Electronic Supplementary Information for

First Synthesis, Full Characterization, and Evidence for the Presence in Human Biological Fluids, of Hydroxycinnamic acid Sulphate and Glucuronide Conjugates, as a Result of Coffee Consumption.

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Part B: Purity control data

Abstract - A systematic investigation of the human metabolism of hydroxycinnamic acid conjugates was carried out. A set of 24 potential human metabolites of coffee polyphenols has been chemically prepared, and used as analytical standards for unequivocal identifications. These included glucuronide conjugates and sulphate esters of caffeic, ferulic, isoferulic, *m*-coumaric and *p*-coumaric acids as well as their dihydro derivatives. A particular focus has been made on caffeic and 3,4-dihydroxyphenylpropionic acid derivatives, especially the sulphate conjugates, for which regioselective preparation was particularly challenging, and have so far never been identified as human metabolites. Ten, out of the 24 synthesized conjugates have been identified in human plasma and/or urine after coffee consumption. A number of these conjugates were synthesized, characterized and detected as hydroxycinnamic acid metabolites for the first time. This was the case of dihydroisoferulic acid 3'-*O*-glucuronide, caffeic acid 3'-sulphate, as well as the sulphate and glucuronide derivatives of 3,4-dihydroxyphenylpropionic acid.

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1. Purity control data of analytical standards 1-24

Table S1: Purity control of analytical standards **1-24**.

No	Compound	LC-HRMS data	HPLC % purity data (nm)	Quantification of sulphate ester content
1a	(*E*)-*p*-Coumaric acid 4'-*O*-β-D-glucuronide	$[M-H]^-$ Calc for $C_{15}H_{15}O_9$: 339.0716; found: 339.0721.	99 (280)	-
2a	(*E*)-*m*-Coumaric acid 3'-*O*-β-D-glucuronide	$[M-H]^-$ calc for $C_{15}H_{15}O_9$: 339.0716; found: 339.0719.	97 (280)	-
3a	(*E*)-Ferulic acid 4'-*O*-β-D-glucuronide	$[M-H]^-$ calc for $C_{16}H_{17}O_{10}$: 369.0821; found: 369.0834.	99 (280)	-
4a + 4b	(*E*)- and (*Z*)-Isoferulic acid 3'-*O*-β-D-glucuronides	$[M-H]^-$ calc for $C_{16}H_{17}O_{10}$: 369.0821; found: 369.0825.	99 (280)	-
13	Dihydro-*p*-coumaric acid 4'-*O*-β-D-glucuronide	$[M-H]^-$ calc for $C_{15}H_{17}O_9$: 341.0872; found: 341.0882.	99 (280)	-
14	Dihydro-*m*-coumaric acid 3'-*O*-β-D-glucuronide	$[M-H]^-$ calc for $C_{15}H_{17}O_9$: 341.0872; found: 341.0878.	99 (210)	-
15	Dihydroferulic acid 4'-*O*-β-D-glucuronide	$[2M-H]^-$ calc for $C_{32}H_{39}O_{20}$: 743.2040; found: 743.2019.	99 (280)	-
16	Dihydroisoferulic acid 3'-*O*-β-D-glucuronide	$[M-H]^-$ calc for $C_{16}H_{19}O_{10}$: 371.0978; found: 371.0974.	98 (280)	-
5a + 5b	(*E*)- and (*Z*)-Caffeic acid 3'-*O*-β-D-glucuronides	$[M-H]^-$ calc for $C_{15}H_{15}O_{10}$: 355.0665; found: 355.0653.	94 (280)	-
6a + 6b	(*E*)- and (*Z*)-Caffeic acid 4'-*O*-β-D-glucuronides	$[M-H]^-$ calc for $C_{15}H_{15}O_{10}$: 355.0665; found: 355.0664.	99 (280)	-
17	Dihydrocaffeic acid 3'-*O*-β-D-glucuronide	$[M-H]^-$ calc for $C_{15}H_{17}O_{10}$: 357.0821; found: 357.0816.	99 (280)	-
18	Dihydrocaffeic acid 4'-*O*-β-D-glucuronide	$[M-H]^-$ calc for $C_{15}H_{17}O_{10}$: 357.0821; found: 357.0824.	98 (280)	-
7	(*E*)-*p*-Coumaric acid 4'-sulphate	$[M-H]^-$ calc for $C_9H_7O_6S$: 242.9963; found: 242.9994	98 (280)	2.81 μmol/mg
8	(*E*)-*m*-Coumaric acid 3'-sulphate	$[M-H]^-$ calc for $C_9H_7O_6S$: 242.9963; found: 243.0001	98 (280)	2.70 μmol/mg
9	(*E*)-Ferulic acid 4'-sulphate	$[M-H]^-$ calc for $C_{10}H_9O_7S$: 273.0069; found: 273.0099	98 (280)	1.93 μmol/mg
10	(*E*)-Isoferulic acid 3'-sulphate	$[M-H]^-$ calc for $C_{10}H_9O_7S$: 273.0069; found: 273.0112	98 (280)	1.57 μmol/mg
11a + 11b	(*E*)- and (*Z*)-Caffeic acid 3'-sulphates	$[M-H]^-$ calc for $C_9H_7O_7S$: 258.9912; found: 258.9960	97 (280)	2.30 μmol/mg
12a + 12b	(*E*)- and (*Z*)-Caffeic acid 4'-sulphates	$[M-H]^-$ calc for $C_9H_7O_7S$: 258.9912; found: 258.9955	95 (280)	1.91 μmol/mg
19	Dihydro-*p*-coumaric acid 4'-sulphate	$[M-H]^-$ calc for $C_9H_9O_6S$: 245.0119; found: 245.0164	98 (210)	2.22 μmol/mg
20	Dihydro-*m*-coumaric acid 3'-sulphate	$[M-H]^-$ calc for $C_9H_9O_6S$: 245.0119; found: 245.0161	98 (210)	2.10 μmol/mg
21	Dihydroferulic acid 4'-sulphate	$[M-H]^-$ calc for $C_{10}H_{11}O_7S$: 275.0225; found: 275.0270	97 (280)	1.57 μmol/mg
22	Dihydroisoferulic acid 3'-sulphate	$[M-H]^-$ calc for $C_{10}H_{11}O_7S$: 275.0225; found: 275.0270	98 (280)	2.15 μmol/mg
23	Dihydrocaffeic acid 3'-sulphate	$[M-H]^-$ calc for $C_9H_9O_7S$: 261.0069; found: 261.0115	98 (280)	1.75 μmol/mg
24	Dihydrocaffeic acid 4'-sulphate	$[M-H]^-$ calc for $C_9H_9O_7S$: 261.0069; found: 261.0108	98 (280)	1.55 μmol/mg

2. HPLC control of analytical standards 1-24 (Method A)

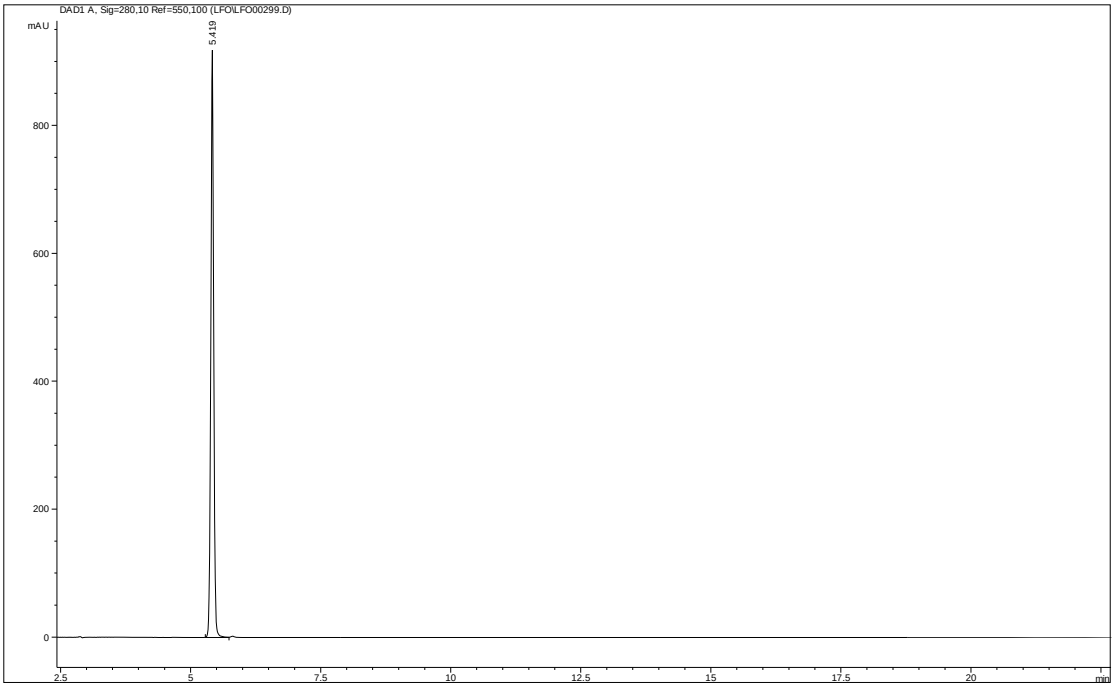

Figure S1: *(E)-p-Coumaric acid 4'-O- β -D-glucuronide* ***1a*** *(280 nm).*

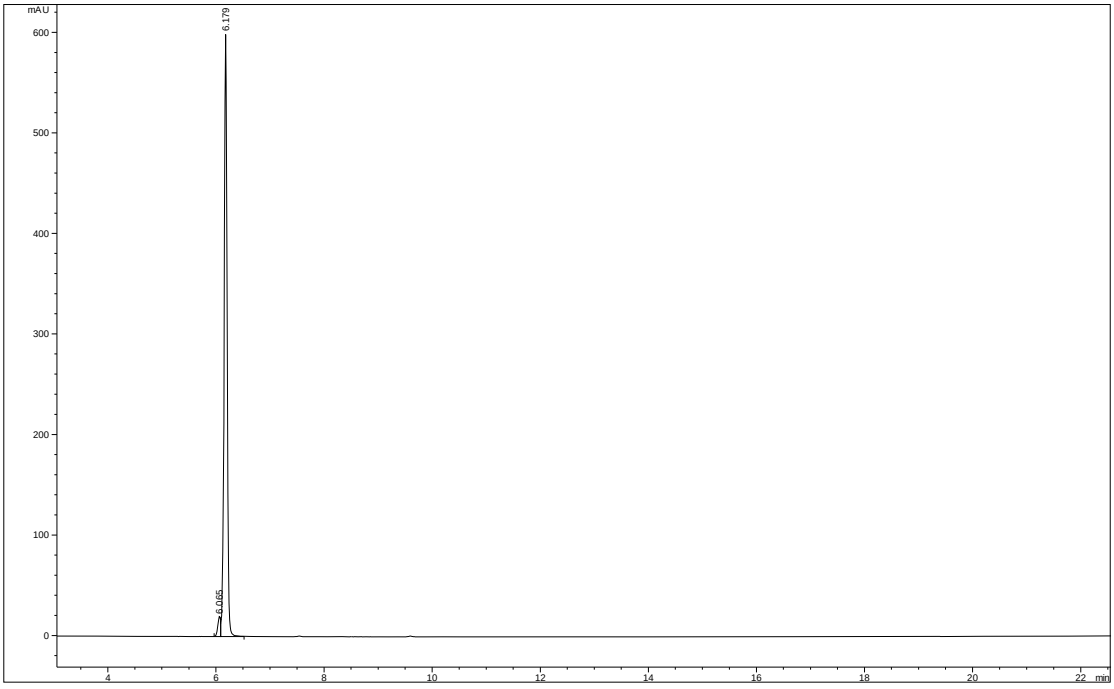

Figure S2: *(E)-m-Coumaric acid 3'-O- β -D-glucuronide* ***2a*** *(280 nm).*

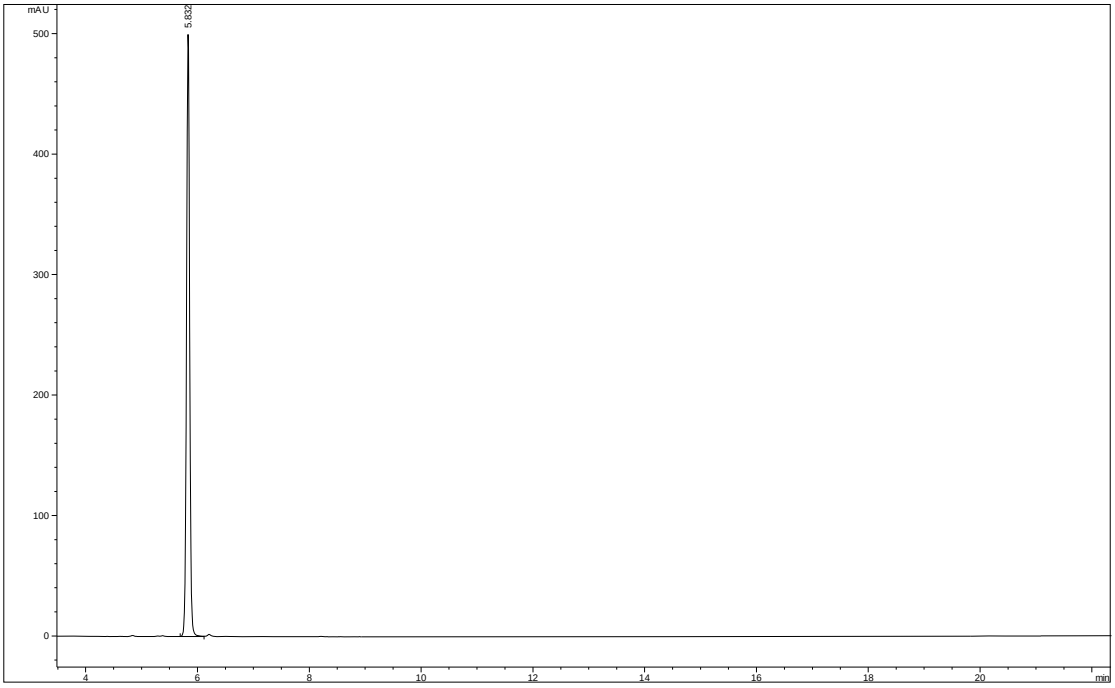

Figure S3: (E)-Ferulic acid 4'-O- β -D-glucuronide ***3a*** *(280 nm).*

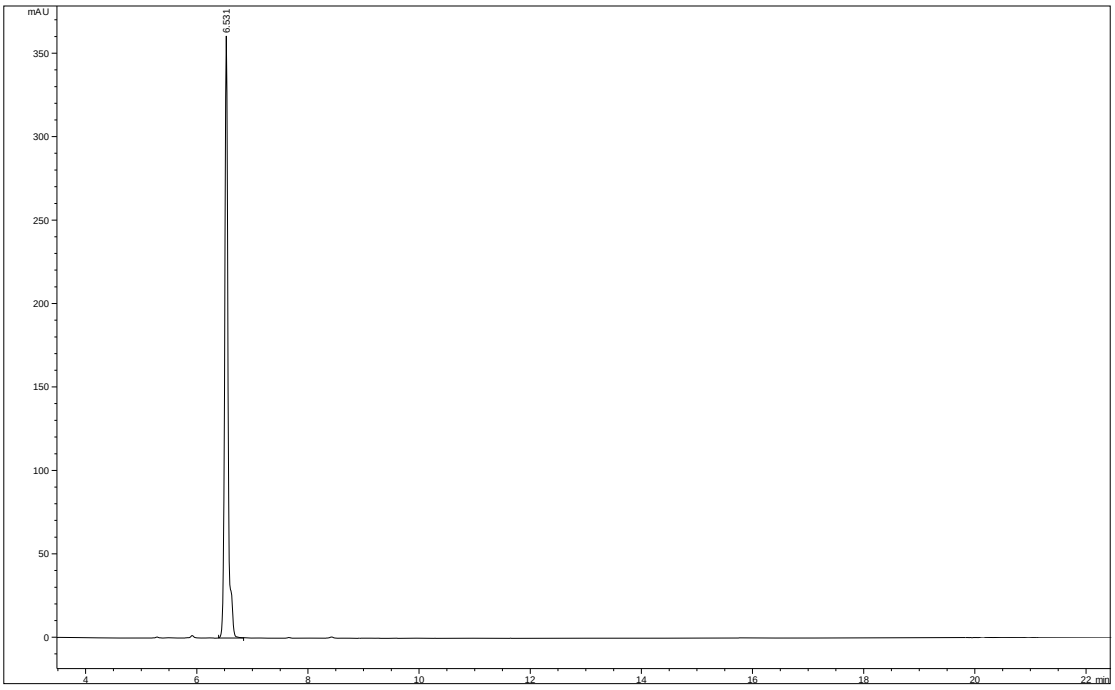

Figure S4: (E)- and (Z)-Isoferulic acid 3'-O-β-D-glucuronides ***4a*** *+* ***4b*** *(280 nm).*

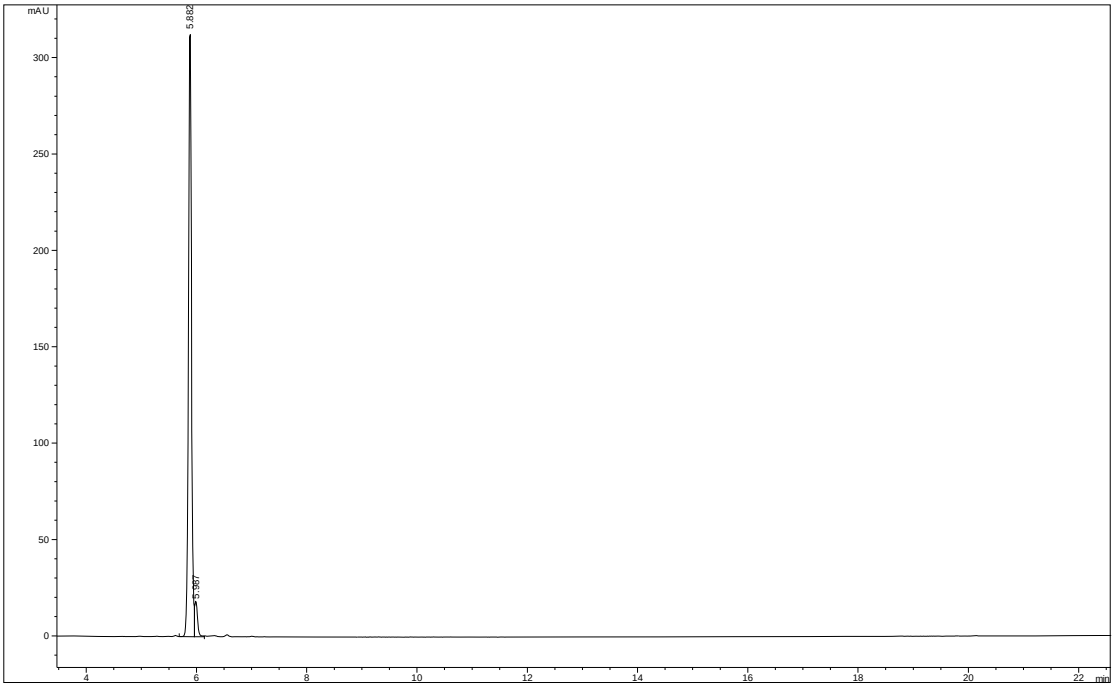

Figure S5: (E)- and (Z)-Caffeic acid 3'-O- β -D-glucuronides ***5a*** *+* ***5b*** *(280 nm).*

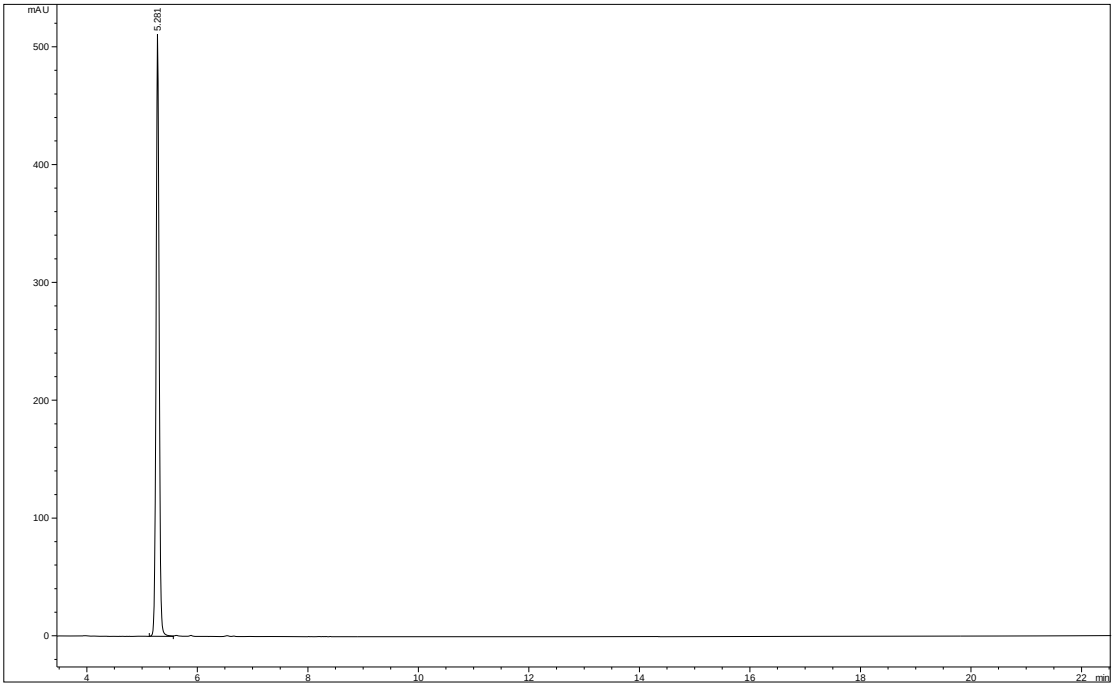

Figure S6: (E)- and (Z)-Caffeic acid 4'-O-β-D-glucuronides ***6a*** *+* ***6b*** *(280 nm).*

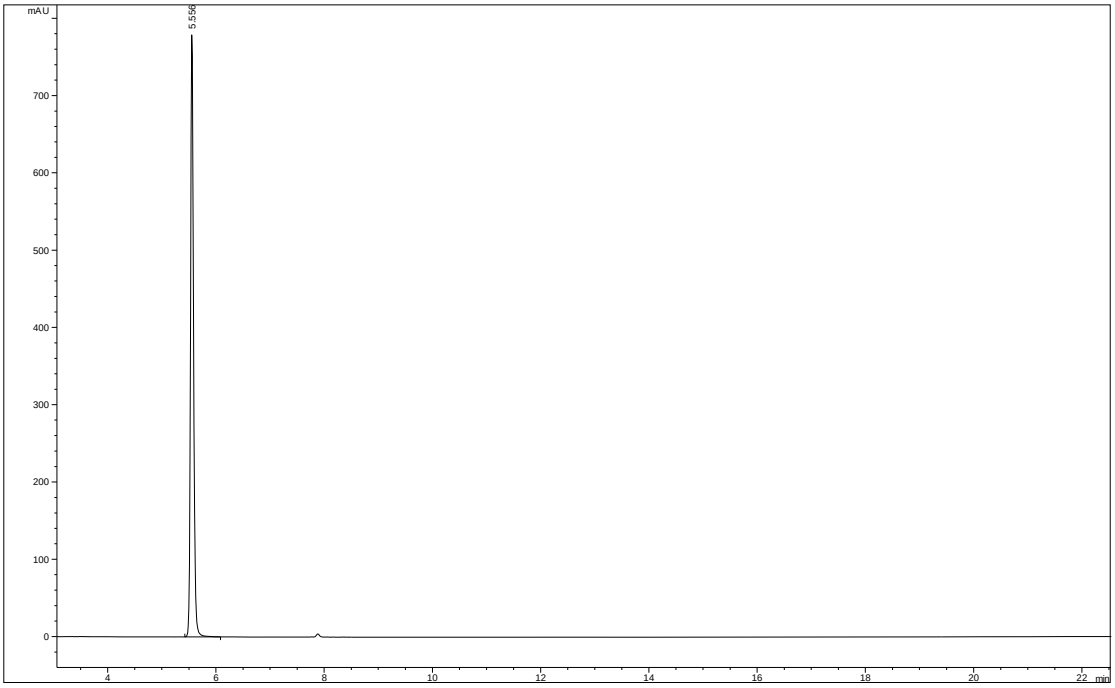

*Figure S7: (E)-p-Coumaric acid 4'-sulphate **7** (280 nm).*

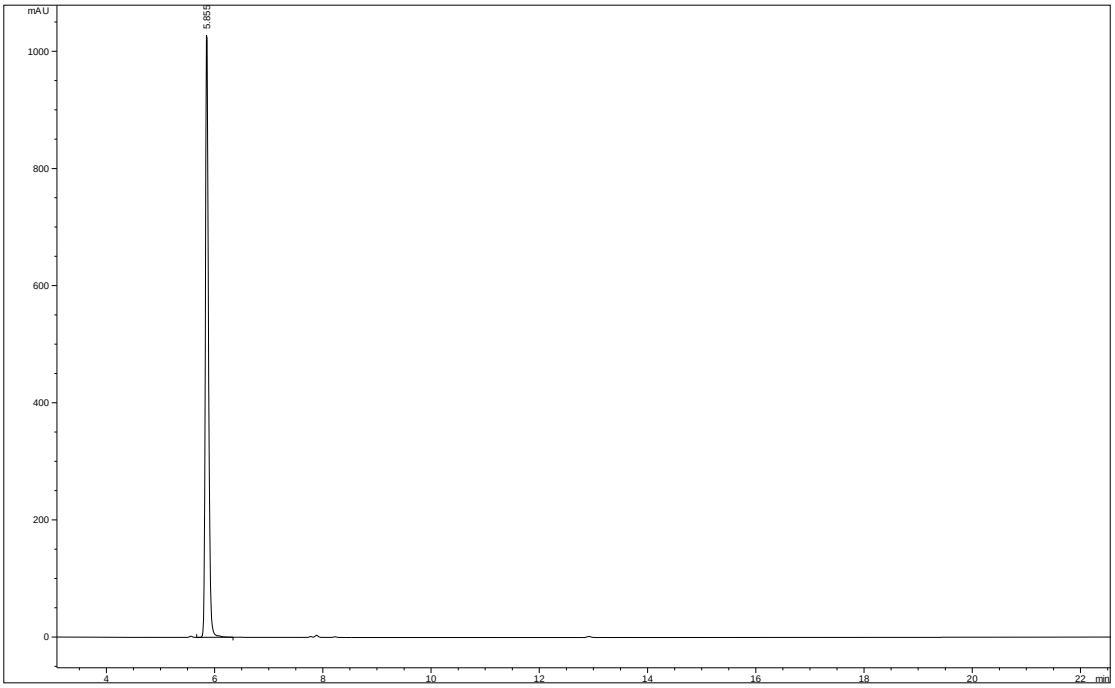

*Figure S8: (E)-m-Coumaric acid 3'-sulphate **8** (280 nm).*

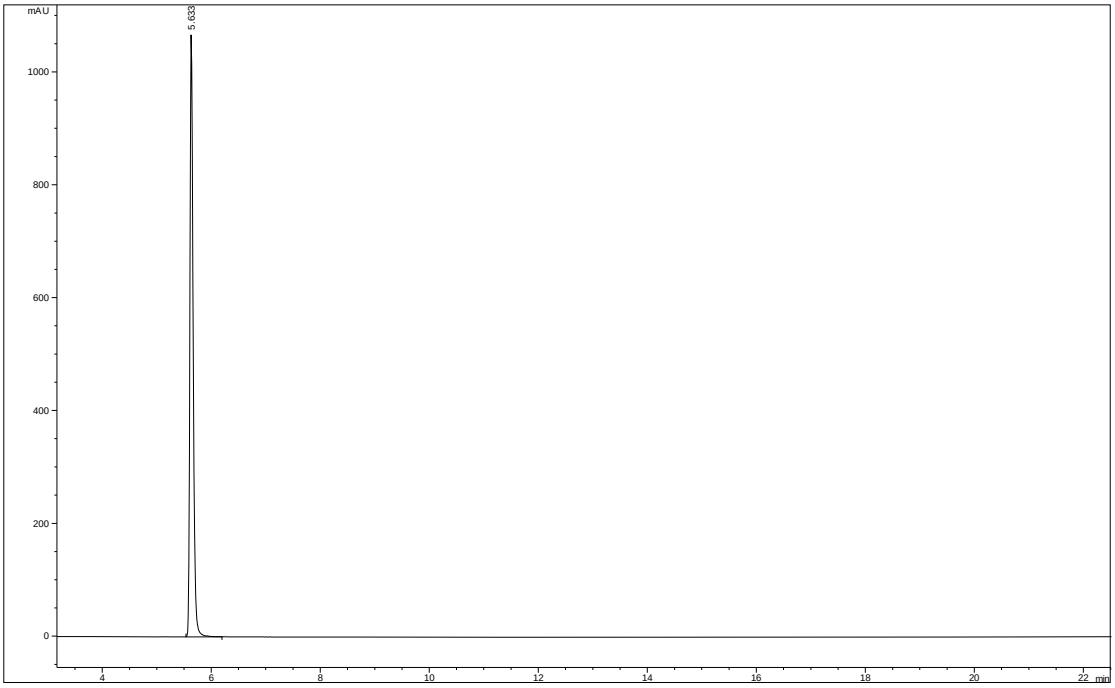

Figure S9: (E)-Ferulic acid 4'-sulphate ***9*** *(280 nm).*

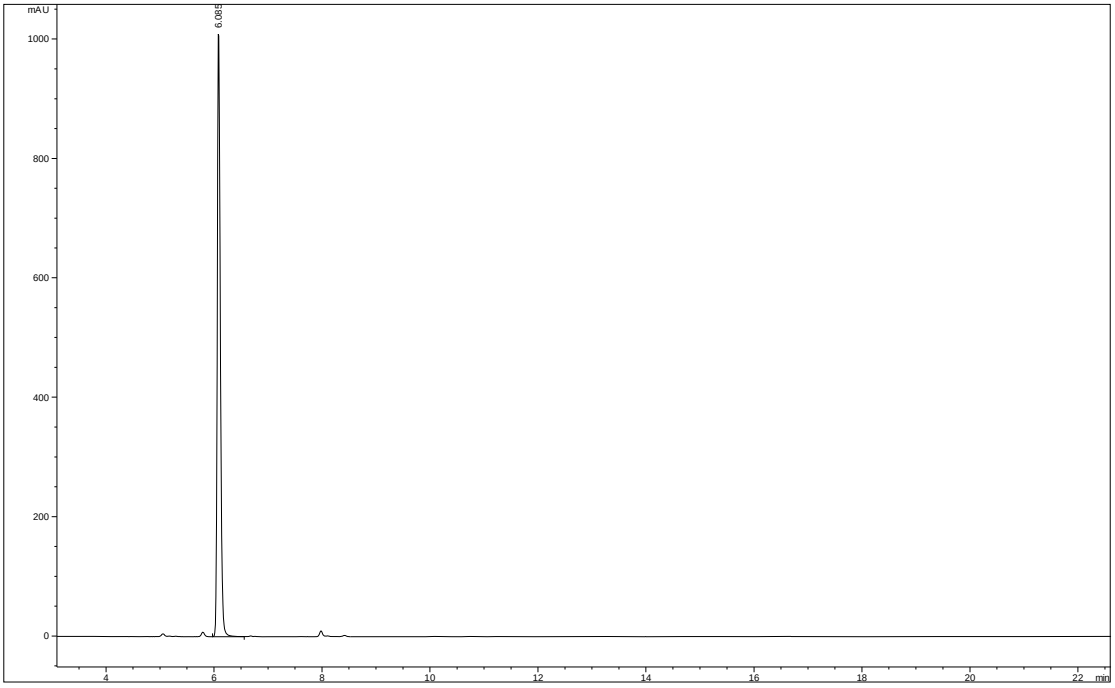

Figure S10: (E)-Isoferulic acid 3'-sulphate ***10*** *(280 nm).*

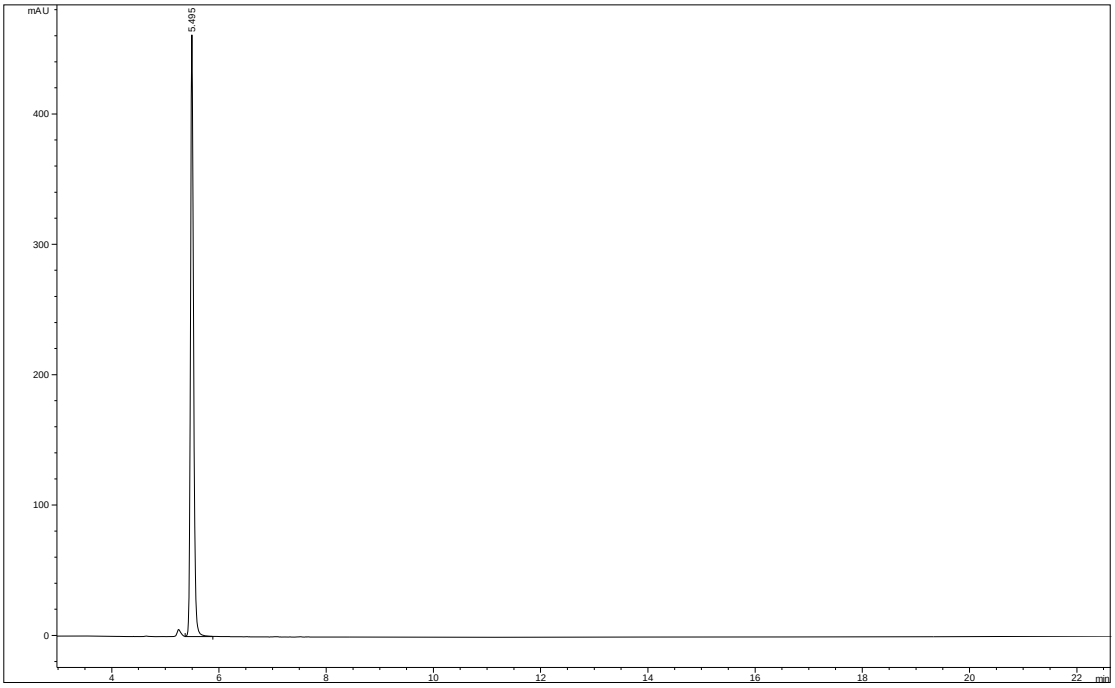

Figure S11: (E)- and (Z)-Caffeic acid 3'-sulphates ***11a*** *+* ***11b*** *(280 nm).*

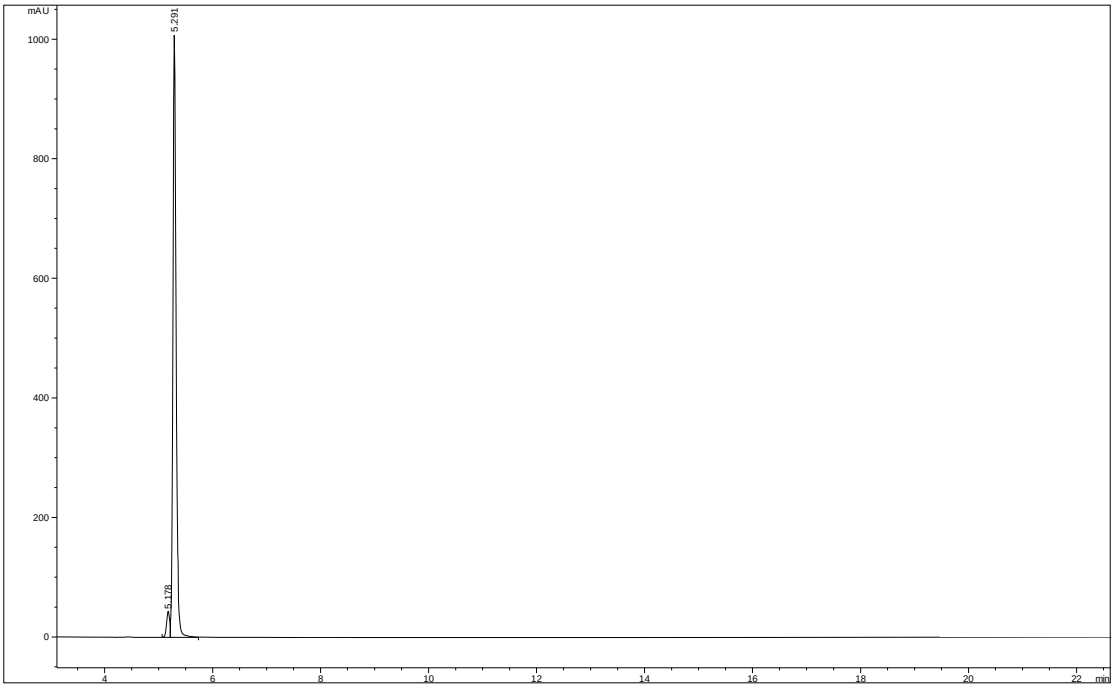

Figure S12: (E)- and (Z)-Caffeic acid 4'-sulphates ***12a*** *+* ***12b*** *(280 nm).*

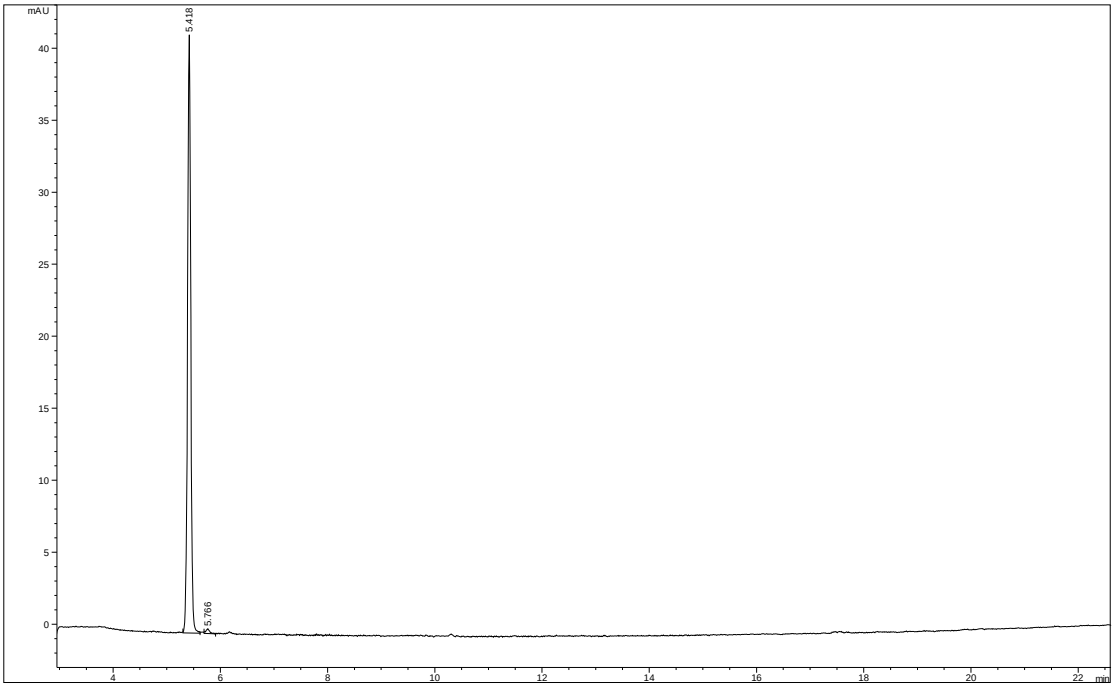

Figure S13: Dihydro-p-coumaric acid 4'-O- β -D-glucuronide ***13*** *(280 nm).*

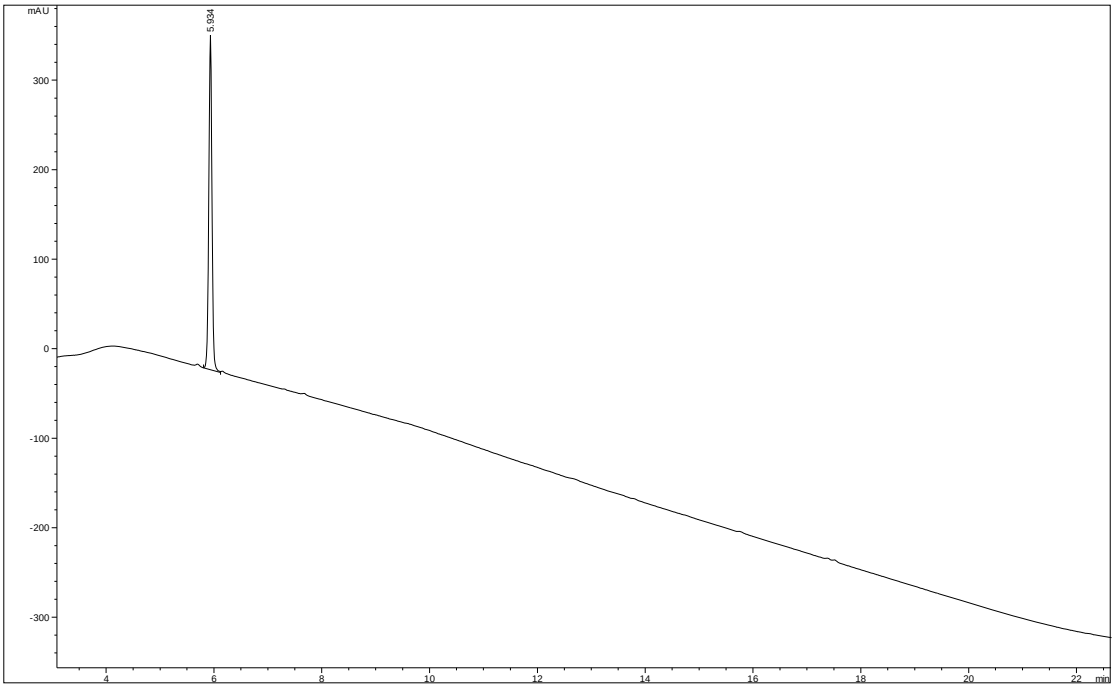

Figure S14: Dihydro-m-coumaric acid 3'-O- β -D-glucuronide ***14*** *(210 nm).*

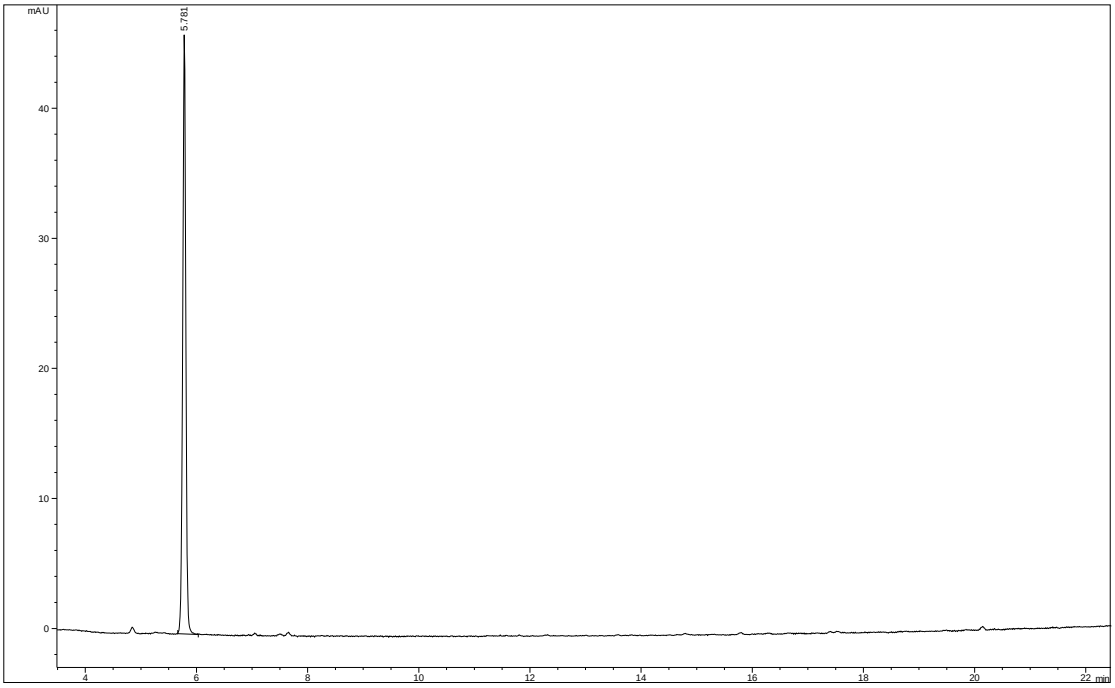

Figure S15: Dihydroferulic acid 4'-O- β -D-glucuronide ***15*** *(280 nm).*

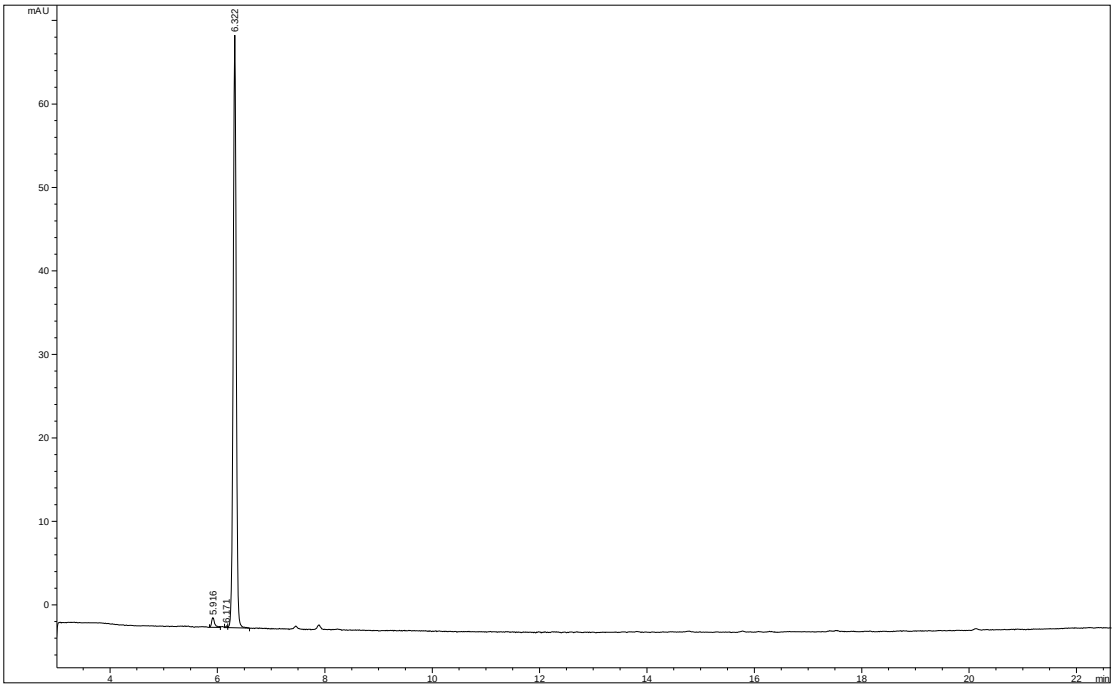

Figure S16: Dihydroisoferulic acid 3'-O- β -D-glucuronide ***16*** *(280 nm).*

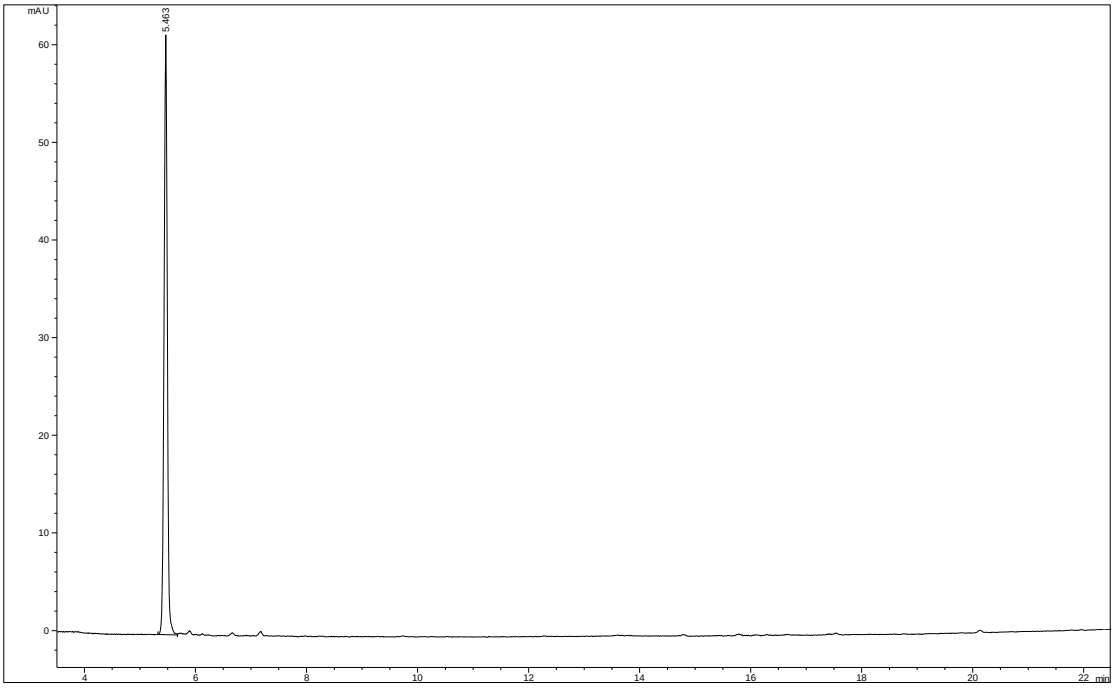

Figure S17: Dihydrocaffeic acid 3'-O- β -D-glucuronide ***17*** *(280 nm).*

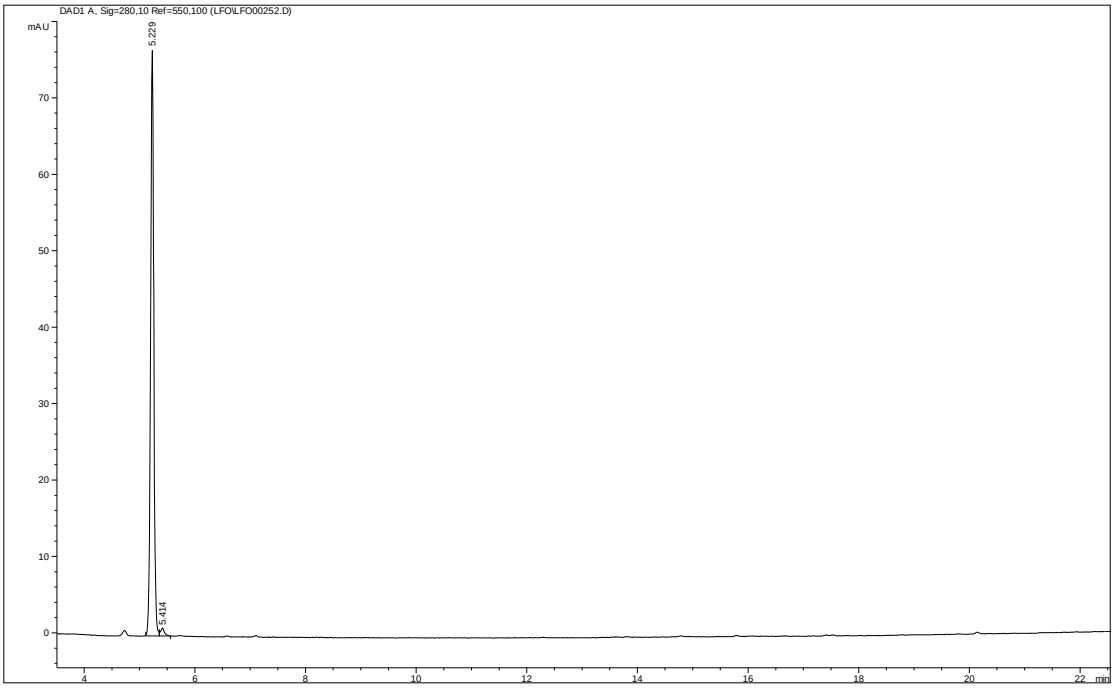

Figure S18: Dihydrocaffeic acid 4'-O- β -D-glucuronide ***18*** *(280 nm).*

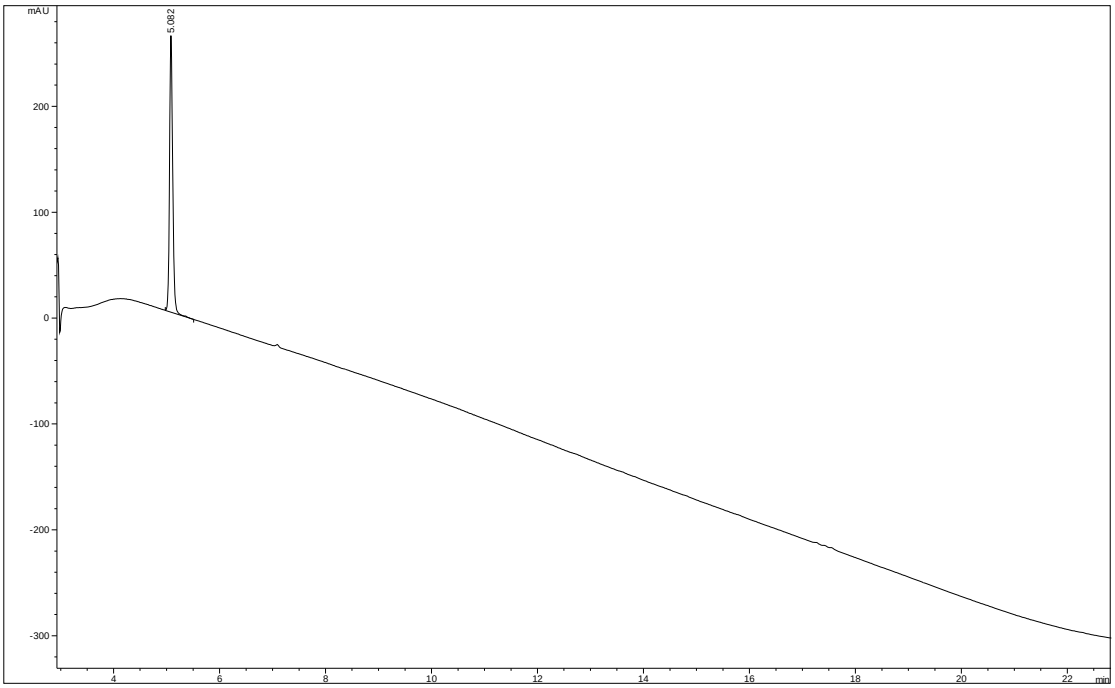

*Figure S19: Dihydro-p-coumaric acid 4'-sulphate **19** (210 nm).*

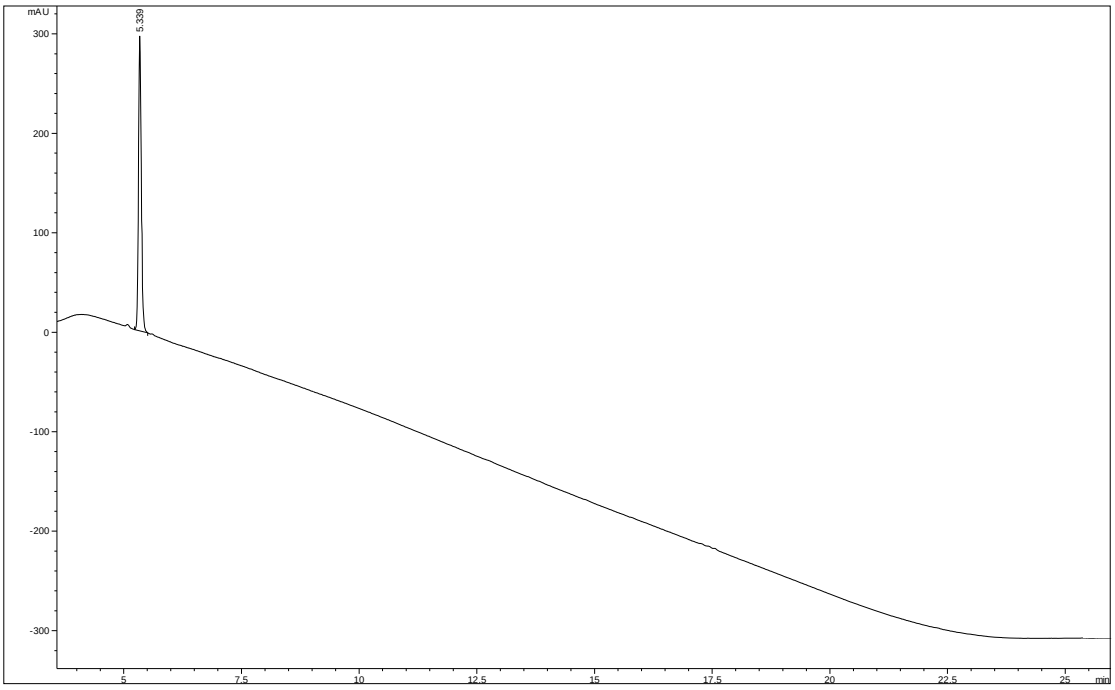

*Figure S20: Dihydro-m-coumaric acid 3'-sulphate **20** (210 nm).*

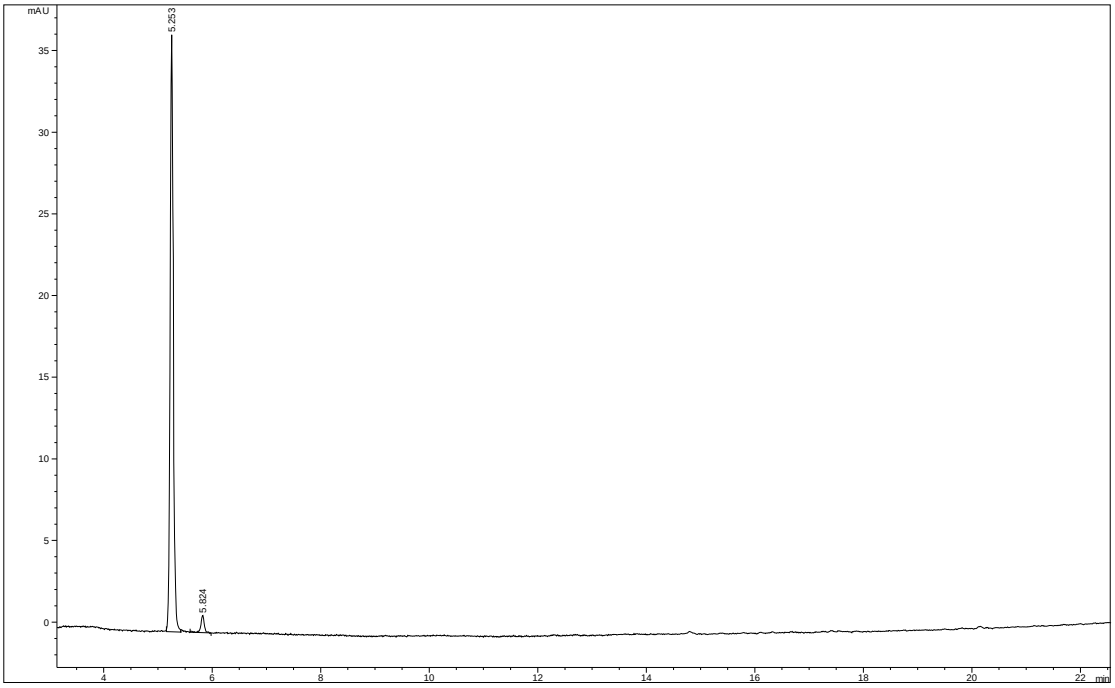

*Figure S21: Dihydroferulic acid 4'-sulphate **21** (280 nm).*

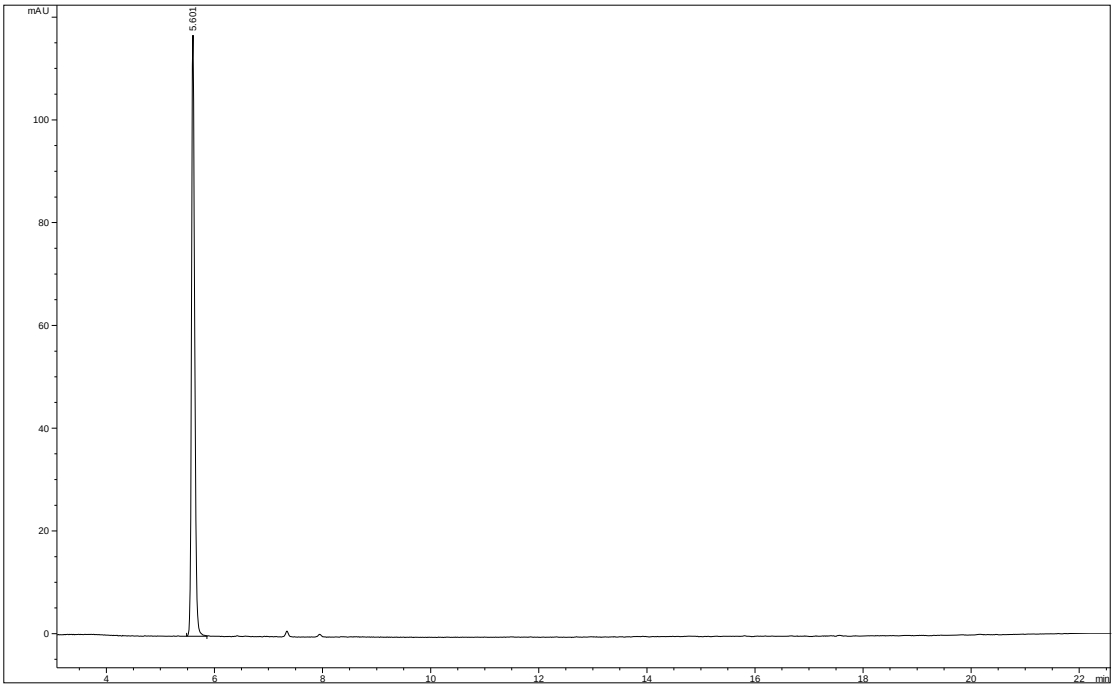

*Figure S22: Dihydroisoferulic acid 3'-sulphate **22** (280 nm).*

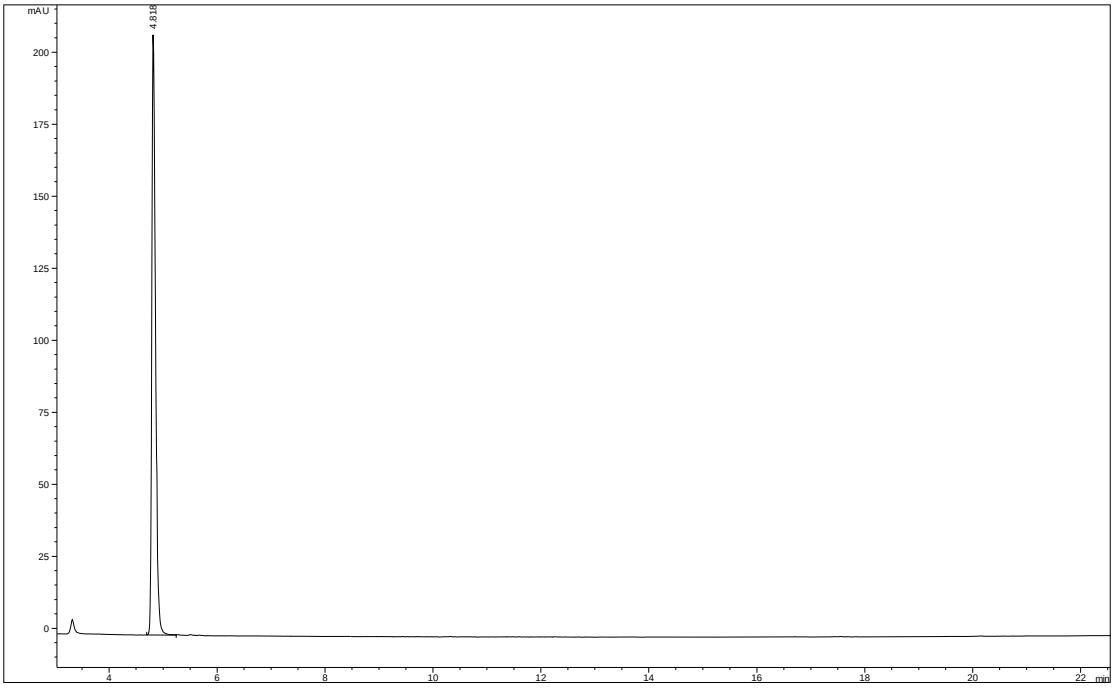

*Figure S23: Dihydrocaffeic acid 3'-sulphate **23** (280 nm).*

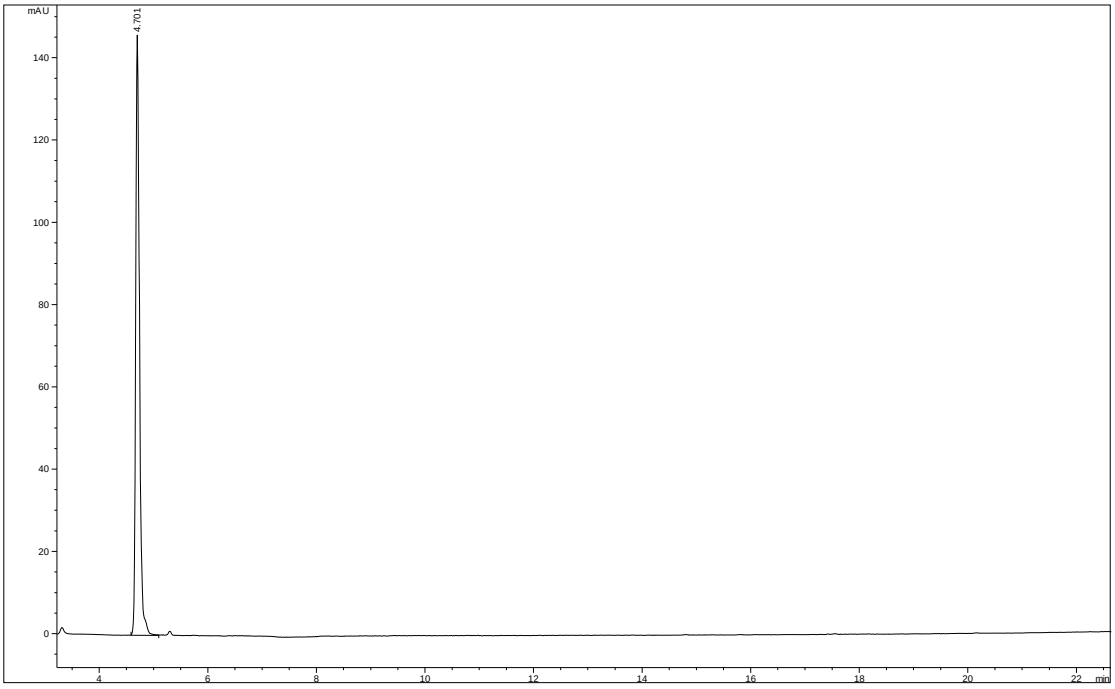

*Figure S24: Dihydrocaffeic acid 4'-sulphate **24** (280 nm).*

3. ^{1}H and ^{13}C NMR spectra of analytical standards 1-24

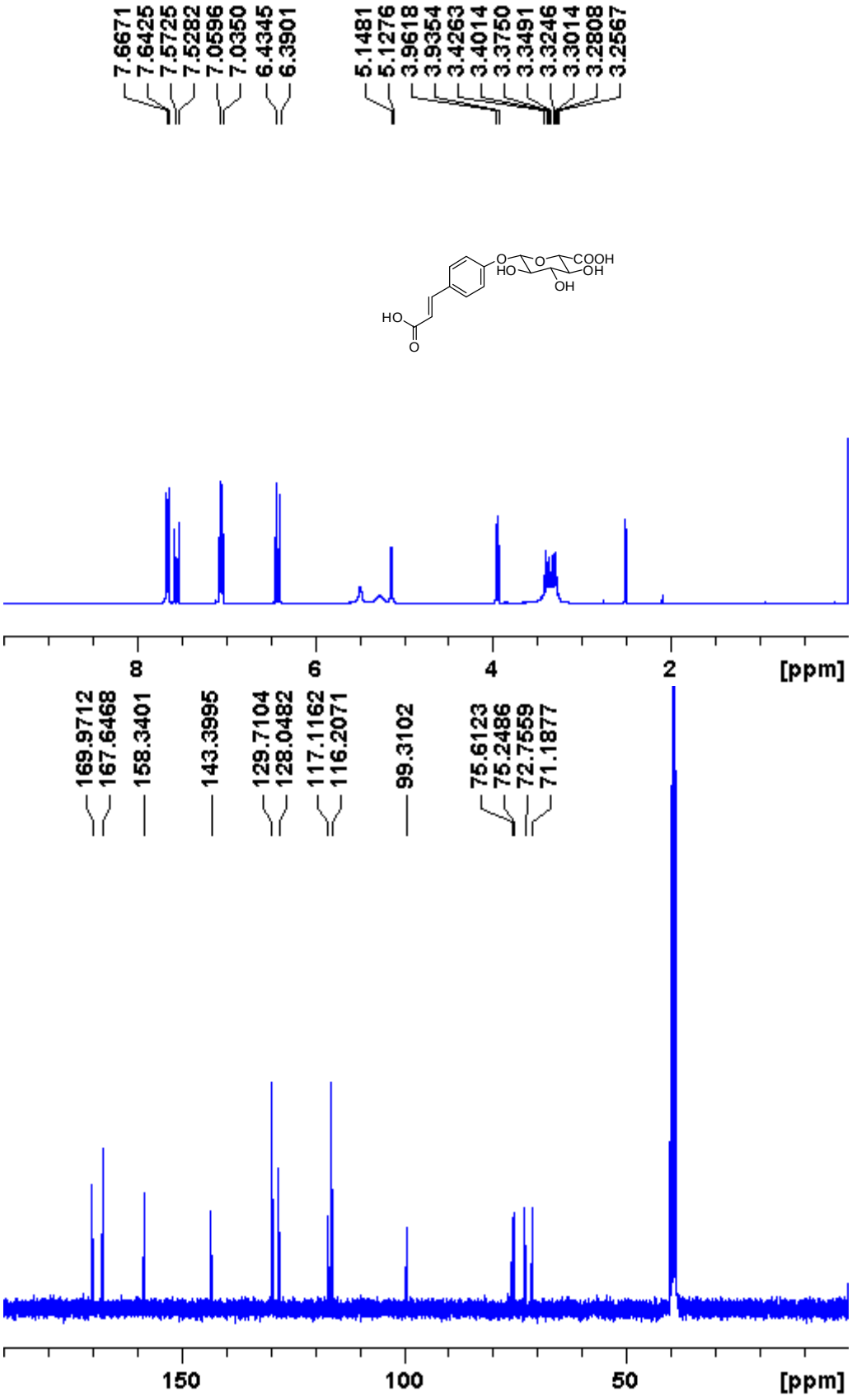

Figure S25: (E)-p-Coumaric acid 4'-O- β -D-glucuronide ***1a***.

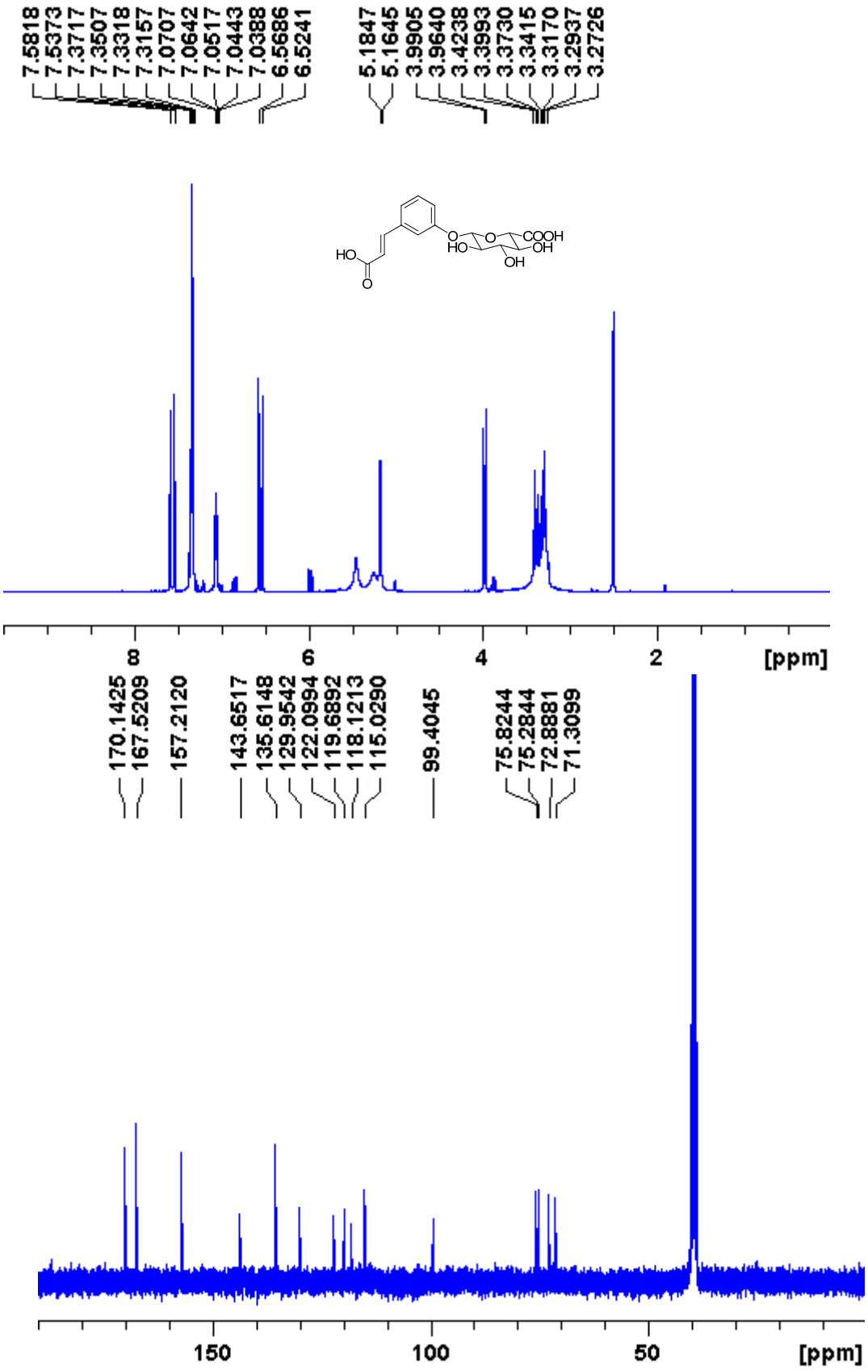

Figure S26: (E)-m-Coumaric acid 3'-O- β -D-glucuronide ***2a***.

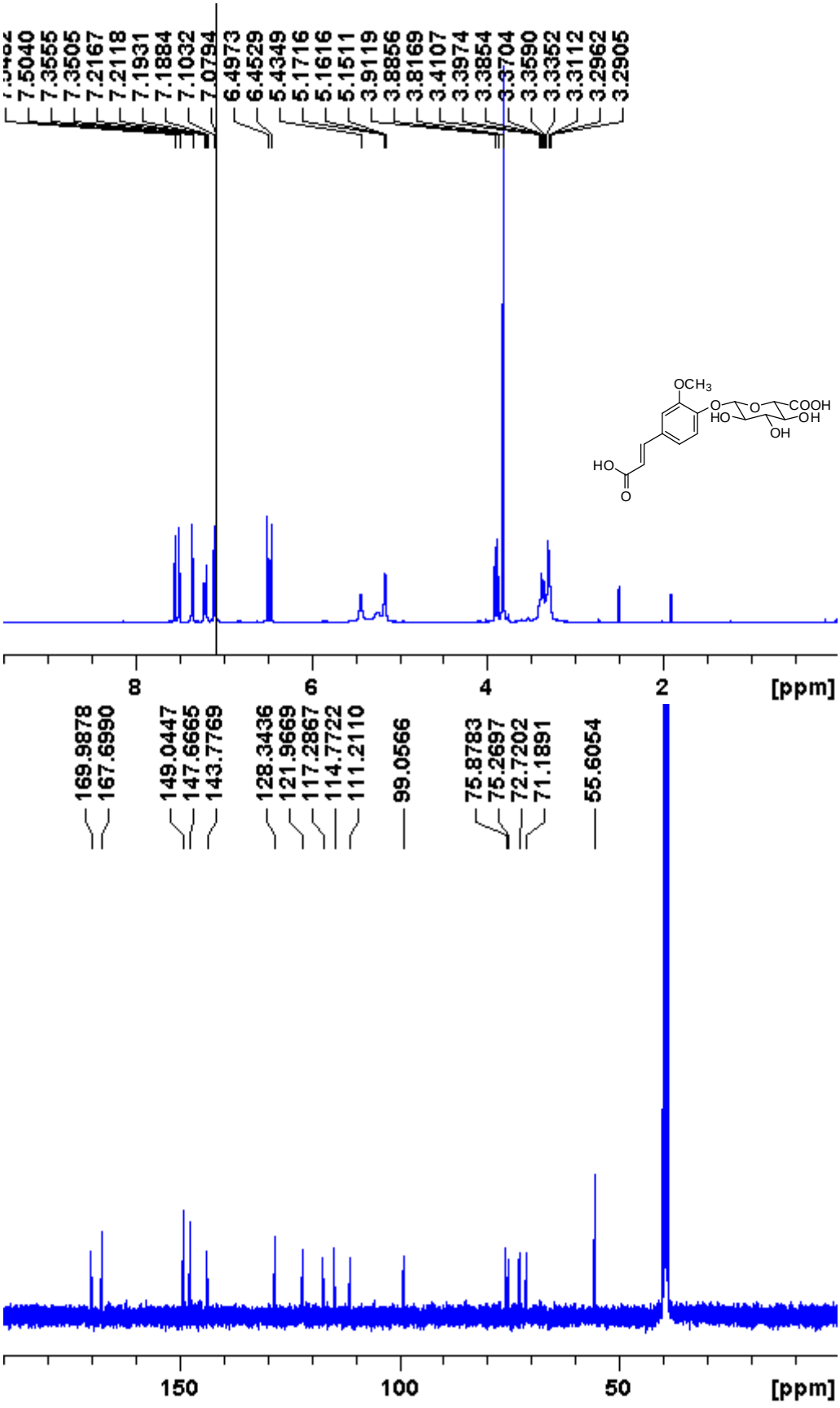

Figure S27: (E)-Ferulic acid 4'-O- β -D-glucuronide ***3a***.

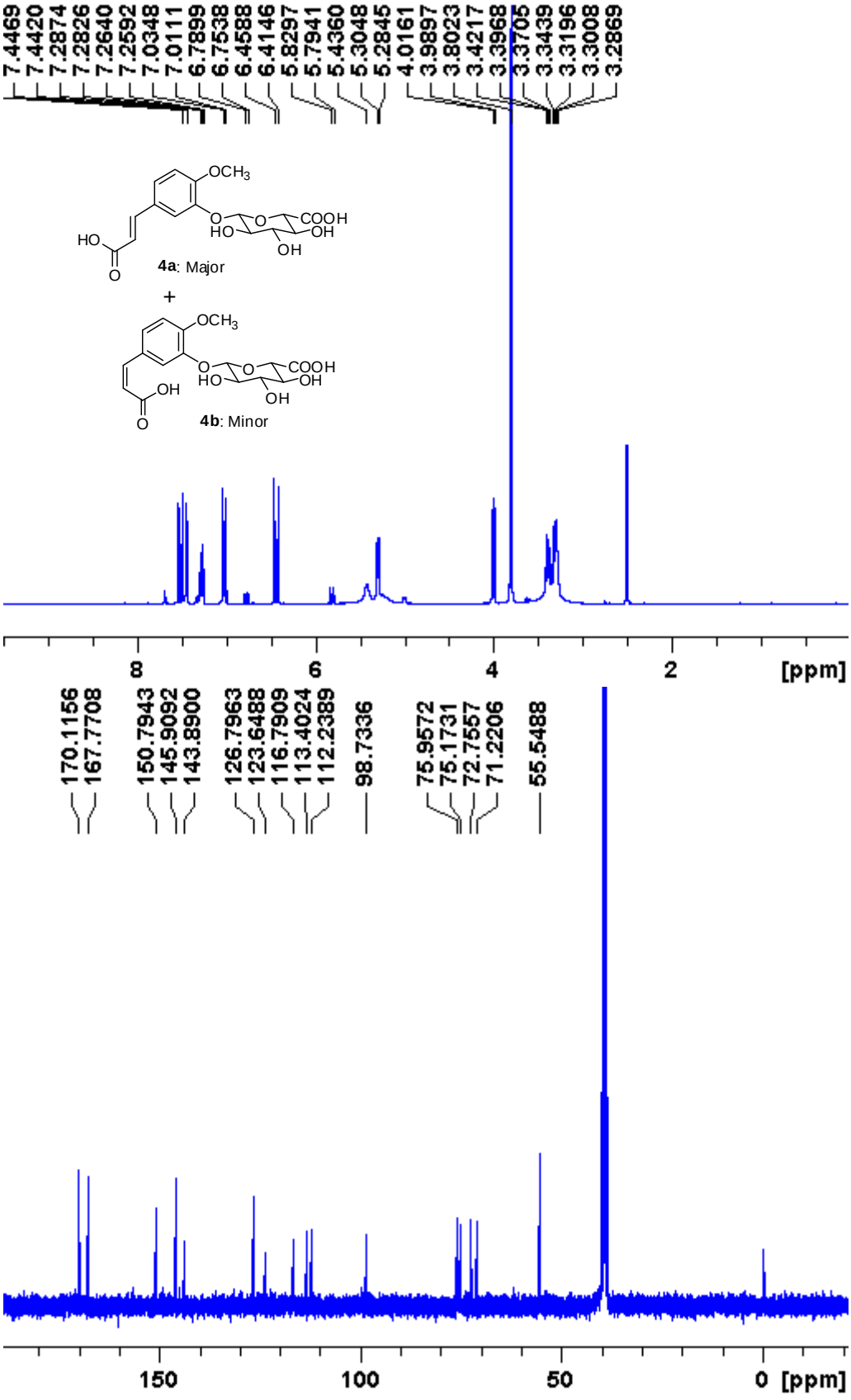

Figure S28: (E)- and (Z)-Isoferulic acid 3'-O- β -D-glucuronides ***4a*** + ***4b***.

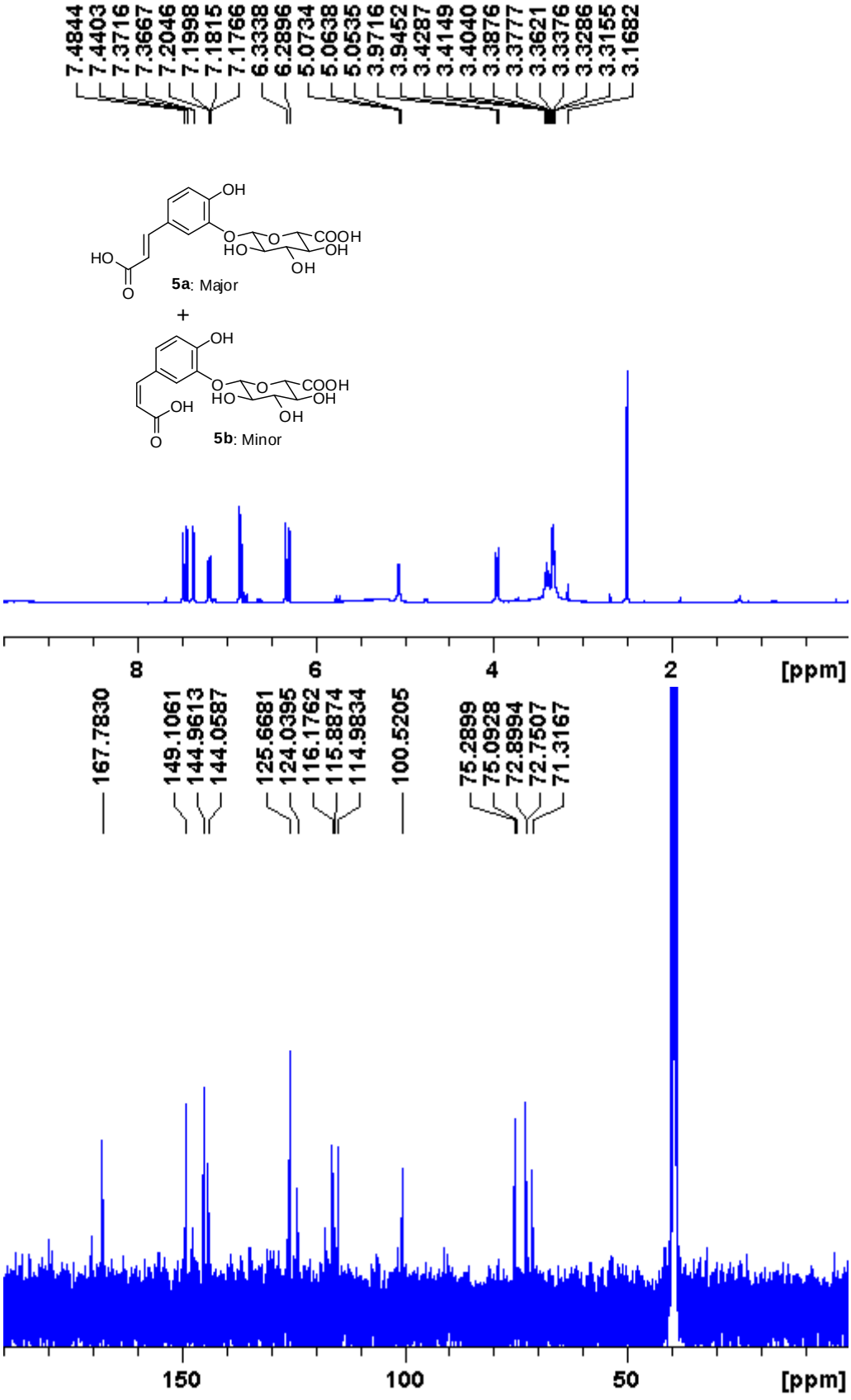

Figure S29: (E)- and (Z)-Caffeic acid 3'-O- β -D-glucuronide ***5a*** *+* ***5b****.*

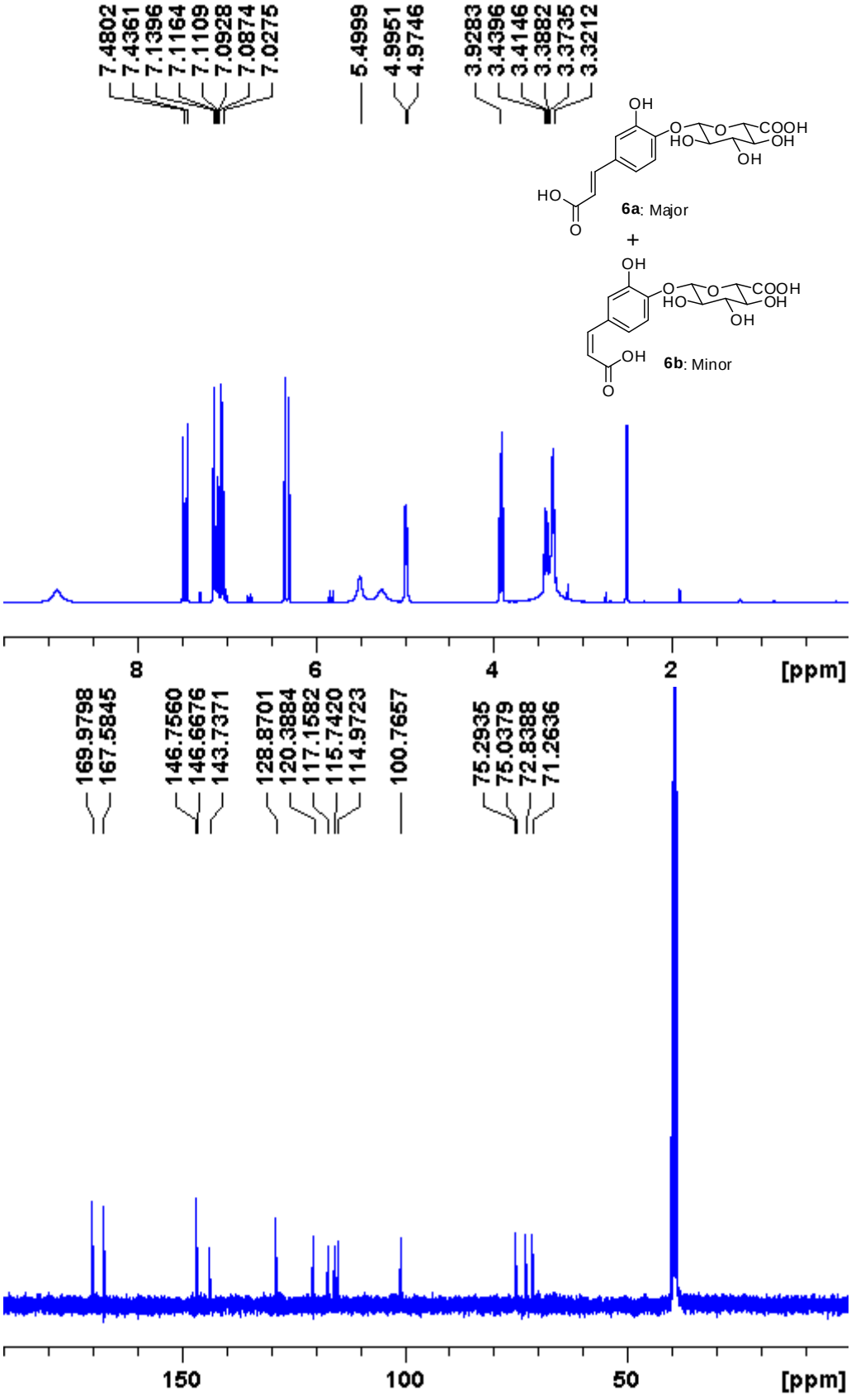

Figure S30: *(E)- and (Z)-Caffeic acid 4'-O- β -D-glucuronide* ***6a*** + ***6b***.

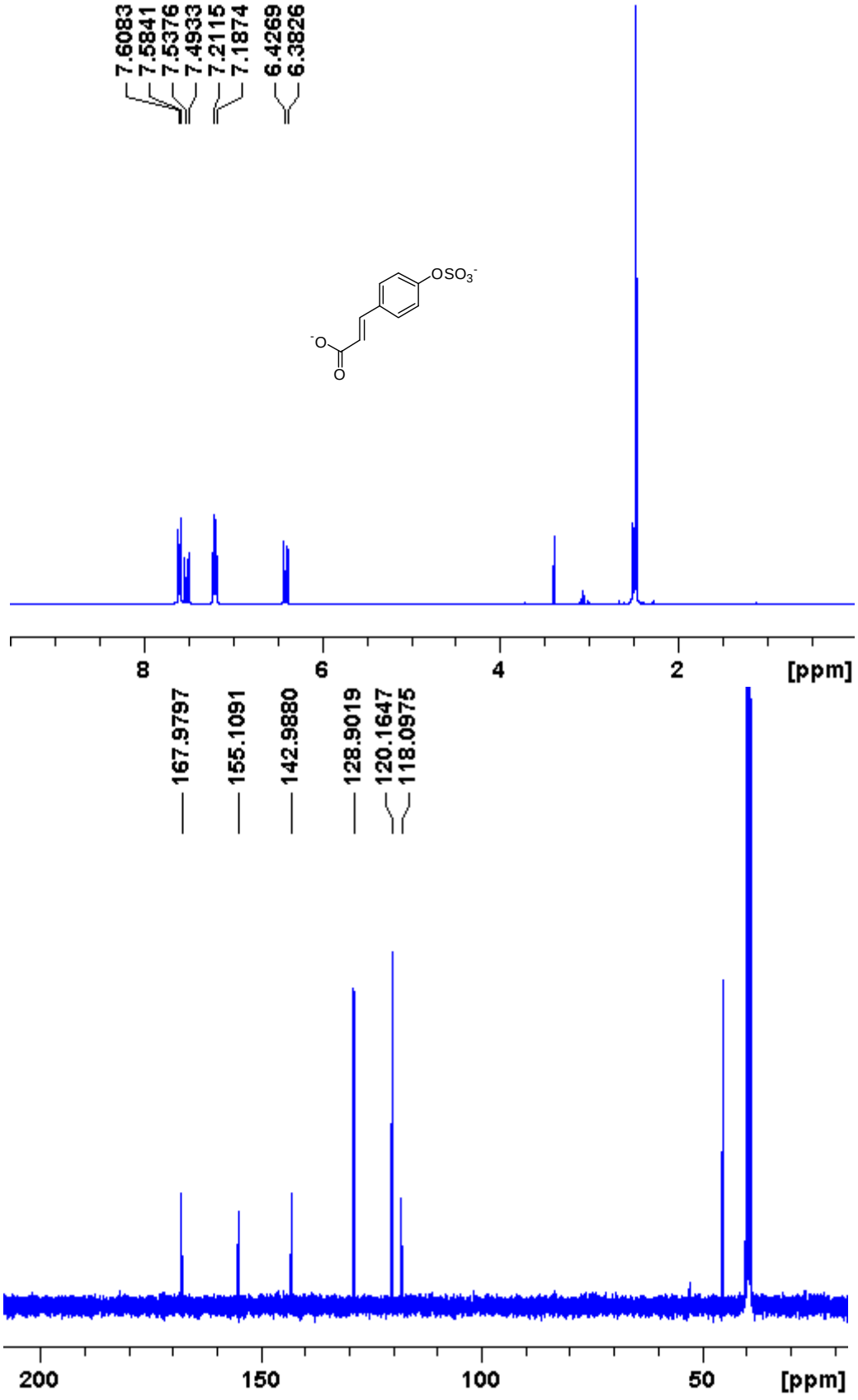

Figure S31: (E)-p-Coumaric acid 4'-sulphate **7**.

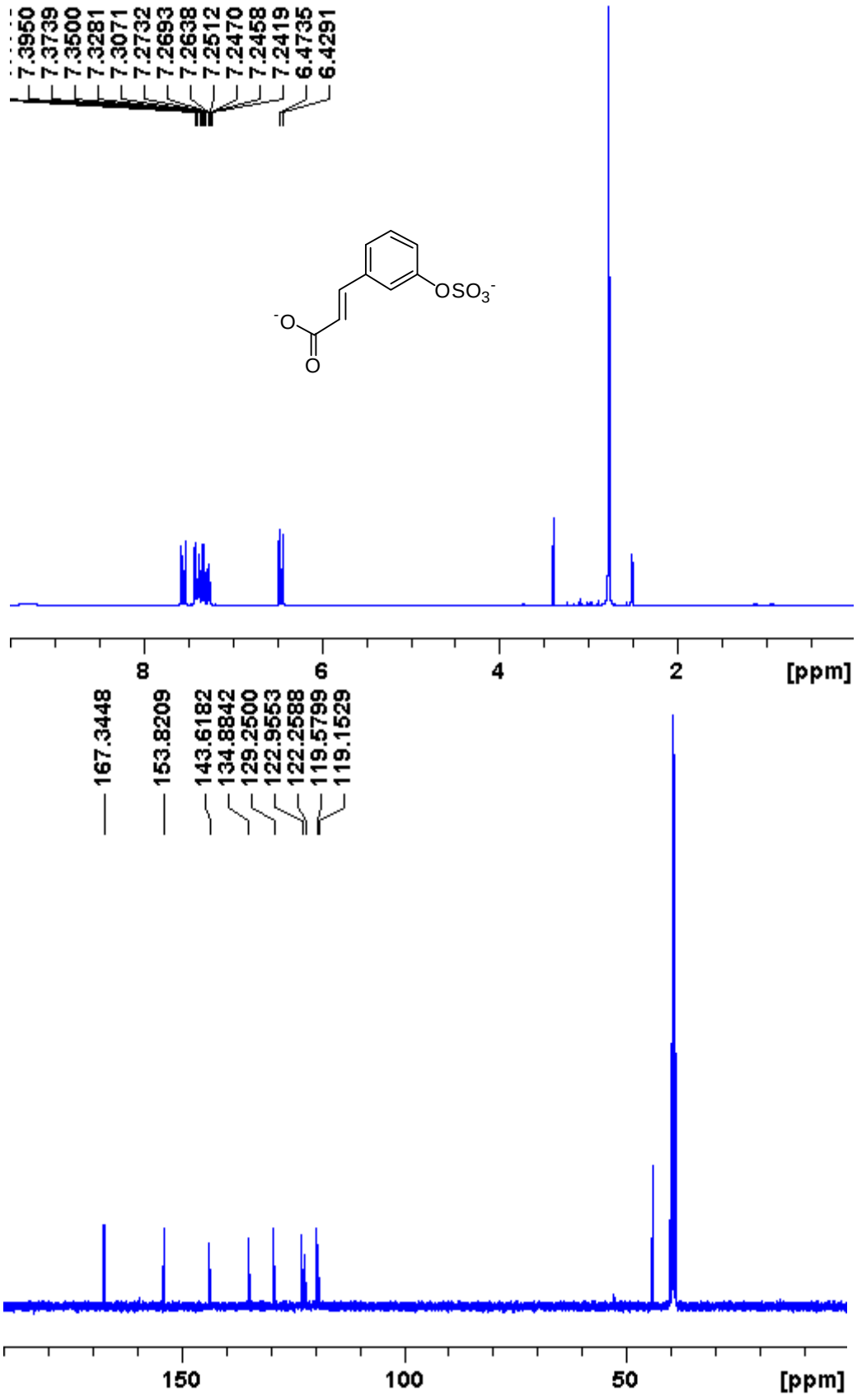

Figure S32: *(E)-m-Coumaric acid 3'-sulphate* ***8***.

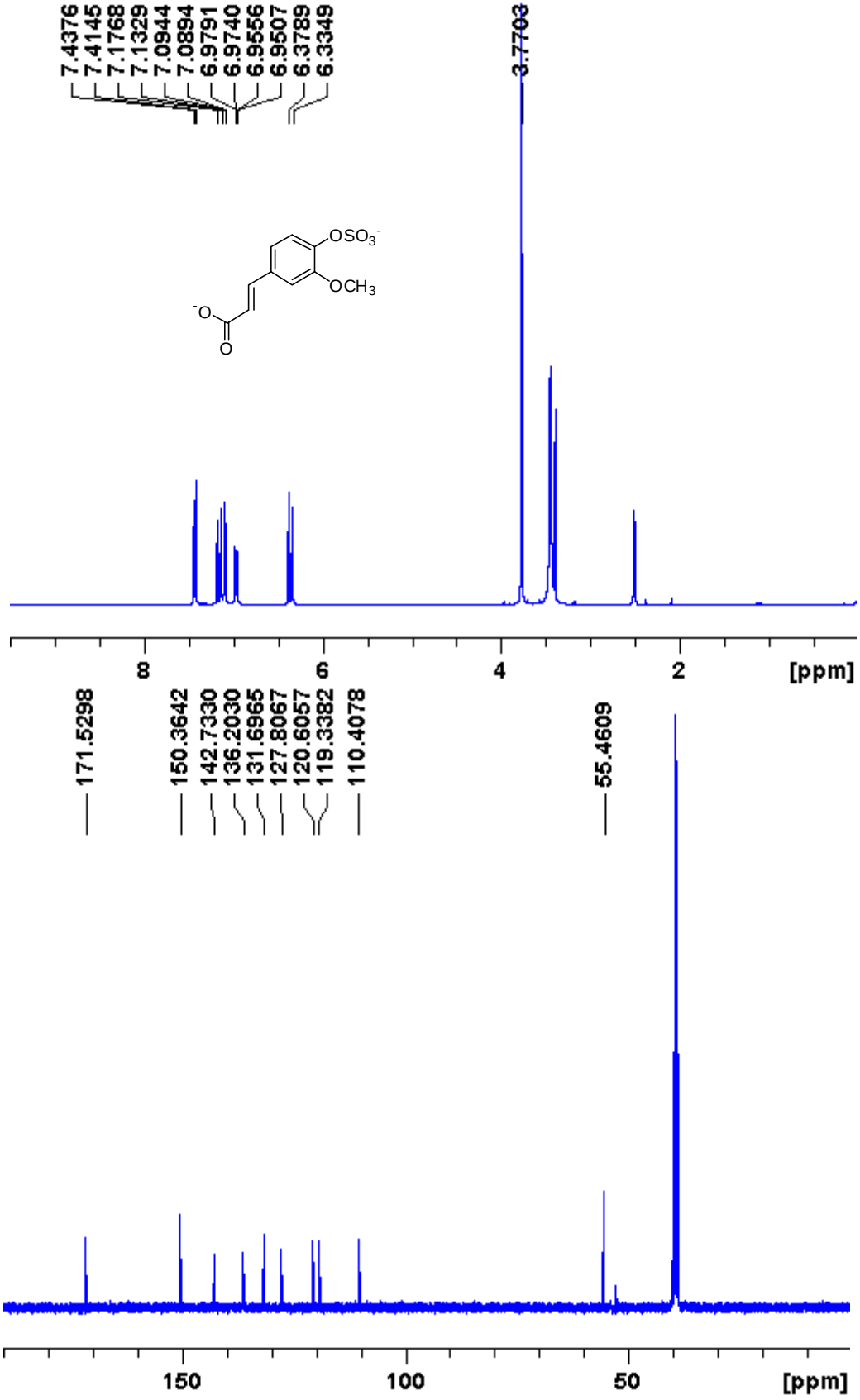

Figure S33: (E)-Ferulic acid 4'-sulphate **9**.

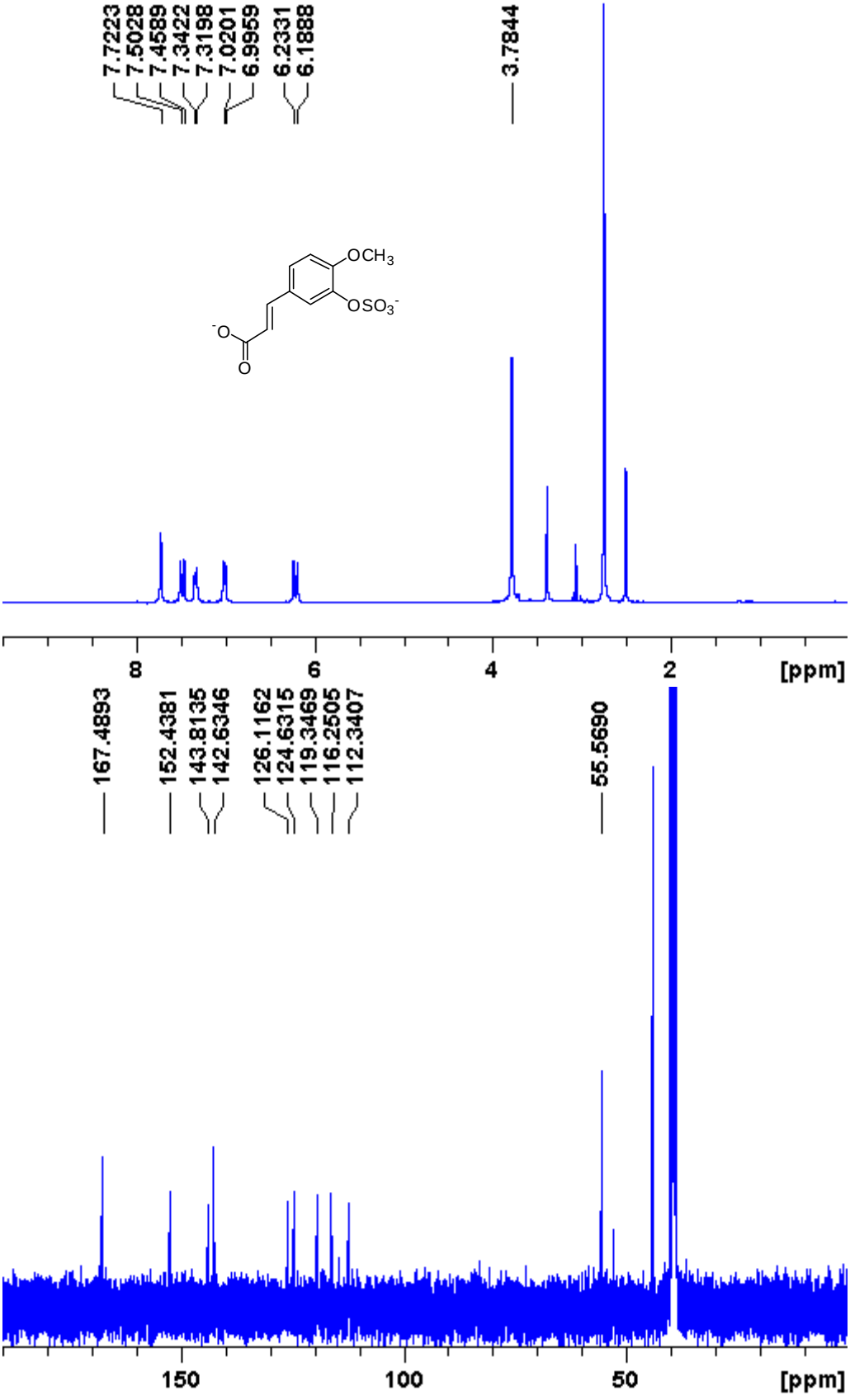

Figure S34: (E)-Isoferulic acid 3'-sulphate ***10***.

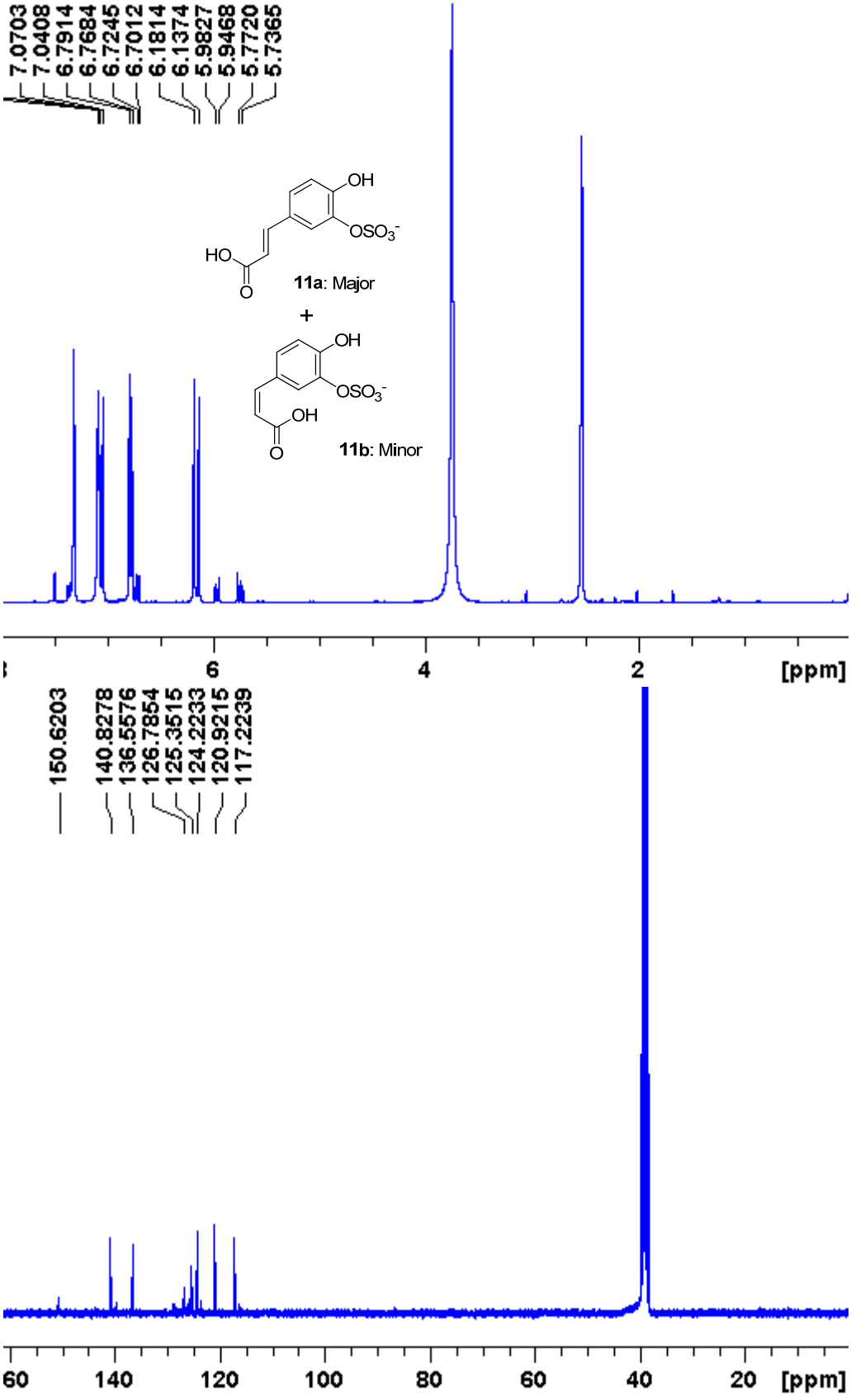

Figure S35: (E)- and (Z)-Caffeic acid 3'-sulphates ***11a*** + ***11b***.

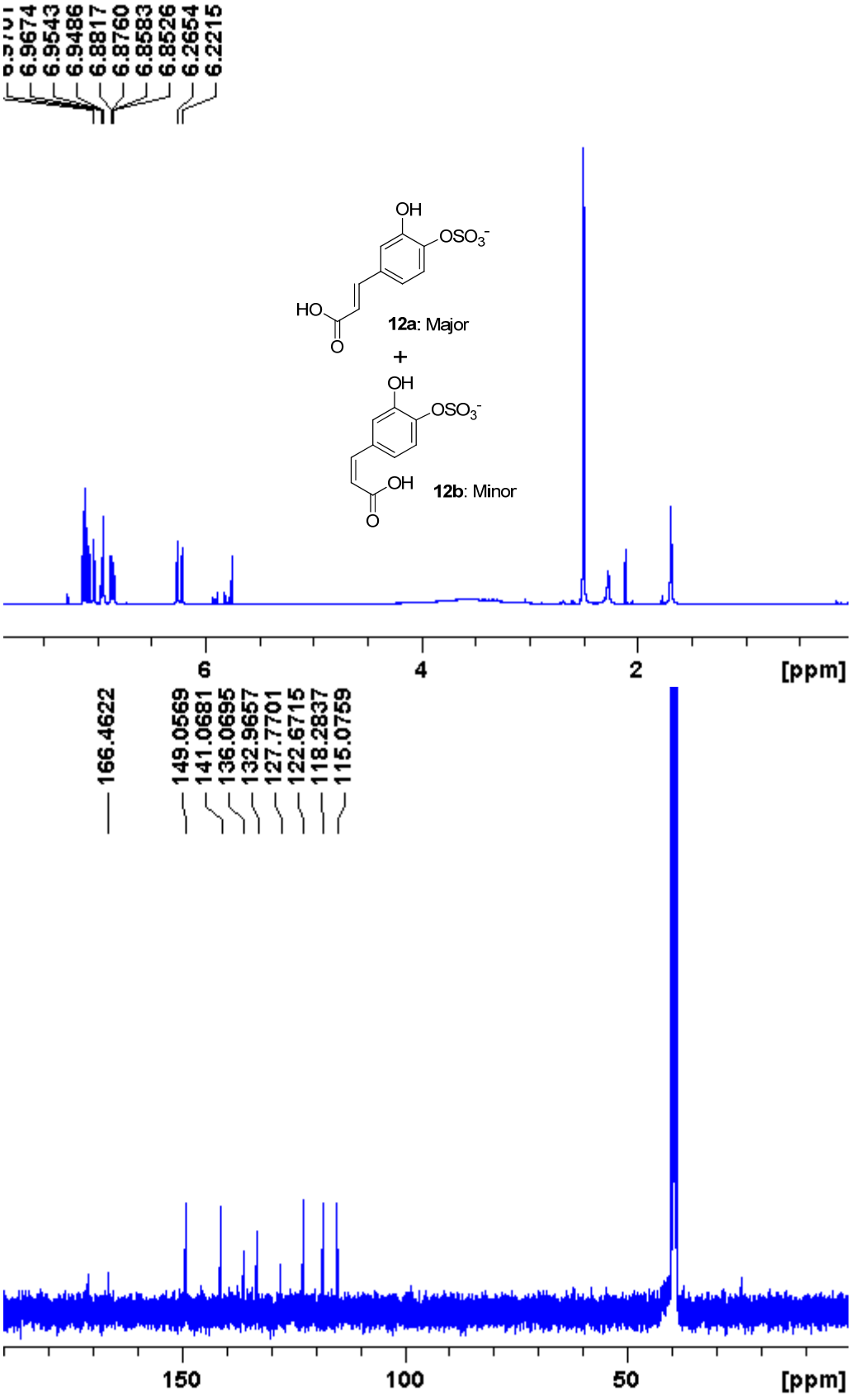

Figure S36: (E)- and (Z)-Caffeic acid 4'-sulphates ***12a*** *+* ***12b****.*

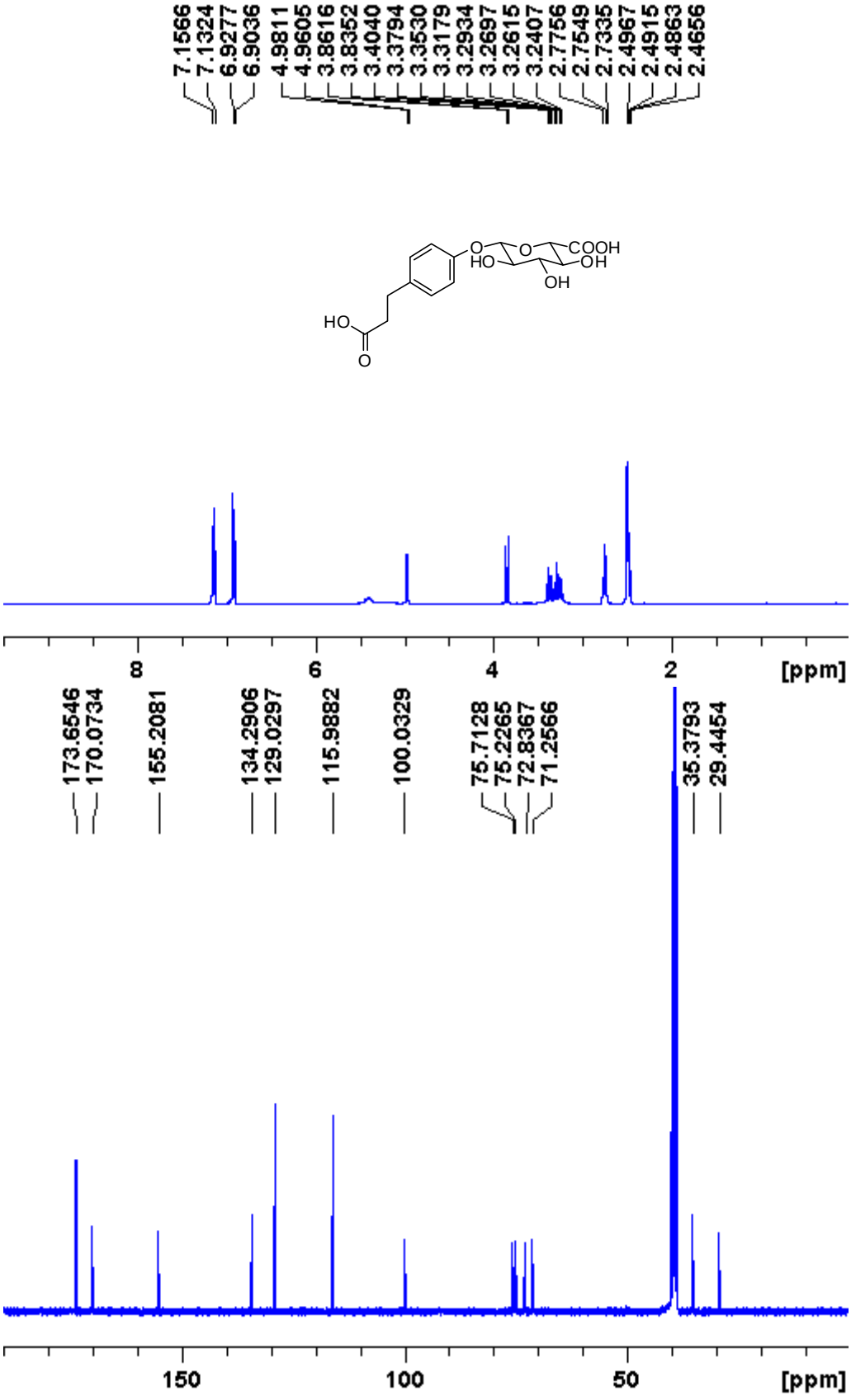

Figure S37: Dihydro-p-coumaric acid 4'-O- β -D-glucuronide ***13***.

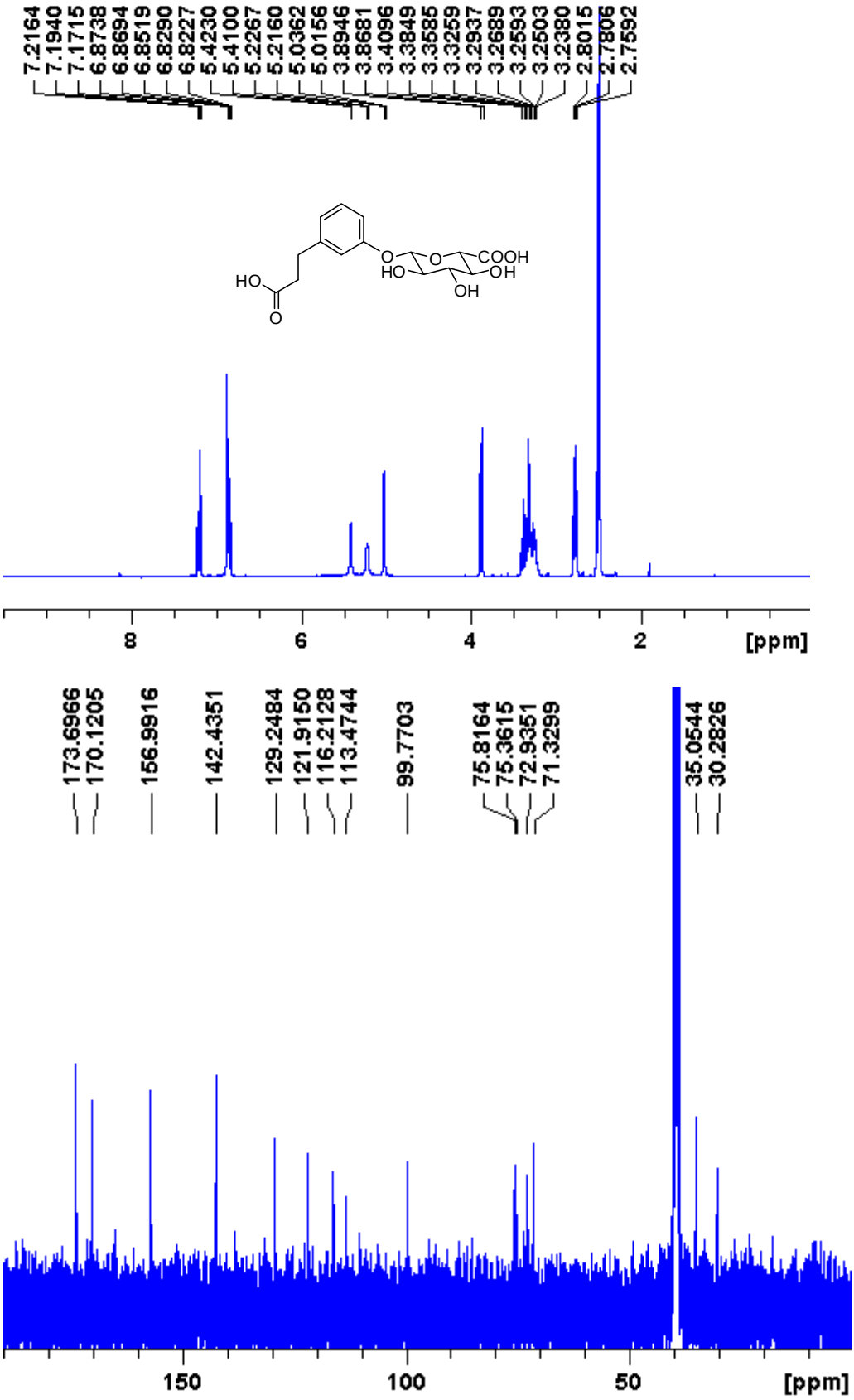

Figure S38: Dihydro-m-coumaric acid 3'-O- β -D-glucuronide ***14***.

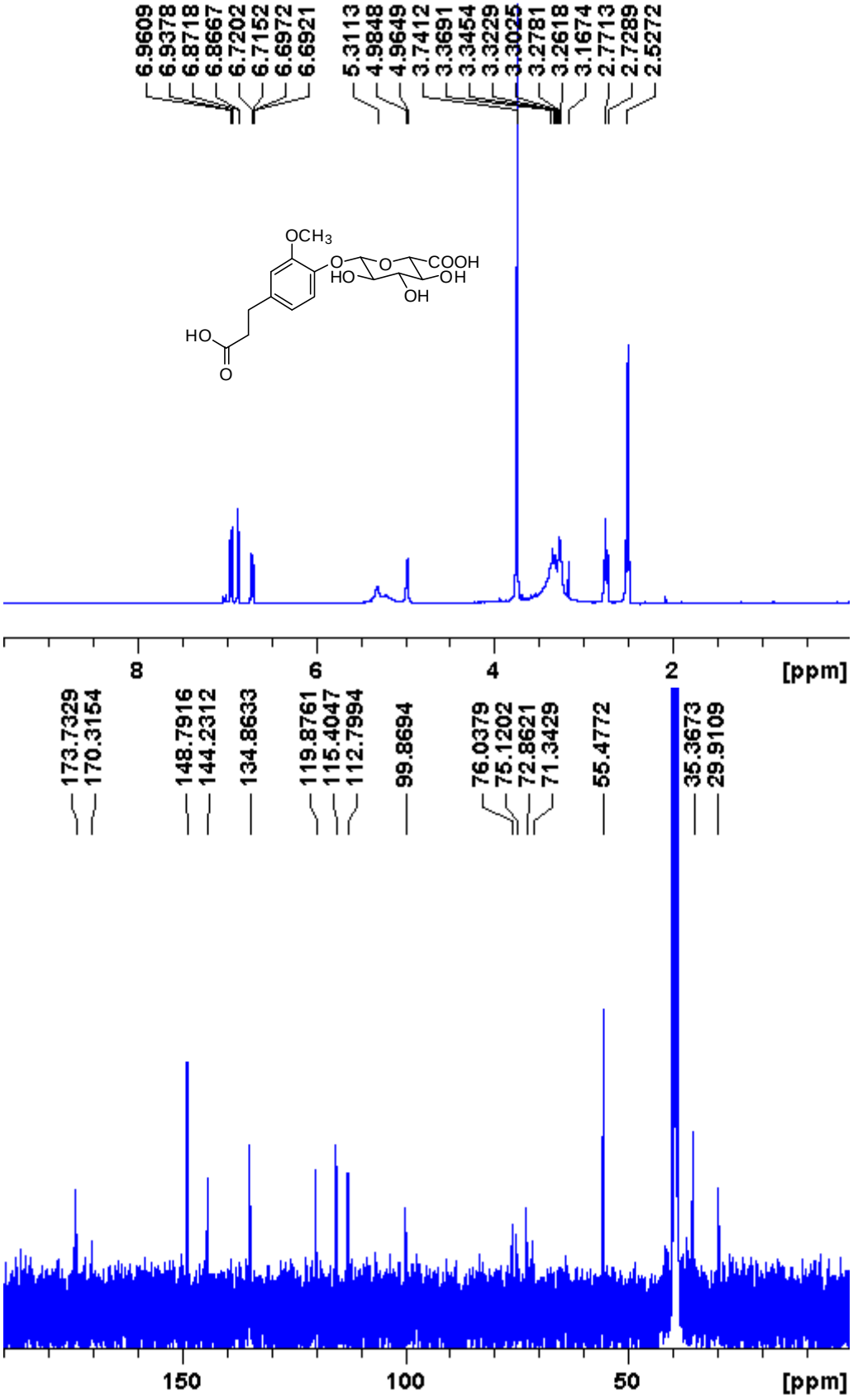

Figure S39: Dihydroferulic acid 4'-O- β -D-glucuronide ***15***.

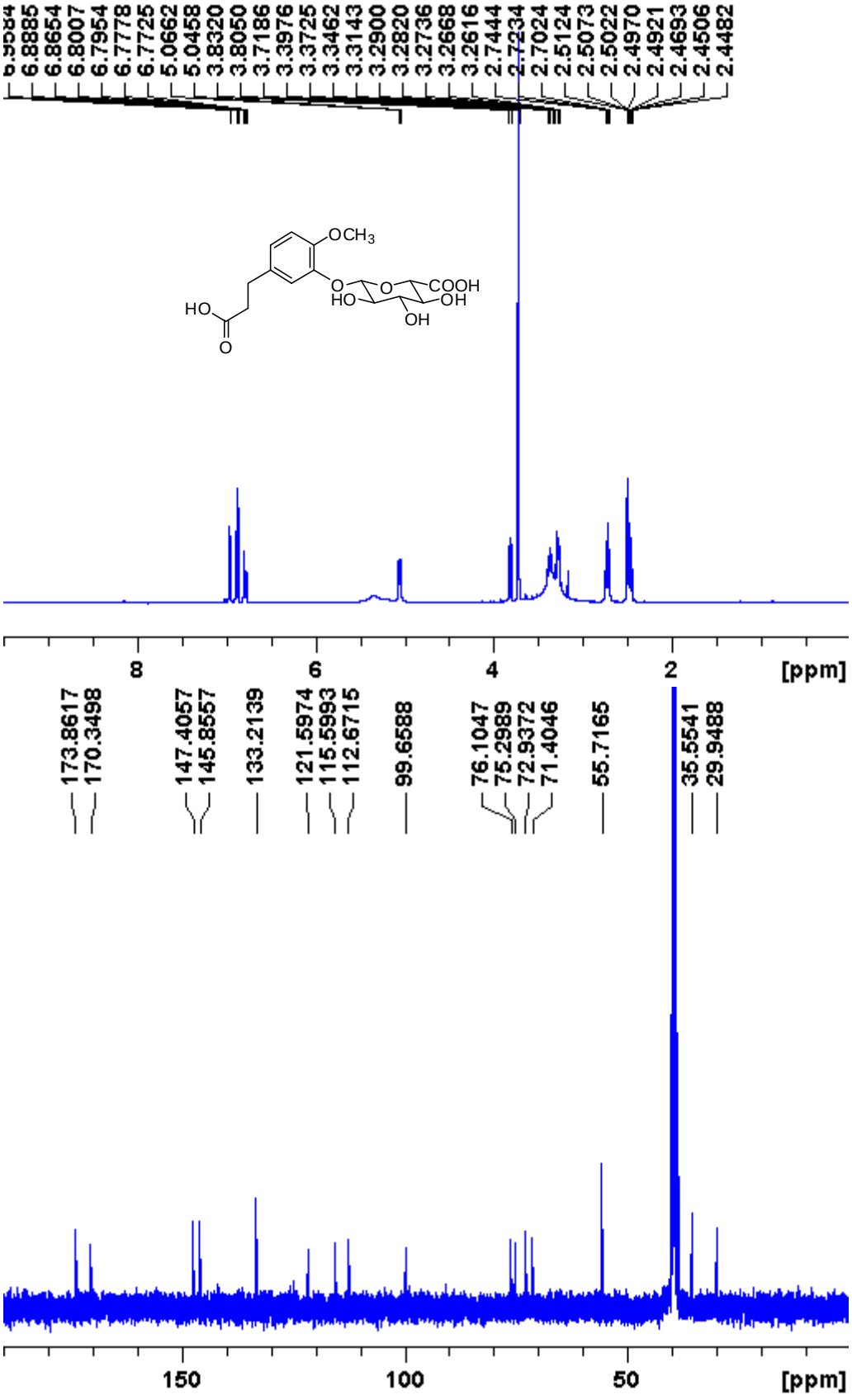

Figure S40: Dihydroisoferulic acid 3'-O- β -D-glucuronide ***16***.

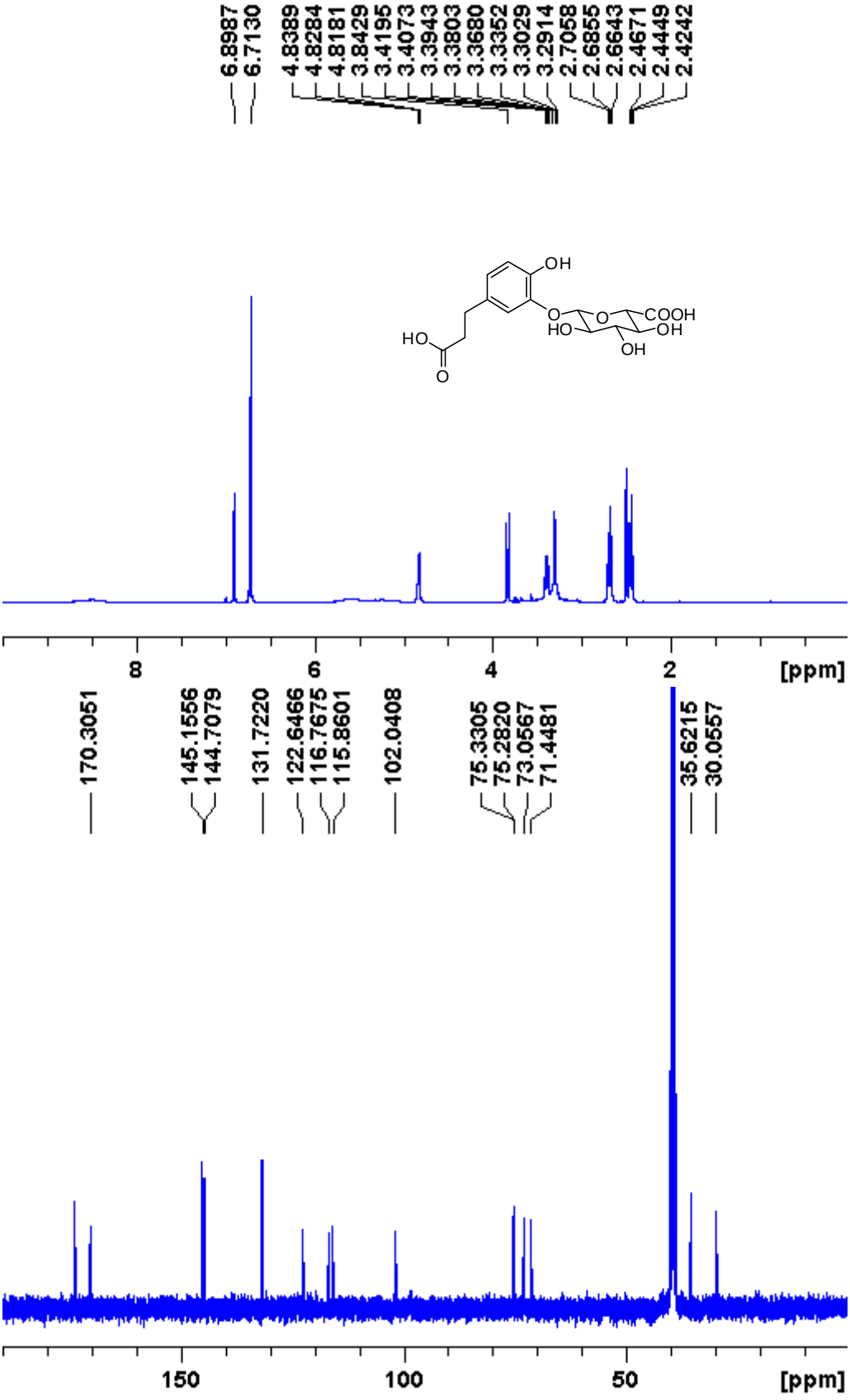

Figure S41: Dihydrocaffeic acid 3'-O- β -D-glucuronide ***17***.

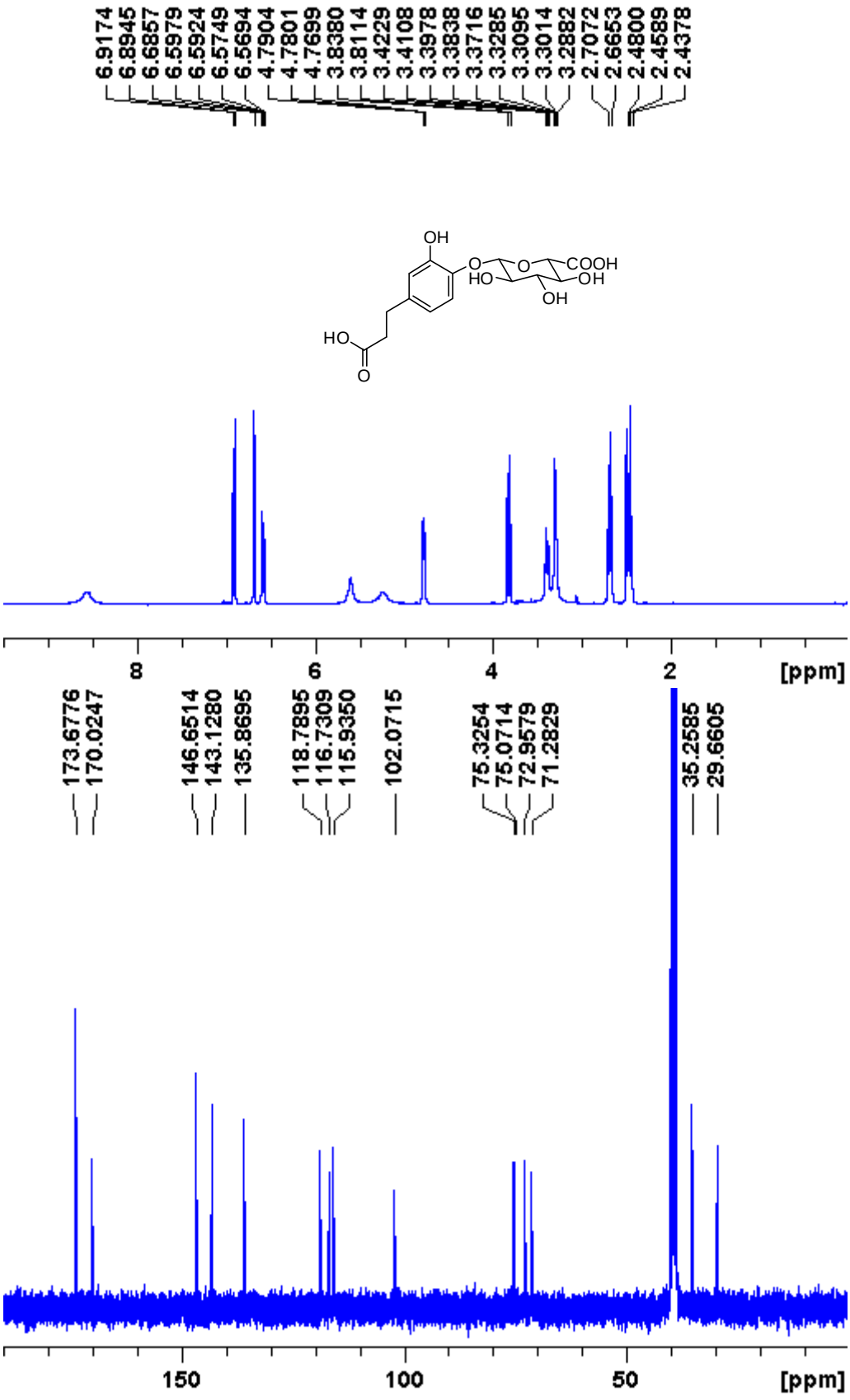

Figure S42: Dihydrocaffeic acid 4'-O- β -D-glucuronide ***18***.

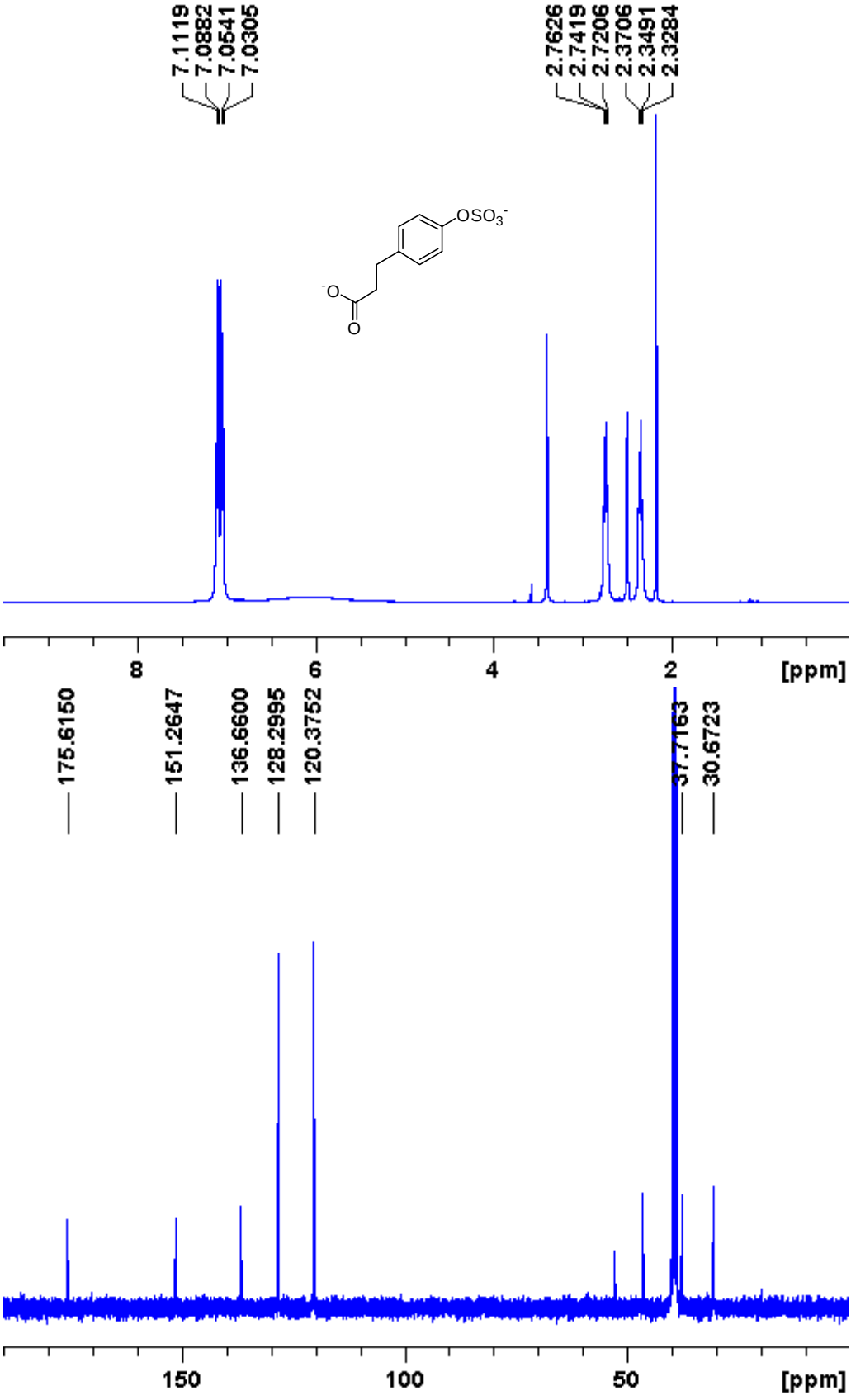

Figure S43: Dihydro-p-coumaric acid 4'-sulphate ***19***.

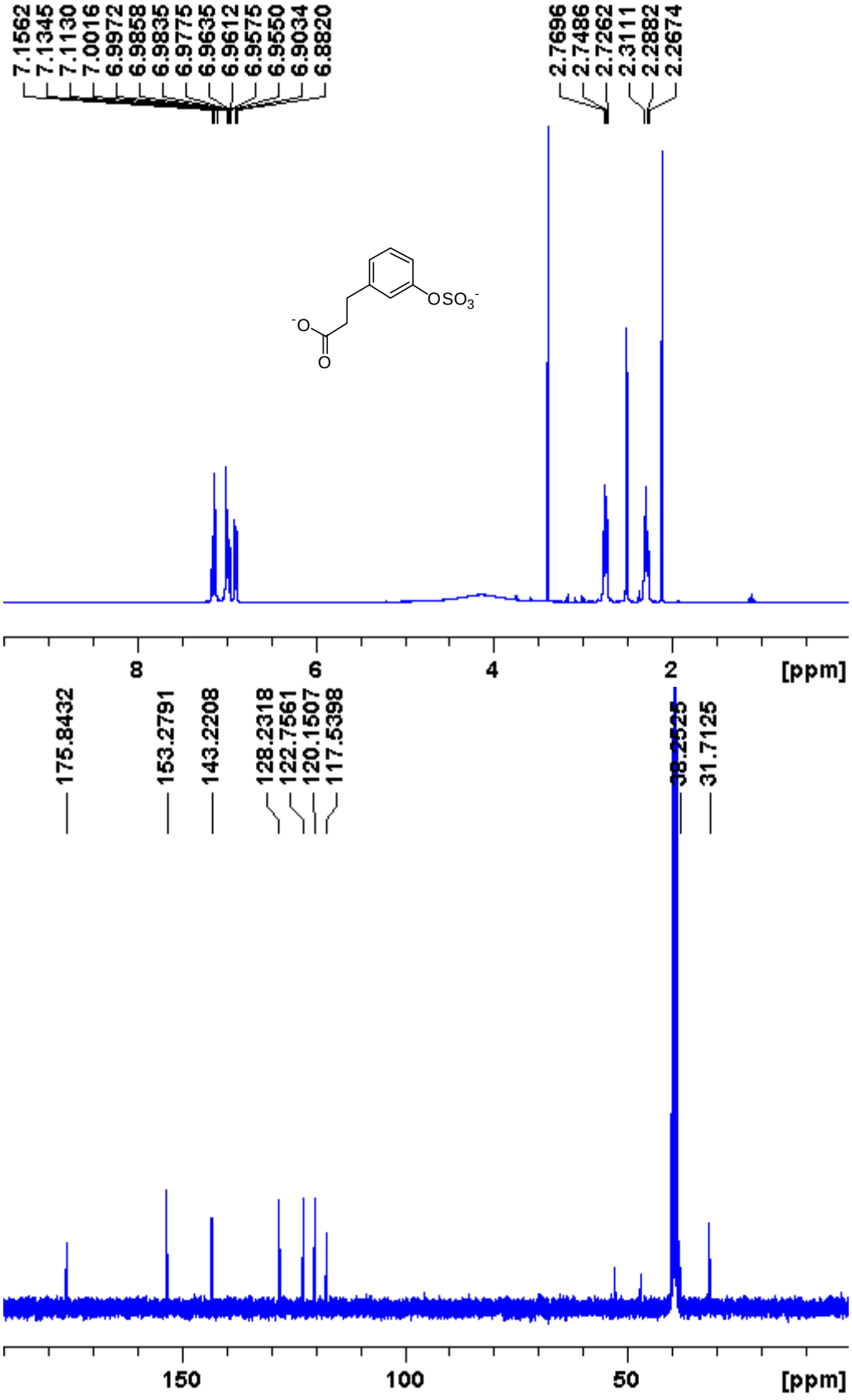

Figure S44: Dihydro-m-coumaric acid 3'-sulphate ***20***.

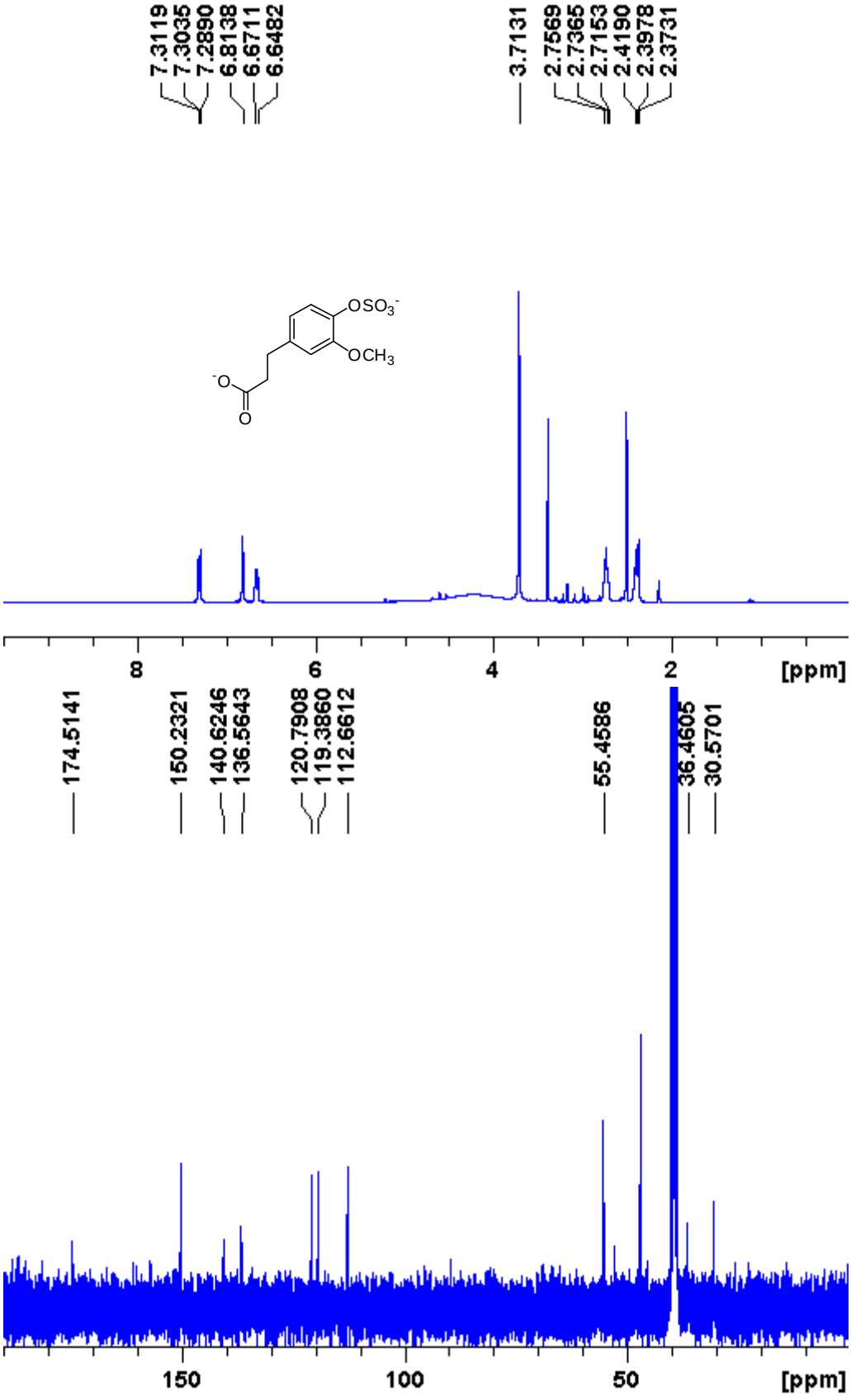

Figure S45: Dihydroferulic acid 4'-sulphate ***21***.

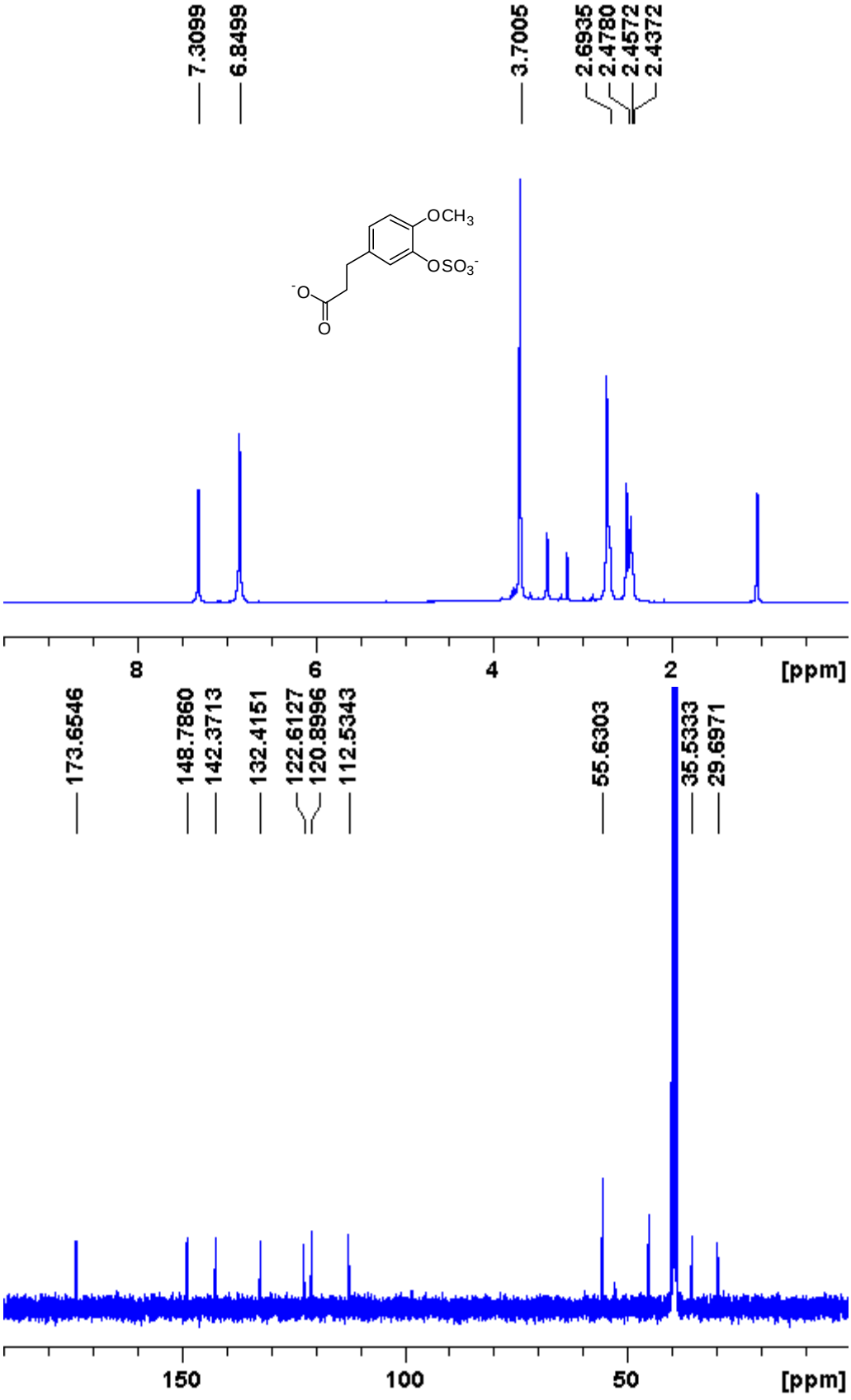

Figure S46: Dihydroisoferulic acid 3'-sulphate ***22***.

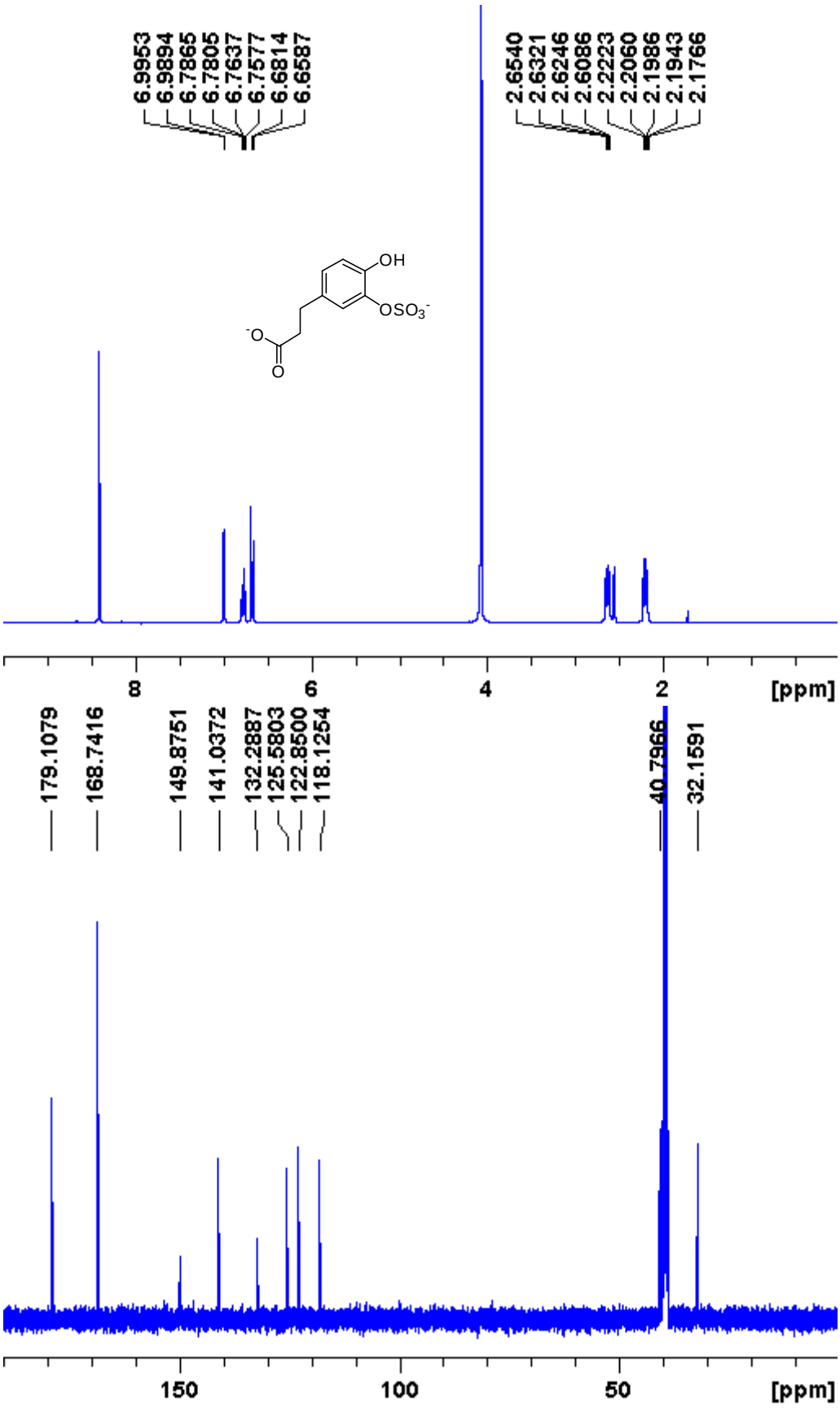

Figure S47: Dihydrocaffeic acid 3'-sulphate ***23***.

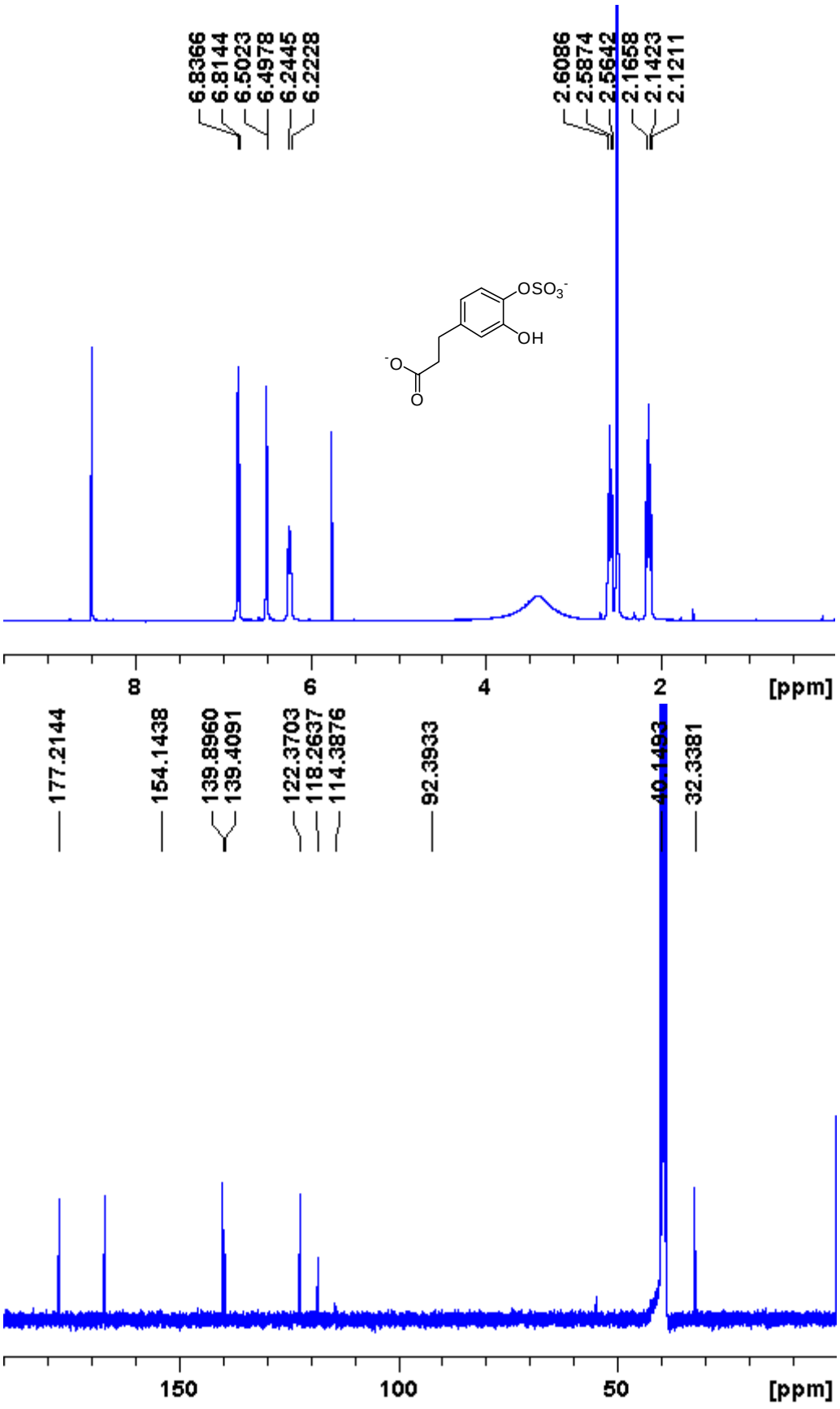

Figure S48: Dihydrocaffeic acid 4'-sulphate ***24***.