



Title	Saponins are responsible for the anti-obesogenic activity of <i>Acacia concinna</i>
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	http://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	article (author version)
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	Eikato_JNatProd_2021_Supplementary material.pdf



[Instructions for use](#)

Supplementary material

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

Zhao Zhuoyue¹, Wijitrapha Ruangaram¹, Eisuke Kato^{2*}

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

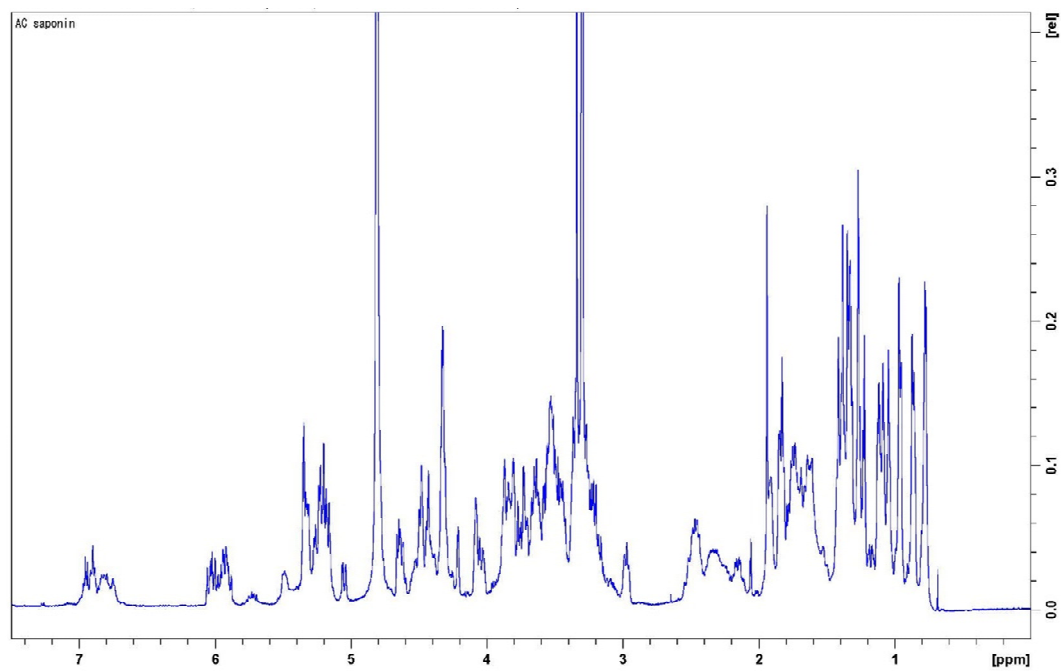
² Division of Fundamental AgriScience and Research, Research Faculty of Agriculture, Hokkaido University, Kita-ku, Sapporo, Hokkaido 060-8589, Japan

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

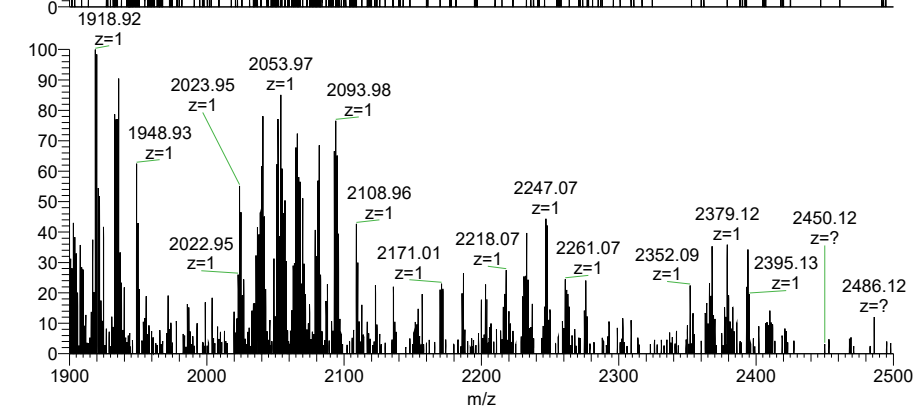
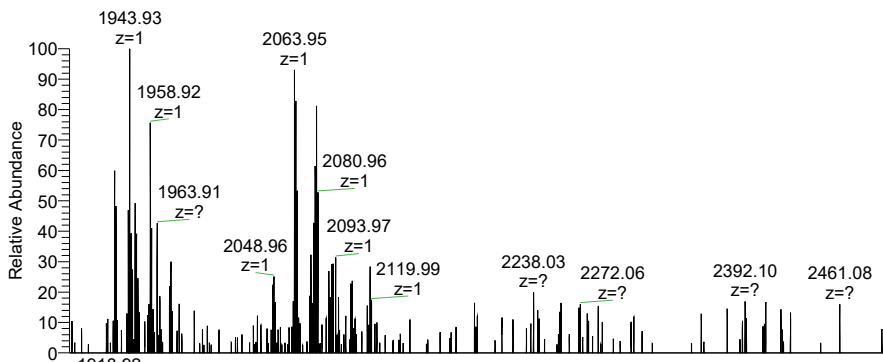
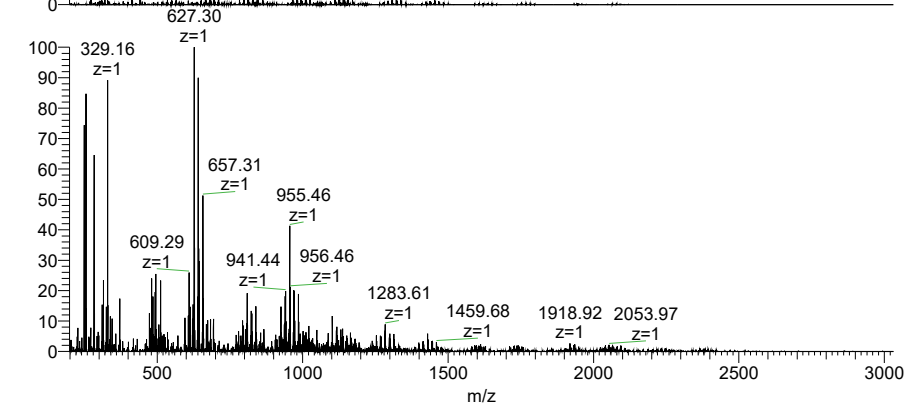
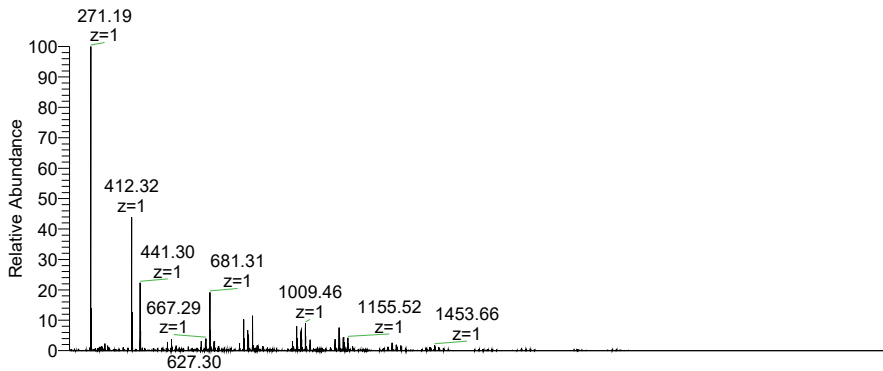
Contents

Supplementary Figure S2. ESI-MS spectrum of the AC saponin	4
Supplementary Table S1. Comparison 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 ₃ OD) . .	6
Supplementary Figure S4. ¹ H-NMR spectrum of compound 2 (500 MHz, CD ₃ OD) . .	7
Supplementary Figure S5. ¹ H-NMR spectrum of compound 3 (500 MHz, CD ₃ OD) . .	8
Supplementary Figure S6. ¹ H-NMR spectrum of compound 4 (500 MHz, CD ₃ OD) . .	9
Supplementary Figure S7. ¹ H-NMR spectrum of compound 5 (500 MHz, Acetone- <i>d</i> ₆)	10
Supplementary Figure S8. ¹³ C-NMR spectrum of compound 5 (500 MHz, Acetone- <i>d</i> ₆)	11
Supplementary Figure S9. COSY spectrum of compound 5 (500 MHz, Acetone- <i>d</i> ₆). .	12
Supplementary Figure S10. HSQC spectrum of compound 5 (500 MHz, Acetone- <i>d</i> ₆)	13
Supplementary Figure S11. HMBC spectrum of compound 5 (500 MHz, Acetone- <i>d</i> ₆)	14
Supplementary Figure S12. ¹ H-NMR spectrum of compound 6 (500 MHz, Acetone- <i>d</i> 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 $[\text{M}+\text{NH}_4]^+$ ion of Glc-Ara attached acacic acid lactone (m/z 782.4)	22
Supplementary Figure S20. MS spectrum of the peak presumed as a $[\text{M}+\text{NH}_4]^+$ ion of Glc-Rha attached acacic acid lactone (m/z 796.4)	22
Supplementary Figure S21. MS spectrum of the peak presumed as a $[\text{M}+\text{NH}_4]^+$ ion of Ara-Rha attached acacic acid lactone (m/z 766.4)	23
Supplementary Figure S22. MS spectrum of the peak presumed as a $[\text{M}+\text{NH}_4]^+$ 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 $[\text{M}+\text{NH}_4]^+$ 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 $[\text{M}+\text{NH}_4]^+$ 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 $[\text{M}+\text{NH}_4]^+$ ion of Glc-Ara-Rha attached acacic acid lactone (m/z 928.5)	25



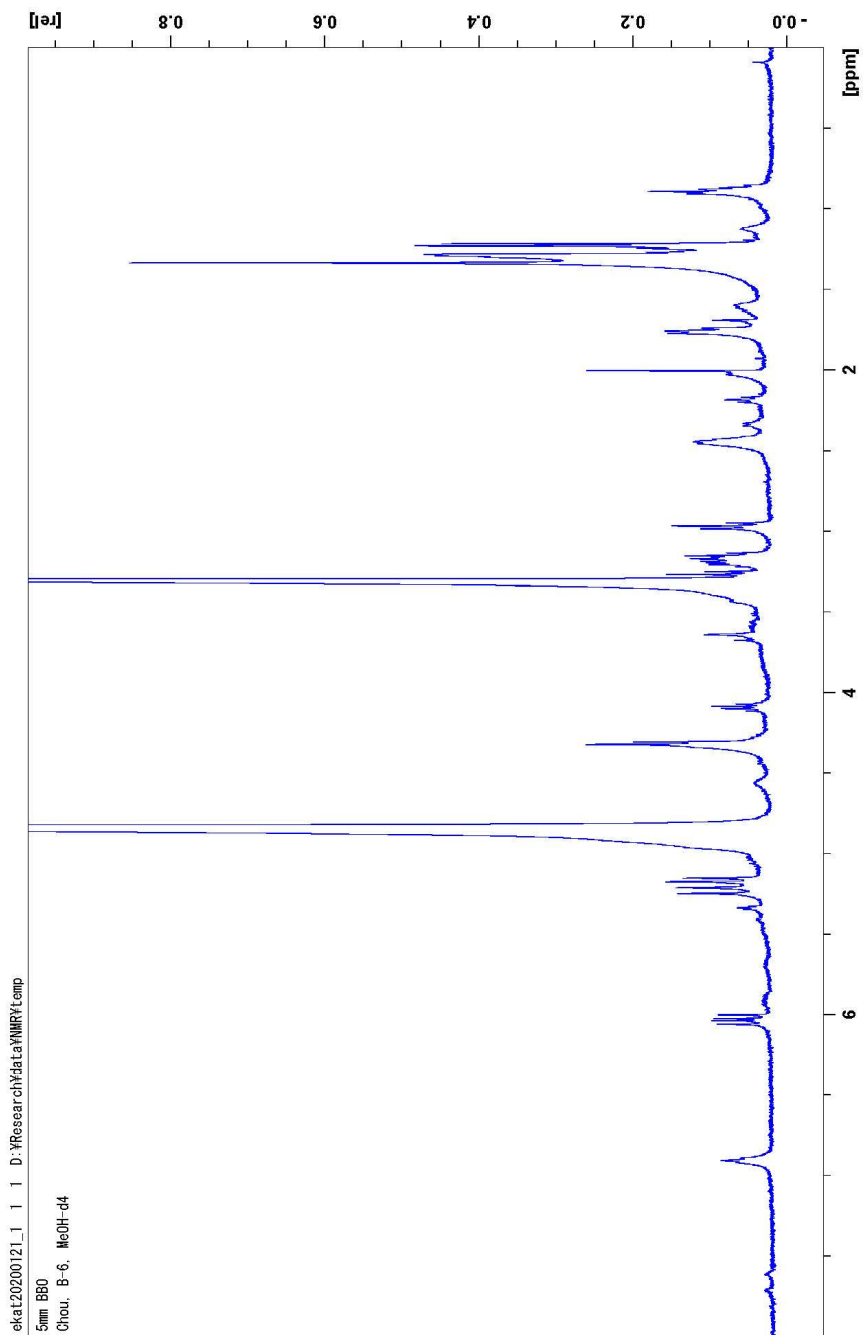
Supplementary Figure S1. $^1\text{H-NMR}$ of the AC saponin



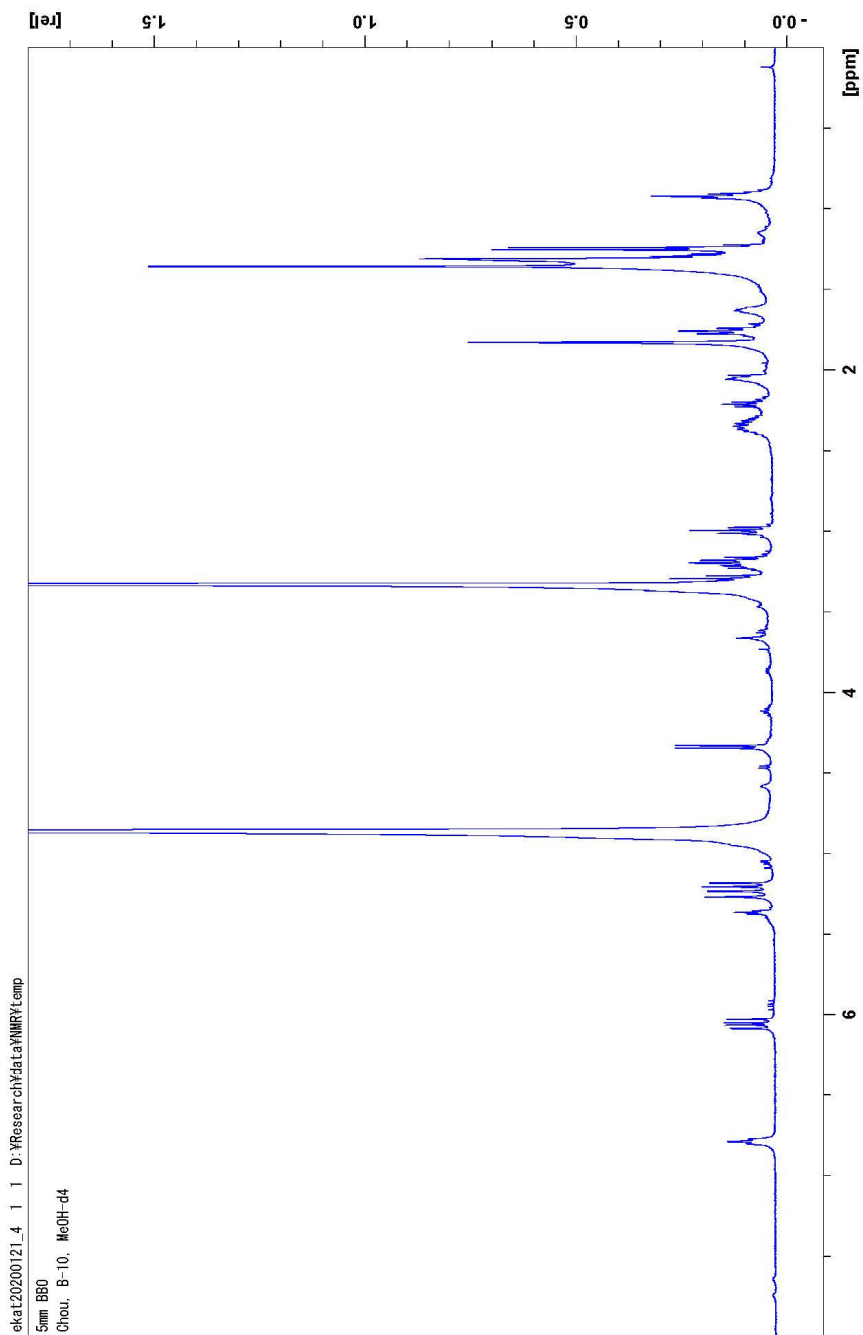
Supplementary Figure S2. ESI-MS spectrum of the AC saponin

Supplementary Table S1. Comparison of ¹H-NMR data of compound 1-4 with literature data

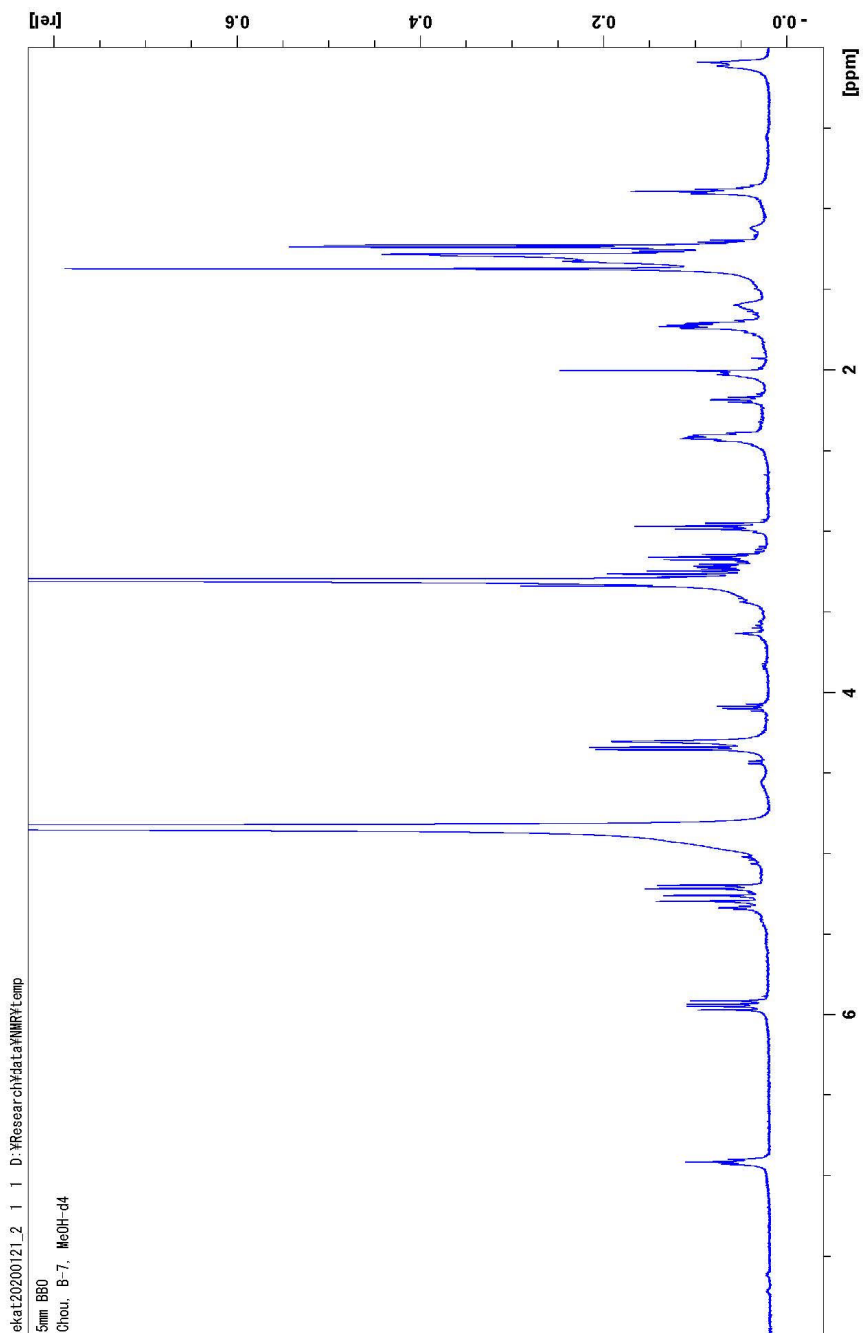
No.	Compound 1		Compound 2		Compound 3		Compound 4	
	literature data[1]	Isolated	literature data[1]	Isolated	literature data[2]	Isolated	literature data[2]	Isolated
3	6.85 (br t, 6.6)	6.91 (br t)	6.85 (br t, 6.6)	6.90 (1H, t)	6.76 (1H, br t, 6.8)	6.76 (1H, t)	6.77 (1H, tq, 7.8, 1.5)	6.79 (1H, tq)
4	2.40 (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)	5.15 (2H, dd)	5.22 (br d, 10.7)	5.20 (2H, dd)	5.17 (1H, dd, 10.7, 1.0)	5.17 (2H, dd)	5.17 (1H, dd, 11.2, 1.0)	5.18 (2H, dd)
9	5.22 (br d, 17.3)		5.28 (br d, 17.1)		5.23 (1H, dd, 17.6, 1.0)		5.23 (1H, dd, 17.8, 1.0)	
10	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)
1	Qui 4.31 (d, 7.8)	4.31 (1H, d)	Qui 4.35 (d, 7.8)	4.33 (1H, d)	Xyl 4.28 (1H, d, 7.3)	4.29 (1H, d)	Qui 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)	3.08 (1H, t)	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)	3.76 (1H, dd, 11.5, 5.6)	3.76 (1H, dd)	1.22 (3H, d, 5.9)	1.24 (2H, d)



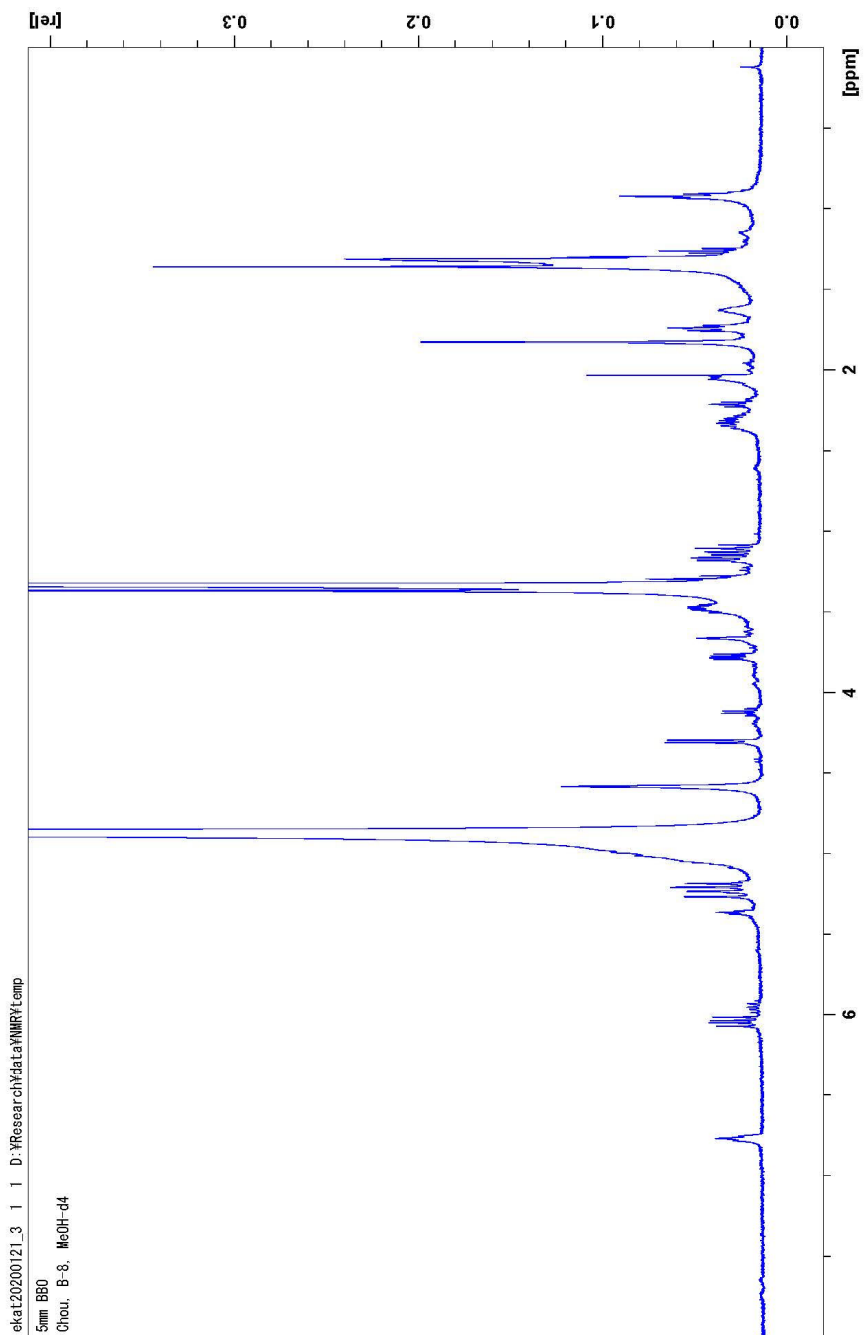
Supplementary Figure S3. ^1H -NMR spectrum of compound **1** (500 MHz, CD_3OD)



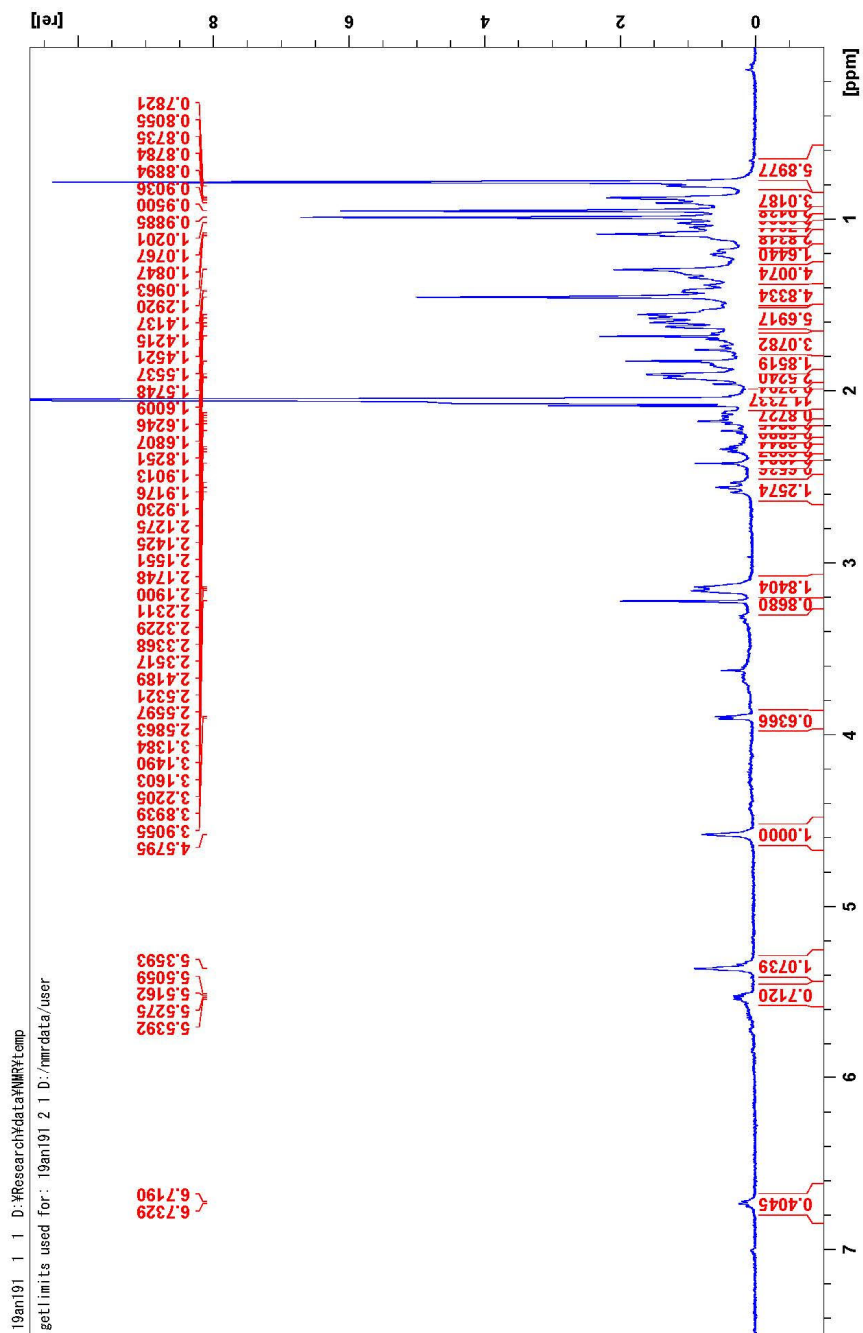
Supplementary Figure S4. ^1H -NMR spectrum of compound **2** (500 MHz, CD_3OD)



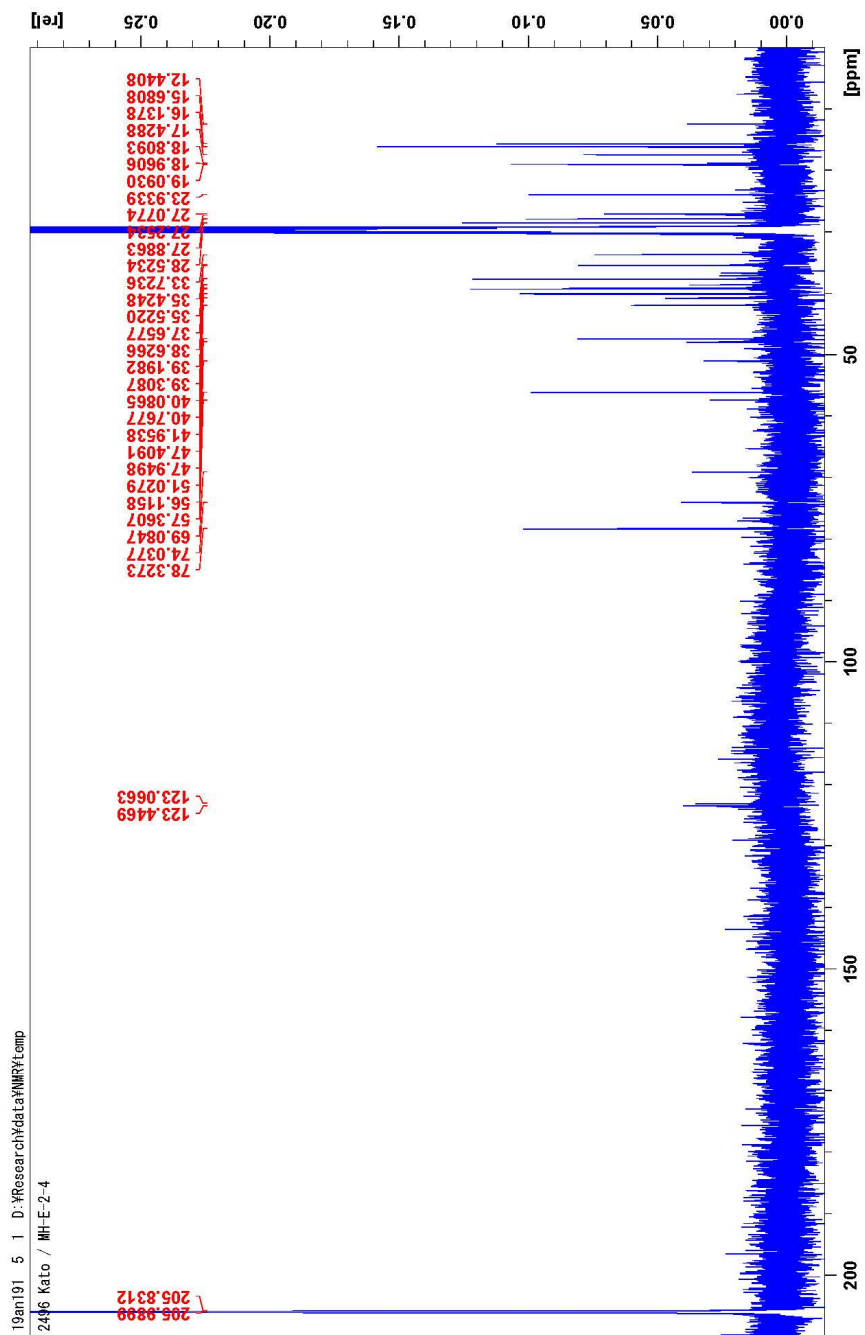
Supplementary Figure S5. $^1\text{H-NMR}$ spectrum of compound **3** (500 MHz, CD_3OD)



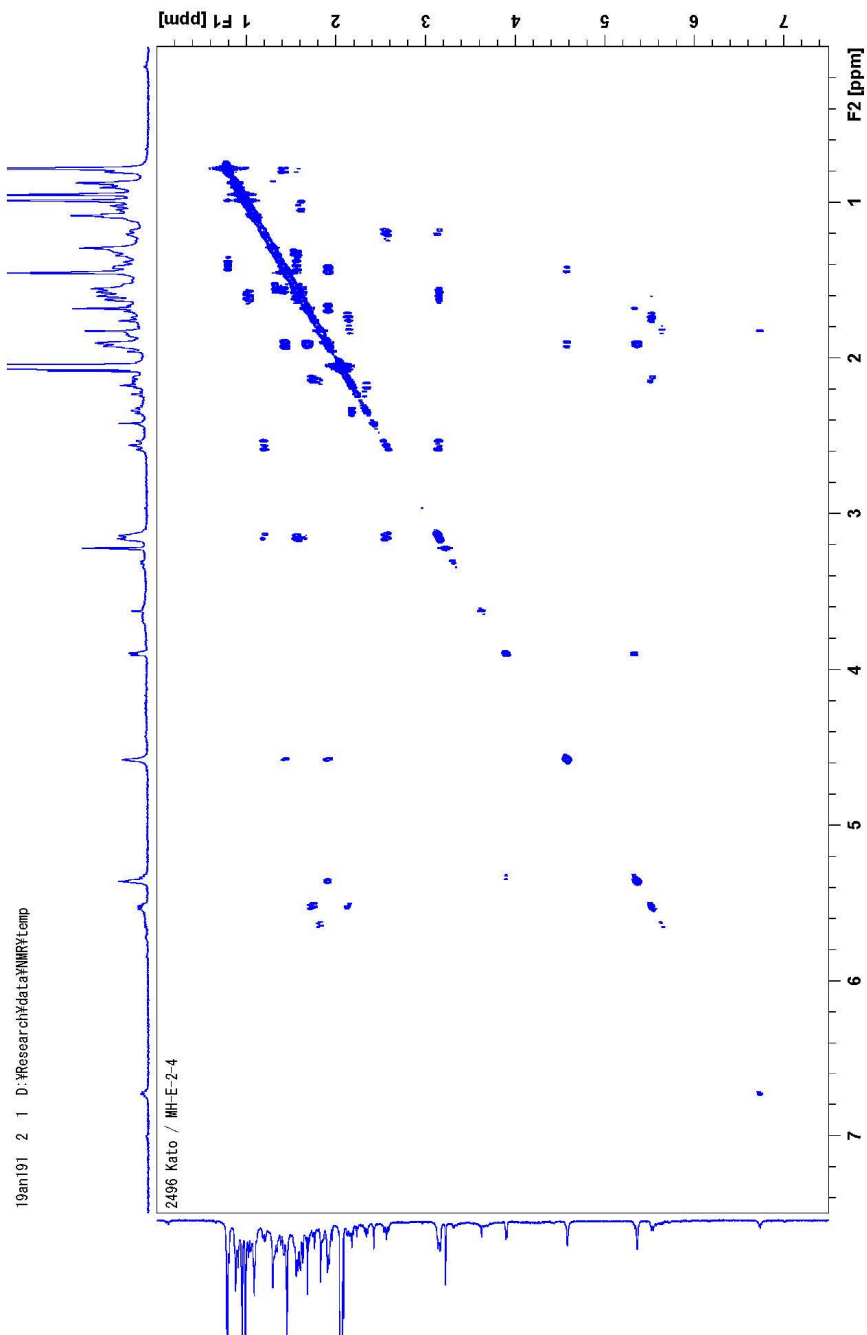
Supplementary Figure S6. ^1H -NMR spectrum of compound 4 (500 MHz, CD_3OD)



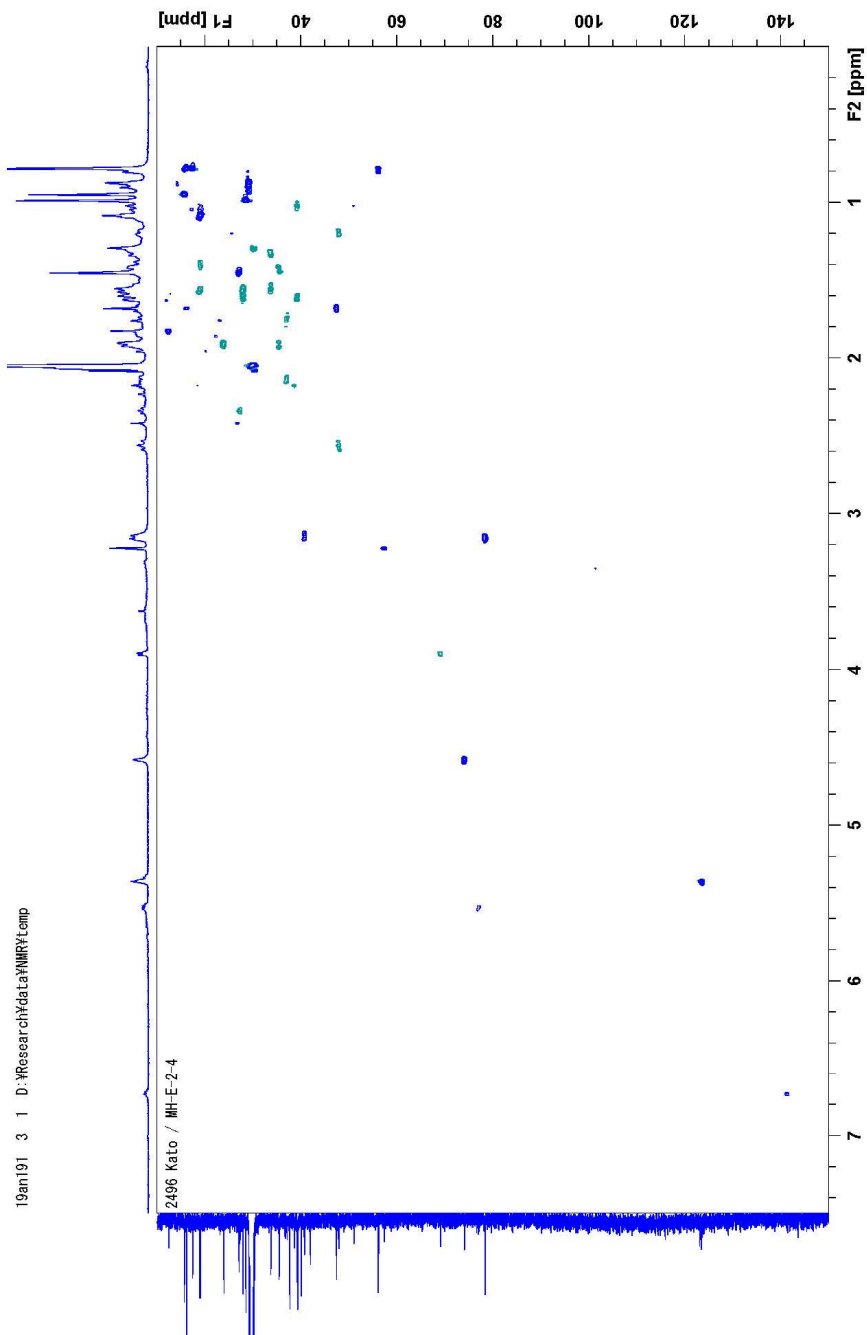
Supplementary Figure S7. ¹H-NMR spectrum of compound **5** (500 MHz, Acetone-*d*₆)



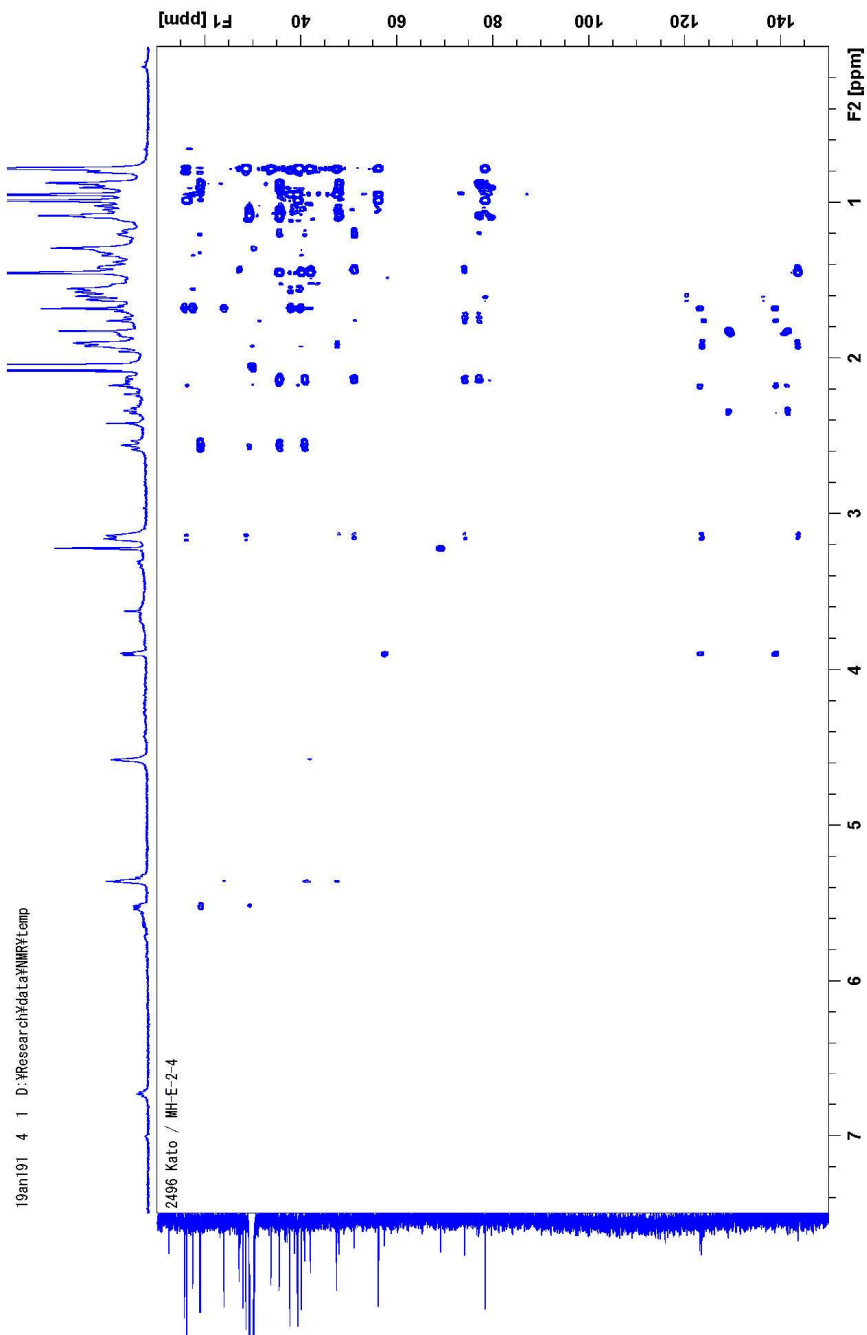
Supplementary Figure S8. ^{13}C -NMR spectrum of compound **5** (500 MHz, Acetone- d_6)



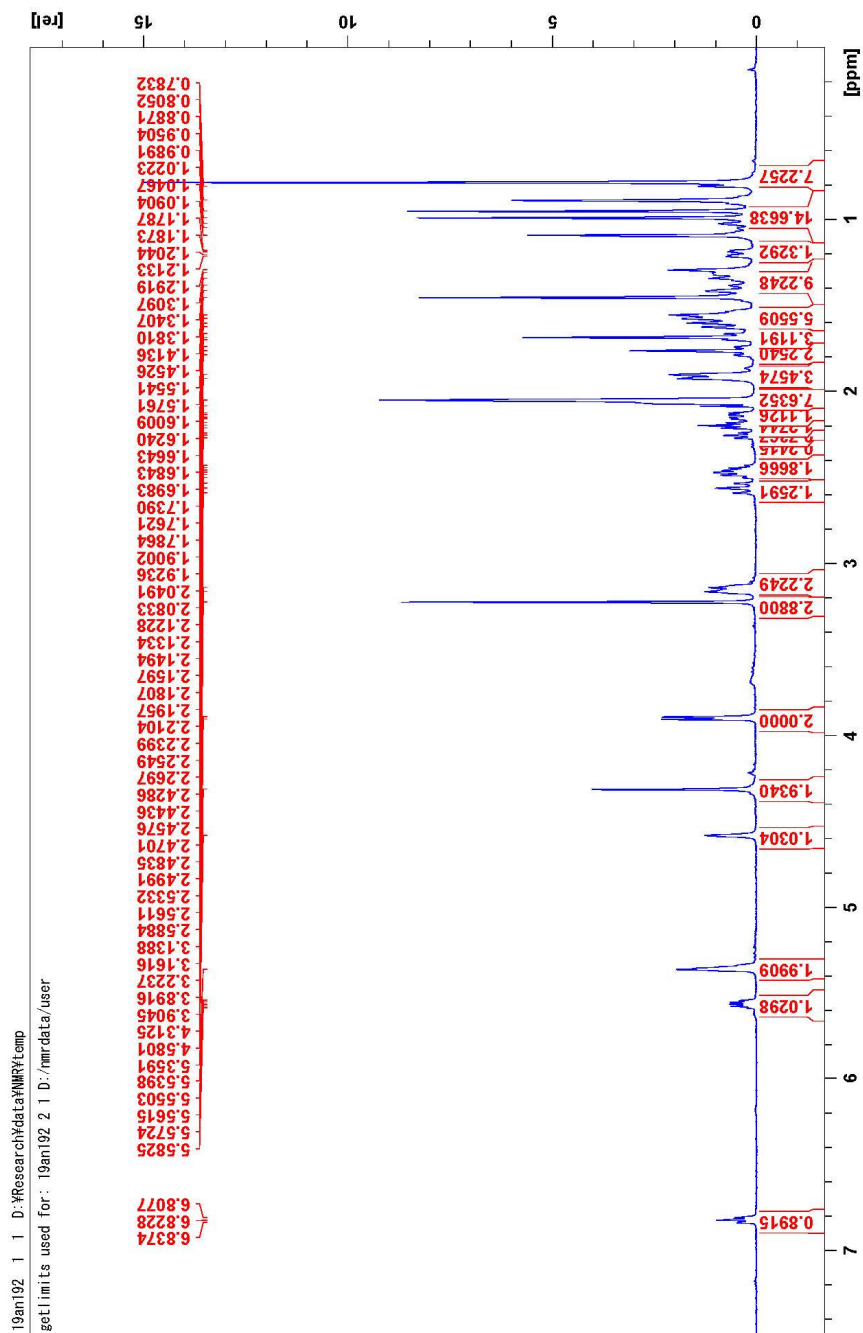
Supplementary Figure S9. COSY spectrum of compound **5** (500 MHz, Acetone- d_6)



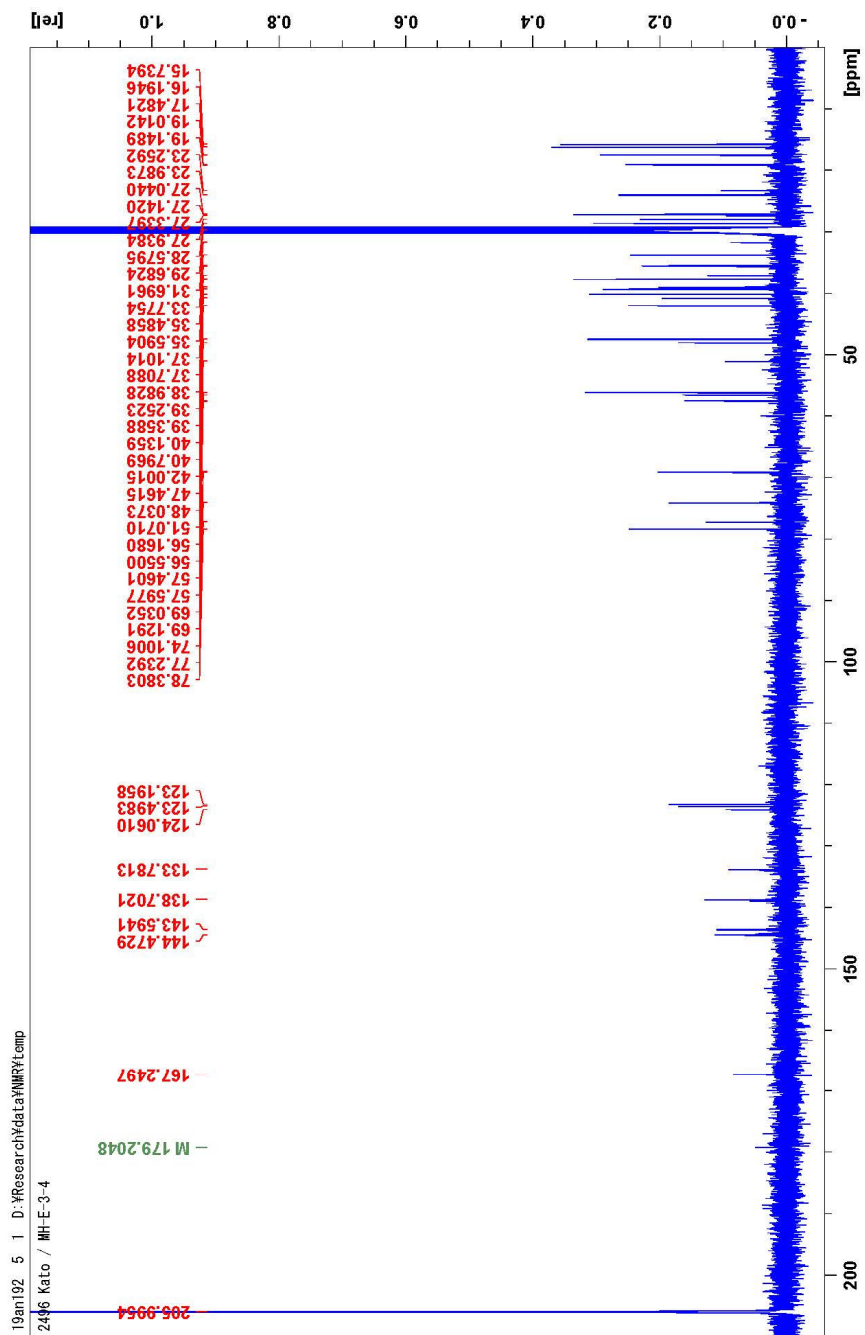
Supplementary Figure S10. HSQC spectrum of compound **5** (500 MHz, Acetone- d_6)



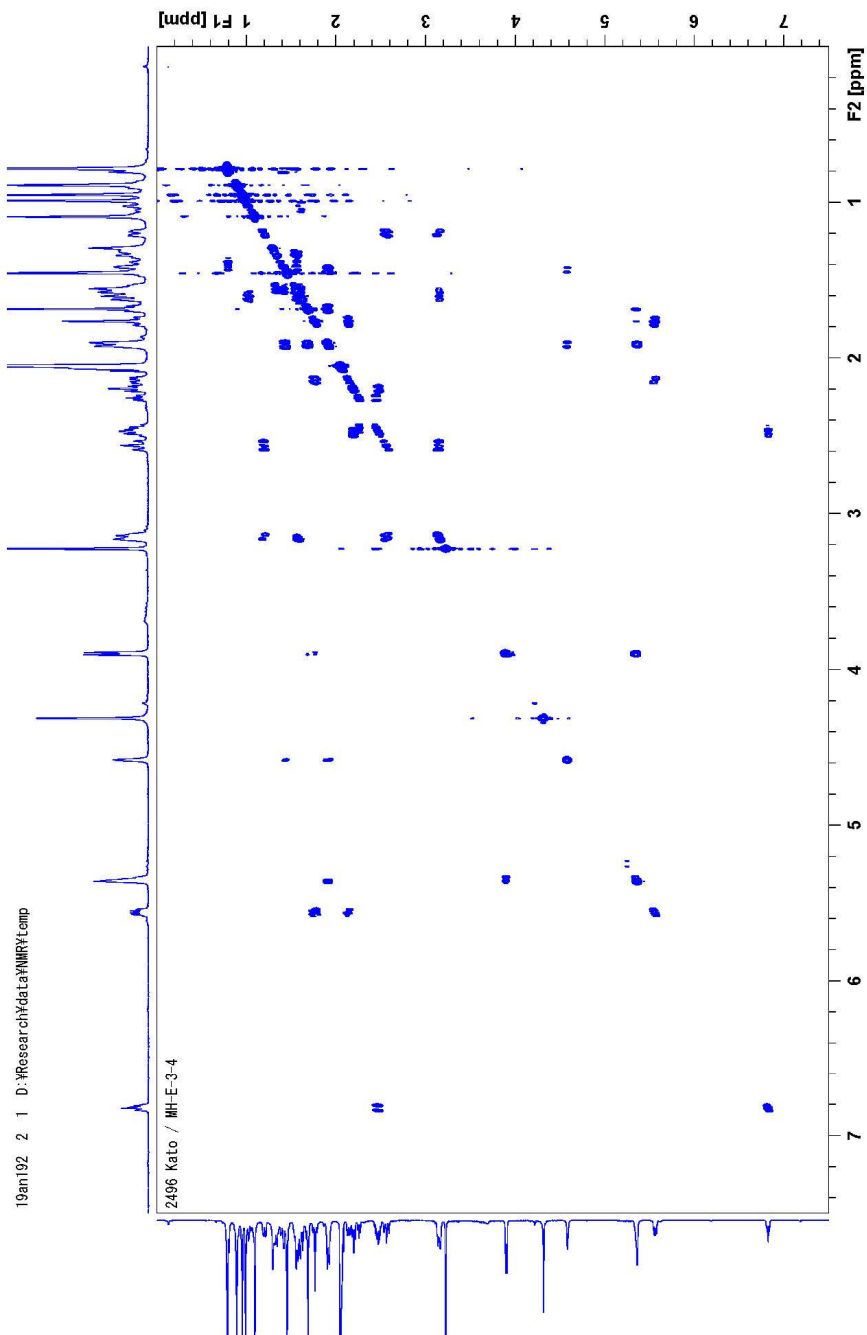
Supplementary Figure S11. HMBC spectrum of compound **5** (500 MHz, Acetone- d_6)



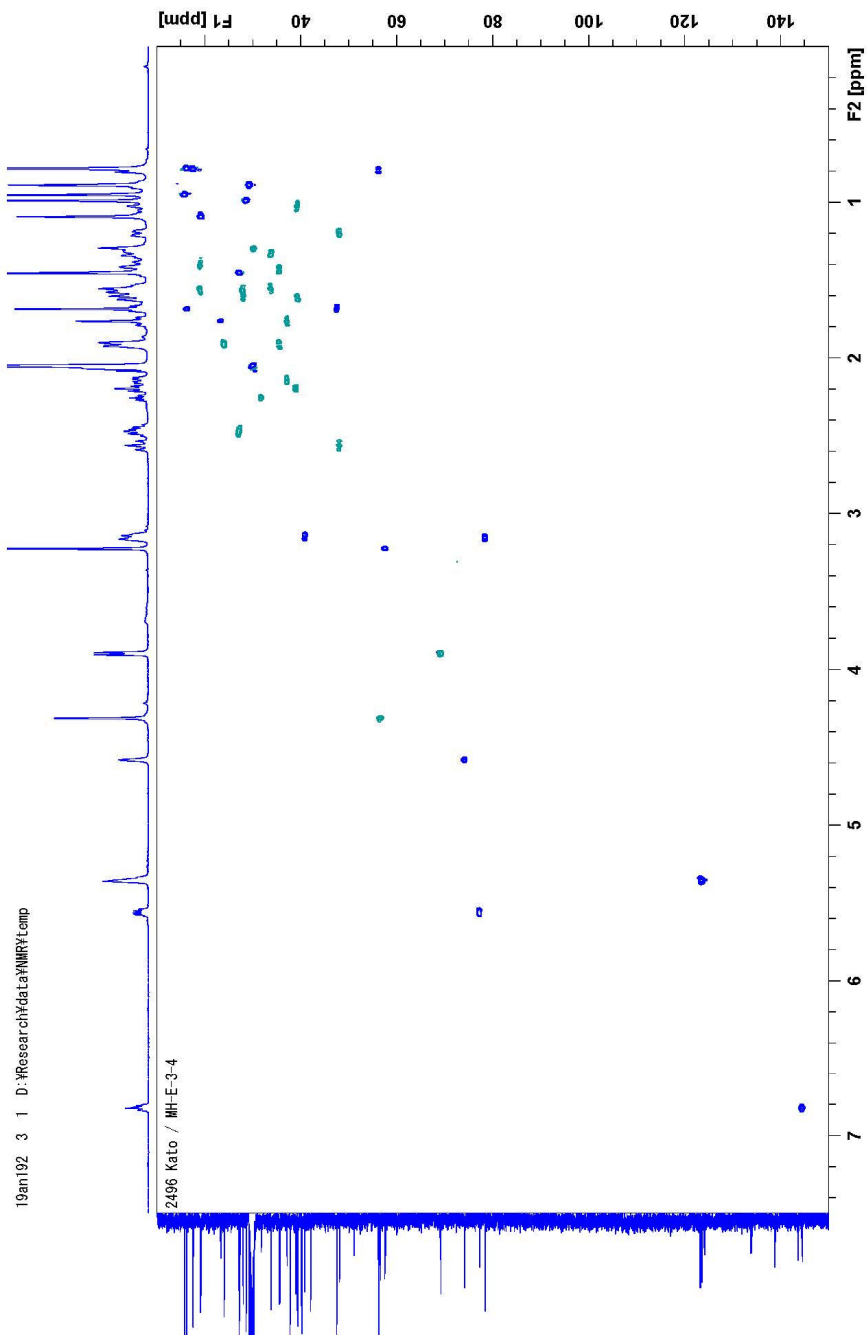
Supplementary Figure S12. ¹H-NMR spectrum of compound 6 (500 MHz, Acetone-*d*₆)



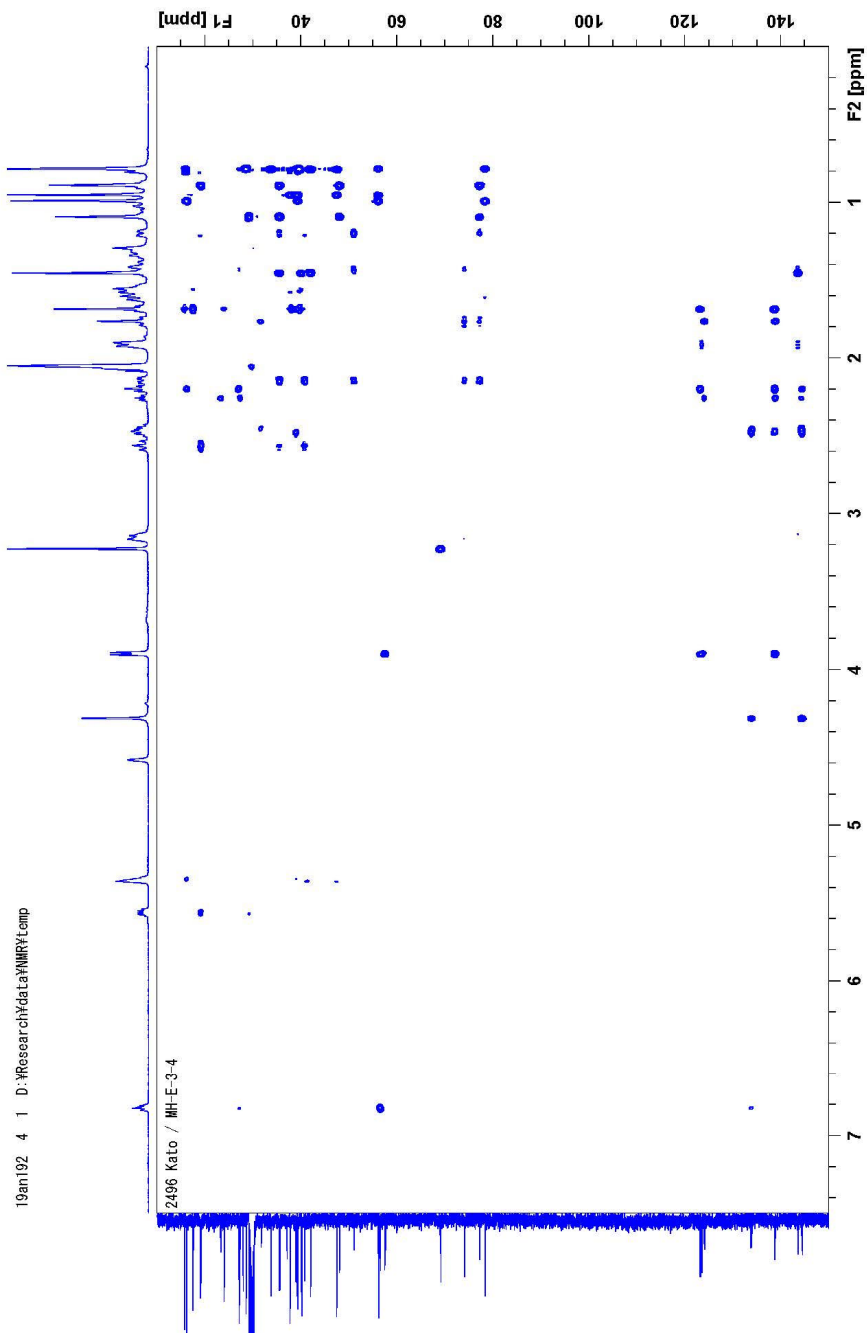
Supplementary Figure S13. ¹³C-NMR spectrum of compound 6 (500 MHz, Acetone-d₆)



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

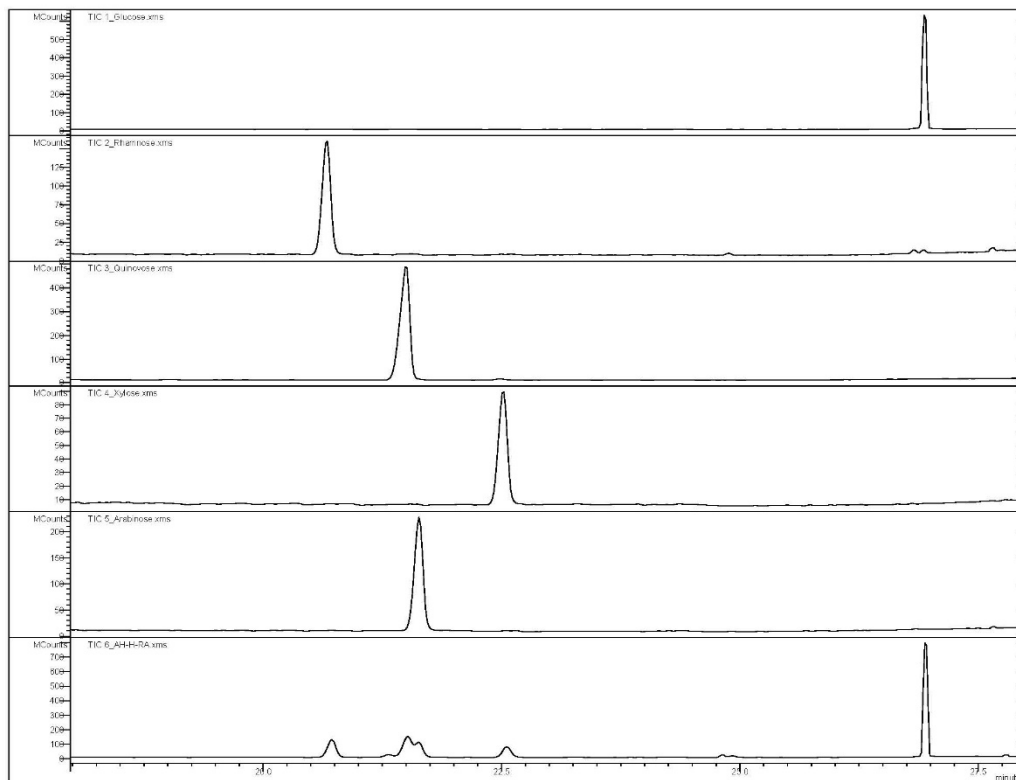


Supplementary Figure S15. HSQC spectrum of compound **6** (500 MHz, Acetone- d_6)



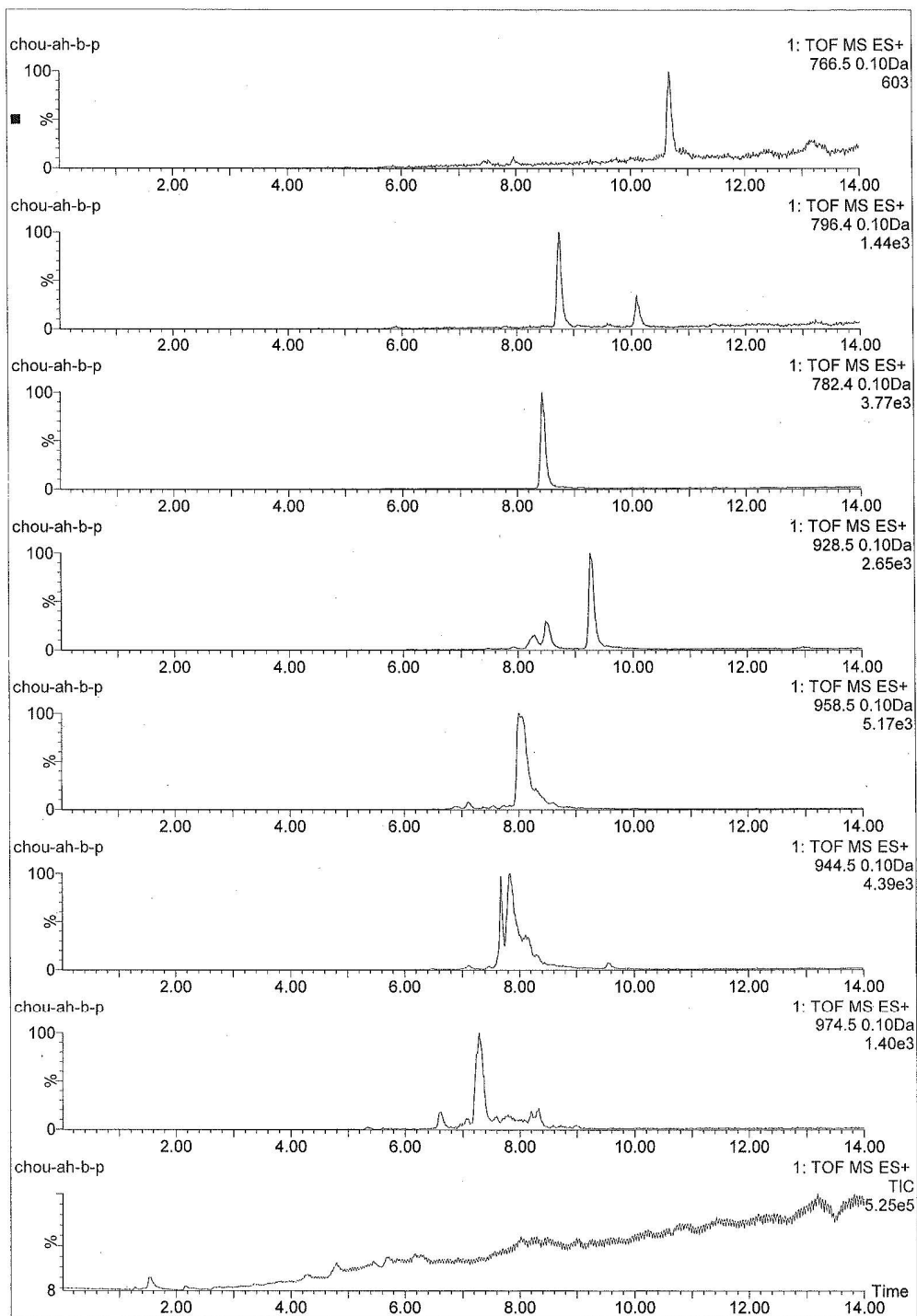
Supplementary Figure S16. HMBC spectrum of compound **6** (500 MHz, Acetone- d_6)

Chromatogram Plots



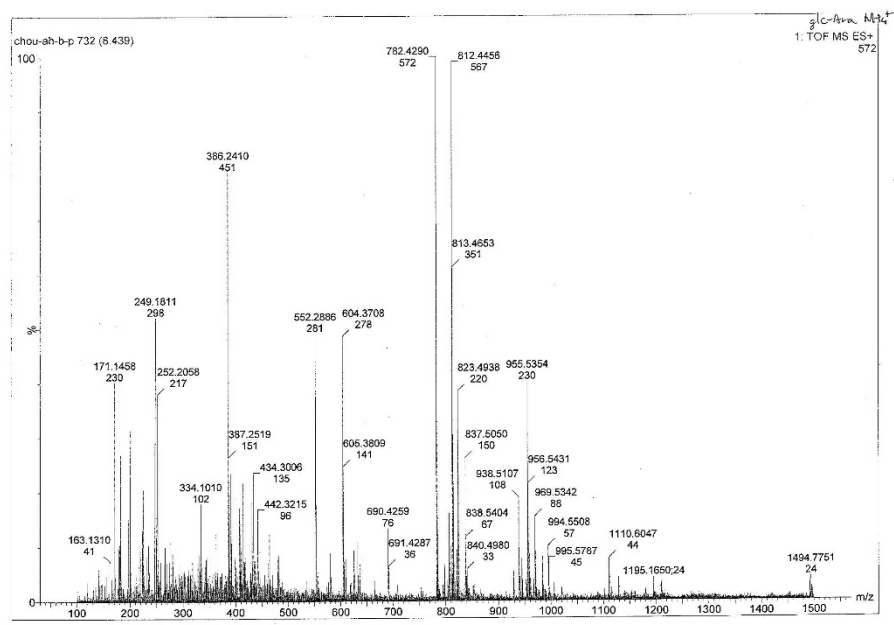
Supplementary Figure S17. GC-MS analysis of the sugars in AC saponin.

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

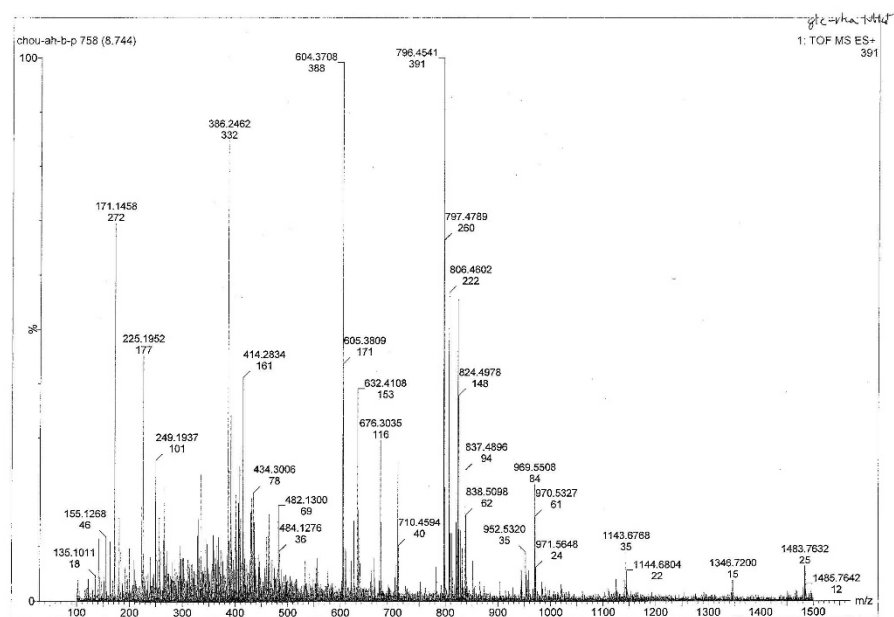


Supplementary Figure S18. LC-MS analysis of alkaline hydrolysis product.

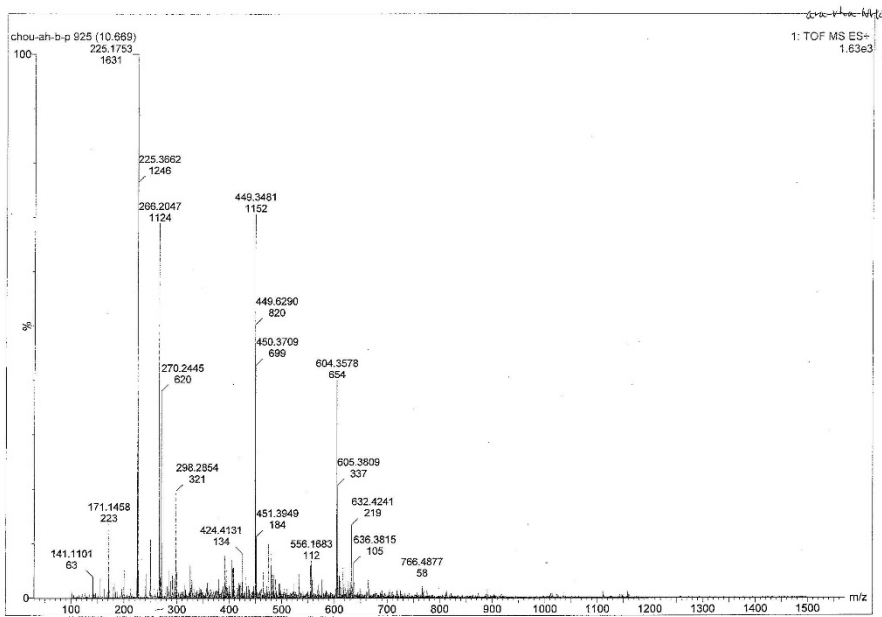
From top to bottom: Extracted ion chromatograms presumed to correspond to $[M+NH_4]^+$ of Glc-Ara: m/z 782.4, Glc-Rha: m/z 796.4, Ara-Rha: m/z 766.4, Glc-Glc-Glc: m/z 974.5, Glc-Glc-Ara: m/z 944.5, Glc-Glc-Rha: m/z 958.5, or Glc-Rra-Rha: m/z 928.5 attached to acacic acid lactone and total ion chromatogram.



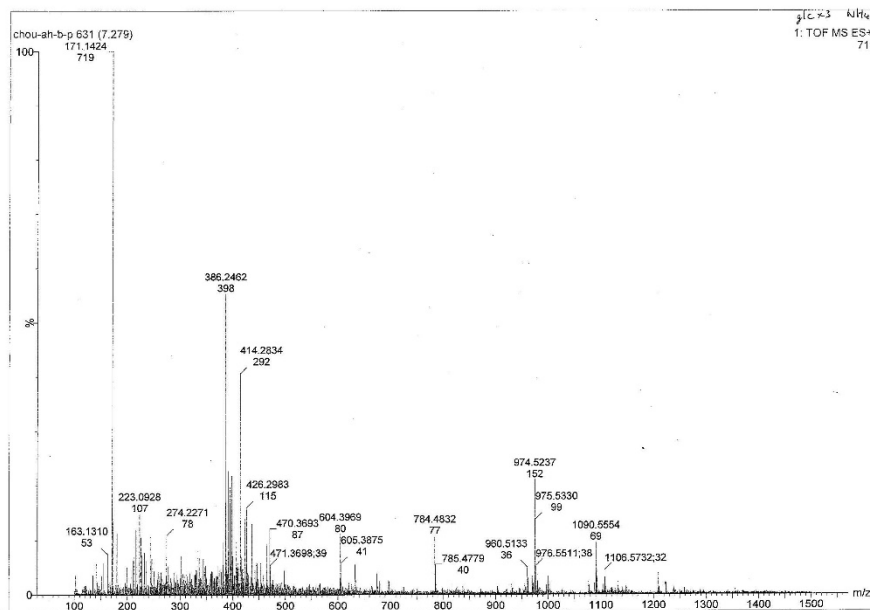
Supplementary Figure S19. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Glc-Ara attached acacic acid lactone (m/z 782.4)



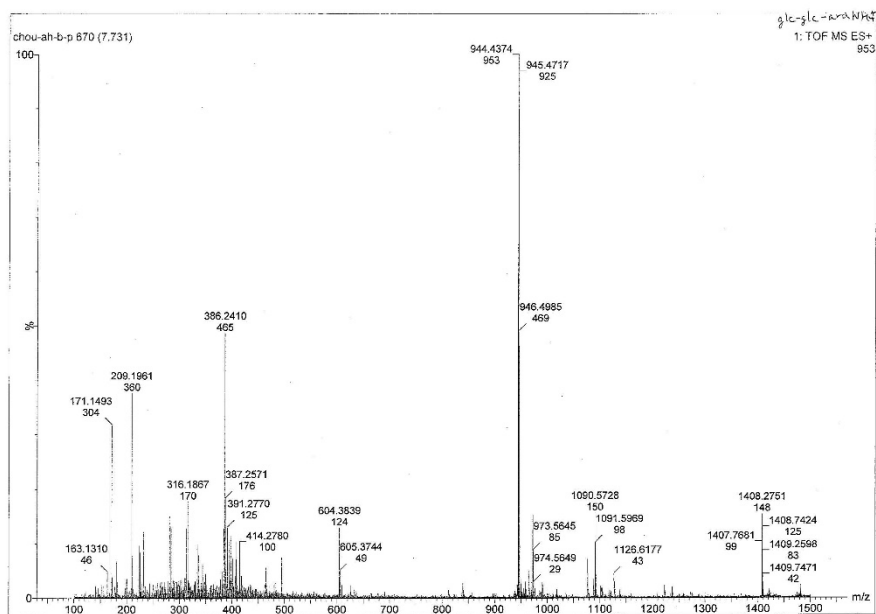
Supplementary Figure S20. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Glc-Rha attached acacic acid lactone (m/z 796.4)



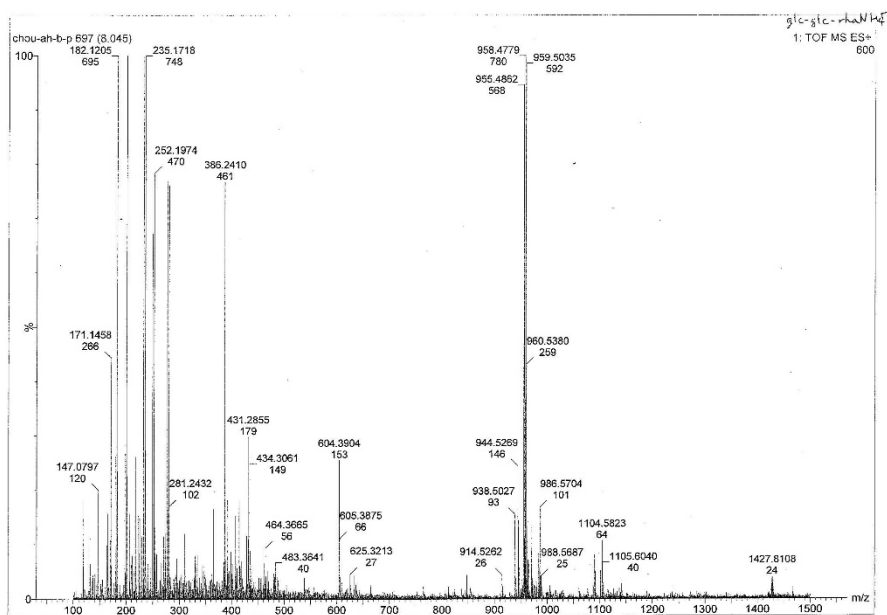
Supplementary Figure S21. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Ara-Rha attached acacic acid lactone (m/z 766.4)



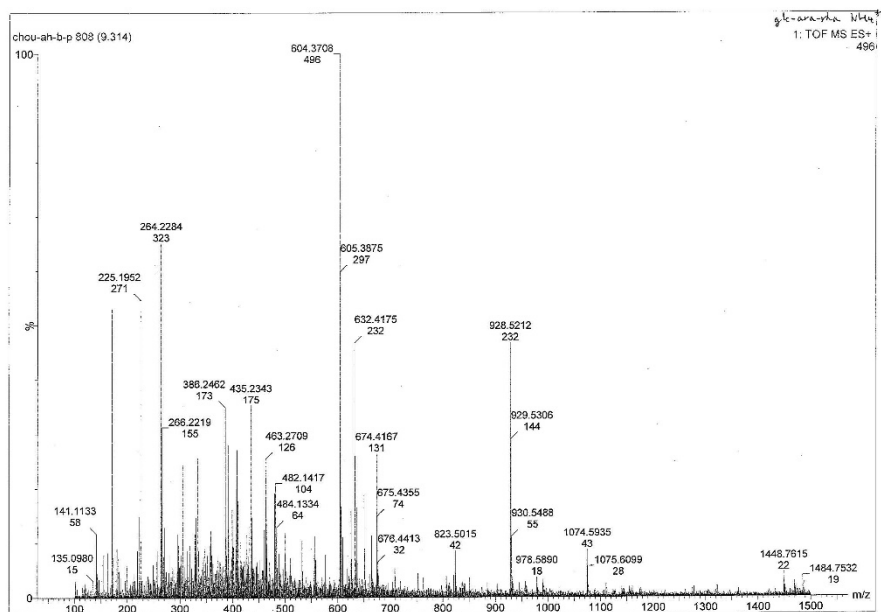
Supplementary Figure S22. MS spectrum of the peak presumed as a $[M+NH_4]^+$ ion of Glc-Glc-Glc attached acacic acid lactone (m/z 974.5)



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 concinna*, 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>