



A New Galloylated Megastigmane Glycoside from the Leaves of *Psidium guajava* and Antimicrobial Activity of the Isolated Compounds

Bui Van Thanh¹, Le Thi Huong², Nguyen Thi Van Anh¹, Nguyen Sinh Khang¹, Do Thi Quynh³, Ninh Khac Ban³, Yoo Been Rho⁴, Hoang Thi Ngoc⁵, Nguyen Thi Minh Hang³, Vu Phuong Nhung¹, Ha Thi Thu Chu¹, Nguyen Phuong Hanh¹, SeonJu Park⁶, and Seung Hyun Kim^{4,*}

¹Institute of Biology, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Nghia Do, Hanoi, Vietnam

²Institute of Physics, VAST, 10 Dao Tan, Giang Vo, Hanoi, Vietnam

³Institute of Chemistry, VAST, 18 Hoang Quoc Viet, Nghia Do, Hanoi, Vietnam

⁴College of Pharmacy, Yonsei Institute of Pharmaceutical Sciences, Yonsei University, Incheon 21983, Republic of Korea

⁵Faculty of Pharmacy and Medical Laboratory Technology, Dong Do University, 60B Nguyen Huy Tuong, Thanh Xuan, Hanoi, Vietnam

⁶Metropolitan Seoul Center, Korea Basic Science Institute, Seoul 02841, Republic of Korea

Abstract – A new galloylated megastigmane glycoside, (3*R*,9*R*)-3-hydroxy-7,8-didehydro- β -ionol 9-*O*-(6'-*O*-galloyl)- β -D-glucopyranoside (**1**), together with four known compounds, mallophenol B (**2**), garcimangosone D (**3**), quercetin-3-*O*- α -L-arabinofuranoside (**4**), and quercetin-3-*O*- α -L-arabinopyranoside (**5**), was isolated from the leaves of *Psidium guajava* L. (Myrtaceae). Their structures were elucidated by NMR spectroscopy and comparison with published data, supported by HR-ESI-MS data where available. The isolated compounds were evaluated for antimicrobial activity against three Gram-positive bacteria, three Gram-negative bacteria, and *Candida albicans*. Compound **1** showed the strongest activity among the isolates, with MIC values of 64 μ g/mL against *Staphylococcus aureus* and *C. albicans*. Compounds **2–5** were less active, and none of the compounds inhibited the tested Gram-negative bacteria at concentrations up to 256 μ g/mL. These findings expand the chemical diversity of *P. guajava* leaves and provide compound-level information on their antimicrobial properties.

Keywords – *Psidium guajava*, Myrtaceae, Megastigmane glycoside, Antimicrobial activity

Introduction

Antimicrobial resistance (AMR) is a major global health problem that reduces the effectiveness of anti-infective therapy and increases morbidity and mortality. In 2021, bacterial AMR was associated with approximately 4.71 million deaths worldwide, including 1.14 million deaths directly attributable to resistance.¹ The World Health Organization's 2025 global surveillance report further indicated that approximately one in six laboratory-confirmed bacterial infections caused by common pathogens in 2023 were resistant to antibiotic treatment.² These trends support continued efforts to identify structurally diverse antimicrobial agents, including natural products from medicinal plants.

Psidium guajava L. (Myrtaceae), commonly known as guava, is cultivated in tropical and subtropical regions and has a long history of medicinal use.³ Preparations of the leaves have traditionally been used for diarrhea, dysentery, gastrointestinal disorders, cough, oral ulcers, and wound care.⁴ Antimicrobial effects have been reported for guava leaf extracts against several bacterial and fungal species,^{5,6} and activity has also been described for the leaf essential oil and its volatile constituents.⁷ These observations indicate that the leaves are a useful source for investigating individual metabolites that may contribute to the reported biological effects.

Phytochemical studies of *P. guajava* leaves have revealed phenolic compounds, flavonoids, triterpenoids, benzophenones, and meroterpenoids.^{8–12} Although the chemistry and antimicrobial properties of crude preparations have been investigated extensively, fewer studies have examined the activities of purified leaf constituents. In the present study, compounds were isolated from *P. guajava* leaves, characterized by spectroscopic methods, and

*Author for correspondence

Seung Hyun Kim, College of Pharmacy, Yonsei Institute of Pharmaceutical Sciences, Yonsei University, Incheon 21983, Republic of Korea

Tel: +82-32-749-4514; E-mail: kimsh11@yonsei.ac.kr

evaluated for antimicrobial activity against selected bacteria and *C. albicans*.

Experimental

General experimental procedures – Preparative HPLC was carried out with an Agilent 1100 HPLC system (Agilent Technologies). UV spectra were recorded using the diode-array detector of the Agilent 1100 HPLC system (Agilent Technologies), with detection wavelengths set at 205, 230, 254, and 280 nm. Silica gel (Kieselgel 60, 230–400 mesh, Merck), Sephadex LH-20 (Merck), and YMC RP-18 resin (YMC Co. Ltd.) were used for column chromatography. NMR spectra were recorded on a Bruker AVANCE NEO 600 spectrometer at 600 MHz for ^1H and 150 MHz for ^{13}C . Optical rotations were measured using a Jasco P-2000 digital polarimeter. HR-ESI-MS spectra were acquired using a Thermo Scientific Orbitrap Exploris 240 mass spectrometer equipped with an electrospray ionization (ESI) source. GC analyses were performed using a Shimadzu GC-2010 gas chromatograph (Shimadzu Corporation).

Plant materials – Leaves of *P. guajava* were collected in Lao Cai, Vietnam, in May 2024. The plant was identified by Dr. Bui Van Thanh, Institute of Biology, VAST. Voucher material was deposited in the Institute of Biology, VAST, under the accession number PG2405.

Extraction and isolation – Dried leaves of *P. guajava* (6.0 kg) were sonicated twice with MeOH (15 L per extraction) for 2 h at 45°C. Evaporation of the extracts under reduced pressure yielded the MeOH extract (PGL, 1.1 kg). The residue was dispersed in water (1.5 L) and sequentially extracted with *n*-hexane and ethyl acetate. After solvent removal, the *n*-hexane and ethyl acetate layers gave PGL1 (75 g) and PGL2 (109 g), respectively; the residual water layer was retained as PGL3.

PGL3 was subjected to Diaion HP-20 column chromatography, beginning with water and followed by 25, 75, and 100% MeOH in water, to obtain PGL3A–PGL3C. PGL3A was separated on silica gel with CH_2Cl_2 –MeOH (20:1, 10:1, 5:1, and 2.5:1, v/v) to yield PGL3A1–PGL3A4. PGL3A2 was further chromatographed on silica gel with CH_2Cl_2 –acetone– H_2O (1:1.5:0.1, v/v/v), followed by Sephadex LH-20 (MeOH– H_2O , 9:1, v/v) and preparative HPLC using 25% ACN in water, to afford compound **4** (12.0 mg, t_{R} = 32.3 min). PGL3B was divided into PGL3B1–PGL3B4 by silica gel chromatography. PGL3B2 was separated with CH_2Cl_2 –acetone– H_2O (1:1:0.05, v/v/v), and PGL3B2C was subsequently chromatographed on RP-18 with acetone– H_2O (1:2, v/v).

Sephadex LH-20 chromatography (MeOH– H_2O , 9:1, v/v) followed by preparative HPLC using 20% ACN in water afforded compound **3** (4.2 mg, t_{R} = 36.9 min). PGL3B3 was successively fractionated on silica gel [CH_2Cl_2 –acetone– H_2O (1:1.5:0.1, v/v/v)], RP-18 [acetone– H_2O (1:2, v/v)], and Sephadex LH-20 [MeOH– H_2O (9:1, v/v)] to give PGL3B3A–PGL3B3C. Preparative HPLC of PGL3B3A with 22.5% ACN in water yielded compounds **1** (4.2 mg, t_{R} = 42.4 min) and **2** (6.0 mg, t_{R} = 27.4 min), whereas PGL3B3C afforded compound **5** (3.8 mg, t_{R} = 54.9 min) under the same HPLC conditions.

Acid hydrolysis – Compound **1** (3.0 mg) was hydrolyzed with 1.0 N HCl in dioxane– H_2O (1:1, v/v) at 80°C for 3 h. After cooling to room temperature, the acidic solution was neutralized with Ag_2CO_3 and evaporated to dryness under a stream of N_2 . The residue was partitioned with H_2O and CHCl_3 , and the aqueous fraction was collected and dried under N_2 . The resulting sugar residue was dissolved in anhydrous pyridine (0.1 mL), treated with L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 mL), and maintained at 60°C for 2 h. Subsequently, trimethylsilylimidazole (0.1 mL) was added, and the reaction was continued at 60°C for 1.5 h to derivatize the sugars. After removing the solvent, the derivatized sugars were partitioned with *n*-hexane and H_2O (0.1 mL each), and the *n*-hexane layer was subjected to GC analysis using a DB-5 column (0.32 mm $\times$ 30 m) equipped with a flame ionization detector (FID). The column, injector, and detector temperatures were maintained at 210, 270, and 300°C, respectively, with He as the carrier gas. The derivatives of authentic D-glucose and L-glucose exhibited retention times (t_{R}) of 14.11 and 14.26 min, respectively. The derivatized sugar released from compound **1** showed a peak at t_{R} = 14.12 min, consistent with authentic D-glucose (14.11 min) and distinct from L-glucose (14.26 min), supporting the D-glucose assignment.

(3R,9R)-3-hydroxy-7,8-didehydro- β -ionol 9-O-(6'-O-galloyl)- β -D-glucopyranoside (1) – White amorphous powder; $[\alpha]_{\text{D}}^{25}$ –8.0 (*c* 0.1, MeOH); $\text{C}_{26}\text{H}_{34}\text{O}_{11}$, HR-ESI-MS m/z : 545.1995 $[\text{M} + \text{Na}]^+$ (calcd for $[\text{C}_{26}\text{H}_{34}\text{O}_{11}\text{Na}]^+$, 545.1993); UV (MeOH) λ_{max} 218 and 277 nm; ^1H - and ^{13}C -NMR (CD_3OD): see Table 1.

Mallophenol B (2) – White amorphous powder; $[\alpha]_{\text{D}}^{25}$ +14.0 (*c* 0.1, MeOH); $\text{C}_{26}\text{H}_{34}\text{O}_{12}$, due to the limited amount of remaining sample, HR-ESI-MS data could not be obtained; ^1H -NMR (CD_3OD , 600 MHz) δ_{H} : 7.10 (2H, s, H-2''/6''), 5.83 (1H, d, J = 1.2 Hz, H-4), 5.82 (2H, overlapped, H-7/H-8), 4.48 (1H, dd, J = 2.4, 12.0 Hz, H_b-6'), 4.43 (1H, d, J = 7.8 Hz, H-1'), 4.40 (1H, m, H-9), 4.38 (1H, dd, J = 5.4, 12.0 Hz, H_a-6'), 3.52 (1H, m, H-5'),

Table 1. ^1H - and ^{13}C -NMR data for compound **1**

Position	1	
	δ_{H} , mult. ^{a,b} (J in Hz)	δ_{C} ^{a,c}
1		37.3
2	1.33, m 1.74, m	47.4
3	3.83, m	65.1
4	1.94, dd (9.6, 17.4) 2.29, dd (5.4, 17.4)	41.8
5		139.2
6		124.4
7		84.5
8		94.2
9	4.78, q (6.6)	67.9
10	1.51, d (6.6)	23.4
11	1.81, s	22.5
12	1.05, s	28.9
13	1.11, s	31.0
9- <i>O</i> -Glc		
1'	4.60, d (7.8)	103.1
2'	3.29, dd (7.8, 8.4)	75.2
3'	3.42, dd (8.4, 9.0)	78.0
4'	3.47, t (9.0)	71.5
5'	3.56, m	75.6
6'	4.42, dd (4.0, 12.0) 4.50, dd (1.8, 12.0)	64.8
6'- <i>O</i> -Gall		
1''		121.5
2'', 6''	7.10, s	110.3
3'', 5''		146.5
4''		139.8
7''		168.4

^a Recorded in CD_3OD , ^b 600 MHz, ^c 150 MHz, Glc: glucopyranoside, Gall: galloyl

3.46 (1H, t, $J = 9.0$ Hz, H-4'), 3.41 (1H, t, $J = 9.0$ Hz, H-3'), 3.24 (1H, dd, $J = 7.8, 9.0$ Hz, H-2'), 2.43 (1H, d, $J = 16.8$ Hz, H_b-2), 2.11 (1H, d, $J = 16.8$ Hz, H_a-2), 1.86 (3H, d, $J = 1.2$ Hz, H-11), 1.30 (3H, d, $J = 6.6$ Hz, H-10), 1.01 (3H, s, H-12), and 0.97 (3H, s, H-13). ^{13}C -NMR (CD_3OD , 150 MHz) δ_{C} : 201.3 (C-3), 168.4 (C-5, C-7''), 146.5 (C-3''/5''), 139.9 (C-4''), 134.9 (C-7), 131.6 (C-8), 127.2 (C-4), 121.5 (C-1''), 110.3 (C-2''/6''), 103.1 (C-1'), 78.0 (C-3'), 77.1 (C-9), 75.5 (C-5'), 75.2 (C-2'), 71.5 (C-4'), 64.7 (C-6'), 50.7 (C-2), 42.5 (C-1), 24.6 (C-13), 23.4 (C-12), 21.2 (C-10), and 19.6 (C-11).

Garcimangosone D (3) – Yellow powder; $[\alpha]_{\text{D}}^{25} -31.0$

(c 0.1, MeOH); $\text{C}_{19}\text{H}_{20}\text{O}_9$, HR-ESI-MS m/z : 415.0998 [$\text{M} + \text{Na}]^+$ (calcd for $[\text{C}_{19}\text{H}_{20}\text{O}_9\text{Na}]^+$, 415.1000); ^1H -NMR (CD_3OD , 600 MHz) δ_{H} : 7.71 (2H, dd, $J = 1.2, 8.4$ Hz, H-2'/6'), 7.54 (1H, t, $J = 7.2$ Hz, H-4'), 7.44 (2H, t, $J = 7.8$ Hz, H-3'/5'), 6.23 (1H, d, $J = 1.8$ Hz, H-5), 6.09 (1H, d, $J = 1.8$ Hz, H-3), 4.84 (1H, m, H-1''), 3.87 (1H, dd, $J = 2.4, 12.0$ Hz, H_b-6''), 3.67 (1H, dd, $J = 6.0, 12.0$ Hz, H_a-6''), 3.35 (1H, m, H-3''), 3.32 (1H, m, H-5''), 3.24 (1H, t, $J = 9.0$ Hz, H-4''), and 2.85 (1H, dd, $J = 7.2, 9.0$ Hz, H-2''). ^{13}C -NMR (CD_3OD , 150 MHz) δ_{C} : 199.5 (C-7), 164.1 (C-4), 162.1 (C-2), 159.7 (C-6), 141.7 (C-1'), 133.1 (C-4'), 130.1 (C-2'/6'), 129.0 (C-3'/5'), 109.1 (C-1), 101.7 (C-1''), 98.2 (C-3), 95.8 (C-5), 78.2 (C-5''), 77.8 (C-3''), 74.6 (C-2''), 71.1 (C-4''), and 62.5 (C-6'').

Quercetin-3-*O*- α -L-arabinofuranoside (4) – Yellow powder; $[\alpha]_{\text{D}}^{25} -17.0$ (c 0.1, MeOH); $\text{C}_{20}\text{H}_{18}\text{O}_{11}$, HR-ESI-MS m/z : 457.0742 [$\text{M} + \text{Na}]^+$ (calcd for $[\text{C}_{20}\text{H}_{18}\text{O}_{11}\text{Na}]^+$, 457.0741); ^1H -NMR (CD_3OD , 600 MHz) δ_{H} : 7.54 (1H, d, $J = 1.8$ Hz, H-2'), 7.51 (1H, dd, $J = 1.8, 8.4$ Hz, H-6'), 6.91 (1H, d, $J = 8.4$ Hz, H-5'), 6.40 (1H, d, $J = 1.8$ Hz, H-8), 6.22 (1H, d, $J = 1.8$ Hz, H-6), 5.49 (1H, s, H-1''), 4.35 (1H, d, $J = 1.8$ Hz, H-2''), 3.93 (1H, dd, $J = 2.4, 5.4$ Hz, H-3''), 3.89 (1H, q, $J = 4.2$ Hz, H-4''), 3.54 (1H, dd, $J = 4.2, 12.0$ Hz, H_b-5''), and 3.52 (1H, dd, $J = 4.8, 12.0$ Hz, H_a-5''). ^{13}C -NMR (CD_3OD , 150 MHz) δ_{C} : 180.0 (C-4), 166.1 (C-7), 163.0 (C-5), 159.3 (C-2), 158.6 (C-9), 149.8 (C-4'), 146.3 (C-3'), 134.9 (C-3), 123.1 (C-6'), 123.0 (C-1'), 116.9 (C-2'), 116.4 (C-5'), 109.5 (C-1''), 105.6 (C-10), 99.9 (C-6), 94.8 (C-8), 88.0 (C-4''), 83.3 (C-2''), 78.7 (C-3''), and 62.5 (C-5'').

Quercetin-3-*O*- α -L-arabinopyranoside (5) – Yellow powder; $[\alpha]_{\text{D}}^{25} -23.0$ (c 0.1, MeOH); $\text{C}_{20}\text{H}_{18}\text{O}_{11}$, HR-ESI-MS m/z : 457.0743 [$\text{M} + \text{Na}]^+$ (calcd for $[\text{C}_{20}\text{H}_{18}\text{O}_{11}\text{Na}]^+$, 457.0741); ^1H -NMR (CD_3OD , 600 MHz) δ_{H} : 7.76 (1H, d, $J = 1.8$ Hz, H-2'), 7.59 (1H, dd, $J = 1.8, 8.4$ Hz, H-6'), 6.89 (1H, d, $J = 8.4$ Hz, H-5'), 6.41 (1H, s, H-8), 6.22 (1H, d, $J = 1.8$ Hz, H-6), 5.17 (1H, d, $J = 6.6$ Hz, H-1''), 3.92 (1H, dd, $J = 6.6, 8.4$ Hz, H-2''), 3.83 (2H, m, H-4''/H_b-5''), 3.66 (1H, m, H-3''), and 3.45 (1H, m, H_a-5''). ^{13}C -NMR (CD_3OD , 150 MHz) δ_{C} : 178.1 (C-4), 164.7 (C-7), 161.6 (C-5), 157.3 (C-9), 157.0 (C-2), 148.6 (C-4'), 144.6 (C-3'), 134.3 (C-3), 121.7 (C-1'), 121.5 (C-6'), 116.1 (C-2'), 114.8 (C-5'), 104.2 (C-10), 103.3 (C-1''), 98.5 (C-6), 93.4 (C-8), 72.7 (C-3''), 70.7 (C-2''), 67.7 (C-4''), and 65.5 (C-5'').

Antimicrobial assay – Compounds **1–5** were subjected to antimicrobial testing against three Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* ATCC 14579), three Gram-negative bacteria (*Escherichia coli* ATCC

25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella enterica* ATCC 13076), and one yeast (*Candida albicans* ATCC 10231). The assay was performed in 96-well plates using a broth-dilution format based on a reported method with minor modifications.¹³ For each test well, 10 μ L of compound solution in DMSO and 190 μ L of a microorganism suspension (2×10^5 CFU/mL) were combined. Plates were maintained at 37°C for 24 h, after which 10 μ L of resazurin solution (0.01%) was added. The plates were further incubated for 1 h until a color change was observed, and the absorbance was subsequently measured at 600 nm. MIC was assigned to the lowest concentration producing at least 97% growth inhibition relative to the untreated control. Streptomycin served as the bacterial positive control, whereas cycloheximide was used for *C. albicans*.

Results and Discussion

Chromatographic separation of the aqueous fraction of *P. guajava* leaves afforded one new megastigmane glycoside (**1**) and four known compounds (**2–5**) (Fig. 1).

Compound **1** was isolated as a white amorphous powder. The molecular formula was established as $C_{26}H_{34}O_{11}$ from the sodium-adduct ion at m/z 545.1995 $[M + Na]^+$ (calcd for $[C_{26}H_{34}O_{11}Na]^+$, 545.1993) in the HR-ESI-MS spectrum. The ^1H -NMR spectrum exhibited a two-proton singlet at δ_H 7.10 (2H, s), assigned to the equivalent galloyl protons H-2'' and H-6'', together with

an anomeric proton at δ_H 4.60 (1H, d, $J = 7.8$ Hz, H-1'), and four methyl signals at δ_H 1.05 (s, H-12), 1.11 (s, H-13), 1.51 (d, $J = 6.6$ Hz, H-10), and 1.81 (s, H-11). The ^{13}C -NMR and HSQC data accounted for 26 carbons, including a galloyl carbonyl, six aromatic carbons, six glucosyl carbons, and 13 carbons attributable to a megastigmane unit. Comparison of the spectral features with those of the reported (3*R*,9*R*)-3-hydroxy-7,8-didehydro- β -ionyl 9-*O*- α -D-arabinopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside¹⁴ showed a key difference at C-6', where compound **1** bears a galloyl group. An HMBC cross-peak from H-6' (δ_H 4.42 and 4.50) to the galloyl carbonyl C-7'' (δ_C 168.4) assigned the galloyl substitution at C-6' (Fig. 2). A further correlation from H-1' (δ_H 4.60) to C-9 (δ_C 67.9) confirmed the 9-*O*-glucosyl linkage. Additional HMBC cross-peaks from H-2/H-4 to C-3, H-10 to C-8/C-9, H-11 to C-5/C-6, and H-12/H-13 to C-6 were consistent with the substitution pattern of the aglycone

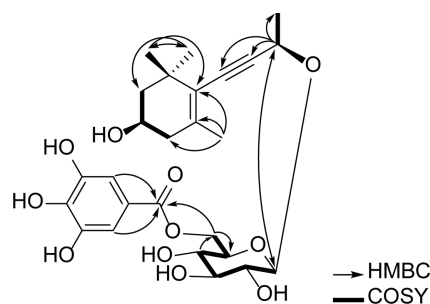


Fig. 2. Key HMBC and COSY correlations of compound **1**.

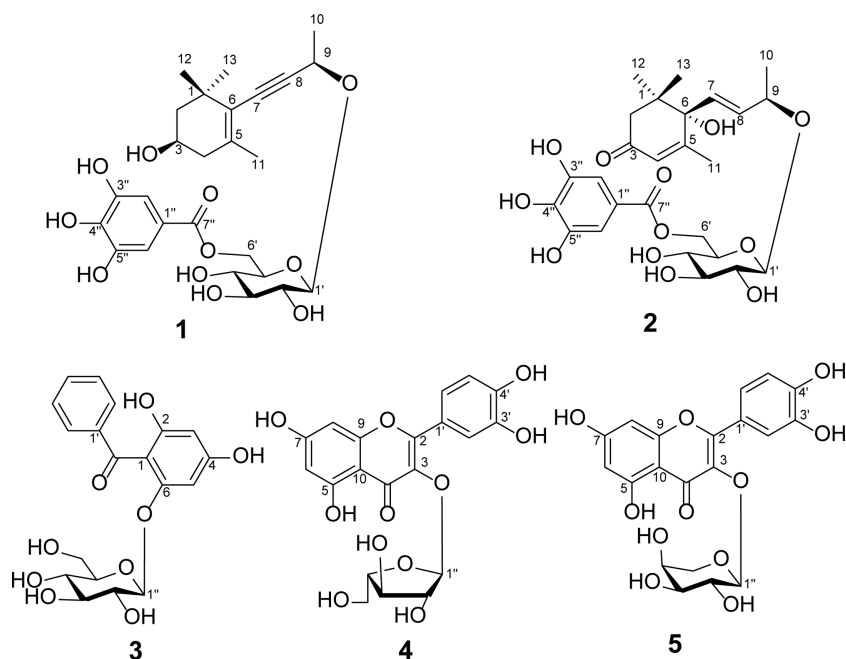


Fig. 1. Chemical structures of compounds **1–5** isolated from *P. guajava* leaves.

(Fig. 2). The large vicinal coupling between H-3 and H-4 ($J = 9.6$ Hz) supported an axial orientation of H-3; comparison with the related glucoside and synthetic stereoisomers supported the 3*R*,9*R* assignment.^{14,15} Accordingly, compound **1** was assigned as (3*R*,9*R*)-3-hydroxy-7,8-didehydro- β -ionol 9-*O*-(6'-*O*-galloyl)- β -D-glucopyranoside.

Compound **2** was characterized by a galloyl singlet at δ_{H} 7.10 (2H) and a β -glucosyl anomeric doublet at δ_{H} 4.43 (1H, $J = 7.8$ Hz), along with signals attributable to a blumenol A-type aglycone.¹⁶ The glucose C-6' signal appeared at δ_{C} 64.7, downfield from that expected for an unsubstituted glucosyl hydroxymethyl carbon, supporting galloylation at C-6'. These spectroscopic features supported its assignment as mallophenol B.¹⁷

Compound **3** showed signals characteristic of a benzophenone framework together with a glucopyranosyl moiety. The carbon data comprised one ketone carbonyl, twelve aromatic carbons corresponding to two benzene rings, and six resonances from a hexose. Agreement with published reference data supported assignment of **3** as garcimangosone D.¹⁸

Compounds **4** and **5** shared the characteristic aromatic resonances of quercetin glycosides, whereas their sugar signals differentiated an α -L-arabinofuranosyl unit in **4** from an α -L-arabinopyranosyl unit in **5**. These data supported assignment of the two compounds as quercetin-3-*O*- α -L-arabinofuranoside¹⁹ and quercetin-3-*O*- α -L-arabinopyranoside,²⁰ respectively.

Antimicrobial activities of the isolated compounds are summarized in Table 2. Compound **1** showed the strongest activity, inhibiting *S. aureus* and *C. albicans* at 64 $\mu\text{g/mL}$ and *E. faecalis* and *B. cereus* at 128 $\mu\text{g/mL}$. Compounds **2** and **3** gave MIC values of 128–256 $\mu\text{g/mL}$ against the susceptible Gram-positive strains and 128 $\mu\text{g/}$

mL against *C. albicans*. Compounds **4** and **5** showed weaker activity against the Gram-positive bacteria (MIC = 256 $\mu\text{g/mL}$) but inhibited *C. albicans* at 128 $\mu\text{g/mL}$. No activity was detected against *E. coli*, *P. aeruginosa*, or *S. enterica* at the highest concentration examined (256 $\mu\text{g/mL}$). Overall, the activity profile was selective and modest, and compound **1** was the most active of the tested isolates.

Garcimangosone D (**3**) and quercetin-3-*O*- α -L-arabinopyranoside (**5**) were previously reported to inhibit *E. coli* and *S. aureus*, with stronger activity against *S. aureus*.²¹ Quercetin-3-*O*- α -L-arabinofuranoside (**4**) has also shown antifungal activity against *Candida* spp., with reported MIC values of 2–32 $\mu\text{g/mL}$ in a study of *Myrcia tomentosa* leaves.²² To the best of our knowledge, no previous antibacterial or antifungal activity of mallophenol B (**2**) has been reported.

Megastigmane glycosides have also been evaluated in antimicrobial studies involving *E. faecalis*, *S. aureus*, and *C. albicans*, although activity varies with structure.²³ A 6'-*O*-galloylated megastigmane glucoside from *Gymnosporia senegalensis* has likewise been evaluated for antibacterial and antifungal activity,²⁴ and antimicrobial effects of megastigmane glycosides have been summarized in a recent review.²⁵ In the present series, both compounds **1** and **2** are 6'-*O*-galloylated megastigmane glucosides; however, compound **1** showed twofold lower MIC values than compound **2** against both *S. aureus* and *C. albicans* (64 $\mu\text{g/mL}$ for compound **1** vs 128 $\mu\text{g/mL}$ for compound **2**). This finding suggests that differences in the megastigmane aglycone, rather than galloylation alone, may contribute to antimicrobial activity. However, given the limited number of compounds examined, this structure–activity relationship should be considered preliminary.

Overall, investigation of *P. guajava* leaves yielded a

Table 2. Antimicrobial activities of compounds **1**–**5**

Compounds	MIC ($\mu\text{g/mL}$)						
	Gram (+)			Gram (–)			Yeast
	<i>E. faecalis</i>	<i>S. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. enterica</i>	<i>C. albicans</i>
1	128	64	128	–	–	–	64
2	128	128	256	–	–	–	128
3	128	128	128	–	–	–	128
4	256	256	256	–	–	–	128
5	256	256	256	–	–	–	128
Streptomycin	256	256	128	32	256	128	
Cycloheximide							32

(–) No antimicrobial activity was observed at the highest tested concentration (MIC > 256 $\mu\text{g/mL}$). Streptomycin and cycloheximide were used as positive controls for bacteria and *C. albicans*, respectively.

new galloylated megastigmane glycoside (**1**) along with four previously reported compounds (**2–5**). Antimicrobial testing showed preferential activity toward Gram-positive bacteria and *C. albicans*, while compound **1** displayed the lowest MIC values in the series. These results add a galloylated megastigmane glycoside to the reported chemistry of *P. guajava* and define the antimicrobial profile of the isolated leaf constituents.

Acknowledgments

This research was funded by the Vietnam Academy of Science and Technology (Grant No. UQĐTCB.06/24-26).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

- (1) GBD 2021 Antimicrobial Resistance Collaborators. *Lancet* **2024**, *404*, 1199–1226.
- (2) World Health Organization. *Global antibiotic resistance surveillance report 2025*; World Health Organization: Geneva, 2025.
- (3) Gutiérrez, R. M. P.; Mitchell, S.; Solis, R. V. *J. Ethnopharmacol.* **2008**, *117*, 1–27.
- (4) Díaz-de-Cerio, E.; Verardo, V.; Gómez-Caravaca, A. M.; Fernández-Gutiérrez, A.; Segura-Carretero, A. *Int. J. Mol. Sci.* **2017**, *18*, 897.
- (5) Das, M.; Goswami, S. *Int. J. Health Sci. Res.* **2019**, *9*, 39–45.
- (6) Dhiman, A.; Nanda, A.; Ahmad, S.; Narasimhan, B. *J. Pharm. Bioallied Sci.* **2011**, *3*, 226–229.
- (7) Soliman, F. M.; Fathy, M. M.; Salama, M. M.; Saber, F. R. *Bull. Fac. Pharm. Cairo Univ.* **2016**, *54*, 219–225.
- (8) Begum, S.; Hassan, S. I.; Ali, S. N.; Siddiqui, B. S. *Nat. Prod. Res.* **2004**, *18*, 135–140.
- (9) Díaz-de-Cerio, E.; Gómez-Caravaca, A. M.; Verardo, V.; Fernández-Gutiérrez, A.; Segura-Carretero, A. *J. Funct. Foods* **2016**, *22*, 376–388.
- (10) Matsuzaki, K.; Ishii, R.; Kobiyama, K.; Kitanaka, S. *J. Nat. Med.* **2010**, *64*, 252–256.
- (11) Shao, M.; Wang, Y.; Huang, X.-J.; Fan, C.-L.; Zhang, Q.-W.; Zhang, X.-Q.; Ye, W.-C. *J. Asian Nat. Prod. Res.* **2012**, *14*, 348–354.
- (12) Shao, M.; Wang, Y.; Liu, Z.; Zhang, D.-M.; Cao, H.-H.; Jiang, R.-W.; Fan, C.-L.; Zhang, X.-Q.; Chen, H.-R.; Yao, X.-S. *Org. Lett.* **2010**, *12*, 5040–5043.
- (13) Gharat, Y. P.; Abdelhamid, A. G.; Yousef, A. E. *Front. Cell. Infect. Microbiol.* **2025**, *15*, 1662291.
- (14) Shao, J.-H.; Chen, J.; Zhao, C.-C.; Shen, J.; Liu, W.-Y.; Gu, W.-Y.; Li, K.-H. *Nat. Prod. Res.* **2019**, *33*, 2662–2667.
- (15) Yamano, Y.; Watanabe, Y.; Watanabe, N.; Ito, M. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2833–2844.
- (16) Erosa-Rejón, G.; Peña-Rodríguez, L. M.; Sterner, O. *J. Mex. Chem. Soc.* **2009**, *53*, 44–47.
- (17) Wei, K.; Li, W.; Koike, K.; Liu, L.; Fu, X.; Lin, L.; Chen, Y.; Nikaido, T. *Chem. Pharm. Bull.* **2004**, *52*, 776–779.
- (18) Huang, Y.-L.; Chen, C.-C.; Chen, Y.-J.; Huang, R.-L.; Shieh, B.-J. *J. Nat. Prod.* **2001**, *64*, 903–906.
- (19) Rodrigues, E. D.; da Silva, D. B.; de Oliveira, D. C. R.; da Silva, G. V. *J. Magn. Reson. Chem.* **2009**, *47*, 1095–1100.
- (20) Schieber, A.; Hilt, P.; Conrad, J.; Beifuss, U.; Carle, R. *J. Sep. Sci.* **2002**, *25*, 361–364.
- (21) Ukwueze, S. E.; Osadebe, P. O.; Okoye, F. B. *Nat. Prod. Res.* **2015**, *29*, 1728–1734.
- (22) da Silva Sa, F. A.; De Paula, J. A. M.; Dos Santos, P. A.; de Almeida Ribeiro Oliveira, L.; de Almeida Ribeiro Oliveira, G.; Liao, L. M.; De Paula, J. R.; do Rosario Rodrigues Silva, M. *Molecules* **2017**, *22*, 1100.
- (23) Nguyen, T. N.; Tam, L. T.; Pham Thi Mai, H.; Tran Thi Hong, H.; Ninh, T. N.; Cuong, D. V.; Nguyen Xuan, C.; Tran, H. Q. *Nat. Prod. Res.* **2023**, *37*, 2862–2870.
- (24) Tatsimo Ndendoung, S. J.; Tamokou, J.-D.-D.; Toume, K.; Havyarimana, L.; Ekom, S. E.; Komatsu, K. *J. Chem. Res.* **2022**, *46*, 17475198211072498.
- (25) Samra, R. M.; Othman, A.; Elsbaey, M.; Amen, Y.; Shimizu, K. *Phytochem. Lett.* **2024**, *60*, 19–89.

Received August 31, 2026

Revised September 20, 2026

Accepted September 21, 2026

SUPPORTING INFORMATION

A New Galloylated Megastigmane Glycoside from the Leaves of *Psidium guajava* and Antimicrobial Activity of the Isolated Compounds

Bui Van Thanh¹, Le Thi Huong², Nguyen Thi Van Anh¹, Nguyen Sinh Khang¹, Do Thi Quynh³, Ninh Khac Ban³, Yoo Been Rho⁴, Hoang Thi Ngoc⁵, Nguyen Thi Minh Hang³, Vu Phuong Nhung¹, Ha Thi Thu Chu¹, Nguyen Phuong Hanh¹, SeonJu Park⁶, and Seung Hyun Kim^{4,*}

¹Institute of Biology, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Nghia Do, Hanoi, Vietnam

²Institute of Physics, VAST, 10 Dao Tan, Giang Vo, Hanoi, Vietnam

³Institute of Chemistry, VAST, 18 Hoang Quoc Viet, Nghia Do, Hanoi, Vietnam

⁴College of Pharmacy, Yonsei Institute of Pharmaceutical Sciences, Yonsei University, Incheon 21983, Republic of Korea

⁵Faculty of Pharmacy and Medical Laboratory Technology, Dong Do University, 60B Nguyen Huy Tuong, Thanh Xuan, Hanoi, Vietnam

⁶Metropolitan Seoul Center, Korea Basic Science Institute, Seoul 02841, Republic of Korea

*Author for correspondence

Seung Hyun Kim, College of Pharmacy, Yonsei Institute of Pharmaceutical Sciences, Yonsei University, Incheon 21983, Republic of Korea

Tel: +82-32-749-4514; E-mail: kimsh11@yonsei.ac.kr

List of supplementary data

Figure S1. HR-ESI-MS of compound 1	3
Figure S2. UV spectrum of compound 1	3
Figure S3. ^1H -NMR spectrum of compound 1	4
Figure S4. ^{13}C -NMR spectrum of compound 1	5
Figure S5. HMBC spectrum of compound 1	6
Figure S6. HSQC spectrum of compound 1	7
Figure S7. COSY spectrum of compound 1	8
Figure S8. GC chromatogram of the TMS-sugar derivative obtained from compound 1	오류! 책갈피가 정의되어 있지 않습니다.

T: FTMS + p ESI Full ms [100.0000-800.0000]

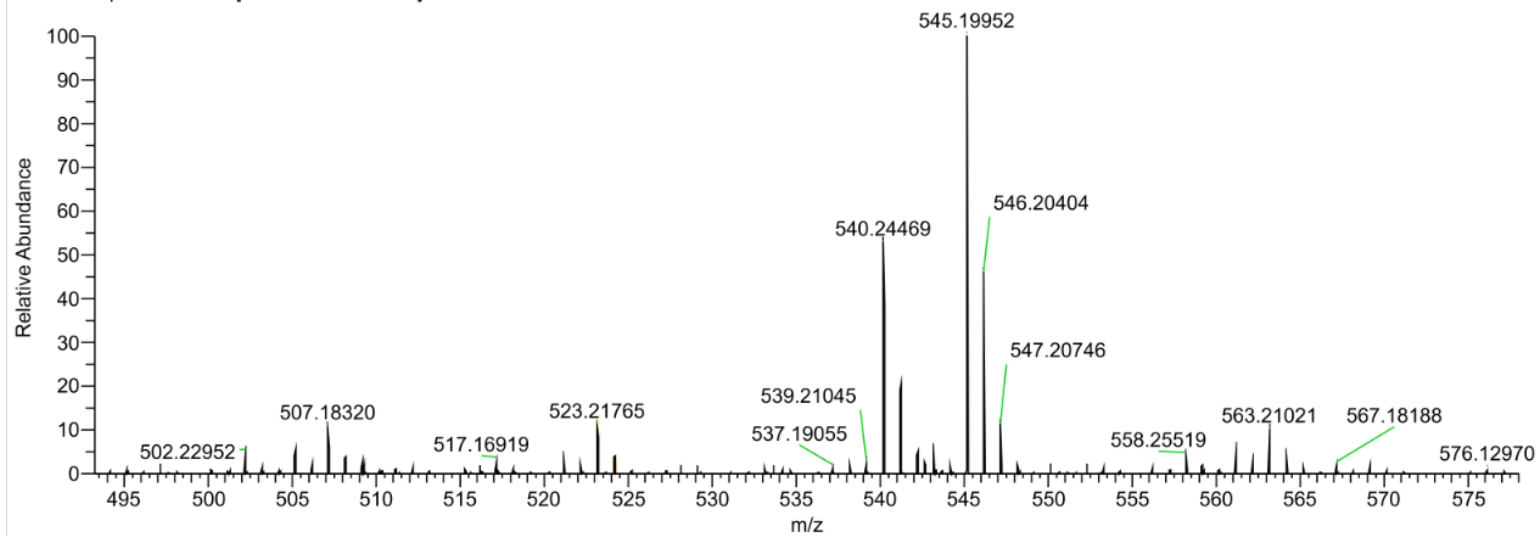


Figure S1. HR-ESI-MS of compound **1**

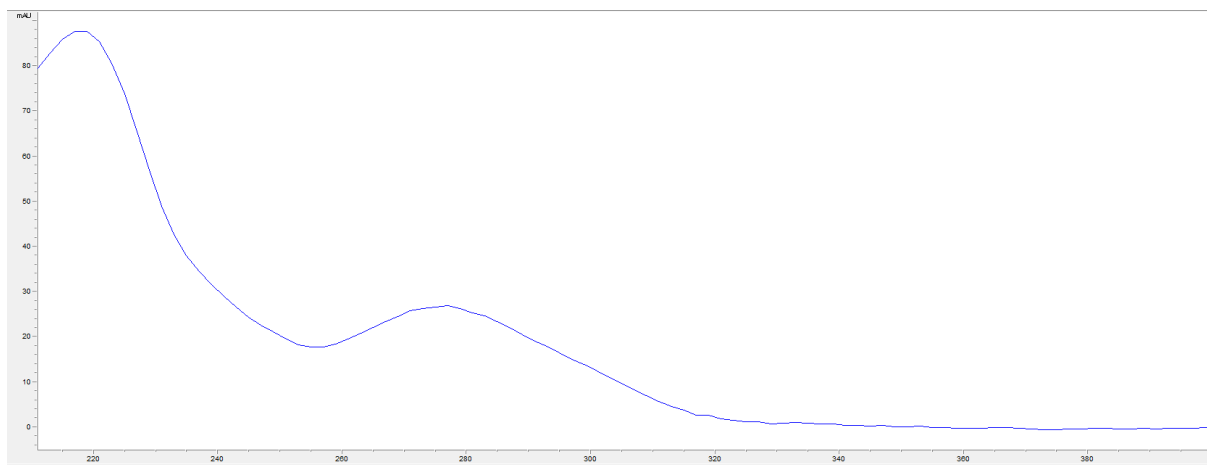


Figure S2. UV spectrum of compound **1**

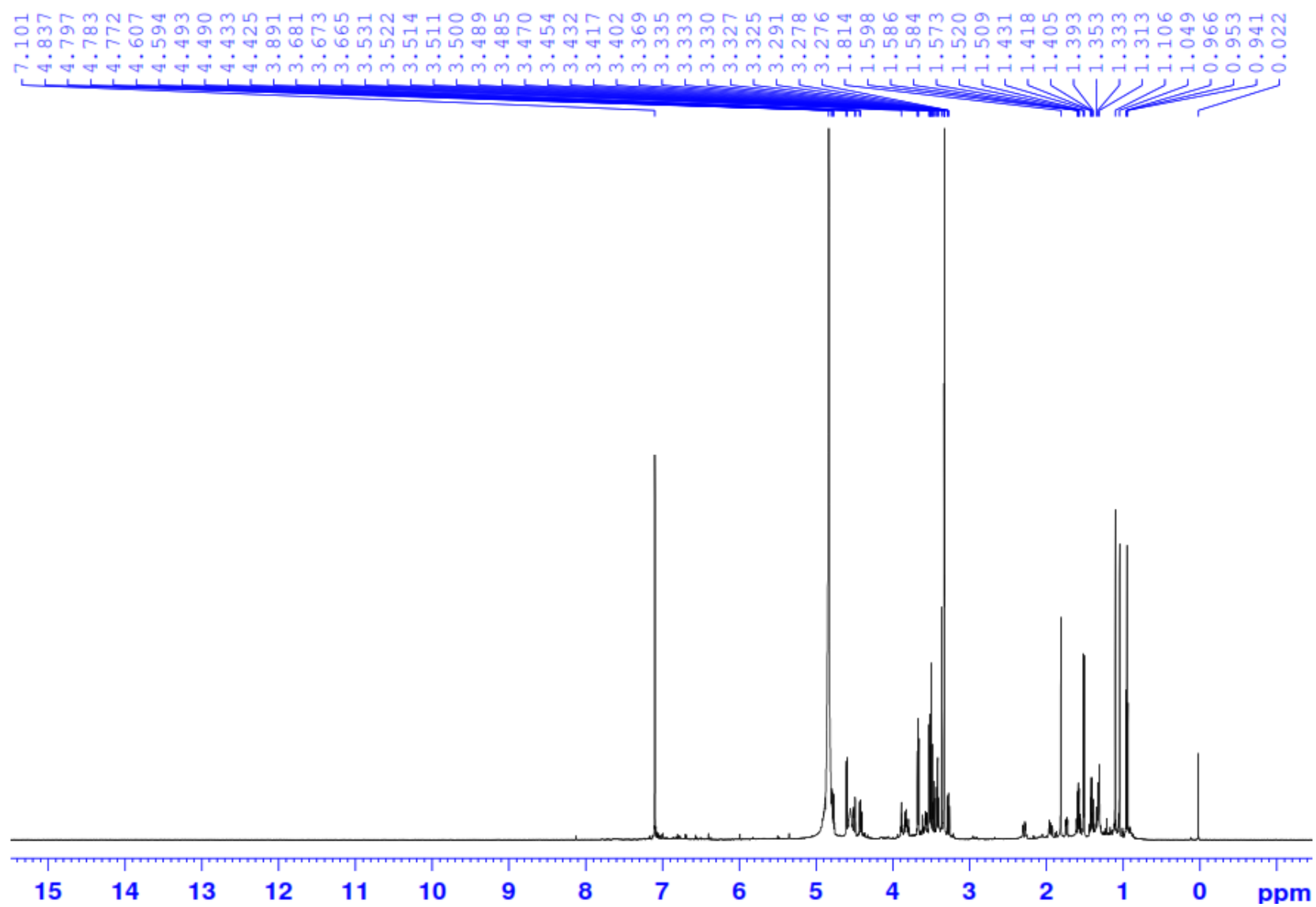


Figure S3. ^1H -NMR spectrum of compound 1

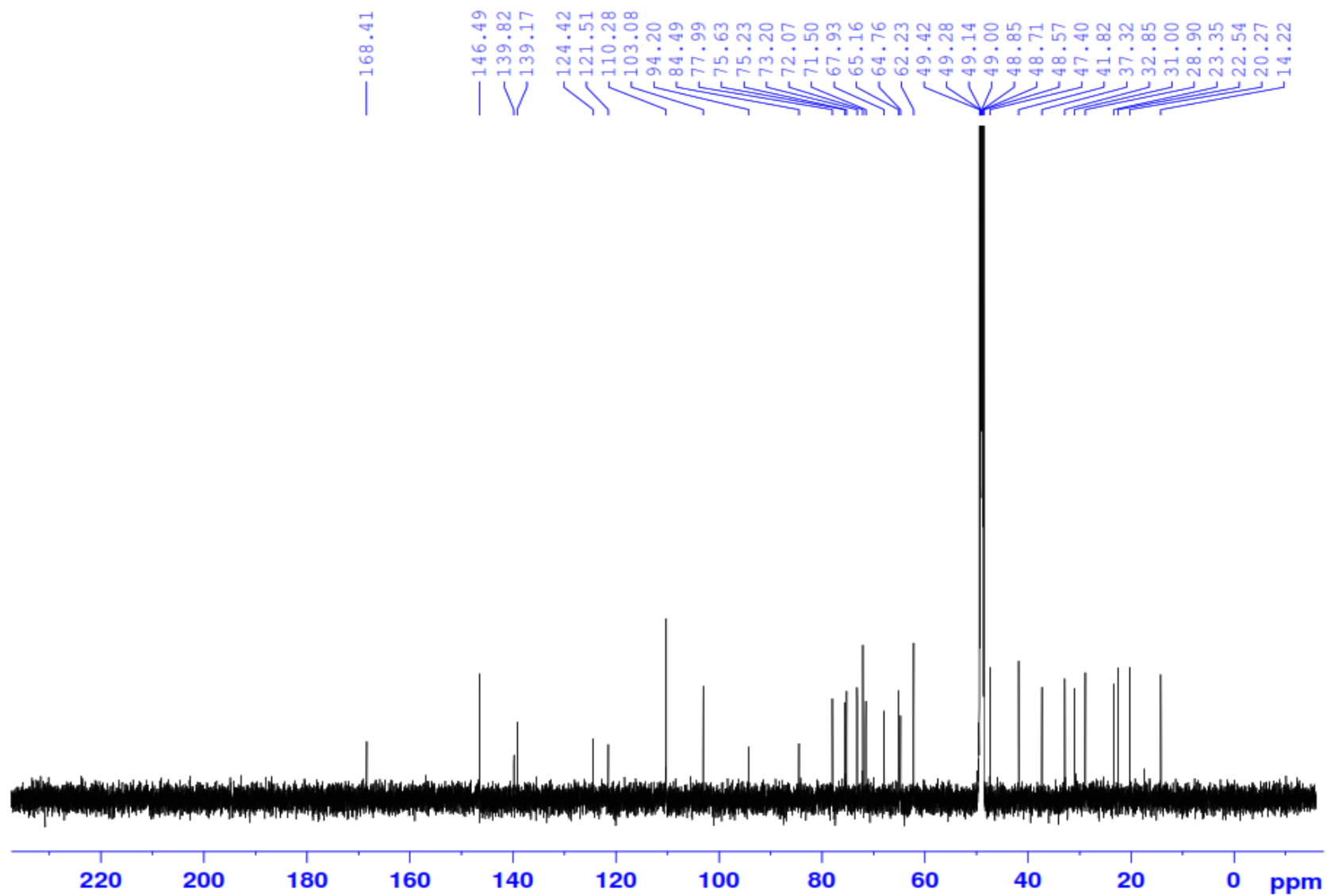


Figure S4. ^{13}C -NMR spectrum of compound **1**

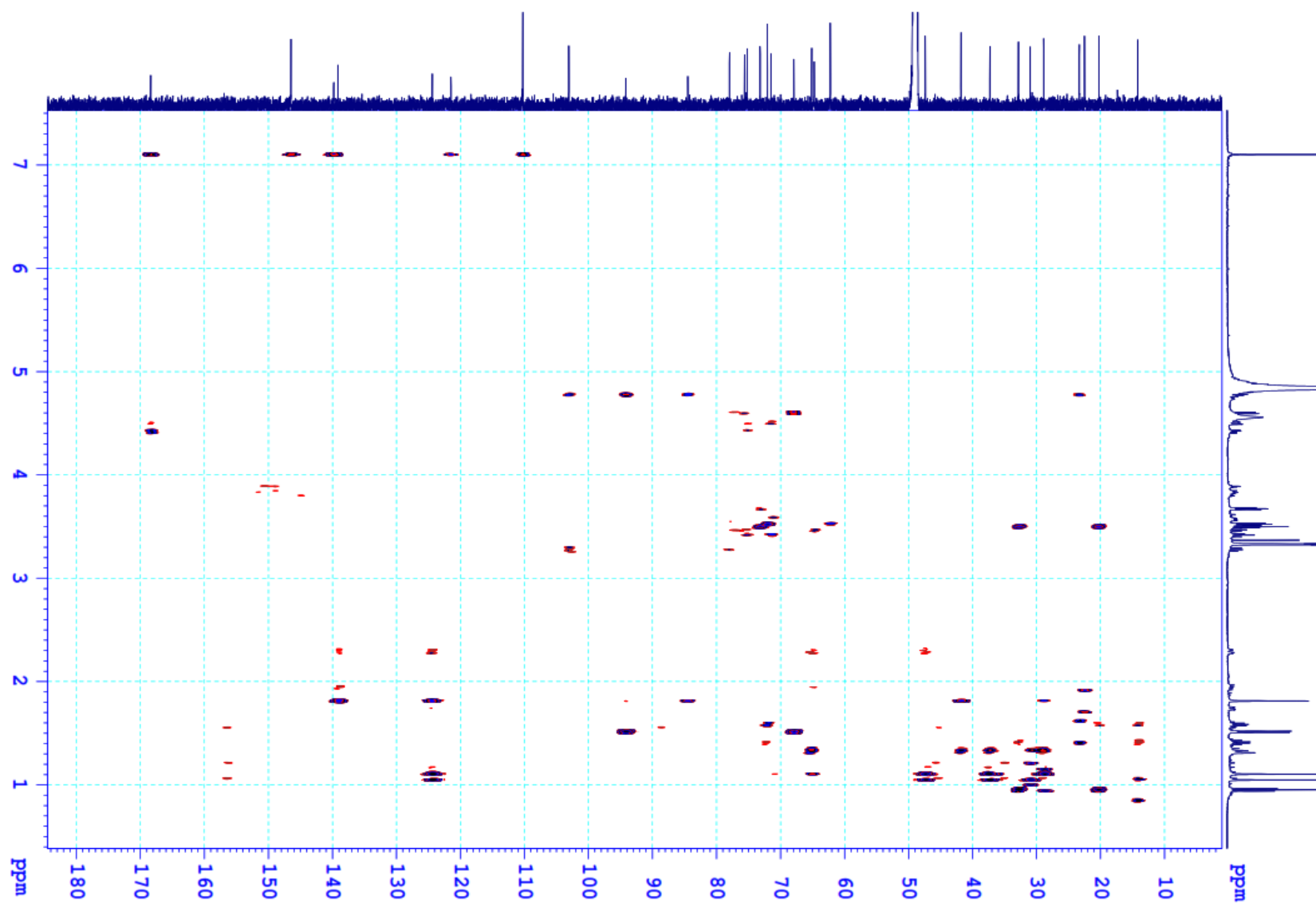


Figure S5. HMBC spectrum of compound **1**

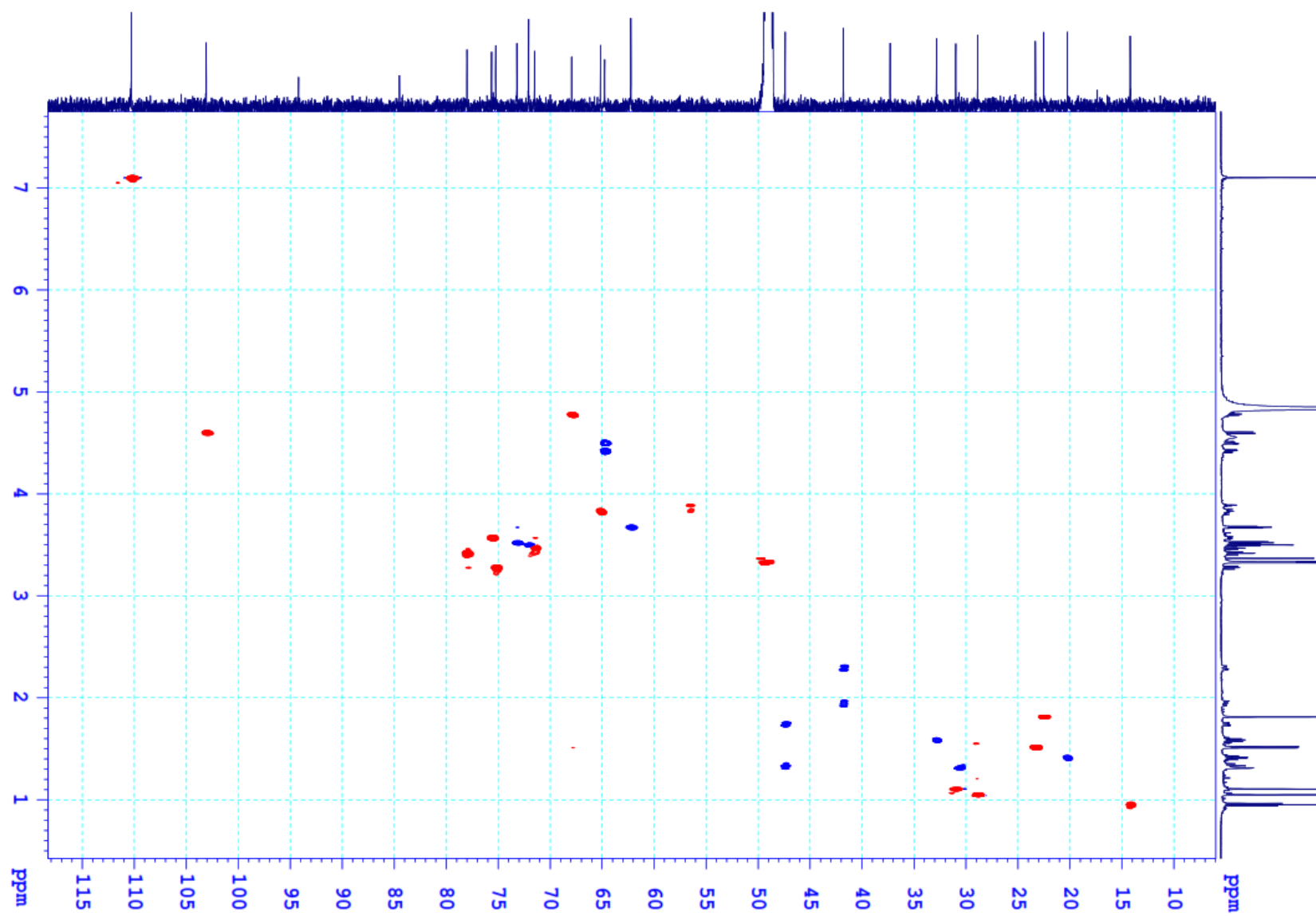


Figure S6. HSQC spectrum of compound 1

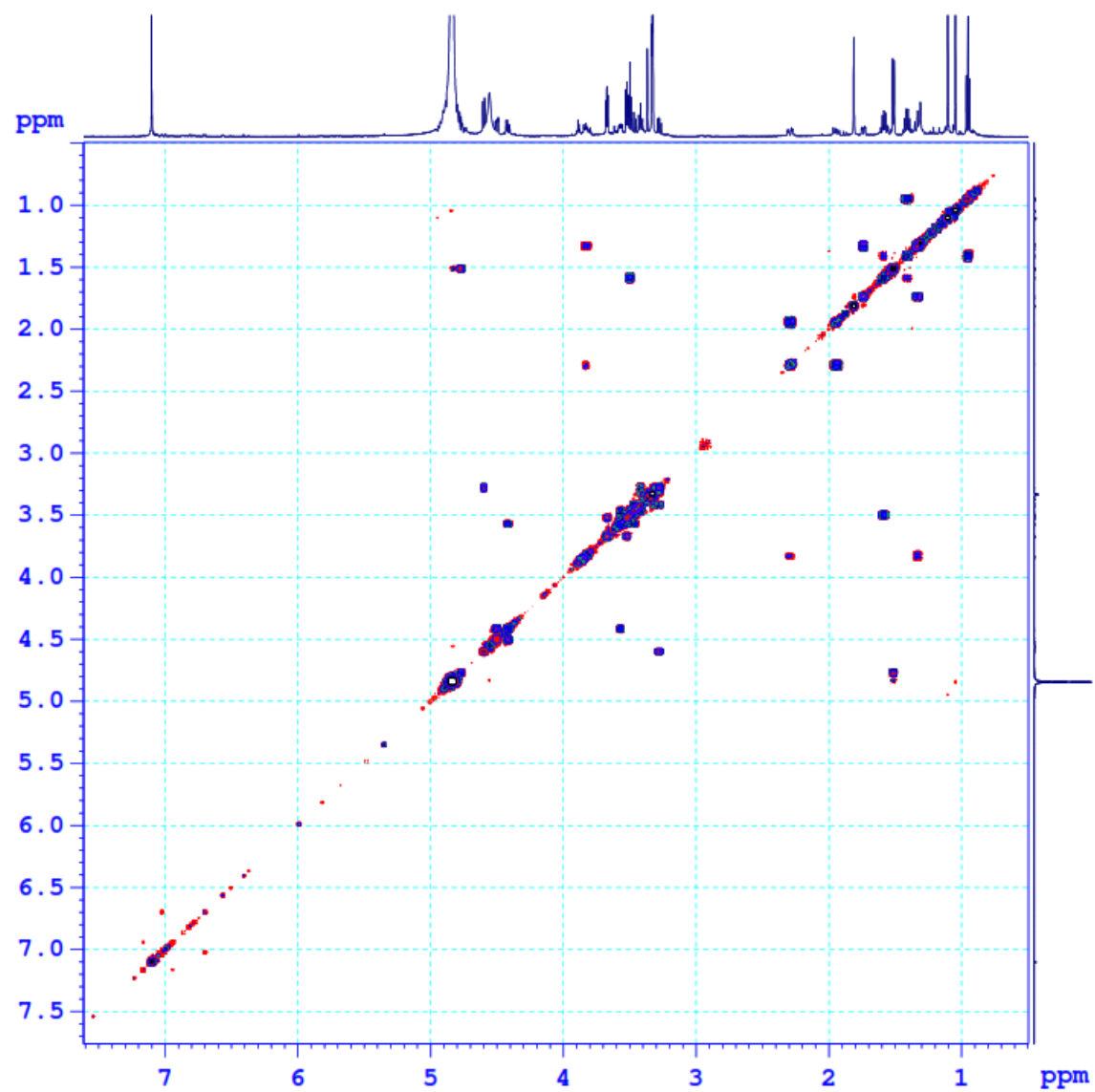


Figure S7. COSY spectrum of compound **1**

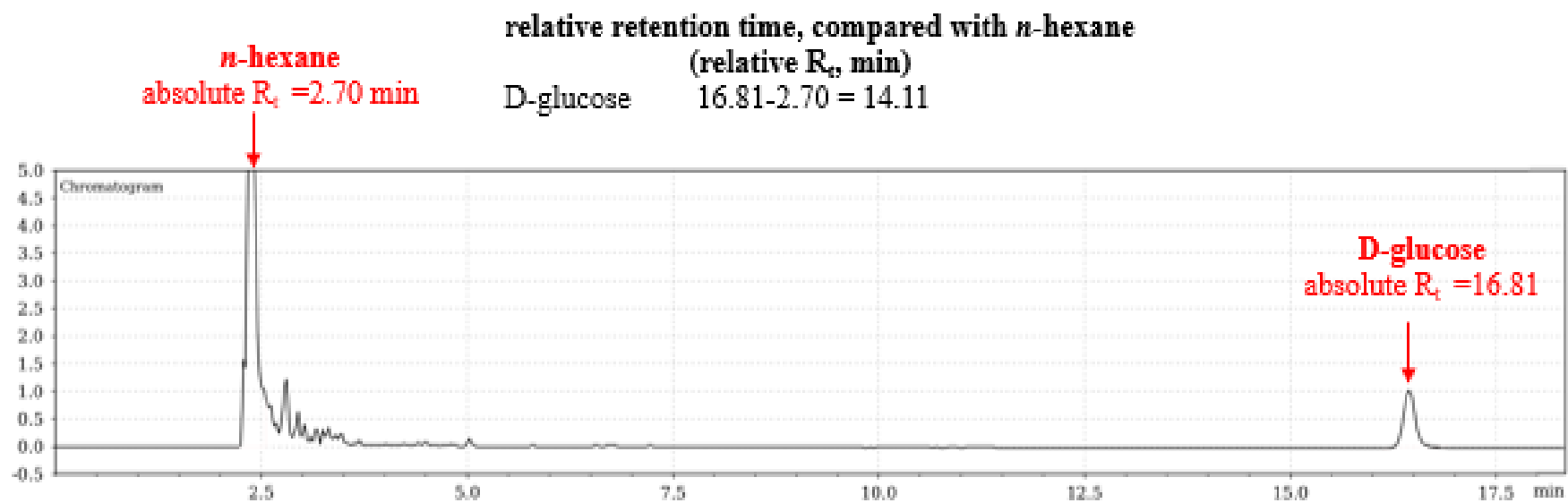


Figure S8. GC chromatogram of the TMS-sugar derivative obtained from compound **1**