

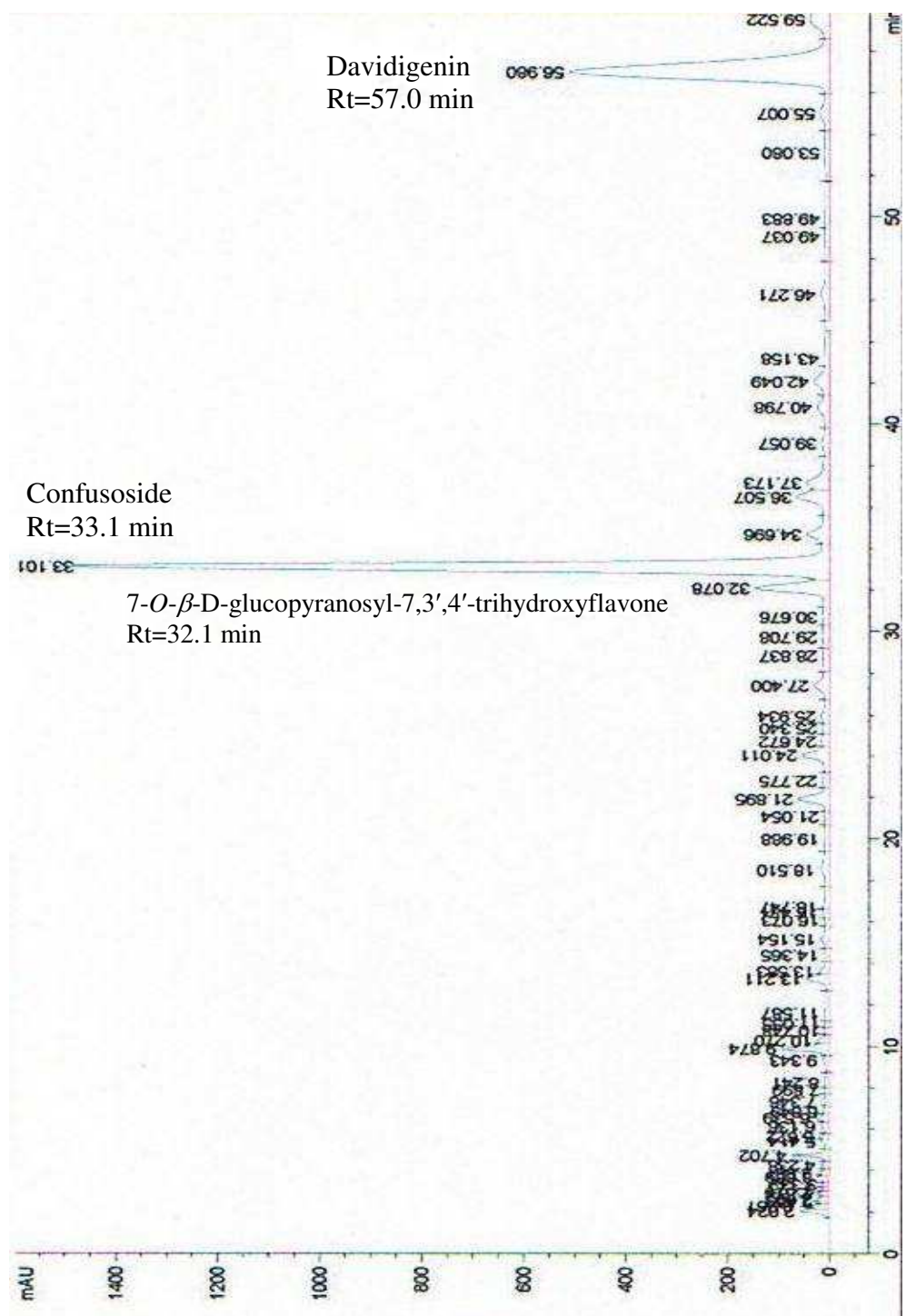
Saponins and Polyphenols from *Fadogia ancylantha* (Makoni Tea)

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Rita Aquino*

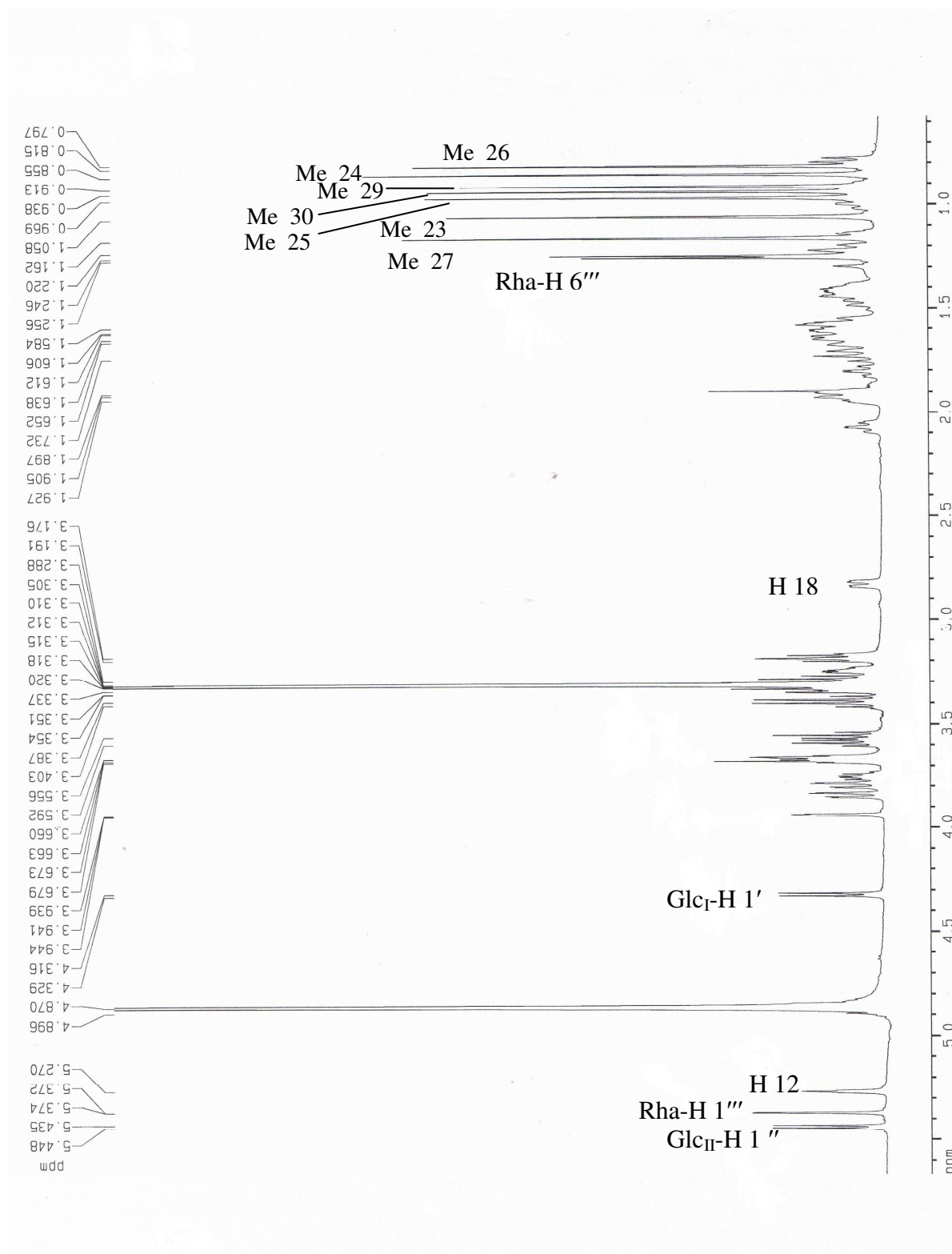
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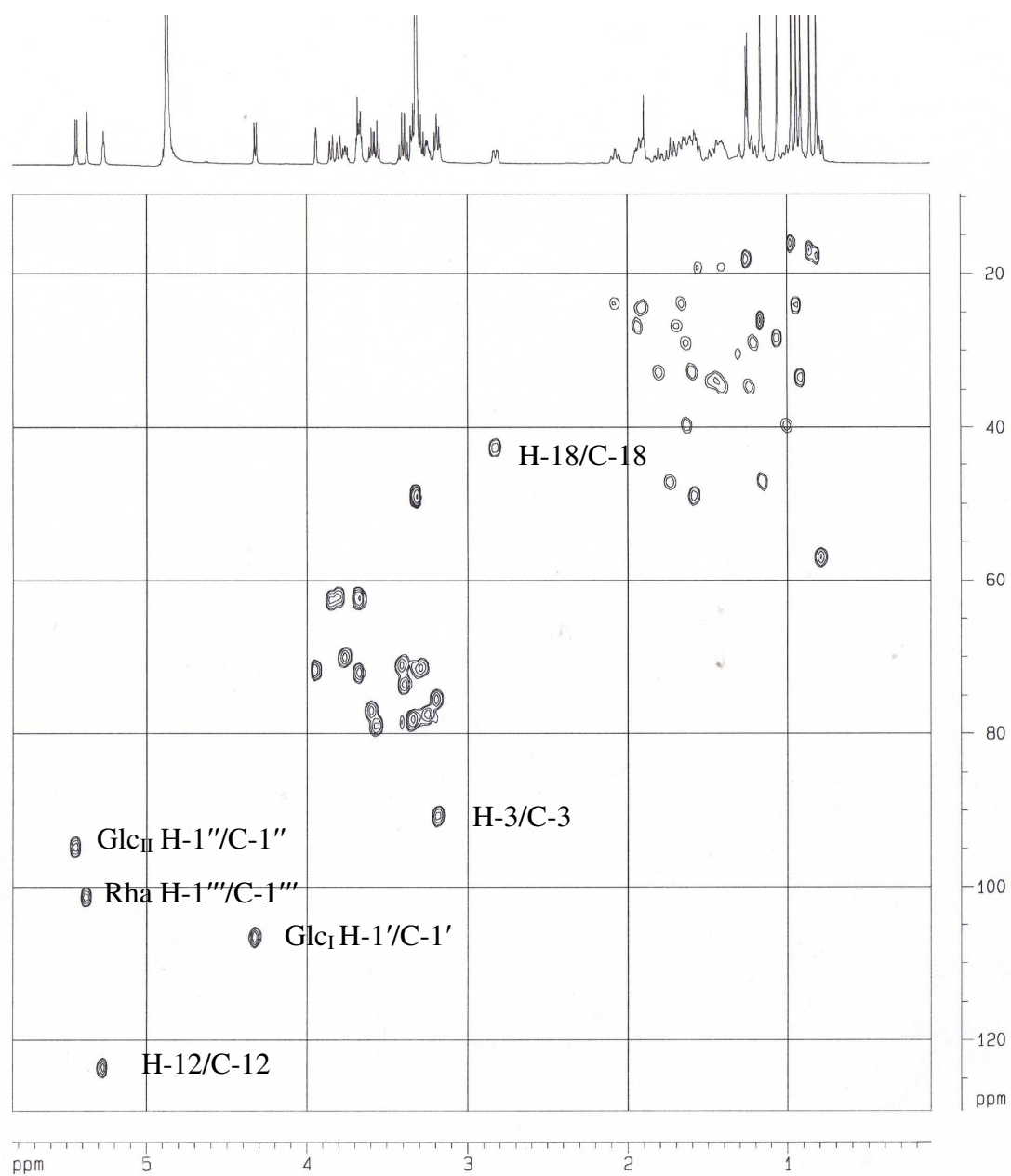
S1. HPLC fingerprint of the plant crude extract (It has been obtained in the same conditions reported in the paper at p 11)



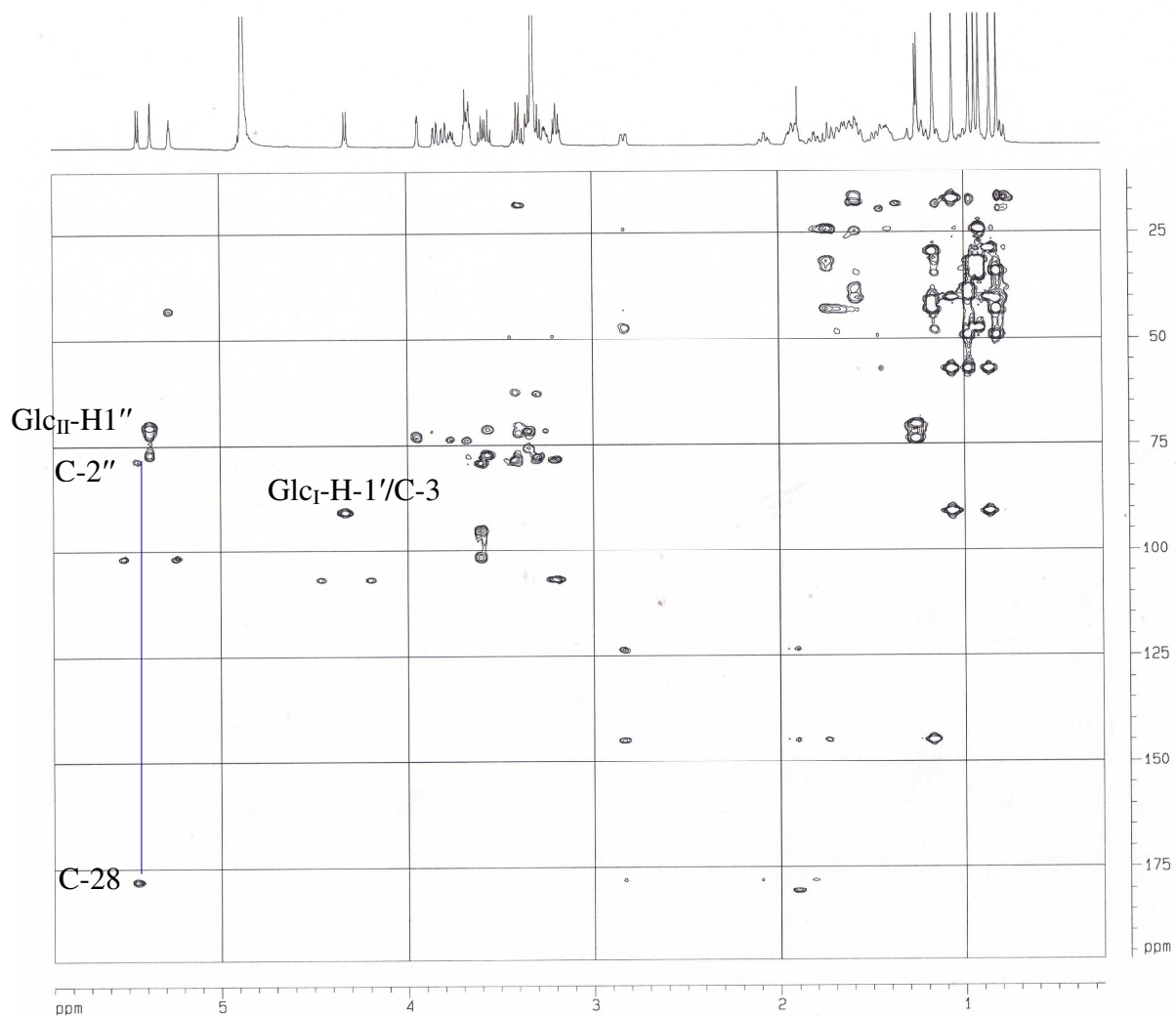
S2. ^1H NMR (600 MHz, CD_3OD) of the new compound **1**



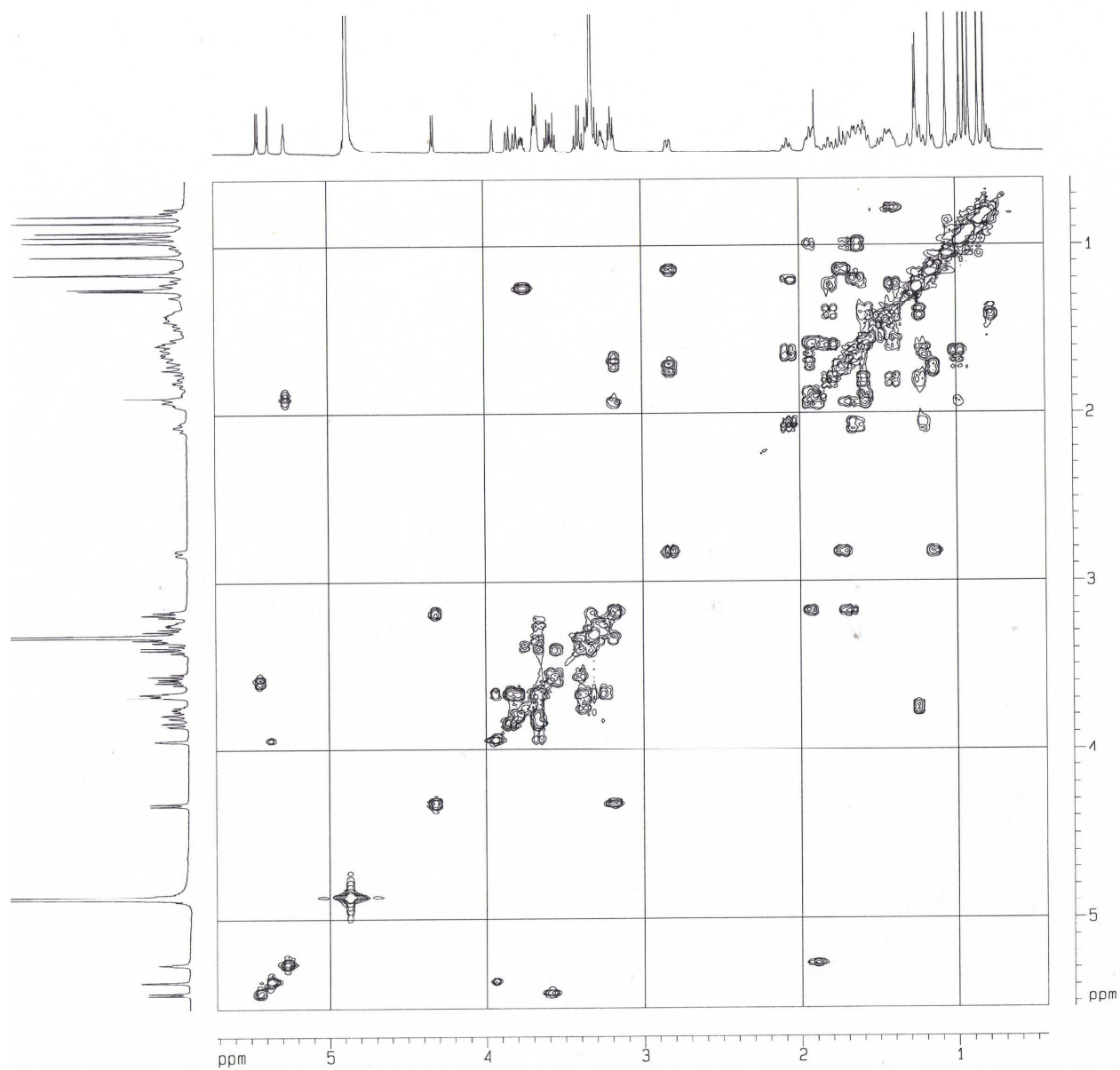
S3. HSQC (600 MHz, CD₃OD) of the new compound **1**



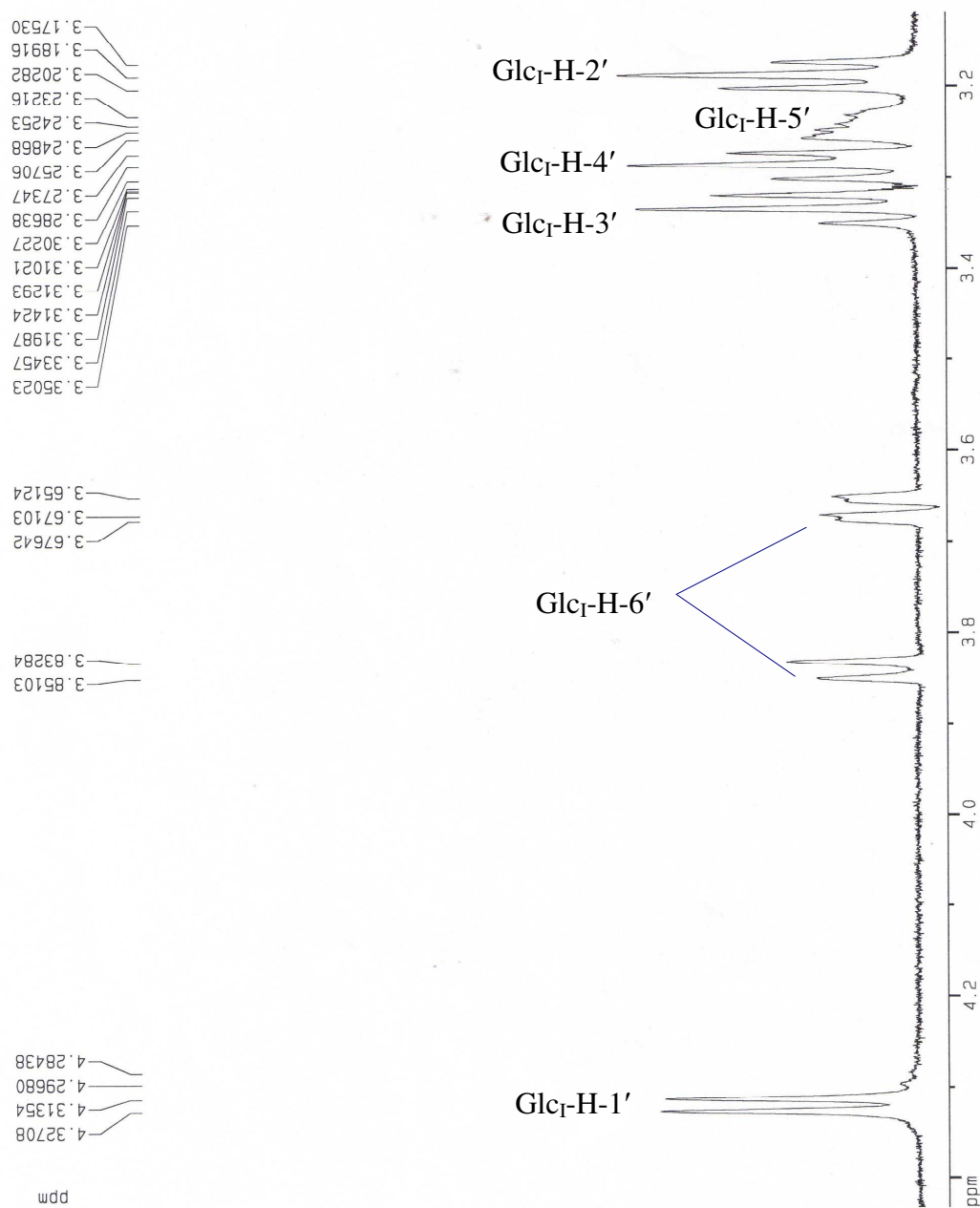
S4. HMBC (600 MHz, CD₃OD) of the new compound **1**



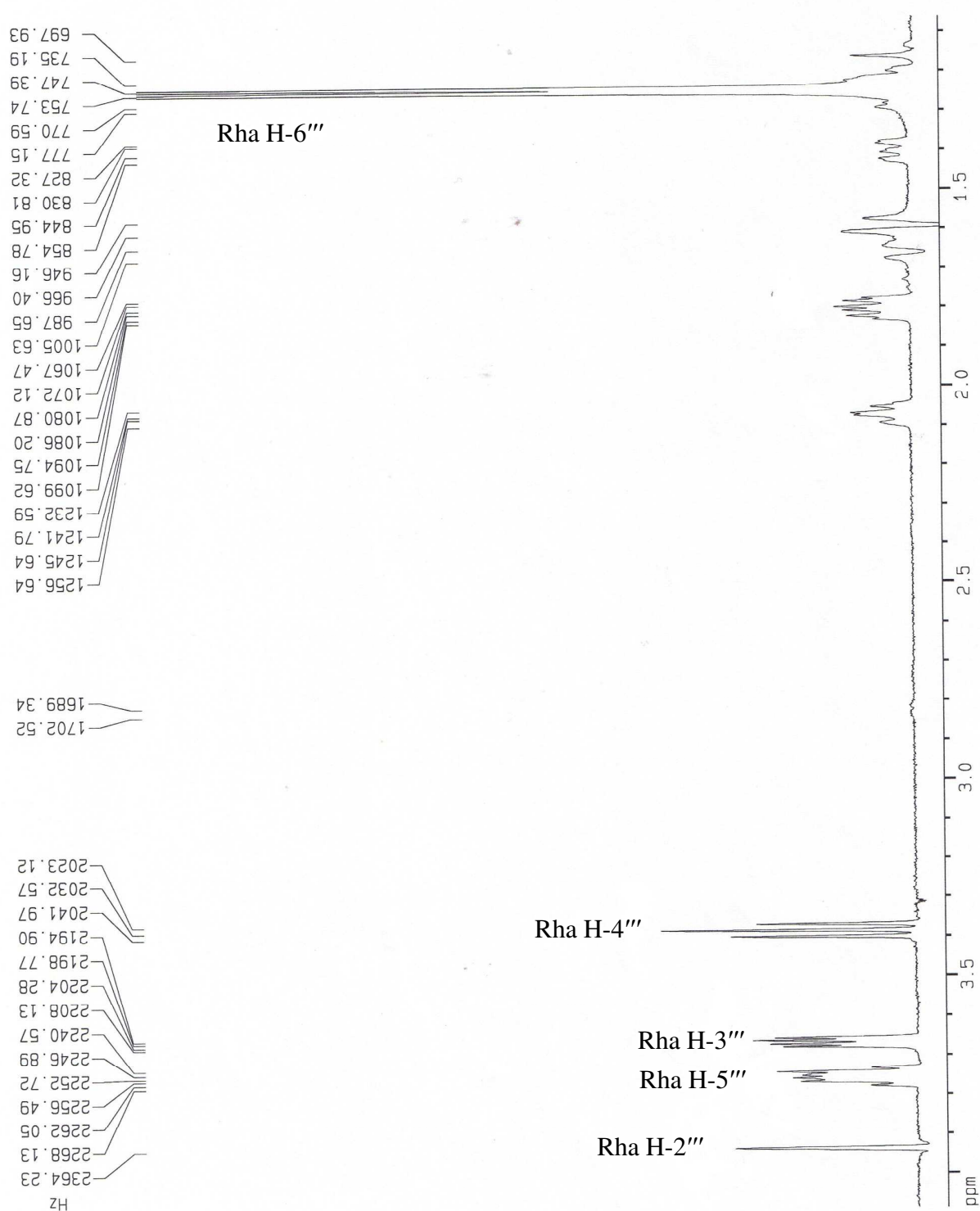
S5. DQF-COSY (600 MHz, CD₃OD) of the new compound **1**



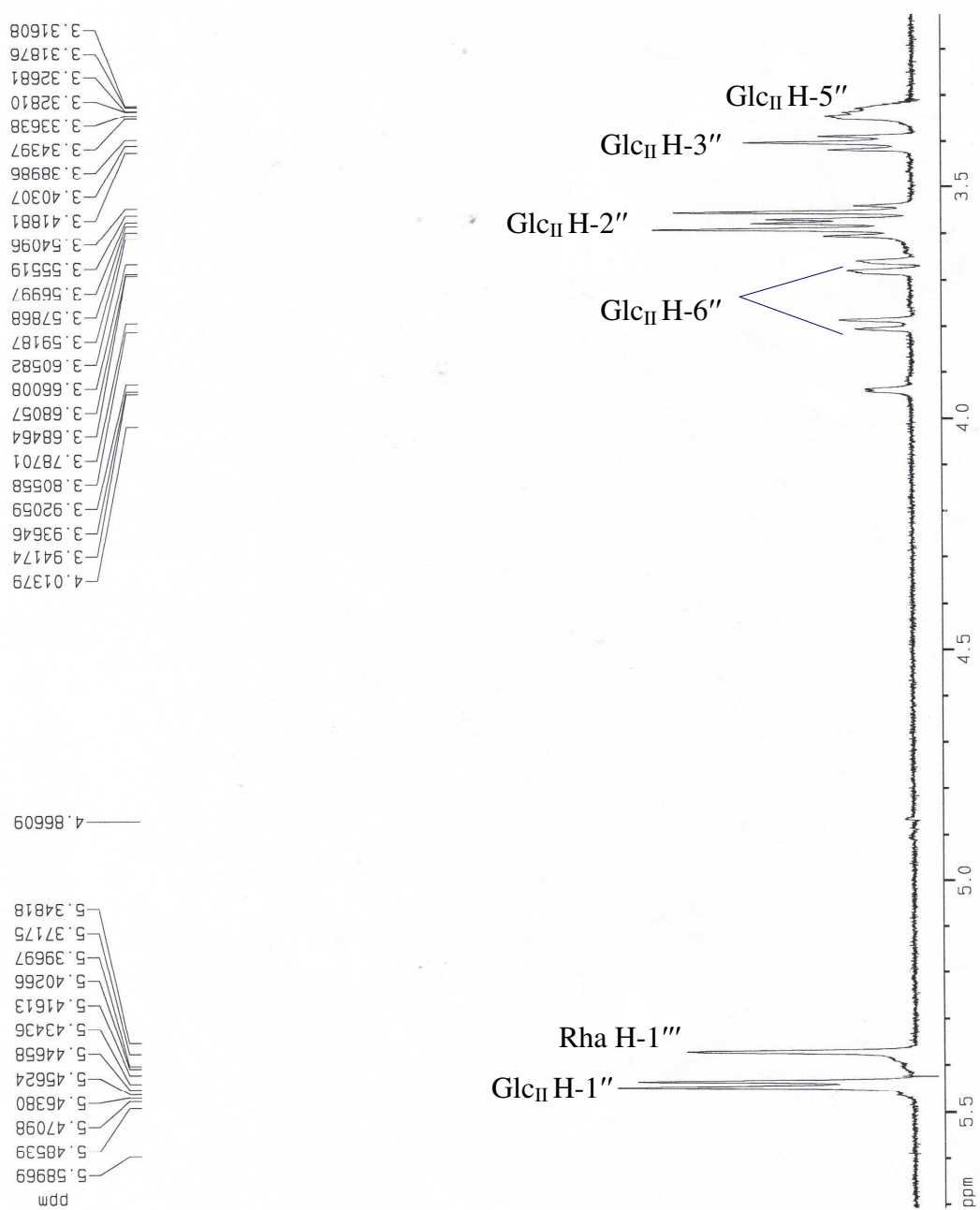
S6. 1D-TOCSY (600 MHz, CD₃OD) of the new compound **1**



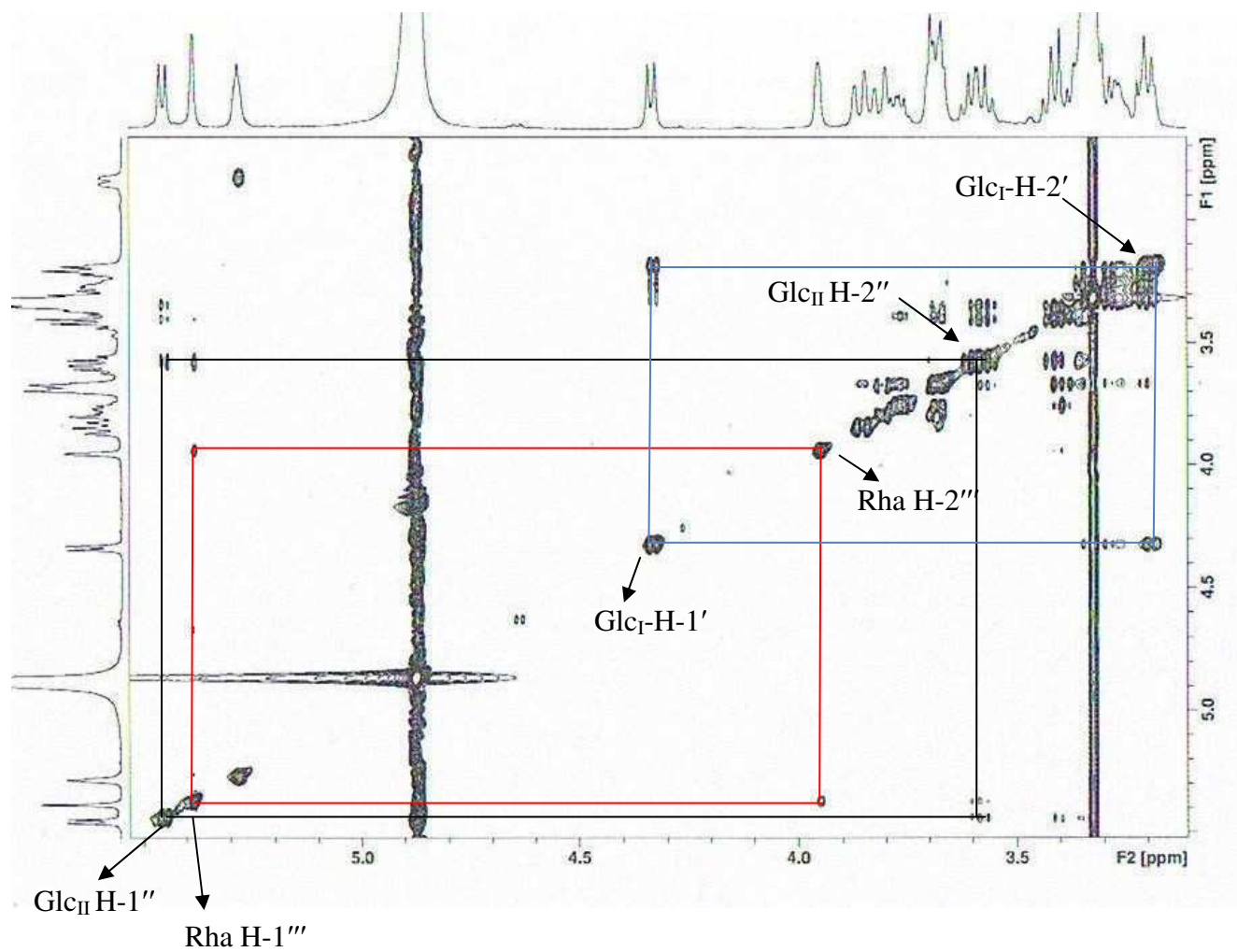
S7. 1D-TOCSY (600 MHz, CD₃OD) of the new compound **1**



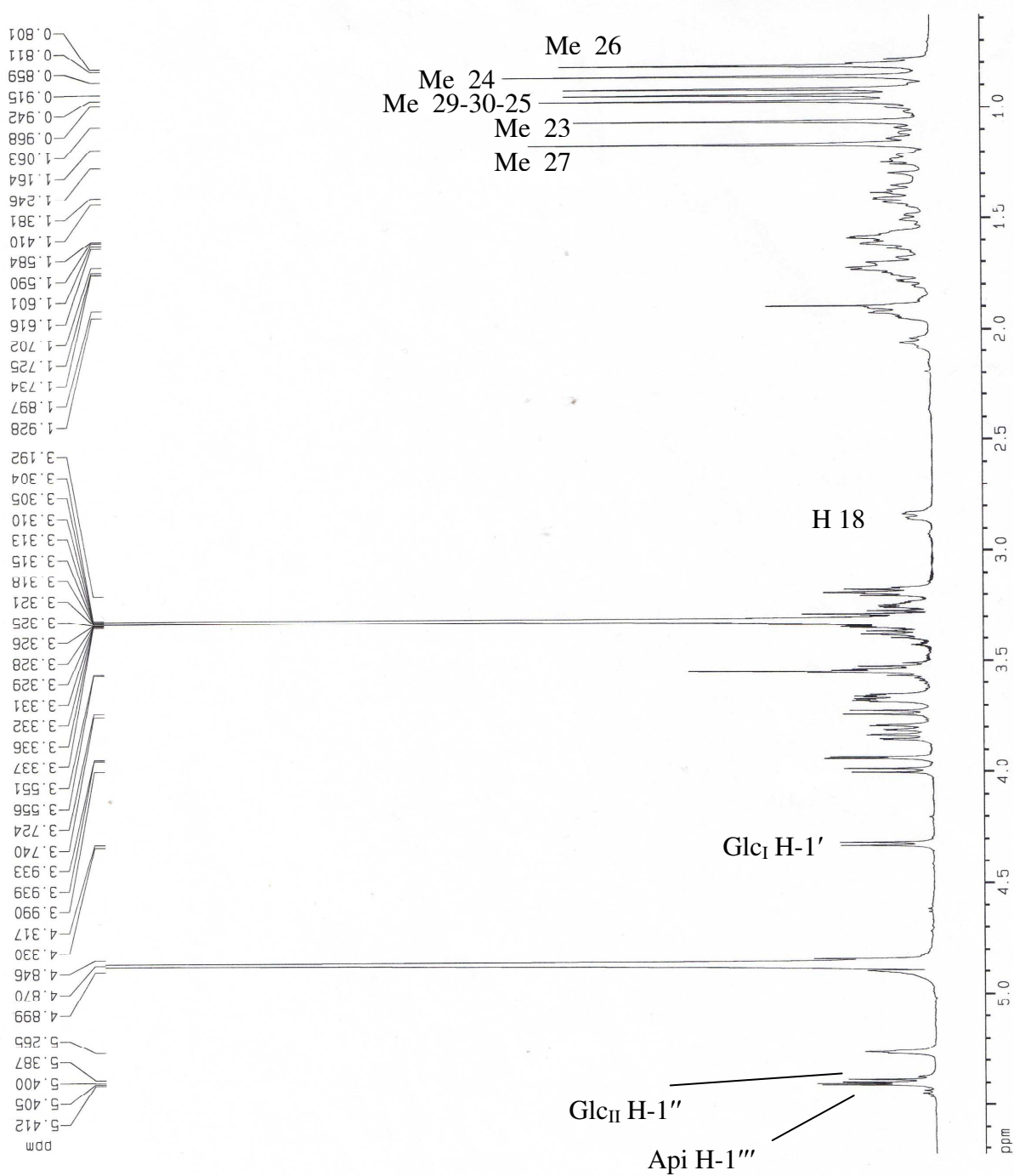
S8. 1D-TOCSY (600 MHz, CD₃OD) of the new compound **1**



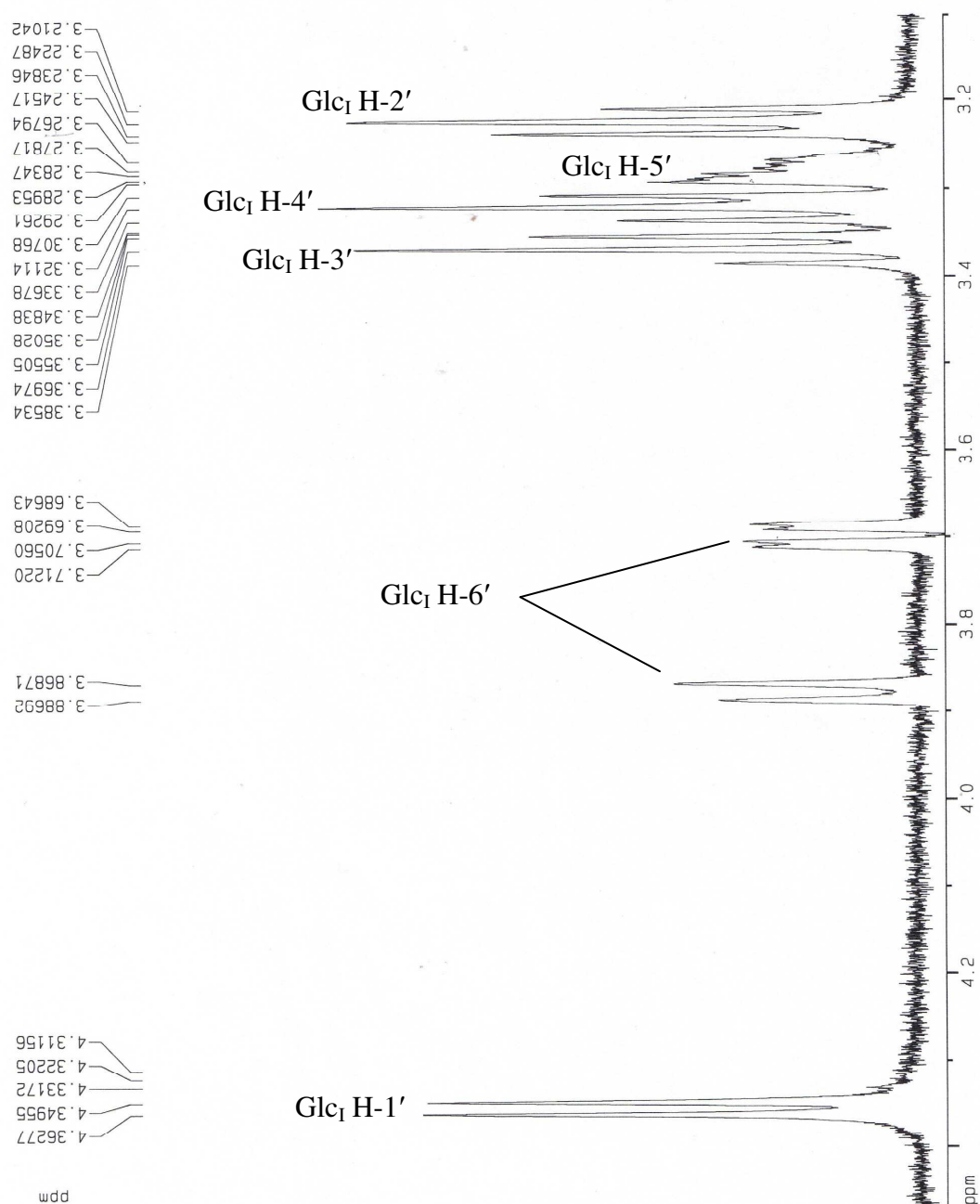
S9. ROESY (600 MHz, CD₃OD) of the new compound **1**



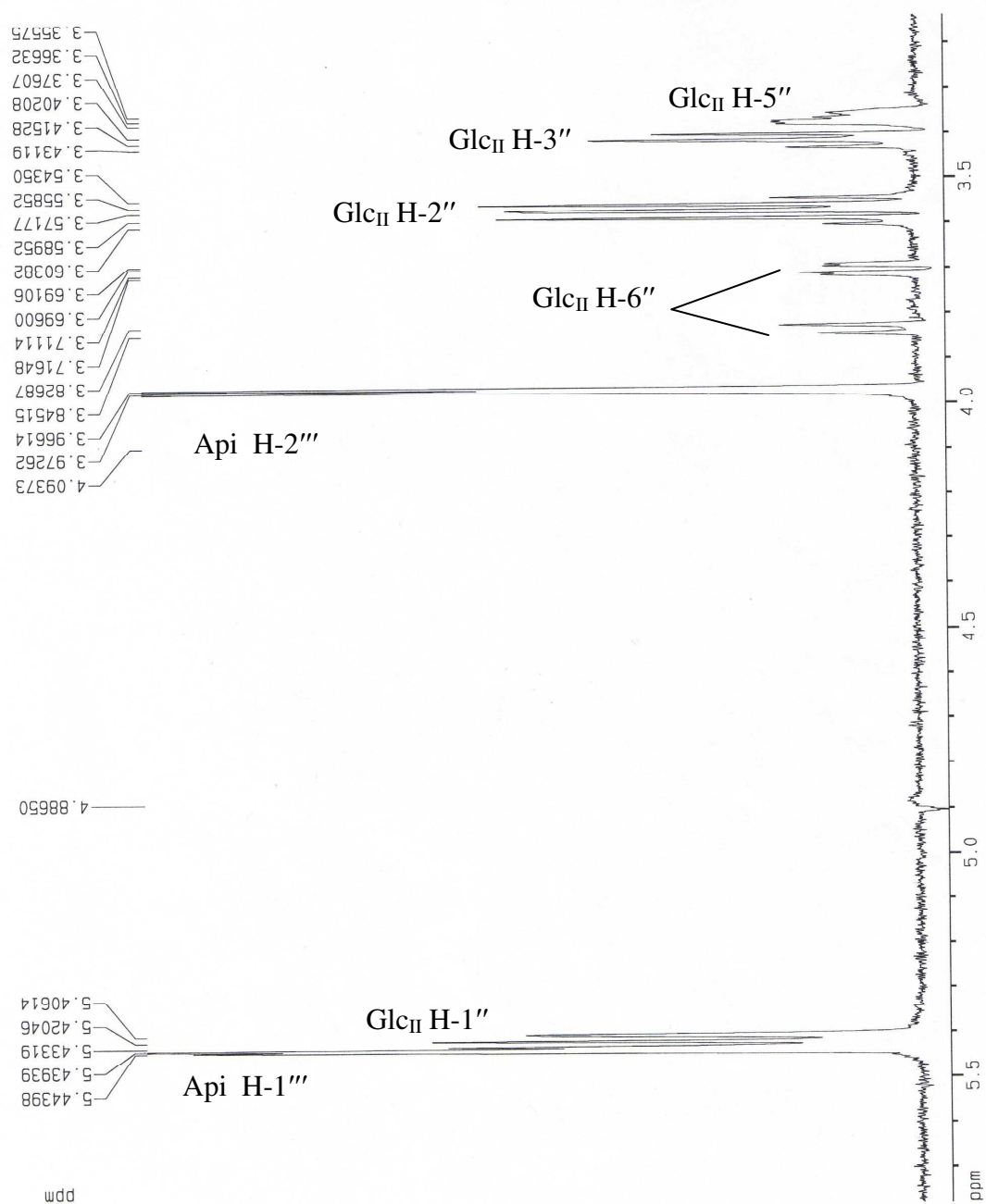
S10. ^1H NMR (600 MHz, CD_3OD) of the new compound **2**



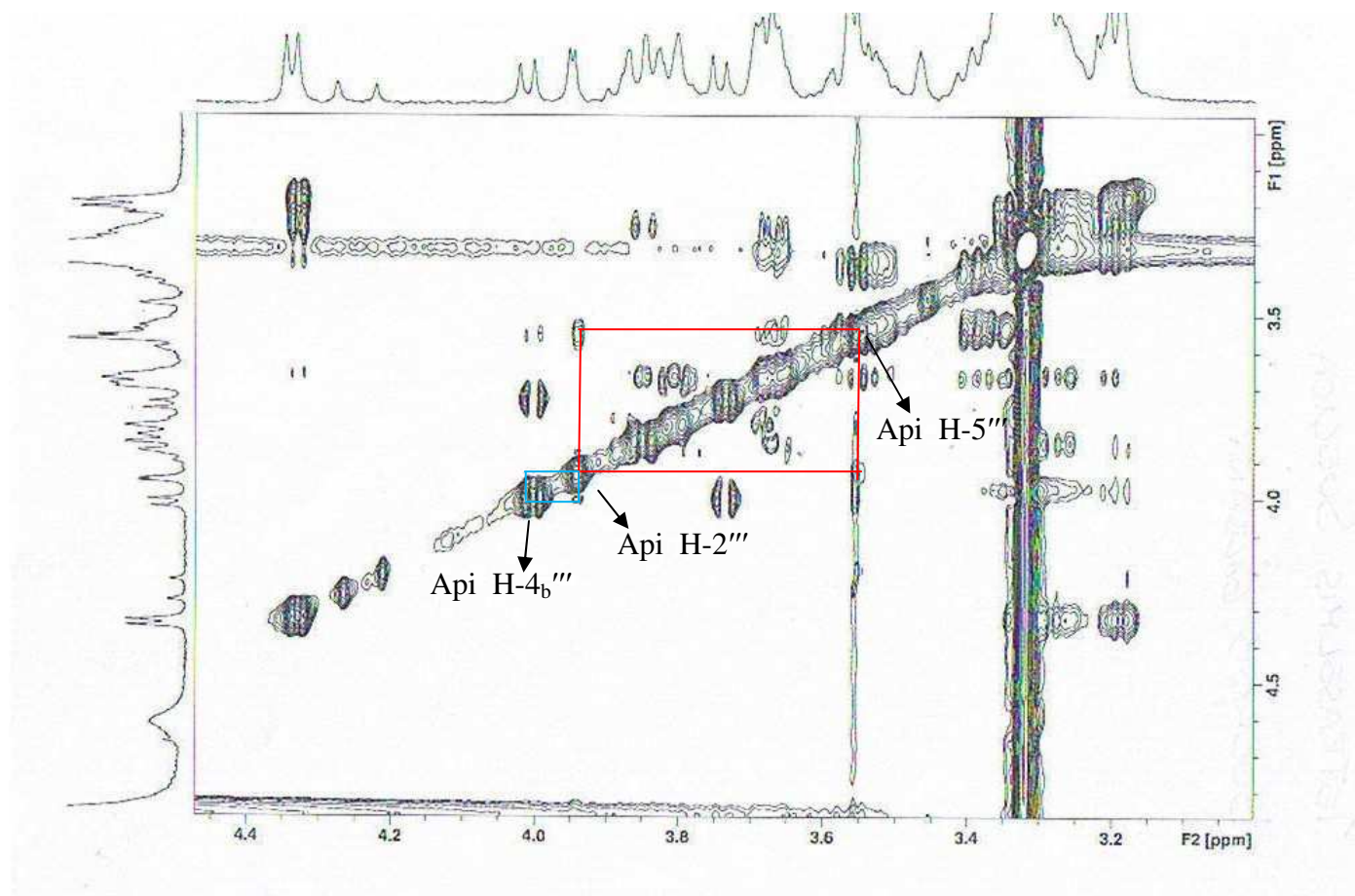
S11. 1D TOCSY (600 MHz, CD₃OD) of the new compound **2**



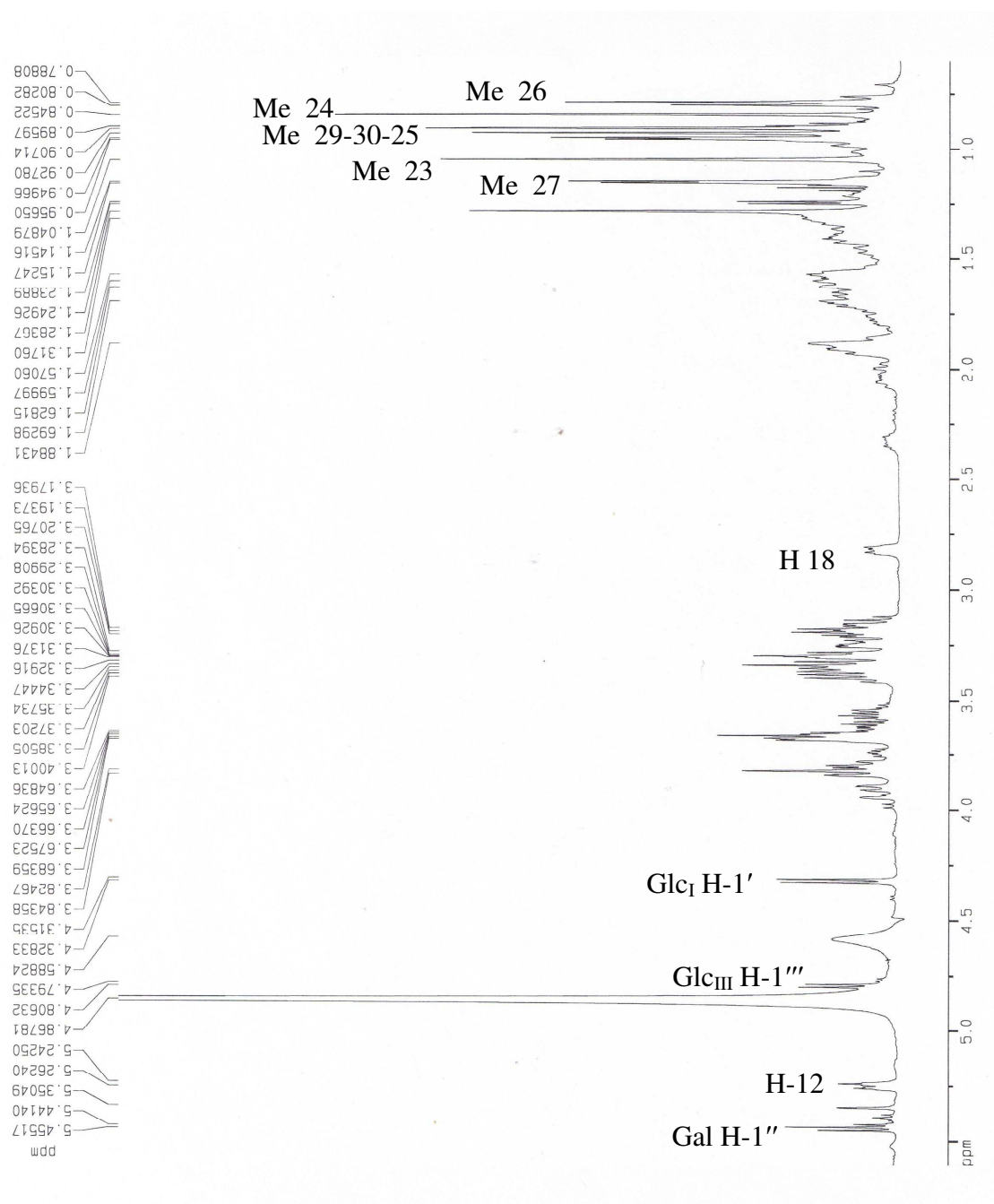
S12. 1D TOCSY (600 MHz, CD₃OD) of the new compound **2**



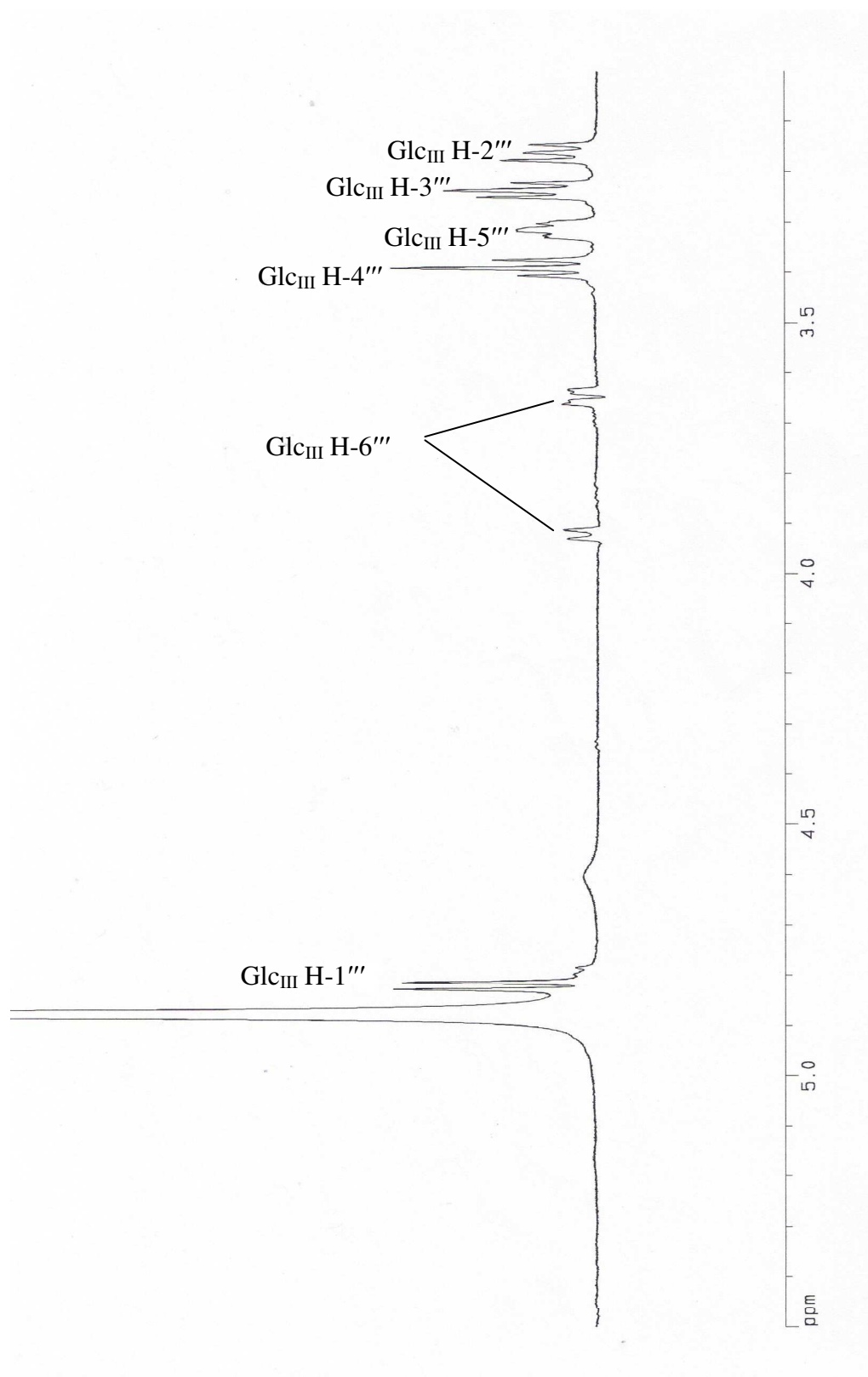
S13. ROESY (600 MHz, CD₃OD) of the new compound **2**



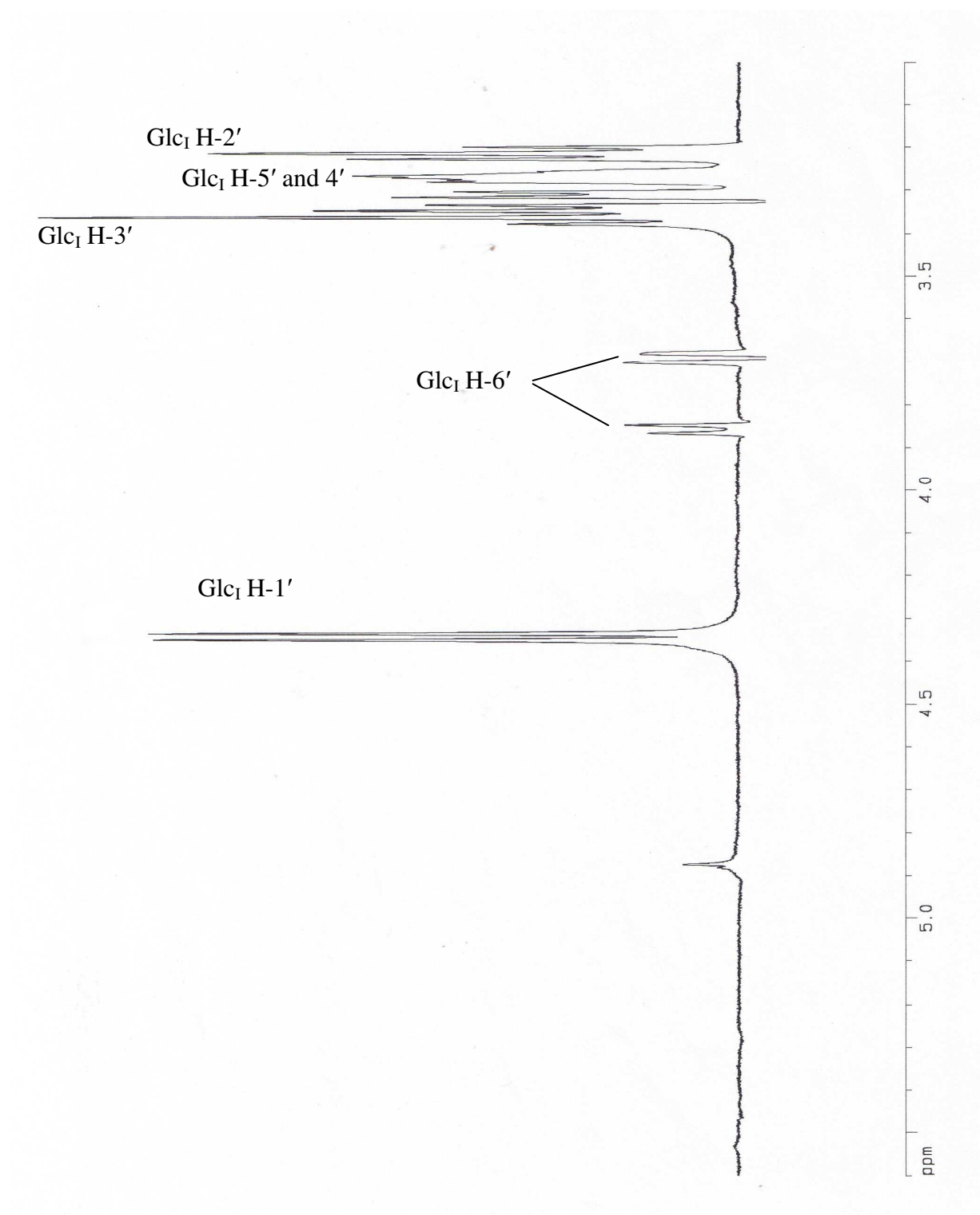
S14. ^1H NMR (600 MHz, CD_3OD) of the new compound **3**



S15. 1D TOCSY (600 MHz, CD₃OD) of the new compound **3**



S16. 1D TOCSY (600 MHz, CD₃OD) of the new compound **3**



S17. 1D TOCSY (600 MHz, CD₃OD) of the new compound **3**

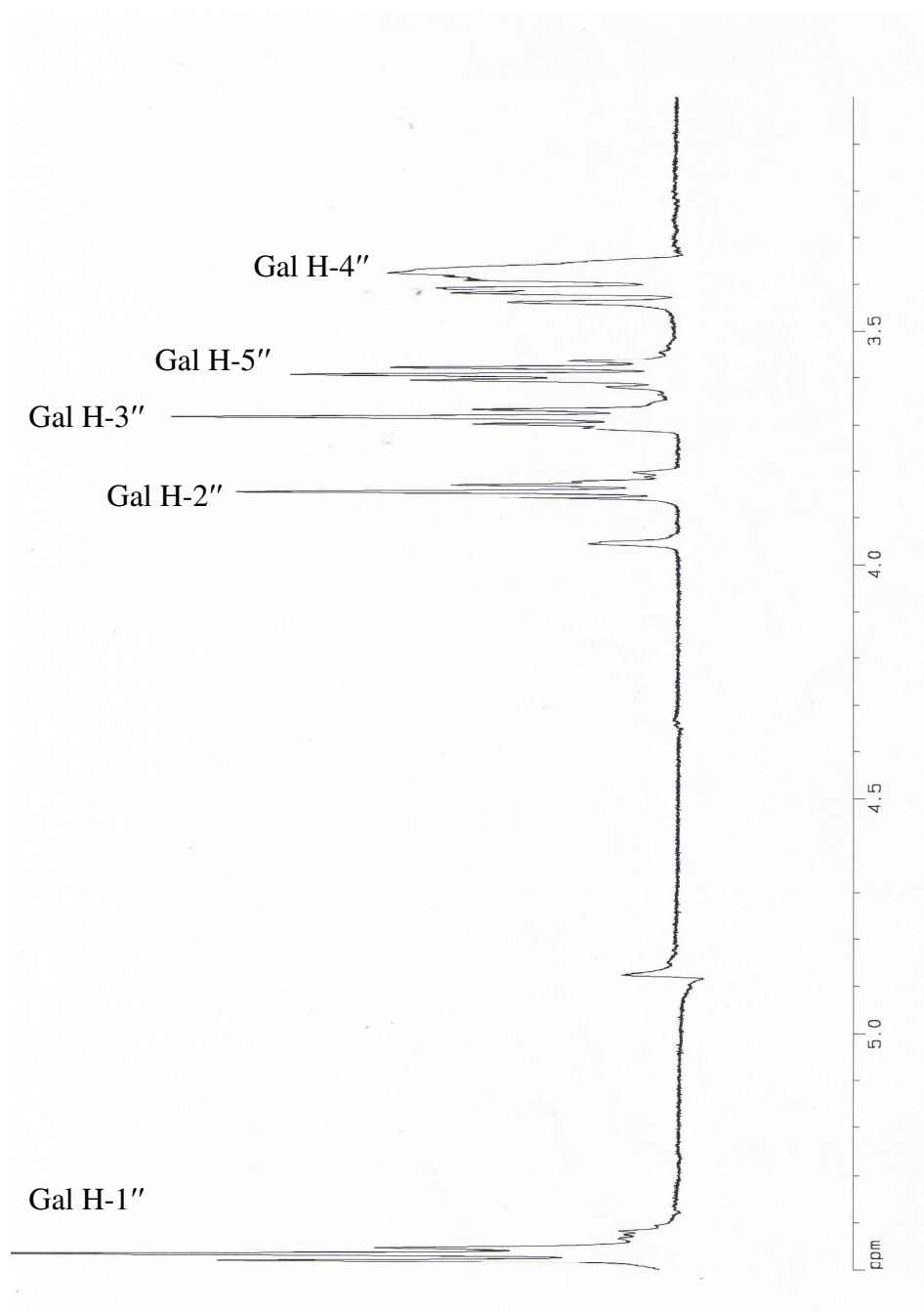


Table **S1**. Total Phenol Content and Free-Radical Scavenging Activity of the Extract and Pure Compounds from the leaves of *F. ancylantha*

extract or compound	phenol content ^a ($\mu\text{g}/\text{mg}$ extract) ^b	DPPH test [EC ₅₀ (μg of extract or compound/mL)]
EtOH-H ₂ O 7:3	283.0 \pm 0.1	80.4 (78.2-82.6) ^c
confusoside		> 1000
davidigenin		> 1000
7-O- β -D-glucopyranosyl-7,3',4'-trihydroxyflavone		28.4 (26.2-30.6) ^c
hirsutrin		20.4 (18.4-22.4) ^c
α -tocopherol ^d		10.1 (8.8-11.4) ^c

^aMean \pm S.D. of three determinations. ^bdavidigenin equivalents. ^c95% confidence limits. ^dPositive control.

S 18. Antimicrobial Activity.

The MeOH extract, confusoside, davidigenin, 7-O- β -D-glucopyranosyl-7,3',4'-trihydroxyflavone from the leaves of *F. ancylantha* were tested for antimicrobial activity using the broth microdilution method in 96-multiwell microtiter plates, in duplicate. The following microorganisms from the American Type Culture Collection (ATCC) were utilized: Gram-positive bacteria (*S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *B. spizizenii* ATCC 6633), Gram-negative bacteria (*P. aeruginosa* ATCC 9027, *E. coli* ATCC 8739), a yeast (*C. albicans* ATCC 10231) and a mold (*A. niger* ATCC 16404). For susceptibility testing, the first step was to place 50 μ L of Triptone soya broth in wells 2-12. The dried centrifuged product, the *n*-BuOH-soluble portion, and rosmarinic acid were initially dissolved in 100 μ L of dimethyl sulfoxide (DMSO) and then in Triptone to a final concentration of 40 mg/mL for the centrifuged product and the *n*-BuOH-soluble portion and 4 mg/mL for rosmarinic acid. Then, 100 μ L of these suspensions were added to the first test well of each microtiter line, and 50 μ L of scalar dilutions were transferred from the second to the 11th well. The 12th tube was considered as growth control, since no test solutions were added. Then, 50 μ L of a suspension of test organism (10^5 colony forming unit-CFU/mL) were added to each well. The final concentration varied from 20 mg/mL (first well) to 0.019 mg/mL (11th well) for the centrifuged product and the *n*-BuOH-soluble portion and from 2 mg/mL to 0.0078 mg/mL for rosmarinic acid. Plates were incubated for 24h at 37 °C and then examined from below using a reflective viewer. MIC was the lowest concentration of extract or compound at which microbial growth was inhibited after 24 h. The minimum bactericidal concentration (MBC) or the minimum fungicidal concentration (MFC) was the lowest concentration of the extract or compound at which survival of any microbial cell was not possible after incubation for 48 h (for bacterial strains) and 5 days (for yeasts and molds) and was determined by inoculating a portion of the broth culture on agar plates, where MIC values were previously defined. A blank control was taken using DMSO alone (100 μ g/mL) added to a series of tubes and the MIC was evaluated as described above. No growth inhibition was observed at DMSO concentrations lower or equal to 25 μ g/mL. The determination of the MICs of known antimicrobial compounds, gentamicin and nystatin, for all the reference strains, were simultaneously carried out (gentamicin, MIC 1 μ g/mL for Gram positive bacteria, 4 μ g/mL for Gram negative bacteria; nystatin, MIC 1 μ g/mL for *C. albicans*).

Table S2. Antimicrobial Activity of the Extract and Pure Compounds from the leaves of *F. ancylantha*.

microorganism	EtOH-H ₂ O 7:3 extract		confusoside		davidigenin		7-O- β -D-glucopyranosyl-7,3',4'-trihydroxyflavone		hirsutrin	
	MIC ^a	MBC ^a	MIC ^a	MBC ^a	MIC ^a	MBC ^a	MIC ^a	MBC ^a	MIC ^a	MBC ^a
<i>S. aureus</i> ATCC 6538	8.00	NA ^b	4.00	4.00	0.12	0.50	2.00	4.00	4.00	4.00
<i>S. epidermidis</i> ATCC 12228	16.00	NA ^b	1.00	2.00	0.25	0.50	0.50	4.00	1.00	4.00
<i>B. spizizenii</i> ATCC 6633	16.00	NA ^b	0.12	1.00	0.12	0.50	1.00	1.00	1.00	2.00
<i>P. aeruginosa</i> ATCC 9027	8.00	NA ^b	NA ^b	NA ^b	2.00	4.00	4.00	NA ^b	NA ^b	NA ^b
<i>E. coli</i> ATCC 8739	16.00	NA ^b	2.00	NA ^b	4.00	NA ^b	NA ^b	NA ^b	NA ^b	NA ^b
<i>C. albicans</i> ATCC 10231	8.00	NA ^b	4.00	NA ^b	1.00	2.00	4.00	NA ^b	4.00	NA ^b
<i>A. niger</i> ATCC 16404	NA ^b	NA ^b	0.12	NA ^b	0.12	NA ^b	1.00	NA ^b	2.00	4.00

^aMinimal inhibitory concentration (MIC) and minimal bactericide concentration (MBC) values are expressed as mg/mL of *Fadogia* extract or pure compound. ^bNA = Not active