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APPENDICES

APPENDIX A

MEDIA

The media were prepared by sterilization in the autoclave at 121°C for 15 minutes

A1. Solid Medium

Potato Dextrose Agar (PDA)

Potato	200.0	g
Dextrose	20.0	g
Agar	15.0	g
DW	1,000	ml

The potatoes were peal and boiled in distilled water until soft. Discharge the pieces of potato by filter. The filtrate was collected and added dextrose and agar. The volume was adjusted 1000 ml. and dissolved by steaming.

A2. Biotransformation medium

Czapex peptone medium

Sucrose	15.0	g
Glucose	15.0	g
Peptone	5.0	g
$FeSO_4 \cdot 7H_2O$	1.0	g
KCl	0.5	g
K_2HPO_4	0.5	g
$MgSO_4$	0.01	g
DW	1000	ml

The pH was adjusted to 7.0 with 1 M HCl.

APPENDIX B

BUFFER FOR ENZYME PREPARATION

B1. Preparation of 0.1 M Sodium Phosphate buffer pH 7.4

0.1 M Sodium phosphate buffer was prepare from 1 M Sodium Phosphate buffer as following,

Desired pH	Volume of 1 M Na_2HPO_4 (ml)	Volume of 1 M $NaHP_2O_4$ (ml)
5.8	7.9	92.1
6.0	12.0	88.0
6.2	17.8	82.2
6.4	25.5	74.5
6.6	35.2	64.8
6.8	46.3	53.7
7.0	57.7	42.3
7.2	68.4	31.6
7.4	77.4	22.6
7.6	84.5	15.5
7.8	89.6	10.4
8.0	93.2	6.8

The solution was made up to 1 liter with distilled water.

B2. Buffer A

0.1 M	Tris-HCL pH 7.6
10 %	Glycerol
1 mM	EDTA
2 mM	DTT
1 mM	PMSF

B3. Buffer B

0.1 M	Tris-HCL pH 7.6
20 %	Glycerol
1 mM	EDTA
2 mM	DTT
1 mM	PMSF

APPENDIX C

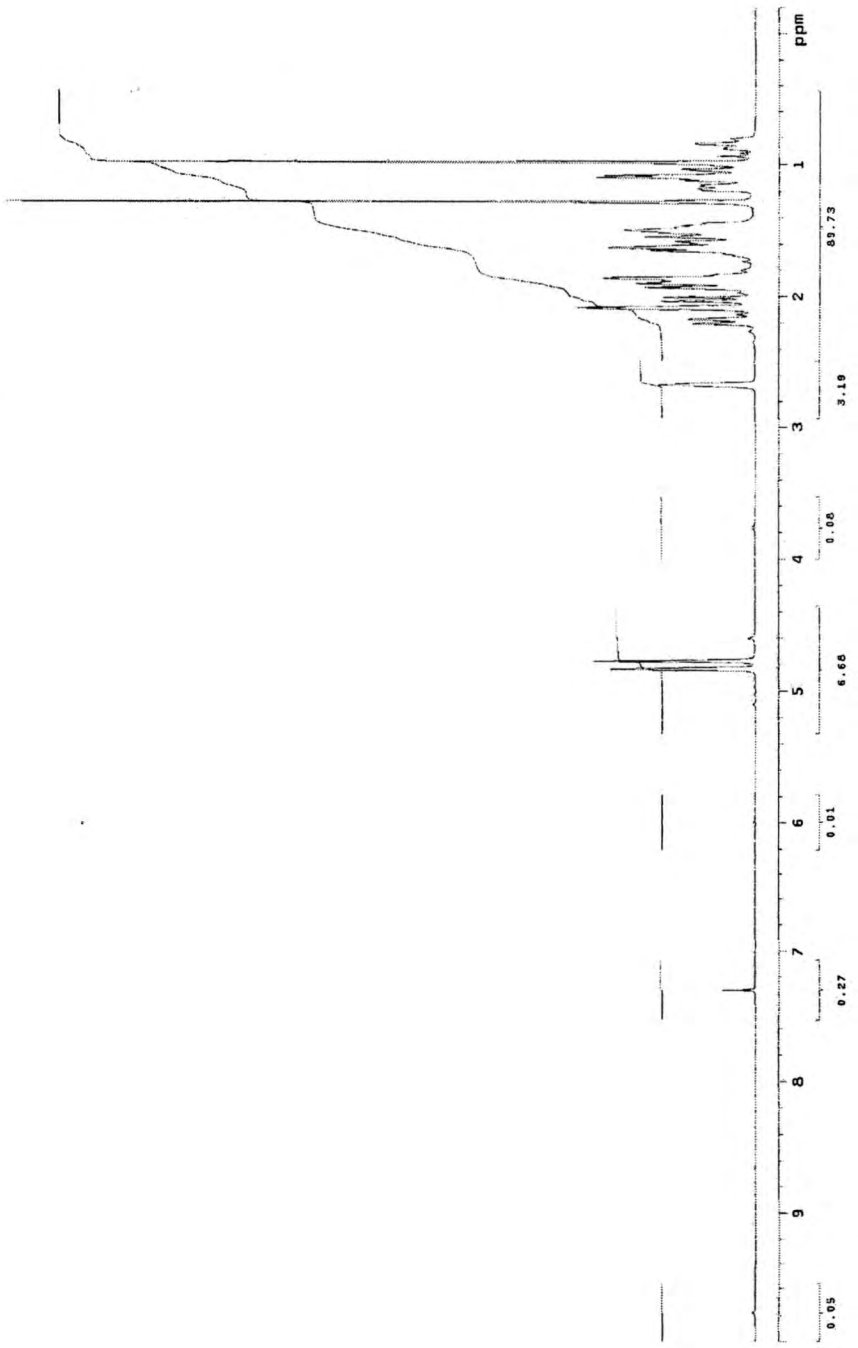


Figure C1. ^{1}H-NMR of compound **1**

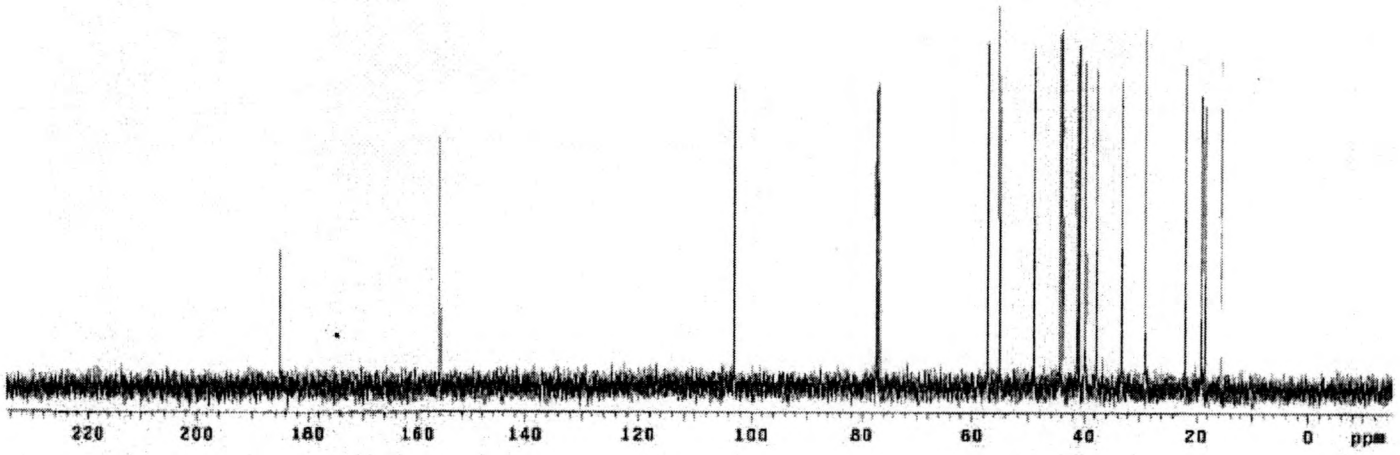


Figure C2. ^{13}C-NMR spectrum of compound **1**

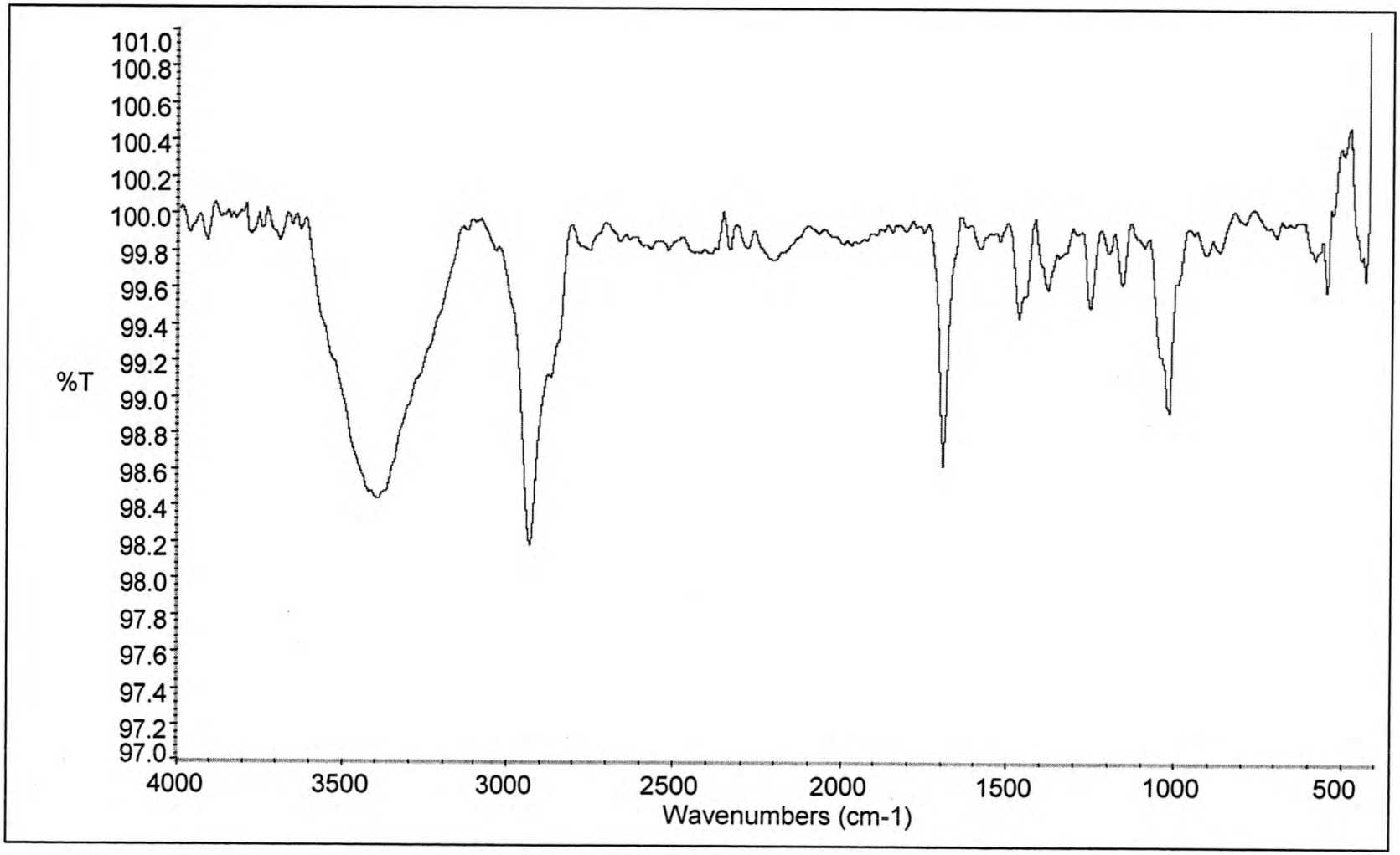


Figure C3. IR spectrum of compound **2**

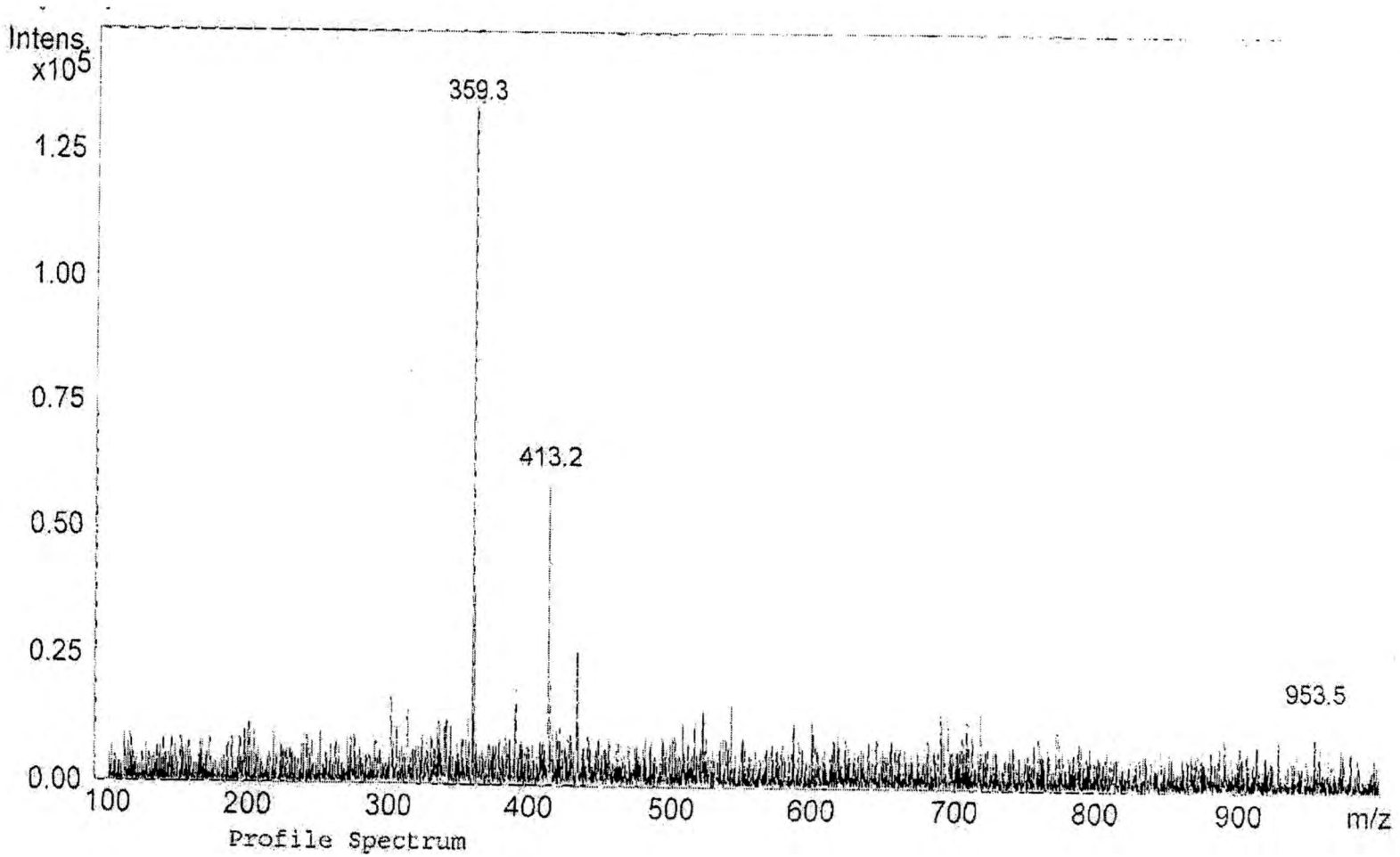


Figure C4. Mass spectrum of compound **2**

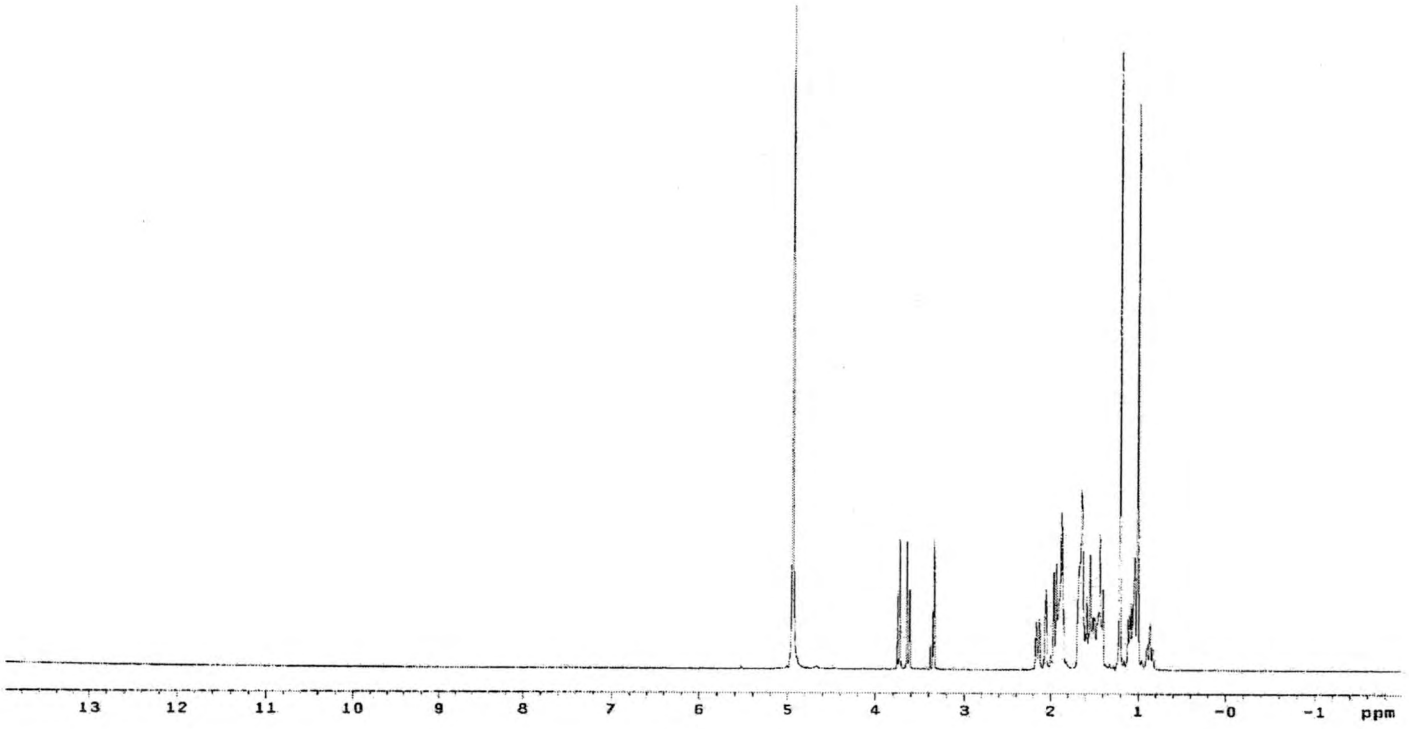


Figure C5. ^{1}H-NMR spectrum of compound **2**

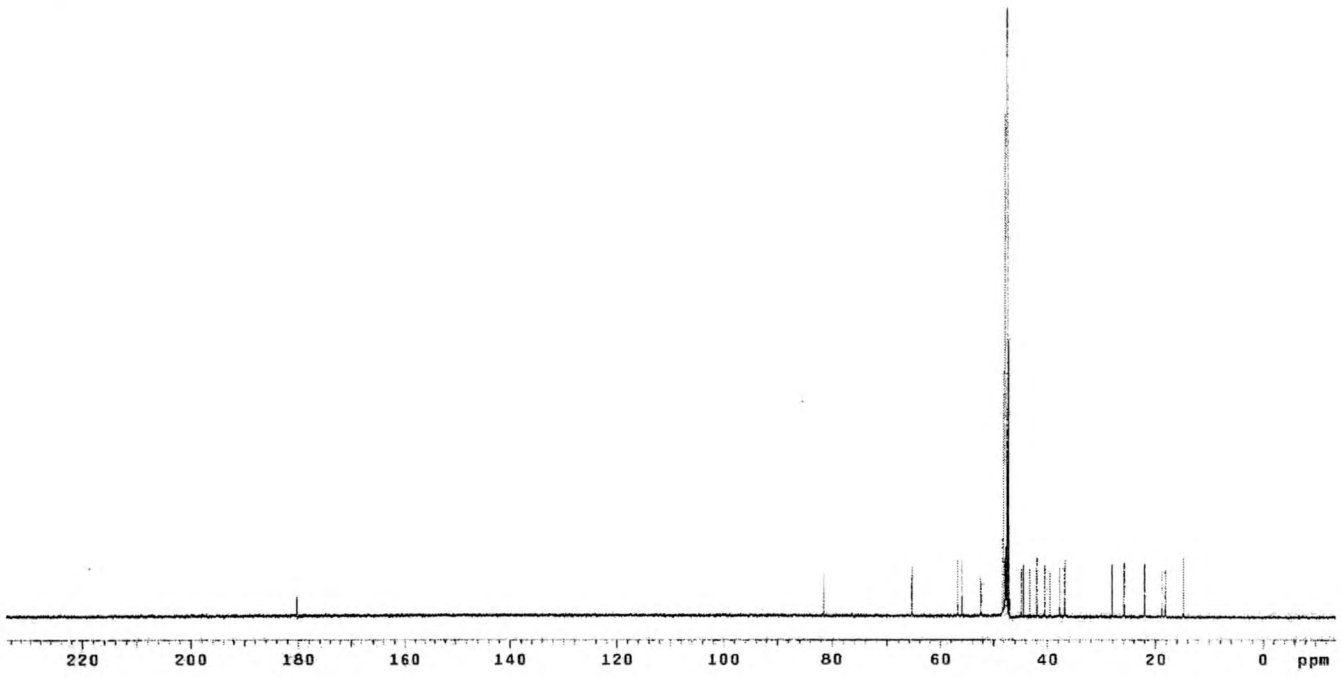


Figure C6. ^{13}C-NMR spectrum of compound **2**

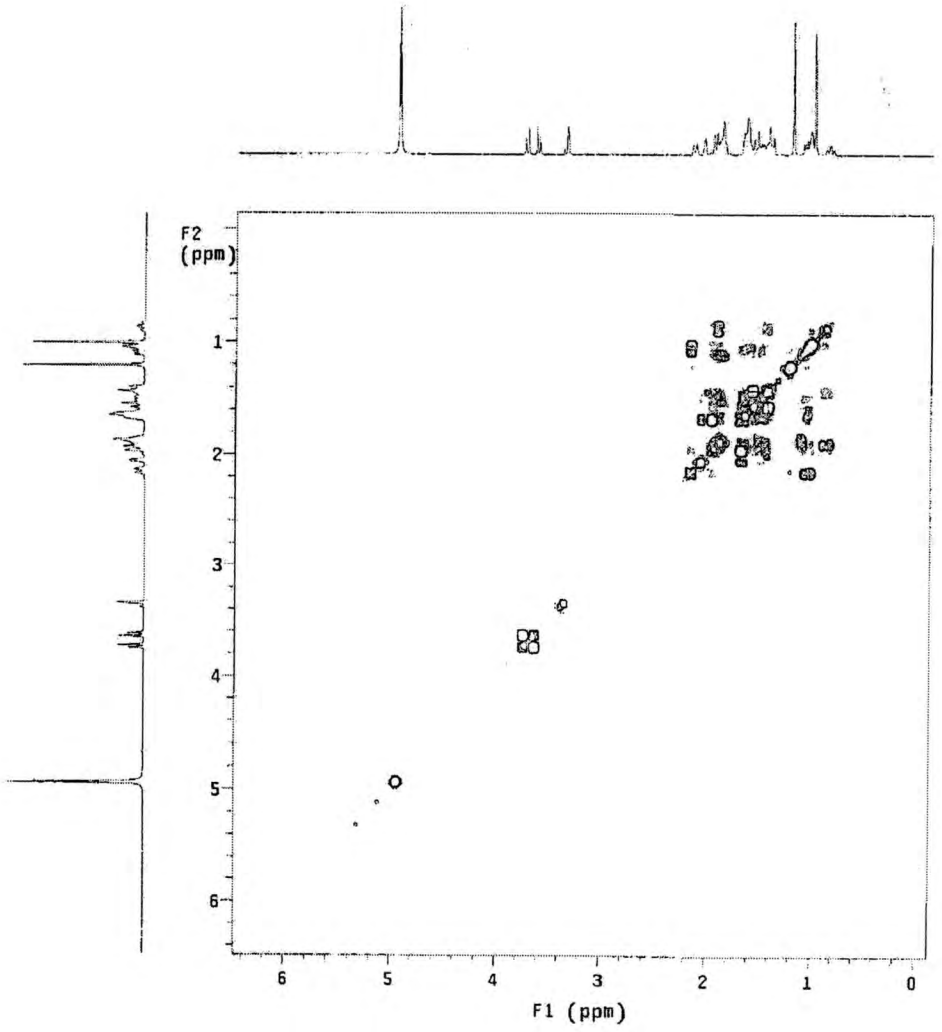


Figure C7. COSY spectrum of compound **2**

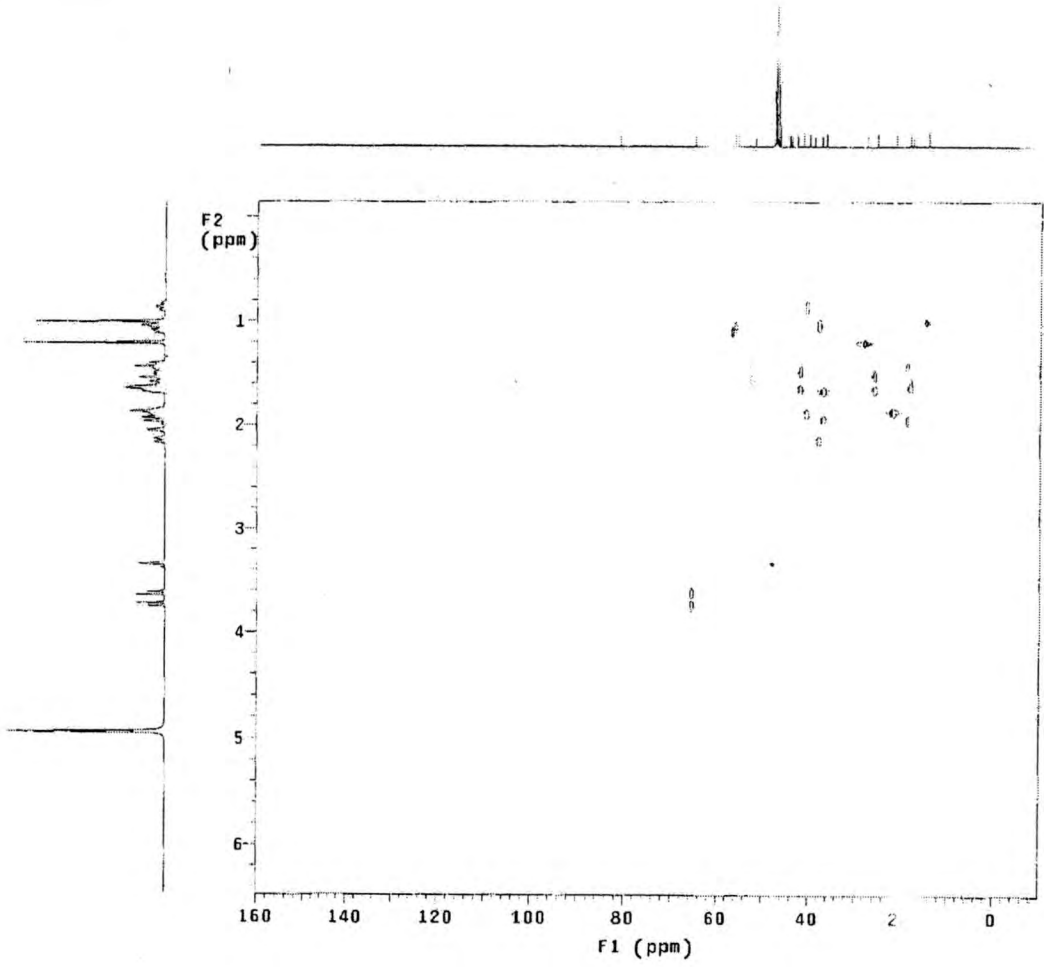


Figure C8. HSQC spectrum of compound **2**

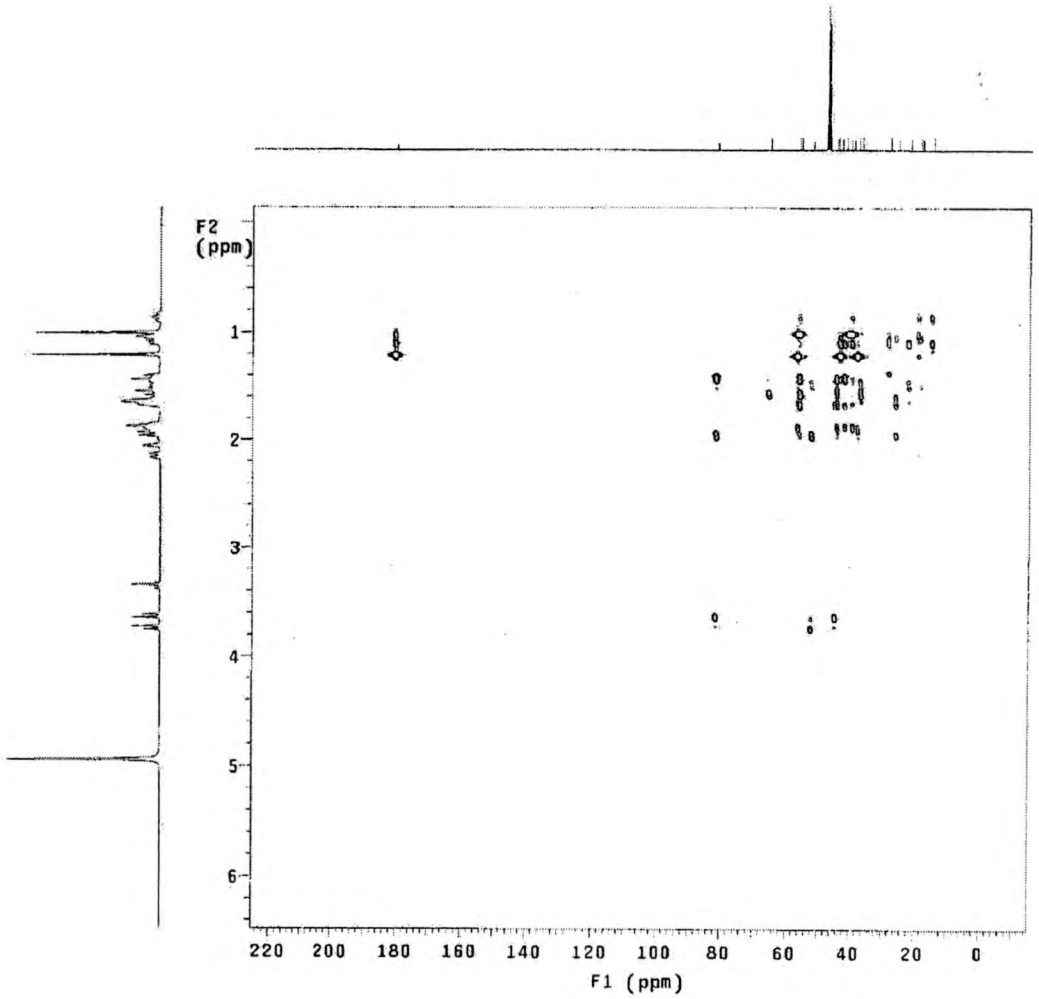


Figure C9. HMBC spectrum of compound **2**

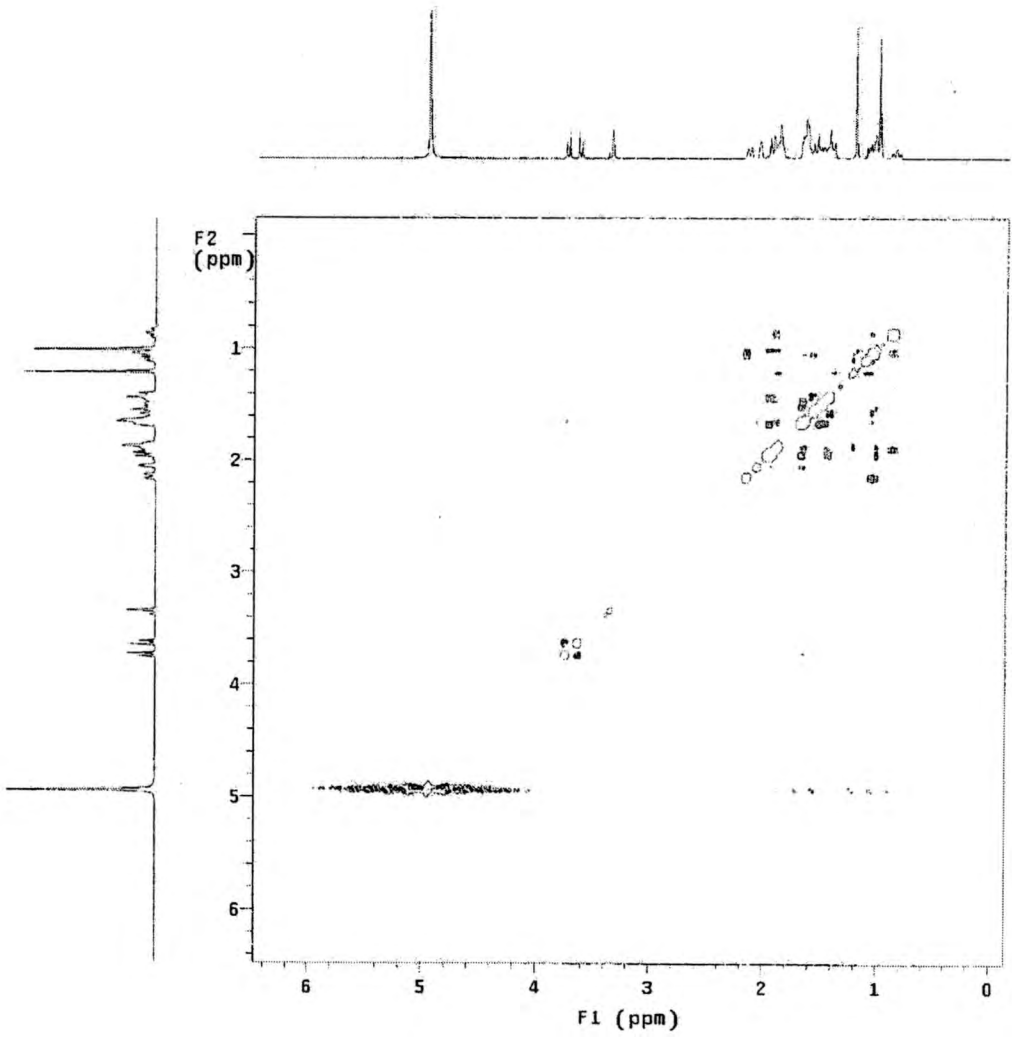


Figure C10. NOSY spectrum of compound **2**

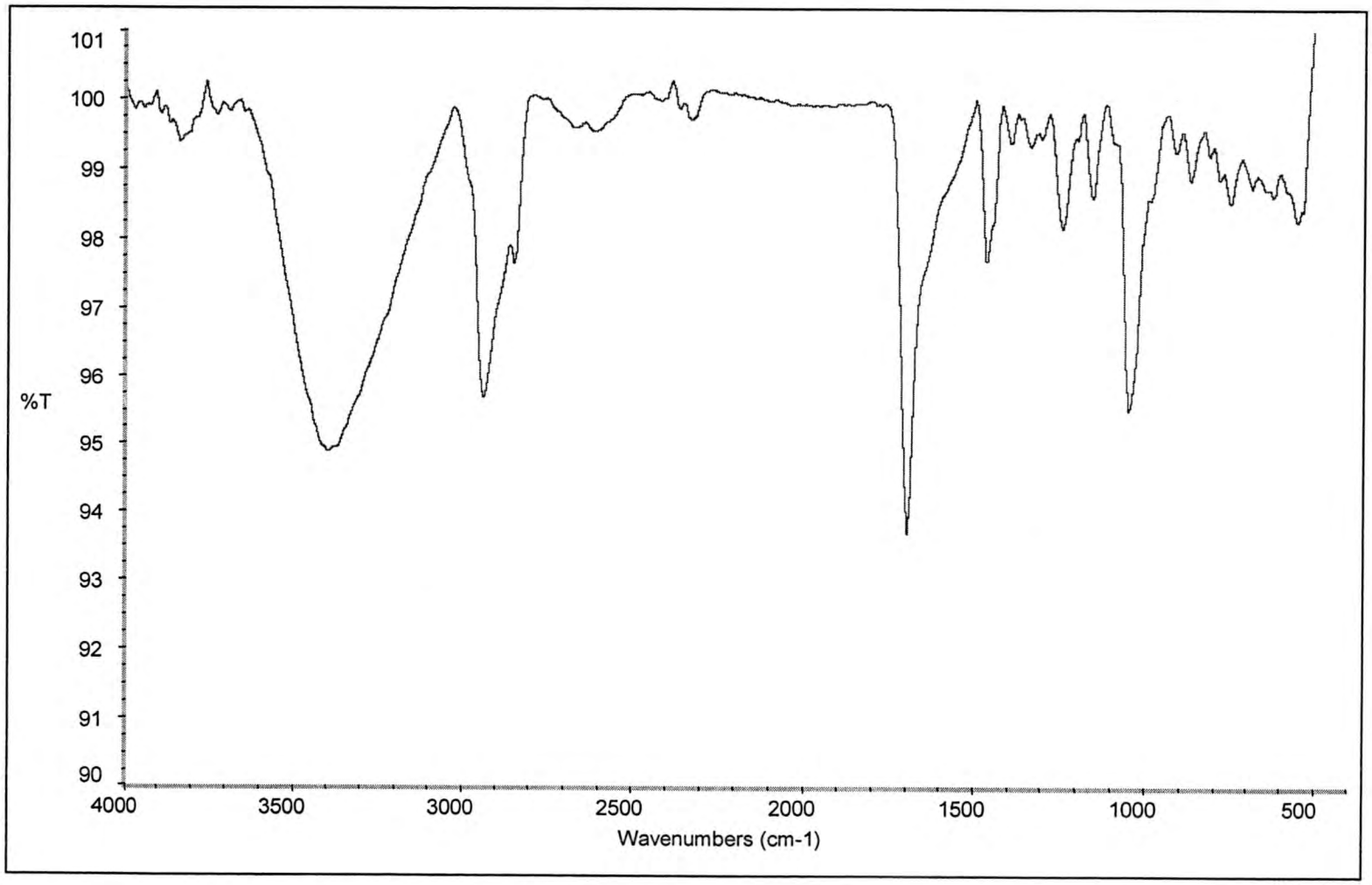


Figure C11. IR Spectrum of compound **3**

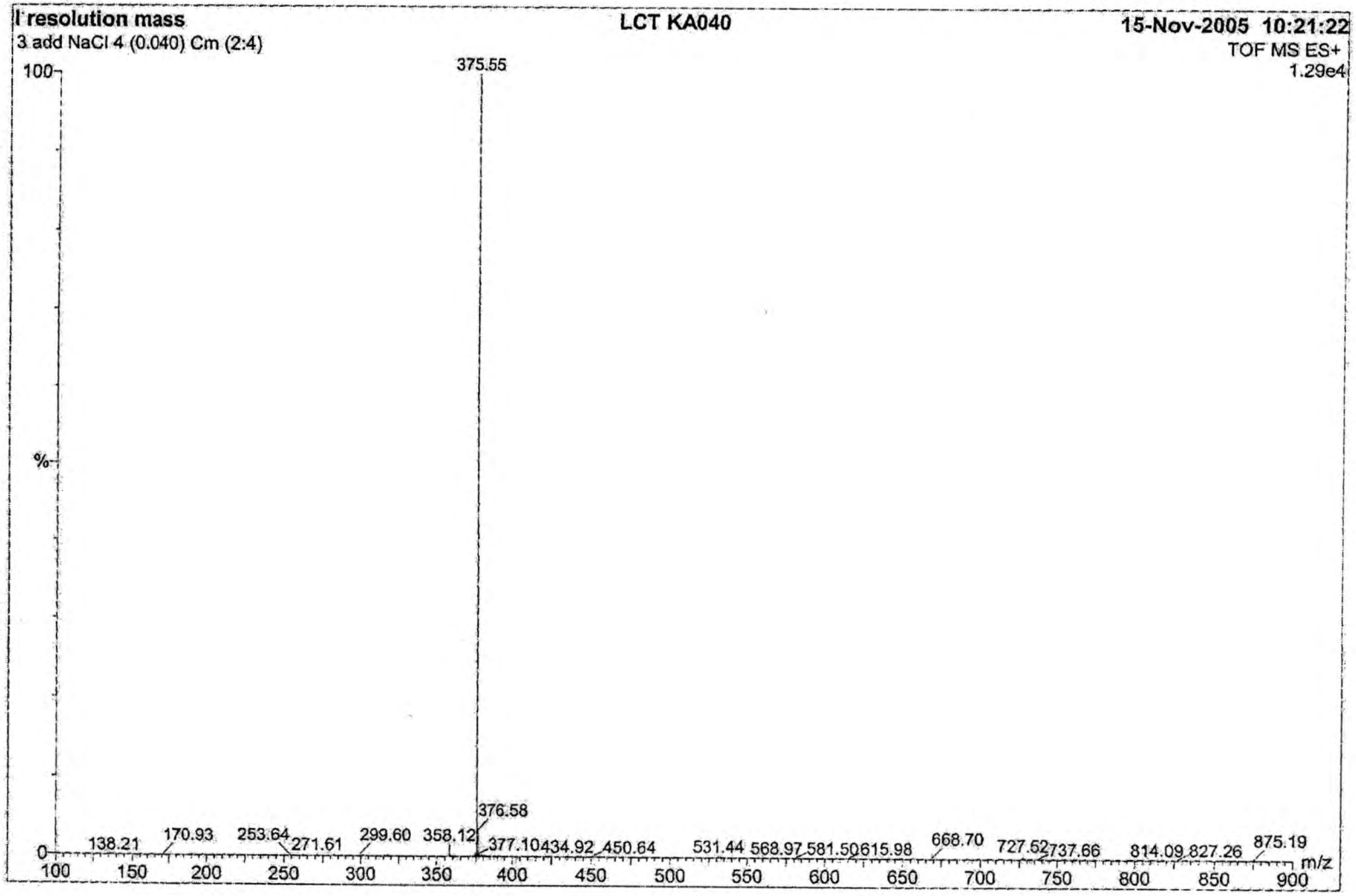


Figure C12. Low resolution mass spectrum of compound **3**

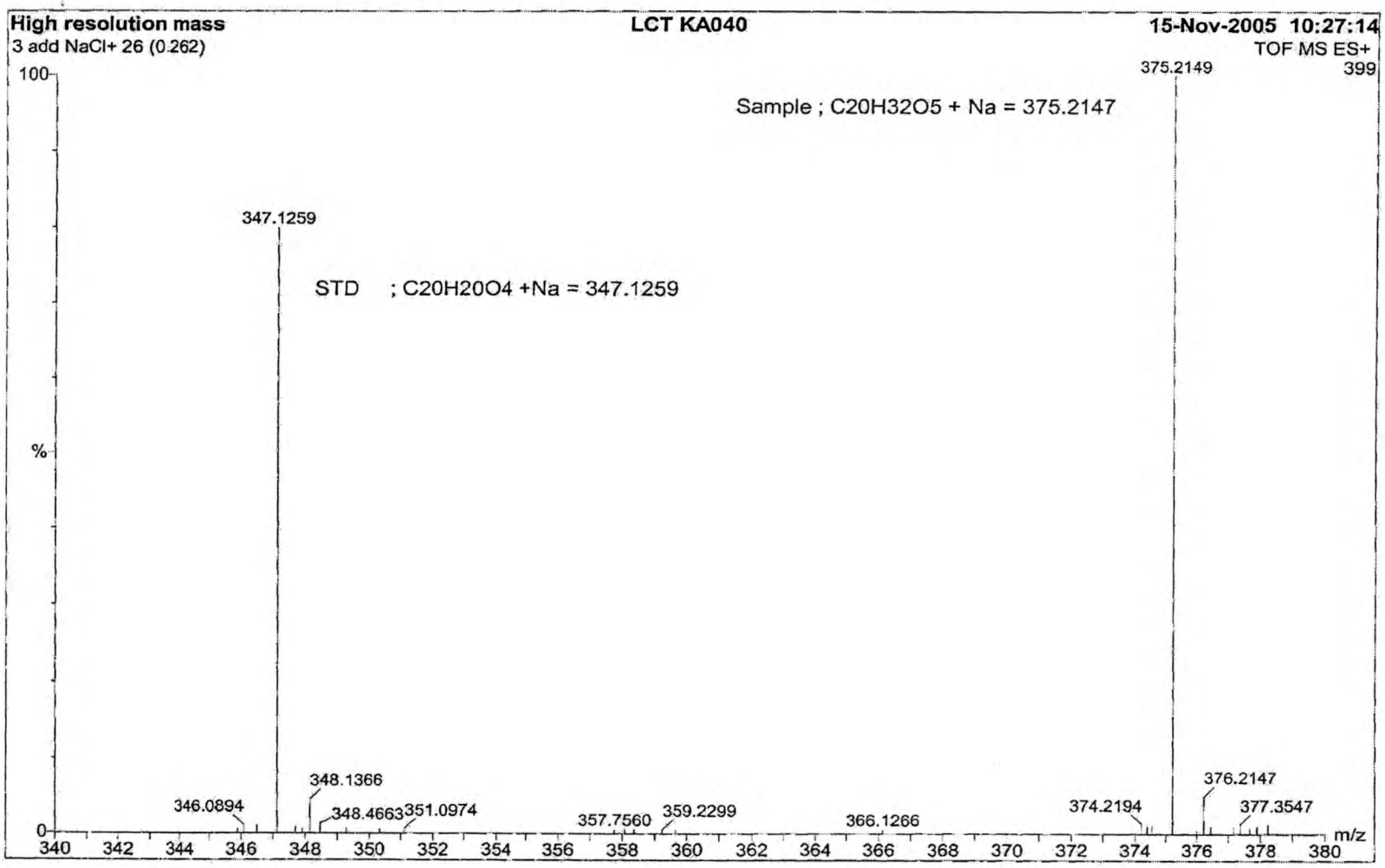


Figure C13. High resolution mass spectrum of compound **3**

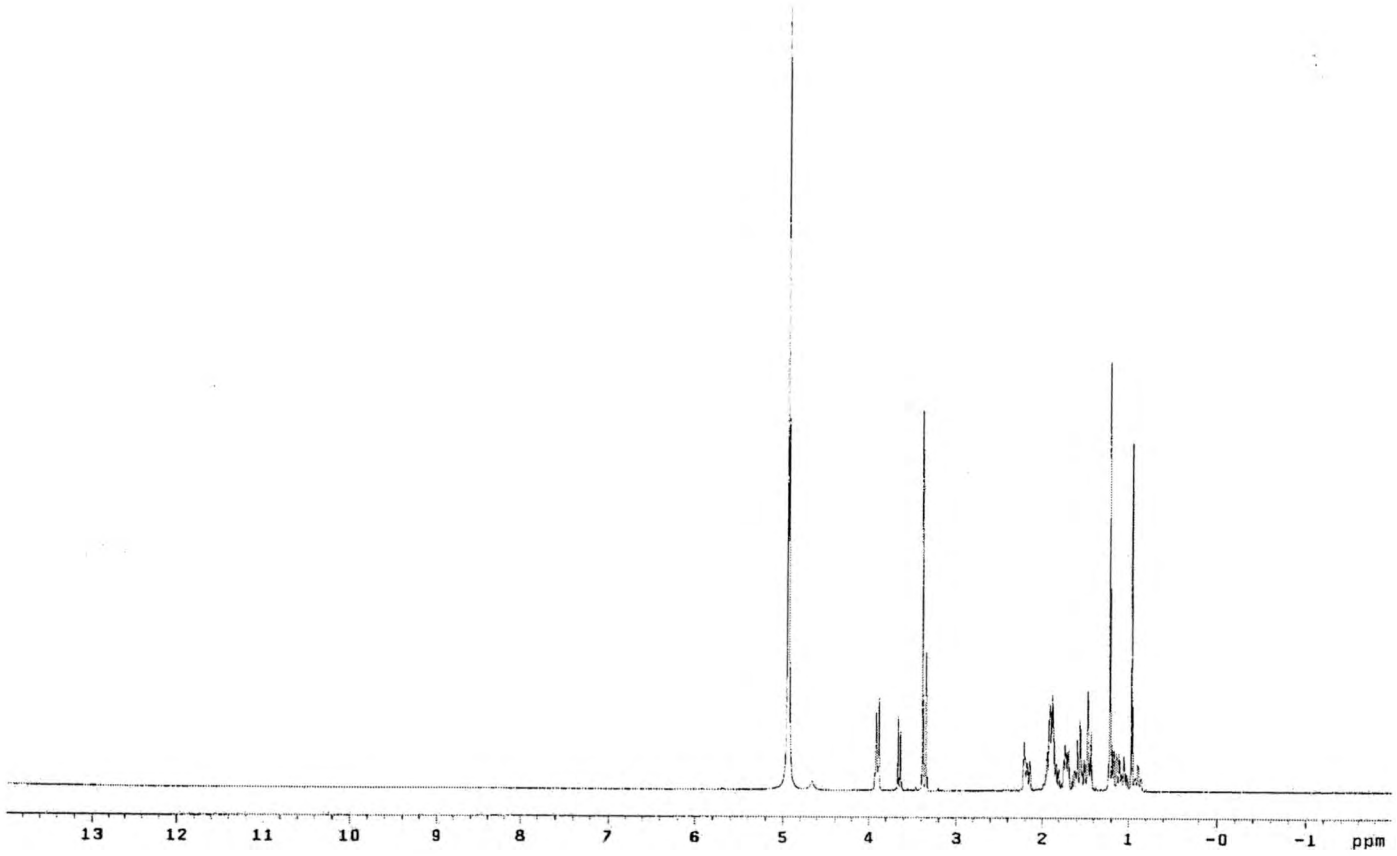


Figure C14. ^{1}H-NMR of compound **3**

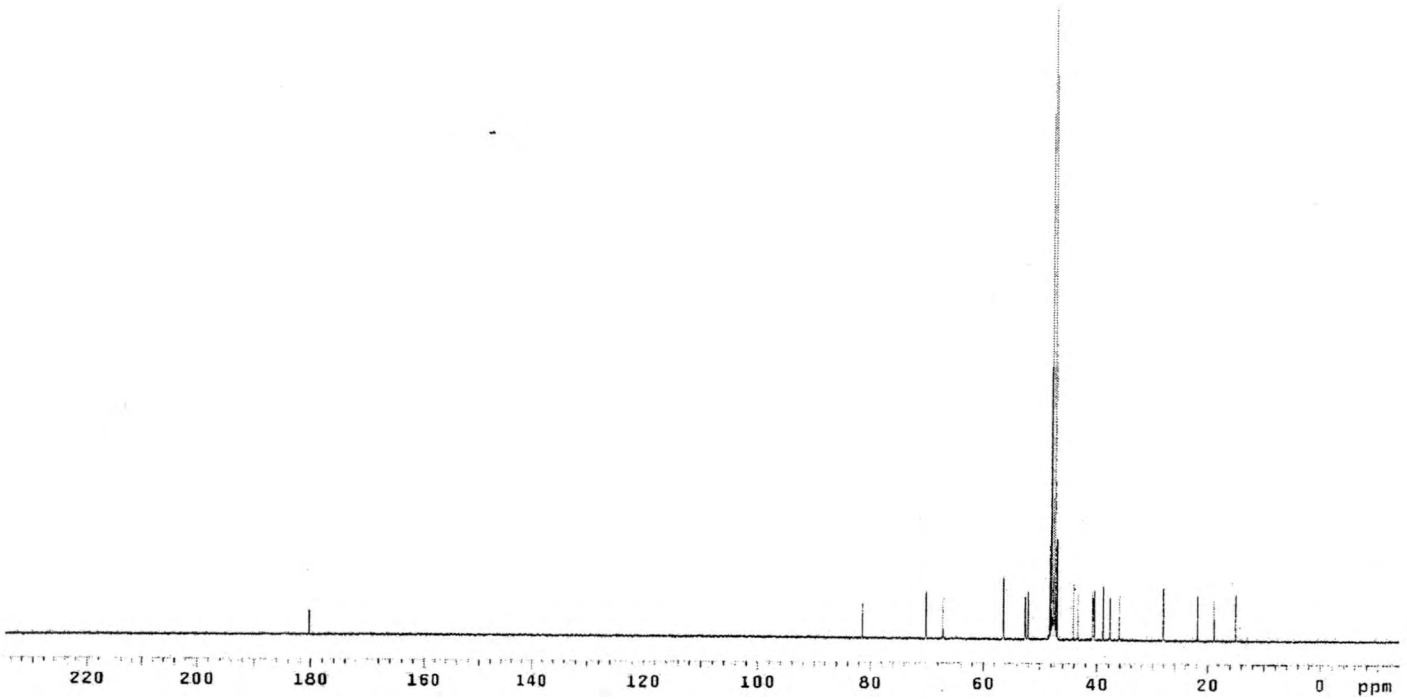


Figure C15. ^{13}C- NMR of compound **3**

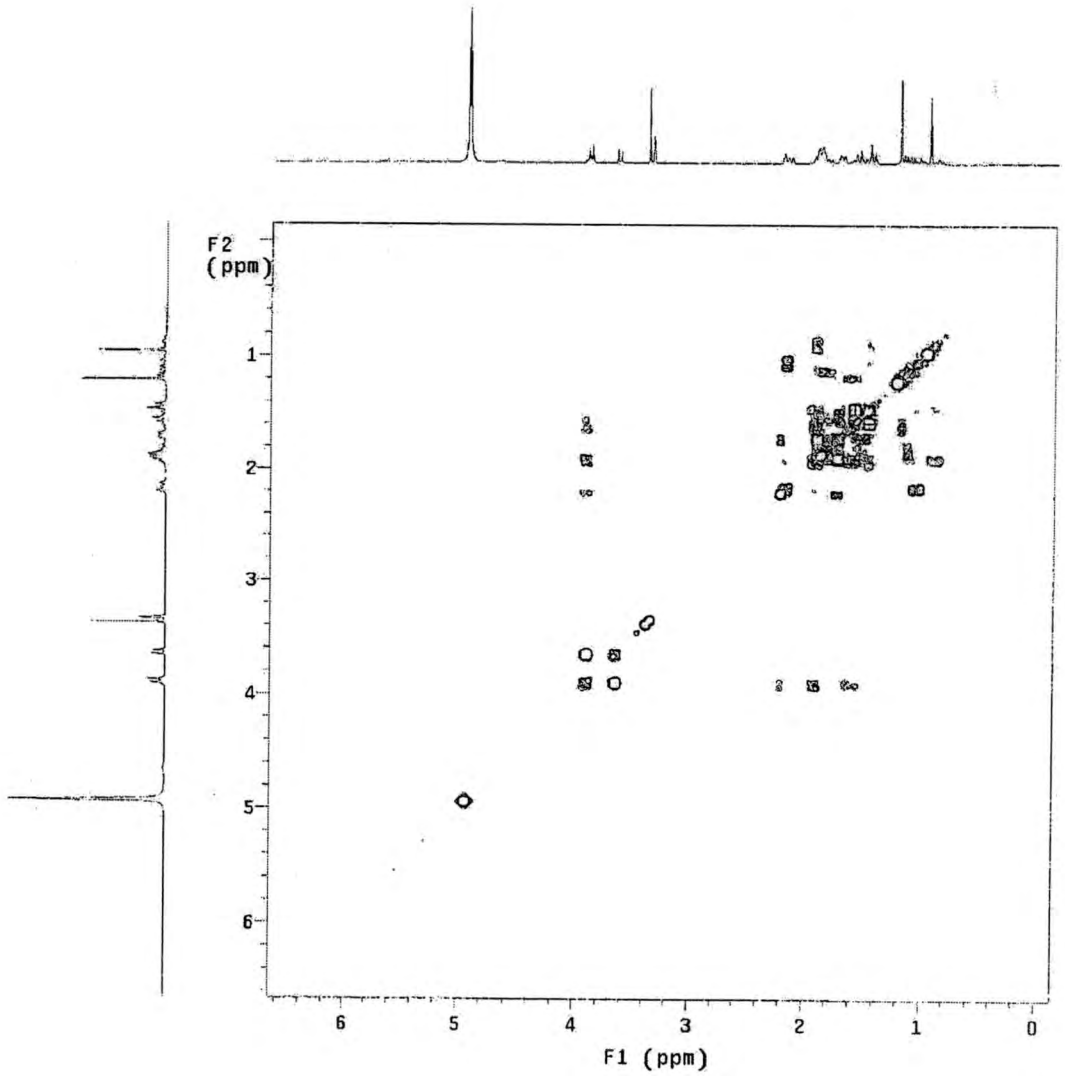


Figure C16. COSY spectrum of compound **3**

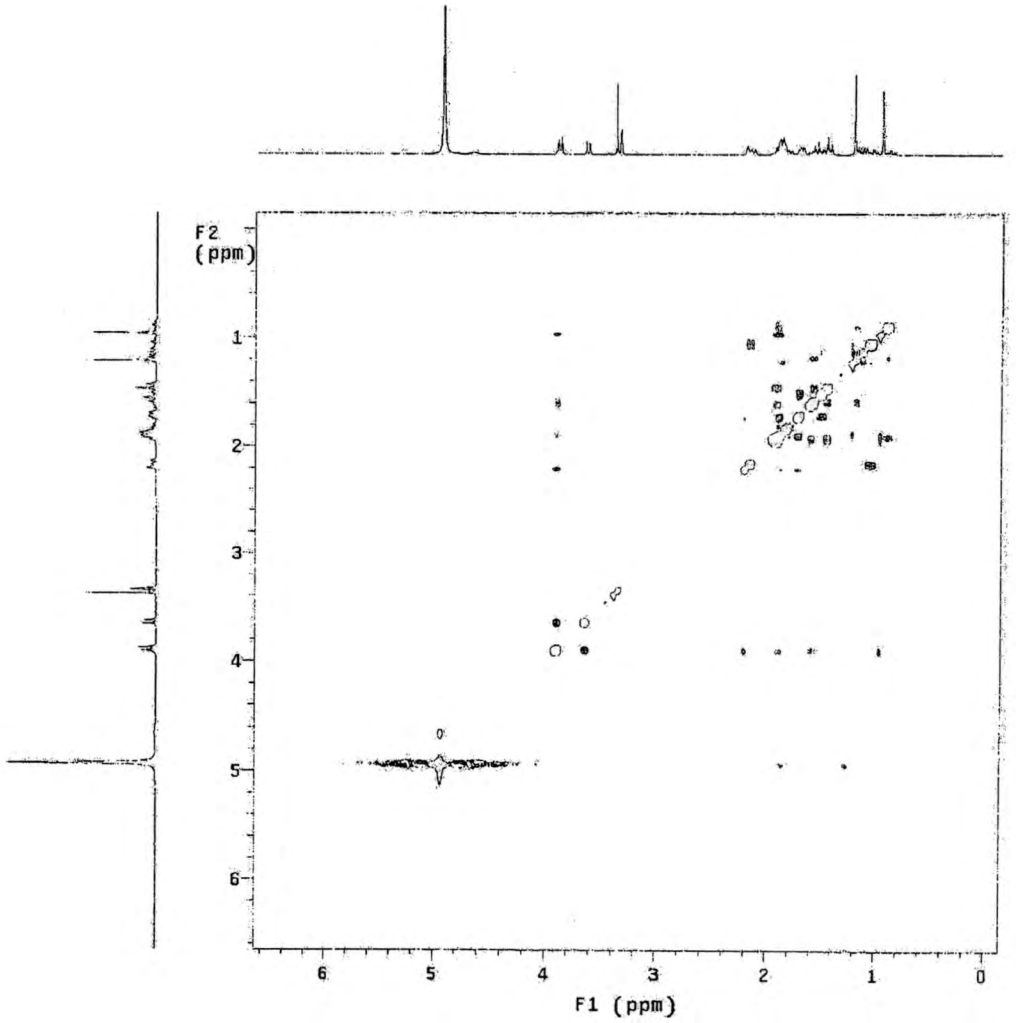


Figure C17. HSQC spectrum of compound **3**

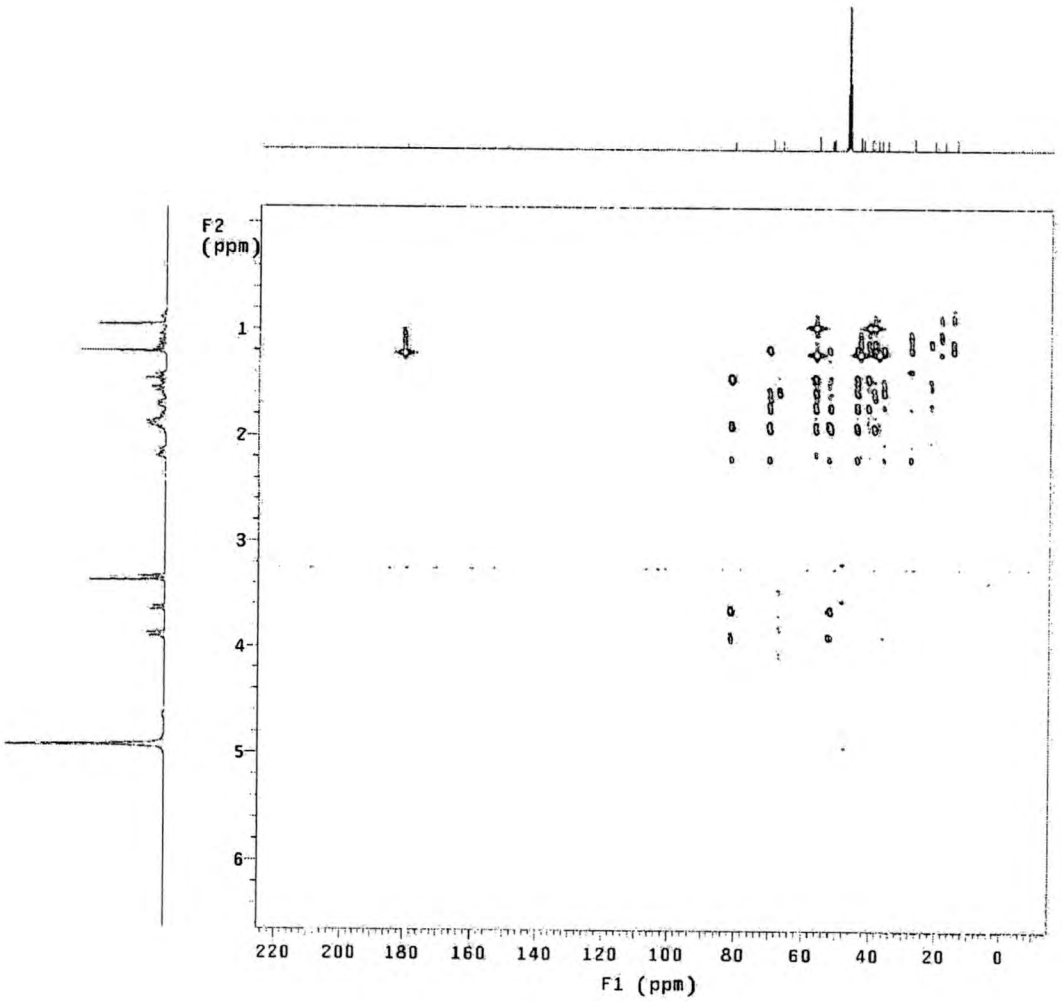


Figure C18. HMBC spectrum of compound **3**

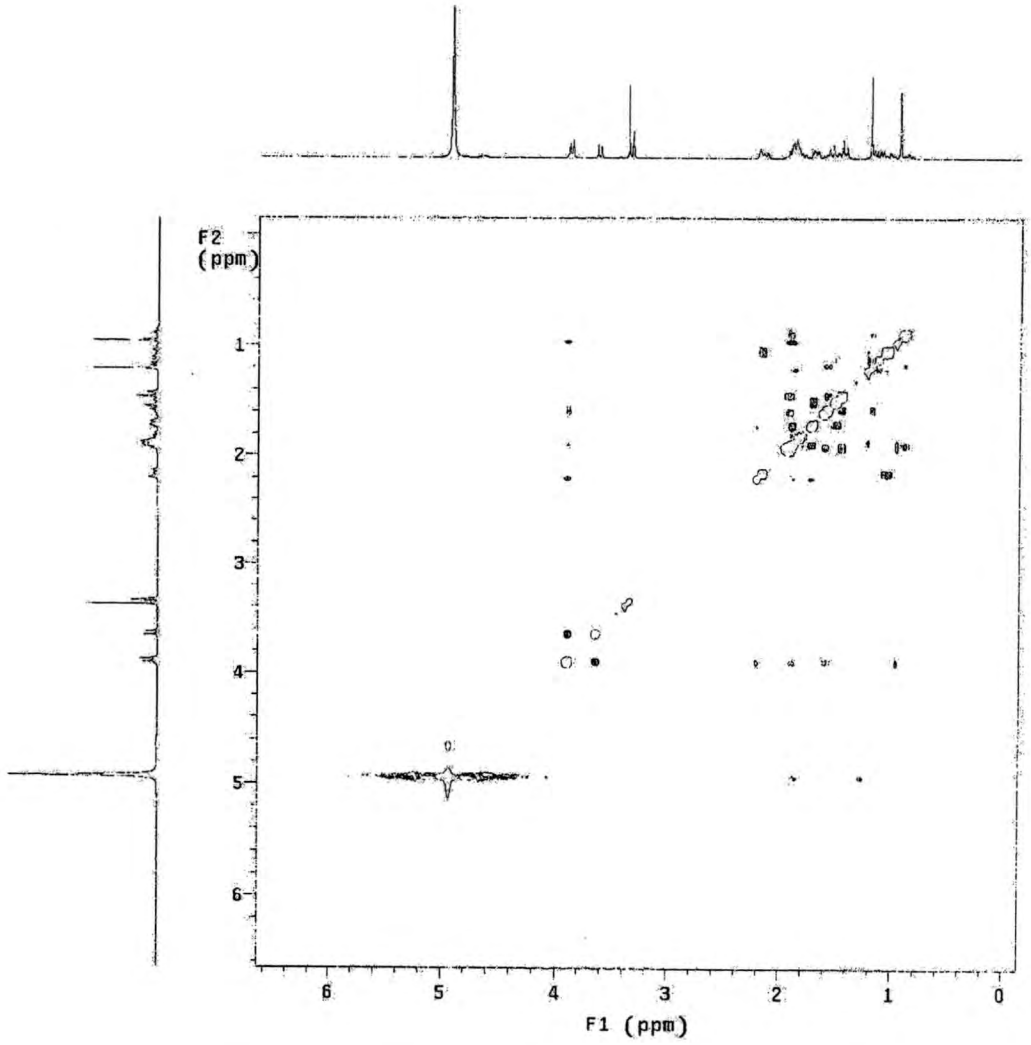


Figure C19. NOESY spectrum of compound **3**

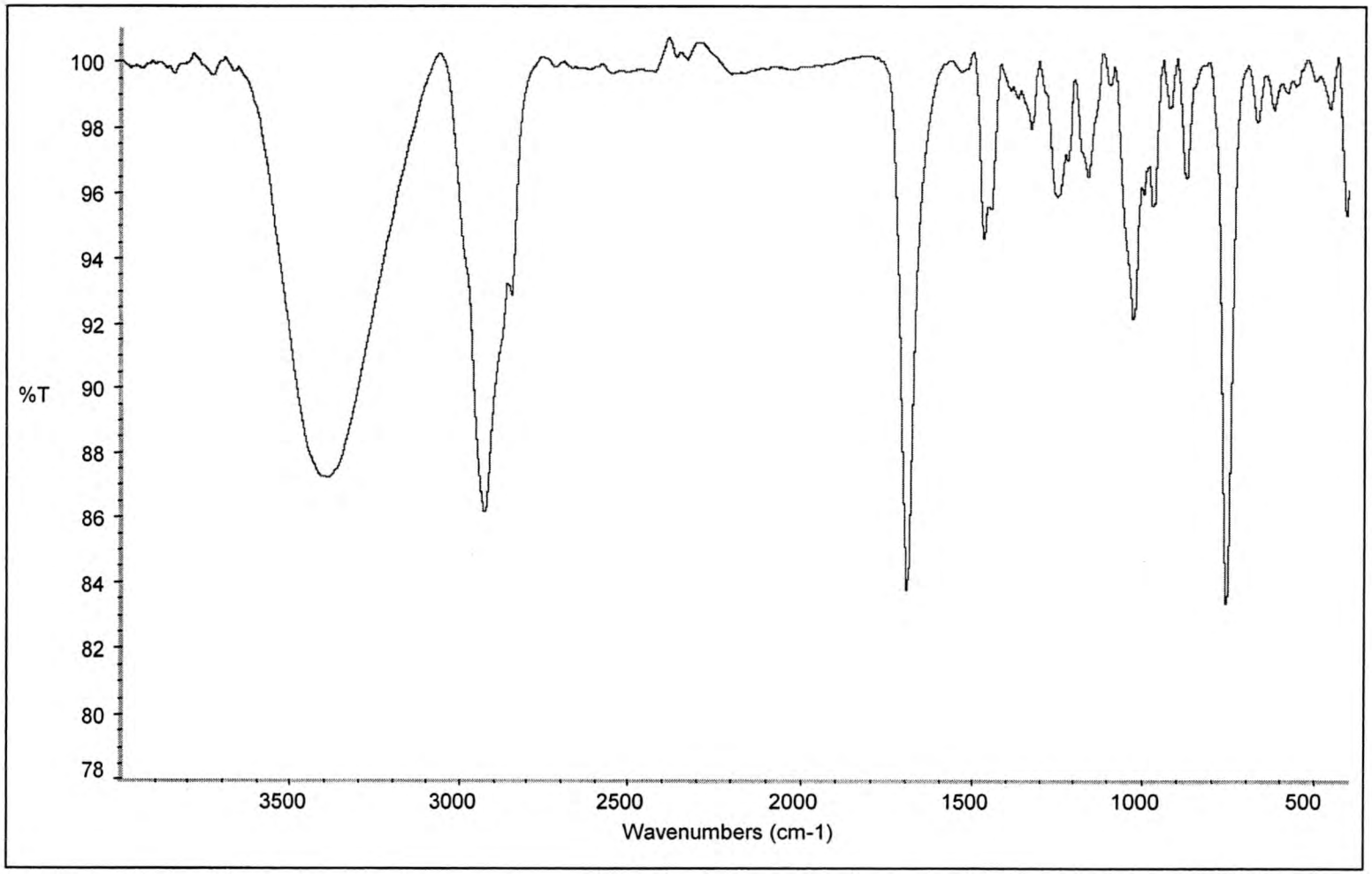


Figure C20. IR Spectrum of compound **4**

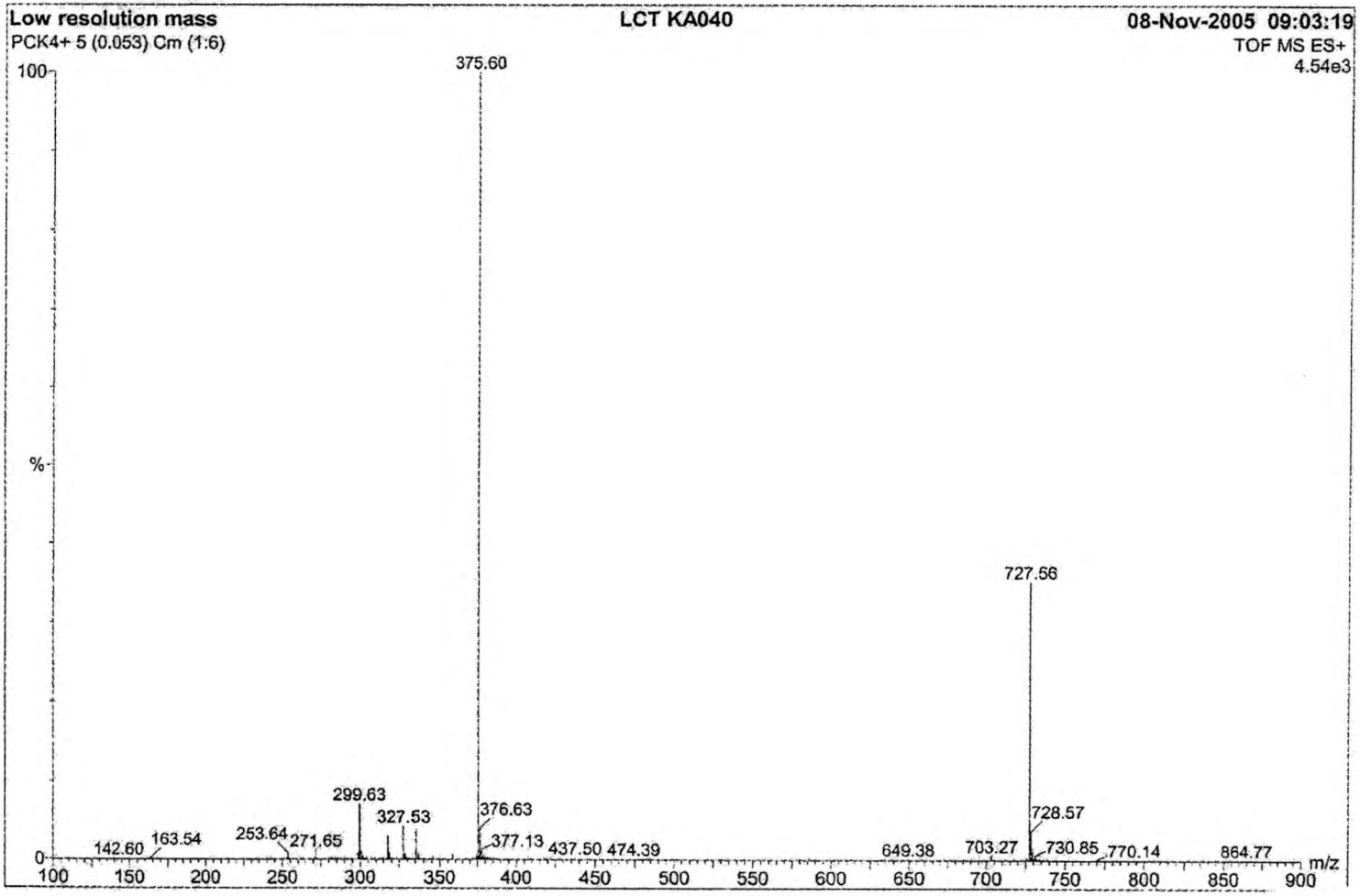


Figure C21. Low resolution mass spectrum of compound **4**

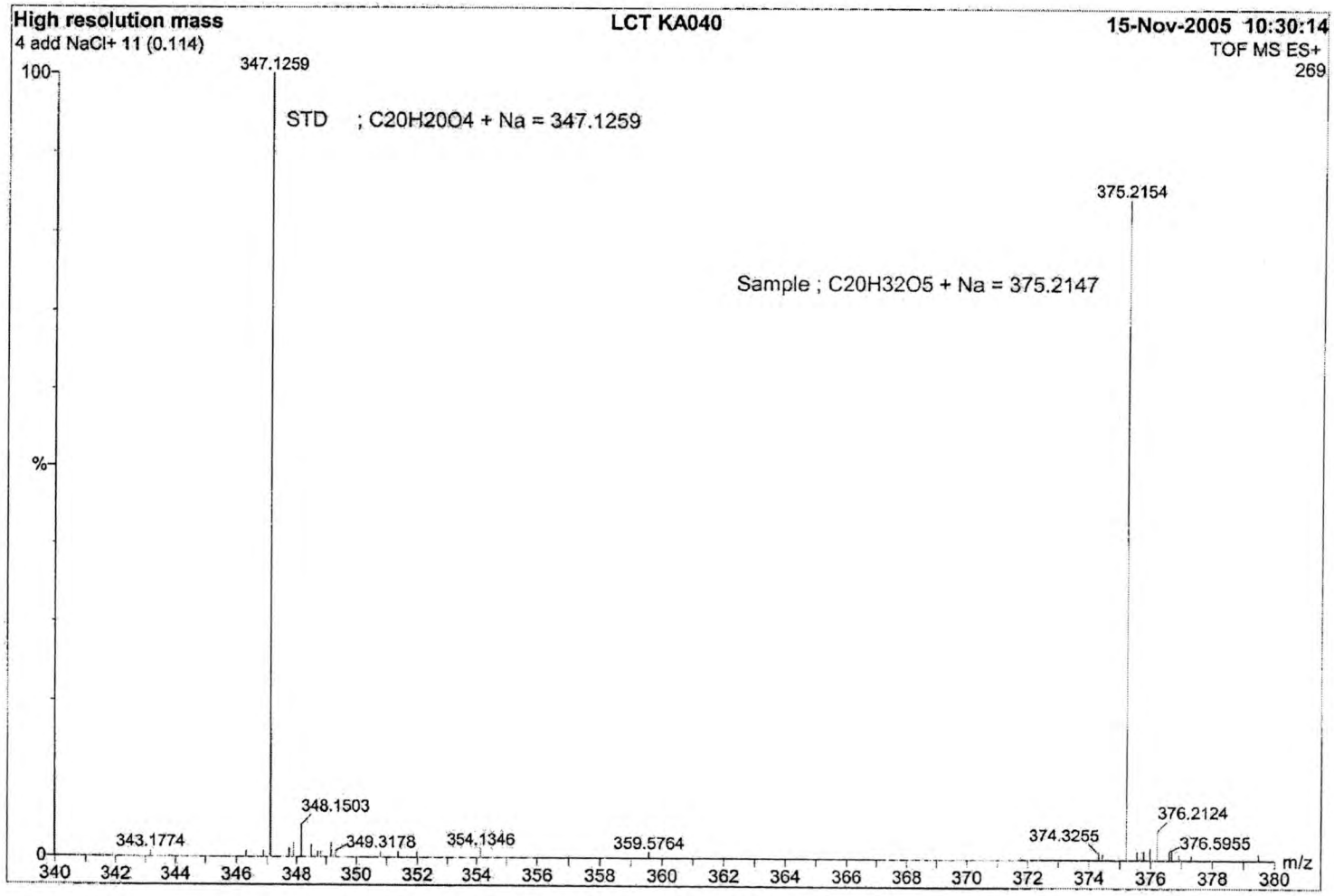


Figure C22. High resolution mass spectrum of compound **4**

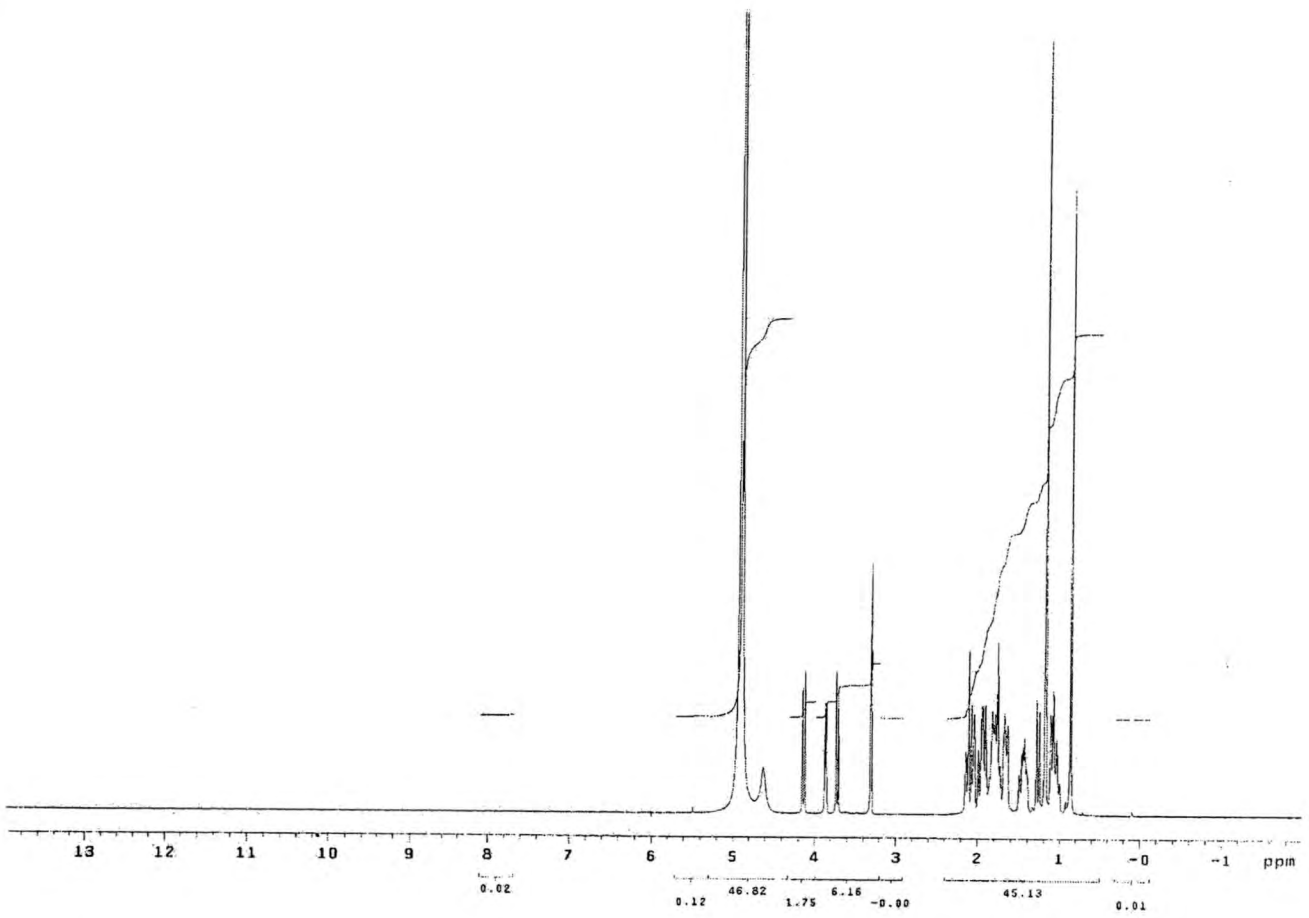


Figure C23. ^{1}H-NMR spectrum of compound **4**

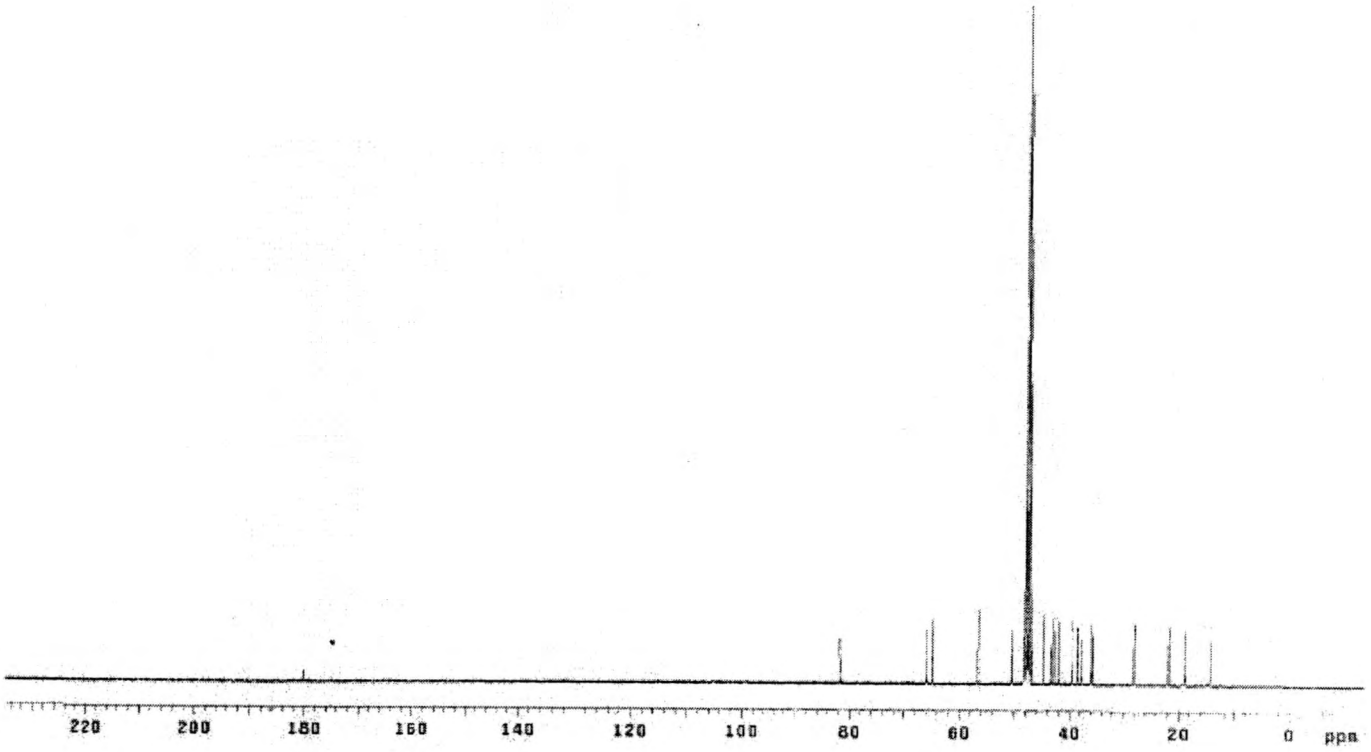


Figure C24. ^{13}C-NMR spectrum of compound **4**

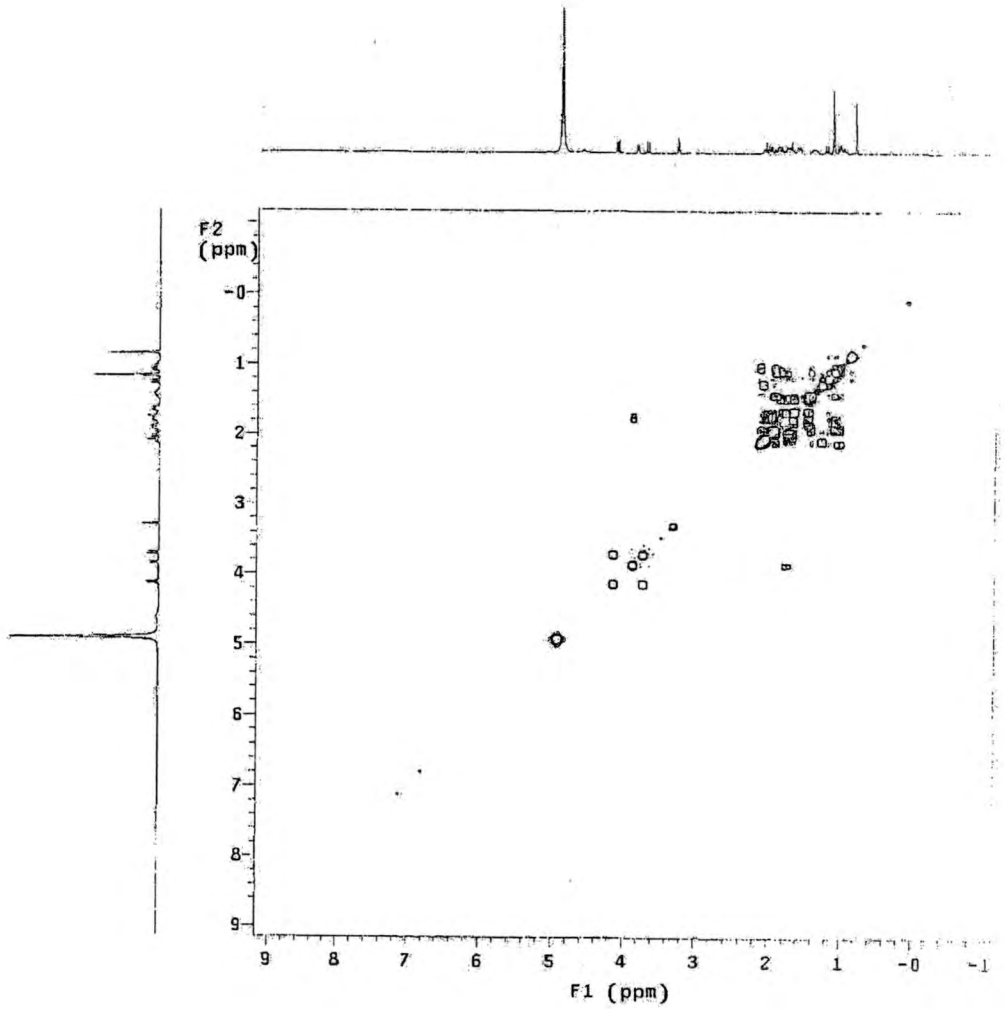


Figure C25. COSY spectrum of compound **4**

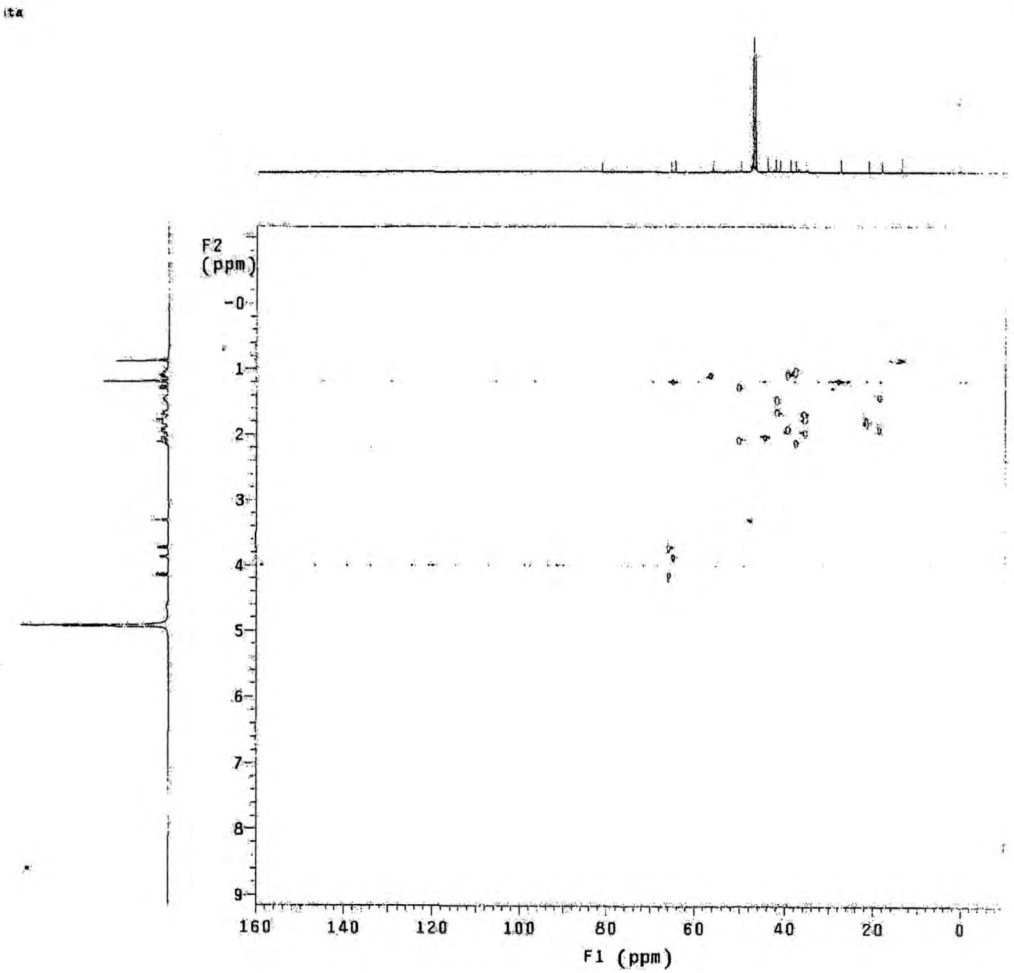


Figure C26. HSQC spectrum of compound **4**

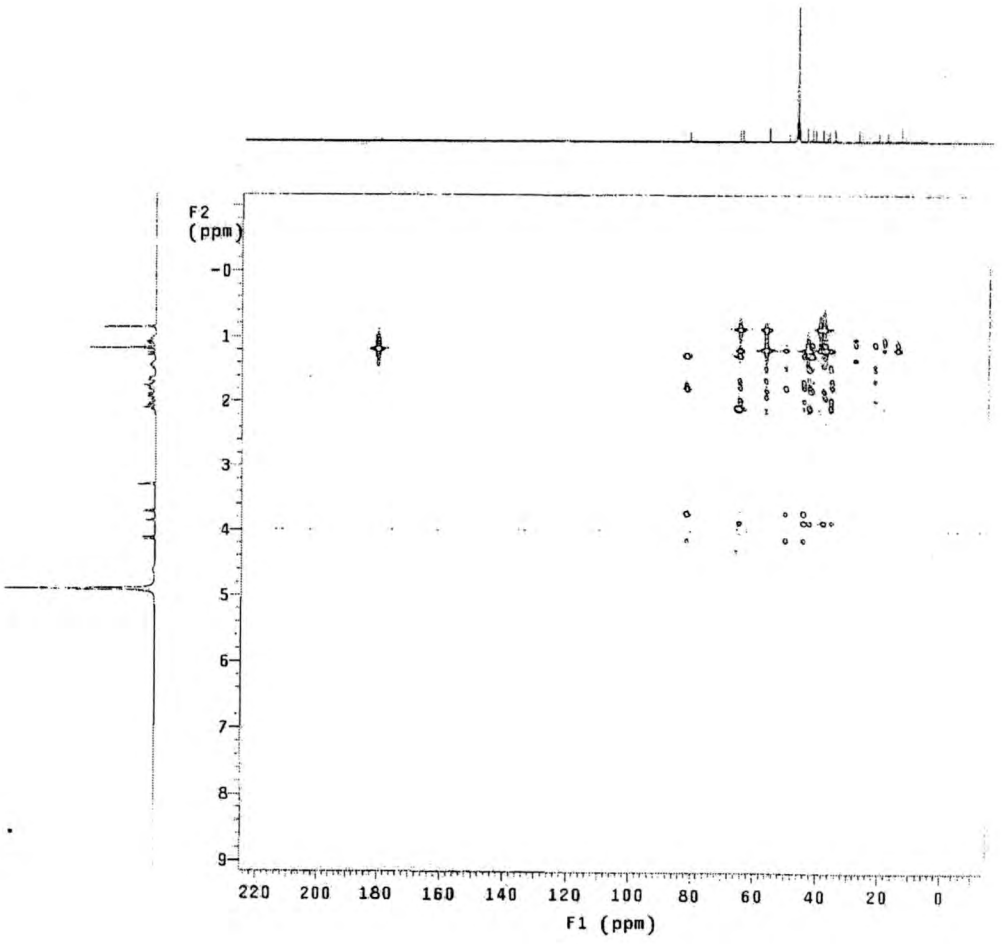


Figure C27. HMBC spectrum of compound **4**

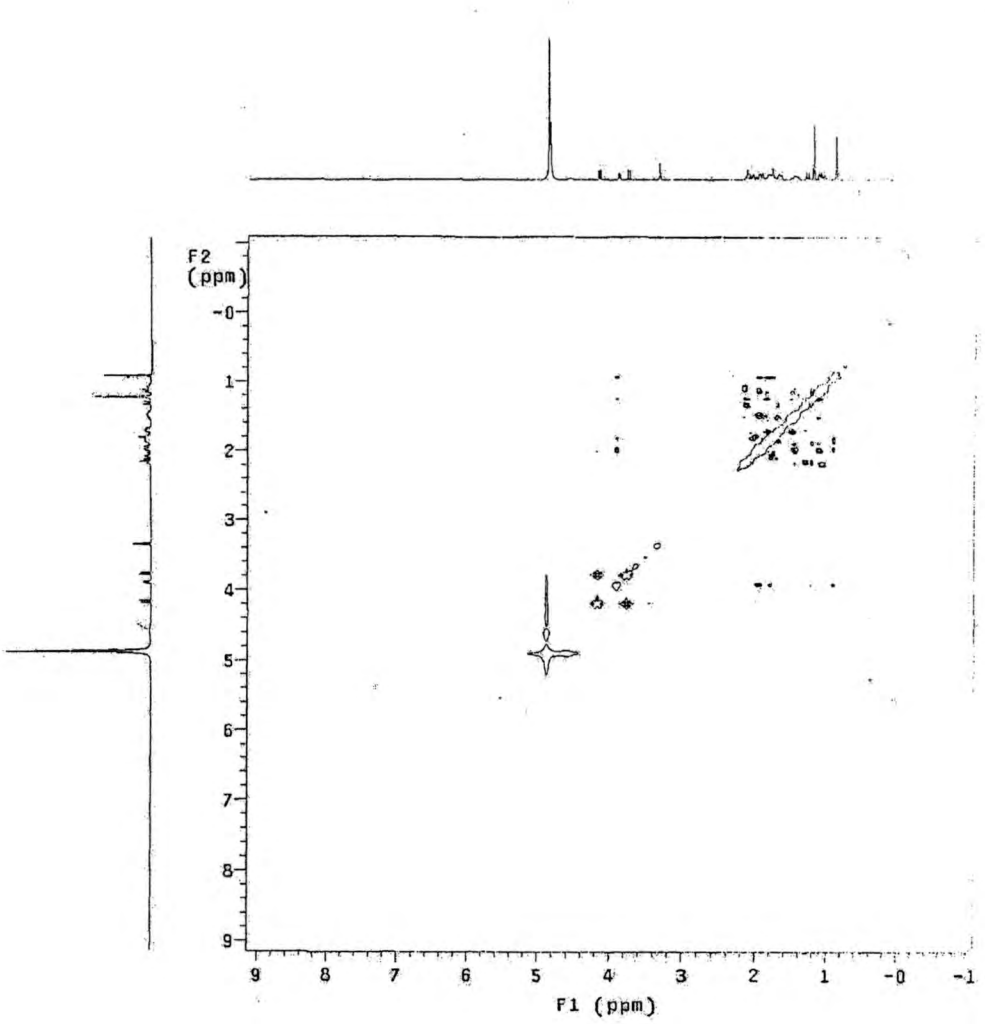


Figure C28. NOSEY NMR spectrum of compound **4**

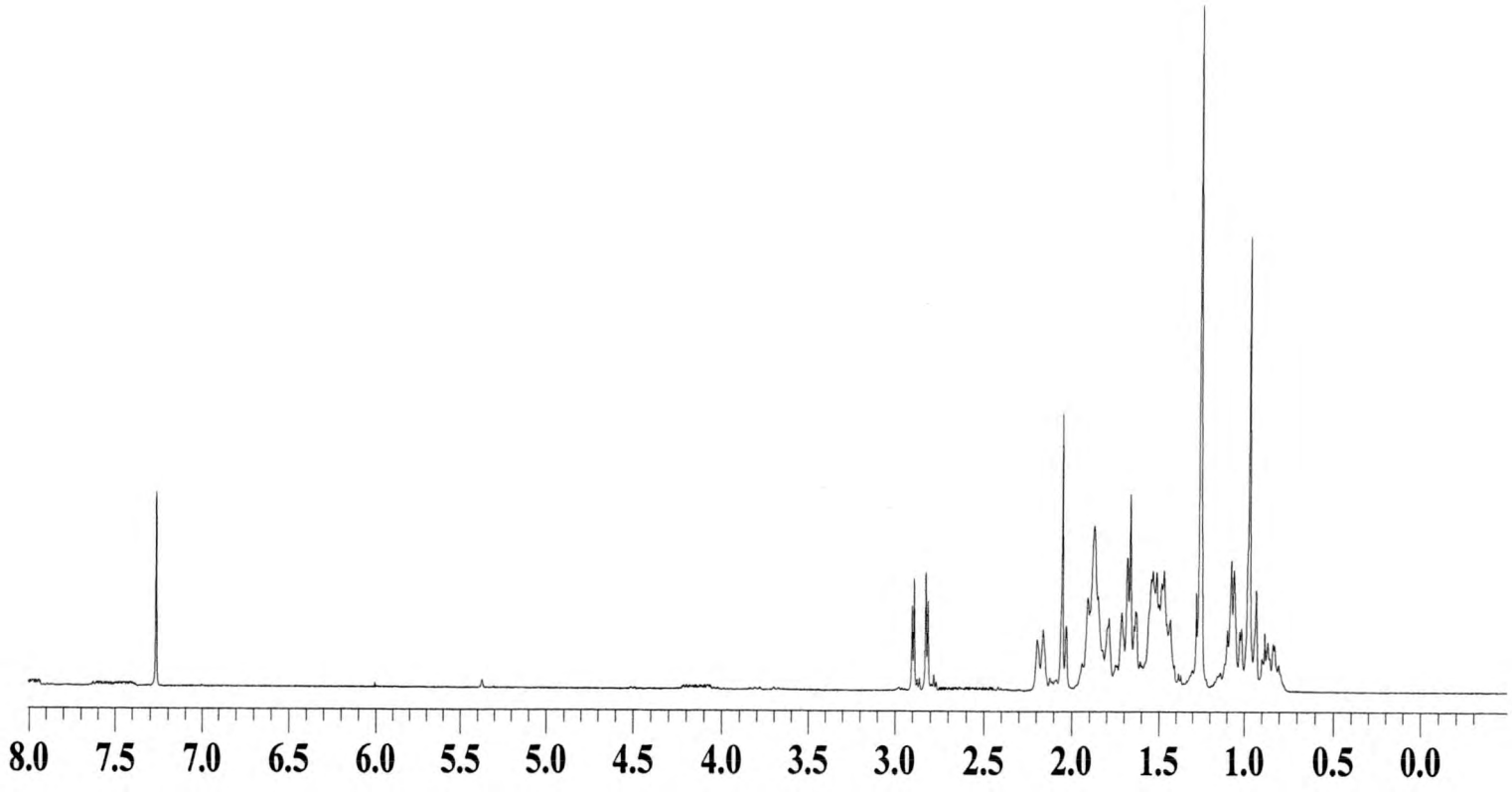


Figure C29. ^{1}H-NMR spectrum of compound **1a**

APPENDIX D

Table D1. Crystal data and structure refinement for compound **3**

Empirical formula	$C_{20}H_{32}O_5$
Formula weight	352
Temperature	293(2) K
Wavelength	0.71073 A
Crystal system, space group	orthorhombic, P212121
Unit cell dimensions	a = 7.50000(10) Å alpha = 90 deg. b = 12.383 (10) Å beta = 90 deg. c = 22.14790 (10) Å gamma = 90 deg.
Volume	2056.86(3) $Å^3$
Z, Calculated density	4,1.306 Mg/m^3
Absorption coefficient	0.100 mm^{-1}
F(000)	880
Theta range for data collection	1.84 to 30.43 deg.
Index ranges	-10<=h<=10, -17<=k<=17, -19<=l<=31
Reflection collection/ unique	15212 / 5767 [R(int) = 0.0231]
Completeness to 2theta=30.43	95.4 %
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	5767 / 0 / 383
Goodness-of-fit on F^2	1.049
Final R indices [I>2sigma (I)]	R1 = 0.0454, wR2 = 0.1180
R indices (all data)	R1 = 0.0540, wR2 = 0.1252
Absolute structure parameter	-0.8(9)
Largest diff. peak and hole	0.335 and -0.489 $e.A^{-3}$

Table D2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for Compound **3**

	X	Y	Z	U(eq)*
C(1)	824(3)	13288(1)	11453(1)	31(1)
C(2)	839(3)	13970(2)	12032(1)	35(1)
C(3)	2733(3)	14278(1)	12204(1)	32(1)
C(4)	3961(2)	13290(1)	12296(1)	25(1)
C(5)	3882(2)	12566(1)	11717(1)	22(1)
C(6)	5152(2)	11597(1)	11703(1)	27(1)
C(7)	5389(2)	11199(2)	11055(1)	29(1)
C(8)	3627(2)	10882(1)	10753(1)	22(1)
C(9)	2206(2)	11784(1)	10838(1)	21(1)
C(10)	1973(2)	12245(1)	11497(1)	22(1)
C(11)	445(2)	11457(1)	10513(1)	26(1)
C(12)	-28(2)	10256(1)	10510(1)	25(1)
C(13)	1593(2)	9505(1)	10433(1)	23(1)
C(14)	2899(2)	9767(1)	10946(1)	24(1)
C(15)	3941(2)	10686(1)	10062(1)	27(1)
C(16)	2760(2)	9727(1)	9869(1)	24(1)
C(17)	1842(3)	9949(2)	9267(1)	31(1)
C(18)	5891(3)	13715(2)	12381(1)	35(1)
C(19)	3533(2)	12742(2)	12899(1)	29(1)
C(20)	1025(2)	11465(2)	11937(1)	29(1)
O(1)	-1323(2)	10099(1)	10045(1)	35(1)
O(2)	3918(2)	8795(1)	9787(1)	31(1)
O(3)	4207(3)	11766(1)	12960(1)	44(1)
O(4)	2754(3)	13192(1)	13303(1)	55(1)
O(5)	761(2)	9071(1)	9063(1)	36(1)

*U(eq) is defined as one third of the trace of the orthogonalized

Table D3. Bond distances (A°) for Compound **3**

Bond Distances	Distances (Å)	Bond Distances	Distances (Å)
C(1)-C(2)	1.534(2)	C(9)-C(10)	1.578(2)
C(1)-C(10)	1.555(2)	C(10)-C(20)	1.546(2)
C(2)-C(3)	1.520(3)	C(11)-C(12)	1.529(2)
C(3)-C(4)	1.544(2)	C(12)-O(1)	1.427(2)
C(4)-C(19)	1.533(2)	C(12)-C(13)	1.540(2)
C(4)-C(18)	1.552(3)	C(13)-C(14)	1.534(2)
C(4)-C(5)	1.565(2)	C(13)-C(16)	1.550(2)
C(5)-C(6)	1.532(2)	C(15)-C(16)	1.542(2)
C(5)-C(10)	1.564(2)	C(16)-O(2)	1.456(2)
C(6)-C(7)	1.528(2)	C(16)-C(17)	1.525(2)
C(7)-C(8)	1.533(2)	C(17)-O(5)	1.429(2)
C(8)-C(14)	1.546(2)	C(19)-O(4)	1.206(2)
C(8)-C(9)	1.554(2)	C(19)-O(3)	1.317(2)
C(8)-C(15)	1.566(2)		

Table D4. Bond angles (deg) for compound **3**

Angles	(Å)	Angles	(Å)
C(2)-C(1)-(10)	113.67(14)	C(20)-C(10)-C(5)	112.53(13)
C(3)-C(2)-C(1)	110.81(16)	C(1)-C(10)-C(5)	108.40(13)
C(2)-C(3)-C(4)	113.03(15)	C(20)-C(10)-C(9)	114.09(13)
C(19)-C(4)-C(3)	109.90(15)	C(1)-C(10)-C(9)	107.76(12)
C(19)-C(4)-C(18)	103.86(14)	C(5)-C(10)-C(9)	106.20(12)
C(3)-C(4)-C(18)	107.67(15)	C(12)-C(11)-C(9)	116.83(13)
C(19)-C(4)-C(5)	116.88(13)	O(1)-C(12)-C(11)	107.05(14)
C(3)-C(4)-C(5)	108.92(13)	O(1)-C(12)-C(13)	112.11(14)
C(18)-C(4)-C(5)	109.19(14)	C(11)-C(12)-C(13)	113.88(13)
C(6)-C(5)-C(10)	111.35(13)	C(14)-C(13)-C(12)	107.17(13)
C(6)-C(5)-C(4)	116.19(13)	C(14)-C(13)-C(16)	101.49(13)
C(10)-C(5)-C(4)	115.81(13)	C(12)-C(13)-C(16)	115.30(13)
C(7)-C(6)-C(5)	110.08(13)	C(13)-C(14)-C(8)	102.04(12)
C(6)-C(7)-C(8)	113.15(14)	C(16)-C(15)-C(8)	107.71(13)
C(7)-C(8)-C(14)	114.34(14)	O(2)-C(16)-C(17)	107.62(13)
C(7)-C(8)-C(9)	110.75(13)	O(2)-C(16)-C(15)	107.64(14)
C(14)-C(8)-C(9)	111.45(12)	C(17)-C(16)-C(15)	111.30(14)
C(7)-C(8)-C(15)	109.71(13)	O(2)-C(16)-C(13)	107.29(13)
C(14)-C(8)-C(15)	100.68(12)	C(17)-C(16)-C(13)	118.77(14)
C(9)-C(8)-C(15)	109.41(13)	C(15)-C(16)-C(13)	103.71(12)
C(8)-C(9)-C(11)	109.83(12)	O(5)-C(17)-C(16)	113.30(15)
C(8)-C(9)-C(10)	116.63(12)	O(4)-C(19)-O(3)	122.24(17)
C(11)-C(9)-C(10)	115.29(13)	O(4)-C(19)-C(4)	122.98(17)
C(20)-C(10)-C(1)	107.65(14)	O(3)-C(19)-C(4)	114.58(15)

APPENDIX E

POLYACRYLAMIDE GEL ELECTROPHORESIS

E1. Stock reagent

Solution A: 30% (w/v) acrylamide, 0.8% (w/v) bis-acrylamide

Acrylamide	29.2	g
Bis-acrylamide	0.8	g

Add distilled water to make 100 ml and stir until completely dissolved

Solution B: (4X Separating Gel Buffer), 100 ml

2 M Tris-HCl (pH 8.8)	75	ml
10 % SDS	4	ml
Distilled water	21	ml

Stable for months in refrigerator

Solution C: (4X Stacking Gel Buffer), 100 ml

1 M Tris-HCl (pH 6.8)	50	ml
10 % SDS	4	ml
Distilled water	46	ml

Stable for months in refrigerator

10% ammonium persulfate, 5 ml

Ammonium persulfate	0.5	g
Distilled water	5	ml

Stable for months in a capped tube in the refrigerator

2M Tris-HCl pH 8.8

Tris (hydroxymethyl)-aminomethane 24.2 g

Adjusted pH to 8.8 with 1M HCl and adjusted volume to 100 ml with distilled water

0.5 M Tris-HCl pH 6.8

Tris (hydroxymethyl)-aminomethane 6.06 g

Adjusted pH to 6.8 with 1M HCl and adjusted volume to 100 ml with distilled water

1 M Tris-HCl pH 6.8

Tris (hydroxymethyl)-aminomethane 6.06 g

Adjusted pH to 6.8 with 1M HCl and adjusted volume to 100 ml with distilled water

E2. SDS-PAGE

12.5% Separating gel

Solution A	4.17	ml
Solution B	2.5	ml
Distilled water	3.33	ml
10% $(NH_4)_2S_2O_8$	50	ml
TEMED	5	ml

5.0% Staking gel

Solution A	0.67	ml
Solution C	1	ml
Distilled water	2.3	ml
10% $(NH_4)_2S_2O_8$	50	ml
TEMED	5	ml

5X Sample Buffer, 10 ml

1 M Tris-HCl (pH 6.8)	0.6	ml
50% glycerol	5	ml
10% SDS	2	ml
2-mercaptocthanol	0.5	ml
1% bromophenol blue	1	ml
H_2O	0.9	ml

One part of sample buffer was added to four parts of sample. The mixture was heat to 5 min in boiling water before loading to gel.

Electrophoresis Buffer, 1 liter

Tris	3	g
Glycine	14.4	g
SDS	1	g

Add water to make 1 liter (pH should be approximately 8.3)

E3. Coomassie blue staining

Staining solution, 100 ml

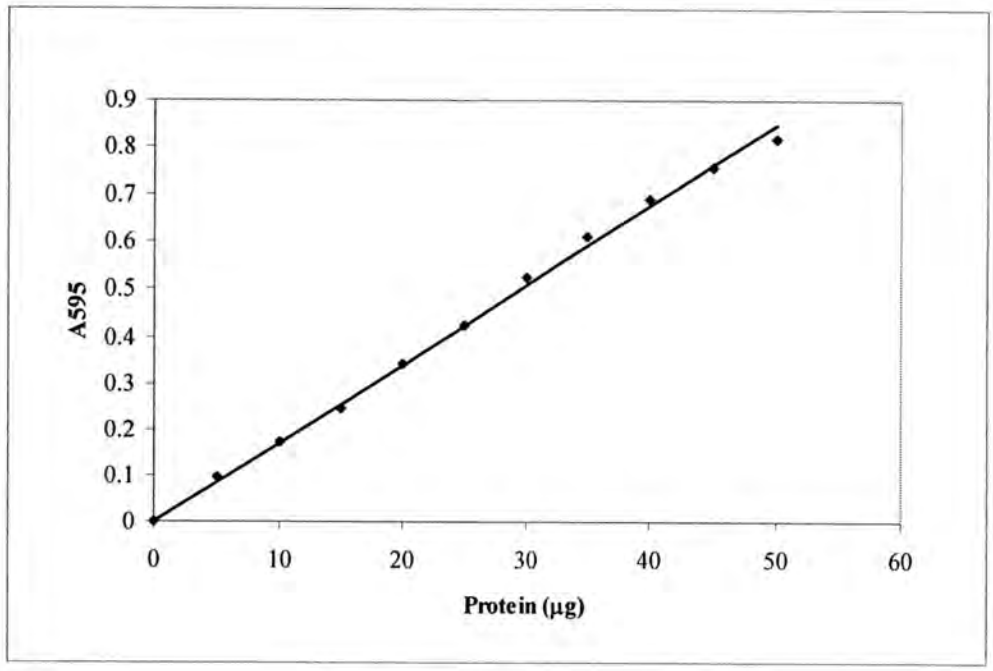
Coomassie brilliant blue R-250	0.1	g
Methanol	45	ml
Acetic acid	10	ml
Distilled water	45	ml

Destaining solution, 100 ml

Methanol	10	ml
Acetic acid	45	ml
Distilled water	80	ml

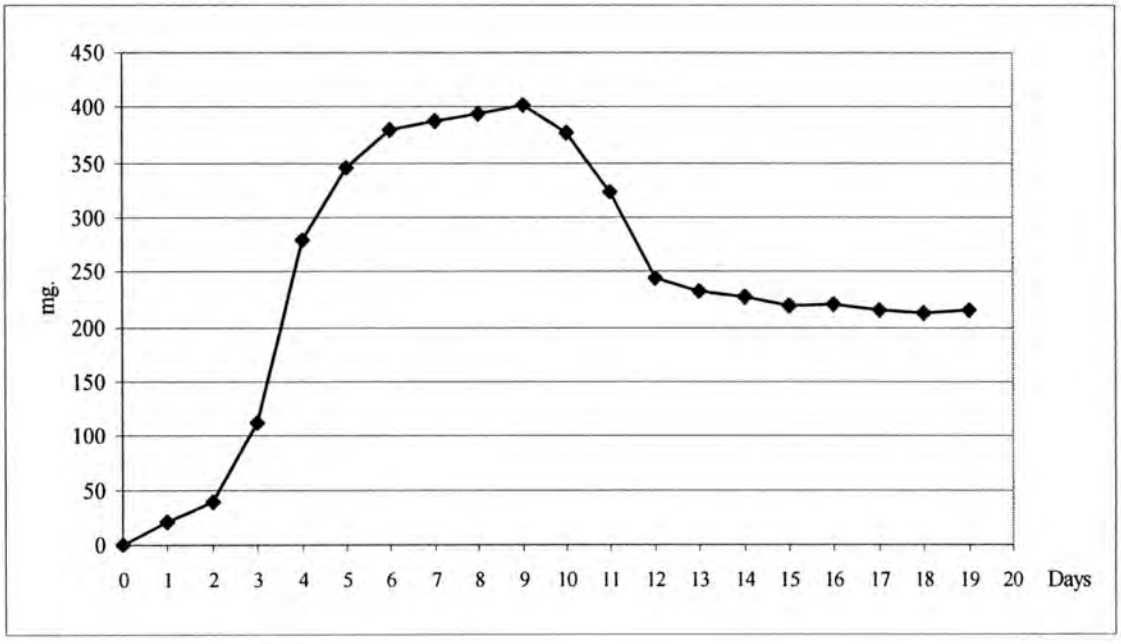
APPENDIX F

Standard curve of protein determination by Bradford's method



APPENDIX G

Growth curve of *P. cubensis* in Czapex Peptone medium



BIOGRAPHY

Miss Jaraslak Pechwang was born on July 11, 1974 in Suratthani province, Thailand. She graduated with a Bachelor's Degree of Science in Medical Technology from the Faculty of Allied Health Science, Chulalongkorn University, Thailand in 1996 and then graduated with a Master's Degree of Science in Biotechnology, Program of Biotechnology, Faculty of Science, Chulalongkorn University in 2001. After graduation M. Sc., she continued her Ph.D. in Biotechnology, Program in Biotechnology, Faculty of Science, Chulalongkorn University. Throughout her Ph.D. studies, she had received the financial support from the Royal Golden Jubilee for Ph.D. Scholarship and partial financial support from the Graduate School, Chulalongkorn University.