Supplementary Material

Antioxidant and Anti-aging Phytoconstituents from *Faucaria tuberculosa*: *in vitro* and in *silico* studies

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Abstract

The research targeting natural cosmeceuticals is now increasing due to the safety and/or limited side effects of natural products that are highly valued in cosmetology. In a research program aimed at exploring botanical sources for valuable skin-care antioxidant components, the current study investigated the phytochemical content and the biological potential of *Faucaria tuberculosa*. Phytochemical investigation of *F. tuberculosa* extract resulted in purification and characterization of six phytoconstituents including one new. The structure of the new constituent was elucidated as catechin-(2→1′,4→2′)-phloroglucinol **(4)**. The structural identity of all isolated compounds were confirmed on the basis of extensive physical and spectral (1D, 2D-NMR and HRESIMS) investigations. The ethanolic extract exhibits a rich content of total phenolics (TPC) and total flavonoids (TFC) estimated as 32±0.034 mg GAE/g and 43± 0.004 mg RE/g, respectively. In addition, the antioxidant (ABTS and FRAP), antihyaluronidase and the antityrosinase activities of

all purified phytoconstituents were evaluated. The results noted phloroglucinol **(1)** and catechin-(2→1′,4→2′)-phloroglucinol (**4**) for remarkable antioxidant activity. While on the other side 3,5-dihydroxyphenyl *β*-D-glucopyranoside (**2**) achieved the most potent inhibitory activity against tyrosinase and hyaluronidase enzymes (IC_{50} 8.637 ± 0.44 µg/ml and 18.04 ± 0.92 µg/ml, respectively) that was comparable to the standard drugs kojic acid (IC_{50} 9.267 µg/ml) and luteolin, (IC_{50} 33.25 ± 1.69 µg/ml). Molecular docking study of 3,5-dihydroxyphenyl *β*-D-glucopyranoside (2) highlights its high potential to bind to the active sites of two enzymes involved in the study.

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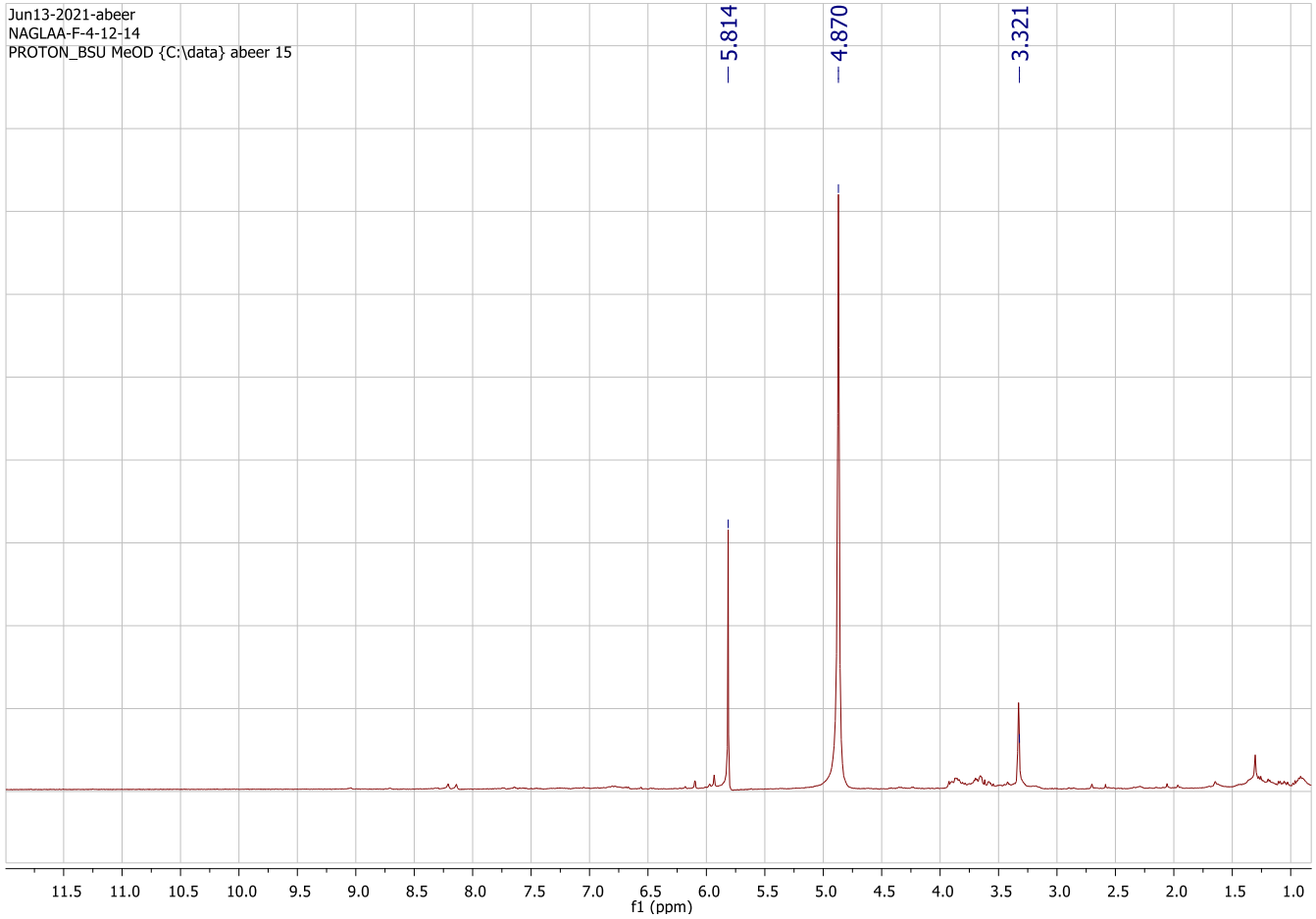
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Figure S1: ^{1}H NMR spectrum of compound 1 (400 MHz, CD_3OD).

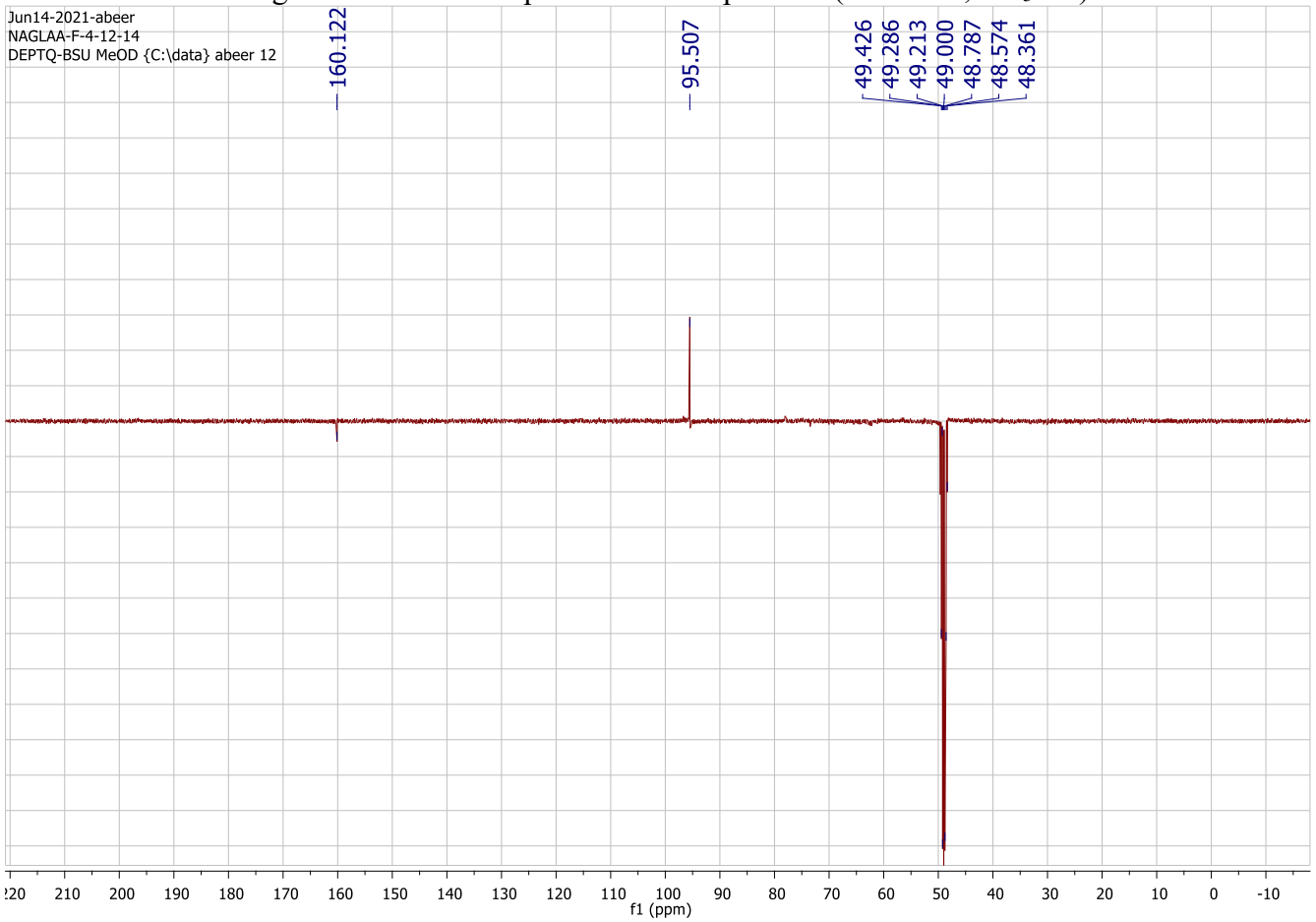

Figure S2: DEPT-Q spectrum of compound 1 (100 MHz, CD_3OD).

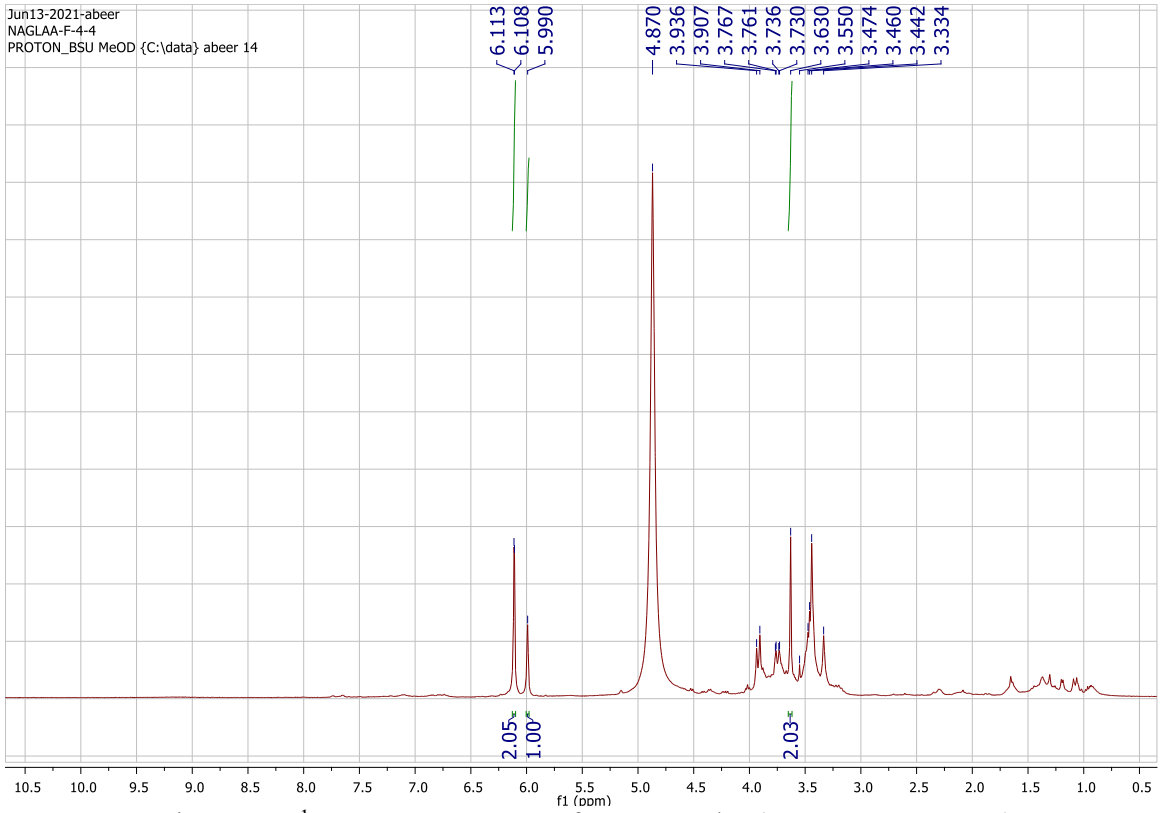

Figure S3: ^{1}H NMR spectrum of compound 2 (400 MHz, *CD_3OD*).

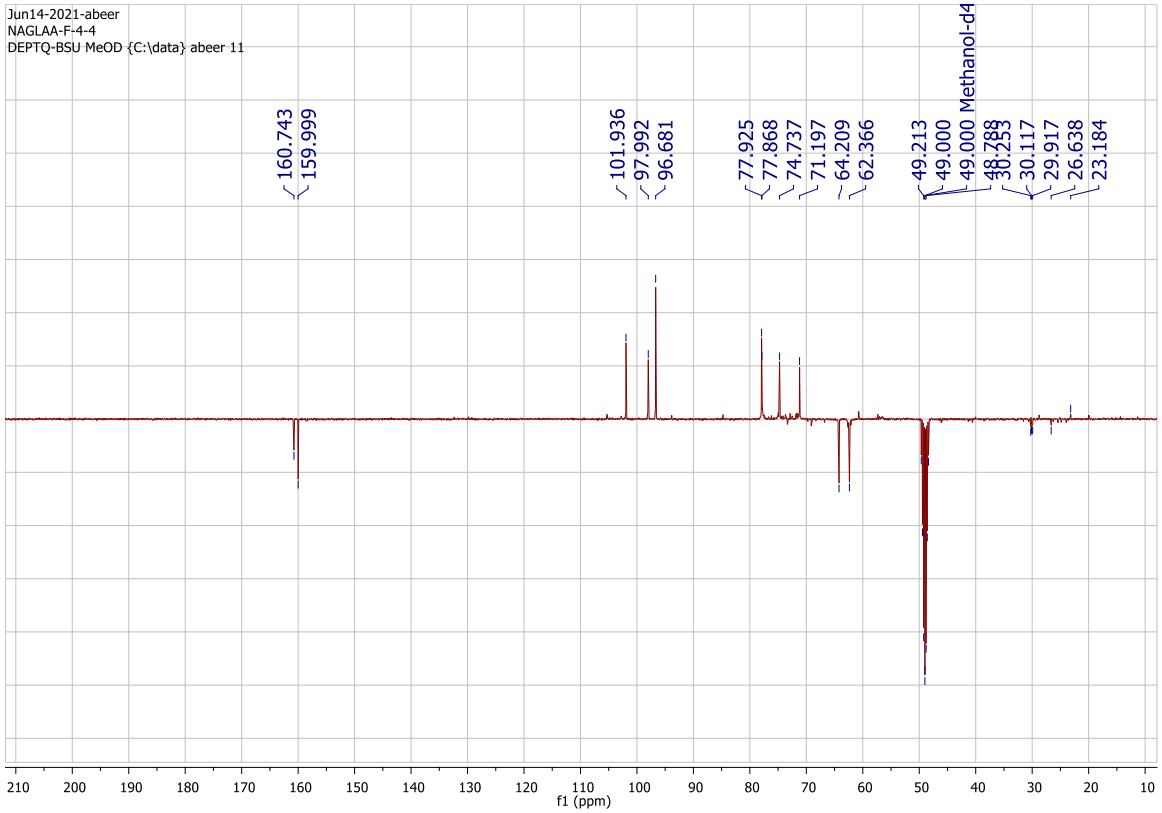

Figure S4: DEPT-Q spectrum of compound **2** (100 MHz, CD_3OD).

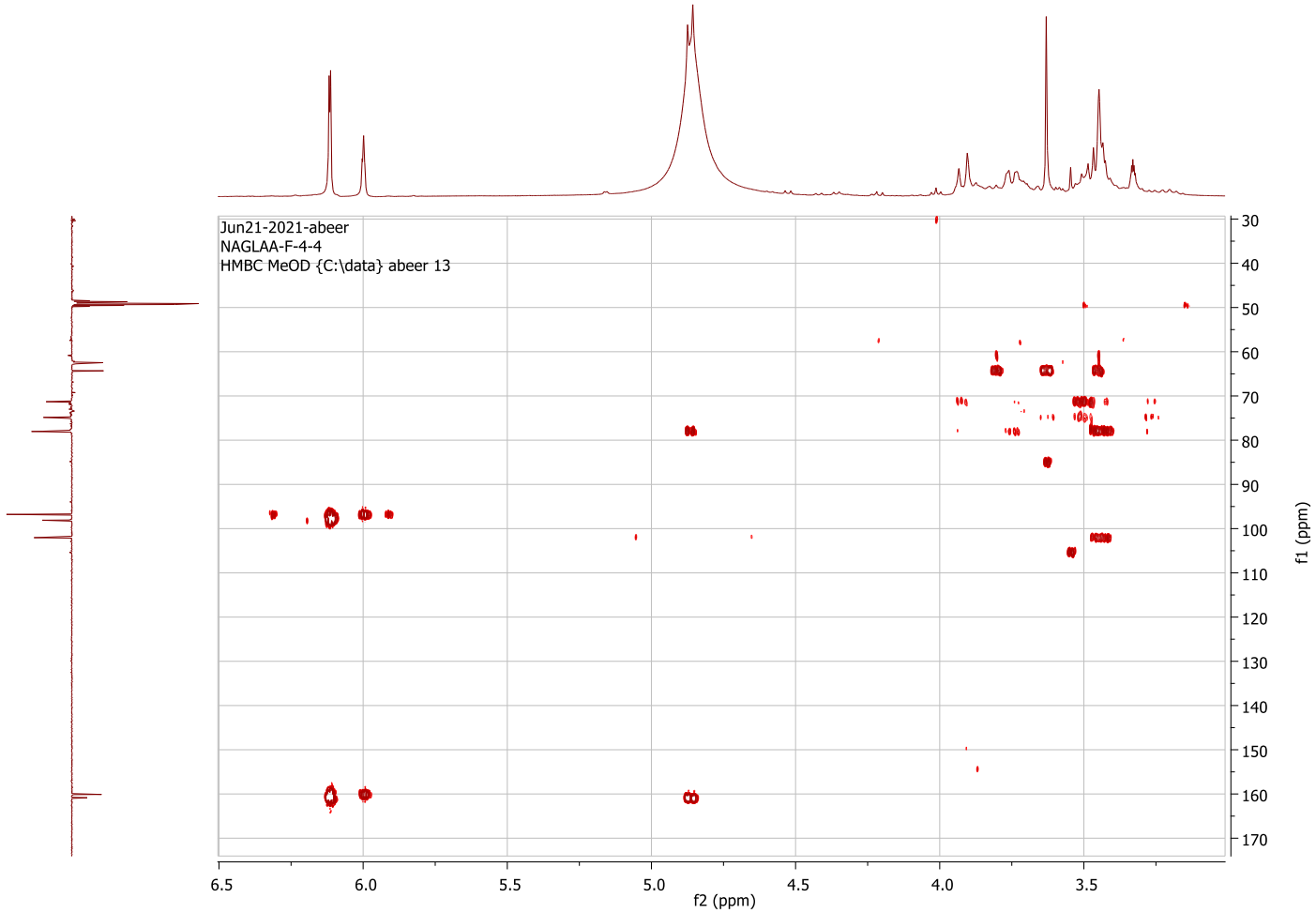

Figure S5: HMBC spectrum of compound **2**

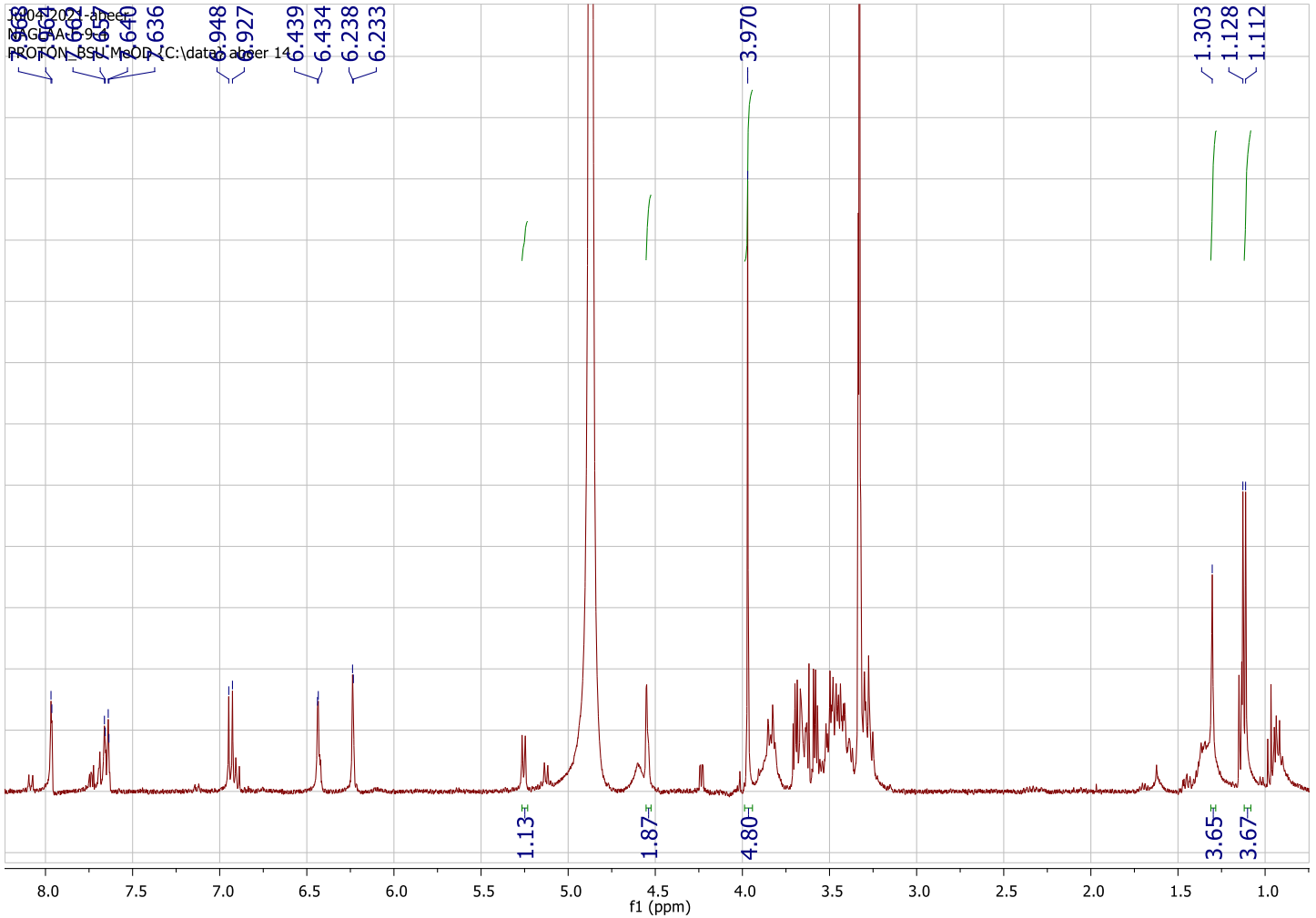

Figure S6: ^{1}H NMR spectrum of compound 3 (400 MHz, CD_3OD).

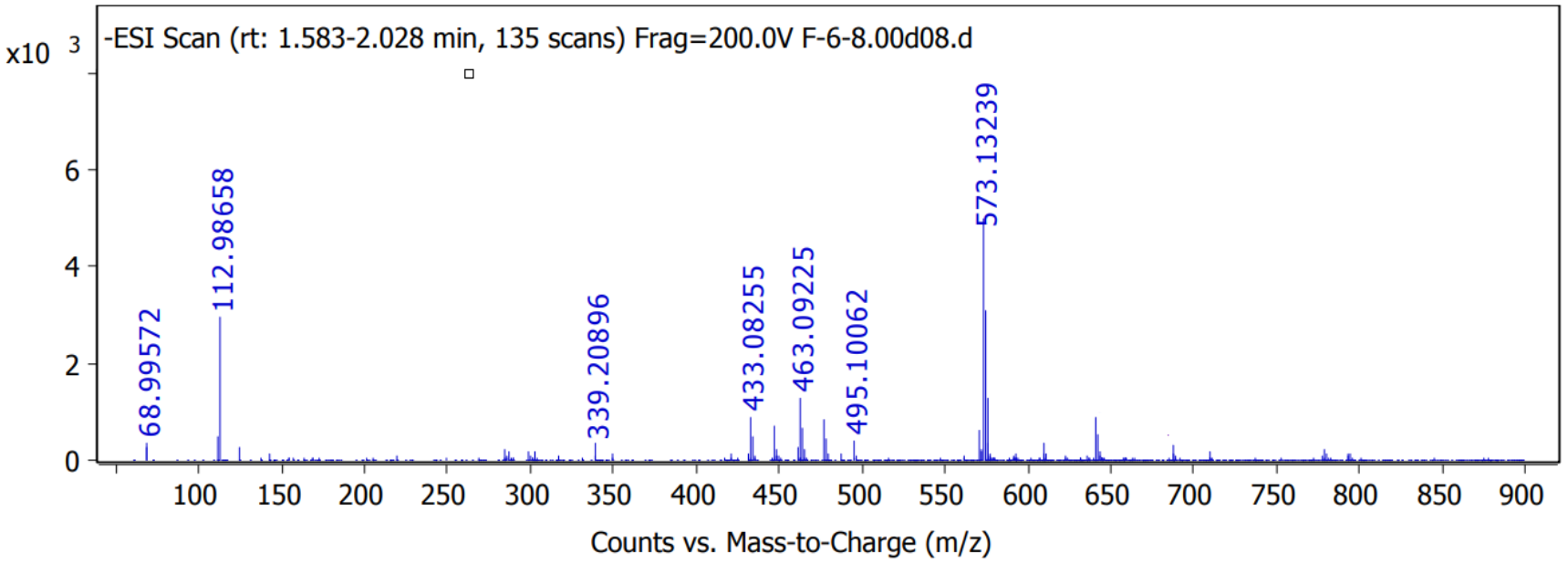

Figure S7: Negative mode HR-ESI- MS spectrum of compound **4**

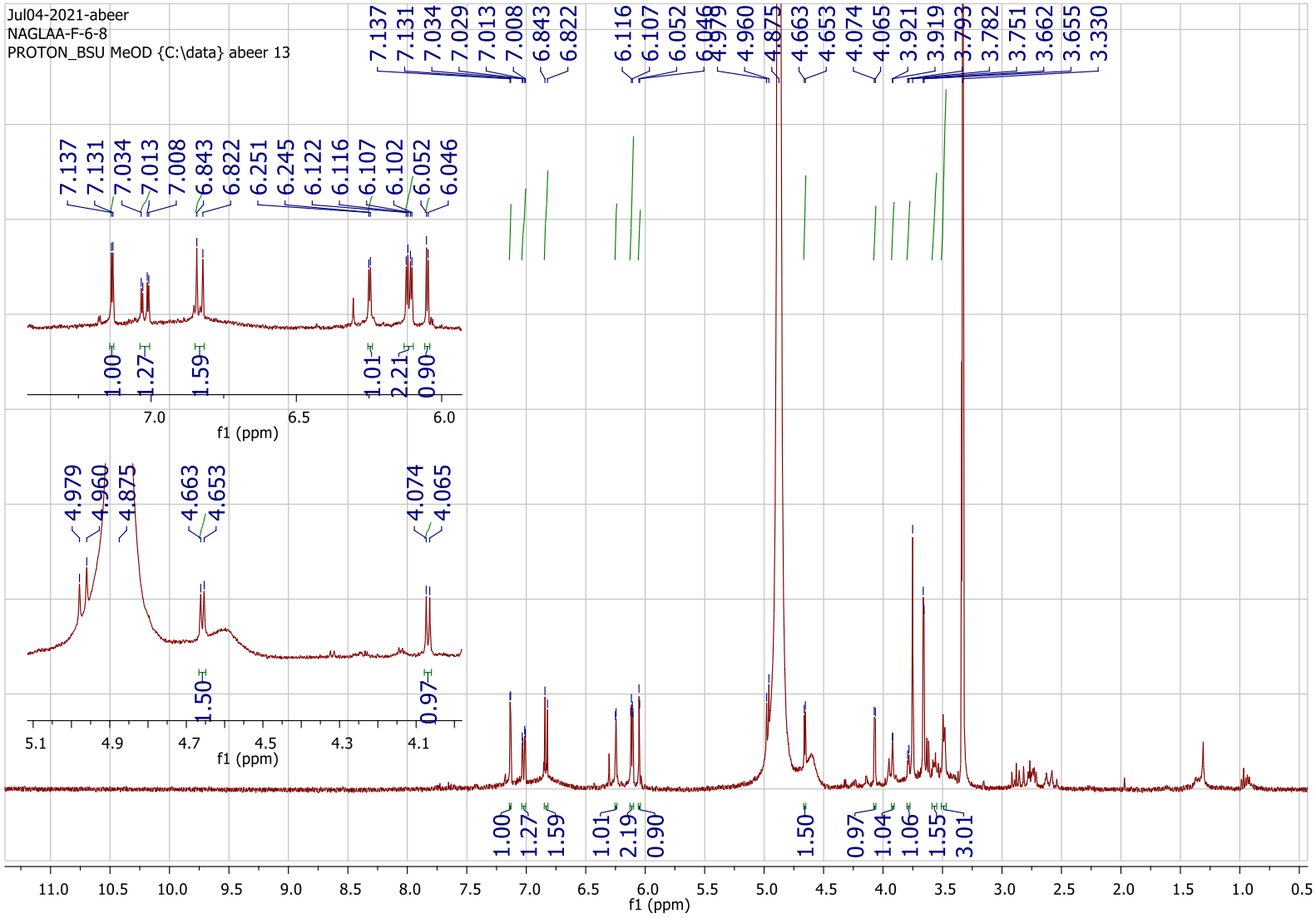

Figure S8: ^{1}H NMR spectrum of compound 4 (400 MHz, CD_3OD).

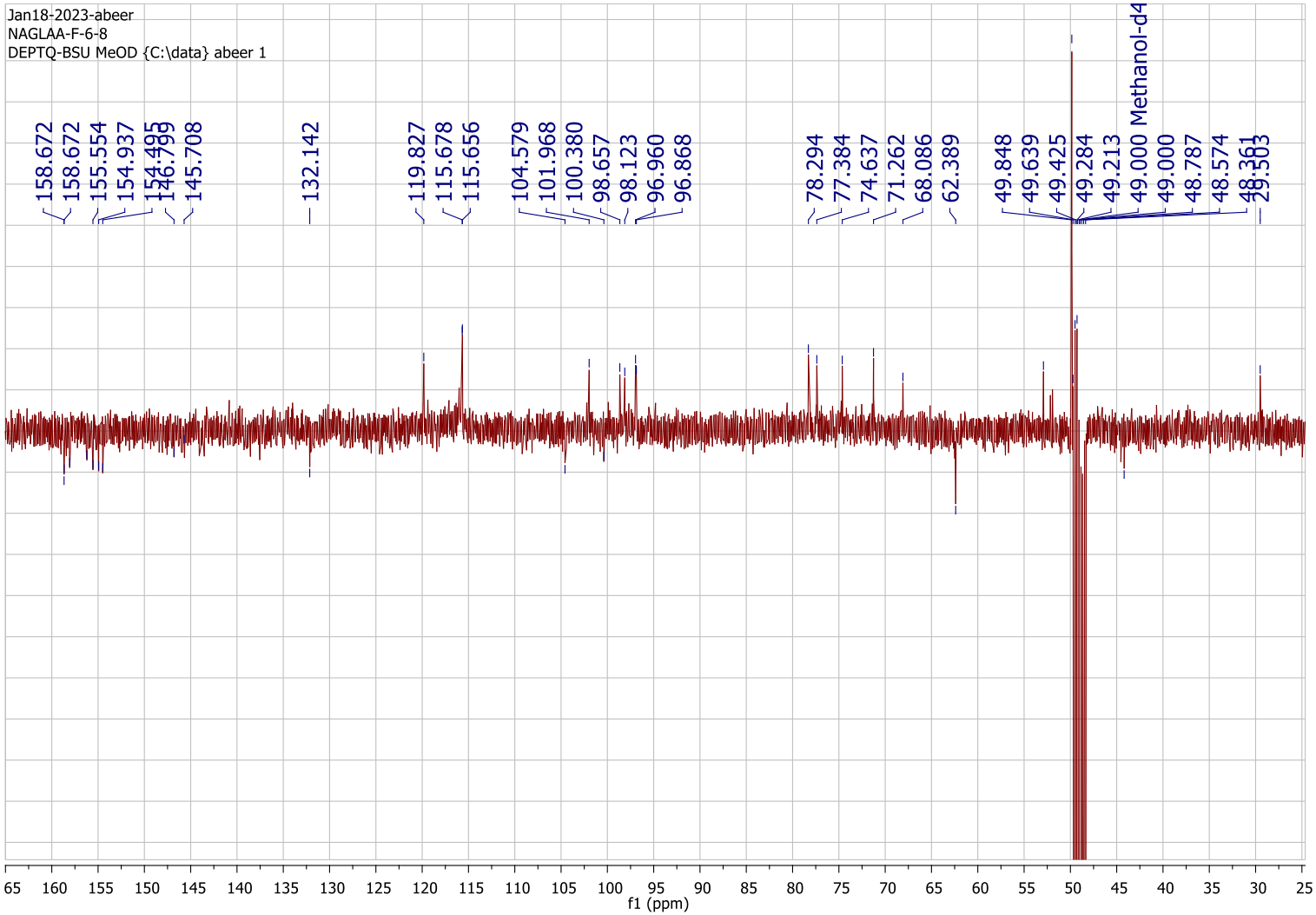

Figure S9: DEPT-Q spectrum of compound **4** (100 MHz, CD_3OD).

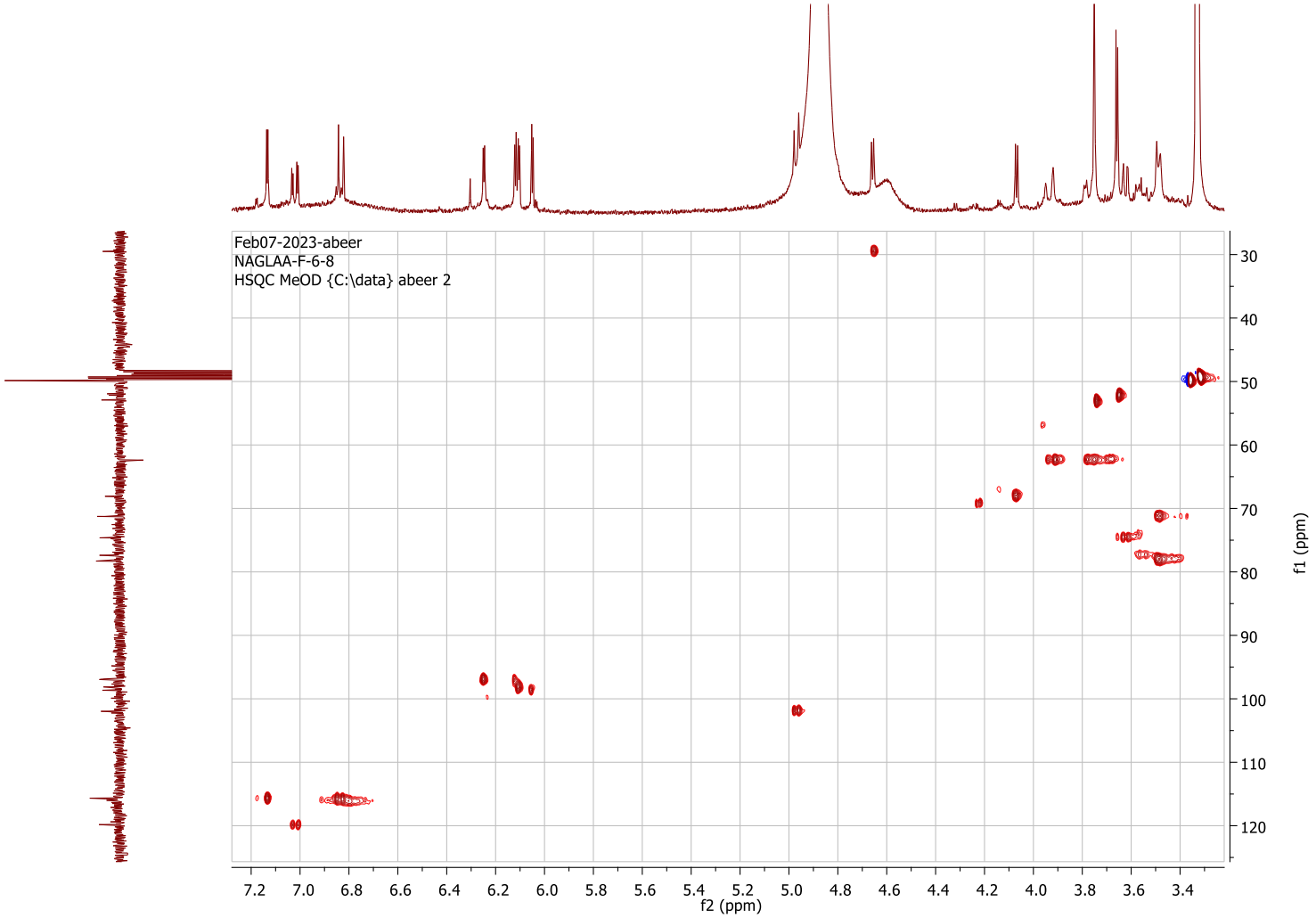

Figure S10: HSQC spectrum of compound **4**

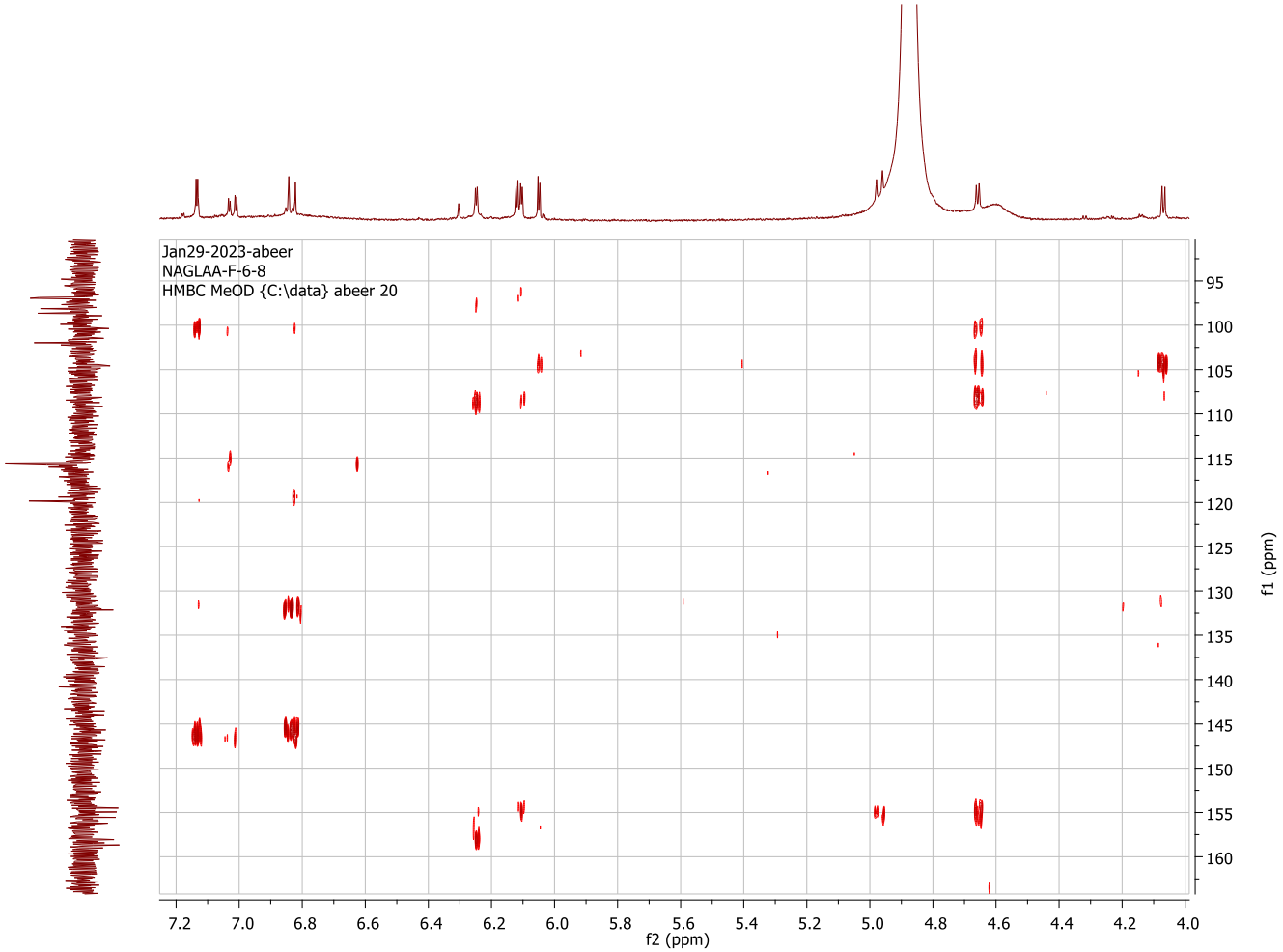

Figure S11: HMBC spectrum of compound **4**

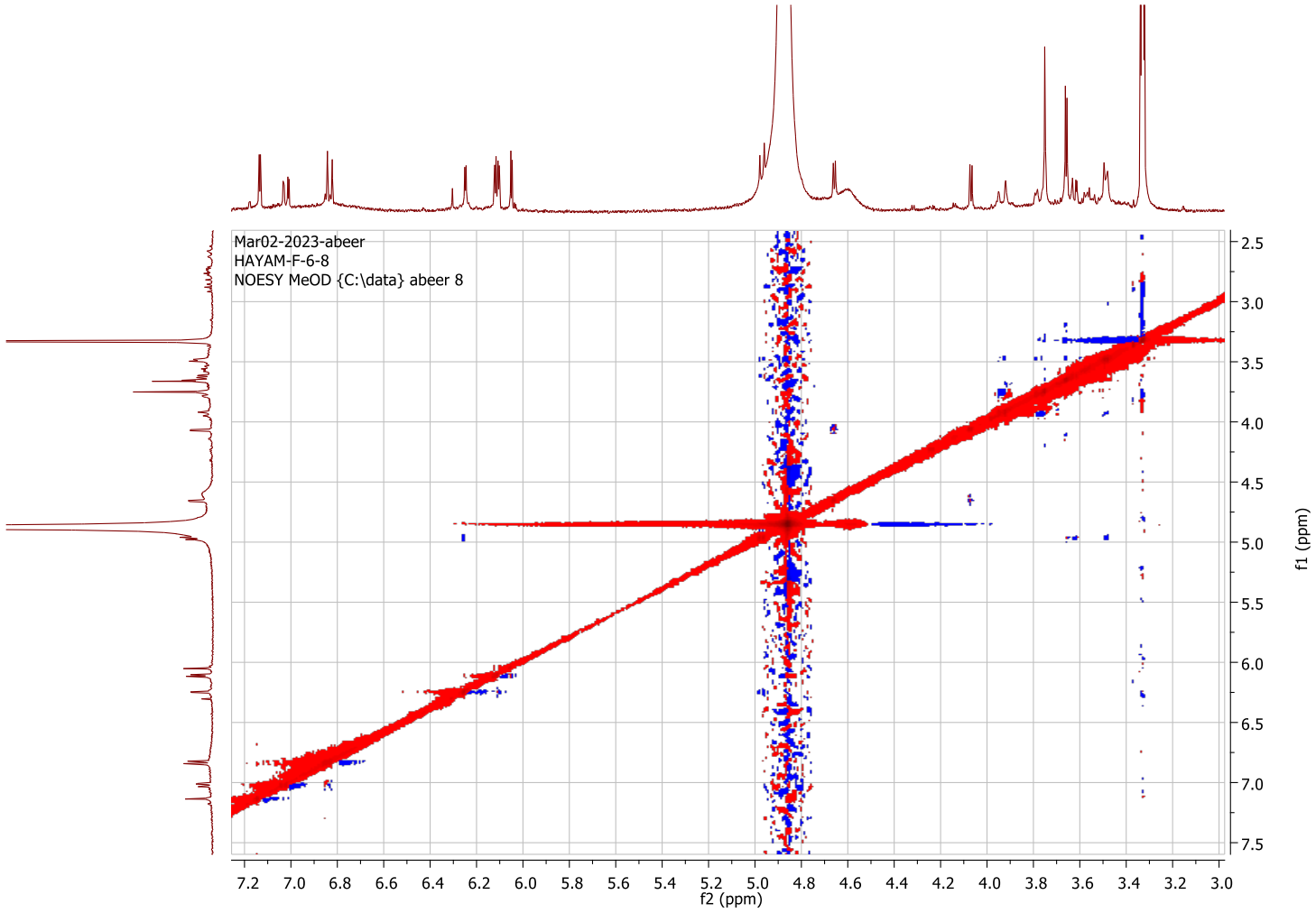

Figure S12: NOESY spectrum of compound **4**

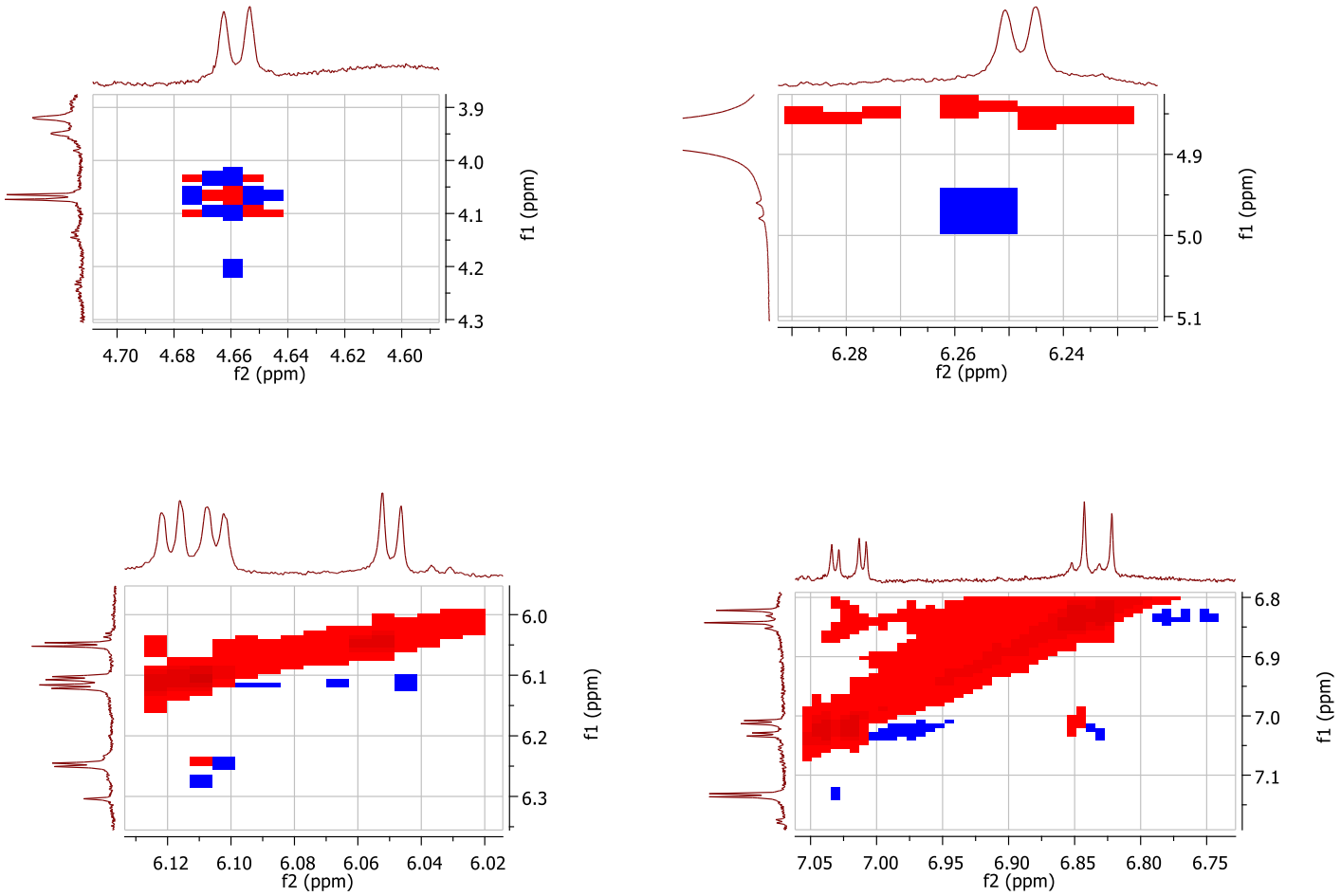

Figure S13: Expanded NOESY spectrum of compound **4**

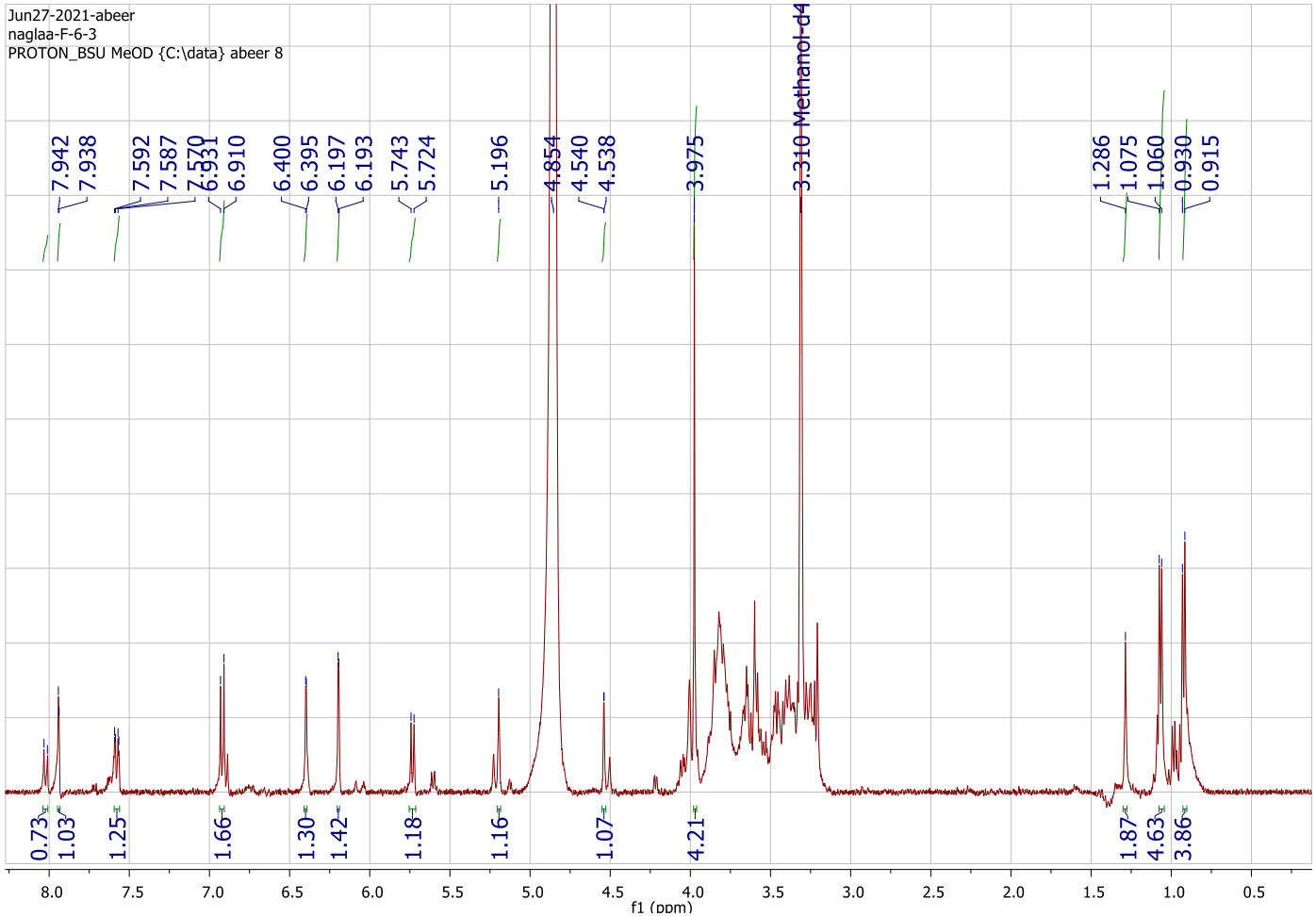

Figure S14: ^{1}H NMR spectrum of compound 5 (400 MHz, CD_3OD).

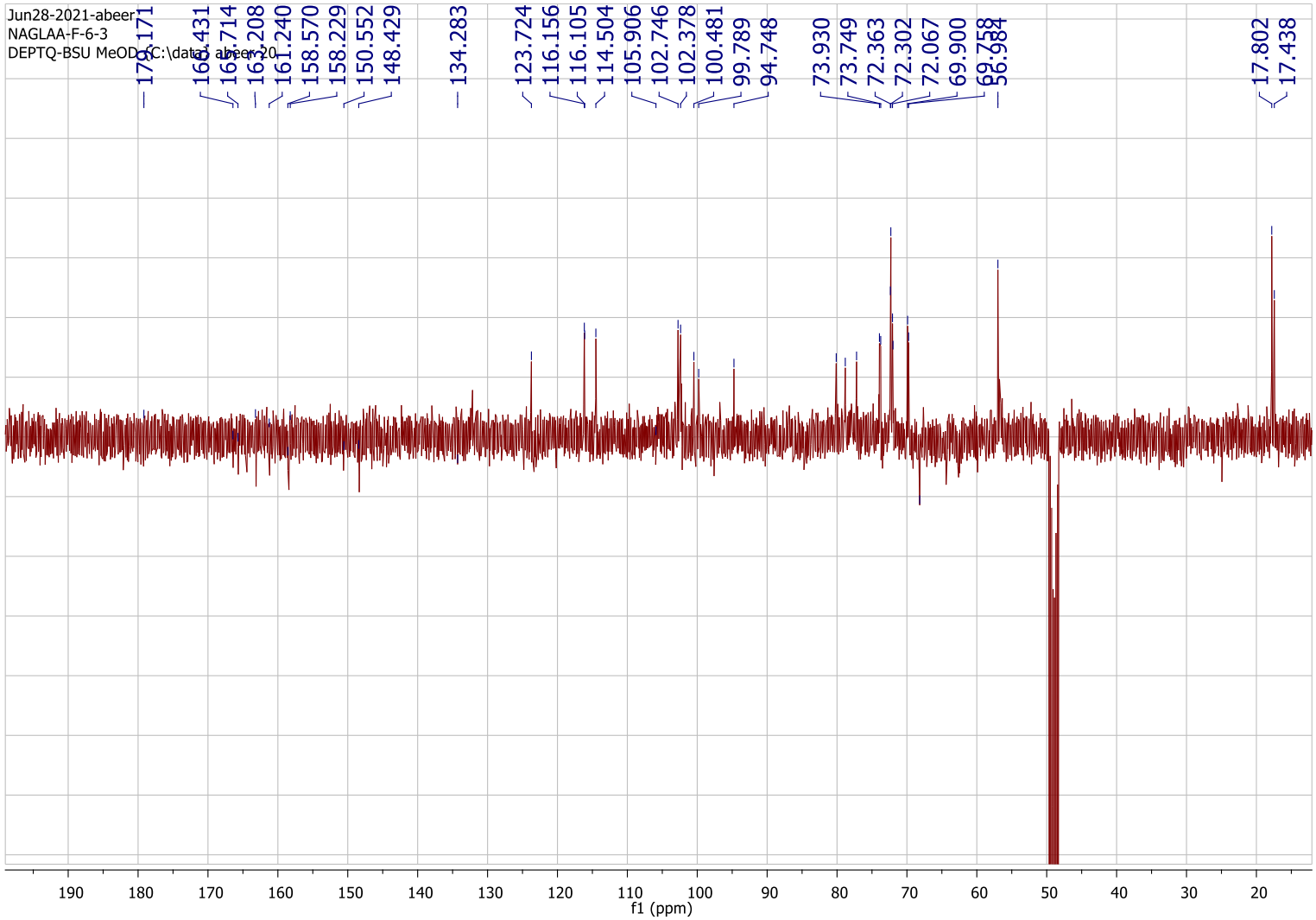

Figure S15: DEPT-Q spectrum of compound 5 (100 MHz, CD_3OD).

NMR data for compounds: **1, 2,3, 5** and **6** were included in supplementary data

Compound 1: Phloroglucinol

^{1}H-NMR (400 MHz, MeOD-d_4); δ_H 5.81 (3H, s, H-2, H-4, H-6. DEPT-Q NMR (101 MHz, MeOD-d_4); δ_C 95.5 (C-1, C-3, C-5), 160.12(C-2, C-4, C-6) (Figure S1 and S2).

Compound 2: 3,5-dihydroxyphenyl *β*-D-glucopyranoside (Phlorin); ^{1}H-NMR (400 MHz, MeOD-d_4); δ_H 6.09 (2H, d, 2, H-2 and H-6), 5.97 (1H, t, 2, H-4), 4.86 (1H, d, 7.6 Hz, H-1′), 3.61-3.42 (4H, m, H-2′,3′,4′,5′), 3.72 (1H, dd, 12.1 and 2.4 Hz, H-6′ a), 3.90 (1H, d, 11.6, H-6′ b); DEPT-Q NMR (101 MHz, MeOD-d_4); δ_C 160.7 (C-1), 159.99 (C-3 and C-5), 97.9 (C-4), 96.6 (C-2 and C-6), 101.9 (C-1′), 77.9 (*d*, C-5′), 77.8 (C-3′), 74.7 (C-2′), 71.2 (C-4′), 62.3 (C-6′) (Figure S3 : S5).

Compound 3: isorhamnetin 3-*O*-rutinoside; ^{1}H-NMR (400 MHz, MeOD-d_4); δ_H 7.94 (1H, d, 1.6, H-2′), 7.63 (1H, dd, 1.6, 8.4, H-6′), 6.91 (1H, d, 8.4, H-5′), 6.41 (1H, d, 2, H-8), 6.21 (1H, d, 2, H-6), 5.23 (1H, d, 7.6, H-1″), 4.53 (1H, br s, H-1‴), 3.2 - 3.7 (H-2″-H-6″, H-2‴-H-5‴), 1.09 (3H, d, 6.4, H-6‴) (Figure S6).

Compound 5: Isorhamnetin 3-*O*-[*α*-rhamnopyranosyl-(1-4)-α-rhamnopyranosyl-(1-6)-*β*-glucopyranoside]; ^{1}H-NMR (400 MHz, MeOD-d_4); δ_H 7.94 (1H, d, 1.6, H-2′), 7.59 (1H, dd, 2, 8.8 , H-6′), 6.92 (1H, d, 8.4, H-5′), 6.39 (1H, d, 2, H-8), 6.19 (1H, d, 2, H-6), 5.73 (1H, d, 7.6, H-1″), 5.19 (1H, br s, H-1‴), 4.54 (1H, br s, H-1⁗), 3.97 (3H, s, OCH_3), 3.00 - 4.00 (H-2″- H-6″, H-2‴-H-5‴, H- 2⁗-H-5⁗), 1.06 (3H, d, 6, H-6‴), 0.92 (3H, d, 6, H-6⁗). Multiplicities of most sugar resonances were not determined, because of signal overlap. DEPT-Q NMR (101 MHz, MeOD-d_4); δ_C 158.5 (C-2), 134.2 (C-3), 179.2 (C-4), 163.2 (C-5), (99.8) (C-6), 166.4(C-7), 94.7 (C-8), 158.2 (C-9), 105.9 (C-10), 123.3 (C-1′), 114.5 (C-2′), 150.6 (C-3′), 148.3 (C-4′), 116.2 (C-5′), 123.7 (C-6′) , 56.9 (OCH_3), 102.7 (C-1″), 73.9 (C-2″), 78.8 (C-3″), 72.0 (C-4″), 77.2 (C-5″), 68.1 (C-6″), 102.3 (C-1‴), 72.3 (C-2‴), 72.3 (C-3‴), 80.1 (C-4‴), 69.8(C-5‴), 17.8 (C-6‴), 100.4 (C- 1⁗), 72.1(C-2⁗), 72.4(C- 3⁗), 73.8 (C-4⁗), 69.9 (C-5⁗), 17.5(C-6⁗) (Figure S14 and S15).

Compound 6: *β*-sitosterol; ^{1}H NMR (400 MHz, $CDCl_3$) signals; δ_H: 5.36 (d, J = 8 Hz, H-6), 3.52 (m, H-3), 1.01 (CH_3-19), 0.93 (CH_3-21), 0.84 (CH_3-26), 0.83 (CH_3-27), 0.81 (CH_3-29), and 0.68 (CH_3-18); DEPT-Q NMR (101 MHz, $CDCl_3$) δ_C: 37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.2 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 50.1 (C-9), 36.5 (C-10), 21.06 (C-11), 39.7 (C-12), 42.19 (C-13), 56.8 (C-14), 24.4 (C-15), 28.9 (C-16), 55.9 (C-17), 12.04 (C-18), 19.5 (C-19), 36.1 (C-20), 18.9 (C-21), 33.9 (C-22), 26.2 (C-23), 45.8 (C-24), 29.3 (C-25), 20.0 (C-26), 19.2 (C-27), 23.3 (C-28), and 12.3 (C-29).