Supplementary data

Chemical Constituents of *Hedyotis diffusa* and Their Anti-Inflammatory Bioactivities

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Appendix A. References for known compounds **8**-**41**.

Figure S1. HRMS spectrum of **1**.

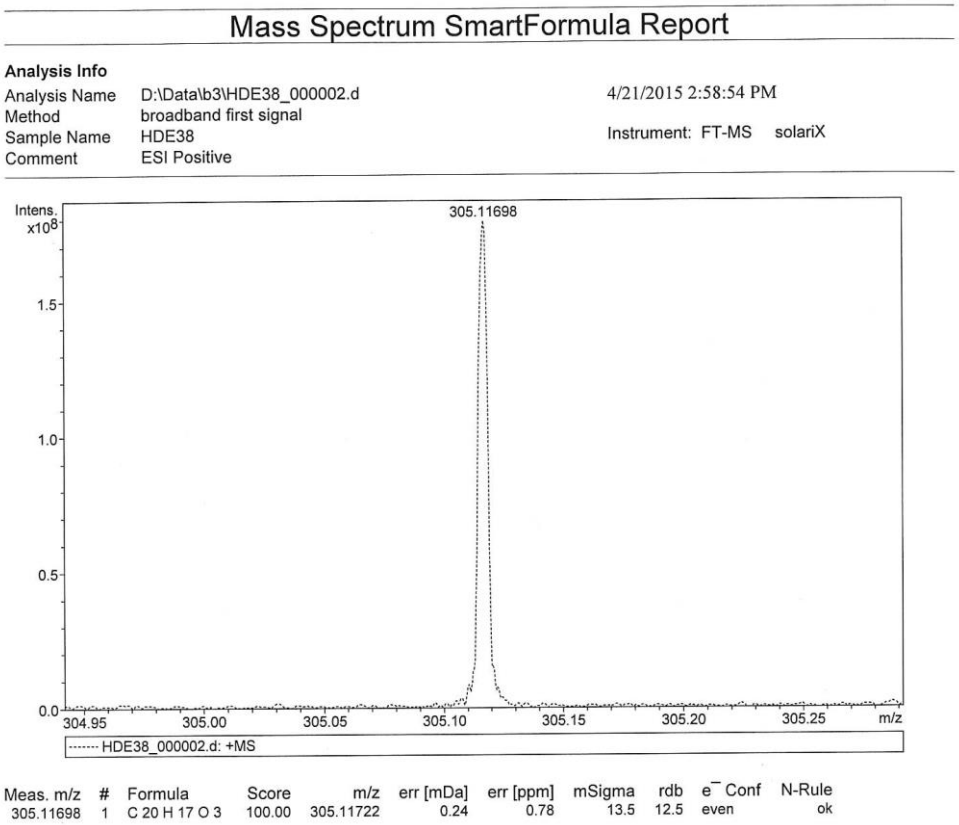


Figure S2. ^{1}H NMR spectrum of **1** ($CDCl_3$, 400 MHz).

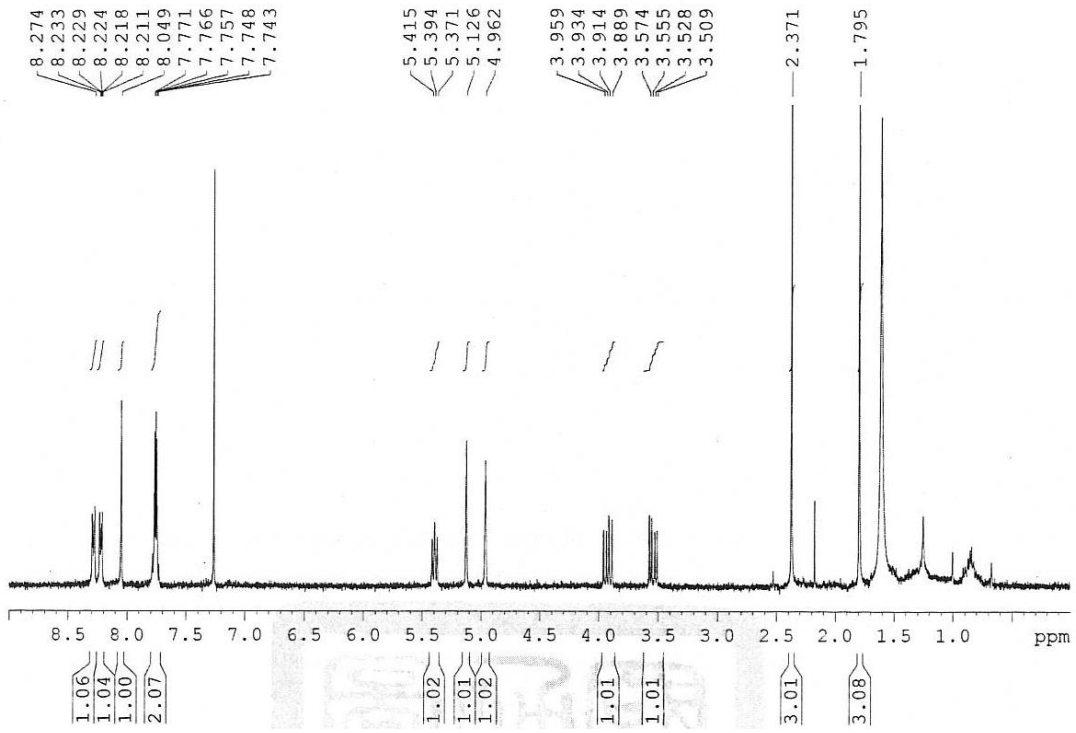


Figure S3. ^{13}C and DEPT NMR spectrum of **1** ($CDCl_3$, 100 MHz).

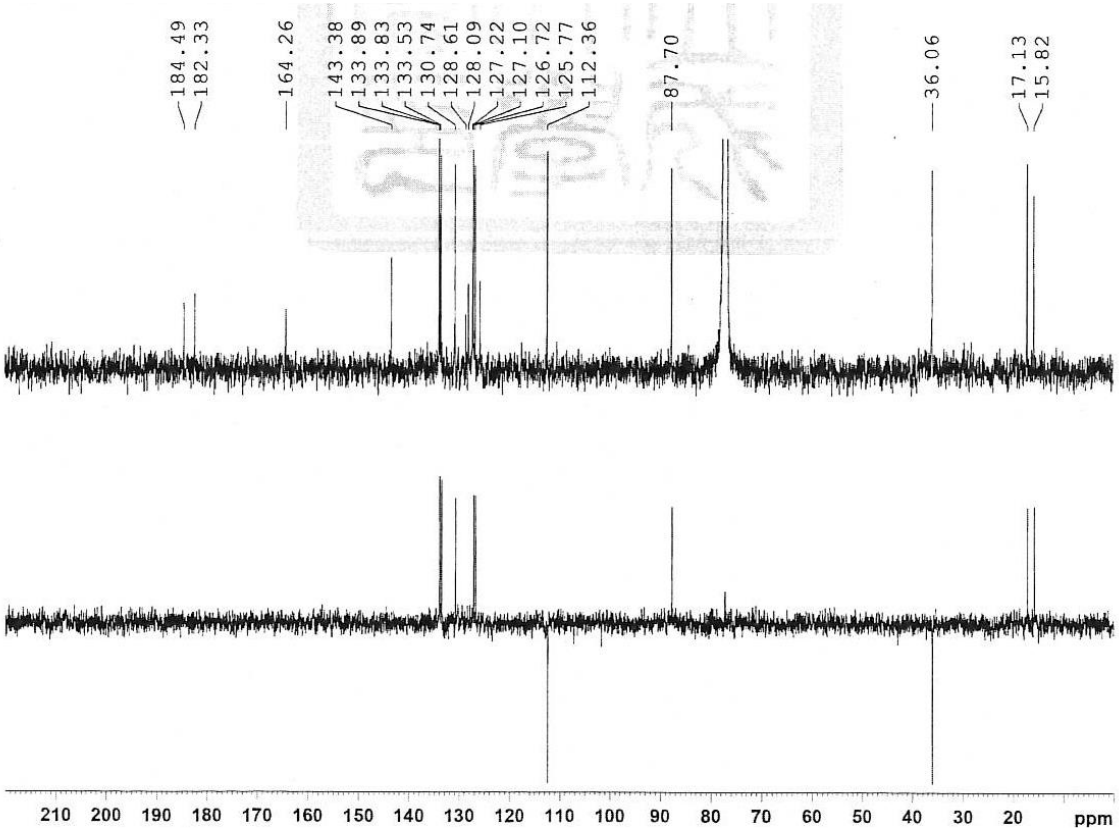


Figure S4. HMBC spectrum of **1** ($CDCl_3$, 400 MHz).

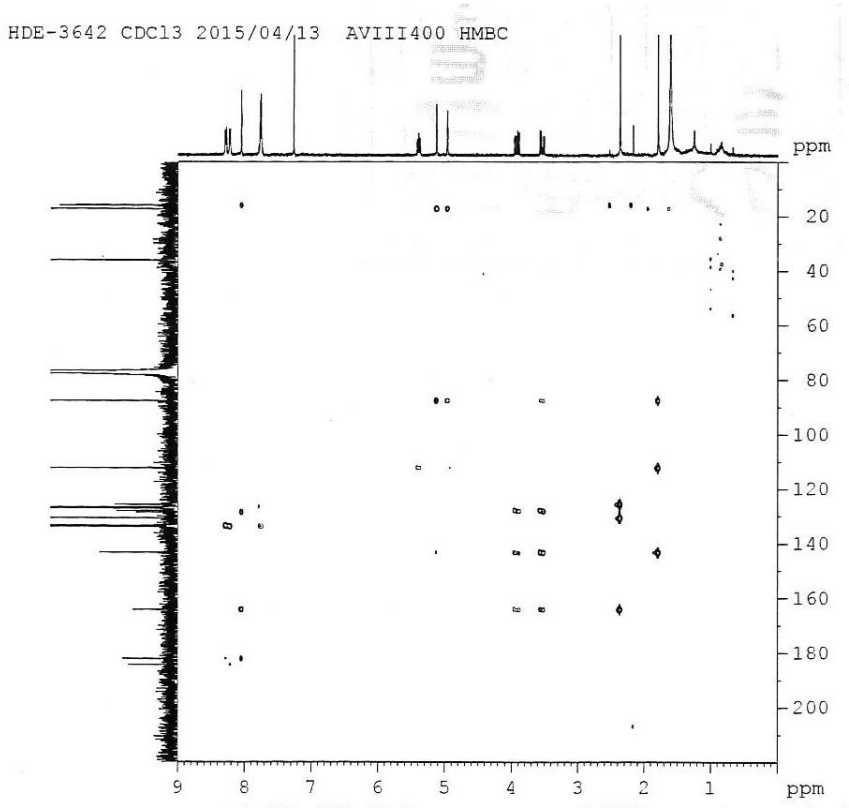


Figure S5. NOESY spectrum of **1** ($CDCl_3$, 400 MHz).

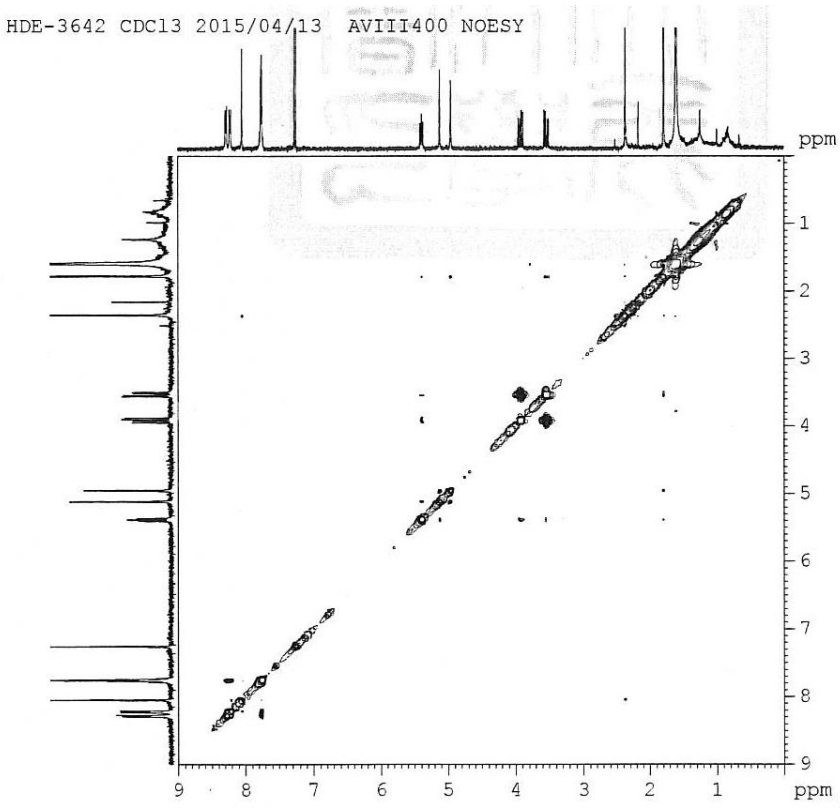


Figure S6. COSY spectrum of **1** ($CDCl_3$, 400 MHz).

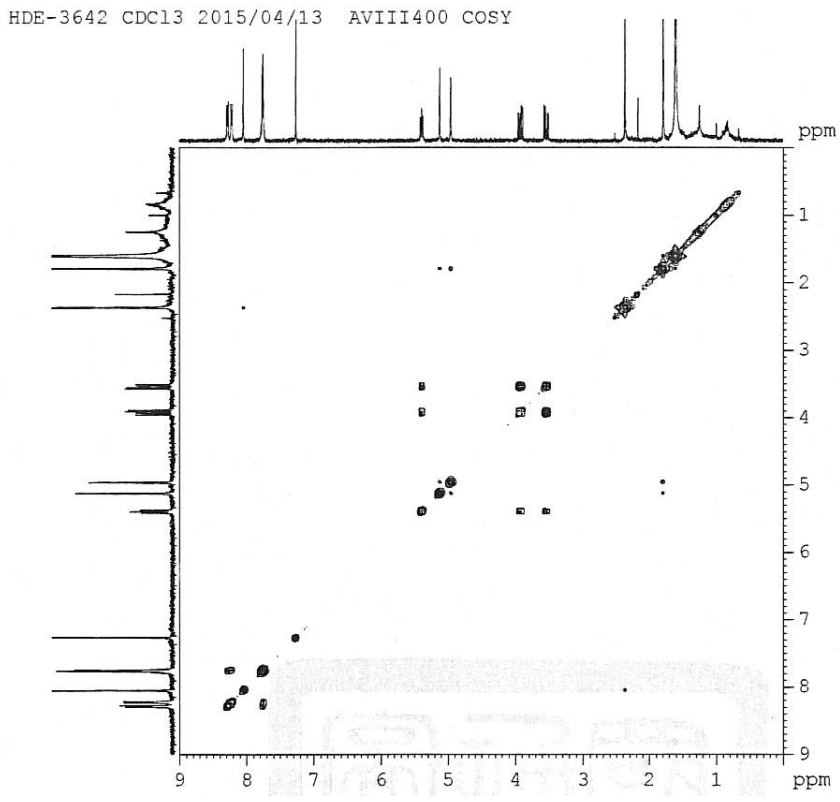


Figure S7. HSQC spectrum of **1** ($CDCl_3$, 400 MHz).

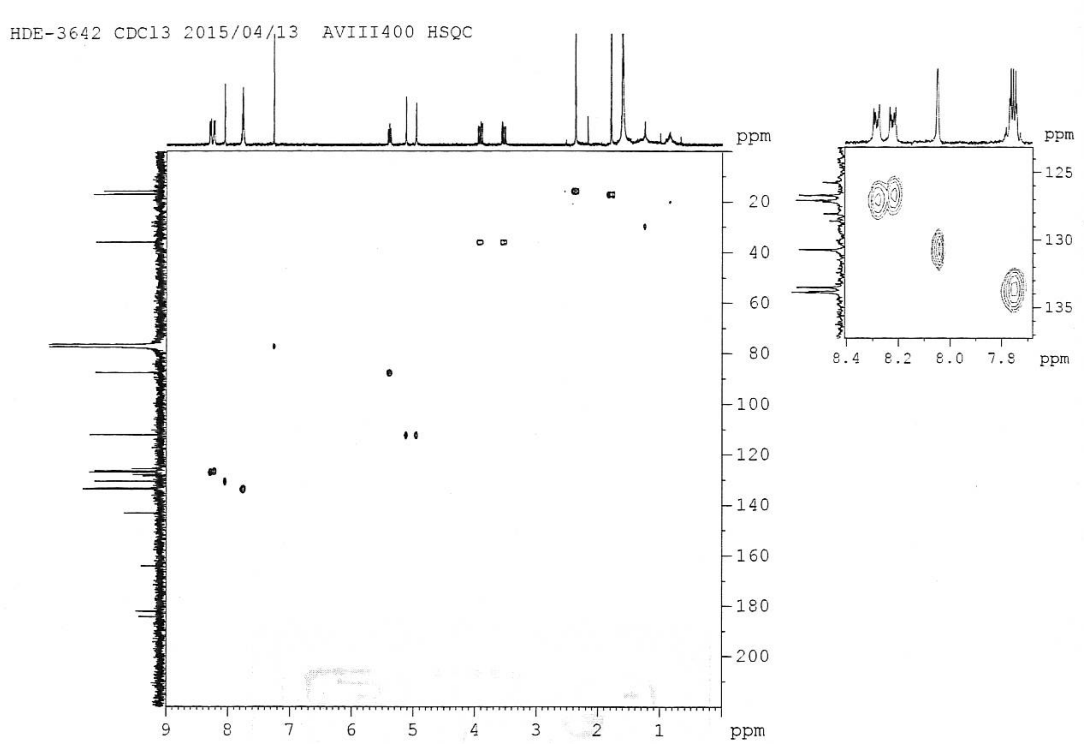


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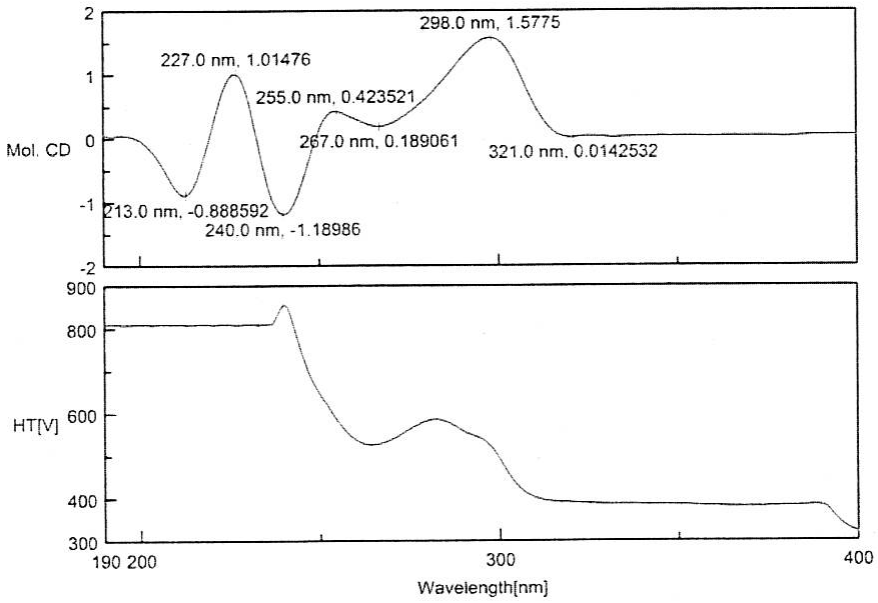


Figure S9. HRMS spectrum of **2**.

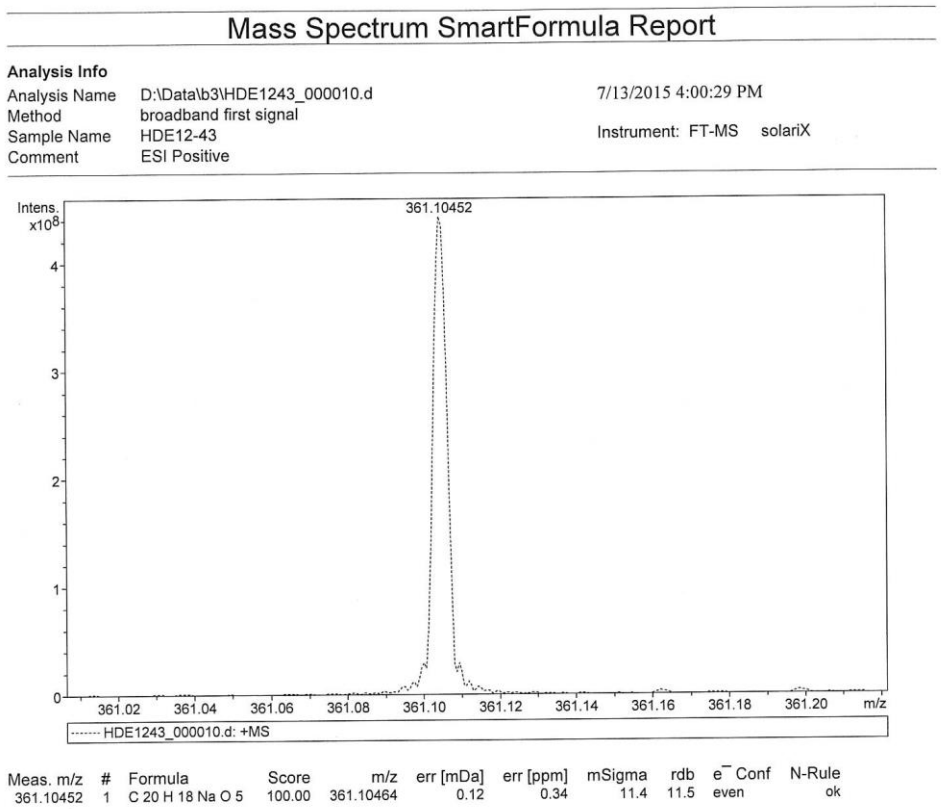


Figure S10. ^{1}H NMR spectrum of **2** (Acetone-d_6, 400 MHz).

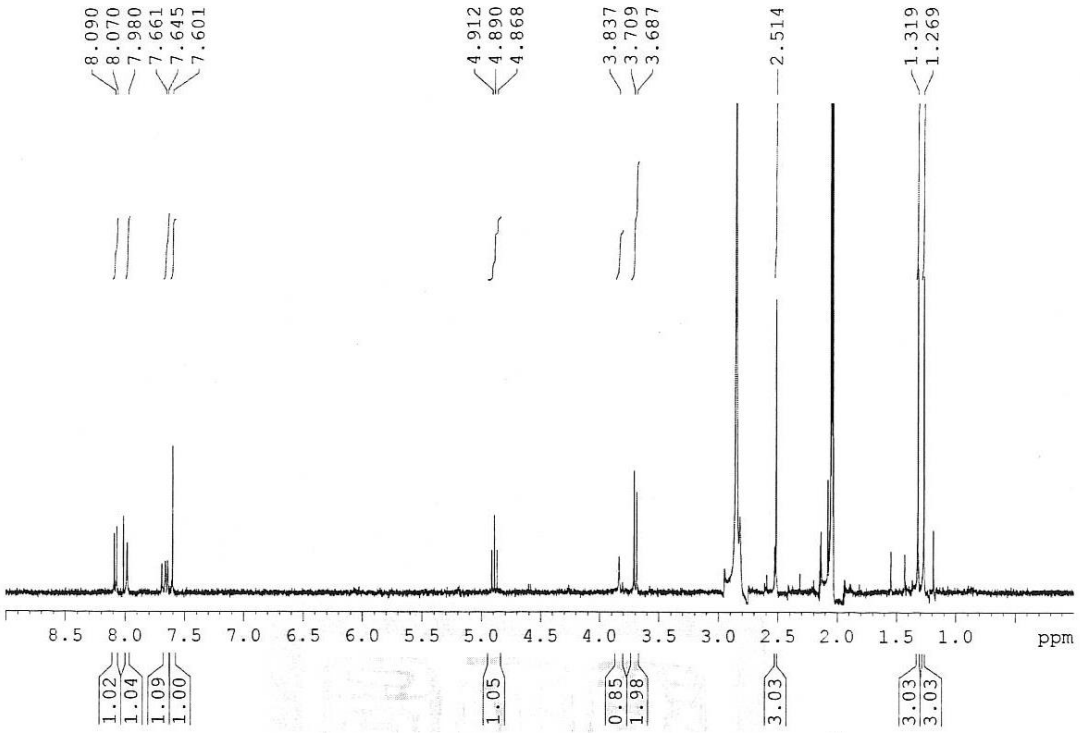


Figure S11. COSY spectrum of **2** (Acetone-d_6, 400 MHz).

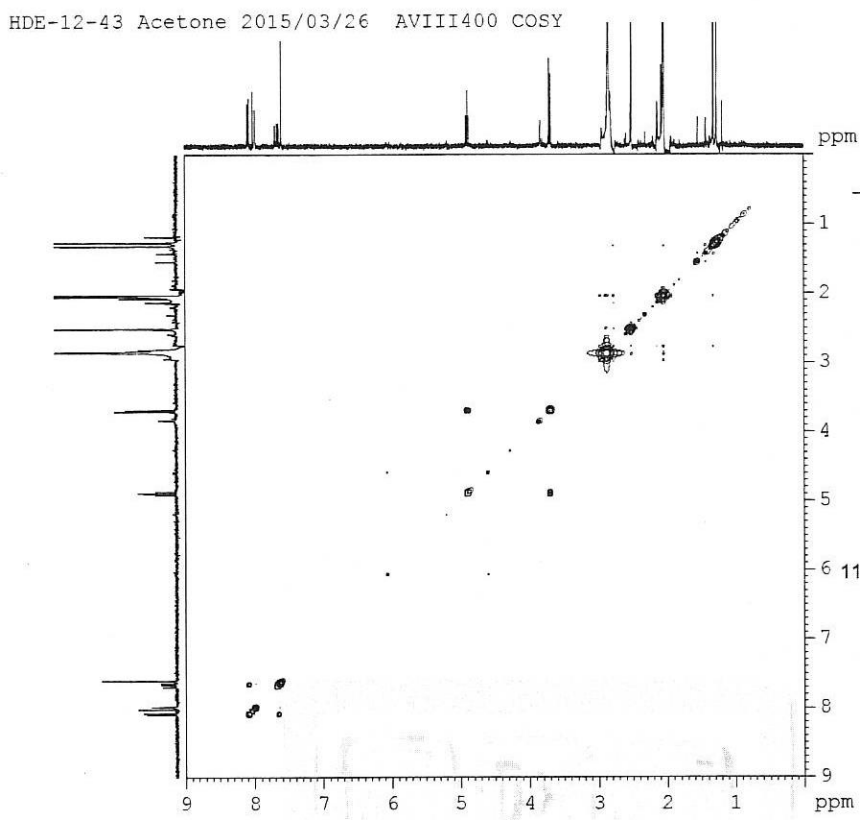


Figure S12. ^{13}C and DEPT NMR spectrum of **2** (Acetone-d_6, 100 MHz).

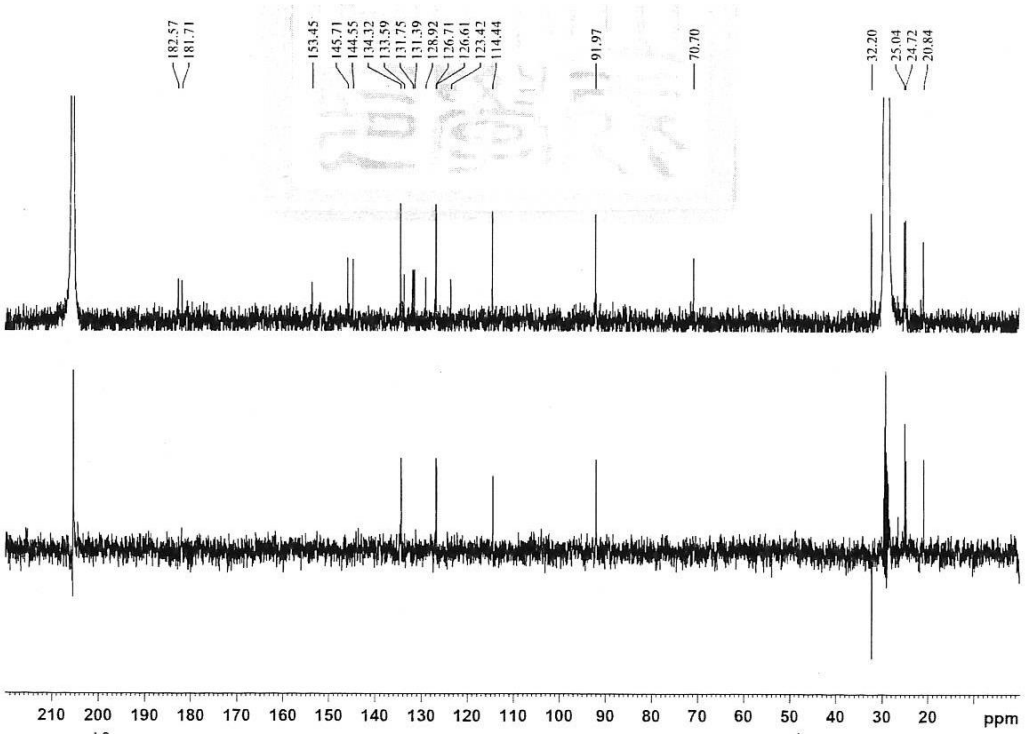


Figure S13. HSQC spectrum of **2** (Acetone-d_6, 400 MHz).

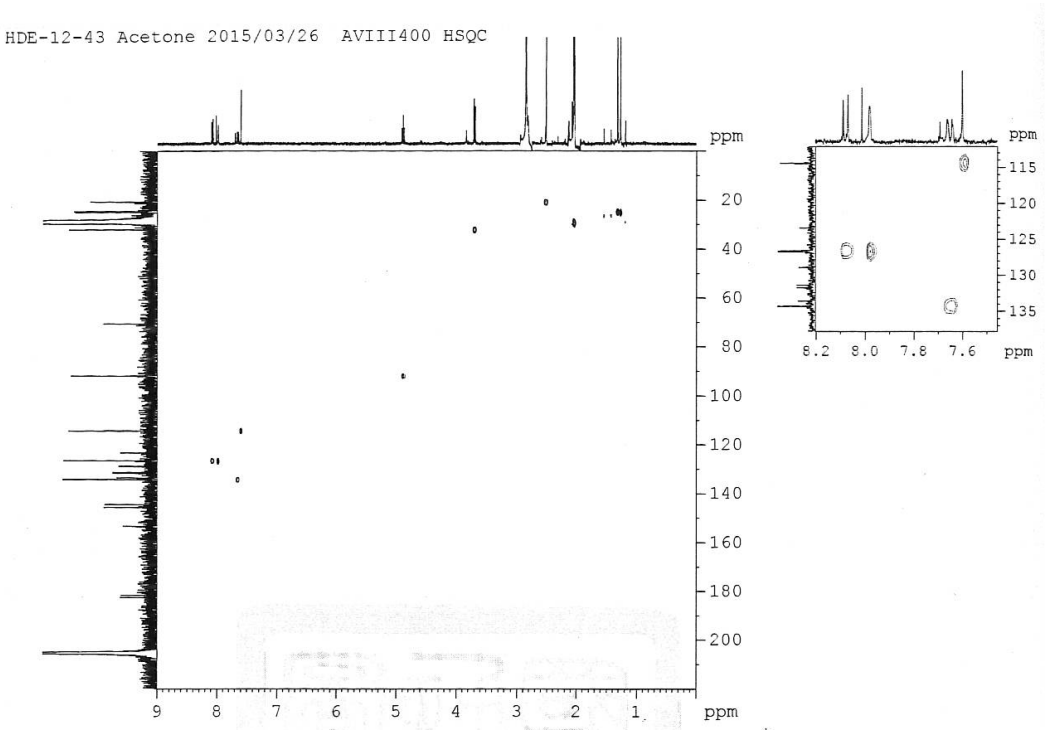


Figure S14. HMBC spectrum of **2** (Acetone-d_6, 400 MHz).

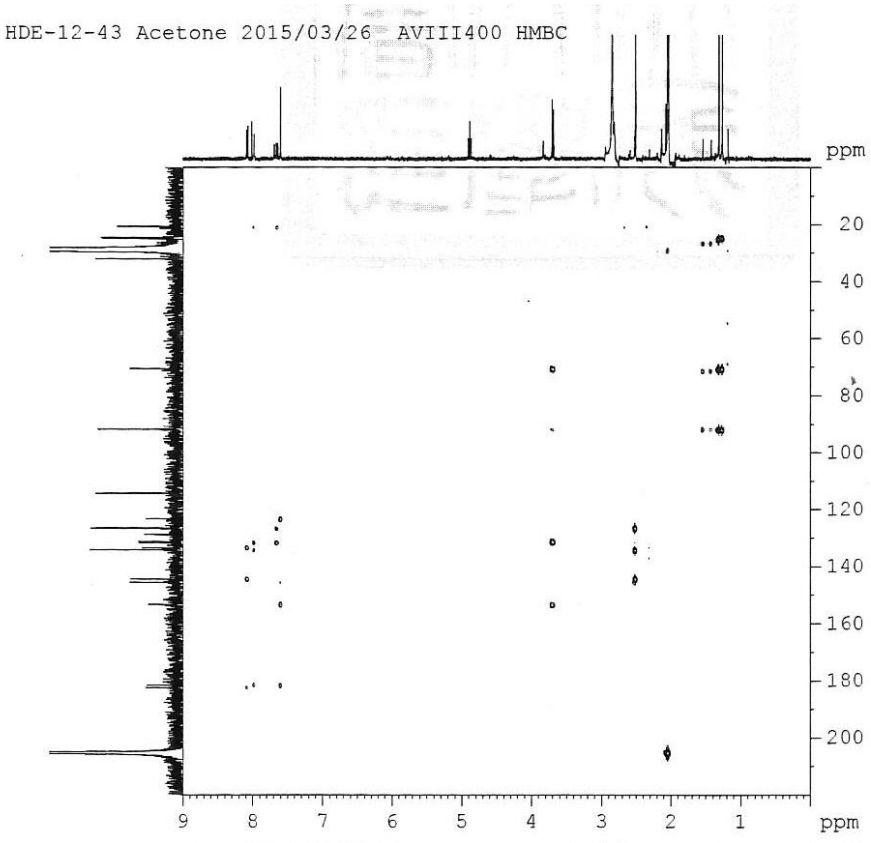


Figure S15. NOESY spectrum of **2** (Acetone-d_6, 400 MHz).

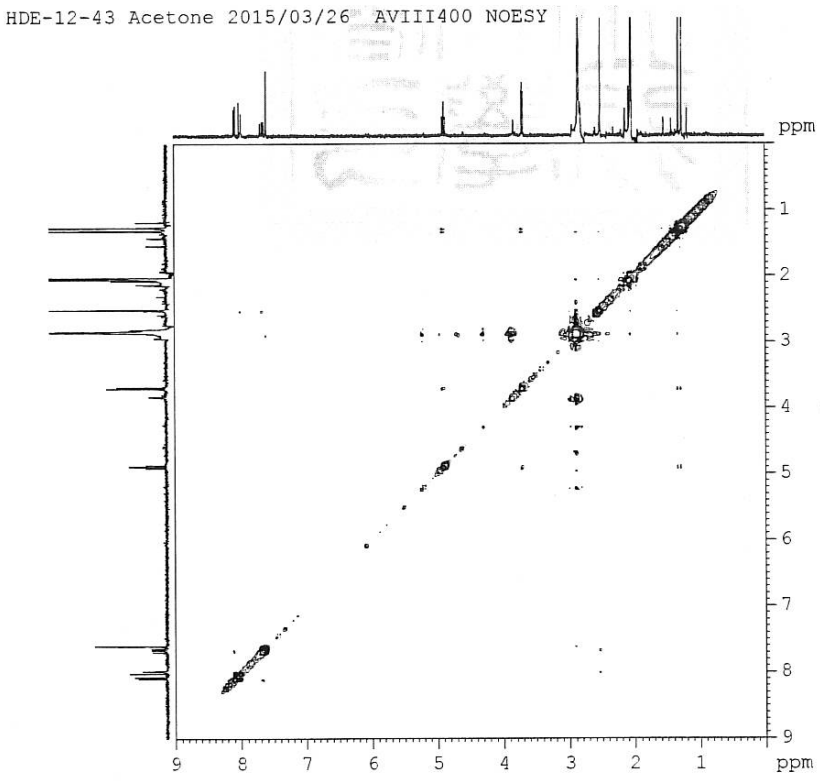


Figure S16. CD spectrum of **2**.

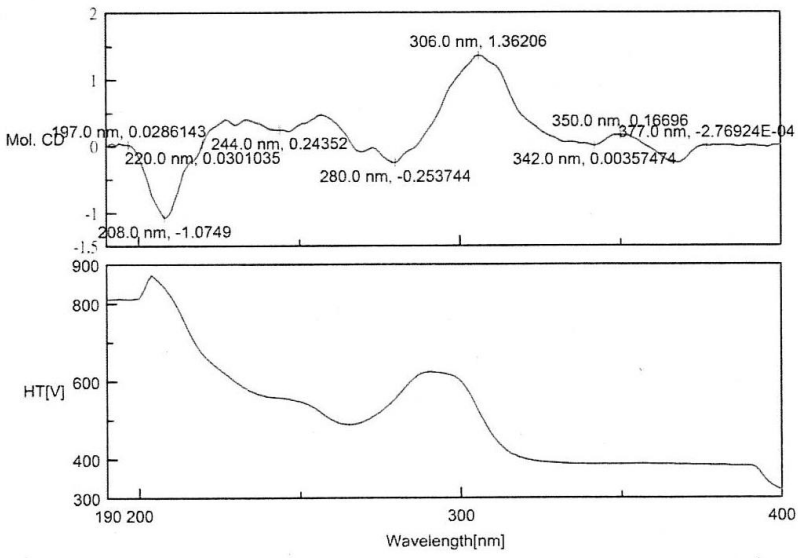


Figure S17. HRMS spectrum of **3**.

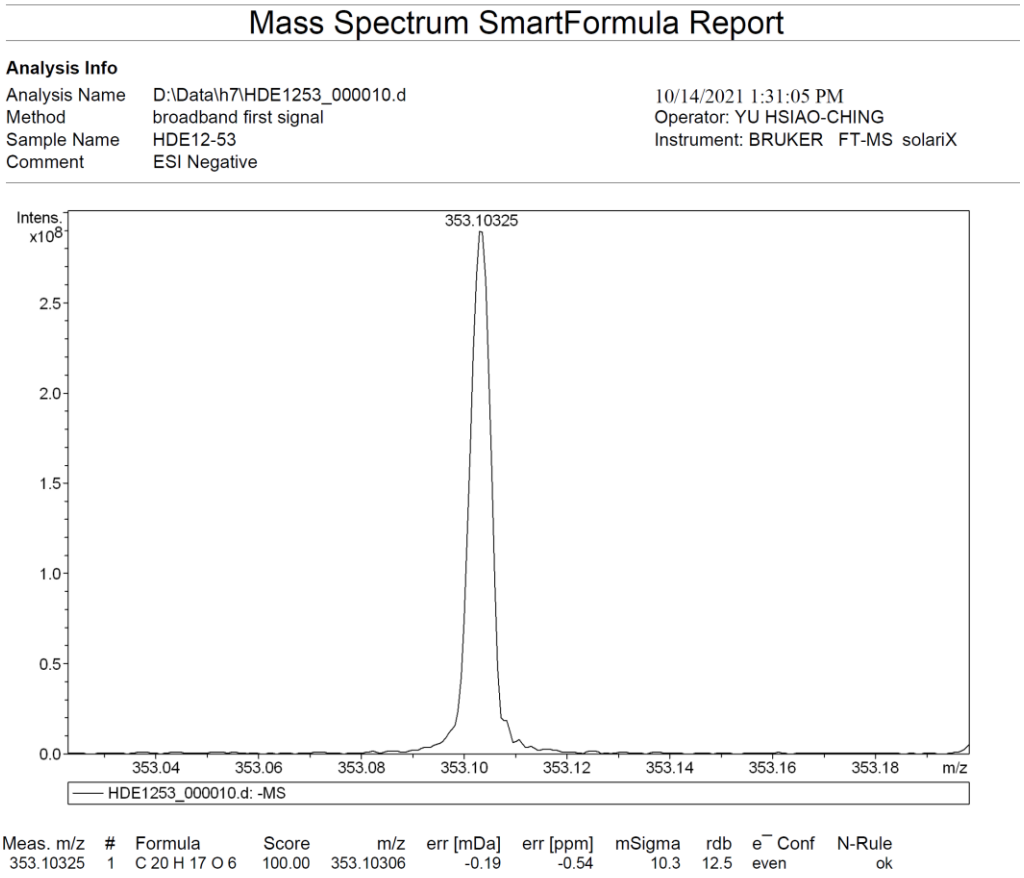


Figure S18. ^{1}H NMR spectrum of **3** (CD_3OD, 400 MHz).

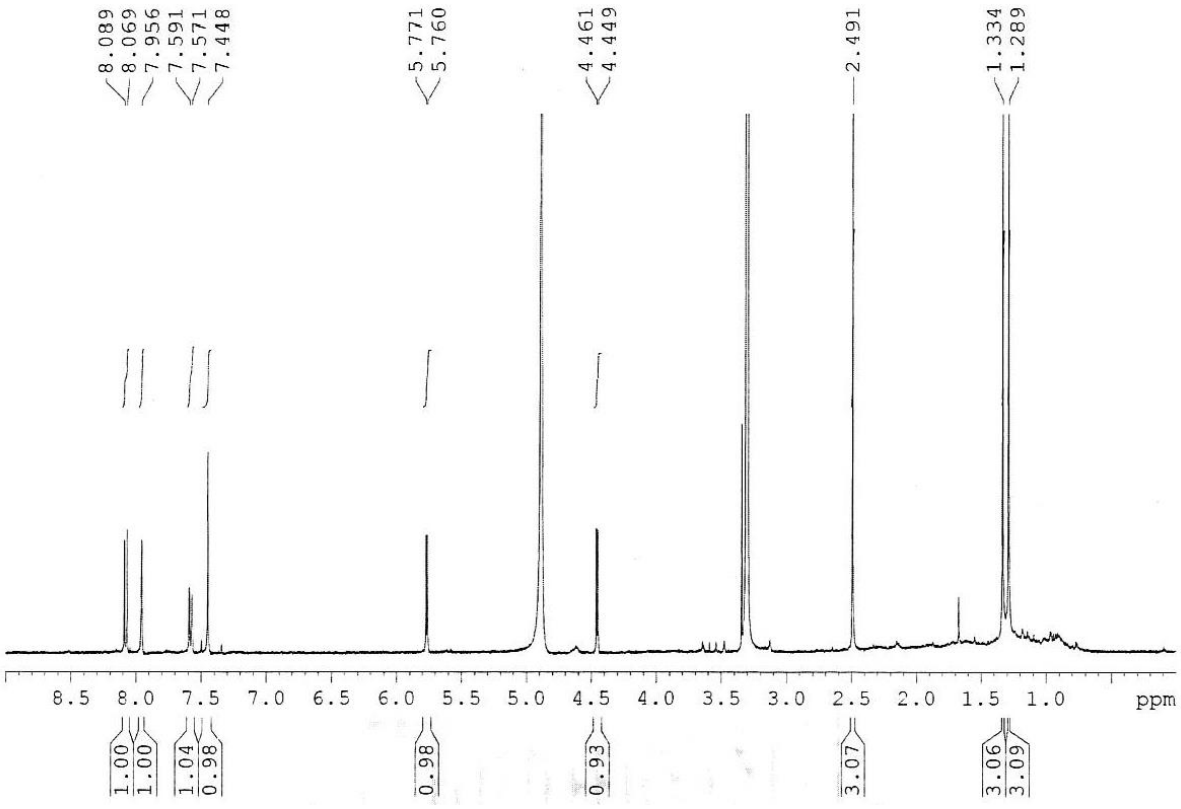


Figure S19. ^{13}C and DEPT NMR spectrum of **3** (CD_3OD, 100

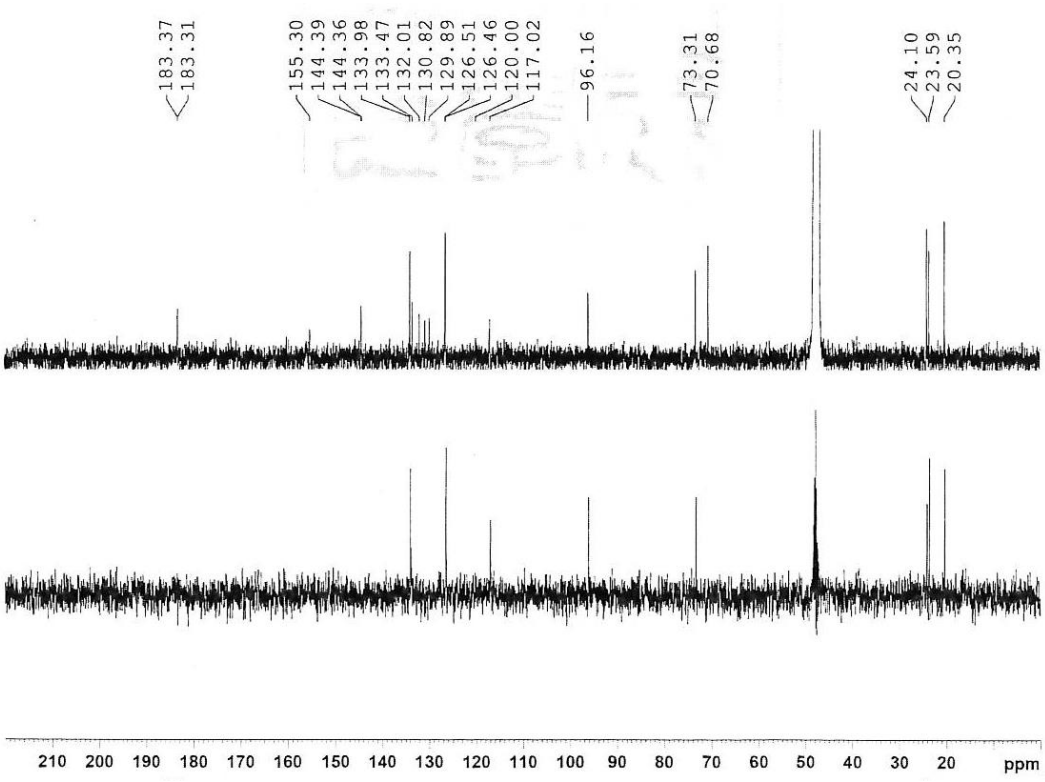


Figure S20. COSY spectrum of **3** (CD_3OD, 400 MHz).

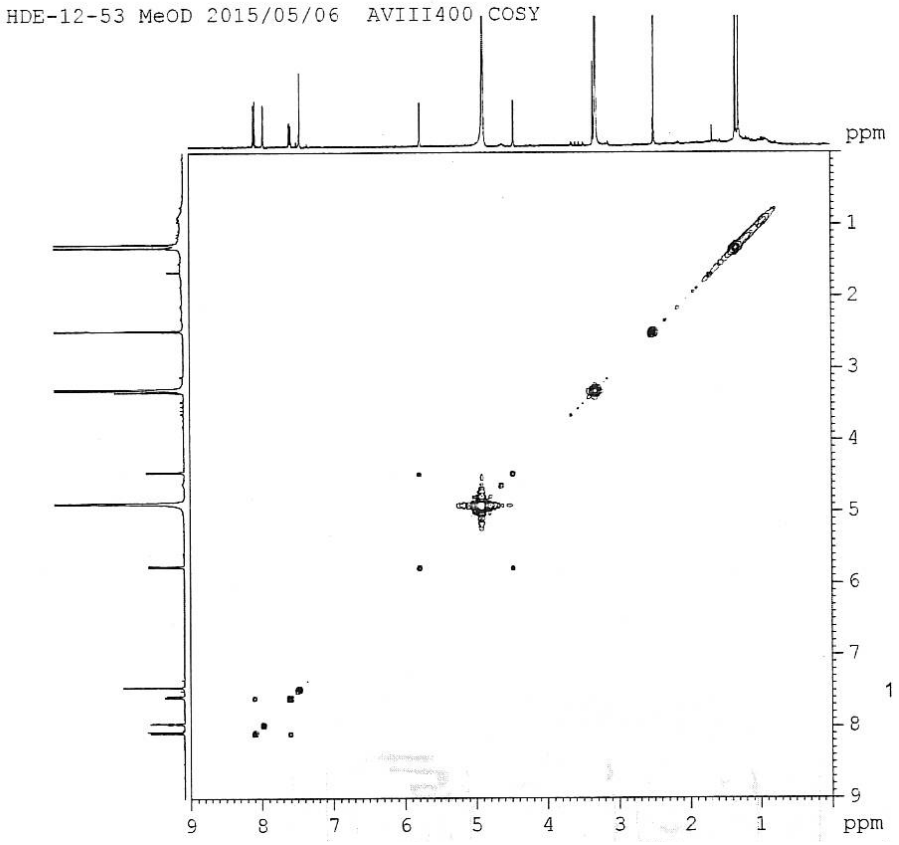


Figure S21. HSQC spectrum of **3** (CD_3OD, 400 MHz).

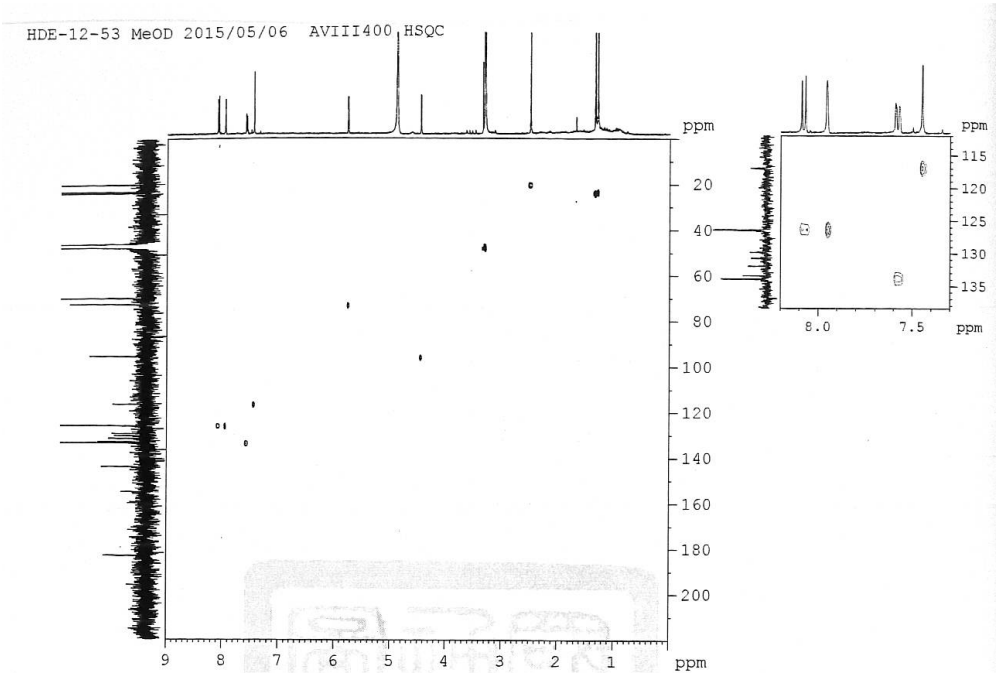


Figure S22. HMBC spectrum of **3** (CD_3OD, 400 MHz).

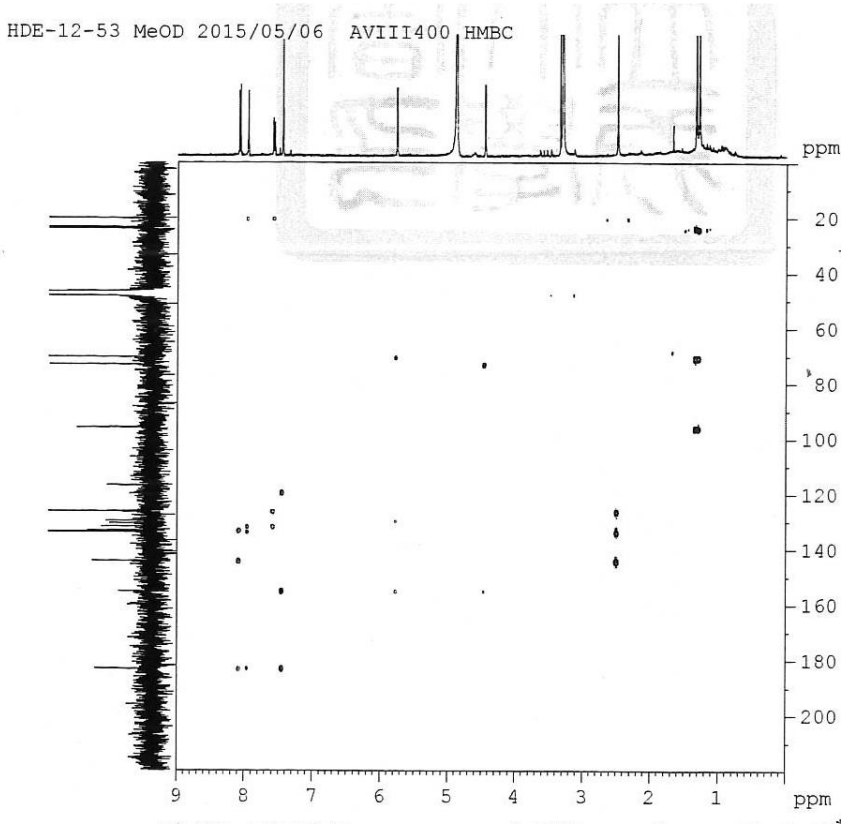


Figure S23. NOESY spectrum of **3** (CD_3OD, 400 MHz).

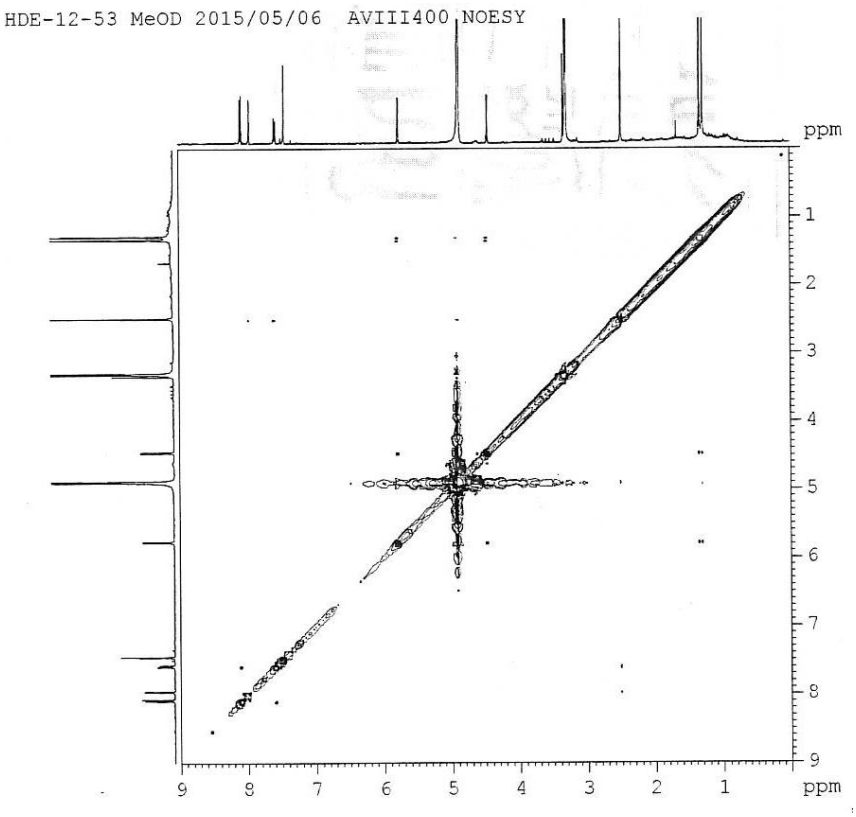


Figure S24. CD spectrum of **3**.

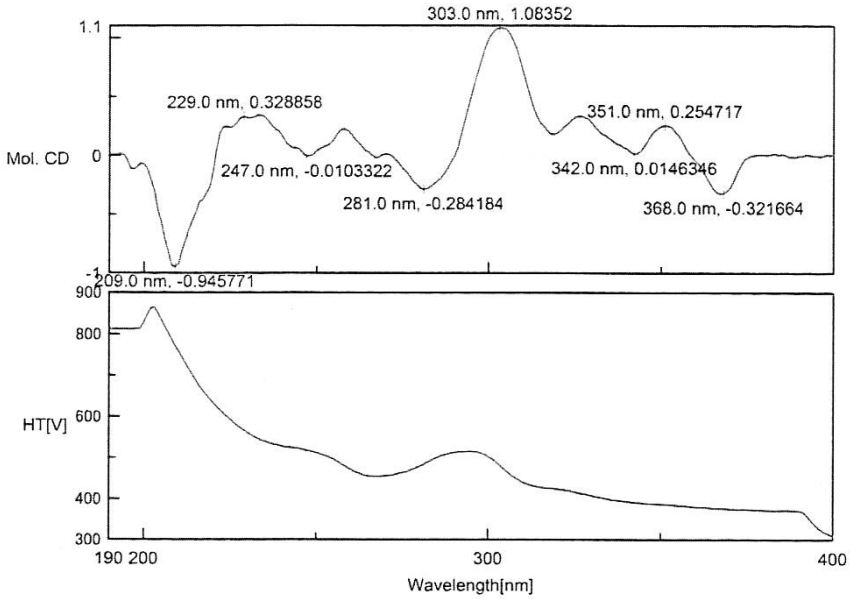


Figure S25. HRMS spectrum of **4**.

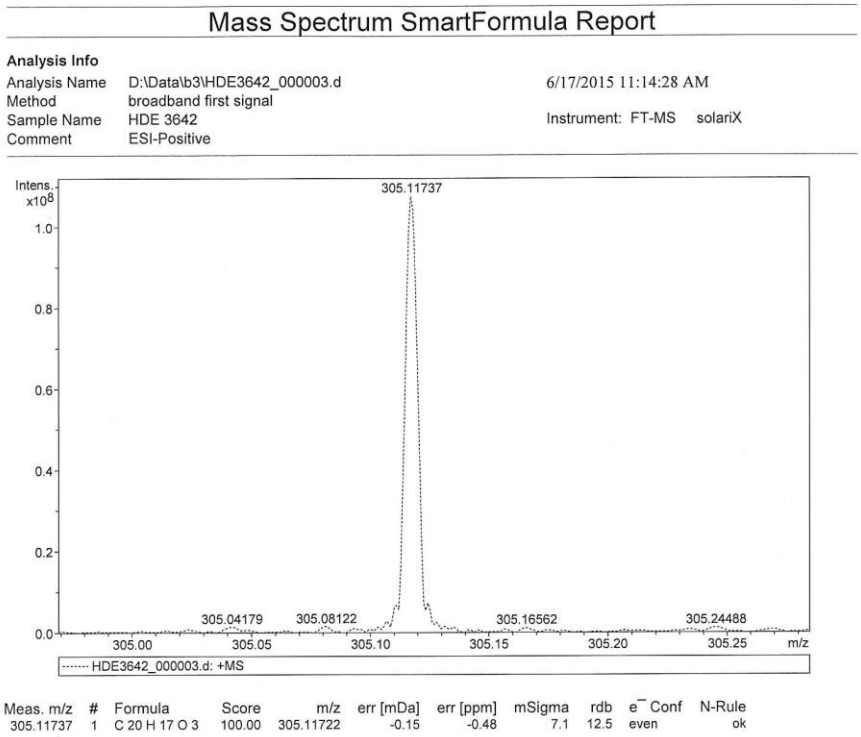


Figure S26. ^{1}H NMR spectrum of **4** ($CDCl_3$, 400 MHz).

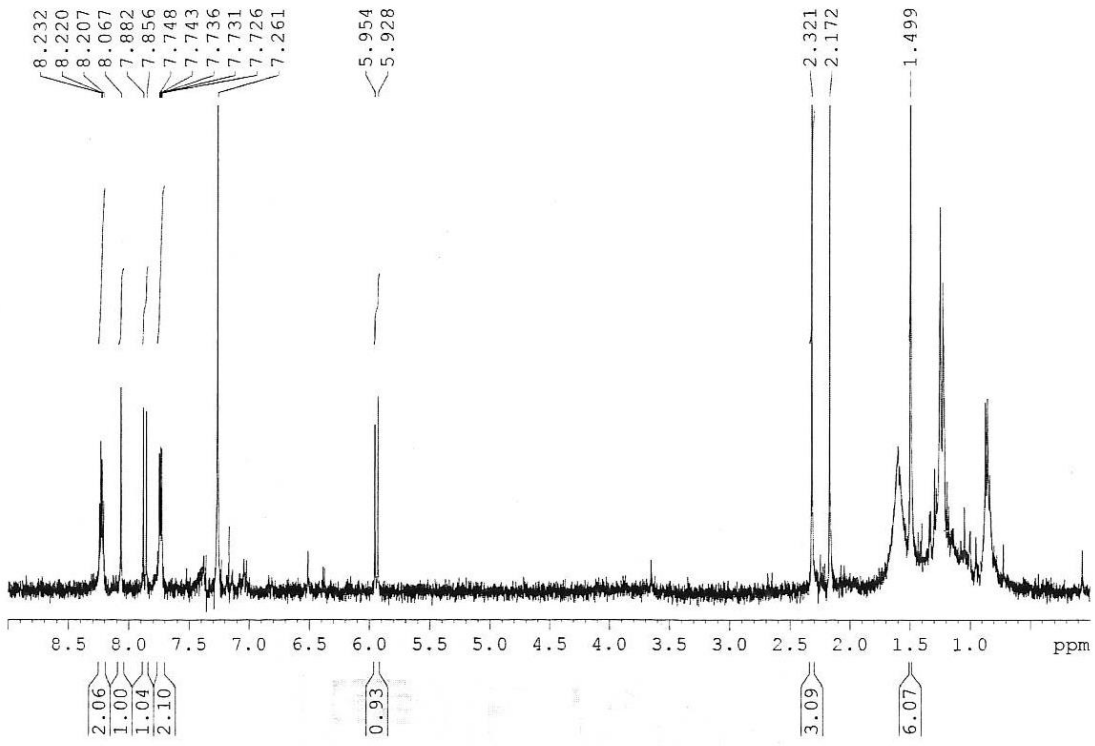


Figure S27. ^{13}C and DEPT NMR spectrum of **4** ($CDCl_3$, 100 MHz).

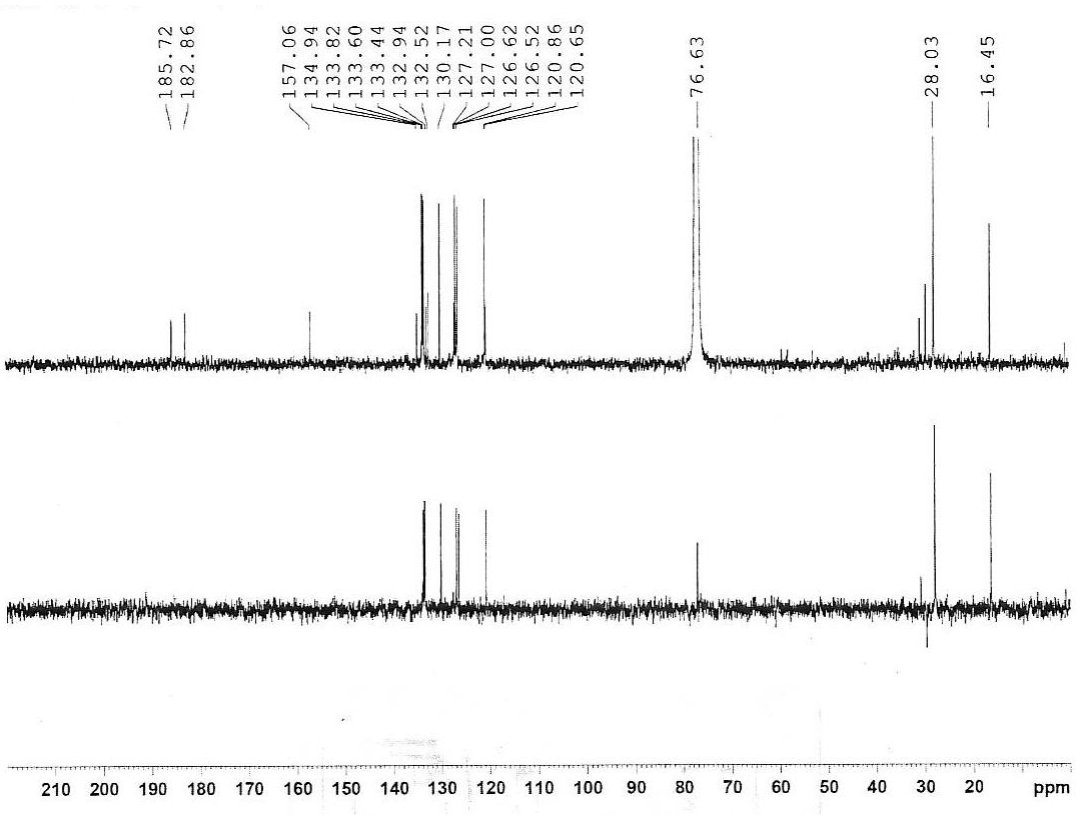


Figure S28. COSY spectrum of **4** ($CDCl_3$, 400 MHz).

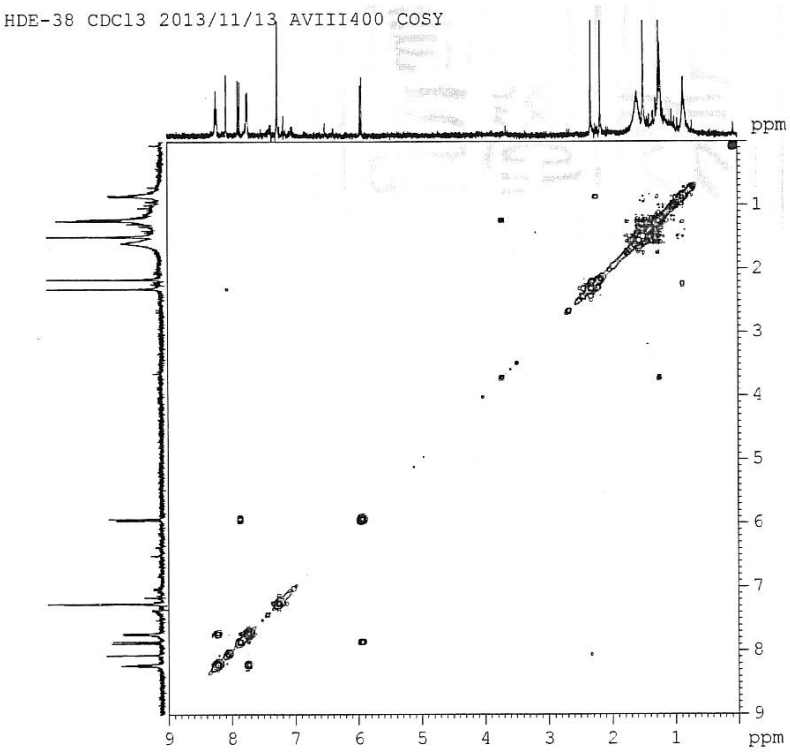


Figure S29. HSQC spectrum of **4** ($CDCl_3$, 400 MHz).

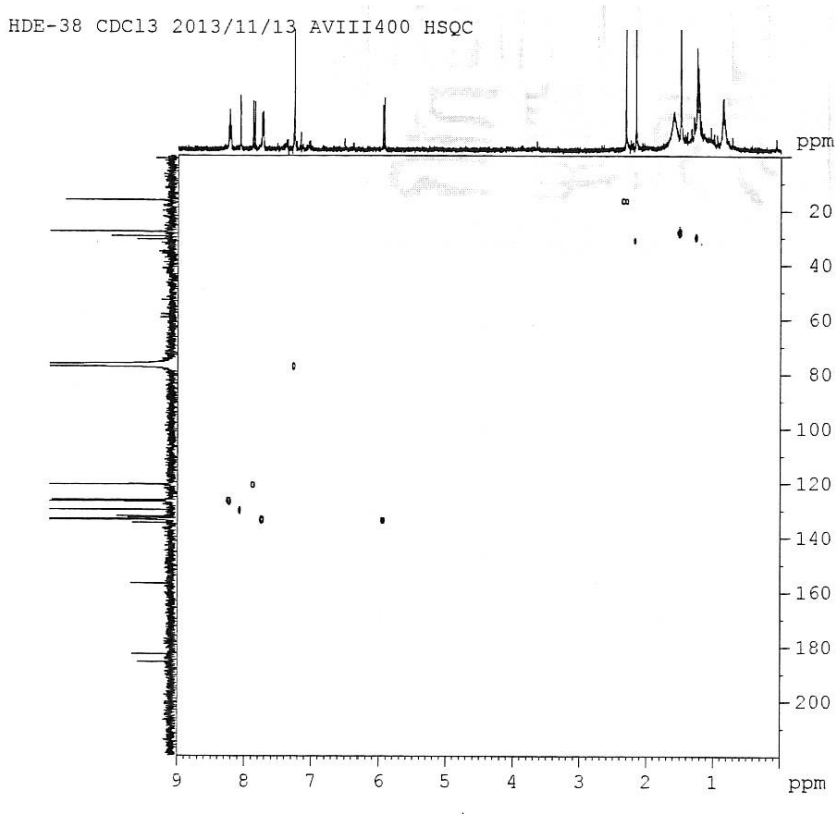


Figure S30. NOESY spectrum of **4** ($CDCl_3$, 400 MHz).

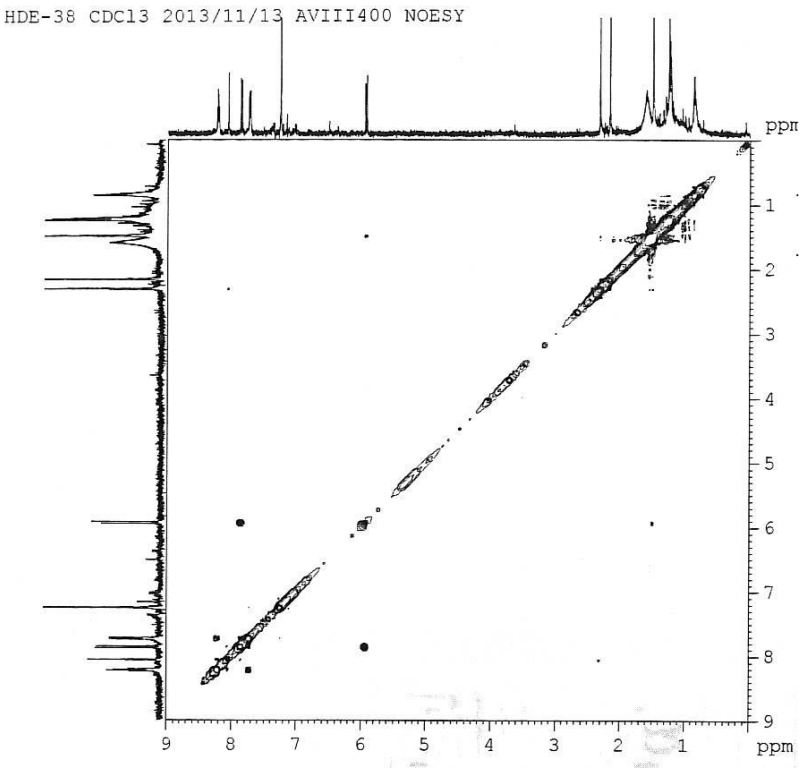


Figure S31. HMBC spectrum of **4** ($CDCl_3$, 400 MHz).

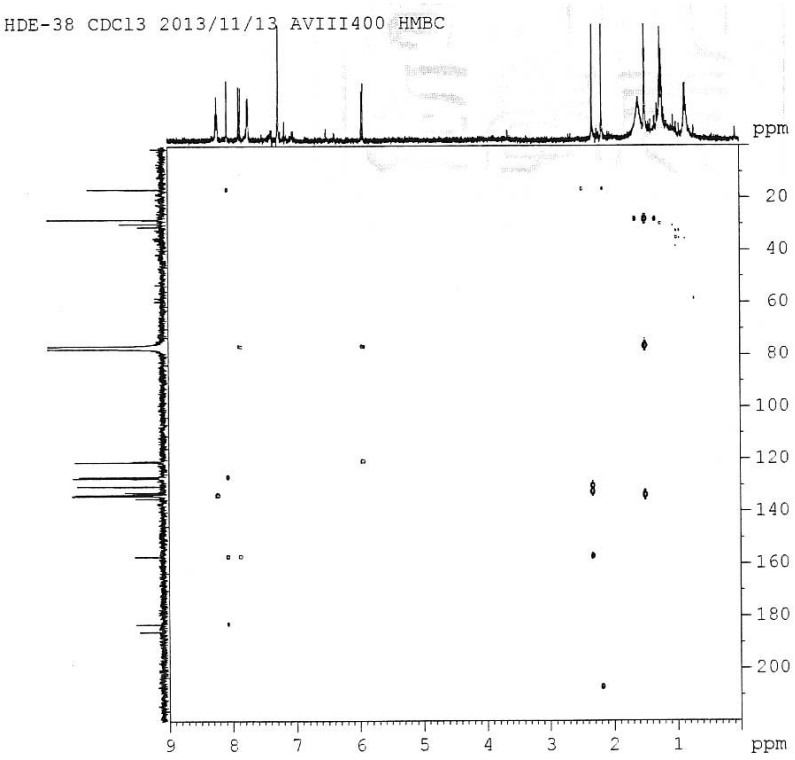


Figure S32. HRMS spectrum of **5**.

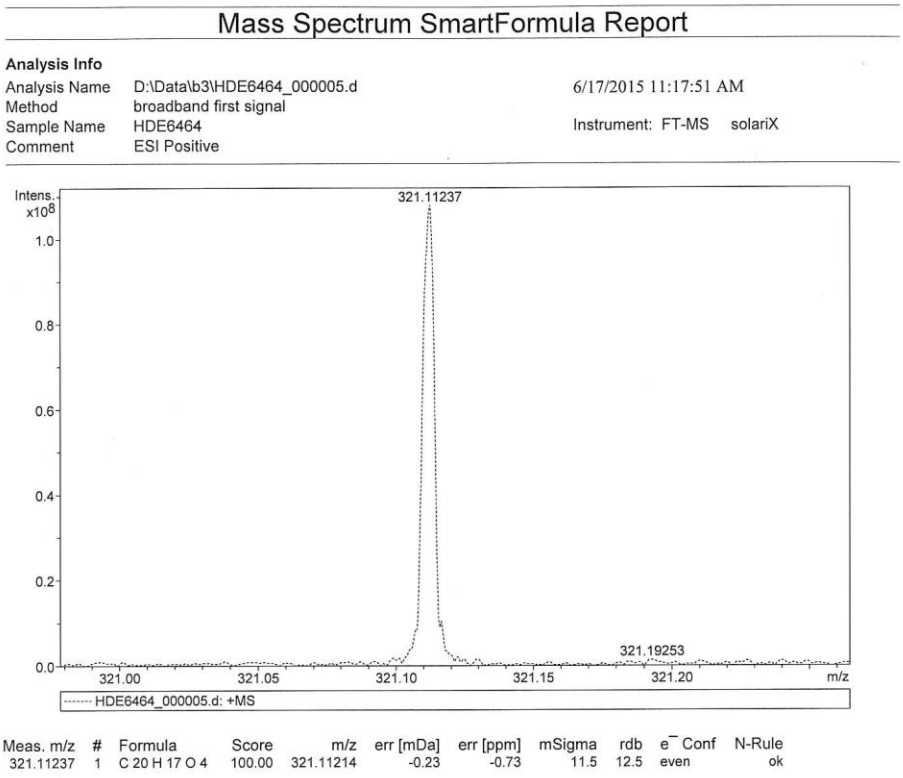


Figure S33. ^{1}H NMR spectrum of **5** ($CDCl_3$, 400 MHz).

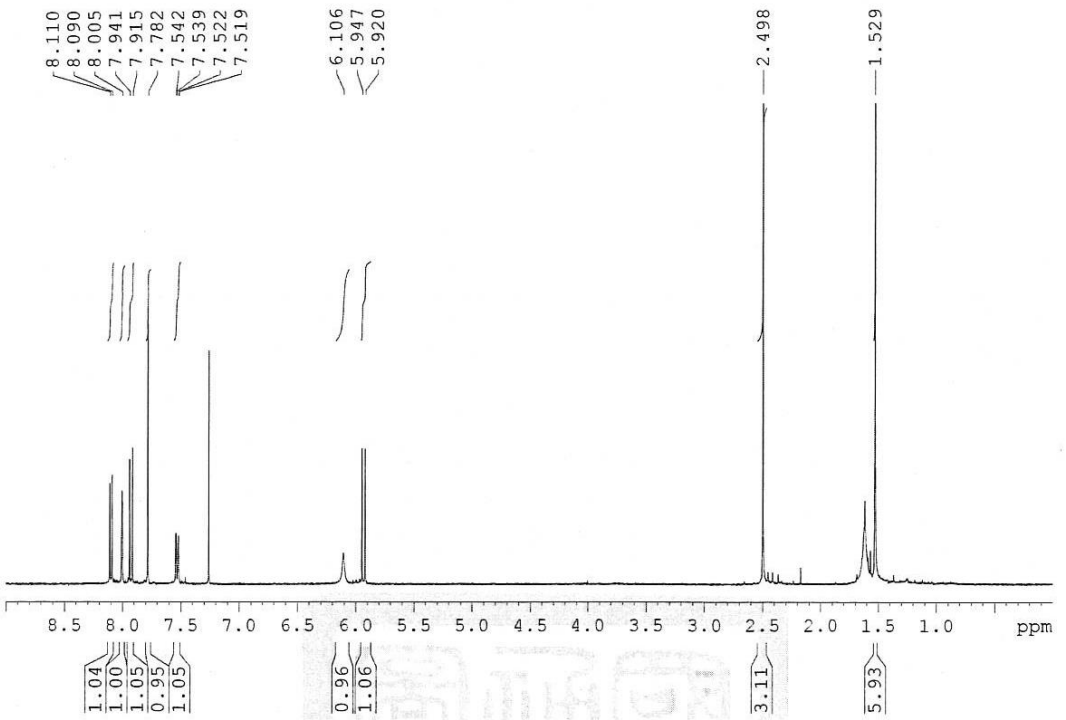


Figure S34. COSY spectrum of **5** ($CDCl_3$, 400 MHz).

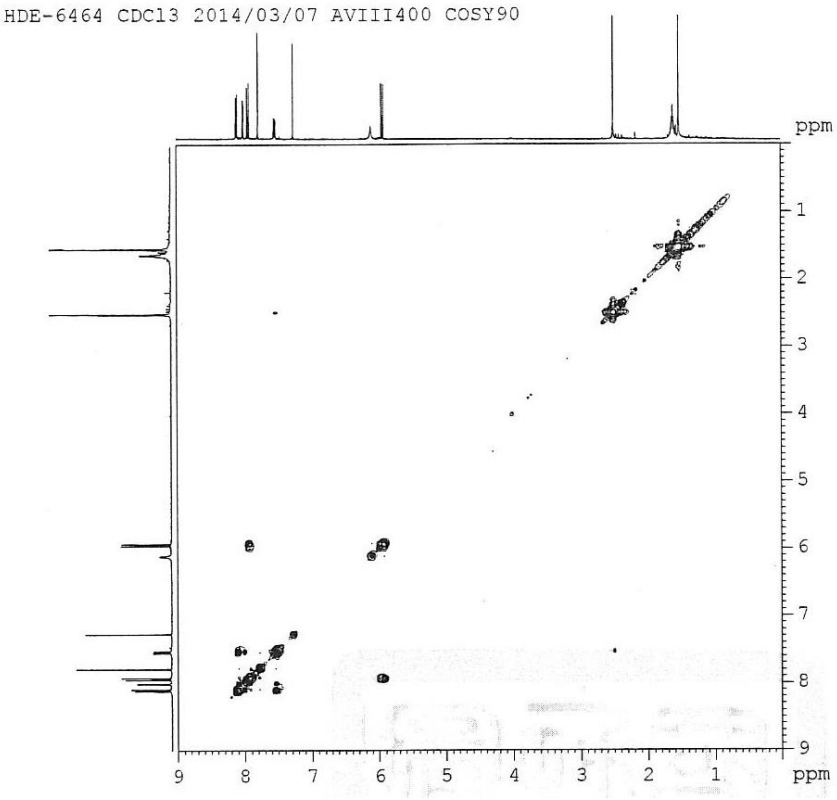


Figure S35. ^{13}C and DEPT NMR spectrum of **5** ($CDCl_3$, 400 MHz).

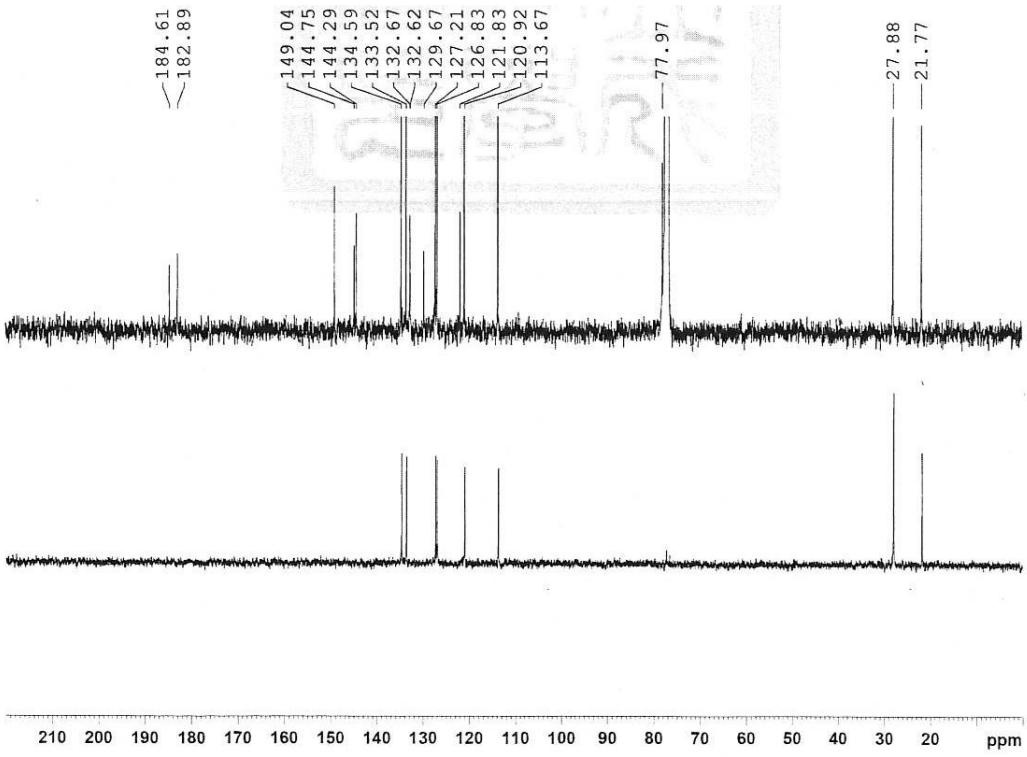


Figure S36. HSQC spectrum of **5** ($CDCl_3$, 400 MHz).

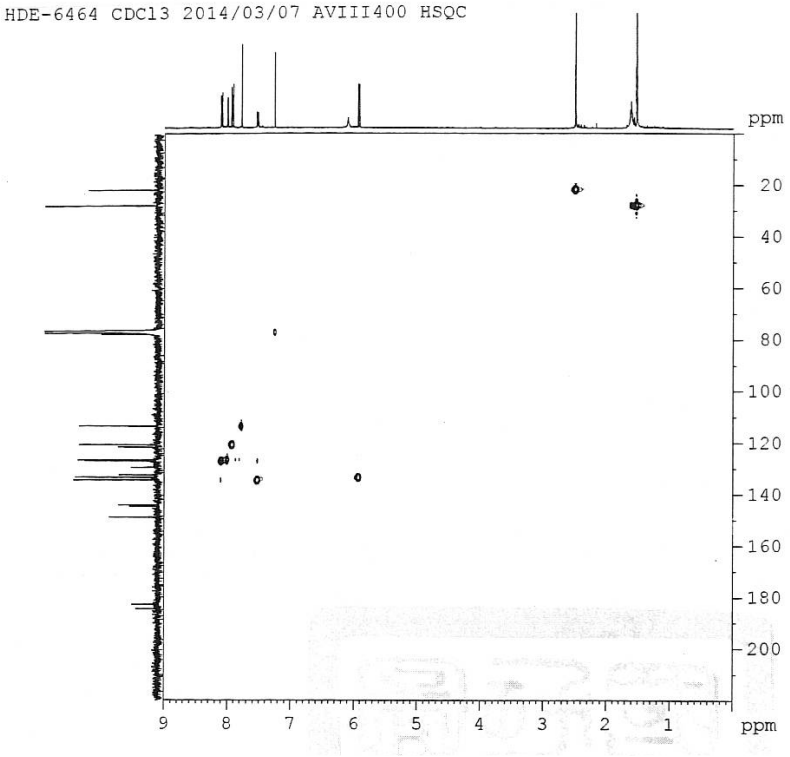


Figure S37. HMBC spectrum of **5** ($CDCl_3$, 400 MHz).

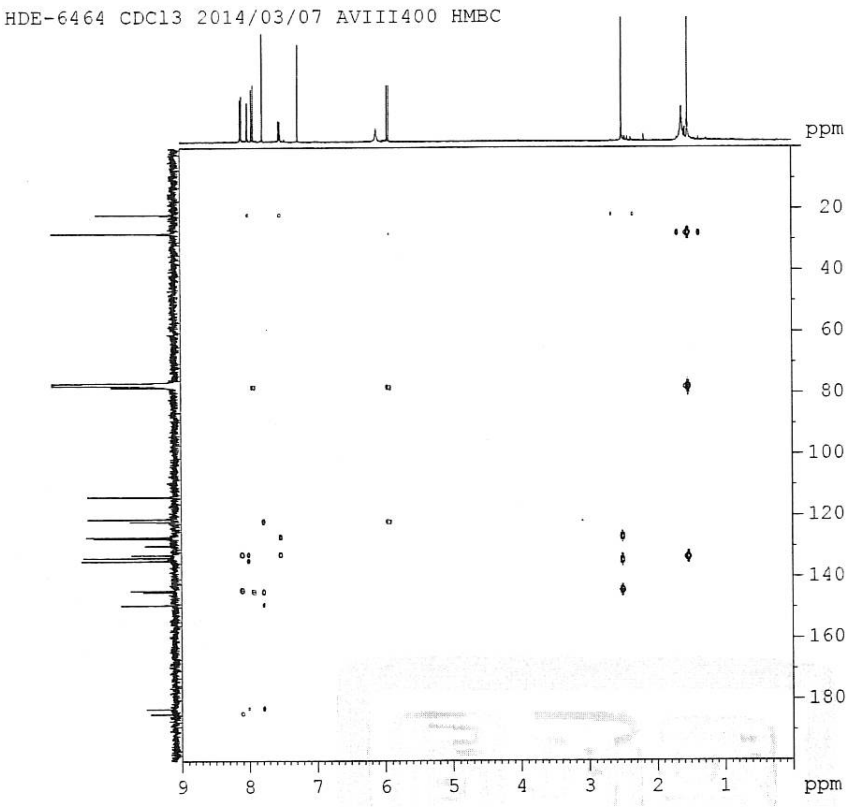


Figure S38. NOESY spectrum of **5** ($CDCl_3$, 400 MHz).

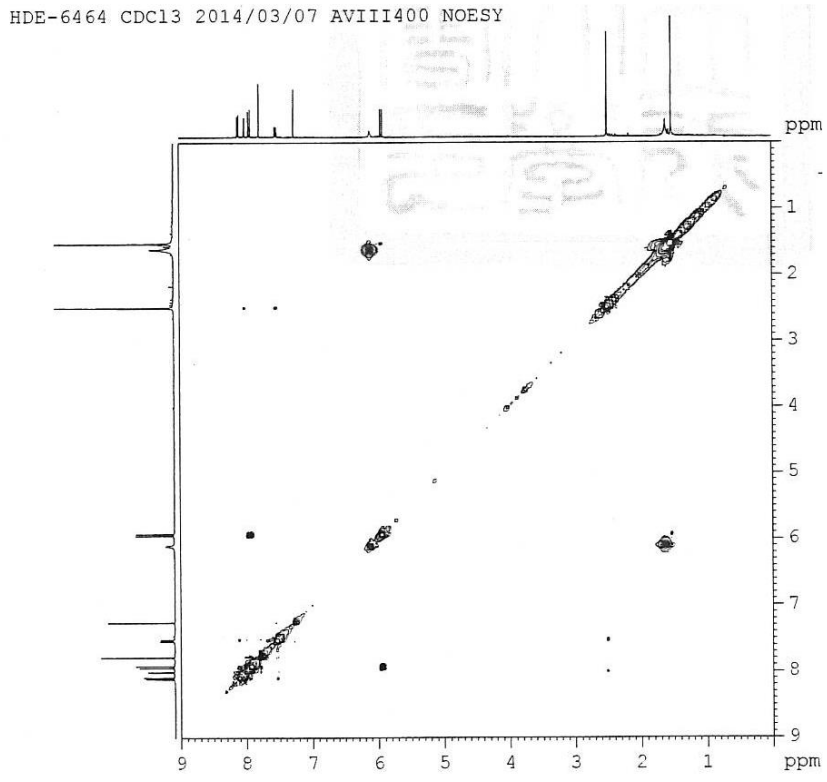


Figure S39. HRMS spectrum of **6**.

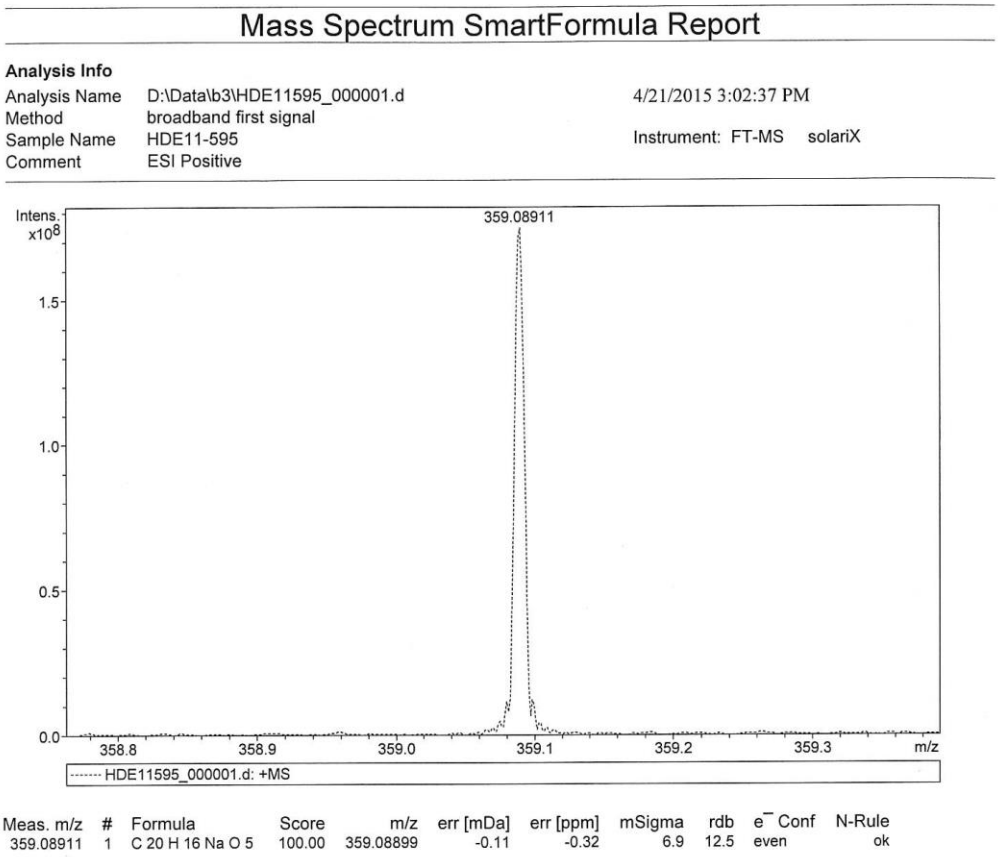


Figure S40. HRMS spectrum of **7**.

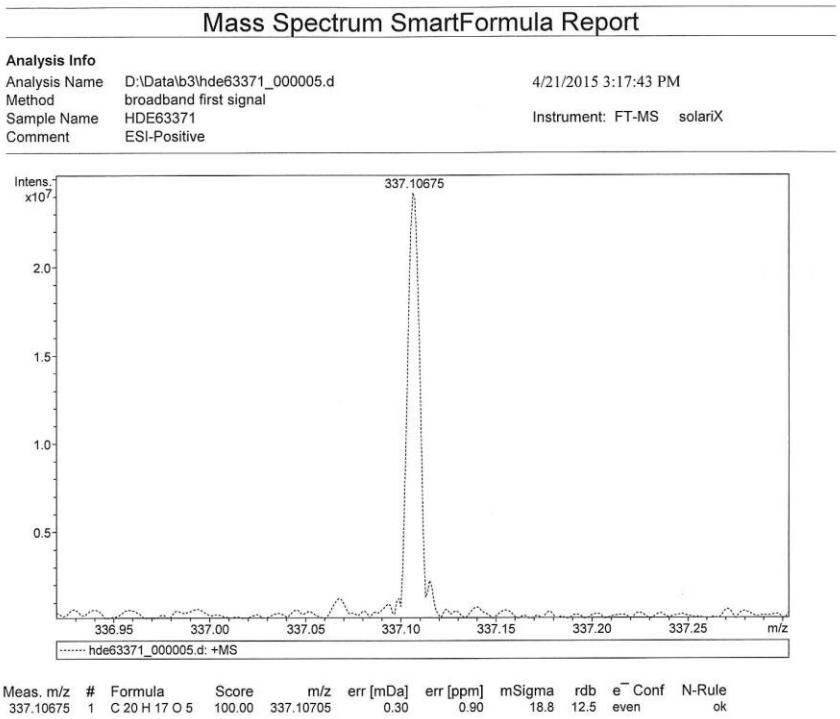


Figure S41. ^{1}H NMR spectrum of **6** (CD_3OD, 700 MHz).

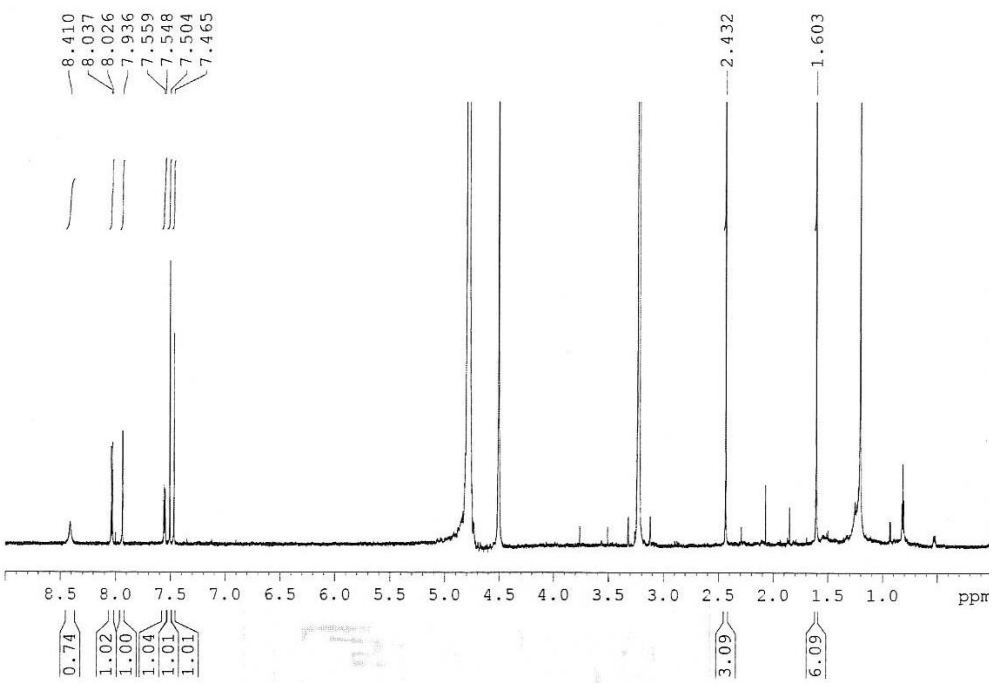


Figure S42. ^{13}C and DEPT NMR spectrum of **6** (CD_3OD, 175 MHz).

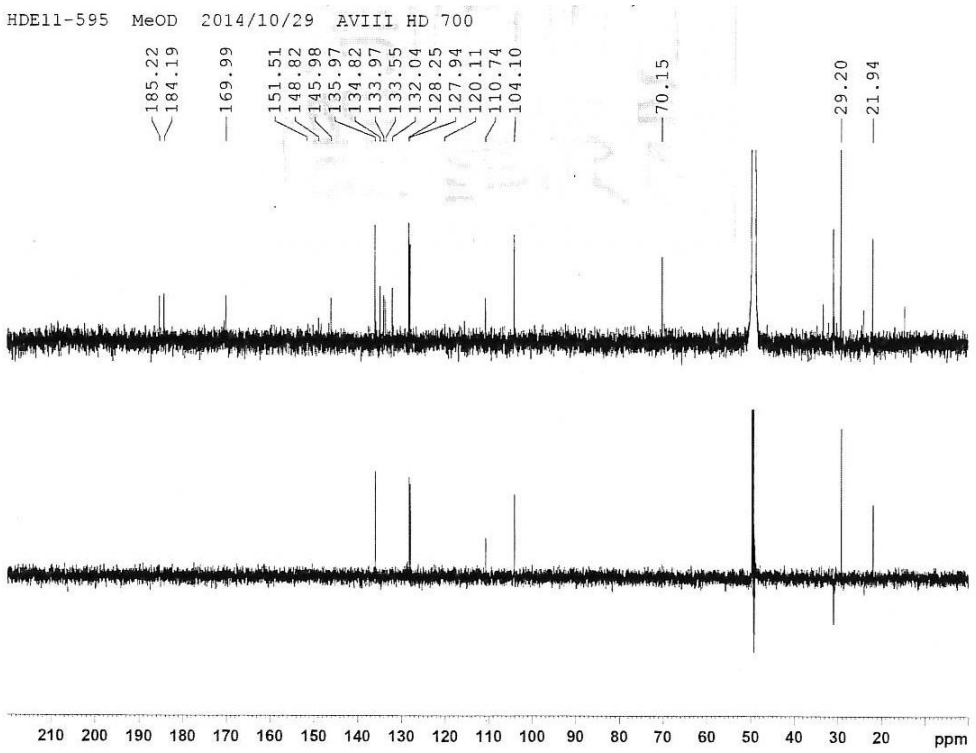


Figure S43. COSY spectrum of **6** (CD_3OD, 700 MHz).

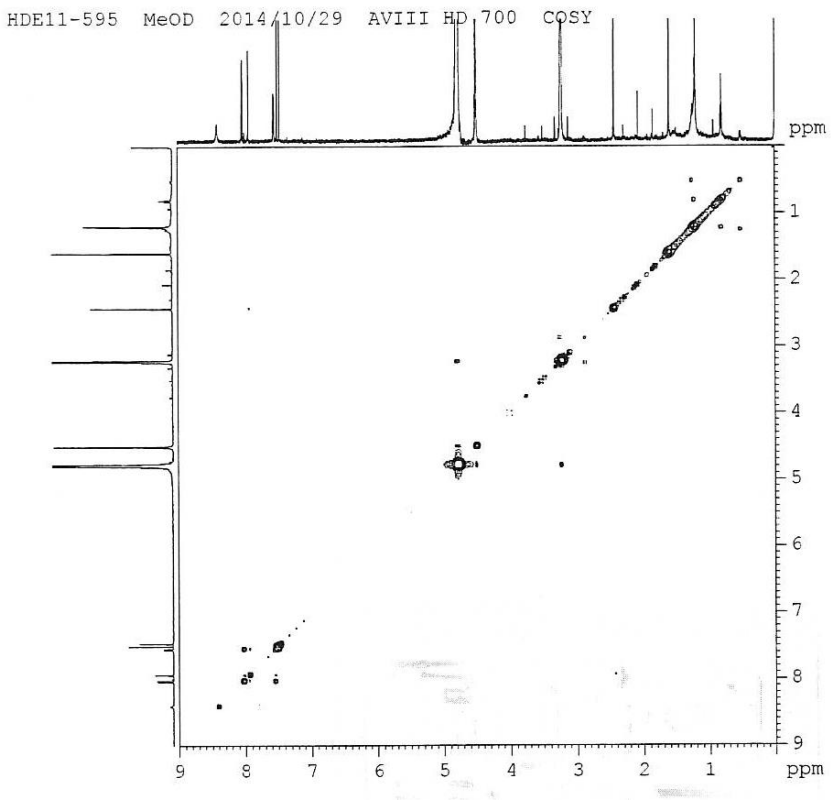


Figure S44. HSQC spectrum of **6** (CD_3OD, 700 MHz).

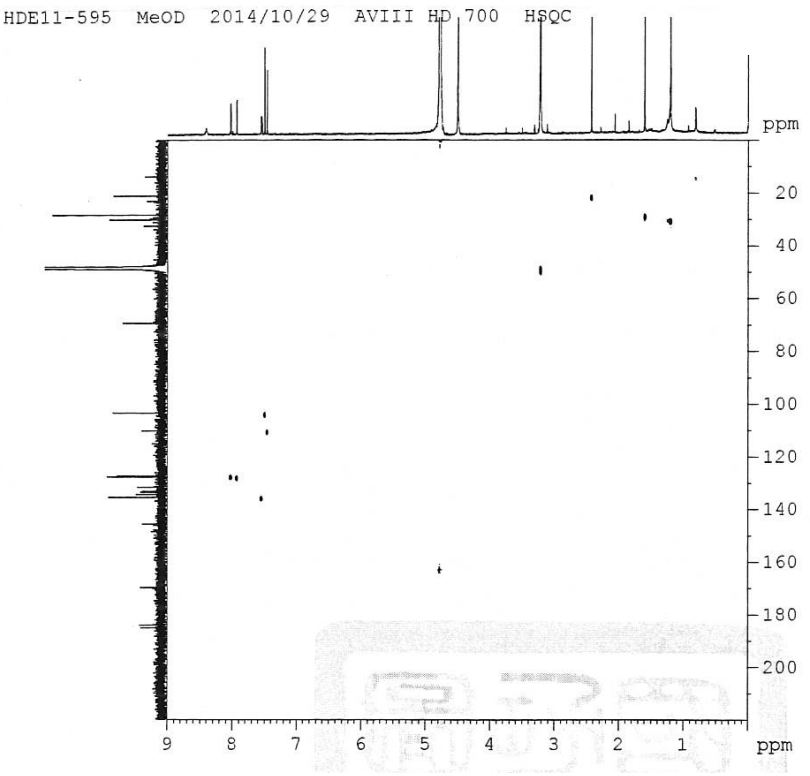


Figure S45. NOESY spectrum of **6** (CD_3OD, 700 MHz).

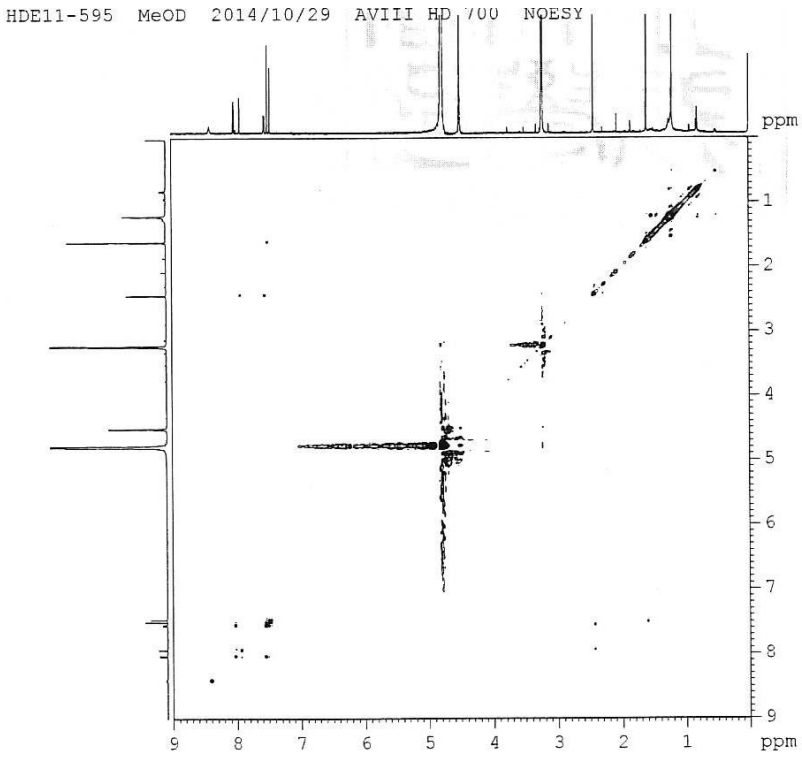


Figure S46. HMBC spectrum of **6** (CD_3OD, 700 MHz).

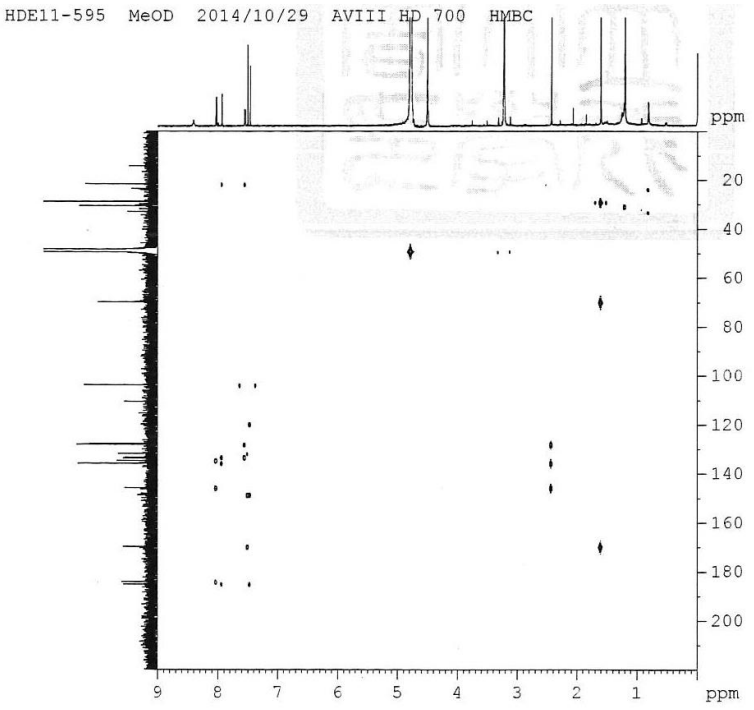


Figure S47. ^1H NMR spectrum of **7** ($CDCl_3$, 400 MHz).

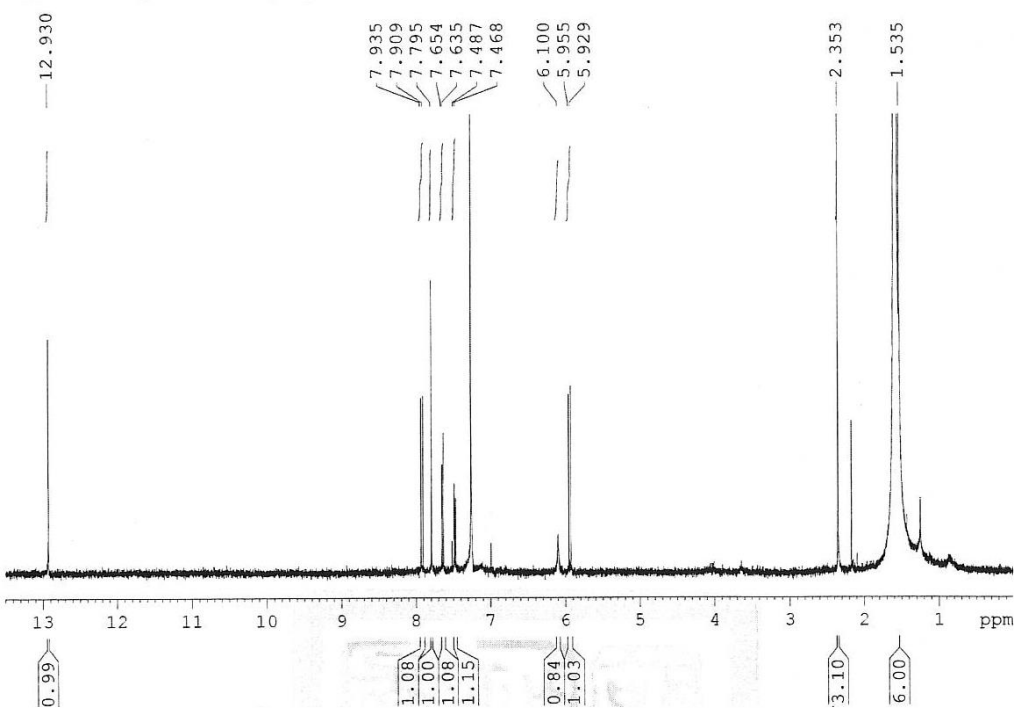


Figure S48. ^{13}C and DEPT NMR spectrum of **7** ($CDCl_3$, 400 MHz).

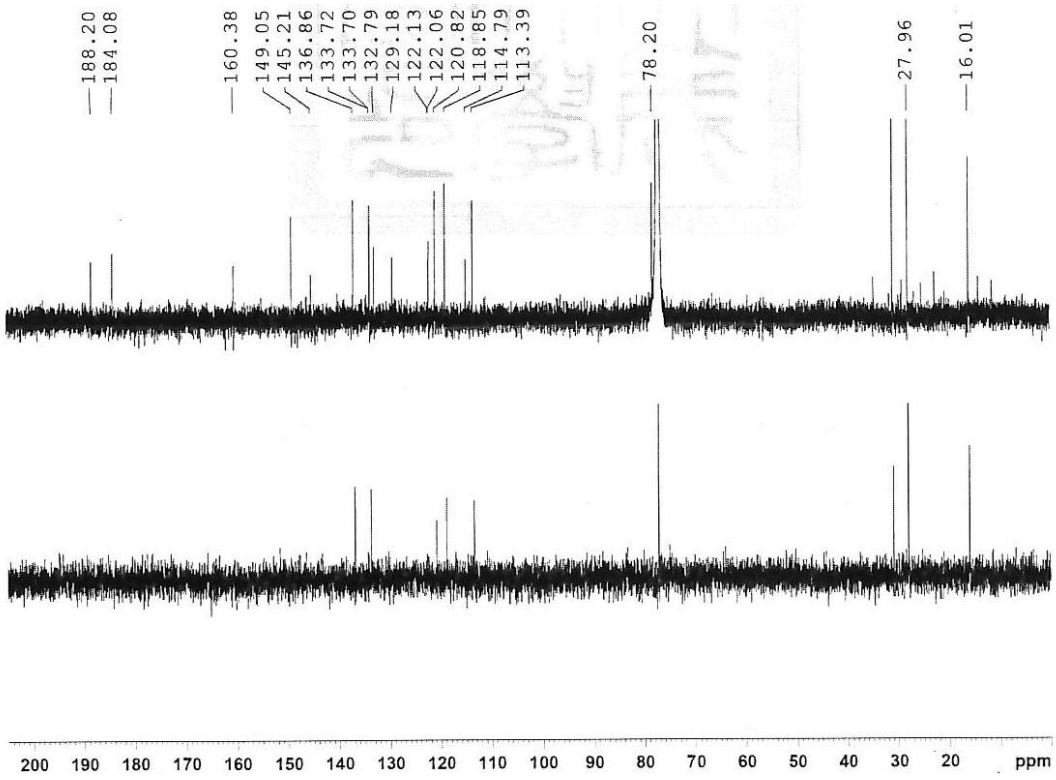


Figure S49. COSY spectrum of **7** ($CDCl_3$, 400 MHz).

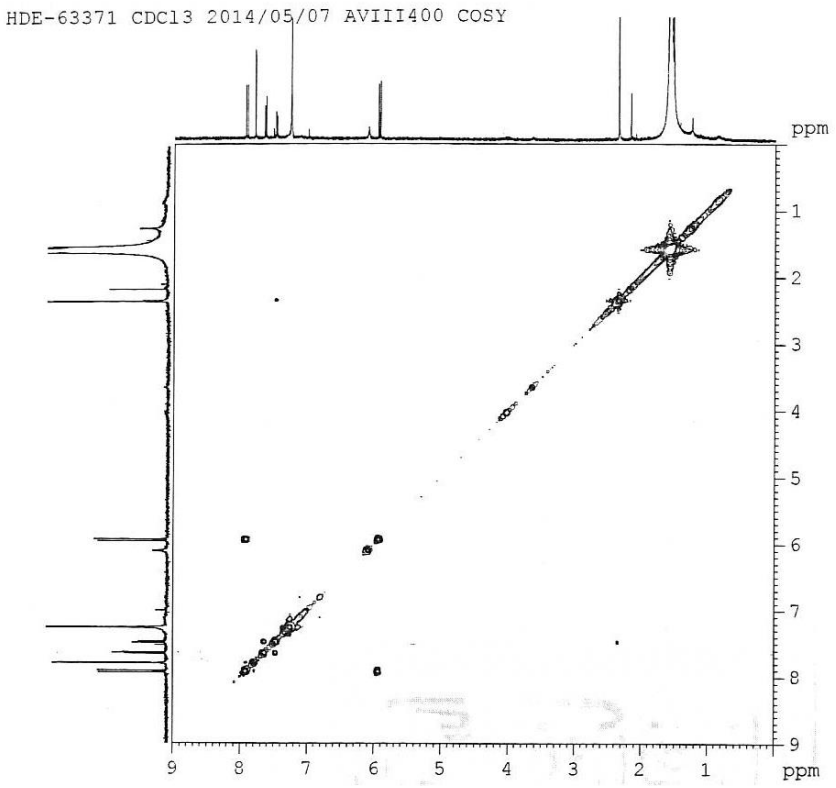


Figure S50. HSQC spectrum of **7** ($CDCl_3$, 400 MHz).

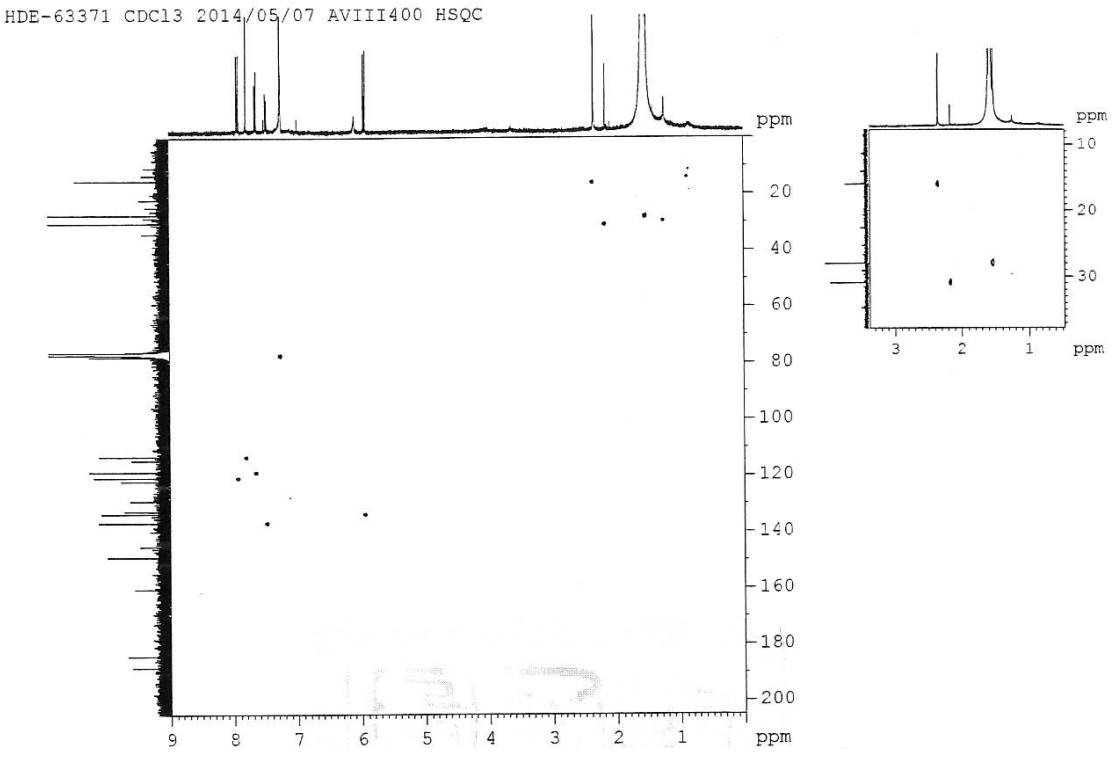


Figure S51. HMBC spectrum of **7** ($CDCl_3$, 400 MHz).

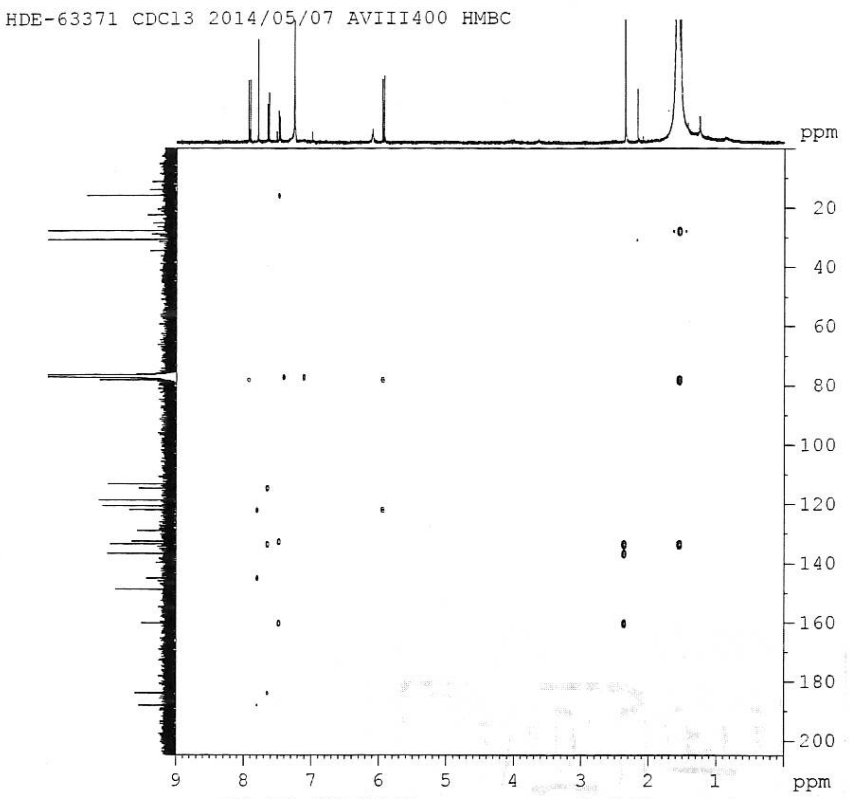
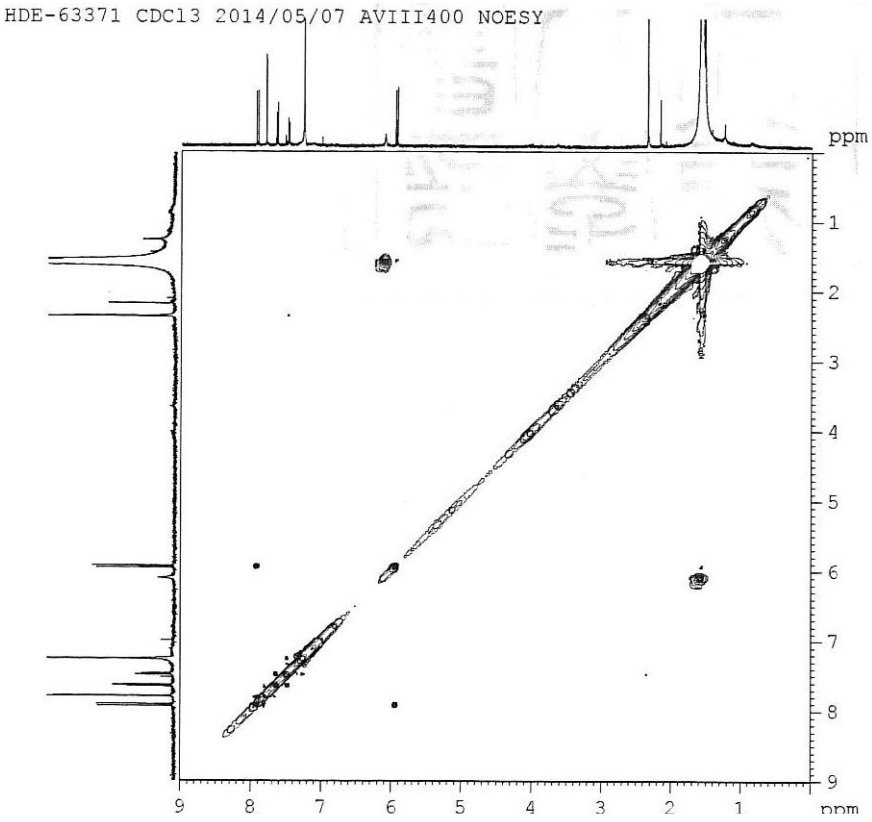


Figure S52. NOESY spectrum of **7** ($CDCl_3$, 400 MHz).



Appendix A. References for Known Compounds

2-hydroxy-6-hydroxymethylanthraquinone (**8**),[1]

tectoquinone (**9**), [2]

2-hydroxymethyl-9,10-anthraquinone (**10**), [3]

2-formyl-9,10-anthraquinone (**11**), [4]

2-hydroxy-3-methyl-9,10-anthraquinone (**12**), [5]

2-methoxy-3-methyl-9,10-anthraquinone (**13**), [6]

digiferruginol (**14**), [7]

2-hydroxy-3-hydroxymethyl-9,10-anthraquinone (**15**), [8]

1-methylalizarin (**16**), [8]

2,6-dihydroxy-3-methyl-9,10-anthraquinone (**17**), [9]

1-hydroxy-2-methoxy-3-methyl-9,10-anthraquinone (**18**), [10]

2-hydroxy-1-methoxy-3-methyl-9,10-anthraquinone (**19**), [5]

3-hydroxy-2-methoxy-6-methyl-9,10-anthraquinone (**20**), [11]

2,3-dimethoxy-6-methyl-9,10-anthraquinone (**21**), [12]

3-hydroxy-2-methoxy-6-hydroxymethyl-9,10-anthraquinone (**22**), [13]

physcion (**23**), [14]

robustaquinone B (**24**), [15]

erythroglaucin (**25**), [16]

capitellataquinone D (**26**), [17]

deacetyl asperulosidic acid methyl ester (**27**), [18]

scandoside methyl ester (**28**), [18]

E-6-*O*-*p*-coumaroyl scandoside methyl ester (**29**), [19]

Z-6-*O*-*p*-coumaroyl scandoside methyl ester (**30**), [19]

E-6-*O*-*p*-methoxycinnamoyl scandoside methyl ester (**31**), [19]

Z-6-*O*-*p*-methoxycinnamoyl scandoside methyl ester (**32**), [19]

E-6-*O*-feruloyl scandoside methyl ester (**33**), [19]

Z-6-*O*-feruloyl scandoside methyl ester (**34**), [19]

4,7-dimethoxy-5-methyl-1,3-benzodioxole (**35**), [20]

p-coumaric acid (**36**), [21]

mixture of ursolic acid (**37**) and oleanolic acid (**38**), [22]

mixture of stigmasterol (**39**) and β-sitosterol (**40**), [23]

aurantiamide acetate (**41**), [24]

References

1. Li, C. K.; Su, X. M.; Li, F. H.; Fu, J.; Wang, H. Q.; Li, B. M.; Chen, R. Y.; Kang J., Cytotoxic quinones from the aerial parts of *Morinda umbellata* L. *Phytochemistry* **2019**, *167*, 112096.
2. Endale, M.; Ekberg, A.; Alao, J. P.; Akala, H. M.; Ndakala, A.; Sunnerhagen, P.; Erdelyi, M.; Yenesew, A., Anthraquinones of the roots of *Pentas micrantha*. *Molecules* **2013**, *18*, 311-321.
3. Mitchell, D.; Lukeman, M.; Lehnherr, D.; Wan, P., Formal intramolecular photoredox chemistry of meta-substituted benzophenones. *Org. Lett.* **2005**, *7*, 3387-3389.
4. Martin, N.; Perez, I.; Sanchez, L.; Seoane, C., Synthesis and properties of the first highly conjugated tetrathiafulvalene analogues covalently attached to [60] fullerene. *J. Org. Chem.* **1997**, *62*, 5690-5695.
5. Zhang, X.; Fox, B. W.; Hadfield, J. A., Preparation of naturally occurring anthraquinones. *Synth. Commun.* **1996**, *26*, 49-62.
6. Sartori, G.; Bigi, F.; Tao, X.; Porta, C.; Maggi, R.; Predieri, G.; Lanfranchi, M.; Pellinghelli, M. A., An investigation of the reaction mechanism of the bis-acylation of aromatics with o-phthaloyl dichorides: regioselective synthesis of anthraquinones. *J. Org. Chem.* **1995**, *60*, 6588-6591.
7. Chang, P.; Lee, K. H., Cytotoxic antileukemic anthraquinones from *Morinda parvifolia*. *Phytochemistry* **1984**, *23*, 1733-1736.
8. Wu, Y. B.; Zheng, C. J.; Qin, L. P.; Sun, L. N.; Han, T.; Jiao, L.; Zhang, Q. Y.; Wu, J. Z., Antiostroporotic activity of anthraquinones from *Morinda officinalis* on osteoblasts and osteoclasts. *Molecules* **2009**, *14*, 573-583.
9. Lei, X. X.; Feng, Y. L.; Yang, S. L.; Xu, L. Z.; Li, Y. Q., A new anthraquinone from *Capparis himalayensis*. *Chem. Nat. Comp.* **2015**, *51*, 40-42.
10. Dhananjeyan, M. R.; Milev, Y. P.; Kron, M. A.; Nair, M. G., Synthesis and activity of substituted anthraquinones against a human filarial parasite, *Brugia malayi*. *J. Med. Chem.* **2005**, *48*, 2822-2830.
11. Nunez Montoya, S. C.; Agnese, A. M.; Cabrera, J. L., Anthraquinone derivatives from *Heterophyllaea pustulata*. *J. Nat. Prod.* **2006**, *69*, 801-803.
12. Punner, F.; Schieven, J.; Hilt, G., Synthesis of fluorenone and anthraquinone derivatives from aryl- and aroyl-substituted propiolates. *Org. Lett.* **2013**, *15*, 4888-4891.
13. Huang, W. H.; Yu, S. H.; Li, Y. B.; Jiang, J. Q., Two new anthraquinones from *Hedyotis diffusa*. *J. Asian Nat. Prod. Res.* **2008**, *10*, 467-471.
14. Mekkado, M.; Madrid, A.; Pena-Cortes, H.; Lopez, R.; Jara, C.; Espinoza, L., Antioxidant activity of anthraquinones isolation from leaves of *Muehlenbeckia hastulata* (J.E. SM.) johnst. (Polygonaceae). *J. Chil. Chem. Soc.* **2013**, *58*, 1767-1770.
15. Han, Y. S.; Heijden, R. V. D.; Lefeber, A. W. M.; Erkelens, C.; Verpoorte, R., Biosynthesis of anthraquinones in cell cultures of Cinchona 'Robusta' proceeds via the methylerythritol 4-phosphate pathway. *Phytochemistry* **2002**, *59*, 45-55.
16. Keller, G.; Steglich, W., 4-Aminophyscion, an anthraquinone derivative from dermocybe (Aqaricales). *Phytochemistry* **1987**, *26*, 2119-2121.
17. Ahmad, R.; Shaari, K.; Lajis, N. H.; Hamzah, A. S.; Ismail, N. H.; Kitajima, M., Anthraquinones from *Hedyotis capitellata*. *Phytochemistry* **2005**, *66*, 1141-1147.
18. Otsuka, H.; Yoshimura, K.; Yamasaki, K.; Cantoria, M. C., Isolation of 10-O-acyl iridoid glucosides from a Philippine medicinal plant, *Oldenlandia corymbosa* L. (Rubiaceae). *Chem. Pharm. Bull.* **1991**, *39*, 2049-2052.
19. Wu, H.; Tao, X.; Chen, Q.; Lao, X., Iridoids from *Hedyotis diffusa*. *J. Nat. Prod.* **1991**, *54*, 254-256.
20. Chen, P. Y.; Wu, J. D.; Tang, K. Y.; Yu, C. C.; Kuo, Y. H.; Zhong, W. B.; Lee, C. K., Isolation and synthesis of a bioactive benzenoid derivative from the fruiting bodies of *Antrodia camphorate*. *Molecules* **2013**, *18*, 7600-7608.
21. Liao, C. R.; Kuo, Y. H.; Ho, Y. L.; Wang, C. Y.; Yang, C.; Lin, C. W.; Chang, Y. S., Studies on cytotoxic constituents from the leaves of *Elaeagnus oldhamii* Maxim, in non-small cell lung cancer A549 cells. *Molecules* **2014**, *19*, 9515-9534.
22. Uddin, G.; Waliullah, B. S. S.; Alam, M.; Sadat, A.; Ahmad, A.; Uddin, A., Chemical constituents and phytotoxicity of solvent extracted fractions of stem bark of *Grewia optiva* Drummond ex Burret. *Middle East J. Sci. Res.* **2011**, *8*, 85-91.
23. Chaturvedula, V. S. P.; Prakash, I., Isolation of stigmasterol and β-sitosterol from dichloromethane extract of *Rubus suavissimus*. *Int. Curr. Pharm. J.* **2012**, *1*, 239-242.
24. Songue, J. L.; Kouam; Dongo, E.; Mpondo, T. N.; White, R. L., Chemical constituents from stem bark and roots of *Clausena anisata*. *Molecules* **2012**, *17*, 13673-13686.