NMR Reveals an Undeclared Constituent in Custom Synthetic Peptides

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Keywords: ^{1}H NMR, peptides, purity, impurity, residual complexity, custom peptide synthesis.

Supporting Material

Table of Contents

QC data provided by the manufacturer of the Synthetic Peptides

DR:

Data Analysis Report:

% of hydrophobic amino acids:	0.00%
% of acidic amino acids:	50.00%
% of basic amino acids	50.00%
% of neutral amino acids	0.00%
MS (M+H)+	288.99 (see **Figure S1**)
Purity (HPLC)	98.44% (see **Figure S2**)
	HPLC, 220 nm, C18linear gradient
	Column: Boston Green ODS, 4.6*250 mm, 5μm
	Solvent A: 0.1% trifluoroacetic in 100% acetonitrile
	Solvent B: 0.1% trifluoroacetic in 100% water
Storage Conditions	-20 °C
Remarks	This product is supplied as trifluoroacetate salt

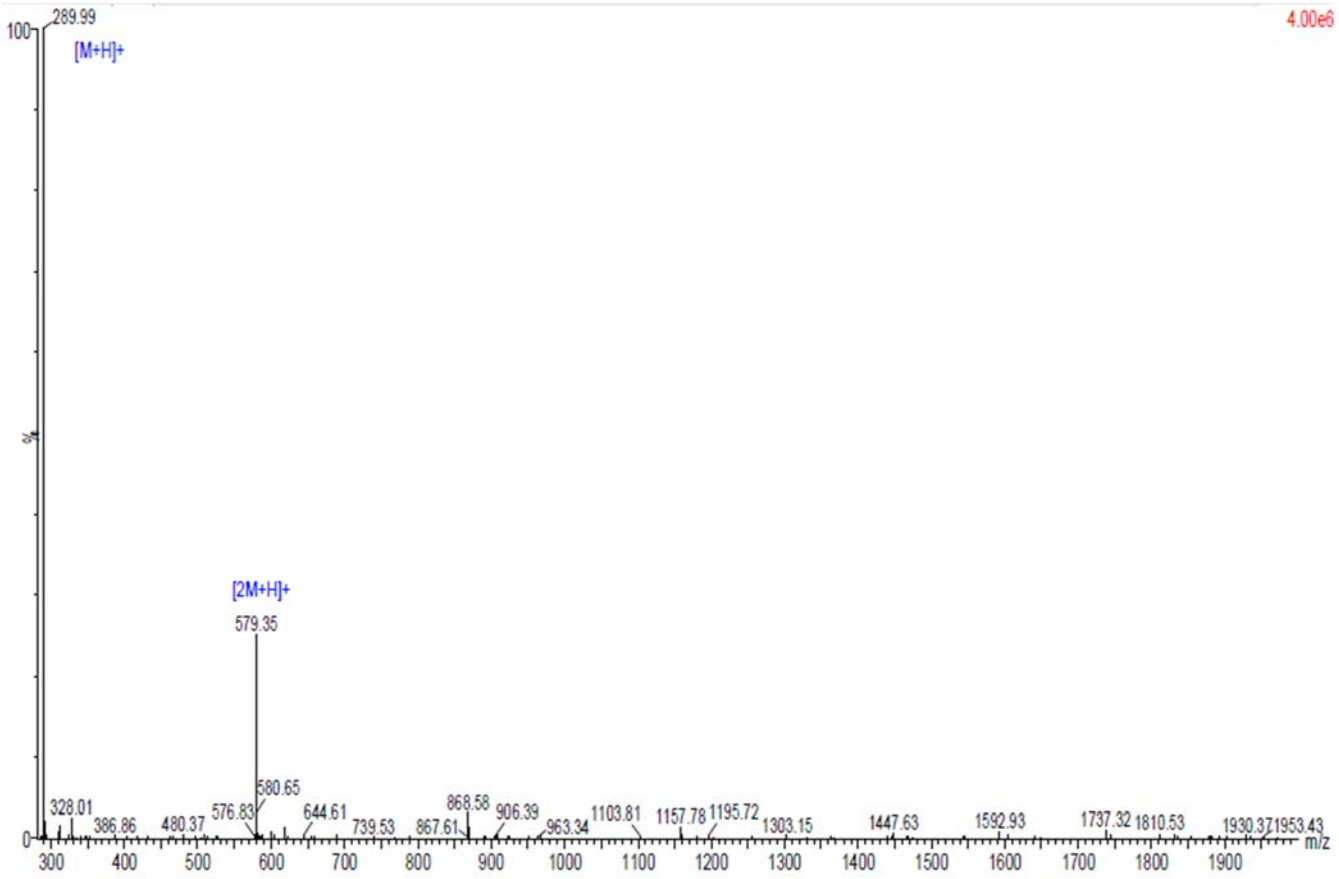


Figure S1. MS data of DR provided by the manufacturer.

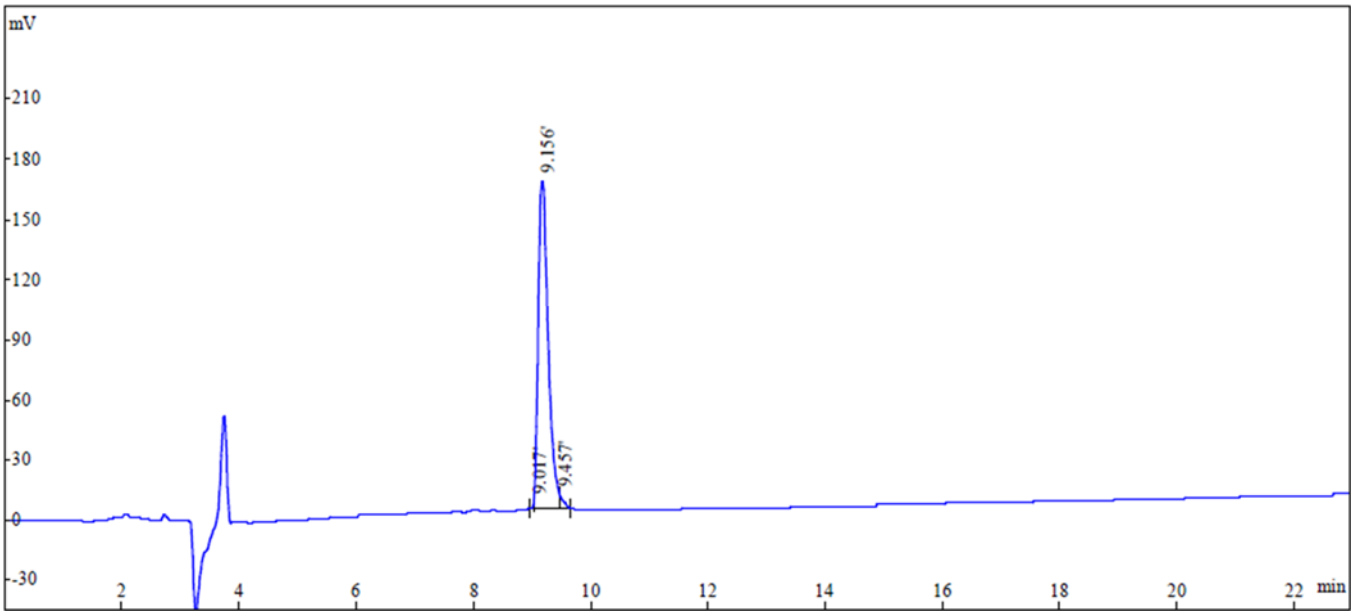


Figure S2. HPLC data of DR provided by the manufacturer.

Table S1. Table of integrated peaks for HPLC data of DR provided by the manufacturer.

Time	**Conc.**	**Area**	**Height**
9.017	0.1245	2254	2790
9.156	98.4496	1782930	162012
9.457	1.4260	25826	6209
Total	100	1811010	171011

DRVYI:

Data Analysis Report:

% of hydrophobic amino acids:	60.00%
% of acidic amino acids:	20.00%
% of basic amino acids	20.00%
% of neutral amino acids	0.00%
MS (M+H)+	664.50 (see **Figure S3**)
Purity (HPLC)	98.34% (see **Figure S4**)
	HPLC, 220 nm, C18linear gradient
	Column: Boston Green ODS, 4.6*250 mm, 5μm
	Solvent A: 0.1% trifluoroacetic in 100% acetonitrile
	Solvent B: 0.1% trifluoroacetic in 100% water
Storage Conditions	-20 °C
Remarks	This product is supplied as trifluoroacetate salt

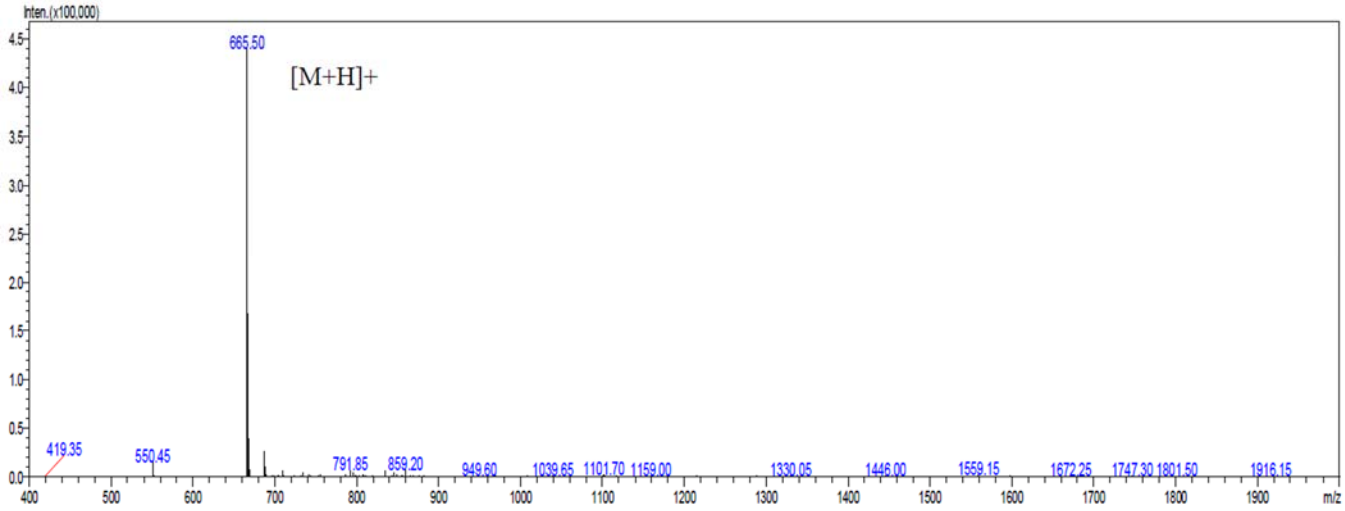


Figure S3. MS data of DRVYI provided by the manufacturer.

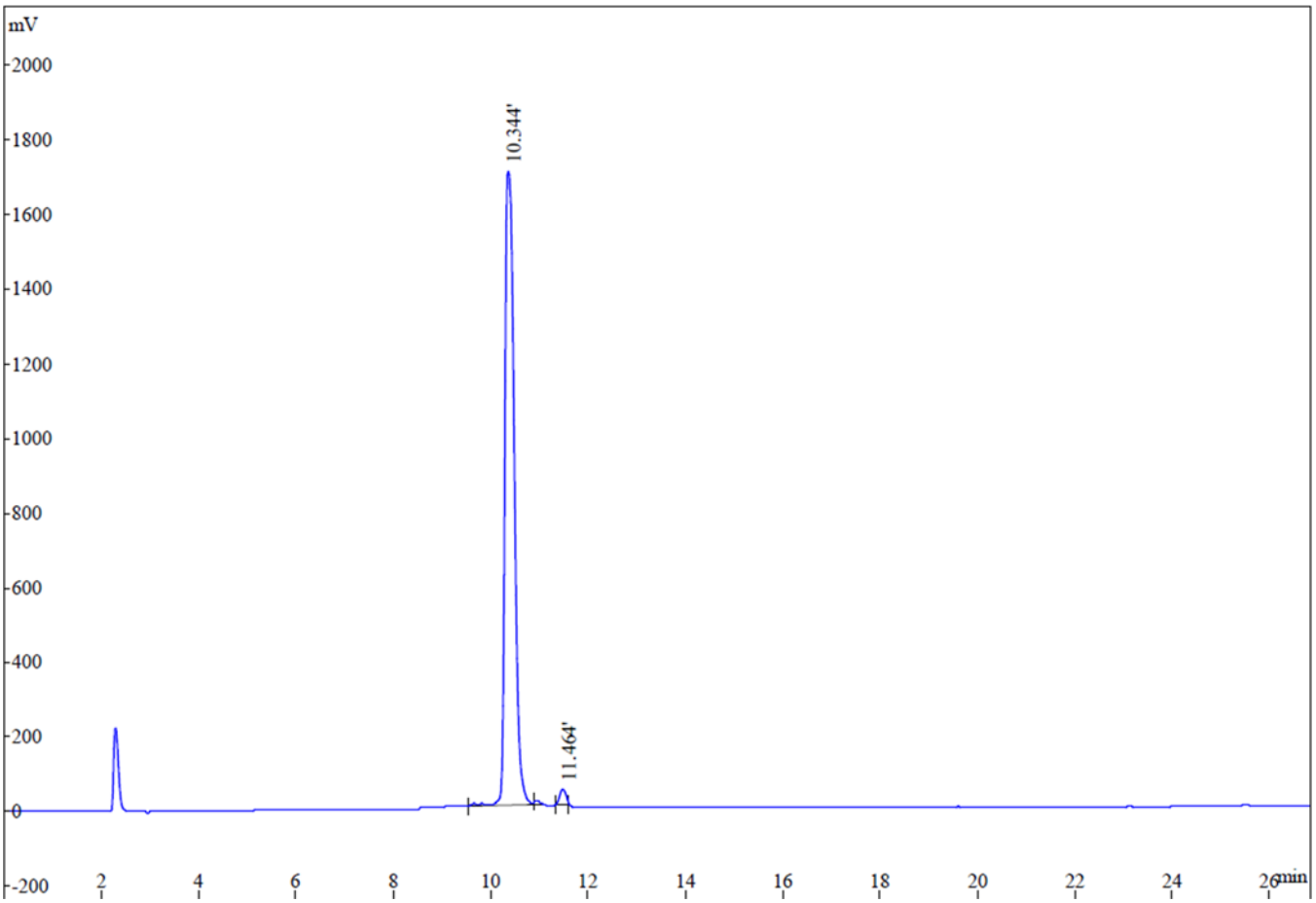


Figure S4. HPLC data of DRVYI provided by the manufacturer.

Table S2. Table of integrated peaks for HPLC data of DRVVYI provided by the manufacturer.

Time	Conc.	Area	Height
10.344	98.3434	19998365	1697217
11.464	1.6566	336869	40936
Total	100	20335234	1738153

800 MHz NMR Data

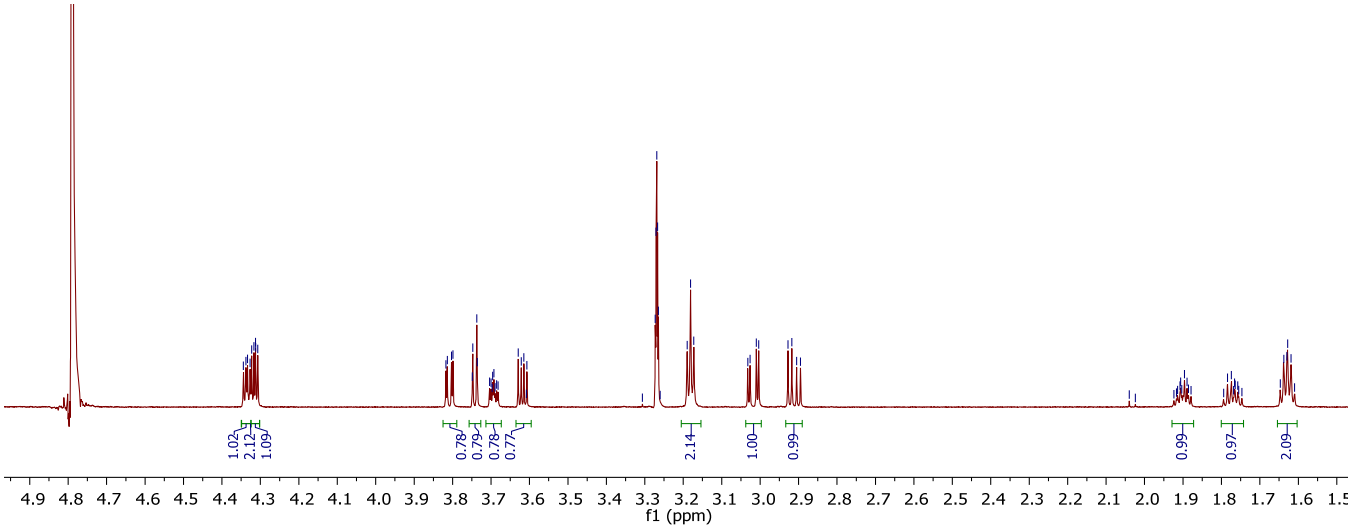


Figure S5. Full ^{1}H NMR spectrum of DR (25 mM, 135 µL D_2O, 40 µL CD_3OD, 3 mm tube) with mannitol impurity at 800 MHz (298K).

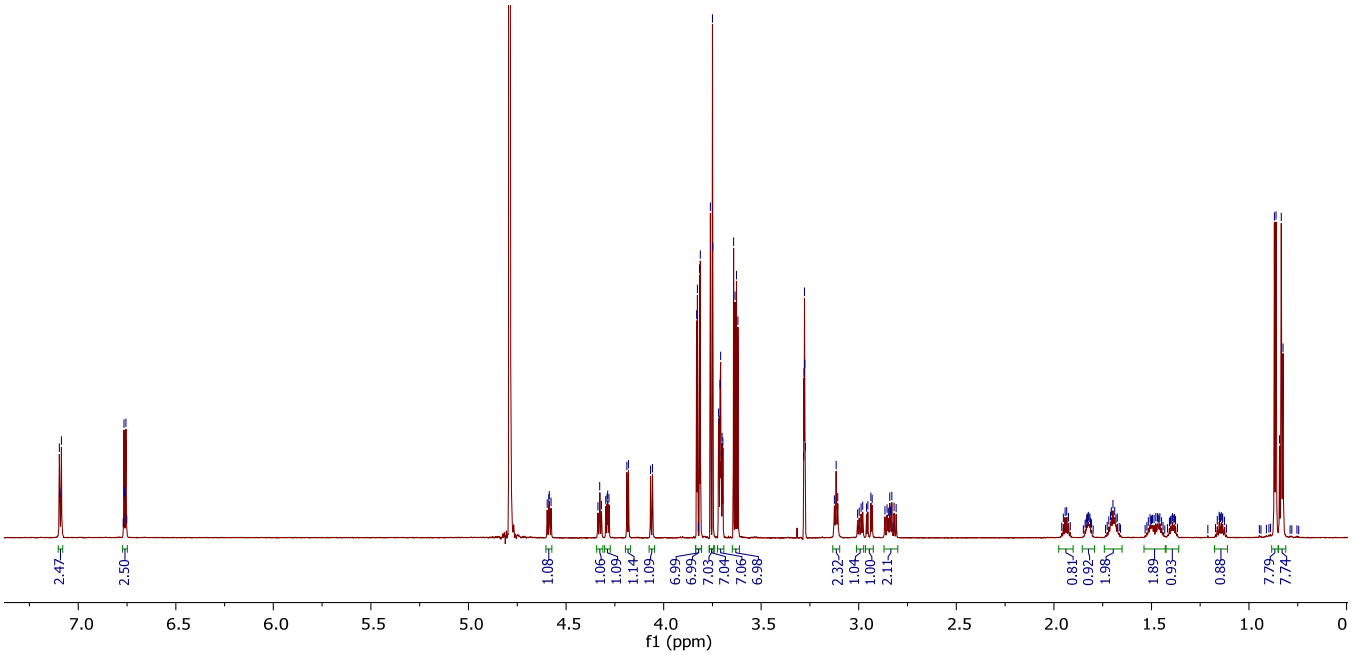


Figure S6. Full ^{1}H NMR spectrum of DRVYI (25 mM, 135 µL D_2O, 40 µL CD_3OD, 3 mm tube) with mannitol impurity at 800 MHz (298K).

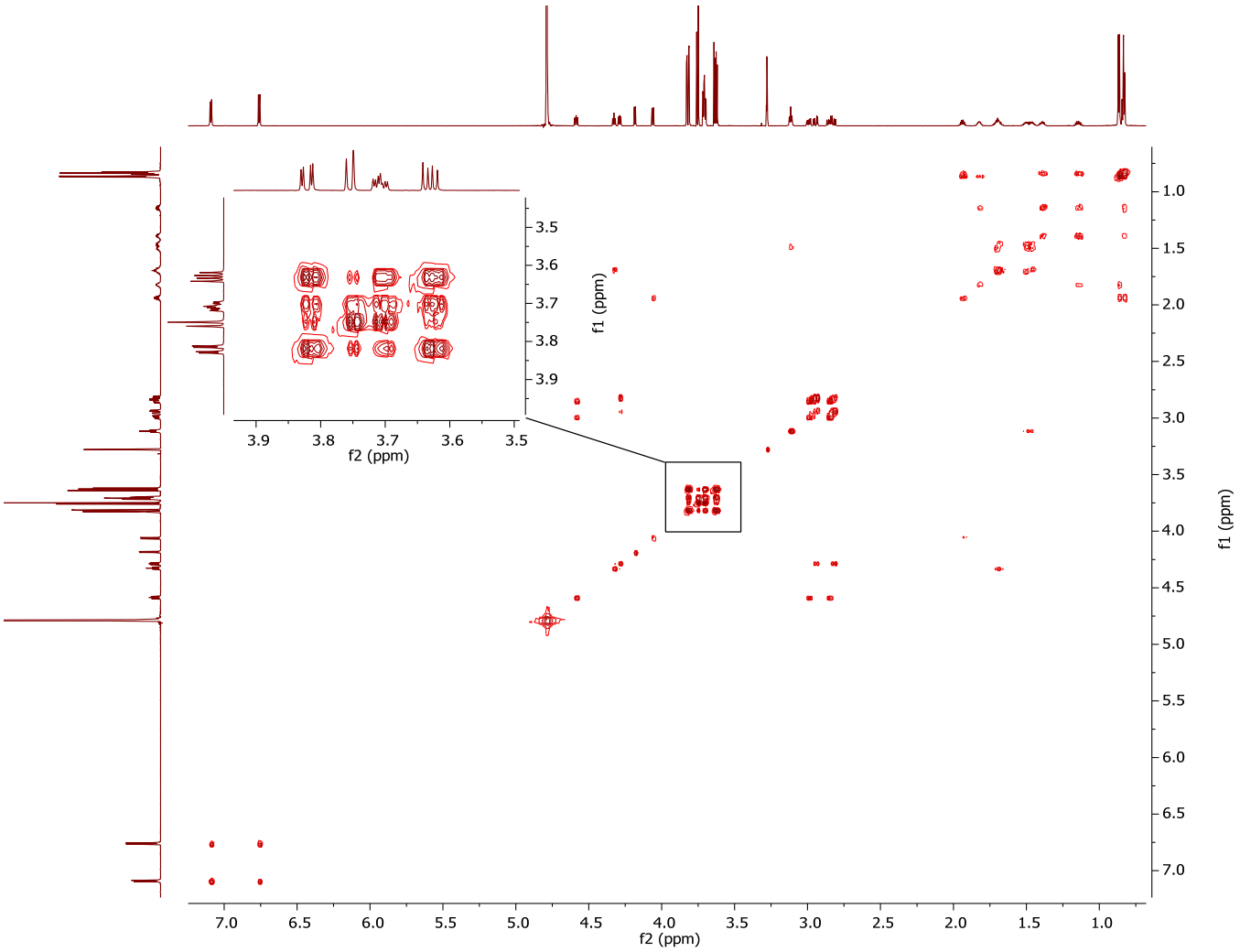


Figure S7. Full $^{1}H/^{1}H$-COSY NMR spectrum of DRVYI (25 mM, 135 µL D_2O, 40 µL CD_3OD, 3 mm tube) at 800 MHz (298K) with region of mannitol impurity expanded.

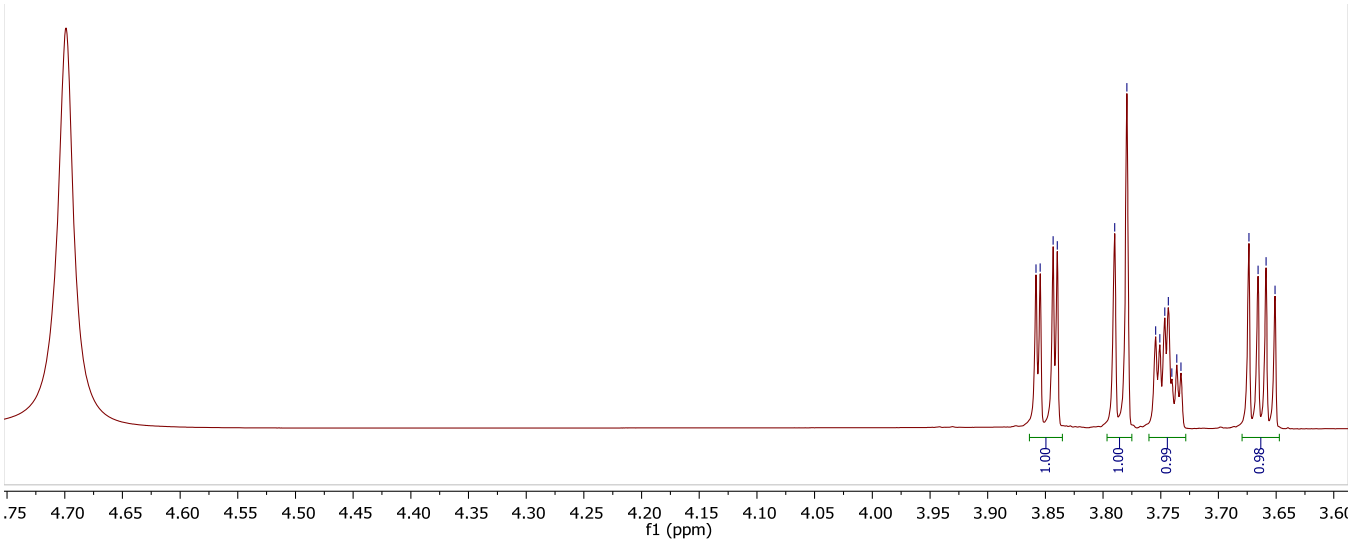


Figure S8. Full ^{1}H NMR spectrum of mannitol (58 mg, 600 µL D_2O, 5 mm tube) at 800 MHz (305K).

900 MHz NMR Data

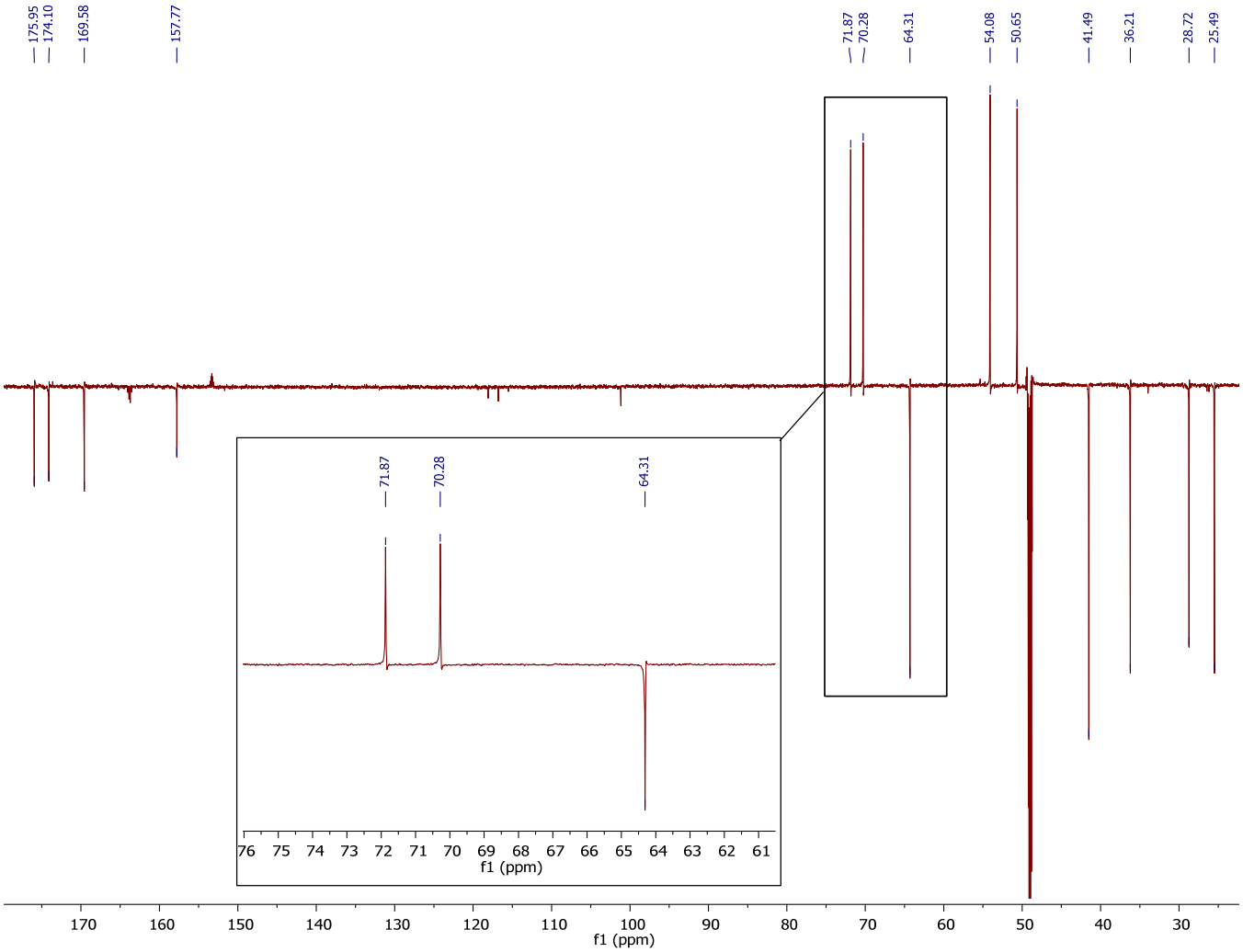


Figure S9. ^{13}C-DEPT-Q-135 NMR spectrum of DR (25 mM, 135 µL D_2O, 40 µL CD_3OD, 3 mm tube) at 225 MHz (298K) with expanded region for the mannitol impurity.

400 MHz NMR Data

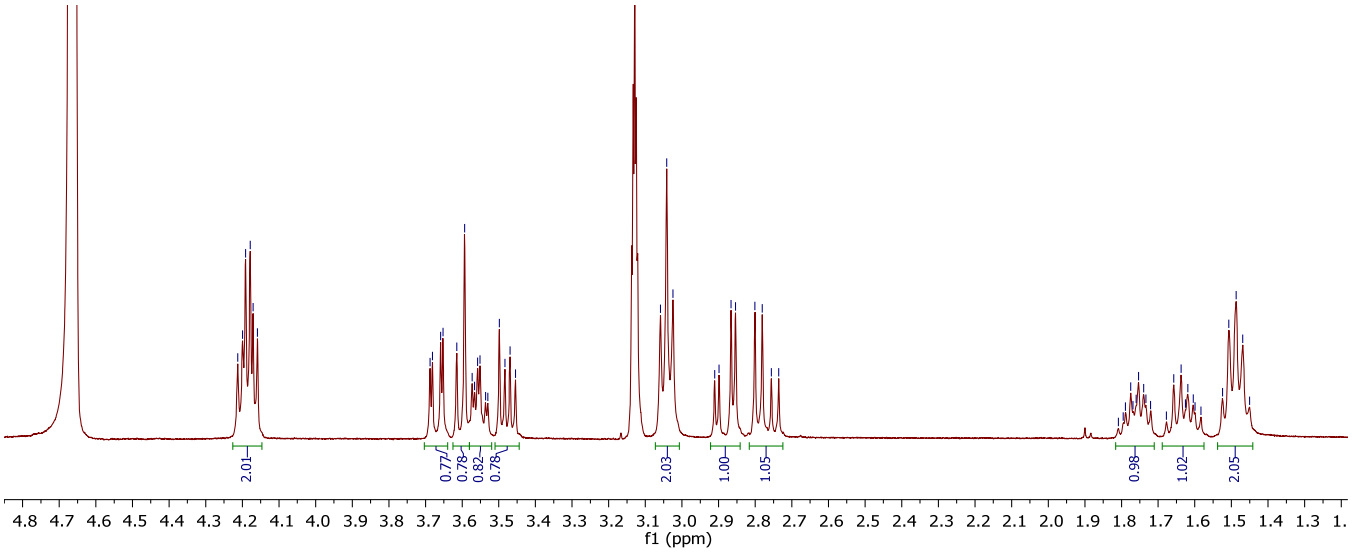


Figure S10. Full ^{1}H NMR spectrum of DR (25 mM, 135 µL D_2O, 40 µL CD_3OD, 3 mm tube) with mannitol impurity at 400 MHz (298K).

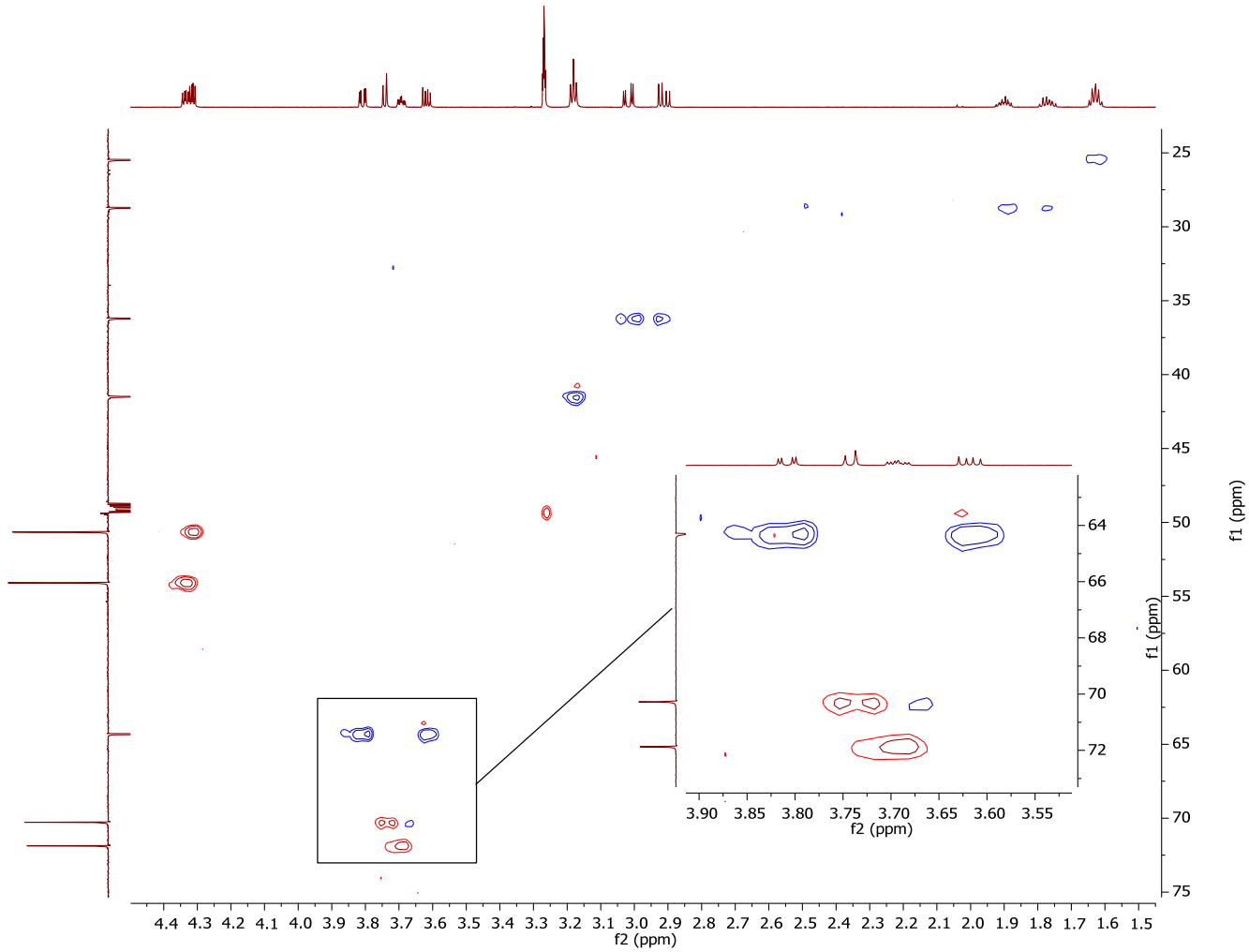


Figure S11. ^{1}H/^{13}C-HSQC NMR spectrum of DR (25 mM, 135 µL D_2O, 40 µL CD_3OD, 3 mm tube) with expansion of the mannitol impurity at 400/100 MHz (298K).

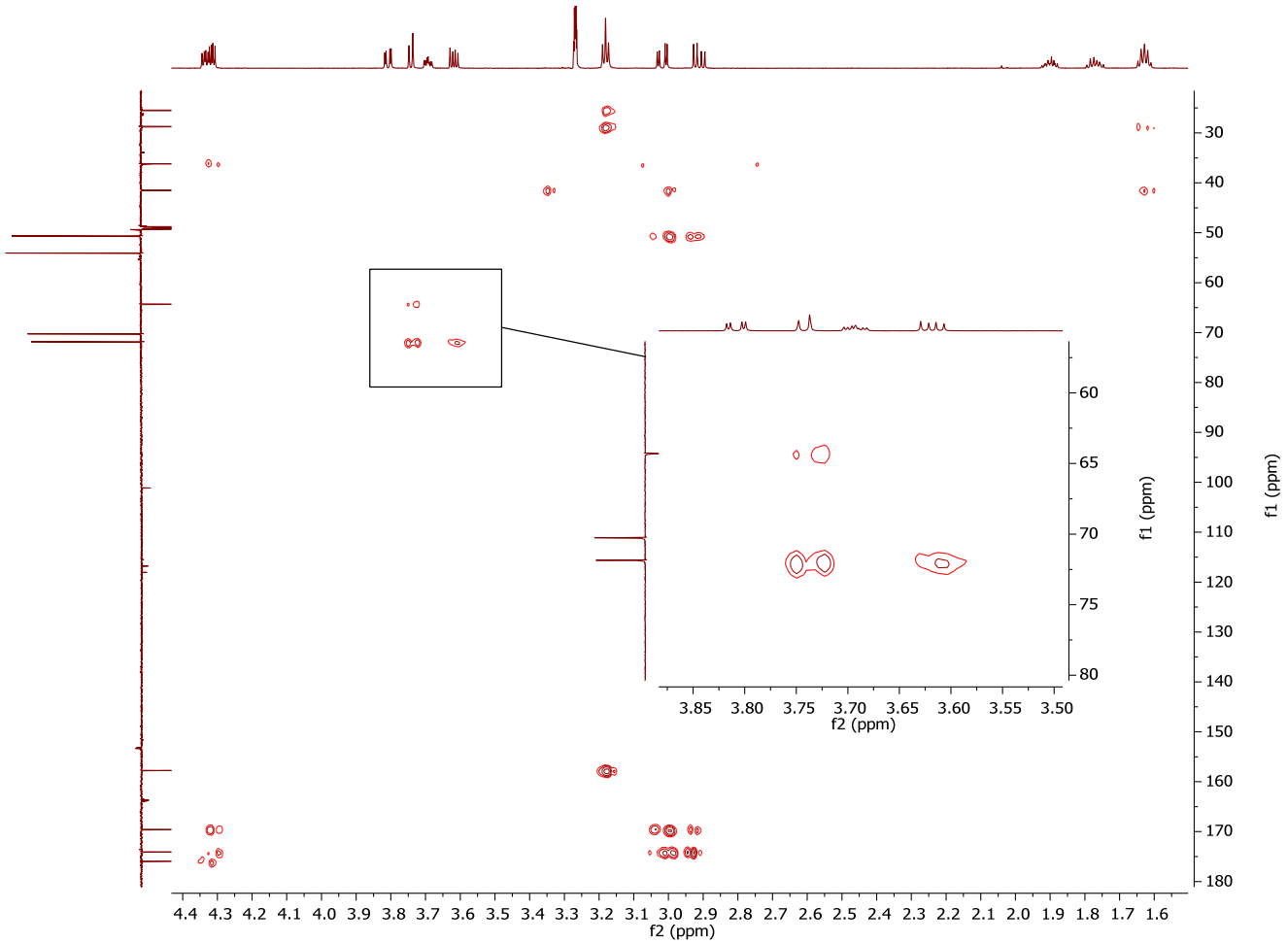


Figure S12. $^{1}H/^{13}C$-HMBC NMR spectrum of DR (25 mM, 135 µL D_2O, 40 µL CD_3OD, 3 mm tube) with expansion of the mannitol impurity at 400/100 MHz (298K).

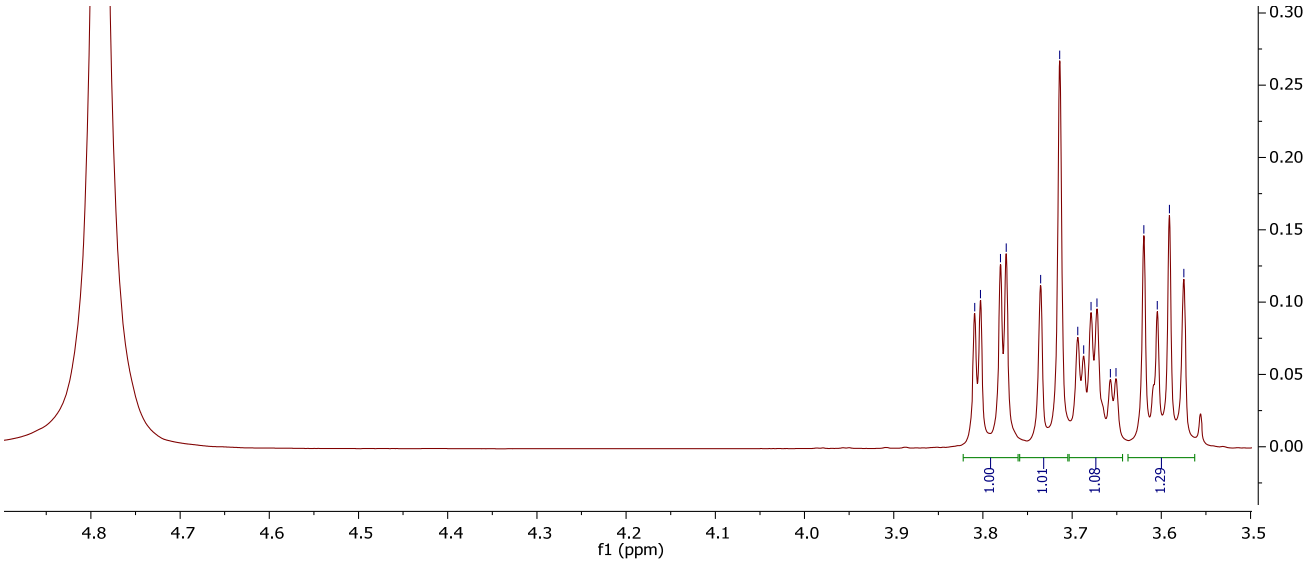


Figure S13. ^{1}H NMR spectrum of mannitol (5.66 mg, 135 µL D_2O, 40 µL CD_3OD, 3 mm tube) at 400 MHz (298K).

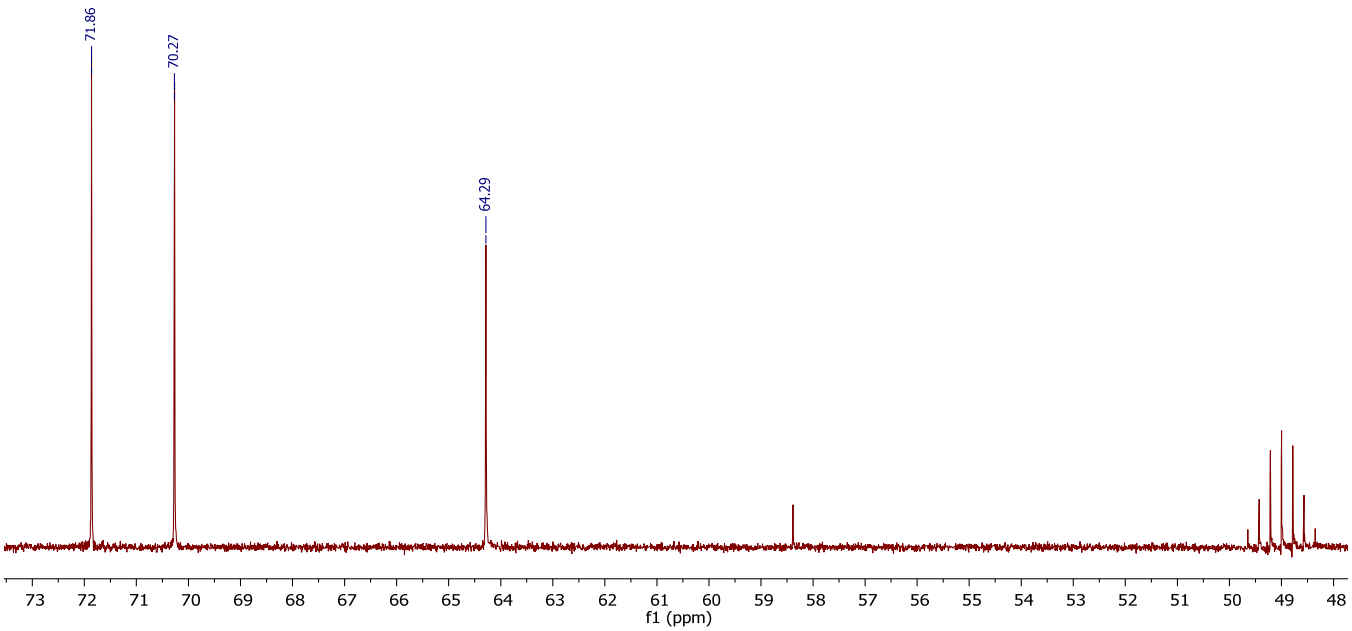


Figure S14. ^{13}C NMR spectrum of mannitol (5.66 mg, 135 µL D_2O, 40 µL CD_3OD, 3 mm tube) at 100 MHz (298K).

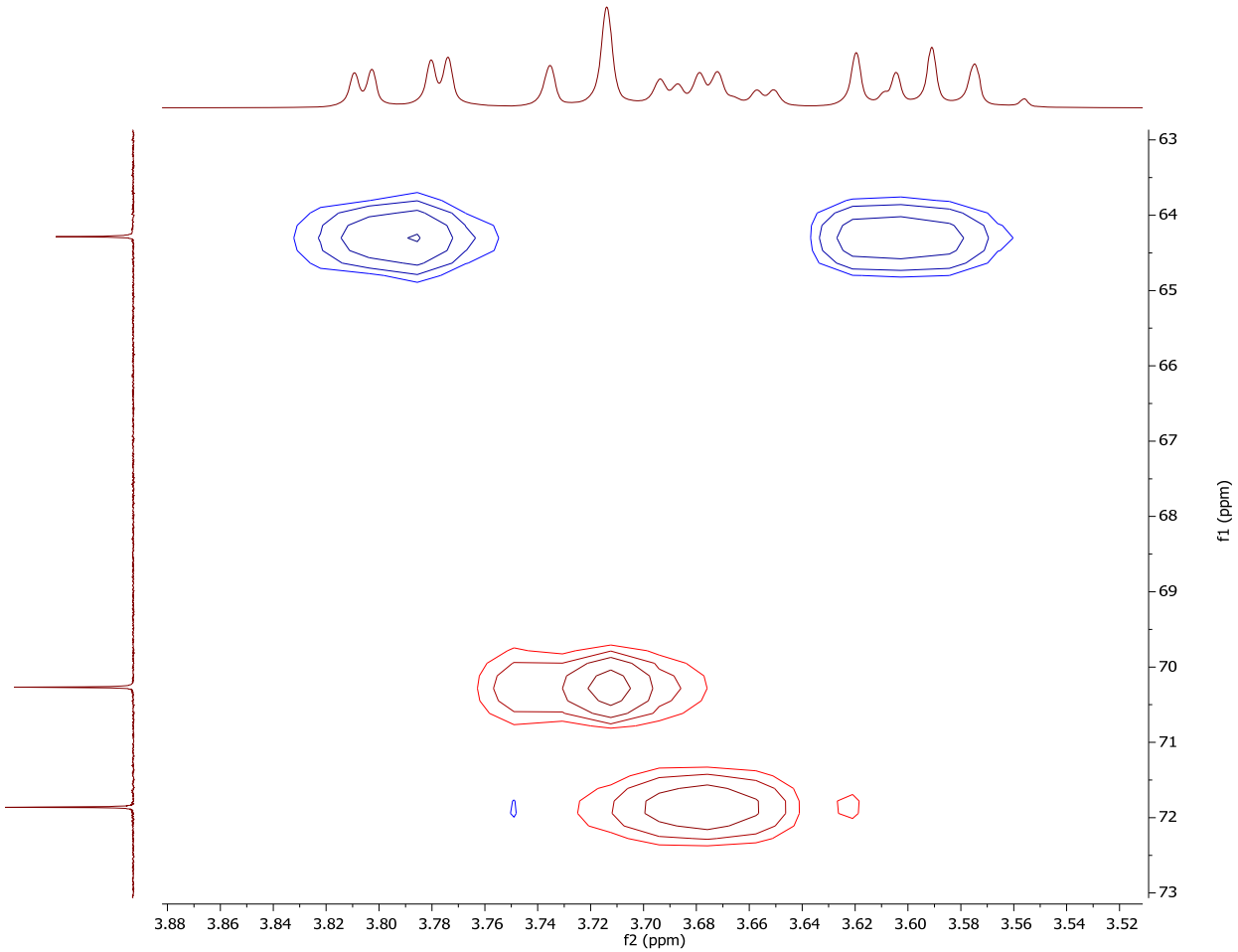


Figure S15. $^{1}H/^{13}C$ HSQC spectrum of mannitol (5.66 mg, 135 µL D_2O, 40 µL CD_3OD, 3 mm tube) at 400/100 MHz (298K).

60 MHz NMR Data

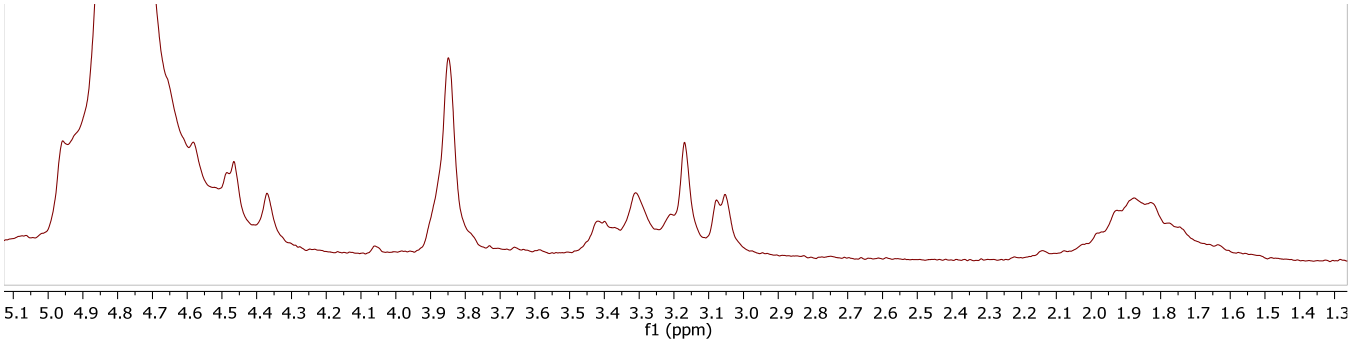


Figure S16. ^{1}H NMR spectra of DR (4 mg, 600 µL, D_2O, 5 mm tube) at 60 MHz (305K).

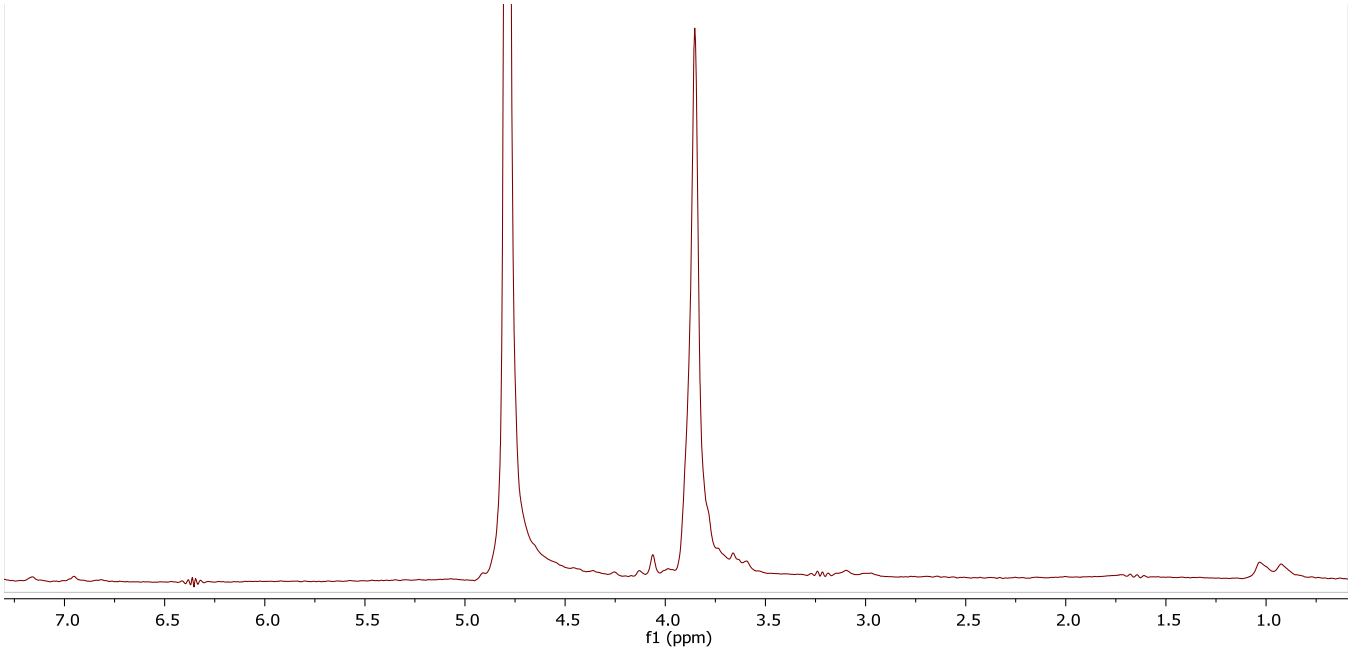


Figure S17. ^{1}H NMR spectra of DRVYI (4 mg, 600 µL, D_2O, 5 mm tube) at 60 MHz (305K).

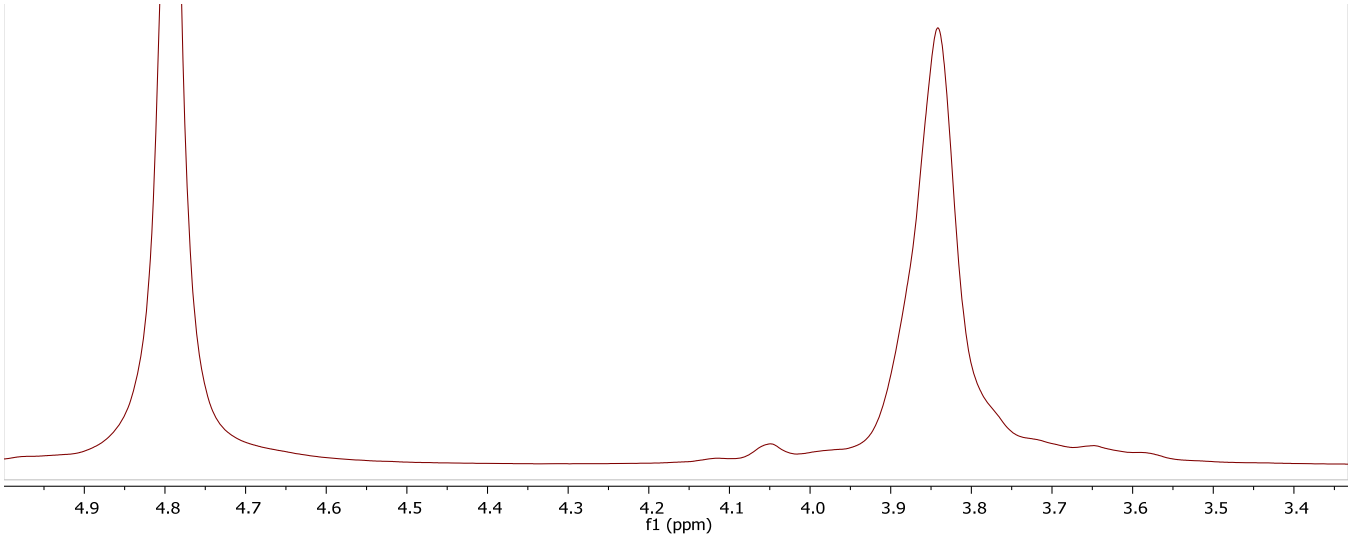


Figure S18. ^{1}H NMR spectra of mannitol (58 mg, 600 µL, D_2O, 5 mm tube) at 60 MHz (305K).

Comparisons

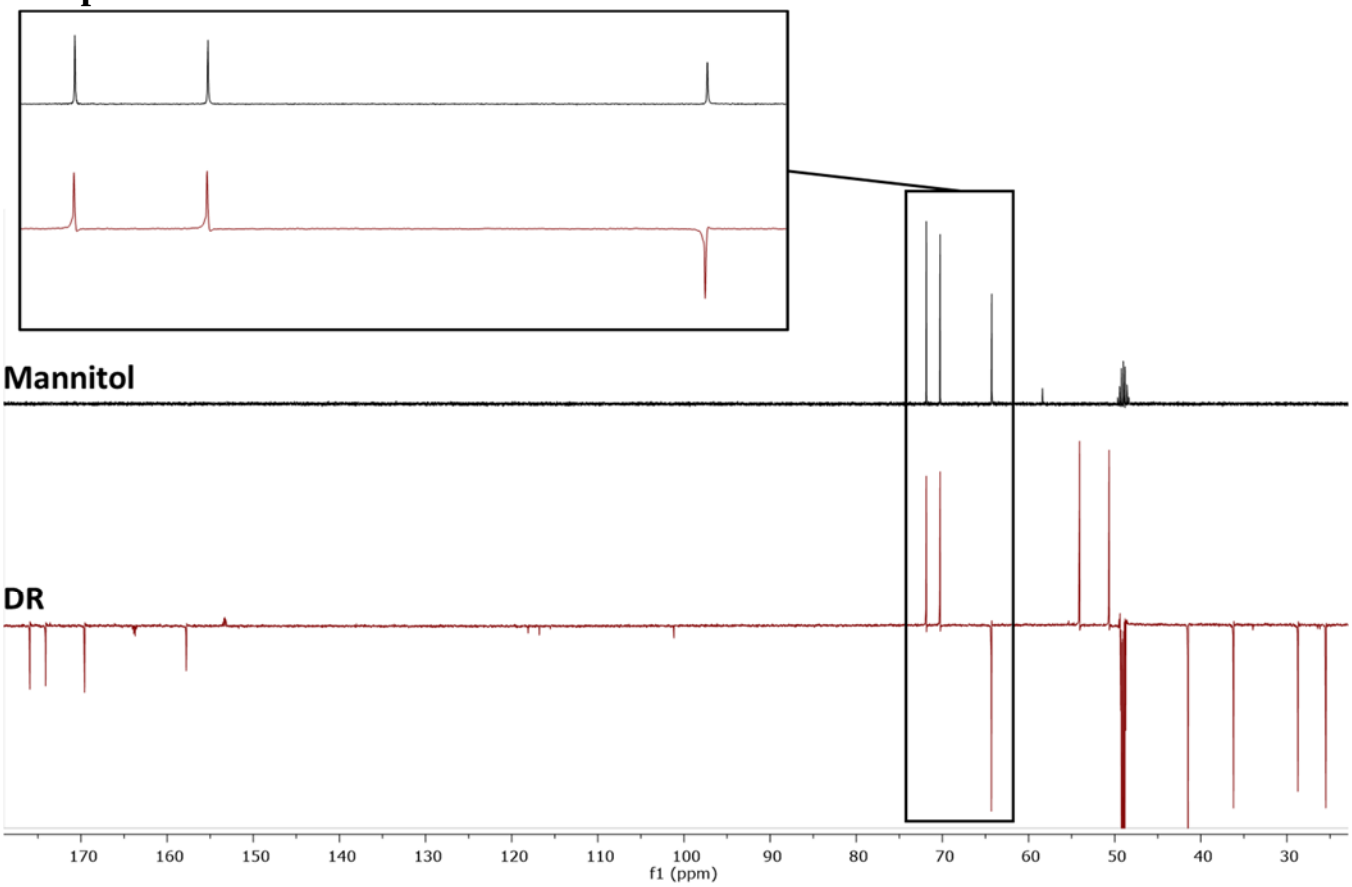


Figure S19. ^{13}C NMR spectrum of mannitol at 100 MHz (top, black), ^{13}C-DEPT-Q-135 NMR spectrum of DR at 225 MHz (bottom, red).

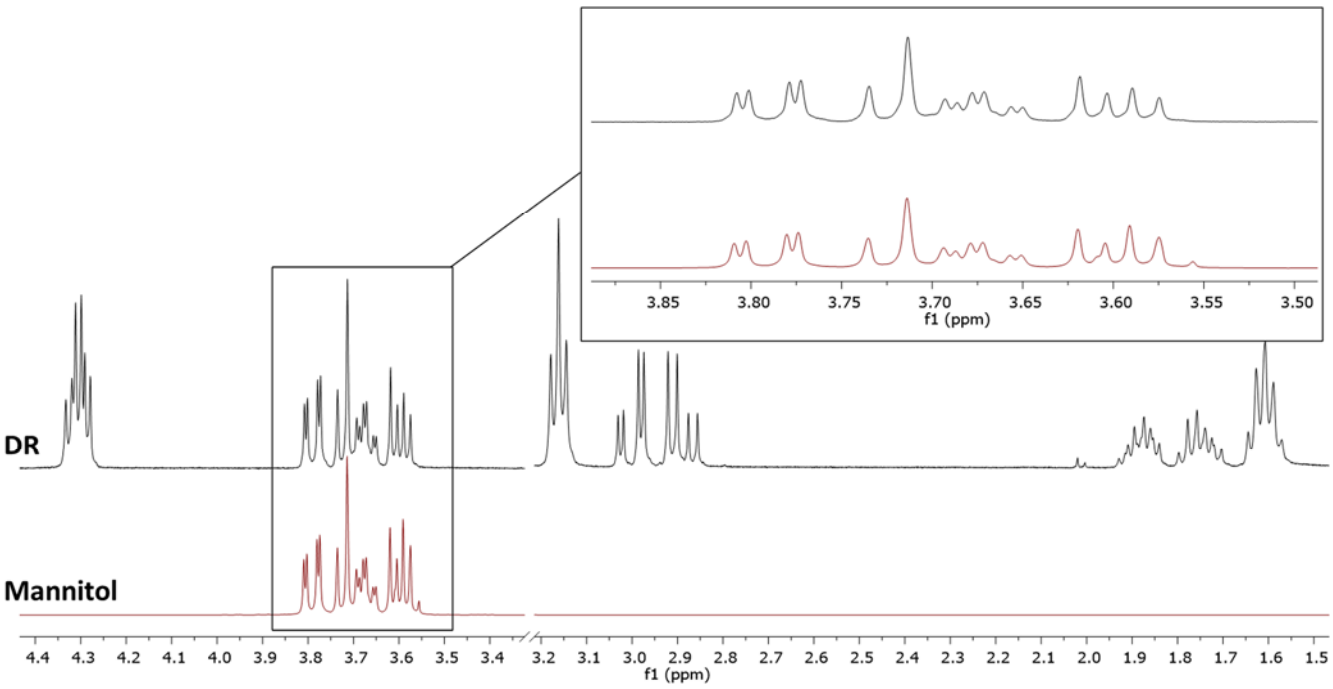


Figure S20. ^{1}H NMR spectrum of DR (top, black) and mannitol (bottom, red) at 400 MHz.

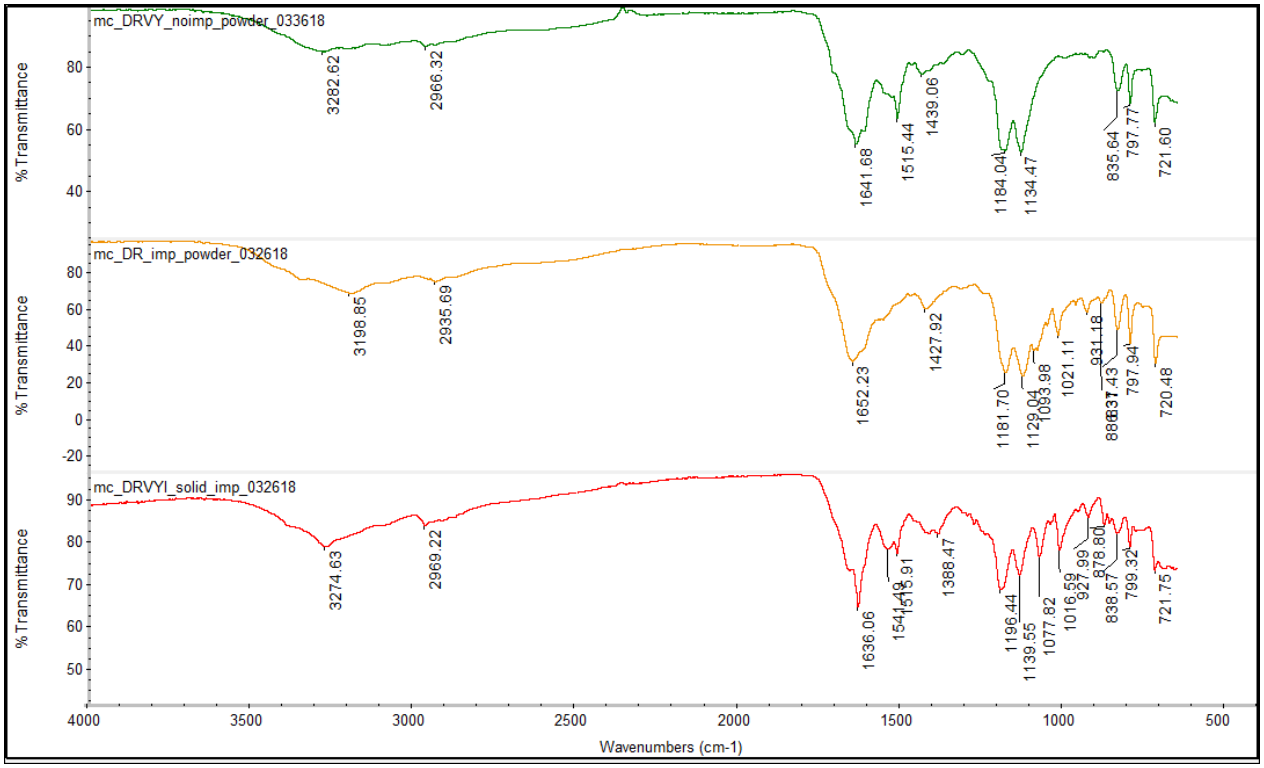


Figure S21. FT-IR chromatogram of DRVY (green) without the impurity versus DR (orange) and DRVYI (red) which both contain the impurity.

HiFSA Analysis

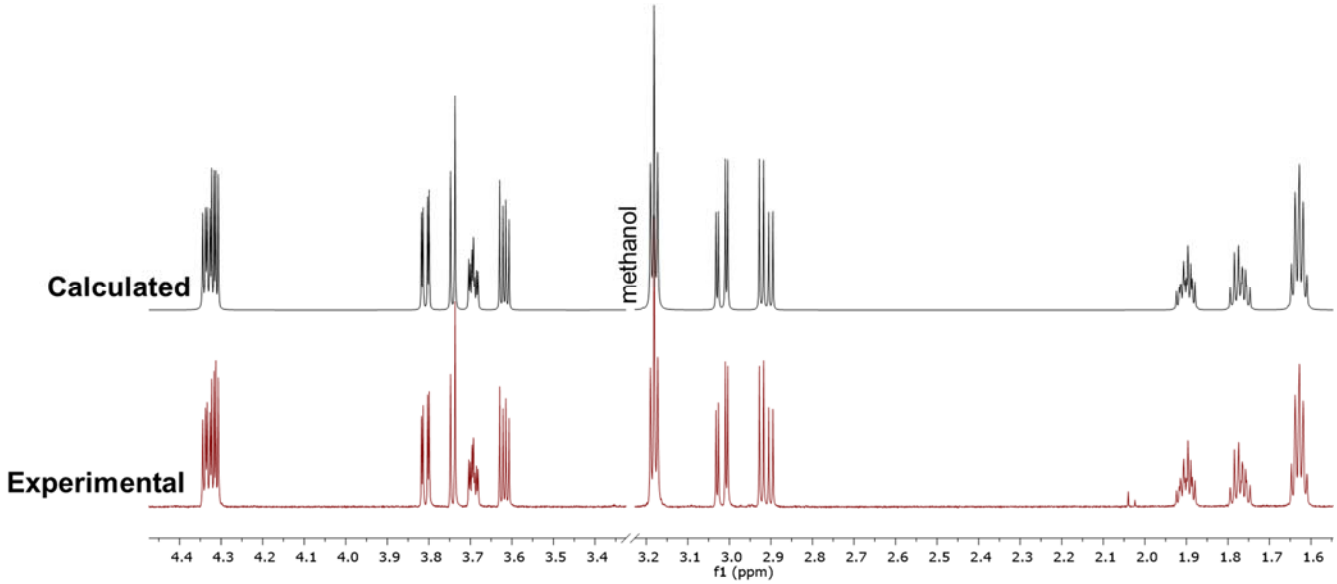


Figure S22. HiFSA calculated ^{1}H NMR spectrum of DR (black, top) versus experimental (red, bottom) at 800 MHz.

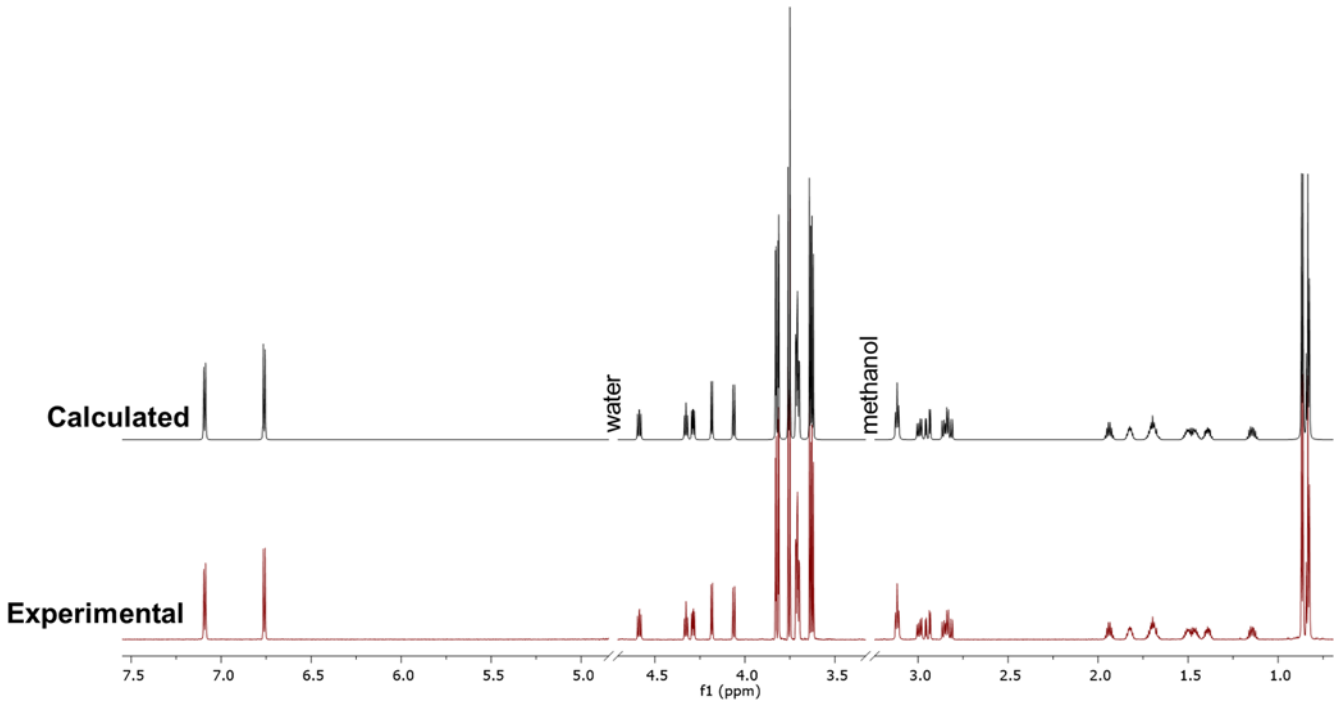


Figure S23. HiFSA calculated ^{1}H NMR spectrum of DRVYI (black, top) versus experimental (red, bottom) at 800 MHz.

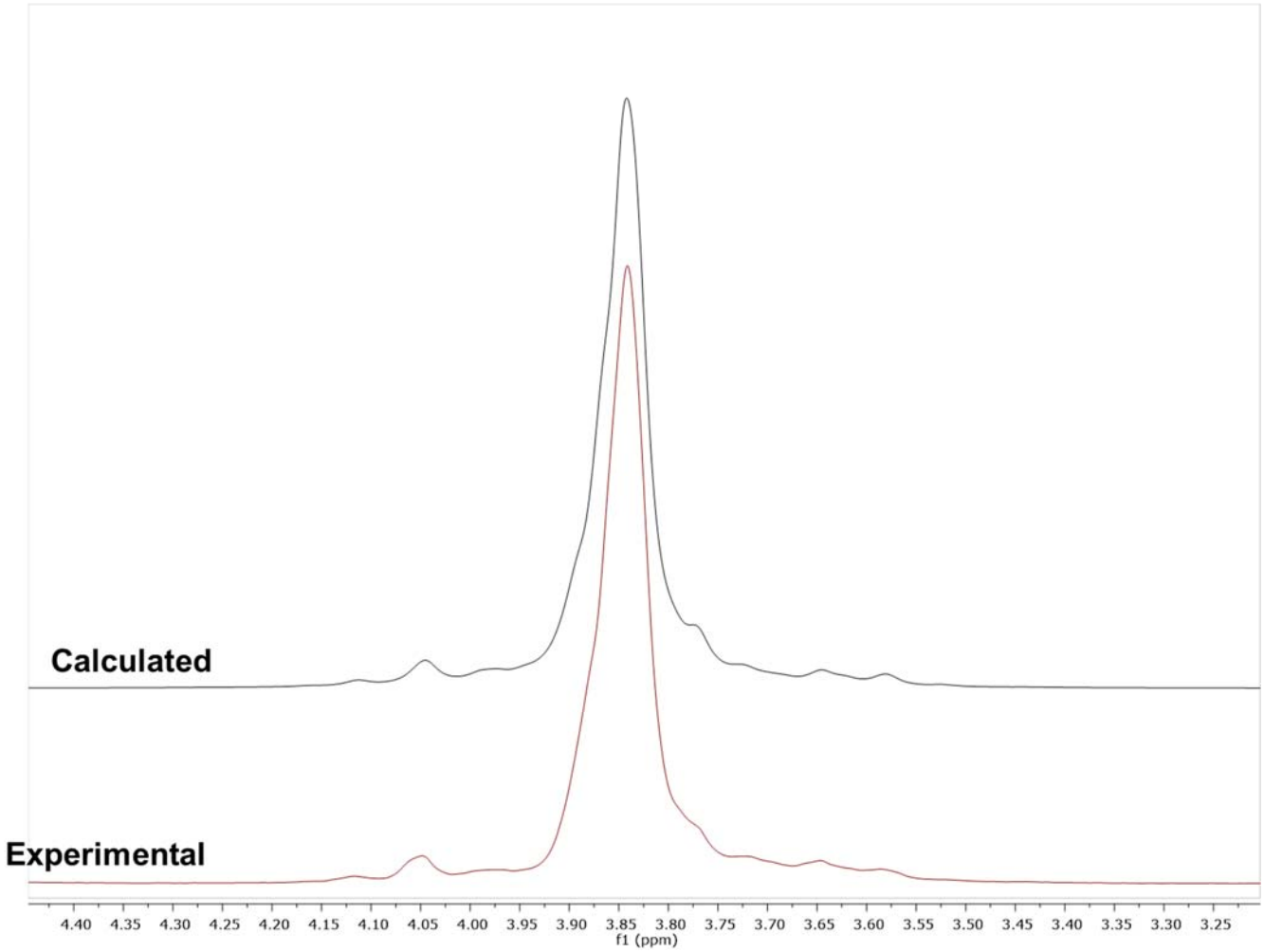


Figure S24. HiFSA calculated ^{1}H NMR spectrum of mannitol (black, top) versus experimental (red, bottom) at 60 MHz.

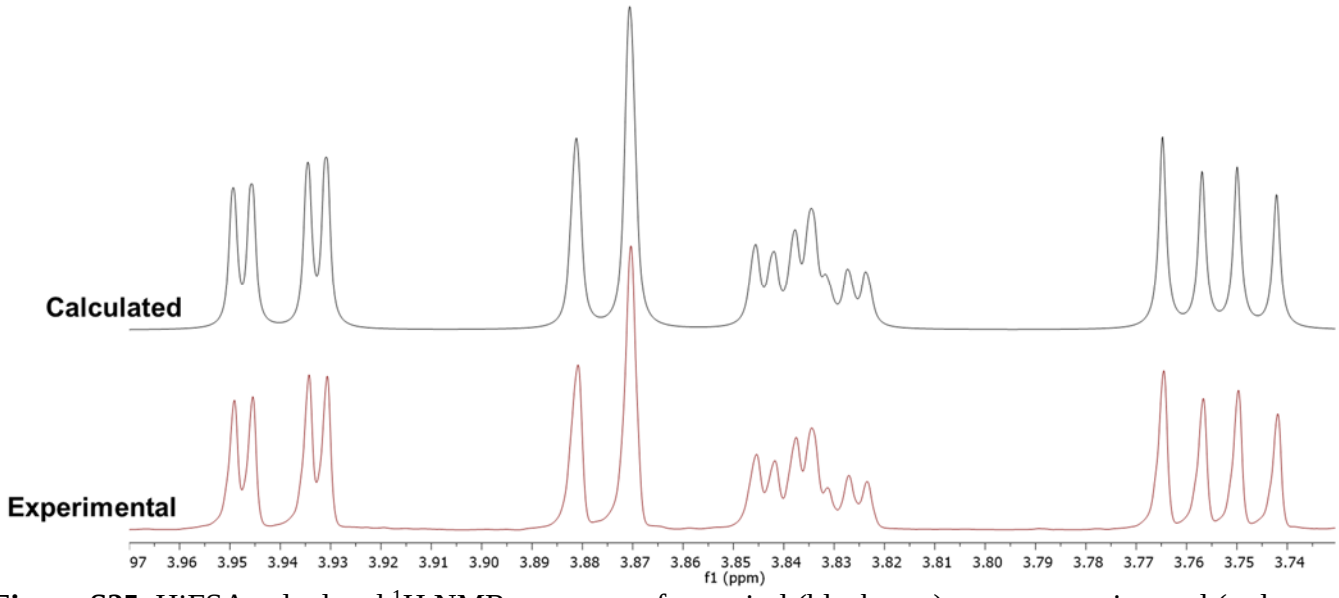
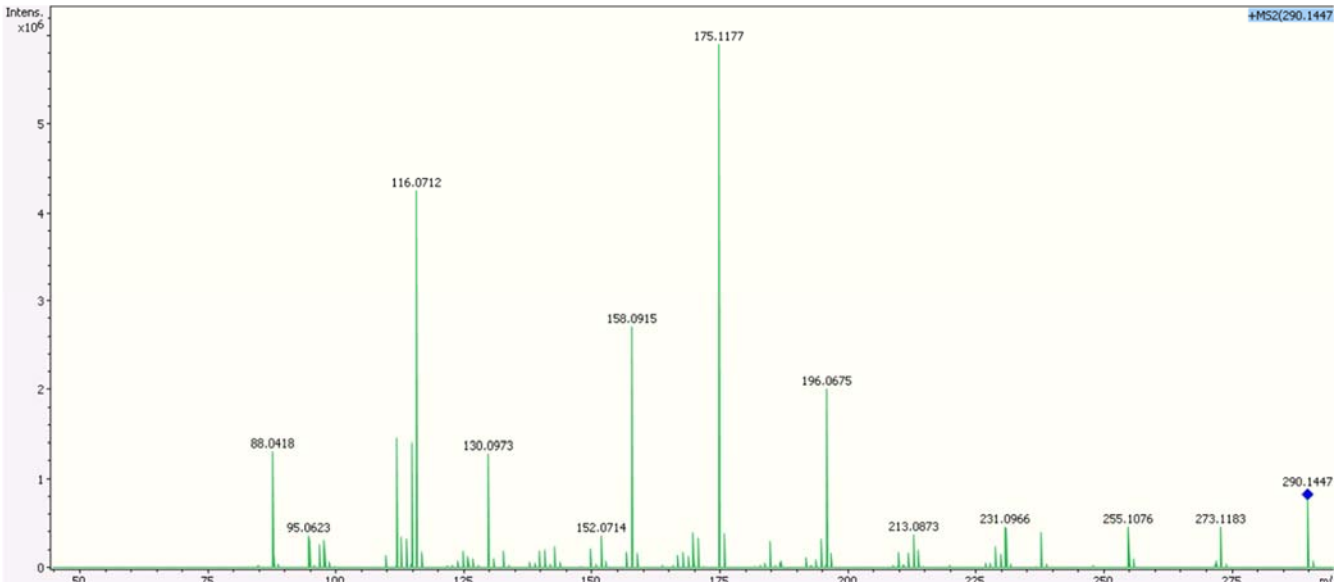


Figure S25. HiFSA calculated ^{1}H NMR spectrum of mannitol (black, top) versus experimental (red, bottom) at 800 MHz.

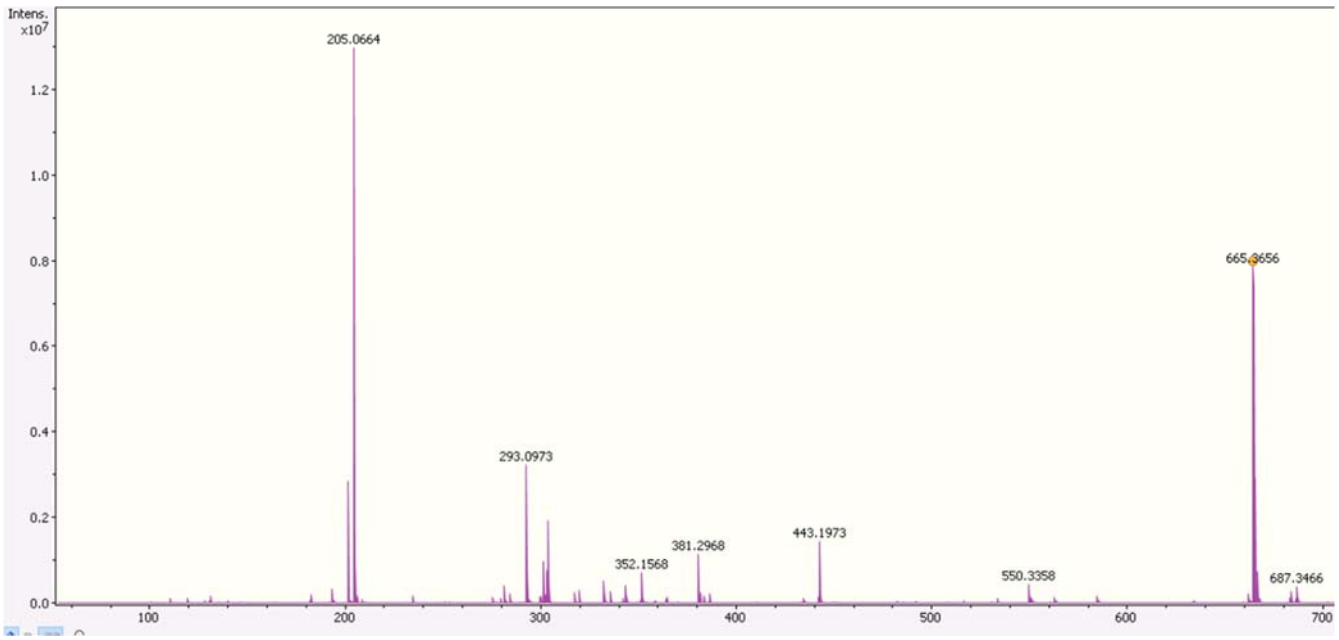
Figure S26. MS analysis of DR.



$C_{10}H_{20}N_5O_5$ Calculated mass 290.1459 [M+H+] Observed mass 290.1451 (err 2.8 ppm)
Visible ions in fragmentation @ 30 eV
a1: 88.0418 (for 88.03935)
a2: 244.1405 (for 244.14046) (minor)
b1: 116.0712 (for 116.03426)
b2: 272.1354 (for 272.13537) (minor)
c1: 133.0616 (for 133.06081) (minor)
x2: 201.0972 (for 201.09826) (minor)
y1: 290.1447 (for 290.14594) (full peptide)
y2: 175.1177 (for 175.11900)
z1: 273.1183 (for 273.12049)
z2: 158.0915 (for 158.09354)

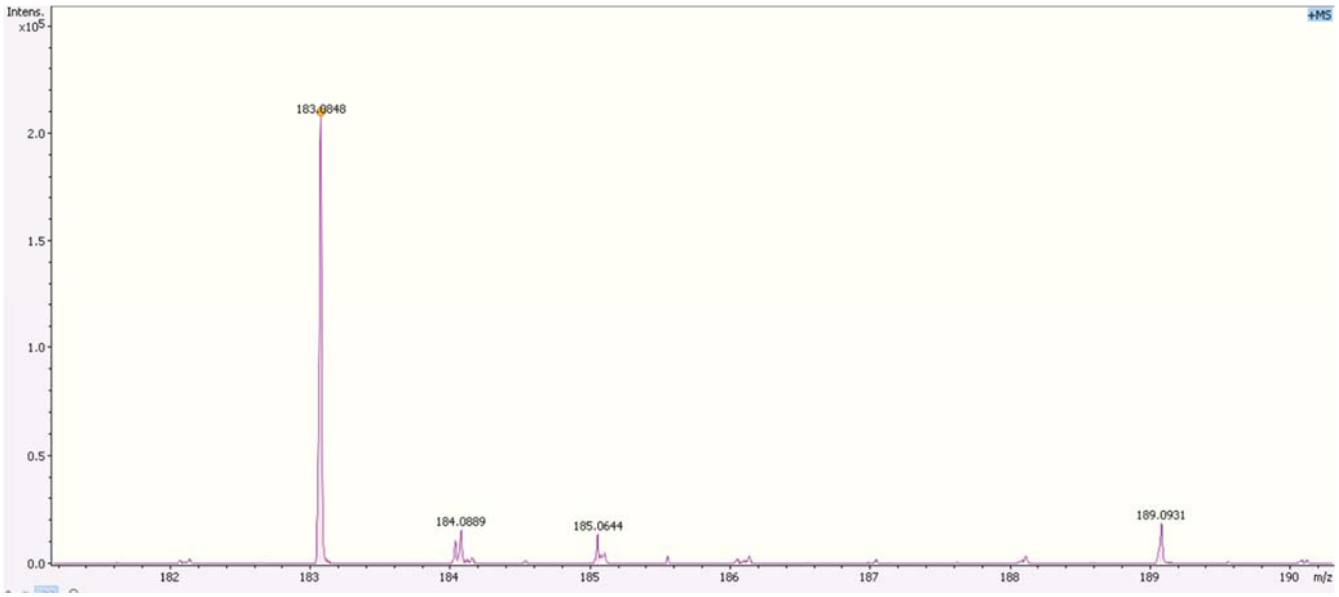
Figure S27. MS analysis of DRVYI with mannitol contaminant.

MS Spectrum



$C_{30}H_{48}N_8O_9$ Calculated mass 665.3617 [M+H+] Observed mass 665.3617 (err 5.8 ppm) [M+H+]

Mannitol region



Mannitol observed:
[M+H] 183.0848 observed for 183.0863 calculated (err 8.0 ppm)
[M+Na] 205.0664 observed for 205.0683 calculated (err 9.0 ppm)

MS^2 (40 eV)
a1 non visible (for 88.03935)
a2 non visible (for 244.14046)
a3 343.1742 (for 343.20887)
a4 non visible (for 506.27220)
a5 non visible (for 619.35627)
b1 non visible (for 116.03426)
b2 272.1182 (for 272.13537)
b3 371.1820 (for 371.20379)
b4 534.2319 (for 534.26712)
b5 647.3218 (for 647.35118)
c1 non visible (for 133.06081)
c2 non visble (for 289.16192)
c3 non visible (for 388.23034)
c4 551.2947 (for 551.29367) (uncertain, in the noise)
x2 non visible (for 576.31407)
x3 non visible (for 420.21296)
x4 non visible (for 321.14454)
x5 non visible (for 158.08121)
y1 665.3303 (for 665.36174) (noisy)
y2 550.3138 (for 550.33480)
y3 394.2109 (for 394.23369)
y4 295.1461 (for 295.16528)
y5 non visible (for 132.10195)
z1 648.2798 (for 648.33629)
z2 unconclusive (for 533.30935)
z3 non visible (for 377.20824)
z4 278.1159 unconclusive (for 278.13983)
z5 non visible (for 114.07650)