Supporting Information

Discovery of Tricyclic Clerodane Diterpenes as Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase Inhibitors and Structure-Activity Relationships

Christian De Ford,[†,‡,§] Carlos Calderón,[#] Pankaj Sehgal,[‖,⊥] Natalya U. Fedosova,[‖,⊥] Renato Murillo,[#] Claus Olesen,[‖,⊥] Poul Nissen,[‖,⊥] Jesper V. Møller,[‖,⊥] and Irmgard Merfort[†,‡,§,*]

[†]Department of Pharmaceutical Biology and Biotechnology, Albert Ludwigs University Freiburg, Stefan-Meier-Strasse 19, D-79104 Freiburg, Germany

[‡]Spemann Graduate School of Biology and Medicine (SGBM), Albert Ludwigs University Freiburg, Albertstrasse 19a, 79104 Freiburg, Germany

[§]Faculty of Chemistry and Pharmacy, Albert Ludwigs University Freiburg, Albertstrasse 25, 79104 Freiburg, Germany

[#]Escuela de Química and CIPRONA, Universidad de Costa Rica, 2060 San José, Costa Rica

[‖]Department of Biomedicine, Ole Worms Allé3, Aarhus University, DK-8000 Aarhus C, Denmark

[⊥]Centre for Membrane Pumps in Cells and Disease (PUMPkin), National Research Foundation, Denmark

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Scheme S1. Hydrolysis reaction of compound **1**

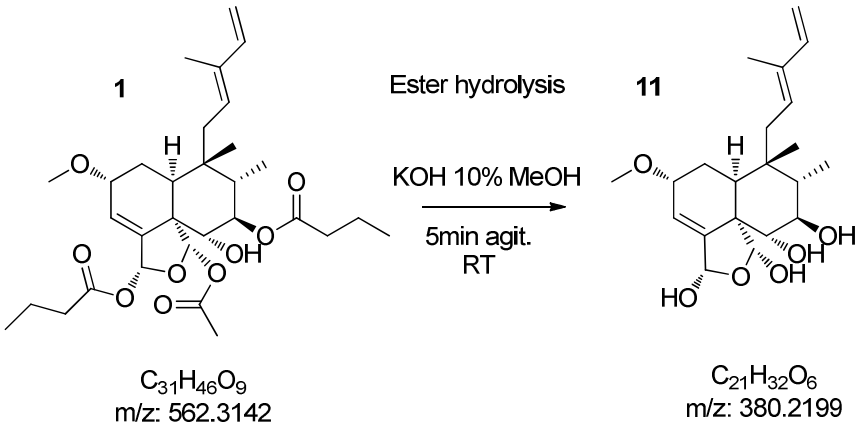


Table S1. Effects of TCDs on the Na^+,K^+-ATPase activity

compound	% NKA inhibition		
	1 μM	10 μM	50 μM
casearin J (**1**)	0.96±0.27	1.92±0.54	4.41±0.27
casearborin D (**2**)	3.13±0.69	14.20±2.88	23.84±1.64
zuelaguidin C (**9**)	3.01±1.34	9.59±4.69	26.3±1.25
7,18,19-deacyl-casearin J (**11**)	0.44±0.41	2.16±0.27	6.55±0.41

NKA: Na^+,K^+-ATPase

CDRUG

Result

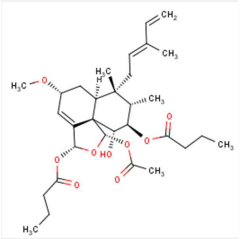
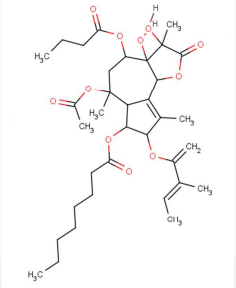
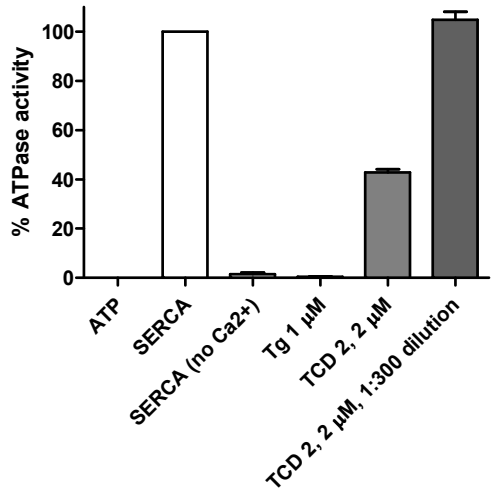
Rank	Query	Matches	Mean_logGI50	HSCORE	P-value
1			-7.727	0.194	0.2355
	Casearin J	Thapsigargin			

Figure S1. CDRUG result for **1**.

a)



b)

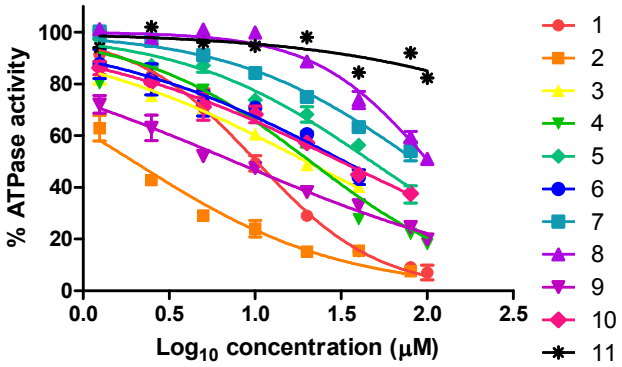


Figure S2. SERCA inhibition assay. A) Assay controls and example of lack of activity of TCDs after dilution, disproving the possibility of being pan-assay interference compounds (PAINS). In this example, compound **2** was tested at 2 µM as final concentration in the assay cuvette, showing 50% of inhibition. On the other hand, by preincubating the protein with the compound at the same concentration and then performing a 1:300 dilution in the assay cuvette, but maintaining the same final concentration of protein, a lack of inhibition is observed. B) Dose-response curves of TCDs on SERCA pump inhibition. The compounds were tested from 1.25-100 µM. Data represent means ± SD of three independent experiments.

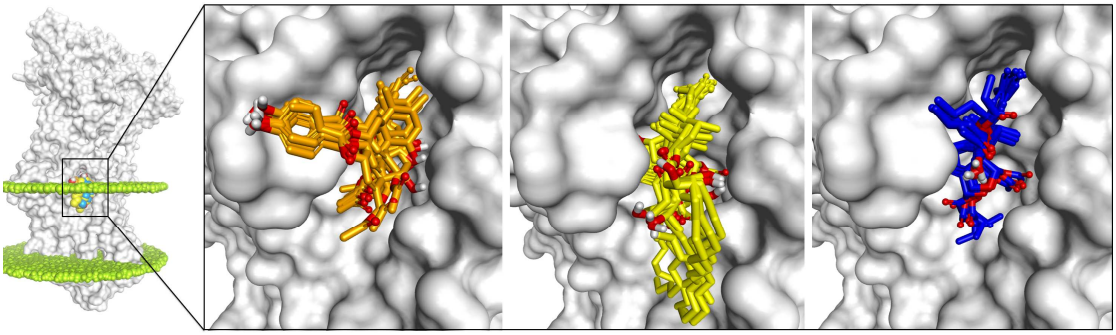


Figure S3. Convergence of solutions for TCDs **1** (blue), **2** (orange), and **9** (yellow) in the transmembrane binding site.

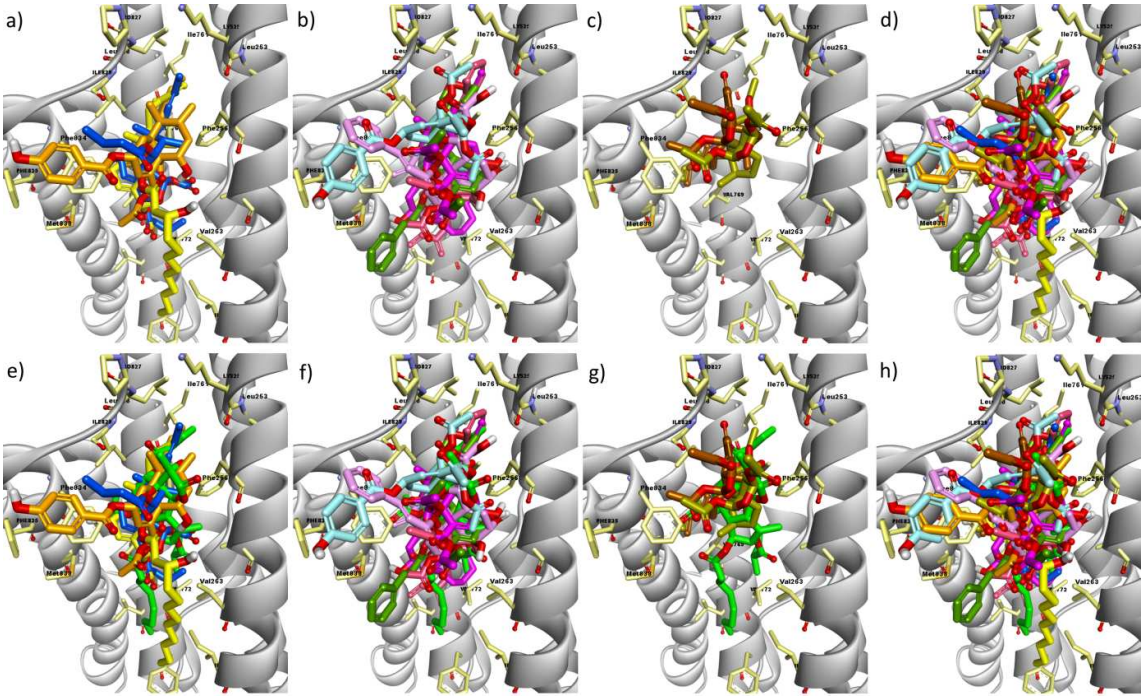


Figure S4. Binding modes of TCDs in the SERCA pump according to the inhibitory activity range. (A) Overlay of best TCDs with KD value > 10 μM, TCDs **1**, **2**, and **9** are shown in blue, yellow and orange, respectively. (B) Overlay of TCDs with KD values between 20-50 μM, TCDs **3**-**6**, and **10** are shown in turquoise, olive green, light pink, dark pink, and purple, respectively. C) TCDs with the worst activity, K_D value > 50 μM, TCDs **7**, and **8** are shown in mustard and brown, respectively. D) Overlay of all TCDs. E) overlay of both TCDs and thapsigargin (Tg). F) Overlay of TCDs with intermediate activity and Tg (green). G) Overlay of the least active TCDs and Tg. H) Overlay of all TCDs and Tg. Tg is shown in green.

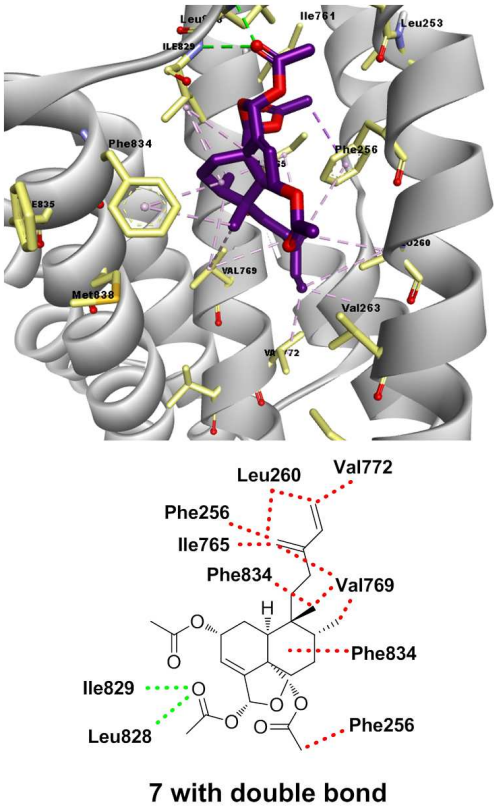


Figure S5. Docking of **7** with the double bond in the transmembrane domain of SERCA1a (PDB ID; 3N5K). The red lines show hydrophobic interactions, the green lines represent hydrogen bonds.

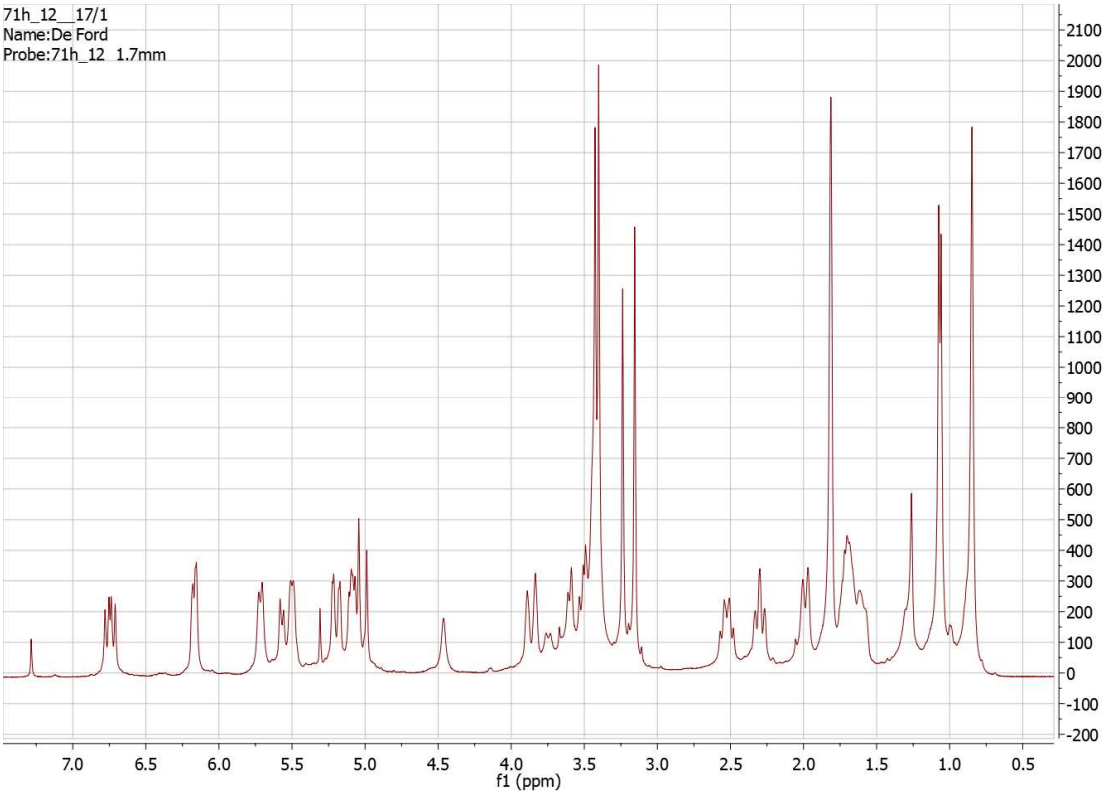


Figure S6. ^{1}H-NMR spectrum of compound **11**. The measure was carried out in$CDCl_3$ at 400 MHz.

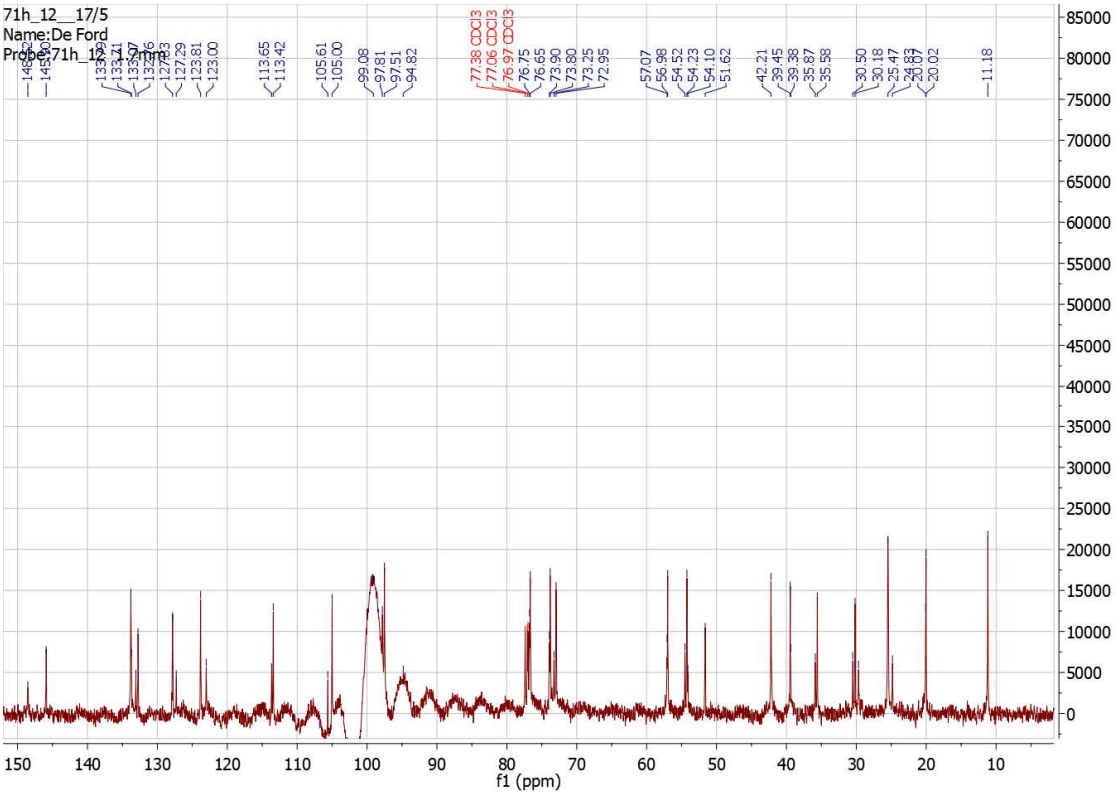


Figure S7. ^{13}C-NMR spectrum of compound **11**. The measure was carried out in$CDCl_3$ at125MHz.

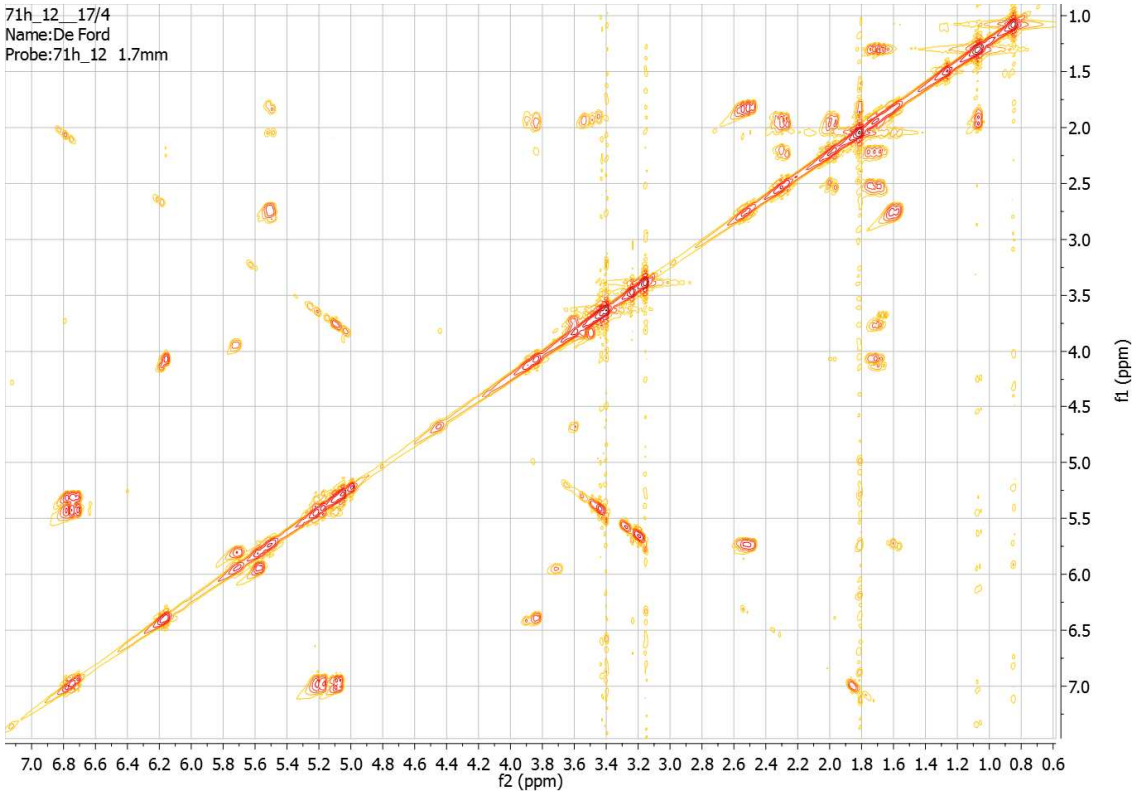


Figure S8. H,H-COSY spectrum of compound **11**.

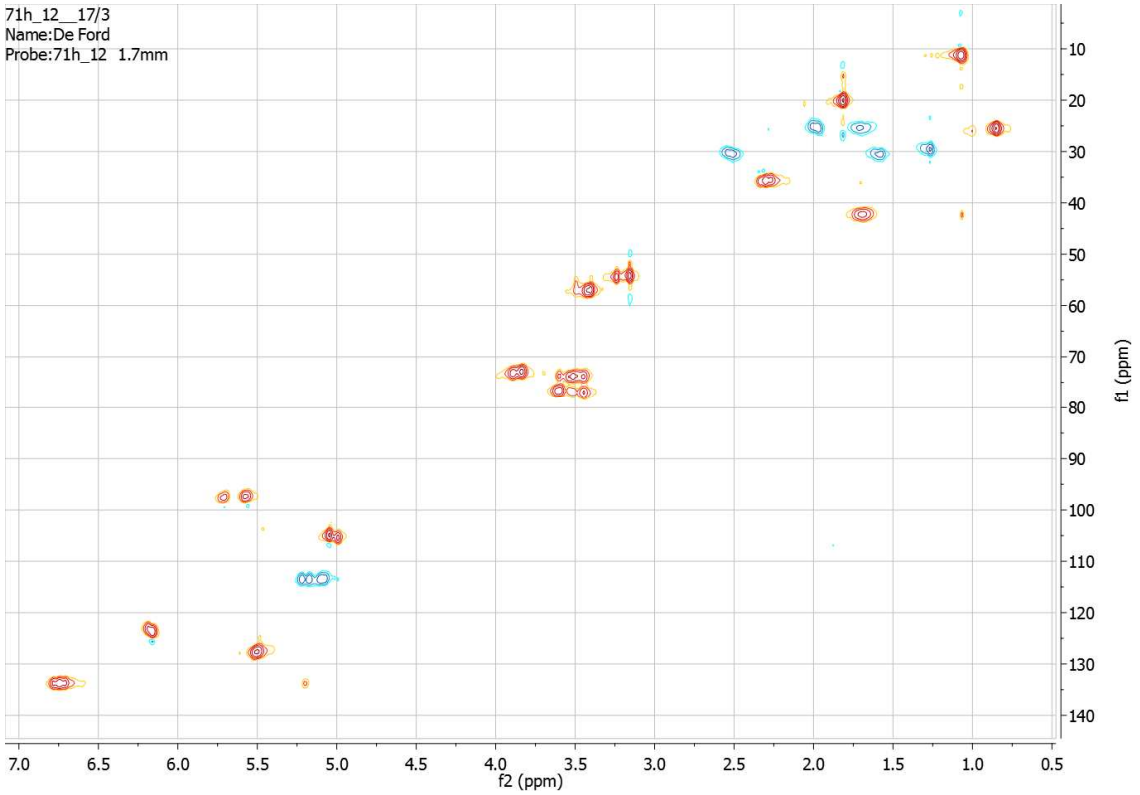


Figure S9. gHSQC spectrum of compound **11**.

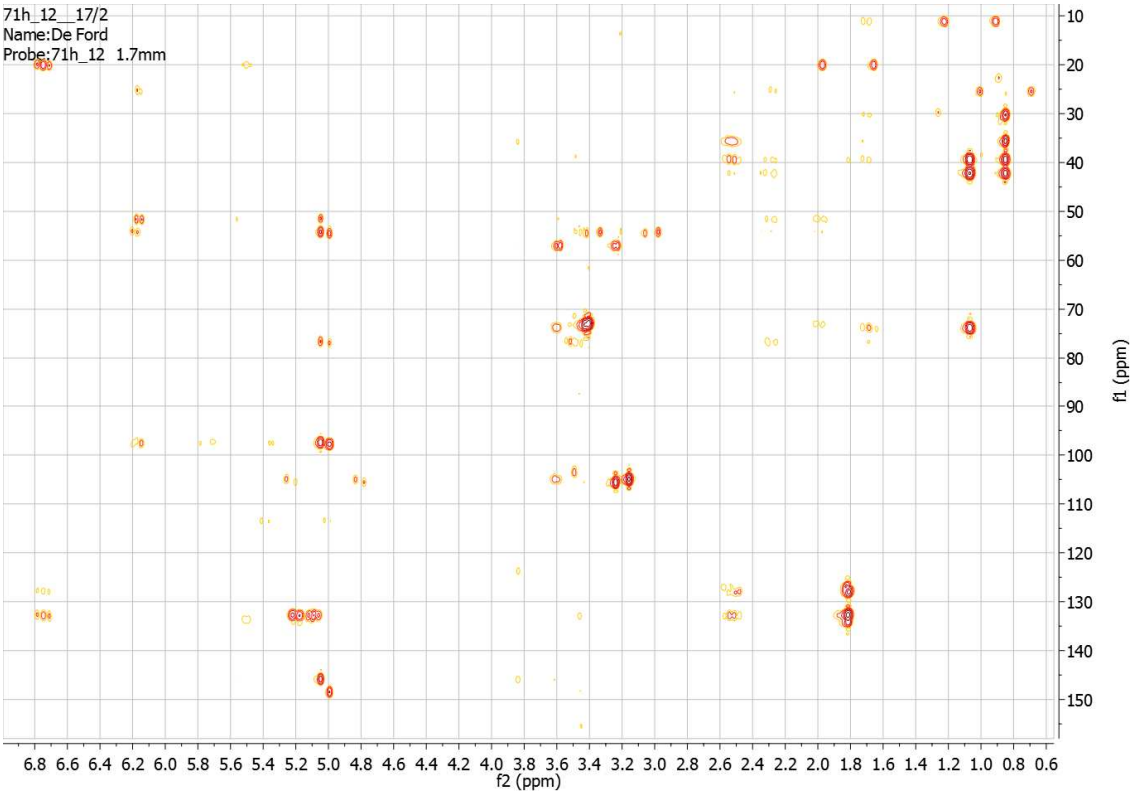


Figure 10. gHMBC spectrum of compound **11**.