4-Formylaminooxyvinylglycine, an Herbicidal Germination-Arrest Factor (GAF) from *Pseudomonas* Rhizosphere Bacteria.

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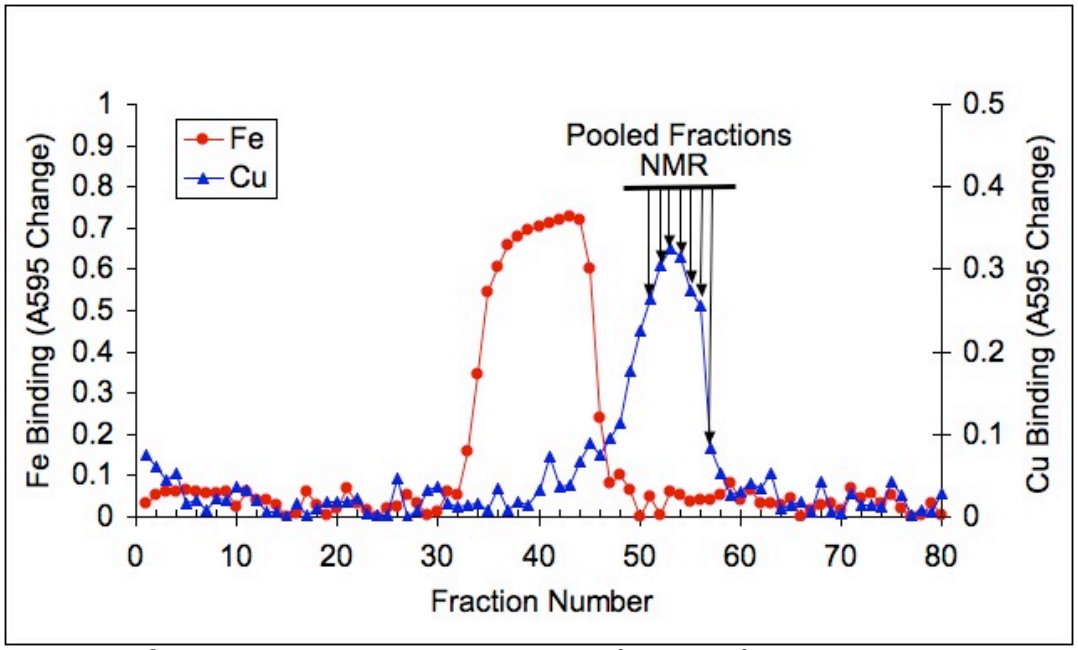


Figure S1. Sephadex® G15 column elution profiles of Fe^{2+} and Cu^{2+} binding activity as measured with the corresponding ChromeAzurol S reagent. For graphical purposes, the changes in absorbance were calculated by subtracting the measured absorbance in each well from the highest well absorbance measured on a given plate. These values are plotted as ΔA_{595}.

Absolute configuration of GAF (1).

Experiments using L-amino acid oxidase (Sigma A9378-5MG, Type IV from *Crotalus atrox*):

The 90% EtOH extract of 330 mL WH6 culture filtrate was resuspended in 33 mL H_2O (~ 10 μM), adjusted to pH 6.8 with NaOH, and distributed in 8 mL aliquots to each of four 25 mL Wheaton bottles (1-4 above). To each bottle was also added 80 units (in 0.9 mL) of commercial bovine liver catalase (Sigma C3155-50MG). To verify AAO enzyme activity, L-Phe (1 mL of 1 mM solution in H_2O) was added to bottles 3 and 4, concurrently with 1 mL H_2O to bottles 1 and 2. Finally, commercial L-AAO stock solution (3.8 units in 0.1 mL) was added to bottles 1 and 3; bottles 2 and 4 were supplemented with 0.1 mL H_2O, for final sample volume of 10 mL in each case. The samples were incubated at 35°C for 30 h, after which the enzymes were precipitated by centrifugation (2 h at 4,000 x g) using Pall Life Sciences Macrosep Centrifugal Devices (10,000 MWCO, 15 mL size) that had been primed beforehand by centrifuging for 1 h with 12 mL H_2O. Each sample filtrate was concentrated in vacuo and redissolved in 76% EtOH at 32X concentration for TLC analysis on Analtech Microcrystalline Cellulose (EtOAc-$PrOH^i$-H_2O 3:6:4) and Analtech GHL Silica (EtOAc-$PrOH^i$-MeOH-H_2O 5:5:18:2) plates.

Experiments using D-amino acid oxidase (Sigma A5222-100UN, from porcine kidney):

The 90% EtOH extract of 360 mL WH6 culture filtrate was resuspended in 36 mL H_2O (~ 10 μM), adjusted to pH 8.5 with NaOH, and distributed in 8 mL aliquots to each of four 25 mL Wheaton bottles (1-4 above). To each bottle was added 380 units (in 0.5 mL) of commercial bovine liver catalase. To verify AAO enzyme activity, D-Ala (1 mL of 1 mM solution in H_2O) was added to bottles 3 and 4, concurrently with 1 mL H_2O to bottles 1 and 2. Finally, commercial D-AAO (3.8 units in 0.5 mL) was added to bottles 1 and 3, while bottles 2 and 4 were supplemented with 0.5 mL H_2O, for final sample volume of 10 mL in each case. The samples were incubated at 35°C for 30 h, after which the enzymes were precipitated by centrifugation (2 hours at 4,000 x g) as described above. Each sample filtrate was analyzed by TLC as described above.

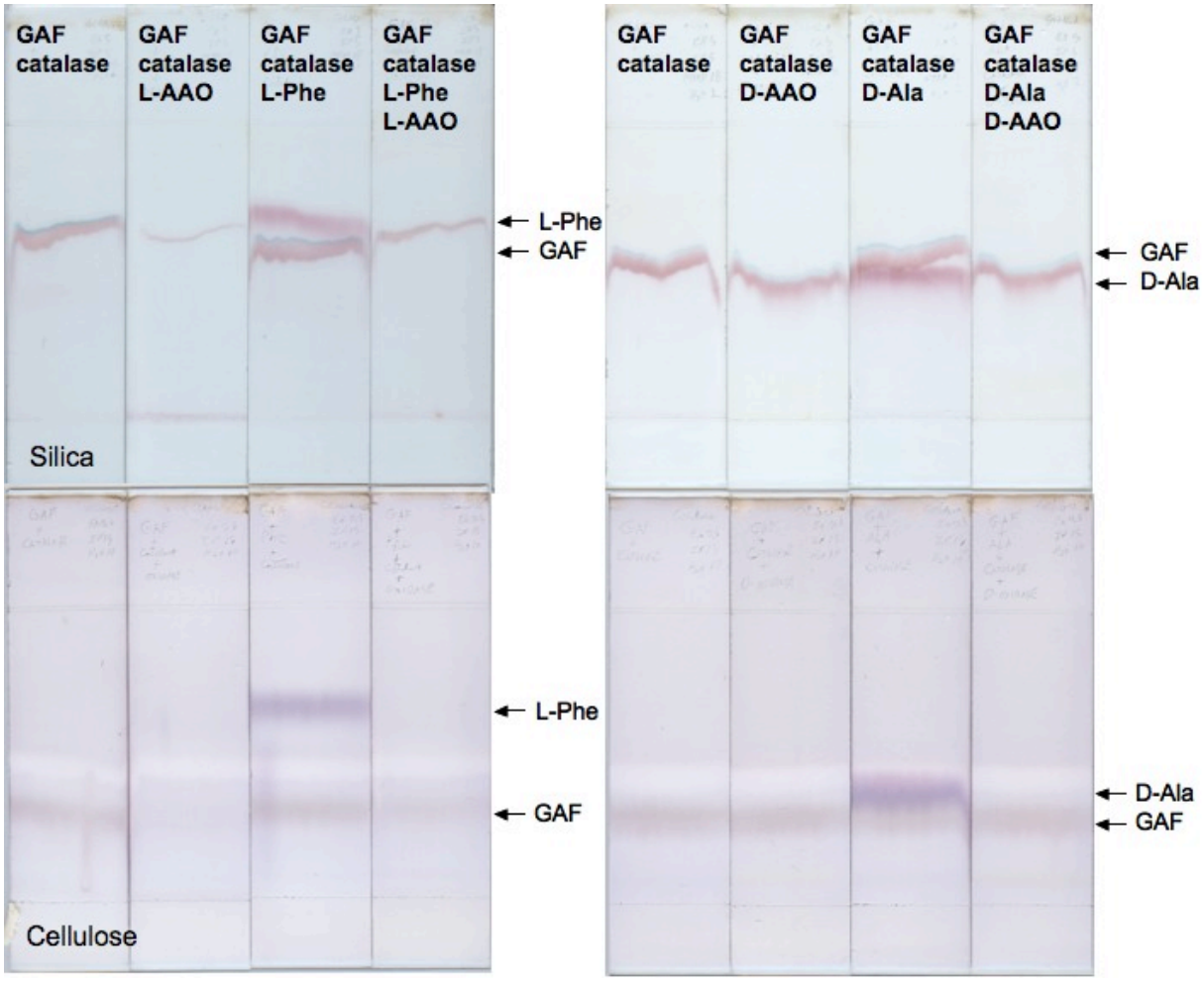
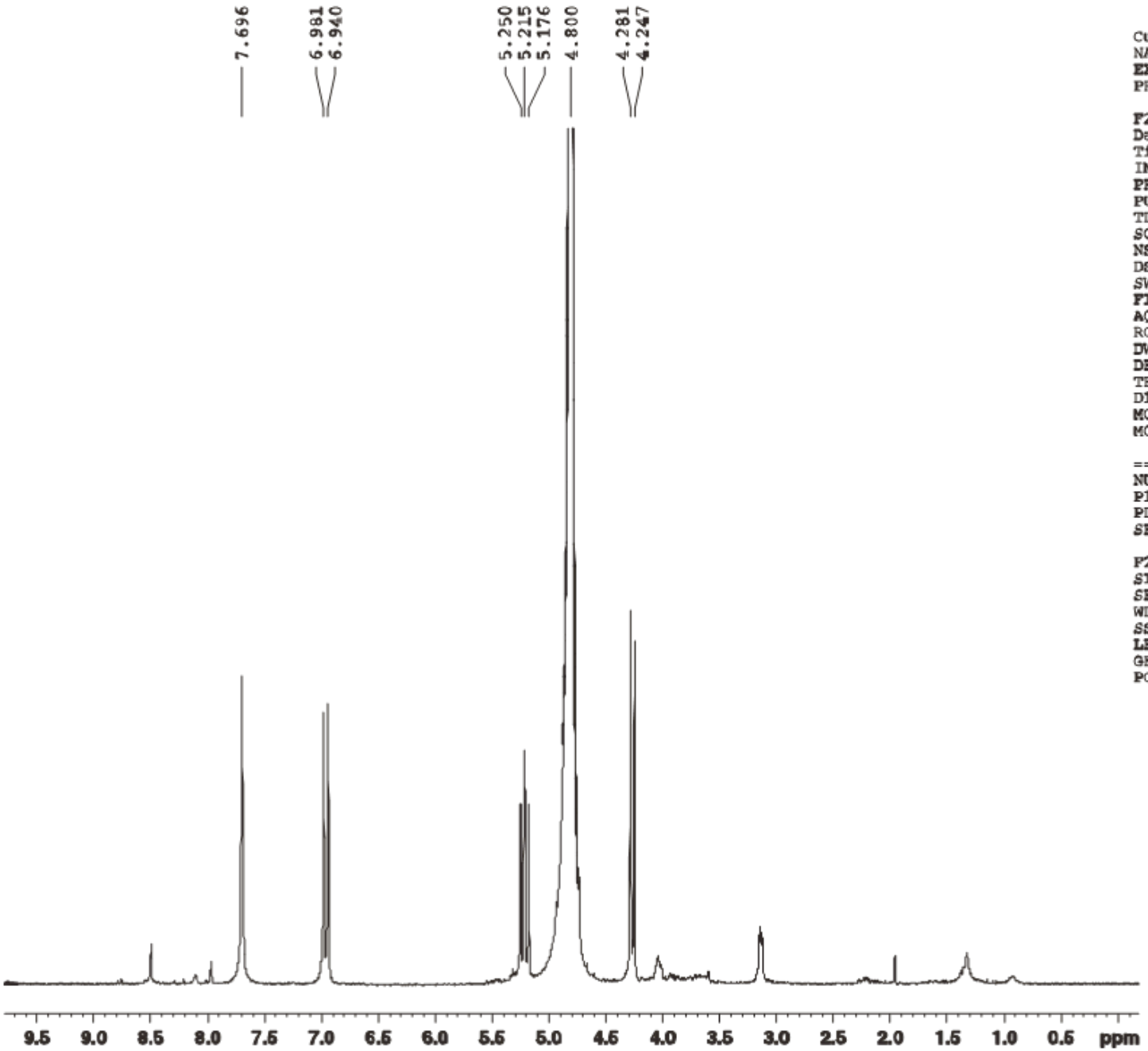


Figure S2. Silica (EtOAc-^{i}PrOH-H_2O 3:6:4) and cellulose (EtOAc-^{i}PrOH-MeOH-H_2O 5:5:18:2) TLC plates for GAF-containing WH6 extract treated with L- and D-amino acid oxidase.

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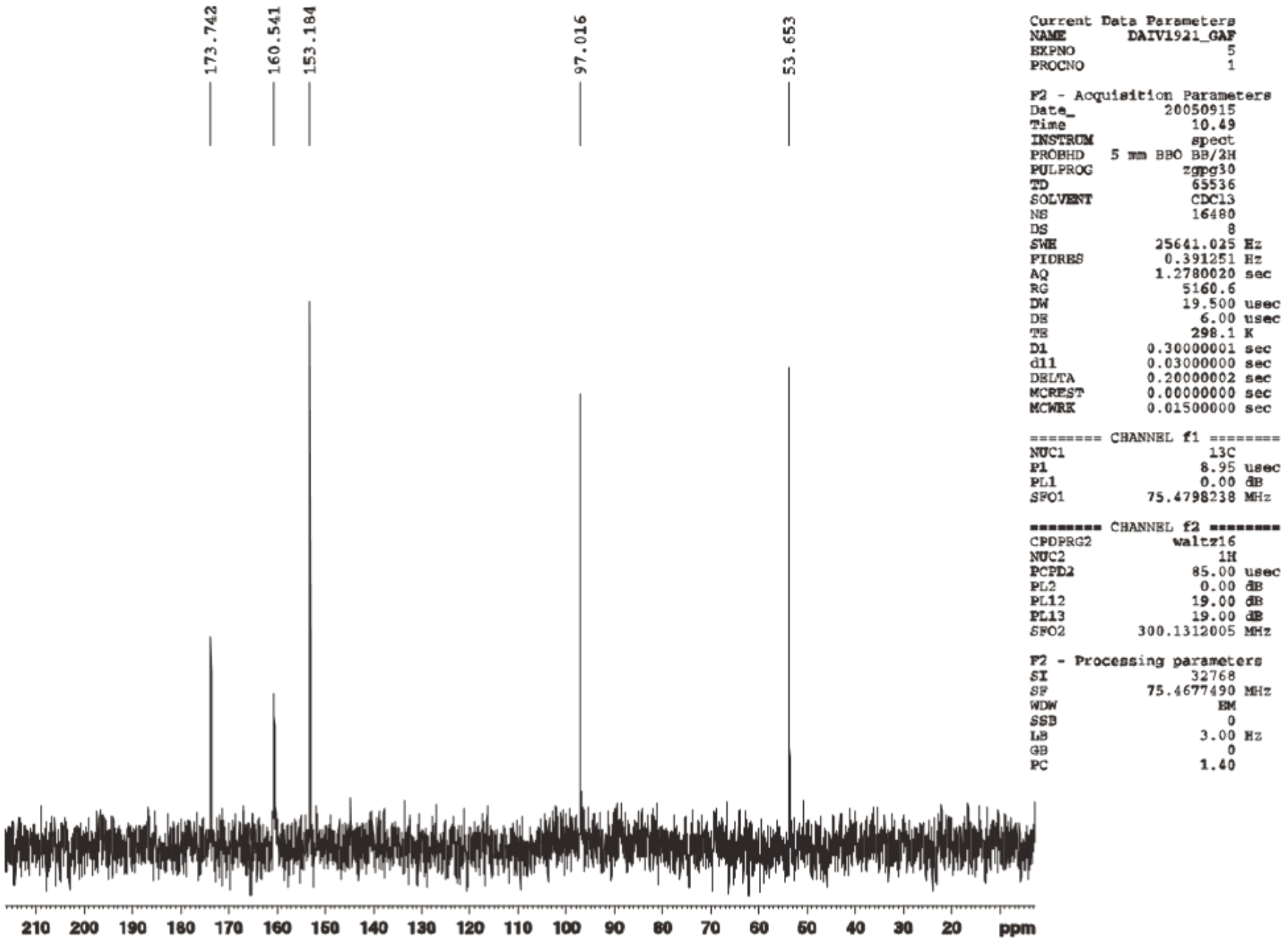

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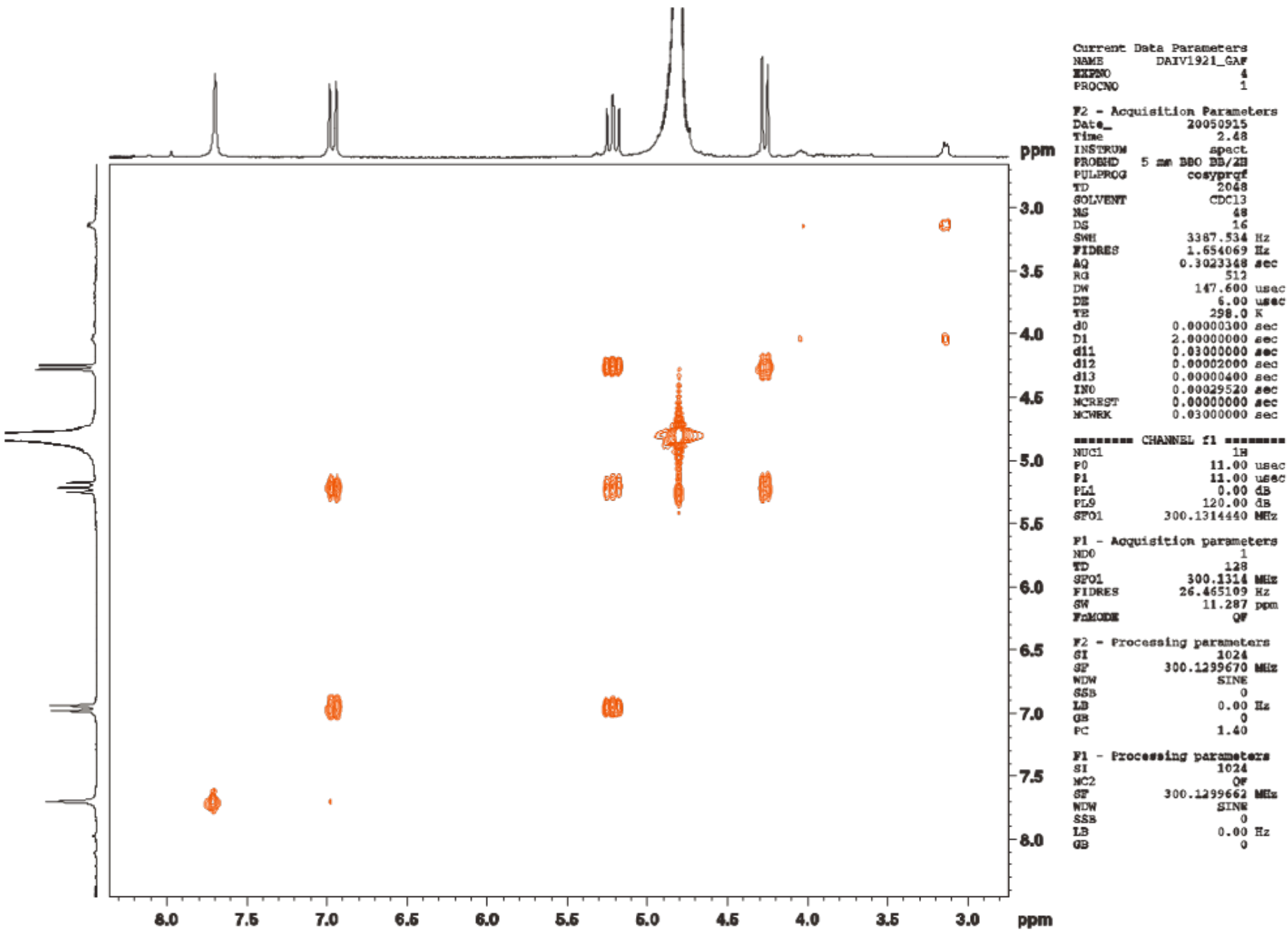

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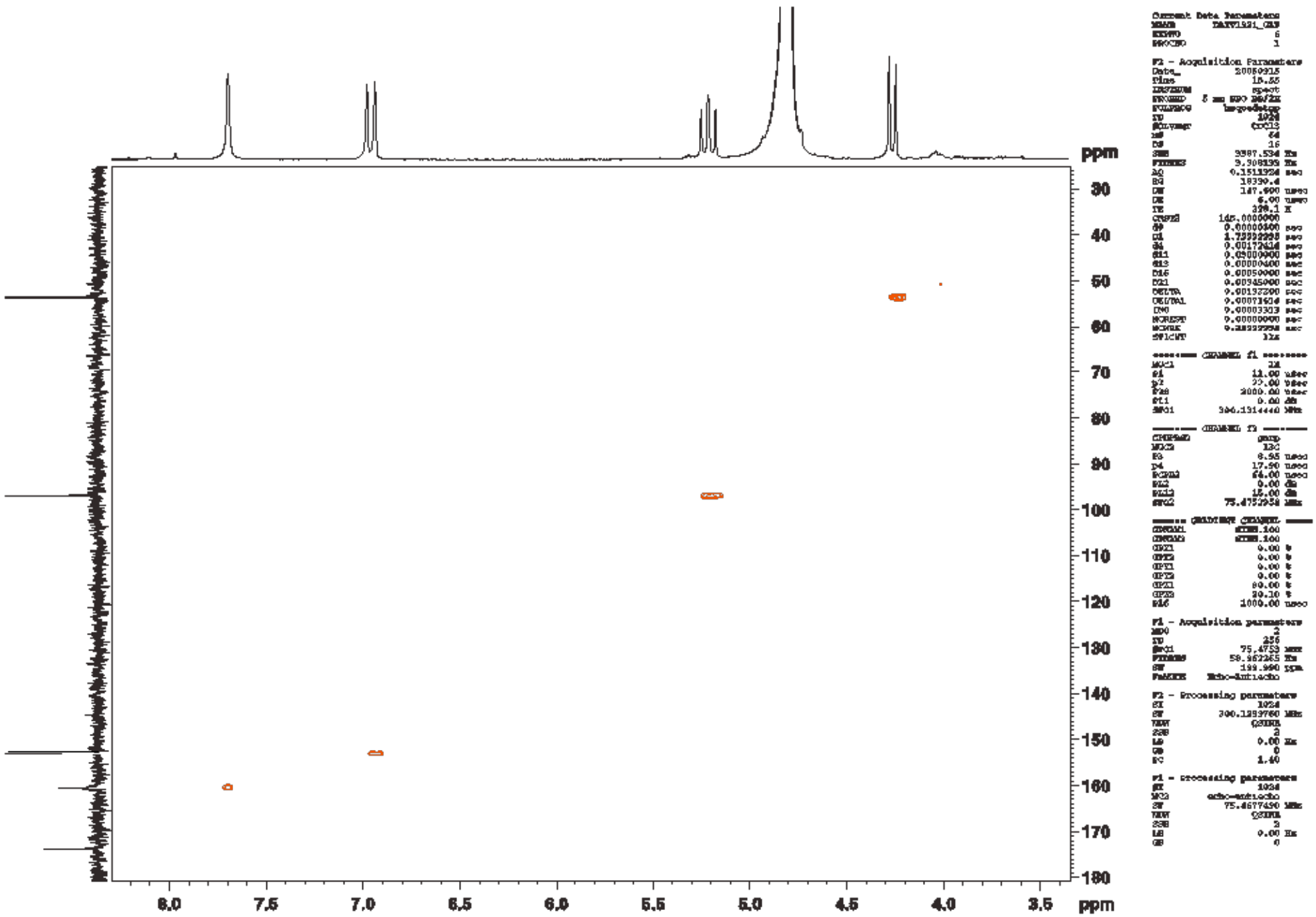

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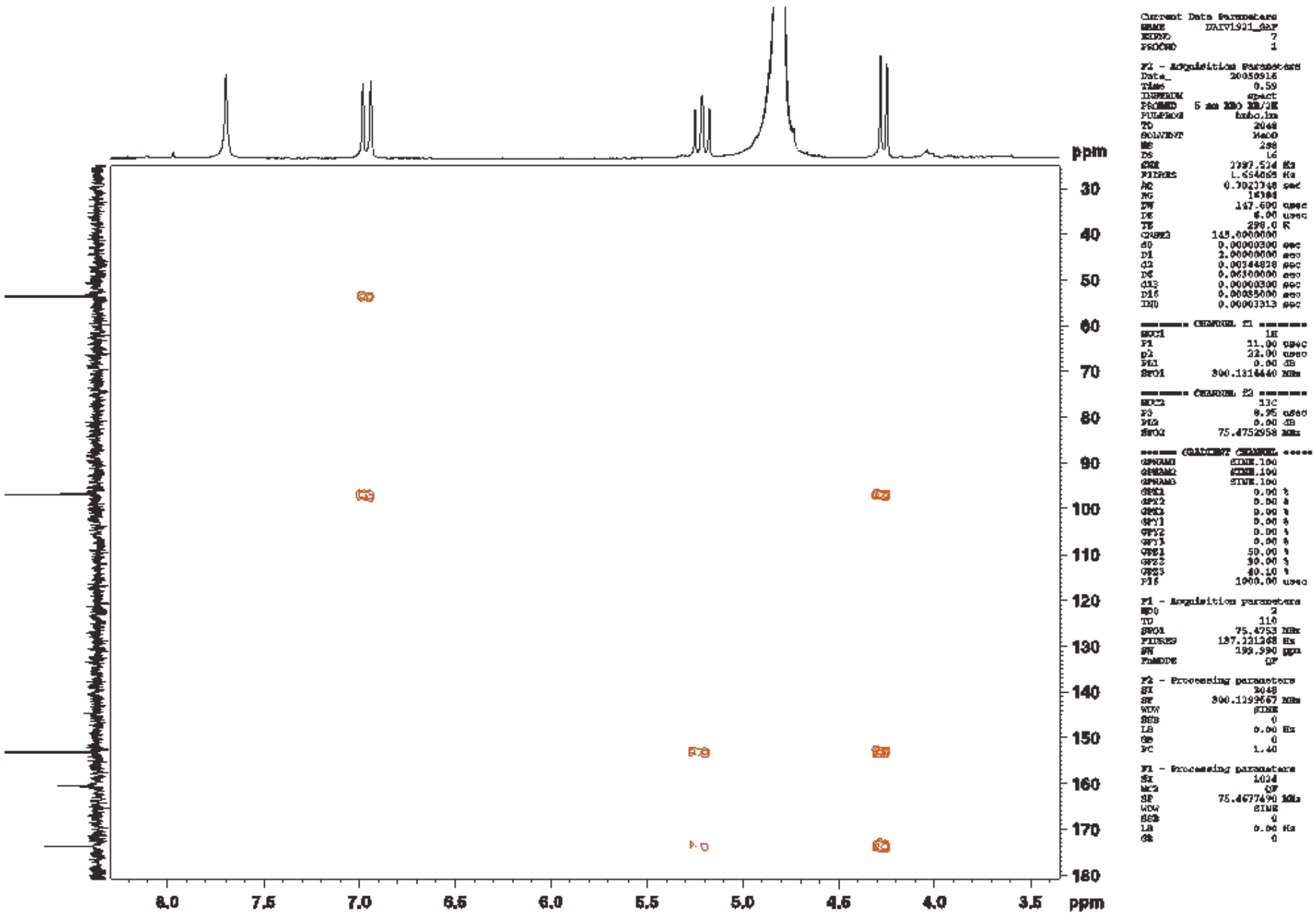

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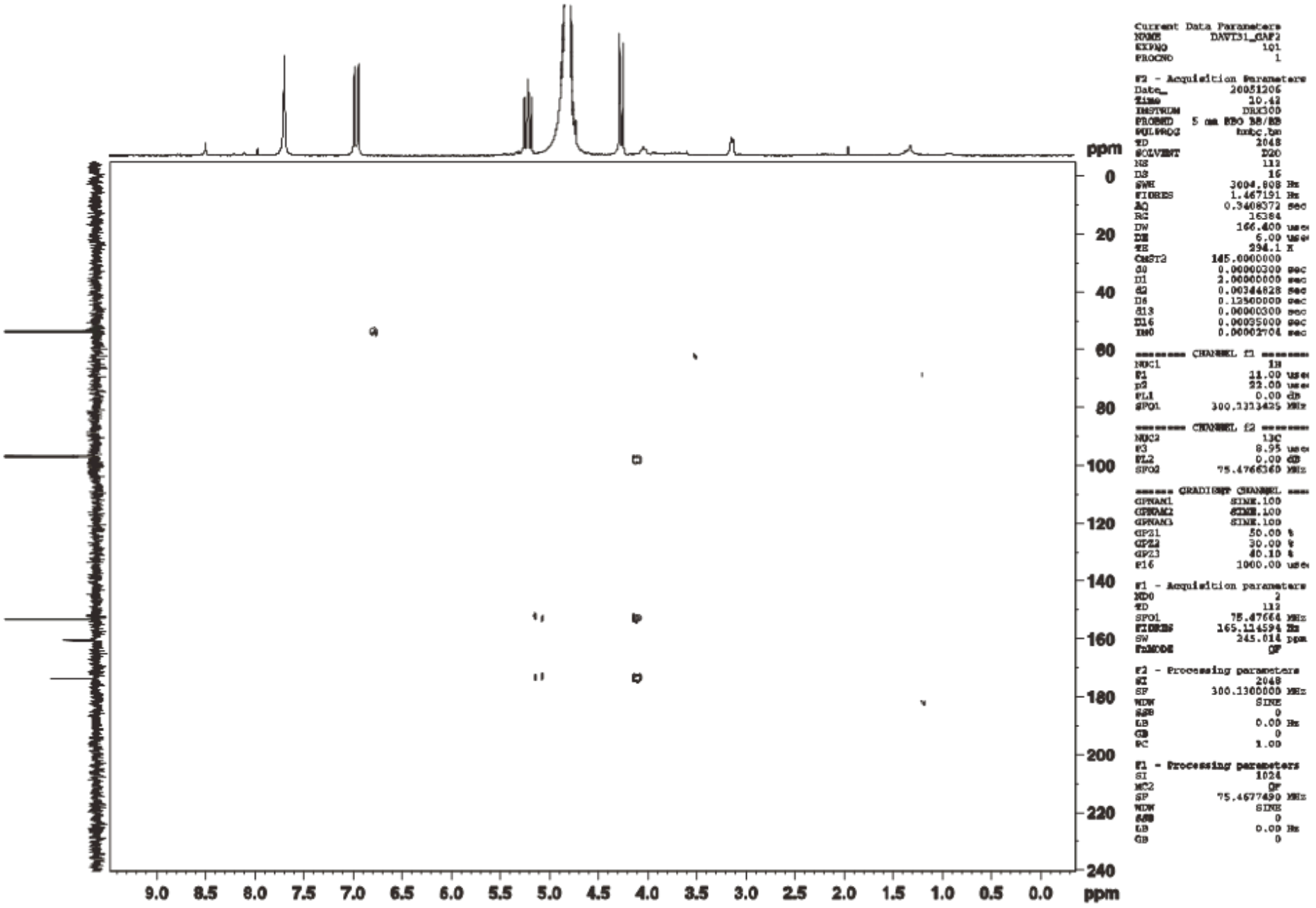

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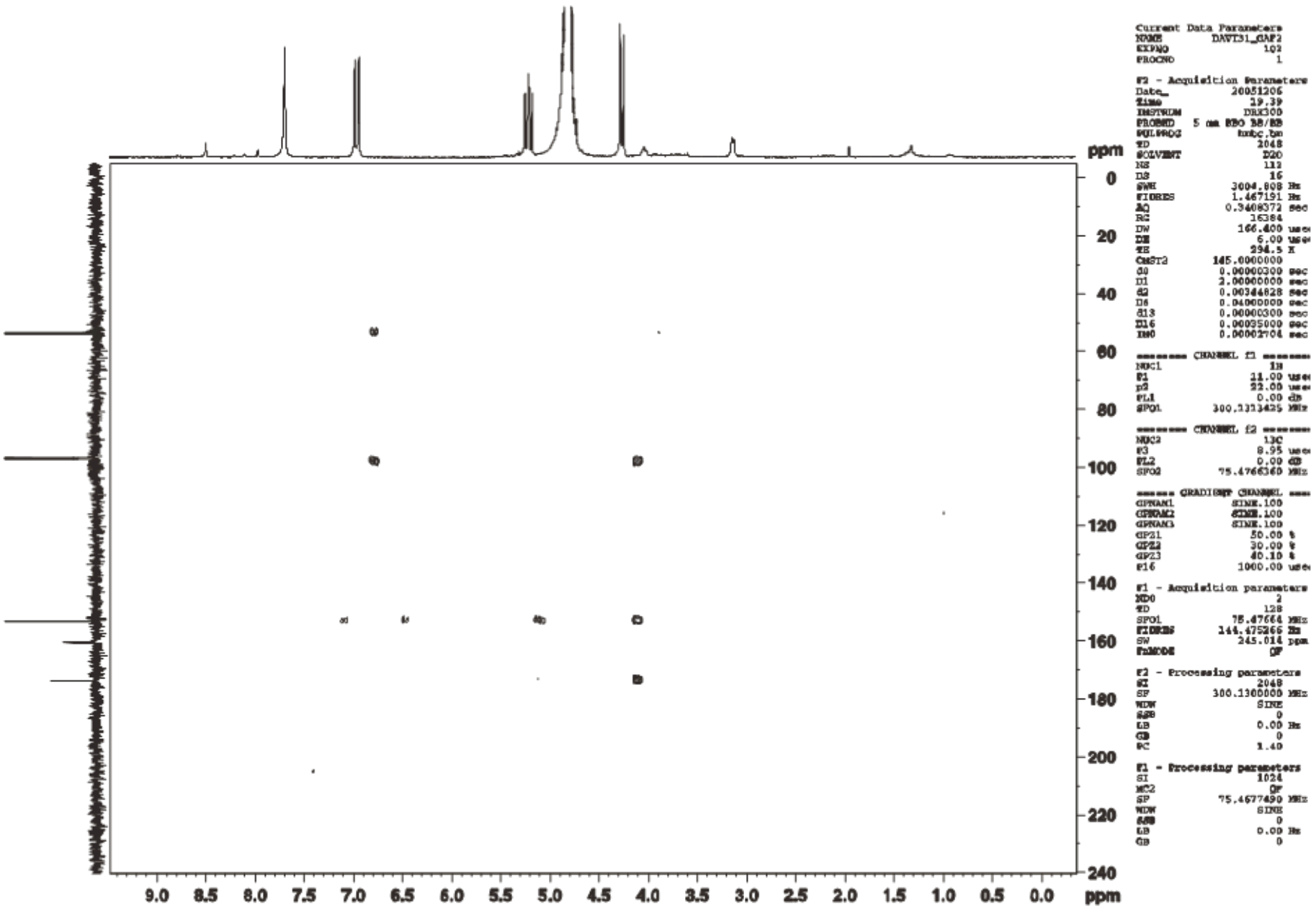

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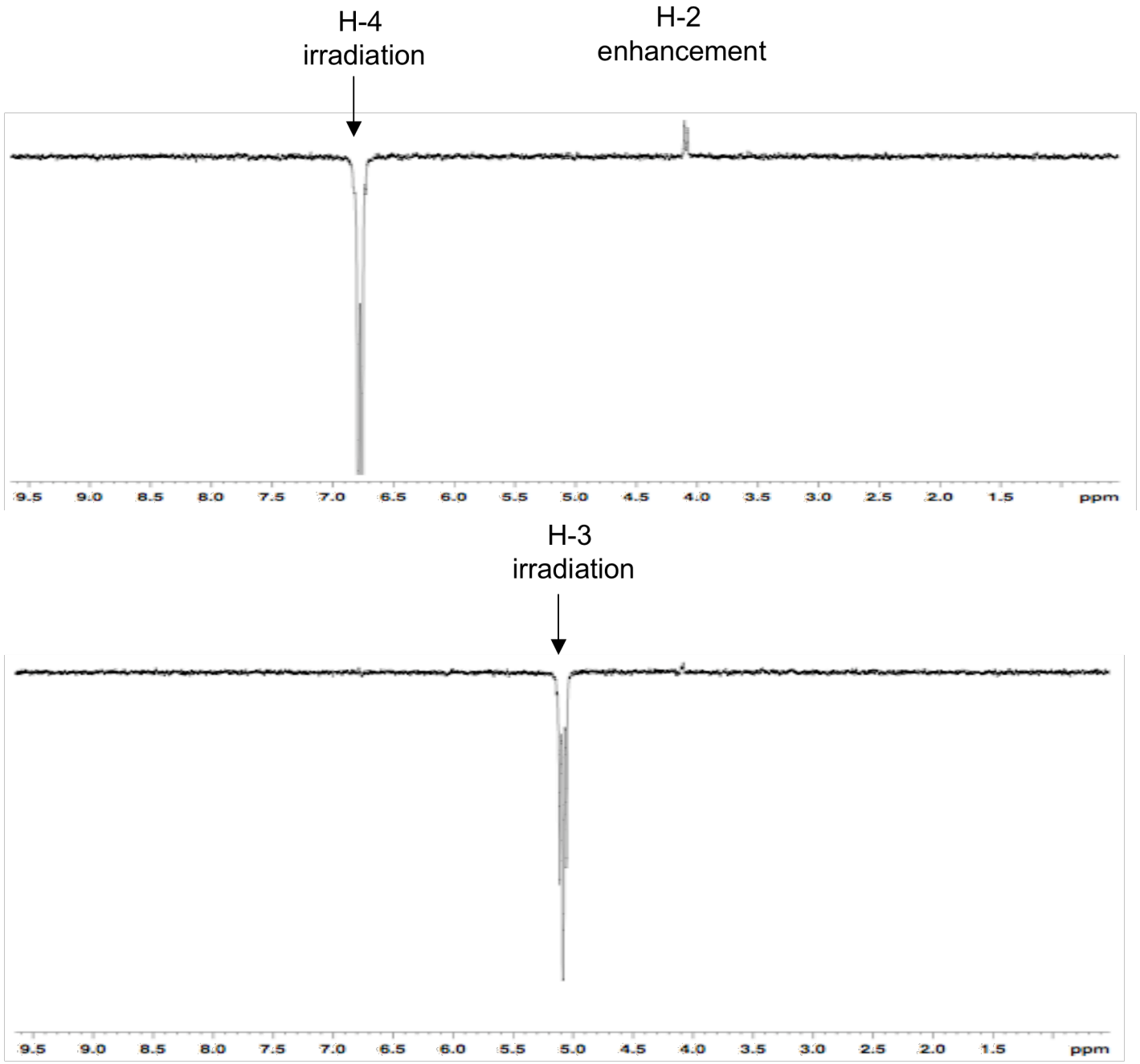
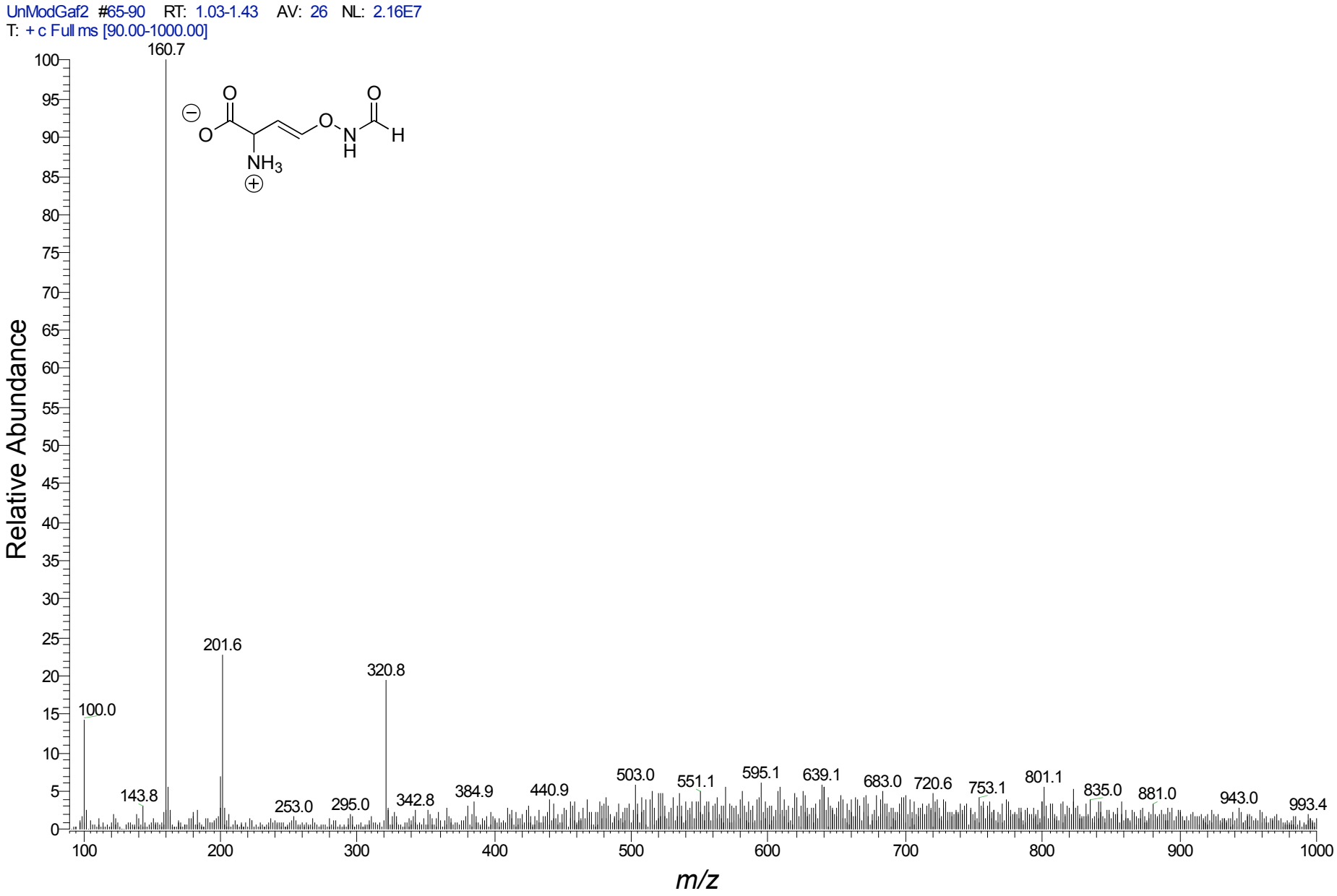


S10. Selective 1D NOESY Spectra (d8 = 500 ms) for 4-formylaminovinylglycine (GAF, **1**)



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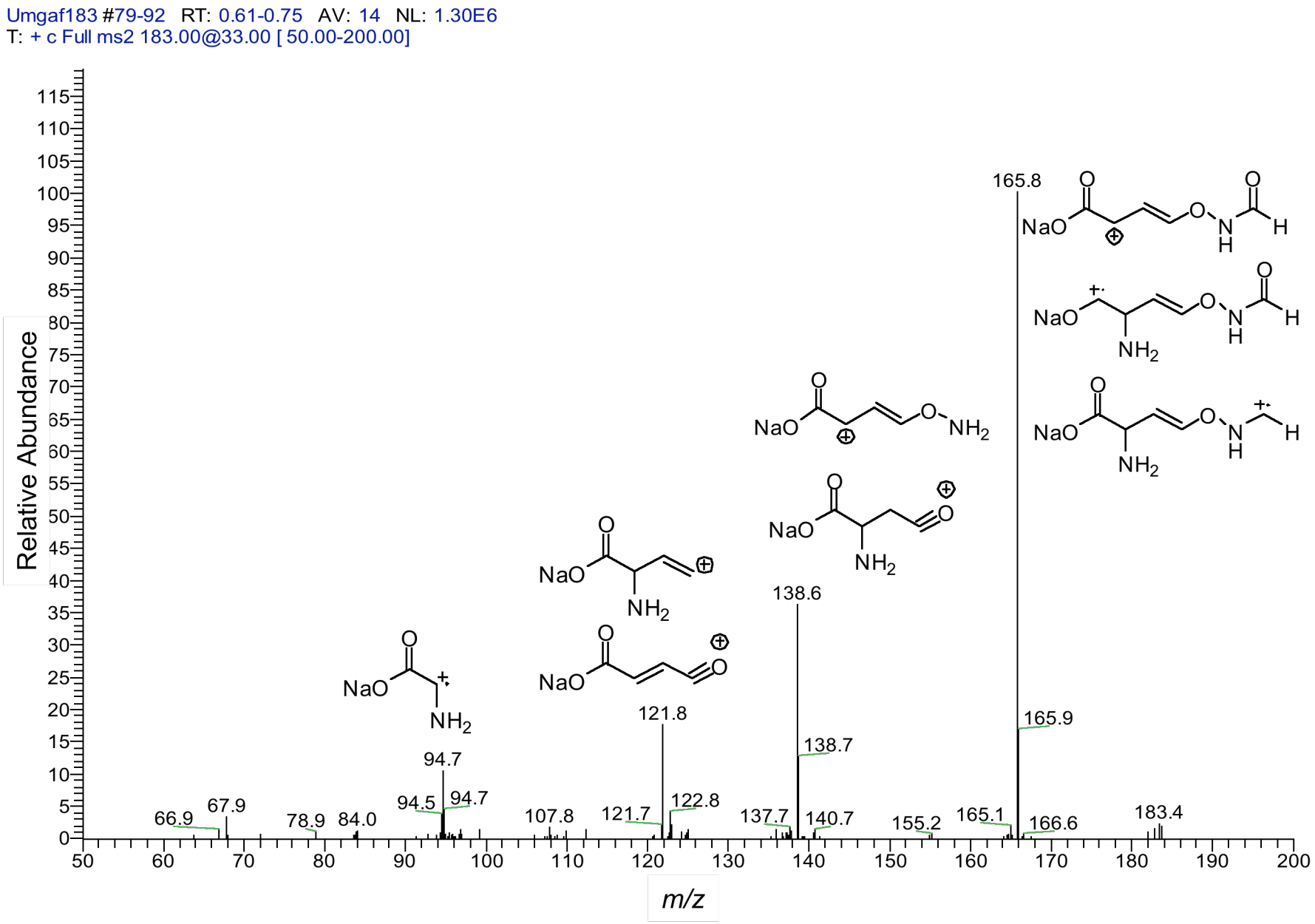


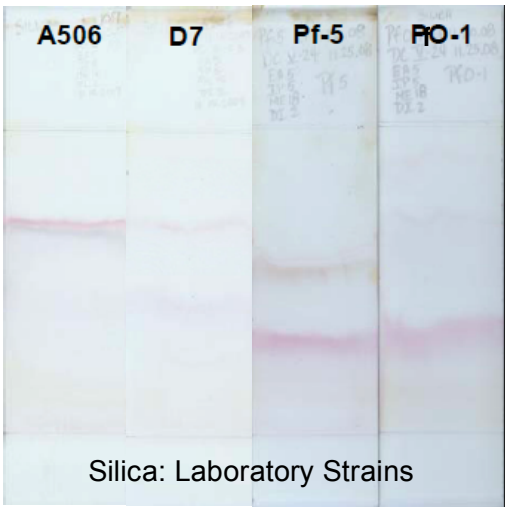
S12. Low resolution ESI-MS^2 of *m/z* 183 $[M+Na]^+$ for 4-formylaminovinylglycine (GAF, **1**)

Table. Taxonomy and Source of USDA-ARS NFSPRC Oregon *Pseudomonas fluorescens* Isolates.

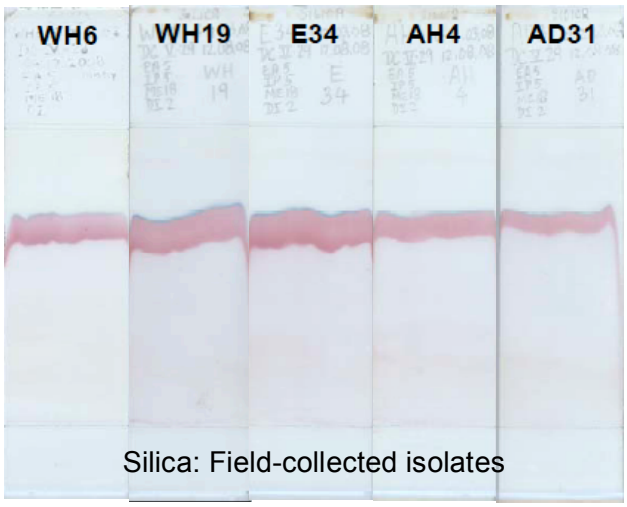
Isolate	NRRL Accession #	Site of Isolation	Rhizosphere or otherwise stated Source of Isolate	*P. fluorescens* Taxonomy	
				Biotype from FAME Analysis (similarity index[a])	rDNA sequence match[b]
A506	N/A	Dr. Joyce Loper, USDA-ARS, OSU, Corvallis	Leaf surfaces of *Pyrus*	A (0.845)	100.0%
D7	N/A	Dr. Ann C. Kennedy, USDA-ARS, WSU, Pullman, WA	*Setaria viridis*	B (0.836)	100.0%
Pf-5	N/A	Dr. Joyce Loper, USDA-ARS, OSU, Corvallis	*Bromus tectorum*	Source ID	
PfO-1	N/A	Dr. Stewart Levy, Tufts University, Medford, MA	*Gossypium hirsutum*	Source ID	
WH6	B-30485	Hyslop Research Farm, OSU, Benton Co.	*Triticum* (wheat)	G (0.888)	99.76%
WH19	B-30484	Hyslop Research Farm, OSU, Benton Co.	*Triticum* (wheat)	A (0.891)	99.77%
E34	B-30481	Hyslop Research Farm, OSU, Benton Co.	Mixed *Poa* species	G (0.885)	99.76%
AH4	B-30482	Disturbed Site, Alsea Valley, Benton Co.	Healthy *Poa* species	A (0.929)	100.0%
AD31	B-30483	Cut Bank, Alsea Valley, Benton County	Dying *Poa* species	B (0.887)	99.68%
AH10	B-50232	Disturbed Site, Alsea Valley, Benton Co.	Healthy *Poa* species	G (0.918)	100.0%
BT1	B-50230	Dept Botany & Plant Pathology Farm, OSU, Linn Co.	*Triticum* (wheat)	A (0.891)	99.76%
E24	B-50229	Hyslop Research Farm, OSU, Benton Co.	Mixed *Poa* species	B (0.807)	100.0%
TDH5	N/A	Organic Vegetable Farm, Corvallis, Benton Co.	Healthy *Poa* species	B (0.849)	99.99%

TR33	B-50220	Dept Botany and Plant Pathology Farm, OSU, Linn Co.	*Triticale*	A (0.912)	99.76%
TR44	B-50219	Dept Botany and Plant Pathology Farm, OSU, Linn Co.	*Triticale*	A (0.913)	99.74%
TR46	B-50218	Dept Botany and Plant Pathology Farm, OSU, Linn Co.	*Triticale*	G (0.940)	99.75%
ALW38	B-50231	Lawn, Alsea Valley, Benton Co.	*Poa* species	G (0.915)	99.79%
G2Y	B-50228	Grower's Field, Linn Co.	*Lolium perenne*	B (0.867)	99.79%
GTR12	B-50227	Organic Vegetable Farm, Philomath, Benton Co.	Grassy weeds, compost pile edge	B (0.890)	99.78%
GTR24	B-50226	Organic Vegetable Farm, Philomath, Benton Co.	Grassy weeds, compost pile edge	B (0.872)	99.78%
A17	N/A	Disturbed Site, Alsea Valley, Benton Co.	*Poa* species	B (0.745)	100.0%
GTR40	B-50223	Organic Vegetable Farm, Philomath, Benton Co.	Grassy weeds, compost pile edge	G (0.910)	99.77%
HB14	B-50224	Lawn, Alsea Valley, Benton Co.	*Poa* species	B (0.868)	99.78%
HB26	B-50223	Lawn, Alsea Valley, Benton Co.	*Poa* species	B (0.884)	99.78%
HB32	B-50222	Lawn, Alsea Valley, Benton Co.	*Poa* species	B (0.867)	99.78%
ST22	B-50221	Hyslop Research Farm, OSU, Benton Co.	*Hordeum vulgare*	G (0.953)	99.78%
W36	B-50217	Hyslop Research Farm, OSU, Benton Co.	*Triticum* with *Poa* species	G (0.892)	99.77%
A3422A	B-50234	Disturbed site, Alsea Valley, Benton Co.	Unknown	B (0.901)	99.79%
AH7	N/A	Disturbed site, Alsea Valley, Benton Co.	*Poa* species	B (0.745)	100%

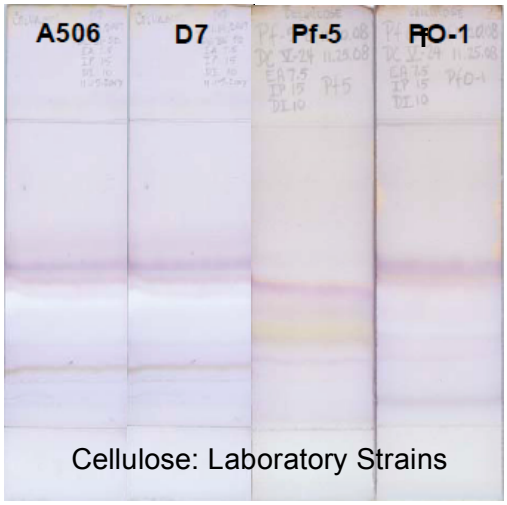
[a] Microcheck Database similarity index of 1.00 = perfect match**;** [b] 16S and LSU D2 % gene sequence match (Microcheck Database)



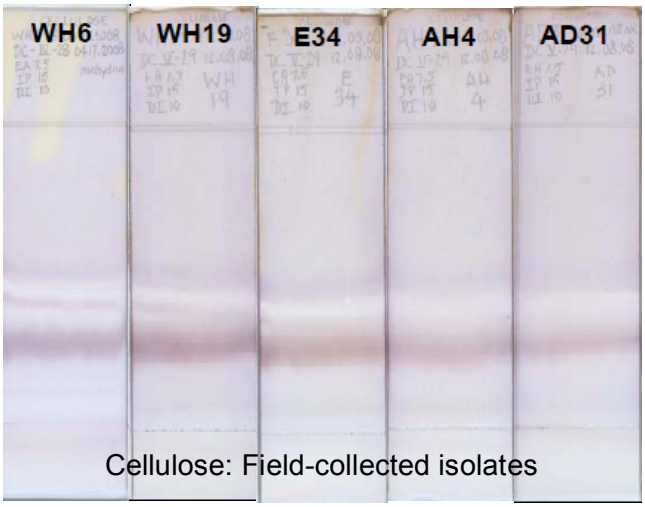
Silica: Laboratory Strains



Silica: Field-collected isolates

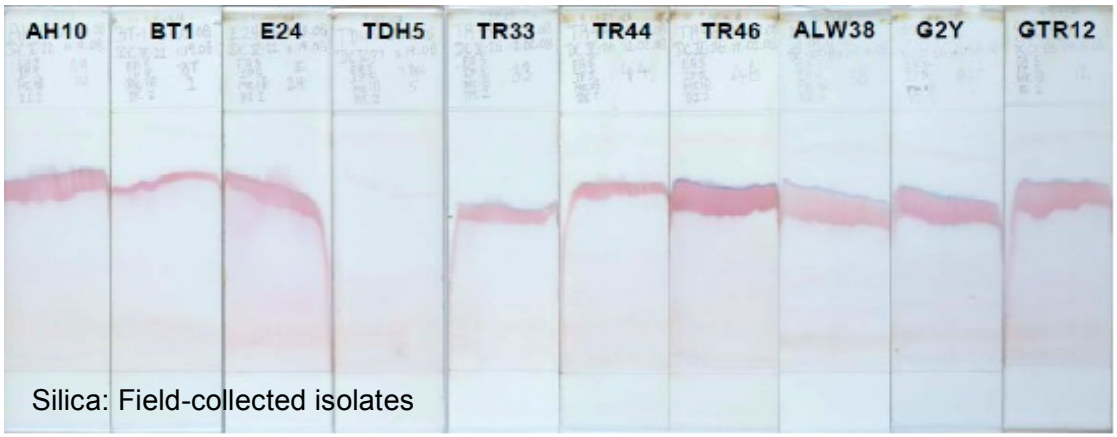


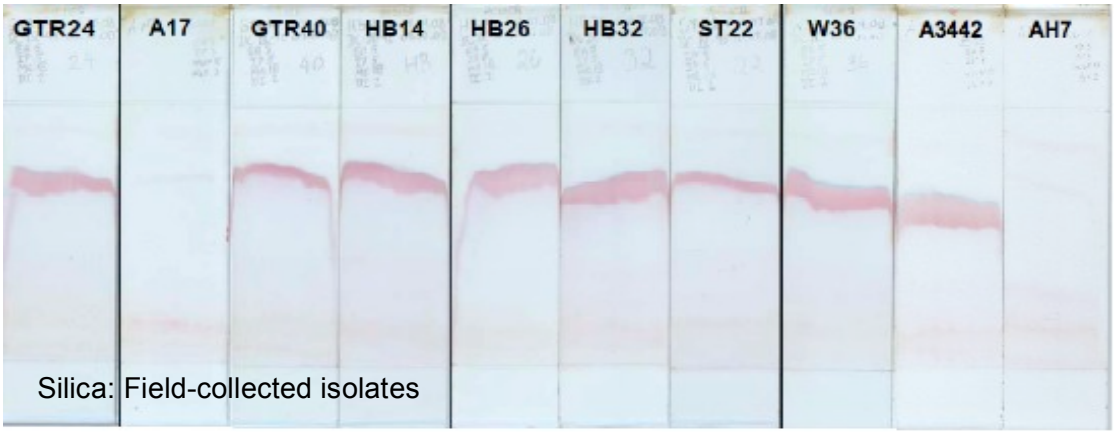
Cellulose: Laboratory Strains

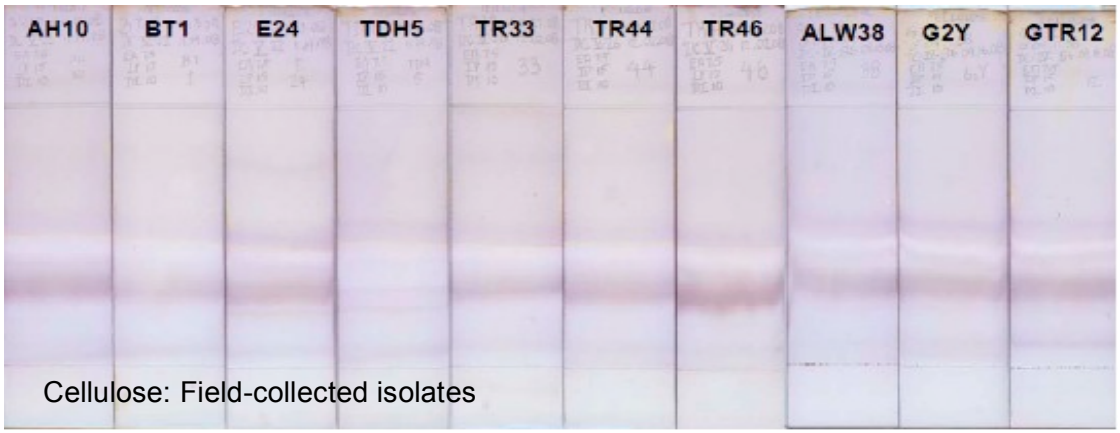


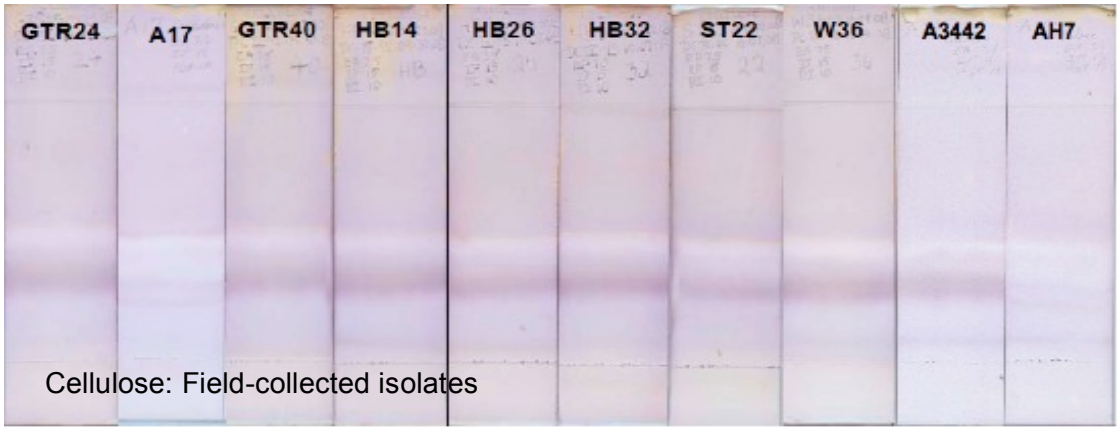
Cellulose: Field-collected isolates

Silica (EtOAc-^{i}PrOH-H_2O 3:6:4) and cellulose (EtOAc-^{i}PrOH-MeOH-H_2O 5:5:18:2) TLC plates for four laboratory strains and the original five field-collected isolates of *P. fluorescens*.

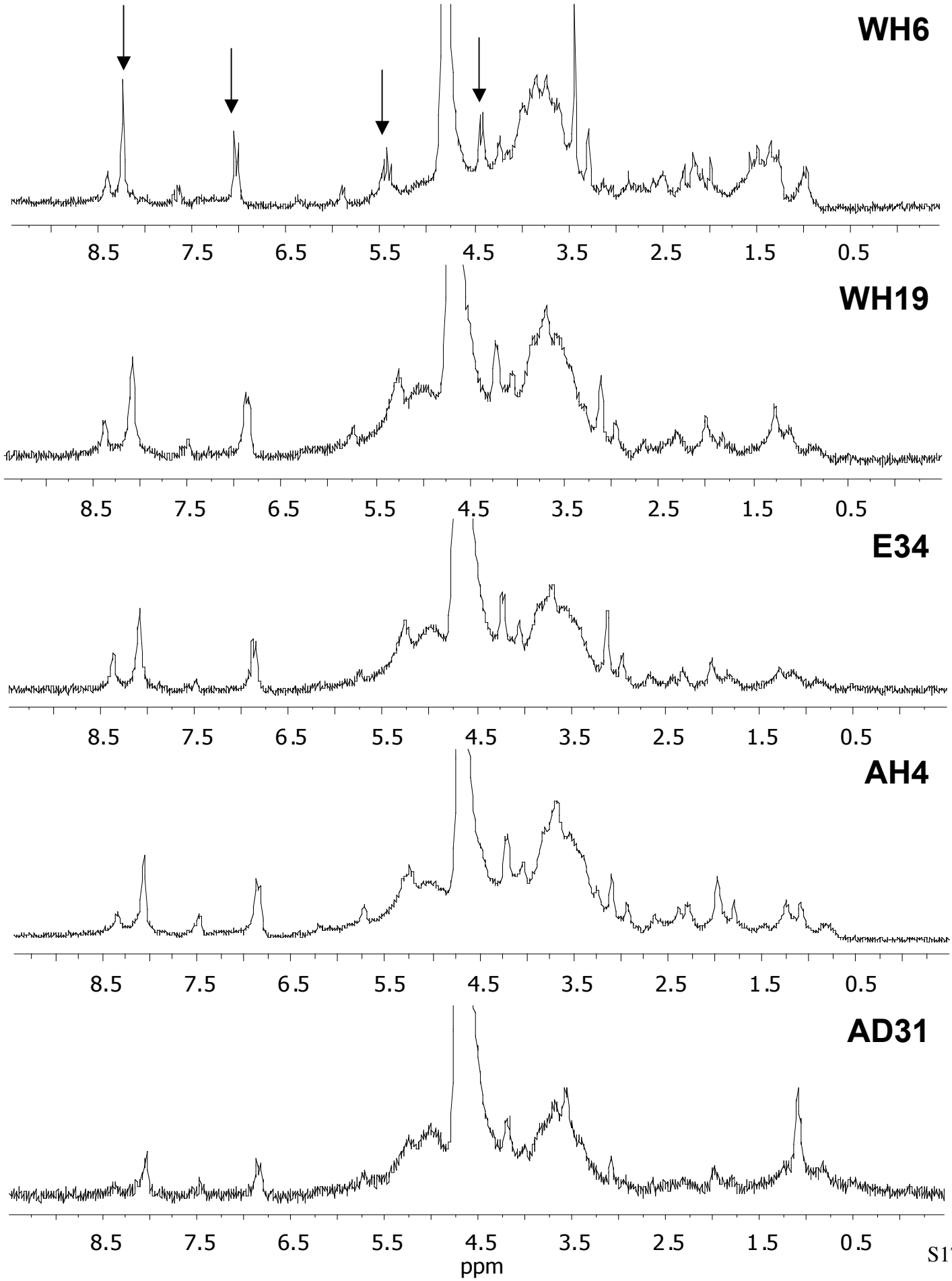








Silica and cellulose TLC plates for twenty additional field-collected *P. fluorescens* isolates.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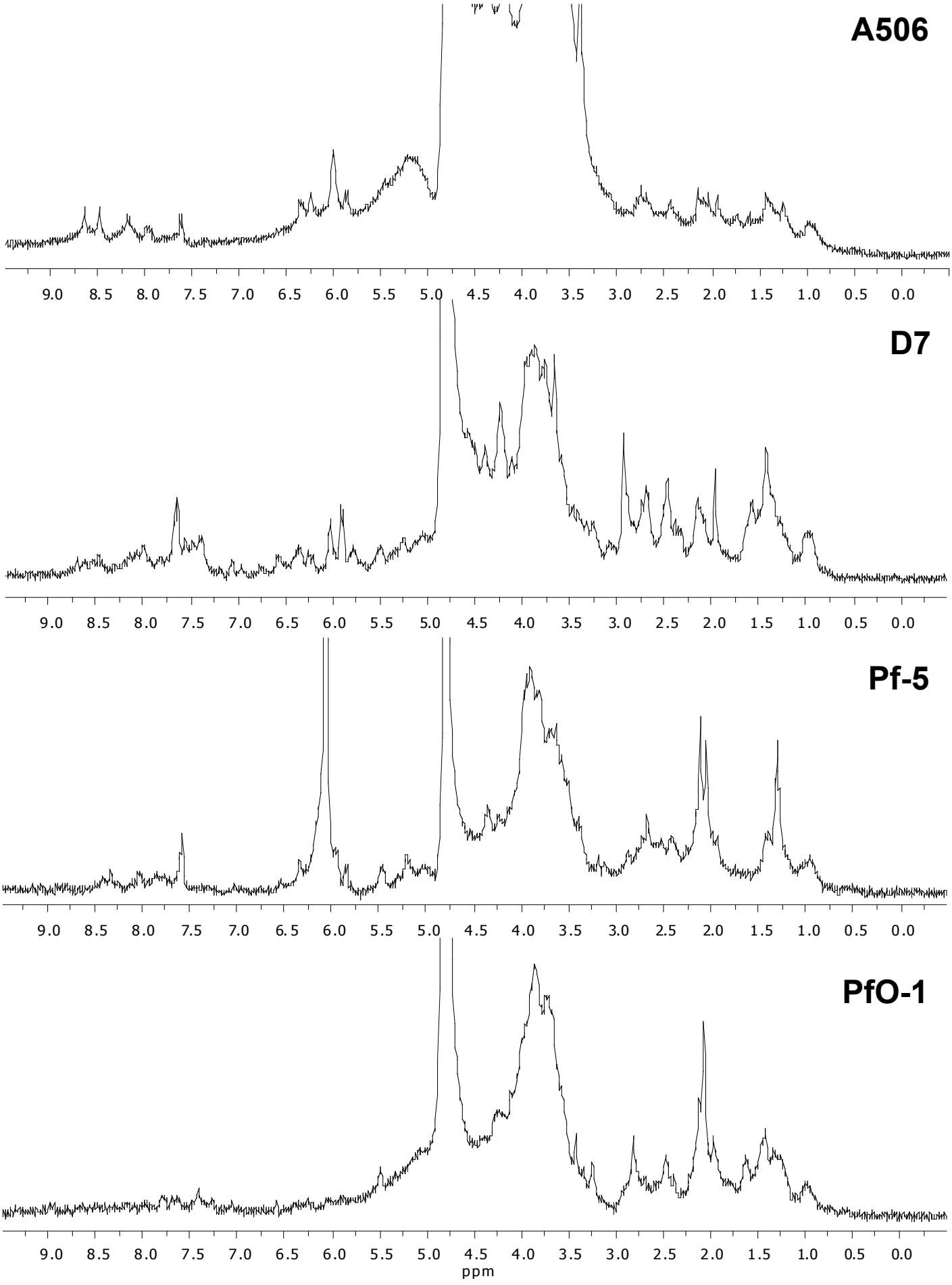
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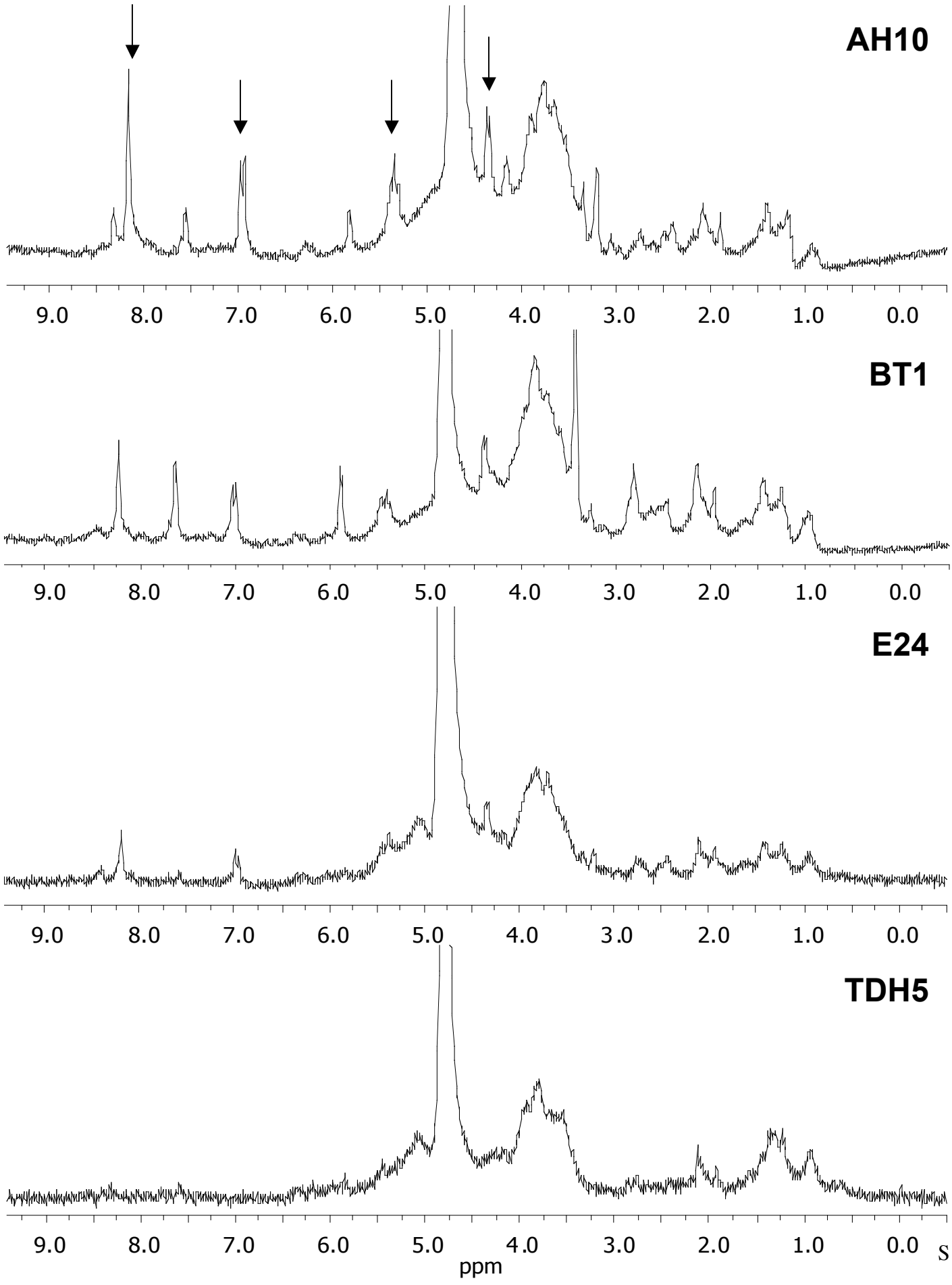
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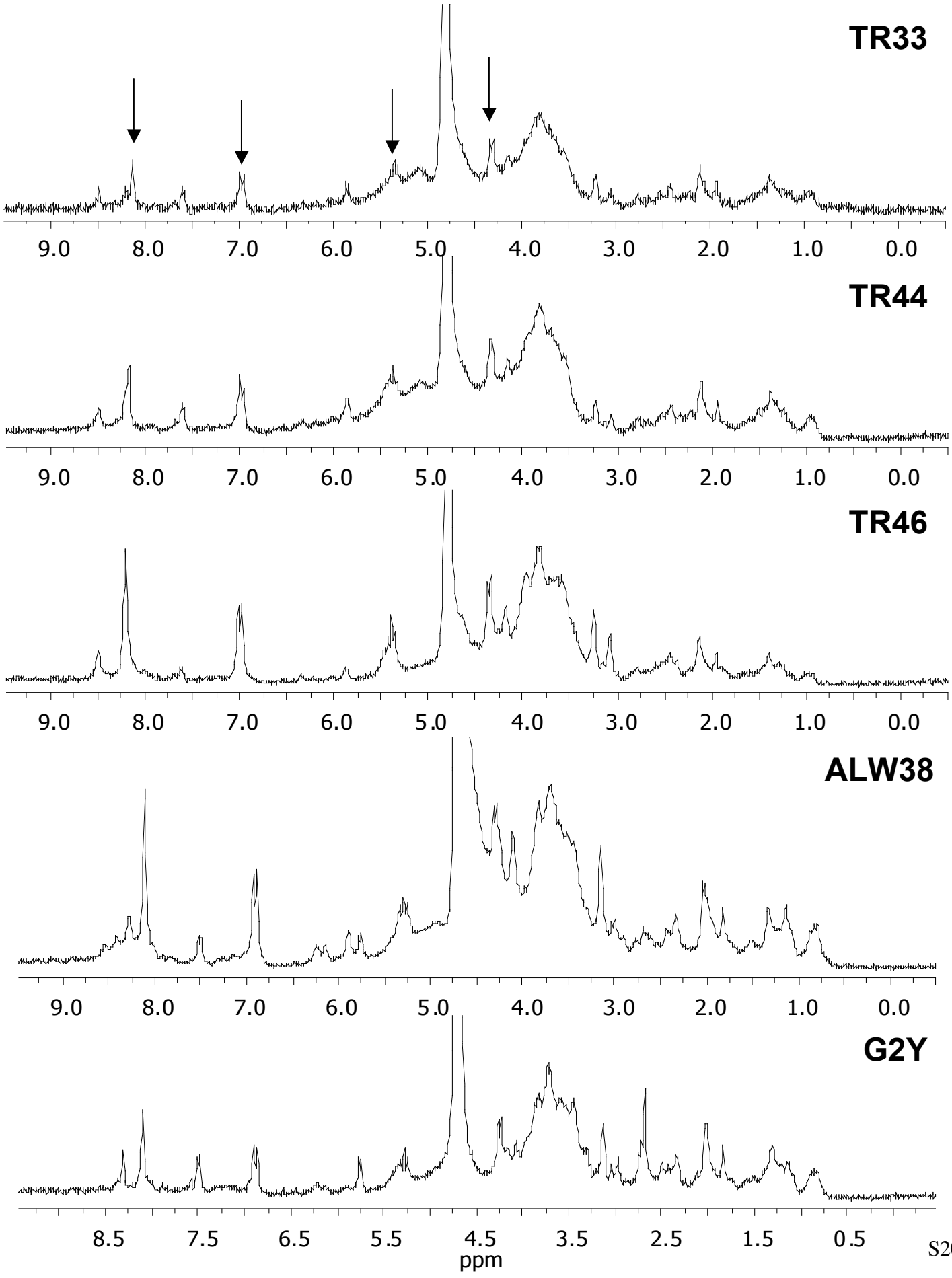
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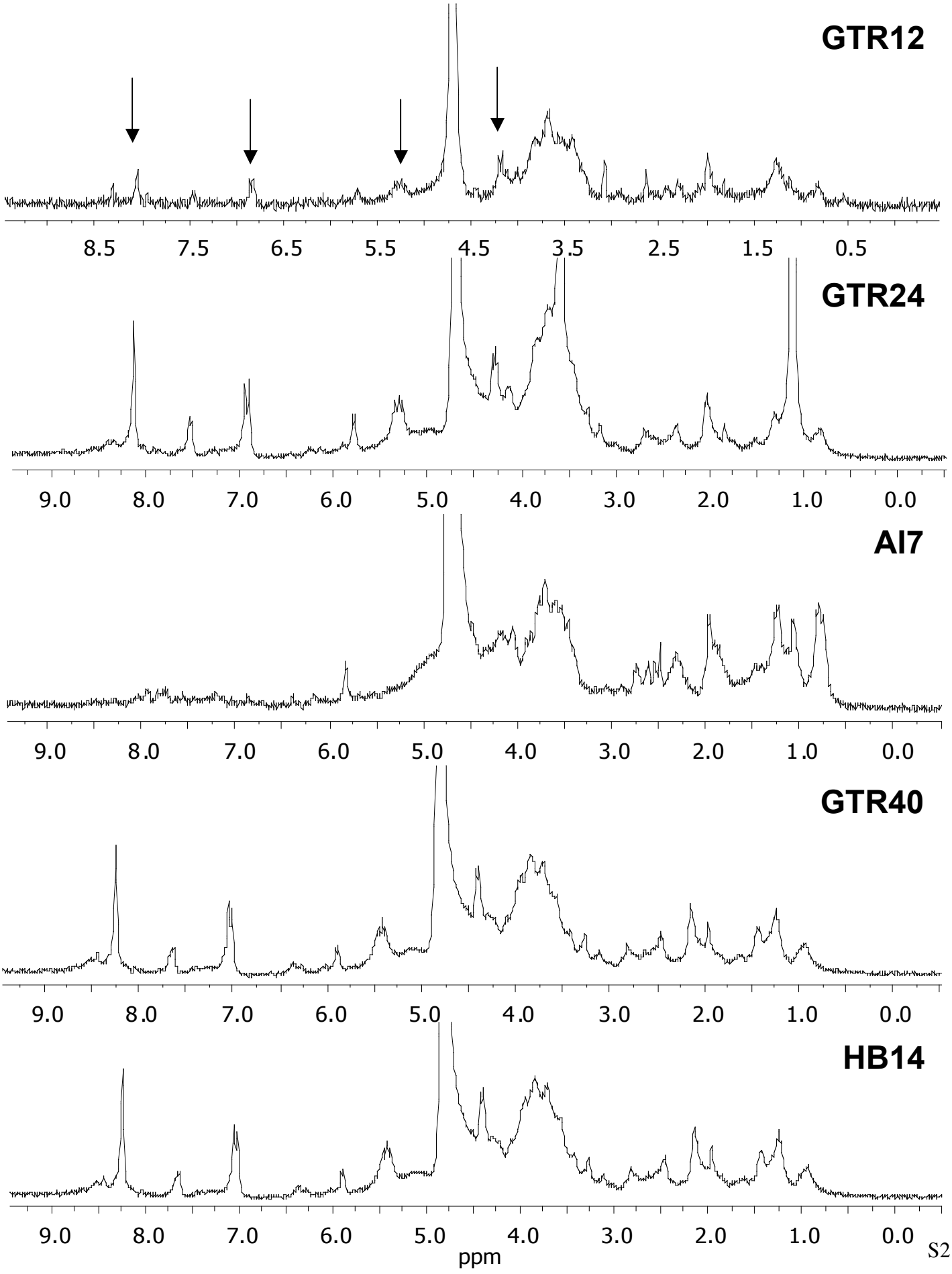
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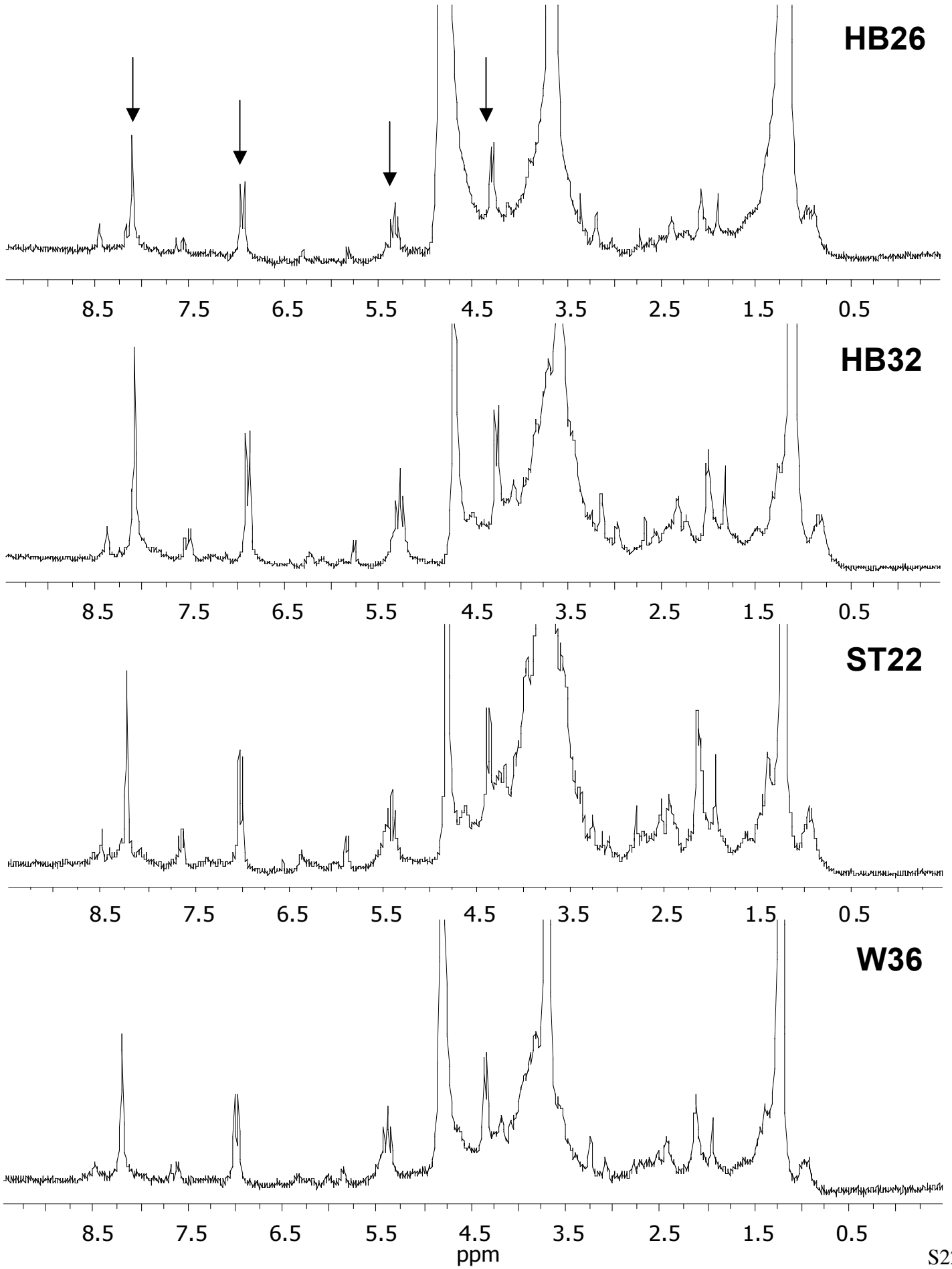
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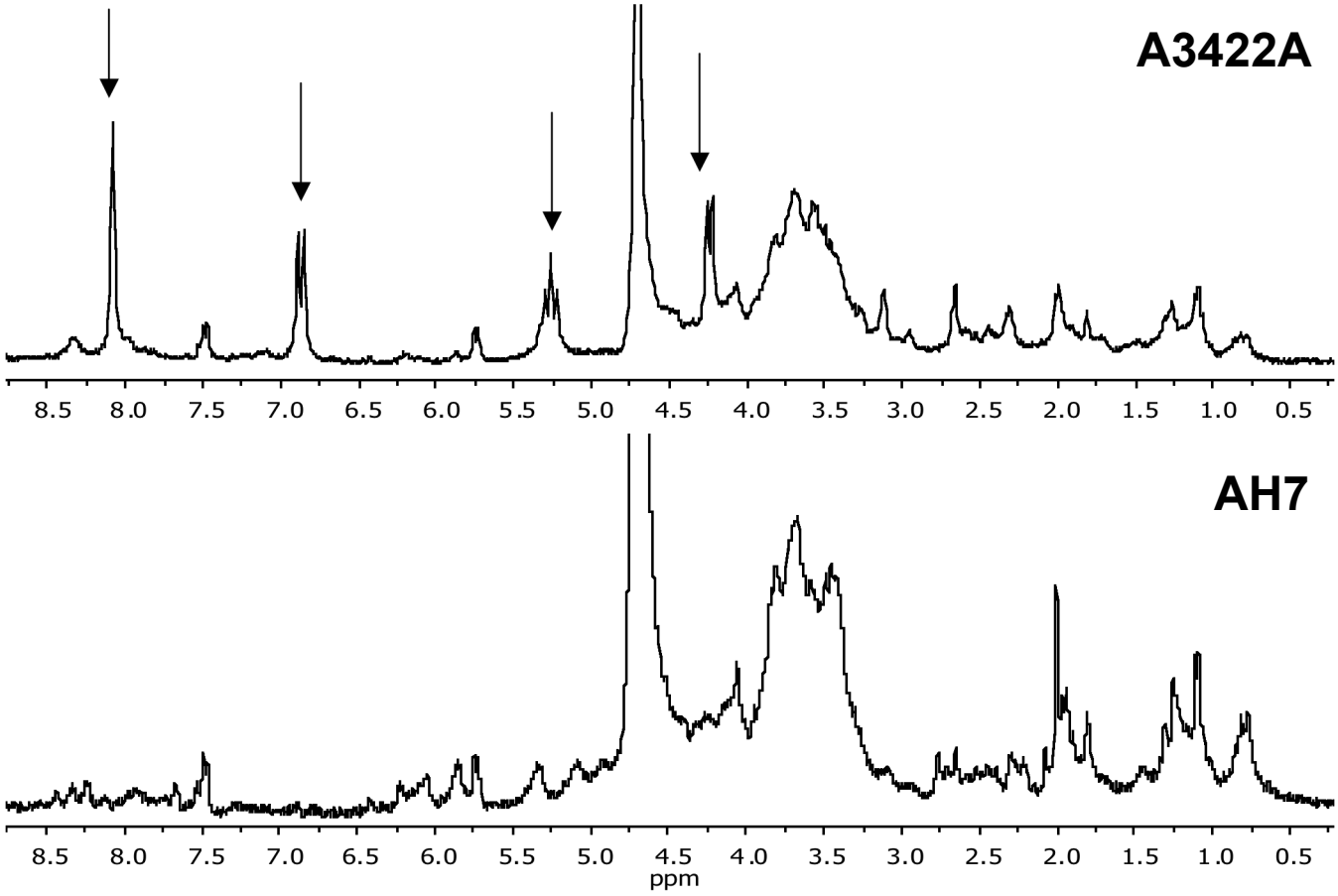
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