# Supplementary Information

# Discovery and Characterization of a Terpene Biosynthetic Pathway featuring a Norbornene-forming Diels-Alderase

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## I Supplementary Notes

### Isolation of compounds

General isolation methods were outlined in the Method section of main text. The source, yield, and analytical data of each compound were listed here.

Isolation of compound **1**

Compound **1** was isolated from 2L of CDST agar plates inoculated with *A. nidulans* expressing SdnA-C-B-H-F-E as 40 mg reddish white solid (20 mg/L). $[\alpha]^{23}{}_{D}$ = -53.4 (c 4.2 MeOH), lit.[1] -55.3 (c 0.19 MeOH); 1H NMR (500 MHz, d5-pyridine) δ 10.26 (s, 1H), 6.16 (dd, J = 3.7, 1.3 Hz, 1H), 4.38 (d, J = 10.7 Hz, 1H), 4.23 (d, J = 10.6 Hz, 1H), 2.98 (t, J = 3.9 Hz, 1H), 2.74 (sept, J = 6.8 Hz, 1H), 2.45 (m, 1H), 2.37-2.27 (m, 2H), 2.08-1.96 (m, 4H), 1.78 (m, 1H), 1.51 (d, J = 12.4 Hz, 1H), 1.11 (t, J = 6.7 Hz, 6H), 1.05 (m, 2H), 0.84 (d, J = 6.9 Hz, 3H); 13C NMR (500 MHz, d5-pyridine) δ 204.7, 175.6, 148.6, 130.7, 73.5, 67.2, 66.6, 59.1, 46.8, 41.7, 41.6, 32.1, 31.3, 29.4, 28.9, 27.9, 26.6, 22.5, 21.0, 17.4; HRMS for $C_{20}H_{28}O_4$ (ESI, $[M+H^+]$) calculated 333.2060, found 333.2072 (deviation 3.49 ppm). The 1H and 13C NMRs of compound **1** match with those previously reported for sordaricin[1]. See Supplementary Table 3, Supplementary Figs. 4, 9-13 for more spectroscopic data.

Isolation of compound **2**

Compound **2** was isolated from 2L of CDST agar plates inoculated with *A. nidulans* expressing SdnA-C-B as 50 mg light yellow solid (25 mg/L). $[\alpha]^{23}{}_{D}$ = 42.5 (c 3.0 DMSO); 1H NMR (500 MHz, $CDCl_3$) δ 5.15 (t, J = 1.0 Hz, 1H), 4.93 (s, 1H), 4.47 (dt, J = 5.3, 1.9 Hz, 1H), 4.35 (d, J = 5.4 Hz, 1H), 3.15 (sept, J = 6.8 Hz, 1H), 2.13 (m, 3H), 2.03 (m, 1H), 1.91-1.77 (m, 2H), 1.67 (m, 2H), 1.62-1.51 (m, 2H), 1.39 (m, 1H), 1.36-1.26 (m, 2H), 0.97 (s, 3H), 0.94 (d, J = 6.9 Hz, 3H), 0.91 (d, J = 6.7 Hz, 3H), 0.78 (d, J = 7.1 Hz, 3H); 13C NMR (500 MHz, $CDCl_3$) δ 155.9, 146.8, 137.8, 106.6, 76.3, 69.1, 50.6, 50.4, 45.6, 43.0, 41.5, 34.6, 34.4, 34.4, 28.4, 27.3, 26.8, 21.8, 20.7, 14.7; HRMS for $C_{20}H_{32}O_2$ (ESI, $[M+H^+-H_2O]$) calculated 287.2369, found 287.2384 (deviation 5.08 ppm). See Supplementary Table 4, Supplementary Figs. 4, 14-19 for more spectroscopic data.

Isolation of compound **3**

Compound **3** was coisolated with compound **2** as 7 mg light yellow solid (3.5 mg/L). 1H NMR (500 MHz, $CDCl_3$) δ 9.97 (s, 1H), 5.09 (q, J = 1.5 Hz, 1H), 4.85 (s, 1H), 4.06-3.94 (m, 2H), 3.45-3.38 (sept, J = 7.0 Hz, 1H), 2.47-2.39 (m, 2H), 2.21-2.12 (m, 2H), 1.89-1.75 (m, 3H), 1.68 (dd, J = 14.1, 9.4 Hz, 1H), 1.59 (m, 1H), 1.57-1.47 (m, 2H), 1.41-1.29 (m, 2H), 1.19 (s, 3H), 1.11 (d, J = 6.9 Hz, 3H), 1.09 (d, J = 6.9 Hz, 3H), 0.87 (d, J = 7.1 Hz, 3H); 13C NMR (500 MHz, $CDCl_3$) δ 188.9, 172.8, 152.2, 142.0, 109.4, 64.6, 50.1, 48.2, 44.9, 36.1, 36.1, 35.6, 33.5, 30.1, 28.9, 26.9, 26.4, 21.7, 21.7, 16.1; HRMS for $C_{20}H_{32}O_2$ (ESI, $[M+Na^+]$) calculated 327.2295, found 327.2306 (derivation 3.51 ppm). See Supplementary Table 5, Supplementary Figs. 4, 20-24 for more spectroscopic data.

Isolation of compound **5**

Compound **5** was isolated from 2L of CDST agar plates inoculated with *A. nidulans* expressing SdnA-C-B-H as 14 mg light yellow solid (9 mg/L). 1H NMR (500 MHz, d5-Pyridine) δ 10.20 (s, 1H), 6.85 (d, J = 5.8 Hz, 1H), 6.49 (dd, J = 5.4 Hz, 1H), 5.55 (t, J = 1.9 Hz, 1H), 5.03 (s, 1H), 4.36 (m, 2H), 3.44 (sept, J = 7.0 Hz, 1H), 2.36 (m, 1H), 2.28 (dd, J = 13.9, 3.1 Hz, 1H), 1.98-1.85 (m, 3H), 1.68 (m, 1H), 1.54-1.48 (m, 1H), 1.37 (s, 3H), 1.29-1.20 (m, 1H), 1.14 (d, J = 6.8 Hz, 3H), 1.11 (d, J = 6.8 Hz, 3H), 0.83 (d, J = 7.0 Hz, 2H); 13C NMR (500 MHz, d5-Pyridine) δ 184.4, 170.0, 157.2, 153.8, 144.0, 128.7, 108.4, 64.5, 57.8, 47.9,

45.0, 36.4, 34.9, 33.3, 30.1, 26.4, 22.8, 22.7, 22.5, 15.5; HRMS for $C_{20}H_{30}O_2$ (ESI, [M+H$^+$]) calculated 303.2319, found 303.2325 (derivation 2.12 ppm). See Supplementary Table 7, Supplementary Figs. 4, 30-34 for more spectroscopic data.

Isolation of compound **6**

Compound **6** was coisolated with compound **5** as 9 mg light yellow solid (4.5 mg/L). 1H NMR (500 MHz, d5-Pyridine) δ 6.72 (d, J = 5.5 Hz, 1H), 6.51 (d, J = 5.4Hz, 1H), 5.54 (s, 1H), 5.16 (s, 1H), 4.46 (d, J = 14.9 Hz, 1H), 4.40 (d, J = 14.9 Hz, 1H), 4.33 (m, 1H), 2.50 (d, J = 13.9, 1H), 2.43 (m, 1H), 2.10-2.02 (m, 1H), 1.93 (m, 2H), 1.76-1.68 (m, 1H), 1.57 (s, 3H), 1.51 (m, 2H), 1.26 (m, 1H), 1.25 (d, J = 6.8 Hz, 3H), 1.19 (d, J = 6.9 Hz, 3H), 0.87 (d, J = 7.1 Hz, 3H); 13C NMR (500 MHz, d5-Pyridine) δ 167.6, 163.7, 153.9, 153.9, 136.6, 128.2, 108.3, 64.4, 58.7, 48.1, 45.2, 36.2, 35.0, 33.3, 29.8, 27.8, 23.3, 22.7, 22.3, 15.4; HRMS for $C_{20}H_{30}O_3$ (ESI, [M+H$^+$-$H_2O$]) calculated 301.2162, found 301.2161 (deviation 0.35 ppm). See Supplementary Table 8, Supplementary Figs. 4, 35-39 for more spectroscopic data.

Isolation of compound **8**

Plasmid XW55 harboring SdnH was transformed into *Saccharomyces cerevisiae* RC01 via Frozen-EZ Yeast Transformation II Kit (Zymo Research)[2]. The transformant was grown in 20 mL uracil drop out media (2% glucose, 0.5% casamino acid, 0.67% yeast nitrogen base w/o amino acid, 0.002% adenine, 0.002% L-tryptophan) overnight which was then used to inoculate 300 mL YPD media (1% yeast extract, 2% peptone, 2% glucose). This culture was shaken at 28 °C 250 rpm overnight before fed with 10 mg compound **2** in DMSO. The culture was left in shaker for additional two days. Cells and media were extracted separately by acetone and ethyl acetate respectively. The organics were combined and dried with a rotary evaporator. The crude extract was then separated by HPLC as stated in main methods. Compound **8** was obtained as 4 mg of light yellow solid (40% isolation yield from **2**). 1H NMR (500 MHz, $CDCl_3$) δ 6.26 (d, J = 5.4 Hz, 1H), 6.02 (d, J = 5.4, 1H), 5.21 (t, J = 1.2 Hz, 1H), 4.90 (s, 1H), 4.68 (d, J = 5.7 Hz, 1H), 4.656 (d, J = 5.7 Hz, 1H), 3.37 (sept, J = 6.8 Hz, 1H), 2.00 (d, J = 14.6 Hz, 1H), 1.97-1.88 (m, 3H), 1.74 (m, 1H), 1.65-1.54 (m, 1H), 1.45 (dd, J = 14.5, 9.6 Hz, 1H), 1.15 (s, 3H), 1.12 (d, J = 6.8 Hz, 3H), 1.10 (d, J = 6.9 Hz, 3H), 0.79 (d, J = 7.1 Hz, 3H), 0.75-0.66 (m, 1H); 13C NMR (500 MHz, $CDCl_3$) δ 155.0, 148.7, 145.4, 141.9, 129.4, 107.8, 75.2, 70.0, 57.1, 48.4, 48.0, 40.1, 38.1, 33.4, 32.5, 27.1, 24.1, 23.4, 21.7, 15.9; HRMS for $C_{20}H_{30}O_2$ (ESI, [M+H +-H2O]) calculated 285.2213, found 285.2220 (deviation 2.48 ppm). See Supplementary Table 10, Supplementary Figs. 4, 45-50 for more spectroscopic data.

Isolation of compound **11**

*A. nidulans* expressing SdnA-C-B-H-F was used to inoculate 3L of CDST liquid culture which was then shaken at 28 °C for 6 days. Cell body and media were separated by filtration and extracted separately by acetone and ethyl acetate respectively. The organics were combined and dried with a rotary evaporator. The crude extract was then separated by normal-phase flash chromatography with a gradient of hexane and ethyl acetate and then by reverse-phase HPLC as stated in main methods. Compound **11** was obtained as 3 mg light yellow solid (1 mg/L). 1H NMR (500 MHz, d5-Pyridine) δ 10.07 (d, J = 1.0 Hz, 1H), 5.97 (d, J = 3.4, 1H), 2.63 (sept, J = 6.8, 1H), 2.23 (m, 2H), 2.05 (t, J = 13.4 Hz, 1H), 1.92-1.78 (m, 4H), 1.53 (m, 1H), 1.39-1.28 (m, 2H), 1.24 (s, 3H), 0.98 (d, J = 6.8 Hz, 3H), 0.95 (d, J = 6.9 Hz, 3H), 0.92-0.81 (m, 2H), 0.68 (d, J = 6.6 Hz, 3H); 13C NMR (500 MHz, d5-Pyridine) δ 204.8, 175.5, 148.8, 131.5, 72.8, 62.1, 58.8, 50.9, 42.1, 41.6, 32.9, 32.3, 31.4, 29.6, 28.1, 26.8, 22.5, 22.4, 21.2, 17.6; HRMS for $C_{20}H_{28}O_3$ (ESI, [M+H$^+$-$H_2O$]) calculated 299.2006, found 299.2009 (deviation 1.15 ppm). See Supplementary Table 13, Supplementary Figs. 4, 62-66 for more spectroscopic data.

Isolation of compound **12**

Compound **12** was coisolated with compound **11** as 3 mg light yellow solid (1 mg/L). 1H NMR (500 MHz, d5-Pyridine) δ 5.97 (d, J = 3.6 1H), 3.74 (d, J = 11.6 Hz, 1H), 3.61 (d, J = 11.6 Hz, 1H), 2.89 (sept, J = 6.8 Hz, 1H), 2.29-2.12 (m, 4H), 2.06-1.95 (m, 3H), 1.79 (m, 1H), 1.52 (d, J = 14.0, 6.6 Hz, 1H), 1.43 (s, 3H), 1.27-1.17 (m, 2H), 1.16 (d, J = 7.0 Hz, 3H), 1.14 (d, J = 7.0 Hz, 3H), 0.83 (d, J = 6.8 Hz, 3H); 0.30 (d, J = 12.3 Hz, 1H); 13C NMR (500 MHz, d5-Pyridine) δ 179.5, 149.8, 130.6, 68.0, 63.4, 50.7, 49.9, 43.3, 42.8, 33.6, 33.0, 32.7, 32.7, 28.8, 27.1, 24.2, 24.1, 23.1, 21.6, 18.3; HRMS for $C_{20}H_{30}O_3$ (ESI, $[M+H^+-H_2O]$) calculated 301.2162, found 301.2167 (deviation 1.64 ppm). See Supplementary Table 14, Supplementary Figs. 4, 67-71 for more spectroscopic data.

Isolation of compound **13**

Compound **13** was coisolated with compound **1** as 5.5 mg light yellow solid (2.3 mg/L). 1H NMR (500 MHz, d5-Pyridine) δ 6.73 (m, 1H), 6.55-6.50 (m, 1H), 4.40-4.32 (m, 1H), 3.15 (m, 1H), 2.63 (d, J = 13.7 Hz, 1H), 2.56-2.48 (m, 1H), 2.02-1.94 (m, 2H), 1.83-1.75 (m, 1H), 1.64-1.53 (m, 2H), 1.58 (s, 3H), 1.53-1.42 (m, 1H), 1.27 (m, 1H), 1.29 (dd, J = 7.1, 3.2 Hz, 3H), 1.27 (d, J = 7.0 Hz, 3H), 1.22 (d, J = 7.0 Hz, 3H), 0.84 (d, J = 7.1 Hz, 3H); 13C NMR (500 MHz, d5-Pyridine) δ 179.6, 167.4, 164.2, 153.9, 128.4, 58.8, 44.8, 43.5, 39.9, 36.7, 35.2, 34.0, 27.9, 23.2, 22.8, 22.1, 15.1, 10.8; HRMS for $C_{20}H_{30}O_4$ (ESI, $[M+H^+-H_2O]$) calculated 317.2111, found 317.2117 (derivation 1.82 ppm). See Supplementary Table 15, Supplementary Figs. 4, 72-76 for more spectroscopic data.

Isolation of compound **14**

Compound **14** was coisolated with compound **1** as 3 mg light yellow solid (1.5 mg/L). 1H NMR (500 MHz, d5-Pyridine) δ 6.72 (m, 1H), 6.52 (m, 1H), 4.35 (m, 1H), 3.75-3.64 (m, 2H), 2.59 (d, J = 13.9, 1H), 2.41-2.28 (m, 1H), 2.07 (m, 1H), 2.01-1.88 (m, 2H), 1.58 (s, 3H), 1.54 (m, 2H), 1.47 (m, 1H), 1.41-1.30 (m, 2H), 1.27 (d, J = 7.0 Hz, 3H), 1.21 (d, J = 7.0 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H), 0.84 (d, J = 7.0 Hz, 3H); 13C NMR (500 MHz, d5-Pyridine) δ 167.4, 164.1, 154.1, 136.4, 136.4, 128.3, 68.2, 58.9, 43.4, 43.4, 36.9, 35.5, 34.3, 27.9, 23.4, 22.8, 22.2, 22.0, 15.2, 11.3; HRMS for $C_{20}H_{32}O_3$ (ESI, $[M+H^+-H_2O]$) calculated 303.2319, found 303.2322 (deviation 2.12 ppm). See Supplementary Table 16, Supplementary Figs. 4, 76-81 for more spectroscopic data.

**Chemical synthesis of compounds 4, 7,** and **10**

General synthetic methods were outlined in the main methods. The source, yield and analytical data of each compound were listed here.

Synthesis of compound **4**

Compound **4** was synthesized from 5 mg of compound **3** with a yield of 100% estimated from UV of the remaining reactant. 1H NMR (500 MHz, $CD_2Cl_2$) δ 9.95 (s, 1H), 9.48 (s, 1H), 6.20 (s, 1H), 5.97 (d, J = 0.8 Hz, 1H), 3.44-3.35 (sept, J = 7.0 Hz, 1H), 2.57 (m, 1H), 2.40 (m, 2H), 2.20 (m, 1H), 1.96-1.84 (m, 2H), 1.80 (m, 1H), 1.76-1.67 (m, 2H), 1.53-1.42 (m, 1H), 1.39 (m, 1H), 1.40-1.32 (m, 1H), 1.29 (dd, J = 14.3, 2.0 Hz, 1H), 1.16 (s, 3H), 1.10 (d, J = 6.9 Hz, 3H), 1.09 (d, J = 6.9 Hz, 3H), 0.89 (d, J = 7.1 Hz, 3H); 13C NMR (126 MHz, $CD_2Cl_2$) δ 195.4, 188.6, 172.5, 154.3, 142.3, 134.7, 50.3, 45.5, 43.2, 36.9, 36.5, 36.1, 33.8, 30.4, 29.6, 27.2, 26.4, 21.8, 21.8, 16.0; HRMS for $C_{20}H_{30}O_2$ (ESI, $[M+H^+]$) calculated 303.2319, found 303.2325 (deviation 2.12 ppm). See Supplementary Table 6, Supplementary Figs. 4, 25-29 for more spectroscopic data.

Synthesis of compound **7**

Compound **7** was synthesized from 3 mg of compound **5** with a yield of 100% estimated from UV of the remaining reactant. 1H NMR (500 MHz, $CD_2Cl_2$) δ 9.90 (s, 1H), 9.45 (s, 1H), 6.77 (d, J = 5.3 Hz, 1H), 6.44 (d, J = 5.3 Hz, 1H), 6.10 (s, 1H), 5.93 (s, 1H), 3.47 (sept, J = 6.8 Hz, 1H), 2.50 (m, 1H), 1.93-1.79 (m, 3H), 1.77-1.66 (m, 2H), 1.25 (m, 3H), 1.23 (d, J = 6.9 Hz, 3H), 1.19 (d, J = 6.9 Hz, 3H), 1.19 (s, 3H), 0.78 (d, J = 7.1 Hz, 3H); 13C NMR (500 MHz, $CD_2Cl_2$) δ 195.6, 184.2, 170.9, 157.0, 153.9, 143.3, 134.4, 128.5, 57.6, 45.4, 42.1, 36.3, 34.3, 33.2, 29.8, 26.5, 22.9, 22.5, 22.4, 15.0; HRMS for $C_{20}H_{28}O_2$ (ESI, $[M+Na^+]$) calculated 301.2162, found 301.2168 (deviation 1.97 ppm). See Supplementary Table 9, Supplementary Figs. 4, 40-44 for more spectroscopic data.

Synthesis of compound **10**

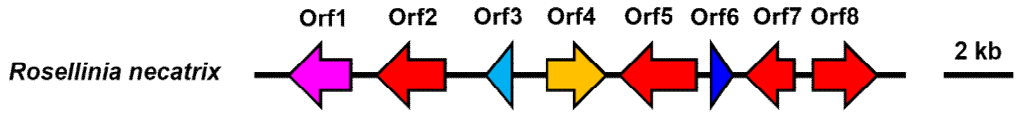
Compound **10** was synthesized from 5 mg of compound **6** with a yield of 95% estimated from UV of the remaining reactant. 1H NMR (500 MHz, d5-Pyridine) δ 9.61 (s, 1H), 6.67 (d, J = 5.4 Hz, 1H), 6.51 (d, J = 5.3 Hz, 1H), 6.33 (s, 1H), 5.93 (s, 1H), 4.31 (sept, J = 6.8 Hz, 1H), 2.79 (m, 1H), 2.33 (d, J = 13.9 Hz, 1H), 2.07-1.76 (m, 4H), 1.69 (m, 1H), 1.51 (s, 3H), 1.40-1.31 (m, 2H), 1.31 (d, J = 6.8 Hz, 3H), 1.19 (d, J = 6.9 Hz, 3H), 0.84 (d, J = 7.0 Hz, 3H); 13C NMR (500 MHz, d5-Pyridine) δ 195.5, 167.2, 164.2, 154.3, 153.7, 136.1, 134.5, 128.4, 58.6, 46.1, 42.2, 36.6, 34.7, 33.7, 30.3, 27.9, 23.2, 22.8, 22.2, 15.4; HRMS for $C_{20}H_{28}O_3$ (ESI, $[M+H^+-H_2O]$) calculated 299.2006, found 299.2009 (deviation 1.15 ppm). See Supplementary Table 12, Supplementary Figs.4, 57-61 for more spectroscopic data.

**Preparation of compound 9**

Compound **9** was a formed by cyclization of compound **7**. During the synthetic reaction of **7**, **9** was only observed as a minor product (less than 5%). However, when a solution of compound **7 (**9 mg) in $CH_2Cl_2$ was evaporated in vacuum, ~ 66% of **9** cyclizes (Supplementary Fig. 51). How the concentration process promotes cyclization of **7** is not fully understood but is possibly caused by increased energy of the system due to higher collision frequency between concentrated molecules. Residue containing compounds **7** and **9** was further separated by HPLC as stated in the main methods to yield 4 mg of compound **9** as light-yellow solid. 1H NMR (500 MHz, MeOD) δ 10.15 (s, 1H), 9.81 (s, 1H), 6.13 (dd, J = 3.4, 1.4 Hz, 1H), 2.43 (dd, J = 4.3, 3.5 Hz, 1H), 2.22-2.12 (m, 1H), 2.14-2.03 (m, 3H), 2.02 (dd, J = 12.6, 4.4 Hz, 1H), 1.84 (m, 1H), 1.77-1.64 (m, 2H), 1.65 (dd, J = 14.3, 6.4 Hz, 1H), 1.32 (d, J = 12.5 Hz, 1H), 1.26-1.19 (m, 1H), 1.16 (s, 3H), 1.05-0.92 (m, 1H), 0.98 (d, J = 6.9 Hz, 3H), 0.93 (d, J = 6.9 Hz, 3H), 0.78 (d, J = 7.0 Hz, 3H); 13C NMR (500 MHz, MeOD) δ 206.9, 206.2, 165.0, 150.1, 134.1, 76.1, 63.3, 61.5, 51.9, 43.1, 42.4, 34.7, 33.2, 32.5, 30.3, 28.9, 27.1, 23.2, 21.8, 21.5, 17.8; HRMS for $C_{20}H_{28}O_2$ (ESI, $[M+H^+]$) calculated 301.2162, found 301.2172 (deviation 3.3 ppm). See Supplementary Table 11, Supplementary Figs. 4, 52-56 for more spectroscopic data.

## II Supplementary Tables

**Supplementary Table 1.** Annotation of the putative sordaricin B cluster.

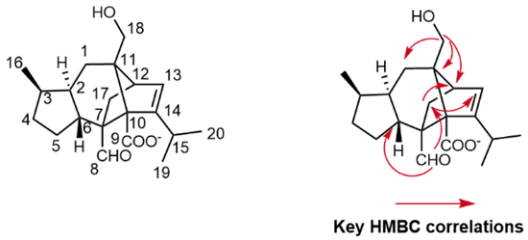



| Gene | GenBank accession | Size (aa) | Proposed function | Homologs in *S. araneosa* (identity/similarity, %) | Homologs in other pathways (identity/similarity, %) |
|---|---|---|---|---|---|
| Orf1 | GAP90682.1 [https://www.ncbi.nlm.nih.gov/protein/GAP90682.1] | 406 | Terpene cyclase | SdnA (66/79) | S0DX56.1 (38/59) |
| Orf2 | GAW26838.1 [https://www.ncbi.nlm.nih.gov/protein/GAW26838.1] | 537 | P450 | SdnB (62/78) | A0A084R1M7.1 (35/56) |
| Orf3 | GAW26839.1 [https://www.ncbi.nlm.nih.gov/protein/GAW26839.1] | 245 | Methyltransferase | SdnD (58/75) | P74838.1 (31/48) |
| Orf4 | GAP90684.1 [https://www.ncbi.nlm.nih.gov/protein/GAP90684.1] | 514 | Glycosyltransferase | SdnJ (60/79) | A0A411KZY6.1 (31/51) |
| Orf5 | GAP90685.1 [https://www.ncbi.nlm.nih.gov/protein/GAP90685.1] | 524 | P450 | SdnH (66/79) | Q6WP51.1 (38/58) |
| Orf6 | GAP90686.1 [https://www.ncbi.nlm.nih.gov/protein/GAP90686.1] | 144 | Hypothetical protein | SdnG (51/68) | Q7V2Q8.1 (29/54) |
| Orf7 | GAP90687.1 [https://www.ncbi.nlm.nih.gov/protein/GAP90687.1] | 433 | P450 | SdnF (71/83) | C9K202.1 (34/53) |
| Orf8 | GAP90688.2 [https://www.ncbi.nlm.nih.gov/protein/GAP90688.2] | 520 | P450 | SdnE (70/82) | C8V7P3.1 (40/62) |

**Supplementary Table 2.** DNA and protein sequence of SdnG (*S. araneosa*) used in this study.

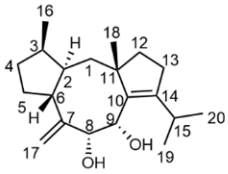
| | |
|---|---|
| DNA sequence | ATGGCTGGCAAGGAAATTCAGACCCCCGATCAGGCCGAGGCATTCGT<br>CGCCAAGGTATTTGACGTCCTCGATTCATATGATTACACCCGTTTCGG<br>TGAGGTGCTTTCCACCGACCTGAAGTACGAAGGTGGTTTGCAAAAGA<br>CCTCTGGGCTGGATAACTTCATCAATGACATCAAGGCATCCACCCAG<br>CGAATGCCAGGGCTCCAGACCTCTCACTCCCGATATCGCACTGAACT<br>CACCGCCGAGGGCACCATCTACAGCGAGGGTCACTCAAACGCATCTT<br>TGGAGTCGAATCCGGGCAAGGTTGTGACTGTACCCATGATTGGCGTG<br>TTCAAGTTGGACAGTGAAGATGGCAAGATCAAGGAGATGCGCATTTA<br>CAAGGATCGTTTGCCATTCTTGGCGCTCCATCAAGCACTCCCTGGGAT<br>GAAGGCGAACAATTAG |
| Protein sequence<br><br>(UniProt accession: A0A1B4XBH4, [https://www.uniprot.org/uniprot/A0A1B4XBH4]) | MAGKEIQTPDQAEAFVAKVFDVLDSYDYTRFGEVLSTDLKYEGGLQKT<br>SGLDNFINDIKASTQRMPGLQTSHSRYRTELTAEGTIYSEGHSNASLESNP<br>GKVVTVPMIGVFKLDSEDGKIKEMRIYKDRLPFLALHQALPGMKANN |

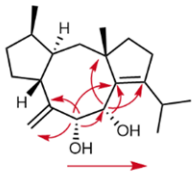
**Supplementary Table 3.** Spectroscopic data of compound **1**.



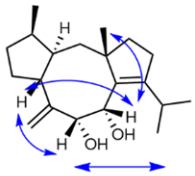

| Position | $\delta_H$ (ppm), mult (J in Hz) | $\delta_C$ (ppm) | COSY |
|---|---|---|---|
| 1 | 2.27, m | 28.8 | |
| 2 | 1.77, m | 41.6 | H1, H3, H6 |
| 3 | 2.00, m | 31.3 | H16 |
| 4 | 1.80, m<br>0.92, m | 32.1 | H3, H4, H5 |
| 5 | 2.00, m<br>1.05, m | 26.6 | H4, H5, H6 |
| 6 | 2.47, m | 41.7 | H2, H5 |
| 7 | | 59.1 | |
| 8 | 10.25 | 204.5 | |
| 9 | | 175.6 | |
| 10 | | 73.4 | |
| 11 | | 67.2 | |
| 12 | 2.98, t (3.8) | 46.8 | H13, H17 |
| 13 | 6.16, d (3.3) | 130.7 | H12, H15 |
| 14 | | 148.6 | |
| 15 | 2.74, septet (6.8) | 27.9 | H13, H19, H20 |
| 16 | 0.84, d (7.0) | 17.4 | H3 |
| 17 | 2.00, m<br>1.52, d (12.4) | 29.4 | H12 |
| 18 | 4.37, d (10.6)<br>4.24, d (10.6) | 66.6 | |
| 19 | 1.11, d (6.7) | 21.1 | H15 |
| 20 | 1.11, d (6.8) | 22.4 | H15 |

**Supplementary Table 4.** Spectroscopic data of compound **2**.

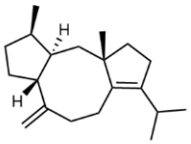




Key HMBC correlations



Key NOSEY correlations

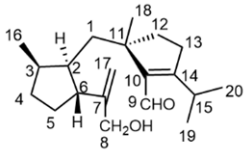


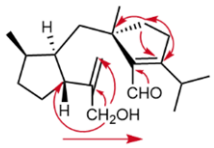
Cycloaraneosene

| Position | $\delta_H$ (ppm), mult (J in Hz) | $\delta_C$ (ppm) | COSY |
|---|---|---|---|
| 1 | 1.57, d (14.5)<br>1.32, dd (14.5, 10.4) | 43.0 | |
| 2 | 1.65, m | 50.4 | H3 |
| 3 | 2.02, m | 41.5 | H2, H16 |
| 4 | 1.39, m<br>1.33, m | 34.4 | H3, H5 |
| 5 | 2.09, m<br>1.65, m | 34.6 | H4, H6 |
| 6 | 1.86, m | 45.6 | H5 |
| 7 | | 155.9 | |
| 8 | 4.47 dt (5.4, 1.6) | 76.3 | H9, H17 |
| 9 | 4.34 d (5.4) | 69.1 | H8 |
| 10 | | 137.8 | |
| 11 | | 50.6 | |
| 12 | 1.58, m<br>1.32, m | 34.4 | H13 |
| 13 | 2.14, m | 26.8 | H12 |
| 14 | | 146.8 | |
| 15 | 3.14, septet (7.0) | 28.4 | H19, H20 |
| 16 | 0.78, d (7.2) | 14.7 | H3 |
| 17 | 5.15, t (1.0)<br>4.93, s | 106.6 | H8 |
| 18 | 0.96, s | 27.3 | |
| 19 | 0.91, d (6.7) | 21.7 | H15 |
| 20 | 0.94, d (6.9) | 20.7 | H15 |

The stereochemistry of **2** was determined by NOESY based on the reported stereochemistry for cycloaraneosene[3,4].

**Supplementary Table 5.** Spectroscopic data of compound **3**

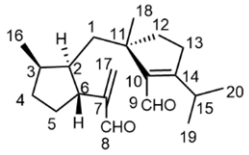


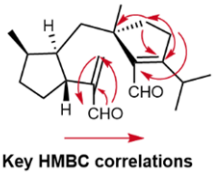




Key HMBC correlations

| Position | $\delta_H$ (ppm), mult (J in Hz) | $\delta_C$ (ppm) | COSY |
|---|---|---|---|
| 1 | 1.69, dd (14.2, 9.5)<br>1.50, m | 36.1 | |
| 2 | 1.60, m | 44.9 | H3 |
| 3 | 2.17, m | 36.1 | H2, H16 |
| 4 | 1.78, m<br>1.37, m | 33.5 | H3 |
| 5 | 1.85, m<br>1.37, m | 28.9 | H6 |
| 6 | 2.17, m | 48.2 | H5 |
| 7 | | 152.2 | |
| 8 | 3.99, m | 64.6 | H17 |
| 9 | 9.97 | 188.9 | |
| 10 | | 142.0 | |
| 11 | | 50.1 | |
| 12 | 1.80, m<br>1.34, m | 35.6 | H13 |
| 13 | 2.43, m | 30.1 | H12 |
| 14 | | 172.8 | |
| 15 | 3.41, septet (7.0) | 26.9 | H19, H20 |
| 16 | 0.86, d (7.1) | 16.1 | H3 |
| 17 | 5.09, q (1.6)<br>4.85, s | 109.4 | H8 |
| 18 | 1.19, s | 26.4 | |
| 19 | 1.11, d (6.9) | 21.7 | H15 |
| 20 | 1.09, d (6.9) | 21.5 | H15 |

**Supplementary Table 6.** Spectroscopic data of compound **4**.

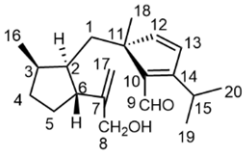


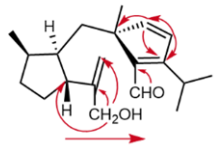




Key HMBC correlations

| Position | $\delta_H$ (ppm), mult (J in Hz) | $\delta_C$ (ppm) | COSY |
|---|---|---|---|
| 1 | 1.71, m<br>1.31, dd (14.2, 1.9) | 36.5 | |
| 2 | 1.79, m | 45.5 | H3, H6 |
| 3 | 2.21, m | 37.0 | H2, H16 |
| 4 | 1.91, m<br>1.37, m | 33.8 | H3 |
| 5 | 1.91, m<br>1.37, m | 29.6 | H6 |
| 6 | 2.58, m | 43.2 | H2, H5 |
| 7 | | 154.3 | |
| 8 | 9.48, s | 195.4 | |
| 9 | 9.95, s | 188.6 | |
| 10 | | 142.3 | |
| 11 | | 50.3 | |
| 12 | 1.71, m<br>1.50, m | 36.1 | H13 |
| 13 | 2.43, m | 30.4 | H12 |
| 14 | | 172.5 | |
| 15 | 3.40, septet (6.9) | 27.2 | H19, H20 |
| 16 | 0.86, d (7.1) | 16.0 | H3 |
| 17 | 6.20<br>5.97, d (0.6) | 134.7 | |
| 18 | 1.16, s | 26.4 | |
| 19 | 1.10, d (6.9) | 21.8 | H15 |
| 20 | 1.09, d (6.9) | 21.8 | H15 |

**Supplementary Table 7.** Spectroscopic data of compound **5**

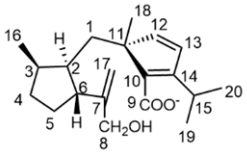


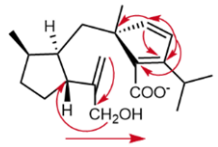




Key HMBC correlations

| Position | $\delta_H$ (ppm), mult (J in Hz) | $\delta_C$ (ppm) | COSY |
|---|---|---|---|
| 1 | 2.27, dd (14.0, 3.0)<br>1.90, m | 34.9 | H2 |
| 2 | 1.49, m | 45.0 | H1 |
| 3 | 1.90, m | 36.4 | H16 |
| 4 | 1.67, m<br>1.24, m | 33.3 | H3, H4, H5 |
| 5 | 1.90, m<br>1.49, m | 30.1 | H4, H5, H6 |
| 6 | 2.35, m | 47.9 | H5 |
| 7 | | 153.8 | |
| 8 | 4.36, m | 64.5 | H17 |
| 9 | 10.2 | 184.4 | |
| 10 | | 144.0 | |
| 11 | | 57.8 | |
| 12 | 6.86, d (5.0) | 157.2 | H13 |
| 13 | 6.49, d (5.2) | 128.7 | H12 |
| 14 | | 170.0 | |
| 15 | 3.45, septet (7.0) | 26.4 | H19, H20 |
| 16 | 0.82, d (7.0) | 15.5 | H3 |
| 17 | 5.55, t (1.9)<br>5.03, s | 108.4 | H8, H17 |
| 18 | 1.37, s | 22.8 | |
| 19 | 1.14, d (6.8) | 22.7 | H15 |
| 20 | 1.11, d (6.8) | 22.5 | H15 |

**Supplementary Table 8.** Spectroscopic data of compound **6**.

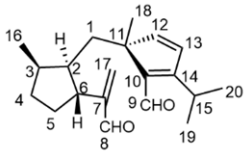


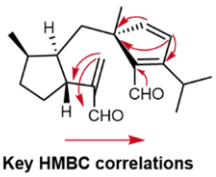




Key HMBC correlations

| Position | $\delta_H$ (ppm), mult (J in Hz) | $\delta_C$ (ppm) | COSY |
|---|---|---|---|
| 1 | 2.52, d (13.4)<br>1.94, m | 34.0 | H1, H2 |
| 2 | 1.49, m | 45.0 | H1 |
| 3 | 2.07, m | 36.2 | H4, H16 |
| 4 | 1.73, m<br>1.26, m | 33.3 | H3, H4, H5 |
| 5 | 1.94, m<br>1.51, m | 29.8 | H4, H5, H6 |
| 6 | 2.44, m | 48.1 | H2, H5 |
| 7 | | 153.9 | |
| 8 | 4.46, d (14.9)<br>4.40, d (14.9) | 64.4 | H8, H17 |
| 9 | | 167.6 | |
| 10 | | 136.6 | |
| 11 | | 58.7 | |
| 12 | 6.73, d (5.3) | 153.9 | H13 |
| 13 | 6.52, d (5.2) | 128.2 | H12 |
| 14 | | 163.7 | |
| 15 | 4.33, m | 27.8 | H19, H20 |
| 16 | 0.88, d (7.2) | 15.4 | H3 |
| 17 | 5.54, s<br>5.16, s | 108.3 | H8, H17 |
| 18 | 1.57, s | 23.3 | |
| 19 | 1.26, d (7.0) | 22.7 | H15 |
| 20 | 1.20, d (7.0) | 22.3 | H15 |

**Supplementary Table 9.** Spectroscopic data of compound **7**.

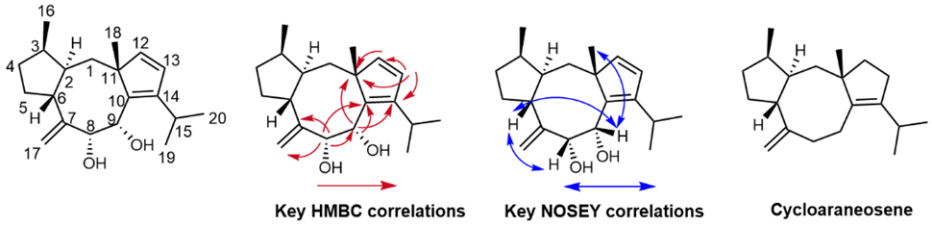



Key HMBC correlations

| Position | $\delta_H$ (ppm), mult (J in Hz) | $\delta_C$ (ppm) | COSY |
|---|---|---|---|
| 1 | 1.90, d (14.1, 2.9)<br>1.73, dd (14.0, 10.1) | 34.3 | |
| 2 | 1.25, m | 45.4 | H3 |
| 3 | 1.85, m | 36.3 | H16 |
| 4 | 1.73, m<br>1.25, m | 33.2 | H5 |
| 5 | 1.88, m<br>1.25, m | 29.8 | H4, H6 |
| 6 | 2.49, m | 42.1 | H5 |
| 7 | | 153.9 | |
| 8 | 9.45 s | 195.5 | |
| 9 | 9.90 s | 184.2 | |
| 10 | | 143.26 | |
| 11 | | 57.6 | |
| 12 | 6.45, d (5.4) | 145.4 | H13 |
| 13 | 6.76, d (5.3) | 157.0 | H12 |
| 14 | | 170.9 | |
| 15 | 3.47, septet (6.7) | 26.5 | H19, H20 |
| 16 | 0.79, d (7.2) | 15.0 | H3 |
| 17 | 6.10, s<br>5.93, s | 134.4 | |
| 18 | 1.19, s | 22.5 | |
| 19 | 1.23, d (6.9) | 22.9 | H15 |
| 20 | 1.19, d (6.9) | 22.4 | H15 |

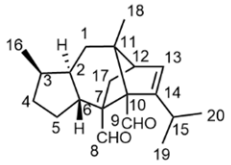
**Supplementary Table 10.** Spectroscopic data of compound **8**.

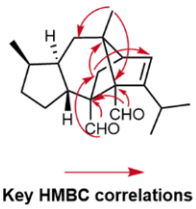



| Position | $\delta_H$ (ppm), mult (J in Hz) | $\delta_C$ (ppm) | COSY |
|---|---|---|---|
| 1 | 1.98, d (14.4)<br>1.43, dd (14.4, 9.4) | 38.1 | |
| 2 | 0.70, q | 48.0 | H3 |
| 3 | 1.94, m | 40.1 | H16 |
| 4 | 1.72, m<br>1.28, m | 33.4 | H3, H5 |
| 5 | 1.94, m<br>1.60, m | 32.5 | H4, H6 |
| 6 | 1.94, m | 48.4 | H5 |
| 7 | | 155.1 | |
| 8 | 4.56 d (5.6) | 75.2 | H9, H17 |
| 9 | 4.67 d (5.6) | 70.0 | H8 |
| 10 | | 141.9 | |
| 11 | | 57.1 | |
| 12 | 6.01, d (5.4) | 145.4 | H13 |
| 13 | 6.25, d (5.5) | 129.4 | H12 |
| 14 | | 148.7 | |
| 15 | 3.37, septet (6.8) | 27.1 | H19, H20 |
| 16 | 0.78, d (7.0) | 15.9 | H3 |
| 17 | 5.20, t (1.2)<br>4.90, s | 107.8 | H8 |
| 18 | 1.15, s | 24.1 | |
| 19 | 1.02, d (6.8) | 23.7 | H15 |
| 20 | 1.00, d (6.7) | 21.4 | H15 |

The stereochemistry of **8** was determined by NOESY based on the reported stereochemistry for cycloaraneosene[3,4]

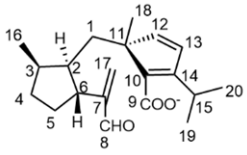
**Supplementary Table 11.** Spectroscopic data of compound **9**.

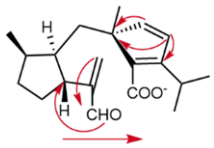



| Position | $\delta_H$ (ppm), mult (J in Hz) | $\delta_C$ (ppm) | COSY |
|---|---|---|---|
| 1 | 2.10, t (13.5)<br>1.65, dd (14.0, 6.5) | 32.9 | H2 |
| 2 | 1.84, m | 43.1 | H1 |
| 3 | 2.07, m | 32.5 | H16 |
| 4 | 2.07, m<br>1.21, m | 33.2 | H5 |
| 5 | 1.70, m<br>0.97, m | 27.1 | H4, H6 |
| 6 | 2.17, m | 42.4 | H5 |
| 7 | | 61.5 | |
| 8 | 9.80, s | 206.1 | |
| 9 | 10.15, s | 206.9 | |
| 10 | | 76.1 | |
| 11 | | 63.3 | |
| 12 | 2.43, dd (4.3, 3.5) | 51.9 | H13, H17 |
| 13 | 6.13, dd (3.3, 1.3) | 134.1 | H12, H15 |
| 14 | | 150.1 | |
| 15 | 2.17, m | 28.9 | H13, H19, H20 |
| 16 | 0.78, d (7.2) | 17.8 | H3 |
| 17 | 2.00, dd (12.5, 4.3)<br>1.31, d (12.6) | 30.3 | H12 |
| 18 | 1.16, s | 21.5 | |
| 19 | 0.93, d (6.8) | 23.2 | H15 |
| 20 | 0.98, d (6.7) | 21.8 | H15 |

**Supplementary Table 12.** Spectroscopic data of compound **10**.

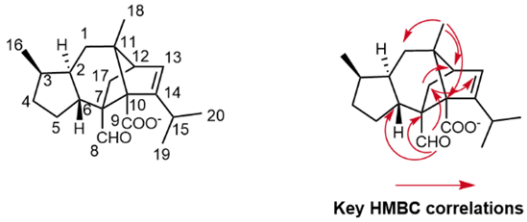



Key HMBC correlations

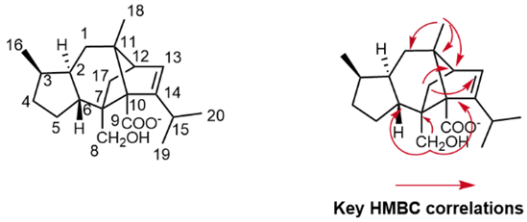
| Position | $\delta_H$ (ppm), mult (J in Hz) | $\delta_C$ (ppm) | COSY |
|---|---|---|---|
| 1 | 2.33, d (13.9)<br>1.89, dd (14, 10.2) | 34.7 | H2 |
| 2 | 1.70, m | 46.1 | H3, H6 |
| 3 | 2.03, m | 36.6 | H2, H16 |
| 4 | 1.81, m<br>1.31, m | 33.6 | H3 |
| 5 | 1.96, m<br>1.36, m | 30.3 | H6 |
| 6 | 2.78, m | 42.2 | H2, H5, H17 |
| 7 | | 154.3 | |
| 8 | 9.61, s | 195.5 | H17 |
| 9 | | 167.2 | |
| 10 | | 136.1 | |
| 11 | | 58.6 | |
| 12 | 6.67, d (5.4) | 153.7 | H13 |
| 13 | 6.50, d (5.4) | 128.4 | H12 |
| 14 | | 164.2 | |
| 15 | 4.31, septet (6.8) | 27.9 | H19, H20 |
| 16 | 0.83, d (7.0) | 15.3 | H3 |
| 17 | 6.33, s<br>5.93, s | 134.5 | H6, H8 |
| 18 | 1.51, s | 23.3 | |
| 19 | 1.31, d (6.8) | 22.8 | H15 |
| 20 | 1.19, d (6.8) | 22.2 | H15 |

**Supplementary Table 13.** Spectroscopic data of compound **11**.



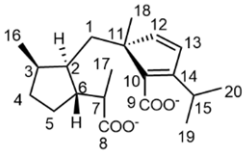

| Position | $\delta_H$ (ppm), mult (J in Hz) | $\delta_C$ (ppm) | COSY |
|---|---|---|---|
| 1 | 2.05, t (13.4)<br>1.35, dd (14.3, 6.4) | 32.9 | |
| 2 | 1.53, m | 42.1 | |
| 3 | 1.87, m | 31.4 | H16 |
| 4 | 1.80, m<br>0.92, m | 32.3 | H3, H5 |
| 5 | 1.87, m<br>0.87, m | 26.8 | H4, H6 |
| 6 | 2.26, m | 41.6 | H5 |
| 7 | | 58.8 | |
| 8 | 10.07, s | 204.8 | |
| 9 | | 175.4 | |
| 10 | | 72.8 | |
| 11 | | 62.1 | |
| 12 | 2.23, m | 50.9 | H13 |
| 13 | 5.97, d (3.4) | 131.4 | H12, H15 |
| 14 | | 148.7 | |
| 15 | 2.63, septet (6.7) | 28.1 | H13, H19, H20 |
| 16 | 0.68, d (6.7) | 17.6 | H3 |
| 17 | 1.80, t (13.4)<br>1.30, d (12.6) | 29.6 | H12 |
| 18 | 1.24, s | 22.5 | |
| 19 | 0.95, d (6.7) | 22.4 | H15 |
| 20 | 0.98, d (6.8) | 21.1 | H15 |

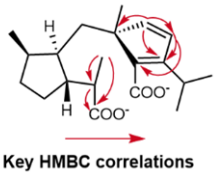
**Supplementary Table 14.** Spectroscopic data of compound **12**.




| Position | $\delta_H$ (ppm), mult (J in Hz) | $\delta_C$ (ppm) | COSY |
|---|---|---|---|
| 1 | 2.19, m<br>1.51, dd (14.3, 6.4) | 33.6 | H2 |
| 2 | 1.79, m | 42.8 | H1 |
| 3 | 2.01, m | 32.7 | H16 |
| 4 | 2.19, m<br>1.20, m | 27.1 | H5 |
| 5 | 2.01, m<br>1.11, m | 32.7 | H4, H6 |
| 6 | 2.23, m | 43.3 | H5 |
| 7 | | 49.9 | |
| 8 | 3.75, d (11.6)<br>3.62, d (11.9) | 68.0 | |
| 9 | | 179.5 | |
| 10 | | 72.6 | |
| 11 | | 63.4 | |
| 12 | 2.23, m | 50.7 | H13 |
| 13 | 5.97, d (3.4) | 130.6 | H12, H15 |
| 14 | | 149.8 | |
| 15 | 2.89, septet (6.8) | 28.8 | H13, H19, H20 |
| 16 | 0.84, d (6.9) | 18.3 | H3 |
| 17 | 2.01, m<br>0.31, d (12.3) | 33.0 | |
| 18 | 1.43, s | 24.1 | |
| 19 | 1.16, d (7.0) | 23.1 | H15 |
| 20 | 1.14, d (7.0) | 21.5 | H15 |

**Supplementary Table 15.** Spectroscopic data of compound **13**.

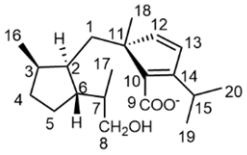


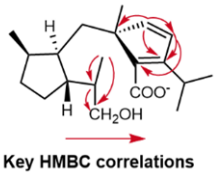




Key HMBC correlations

| Position | $\delta_H$ (ppm), mult (J in Hz) | $\delta_C$ (ppm) | COSY |
|---|---|---|---|
| 1 | 2.64, d (13.4)<br>1.97, m | 35.2 | H2 |
| 2 | 1.45, m | 43.5 | H1 |
| 3 | 1.98, m | 36.7 | H4, H16 |
| 4 | 1.57, m<br>1.27, m | 34.0 | H3, H5 |
| 5 | 1.79, m<br>1.65, m | 23.2 | H4, H6 |
| 6 | 2.52, m | 44.8 | H2, H5 |
| 7 | 3.15, m | 39.9 | H6, H17 |
| 8 | | 179.6 | |
| 9 | | 167.4 | |
| 10 | | 135.9 | |
| 11 | | 58.8 | |
| 12 | 6.73, m | 153.9 | H13 |
| 13 | 6.52, m | 128.3 | H12 |
| 14 | | 164.2 | |
| 15 | 4.36, m | 27.9 | H19, H20 |
| 16 | 0.85, d (7.1) | 15.1 | H3 |
| 17 | 1.29, dd (7.1, 3.2) | 10.8 | H7 |
| 18 | 1.57, s | 23.2 | |
| 19 | 1.27, d (7.0) | 22.8 | H15 |
| 20 | 1.22, d (7.0) | 22.1 | H15 |

**Supplementary Table 16.** Spectroscopic data of compound **14**.

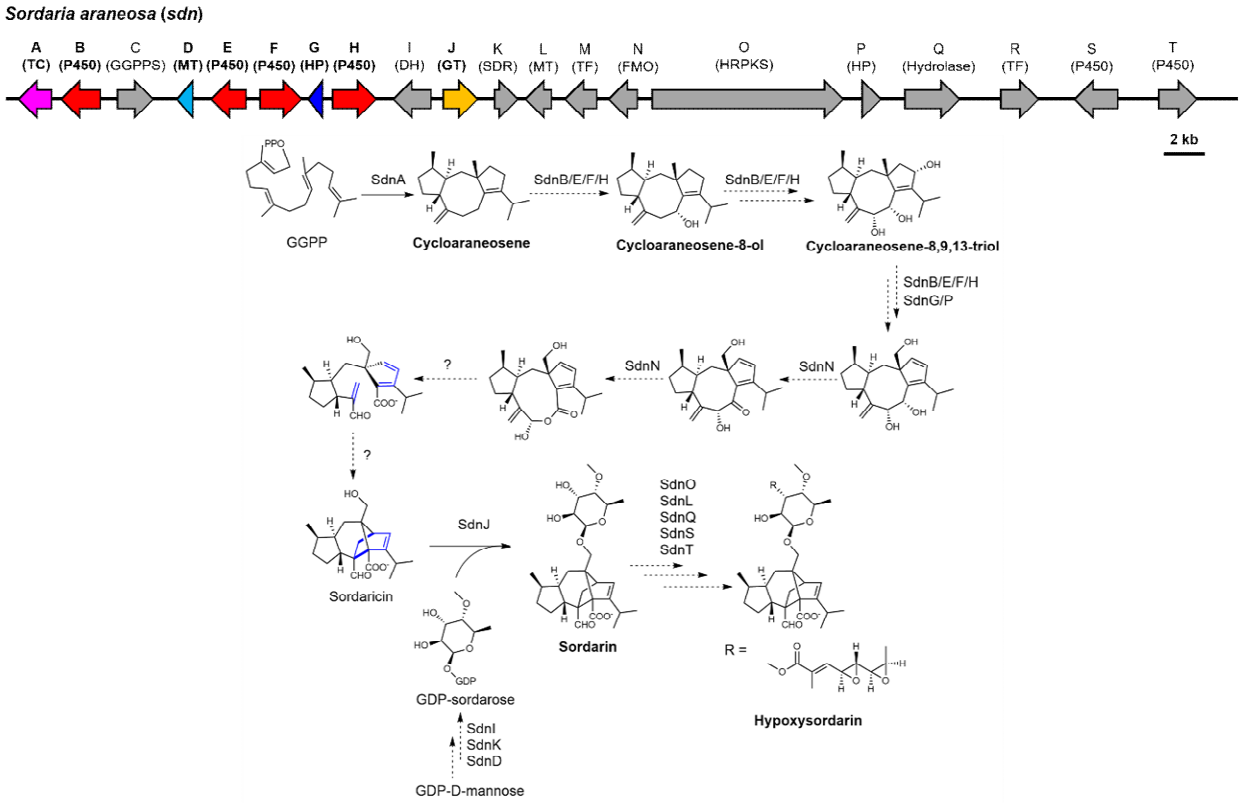


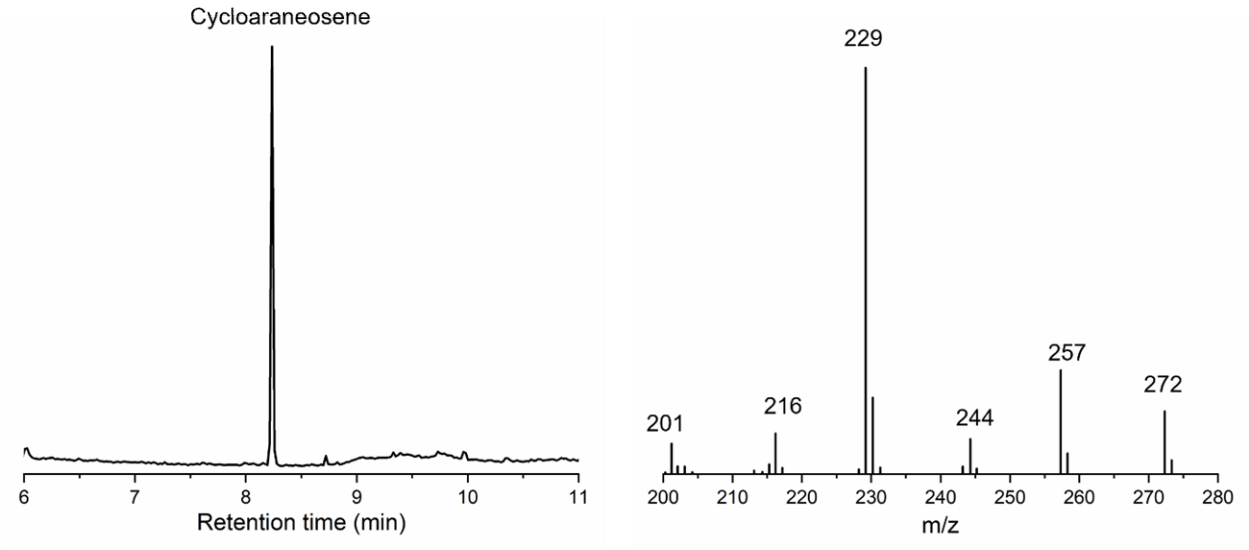

Key HMBC correlations

| Position | $\delta_H$ (ppm), mult (J in Hz) | $\delta_C$ (ppm) | COSY |
|---|---|---|---|
| 1 | 2.60, d (13.8)<br>1.92, m | 35.5 | H2 |
| 2 | 1.47, m | 43.4 | H1 |
| 3 | 1.92, m | 36.9 | H4, H16 |
| 4 | 1.54, m<br>1.35, m | 34.3 | H3, H5 |
| 5 | 1.54, m<br>1.35, m | 22.0 | H4 |
| 6 | 2.08, m | 43.4 | H7 |
| 7 | 2.33, m | 36.9 | H6, H8, H17 |
| 8 | 3.69, m | 68.2 | H7 |
| 9 | | 167.4 | |
| 10 | | 136.4 | |
| 11 | | 58.9 | |
| 12 | 6.73, m | 154.1 | H13 |
| 13 | 6.52, m | 128.3 | H12 |
| 14 | | 164.1 | |
| 15 | 4.35, m | 27.9 | H19, H20 |
| 16 | 0.84, d (7.1) | 15.2 | H3 |
| 17 | 0.96, d (6.8) | 11.3 | H7 |
| 18 | 1.58, s | 23.4 | |
| 19 | 1.27, d (7.0) | 22.8 | H15 |
| 20 | 1.21, d (7.0) | 22.2 | H15 |

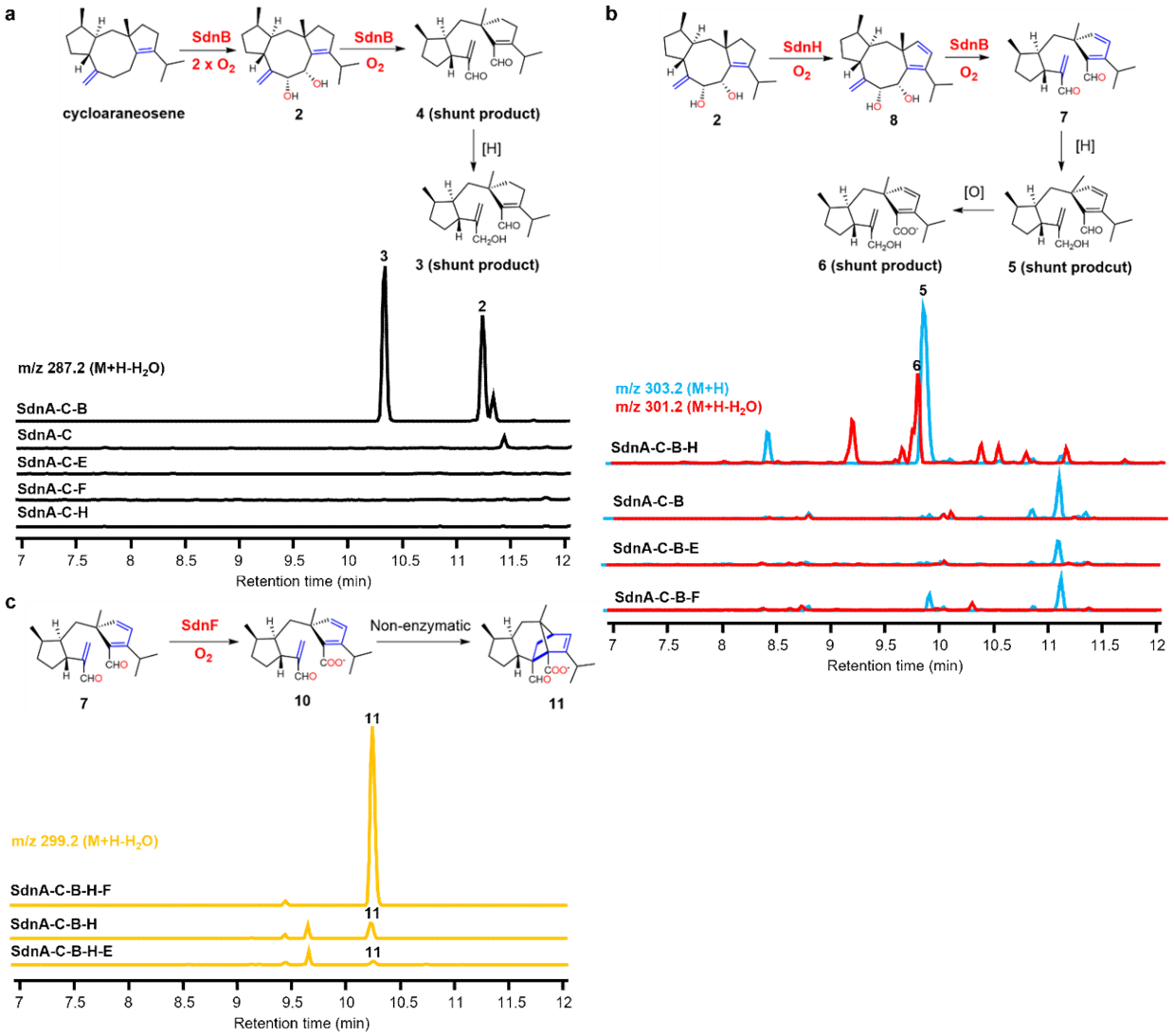
## III Supplementary Figures




**Supplementary Fig 1.** Proposed biosynthesis of sordaricin, sordarin, and hypoxysordarin by Kudo, et al[19]. Genes in *sdn* but absent from the sordarin B cluster are colored gray. Solid arrows represent experimentally characterized steps while dashed arrows represent proposed reactions. Five structures named in bold were isolated from *S. araneosa* and characterized by the same study. TC, terpene cyclase; GGPPS, geranylgeranyl pyrophosphate synthase; MT, methyl transferase; HP, hypothetical protein; DH, dehydratase; GT, glycosyl transferase; SDR, short chain reductase; TF, transcription factor; FMO, flavin-dependent monooxygenase; HRPKS, highly reducing polyketide synthase.



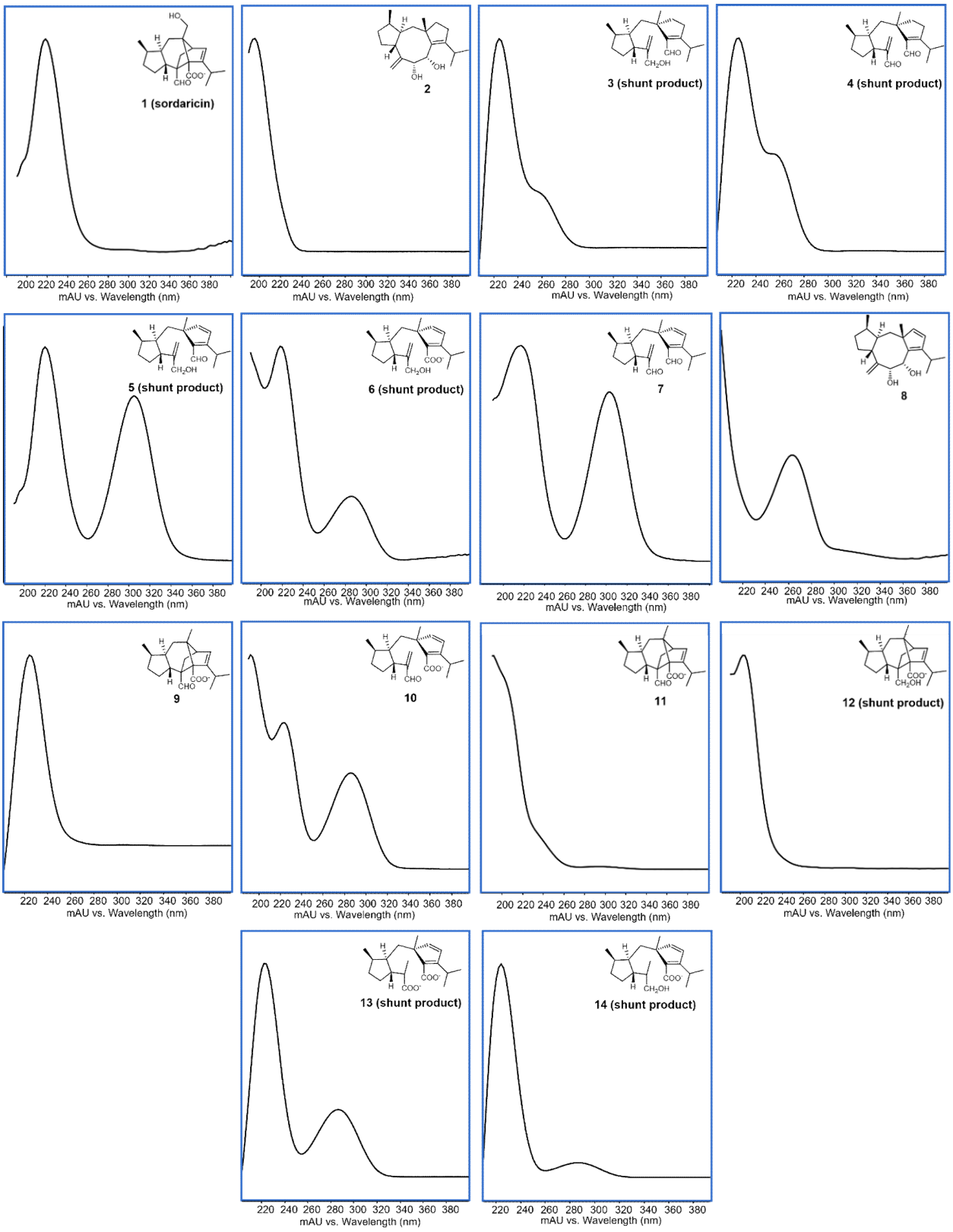

**Supplementary Fig 2.** GC-MS analysis of *A. nidulans* transformed with SdnA-C. The fragmentation pattern of cycloaraneosene (MW 272) matches with that reported by Kudo, et al[4].




546

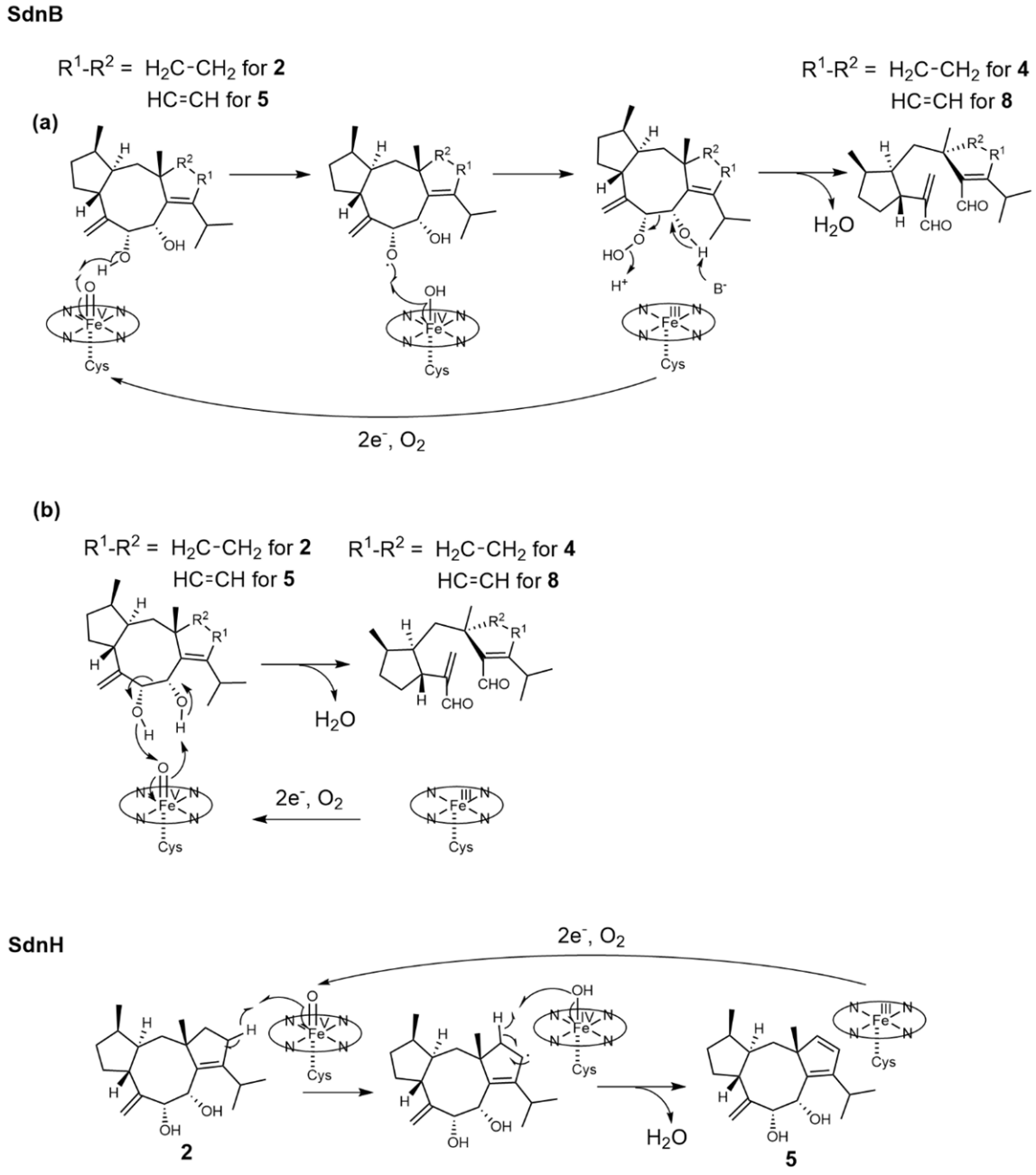
547 **Supplementary Fig 3.** Stepwise reconstitution of genes involved in sordaricin biosynthesis. **a**, metabolic
548 profiles of *A.nidulans* coexpressing SdnA-C with SdnB/E/F/H individually. **b**, metabolic profiles of
549 *A.nidulans* coexpressing SdnA-C-B with SdnE/F/H individually. **c**, metabolic profiles of *A.nidulans*
550 coexpressing SdnA-C-B-H with SdnE/F individually. The trace mount of **11** in *A.nidulans* expressing
551 SdnA-C-B-H and A-C-B-H-E is likely a result of air or host oxidation of compound **7** to **10** which then
552 undergo non-enzymatic cyclization to form **11**. The chromatograms in all cases are extracted from mass
553 spectra of the base peak for each compound.

554

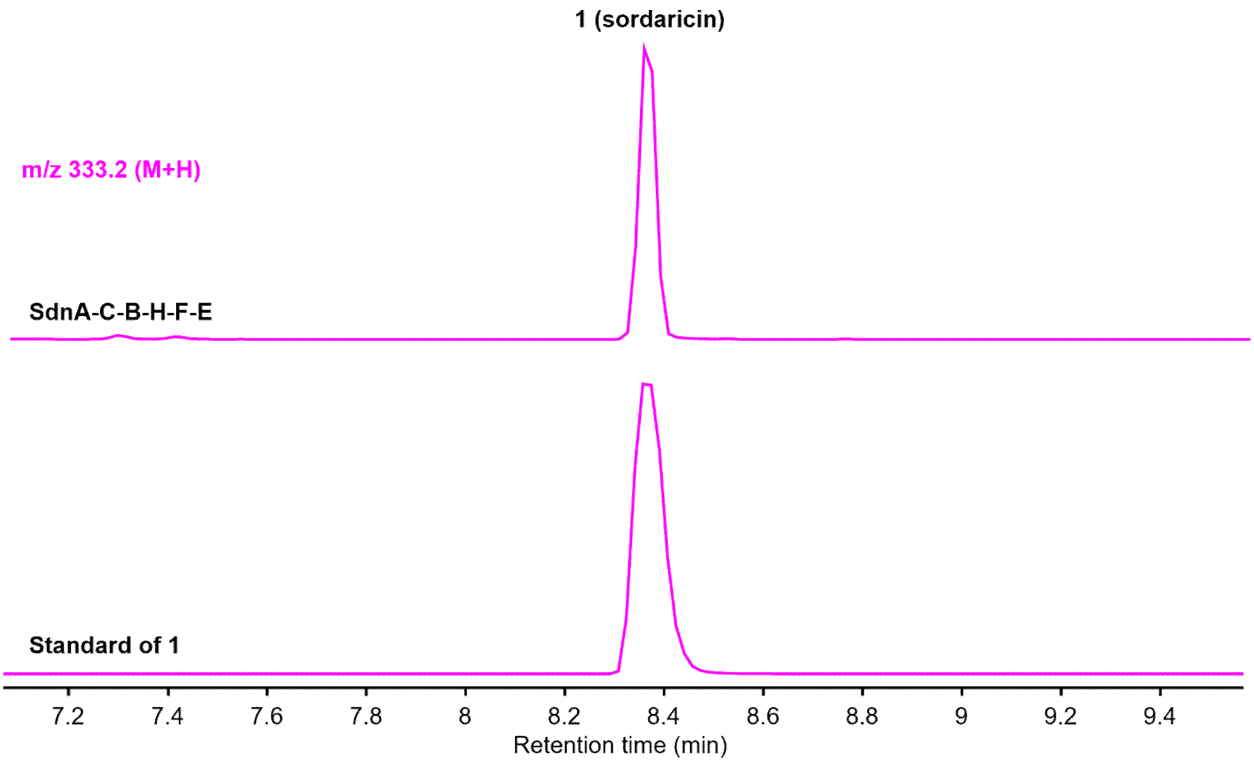



555

556 **Supplementary Fig 4.** UV-vis spectra of purified compounds. All spectra were measured with an Agilent
557 1260 Infinity II LC system equipped with a variable wavelength detector.




**Supplementary Fig 5.** Proposed mechanism of SdnB (diol cleavage[5]) and SdnH (desaturation[6]).

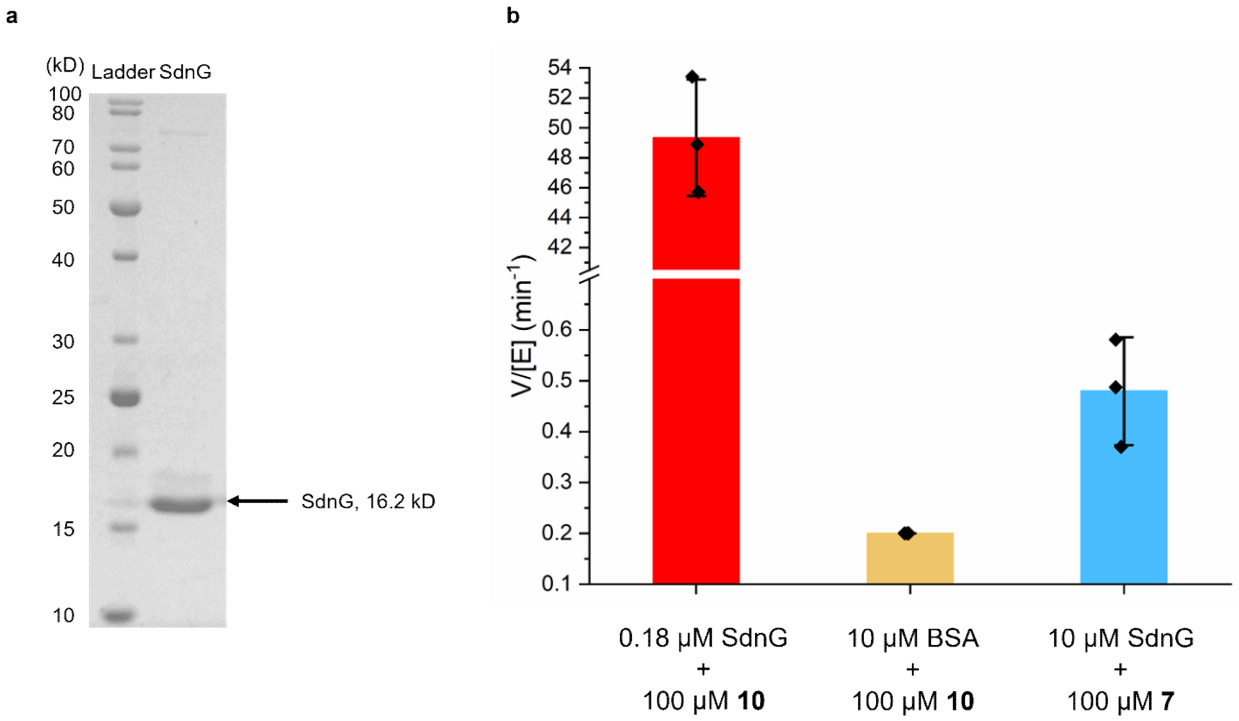



562

563 **Supplementary Fig 6.** Reconstitution of sordaricin biosynthesis in *S. cerevisiae* RC01.

| | Description | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|
| | Eutypa lata UCREL1 contig_2090, whole genome shotgun sequence | 177 | 177 | 87% | 9e-51 | 54.44% | 69956 | AORF01002090.1 |
| | Scedosporium aurantiacum strain WM 09.24 scaffold-2, whole genome shotgun sequence | 117 | 229 | 93% | 8e-47 | 70.73% | 314905 | JUDQ01000002.1 |
| | Reticulascus tulasneorum strain NRRL18230 unitig_0, whole genome shotgun sequence | 110 | 199 | 100% | 6e-44 | 69.23% | 5257612 | LSAX01000001.1 |
| | Reticulascus tulasneorum strain NRRL18230 scaffold00019, whole genome shotgun sequence | 110 | 199 | 100% | 6e-44 | 69.23% | 948340 | LSAY01000019.1 |
| | Trichoderma pleuroti strain TPhu1 contig118, whole genome shotgun sequence | 157 | 157 | 99% | 1e-43 | 44.12% | 69223 | MDJU01000118.1 |
| | Neopestalotiopsis sp. 37M scaffold_3, whole genome shotgun sequence | 102 | 188 | 98% | 9e-41 | 67.12% | 1371024 | SWKT01000003.1 |
| | Periconia macrospinosa strain DSE2036 DM02scaffold_371_Cont1150, whole genome shotgun sequence | 101 | 186 | 87% | 4e-40 | 65.75% | 30277 | PCYO01001150.1 |
| | Talaromyces borbonicus strain SV-2017a Contig0000011, whole genome shotgun sequence | 100 | 206 | 99% | 8e-40 | 69.44% | 718236 | NBSA01000011.1 |
| | Trichoderma afroharzianum cultivar MRI349 Contig615, whole genome shotgun sequence | 136 | 136 | 99% | 2e-36 | 43.43% | 43395 | JAEKOX010000615.1 |
| | Trichoderma harzianum strain T6776 Scaffolds0269.1, whole genome shotgun sequence | 136 | 136 | 99% | 2e-36 | 43.43% | 49334 | JOKZ01000269.1 |
| | Trichoderma harzianum strain T11_W Scaffold7_1, whole genome shotgun sequence | 136 | 136 | 99% | 2e-36 | 43.43% | 1590712 | WUWT01000010.1 |
| | Calcarisporium arbuscula strain NRRL 3705 Contig4, whole genome shotgun sequence | 118 | 173 | 86% | 2e-36 | 55.36% | 2470260 | WBSA01000004.1 |
| | Trichoderma harzianum strain Tr1 Tr1_contig_267, whole genome shotgun sequence | 105 | 172 | 98% | 5e-36 | 51.38% | 210298 | MTYI01000253.1 |
| | Talaromyces verruculosus strain TS63-9 Scaffold63, whole genome shotgun sequence | 117 | 199 | 100% | 2e-29 | 41.50% | 172023 | LHCL01000063.1 |
| | Xylaria longipes strain IHI A66 Contig_4, whole genome shotgun sequence | 115 | 115 | 97% | 3e-29 | 39.46% | 259134 | NQIL01000004.1 |
| | Talaromyces adpressus strain CBS 142503 Contig0000575, whole genome shotgun sequence | 113 | 198 | 100% | 2e-28 | 41.50% | 184356 | NHZS01000561.1 |
| | Talaromyces thailandensis strain OC-R06-P5 OC-R06-P5_contig_60, whole genome shotgun sequence | 68.9 | 131 | 86% | 7e-24 | 46.58% | 288724 | JACVQZ010000060.1 |
| | Xylaria multiplex strain DSM 110363 Contig_156, whole genome shotgun sequence | 69.3 | 129 | 82% | 4e-23 | 43.04% | 93426 | WUBL01000156.1 |
| | Neopestalotiopsis clavispora strain IHI 201606 Contig_4, whole genome shotgun sequence | 97.8 | 184 | 98% | 9e-23 | 63.01% | 420075 | JAANBA010000004.1 |
| | Fungal sp. No.14919 DNA, contig: Contig0070, whole genome shotgun sequence | 65.9 | 127 | 80% | 1e-22 | 38.96% | 217582 | BDMC01000070.1 |
| | Xylaria hypoxylon strain DSM 108379 Contig_44, whole genome shotgun sequence | 95.1 | 95.1 | 84% | 7e-22 | 39.26% | 191544 | SKBN01000044.1 |
| | Xylaria hypoxylon strain CBS 122620 genome assembly, contig: tig00000792, whole genome shotgun sequence | 95.1 | 95.1 | 84% | 7e-22 | 39.26% | 1026764 | CADCXB010000050.1 |
| | Xylaria grammica strain EL000614 Contig15, whole genome shotgun sequence | 62.0 | 123 | 80% | 2e-21 | 53.12% | 3880253 | NGZP02000014.1 |
| | Xylaria grammica strain IHI A82 Contig_294, whole genome shotgun sequence | 62.0 | 123 | 80% | 2e-21 | 53.12% | 51925 | RYZI01000294.1 |
| | Rosellinia necatrix DNA, contig: Contig87_1, strain: W97, whole genome shotgun sequence | 91.7 | 91.7 | 84% | 1e-20 | 37.72% | 91525 | BBSO02000556.1 |
| | Pestalotiopsis sp. CR013 tig00037640_pilon_pilon_pilon, whole genome shotgun sequence | 91.3 | 181 | 86% | 2e-20 | 75.00% | 7274155 | JACFXT010000010.1 |
| | Lecanicillium fungicola 150-1 genome assembly, contig: contig_232, whole genome shotgun sequence | 82.0 | 131 | 85% | 2e-17 | 37.88% | 186091 | FWCC01000232.1 |
| | Hyphodiscus hymeniophilus strain ATCC 34498 Hyd_11, whole genome shotgun sequence | 78.6 | 137 | 69% | 4e-16 | 53.62% | 1583289 | VNKQ01000012.1 |
| * | Chalara longipes BDJ N431scaffold_16_Cont117, whole genome shotgun sequence | 50.8 | 99.0 | 74% | 2e-06 | 48.94% | 2141128 | VKGA01000117.1 |

**Supplementary Fig 7.** Blast research results for SdnG homologs in NCBI genomic database. SdnG from *S. araneosa* was used as query. All identified homologs of SdnG cluster with other Sdn enzymes responsible for sordaricin biosynthesis except for the last entry (highlighted with *) which is an orphan gene.




570

571 **Supplementary Fig 8.** Characterization of SdnG. **a**, SDS-PAGE (12%) of purified SdnG. **b**, Rates of
572 cyclization of **10** and **7** (100 μM) in the presence of SdnG or BSA. The bars and error bars represent the
573 mean and standard deviation of n=3 independent measurements respectively. The rate with 10 μM BSA is
574 within the detection limit of the assay (0.2 $min^{-1}$). Source data are provided as a Source Data file.

575

576

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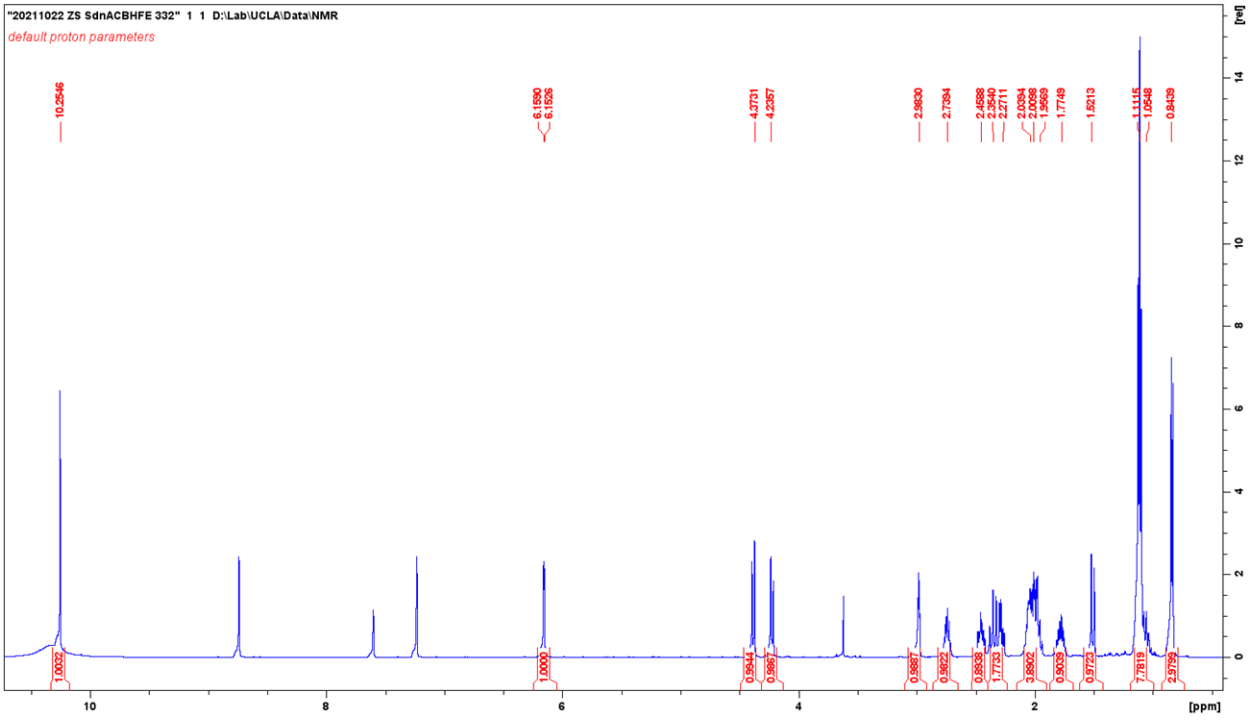
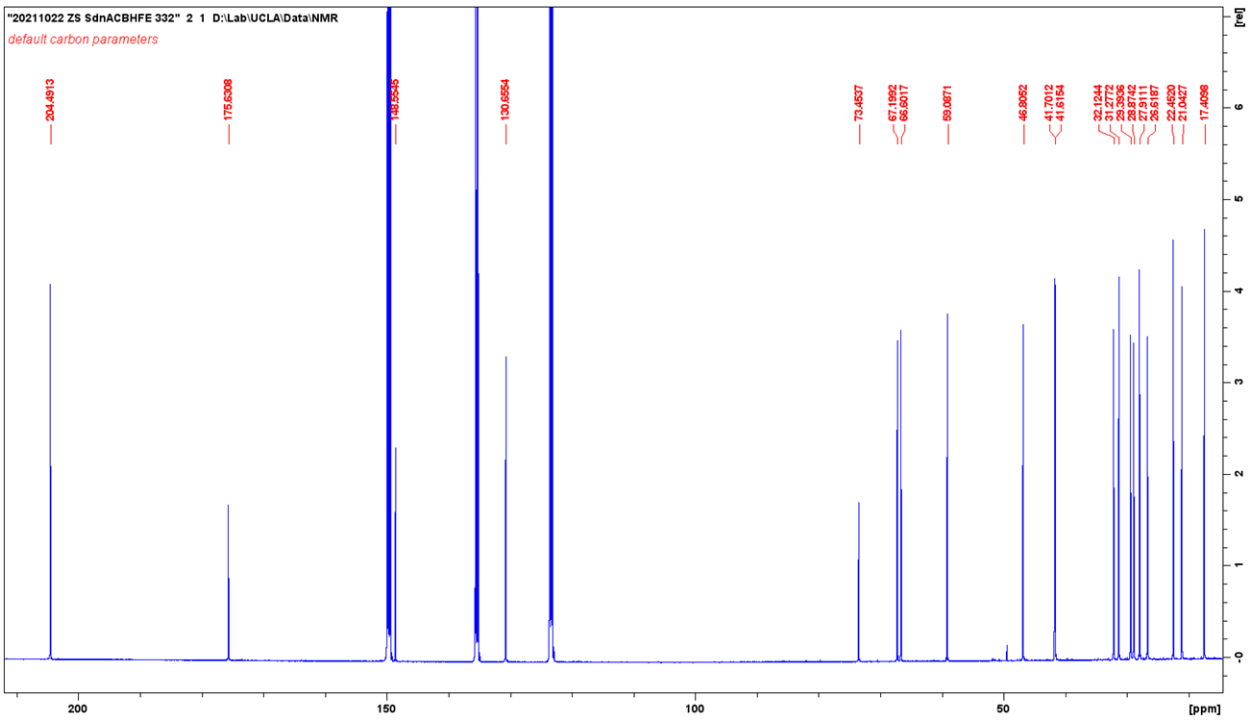
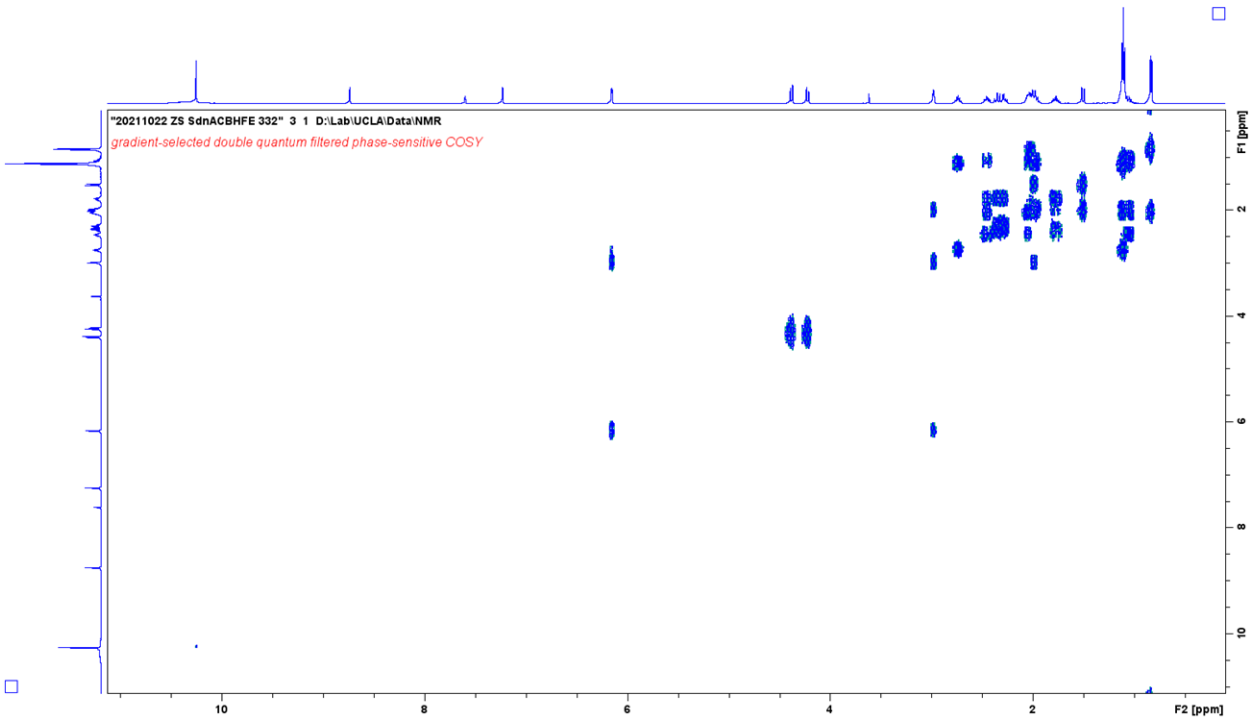



**Supplementary Fig 9.** $^{1}$H NMR of compound **1** in d5-pyridine, 500 MHz.

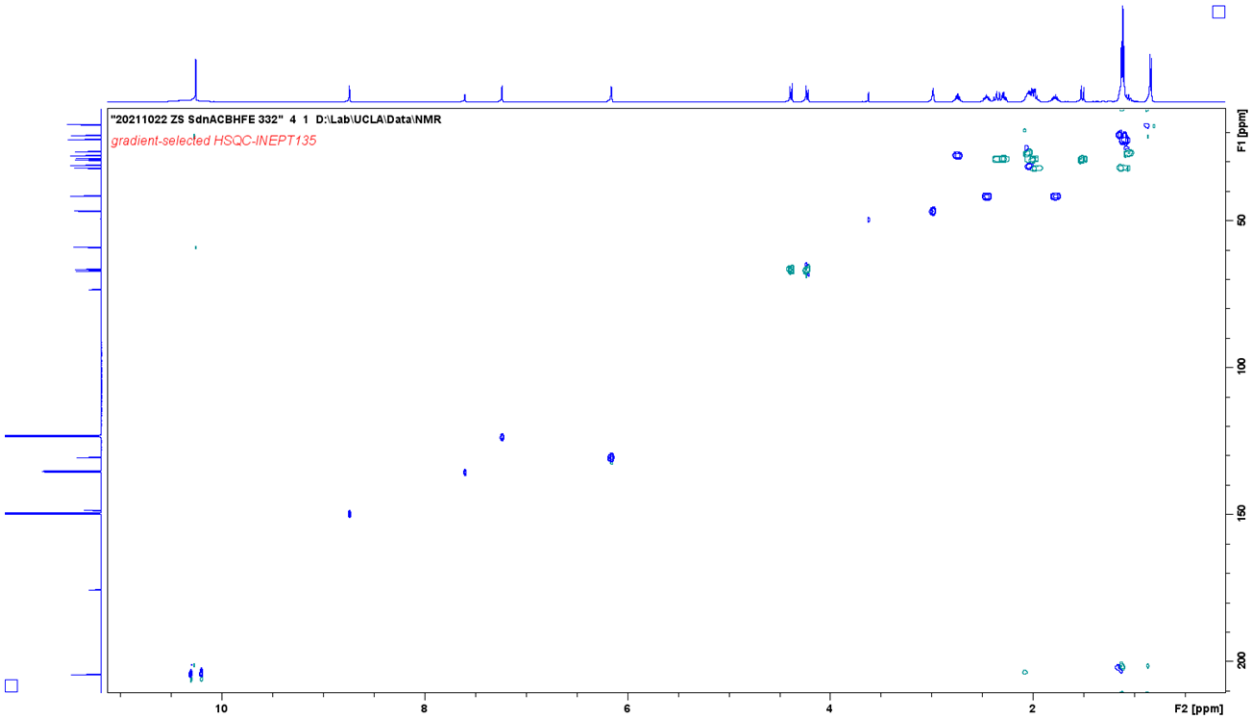



**Supplementary Fig 10.** $^{13}$C NMR of compound **1** in d5-pyridine, 500 MHz.




585

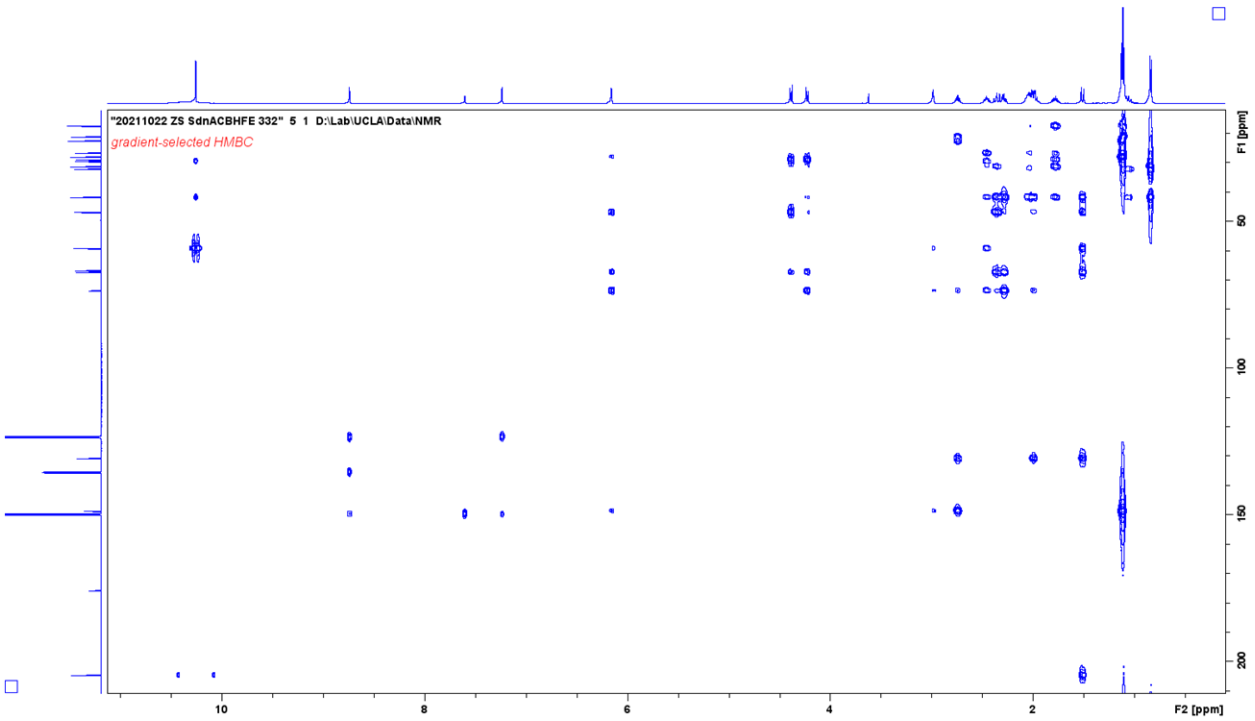
586 **Supplementary Fig 11.** $^{1}$H-$^{1}$H COSY of compound **1** in d5-pyridine, 500 MHz.



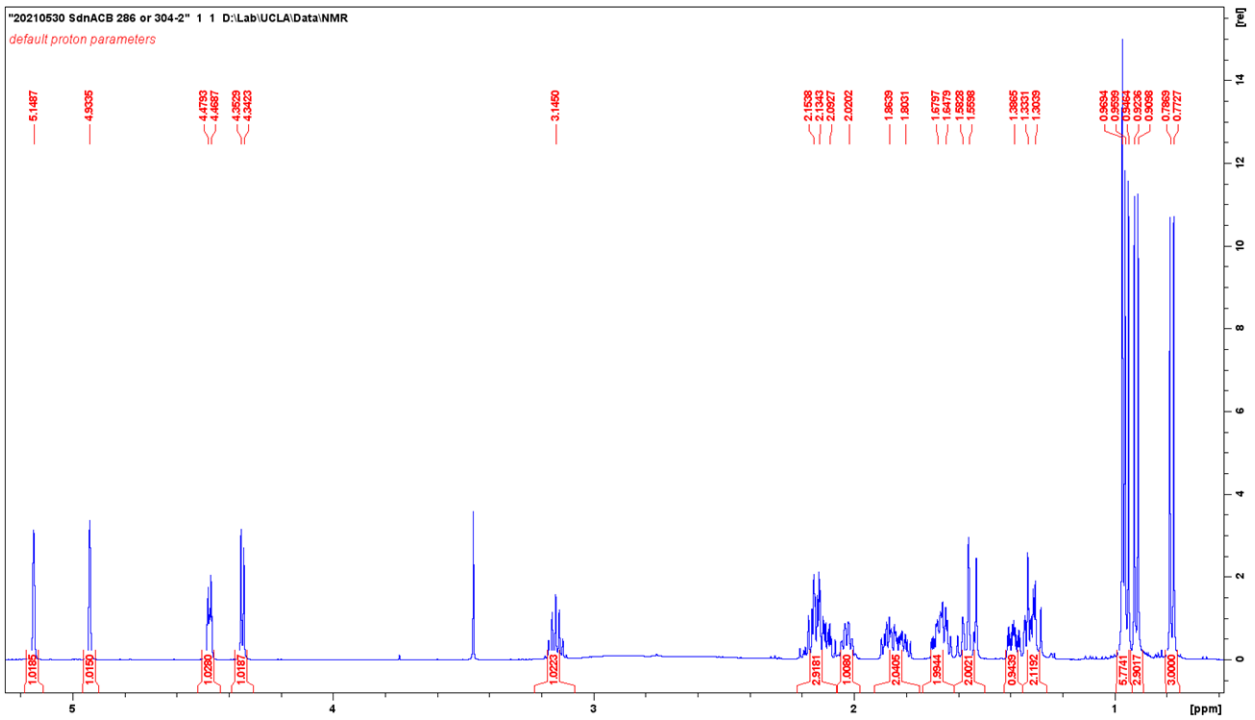

587

588 **Supplementary Fig 12.** $^{1}$H-$^{13}$C HSQC of compound **1** in d5-pyridine, 500 MHz.

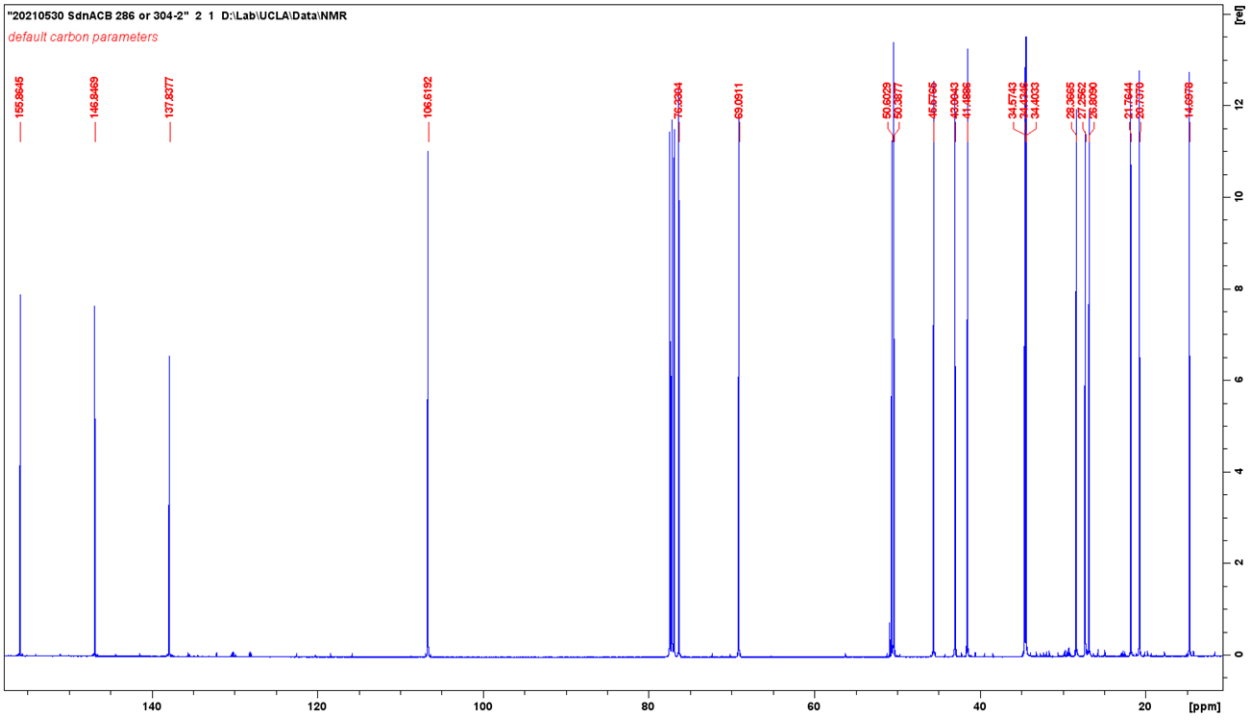
589




**Supplementary Fig 13.** $^{1}H$-$^{13}C$ HMBC of compound **1** in d5-pyridine, 500 MHz.

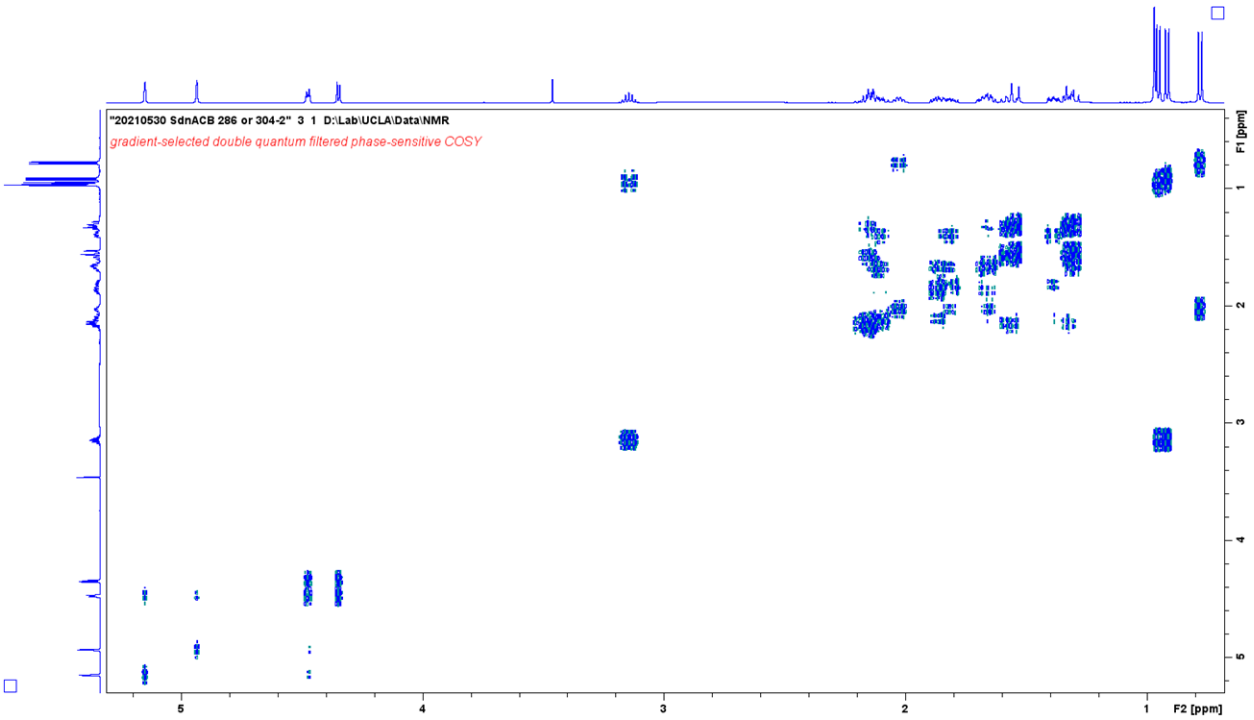



**Supplementary Fig 14.** $^{1}H$ NMR of compound **2** in $CDCl_3$, 500 MHz.




597

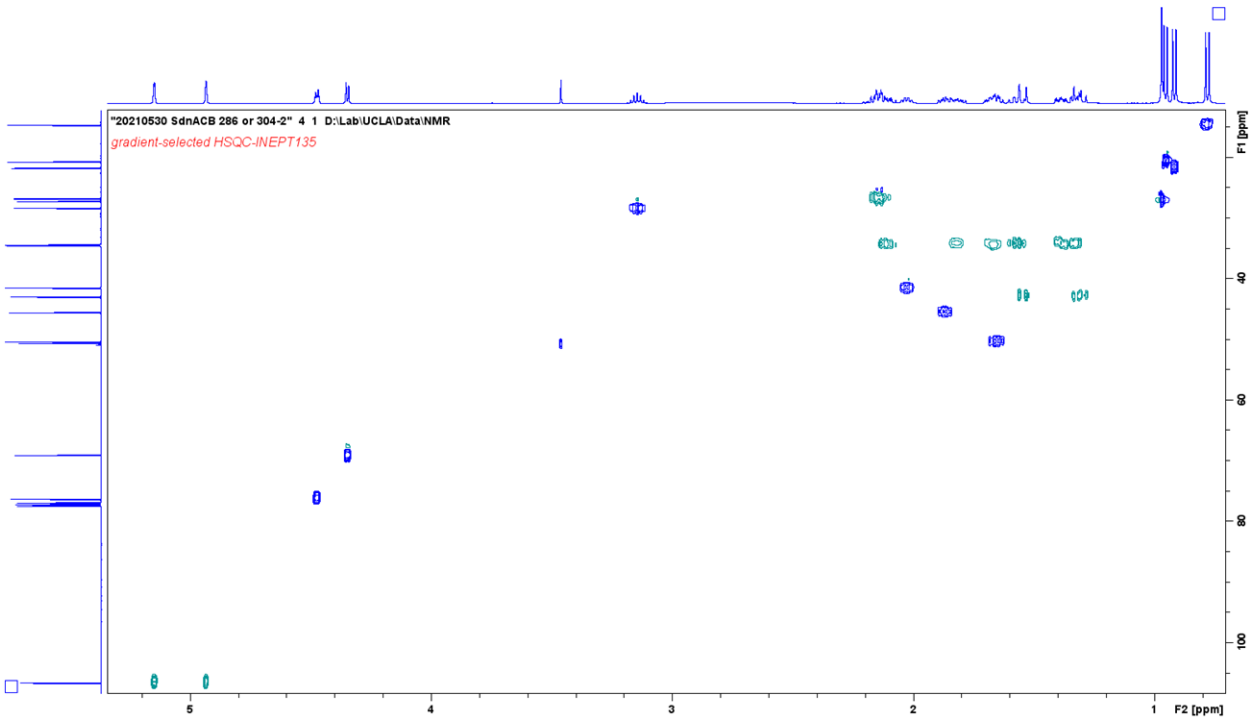
598 **Supplementary Fig 15.** $^{13}C$ NMR of compound **2** in $CDCl_3$, 500 MHz.



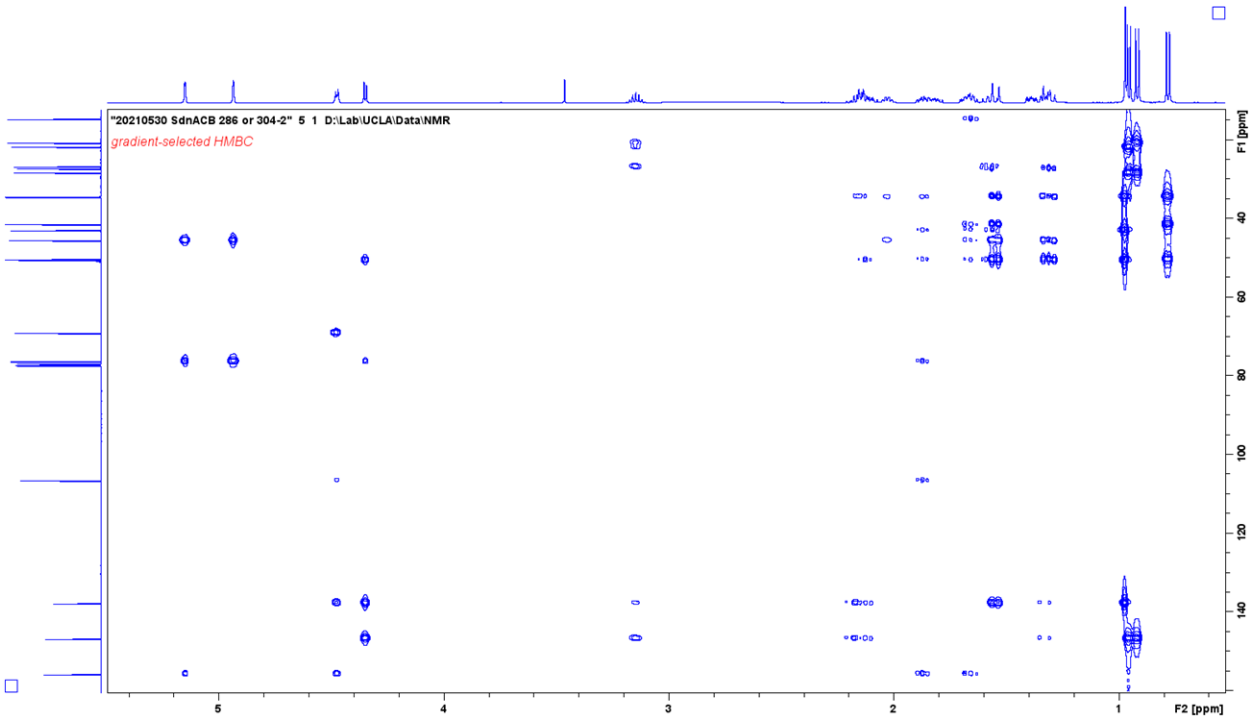

599

600 **Supplementary Fig 16.** $^{1}H$-$^{1}H$ COSY of compound **2** in $CDCl_3$, 500 MHz.

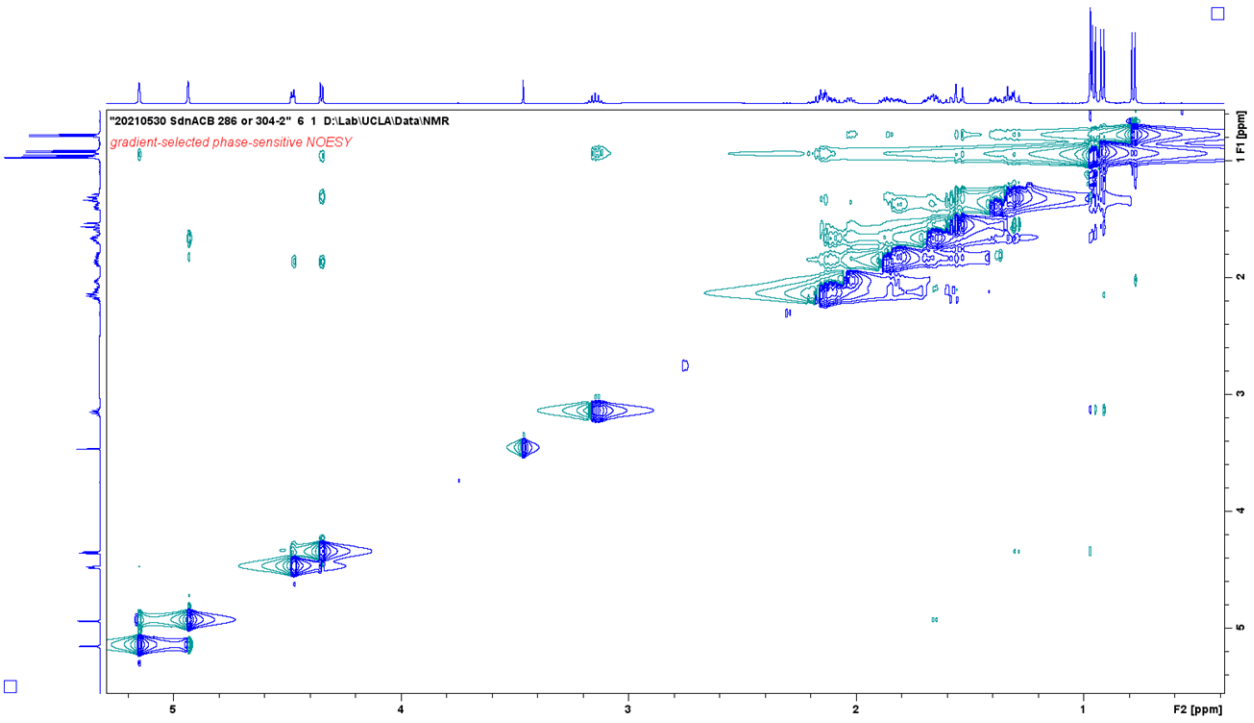
601




**Supplementary Fig 17.** $^{1}H$-$^{13}C$ HSQC of compound **2** in $CDCl_3$, 500 MHz.



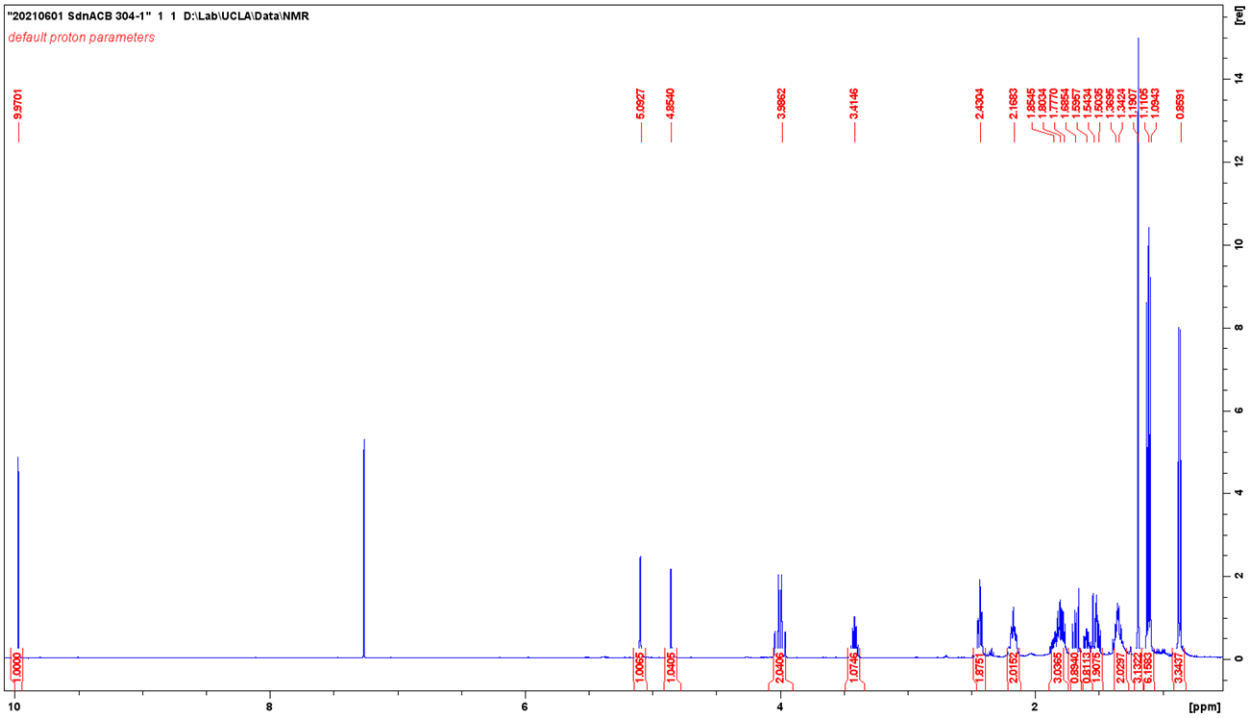

**Supplementary Fig 18**. $^{1}H$-$^{13}C$ HMBC of compound **2** in $CDCl_3$, 500 MHz.




608

609 **Supplementary Fig 19.** $^{1}$H-$^{1}$H NOESY of compound **2** in $CDCl_3$, 500 MHz.

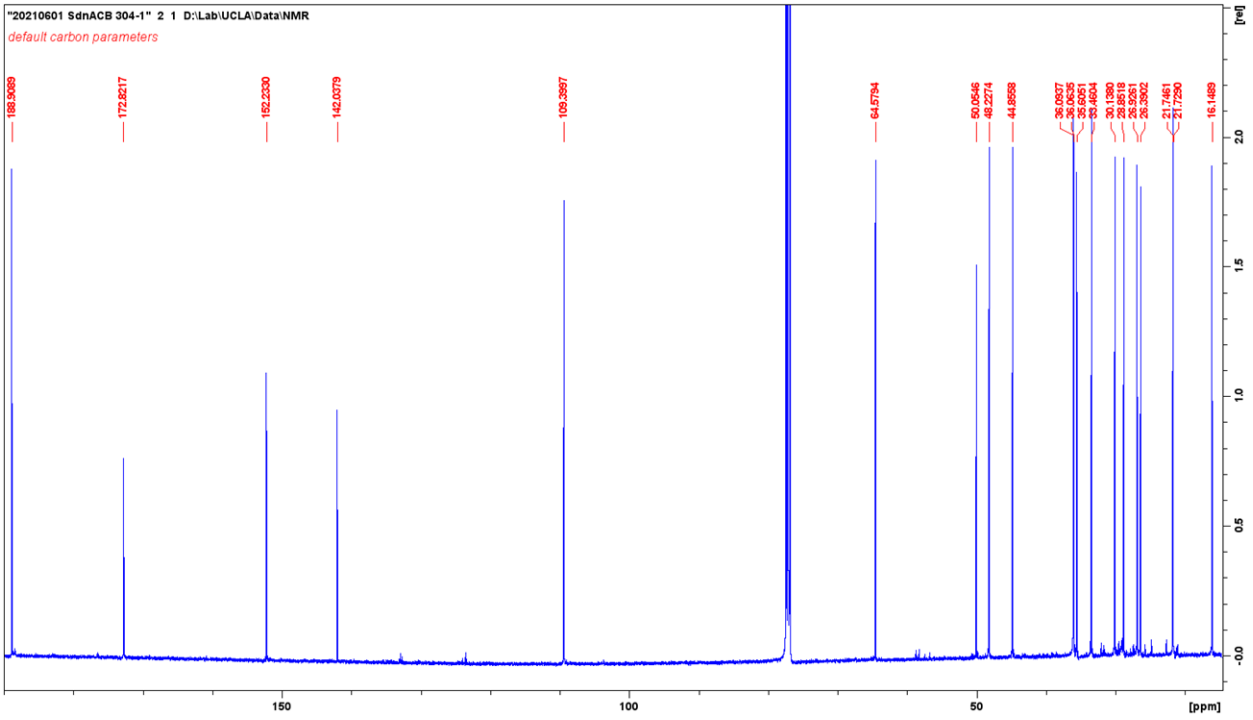
610



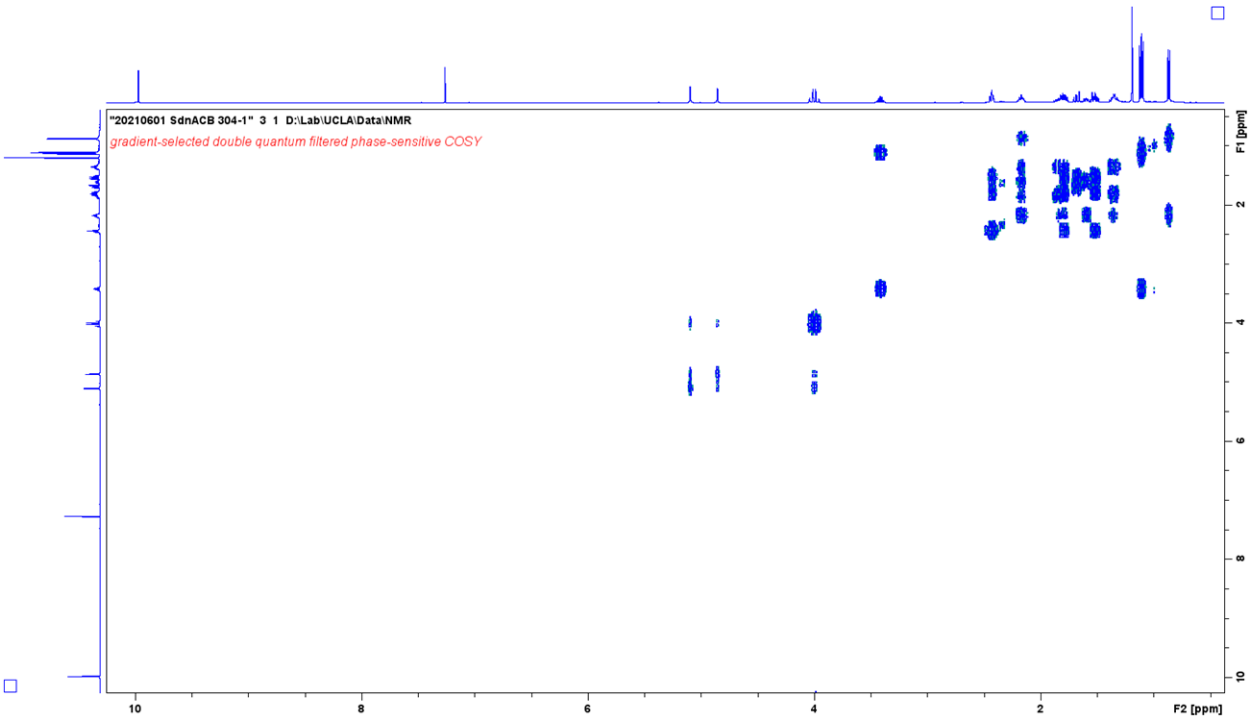

611

612 **Supplementary Fig 20.** $^{1}$H NMR of compound **3** in $CDCl_3$, 500 MHz.

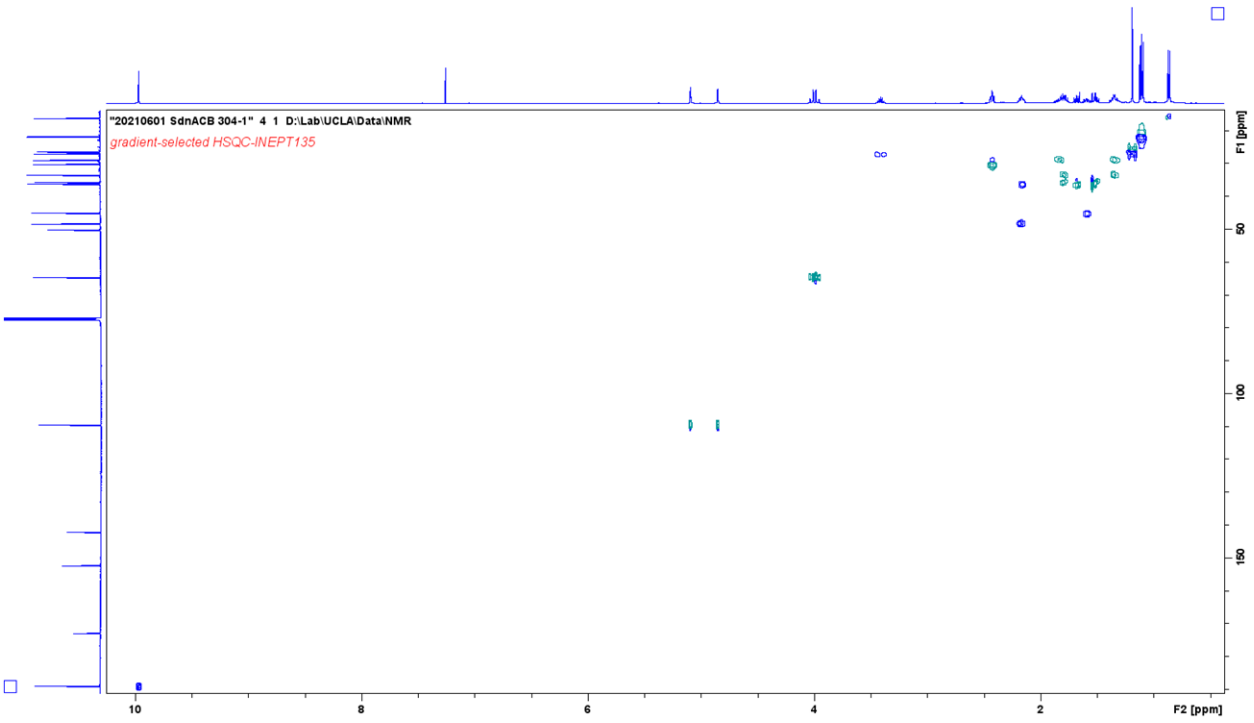
613



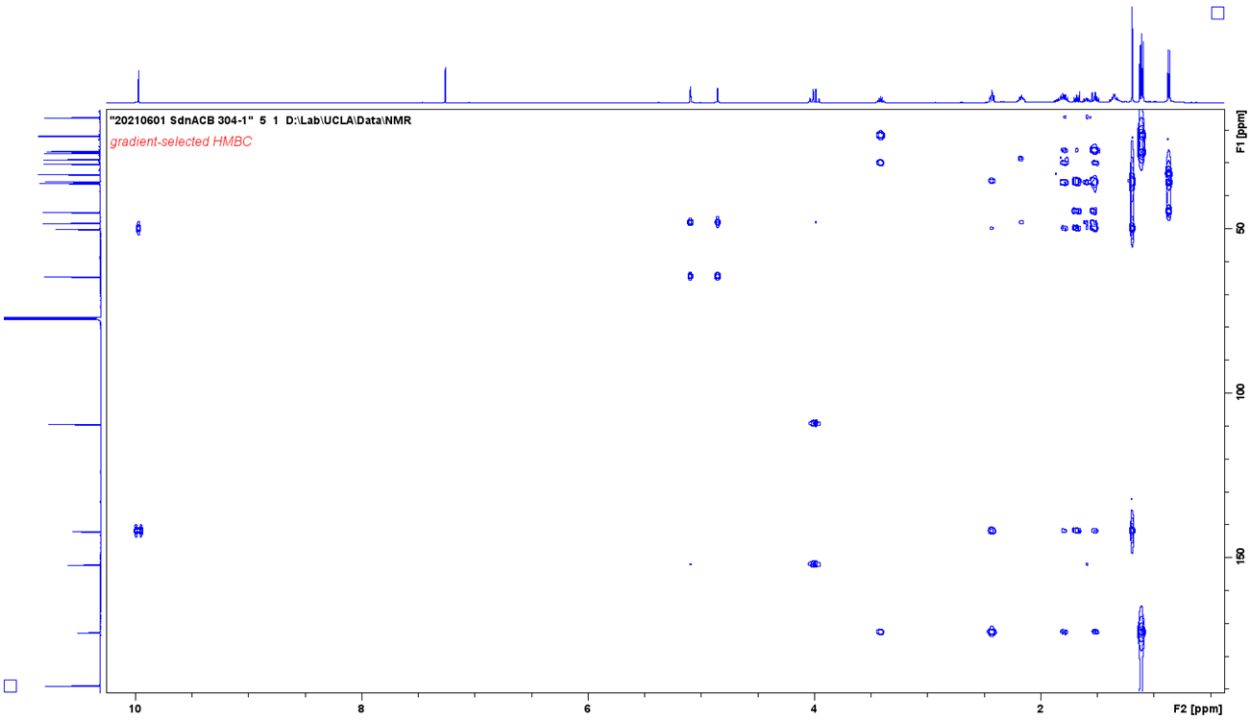

**Supplementary Fig 21.** $^{13}C$ NMR of compound **3** in $CDCl_3$, 500 MHz.



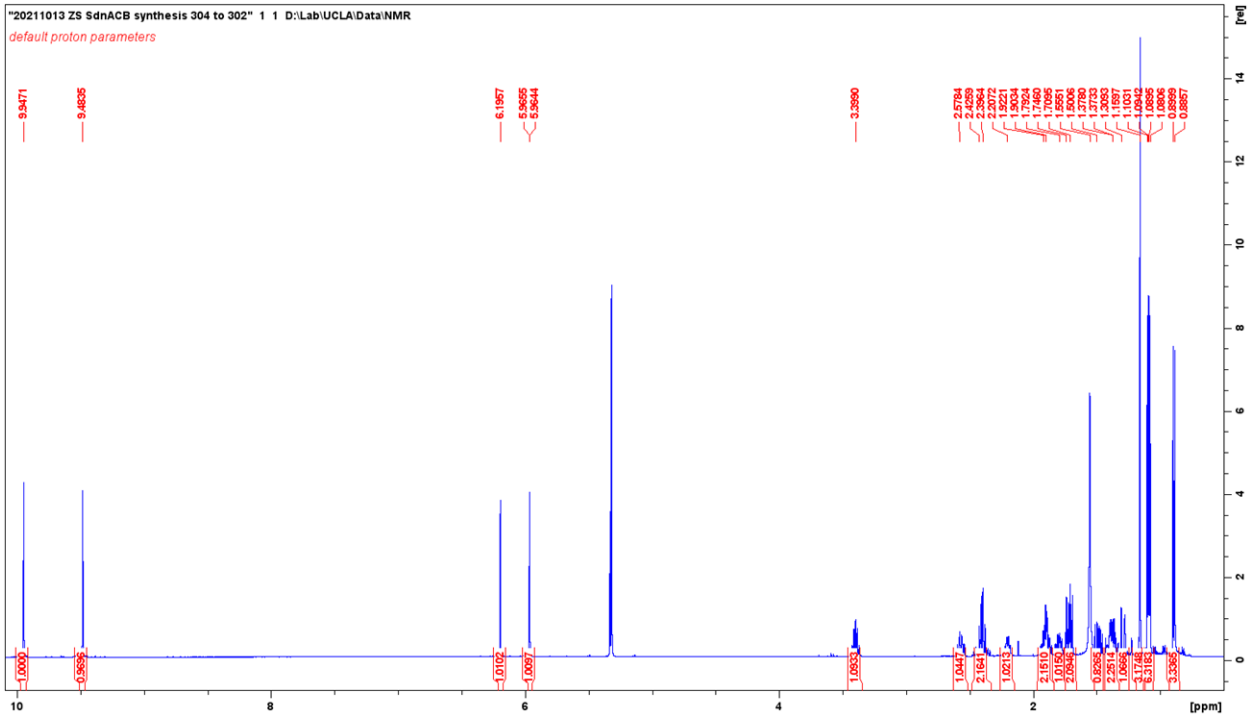

**Supplementary Fig 22.** $^{1}H$-$^{1}H$ COSY of compound **3** in $CDCl_3$, 500 MHz.




**Supplementary Fig 23.** $^{1}H$-$^{13}C$ HSQC of compound **3** in $CDCl_3$, 500 MHz.



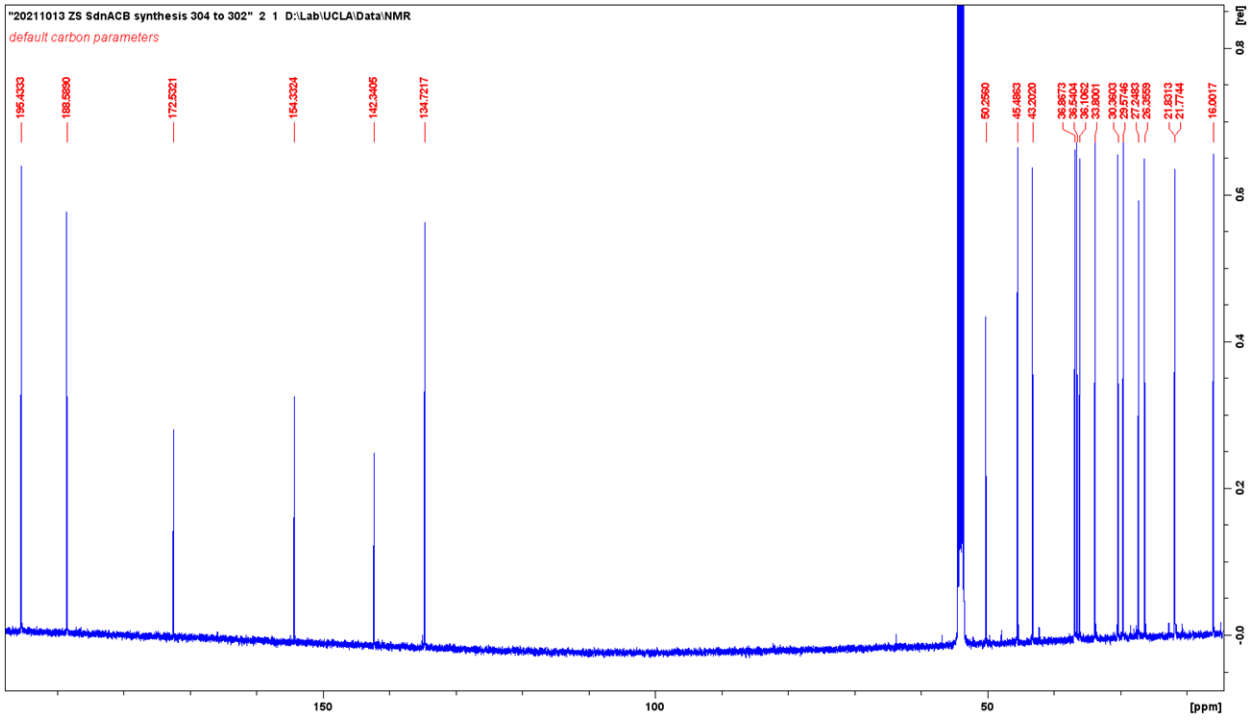

**Supplementary Fig 24.** $^{1}H$-$^{13}C$ HMBC of compound **3** in $CDCl_3$, 500 MHz.




626

627 **Supplementary Fig 25.** $^{1}$H NMR of compound **4** in $CD_2Cl_2$, 500 MHz.

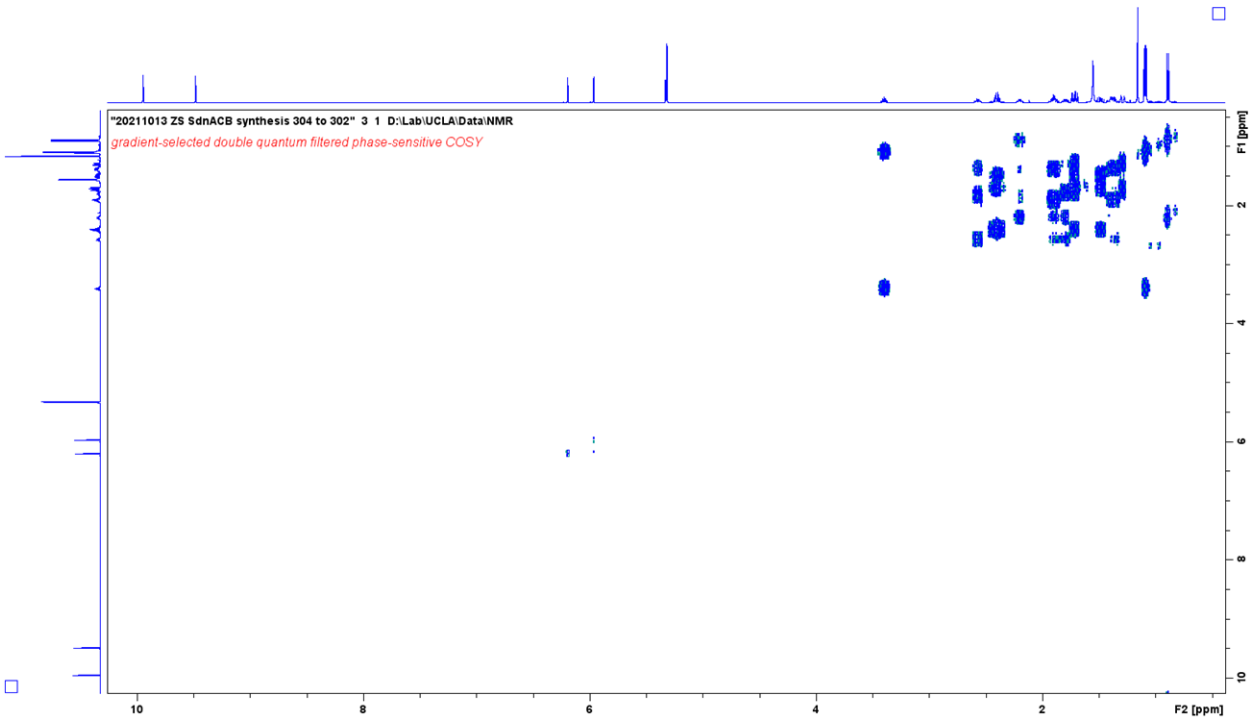
628



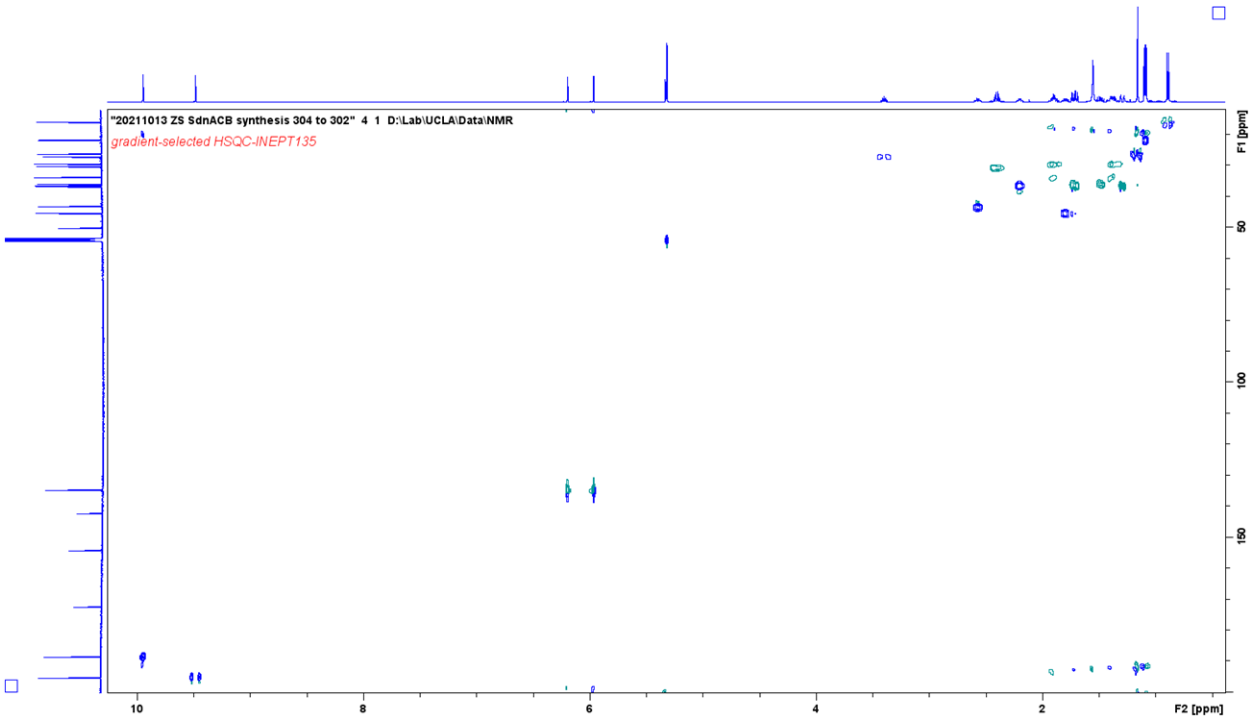

629

630 **Supplementary Fig 26.** $^{13}$C NMR of compound **4** in $CD_2Cl_2$, 500 MHz.

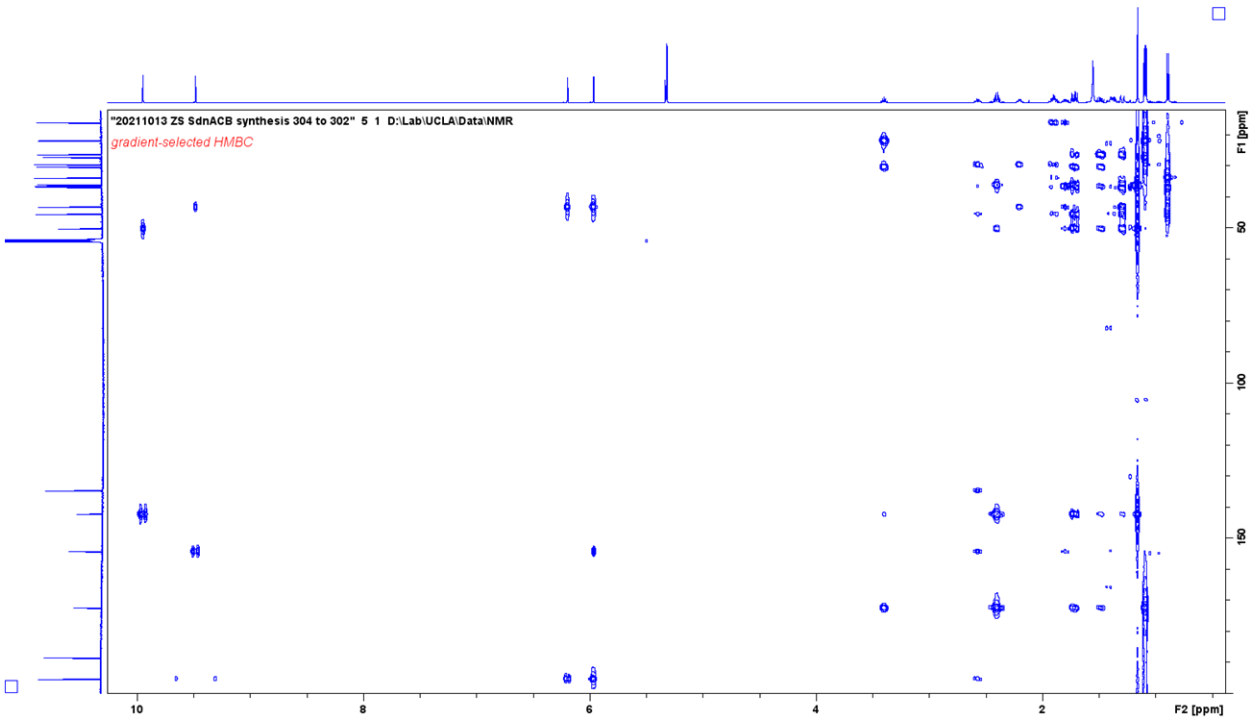
631



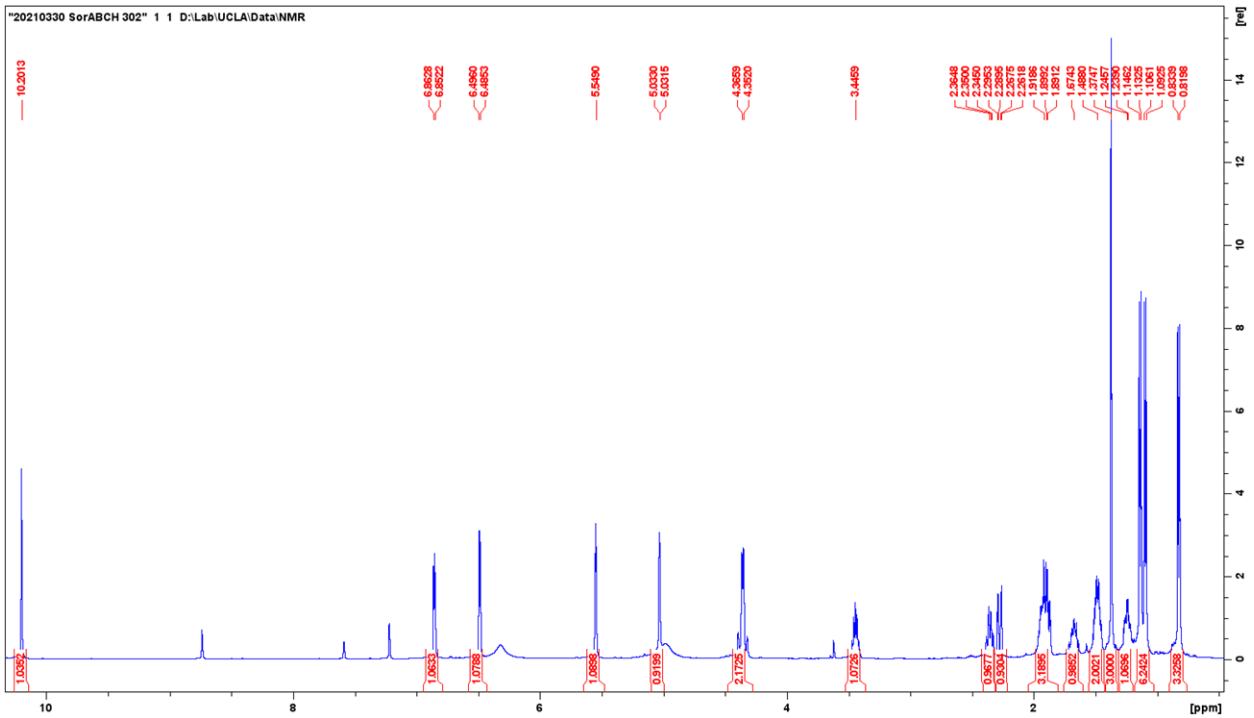

**Supplementary Fig 27.** $^{1}$H-$^{1}$H COSY of compound **4** in $CD_2Cl_2$, 500 MHz.



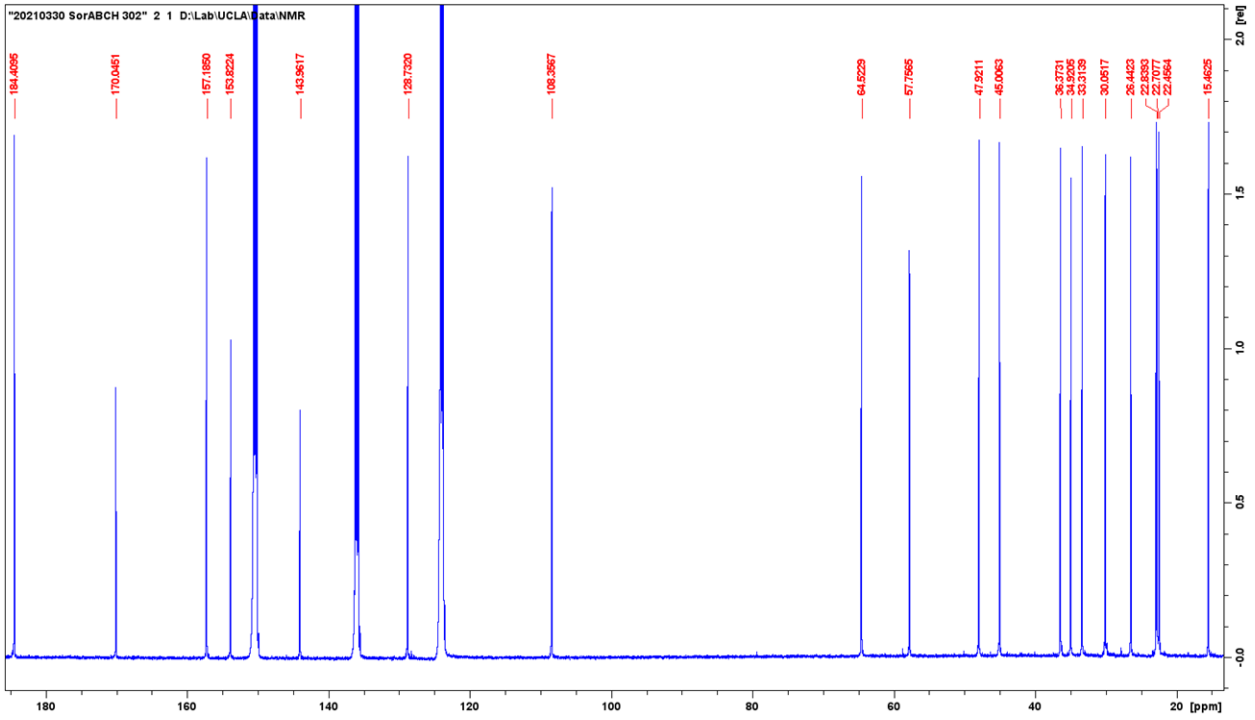

**Supplementary Fig 28.** $^{1}$H-$^{13}$C HSQC of compound **4** in $CD_2Cl_2$, 500 MHz.



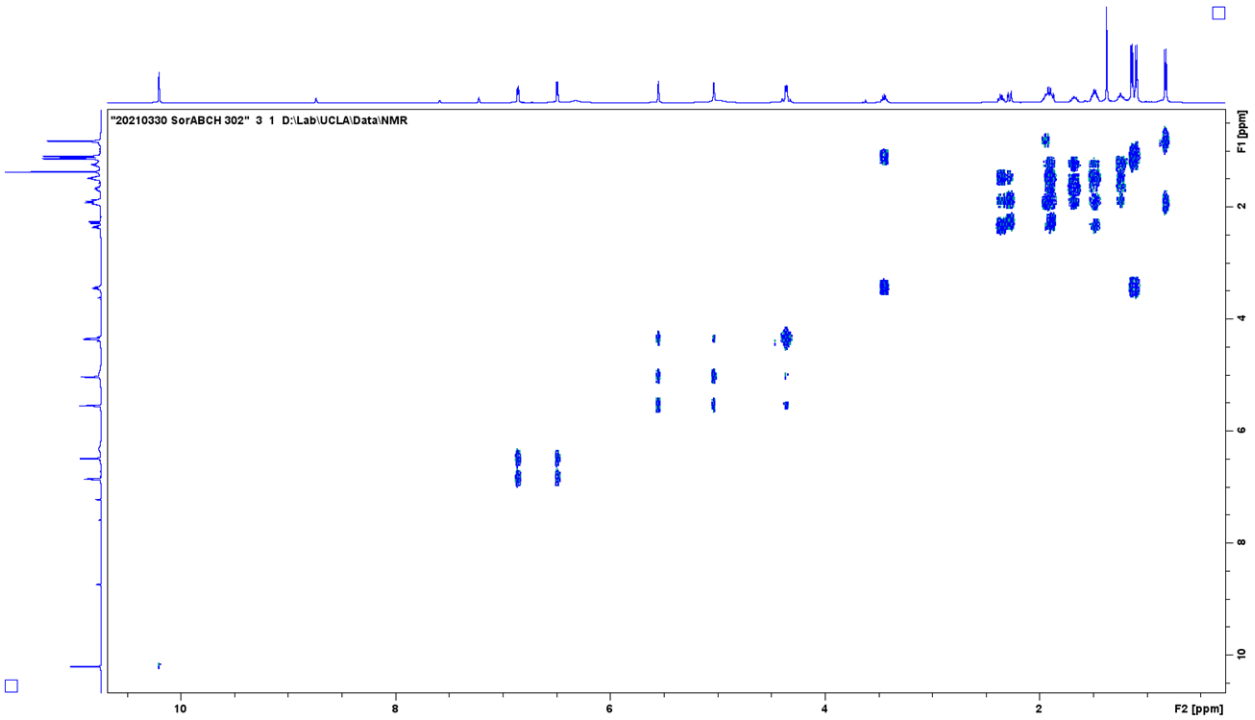

**Supplementary Fig 29.** $^{1}H$-$^{13}C$ HMBC of compound **4** in $CD_2Cl_2$, 500 MHz.



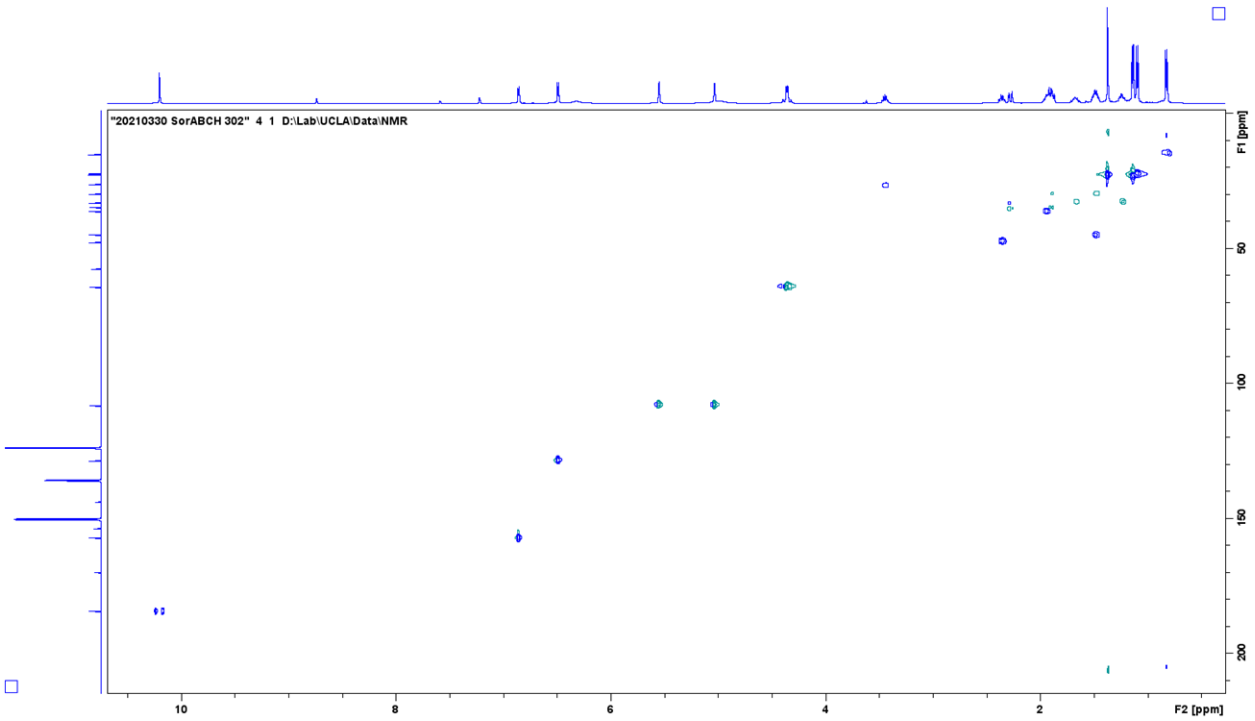

**Supplementary Fig 30.** $^{1}H$ NMR of compound **5** in d5-pyridine, 500 MHz.



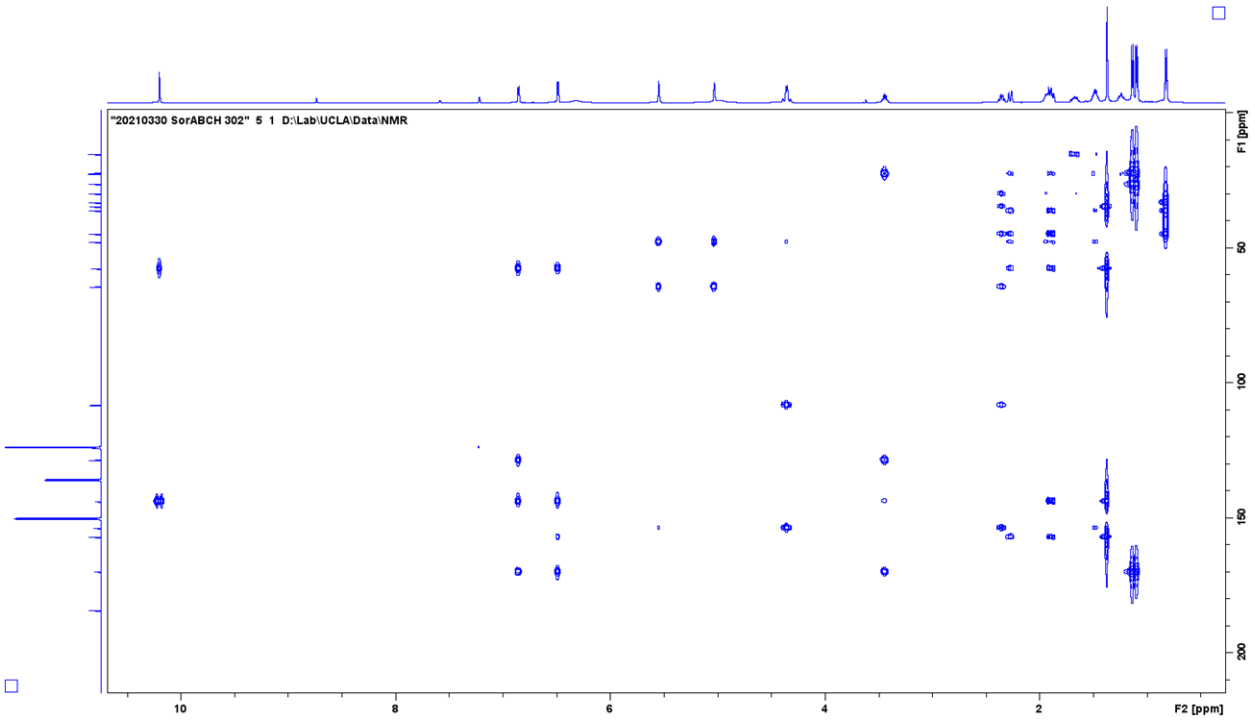

**Supplementary Fig 31.** $^{13}$C NMR of compound **5** in d5-pyridine, 500 MHz.



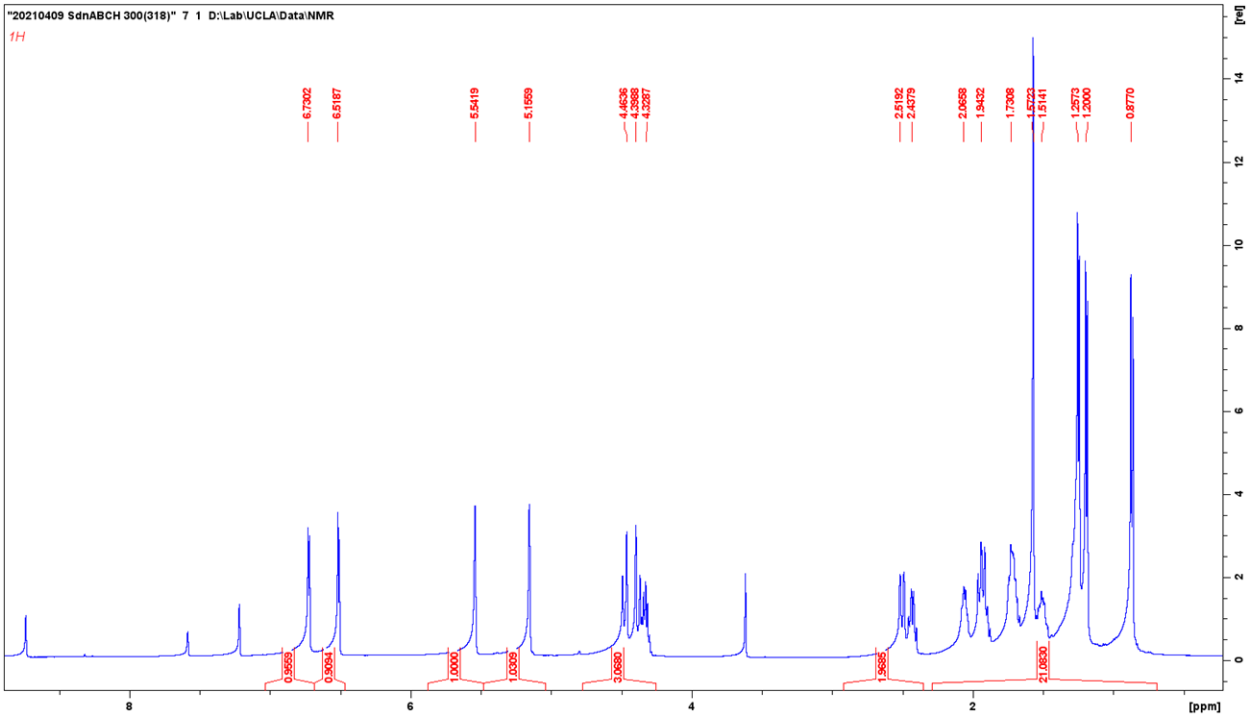

**Supplementary Fig 32.** $^{1}$H-$^{1}$H COSY of compound **5** in d5-pyridine, 500 MHz.



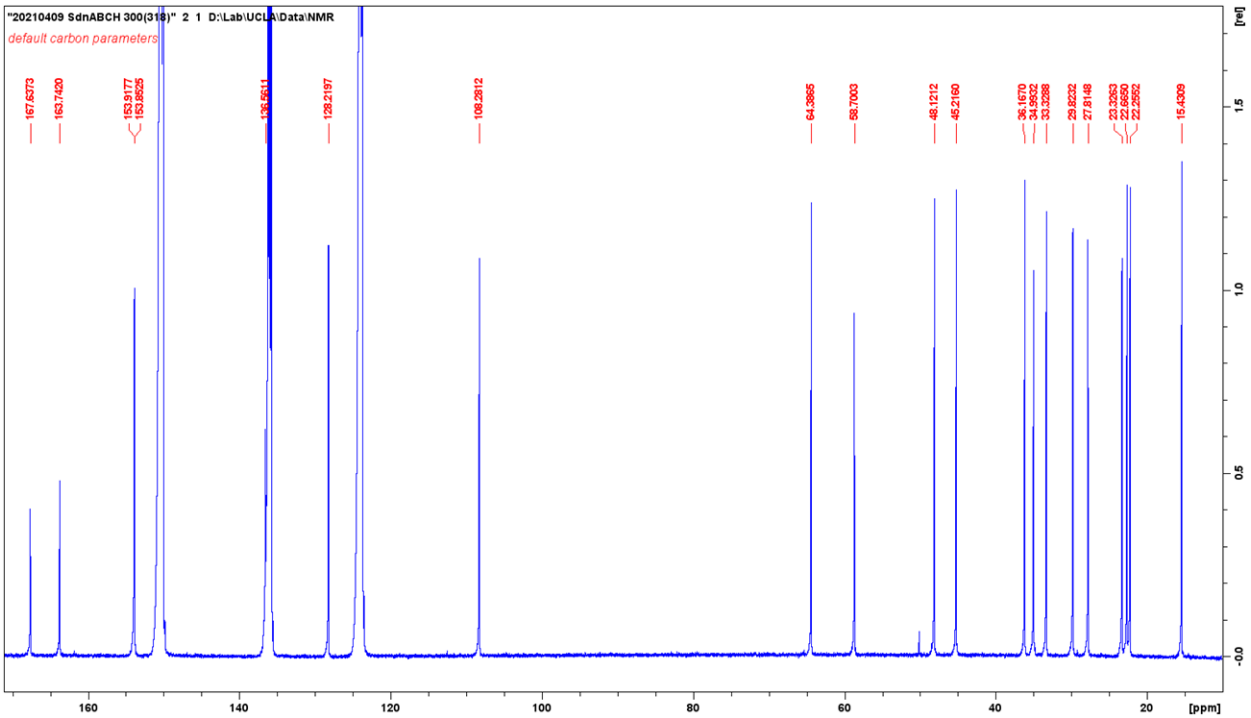

**Supplementary Fig 33.** $^{1}H$-$^{13}C$ HSQC of compound **5** in d5-pyridine, 500 MHz.



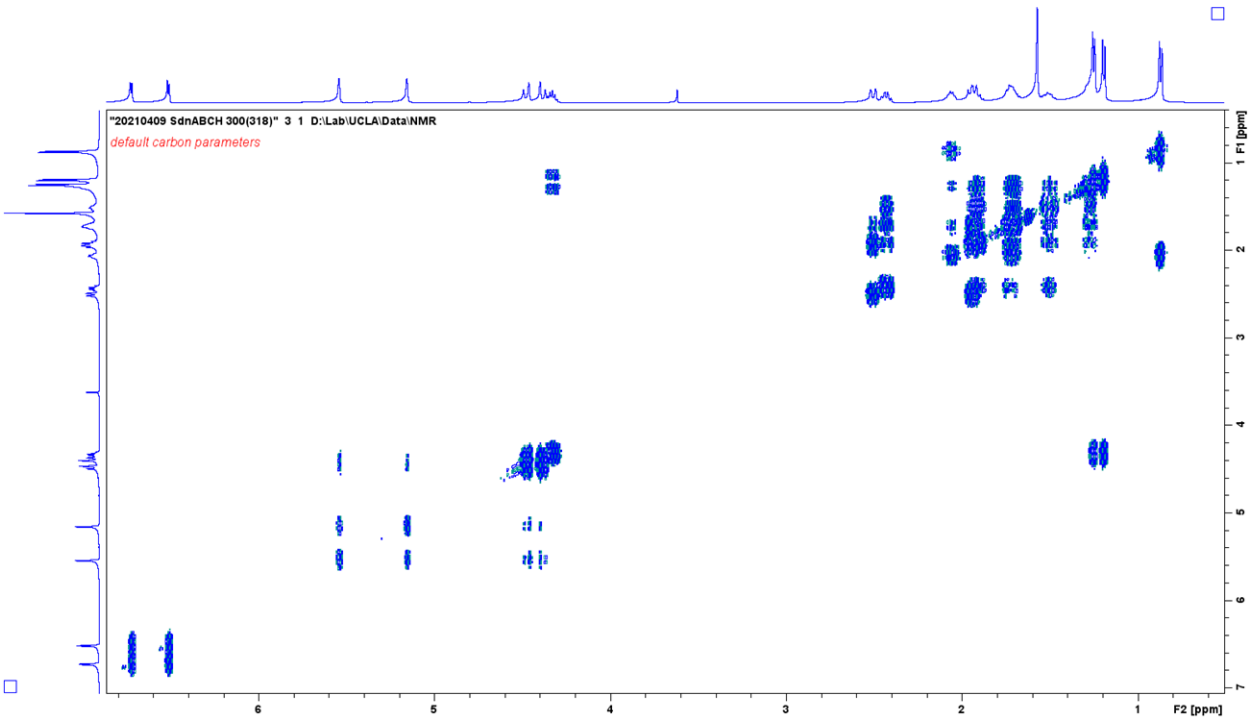

**Supplementary Fig 34.** $^{1}H$-$^{13}C$ HMBC of compound **5** in d5-pyridine, 500 MHz.




**Supplementary Fig 35.** $^{1}$H NMR of compound **6** in d5-pyridine, 500 MHz.



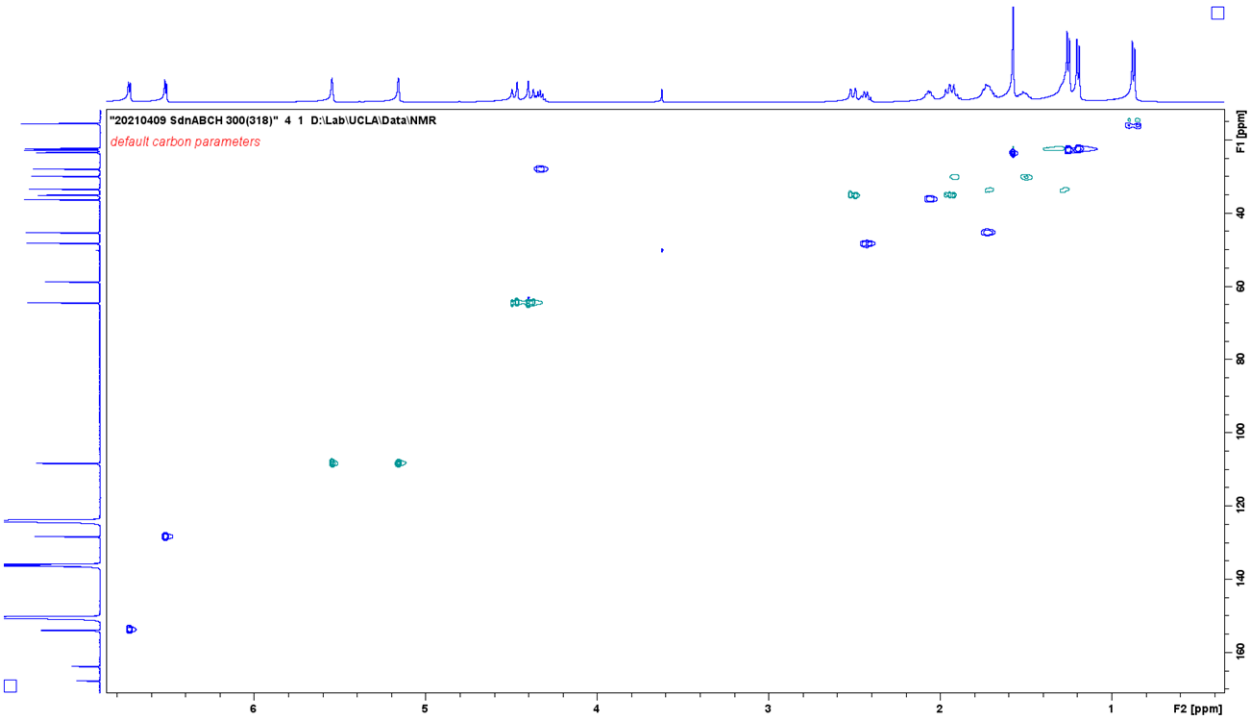

**Supplementary Fig 36.** $^{13}$C NMR of compound **6** in d5-pyridine, 500 MHz.




662

663 **Supplementary Fig 37.** $^{1}$H-$^{1}$H COSY of compound **6** in d5-pyridine, 500 MHz.

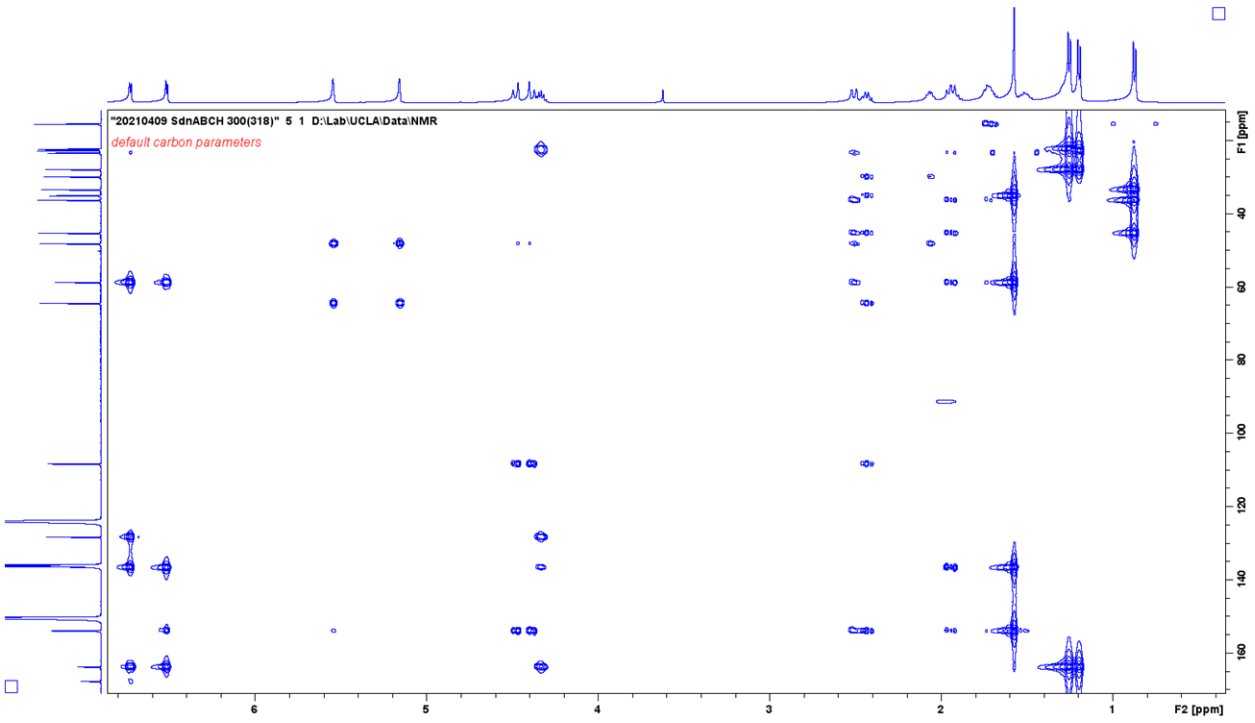
664



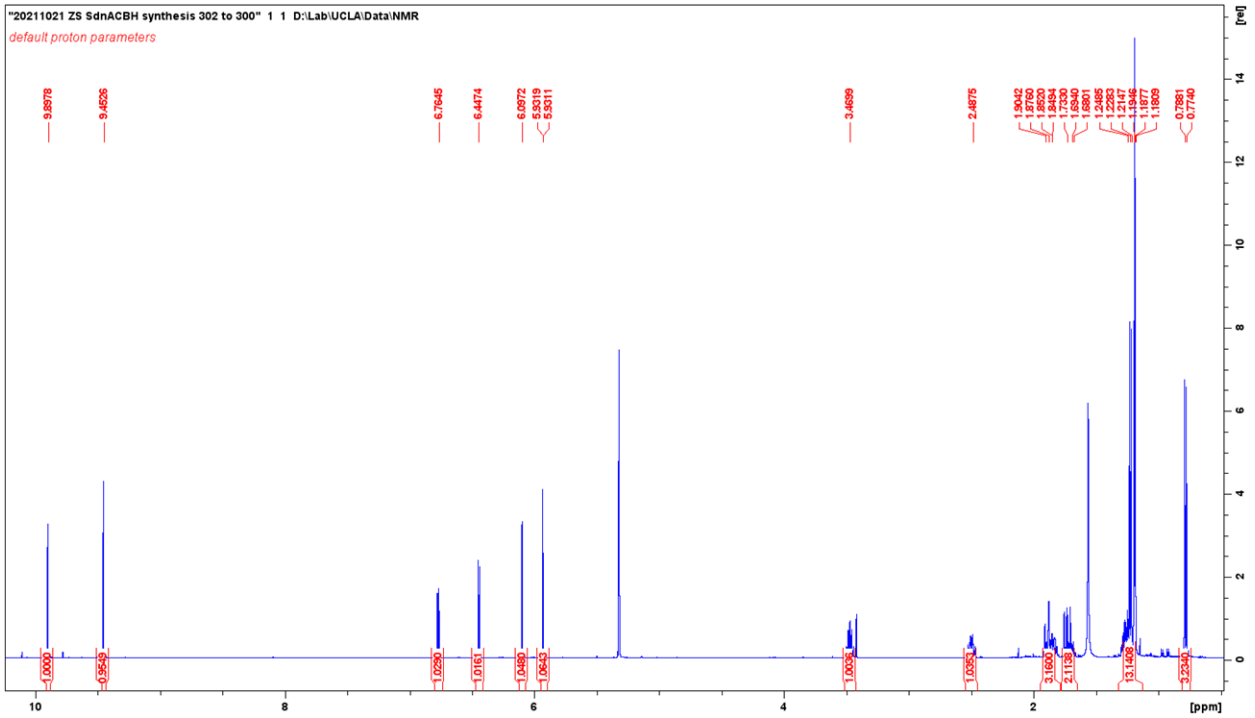

665

666 **Supplementary Fig 38.** $^{1}$H-$^{13}$C HSQC of compound **6** in d5-pyridine, 500 MHz.

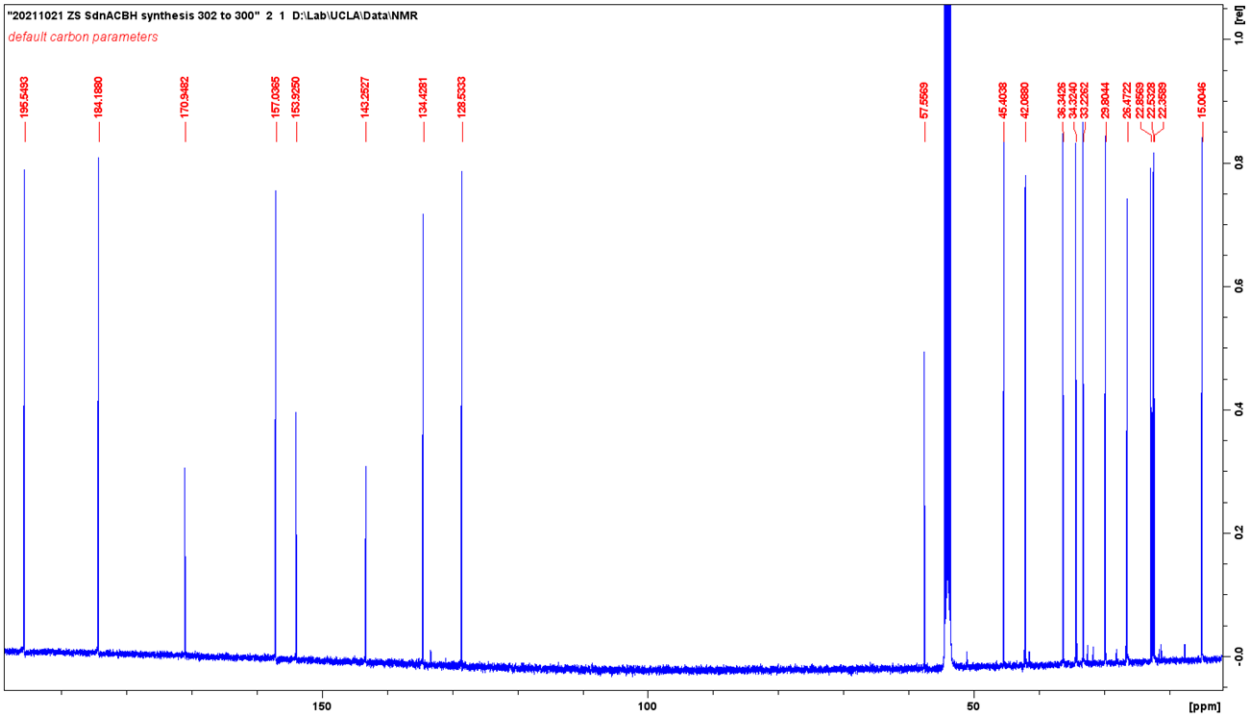
667



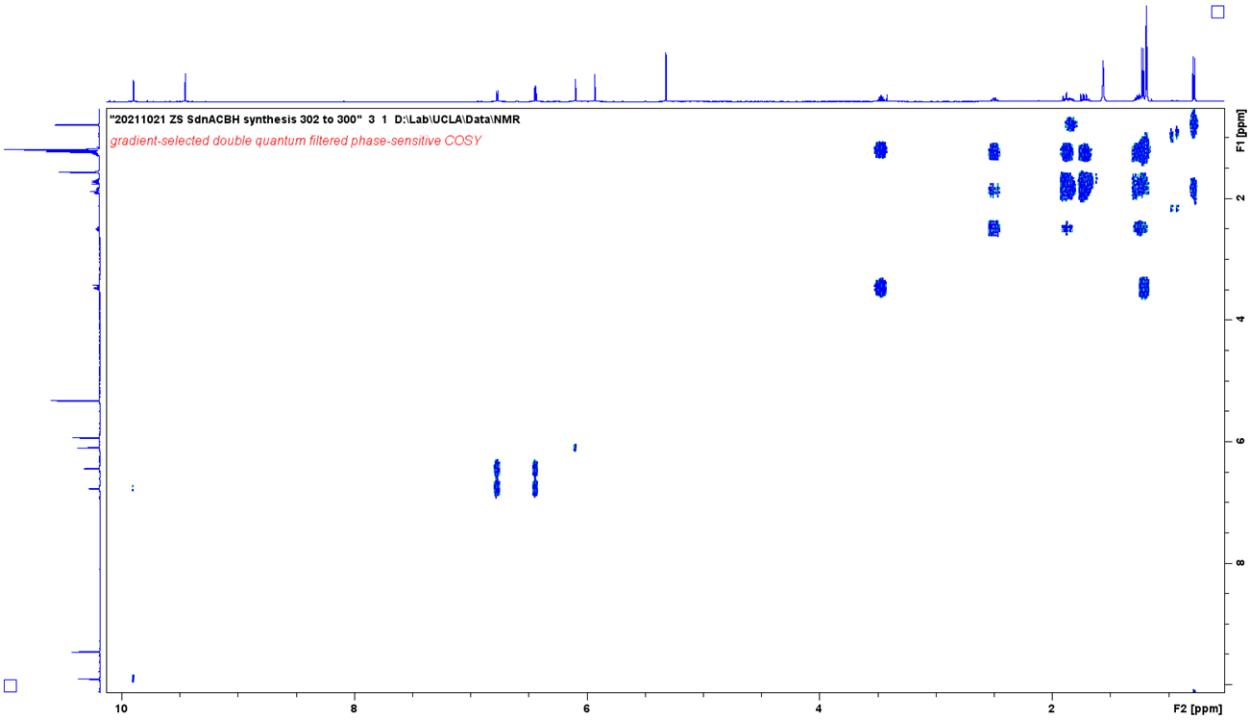

**Supplementary Fig 39.** $^{1}H$-$^{13}C$ HMBC of compound **6** in d5-pyridine, 500 MHz.



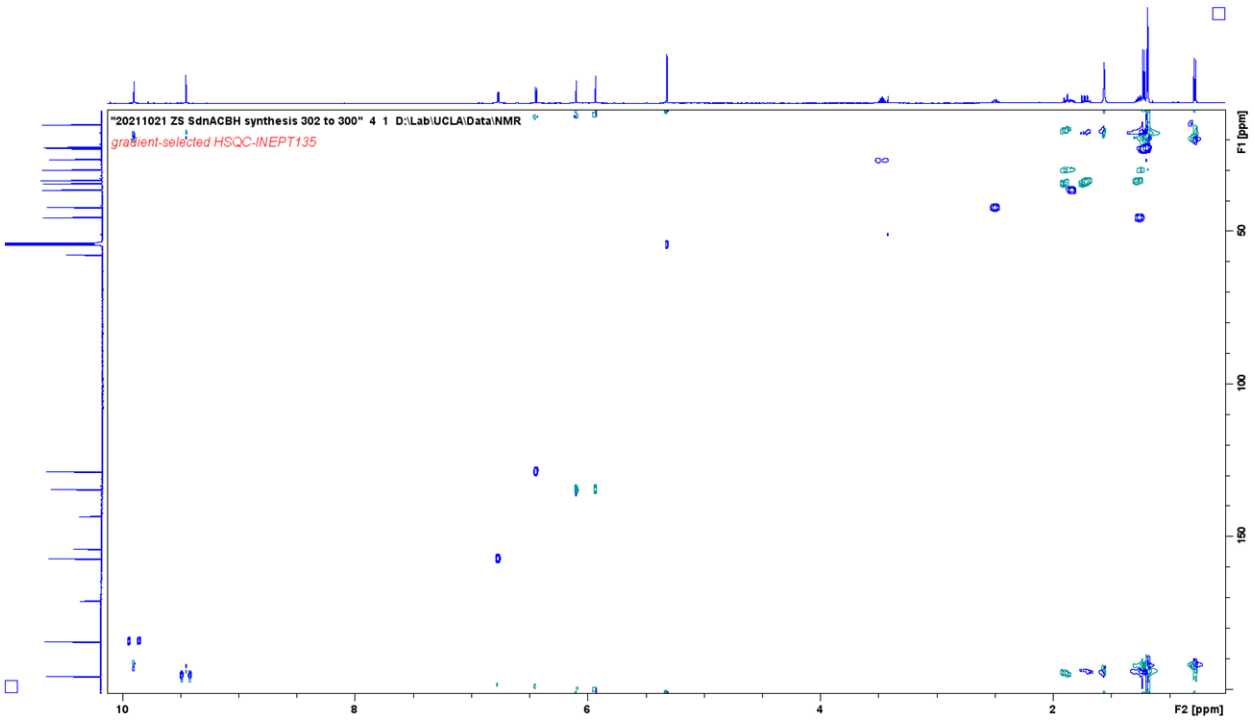

**Supplementary Fig 40.** $^{1}H$ NMR of compound **7** in $CD_2Cl_2$, 500 MHz.




**Supplementary Fig 41.** $^{13}C$ NMR of compound **7** in $CD_2Cl_2$, 500 MHz.

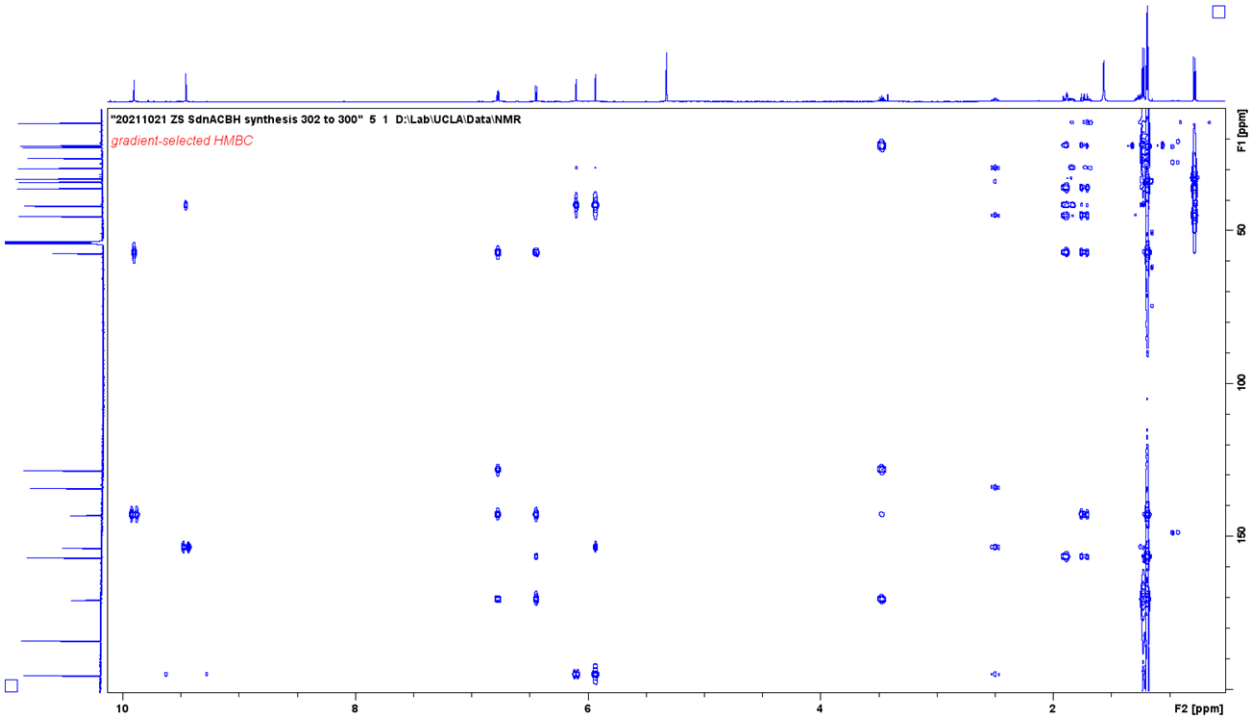



**Supplementary Fig 42.** $^{1}H$-$^{1}H$ COSY of compound **7** in $CD_2Cl_2$, 500 MHz.




680

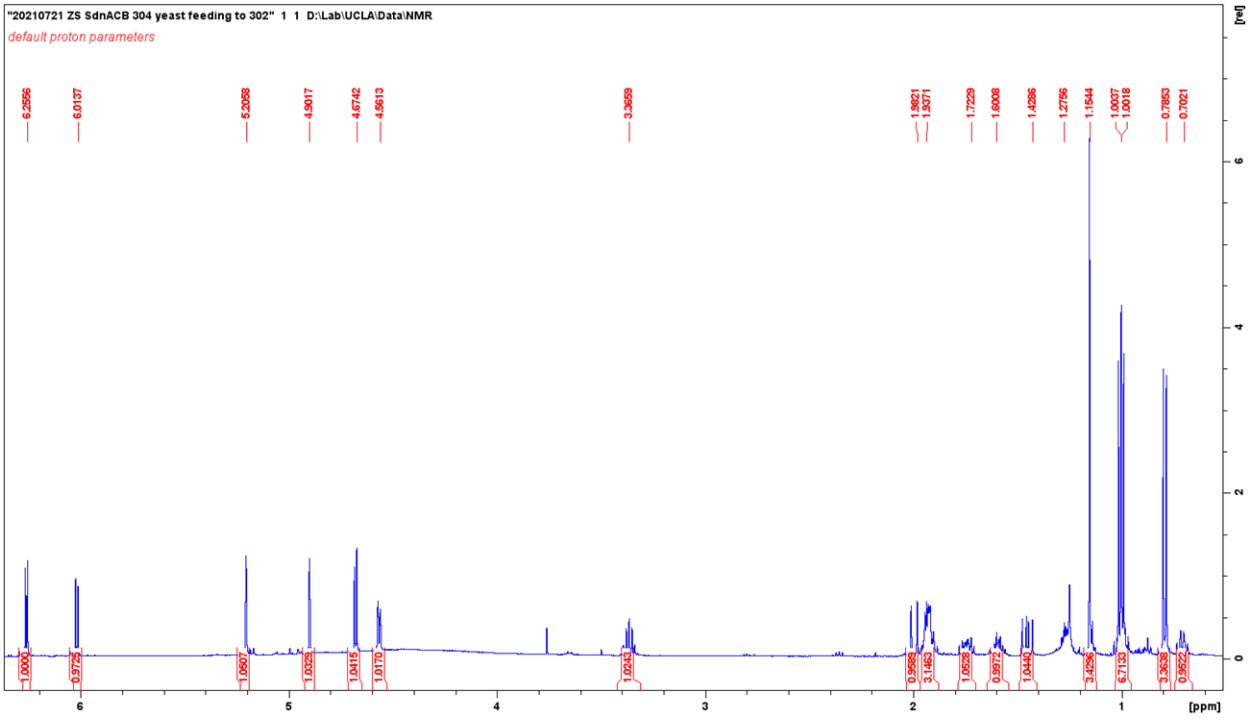
681 **Supplementary Fig 43.** $^{1}H$-$^{13}C$ HSQC of compound **7** in $CD_2Cl_2$, 500 MHz.




682

683 **Supplementary Fig 44.** $^{1}H$-$^{13}C$ HMQC of compound **7** in $CD_2Cl_2$, 500 MHz.

684

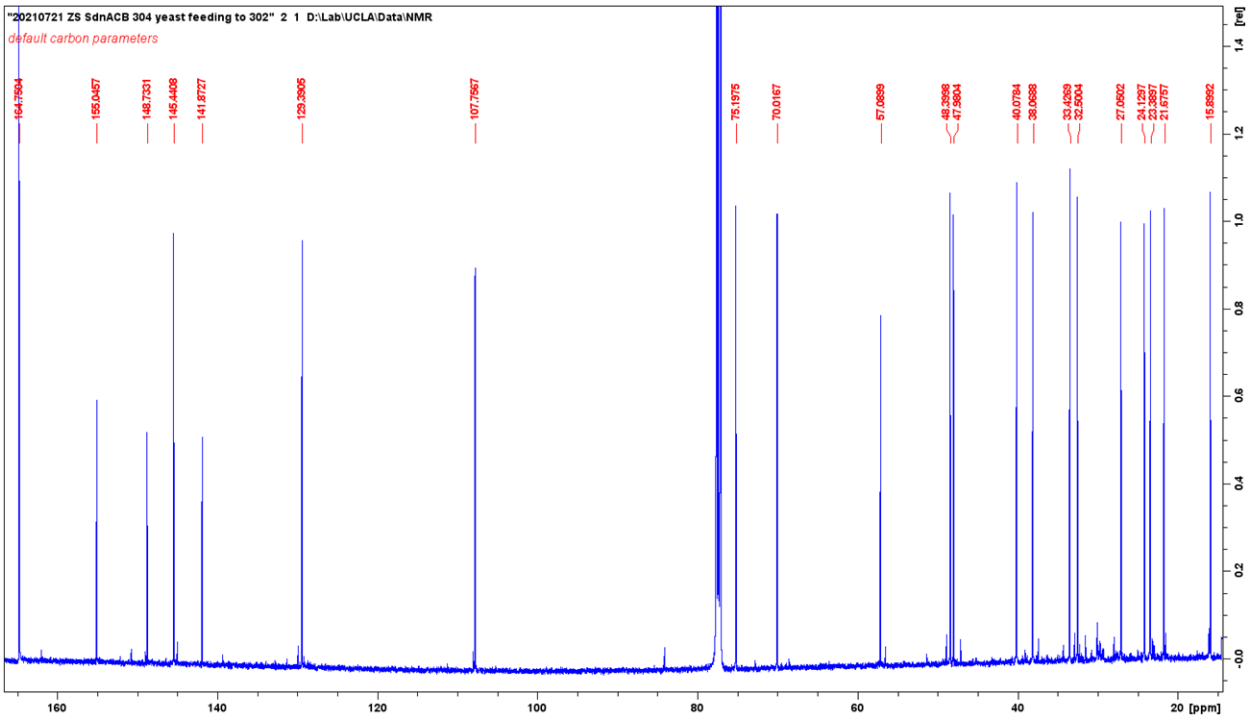



685

686 **Supplementary Fig 45.** $^{1}$H NMR of compound **8** in $CDCl_3$, 500 MHz.

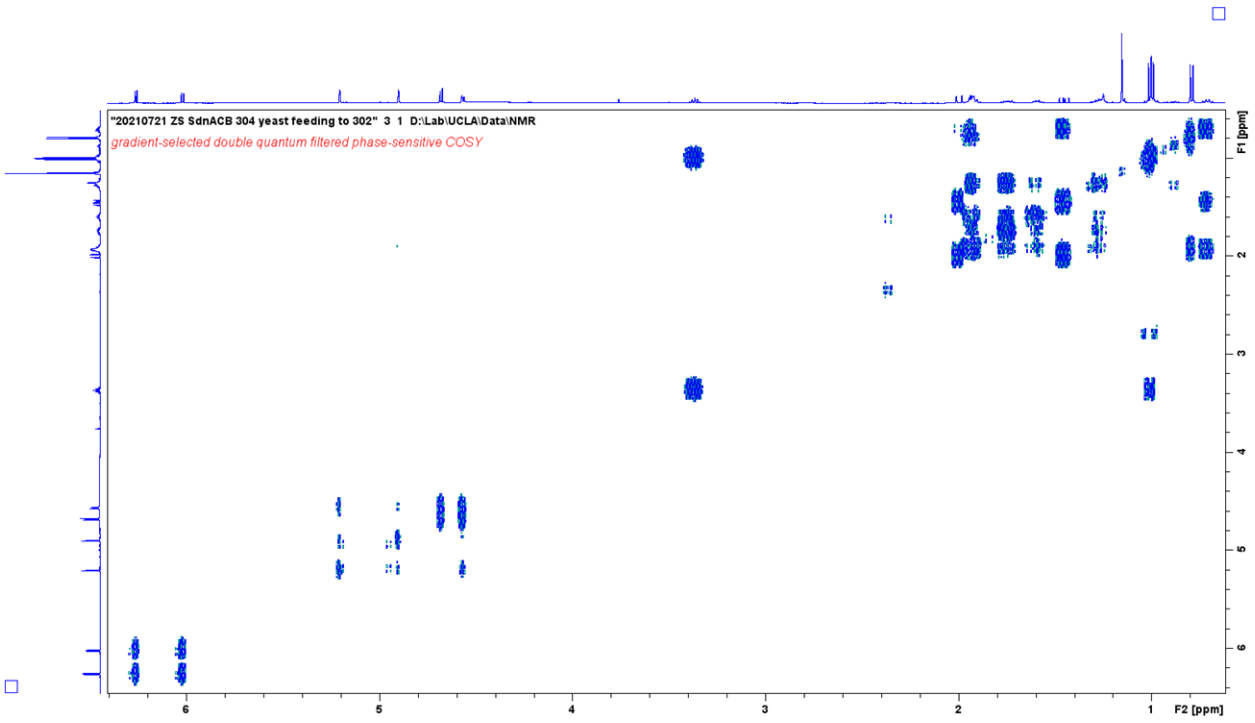
687

688




689

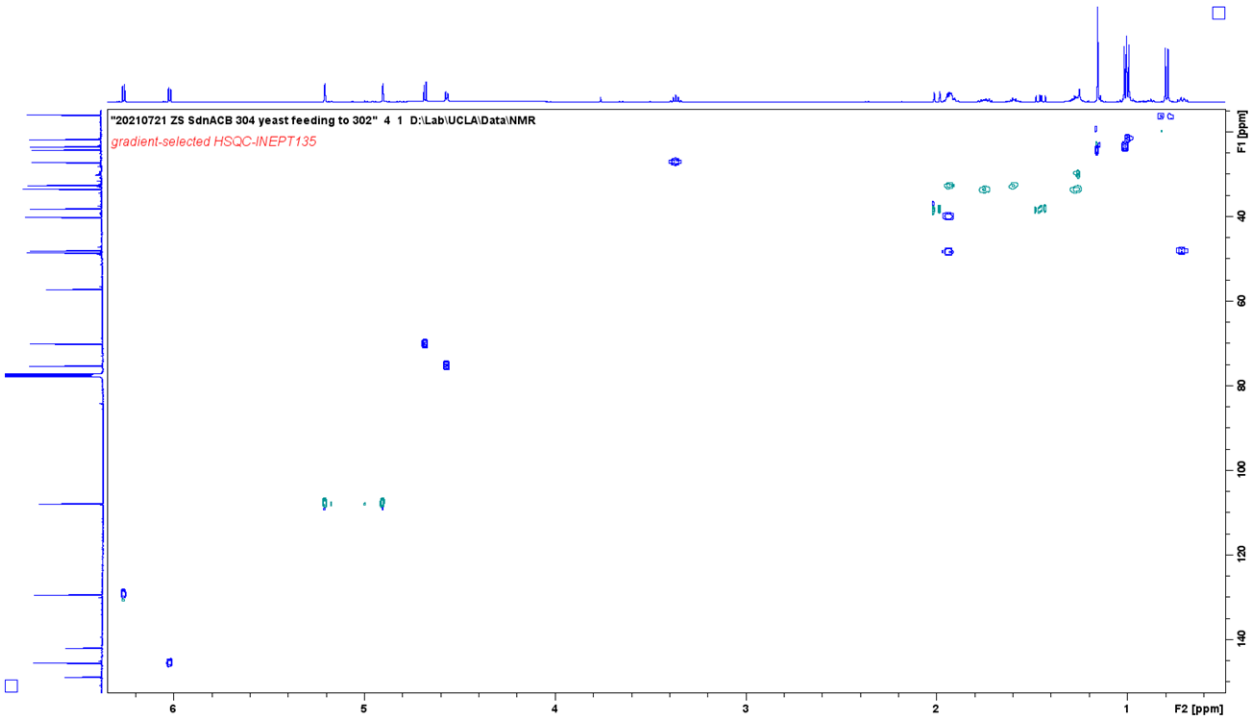
690 **Supplementary Fig 46.** $^{13}$C NMR of compound **8** in $CDCl_3$, 500 MHz.




691

692 **Supplementary Fig 47.** $^{1}$H-$^{1}$H COSY of compound **8** in $CDCl_3$, 500 MHz.

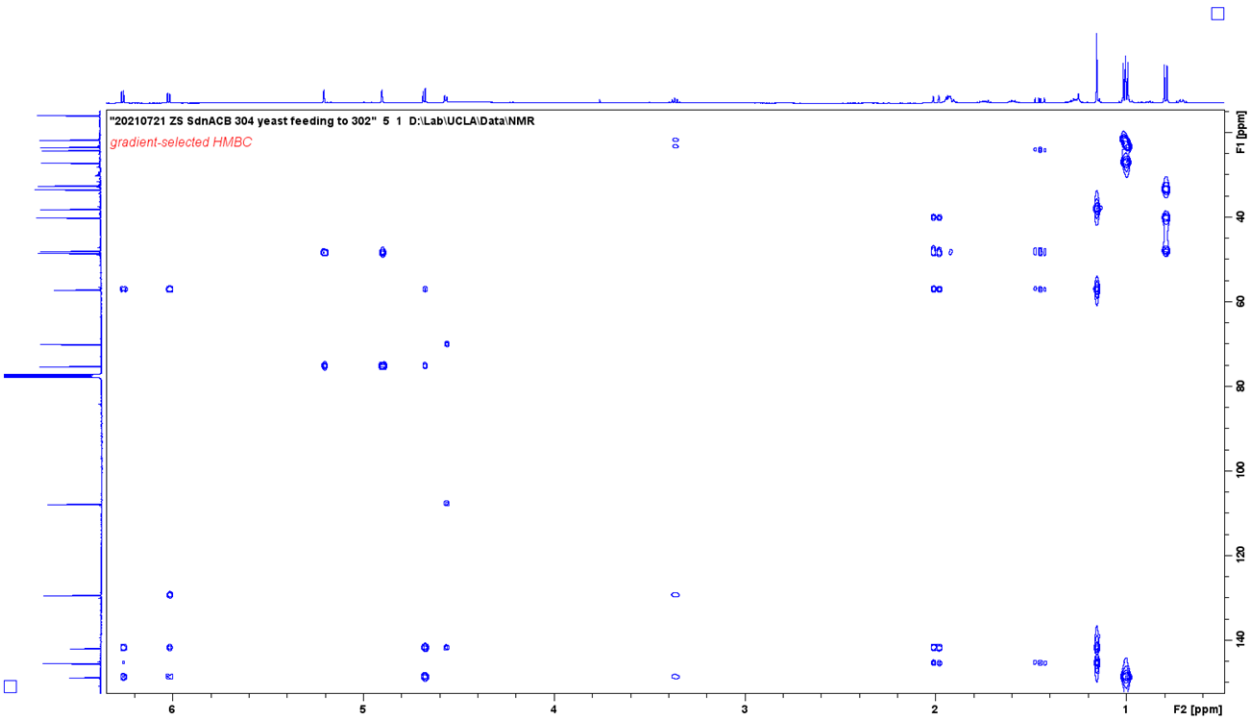
693



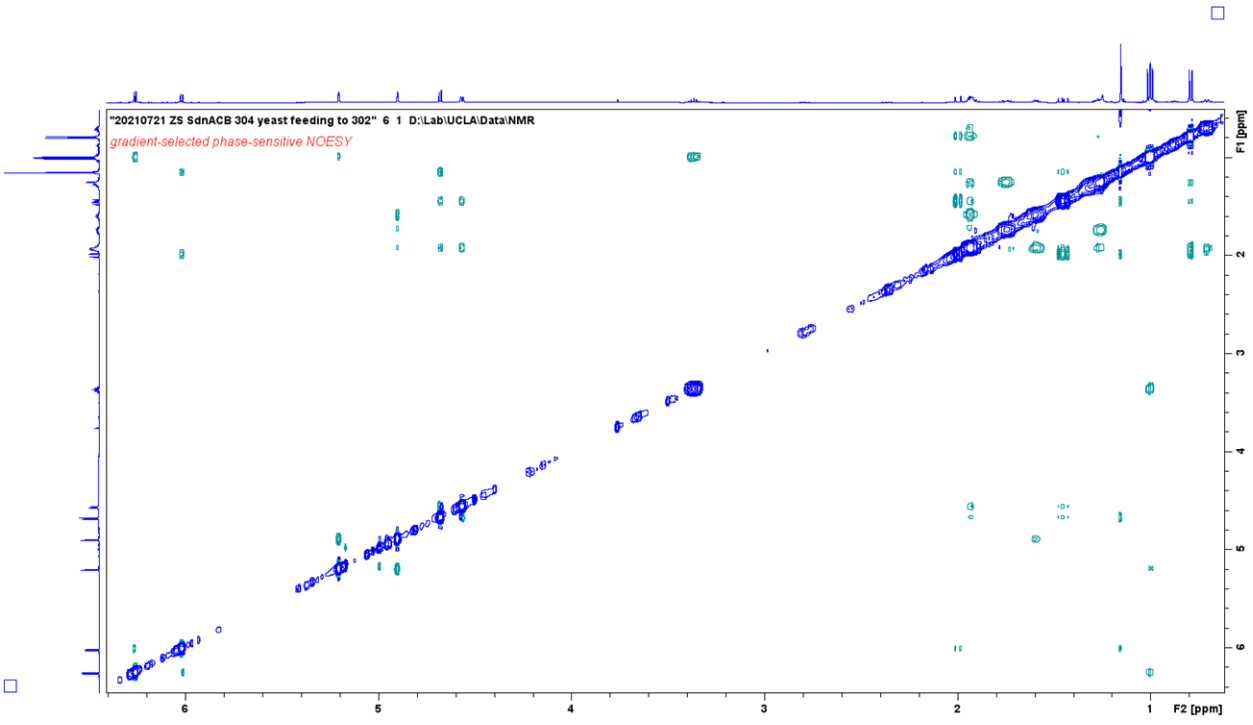

694

695 **Supplementary Fig 48.** $^{1}$H-$^{13}$C HSQC of compound **8** in $CDCl_3$, 500 MHz.

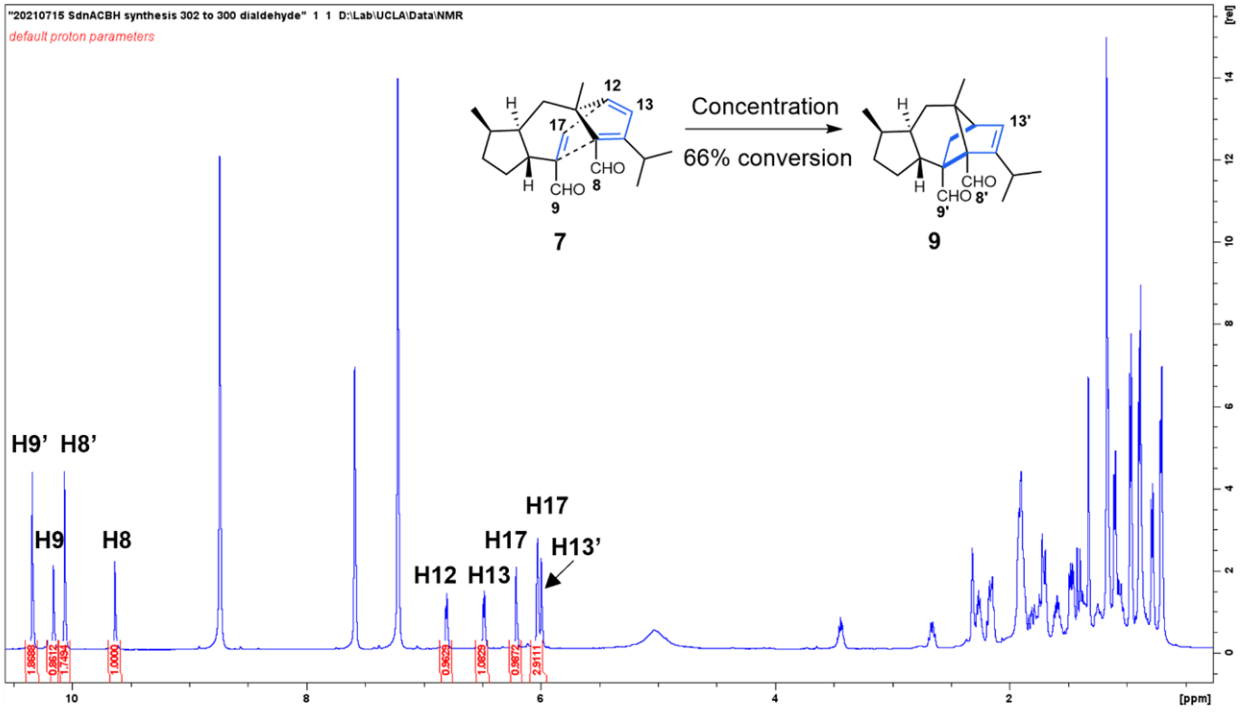
696




**Supplementary Fig 49.** $^{1}H$-$^{13}C$ HMBC of compound **8** in $CDCl_3$, 500 MHz.



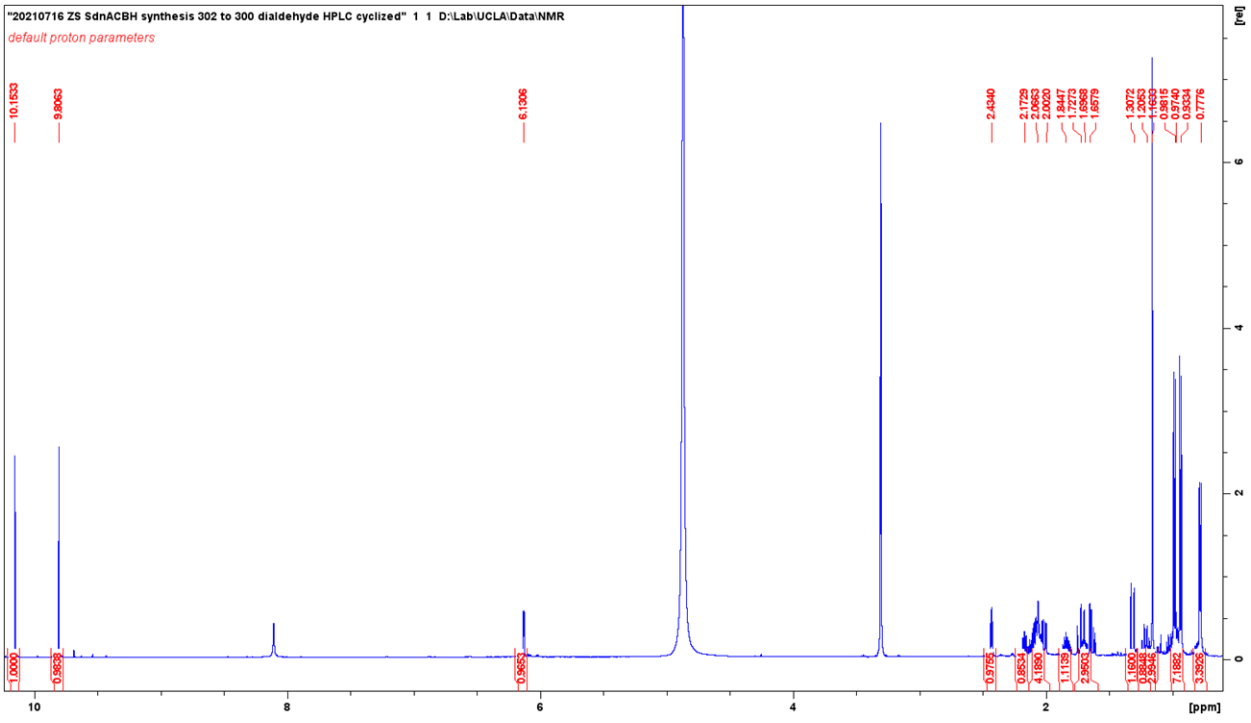

**Supplementary Fig 50.** $^{1}H$-$^{1}H$ NOESY of compound **8** in $CDCl_3$, 500 MHz.




703

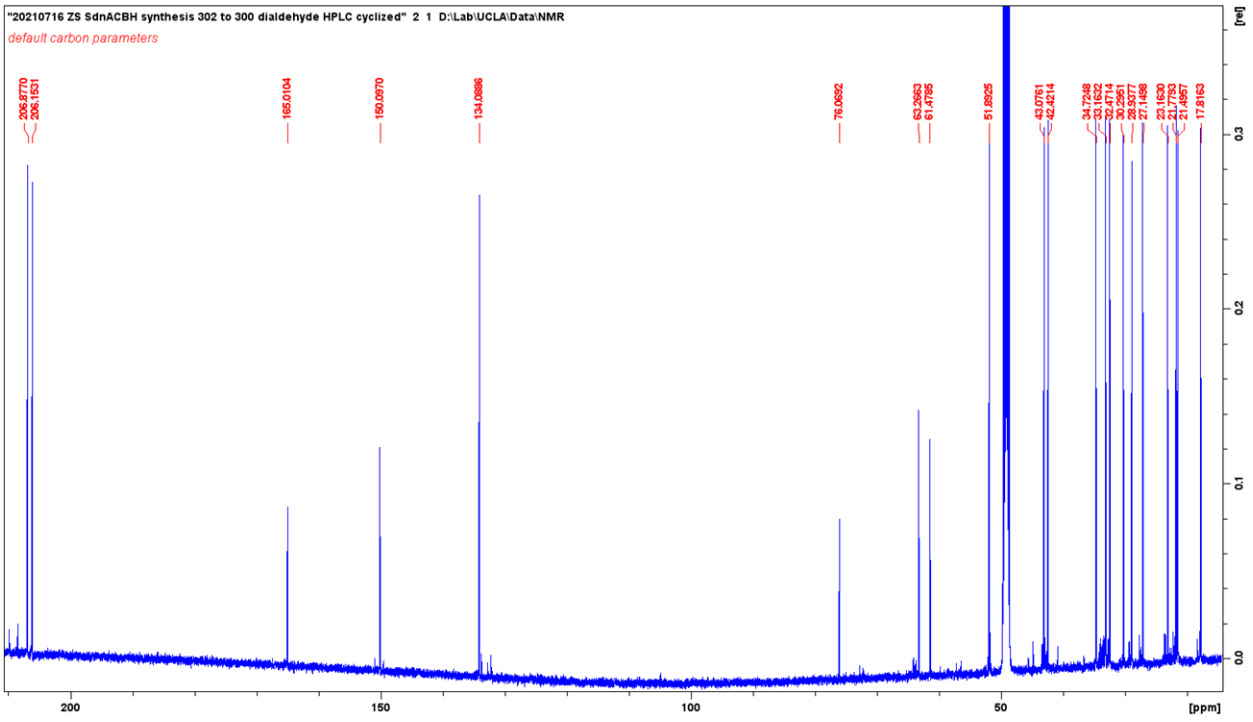
704 **Supplementary Fig 51.** $^{1}$H NMR of mixed compounds **7** and **9** in d5-pyridine, 500 MHz. The spectrum
705 was taken after a pure sample of compound **7** in $CDCl_2$ was dried in vacuum and redissolved in d5-
706 pyridine. Concentration promoted cyclization of **7** to form a 1 to 2 mixture of **7** and **9**.

707

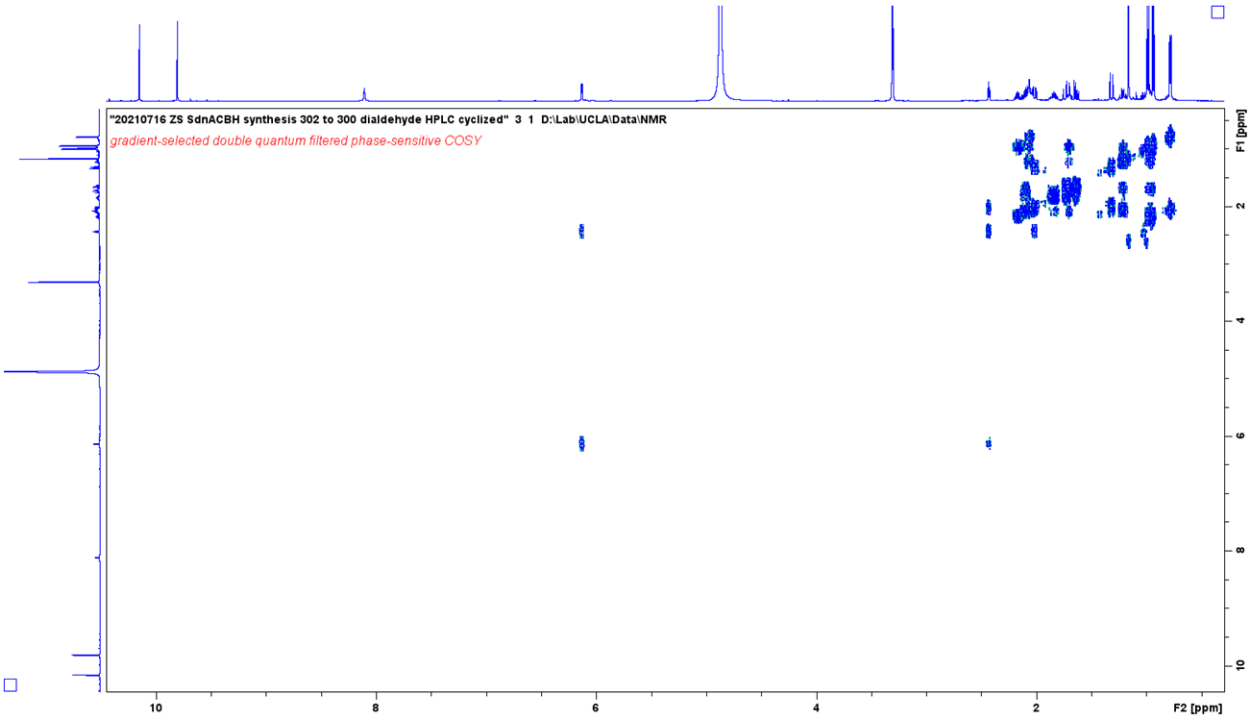



708

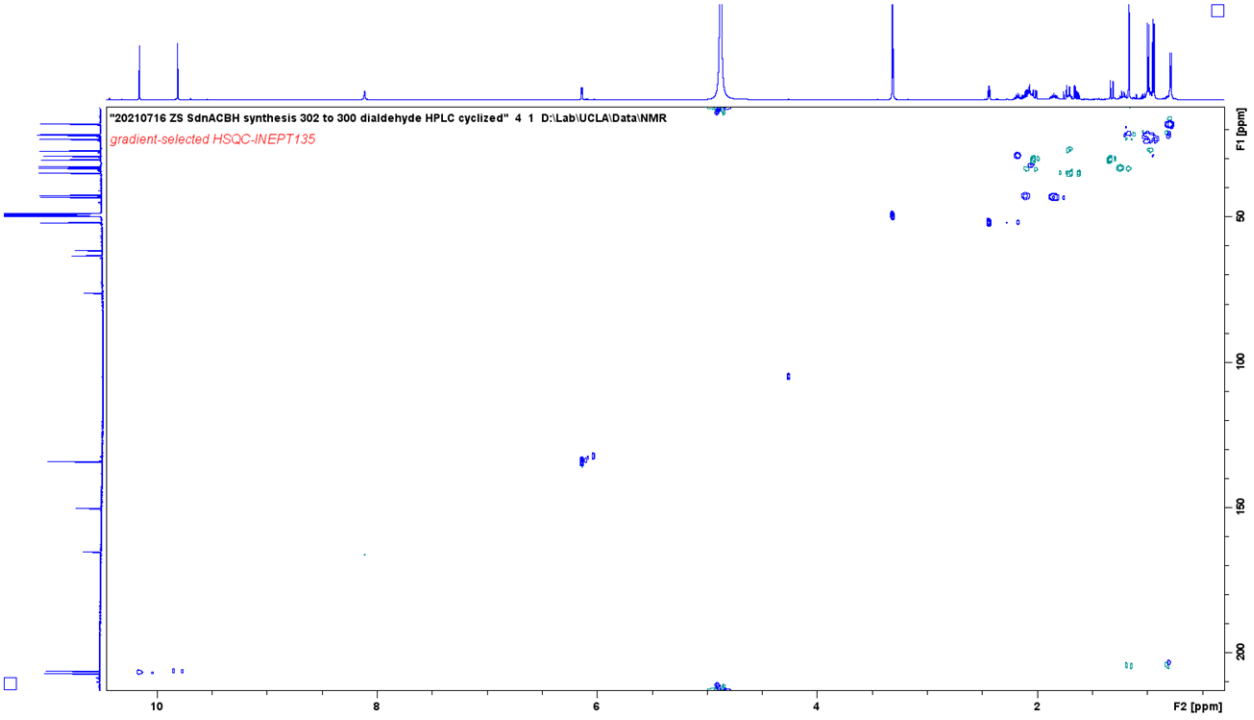
709 **Supplementary Fig 52.** $^{1}$H NMR of compound **9** in $CDCD_3$, 500 MHz.



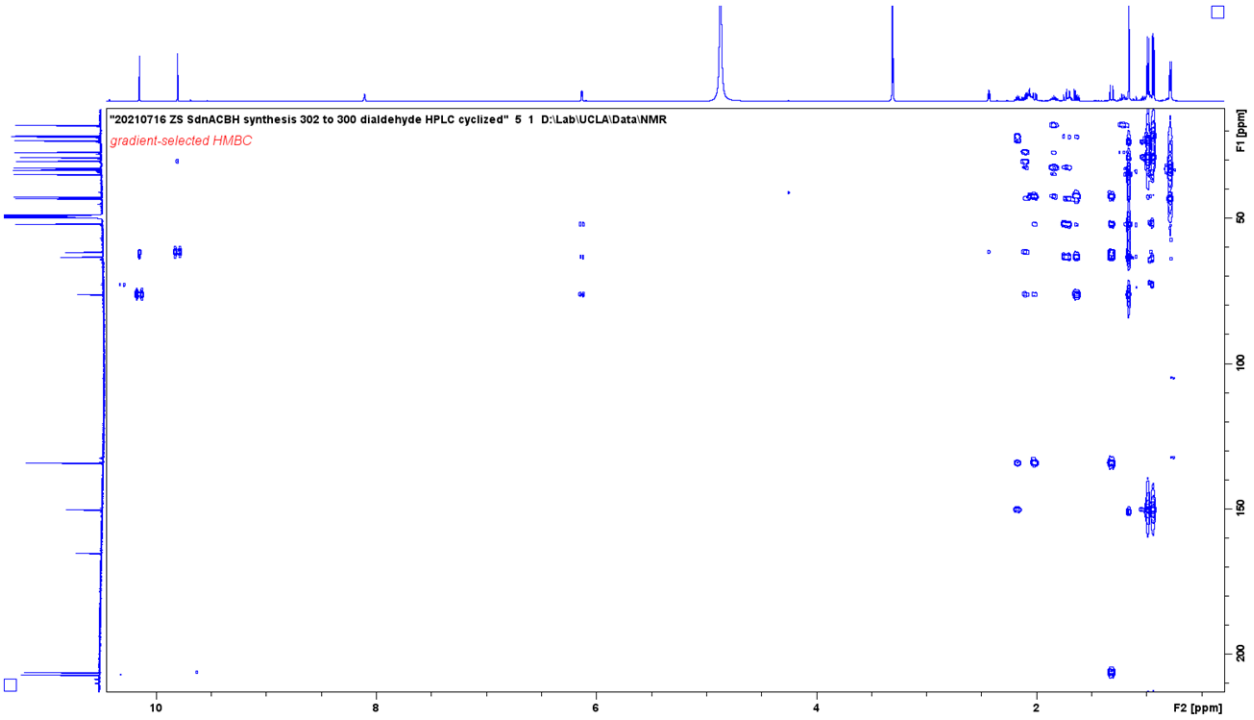

**Supplementary Fig 53.** $^{13}C$ NMR of compound **9** in $CDCD_3$, 500 MHz.



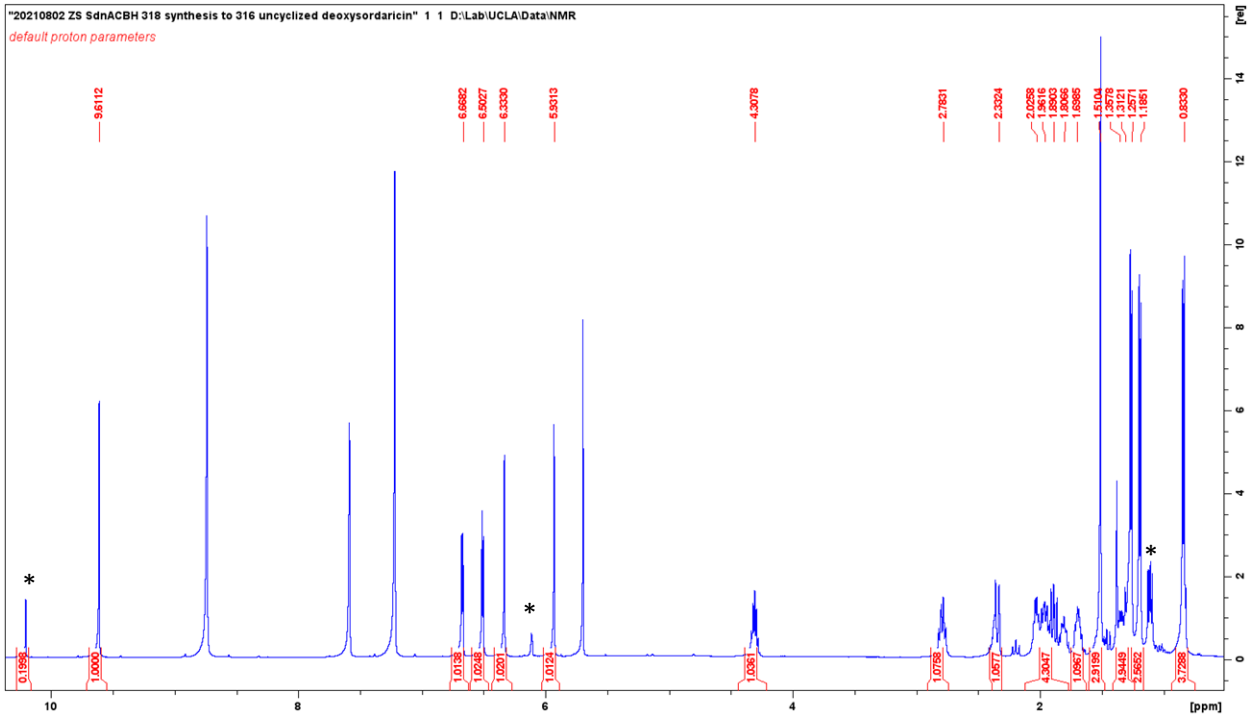

**Supplementary Fig 54.** $^{1}H$-$^{1}H$ COSY of compound **9** in $CDCD_3$, 500 MHz.



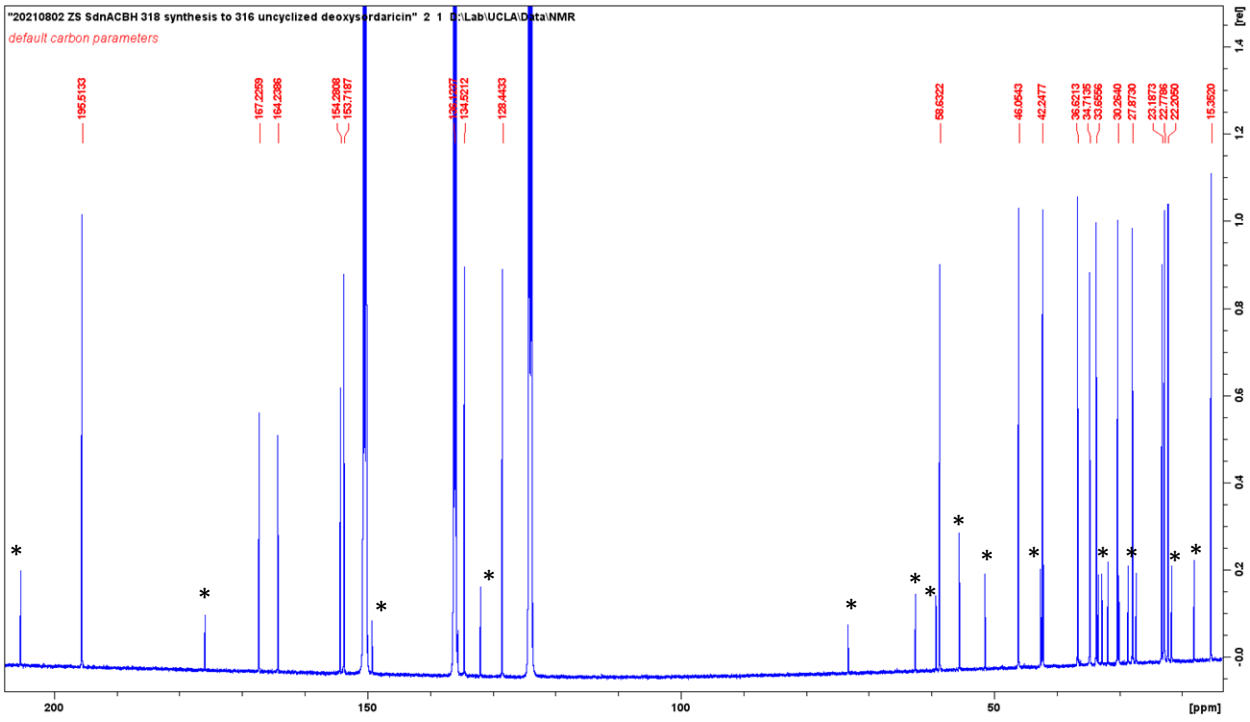

**Supplementary Fig 55.** $^{1}H$-$^{13}C$ HSQC of compound **9** in $CDCD_3$, 500 MHz.



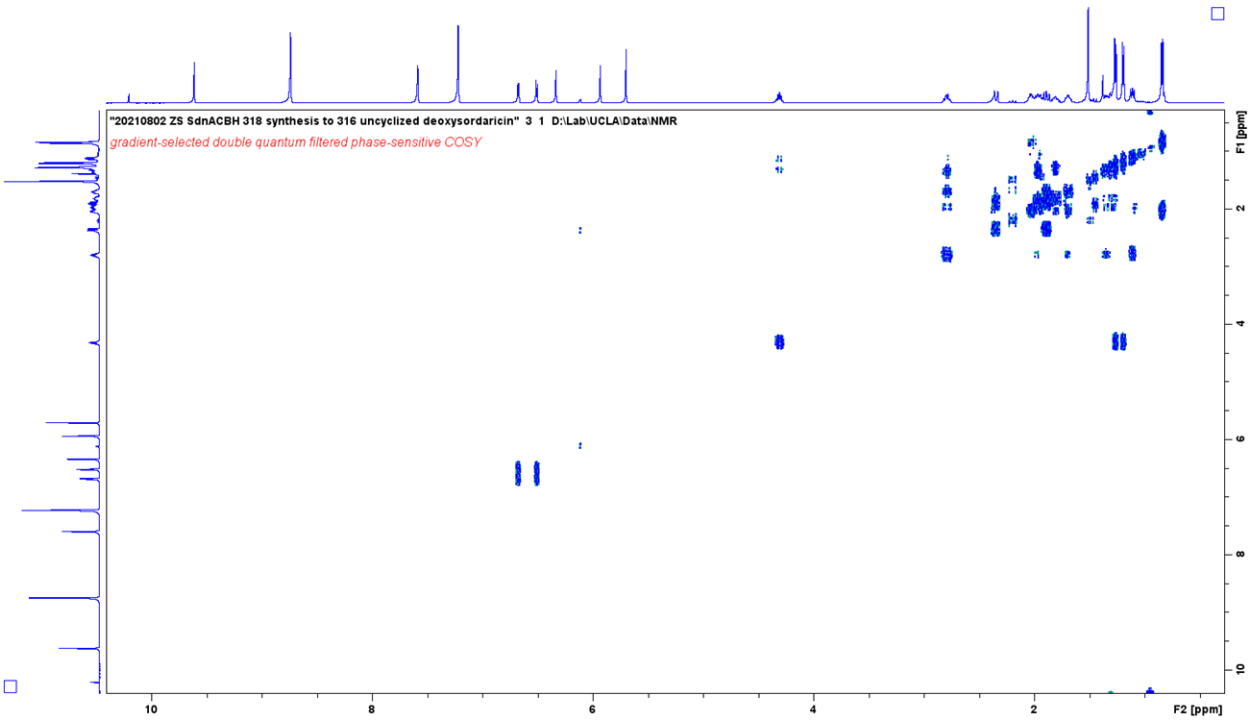

**Supplementary Fig 56.** $^{1}H$-$^{13}C$ HMBC of compound **9** in $CDCD_3$, 500 MHz.




**Supplementary Fig 57.** $^{1}$H NMR of compound **10** in $CD_2Cl_2$, 500 MHz. Signals labeled with * are from compound **11**, which formed via the cyclization of **10** during the synthesis and reaction workup.



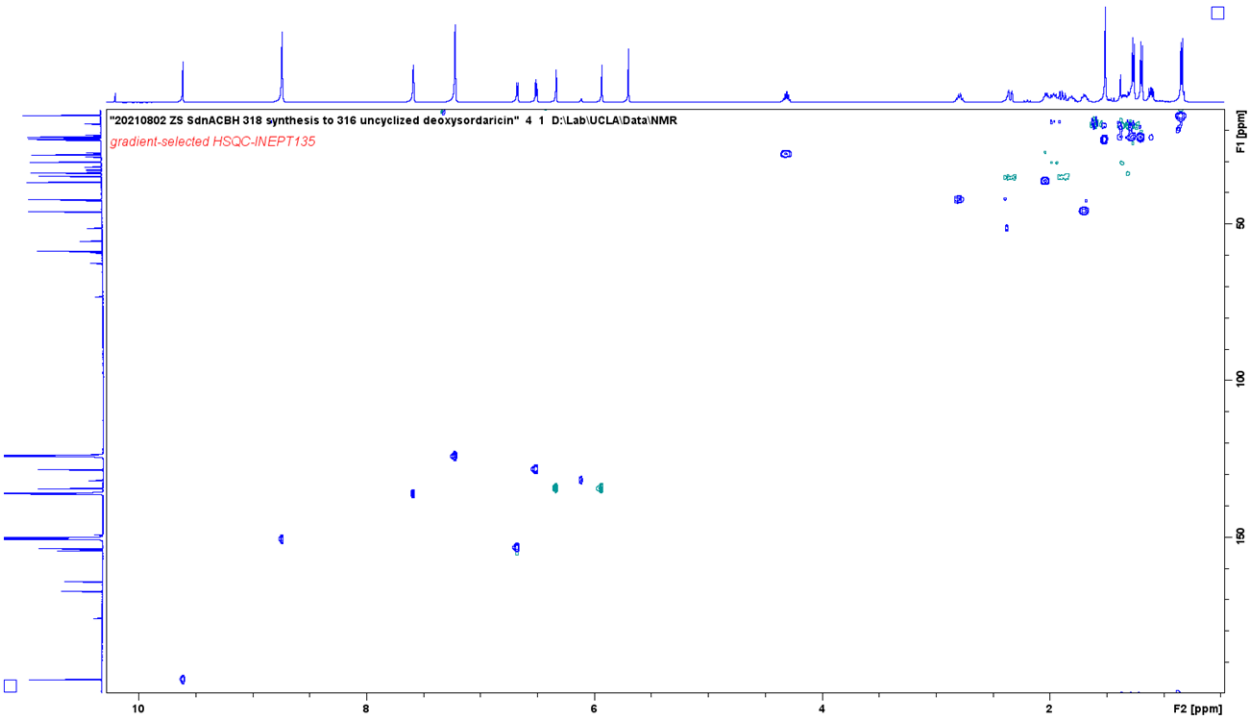

**Supplementary Fig 58.** $^{13}$C NMR of compound **10** in $CD_2Cl_2$, 500 MHz. Signals labeled with * are from compound **11**, which formed via the cyclization of **10** during the synthesis and reaction workup.




729

730 **Supplementary Fig 59.** $^{1}H$-$^{1}H$ COSY of compound **10** in $CD_2Cl_2$, 500 MHz.

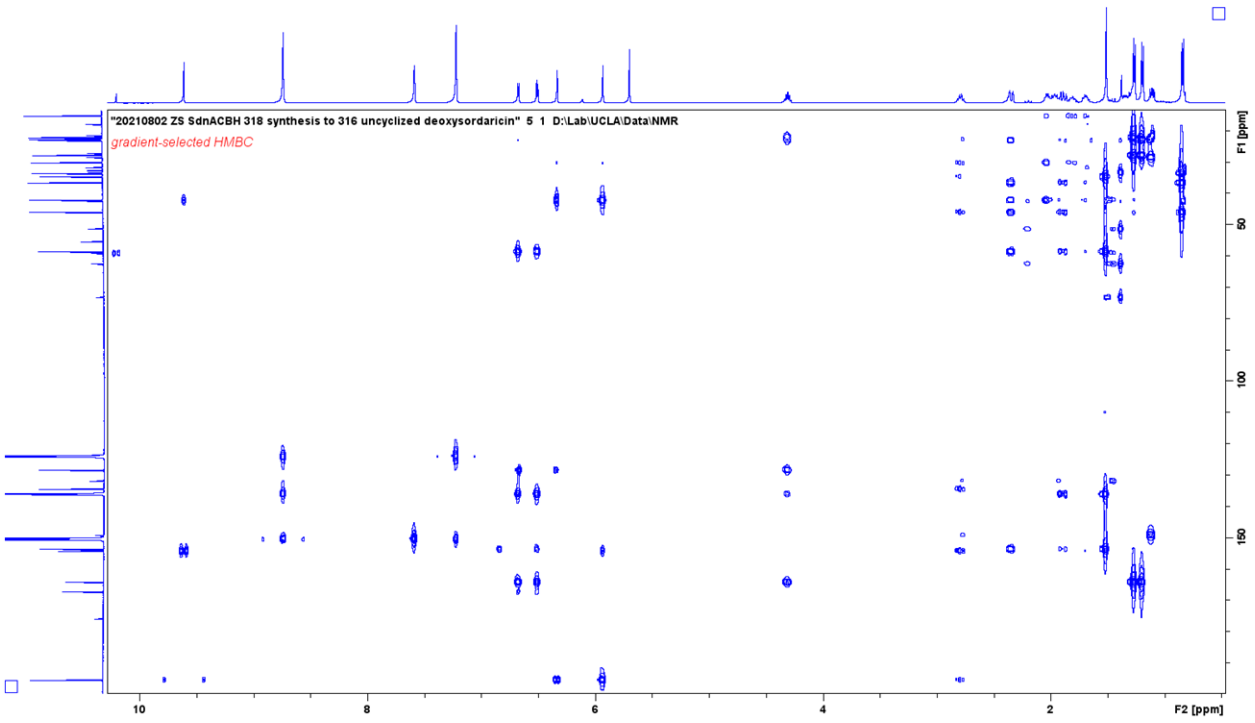
731




732

733 **Supplementary Fig 60.** $^{1}H$-$^{13}C$ HSQC of compound **10** in $CD_2Cl_2$, 500 MHz.

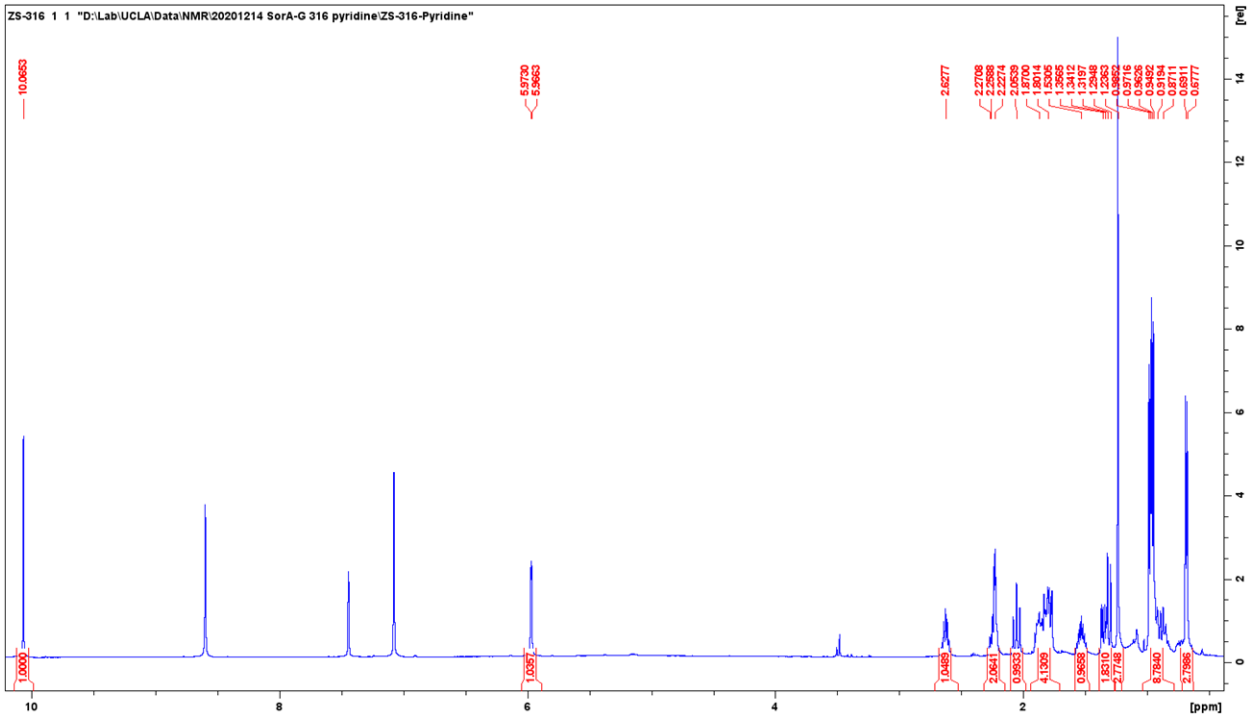
734




735

736 **Supplementary Fig 61.** $^{1}H$-$^{13}C$ HMBC of compound **10** in $CD_2Cl_2$, 500 MHz.

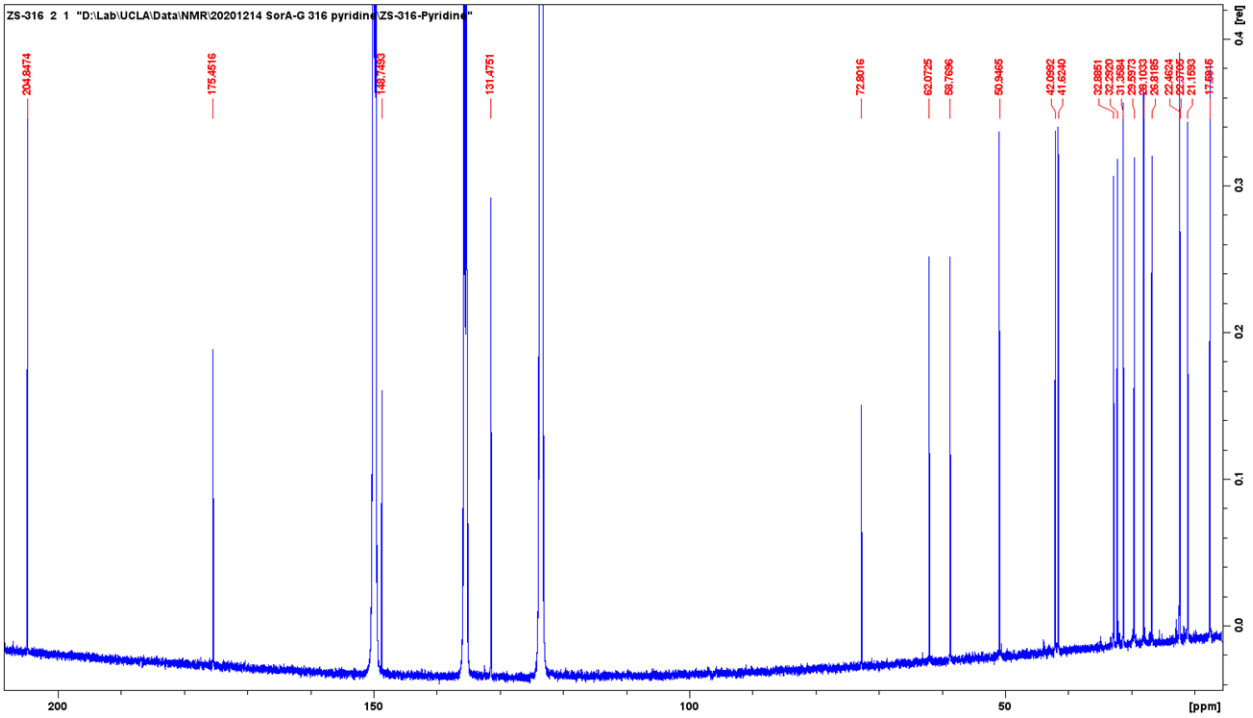
737




738

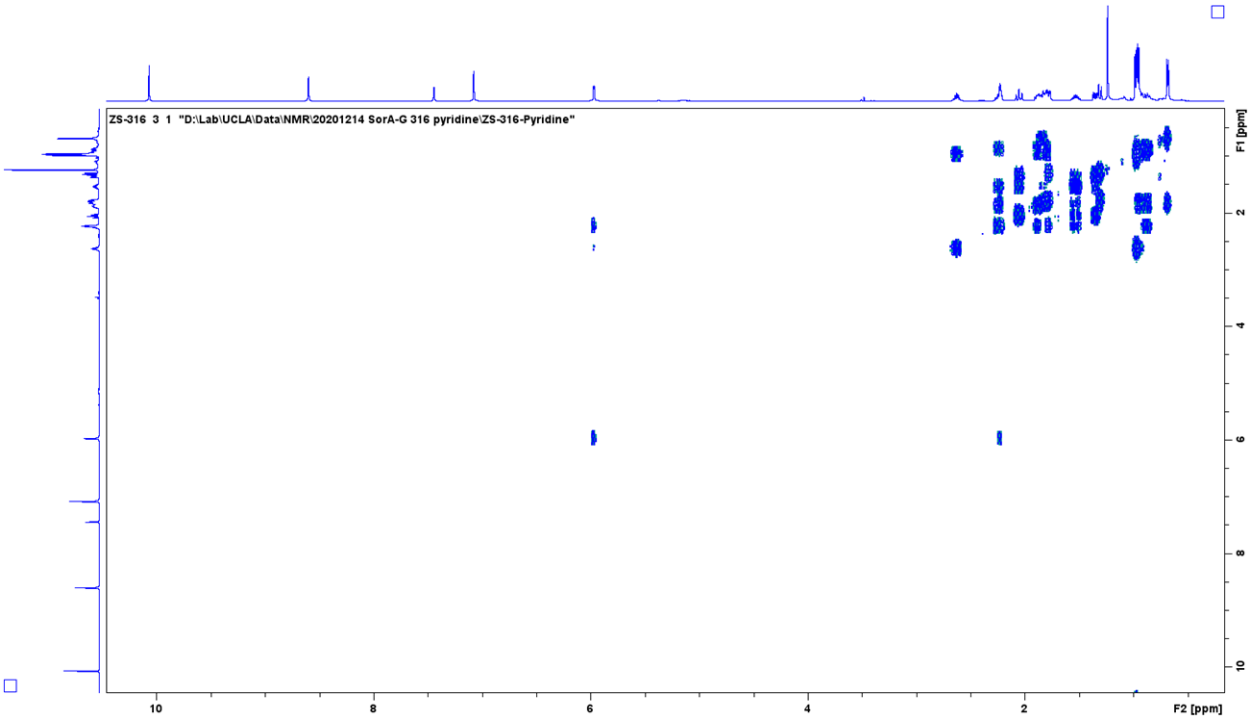
739 **Supplementary Fig 62.** $^{1}H$ NMR of compound **11** in d5-pyridine, 500 MHz.

740




741

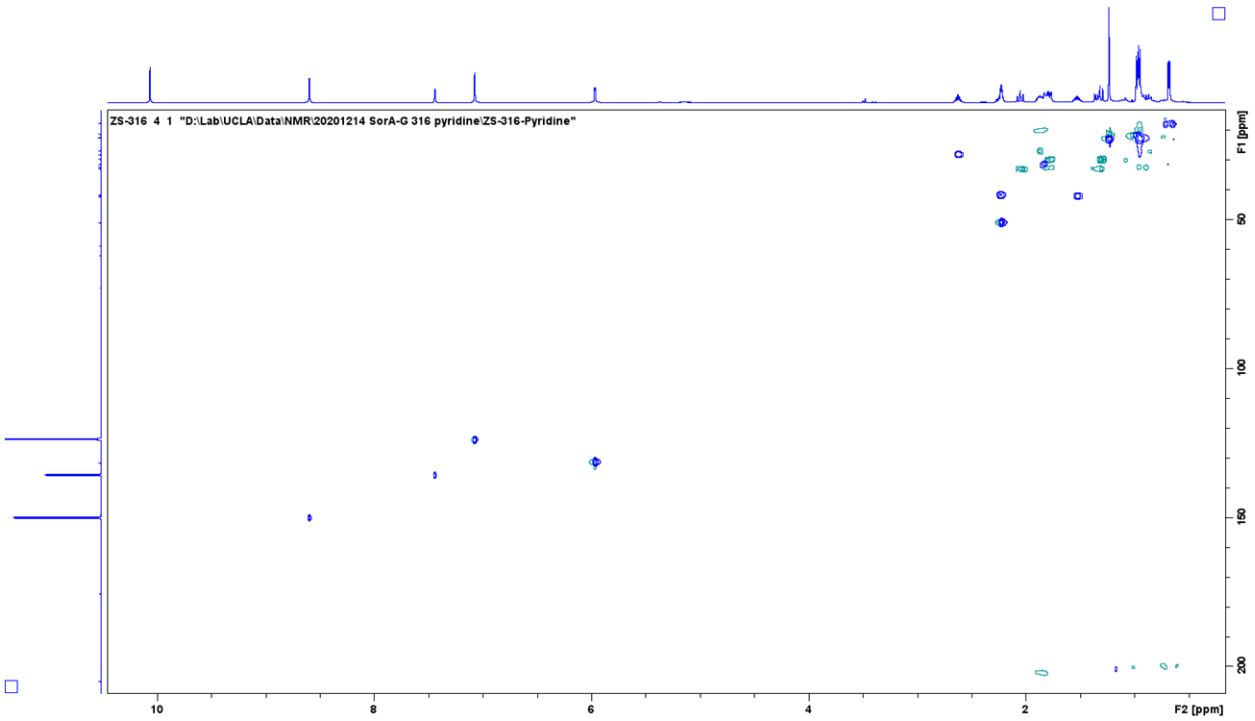
742 **Supplementary Fig 63.** $^{13}C$ NMR of compound **11** in d5-pyridine, 500 MHz.



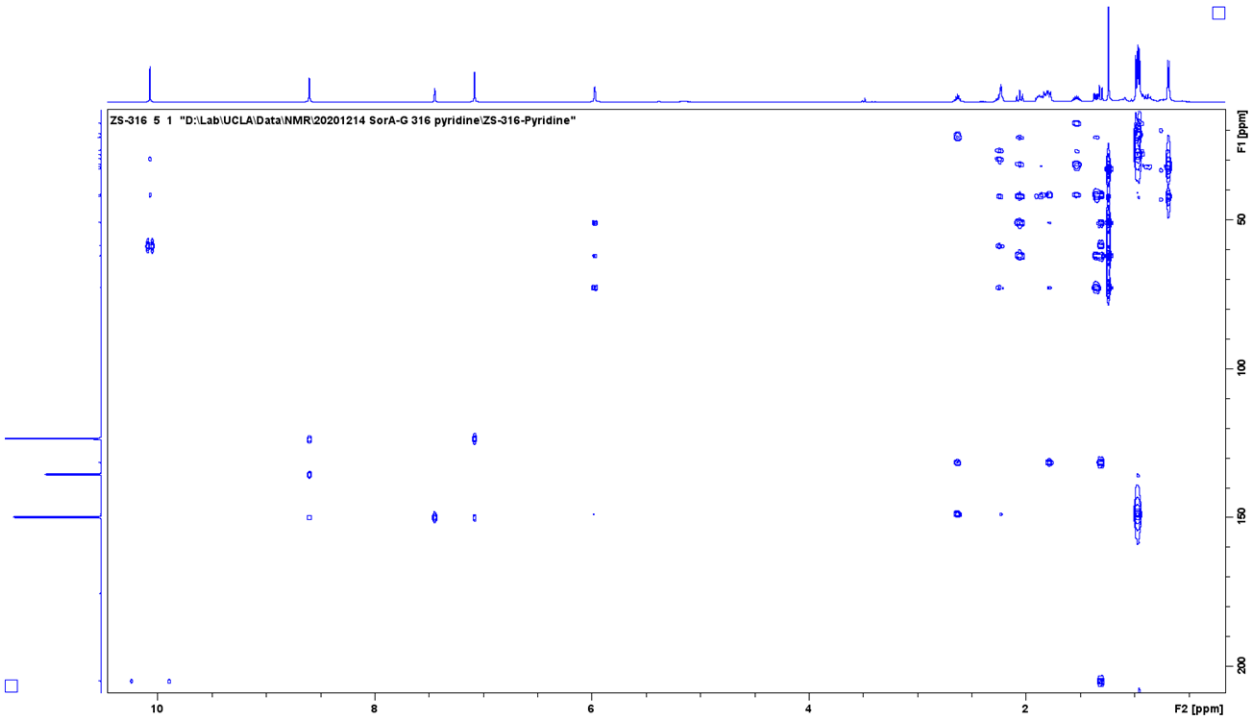

743

744 **Supplementary Fig 64.** $^{1}H$-$^{1}H$ COSY of compound **11** in d5-pyridine, 500 MHz.

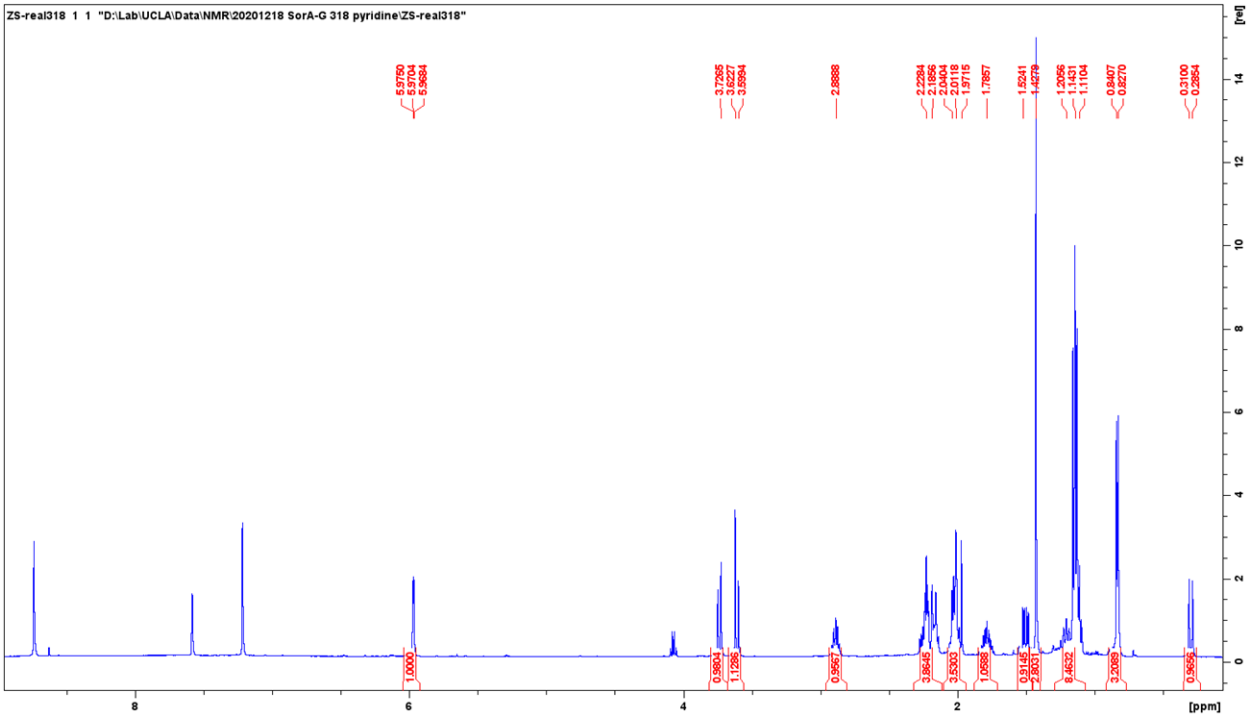
745




**Supplementary Fig 65.** $^{1}H$-$^{13}C$ HSQC of compound **11** in d5-pyridine, 500 MHz.



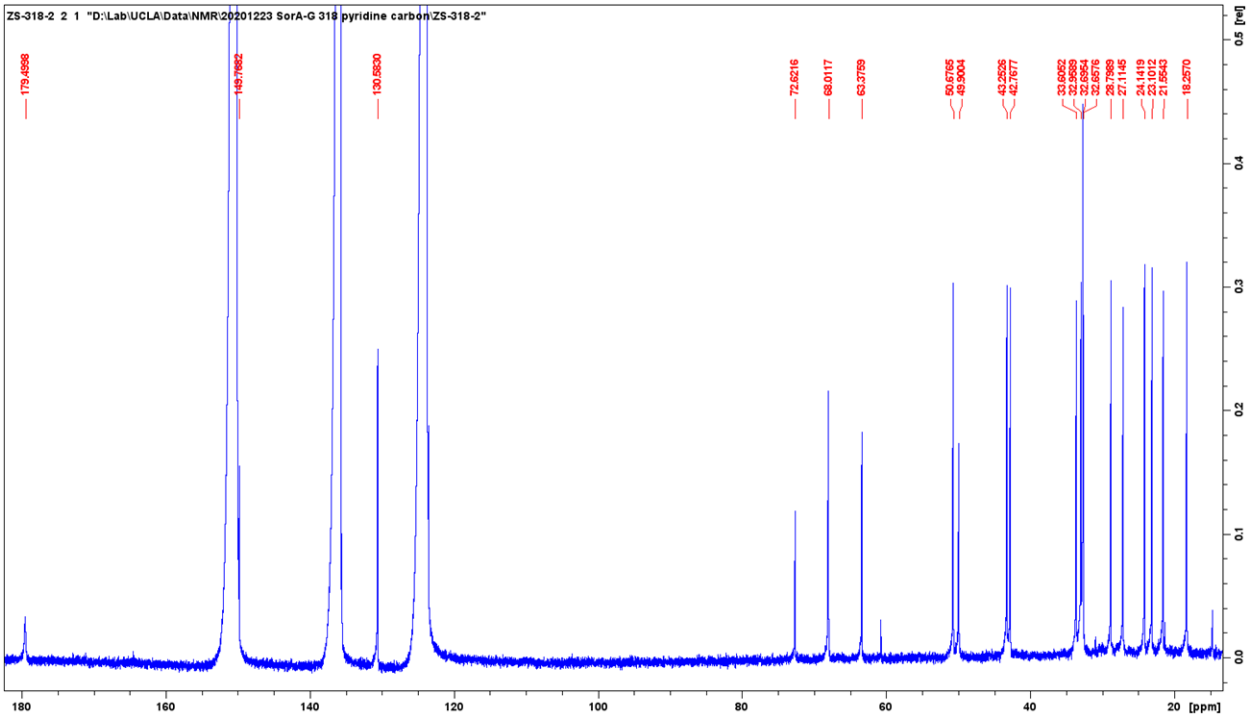

**Supplementary Fig 66.** $^{1}H$-$^{13}C$ HMBC of compound **11** in d5-pyridine, 500 MHz.




752

753 **Supplementary Fig 67.** $^{1}$H NMR of compound **12** in d5-pyridine, 500 MHz.

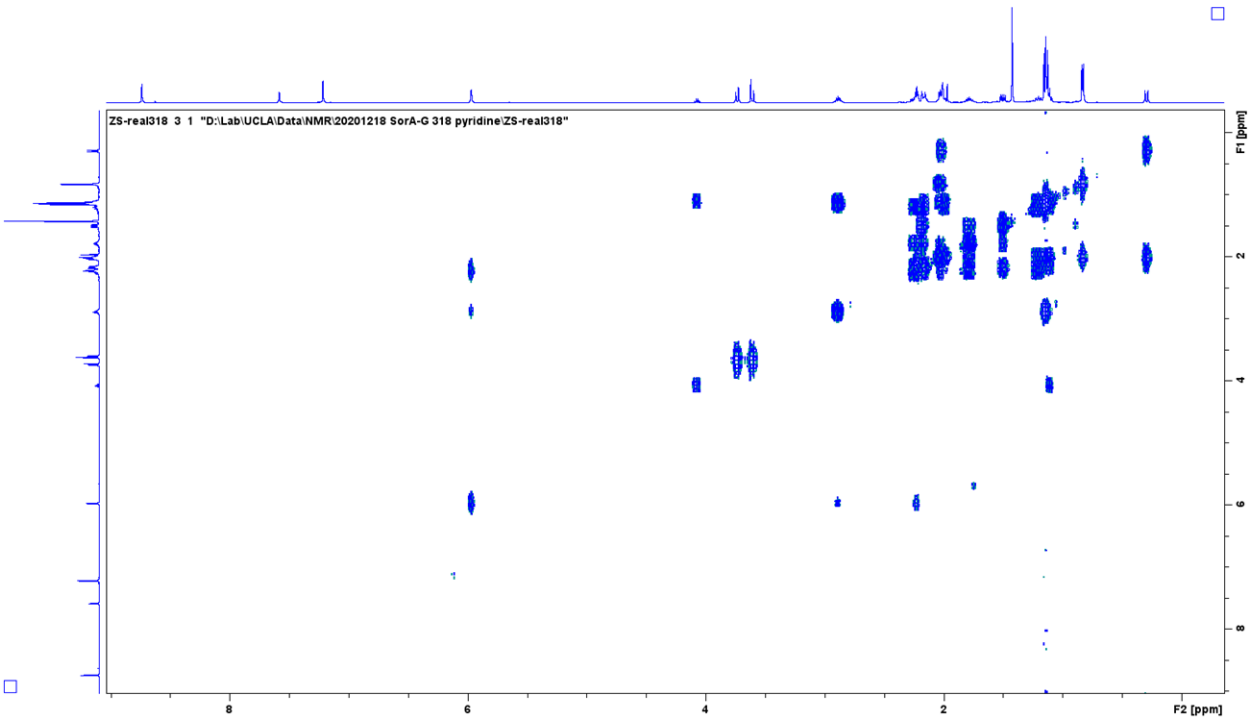
754



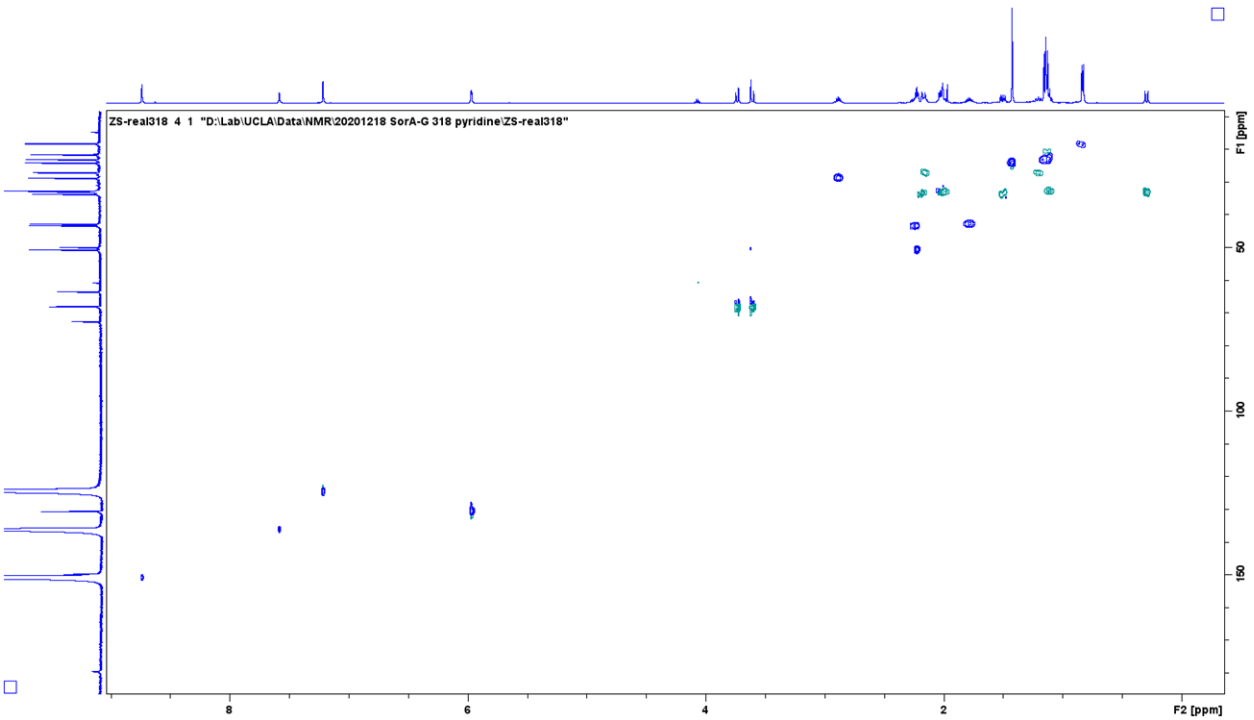

755

756 **Supplementary Fig 68.** $^{13}$C NMR of compound **12** in d5-pyridine, 500 MHz.

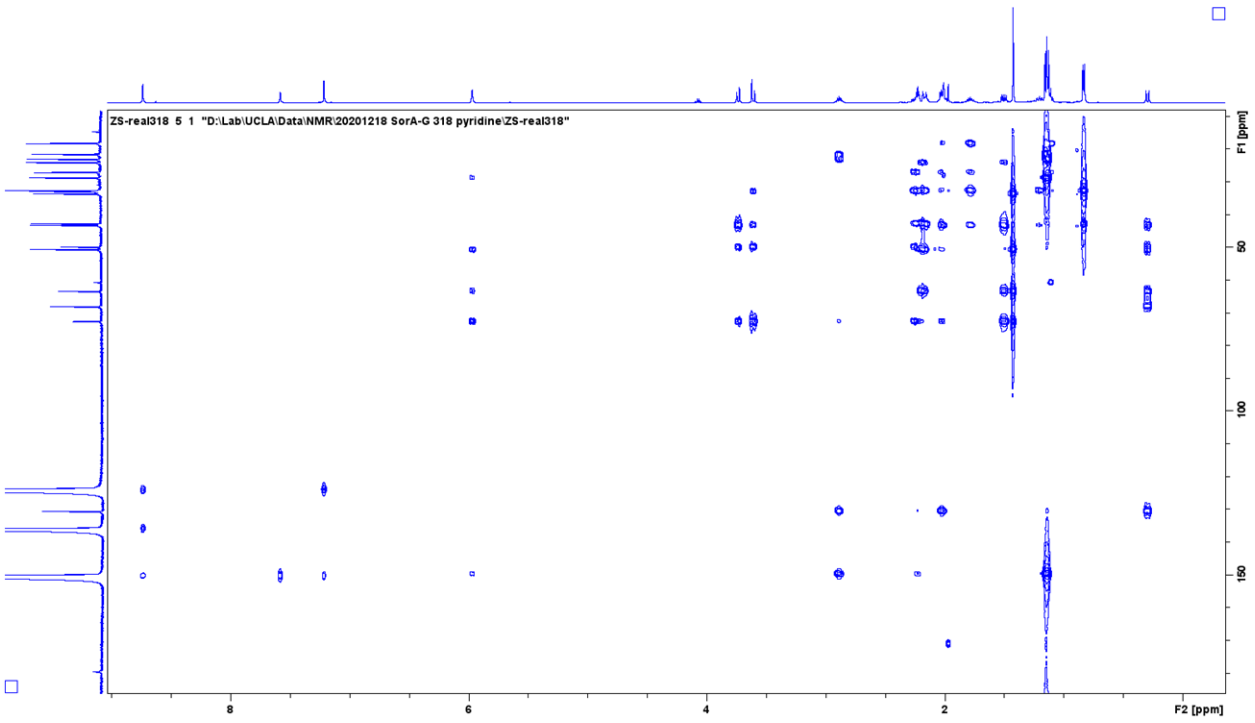
757



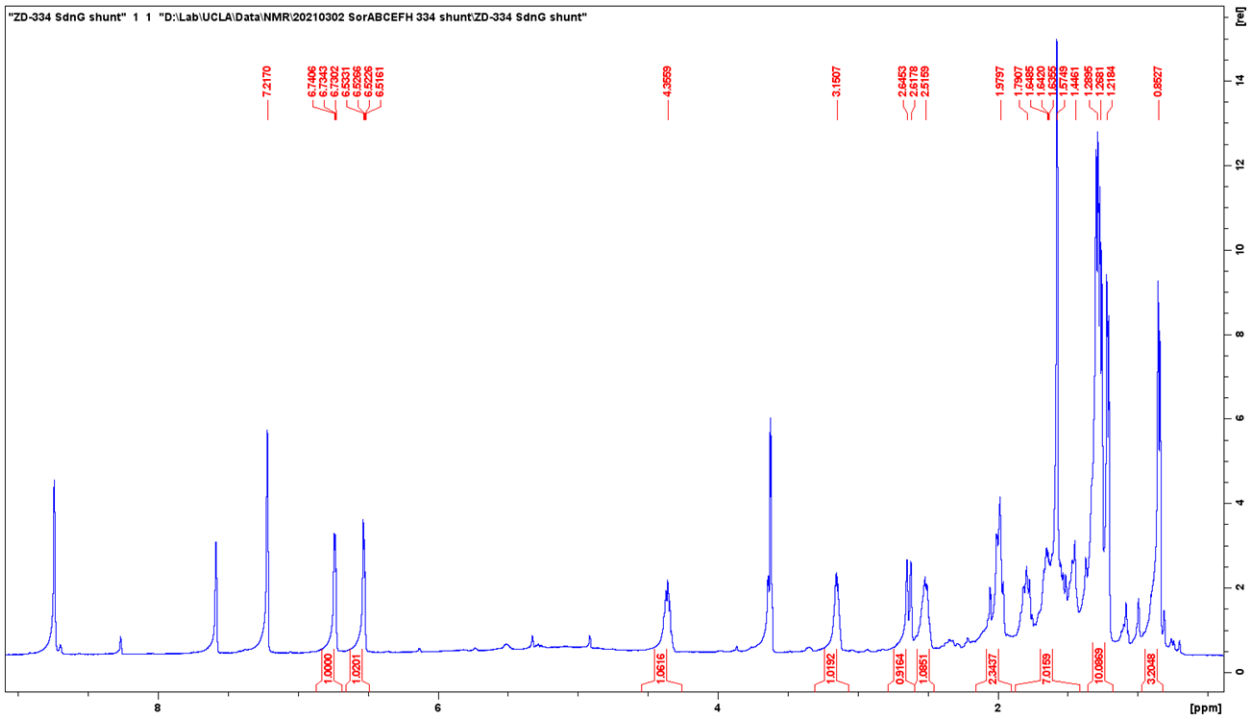

**Supplementary Fig 69.** $^{1}$H-$^{1}$H COSY of compound **12** in d5-pyridine, 500 MHz.



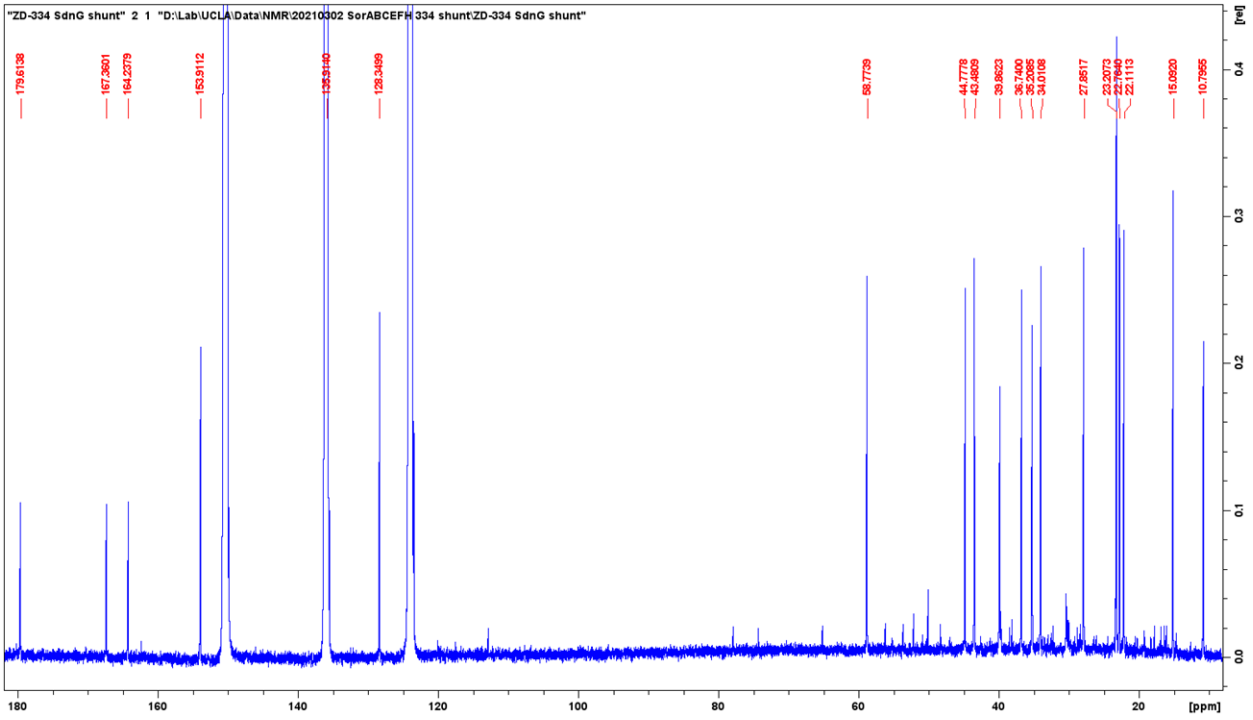

**Supplementary Fig 70.** $^{1}$H-$^{13}$HSQC of compound **12** in d5-pyridine, 500 MHz.




**Supplementary Fig 71.** $^{1}$H-$^{13}$HMBC of compound **12** in d5-pyridine, 500 MHz.

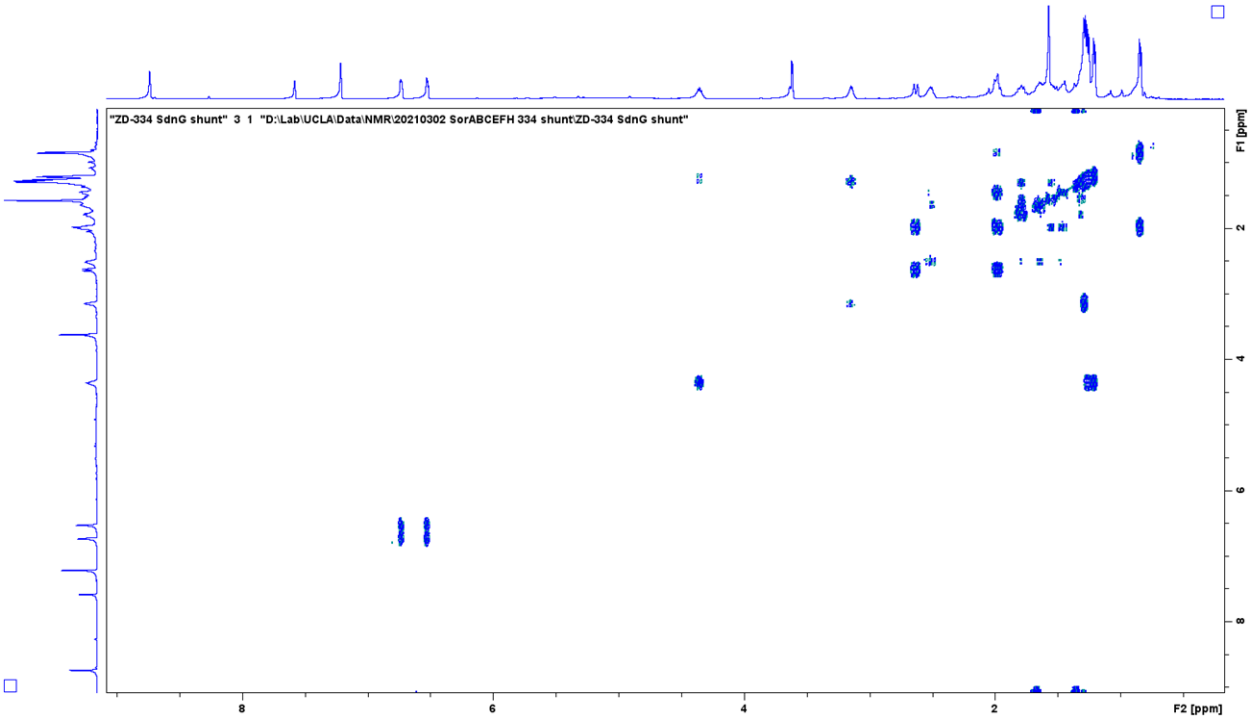



**Supplementary Fig 72.** $^{1}$H NMR of compound **13** in d5-pyridine, 500 MHz.




770

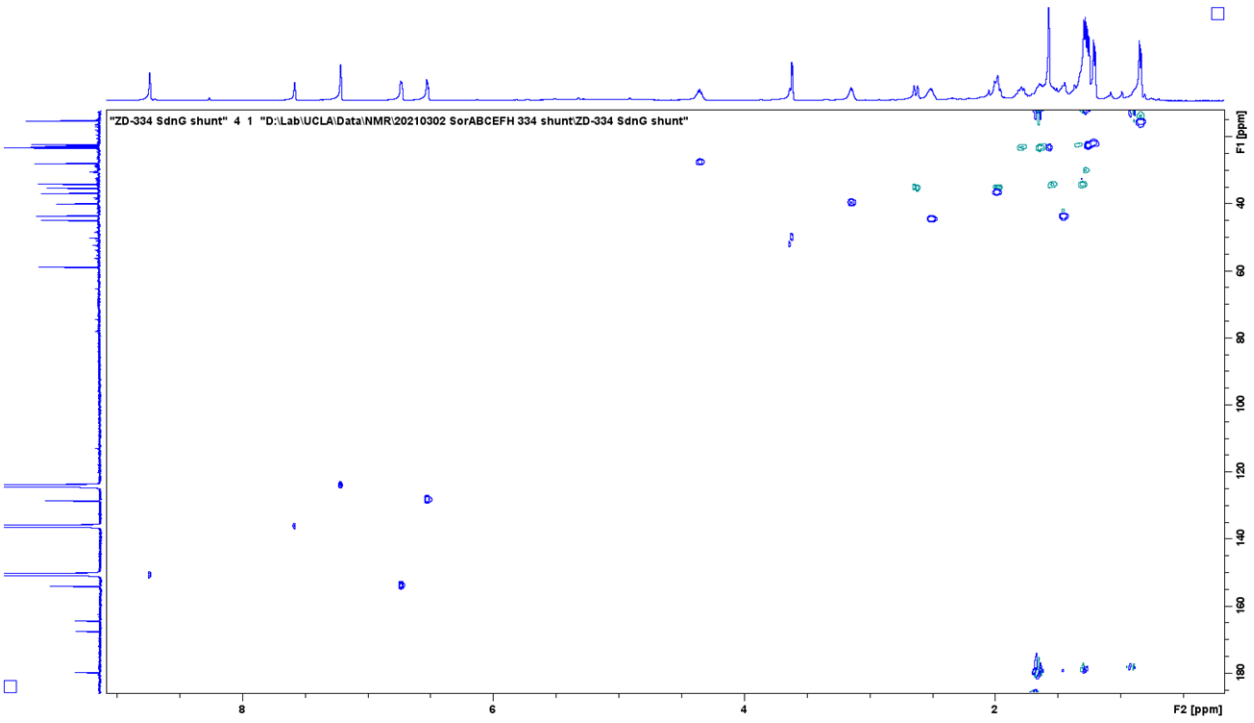
771 **Supplementary Fig 73.** $^{13}$C NMR of compound **13** in d5-pyridine, 500 MHz.



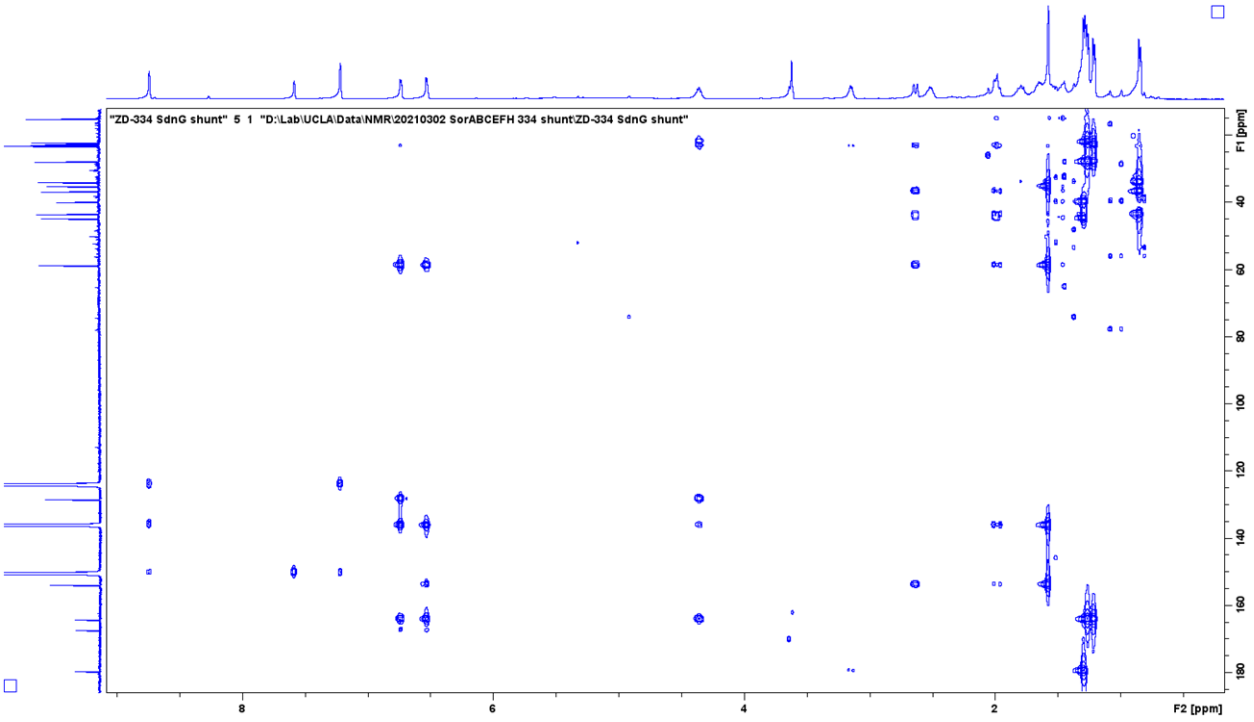

772

773 **Supplementary Fig 74.** $^{1}$H-$^{1}$H COSY of compound **13** in d5-pyridine, 500 MHz.

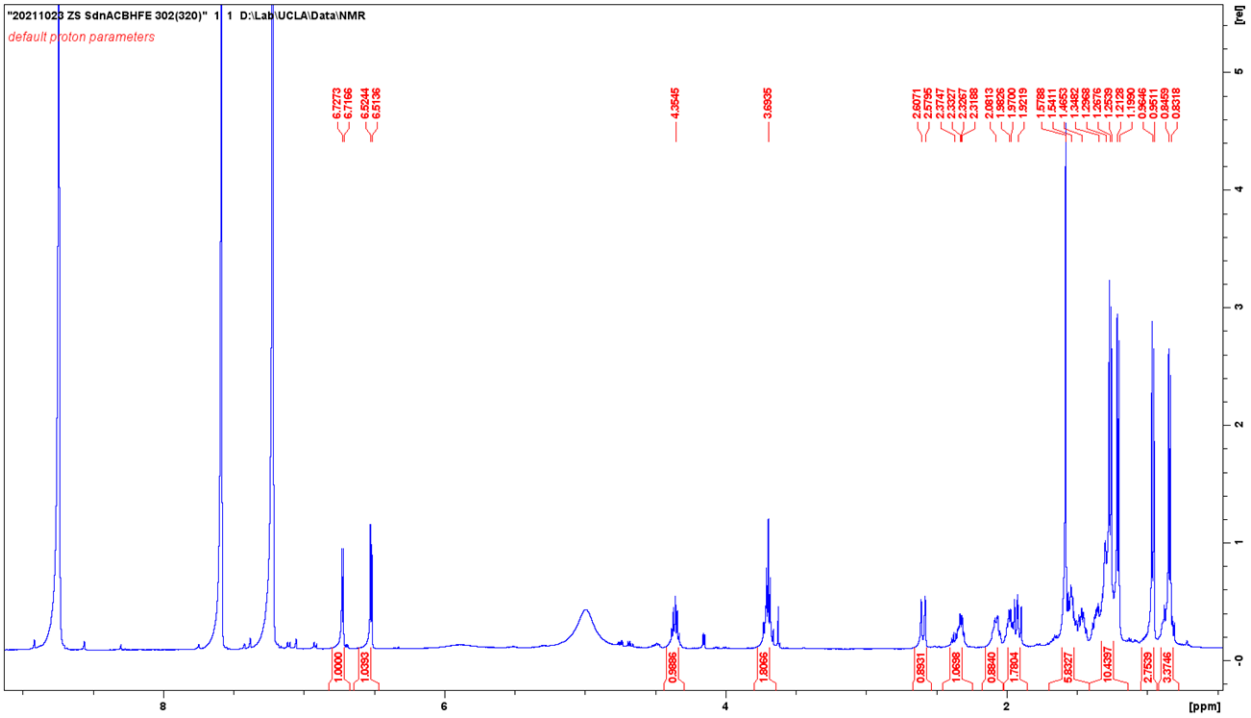
774




**Supplementary Fig 75.** $^{1}H$-$^{13}C$ HSQC of compound **13** in d5-pyridine, 500 MHz.

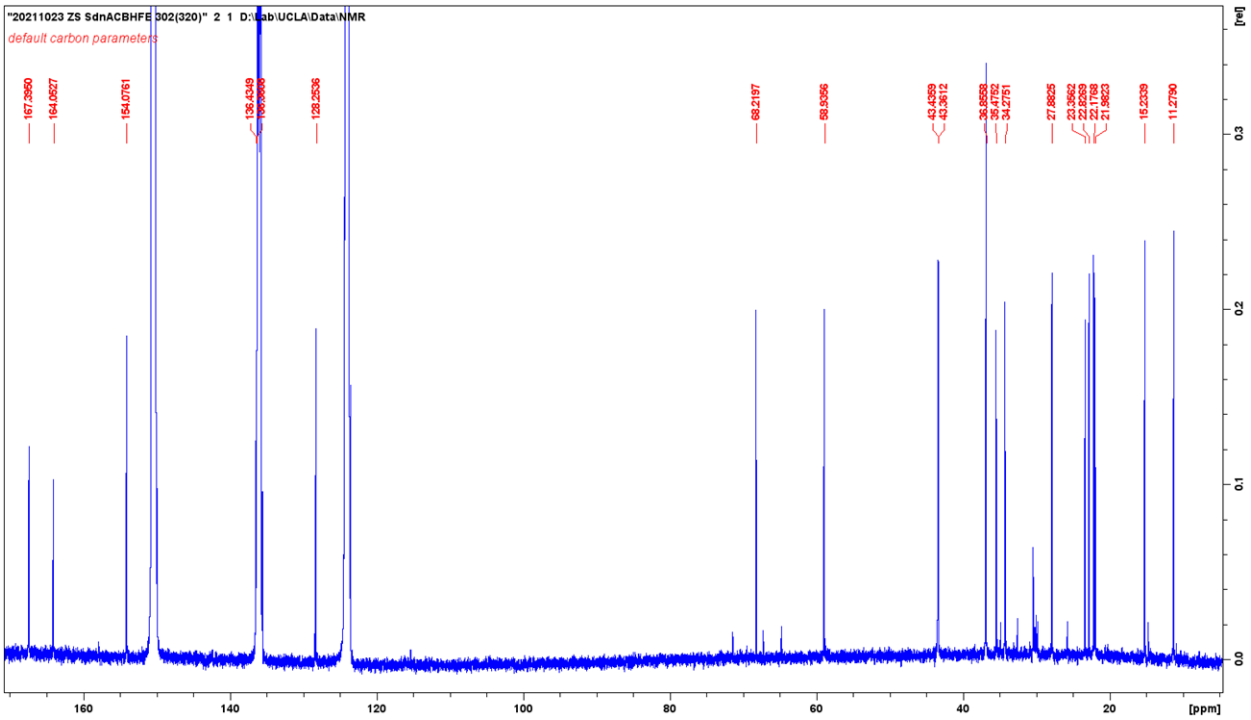



**Supplementary Fig 76.** $^{1}H$-$^{13}C$ HSQC of compound **13** in d5-pyridine, 500 MHz.



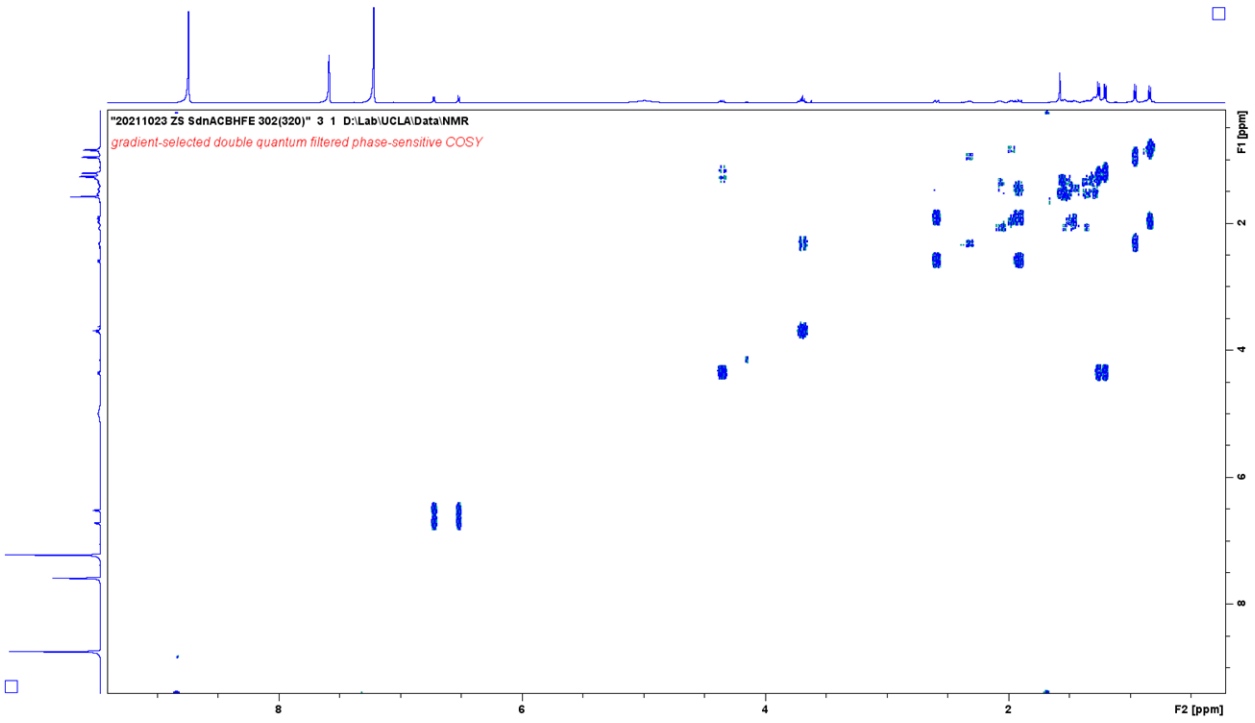

779

780 **Supplementary Fig 77.** $^{1}$H NMR of compound **14** in d5-pyridine, 500 MHz.



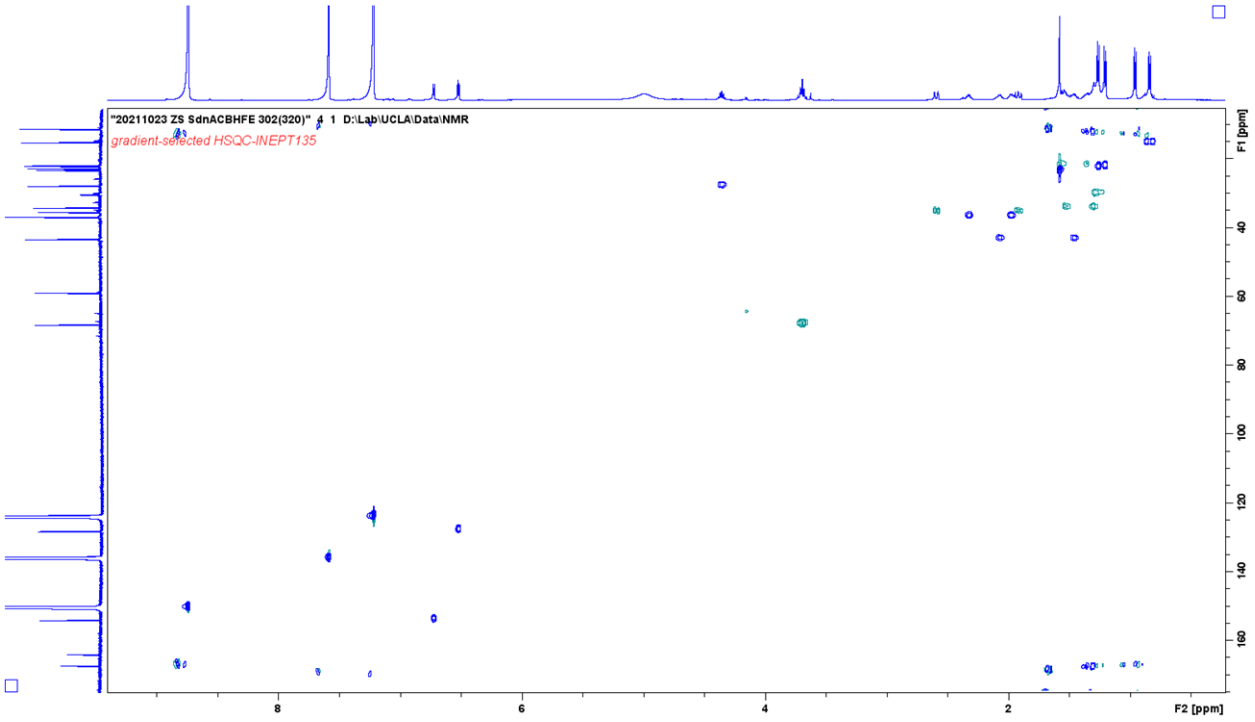

781

782 **Supplementary Fig 78.** $^{13}$C NMR of compound **14** in d5-pyridine, 500 MHz.



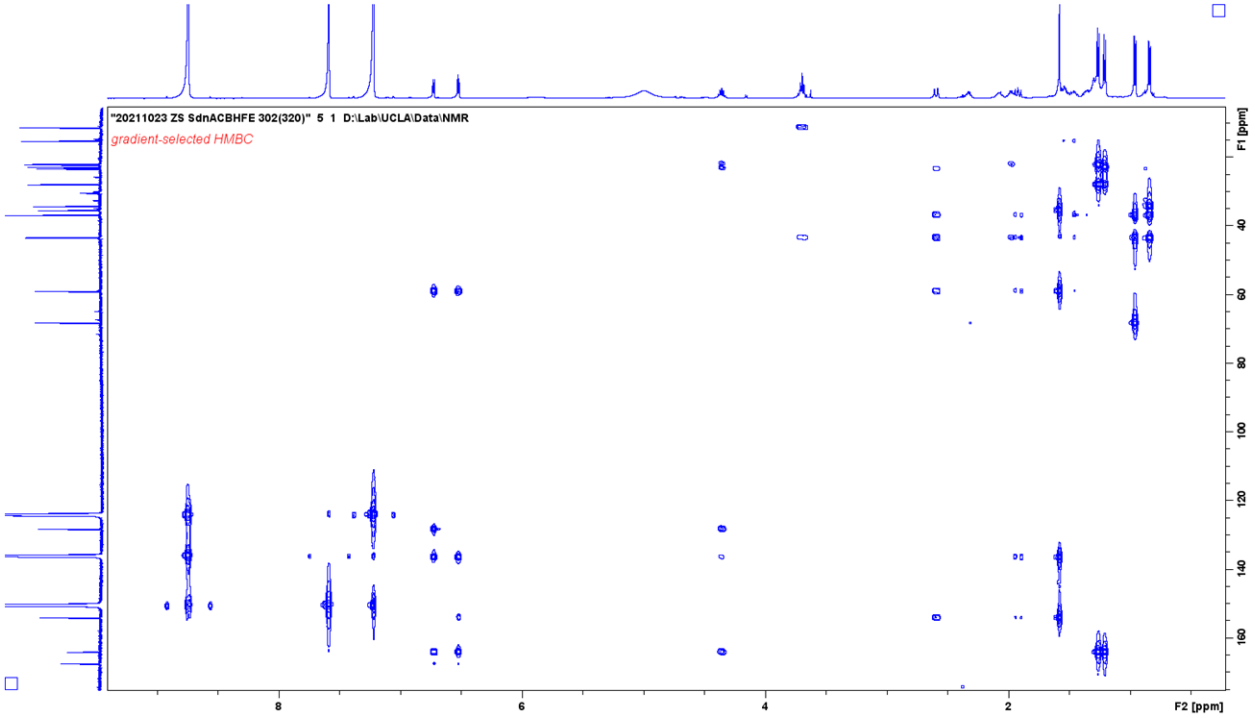

783

784 **Supplementary Fig 79.** $^{1}$H-$^{1}$H COSY of compound **14** in d5-pyridine, 500 MHz.

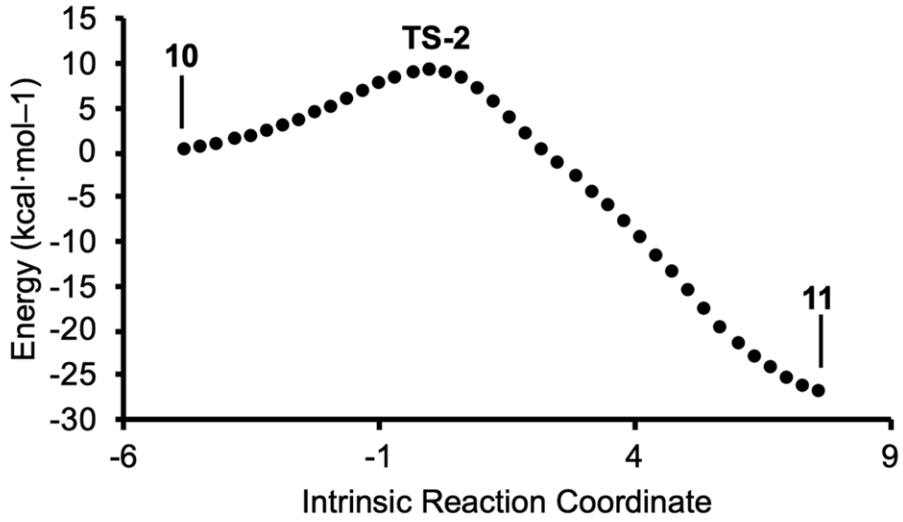



785

786 **Supplementary Fig 80.** $^{1}$H-$^{13}$C HSQC of compound **14** in d5-pyridine, 500 MHz.




**Supplementary Fig 81.** $^{1}H$-$^{13}C$ HMBC of compound **14** in d5-pyridine, 500 MHz.




**Supplementary Fig 82.** Intrinsic reaction coordinate calculation initiated from TS-2.

## IV Supplementary references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## V Uncropped scan of SDS-PAGE in Supplementary Fig 8.