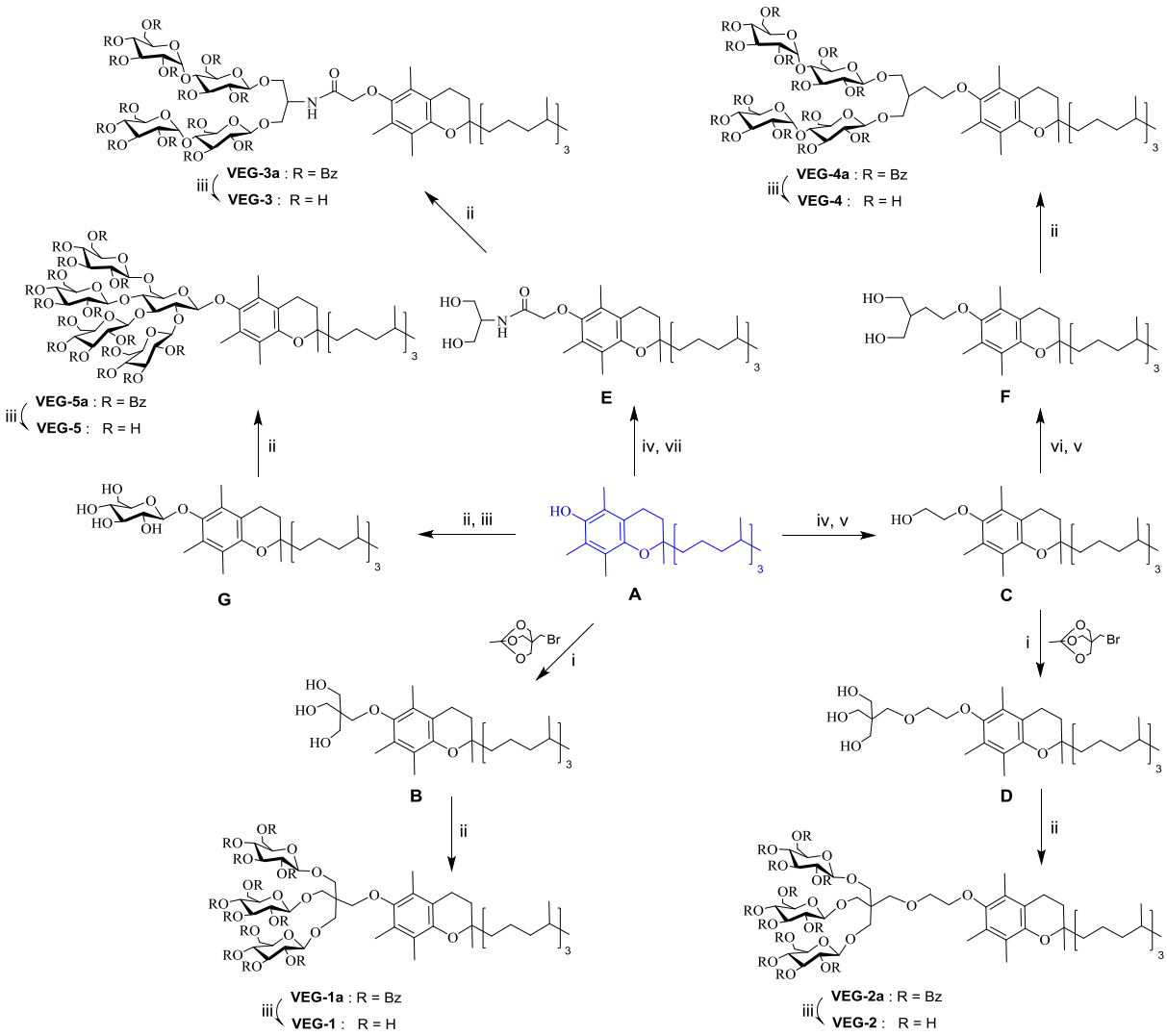
Negative stain EM analysis of β_2AR-G_s solubilized in VEG-3: β_2AR-G_s was prepared for electron microscopy using the conventional negative staining protocol,[5] and imaged at room temperature with a Tecnai T12 electron microscope operated at 120 kV using low-dose procedures. Images were recorded at a magnification of 71,138x and a defocus value of ~1.4 μm on a Gatan US4000 CCD camera. All images were binned (2x2 pixels) to obtain a pixel size of 4.16 Å at the specimen level. Particles were manually excised using e2boxer (part of the EMAN2 software suite).[6] 2D reference-free alignment and classification of particle projections was performed using ISAC.[7] 10377 projections of β_2AR-G_s were subjected to ISAC, producing 149 classes consistent over two-way matching and accounting for 10217 particle projections.

LeuT stability assay

Purification of the wild type of the leucine transporter (LeuT) from *Aquifex aeolicus* was performed according to the protocol described previousely.[8] LeuT was expressed in *E. coli* C41(DE3) transformed with pET16b encoding C-terminally 8xHis-tagged transporter (expression plasmid was kindly provided by Dr. E. Gouaux, Vollum Institute, Portland, Oregon, USA). After isolation of

bacterial membranes, the protein was solubilized by treatment of 1.0% DDM. The DDM-solubilized protein was bound to Ni^{2+}-NTA resin (Life Technologies, Denmark) and eluted in 20 mM Tris-HCl (pH 8.0), 1 mM NaCl, 199 mM KCl, 0.05% DDM and 300 mM imidazole. Subsequently, approx. 1.5 mg/ml protein stock was diluted ten-fold in identical buffer without DDM and imidazole, but supplemented with a VEG (VEG-1, VEG-2, VEG-3, VEG-4, or VEG-5), or DDM (control). The final detergent concentrations were CMC + 0.04 wt%. The resulting protein samples were stored for 10 days at room temperature and after centrifugation, ligand binding activity of the transporter was measured at the indicated time points *via* scintillation proximity assay (SPA) using [^{3}H]-Leucine.[9] The assay was performed with buffer containing 450 mM NaCl and the respective VEGs at the concentrations indicated above. SPA reaction was carried out in the presence of 20 nM [^{3}H]-Leu and 1.25 mg/ml copper chelate (His-Tag) YSi beads (both from PerkinElmer, Denmark). Total [^{3}H]-Leu binding for the respective samples was measured using a MicroBeta liquid scintillation counter (PerkinElmer).

Synthetic scheme I



i) NaH, DMF:THF (1:1), 100 ℃; CH_2Cl_2:MeOH (1:1), conc. HCl, 60 ℃; ii) 2,4,6-collidine, AgOTf, perbenzoylated glucosyl/maltosyl bromide, CH_2Cl_2, -45 ℃ to RT; iii) NaOMe, MeOH, RT; iv) methyl bromoacetate, anhydrous K_2CO_3, KI, acetone, reflux; v) $LiAlH_4$, THF, RT; vi) CBr_4, PPh_3, CH_2Cl_2; diethyl malonate, NaH, EtOH, reflux; vii) serinol, anhydrous K_2CO_3, DMSO, 25 ℃.

Synthetic procedures

Compound B synthesis: This reaction was carried out according to a literature method[10] with some modifications. Briefly, a mixture of vitamin E (compound **A**; DL-*α*-tocopherol) (1.0 equiv.) was treated with NaH (3.0 equiv.) in DMF (12 ml) and the reaction mixture was left under vigorous stirring for 15 mins at room temperature. And then a 4-(bromomethyl)-methyl-2,6,7-trioxabicyclo[2.2.2]-octane (1.8 equiv.) dissolved in THF (12 ml) was added dropwise to the reaction mixture. The resulting mixture was heated at 100 ℃ for 24 hours under nitrogen. After quenching with methanol, organic solvents were removed under reduced pressure. The solid residue was dissolved in CH_2Cl_2 and the organic solution was washed with brine and dried over anhydrous

Na_2SO_4. After concentration of the organic solvent, the residue was dissolved in CH_2Cl_2/MeOH mixture. To this solution was added a few drops of conc. HCl and the resulting mixture was heated at 50 ℃ for 4 hours. After neutralization with NaOH and concentration of the reaction mixture, the residue was purified by column chromatography (EtOAc/hexane) to afford the desired product (**B**) in 78 % yield (two steps). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.92 (s, 6H), 3.66 (s, 2H), 3.05 (br s, 3H), 2.56 (t, *J* = 6.8 Hz, 2H), 2.16 (s, 3H), 2.12 (s, 3H), 2.07 (s, 3H), 1.88-1.72 (m, 2H), 1.54-1.50 (m, 3H), 1.43-1.05 (m, 21H), 0.88-0.84 (m, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 148.4, 147.1, 127.7, 125.8, 123.4, 118.0, 75.1, 64.8, 45.4, 40.2, 40.1, 39.6, 37.7, 37.6, 37.5, 32.9, 32.8, 31.4, 28.1, 25.0, 24.6, 24.0, 22.9, 22.8, 21.3, 20.8, 19.9, 19.8, 12.8, 12.0, 11.9; **HRMS (FAB$^+$)**: calcd. for $C_{34}H_{60}O_5$ $[M+H]^+$ 549.4519, observed 549.4516.

Compound C synthesis: A mixture of Vitamin E (compound **A;** DL-*α*-tocopherol) (16 mmol), methyl bromoacetate (22 mmol), anhydrous K_2CO_3 (35 mmol), KI (8 mmol) in anhydrous acetone was stirred under argon atmosphere and refluxed for overnight. After removing the solvent, the residue was dissolved in CH_2Cl_2 and extracted with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. After complete evaporation of the solvent, $LiAlH_4$ (14.0 mmol) was added slowly to the residue dissolved in THF at 0 ℃. The mixture was stirred at room temperature for 4 hours, quenched with MeOH, water, a 1.0 N aqueous HCl solution successively at 0 ℃ and then extracted with CH_2Cl_2 twice. The combined organic layer was washed with brine and dried with anhydrous Na_2SO_4. The residue was purified by silica gel column chromatography (EtOAc/hexane) providing a desired compound (**C**) in 85 % yield (two steps). The ^{1}H and ^{13}C NMR spectra of the product were consistent with reported ones.[11]

Compound D synthesis: The compound **D** was prepared following the synthetic procedure for compound B using compound C as a starting material (80 % yield; two steps). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.78-3.76 (m, 2H), 3.70 (s, 6H), 3.60 (s, 2H), 3.50 (s, 2H), 2.55 (t, *J* = 6.8 Hz, 2H), 2.15 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 1.88-1.72 (m, 2H), 1.54-1.50 (m, 3H), 1.43-1.05 (m, 21H), 0.87-0.83 (m, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 148.1, 147.7, 127.8, 125.9, 123.1, 117.8, 75.0, 73.1. 72.0, 71.2, 64.1, 45.4, 40.4, 40.3, 39.5, 37.7, 37.6, 37.5, 37.4, 33.0, 32.9, 32.8, 31.4, 31.3, 28.1, 25.0, 24.9, 24.6, 24.0, 22.9, 22.8, 21.2, 20.8, 20.0, 19.9, 19.8, 19.7, 19.6, 12.8, 11.9; **HRMS (FAB$^+$)**: calcd. for $C_{36}H_{64}O_6$ $[M+H]^+$ 593.4781, observed 593.4776.

Compound E synthesis: A mixture of Vitamin E (compound **A**; DL-*α*-tocopherol) (16 mmol), methyl bromoacetate (22 mmol), anhydrous K_2CO_3 (35 mmol), KI (8 mmol) in anhydrous acetone was stirred under argon atmosphere and refluxed overnight. After removing the solvent, the residue was dissolved in CH_2Cl_2 and extracted with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to provide a crude colorless oil. The crude oil was treated with serinol (25 mmol) and anhydrous K_2CO_3 (35 mmol) dissolved in redistilled Me_2SO (20 mL) and stirred at 25 ℃ for 6 hours. The reaction mixture was diluted with water and then extracted with Et_2O. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. After complete evaporation of the solvent, the residue was purified by flash column chromatography (EtOAc/hexane) providing desired product (**E**) as a white solid in 85 % yield. **^{1}H NMR** (400 MHz,

$CDCl_3$): δ 7.68 (d, *J* = 8.0 Hz, 1H), 4.20 (br s, 2H), 4.09-4.01 (m, 1H), 3.88-3.85 (m, 2H), 3.78-3.75 (m, 2H), 2.55 (t, *J* = 6.8 Hz, 2H), 2.15 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 1.88-1.72 (m, 2H), 1.54-1.50 (m, 3H), 1.43-1.05 (m, 21H), 0.87-0.83 (m, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.3, 148.5, 146.8, 127.4, 125.5, 123.3, 117.8, 75.0, 71.1, 61.8, 52.3, 40.3, 40.2, 39.5, 37.7, 37.6, 37.6, 37.4, 32.9, 32.8, 31.2, 28.1, 24.9, 24.6, 23.8, 22.8, 22.7, 21.1, 20.7, 20.0, 19.8, 19.7, 19.6, 12.8, 11.9; **HRMS (FAB$^+$)**: calcd. for $C_{34}H_{59}NO_5$ $[M+H]^+$ 562.4471, observed 562.4474.

Compound F synthesis: A mixture of compound **C** (1.0 equiv), PPh_3 (1.5 equiv.), and CBr_4 (1.2 equiv.) in dry CH_2Cl_2 was stirred at room temperature for 4 hours under Ar. The solution was washed with $NaHCO_3$ and brine, dried over Na_2SO_4 and filtered. The filtrate was evaporated to give a crude product, which was used in the next step without further purification. This crude product was added to a stirred solution of diethyl malonate (1.0 equiv.) and NaH (1.0 equiv.) dissolved in ethanol. The resulting mixture was heated at reflux for 4 hours. Upon cooling to room temperature, water was added and the product was extracted into Et_2O, dried over Na_2SO_4 and the solvent was removed by rotary evaporation to afford the crude product which was further treated with $LiAlH_4$ (3.5 equiv.) in dry THF for 4 hours at room temperature. After completion of reaction, water was added dropwise into the solution and was extracted with CH_2Cl_2 twice. The combined extract was washed with 1.0 M HCl and brine, dried over Na_2SO_4 and concentrated in *vacuo*. The residue was purified by silica gel column chromatography (EtOAc/hexane) providing a desired product (**F**) in 80 % yield (two steps). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.83-3.78 (m, 2H), 3.75-3.70 (m, 4H), 3.05 (br s, 2H), 2.56 (t, *J* = 6.8 Hz, 2H), 2.15 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 1.88-1.76 (m, 2H), 1.74-1.70 (m, 2H), 1.69-1.67 (m, 1H), 1.54-1.50 (m, 3H), 1.43-1.05 (m, 21H), 0.87-0.83 (m, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 148.2, 148.1, 127.8, 125.9, 123.4, 117.7, 75.0, 71.3, 65.7, 40.5, 40.3, 40.2, 39.5, 37.7, 37.6, 37.5, 37.4, 33.0, 32.9, 32.8, 31.4, 31.3, 29.1, 28.1, 25.0, 24.6, 24.0, 22.9, 22.8, 21.2, 20.8, 19.9, 19.8, 19.7, 19.6, 13.0, 12.2, 12.0; **HRMS (FAB$^+$)**: calcd. for $C_{34}H_{60}O_4$ $[M+H]^+$ 532.4492, observed 532.4494.

General procedure for glycosylation

This reaction was carried out according to a literature method[12] with some modifications. Briefly, a mixture of an alcohol (compound **A**, **B**, **D**, **E**, **F**, or **G**[13]), AgOTf, 2,4,6-collidine in anhydrous CH_2Cl_2 was stirred at -45 ℃. Then perbenzoylated glucosylbromide/perbenzoylated maltosylbromide in CH_2Cl_2 was transferred via cannula to a solution over time of 0.5 hours. The reaction was left to warm to 0 ℃ for 1.5 hours. The reaction was monitored by TLC. After completion of reaction (as detected by TLC), pyridine was added to the reaction mixture. The reaction mixture was diluted with CH_2Cl_2 before being filtered over celite. The filtrate was washed successively with a 1.0 M aqueous $Na_2S_2O_3$ solution, a 0.1 M aqueous HCl solution, and brine. Then the organic layer was dried with anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation. The residue was purified by silica gel column chromatography (EtOAc/hexane) providing a desired product as a glassy solid.

VEG-1a was prepared in 80% yield according to the general procedure for glycosylation reactions. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.10-7.83 (m, 18H), 7.62-7.15 (m, 42H), 5.66 (t, *J* = 9.6 Hz, 3H), 5.56 (t, *J* = 9.6 Hz, 3H), 5.41 (t, *J* = 8.0 Hz, 3H), 4.42-4.39 (m, 3H), 4.33-4.30 (m, 3H), 4.12 (d, *J* = 8.0 Hz, 3H), 4.02 (d, *J* = 8.0 Hz, 3H), 3.73 (d, *J* = 8.0 Hz, 1H), 3.54 (d, *J* = 8.0 Hz, 1H), 3.34 (m, 6H), 2.56 (t,

J = 6.8 Hz, 2H), 2.16 (s, 3H), 2.12 (s, 3H), 2.07 (s, 3H), 1.88-1.72 (m, 2H), 1.54-1.50 (m, 3H), 1.43-1.05 (m, 21H), 0.88-0.84 (m, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.1, 165.9, 165.1, 164.7, 147.7, 147.3, 133.6, 133.5, 133.3, 133.1, 130.0, 129.8, 129.6, 129.1, 128.9, 128.8, 128.6, 128.5, 128.4, 128.1, 126.1, 122.5, 117.3, 101.23, 74.6, 72.6, 71.9, 71.8, 69.5, 68.1, 62.9, 45.0, 39.4, 37.7, 37.5, 37.4, 32.8, 28.0, 24.9, 24.5, 23.8, 22.8, 22.7, 21.2, 19.9, 12.45, 11.8, 12.4, 11.8, 11.6.

VEG-2a was prepared in 85% yield according to the general procedure for glycosylation reactions. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.10-7.80 (m, 18H), 7.60-7.10 (m, 42H), 5.66 (t, *J* = 9.6 Hz, 3H), 5.56 (t, *J* = 9.6 Hz, 3H), 5.41 (t, *J* = 8.0 Hz, 3H), 4.42-4.39 (m, 3H), 4.16-4.14 (m, 3H), 3.88 (d, *J* = 8.0 Hz, 3H), 3.64 (d, *J* = 8.0 Hz, 3H), 3.36-3.46 (m, 4H), 3.38-3.25 (m, 4H), 3.22-3.15 (m, 4H), 2.55 (t, *J* = 6.8 Hz, 2H), 2.15 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 1.88-1.72 (m, 2H), 1.54-1.50 (m, 3H), 1.43-1.05 (m, 21H), 0.87-0.83 (m, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.1, 165.8, 165.1, 164.8, 147.9, 147.7, 133.6, 133.4, 133.3, 133.1, 130.1, 129.8, 129.7, 129.6, 129.1, 129.0, 128.9, 128.5, 128.4, 128.3, 128.0, 127.9, 126.0, 122.8, 117.6, 101.4, 74.8, 72.7, 72.0, 71.6, 70.7, 69.7, 67.6, 63.0, 45.3, 40.4, 39.5, 37.7, 37.6, 37.5, 37.4, 37.3, 32.9, 32.8, 31.4, 28.0, 25.0, 24.5, 23.8, 23.7, 22.8, 22.7, 21.7, 20.7, 19.9, 19.7, 12.8, 12.0, 11.9.

VEG-3a was prepared in 80% yield according to the general procedure for glycosylation reactions. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.09-7.93 (m, 14H), 7.89-7.86 (m, 4H), 7.82-7.80 (m, 4H), 7.76-7.71 (m, 4H), 7.64-7.58 (m, 2H), 7.53-7.16 (m, 42H), 6.18 (t, *J* = 8.0 Hz, 2H), 5.73-5.64 (m, 4H), 5.40-5.33 (m, 2H), 5.21-5.13 (m, 4H), 4.72-4.56 (m, 4H), 4.39-4.10 (m, 10H), 3.85-3.80 (m, 2H), 3.39 (d, *J* = 8.0 Hz, 1H), 3.33 (t, *J* = 8.0 Hz, 2H), 3.07 (d, *J* = 8.0 Hz, 2H), 2.91 (t, *J* = 8.0 Hz, 1H), 2.51 (t, *J* = 6.8 Hz, 2H), 2.14 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 1.86-1.72 (m, 2H), 1.54-1.50 (m, 3H), 1.43-1.05 (m, 21H), 0.87-0.84 (m, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 168.7, 166.2, 166.1, 166.0, 165.9, 165.8, 165.6, 165.5, 165.1, 165.0, 164.9, 164.8, 148.4, 147.1, 134.0, 133.7, 133.6, 133.5, 133.4, 130.1, 130.0, 129.9, 129.8, 129.7, 129.5, 129.3, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.3, 127.5, 125.6, 123.2, 117.7, 100.9, 95.7, 74.9, 72.0, 71.4, 69.8, 69.1, 69.0, 62.6, 39.4, 37.6, 37.5, 37.4, 37.3, 32.8, 28.0, 24.9, 24.5, 24.0, 23.8, 22.8, 22.7, 21.1, 19.9, 19.8, 12.0, 11.9.

VEG-4a was prepared in 78% yield according to the general procedure for glycosylation reactions. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.11 (d, *J* = 8.0 Hz, 2H), 8.05-7.93 (m, 12H), 7.87-7.85 (m, 6H), 7.81-7.78 (m, 2H), 7.74-7.72 (m, 6H), 7.53-7.18 (m, 42H), 6.15 (t, *J* = 8.0 Hz, 2H), 5.81 (t, *J* = 4.0 Hz, 2H), 5.71-5.62 (m, 4H), 5.37-5.24 (m, 4H), 5.19-5.09 (m, 2H), 4.77-4.08 (m, 12H), 3.74-3.72 (m, 2H), 3.60-3.44 (m, 4H), 3.30-3.27 (m, 2H), 3.16 (d, *J* = 4.0 Hz, 1H), 3.05 (d, *J* = 8.0 Hz, 1H), 2.99 (d, *J* = 8.0 Hz, 1H), 2.85 (d, *J* = 8.0 Hz, 1H), 2.53 (t, *J* = 6.8 Hz, 2H), 2.15 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 1.88-1.76 (m, 2H), 1.74-1.70 (m, 2H), 1.69-1.67 (m, 1H), 1.54-1.50 (m, 3H), 1.43-1.05 (m, 21H), 0.88-0.84 (m, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.2, 166.1, 165.9, 165.8, 165.5, 165.2, 165.1, 164.0, 164.9, 148.3, 147.6, 133.7, 133.6, 133.5, 133.4, 133.3, 133.2, 130.1, 130.0, 129.9, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 127.8, 125.8, 122.7, 117.5, 74.7, 72.3, 72.1. 71.3, 70.2, 69.8, 69.1, 69.0, 39.4, 37.5, 37.4, 37.3, 32.8, 32.7, 28.0, 25.0, 24.5, 23.9, 22.8, 22.7, 21.1, 20.6, 20.0, 19.8, 12.8, 12.0, 11.8.

VEG-5a was prepared in 70% yield according to the general procedure for glycosylation reactions.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (d, *J* = 8.0 Hz, 2H), 8.15-7.80 (m, 24H), 7.72-6.67 (m, 5H), 7.66-7.59 (m, 3H), 7.58-7.12 (m, 46H), 5.90-5.75 (m, 4H), 5.71-4.45 (m, 7H), 5.34 (t, *J* = 8.0 Hz, 1H), 4.95 (d, *J* = 8.0 Hz, 1H), 4.82-4.75 (m, 4H), 4.62-4.56 (m, 3H), 4.47-4.35 (m, 4H), 4.16-4.08 (m, 2H), 4.04-4.01 (m, 1H), 3.92-3.85 (m, 3H), 3.80-3.68 (m, 2H), 3.02 (br s, 1H), 2.56 (t, *J* = 6.8 Hz, 2H), 2.17 (s, 3H), 2.16 (s, 3H), 2.02 (s, 3H), 1.88-1.75 (m, 2H), 1.74-1.70 (m, 2H),1.69-1.67 (m, 1H), 1.54-1.50 (m, 3H), 1.43-1.05 (m, 21H), 0.87-0.84 (m, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): 166.2, 165.9, 165.3, 165.2, 165.1, 133.7, 133.5, 133.2, 130.2, 129.9, 129.8, 129.7, 129.6, 129.5, 129.3, 129.0, 128.8, 128.6, 128.5, 128.4, 128.0, 122.4, 74.8, 74.6, 74.2, 72.5, 72.4, 72.3, 72.1, 69.8, 69.1, 39.5, 37.6, 37.5, 32.9, 28.1, 25.0, 24.6, 22.9, 22.8, 19.9, 19.8, 14.2, 13.3, 12.1, 11.9.

General procedure for de-O-benzoylation under Zemplén's condition

A glycosylated product was dissolved in MeOH and then treated with the required amount of a methanolic solution of 0.5 M NaOMe such that the final concentration of NaOMe was 0.05 M. The reaction mixture was left stirring for 6 hours at room temperature, and then neutralized with amberlite IR-120 (H^+ form) resin. The resin was removed by filtration and washed with MeOH, and solvent was removed from the combined filtrate *in vacuo*. The residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$) to give a product as a white solid.

VEG-1 was synthesized according to the general procedure for de-*O*-benzoylation. Yield: 92%; **^{1}H NMR** (400 MHz, CD_3OD): δ 4.33 (d, *J* = 8.0 Hz, 3H), 4.17 (d, *J* = 8.0 Hz, 3H), 3.83-3.74 (m, 8H), 3.61-3.59 (m, 5H), 3.34 (t, *J* = 8.0 Hz, 3H), 3.25-3.22 (m, 8H), 3.17 (t, *J* = 8.0 Hz, 4H), 2.50 (t, *J* = 8.0 Hz, 2H), 2.11 (s, 3H), 2.07 (s, 3H), 1.97 (s, 3H), 1.71-1.66 (m, 2H), 1.49-1.32 (m, 8H), 1.24-1.21 (m, 8H), 1.13-1.04 (m, 11H), 0.82-0.79 (m, 12H); **^{13}C NMR** (100 MHz, CD_3OD): δ 149.0, 148.9, 129.14, 127.3, 123.7, 118.7, 104.8, 78.0, 77.8, 75.7, 75.3, 72.9, 71.8, 70.0, 62.9, 46.6, 41.0, 40.6, 38.8, 38.7, 38.6, 38.5, 38.4, 34.0, 33.9, 33.8, 32.7, 29.2, 26.0, 25.5, 24.2, 24.1, 23.3, 23.2, 22.1, 21.7, 20.4, 20.3, 13.4, 12.5, 12.2; **HRMS (FAB$^+$)**: calcd. for $C_{52}H_{90}O_{20}$ $[M+Na]^+$ 1057.5923, observed 1057.5920.

VEG-2 was synthesized according to the general procedure for de-*O*-benzoylation. Yield: 92%; **^{1}H NMR** (400 MHz, CD_3OD): δ 4.35 (d, *J* = 8.0 Hz, 3H), 4.02 (d, *J* = 8.0 Hz, 3H), 3.83 (d, *J* = 8.0 Hz, 4H), 3.75-3.74 (m, 4H), 3.68-3.62 (m, 8H), 3.37-3.17 (m, 15H), 2.55 (t, *J* = 8.0 Hz, 2H), 2.15 (s, 3H), 2.11 (s, 3H), 2.02 (s, 3H), 1.76-1.71 (m, 2H), 1.53-1.36 (m, 8H), 1.28-1.18 (m, 12H), 1.14-1.07 (m, 7H), 0.86-0.83 (m, 12H); **^{13}C NMR** (100 MHz, CD_3OD): δ 149.2, 149.1, 128.8, 127.0, 123.8, 118.9, 105.1, 78.1, 77.8, 75.8, 75.2, 73.5, 72.0, 71.7, 70.8, 69.9, 62.8, 46.7, 41.1, 41.0, 40.6, 38.8, 38.6, 38.5, 38.4, 34.0, 33.9, 32.7, 29.2, 26.0, 25.6, 24.2, 23.3, 23.2, 22.2, 21.7, 20.4, 20.3, 13.3, 12.4, 12.2; **HRMS (FAB$^+$)**: calcd. for $C_{54}H_{94}O_{21}$ $[M+Na]^+$ 1101.6185, observed 1101.6189.

VEG-3 was synthesized according to the general procedure for de-*O*-benzoylation. Yield: 90%; **^{1}H NMR** (400 MHz, CD_3OD): δ 5.13 (d, *J* = 4.0 Hz, 2H), 4.42-4.39 (m, 1H), 4.36 (t, *J* = 8.0 Hz, 2H), 4.14 (s, 2H), 4.04-3.98 (m, 2H), 3.91-3.86 (m, 3H), 3.81-3.74 (m, 6H), 3.68-3.57 (m, 10H), 3.49 (t, *J* = 8.0 Hz, 2H), 3.44-3.38 (m, 5H), 3.32-3.23 (m, 6H), 2.56 (t, *J* = 8.0 Hz, 2H), 2.12 (s, 3H), 2.09 (s, 3H), 2.02 (s, 3H), 1.78-1.73 (m, 2H), 1.53-1.34 (m, 8H), 1.28-1.19 (m, 12H), 1.13-1.05 (m, 7H), 0.86-

0.83 (m, 12H); **^{13}C NMR** (100 MHz, CD_3OD): δ 171.8, 149.6, 148.6, 128.5, 126.8, 124.1, 119.1, 104.8, 102.9, 81.3, 77.8, 76.7, 76.0, 75.1, 74.9, 74.8, 74.2, 72.3, 71.5, 69.4, 62.8, 62.3, 50.6, 41.0, 40.6, 38.6, 38.5, 38.4, 34.0, 33.9, 33.8, 32.7, 29.2, 26.0, 25.5, 24.2, 23.3, 23.2, 22.2, 21.2, 20.8, 19.9, 19.8, 19.7, 19.6, 13.0, 12.2, 12.0; **HRMS (FAB$^+$)**: calcd. for $C_{58}H_{99}NO_{25}$ $[M+Na]^+$ 1232.6404, observed 1232.6410.

VEG-4 was synthesized according to the general procedure for de-*O*-benzoylation. Yield: 90%; **^{1}H NMR** (400 MHz, CD_3OD): δ 5.16 (d, *J* = 4.0 Hz, 2H), 4.35 (d, *J* = 8.0 Hz, 2H), 4.02-3.96 (d, *J* = 4.0 Hz, 2H), (m, 2H), 3.90-3.58 (m, 22H), 3.52 (t, *J* = 8.0 Hz, 2H), 3.45-3.42 (m, 2H), 3.39-3.33 (m, 4H), 3.30-3.23 (m, 6H), 2.56 (t, *J* = 8.0 Hz, 2H), 2.26-2.22 (m, 1H), 2.13 (s, 3H), 2.09 (s, 3H), 2.02 (s, 3H), 1.91-1.85 (m, 2H), 1.78-1.73 (m, 2H), 1.58-1.35 (m, 8H), 1.29-1.19 (m, 12H), 1.16-1.05 (m, 7H), 0.87-0.84 (m, 12H); **^{13}C NMR** (100 MHz, CD_3OD): δ 149.6, 149.0, 128.7, 126.9, 123.8, 118.8, 104.9, 104.7, 103.0, 81.4, 77.9, 76.6, 75.8, 74.8, 74.2, 72.2, 71.6, 71.3, 70.8, 62.8, 62.3, 40.9, 40.6, 38.6, 38.5, 38.4, 38.1, 38.0, 34.0, 33.9, 32.8, 30.2, 29.2, 26.0, 25.5, 24.3, 23.3, 23.2, 22.1, 21.7, 20.4, 20.3, 13.3, 12.4, 12.2; **HRMS (FAB$^+$)**: calcd. for $C_{58}H_{100}O_{24}$ $[M+Na]^+$ 1203.6502, observed 1203.6504.

VEG-5 was synthesized according to the general procedure for de-*O*-benzoylation. Yield: 88%**;** **^{1}H NMR** (400 MHz, CD_3OD): δ 4.99 (d, *J* = 8.0 Hz, 1H), 4.93 (d, *J* = 8.0 Hz, 1H), 4.72 (d, *J* = 8.0 Hz, 1H), 4.65 (d, *J* = 8.0 Hz, 1H), 4.26 (d, *J* = 8.0 Hz, 1H), 4.20-4.09 (m, 4H), 3.87-3.75 (m, 5H), 3.69-3.55 (m, 5H), 3.41-3.12 (m, 22H), 2.57 (t, *J* = 8.0 Hz, 2H), 2.20 (s, 3H), 2.17 (s, 3H), 2.01 (s, 3H), 1.78-1.73 (m, 2H), 1.52-1.36 (m, 8H), 1.28-1.18 (m, 12H), 1.12-1.07 (m, 7H), 0.85-0.82 (m, 12H); **^{13}C NMR** (100 MHz, CD_3OD): δ 149.6, 147.5, 123.7, 123.6, 118.8, 104.8, 104.5, 103.8, 103.0, 102.4, 81.4, 79.9, 78.1, 78.0, 77.9, 77.8, 75.9, 75.3, 75.2, 71.9, 71.8, 71.6, 71.5, 69.6, 62.9, 62.7, 41.1, 40.6, 38.8, 38.6, 38.5, 38.4, 34.0, 33.9, 29.2, 26.0, 25.5, 24.2, 23.3, 23.2, 22.2, 21.8, 20.4, 20.3, 20.2, 14.6, 14.5, 13.7, 12.1, 12.0; **HRMS (FAB$^+$)**: calcd. for $C_{59}H_{100}O_{27}$ $[M+Na]^+$ 1263.6350, observed 1263.6353.

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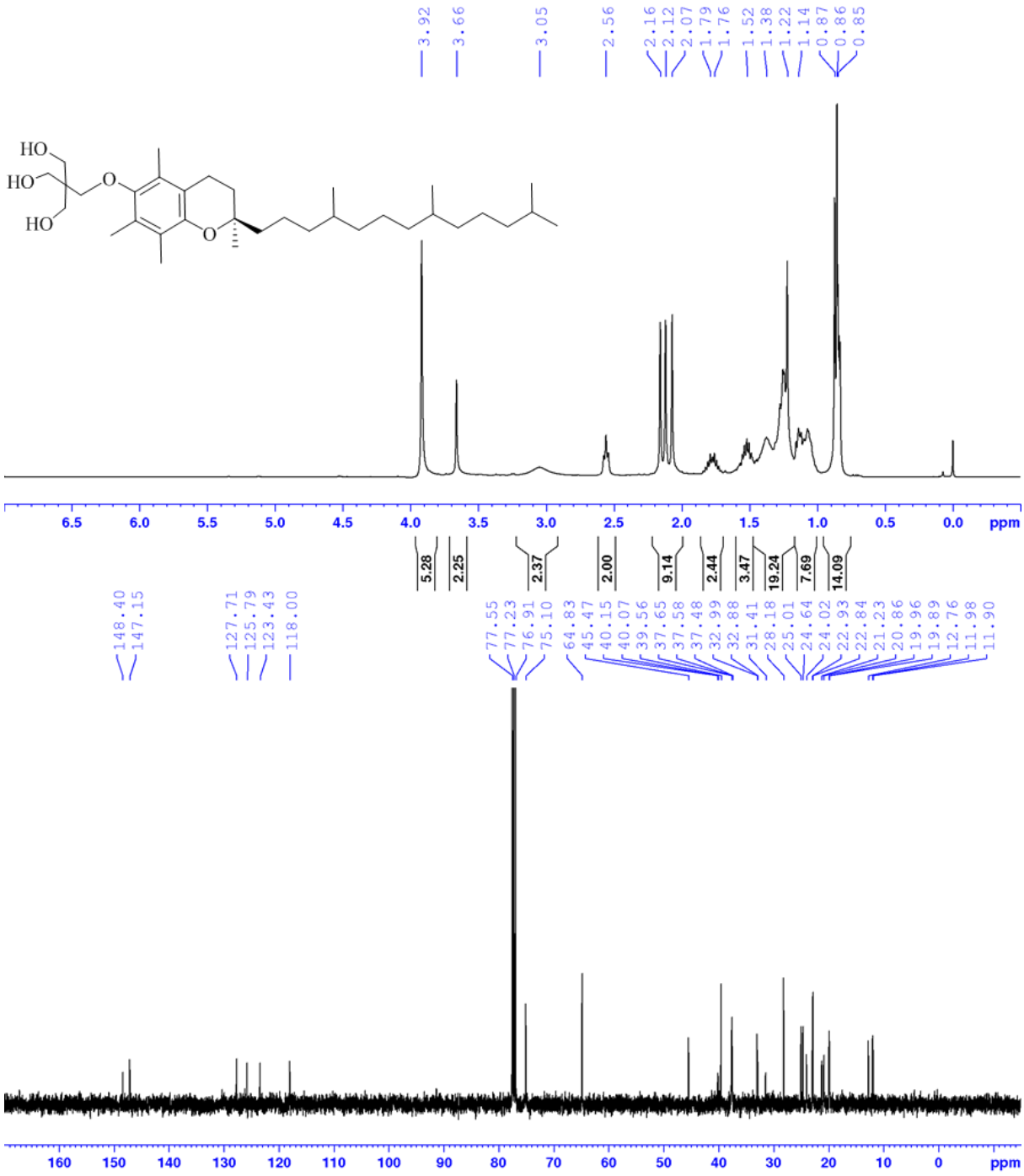

Figure S7. ^{1}H & ^{13}C NMR spectra ($CDCl_3$, 298 K) of compound **B**.

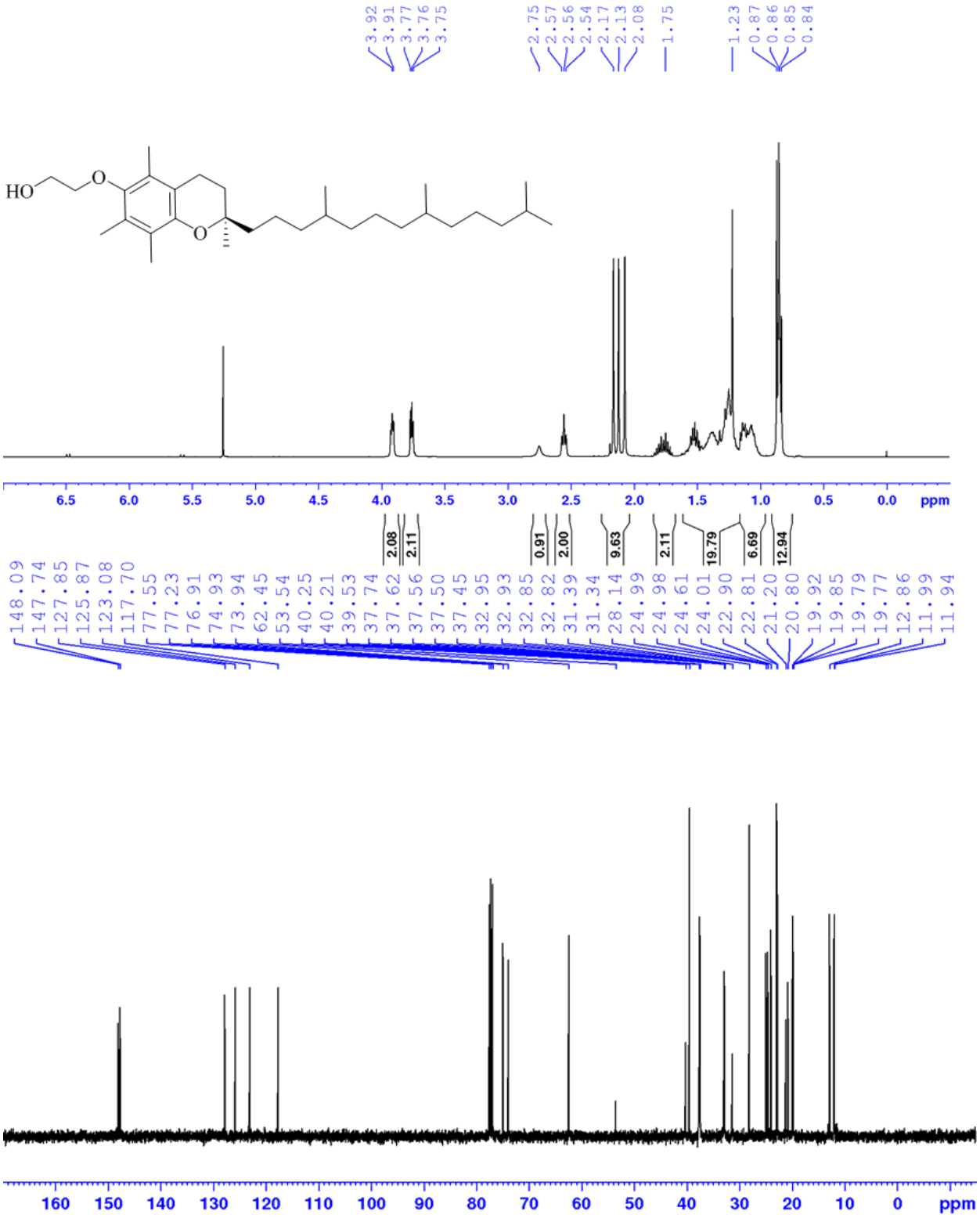

Figure S8. ^{1}H & ^{13}C NMR spectra ($CDCl_3$, 298 K) of compound **C**.

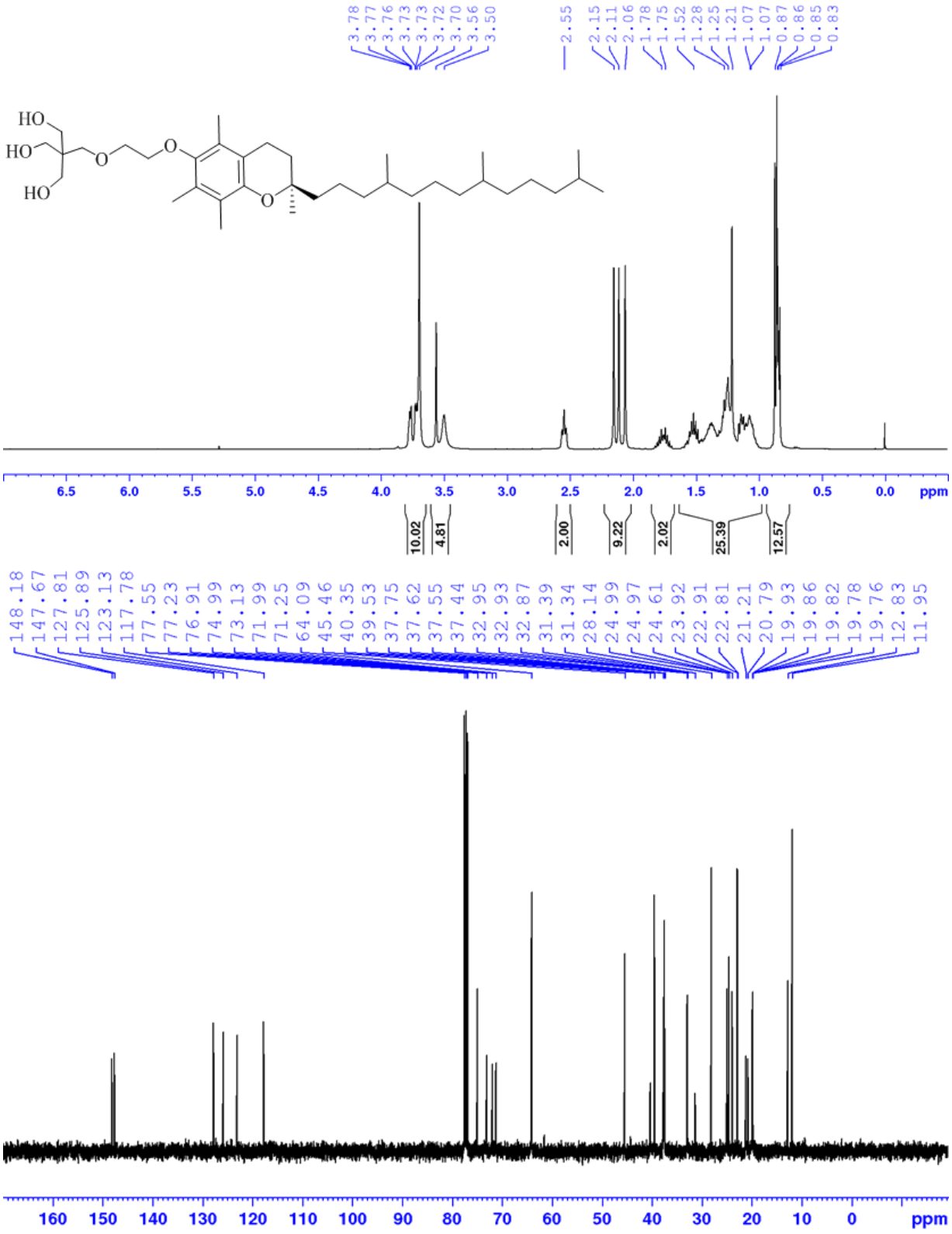

Figure S9. ^{1}H & ^{13}C NMR spectra ($CDCl_3$, 298 K) of compound **D**.

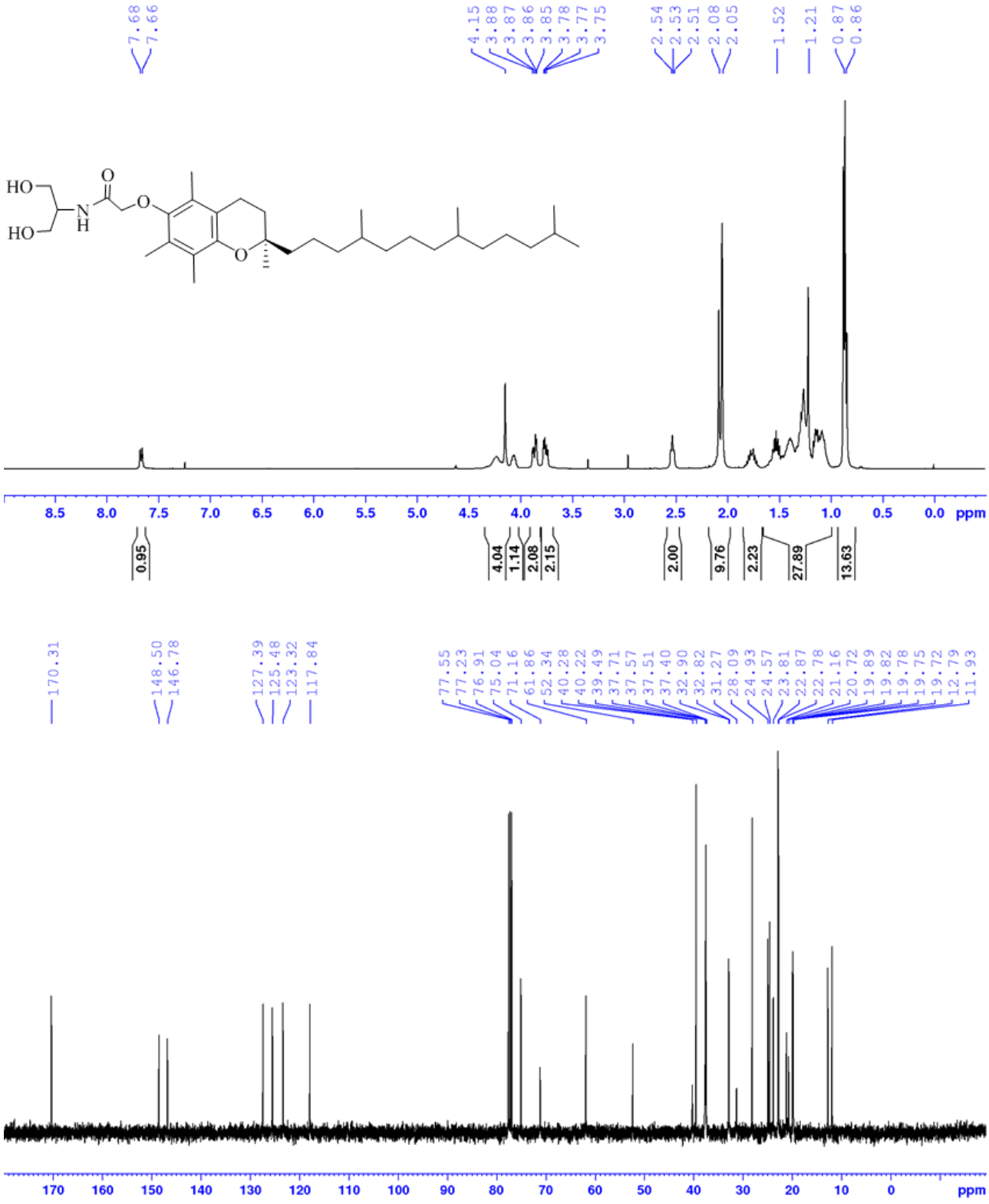

Figure S10. ^{1}H & ^{13}C NMR spectra ($CDCl_3$, 298 K) of compound **E**.

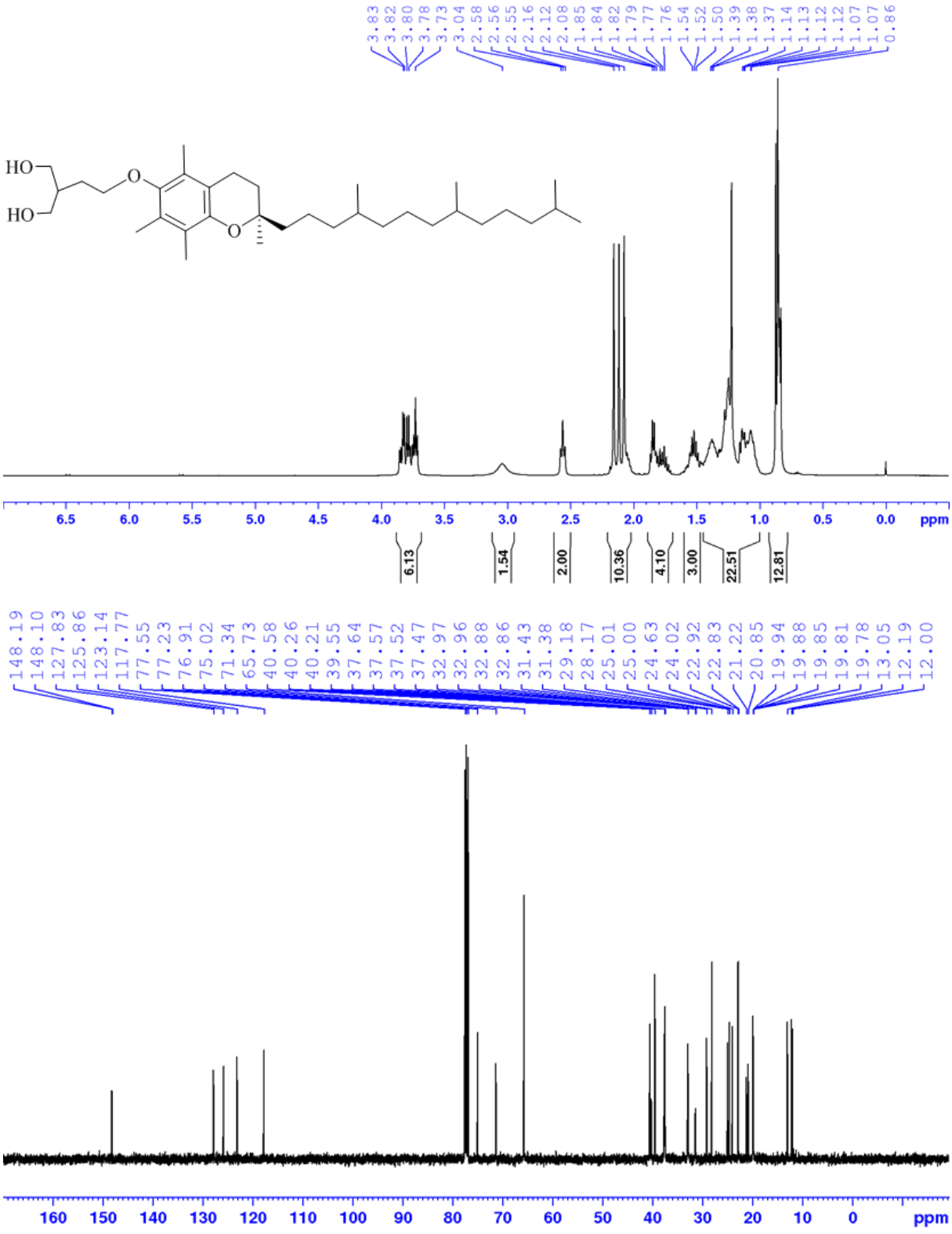

Figure S11. ^{1}H & ^{13}C NMR spectra ($CDCl_3$, 298 K) of compound **F**.

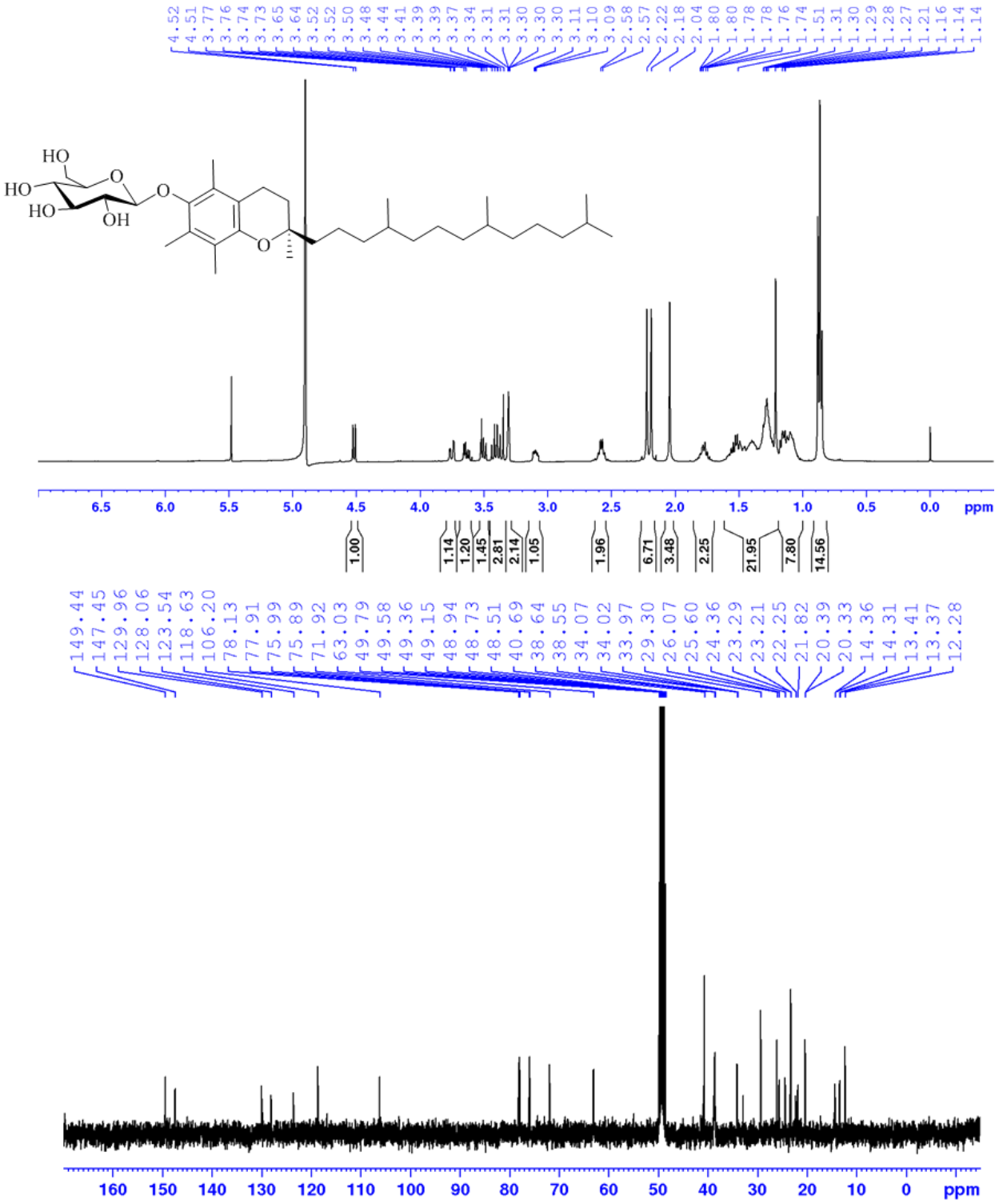

Figure S12. ^{1}H & ^{13}C NMR spectra (CD_3OD, 298 K) of compound **G**.

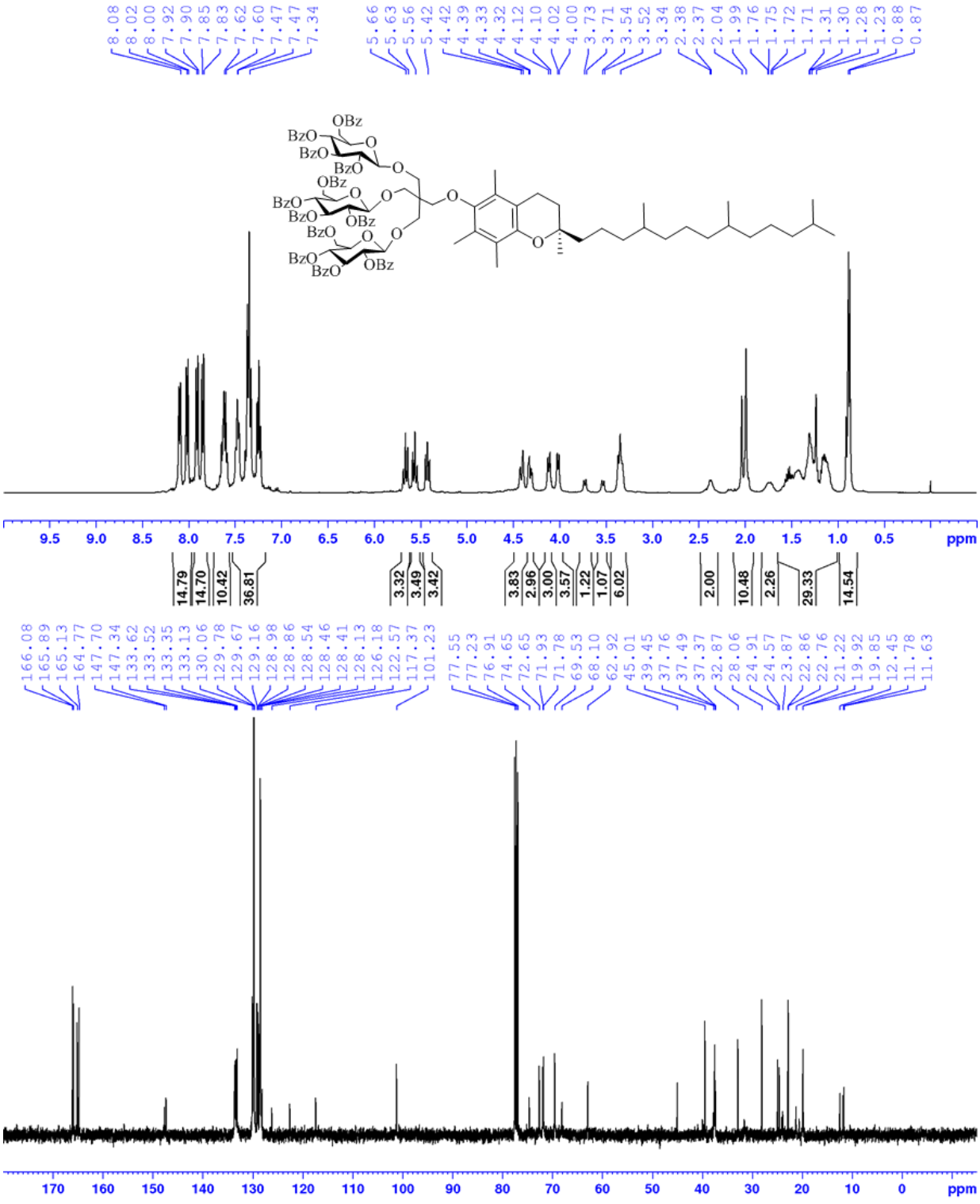

Figure S13. ^{1}H & ^{13}C NMR spectra ($CDCl_3$, 298 K) of compound **VEG-1a**.

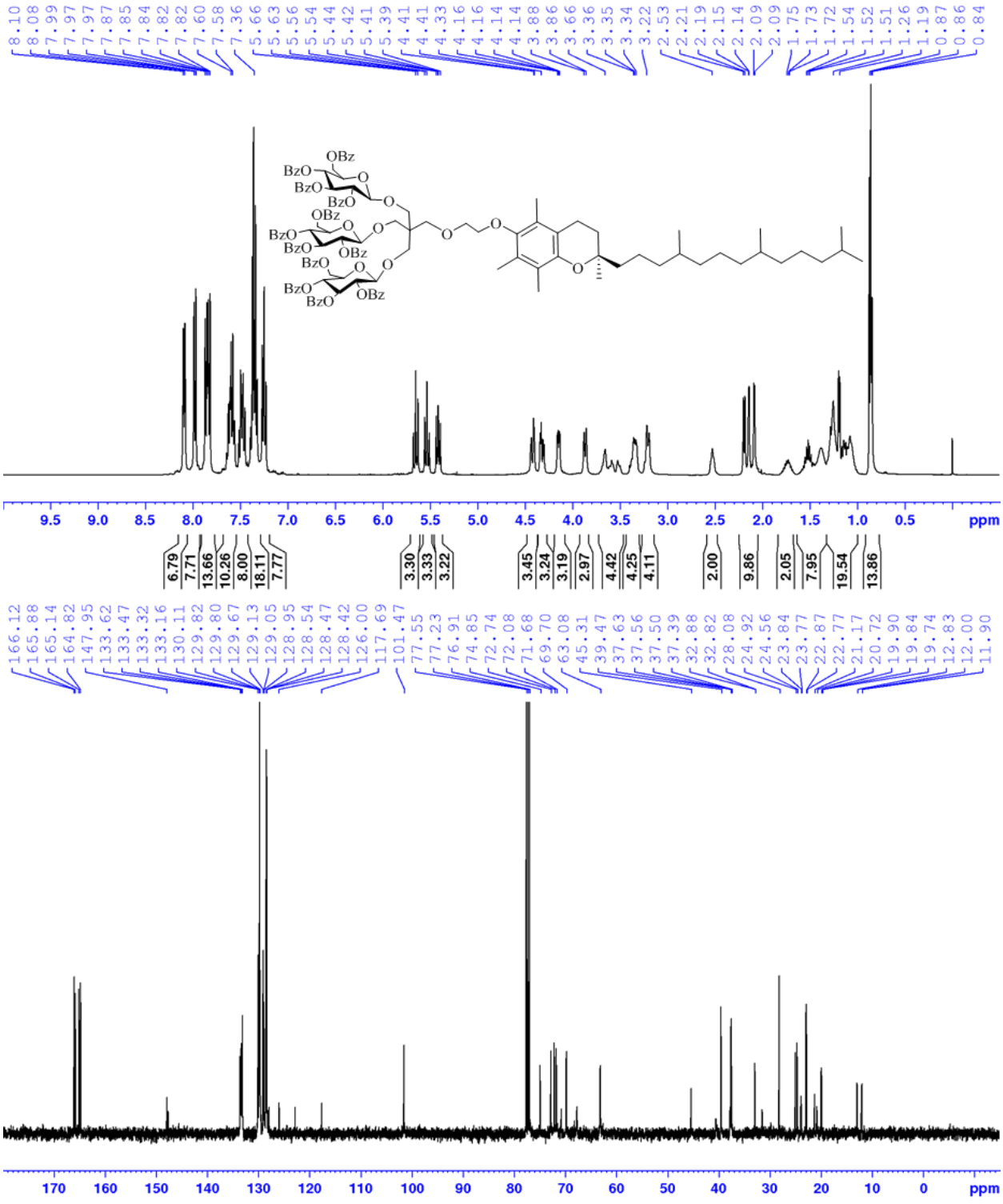

Figure S14. ^{1}H & ^{13}C NMR spectra ($CDCl_3$, 298 K) of compound **VEG-2a**.

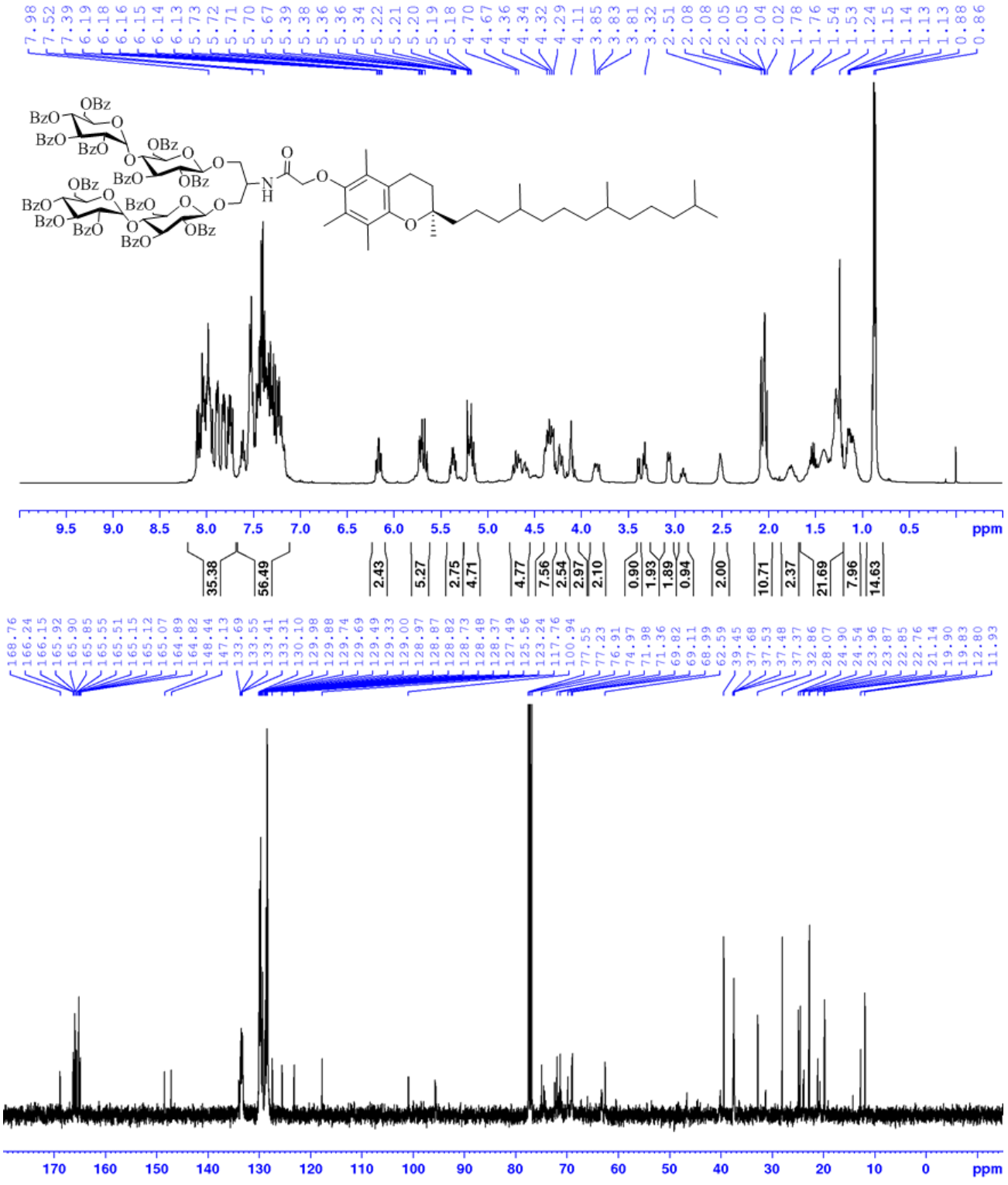

Figure S15. ^{1}H & ^{13}C NMR spectra ($CDCl_3$, 298 K) of compound **VEG-3a**.

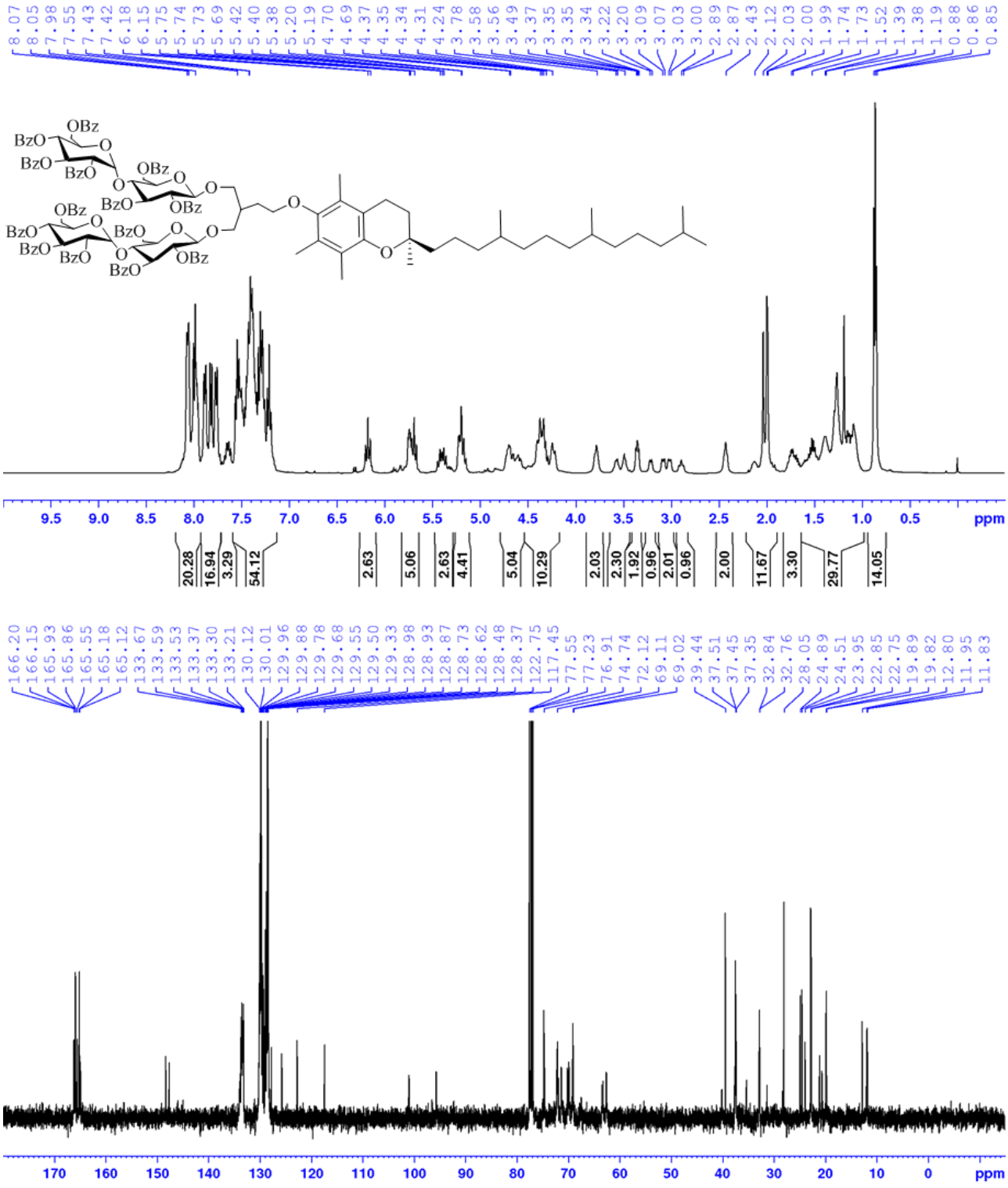

Figure S16. ^{1}H & ^{13}C NMR spectra ($CDCl_3$, 298 K) of compound **VEG-4a**.

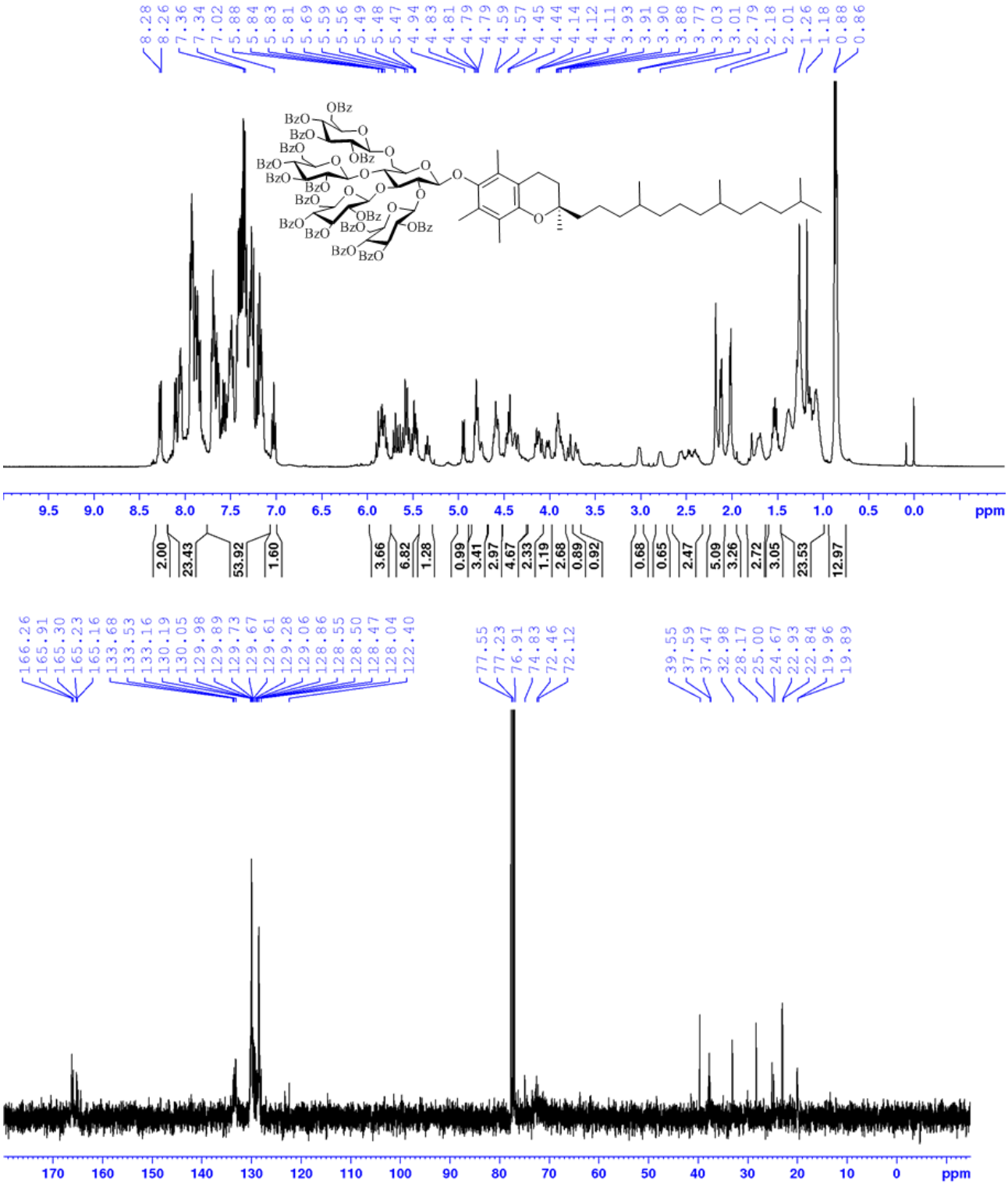

Figure S17. ^{1}H & ^{13}C NMR spectra ($CDCl_3$, 298 K) of compound **VEG-5a**

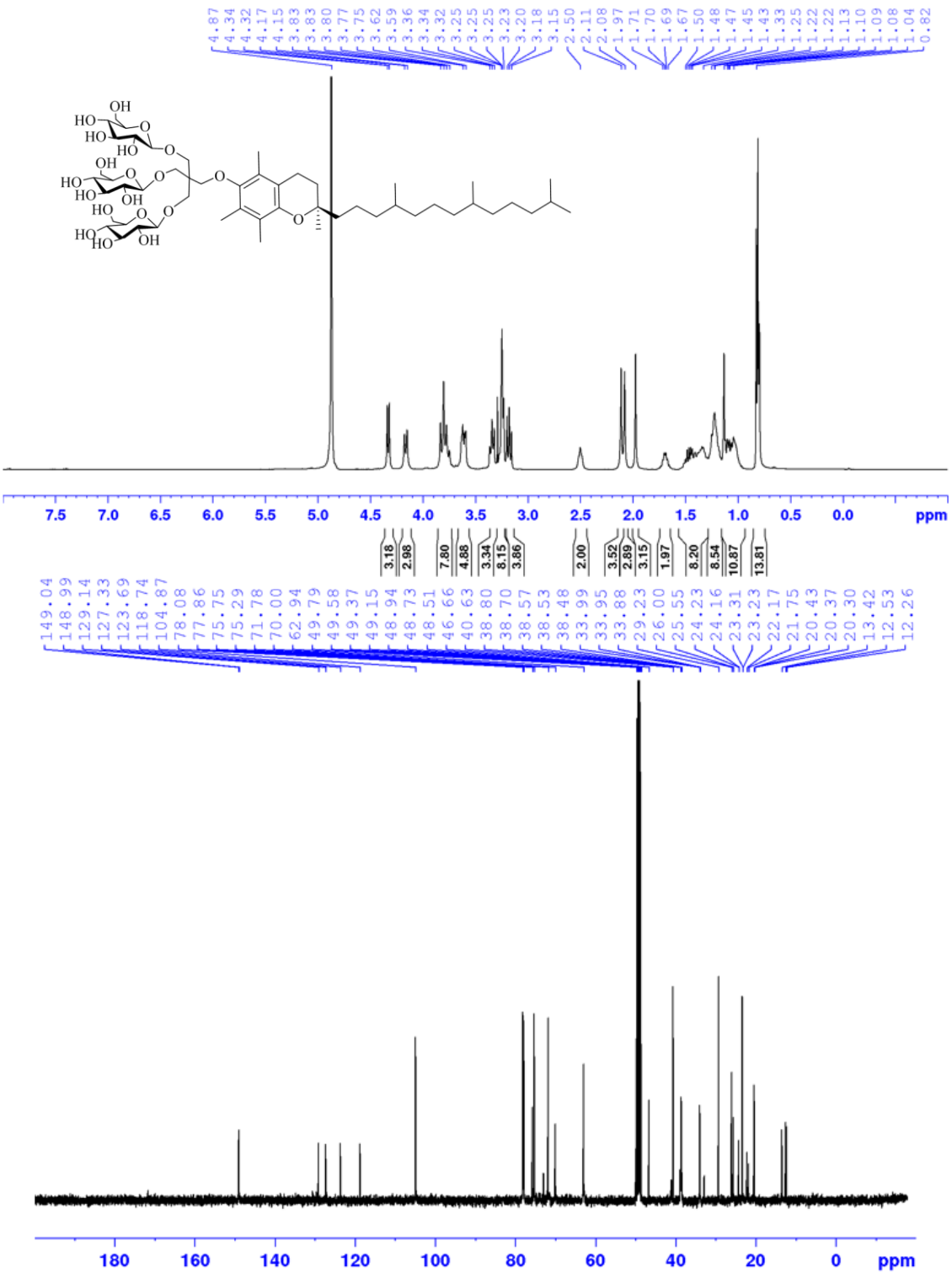

Figure S18. ^{1}H & ^{13}C NMR spectra (CD_3OD, 298 K) of **VEG-1**.

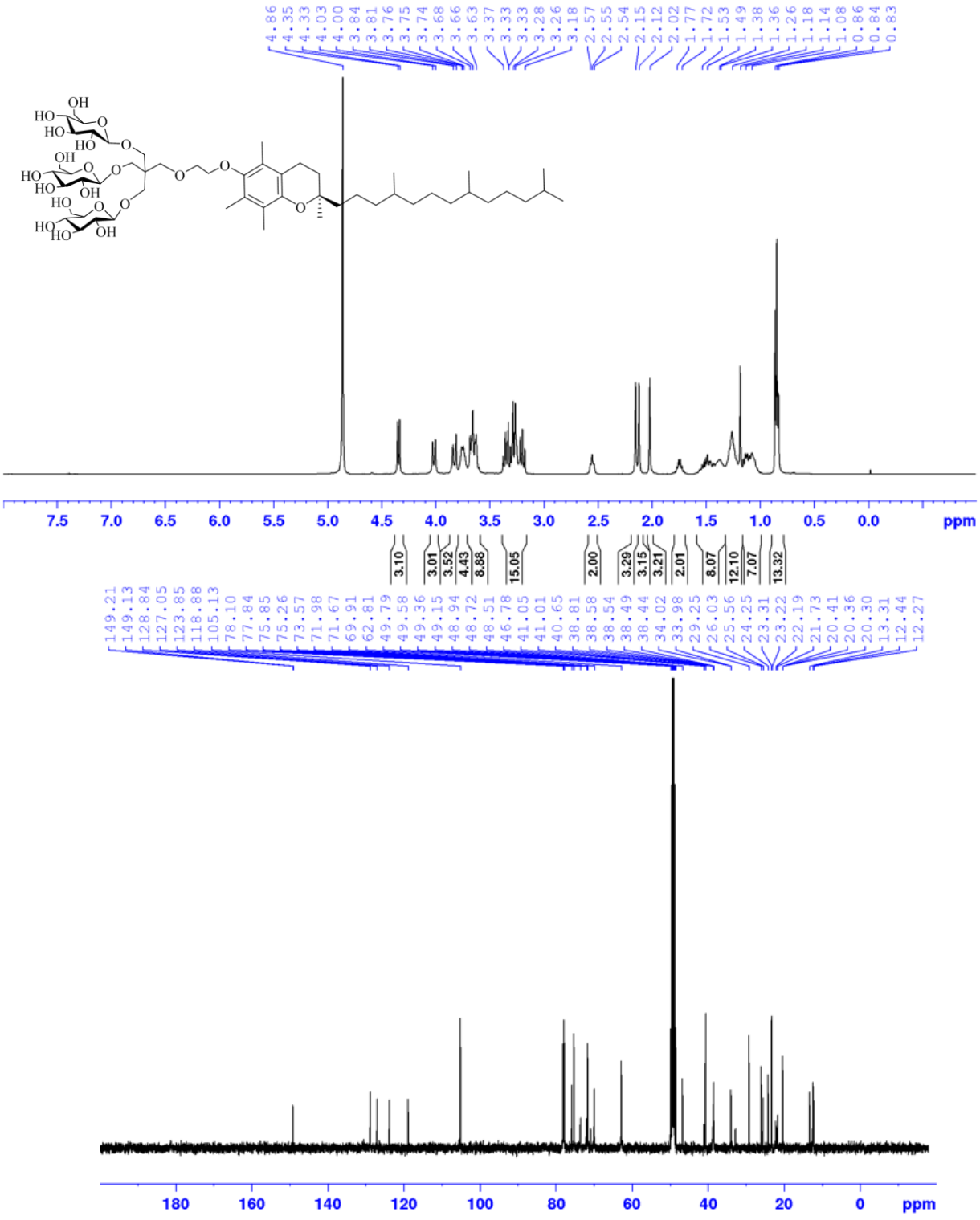

Figure S19. ^{1}H & ^{13}C NMR spectra (CD_3OD, 298 K) of **VEG-2**.

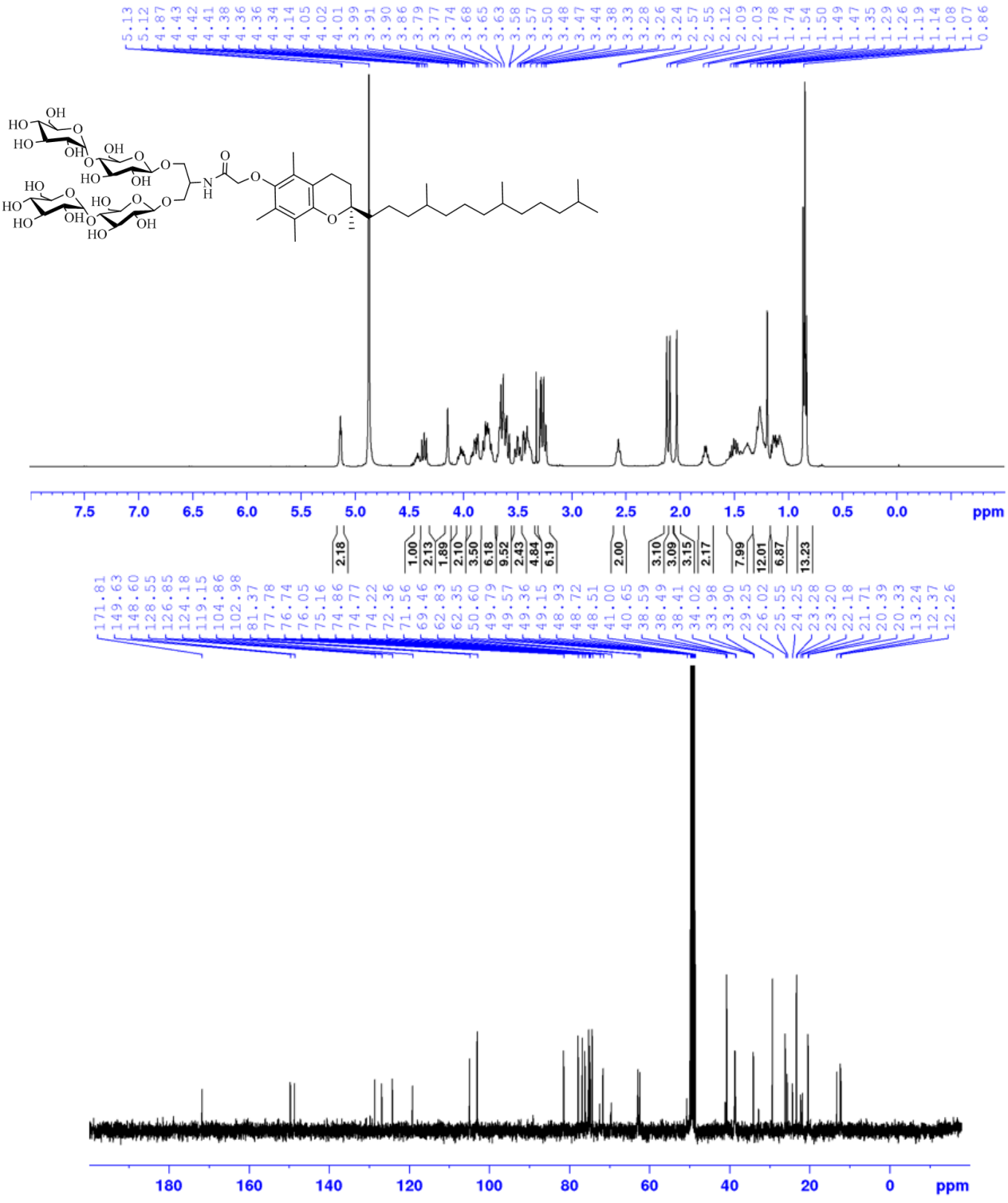

Figure S20. ^{1}H & ^{13}C NMR spectra (CD_3OD, 298 K) of **VEG-3**.

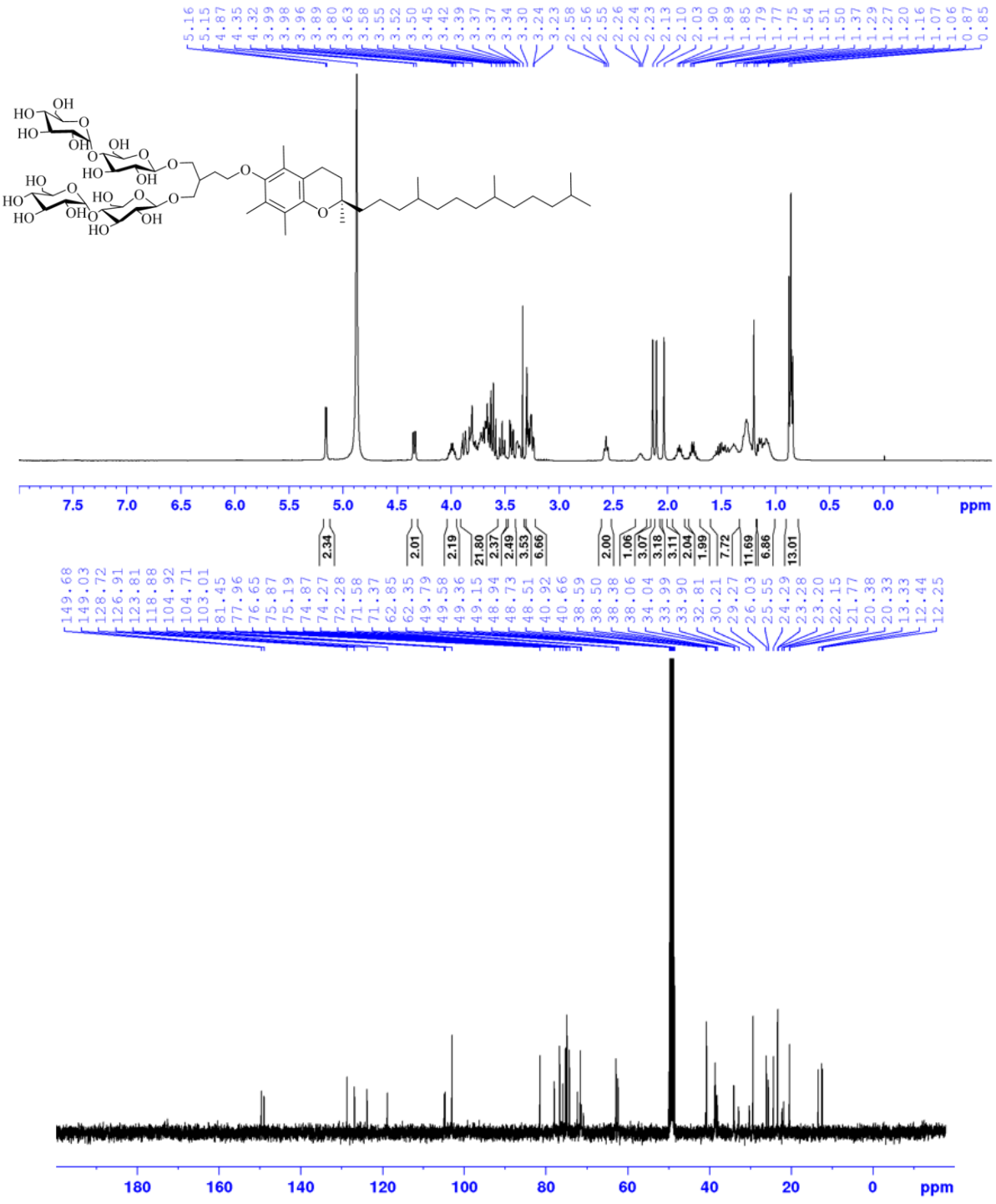

Figure S21. ^{1}H & ^{13}C NMR spectra (CD_3OD, 298 K) of **VEG-4**.

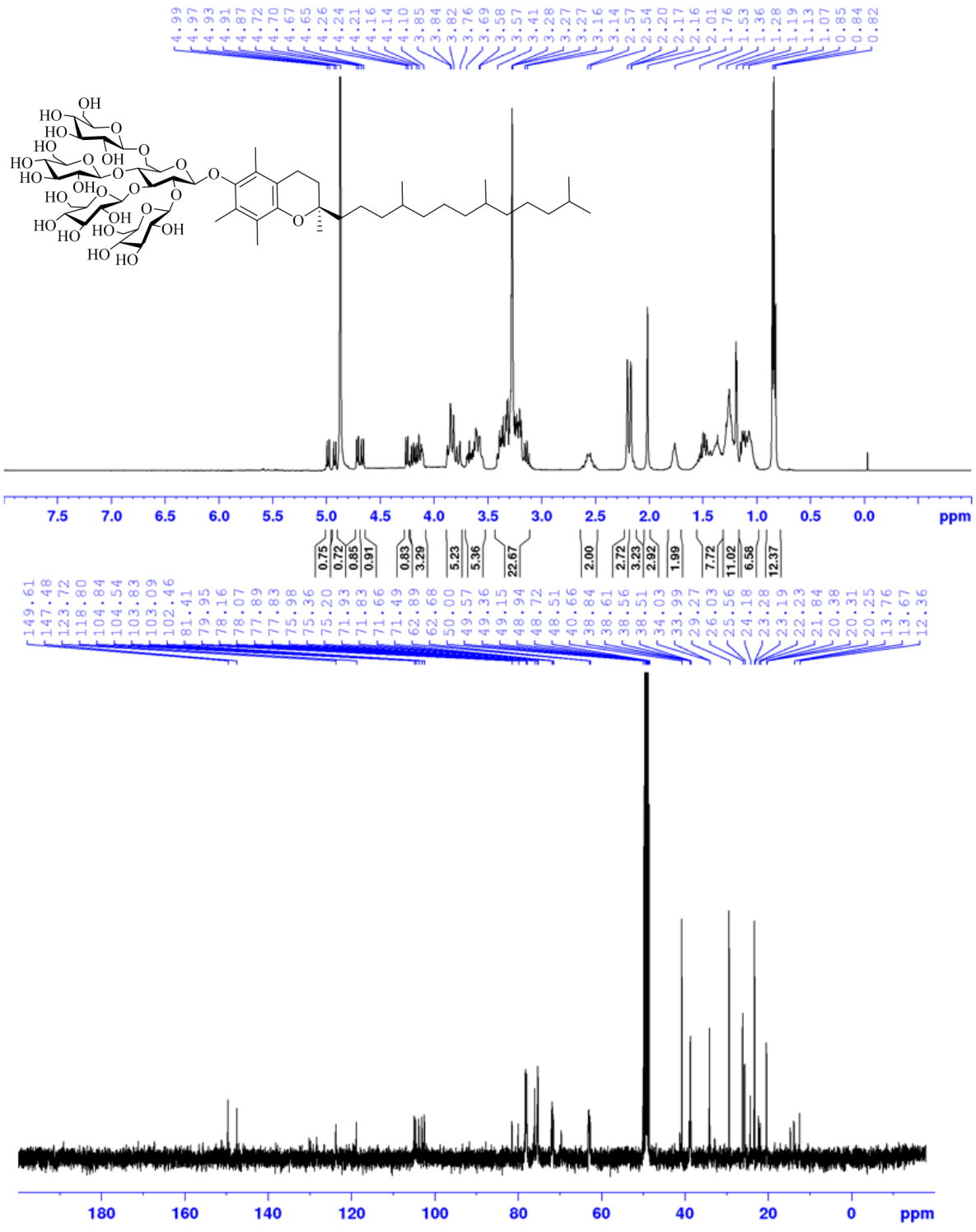

Figure S22. ^{1}H & ^{13}C NMR spectra (CD_3OD, 298 K) of **VEG-5**.