

Synthesis of integerrimide **A** by an on-resin tandem Fmoc-deprotection– macrocyclisation Approach

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Determination of loading efficiency

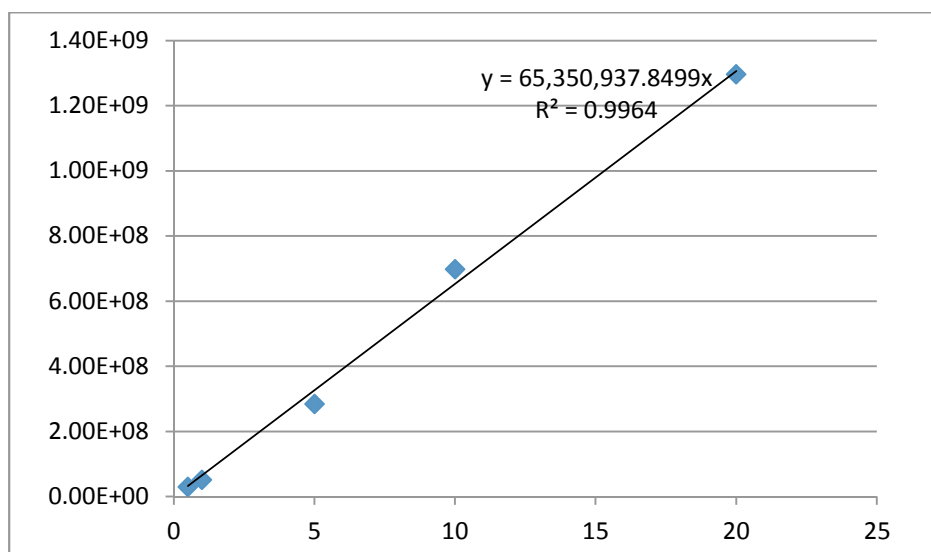
Loading efficiency was determined by UV analysis of the Fmoc group removed into solution upon treating dried resin **4** (1-2 mg) with 20% piperidine in DMF (1 ml) for 30 min using an Agilent 8453 UV-visible spectrophotometer at fixed wavelengths of 301, 302 and 304 nm. Each sample was prepared by diluting 250 µl of its corresponding reaction mixture with DMF to reach a volume of 5 ml (volumetric flask). The sample volume of 3 ml was held in a 4-ml cuvette for analysis. A solvent blank (3 ml), from 150 µl of 20% piperidine in DMF and 2850 µl of DMF, was used as a reference.

$$loading = \frac{Abs \times 20}{\epsilon^{301} \times m^{resin}}$$

The above formula was used to acquire glycine loading efficiency in mol·g⁻¹, where Abs is an average absorption of 10 scans measured relative to the solvent blank recorded at 301 nm; 20 is the dilution factor; ϵ^{301} is 7800, which is a known molar extinction coefficient for a dibenzofulvene adduct (the Fmoc fragment removed into solution) at 301 nm;¹ m^{resin} is mass of dried resin **4** used in mg.

Calibration curve²

The linear equation obtained is $y = 6.5351 \times 10^7 x$, where x = concentration and y = the number of counts of the sum of $[M+H]^+$ and $[M+Na]^+$ ions of **8** at m/z 937.6 and 959.6, respectively.



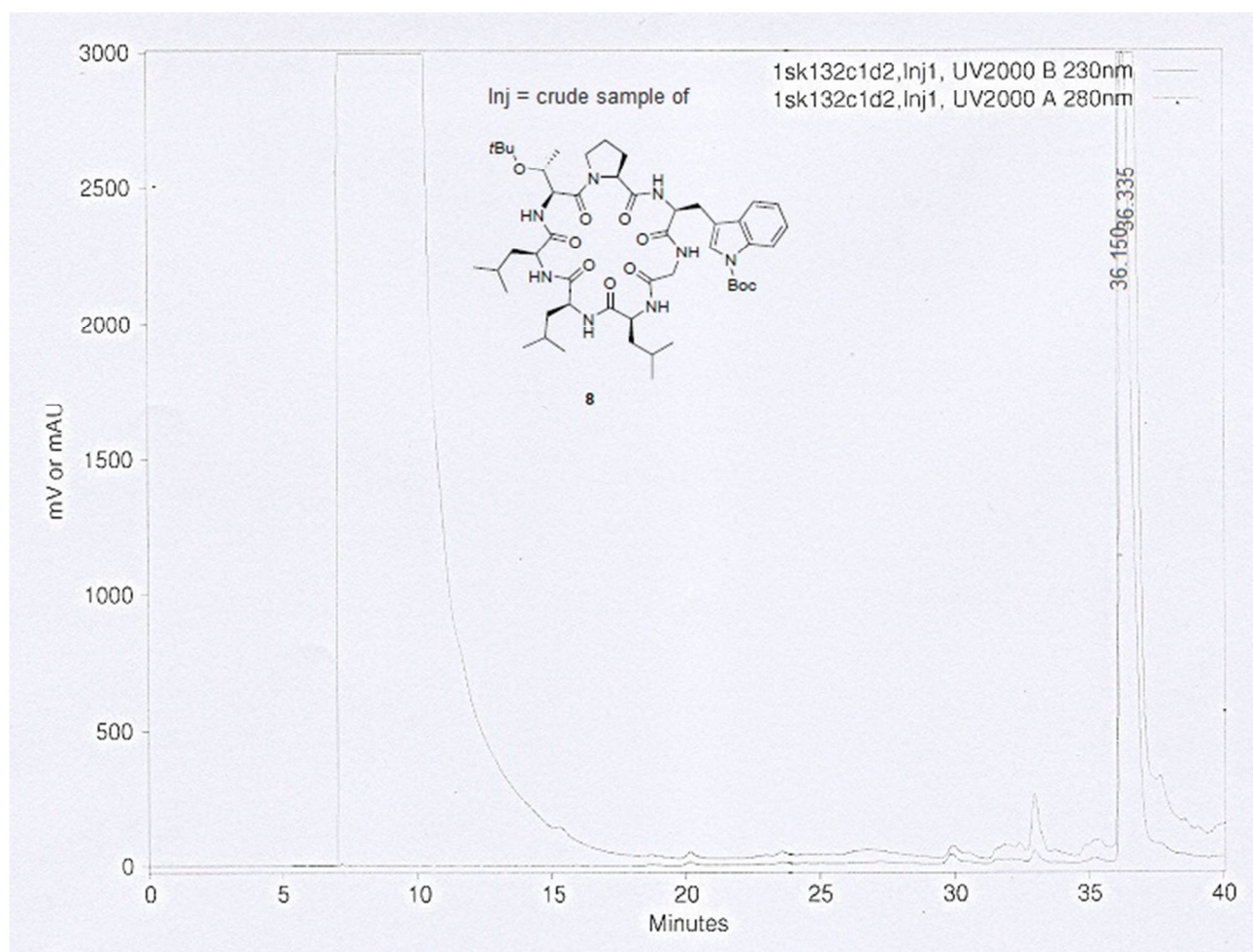
Note on oxidation of integerrimide A (**1**) in DMSO-d₆

When integerrimide A (**1**) was left as a solution of DMSO-d₆ at room temperature for 7 d, it was found to have converted into its oxidised derivatives. After removing DMSO-d₆, the resulting residue was subjected to RP-HPLC (10-70% MeCN in H₂O, 40 min, flow rate 11 ml/min, on a Vydac® 218TP™ C18 column, 10 µm, 250 × 22 mm) and the eluates obtained analysed by HRMS. None of m/z that corresponded to the $[M+Na]^+$ ions of **1** (C₄₀H₆₀N₈O₈Na⁺) was observed but the following m/z of $[M+Na]^+$ that corresponded to C₄₀H₆₀N₈O₉Na⁺, C₄₀H₆₀N₈O₁₀Na⁺ (major), C₄₁H₆₂N₈O₁₀Na⁺, C₄₀H₅₈N₈O₉Na⁺, and C₄₀H₅₉ClN₈O₉Na⁺. This was likely to be due to irreversible oxidation of the unprotected indole unit of Trp in the natural product.³

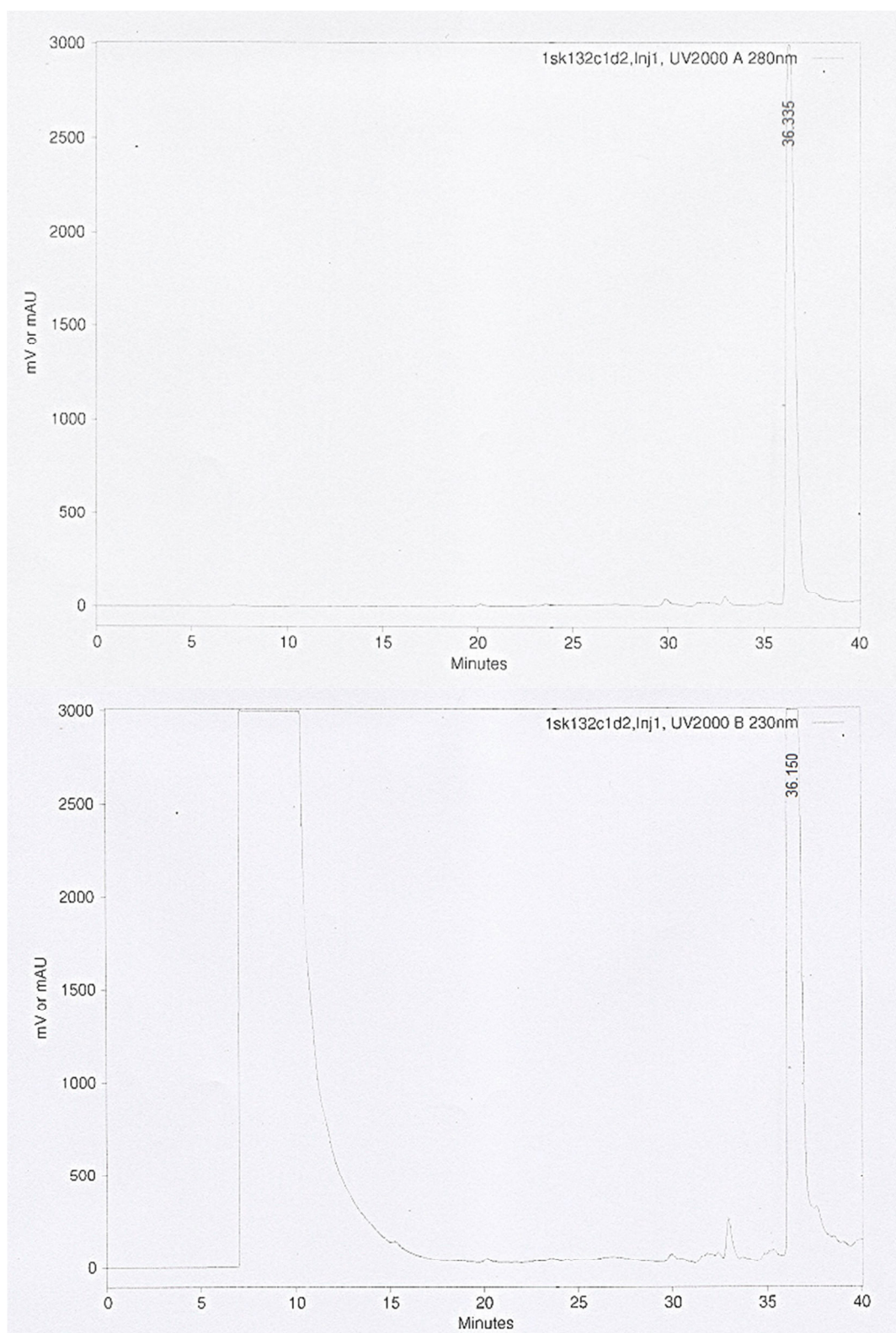
References

1. W. C. Saxinger, in *Phospho-Proteomics : Methods and Protocols*, ed. M. de Graauw, Humana Press, New York, 2009, vol. 527, pp. 257-267.
2. J. C. Miller and J. N. Miller, *Statistics for Analytical Chemistry*, 3 edn., Ellis Horwood, London, 1993.
3. G. A. Grant, *Synthetic Peptides : A User's Guide*, 2 edn., Oxford University Press, New York, 2002.

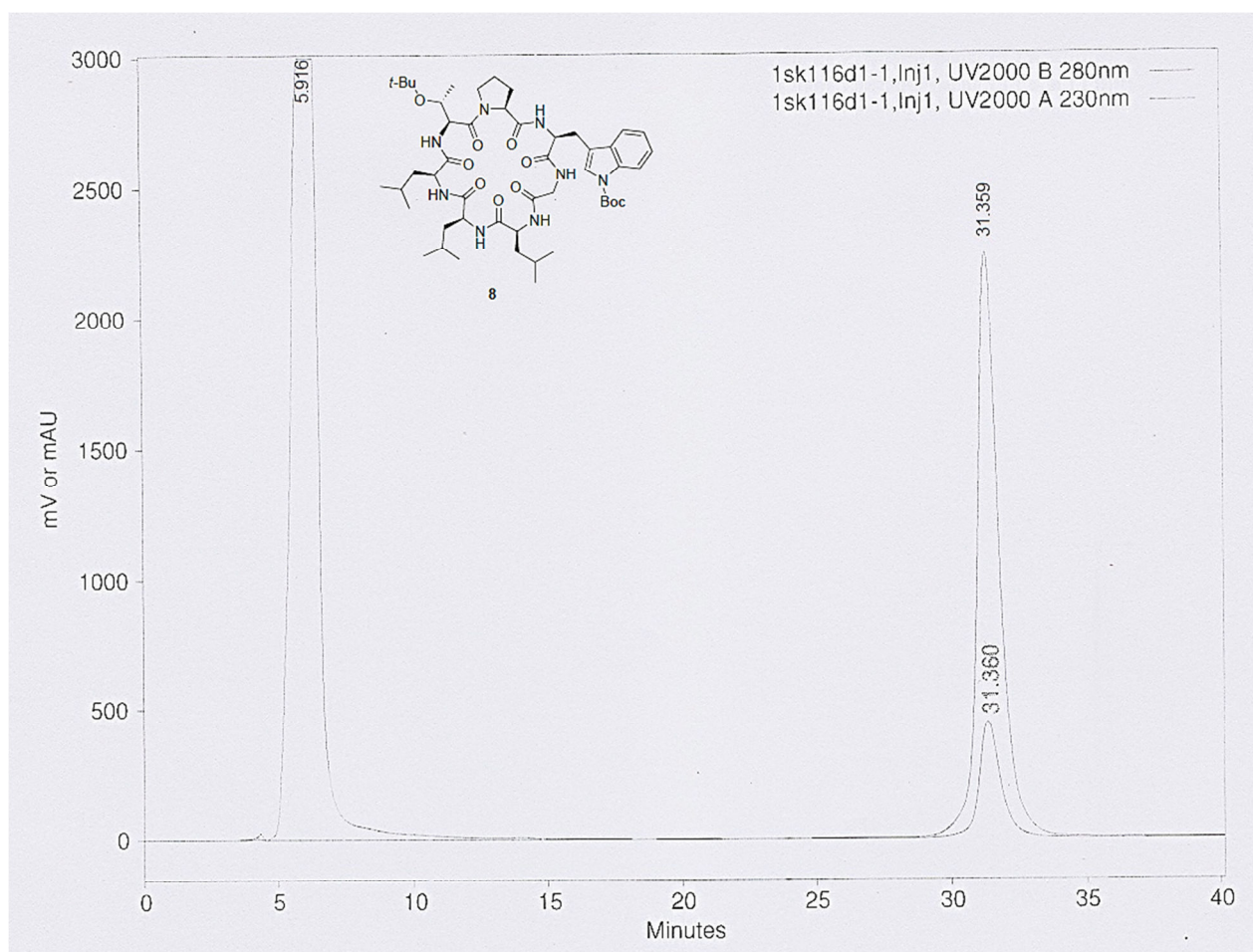
Preparative RP-HPLC of crude material of intermediate **8** (loaded as a solution in DMF): 0-100% MeCN in H₂O, 40 min, flow rate 11 ml/min, on a Vydac® 218TP™ C18 column (10 µm, 250 × 22 mm). t_R = 36 min.



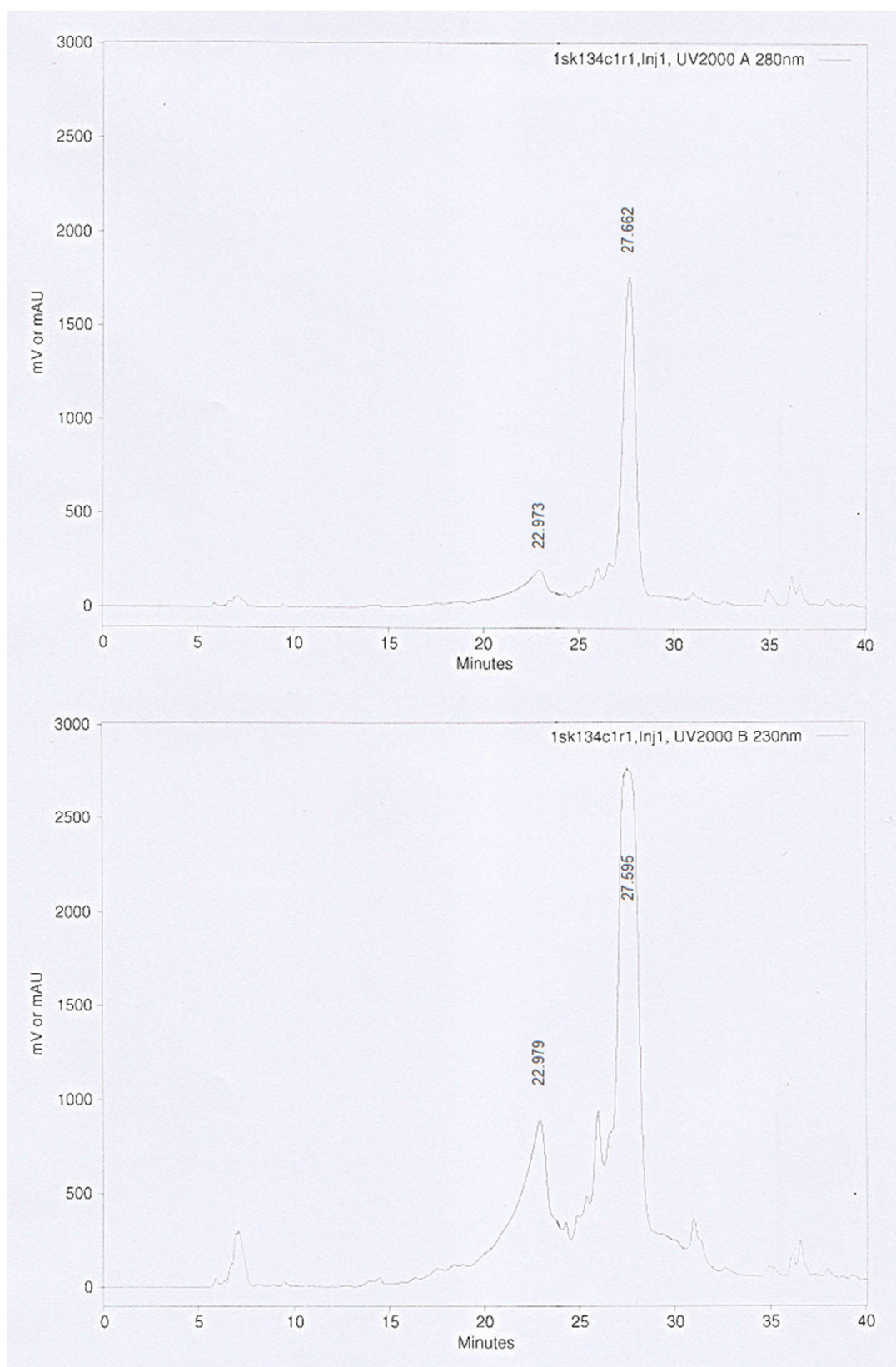
Preparative RP-HPLC of crude material of intermediate **8** (loaded as a solution in DMF): 0-100% MeCN in H₂O, 40 min, flow rate 11 ml/min, on a Vydac® 218TP™ C18 column (10 μm, 250 × 22 mm). t_R = 36 min.

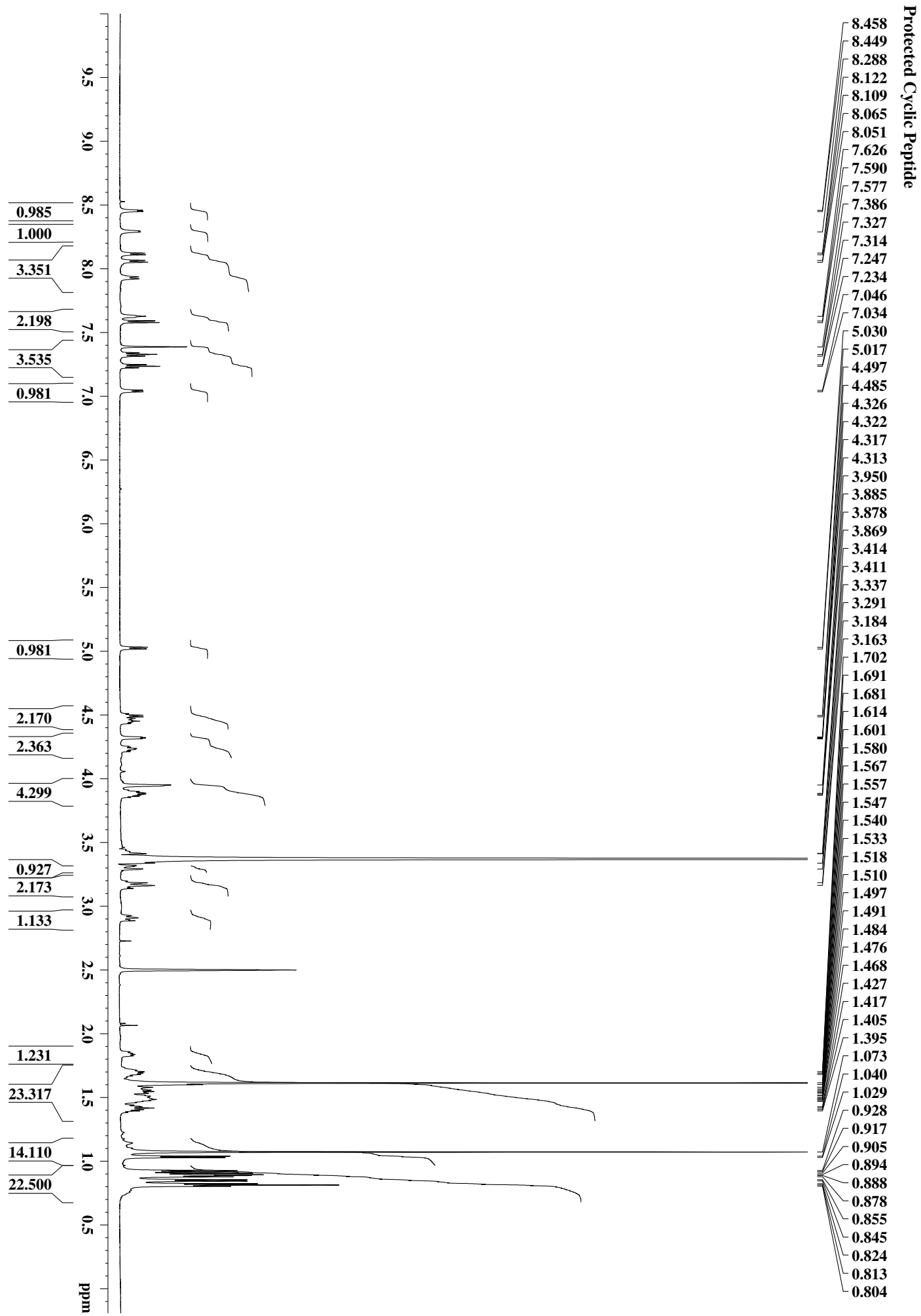


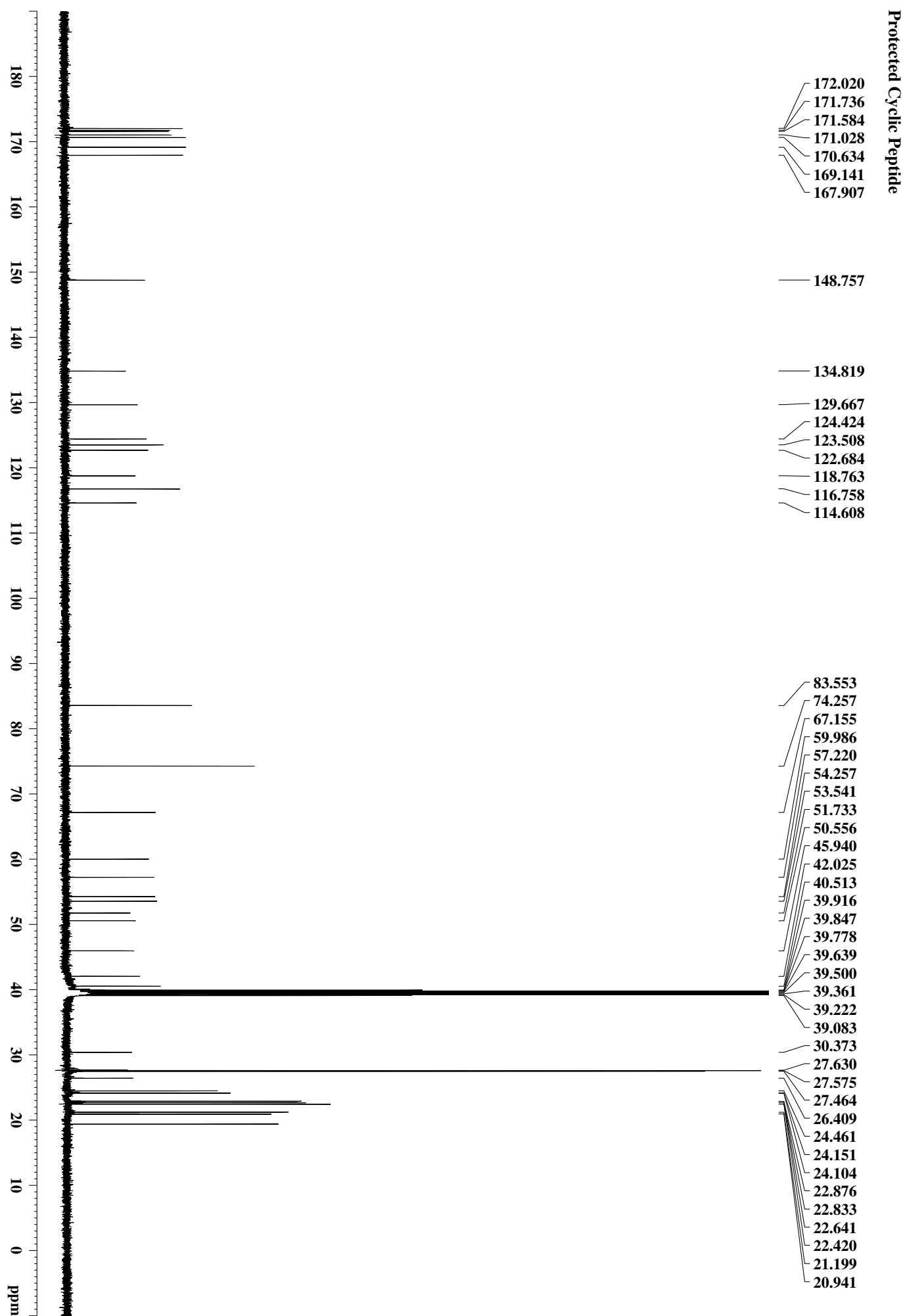
Preparative RP-HPLC of intermediate **8** (loaded as a solution in DMF): 50-70% MeCN in H₂O, 40 min, flow rate 11 mL/min, on a Vydac® 218TP™ C18 column (10 µm, 250 × 22 mm). t_R = 31 min.



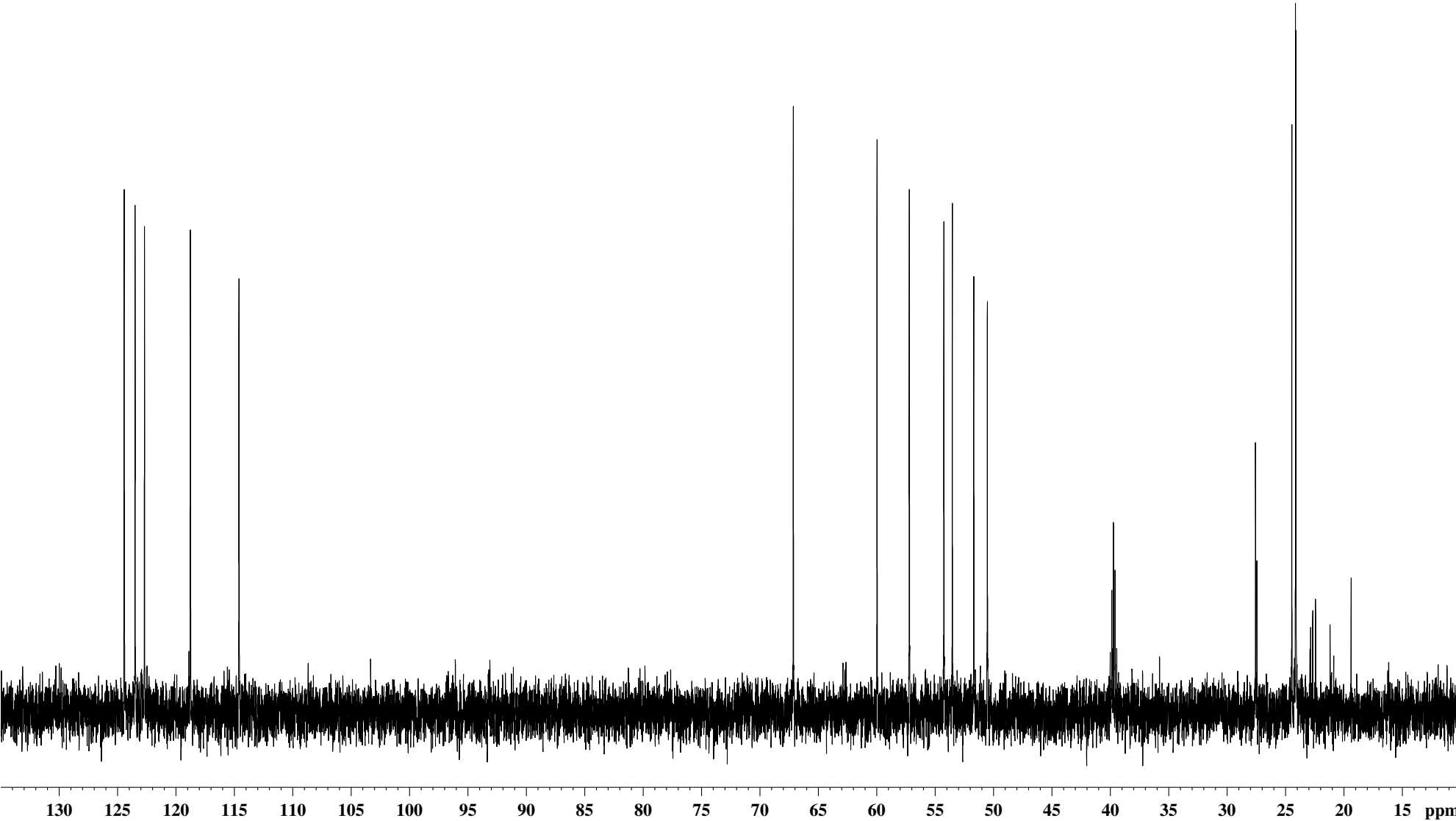
Preparative RP-HPLC of crude material of integerrimide A (**1**): 0-100% MeCN in H₂O, 40 min, flow rate 11 ml/min, on a Vydac® 218TP™ C18 column (10 µm, 250 × 22 mm). t_R = 23 and 28 min.



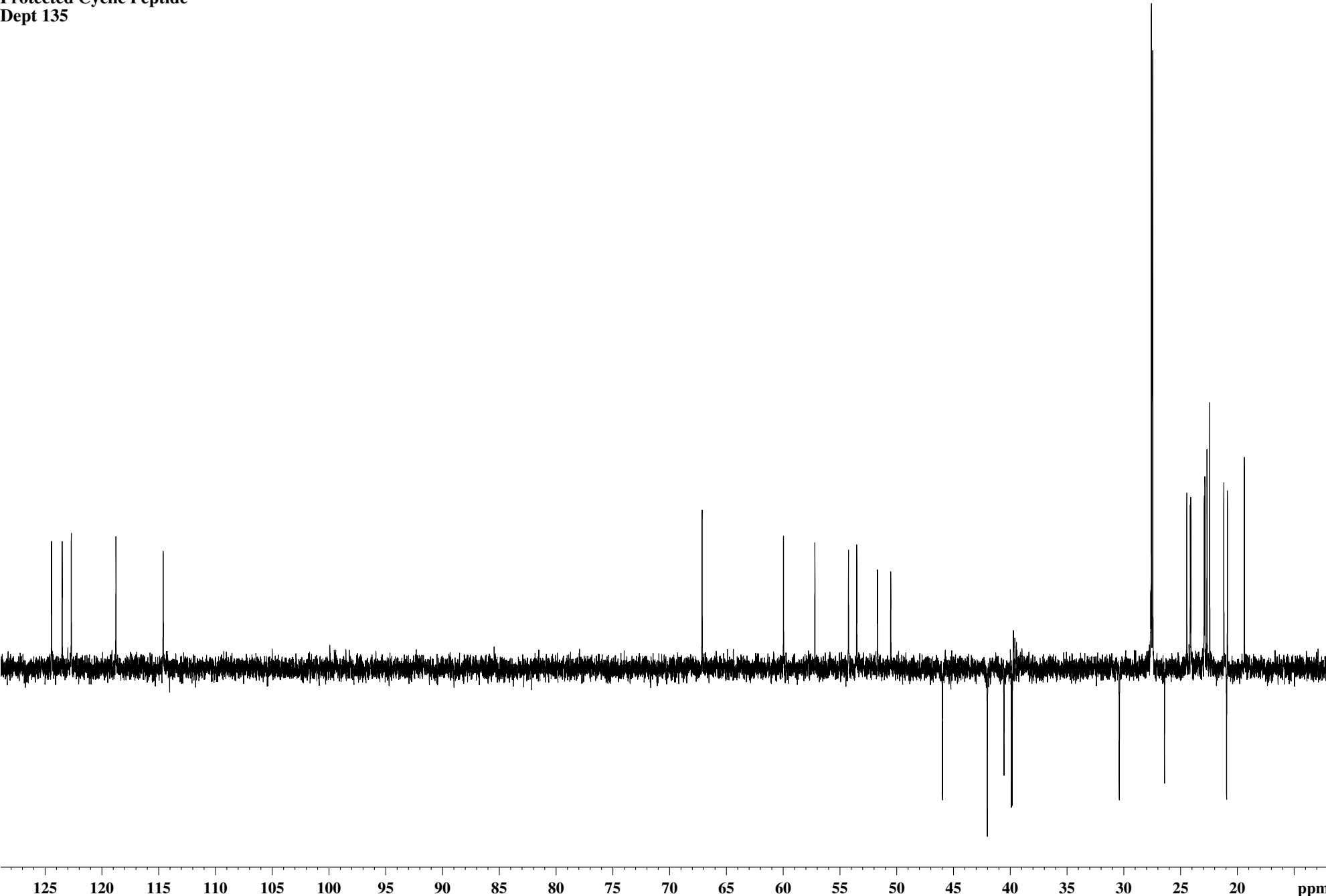




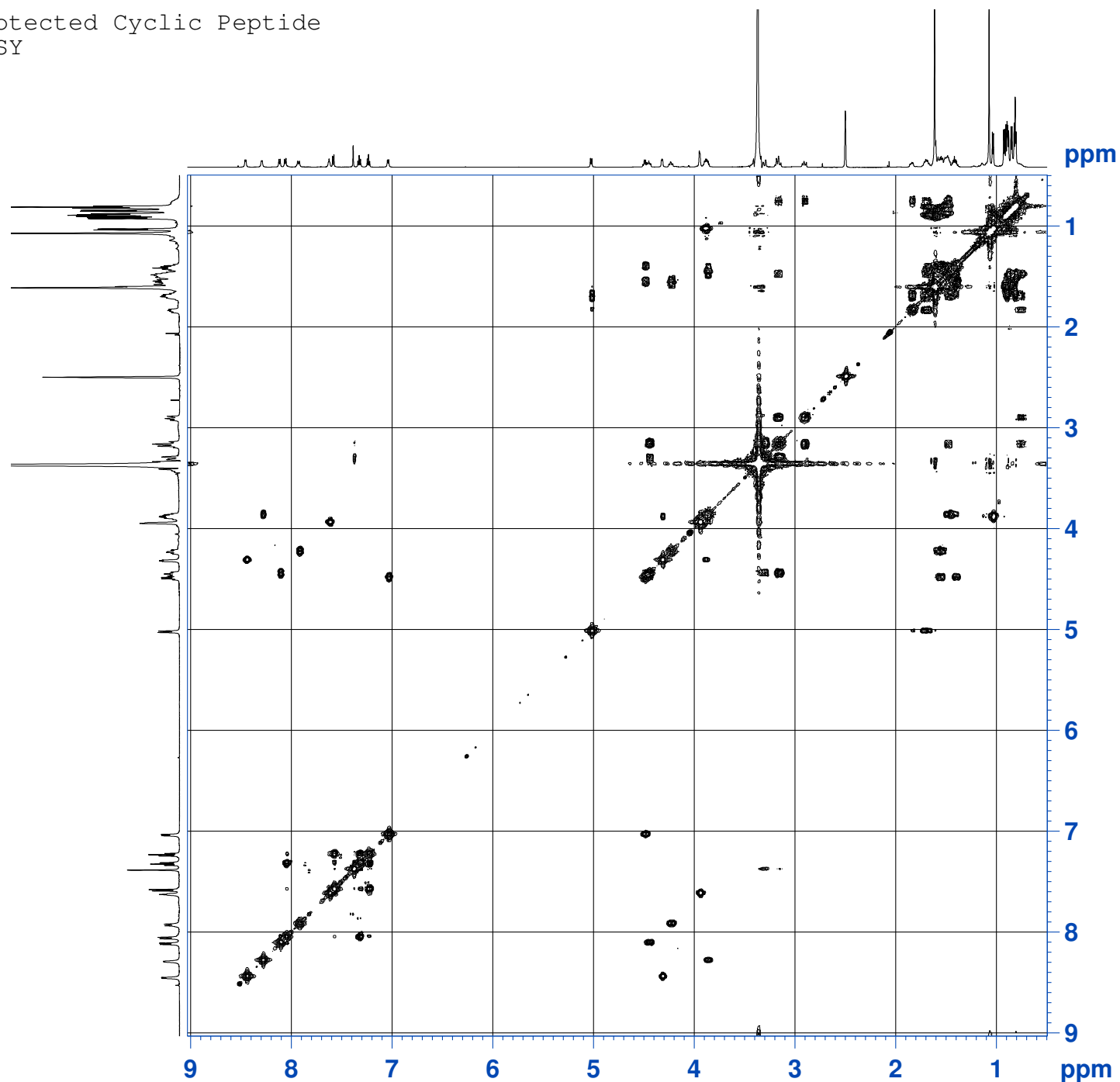
Protected Cyclic Peptide
Dept 90



Protected Cyclic Peptide
Dept 135



Protected Cyclic Peptide
COSY



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PROCNO 1

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TD 2048
SOLVENT DMSO
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DS 8
SWH 5122.951 Hz
FIDRES 2.501441 Hz
AQ 0.2000324 sec
RG 181
DW 97.600 usec
DE 6.00 usec
TE 299.0 K
d0 0.00000300 sec
D1 2.00000000 sec
d13 0.00000400 sec
D16 0.00020000 sec
INO 0.00019520 sec

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PL1 -3.50 dB
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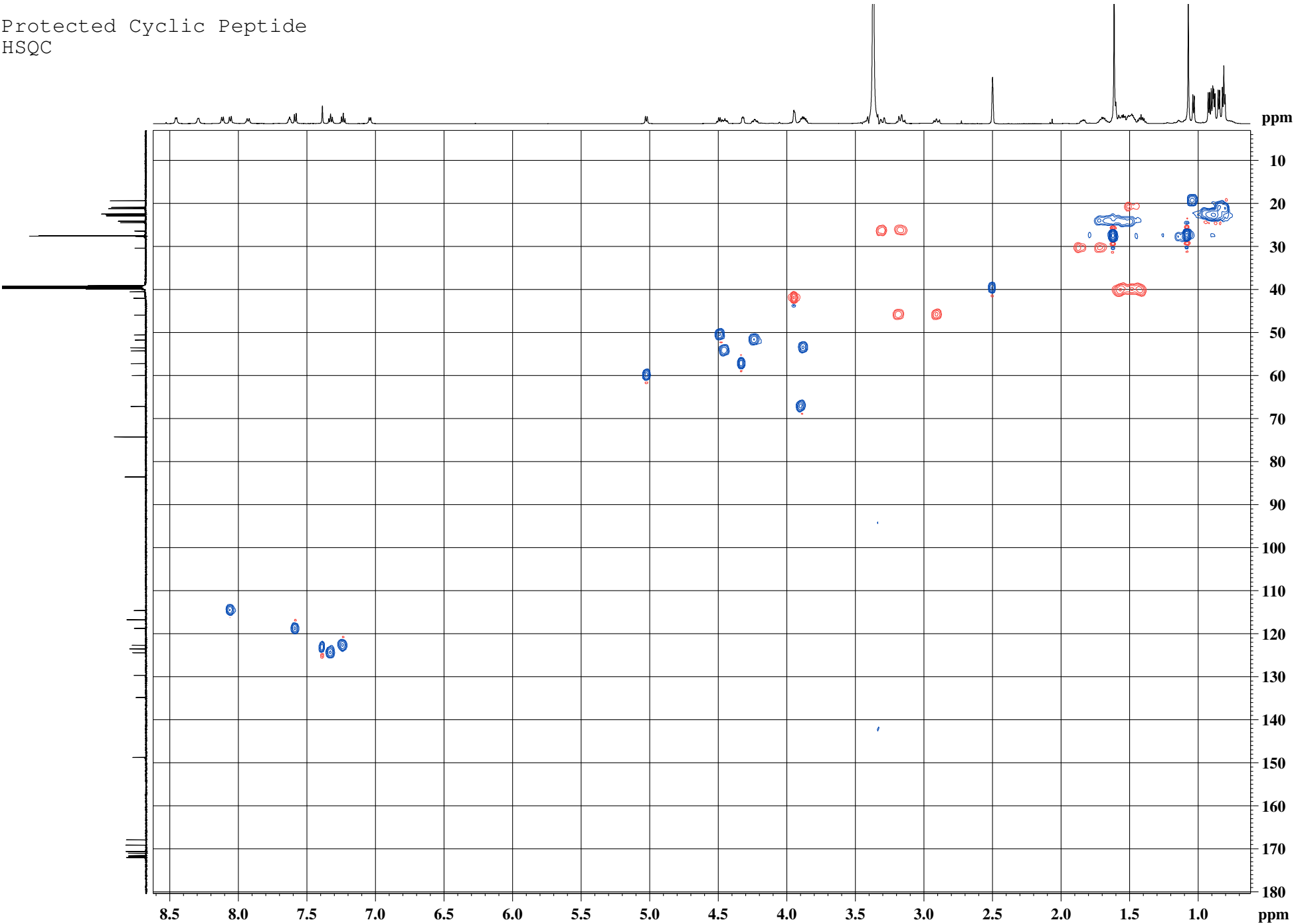
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FnMODE QF

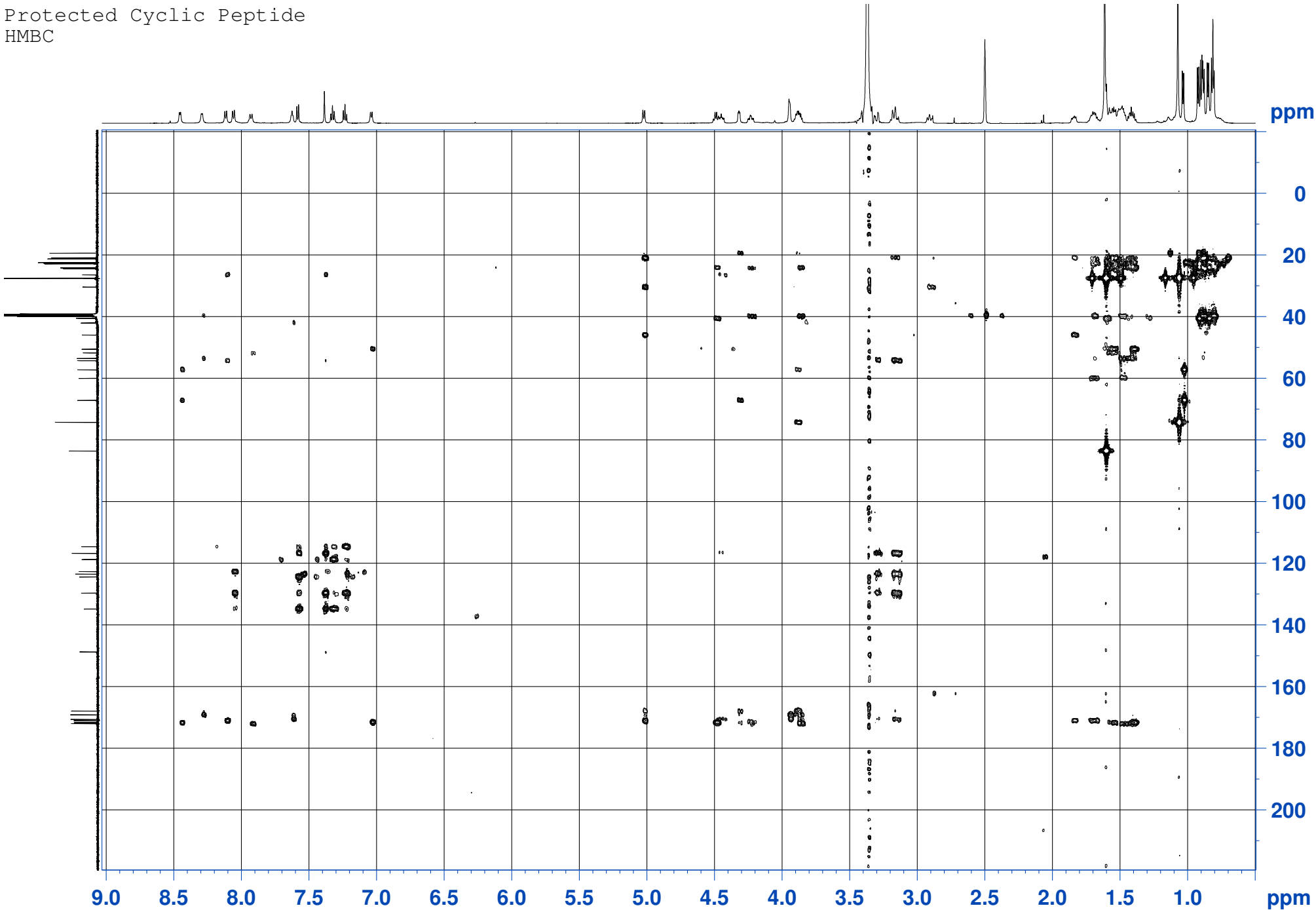
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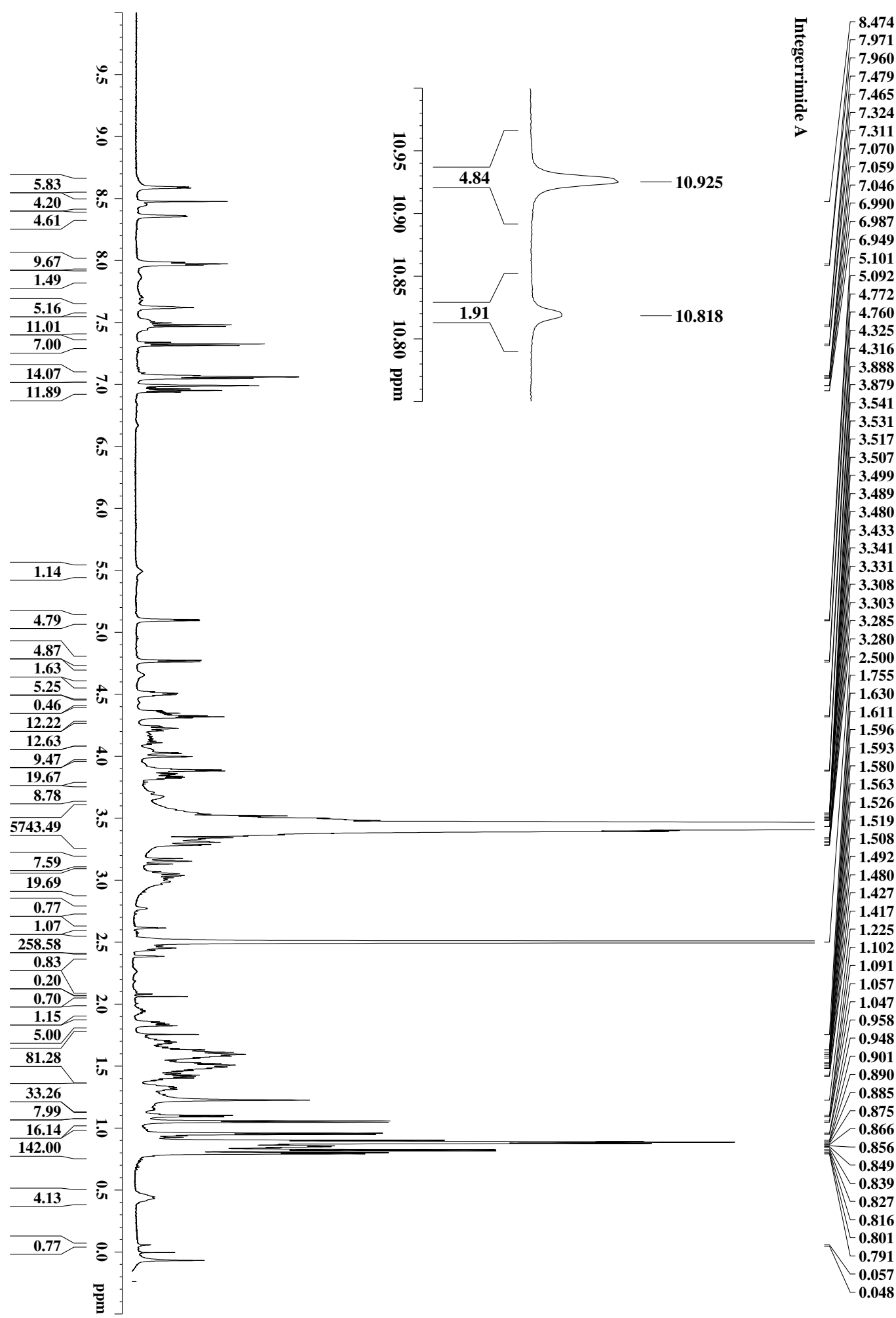
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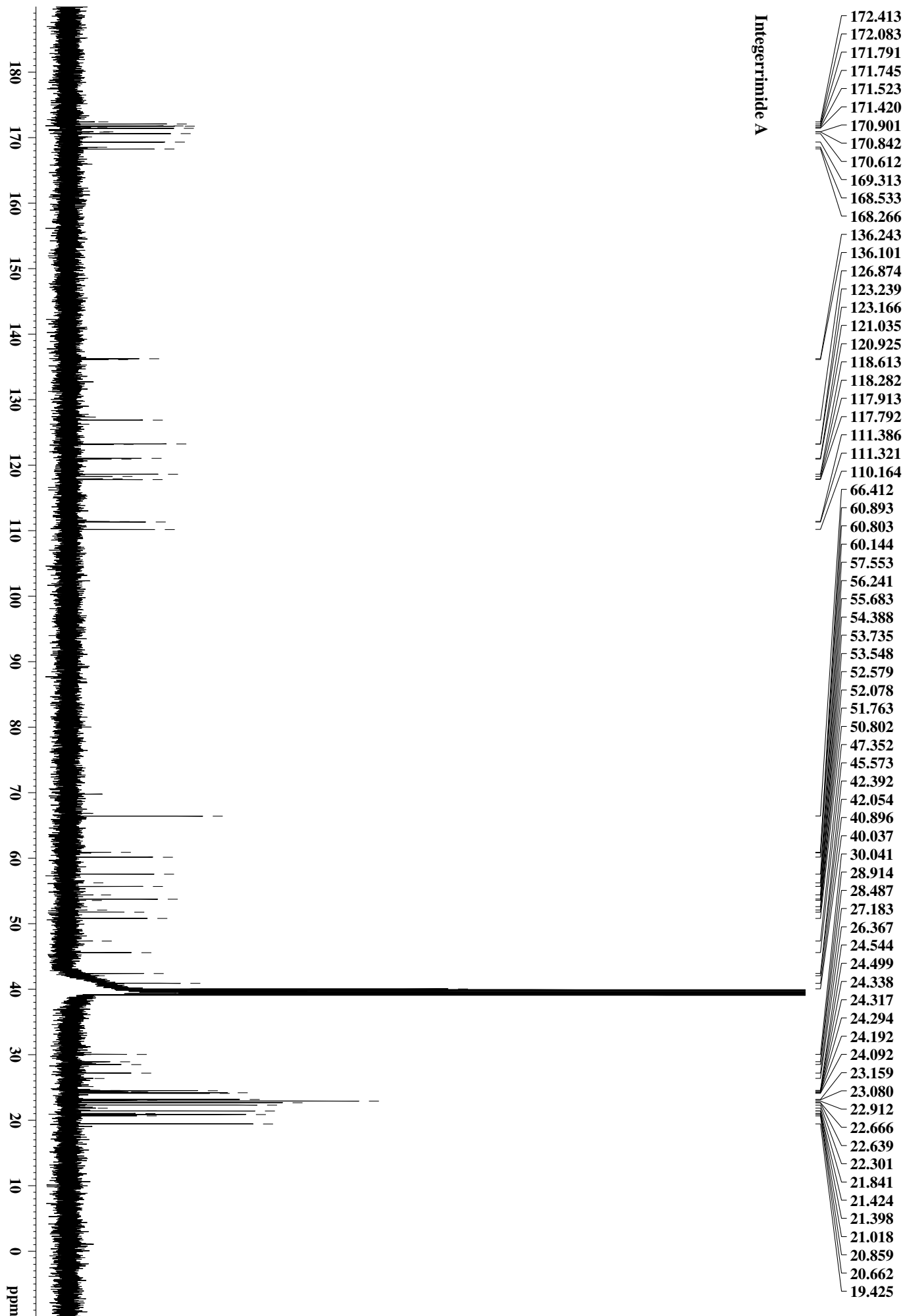
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HSQC



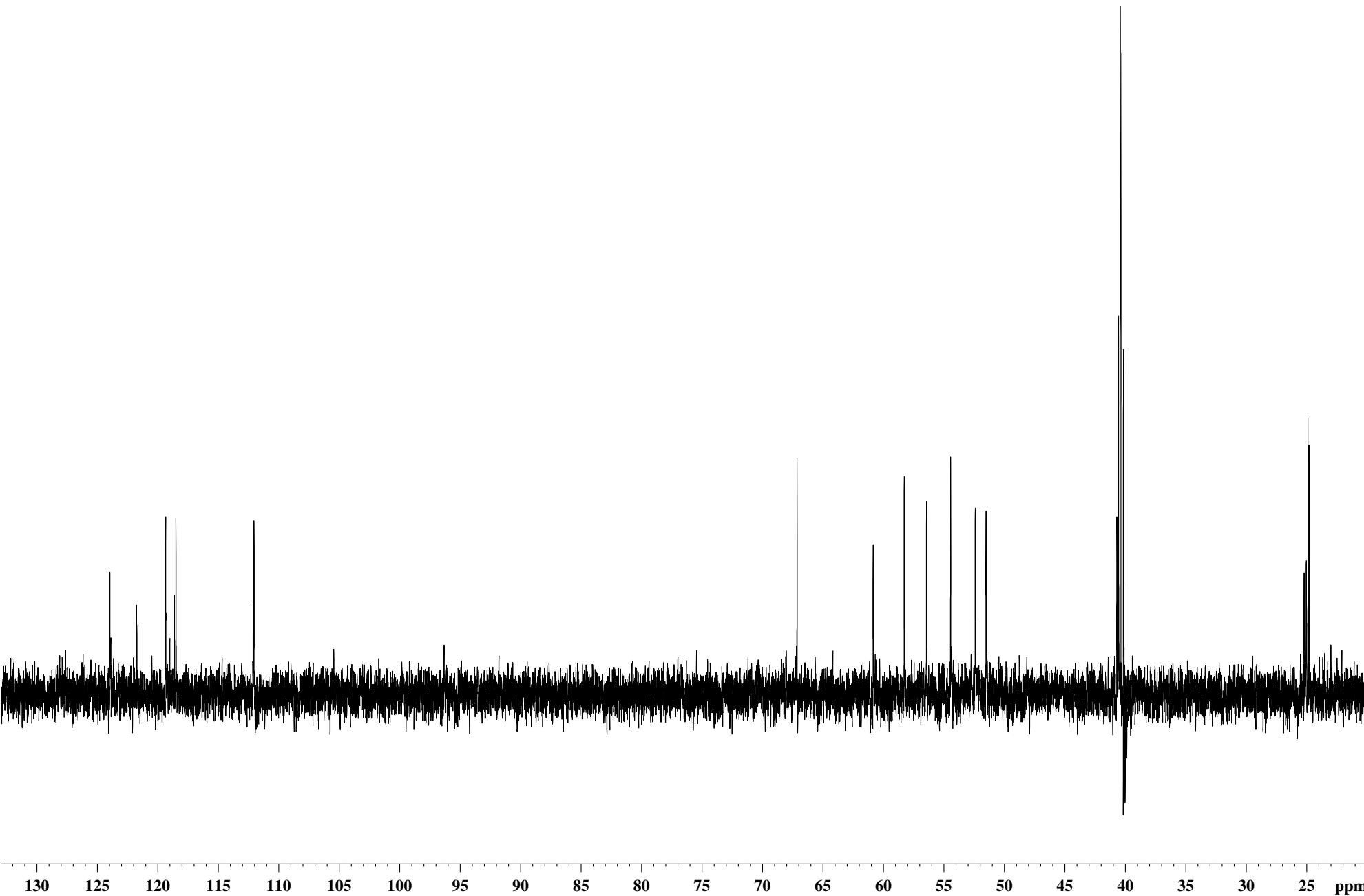
Protected Cyclic Peptide
HMBC



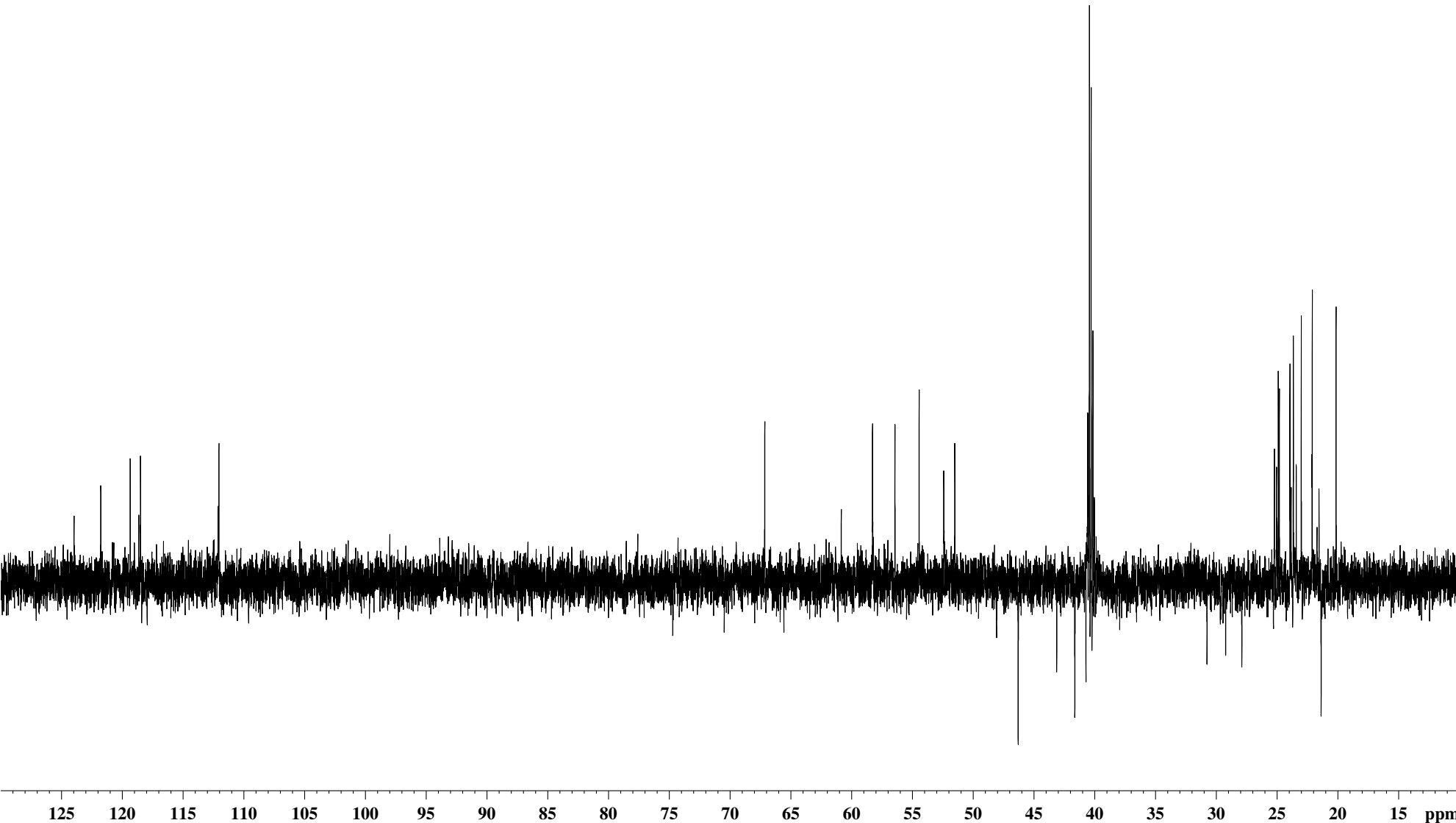




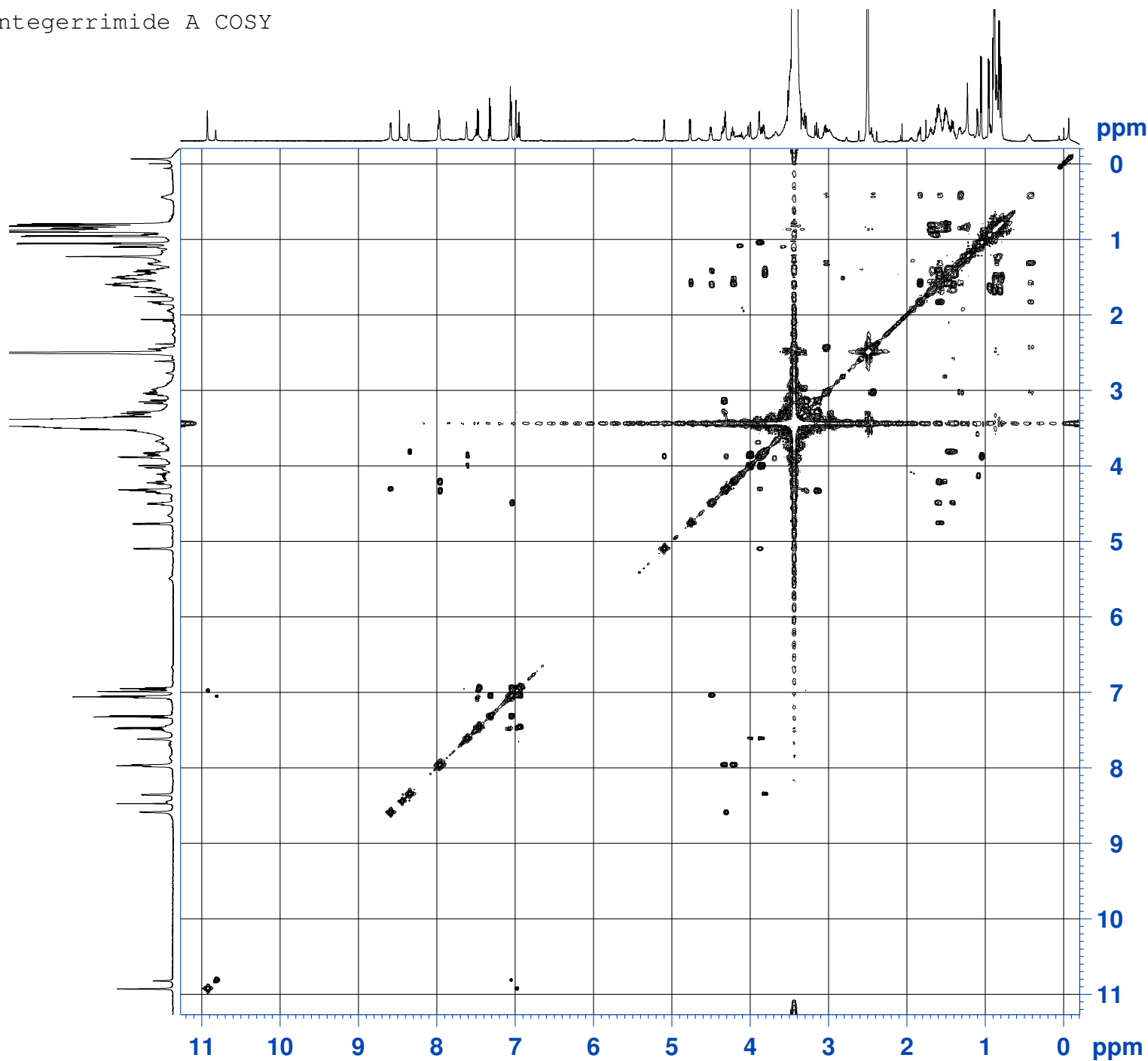
Integerrimide A
DEPT 90



Integerrimide A
DEPT 135



Integerrimide A COSY



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EXPNO     7
PROCNO    1

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SOLVENT   DMSO
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DS         8
SWH        6887.052 Hz
FIDRES     3.362818 Hz
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RG         64
DW         72.600 usec
DE         6.00 usec
TE         300.5 K
d0         0.00000300 sec
D1         2.00000000 sec
d13        0.00000400 sec
D16        0.00010000 sec
IN0        0.00014520 sec

===== CHANNEL f1 =====
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P1         8.00 usec
PL1        -2.00 dB
SFO1       600.1333265 MHz

===== GRADIENT CHANNEL =====
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GPZ2       15.00 %
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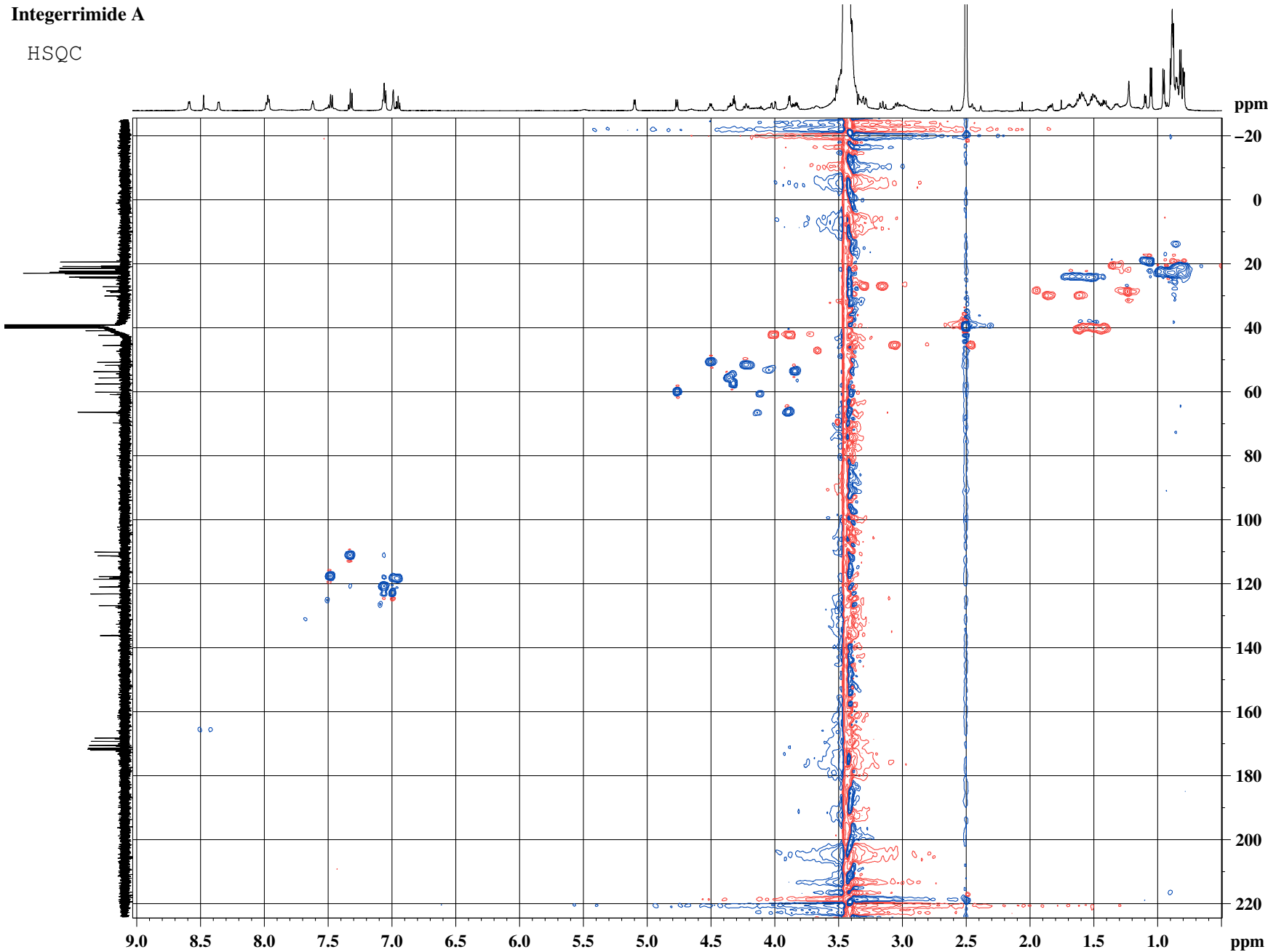
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FnMODE     QF

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SSB        0
LB         0 Hz
GB         0
PC         1.40

F1 - Processing parameters
SI         1024
MC2        QF
SF         600.1300064 MHz
WDW        echo-antiecho
SSB        0
LB         0 Hz
GB         0
    
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Integerrimide A

HSQC



Integerrimide A

HMBC

