frontiers

Supplementary material

^{1}H NMR-Based Chemometrics to Gain Insights into the Bran of Radiation-Induced Colored Wheat Mutant

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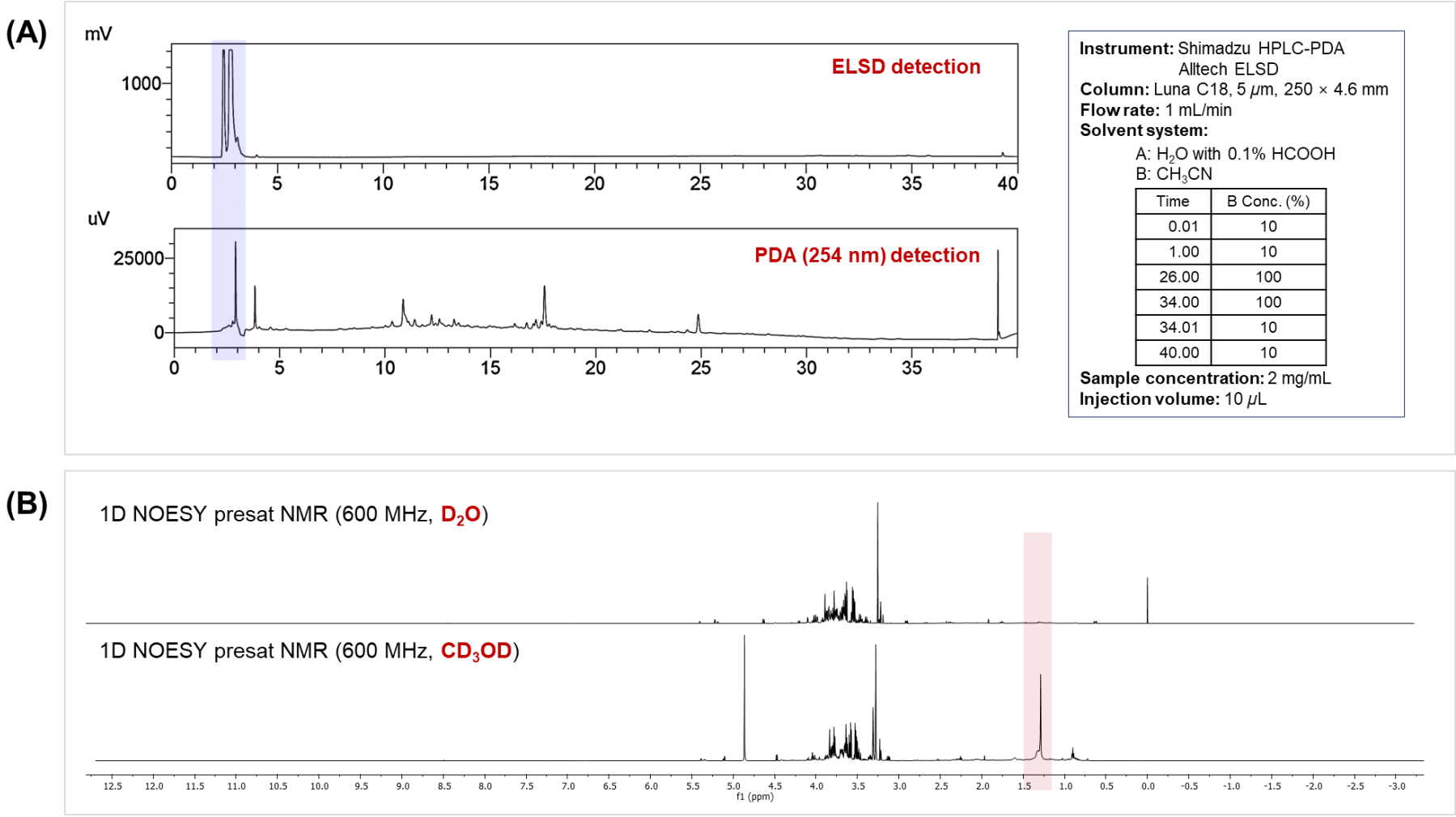
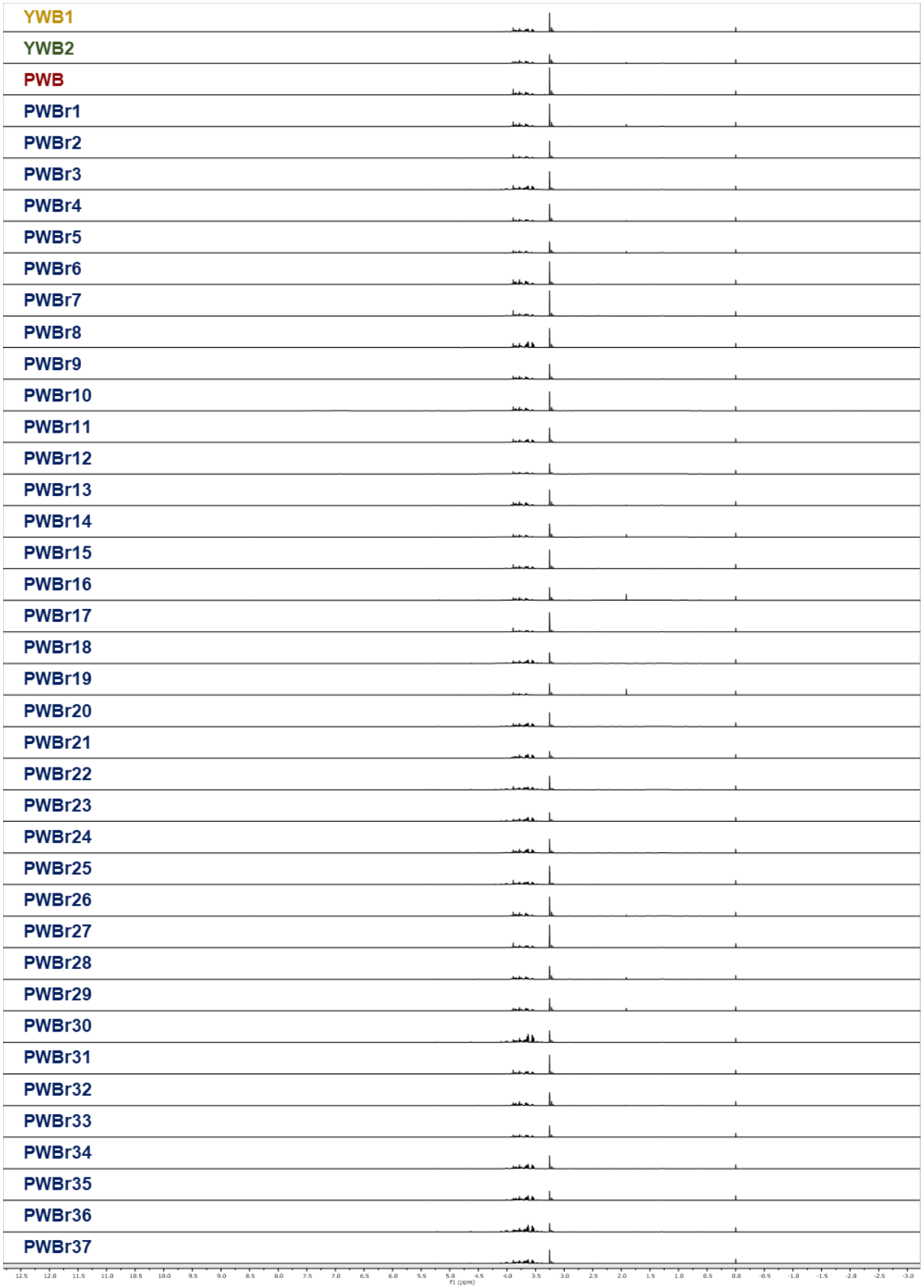


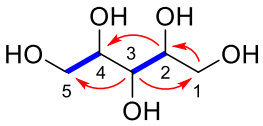
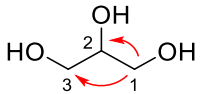
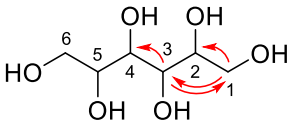
Supplementary Figure 1. Preliminary data of **PWBr18**: (A) comparison of HPLC analysis data under detection of ELSD and PDA (254 nm), and (B) comparison of ^{1}H NMR (600 MHz) spectra in D_2O and CD_3OD.



Supplementary Figure 2. ^{1}H NMR (600 MHz, D_2O) spectra of 40 wheat bran samples (1D NOESY presat pulse sequence with δ_H 4.70 of a presaturation frequency for water suppression)

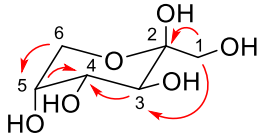
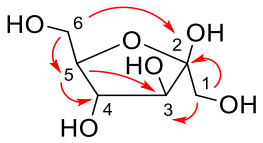
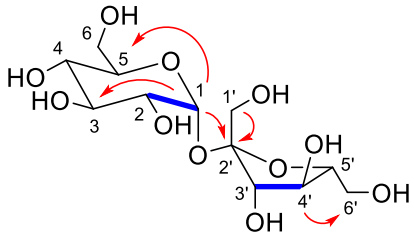
Supplementary Table 1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data of the identified metabolites with ^{1}H-^{1}H COSY (**blue bold**) and ^{1}H-^{13}C HMBC (→) correlations in three representatives, **PWB**, **PWBr3**, or **PWBr19**.

(NOTE) This table includes the assignments of ^{1}H and ^{13}C NMR resonances supported by 2D NMR correlations, with aids of the BMRD NMR data base. The ^{1}H NMR peak assignments, performed only by the peak shape-based assessment using compound information from the Chenomx-embedded library (i.e. no sufficient evidences from 2D NMR correlation), were labeled "LB" in a meaning of "library based". The ^{13}C NMR assignment was still given when the ^{13}C NMR signal was significantly detectable in the ^{13}C NMR data of the extract samples and unambiguous assignment was allowed in comparison with the BMRD reference or previously reported data. "ND" (not determined) indicates the case where the ^{13}C NMR signal was not determined due to low signal intensity or signal overlap. The 2D NMR correlations with substantial signal overlap, were not included due to uncertainty in the assignment.

metabolite (ID)	position	δ_H, mult (*J* in Hz)	δ_C	COSY (**blue bold**)	HMBC (H → C)	Reference sample code	MW (Da)
Sugar Alcohols							
 arabinitol (**Ara**)	1	3.83, dd (12.0, 3.0), Ha 3.65[a], Hb	65.7	Ha/Hb	2	**PWB**	152.15
	2	3.74[a]	73.6	3	1, 3, 4		
	3	3.56, dd (8.4, 2.2)	73.2	2	1, 2, 4, 5		
	4	3.92, ddd (7.4, 5.5, 2.2)	73.0	5	5		
	5	3.66[a]	65.8	4			
 glycerol (**Glo**)	1	3.64, dd (11.6, 4.7), Ha 3.55, dd (11.6, 6.5), Hb	65.2	Ha/Hb	2, 3	**PWB**	104.17
	2	3.77[a]	74.8				
	3	3.64, dd (11.6, 4.7), Ha 3.55, dd (11.6, 6.5), Hb	65.2	Ha/Hb	1, 2		
 mannitol (**Man**)	1	3.86, dd (11.8, 2.8), Ha 3.66, dd (11.8, 6.2), Hb	65.9	Ha/Hb	2, 3	**PWB**	182.17
	2	3.75, ddd (8.5, 6.2, 2.8)	73.5		1, 4		
	3	3.79, d (8.5)	72.0		1, 4, 5		
	4	3.79, d (8.5)	72.0		2, 3, 6		
	5	3.75, ddd (8.5, 6.2, 2.8)	73.5		3, 6		
	6	3.86, dd (11.8, 2.8), Ha 3.66, dd (11.8, 6.2), Hb	65.9	Ha/Hb	4, 5		

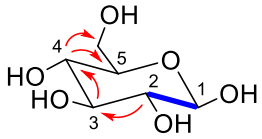
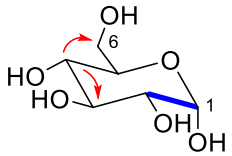
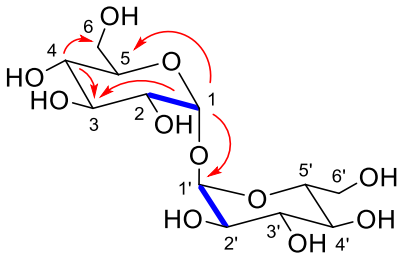
[a]Multiplicity was not determined due to signal overlap.

(**Supplementary Table 1.** continued)

metabolite (ID)	position	δ_H, mult (*J* in Hz)	δ_C	COSY (**blue bold**)	HMBC (H → C)	Reference sample code	MW (Da)
Sugars							
fructose (**Frc**)							
	1	3.70, d (11.8), Ha 3.55, d (11.8), Hb	66.6	Ha/Hb	2, 3		
	2		100.8				
	3	3.78, d (10.0)	70.3				
	4	3.88, dd (10.0, 3.5)	72.4		3		
	5	3.98, dt (3.5, 1.3)	71.9		3, 4, 6		
	6	4.01, dd (12.7, 1.3), Ha 3.69, dd (12.7, 1.3), Hb	66.1	Ha/Hb	2, 5	**PWBr3**	180.16
β-fructopyranose							
	1	3.58, d (12.1), Ha 3.54[a], Hb	65.4		2, 3		
	2		104.2				
	3	4.10[a]	78.1		4		
	4	4.10[a]	77.2		3		
	5	3.82[a]	83.4		4		
	6	3.80[a], Ha 3.66[a], Hb	65.1		5 (only from Hb)		
β-fructofuranose							
	Glc						
	1	5.40, d (3.9)	94.9	2	3, 5, 2'		
	2	3.55[a]	73.8	1			
	3	LB	75.3				
	4	LB	ND				
	5	LB	75.1				
	6	LB	62.8				
	Frc					**PWBr3**	342.30
	1'	3.67[a]	64.0		2'		
	2'		106.4				
	3'	4.21, d (8.8)	79.1	4'	4'		
	4'	4.04, t (8.6)	76.7	3'	3', 6'		
	5'	3.88[a]	84.1				
	6'	LB	65.1				
sucrose (**Suc**)							

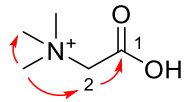
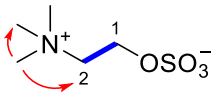
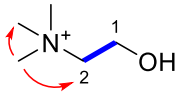
[a]Multiplicity was not determined due to signal overlap.

(**Supplementary Table 1.** continued)

metabolite (ID)	position	δ_H, mult (*J* in Hz)	δ_C	COSY (**blue bold**)	HMBC (H → C)	Reference sample code	MW (Da)
Sugars							
glucose (**Glc**)							
	1	4.63, d (8.0)	98.6	2			
	2	3.23, dd (9.4, 8.0)	76.8	1	1, 3		
	3	3.48, t (9.4)	78.5		2, 4		
	4	3.39, t (9.4)	72.3		5, 6		
	5	LB	78.7				
	6	LB	63.5				
β-glucopyranose						**PWBr3**	180.16
	1	5.22, d (3.8)	94.8	2			
	2	3.52[a]	74.2	1			
	3	LB	75.5				
	4	3.40, t (9.6)	72.4		3, 6		
	5	LB	74.2				
	6	LB	63.3				
α-glucopyranose							
	1	5.18, d (3.8)	95.9	2	3, 5, 1'		
	2	3.64[a]	73.8	1			
	3	LB	75.2				
	4	3.44, t (9.7)	72.4		3, 6		
	5	LB	74.9				
	6	3.84, 3.75[a]	63.2				
	1'	5.18, d (3.8)	95.9	2'	1, 3', 5'		
	2'	3.64[a]	73.8	1'			
	3'	LB	75.2				
	4'	3.44, t (9.7)	72.4		3', 6'		
	5'	LB	74.9				
	6'	3.84, 3.75[a]	63.2				
trehalose (**Tre**)						**PWB**	342.30

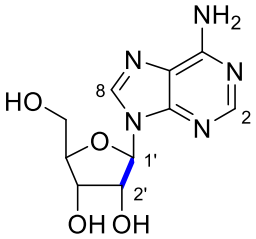
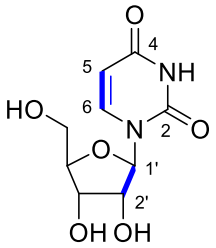
[a]Multiplicity was not determined due to signal overlap.

(**Supplementary Table 1.** continued)

metabolite (ID)	position	δ_H, mult (*J* in Hz)	δ_C, mult (*J* in Hz)	COSY (**blue bold**)	HMBC (H → C)	Reference sample code	MW (Da)
Choline derivatives							
 betaine (**Bt**)	1	-	172.0			**PWB**	117.15
	2	3.89, s	68.89, t (3.00)		1, NCH_3		
	$N(CH_3)_3$	3.25, s	56.08, t (3.75)		2, NCH_3		
 choline sulfate (**CS**)	1	4.49, m	64.7	2		**PWB**	183.23
	2	3.75[a]	67.50, t (3.00)	1	NCH_3		
	$N(CH_3)_3$	3.22, s	56.65, t (3.75)		2, NCH_3		
 choline (**Cho**)	1	4.05[a]	58.3	2		**PWB**	104.17
	2	3.51, m	70.13, t (3.00)	1	NCH_3		
	$N(CH_3)_3$	3.19, s	56.59, t (3.75)		2, NCH_3		

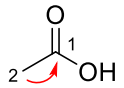
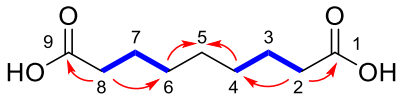
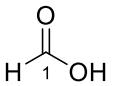
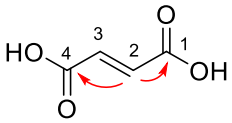
[a]Multiplicity was not determined due to signal overlap.

(**Supplementary Table 1.** continued)

metabolite (ID)	position	δ_H, mult (J in Hz)	δ_C	COSY (**blue bold**)	HMBC (H → C)	Reference sample code	MW (Da)
Nucleosides							
 adenosine (**Ado**)	2	LB	ND			**PWB**	267.24
	8	LB	ND				
	1'	6.06 (d, J = 6.0 Hz)	ND	2'			
	2'	4.79	ND				
	3'	LB	ND				
	4'	LB	ND				
	5'	LB	ND				
 uridine (**Urd**)	5	5.89, d (8.0)	ND	6		**PWB**	244.20
	6	7.86, d (8.1)	ND	5			
	1'	5.90, d (4.5)	ND	2'			
	2'	4.34[a]	ND	1'			
	3'	LB	ND				
	4'	LB	ND				
	5'	LB	ND				

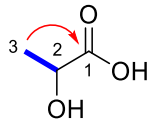
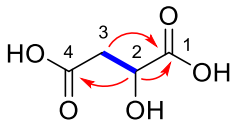
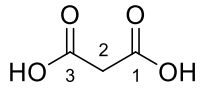
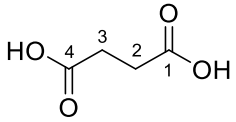
[a]Multiplicity was not determined due to signal overlap.

(**Supplementary Table 1.** continued)

metabolite (ID)	position	δ_H, mult (J in Hz)	δ_C	COSY (**blue bold**)	HMBC (H → C)	Reference sample code	MW (Da)
Organic acids							
 acetate (**AcA**)	1		184.0				
	2	1.91, s	25.8		1	**PWBr19**	60.05
 azelate (**AzA**)	1		186.5				
	2	2.18, t (7.5)	40.0	3	1, 3, 4		
	3	1.54[a]	28.4	2, 4	4		
	4	1.30[a]	31.3	3	5		
	5	1.30[a]	31.0			**PWB**	188.22
	6	1.30[a]	31.3	7			
	7	1.54[a]	28.4	6, 8			
	8	2.18, t (7.5)	40.0	7	6, 7, 9		
	9		186.5				
 formate (**FA**)	1	8.44, s	173.8			**PWB**	46.03
 fumarate (**FmA**)	1		177.4				
	2	6.51, s	138.0	1, 4			
	3	6.51, s	138.0	1, 4		**PWB**	116.07
	4		177.4				

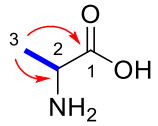
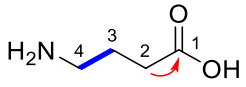
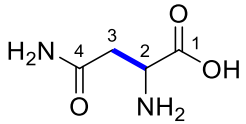
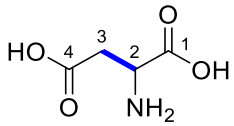
[a]Multiplicity was not determined due to signal overlap.

(**Supplementary Table 1.** continued)

metabolite (ID)	position	δ_H, mult (J in Hz)	δ_C	COSY (**blue bold**)	HMBC (H → C)	Reference sample code	MW (Da)
Organic acids							
 lactate (**LA**)	1		185.3[b]				
	2	4.10[a]	71.2	3			
	3	1.32, d (7.0)	22.8	2	1	**PWB**	90.08
 malate (**MA**)	1		182.2				
	2	4.29, dd (10.0, 3.1)	73.0	3	1, 3, 4		
	3	2.67, dd (15.5, 3.1), Ha 2.38, dd (15.5. 10.0), Hb	45.1	2, Ha/Hb	1, 2	**PWB**	134.09
	4		183.6				
 malonate (**MnA**)	1		ND				
	2	3.13, s	51.0[b]			**PWB**	104.06
	3		ND				
 succinate (**SA**)	1		ND				
	2	2.42, s	36.3				
	3	2.42, s	36.3			**PWBr19**	118.09
	4		ND				

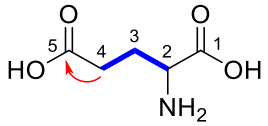
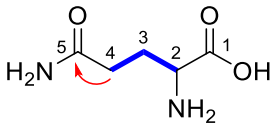
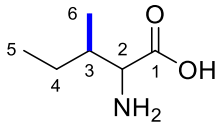
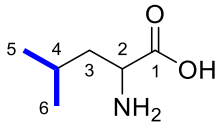
[a]Multiplicity was not determined due to signal overlap. [b]chemical shift was extracted from HMBC correlation.

(**Supplementary Table 1.** continued)

metabolite (ID)	position	δ_H, mult (J in Hz)	δ_C	COSY (**blue bold**)	HMBC (H → C)	Reference sample code	MW (Da)
Amino acids							
 alanine (**Ala**)	1		178.5				
	2	3.77[a]	53.2	3			
	3	1.47, d (7.3)	18.9	2	1, 2	**PWB**	89.09
 γ-aminobutyrate (**GABA**)	1		184.2[b]				
	2	2.29, t (7.4)	37.0	3	1		
	3	1.89[a]	26.3	2, 4		**PWB**	103.12
	4	3.00, br t (7.6)	42.0	3			
 asparagine (**Asn**)	1		ND				
	2	4.00[a]	ND	3			
	3	2.94, dd (16.9, 4.2), Ha 2.85, dd (16.9, 7.8), Hb	ND	2		**PWB**	132.12
	4		ND				
 aspartate (**Asp**)	1		ND				
	2	3.90[a]	ND	3			
	3	2.80, dd (17.5, 3.8), Ha 2.67[a], Hb	ND	2, Ha/Hb		**PWB**	133.10
	4		ND				

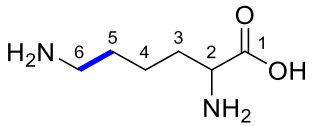
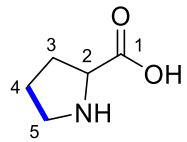
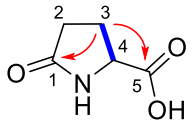
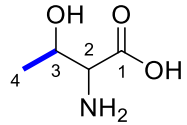
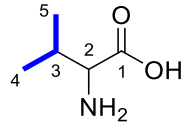
[a]Multiplicity was not determined due to signal overlap. [b]chemical shift was extracted from HMBC correlation.

(**Supplementary Table 1.** continued)

metabolite (ID)	position	δ_H, mult (J in Hz)	δ_C	COSY (**blue bold**)	HMBC (H → C)	Reference sample code	MW (Da)
Amino acids							
 glutamate (**Glu**)	1 2 3 4 5	 3.75[a] 2.11, 2.06[a] 2.34[a] 	ND 57.3 29.6 36.1 183.8[b]	 3 2, 4 3 	 5 	**PWB**	147.13
 glutamine (**Gln**)	1 2 3 4 5	 3.77[a] 2.13[a] 2.45[a] 	ND 56.8 28.9 33.5 180.4[b]	 3 2, 4 3 	 5 	**PWB**	146.14
 isoleucine (**Ile**)	1 2 3 4 5 6	 LB 1.97[a] LB LB 1.00, d (7.0)	ND ND ND ND ND ND	 6 3	 	**PWB**	131.17
 leucine (**Leu**)	1 2 3 4 5, 6	 LB LB 1.71[a] 0.95[a]	ND ND ND ND ND	 5, 6 4	 	**PWB**	131.17

[a]Multiplicity was not determined due to signal overlap. [b]chemical shift was extracted from HMBC correlation.

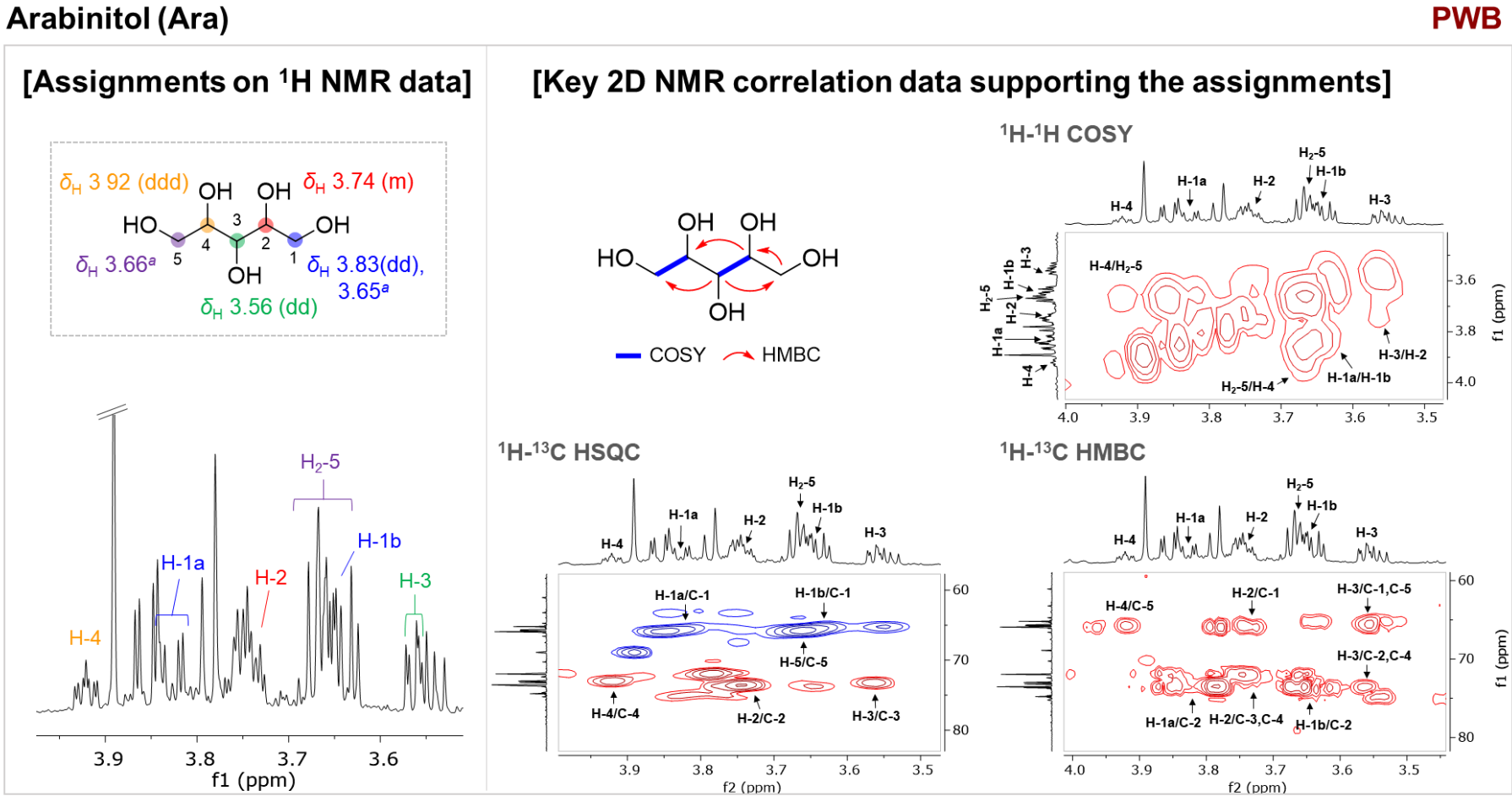
(**Supplementary Table 1.** continued)

metabolite (ID)	position	δ_H, mult (J in Hz)	δ_C	COSY (**blue bold**)	HMBC (H → C)	Reference sample code	MW (Da)
Amino acids							
 lysine (**Lys**)	1	LB	ND			**PWB**	146.19
	2	LB	ND				
	3	LB	ND				
	4	LB	ND				
	5	1.71[a]	ND	6			
	6	3.01[a]	ND	5			
 proline (**Pro**)	1		ND			**PWB**	115.13
	2	4.12, d (9.1, 6.7)	63.9				
	3	LB	31.7				
	4	1.99[a]	26.5	5			
	5	3.41, 3.33[a]	48.8	4			
 pyroglutamate (**Glp**)	1		184.5			**PWB**	129.11
	2	2.39[a]	32.4				
	3	2.50, m, Ha 2.02, m, Hb	28.1	4	1, 5		
	4	4.16, dd (9.0, 5.8)	61.1	3			
	5		183.1				
 threonine (**Thr**)	1		ND			**PWB**	119.12
	2	LB	ND				
	3	4.24[a]	ND	4			
	4	1.32[a]	ND	3			
 valine (**Val**)	1		ND			**PWB**	117.15
	2	LB	ND				
	3	2.27[a]	ND	4, 5			
	4	1.03, d (7.0)	20.7	3			
	5	0.98, d (7.0)	19.4	3			

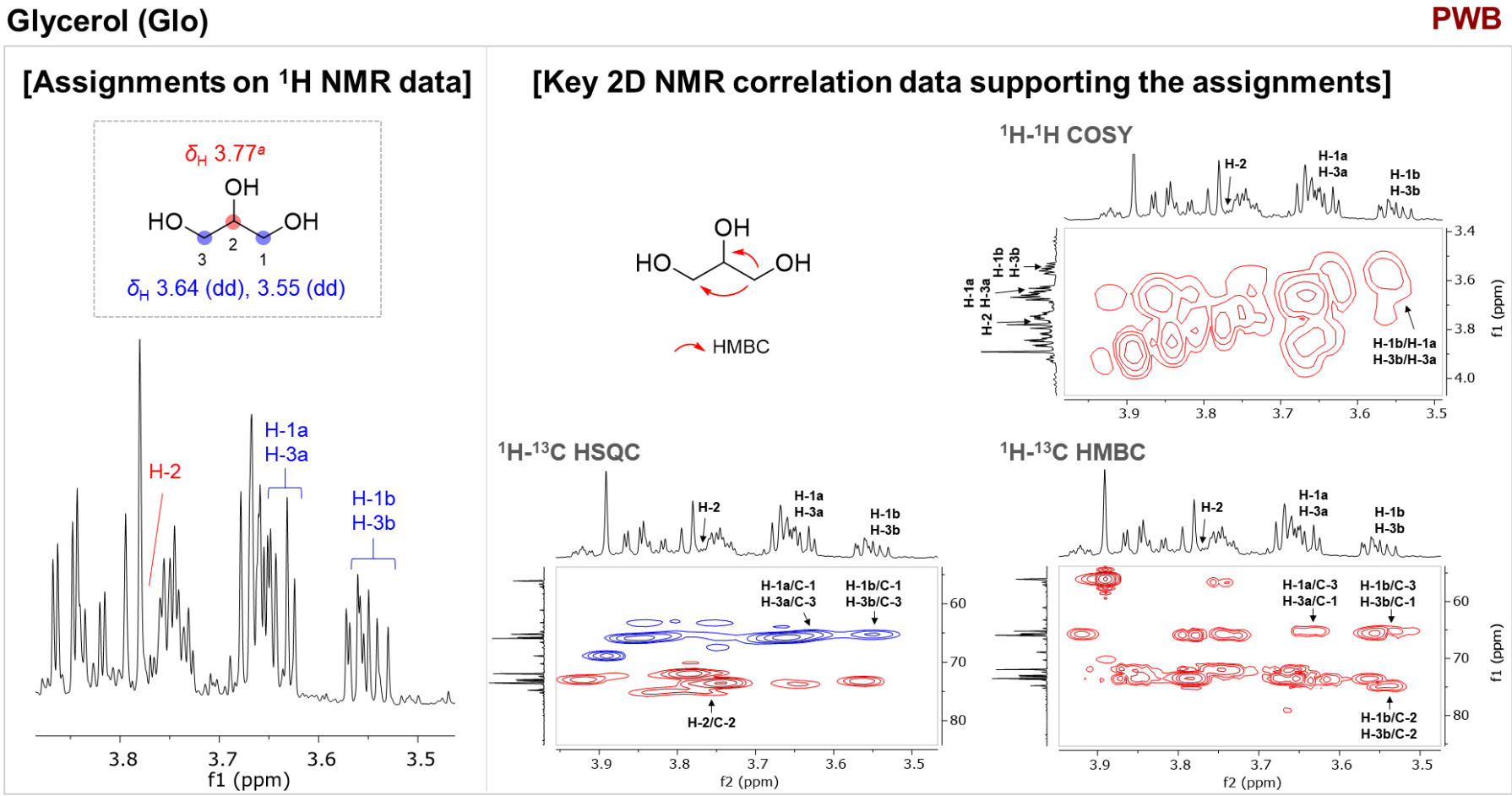
[a]Multiplicity was not determined due to signal overlap.

Supplementary Table 2. BMRD codes of the identified metabolites

metabolite name	BMRD code	metabolite name	BMRD code
Sugar alcohols		**Organic acids**	
arabinitol (Ara)	bmse000068	acetate (AcA)	bmse000857
glycerol (Glo)	bmse000184	azelate (AzA)	not available
mannitol (Man)	bmse000099	formate (FA)	bmse000203
Sugars		fumarate (FmA)	bmse000083
fructose (Frc)	bmse000010	lactate (LA)	bmse000208
glucose (Glc)	bmse000015	malate (MA)	bmse000046
sucrose (Suc)	bmse000119	malonate (MnA)	bmse000386
trehalose (Tre)	bmse000125	succinate (SA)	bmse000183
Choline derivatives		**Amino acids**	
betaine (Bt)	bmse000069	alanine (Ala)	bmse000028
choline (Cho)	bmse000285	γ-aminobutyrate (GABA)	bmse000340
choline sulfate (CS)	-	asparagine (Asn)	bmse000030
Nucleosides		aspartate (Asp)	bmse000875
adenosine (Ado)	bmse000061	glutamate (Glu)	bmse000037
uridine (Urd)	bmse000158	glutamine (Gln)	bmse000038
		isoleucine (Ile)	bmse000041
		leucine (Leu)	bmse000042
		lysine (Lys)	bmse000043
		proline (Pro)	bmse000047
		pyroglutamate (Glp)	not available
		threonine (Thr)	bmse000049
		valine (Val)	bmse000052



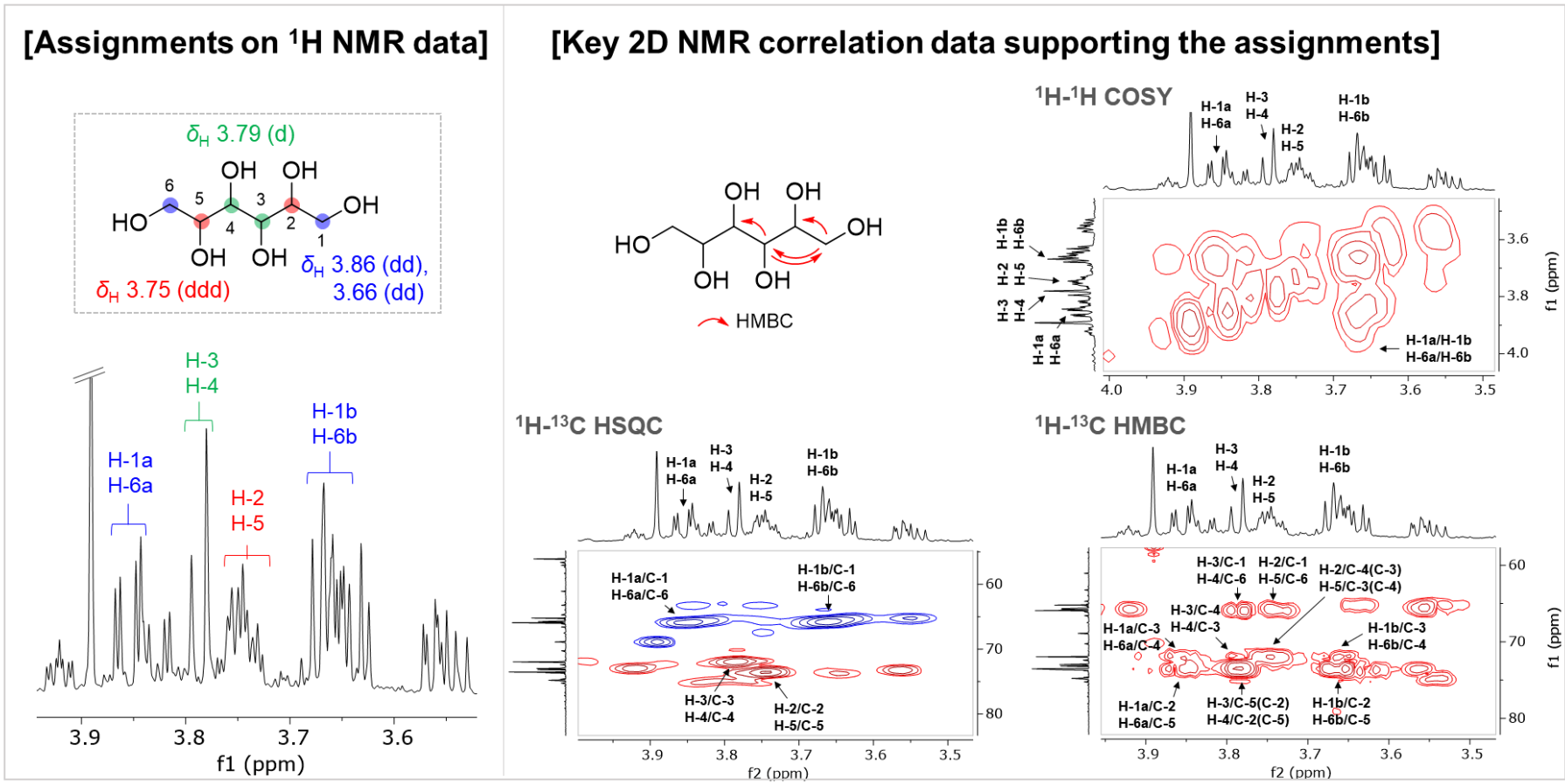
Supplementary Figure 3. Detailed 2D NMR (600 MHz, D_2O) data analysis of arabinitol (Ara) in **PWB**



[a]Multiplicity was not determined due to signal overlapping.

Biological Magnetic Resonance Data Bank (BMRD) code: bmse0000184

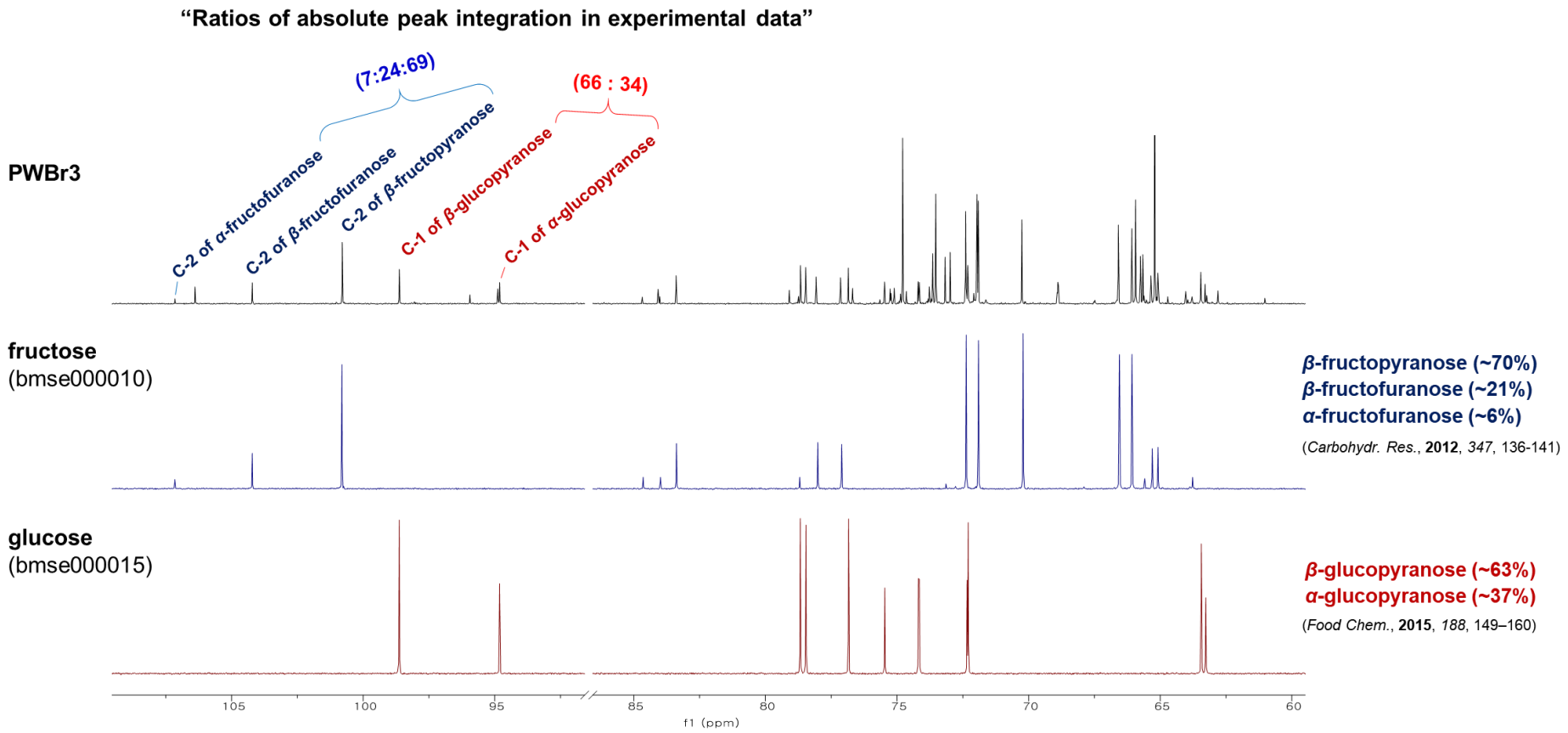
Supplementary Figure 4. Detailed 2D NMR (600 MHz, D_2O) data analysis of glycerol (Glo) in **PWB**



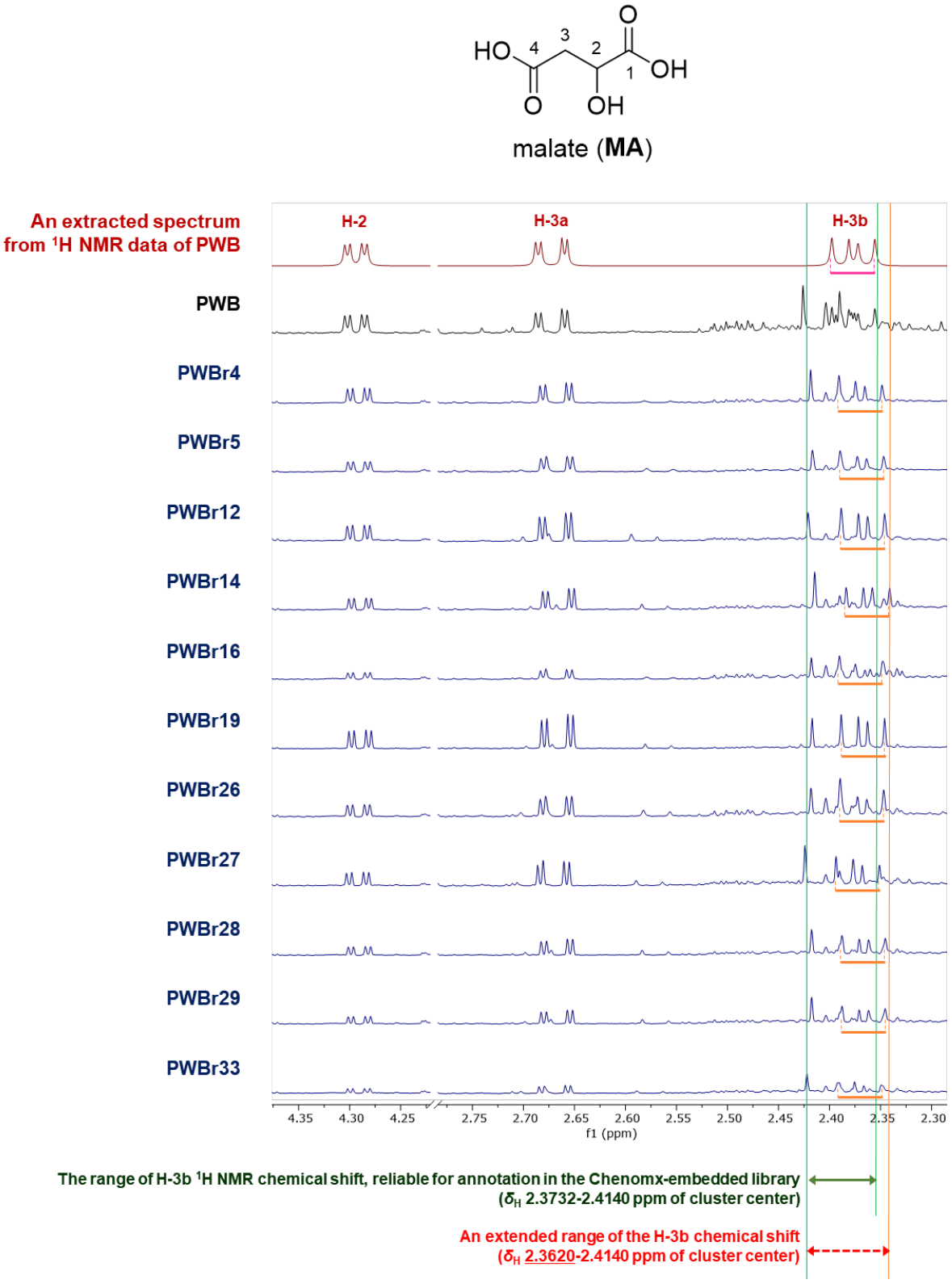
[a]Multiplicity was not determined due to signal overlapping.

Biological Magnetic Resonance Data Bank (BMRD) code: bmse000099

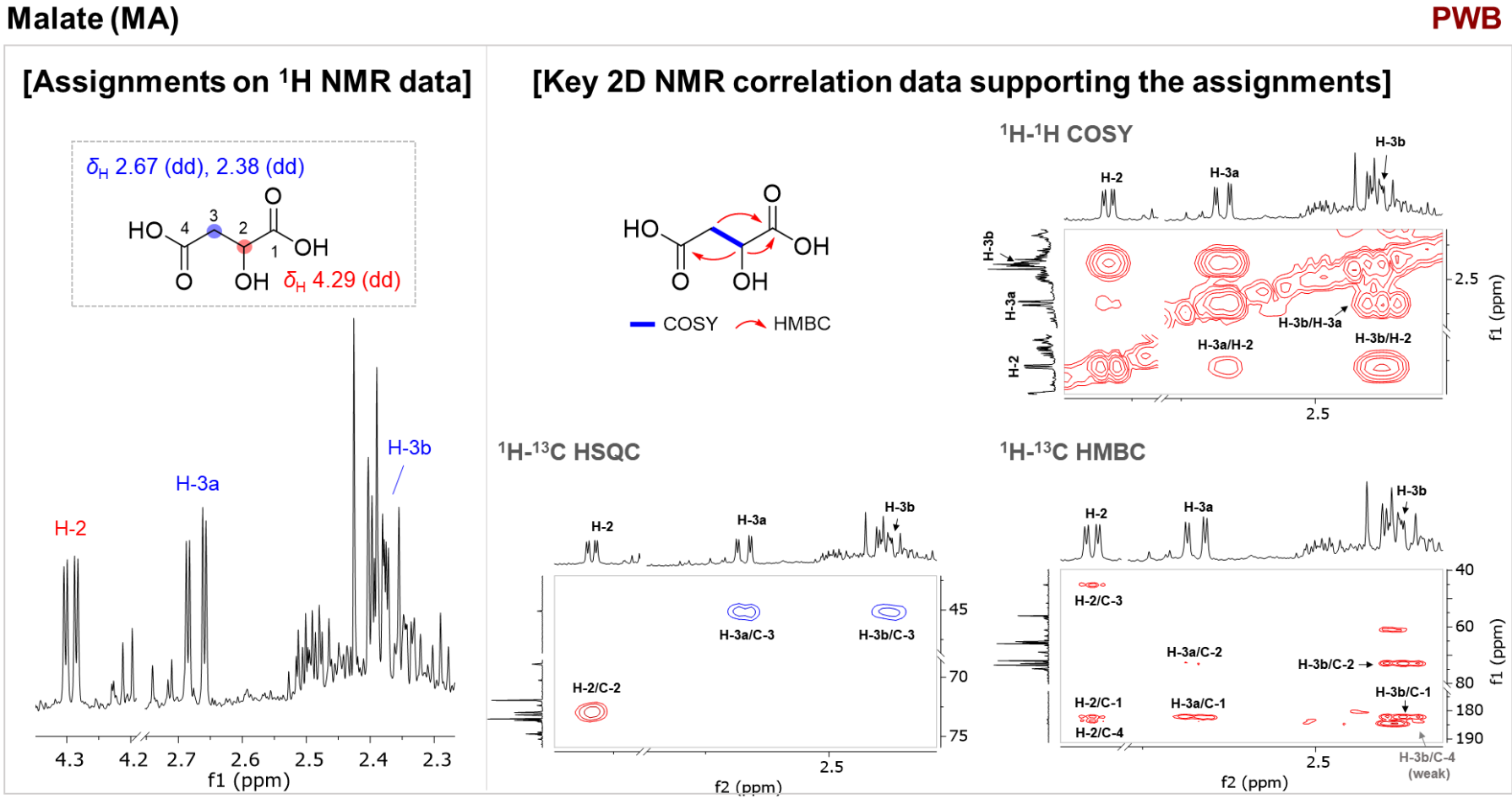
Supplementary Figure 5. Detailed 2D NMR (600 MHz, D_2O) data analysis of mannitol (Man) in **PWB**



Supplementary Figure 6. ^{13}C NMR data (150 MHz, D_2O) of **PWBr3** with BMRD reference data of fructose (bmse000010) and glucose (bmse000015)

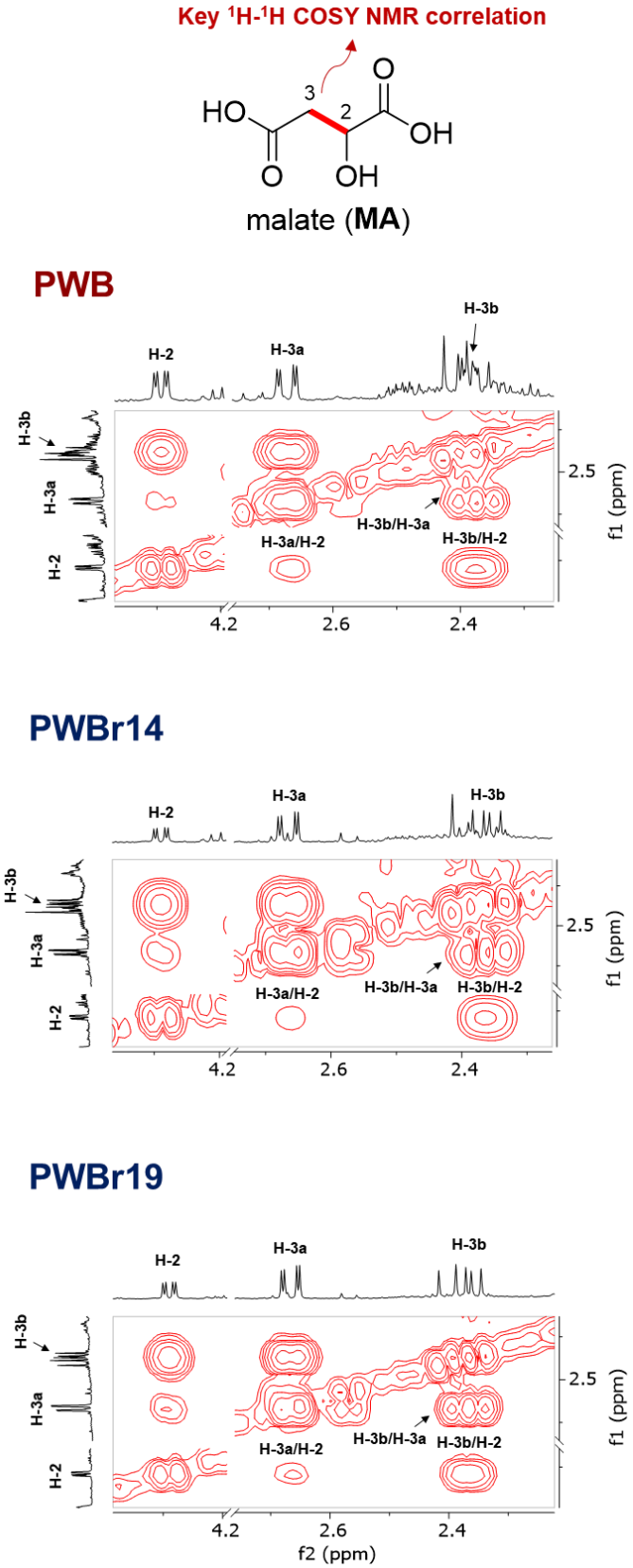


Supplementary Figure 7. Stacked ^{1}H NMR (600 MHz, D_2O) spectra in an expansion for malate (MA) signals

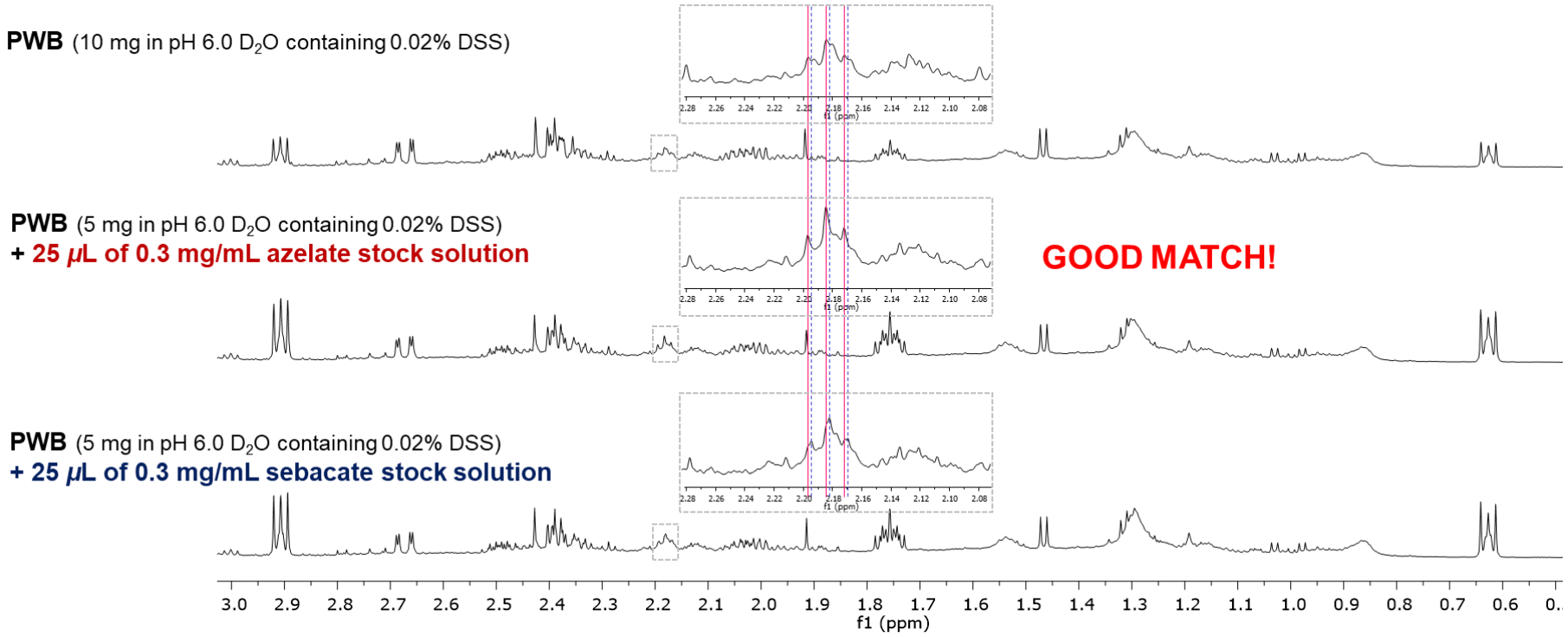


[a]Multiplicity was not determined due to signal overlapping. Biological Magnetic Resonance Data Bank (BMRD) code: bmse000046

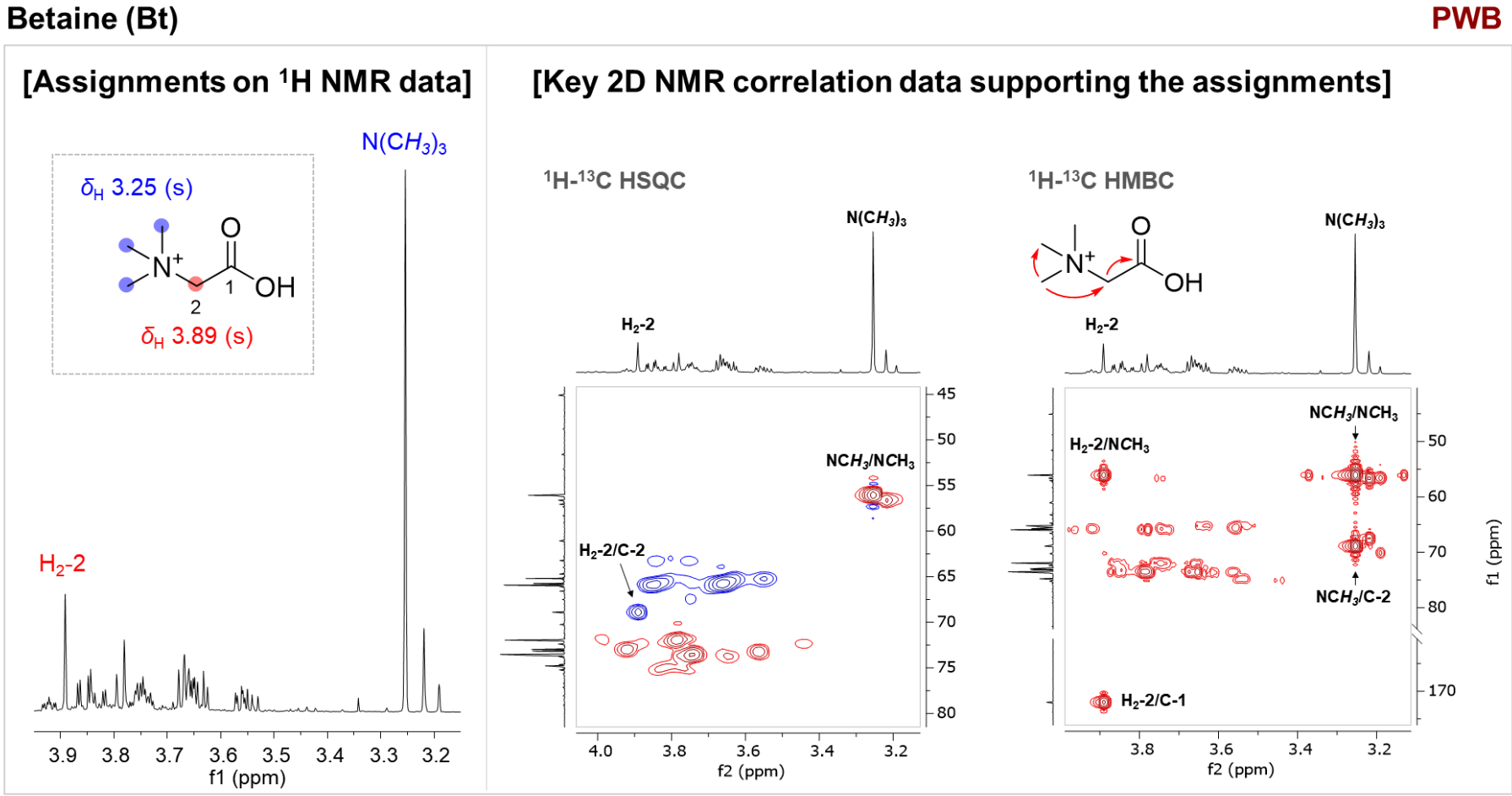
Supplementary Figure 8. Detailed 2D NMR (600 MHz, D_2O) data analysis of malate (MA) in **PWB**



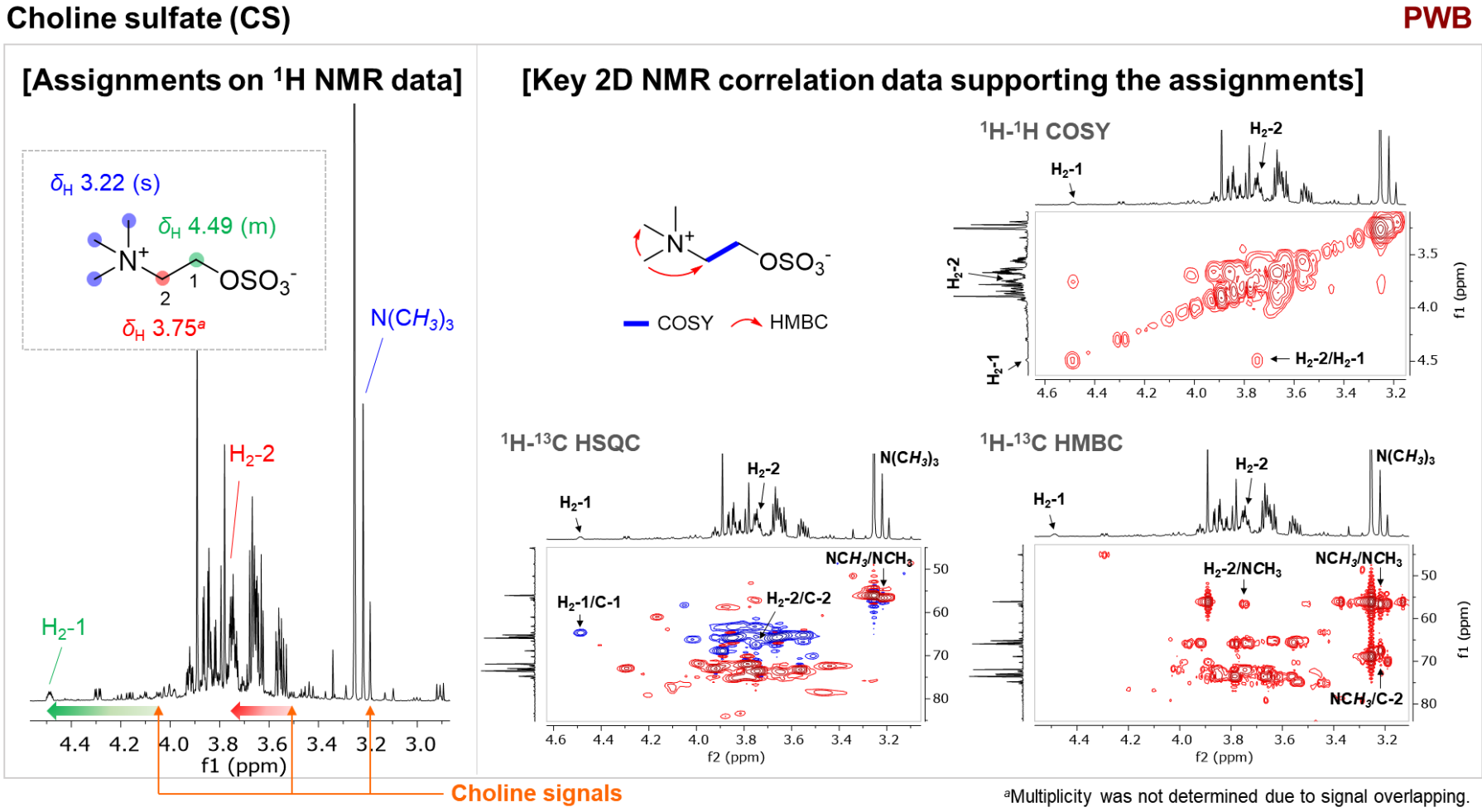
Supplementary Figure 9. Key ^{1}H-^{1}H COSY (600 MHz, D_2O) correlations of malate in **PWB**, **PWBr14**, and **PWBr19**



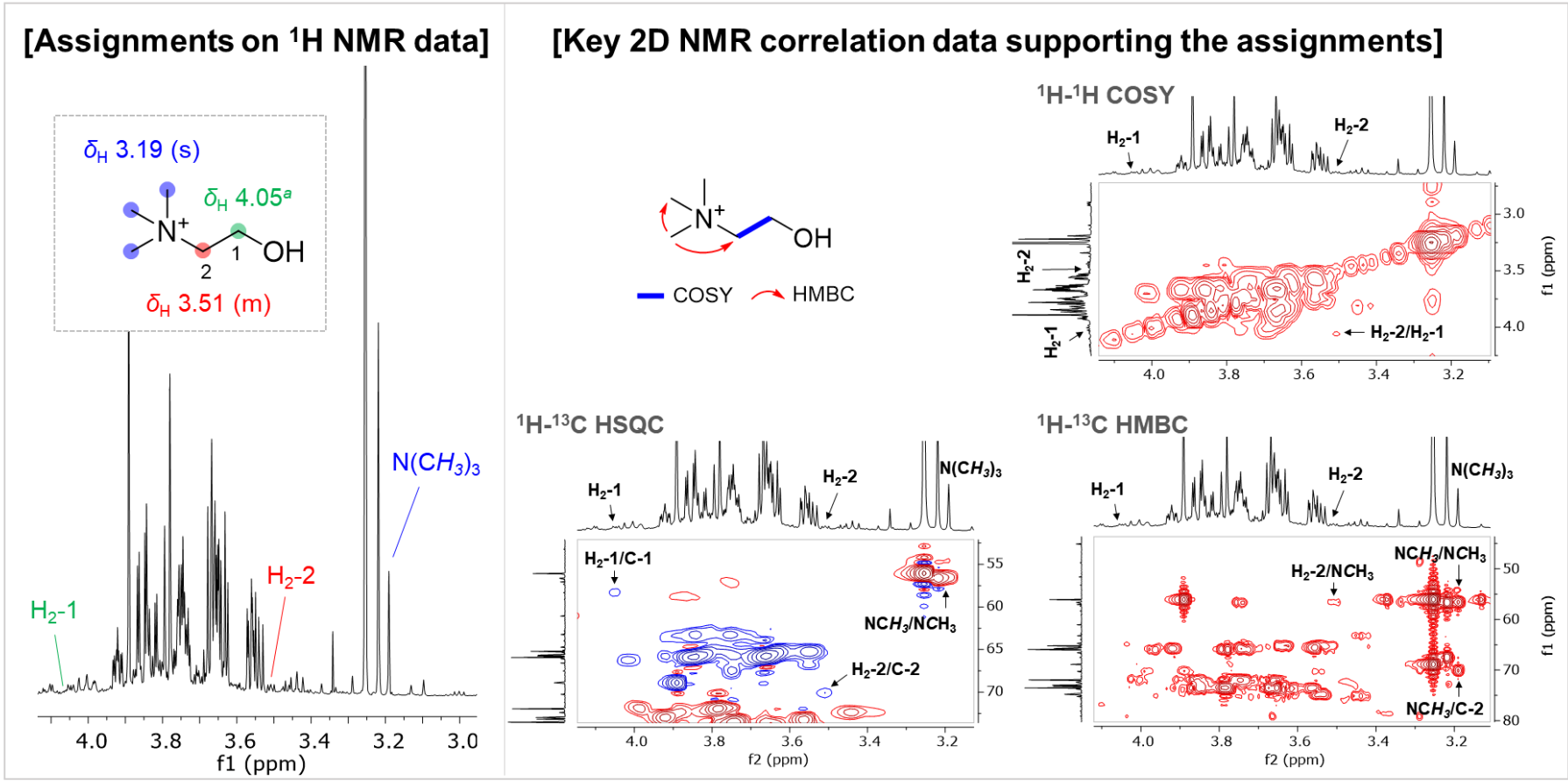
Supplementary Figure 10. Spiking ^{1}H NMR experiments (600 MHz, D_2O) with commercial standards of azelate and sebacate



Supplementary Figure 11. Detailed 2D NMR (600 MHz, D_2O) data analysis of betaine (Bt) in **PWB**



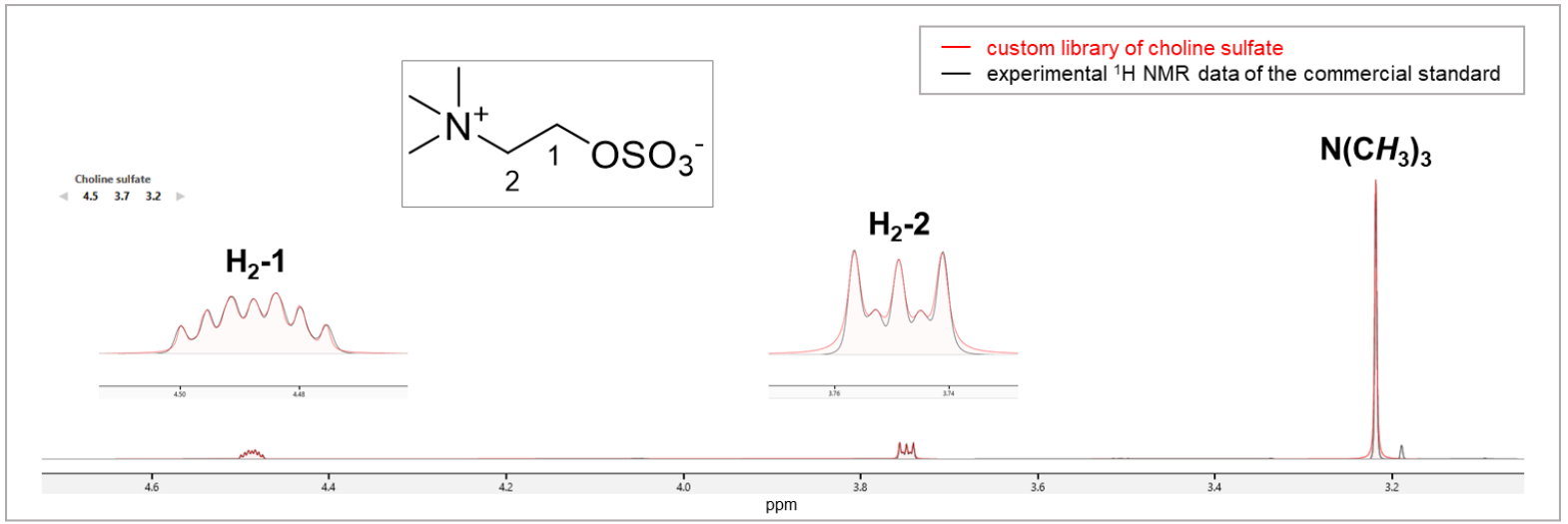
Supplementary Figure 12. Detailed 2D NMR (600 MHz, D_2O) data analysis of choline sulfate (CS) in **PWB**



[a]Multiplicity was not determined due to signal overlapping.

Biological Magnetic Resonance Data Bank (BMRD) code: bmse000285

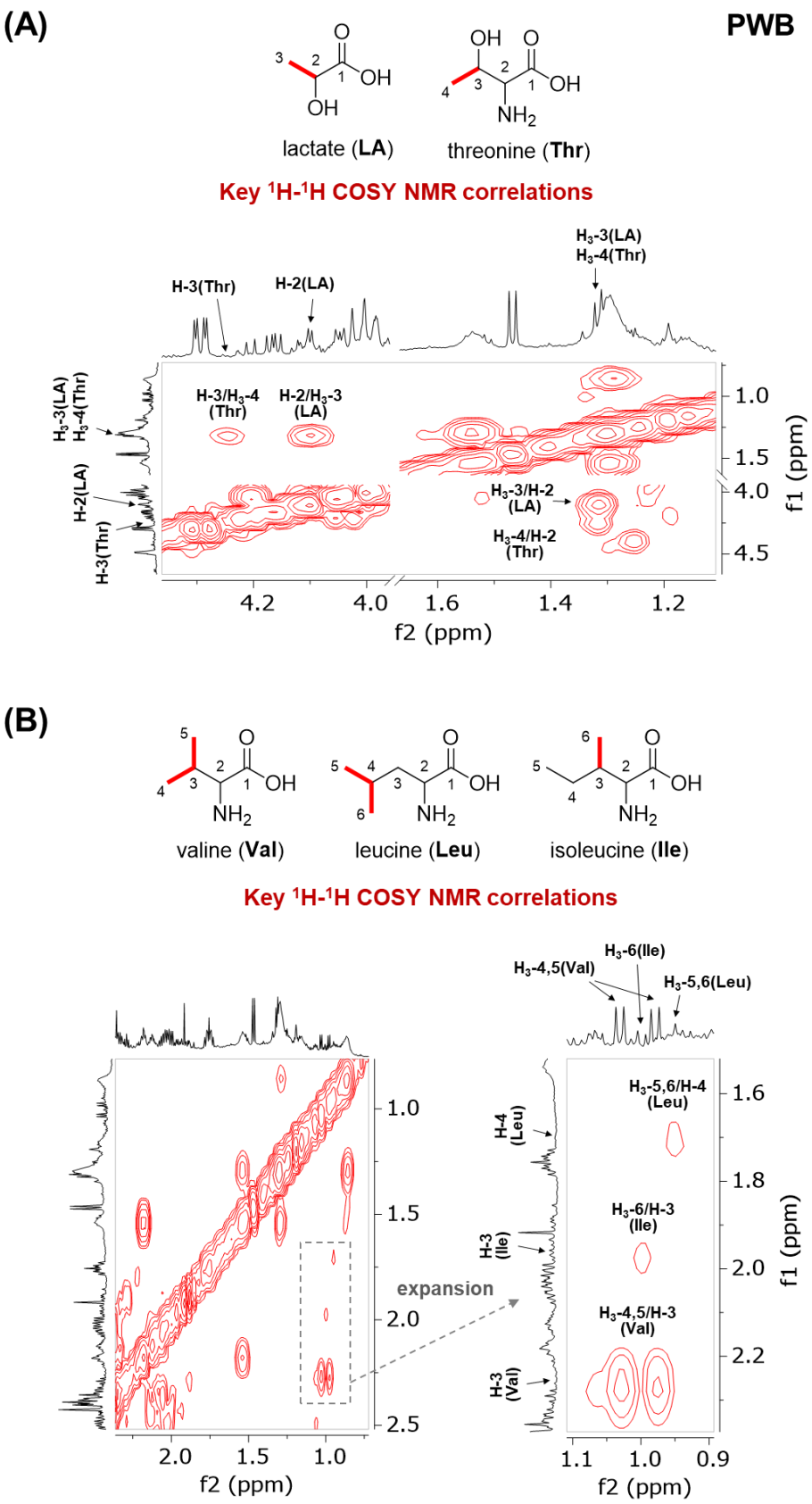
Supplementary Figure 13. Detailed 2D NMR (600 MHz, D_2O) data analysis of choline (Cho) in **PWB**



- **Sample information**: 2.00 mg/mL D_2O solution (10.7 mM of choline sulfate of 98% standard sample purity)
- **^{1}H NMR data acquisition parameter:** 1D NOESY presat pulse sequence (noesypr1d; presaturation frequency, δ_H 4.70 ppm) calibrated 90 degrees pulse (P1), D1 2 s, AQ 4 s, RG 64; NS 64; DS 2
- **^{1}H NMR data processing parameter:** Fifth-order polynomial fitting, Lorentzian-to-Gaussian apodization (exponential factor -0.3, Gaussian factor 0.05)

* The prepared custom Chenomx compound library is provided as xcpd file. Please cite this paper for use.

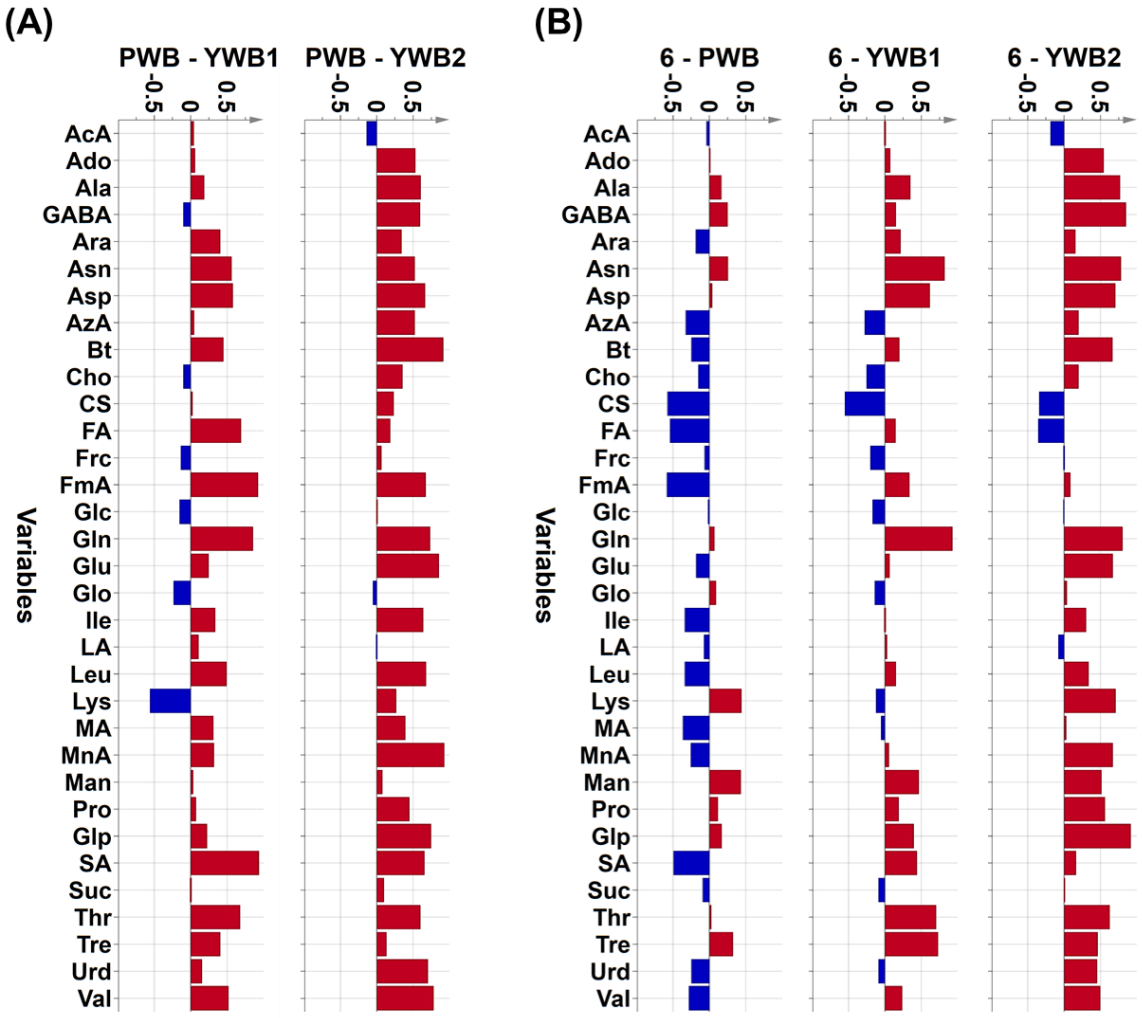
Supplementary Figure 14. Preparation of the custom compound library for choline sulfate



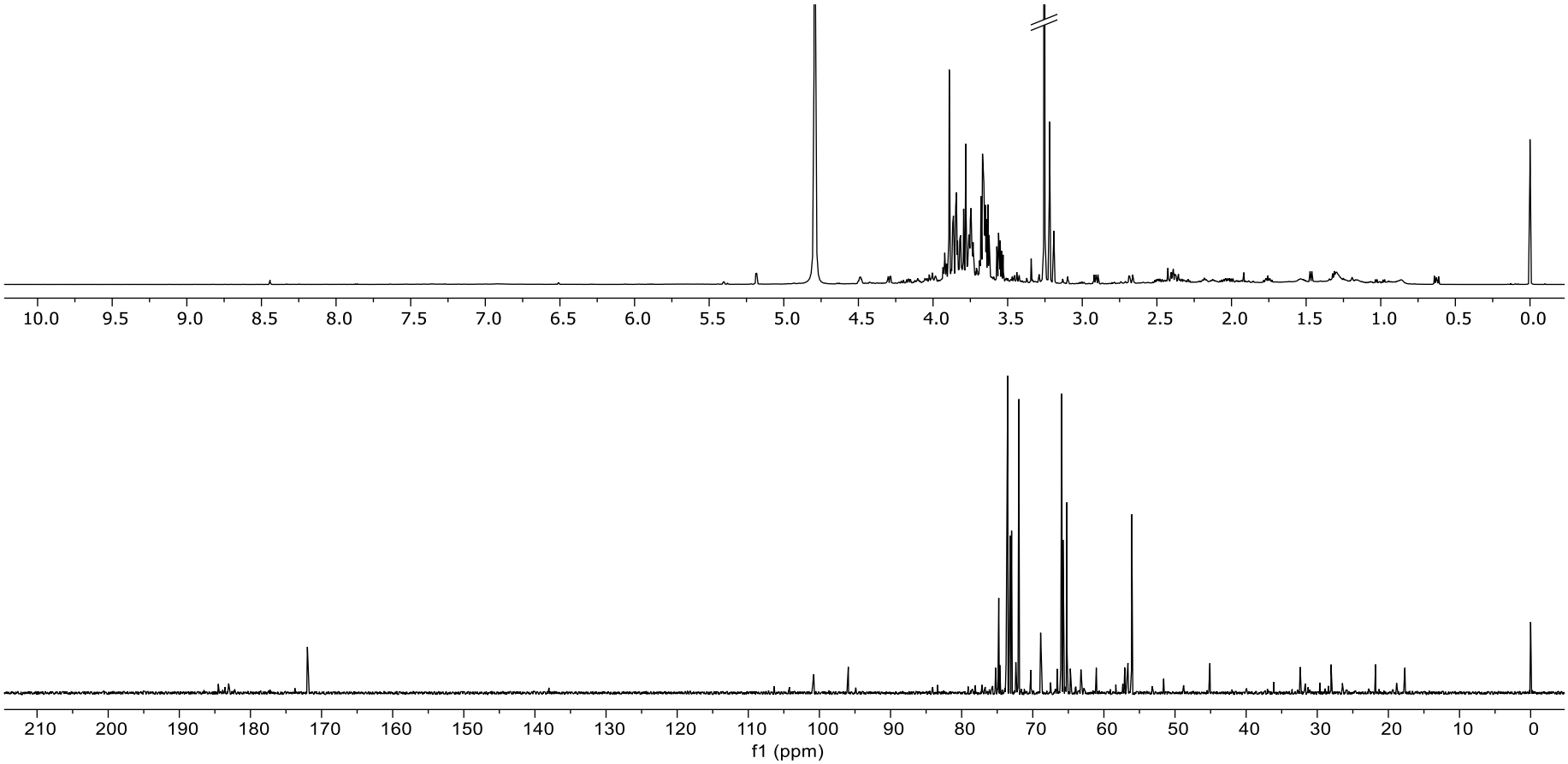
Supplementary Figure 15. Key ^{1}H-^{1}H COSY (600 MHz, D_2O) correlations for identification of (A) lactate (LA) and threonine (Thr), and (B) valine (Val), leucine (Leu), and isoleucine (Ile) in **PWB**

Supplementary Table 3. Concentrations (mg/g dried extract) of the identified metabolites of the wheat bran samples

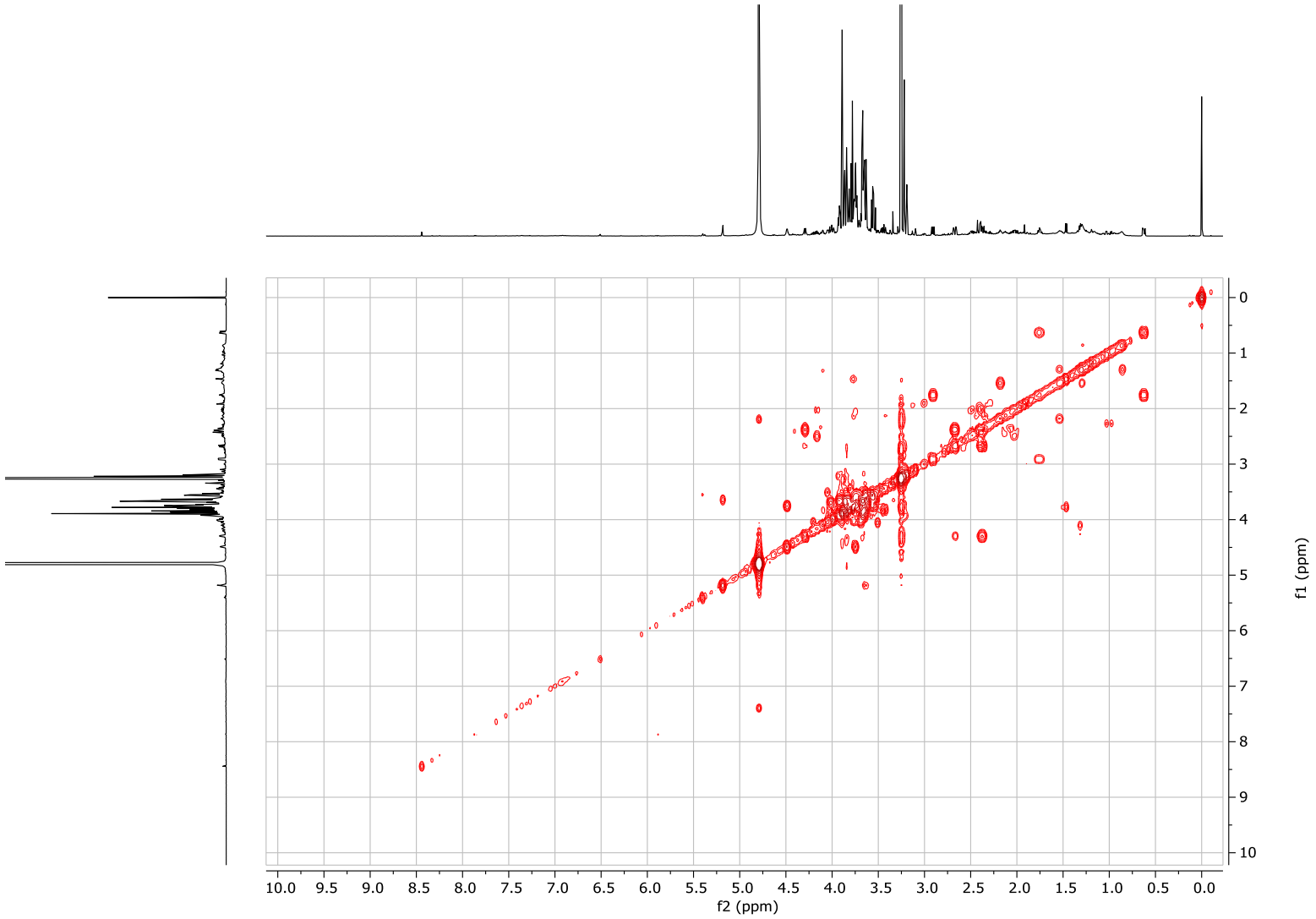
Samples	GABA	AcA	Ado	Ala	Ara	Asn	Asp	AzA	Bt	Cho	CS	FA	Frc	FmA	Glc	Gln	Glu	Glo	Ile	LA	Leu	Lys	MA	MnA	Man	Pro	Glp	SA	Suc	Thr	Tre	Urd	Val
YWB1	1.671	0.080	0.451	2.078	35.578	0.318	0.665	2.300	39.136	2.945	5.718	0.100	27.330	0.032	11.086	1.454	3.250	59.023	0.440	0.845	0.436	1.266	7.483	0.962	60.054	2.650	7.349	0.234	5.673	0.378	9.276	0.835	0.646
YWB2	0.314	3.655	0.247	0.983	37.223	0.373	0.541	1.114	19.346	2.080	5.611	0.748	8.280	0.147	0.968	1.906	1.363	26.917	0.302	0.943	0.349	0.221	5.712	0.429	57.148	0.930	2.010	0.603	2.331	0.465	13.787	0.527	0.382
PWB	1.475	0.926	0.478	2.566	46.353	1.113	1.468	2.424	58.195	2.754	4.783	0.989	14.403	0.458	1.397	4.635	4.021	17.450	0.596	0.940	0.665	0.559	14.130	1.240	62.304	3.003	9.682	1.475	5.760	1.130	16.046	0.925	1.175
PWBr1	0.567	6.063	0.397	1.355	45.787	0.217	0.825	2.334	47.096	2.159	5.812	1.166	7.626	0.227	0.835	1.775	3.047	20.364	0.351	1.097	0.442	0.232	7.504	0.904	64.978	2.014	4.118	0.861	1.427	0.294	15.457	0.686	0.504
PWBr2	0.248	0.540	0.209	1.162	30.856	0.169	0.420	3.481	41.697	1.964	3.697	0.745	14.173	0.196	4.353	1.975	2.235	21.002	0.405	1.212	0.405	0.099	13.044	0.893	36.659	2.232	3.993	1.047	8.252	1.027	6.428	0.439	0.455
PWBr3	0.797	0.078	0.386	1.628	28.616	0.396	0.540	2.567	41.445	2.488	2.945	0.193	68.011	0.054	44.217	2.378	2.673	91.647	0.359	0.854	0.430	0.378	6.841	1.013	40.559	3.184	3.855	1.235	29.192	0.559	10.544	0.696	0.378
PWBr4	0.109	2.545	0.188	0.805	34.677	0.106	0.503	2.302	38.319	1.608	4.466	1.378	8.307	0.473	1.937	1.492	1.596	23.963	0.232	1.122	0.333	0.023	13.013	0.713	41.605	1.166	3.100	1.123	2.043	0.479	9.998	0.426	0.294
PWBr5	0.108	6.281	0.223	0.878	30.287	0.066	0.635	2.632	27.336	1.952	4.268	1.342	4.201	0.233	0.719	1.659	1.645	10.778	0.265	0.873	0.409	0.067	11.241	0.550	46.284	1.951	2.542	0.887	1.279	0.869	8.013	0.458	0.340
PWBr6	1.964	0.088	0.484	2.999	41.377	1.477	1.522	1.609	47.635	2.458	3.420	0.292	7.963	0.187	0.202	4.895	3.452	33.791	0.441	0.872	0.507	1.112	6.352	1.014	90.649	3.540	11.410	0.820	2.618	1.161	21.479	0.784	0.888
PWBr7	0.505	0.306	0.402	1.675	34.478	0.324	0.422	3.018	51.547	2.476	3.944	0.518	39.015	0.156	12.042	2.053	2.448	30.318	0.447	1.301	0.445	0.263	24.330	1.077	48.289	2.628	5.663	1.558	21.766	0.261	9.023	0.679	0.640
PWBr8	0.486	0.159	0.197	1.031	45.209	0.203	0.459	1.463	40.145	2.244	4.593	0.128	23.021	0.030	24.821	1.548	2.380	128.754	0.333	0.905	0.337	0.238	4.414	0.901	71.347	2.236	3.755	1.132	3.388	0.350	14.074	0.746	0.333
PWBr9	0.202	1.305	0.299	0.784	43.407	0.094	0.462	2.091	33.441	1.781	3.658	0.662	6.839	0.098	3.028	1.895	1.656	22.834	0.258	1.042	0.292	0.104	5.194	0.688	58.021	1.431	2.743	0.906	1.263	0.501	16.221	0.529	0.247
PWBr10	0.835	0.080	0.282	1.453	42.343	0.300	0.635	1.712	40.605	2.368	4.815	0.123	10.795	0.043	5.736	2.121	2.076	25.024	0.338	0.717	0.364	0.544	3.344	0.852	71.857	2.188	3.520	0.802	7.569	0.513	10.596	0.587	0.437
PWBr11	0.504	0.213	0.208	1.045	36.451	0.222	0.796	1.714	33.087	1.803	3.883	0.275	24.752	0.011	25.314	1.659	2.410	71.504	0.307	0.894	0.341	0.279	6.739	0.813	48.454	2.274	3.644	0.960	8.612	0.407	10.791	0.551	0.331
PWBr12	0.200	0.404	0.091	0.704	24.332	0.073	0.348	1.417	22.952	1.045	2.457	0.597	20.456	0.140	10.182	1.877	2.173	23.363	0.214	0.811	0.208	0.083	18.342	0.502	31.793	1.436	2.002	1.024	6.979	0.204	8.530	0.366	0.228
PWBr13	0.586	1.699	0.368	1.725	45.402	0.503	1.039	2.178	33.997	2.281	4.924	0.969	6.208	0.246	0.217	3.669	2.339	21.210	0.339	0.752	0.422	0.371	7.059	0.723	76.180	1.517	4.977	0.747	1.597	1.077	15.252	0.632	0.573
PWBr14	0.153	8.060	0.143	0.825	37.127	0.149	0.624	2.177	27.712	1.787	4.196	0.966	5.666	0.142	1.589	2.893	2.332	13.413	0.244	1.007	0.367	0.071	12.507	0.574	44.009	1.977	2.916	1.199	4.229	0.356	11.181	0.470	0.321
PWBr15	1.046	0.478	0.408	2.320	37.618	0.683	0.909	1.847	39.272	2.760	3.737	0.382	32.744	0.118	9.200	1.939	3.250	42.118	0.450	1.035	0.480	0.484	12.391	0.937	50.206	5.336	7.007	0.923	24.325	0.278	14.542	0.828	0.720
PWBr16	0.267	19.150	0.365	1.405	33.640	0.331	0.762	1.222	29.146	2.127	4.438	0.943	3.585	0.238	0.061	4.847	2.462	14.055	0.290	0.991	0.393	0.037	6.703	0.551	61.059	1.080	4.497	0.731	1.837	0.620	18.013	0.612	0.536
PWBr17	0.341	0.330	0.142	1.182	26.128	0.196	0.543	2.878	42.570	1.753	2.577	0.432	15.780	0.150	3.963	1.486	1.724	23.529	0.231	0.886	0.368	0.144	13.272	0.845	27.922	2.714	4.556	1.058	7.030	0.584	4.834	0.394	0.453
PWBr18	0.291	1.235	0.065	0.769	25.166	0.163	0.509	1.193	23.174	1.488	2.575	0.351	46.332	0.035	49.102	1.190	1.696	82.814	0.213	0.554	0.308	0.113	6.378	0.540	45.517	2.831	2.821	0.735	18.406	0.277	10.704	0.464	0.261
PWBr19	0.026	17.552	0.162	0.384	29.978	0.103	0.136	1.133	24.697	1.289	3.717	1.166	1.888	0.313	0.452	1.212	0.919	6.591	0.138	0.437	0.200	0.009	19.768	0.447	33.677	0.791	1.179	0.981	1.649	0.254	5.568	0.359	0.161
PWBr20	0.493	0.198	0.262	1.138	46.716	0.271	0.641	1.699	31.009	2.062	2.931	0.374	58.016	0.117	20.476	1.639	2.129	81.132	0.274	1.175	0.463	0.256	7.094	0.735	58.113	2.886	2.736	0.868	8.553	0.279	15.515	0.596	0.407
PWBr21	0.176	0.125	0.155	0.879	50.303	0.160	0.455	0.991	15.786	1.887	3.642	0.282	22.187	0.076	8.758	1.240	1.819	84.216	0.324	1.041	0.325	0.159	3.006	0.369	62.813	1.323	1.732	0.636	16.257	0.236	12.792	0.456	0.303
PWBr22	0.499	0.140	0.212	1.385	33.562	0.081	0.394	2.579	29.406	2.681	1.993	0.302	89.769	0.058	50.026	1.773	2.258	57.311	0.349	1.095	0.391	0.373	7.221	0.670	25.560	3.867	2.844	0.923	35.113	0.149	5.381	0.523	0.510
PWBr23	0.146	0.162	0.050	0.645	38.926	0.124	0.407	1.995	19.205	1.574	2.488	0.829	76.267	0.225	40.217	1.713	1.773	92.037	0.196	1.085	0.293	0.092	8.368	0.448	42.515	2.675	1.978	1.074	7.454	0.162	8.939	0.471	0.232
PWBr24	0.707	0.139	0.269	1.468	50.894	0.288	0.552	1.670	30.185	2.185	3.201	0.138	45.344	0.071	12.467	1.853	2.226	83.989	0.383	0.885	0.367	0.665	6.898	0.698	65.487	2.224	3.970	0.928	4.720	0.190	10.688	0.600	0.458
PWBr25	0.840	0.230	0.363	1.932	29.805	0.625	0.612	1.982	39.510	2.863	2.093	0.248	78.831	0.104	34.820	1.313	3.142	53.963	0.296	0.962	0.383	0.388	18.161	0.944	39.709	5.407	5.414	1.477	29.499	0.234	7.899	0.667	0.550
PWBr26	0.473	3.789	0.298	1.608	47.439	0.572	0.993	2.562	42.138	2.358	5.861	1.311	7.832	0.385	0.549	3.385	2.494	21.367	0.348	1.103	0.432	0.320	13.484	0.880	70.601	1.625	5.607	1.034	3.733	1.016	13.601	0.558	0.590
PWBr27	0.335	0.830	0.266	1.205	33.798	0.266	0.754	2.522	48.649	2.037	3.584	0.897	20.636	0.167	6.841	1.493	2.068	37.308	0.286	1.228	0.345	0.152	14.878	0.975	42.525	2.310	2.938	1.245	7.921	0.154	7.454	0.545	0.392
PWBr28	0.181	6.346	0.330	0.986	40.253	0.152	0.519	1.768	30.044	2.015	5.338	1.185	7.920	0.213	1.055	2.390	2.239	22.634	0.250	1.071	0.316	0.089	10.540	0.599	64.175	1.319	1.983	0.896	2.222	0.368	12.741	0.489	0.321
PWBr29	0.140	7.594	0.253	0.891	43.944	0.144	0.587	1.837	26.339	1.822	4.706	1.382	13.238	0.203	2.423	1.855	2.236	35.011	0.308	1.211	0.364	0.097	8.692	0.540	65.402	1.370	2.098	0.880	2.716	0.178	13.041	0.383	0.327
PWBr30	0.360	0.111	0.228	1.100	41.688	0.184	0.298	1.187	24.308	1.639	2.874	0.265	91.741	0.164	62.779	1.895	1.658	183.155	0.196	0.869	0.250	0.219	6.784	0.576	56.751	3.100	2.528	1.022	13.535	0.160	12.539	0.553	0.203
PWBr31	0.518	0.181	0.320	1.587	41.749	0.348	0.608	1.913	41.593	2.218	3.388	0.357	27.357	0.140	7.608	1.872	3.086	48.318	0.362	1.244	0.434	0.321	11.583	0.882	60.228	2.389	4.752	1.342	13.326	0.341	10.628	0.565	0.592
PWBr32	0.233	1.425	0.288	0.905	40.007	0.085	0.500	1.367	28.094	2.216	5.957	1.049	14.050	0.105	4.179	1.687	1.897	27.894	0.312	1.128	0.293	0.145	4.503	0.578	80.762	1.138	2.725	0.808	6.158	0.147	10.456	0.544	0.323
PWBr33	0.140	0.332	0.249	0.778	41.197	0.084	0.301	2.033	25.493	1.636	3.277	0.679	12.773	0.107	3.543	1.310	1.547	33.621	0.214	1.214	0.222	0.049	5.435	0.499	48.199	0.985	1.984	0.701	2.786	0.329	8.624	0.498	0.249
PWBr34	0.353	0.110	0.282	1.197	50.270	0.190	0.364	1.828	28.506	1.994	3.298	0.287	47.938	0.085	19.306	1.340	2.053	101.679	0.265	1.093	0.303	0.189	4.147	0.616	69.984	2.361	2.659	0.600	5.510	0.175	15.021	0.550	0.324
PWBr35	0.231	0.103	0.181	0.958	50.368	0.070	0.273	2.436	18.610	1.719	3.251	0.138	41.922	0.018	27.738	1.501	1.654	94.144	0.249	1.000	0.276	0.281	3.155	0.399	65.426	2.008	2.604	0.515	13.908	0.235	10.837	0.384	0.226
PWBr36	0.216	0.087	0.196	0.698	42.931	0.161	0.336	1.256	17.858	1.330	2.016	0.297	97.292	0.128	60.665	1.472	1.087	145.706	0.204	0.912	0.233	0.155	4.518	0.431	52.231	1.662	1.429	0.760	9.738	0.052	11.768	0.439	0.181
PWBr37	0.210	0.090	0.155	0.917	39.922	0.113	0.205	2.335	27.633	1.594	2.571	0.222	87.765	0.086	51.495	1.487	1.479	80.592	0.221	1.007	0.247	0.204	8.806	0.577	44.112	1.644	1.903	1.191	23.583	0.210	7.605	0.474	0.218



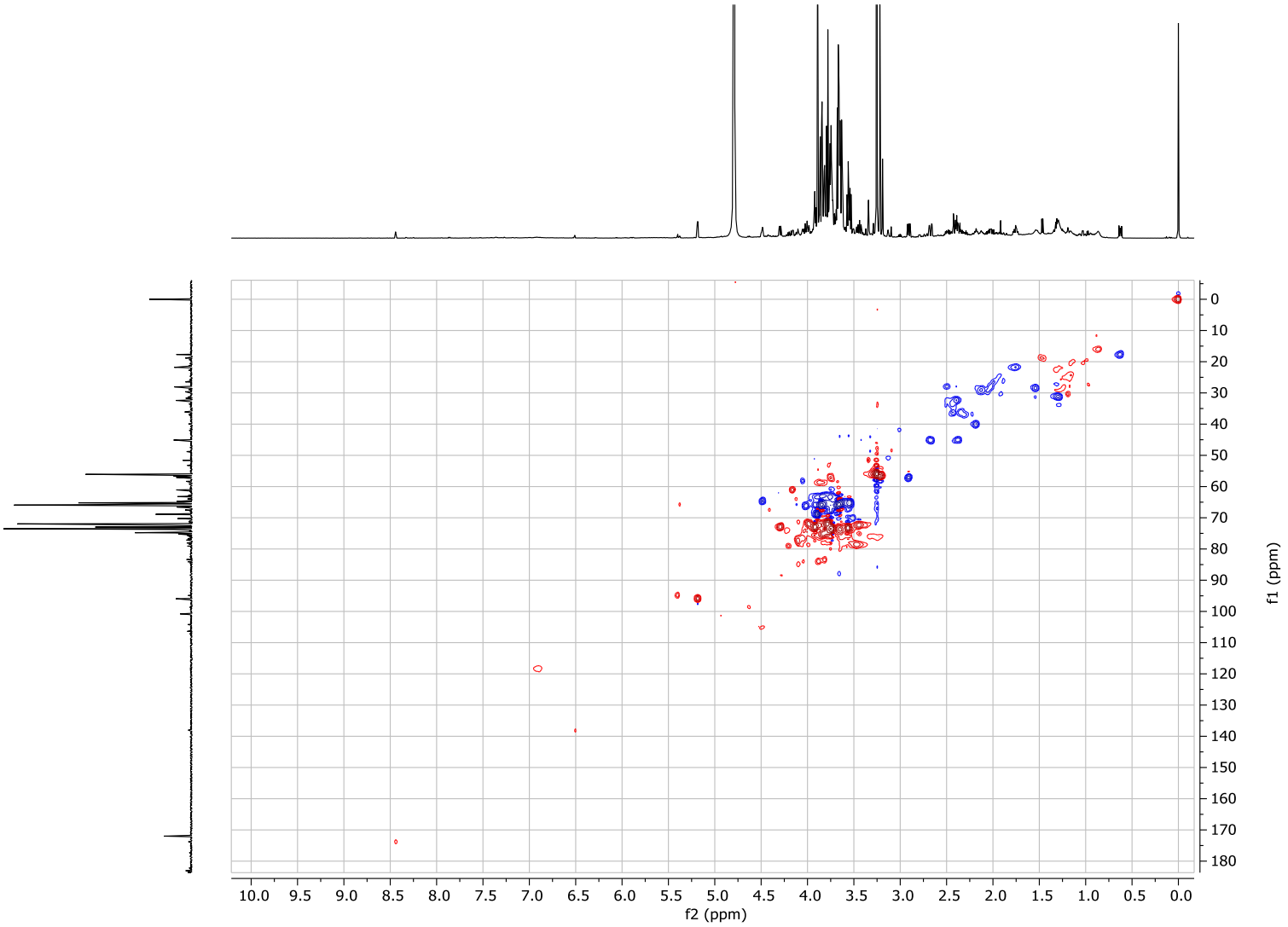
Supplementary Figure 16. Subtraction plots of (A) **PWB** with **YWB1** (left) or **YWB2** (right), and (B) **PWBr6** with **PWB**, **YWB1**, or **YWB2** (left to right) for 33 variables (quantification data were rescaled by min-max normalization to [0, 1] for plotting; positive or negative values were colored red or blue, respectively)



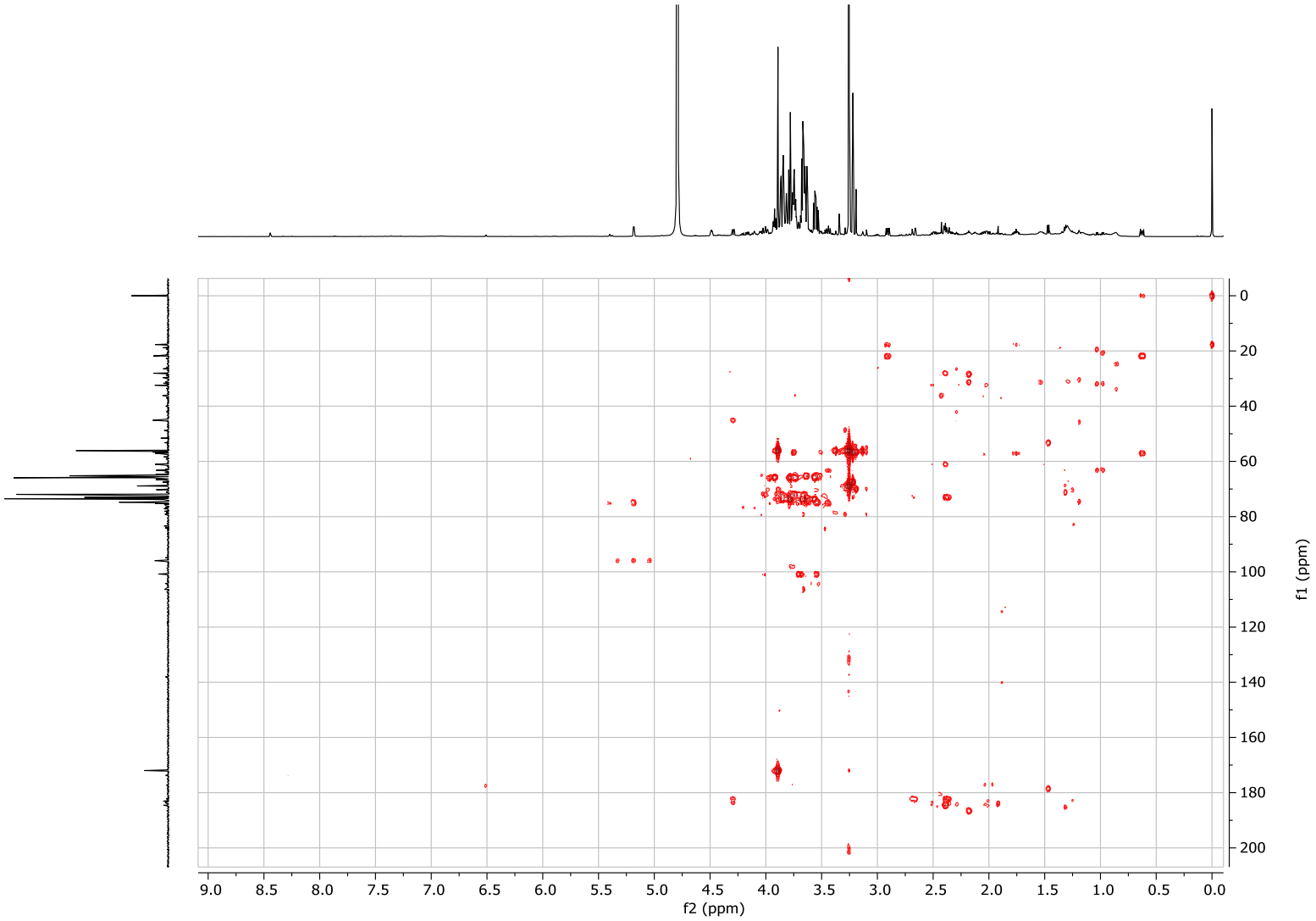
Supplementary Figure 17. ^{1}H (top, 600 MHz) and ^{13}C (bottom, 150 MHz) NMR spectra (D_2O) of **PWB**



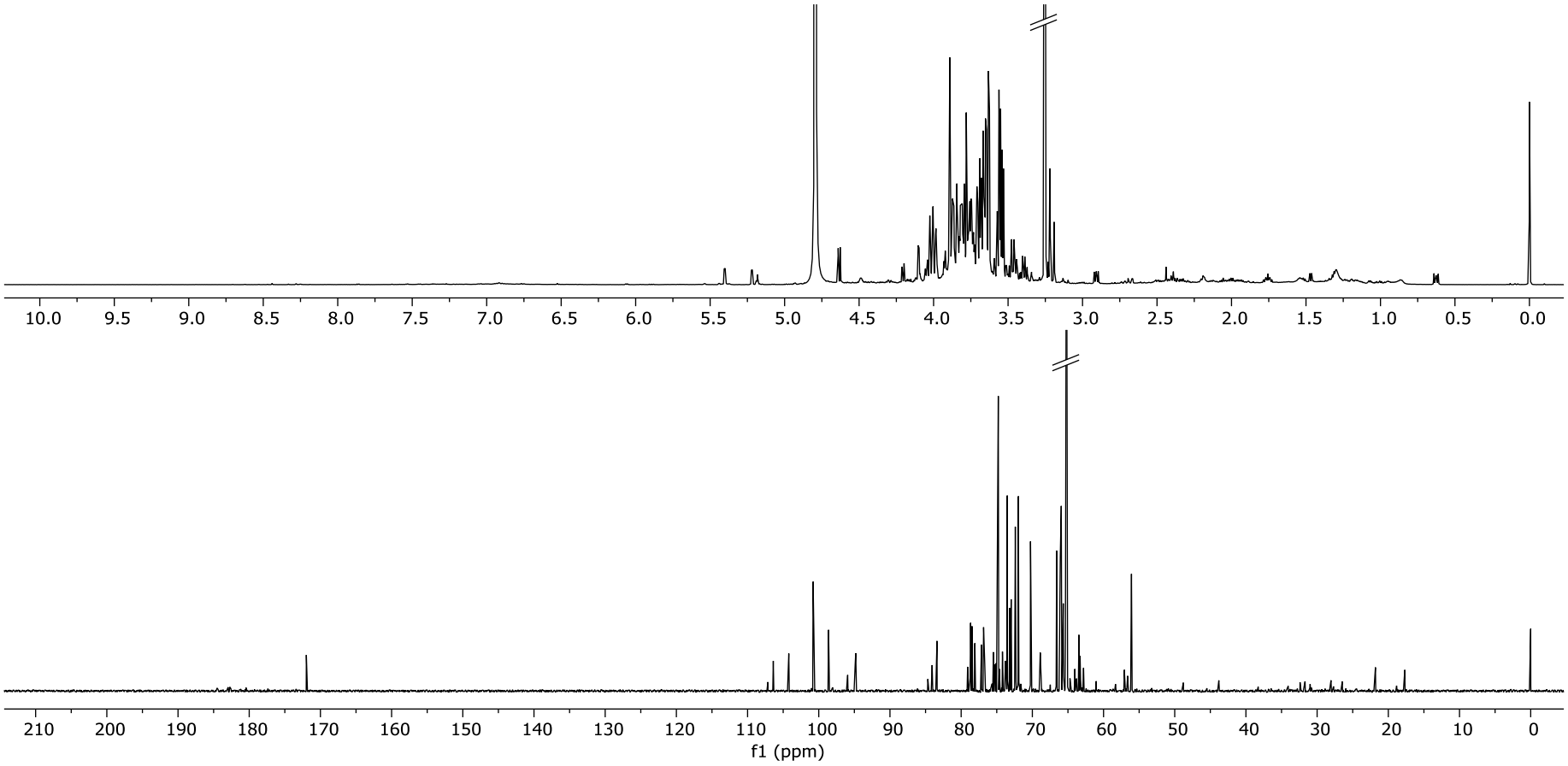
Supplementary Figure 18. ^{1}H-^{1}H COSY spectrum (D_2O) of **PWB**



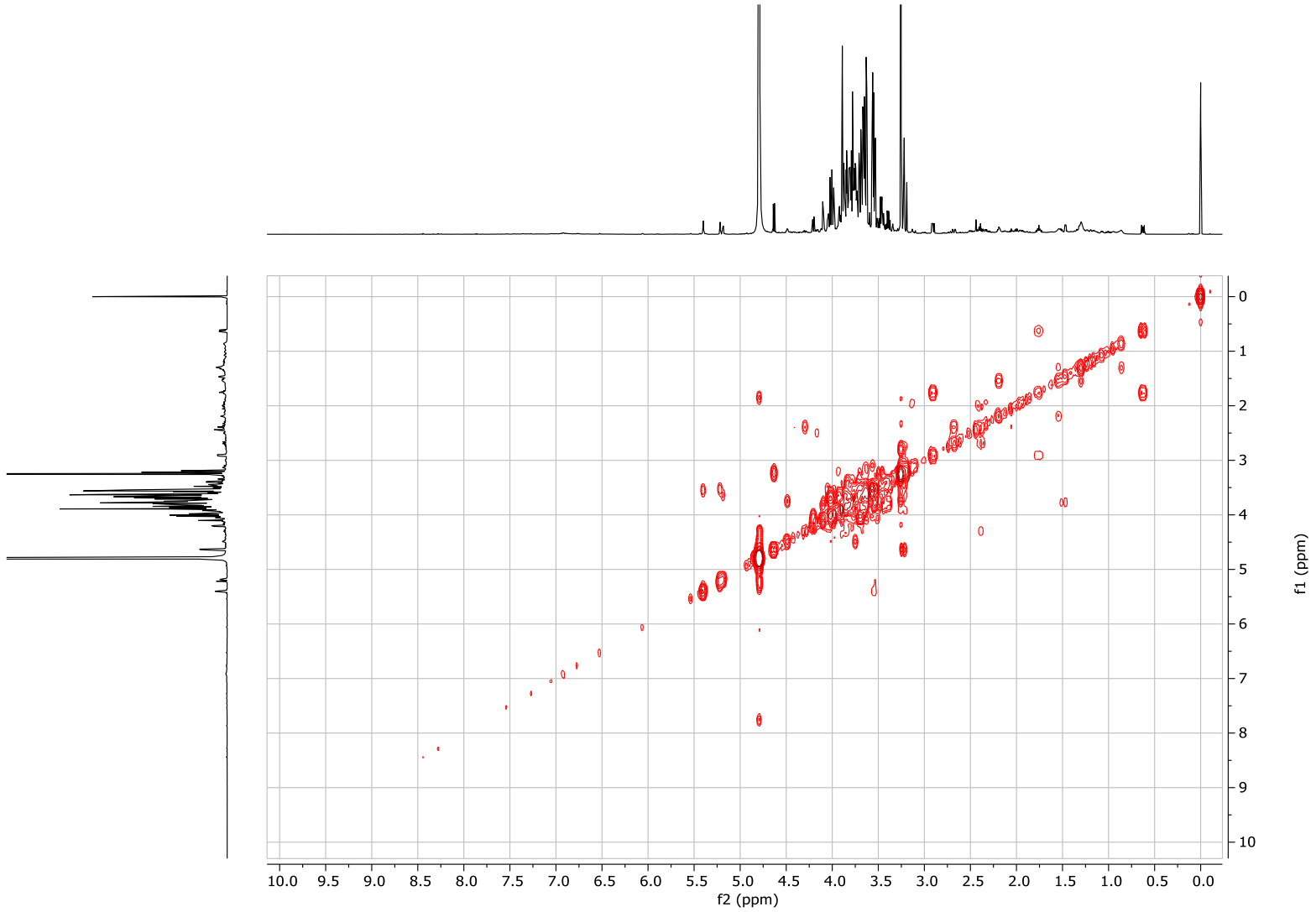
Supplementary Figure 19. ^{1}H-^{13}C HSQC spectrum (D_2O) of **PWB**



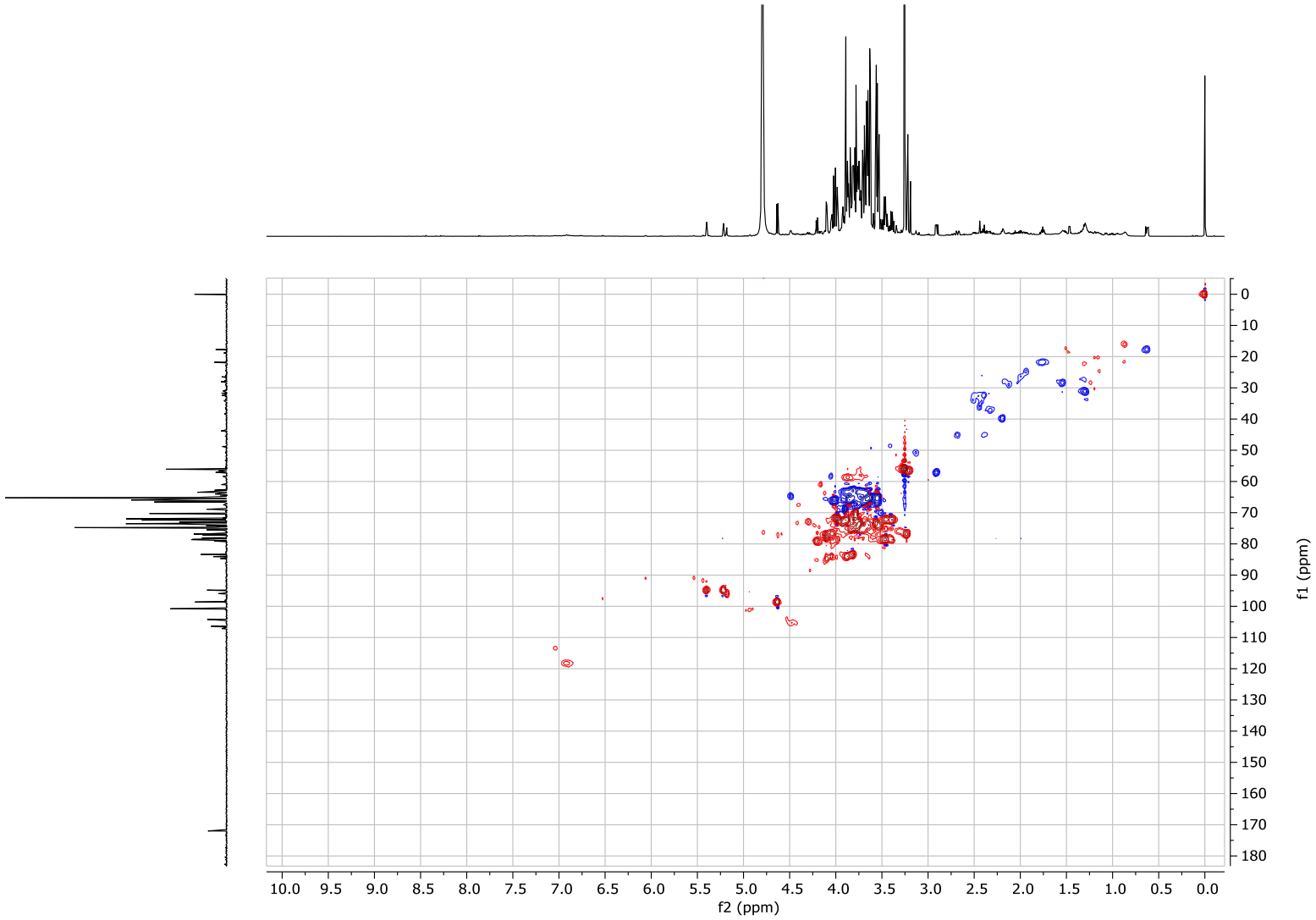
Supplementary Figure 20. ^{1}H-^{13}C HMBC spectrum (D_2O) of **PWB**



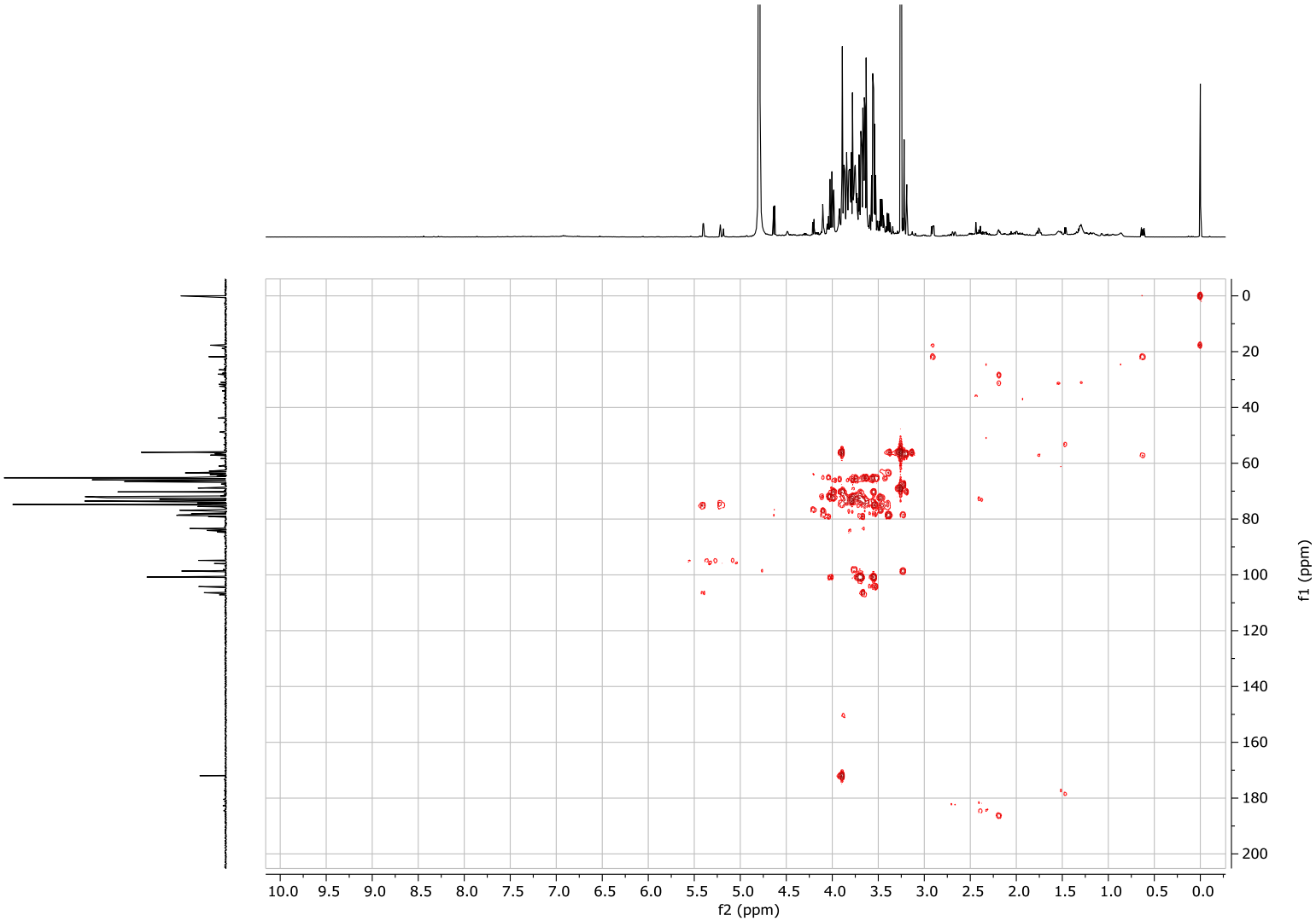
Supplementary Figure 21. ^{1}H (top, 600 MHz) and ^{13}C (bottom, 150 MHz) NMR spectra (D_2O) of **PWBr3**



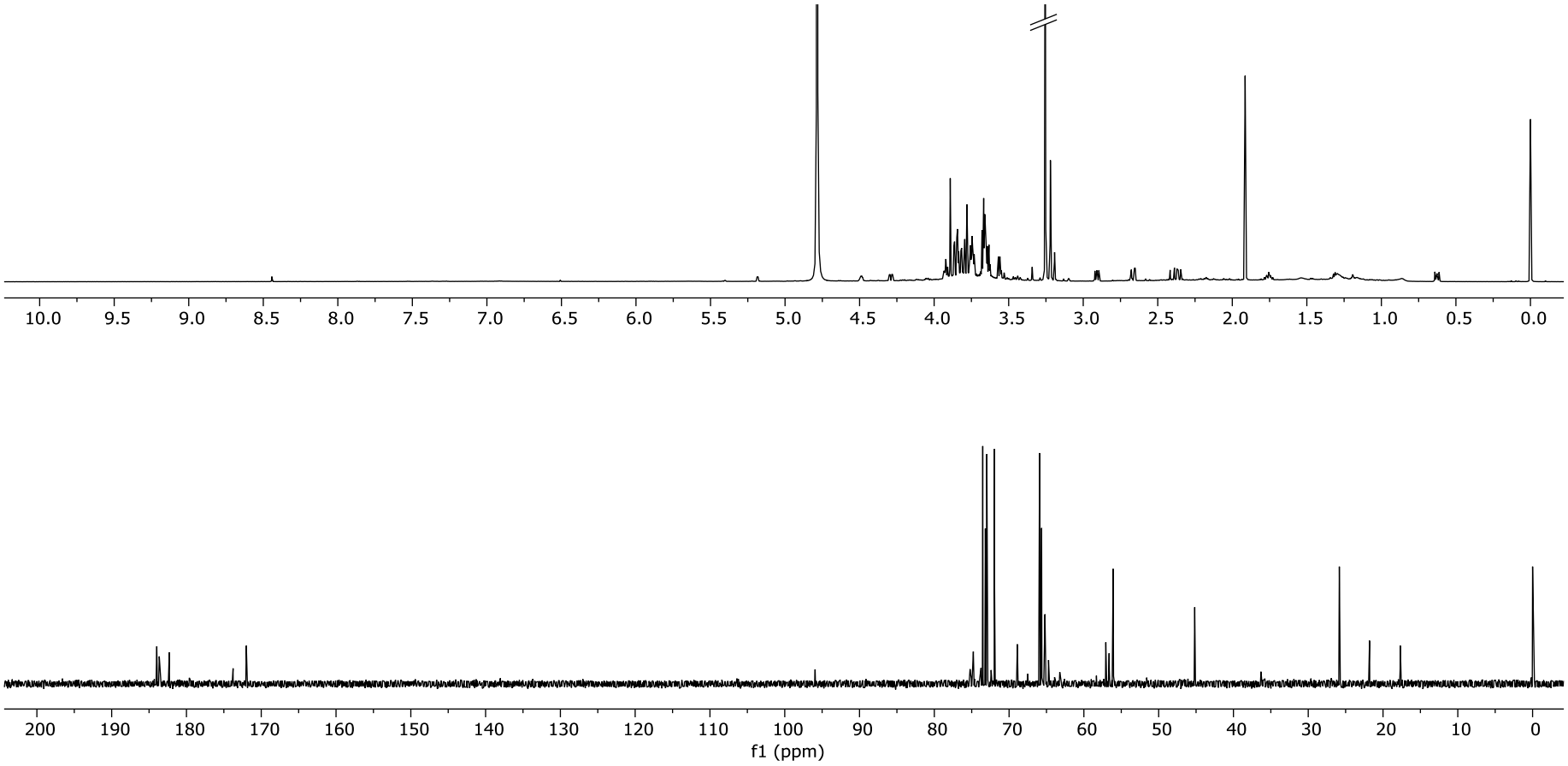
Supplementary Figure 22. ^{1}H-^{1}H COSY spectrum (D_2O) of **PWBr3**



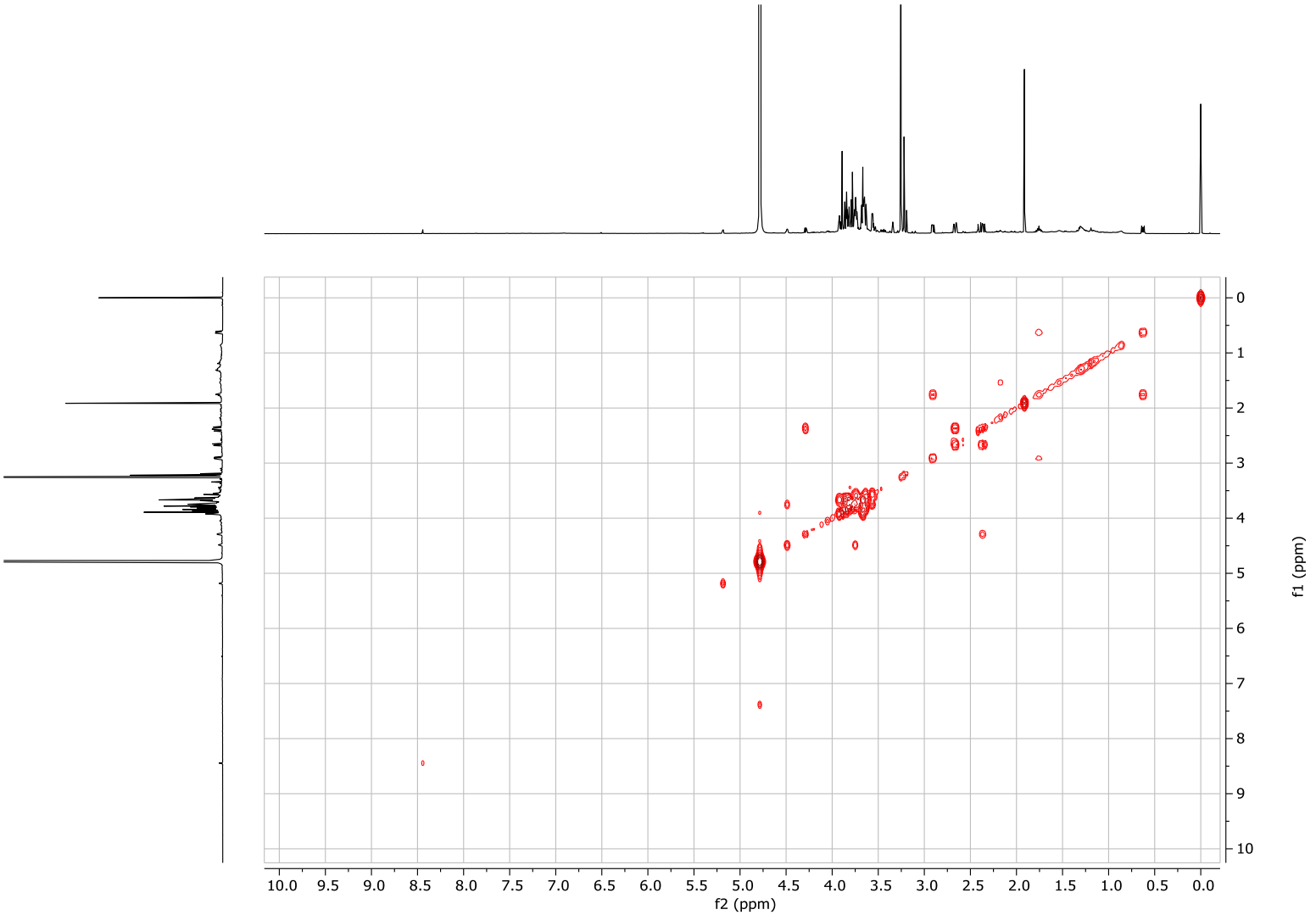
Supplementary Figure 23. ^{1}H-^{13}C HSQC spectrum (D_2O) of **PWBr3**



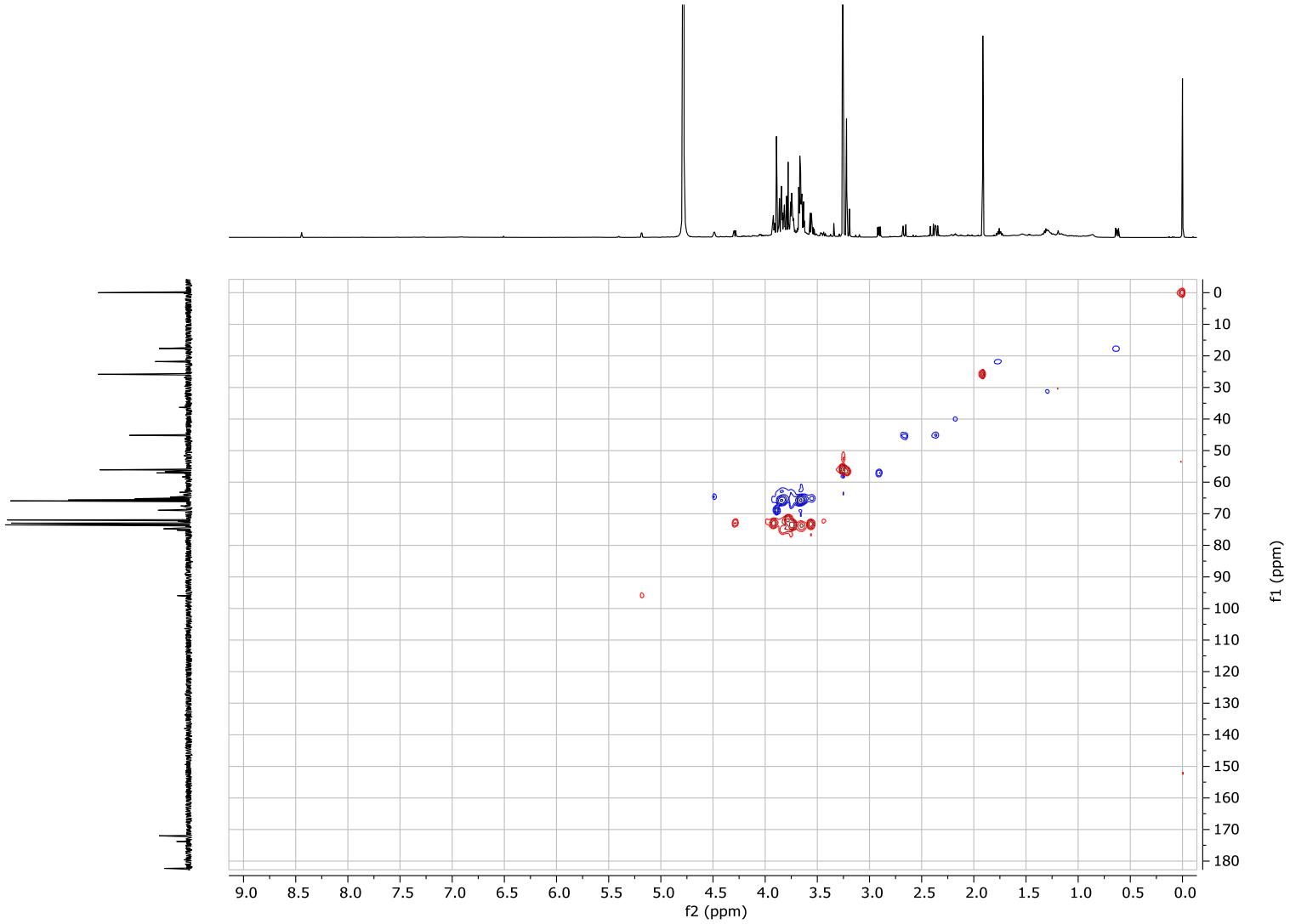
Supplementary Figure 24. ^{1}H-^{13}C HMBC spectrum (D_2O) of **PWBr3**



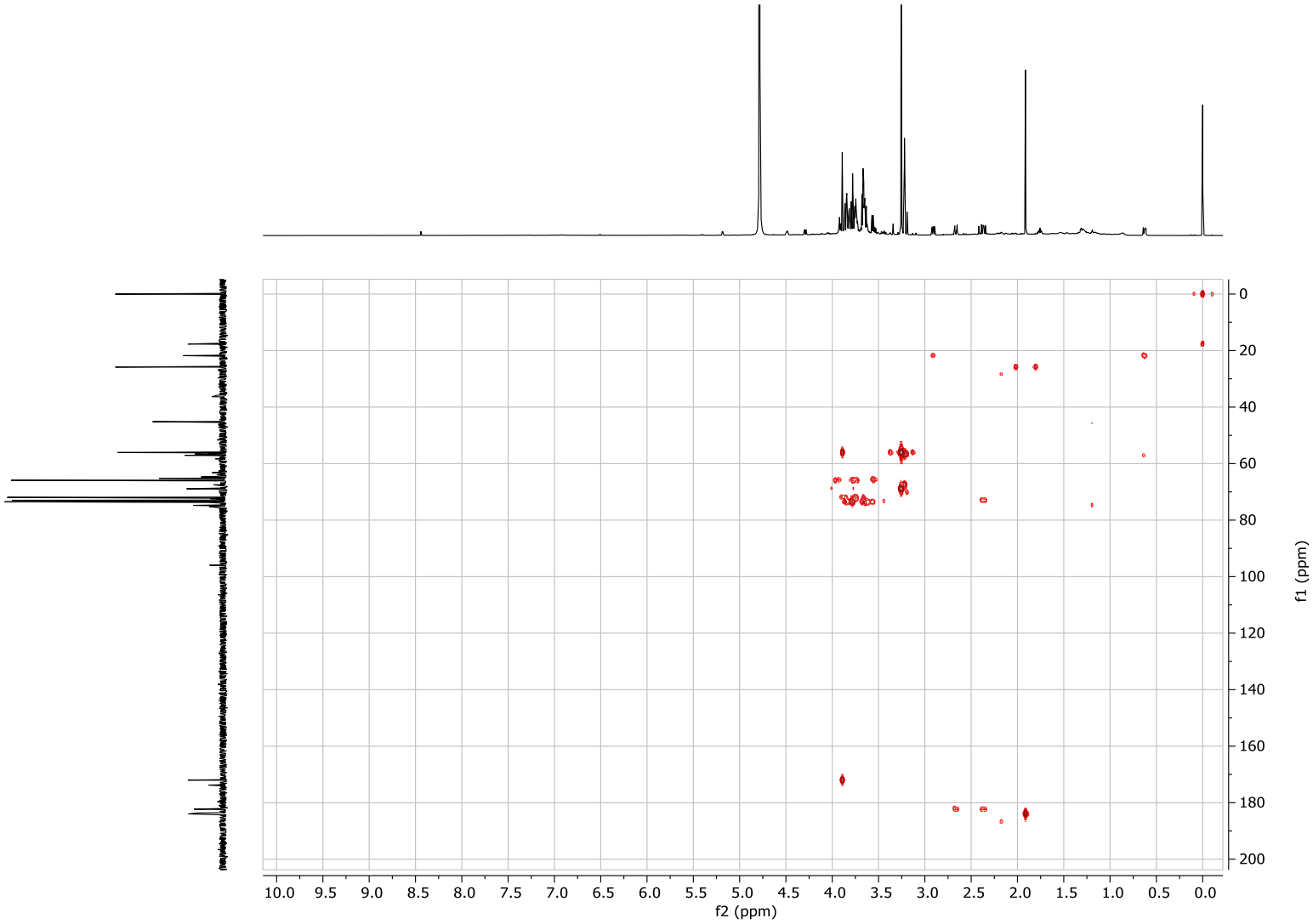
Supplementary Figure 25. ^{1}H (top, 600 MHz) and ^{13}C (bottom, 150 MHz) NMR spectra (D_2O) of **PWBr19**



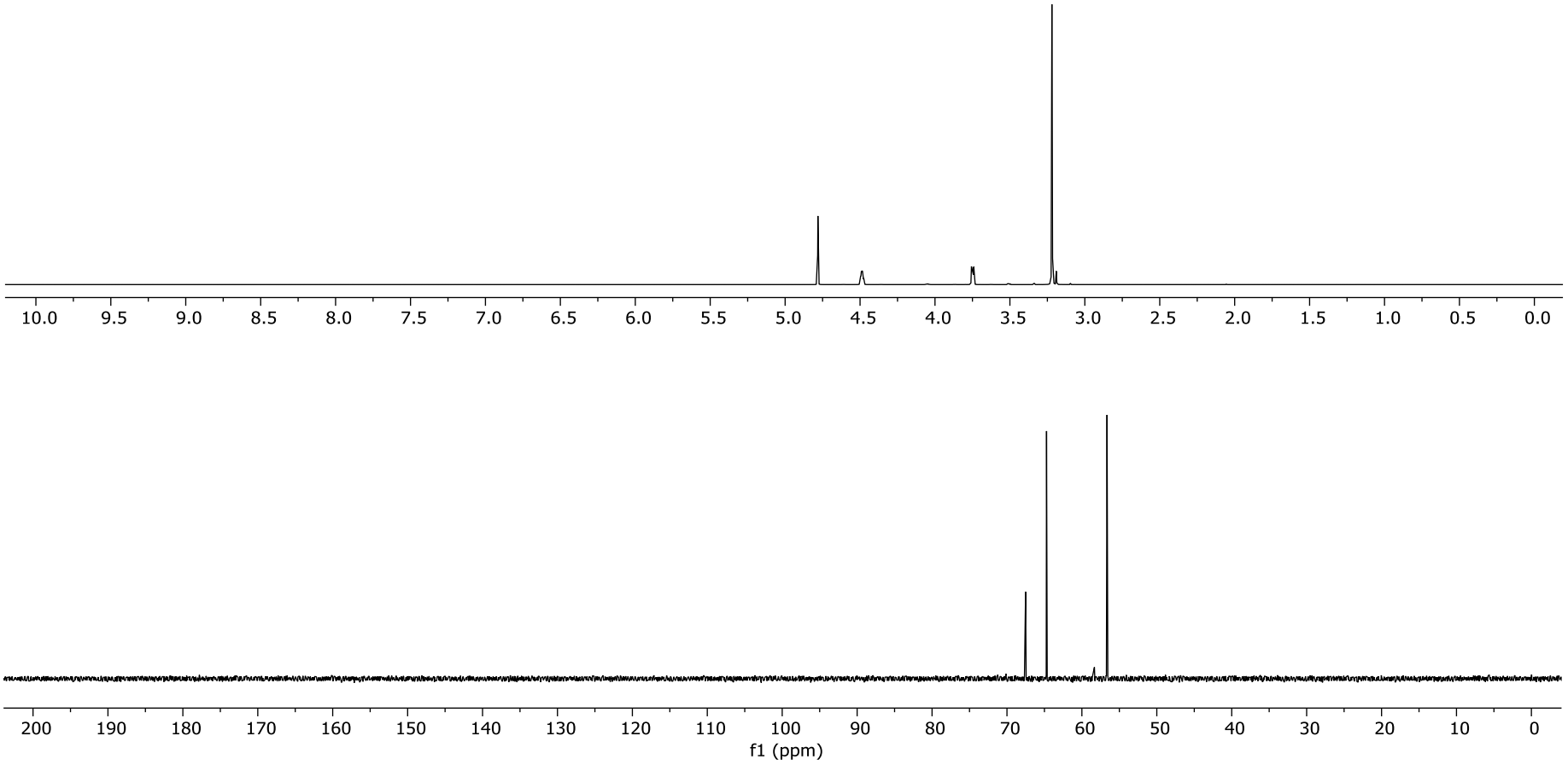
Supplementary Figure 26. ^{1}H-^{1}H COSY spectrum (D_2O) of **PWBr19**



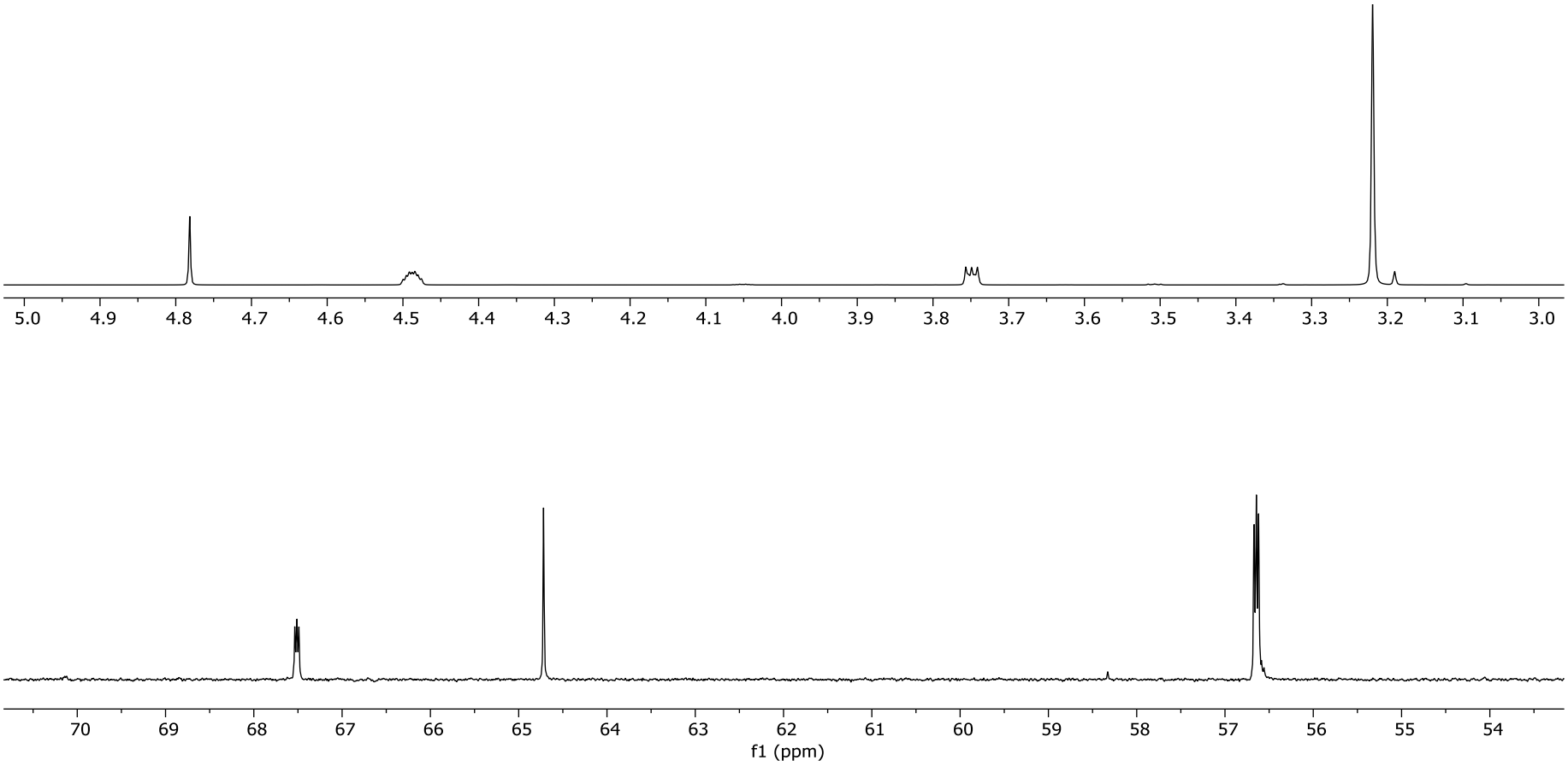
Supplementary Figure 27. ^{1}H-^{13}C HSQC spectrum (D_2O) of **PWBr19**



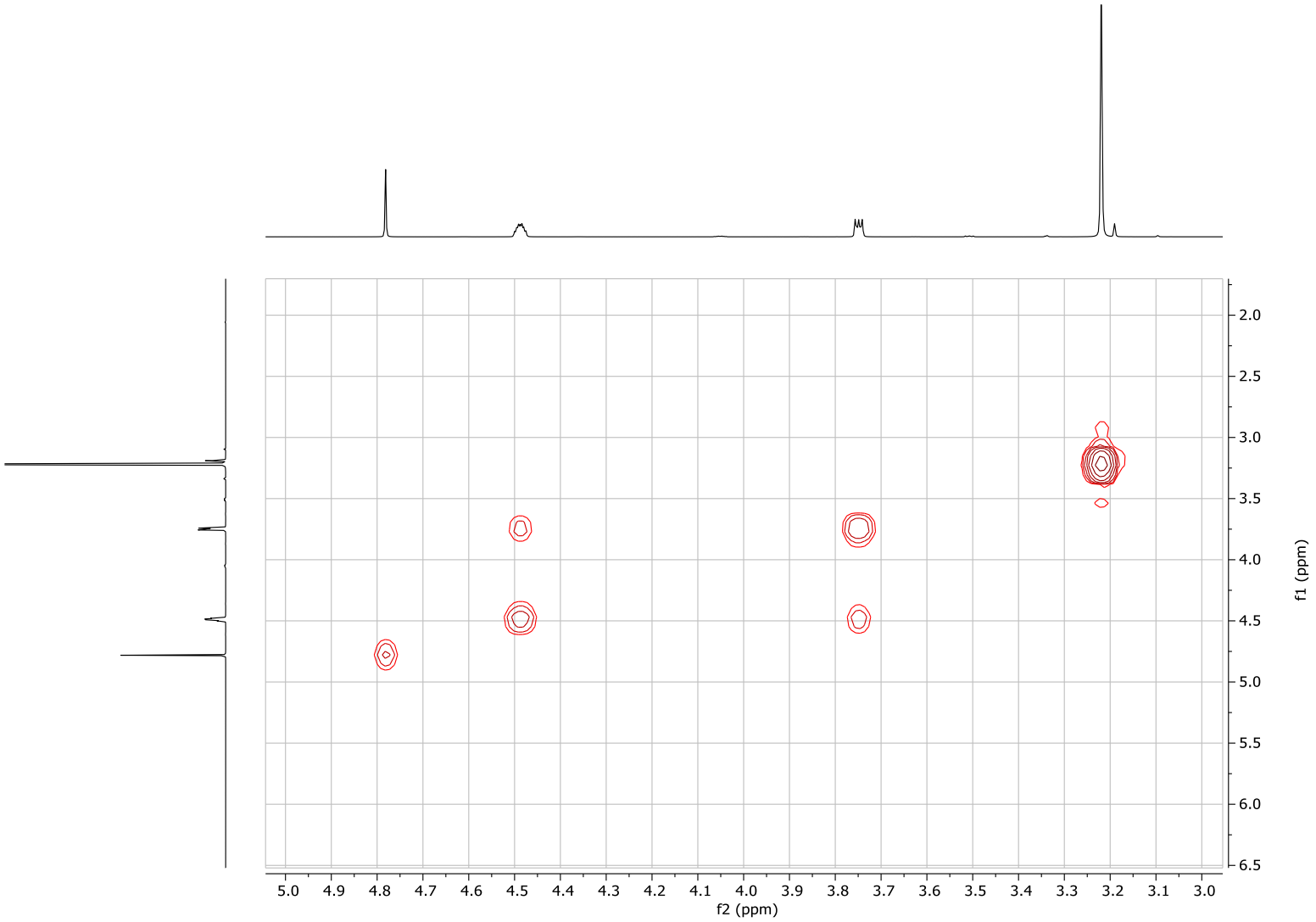
Supplementary Figure 28. ^{1}H-^{13}C HMBC spectrum (D_2O) of **PWBr19**



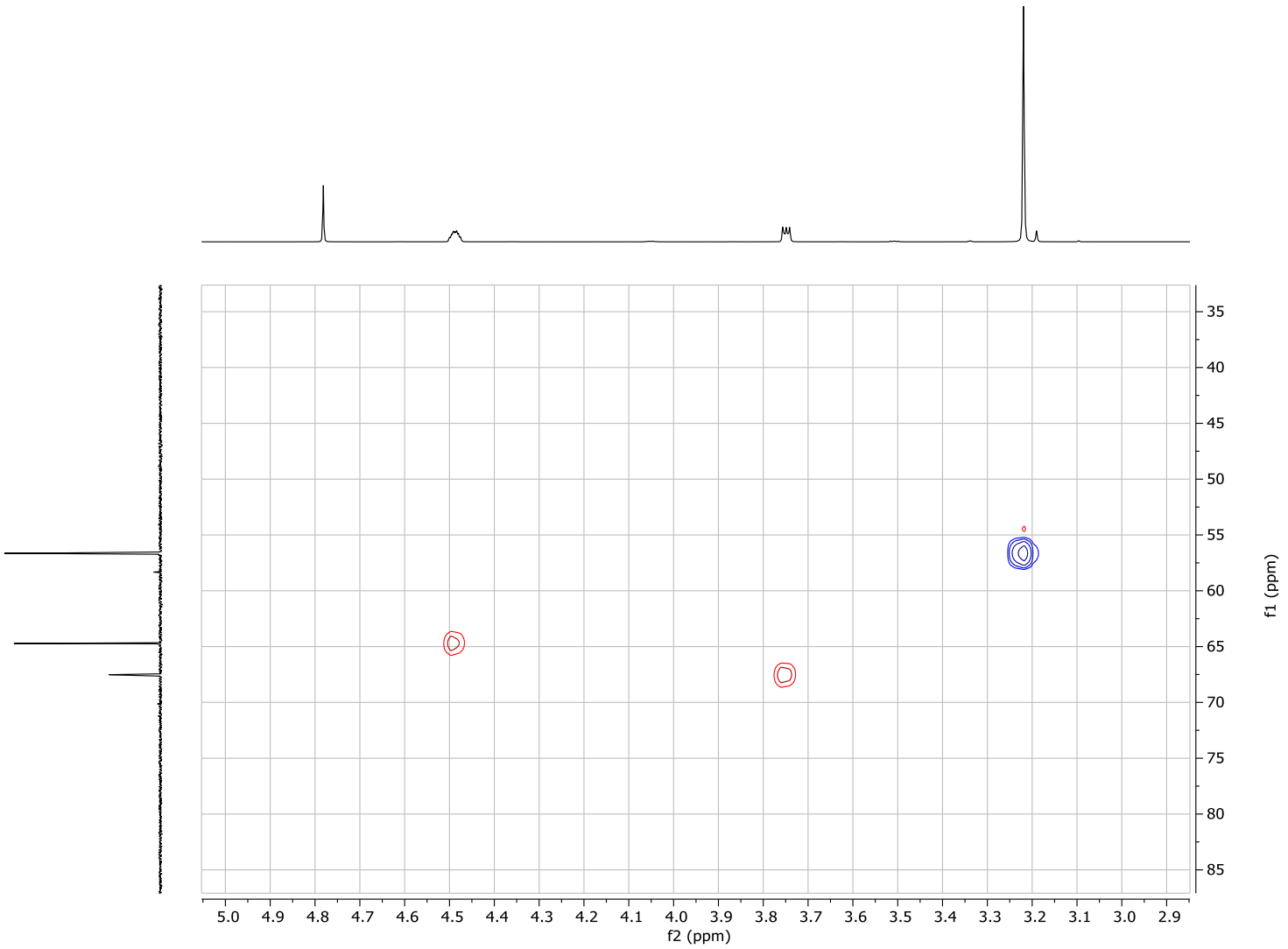
Supplementary Figure 29. ^{1}H (top, 600 MHz) and ^{13}C (bottom, 150 MHz) NMR spectra (D_2O) of choline sulfate (CS)



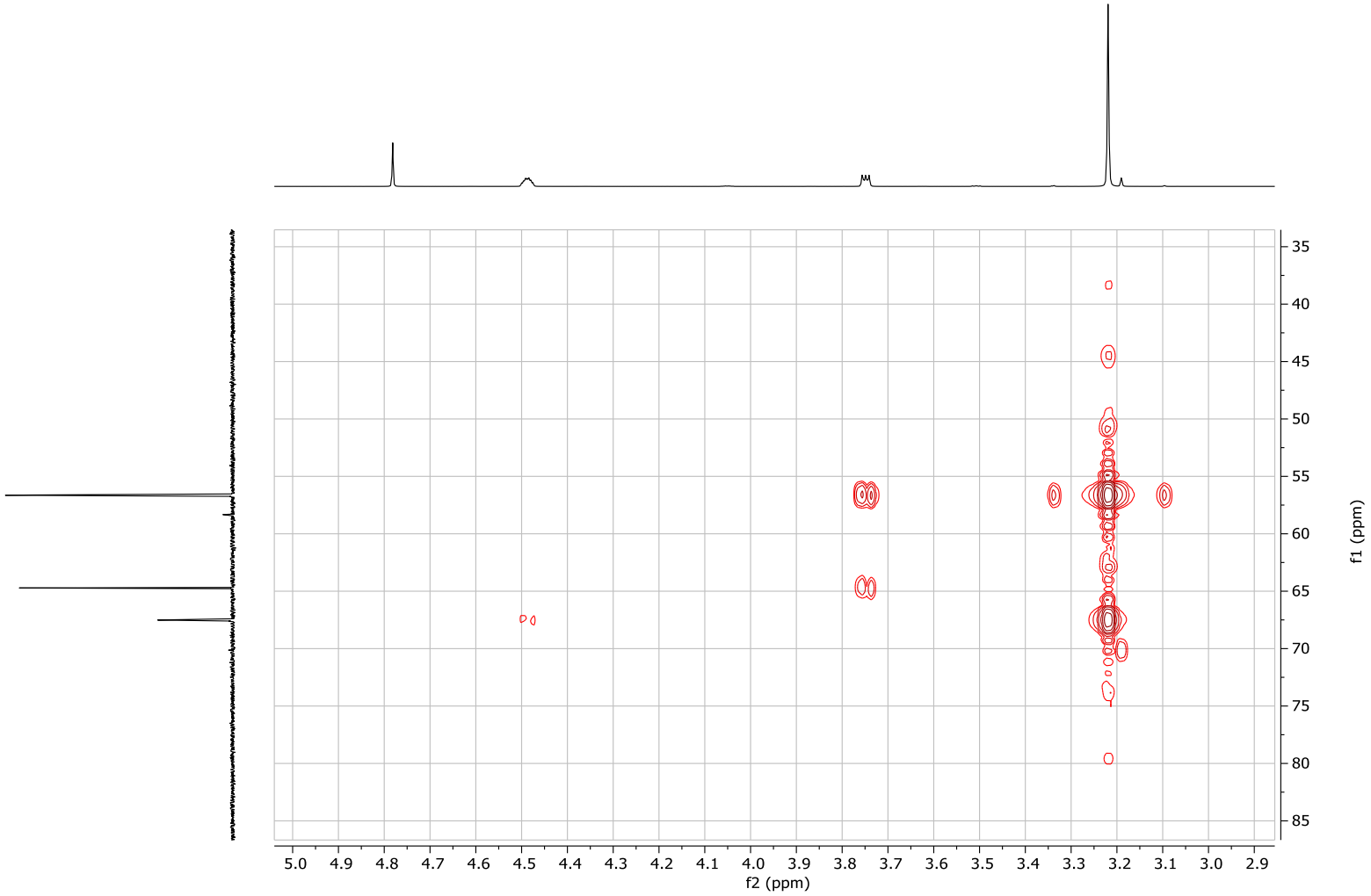
Supplementary Figure 30. Expanded ^{1}H (top, 600 MHz) and ^{13}C (bottom, 150 MHz) NMR spectra (D_2O) of choline sulfate (CS)



Supplementary Figure 31. ^{1}H-^{1}H COSY spectrum (D_2O) of choline sulfate (CS)



Supplementary Figure 32. ^{1}H-^{13}C HSQC spectrum (D_2O) of choline sulfate (CS)



Supplementary Figure 33. ^{1}H-^{13}C HMBC spectrum (D_2O) of choline sulfate (CS)