

Supplementary Material for:

Polyoxypregnane Glycosides from Root of *Marsdenia tenacissima* and Inhibited Nitric Oxide Levels in LPS Stimulated RAW 264.7 cells

Zhi Na ^{1,*}, PianChou Gongpan ¹, Qingfei Fan ^{2,*}

¹ Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla 666303, China; gongpan@xtbg.ac.cn (P.G.)

² College of Science, Yunnan Agricultural University, Kunming 650201, China

* Correspondence: nazhi@xtbg.org.cn (Z. N.), fanqingfei@xtbg.ac.cn (Q.F.)

Contents

Figures

Figure S1. HRESIMS spectrum of compound 1	4
Figure S2. ¹ H-NMR (500 MHz, CDCl ₃) spectrum of compound 1	5
Figure S3. ¹³ C-NMR and DEPT (125 MHz, CDCl ₃) spectrum of compound 1	5
Figure S4. ¹ H- ¹ H COSY spectrum of compound 1	6
Figure S5. HSQC spectrum of compound 1	6
Figure S6. HMBC spectrum of compound 1	7
Figure S7. ROESY spectrum of compound 1	7
Figure S8. HREIMS spectrum of compound 2	8
Figure S9. ¹ H-NMR (500 MHz, CDCl ₃) spectrum of compound 2	9
Figure S10. ¹³ C-NMR and DEPT (125 MHz, CDCl ₃) spectrum of compound 2	9
Figure S11. ¹ H- ¹ H COSY spectrum of compound 2	10
Figure S12. HSQC spectrum of compound 2	10
Figure S13. HMBC spectrum of compound 2	11
Figure S14. ROESY spectrum of compound 2	11
Figure S15. HRESIMS spectrum of compound 3	12
Figure S16. ¹ H-NMR (500 MHz, CDCl ₃) spectrum of compound 3	13
Figure S17. ¹³ C-NMR and DEPT (125 MHz, CDCl ₃) spectrum of compound 3	13
Figure S18. ¹ H- ¹ H COSY spectrum of compound 3	14
Figure S19. HSQC spectrum of compound 3	14
Figure S20. HMBC Spectrum of compound 3	15
Figure S21. ROESY Spectrum of compound 3	15
Figure S22. HRESIMS spectrum of compound 4	16
Figure S23. ¹ H-NMR (500 MHz, pyridine- <i>d</i> ₅) spectrum of compound 4	17

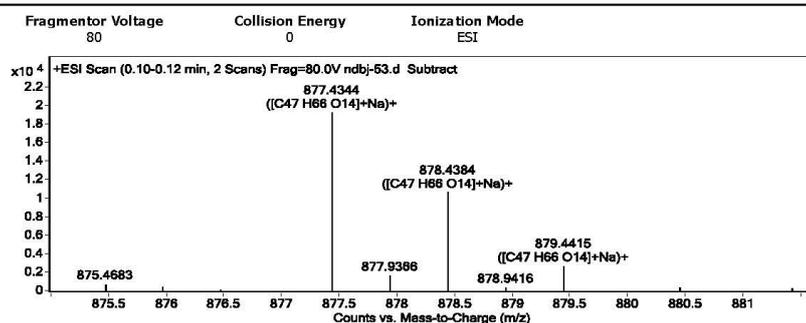
Figure S24. ^{13}C -NMR and DEPT (125 MHz, pyridine- d_5) spectrum of compound 4	17
Figure S25. ^1H - ^1H COSY spectrum of compound 4	18
Figure S26. HSQC spectrum of compound 4	18
Figure S27. HMBC spectrum of compound 4	19
Figure S28. ROESY spectrum of compound 4	19
Figure S29. HRESIMS spectrum of compound 5	20
Figure S30. ^1H -NMR (500 MHz, pyridine- d_5) spectrum of compound 5	21
Figure S31. ^{13}C -NMR and DEPT (125 MHz, pyridine- d_5) spectrum of compound 5	21
Figure S32. ^1H - ^1H COSY spectrum of compound 5	22
Figure S33. HSQC spectrum of compound 5	22
Figure S34. HMBC spectrum of compound 5	23
Figure S35. ROESY spectrum of compound 5	23
Figure S36. HRESIMS spectrum of compound 6	24
Figure S37. ^1H -NMR (500 MHz, pyridine- d_5) spectrum of compound 6	25
Figure S38. ^{13}C -NMR and DEPT (125 MHz, pyridine- d_5) spectrum of compound 6	25
Figure S39. ^1H - ^1H COSY spectrum of compound 6	26
Figure S40. HSQC spectrum of compound 6	26
Figure S41. HMBC spectrum of compound 6	27
Figure S42. ROESY spectrum of compound 6	27
Figure S43. HRESIMS spectrum of compound 7	28
Figure S44. ^1H -NMR (500 MHz, pyridine- d_5) spectrum of compound 7	29
Figure S45. ^{13}C -NMR and DEPT (125 MHz, pyridine- d_5) spectrum of compound 7	29
Figure S46. The ^1H - ^1H COSY spectrum of compound 7	30
Figure S47. HSQC spectrum of compound 7	30
Figure S48. HMBC spectrum of compound 7	31
Figure S49. ROESY spectrum of compound 7	31
Figure S50. Effects of compounds 1–7 on cell viability.....	32

Qualitative Analysis Report

Data Filename	ndbj-53.d	Sample Name	ndbj-53
Sample Type	Sample	Position	P1-F2
Instrument Name	Instrument 1	User Name	
Acq Method	s.m	Acquired Time	10/28/2020 2:27:28 PM
IRM Calibration Status	Success	DA Method	Default.m
Comment			

Sample Group	Info.
Acquisition SW	6200 series TOF/6500 series
Version	Q-TOF B.05.01 (B5125.2)

User Spectra



Peak List

m/z	z	Abund	Formula	Ion
551.301	1	1890.17		
673.2979	1	2904.21		
872.4793	1	3755.74		
877.4344	1	19341.66	C47 H66 O14	(M+Na)+
878.4384	1	10693.28	C47 H66 O14	(M+Na)+
879.4415	1	2738.25	C47 H66 O14	(M+Na)+
893.4087	1	14138.9		
894.4116	1	6676		
895.4125	1	3179.64		
1019.5192	1	3404.45		

Formula Calculator Element Limits

Element	Min	Max
C	3	120
H	0	240
O	0	30

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C47 H66 O14	854.4453	877.4345	877.4344	0.10	0.11	15.0000

--- End Of Report ---

Figure S1 HRESIMS spectrum of compound 1

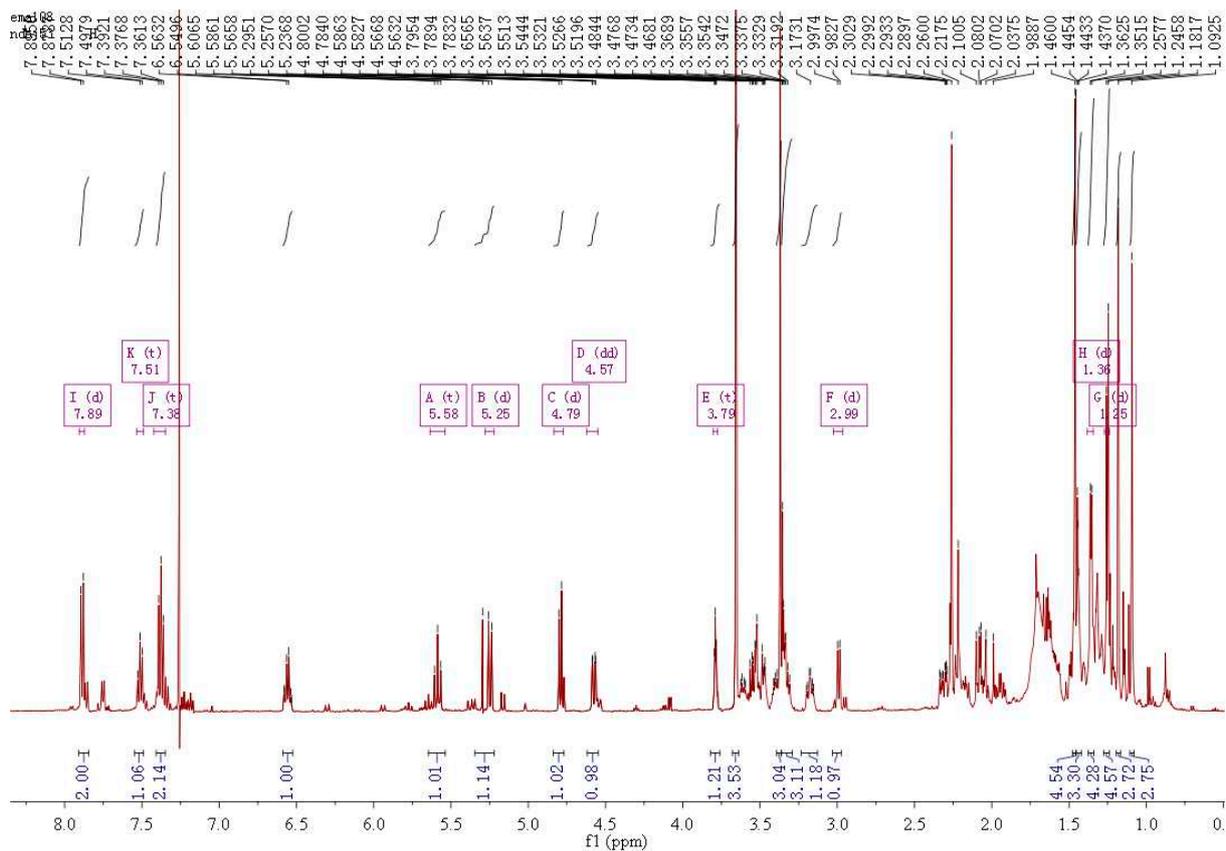


Figure S2 $^1\text{H-NMR}$ (500 MHz, CDCl_3) spectrum of compound **1**

ndbj53
ndbj53 hsqc

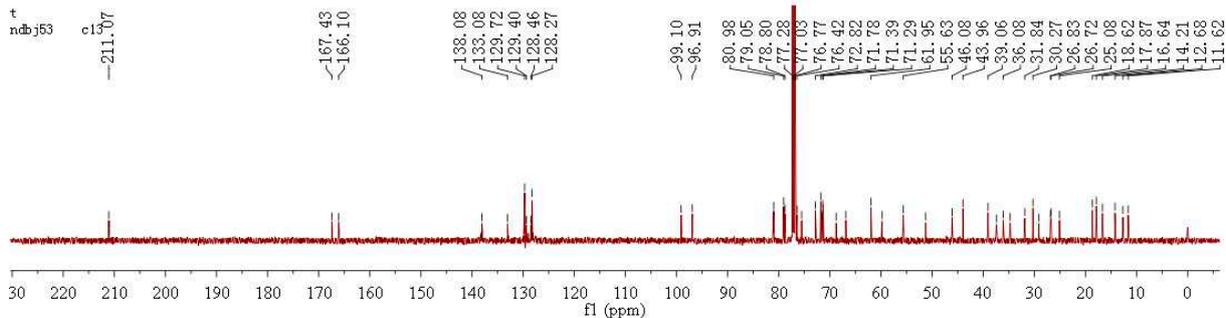


Figure S3 $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) and DEPT spectrum of compound **1**

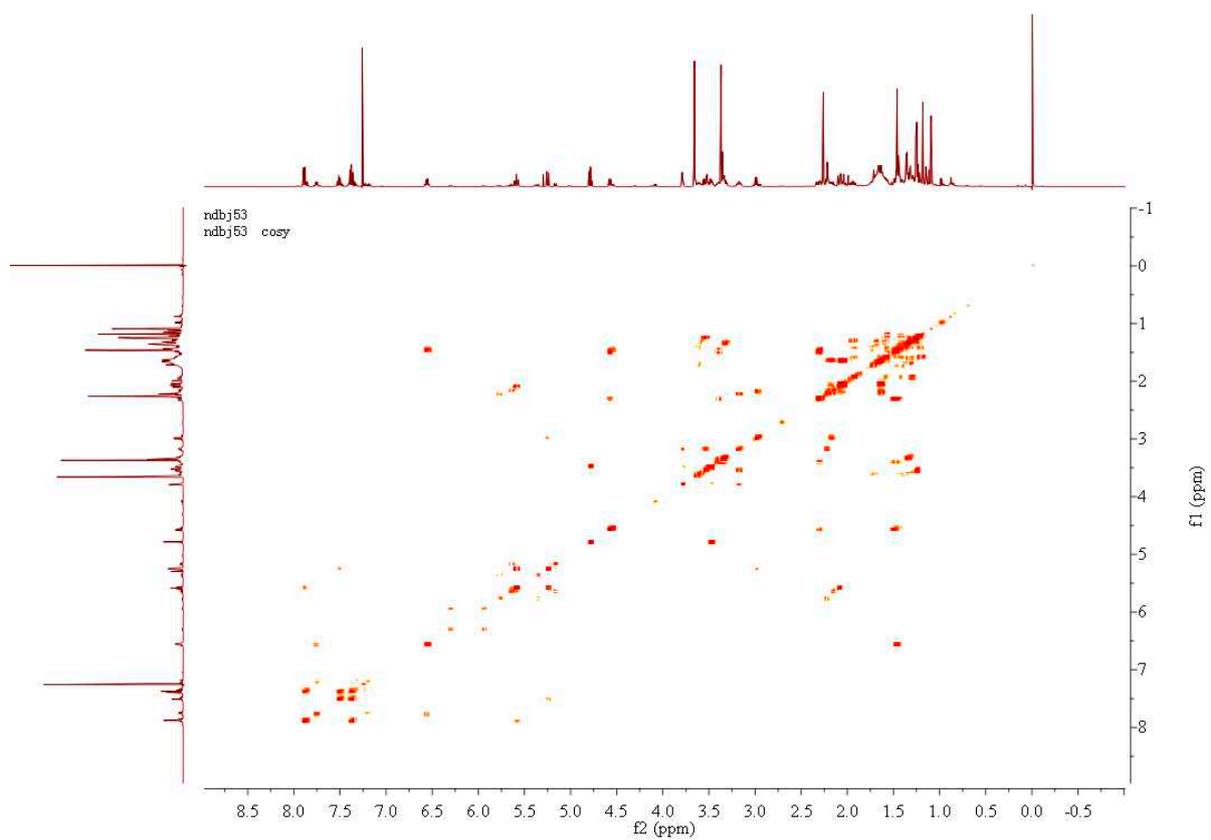


Figure S5 ^1H - ^1H COSY spectrum of compound **1**

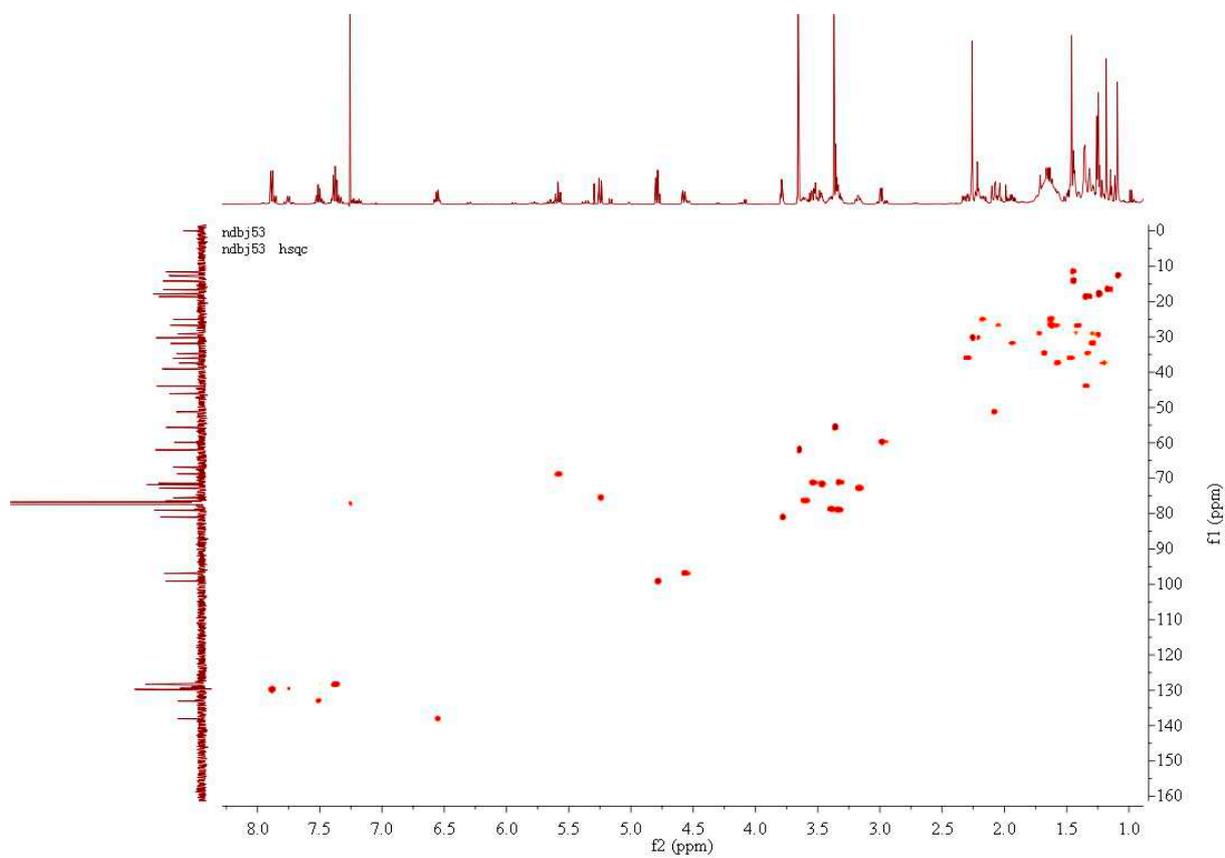


Figure S5 HSQC spectrum of compound **1**

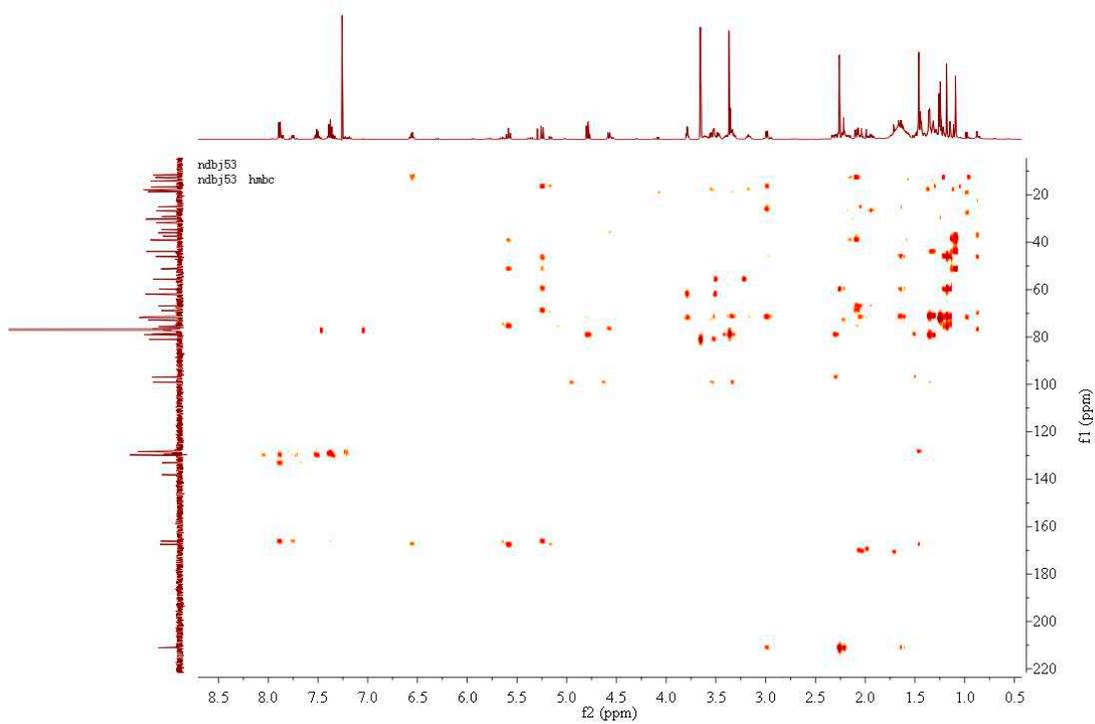


Figure S6 HMBC spectrum of compound **1**

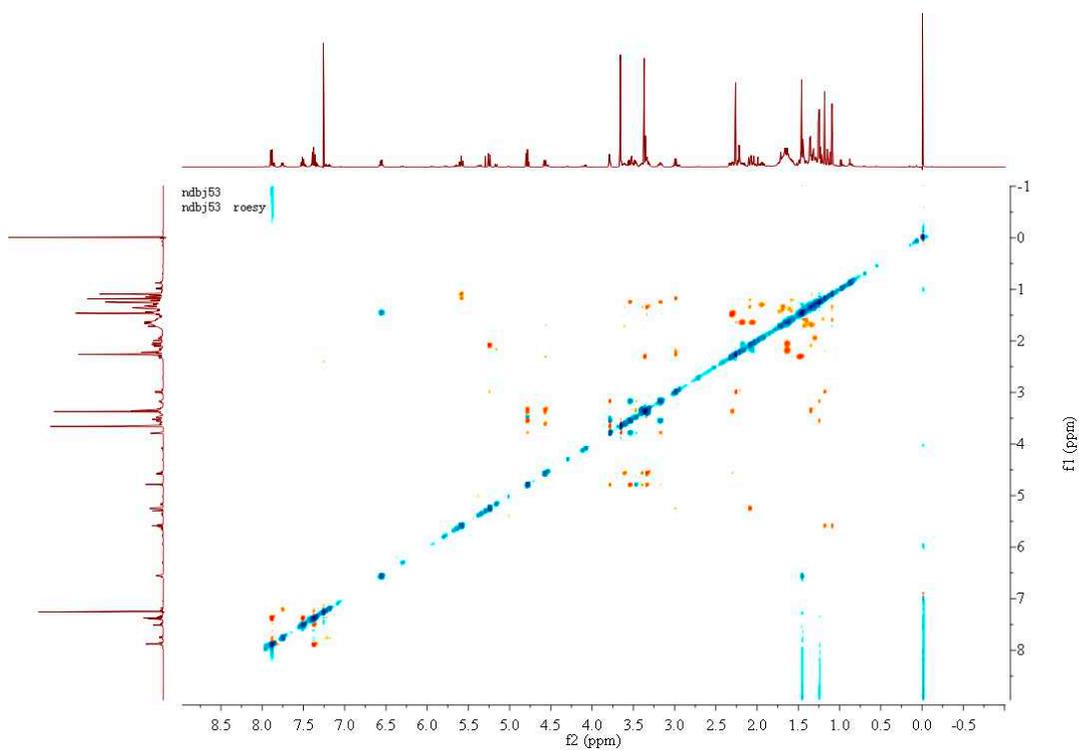


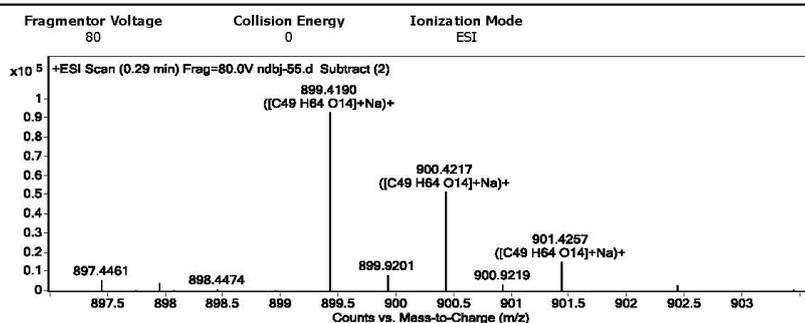
Figure S7 ROESY Spectrum of Compound **1**

Qualitative Analysis Report

Data Filename	ndbj-55.d	Sample Name	ndbj-55
Sample Type	Sample	Position	P1-F3
Instrument Name	Instrument 1	User Name	
Acq Method	s.m	Acquired Time	10/28/2020 2:28:39 PM
IRM Calibration Status	Success	DA Method	Default.m
Comment			

Sample Group	Info.
Acquisition SW	6200 series TOF/6500 series
Version	Q-TOF B.05.01 (B5125.2)

User Spectra



Peak List

m/z	z	Abund	Formula	Ion
377.2662	1	27443.59		
409.2559	1	15374.56		
673.298	1	10130.33		
865.4335	1	18937.1		
894.4545	1	11253.08		
899.419	1	93438.41	C49 H64 O14	(M+Na)+
900.4217	1	51815.04	C49 H64 O14	(M+Na)+
901.4257	1	15371.9	C49 H64 O14	(M+Na)+
915.3934	1	14416.72		
916.3973	1	9985.13		

Formula Calculator Element Limits

Element	Min	Max
C	3	120
H	0	240
O	0	30

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C49 H64 O14	876.4296	899.4188	899.4190	-0.20	-0.22	18.0000

--- End Of Report ---

Figure S8 HRESIMS spectrum of compound 2

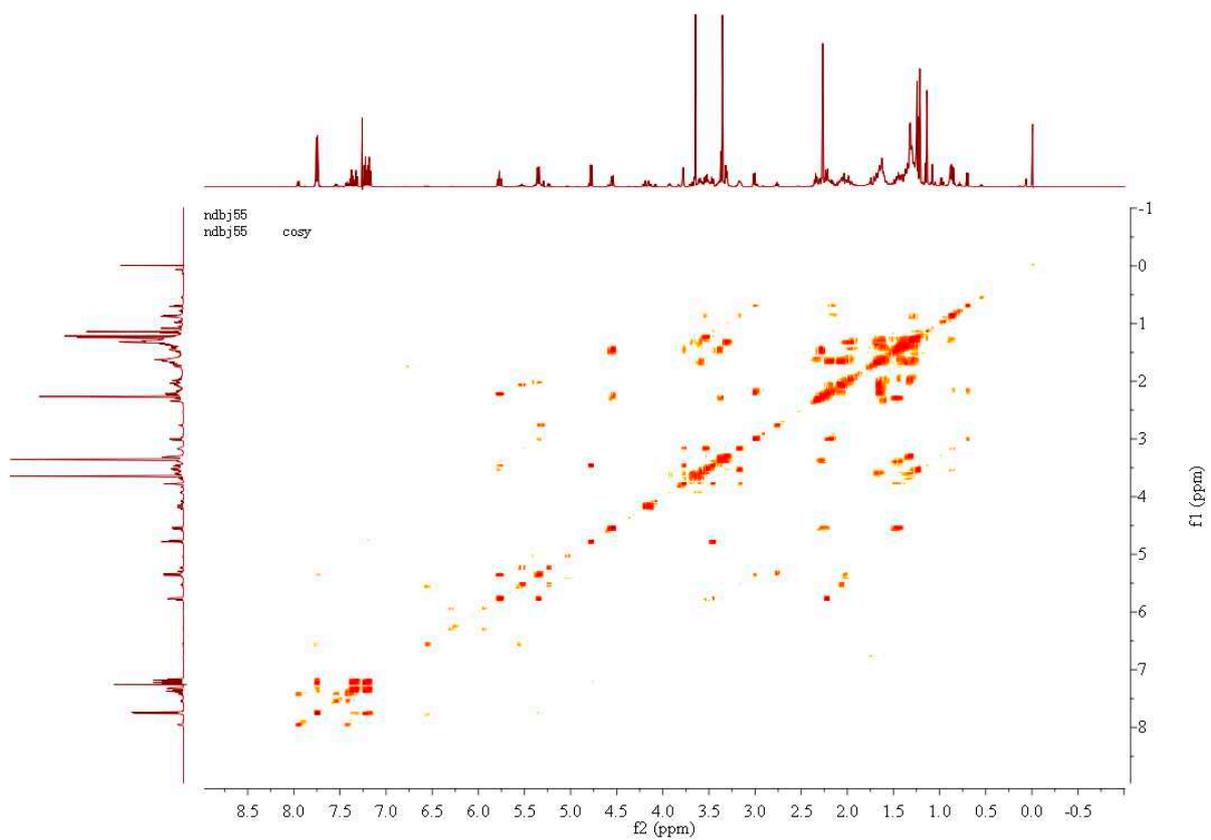


Figure S11 ^1H - ^1H COSY spectrum of compound **2**

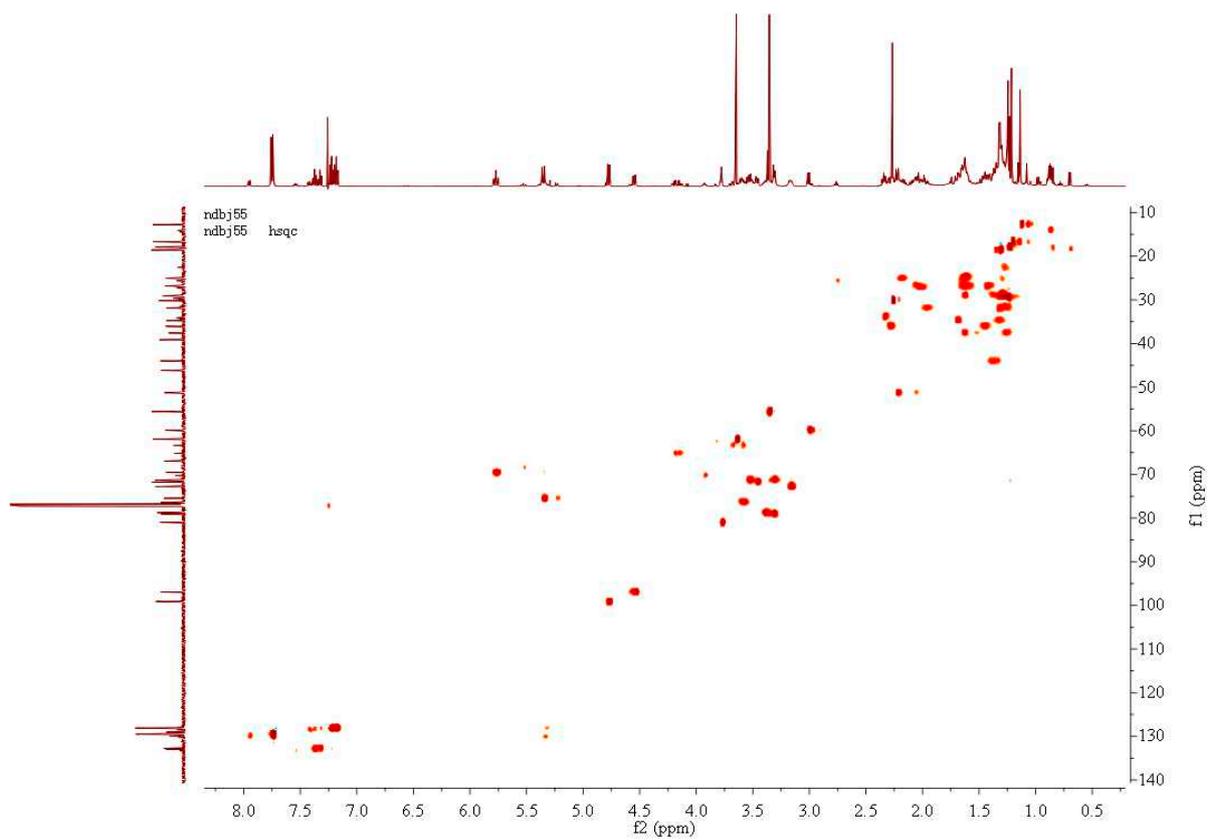


Figure S12 HSQC spectrum of compound **2**

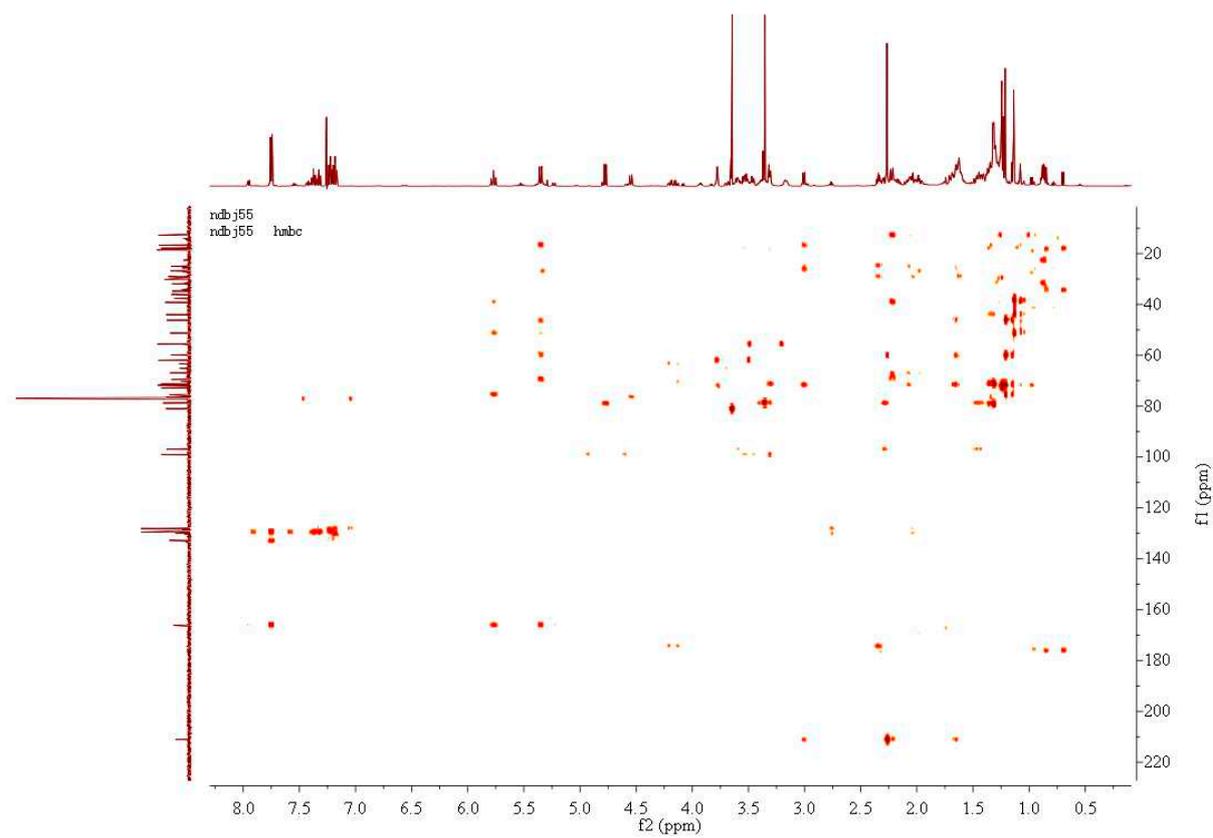


Figure S13 HMBC spectrum of compound **2**

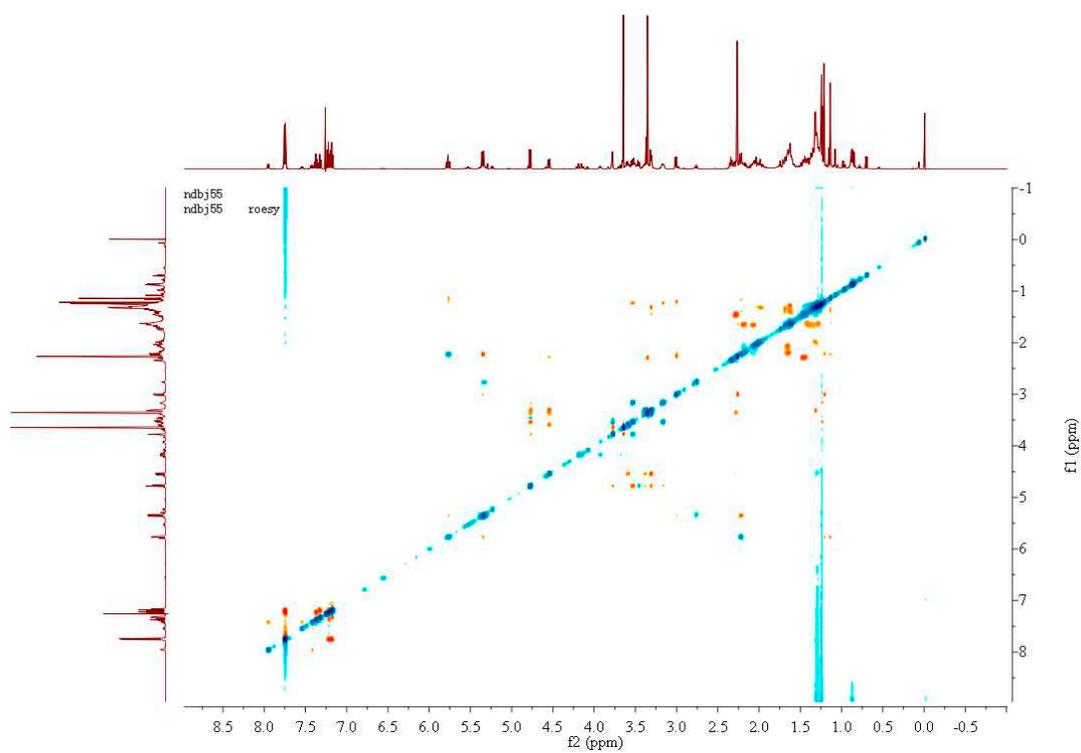


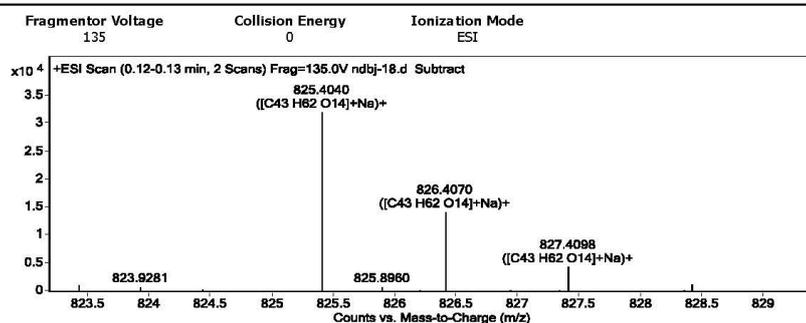
Figure S14 ROESY spectrum of compound **2**

Qualitative Analysis Report

Data Filename	ndbj-18.d	Sample Name	ndbj-18
Sample Type	Sample	Position	P1-B1
Instrument Name	Instrument 1	User Name	
Acq Method	s.m	Acquired Time	12/25/2020 3:23:45 PM
IRM Calibration Status	Success	DA Method	Default.m
Comment			

Sample Group	Info.
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.05.01 (B5125.2)

User Spectra



Peak List

m/z	z	Abund	Formula	Ion
347.2216	1	6409.36		
365.2326	1	7015		
499.2692	1	48087.03		
500.2729	1	15768.36		
691.3668	1	15865.02		
692.3689	1	6742.21		
820.4472	1	8841.49		
825.404	1	32109.97	C43 H62 O14	(M+Na)+
826.407	1	14198.66	C43 H62 O14	(M+Na)+
1359.7437	1	5780.44		

Formula Calculator Element Limits

Element	Min	Max
C	3	120
H	0	240
O	0	30

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C43 H62 O14	802.4140	825.4032	825.4040	-0.80	-0.97	13.0000

--- End Of Report ---

Figure S15 HRESIMS spectrum of compound 3

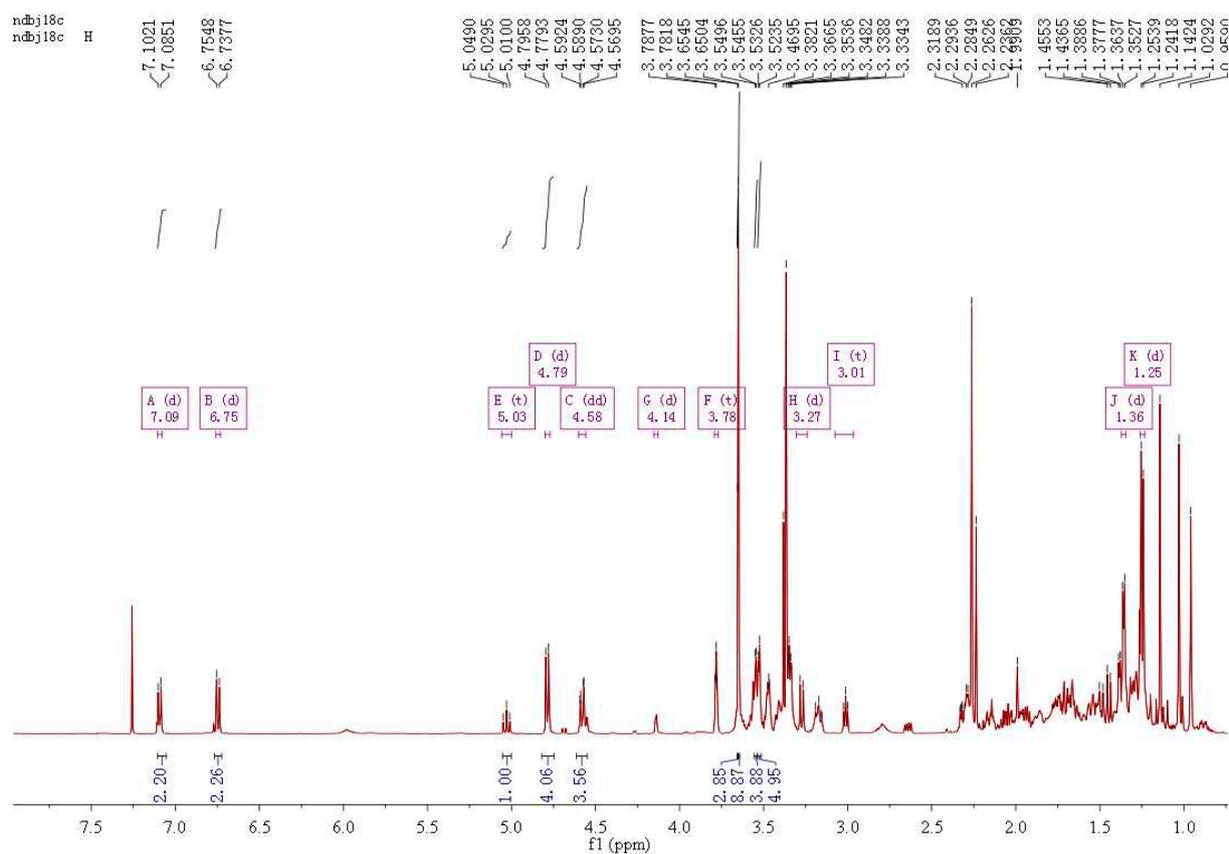


Figure S16 $^1\text{H-NMR}$ (500 MHz, CDCl_3) spectrum of compound 3

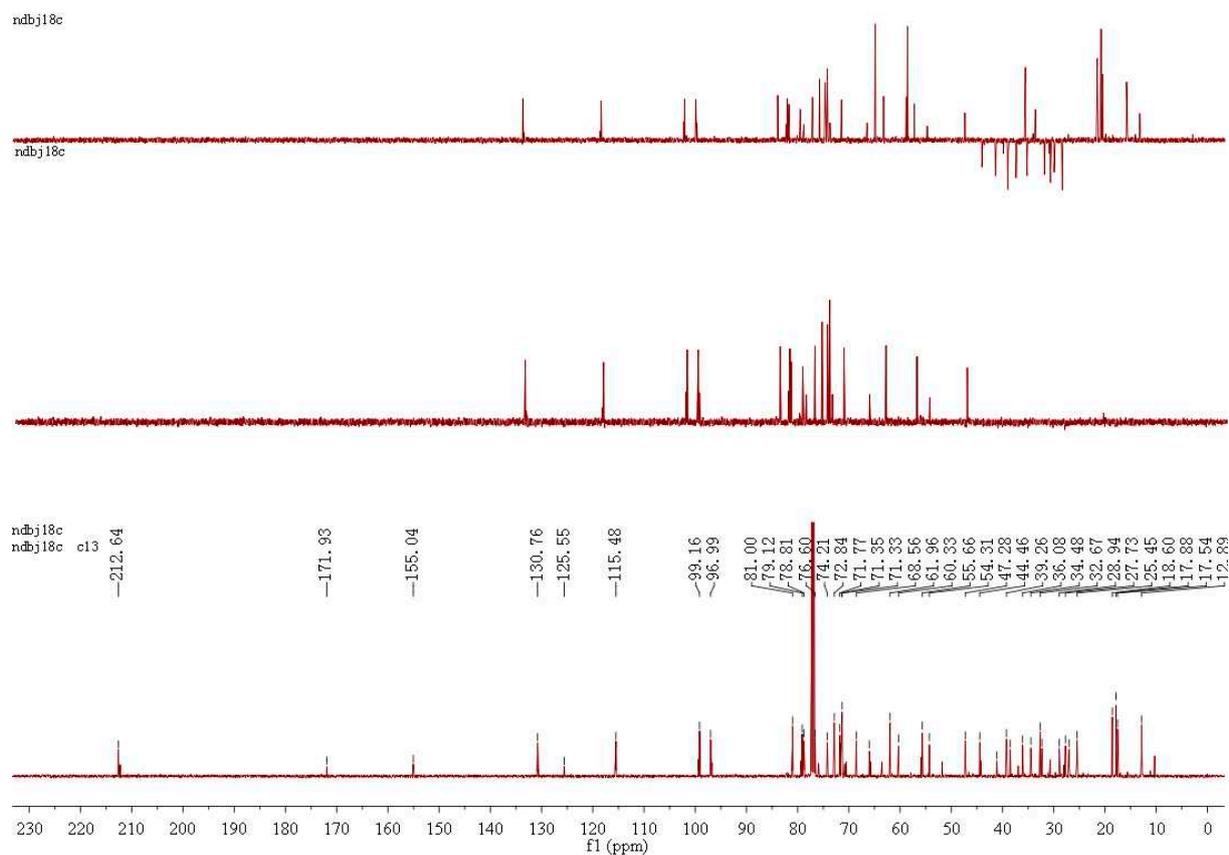


Figure S17 $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) and DEPT spectrum of compound 3

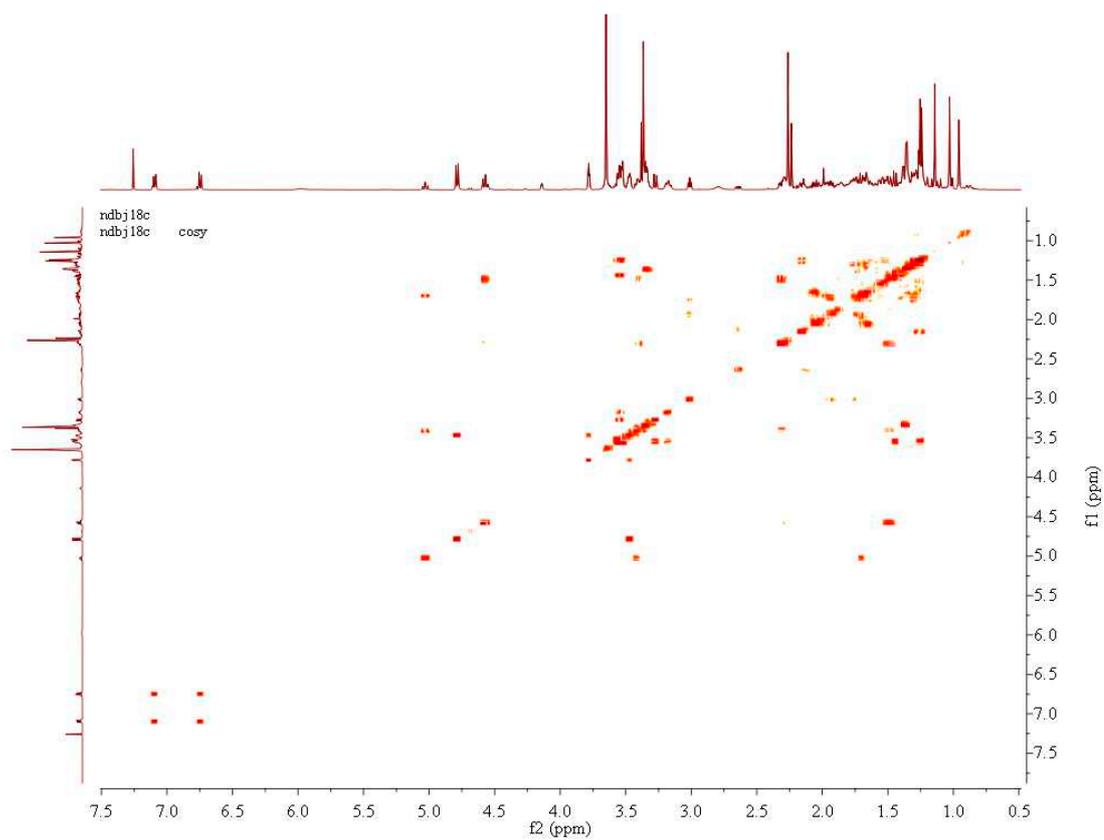


Figure S18 ^1H - ^1H COSY spectrum of compound **3**

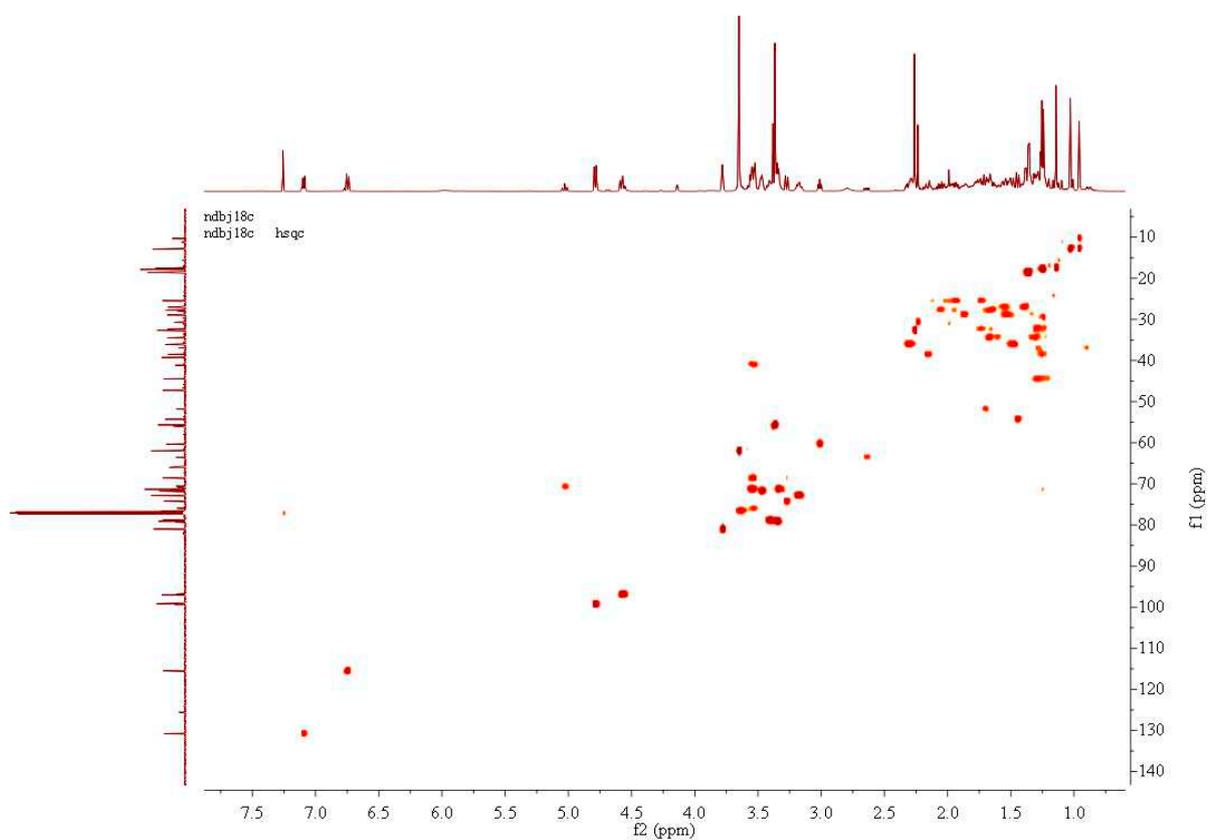


Figure S19 HSQC spectrum of compound **3**

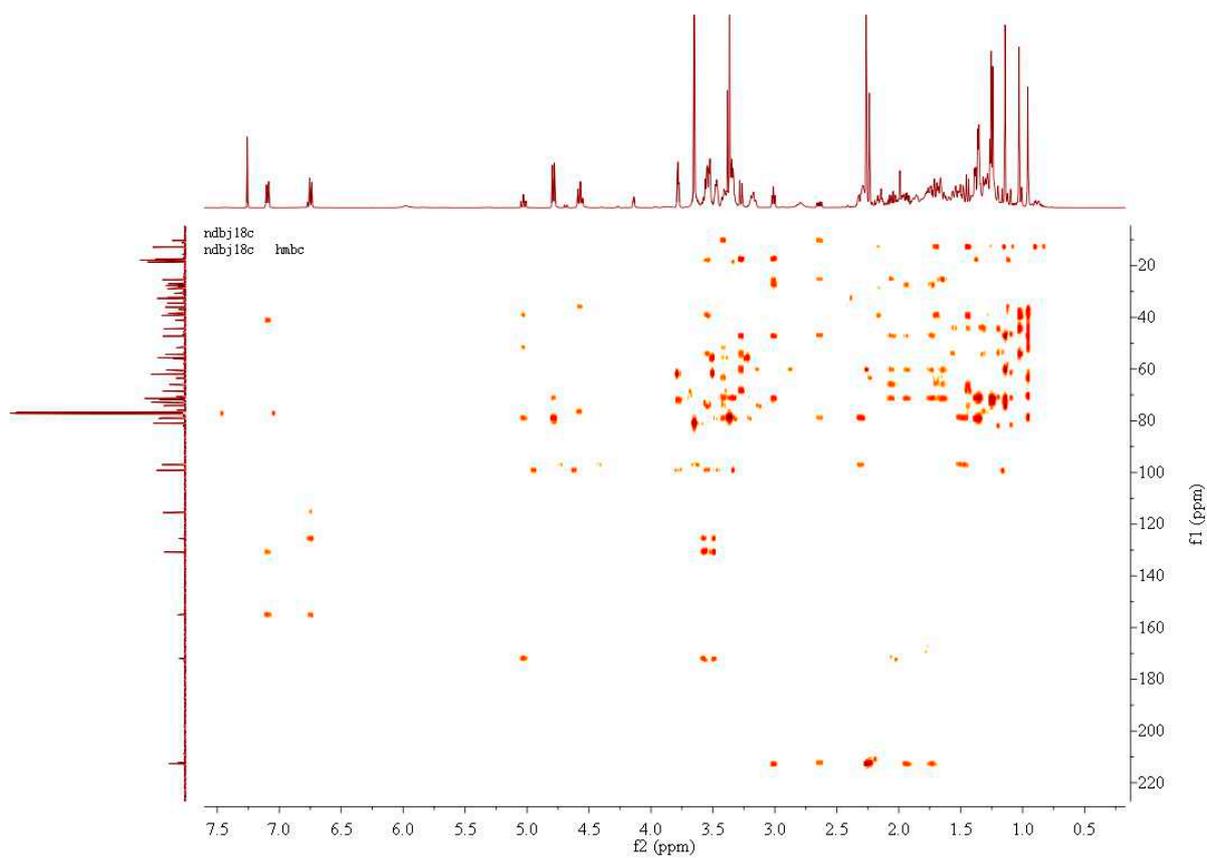


Figure S20 HMBC Spectrum of Compound **3**

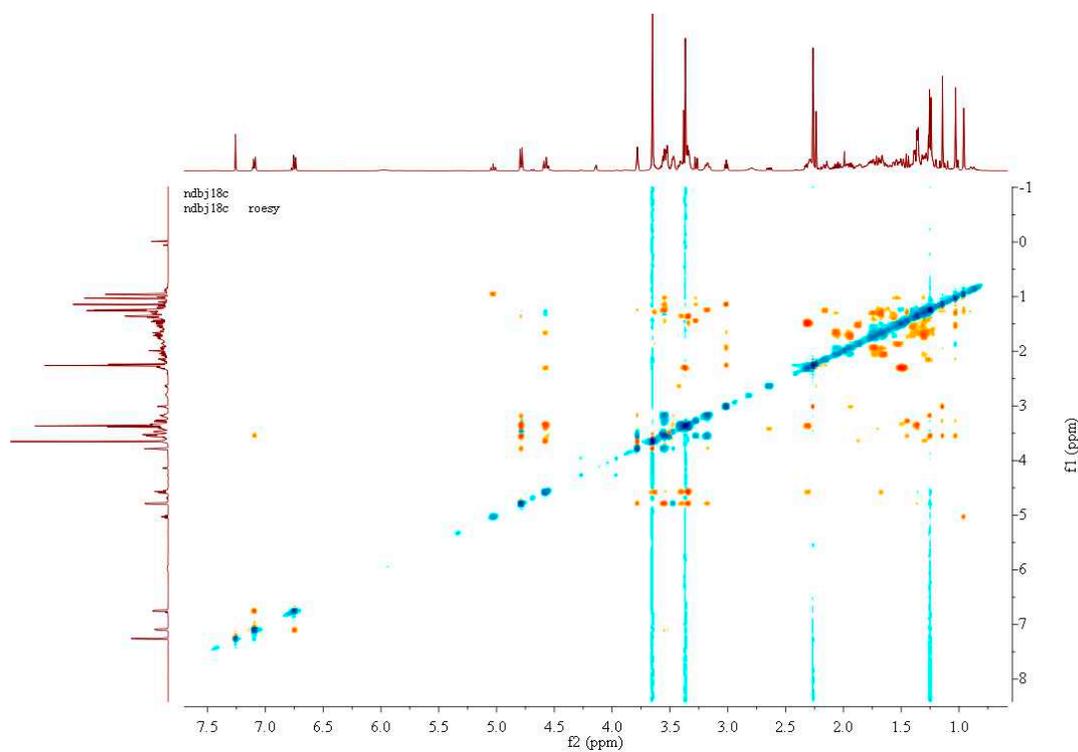


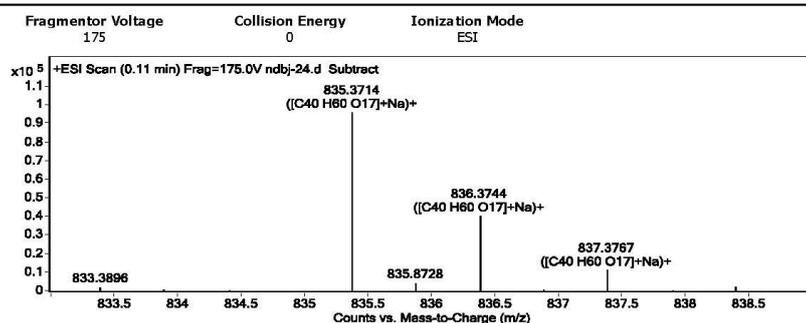
Figure S21 ROESY spectrum of compound **3**

Qualitative Analysis Report

Data Filename	ndbj-24.d	Sample Name	ndbj-24
Sample Type	Sample	Position	P1-A2
Instrument Name	Instrument 1	User Name	
Acq Method	s.m	Acquired Time	11/26/2020 3:37:32 PM
IRM Calibration Status	Success	DA Method	Default.m
Comment			

Sample Group	Info.
Acquisition SW	6200 series TOF/6500 series
Version	Q-TOF B.05.01 (B5125.2)

User Spectra



Peak List

m/z	z	Abund	Formula	Ion
830.4153	1	48589.8		
831.4181	1	21074.41		
835.3714	1	96274.69	C40 H60 O17	(M+Na)+
836.3744	1	40454.15	C40 H60 O17	(M+Na)+
953.4694	1	16813.27		
955.4812	1	11500.18		
995.4805	1	33026.6		
996.4838	1	16801.98		
1647.7493	1	24481.94		
1648.7539	1	21411.65		

Formula Calculator Element Limits

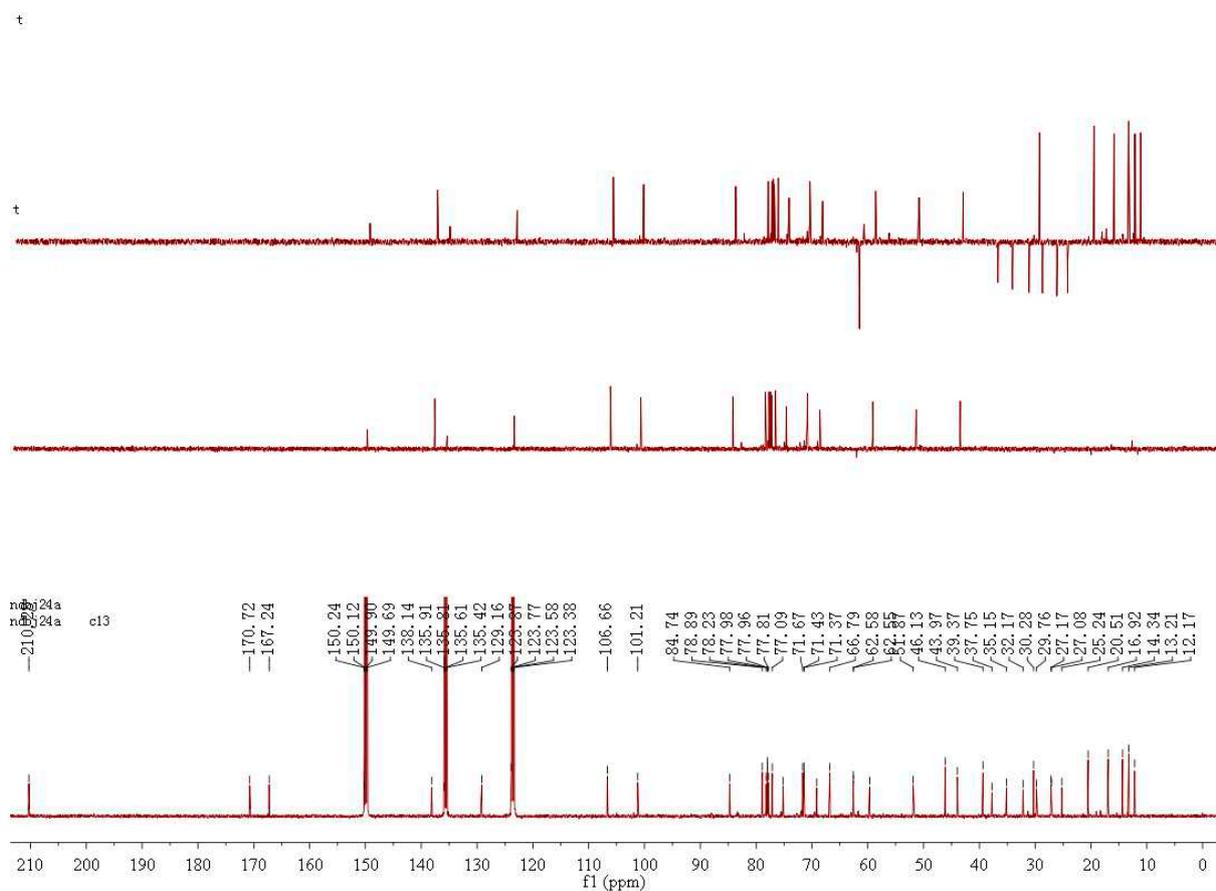
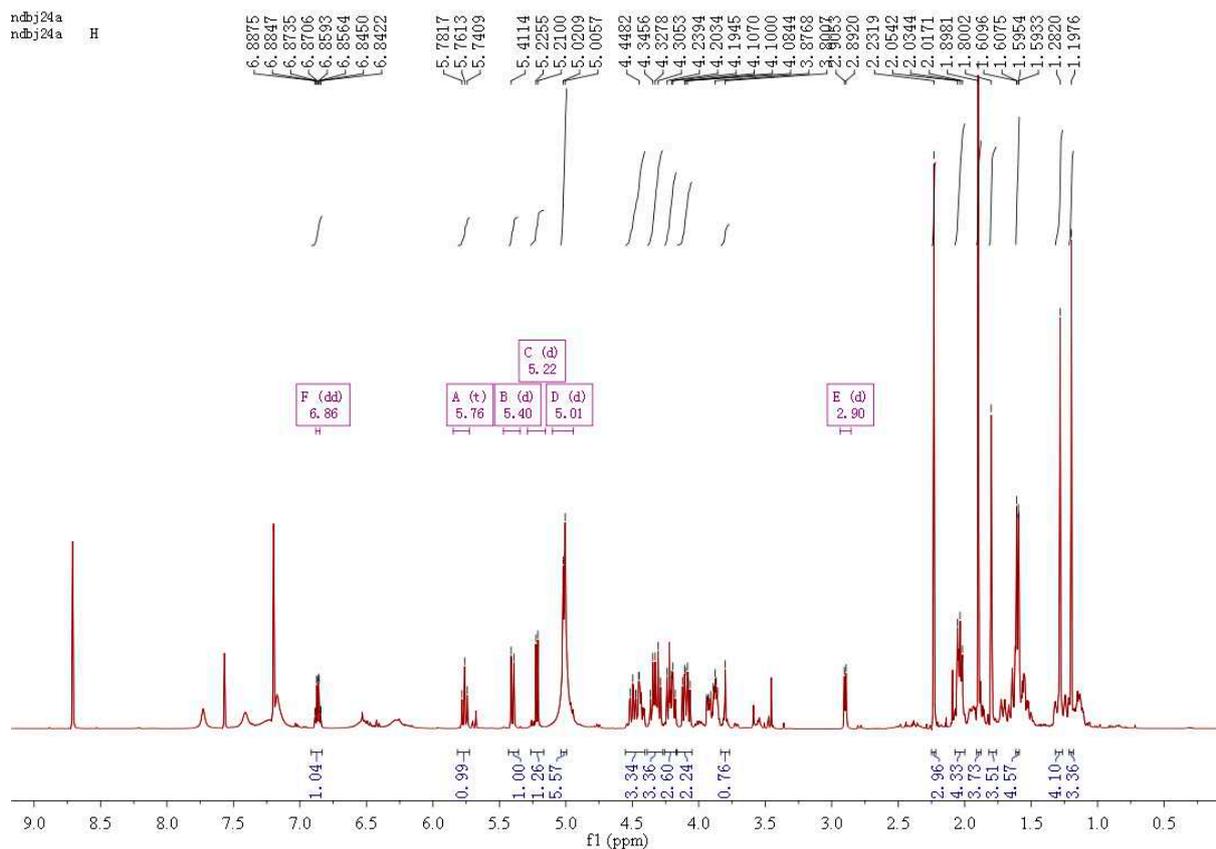
Element	Min	Max
C	3	60
H	0	120
O	0	30

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C40 H60 O17	812.3831	835.3723	835.3714	0.90	1.08	11.0000

--- End Of Report ---

Figure S22 HRESIMS spectrum of compound 4



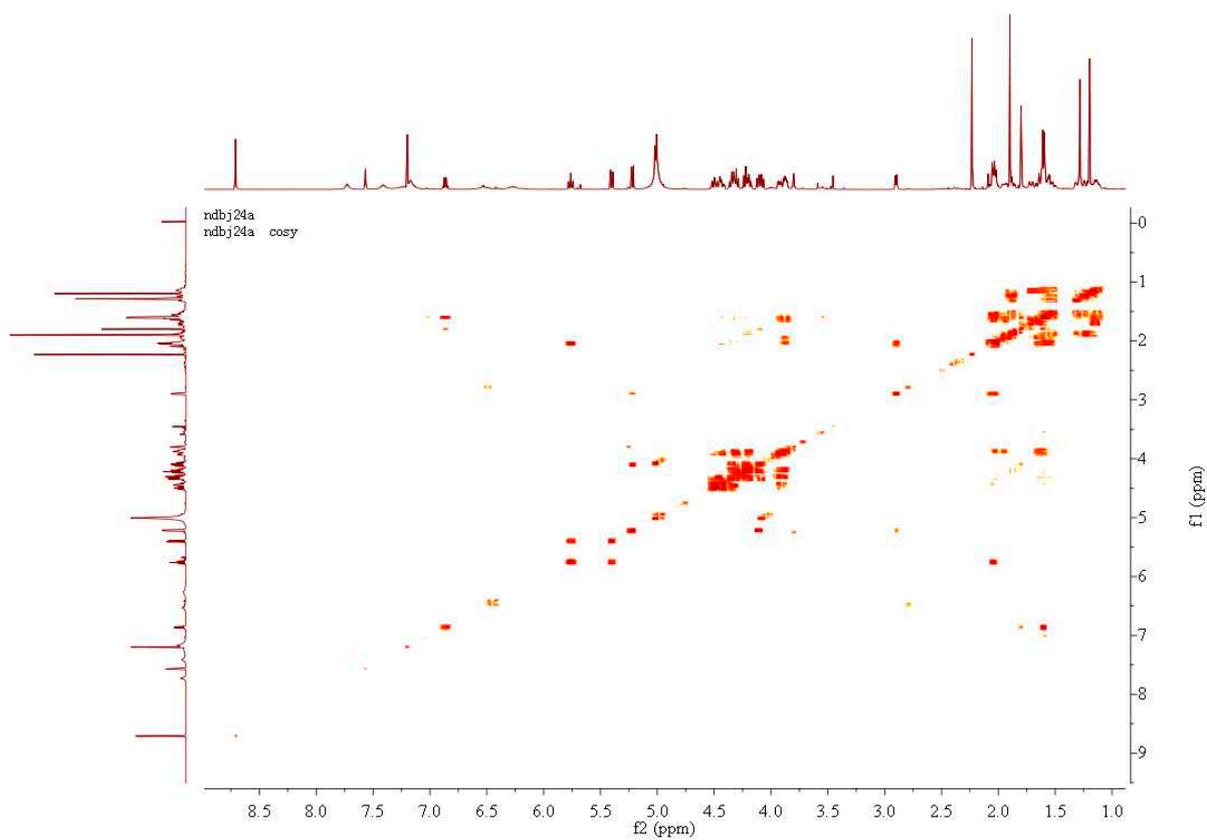


Figure S25 ^1H - ^1H COSY spectrum of compound 4

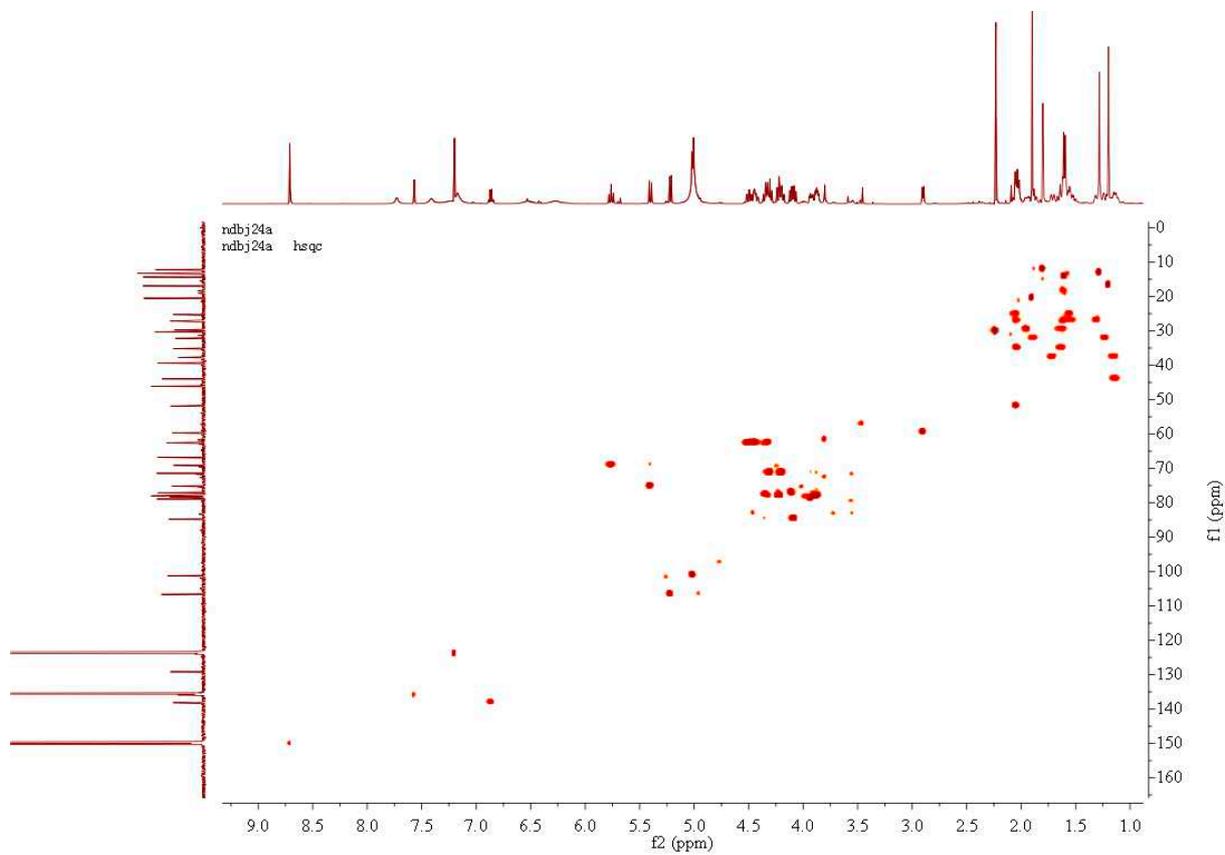


Figure S26 HSQC spectrum of compound 4

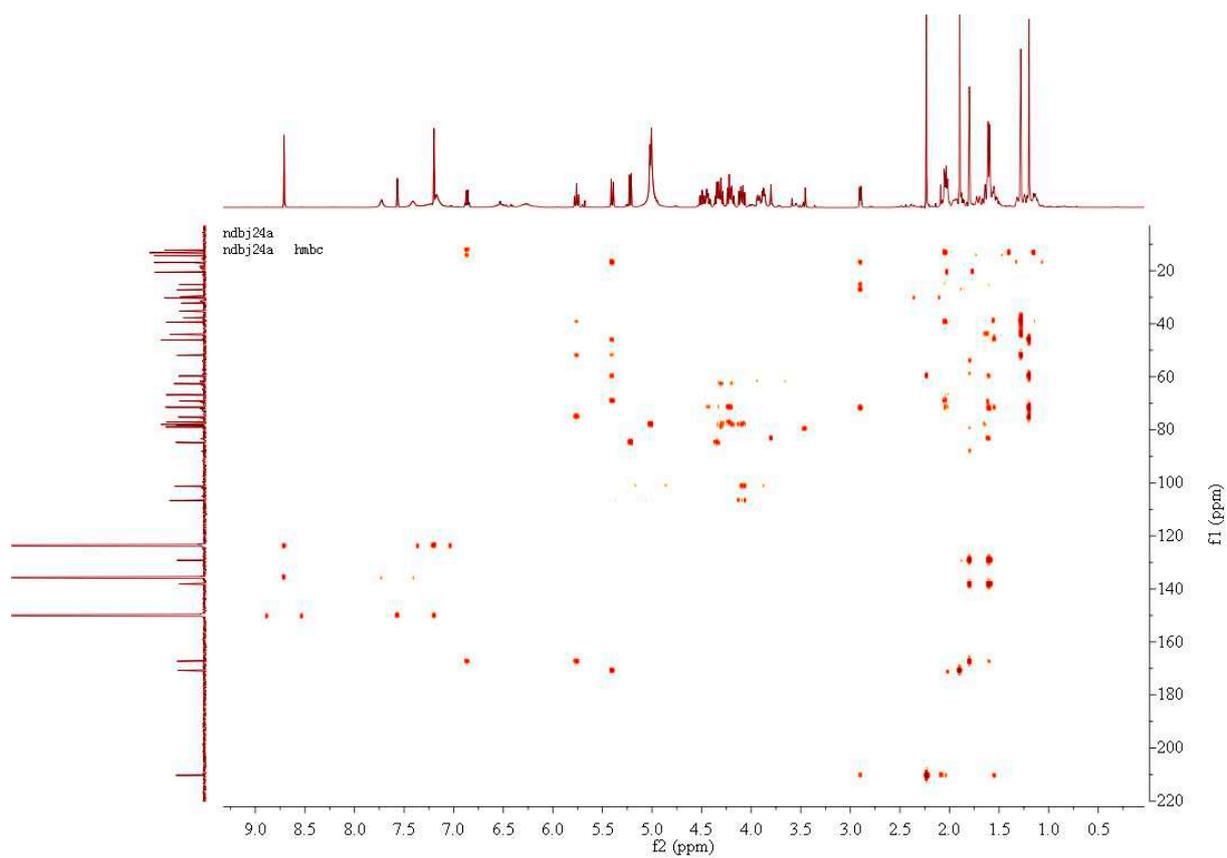


Figure S27 The HMBC spectrum of compound **4**

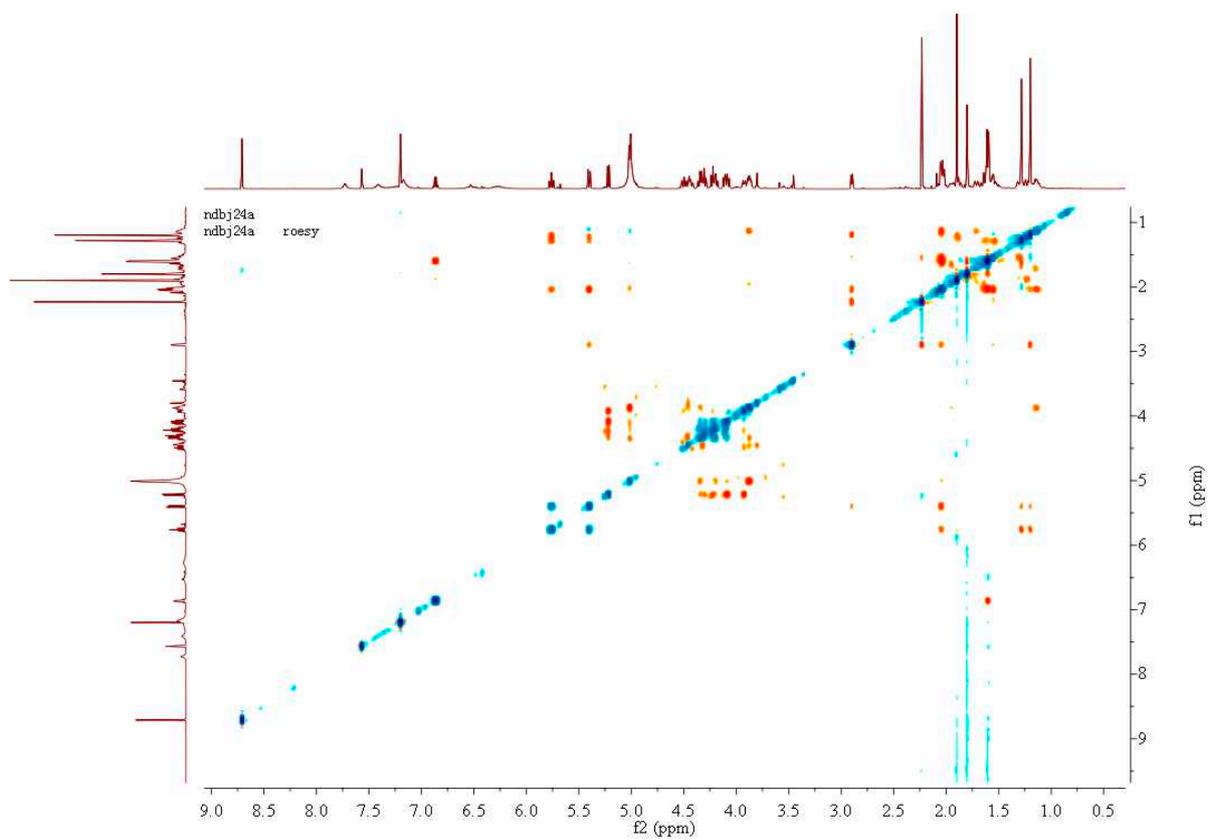


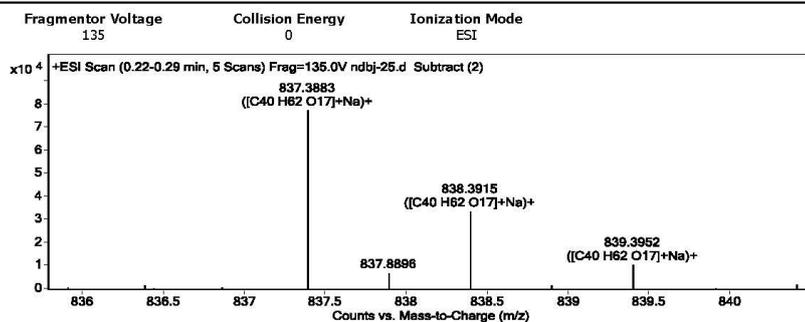
Figure S28 ROESY spectrum of compound **4**

Qualitative Analysis Report

Data Filename	ndbj-25.d	Sample Name	ndbj-25
Sample Type	Sample	Position	P1-C1
Instrument Name	Instrument 1	User Name	
Acq Method	s.m	Acquired Time	11/27/2020 3:26:00 PM
IRM Calibration Status	Success	DA Method	Default.m
Comment			

Sample Group	Info.
Acquisition SW	6200 series TOF/6500 series
Version	Q-TOF B.05.01 (B5125.2)

User Spectra



Peak List

m/z	z	Abund	Formula	Ion
112.1875		30322.51		
832.4326	1	52515.62		
837.3883	1	77573.45	C40 H62 O17	(M+Na)+
838.3915	1	33777	C40 H62 O17	(M+Na)+
853.4192	1	148654.5		
854.4221	1	65545.1		
955.4875	1	100281.19		
956.4909	1	50290.4		
1651.7858	1	27988.96		
1652.7894	1	24435.12		

Formula Calculator Element Limits

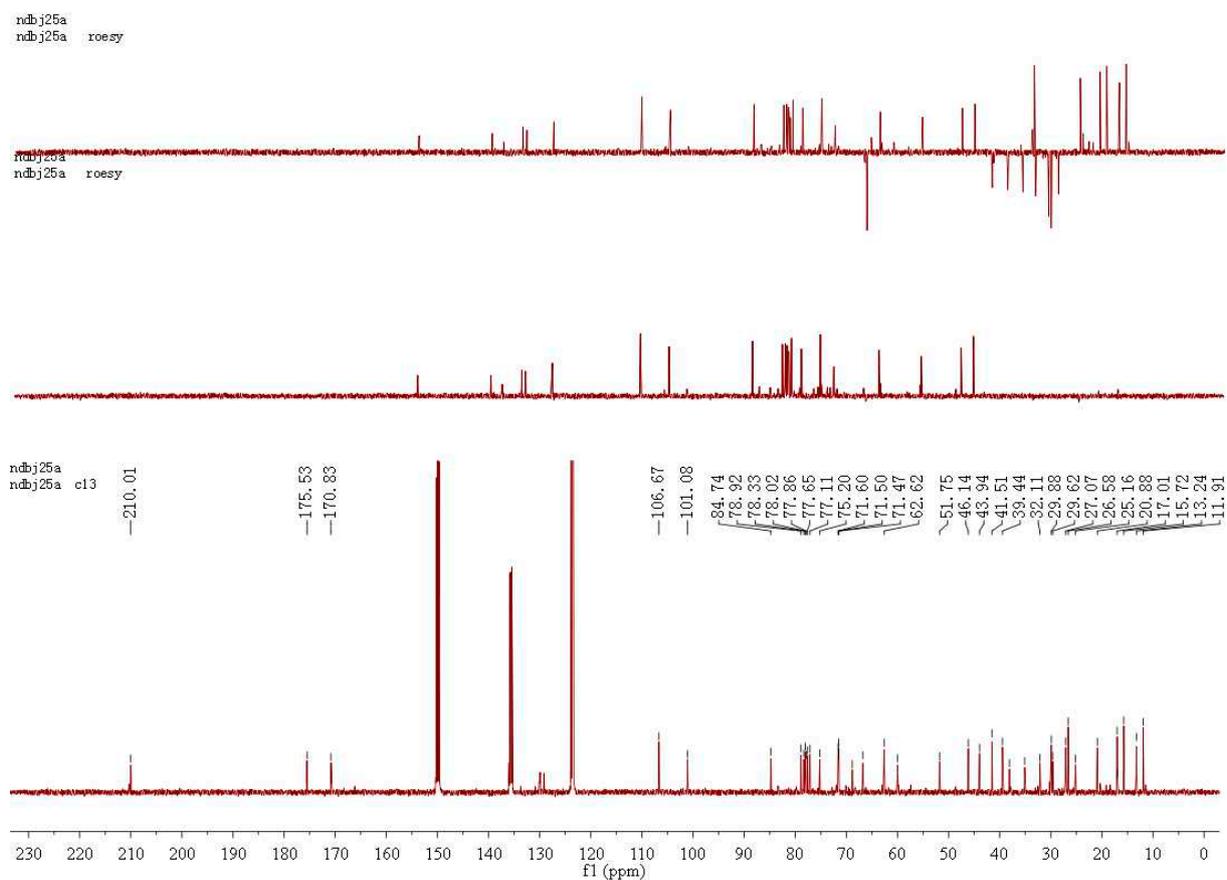
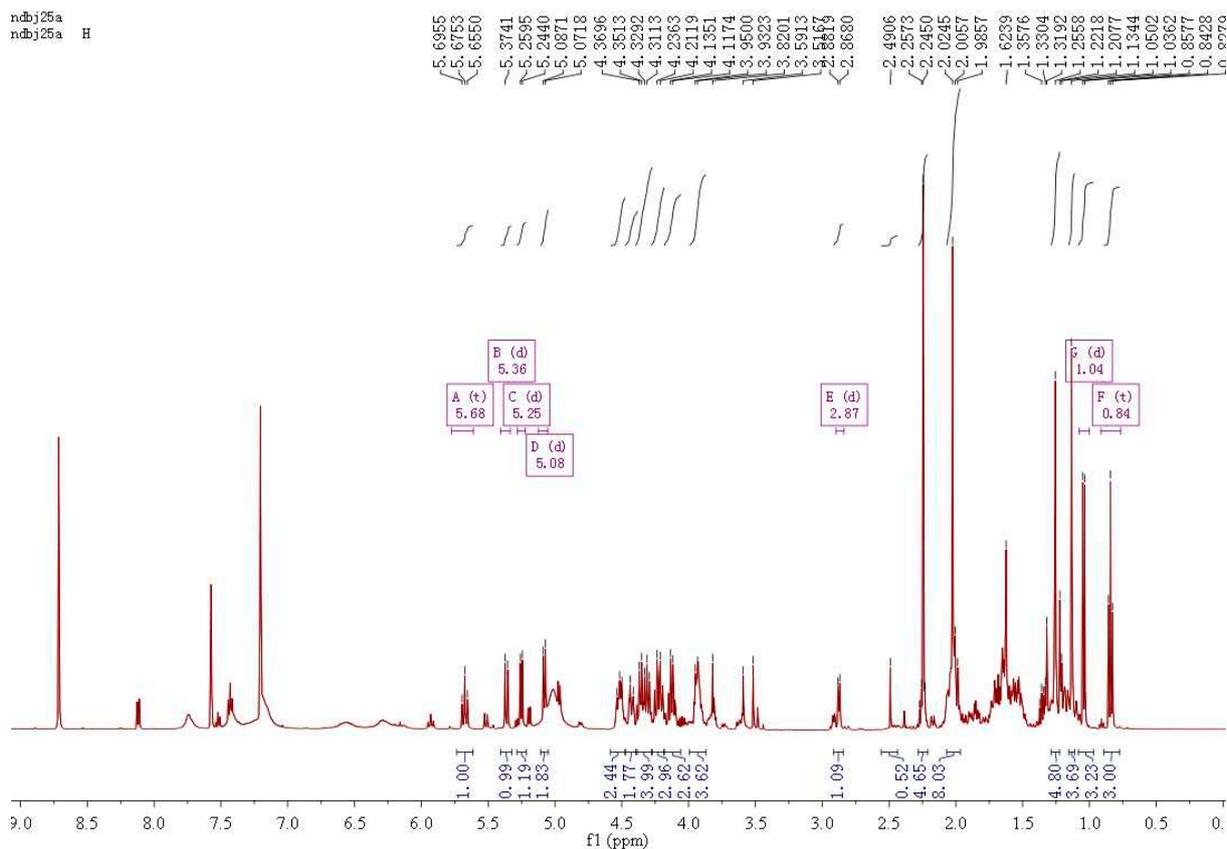
Element	Min	Max
C	3	60
H	0	120
O	0	30

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C40 H62 O17	814.3987	837.3879	837.3883	-0.40	-0.48	10.0000

--- End Of Report ---

Figure S29 HRESIMS spectrum of compound 5



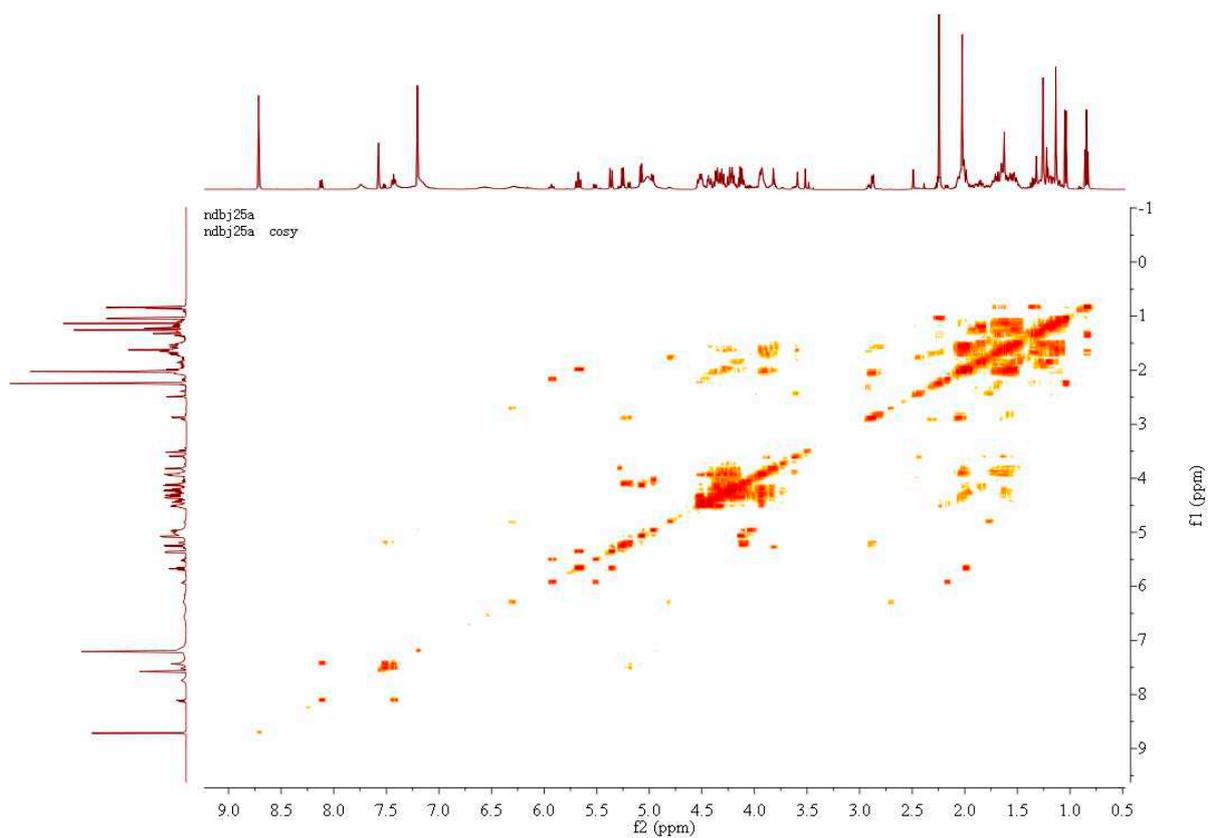


Figure S32 ^1H - ^1H COSY spectrum of compound **5**

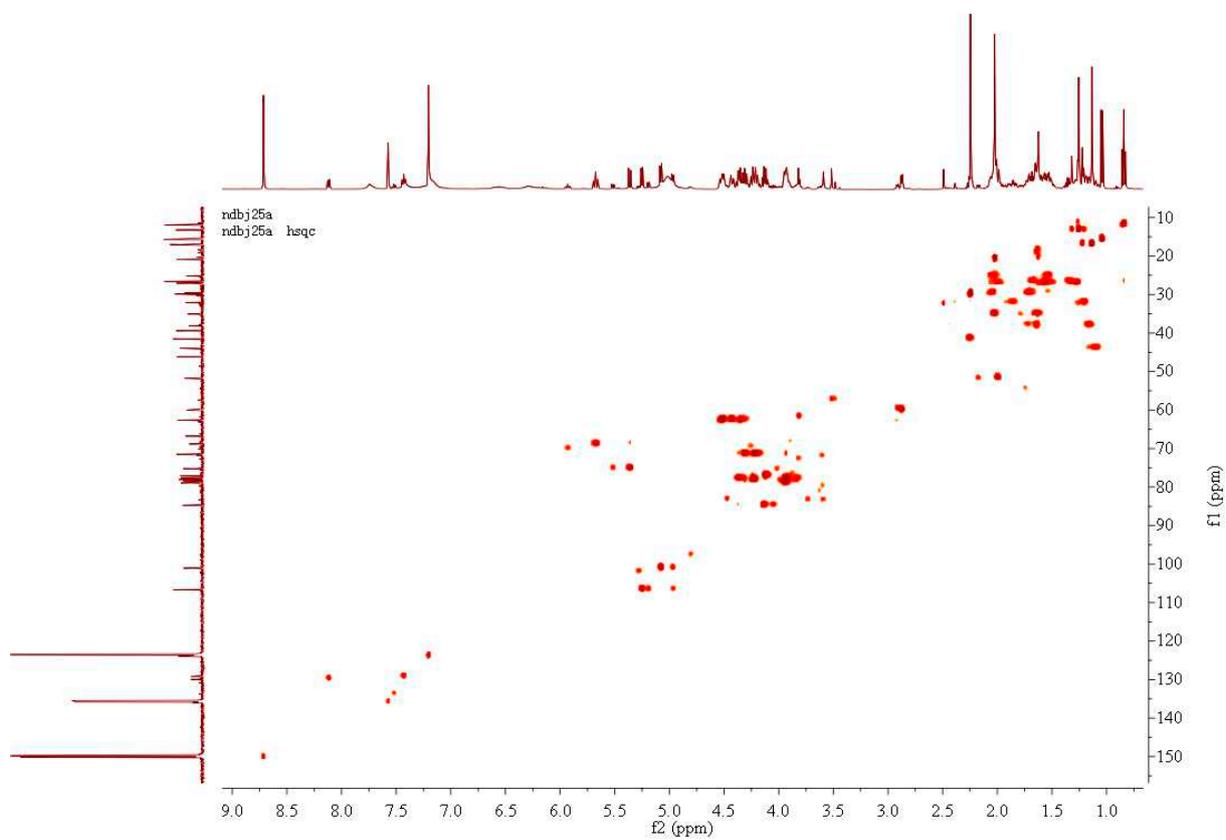


Figure S33 HSQC spectrum of compound **5**

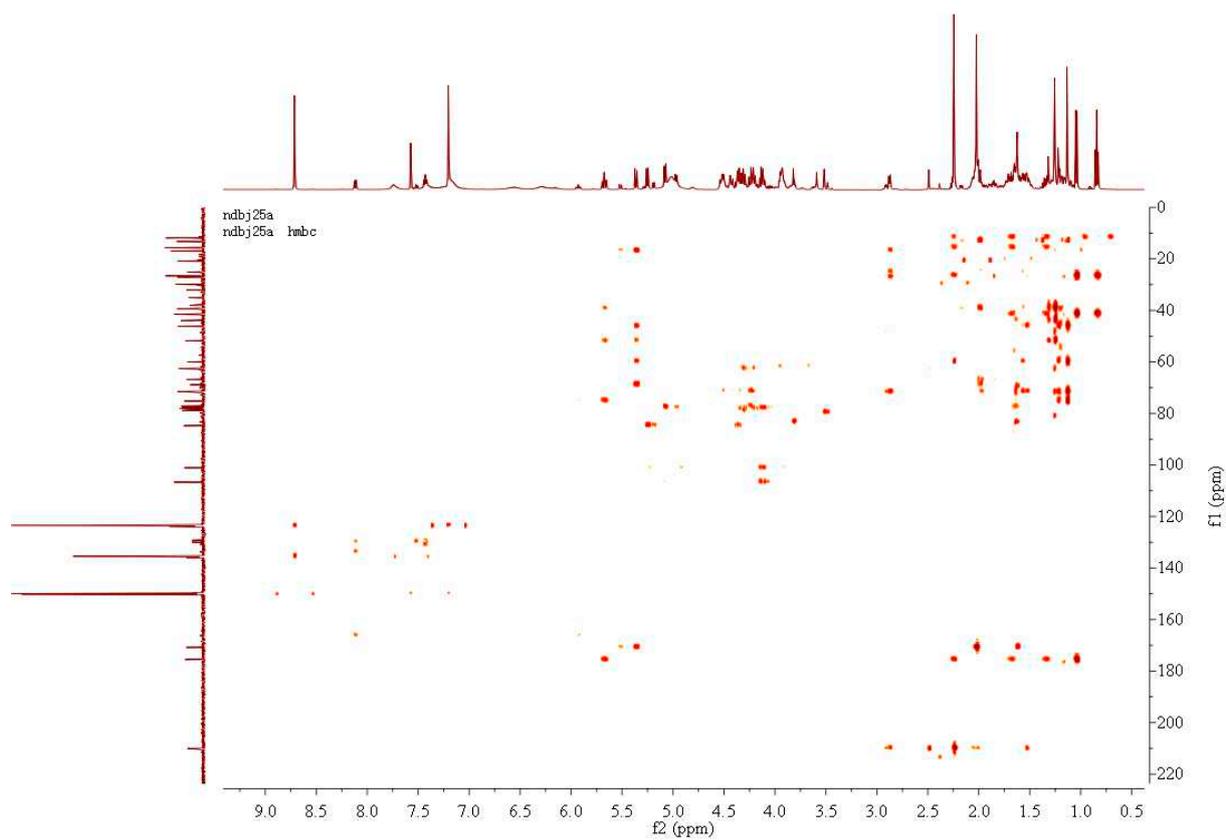


Figure S34 HMBC spectrum of compound **5**

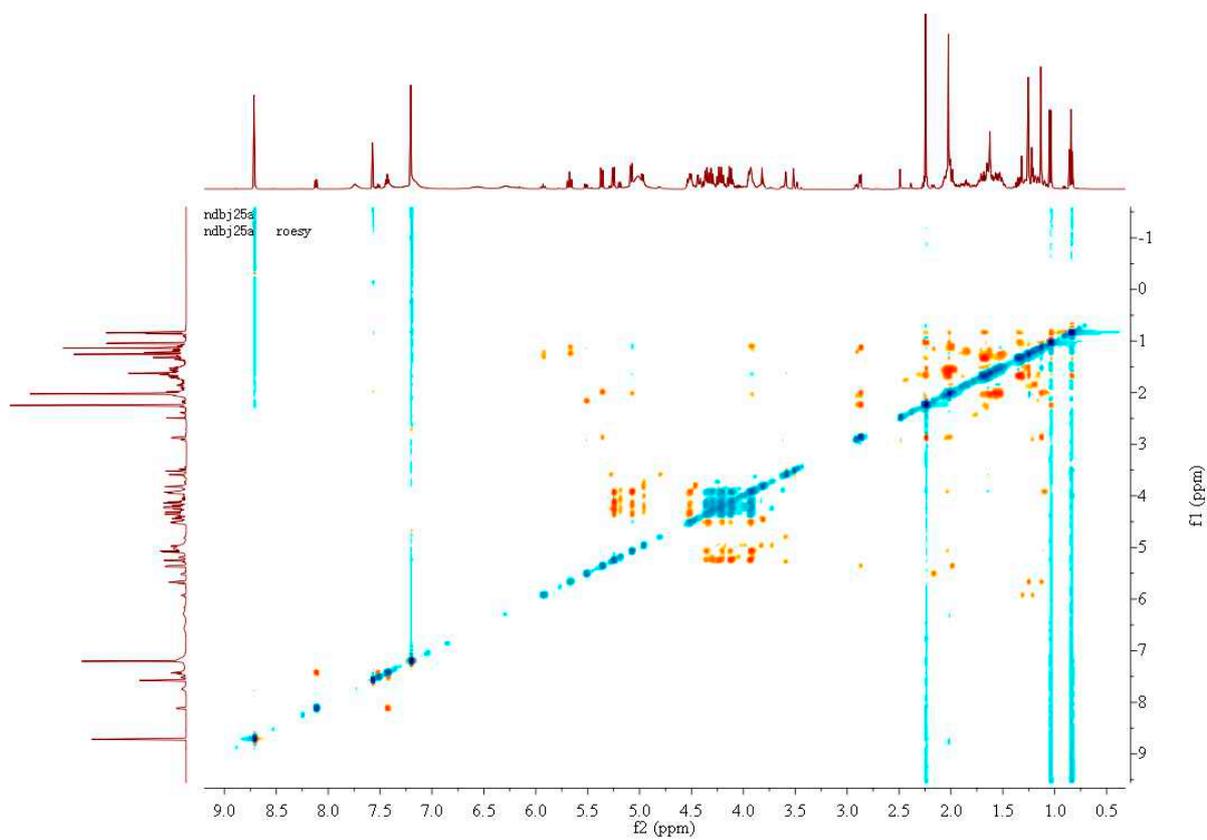


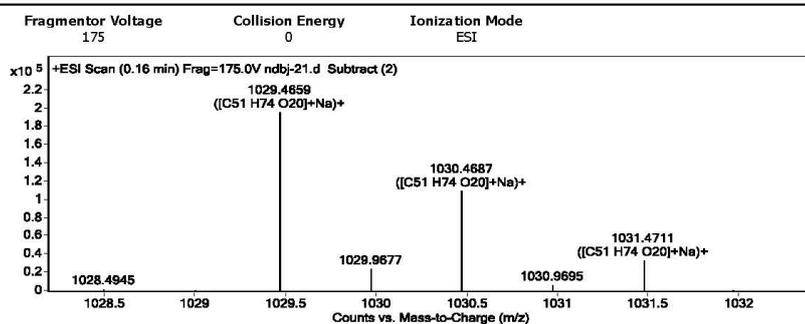
Figure S35 ROESY spectrum of compound **5**

Qualitative Analysis Report

Data Filename	ndbj-21.d	Sample Name	ndbj-21
Sample Type	Sample	Position	P1-A1
Instrument Name	Instrument 1	User Name	
Acq Method	s.m	Acquired Time	11/26/2020 3:36:21 PM
IRM Calibration Status	Success	DA Method	Default.m
Comment			

Sample Group	Info.
Acquisition SW	6200 series TOF/6500 series
Version	Q-TOF B.05.01 (B5125.2)

User Spectra



Peak List

m/z	z	Abund	Formula	Ion
541.2782	1	12301.04		
937.4756	1	40547.91		
938.4793	1	18861.75		
1024.5098	1	42988.28		
1025.5133	1	23825.68		
1029.4659	1	196133.84	C51 H74 O20	(M+Na)+
1029.9677	1	24163.45		
1030.4687	1	109558.27	C51 H74 O20	(M+Na)+
1031.4711	1	33537.78	C51 H74 O20	(M+Na)+
1045.4396	1	14389.06		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C51 H74 O20	1006.4773	1029.4666	1029.4659	0.70	0.68	15.0000

--- End Of Report ---

Figure S36 HRESIMS spectrum of compound 6

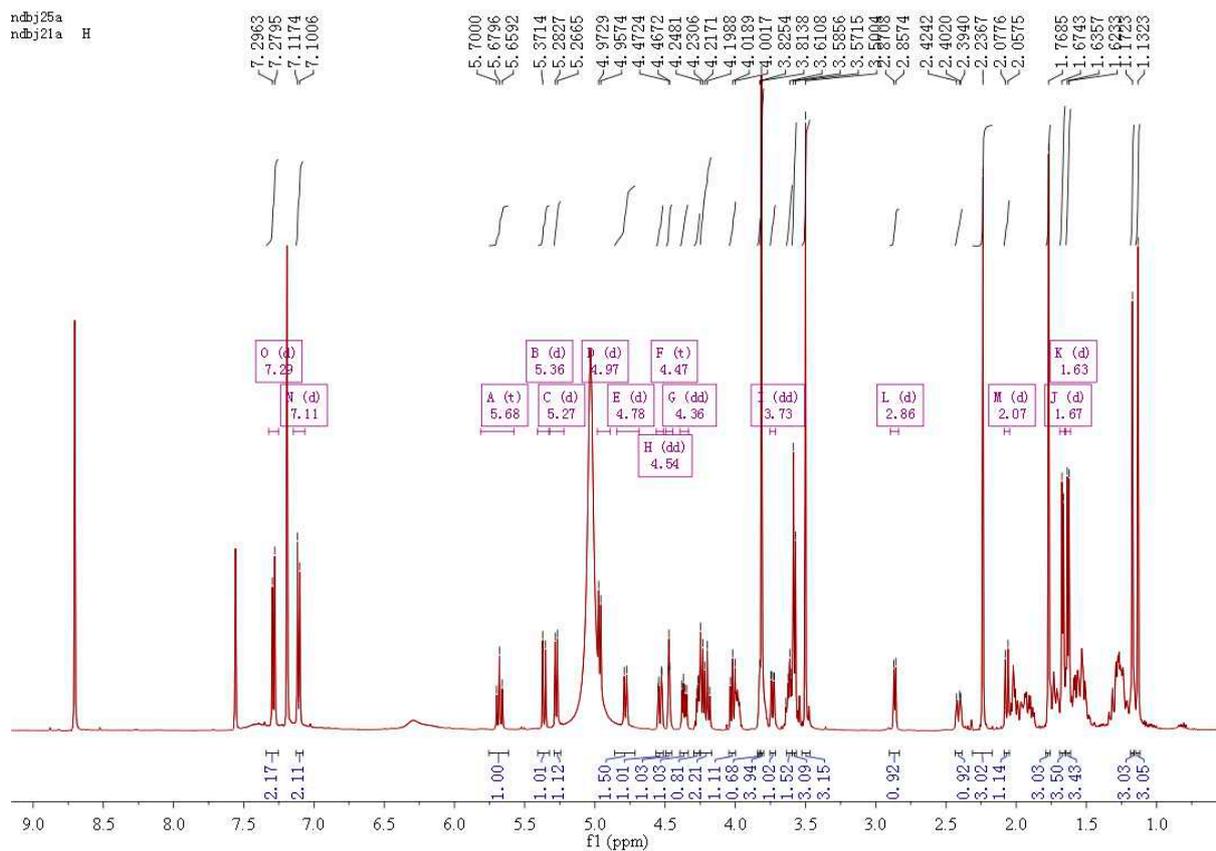


Figure S37 $^1\text{H-NMR}$ (500 MHz, pyridine- d_5) spectrum of compound **6**

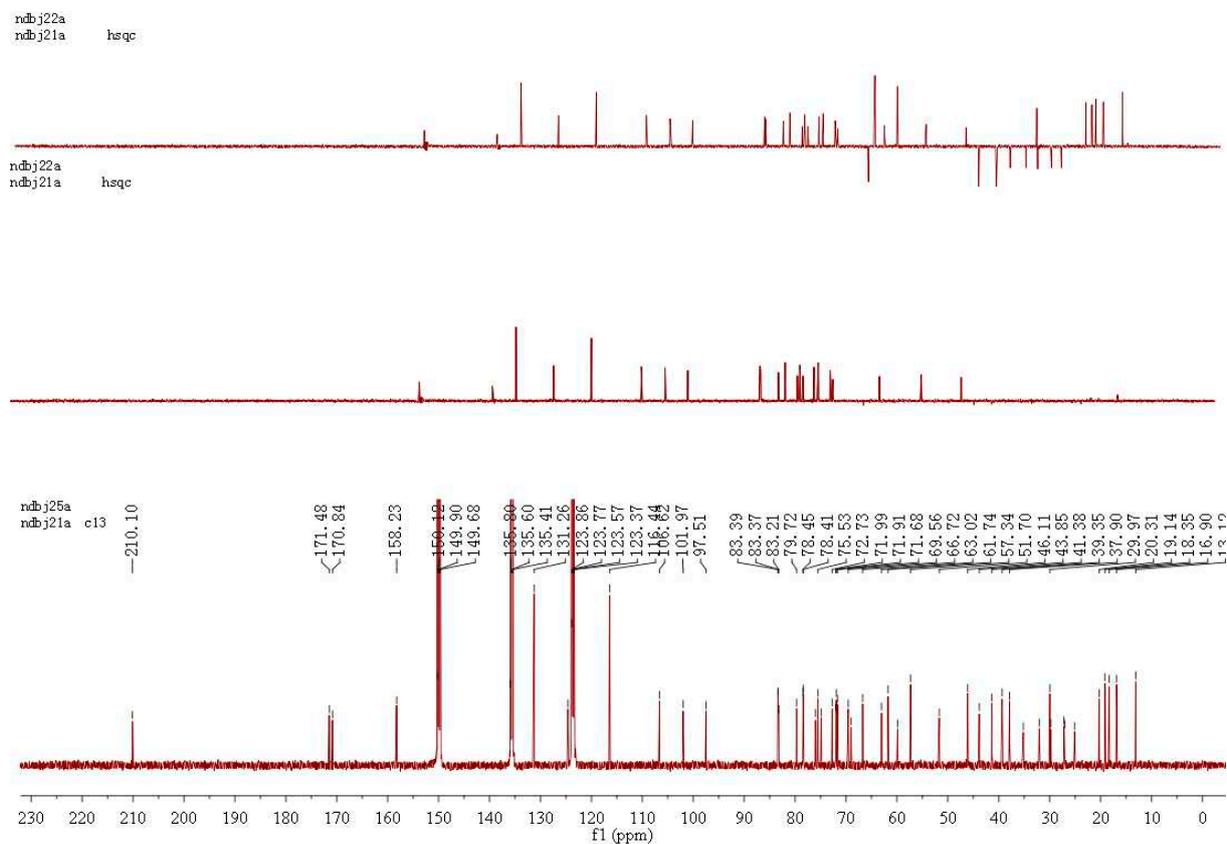


Figure S38 $^{13}\text{C-NMR}$ and DEPT (125 MHz, pyridine- d_5) spectrum of compound **6**

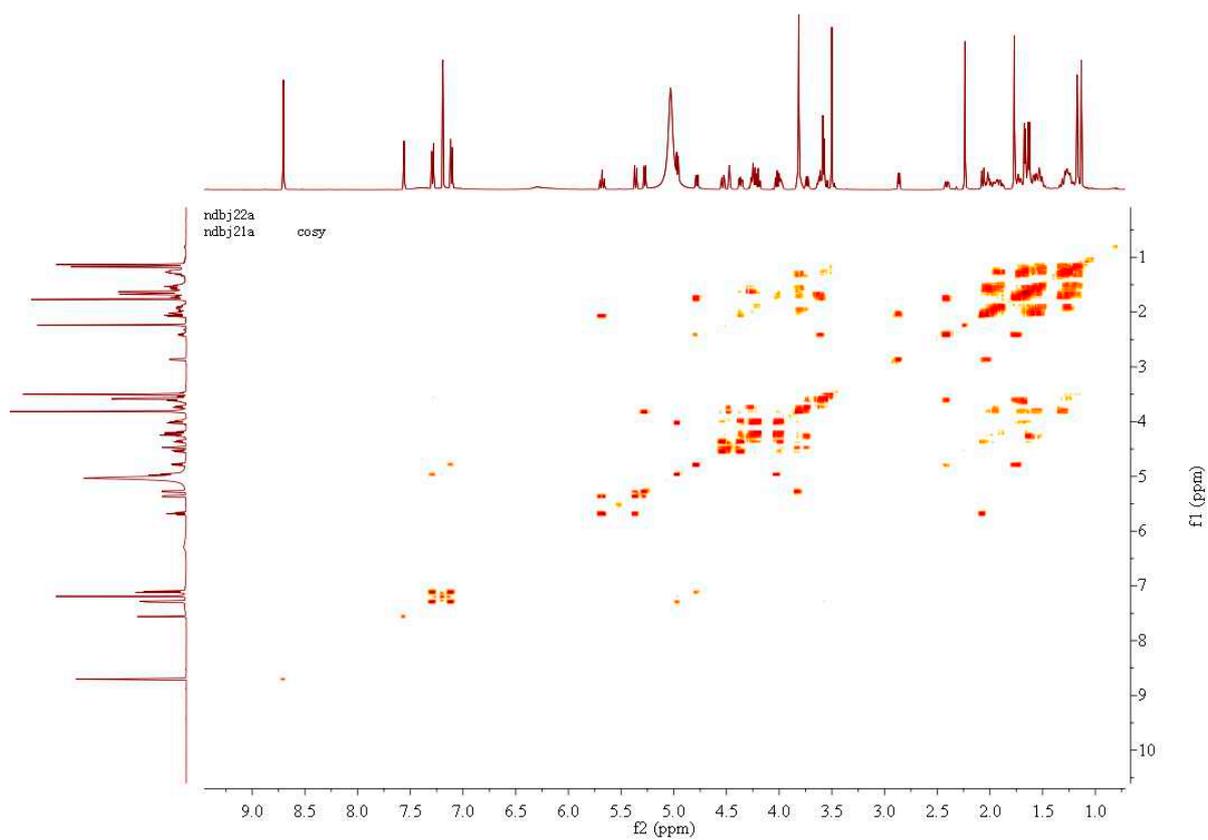


Figure S39 ^1H - ^1H COSY spectrum of compound **6**

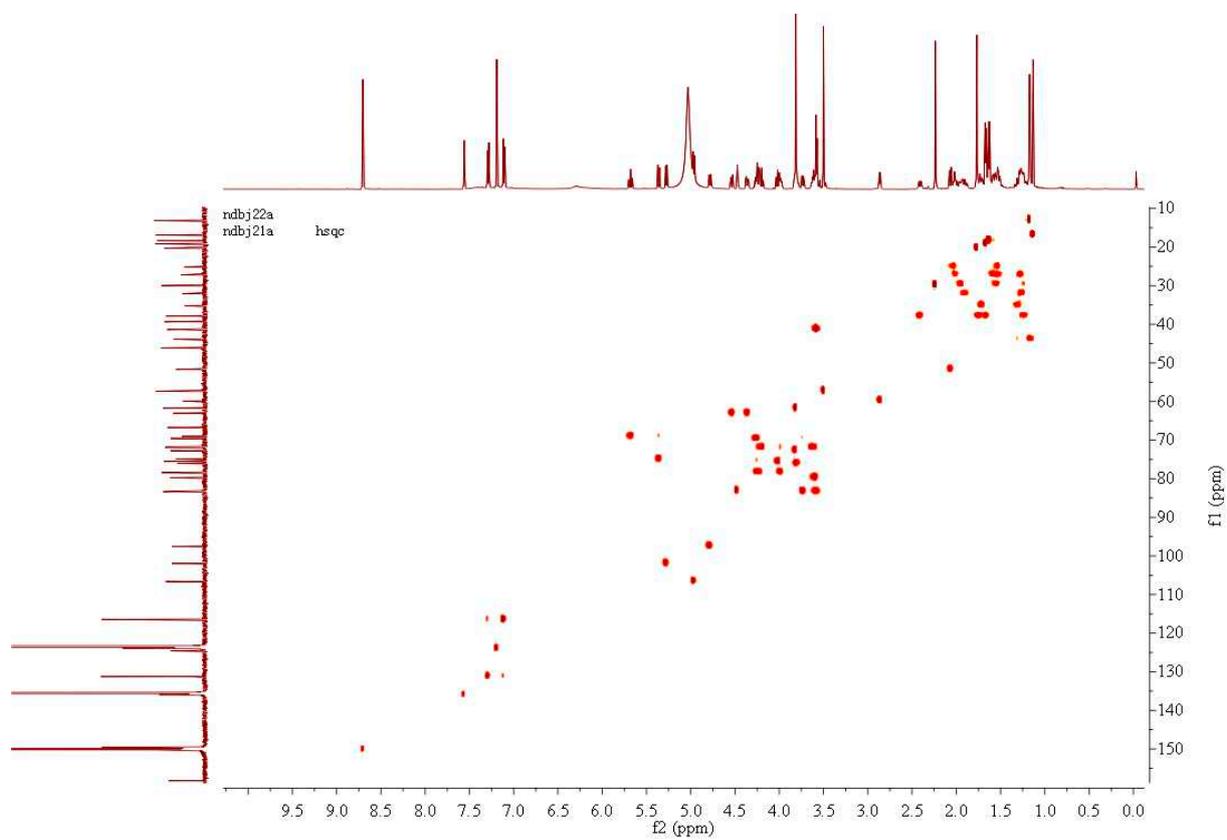


Figure S40 HSQC spectrum of compound **6**

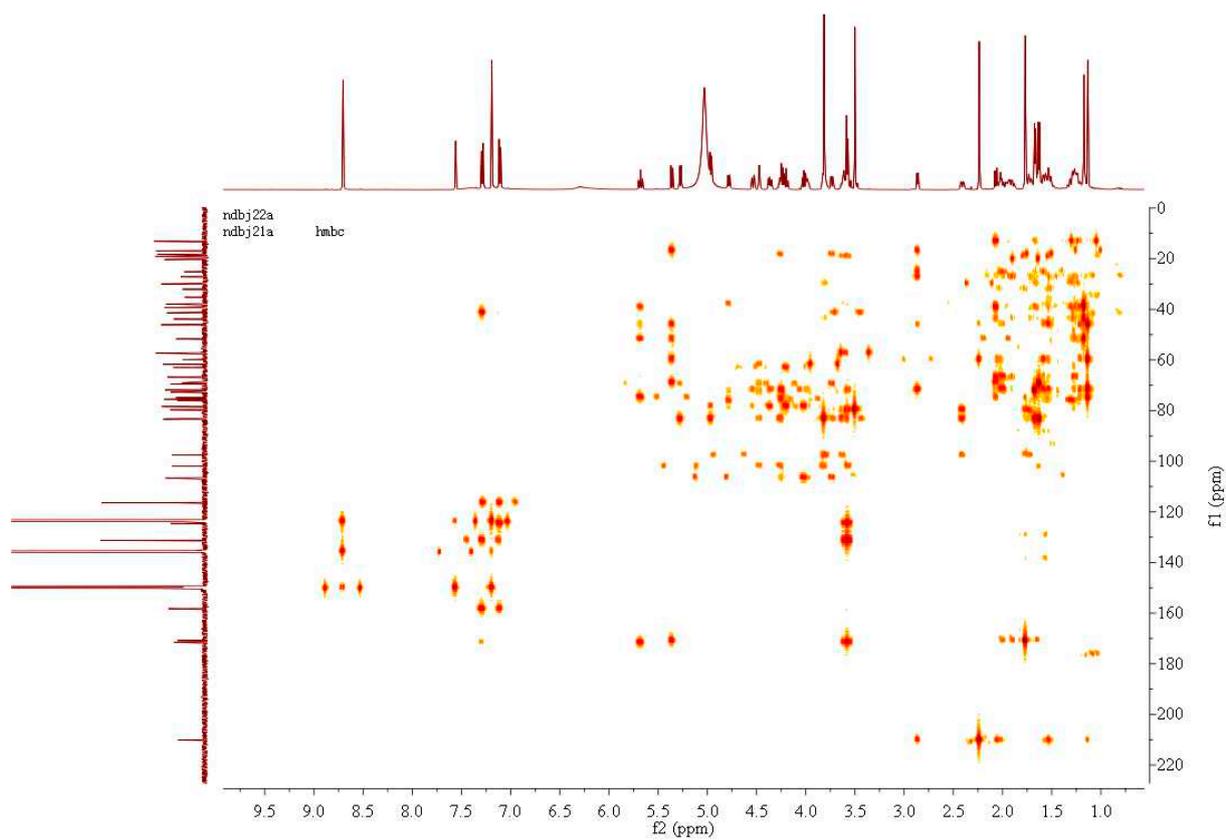


Figure S41 HMBC spectrum of compound **6**

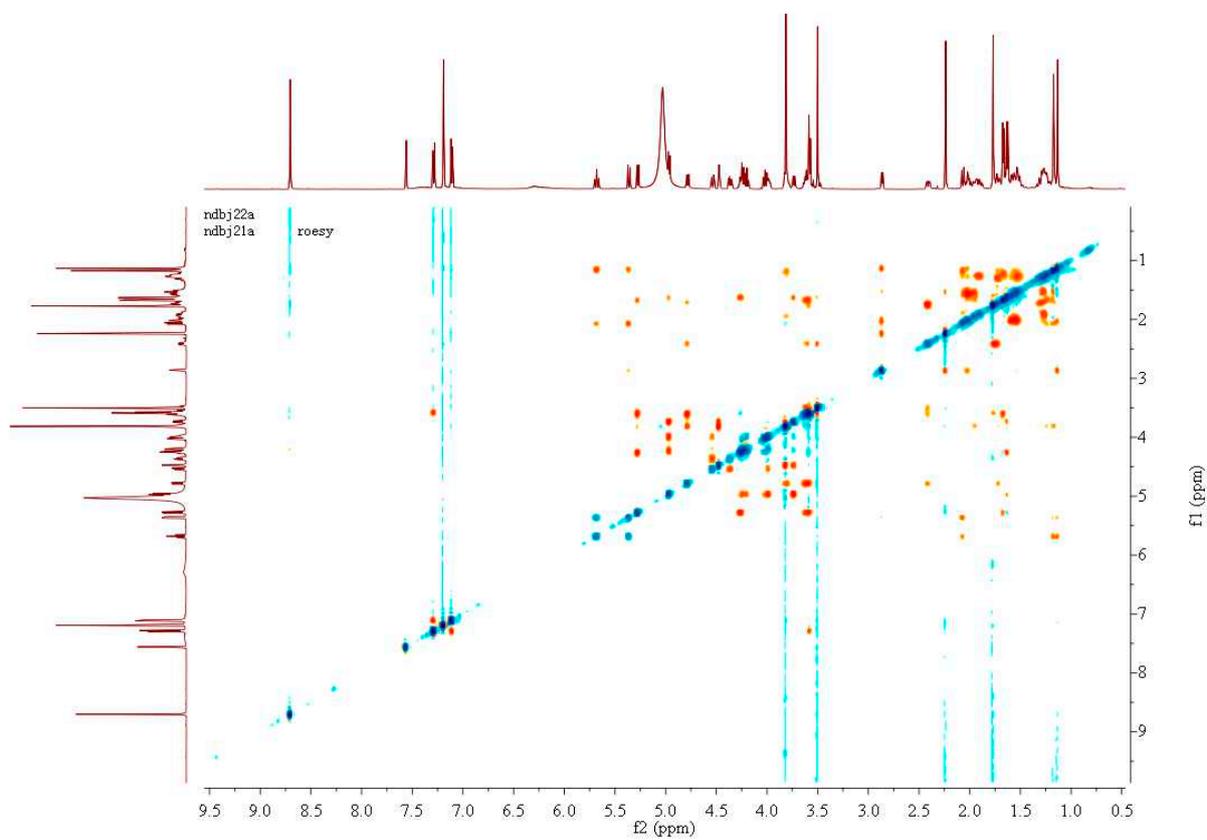
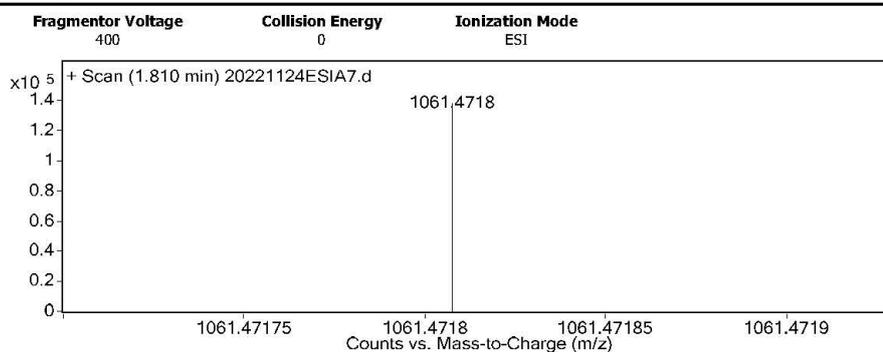


Figure S42 ROESY spectrum of compound **6**

Qualitative Analysis Report

Data Filename	20221124ESIA7.d	Sample Name	ndbj-43
Sample Type	Sample	Position	
Instrument Name	Agilent G6230 TOF MS	User Name	KIB
Acq Method	ESI.m	Acquired Time	11/24/2022 4:10:23 PM
IRM Calibration Status	Success	DA Method	ESI.m
Comment			
Sample Group		Info.	
Acquisition SW	6200 series TOF/6500 series		
Version	Q-TOF B.05.01 (B5125.2)		

User Spectra



Peak List

m/z	z	Abund	Formula	Ion
857.4657	1	33723.2		
922.0098	1	48368.4		
1019.5185	1	54652.85		
1020.5211	1	28420.66		
1027.4868	1	36759.5		
1028.4885	1	20096.37		
1061.4718	1	137733.5	C55 H74 Na O19	M+
1062.4747	1	78992.97	C55 H74 Na O19	M+
1063.477	1	23792.72	C55 H74 Na O19	M+
1181.5688	1	20248		

Formula Calculator Element Limits

Element	Min	Max
C	0	200
H	0	400
O	15	23
Na	1	1

Formula Calculator Results

Formula	CalculatedMass	Mz	Diff.(mDa)	Diff. (ppm)	DBE
C55 H74 Na O19	1061.4722	1061.4718	0.4	0.4	18.5

--- End Of Report ---

Figure S43 HRESIMS spectrum of compound 7

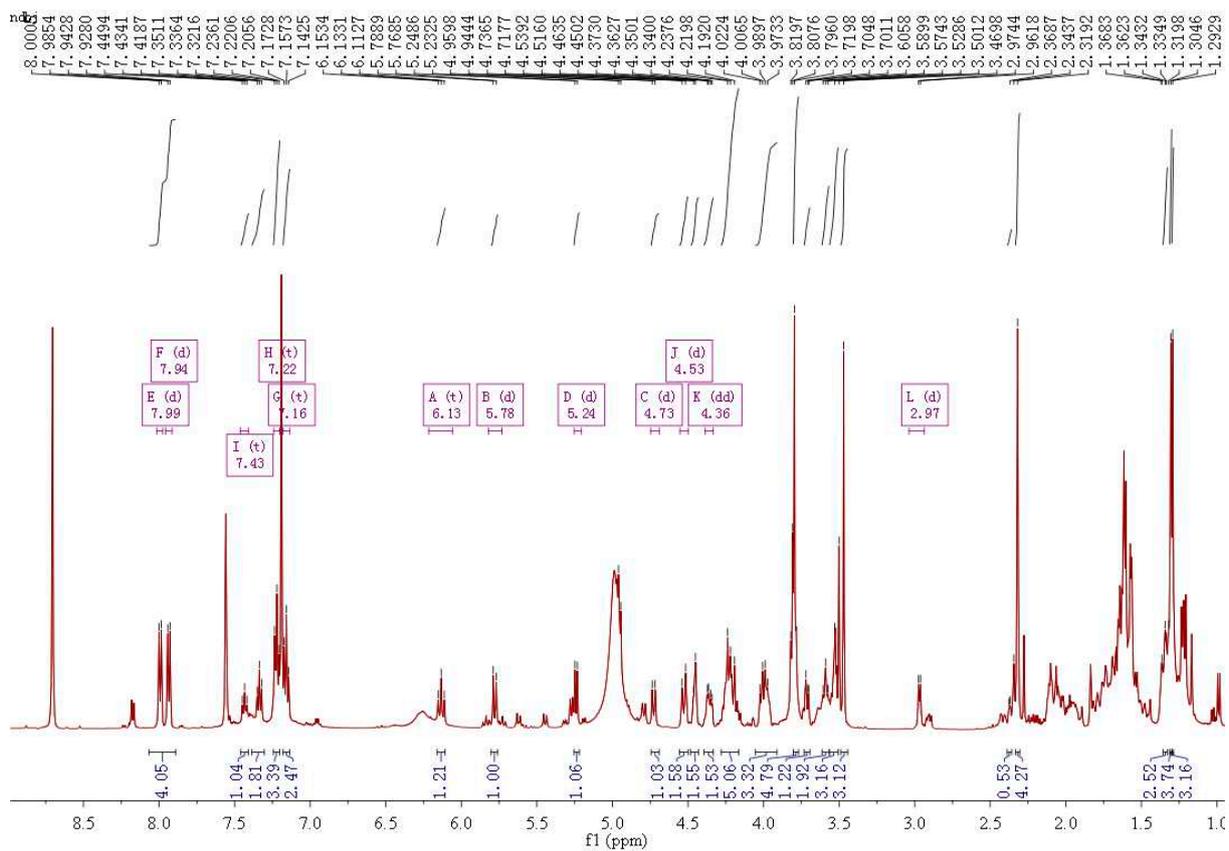


Figure S44 $^1\text{H-NMR}$ (500 MHz, pyridine- d_5) spectrum of compound 7

stq-120a

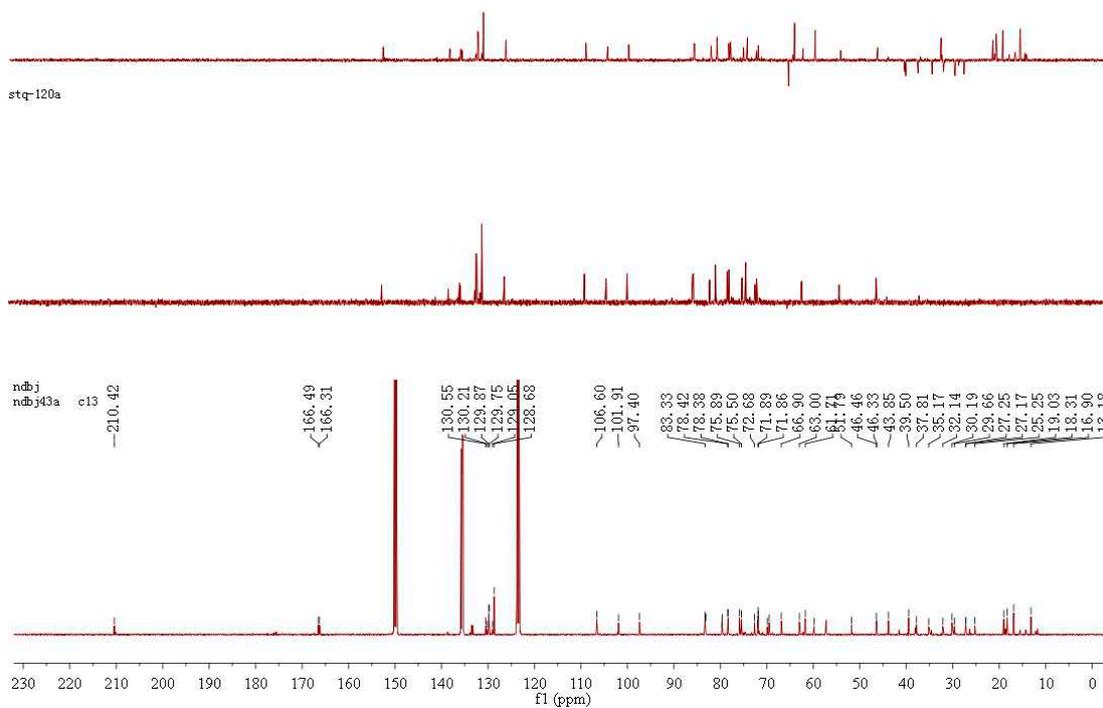


Figure S45 $^{13}\text{C-NMR}$ and DEPT (125 MHz, pyridine- d_5) spectrum of compound 7

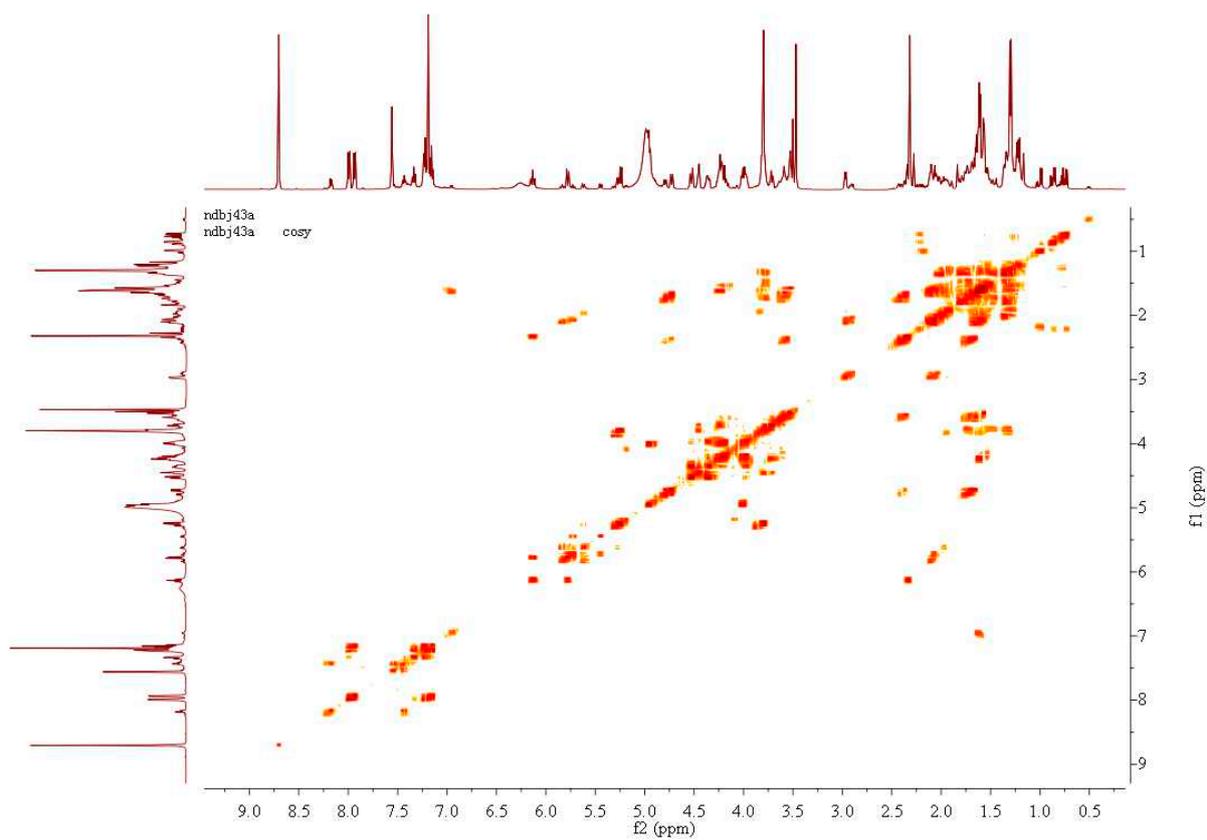


Figure S46 ^1H - ^1H COSY spectrum of compound **7**

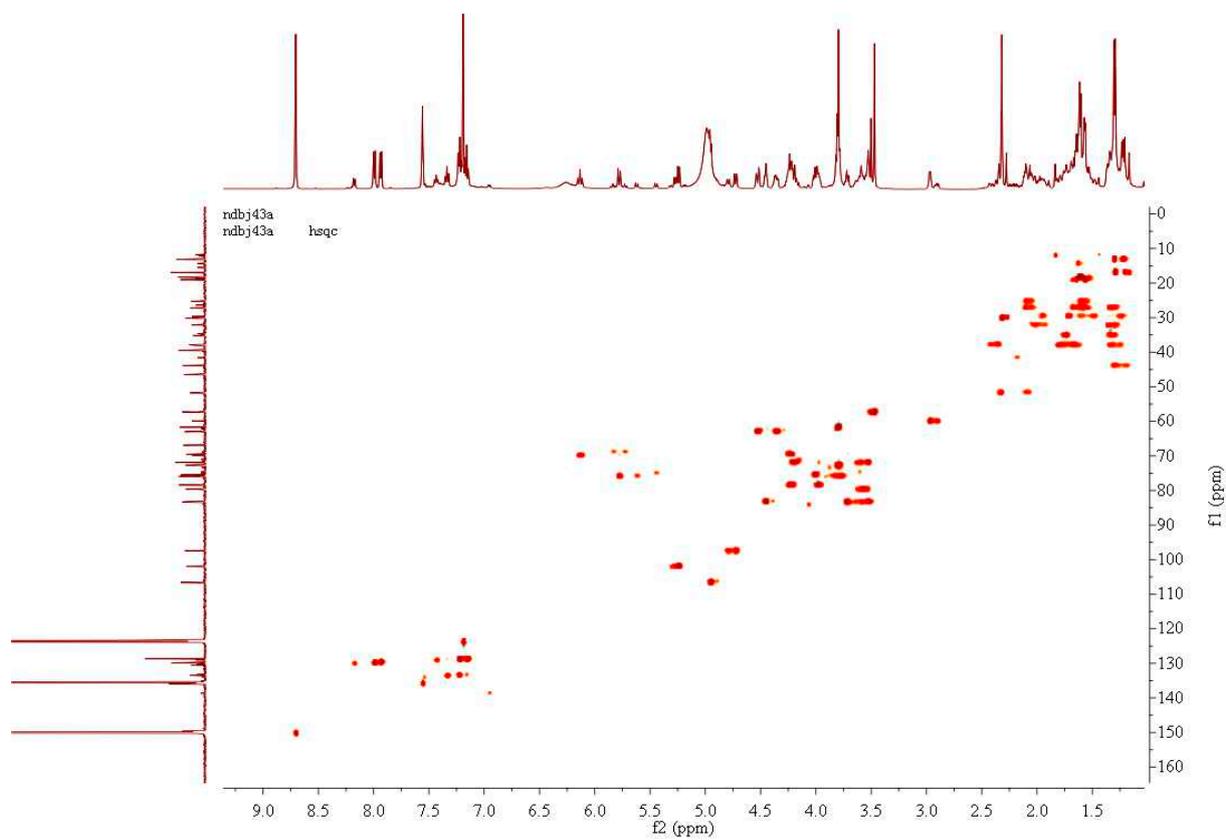


Figure S47 HSQC spectrum of compound **7**

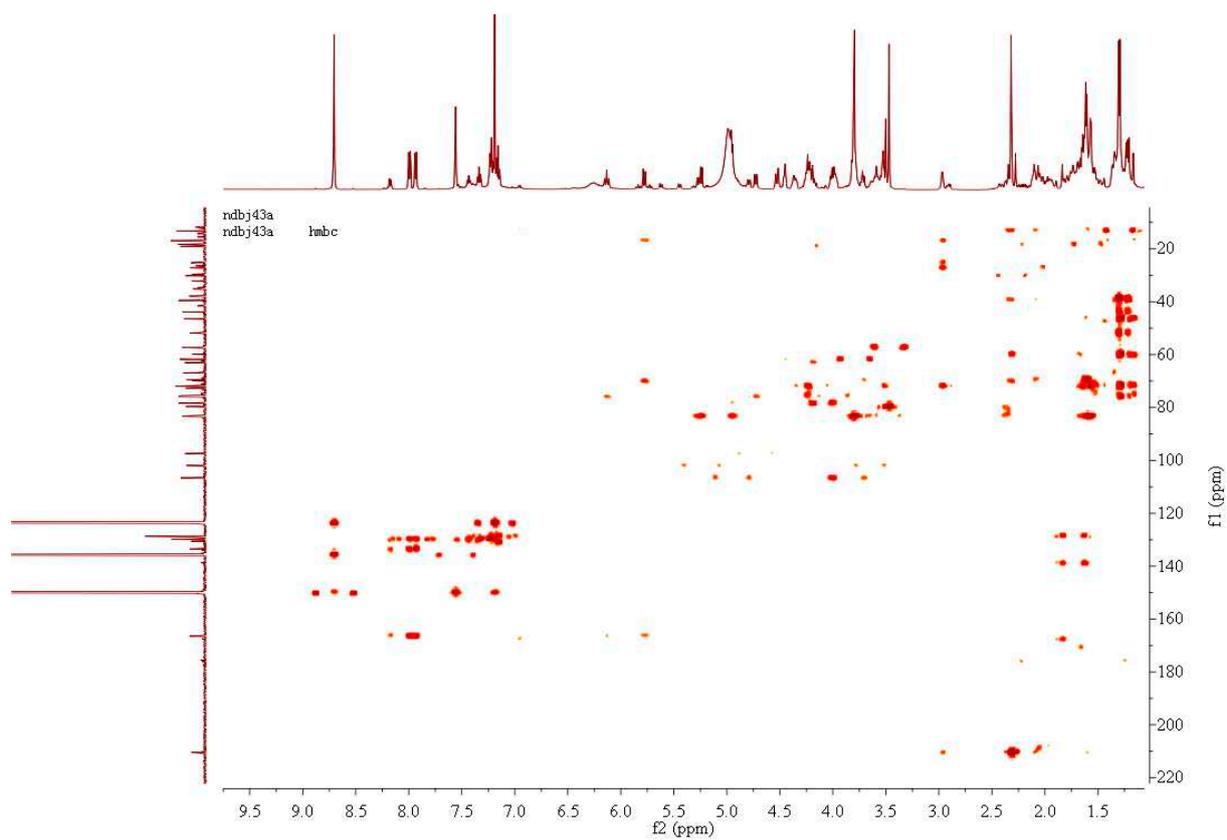


Figure S48 HMBC spectrum of compound **7**

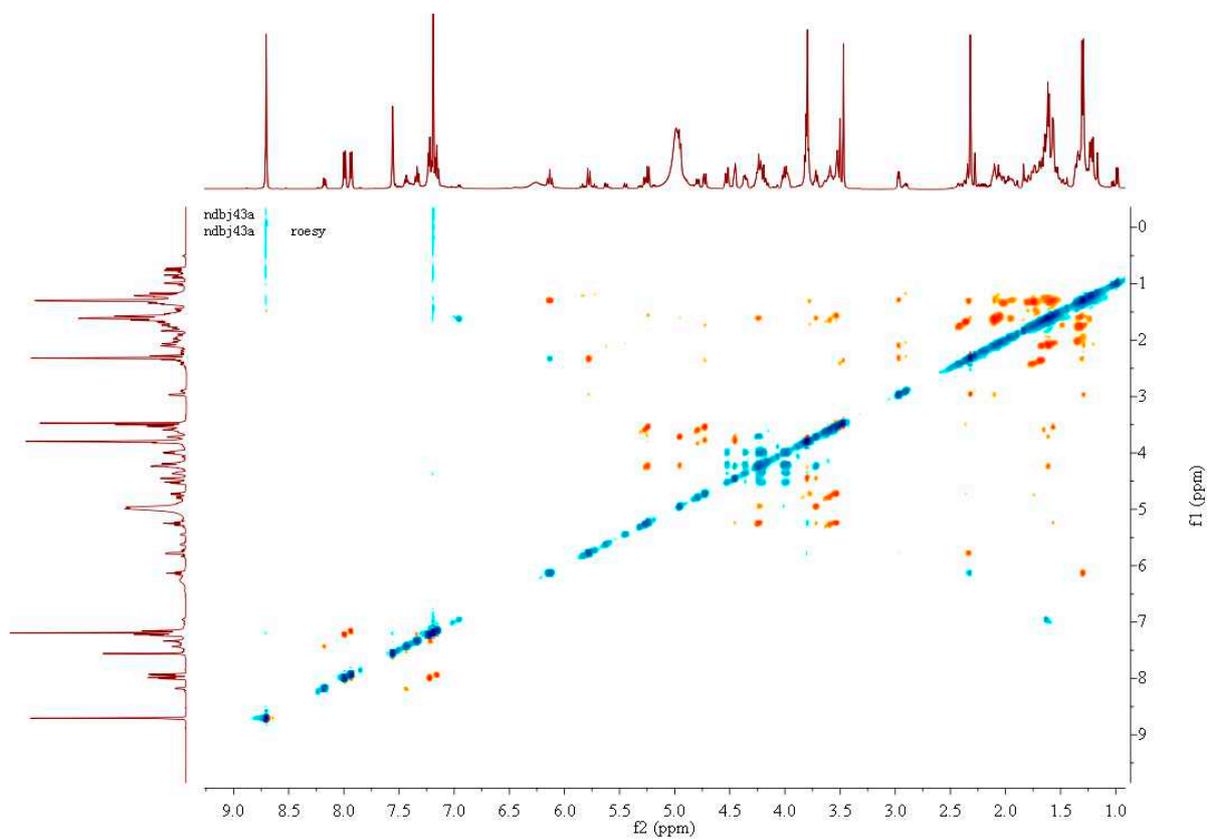


Figure S49 ROESY spectrum of compound **7**

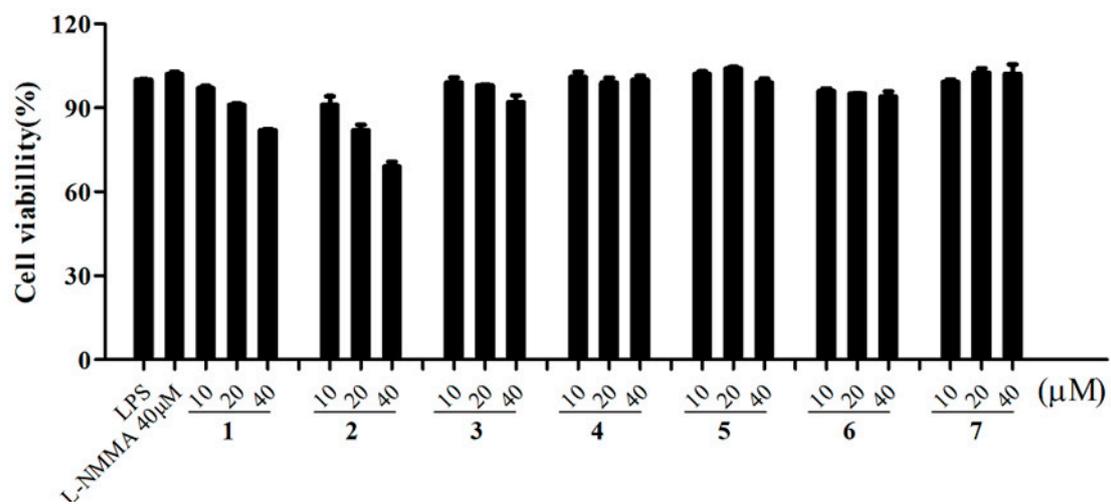


Figure S50 Effects of compounds 1–7 on cell viability.