Supporting Information

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A Trimeric Proanthocyanidin from the Bark of *Acacia leucophloea* Willd.

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Table of Contents **Page**

Experimental

Instrumental

The optical rotations were measured on a JASCO DIP-370 polarimeter. UV spectra were measured in MeOH on a Hitachi 150-20 Double Beam Spectrophotometer. The CD spectra were recorded on a J-720 spectropolarimeter. ^{1}H, ^{13}C NMR, and 2D NMR spectra were obtained in methanol-d_4 (δ_H 3.30 and δ_C 49.0) and chloroform-d_3 (δ_H 7.24 and δ_C 77.0) on a Bruker Avance III 600 NMR spectrometer, equipped with a 5 mm cryoprobe using standard pulse programs. The ESI-MS data were obtained on an Esquire 2000 ion trap mass spectrometer (Bruker Daltonik, Bremen, Germany). The HR-ESI-MS data were measured on a micrOTOF orthogonal ESI-TOF mass spectrometer (Bruker, Daltonik, Bremen, Germany). TLC analyses were carried out on silica gel plates (KG60-F_{254}, Merck). Semi-preparative HPLC was performed on a Phenomenex® RP-18 column (Prodigy ODS-3, 250 × 10 mm, 5 μm). The microplate spectrophotometer for bioassay was SPECTRAmax® PLUS (Molecular Devices, U.S.A).

Chemicals and reagents

CH_3CN (HPLC grade) and MeOH were purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA) and deionized water was prepared from a Barnstead water purification system (Dubuque, IA, USA). Chloroform-d_3 (99.8%) was purchased from Cambridge Isotope Lab. Inc. (Andover, MA, USA). Methanol-d_4 (99.8%) was purchased from Merck KGaA (Darmstadt, Germany). α-Glucosidase type IV from *Bacillus stearothermophilus*, *p*-nitrophenyl α-D-glucopyranoside, K_2HPO_4 and KH_2PO_4 were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Extraction and Isolation

The EtOH extract (174.5 g) of the dry bark of *A. leucophloea* (1.64 Kg) was triturated with solvents in sequence (500mL ×3) to give fractions soluble in $CHCl_3$, EtOAc, acetone and EtOH. The EtOAc fraction (50 g out of 55 g) was subjected to silica gel column eluted by different solvent systems in increasing order of polarity. EtOAc eluent of column to give fraction AL-1. AL-1 (1.01 g) was dissolved in 25% MeOH–H_2O (10 ml) and the soluble part (623.5 mg) was separated on a Lobar RP-18 column (LiChroprep RP-18, size B, 310 × 25 mm; 40-63 μm, Merck), delivered by a stepwise gradient of MeOH–H_2O from 15:85 to 75:25, to give six subfractions. Part of subfr. 3 (fr.AL-1-3) (31.7 mg out of 114.4 mg) was separated on a semi-preparative HPLC column, 3.17 mg (0.1 ml MeOH) × 10, delivered by MeCN−H_2O (18:82) with a flow rate of 2.5 mL/min and detection at 300 nm. The fraction (6.9 mg) containing the major peak (t_R = 52.1 min) was further purified on a Sephadex LH-20 column (75 × 1.5 cm, MeOH) to give **1** (1.8 mg). The 25% $MeOH_{aq.}$ insoluble fraction (387 mg) of AL-1 was chromatographed on a Sephadex LH-20 column (72 × 2.5 cm, MeOH–H_2O 7:3) to give **1** (17.9 mg).

O-Methylation and Acetylation of compound 1

To the solution of compound **1** (3.0 mg) in acetone (1 mL) was added dimethyl sulfate (200 μL), K_2CO_3 (50 mg) and Cs_2CO_3 (20 mg). The mixture was refluxed (65 °C) under N_2 for 4 hours and the reaction mixture was evaporated to give a residue which was partitioned between H_2O (10 mL) and $CHCl_3$ (10 mL × 3). The $CHCl_3$ extract was purified on a silica gel column (10–55% acetone–hexane) to give **1c** (0.4 mg). The solution of **1c** in acetic anhydride (200 μL) and anhydrous pyridine (100 μL) was stirred for 4 h at room temperature. After quenching with EtOH (200 μL) for 0.5 h, the reaction mixture was evaporated to dryness and the residue was further purified by a Sephadex LH-20 column (7:3, MeOH–$CHCl_3$) to give **1d** (0.4 mg).

(–)-Fisetinidol-(4*α*,8)-[(–)-fisetinidol-(4*α*,6)]-(+)-catechin (1)

White amorphous powder; $[\alpha]^{27}_{D}$ +83.8 (c 0.50, MeOH); UV (MeOH) λmax (log ε): 281.5 (4.29); CD (MeOH) Δε215 −49.32; ^{1}H and ^{13}C NMR, see Table 1; HMBC, see Figure 2; (+)ESIMS m/z (rel int %) 857.2 (100, $[M+Na]^+$); (+) HRESIMS m/z 857.2037 [M+Na]+, calcd for $C_{45}H_{38}NaO_{16}$, 857.2058.

(*2R,3S*)-2,3-*trans*-3-Hydroxy-6,8-bi-[(*2R,3S,4S)*-2,3-*trans*-3,4-*trans*-3-hydroxy-3',4',7-trimethoxyflavan-4-yl]-3',4',5,7-tetramethoxyflavan (1c)

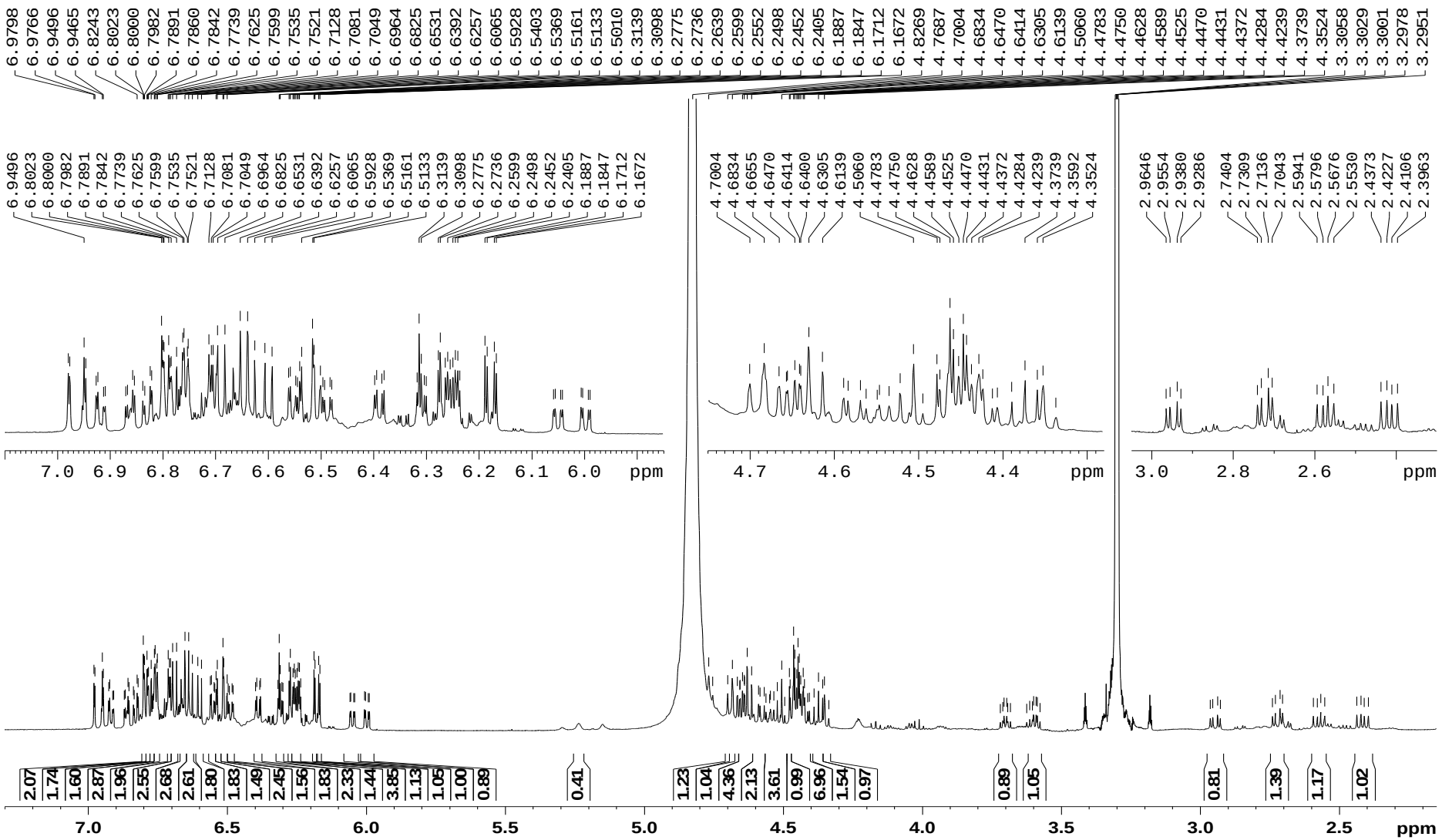
White amorphous powder; CD (MeOH) Δε223 –CE, Δε235 –CE; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.99, 3.94, 3.90, 3.86, 3.86, 3.85, 3.73, 3.73, 3.72, 3.59 (s, OCH_3); (+) ESIMS m/z (rel int %) 997.4 (100, $[M+Na]^+$).

(*2R,3S*)-2,3-*trans*-3-acetoxy-6,8-bi-[(*2R,3S,4S*)-2,3-*trans*-3,4-*trans*-3-acetoxy-3',4',7-trimethoxyflavan-4-yl]-3',4',5,7-tetramethoxyflavan (1d)

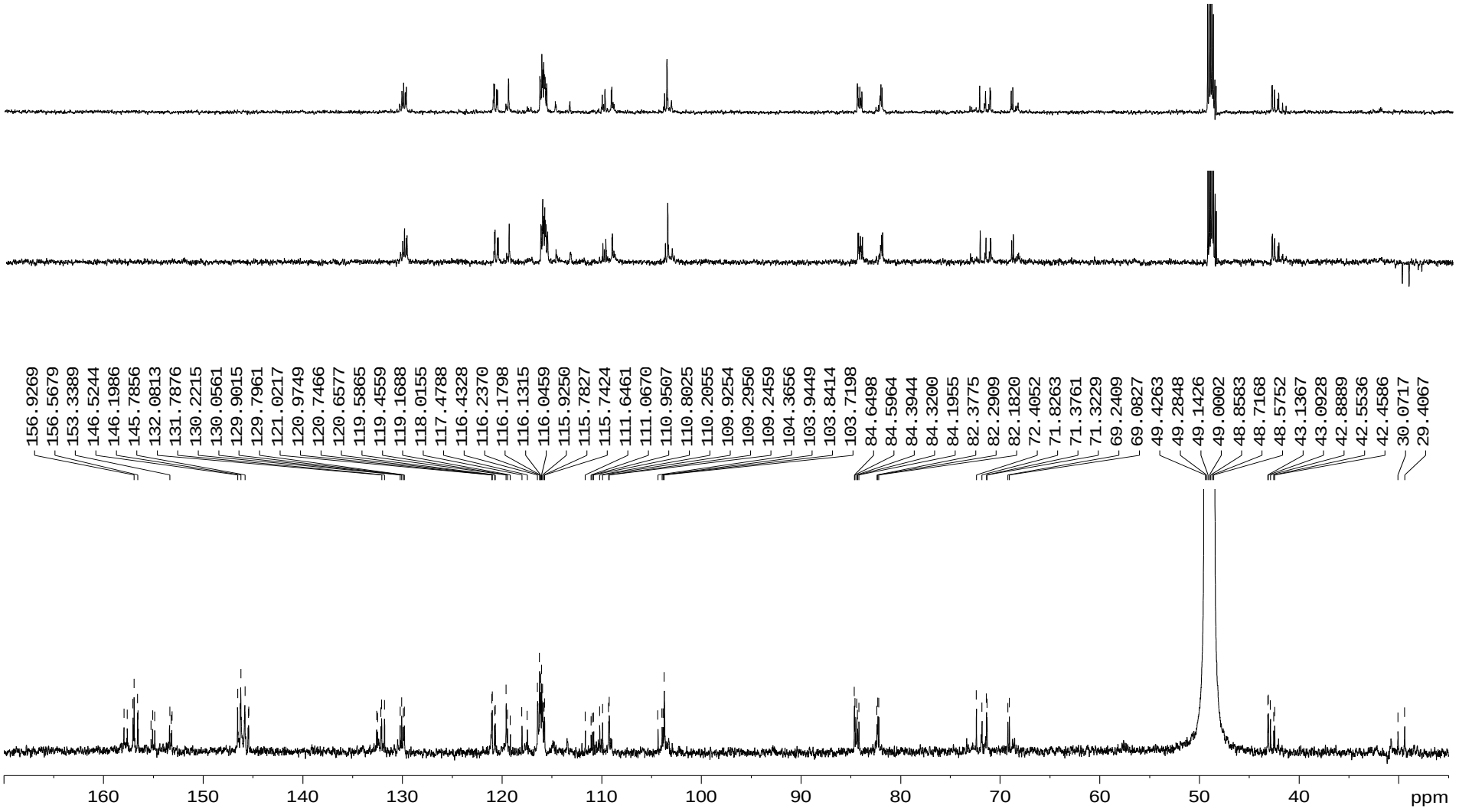
White amorphous powder; CD (MeOH) Δε223 – CE, Δε234 – CE; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.85, 1.82 (s, $COCH_3$), 1.71, 1.67 (s, 3H, $COCH_3$), 1.54, 1.44 (s, 3H, $COCH_3$); (+) ESIMS m/z (rel int %) 1123.4 (68, $[M+Na]^+$), 1139.4 (100, $[M+K]^+$).

Assay for α-Glucosidase Activity

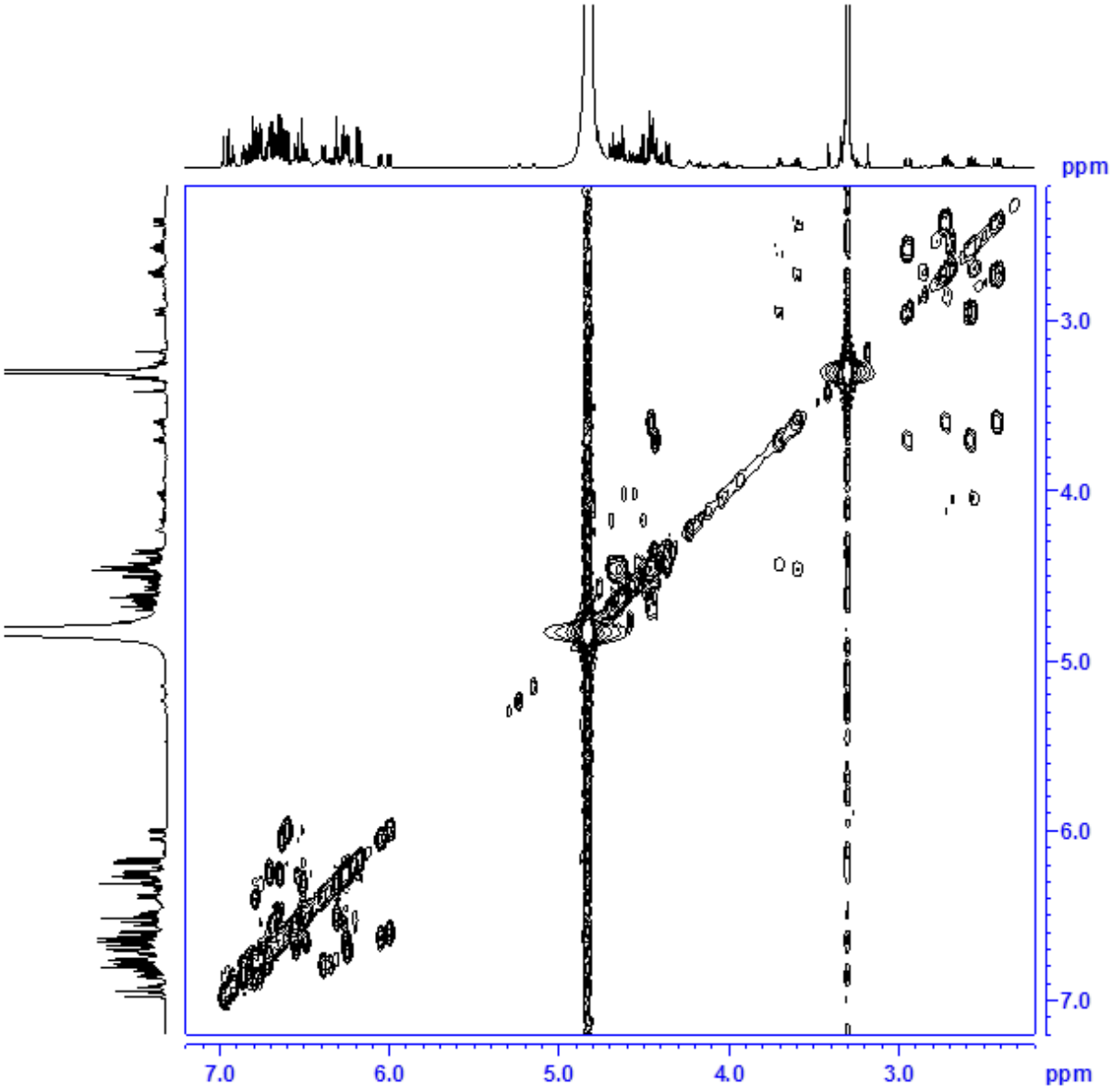
The inhibitory activities against α-glucosidase type IV from *Bacillus stearothermophilus* were performed following the reported method [1]. Compound **1** was dissolved in 10% MeOH. Acarbose (Bayer) was chosen as the positive control with the IC_{50} value against the same enzyme of 0.049 μM.



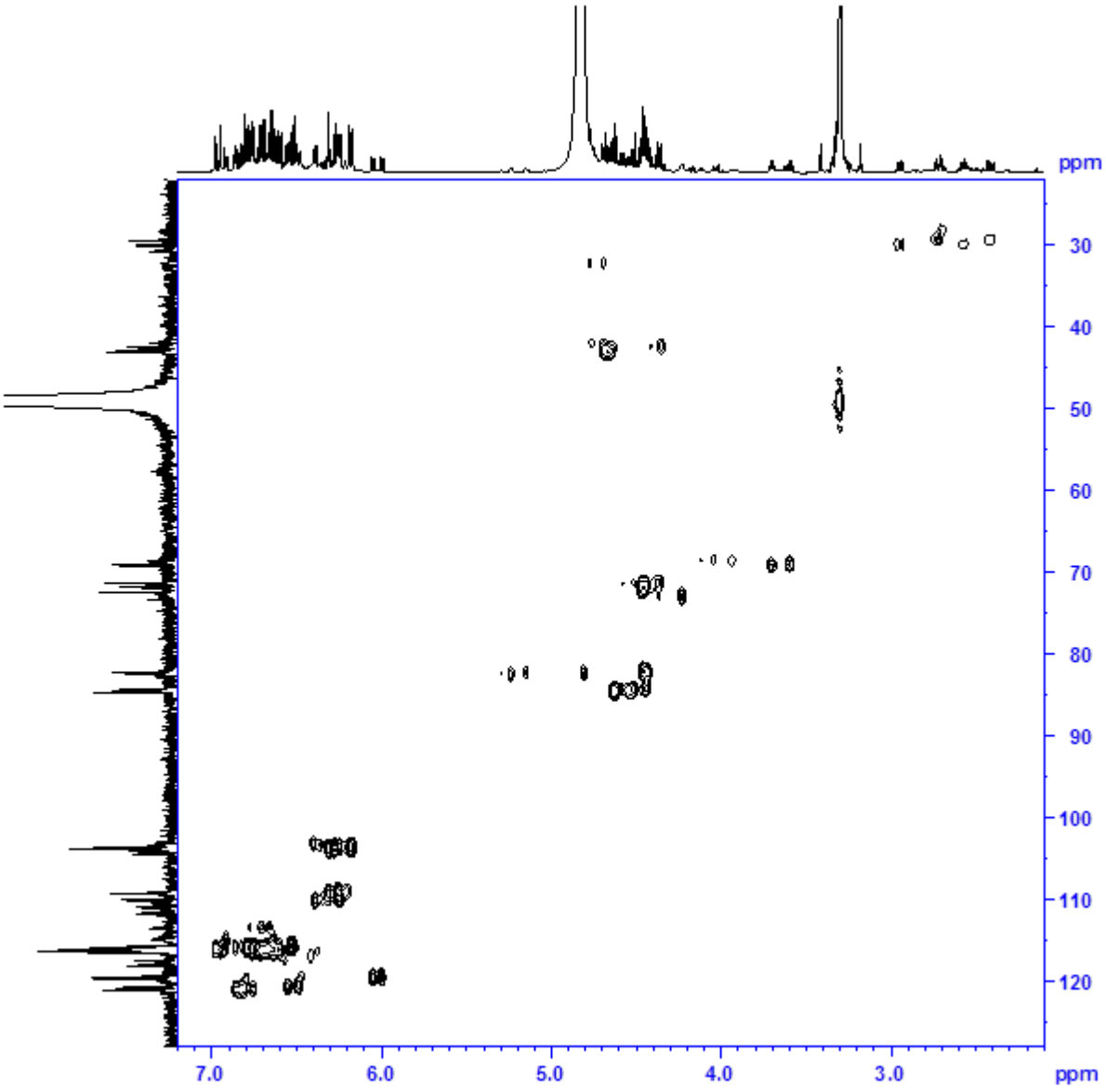
S1: ^{1}H NMR (600 MHz, methanol-d_4) Spectrum of Compound **1**



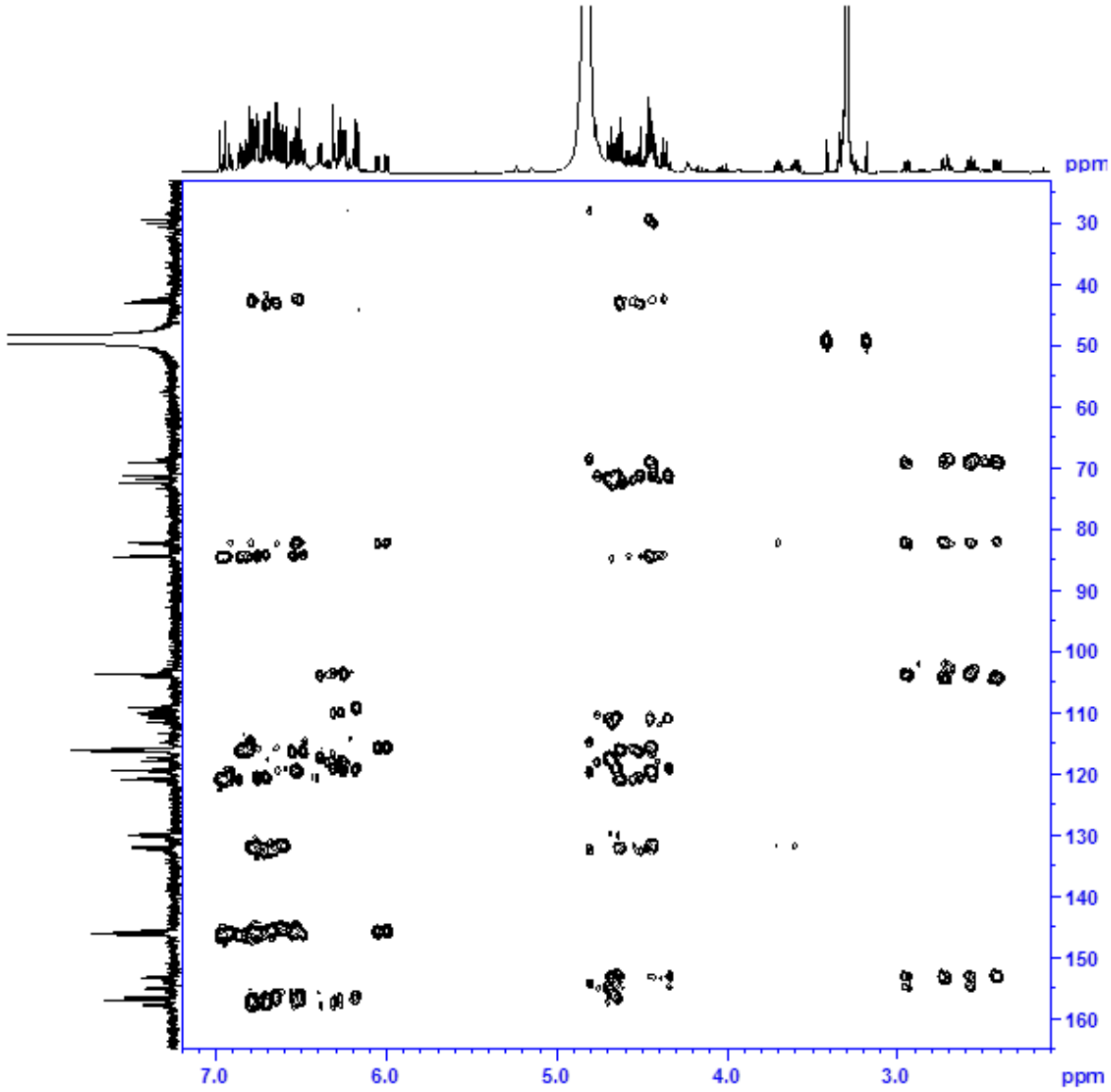
S2: ^{13}C NMR (600 MHz, methanol-d_4) Spectrum of Compound **1**



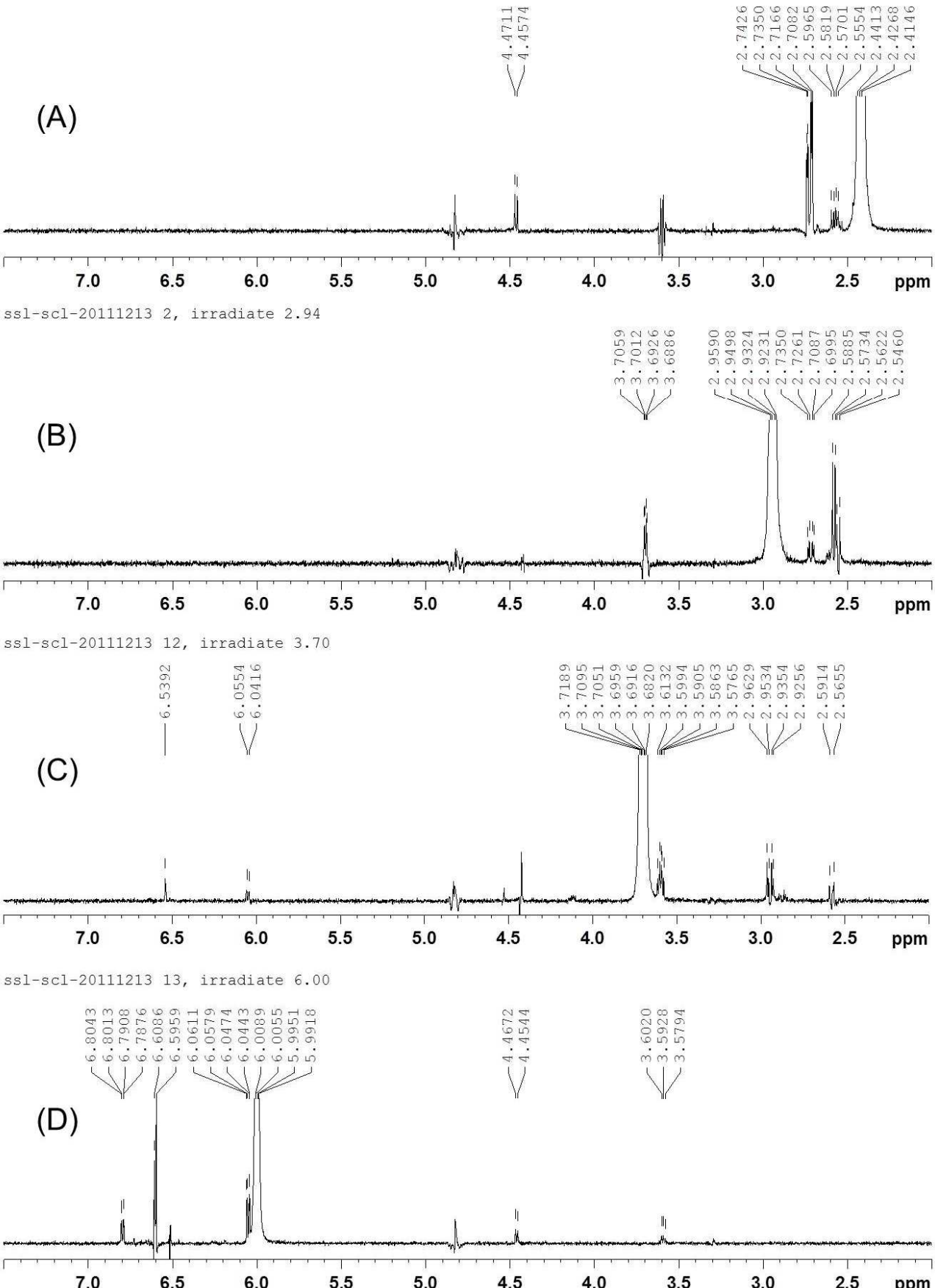
S3: COSY (600 MHz, methanol-d_4) Spectrum of Compound **1**



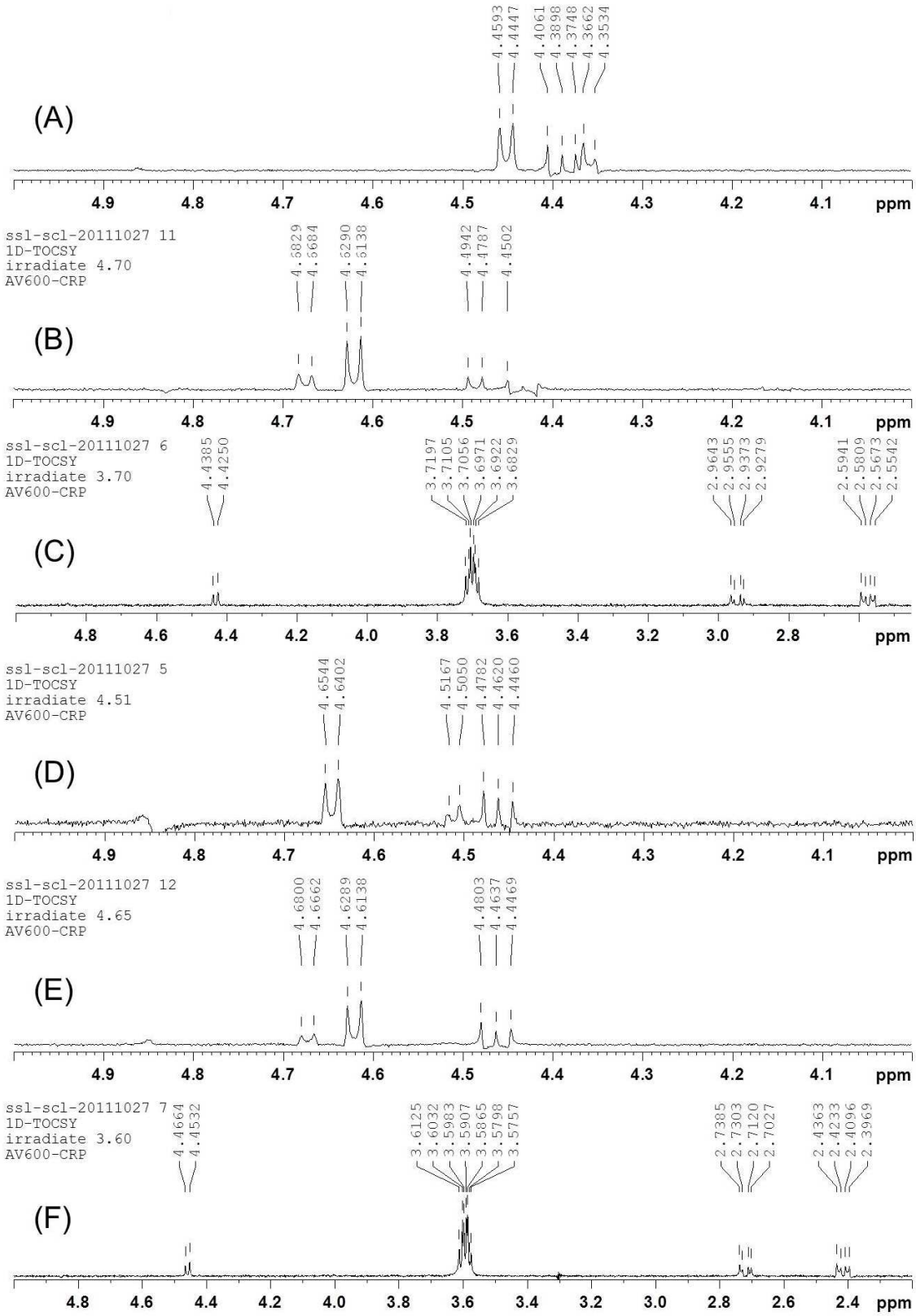
S4: HSQC (600 MHz, methanol-d_4) Spectrum of Compound **1**



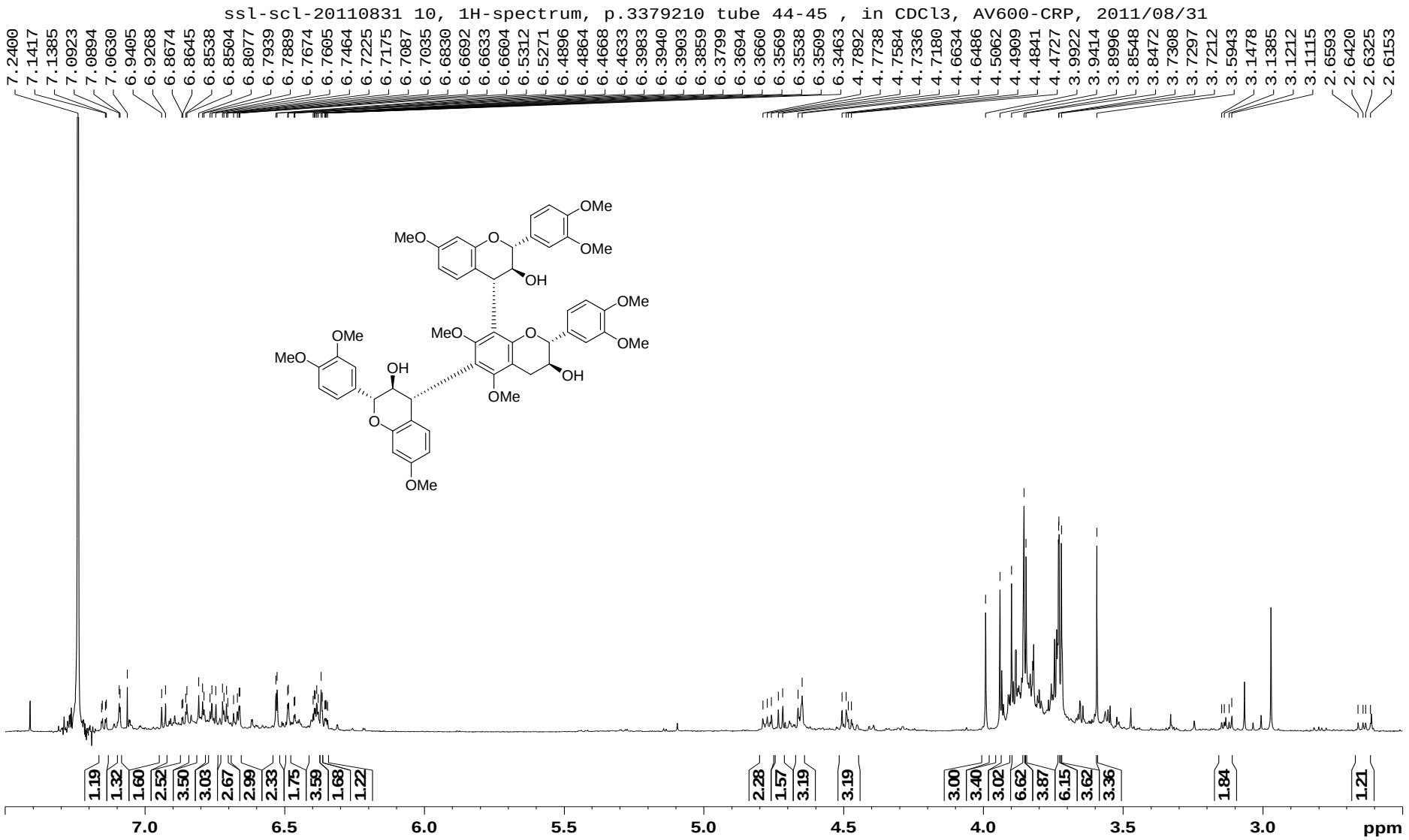
S5: HMBC (600 MHz, methanol-d_4) Spectrum of Compound **1**



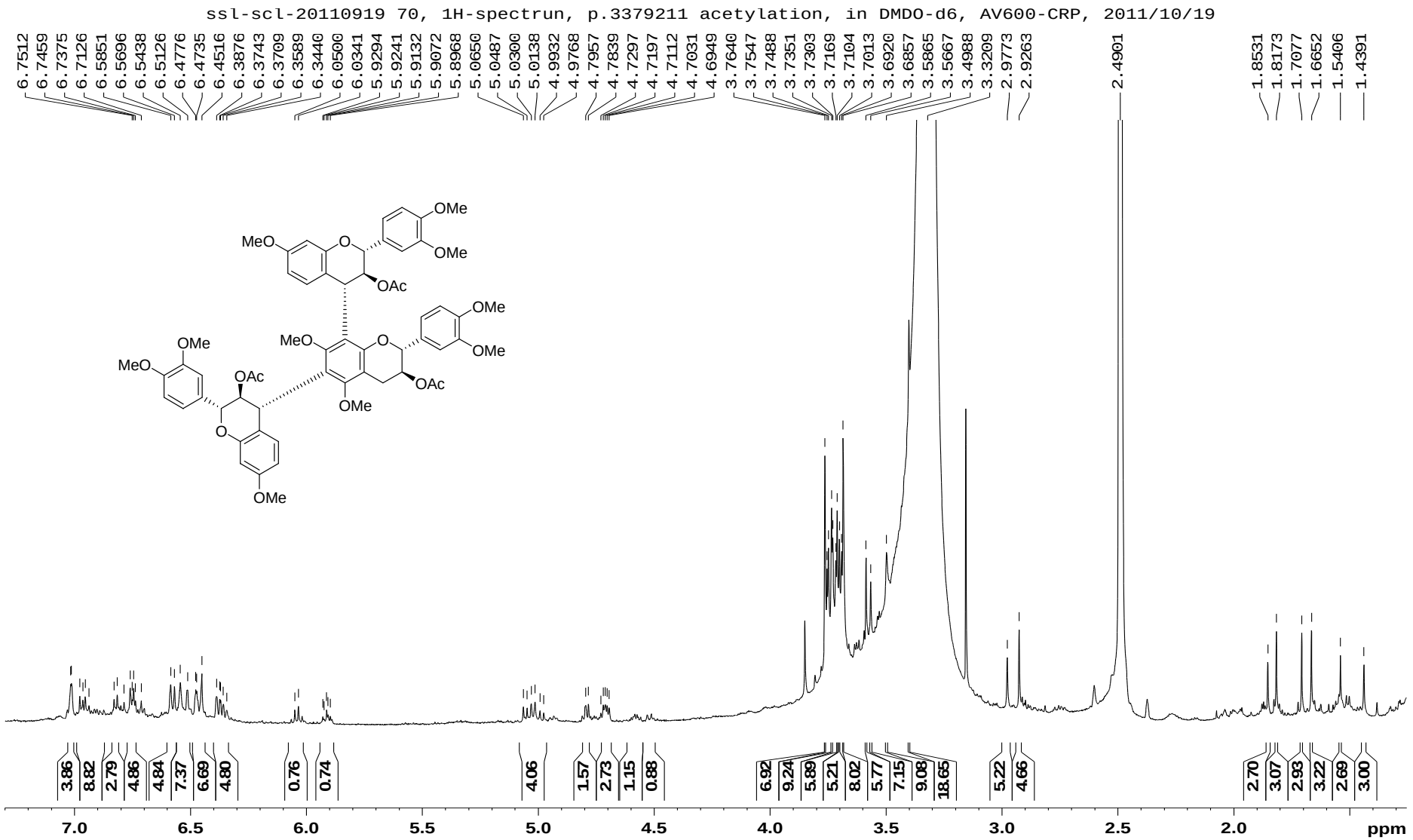
S6: Selected NOESY (600 MHz, methanol-d_4) Spectrum of Compound **1** at δ 2.42 (A), 2.94 (B), 2.37 (C), and 6.00 (D)



S7: Selected TOCSY (600 MHz, methanol-d_4) Spectrum of Compound **1** at δ 4.36 (A), 4.70 (B), 3.70 (C), 4.51 (D), 4.65 (E), and 3.60 (F)



S8: ^{1}H NMR (600 MHz, chloroform-d_3) Spectrum of Compound **1c**



S9: ^{1}H NMR (600 MHz, DMSO-d_6) Spectrum of Compound **1d**

Reference

[1] S. S. Lee, H. C. Lin and C. K. Chen (2008). Acylated flavonol monorhamnosides, α-glucosidase inhibitors, from *Machilus philippinensis, Phytochemistry.* **69**, 2347–2353.