Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

An ortho *C*-methylation/*O*-glycosylation motif on a hydroxy-coumarin scaffold, selectively installed by biocatalysis

Alexander Gutmann,[a] Margaretha Schiller,[a] Mandana Gruber-Khadjawi[b,c] and Bernd Nidetzky[a,b]*

[a] Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, NAWI Graz, Petersgasse 12, 8010 Graz, Austria
Fax: (+43)-316-873-8434; phone:(+43)-316-873-8400; e-mail: bernd.nidetzky@tugraz.at

[b] Austrian Centre of Industrial Biotechnology, Petersgasse 14, 8010 Graz, Austria

[c] Institute of Organic Chemistry, Graz University of Technology, NAWI Graz, Stremayrgasse 9, 8010 Graz, Austria

Table of contents

1. Experimental Section

Solubility of 4,5,7-trihydroxy-3-phenyl-coumarin (1) in presence of DMSO

The solubility of **1** was tested in 50 mM phosphate buffer (pH 6.5) supplemented with 5, 10 or 20% DMSO. At each DMSO concentration **1** was added to final concentrations of 0.5, 1 and 2 mM. After overnight incubation on a thermomixer at 1000 rpm and 35°C, samples were centrifuged for 15 min at 13200 rpm to remove undissolved **1**. The concentration of dissolved **1** in the supernatant was determined by HPLC.

Substrate inhibition kinetics

The dependence of NovO and UGT71A15 reaction rates on **1** concentration was fitted by Equation 1 to account for the effect of substrate inhibition. Experimentally determined reaction rates (r) at distinct concentrations of **1** (c_1) were used. The theoretically attainable maximum rate in absence of substrate inhibition (r_{max}), the Michaelis–Menten constant (K_M) and the inhibition constant (K_I) were fitted. IC_{50} values represent concentrations of **1** where inhibition by **1** decreased the reaction rate to 50% of the highest experimentally observed rate.

$$(1) \qquad r = \frac{r_{max} + c_1}{K_M + c_1 \cdot \left(1 + \frac{c_1}{K_I}\right)}$$

Equilibrium of the UGT71A15 reaction

The equilibrium constant (K_{eq}) of **1** glucosylation by UGT71A15 was calculated according to Equation 2. Concentrations of **1** (c_1) and **2** (c_2) were directly measured by HPLC and those for UDP and UDP-glc were inferred from conversion of **1** and reaction stoichiometry.

$$(2) \qquad K_{eq} = \frac{c_2 \cdot c_{UDP}}{c_1 \cdot c_{UDP-glc}}$$

2. Results

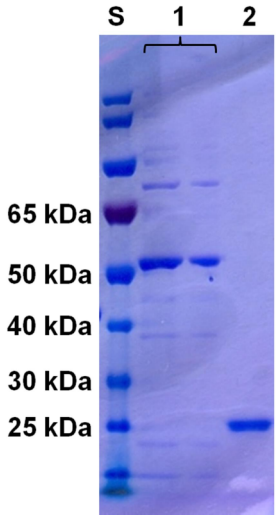

Fig. S1 Strep-tag affinity purified enzymes from *E. coli* overexpression cultures were analyzed by SDS-PAGE. S: PageRuler^{TM} Prestained Protein Ladder (Thermo Scientific); 1: UGT71A15 (53.9 kDa); 2: NovO (25.4 kDa).

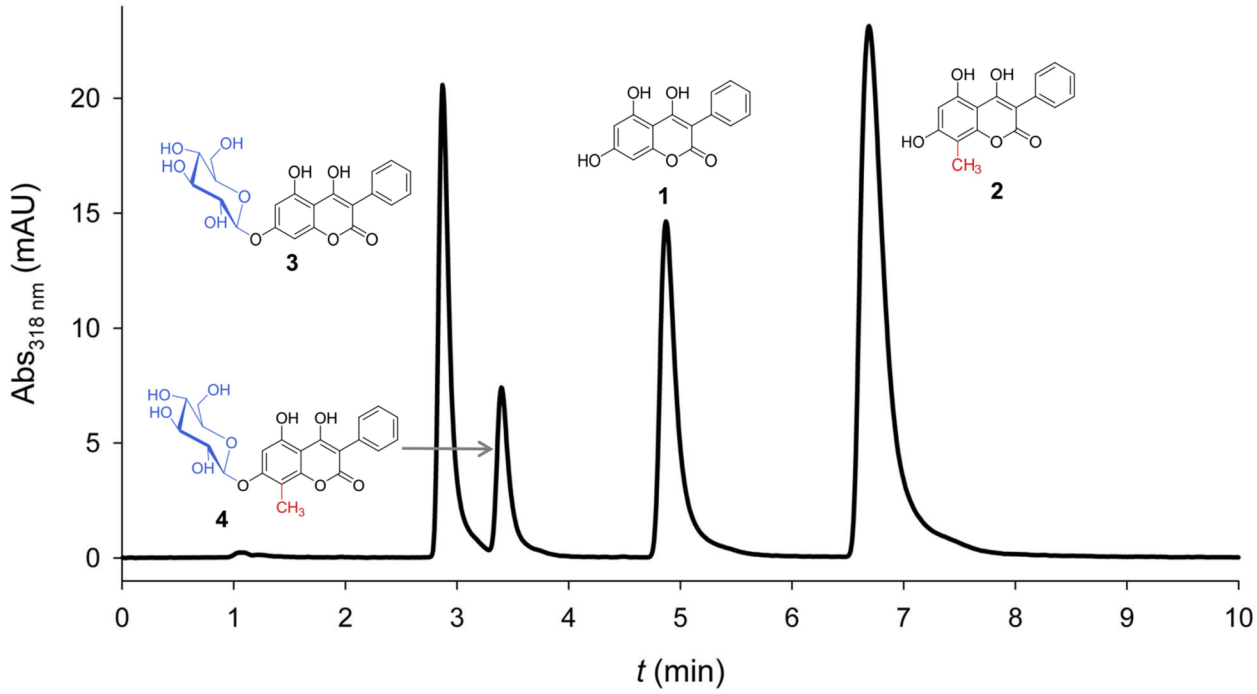

Fig. S2 Reversed phase C-18 HPLC with UV-detection at 318 nm was used to quantify compounds **1**-**4**.

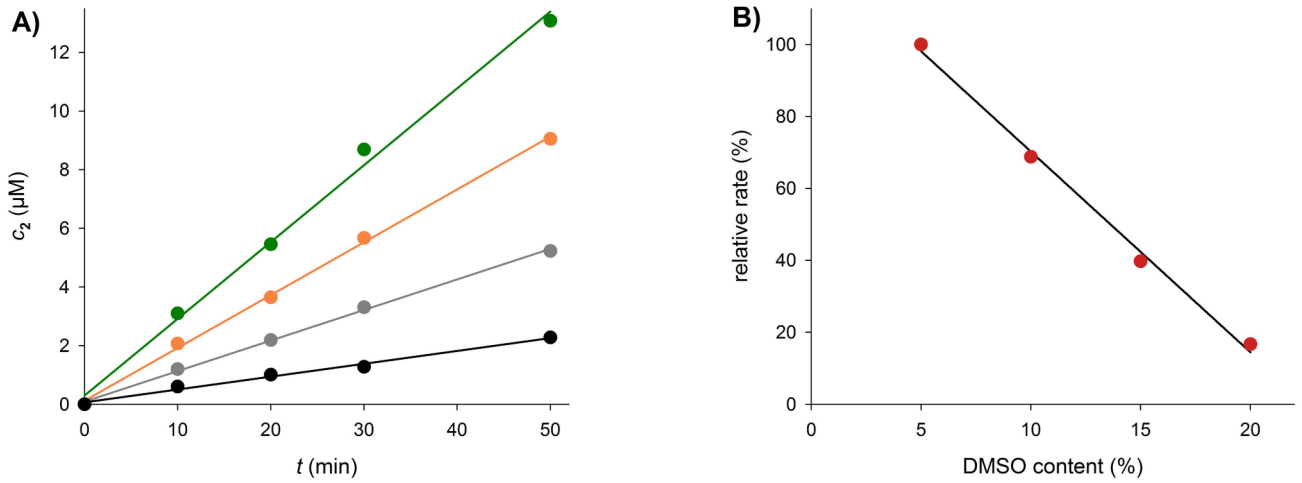

Fig. S3 The influence of DMSO on methylation of **1** by NovO was studied (100 µM **1**, 1 mM SAM, 5 µg mL^{-1} NovO, pH 6.5). A) Time courses of **2** formation are shown. The DMSO content was 5% (green), 10% (orange), 15% (grey) or 20% (black). Solid lines represent linear fits of the data. B) Relative reaction rates at various DMSO concentrations were calculated from the time courses shown in (A). The solid line represents a linear fit of the data.

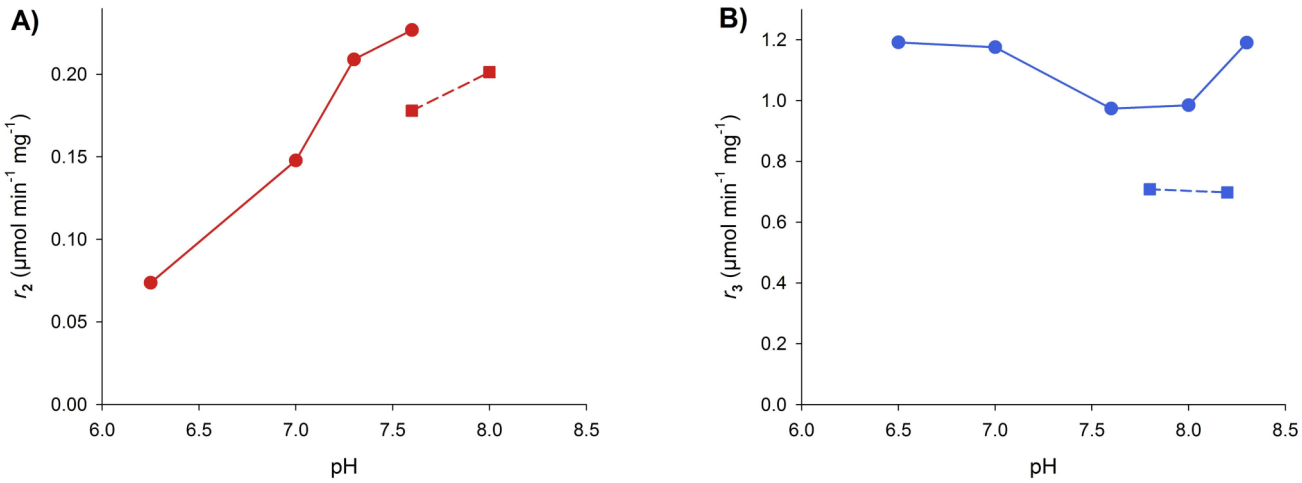

Fig. S4 The pH-activity profiles of NovO (A) and UGT71A15 (B) are shown. Either phosphate (circles, solid lines) or HEPES buffer (squares, dashed lines) was used. A) 100 µM **1** were methylated from 1 mM SAM by 5 µg mL^{-1} NovO. B) 1 mM **1** were glucosylated from 1.5 mM UDP-glc by 1 µg mL^{-1} UGT71A15.

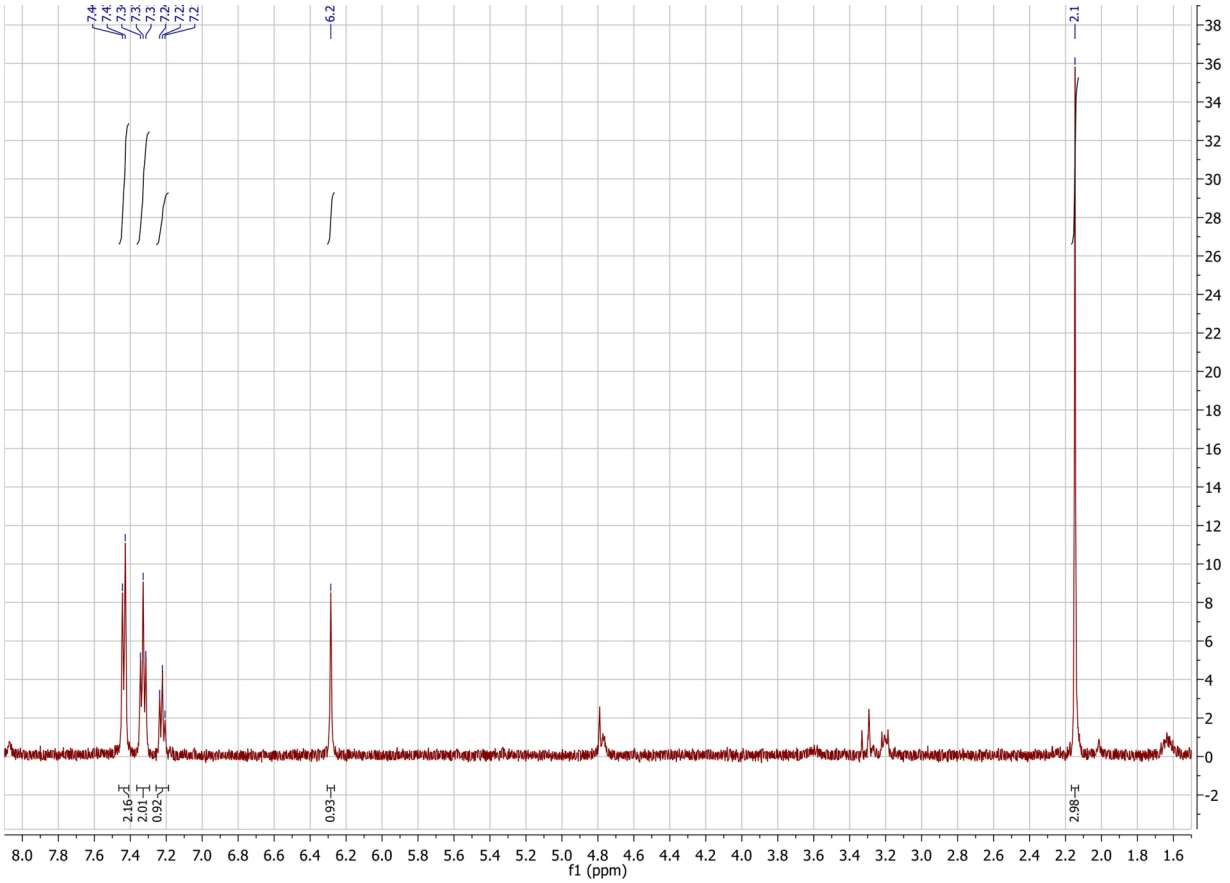

Fig. S5 ^{1}H-NMR of purified **2**.

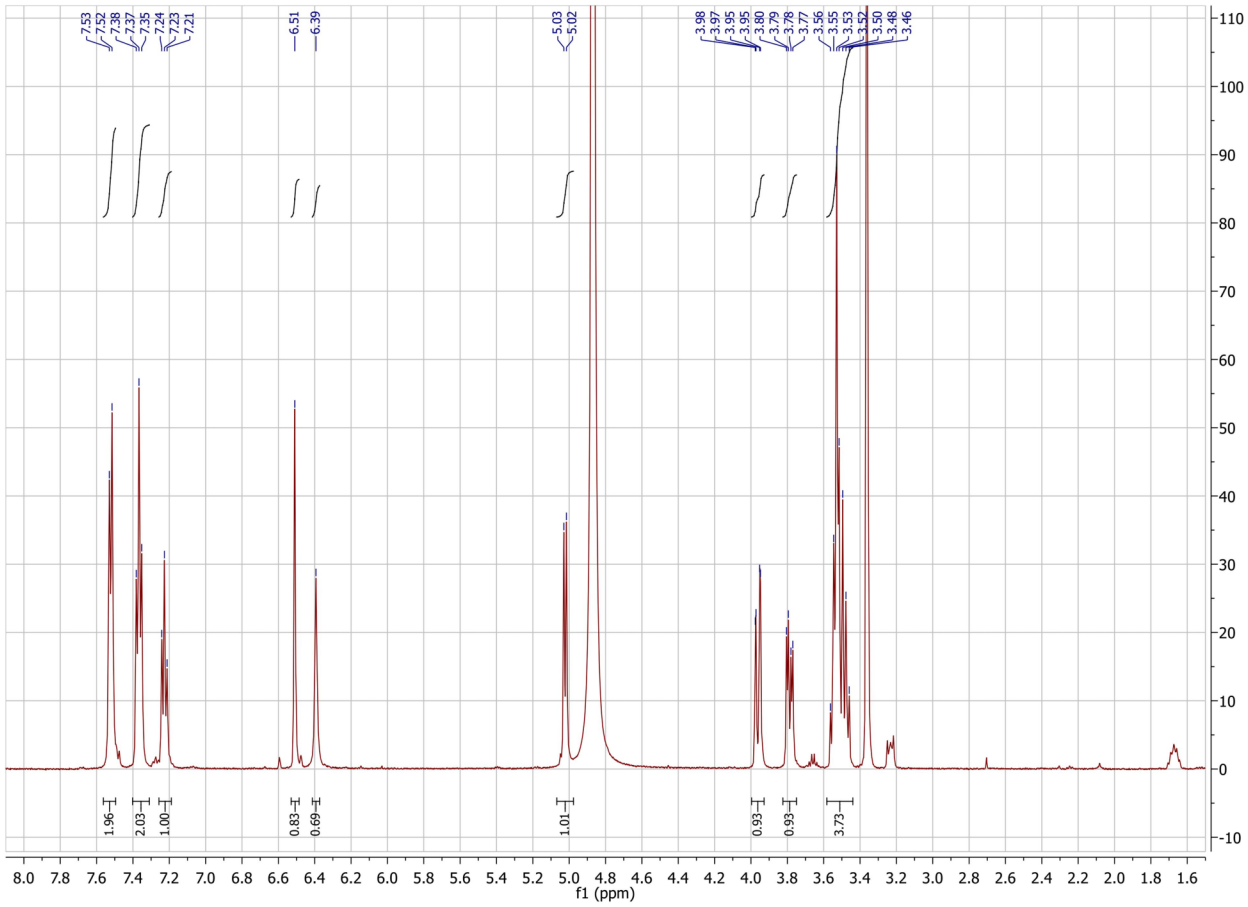

Fig. S6 ^{1}H-NMR of purified **3**.

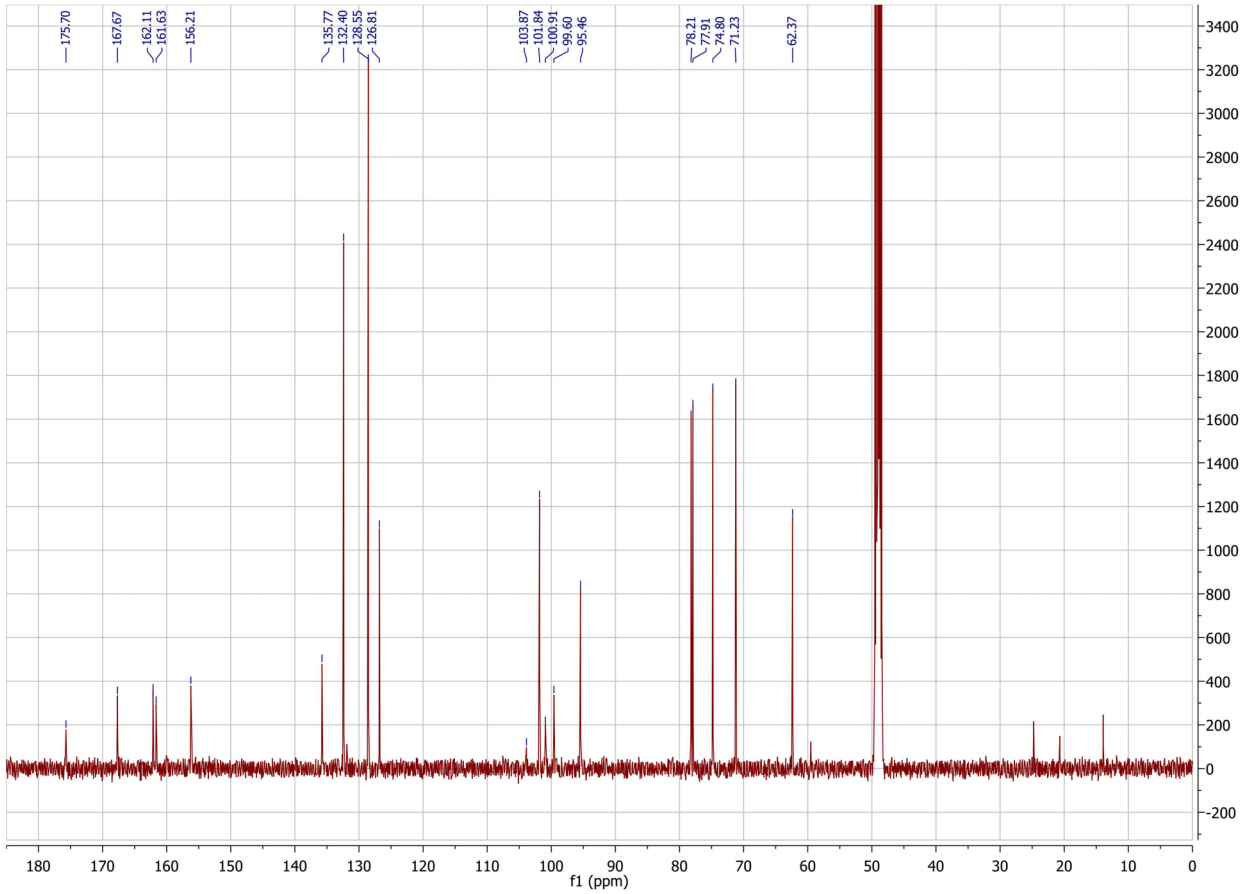

Fig. S7 ^{13}C-NMR of purified **3**.

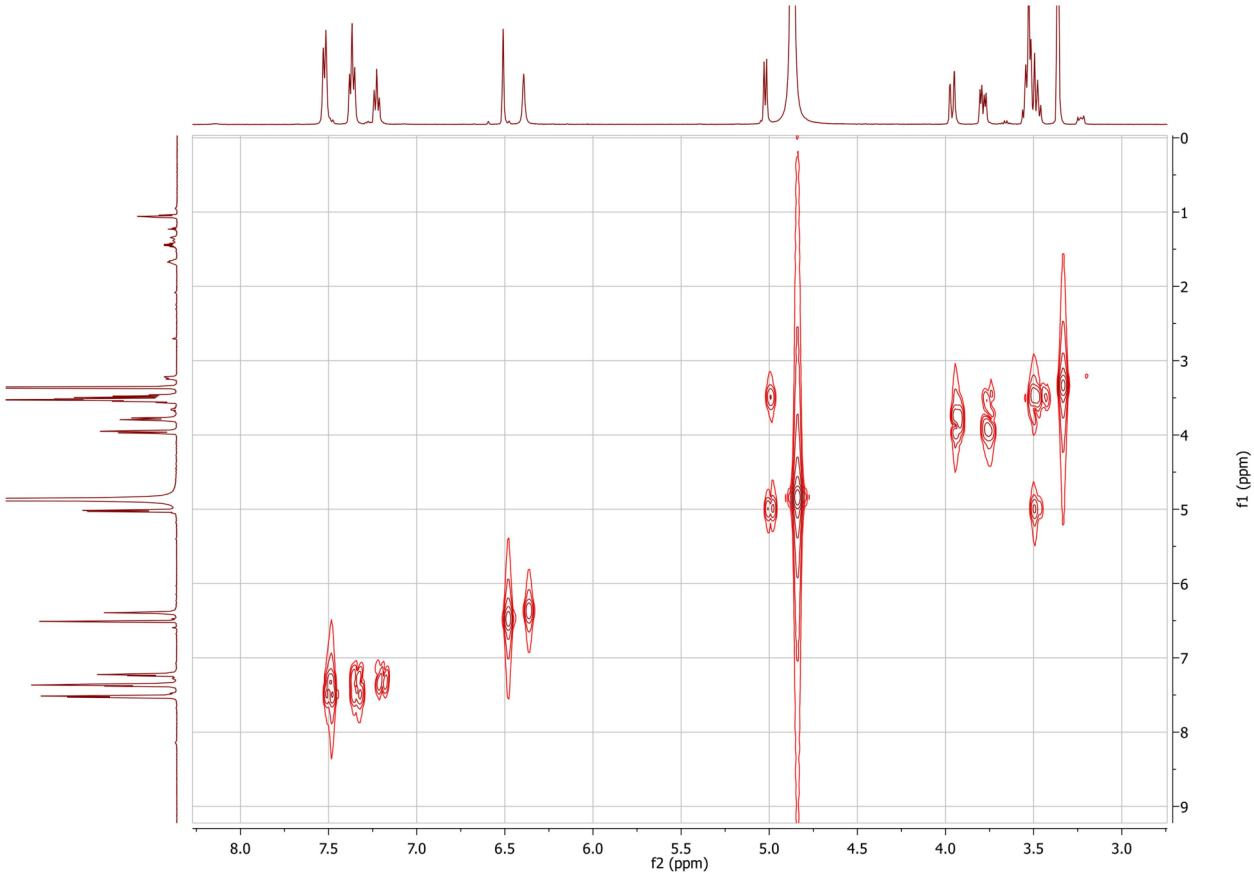

Fig. S8 2D COSY-NMR of purified **3**.

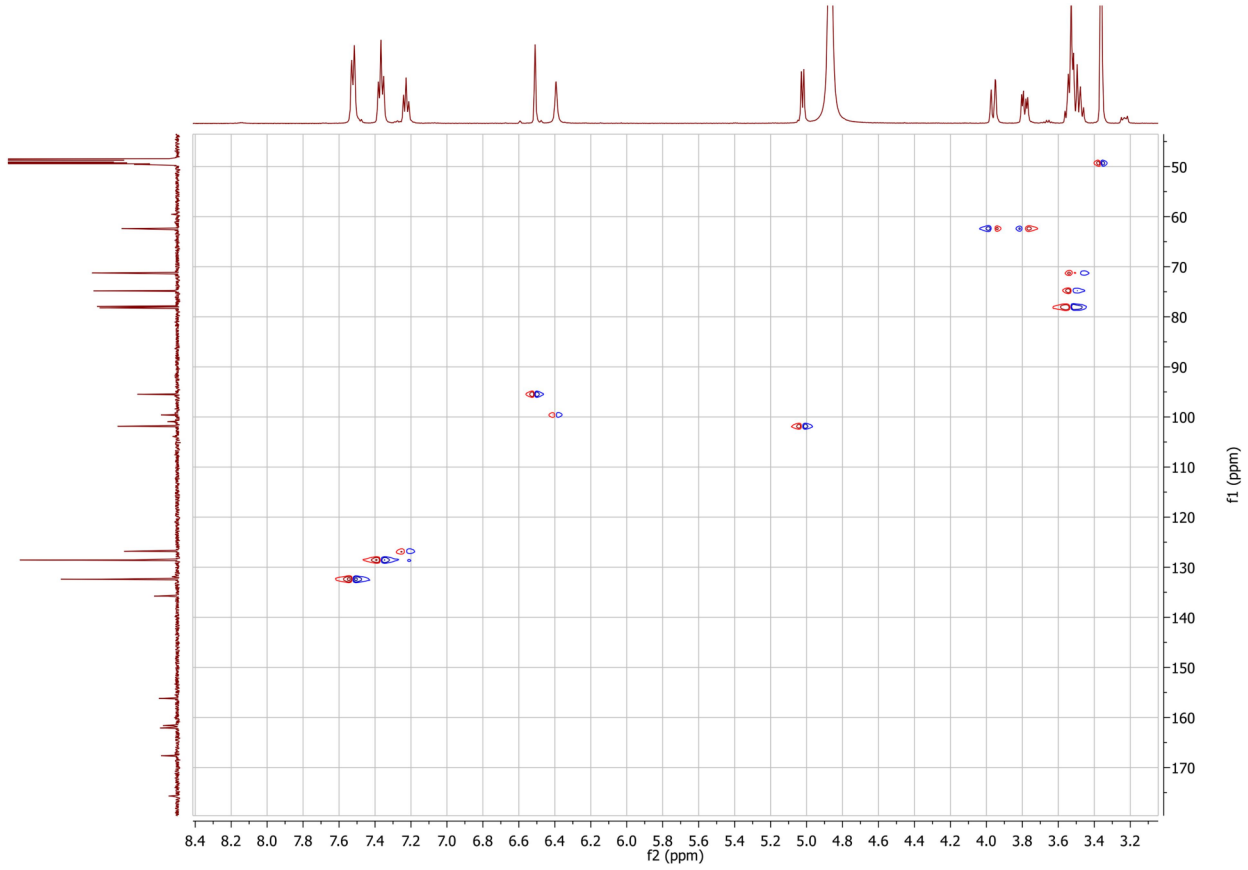

Fig. S9 2D HSQC-NMR of purified **3**.

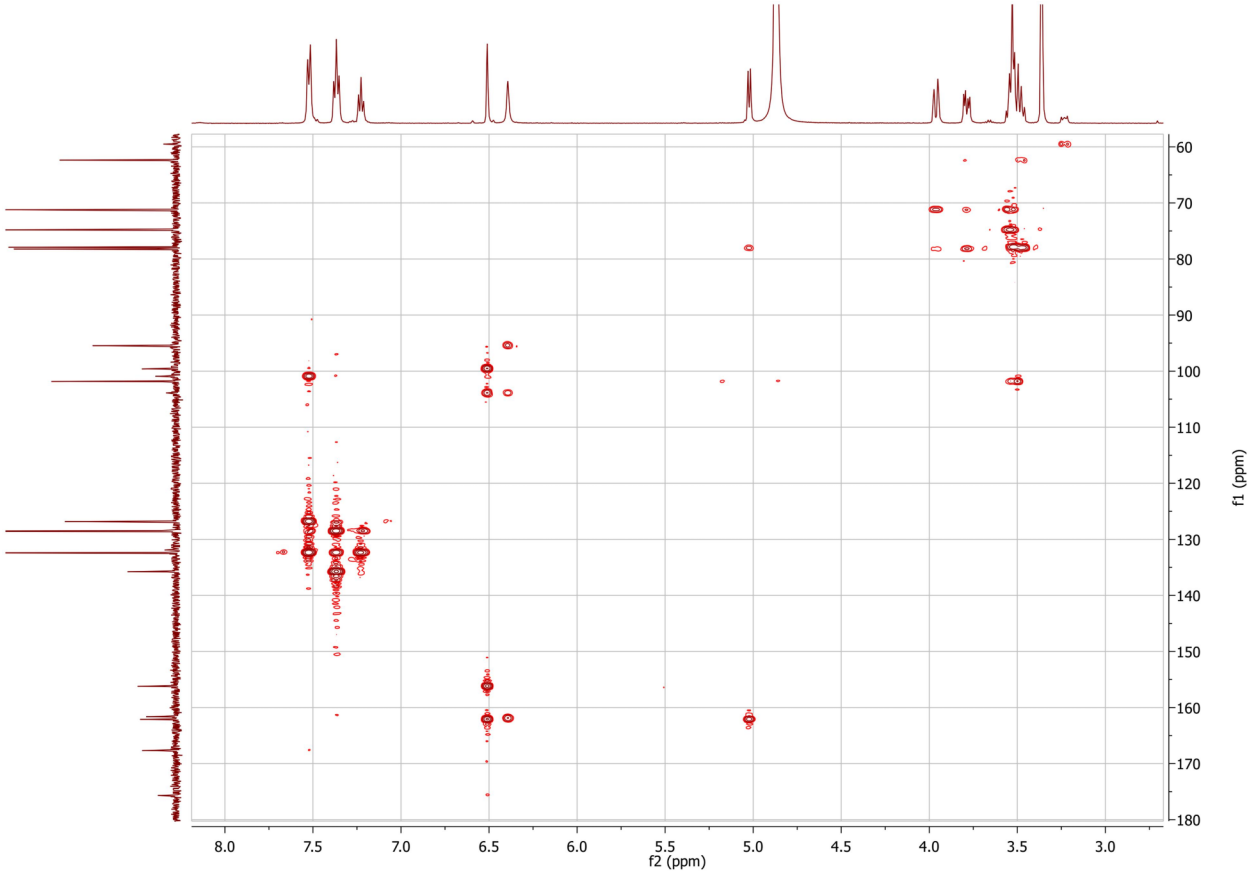

Fig. S10 2D HMBC-NMR of purified **3**.

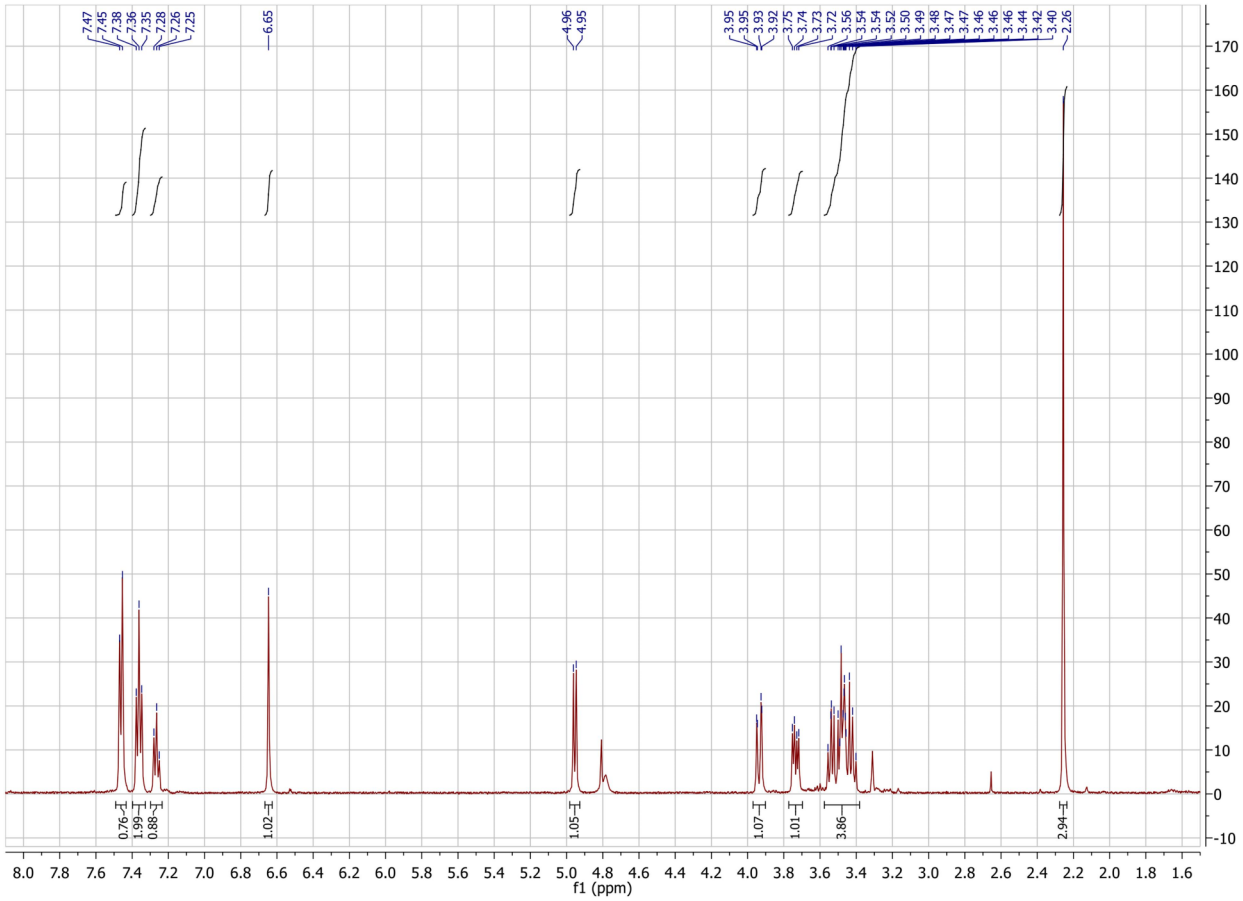

Fig. S11 ^{1}H-NMR of purified **4**.

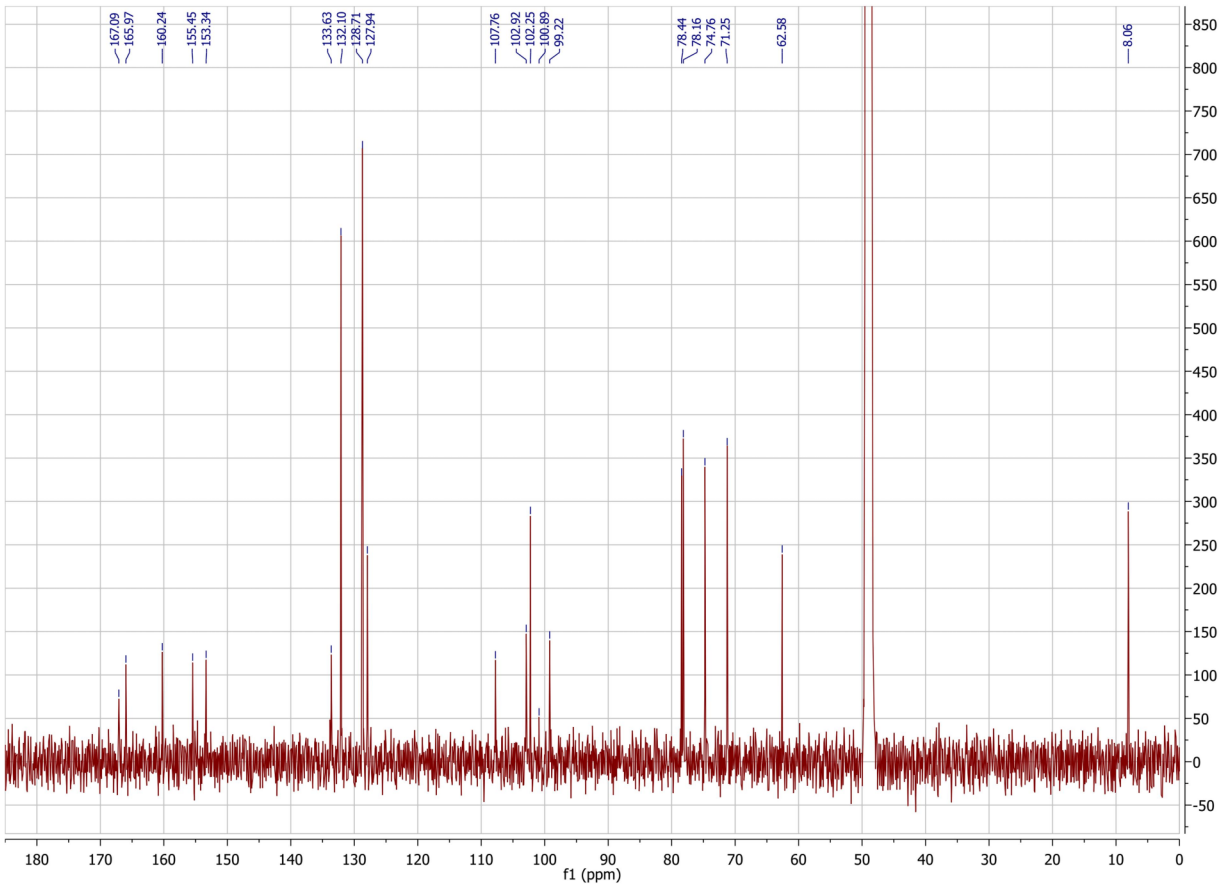

Fig. S12 ^{13}C-NMR of purified **4**.

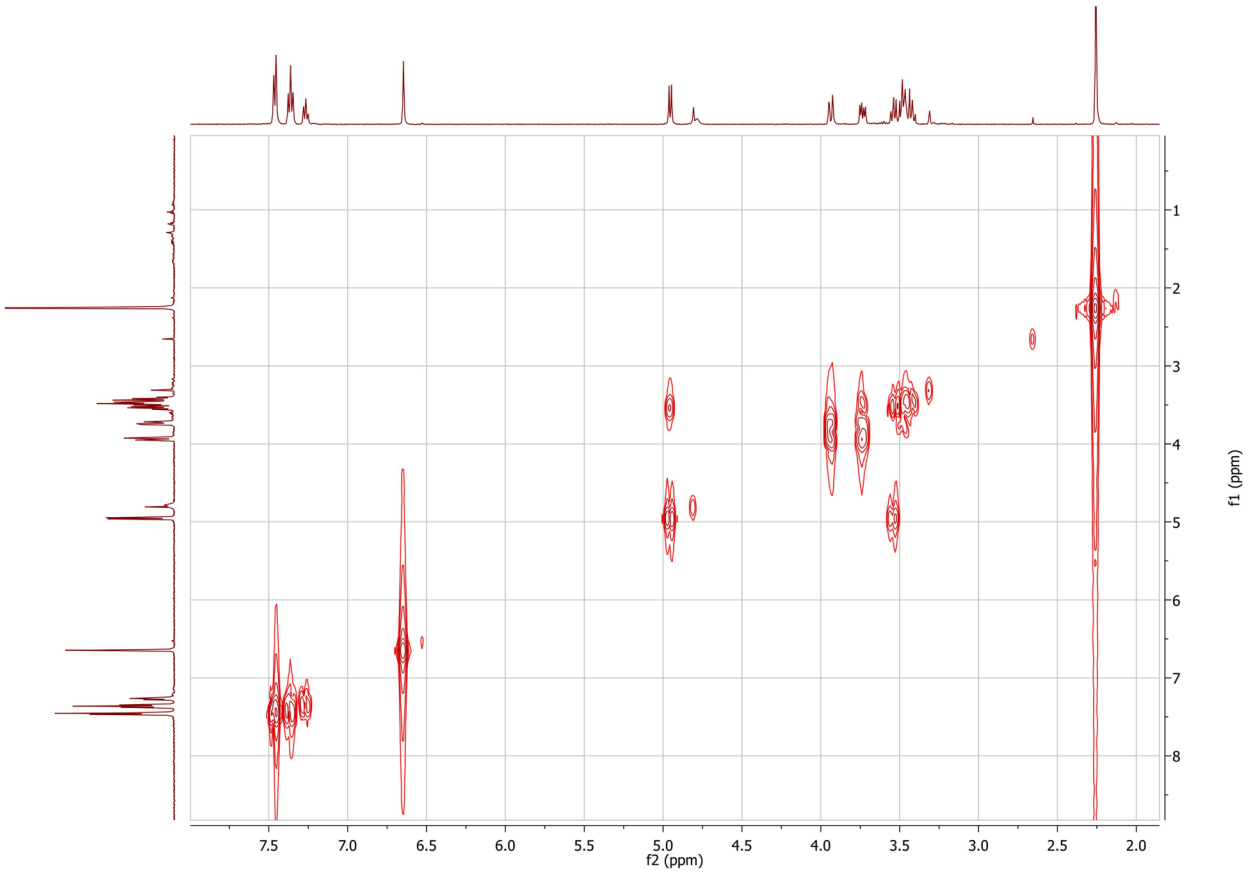

Fig. S13 2D COSY-NMR of purified **4**.

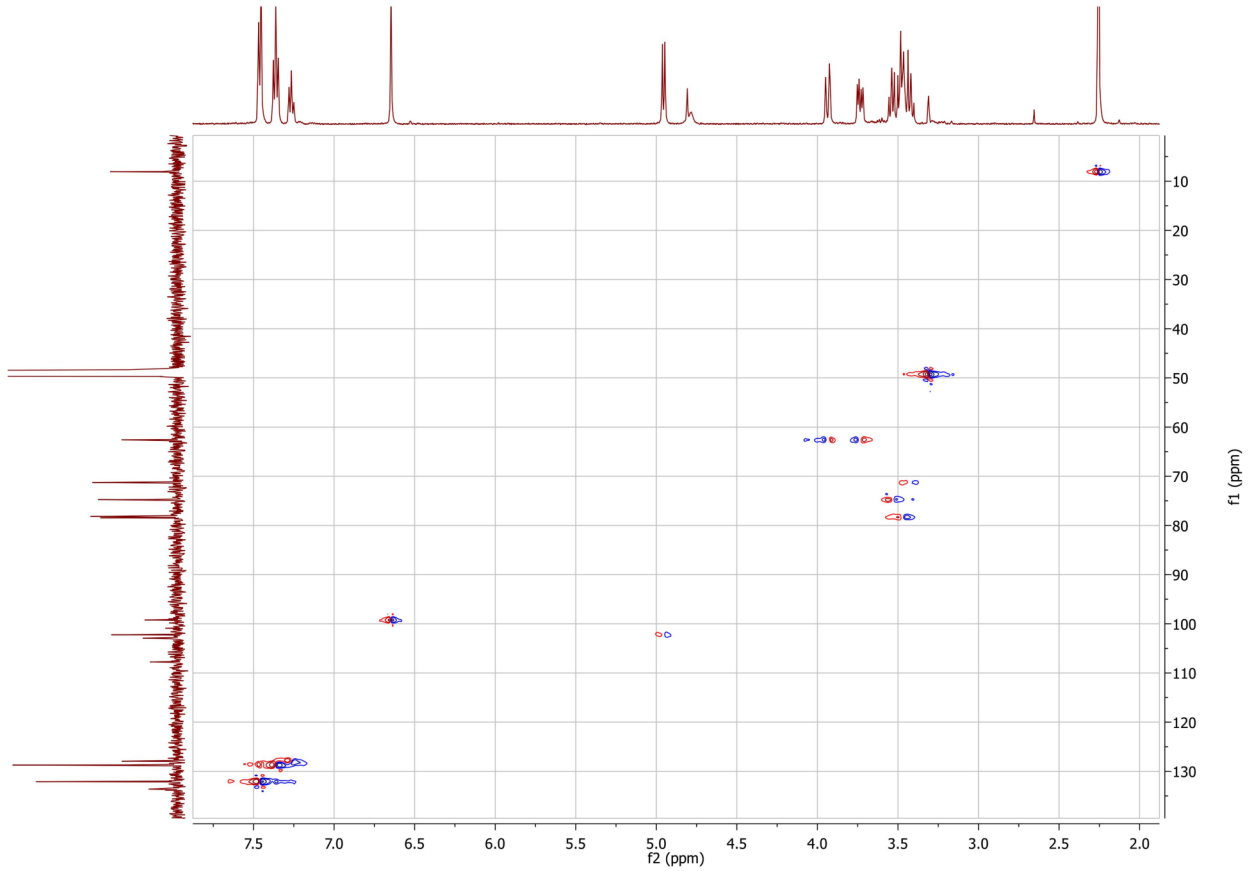

Fig. S14 2D HSQC-NMR of purified **4**.

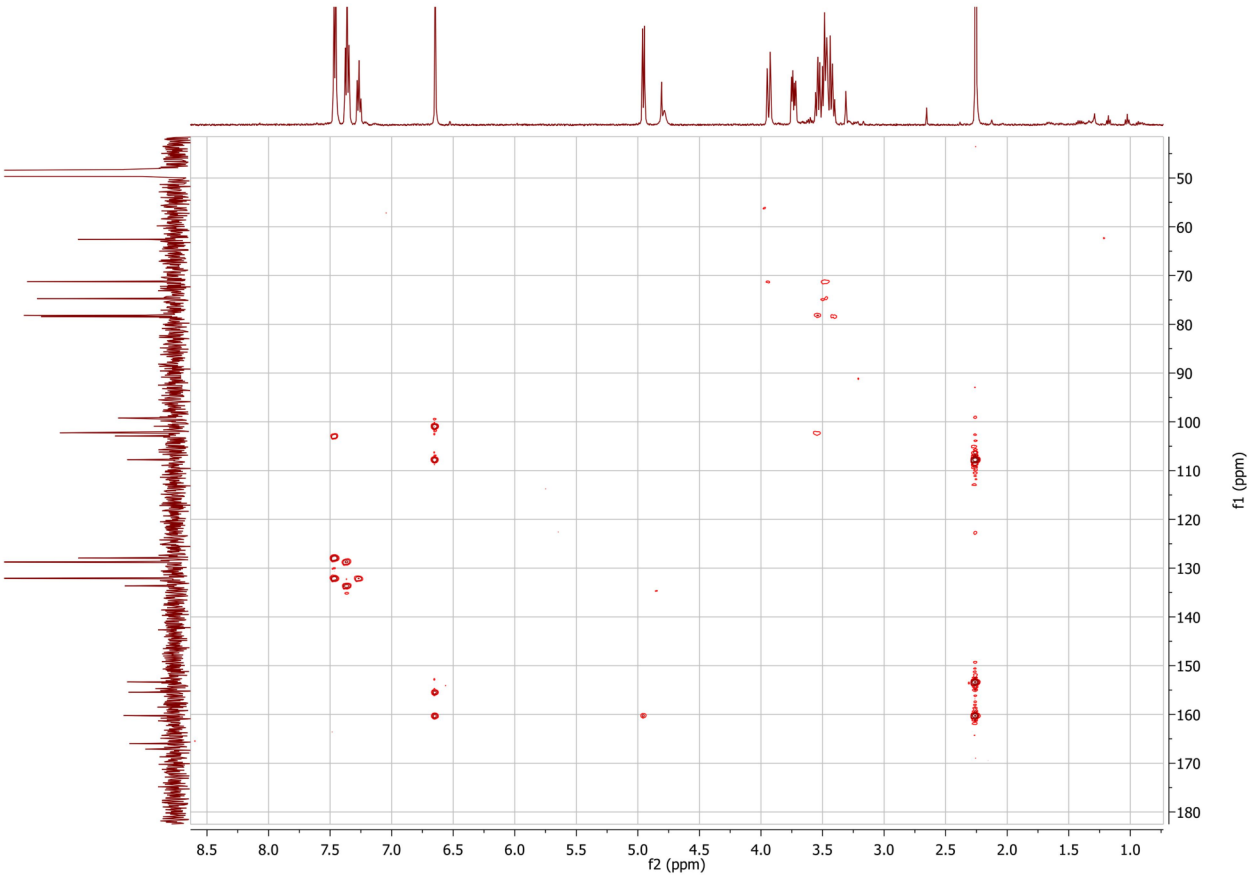

Fig. S15 2D HMBC-NMR of purified **4**.