

Supporting Information

Rec. Nat. Prod. 16:4 (2022) 404-408

A New Isoflavone Glucoside from the Roots of *Astragalus membranaceus* var. *mongholicus*

Yadong Wang¹, Chao Wang², Yuanhao Wang^{2*}, Qing Hu^{2*}, and Fenghuan Wang¹

¹ School of Light Industry, Beijing Technology and Business University, Beijing 100048, China

² SUSTech Engineering Innovation Center, School of Environmental Science and Engineering, Southern University of Science and Technology, Shenzhen 518055, China

| Table of Contents | Page |
|--|------|
| 1. General experimental procedures | 2 |
| 2. Plant material | 2 |
| 3. Extraction and isolation | 2 |
| 4. Crystallographic data of compound 1a | 3 |
| 5. Cell culture and viability assay | 3 |
| 6. Measurement of NO production | 3 |
| Figure S1: Scifinder search of new compound 1 | 4 |
| Figure S2: HR-ESI-MS spectrum of 1 | 5 |
| Figure S3: ¹ H-NMR (500 MHz, CD ₃ OD) spectrum of 1 | 6 |
| Figure S4: ¹³ C-NMR (125 MHz, CD ₃ OD) spectrum of 1 | 7 |
| Figure S5: DEPT135 (125 MHz, CD ₃ OD) spectrum of 1 | 8 |
| Figure S6: HSQC spectrum of 1 | 9 |
| Figure S7: HSQC spectrum of 1 (From δ_C 5 ppm to δ_C 90 ppm) | 10 |
| Figure S8: HSQC spectrum of 1 (From δ_C 95 ppm to δ_C 160 ppm) | 11 |
| Figure S9: HMBC spectrum of 1 | 12 |
| Figure S10: ¹ H- ¹ H COSY spectrum of 1 | 13 |

1. General experimental procedures

X-ray data were collected using an Agilent Xcalibur Nova X-ray diffractometer. Optical rotations were measured on a Rodolph Research Analytical Autopol I Automatic Polarimeter. UV data were obtained using Shimadzu UV-2450 spectrophotometry, and IR spectra were obtained using a Bruker Tensor 27 spectrometer. 1D and 2D NMR spectra were measured on a Bruker AM-500 spectrometer. HRESIMS were performed on a Waters Micromass Q-TOF spectrometer. Semipreparative HPLC separations were carried out by photodiode array (PDA) analysis using a Wufeng LC-100 apparatus with a Kromasil 100-5 C₁₈ column (250 × 10 mm, 5 μm). TLC analysis was carried out on silica gel plates (Marine Chemical Ltd., Qingdao, China). Silica gel (300–400 mesh, Qingdao Haiyang Chemical Co., Ltd.), reversed-phase C₁₈ (RP-C₁₈) silica gel (Fuji, 40–75 μm), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden) were used for column chromatography (CC).

2. Plant material

The roots of *A. membranaceus var. mongholicus* were collected from Gansu Province, China, in October 2019. The identification of the plant was done by one of the author (Y. Wang), and a voucher specimen (201910-HQ) was deposited at School of Environmental Science and Engineering, Southern University of Science and Technology.

3. Extraction and isolation

The dried roots of *A. membranaceus var. mongholicus*. (15 kg) were powdered and extracted with 80% EtOH (45 L) at room temperature three times. After the evaporation of solvent under reduced pressure, the crude extract was suspended in water partitioned with EtOAc. The EtOAc portion (165 g) was subjected to silica gel CC with a gradient of CH₂Cl₂/MeOH (100:1 → 5:1, v/v) to yield five fractions (Fr. A–Fr. E). Fr. B (29 g) was applied to RP-C₁₈ silica gel column with MeOH/H₂O (30:70 → 100:0) as eluents to afford six subfractions (Fr. B1–Fr. B6). Fr. B2 (210 mg) further subjected to semipreparative HPLC separation (MeCN/H₂O, 30:70) to afford compound **7** (24 mg, *t_R* 16 min). Fr. B3 (800 mg) was purified using semipreparative HPLC (MeCN/H₂O, 45:55) to afford compounds **4** (60 mg, *t_R* 14.5 min), **6** (88 mg, *t_R* 15.3 min), **5** (75 mg, *t_R* 16.5 min). Fr. B5 (250 mg) was loaded onto a Sephadex LH-20 column using MeOH as eluent to give compound **1** (15 mg). Fr. C (8 g) was subjected to separation over a Sephadex LH-20 column (MeOH) to give one subfraction, which was further purified by semipreparative HPLC (MeCN/H₂O, 50:50) to yield **2** (160 mg, *t_R* 12.5 min) and **3** (85 mg, *t_R* 13 min).

4. Crystallographic data of compound 1a

monoclinic, space group I2 (no. 5), $a = 5.03499(5) \text{ \AA}$, $b = 10.24226(11) \text{ \AA}$, $c = 46.0558(4) \text{ \AA}$, $\beta = 92.4946(8)^\circ$, $V = 2372.83(4) \text{ \AA}^3$, $Z = 4$, $T = 100.00(10) \text{ K}$, $\mu (\text{Cu K}\alpha) = 0.864 \text{ mm}^{-1}$, $D_{\text{calc}} = 1.300 \text{ g/cm}^3$, 24799 reflections measured ($7.686^\circ \leq 2\Theta \leq 158.27^\circ$), 5026 unique ($R_{\text{int}} = 0.0485$, $R_{\text{sigma}} = 0.0310$) which were used in all calculations. The final R_1 was 0.0536 ($I > 2\sigma(I)$) and wR_2 was 0.1495 (all data). Crystallographic data for **1** have been deposited in the Cambridge Crystallographic Data Center (CCDC number: 2108374).

5. Cell culture and viability assay

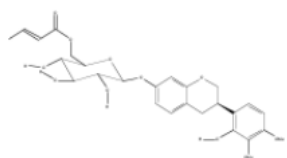
RAW 264.7 cells were obtained from Cell Bank of Chinese Academy of Sciences (Shanghai, China). Cells were cultivated in DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin at 37 °C in a humidified incubator containing 5% CO₂. The cell viability assay was detected by using MTT method.

6. Measurement of NO production

The NO concentration was measured by the Griess reagent. Briefly, RAW264.7 cells were treated with LPS (1.0 $\mu\text{g/mL}$) and compounds for 24 h. After that, the 50 μL of culture supernatant was collected to react with the same volume of Griess reagent for 10 min at room temperature in the dark. Then, the absorbance was measured at 540 nm using a microplate reader. Inhibition (%) = $(1 - (A_{\text{LPS} + \text{sample}} - A_{\text{untreated}}) / (A_{\text{LPS}} - A_{\text{untreated}})) \times 100$. The experiments were performed in triplicates. Quercetin was used as a positive control.

SUBSTANCES: CHEMICAL STRUCTURE

Structure Editor:



Search Type:

- Exact Structure
- Substructure
- Similarity


Show precision analysis

Click image to change structure or view detail.

Import CXF

Search

Advanced Search



Launch a SciFinder/SciFinderⁿ substance or reaction search directly from the latest version of ChemDraw. [Learn More](#)

Chemical Structure similarity

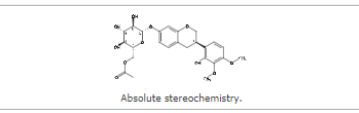
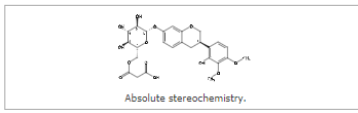
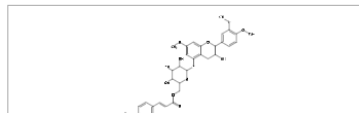
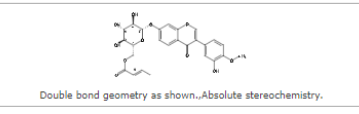
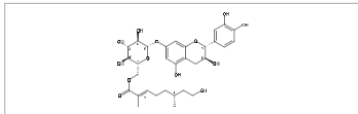
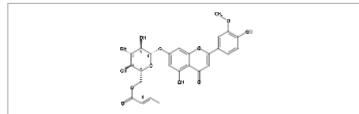
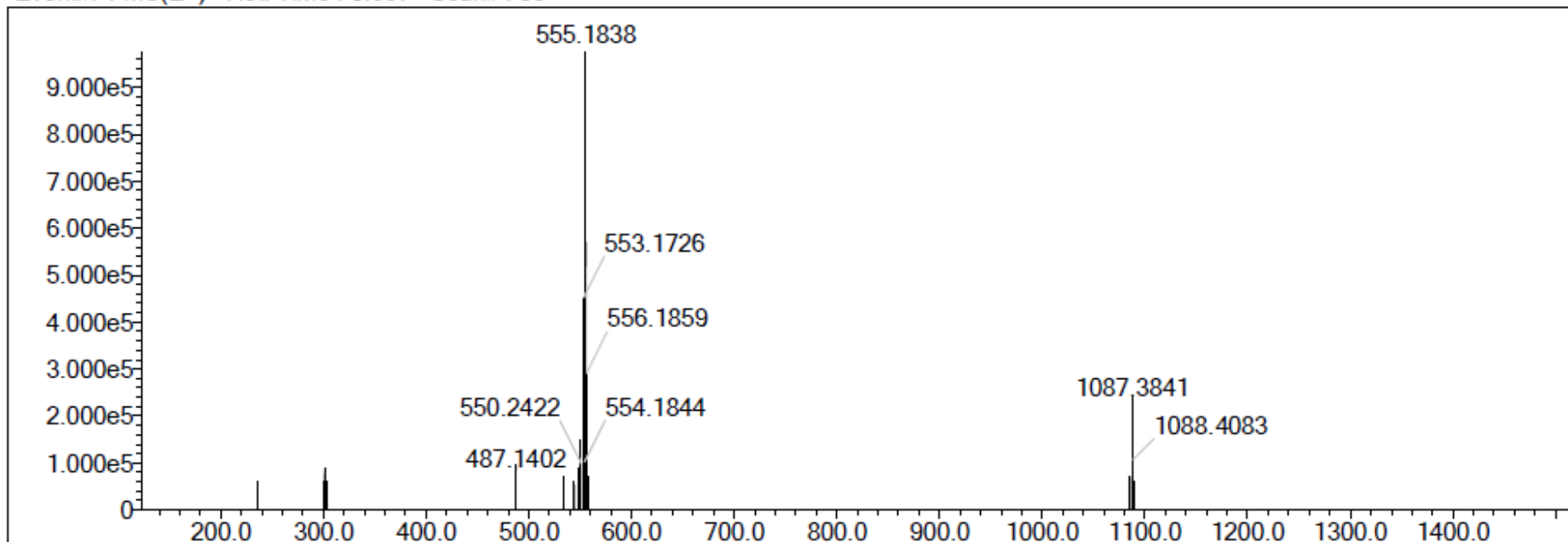
| SUBSTANCES | | | | | | | | | | | | | | | | | | | | | |
|---|--|---|------------|--|---|--------------------------------|---|--------------------------------|---|--------------------------------|----|--------------------------------|-----|--------------------------------|------|--------------------------------|-------|--------------------------------|-------|---|-------|
| <p>Select All Deselect All</p> <p>0 of 7 Similarity Candidates Selected</p> <table border="1"> <thead> <tr> <th>Similarity Range</th> <th>Substances</th> </tr> </thead> <tbody> <tr> <td><input type="checkbox"/> ≥ 99 (most similar)</td> <td>0</td> </tr> <tr> <td><input type="checkbox"/> 95-98</td> <td>0</td> </tr> <tr> <td><input type="checkbox"/> 90-94</td> <td>2</td> </tr> <tr> <td><input type="checkbox"/> 85-89</td> <td>37</td> </tr> <tr> <td><input type="checkbox"/> 80-84</td> <td>535</td> </tr> <tr> <td><input type="checkbox"/> 75-79</td> <td>3171</td> </tr> <tr> <td><input type="checkbox"/> 70-74</td> <td>12163</td> </tr> <tr> <td><input type="checkbox"/> 65-69</td> <td>28648</td> </tr> <tr> <td><input type="checkbox"/> 0-64 (least similar)</td> <td>74370</td> </tr> </tbody> </table> <p>Get Substances</p> | | Similarity Range | Substances | <input type="checkbox"/> ≥ 99 (most similar) | 0 | <input type="checkbox"/> 95-98 | 0 | <input type="checkbox"/> 90-94 | 2 | <input type="checkbox"/> 85-89 | 37 | <input type="checkbox"/> 80-84 | 535 | <input type="checkbox"/> 75-79 | 3171 | <input type="checkbox"/> 70-74 | 12163 | <input type="checkbox"/> 65-69 | 28648 | <input type="checkbox"/> 0-64 (least similar) | 74370 |
| Similarity Range | Substances | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> ≥ 99 (most similar) | 0 | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> 95-98 | 0 | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> 90-94 | 2 | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> 85-89 | 37 | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> 80-84 | 535 | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> 75-79 | 3171 | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> 70-74 | 12163 | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> 65-69 | 28648 | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> 0-64 (least similar) | 74370 | | | | | | | | | | | | | | | | | | | | |
| <p>Score: 92</p> <p><input type="checkbox"/> 1. 1070872-24-0</p>  <p>Absolute stereochemistry.</p> <p>C25 H30 O11 β-D-Glucopyranoside, (3<i>R</i>)-3,4-dihydro-3-(2-hydroxy-3,4-dimethoxyphenyl)-2<i>H</i>-1-benzopyran-7-yl, 6-acetate</p> <p>Key Physical Properties</p> | <p>Score: 90</p> <p><input type="checkbox"/> 2. 285128-29-2</p>  <p>Absolute stereochemistry.</p> <p>C26 H30 O13 β-D-Glucopyranoside, (3<i>R</i>)-3,4-dihydro-3-(2-hydroxy-3,4-dimethoxyphenyl)-2<i>H</i>-1-benzopyran-7-yl, 6-(hydrogen propanoate)</p> <p>Key Physical Properties</p> | <p>Score: 89</p> <p><input type="checkbox"/> 3. 103215-63-0</p>  <p>C34 H38 O13 β-D-Glucopyranoside, 2-(3,4-dimethoxyphenyl)-3,4-dihydro-3-hydroxy-7-methoxy-2<i>H</i>-1-benzopyran-5-yl, 6-[3-(4-methoxyphenyl)-2-propenoate], (2<i>R</i>,<i>trans</i>)- (9<i>C</i>1)</p> <p>Key Physical Properties</p> | | | | | | | | | | | | | | | | | | | |
| <p>Score: 89</p> <p><input type="checkbox"/> 4. 1024025-73-7</p>  <p>Double bond geometry as shown..Absolute stereochemistry.</p> <p>C26 H26 O11 4<i>H</i>-1-Benzopyran-4-one, 3-(3-hydroxy-4-methoxyphenyl)-7-[[6-<i>O</i>-[(2<i>E</i>)-1-oxo-2-buten-1-yl]-β-D-glucopyranosyl]oxy]</p> <p>Key Physical Properties</p> | <p>Score: 89</p> <p><input type="checkbox"/> 5. 2099167-54-9</p>  <p>Absolute stereochemistry..Rotation (+)..Double bond geometry as shown.</p> <p>C31 H40 O13 β-D-Glucopyranoside, (2<i>R</i>,3<i>S</i>)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-3,5-dihydroxy-2<i>H</i>-1-benzopyran-7-yl, 6-[[2<i>E</i>,6<i>R</i>]-8-hydroxy-2,6-dimethyl-2-octenoate]</p> <p>Key Physical Properties</p> | <p>Score: 87</p> <p><input type="checkbox"/> 6. 123656-61-1</p>  <p>Double bond geometry as shown..Absolute stereochemistry.</p> <p>C26 H36 O12 4<i>H</i>-1-Benzopyran-4-one, 5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-[[6-<i>O</i>-[1-oxo-2-butenyl]-β-D-glucopyranosyl]oxy]-, (<i>E</i>)- (9<i>C</i>1)</p> <p>Key Physical Properties Spectra</p> | | | | | | | | | | | | | | | | | | | |

Figure S1: Scifinder search of new compound 1

Event#: 1 MS(E+) Ret. Time : 0.637 Scan#: 95



| Formula (M) | Ion | Meas. m/z | Pred. m/z | Df. (mDa) | Df. (ppm) |
|---|---------------------|-----------|-----------|-----------|-----------|
| C ₂₇ H ₃₂ O ₁₁ | [M+Na] ⁺ | 555.1838 | 555.1837 | 0.1 | 0.18 |

Figure S2: HR-ESI-MS spectrum of 1

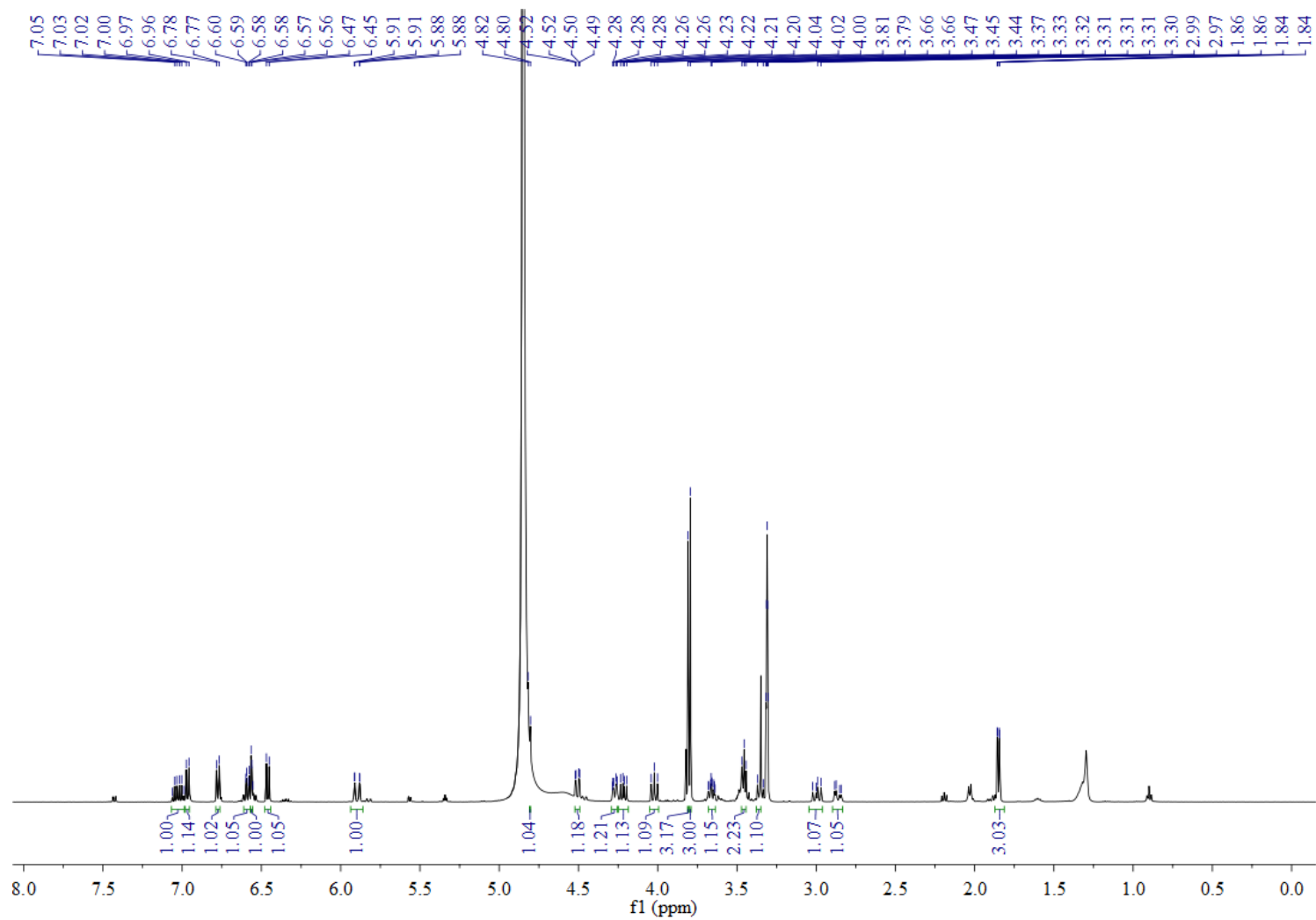


Figure S3: ¹H-NMR (500 MHz, CD₃OD) spectrum of 1

© 2021 ACG Publications. All rights reserved.

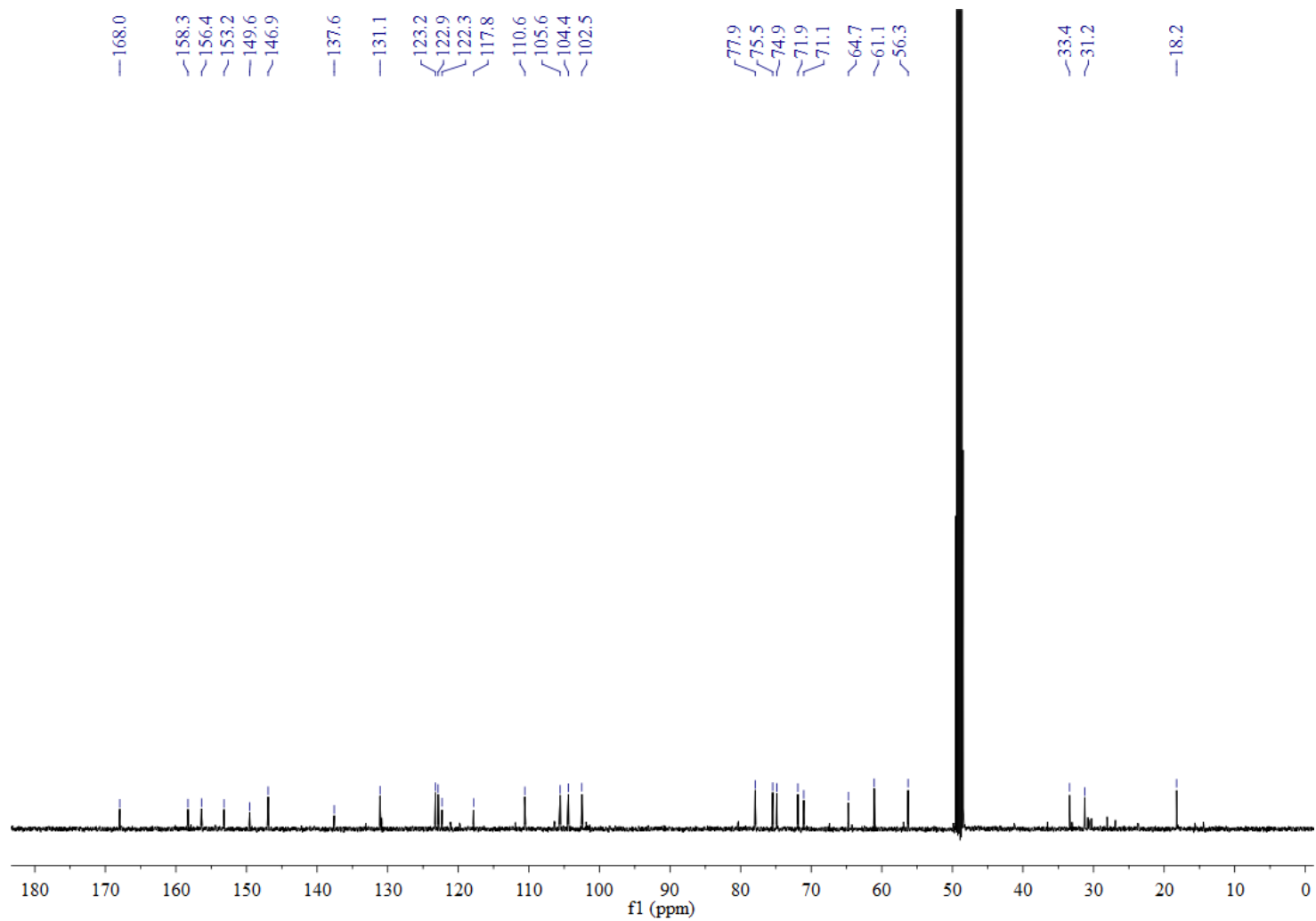


Figure S4: ¹³C-NMR (125 MHz, CD₃OD) spectrum of **1**

© 2021 ACG Publications. All rights reserved.

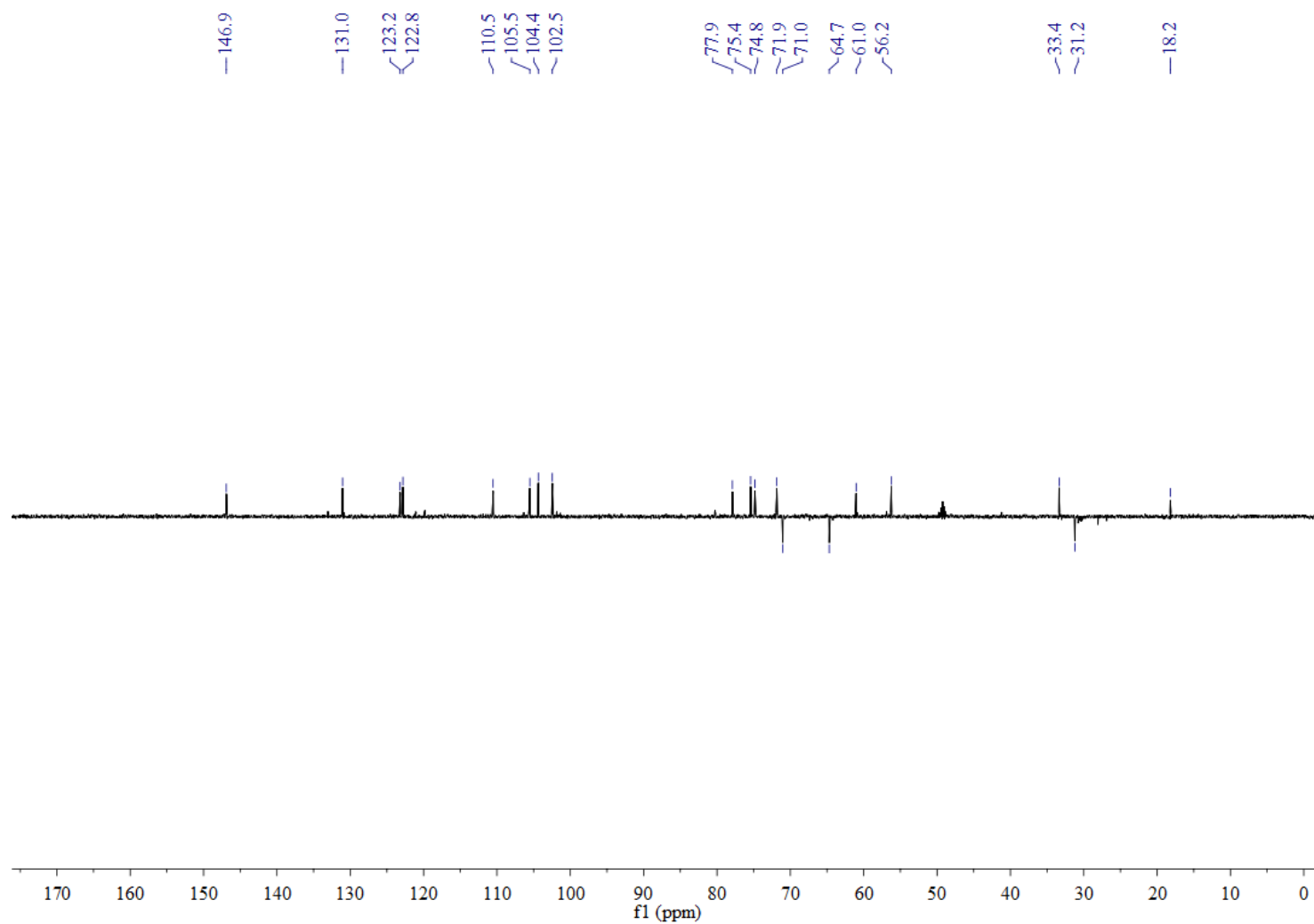


Figure S5: DEPT135 (125 MHz, CD₃OD) spectrum of **1**

© 2021 ACG Publications. All rights reserved.

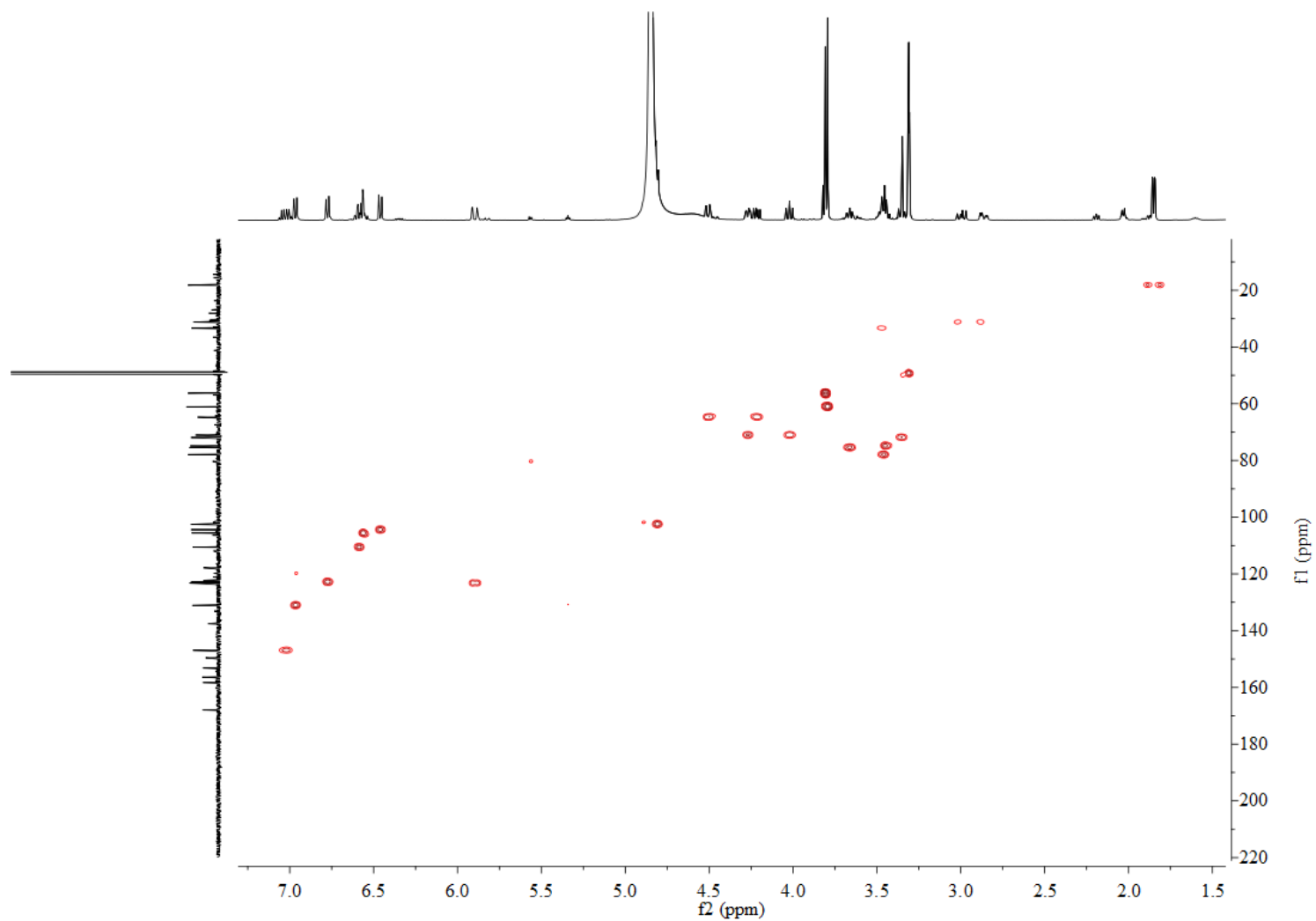


Figure S6: HSQC spectrum of **1**

© 2021 ACG Publications. All rights reserved.

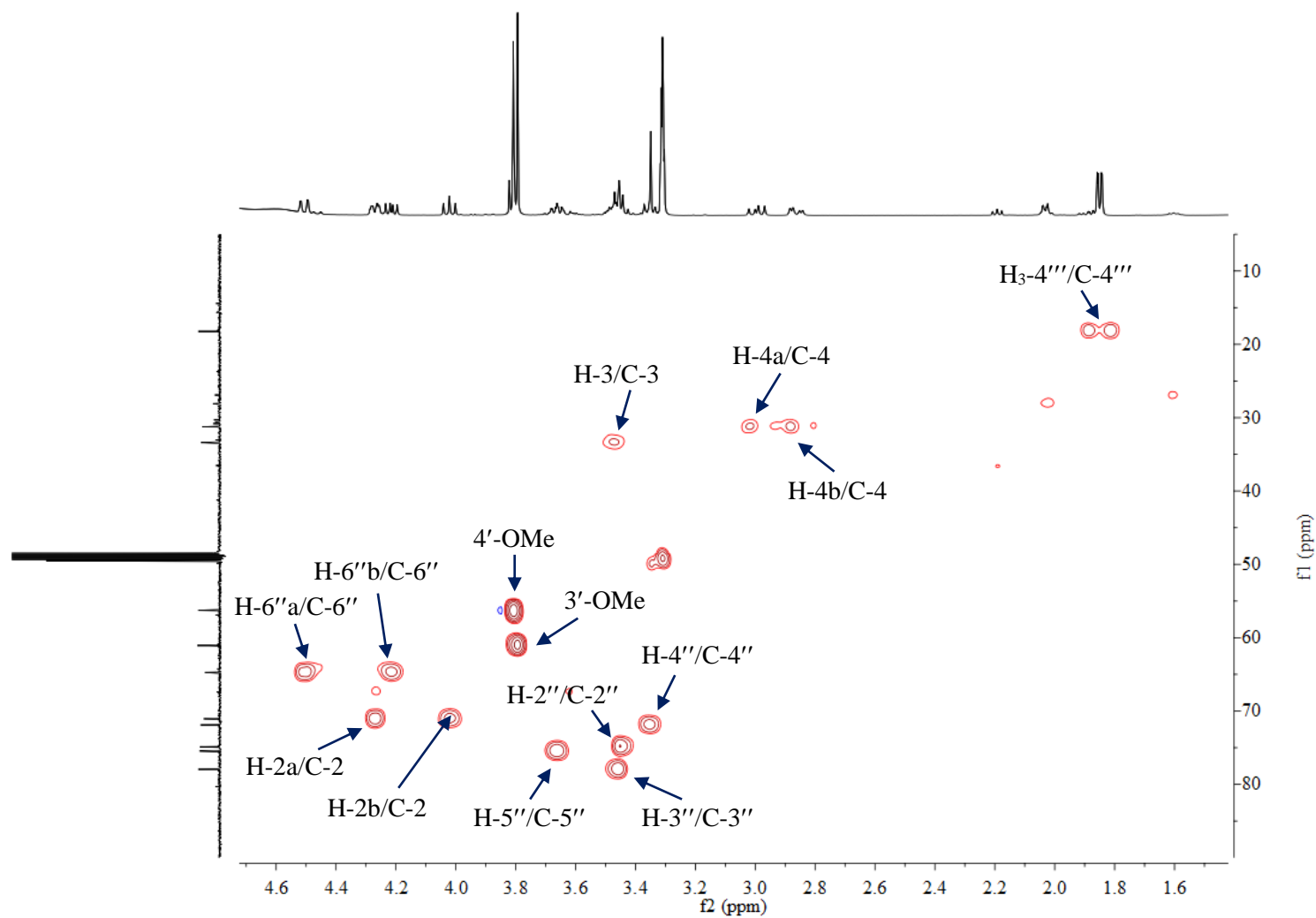


Figure S7: HSQC spectrum of **1** (From δ_{C} 5 ppm to δ_{C} 90 ppm)

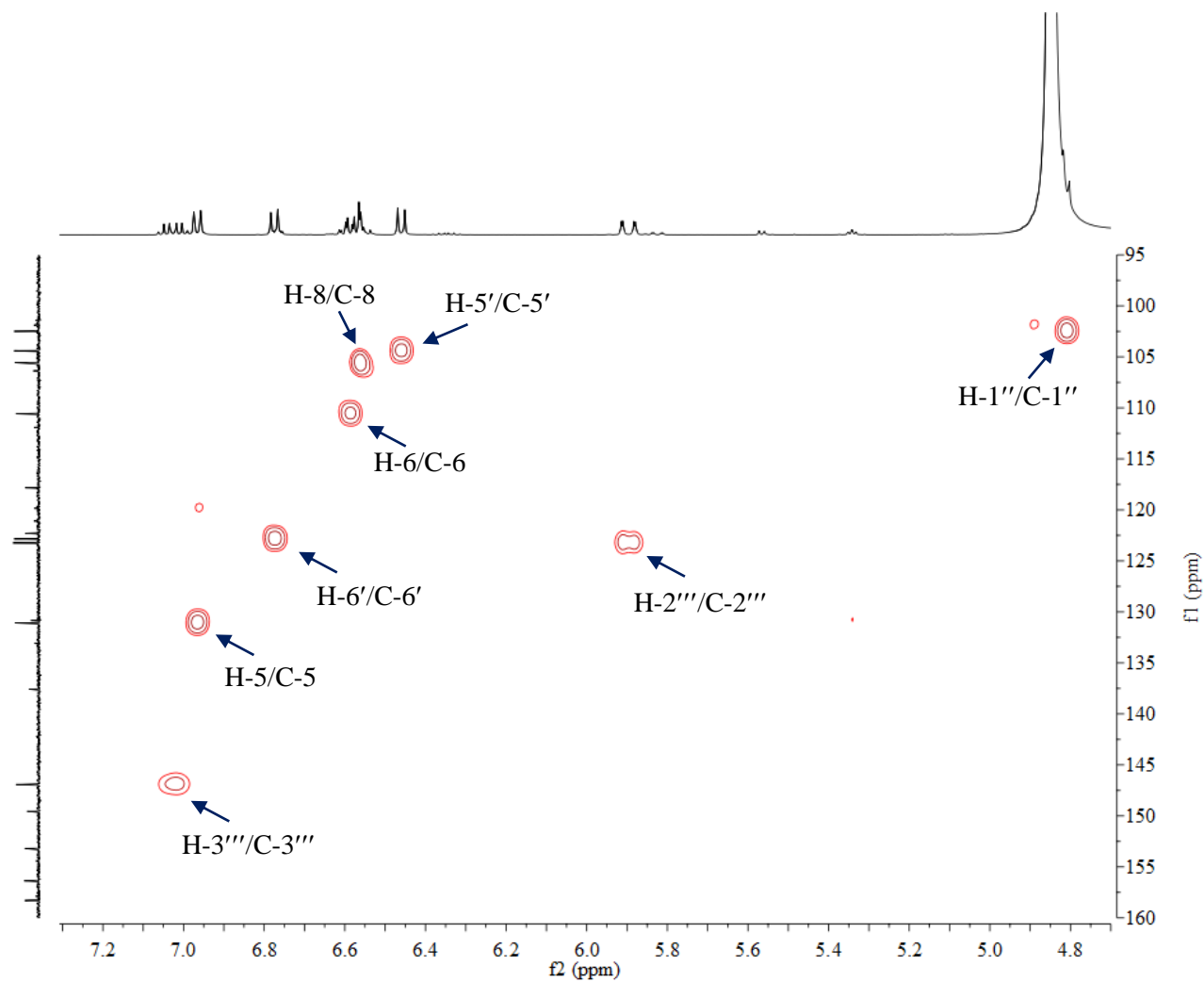


Figure S8: HSQC spectrum of **1** (From δ_{C} 95 ppm to δ_{C} 160 ppm)

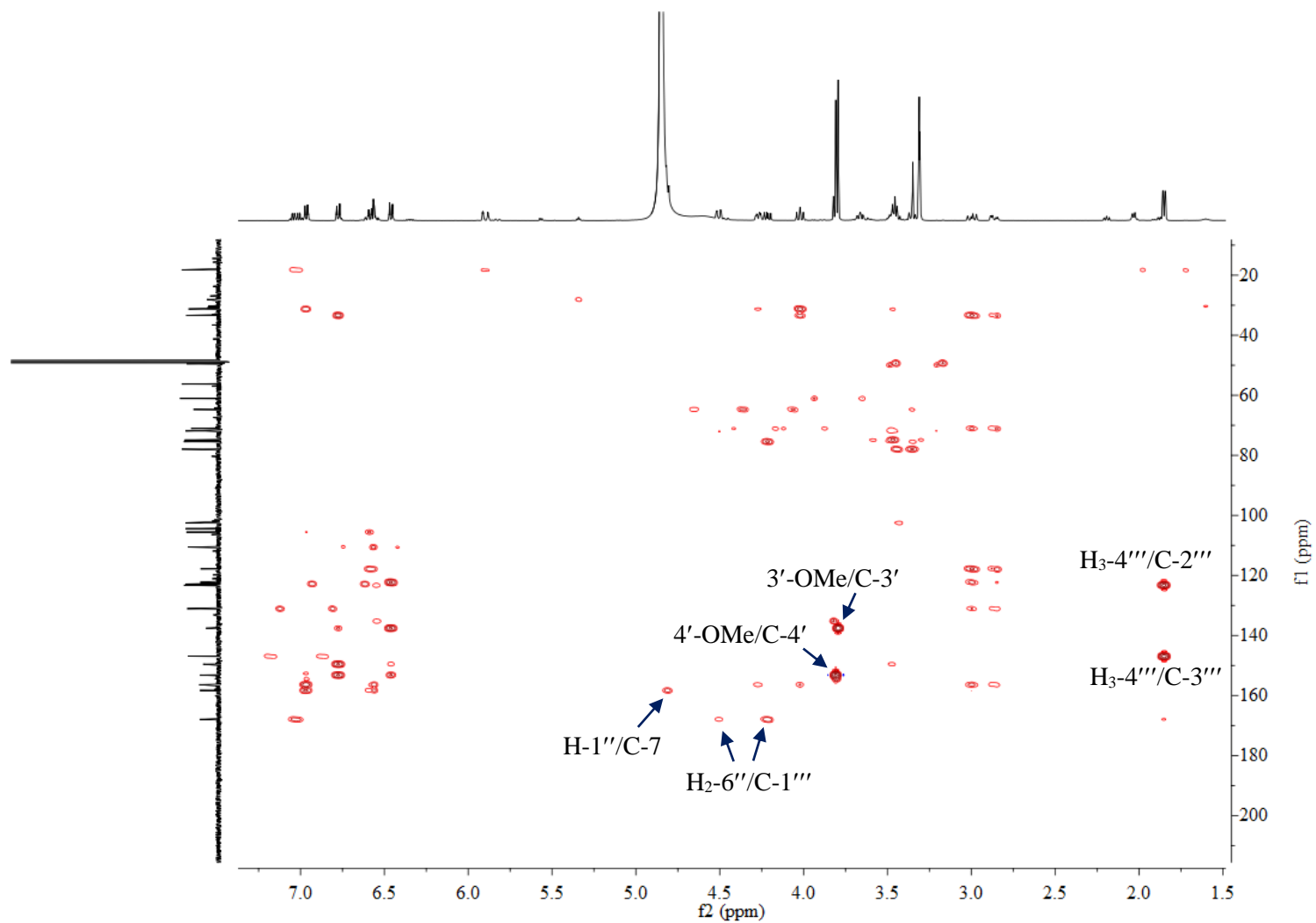


Figure S9: HMBC spectrum of **1**

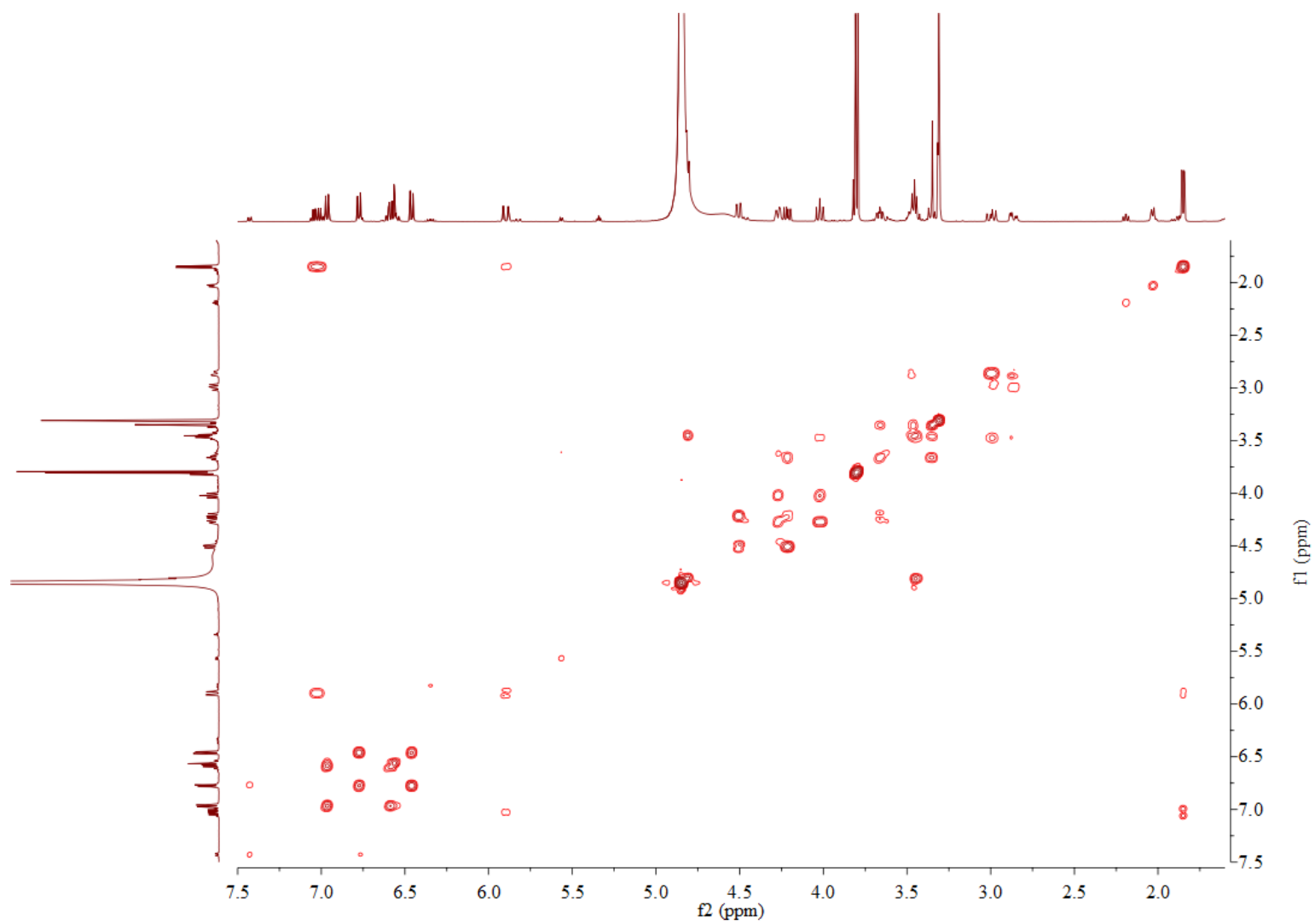


Figure S10: ^1H - ^1H COSY spectrum of **1**

© 2021 ACG Publications. All rights reserved.