Supporting Information

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A new acylated flavonol glycoside from *Chenopodium foliosum.*

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S1: Experimental

General: Optical rotations (OR) were measured on a Schmidt + Haensch UniPol L1000. Infrared Infrared (IR) spectra were recorded on a Thermo Scientific Nicolet iS10 spectrometer in KBr. UV spectra were run in MeOH or with the standard shift reagents on a Libra S-70 spectrophotometer (Biochrom). HRESI-MS spectra were recorded on an Agilent Q-TOF 6540 UHD. NMR spectra were recorded on a Bruker BioSpin (Rheinstetten, Germany) Avance III 600 spectrometer at 600 MHz (^{1}H) and 150 MHz (^{13}C) in DMSO-d_6.Chemical shifts were referenced to the solvent signals (residual DMSO-d_6 at δ_H 2.50 and δ_C 39.5). The raw NMR data was proceeded using Spinworks ver 3.1.8.1 (http://home.cc.umanitoba.ca/~wolowiec/spinworks/index.html). Column chromatography (CC) was carried out with Diaion HP-20, MCI-gel (Supelco, USA) and LiChroprep C-18 (40–63 mm, using an over-pressure of 0.8– 1.0 bar, Merck, Darmstadt, Germany) as stationary phase. Semi-preparative high performance liquid chromatography (HPLC) was performed on a Waters (Milford MA, USA) Breeze 2 high pressure binary gradient system consisting of a pump model 1525EF, manual injector 7725i and an UV detector model 2489. Separations were achieved on a semi-preparative HPLC column Kromasil C18 (250 mm, 21.6 mm, 10 mm) purchased from Eka Chemicals AB (Bohus, Sweden). Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck) using following mobile phase: EtOAc–AcOH–HCOOH–H_2O (25:3:3:7). The chromatograms were observed under an UV light (254 and 366 nm) before and after spraying with 1% Natural Product Reagent A (Carl Roth, Germany) in MeOH.

Chemicals*:* Pentobarbital sodium (Sanofi, France); lactate dehydrogenase (LDH) kit (Randox, UK); DMSO and trichloroacetic acid (TCA) (Valerus, Bulgaria); NaCl, KCl, D-glucose, $NaHCO_3$, $CaCl_2.2H_2O$ and 2,2'-dinitro-5,5'-dithiodibenzoic acid (DTNB) (Merck, Darmstadt, Germany); KH_2PO_4 (Scharlau Chemie SA, Spain); HEPES, $MgSO_4.7H_2O$, collagenase from *Clostridium histolyticum* type IV, albumin, bovine serum fraction V, minimum 98%, EGTA, 2-thiobarbituric acid (4,6-dihydroxypyrimidine-2-thiol; TBA), 2,2′-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazine-6-sulfonic acid) (ABTS), sulfanilamide, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride x $6H_2O$ (Sigma-Aldrich, Taufkirchen, Germany). All solvents were of HPLC grade and were purchased from Merck or Sigma-Aldrich (Taufkirchen, Germany). All reagents were of analytical grades.

Extraction and isolation: The aerial parts of *C. foliosum* were dried in the shade and powdered plant material (857 g) was extracted with CH_2Cl_2 (7×3 L). After filtration, the extracts were combined and the solvent was evaporated under reduced pressure to give 31.3 g of greenish waxy residue. Subsequently, the plant material was extracted with MeOH (7×3 L), 70% aq. MeOH (6×2 L) and 50% aq. MeOH (2×2 L). The resulting extracts were combined, concentrated under vacuo until most of the MeOH was removed and the aq. residue was exhaustively extracted with CH_2Cl_2 (8×300 mL). The aq. layer was conc. to 200 mL and then subjected to CC over Diaion HP-20 (7×75 cm) with eluent H_2O-MeOH (100:0→0:100) to obtain 86 fractions (500 mL each) that were combined into 23 pooled fractions (I-XXIII) on basis of the TLC profiles. The fractions XVI (5.18 g, 70% MeOH) was separately subjected to CC over MCI gel (4×30 cm, 100 mL) with eluent H_2O-MeOH (50:50→0:100). The sub-fraction 39-46 (1 g, 55% MeOH) of XVI was further subjected to LPLC on RP-18 (2.5×48 cm, 50 mL) using 50% MeOH as a mobile phase. An isocratic semi-prep. HPLC purification of sub-fraction 12-18 (531 mg, 50% MeOH) with MeOH–H_2O (43:57, 19.5 mL min^{-1}, 280 nm) as eluent gave pure **1** (64.5 mg).

Sugar analysis: Compound **1** (4 mg) was refluxed with 2 mL mixture of 2N HCl-MeOH (1:1) for 2 h. The reaction mixture was filtered through a Diaion HP-20SS followed by subsequent elution with H_2O and MeOH. Water portion was filtered through Amberlite IRC-86 resin and then was evaporated to dryness. The absolute configuration of sugar was established using the method of Tanaka et al. with some modifications [1]. Briefly, the dry water eluate was treated with a solution (0.1 mL) of L-cycteine methyl ester in pyridine (5 mg/mL) at 60 °C for 1 h. A solution (0.1 mL) of o-tolylisothiocyanate in pyridine (5 mg/mL) was added to the mixture and heated at 60 °C for 1 h. The resulting solution was analyzed using HPLC [Purospher STAR RP-18e 5 µm column (Merck; 4.6×250 mm) with 25% MeCN in 50mM H_3PO_4, flow rate 1 mL/min, UV detection at 250 nm]. The occurrence of D-glucose and D-apiose (t_R values of the tolylthiocarbamoyl-thiazolidine derivatives were 18.7 and 32.2 min) were found in the residue.

DPPH radical scavenging activity: Free radical scavenging activity was measured by using DPPH method [2]. Different concentrations (1 mL) of compound in MeOH were added to 1 mL methanol solution of 2,2′-diphenyl-1-picrylhydrazyl (DPPH) (0.05 mM). The absorbance was measured at 517 nm after 30 min. Results were evaluated as percentage scavenging of radical:

$$\%\text{DPPH radical scavenging activity} = \frac{\text{Abs}_{control} - \text{Abs}_{sample}}{\text{Abs}_{control}} \times 100 \ ,$$

where $\text{Abs}_{control}$ is the absorbance of DPPH radical in MeOH, Abs_{sample} is the absorbance of DPPH radical solution mixed with sample. IC_{50} value (concentration of sample where absorbance of DPPH decreases 50 % with respect to absorbance of blank) of the sample was determined. BHT was used as a positive control. All determinations were performed in triplicate (n=3).

ABTS radical scavenging assay: For ABTS assay, the procedure followed the method of Arnao *et al*. [3] with some modifications. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulphate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 14 h at room temperature in the dark. The solution was then diluted by mixing 1 mL ABTS solution with 30 mL methanol to obtain an absorbance of 0.70 ± 0.01 units at 734 nm using a spectrophotometer. A fresh ABTS solution was prepared for each assay. Different concentrations (1 mL) of compounds were allowed to react with 1 mL of the ABTS solution and the absorbance was taken at 734 nm after 7 min. The ABTS scavenging capacity of the compound was calculated as:

$$\%\text{ABTS radical scavenging activity} = \frac{\text{Abs}_{control} - \text{Abs}_{sample}}{\text{Abs}_{control}} \times 100 \ ,$$

where $\text{Abs}_{control}$ is the absorbance of ABTS radical in methanol; Abs_{sample} is the absorbance of an ABTS radical solution mixed with sample. IC_{50} value (concentration of sample where absorbance of ABTS decreases 50 % with respect to absorbance of blank) of the sample was determined. BHT was used as a positive control. All determinations were performed in triplicate (n=3).

Ferric reducing/antioxidant power (FRAP): The FRAP assay was done according to the method described by Benzie and Strain [4] with some modifications. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM

$FeCl_3 \times 6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3 \times 6H_2O$ solution and then warmed at 37 °C before using. 0.6 mL of compound in MeOH was allowed to react with 2.8 mL of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were then taken at 593 nm. Results are expressed in μM Trolox equivalent (TE). BHT was used as a positive control. All determinations were performed in triplicate (n=3).

Determination of antioxidant activity in linoleic acid system by the FTC method: The antioxidant activity of studied compound (0.2 mM) against lipid peroxidation was measured through ammonium thiocyanate assay, as described by Takao *et al*. [5], with some modifications. The reaction solution, containing 0.2 ml of extract (1 mg/mL dry weight in MeOH), 0.2 ml of linoleic acid emulsions (25 mg/ml in 99% ethanol), and 0.4 ml of 50 mM phosphate buffer (pH 7.4), was incubated in the dark at 40°C. A 0.1 ml aliquot of the reaction solution was then added to 3 ml of 70% (v/v) ethanol and 0.05 mL of 30% (w/v) ammonium thiocyanate. Precisely 3 min after the addition of 0.05 mL of 20 mM ferrous chloride in 3.5% (v/v) hydrochloric acid to the reaction mixture, the absorbance of the resulting red color was measured at 500 nm. Aliquots were assayed every 24 h until the day after the absorbance of the control solution (without compound) reached maximum value. BHT (0.2 mM) was used as a positive control.

Experimental animals: Male Wistar rats (body weight, 200-250 g) were used. Rats were housed in plexiglass cages (3 per cages) in a 12/12 light/dark cycle, temperature 20 ± 2°C. Food and water were provided *ad libitum*. Animals were purchased from the National Breeding Centre, Sofia, Bulgaria. All experiments were performed after at least one week of adaptation to this environment. The experimental procedures were approved by the Institutional Animal Care and Use Committee at the Medical University-Sofia, Bulgaria. The principles stated in the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123) were followed strictly throughout the experiment.

Isolation and incubation of hepatocytes: Rats were anesthetized with sodium pentobarbital (0.2 ml/100 g). *In situ* liver perfusion and cell isolation were performed as described by Fau et al. [6], with modifications [7]. Cells were counted under the microscope and the viability was assessed by trypan blue exclusion (0.05 %) [6]. Hepatocytes were incubated with 10 μg/ml, 100 μg/ml of compound **1** and Silymarin [8] and 86 μM carbon tetrachloride [9].

Lactate dehydrogenase release: Lactate dehydrogenase release in isolated rat hepatocytes was measured as described by Bergmeyer et al. [10].

GSH depletion and MDA assay: GSH depletion and MDA production in isolated rat hepatocytes were measured spectrophotometricaly at 412 nm and 535 nm, respectively [6].

Statistical analysis: Statistical analysis was performed using statistical programme 'MEDCALC'. The results are expressed as the mean ± SD of 6 experiments. Three parallel samples were used. The significance of the data was assessed using the nonparametric Mann–Whitney test. Values of $P \leq 0.05$ were considered statistically significant.

S2: References

[1] T. Tanaka, T. Nakashima, T. Ueda, K. Tomii and I. Kouno (2007). Facile discrimination of aldose enantiomers by reversed-phase HPLC. *Chem. Pharm. Bull.* **55**, 899-901.

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[4] I. Benzie and J. Strain (1996) The ferric reducing ability of plasma (FRAP) as a measure of ‘‘antioxidant power’’: The FRAP assay. *Anal. Biochem.* **239**, 70-76.

[5] T. Takao, F. Kitatani, N. Watanabe, A. Yagi and K. Sakata (1994). A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. *Biosci. Biotechnol. Biochem.* **58**, 1780-1783.

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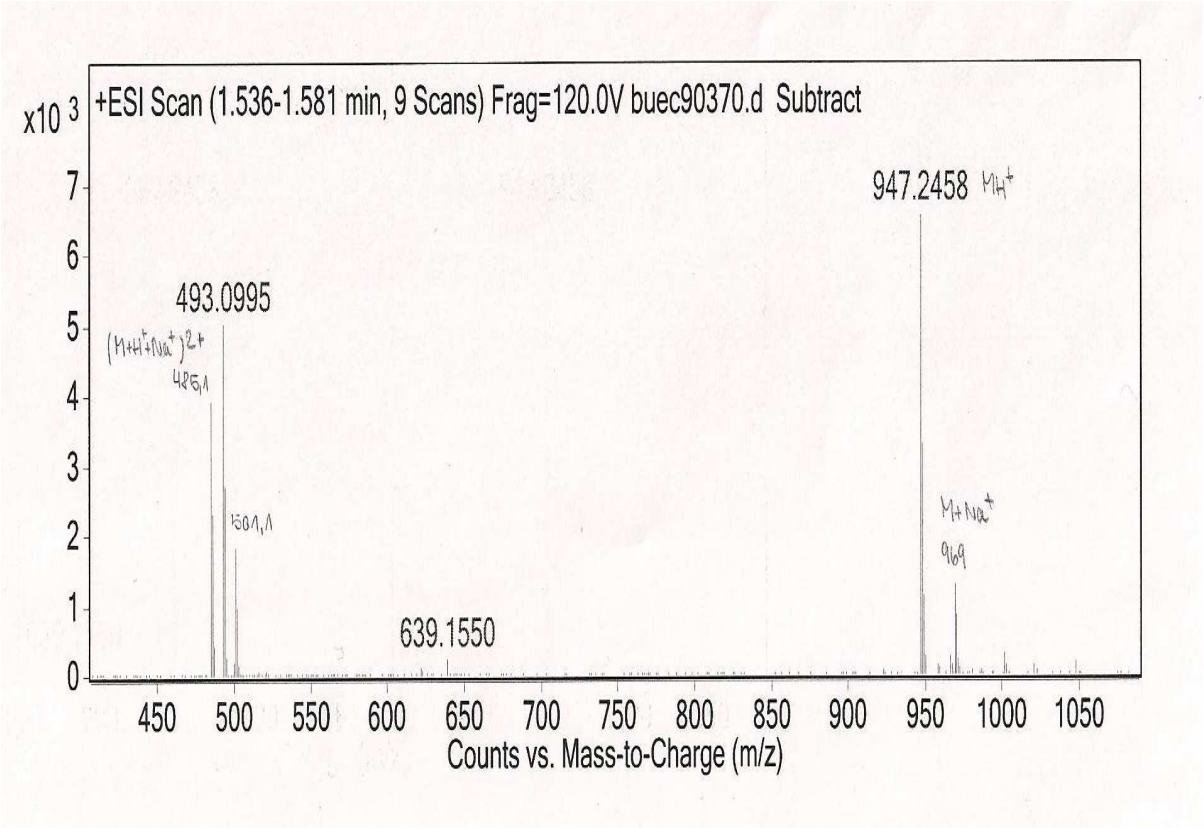
[7] M. Mitcheva, M. Kondeva, V. Vitcheva, P. Nedialkov and G. Kitanov (2006). Effect of benzophenones from *Hypericum annulatum* on carbon tetrachloride-induced toxicity in freshly isolated rat hepatocytes. *Redox Rep.* **11,** 1-8.

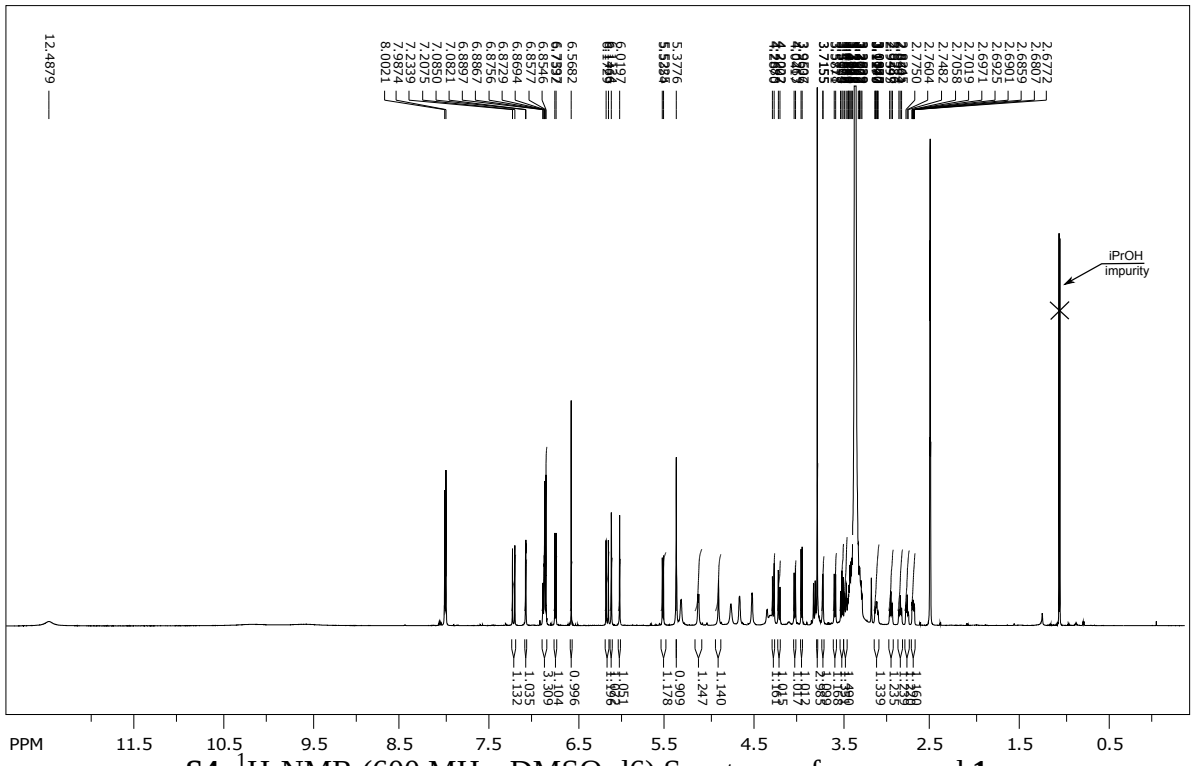
[8] M. Mitcheva, M. Kondeva-Burdina, V. Vitcheva, I. Krasteva and S. Nikolov (2008). Effect of purified saponin mixture from *Astragalus corniculatus* on toxicity models in isolated rat hepatocytes. *Pharm. Biol.* **46**, 866-870.

[9] M. U. Dianzani, G. Poli, E. Gravela, E. Chiarpotto and E. Albano (1981). Influence of lipid peroxidation on lipoprotein secretion by isolated hepatocytes. *Lipids* **16**, 823-829.

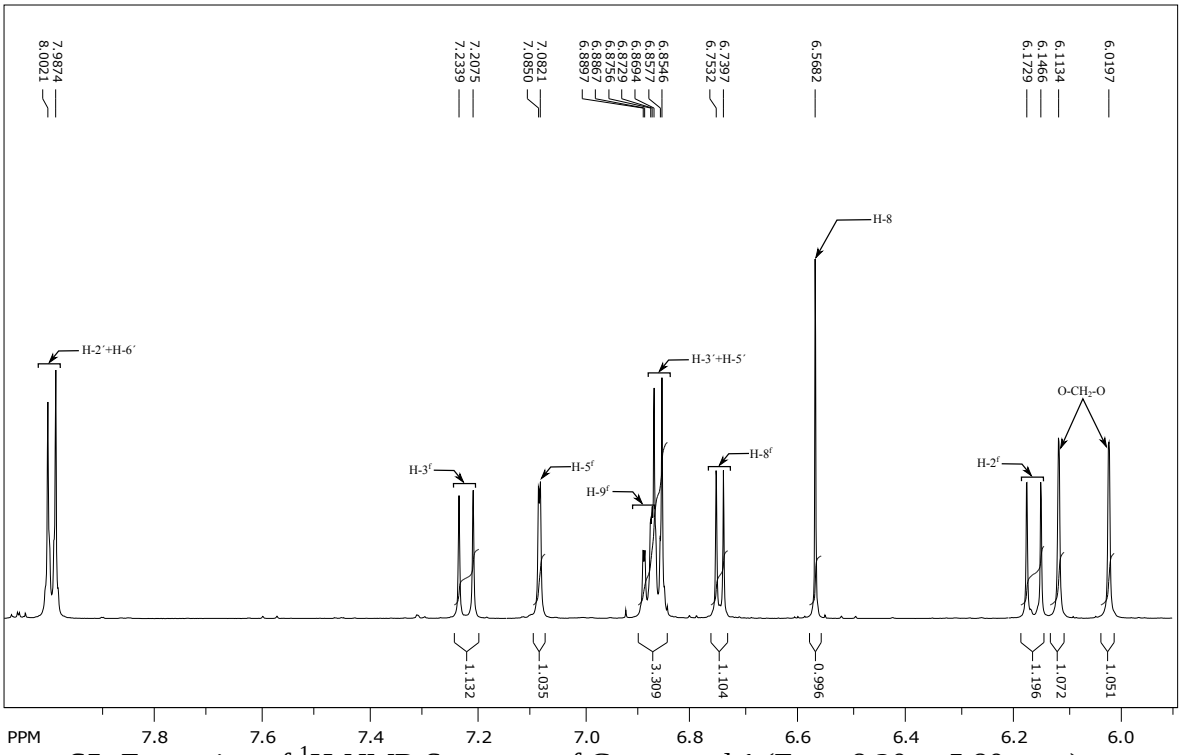
[10] H.U. Bergmeyer, K. Gawehn and M. Grassl (1974). *Methods of Enzymatic Analysis.* Bergmeyer HU (Eds.), Weinheim: Verlag Chemie, 1, 481-482.



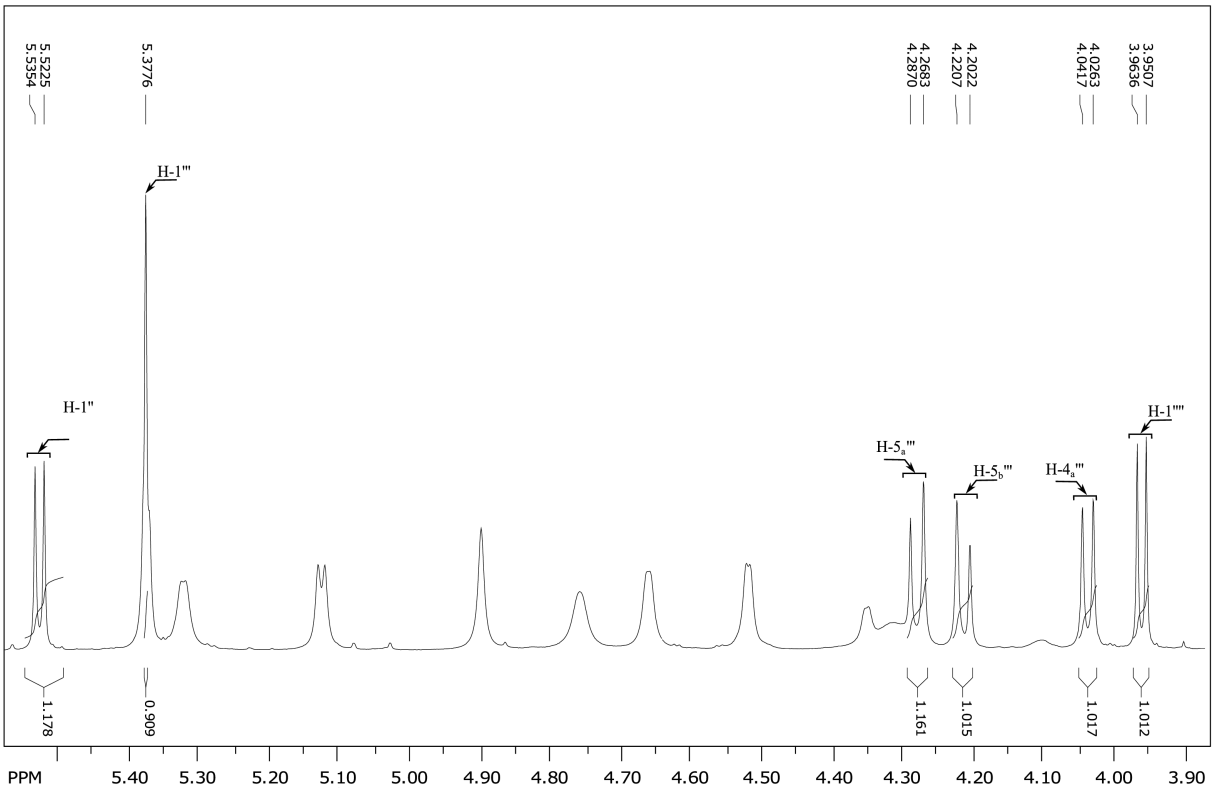
S3: HRESI-MS Spectrum of Compound **1**



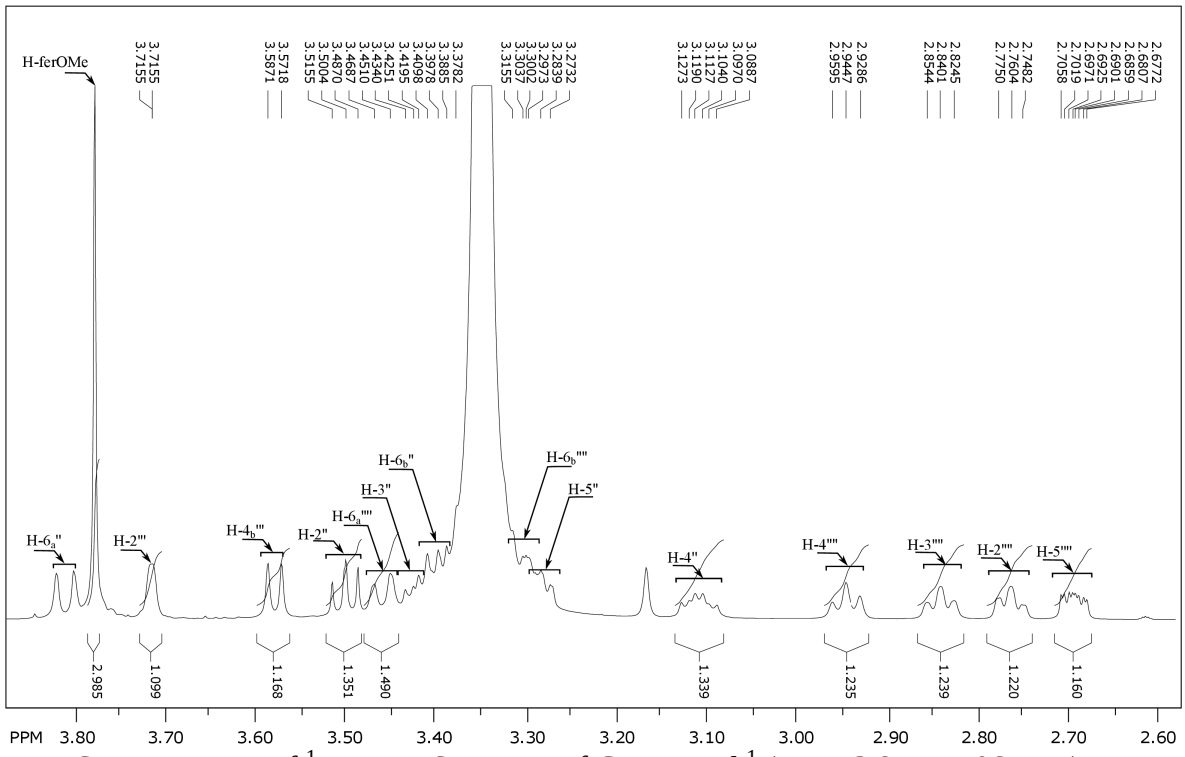
S4: ^{1}H-NMR (600 MHz, DMSO-d6) Spectrum of compound **1**



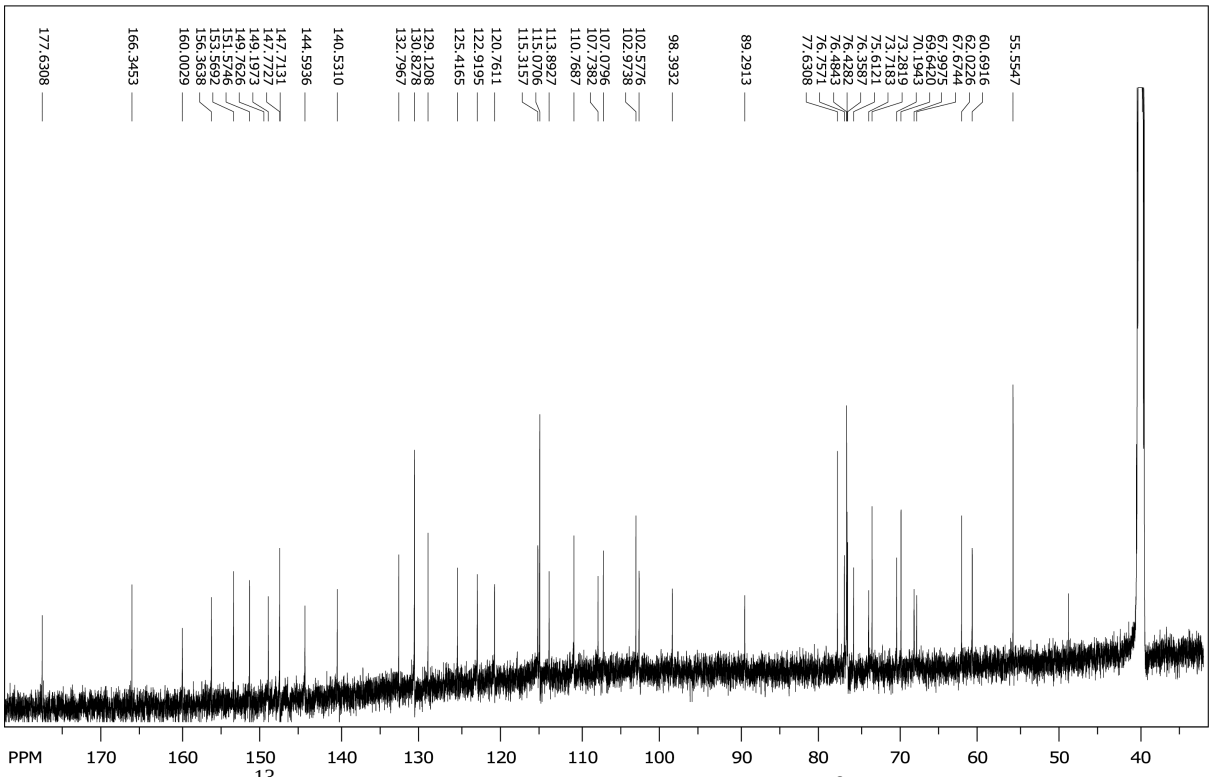
S5: Expansion of ^{1}H-NMR Spectrum of Compound **1** (From 8.20 to 5.80 ppm)



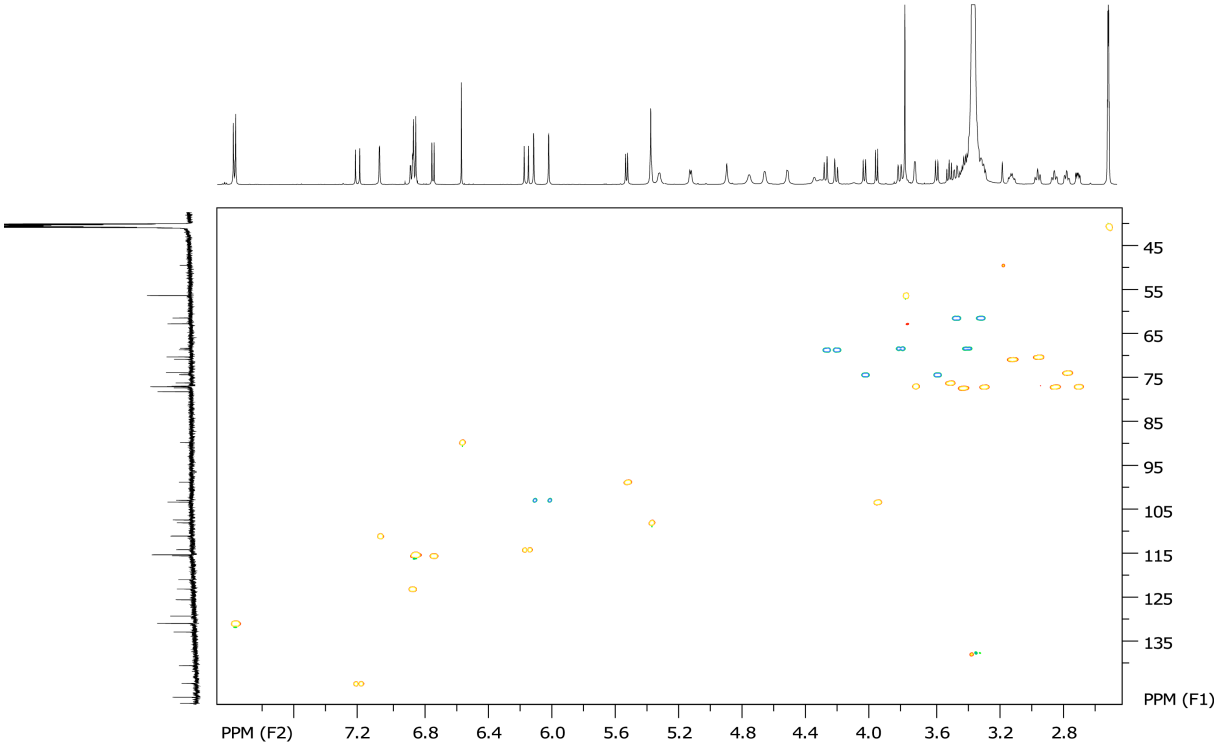
S6: Expansion of ^{1}H-NMR Spectrum of Compound **1** (From 5.80 to 3.90 ppm)



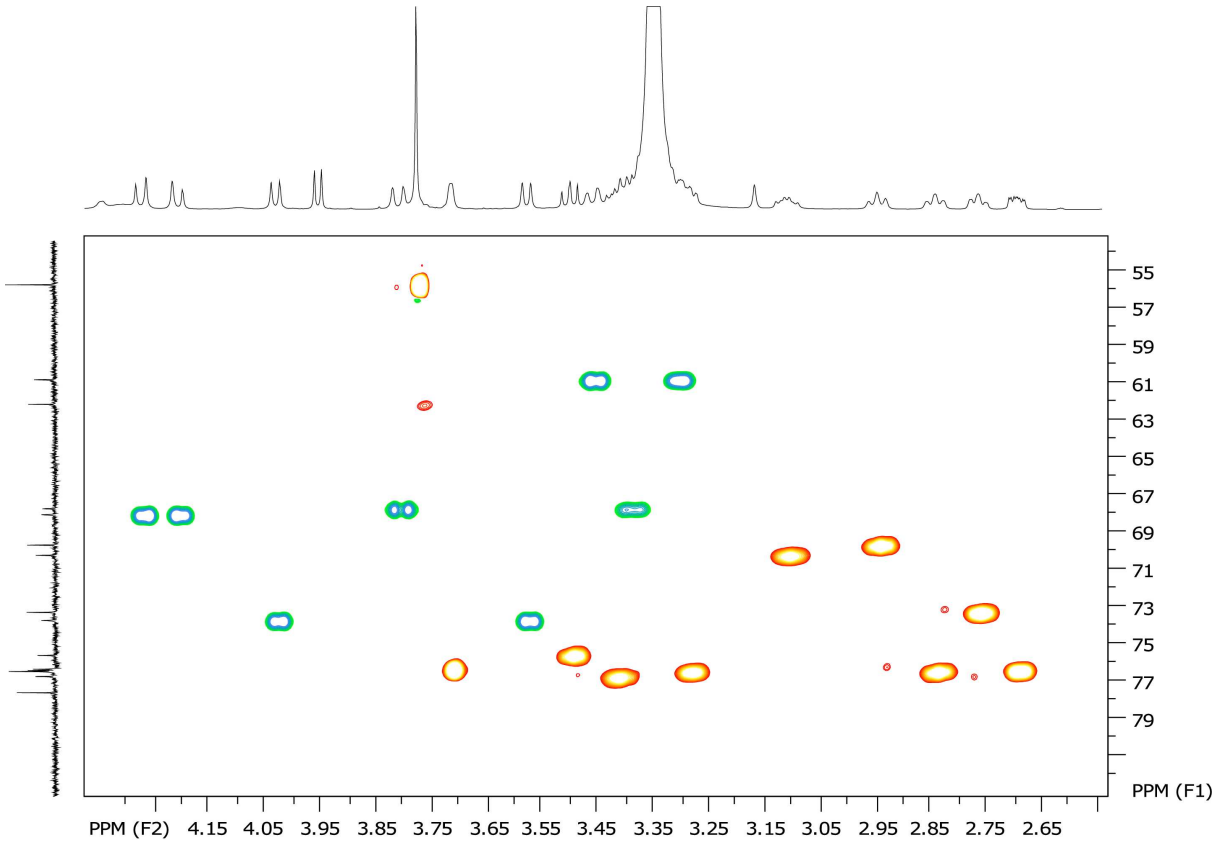
S7: Expansion of ^{1}H-NMR Spectrum of Compound ^{1} (From 3.85 to 2.06 ppm)



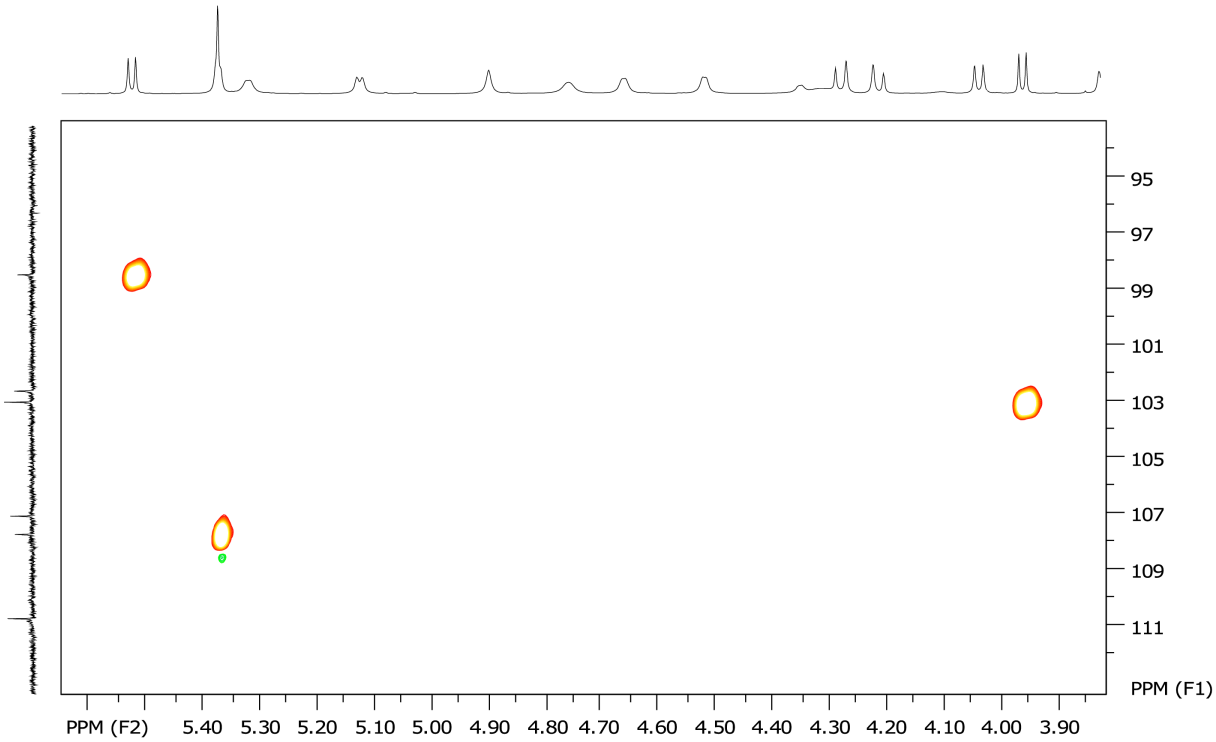
S8: ^{13}C-NMR (150 MHz, DMSO-d_6) Spectrum of Compound **1**



S9: HSQC Spectrum of Compound **1**



S10: Expansion of HSQC Spectrum of Compound **1** (sugar and OCH_3 atoms)



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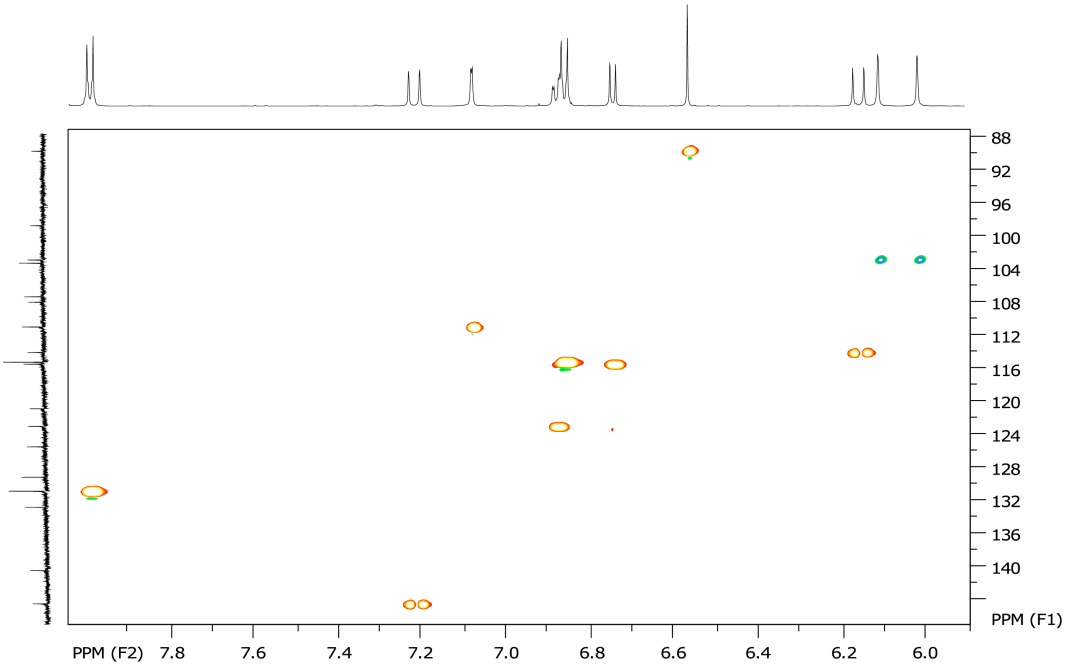

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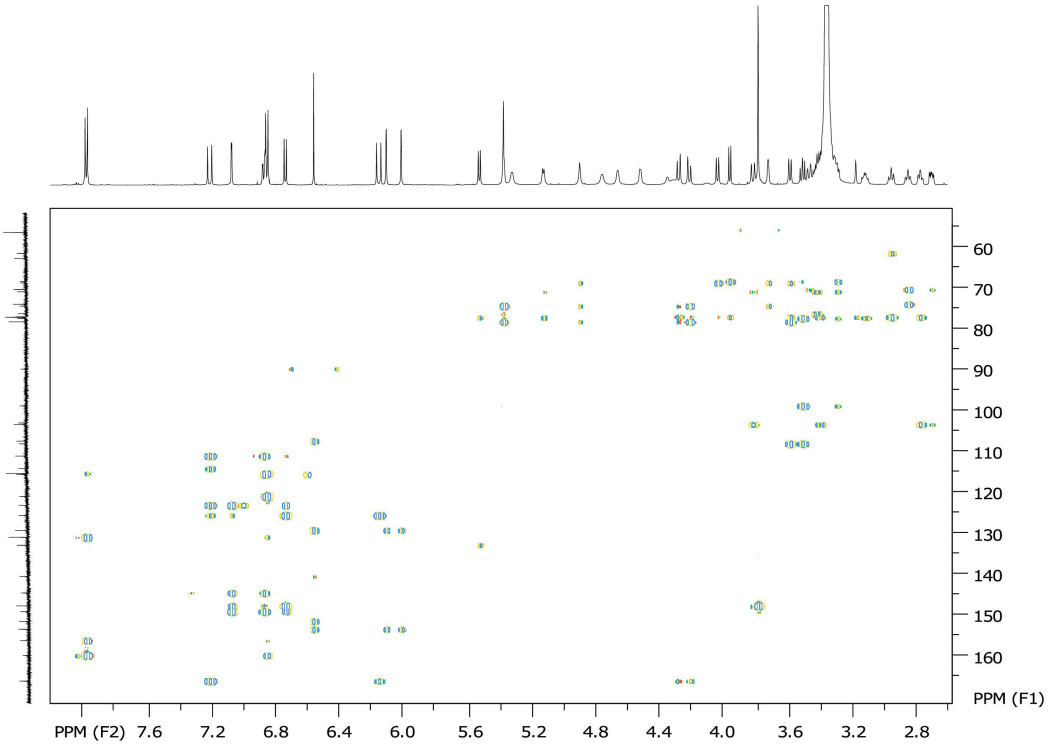

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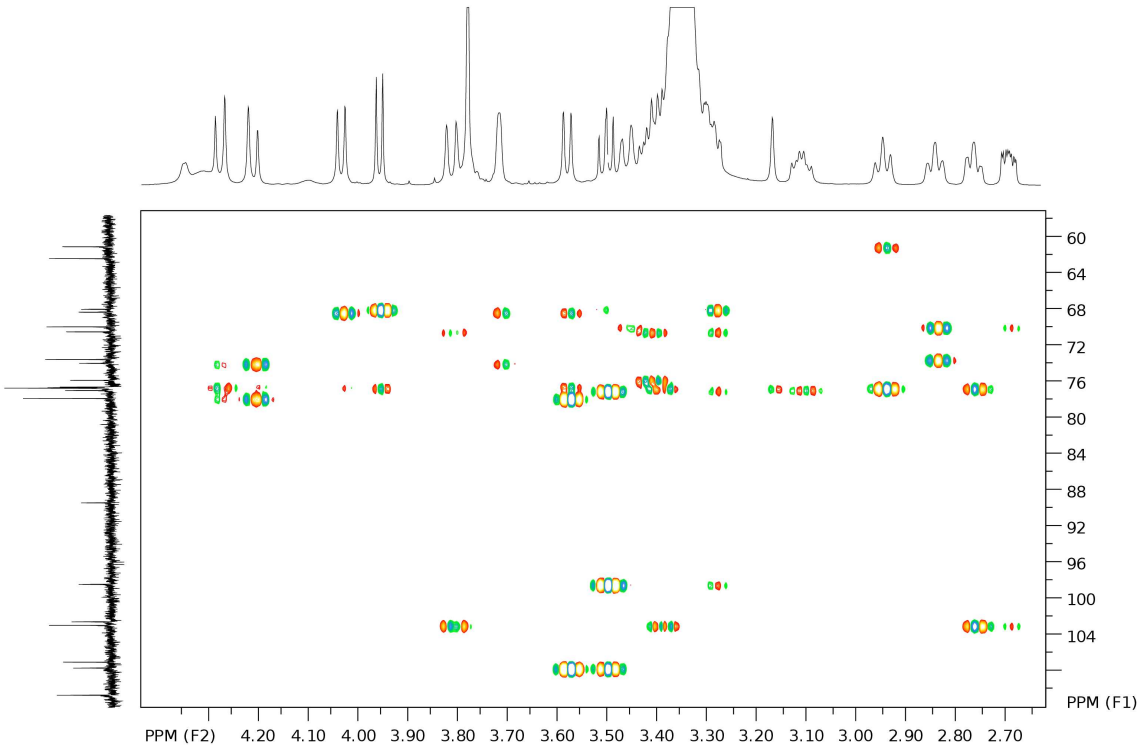

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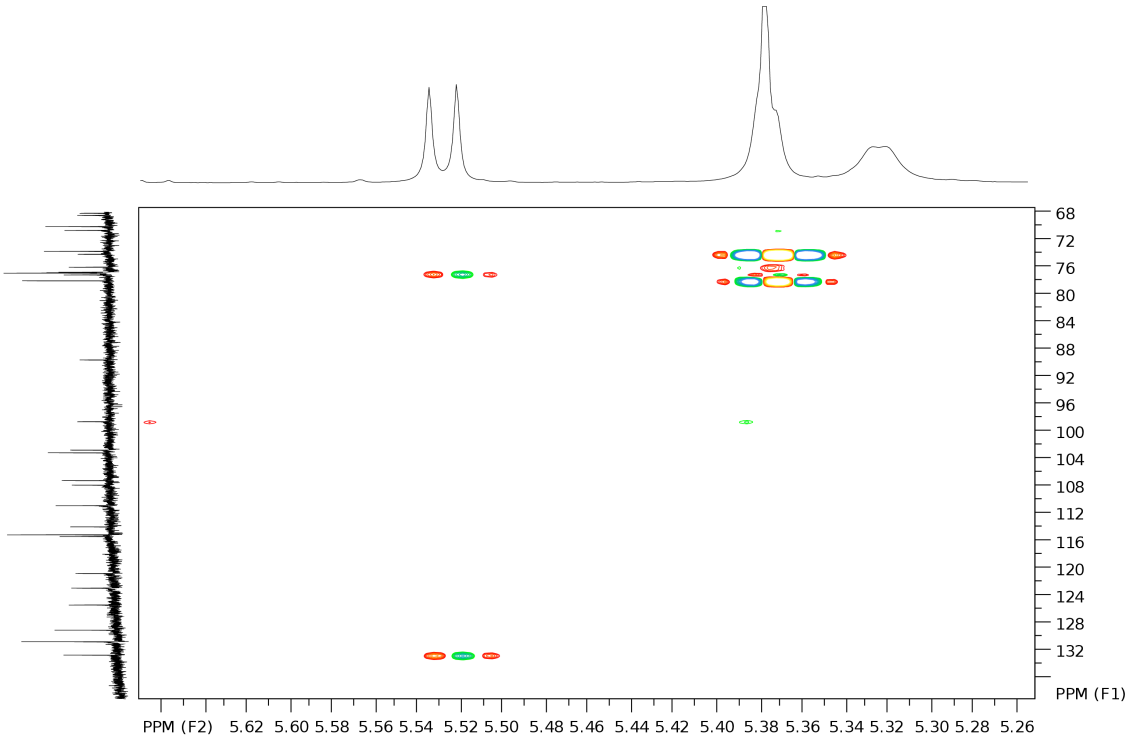

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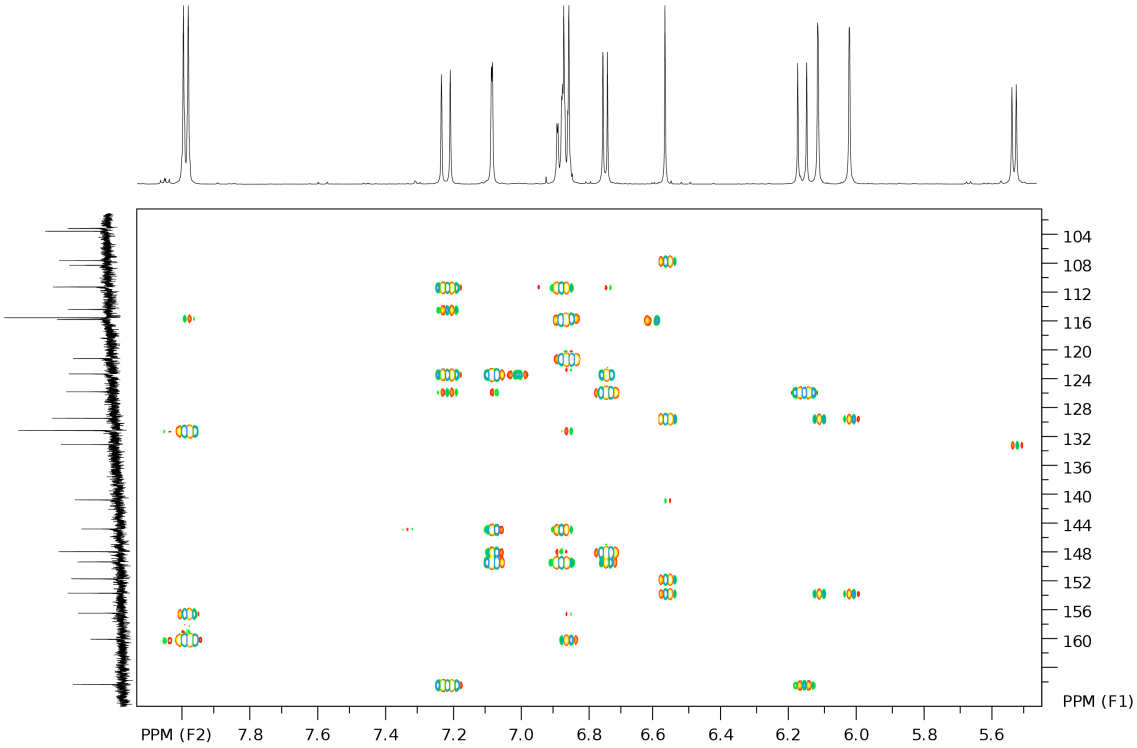

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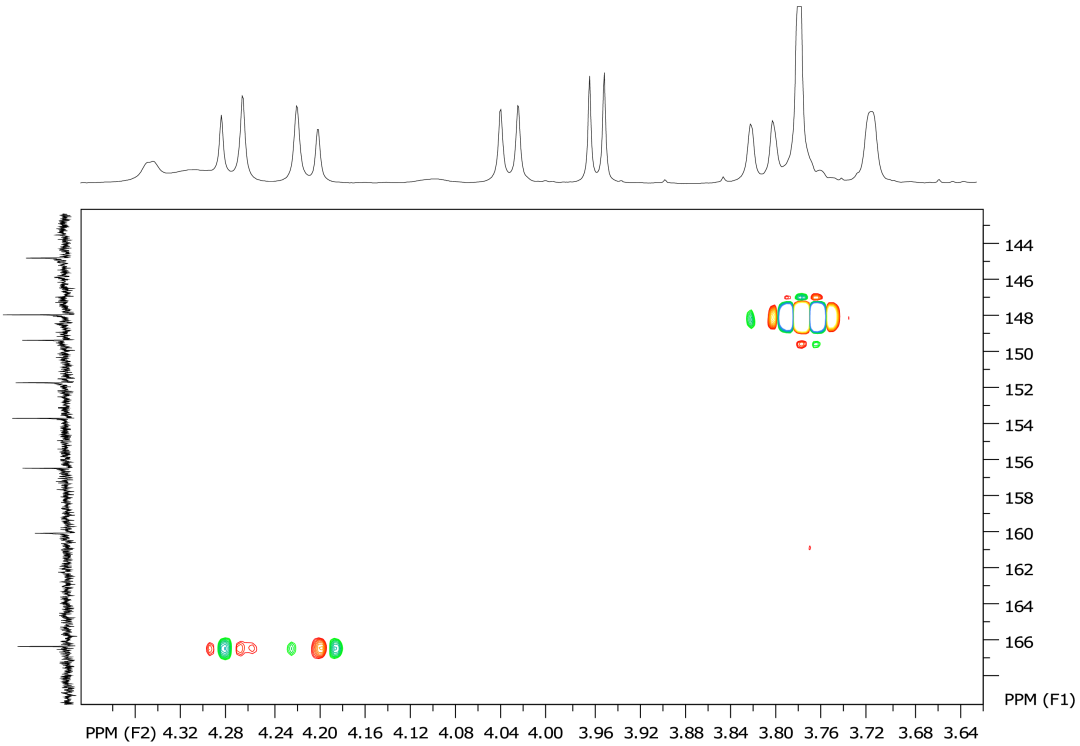

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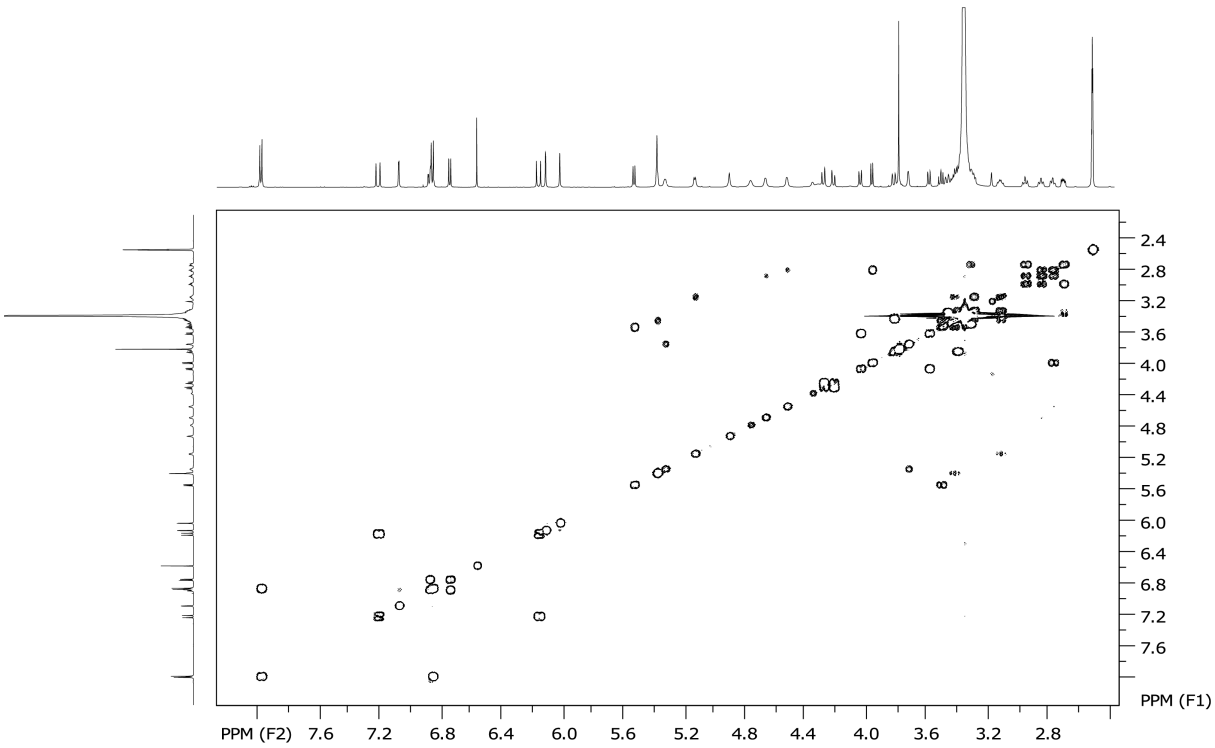
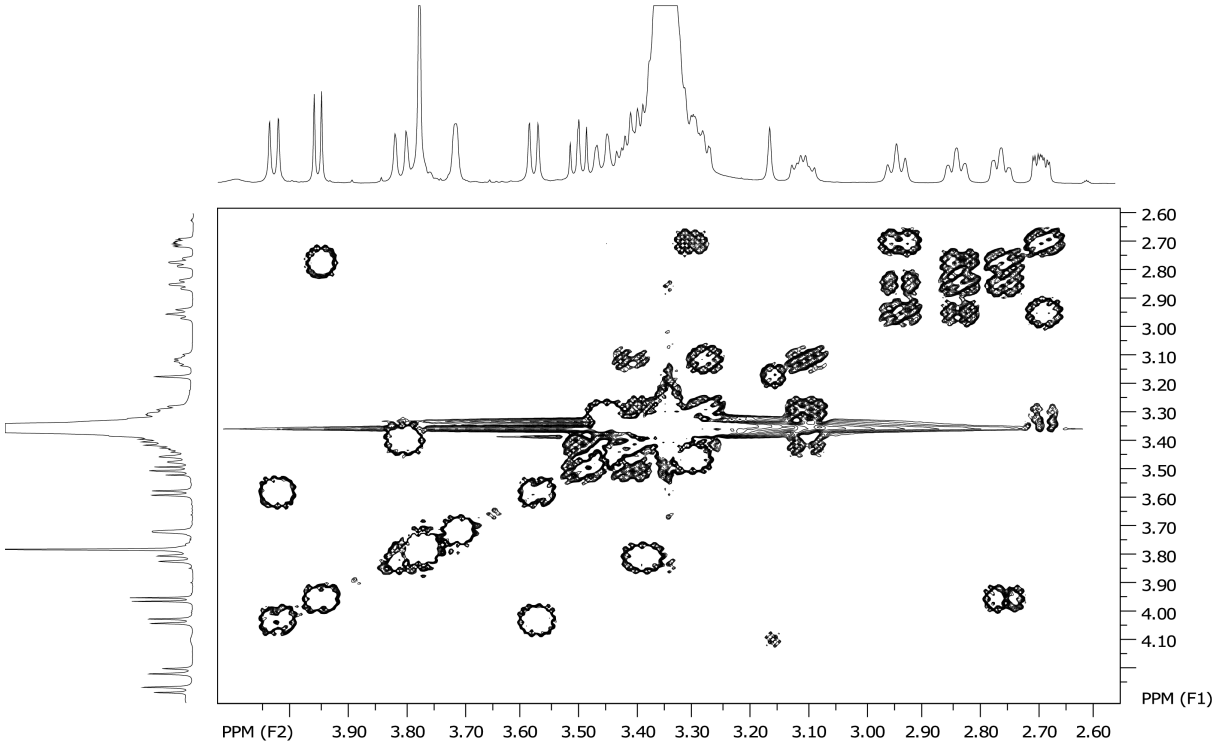
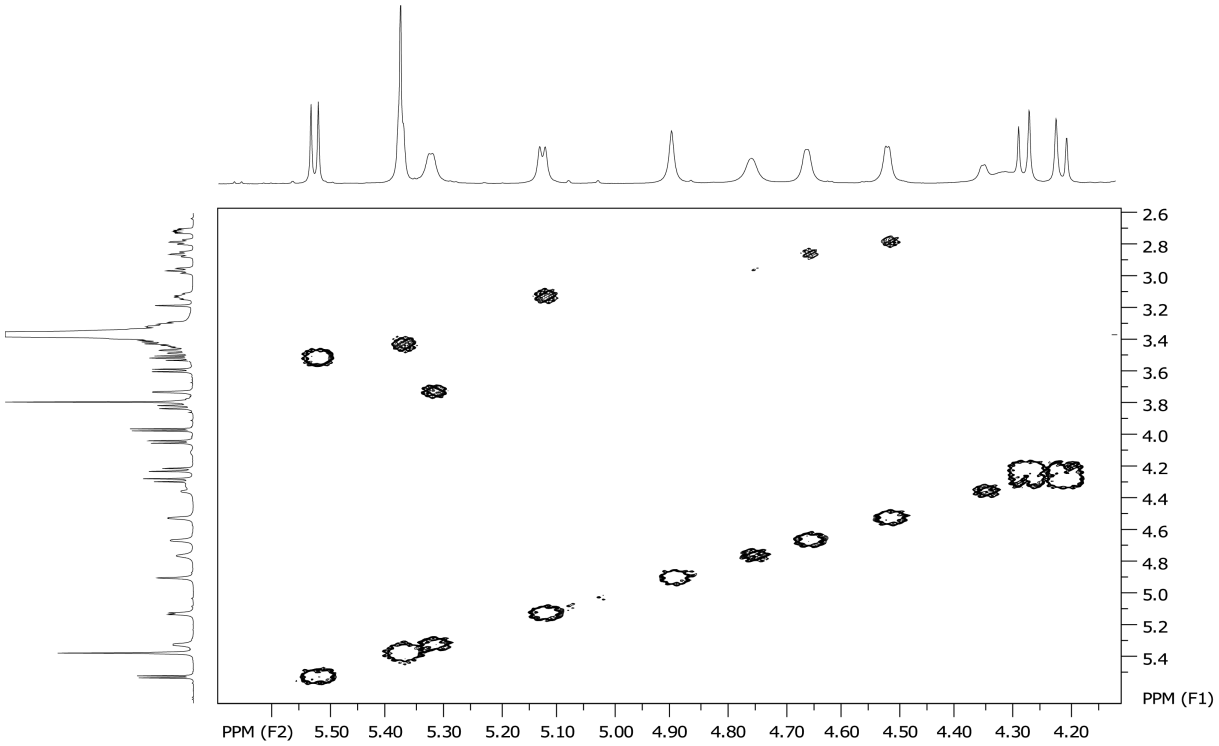


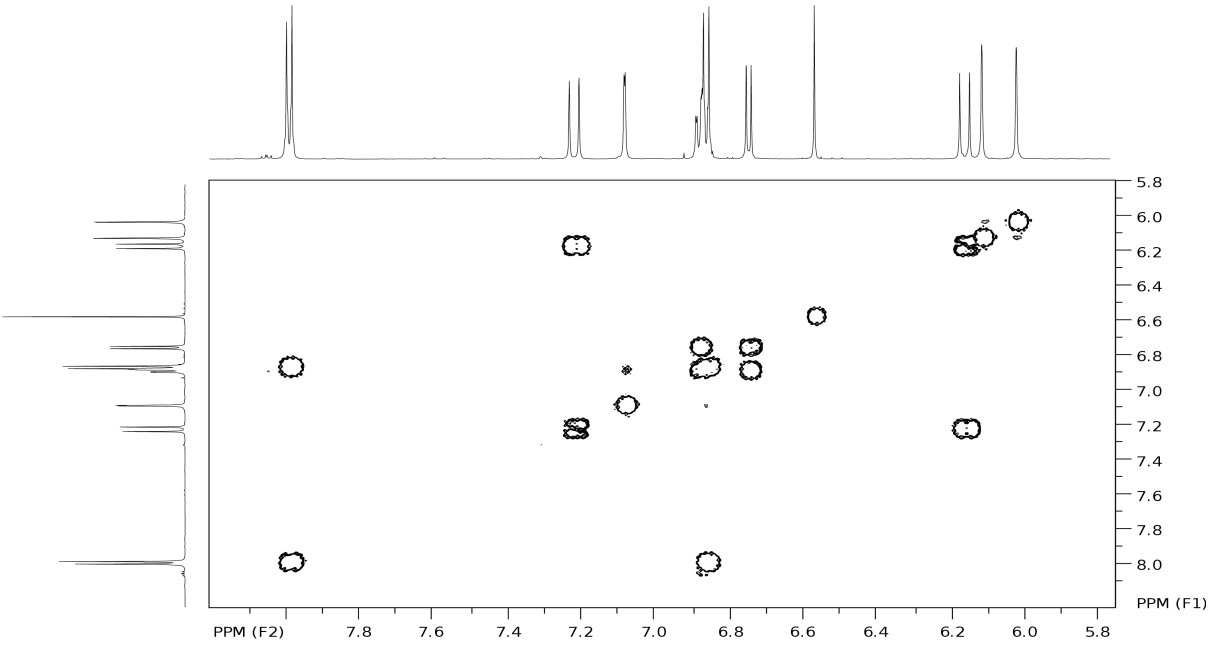
S18: COSY Spectrum of Compound **1**



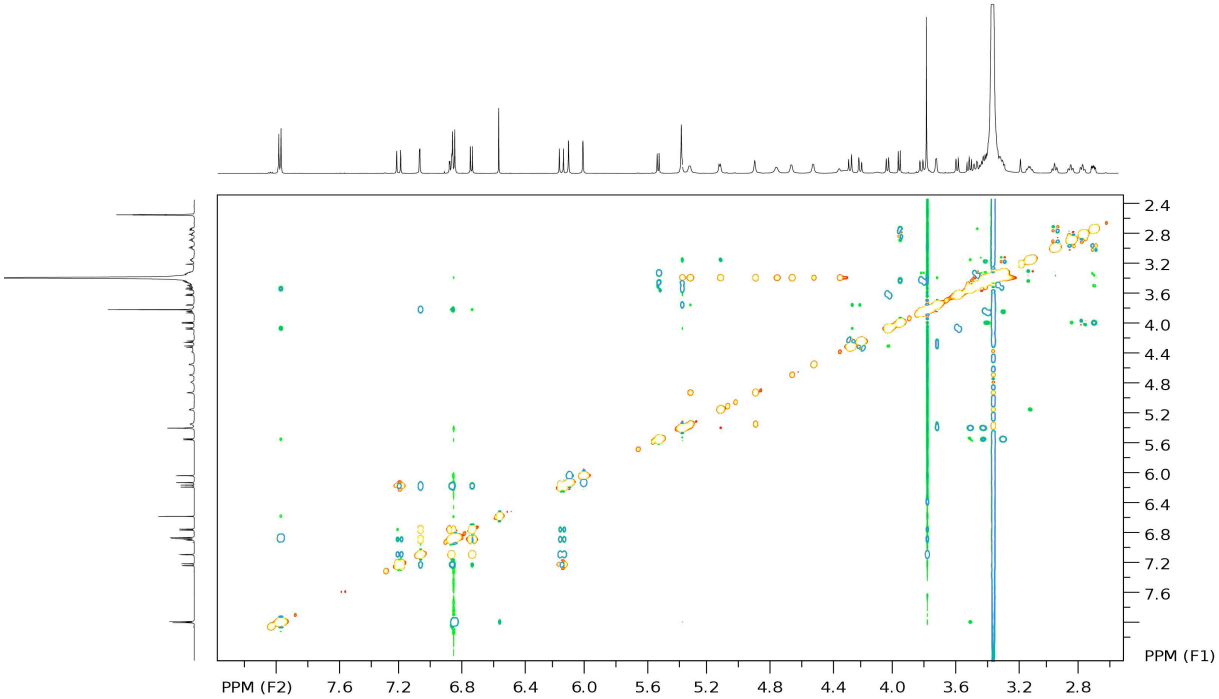
S19: Expansion of COSY Spectrum of Compound **1** (sugar protons region)



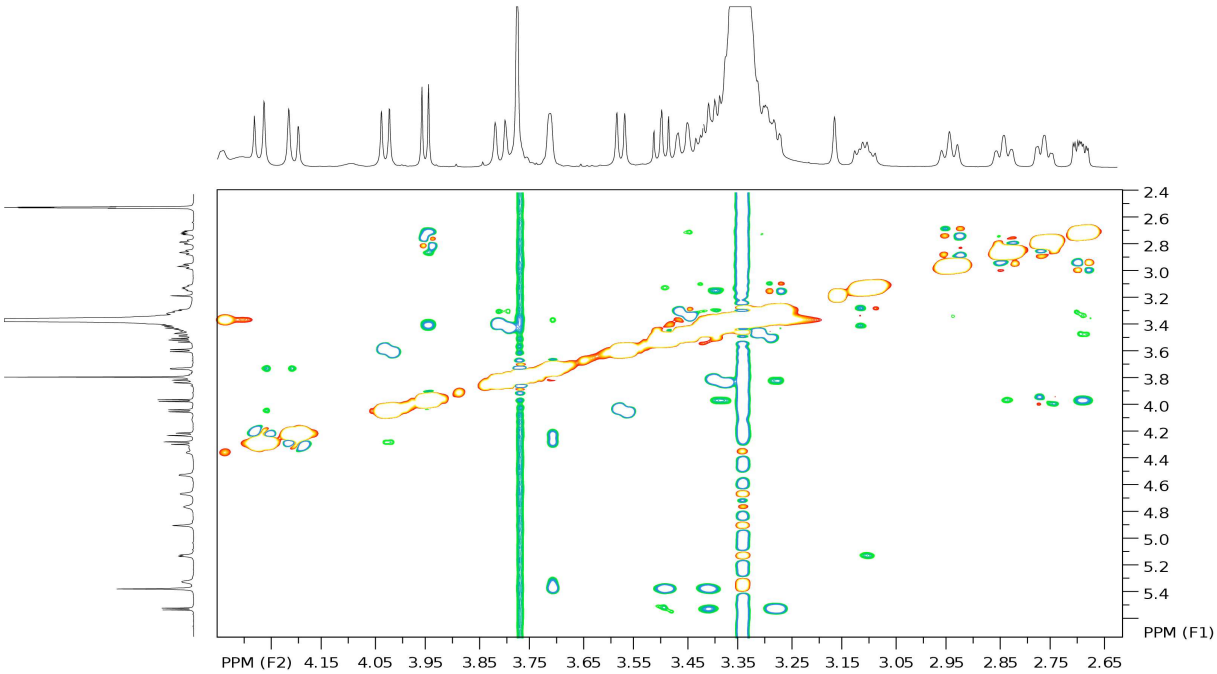
S20: Expansion of COSY Spectrum of Compound **1** (anomeric sugar protons H-1" and H-1'")



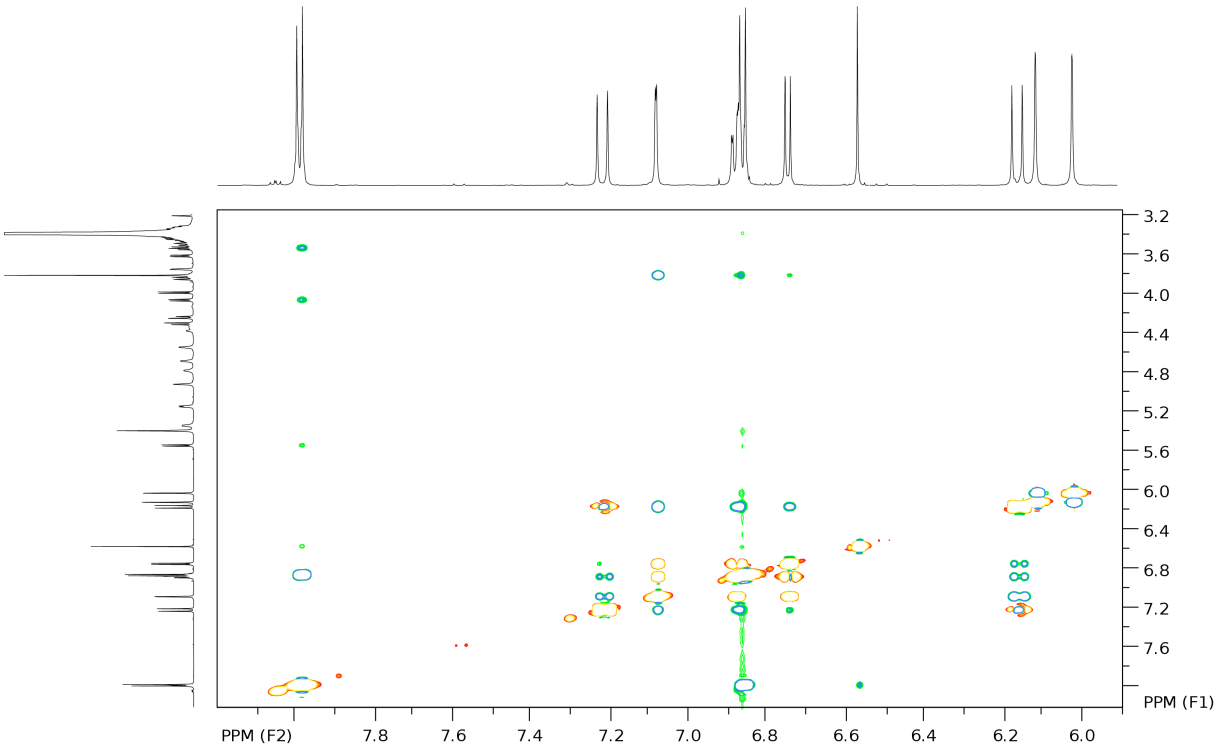
S21: Expansion of COSY Spectrum of Compound **1** (aglycone protons region)



S22: ROESY Spectrum of Compound **1**



S23: Expansion of ROESY Spectrum of Compound **1** (sugar protons region)



S24: Expansion of ROESY Spectrum of Compound **1** (aglycone and feruloyl protons region)