*Supplementary Materials*

# Antimicrobial Properties of Flavonoid Derivatives with Bromine, Chlorine, and Nitro Group Obtained by Chemical Synthesis and Biotransformation Studies

**Martyna Perz[1,*], Daria Szymanowska[2,3], Tomasz Janeczko[1], and Edyta Kostrzewa-Susłow[1,*]**

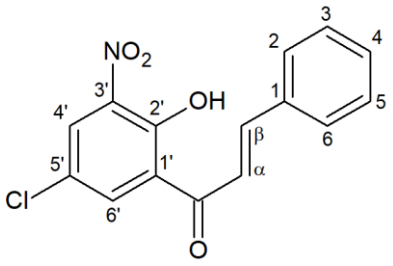
[1] Department of Food Chemistry and Biocatalysis, Faculty of Biotechnology and Food Science, Wrocław University of Environmental and Life Sciences, 50-375 Wrocław, Poland;

[2] Department of Biotechnology and Food Microbiology, Faculty of Food Science and Nutrition, Poznań University of Life Sciences, 60-627 Poznan, Poland;

[3] Department of Pharmacognosy and Biomaterials, Faculty of Pharmacy, Poznań University of Medical Sciences, Rokietnicka 3, 60-806 Poznań, Poland;

* Correspondence: martyna.perz@upwr.edu.pl (M.P.); edyta.kostrzewa-suslow@upwr.edu.pl (E.K.-S.)

Compound name: 5′-chloro-2′-hydroxy-3′-nitrochalcone
Molecular Formula: $C_{15}H_{10}ClNO_4$
Formula Weight: 303.697
Ionization mode: positive
Precursor $[M + H]^+$: 304.029
Monoisotopic Mass: 303.029
Collision energy (CE): -15.0



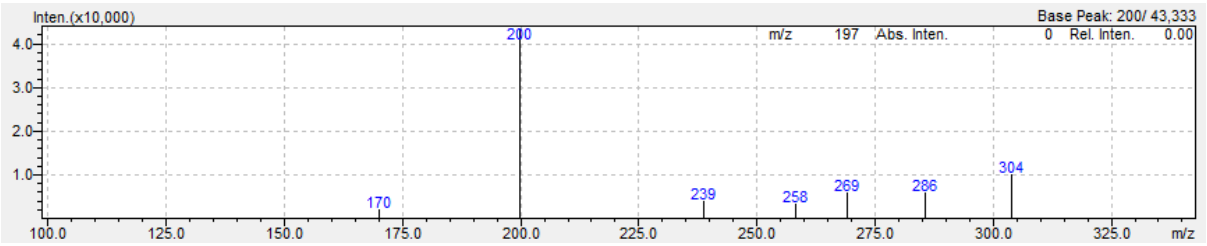




*Figure S1. LC-MS spectrum of 5′-chloro-2′-hydroxy-3′-nitrochalcone (4).*

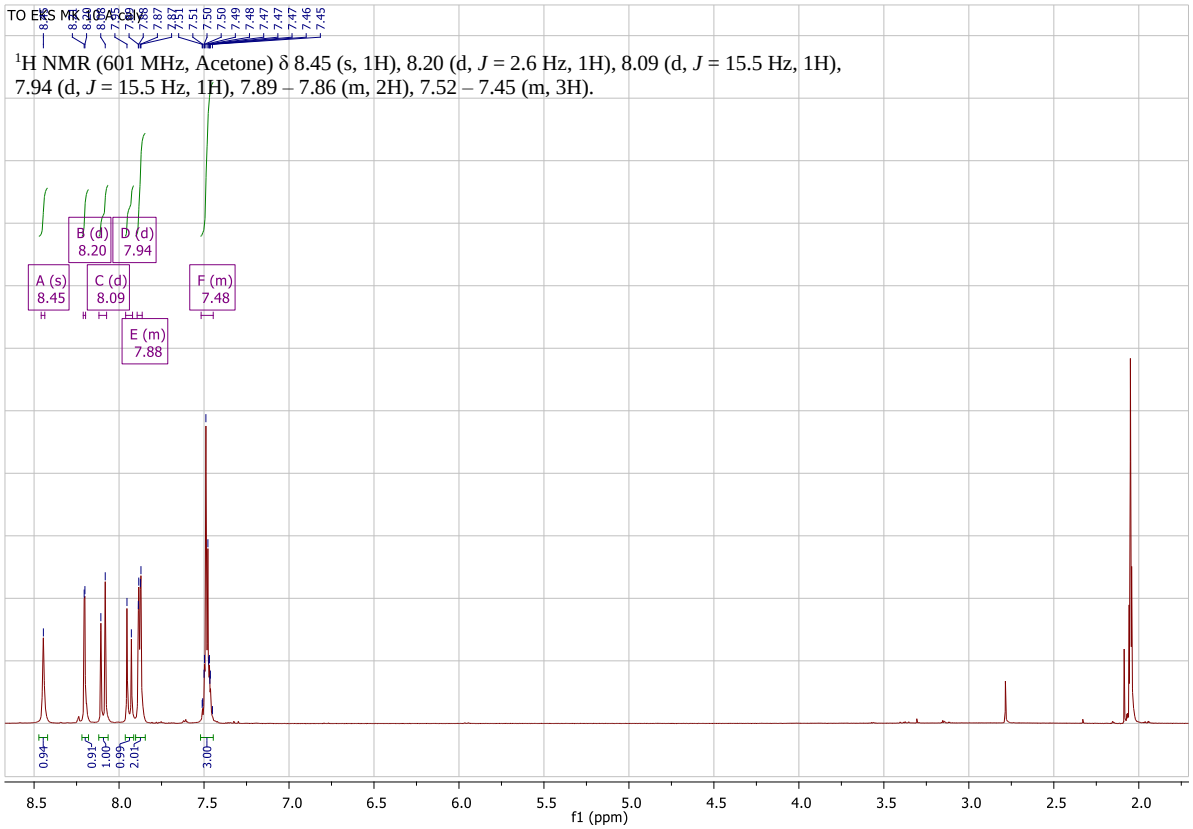



*Figure S2. ¹H NMR spectrum of 5′-chloro-2′-hydroxy-3′-nitrochalcone (4).*

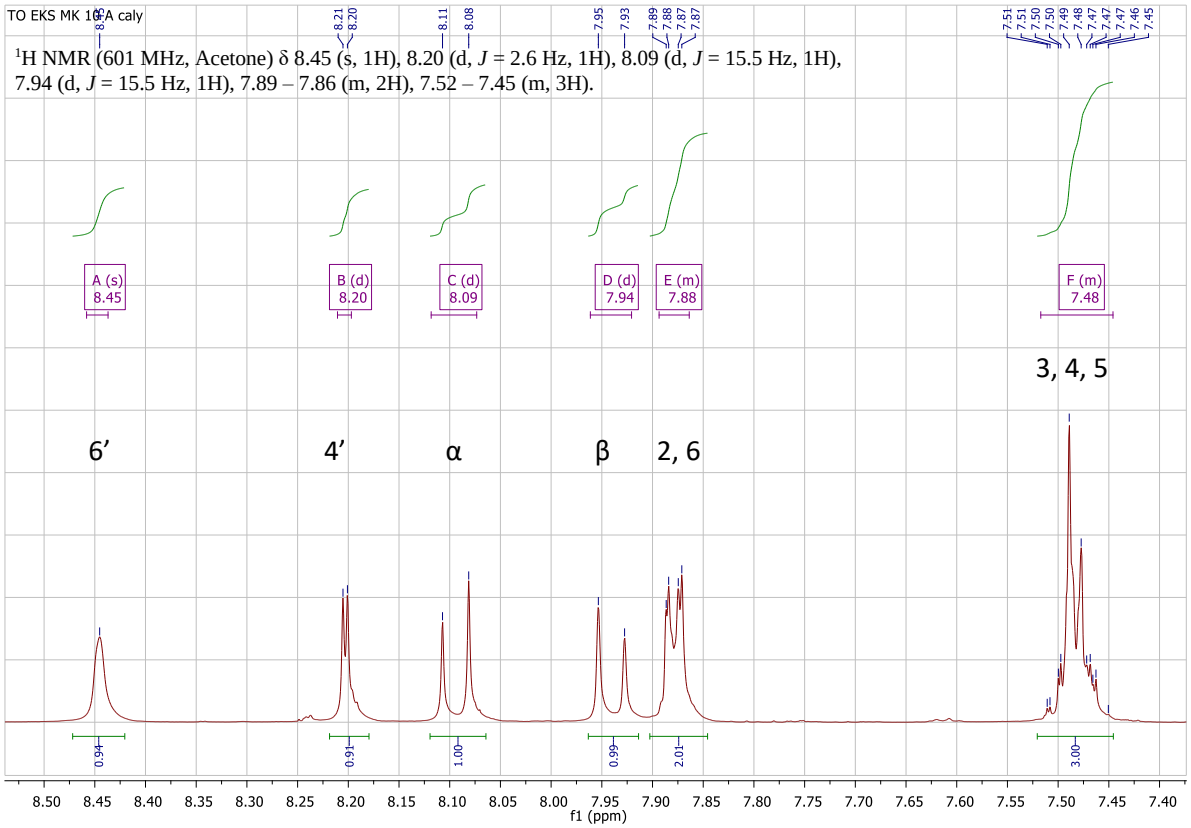



*Figure S3. ¹H NMR spectrum expansion of 5′-chloro-2′-hydroxy-3′-nitrochalcone (4).*

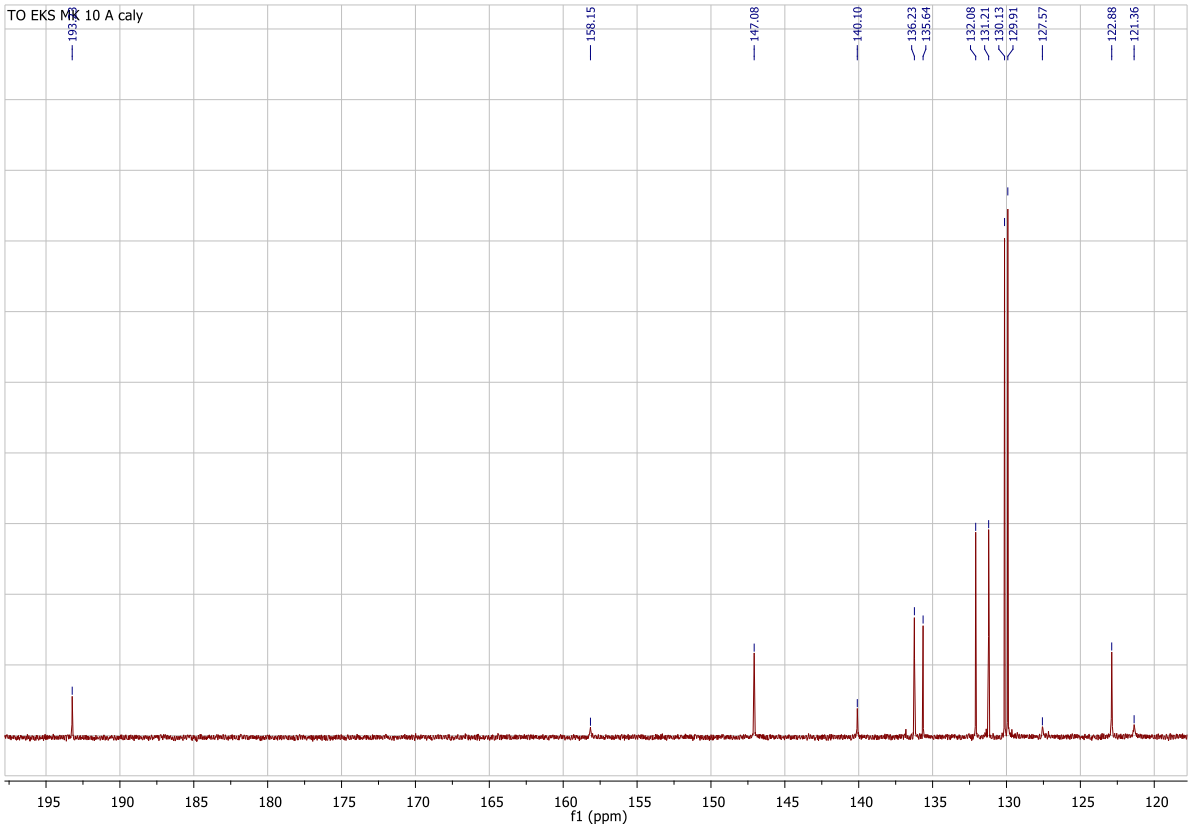



*Figure S4. $^{13}C$ NMR of 5′-chloro-2′-hydroxy-3′-nitrochalcone (4).*

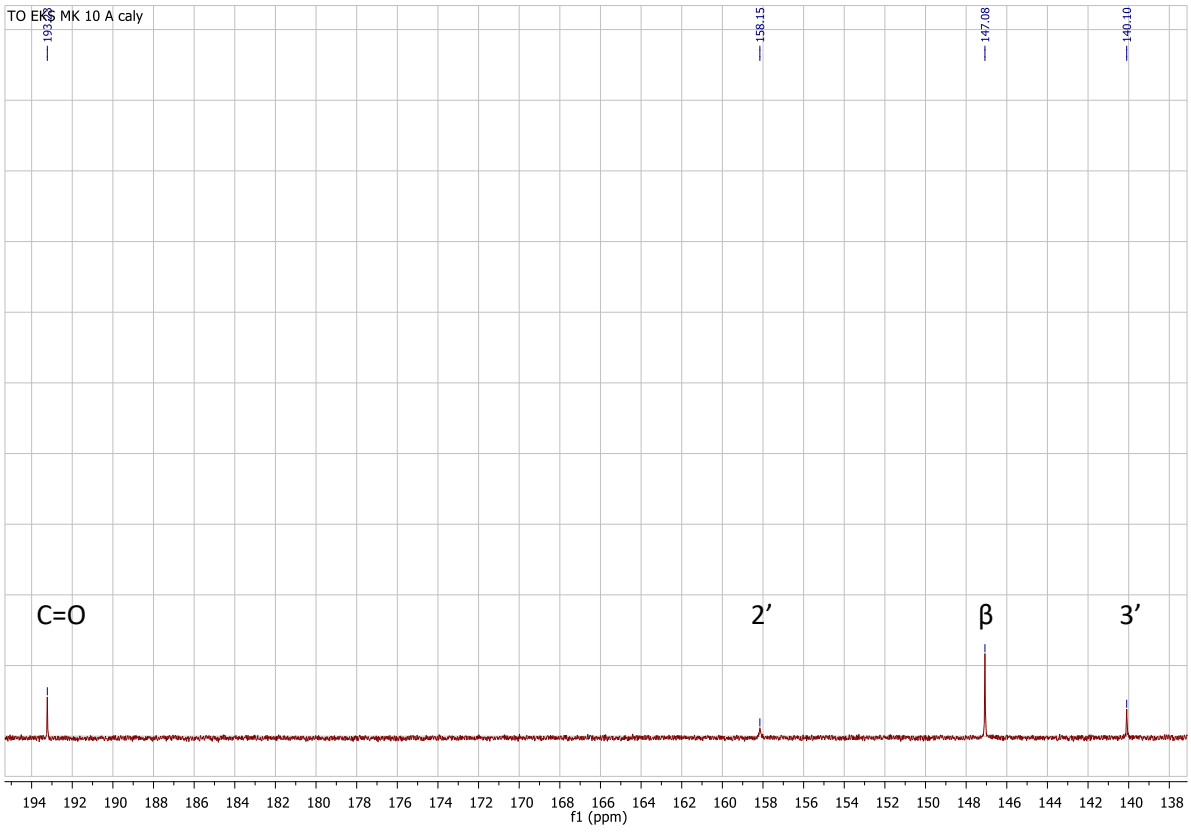



*Figure S5. $^{13}C$ NMR spectrum expansion of 5′-chloro-2′-hydroxy-3′-nitrochalcone (4).*

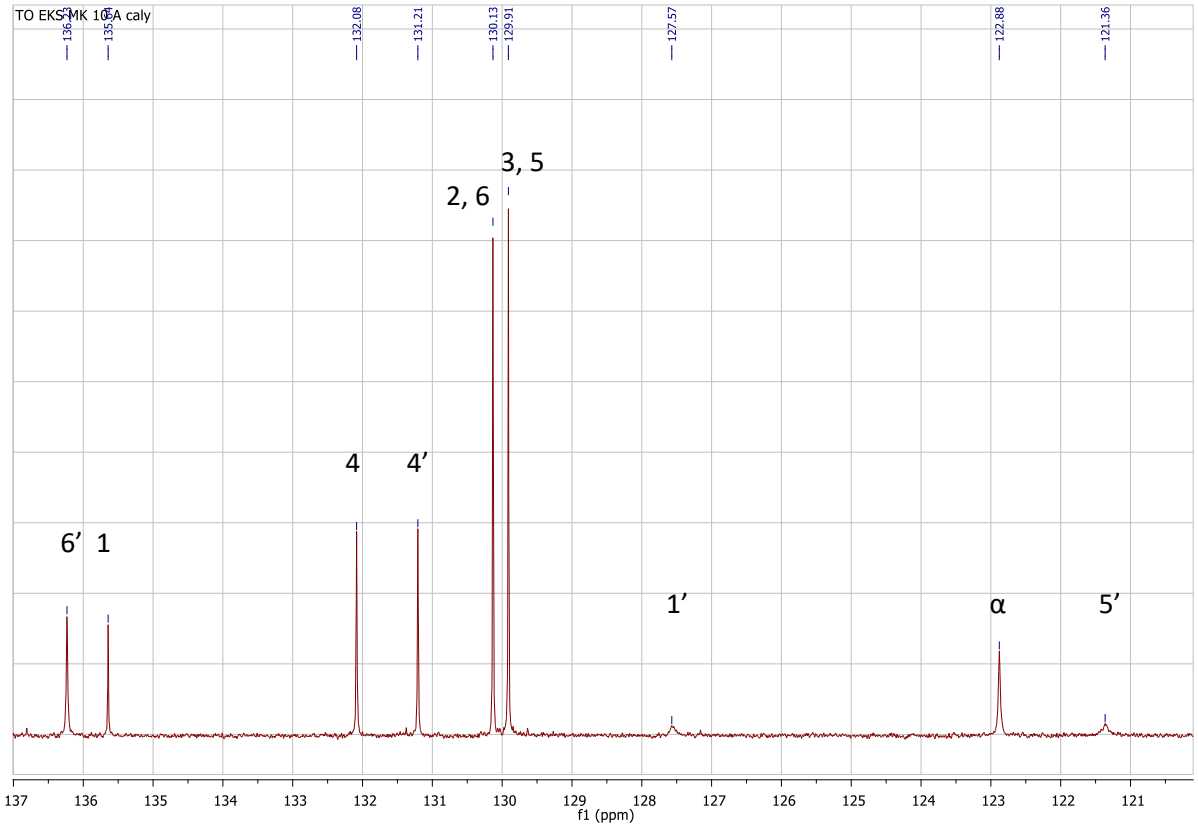



*Figure S6. $^{13}C$ NMR spectrum expansion of 5′-chloro-2′-hydroxy-3′-nitrochalcone (4).*

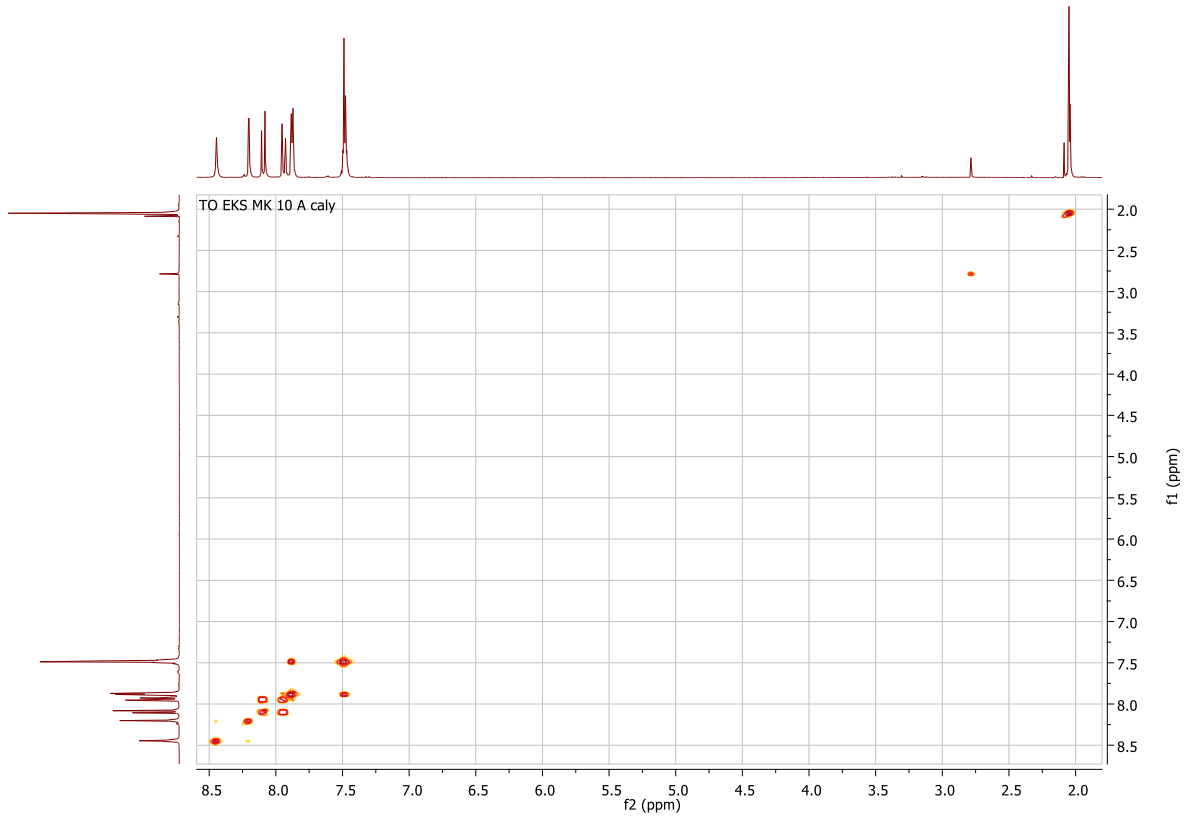



*Figure S7. COSY contour map – $^{1}H$ x $^{1}H$ of 5′-chloro-2′-hydroxy-3′-nitrochalcone (4).*

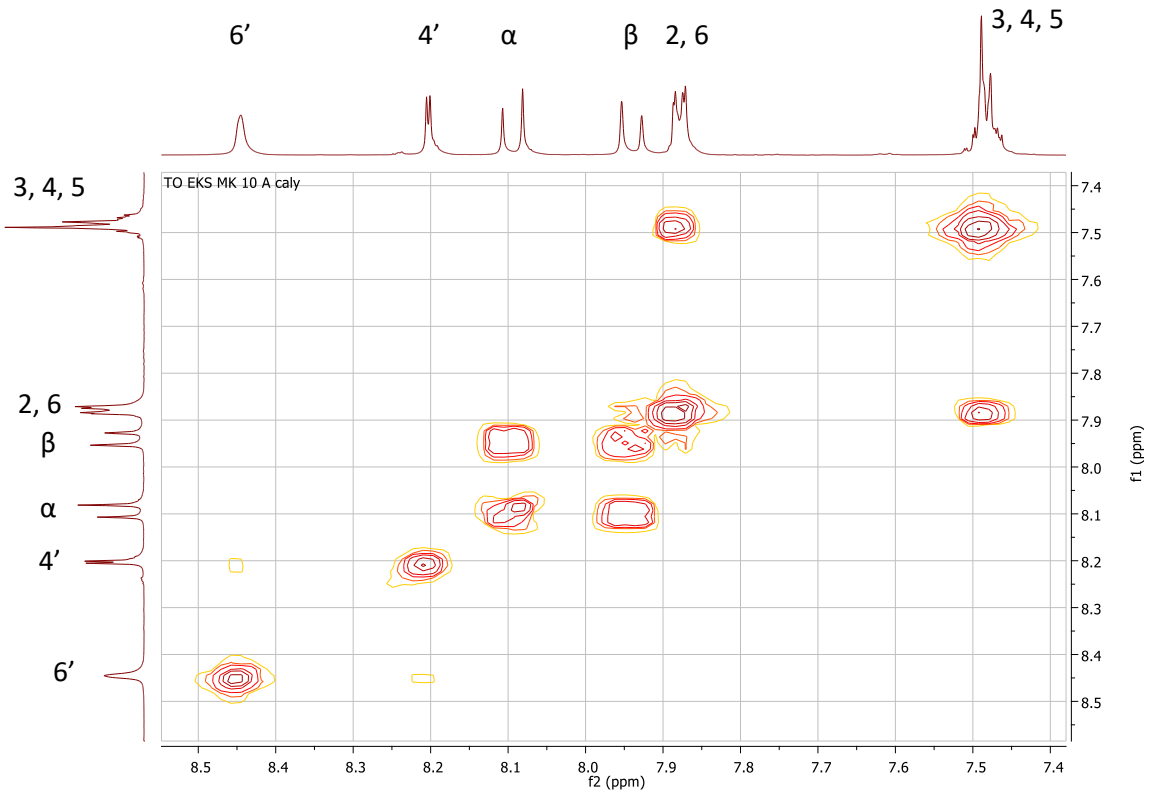



*Figure S8. COSY contour map – $^{1}H$ x $^{1}H$ expansion of 5′-chloro-2′-hydroxy-3′-nitrochalcone (4).*

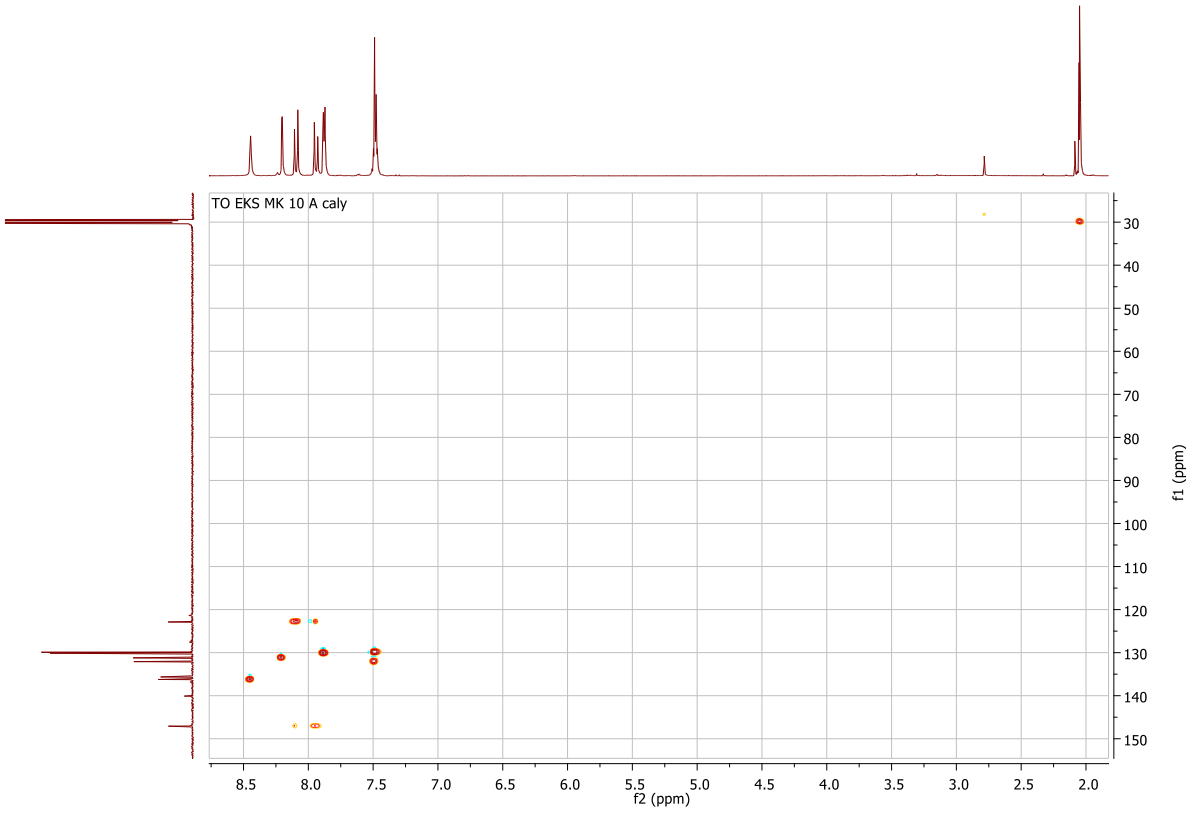



*Figure S9. HSQC contour map – $^{1}H$ x $^{13}C$ of 5′-chloro-2′-hydroxy-3′-nitrochalcone (4).*

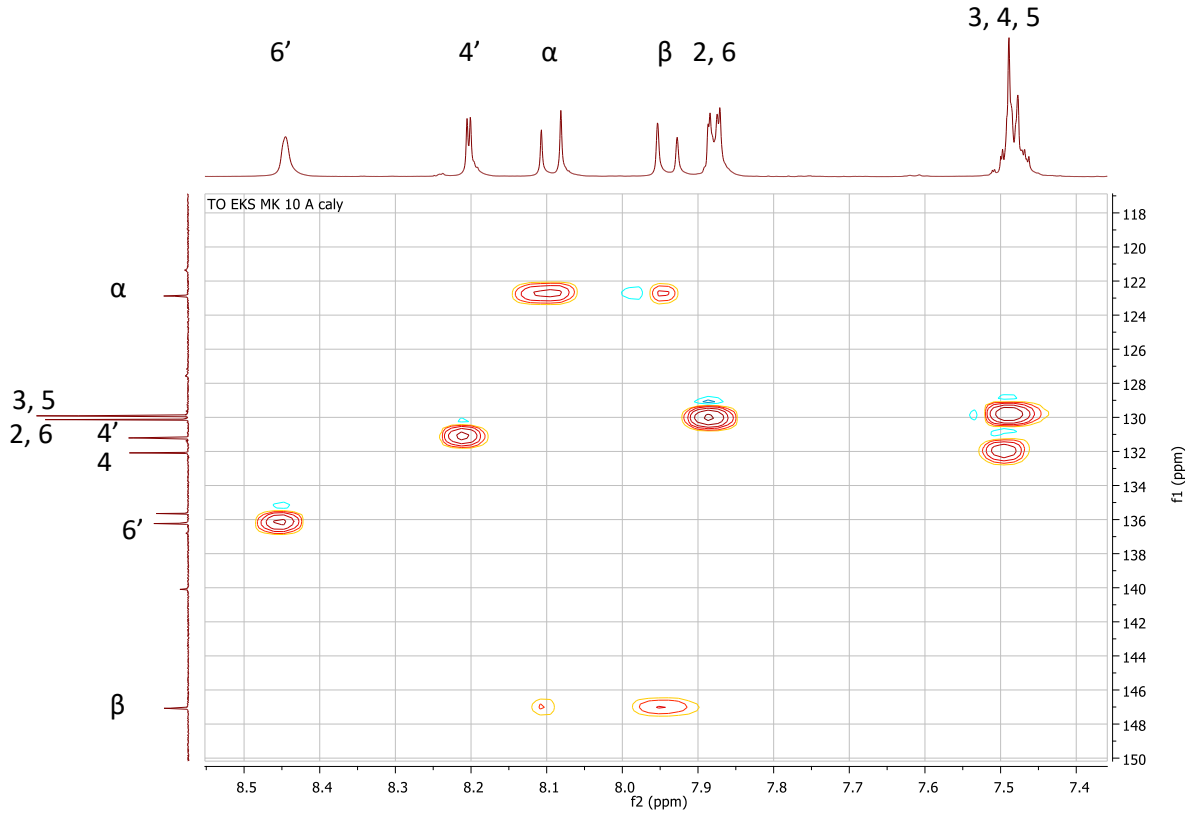



*Figure S10. HSQC contour map – $^{1}H$ x $^{13}C$ expansion of 5′-chloro-2′-hydroxy-3′-nitrochalcone (4).*

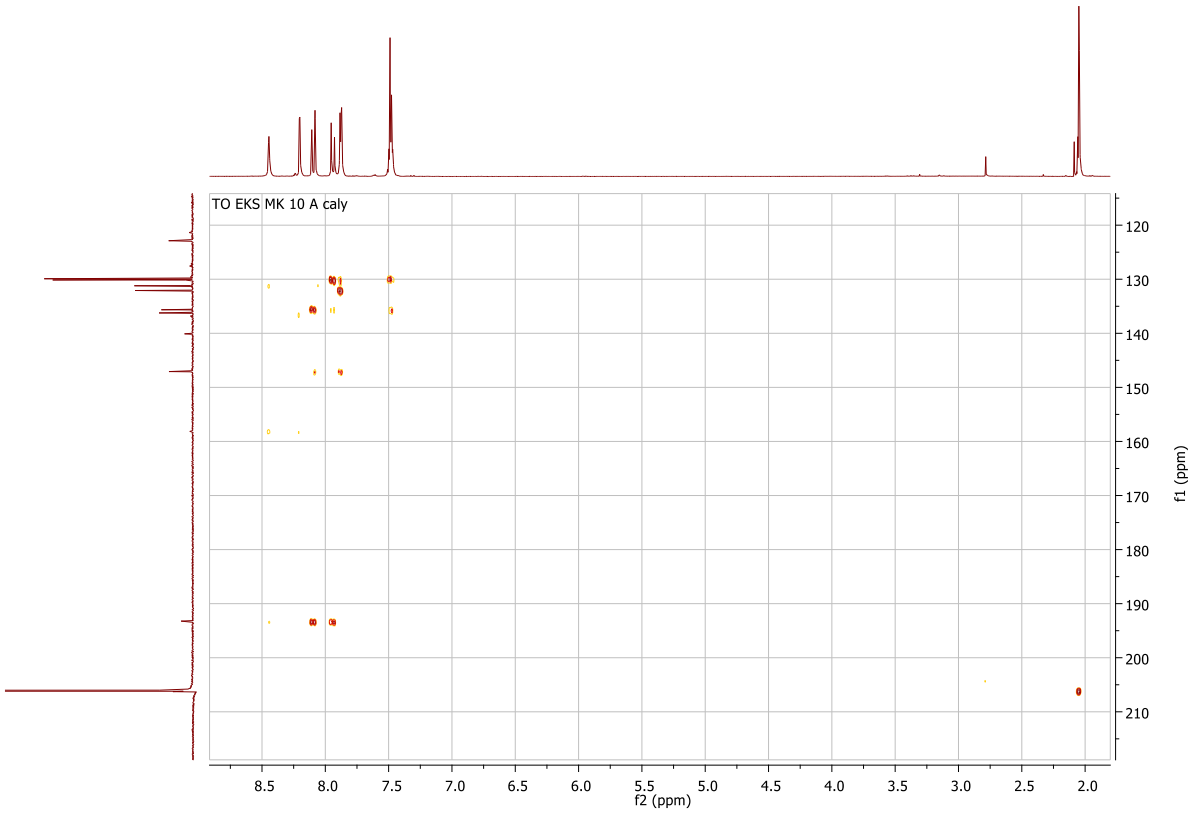



*Figure S11. HMBC contour map – $^{1}H$ x $^{13}C$ of 5′-chloro-2′-hydroxy-3′-nitrochalcone (4).*

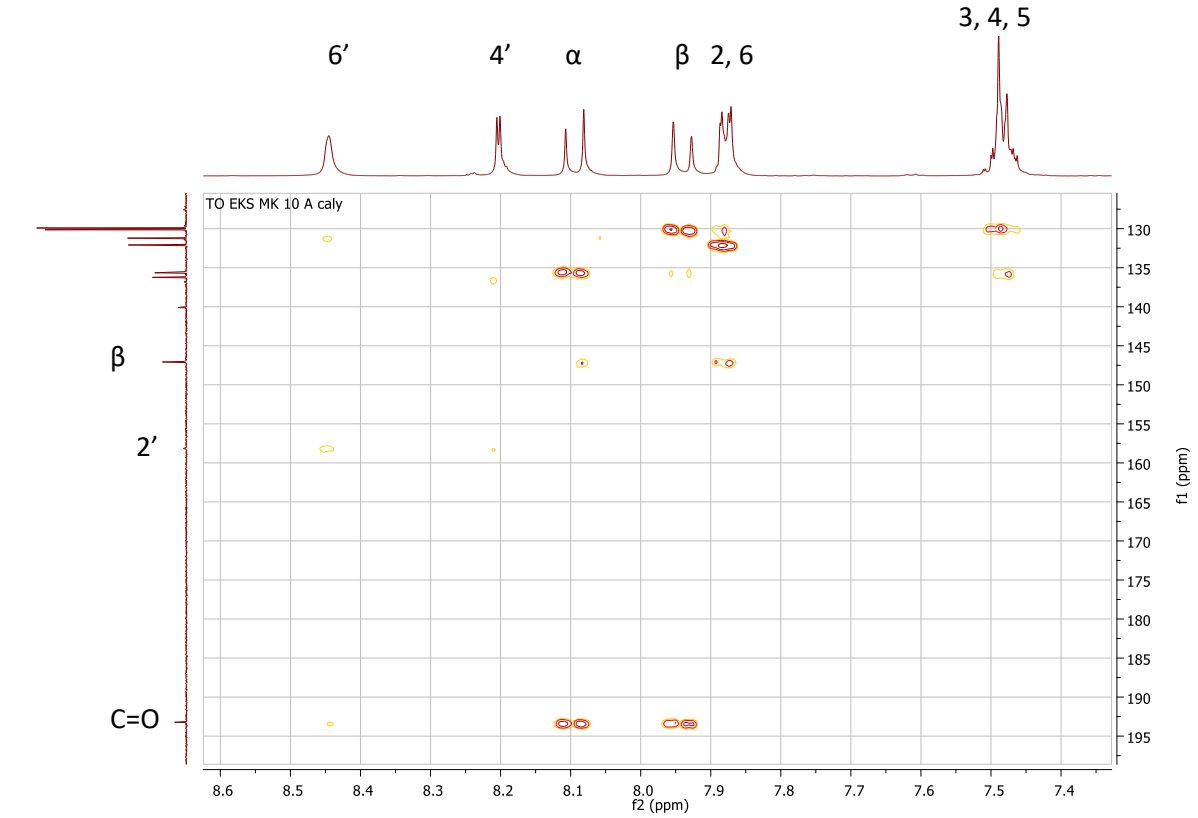



*Figure S12. HMBC contour map – $^{1}H$ x $^{13}C$ expansion of 5’-chloro-2’-hydroxy-3’-nitrochalcone (4).*

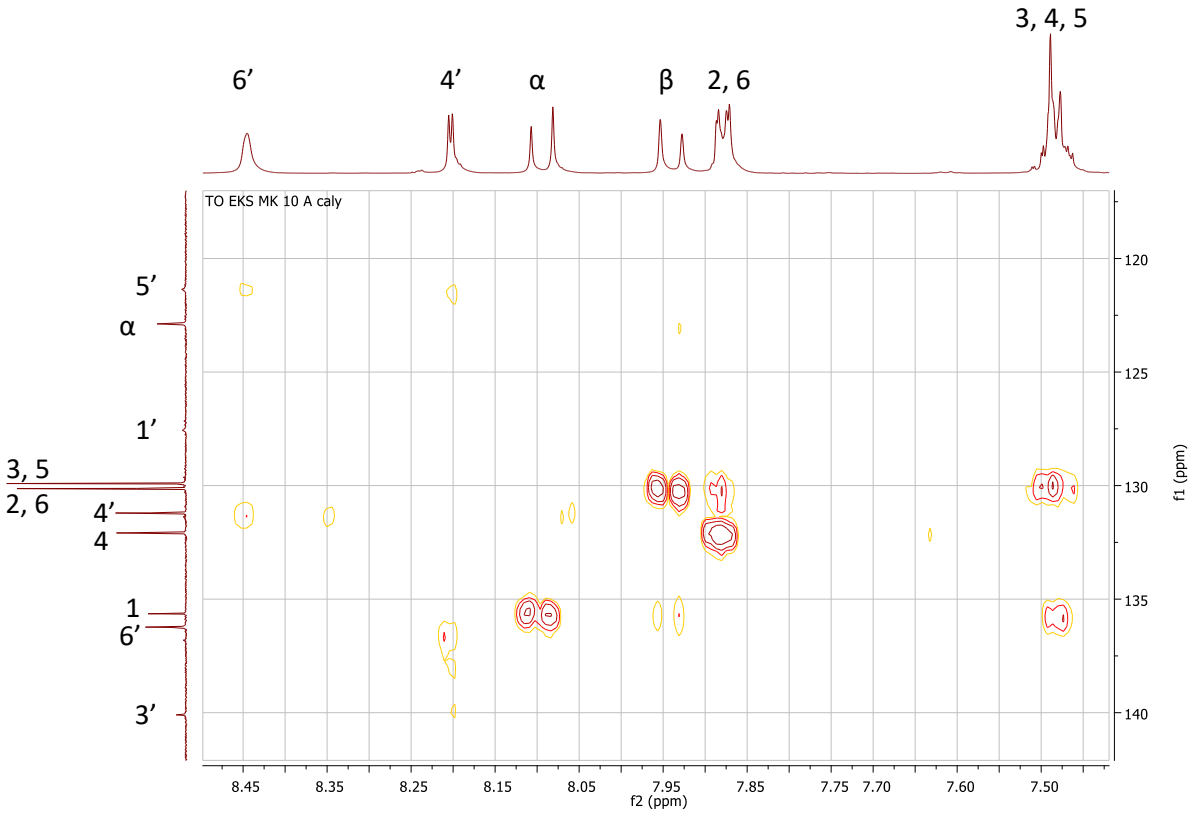
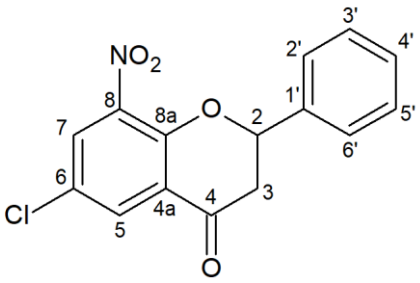



*Figure S13. HMBC contour map – $^{1}H$ x $^{13}C$ expansion of 5’-chloro-2’-hydroxy-3’-nitrochalcone (4).*

Compound name: 6-chloro-8-nitroflavanone
Molecular Formula: $C_{15}H_{10}ClNO_4$
Formula Weight: 303.697
Ionization mode: negative
Precursor $[M + H]^+$: 302.029
Monoisotopic Mass: 303.029
Collision energy (CE): 25.0



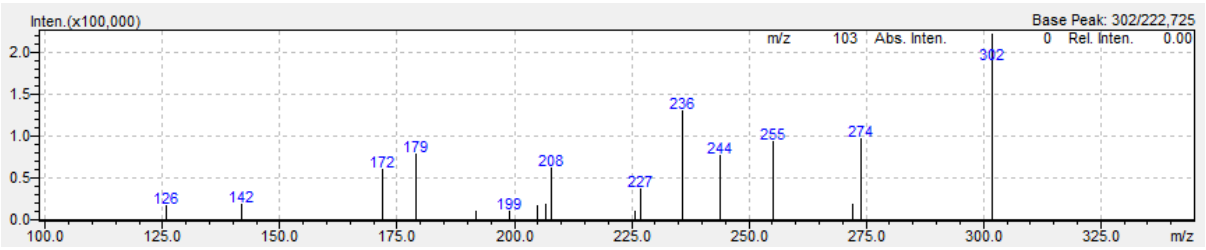




*Figure S14. LC-MS spectrum of 6-chloro-8-nitroflavanone.*

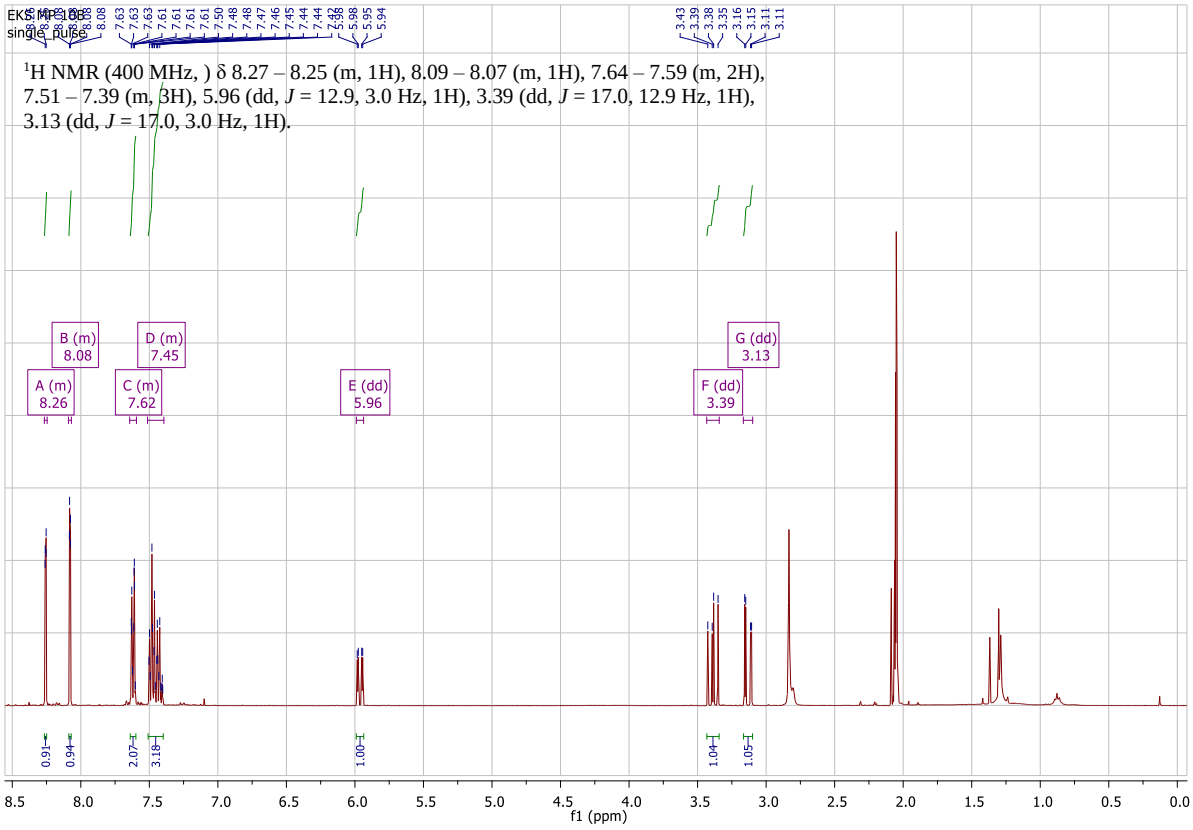



*Figure S15. $^1$H NMR spectrum of 6-chloro-8-nitroflavanone.*

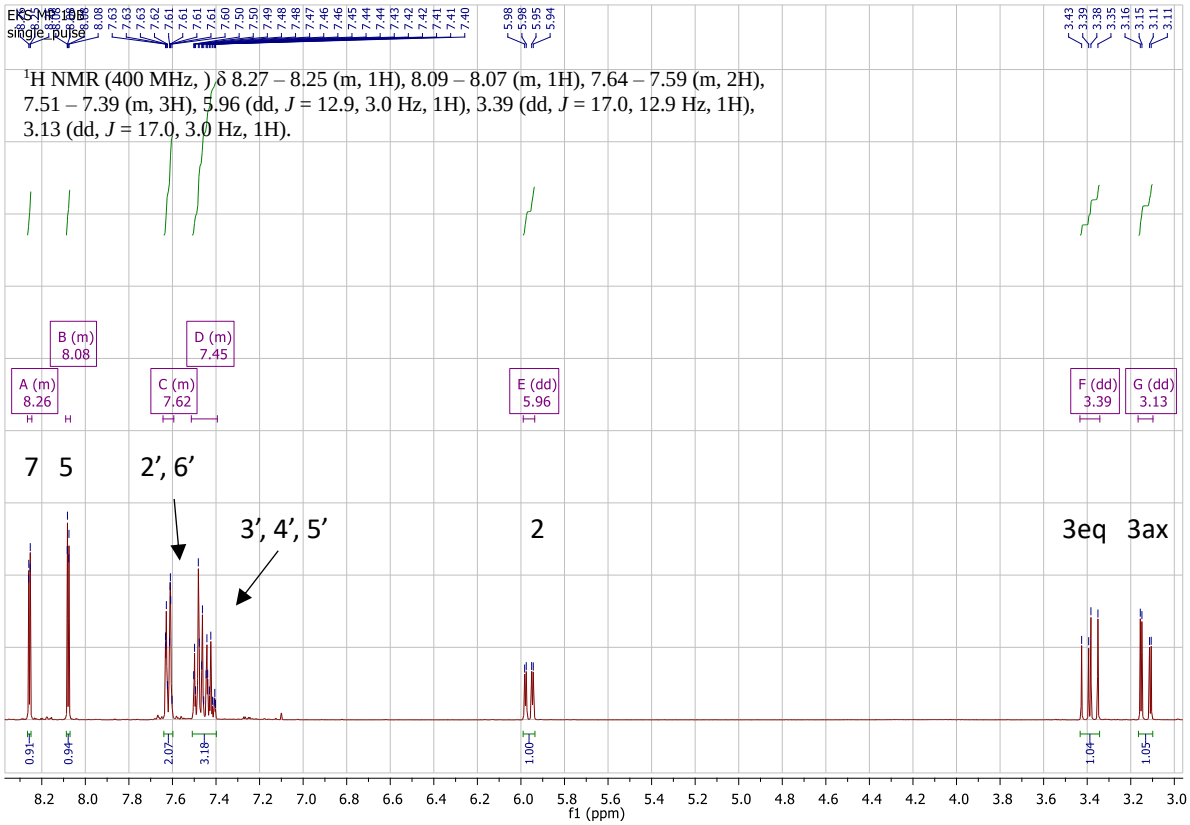



*Figure S16. $^1$H NMR spectrum expansion of 6-chloro-8-nitroflavanone.*

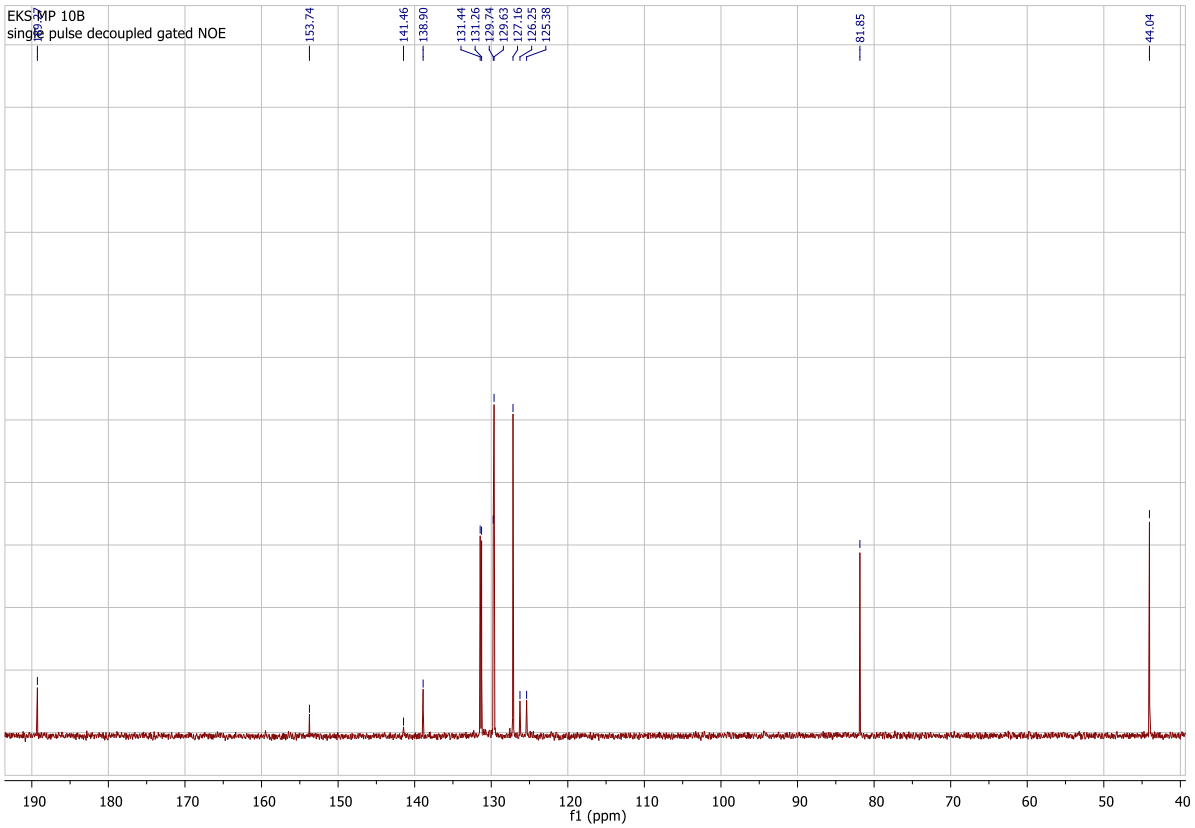



*Figure S17. $^{13}$C NMR spectrum of 6-chloro-8-nitroflavanone.*

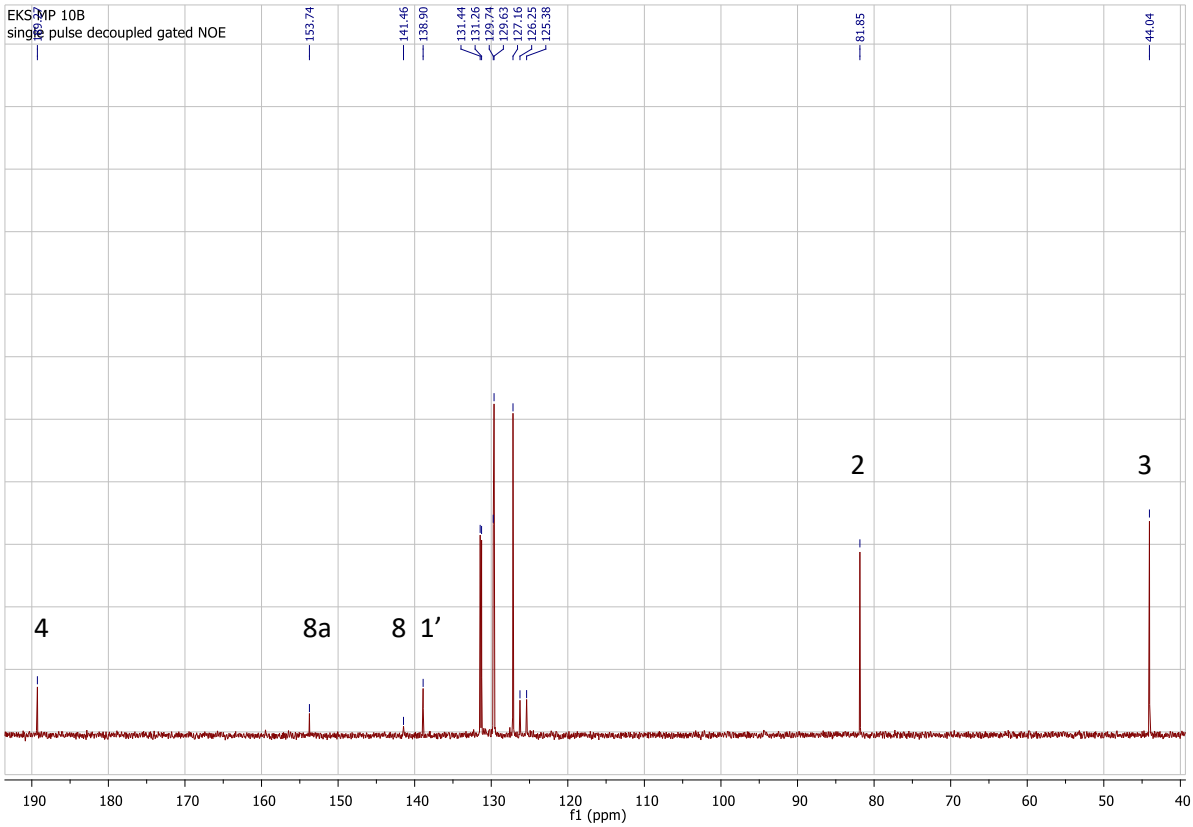



*Figure S18. [13]C NMR spectrum expansion of 6-chloro-8-nitroflavanone.*

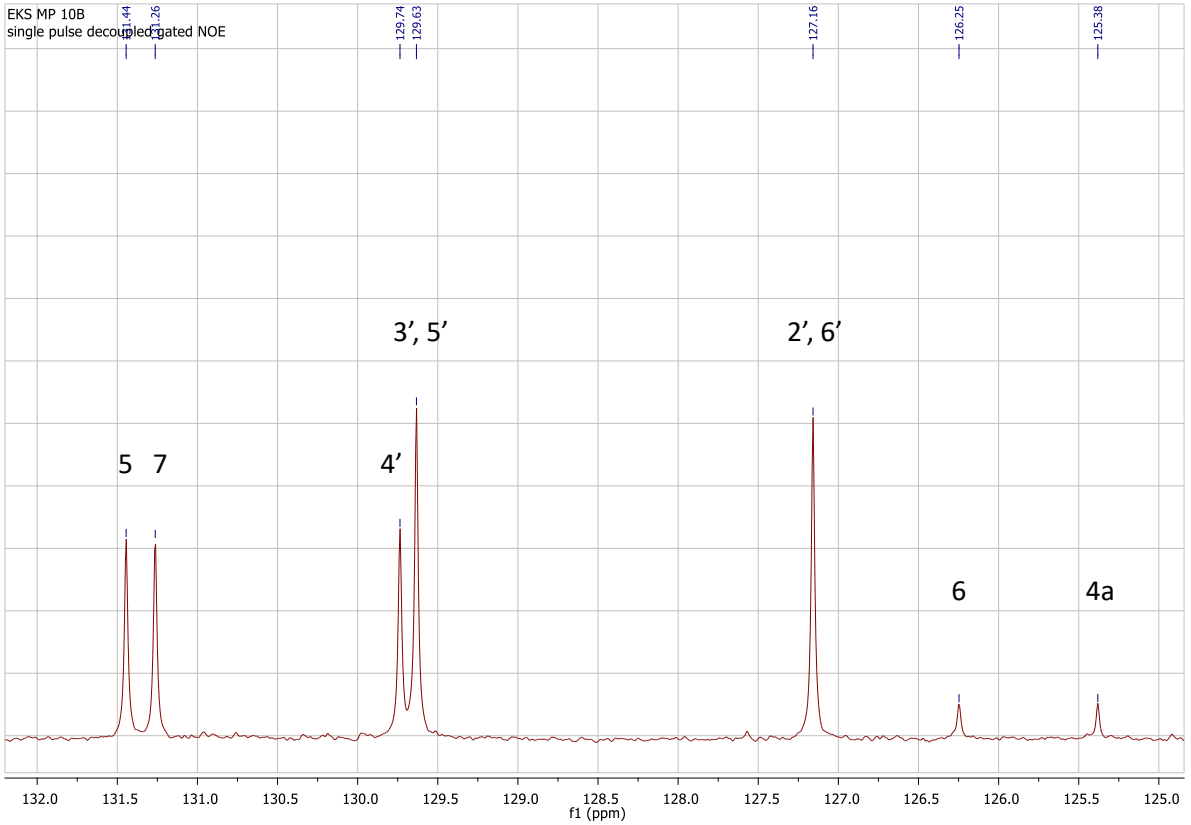



*Figure S19. [13]C NMR spectrum expansion of 6-chloro-8-nitroflavanone*

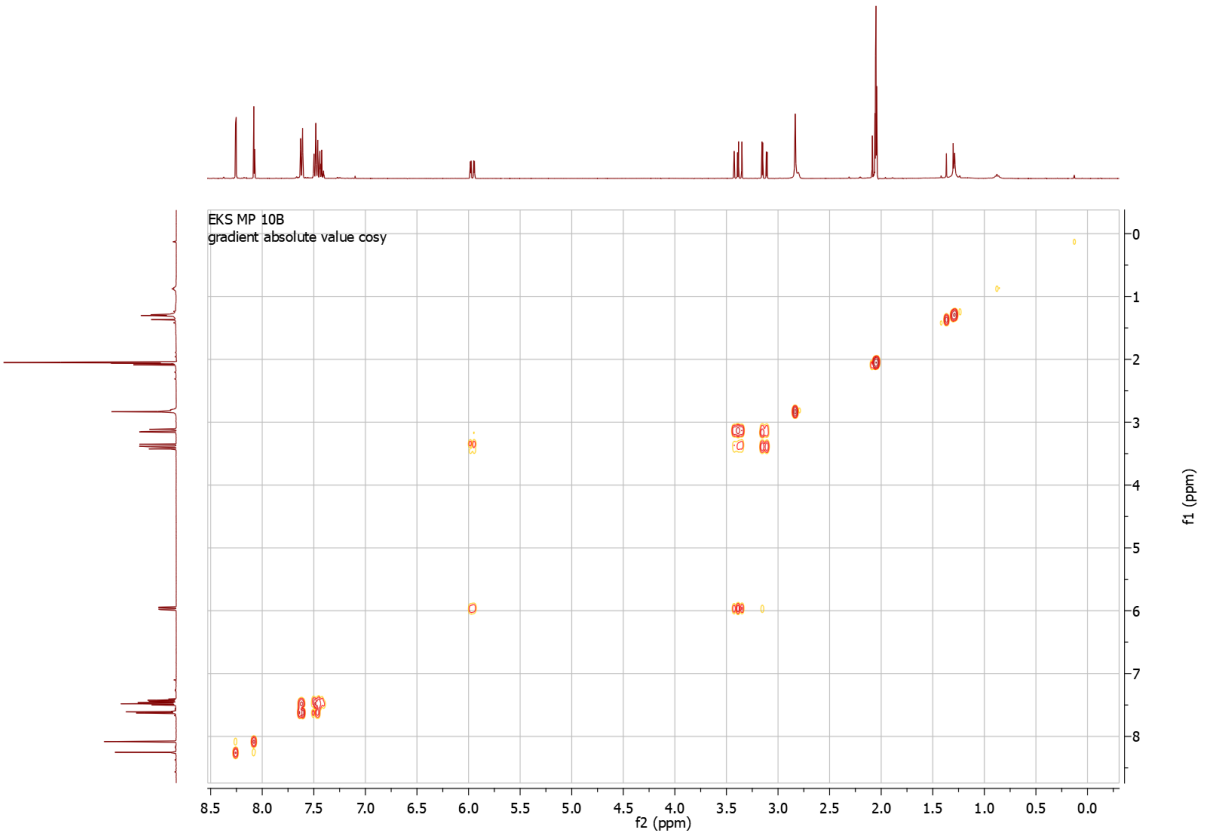



*Figure S20. COSY contour map – $^1$H x $^1$H expansion of 6-chloro-8-nitroflavanone.*

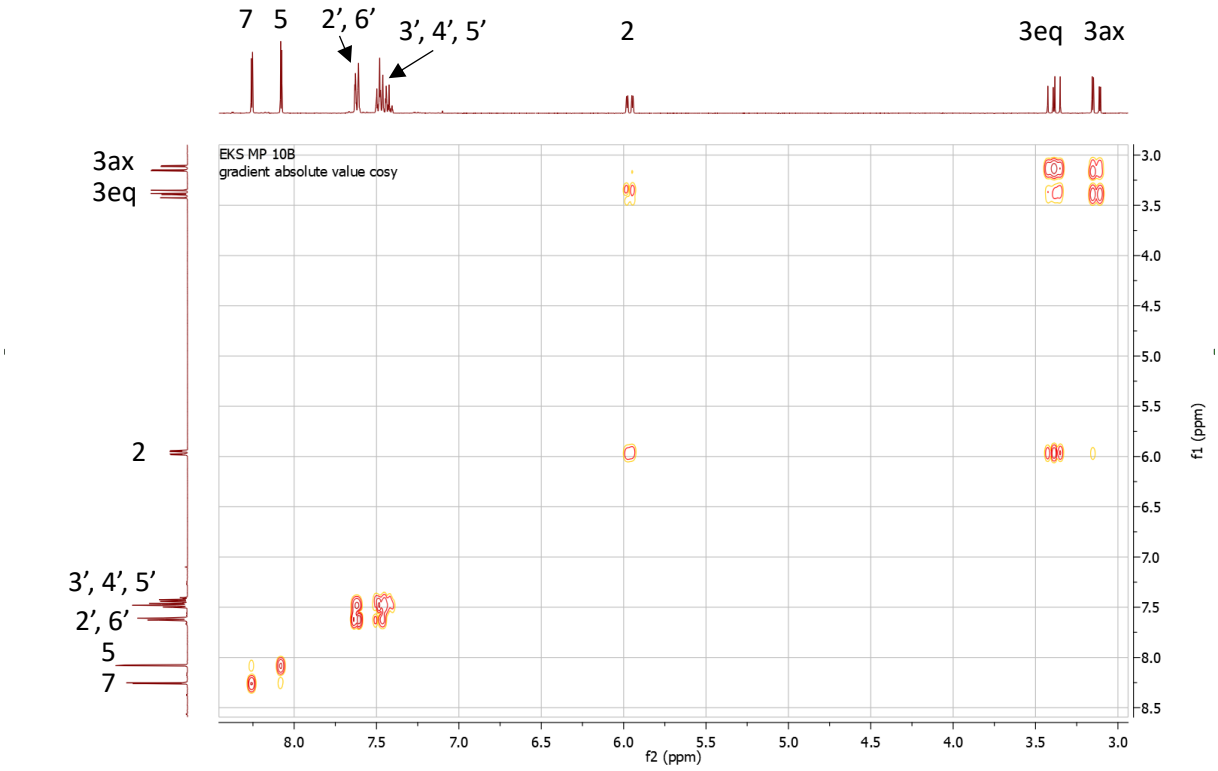



*Figure S21. COSY contour map – $^1$H x $^1$H expansion of 6-chloro-8-nitroflavanone.*

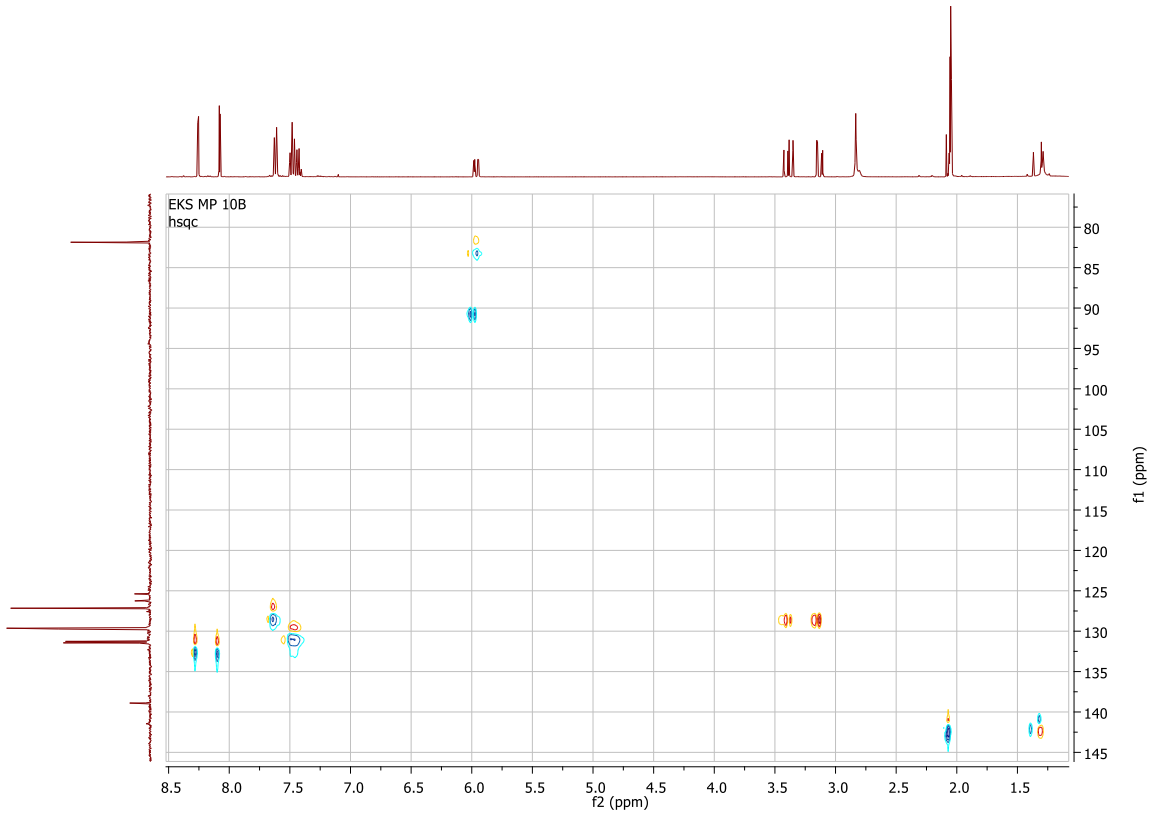



*Figure S22. HSQC contour map – $^{1}H$ x $^{13}C$ of 6-chloro-8-nitroflavanone.*

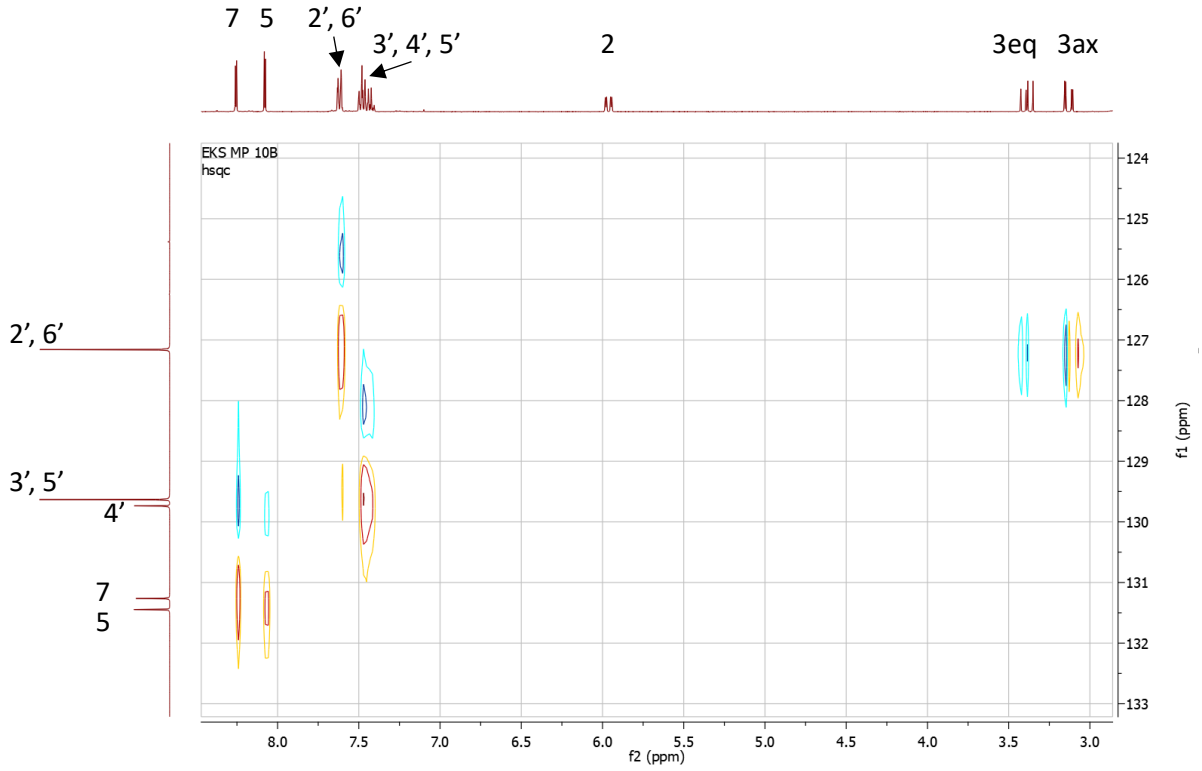



*Figure S23. HSQC contour map – $^{1}H$ x $^{13}C$ expansion of 6-chloro-8-nitroflavanone.*

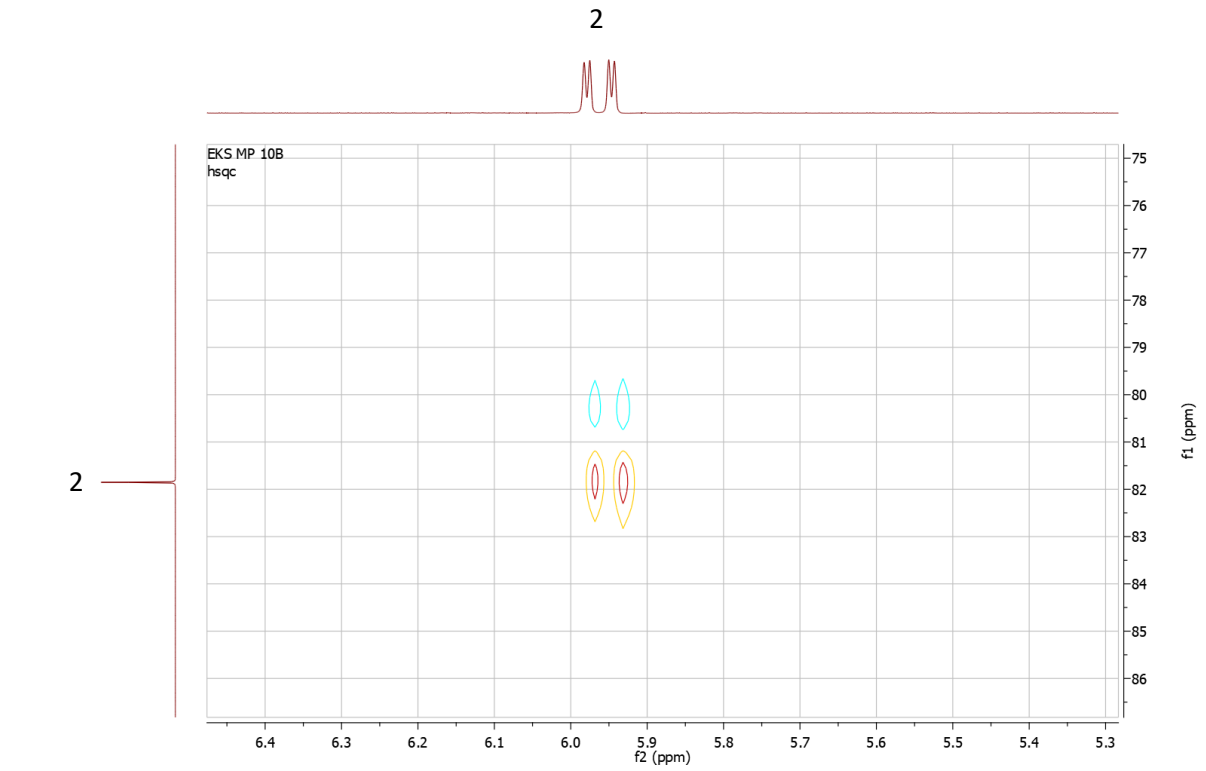



*Figure S24. HSQC contour map – $^{1}H$ x $^{13}C$ expansion of 6-chloro-8-nitroflavanone.*

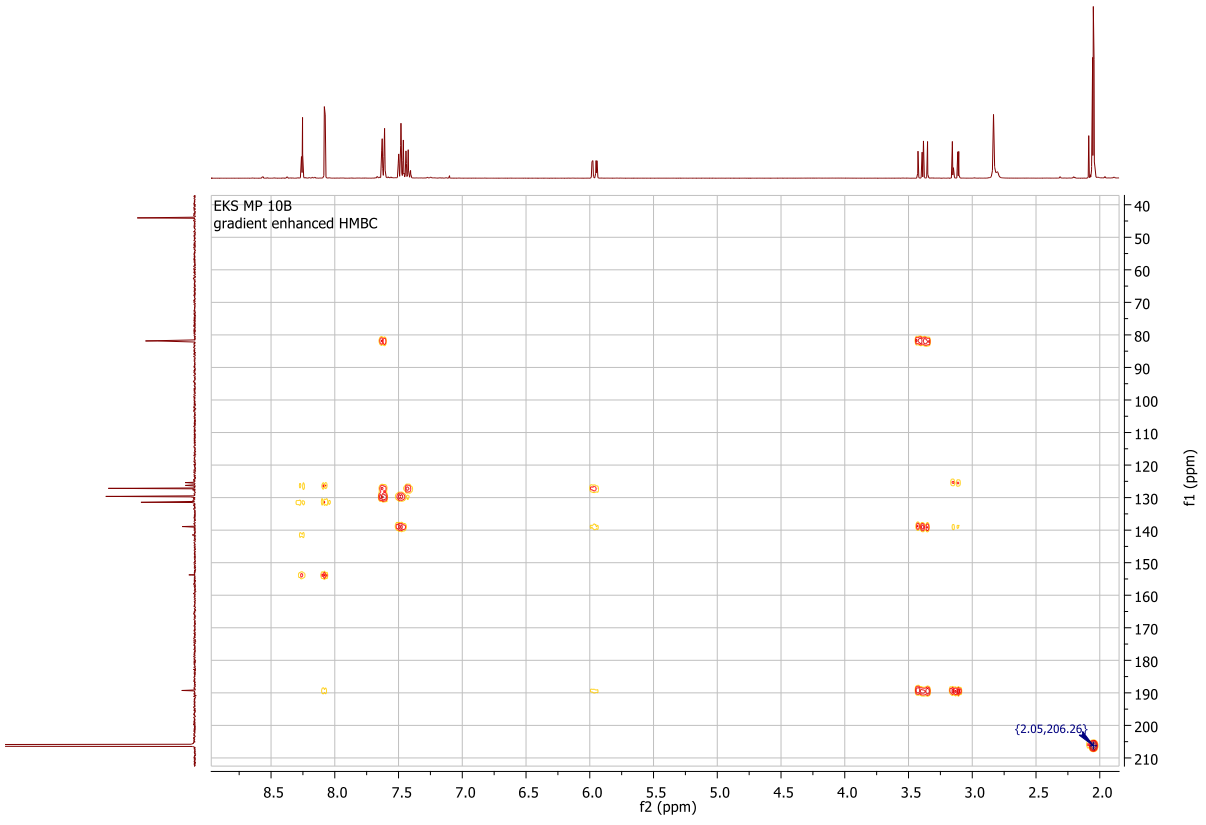



*Figure S25. HMBC contour map – $^{1}H$ x $^{13}C$ of 6-chloro-8-nitroflavanone.*

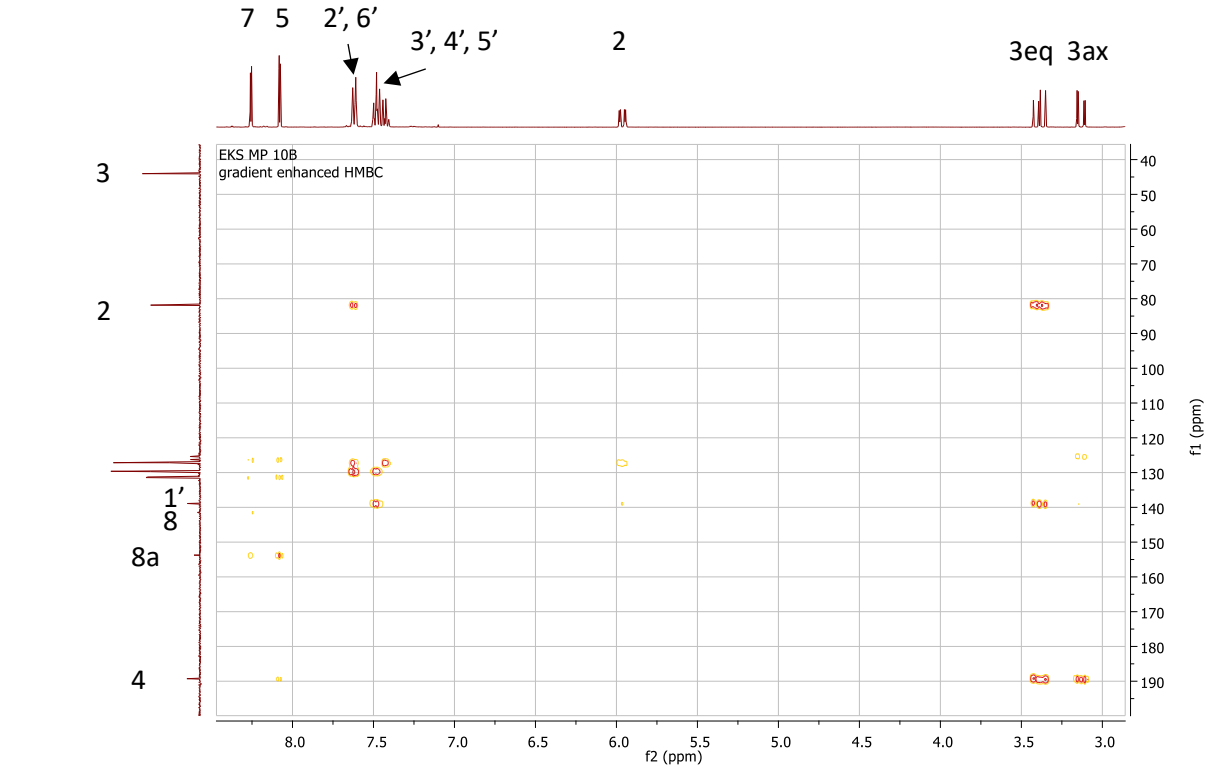



*Figure S26. HMBC contour map – $^{1}H$ x $^{13}C$ expansion of 6-chloro-8-nitroflavanone.*

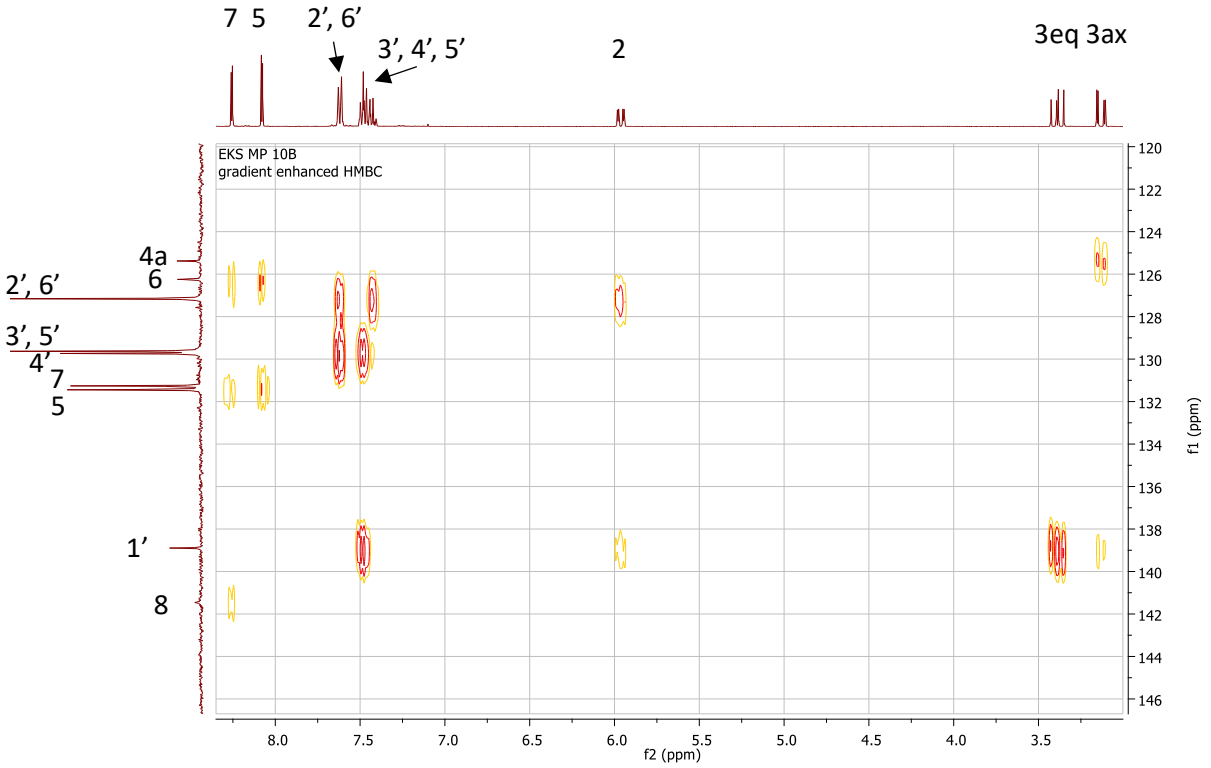
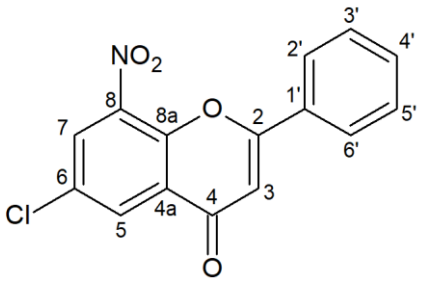



*Figure S27. HMBC contour map – $^{1}H$ x $^{13}C$ expansion of 6-chloro-8-nitroflavanone.*

Compound name: 6-chloro-8-nitroflavone
Molecular Formula: $C_{15}H_8ClNO_4$
Formula Weight: 301.681
Ionization mode: positive
Precursor $[M + H]^+$: 302.014
Monoisotopic Mass: 301.014
Collision energy (CE): -25.0



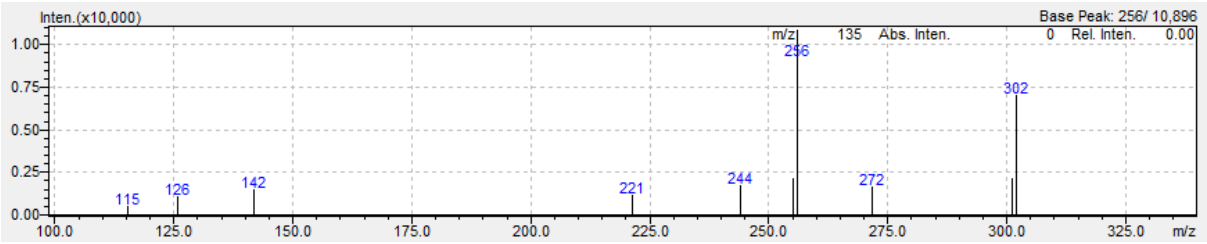




*Figure S28. LC-MS spectrum of 6-chloro-8-nitroflavone.*

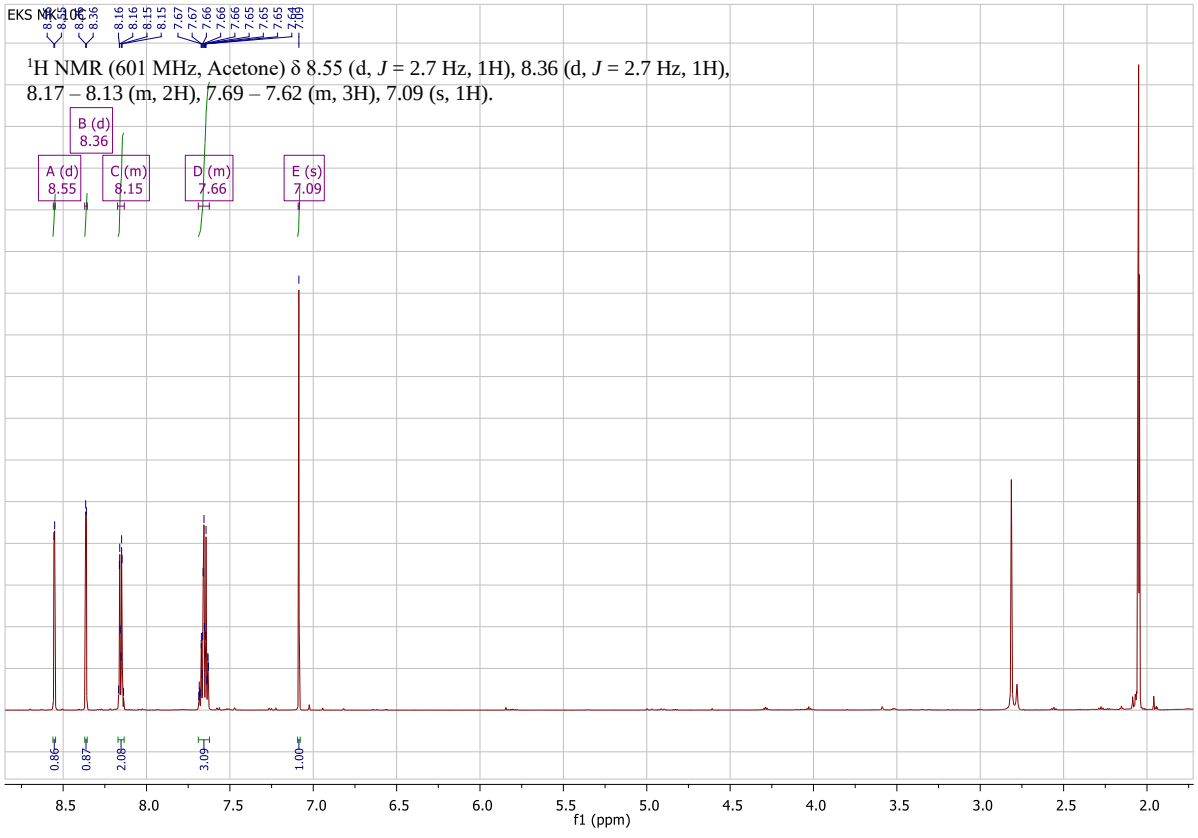



*Figure S29. ¹H NMR spectrum of 6-chloro-8-nitroflavone.*

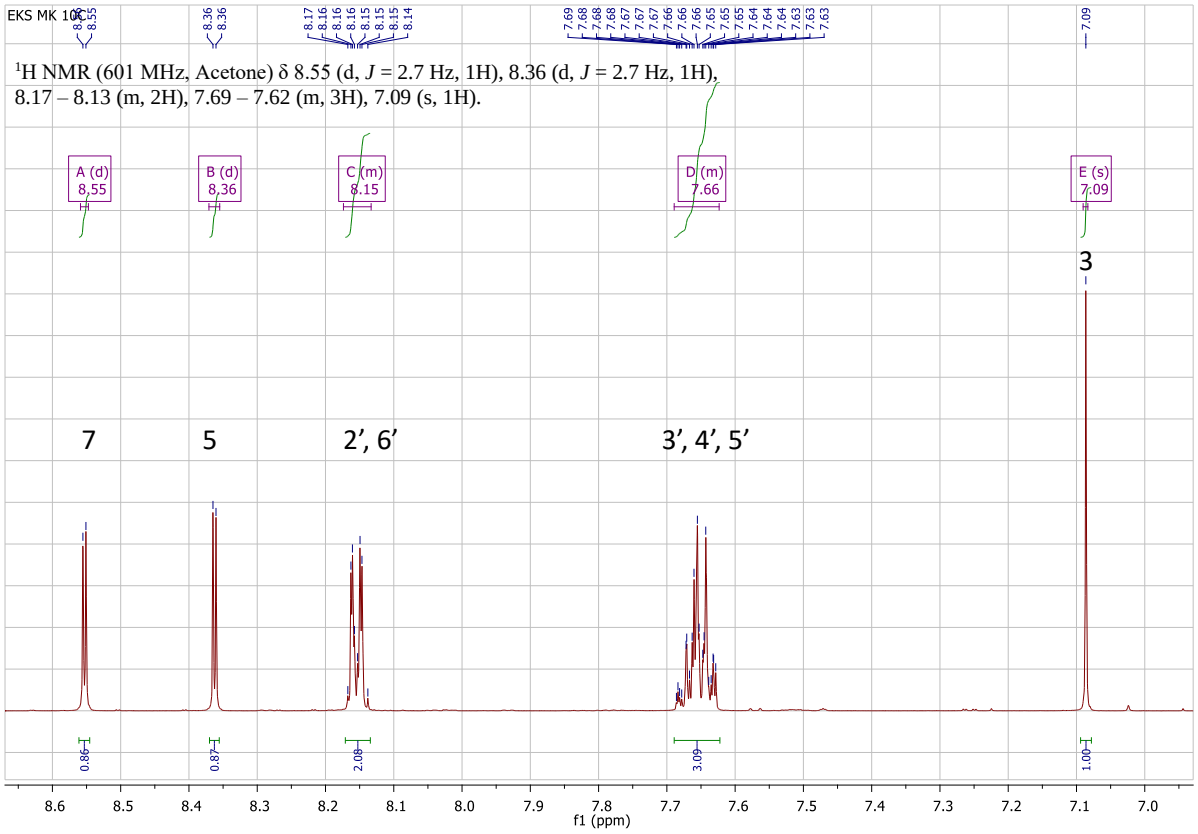



*Figure S30. $^1$H NMR spectrum expansion of 6-chloro-8-nitroflavone.*

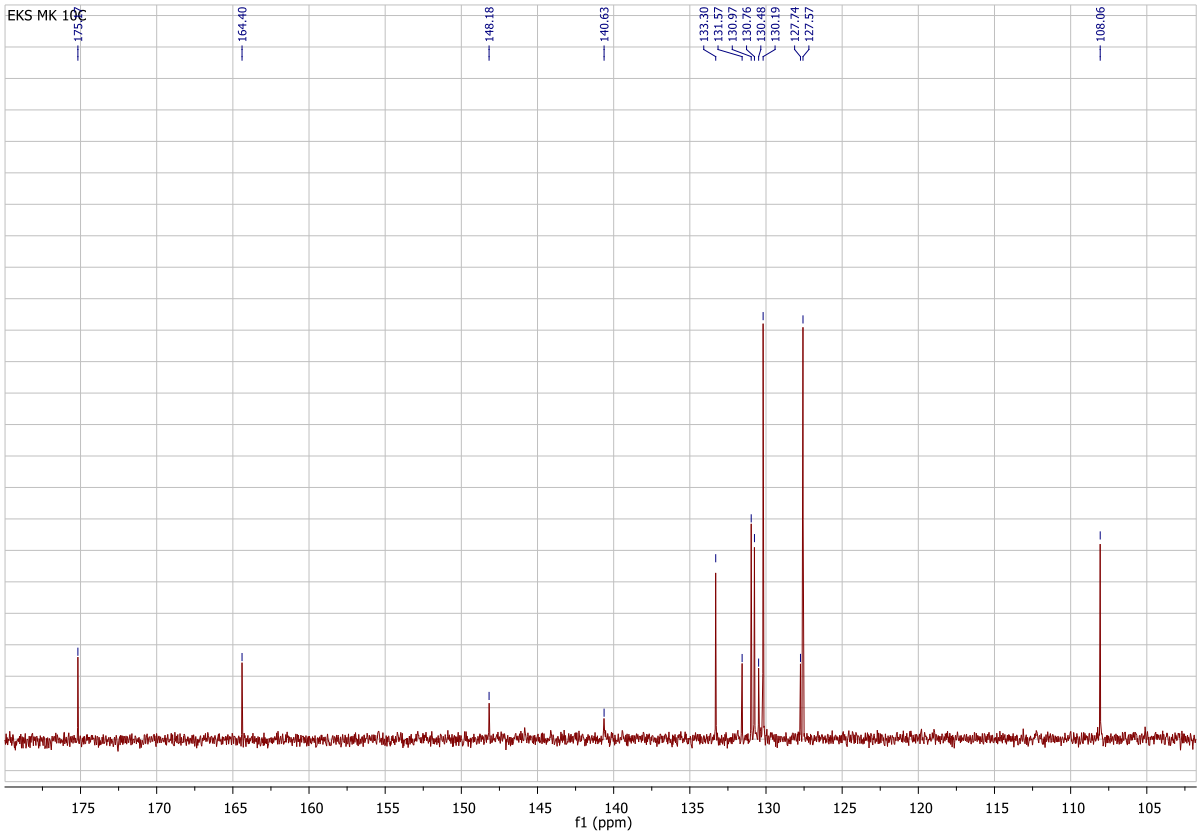



*Figure S31. $^{13}$C NMR spectrum of 6-chloro-8-nitroflavone.*

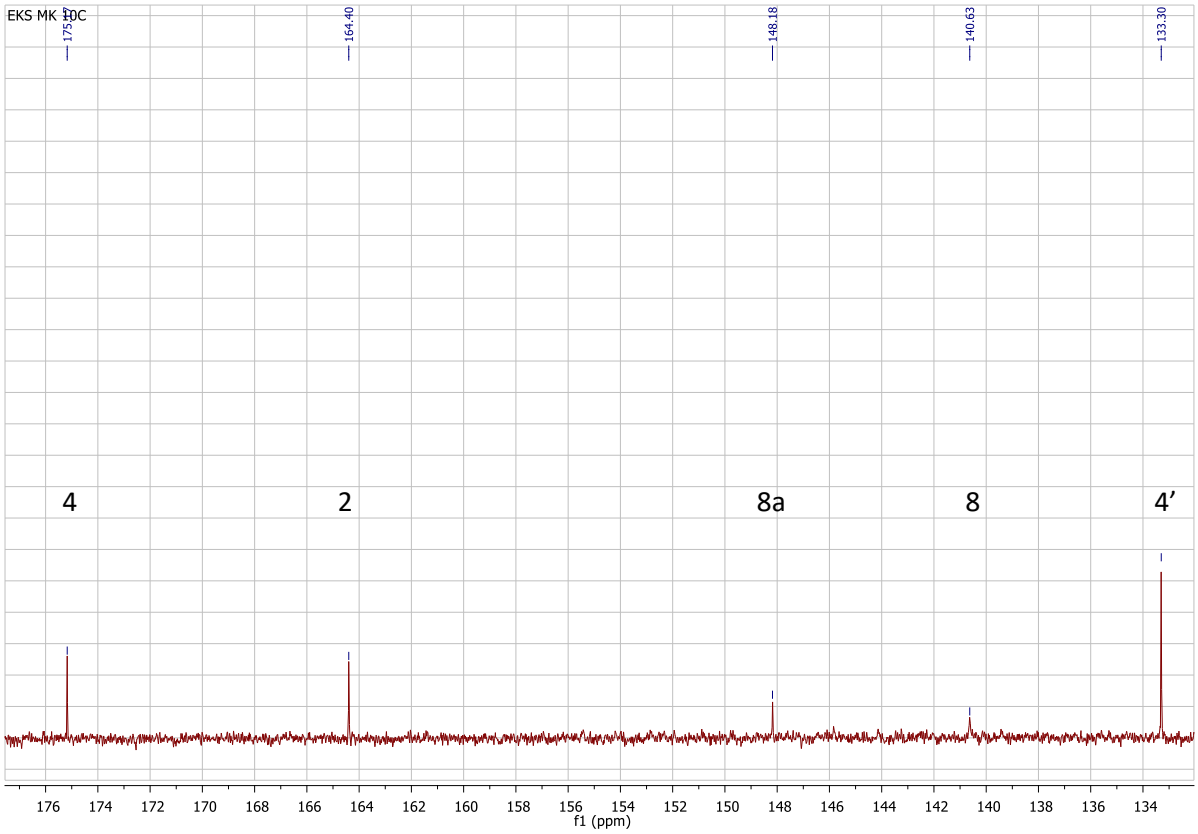



*Figure S32. [13]C NMR spectrum expansion of 6-chloro-8-nitroflavone.*

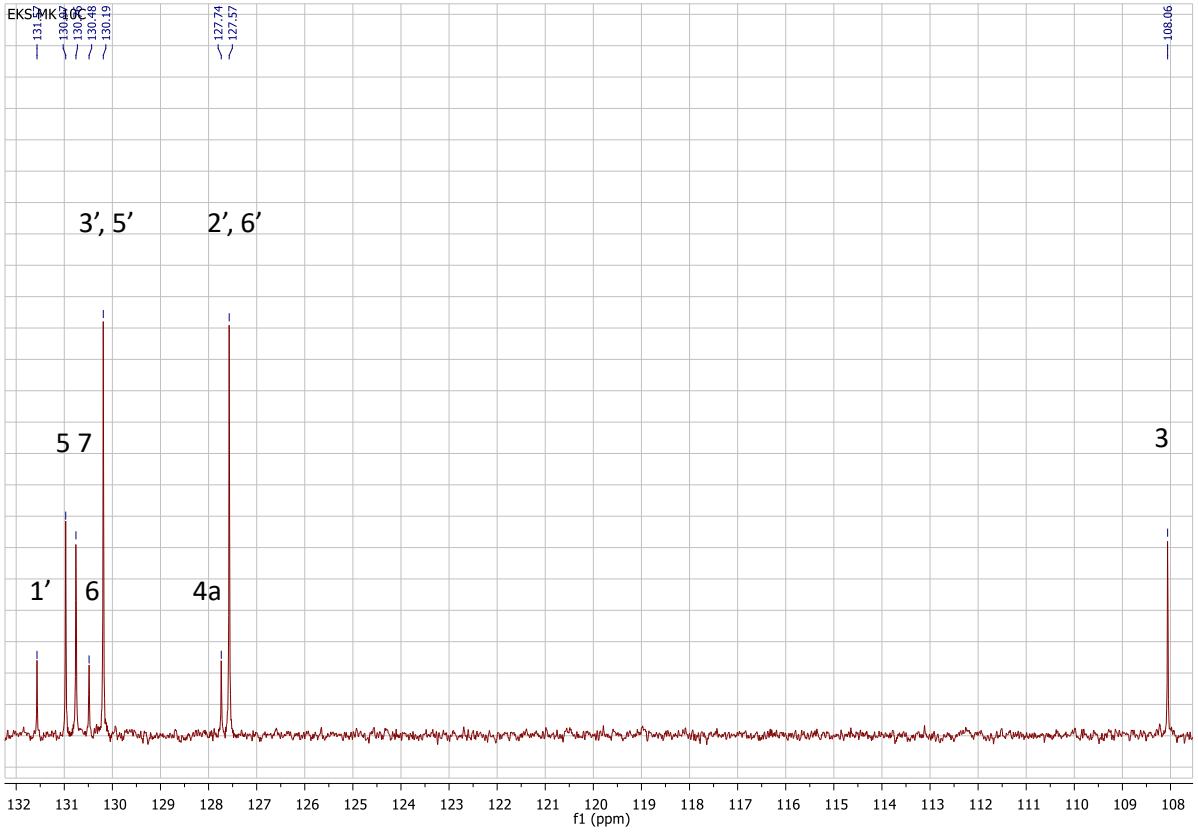



*Figure S33. [13]C NMR spectrum expansion of 6-chloro-8-nitroflavone.*

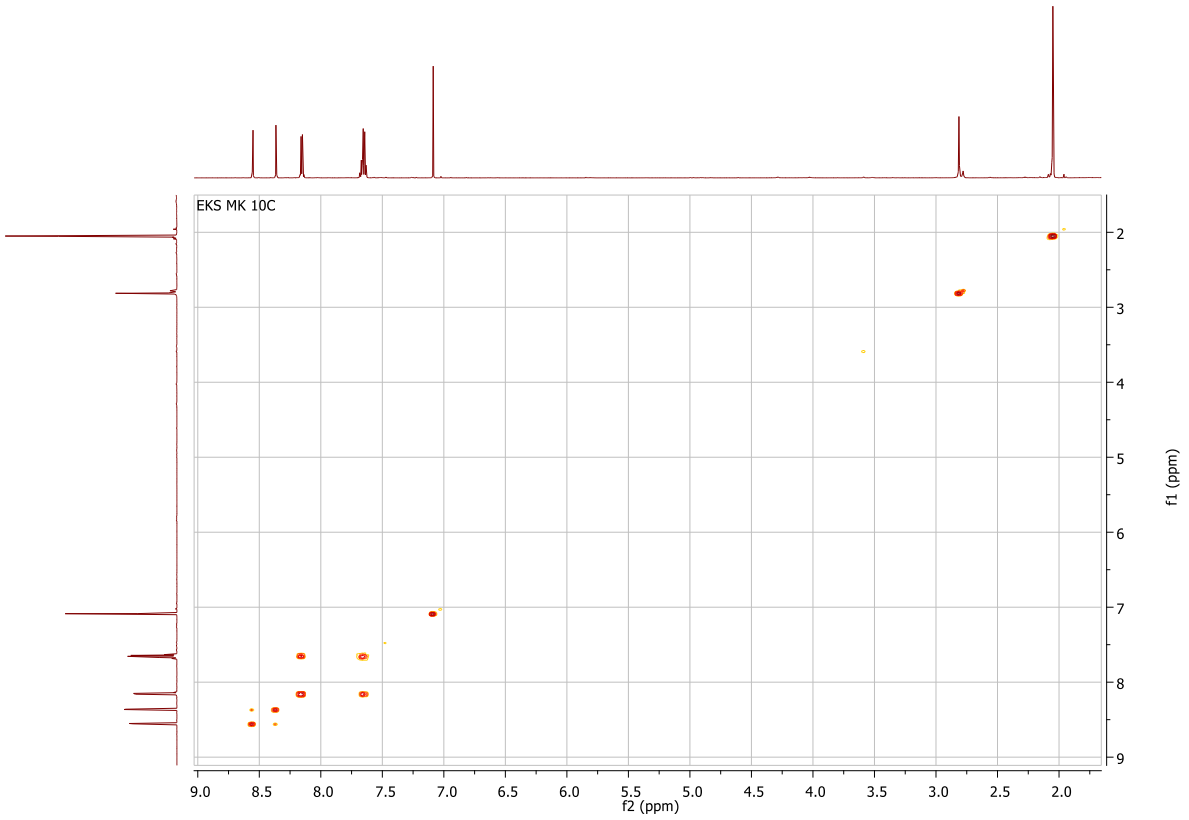



*Figure S34. COSY contour map – $^{1}H$ x $^{1}H$ of 6-chloro-8-nitroflavone.*

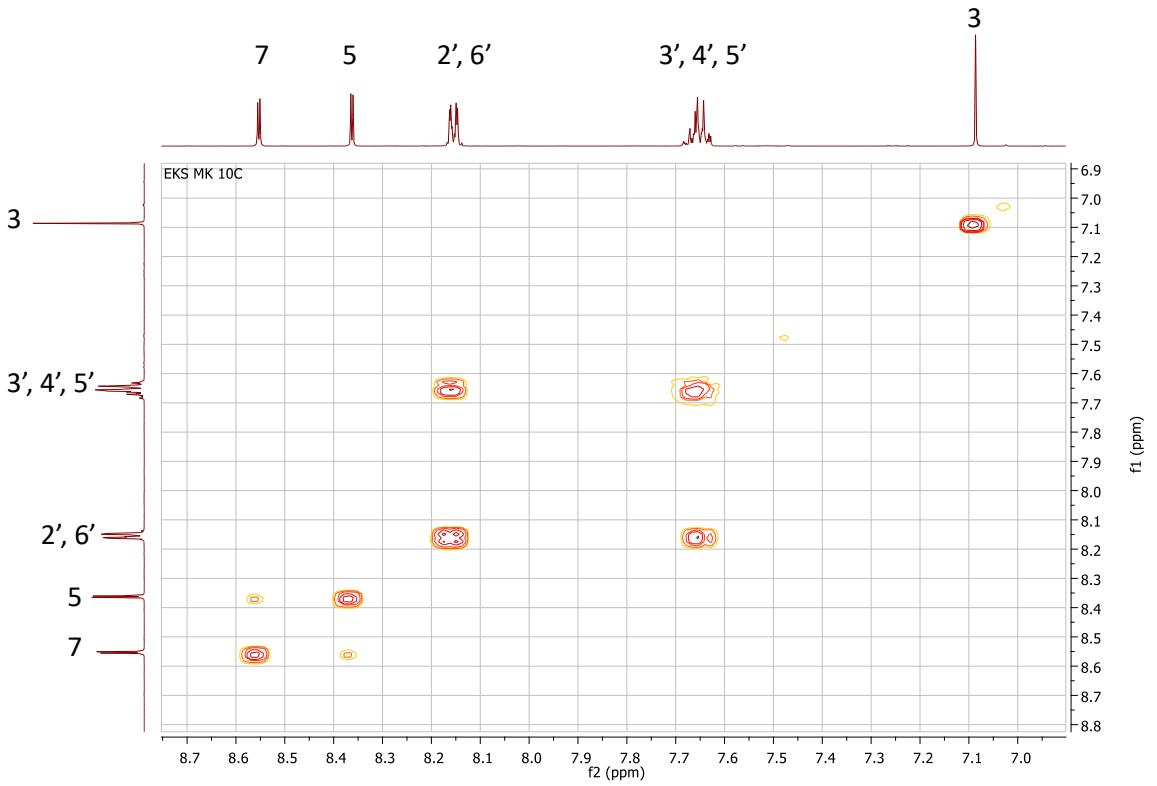



*Figure S35. COSY contour map – $^{1}H$ x $^{1}H$ expansion of 6-chloro-8-nitroflavone.*

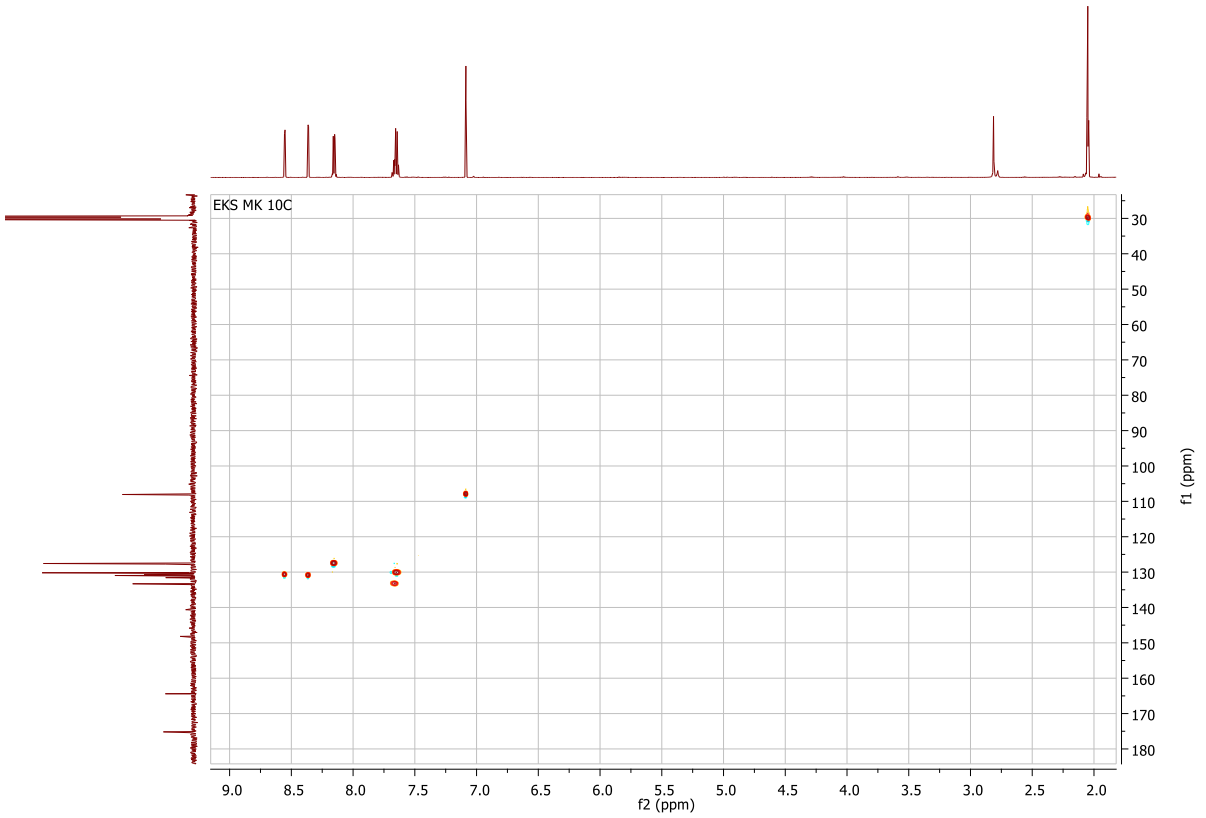



*Figure S36. HSQC contour map – $^{1}H$ x $^{13}C$ of 6-chloro-8-nitroflavone.*

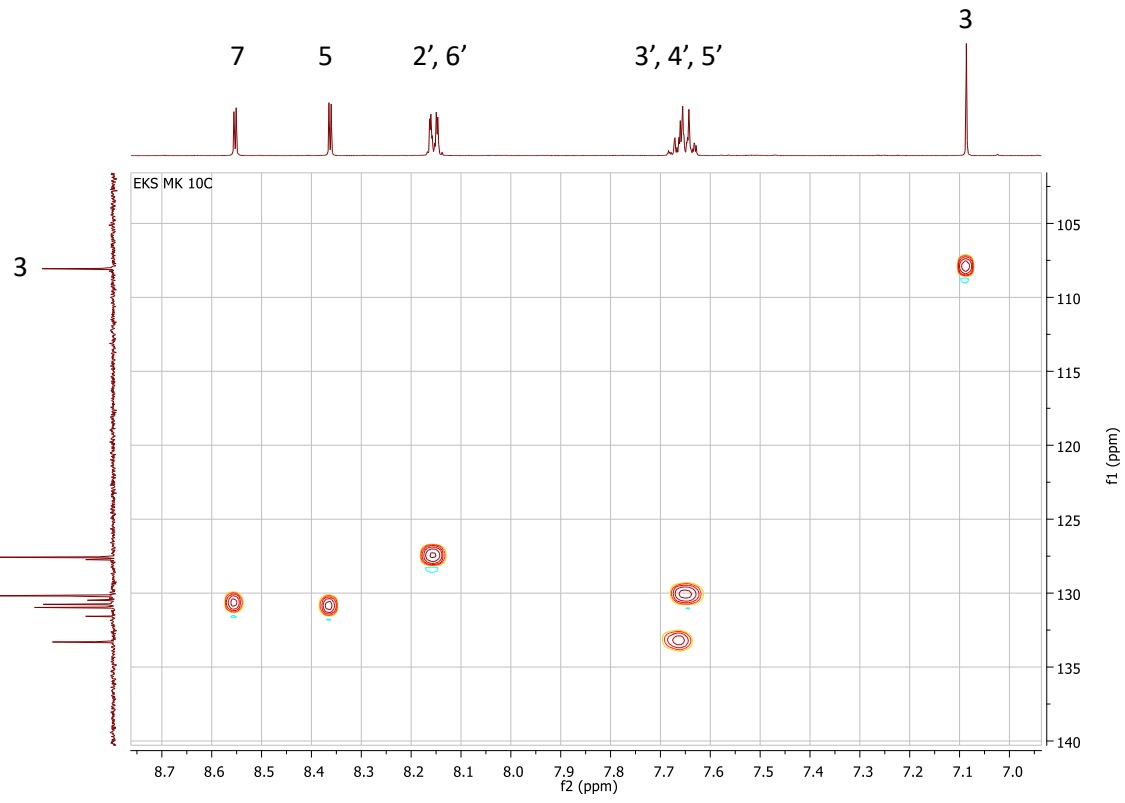



*Figure S37. HSQC contour map – $^{1}H$ x $^{13}C$ expansion of 6-chloro-8-nitroflavone.*

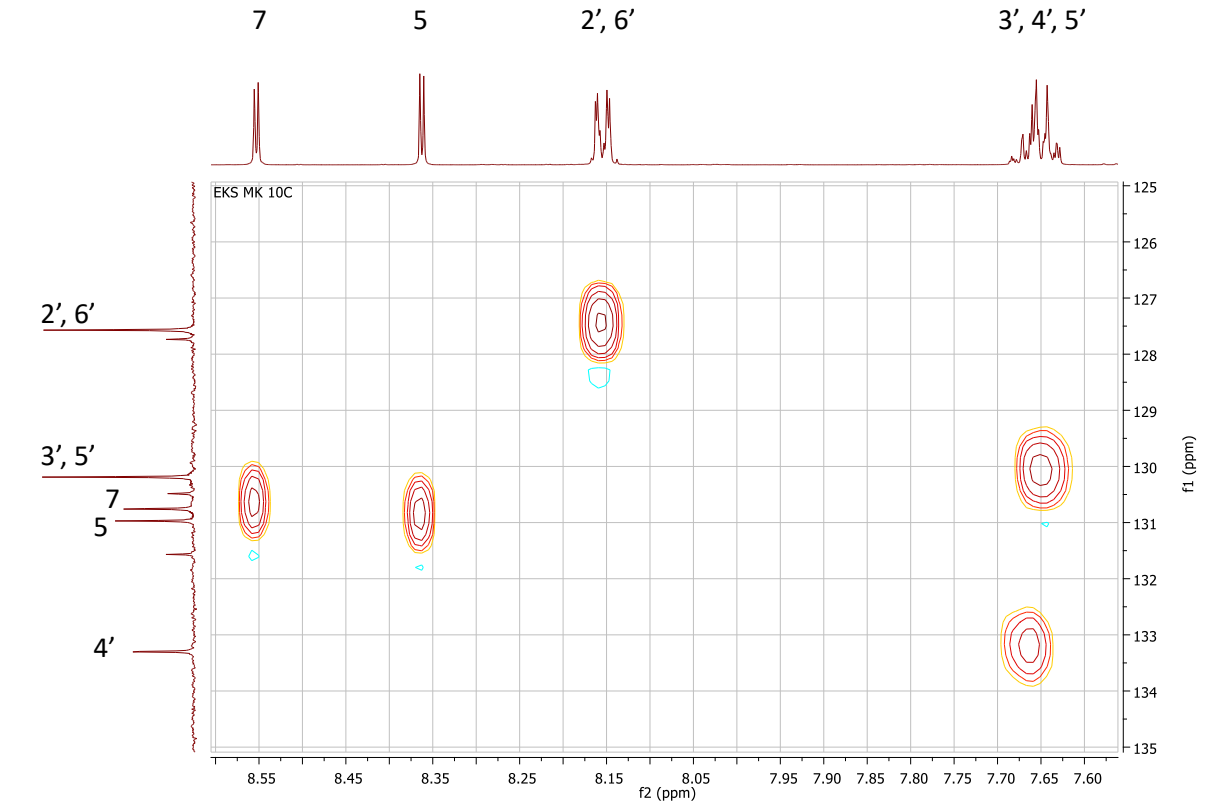



*Figure S38. HSQC contour map – $^{1}H$ x $^{13}C$ expansion of 6-chloro-8-nitroflavone.*

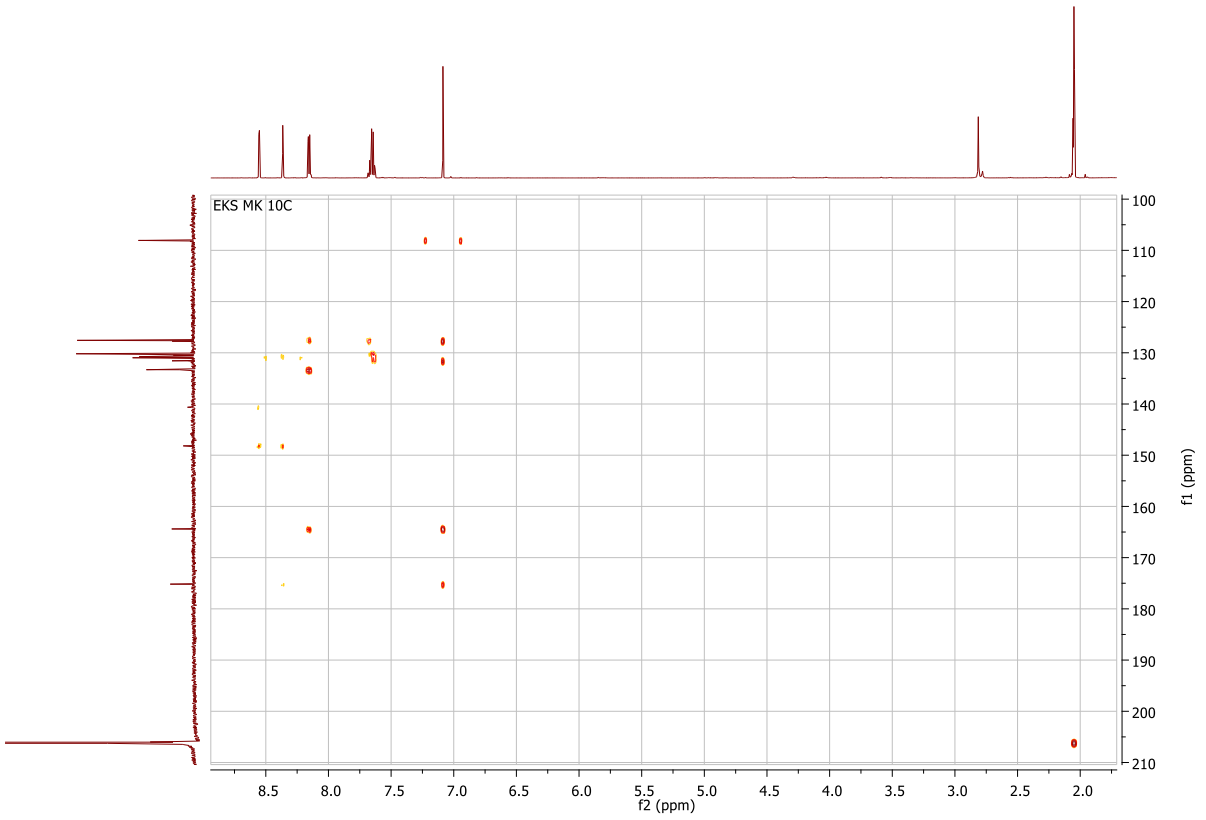



*Figure S39. HMBC contour map – $^{1}H$ x $^{13}C$ of 6-chloro-8-nitroflavone.*

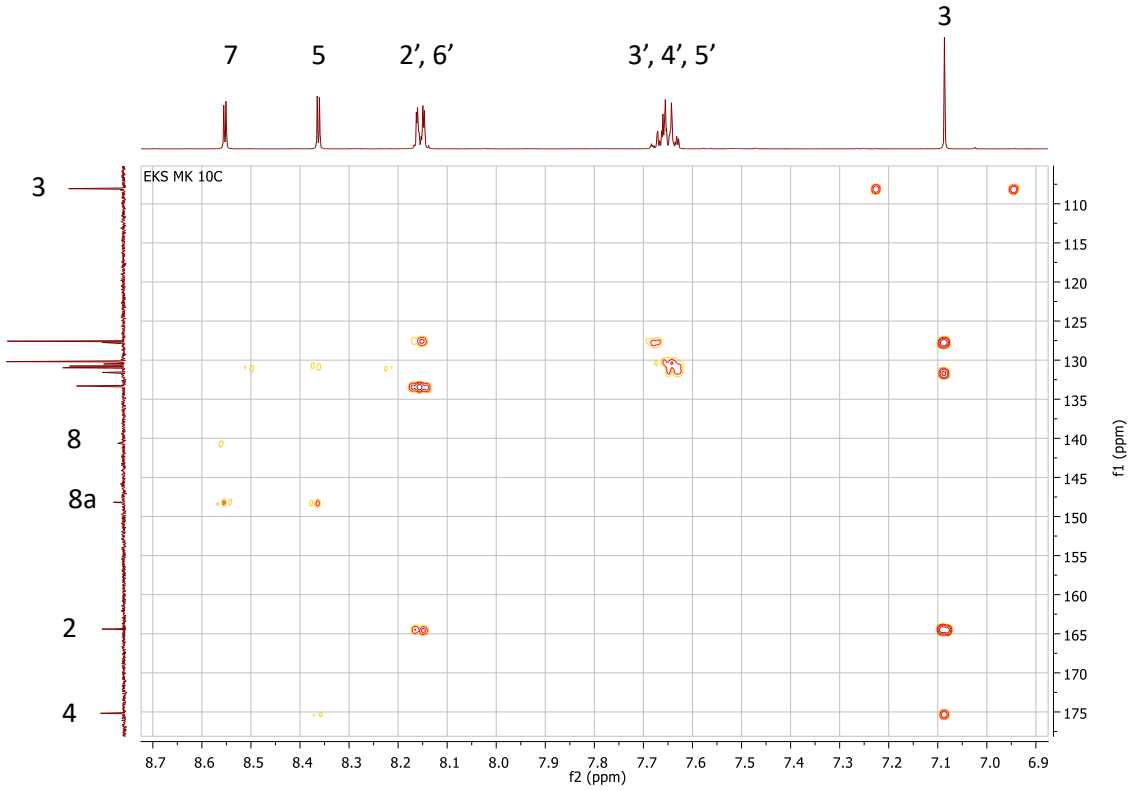



*Figure S40. HMBC contour map – $^{1}H$ x $^{13}C$ expansion of 6-chloro-8-nitroflavone.*

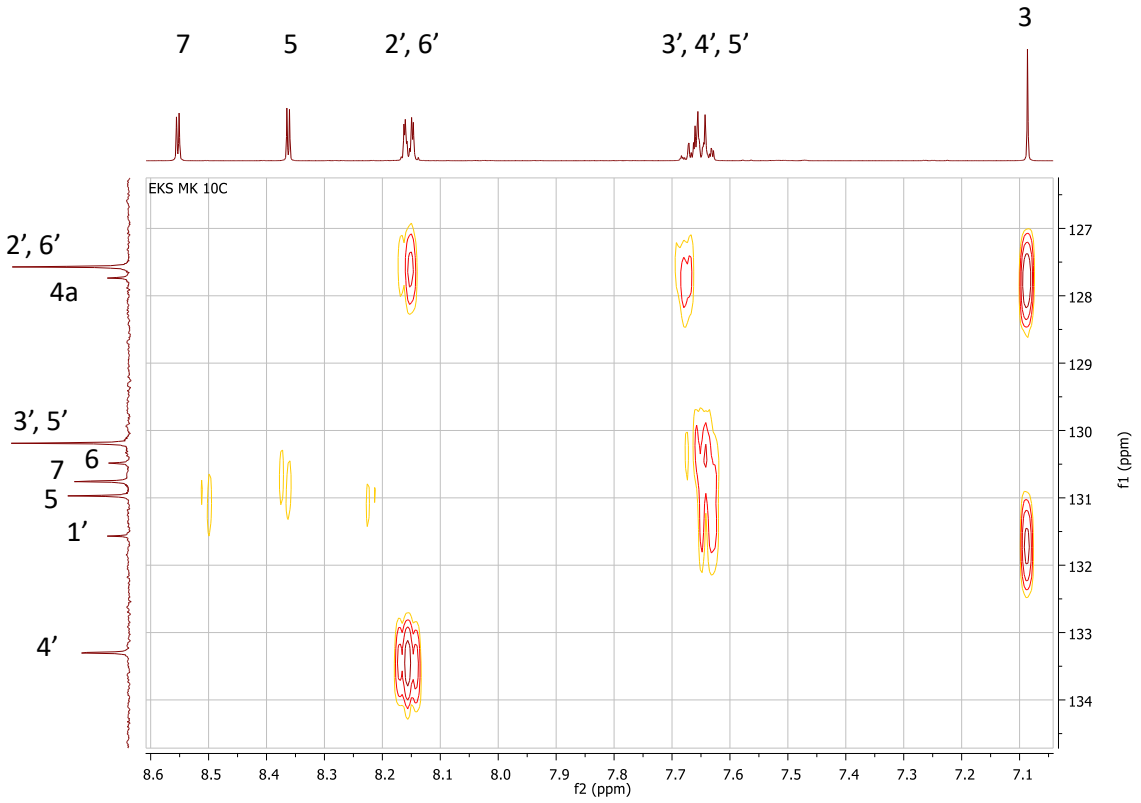
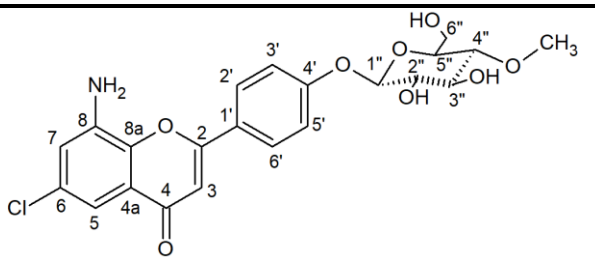



*Figure S41. HMBC contour map – $^{1}H$ x $^{13}C$ expansion of 6-chloro-8-nitroflavone.*

Compound name: 8-amino-6-chloroflavone 4′-*O*-*β*-D-(4″-*O*-methyl)-glucopyranoside
Molecular Formula: $C_{22}H_{22}ClNO_8$
Formula Weight: 463.865
Ionization mode: positive
Precursor $[M + H]^+$: 464.103
Monoisotopic Mass: 463.103
Collision energy (CE): -15.0



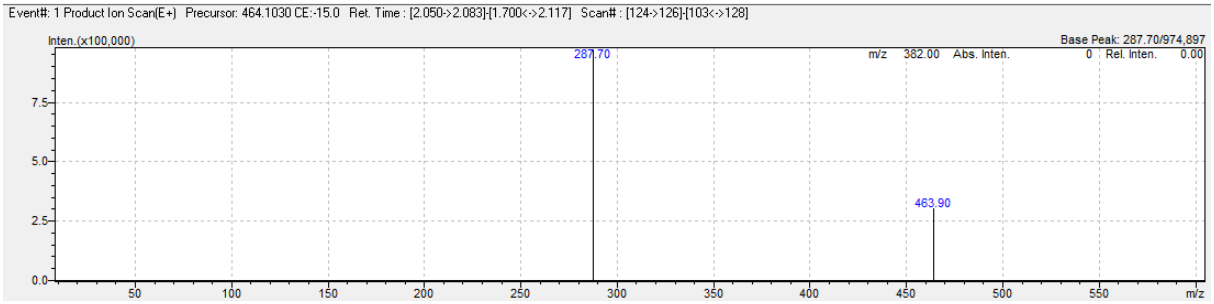




*Figure S42. LC-MS spectrum of 8-amino-6-chloroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

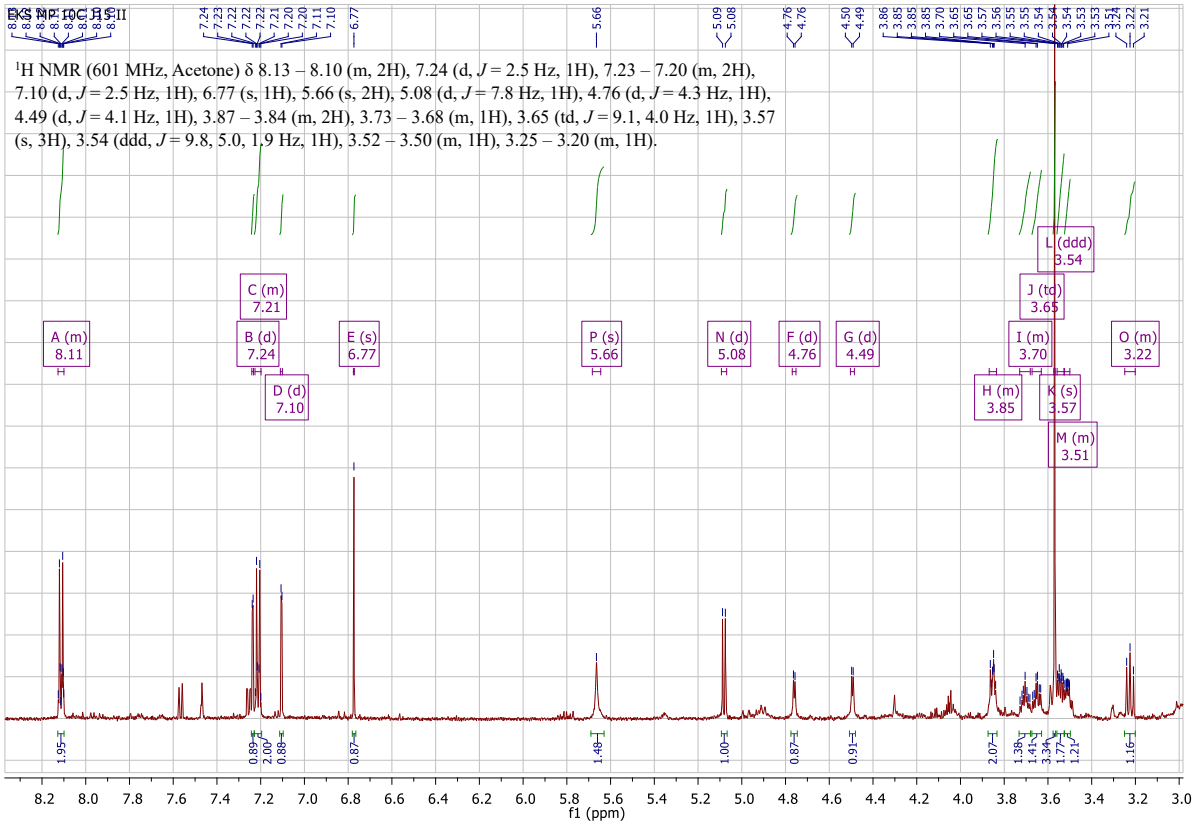



*Figure S43. ¹H NMR spectrum of 8-amino-6-chloroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

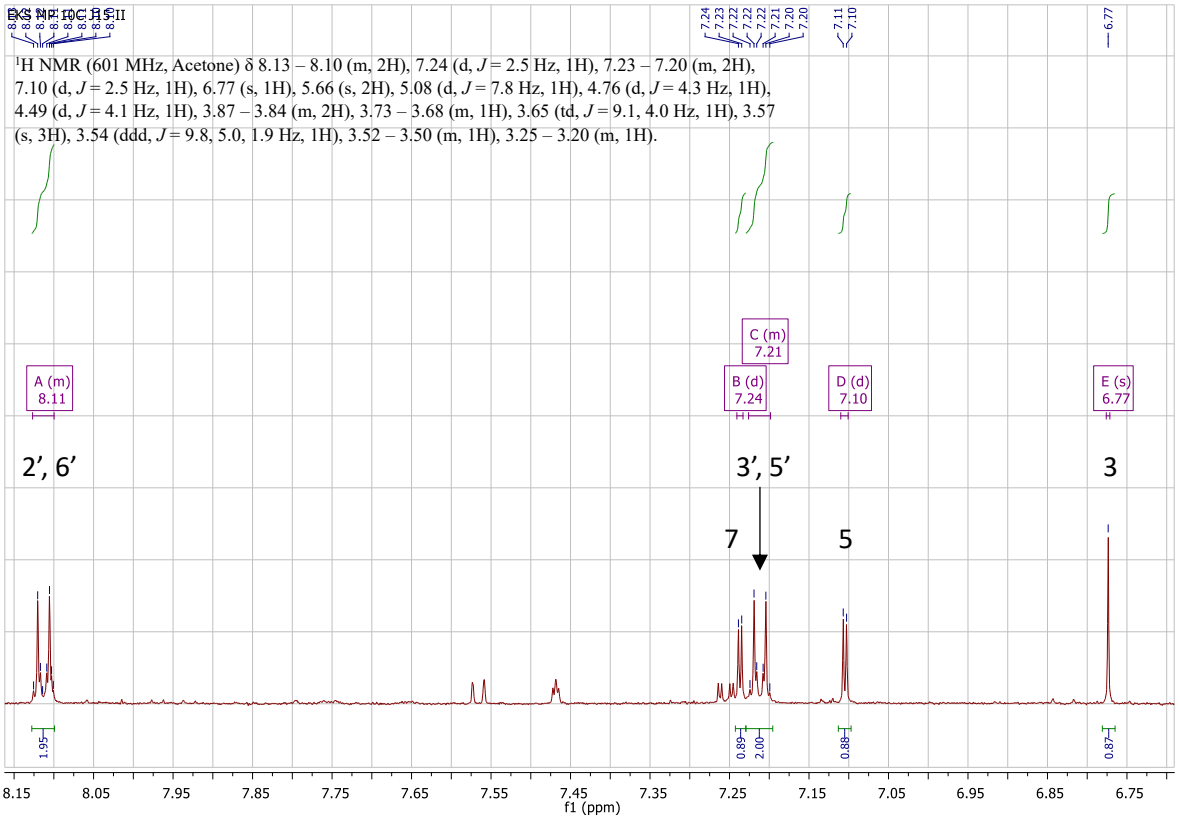



*Figure S44. ¹H NMR spectrum expansion of 8-amino-6-chloroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

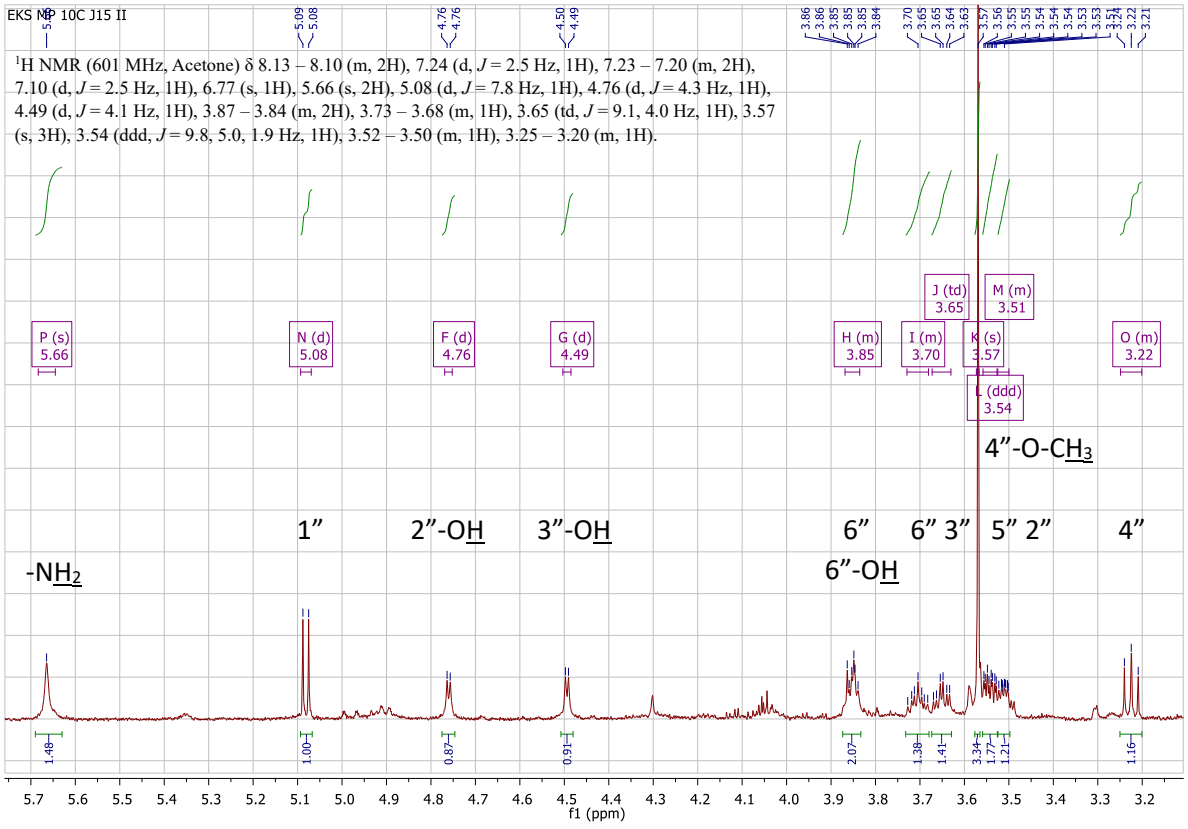



*Figure S45. ¹H NMR spectrum expansion of 8-amino-6-chloroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*




*Figure S46. [13]C NMR spectrum of 8-amino-6-chloroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

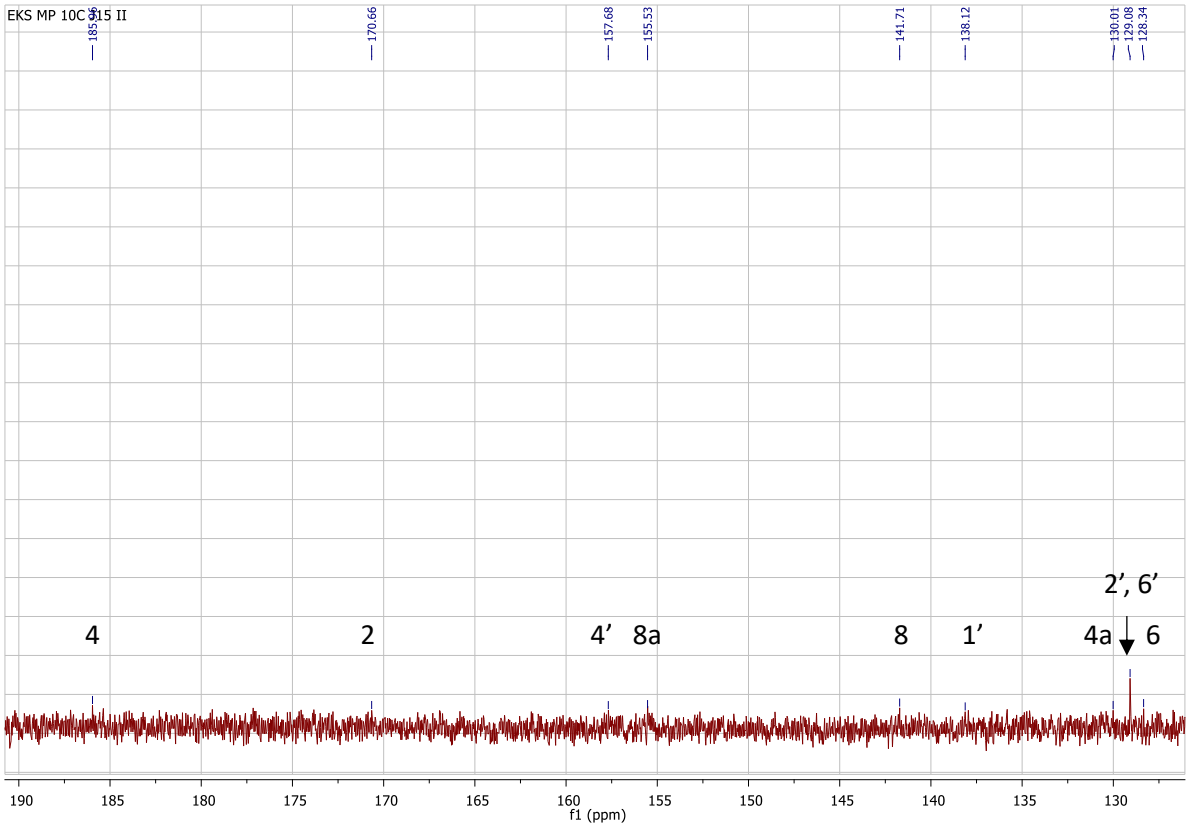



*Figure S47. [13]C NMR spectrum expansion of 8-amino-6-chloroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

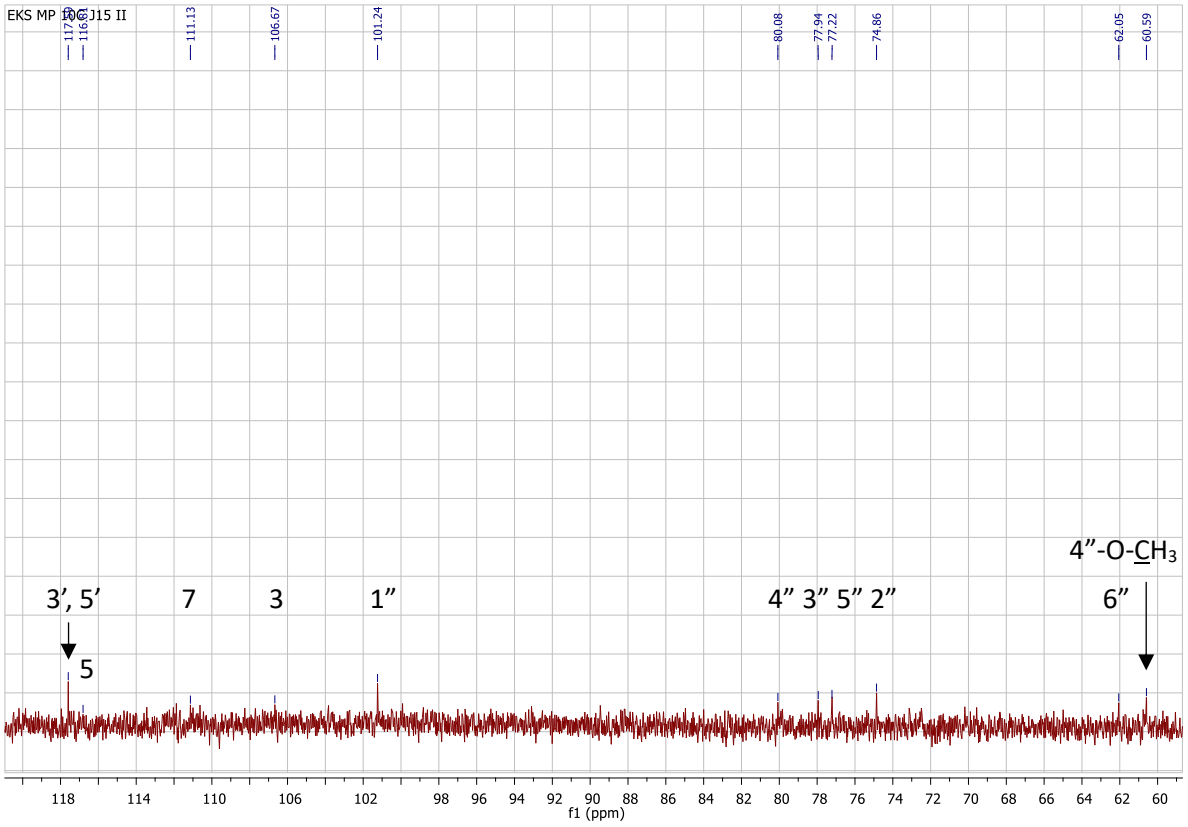



*Figure S48. $^{13}C$ NMR spectrum expansion of 8-amino-6-chloroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

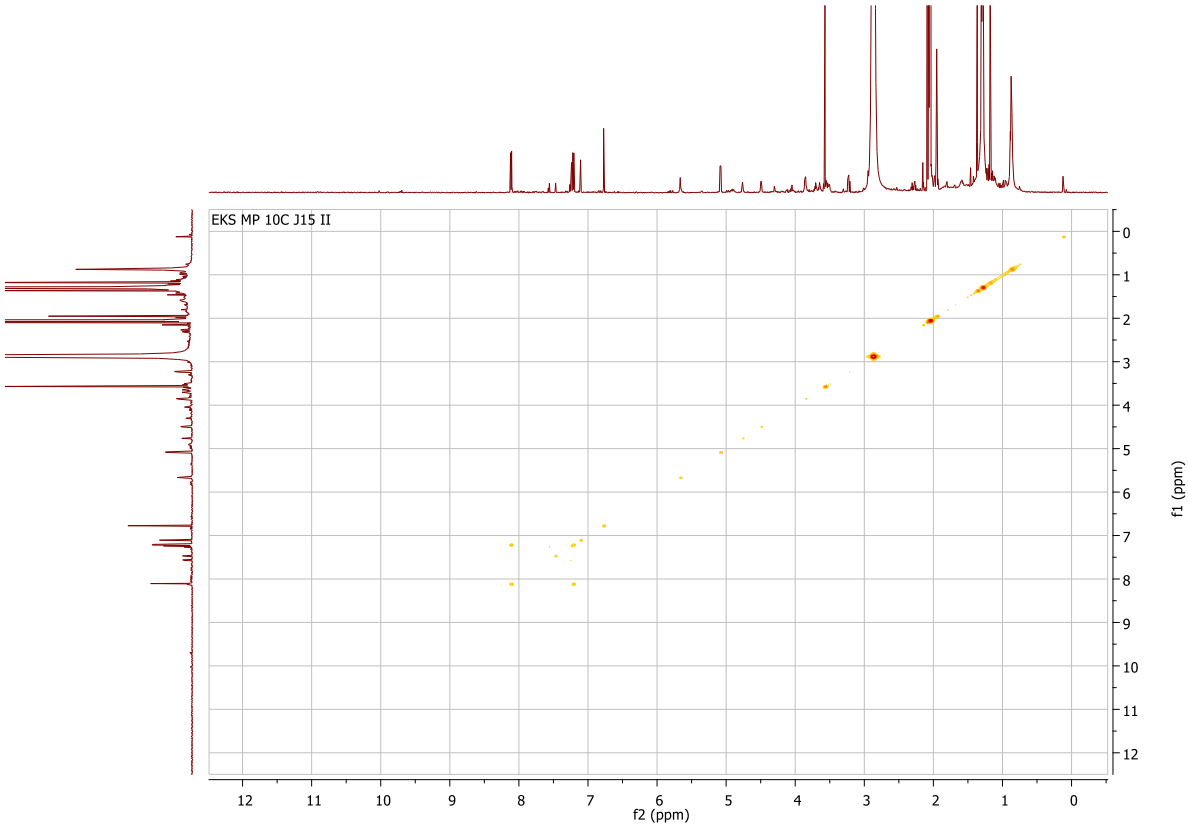



*Figure S49. COSY contour map – $^{1}H$ x $^{1}H$ of 8-amino-6-chloroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

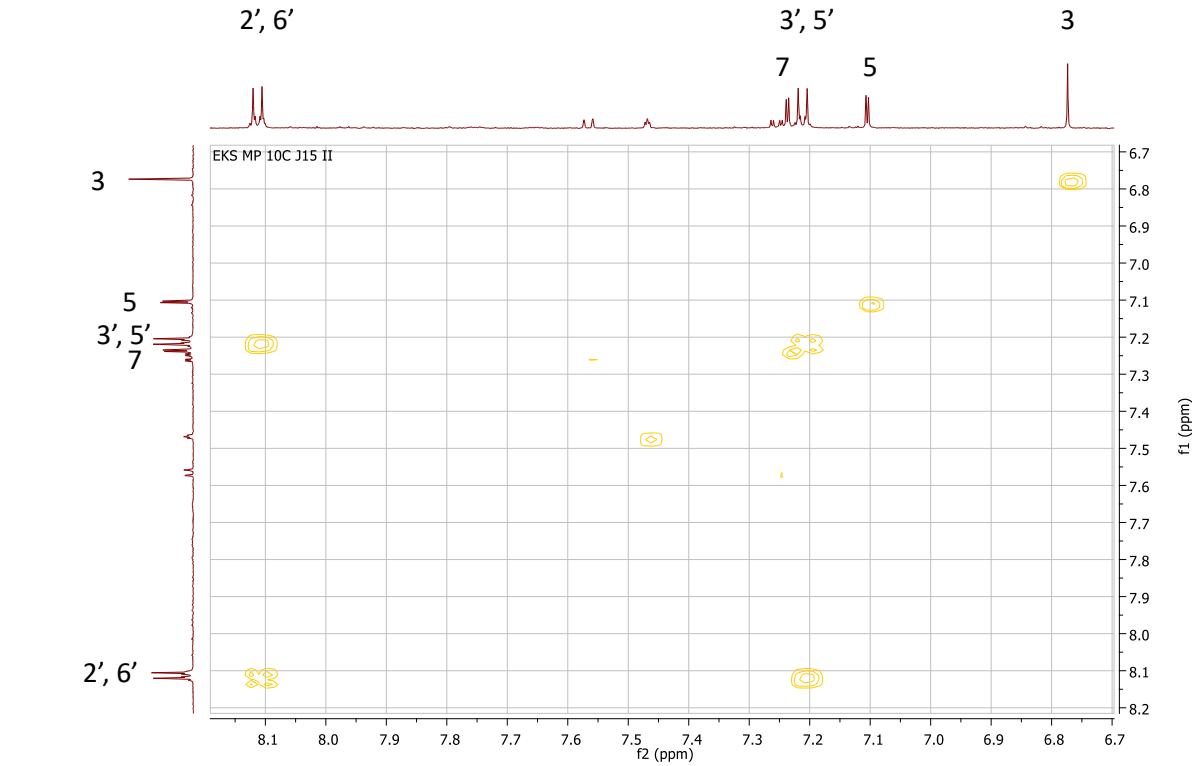



*Figure S50. COSY contour map – $^1H$ x $^1H$ expansion of 8-amino-6-chloroflavone 4'-O-β-D-(4"-O-methyl)-glucopyranoside.*

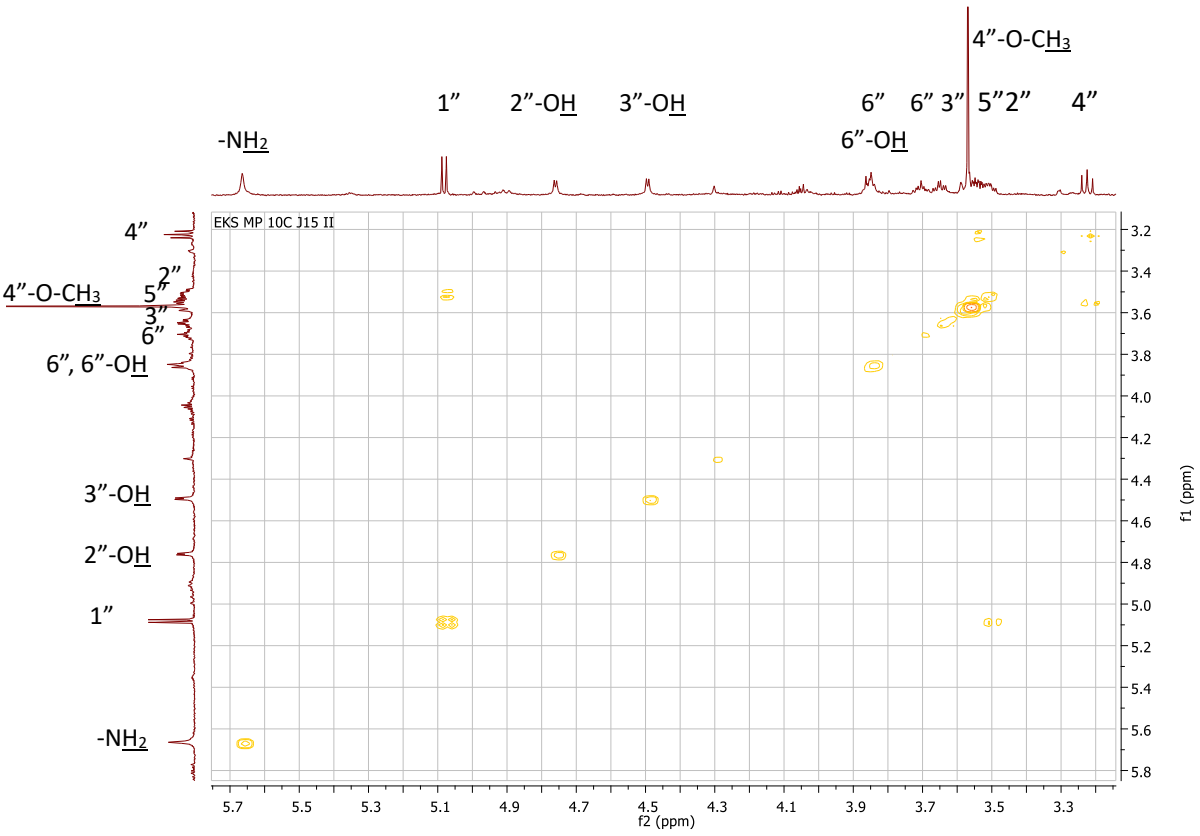



*Figure S51. COSY contour map – $^1H$ x $^1H$ expansion of 8-amino-6-chloroflavone 4'-O-β-D-(4"-O-methyl)-glucopyranoside.*

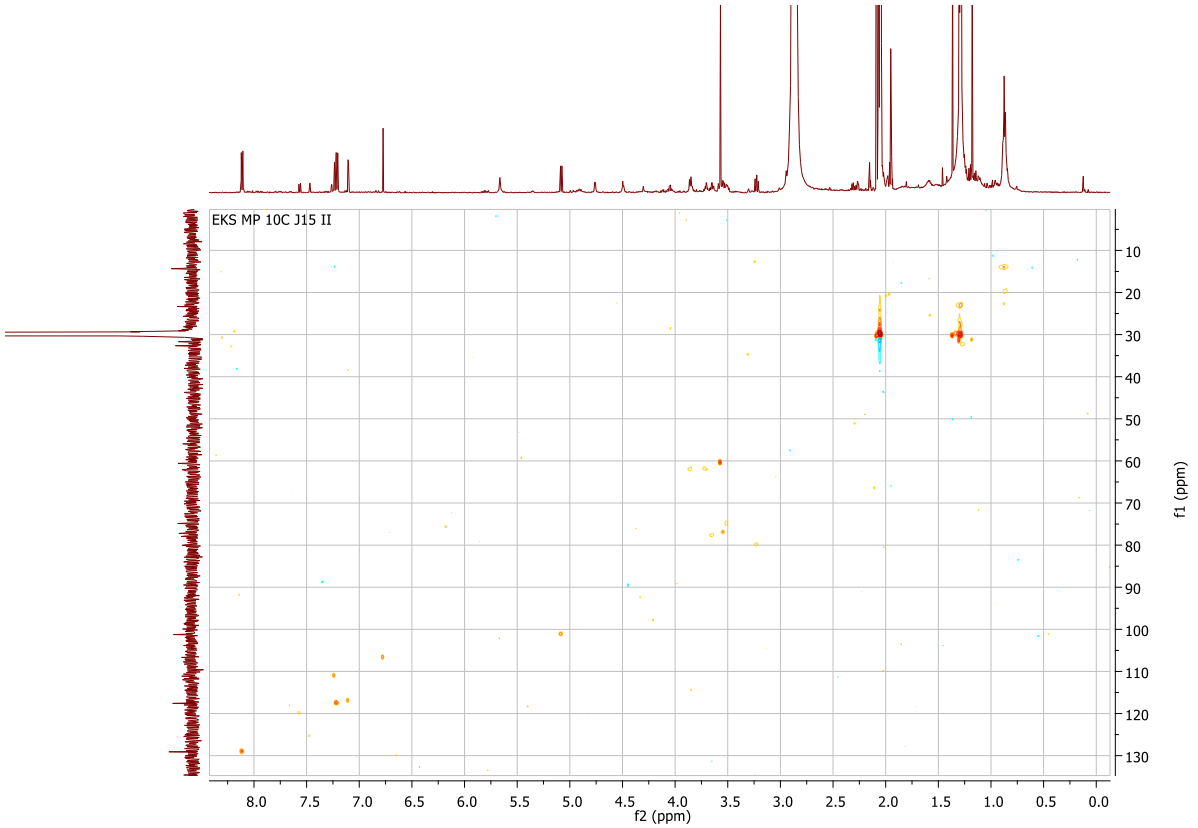



*Figure S52. HSQC contour map – $^{1}H$ x $^{13}C$ of 8-amino-6-chloroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

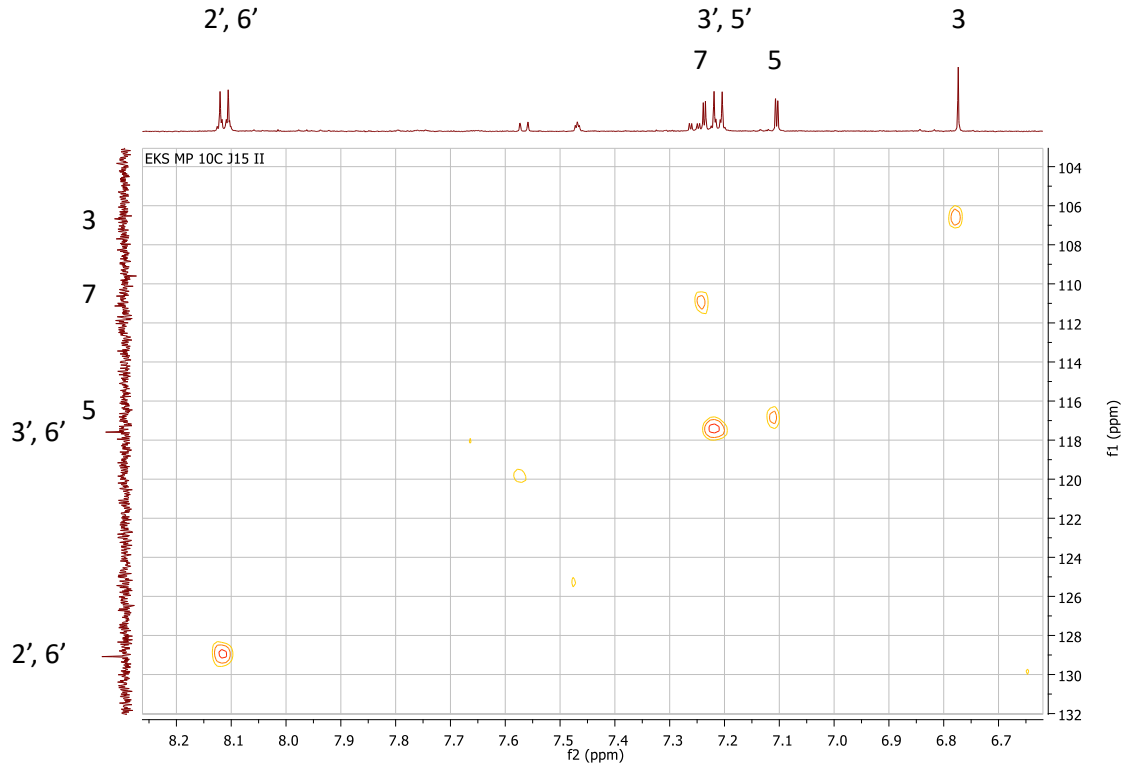



*Figure S53. HSQC contour map – $^{1}H$ x $^{13}C$ expansion of 8-amino-6-chloroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

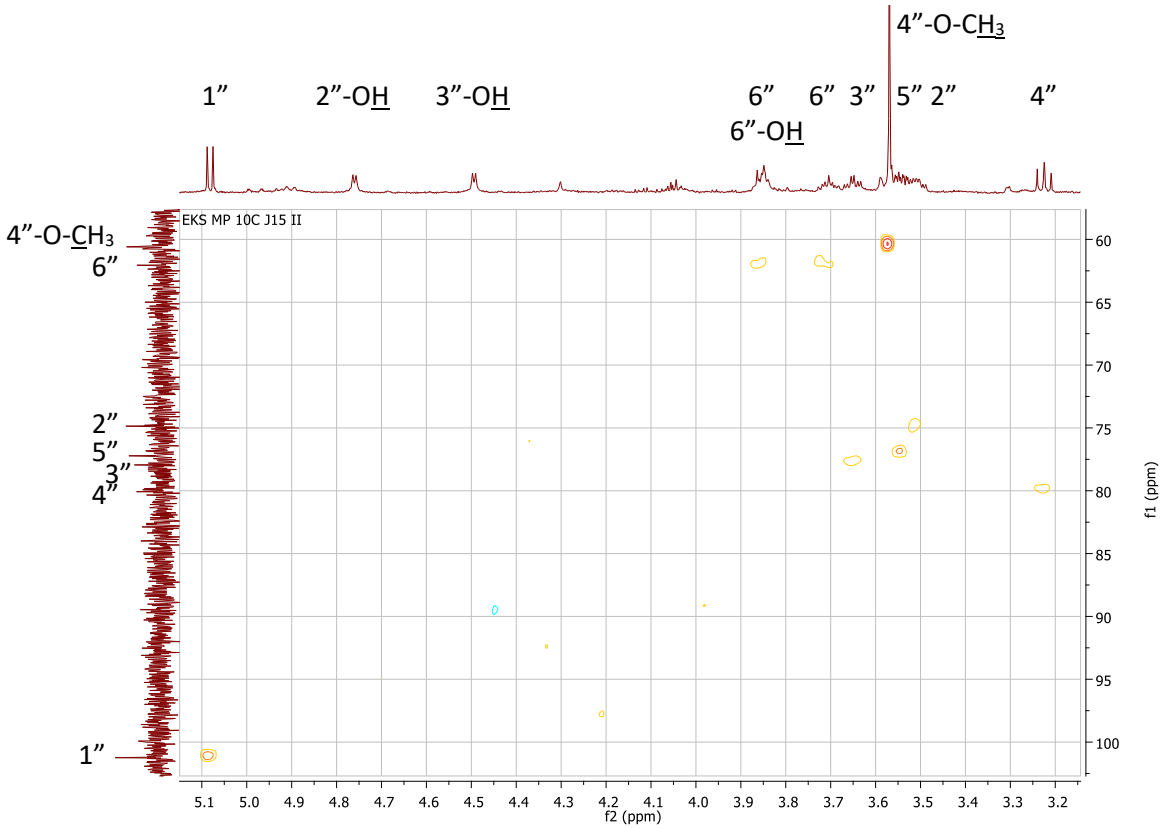



*Figure S54. HSQC contour map – $^{1}H$ x $^{13}C$ expansion of 8-amino-6-chloroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

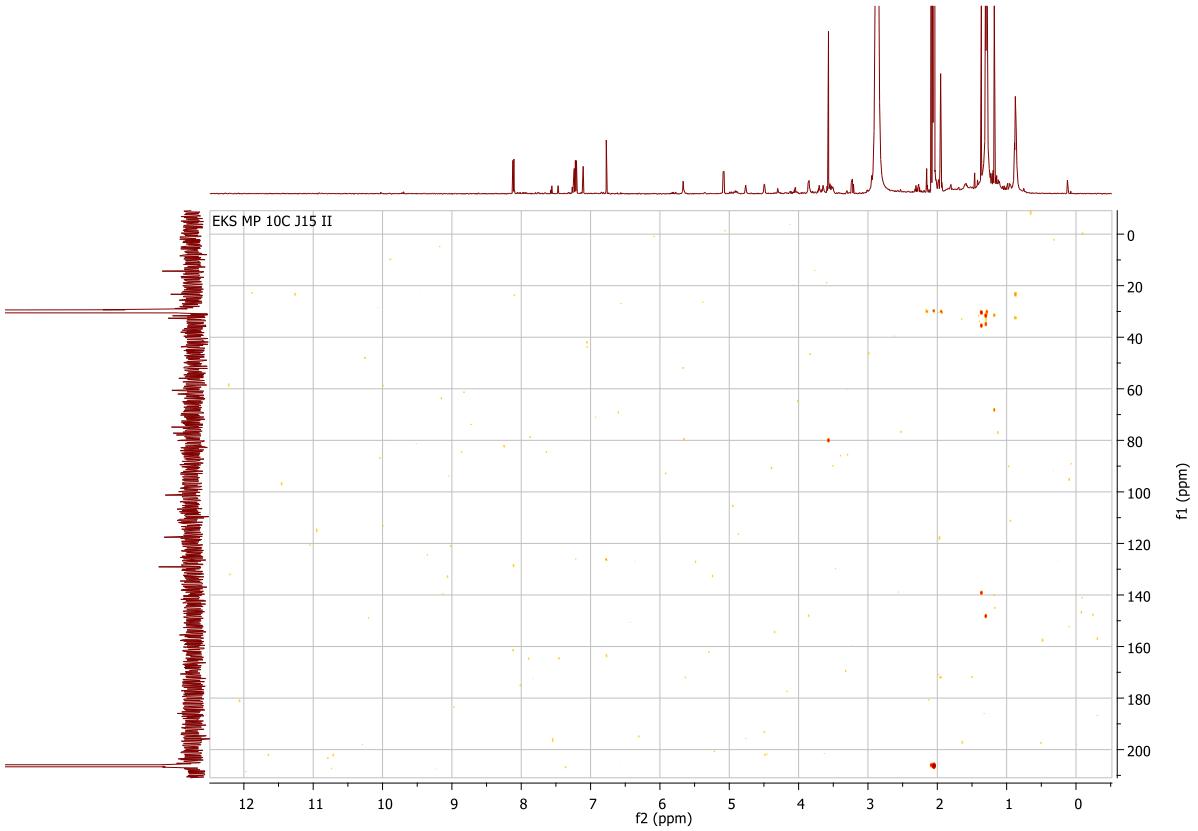
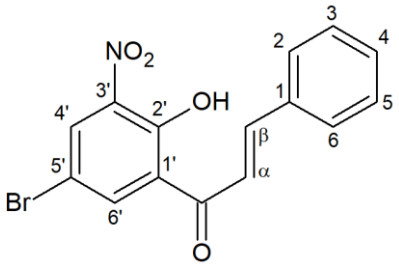



*Figure S55. HMBC contour map – $^{1}H$ x $^{13}C$ of 8-amino-6-chloroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

Compound name: 5′-bromo-2′-hydroxy-3′-nitrochalcone
Molecular Formula: $C_{15}H_{10}BrNO_4$
Formula Weight: 348.148
Ionization mode: positive
Precursor $[M + H]^+$: 347.979
Monoisotopic Mass: 346.979
Collision energy (CE): -15.0



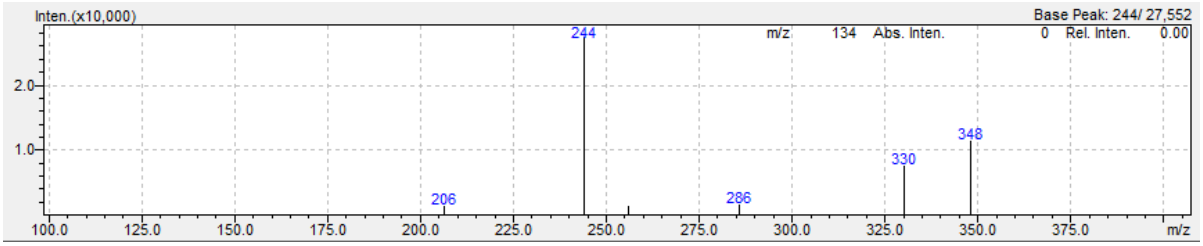




*Figure S56. LC-MS spectrum of 5′-bromo-2′-hydroxy-3′-nitrochalcone.*

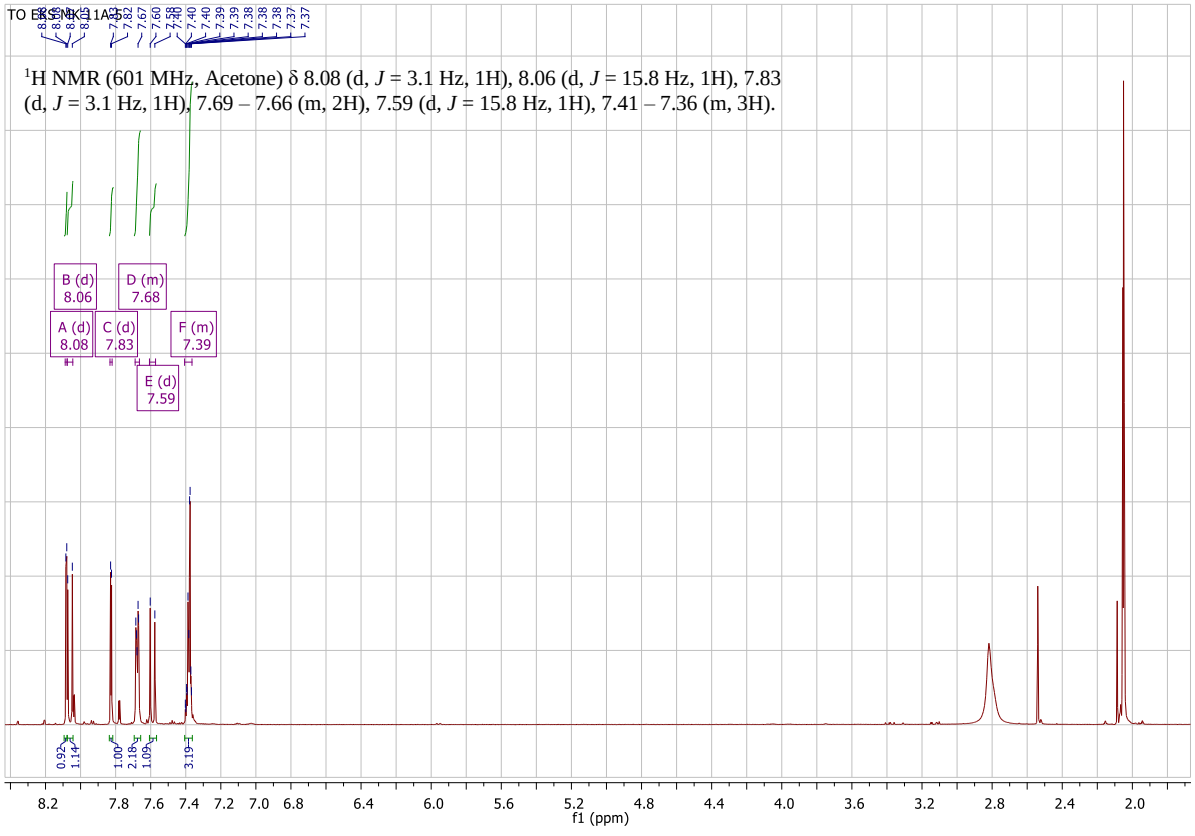



*Figure S57. $^1$H NMR spectrum of 5′-bromo-2′-hydroxy-3′-nitrochalcone.*

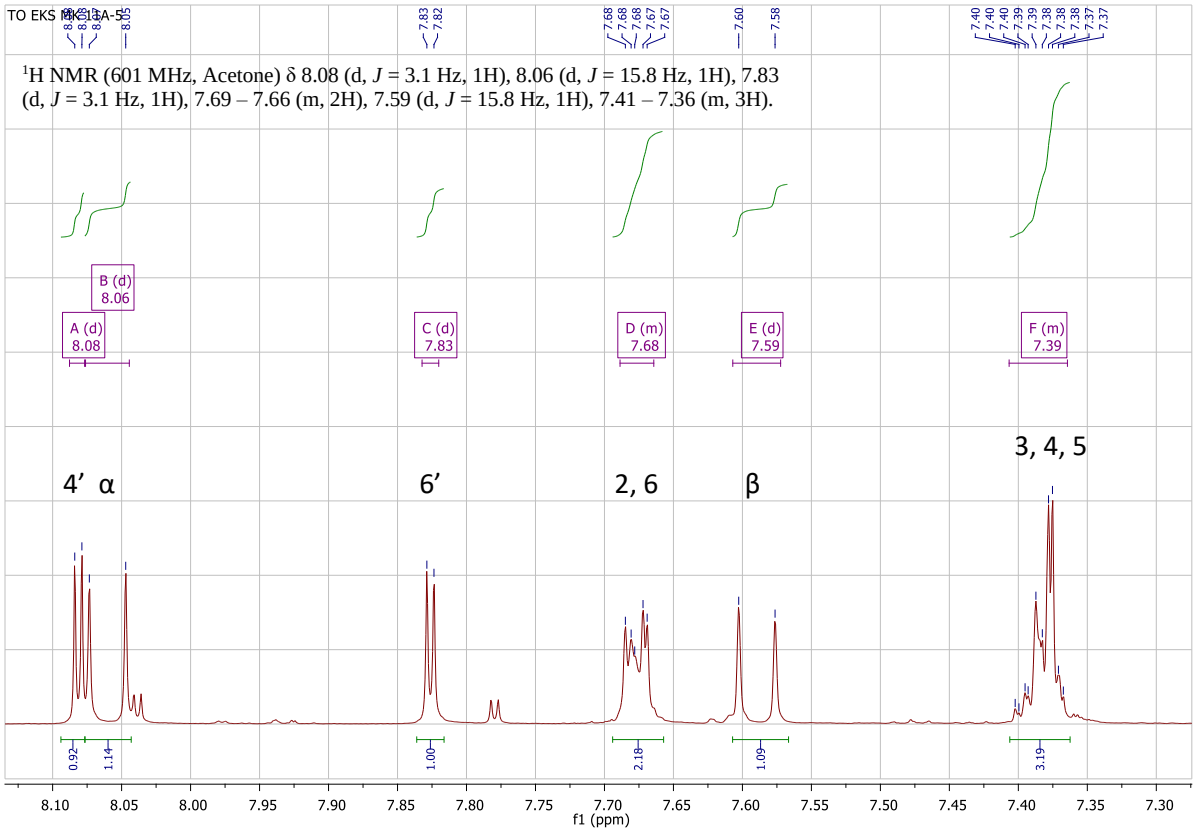



*Figure S58. $^1$H NMR spectrum expansion of 5′-bromo-2′-hydroxy-3′-nitrochalcone.*

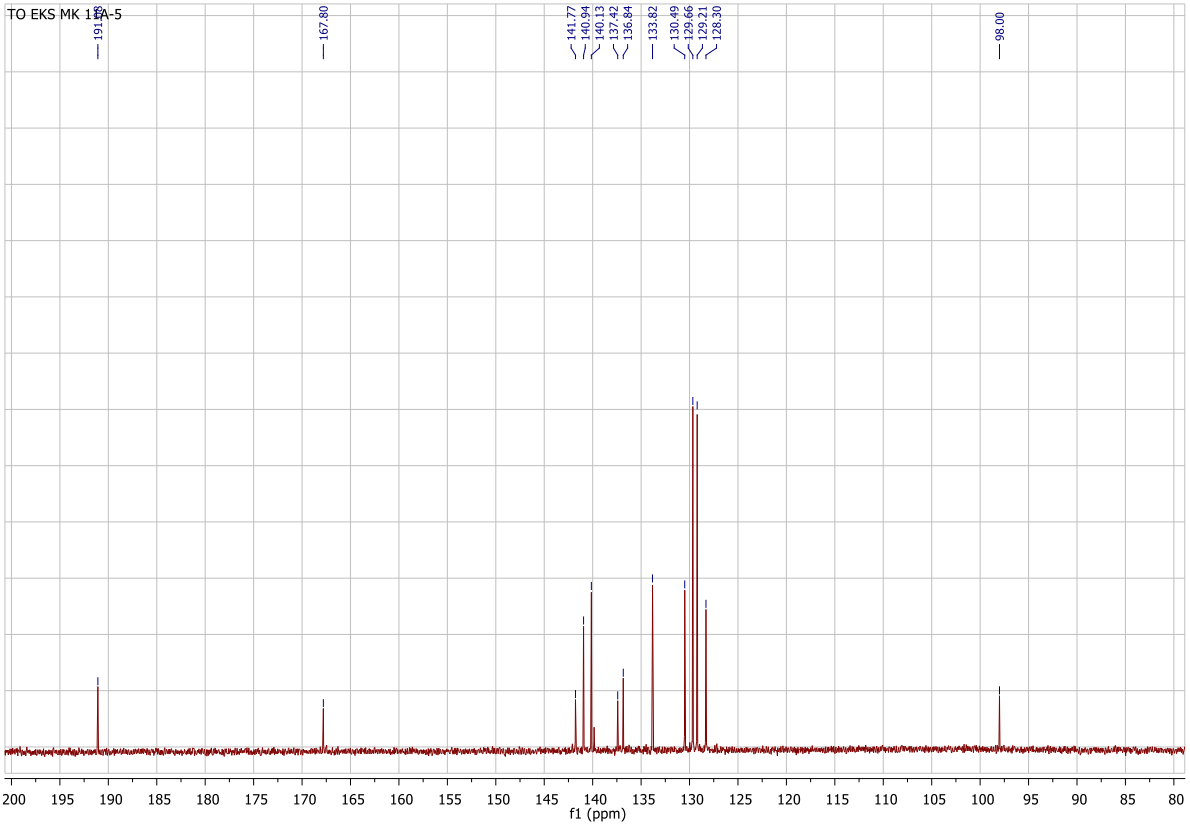



*Figure S59. $^{13}$C NMR spectrum of 5′-bromo-2′-hydroxy-3′-nitrochalcone.*

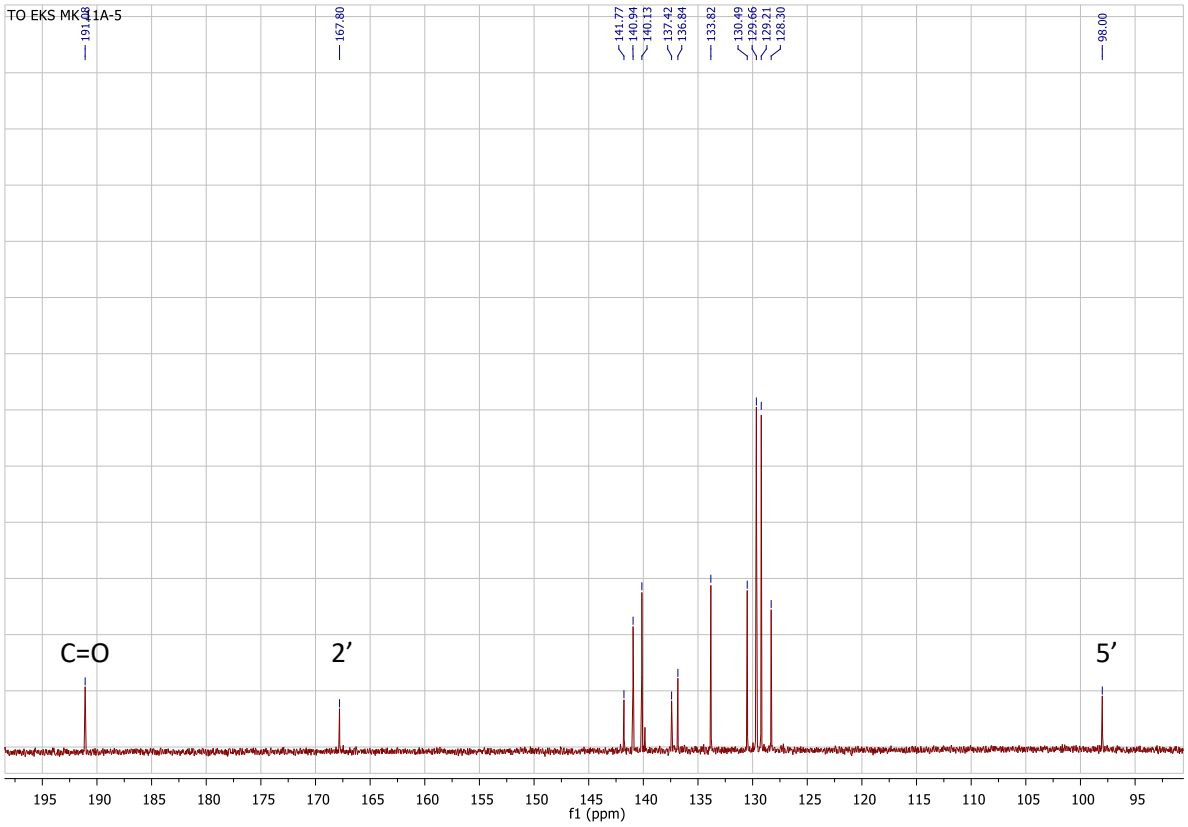



*Figure S60. $^{13}C$ NMR spectrum expansion of 5′-bromo-2′-hydroxy-3′-nitrochalcone.*

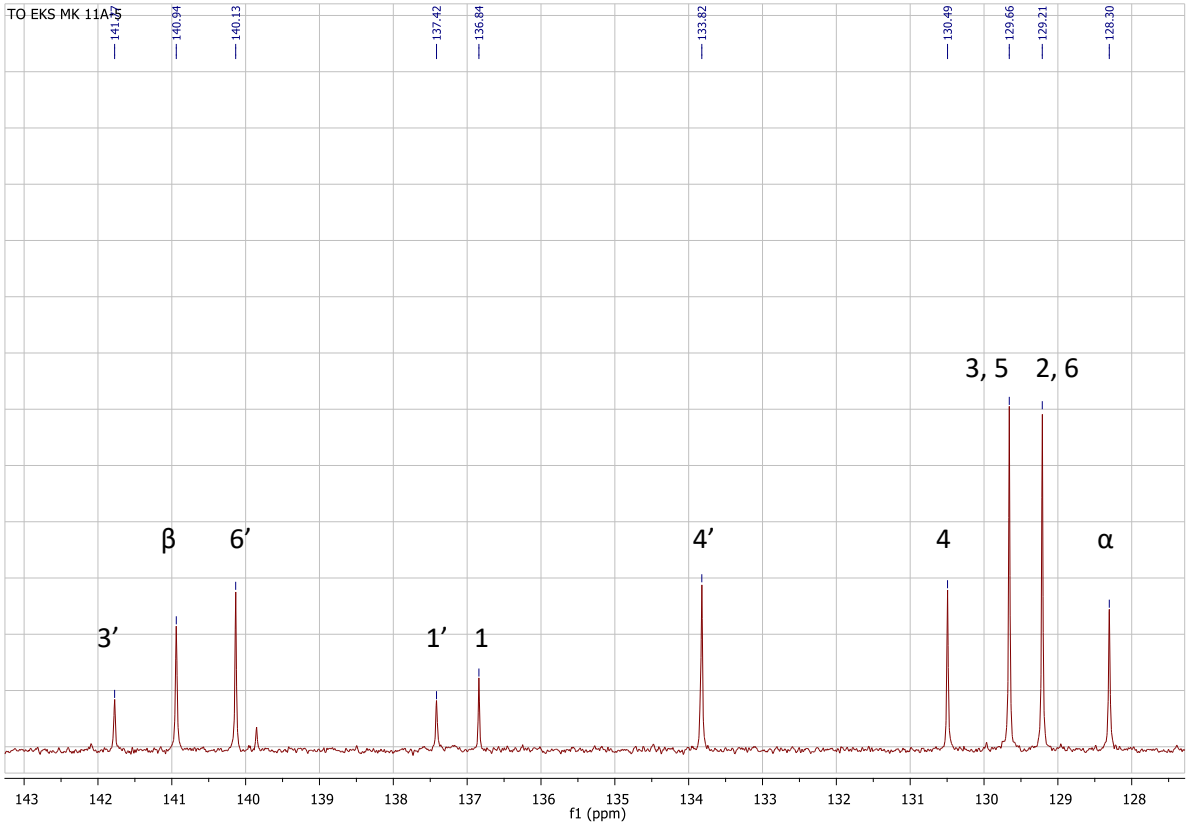



*Figure S61. $^{13}C$ NMR spectrum expansion of 5′-bromo-2′-hydroxy-3′-nitrochalcone.*

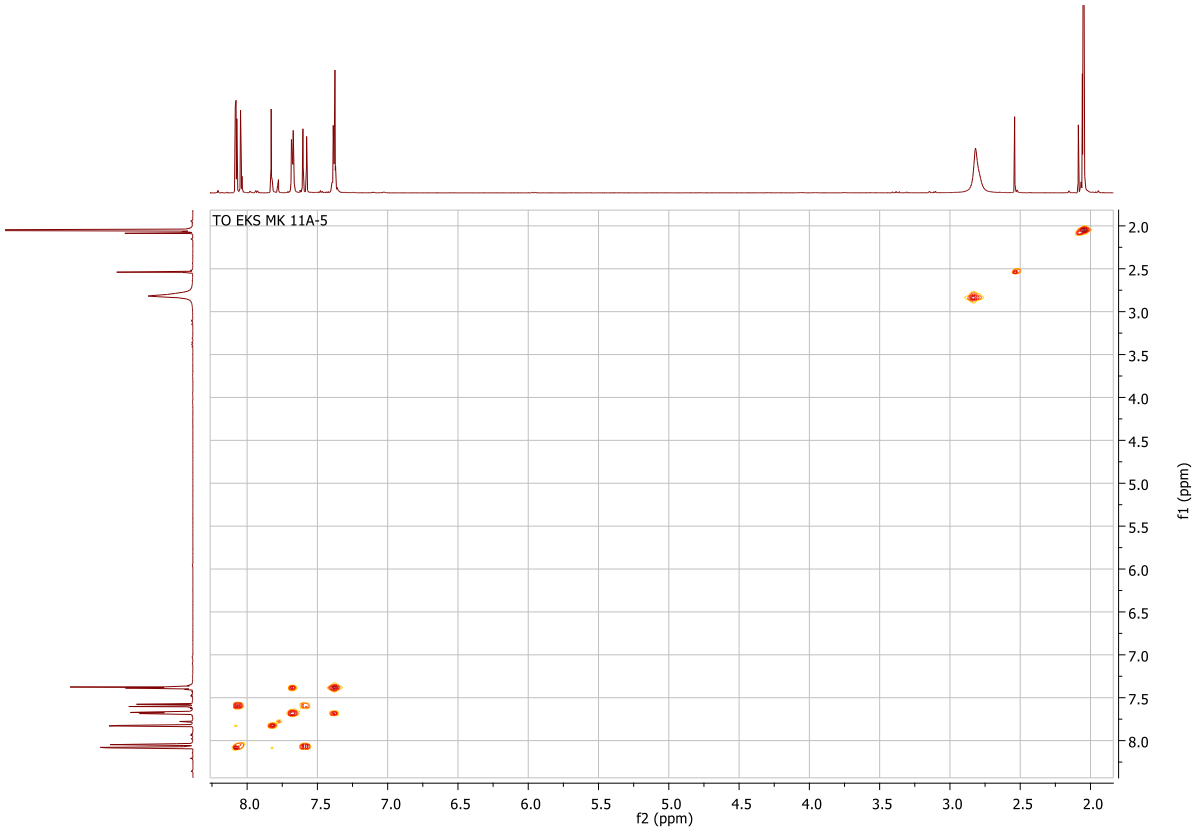



*Figure S62. COSY contour map – $^1H$ x $^1H$ of 5′-bromo-2′-hydroxy-3′-nitrochalcone.*

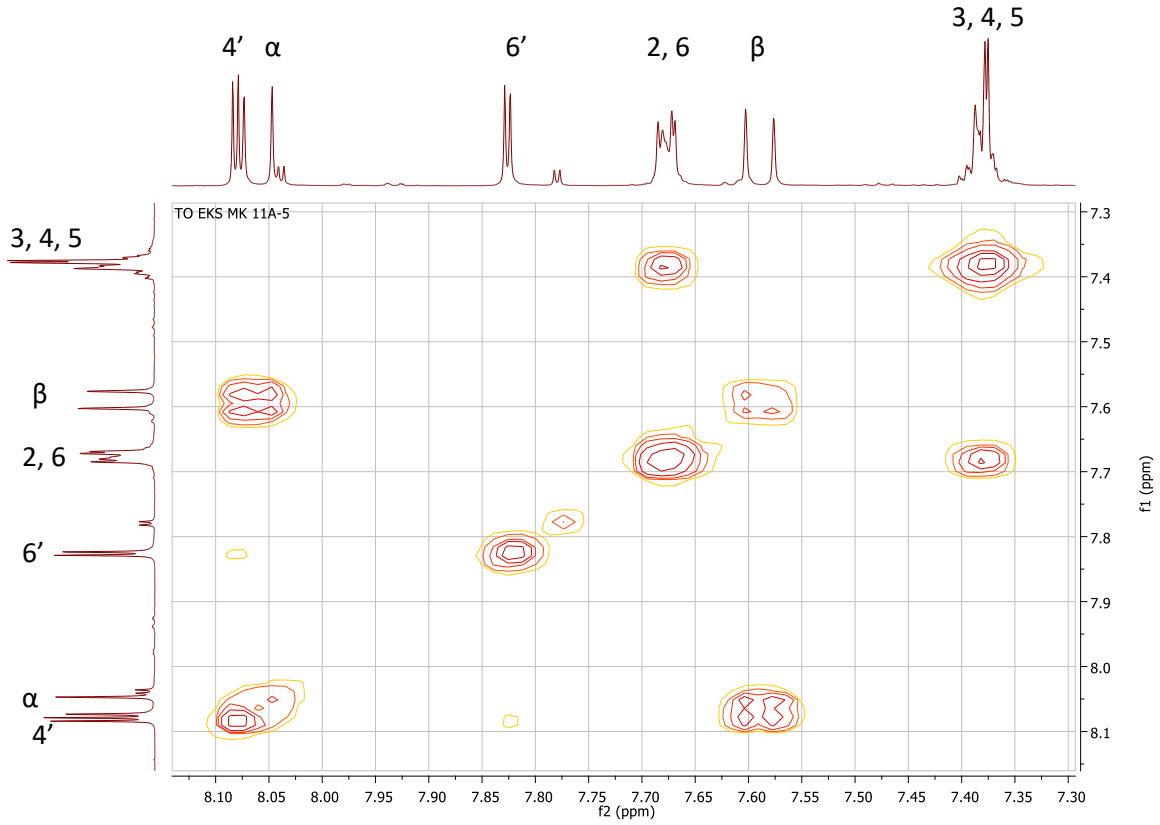



*Figure S20. COSY contour map – $^1H$ x $^1H$ expansion of 5′-bromo-2′-hydroxy-3′-nitrochalcone.*

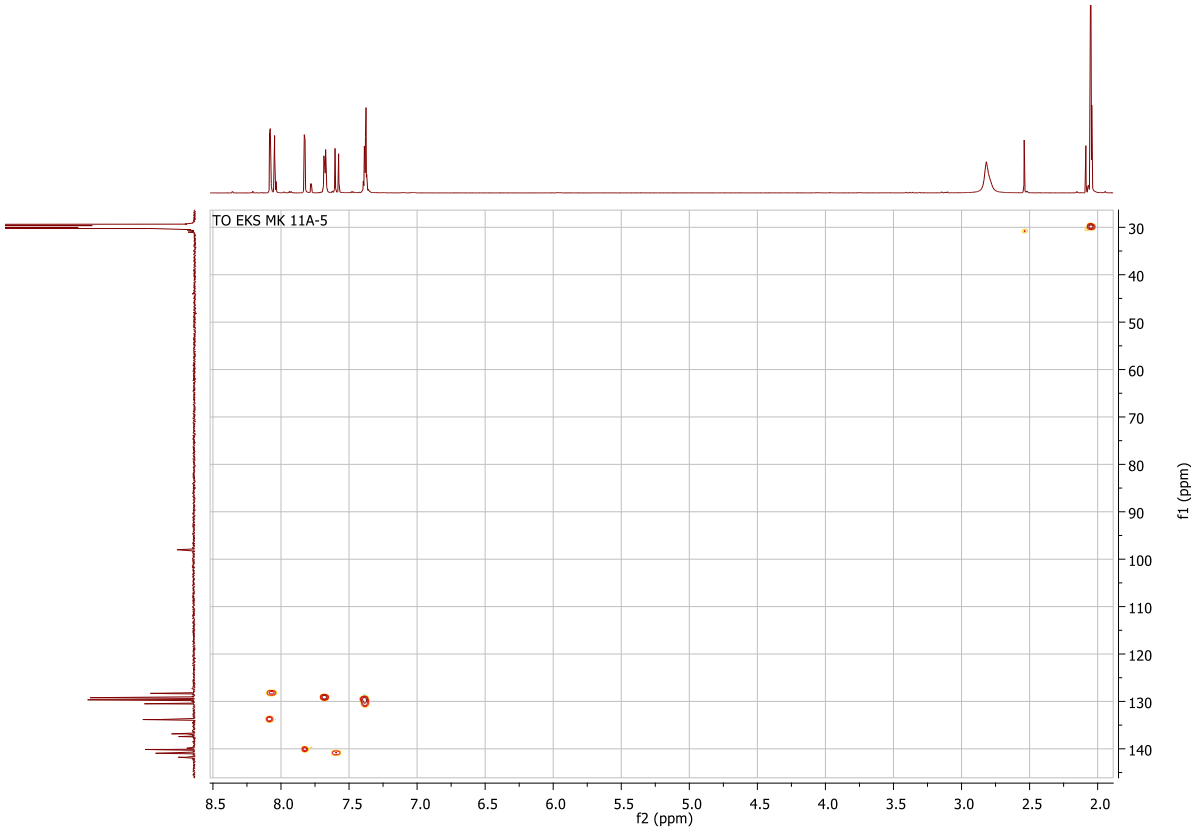



*Figure S64. HSQC contour map – $^{1}H$ x $^{13}C$ of 5′-bromo-2′-hydroxy-3′-nitrochalcone.*

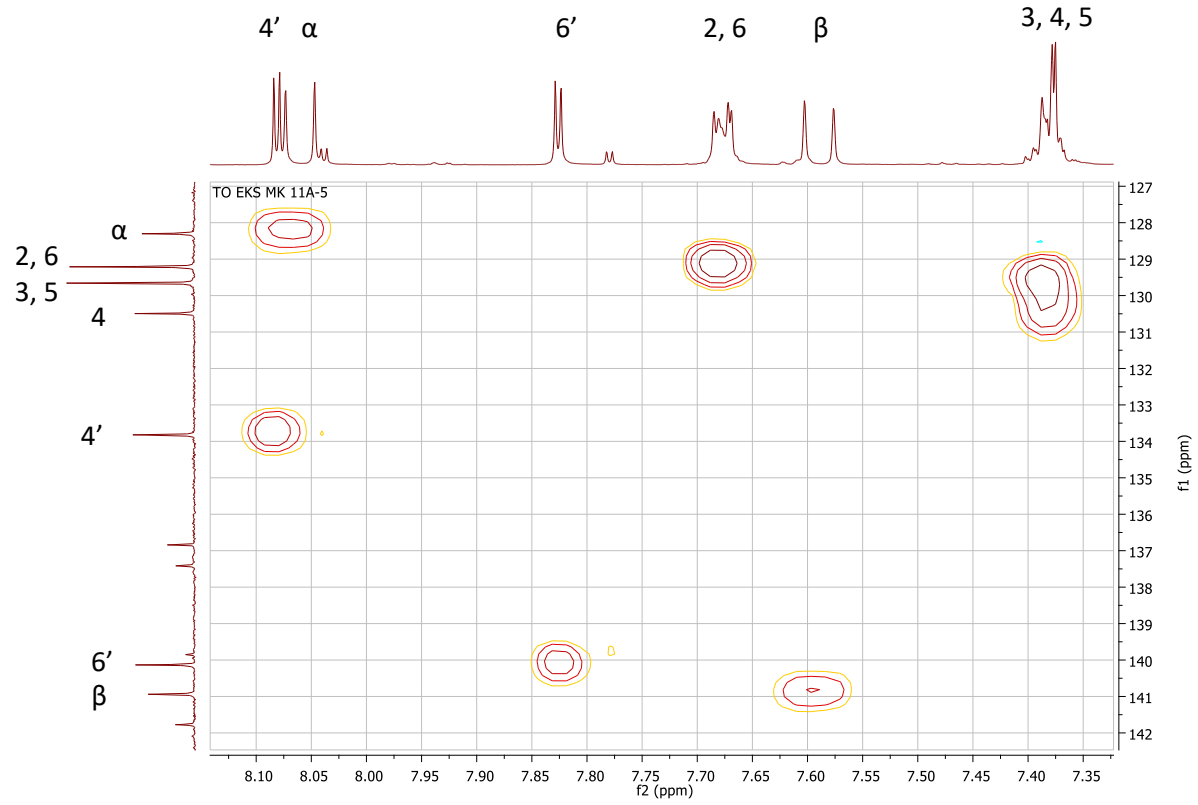



*Figure S65. HSQC contour map – $^{1}H$ x $^{13}C$ expansion of 5′-bromo-2′-hydroxy-3′-nitrochalcone.*

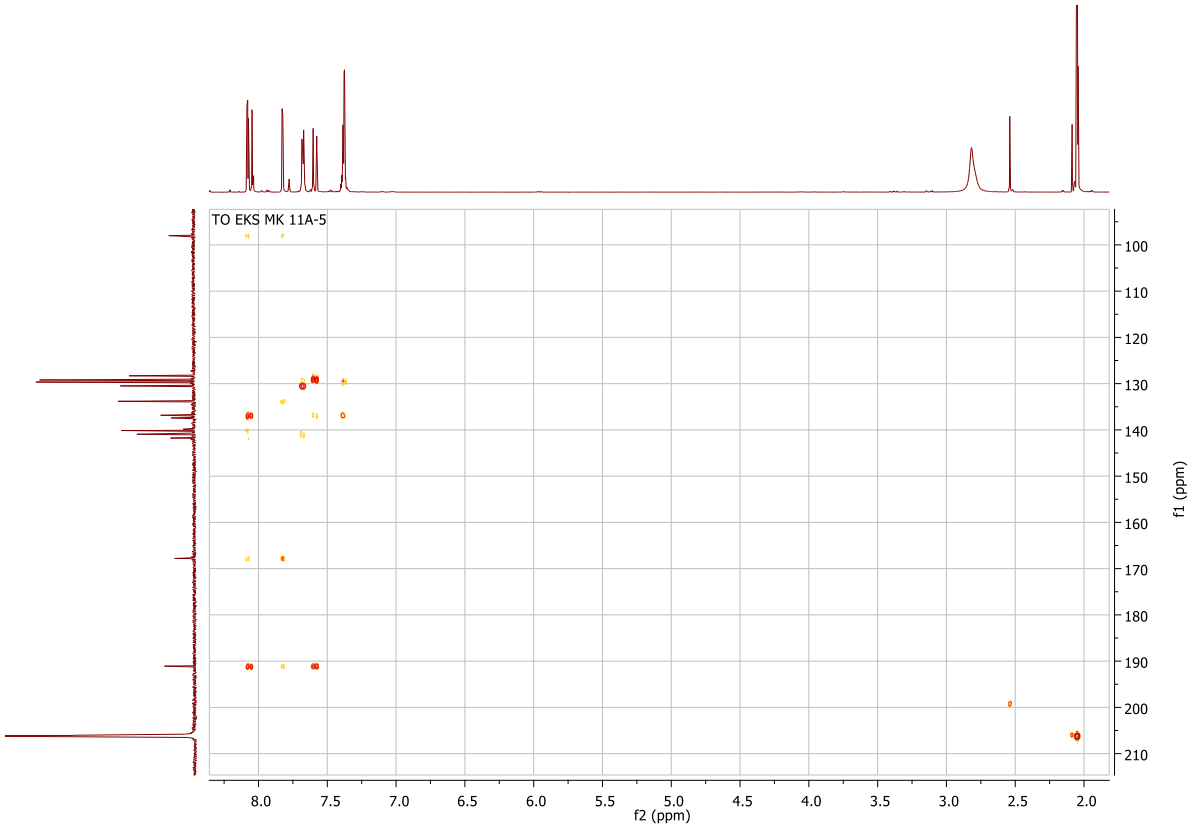



*Figure S66. HMBC contour map – $^{1}H$ x $^{13}C$ of 5′-bromo-2′-hydroxy-3′-nitrochalcone.*

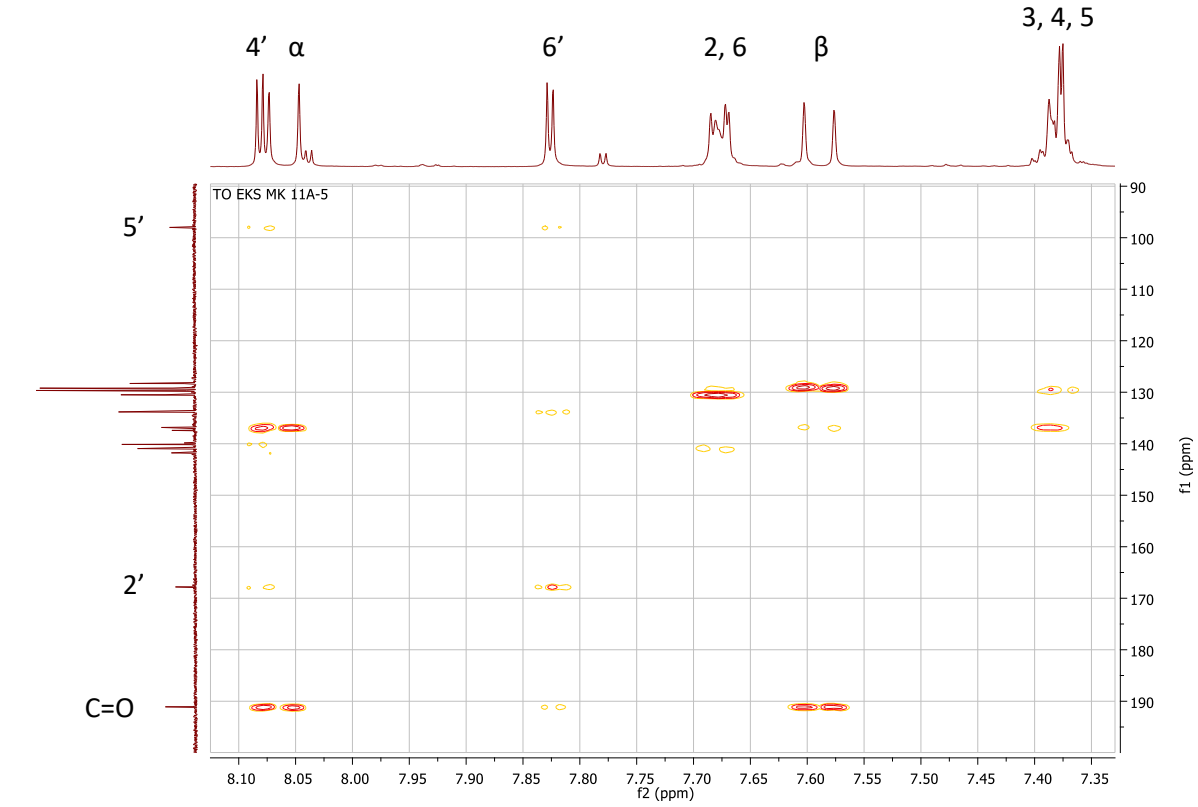



*Figure S67. HMBC contour map – $^{1}H$ x $^{13}C$ expansion of 5′-bromo-2′-hydroxy-3′-nitrochalcone.*

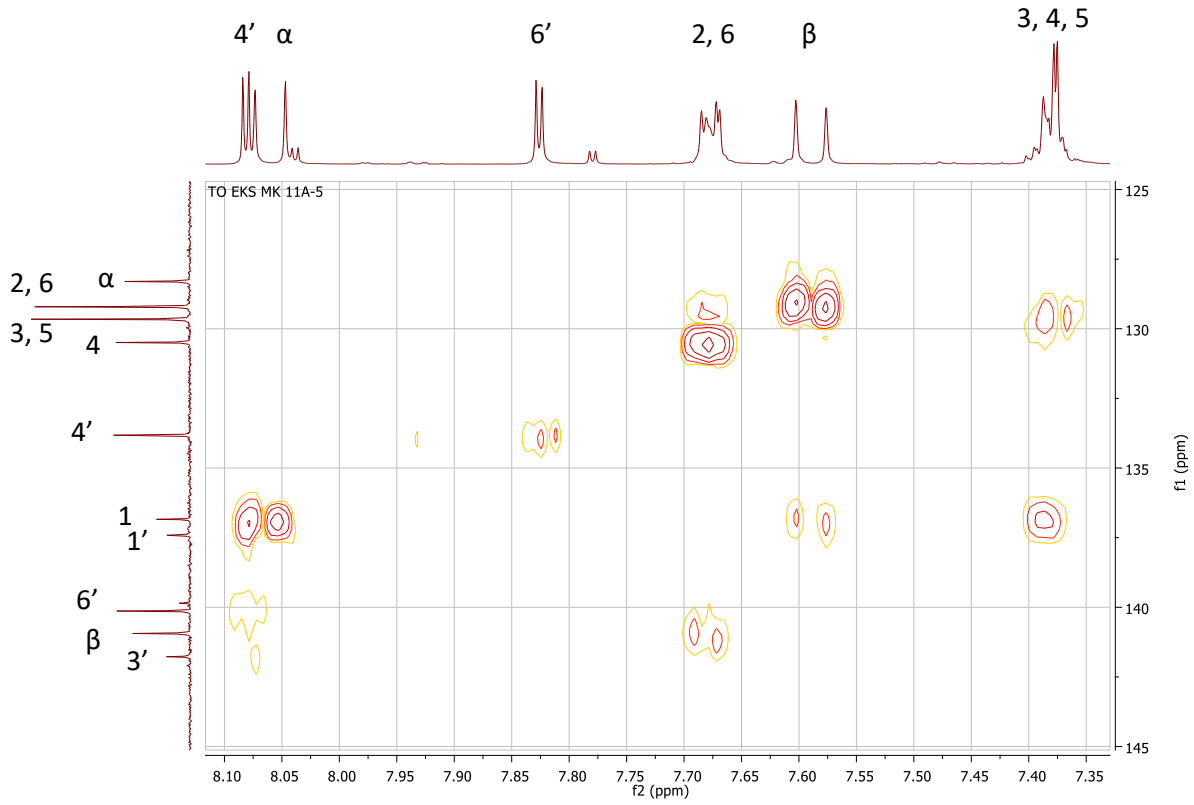
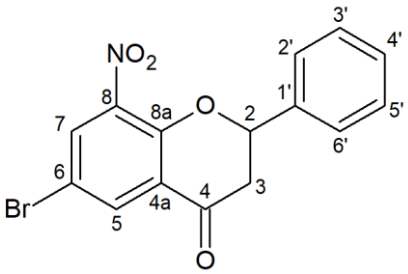



*Figure S68. HMBC contour map – $^{1}H$ x $^{13}C$ expansion of 5′-bromo-2′-hydroxy-3′-nitrochalcone.*

Compound name: 6-bromo-8-nitroflavanone
Molecular Formula: $C_{15}H_{10}BrNO_4$
Formula Weight: 348.148
Ionization mode: negative
Precursor $[M + H]^+$: 345.979
Monoisotopic Mass: 346.979
Collision energy (CE): +25.0



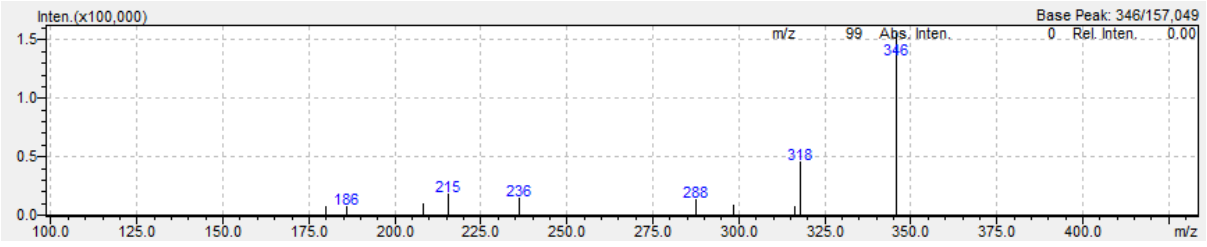




*Figure S69. LC-MS spectrum of 6-bromo-8-nitroflavanone.*

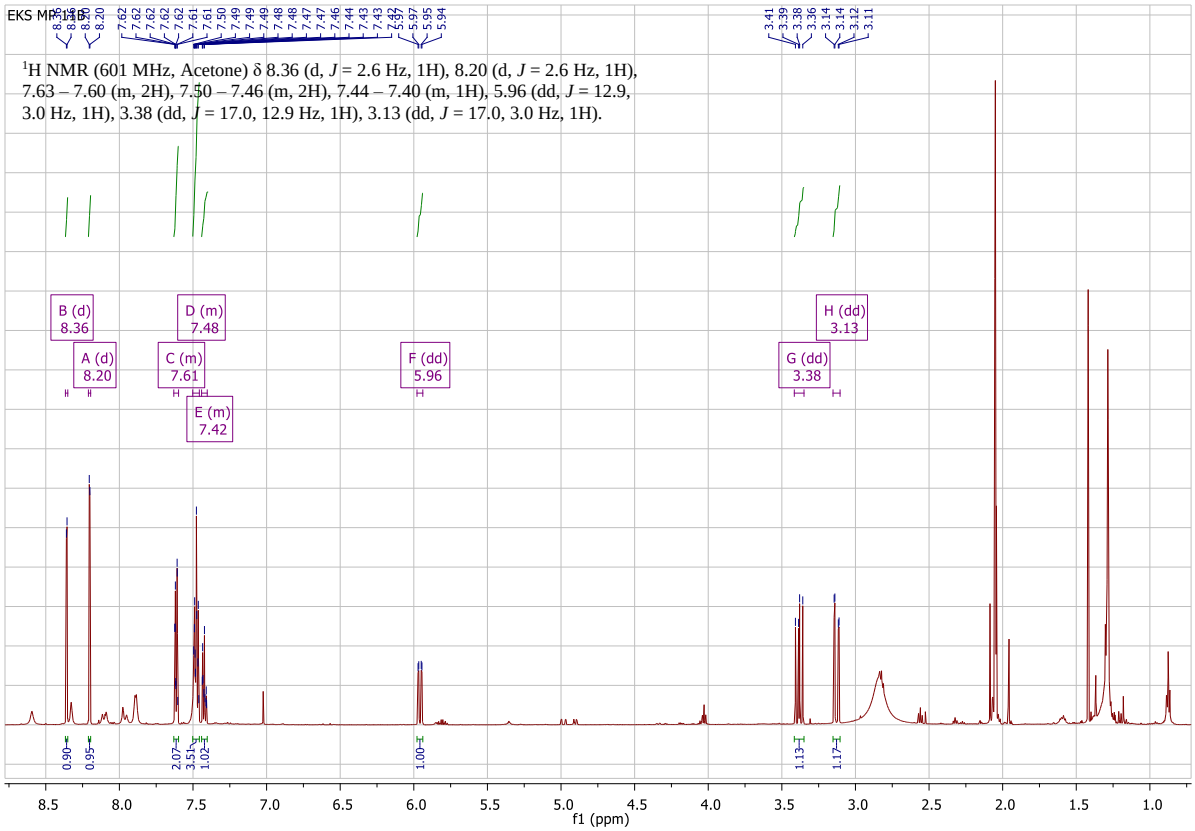



*Figure S70. $^1$H NMR spectrum of 6-bromo-8-nitroflavanone.*

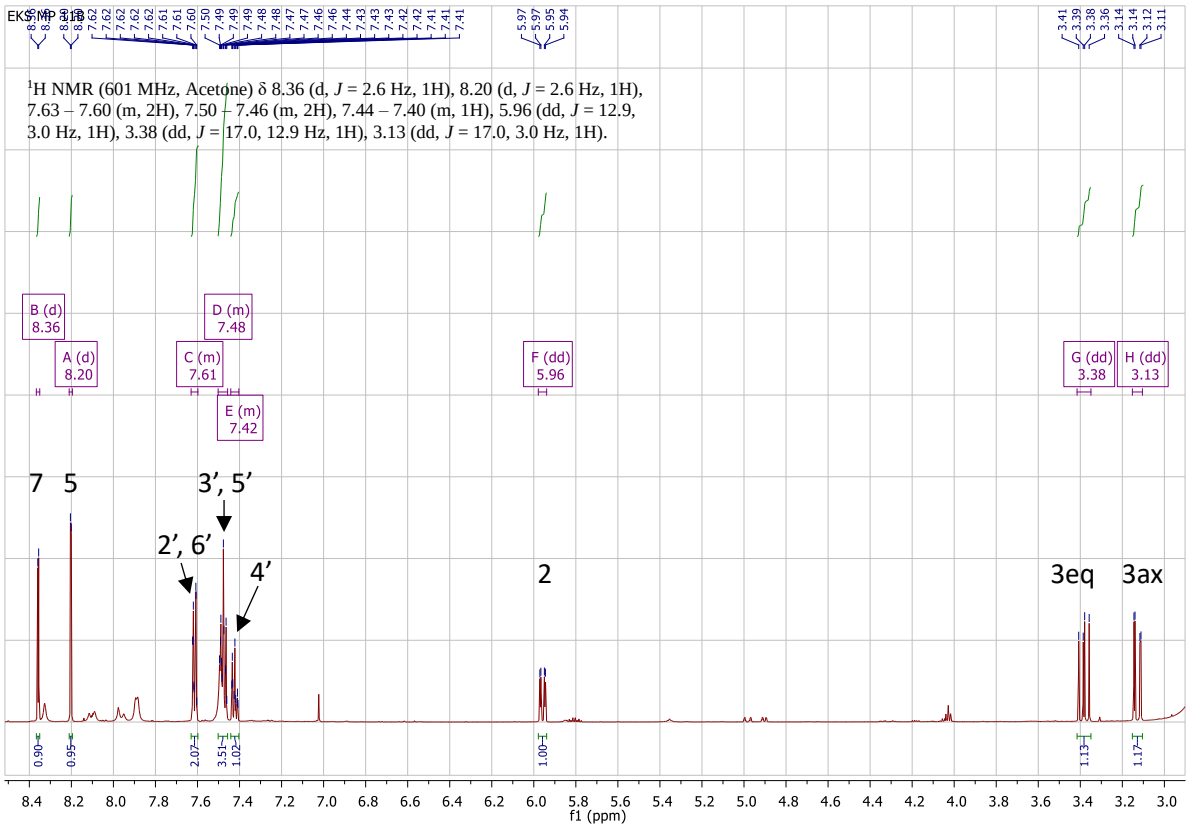



*Figure S71. $^1$H NMR spectrum expansion of 6-bromo-8-nitroflavanone.*

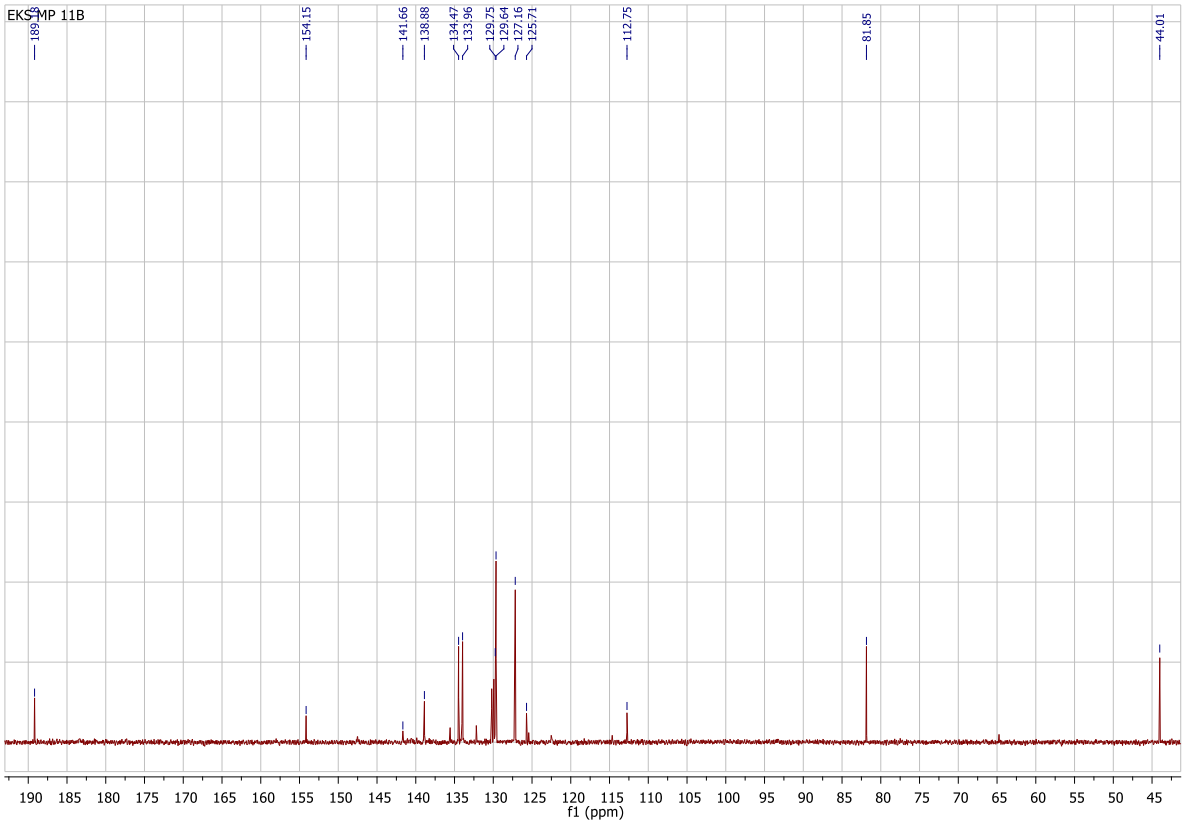



*Figure S72. $^{31}$C NMR spectrum of 6-bromo-8-nitroflavanone.*

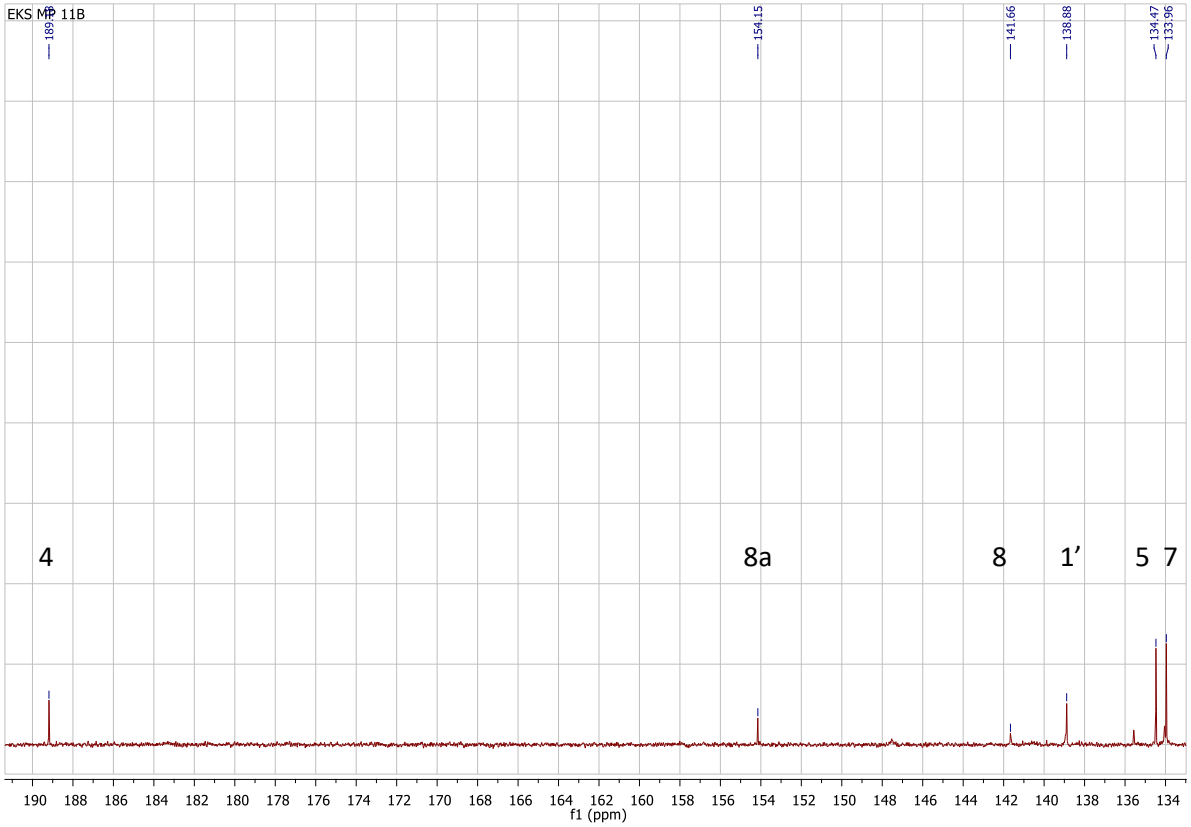



*Figure S73. $^{31}$C NMR spectrum expansion of 6-bromo-8-nitroflavanone.*

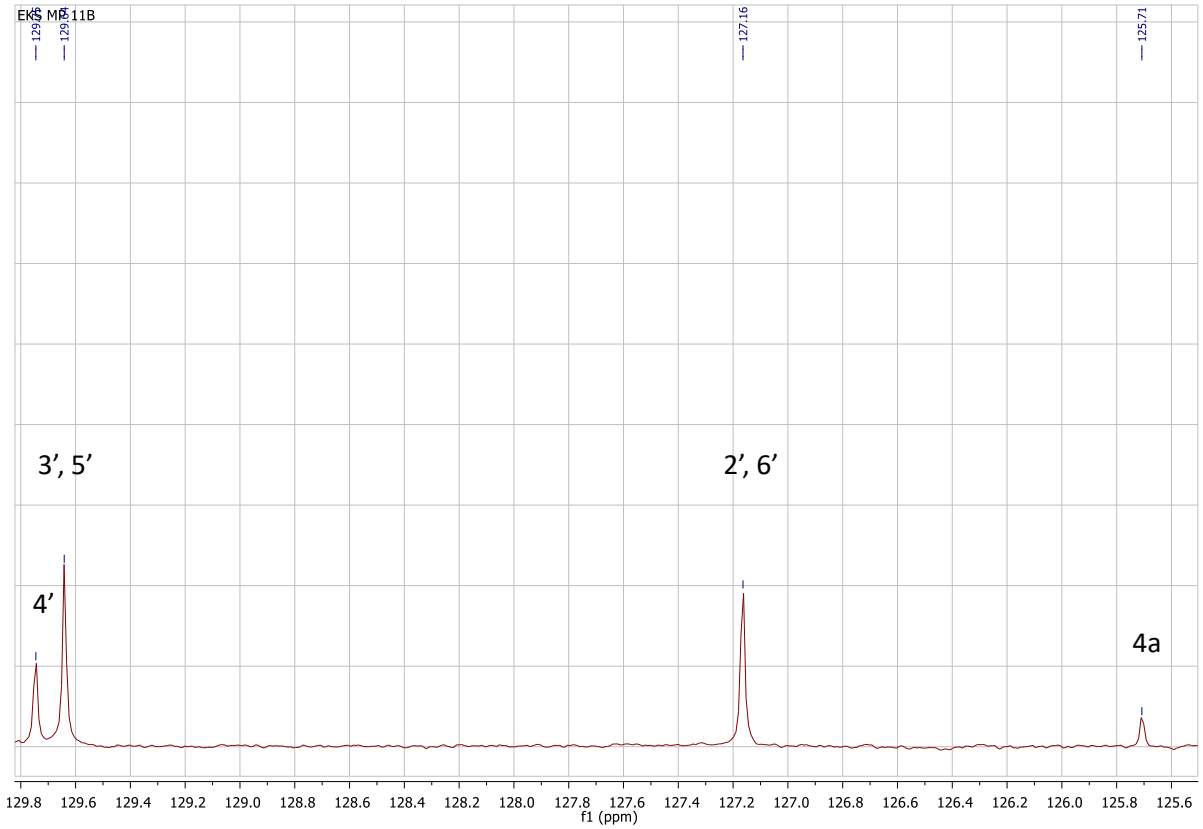



*Figure S74. $^{31}$C NMR spectrum expansion of 6-bromo-8-nitroflavanone.*

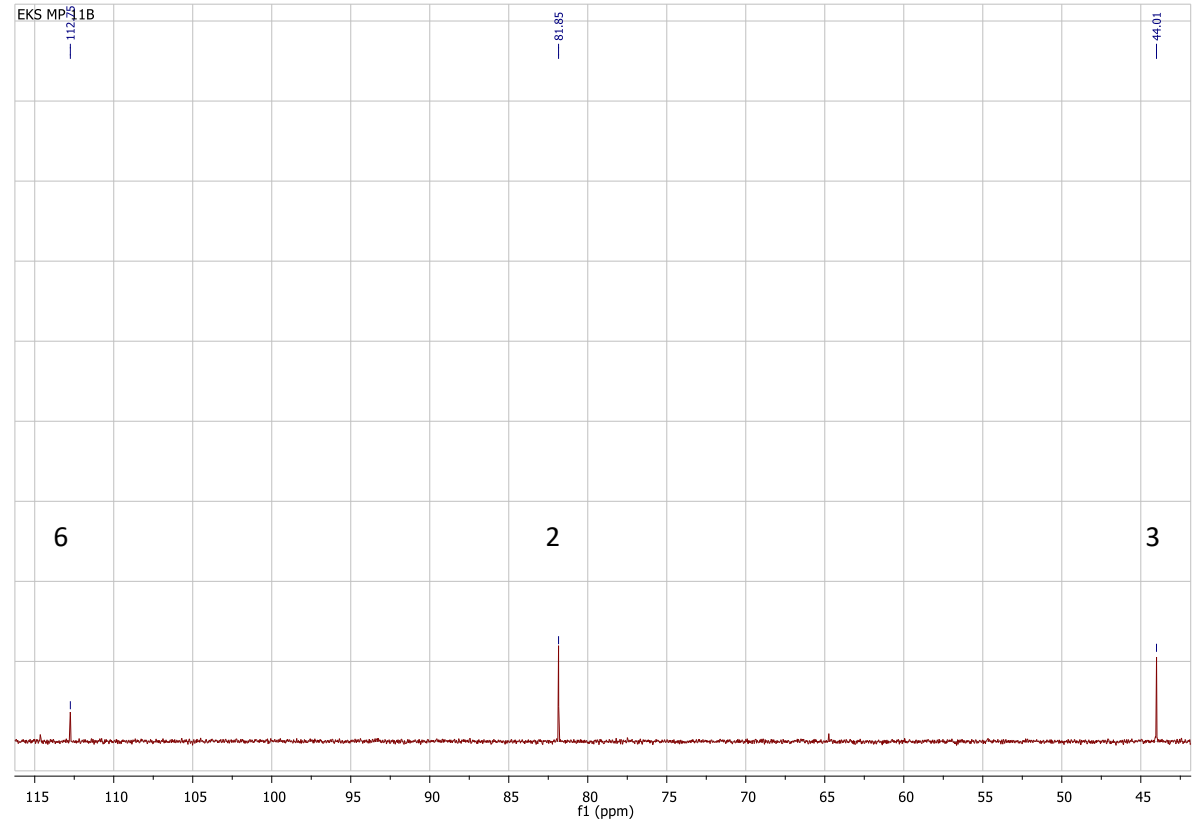



*Figure S75. $^{31}$C NMR spectrum expansion of 6-bromo-8-nitroflavanone.*

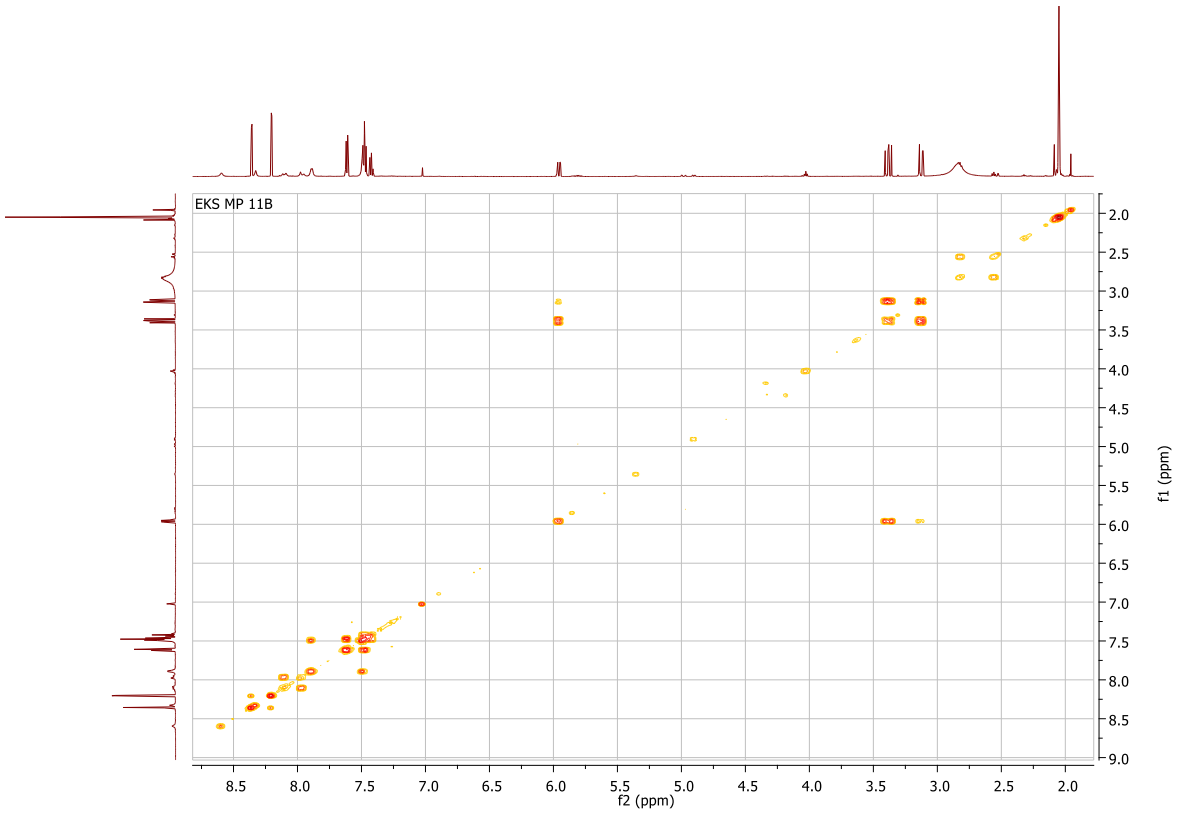



*Figure S76. COSY contour map – $^1H$ x $^1H$ of 6-bromo-8-nitroflavanone.*

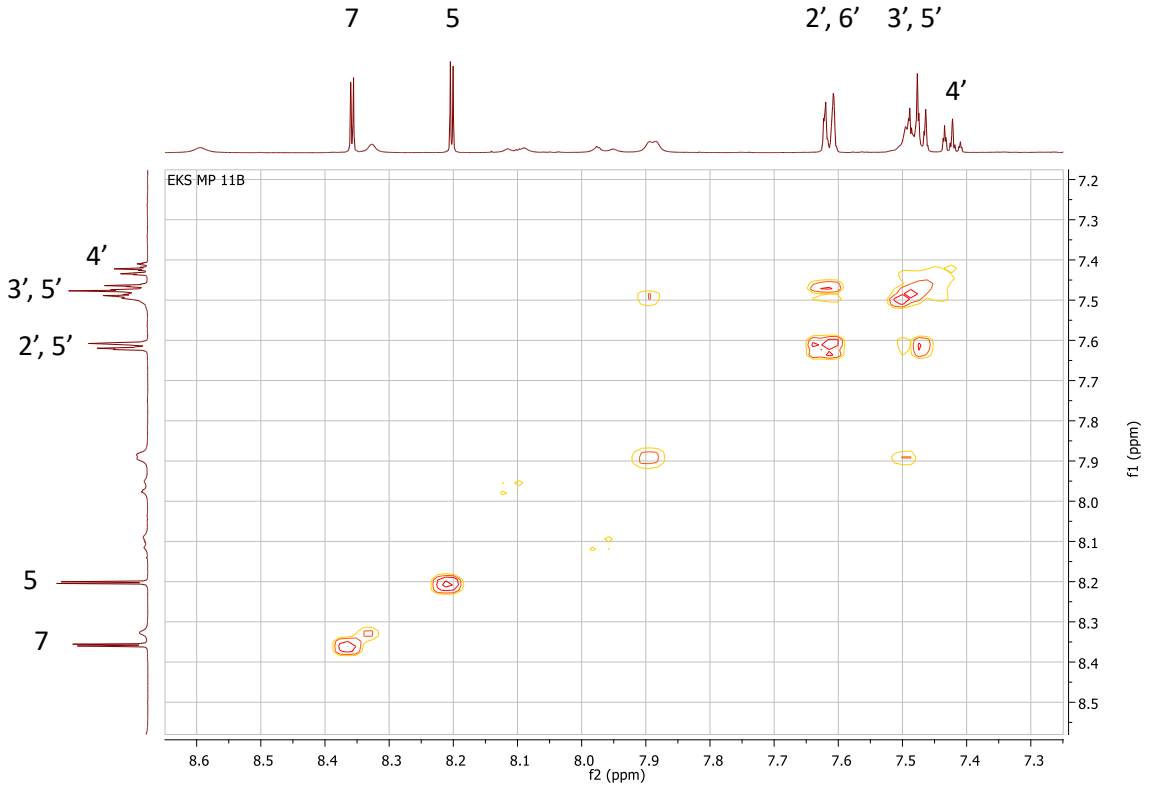



*Figure S77. COSY contour map – $^1H$ x $^1H$ expansion of 6-bromo-8-nitroflavanone.*

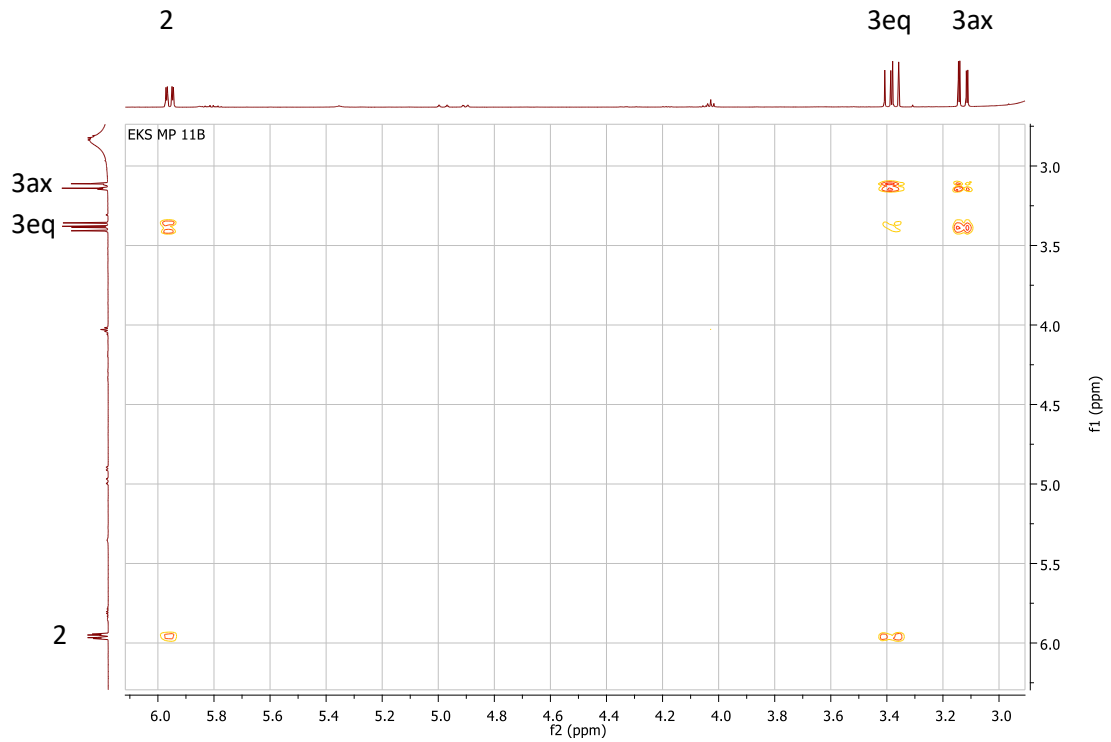



*Figure S78. COSY contour map – $^{1}H$ x $^{1}H$ expansion of 6-bromo-8-nitroflavanone.*

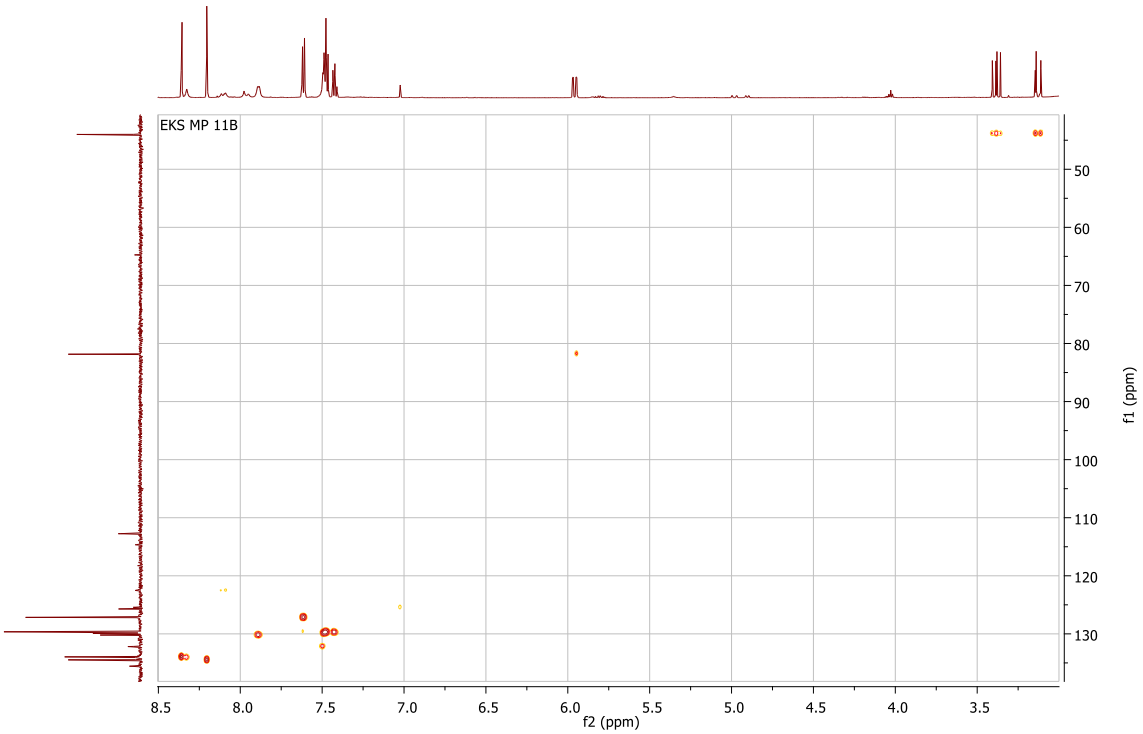



*Figure S79. HSQC contour map – $^{1}H$ x $^{13}C$ of 6-bromo-8-nitroflavanone.*

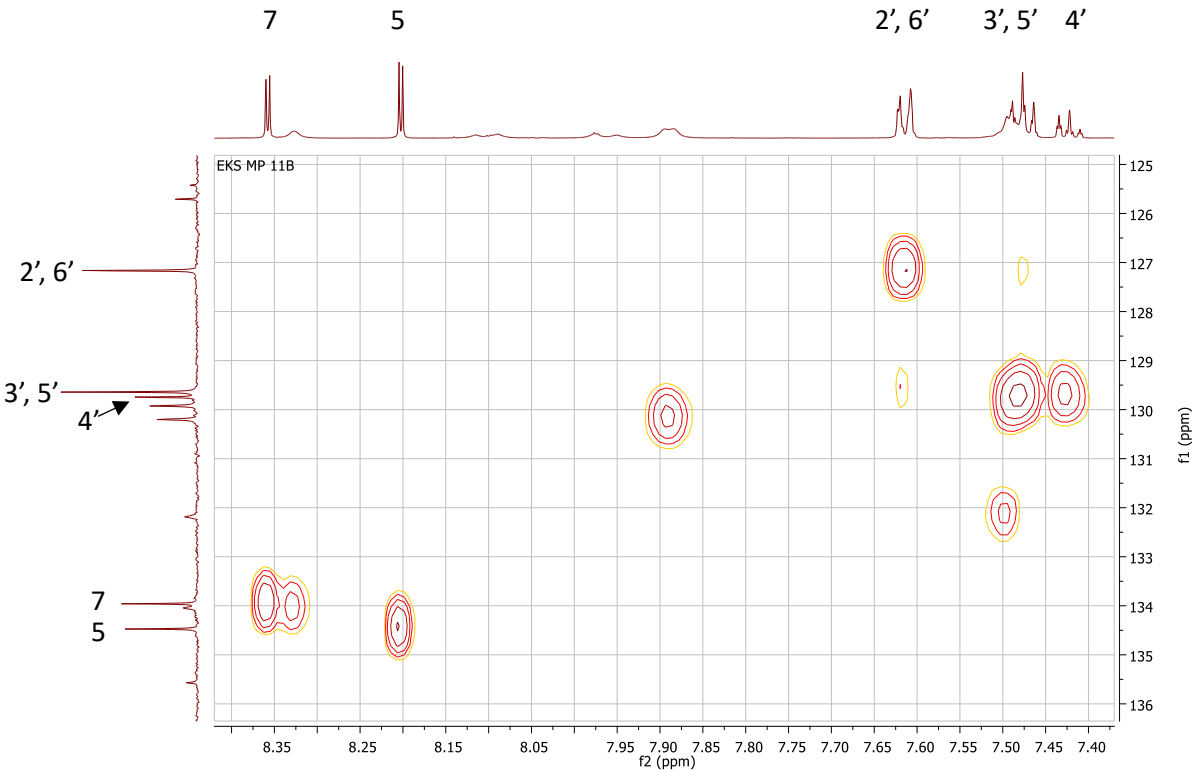



*Figure S80. HSQC contour map – $^{1}H$ x $^{13}C$ expansion of 6-bromo-8-nitroflavanone.*

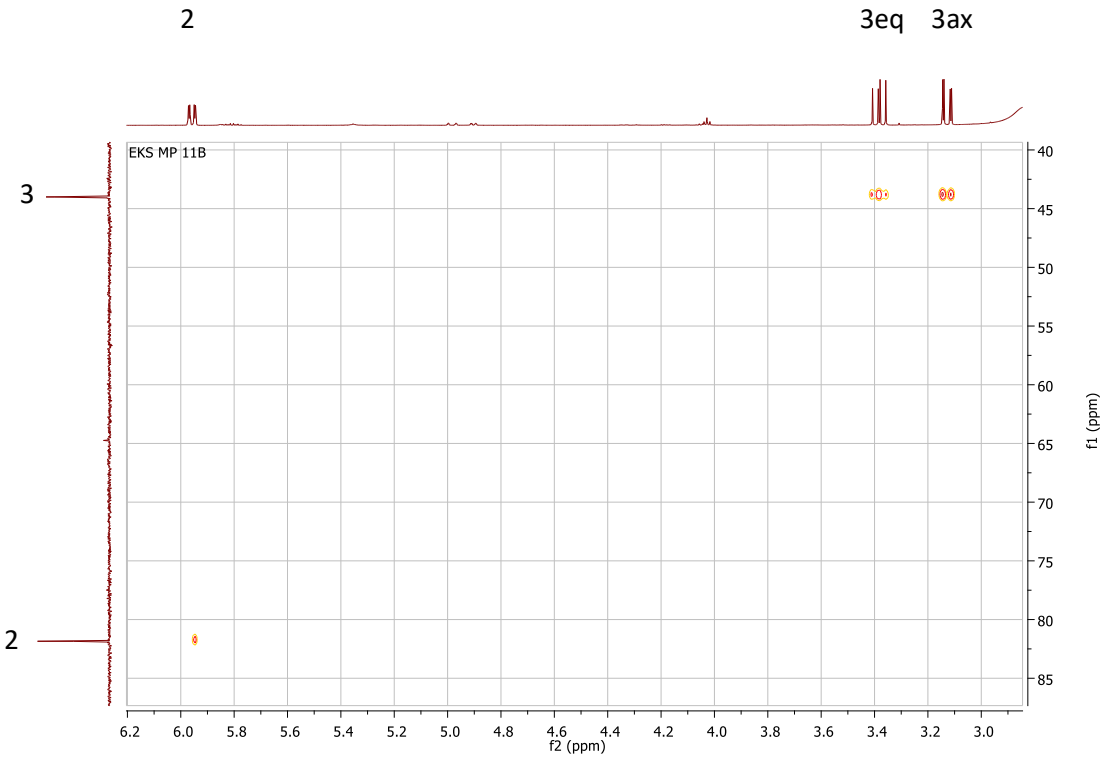



*Figure S81. HSQC contour map – $^{1}H$ x $^{13}C$ expansion of 6-bromo-8-nitroflavanone.*

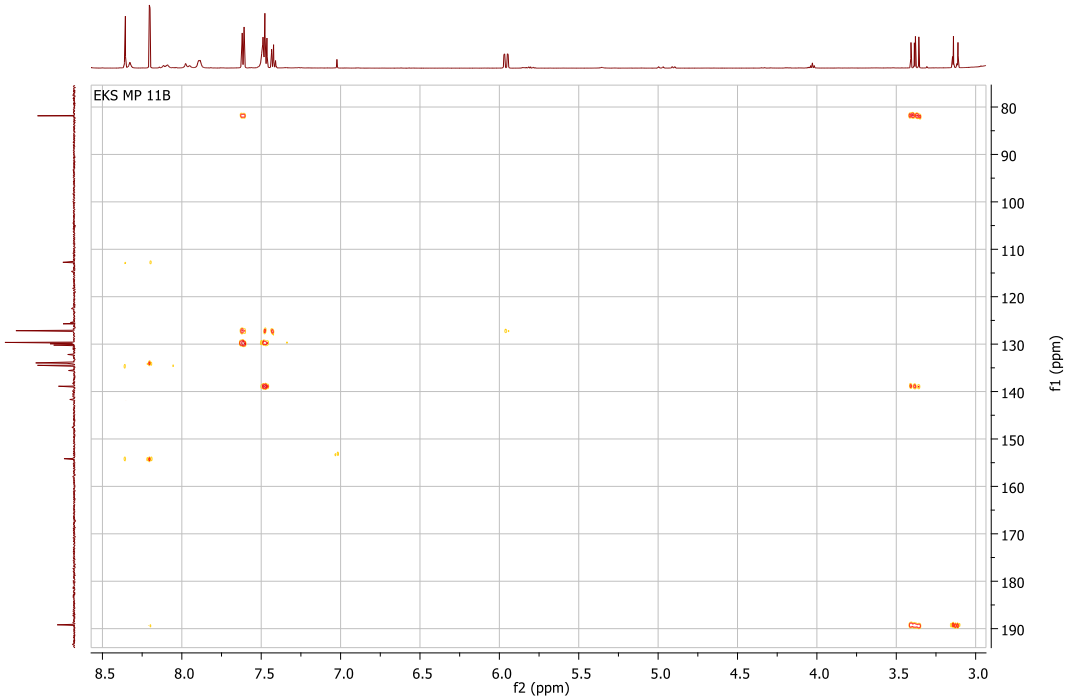



*Figure S82. HMBC contour map – $^{1}H$ x $^{13}C$ of 6-bromo-8-nitroflavanone.*

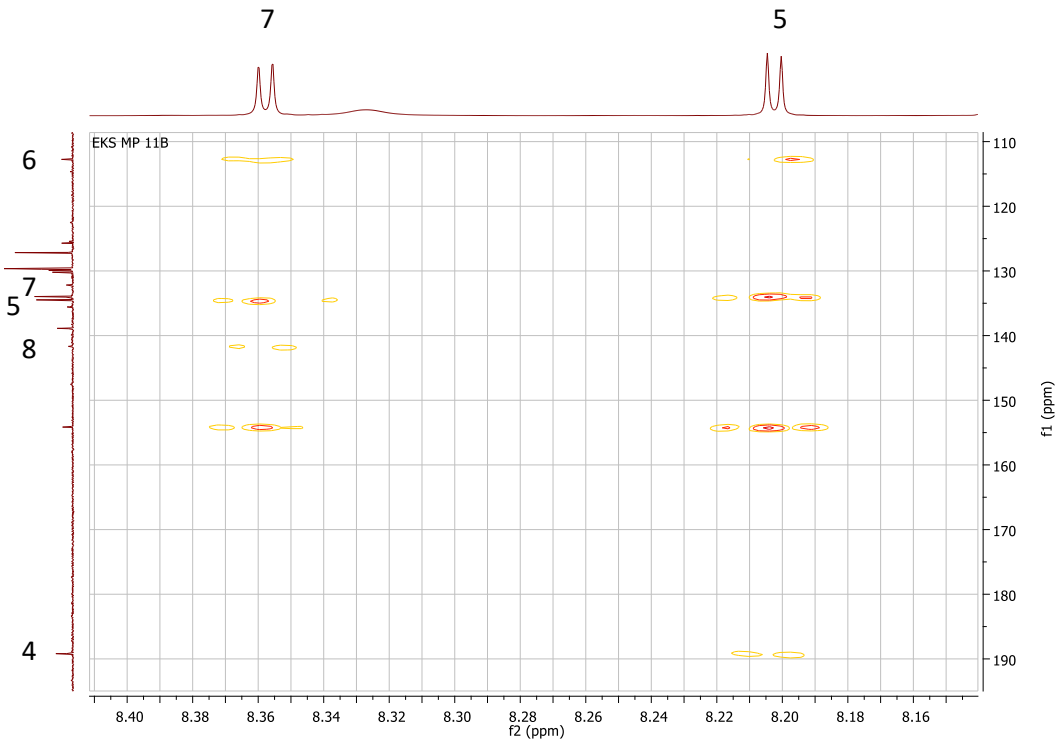



*Figure S83. HMBC contour map – $^{1}H$ x $^{13}C$ expansion of 6-bromo-8-nitroflavanone.*

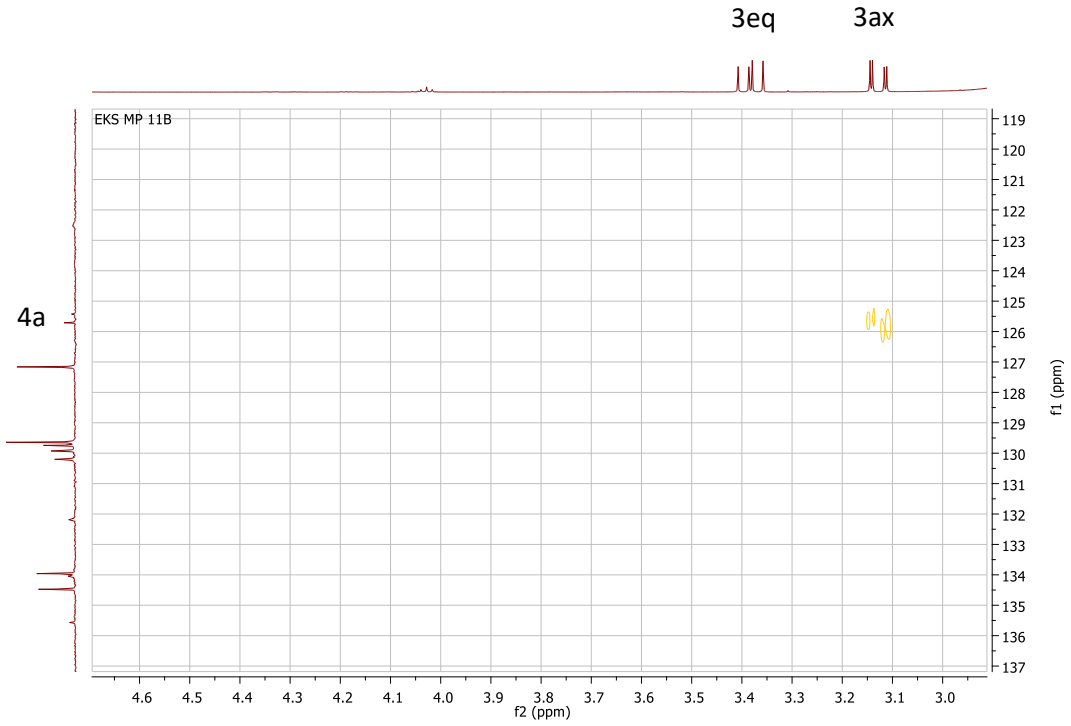
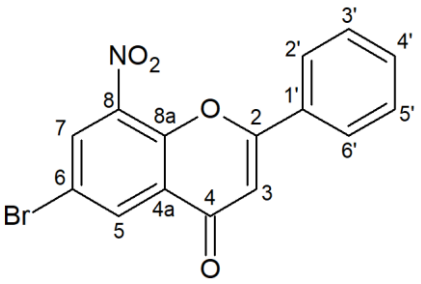



*Figure S84. HMBC contour map – $^{1}H$ x $^{13}C$ expansion of 6-bromo-8-nitroflavanone.*

Compound name: 6-bromo-8-nitroflavone
Molecular Formula: $C_{15}H_8BrNO_4$
Formula Weight: 346.132
Ionization mode: positive
Precursor $[M + H]^+$: 345.964
Monoisotopic Mass: 344.964
Collision energy (CE): -25.0



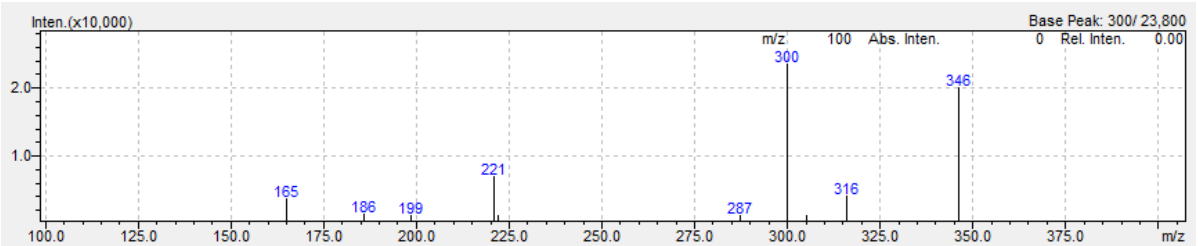




*Figure S85. LC-MS spectrum of 6-bromo-8-nitroflavone.*

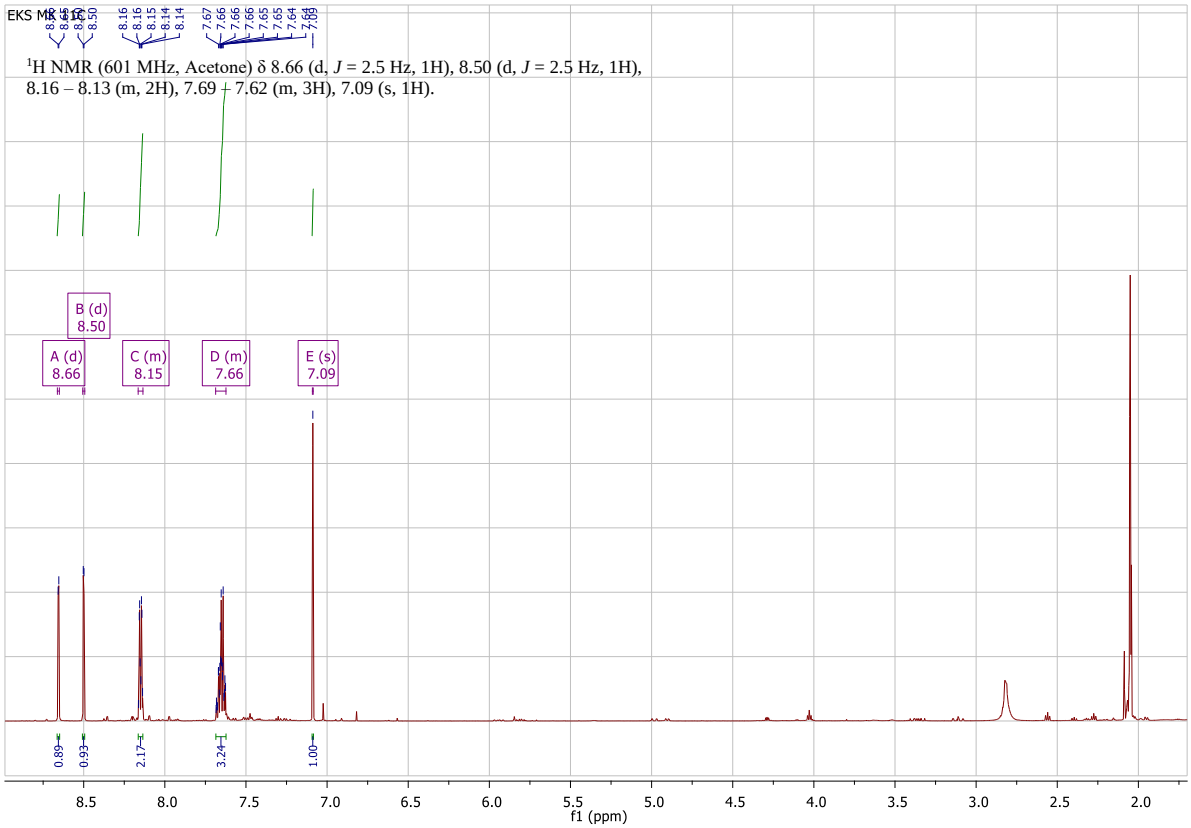



*Figure S86. $^1$H NMR spectrum of 6-bromo-8-nitroflavone.*

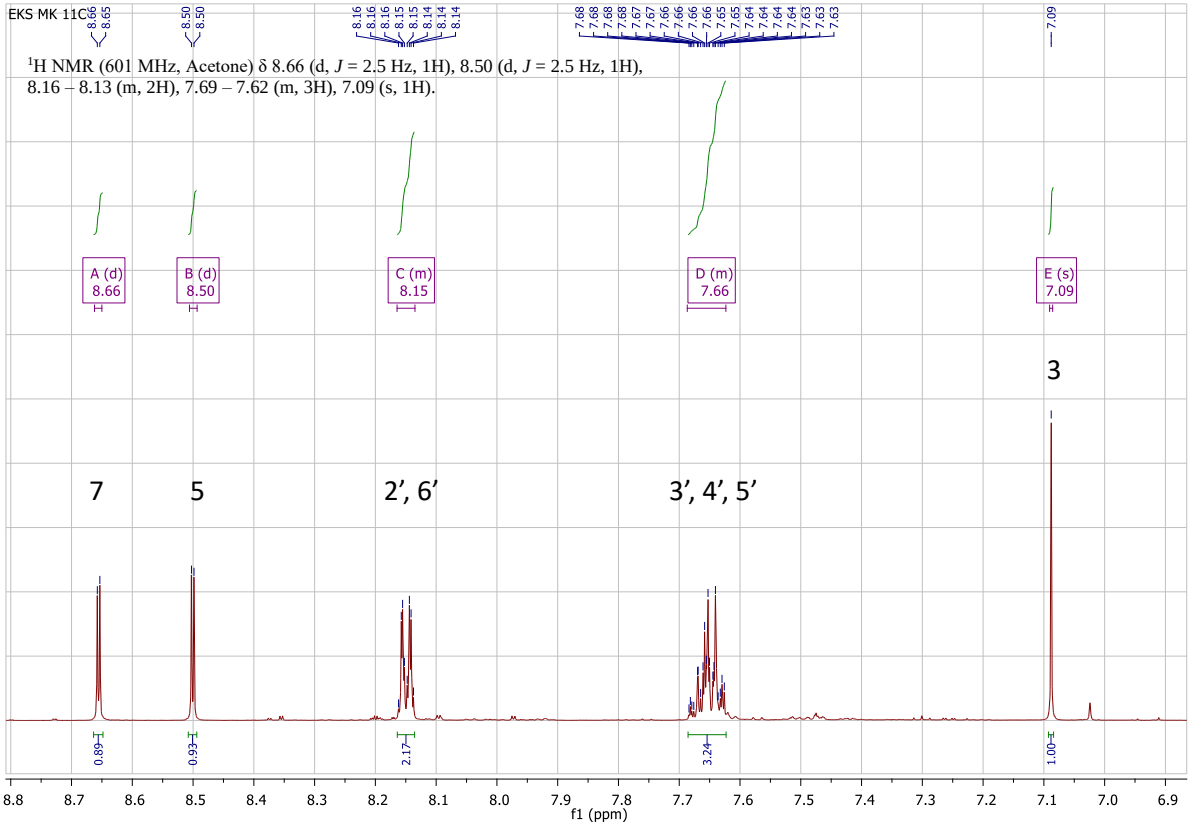



*Figure S87. $^1$H NMR spectrum expansion of 6-bromo-8-nitroflavone.*

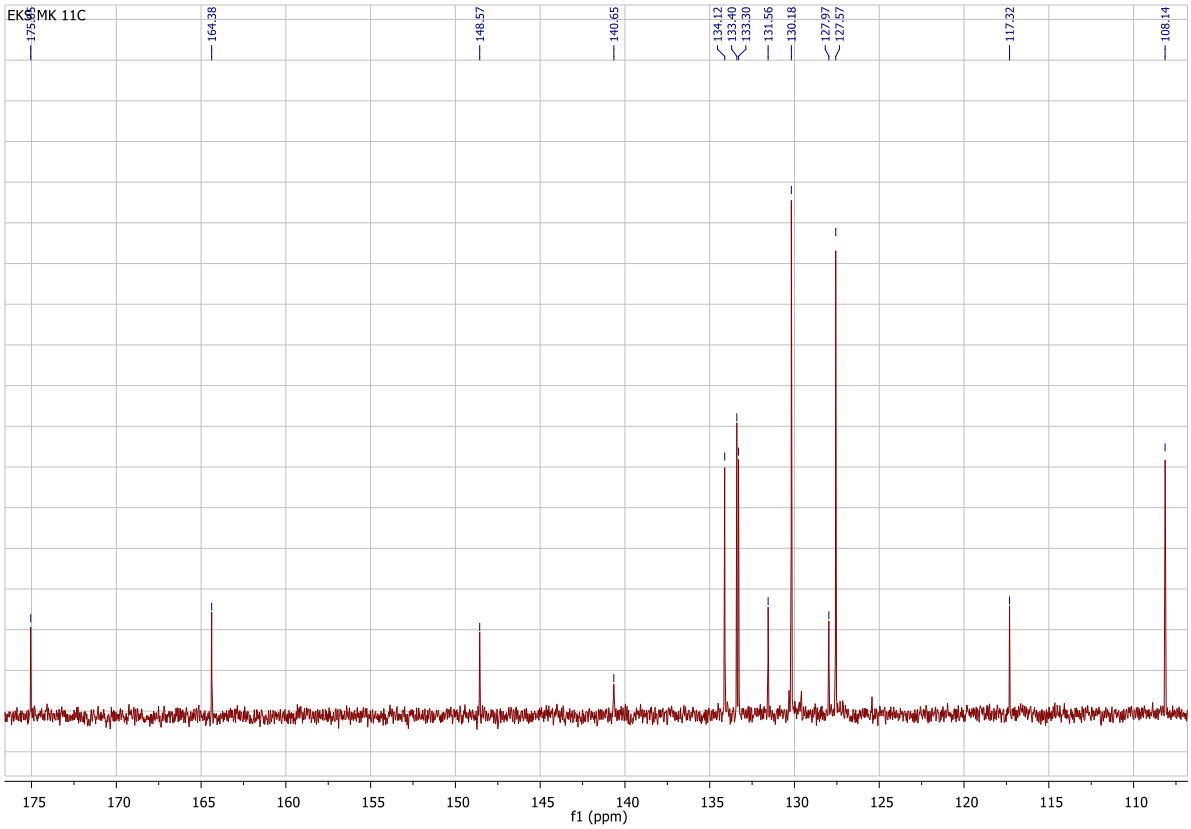



*Figure S88. $^{13}C$ NMR spectrum of 6-bromo-8-nitroflavone.*

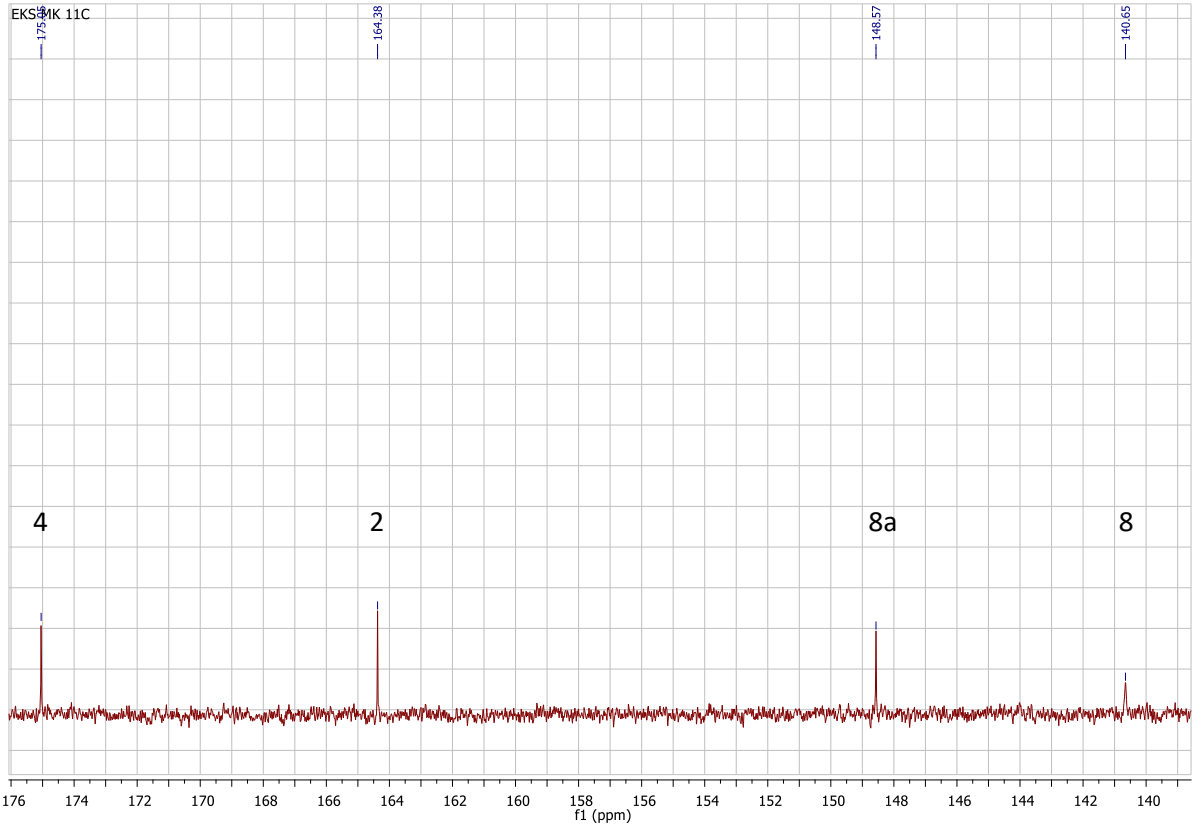



*Figure S89. $^{13}C$ NMR spectrum expansion of 6-bromo-8-nitroflavone.*

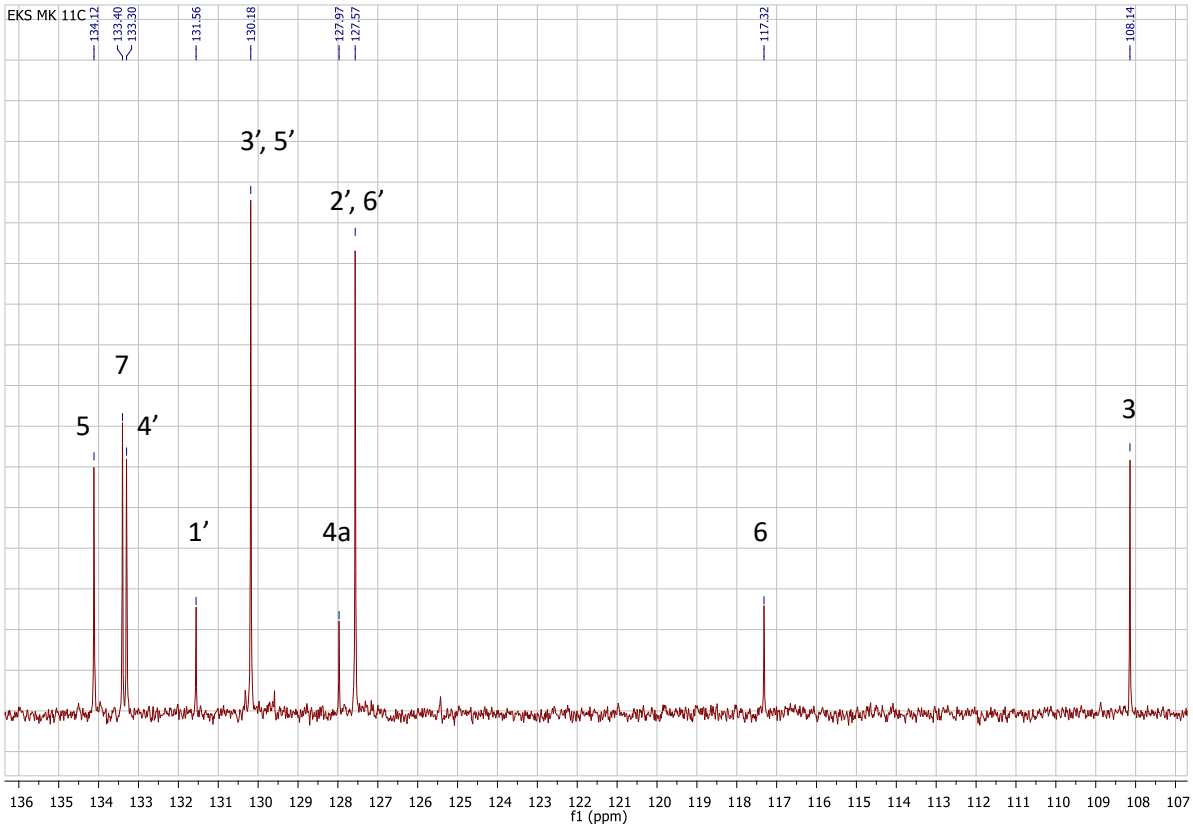



*Figure S90. $^{13}C$ NMR spectrum expansion of 6-bromo-8-nitroflavone.*

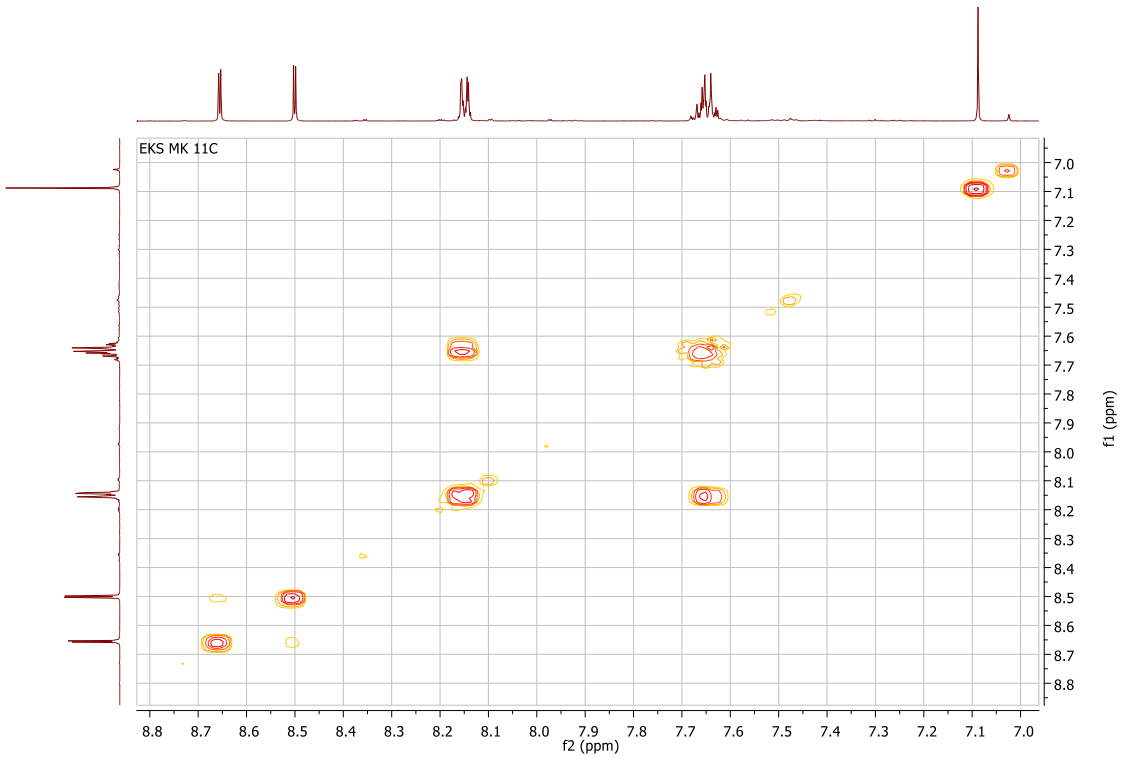



*Figure S91. COSY contour map – $^{1}H$ x $^{1}H$ of 6-bromo-8-nitroflavone.*

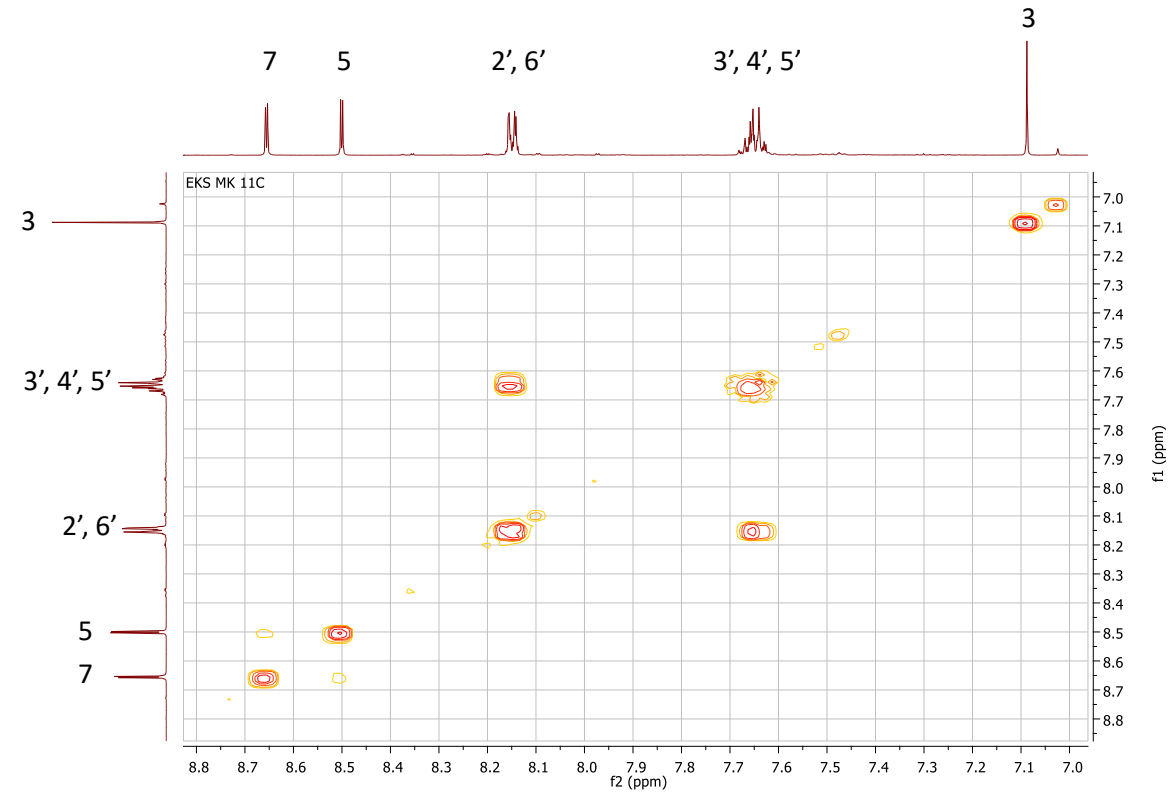



*Figure S92. COSY contour map – $^{1}H$ x $^{1}H$ expansion of 6-bromo-8-nitroflavone.*

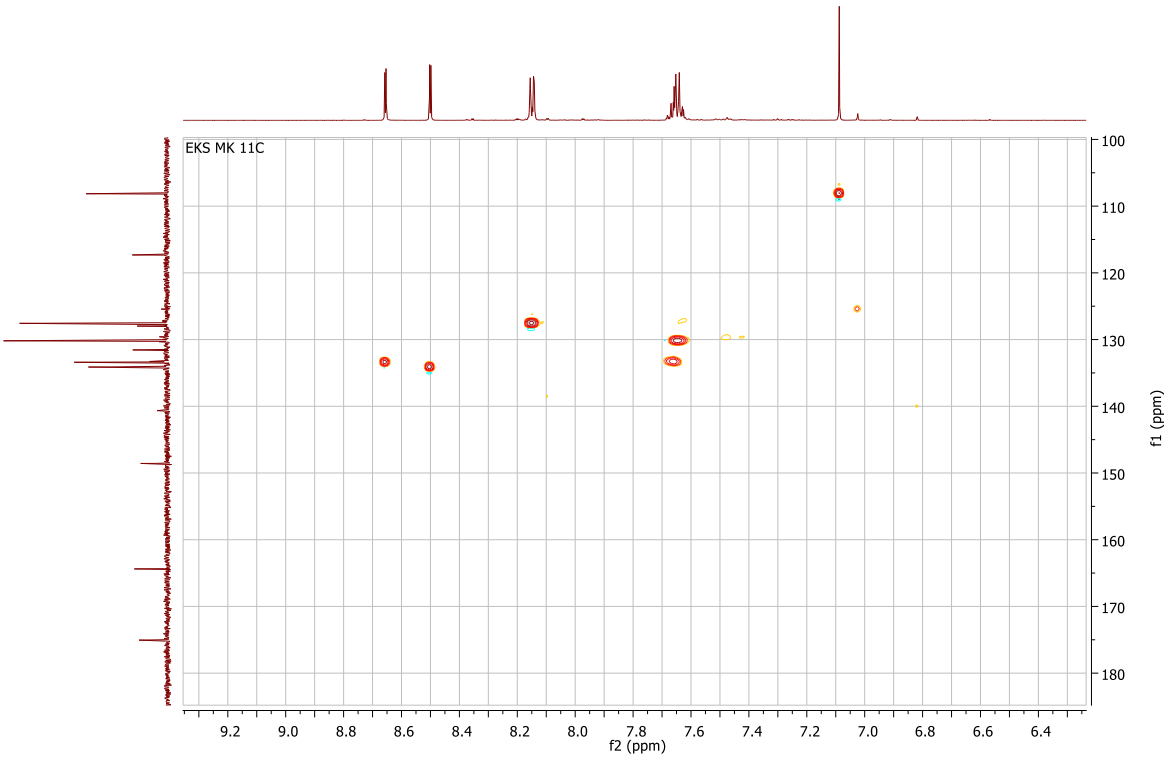



*Figure S93. HSQC contour map – $^{1}H$ x $^{13}C$ of 6-bromo-8-nitroflavone.*

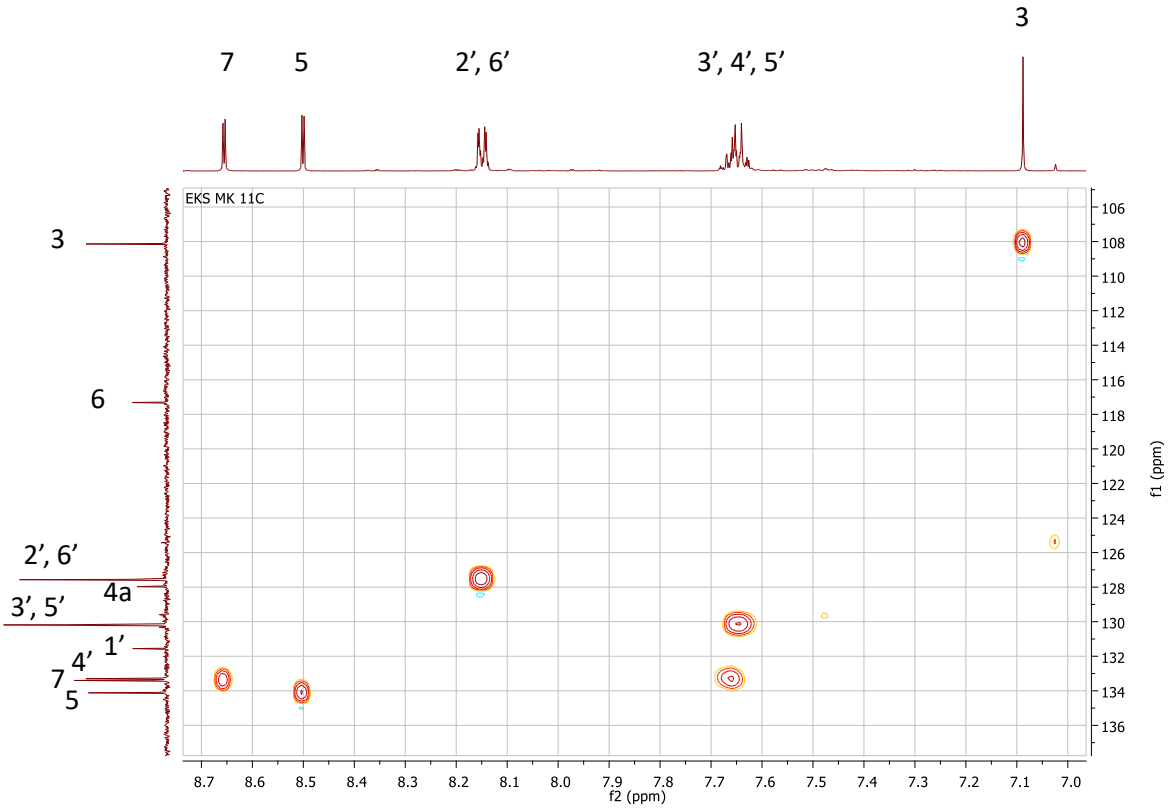



*Figure S94. HSQC contour map – $^{1}H$ x $^{13}C$ expansion of 6-bromo-8-nitroflavone.*

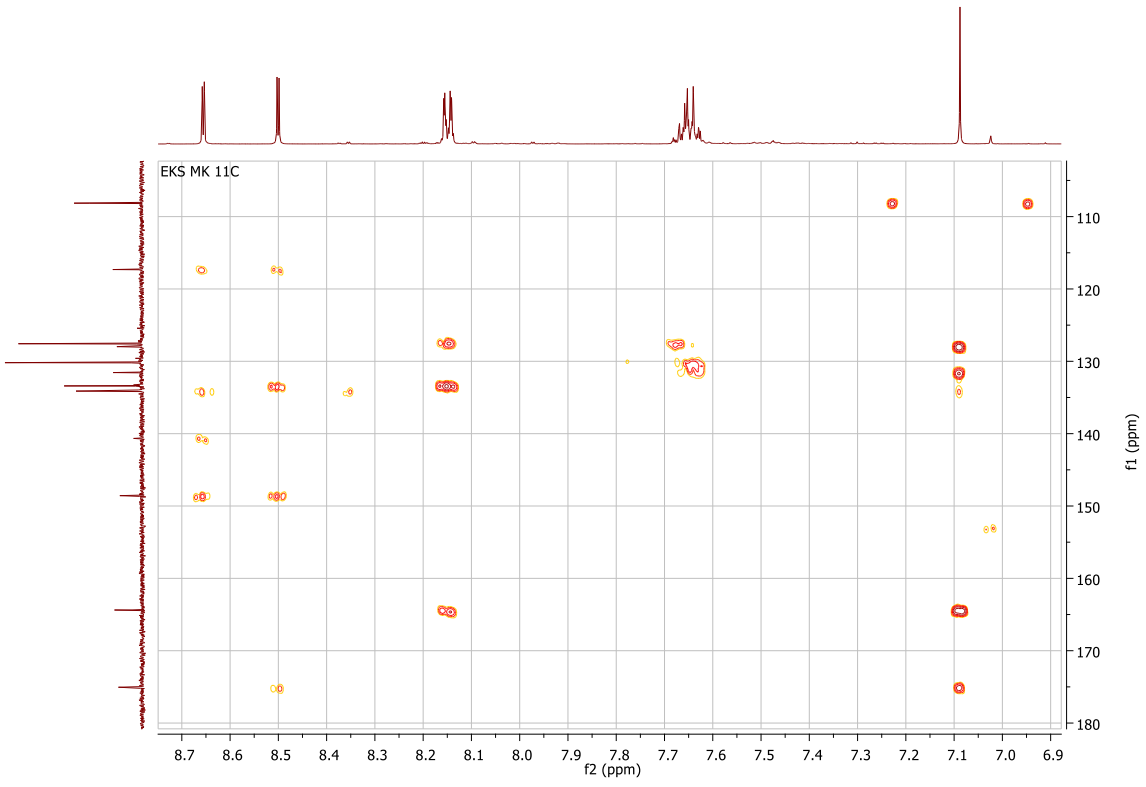



*Figure S95. HMBC contour map – $^{1}H$ x $^{13}C$ of 6-bromo-8-nitroflavone.*

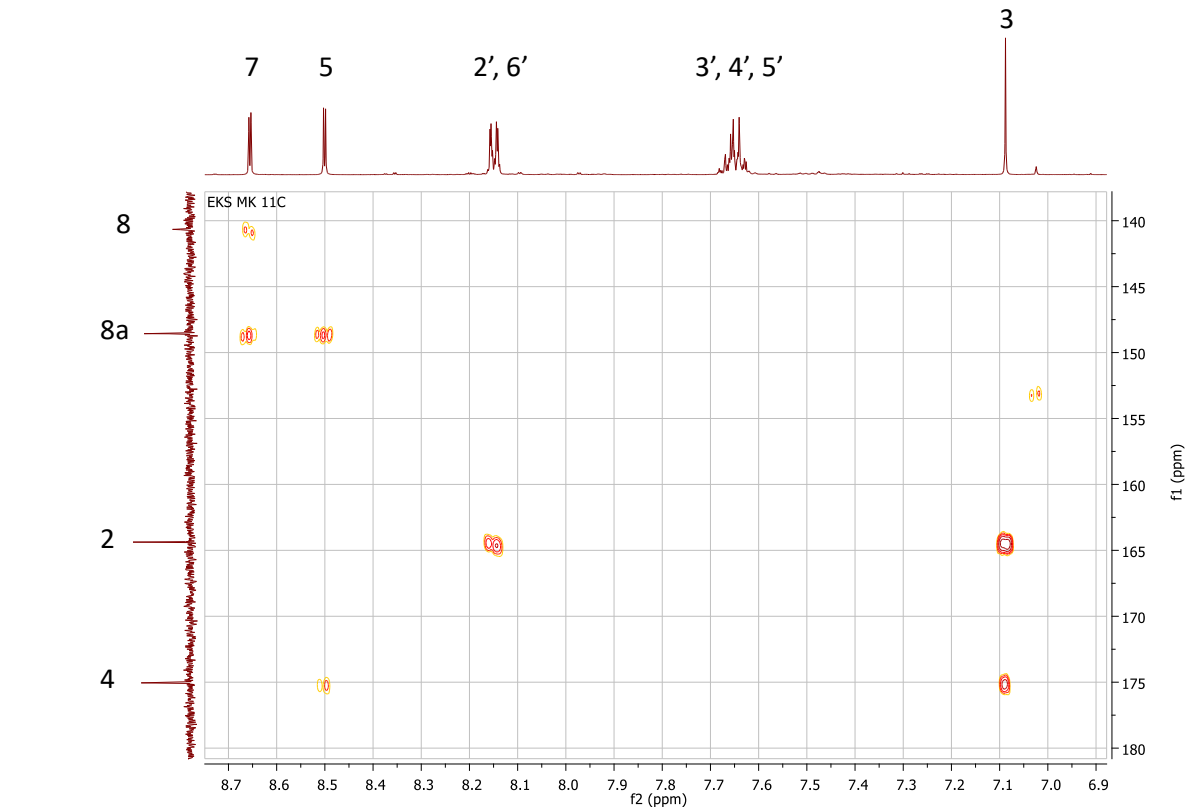



*Figure S96. HMBC contour map – $^{1}H$ x $^{13}C$ expansion of 6-bromo-8-nitroflavone.*

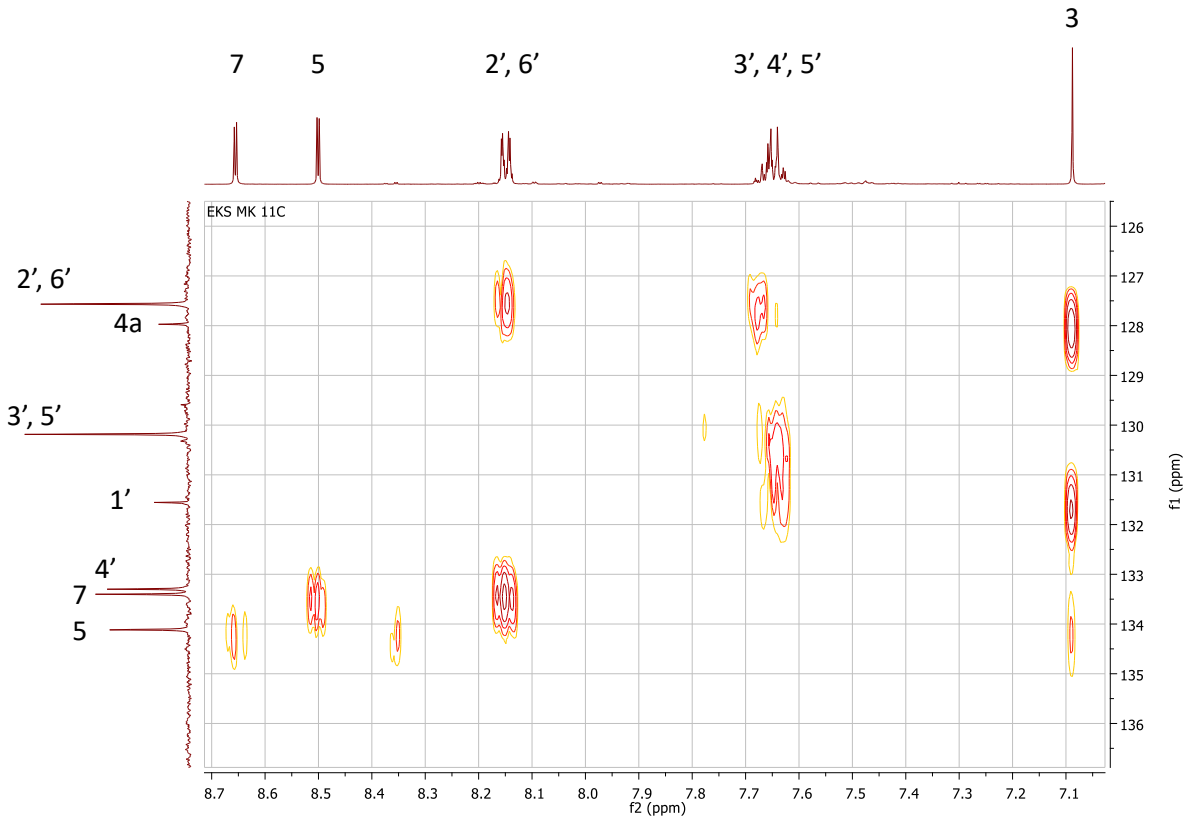



*Figure S97. HMBC contour map – $^{1}H$ x $^{13}C$ expansion of 6-bromo-8-nitroflavone.*

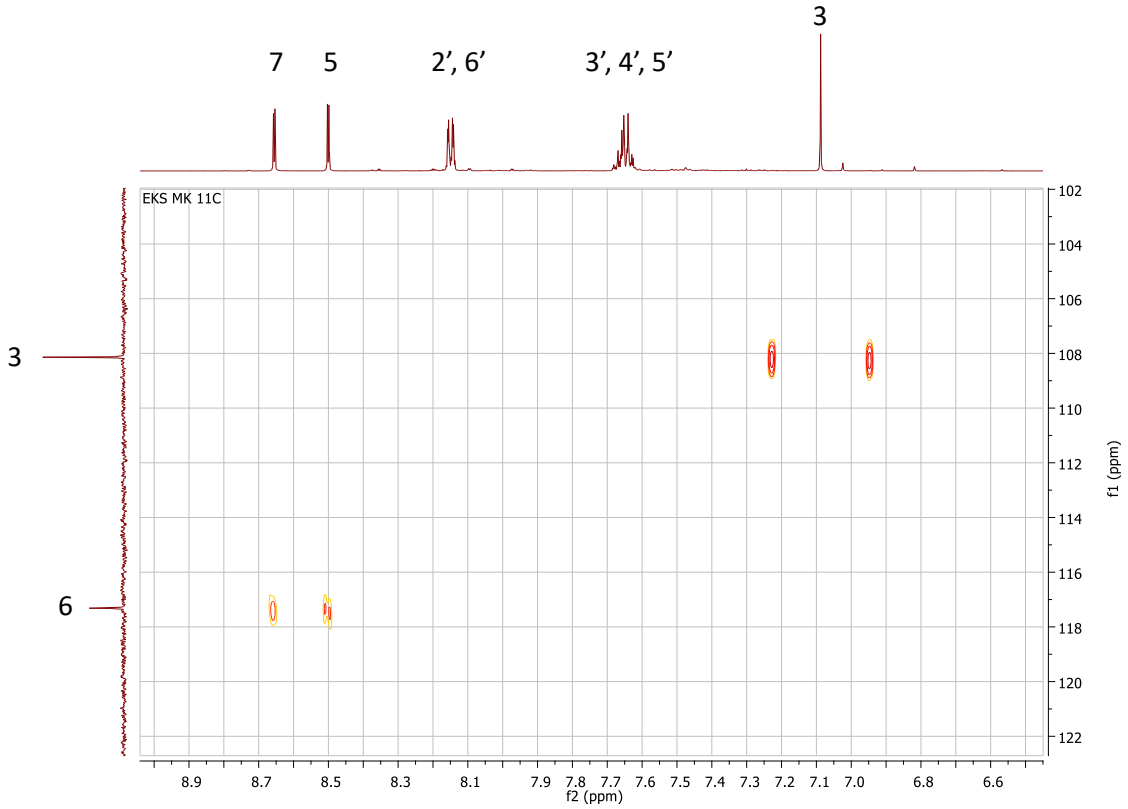
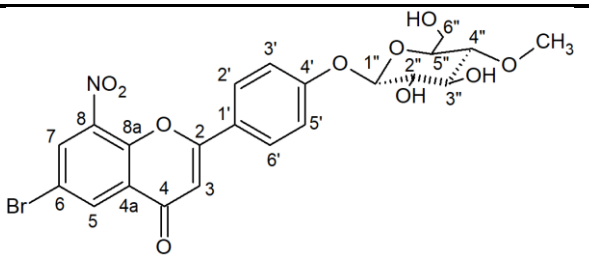



*Figure S98. HMBC contour map – $^{1}H$ x $^{13}C$ expansion of 6-bromo-8-nitroflavone.*

Compound name: 6-Bromo-8-nitroflavone 4′-*O*-*β*-D-(4″-O-methyl)-glucopyranoside
Molecular Formula: $C_{22}H_{20}BrNO_{10}$
Formula Weight: 538.299
Ionization mode: positive
Precursor $[M + H]^+$: 539.030
Monoisotopic Mass: 537.027
Collision energy (CE): -25.0



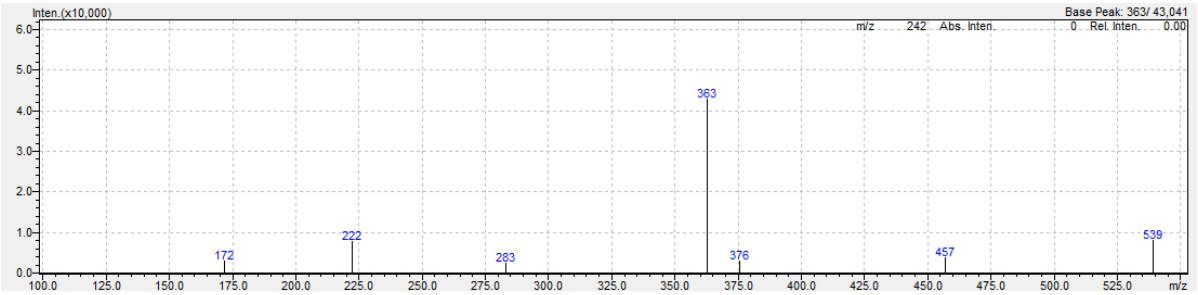



*Figure S99. LC-MS spectrum of 6-bromo-8-nitroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

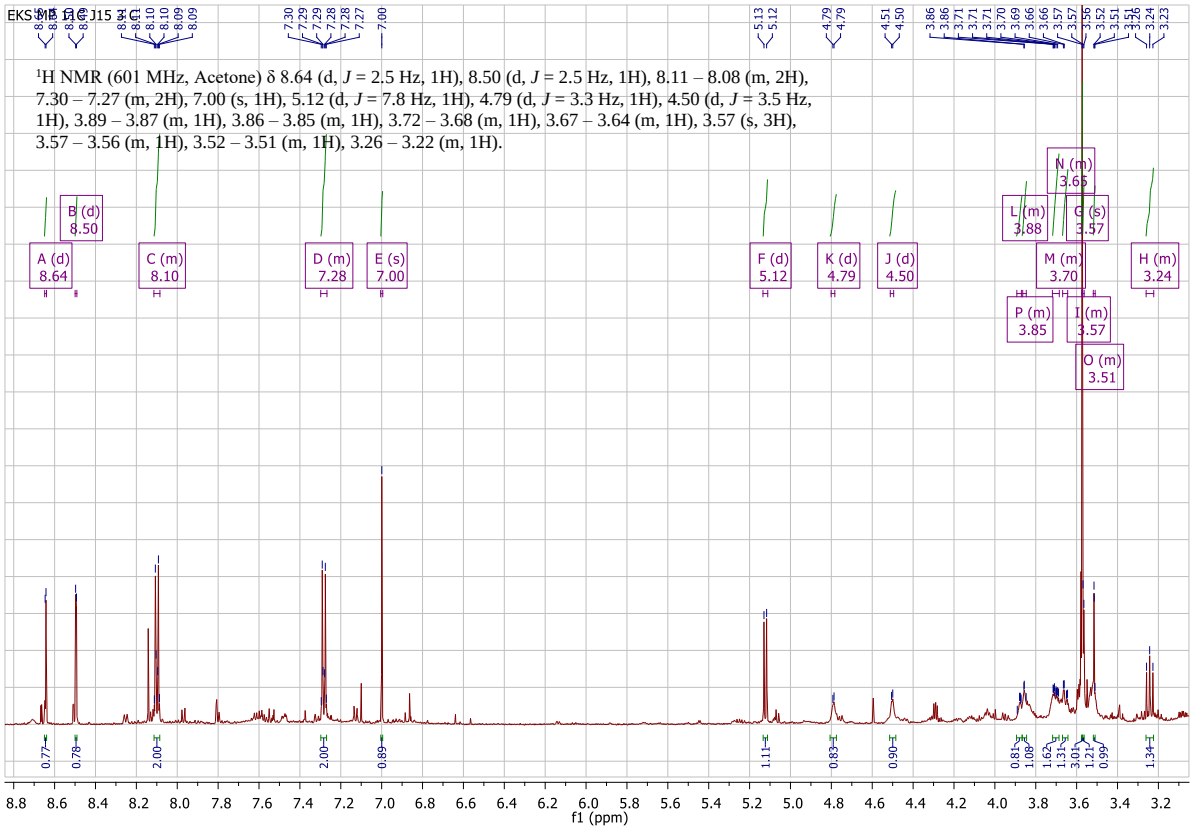



*Figure S100. ¹H NMR spectrum of 6-bromo-8-nitroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

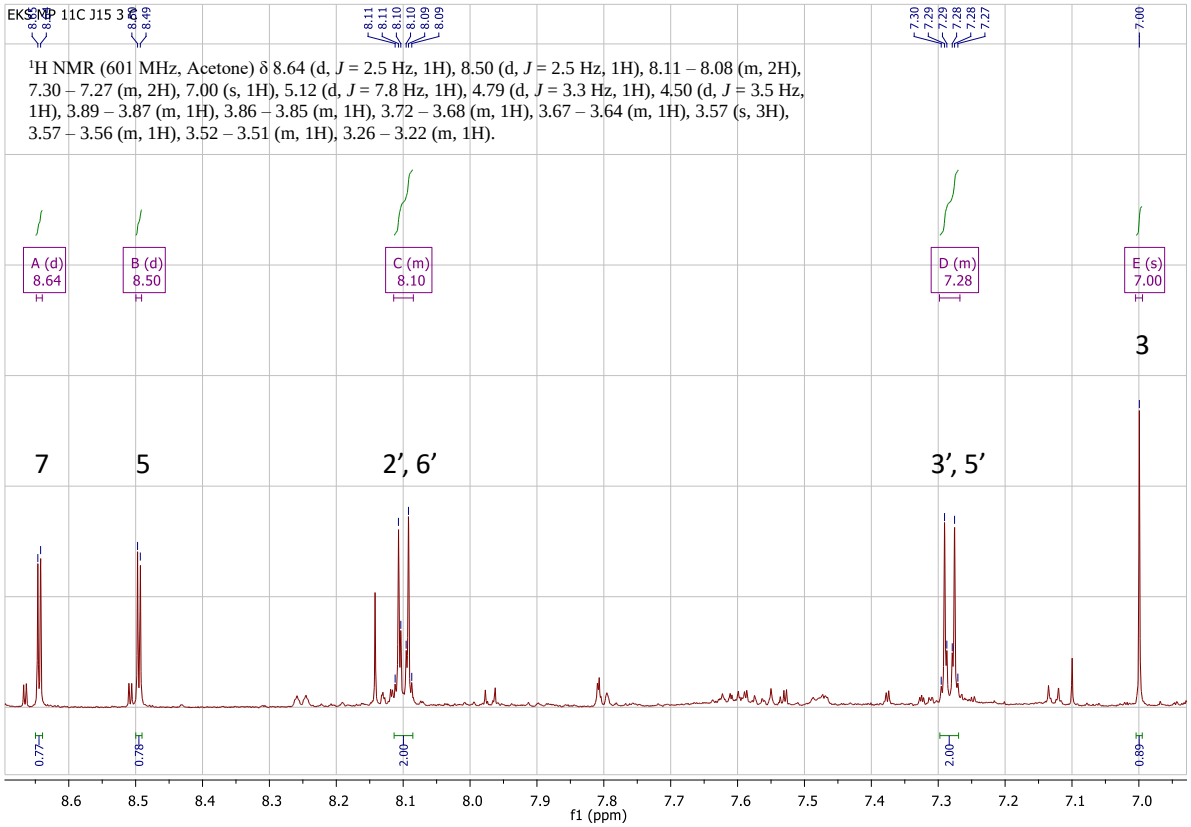



*Figure S101. ¹H NMR spectrum expansion of 6-bromo-8-nitroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

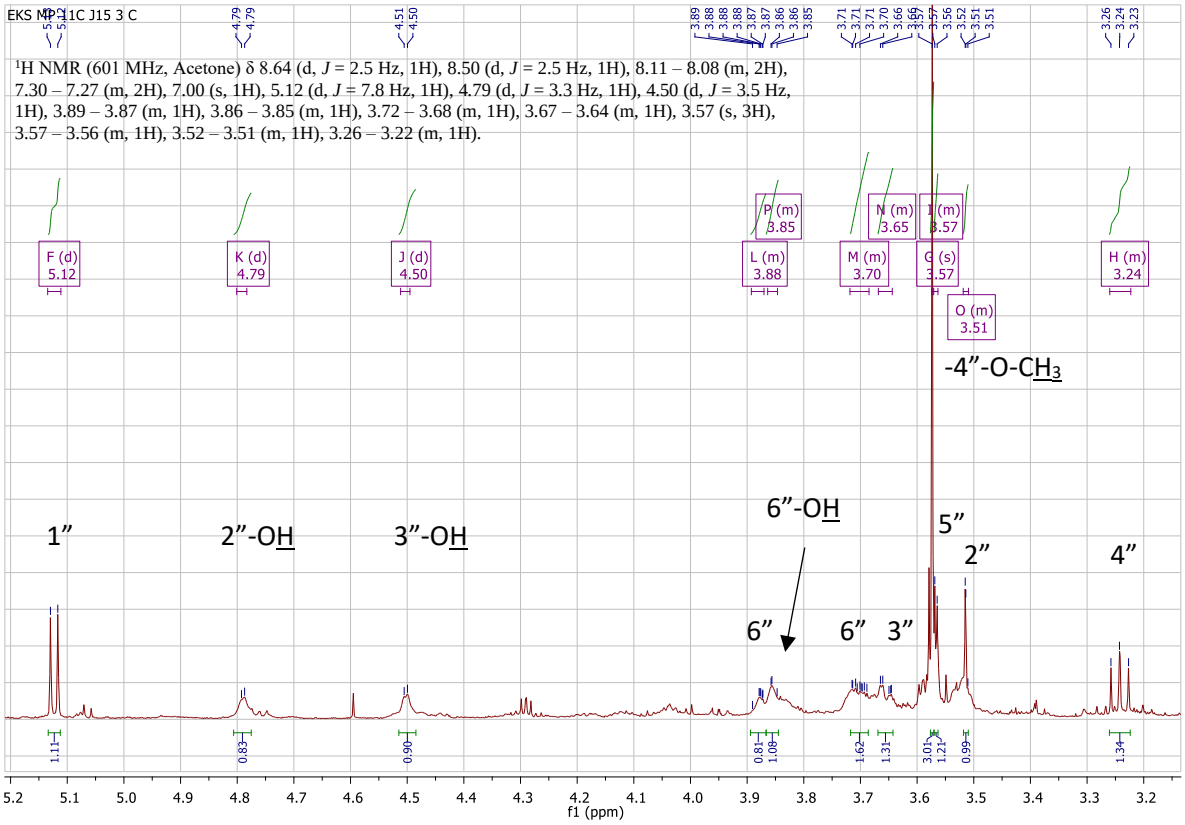



*Figure S102. [1]H NMR spectrum expansion of 6-bromo-8-nitroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

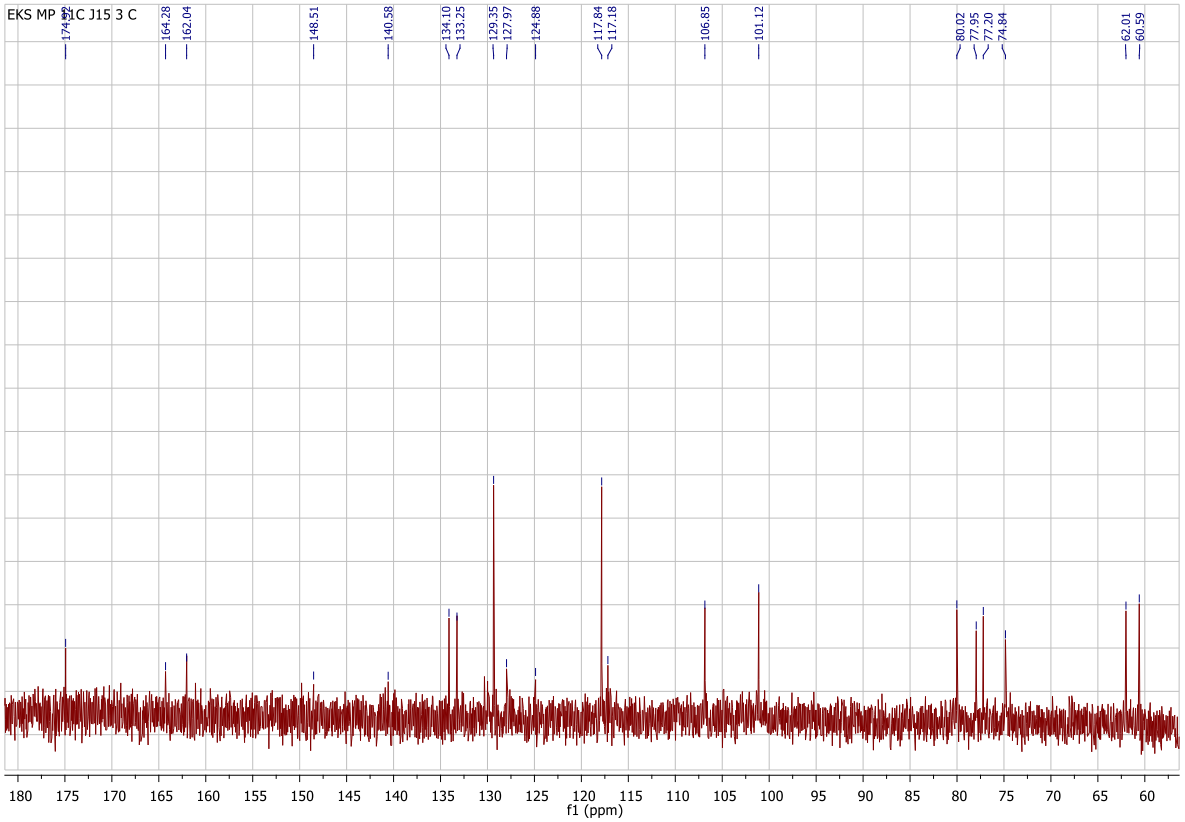



*Figure S103. [13]C NMR spectrum of 6-bromo-8-nitroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

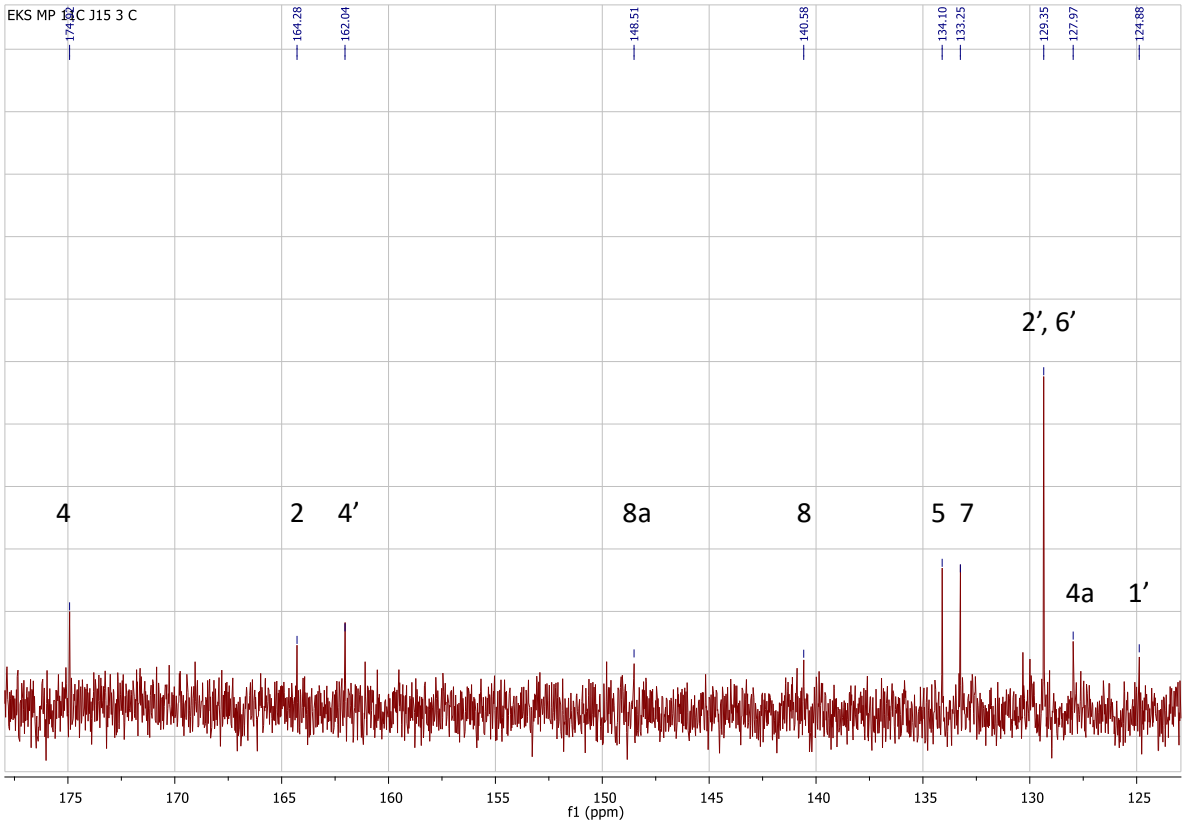



*Figure S104. $^{13}C$ NMR spectrum expansion of 6-bromo-8-nitroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

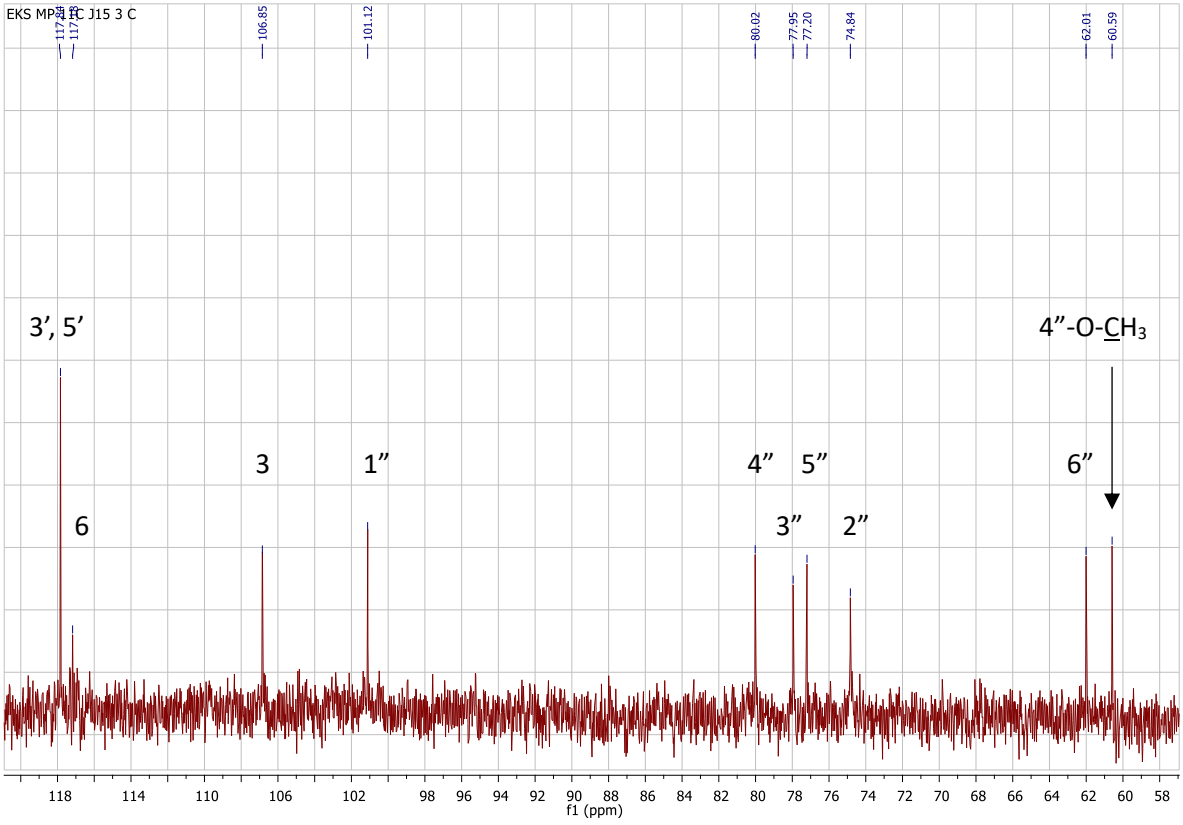



*Figure S105. $^{13}C$ NMR spectrum expansion of 6-bromo-8-nitroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

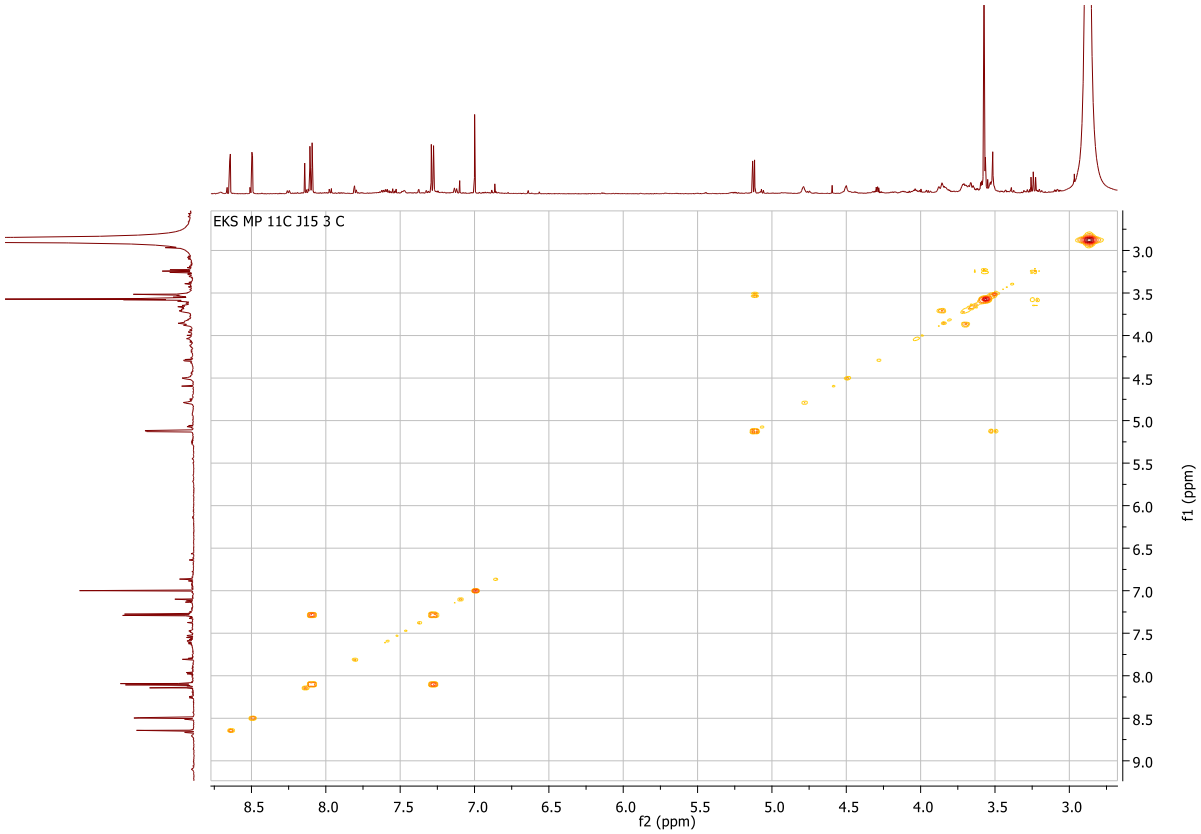



*Figure S106. COSY contour map – $^{1}H$ x $^{1}H$ of 6-bromo-8-nitroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

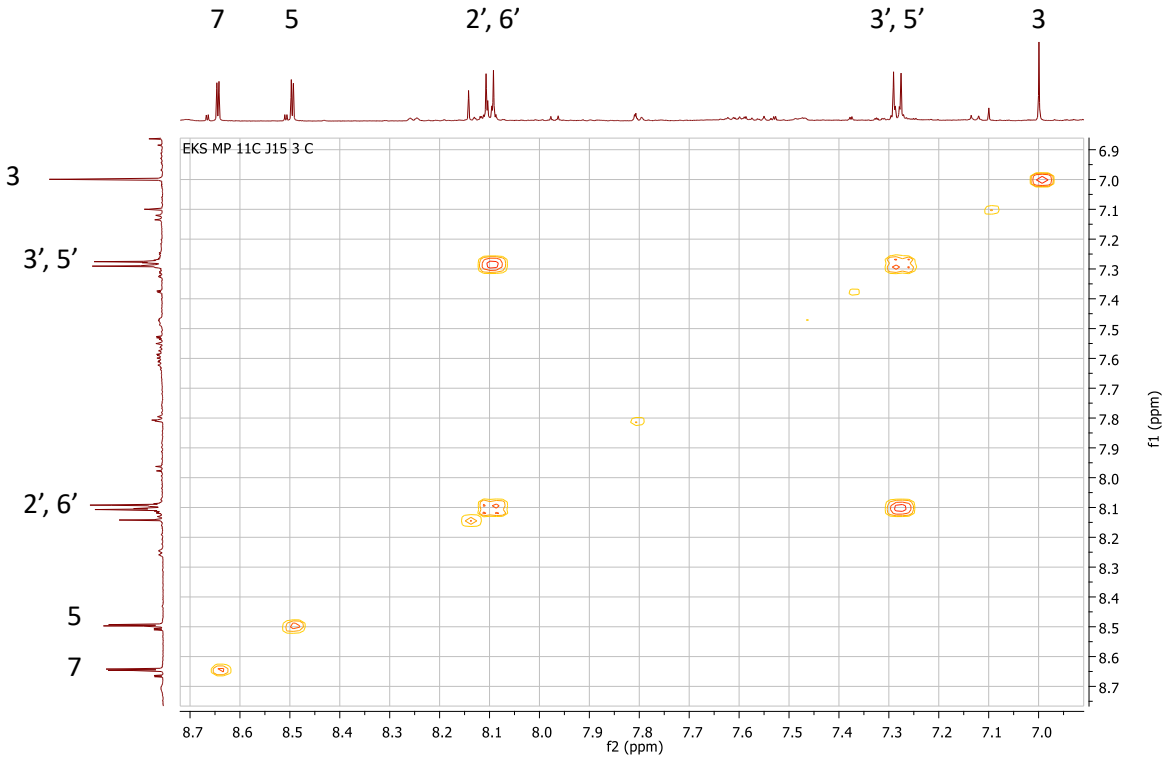



*Figure S107. COSY contour map – $^{1}H$ x $^{1}H$ expansion of 6-bromo-8-nitroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

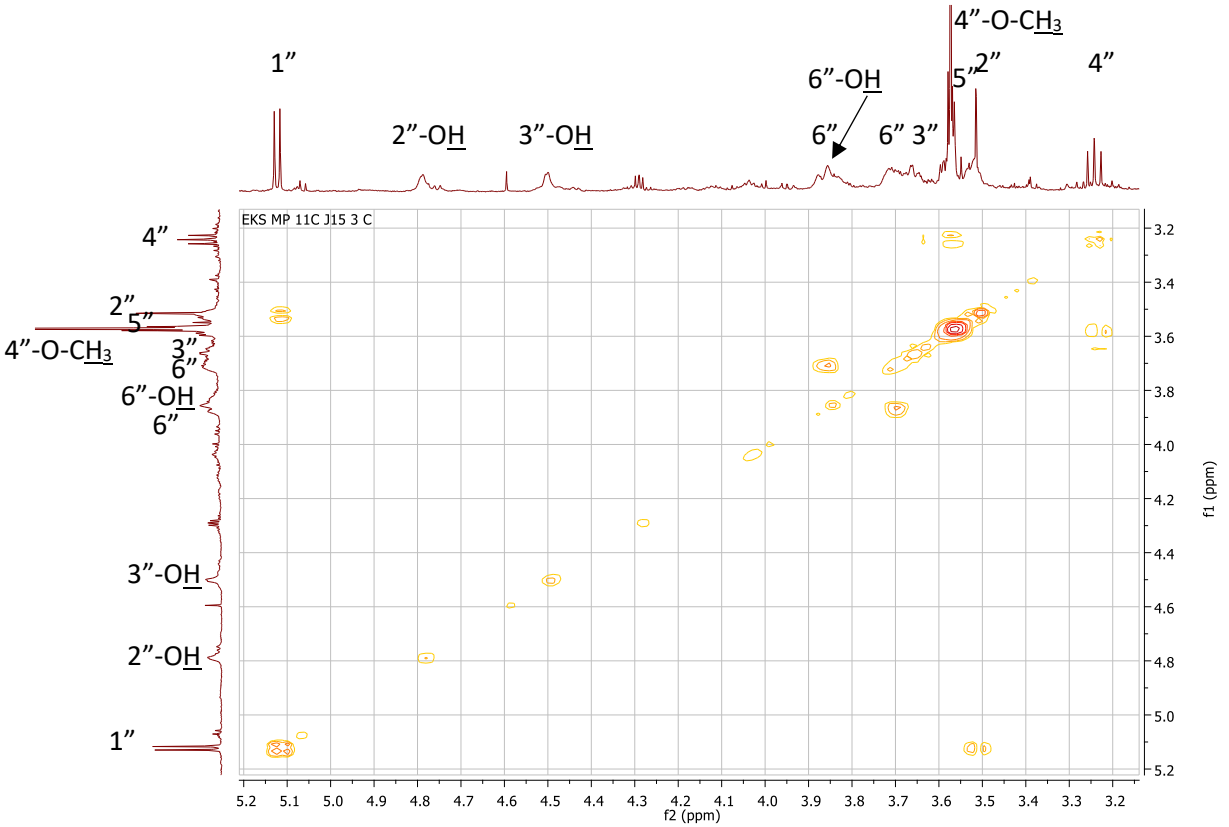



*Figure S108. COSY contour map – $^{1}H$ x $^{1}H$ expansion of 6-bromo-8-nitroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

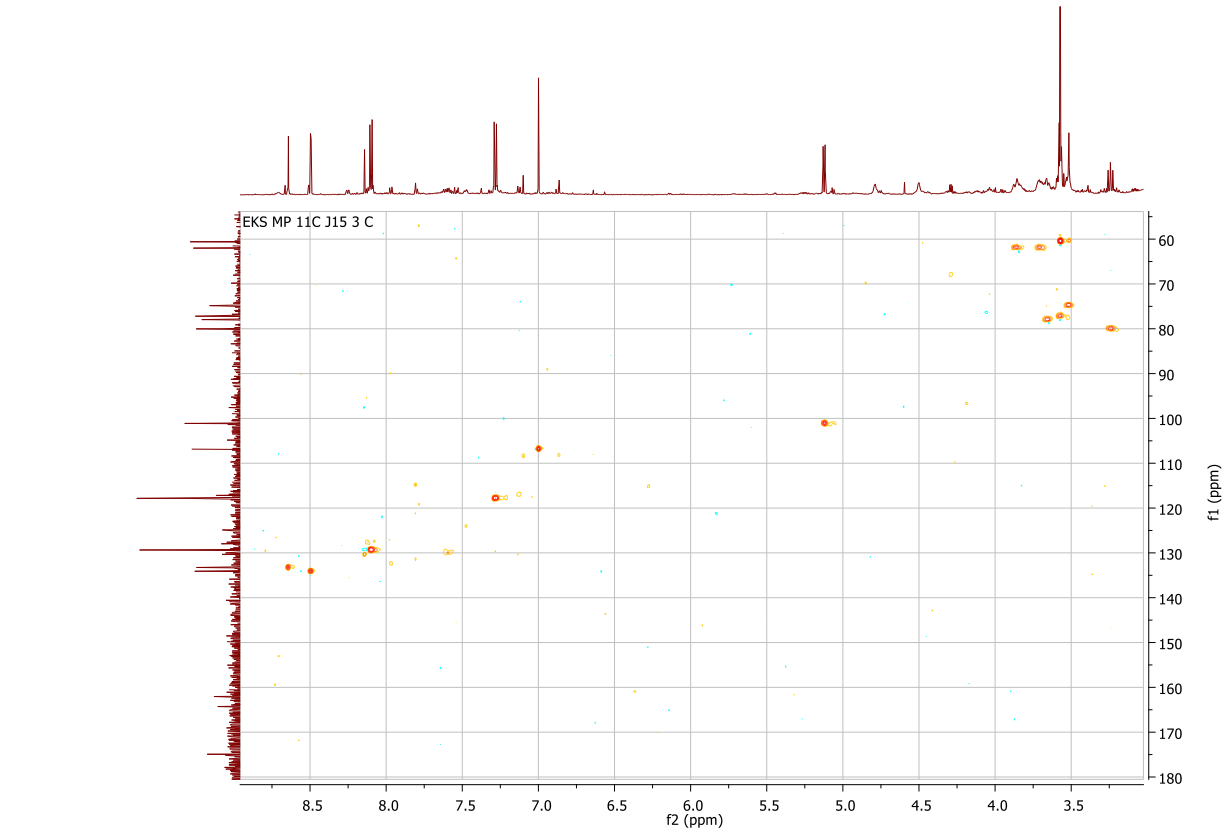



*Figure S109. HSQC contour map – $^{1}H$ x $^{13}C$ of 6-bromo-8-nitroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

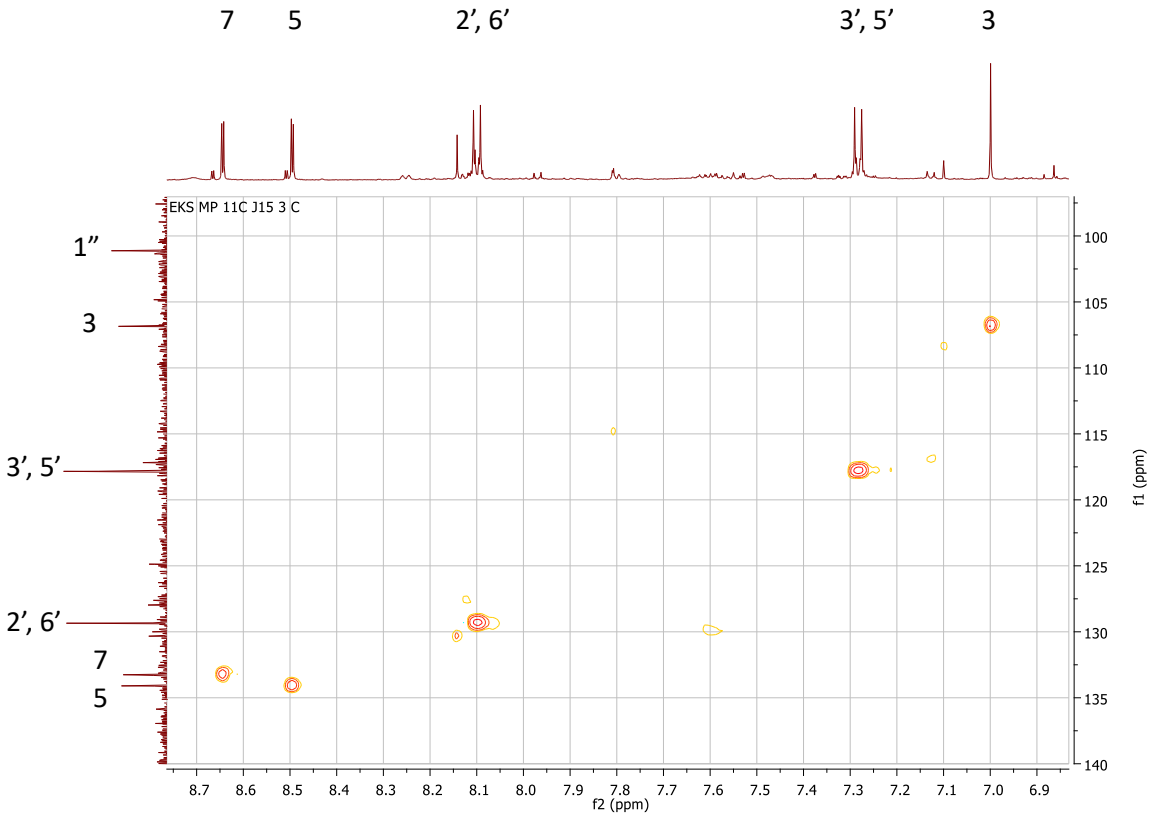



*Figure S110. HSQC contour map – $^{1}H$ x $^{13}C$ expansion of 6-bromo-8-nitroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

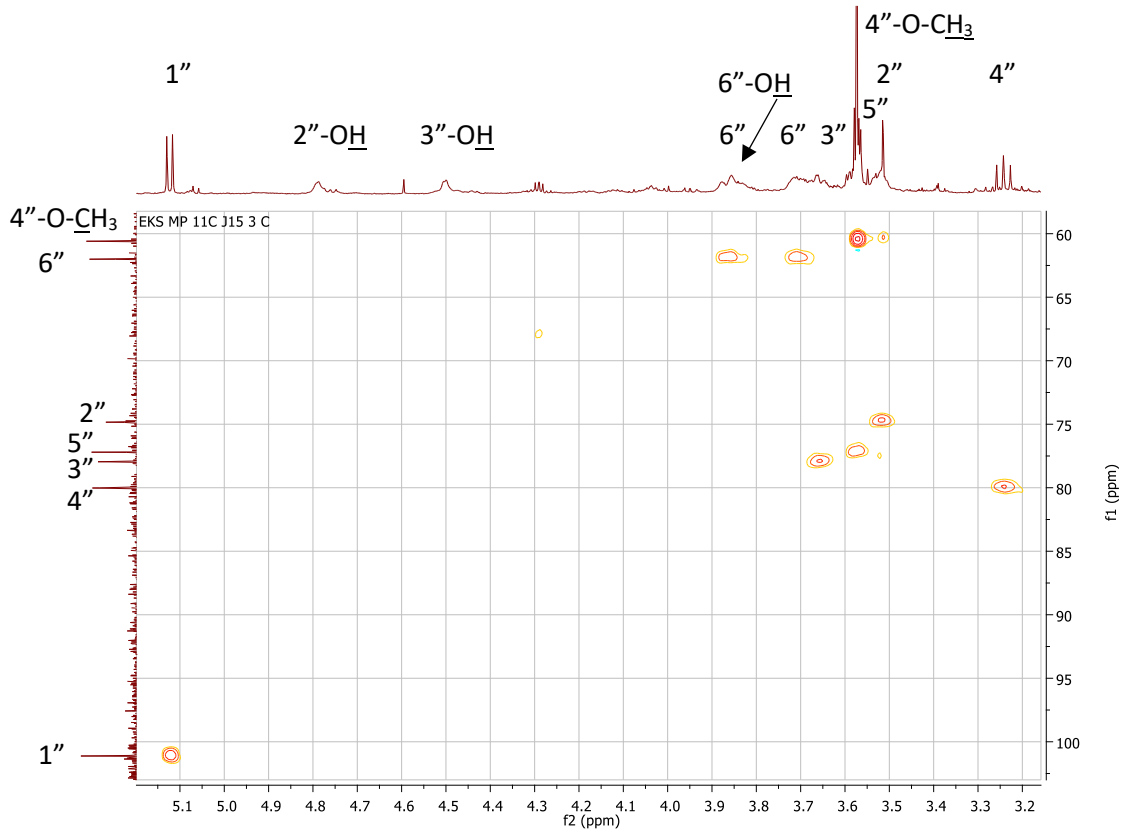



*Figure S111. HSQC contour map – $^{1}H$ x $^{13}C$ expansion of 6-bromo-8-nitroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

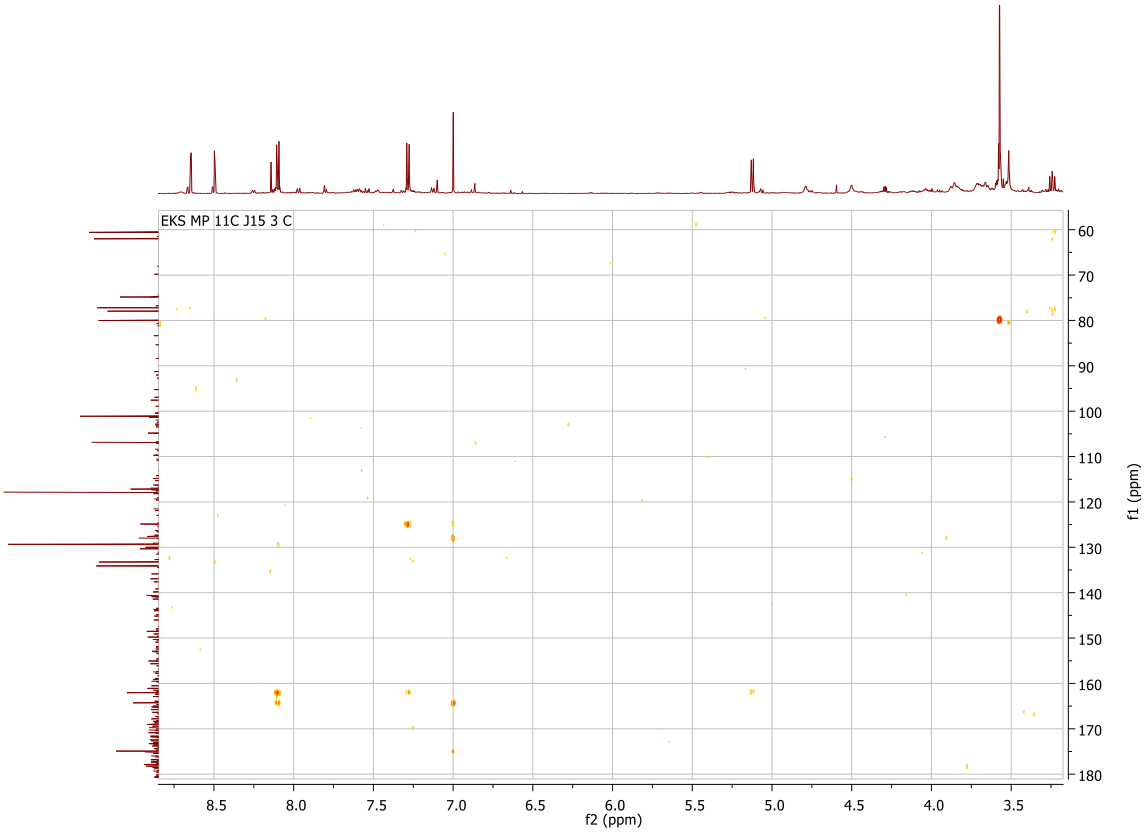



*Figure S112. HMBC contour map – $^{1}H$ x $^{13}C$ of 6-bromo-8-nitroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

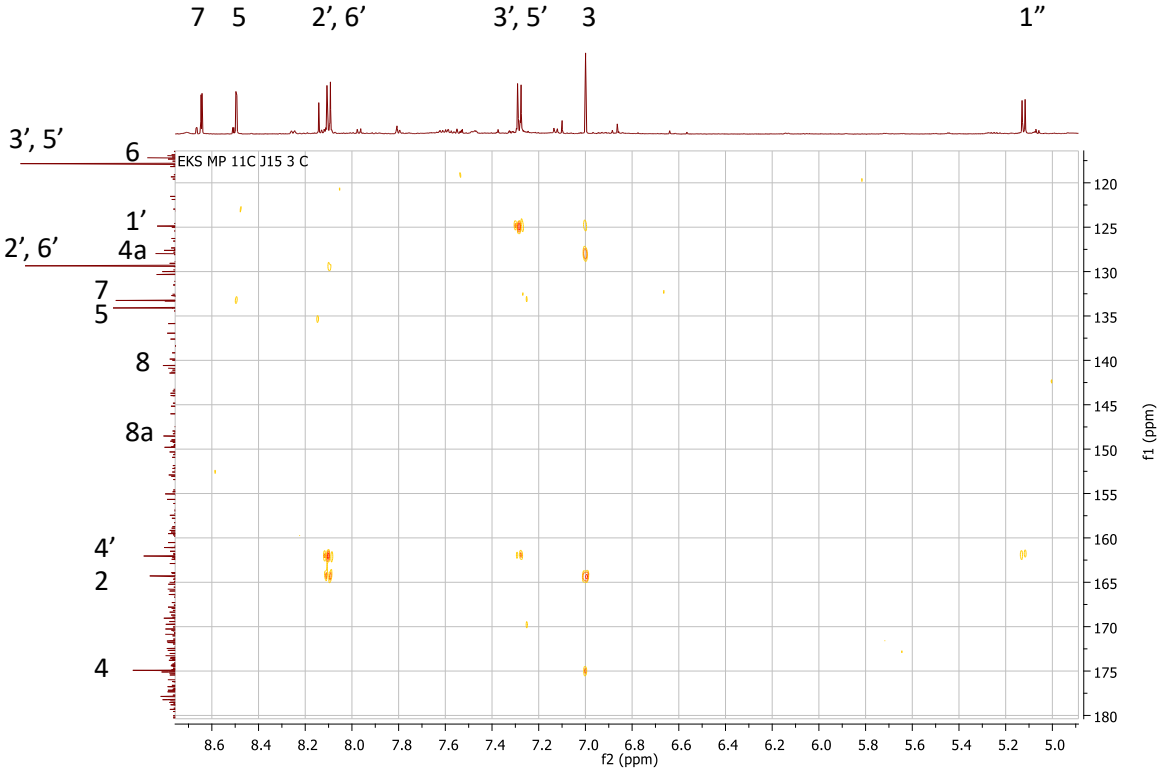



*Figure S113. HMBC contour map – $^{1}H$ x $^{13}C$ expansion of 6-bromo-8-nitroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*

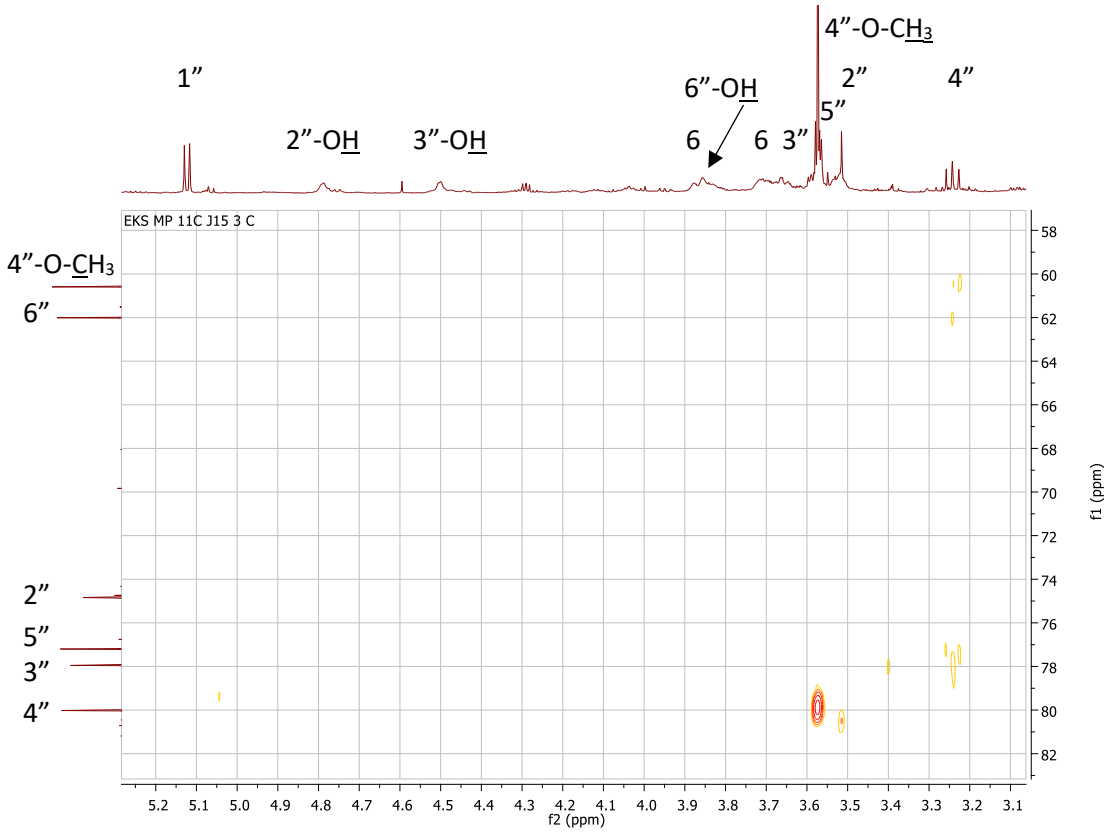



*Figure S114. HMBC contour map – $^{1}H$ x $^{13}C$ expansion of 6-bromo-8-nitroflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside.*