Organic Letters

Supporting Information to Accompany:

Isoapoptolidin: Structure and Activity of the Ring Expanded Isomer of Apoptolidin

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Data for isoapoptolidin (2):

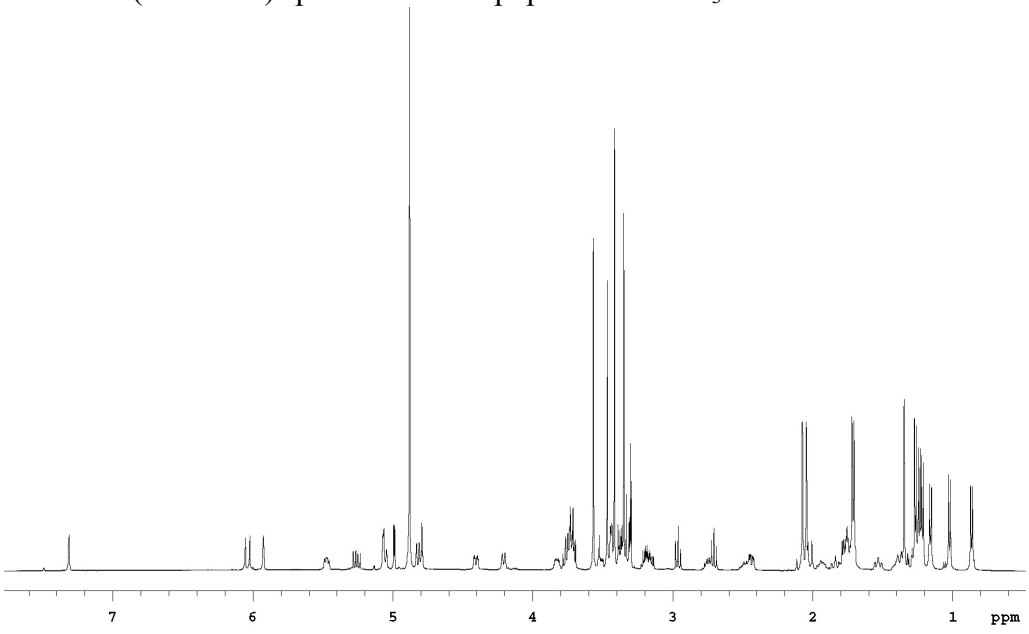
$[\alpha]_D$ = -187 (c=0.41, MeOH)

UV/Vis λ_{max} = 234 (39,500), 304 (19,400)

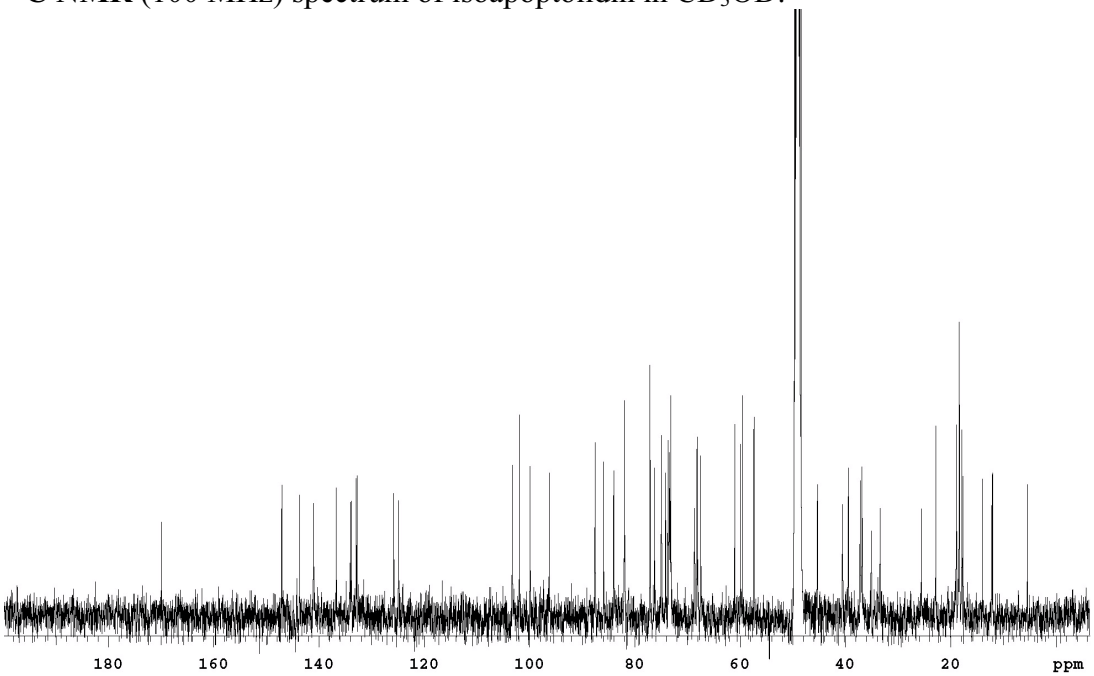
Melting Point: 134 – 136 ºC

Mass Spectrum (+ESMS) *m/z* 1151.7 (1151.6 calculated for $C_{58}H_{96}O_{21}$ + Na)

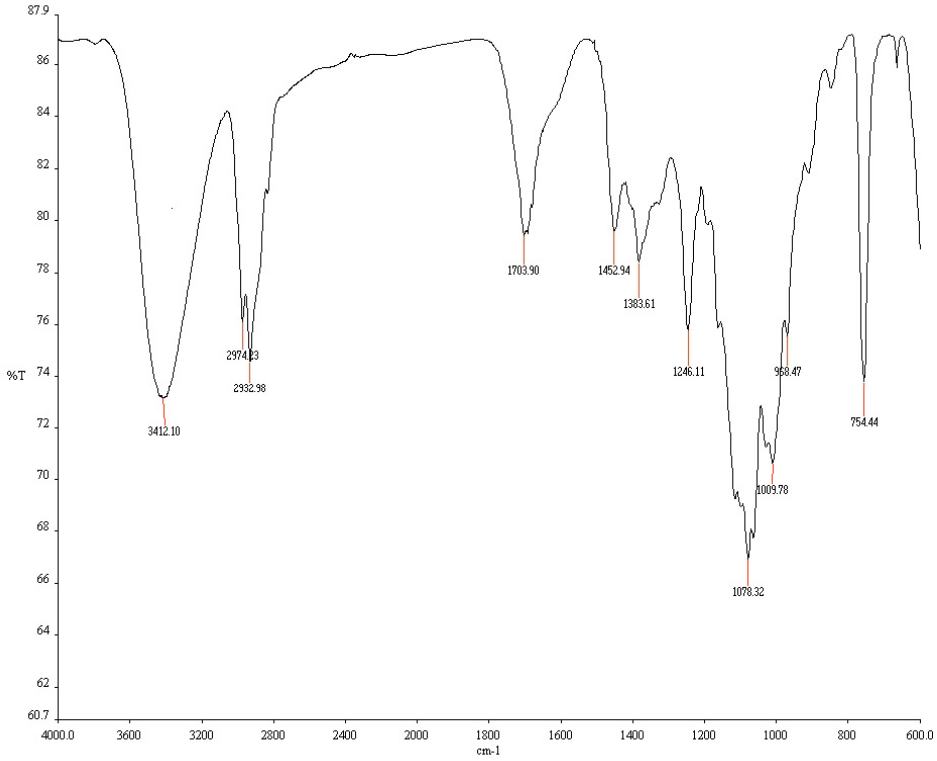
^{1}H NMR (500 MHz) spectrum of isoapoptolidin in CD_3OD:



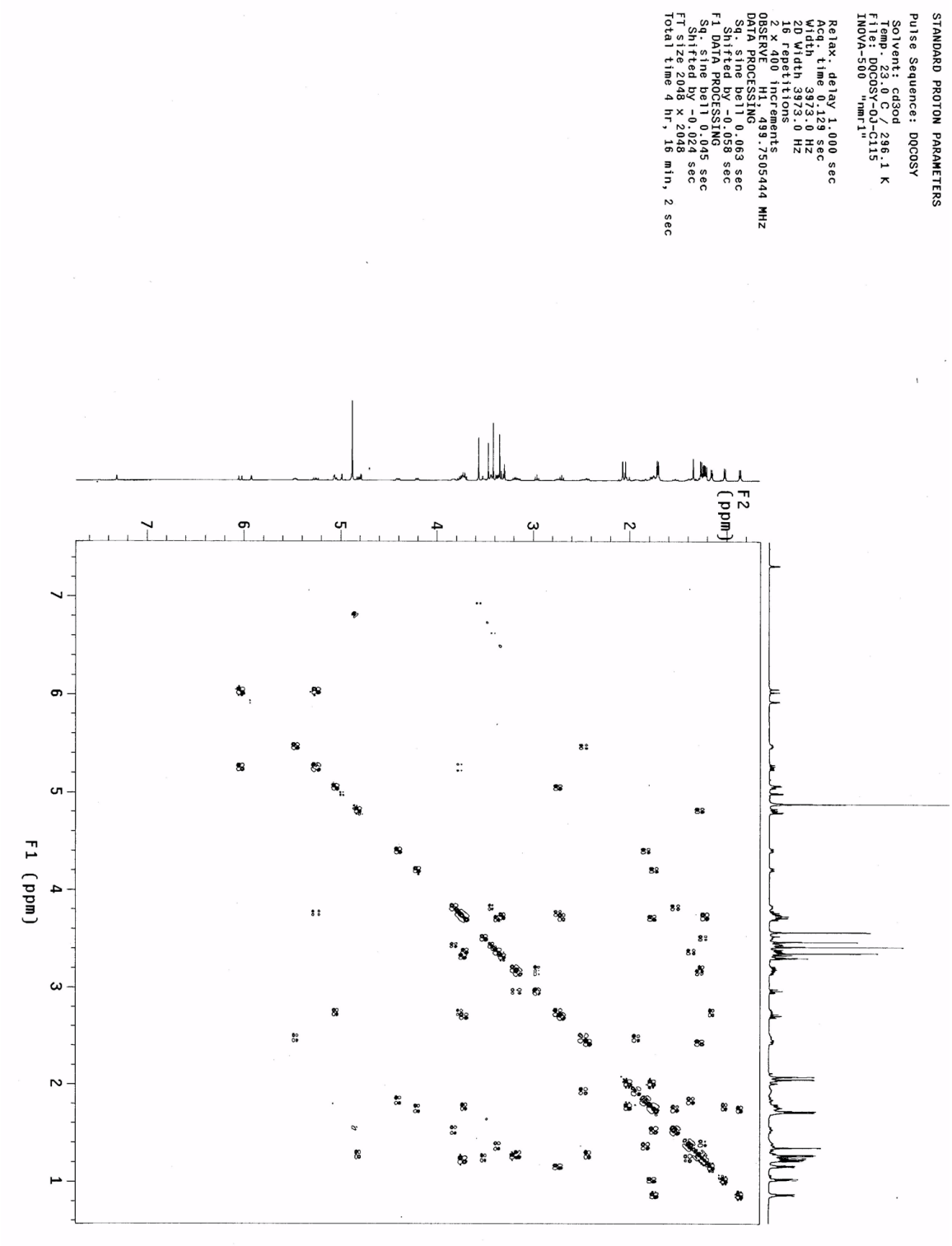
^{13}C NMR (100 MHz) spectrum of isoapoptolidin in CD_3OD:



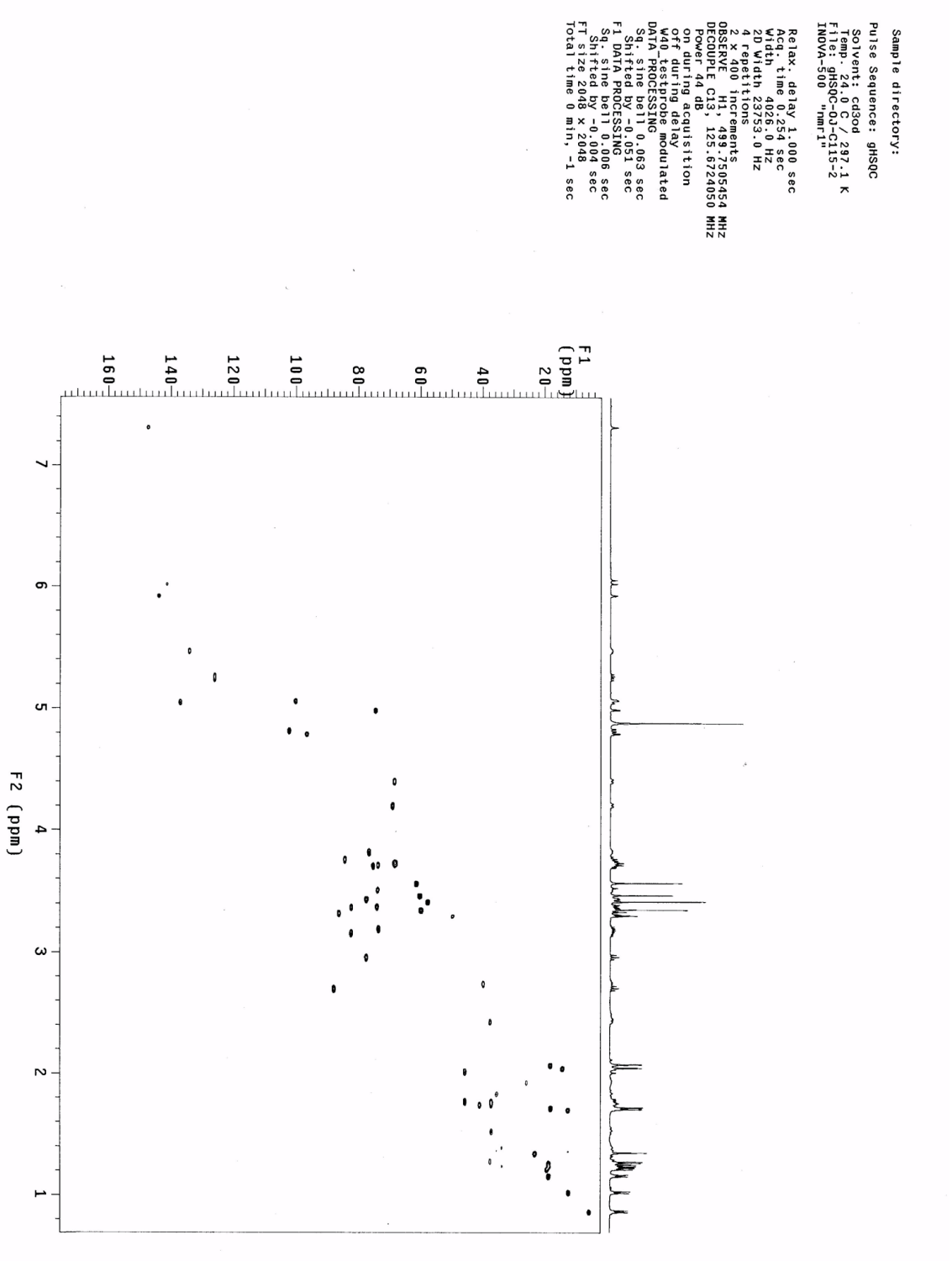
FTIR spectrum of isoapoptolidin (thin film)



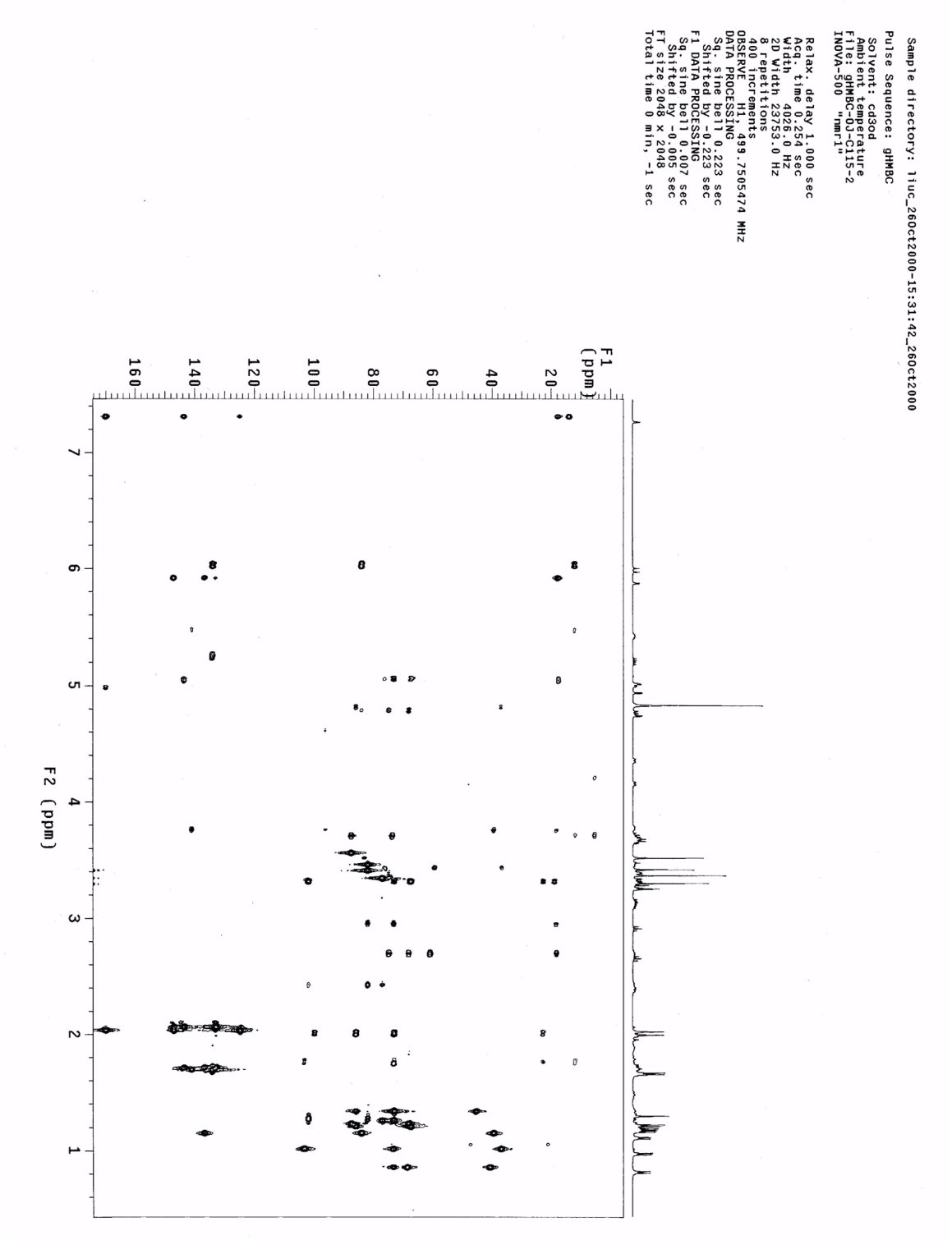
DQ-COSY (500 MHz) spectrum of isoapoptolidin in CD_3OD:



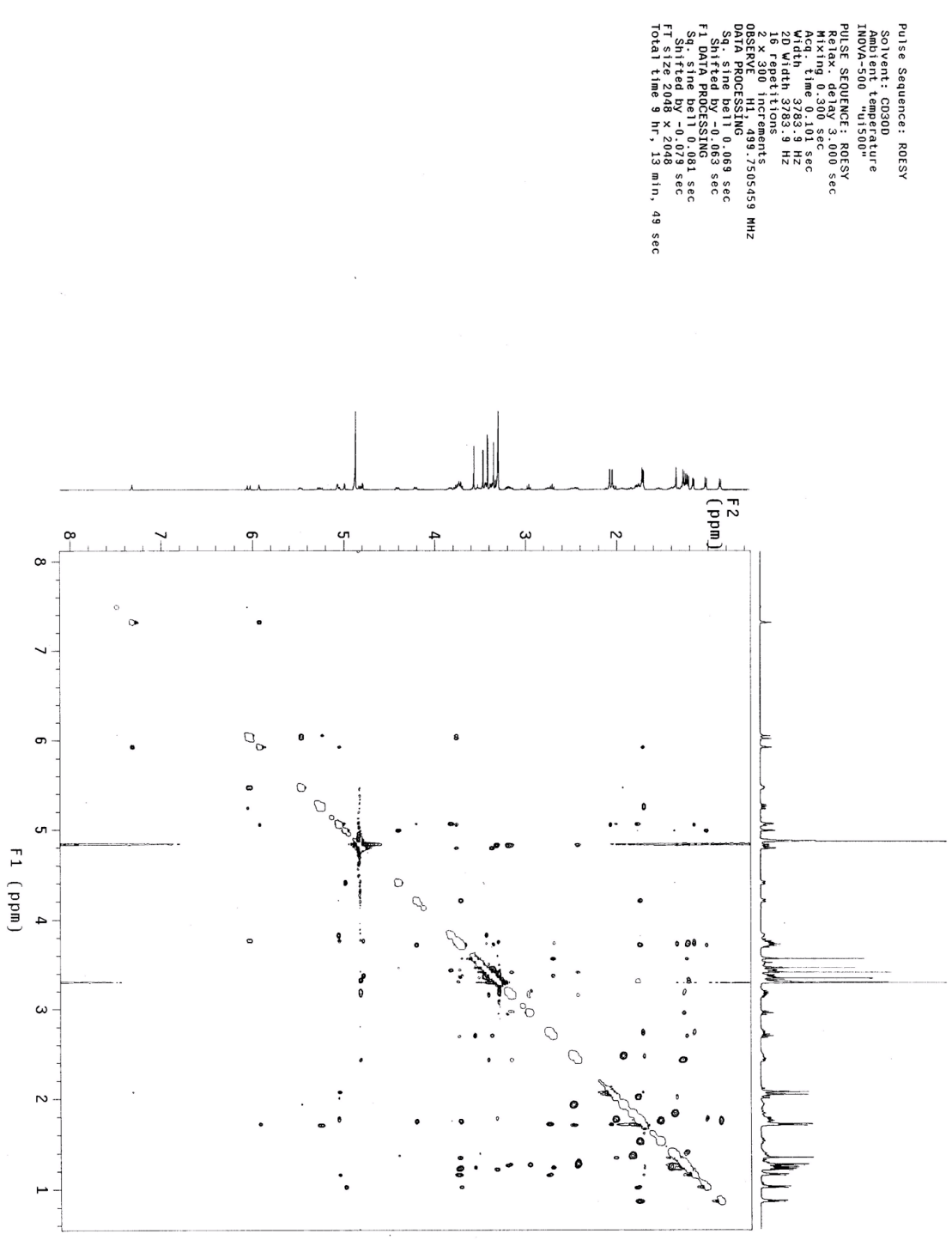
HSQC (500 MHz) spectrum of isoapoptolidin in CD_3OD:



HMBC (500 MHz) spectrum of isoapoptolidin in CD_3OD:



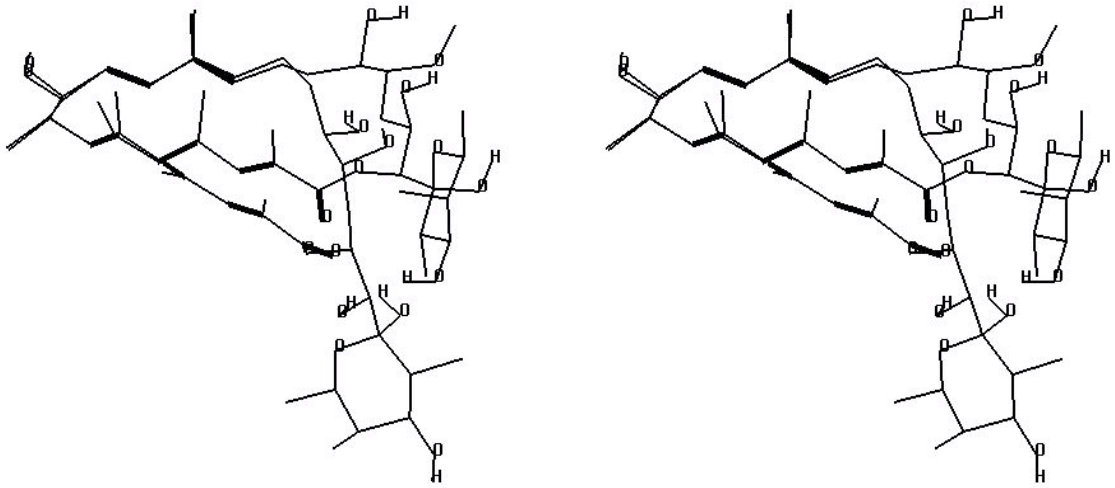
ROESY (500 MHz) spectrum of isoapoptolidin in CD_3OD:



ROESY correlations used in isoapoptolidin solution conformation calculations:

H(F1)	*H(F2)*	ROESY Volume
3	15b	*Weak*
3	18b	*Weak*
5	3	*Strong*
6-Me	8	*Strong*
7	5	*Strong*
8	10	*Strong*
9	11	*Strong*
9	7	*Strong*
12-Me	10	*Strong*
12-Me	14b	*Medium*
13	11	*Strong*
14a	13	*Strong*
15b	13	*Strong*
15b	16	*Strong*
16	13	*Medium*
17	19	*Medium*
17-Me	19	*Weak*
18a	19	*Strong*
18a	20	*Medium*
19	20	*Strong*
22	20	*Medium*
22	2-Me	*Weak*
22-Me	20	*Medium*

Calculated solution conformations for the core structures of isoapoptolidin (upper overlaid structure) and apoptolidin. Both calculations were performed with Macromodel 7.0 using similar parameters.

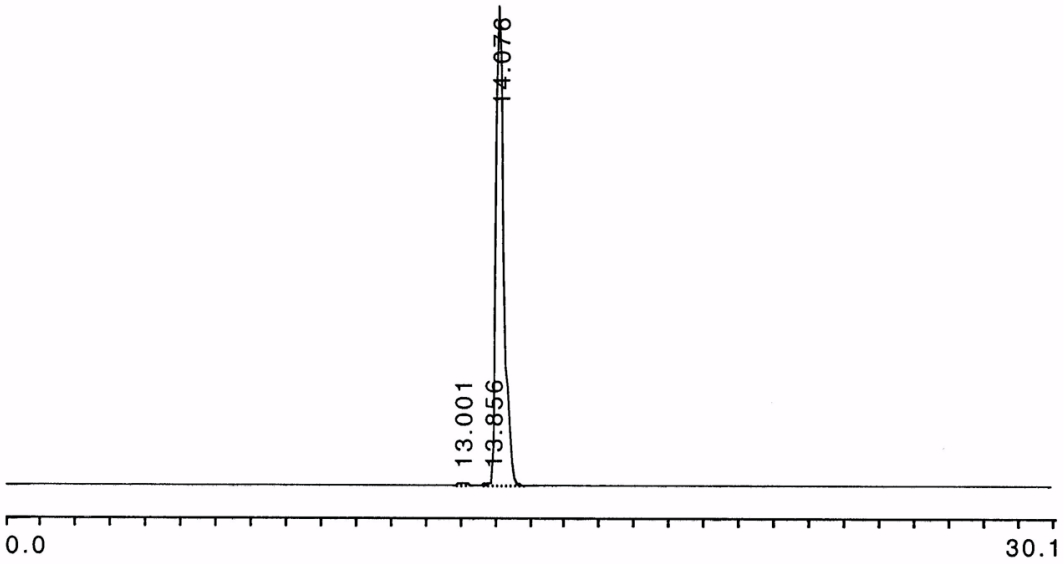


HPLC trace of purified isoapoptolidin (reverse-phase):

Sample: ISOAPOPTOLIDIN
4mg/ml MeOH Solution
5uL Inject Volume
254 nm
Alltech Altima C18 5u
150mm x 4.6mm

Method: Anal RP 40-84%
Inject Vol: 5
Internal Std: 0
Sampling Int: 0.1 Seconds

Data:



Analysis: Channel A

Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
1	13.001	N	3405	27885	0.699
2	13.856	N1	1446	11893	0.298
3	14.076	N2	332585	3946876	99.002
Total Area				3986654	99.999

ATPase inhibition assay. The mitochodrial ATPase inhibition assay has been modified from the original published procedure to be compatible with a 96-well plate-reader format. Numerical values for ATPase inhibition are comparable to the original procedure.

All solutions are prepared using deionized water. A reaction buffer (solution A) is prepared containing the following components: $MgCl_2 \cdot 6H_2O$ (3.3 mM), antimycin A (2 μg/ml) and Tris (50 mM). The buffer is adjusted to pH = 8 using HCl (1.0 M). A separate solution (solution B) is prepared containing lactate dehydrogenase (500 U/ml) and pyruvate kinase (250 U/ml). A third solution (solution C) is prepared containing phosphoenol pyruvate (100 mM) and NADH (30 mM). A fourth solution (Solution D) is prepared containing ATP (50 mM).

Because the activity of ATPase in isolated mitochondria varies by batch, the amount used is calibrated to achieve a specified turnover rate for the null control. In general, all NADH should be consumed in the null control within 10 to 20 minutes of the initiation of the reaction. The volume of mitochondria suspension required to achieve this should not be more than 5% of the total reaction volume.

The total reaction volume for a single well in a 96-well plate is set to be 200 μL. A series of dilutions of the compound to be tested is first added to each of the relevant wells, along with 5μL of solution D. The reaction mixture is then prepared separately corresponding to 180 μL of solution A, 2 μL of solution B, and 5 μL of solution C per well used. A sufficient amount of isolated mitochondria is then added to achieve the desired reaction rate. The solution is thoroughly mixed, then transferred to individual wells to bring the total well volume to 200μL. The absorbance at each well is measured at 360 nm in 15 second intervals for a total of 25 minutes using a VERSAmax tunable microplate reader (Molecular Devices).

After the data acquisition is complete, the reaction rate is calculated for each well over its linear range. This rate is then normalized with respect to the reaction rate for the null well, and plotted as a function of concentration. IC50 values are reported as the concentration where the inhibited rate is 50% of the uninhibited rate. All data-points are collected in triplicate, and averaged after rates have been calculated.