

## Supporting Information

### Total Synthesis and Metabolic Stability of Hispidulin and its *d*-Labelled Derivative

Liang-Chieh Chen, Kai-Cheng Hsu, Lih-Chu Chiou, Hui-Ju Tseng, and Wei-Jan Huang\*

## Table of Contents

|                                                                                                                   |    |
|-------------------------------------------------------------------------------------------------------------------|----|
| <b>Table S1.</b> Comparison of $^1\text{H}$ and $^{13}\text{C}$ NMR data of synthesis and natural hispidulin..... | 4  |
| <b>Figure S1.</b> $^1\text{H}$ NMR ( $\text{CDCl}_3$ , 300 MHz) spectrum of compound <b>20</b> .....              | 5  |
| <b>Figure S2.</b> $^{13}\text{C}$ NMR ( $\text{CDCl}_3$ , 125 MHz) spectrum of compound <b>20</b> .....           | 5  |
| <b>Figure S3.</b> $^1\text{H}$ NMR ( $\text{CDCl}_3$ , 300 MHz) spectrum of compound <b>21</b> .....              | 6  |
| <b>Figure S4.</b> $^{13}\text{C}$ NMR ( $\text{CDCl}_3$ , 125 MHz) spectrum of compound <b>21</b> .....           | 6  |
| <b>Figure S5.</b> ROESY spectrum of compound <b>21</b> .....                                                      | 7  |
| <b>Figure S6.</b> $^1\text{H}$ NMR ( $\text{CDCl}_3$ , 300 MHz) spectrum of compound <b>22</b> .....              | 7  |
| <b>Figure S7.</b> $^{13}\text{C}$ NMR ( $\text{CDCl}_3$ , 125 MHz) spectrum of compound <b>22</b> .....           | 8  |
| <b>Figure S8.</b> $^1\text{H}$ NMR ( $\text{CDCl}_3$ , 300 MHz) spectrum of compound <b>14</b> .....              | 8  |
| <b>Figure S9.</b> $^{13}\text{C}$ NMR ( $\text{CDCl}_3$ , 125 MHz) spectrum of compound <b>14</b> .....           | 9  |
| <b>Figure S10.</b> $^1\text{H}$ NMR ( $\text{CDCl}_3$ , 300 MHz) spectrum of compound <b>23a</b> .....            | 9  |
| <b>Figure S11.</b> $^{13}\text{C}$ NMR ( $\text{CDCl}_3$ , 125 MHz) spectrum of compound <b>23a</b> .....         | 10 |
| <b>Figure S12.</b> $^1\text{H}$ NMR ( $\text{CDCl}_3$ , 300 MHz) spectrum of compound <b>23b</b> .....            | 10 |
| <b>Figure S13.</b> $^{13}\text{C}$ NMR ( $\text{CDCl}_3$ , 125 MHz) spectrum of compound <b>23b</b> .....         | 11 |
| <b>Figure S14.</b> $^1\text{H}$ NMR ( $\text{CDCl}_3$ , 300 MHz) spectrum of compound <b>24a</b> .....            | 11 |
| <b>Figure S15.</b> $^{13}\text{C}$ NMR ( $\text{CDCl}_3$ , 125 MHz) spectrum of compound <b>24a</b> .....         | 12 |
| <b>Figure S16.</b> $^1\text{H}$ NMR ( $\text{DMSO}-d_6$ , 300 MHz) spectrum of compound <b>24b</b> .....          | 12 |
| <b>Figure S17.</b> $^{13}\text{C}$ NMR ( $\text{DMSO}-d_6$ , 125 MHz) spectrum of compound <b>24b</b> .....       | 13 |
| <b>Figure S18.</b> $^1\text{H}$ NMR ( $\text{CDCl}_3$ , 300 MHz) spectrum of compound <b>25a</b> .....            | 13 |
| <b>Figure S19.</b> $^{13}\text{C}$ NMR ( $\text{DMSO}-d_6$ , 125 MHz) spectrum of compound <b>25a</b> .....       | 14 |
| <b>Figure S20.</b> $^1\text{H}$ NMR ( $\text{DMSO}-d_6$ , 300 MHz) spectrum of compound <b>25b</b> .....          | 14 |
| <b>Figure S21.</b> $^{13}\text{C}$ NMR ( $\text{DMSO}-d_6$ , 125 MHz) spectrum of compound <b>25b</b> .....       | 15 |
| <b>Figure S22.</b> $^1\text{H}$ NMR ( $\text{DMSO}-d_6$ , 500 MHz) spectrum of hispidulin.....                    | 15 |
| <b>Figure S23.</b> $^{13}\text{C}$ NMR ( $\text{DMSO}-d_6$ , 125 MHz) spectrum of hispidulin.....                 | 16 |
| <b>Figure S24.</b> ROESY spectrum of hispidulin.....                                                              | 16 |
| <b>Figure S25.</b> HSQC spectrum of hispidulin.....                                                               | 17 |

|                                                                                                               |    |
|---------------------------------------------------------------------------------------------------------------|----|
| Figure S26. HMBC spectrum of hispidulin.....                                                                  | 17 |
| Figure S27. HMBC spectrum of hispidulin.....                                                                  | 18 |
| Figure S28. HMBC spectrum of hispidulin.....                                                                  | 18 |
| Figure S29. <sup>1</sup> H NMR (DMSO- <i>d</i> <sub>6</sub> , 500 MHz) spectrum of <i>d</i> -hispidulin.....  | 19 |
| Figure S30. <sup>13</sup> C NMR (DMSO- <i>d</i> <sub>6</sub> , 125 MHz) spectrum of <i>d</i> -hispidulin..... | 19 |
| Figure S31. ROESY spectrum of <i>d</i> -hispidulin.....                                                       | 20 |
| Figure S32. HSQC spectrum of <i>d</i> -hispidulin.....                                                        | 20 |
| Figure S33. HSQC spectrum of <i>d</i> -hispidulin.....                                                        | 21 |
| Figure S34. HMBC spectrum of <i>d</i> -hispidulin.....                                                        | 21 |
| Figure S35. HMBC spectrum of <i>d</i> -hispidulin.....                                                        | 22 |
| Figure S36. HMBC spectrum of <i>d</i> -hispidulin.....                                                        | 22 |
| Figure S37. HPLC chromatogram of compound 20.....                                                             | 23 |
| Figure S38. HPLC chromatogram of compound 21.....                                                             | 23 |
| Figure S39. HPLC chromatogram of compound 22.....                                                             | 23 |
| Figure S40. HPLC chromatogram of compound 14.....                                                             | 24 |
| Figure S41. HPLC chromatogram of compound 23a.....                                                            | 24 |
| Figure S42. HPLC chromatogram of compound 23b.....                                                            | 24 |
| Figure S43. HPLC chromatogram of compound 24a.....                                                            | 25 |
| Figure S44. HPLC chromatogram of compound 24b.....                                                            | 25 |
| Figure S45. HPLC chromatogram of compound 25a.....                                                            | 25 |
| Figure S46. HPLC chromatogram of compound 25b.....                                                            | 26 |
| Figure S47. HPLC chromatogram of hispidulin.....                                                              | 26 |
| Figure S48. HPLC chromatogram of <i>d</i> -hispidulin.....                                                    | 26 |

**Table S1.** Comparison of  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of hispidulin synthesized in this study and in literature.

| Position | This study <sup>1</sup>           |                     | Literature <sup>2</sup>           |                     |
|----------|-----------------------------------|---------------------|-----------------------------------|---------------------|
|          | $\delta_{\text{H}}$ , m<br>(J/Hz) | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ , m<br>(J/Hz) | $\delta_{\text{C}}$ |
| 2        |                                   | 163.8, qC           |                                   | 163.8, qC           |
| 3        | 6.77, s                           | 102.4, CH           | 6.75, s                           | 102.4, CH           |
| 4        |                                   | 182.2, qC           |                                   | 182.1, qC           |
| 5        |                                   | 152.8, qC           |                                   | 152.8, qC           |
| 5-OH     | 13.07, s                          |                     | 13.05                             |                     |
| 6        |                                   | 131.4, qC           |                                   | 131.4, qC           |
| 6-OMe    | 3.74, s                           | 60.0                | 3.73, s                           | 59.9                |
| 7        |                                   | 157.3, qC           |                                   | 157.3, qC           |
| 7-OH     | 10.73, s                          |                     |                                   |                     |
| 8        | 6.59, s                           | 94.3, CH            | 6.57, s                           | 94.2, CH            |
| 9        |                                   | 152.4, qC           |                                   | 152.4, qC           |
| 10       |                                   | 104.1, qC           |                                   | 104.0, qC           |
| 1'       |                                   | 121.2, qC           |                                   | 121.2, qC           |
| 2', 6'   | 7.92, d (8.9)                     | 128.5, CH           | 7.90, d (8.8)                     | 128.4, CH           |
| 3', 5'   | 6.92, d (8.9)                     | 116.0, CH           | 6.90, d (8.8)                     | 115.9, CH           |
| 4'       |                                   | 161.2, qC           |                                   | 161.2, qC           |
| 4'-OH    | 10.38, s                          |                     |                                   |                     |

<sup>1</sup> $^1\text{H}$  NMR (500 MHz, DMSO-*d*<sub>6</sub>); <sup>13</sup> $^{13}\text{C}$  NMR (125 MHz, DMSO-*d*<sub>6</sub>); <sup>2</sup>  $^1\text{H}$  NMR (400 MHz, DMSO-*d*<sub>6</sub>);  $^{13}\text{C}$  NMR (100 MHz, DMSO-*d*<sub>6</sub>); data obtained from reference 33.

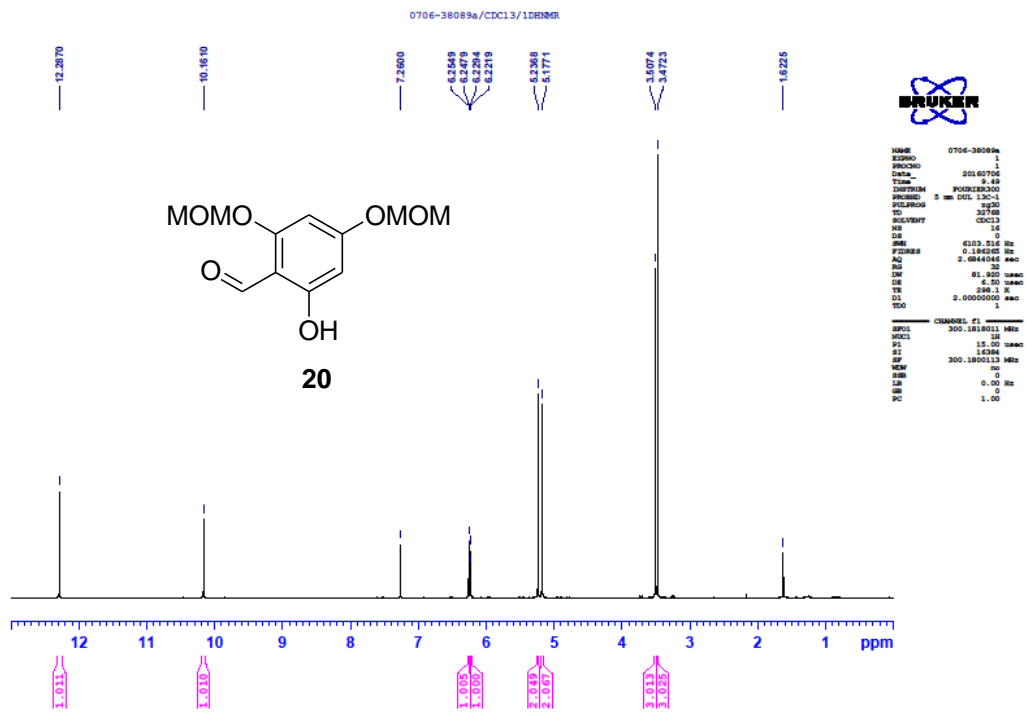


Figure S1. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of compound 20

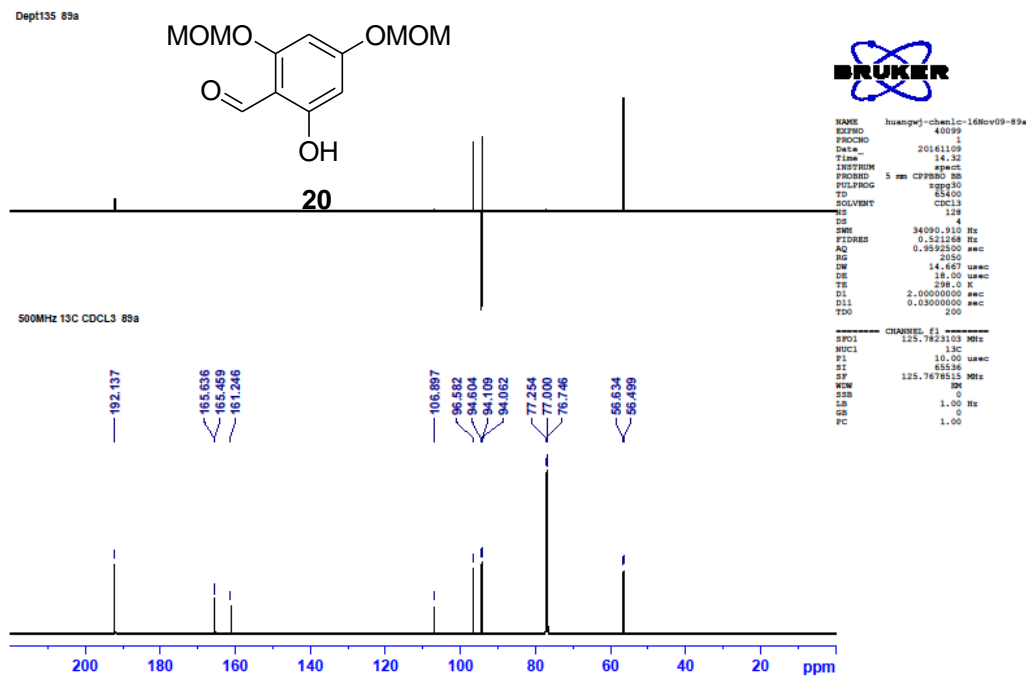


Figure S2. <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) spectrum of compound 20

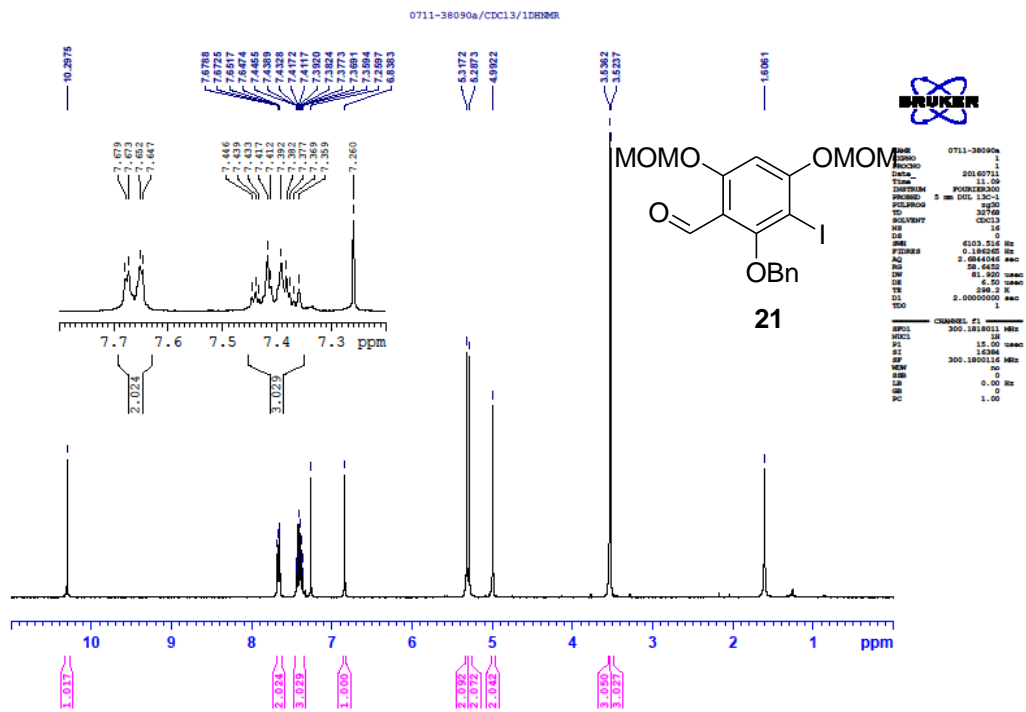


Figure S3. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of compound 21

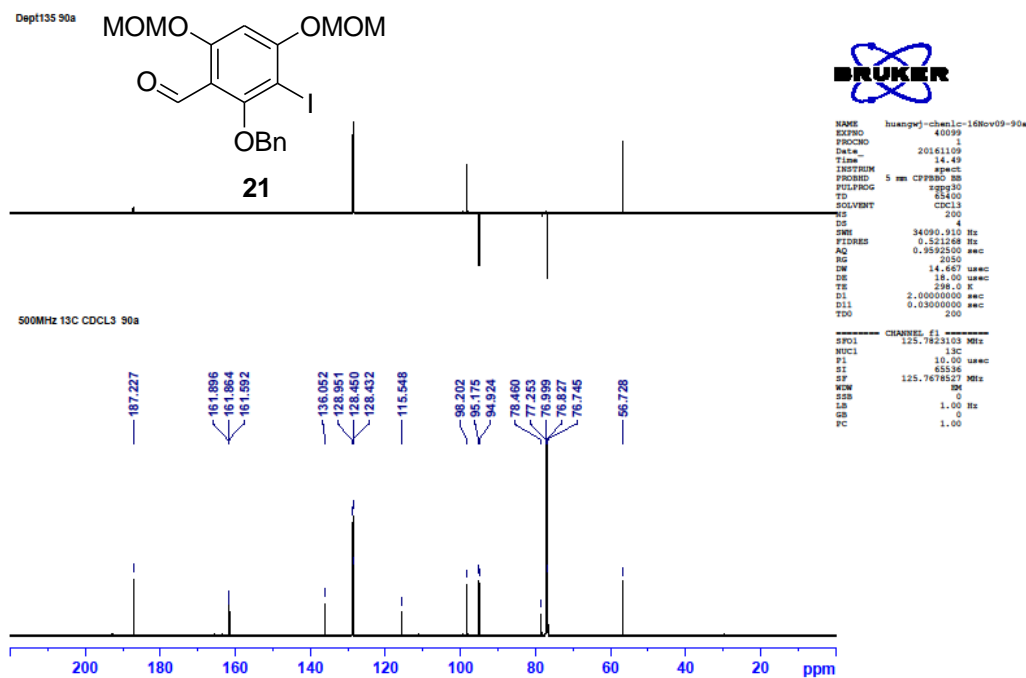
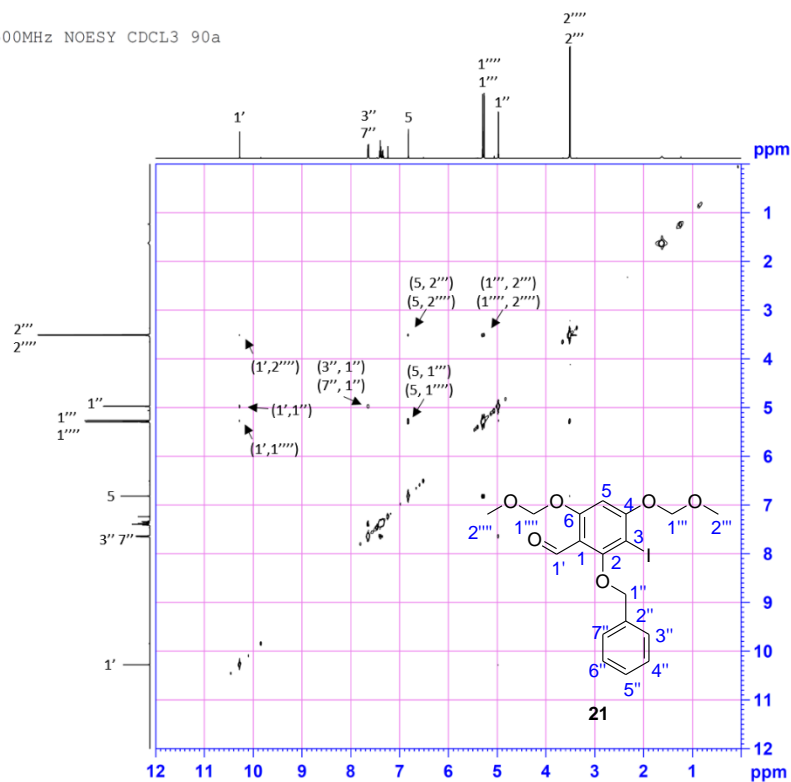


Figure S4. <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) spectrum of compound 21

500MHz NOESY CDCl<sub>3</sub> 90a

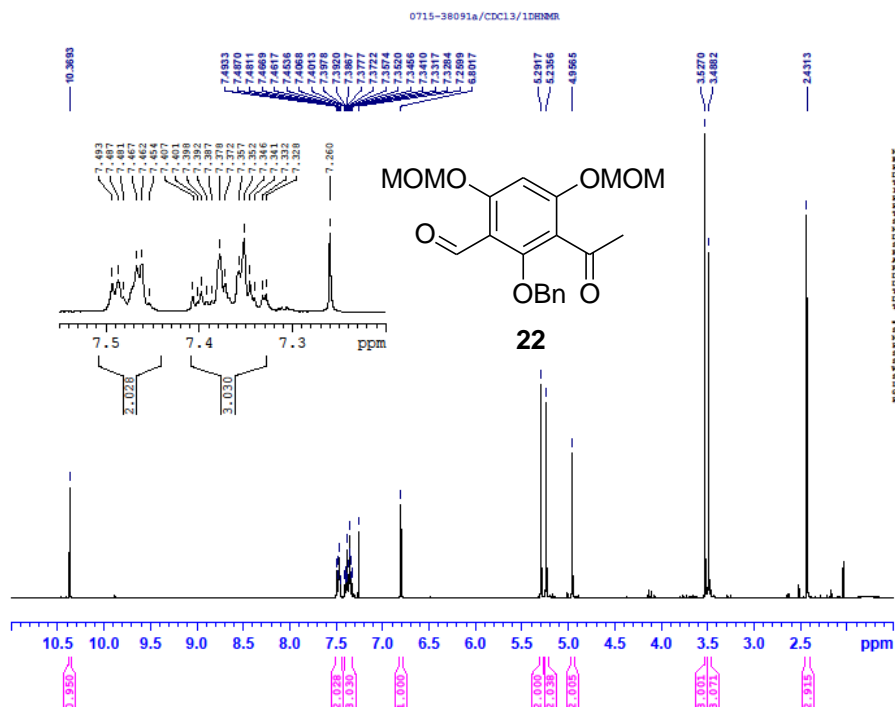


```

NAME huangwj-chen1c-16Nov09-90a
EXPNO 71
PROCNO 1
Date_ 20161111
Time 14.33
INSTRUM spect
PROBHD 5 mm CPMAS BB
PULPROG noesypphpgp
TD 2048
SOLVENT CDCl3
NS 8
DS 80
SWH 7002.801 Hz
FIDRES 3.419337 Hz
AQ 0.1462772 sec
RG 22.6
DM 71.400 usec
DE 22.00 usec
TE 298.0 K
D0 0.00005638 sec
D1 2.00000000 sec
D2 0.40000001 sec
D11 0.03000000 sec
D12 0.00020000 sec
D16 0.00020000 sec
INO 0.00014280 sec

===== CHANNEL f1 =====
SFO1 500.170010 MHz
NUC1 1H
P1 11.80 usec
F2 23.60 usec
PI7 2500.00 usec
NDO 1
TD 512
SFO1 500.173 MHz
FIDRES 13.477346 Hz
SW 14.001 ppm
PRMODE States-TFPI
SI 2048
SF 500.170020 MHz
WAW QSIINE
SSB 0
LB 0.00 Hz
GB 0
PC 1.00
SI 2048
MC2 States-TFPI
SF 500.170020 MHz
WAW QSIINE
SSB 0
LB 0.00 Hz
GB 0
  
```

Figure S5. ROESY spectrum of compound 21



```

NAME 0715-38091a
EXPNO 1
PROCNO 1
Date_ 20160715
Time 10.26
INSTRUM spect
PROBHD 5 mm DUL 13C-1
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 16
DS 16
SWH 6103.516 Hz
FIDRES 0.186026 Hz
AQ 2.6844046 sec
RG 256
DM 01.920 usec
DE 6.50 usec
TE 298.0 K
D0 2.00000000 sec
D1 1
D11 0
D12 0
D16 0
INO 0

===== CHANNEL f1 =====
SFO1 300.188011 MHz
NUC1 13C
P1 15.00 usec
SI 16384
SF 300.188011 MHz
WAW 0
SSB 0
LB 0.00 Hz
GB 1.00
  
```

Figure S6. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of compound 22

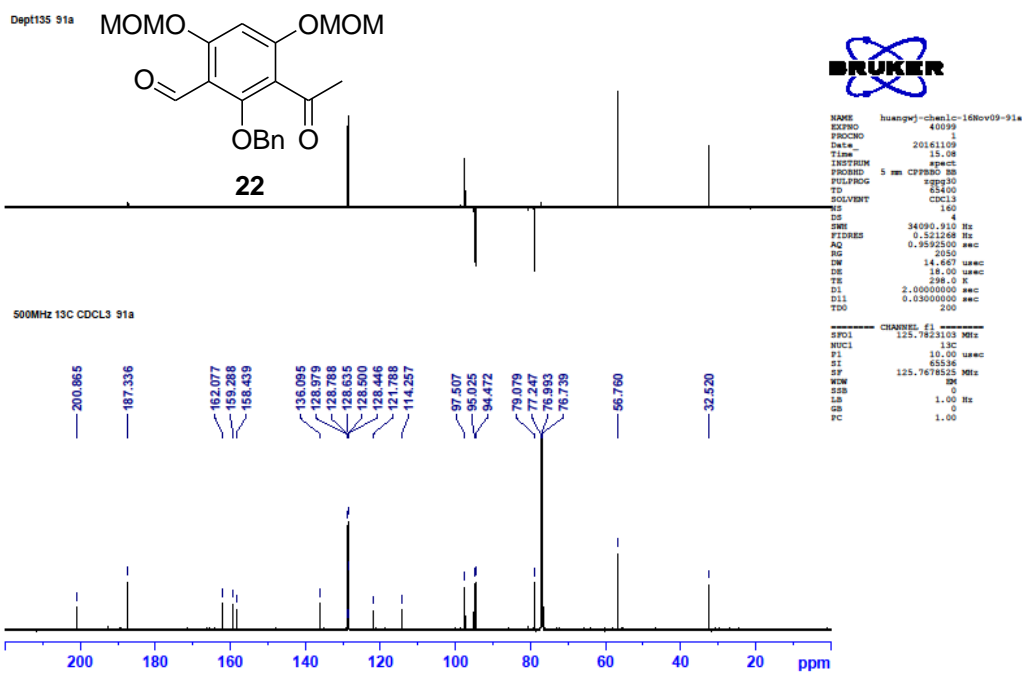


Figure S7. <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) spectrum of compound **22**

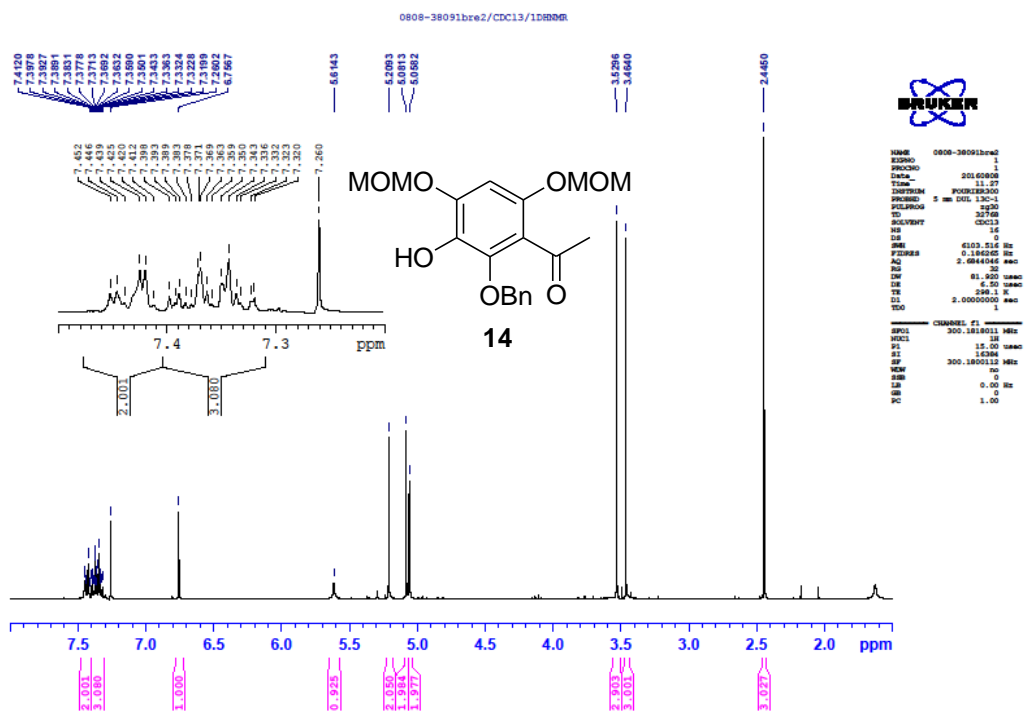


Figure S8. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of compound **14**



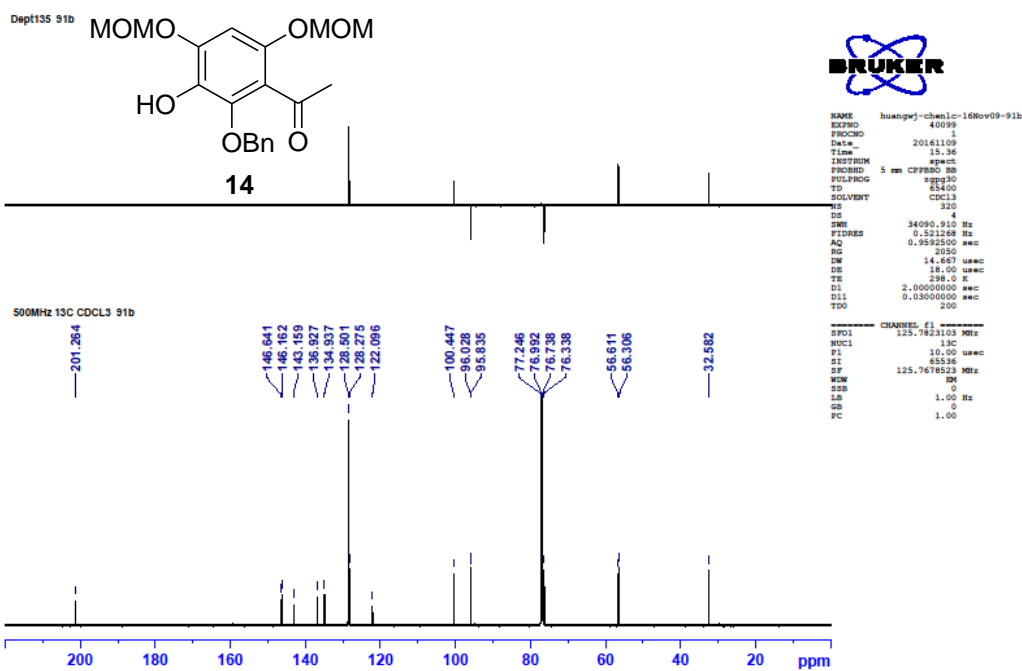


Figure S9. <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) spectrum of compound 14

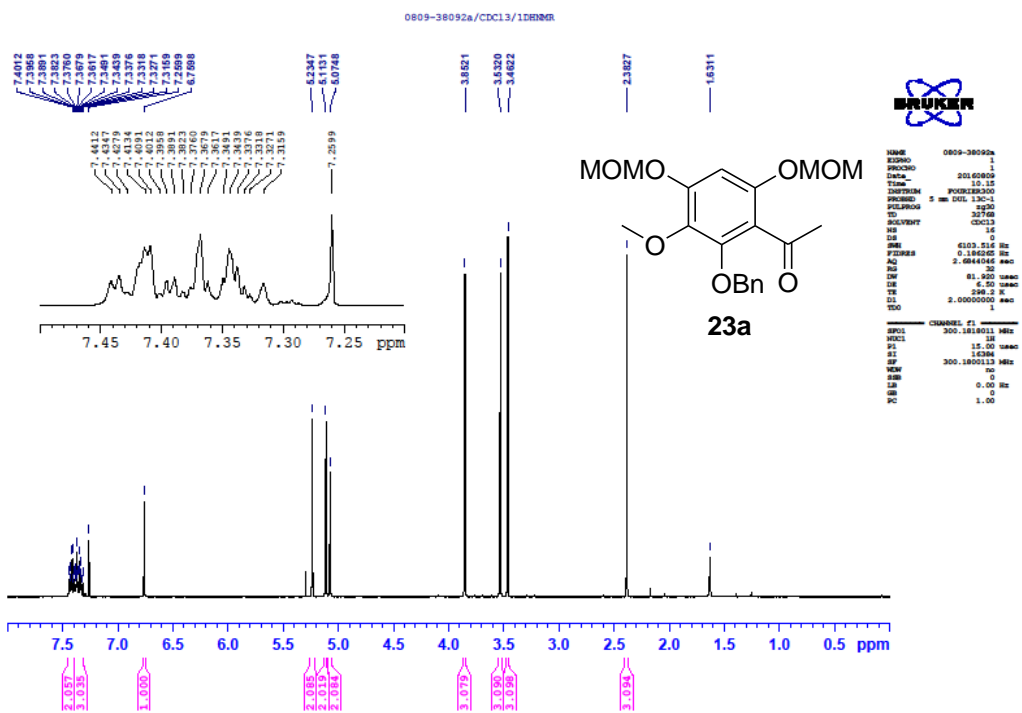


Figure S10. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of compound 23a

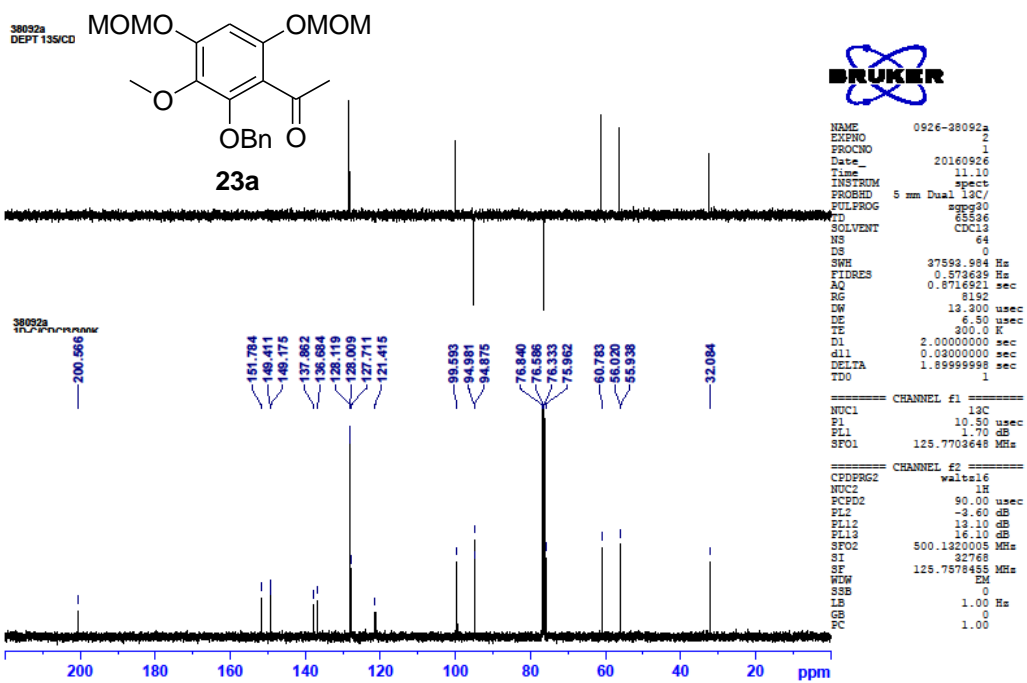


Figure S11. <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) spectrum of compound **23a**

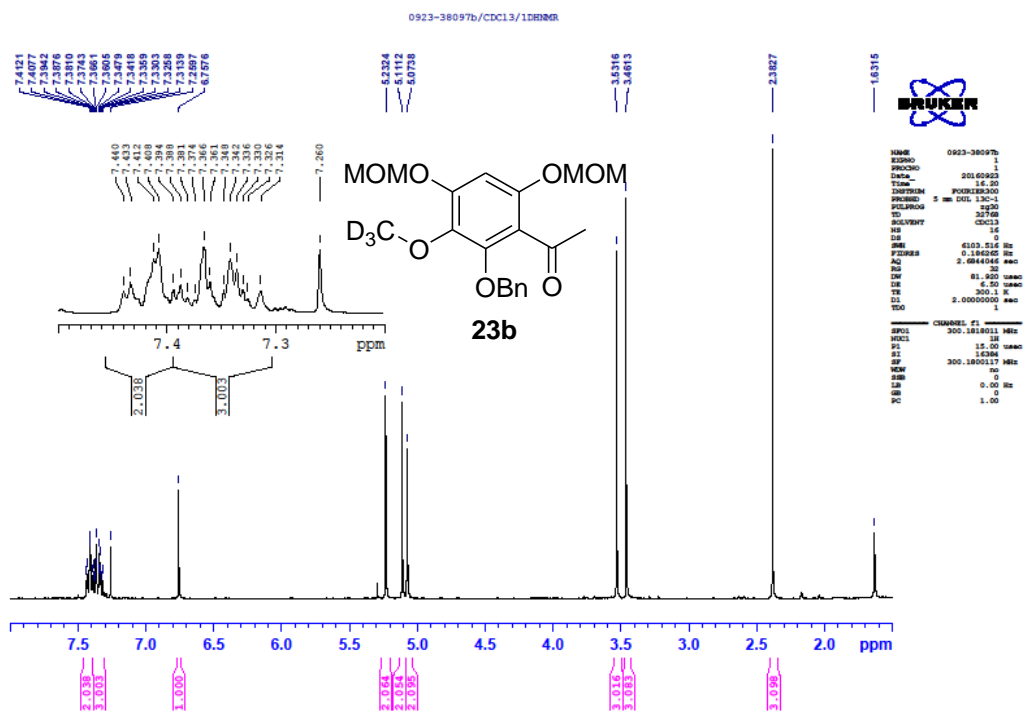


Figure S12. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of compound **23b**

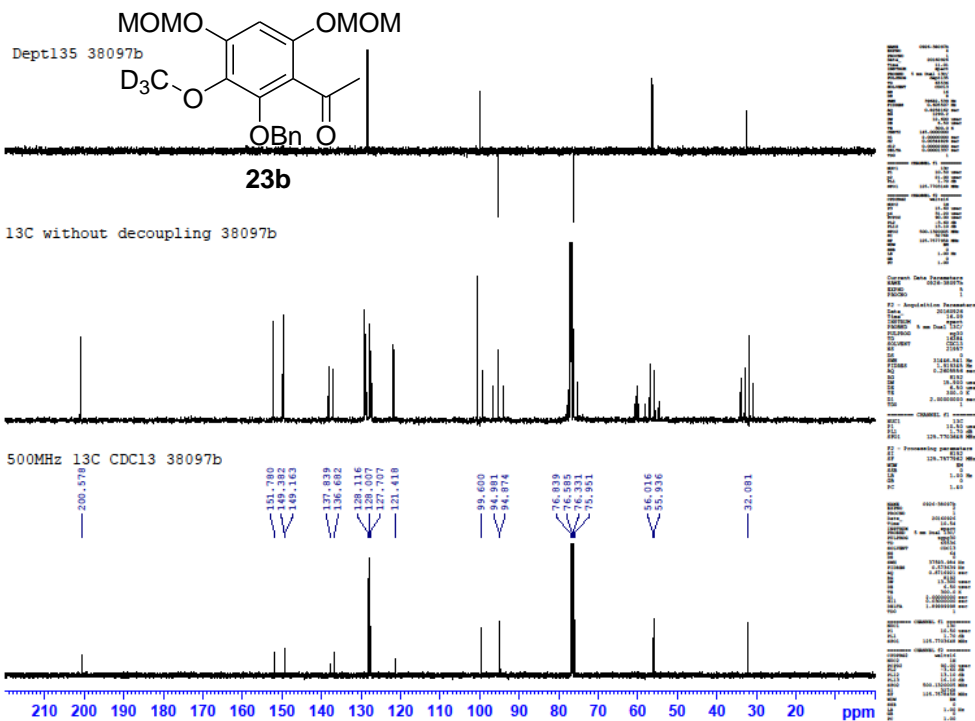


Figure S13. <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) spectrum of compound **23b**

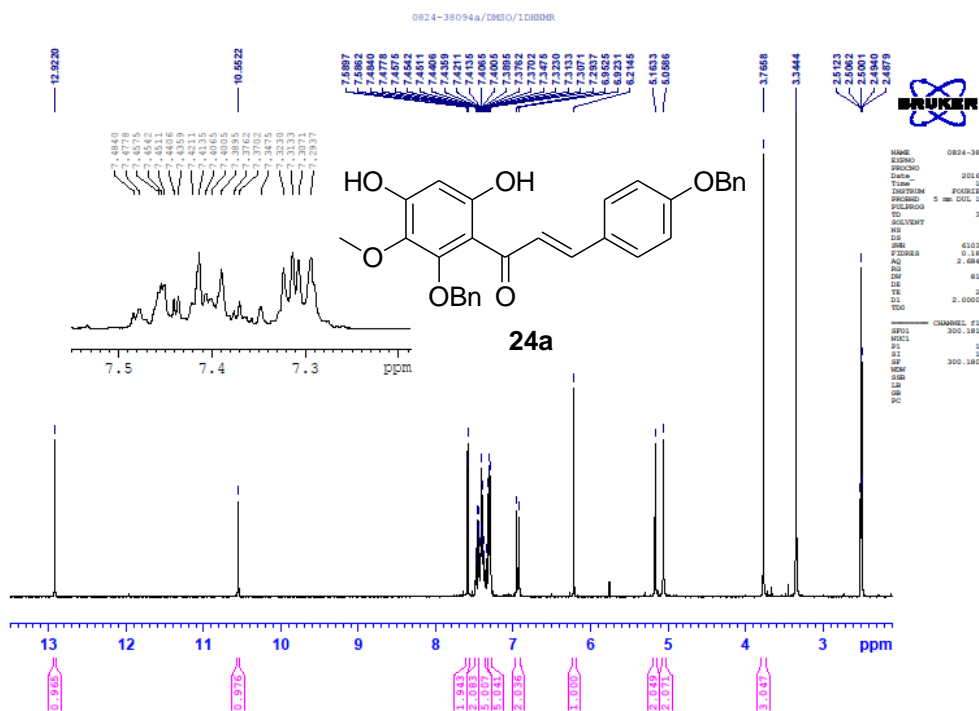


Figure S14. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of compound **24a**

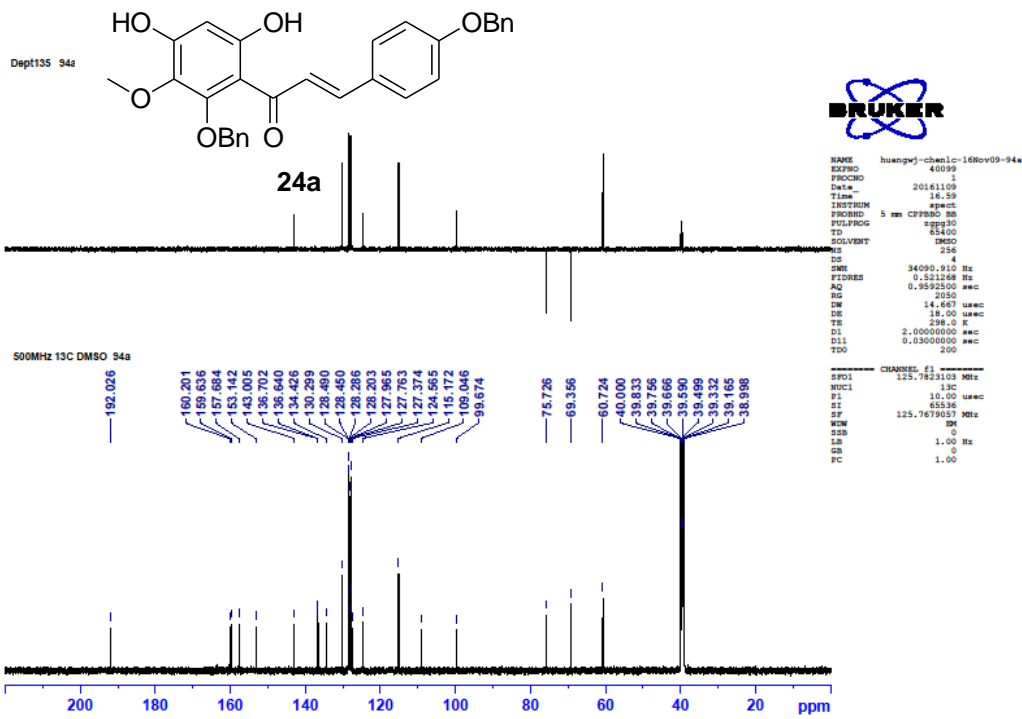


Figure S15. <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) spectrum of compound 24a

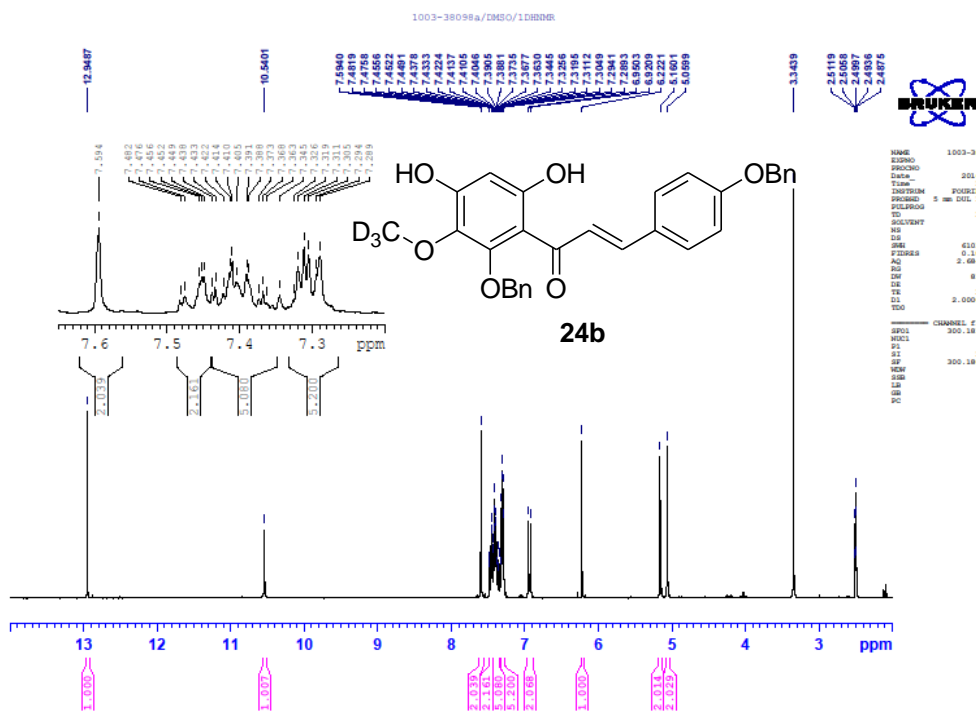


Figure S16. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz) spectrum of compound 24b

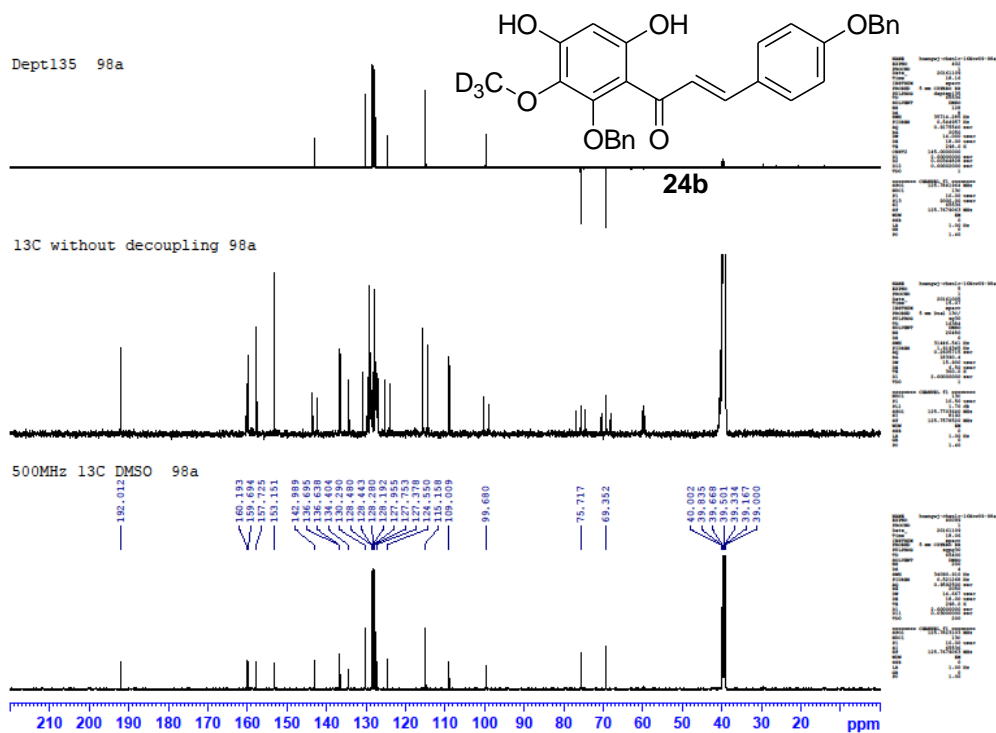


Figure S17.  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 125 MHz) spectrum of compound 24b

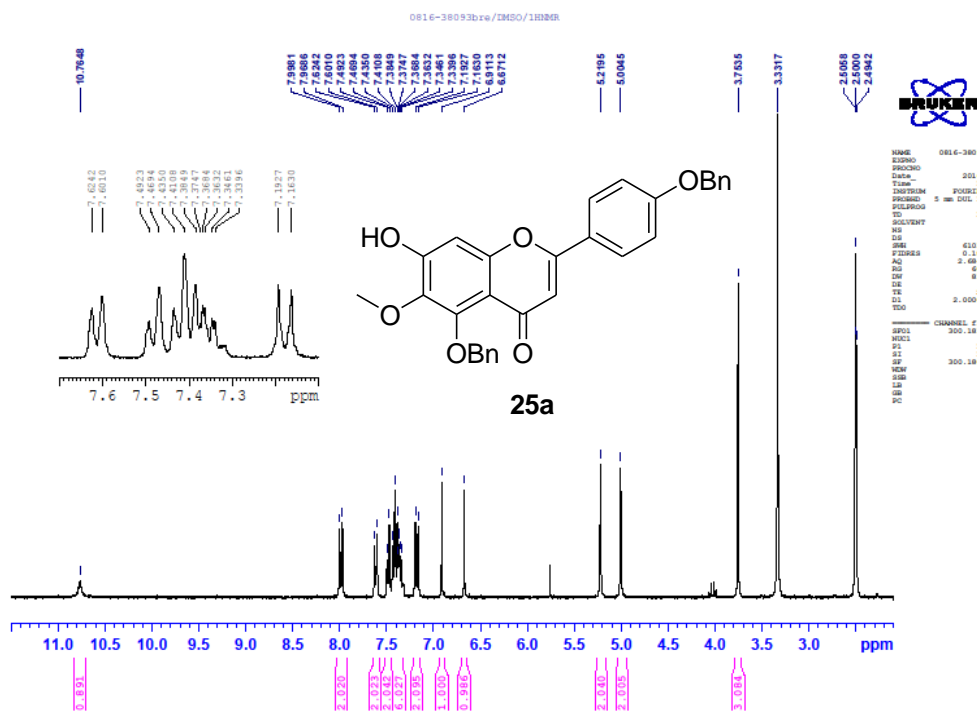


Figure S18.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz) spectrum of compound 25a



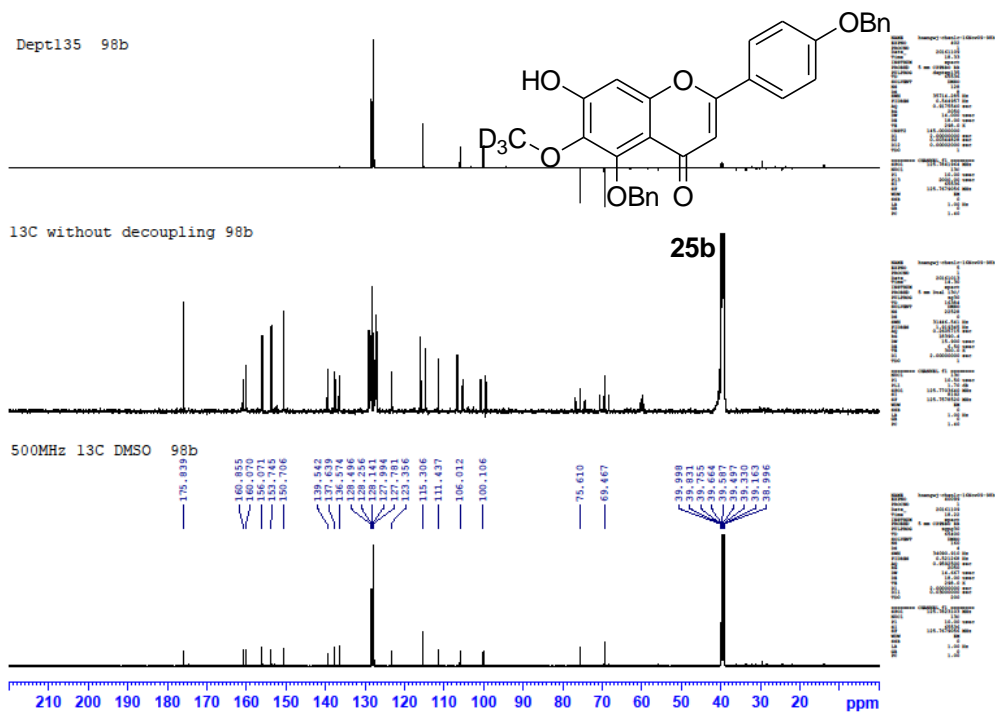


Figure S21. <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 125 MHz) spectrum of compound 25b

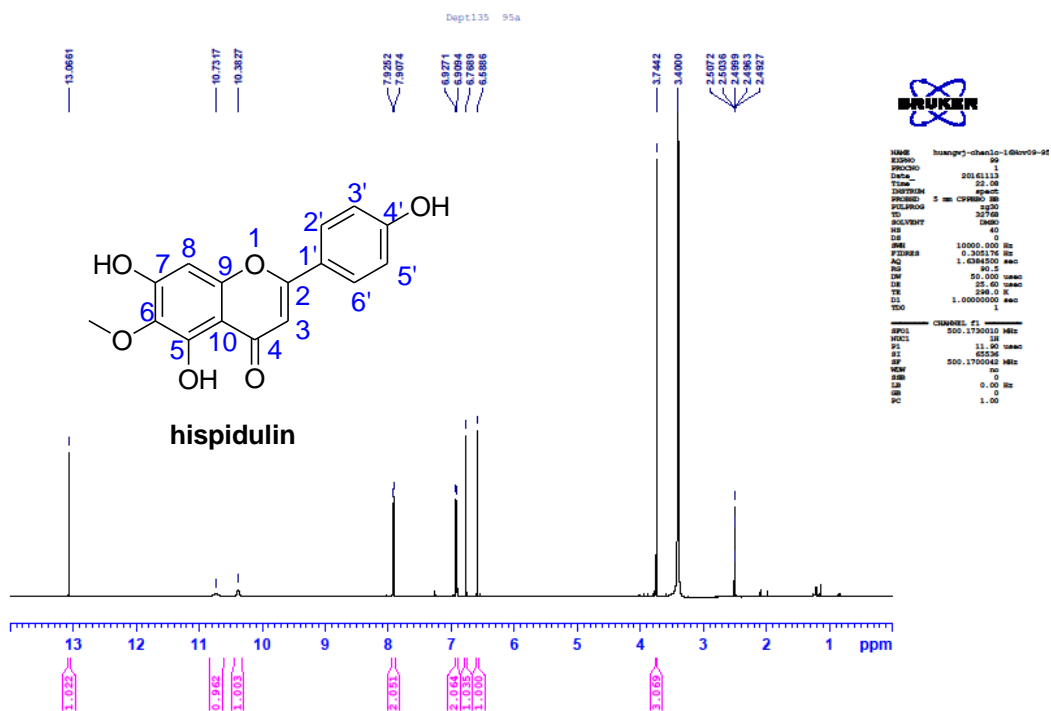


Figure S22. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz) spectrum of hispidulin

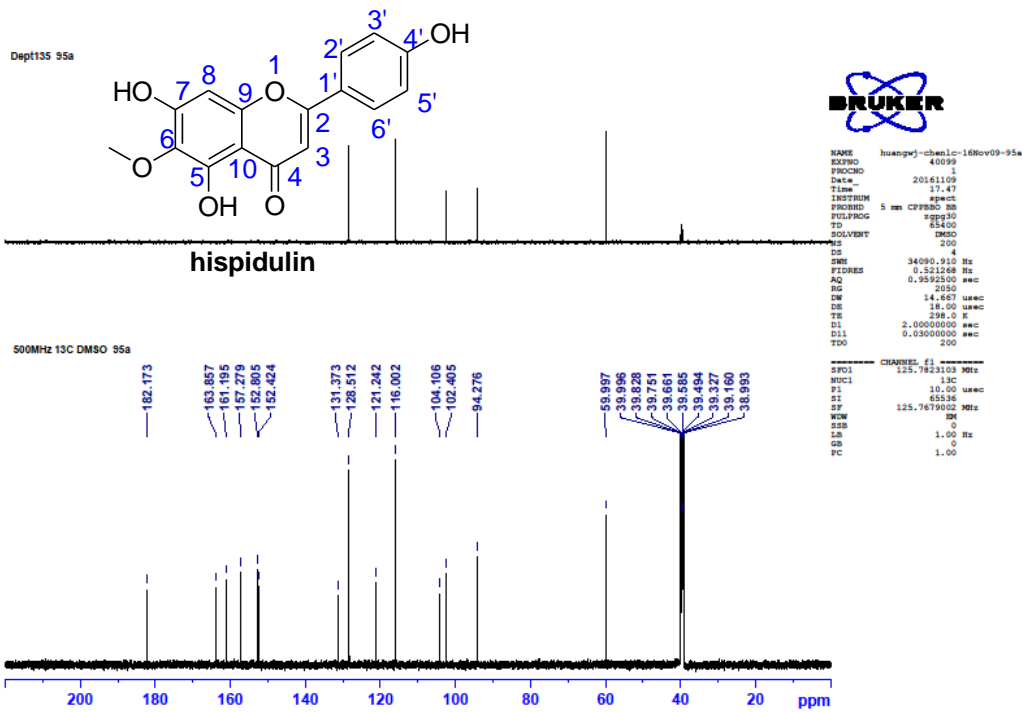


Figure S23.  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 125 MHz) spectrum of hispidulin

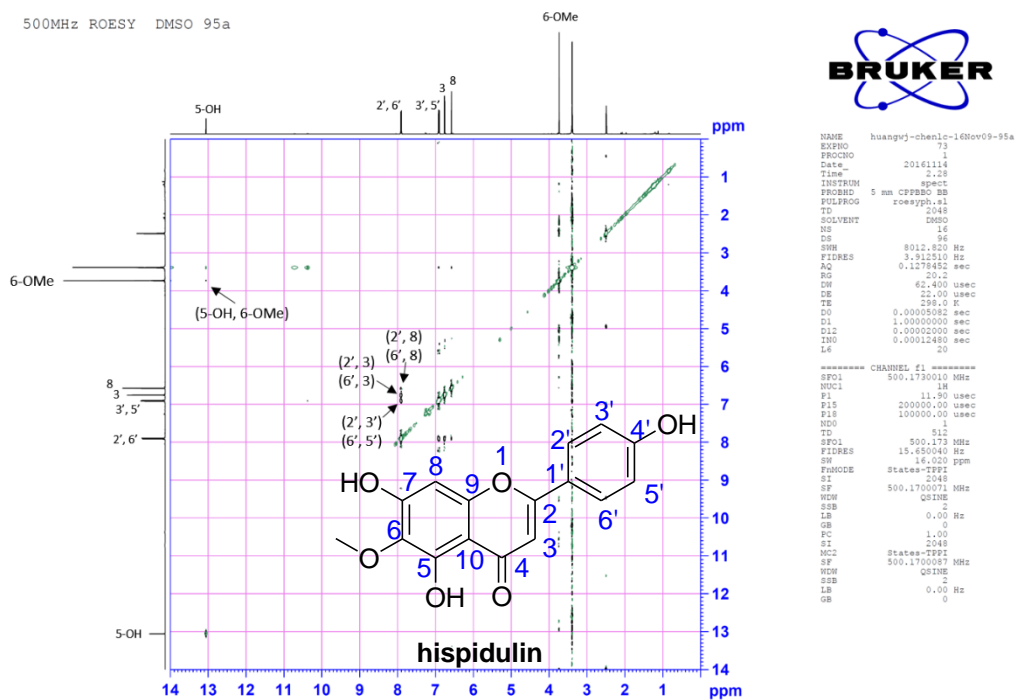


Figure S24. ROESY spectrum of hispidulin



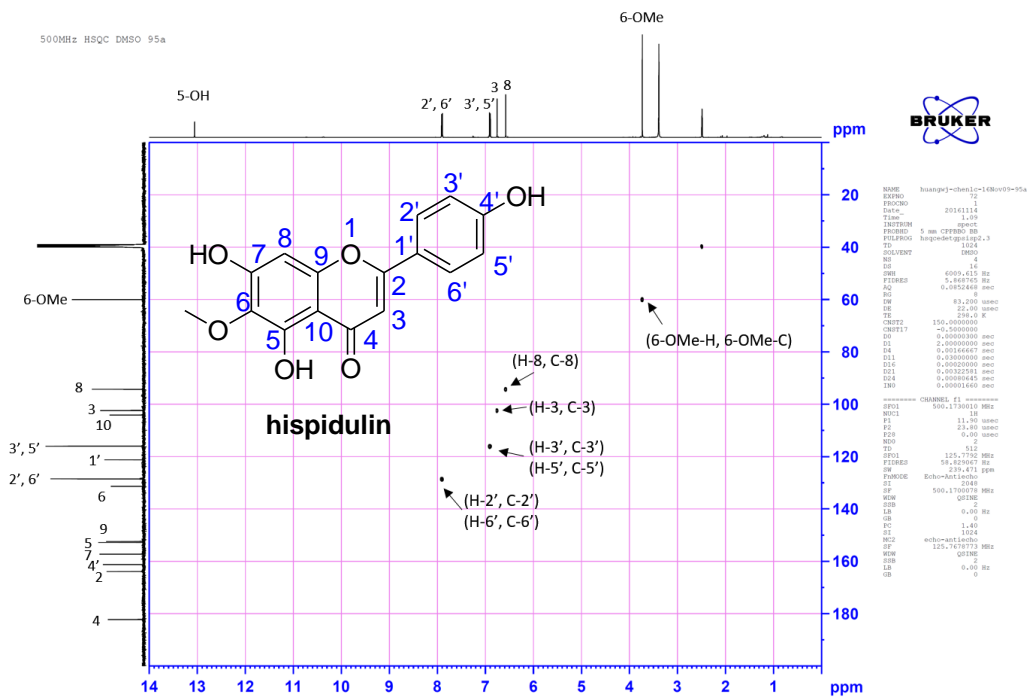


Figure S25. HSQC spectrum of hispidulin

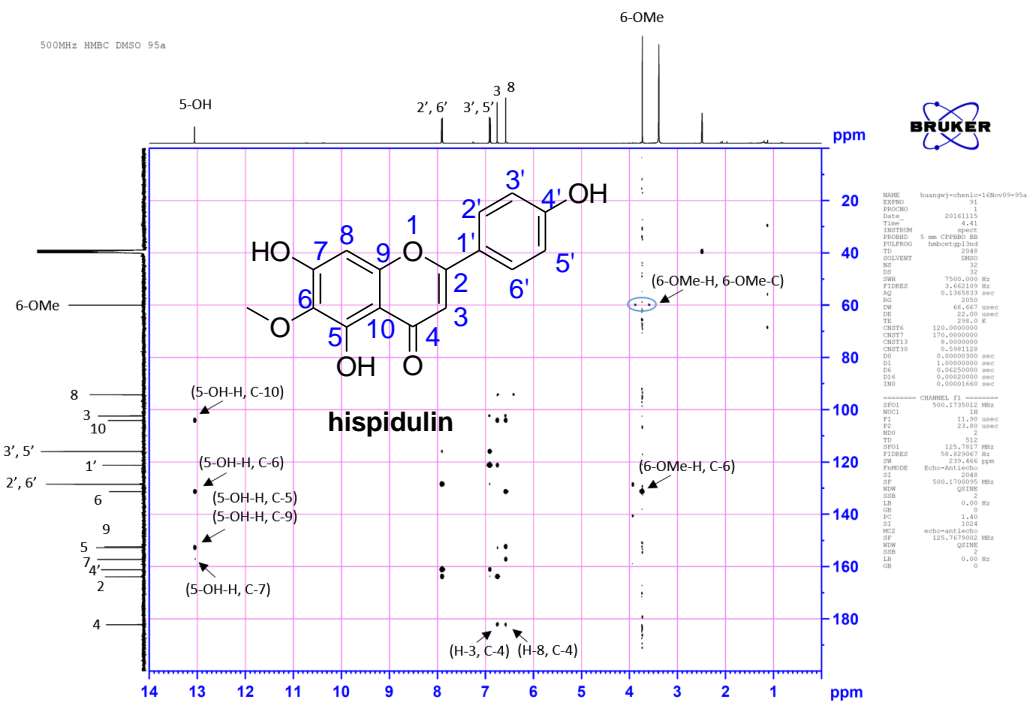


Figure S26. HMBC spectrum of hispidulin

500MHz HMBC DMSO 95a

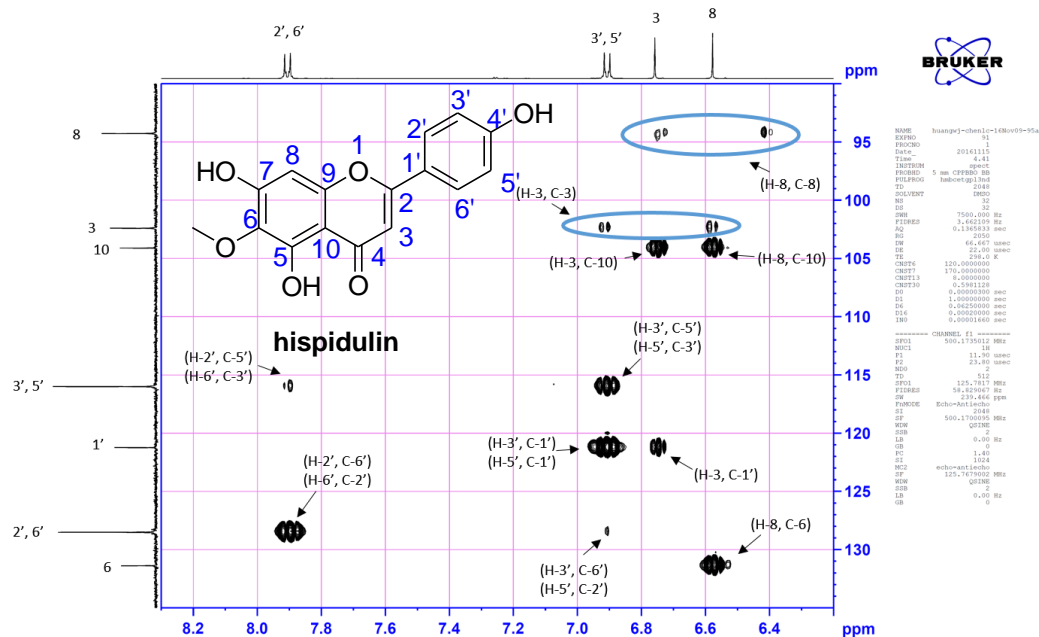


Figure S27. HMBC spectrum of hispidulin

500MHz HMBC DMSO 95a

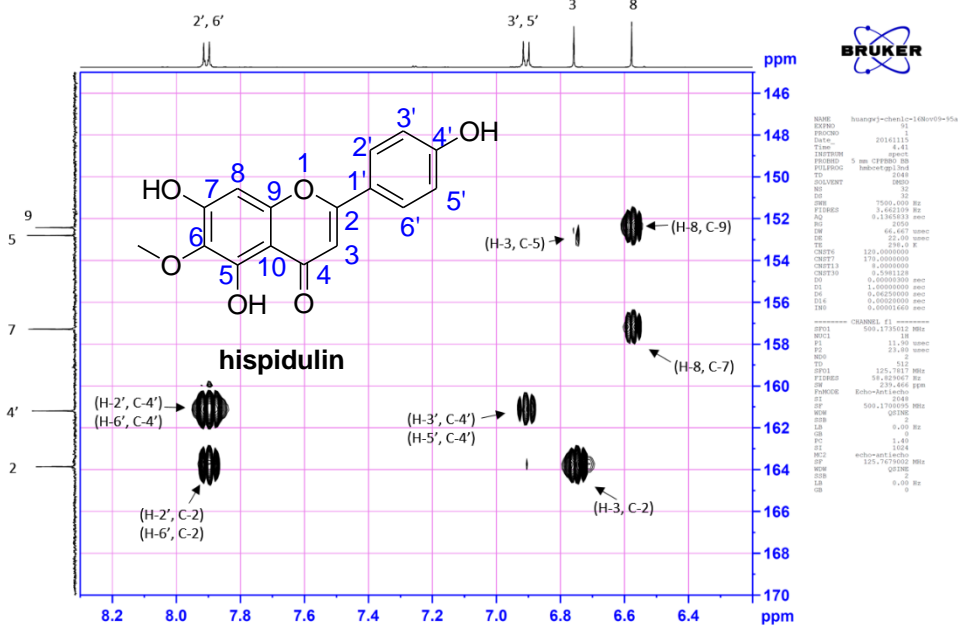


Figure S28. HMBC spectrum of hispidulin

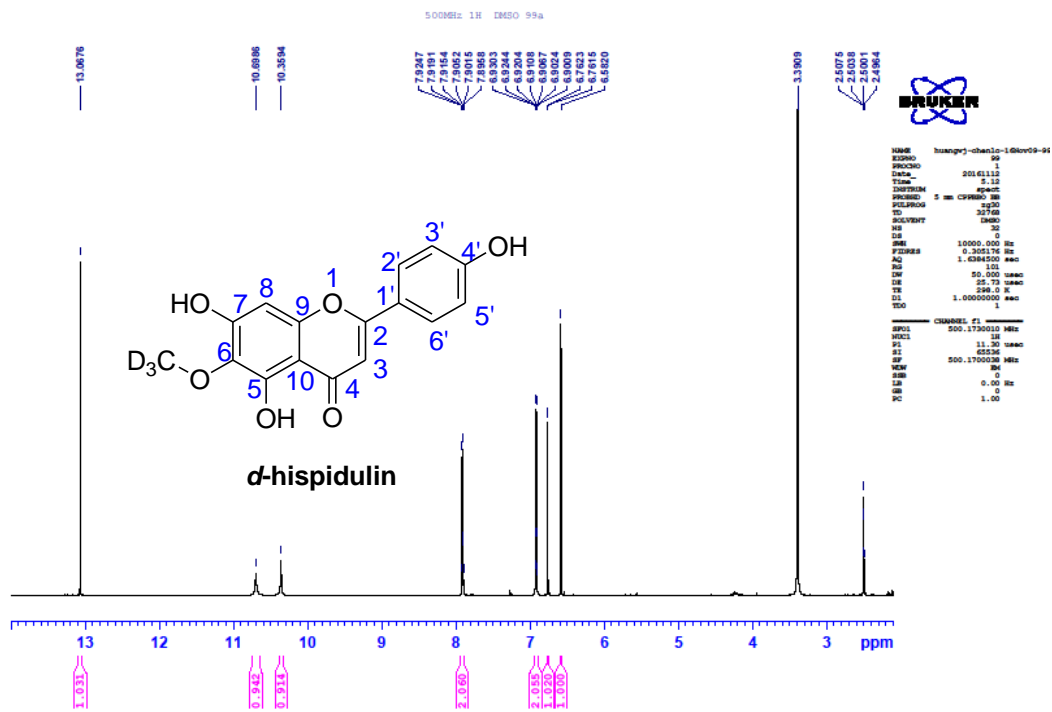


Figure S29. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz) spectrum of *d*-hispidulin

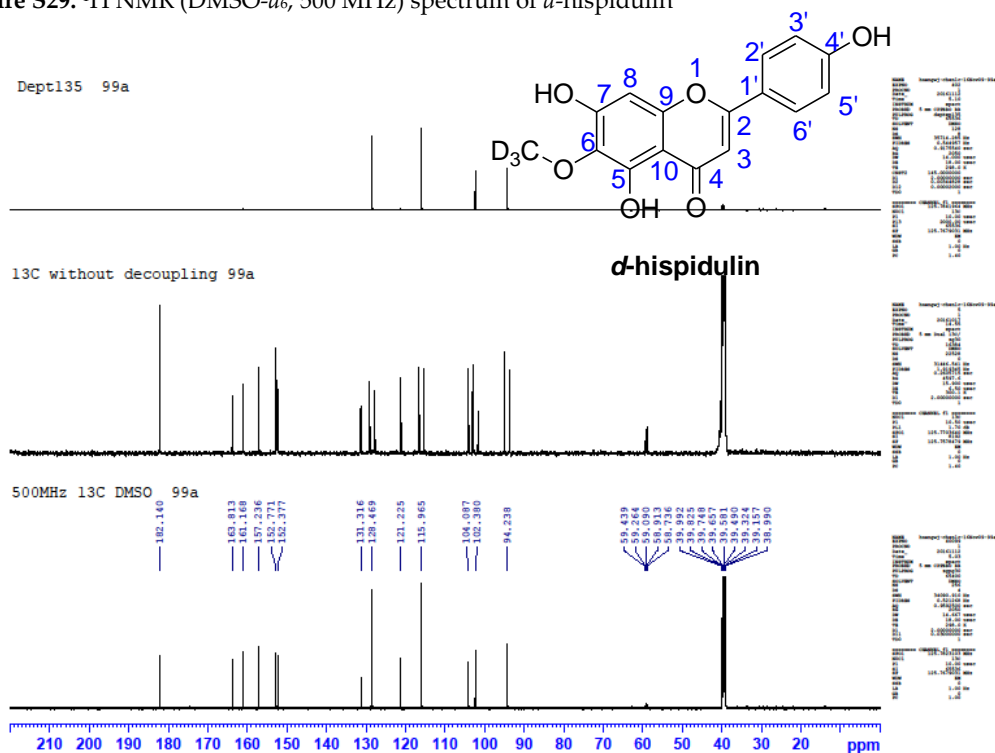
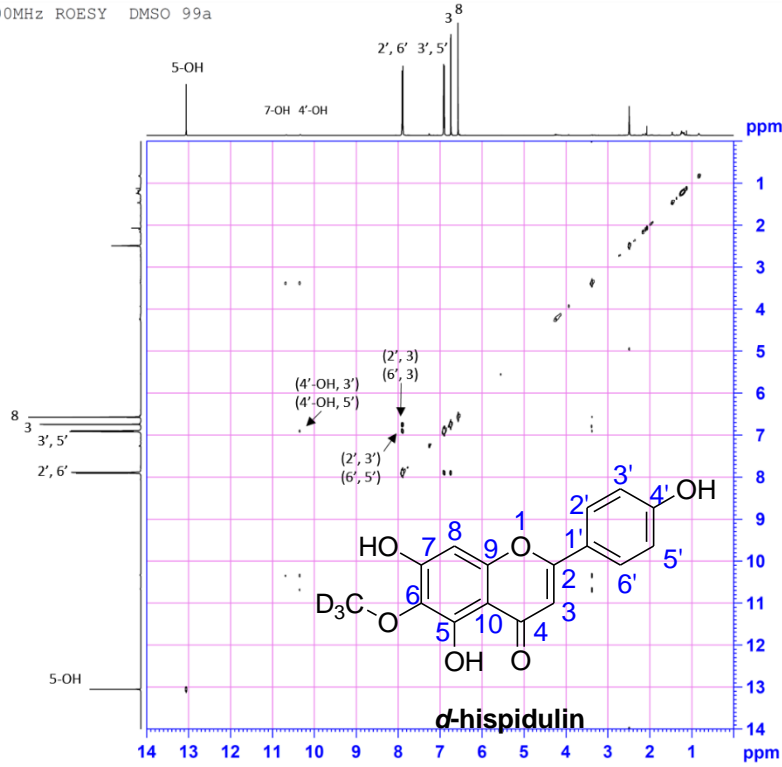


Figure S30. <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 125 MHz) spectrum of *d*-hispidulin

500MHz ROESY DMSO 99a



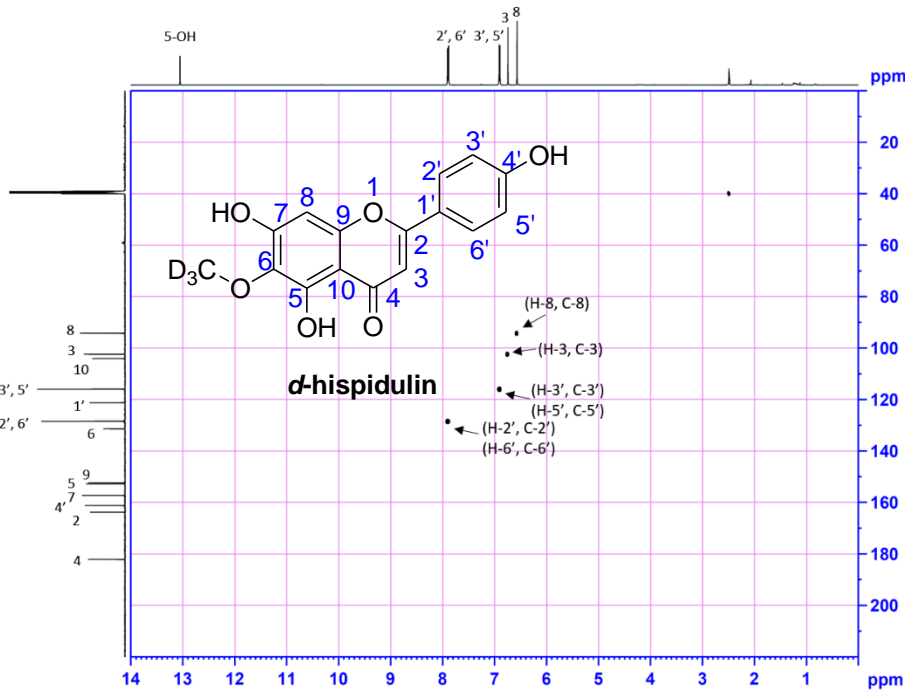
```

NAME      huangwj-chenjic-16Nov09-99a
EXPNO    73
PROCNO   1
Date_    20161113
Time     17.04
INSTRUM  spect
PROBHD   5 mm CPBEBB BB
PULPROG  roesyph.s1
TD        2048
SOLVENT  DMSO
NS        8
DS        96
SWH       8012.820 Hz
FIDRES   3.912510 Hz
AQ        0.1278452 sec
RG        18
DW        62.400 usec
DE        22.00 usec
TE        298.0 K
DO        0.00005076 sec
D1        1.00000000 sec
D12       0.00002000 sec
IN0       0.00012480 sec
L6        20

===== CHANNEL f1 =====
SFO1     500.173010 MHz
NUC1     1H
P1       12.00 usec
P15      200000.00 usec
P18      100000.00 usec
NS0      1
TD        512
SFO1     500.173 MHz
FIDRES   15.650400 Hz
SW        16.020 ppm
F2MODE   States-TPPI
SI        2048
SF        500.170088 MHz
WDW       QSI
SSB       2
LB        0.00 Hz
GB        1.00
SI        2048
MC2      States-TPPI
SF        500.170088 MHz
WDW       QSI
SSB       2
LB        0.00 Hz
GB        0
    
```

Figure S31. ROESY spectrum of *d*-hispidulin

500MHz HSQC DMSO 99a



```

NAME      huangwj-chenjic-16Nov09-99a
EXPNO    72
PROCNO   1
Date_    20161112
Time     15.55
INSTRUM  spect
PROBHD   5 mm CPBEBB BB
PULPROG  hsqcphs1
TD        65536
SOLVENT  DMSO
NS        8
DS        16
SWH       6069.415 Hz
FIDRES   0.948765 Hz
AQ        0.0852468 sec
RG        655
DW        83.200 usec
DE        22.00 usec
TE        298.0 K
CHFT2    150.000000 sec
CHFT17   -0.50000000 sec
D1        0.00000000 sec
D11       0.00000000 sec
D14       0.00000000 sec
D15       0.00000000 sec
D16       0.00000000 sec
D17       0.00000000 sec
D18       0.00000000 sec
D19       0.00000000 sec
D20       0.00000000 sec
D21       0.00000000 sec
D22       0.00000000 sec
D23       0.00000000 sec
D24       0.00000000 sec
D25       0.00000000 sec
D26       0.00000000 sec
D27       0.00000000 sec
D28       0.00000000 sec
D29       0.00000000 sec
D30       0.00000000 sec
D31       0.00000000 sec
D32       0.00000000 sec
D33       0.00000000 sec
D34       0.00000000 sec
D35       0.00000000 sec
D36       0.00000000 sec
D37       0.00000000 sec
D38       0.00000000 sec
D39       0.00000000 sec
D40       0.00000000 sec
D41       0.00000000 sec
D42       0.00000000 sec
D43       0.00000000 sec
D44       0.00000000 sec
D45       0.00000000 sec
D46       0.00000000 sec
D47       0.00000000 sec
D48       0.00000000 sec
D49       0.00000000 sec
D50       0.00000000 sec
D51       0.00000000 sec
D52       0.00000000 sec
D53       0.00000000 sec
D54       0.00000000 sec
D55       0.00000000 sec
D56       0.00000000 sec
D57       0.00000000 sec
D58       0.00000000 sec
D59       0.00000000 sec
D60       0.00000000 sec
D61       0.00000000 sec
D62       0.00000000 sec
D63       0.00000000 sec
D64       0.00000000 sec
D65       0.00000000 sec
D66       0.00000000 sec
D67       0.00000000 sec
D68       0.00000000 sec
D69       0.00000000 sec
D70       0.00000000 sec
D71       0.00000000 sec
D72       0.00000000 sec
D73       0.00000000 sec
D74       0.00000000 sec
D75       0.00000000 sec
D76       0.00000000 sec
D77       0.00000000 sec
D78       0.00000000 sec
D79       0.00000000 sec
D80       0.00000000 sec
D81       0.00000000 sec
D82       0.00000000 sec
D83       0.00000000 sec
D84       0.00000000 sec
D85       0.00000000 sec
D86       0.00000000 sec
D87       0.00000000 sec
D88       0.00000000 sec
D89       0.00000000 sec
D90       0.00000000 sec
D91       0.00000000 sec
D92       0.00000000 sec
D93       0.00000000 sec
D94       0.00000000 sec
D95       0.00000000 sec
D96       0.00000000 sec
D97       0.00000000 sec
D98       0.00000000 sec
D99       0.00000000 sec
D100      0.00000000 sec

===== CHANNEL f1 =====
SFO1     500.173010 MHz
NUC1     1H
P1       11.80 usec
P2       23.40 usec
P28      0.00 usec
NS0      2
TD        512
SFO1     125.7700 MHz
FIDRES   58.829667 Hz
SW        239.471 ppm
F2MODE   Echo-AntiEcho
SI        2048
SF        500.170072 MHz
WDW       QSI
SSB       2
LB        0.00 Hz
GB        1.40
SI        1024
MC2      echo-anti-echo
SF        125.767800 MHz
WDW       QSI
SSB       2
LB        0.00 Hz
GB        0
    
```

Figure S32. HSQC spectrum of *d*-hispidulin

500MHz HSQC DMSO 99a

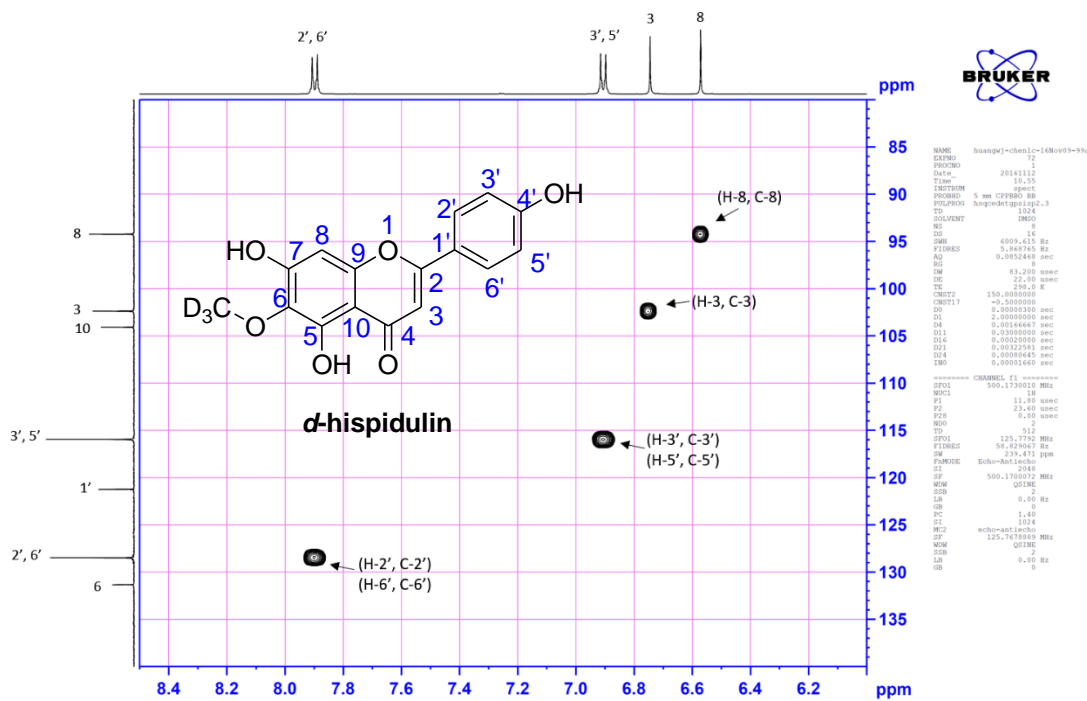


Figure S33. HSQC spectrum of *d*-hispidulin

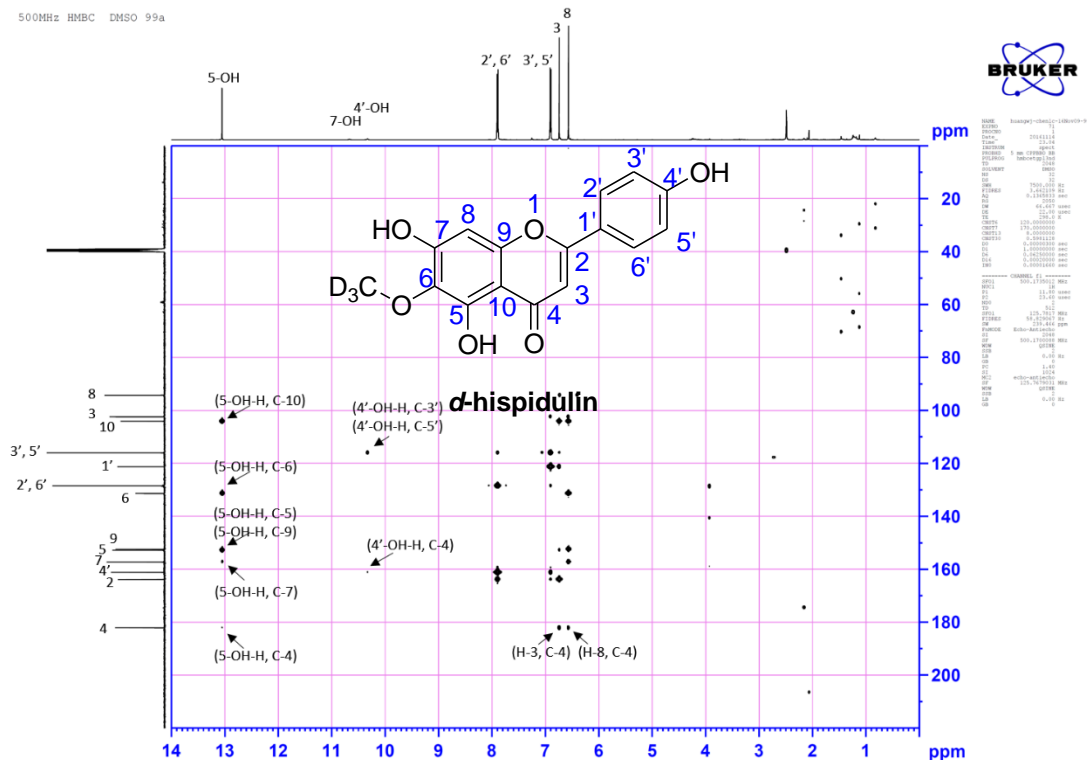


Figure S34. HMBC spectrum of *d*-hispidulin

500MHz HMBC DMSO 99a

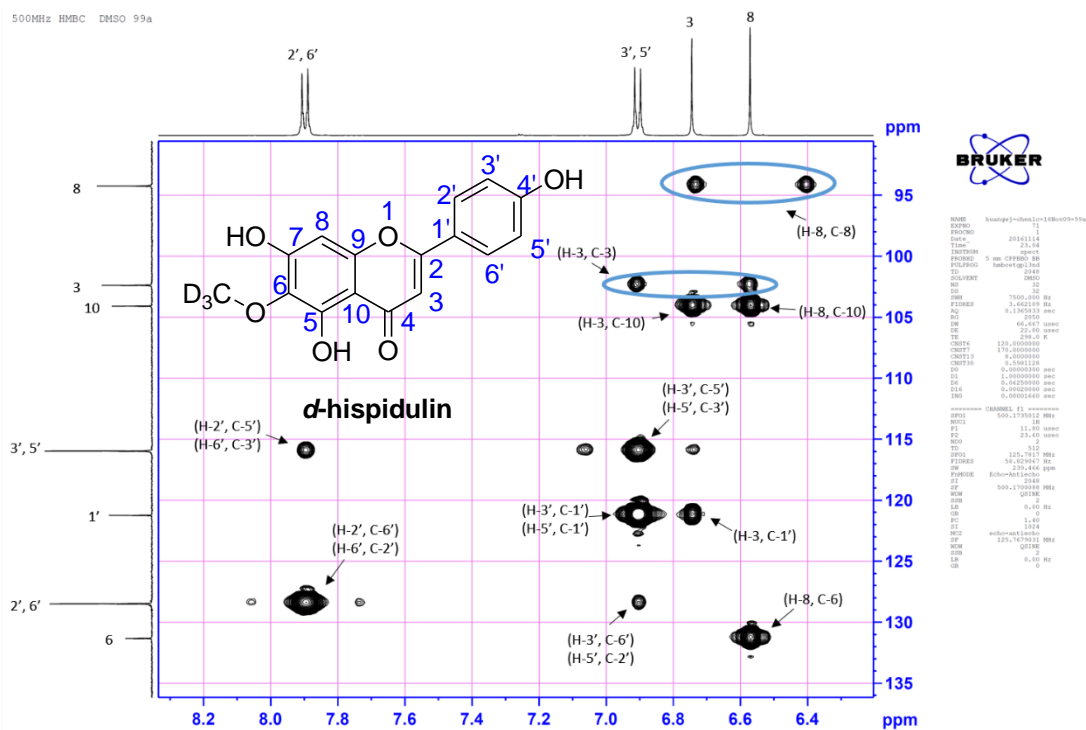


Figure S35. HMBC spectrum of *d*-hispidulin

500MHz HMBC DMSO 99a

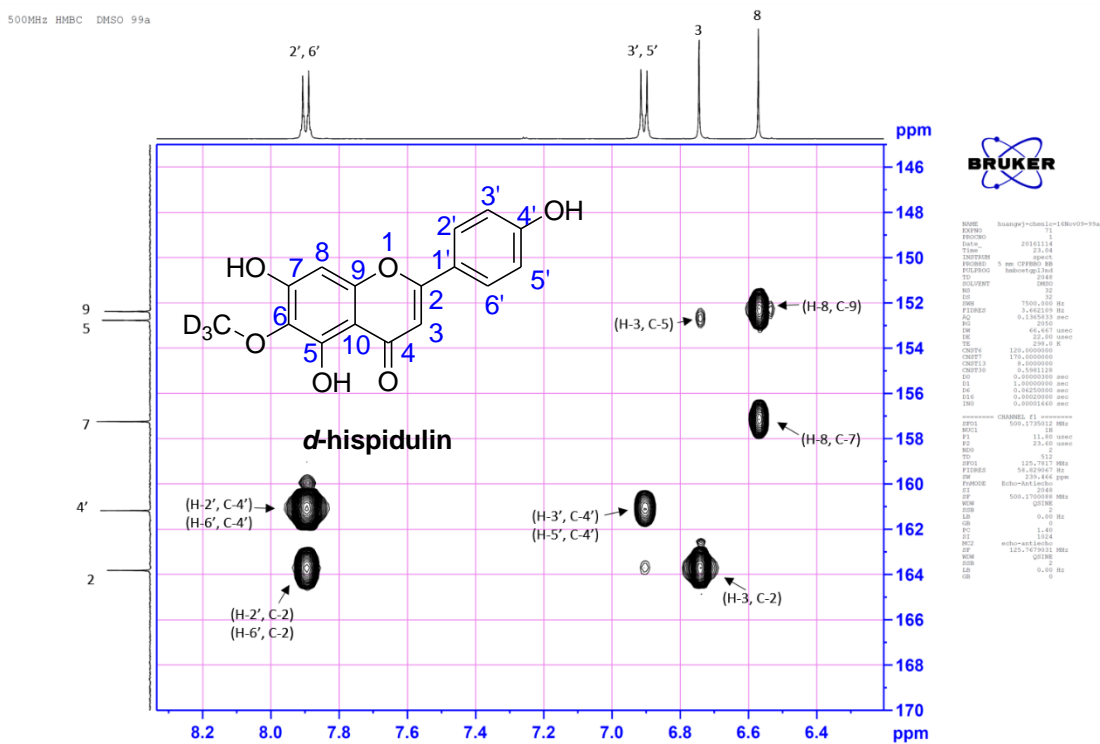


Figure S36. HMBC spectrum of *d*-hispidulin

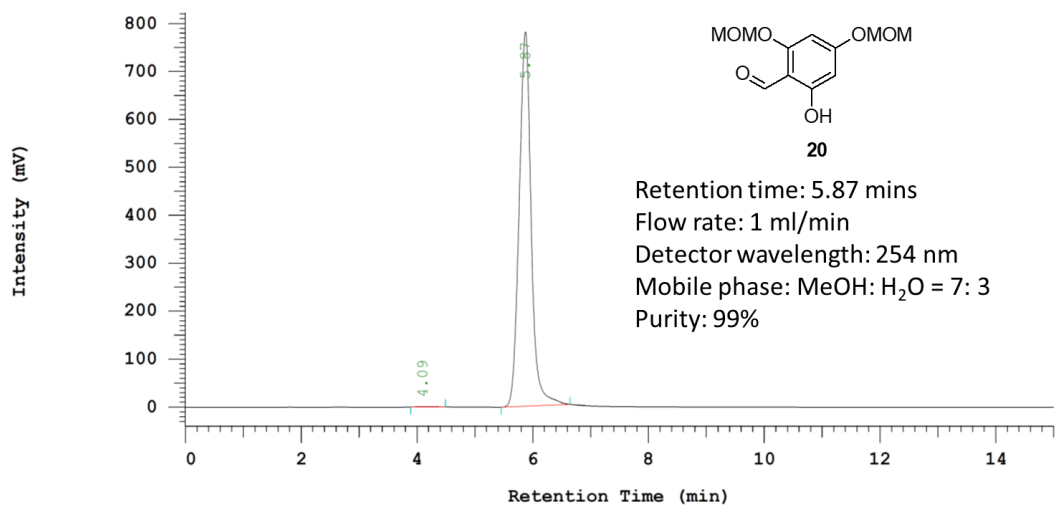


Figure S37. HPLC chromatogram of compound 20

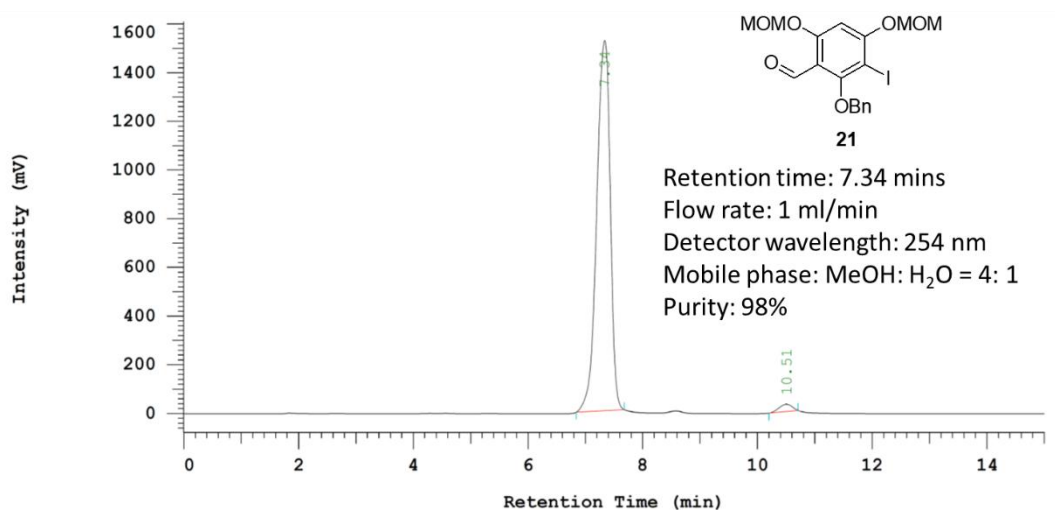


Figure S38. HPLC chromatogram of compound 21

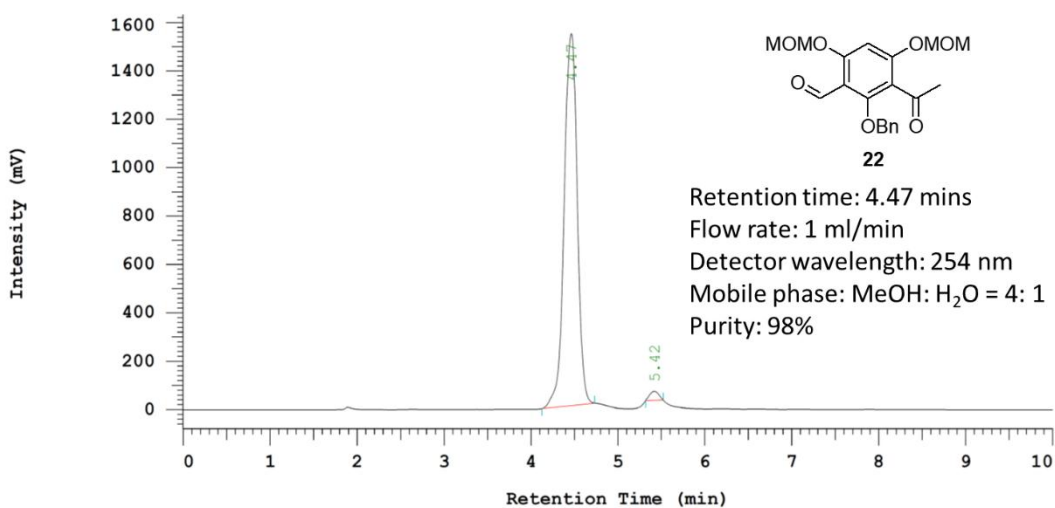


Figure S39. HPLC chromatogram of compound 22

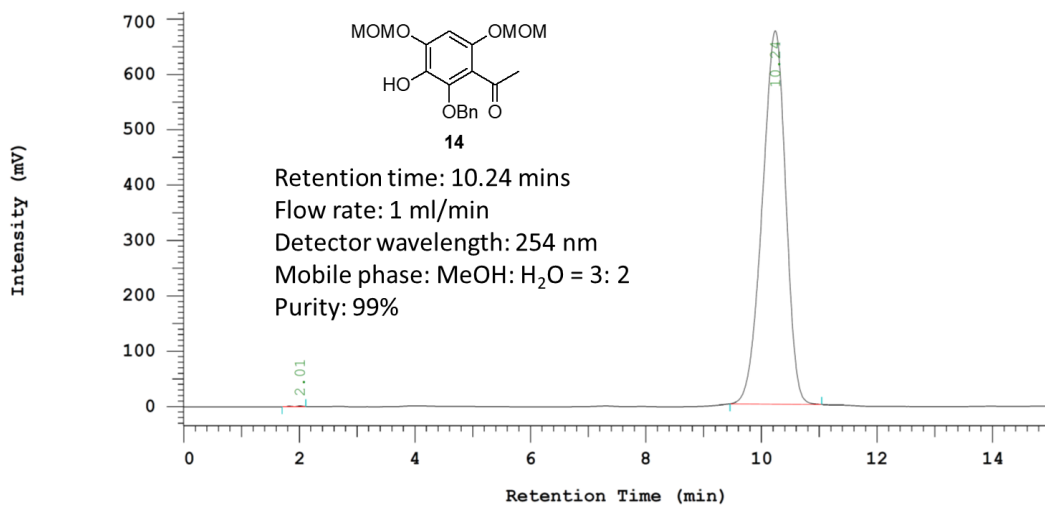


Figure S40. HPLC chromatogram of compound 14

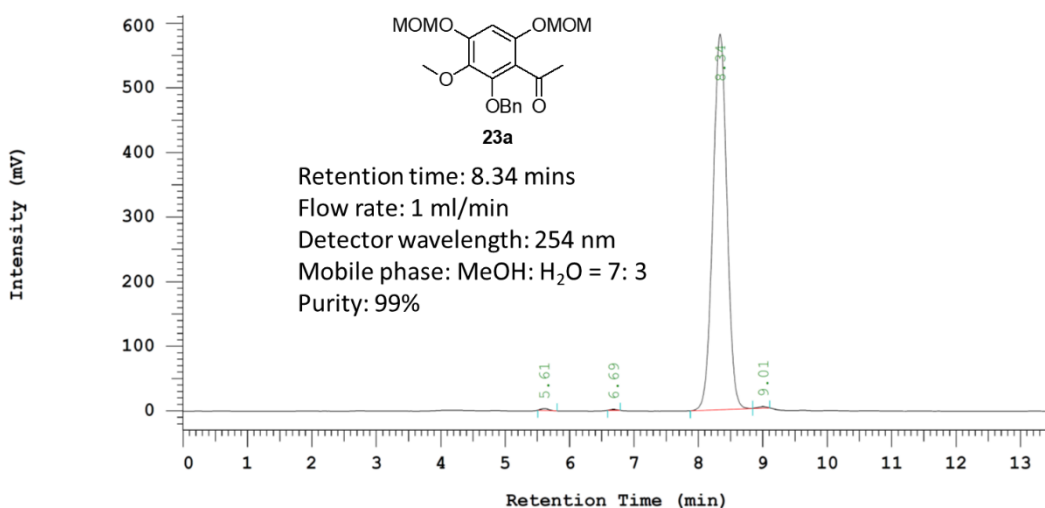


Figure S41. HPLC chromatogram of compound 23a

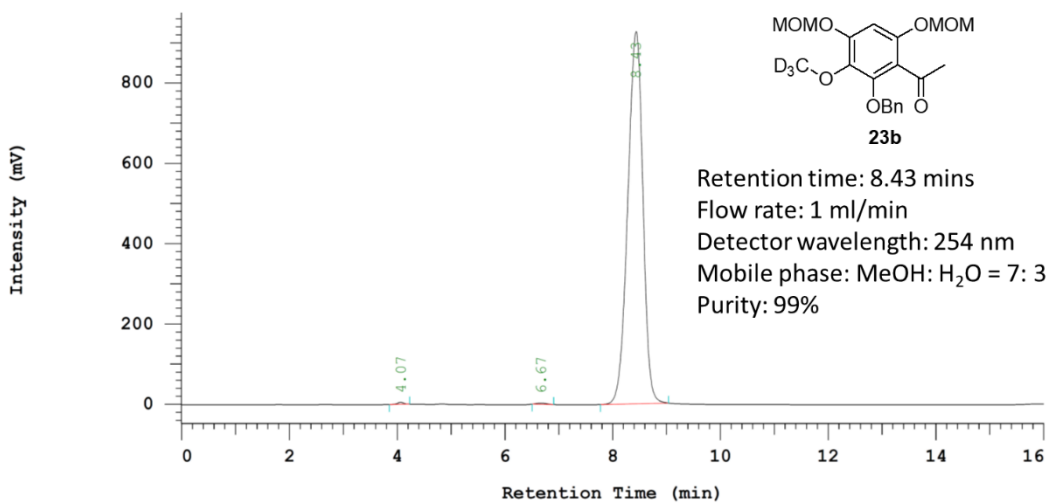


Figure S42. HPLC chromatogram of compound 23b



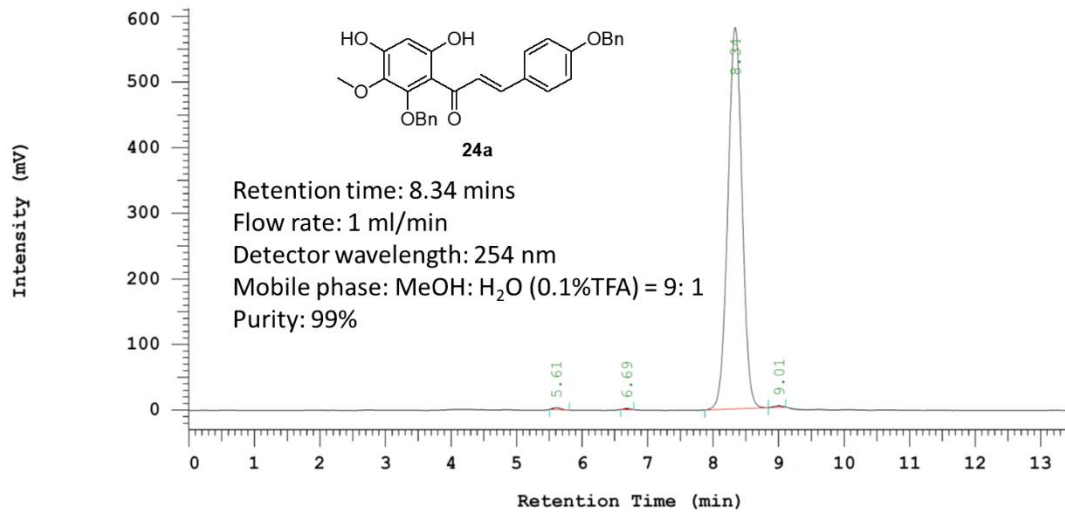


Figure S43. HPLC chromatogram of compound 24a

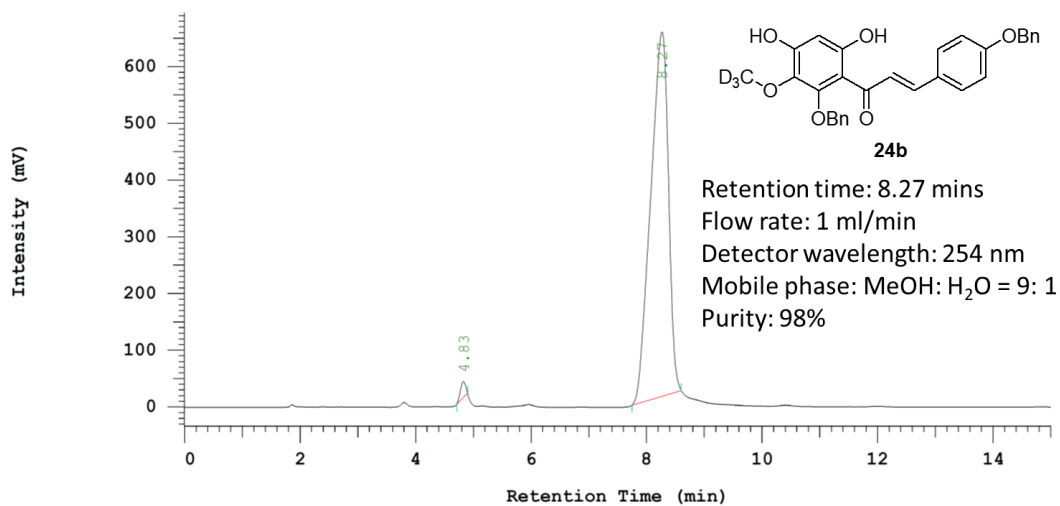


Figure S44. HPLC chromatogram of compound 24b

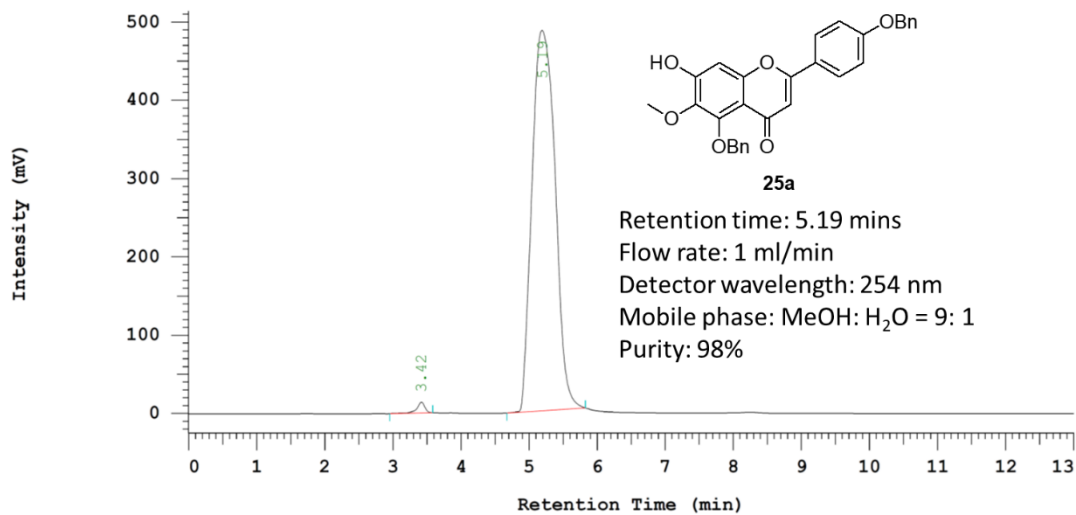


Figure S45. HPLC chromatogram of compound 25a

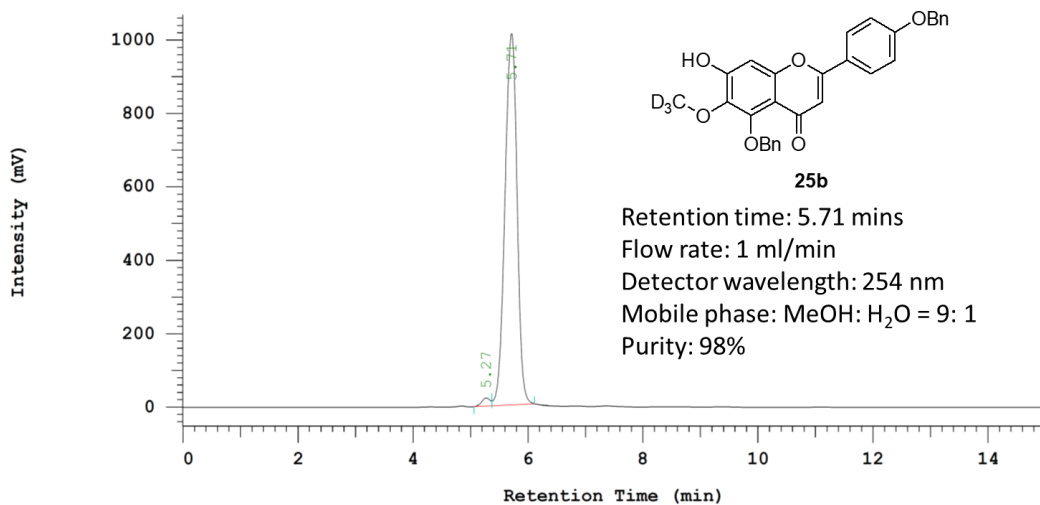


Figure S46. HPLC chromatogram of compound 25b

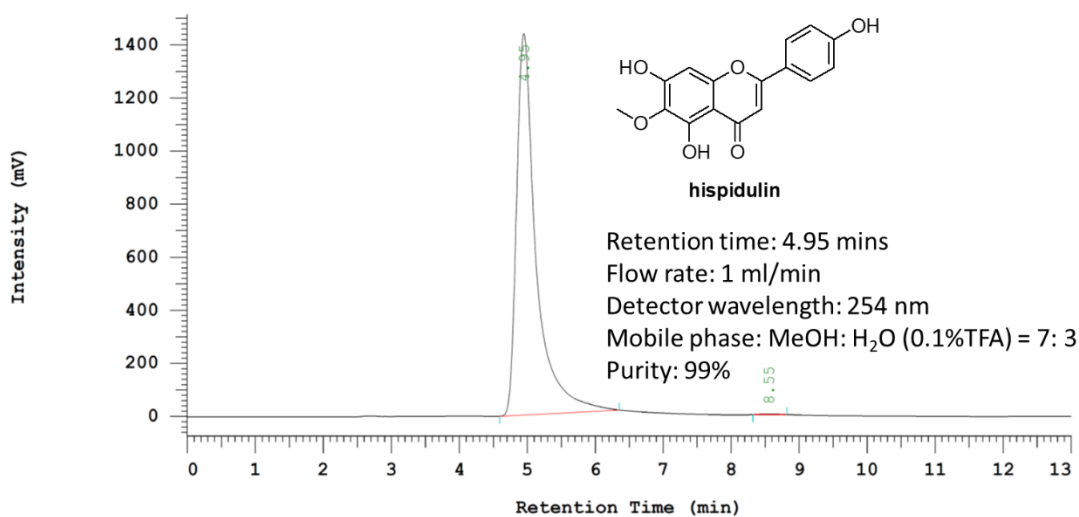


Figure S47. HPLC chromatogram of hispidulin

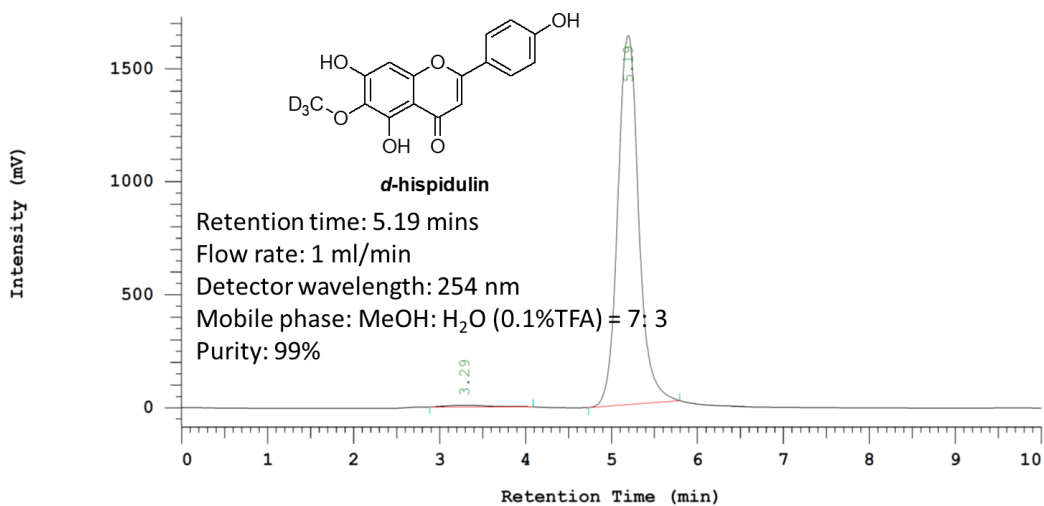


Figure S48. HPLC chromatogram of *d*-hispidulin