Supplementary Material (ESI) for Chemical Communications


# D-CYSTEINE OCCURENCE IN THIOSTREPTON MAY NOT NECESSITATE AN EPIMERASE

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**Supporting Information: 6 pages**

**Analytical HPLC methods**

Varian ProStar, Diode Array Detector, flow = 1 mL /min, ternary gradient (A/B/C in v/v/v): A = bidest. $H_2O$, B = $CH_3CN$, C = 2% TFA in bidest. $H_2O$.

A/B/C = 90/10/0 for 1 min, then to 0/70/0 in 16 min, then 0/100/0 for 2 min.
Column: 125 x 4 mm Nucleodur 5 µm C4 (Macherey & Nagel).

**Preparation of thiostrepton derivatives**

To a stirred solution of thiostrepton **1** (1.0 g, 0.601 mmol) in $CHCl_3$ (50 ml) was added $HNEt_2$ (5 ml) dropwise at 0°C. After 5 min the reaction was continued at r.t. for 3 hrs. The volatiles were coevaporated with toluene and the residue was purified by silica gel column chromatography (2-5 % MeOH in $CHCl_3$) to yield epimer **2**.
Purification of fractions containing the *L*-epimer **3** was achieved by additional preparative HPLC of appropriate fractions (C4 column, $CH_3CN/H_2O$) followed by lyophilization to provide **3**.

***D*-epimer (2)**: 70 % isolated yield, colorless powder, m.p. > 240 °C decomp., $R_f$ = 0.35 ($CHCl_3$/MeOH 9:1). HPLC: $t_R$ = 14.1 min. $^1$H NMR (400 MHz, $CDCl_3$/MeOD 4:1) δ = 8.12 (s, 1H), 8.10 (s, 1H), 8.00 (s, 1H), 7.40 (s, 1H), 7.14 (s, 1H), 6.73 (d, *J* = 10.1 Hz, 1H), 6.46 (d, *J* = 1.7 Hz, 1H), 6.25 – 6.15 (m, 2H), 6.06 (q, *J* = 7.1 Hz, 1H), 5.67 (d, *J* = 2.1 Hz, 1H), 5.63 – 5.57 (m, 2H), 5.48 (d, *J* = 1.6 Hz, 1H), 5.18 (s, 1H), 5.16 – 5.12 (m, 2H), 4.81 (dd, *J* = 12.9, 8.9 Hz, 1H), 4.60 – 4.52 (m, 1H), 4.28 – 4.23 (m, 2H), 3.93 – 3.86 (m, 1H), 3.68 – 3.60 (m, 2H), 3.51 – 3.43 (m, 2H), 3.36 – 3.25 (m, 1H), 3.00 (dd, *J* = 13.0, 11.4 Hz, 1H), 2.80 (d, *J* = 4.3 Hz, 1H), 2.78 – 2.71 (m, 1H), 2.13 (t, *J* = 12.9 Hz, 1H), 1.79 – 1.71 (m, 1H), 1.55 (d, *J* = 6.6 Hz, 3H), 1.45 (d, *J* = 7.1 Hz, 3H), 1.42 – 1.37 (m, 1H), 1.26 (d, *J* = 6.6 Hz, 3H), 1.21 (d, *J* = 6.6 Hz, 3H), 1.13 (d, *J* = 6.4 Hz, 3H), 1.03 – 0.97 (m, 6H), 0.95 – 0.86 (m, 1H), 0.83 (d, *J* = 6.9 Hz, 3H), 0.72 (t, *J* = 7.3 Hz, 3H), 0.65 (d, *J* = 6.2 Hz, 3H). HRMS calcd. for [$C_{69}H_{83}N_{18}O_{17}S_5$+$H^+$] 1595.478, found (ESI) 1595.482.

***L*-epimer (3)**: 0.7 % isolated yield, colorless powder, m.p. > 240 °C decomp., $R_f$ = 0.31 ($CHCl_3$/MeOH 9:1). HPLC: $t_R$ = 12.1 min. $^1$H NMR (400 MHz, $CDCl_3$/MeOD 4:1) δ = 8.07 (s, 2H), 8.04 (s, 1H), 7.38 (s, 1H), 7.11 (s, 1H), 6.69 (d, *J* = 10.2 Hz, 1H), 6.45 (d, *J* = 1.7 Hz, 1H), 6.25 – 6.14 (m, 2H), 6.11 – 6.03 (m, 1H), 5.70 – 5.64 (m, 2H), 5.49 – 5.45 (m, 1H), 5.35 (d, *J* = 8.5 Hz, 3H), 5.24 (s, 1H), 5.16 (s, 1H), 5.14 – 5.09 (m, 1H), 4.62 (dd, *J* = 13.4, 8.7 Hz, 1H), 4.57 – 4.53 (m, 1H), 4.29 (dd, *J* = 7.7, 4.0 Hz, 1H), 4.16 (s, 1H), 3.90 – 3.81 (m, 1H), 3.63 (q, *J* = 6.4 Hz, 1H), 3.60 – 3.56 (m, 1H), 3.45 (dd, *J* = 5.6, 2.1 Hz, 1H), 3.40 (dd, *J* = 11.2, 8.7 Hz, 1H), 3.13 – 3.07 (m, 1H), 2.78 (d, *J* = 4.7 Hz, 1H), 2.76 – 2.69 (m, 1H), 2.14 –

2.05 (m, 1H), 1.95 – 1.85 (m, 1H), 1.71 – 1.61 (m, 1H), 1.51 (dd, *J* = 11.5, 6.7 Hz, 6H), 1.23 (dd, *J* = 14.8, 6.5 Hz, 6H), 1.18 – 1.11 (m, 1H), 1.05 (d, *J* = 6.5 Hz, 3H), 1.02 – 0.96 (m, 6H), 0.95 – 0.86 (m, 1H), 0.81 (d, *J* = 6.9 Hz, 3H), 0.70 (t, *J* = 7.4 Hz, 4H), 0.56 (d, *J* = 6.4 Hz, 3H). HRMS calcd. for [$C_{59}H_{82}N_{18}O_{17}S_5$+$H^+$] 1594.471, found (ESI) 1595.479.

**Preparation of ring-opened truncated thiostrepton**

Compound **2** (150 mg, 0.09 mmol) was dissolved in 11 ml anhydrous MeOH/THF (8:3), followed by the addition of 60 µl NaOMe solution (1 M in MeOH) at 0°C. The reaction was continued for 8 hours while the temperature was allowed to rise to rt. The volatiles were removed and unreacted starting material was removed from the crude mixture (79 % conversion) by silica gel column chromatography (5 % MeOH in $CHCl_3$) to give 114 mg of the isomer mixture containing compounds **5** and **6** in approx 1:1 ratio (HPLC). Separation of the two isomers was achieved by preparative HPLC (C4 column, $CH_3CN/H_2O$).

**5:** isolated 6.6 mg, colorless powder, m.p. > 240 °C decomp., $R_f$ = 0.16 ($CHCl_3$/MeOH 9:1). HPLC: $t_R$ = 10.9 min. $^1$H NMR (600 MHz, $CDCl_3$/MeOD 4:1) δ = 8.06 (s, 1H), 8.02 (s, 1H), 8.02 (s, 1H), 6.84 (s, 1H), 6.66 (d, *J* = 9.7 Hz, 1H), 6.43 (s, 1H), 6.07 (s, 1H), 6.03 (d, *J* = 8.3 Hz, 1H), 5.53 (s, 1H), 5.49 (s, 2H), 5.46 – 5.41 (m, 1H), 5.29 (s, 1H), 4.98 – 4.85 (m, 3H), 4.77 (d, *J* = 11.7 Hz, 1H), 4.17 – 4.11 (m, *J* = 7.1 Hz, 3H), 3.85 – 3.81 (m, 2H), 3.76 (s, 3H), 3.66 (dd, *J* = 12.5, 6.1 Hz, 3H), 3.52 – 3.47 (m, 1H), 3.36 – 3.25 (m, 1H), 2.92 – 2.85 (m, 1H), 2.77 (s, 1H), 1.85 – 1.75 (m, 2H), 1.61 (t, *J* = 8.7 Hz, 3H), 1.46 – 1.37 (m, 3H), 1.31 (d, *J* = 7.2 Hz, 3H), 1.29 – 1.22 (m, 6H), 1.22 – 1.15 (m, 1H), 1.09 (d, *J* = 6.4 Hz, 3H), 1.04 (d, *J* = 6.3 Hz, 3H), 0.97 (s, 3H), 0.88 (d, *J* = 6.9 Hz, 3H), 0.78 (s, 3H). LCMS (ESI) found 1627.19. HRMS calcd. for [$C_{70}H_{86}O_{18}N_{18}S_5$+$H^+$] 1627.504, found (ESI) 1627.505.

**6:** isolated 5.6 mg, colorless powder, m.p. > 240 °C decomp., $R_f$ = 0.18 ($CHCl_3$/MeOH 9:1). HPLC: $t_R$ = 11.2 min. $^1$H NMR (600 MHz, $CDCl_3$/MeOD 4:1) δ = 8.07 (s, 1H), 8.01 (s, 2H), 7.81 (s, 1H), 6.72 (s, 1H), 6.67 (d, *J* = 10.2 Hz, 1H), 6.42 (s, 1H), 6.18 (s, 1H), 6.03 (d, *J* = 10.3 Hz, 1H), 5.72 – 5.64 (m, 1H), 5.48 (d, *J* = 1.3 Hz, 2H), 5.45 – 5.40 (m, 1H), 5.25 (s, 1H), 4.90 (q, *J* = 6.4 Hz, 2H), 4.85 – 4.79 (m, 1H), 4.75 (d, *J* = 11.9 Hz, 1H), 4.40 (s, 1H), 4.36 – 4.31 (m, 1H), 4.20 – 4.13 (m, 4H), 3.84 – 3.81 (m, 1H), 3.77 (s, 3H), 3.62 – 3.53 (m, 3H), 3.46 – 3.39 (m, 3H), 2.92 – 2.85 (m, 1H), 1.84 – 1.76 (m, 1H), 1.67 (d, *J* = 6.8 Hz, 3H), 1.46 – 1.39 (m, *J* = 6.6 Hz, 2H), 1.29 (d, *J* = 7.2 Hz, 3H), 1.27 (d, *J* = 6.5 Hz, 3H), 1.25 – 1.21 (m, 3H), 1.20 (d, *J* = 7.2 Hz, 3H), 1.12 – 1.07 (m, 3H), 1.04 (d, *J* = 5.8 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 3H), 0.77 (dd, *J* = 9.3, 5.4 Hz, 3H). LCMS (ESI) found 1627.19. HRMS calcd. for [$C_{70}H_{86}O_{18}N_{18}S_5$+$H^+$] 1627.504, found (ESI) 1627.505.

**NMR titrations / Kinetics**

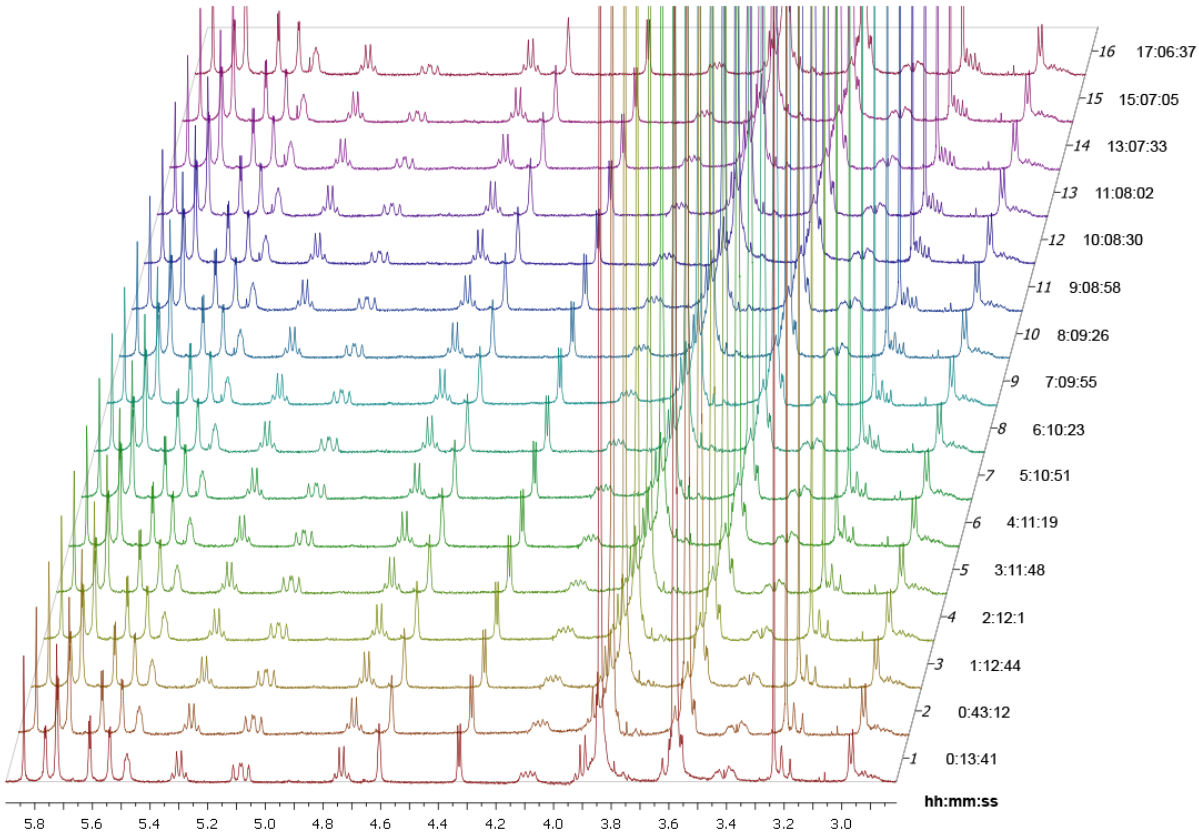
10 mg thiostrepton **1** was dissolved in 500 µl THF-$d_8$ followed by the addition of 95 µl buffer pH 12 (50 mM $Na_2CO_3$ in $D_2O$). 1D-1H-NMR spectra were recorded on a Varian 400 MHz spectrometer at different time intervals. The raw data was processed and the peak corresponding to the α-proton of the thiazoline (at 5.1 ppm) was integrated for the different time points. The values were normalized to non-exchanging protons of the molecule, and plotted against the time.

The data was fitted to a bi-exponential decay model according to the equation:

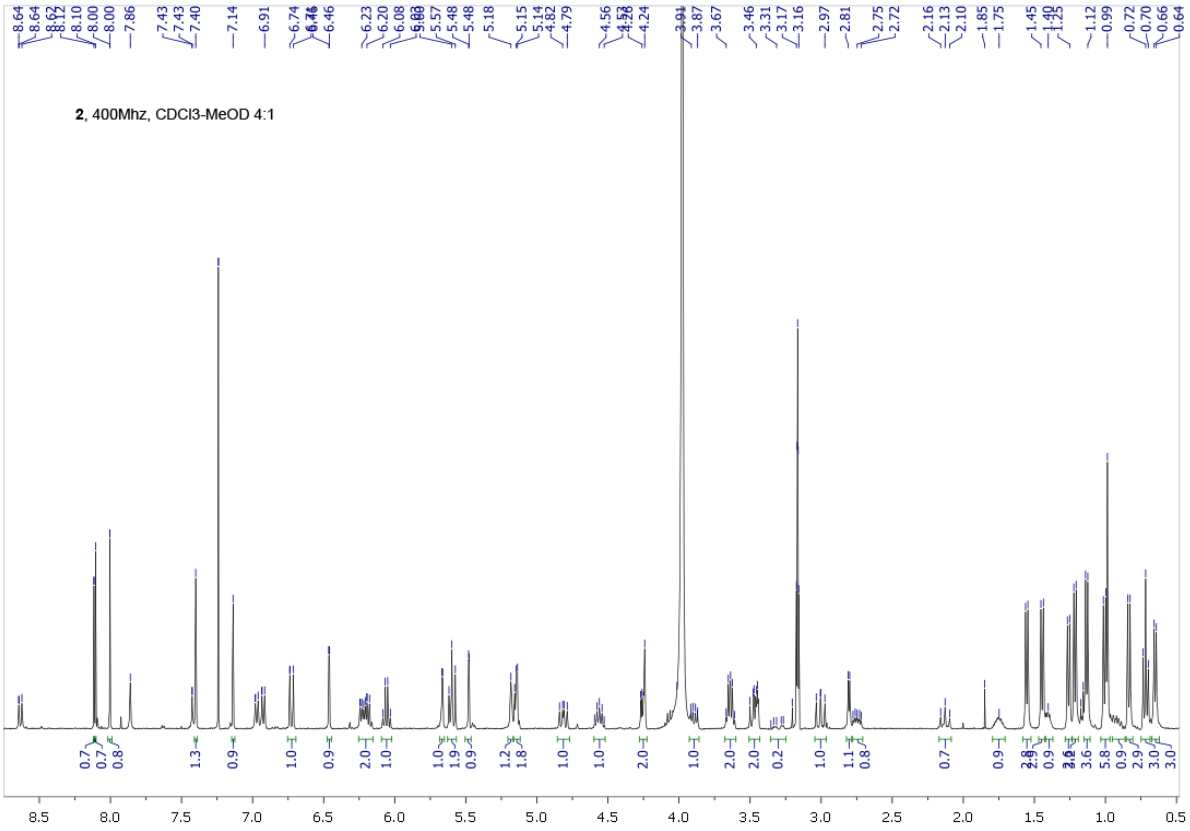
y = A1*exp(-x/t1) + A2*exp(-x/t2) + y0

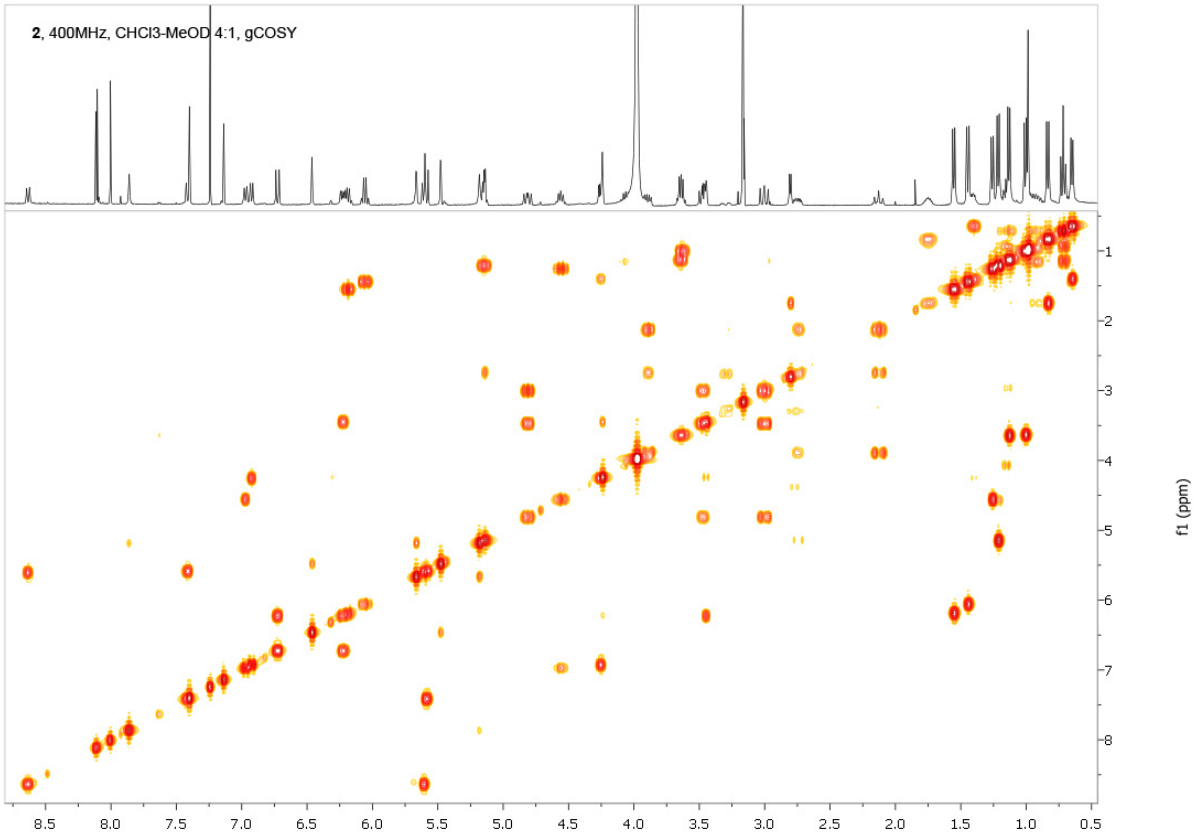
| $Chi^2$ | $R^2$ |
|---|---|
| 0.00046 | 0.99334 |

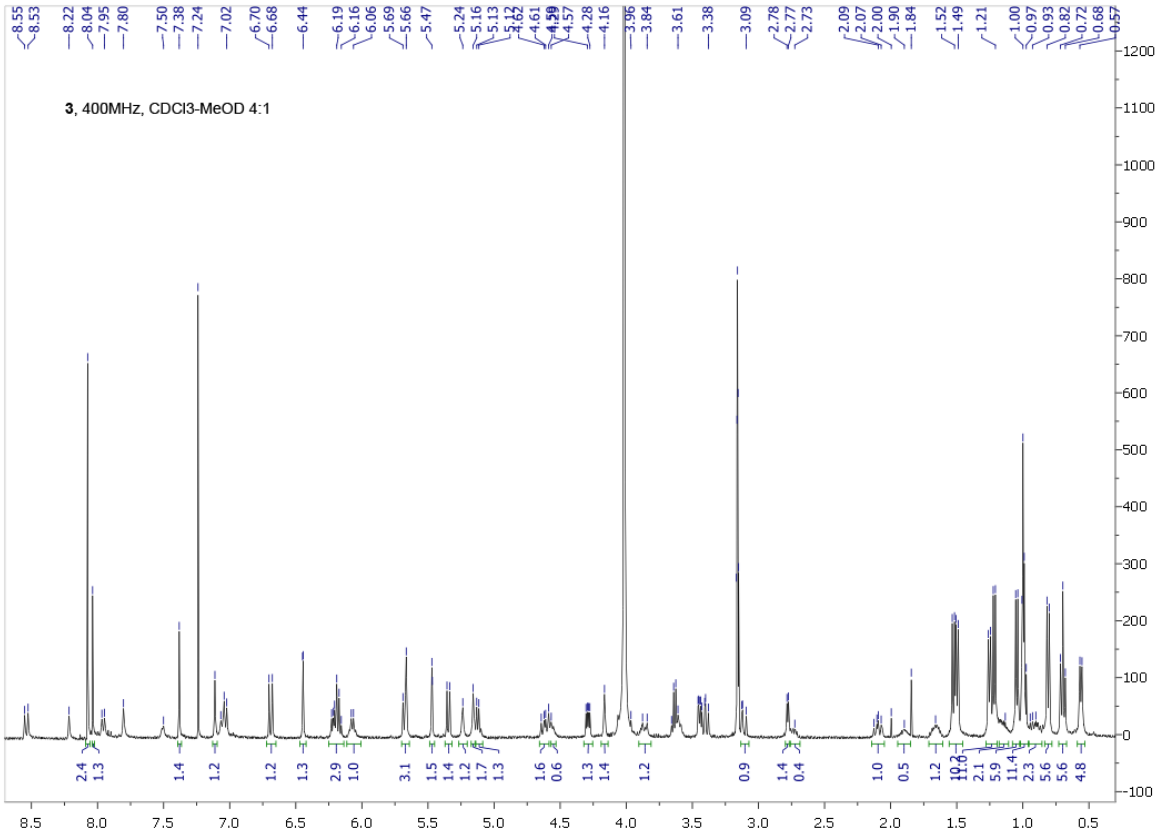
| Parameter | Value | Error |
|---|---|---|
| y0 | -1.13845 | 32.40731 |
| x0 | -6.8244E-8 | -- |
| A1 | 0.5576 | -- |
| t1 | 12.94255 | 5.25693 |
| A2 | 1.55263 | -- |
| t2 | 644.20996 | 15366.80749 |



## Selected NMR spectra

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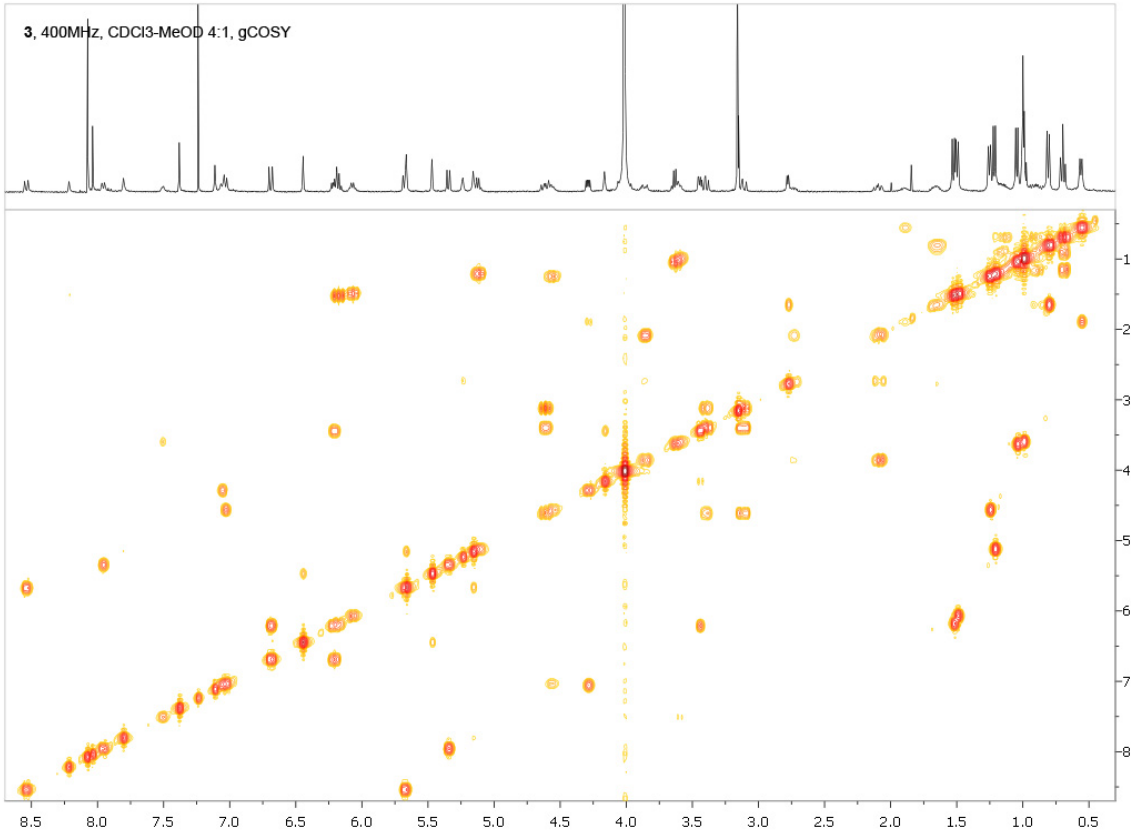
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