HOKKAIDO UNIVERSITY

Title	Saponins are responsible for the anti-obesogenic activity of Acacia concinna
Author(s)	Zhuoyue, Zhao; Ruangaram, Wijitrapha; Kato, Eisuke
Citation	Journal of natural medicines, 75, 1005-1013 https://doi.org/10.1007/s11418-021-01530-0
Issue Date	2021-09
Doc URL	https://hdl.handle.net/2115/86646
Rights	This is a post-peer-review, pre-copyedit version of an article published in Journal of natural medicines. The final authenticated version is available online at: http://dx.doi.org/10.1007/s11418-021-01530-0
Type	journal article
File Information	Eikato_JNatProd_2021_Supplementary material.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Supplementary material

Saponins are responsible for the anti-obesogenic activity of *Acacia concinna*

Zhao Zhuoyue[1], Wijitrapha Ruangaram[1], Eisuke Kato[2*]

1 Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita-ku, Sapporo, Hokkaido 060-8589, Japan

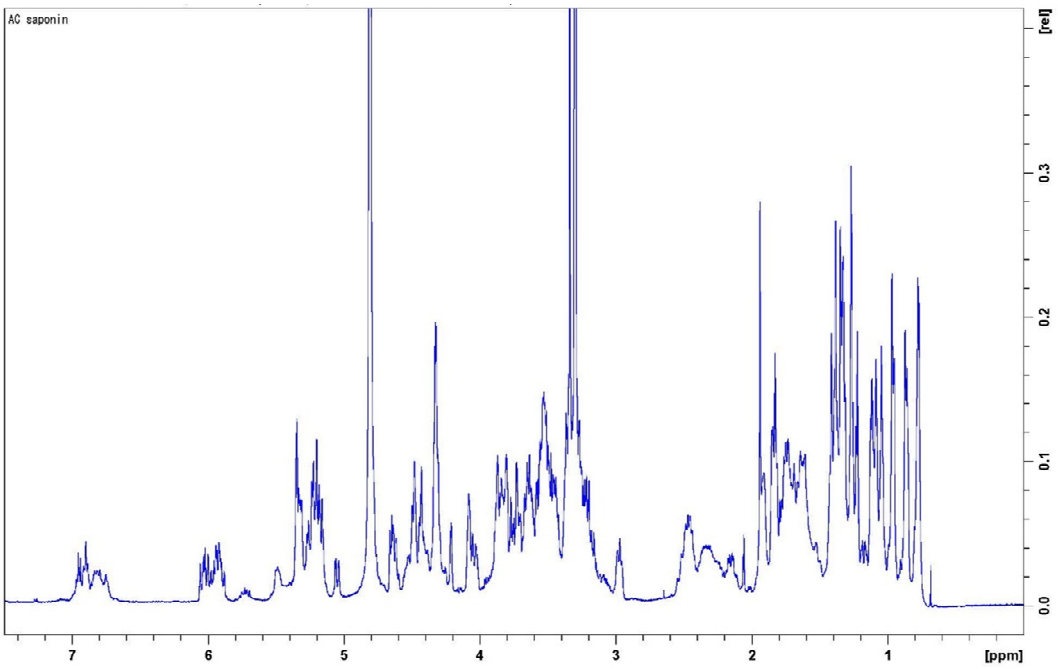
2 Division of Fundamental AgriScience and Research, Research Faculty of Agriculture, Hokkaido University, Kita-ku, Sapporo, Hokkaido 060-8589, Japan

*Tel: +81-11-706-2496, E-mail: eikato@chem.agr.hokudai.ac.jp

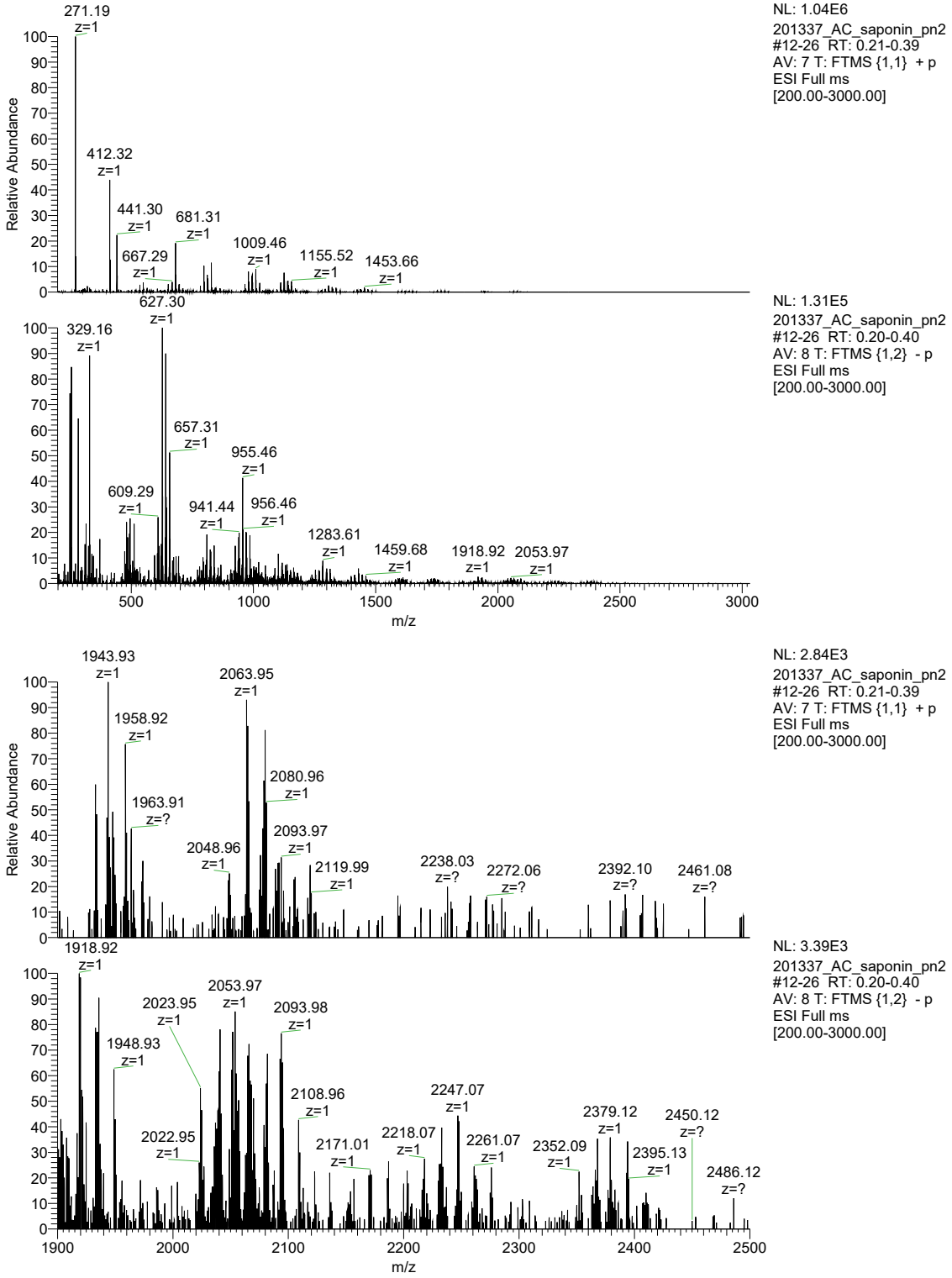
Contents

Supplementary Figure S2. ESI-MS spectrum of the AC saponin 4
Supplementary Table S1. Comparation of ^{1}H-NMR data of compound **1**-**4** with literature data 5
Supplementary Figure S3. ^{1}H-NMR spectrum of compound **1** (500 MHz, CD_3OD) . . 6
Supplementary Figure S4. ^{1}H-NMR spectrum of compound **2** (500 MHz, CD_3OD) . . 7
Supplementary Figure S5. ^{1}H-NMR spectrum of compound **3** (500 MHz, CD_3OD) . . 8
Supplementary Figure S6. ^{1}H-NMR spectrum of compound **4** (500 MHz, CD_3OD) . . 9
Supplementary Figure S7. ^{1}H-NMR spectrum of compound **5** (500 MHz, Acetone-d_6) 10
Supplementary Figure S8. ^{13}C-NMR spectrum of compound **5** (500 MHz, Acetone-d_6) 11
Supplementary Figure S9. COSY spectrum of compound **5** (500 MHz, Acetone-d_6). 12
Supplementary Figure S10. HSQC spectrum of compound **5** (500 MHz, Acetone-d_6) 13
Supplementary Figure S11. HMBC spectrum of compound **5** (500 MHz, Acetone-d_6) 14
Supplementary Figure S12. ^{1}H-NMR spectrum of compound **6** (500 MHz, Acetone-d_6) 15

Supplementary Figure S13. ^{13}C-NMR spectrum of compound **6** (500 MHz, Acetone-d_6) 16
Supplementary Figure S14. COSY spectrum of compound **6** (500 MHz, Acetone-d_6) 17
Supplementary Figure S15. HSQC spectrum of compound **6** (500 MHz, Acetone-d_6) 18
Supplementary Figure S16. HMBC spectrum of compound **6** (500 MHz, Acetone-d_6) 19
Supplementary Figure S17. GC-MS analysis of the sugars in AC saponin. 20
Supplementary Figure S18. LC-MS analysis of alkaline hydrolysis product. 21
Supplementary Figure S19. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Glc-Ara attached acacic acid lactone (m/z 782.4) 22
Supplementary Figure S20. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Glc-Rha attached acacic acid lactone (m/z 796.4) 22
Supplementary Figure S21. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Ara-Rha attached acacic acid lactone (m/z 766.4) 23
Supplementary Figure S22. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Glc-Glc-Glc attached acacic acid lactone (m/z 974.5) 23
Supplementary Figure S23. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Glc-Glc-Ara attached acacic acid lactone (m/z 944.5) 24
Supplementary Figure S24. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Glc-Glc-Rha attached acacic acid lactone (m/z 958.5) 24
Supplementary Figure S25. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Glc-Ara-Rha attached acacic acid lactone (m/z 928.5) 25



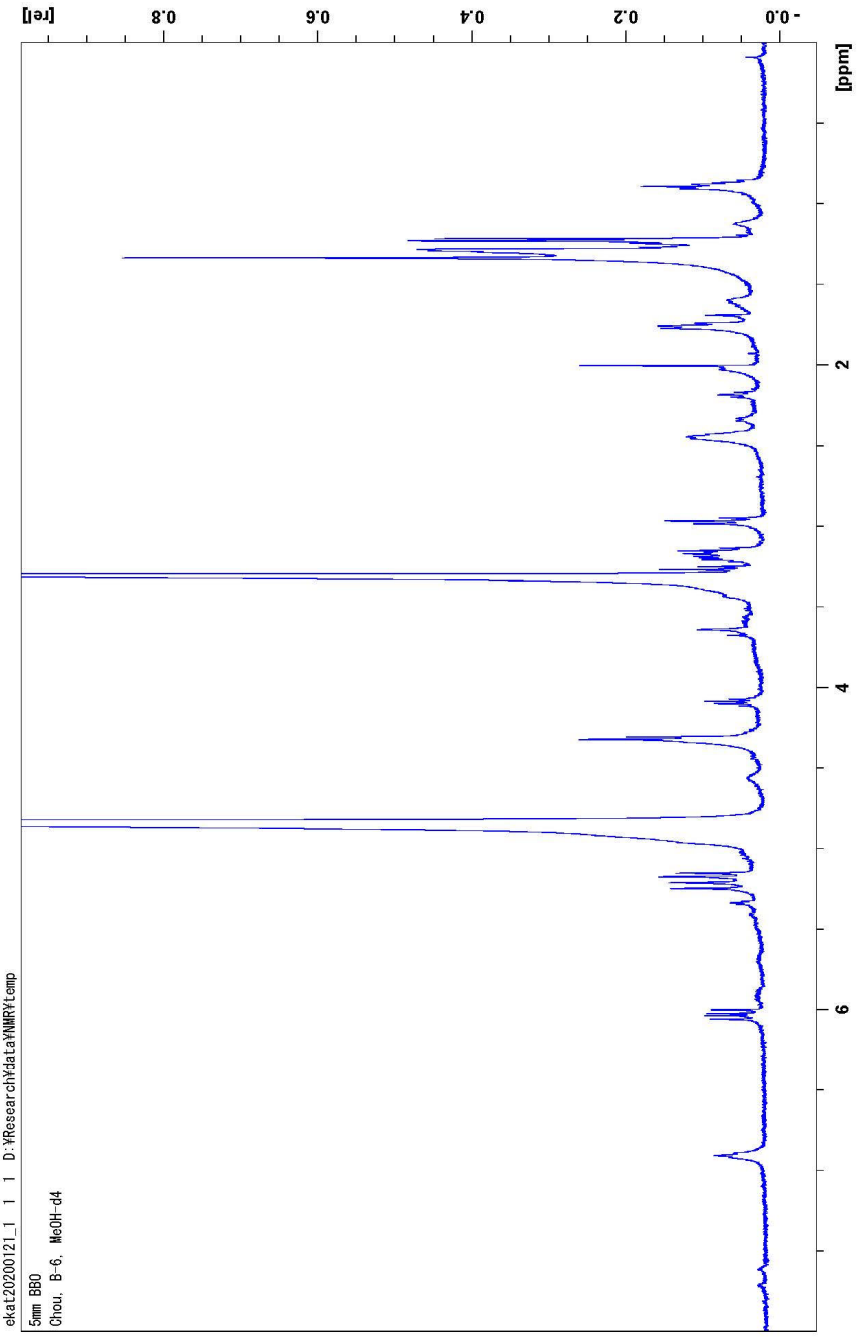
Supplementary Figure S1. ^{1}H-NMR of the AC saponin



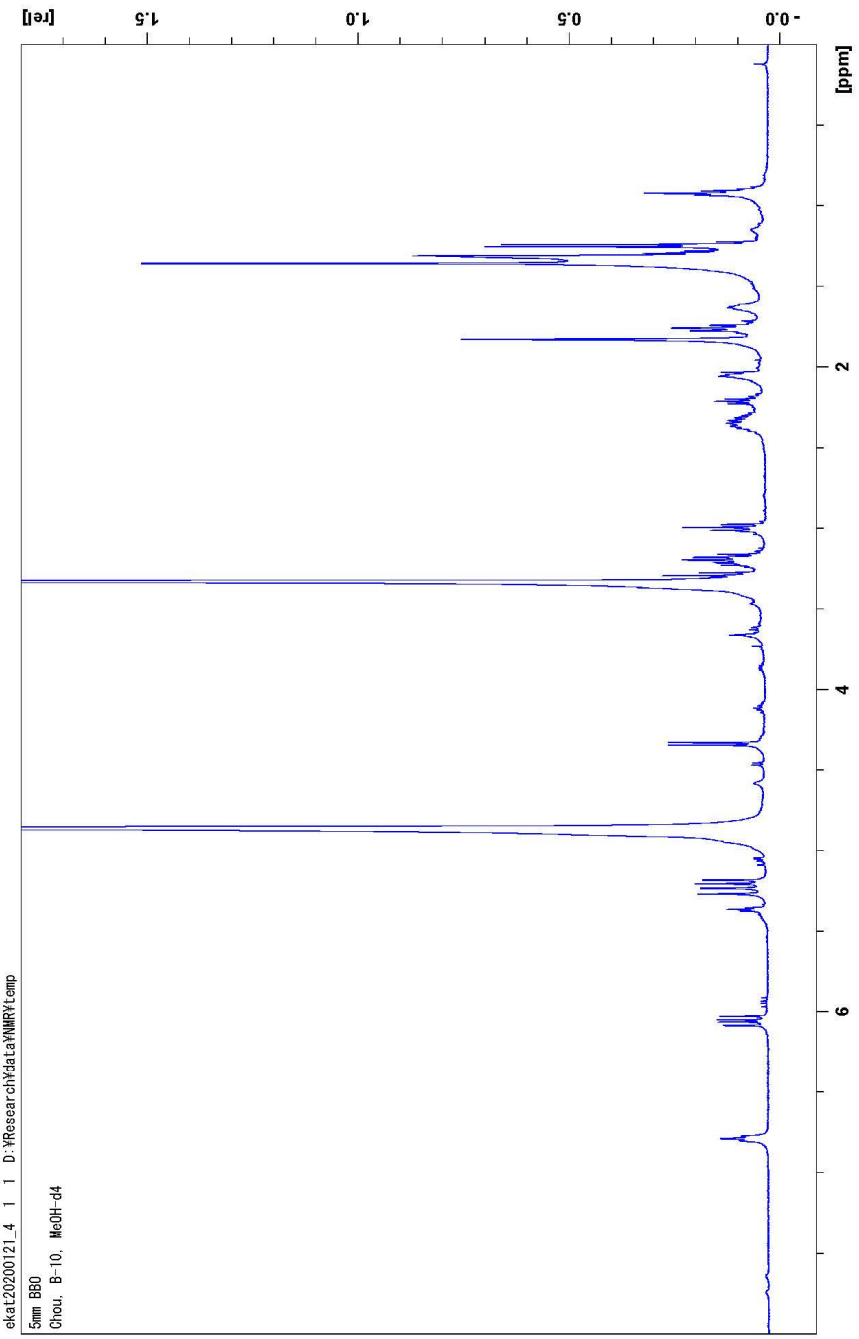
Supplementary Figure S2. ESI-MS spectrum of the AC saponin

Supplementary Table S1. Comparation of ^{1}H-NMR data of compound **1**-**4** with literature data

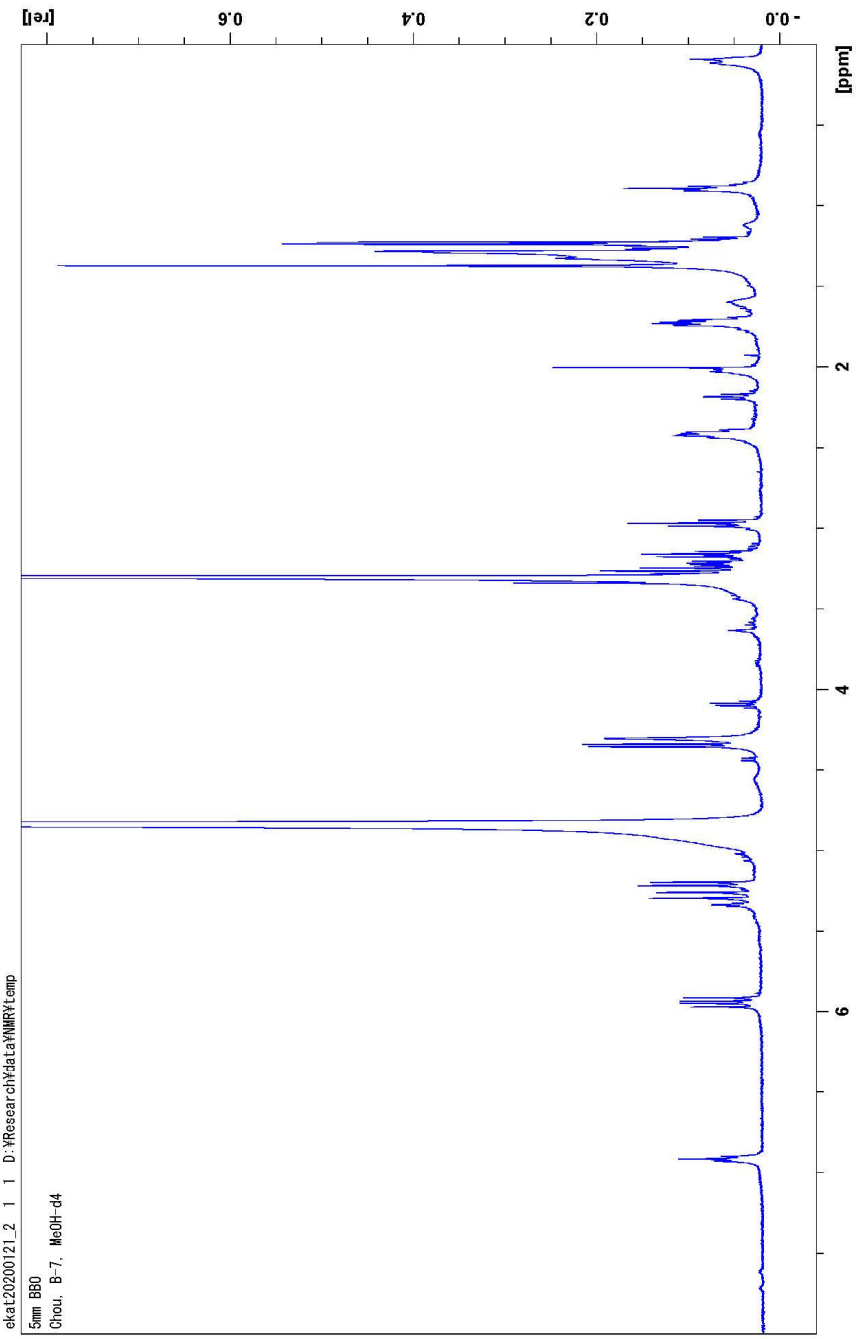
	Compound 1		**Compound 2**		**Compound 3**		**Compound 4**	
No.	literature data[1]	Isolated	literature data[1]	Isolated	literature data[2]	Isolated	literature data[2]	Isolated
3	6.85 (br t, 6.6)	6.91 (br t)	6.85 (br t, 6.6)	6.90 (1H, t)	6.76 (1H, br t, 6.8)	6.76 (1H, t)	6.77 (1H, tq, 7.8, 1.5)	6.79 (1H, tq)
4	2.40 (m)	2.44 (2H, m)	2.40 (m)	2.39 (2H, m)	2.30 (2H, m)	2.27 (2H, m)	2.32 (2H, m)	2.30 (2H, m)
5	1.73 (m)	1.76 (2H, m)	1.73 (m)	1.70 (2H, m)	1.71 (2H, m)	1.70 (2H, m)	1.73 (2H, t, 8.3)	1.75 (2H, m)
7	6.03 (dd, 17.3, 11.0)	6.01 (1H, dd)	5.94 (dd, 17.3, 11.0)	5.91 (1H, dd)	6.01 (1H, dd, 17.6, 10.7)	6.01 (1H, dd)	6.03 (1H, dd, 17.8, 11.2)	6.03 (1H, dd)
8	5.16 (br d, 11.0) 5.22 (br d, 17.3)	5.15 (2H, dd)	5.22 (br d, 10.7) 5.28 (br d, 17.1)	5.20 (2H, dd)	5.17 (1H, dd, 10.7, 1.0) 5.23 (1H, dd, 17.6, 1.0)	5.17 (2H, dd)	5.17 (1H, dd, 11.2, 1.0) 5.23 (1H, dd, 17.8, 1.0)	5.18 (2H, dd)
9	4.31 (br s)	4.31 (2H, s)	4.31 (s)	4.30 (1H, s)	1.80 (3H, s)	1.80 (2H, s)	1.80 (3H, d, 1.5)	1.82 (2H, s)
10	1.34 (s)	1.33 (6H, s)	1.37 (s)	1.37 (2H, s)	1.33 (3H, s)	1.33 (3H, s)	1.33 (3H, s)	1.36 (3H, s)
	Qui		Qui		Xyl		Qui	
1	4.31 (d, 7.8)	4.31 (1H, d)	4.35 (d, 7.8)	4.33 (1H, d)	4.28 (1H, d, 7.3)	4.29 (1H, d)	4.31 (1H, d, 7.8)	4.31 (1H, d)
2	3.16 (dd, 9.1, 7.8)	3.19 (m)	3.16 (dd, 9.1, 7.8)	3.14 (1H, dd)	3.14 (1H, dd, 9.3, 7.8)	3.16 (1H, dd)	3.16 (1H, dd, 9.3, 7.8)	3.16 (1H, dd)
3	3.28 (t, 9.1)	3.27 (1H, t)	3.28 (t, 9.1)	3.27 (1H, t)	3.28 (1H, t, 9.0)	3.28 (1H, t)	3.27 (1H, t, 9.3)	3.27 (1H, t)
4	2.98 (t, 9.1)	2.95 (1H, t)	2.98 (t, 9.1)	2.97 (1H, t)	3.46 (1H, m)	3.46 (1H, m)	2.97 (1H, t, 9.3)	2.97 (1H, t)
5	3.18 (m)	3.19 (m)	3.18 (m)	3.23 (1H, m)	3.08 (1H. t, 11.0) 3.76 (1H, dd, 11.5, 5.6)	3.08 (1H, t) 3.76 (1H, dd)	3.19 (1H, dq, 9.3, 5.9)	3.19 (1H, dq)
6	1.23 (d, 5.9)	1.22 (3H, d)	1.22 (d, 5.3)	1.23 (3H, d)			1.22 (3H, d, 5.9)	1.24 (2H, d)



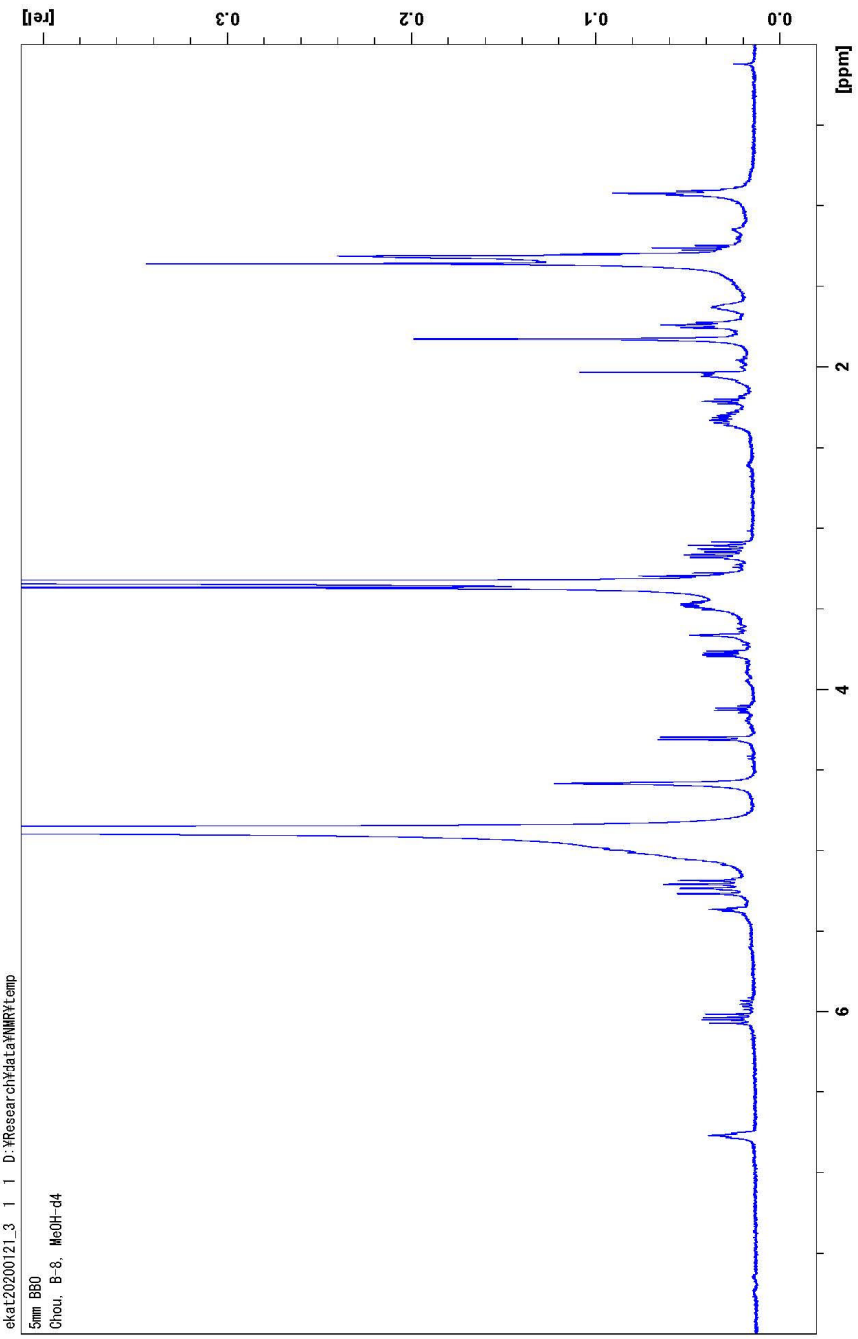
Supplementary Figure S3. ^{1}H-NMR spectrum of compound **1** (500 MHz, CD_3OD)



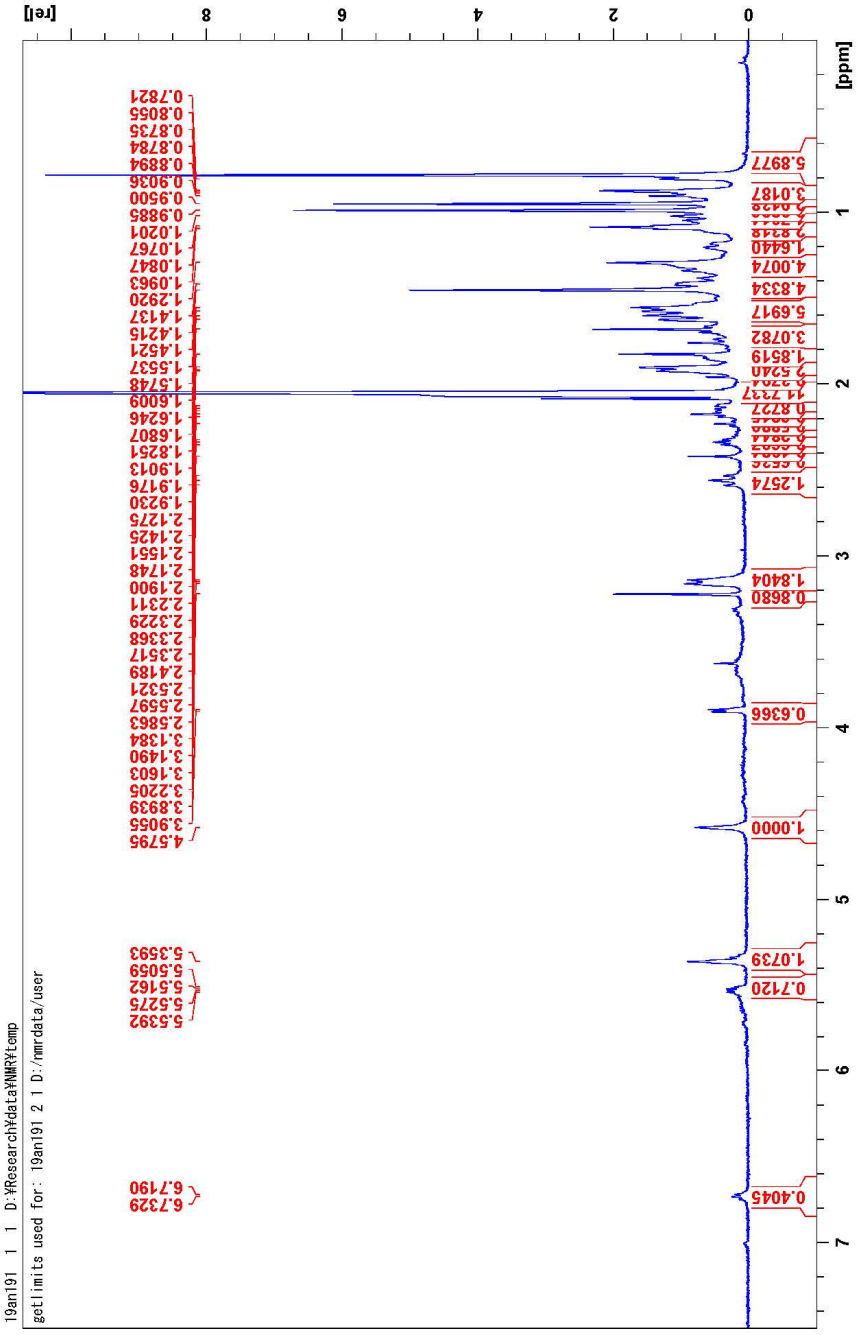
Supplementary Figure S4. ^{1}H-NMR spectrum of compound **2** (500 MHz, CD_3OD)



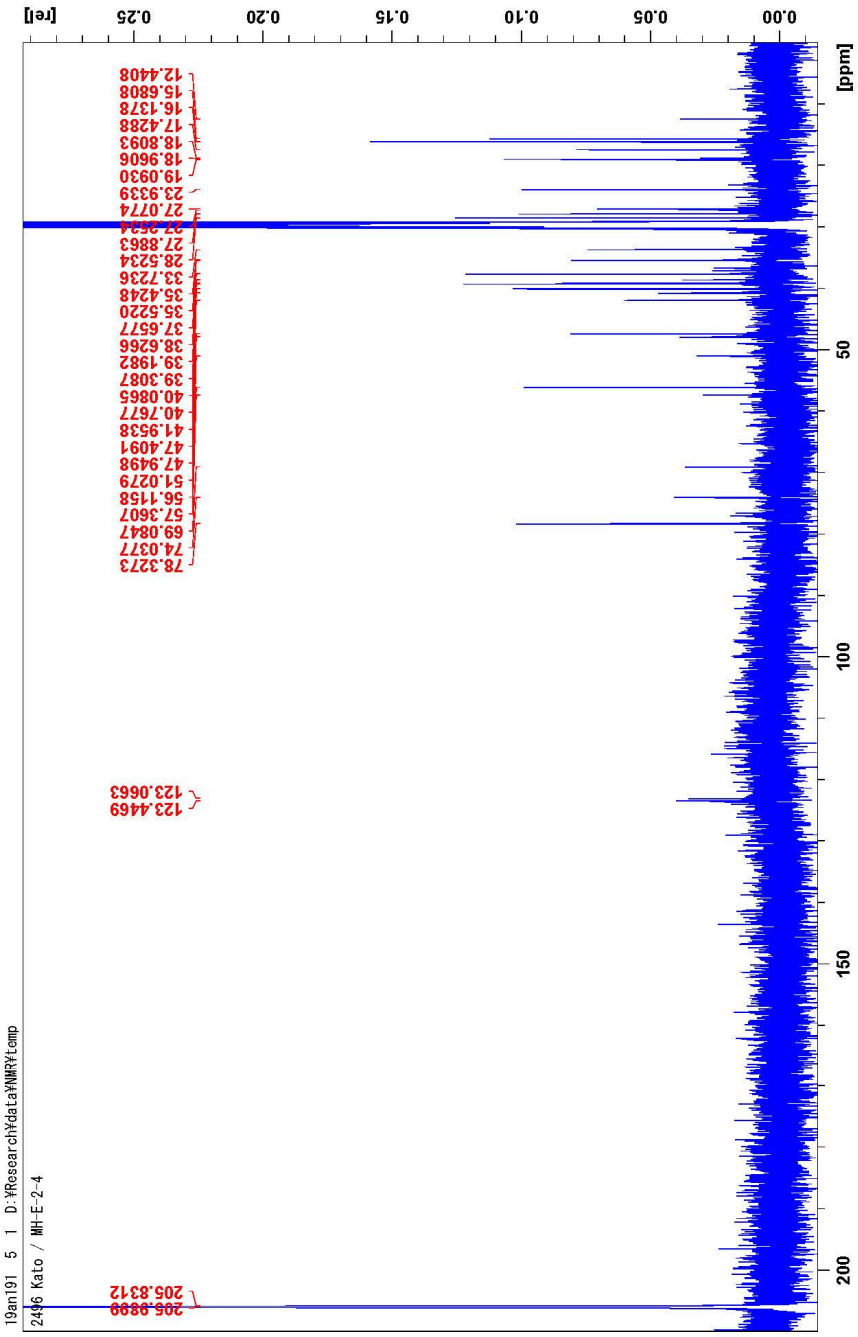
Supplementary Figure S5. ^{1}H-NMR spectrum of compound **3** (500 MHz, CD_3OD)



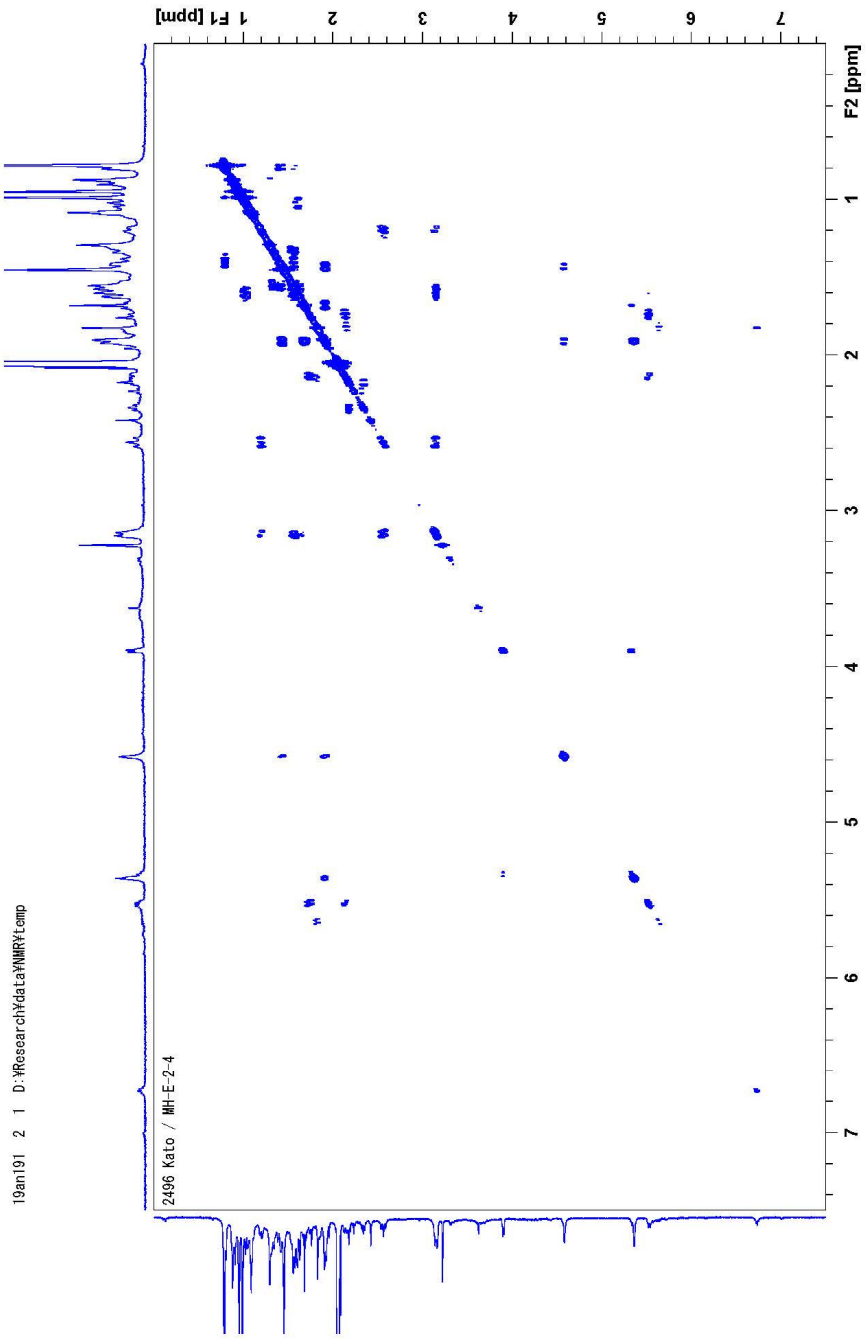
Supplementary Figure S6. ^{1}H-NMR spectrum of compound **4** (500 MHz, CD_3OD)



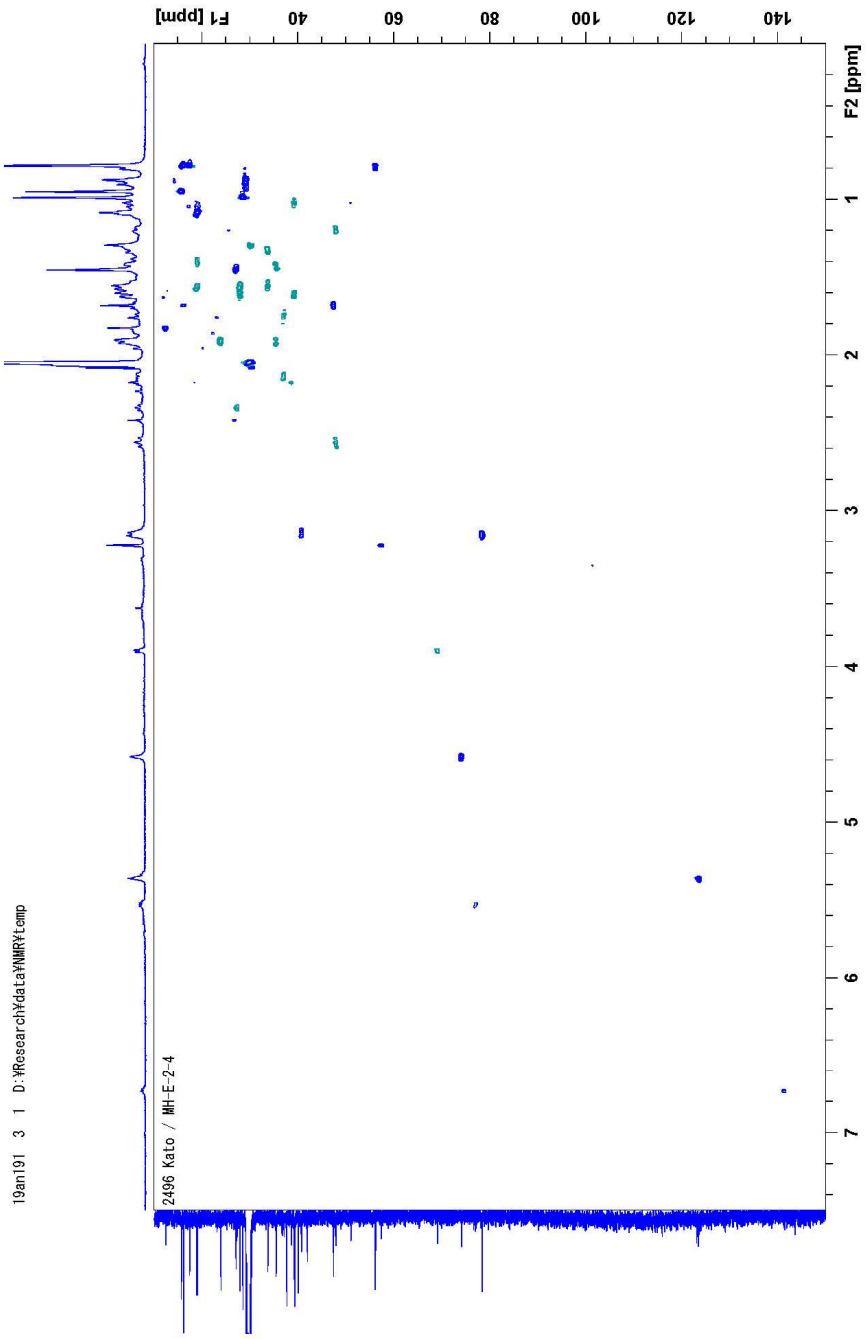
Supplementary Figure S7. ^{1}H-NMR spectrum of compound **5** (500 MHz, Acetone-d_6)



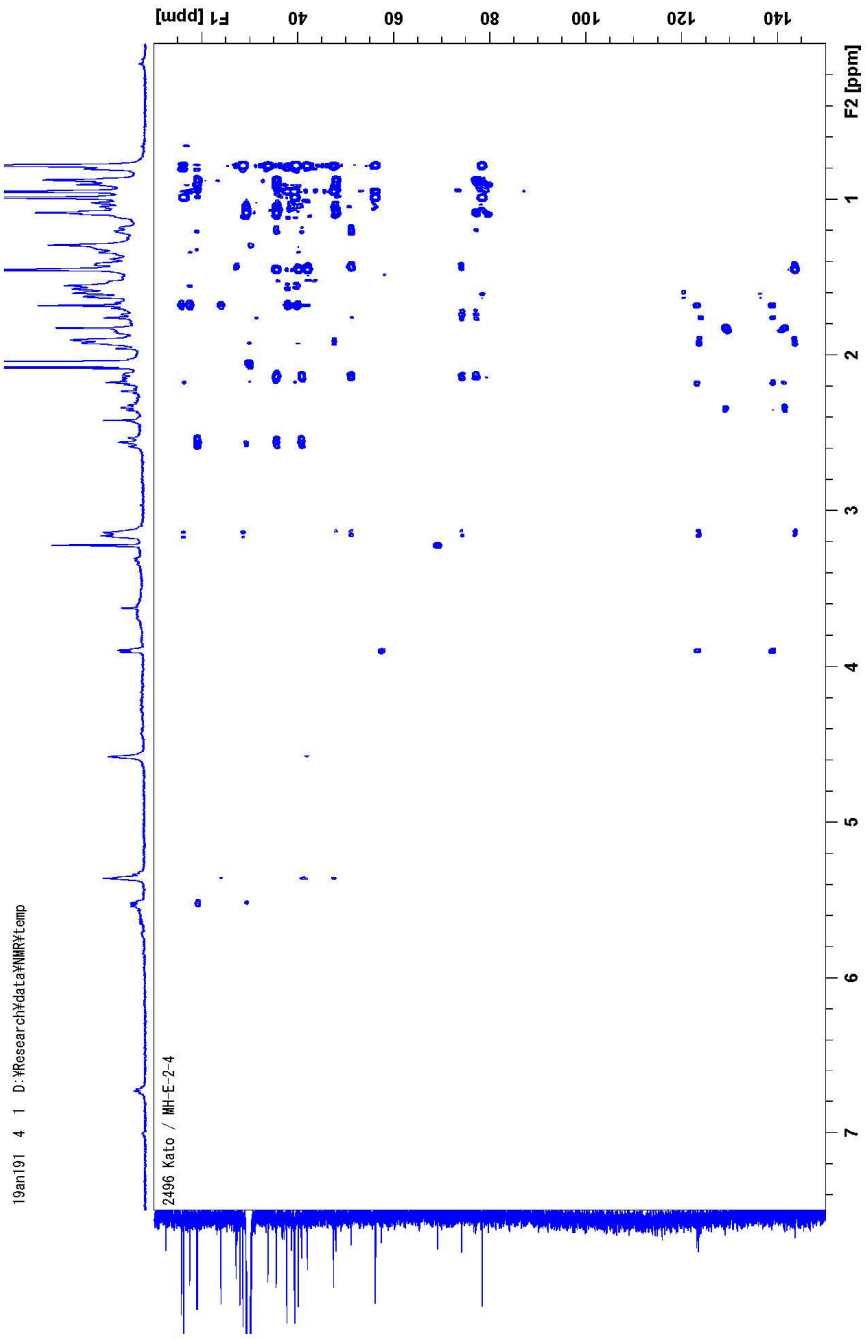
Supplementary Figure S8. ^{13}C-NMR spectrum of compound **5** (500 MHz, Acetone-d_6)



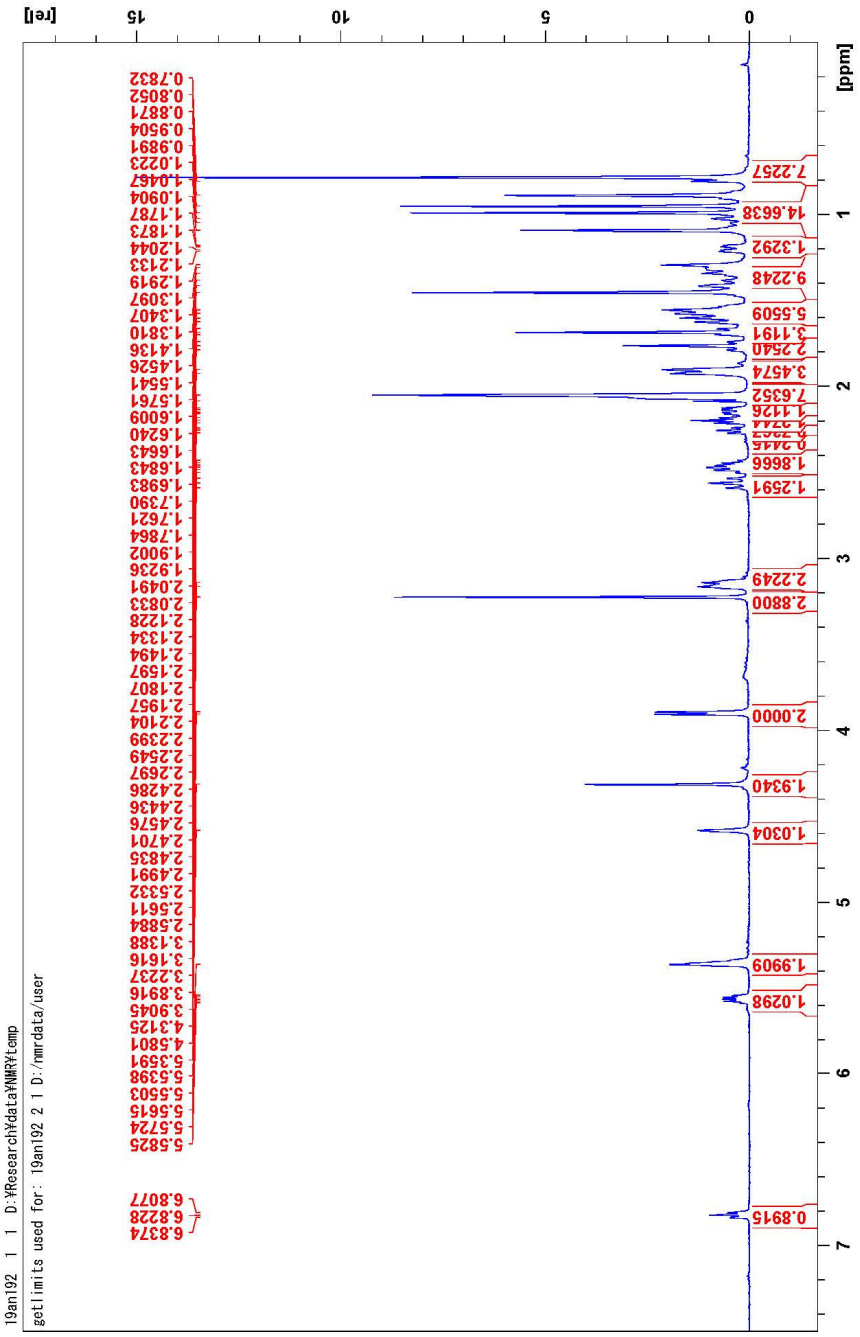
Supplementary Figure S9. COSY spectrum of compound **5** (500 MHz, Acetone-d_6)



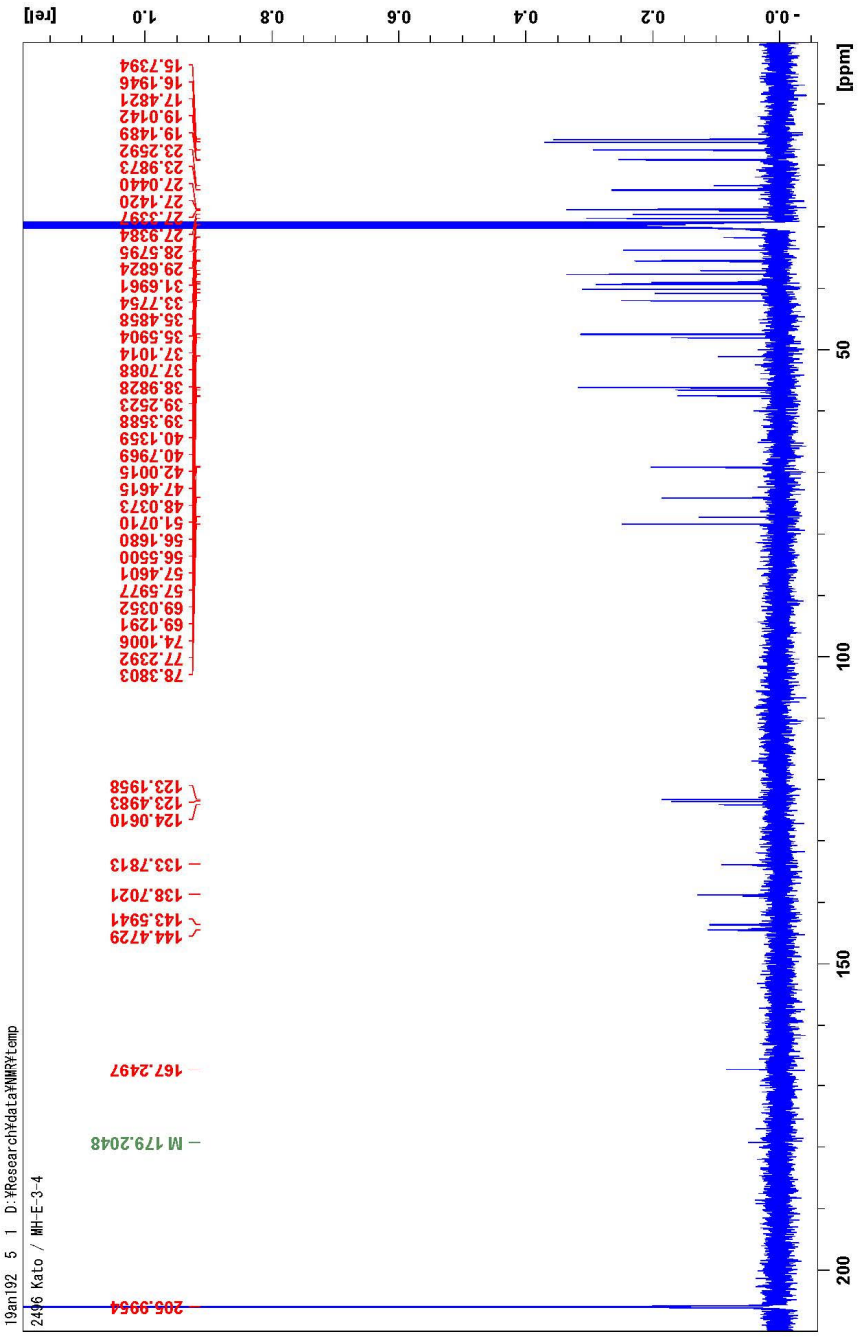
Supplementary Figure S10. HSQC spectrum of compound **5** (500 MHz, Acetone-d_6)



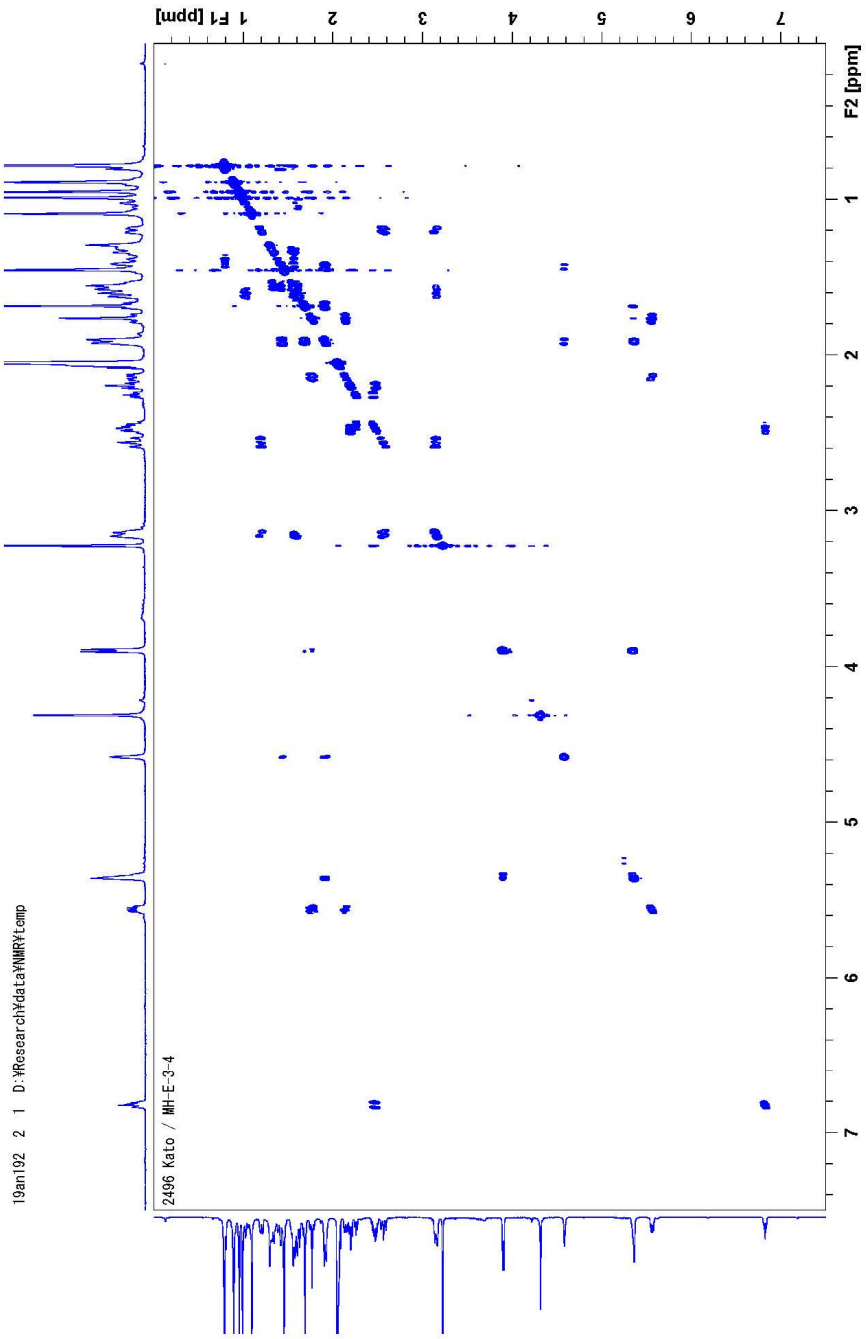
Supplementary Figure S11. HMBC spectrum of compound **5** (500 MHz, Acetone-d_6)



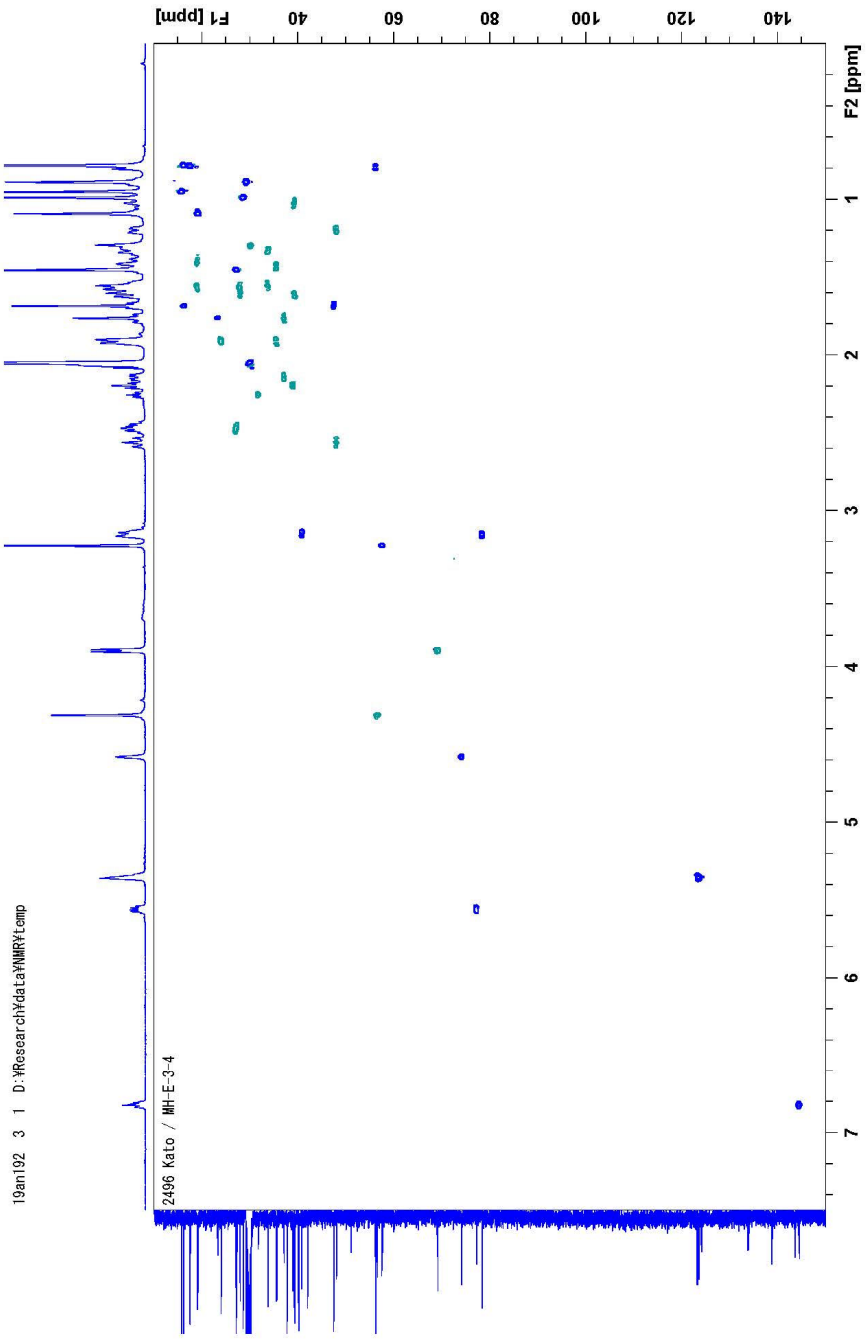
Supplementary Figure S12. ^{1}H-NMR spectrum of compound **6** (500 MHz, Acetone-d_6)



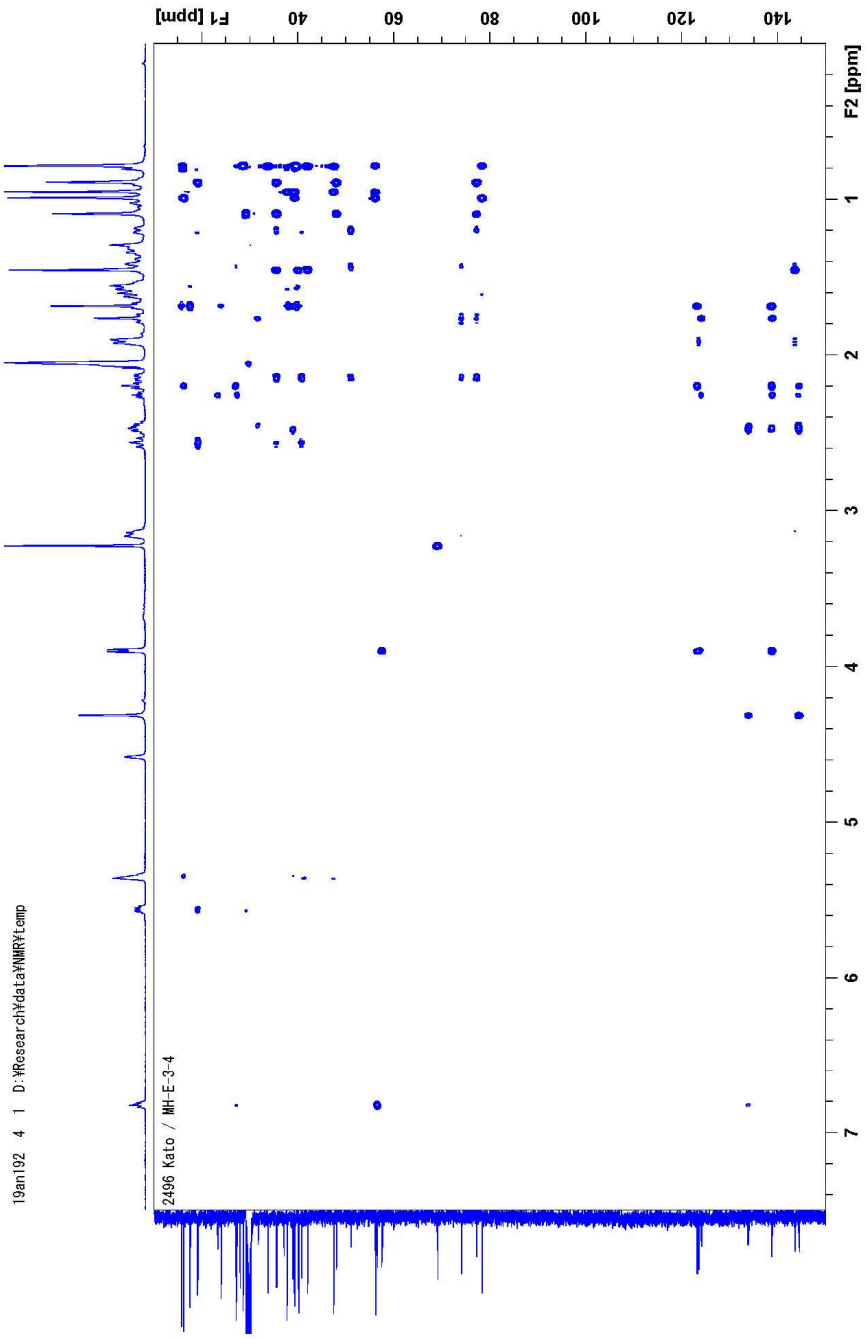
Supplementary Figure S13. ^{13}C-NMR spectrum of compound **6** (500 MHz, Acetone-d_6)



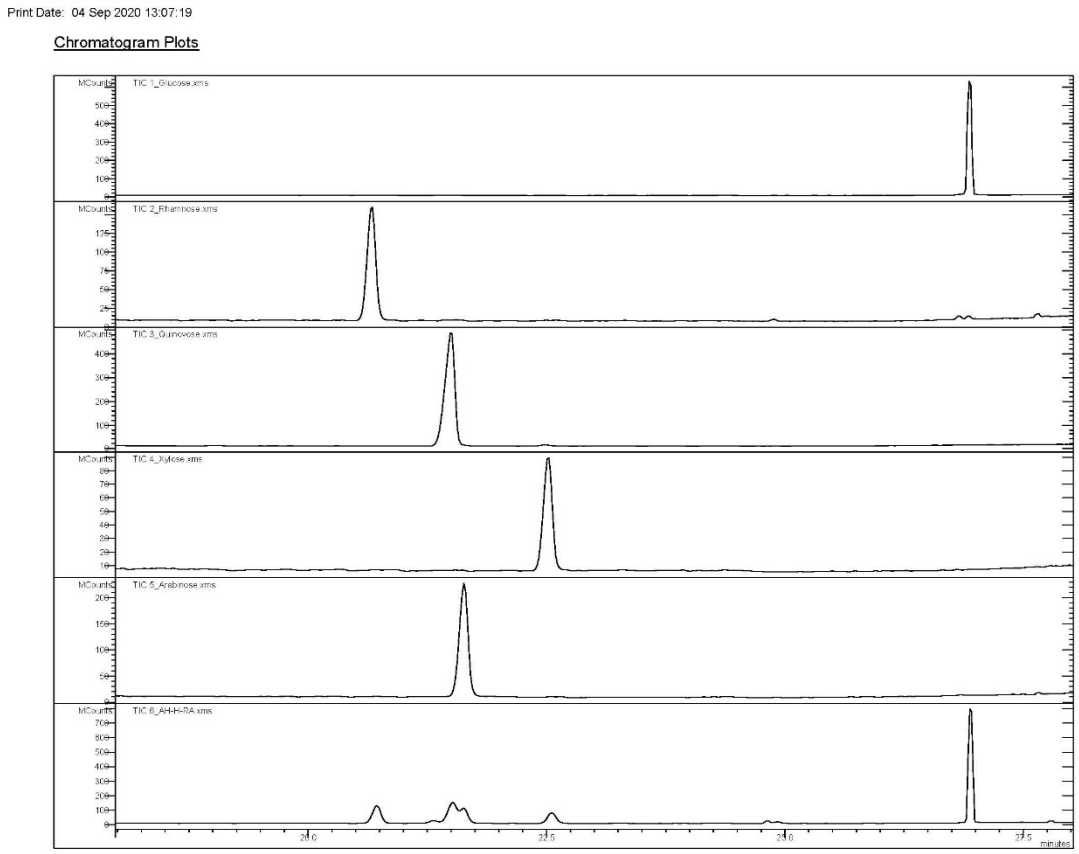
Supplementary Figure S14. COSY spectrum of compound **6** (500 MHz, Acetone-d_6)



Supplementary Figure S15. HSQC spectrum of compound **6** (500 MHz, Acetone-d_6)

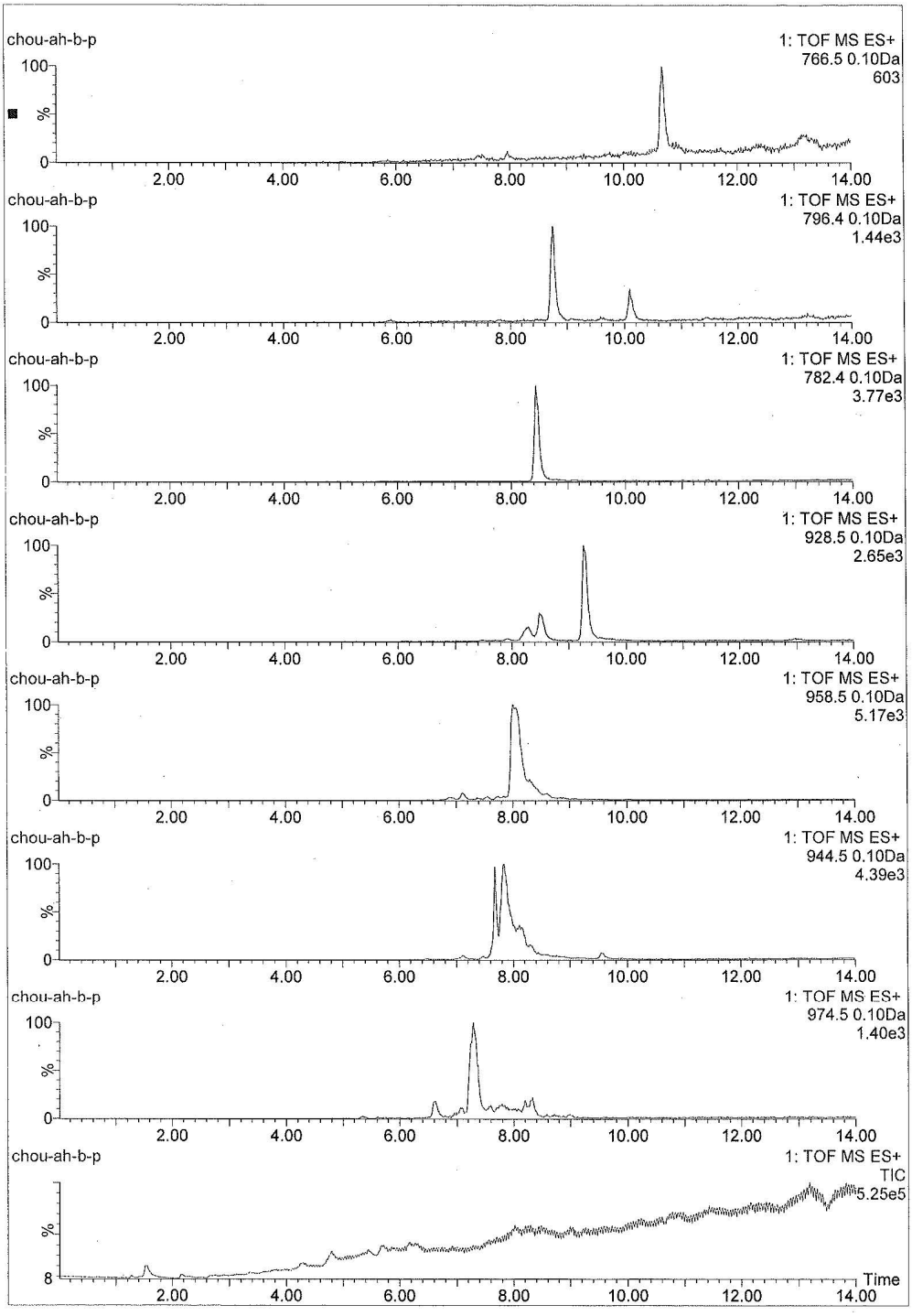


Supplementary Figure S16. HMBC spectrum of compound **6** (500 MHz, Acetone-d_6)



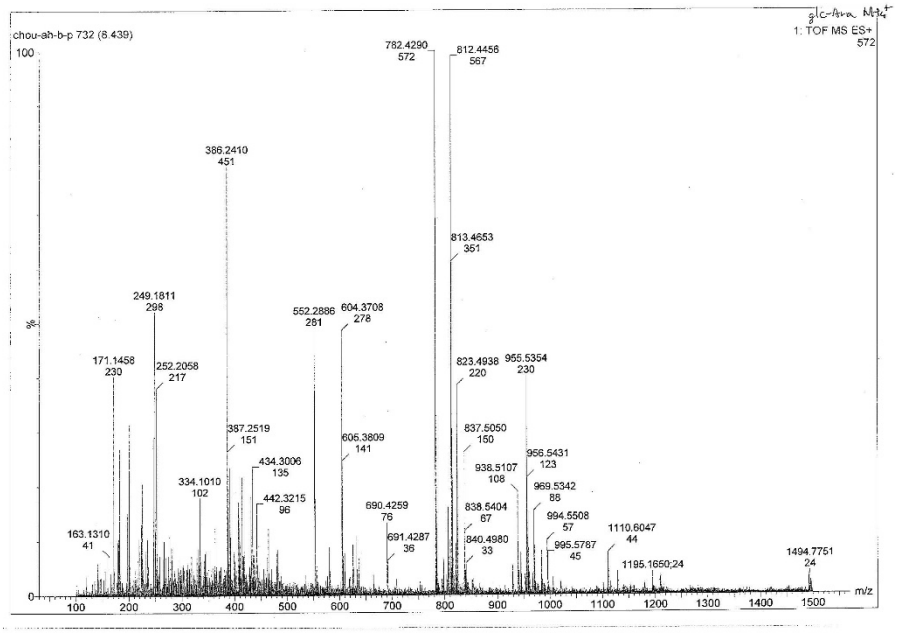
Supplementary Figure S17. GC-MS analysis of the sugars in AC saponin.

From top to bottom: D-glucose, L-rhamnose, D-quinovose, D-xylose, D-arabinose, acid hydrolyzed AC saponin

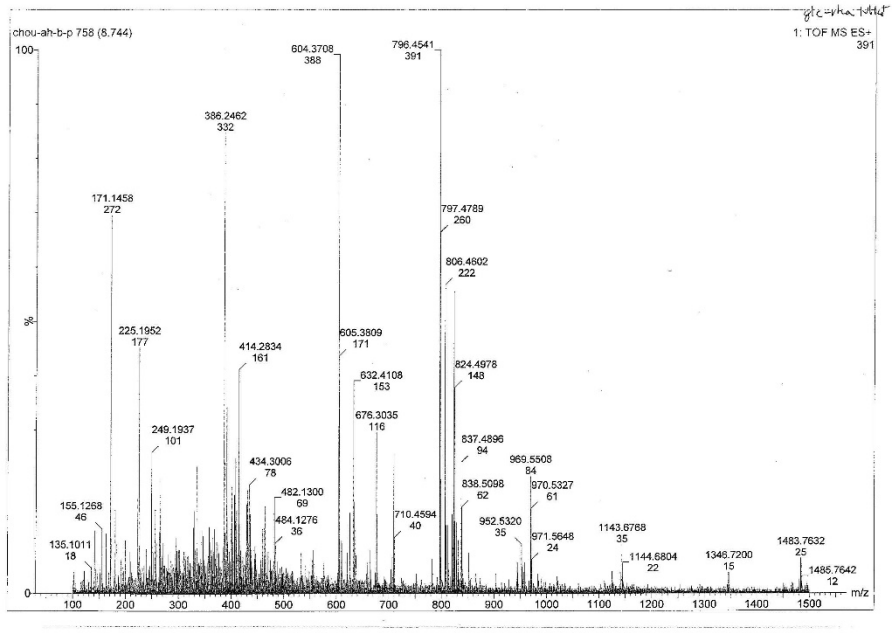


Supplementary Figure S18. LC-MS analysis of alkaline hydrolysis product.

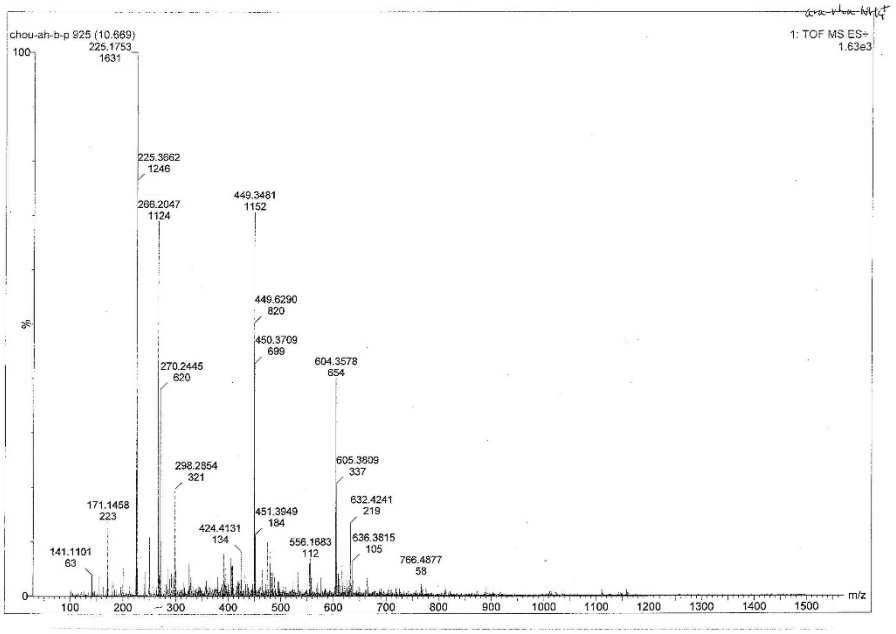
From top to bottom: Extracted ion chromatograms presumed to correspond to $[M+NH_4]^+$ of Glc-Ara: m/z 782.4, Glc-Rha: m/z 796.4, Ara-Rha: m/z 766.4, Glc-Glc-Glc: m/z 974.5, Glc-Glc-Ara: m/z 944.5, Glc-Glc-Rha: m/z 958.5, or Glc-Rra-Rha: m/z 928.5 attached to acacic acid lactone and total ion chromatogram.



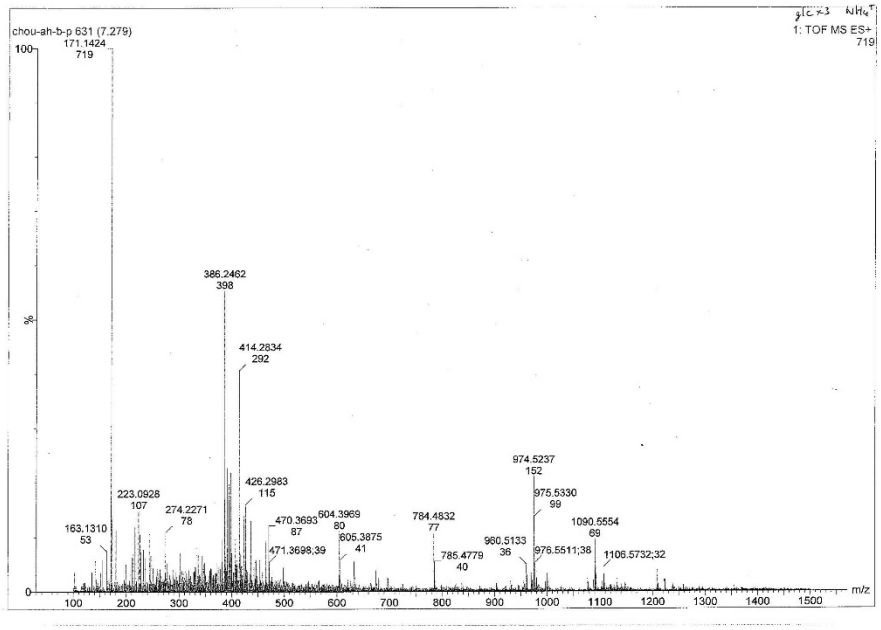
Supplementary Figure S19. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Glc-Ara attached acacic acid lactone (m/z 782.4)



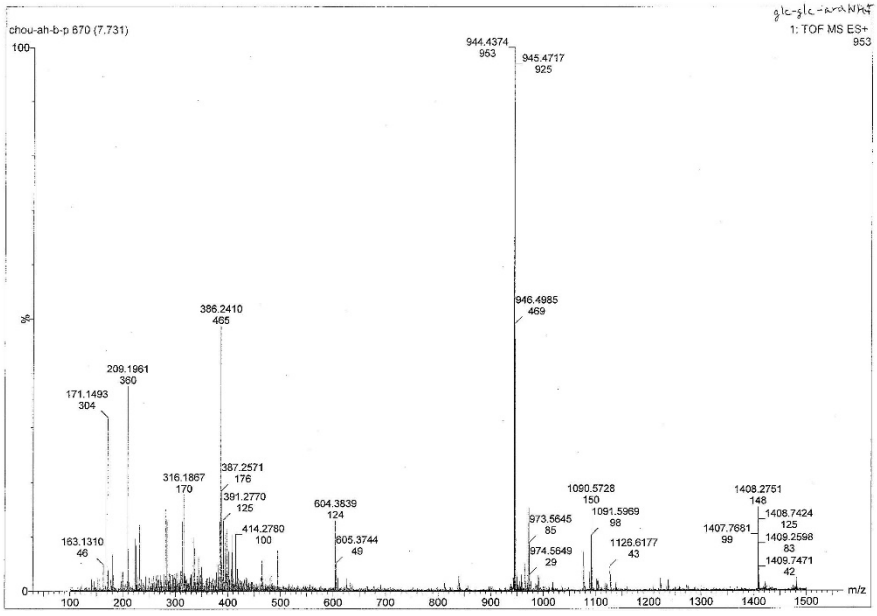
Supplementary Figure S20. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Glc-Rha attached acacic acid lactone (m/z 796.4)



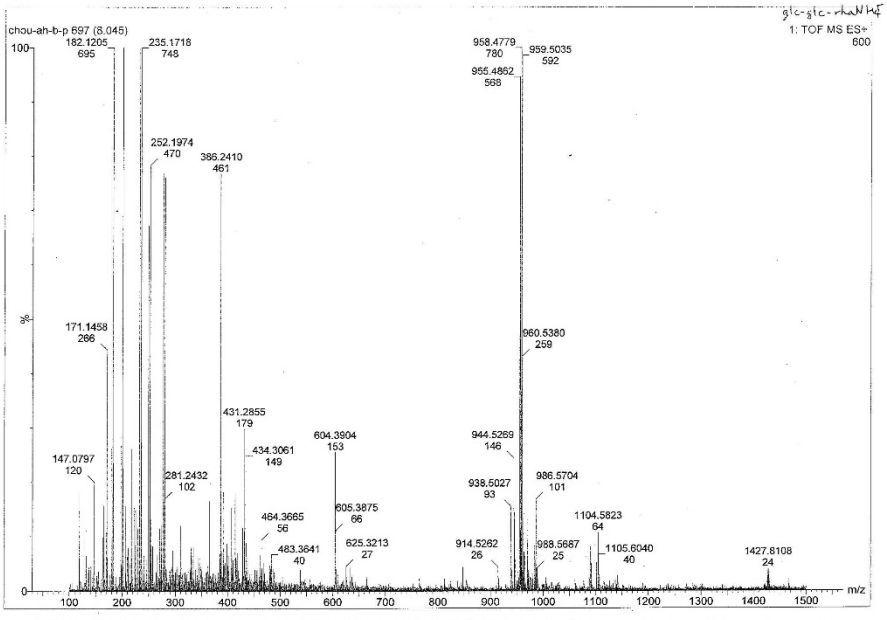
Supplementary Figure S21. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Ara-Rha attached acacic acid lactone (m/z 766.4)



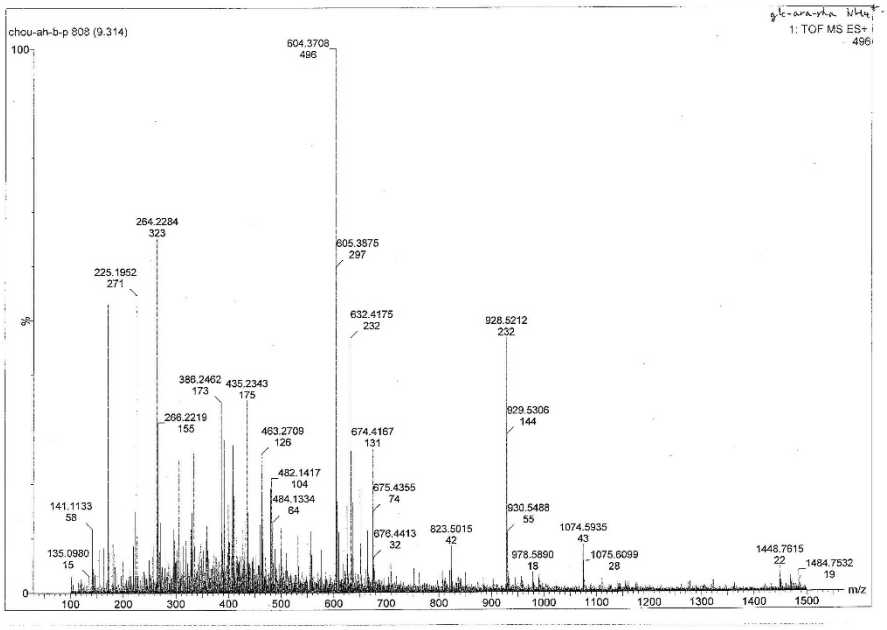
Supplementary Figure S22. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Glc-Glc-Glc attached acacic acid lactone (m/z 974.5)



Supplementary Figure S23. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Glc-Glc-Ara attached acacic acid lactone (m/z 944.5)



Supplementary Figure S24. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Glc-Glc-Rha attached acacic acid lactone (m/z 958.5)



Supplementary Figure S25. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Glc-Ara-Rha attached acacic acid lactone (m/z 928.5)

References

1. Tezuka Y, Honda K, Banskota AH, et al (2000) Kinmoonosides A−C, Three New Cytotoxic Saponins from the Fruits of Acacia c oncinna , a Medicinal Plant Collected in Myanmar. J Nat Prod 63:1658–1664. https://doi.org/10.1021/np000347f
2. Kiuchi F, Gafur MA, Obata T, et al (1997) Acacia concinna Saponins. II. Structures of Monoterpenoid Glycosides in the Alkaline Hydrolysate of the Saponin Fraction. Chem Pharm Bull (Tokyo) 45:807–812. https://doi.org/10.1248/cpb.45.807