Supplementary Material

Microbial Transformation of Yakuchinone A and Cytotoxicity Evaluation of Its Metabolites

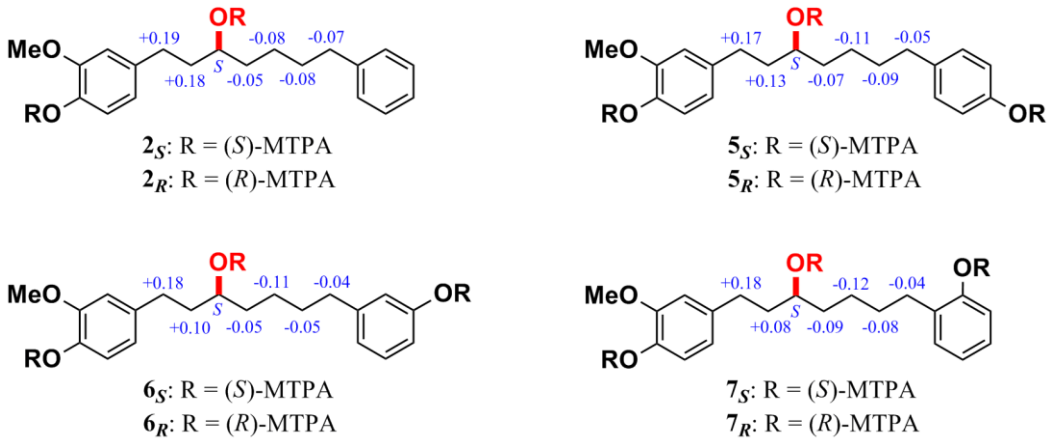
Chen Huo [1,†], Fubo Han [1,†], Yina Xiao [1], Hyun Jung Kim [2,*] and Ik-Soo Lee [1,*]

[1] College of Pharmacy, Chonnam National University, Gwangju 61186, Korea; huochen_0213@163.com (C.H.); hanfubo0306@gmail.com (F.H.); yogurtxiao@163.com (Y.X.)
[2] College of Pharmacy and Natural Medicine Research Institute, Mokpo National University, Muan, Jeonnam 58554, Korea
* Correspondence: hyunkim@mokpo.ac.kr (H.J.K.); islee@chonnam.ac.kr (I.-S.L.); Tel.: +82-62-530-2932
† These authors contributed equally to this work.

Table S1. Twenty-two microorganisms were incubated for screening of yakuchinone A.

Microorganisms	Metabolite production [a]
Absidia coerulea KCTC 6936	(+)
Alternaria alternata KCTC 6005	(-)
Aspergillus fumigatus KCTC 6145	(-)
Aspergillus niger KCCM 60332	(-)
Aspergillus oryzae KCCM 60345	(-)
Cunninghamella elegans var. *elegans* KCTC 6992	(+)
Filobasidium neoformans KCTC 7902	(-)
Fusarium merismoides KCTC 6153	(-)
Gliocladium deliquescens KCTC 6173	(-)
Glomerella cingulata KCTC 6075	(-)
Hormoconis resinae KCTC 6966	(+)
Kluyveromyces marxianus KCTC 7155	(-)
Mortierella ramanniana var. *angulispora* KCTC 6137	(-)
Monascus rubber KCTC 6122	(-)
Mucor hiemalis KCTC 26779	(+++)
Mucor plumbeus KCCM 60265	(++)
Penicillium chrysogenum KCTC 6933	(-)
Rhizopus oryzae KCCM 60556	(+)
Saccharomycodes ludwigii KCTC 7126	(-)
Torulaspora delbrueckii KCTC 7116	(-)
Trichoderma koningii KCTC 6042	(-)
Tremella mesenterica KCTC 7131	(-)

[a] Metabolite production denoted by (+) indicted that metabolites were produced as shown by TLC analysis.



$\mathbf{2}_S$: R = (*S*)-MTPA
$\mathbf{2}_R$: R = (*R*)-MTPA

$\mathbf{5}_S$: R = (*S*)-MTPA
$\mathbf{5}_R$: R = (*R*)-MTPA

$\mathbf{6}_S$: R = (*S*)-MTPA
$\mathbf{6}_R$: R = (*R*)-MTPA

$\mathbf{7}_S$: R = (*S*)-MTPA
$\mathbf{7}_R$: R = (*R*)-MTPA

Figure S1. $\Delta\delta_H$ (=δ_S–δ_R) values for the Mosher ester derivatives of **2**, **5**, **6**, and **7**.

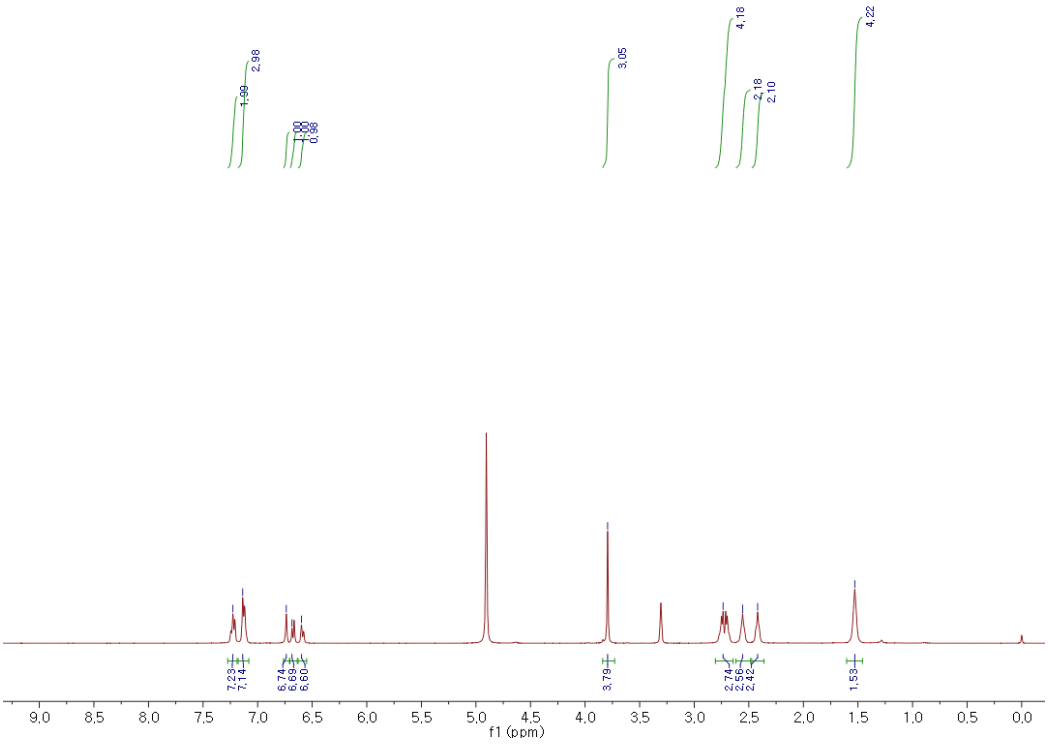
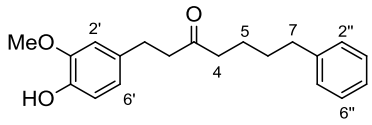


Figure S2. ^{1}H-NMR (CD_3OD, 400 MHz) spectrum of yakuchinone A (**1**).



Yakuchinone A (**1**)

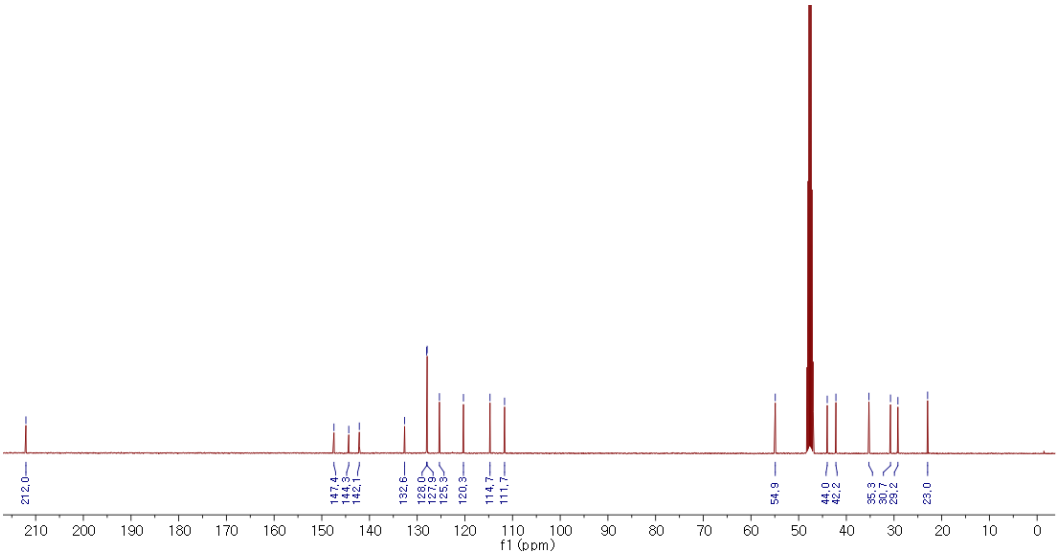


Figure S3. ^{13}C-NMR (CD_3OD, 100 MHz) spectrum of yakuchinone A (**1**).

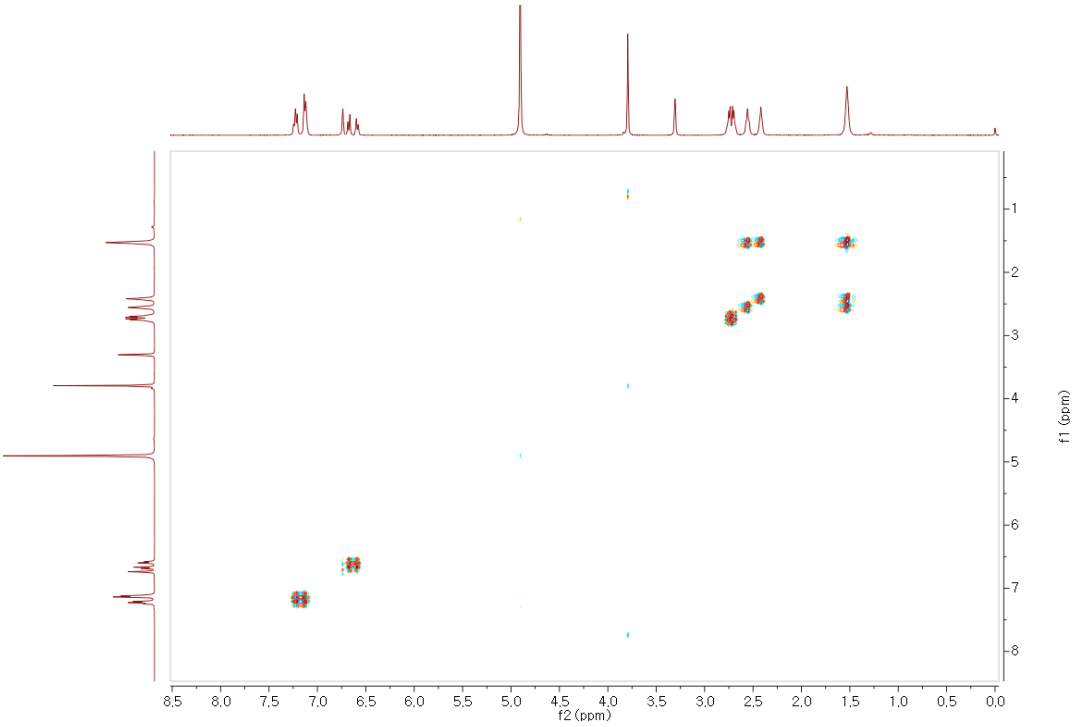


Figure S4. COSY spectrum of yakuchinone A (**1**).

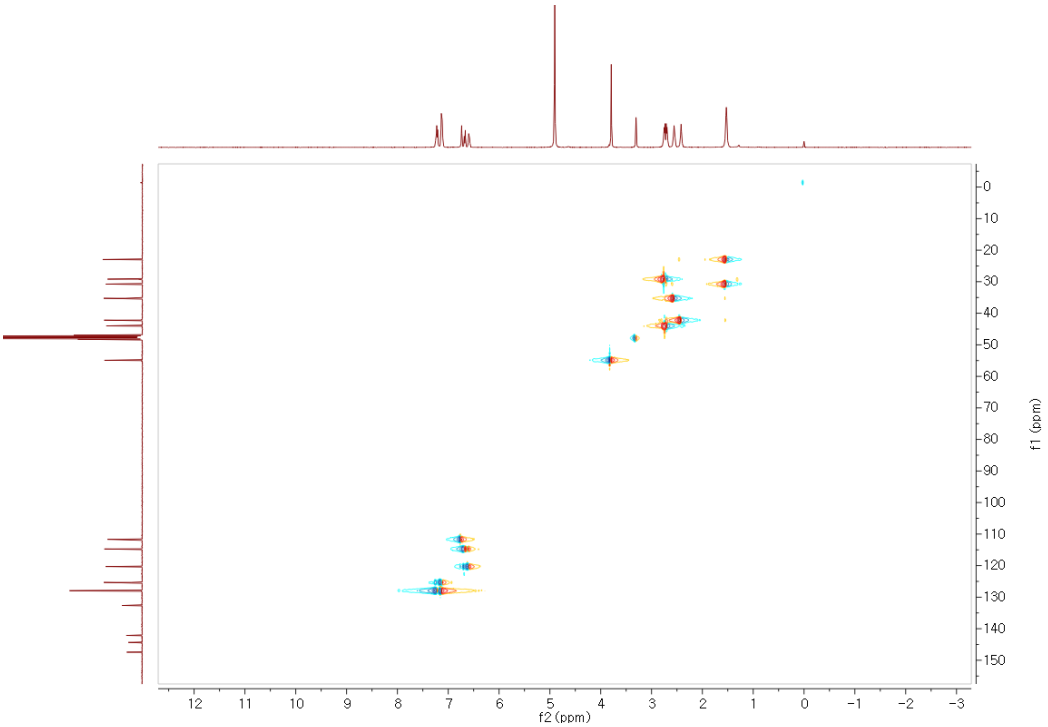


Figure S5. HSQC spectrum of yakuchinone A (**1**).

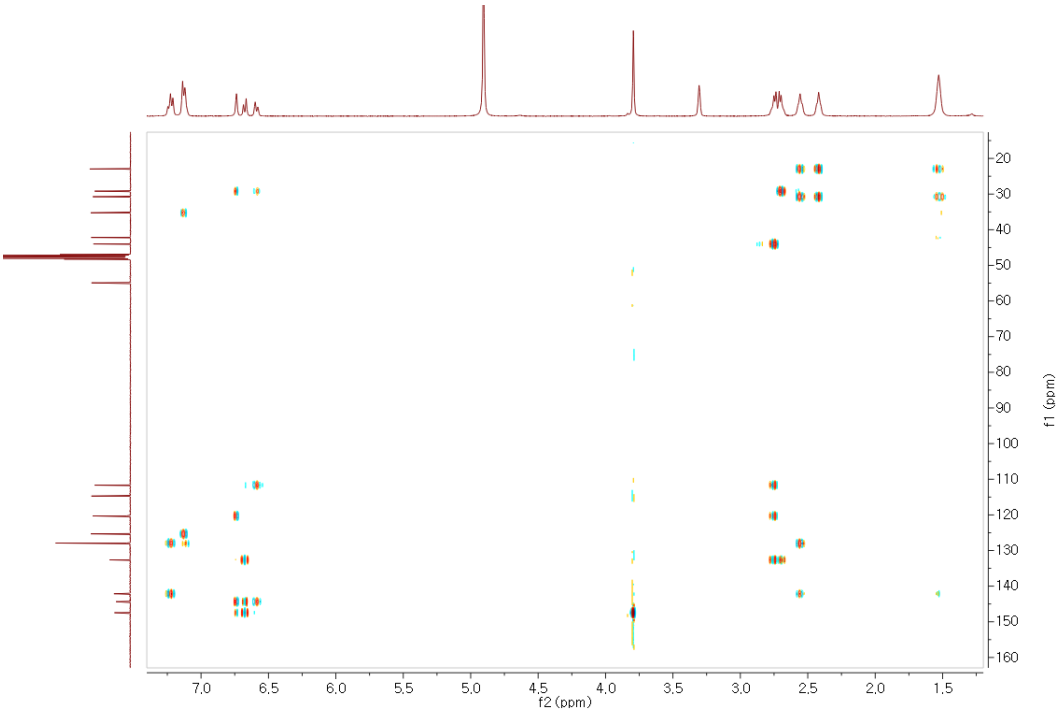


Figure S6. HMBC spectrum of yakuchinone A (**1**).

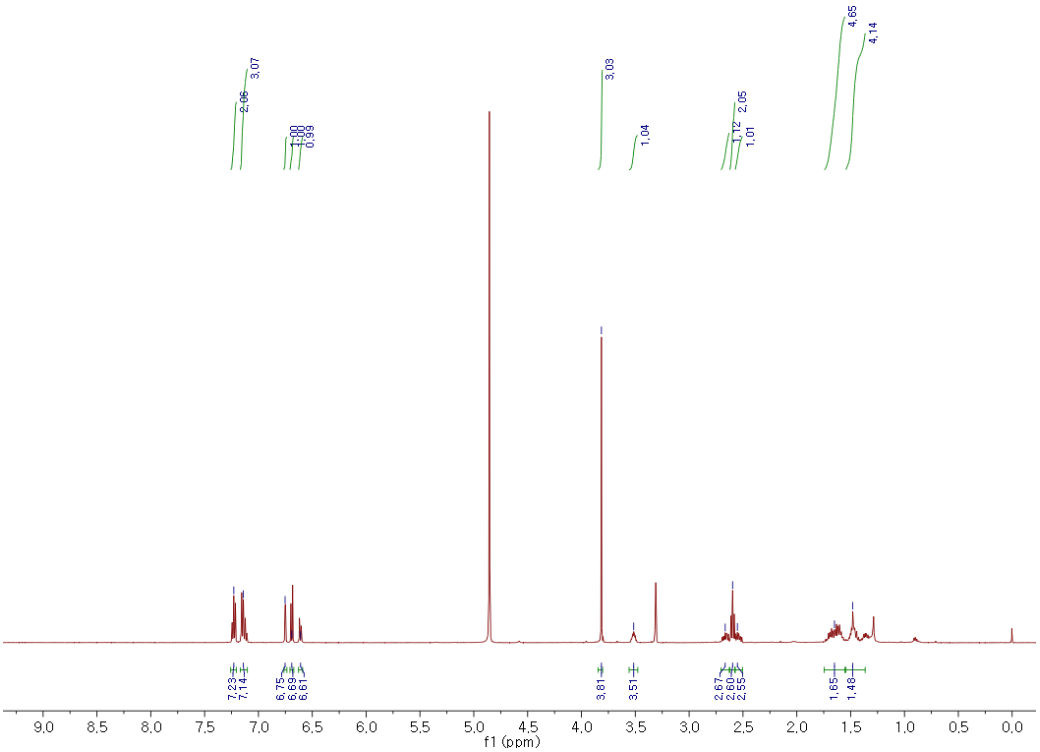


Figure S7. ^{1}H-NMR (CD_3OD, 500 MHz) spectrum of **2**.

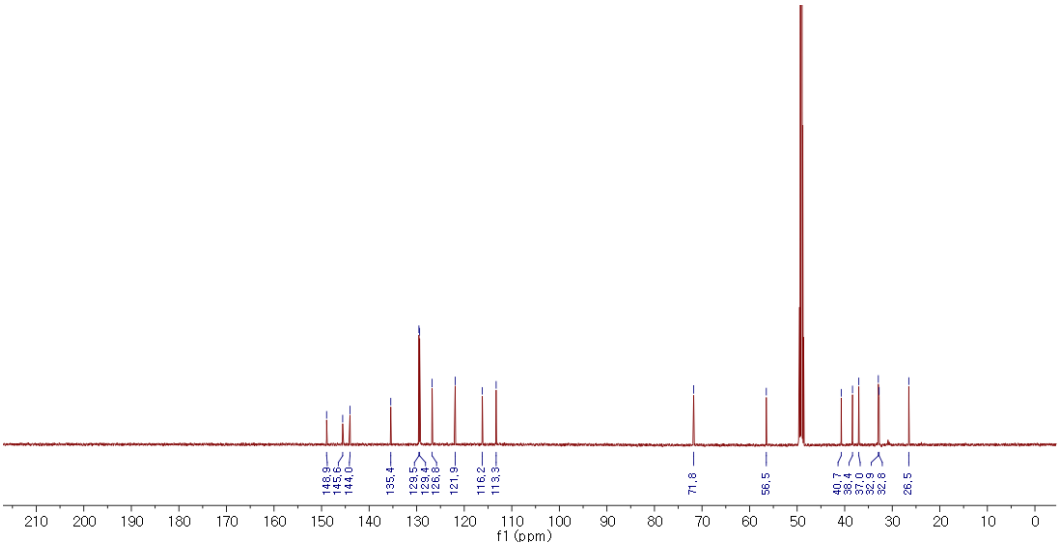


Figure S8. ^{13}C-NMR (CD_3OD, 125 MHz) spectrum of **2**.

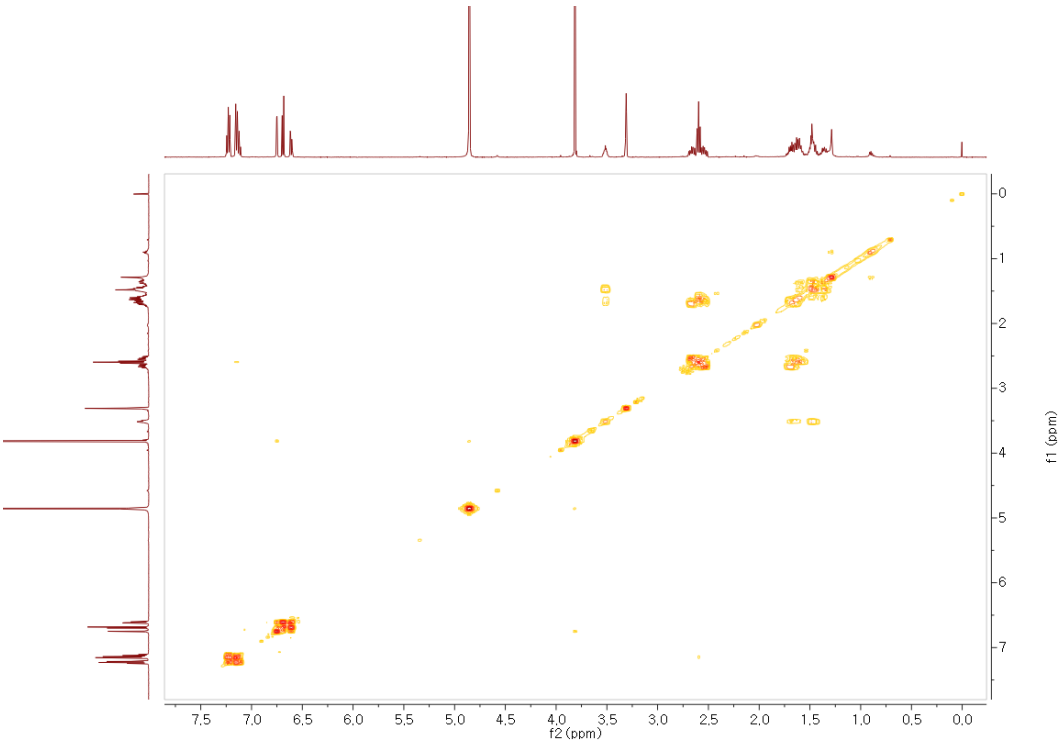


Figure S9. COSY spectrum of **2**.

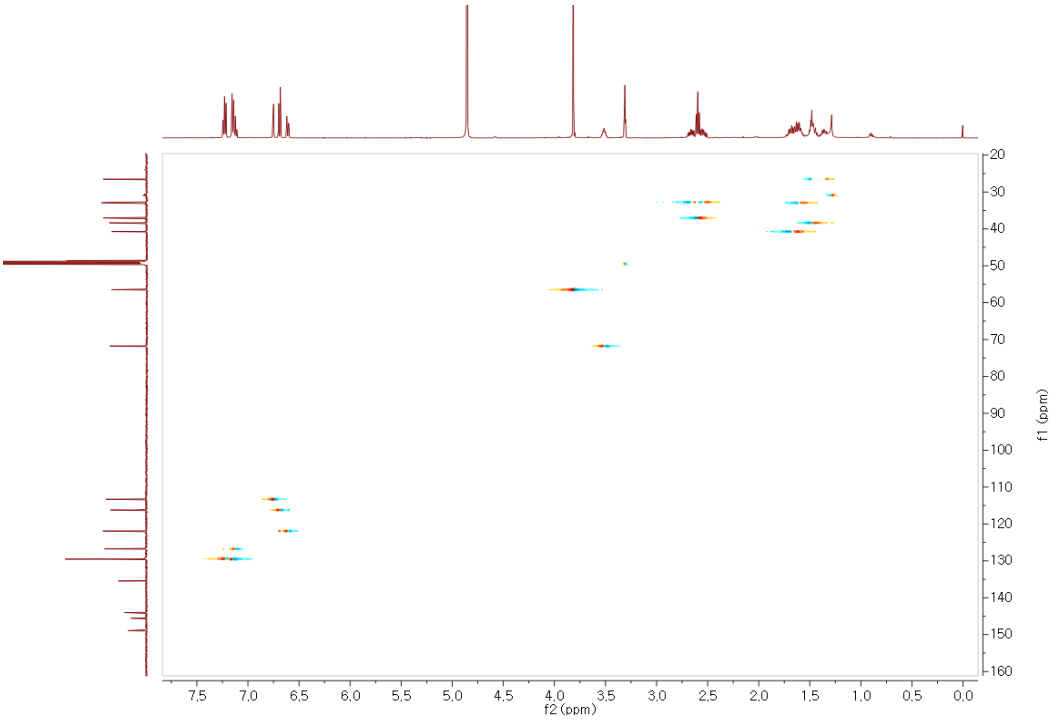


Figure S10. HSQC spectrum of **2**.

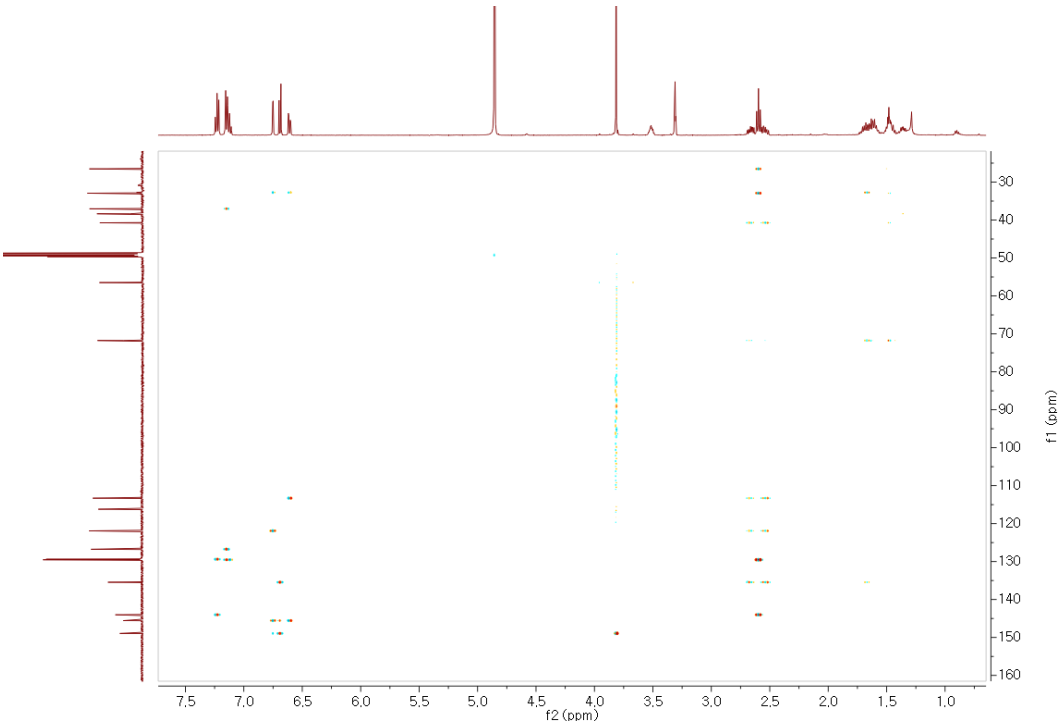
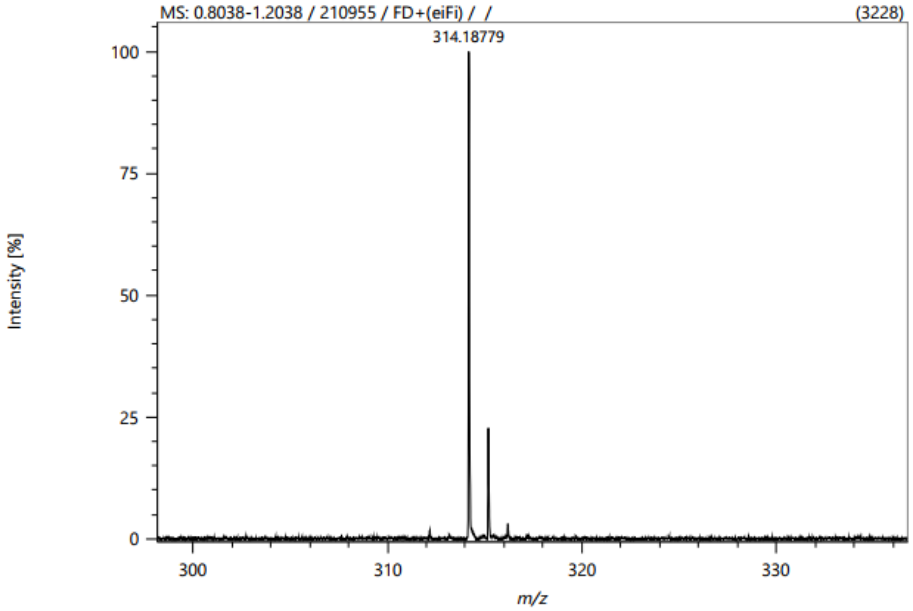


Figure S11. HMBC spectrum of **2**.



Elemental Composition

Parameters		Elements Set 1:			
Tolerance:	30.00 mDa	Symbol	C	H	O
Electron:	Odd/Even	Min	1	5	1
Charge:	+1	Max	20	26	3
DBE:	-90.0 - 90.0				

Results

Mass	Intensity	Formula	Calculated Mass	Mass Difference [mDa]	Mass Difference [ppm]	DBE
314.18779	3228.10	C20 H26 O3	314.18765	0.15	0.46	8.0

Figure S12. HRFDMS spectrum of **2**.

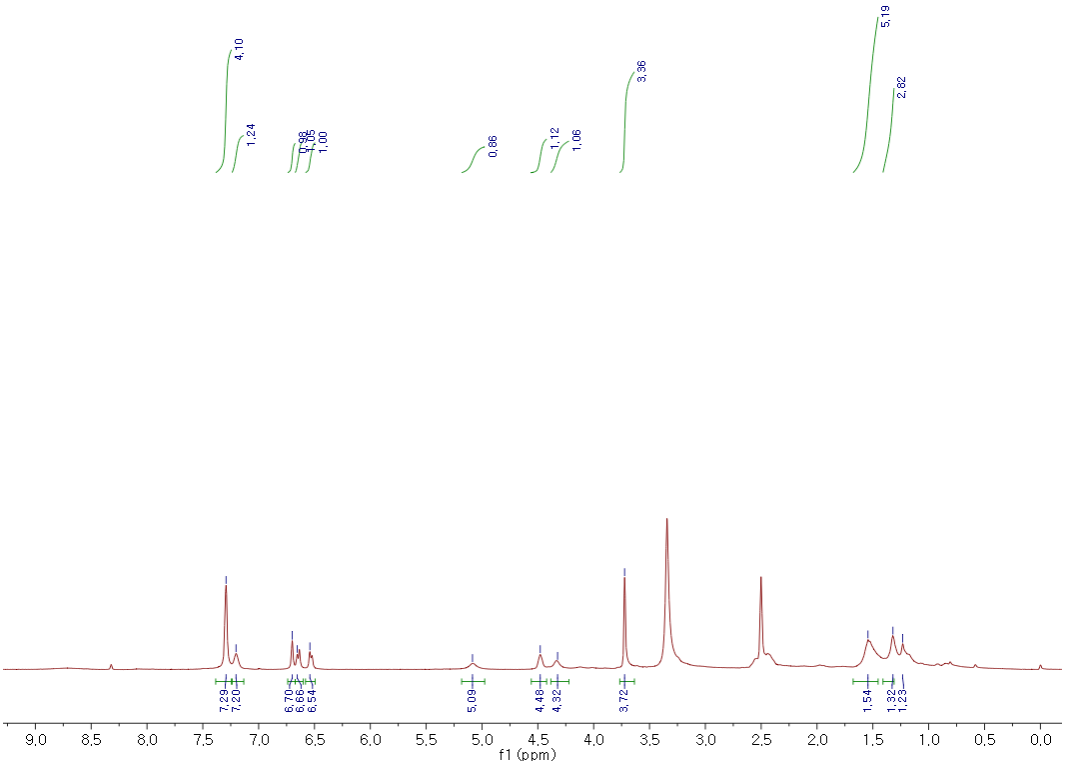


Figure S13. [1]H-NMR (DMSO-*d*$_6$, 400 MHz) spectrum of **3**.

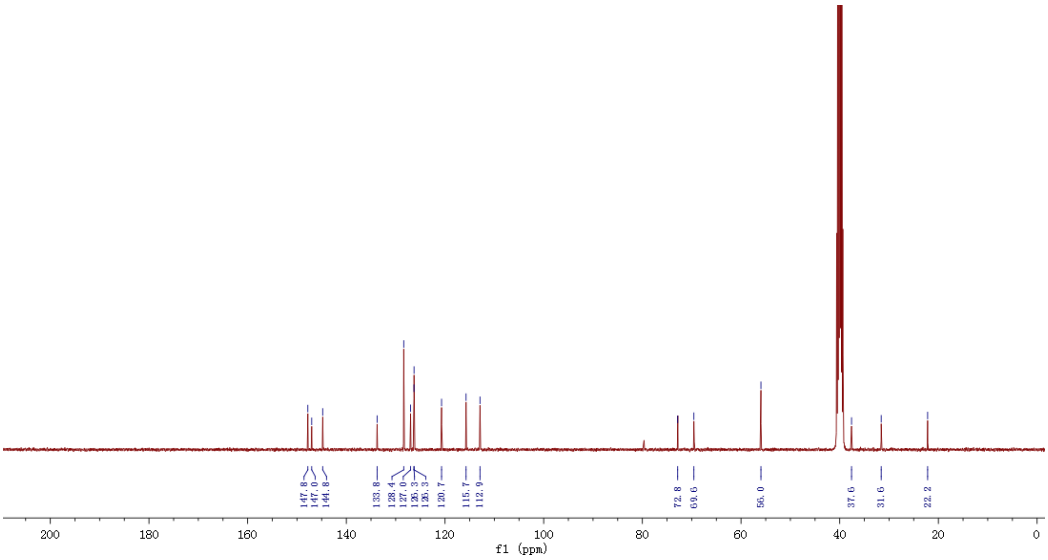


Figure S14. [13]C-NMR (DMSO-*d*$_6$, 100 MHz) spectrum of **3**.

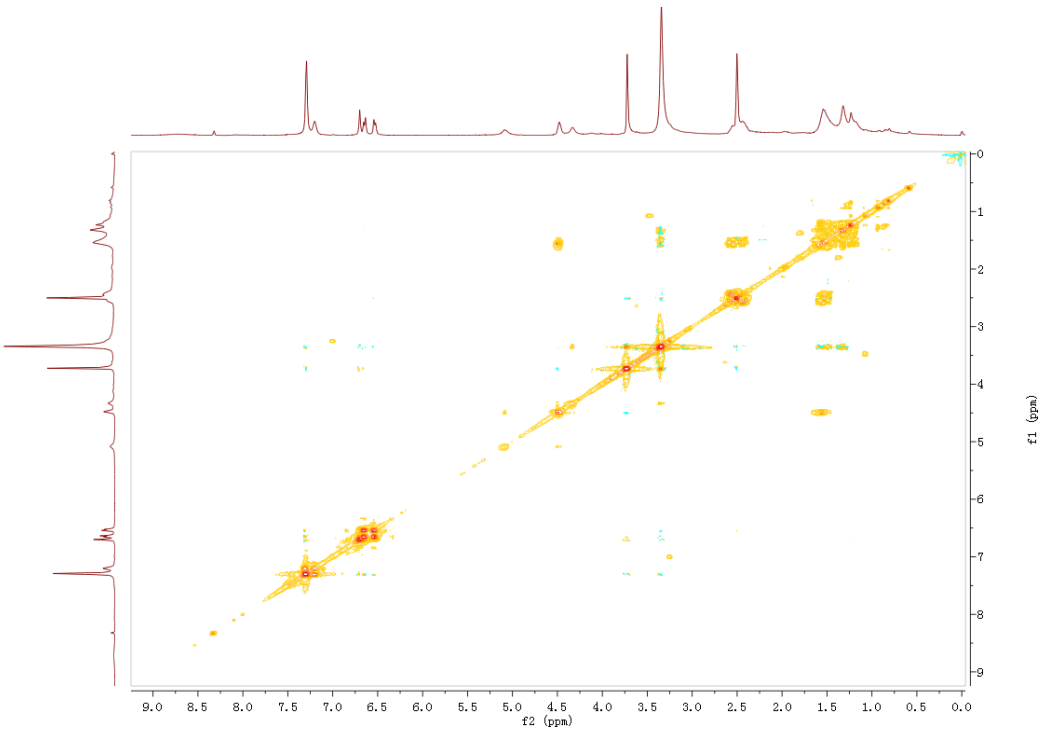


Figure S15. COSY spectrum of **3**.

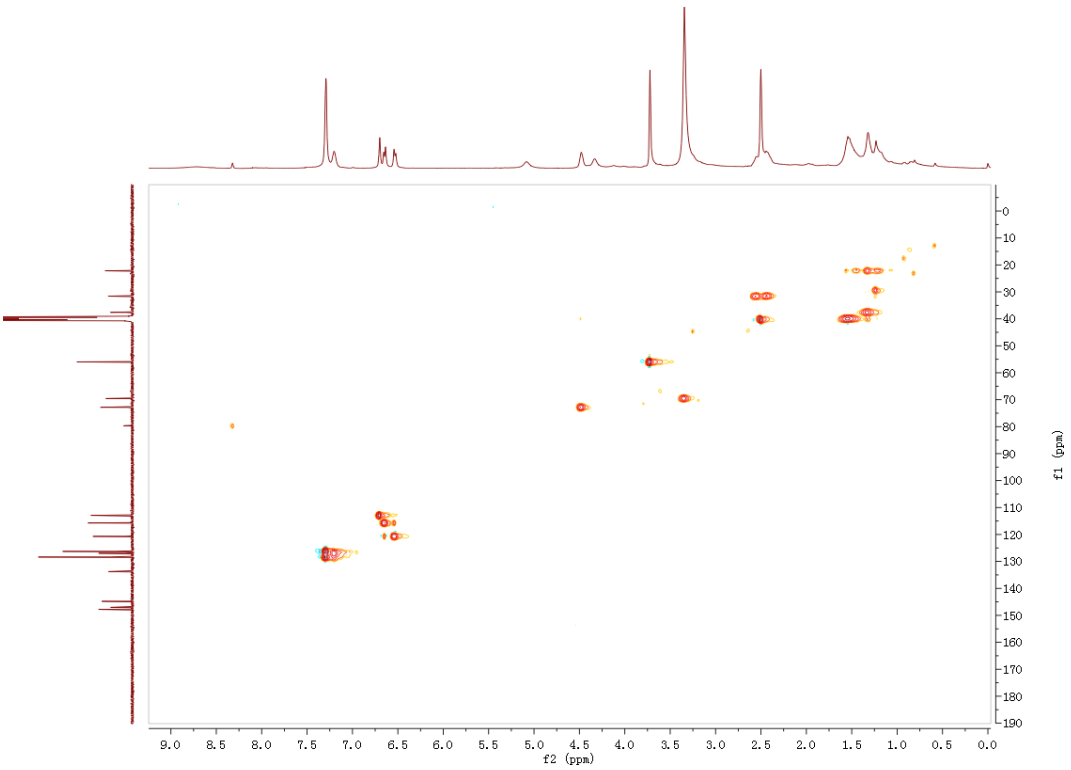


Figure S16. HSQC spectrum of **3**.

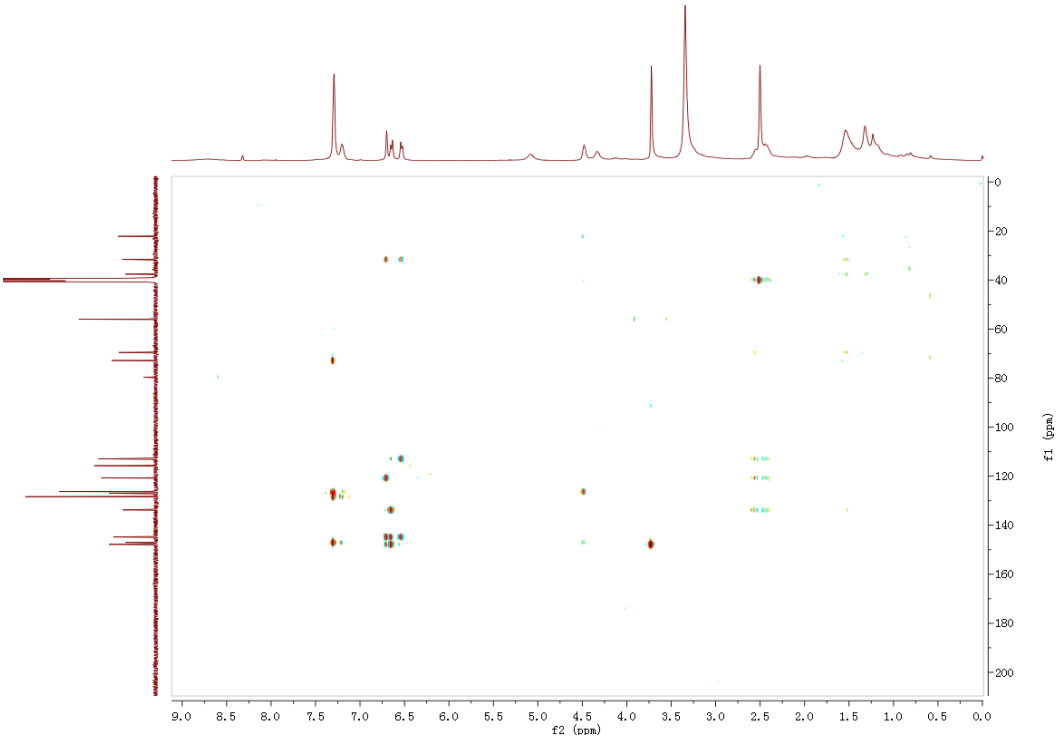
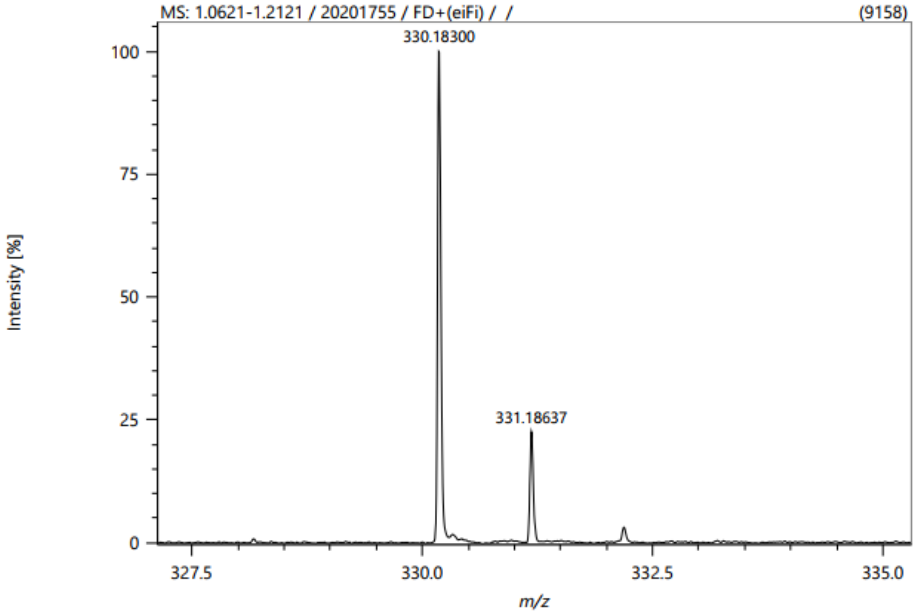


Figure S17. HMBC spectrum of **3**.



Elemental Composition

Parameters		Elements Set 1:			
Tolerance:	30.00 mDa	Symbol	C	H	O
Electron:	Odd/Even	Min	5	5	1
Charge:	+1	Max	20	26	4
DBE:	-90.0 - 90.0				

Results

Mass	Intensity	Formula	Calculated Mass	Mass Difference [mDa]	Mass Difference [ppm]	DBE
330.18300	9158.13	C20 H26 O4	330.18256	0.44	1.35	8.0

Figure S18. HRFDMS spectrum of **3**.

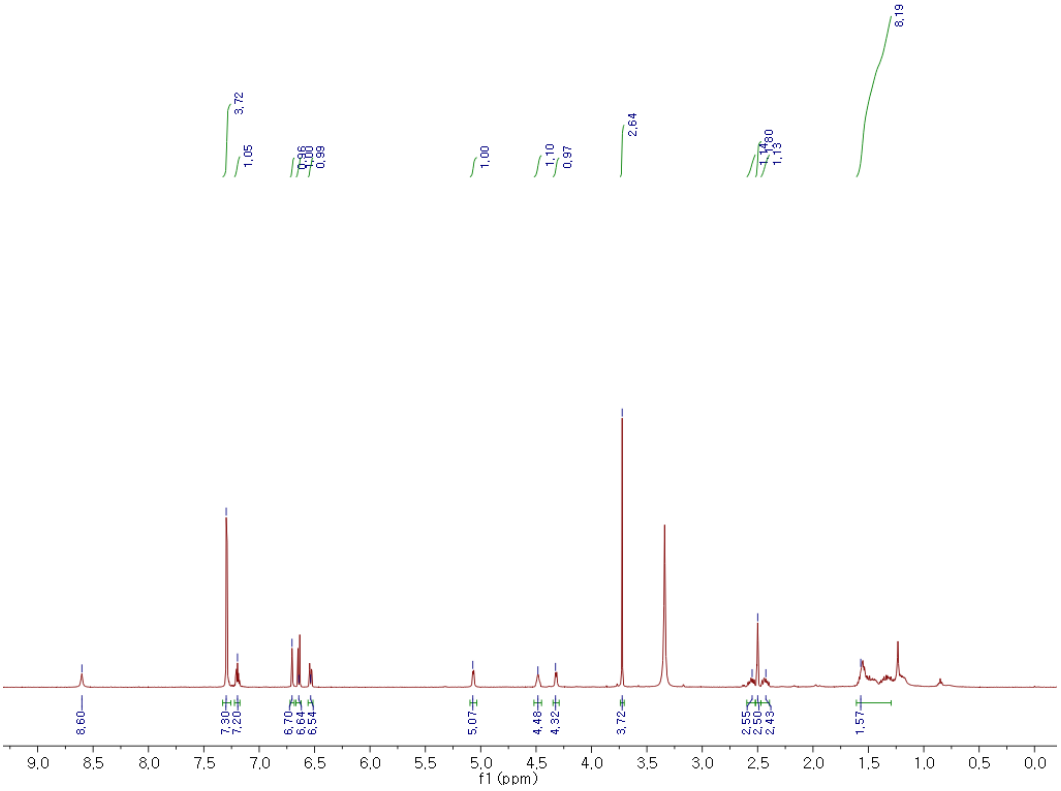


Figure S19. [1]H-NMR (DMSO-*d*$_6$, 500 MHz) spectrum of **3a**.

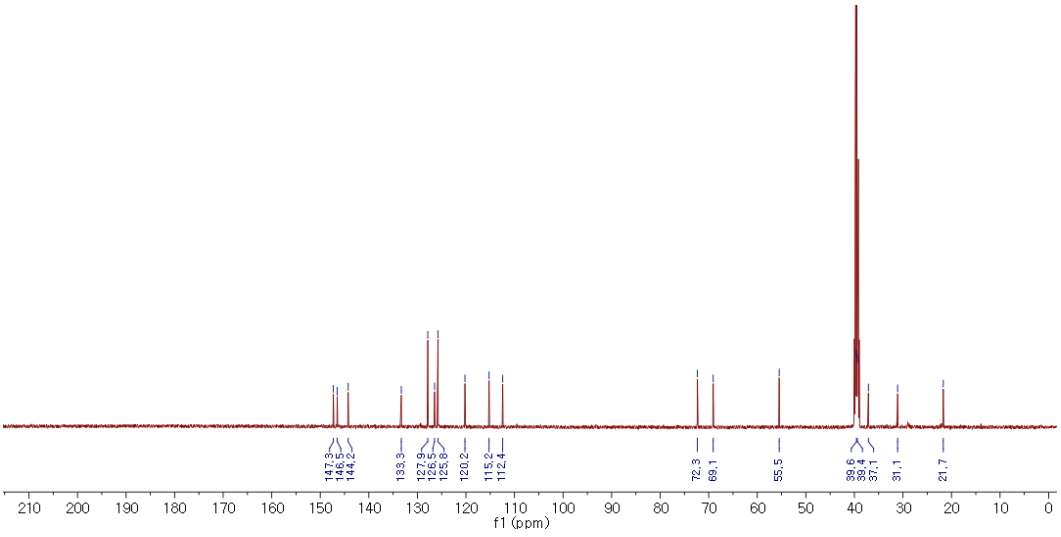
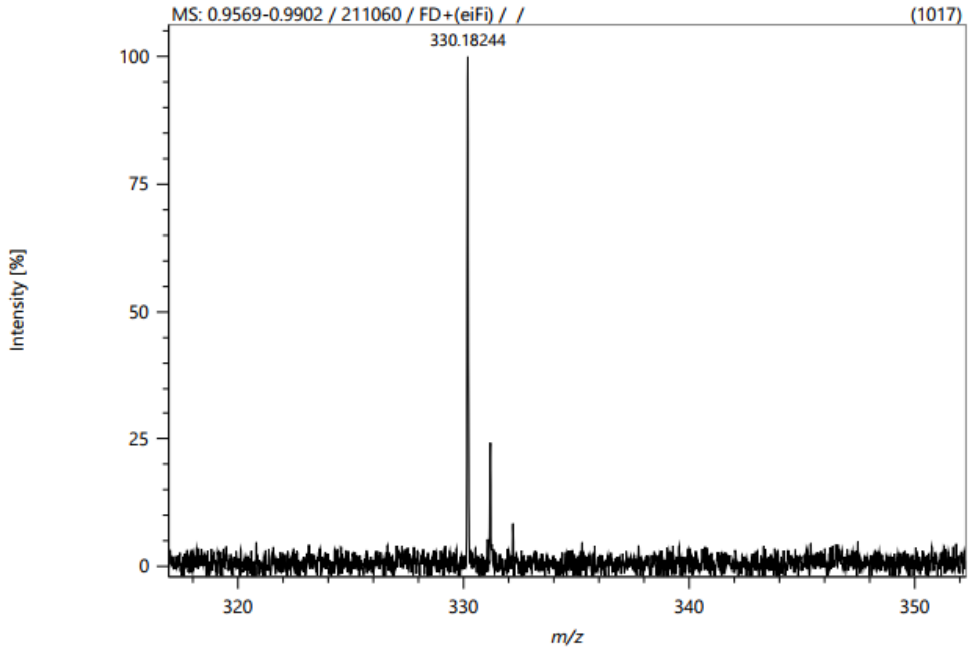


Figure S20. [13]C-NMR (DMSO-*d*$_6$, 125 MHz) spectrum of **3a**.



Elemental Composition

Parameters		Elements Set 1:			
Tolerance:	30.00 mDa	Symbol	C	H	O
Electron:	Odd/Even	Min	1	5	1
Charge:	+1	Max	20	26	4
DBE:	-90.0 - 90.0				

Results

Mass	Intensity	Formula	Calculated Mass	Mass Difference [mDa]	Mass Difference [ppm]	DBE
330.18244	1016.67	C20 H26 O4	330.18256	-0.13	-0.38	8.0

Figure S21. HRFDMS spectrum of **3a**.

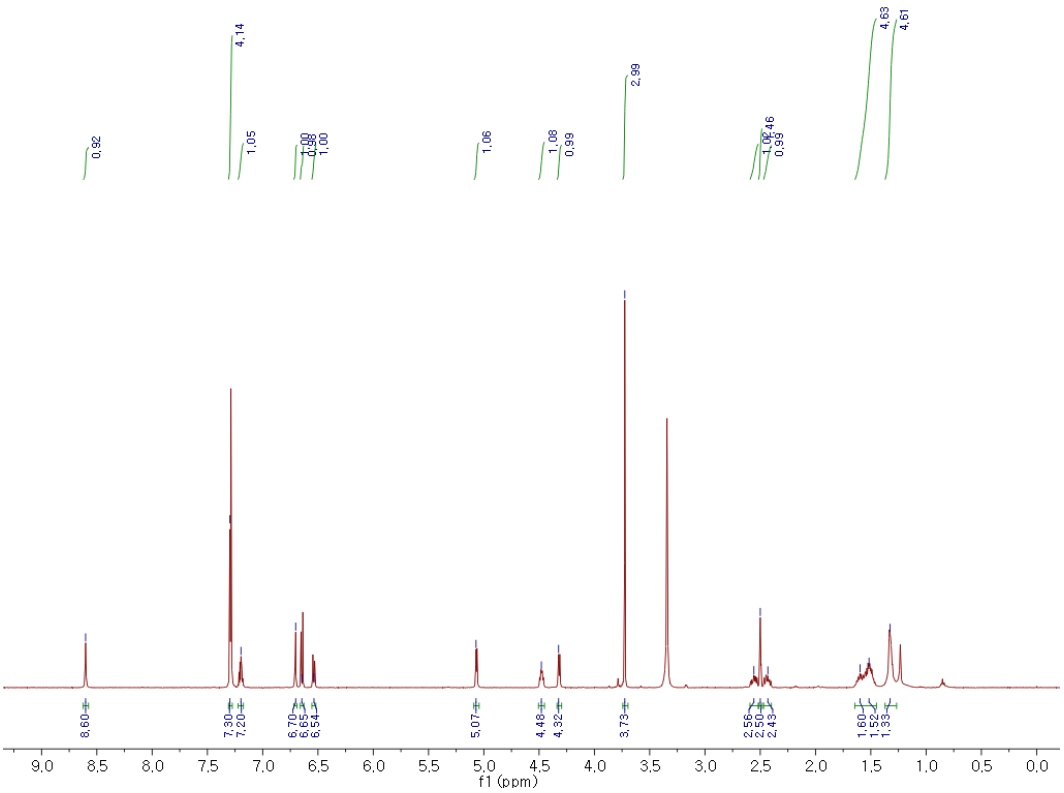


Figure S22. [1]H-NMR (DMSO-*d*$_6$, 500 MHz) spectrum of **3b**.

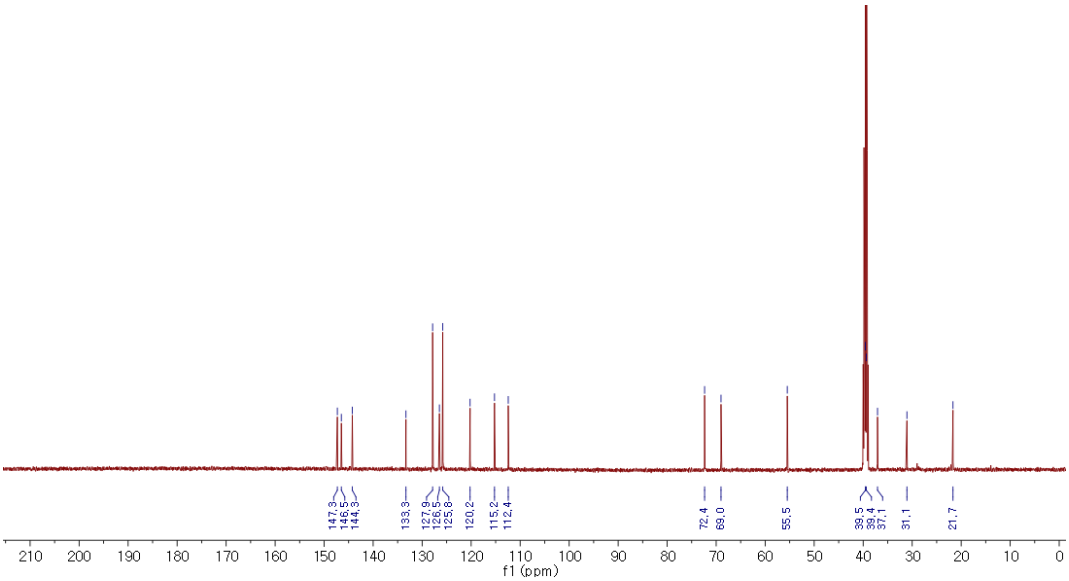


Figure S23. [13]C-NMR (DMSO-*d*$_6$, 125 MHz) spectrum of **3b**.

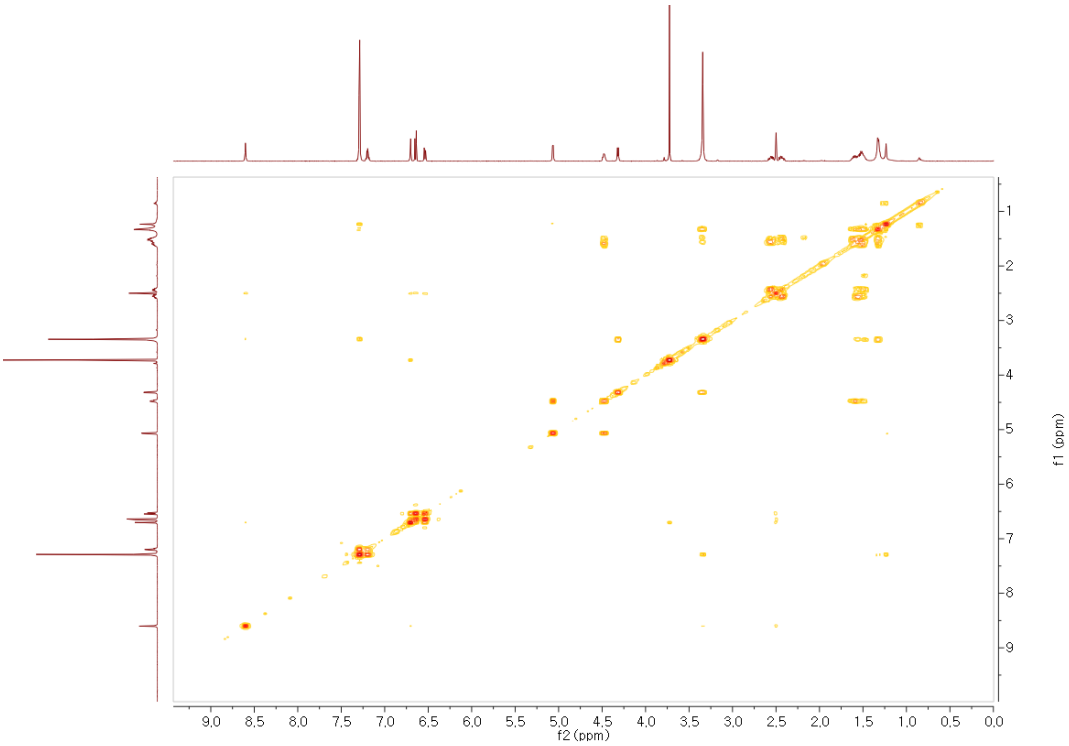


Figure S24. COSY spectrum of **3b**.

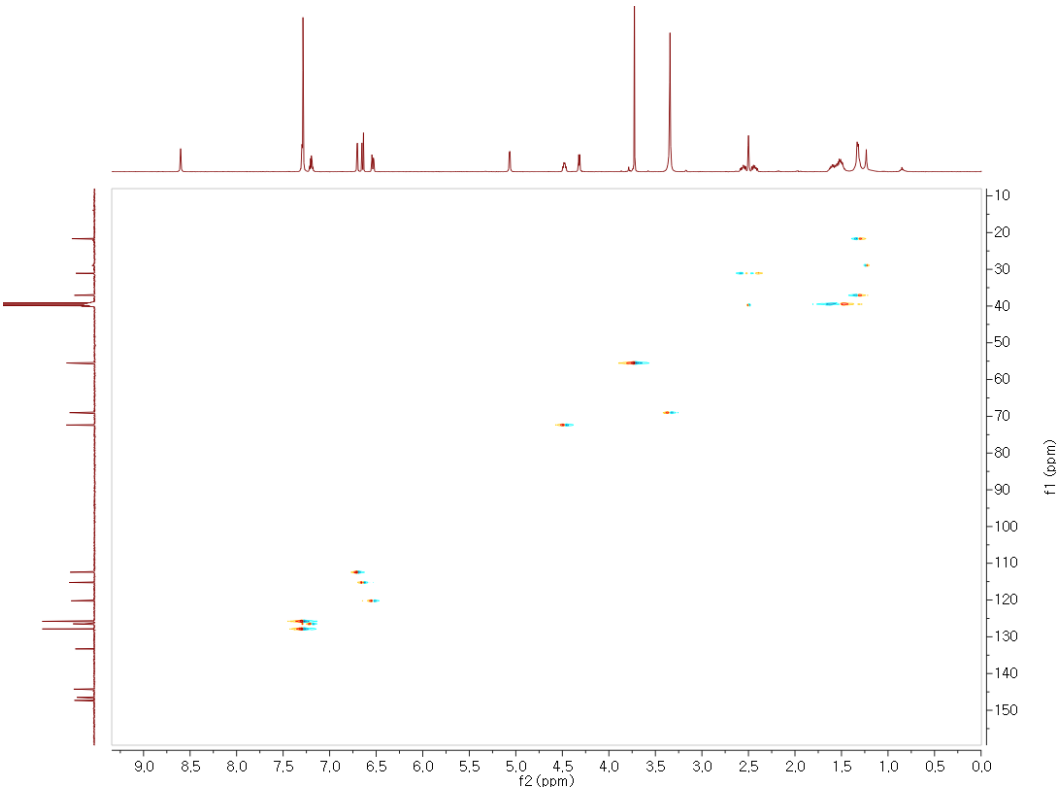


Figure S25. HSQC spectrum of **3b**.

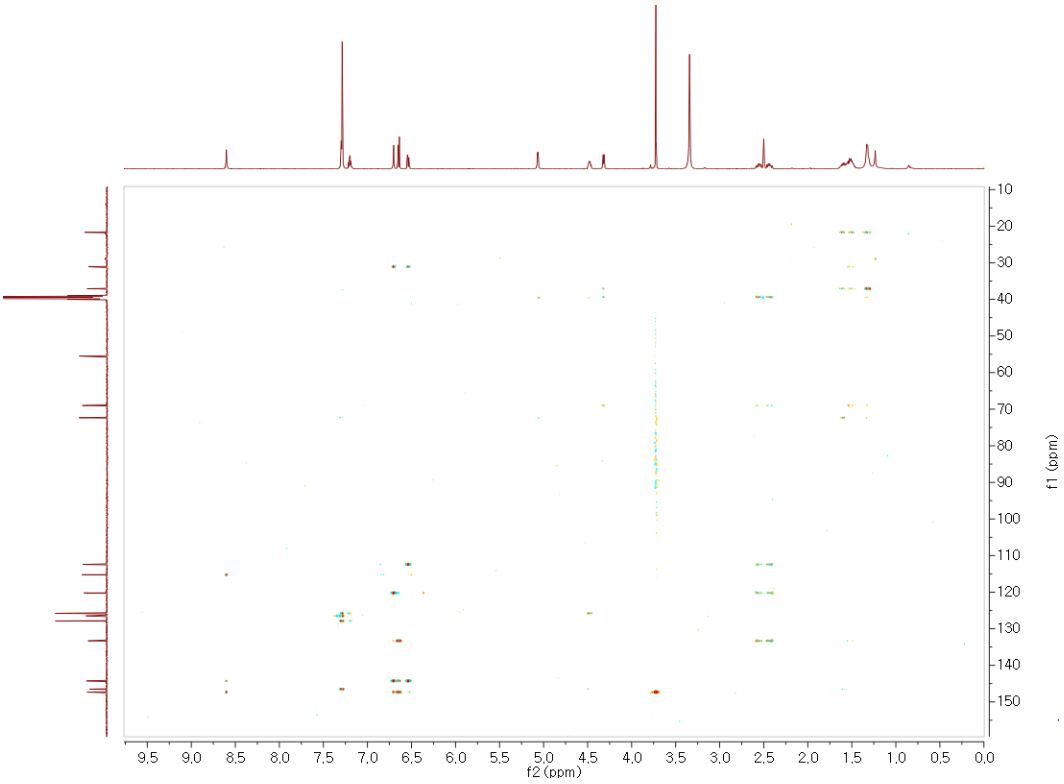
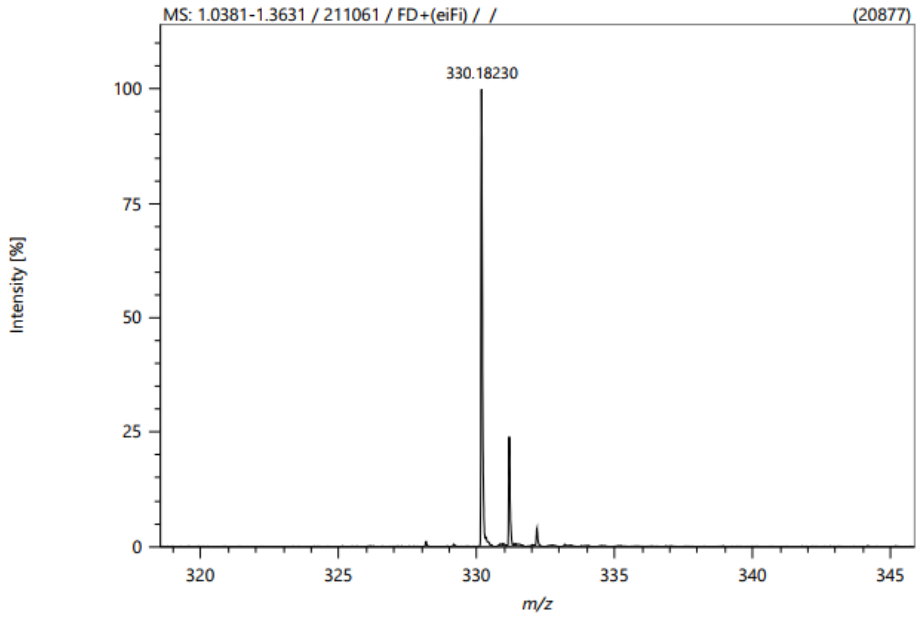


Figure S26. HMBC spectrum of **3b**.



Elemental Composition

Parameters		Elements Set 1:			
Tolerance:	30.00 mDa	Symbol	C	H	O
Electron:	Odd/Even	Min	1	5	1
Charge:	+1	Max	20	26	4
DBE:	-90.0 - 90.0				

Results

Mass	Intensity	Formula	Calculated Mass	Mass Difference [mDa]	Mass Difference [ppm]	DBE
330.18230	20876.99	C20 H26 O4	330.18256	-0.26	-0.80	8.0

Figure S27. HRFDMS spectrum of **3b**.

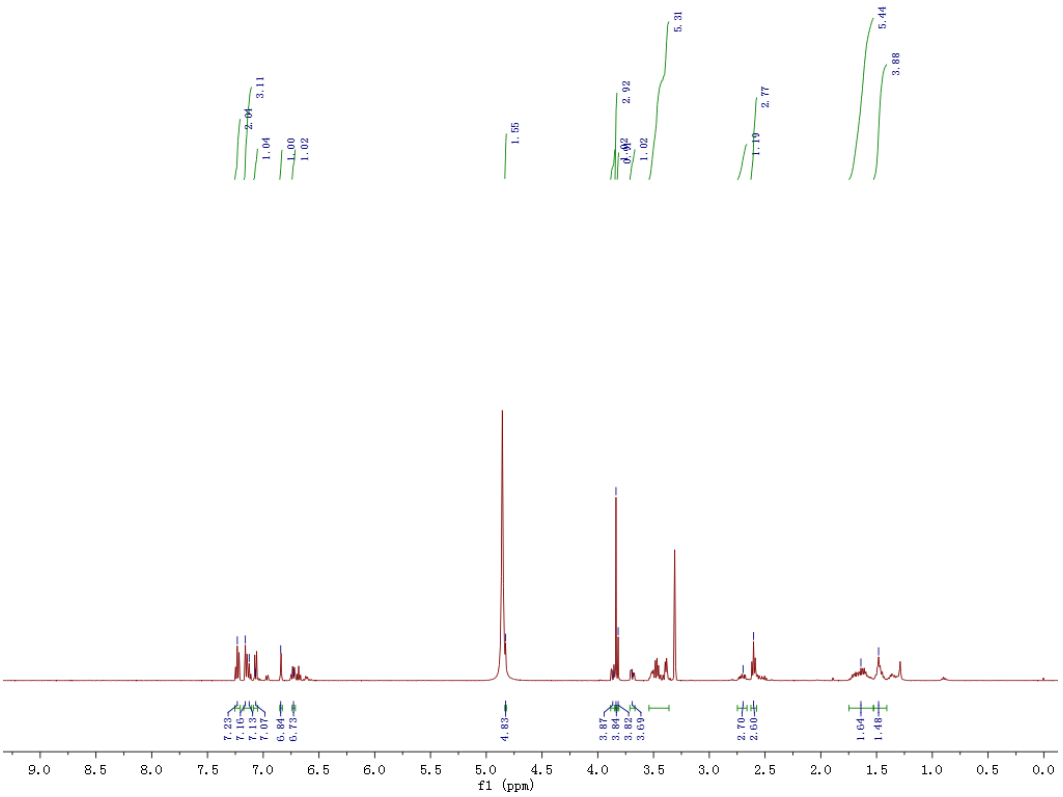


Figure S28. ^{1}H-NMR (CD_3OD, 500 MHz) spectrum of **4**.

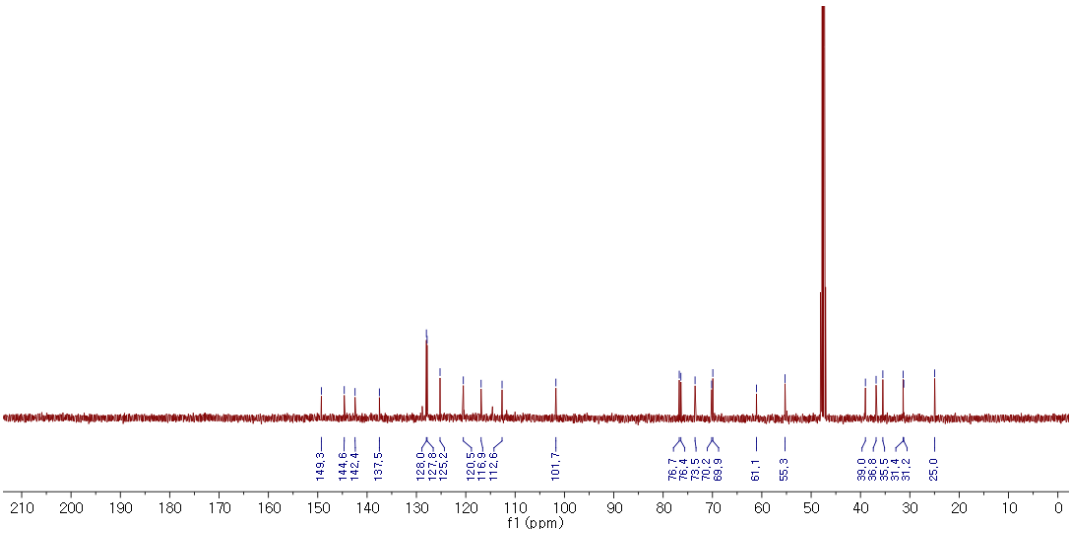


Figure S29. ^{13}C-NMR (CD_3OD, 125 MHz) spectrum of **4**.

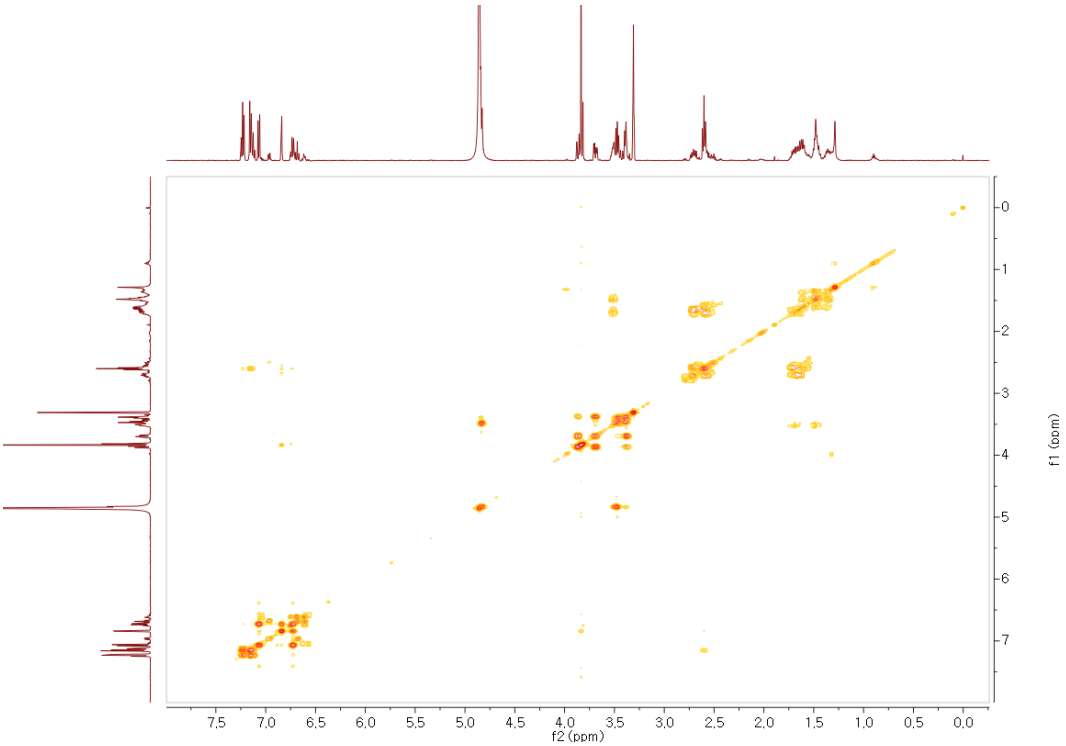


Figure S30. COSY spectrum of **4**.

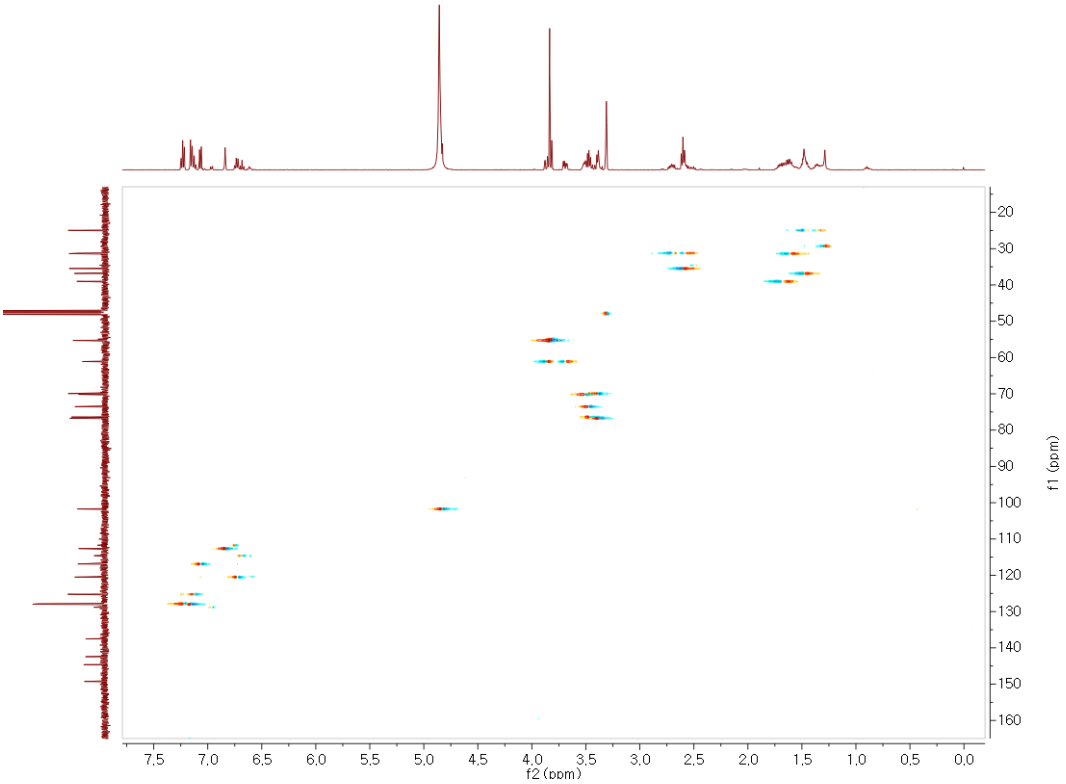


Figure S31. HSQC spectrum of **4**.

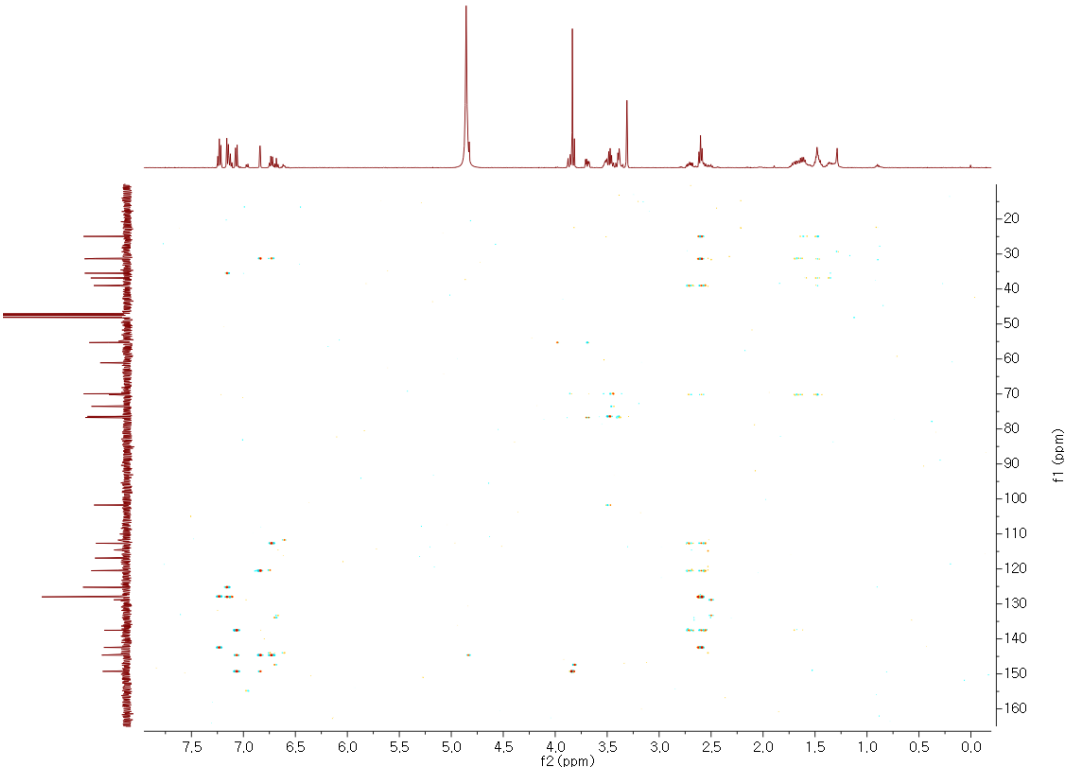
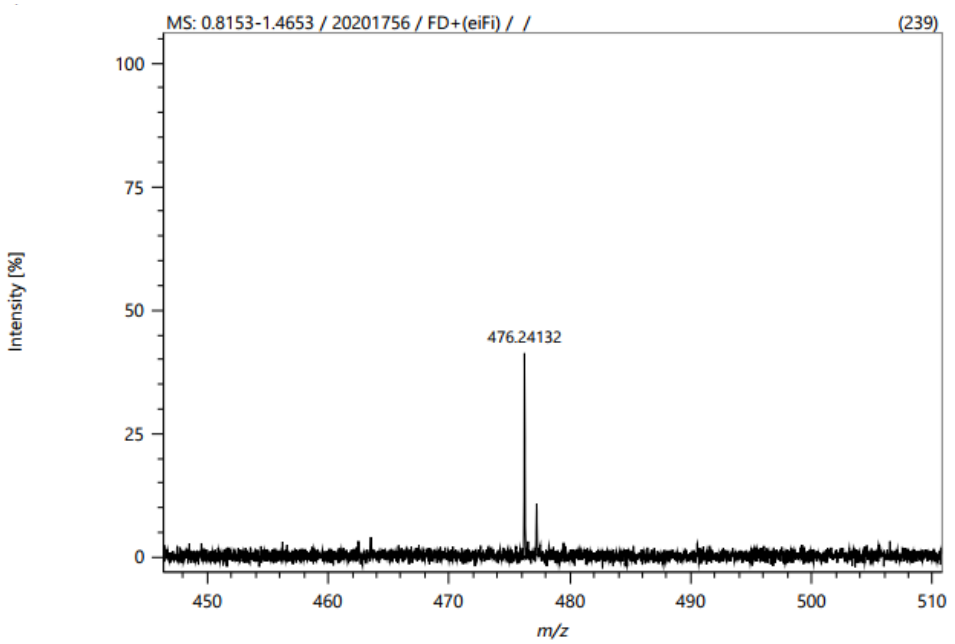


Figure S32. HMBC spectrum of **4**.



Elemental Composition

Parameters		Elements Set 1:			
Tolerance:	30.00 mDa	Symbol	C	H	O
Electron:	Odd/Even	Min	5	5	1
Charge:	+1	Max	26	36	8
DBE:	-90.0 - 90.0				

Results

Mass	Intensity	Formula	Calculated Mass	Mass Difference [mDa]	Mass Difference [ppm]	DBE
476.24132	238.71	C26 H36 O8	476.24047	0.85	1.78	9.0

Figure S33. HRFDMS spectrum of **4**.

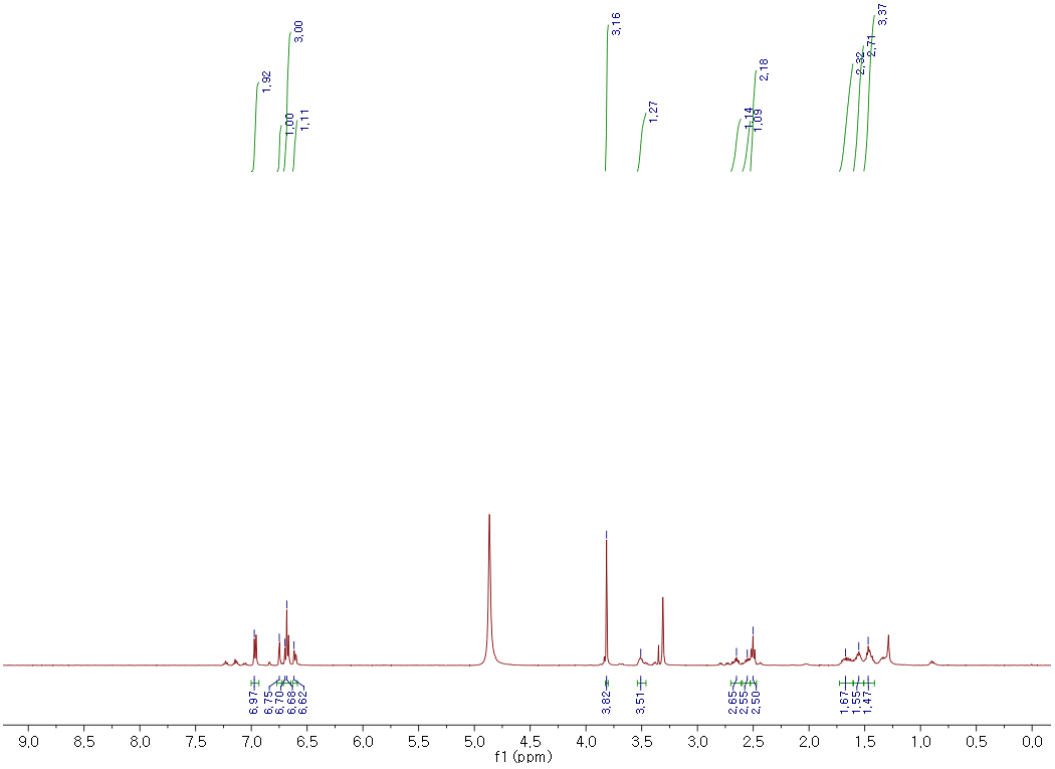


Figure S34. ^{1}H-NMR (CD_3OD, 500 MHz) spectrum of **5**.

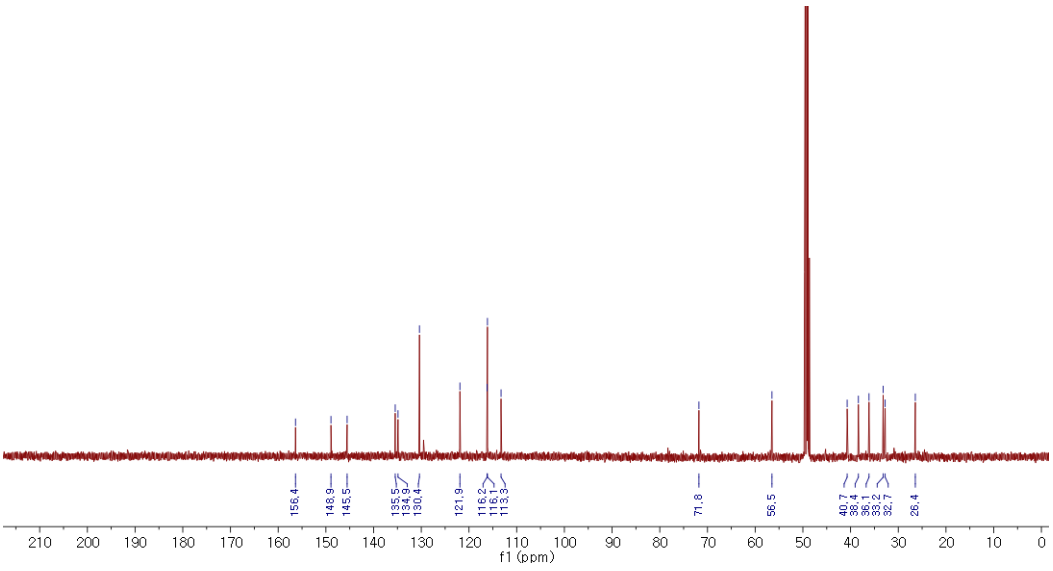


Figure S35. ^{13}C-NMR (CD_3OD, 125 MHz) spectrum of **5**.

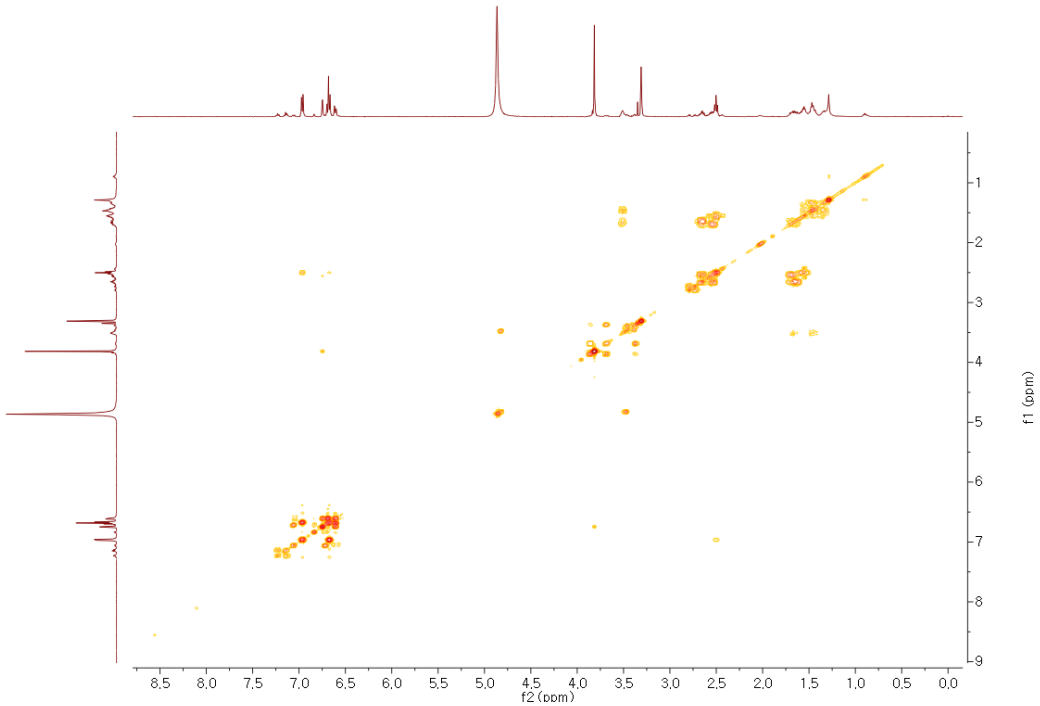


Figure S36. COSY spectrum of **5**.

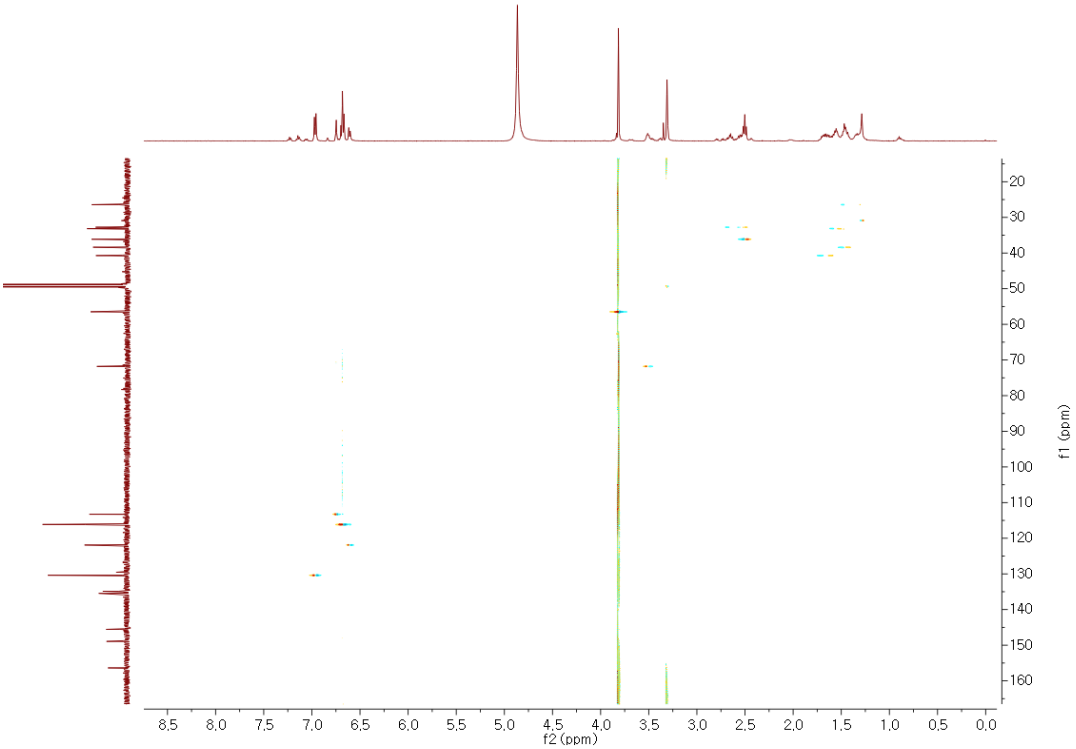


Figure S37. HSQC spectrum of **5**.

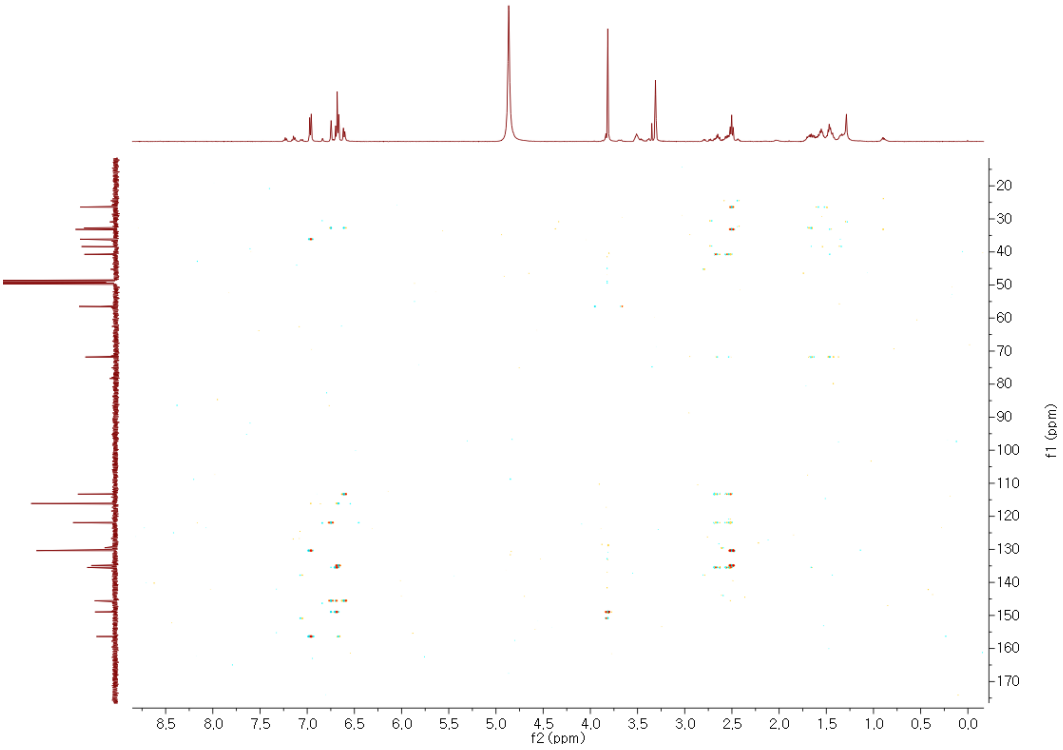
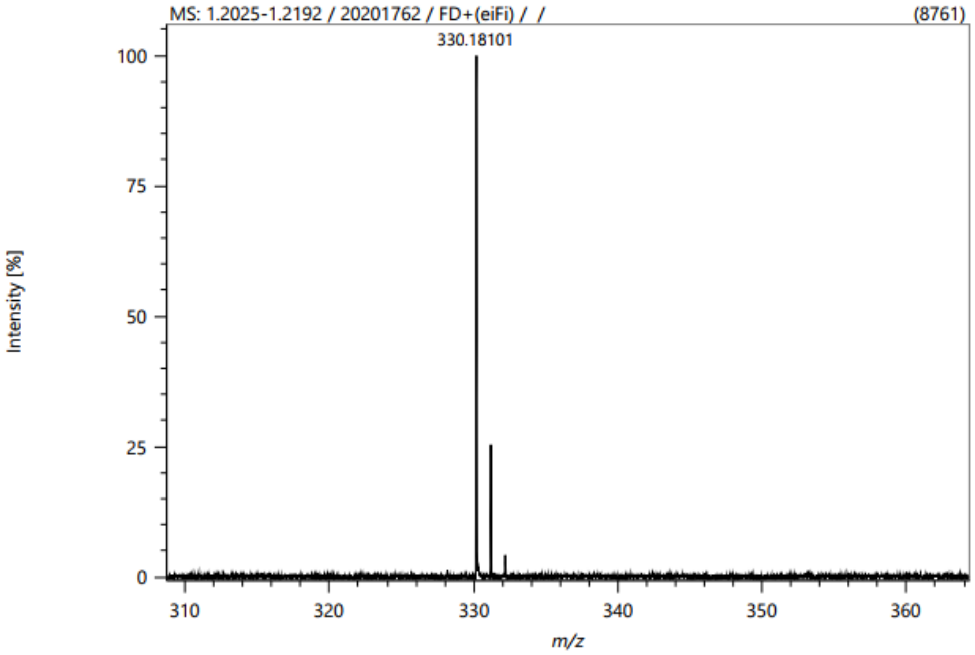


Figure S38. HMBC spectrum of **5**.



Elemental Composition

Parameters		Elements Set 1:			
Tolerance:	30.00 mDa	Symbol	C	H	O
Electron:	Odd/Even	Min	5	5	1
Charge:	+1	Max	20	26	4
DBE:	-90.0 - 90.0				

Results

Mass	Intensity	Formula	Calculated Mass	Mass Difference [mDa]	Mass Difference [ppm]	DBE
330.18101	8760.60	C20 H26 O4	330.18256	-1.55	-4.69	8.0

Figure S39. HRFDMS spectrum of **5**.

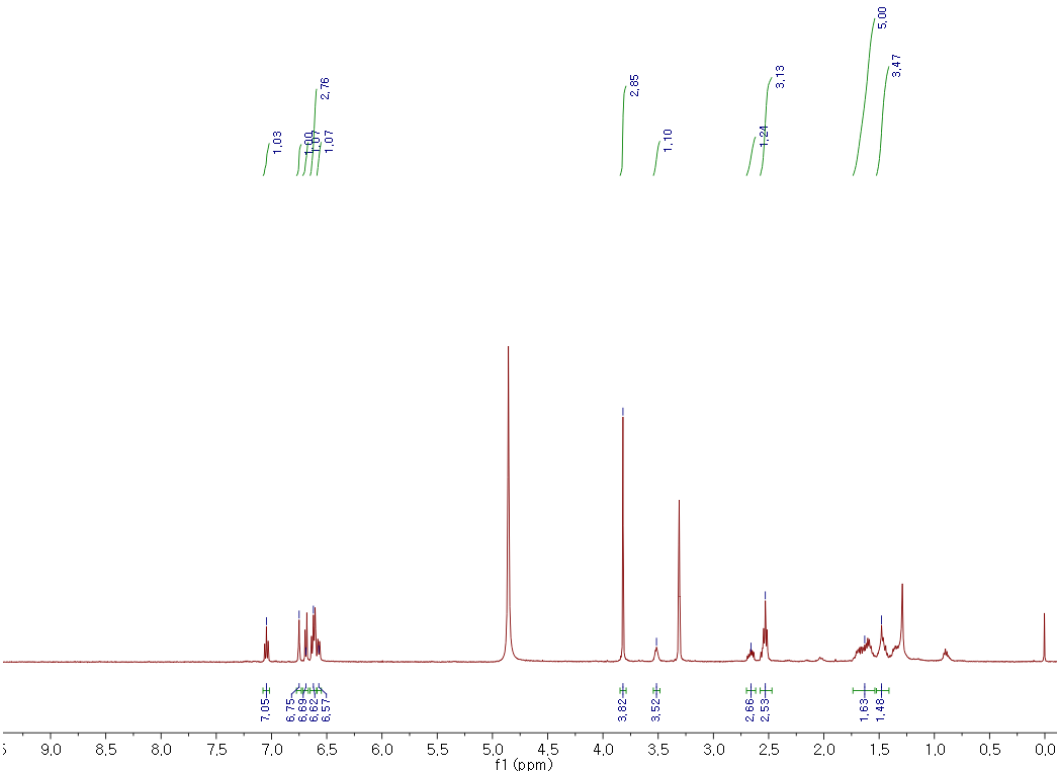


Figure S40. ^{1}H-NMR (CD_3OD, 500 MHz) spectrum of **6**.

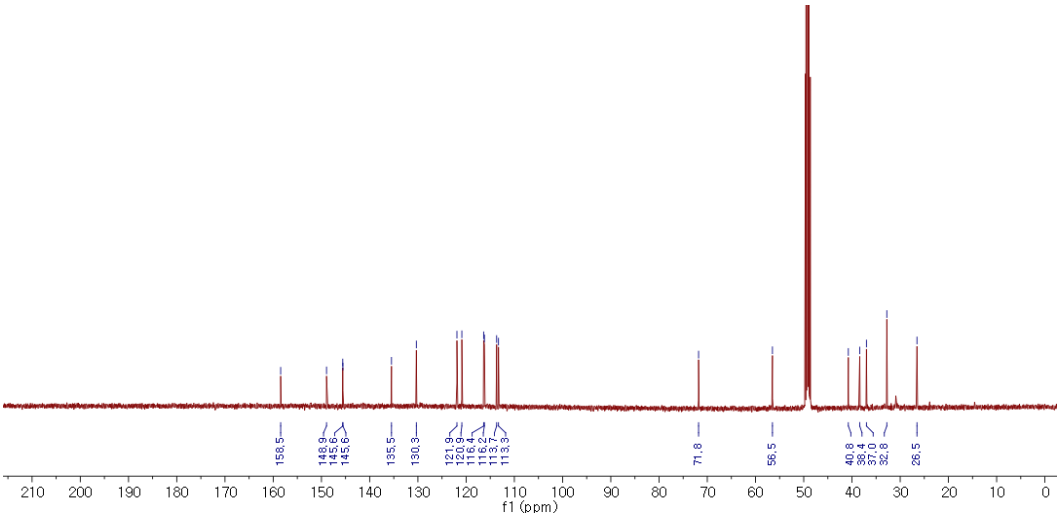


Figure S41. ^{13}C-NMR (CD_3OD, 125 MHz) spectrum of **6**.

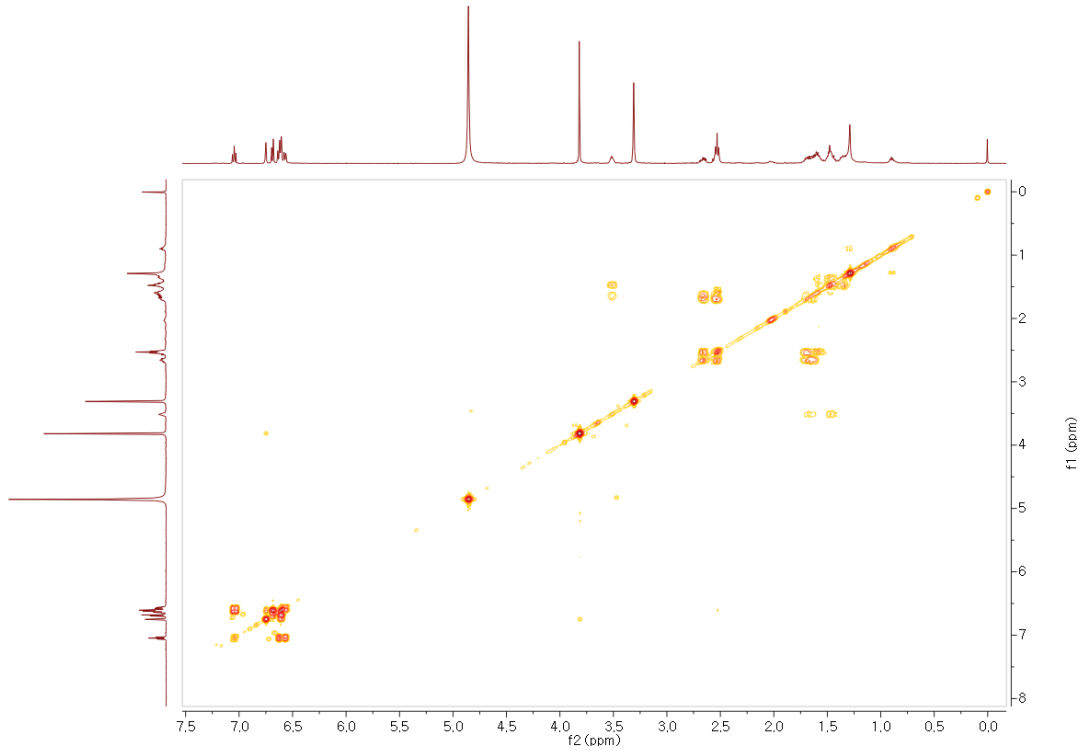


Figure S42. COSY spectrum of **6**.

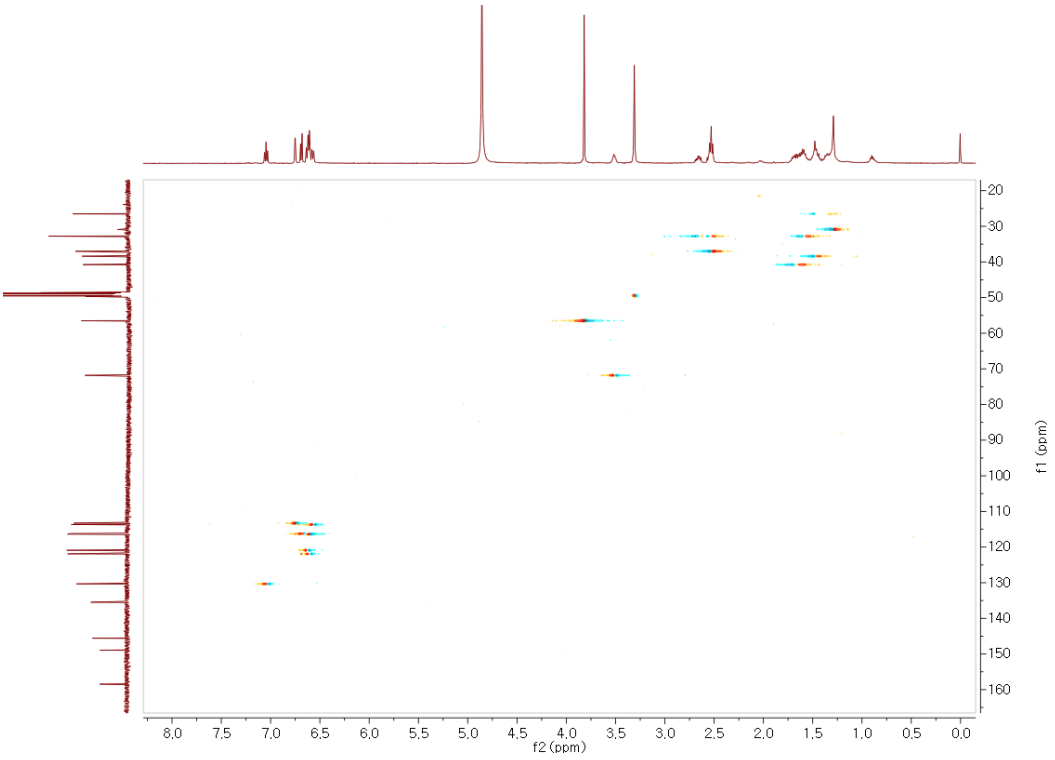


Figure S43. HSQC spectrum of **6**.

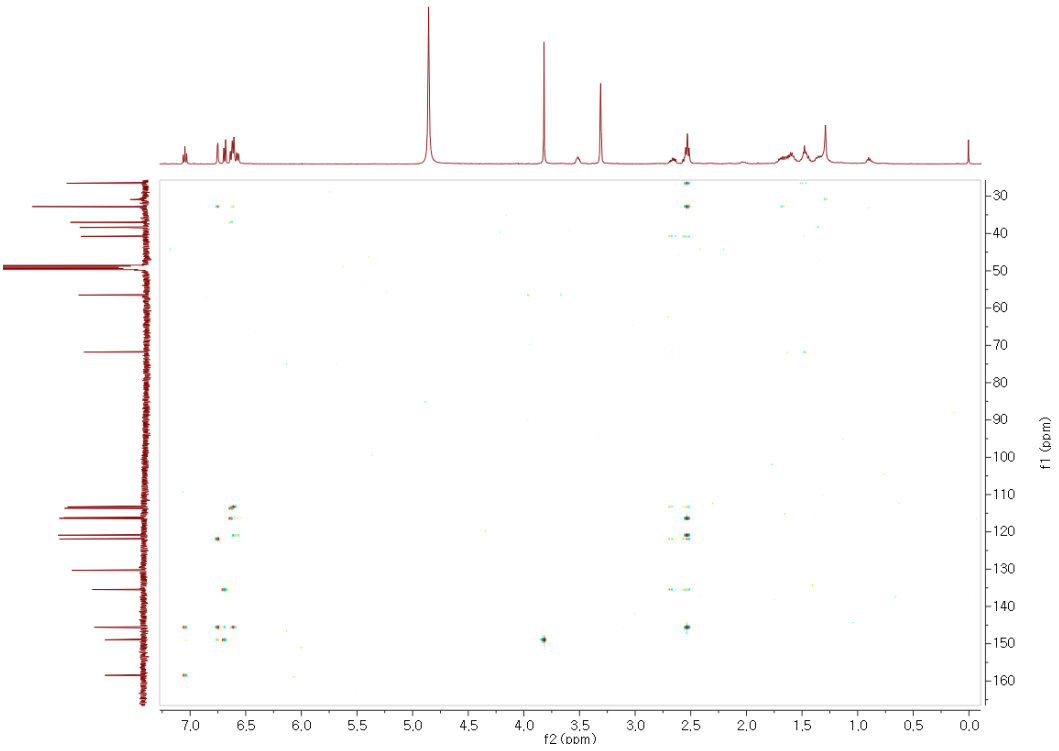
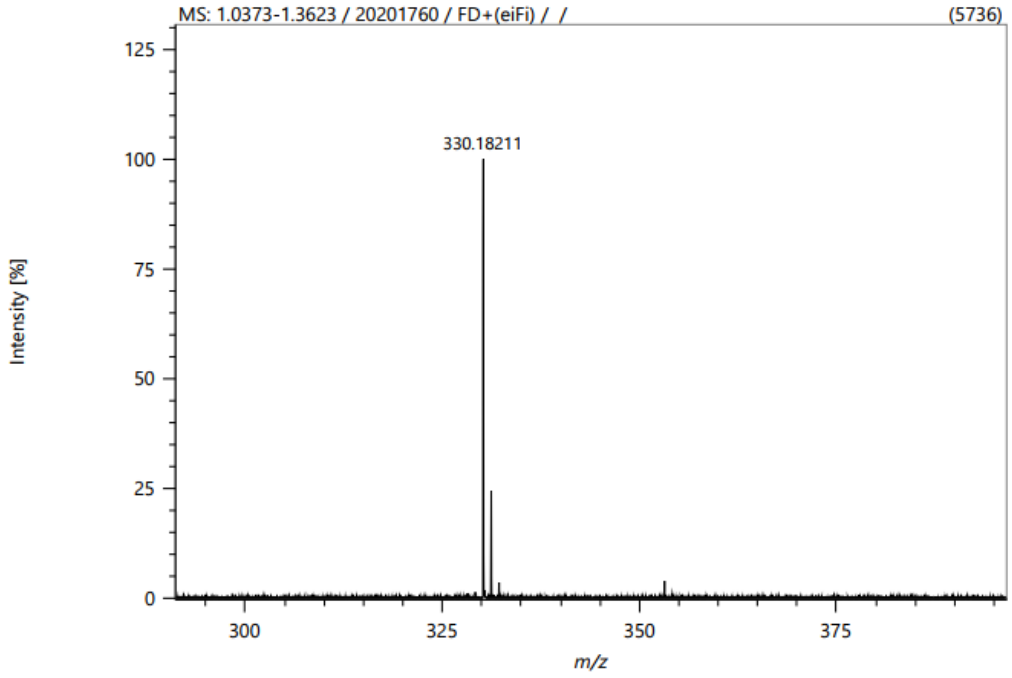


Figure S44. HMBC spectrum of **6**.



Elemental Composition

Parameters		Elements Set 1:			
Tolerance:	30.00 mDa	Symbol	C	H	O
Electron:	Odd/Even	Min	5	5	1
Charge:	+1	Max	20	26	4
DBE:	-90.0 - 90.0				

Results

Mass	Intensity	Formula	Calculated Mass	Mass Difference [mDa]	Mass Difference [ppm]	DBE
330.18211	5736.45	C20 H26 O4	330.18256	-0.45	-1.36	8.0

Figure S45. HRFDMS spectrum of **6**.

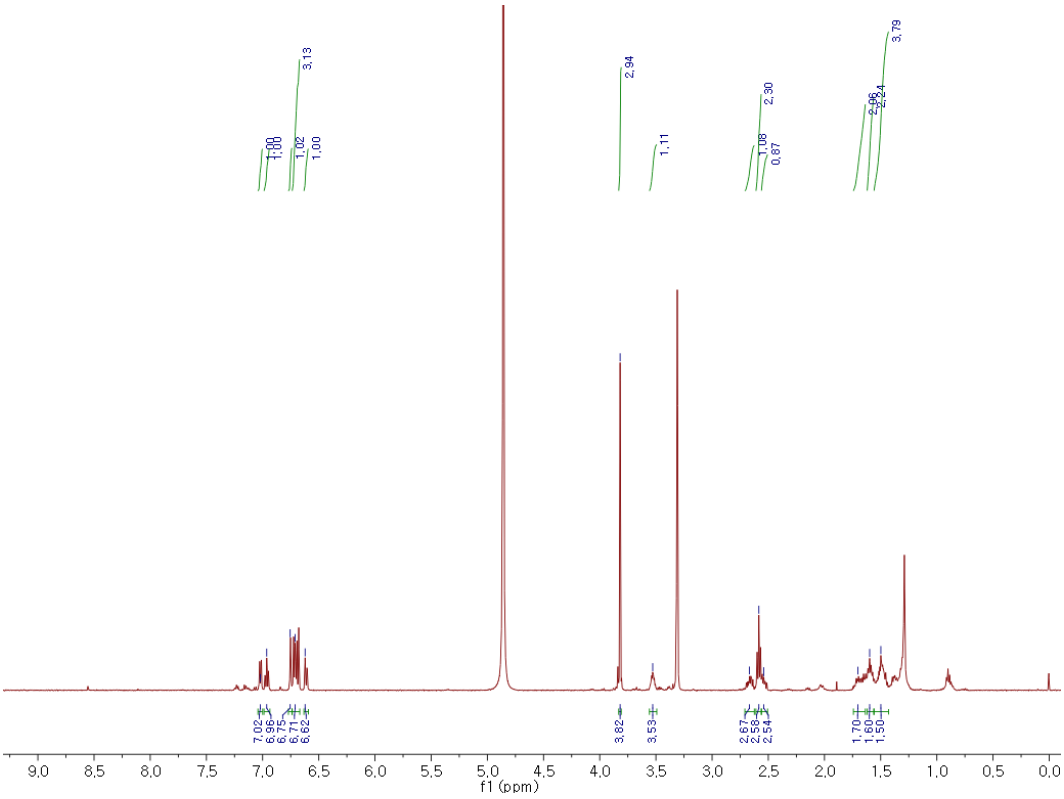


Figure S46. ^{1}H-NMR (CD_3OD, 500 MHz) spectrum of **7**.

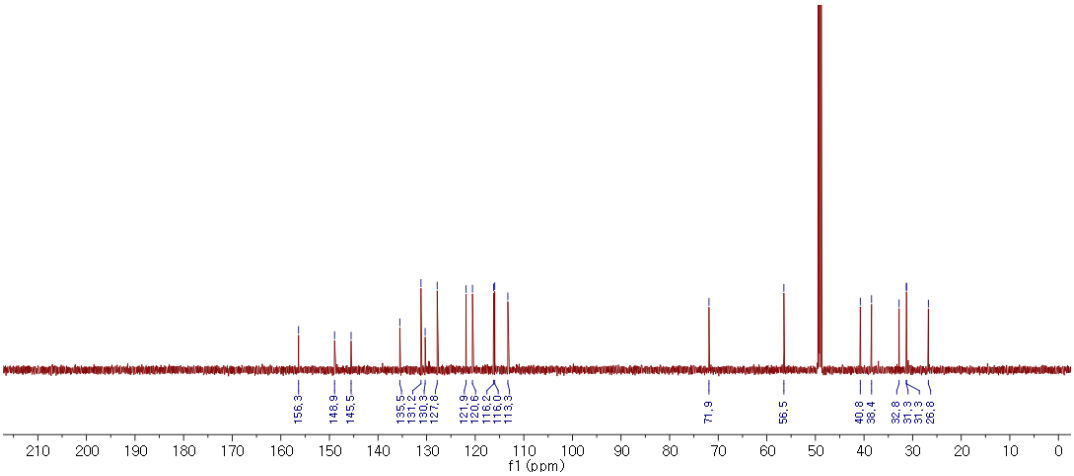


Figure S47. ^{13}C-NMR (CD_3OD, 125 MHz) spectrum of **7**.

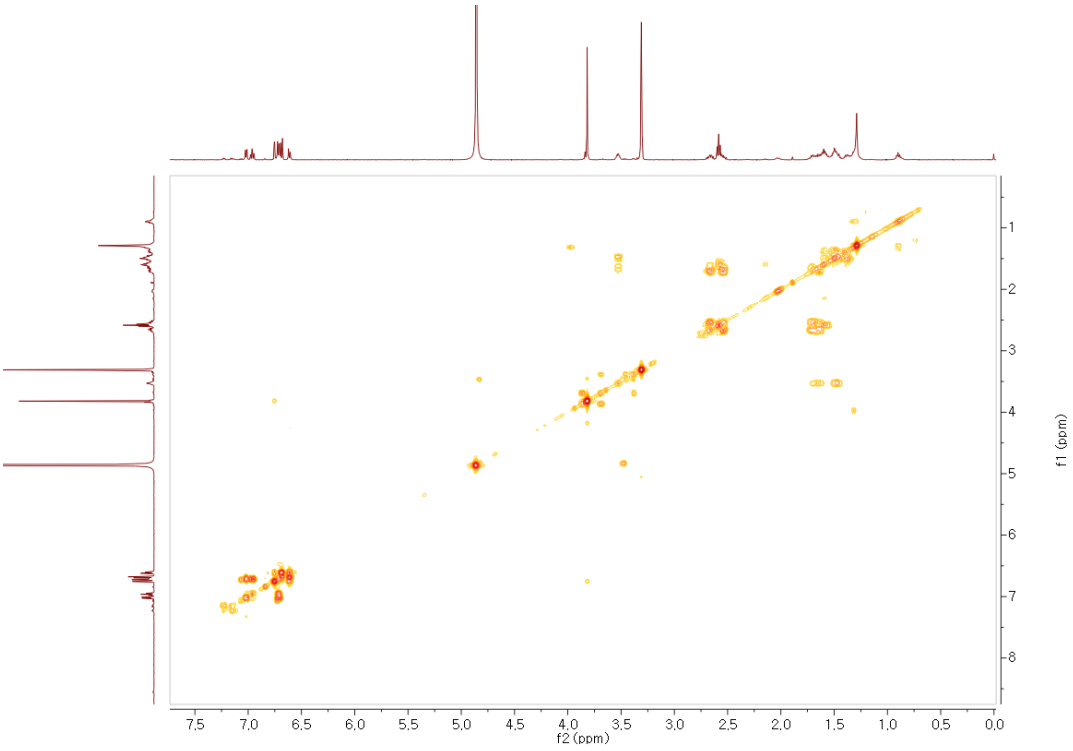


Figure S48. COSY spectrum of **7**.

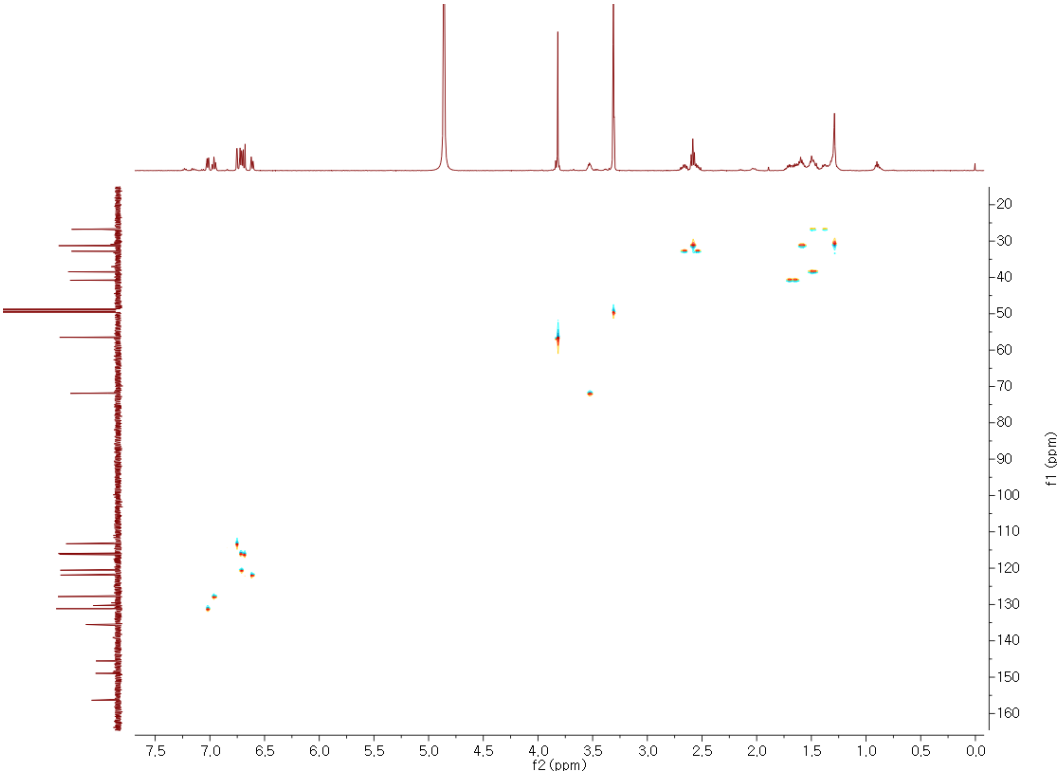


Figure S49. HSQC spectrum of **7**.

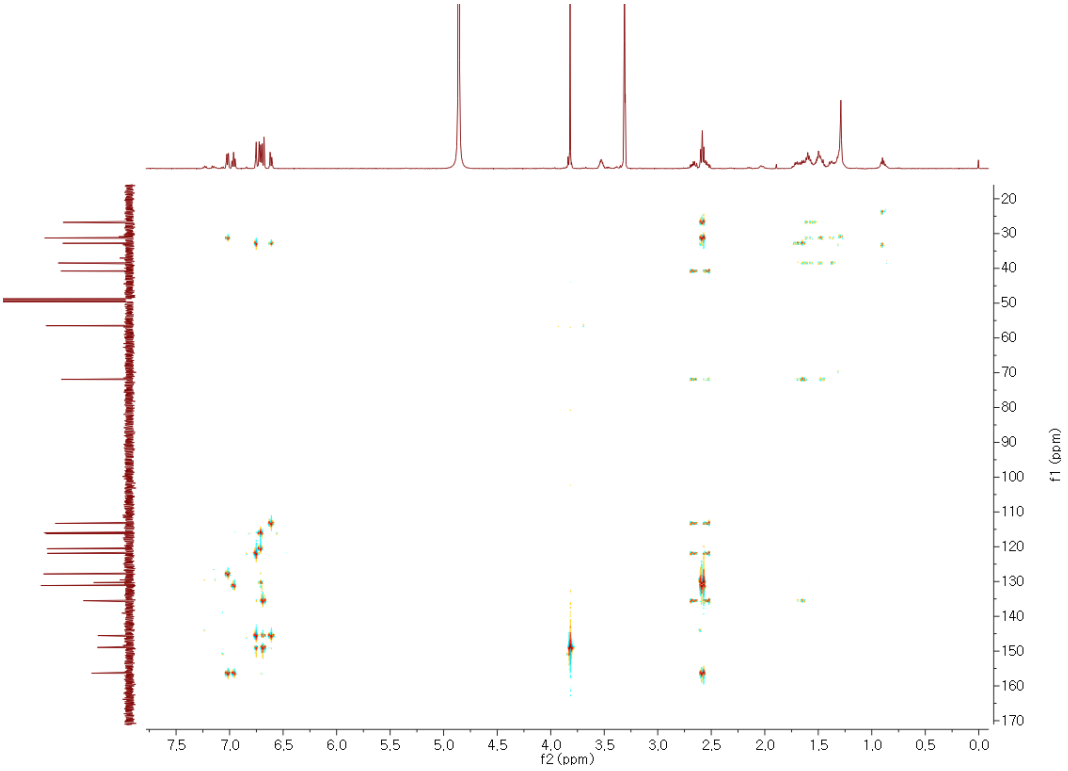
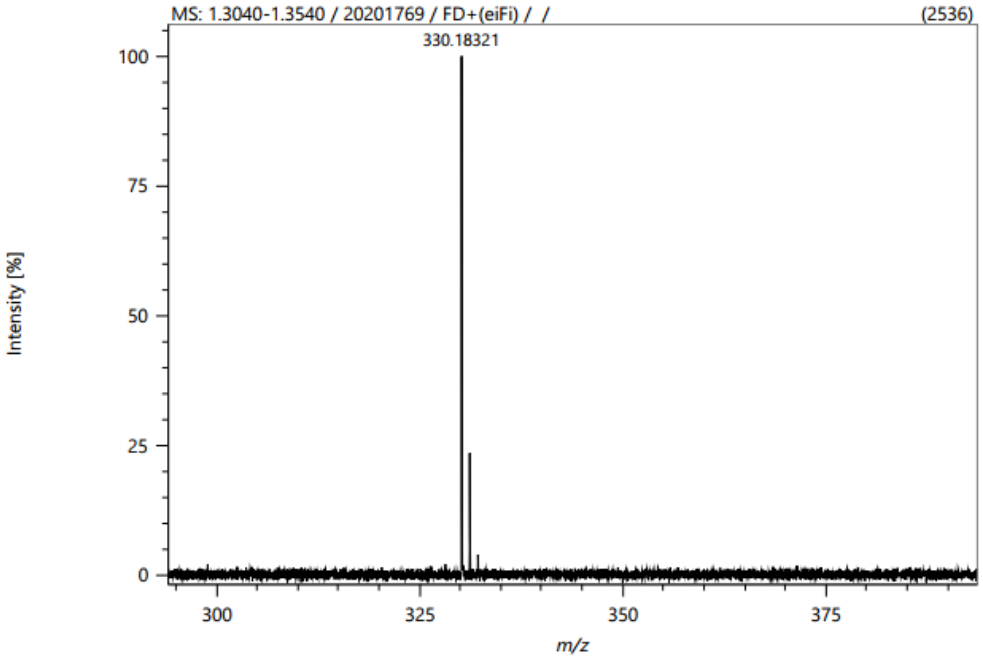


Figure S50. HMBC spectrum of **7**.



Elemental Composition

Parameters		Elements Set 1:			
Tolerance:	30.00 mDa	Symbol	C	H	O
Electron:	Odd/Even	Min	5	5	1
Charge:	+1	Max	20	26	4
DBE:	-90.0 - 90.0				

Results

Mass	Intensity	Formula	Calculated Mass	Mass Difference [mDa]	Mass Difference [ppm]	DBE
330.18321	2535.95	C20 H26 O4	330.18256	0.65	1.97	8.0

Figure S51. HRFDMS spectrum of **7**.

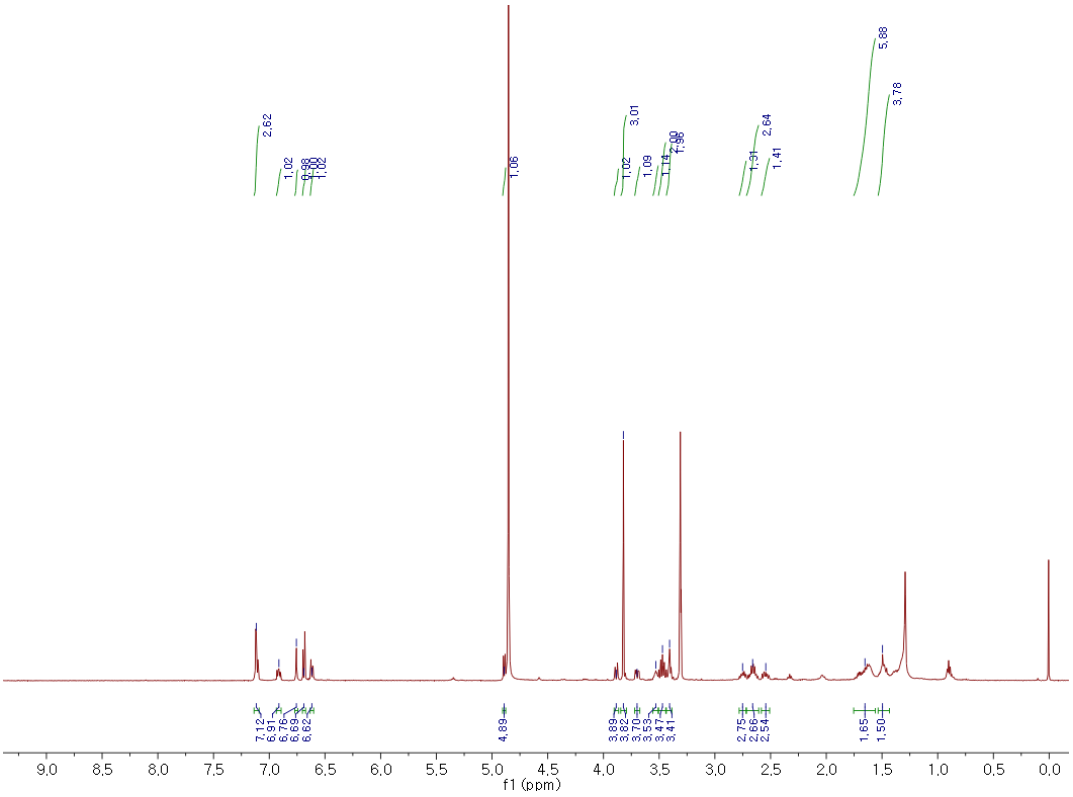


Figure S52. ^{1}H-NMR (CD_3OD, 500 MHz) spectrum of **8**.

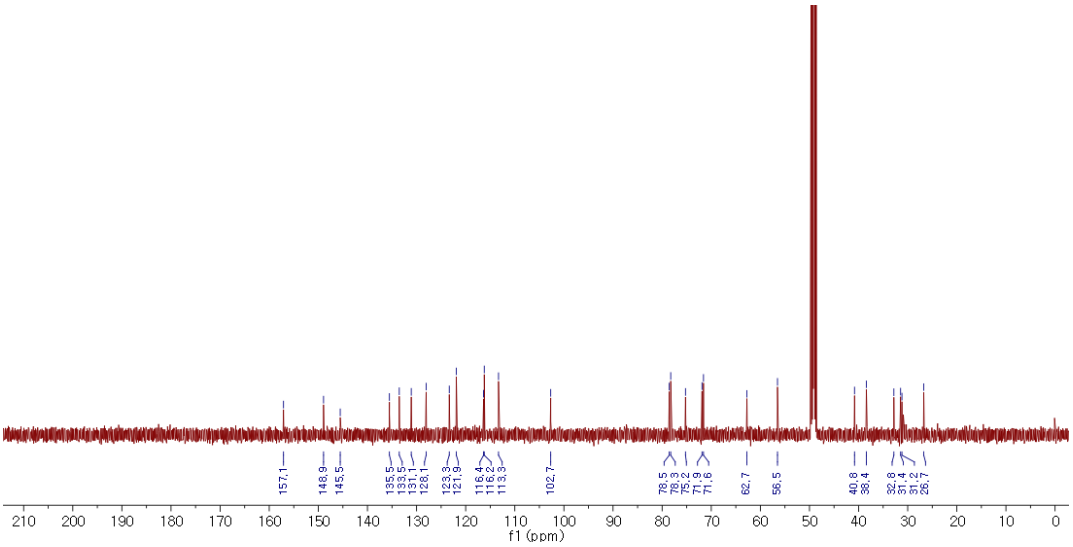


Figure S53. ^{13}C-NMR (CD_3OD, 125 MHz) spectrum of **8**.

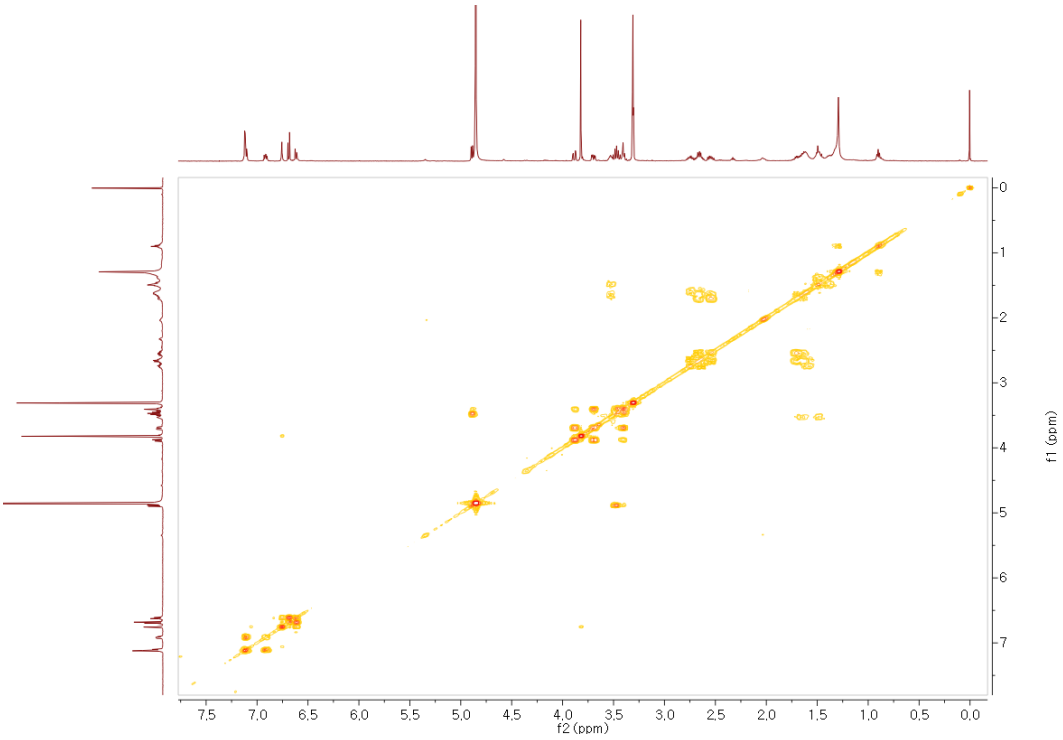


Figure S54. COSY spectrum of **8**.

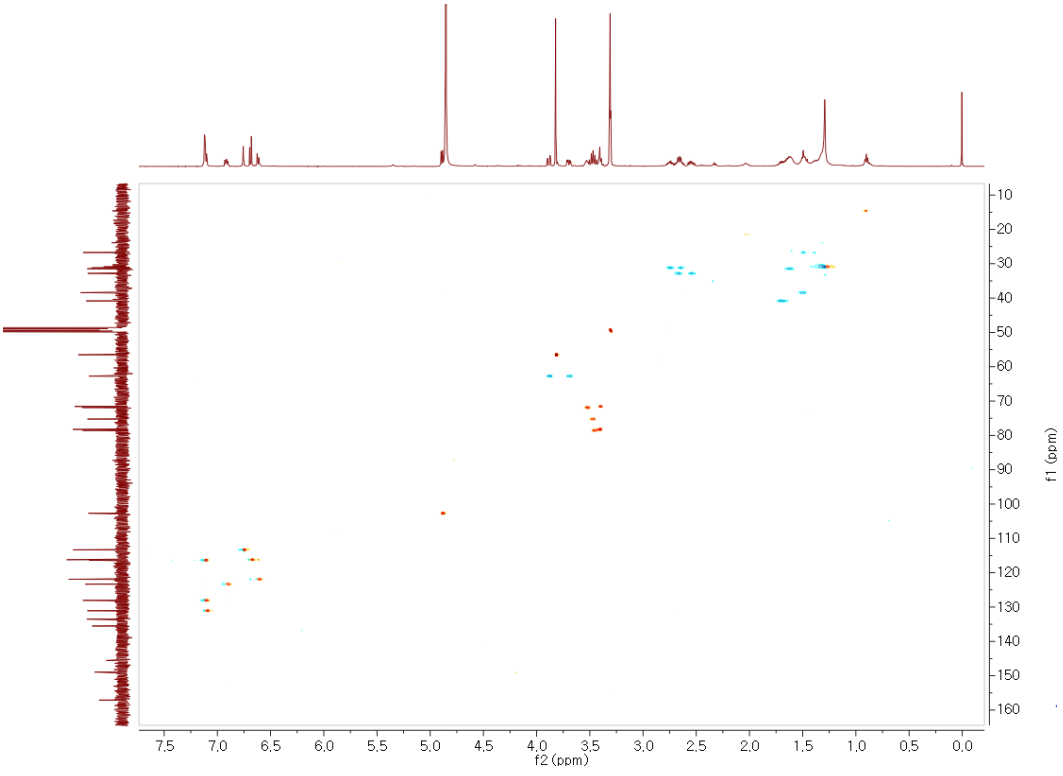


Figure S55. HSQC spectrum of **8**.

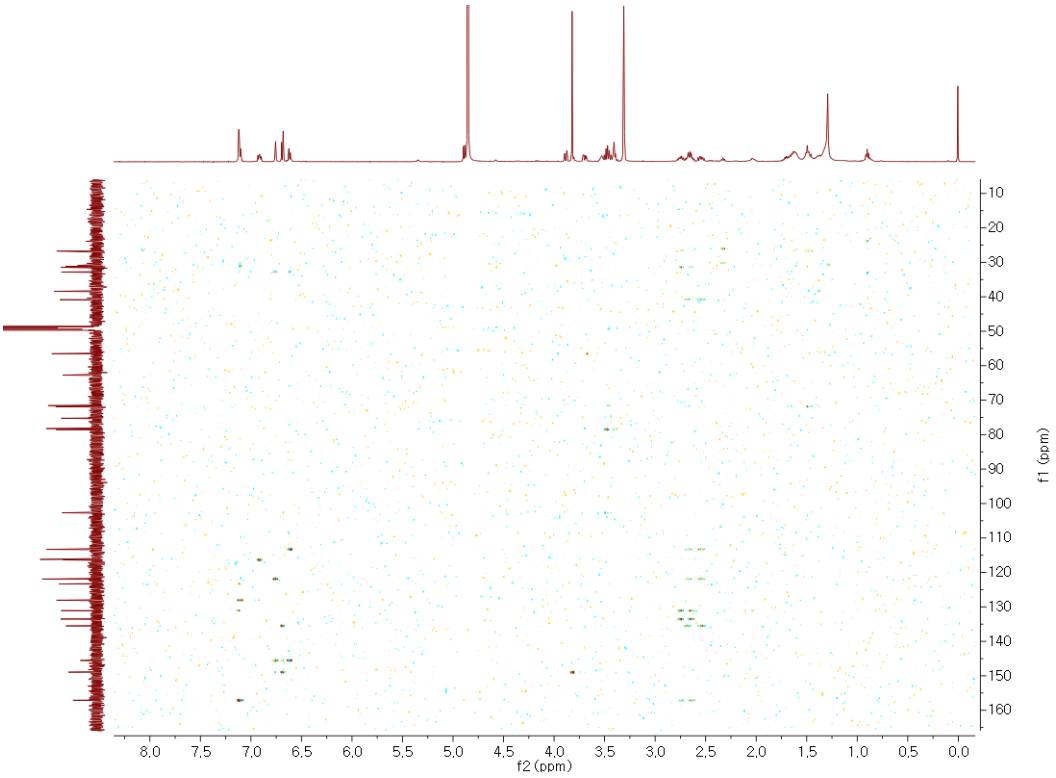
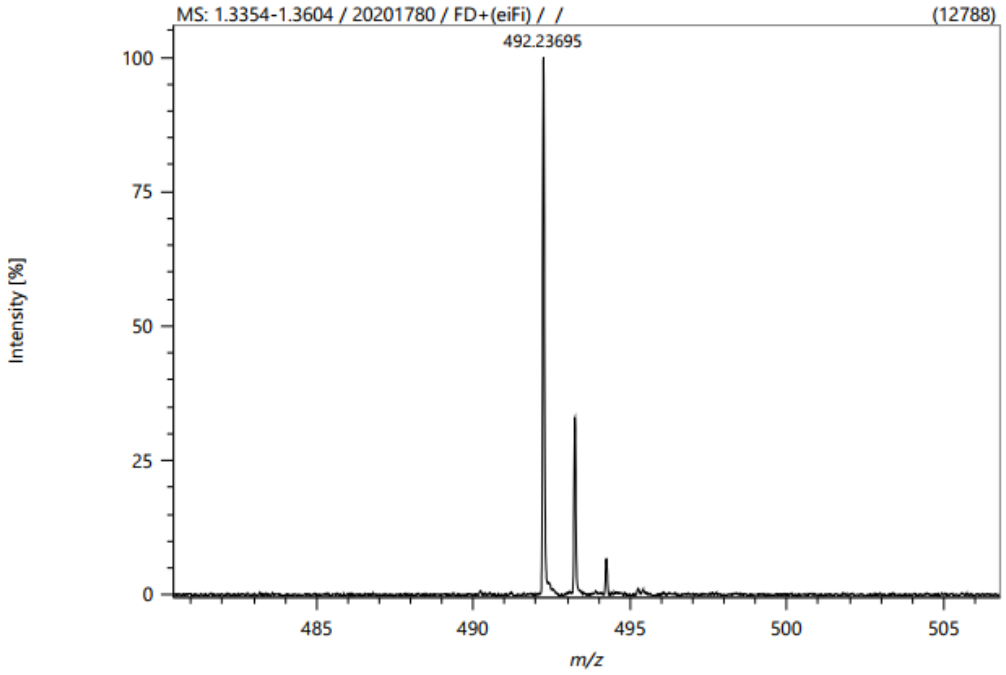


Figure S56. HMBC spectrum of **8**.



Elemental Composition

Parameters		Elements Set 1:			
Tolerance:	30.00 mDa	Symbol	C	H	O
Electron:	Odd/Even	Min	5	5	1
Charge:	+1	Max	26	36	9
DBE:	-90.0 - 90.0				

Results

Mass	Intensity	Formula	Calculated Mass	Mass Difference [mDa]	Mass Difference [ppm]	DBE
492.23695	12787.86	C26 H36 O9	492.23538	1.56	3.17	9.0

Figure S57. HRFDMS spectrum of **8**.

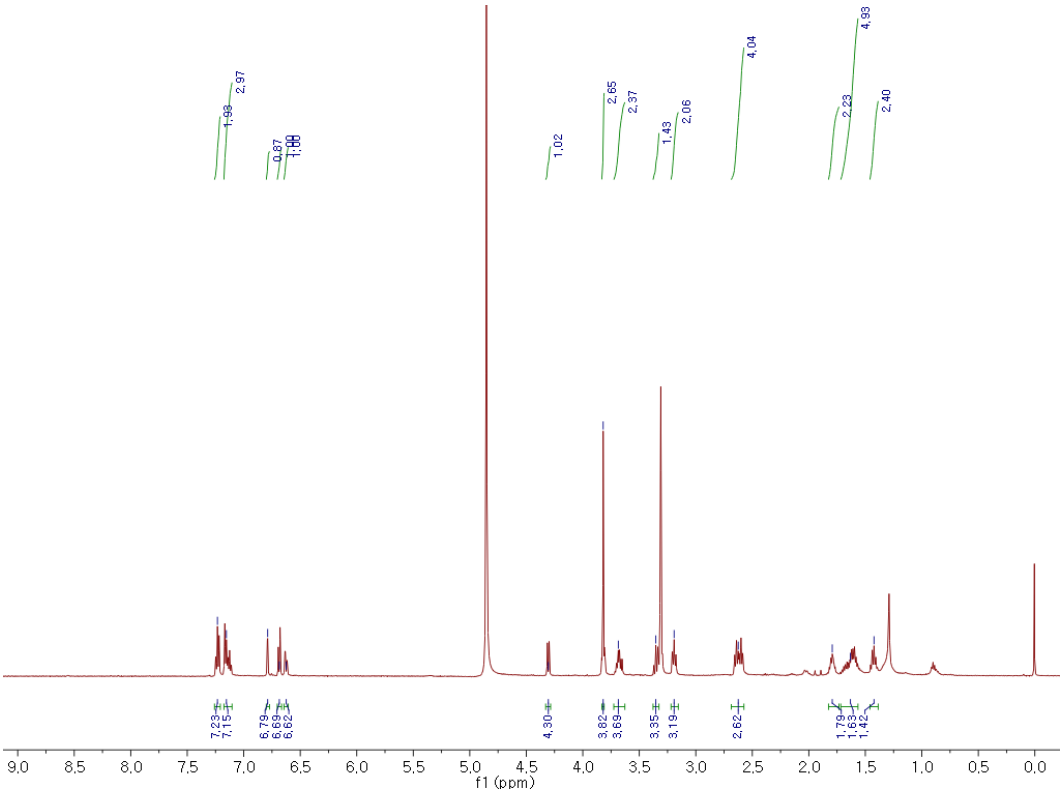


Figure S58. ^{1}H-NMR (CD_3OD, 500 MHz) spectrum of **9**.

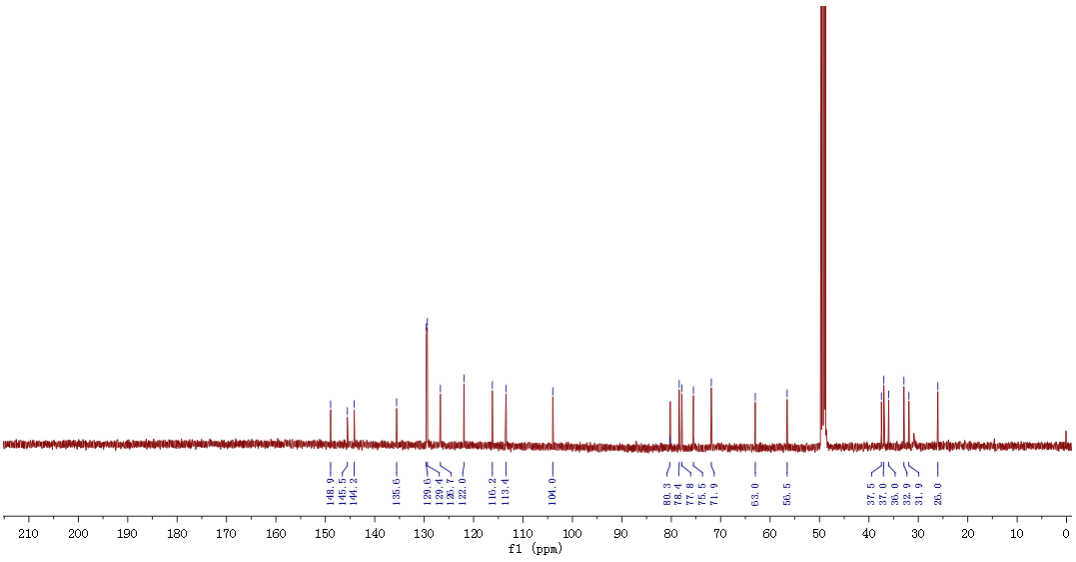


Figure S59. ^{13}C-NMR (CD_3OD, 125 MHz) spectrum of **9**.

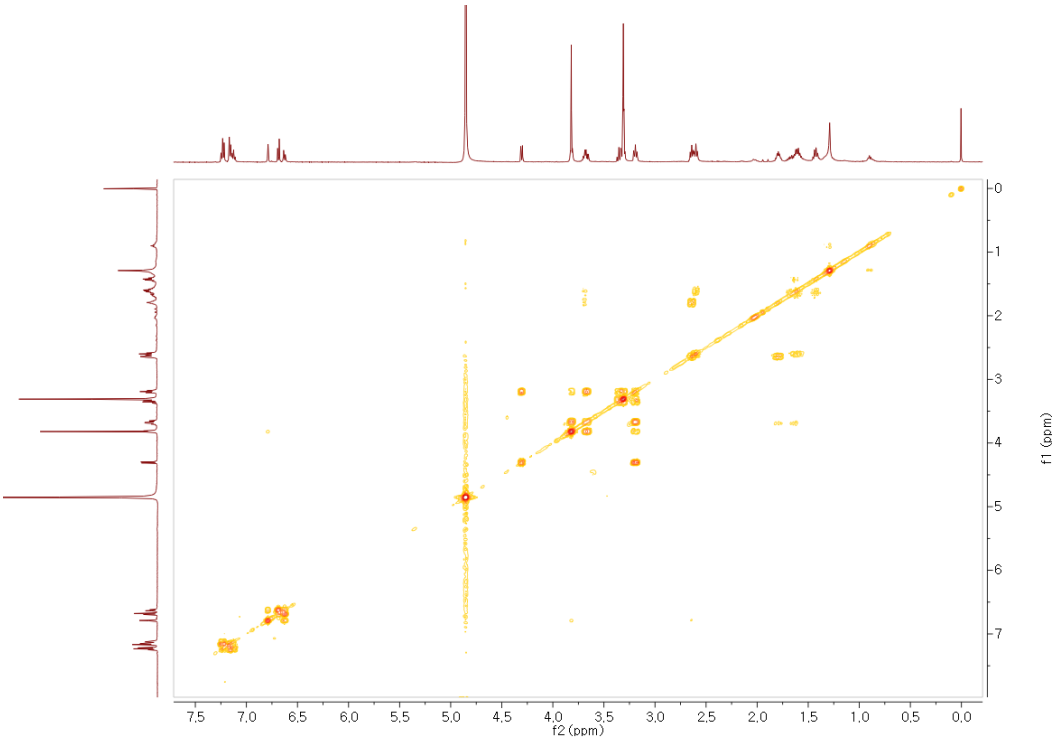


Figure S60. COSY spectrum of **9**.

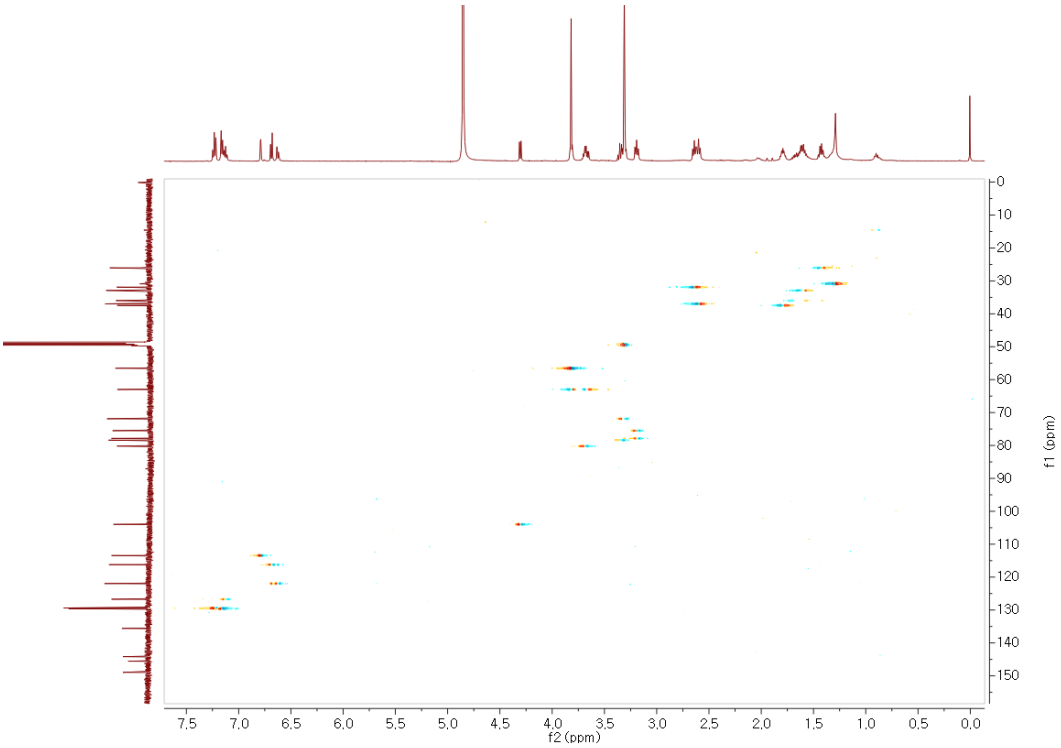


Figure S61. HSQC spectrum of **9**.

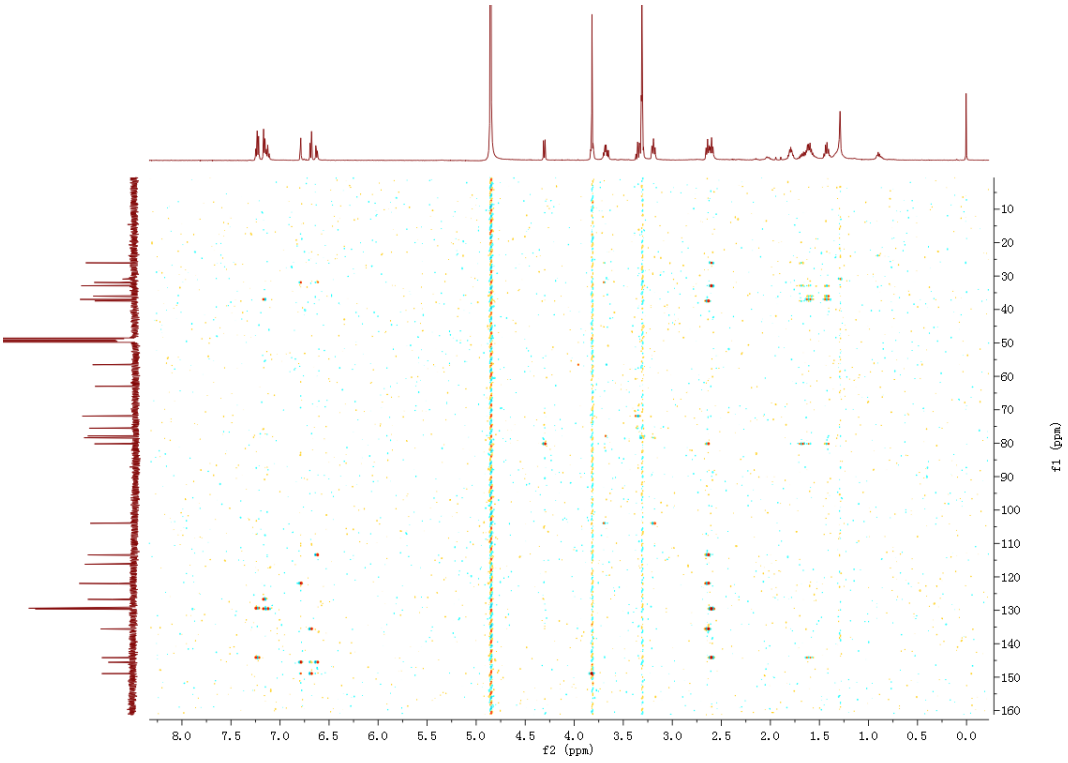
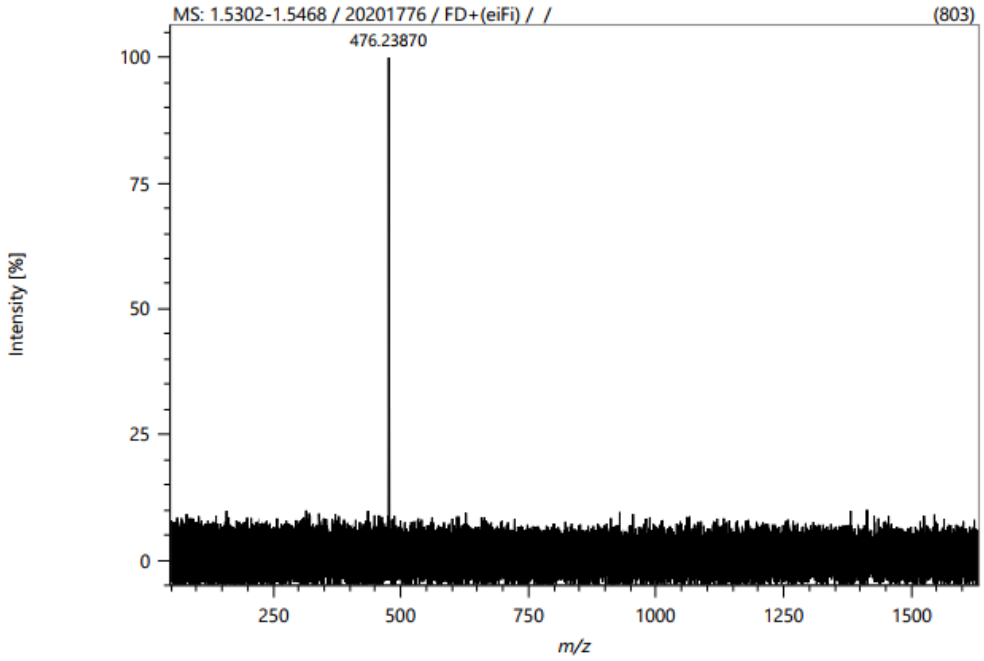


Figure S62. HMBC spectrum of **9**.



Elemental Composition

Parameters		Elements Set 1:			
Tolerance:	30.00 mDa	Symbol	C	H	O
Electron:	Odd/Even	Min	5	5	1
Charge:	+1	Max	26	36	8
DBE:	-90.0 - 90.0				

Results

Mass	Intensity	Formula	Calculated Mass	Mass Difference [mDa]	Mass Difference [ppm]	DBE
476.23870	802.98	C26 H36 O8	476.24047	-1.77	-3.72	9.0

Figure S63. HRFDMS spectrum of **9**.

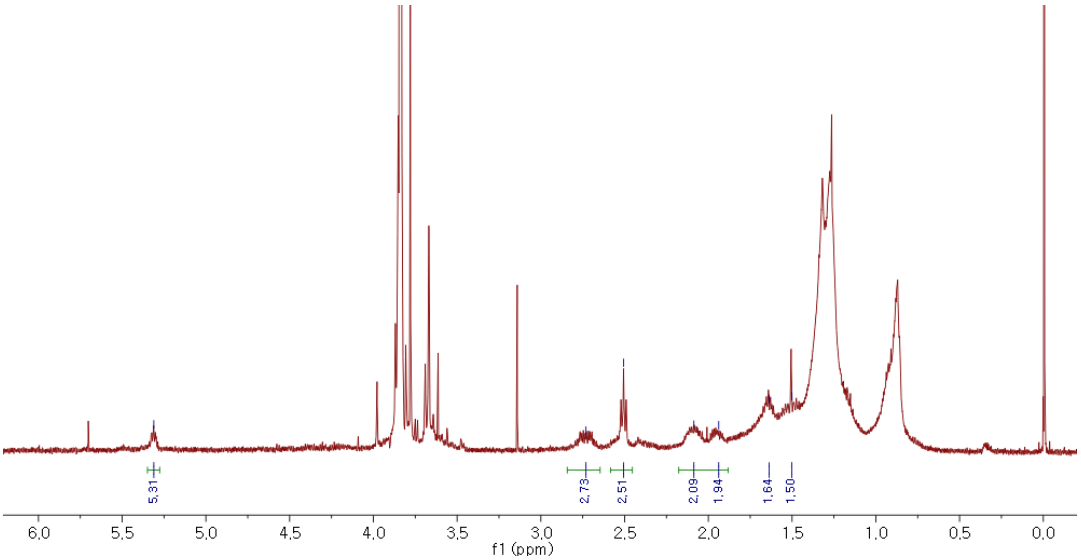


Figure S64. ^{1}H-NMR (pyridine-d_5, 500 MHz) spectrum of (*S*)-MTPA ester of **2**.

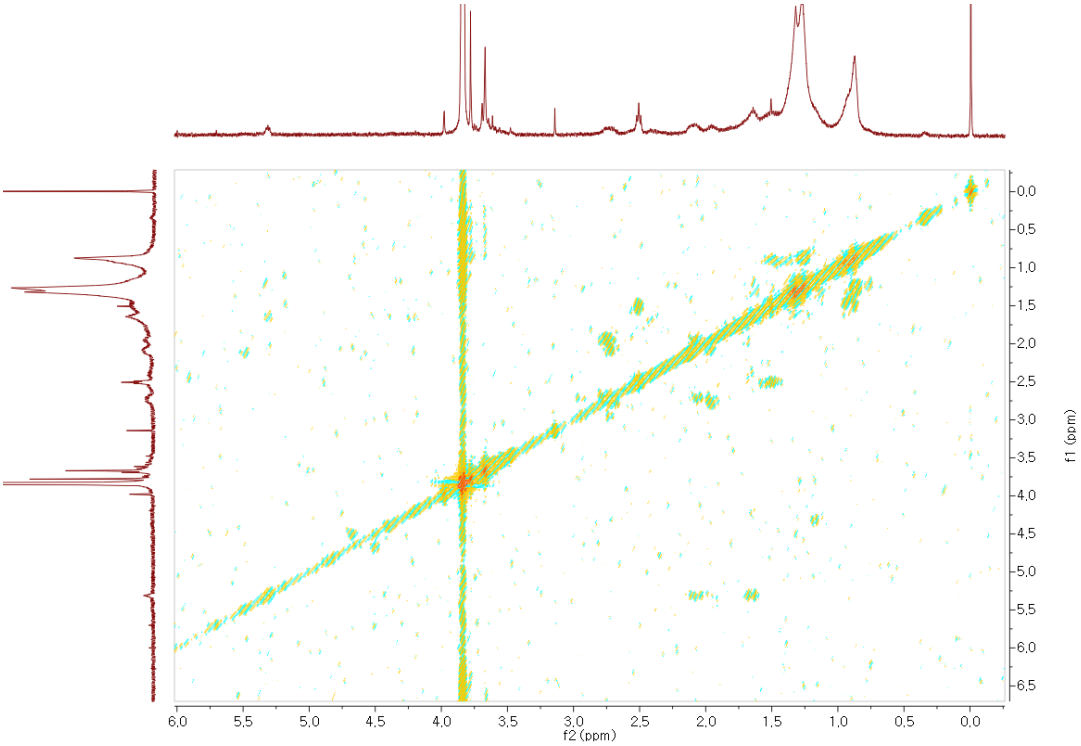


Figure S65. COSY spectrum of (*S*)-MTPA ester of **2**.

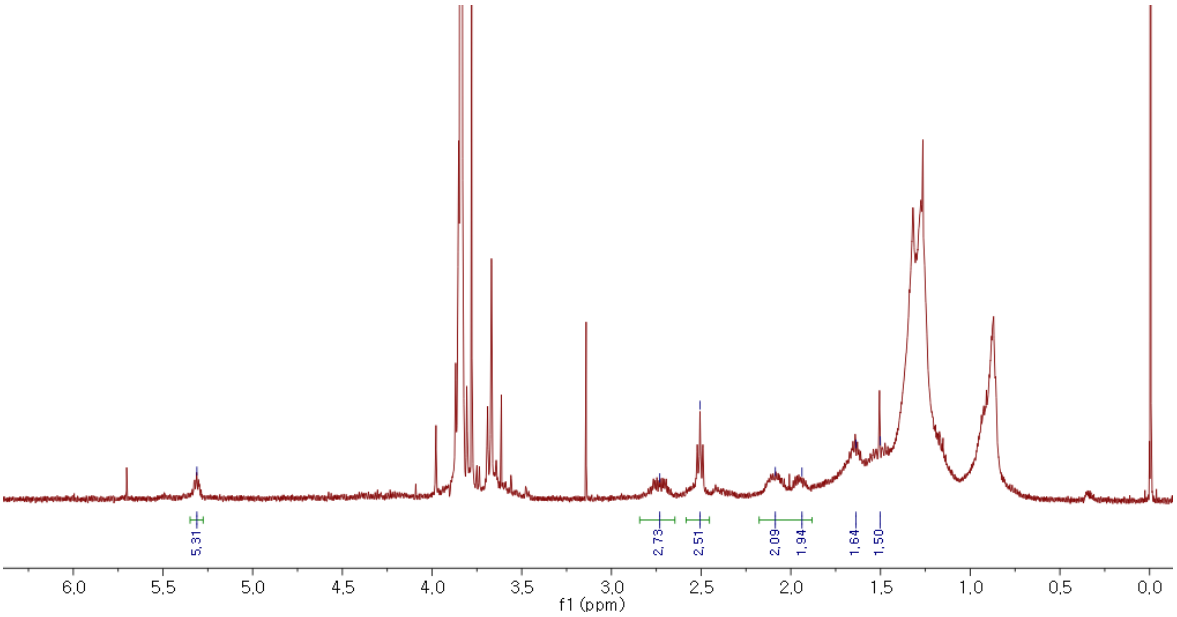


Figure S66. ^{1}H-NMR (pyridine-d_5, 500 MHz) spectrum of (*R*)-MTPA ester of **2**.

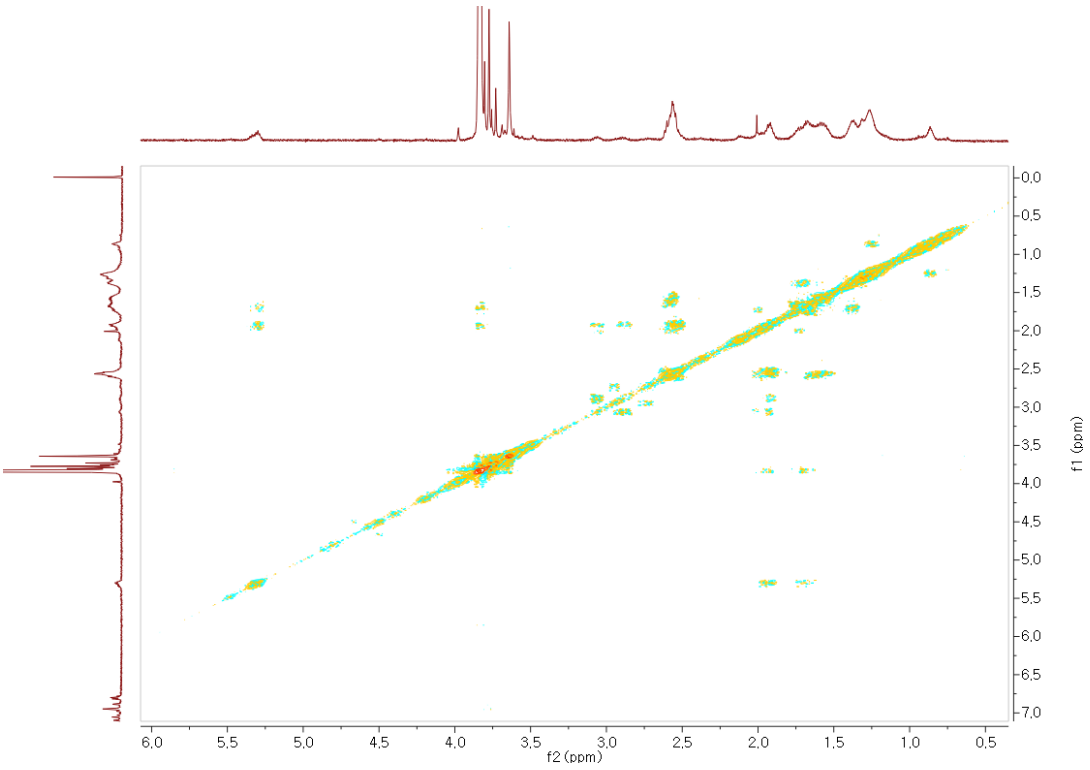


Figure S67. COSY spectrum of (*R*)-MTPA ester of **2**.

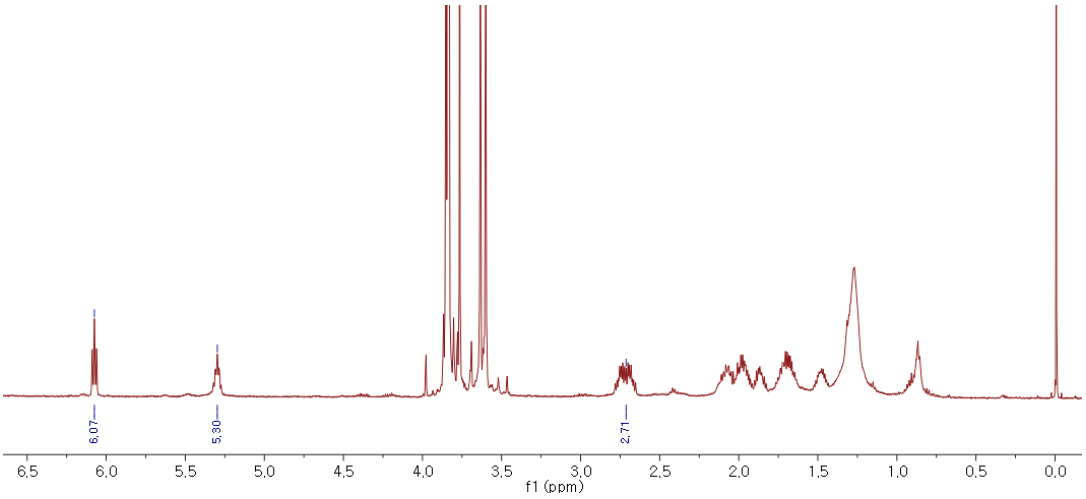


Figure S68. ^{1}H-NMR (pyridine-d_5, 500 MHz) spectrum of (*S*)-MTPA ester of **3a**.

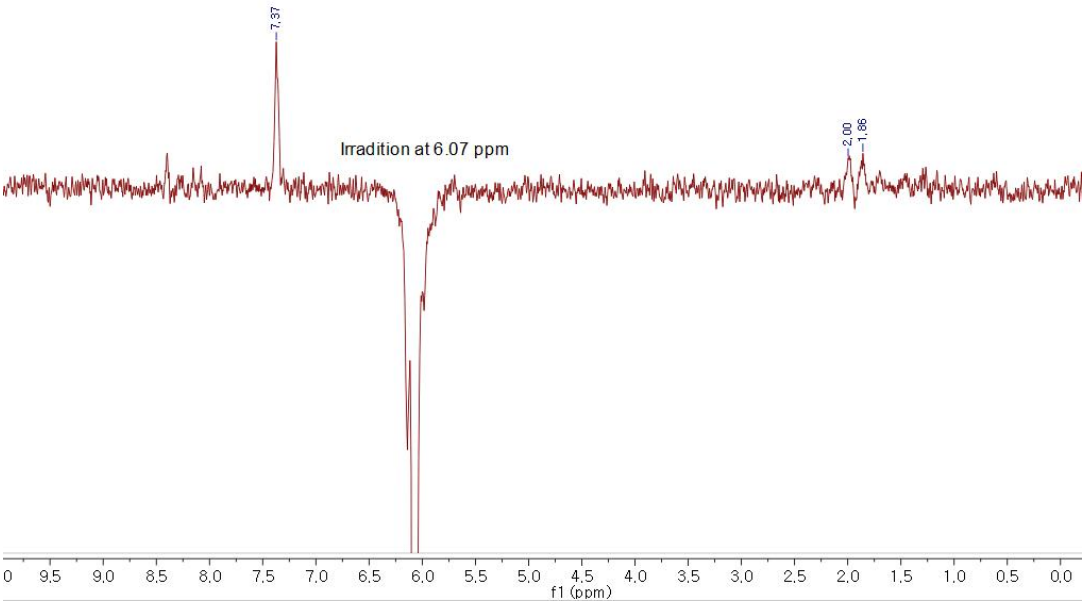


Figure S69. NOE spectrum of (*S*)-MTPA ester of **3a**.

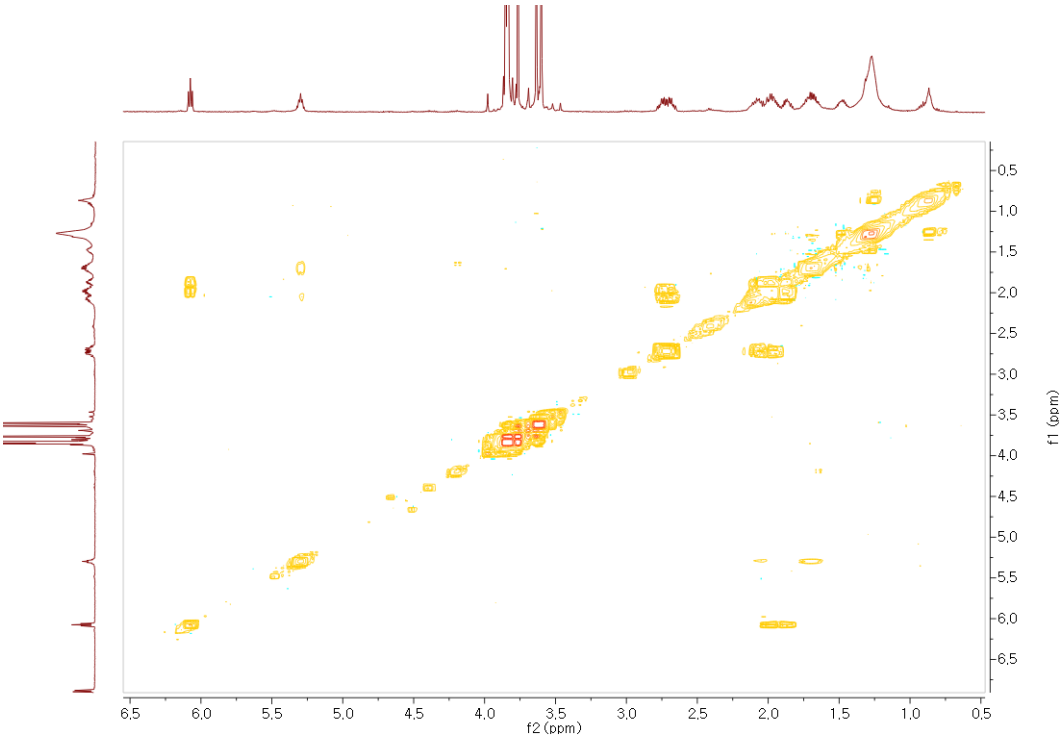


Figure S70. COSY spectrum of (*S*)-MTPA ester of **3a**.

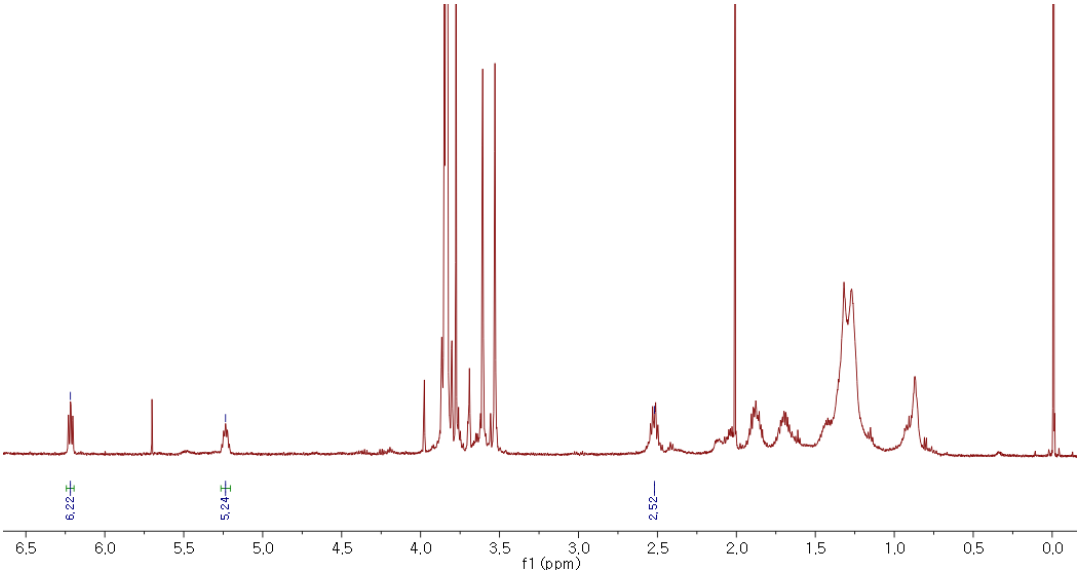


Figure S71. ^{1}H-NMR (pyridine-d_5, 500 MHz) spectrum of (*R*)-MTPA ester of **3a**.

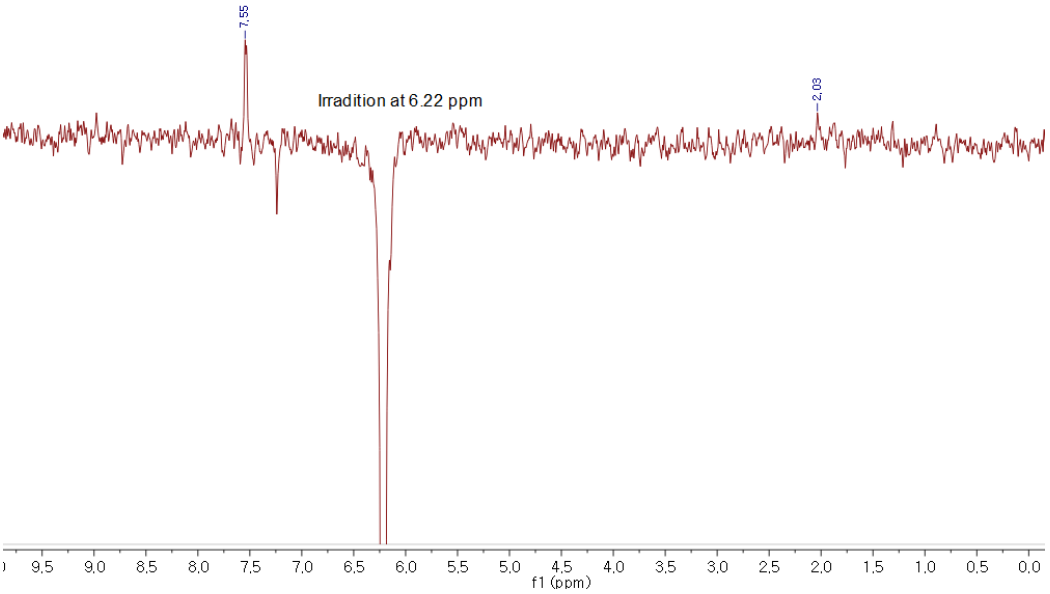


Figure S72. NOE spectrum of (*R*)-MTPA ester of **3a**.

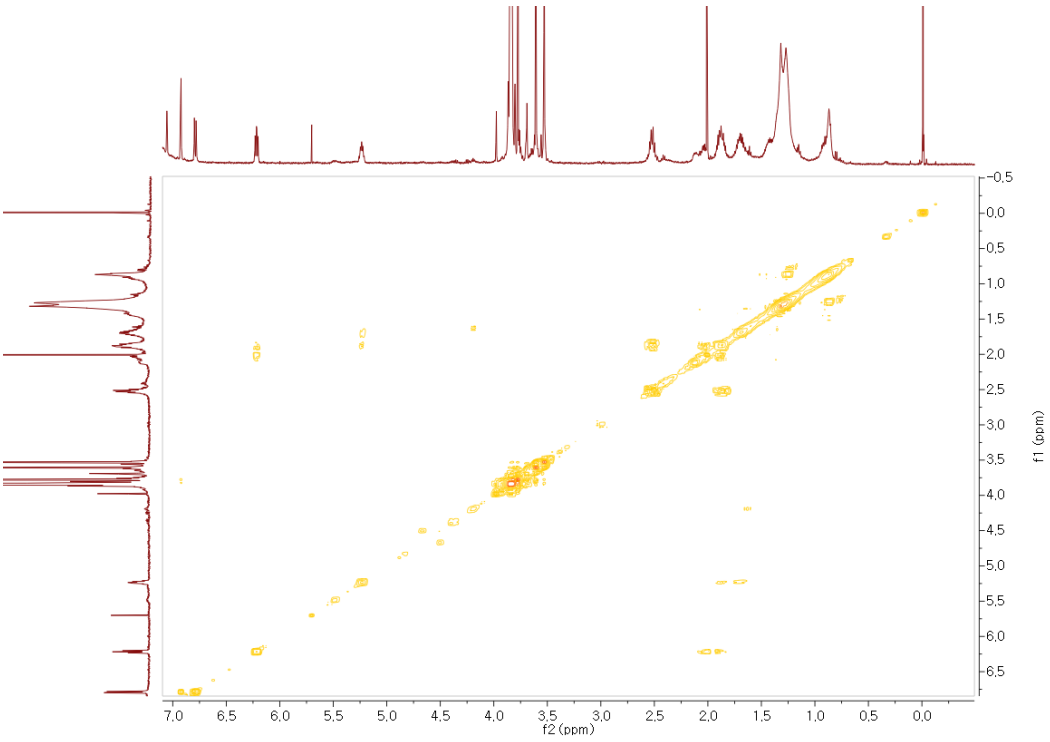


Figure S73. COSY spectrum of (*R*)-MTPA ester of **3a**.

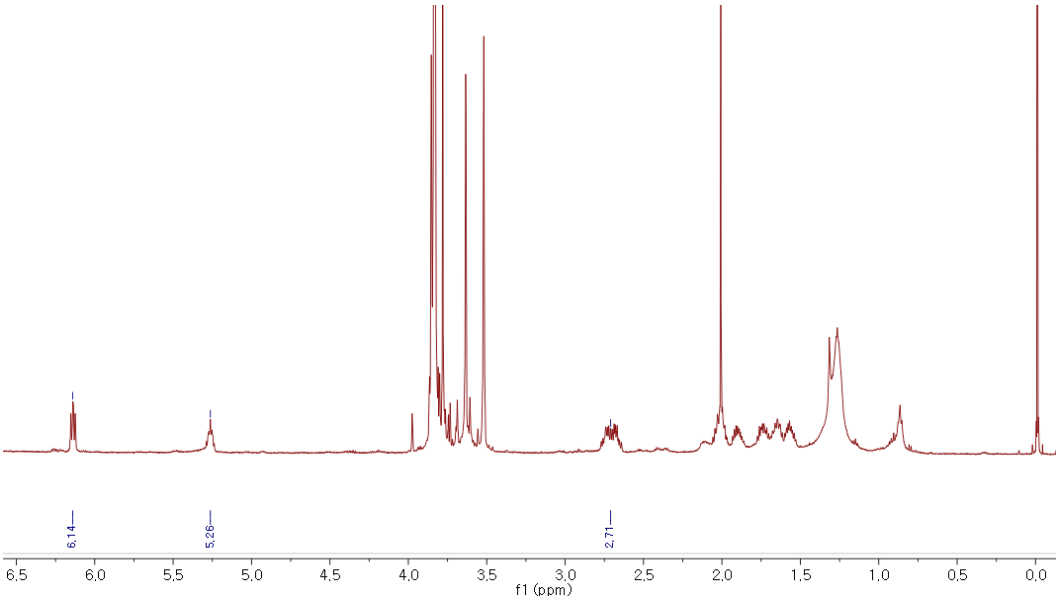


Figure S74. ^{1}H-NMR (pyridine-d_5, 500 MHz) spectrum of (*S*)-MTPA ester of **3b**.

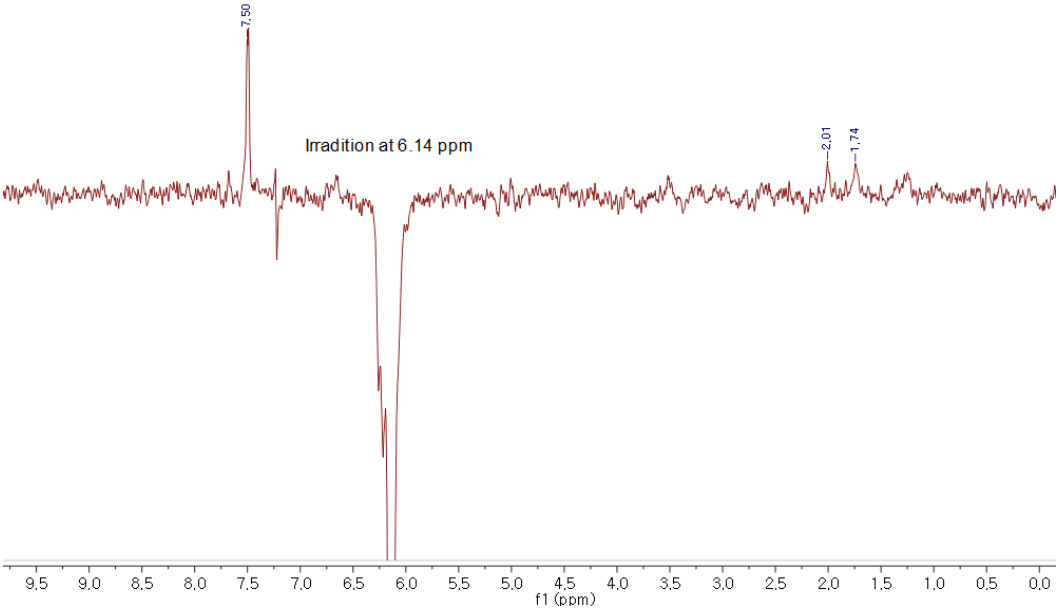


Figure S75. NOE spectrum of (*S*)-MTPA ester of **3b**.

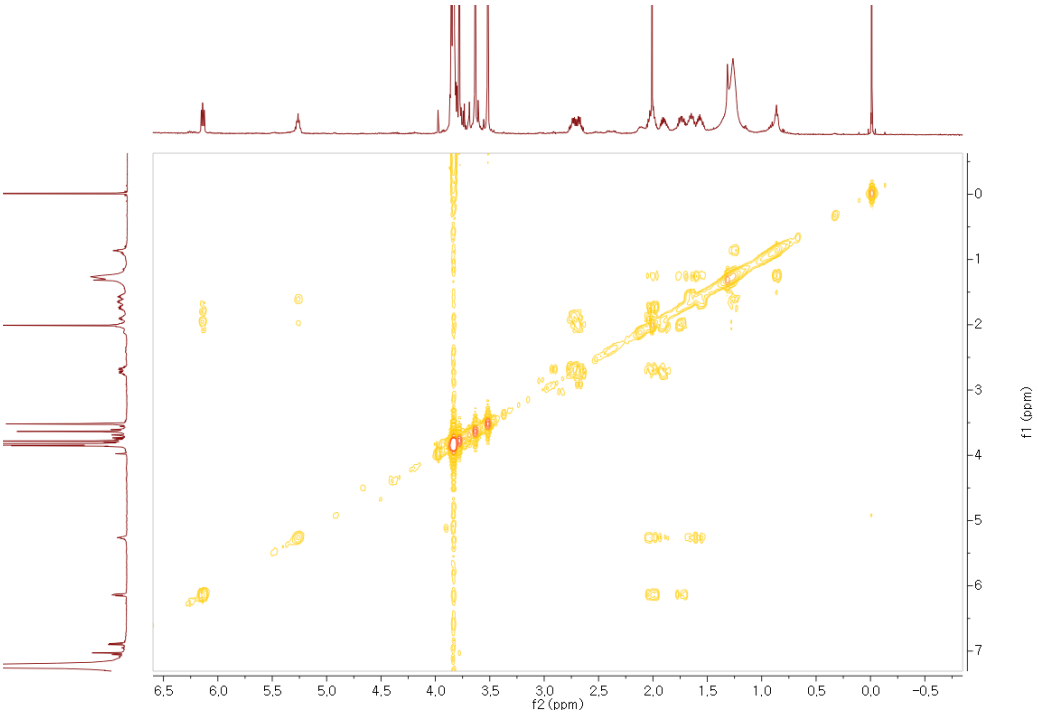


Figure S76. COSY spectrum of (*S*)-MTPA ester of **3b**.

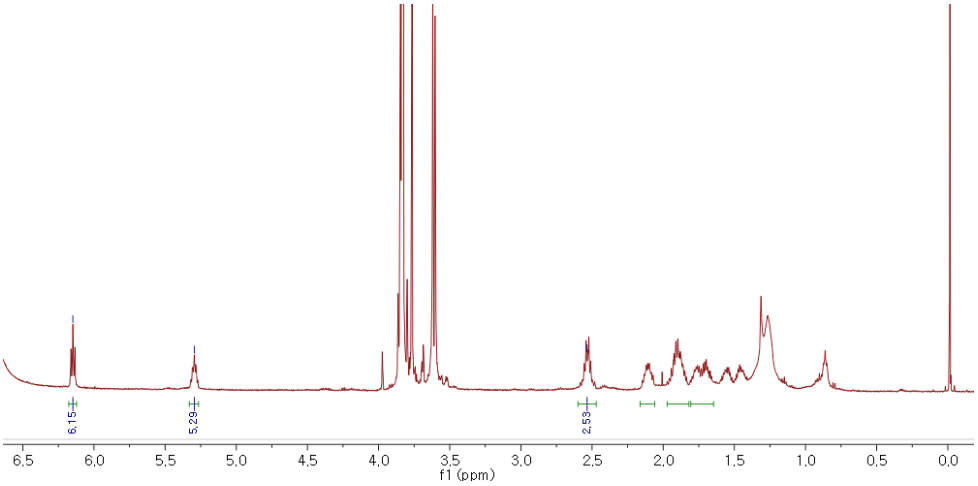


Figure S77. ^{1}H-NMR (pyridine-d_5, 500 MHz) spectrum of (*R*)-MTPA ester of **3b**.

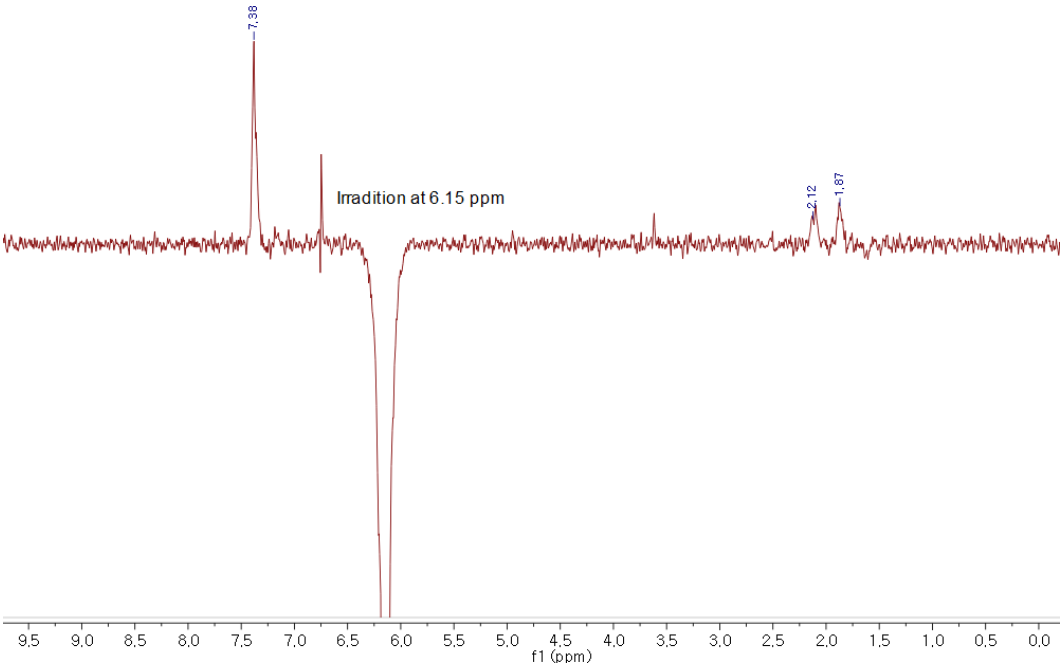


Figure S78. NOE spectrum of (*R*)-MTPA ester of **3b**.

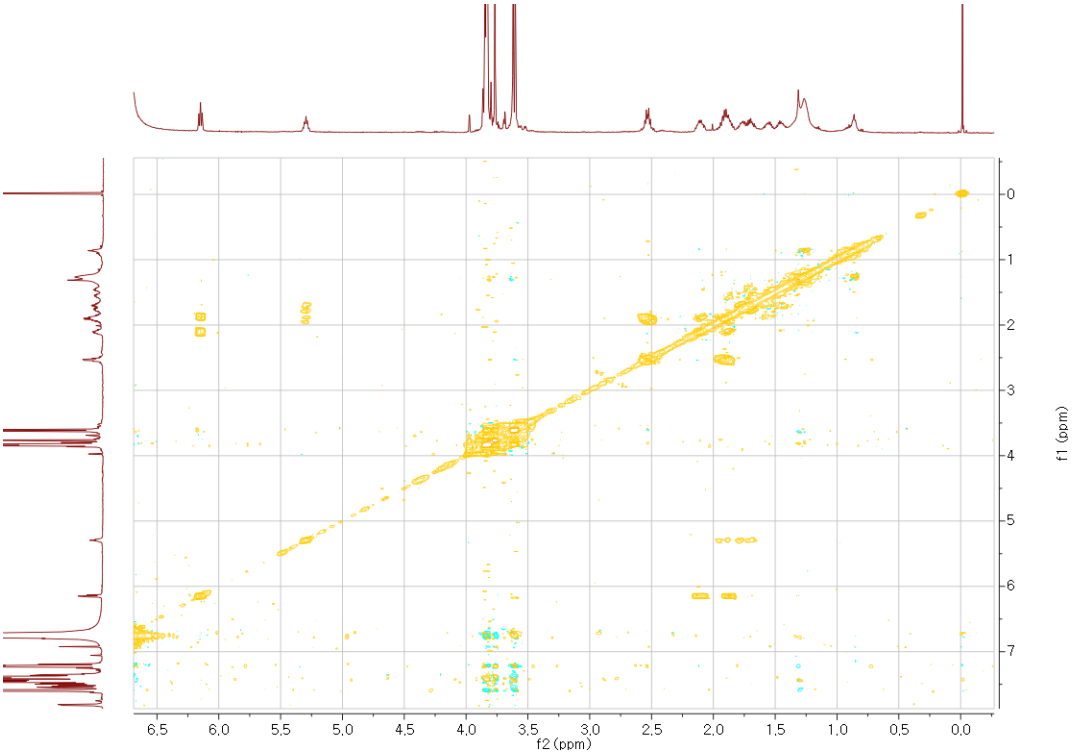


Figure S79. COSY spectrum of (*R*)-MTPA ester of **3b**.

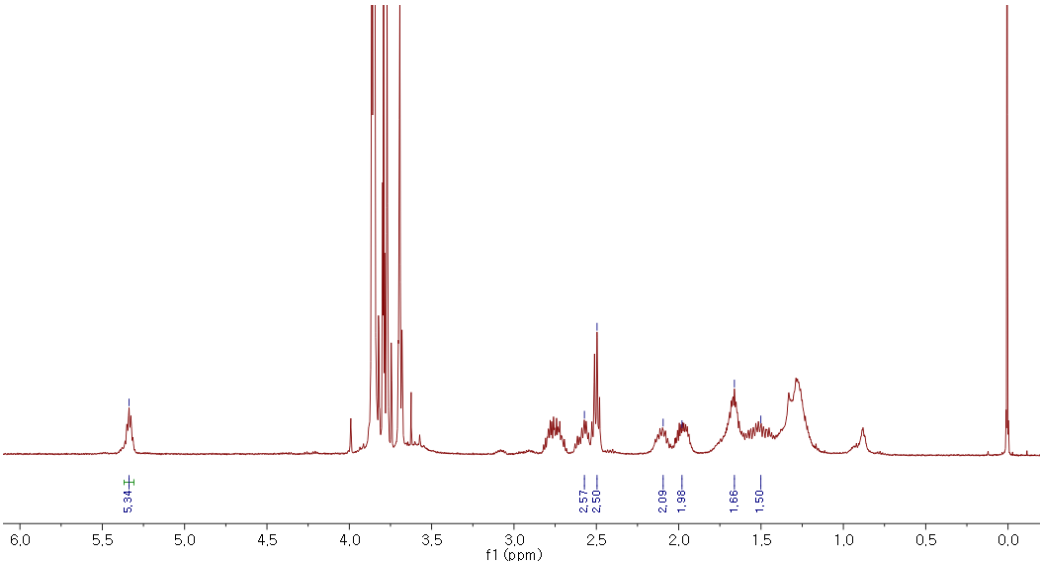


Figure S80. ^{1}H-NMR (pyridine-d_5, 500 MHz) spectrum of (*S*)-MTPA ester of **5**.

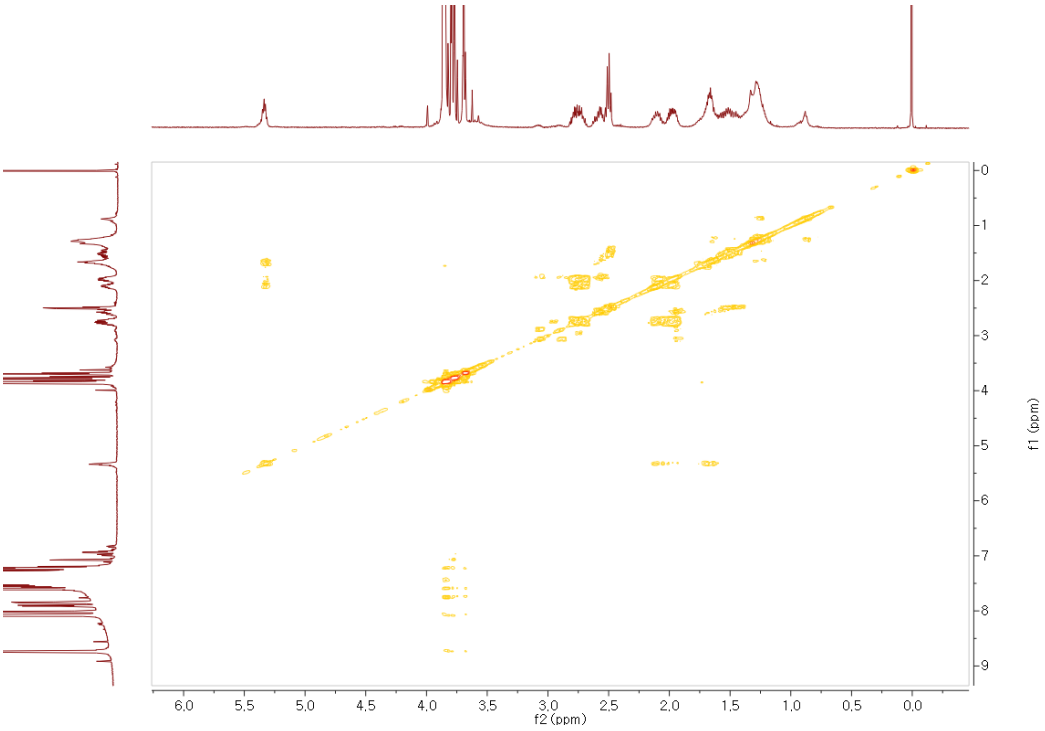


Figure S81. COSY spectrum of (*S*)-MTPA ester of **5**.

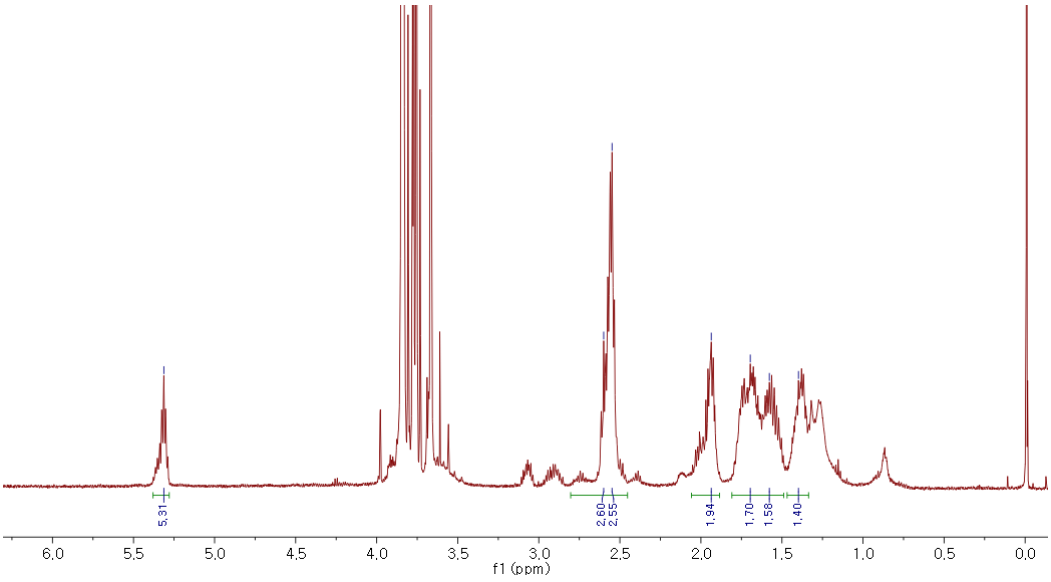


Figure S82. ^{1}H-NMR (pyridine-d_5, 500 MHz) spectrum of (*R*)-MTPA ester of **5**.

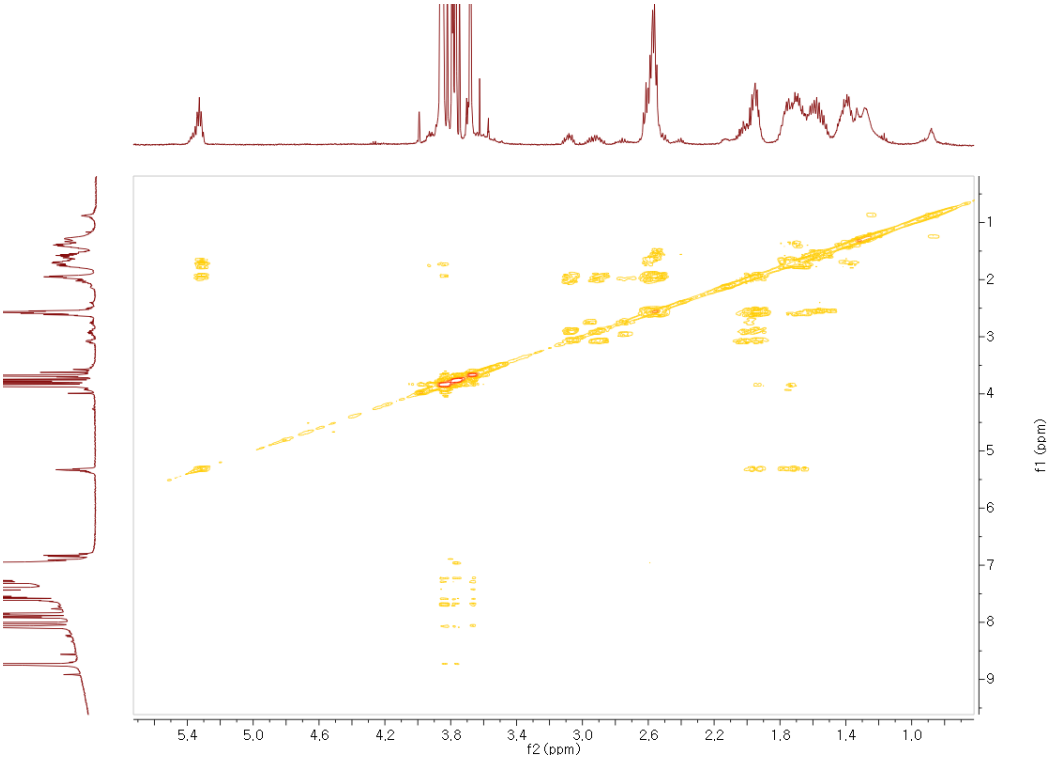


Figure S83. COSY spectrum of (*R*)-MTPA ester of **5**.

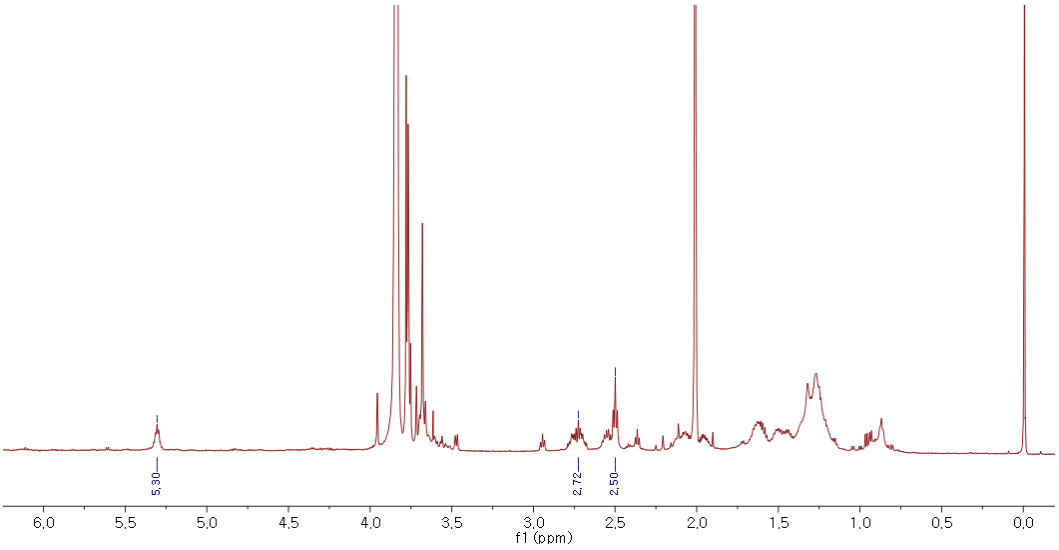


Figure S84. ^{1}H-NMR (pyridine-d_5, 500 MHz) spectrum of (*S*)-MTPA ester of **6**.

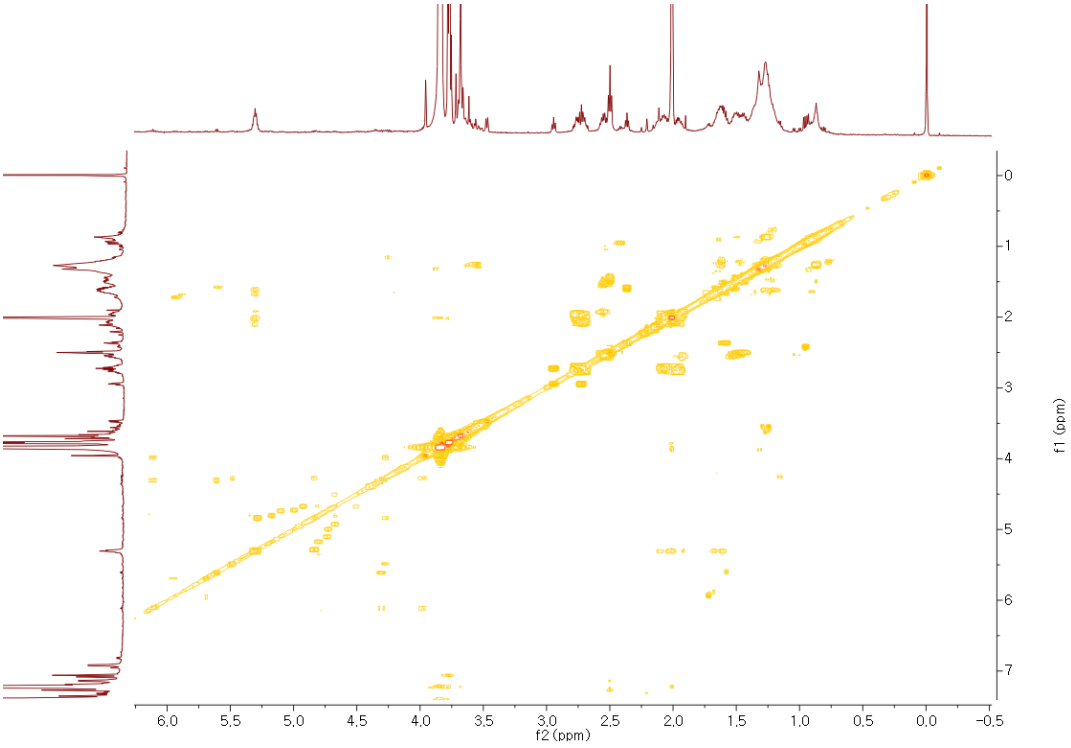


Figure S85. COSY spectrum of (*S*)-MTPA ester of **6**.

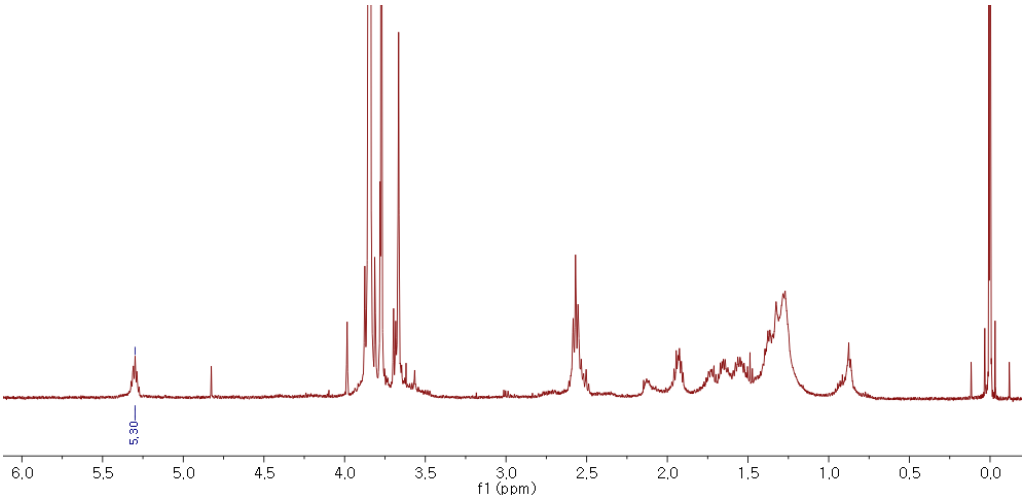


Figure S86. ^{1}H-NMR (pyridine-d_5, 500 MHz) spectrum of (*R*)-MTPA ester of **6**.

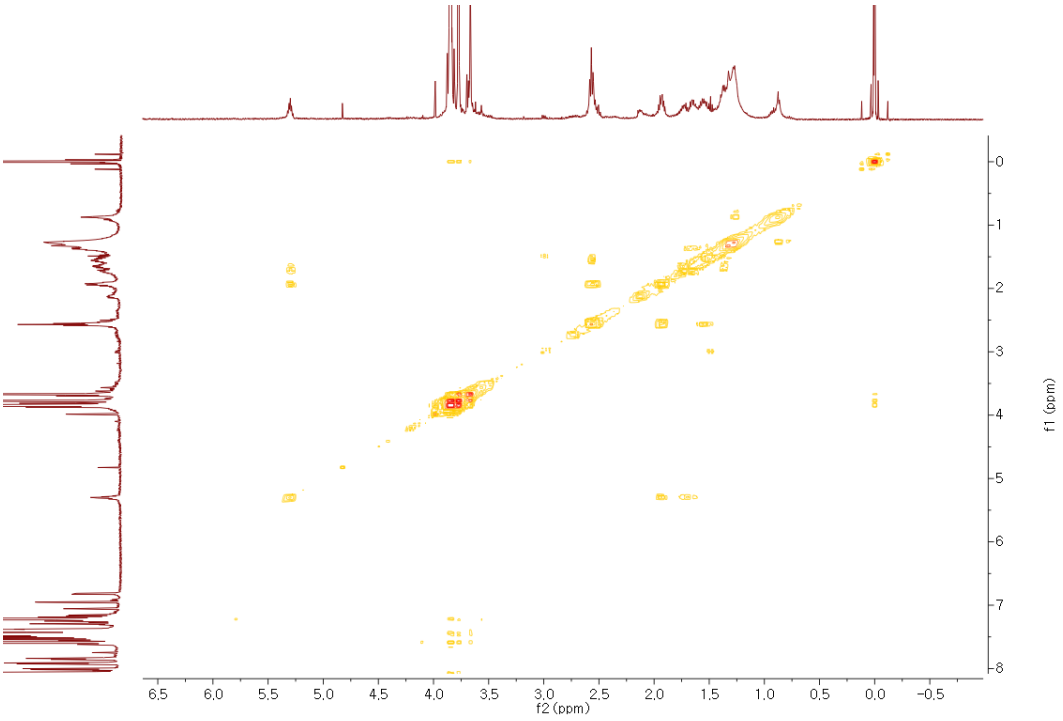


Figure S87. COSY spectrum of (*R*)-MTPA ester of **6**.

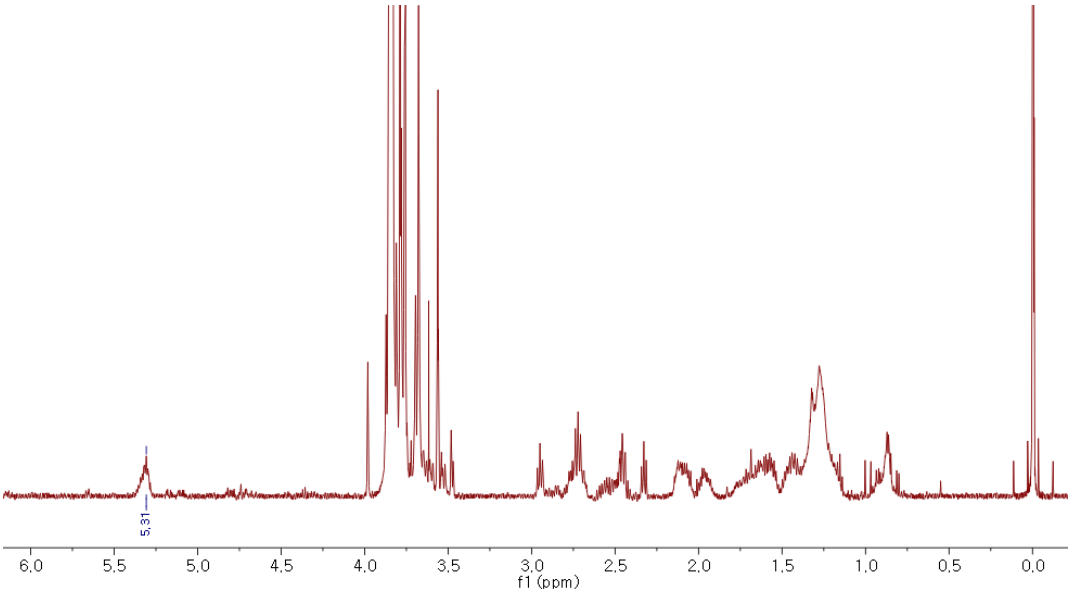


Figure S88. ^{1}H-NMR (pyridine-d_5, 500 MHz) spectrum of (*S*)-MTPA ester of **7**.

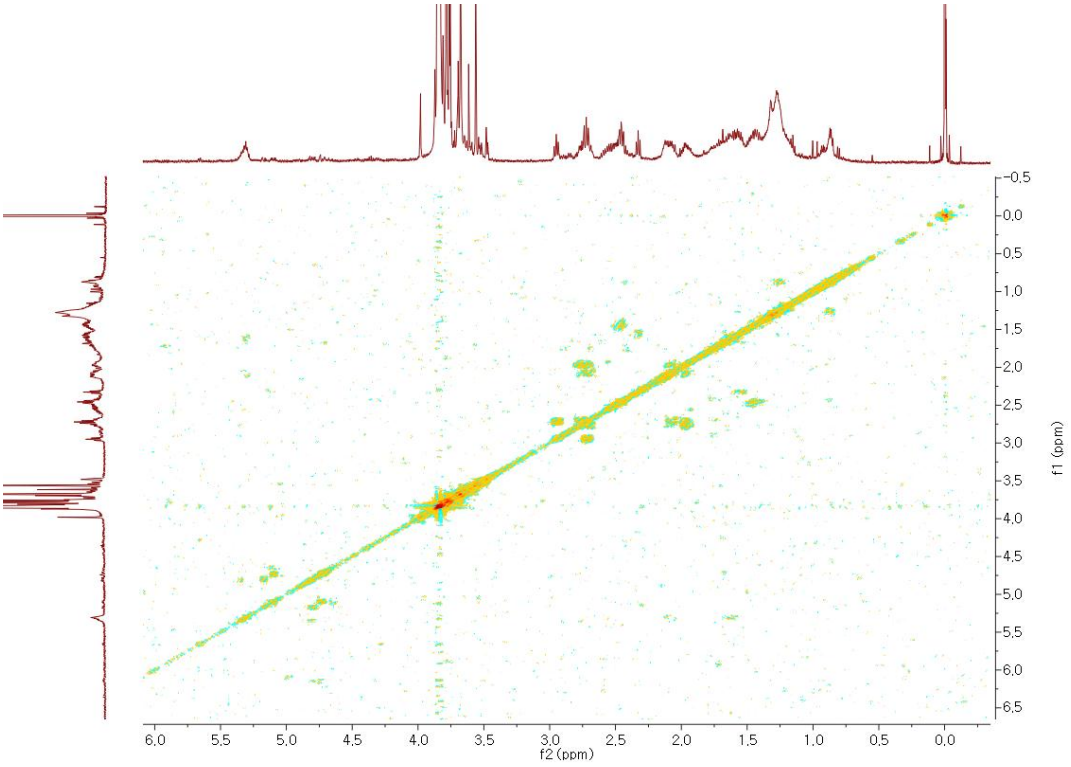


Figure S89. COSY spectrum of (*S*)-MTPA ester of **7**.

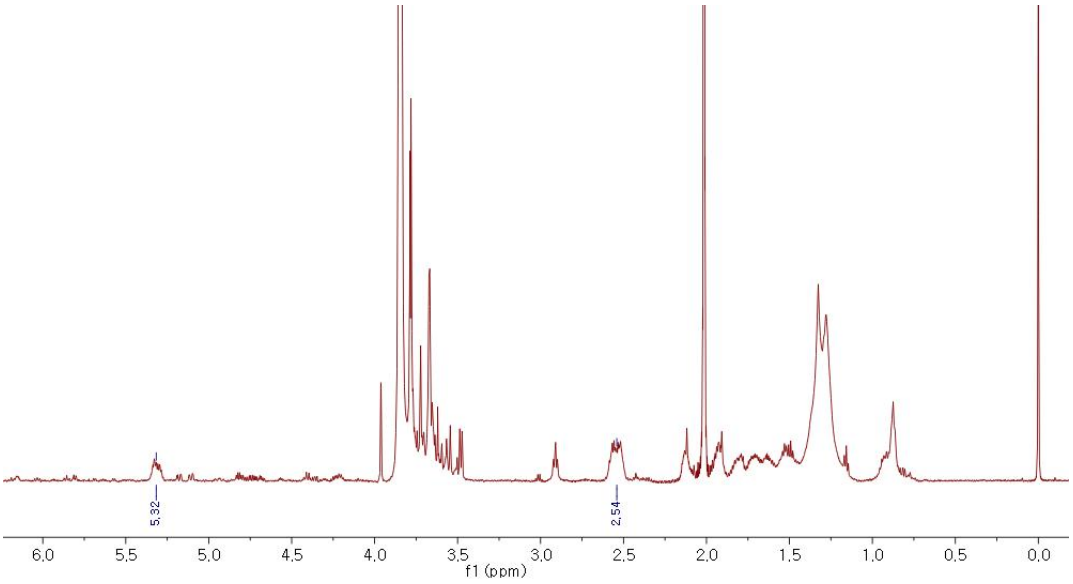


Figure S90. ^{1}H-NMR (pyridine-d_5, 500 MHz) spectrum of (*R*)-MTPA ester of **7**.

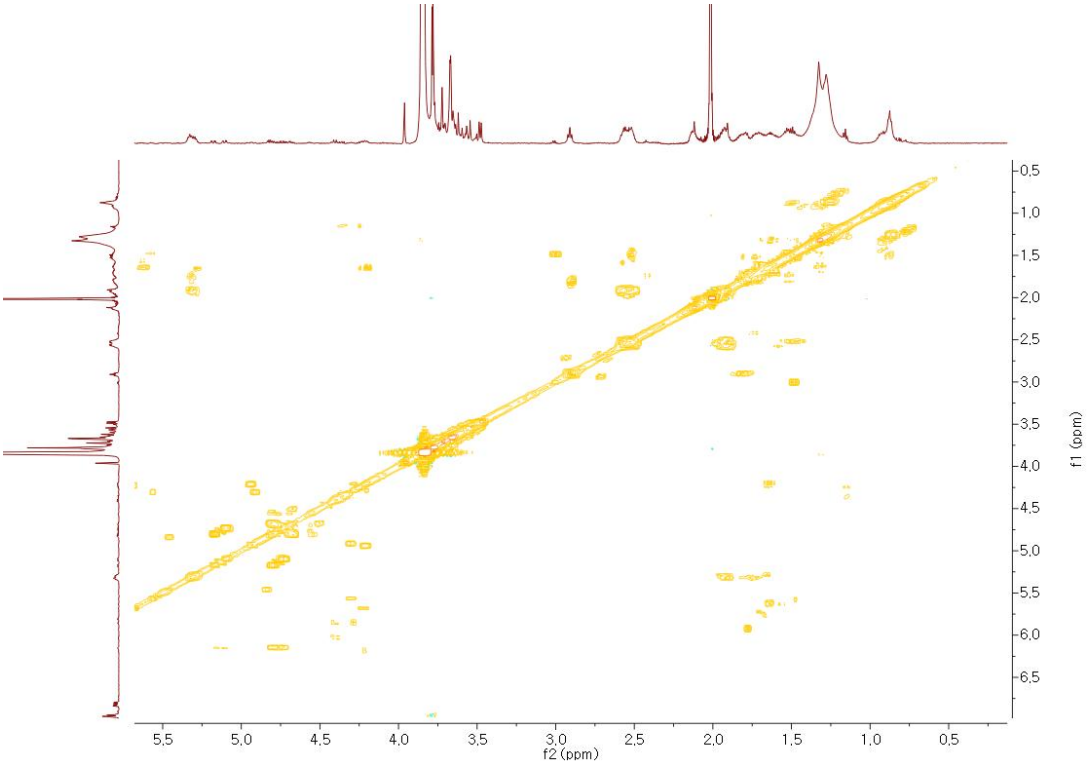


Figure S91. COSY spectrum of (*S*)-MTPA ester of **7**.