**ELECTRONIC SUPPORTING INFORMATION**

# Simultaneous C7- and N1-prenylation of cyclo-L-Trp-L-Trp catalyzed by a prenyltransferase from *Aspergillus oryzae*

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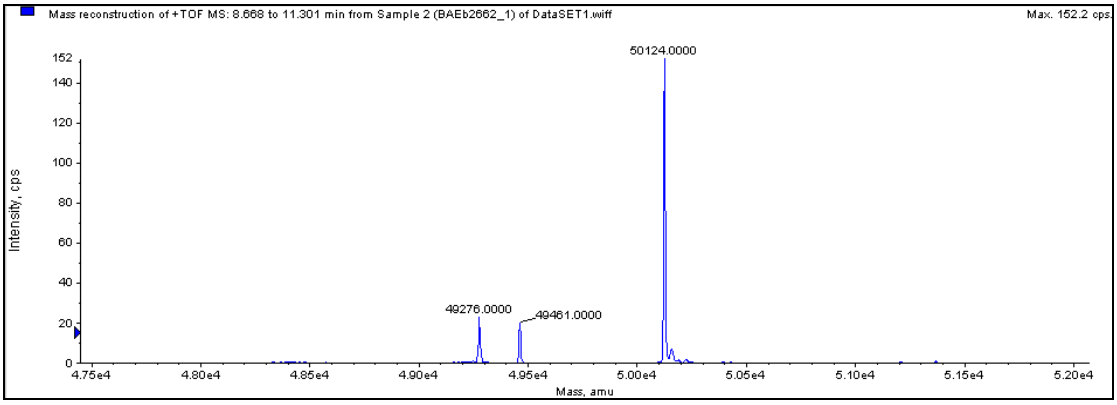
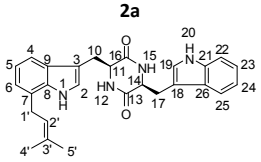
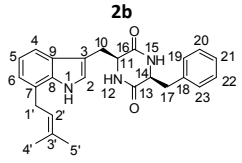
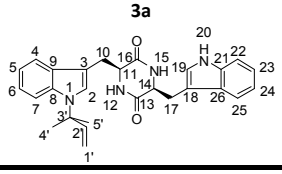
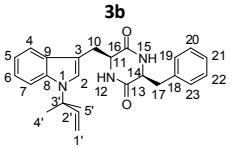



**Figure S1**: LC-ESI-TOF-MS of $His_6$-CTrpPT.

For a complete desalting of the proteins by HPLC using an Agilent 1100 system, samples were applied to a monolithic 50/1 ProSwift RP-4H column (Dionex). Desalted proteins were eluted by the following gradient of buffer A (water/0.05% formic acid) and buffer B (acetonitrile/0.045% formic acid) at a column temperature of 40°C and a flow rate of 0.2 mL/min: Isocratic elution with 5% A for two minutes, followed by a linear gradient to 95% B within 8 minutes and holding 95% B for additional 4 minutes.

Online mass spectrometric analysis was done with a Qstar Pulsar i mass spectrometer (Applied Biosystems) equipped with an ESI source. Parameters were as follows: DP1 75, FP 265, DP2 15, CAD 2, GS1 65, CUR 35. The voltage applied was 5500 V. Positive ions within the mass range of 500-2000 m/z were detected. For better performance, the "Enhance All" mode was activated.

**Table S1**: $^{1}$H- and $^{13}$C-NMR data of enzyme products ($CDCl_3$)

| Compound | **2a**  | | **2b**  | | **3a**  | | **3b**  | |
|---|---|---|---|---|---|---|---|---|
| Position | $\delta_C$ | $\delta_H$, multi., J in Hz | $\delta_C$ | $\delta_H$, multi., J in Hz | $\delta_C$ | $\delta_H$, multi., J in Hz | $\delta_C$ | $\delta_H$, multi., J in Hz |
| 1 | - | 8.03, s | - | 8.12, s | - | - | - | - |
| 2 | 123.6 | 6.54, s | 123.4 | 7.00, d, 2.3 | 124.9 | 6.71, s | | 7.10, s |
| 3 | 109.2 | - | 109.5 | - | 106.6 | - | | - |
| 4 | 116.7 | 7.41, d, 7.9 | 116.8 | 7.48, d, 8.0 | 119.0 | 7.54, d, 7.7 | | 7.61, d, 7.1 |
| 5 | 120.1 | 7.08, t, 7.4 | 120.2 | 7.11, t, 7.5 | 119.2 | 7.10, t, 7.2 | | 7.14, t, 7.2 |
| 6 | 122.0 | 7.02, d, 7.1 | 122.1 | 7.05, d, 7.1 | 121.1 | 7.14, t, 7.9 | | 7.17, t, 7.7 |
| 7 | 124.2 | - | 124.4 | - | 113.8 | 7.49, d, 8.1 | | 7.52, d, 7.6 |
| 8 | 135.4 | - | 135.6 | - | 135.5 | - | | - |
| 9 | 126.5 | - | 126.7 | - | 128.5 | - | | - |
| 10 | 30.3 | 3.24, dd, 14.3, 3.2<br>2.40, dd, 14.4, 8.8 | 30.4 | 3.29, dd, 14.5, 3.5<br>2.64, dd, 14.6, 8.6 | 30.2 | 3.20, dd, 14.6, 3.4<br>2.46, dd, 14.5, 8.3 | | 3.26, dd, 14.7, 3.7<br>2.80, dd, 14.6, 8.0 |
| 11 | 54.9 | 4.19, overlapped | 54.9 | 4.22, m | 55.1 | 4.19, d, 7.0 | | 4.25, br d, 7.8 |
| 12 | - | 5.71, s | - | 5.76, s | - | 5.70, s | | 5.78, s |
| 13 | 166.8 | - | 166.8 | - | 166.9 | - | | - |
| 14 | 54.9 | 4.19, overlapped | 56.2 | 4.11, m | 54.8 | 4.19, d, 7.0 | | 4.08, m |
| 15 | - | 5.73, s | - | 5.57, s | - | 5.77, s | | 5.53, s |
| 16 | 166.8 | - | 166.4 | - | 166.9 | - | | - |
| 17 | 30.1 | 3.24, dd, 14.3, 3.2<br>2.52, dd, 14.4, 8.4 | 39.8 | 3.10, dd, 13.7, 3.5<br>2.17, dd, 13.6, 9.1 | 30.5 | 3.23, td, 14.3, 3.3<br>2.39, dd, 14.4, 8.8 | | 3.12, dd, 13.4, 3.6<br>2.04, dd, 13.9, 9.8 |
| 18 | 108.9 | - | 135.1 | - | 108.8 | - | | - |
| 19 | 123.9 | 6.60, s | 129.6 | 6.88, d, 7.1 | 124.0 | 6.47, s | | 6.80, d, 6.5 |
| 20 | - | 8.07, s | 128.8 | 7.29, t, 7.3 | - | 8.04, s | | 7.26, overlapped |
| 21 | 136.1 | - | 127.4 | 7.26, overlapped | 136.0 | - | | 7.26, overlapped |
| 22 | 111.2 | 7.36, d, 8.0 | 128.9 | 7.29, t, 7.3 | 111.2 | 7.36, d, 8.0 | | 7.26, overlapped |
| 23 | 122.4 | 7.23, t, 7.7 | 129.6 | 6.88, d, 7.1 | 122.4 | 7.21, t, 7.2 | | 6.80, d, 6.5 |
| 24 | 119.9 | 7.16, t, 7.5 | - | - | 119.9 | 7.14, t, 7.9 | | |
| 25 | 118.8 | 7.58, d, 7.8 | - | - | 118.8 | 7.57, d, 7.9 | | |
| 26 | 126.6 | - | - | - | 126.5 | - | | |
| 1’ | 30.5 | 3.52, d, 7.1 | 30.6 | 3.54, d, 7.1 | 113.5 | 5.19, d, 10.7<br>5.16, d, 17.5 | | 5.22, d, 10.7<br>5.19, d, 17.4 |
| 2’ | 121.7 | 5.30, t, 7.1 | 121.8 | 5.31, t, 7.2 | 143.7 | 6.07, dd, 17.6, 10.8 | | 6.10, dd, 17.5, 10.7 |
| 3’ | 133.3 | - | 133.4 | - | 58.9 | - | | - |
| 4’ | 17.7 | 1.78, s | 17.7 | 1.79, s | 27.8 | 1.71, s | | 1.76, s |
| 5’ | 25.4 | 1.71, s | 25.4 | 1.71, s | 27.6 | 1.69, s | | 1.73, s |

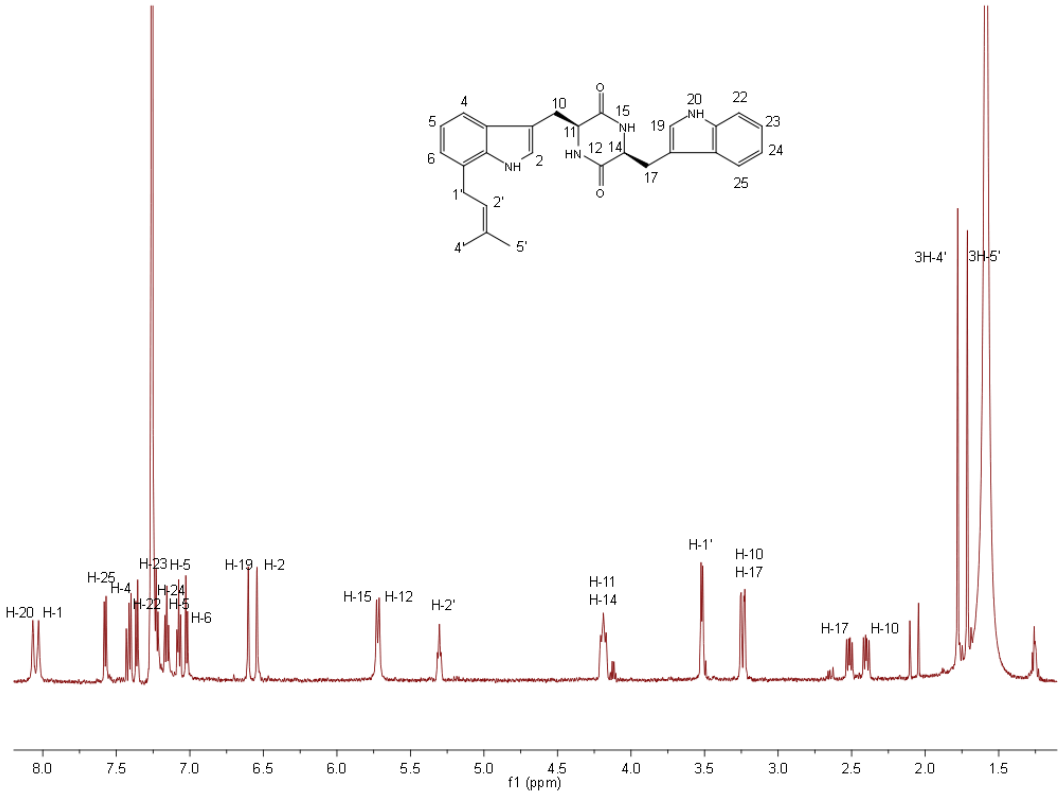



**Figure S2.1**: $^{1}$H-NMR spectrum of **2a** in $CDCl_3$.

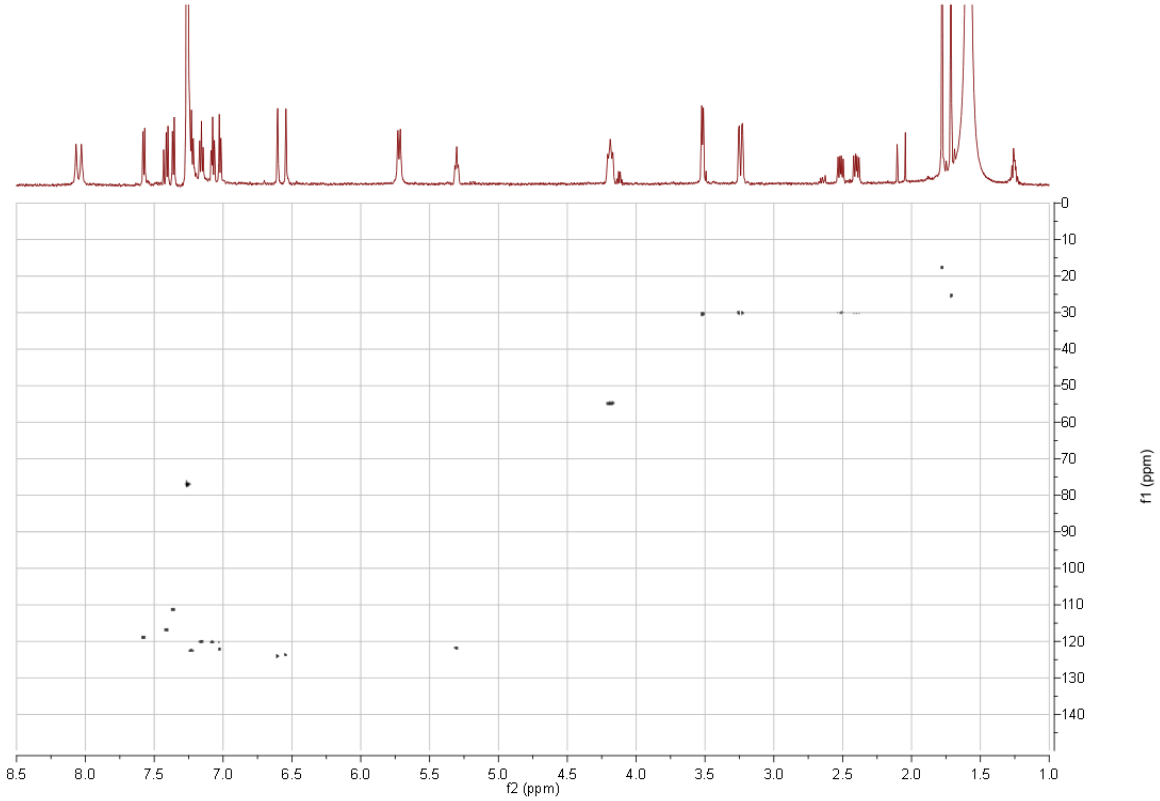



**Figure S2.2**: HSQC spectrum of **2a** in $CDCl_3$.

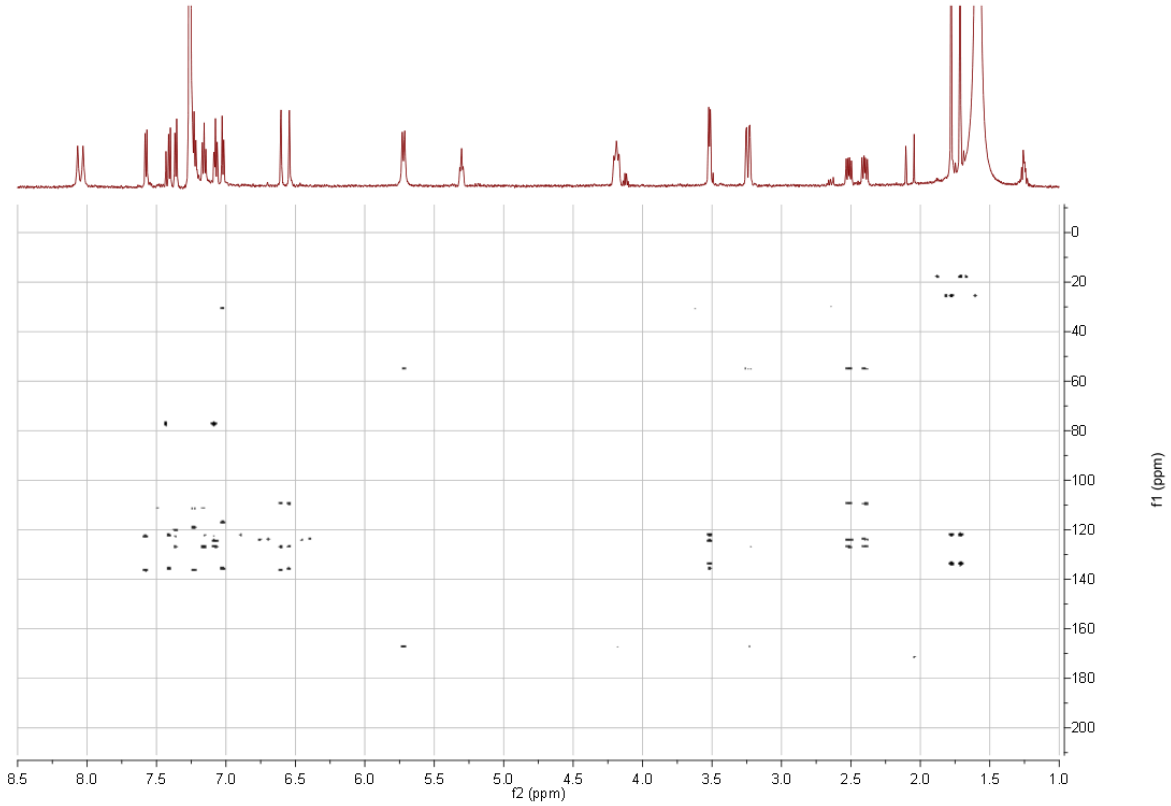



**Figure S2.3**: HMBC spectrum of **2a** in $CDCl_3$.

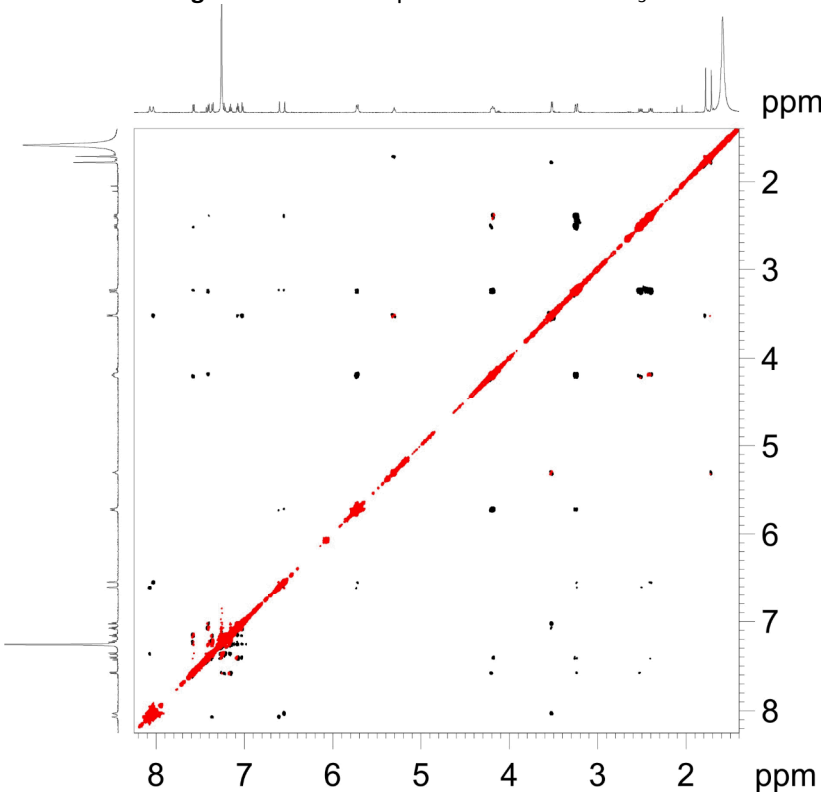



**Figure S2.4**: ROESY spectrum of **2a** in $CDCl_3$.

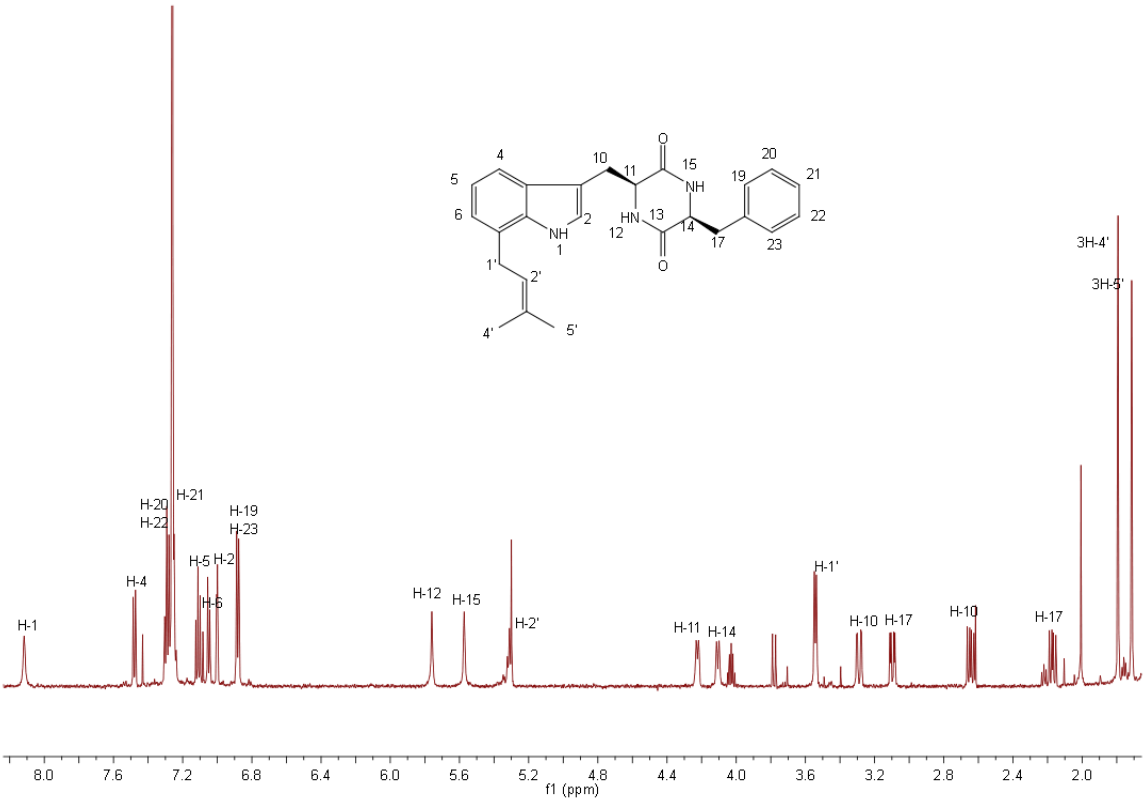



**Figure S3.1**: $^{1}$H-NMR spectrum of **2b** in $CDCl_3$.

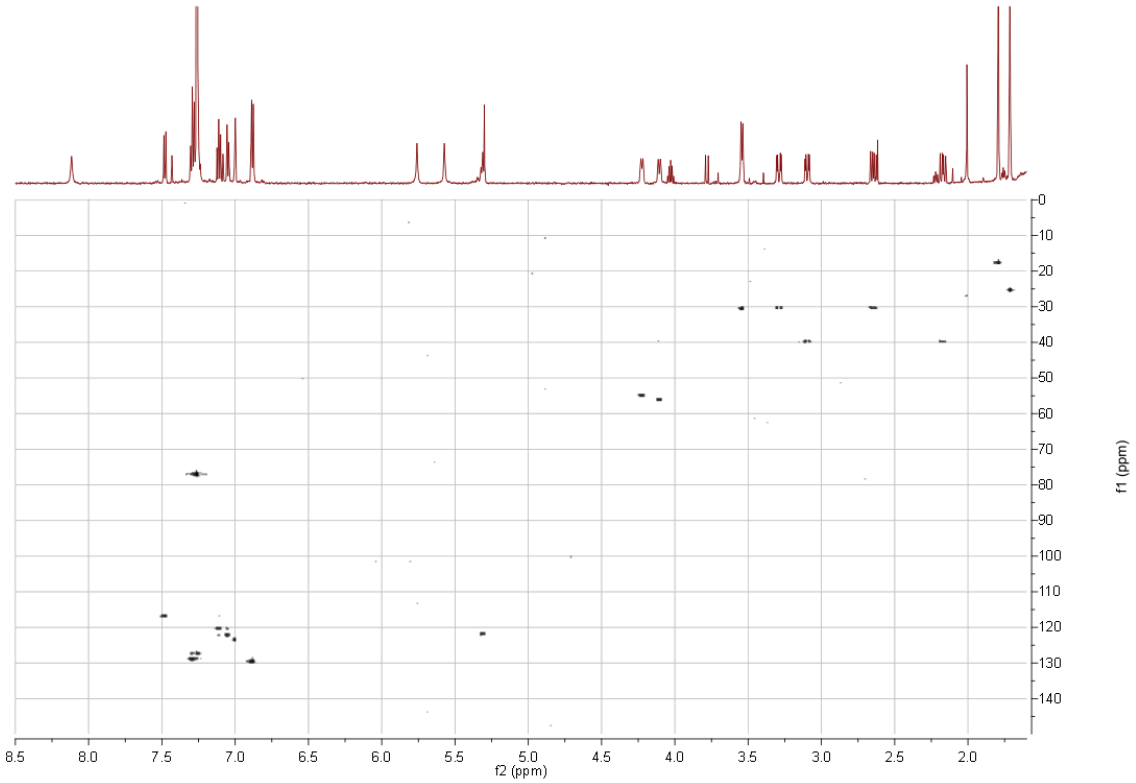



**Figure S3.2**: HSQC spectrum of **2b** in $CDCl_3$.

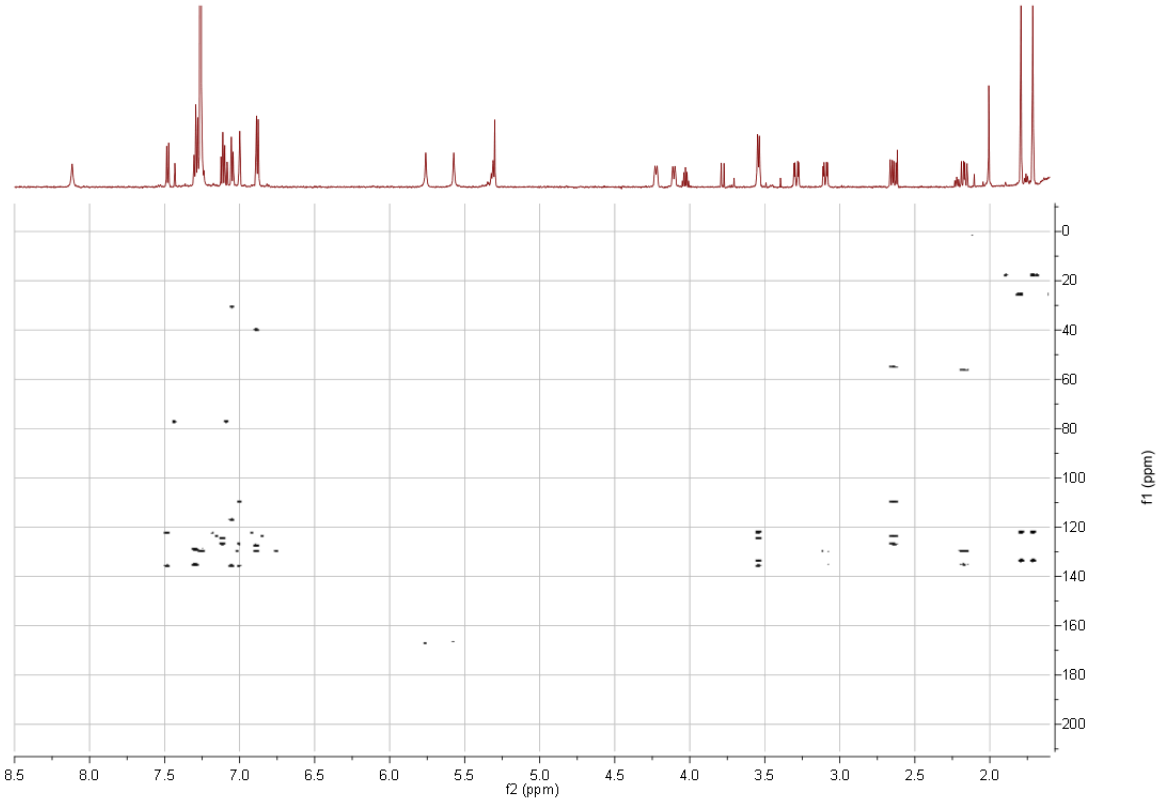



**Figure S3.3**: HMBC spectrum of **2b** in $CDCl_3$.

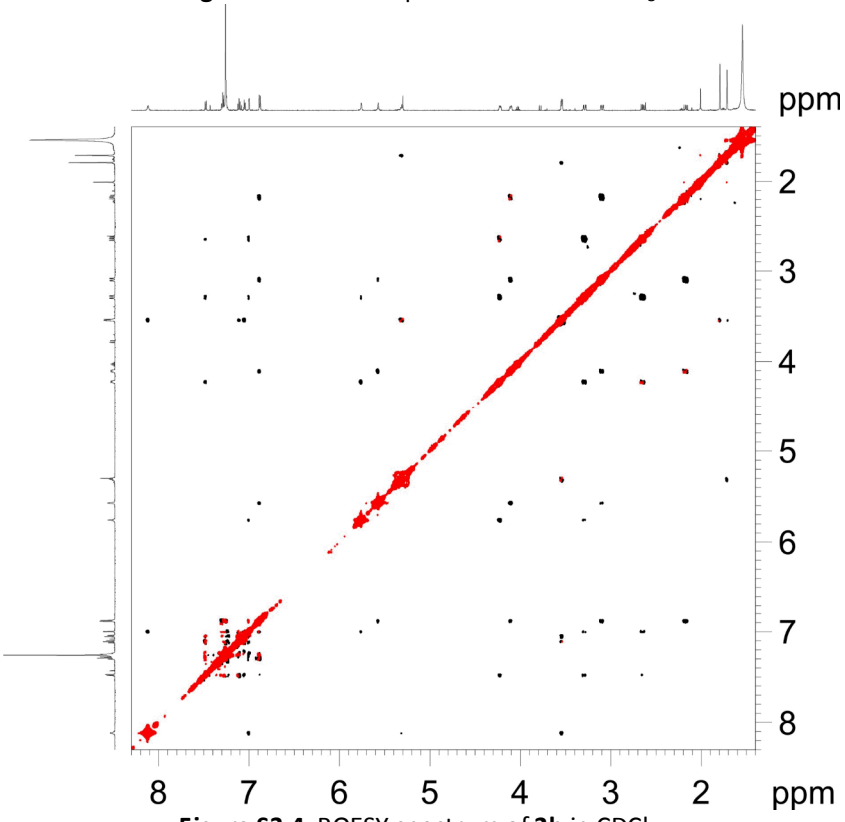



**Figure S3.4**: ROESY spectrum of **2b** in $CDCl_3$.

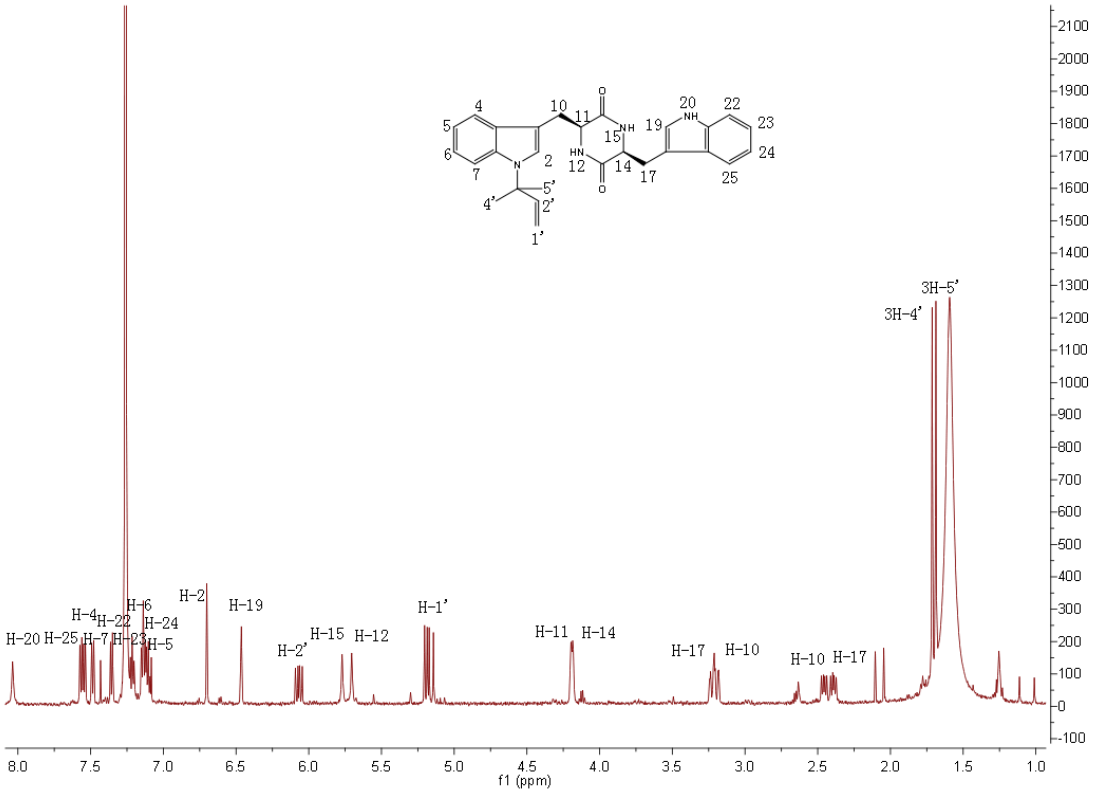



**Figure S4.1**: $^{1}$H-NMR spectrum of **3a** in $CDCl_3$.

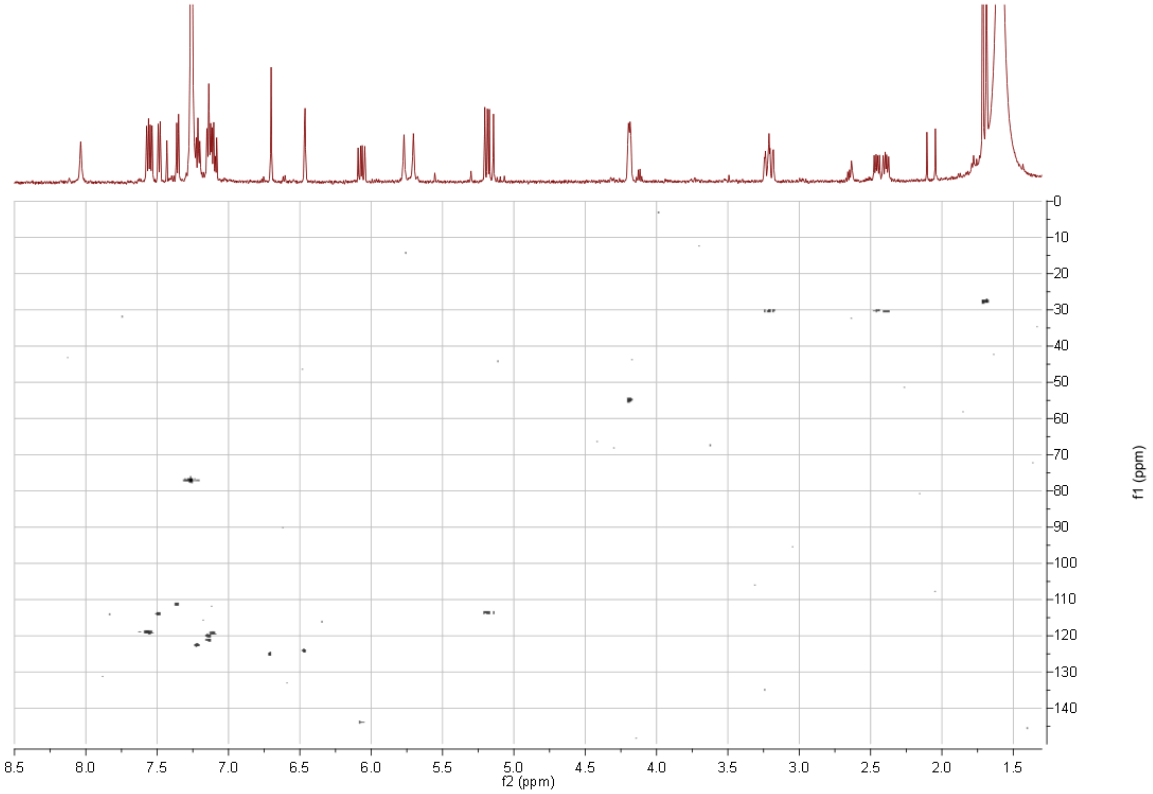



**Figure S4.2**: HSQC spectrum of **3a** in $CDCl_3$.

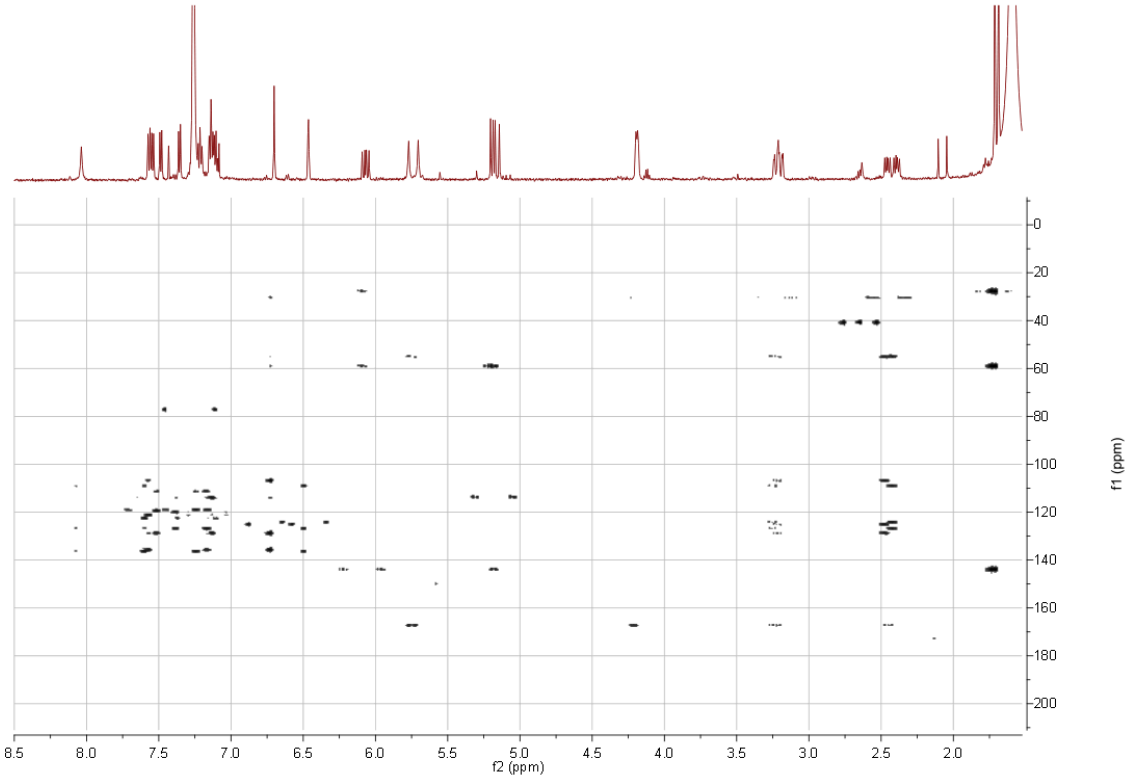



**Figure S4.3**: HMBC spectrum of **3a** in $CDCl_3$.

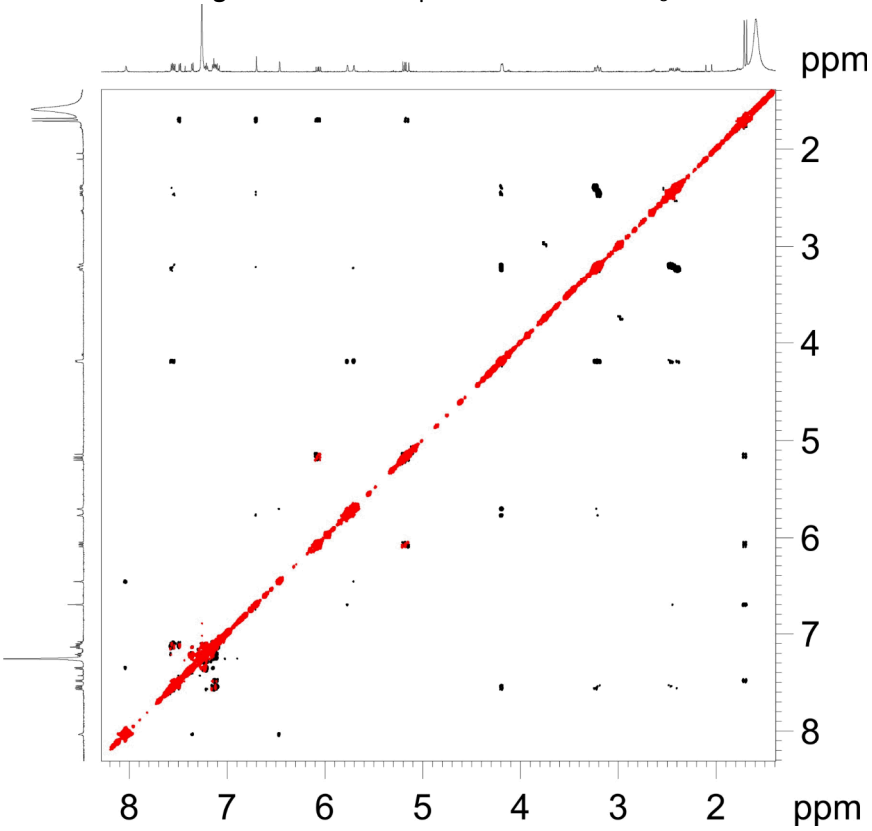



**Figure S4.4**: ROESY spectrum of **3a** in $CDCl_3$.

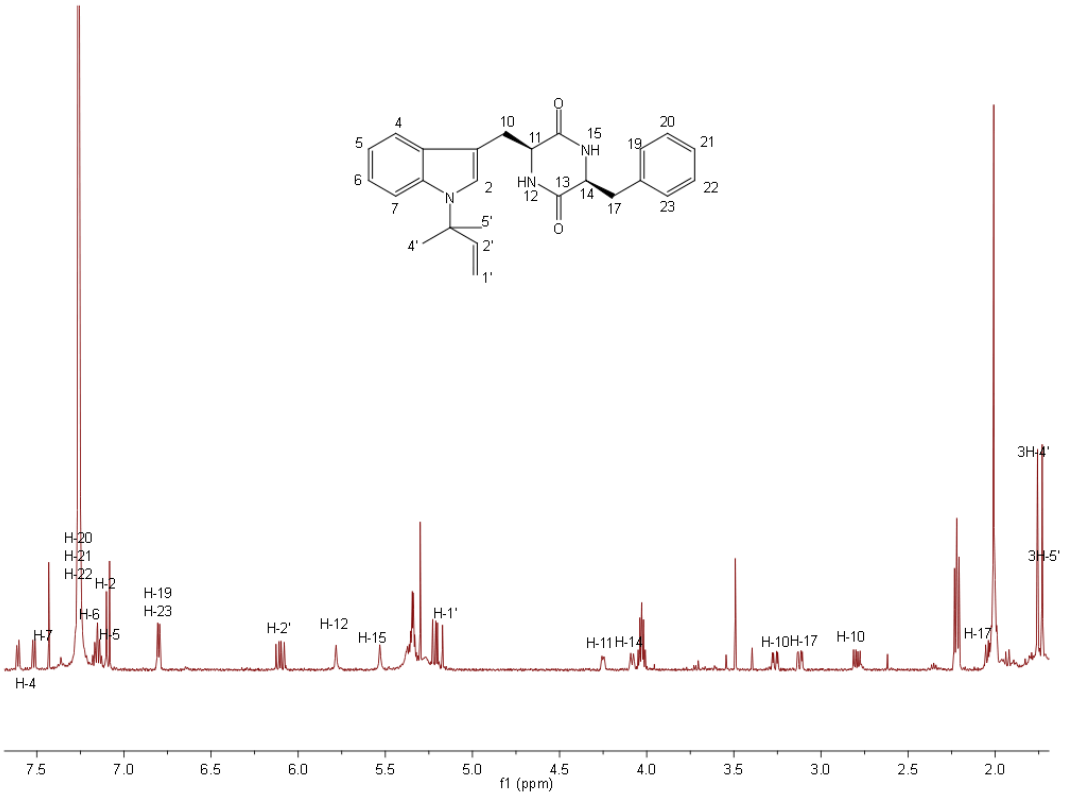



**Figure S5.1**: $^{1}$H-NMR spectrum of **3b** in $CDCl_3$.

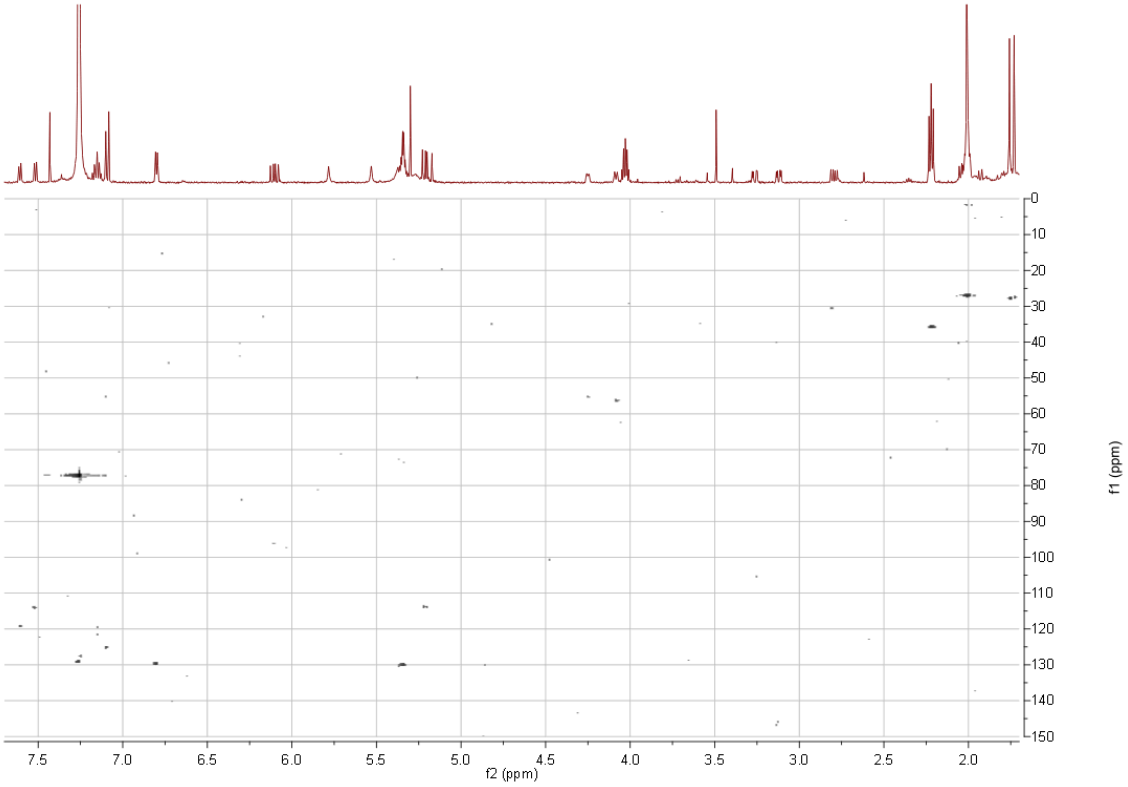



**Figure S5.2**: HSQC spectrum of **3b** in $CDCl_3$.

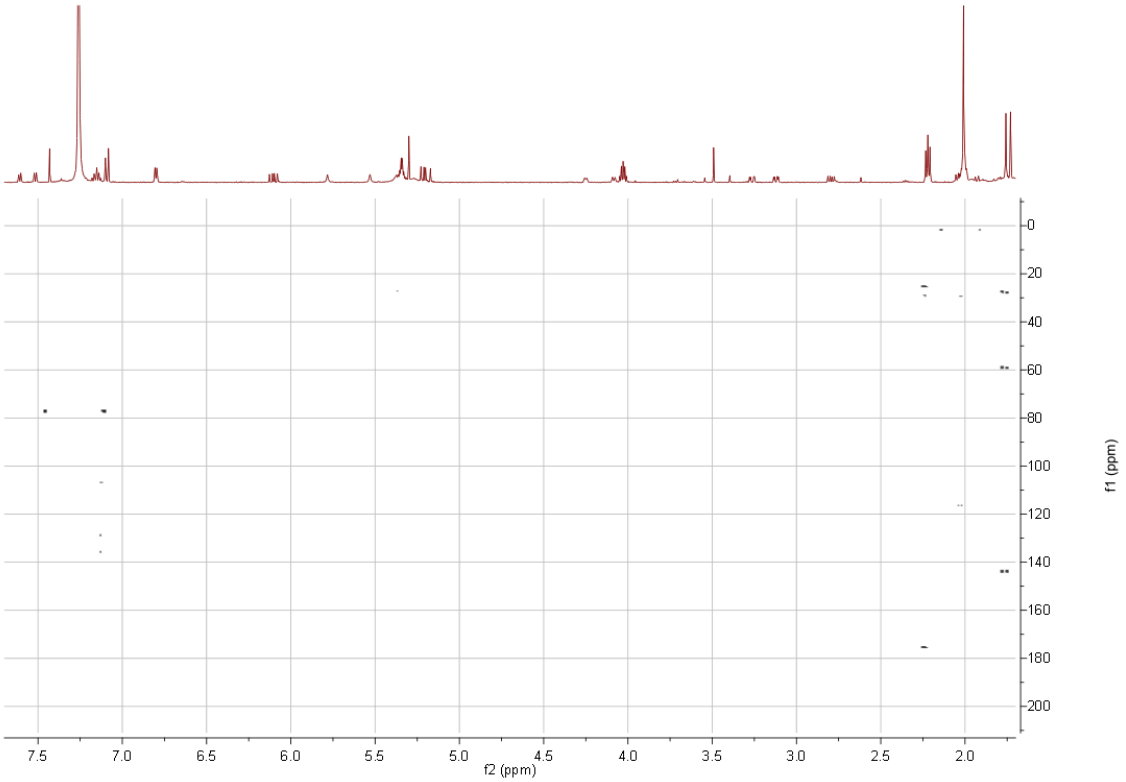



**Figure S5.3**: HMBC spectrum of **3b** in $CDCl_3$.

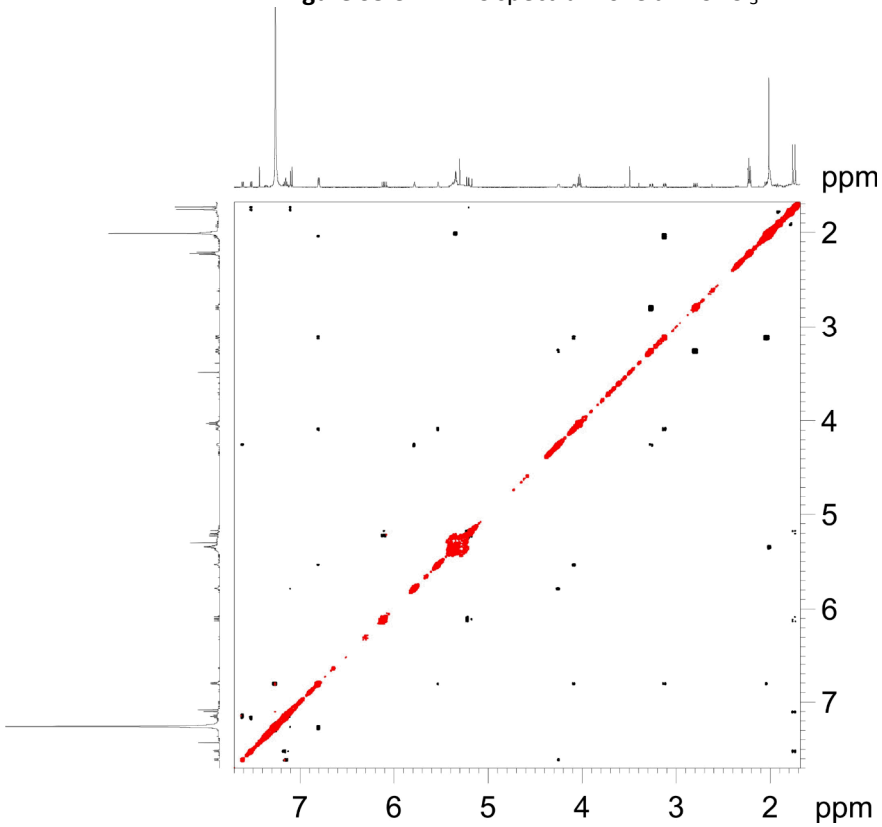



**Figure S5.4**: NOESY spectrum of **3b** in $CDCl_3$.