

• Supporting Information

Synthesis of bisdesmosidic oleanolic acid saponins via a glycosylation-deprotection sequence under continuous microfluidic/batch conditions.

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I. Set up for microflow reactor.

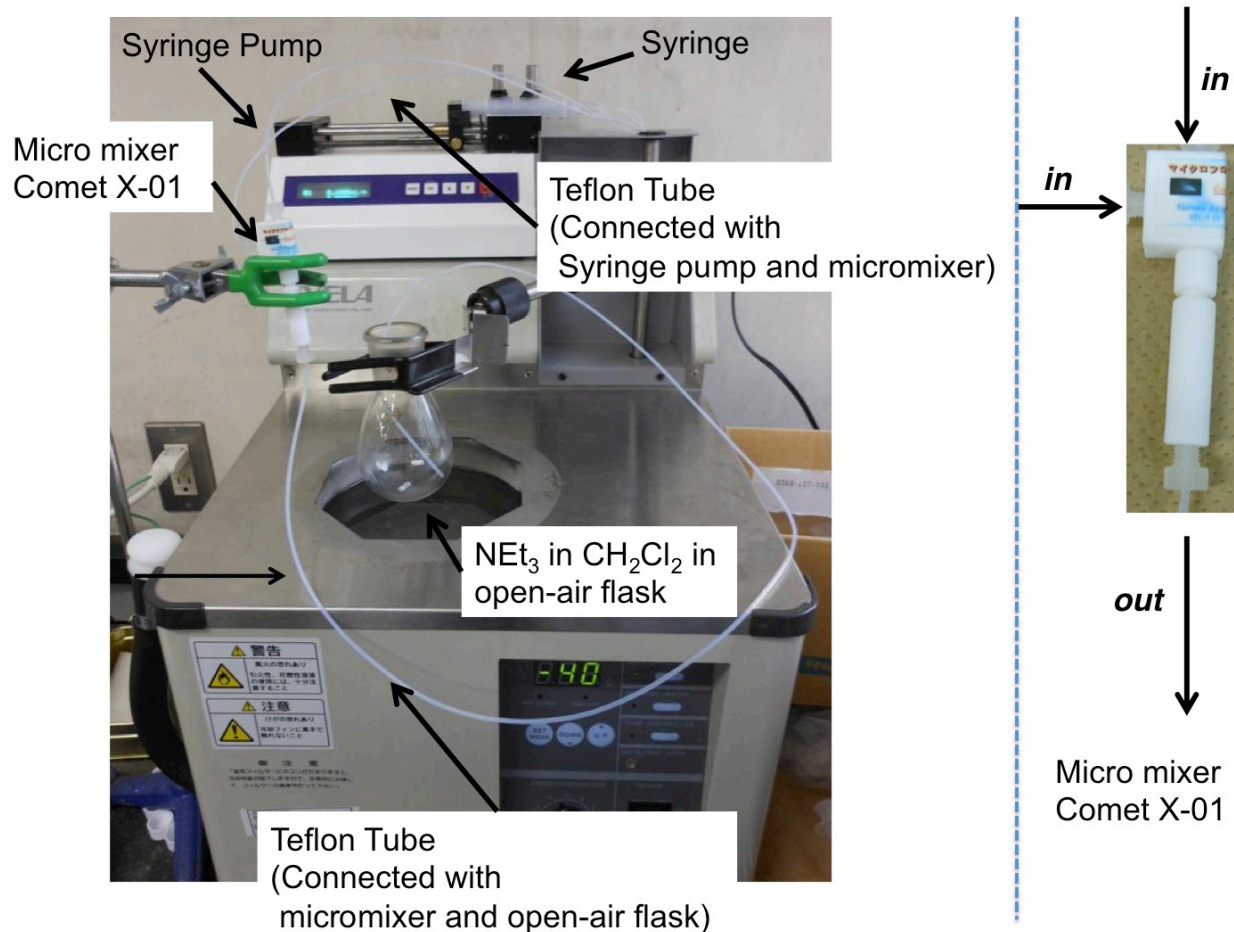


Figure S1 Micro-flow system

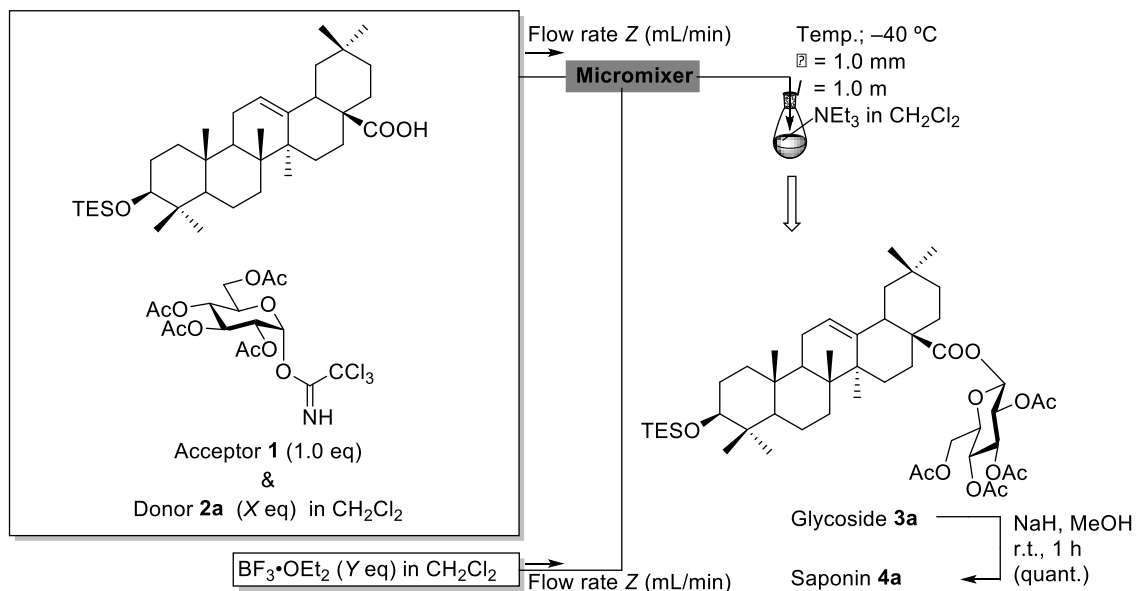
Comet X-01, microflow reactor was purchased from Techno Applications Co. Ltd. (E-mail: yukio-matsubara@nifty.com). Teflon tube with inner diameter 1.0 mm was purchased from Senshu Scientific Co. Ltd. and Techno Applications Co. Ltd. The Comet X-01 mixers and Teflon tube were connected with PEEK fittings purchased from Techno Applications Co. Ltd. Solutions were introduced to microflow system with the syringe pumps (Catamaran HII-10, Techno Applications Co. Ltd.) equipped disposable syringes [a syringe/polypropylene (PP), a plunger and a seal part/polyethylene (PE), AS ONE]. The disposable syringes and Teflon tube were connected with joints purchased from Techno Applications Co. Ltd.

The employed microflow system is shown in SI-Figure 1. The disposable syringes and Comet X-01 were connected with Teflon tube (inner diameter: 1.0 mm, length: 1000 mm, volume 785 μ L).

The Comet X-01 micromixer and open-air flask were connected with Teflon tube (inner diameter: 1.0 mm, length: 1000 mm, volume 785 CL). In the open-air flask, triethylamine (3.0 μ L) diluted in CH_2Cl_2 (1 mL) were prepared for quenching the reaction.

II. Procedure for C-28 glycosylation of **1** with acetylated donor **2a** using microflow reactor.

Table S1 The detail result of synthesis of glycoside **3a** under microfluidic system at various reaction condition.

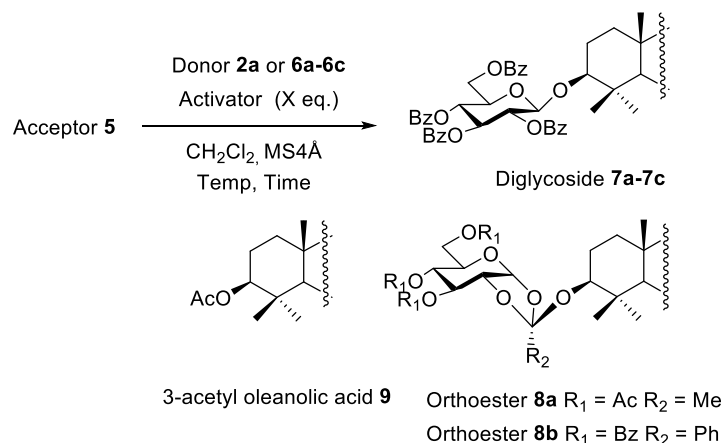


Entry	Donor 2a (eq.)	$\text{BF}_3 \cdot \text{OEt}_2$ (eq.)	Flow rate (mL/min)	Result (%)
1	1.0	0.1	0.1	no reaction
2	1.0	0.1	0.2	no reaction
3	1.0	0.1	0.3	no reaction
4	1.0	0.1	0.4	no reaction
5	1.0	0.1	0.5	no reaction
6	1.0	0.1	0.6	no reaction
7	1.0	0.1	0.7	no reaction
8	1.0	0.1	0.8	no reaction
9	1.0	0.1	0.9	no reaction
10	1.0	0.5	0.7	3.6
11	1.0	0.5	0.6	48
12	1.0	0.5	0.5	17
13	1.0	0.5	0.4	4.9
14	1.0	0.5	0.1	trace
15	1.0	0.5	0.05	trace
16	1.0	0.5	0.1	trace
17	1.0	1.0	0.7	4.5

18	1.0	1.0	0.6	51
19	1.0	1.0	0.5	4.5
20	1.0	1.0	0.4	2.6
21	2.0	0.5	0.6	quant.
22	2.0	1.0	0.6	quant.
23	2.0	0.5	0.7	quant.
24	2.0	0.5	0.5	quant.
25	2.0	0.5	0.4	quant.
26	2.0	0.5	0.1	quant.
27	2.0	1.0	0.5	quant.
28	2.0	1.0	0.4	76
29	2.0	1.0	0.1	quant.
30	2.0	1.0	0.7	quant.
31	1.5	0.1	0.6	0.9
32	1.5	0.5	0.6	59
33	1.5	1.0	0.6	66
34	2.0	0.1	0.6	11
35	2.0	0.5	0.8	75

III. Procedure for C-3 glycosylation of **5** with acetylated donor **2a** and benzoylated donor **6a**, **6b**, **6c** using batch apparatus.

Table S2. The study of glycosylation at C-3 in batch reaction toward synthesis of diglycoside **7a-7c**.



Entry	eq.	Donor Structure	Activator (eq.)	Temp (°C)	Time (min)	Product	Result (%)
1	1.0		$\text{BF}_3 \cdot \text{OEt}_2$ (1.0)	-40	60	9	-
2	1.0		TMSOTf (1.0)	0	10	-	no reaction
3	3.0		TMSOTf (0.1)	-40	10	Orthoester 8a	21 ~ 63
4	2.0	2a	TMSOTf (0.1)	r.t.	10	9	-
5	1.5		TMSOTf (0.1)	r.t.	50	Diglycoside 7a (β only) Orthoester 8b	7a : 26 8b : 43
6	1.5		TMSOTf (1.0)	r.t.	10	Diglycoside 7a (β only)	69
7	3.0	6a	TMSOTf (1.0)	r.t.	10	Diglycoside 7a (β only)	70
8	3.0		TMSOTf (1.0)	r.t.	20	Diglycoside 7b (β only)	64
9	3.0		TMSOTf (1.0)	r.t.	20	Diglycoside 7c (β only)	72

Under a variety of reaction conditions, The glycosylation using acetylated **2a** did not give the protected diglycoside **7a** (entries 1-4); instead, the acyloxonium ion intermediate derived from the acetyl imidate donor produced orthoester **8a**^{SI-i}, which then rearranged to acetylated oleanolic acid **9**. The structure of orthoester **8a** was determined by a chemical shift on the ¹H NMR at anomeric proton compared to β glycosidic bond.

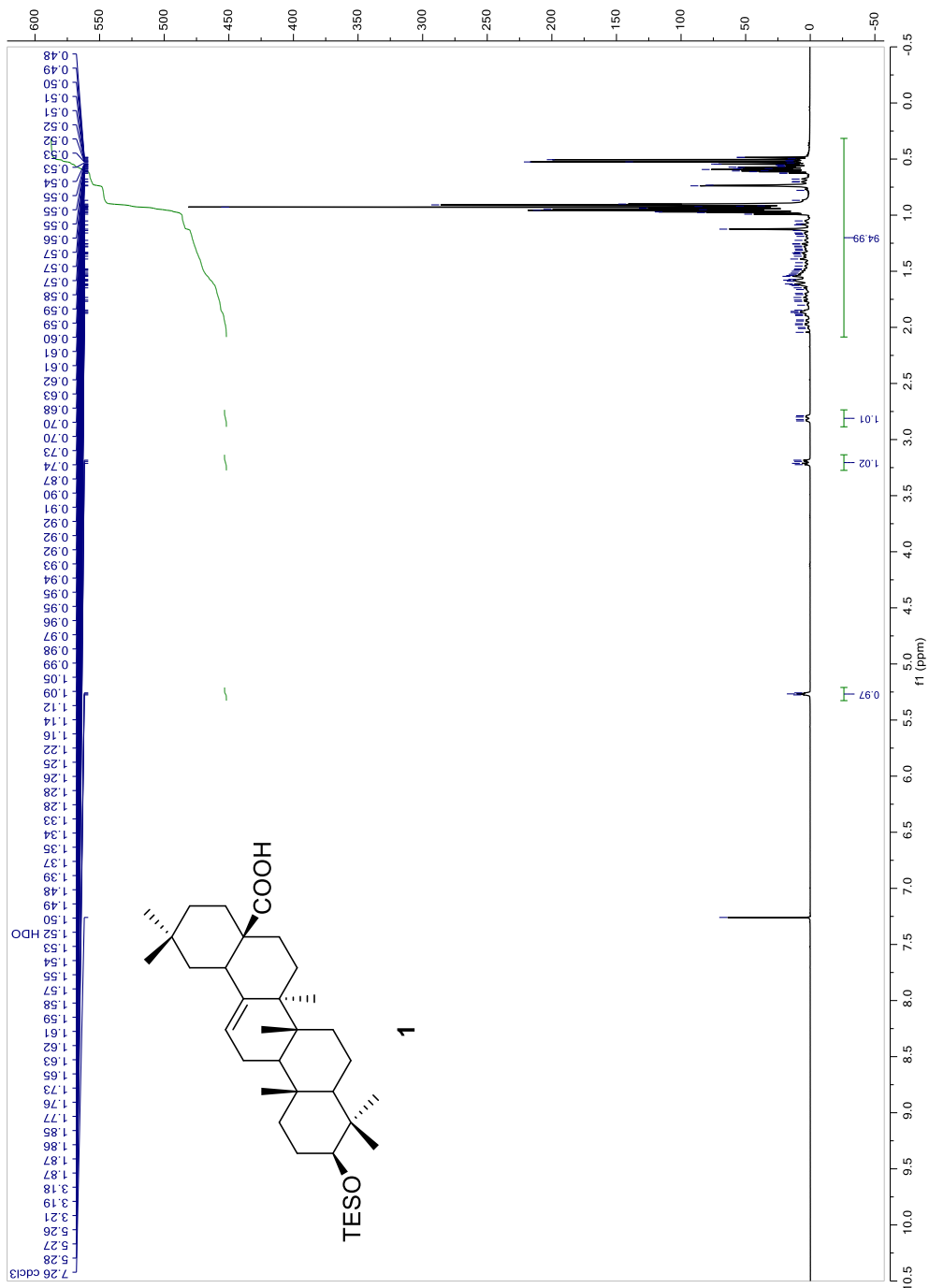
In order to minimize orthoester formation, the glycosidic linkage at C-3 was constructed using benzoylated glucosyl imidate donor **6a**^{SI-ii}. The glycosylation reaction in the presence of a catalytic amount of TMSOTf afforded diglycoside **7a** in low yield along with orthoester **8b** in moderate yield (entry 5). On the other hand, when a stoichiometric amount of TMSOTf was used, **8b** was not detected and **7a** was the only product (entry 6). Using 3.0 eq of glycosyl donor **6a** did not improve the yield of **7a** as compared to the reaction using 1.5 eq of **6a** (entries 6 and 7).

SI-i a) Zhu, X.; Yu, B.; Hui, Y.; Schmidt, R. R. *Eur. J. Org. Chem.* **2004**, 2004, 965–973. b) Kuczynska, K.; Pakulski, Z. *Tetrahedron* **2015**, 71, 2900–2905.

SI-ii Lee, W.-S.; Kim, W.-I.; Kim, K.-T.; Chung, S.-K. *Bull. Korean Chem. Soc.* **2011**, 32, 2286–2300.

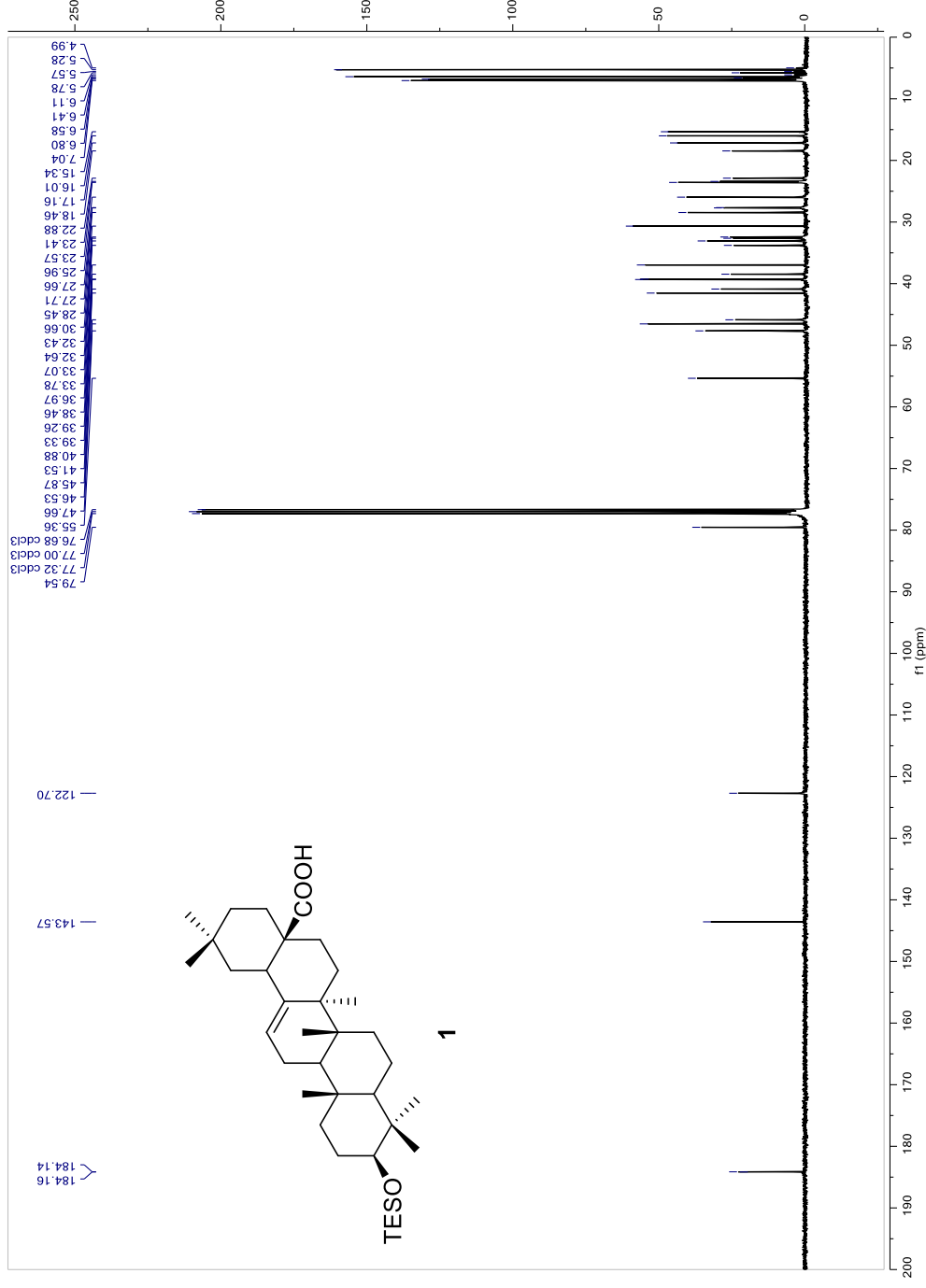
IV-1. ^1H and ^{13}C NMR chart of prepared compounds

400-MR (400 MHz, CDCl_3)



The assignments were confirmed
by ^1H - ^1H COSY, HSQC and HMBC.

400-MR (400 MHz, CDCl₃)

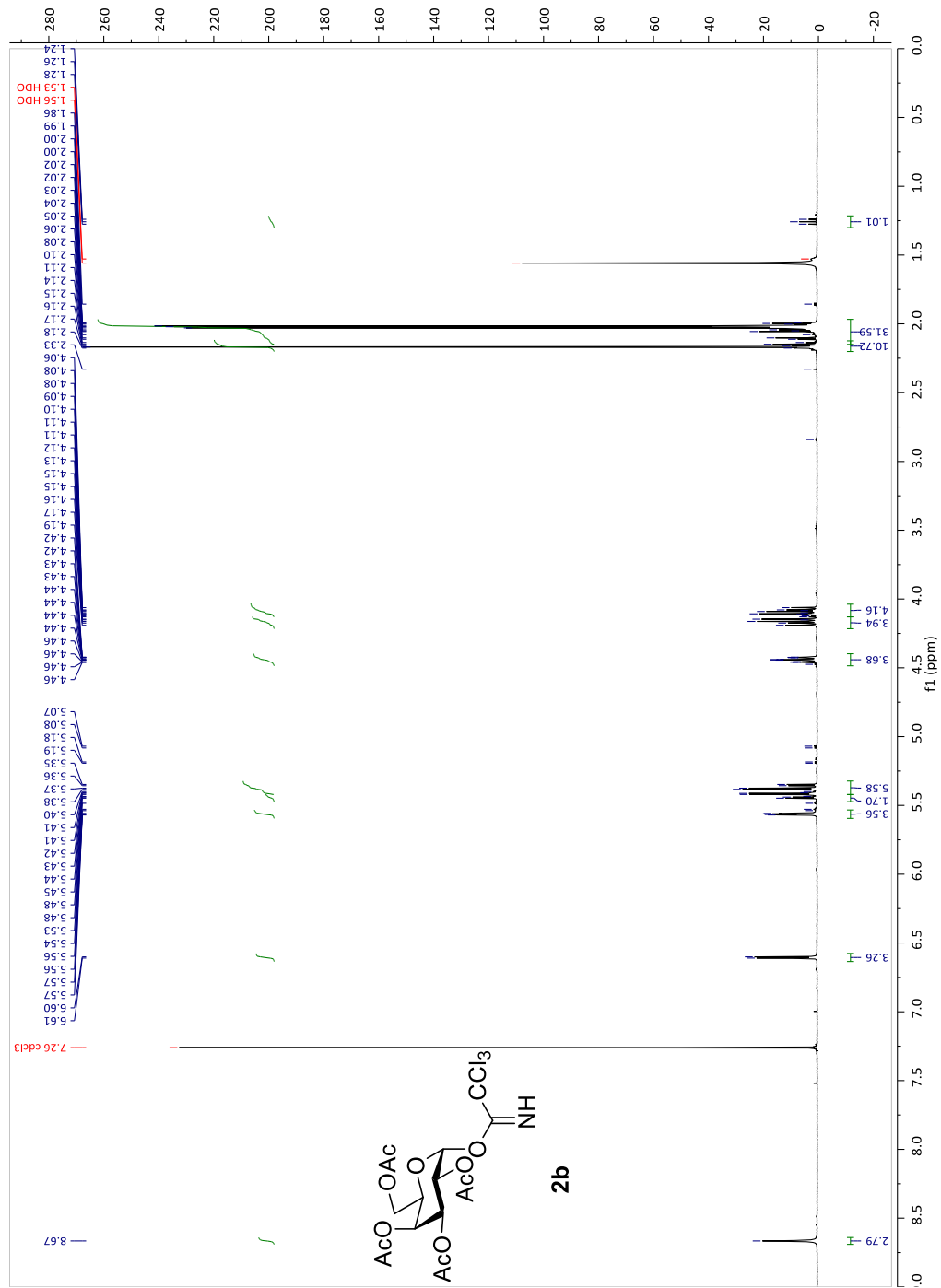


S9

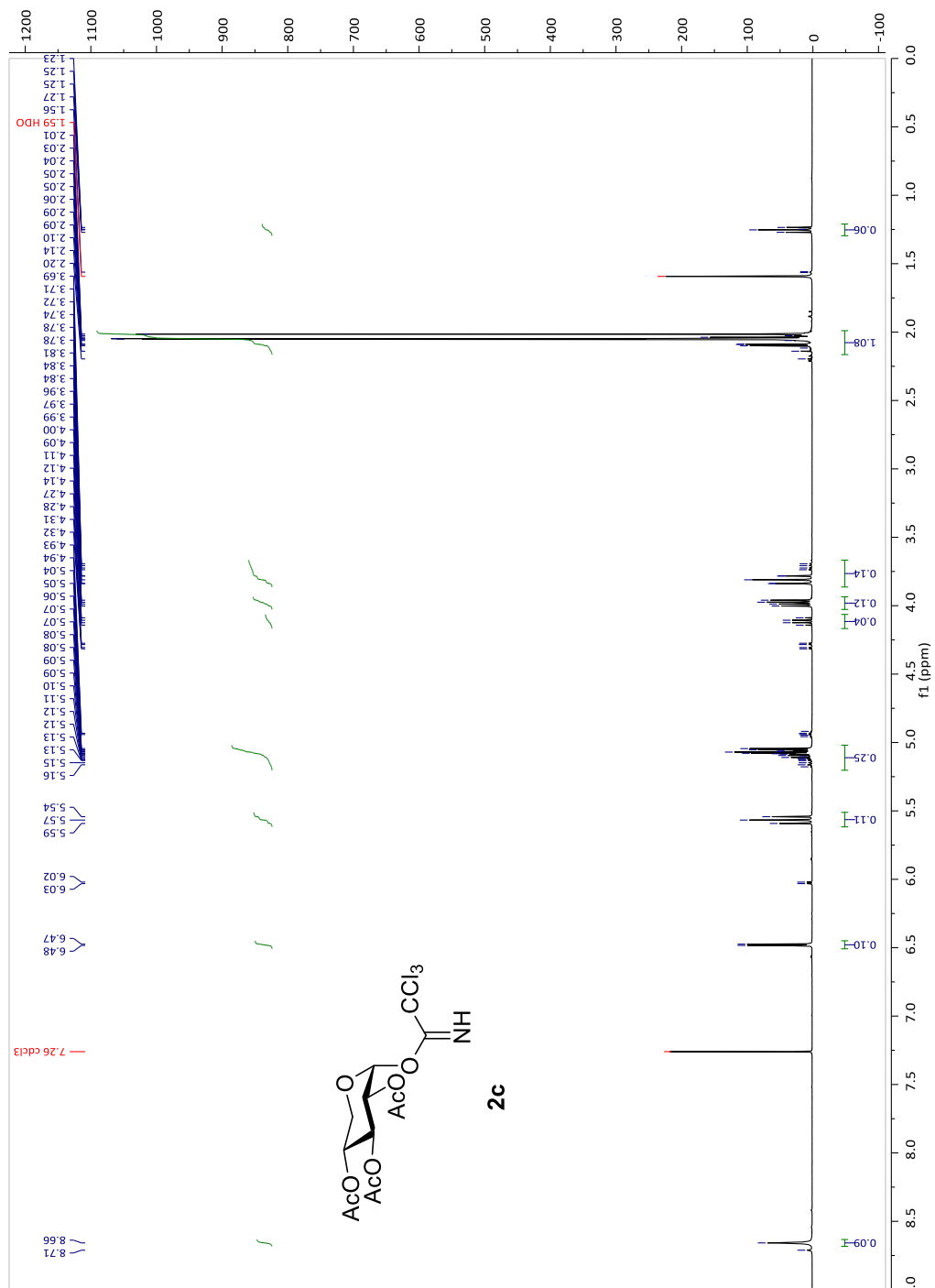
The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

9

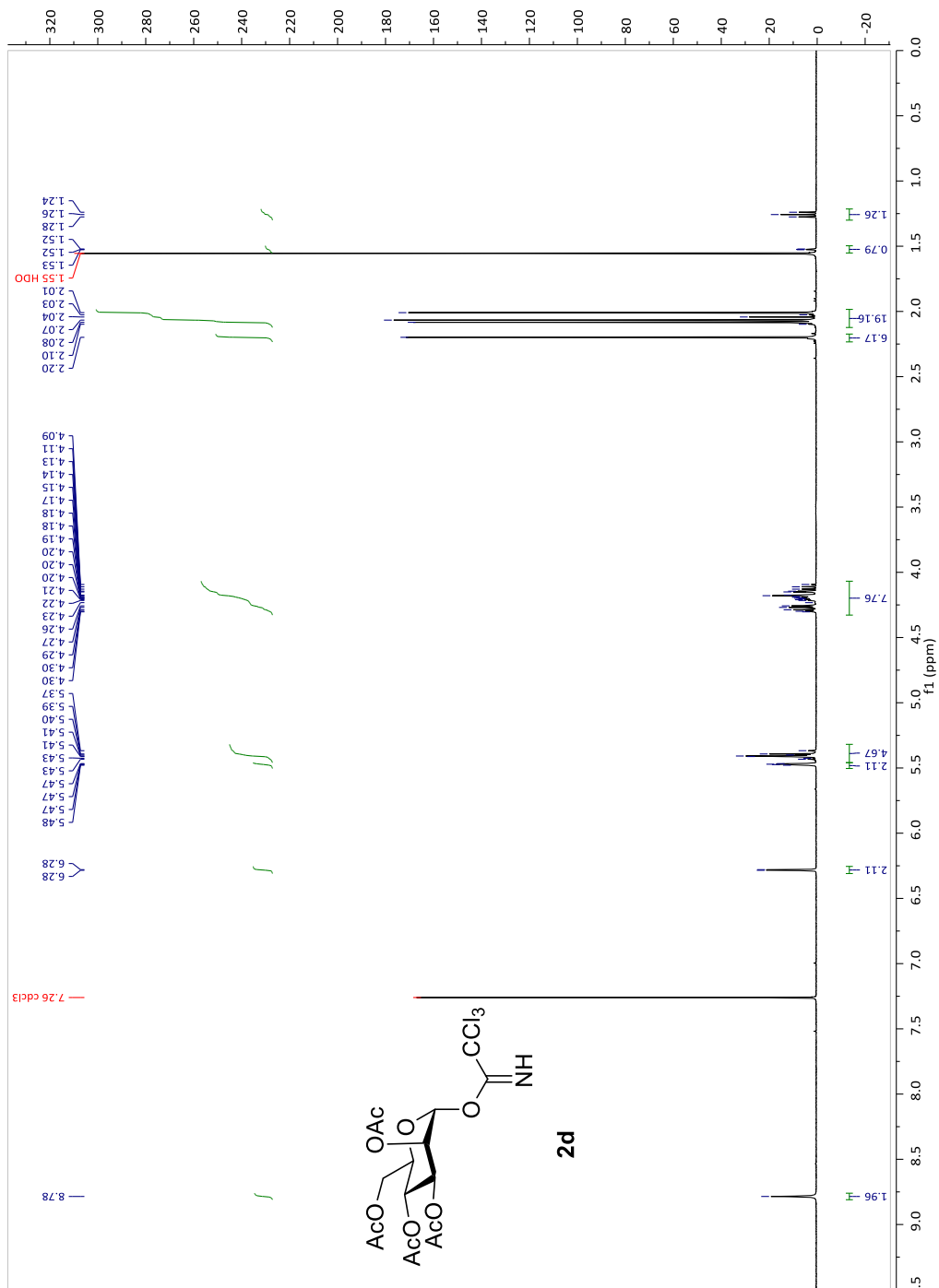
400-MR DD2 (400 MHz, CDCl₃)



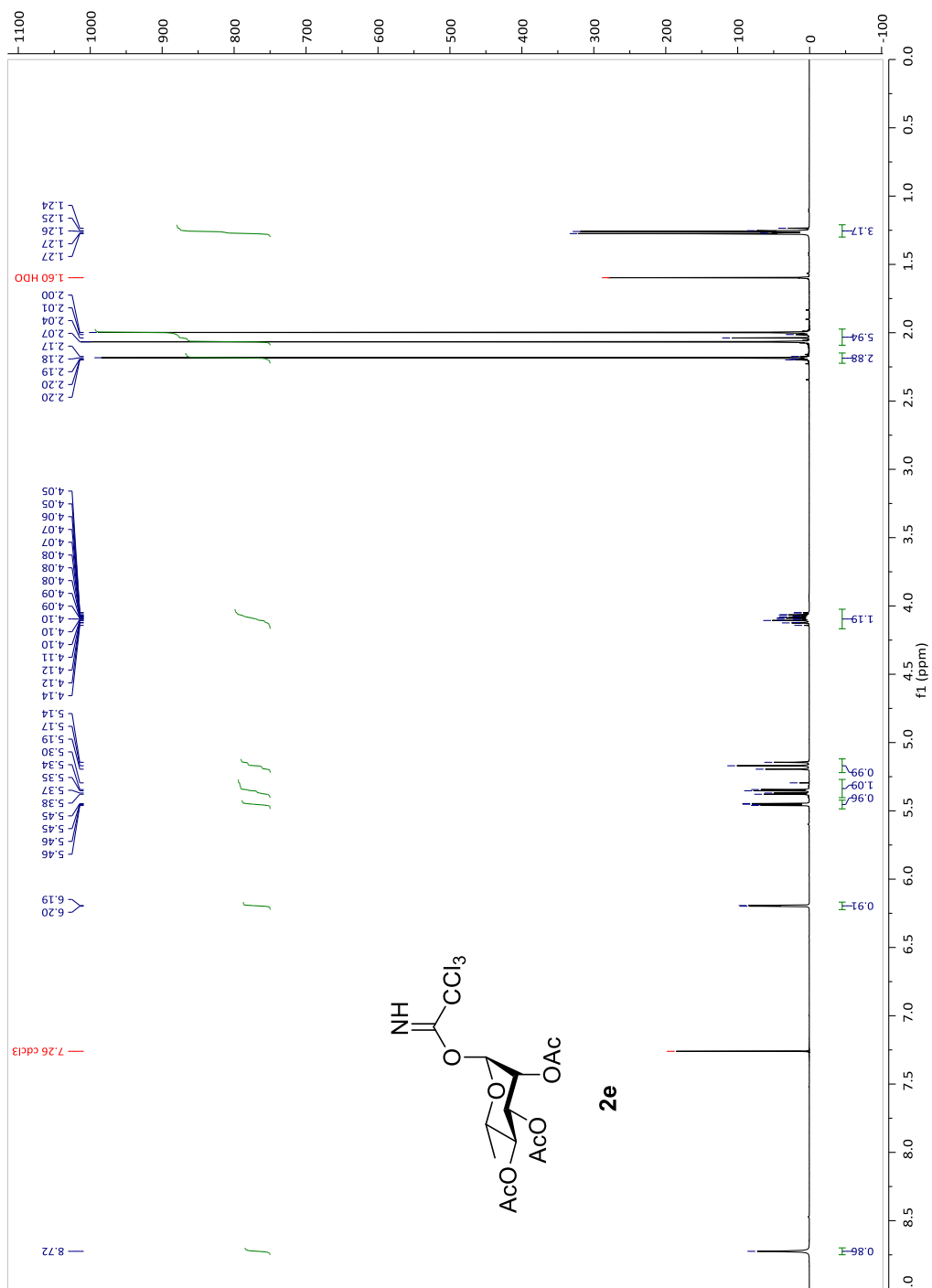
400-MR DD2 (400 MHz, CDCl₃)



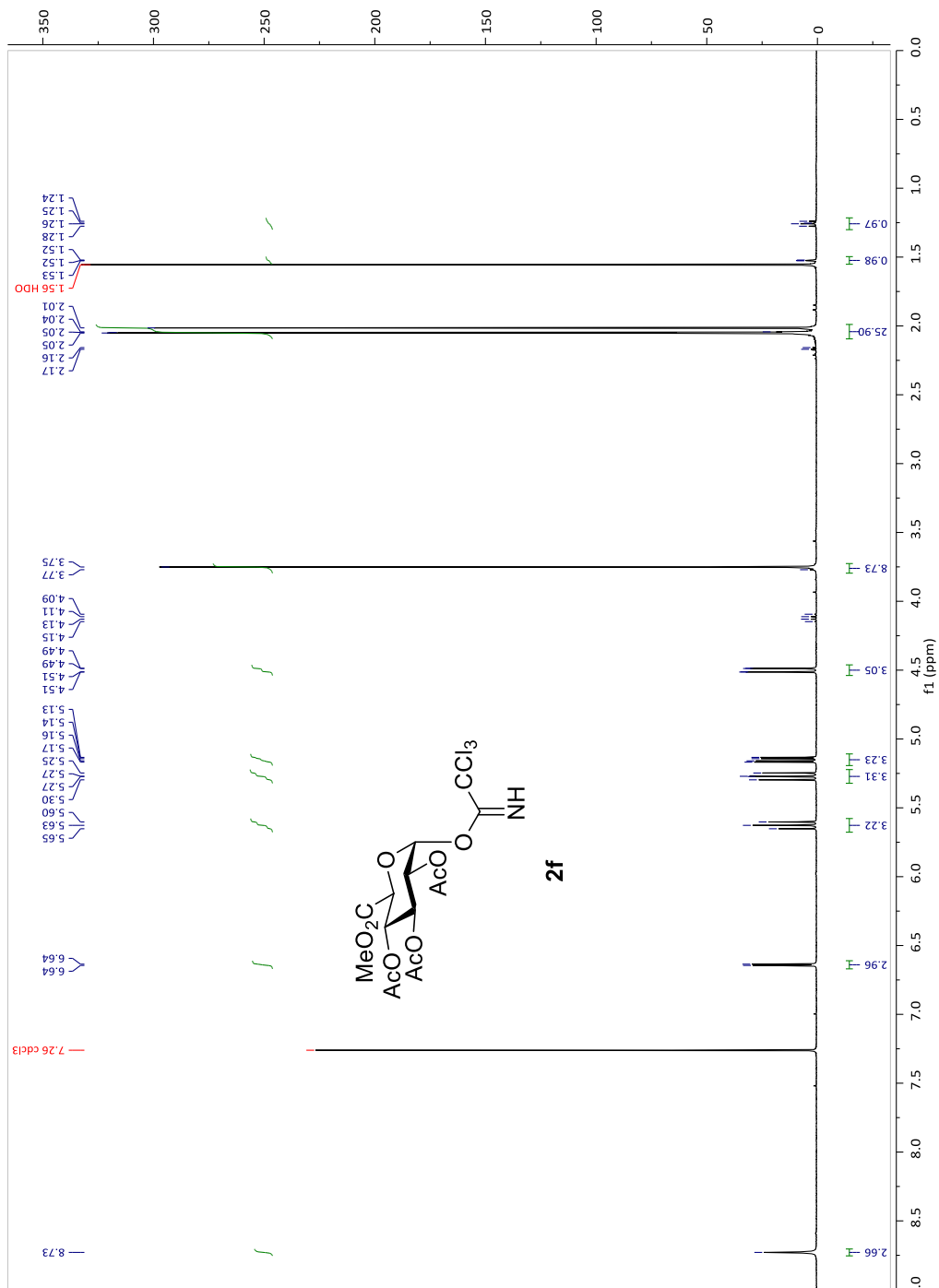
400-MR DD2 (400 MHz, CDCl₃)

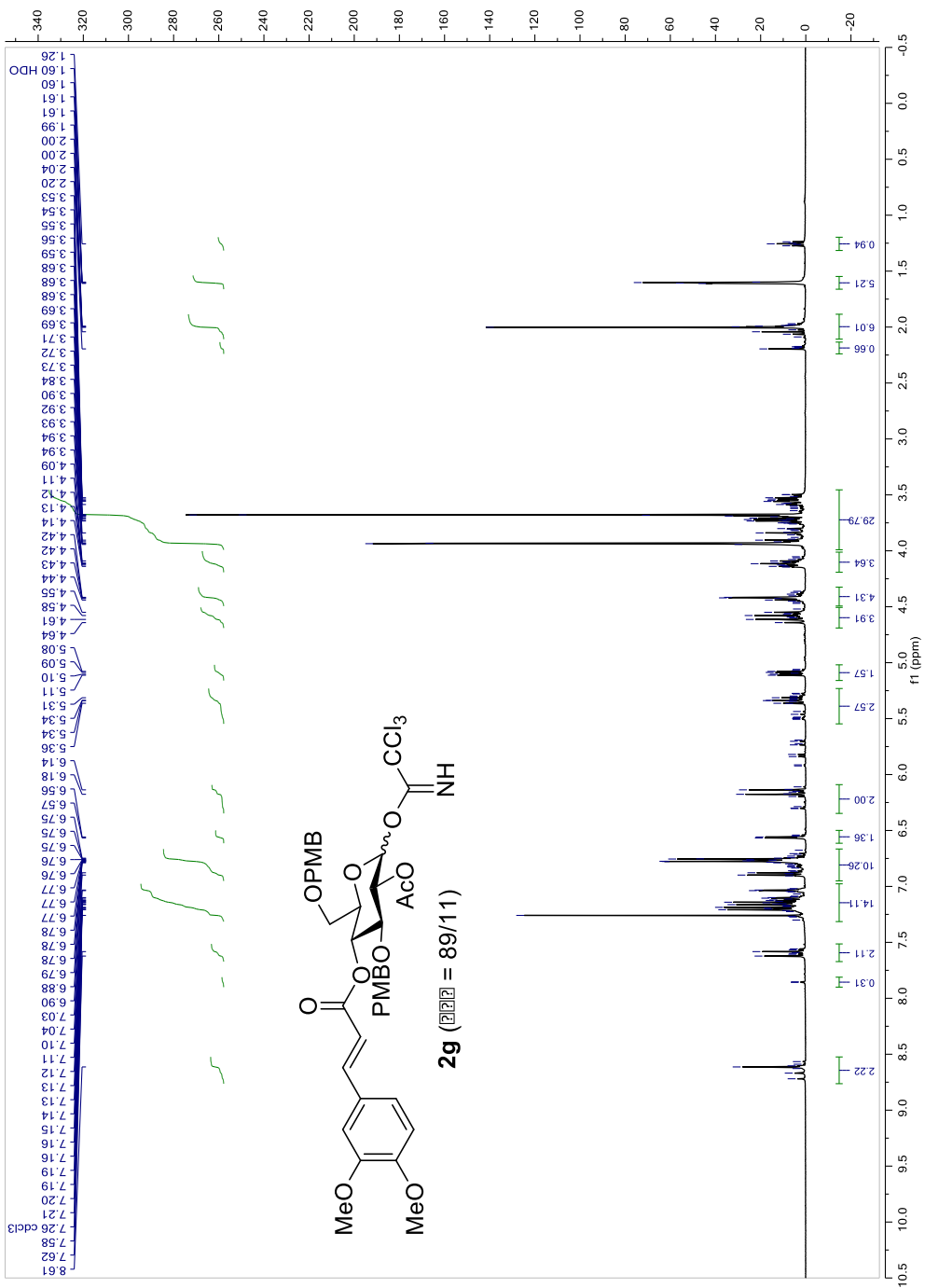


400-MR DD2 (400 MHz, CDCl₃)

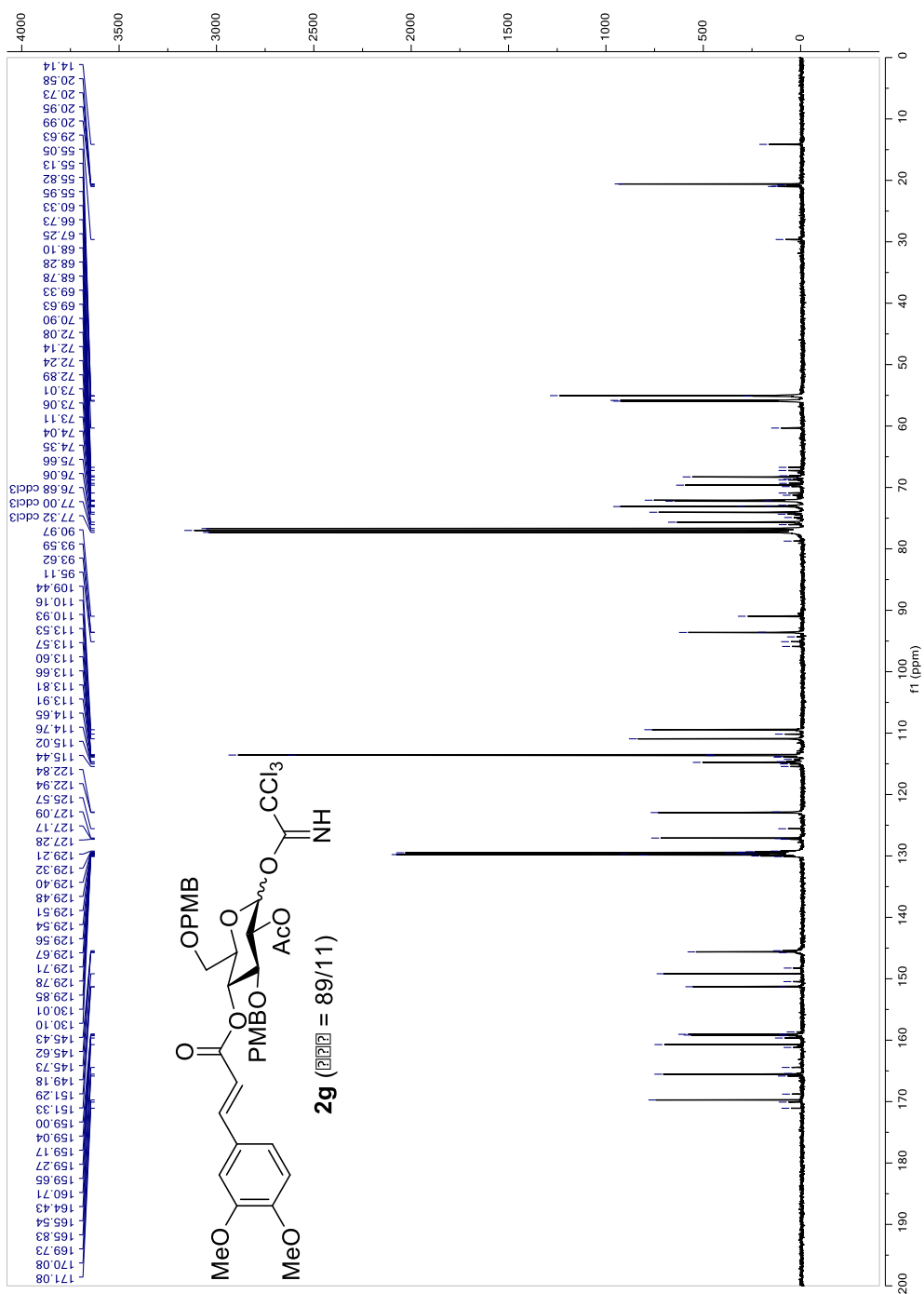


400-MR DD2 (400 MHz, CDCl₃)

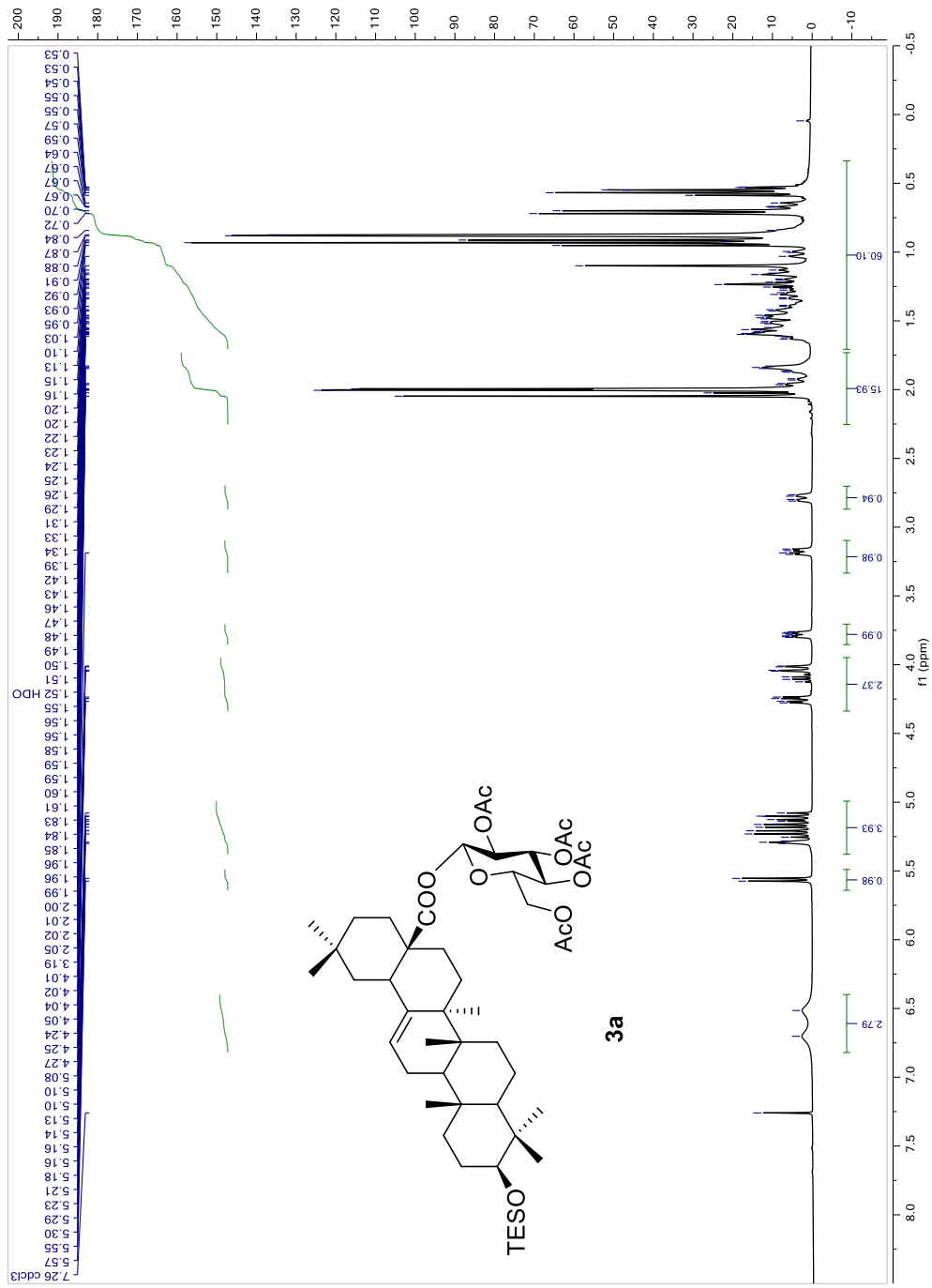


400-MR (400 MHz, CDCl_3)

400-MR (400 MHz, CDCl₃)

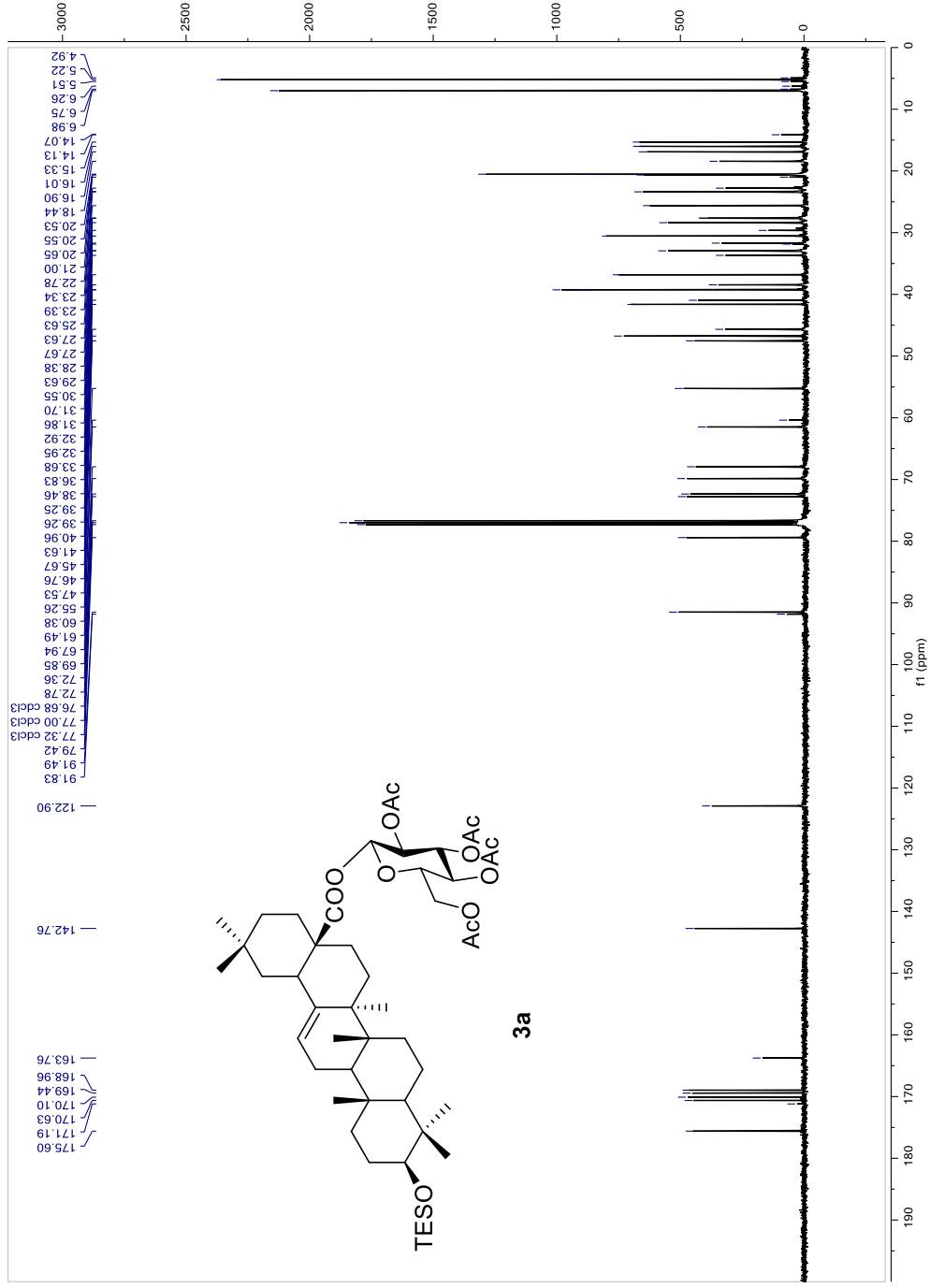


400-MR (400 MHz, CDCl₃)



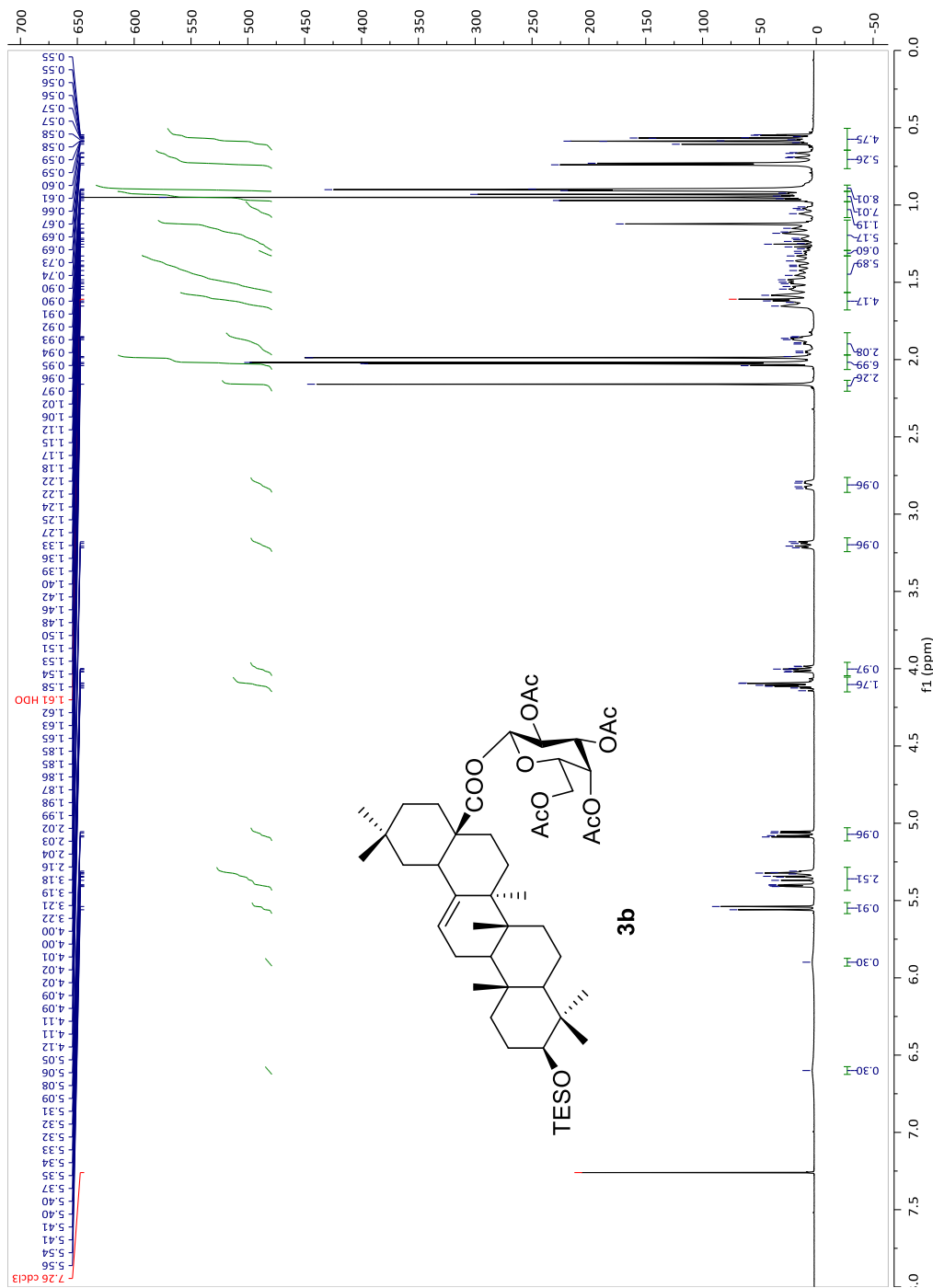
The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

400-MR (400 MHz, CDCl₃)



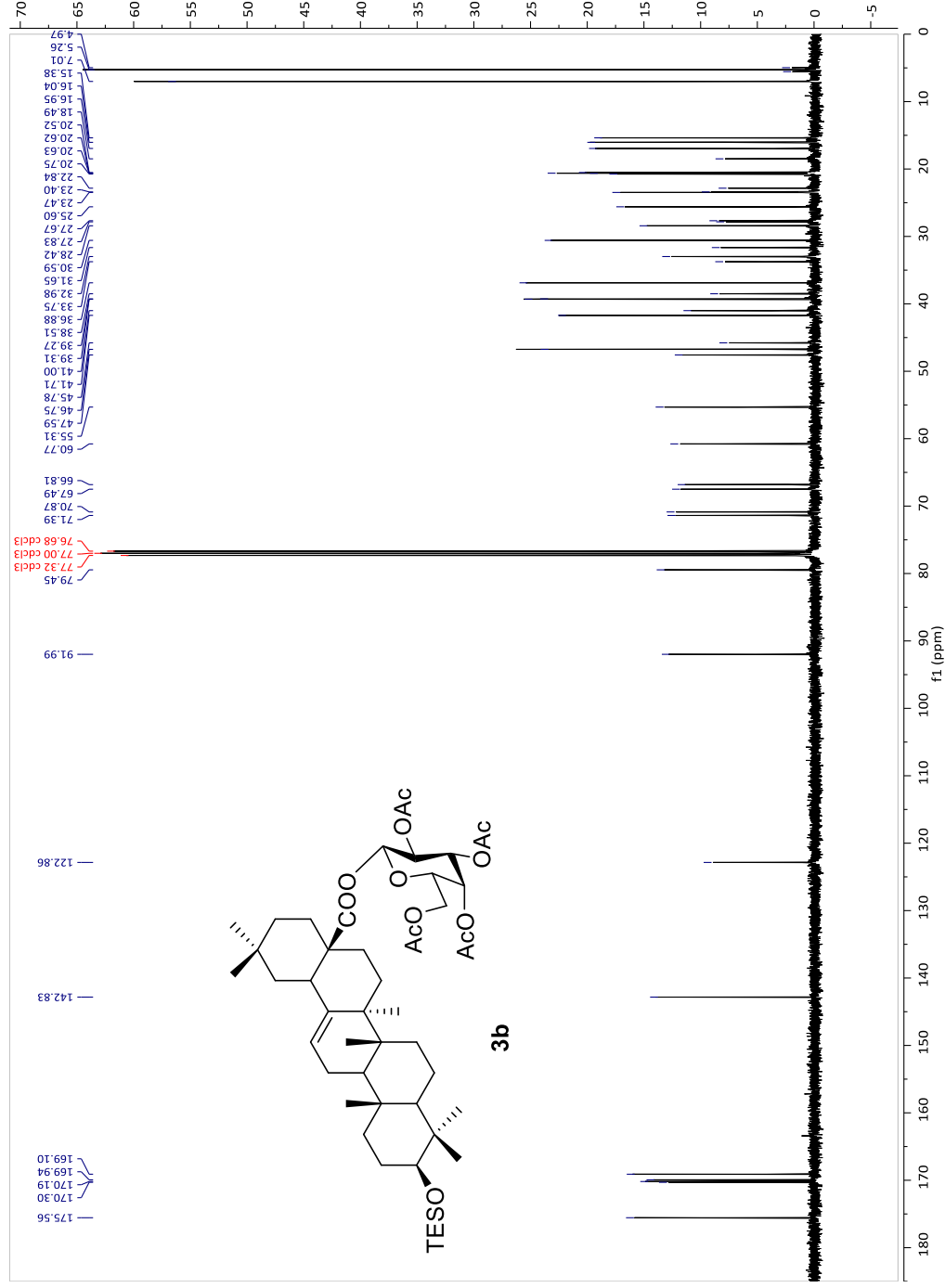
The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, CDCl₃)



The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, CDCl₃)



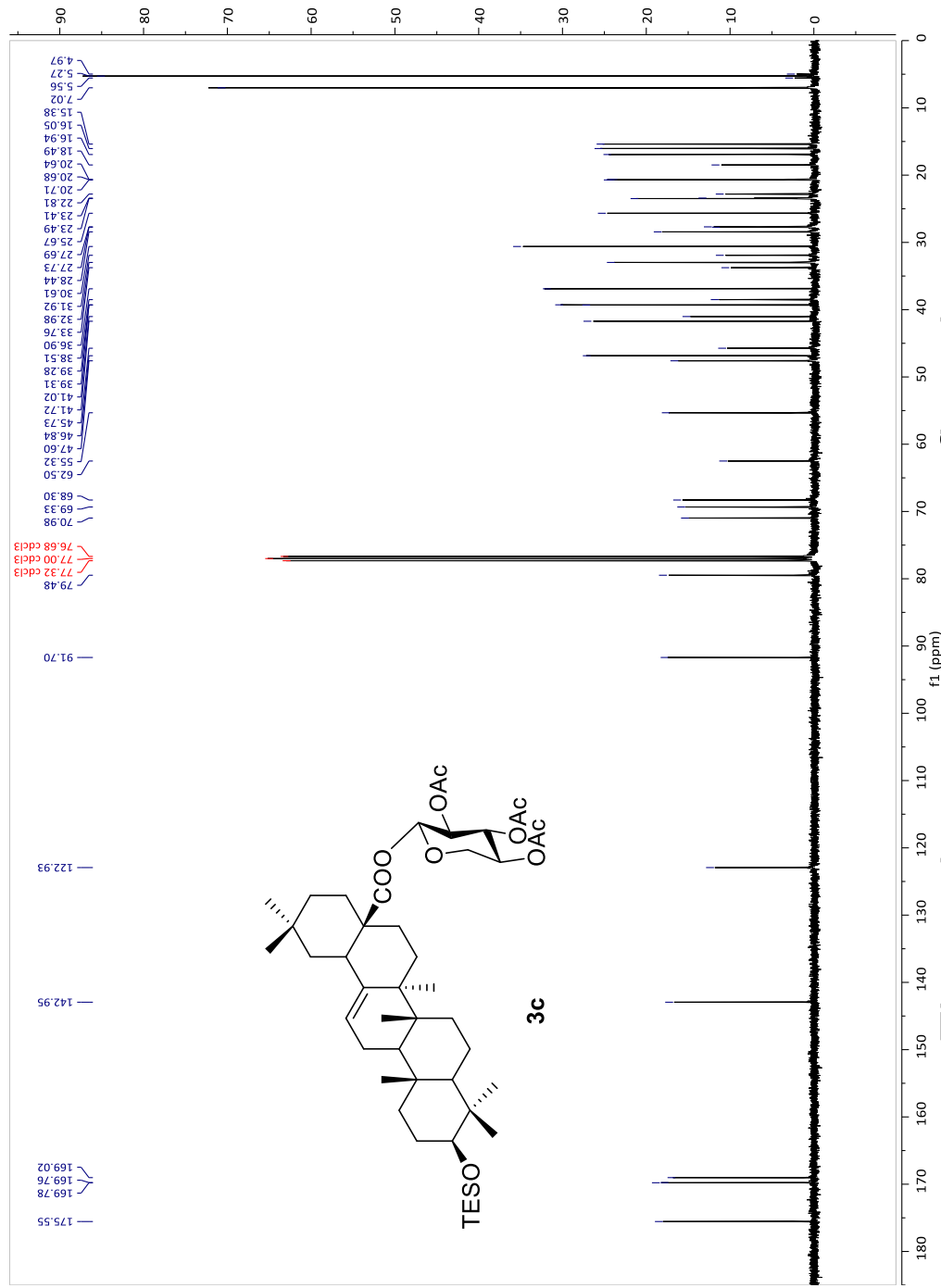
The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

S21



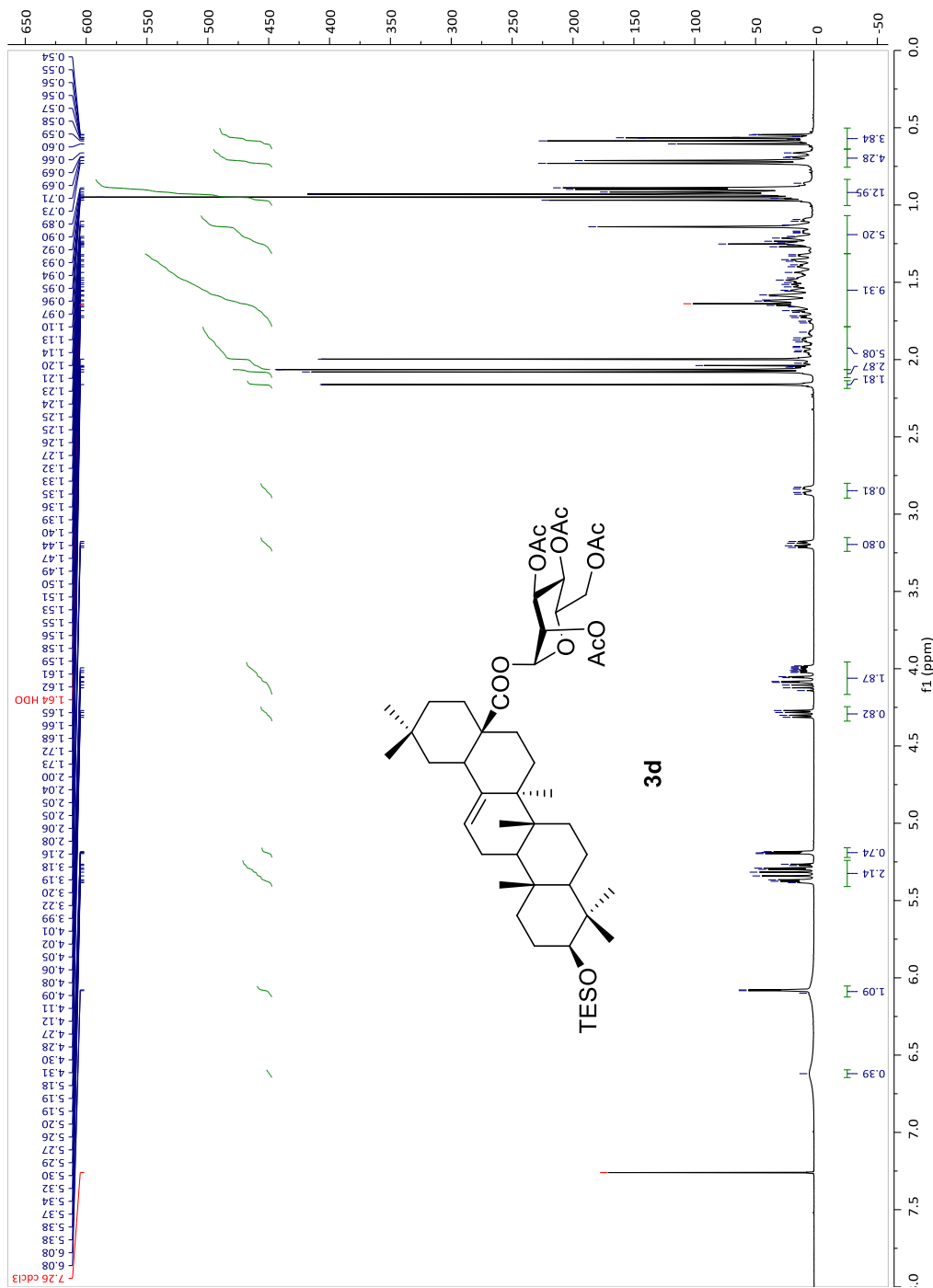
The assignments were confirmed by ^1H - ^1H COSY, HSQC and HMQC.

400-MR DD2 (400 MHz, CDCl₃)



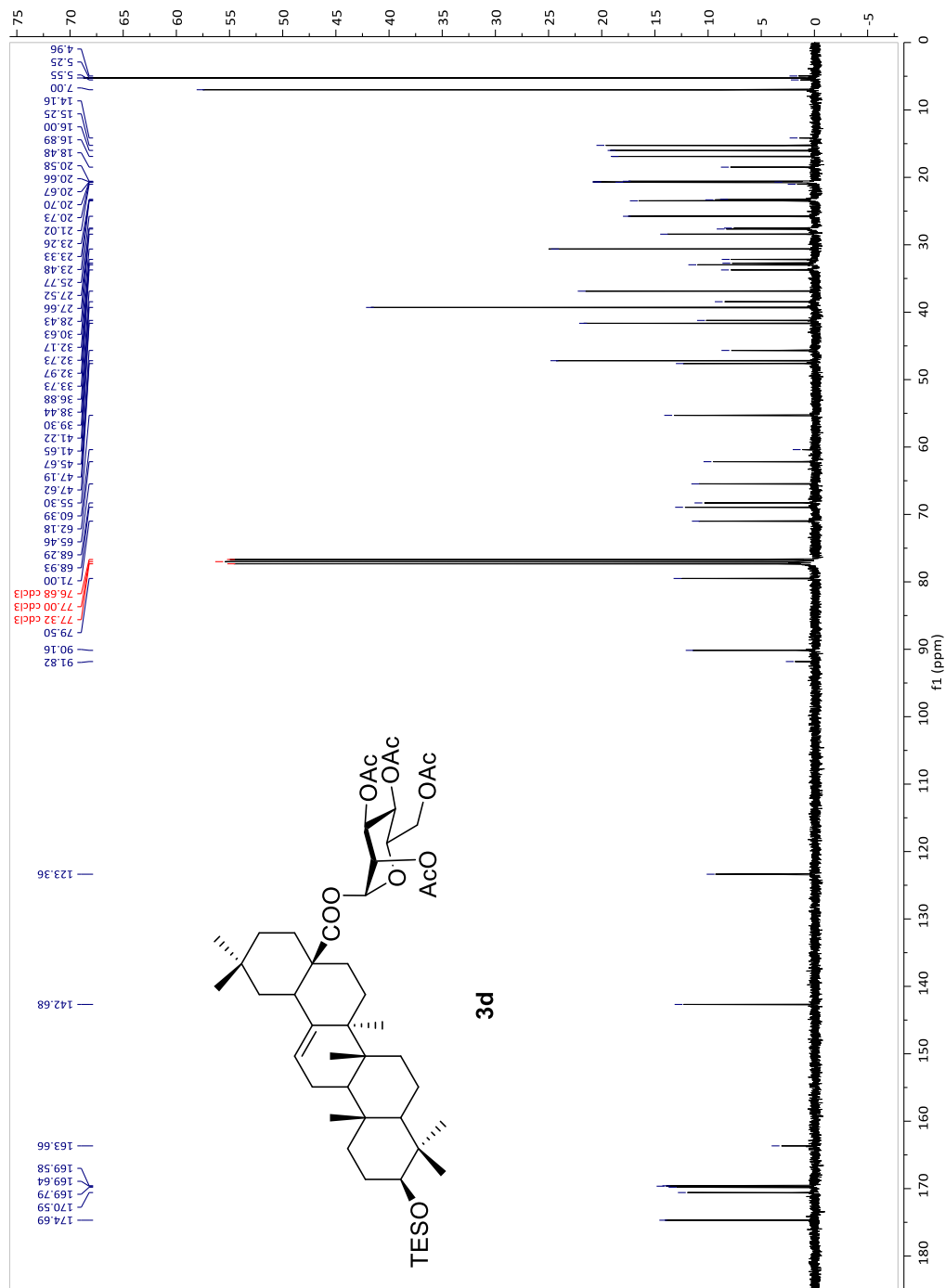
The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, CDCl₃)



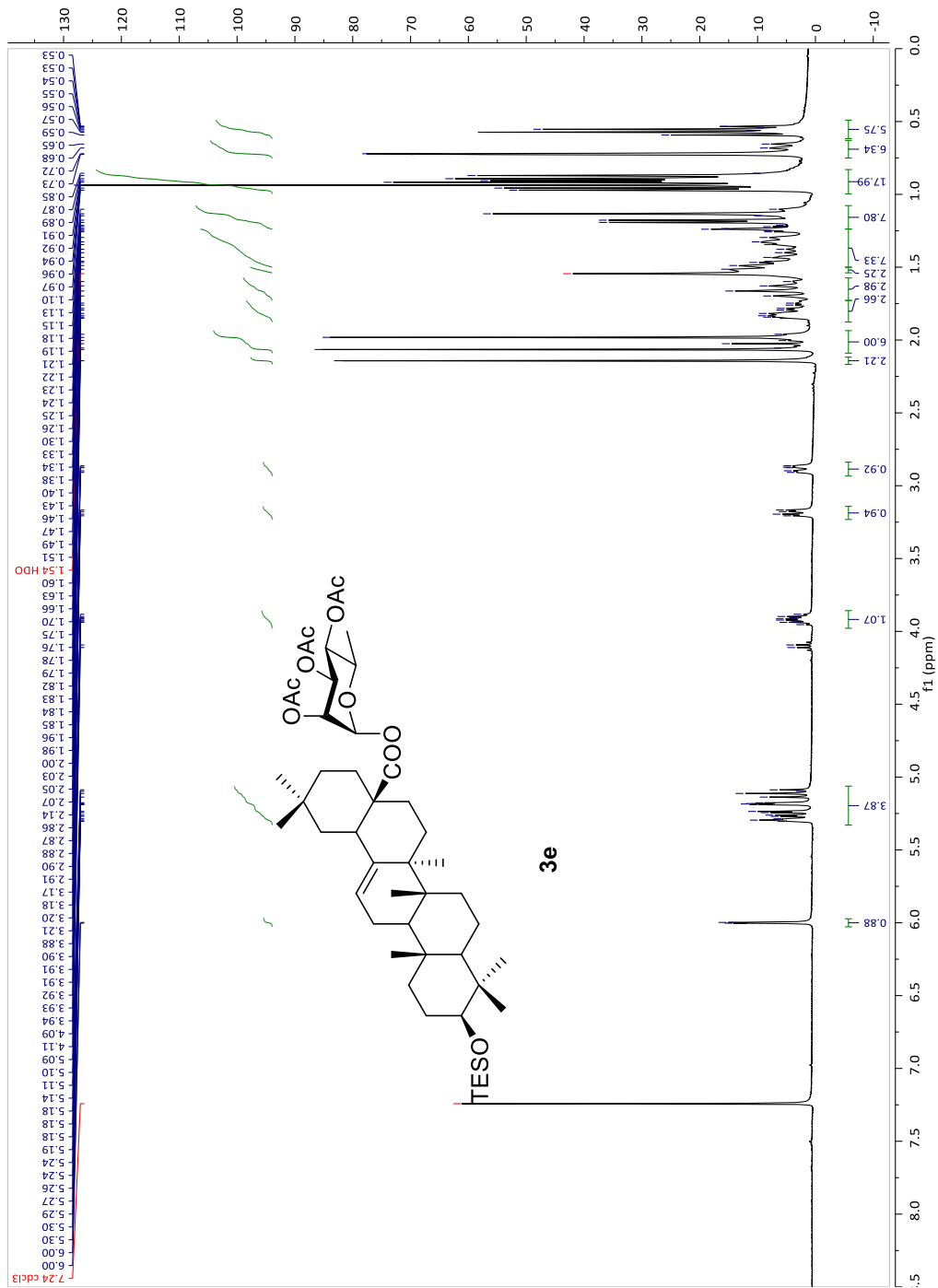
The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, CDCl₃)

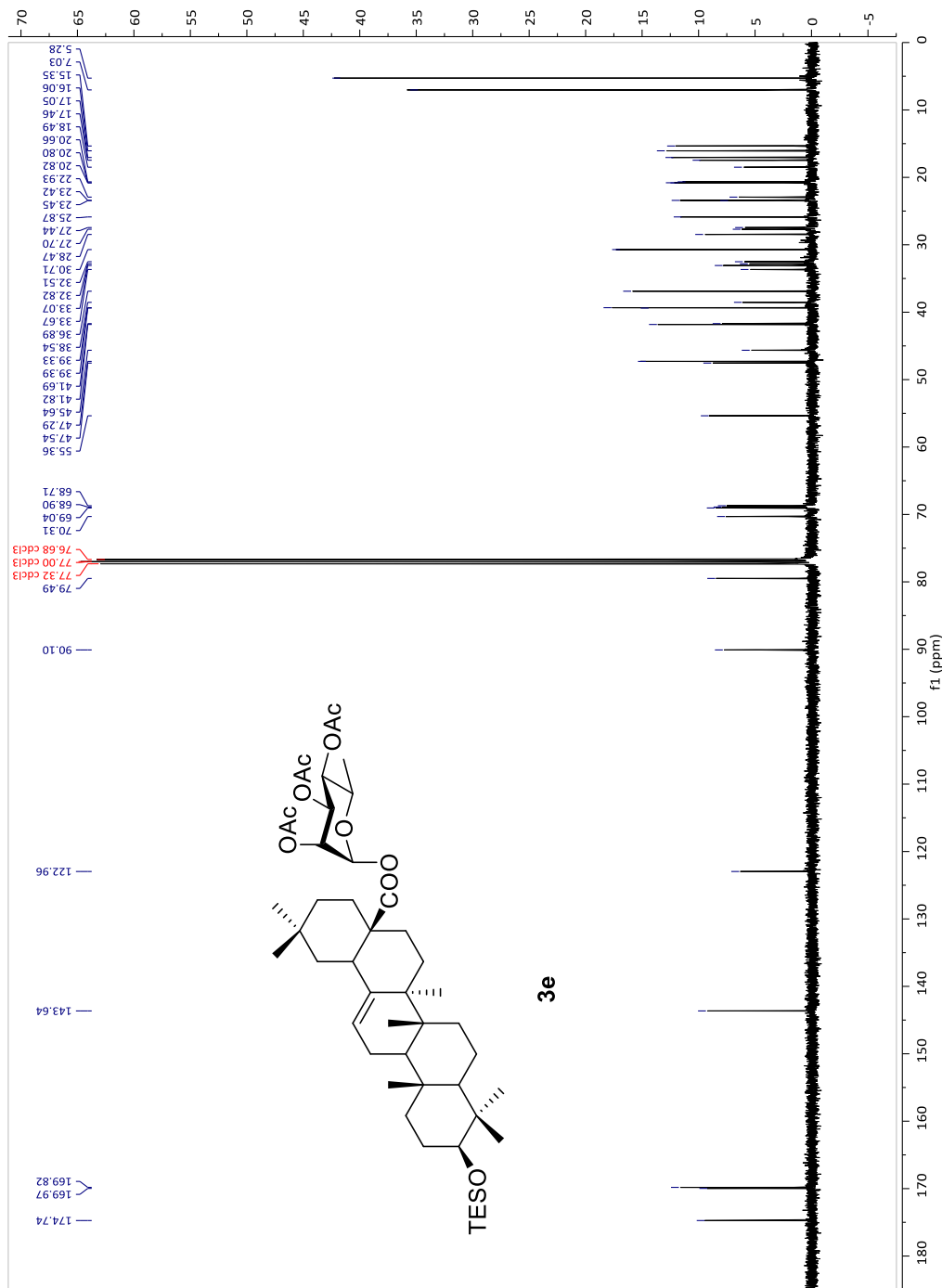


The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, CDCl₃)

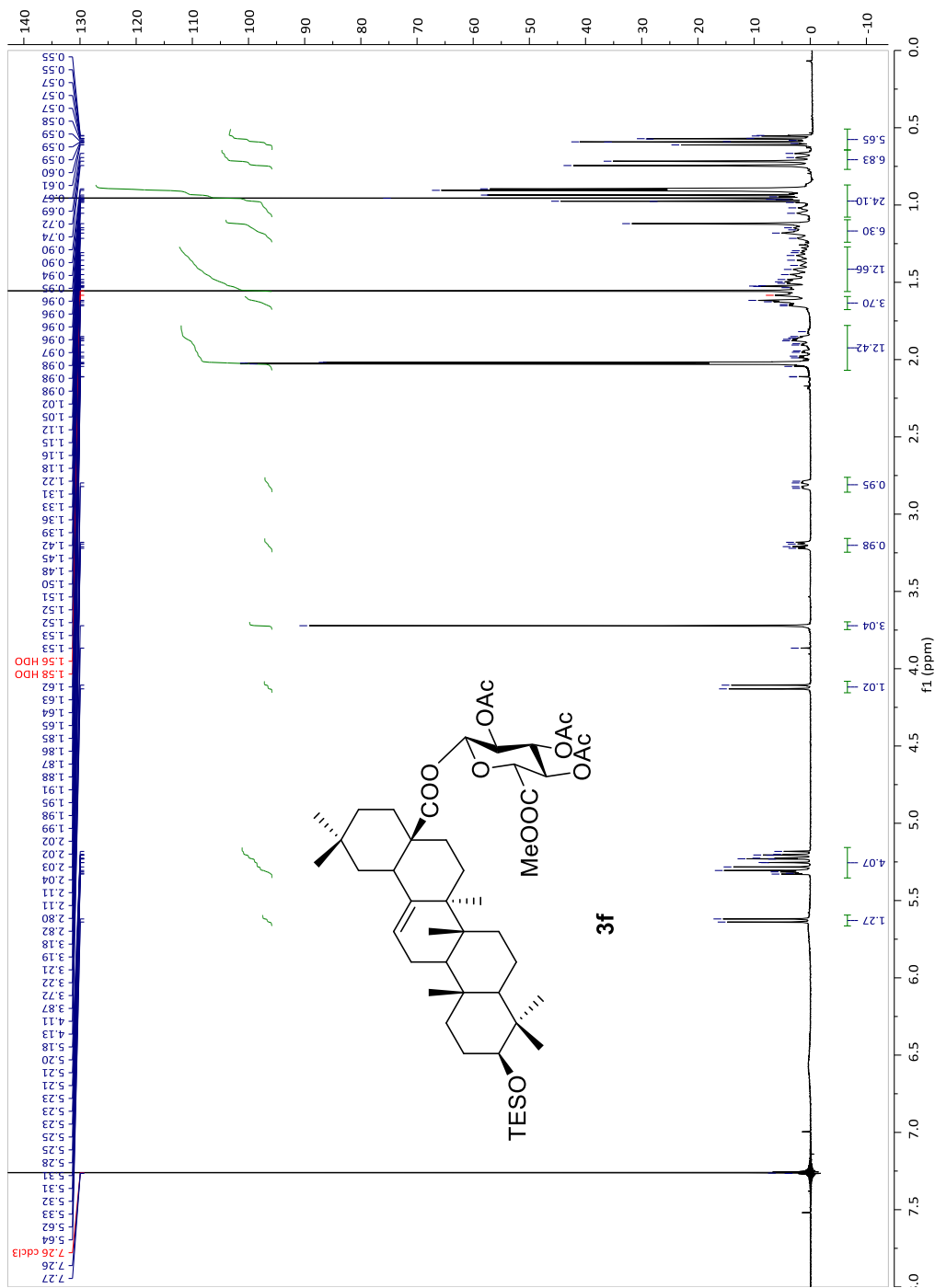


400-MR DD2 (400 MHz, CDCl₃)



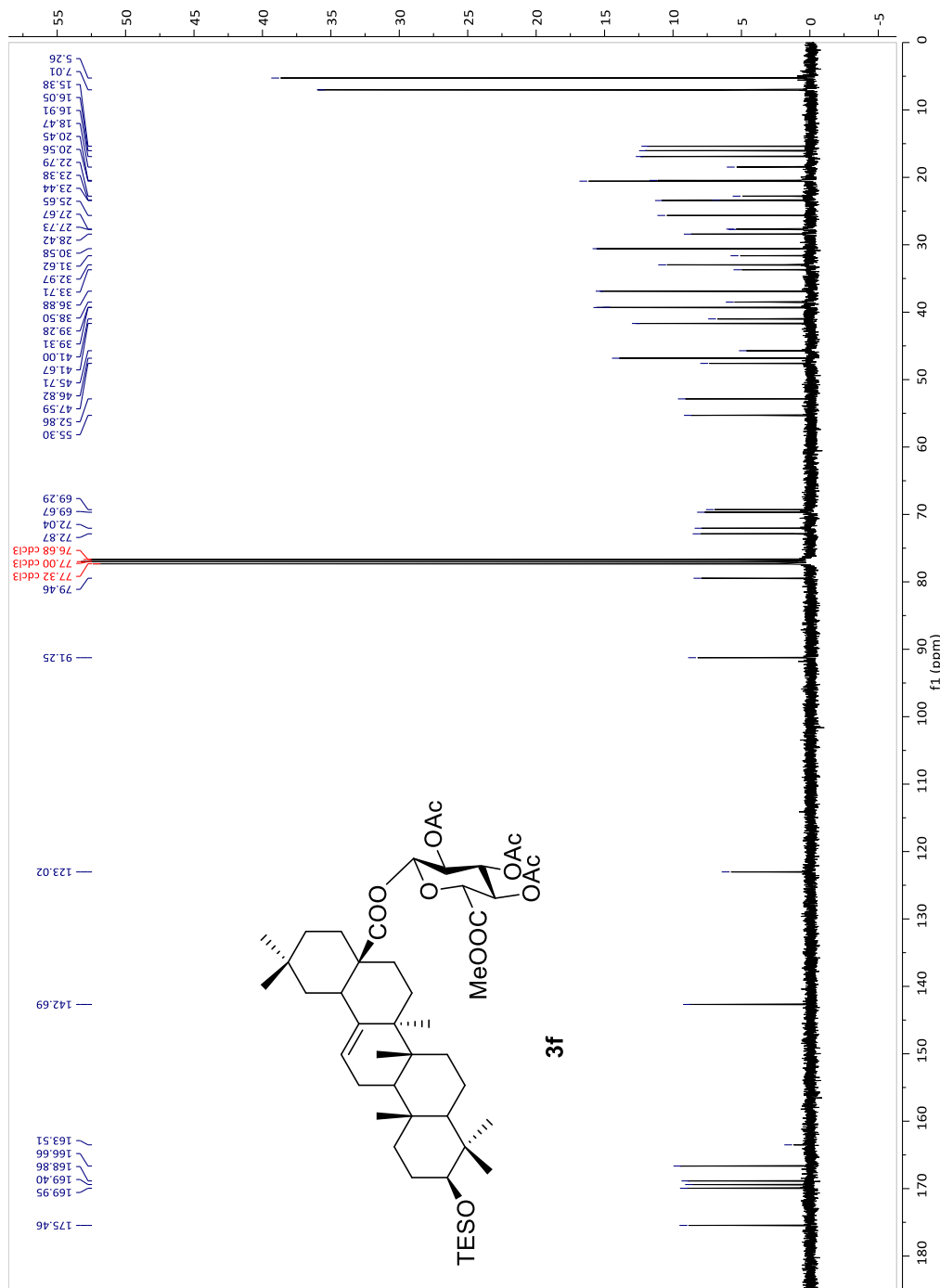
The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, CDCl₃)



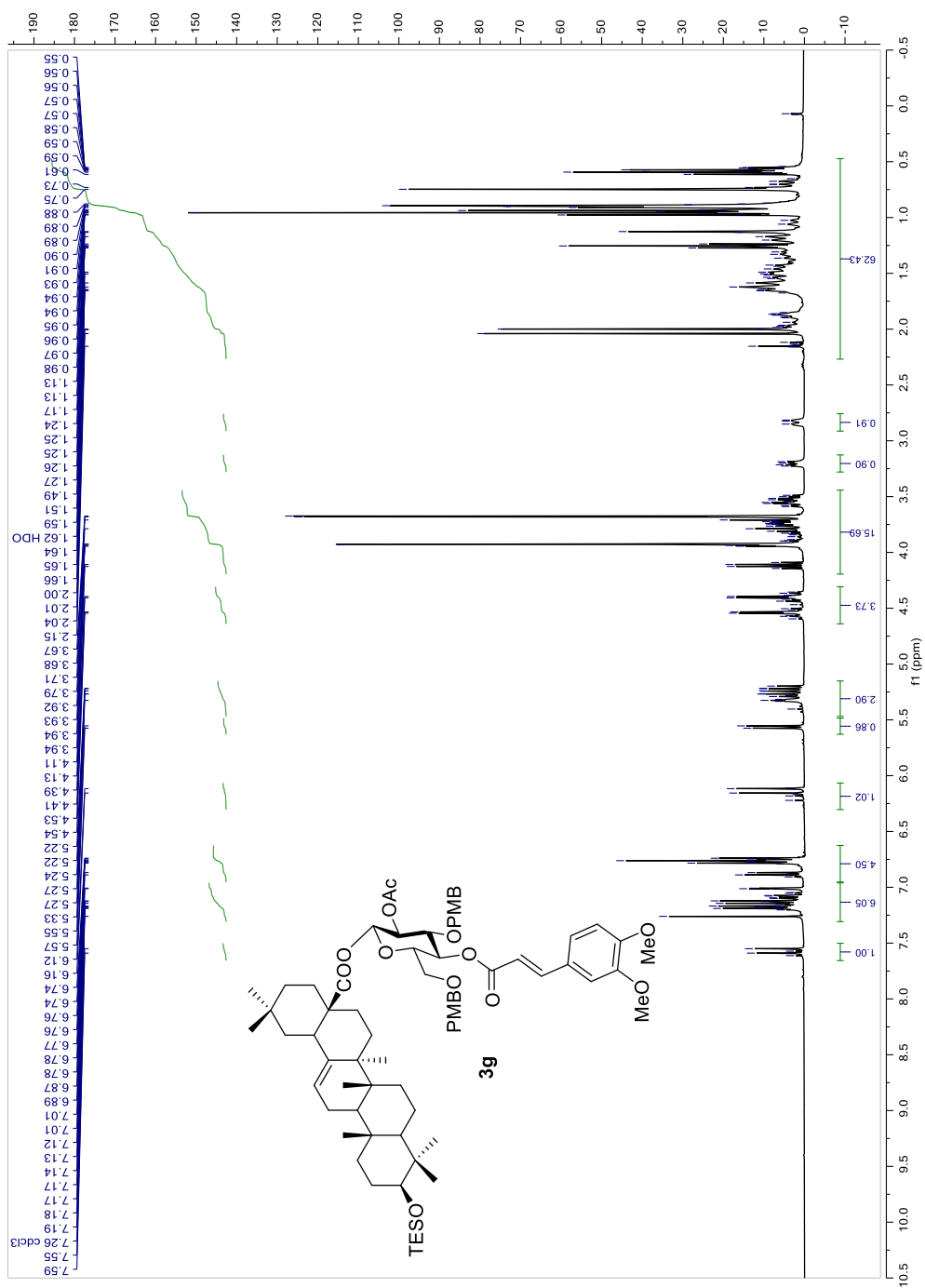
The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, CDCl₃)



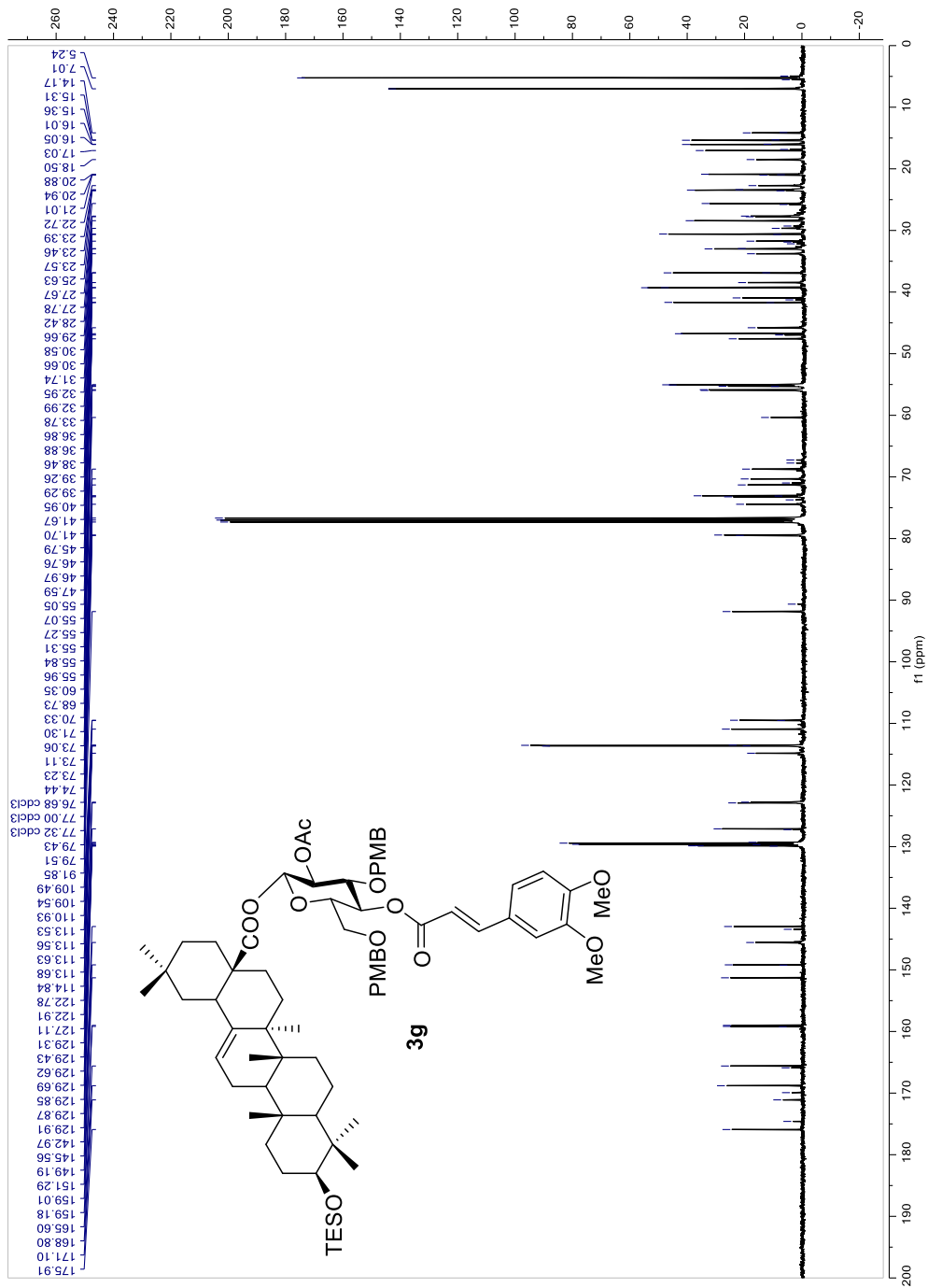
The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

400-MR (400 MHz, CDCl₃)



The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

400-MR (400 MHz, CDCl₃)

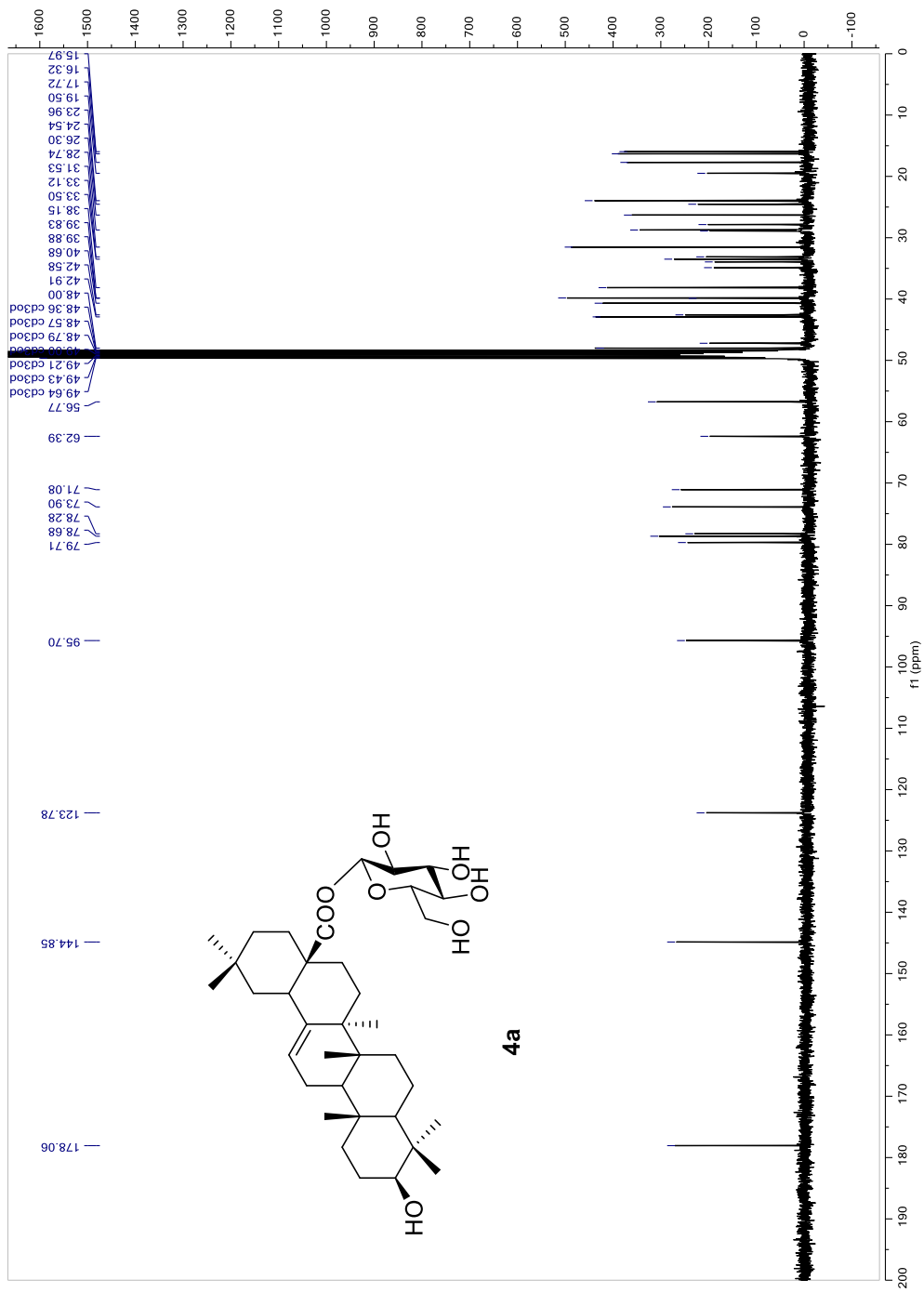


The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

[illegible]

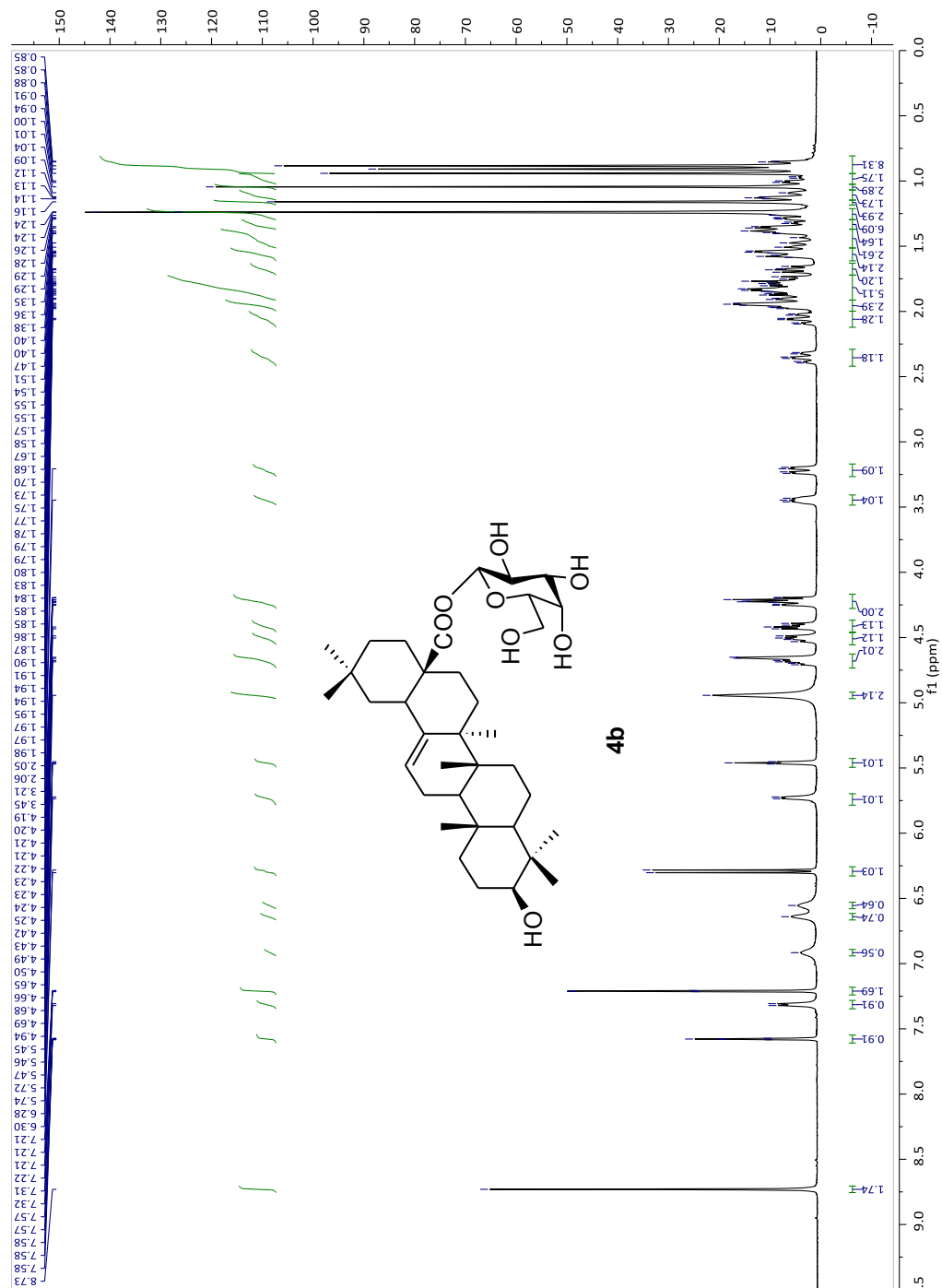
31

400-MR (400 MHz, CD₃OD)



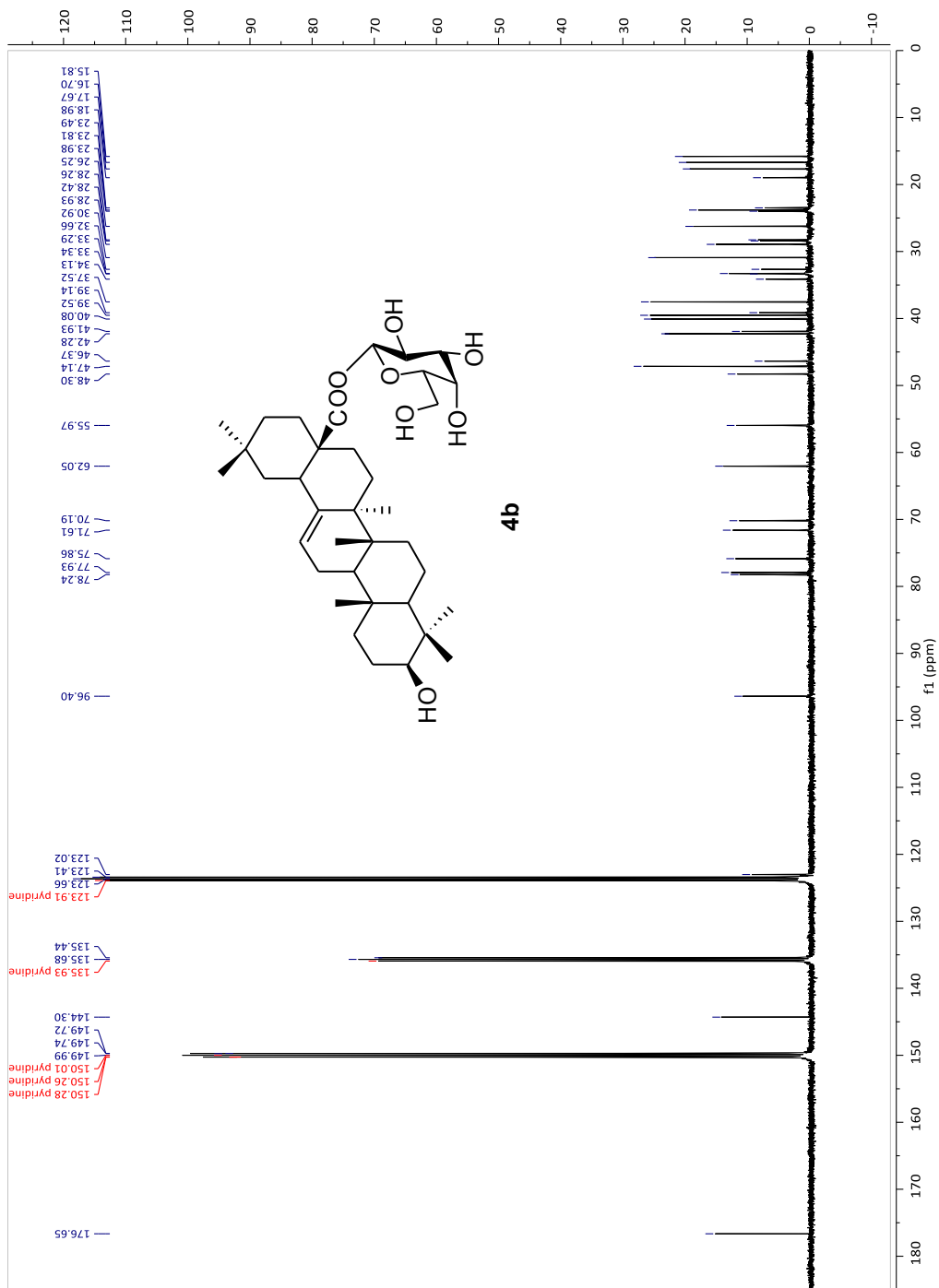
The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, pyridine- d_5)



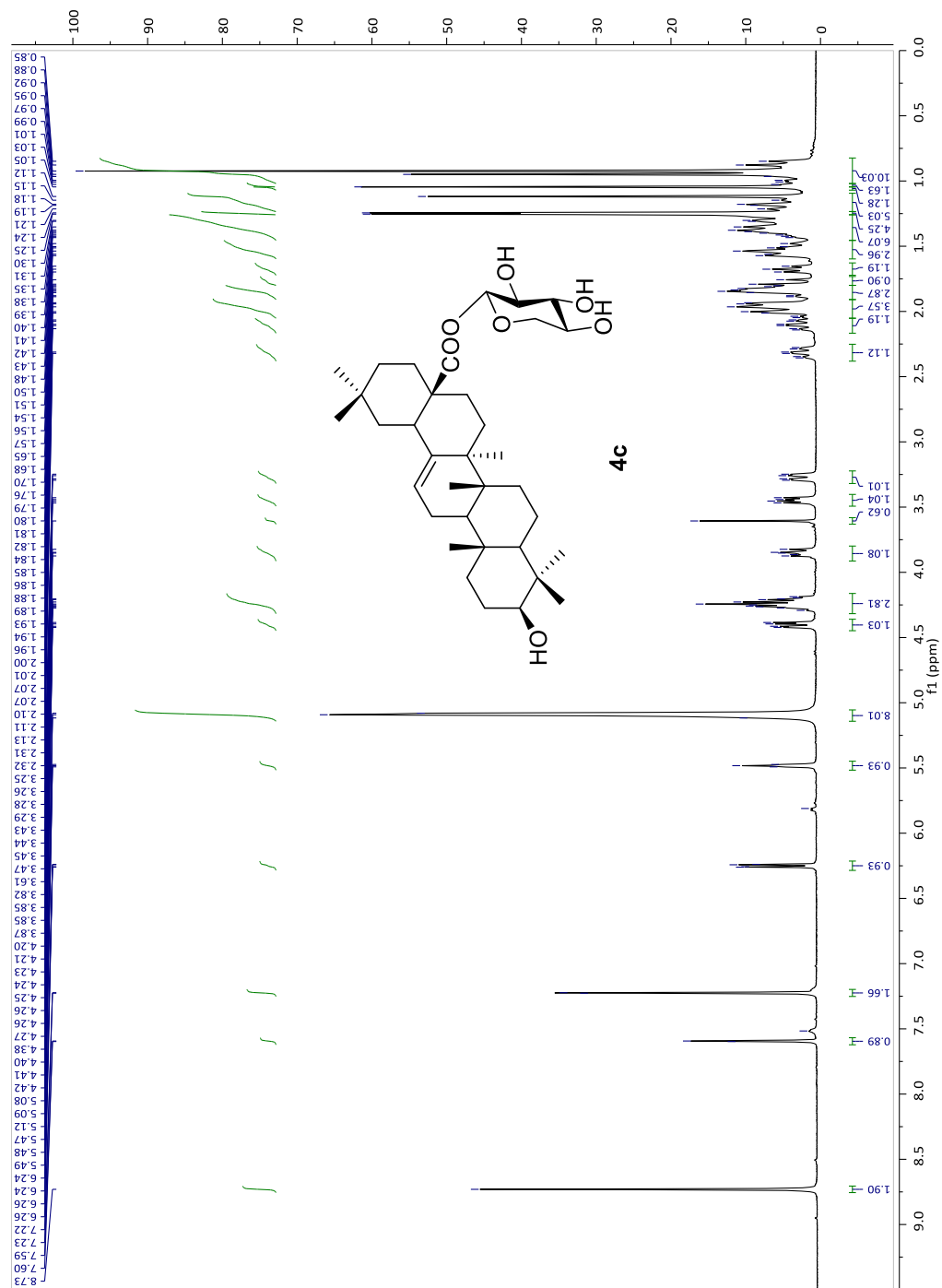
The assignments were confirmed by ^1H - ^1H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, pyridine- d_5)



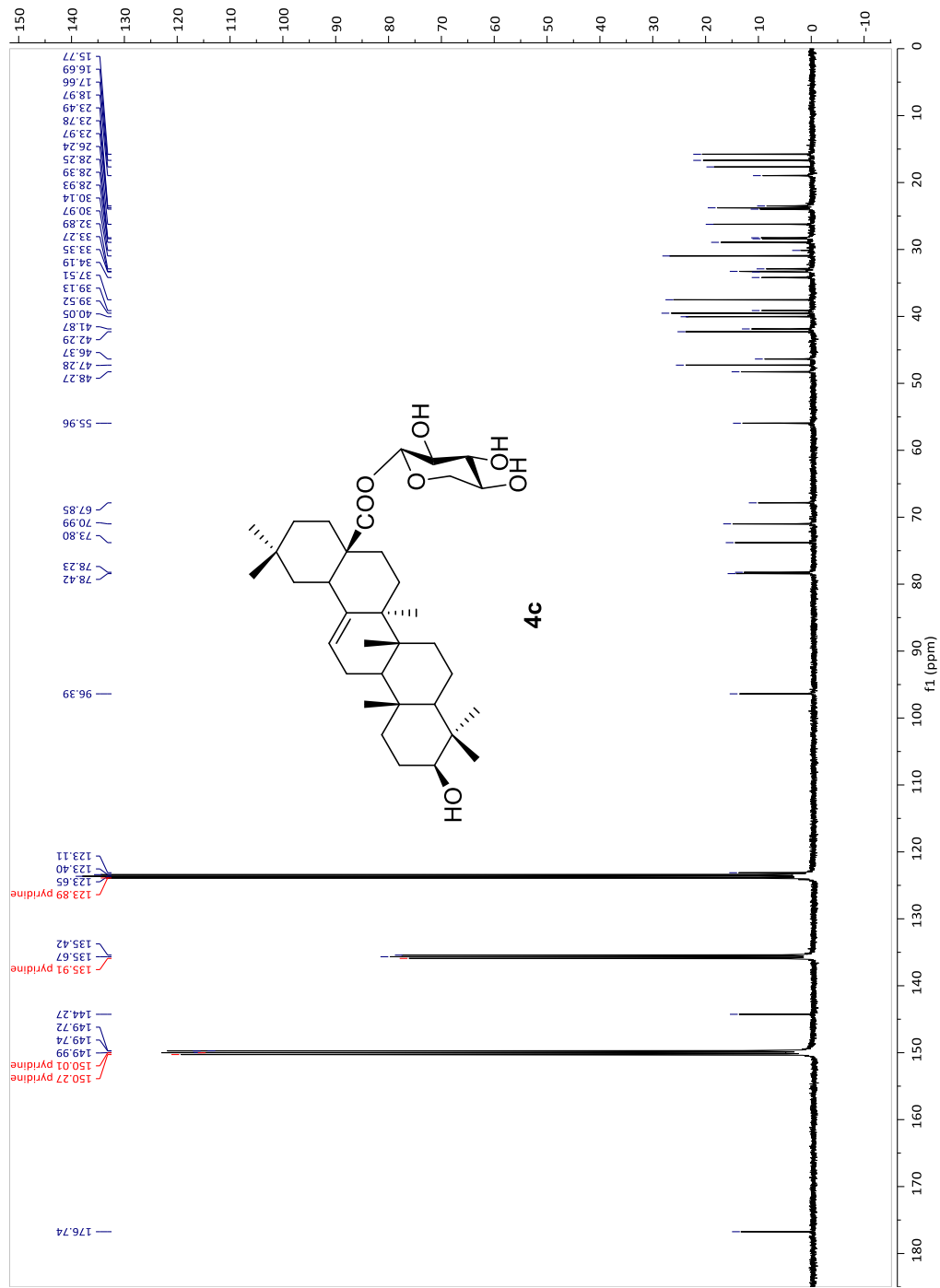
The assignments were confirmed by ^1H - ^1H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, pyridine- d_5)



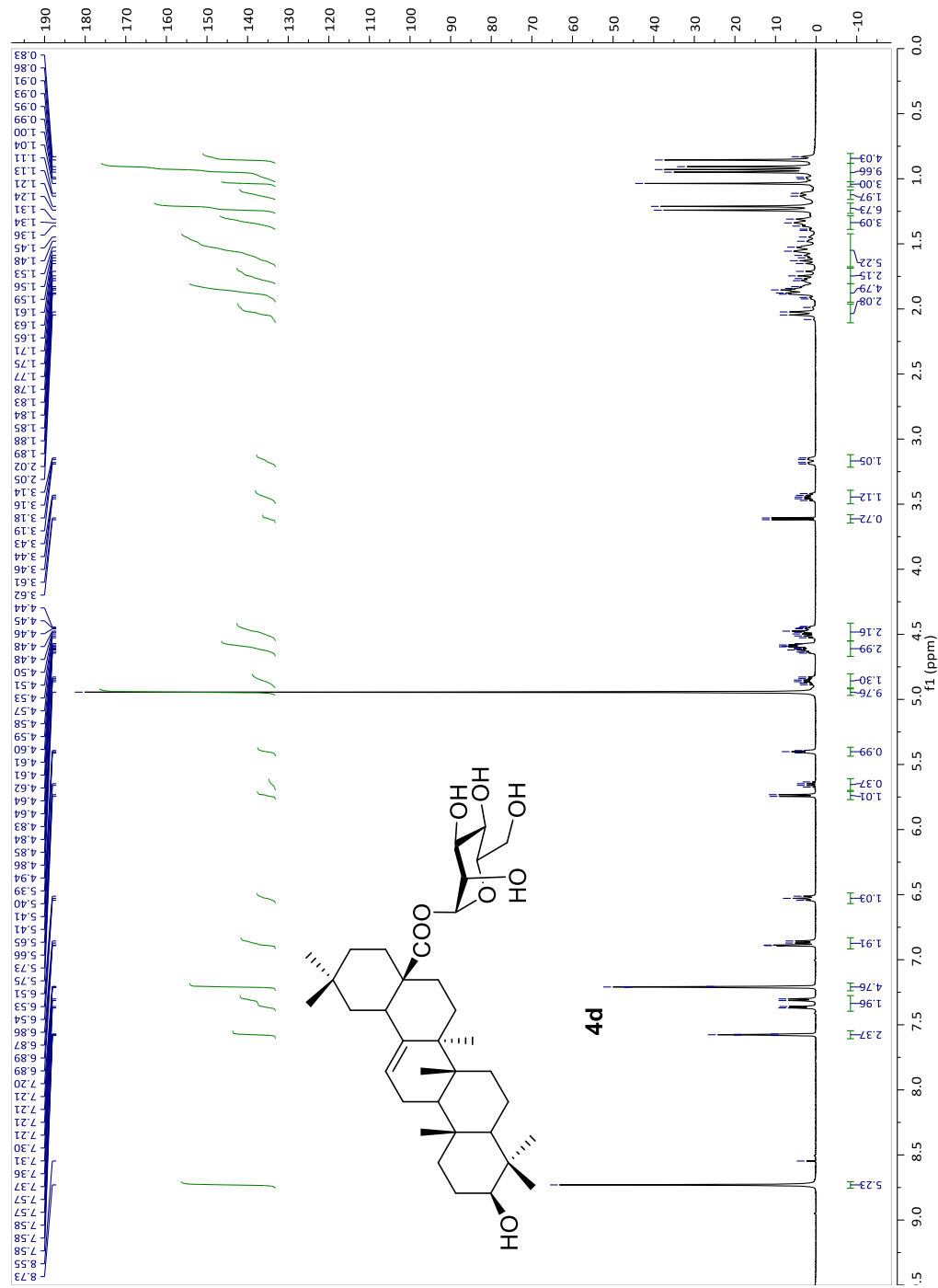
The assignments were confirmed by ^1H - ^1H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, pyridine- d_5)



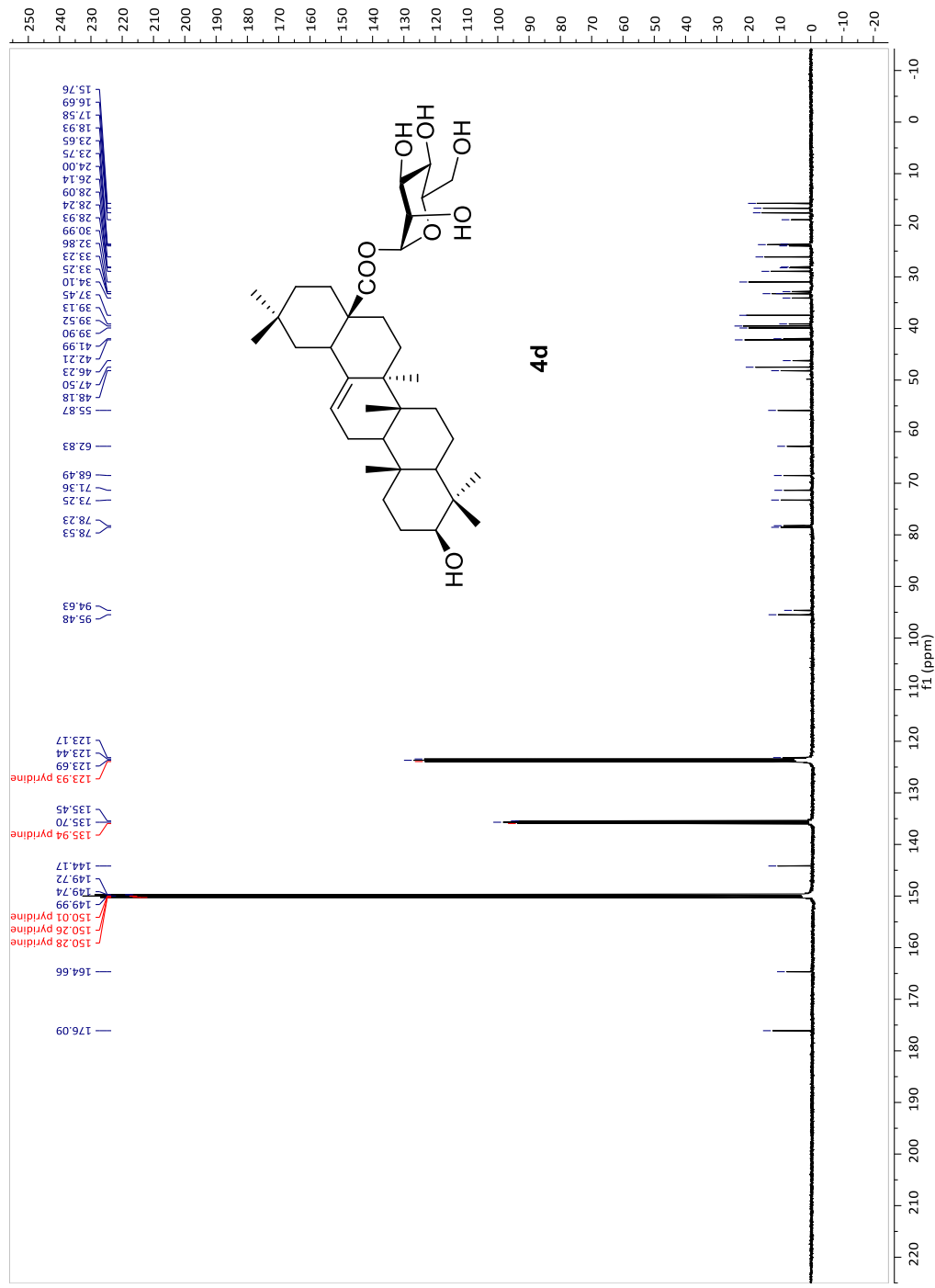
The assignments were confirmed by ^1H - ^1H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, pyridine-*d*₅)



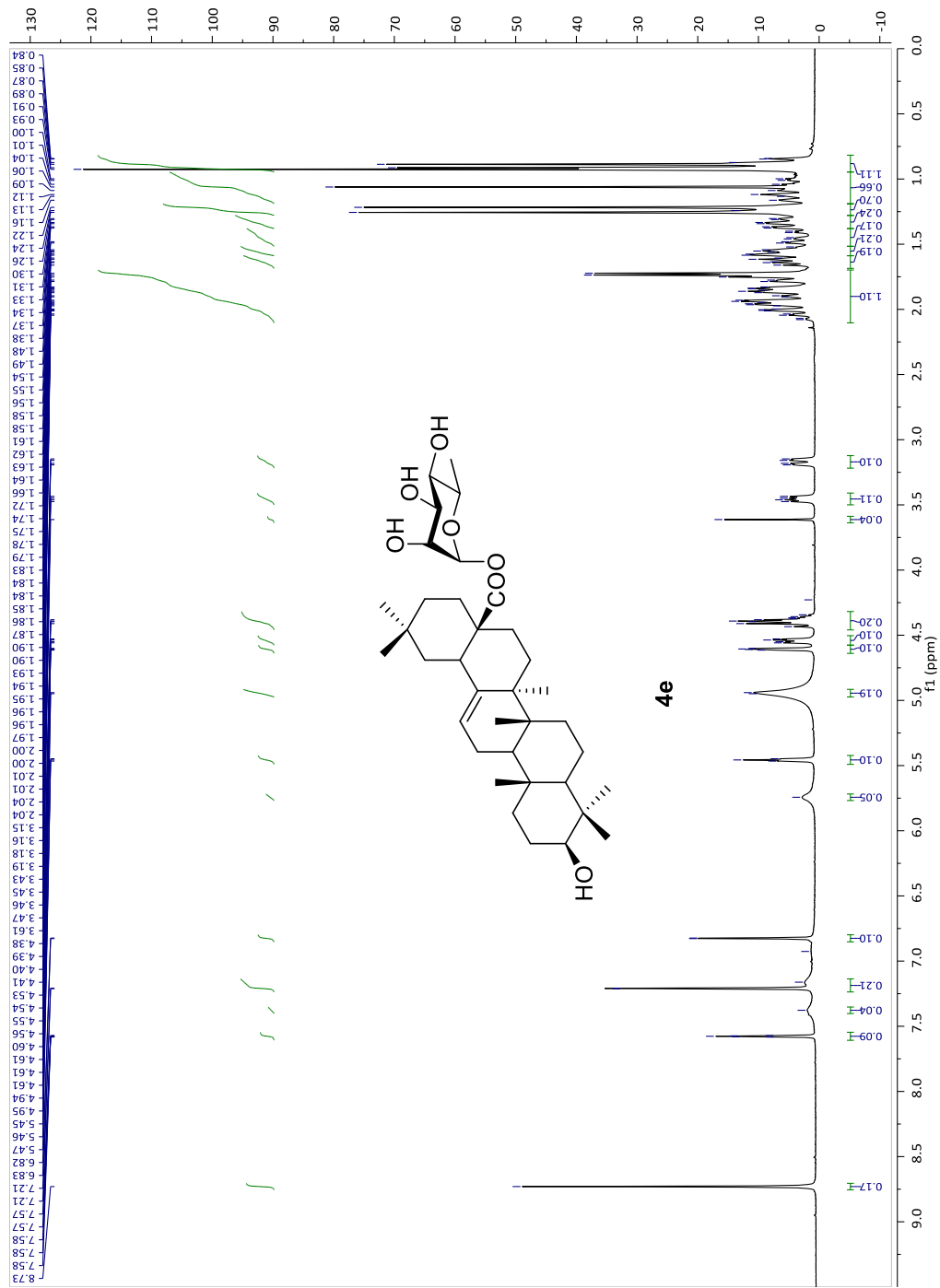
The assignments were confirmed by ¹H-¹H COSY, GATE1, HSQC and HMBC.

400-MR DD2 (400 MHz, pyridine- d_5)



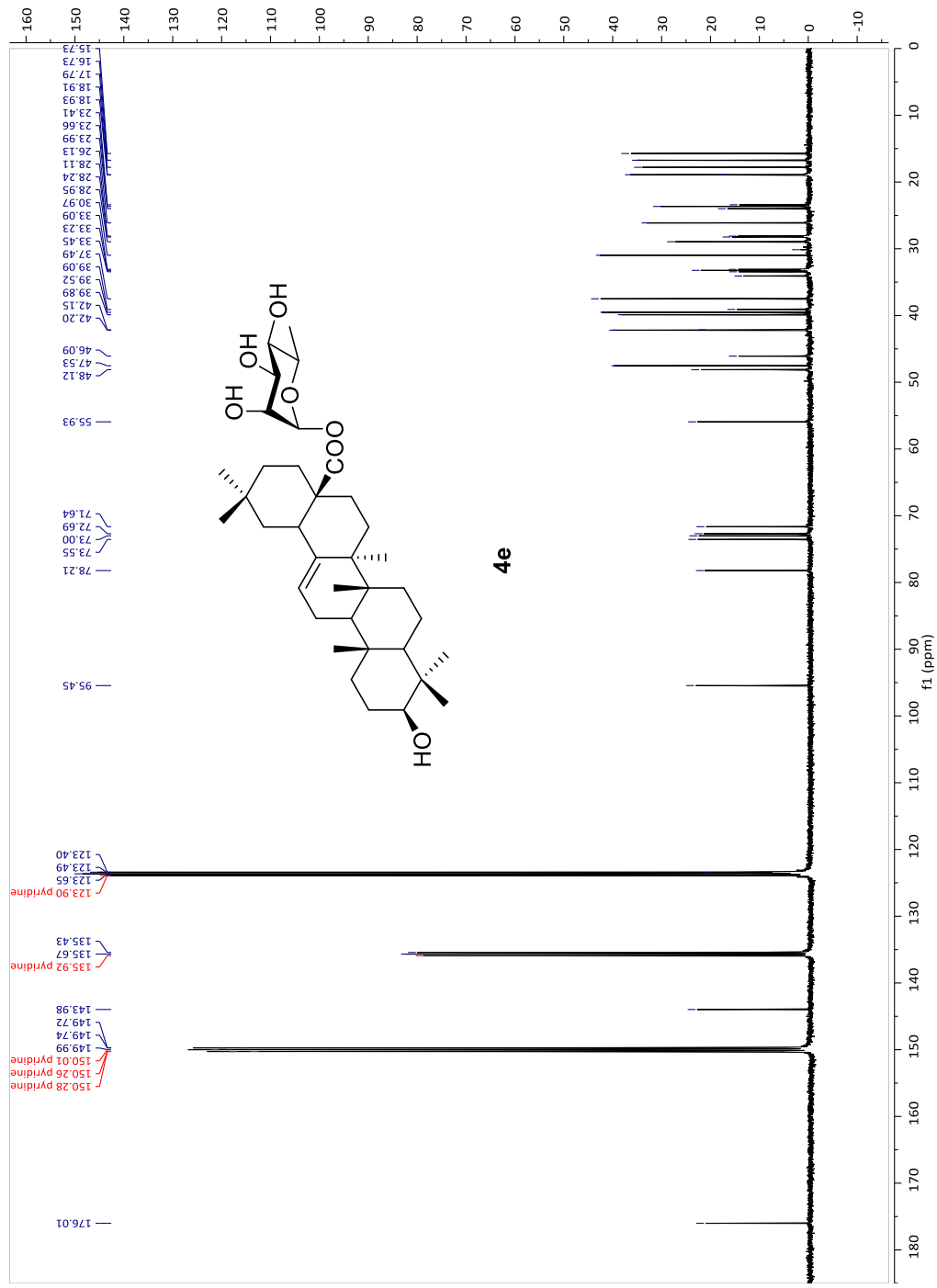
The assignments were confirmed by ^1H - ^1H COSY, GATE1, HSQC and HMBC.

400-MR DD2 (400 MHz, pyridine- d_5)



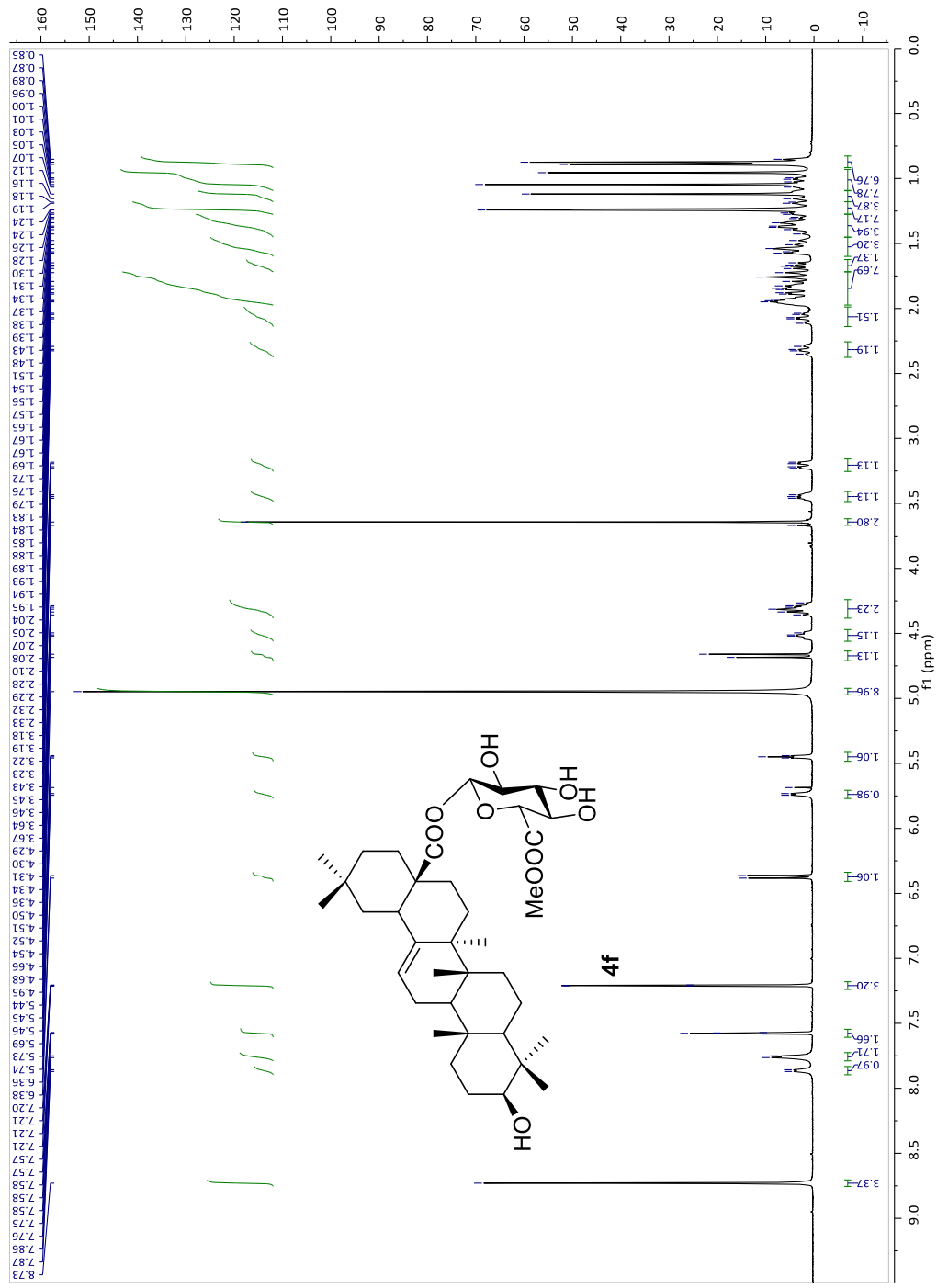
The assignments were confirmed by ^1H - ^1H COSY, GATE1, HSQC and HMBC.

400-MR DD2 (400 MHz, pyridine- d_5)



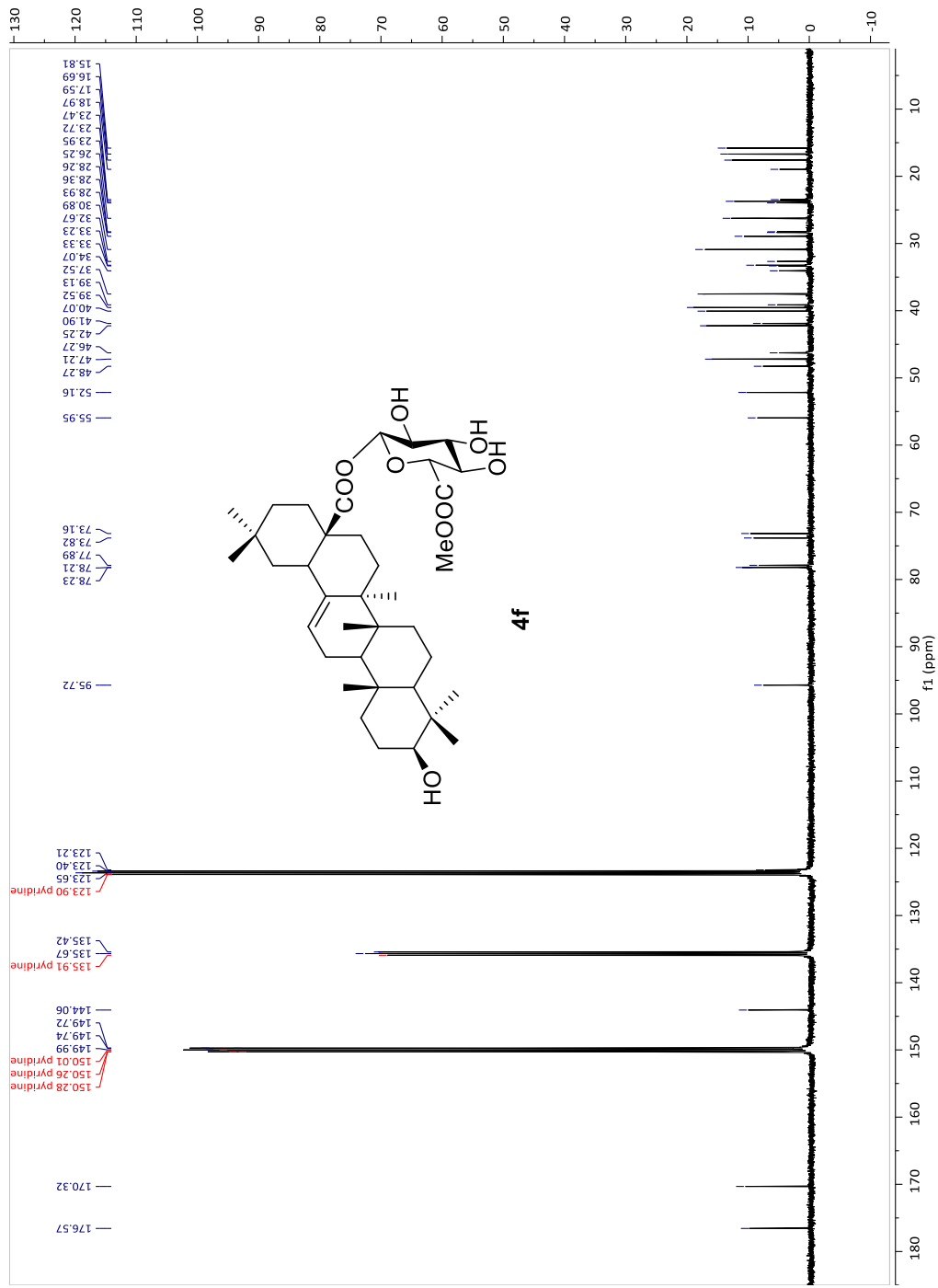
The assignments were confirmed
by ^1H - ^1H COSY, GATE1, HSQC and HMBC.

400-MR DD2 (400 MHz, pyridine- d_5)



The assignments were confirmed by ^1H - ^1H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, pyridine- d_5)



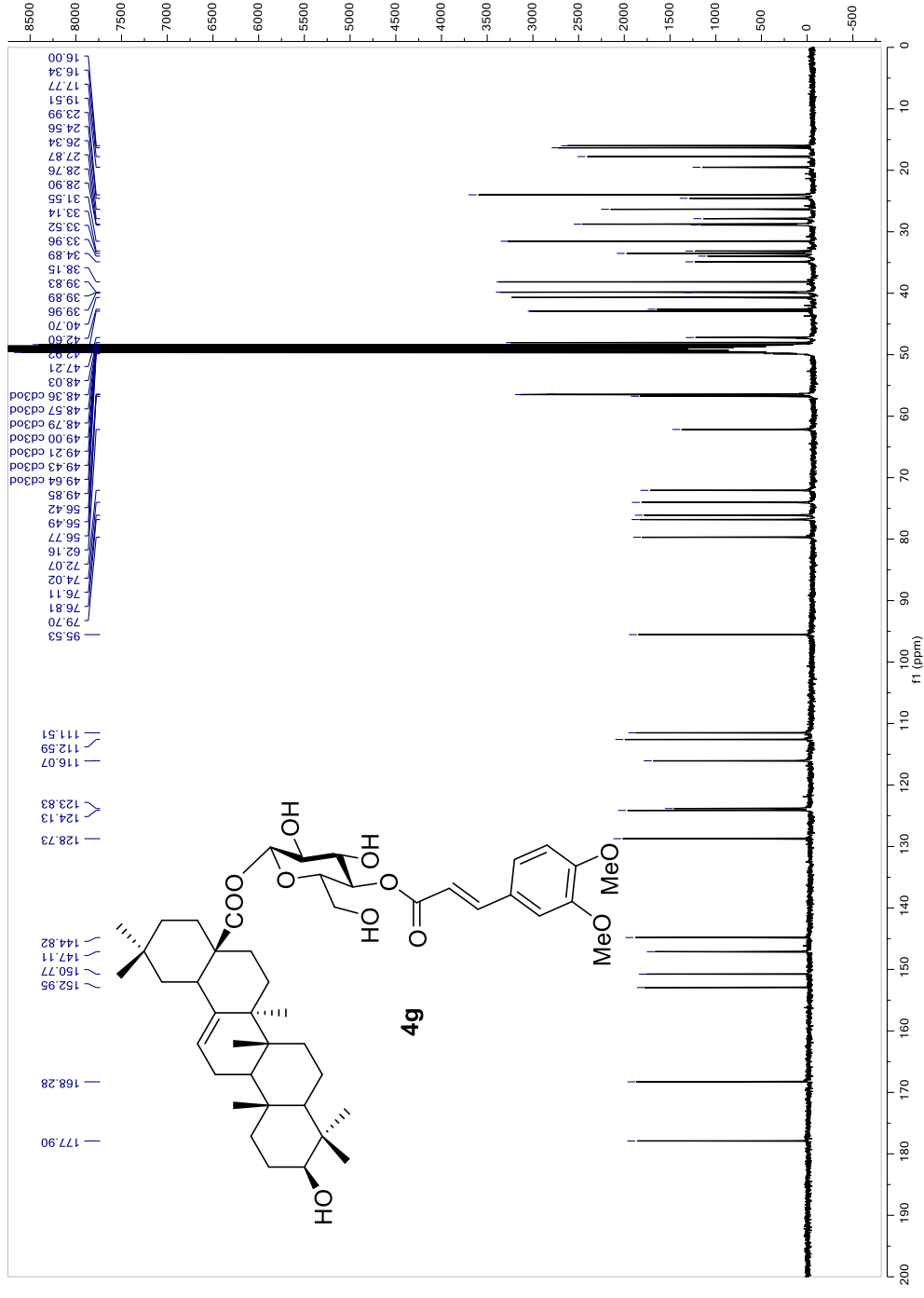
The assignments were confirmed by ^1H - ^1H COSY, HSQC and HMBC.

S43



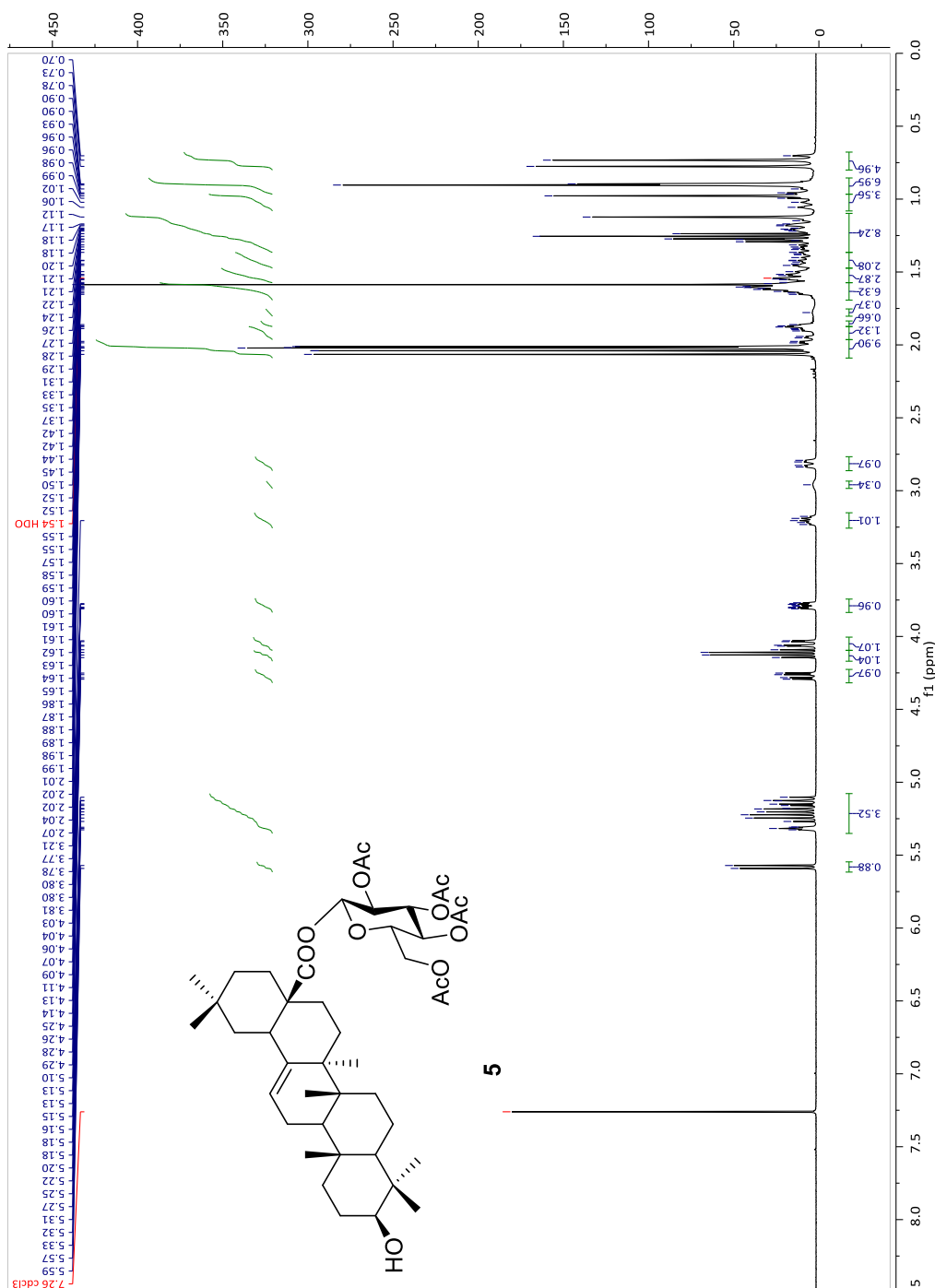
The assignments were confirmed by ^1H - ^1H COSY, HSQC and HMQC.

400-MR (400 MHz, CD₃OD)



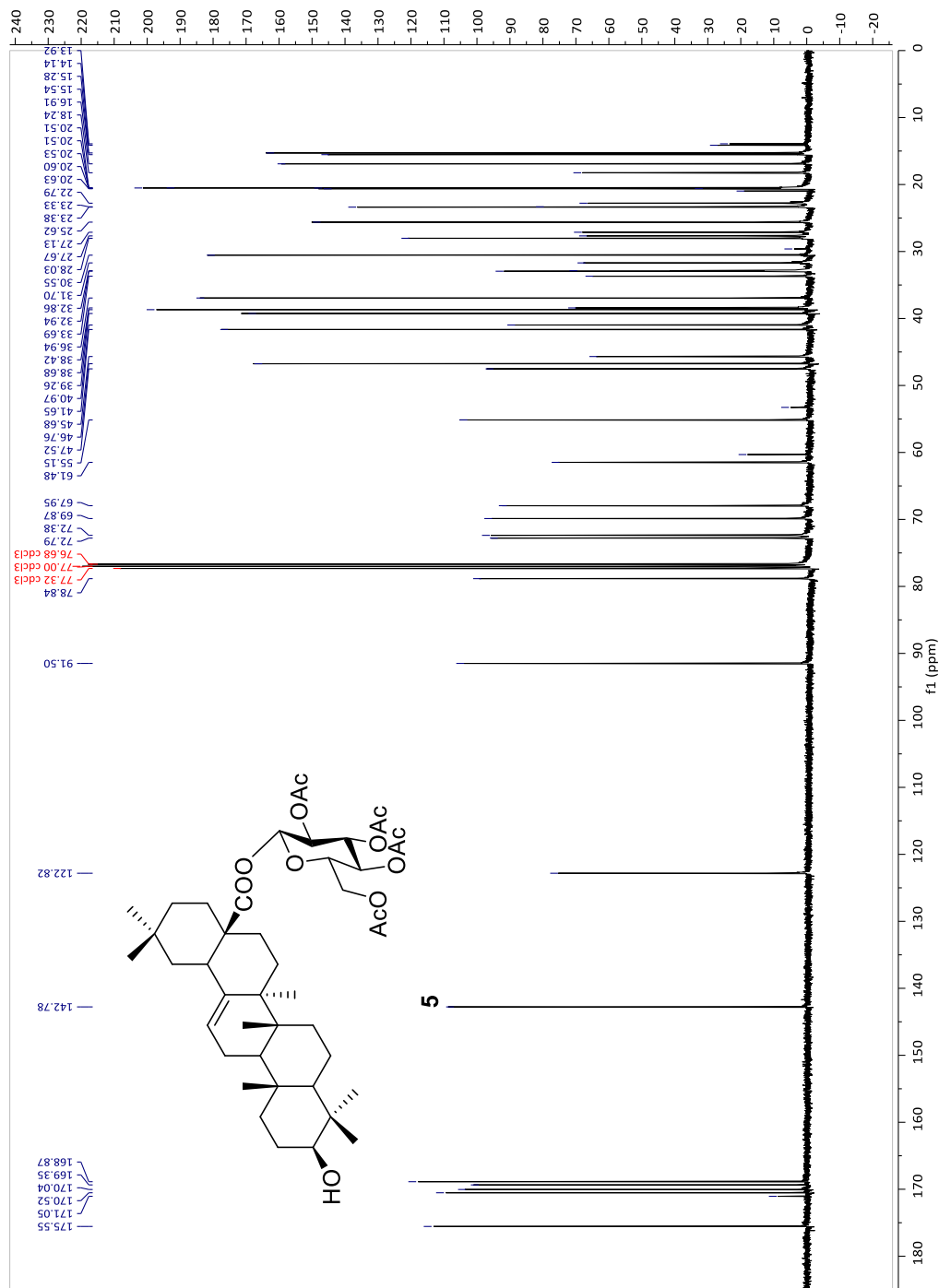
The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, CDCl₃)



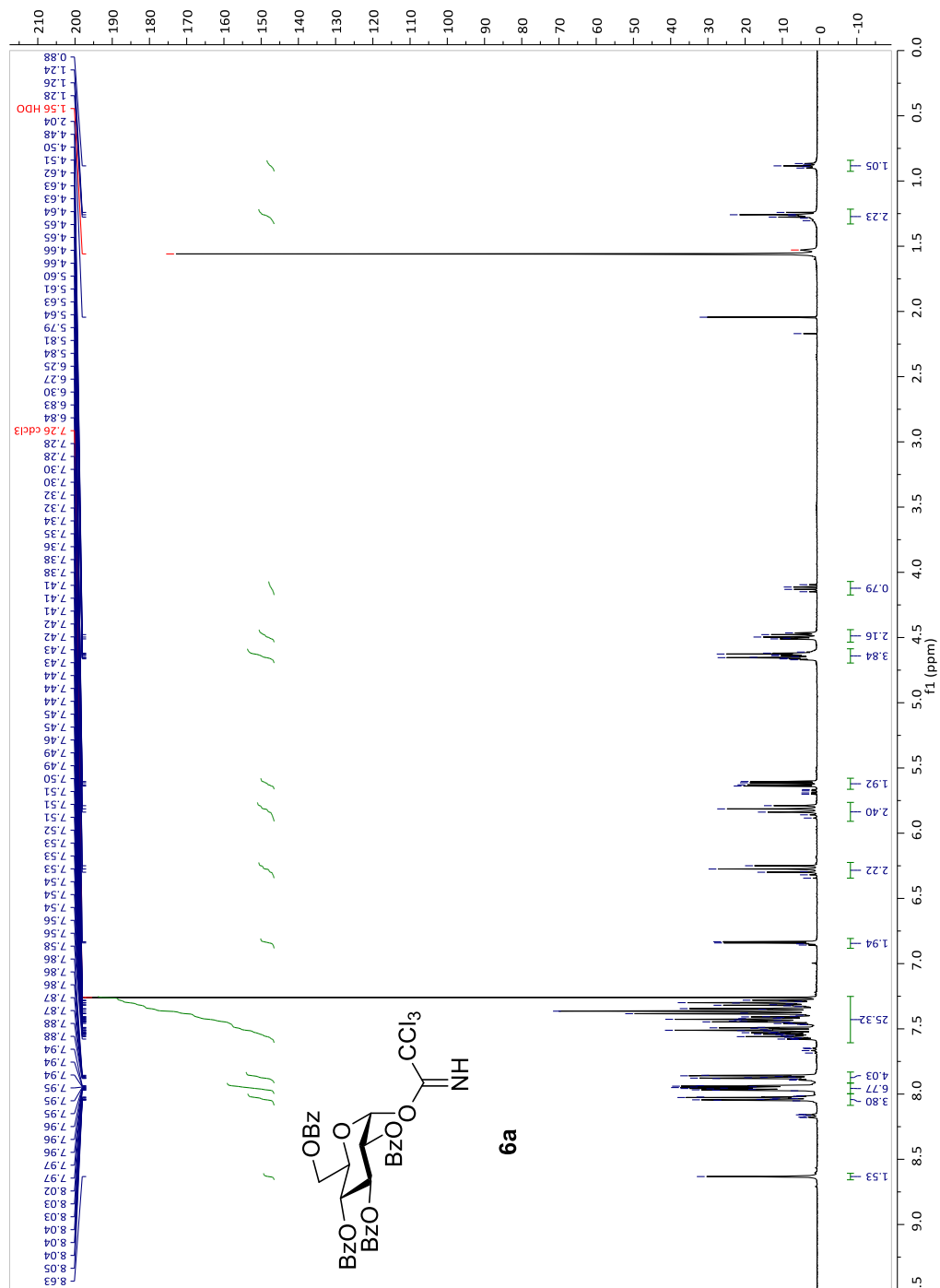
The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, CDCl₃)



The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, CDCl₃)



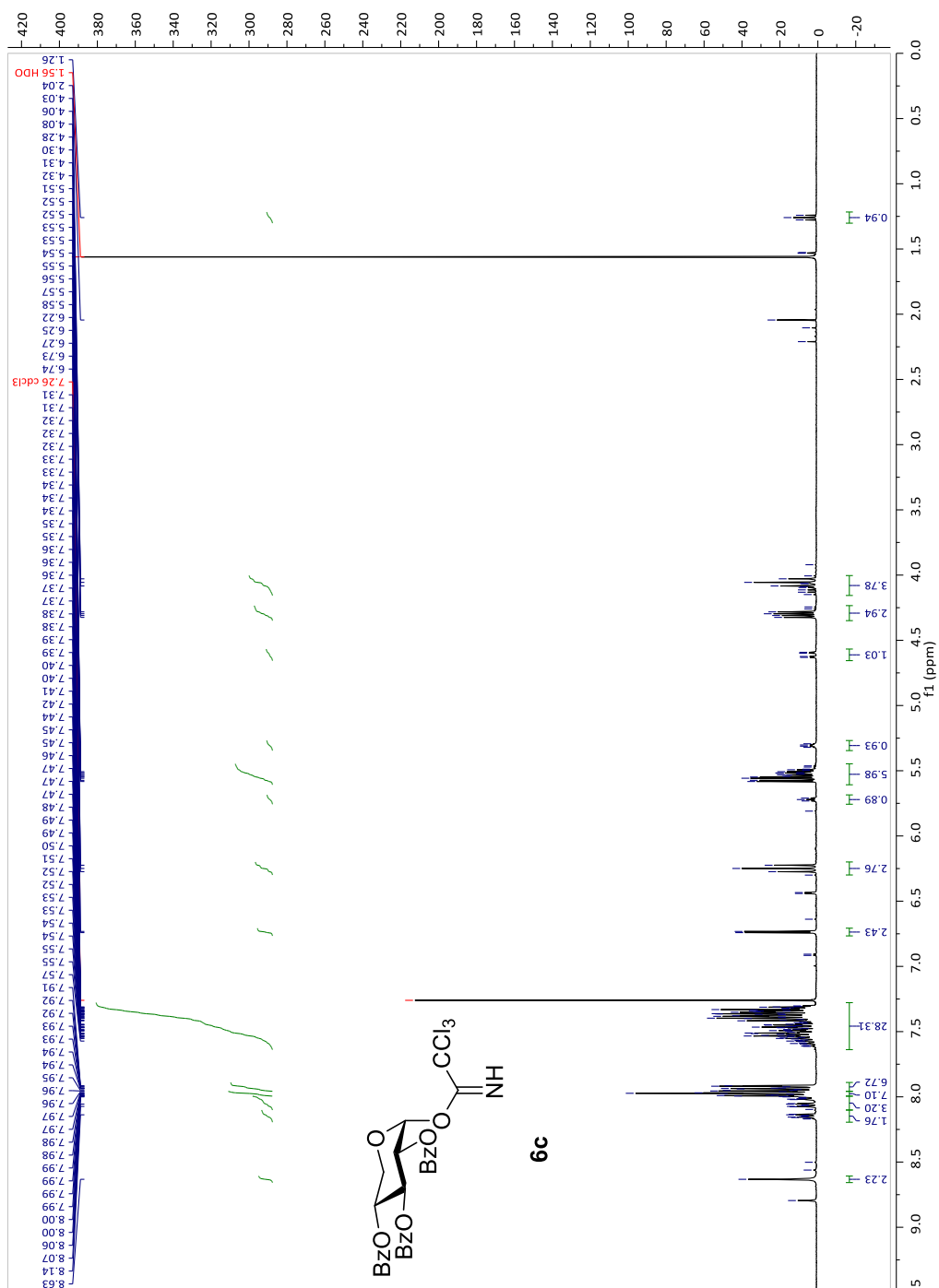
6b

O=C(N)OC1C(OC(=O)c2ccccc2)OC(OC(=O)c3ccccc3)C1

¹H NMR spectrum (CDCl₃) of compound **6b**. The x-axis represents the chemical shift in ppm (f1), ranging from 0.0 to 10.0. The y-axis represents the intensity. Key peaks are labeled with their chemical shifts and integration values:

- ~7.81 ppm (broad, integration 12.82)
- ~7.97 ppm (multiplet, integration 3.67)
- ~7.99 ppm (multiplet, integration 15.19)
- ~7.99 ppm (multiplet, integration 3.21)
- ~7.99 ppm (multiplet, integration 26.23)
- ~7.99 ppm (multiplet, integration 12.82)
- ~7.99 ppm (multiplet, integration 3.06)
- ~7.99 ppm (multiplet, integration 3.16)
- ~7.99 ppm (multiplet, integration 3.84)
- ~7.99 ppm (multiplet, integration 3.59)
- ~7.99 ppm (multiplet, integration 0.97)
- ~7.99 ppm (multiplet, integration 0.80)
- ~7.99 ppm (multiplet, integration 1.90)
- ~7.99 ppm (multiplet, integration 1.97)
- ~7.99 ppm (multiplet, integration 0.80)

400-MR DD2 (400 MHz, CDCl₃)

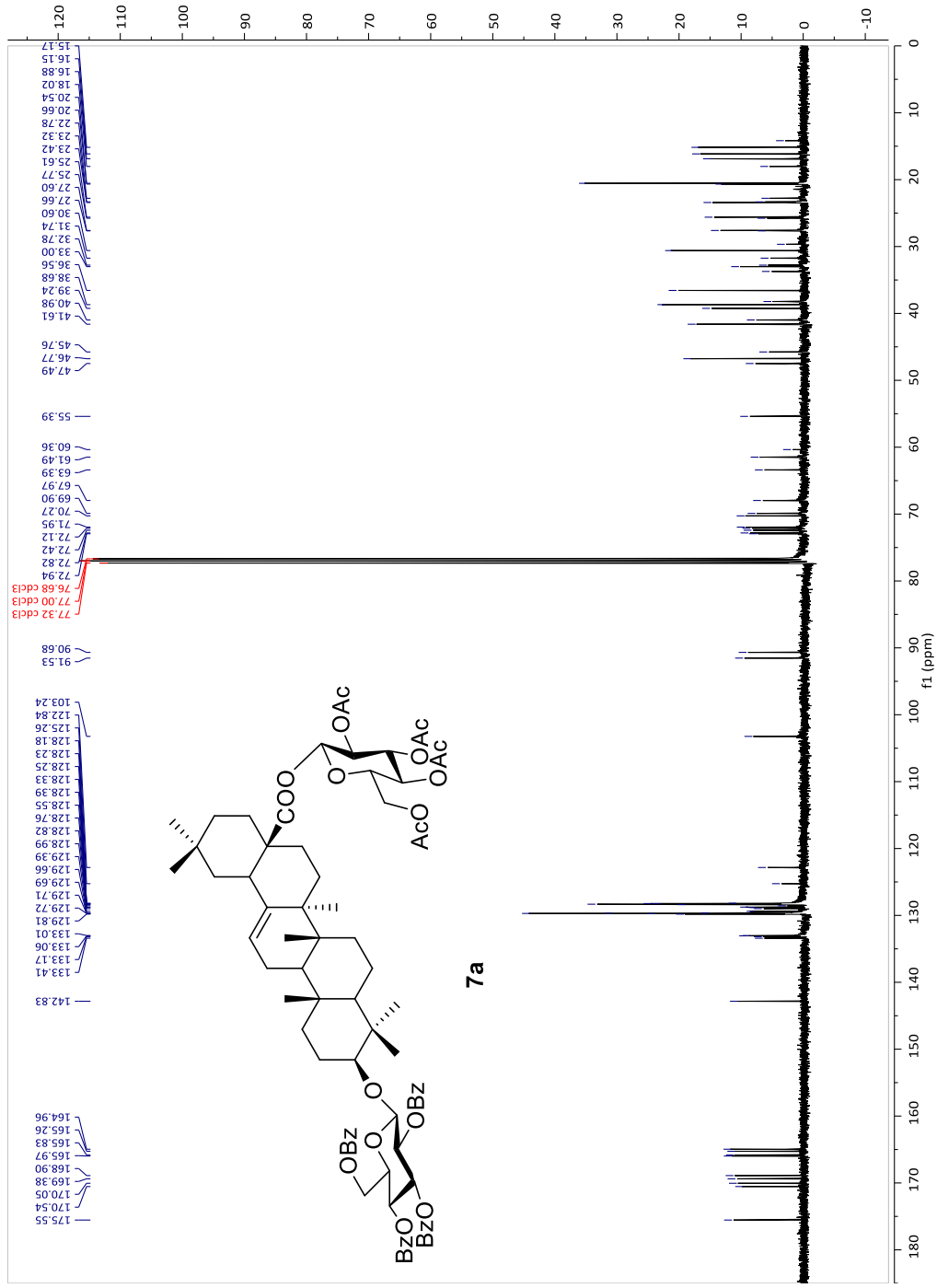


S50



The assignments were confirmed by ^1H - ^1H COSY, HSQC and HMQC.

400-MR DD2 (400 MHz, CDCl₃)



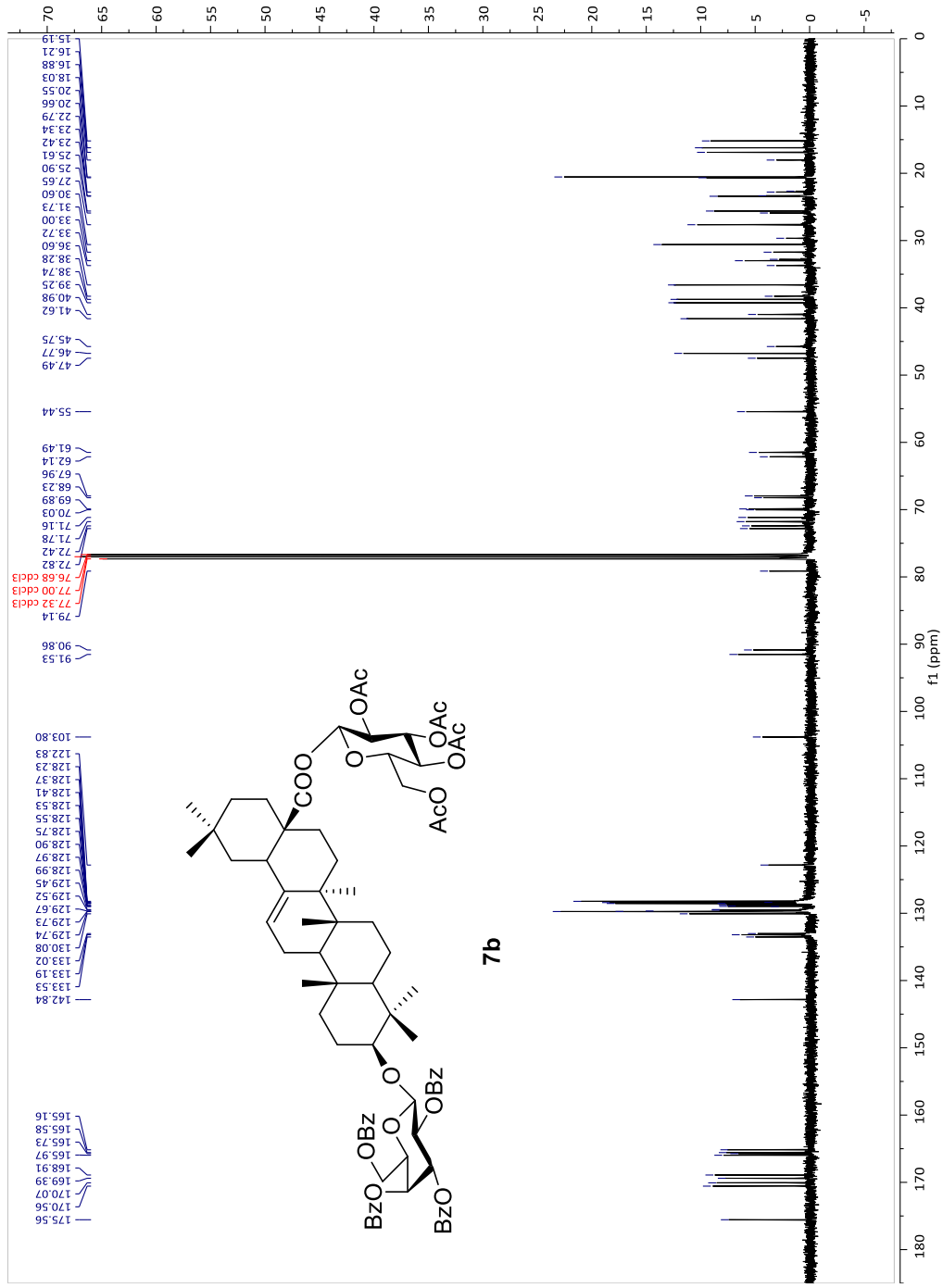
The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

S52



The assignments were confirmed by ^1H - ^1H COSY, HSQC and HMQC.

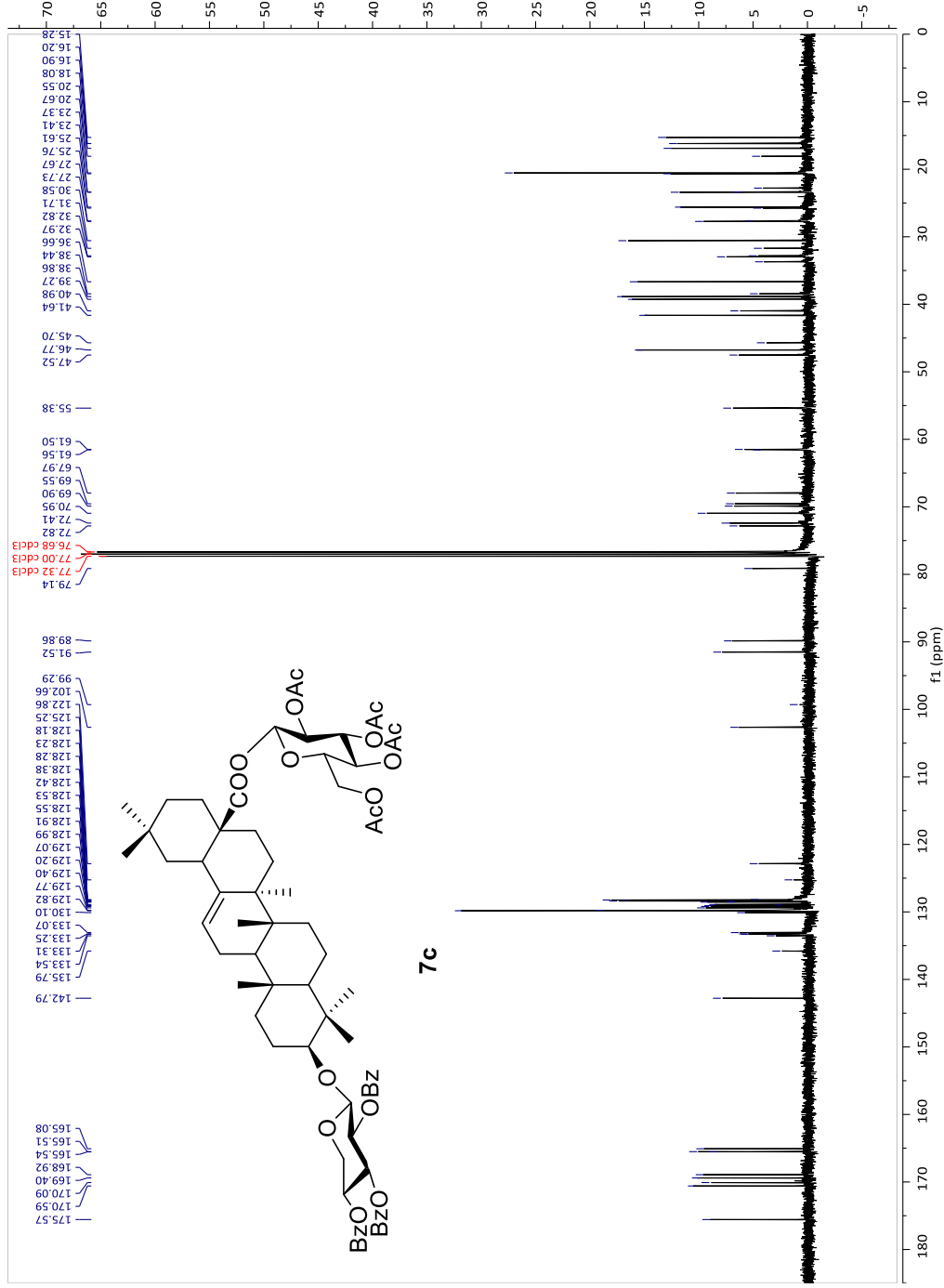
400-MR DD2 (400 MHz, CDCl₃)



The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

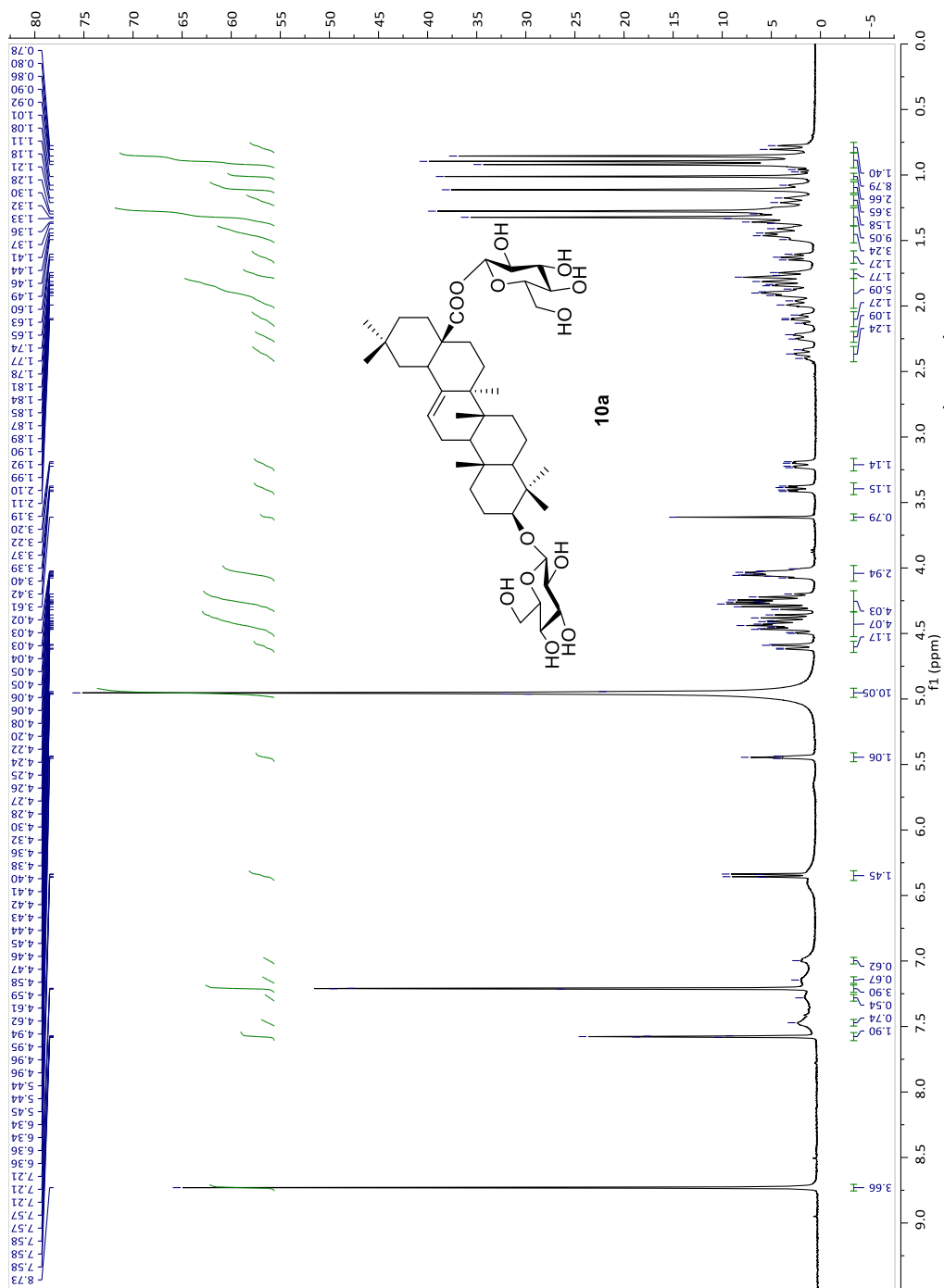
54

400-MR DD2 (400 MHz, CDCl₃)



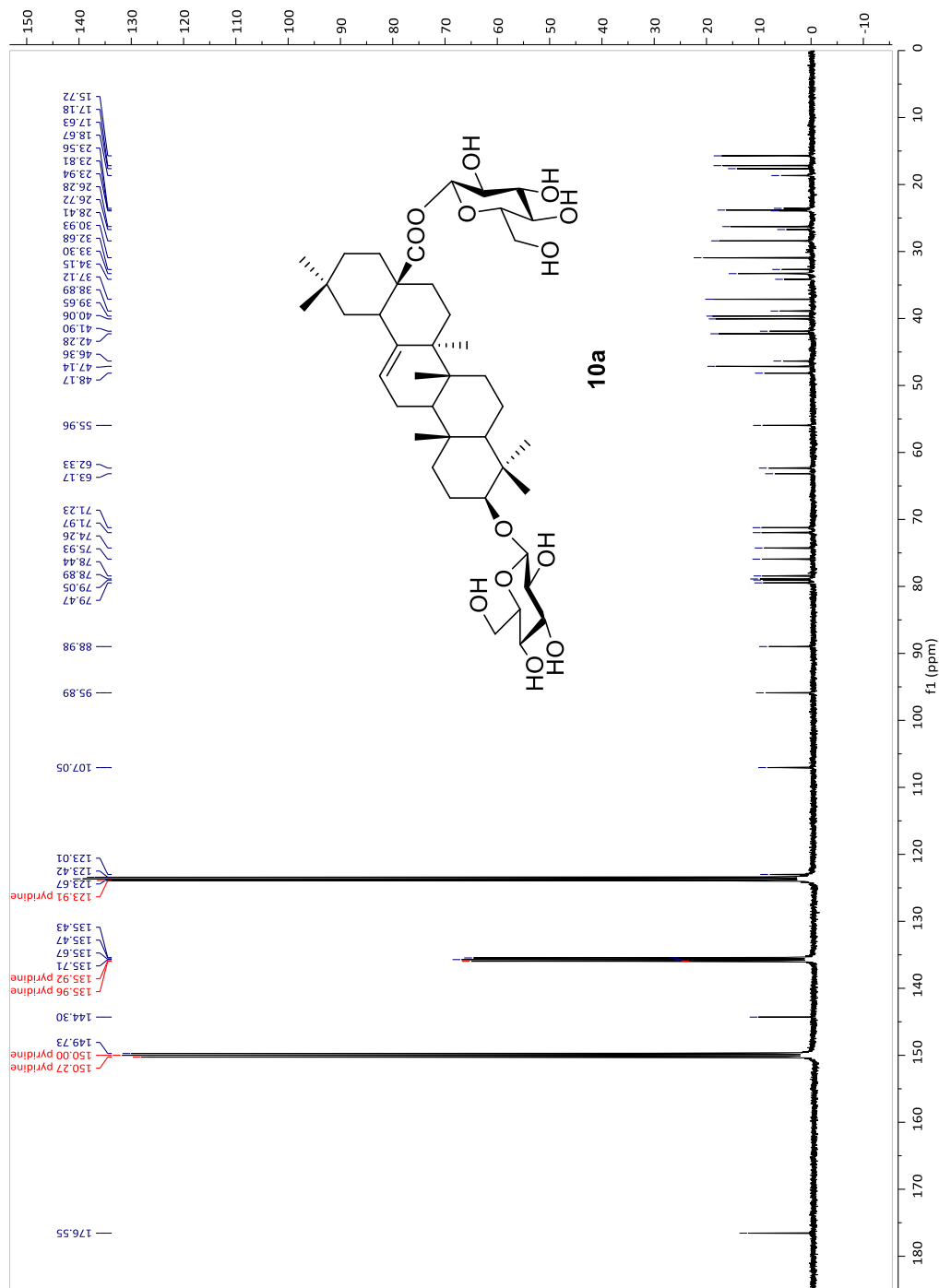
The assignments were confirmed by ¹H-¹H COSY, HSQC and HMBC.

400-MR DD2 (400 MHz, pyridine- d_5)



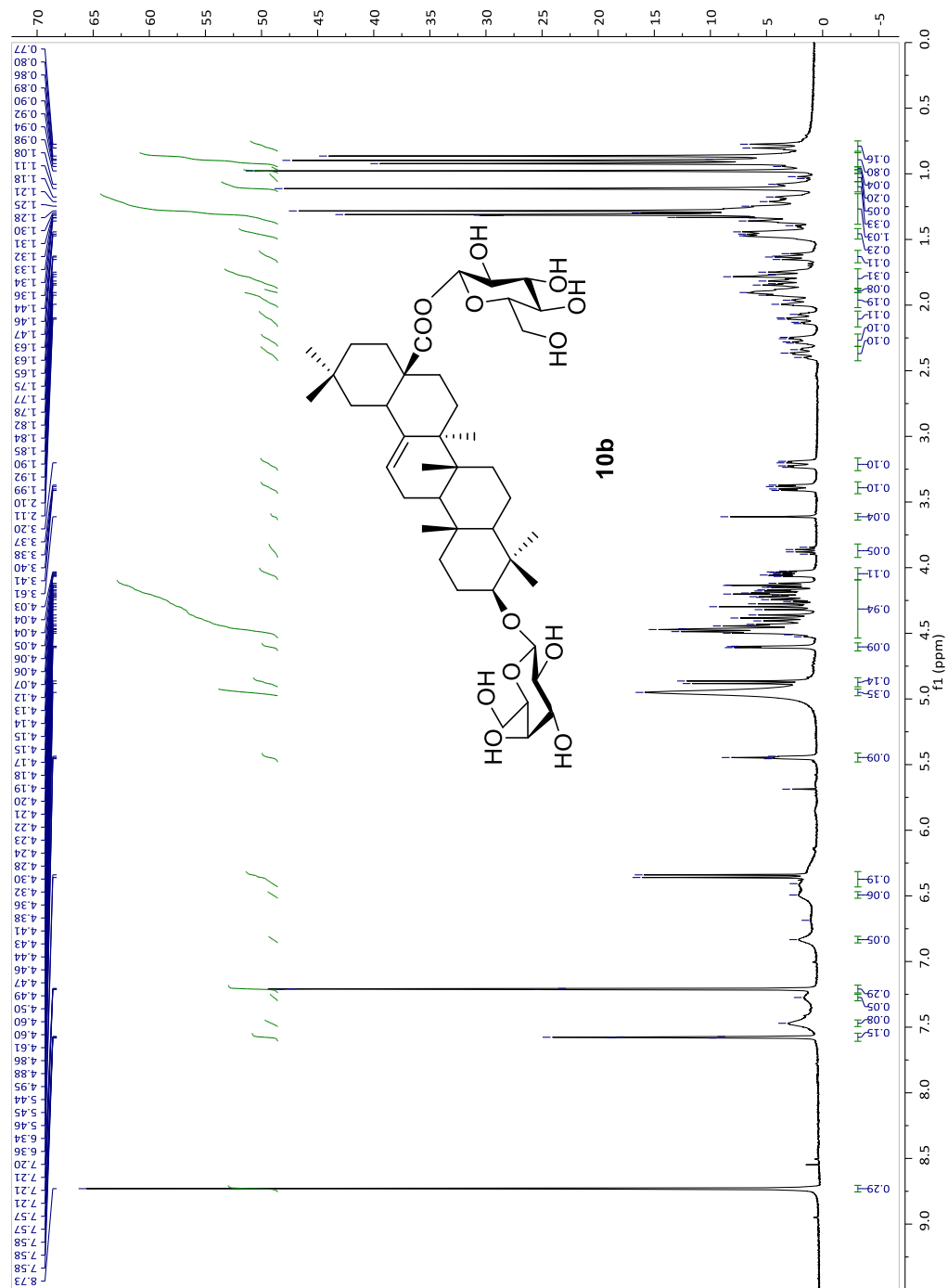
The assignments were confirmed by ^1H - ^1H COSY, HSQC, HMBC, 1D TOCSY and HSQC-TOCSY.

400-MR DD2 (400 MHz, pyridine- d_5)



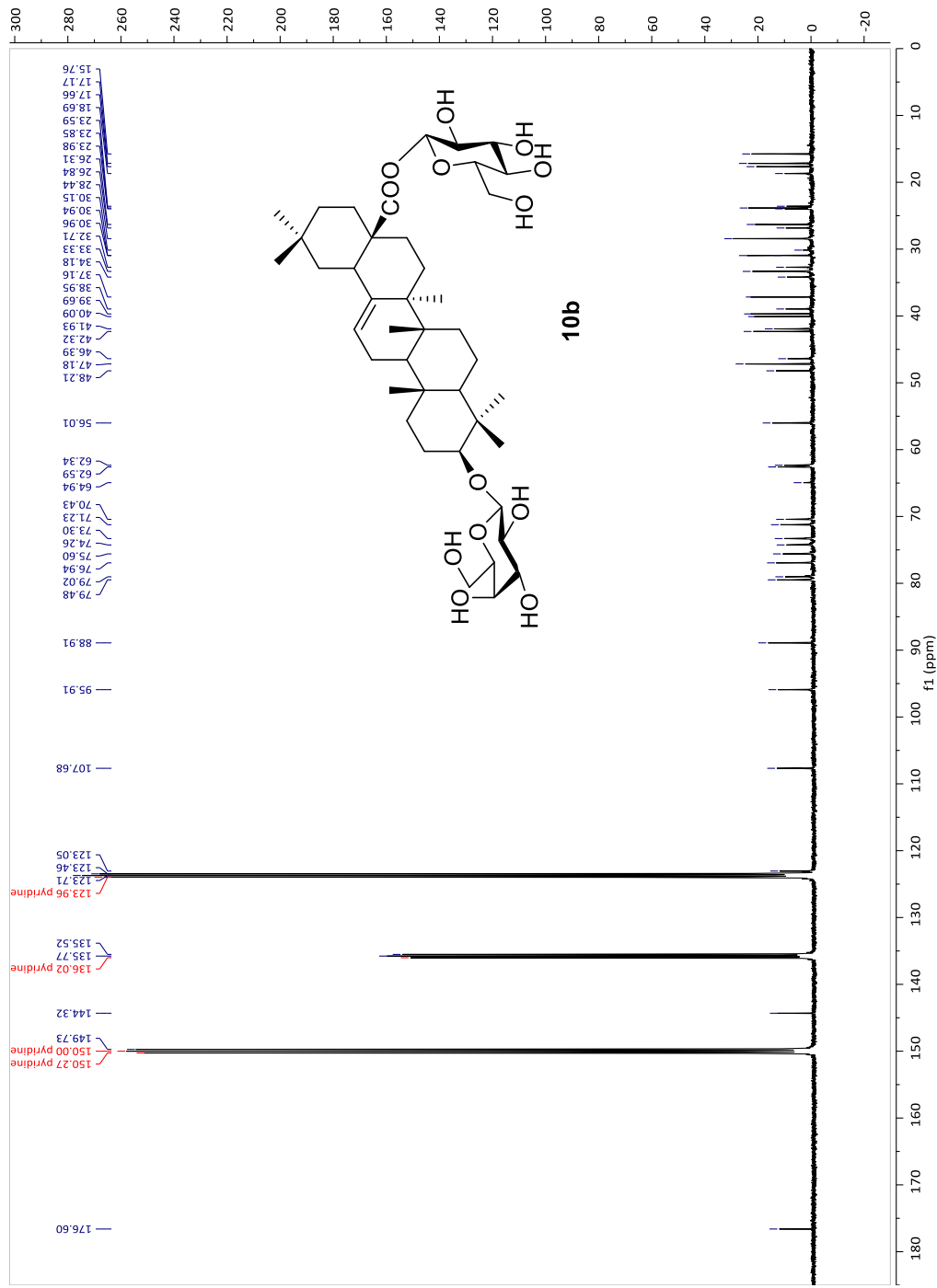
The assignments were confirmed by ^1H - ^1H COSY, HSQC, HMBC, 1D TOCSY and HSQC-TOCSY.

400-MR DD2 (400 MHz, pyridine- d_5)



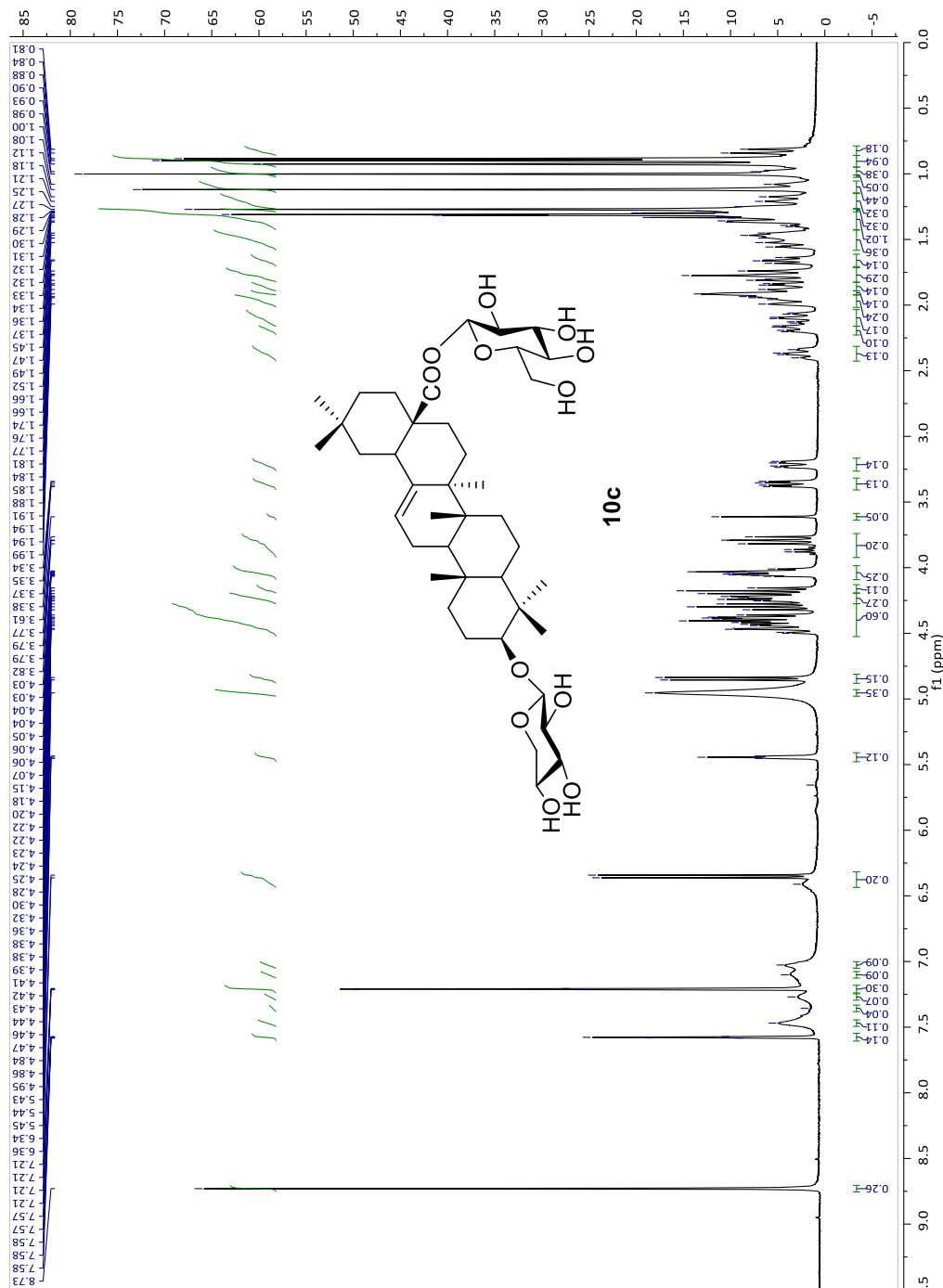
The assignments were confirmed by ^1H - ^1H COSY, HSQC, HMBC, 1D TOCSY and HSQC-TOCSY.

400-MR DD2 (400 MHz, pyridine- d_5)

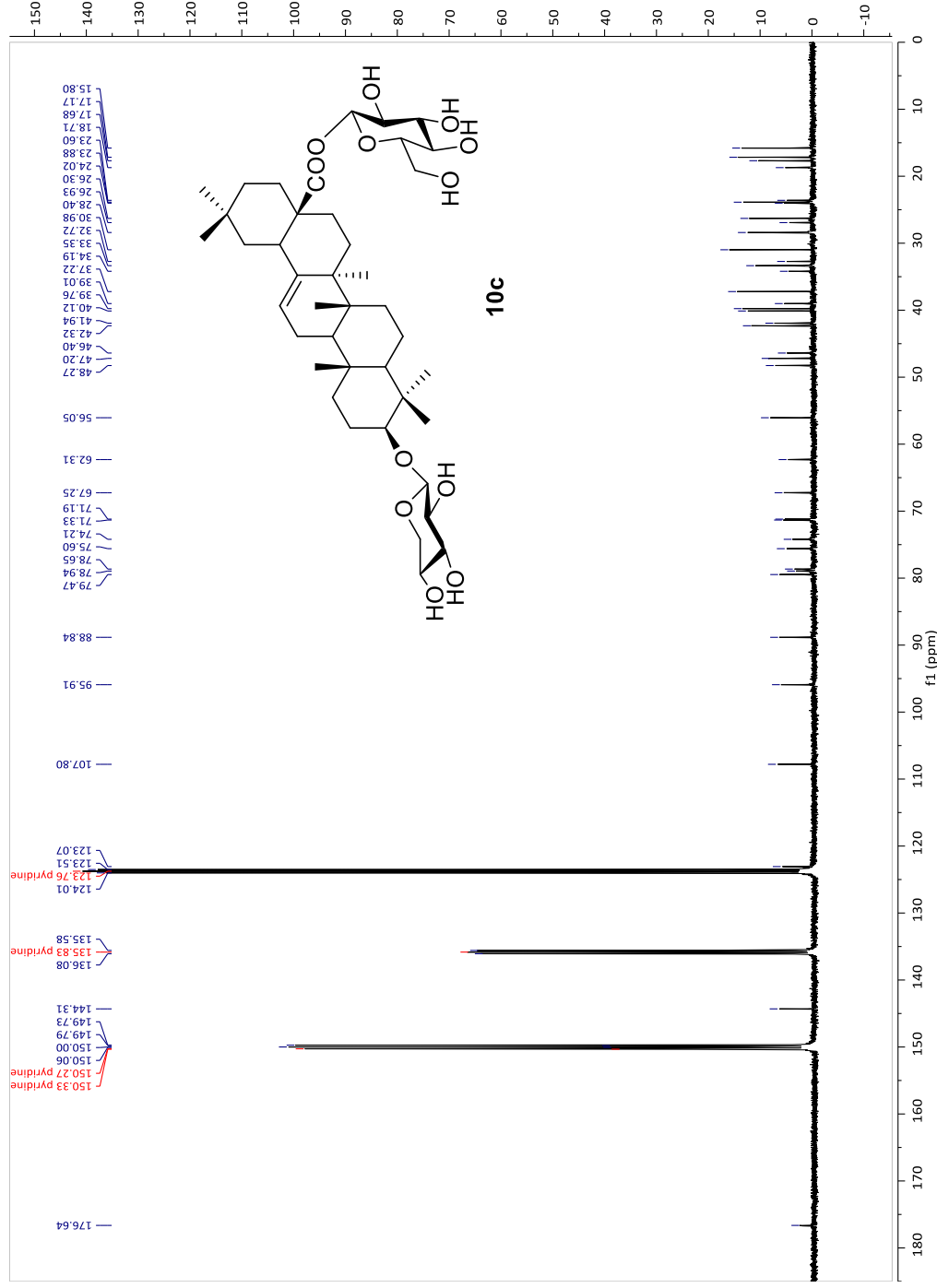


The assignments were confirmed by ^1H - ^1H COSY, HSQC, HMBC, 1D TOCSY and HSQC-TOCSY.

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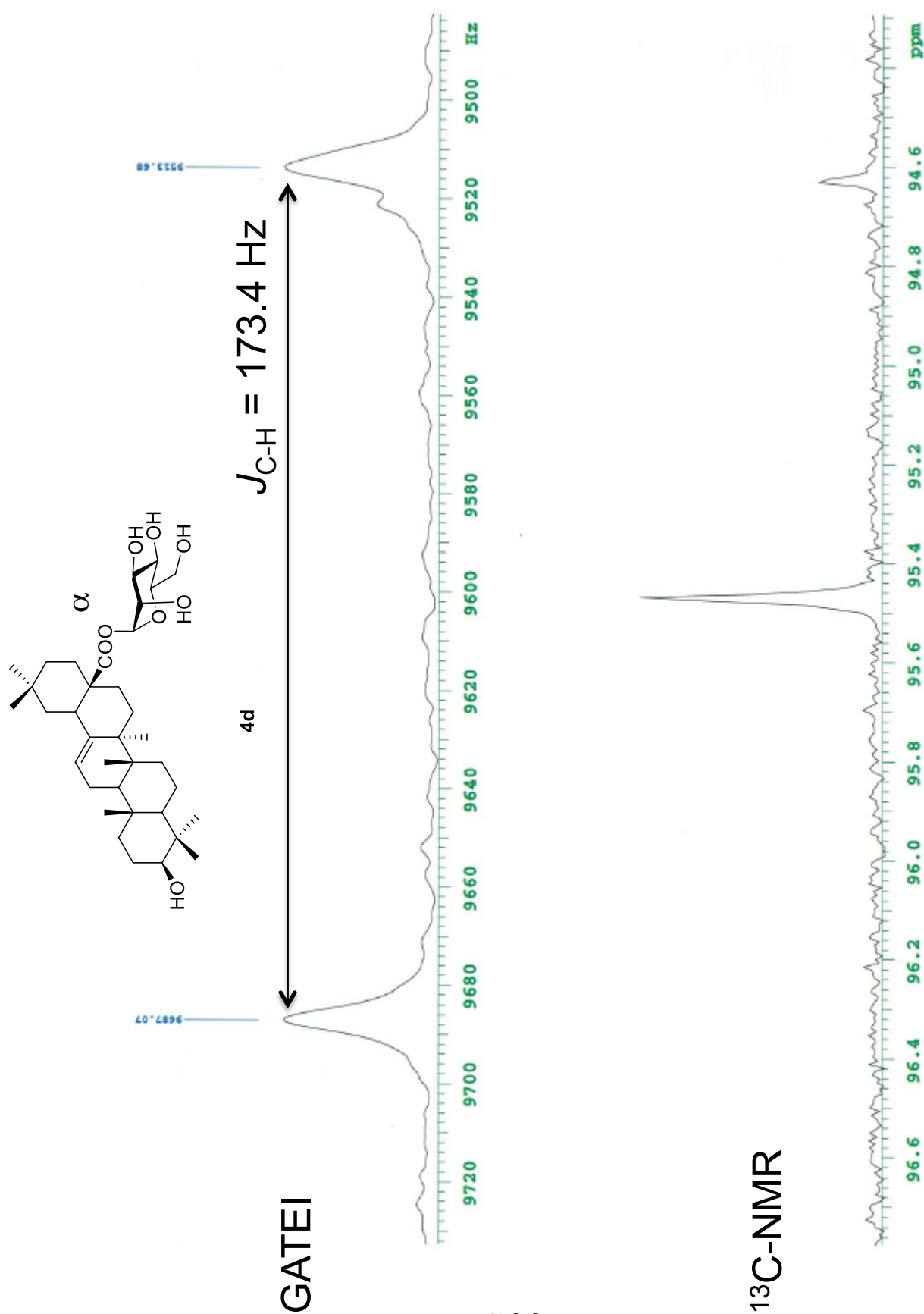


400-MR DD2 (400 MHz, pyridine- d_5)

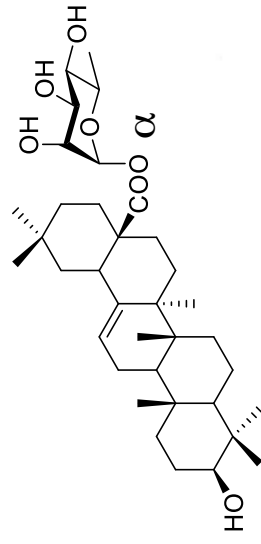


The assignments were confirmed by ^1H - ^1H COSY, HSQC, HMBC, 1D TOCSY and HSQC-TOCSY.

IV-2. GATEI chart of prepared compounds



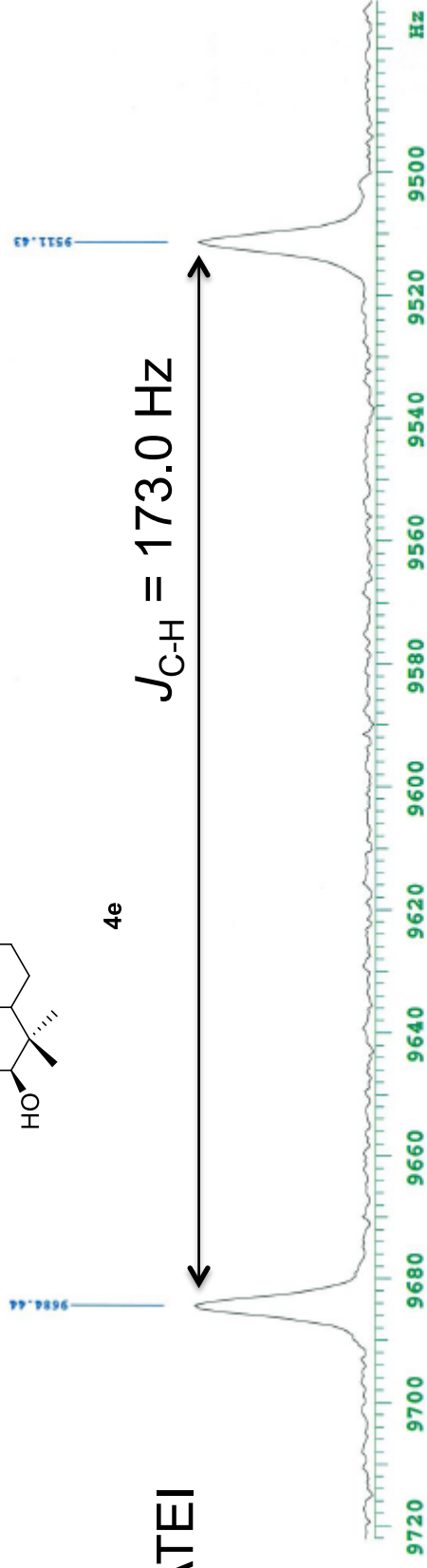
S62



4e

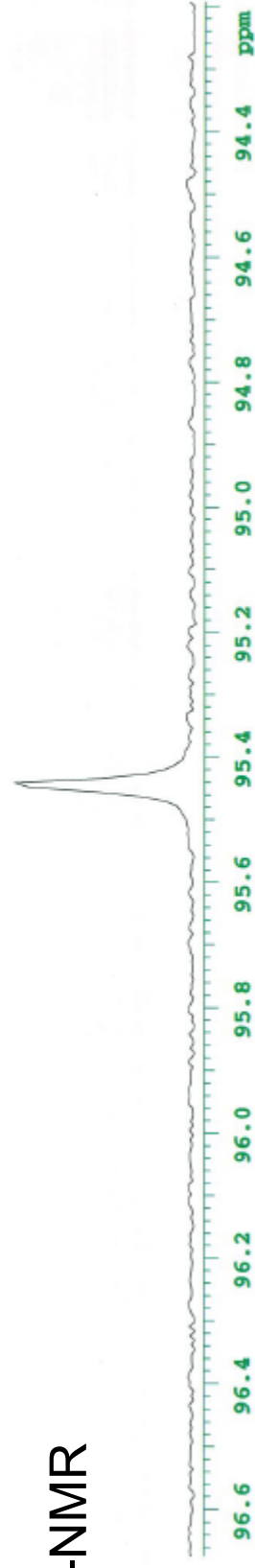
$$J_{C-H} = 173.0 \text{ Hz}$$

GATEI



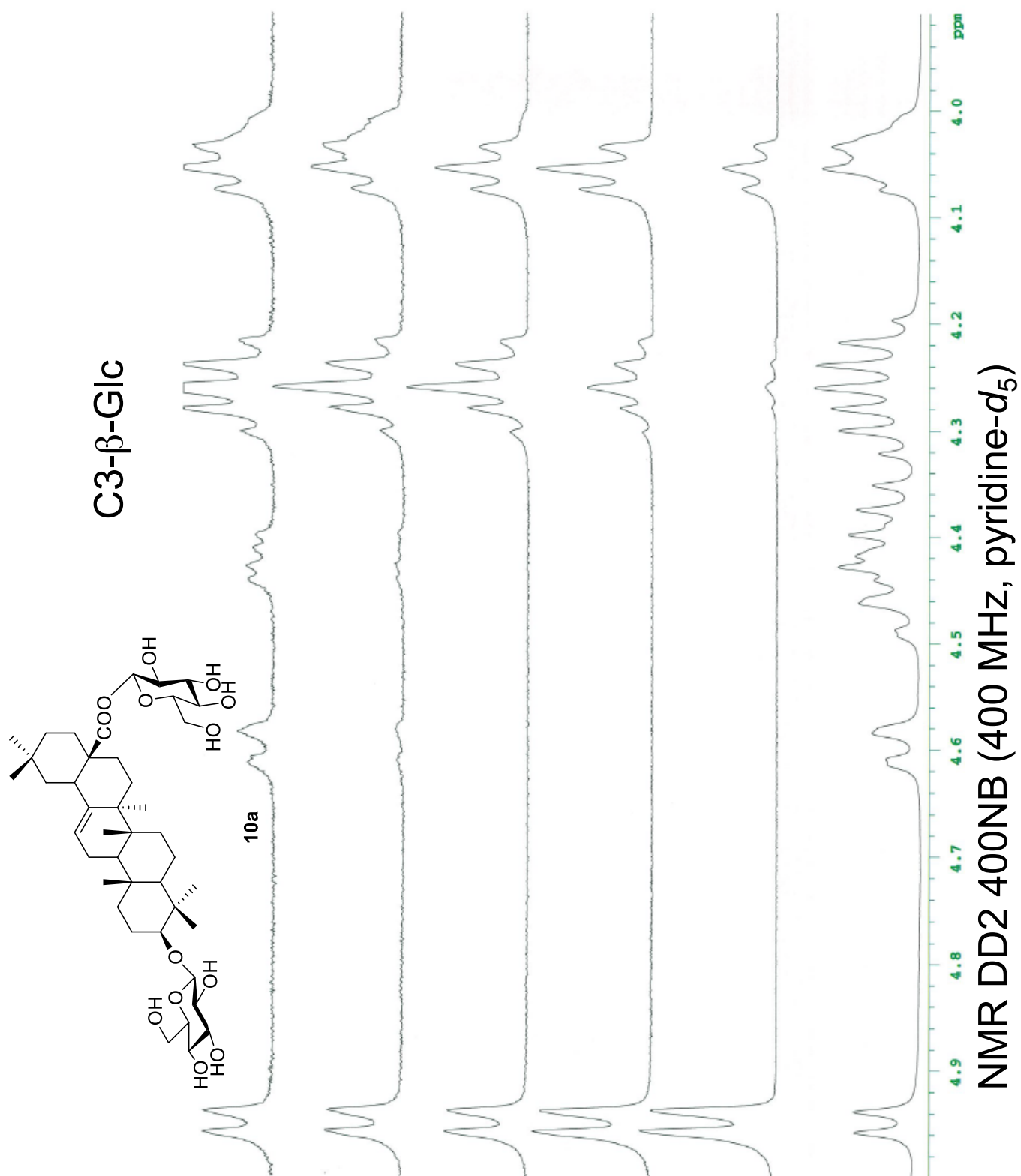
S63

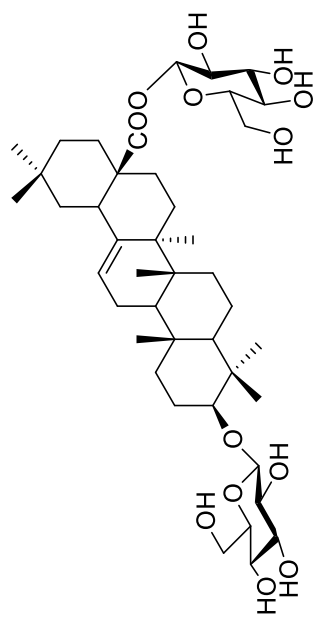
¹³C-NMR



NMR DD2 400NB (400 MHz, pyridine-*d*₅)

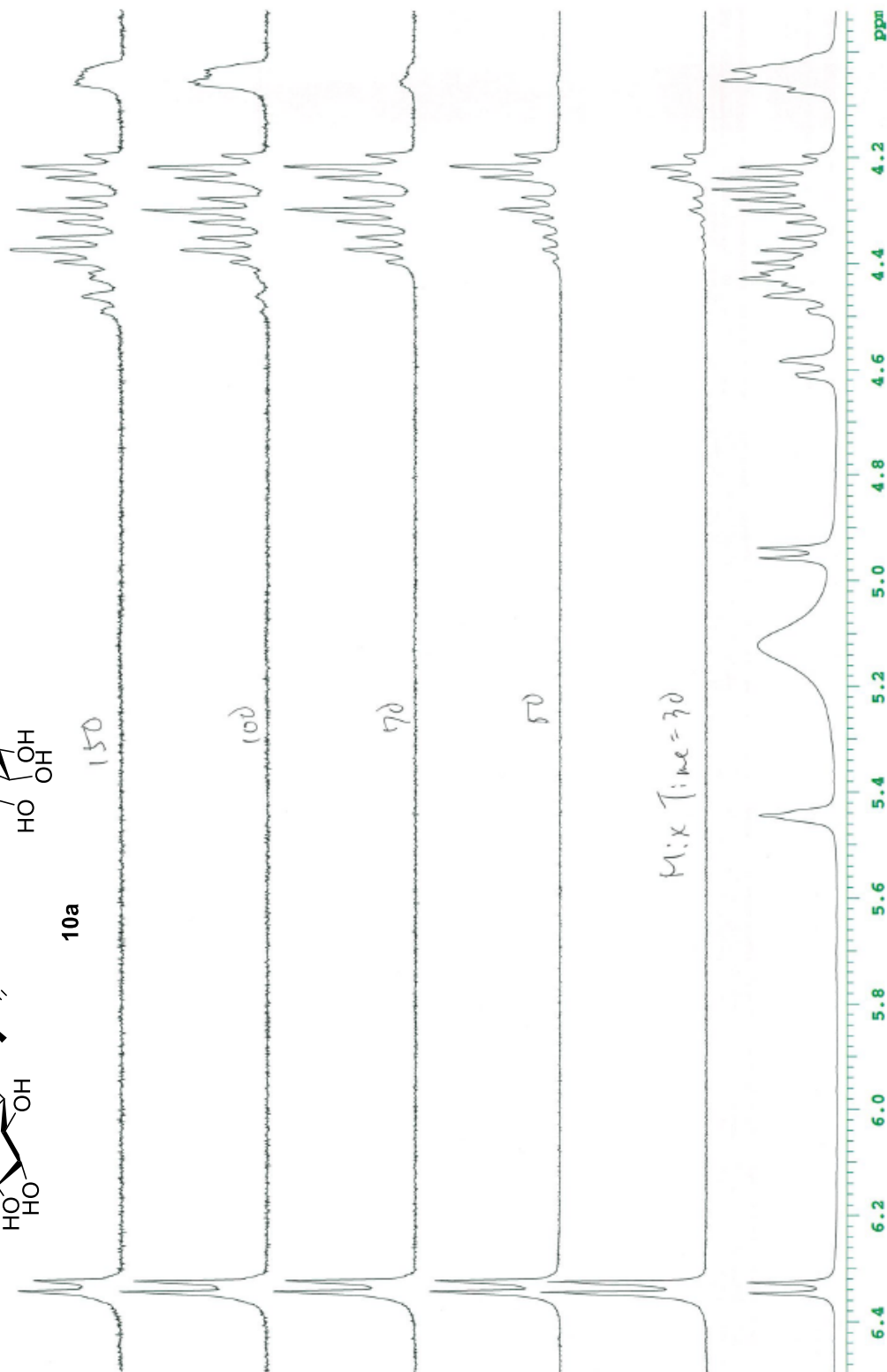
IV-3. TOCSY chart of prepared compounds





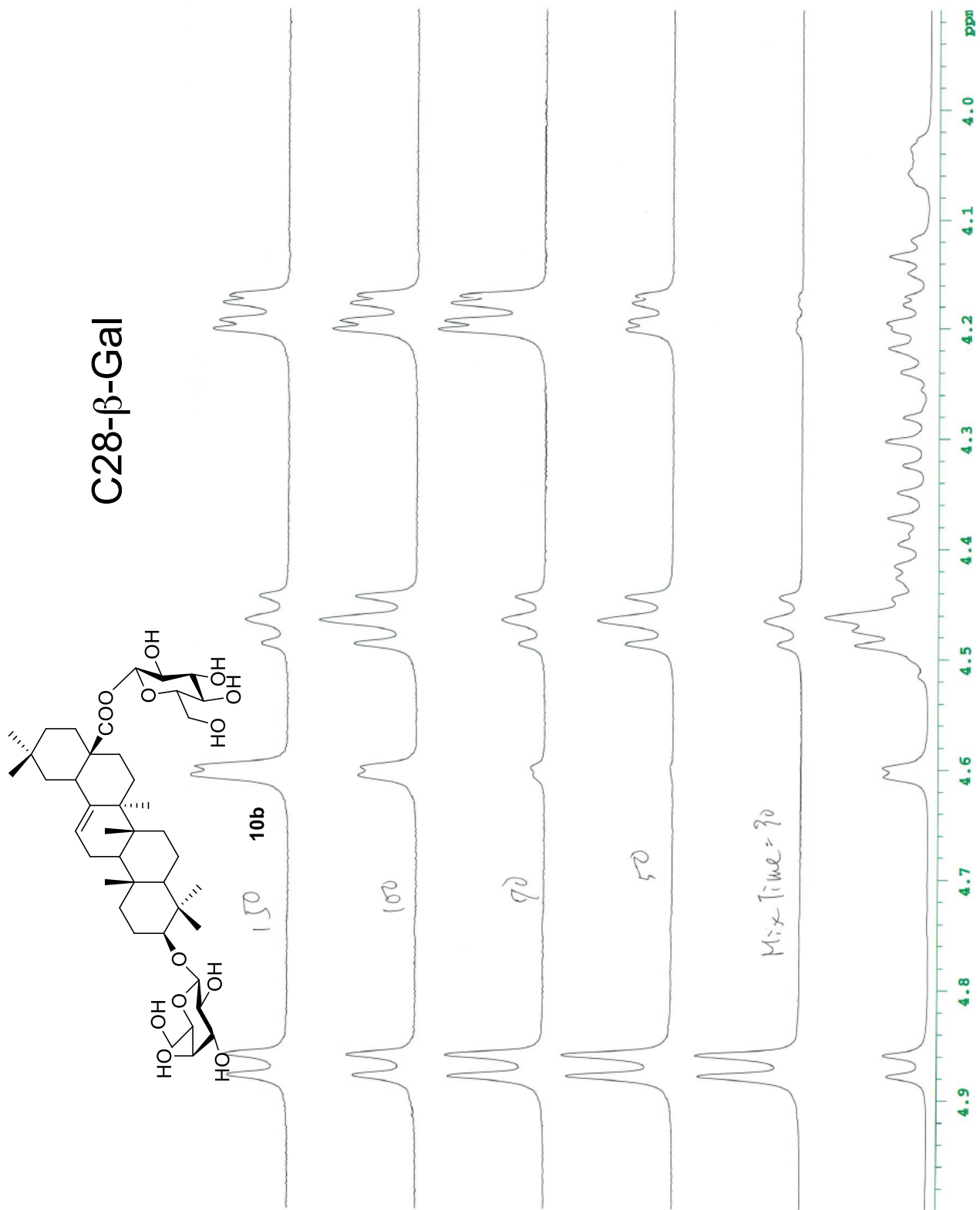
10a

C28-β-Glc



S65

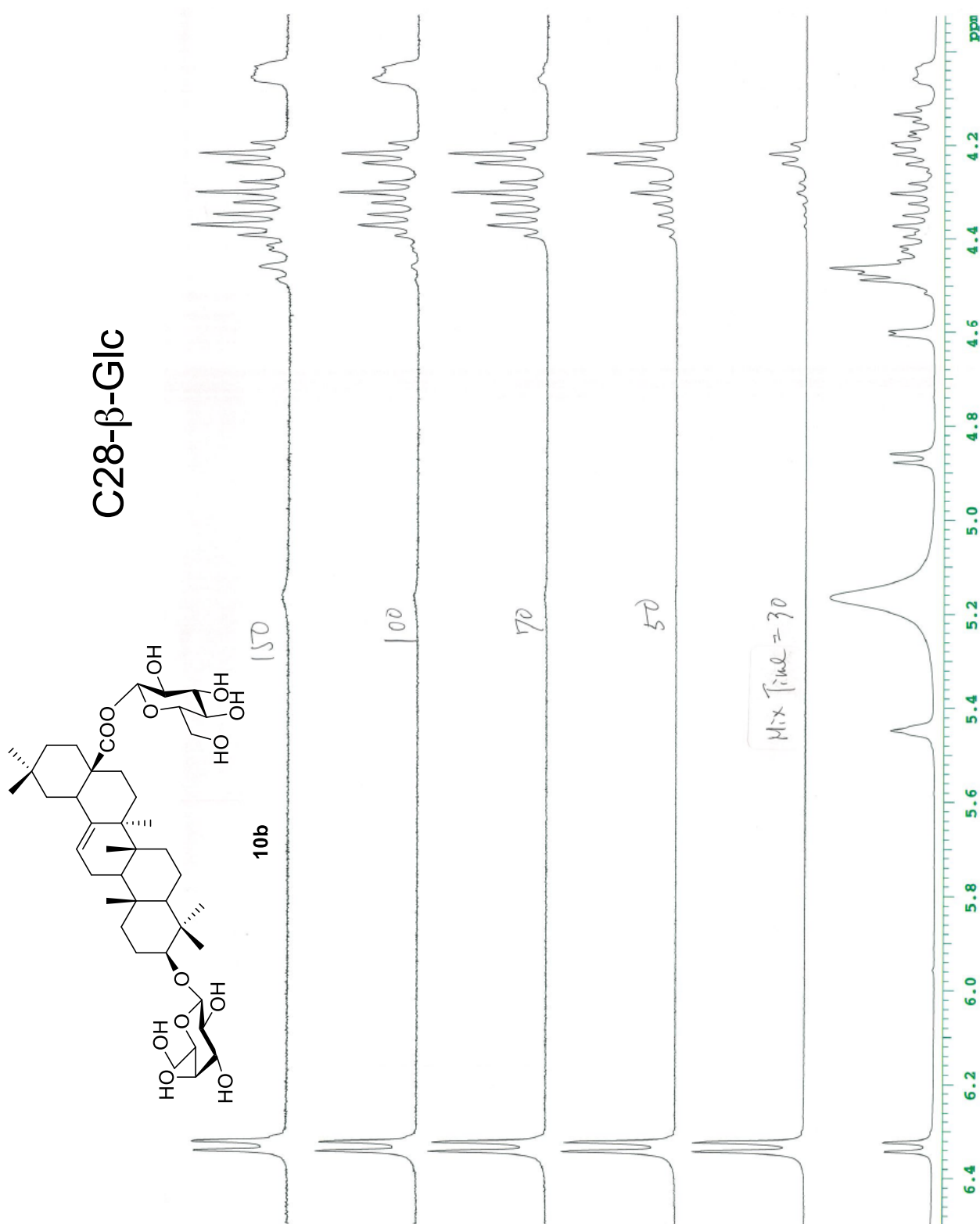
NMR DD2 400NB (400 MHz, pyridine- d_5)



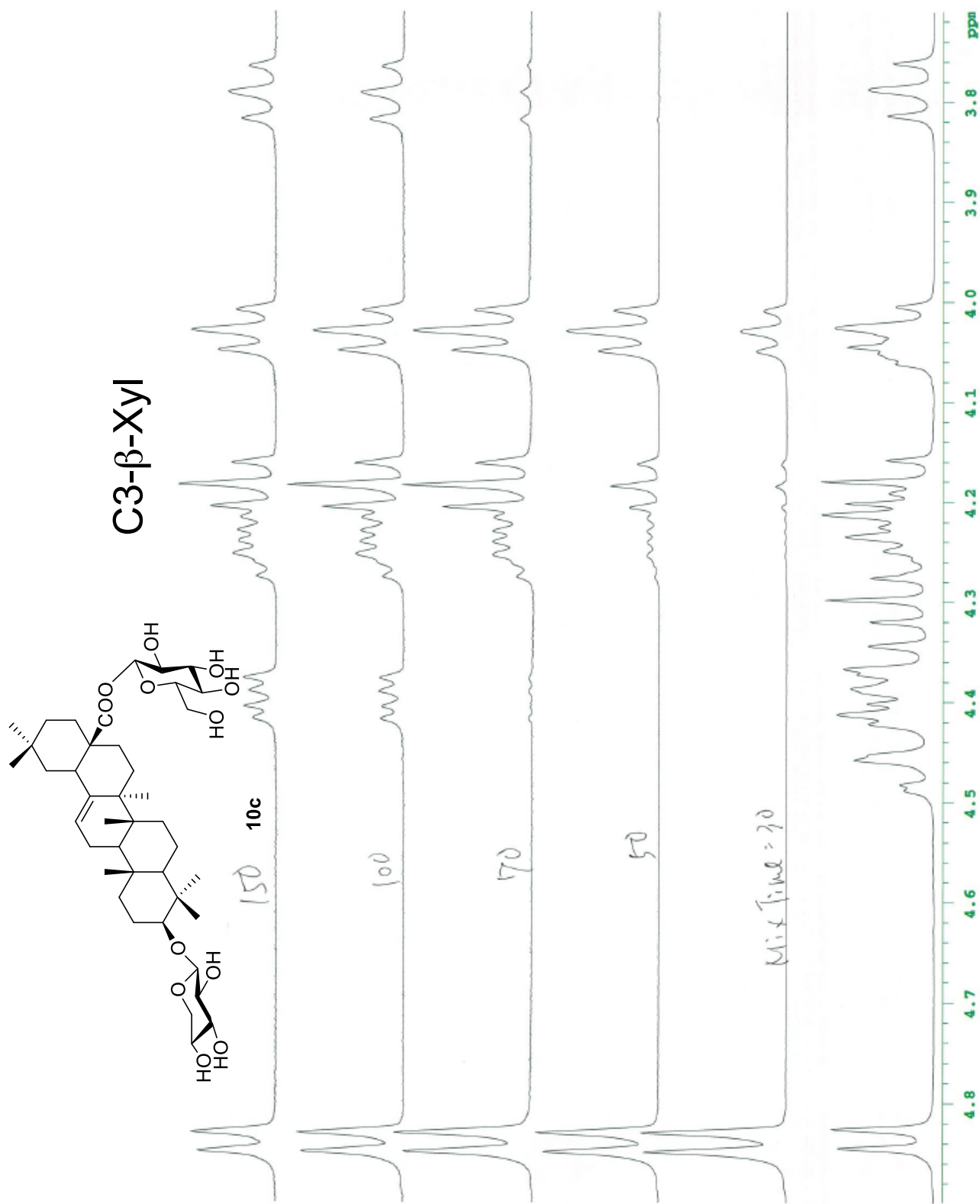
S66

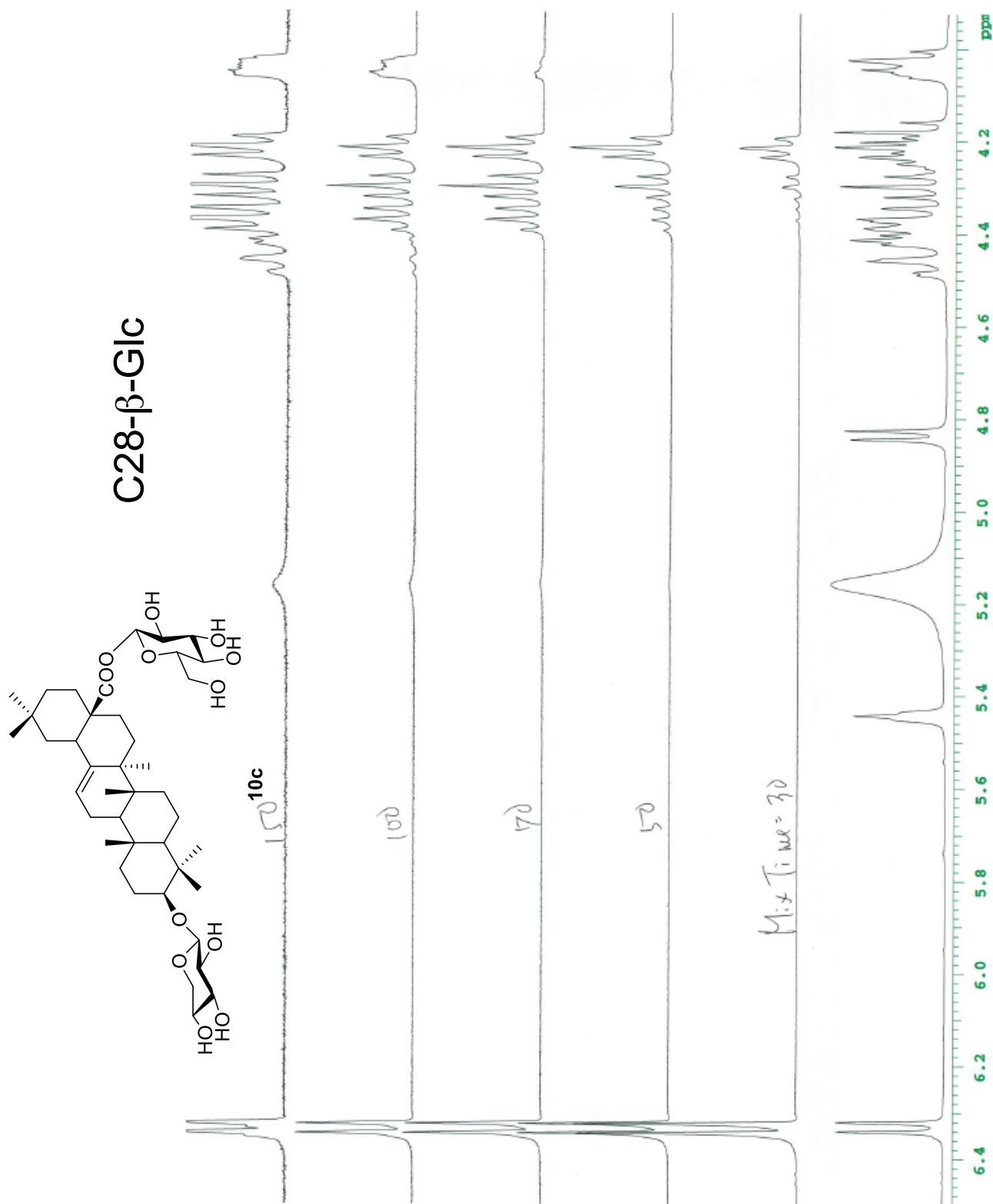
66

NMR DD2 400NB (400 MHz, pyridine- d_5)



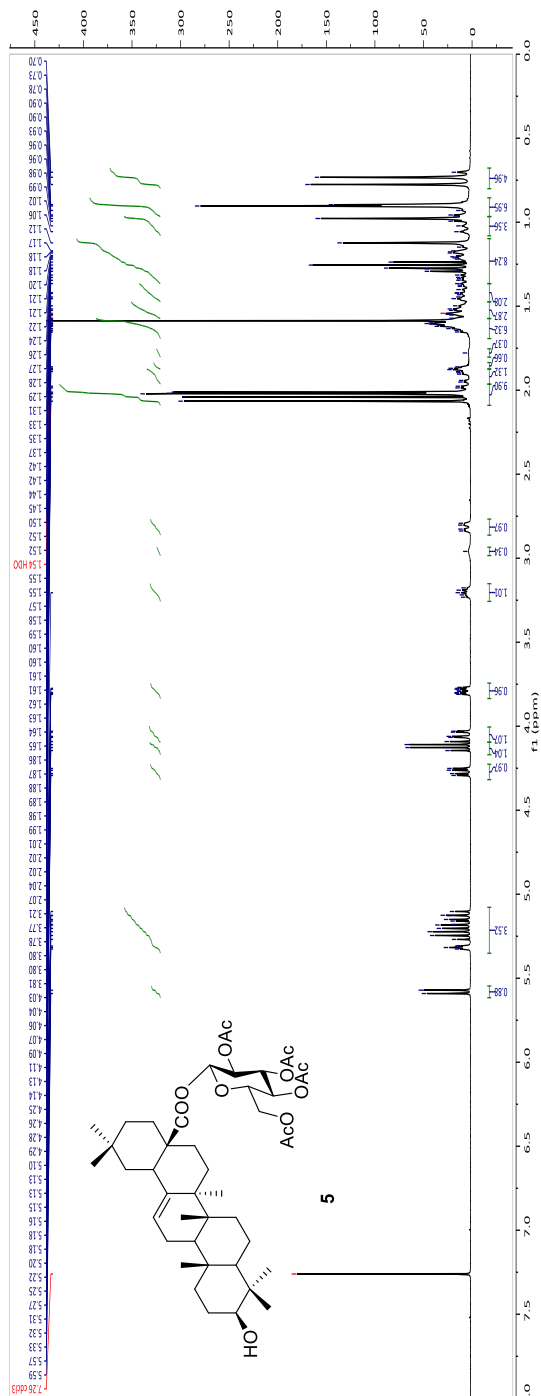
NMR DD2 400NB (400 MHz, pyridine- d_5)





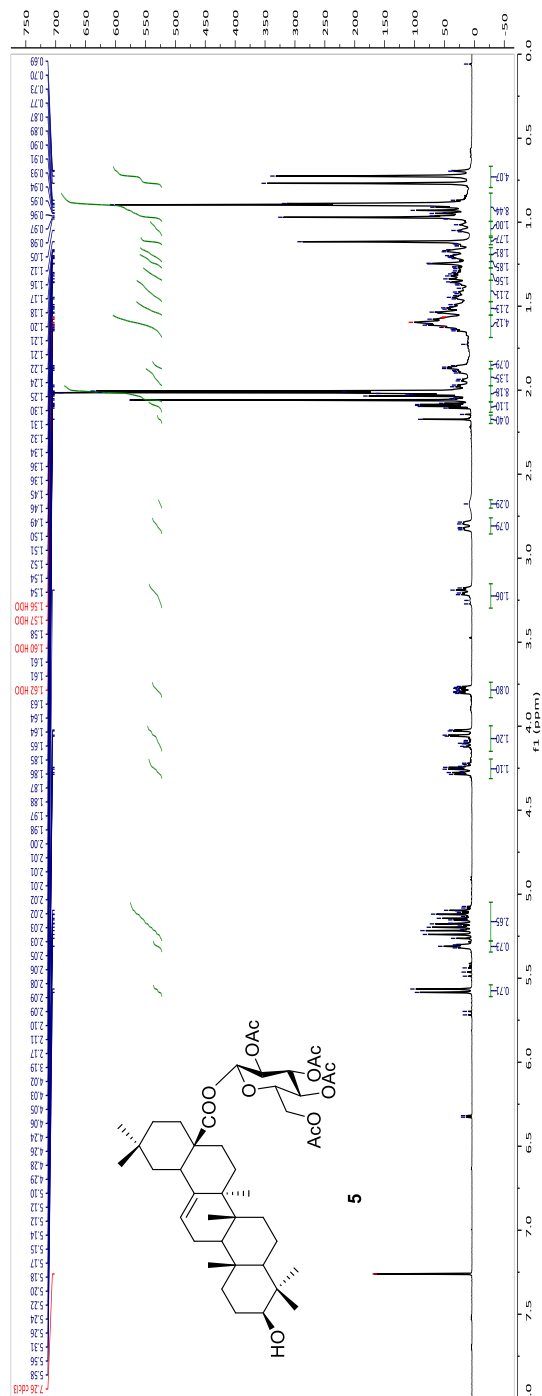
NMR DD2 400NB (400 MHz, pyridine- d_5)

IV-4. ¹H NMR chart of prepared compound with continuous microfluidic glycosylation-batch deprotection



Stepwise
synthesis
400-MR
DD2
400 MHz
CDCl₃

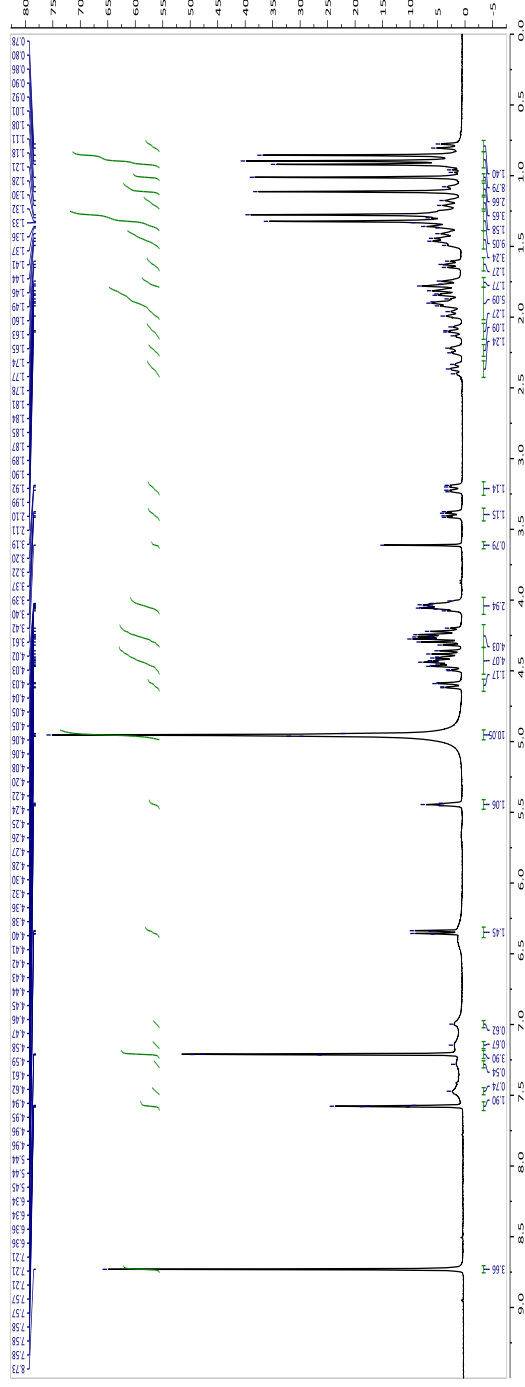
S70



Cotinuuous
synthesis
400-MR
DD2
400 MHz
CDCl₃

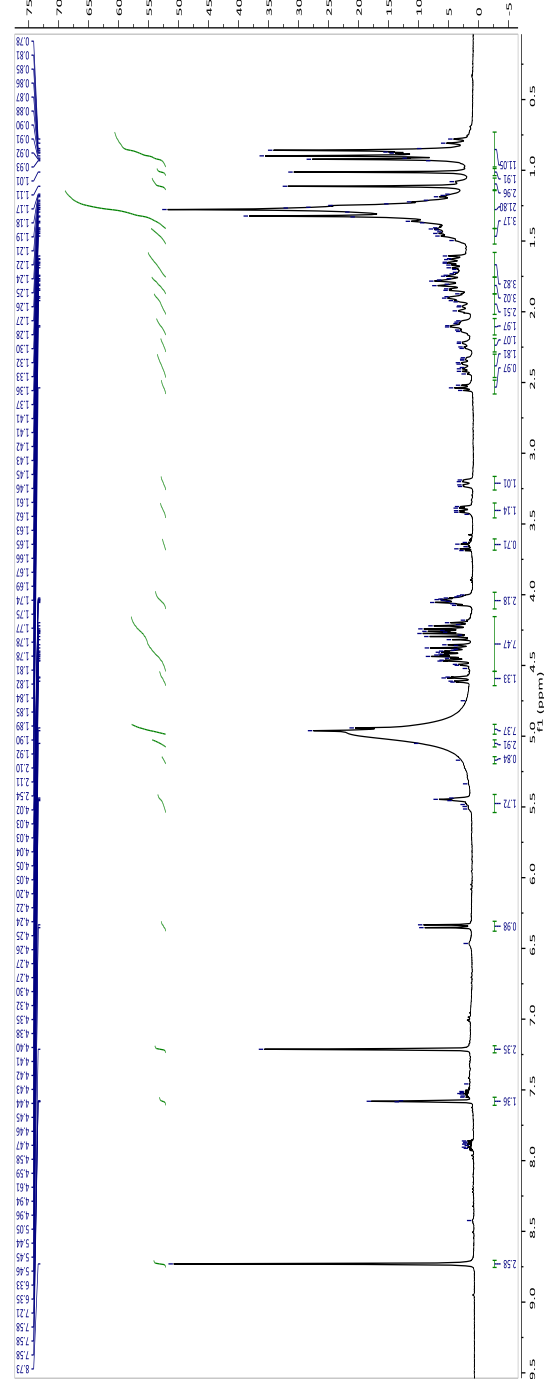
Stepwise
synthesis
400-MR
DD2
400 MHz
pyridine-*d*₅

S71



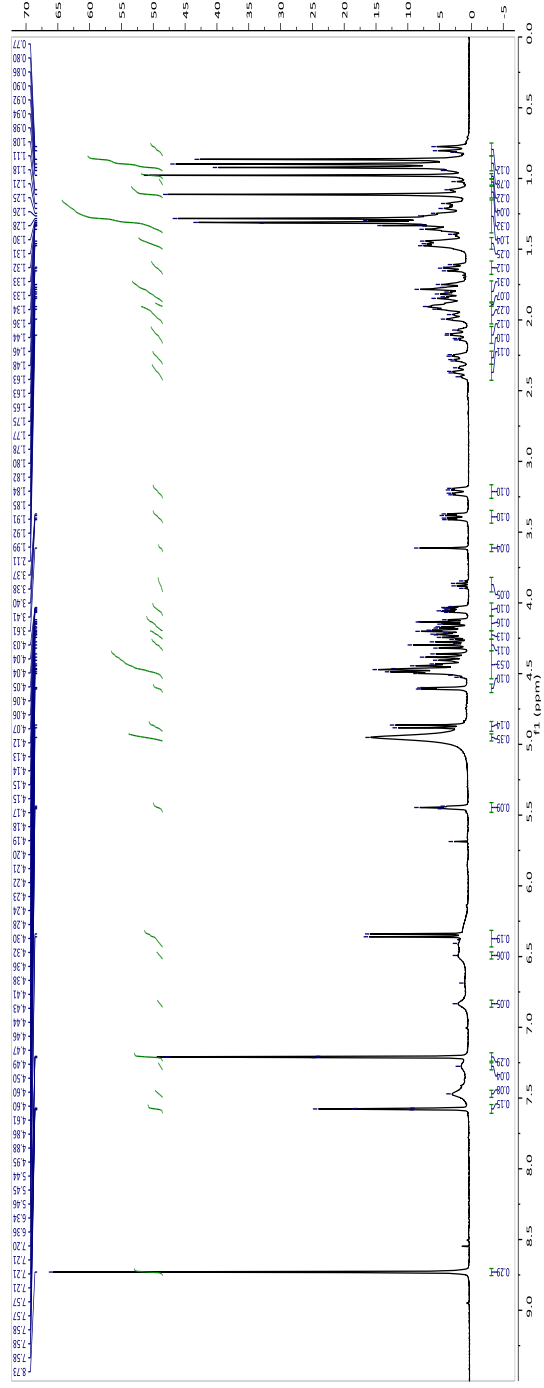
Cotinuuous
synthesis
400-MR
DD2
400 MHz
pyridine-*d*₅

71



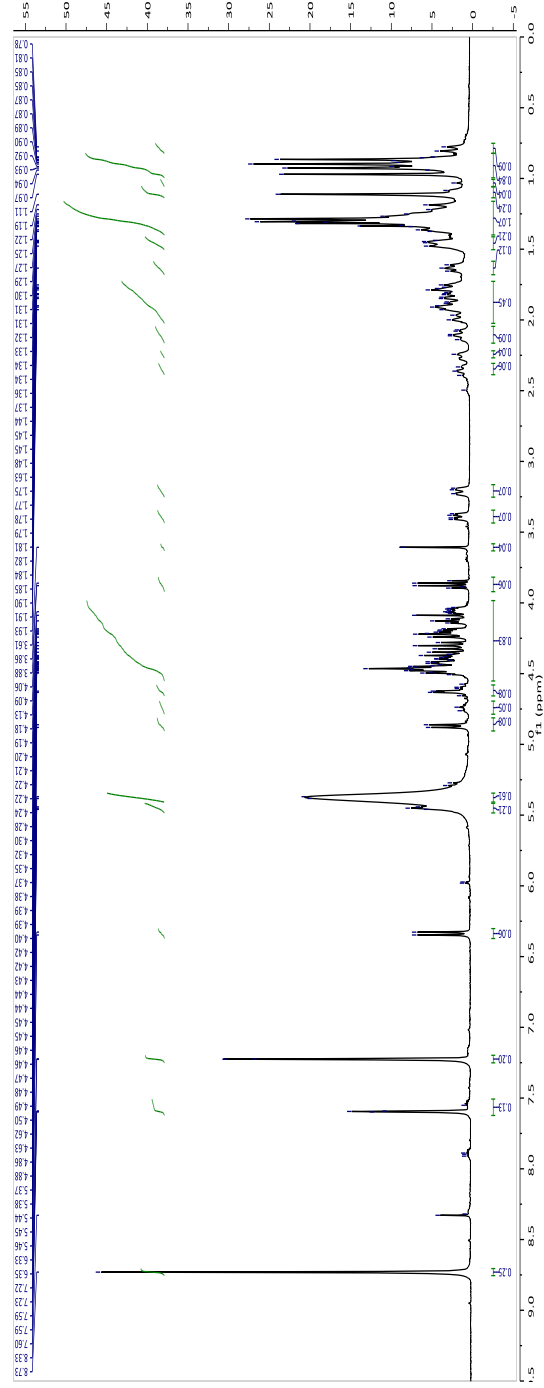
Stepwise
synthesis
400-MR
DD2
400 MHz
pyridine-*d*₅

S72

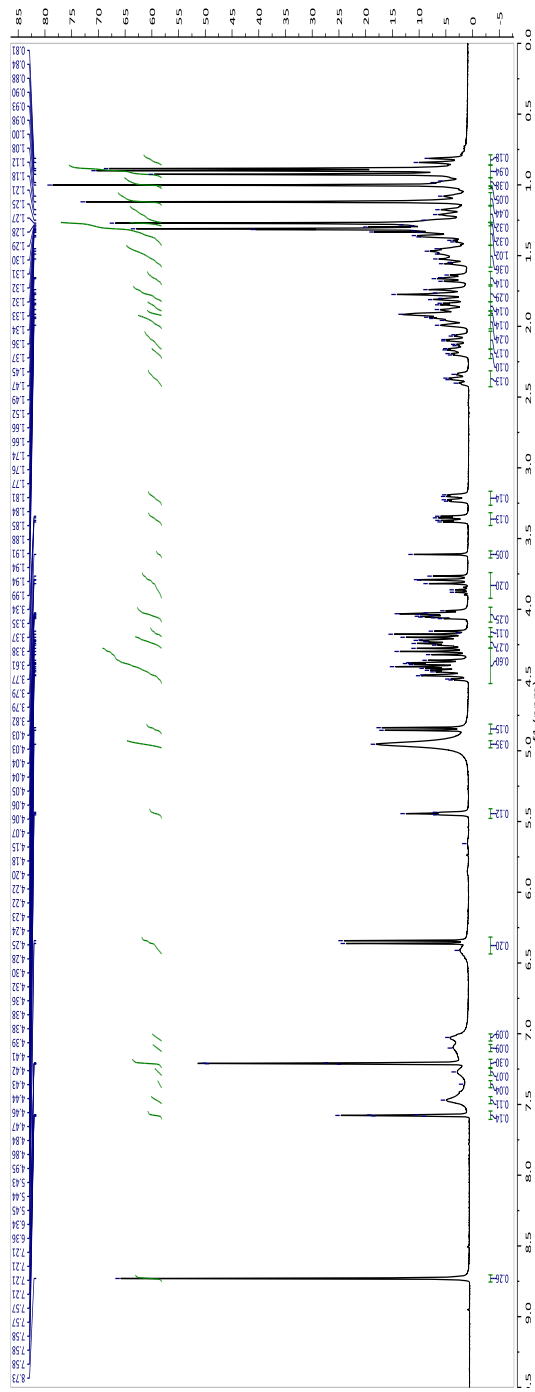


Cotinuous
synthesis
400-MR
DD2
400 MHz
pyridine-*d*₅

72



Stepwise
synthesis
400-MR
DD2
400 MHz
pyridine-*d*₅



S73

Cotinuuous
synthesis
400-MR
DD2
400 MHz
pyridine-*d*₅

