**Supplementary Materials**

# 4-*O*-glucosylation of trichothecenes by *Fusar*ium species: a phase II xenobiotic metabolism for t-type trichothecene producers

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**Table S1.** NMR spectroscopic data for **1** and **2**.

| TDmol-glc (**1**) | | | | TCC-glc (**2**) | | | |
|---|---|---|---|---|---|---|---|
| position | | $\delta_H$ (ppm) | $\delta_C$ (ppm) | position | | $\delta_H$ (ppm) | $\delta_C$ (ppm) |
| 2 | CH | 3.69 (d, 5.4) | 80.5 | 2 | CH | 3.83 (d, 5.2) | 80.9 |
| 3 | $CH_a$ | 2.51 (dd, 15.2, 8.0) | 38.8 | 3 | $CH_a$ | 2.60 (dd, 15.5, 7.9) | 38.9 |
| 3 | $CH_b$ | 2.00 (ddd, 15.2, 5.4, 3.7) | | 3 | $CH_b$ | 2.15 (ddd, 15.5, 5.2, 3.9) | |
| 4 | CH | 4.49 (dd, 8.0, 3.7) | 79.4 | 4 | CH | 4.46 (dd, 7.9, 3.9) | 78.8 |
| 5 | C | - | 51.0 | 5 | C | - | 51.0 |
| 6 | C | - | 41.2 | 6 | C | - | 44.5 |
| 7 | $CH_a$ | 1.88 (dt, 12.6, 5.7) | 25.3 | 7 | $CH_a$ | 2.87 (dd, 15.4, 0.9) | 43.0 |
| 7 | $CH_b$ | 1.47 (ddt, 12.6, 5.4, 1.7) | | 7 | $CH_b$ | 2.26 (d, 15.4) | |
| 8 | $CH_2$ | 1.90-2.10 (m) | 29.0 | 8 | $CH_2$ | - | 201.0 |
| 9 | C | - | 141.3 | 9 | C=O | - | 138.6 |
| 10 | CH | 5.35 (brd, 4.9) | 119.8 | 10 | CH | 6.53 (brd, 5.9) | 139.5 |
| 11 | CH | 3.58-3.63** (m) | 71.8 | 11 | CH | 3.98 (brd, 5.9) | 71.1 |
| 12 | C | - | 67.4 | 12 | C | - | 67.3 |
| 13 | $CH_a$ | 3.11 (d, 4.0) | 49.1*** | 13 | $CH_a$ | 3.14 (d, 3.7) | 48.6 |
| 13 | $CH_b$ | 2.95 (d, 4.0) | | 13 | $CH_b$ | 2.98 (d, 3.7) | |
| 14 | $CH_3$ | 0.85 (s) | 6.8 | 14 | $CH_3$ | 0.85 (s) | 6.5 |
| 15 | $CH_3$ | 0.91 (s) | 16.4 | 15 | $CH_3$ | 1.00 (d, 0.9) | 18.7 |
| 16 | $CH_3$ | 1.70 (brs) | 23.3 | 16 | $CH_3$ | 1.77 (brs) | 15.3 |
| 1' | CH | 4.86* (m) | 100.8 | 1' | CH | 4.86 (d, 3.7) | 101.0 |
| 2' | CH | 3.36 (dd, 9.7, 3.7) | 74.1 | 2' | CH | 3.36 (dd, 9.7, 3.7) | 74.0 |
| 3' | CH | 3.61-3.89** (m) | 75.0 | 3' | CH | 3.61-3.70**** (m) | 74.8 |
| 4' | CH | 3.24 (t, 9.3) | 72.1 | 4' | CH | 3.25 (t, 9.3) | 72.1 |
| 5' | CH | 3.61-3.89** (m) | 74.4 | 5' | CH | 3.61-3.70**** (m) | 74.4 |
| 6' | $CH_a$ | 3.86 (brd, 9.7) | 62.9 | 6' | $CH_a$ | 3.84-3.89 (m) | 62.9 |
| 6' | $CH_b$ | 3.61-3.89** (m) | | 6' | $CH_b$ | 3.61-3.70**** (m) | |

*Overlapped peak of CH and $H_2O$.
**Overlapped peak of CH and three kinds of CH on the structure of sugar.
***Overlapped peak of C and solvent.
****Overlapped peak of three kinds of CH on the structure of sugar.

**Table S2.** NMR spectroscopic data for **3**.

| HT-2-glc (**3**) | | | |
|---|---|---|---|
| position | | $\delta_H$ (ppm) | $\delta_C$ (ppm) |
| 2 | CH | 3.49 (d, 4.9) | 80.6 |
| 3 | CH | 4.28 (dd, 4.9, 3.2) | 79.1 |
| 4 | CH | 4.57 (d, 3.2) | 87.8 |
| 5 | C | - | 50.5 |
| 6 | C | - | 44.1 |
| 7 | $CH_a$ | 1.89 (brd, 15.4) | 29.1 |
| 7 | $CH_b$ | 2.40 (dd,15.4, 5.7) | |
| 8 | CH | 5.32 (d,5.7) | 69.5 |
| 9 | C | - | 137.2 |
| 10 | CH | 5.75 (brd, 5.7) | 125.2 |
| 11 | CH | 4.27 (brd, 5.7) | 68.5 |
| 12 | C | - | 66.1**** |
| 13 | $CH_a$ | 2.89 (d, 4.0) | 48.2 |
| 13 | $CH_b$ | 3.04 (d, 4.0) | 7.5 |
| 14 | $CH_3$ | 0.86 (s) | |
| 15 | $CH_a$ | 3.99 (d, 12.6) | 66.1**** |
| 15 | $CH_b$ | 4.42 (d,12.6) | |
| 16 | $CH_3$ | 1.74 (brs) | 20.4 |
| 1' | C=O | - | 172.2 |
| 2' | $CH_a$ | 2.17 (dd, 14.6, 6.6) | 44.5 |
| 2' | $CH_b$ | 2.21 (dd, 14.6, 7.7) | |
| 3' | CH | 2.04-2.14* (m) | 26.9 |
| 4' / 5' | $CH_3$ | 0.98 (d, 6.6) | 22.8 |
| 5' / 4' | $CH_3$ | 0.97 (d, 6.6) | 22.7 |
| 1" | C=O | - | 174.0 |
| 2" | $CH_3$ | 2.08 (s) | 21.1 |
| 1"' | CH | 4.91 (d, 4.0) | 101.3 |
| 2"' | CH | 3.41 (dd, 10.0, 4.0) | 74.0 |
| 3"' | CH | 3.61-3.71** (m) | 75.0 |
| 4"' | CH | 3.26-3.33*** (m) | 72.0 |
| 5"' | CH | 3.79 (ddd, 9.9, 6.4, 2.0) | 74.3 |
| 6"' | $CH_a$ | 3.61-3.71** (m) | 62.6 |
| 6"' | $CH_b$ | 3.91 (dd, 11.5, 2.0) | |

*Overlapped peak of CH and $CH_3$.
**Overlapped peak of two kinds of CH on the structure of sugar.
***Overlapped peak of CH and solvent.
****Overlapped peak of two kinds of carbon.

**Table S3.** NMR spectroscopic data for **4**.

| THA-glc (**4**) | | | |
|---|---|---|---|
| position | | $\delta_H$ (ppm) | $\delta_C$ (ppm) |
| 2 | CH | 3.98 (dd, 11.7, 6.3) | 79.0 |
| 3 | $CH_a$ | 1.81 (q, 11.7) | 40.2 |
| 3 | $CH_b$ | 2.44-2.53 (m) | |
| 4 | CH | 3.89 (dd, 11.7, 5.4) | 80.7 |
| 5 | C | - | 60.2 |
| 6 | C | - | 44.5 |
| 7 | $CH_a$ | 1.31 (dd, 12.6, 5.7) | 26.8 |
| 7 | $CH_b$ | 1.67 (dd, 12.6, 6.0) | |
| 8 | $CH_a$ | 1.92 (dd, 18.3, 5.7) | 29.3 |
| 8 | $CH_b$ | 2.02-3.13 (m) | |
| 9 | C | - | 141.9 |
| 10 | CH | 5.50 (dm, 4.3) | 119.5 |
| 11 | CH | 3.70-3.74* (m) | 78.6 |
| 12 | C | - | 91.5 |
| 13 | $CH_a$ | 3.71 (d,11.7) | 65.6 |
| 13 | $CH_b$ | 3.78 (d,11.7) | |
| 14 | $CH_3$ | 1.10 (s) | 10.6 |
| 15 | $CH_3$ | 0.89 (s) | 15.0 |
| 16 | $CH_3$ | 1.71 (brs) | 23.8 |
| 1' | CH | 4.87 (d,3.7) | 101.9 |
| 2' | CH | 3.36 (dd, 9.7, 3.7) | 74.0 |
| 3' | CH | 3.63 (dd, 9.7, 8.9) | 75.2 |
| 4' | CH | 3.29-3.34** (m) | 72.2 |
| 5' | CH | 3.59-3.64*** (m) | 74.4 |
| 6' | $CH_a$ | 3.69 (dd, 12.0, 5.2) | 62.9 |
| 6' | $CH_b$ | 3.77 (d, 12.0, 2.6) | |

*Overlapped peak of two kinds of CH.
**Overlapped peak of CH and solvent.
***Overlapped peak of two kinds of CH on the structure of sugar.

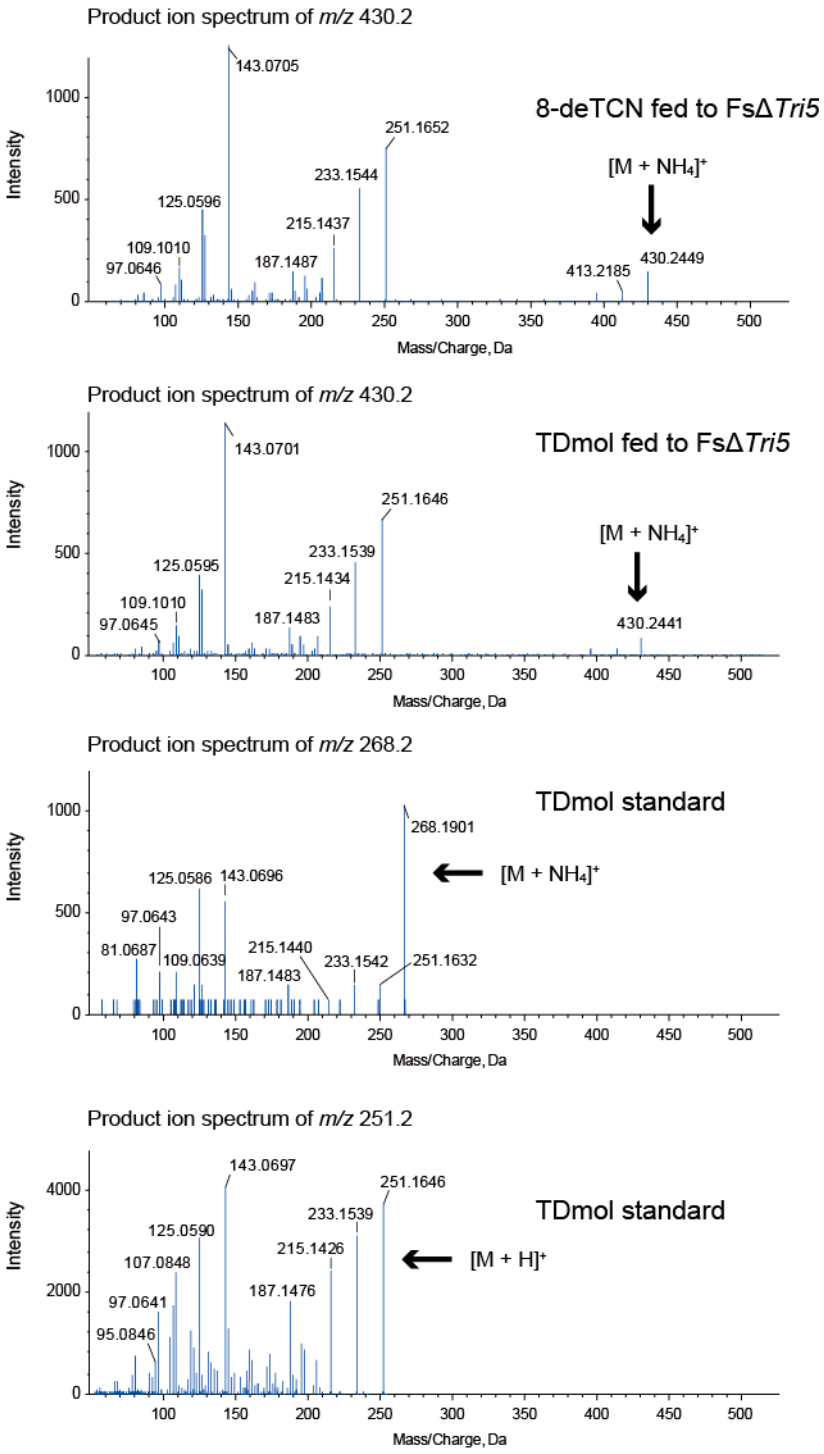



**Figure S1.** LC-MS/MS analysis of the acetonitrile extract of the culture fed with trichodermol (TDmol) and 8-deoxytrichothecin (8-deTCN). The trichothecenes fed to the culture of strain FsΔ*Tri5* were recovered at 48 h.

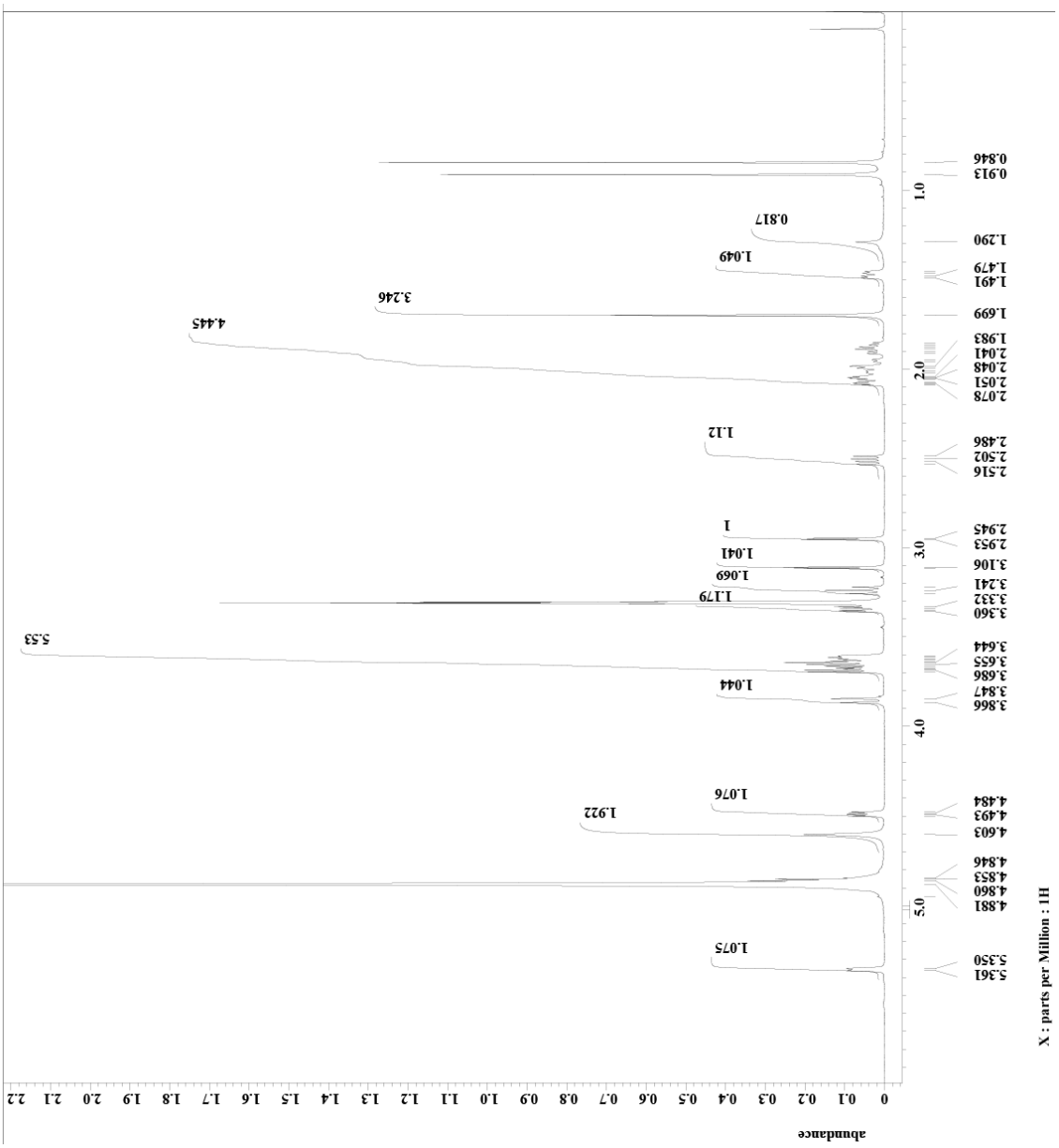



**Figure S2-a.** $^{1}$H-NMR spectrum of **1**.

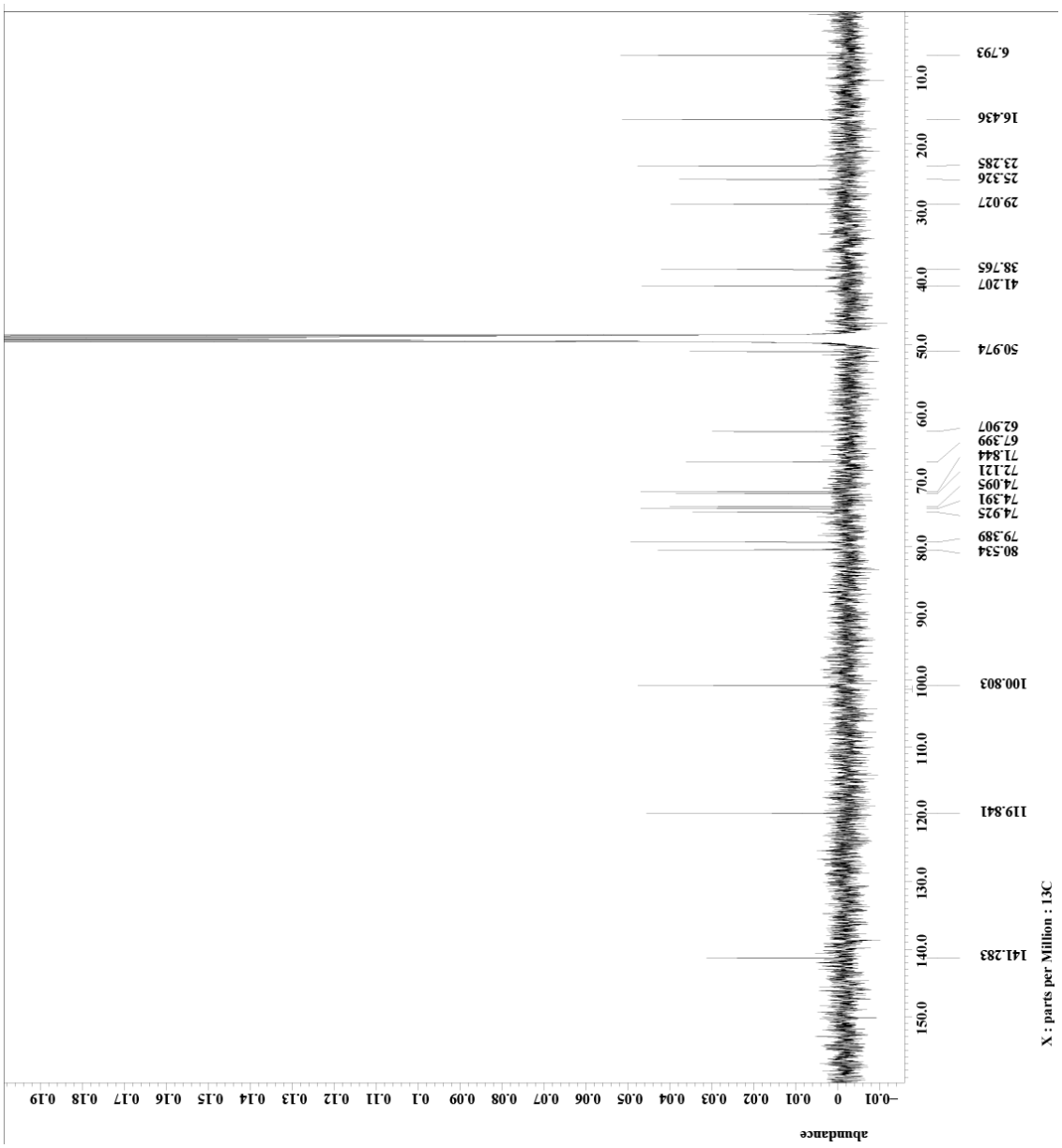



**Figure S2-b.** $^{13}$C NMR spectrum of **1**.

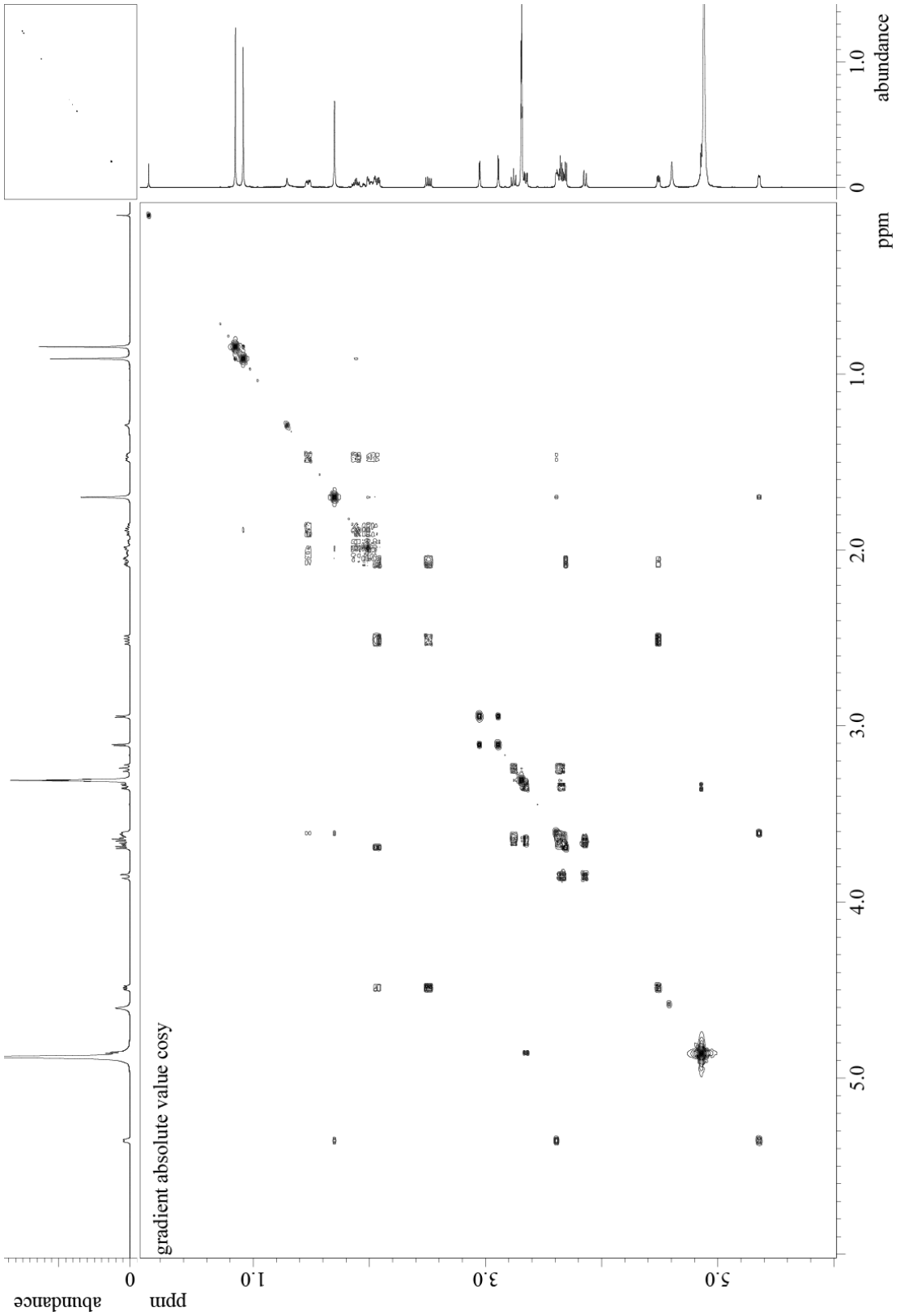



**Figure S2-c.** Correlation spectroscopy (COSY) spectrum of **1**.

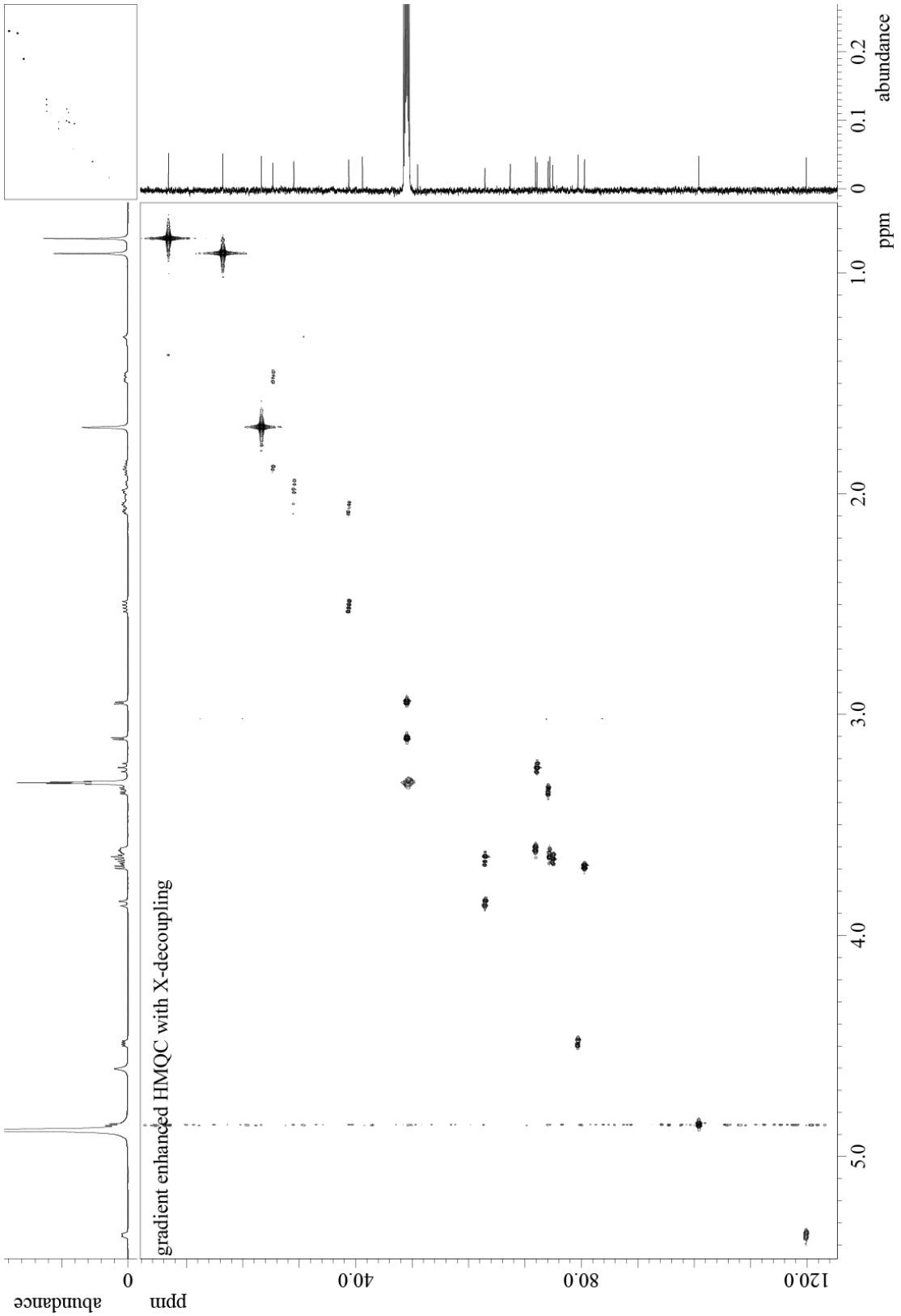



**Figure S2-d.** Heteronuclear multiple quantum coherence (HMQC) spectrum of **1**.

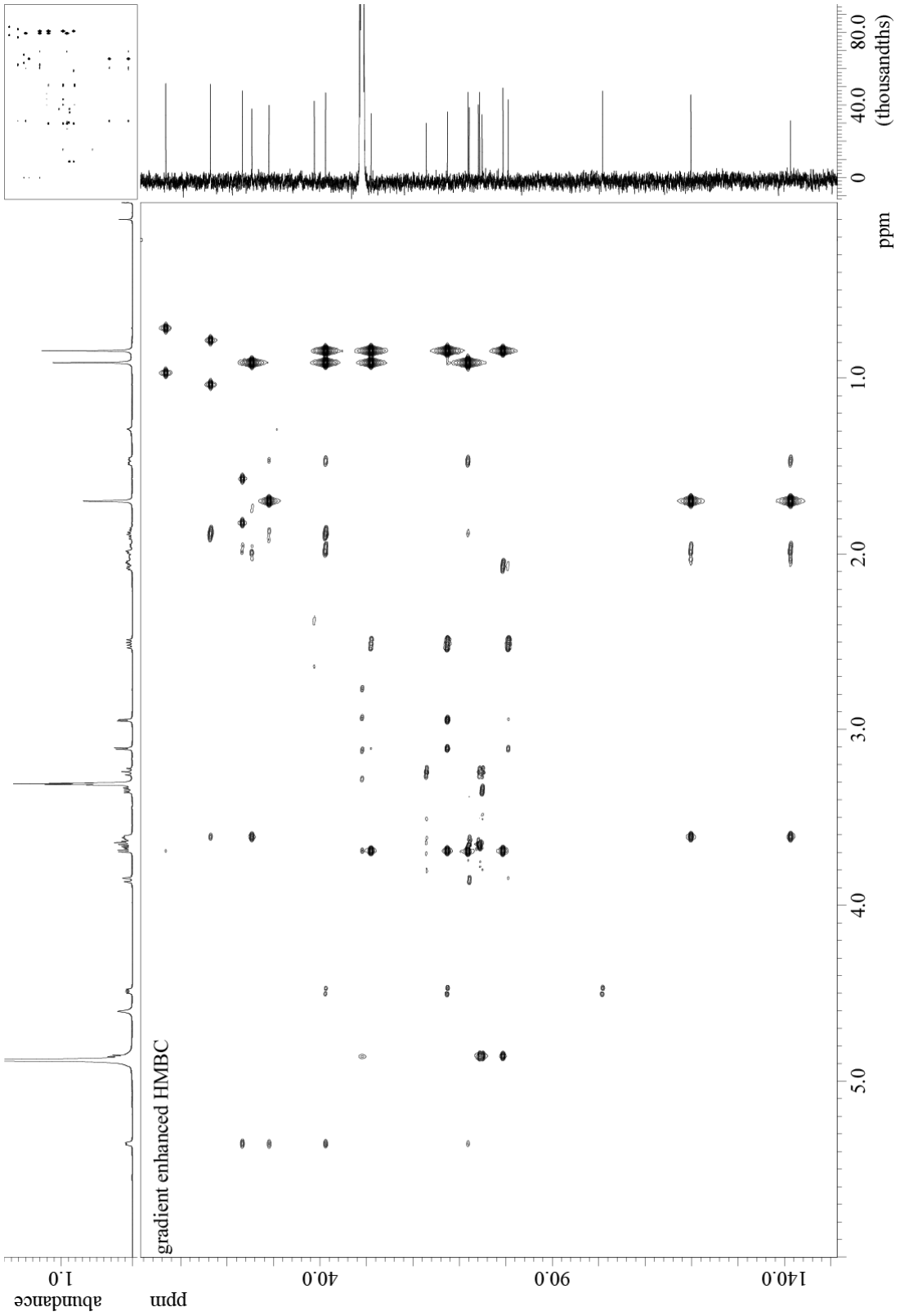



**Figure S2-e.** Heteronuclear multiple bond coherence (HMBC) spectrum of **1**.

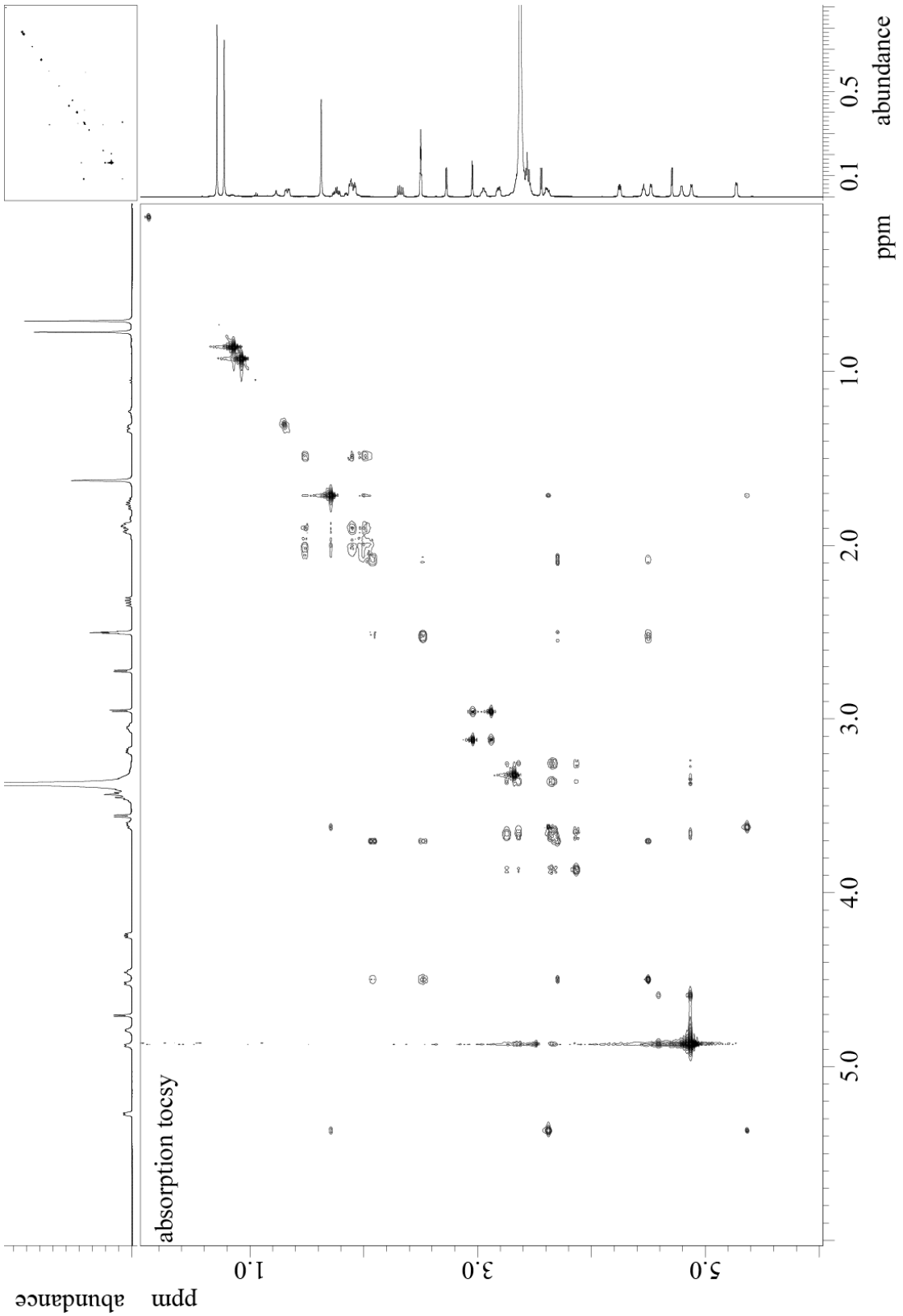



**Figure S2-f.** Total correlation spectroscopy (TOCSY) spectrum of **1**.

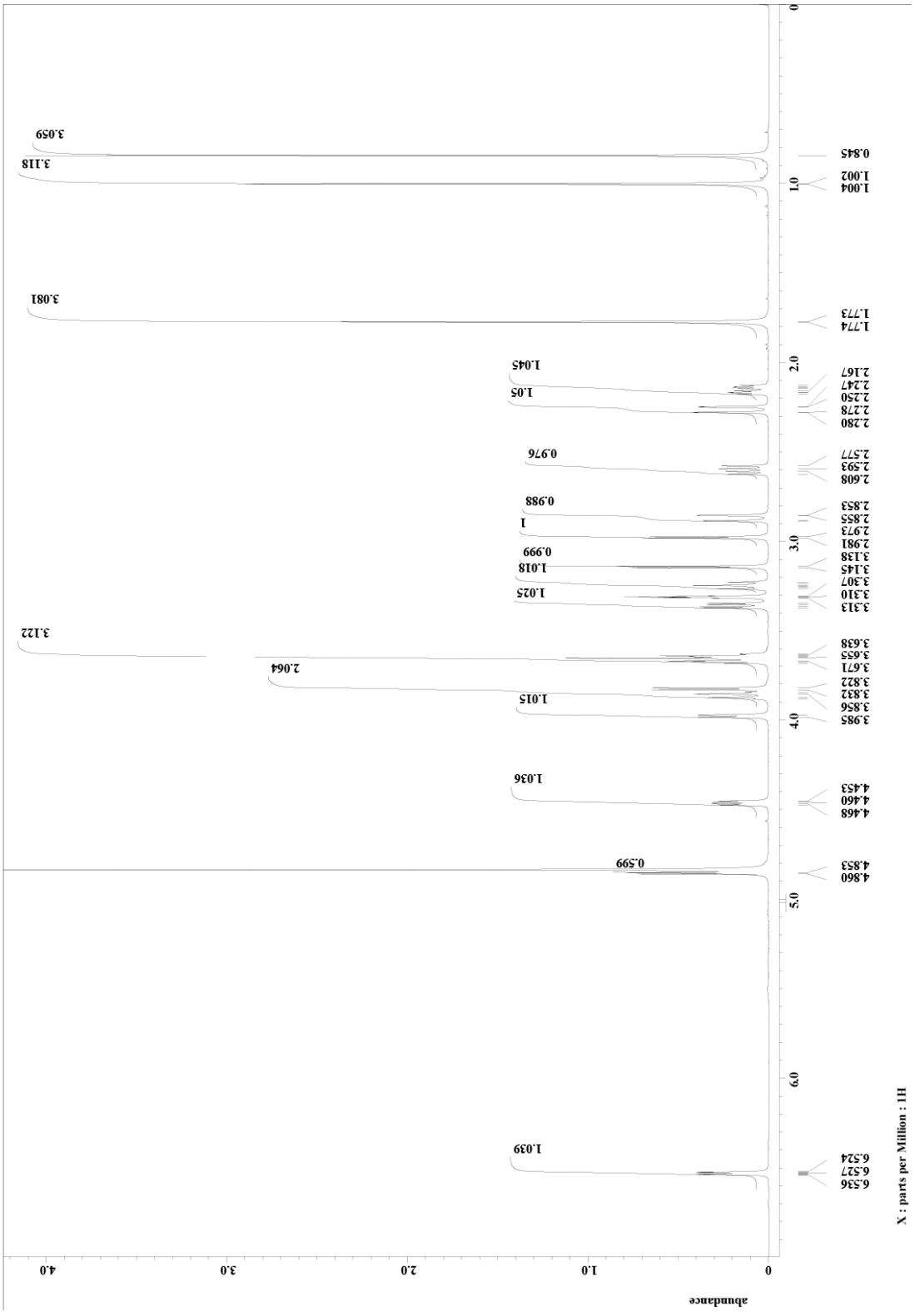



**Figure S3-a.** $^{1}$H NMR spectrum of **2**.

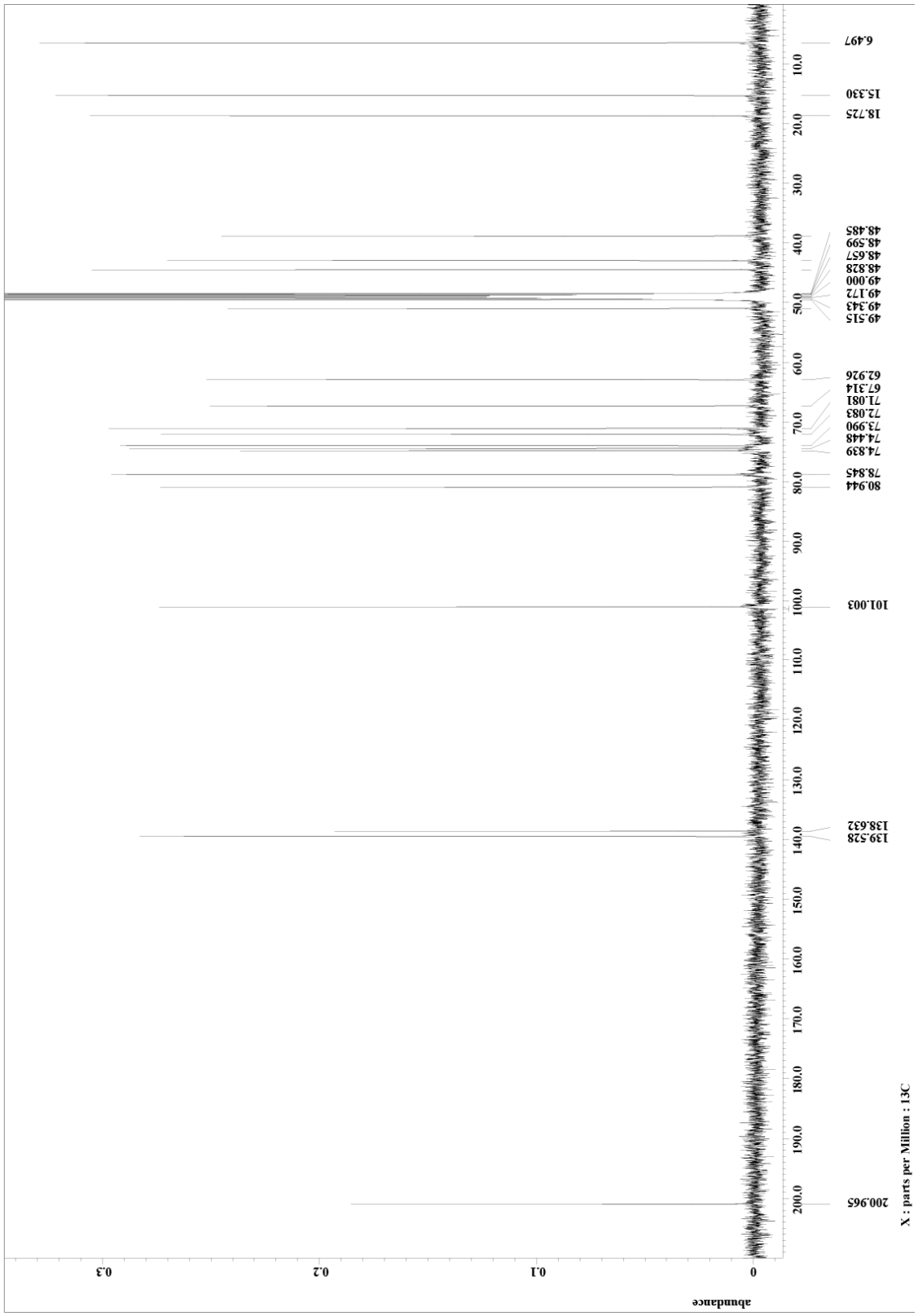



**Figure S3-b.** $^{13}$C NMR spectrum of **2**.

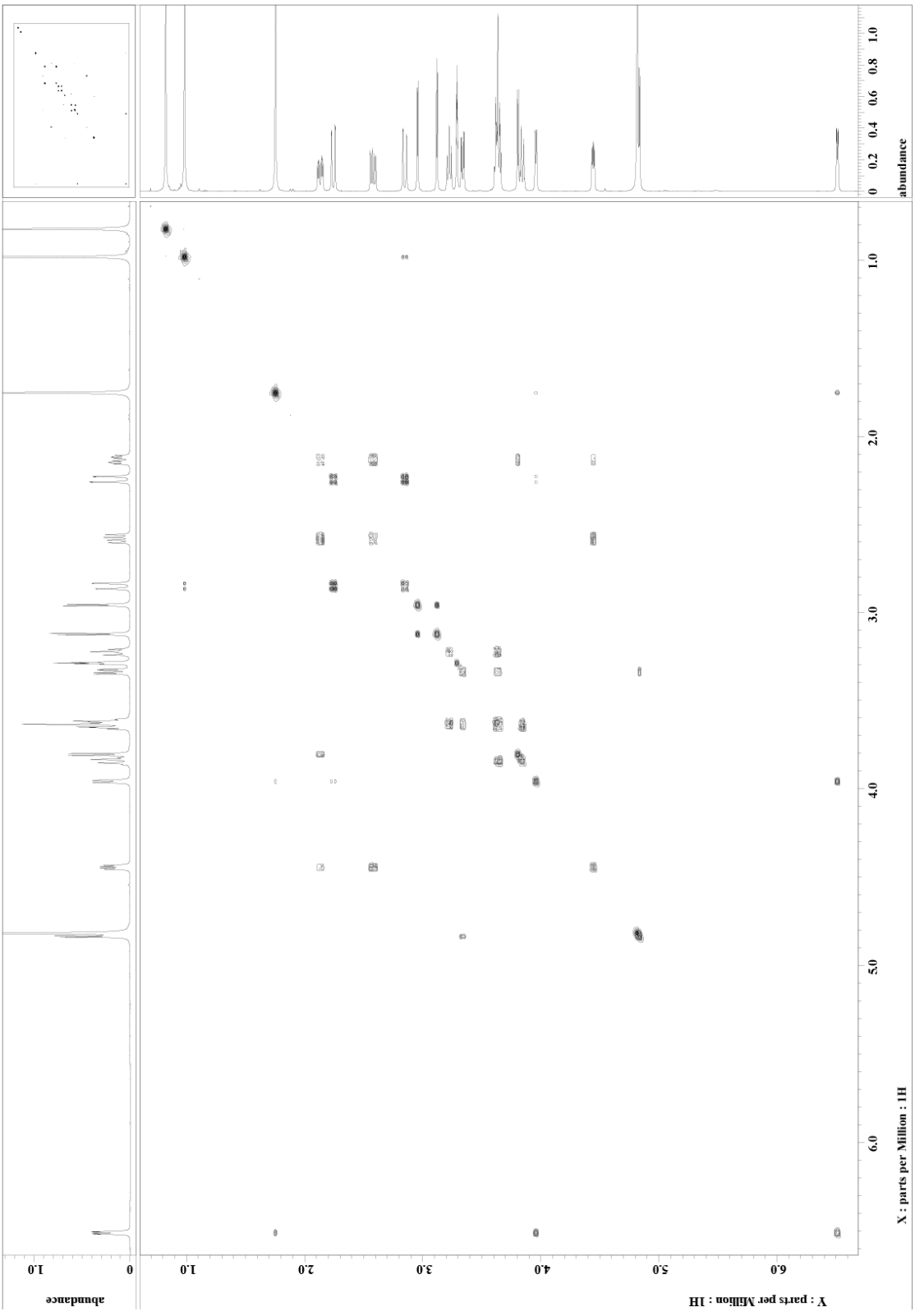



**Figure S3-c.** Correlation spectroscopy (COSY) spectrum of **2**.

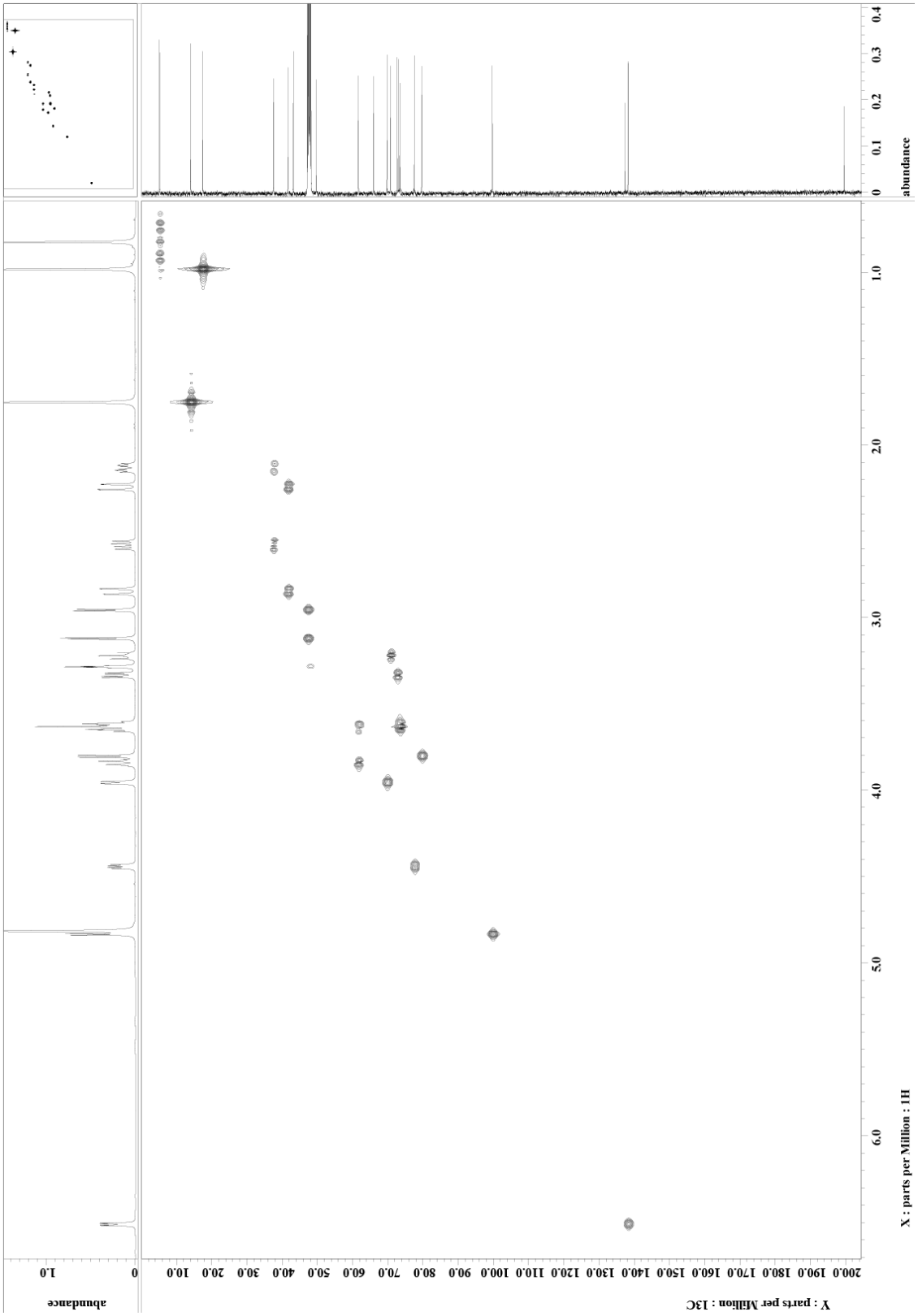



**Figure S3-d.** Heteronuclear multiple quantum coherence (HMQC) spectrum of **2**.

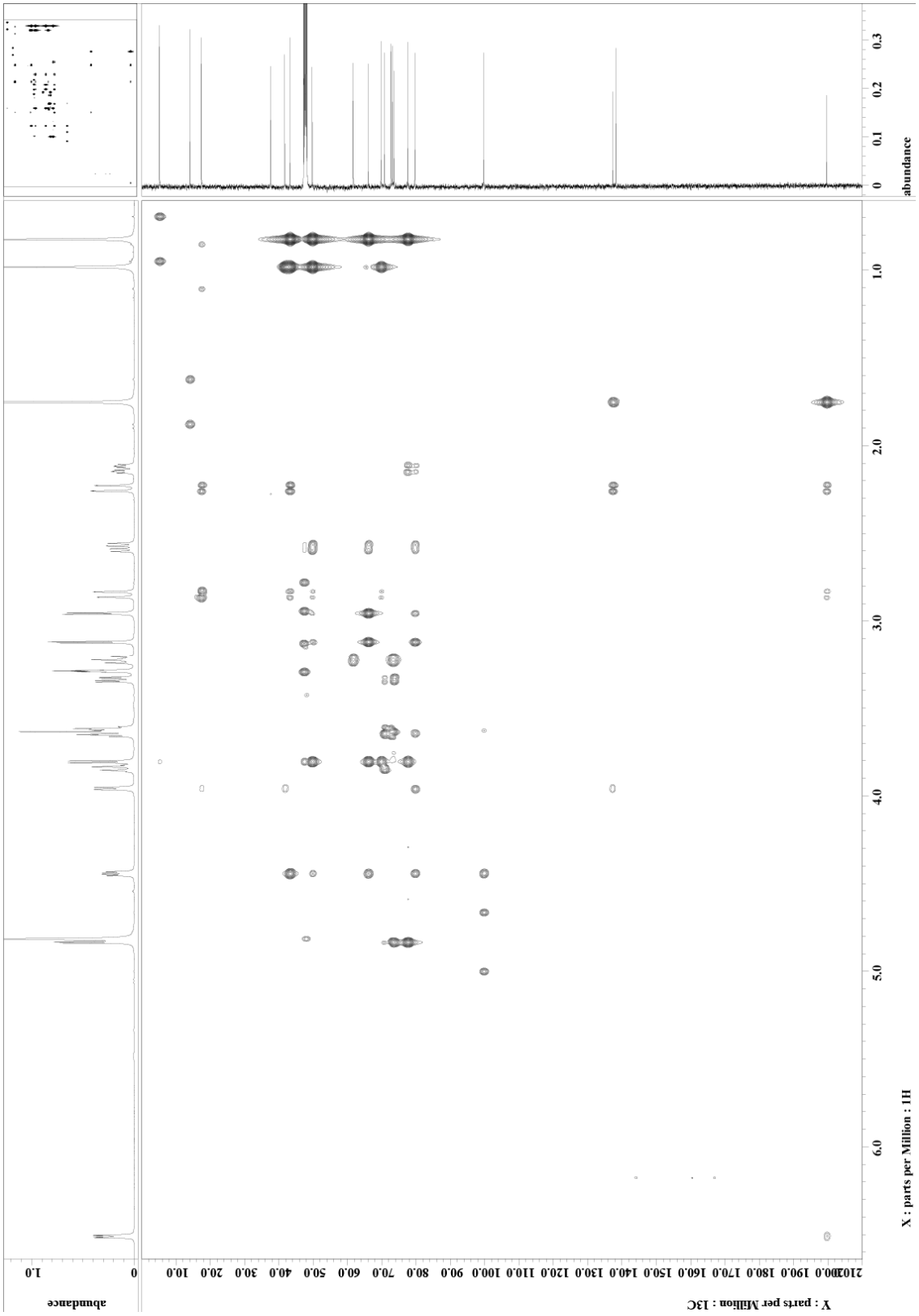



**Figure S3-e.** Heteronuclear multiple bond coherence (HMBC) spectrum of **2**.

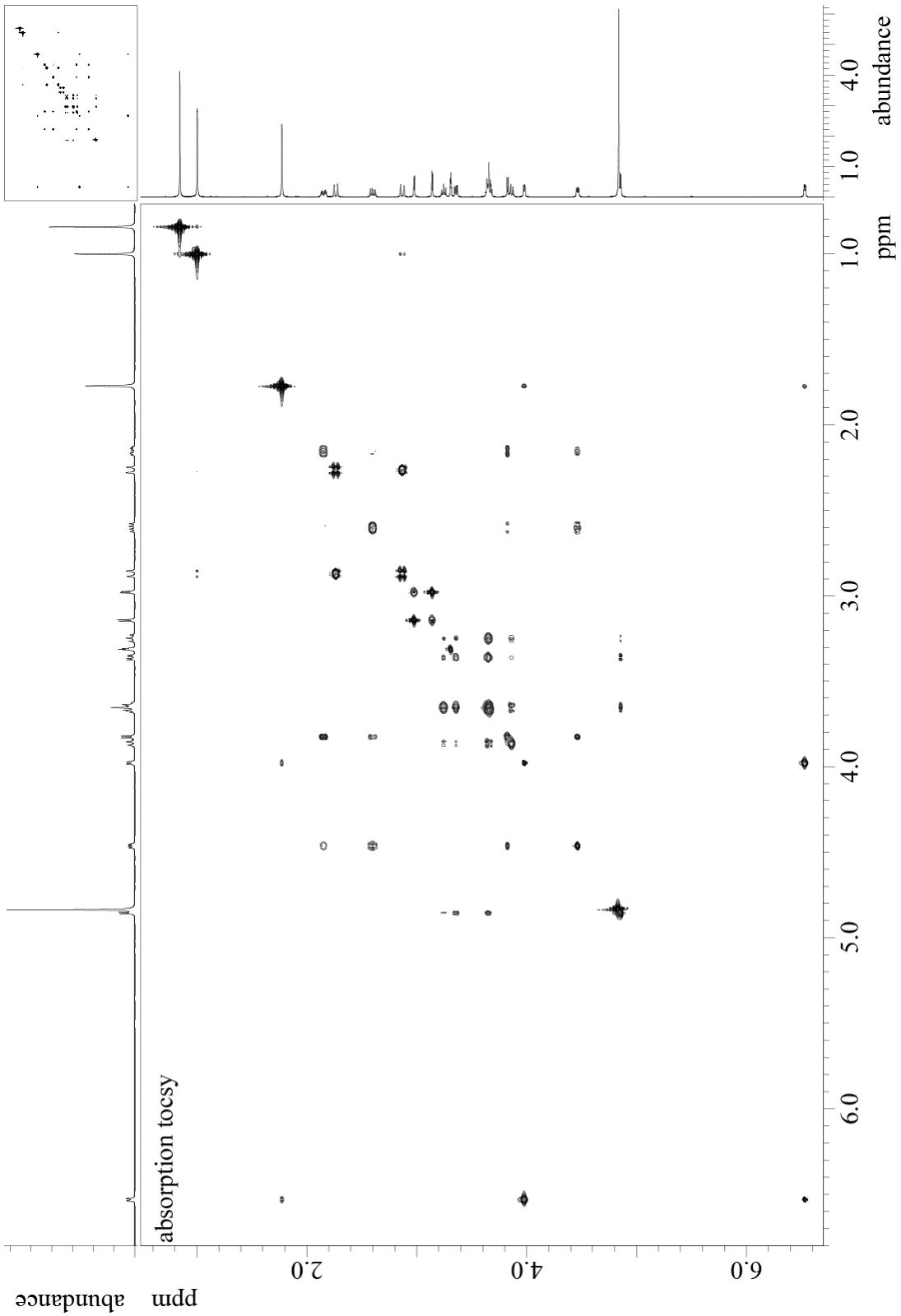



**Figure S3-f.** Total correlation spectroscopy (TOCSY) spectrum of **2**.

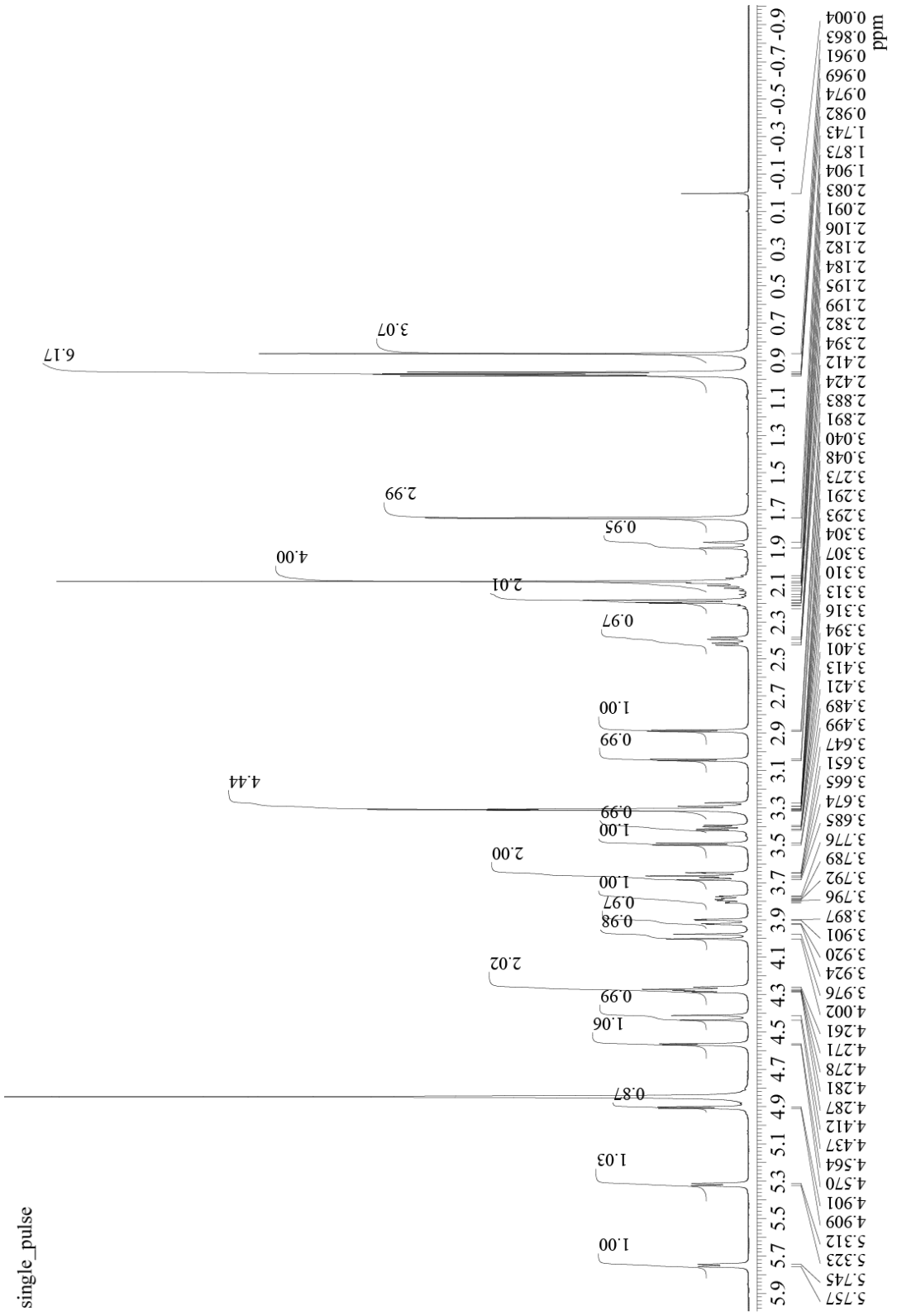



**Figure S4-a.** $^{1}$H NMR spectrum of **3**.

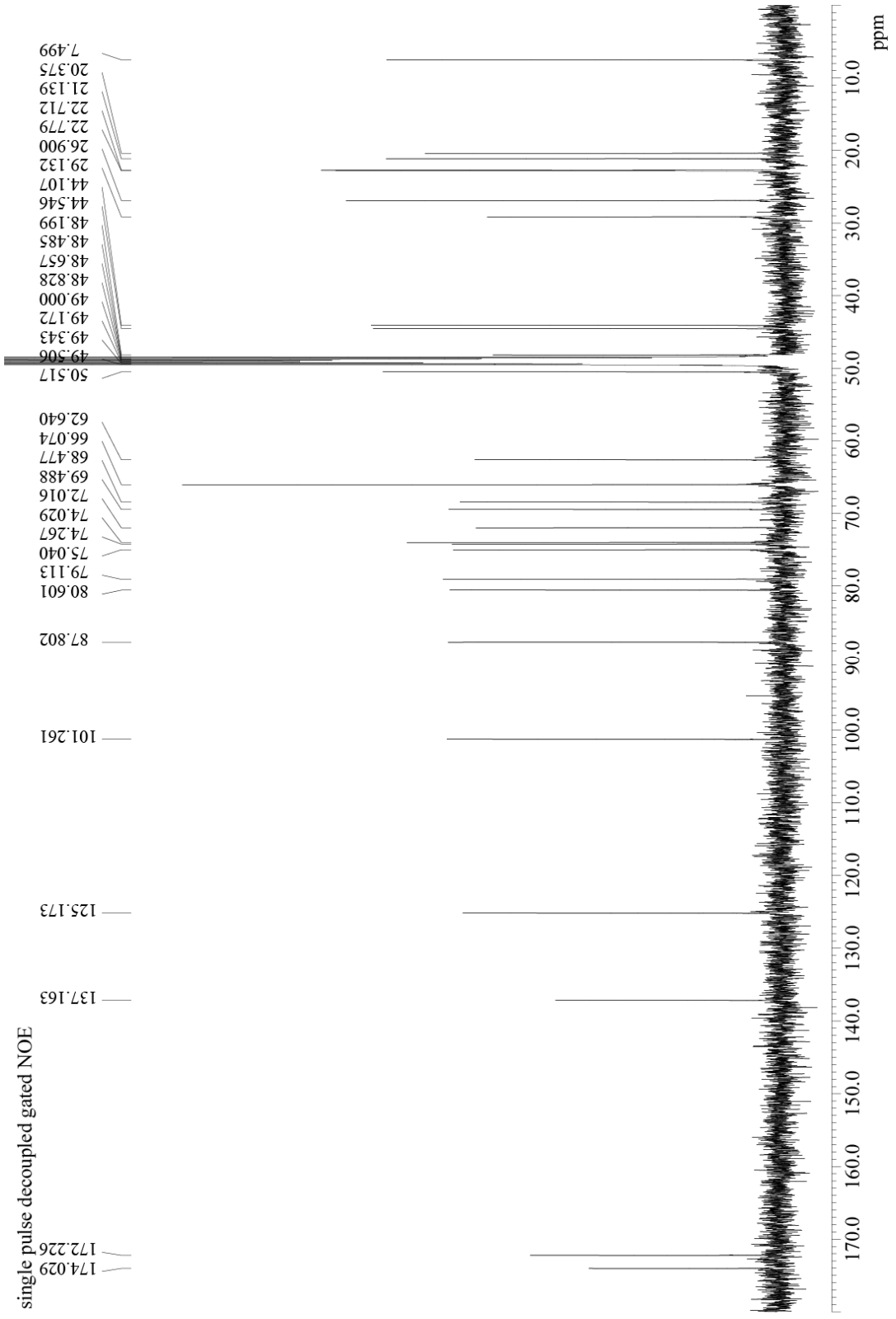



**Figure S4-b.** $^{13}$C NMR spectrum of **3**.

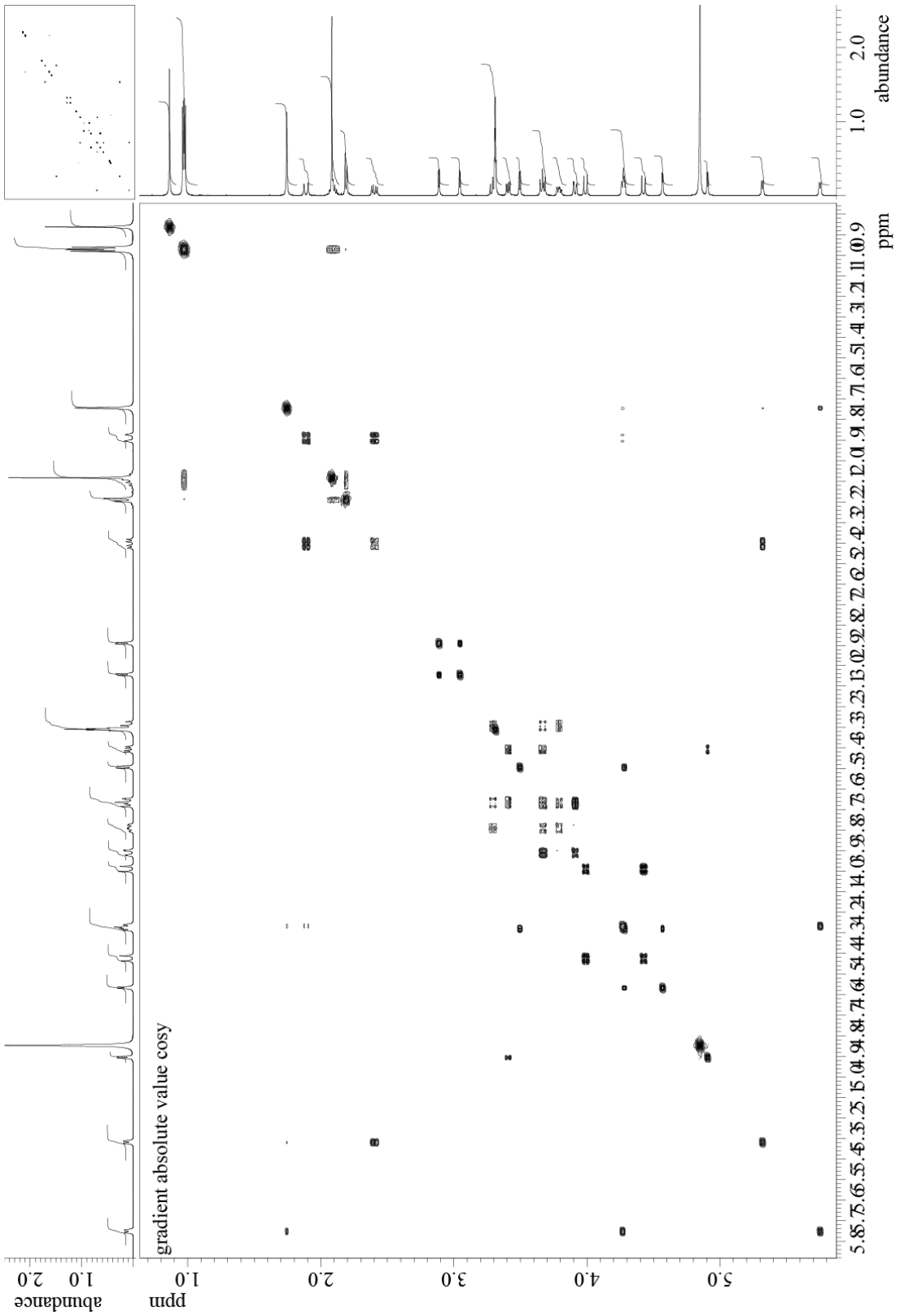



**Figure S4-c.** Correlation spectroscopy (COSY) spectrum of **3**.

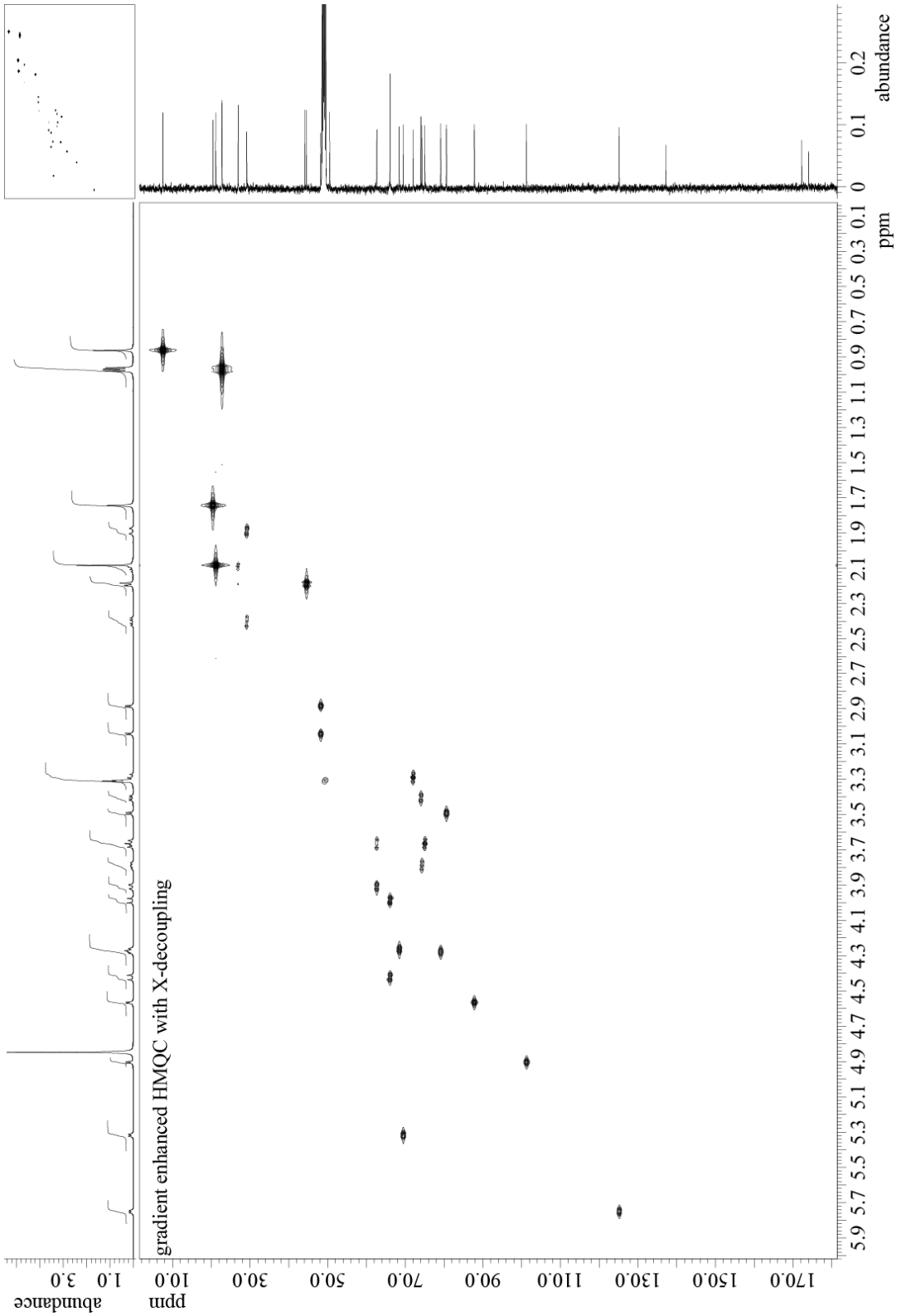



**Figure S4-d.** Heteronuclear multiple quantum coherence (HMQC) spectrum of **3**.

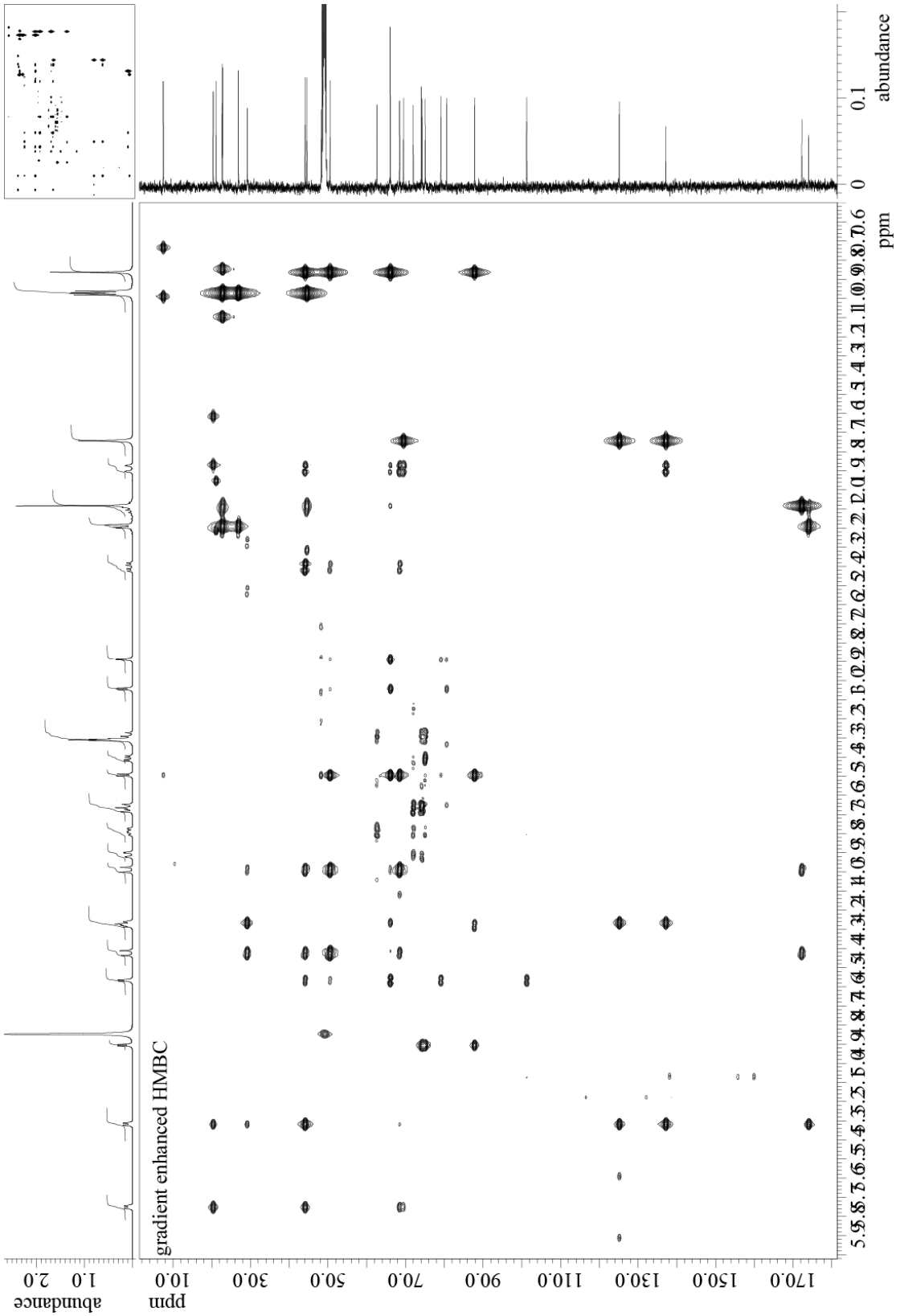



**Figure S4-e.** Heteronuclear multiple bond coherence (HMBC) spectrum of **3**.

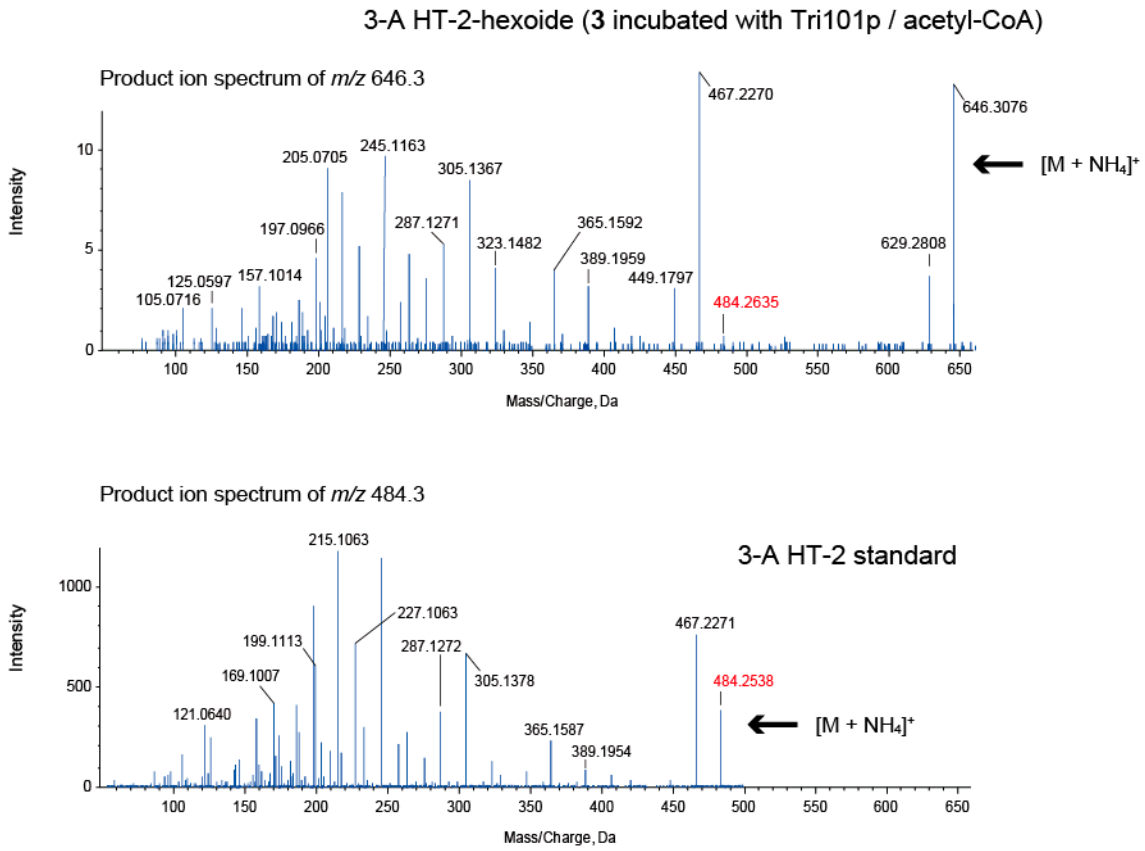



**Figure S5.** LC-MS/MS analysis of the Tri101p reaction mixture of HT-2-toxin-4-*O*-α-glucopyranoside (HT-2-4-glc) and acetyl CoA. MS/MS spectrum corresponding to *m*/*z* 646.3069 was detected from the mixture after 6 h of incubation (upper penal). MS/MS spectrum of purified 3-acetyl HT-2 toxin (3-A HT-2) standard, detected as [3-A HT-2 + $NH_4$]$^+$, is shown for comparison.

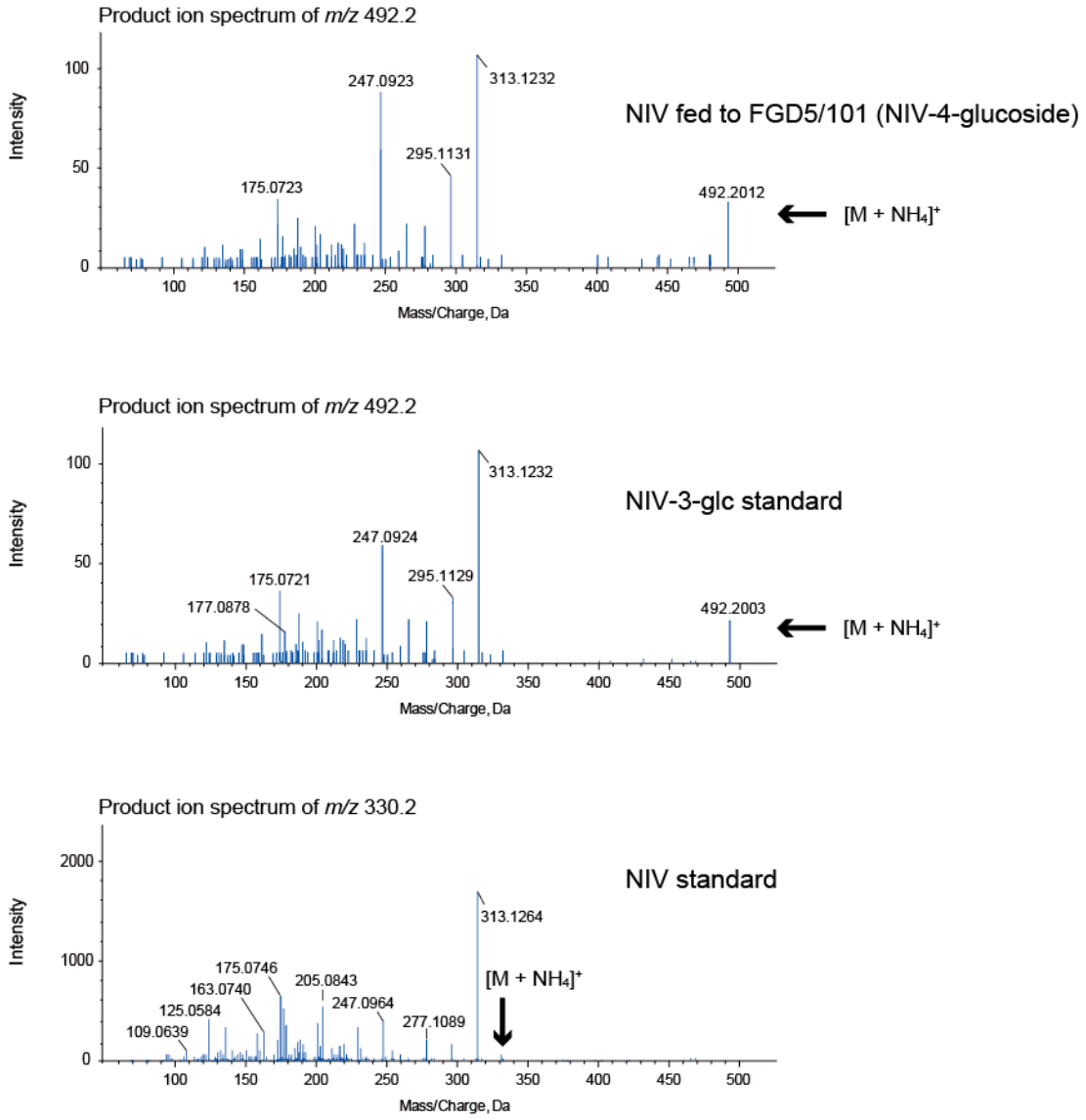



**Figure S6.** Comparison of MS/MS spectra of putative NIV-4-*O*-glucoside and NIV-3-*O*-β-glucopyranoside (glc) standard. Putative NIV-4-glucoside (upper panel) and NIV-3-glc standard (middle panel) were analyzed by LC-MS/MS in a set of experiment. As a reference, NIV standard was also analyzed under the same condition (lower panel).

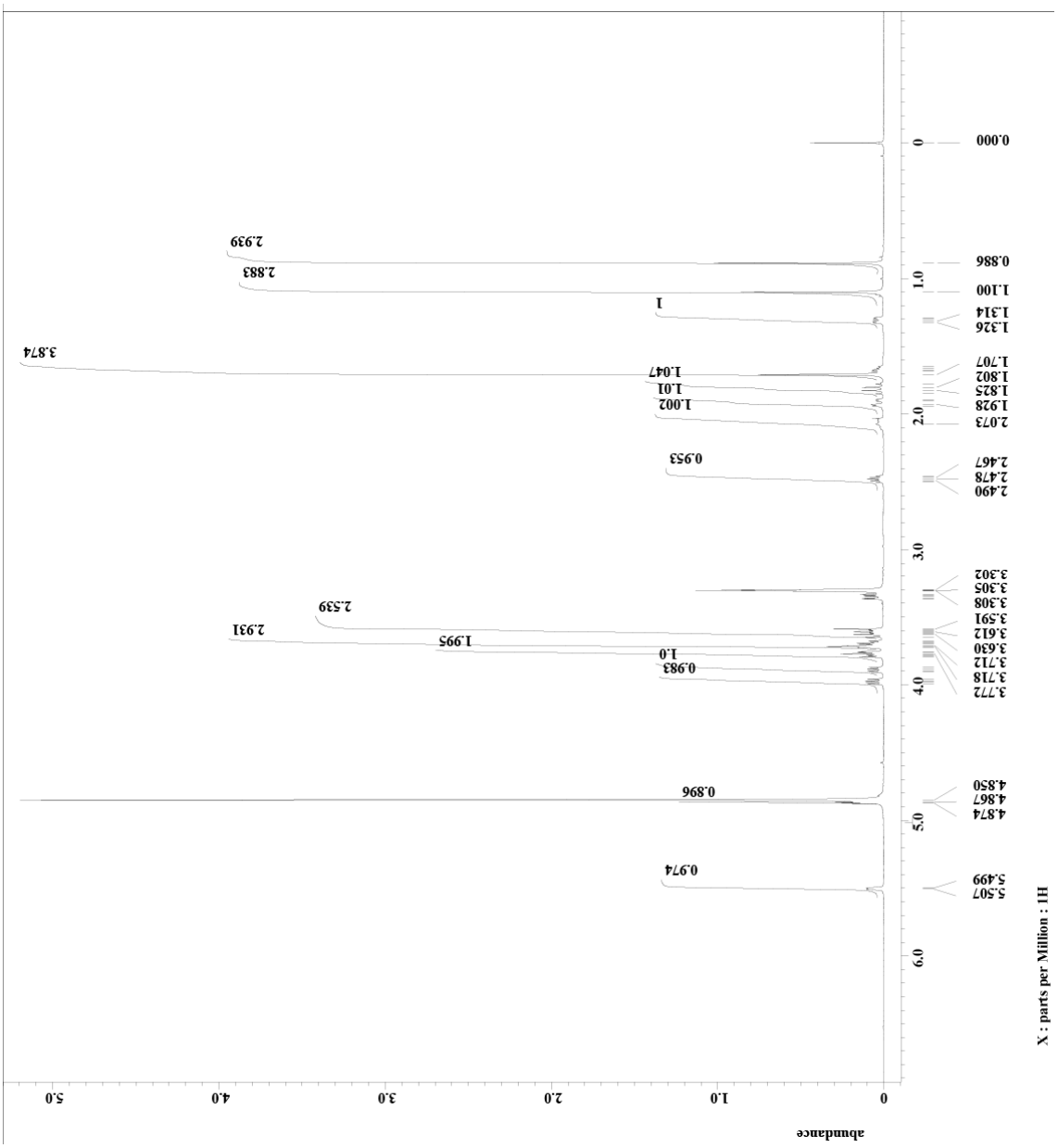



**Figure S7-a.** $^{1}$H NMR spectrum of **4**.

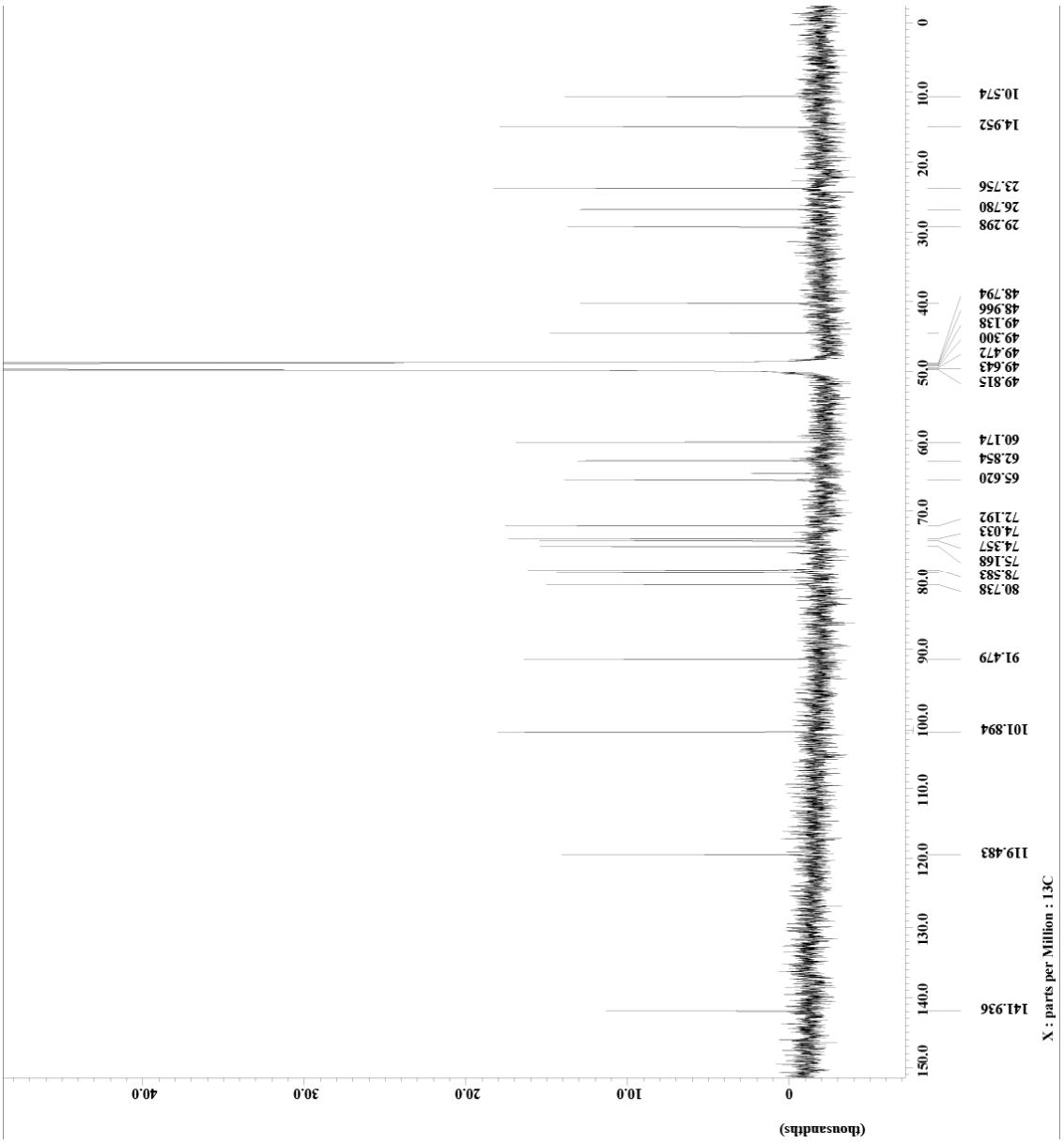



**Figure S7-b.** $^{13}C$ NMR spectrum of **4**.

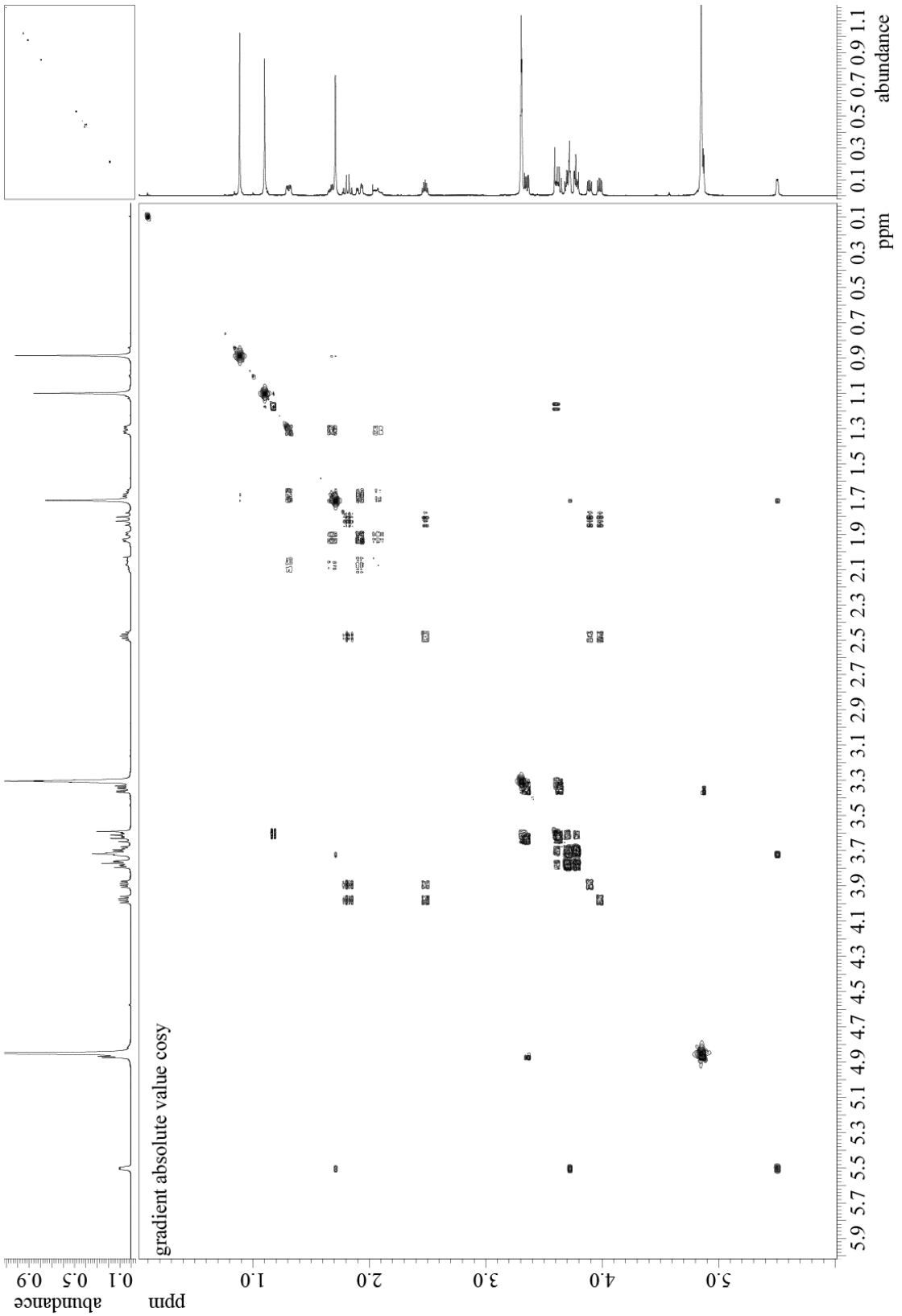



**Figure S7-c.** Correlation spectroscopy (COSY) spectrum of **4**.

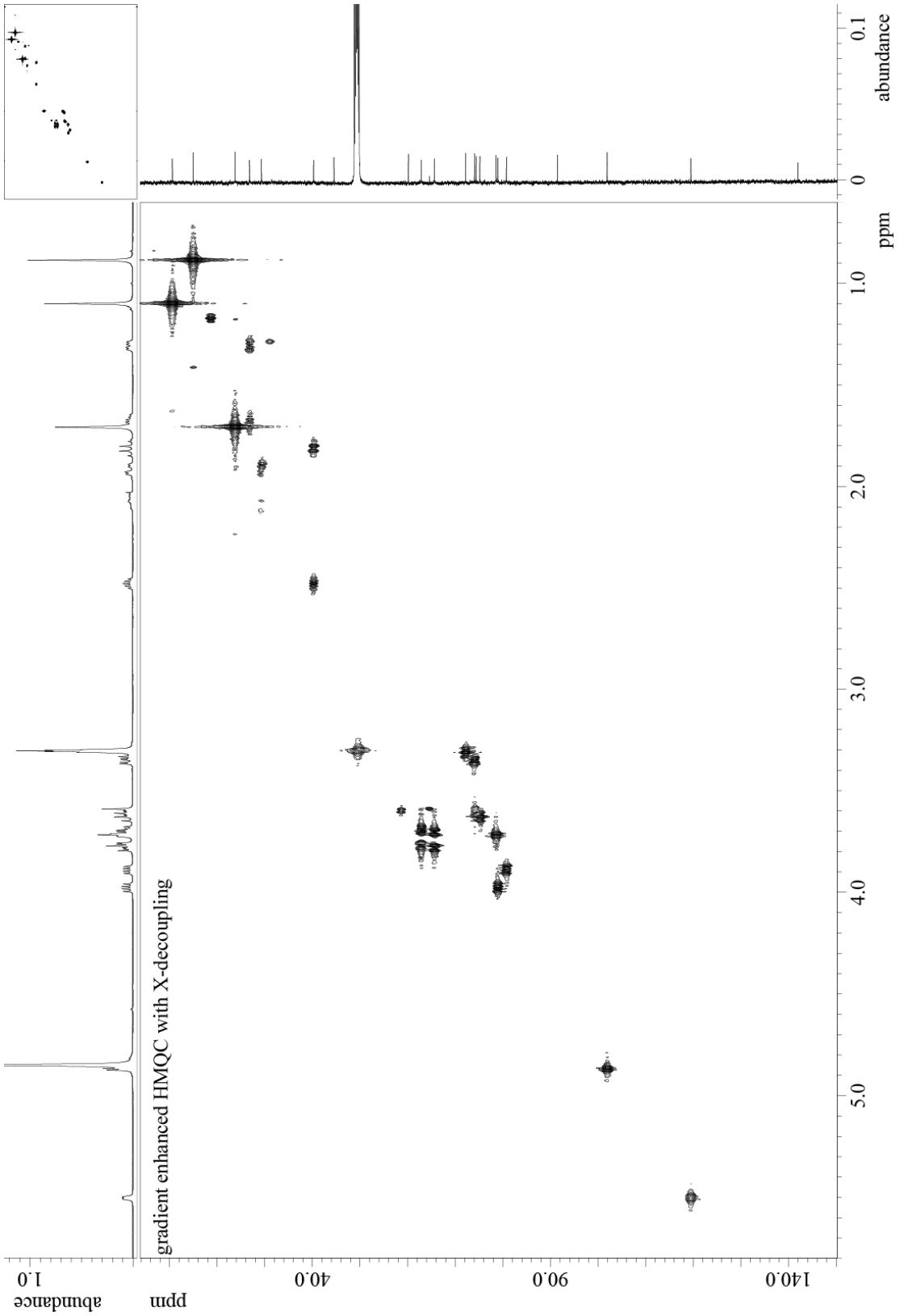



**Figure S7-d.** Heteronuclear multiple quantum coherence (HMQC) spectrum of **4**.

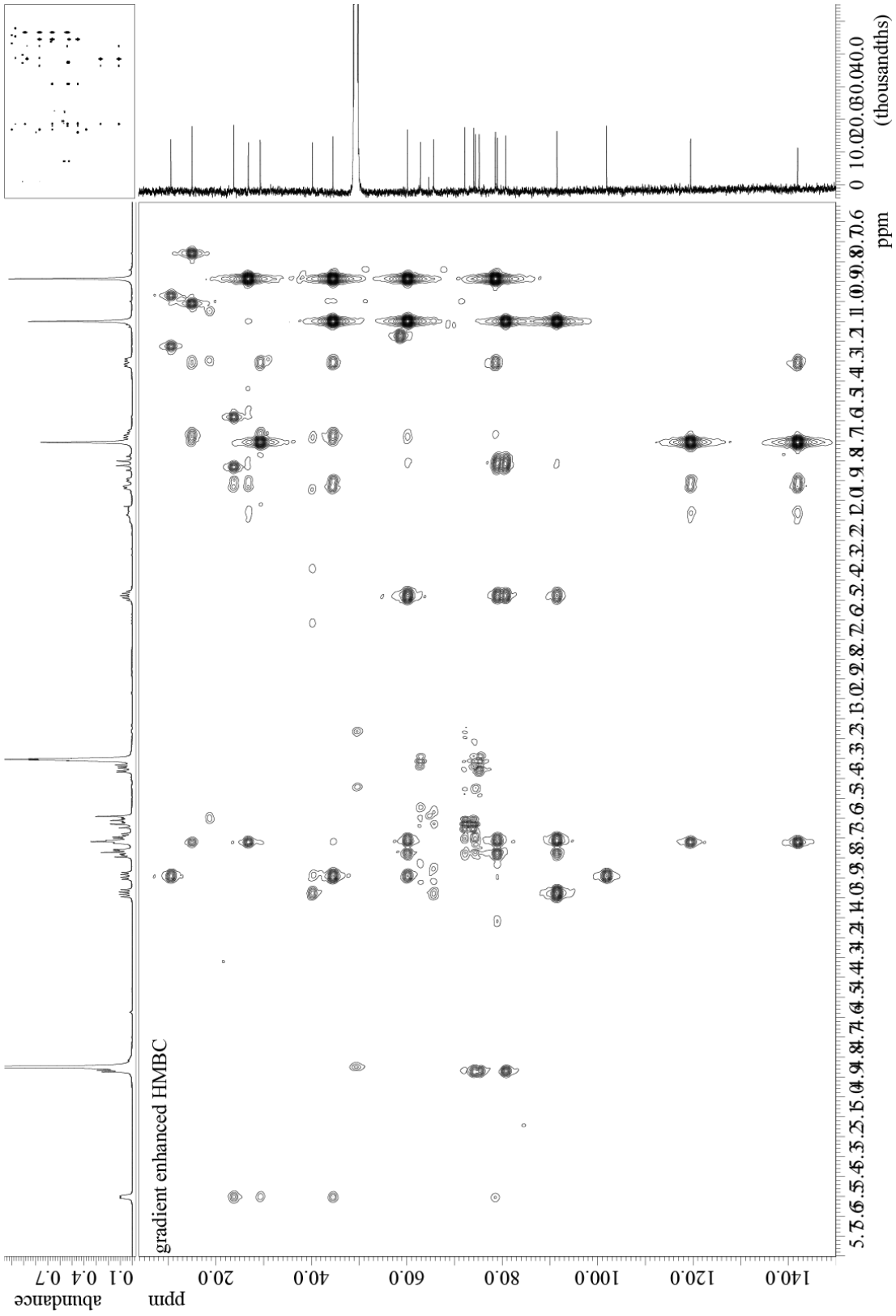



**Figure S7-e.** Heteronuclear multiple bond coherence (HMBC) spectrum of **4**.

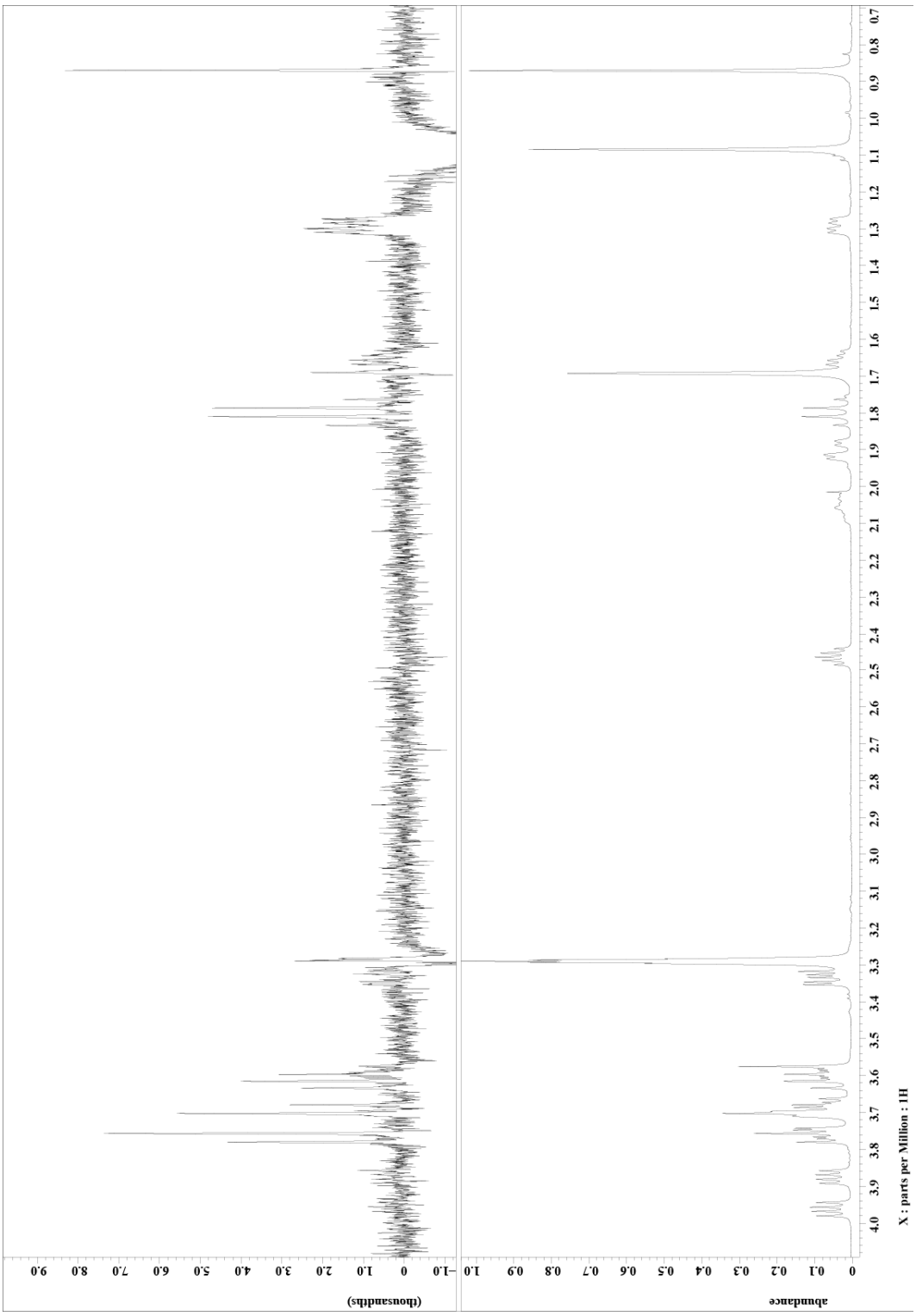



**Figure S7-f.** 1D-nuclear Overhauser effect (1D-NOE) and $^{1}$H NMR spectrum of **4**. The upper is 1D-NOE spectrum obtained by pulse irradiation at 1.10 ppm. The under is $^{1}$H-NMR spectrum.

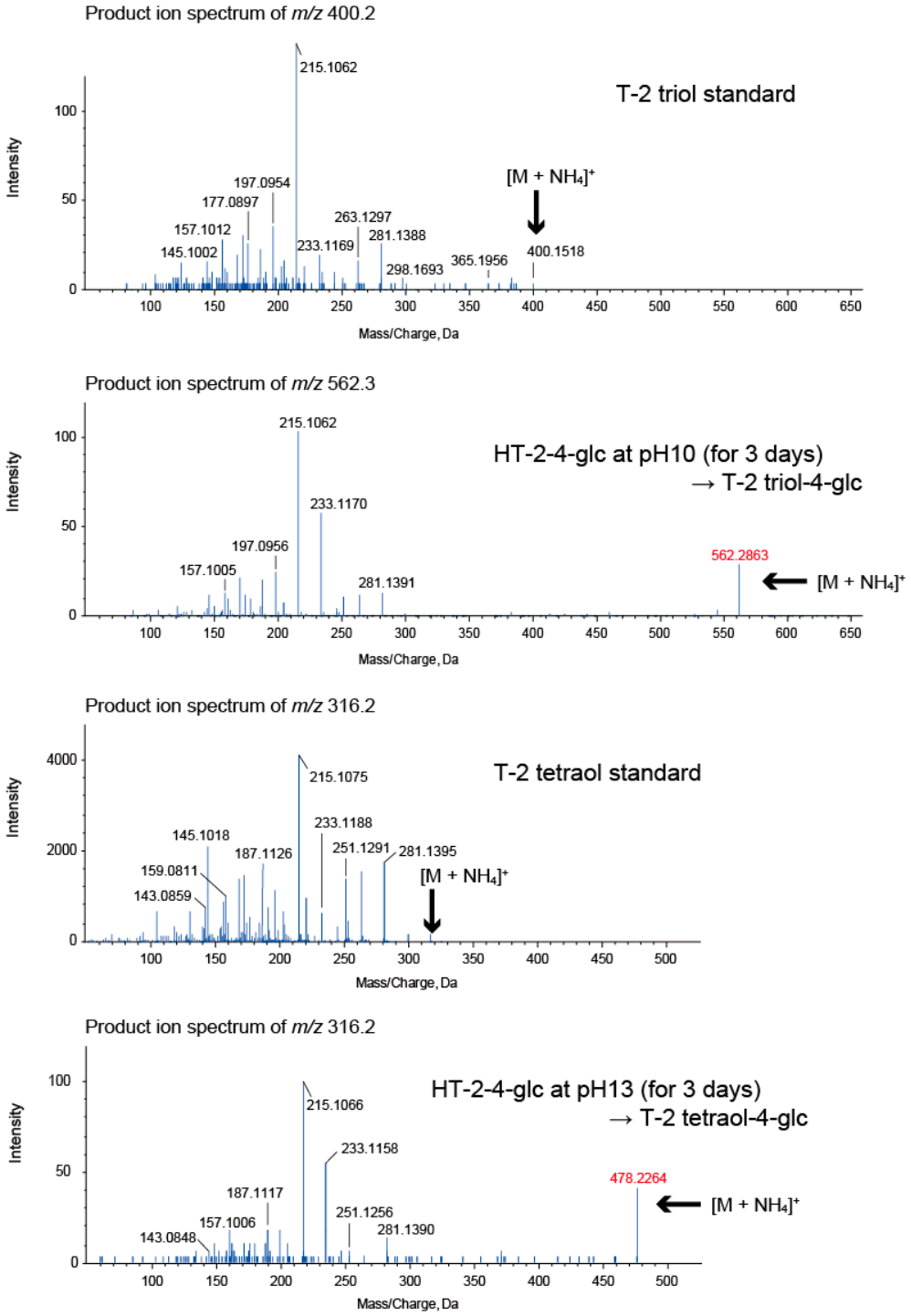



**Figure S8.** Stability of **3** at alkaline pH. MS/MS spectra of deacetylated products, corresponding to [T-2 triol-hexoside + $NH_4$]$^+$ (panel 2; pH10 for 3 days) and [T-2 tetraol-hexoside + $NH_4$]$^+$ (panel 4; pH13 for 3 days), are shown, together with those of their aglycons (panel 1 and 3).