**Supporting information for:**

# Cytotoxic Stilbenoids, Hetero- and Homodimers of Homoisoflavonoids from *Prospero autumnale*

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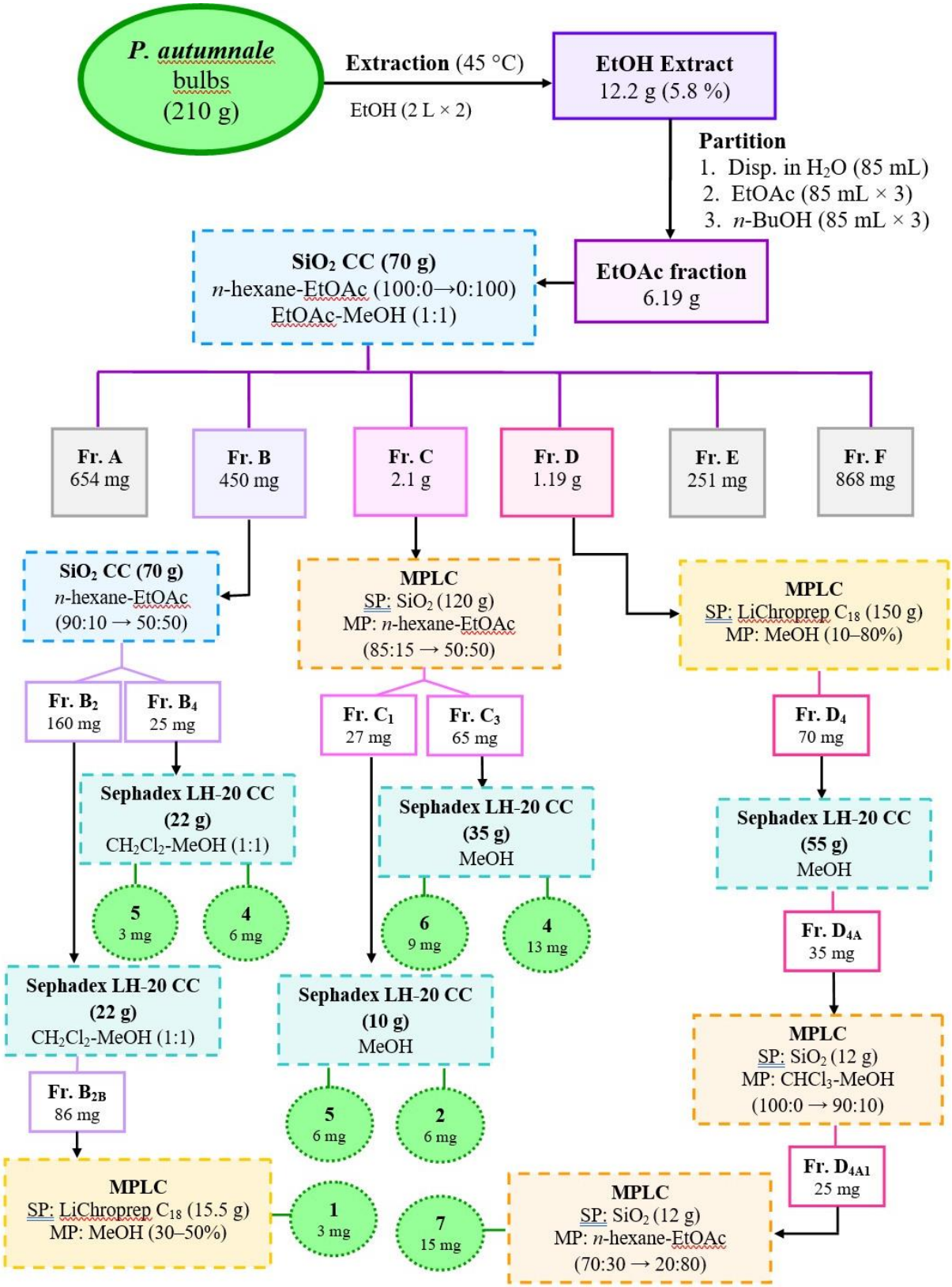
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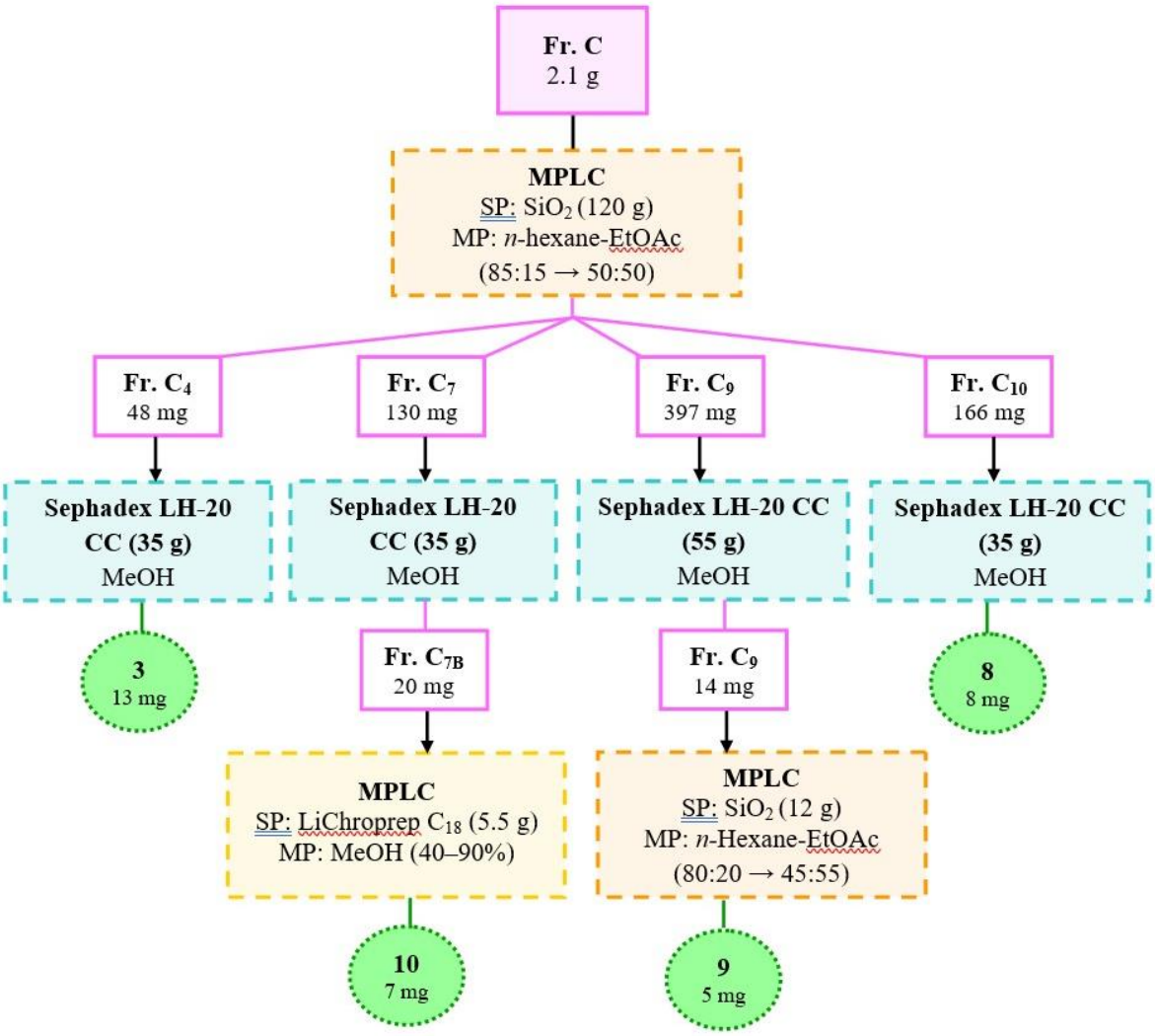
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**Scheme S1.1** Isolation of compounds from *Prospero autumnale*.



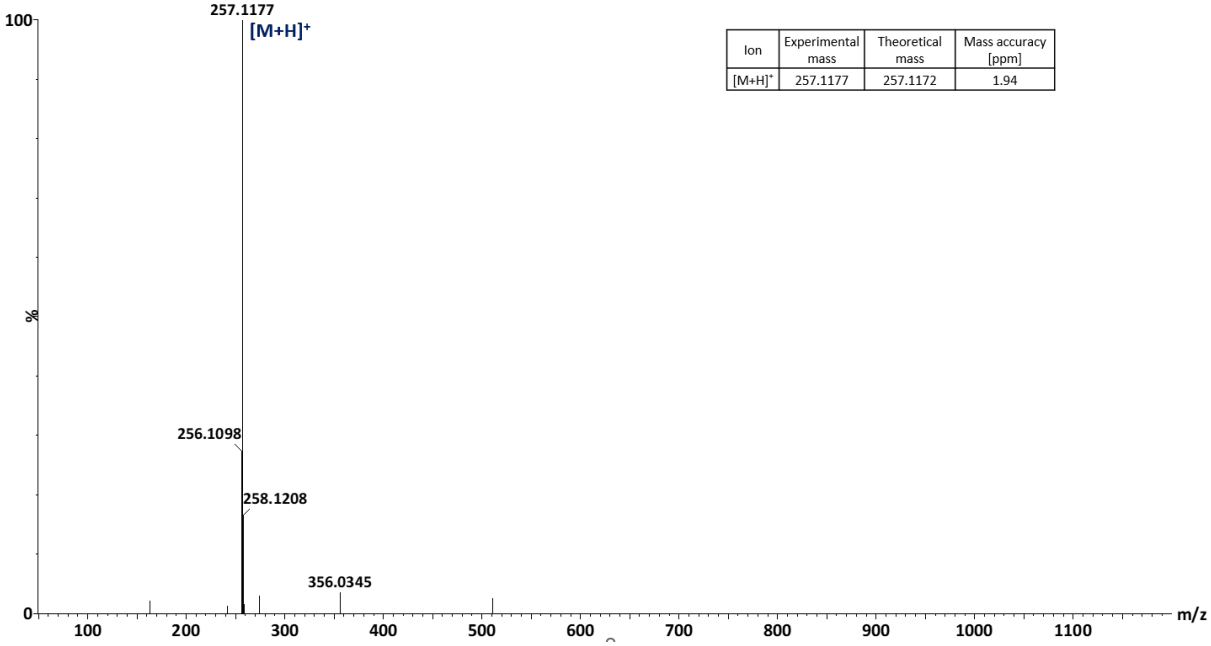

**Scheme S1.2** Isolation of compounds from *Prospero autumnale.*

**Table S1.** Cytotoxic activities of the extract and fractions[a]

| | **Extract/Fraction**<br><br>**$IC_{50}$ ± SEM [$\mu$g/mL][a]** | | |
|---|---|---|---|
| **Cell Lines** | **EtOH extr.** | **EtOAc fraction** | **Doxorubicin[b]** |
| HCT116 | 59.99±2.76 | 22.01±5.13 | 0.86±0.14 |
| LoVo | 86.13±5.14 | 11.79±1.67 | 2.40±0.19 |
| MCF7 | 81.52±2.10 | 16.92±1.08 | 2.32±0.28 |
| MDA-MB-231 | >100 | 20.13±1.34 | 2.33±0.33 |
| PC3 | 15.11±1.64 | 19.22±1.01 | 0.88±0.08 |
| DU145 | 69.93±1.16 | 34.04±1.49 | 5.93±0.38 |
| HEP3B | 26.72±2.02 | 22.23±0.99 | 1.10±0.07 |
| HEPG2 | 56.15±8.73 | 27.34±2.84 | 4.50±0.27 |
| L929 | >100 | 21.52±1.97 | >100 |

[a]$IC_{50}$ for *n*-BuOH and $H_2O$ fractions: >100 $\mu$g/mL. $IC_{50}$ values were calculated from the cell growth inhibition curves obtained from the treatments with increasing concentrations of extracts or fractions for 48 h. Experiments were carried out in triplicate. [b] Positive control.

**Figure S1.** HRESIMS of prospestilbene (**1**)




| Ion | Experimental mass | Theoretical mass | Mass accuracy [ppm] |
|---|---|---|---|
| $[M+H]^+$ | 257.1177 | 257.1172 | 1.94 |

**Figure S2.** $^1$H NMR (500 MHz) spectrum of prospestilbene (**1**) in $CD_3OD$

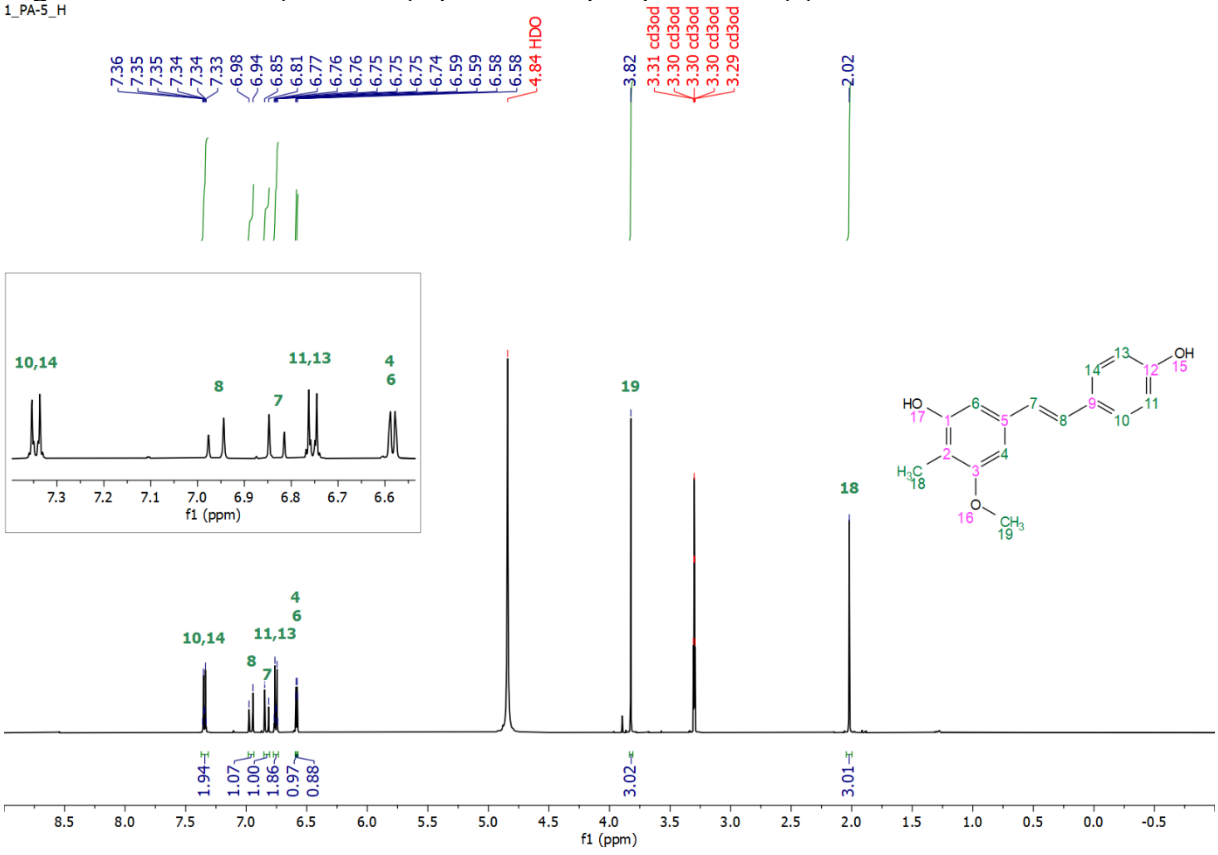


**Figure S3.** $^{13}C$ NMR (125.7 MHz) spectrum of prospestilbene (**1**) in $CD_3OD$

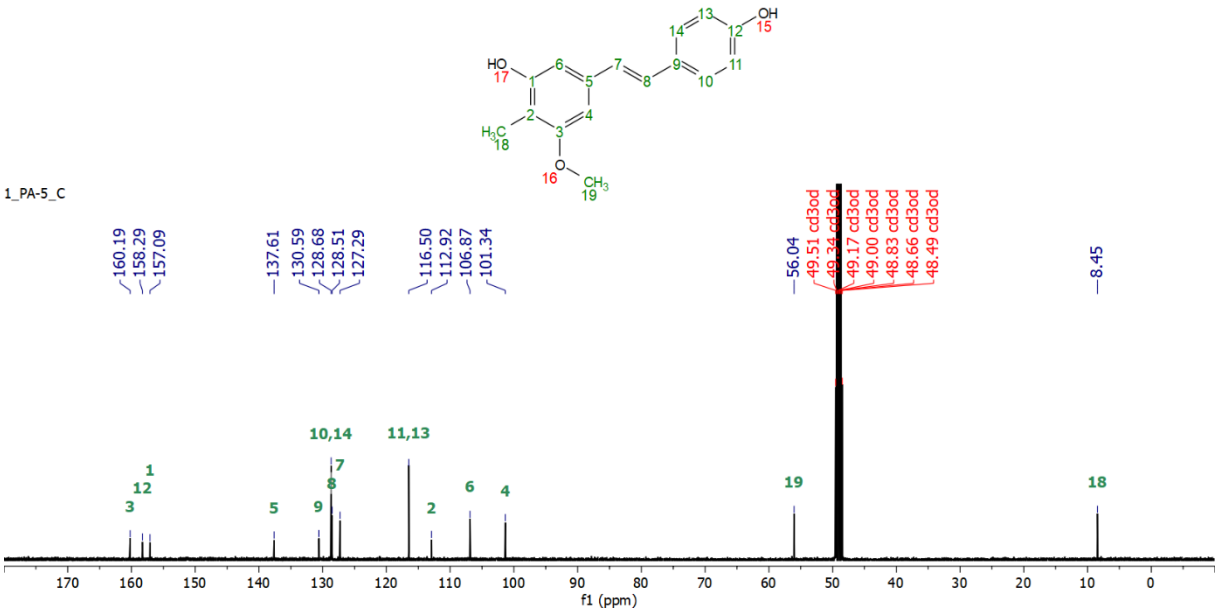



**Figure S4.** HSQC spectrum of prospestilbene (**1**)

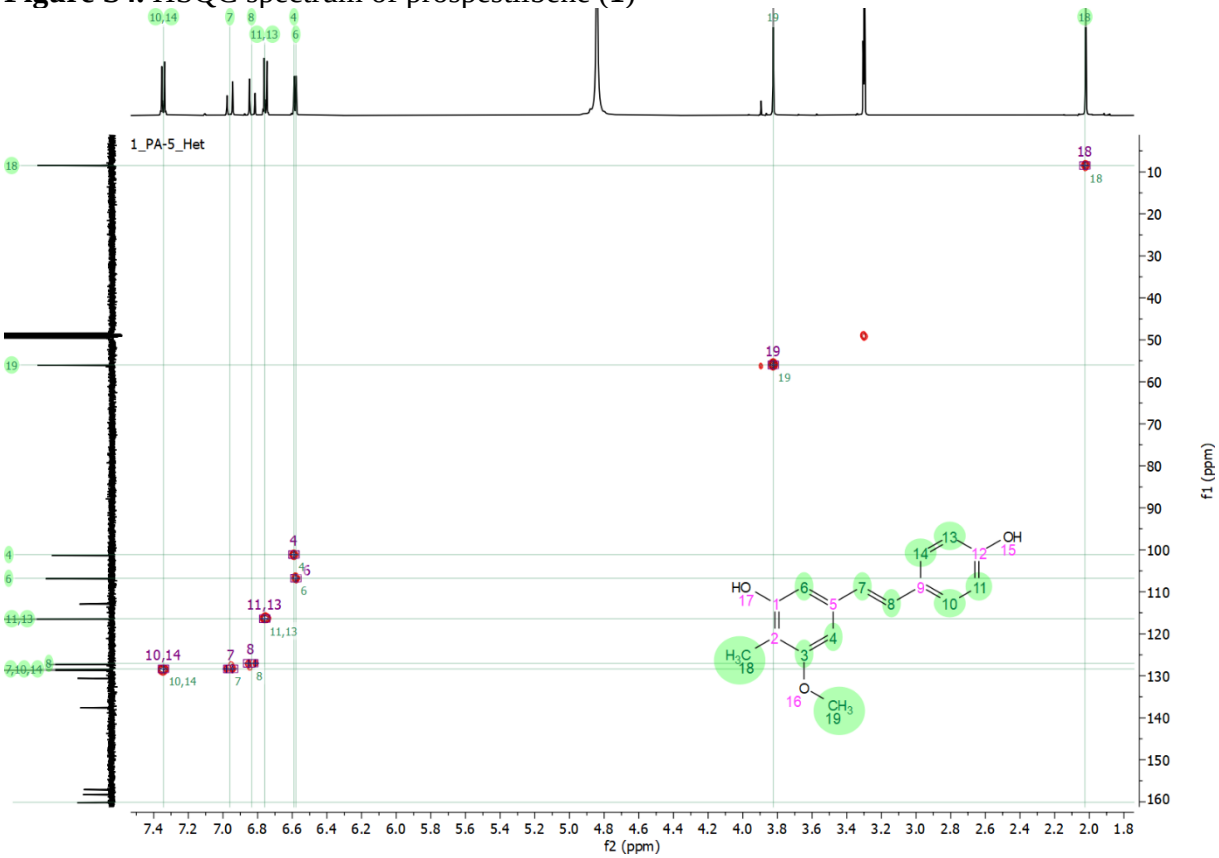


**Figure S5.** HMBC spectrum of prospestilbene (**1**)

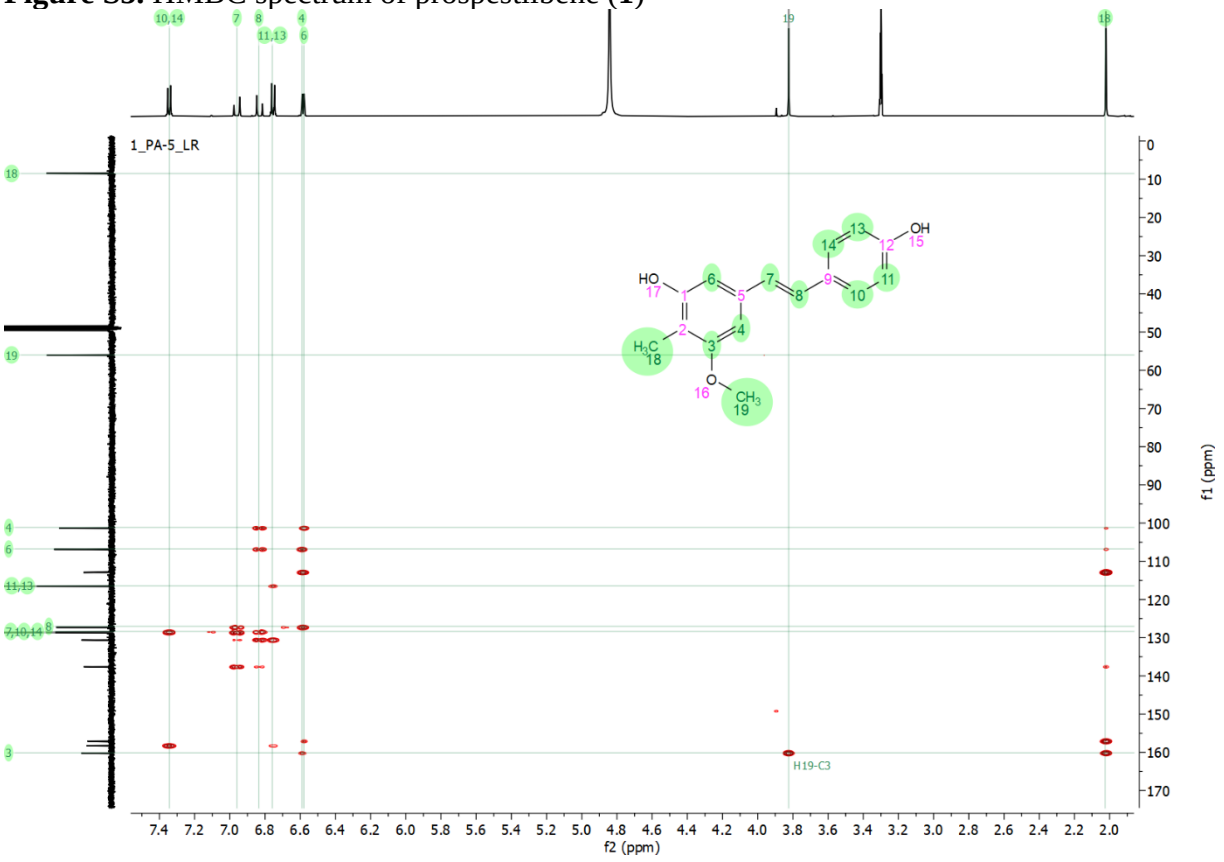


**Figure S6.** NOESY spectrum of prospestilbene (**1**)

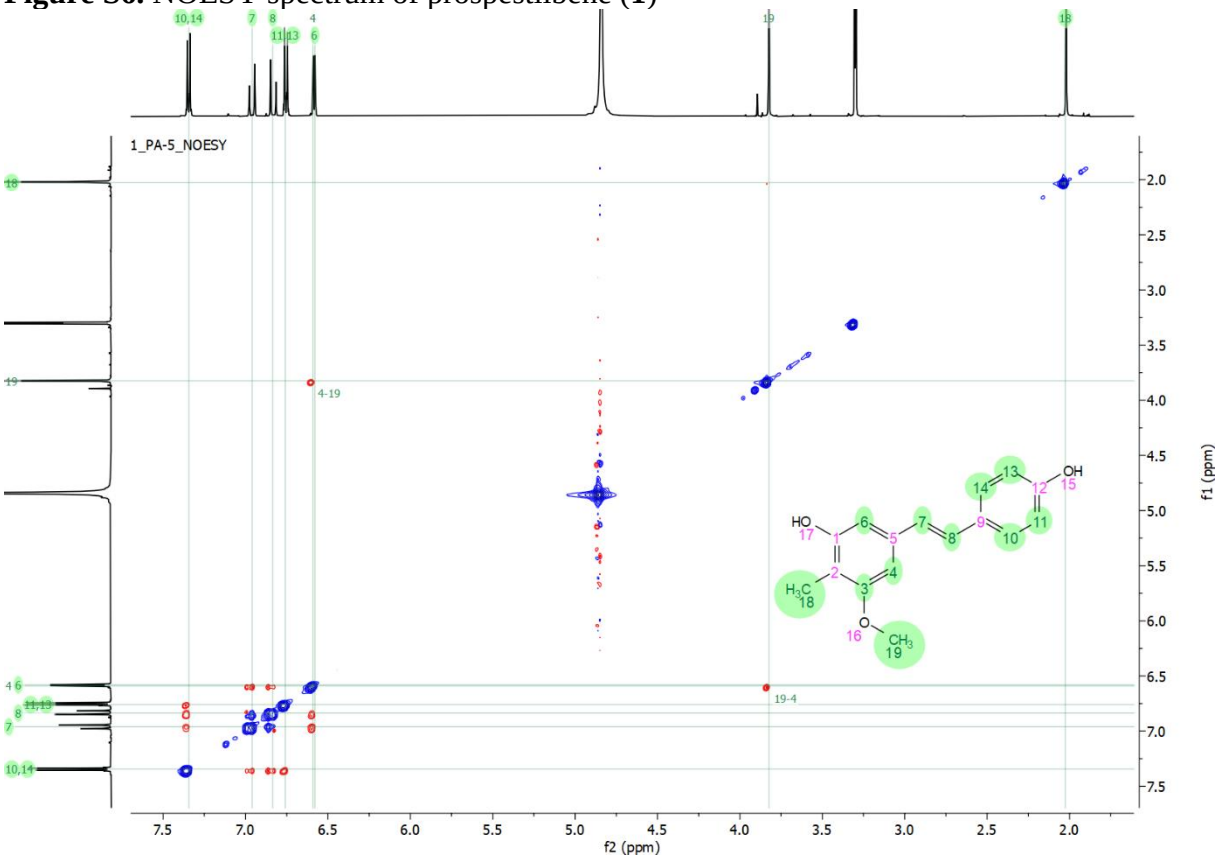



**Figure S7.** IR spectrum of prospestilbene (**1**)

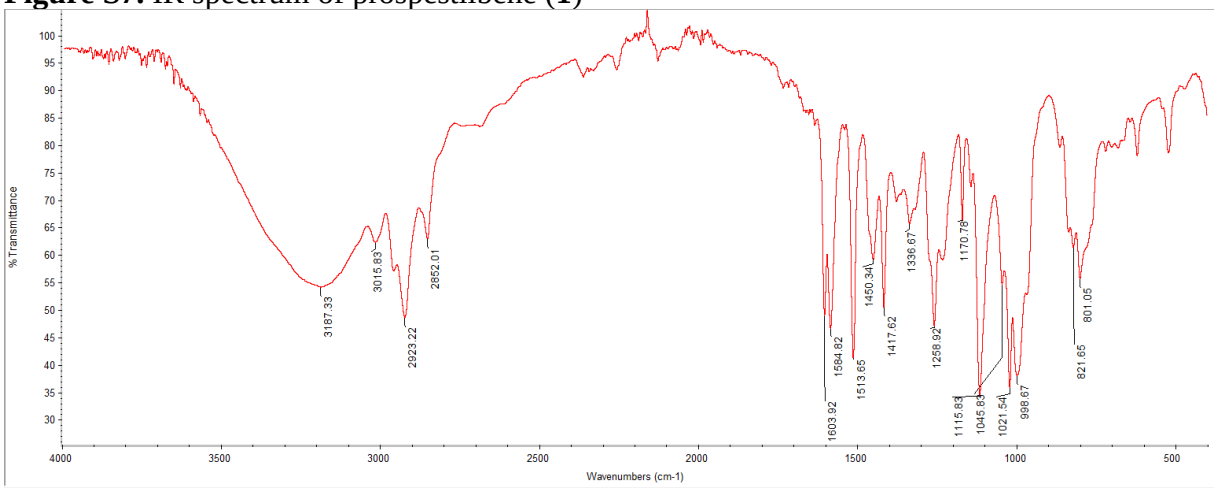


**Figure S8.** UV spectrum of prospestilbene (**1**)

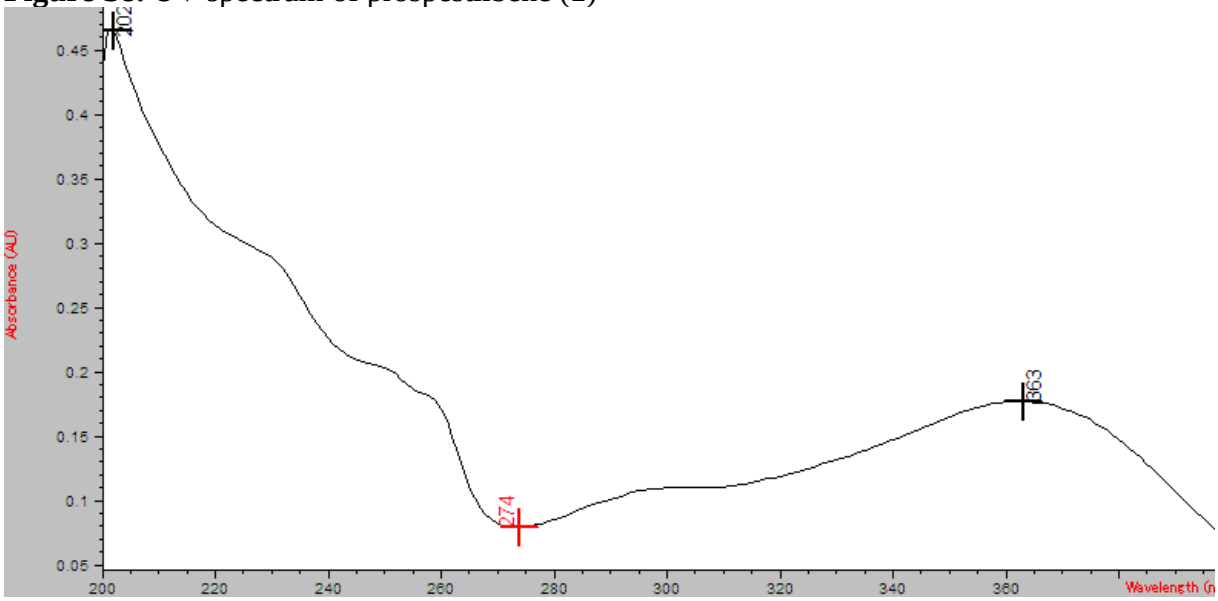
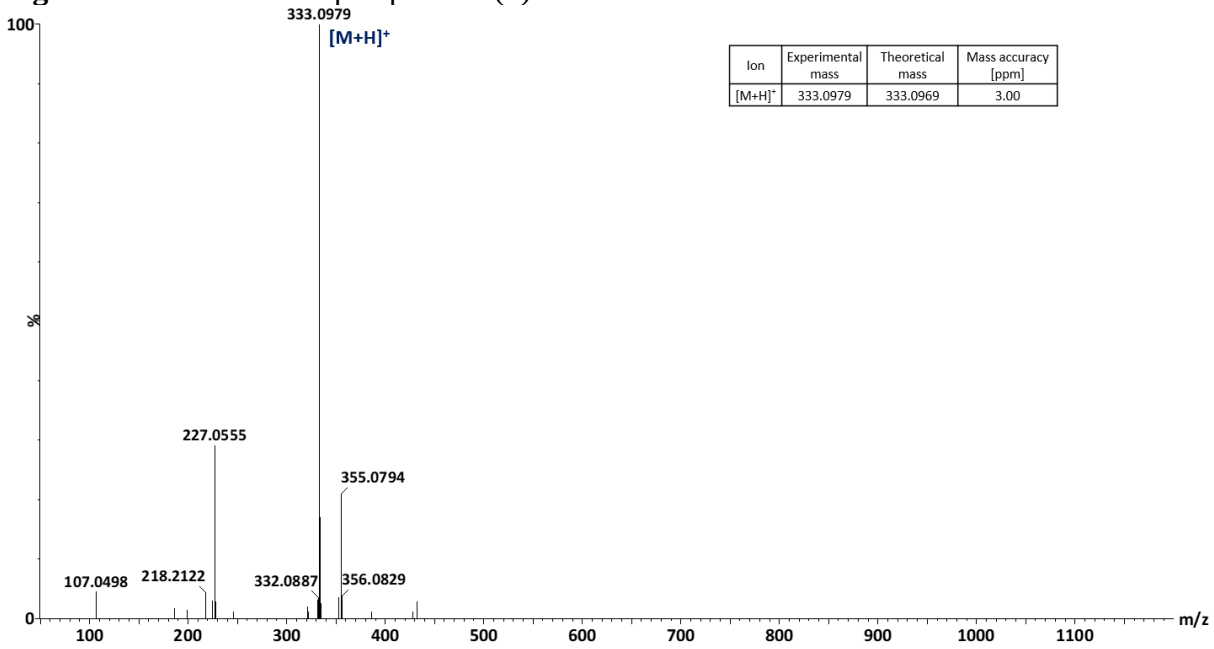


**Figure S9.** HRESIMS of prosperin A (**8**)




| Ion | Experimental mass | Theoretical mass | Mass accuracy [ppm] |
|---|---|---|---|
| $[M+H]^+$ | 333.0979 | 333.0969 | 3.00 |

**Figure S10.** $^1$H NMR (500 MHz) spectrum of prosperin A (**8**) in $CD_3COCD_3$

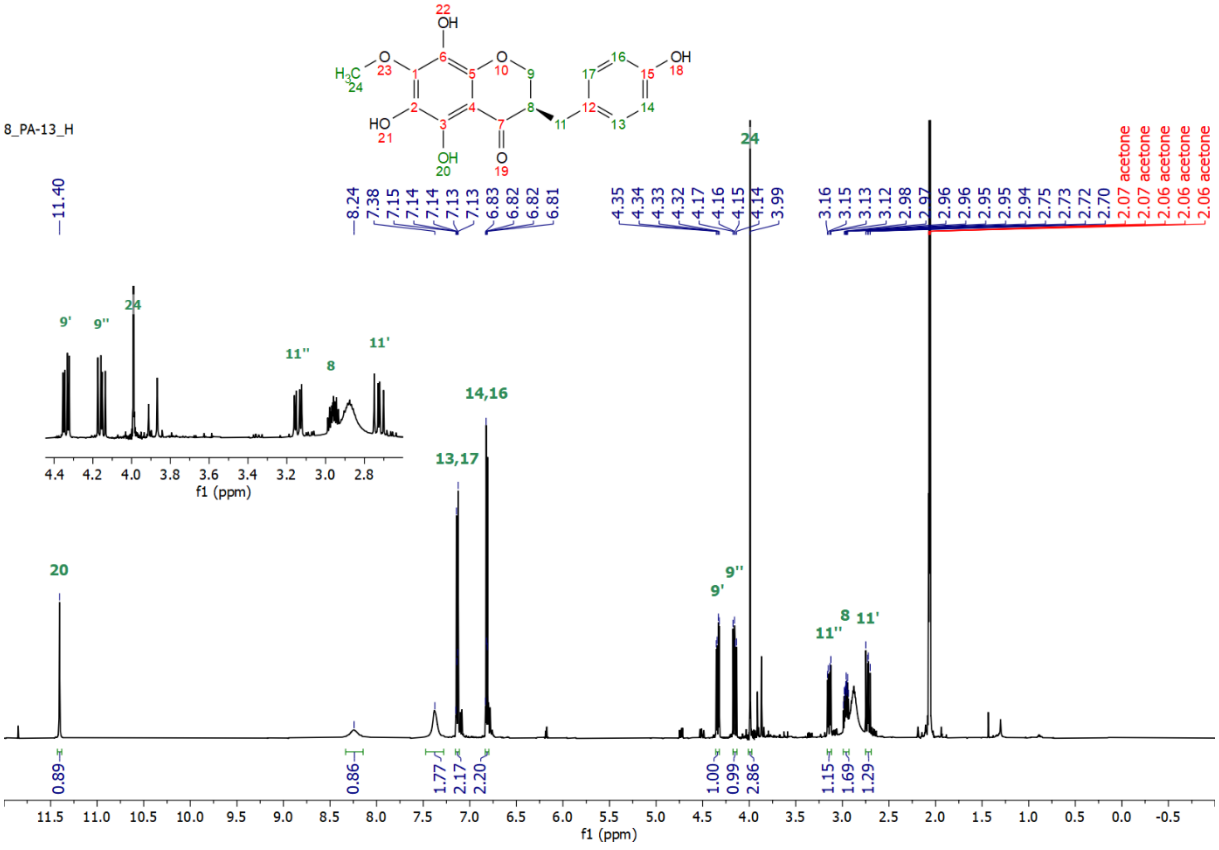


**Figure S11.** $^{13}C$ NMR (125.7 MHz) spectrum of prosperin A (**8**) in $CD_3COCD_3$

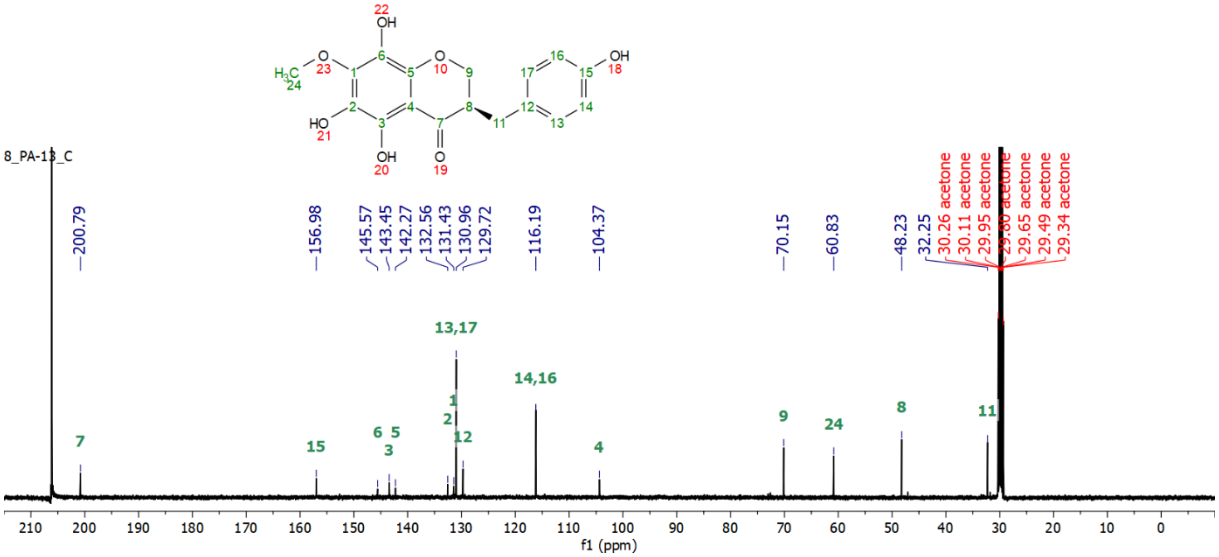



**Figure S12.** HSQC spectrum of prosperin A (**8**)

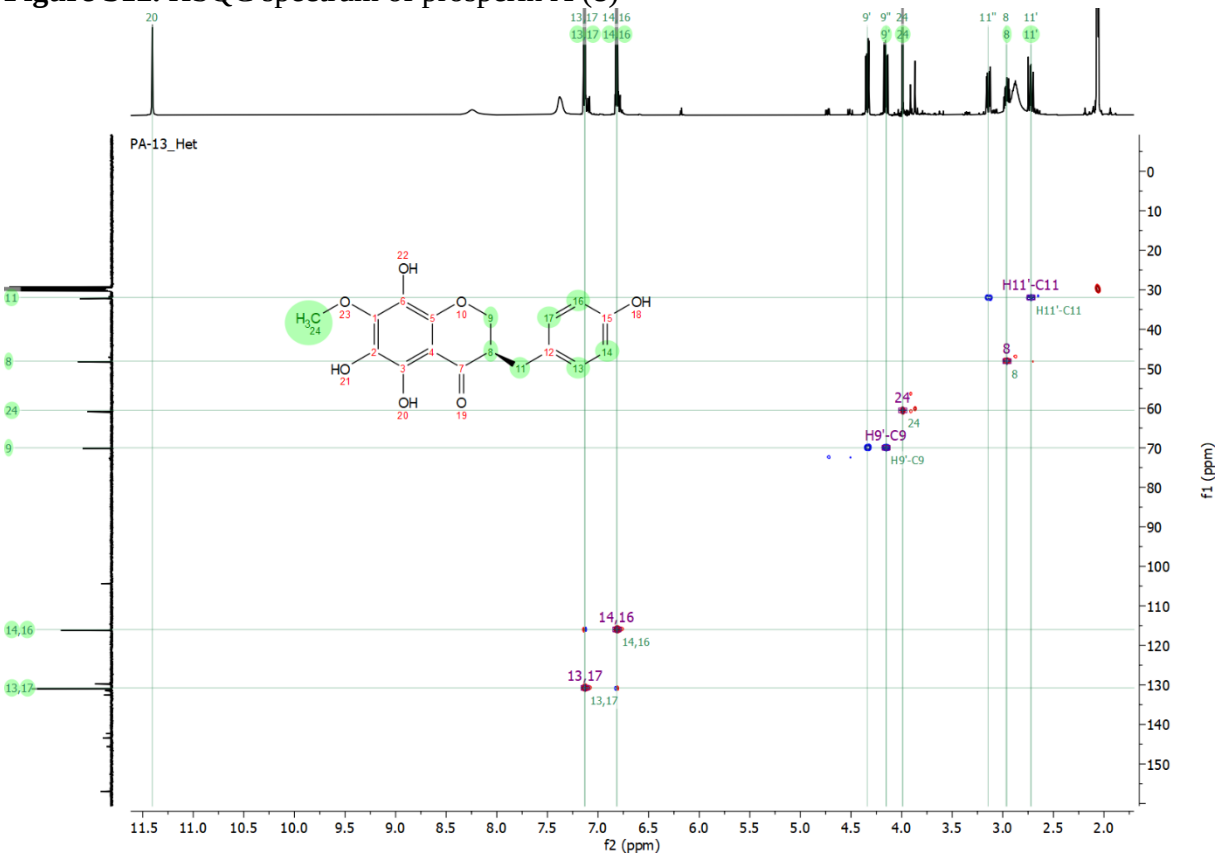


**Figure S13.** HMBC spectrum of prosperin A (**8**)

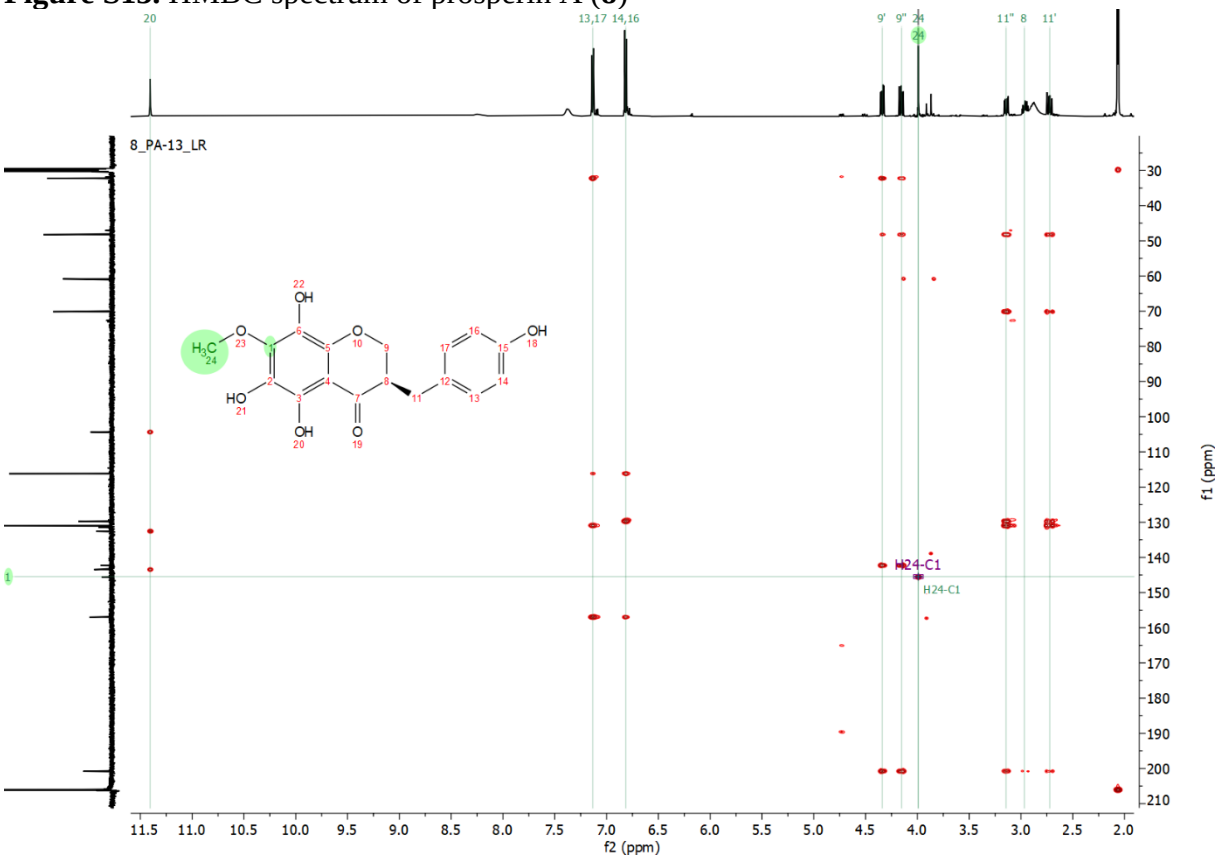



**Figure S14.** ROESY spectrum of prosperin A (**8**)

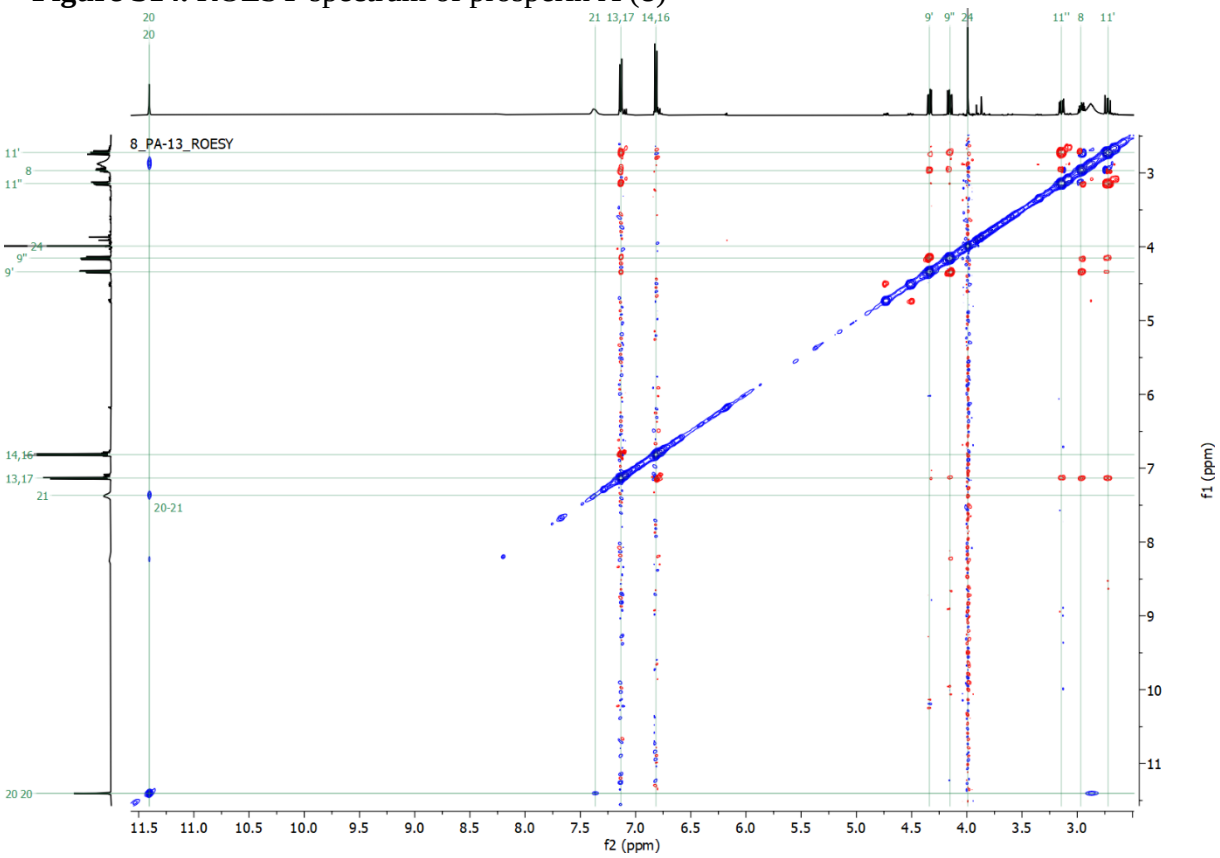


**Figure S15.** $^{1}H$ NMR (600 MHz) spectrum of prosperin A (**8**) in $CDCl_3$

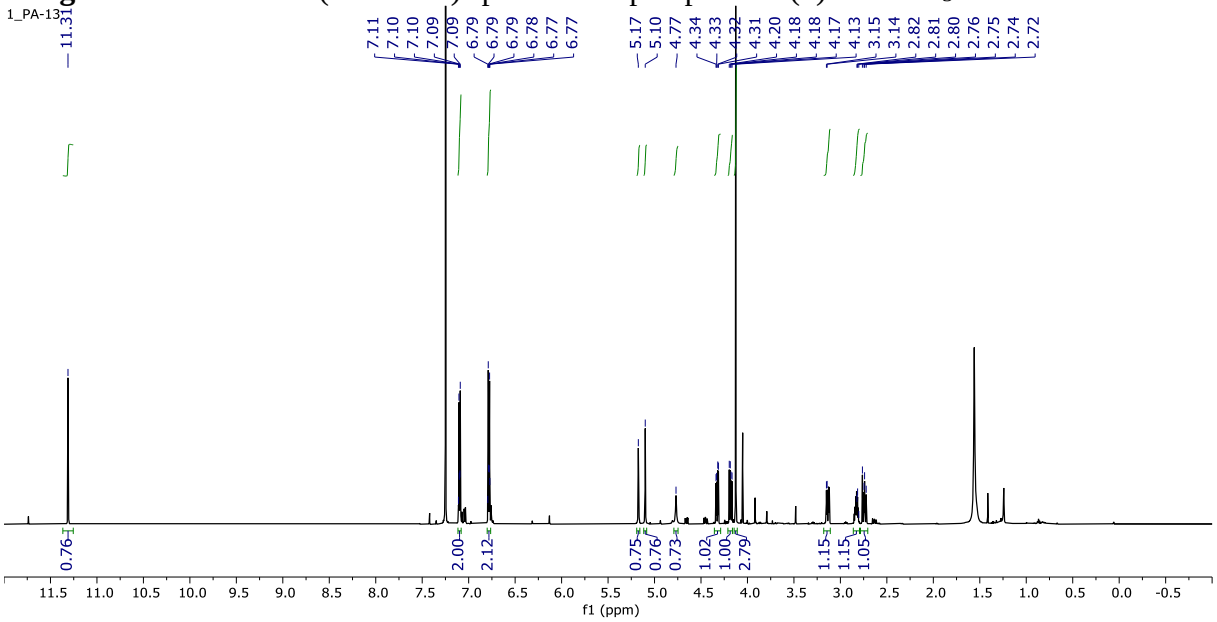



**Figure S16.** $^{13}C$ NMR (150 MHz) spectrum of prosperin A (**8**) in $CDCl_3$

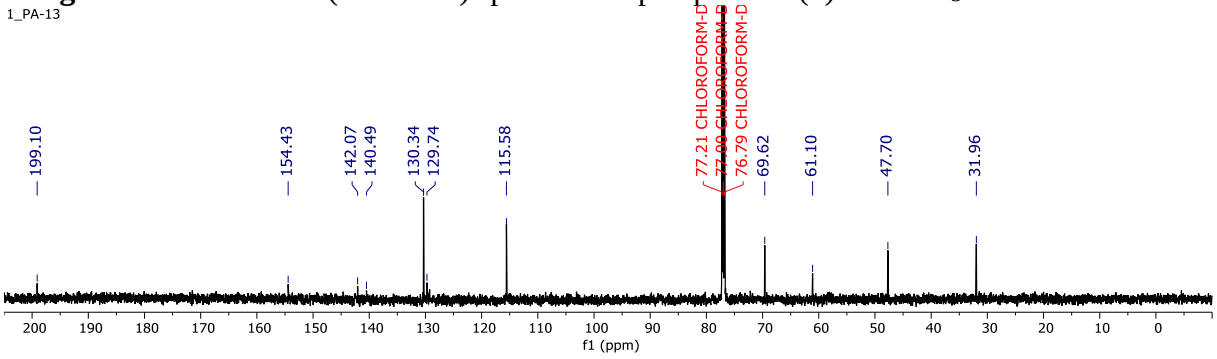
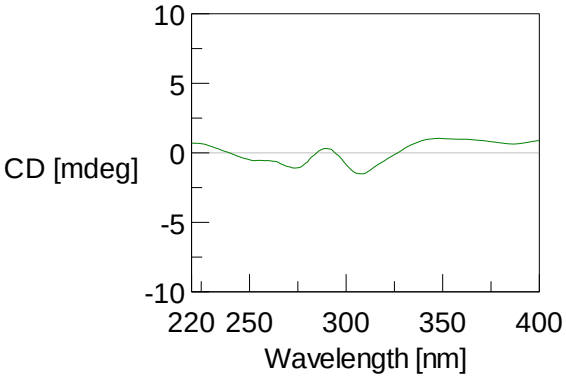


**Figure S17.** ECD spectrum of prosperin A (**8**) in MeOH




ECD (c 0.2 mg/ml, MeOH)

$\Delta\varepsilon_{273} = -0.55$ $[\theta]_{273} = -1826$

$\Delta\varepsilon_{308} = -0.76$ $[\theta]_{308} = -2507$

**Figure S18.** IR spectrum of prosperin A (**8**)

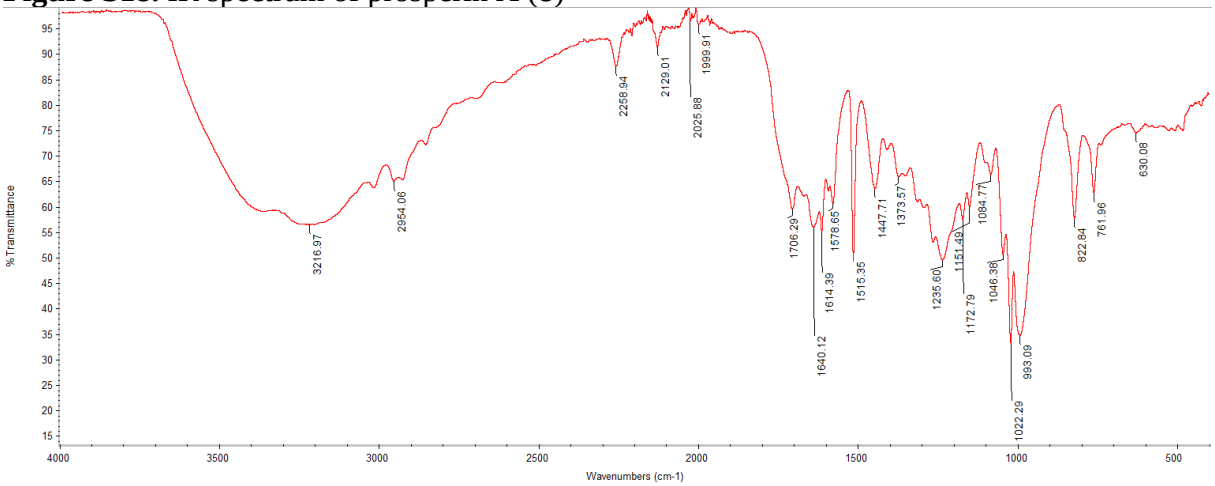



**Figure S19.** UV spectrum of prosperin A (**8**)

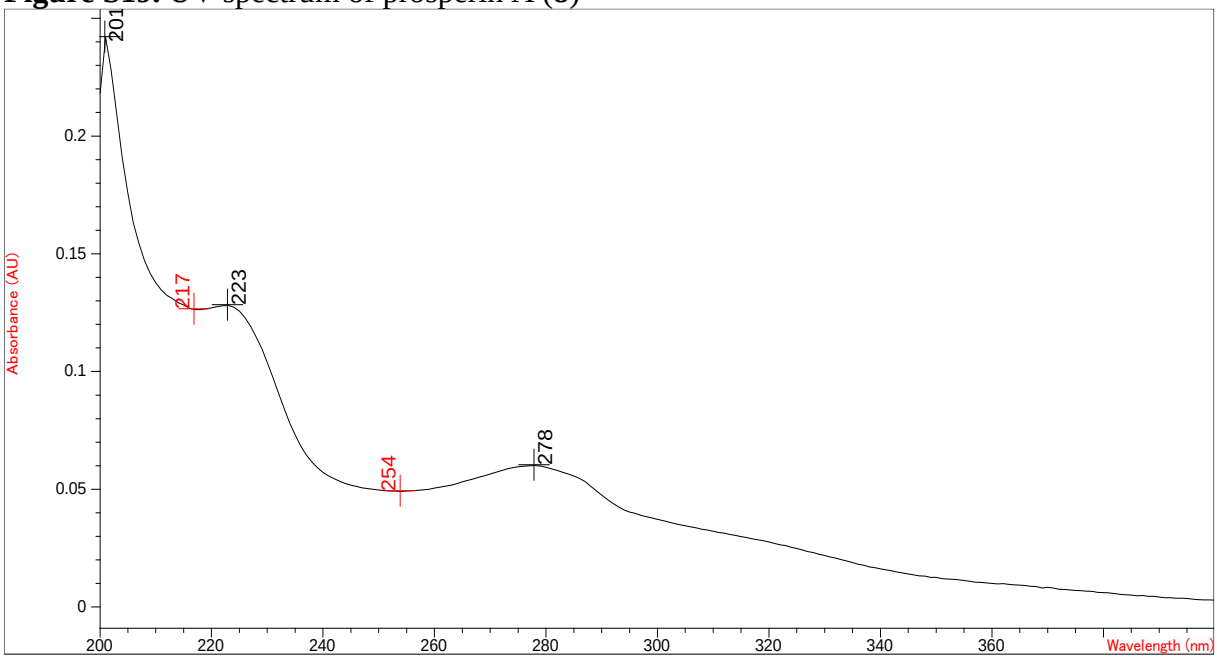
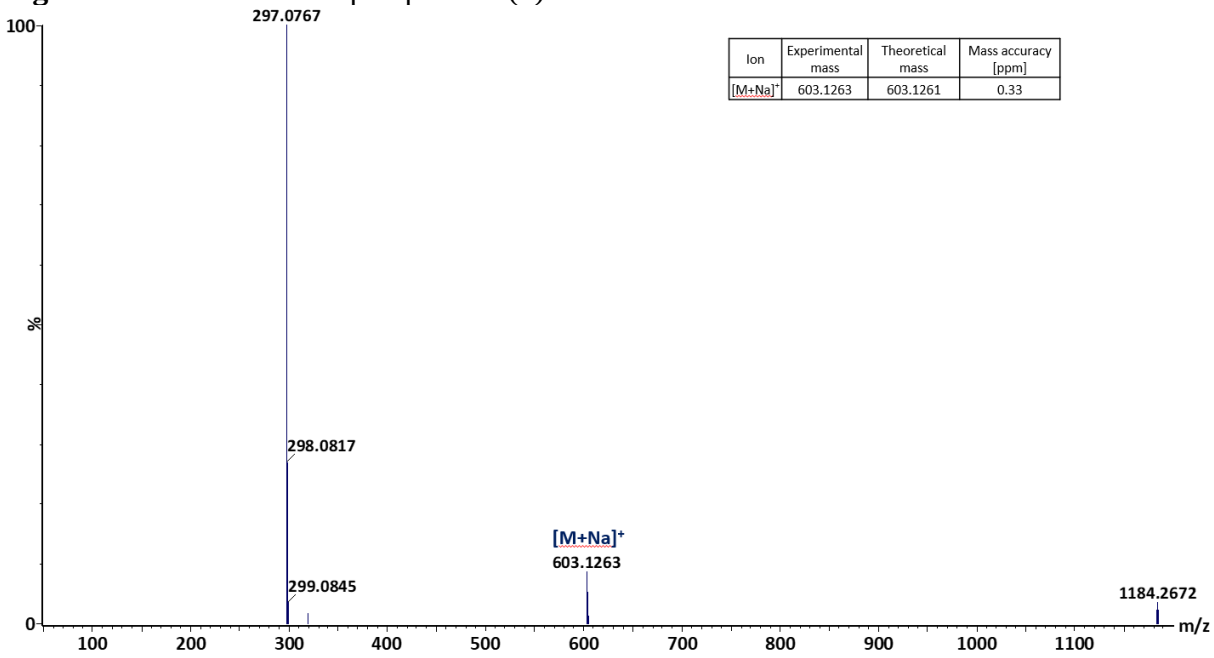


**Figure S20.** HRESIMS of prosperin B (**9**)




| Ion | Experimental mass | Theoretical mass | Mass accuracy [ppm] |
|---|---|---|---|
| $[M+Na]^+$ | 603.1263 | 603.1261 | 0.33 |

**Figure S21.** $^1$H NMR (500 MHz) spectrum of prosperin B (**9**) in $CD_3COCD_3$

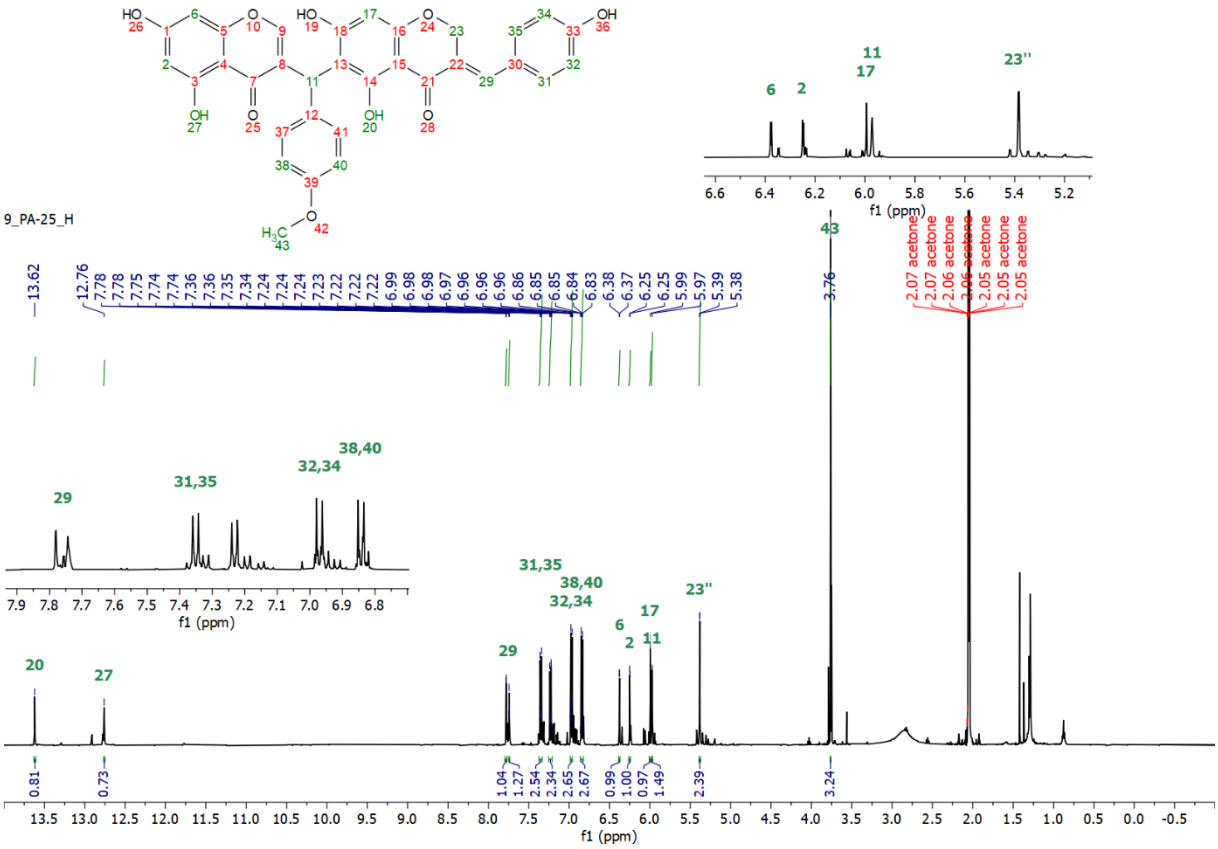


**Figure S22.** $^{13}C$ NMR (125.7 MHz) spectrum of prosperin B (**9**) in $CD_3COCD_3$

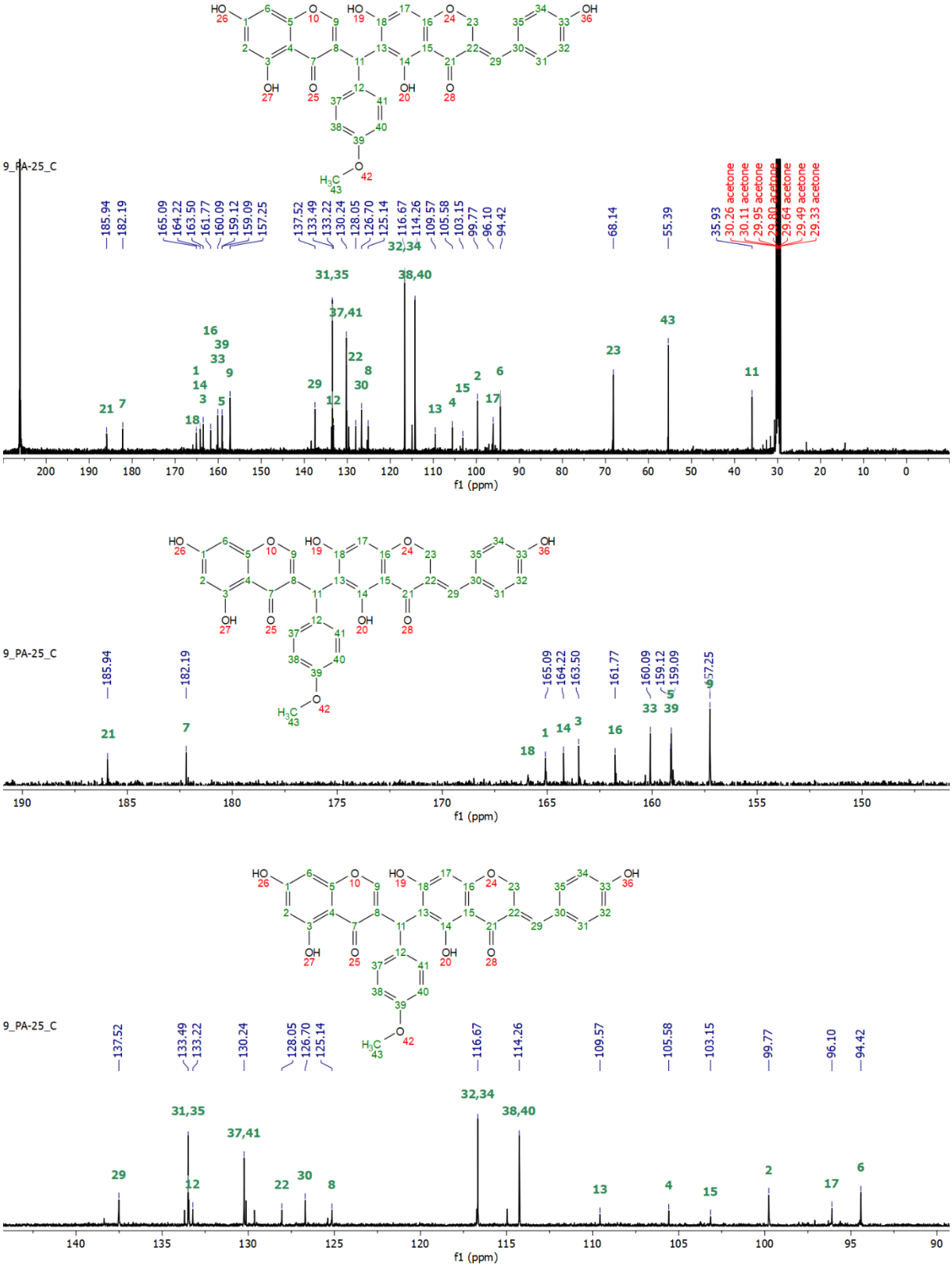


**Figure S23.** HSQC spectrum of prosperin B (**9**)

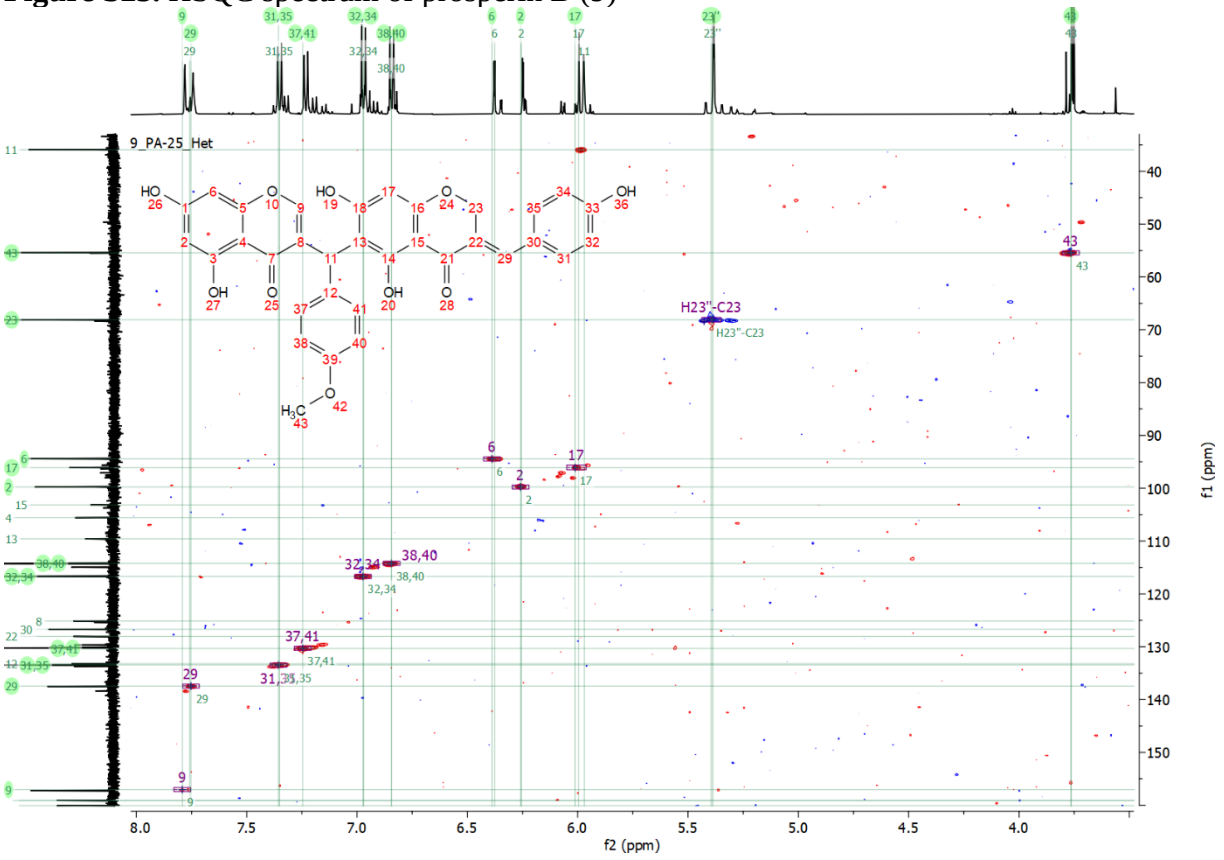



**Figure S24.** COSY spectrum of prosperin B (**9**)

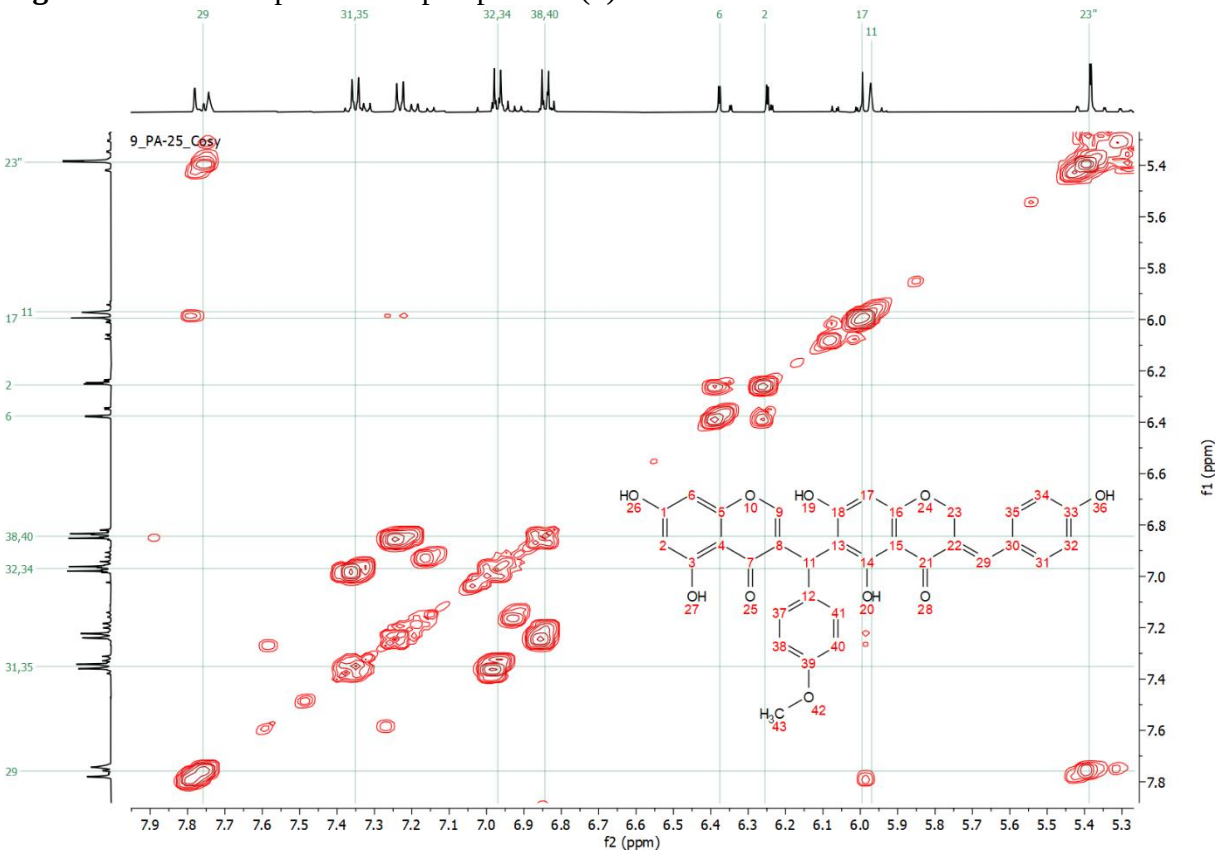


**Figure S25.** HMBC spectrum of prosperin B (**9**)

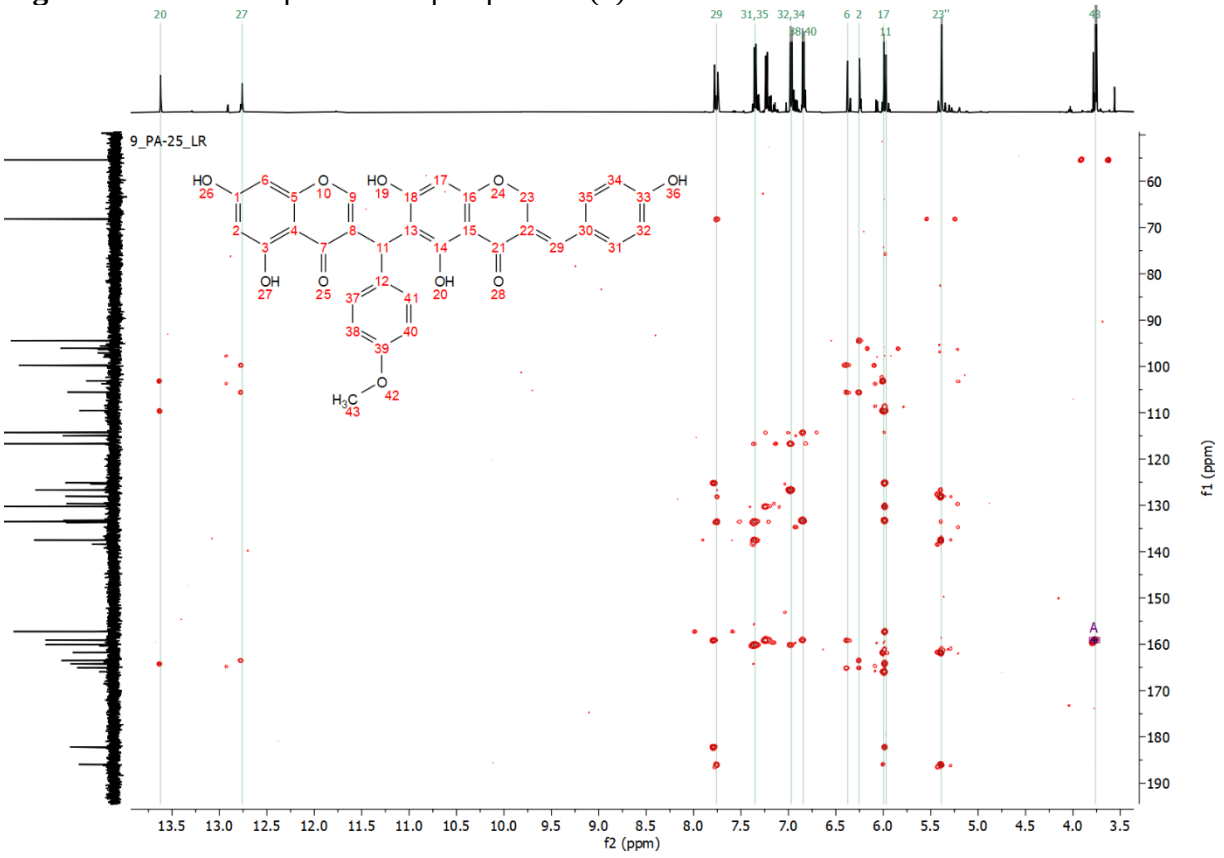



**Figure S26.** NOESY spectrum of prosperin B (**9**)

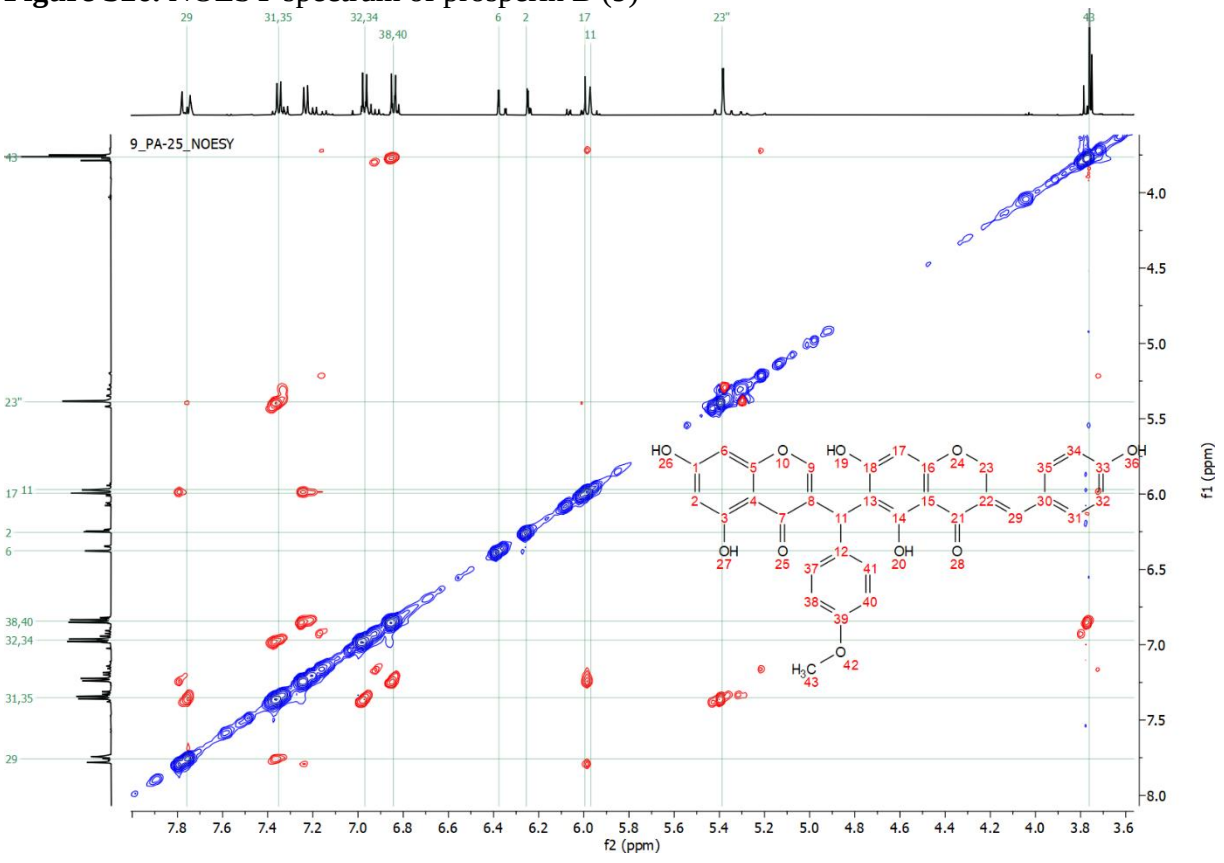
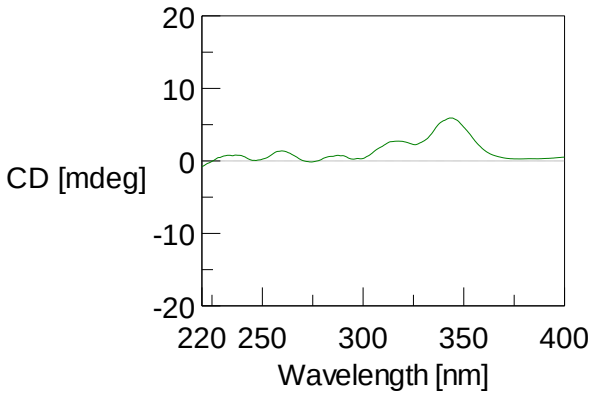


**Figure S27.** ECD spectrum of prosperin B (**9**) in MeOH




ECD (c 0.2 mg/ml, MeOH)

| | |
|---|---|
| $\Delta\varepsilon_{237}$ = +0.69 | $[\theta]_{237}$ = +2291 |
| $\Delta\varepsilon_{260}$ = +1.20 | $[\theta]_{260}$ = +3974 |
| $\Delta\varepsilon_{287}$ = +0.66 | $[\theta]_{287}$ = +2175 |
| $\Delta\varepsilon_{317}$ = +2.38 | $[\theta]_{317}$ = +7861 |
| $\Delta\varepsilon_{343}$ = +5.20 | $[\theta]_{343}$ = +17143 |

**Figure S28.** IR spectrum of prosperin B (**9**)

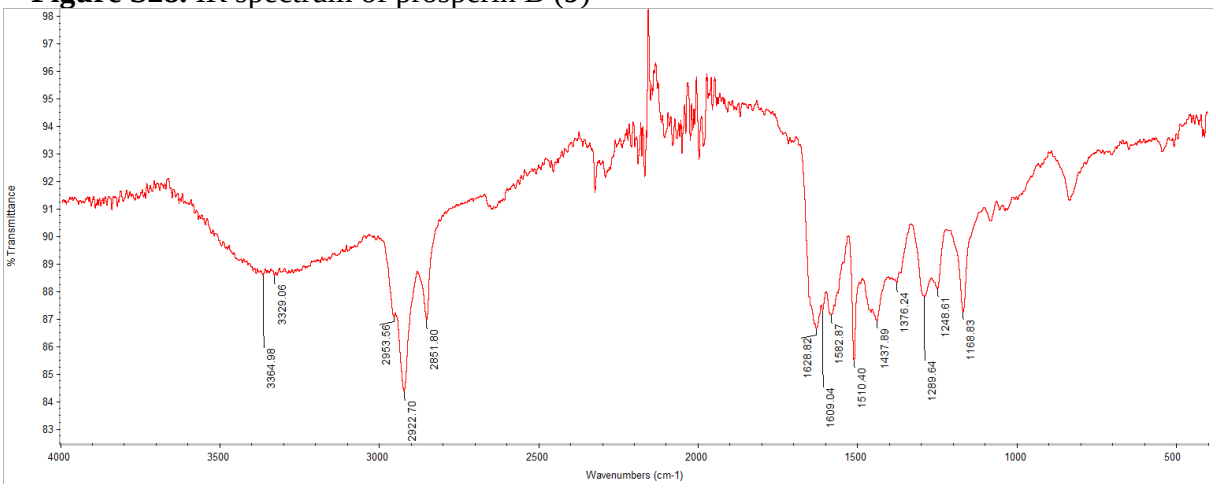



**Figure S29.** UV spectrum of prosperin B (**9**)

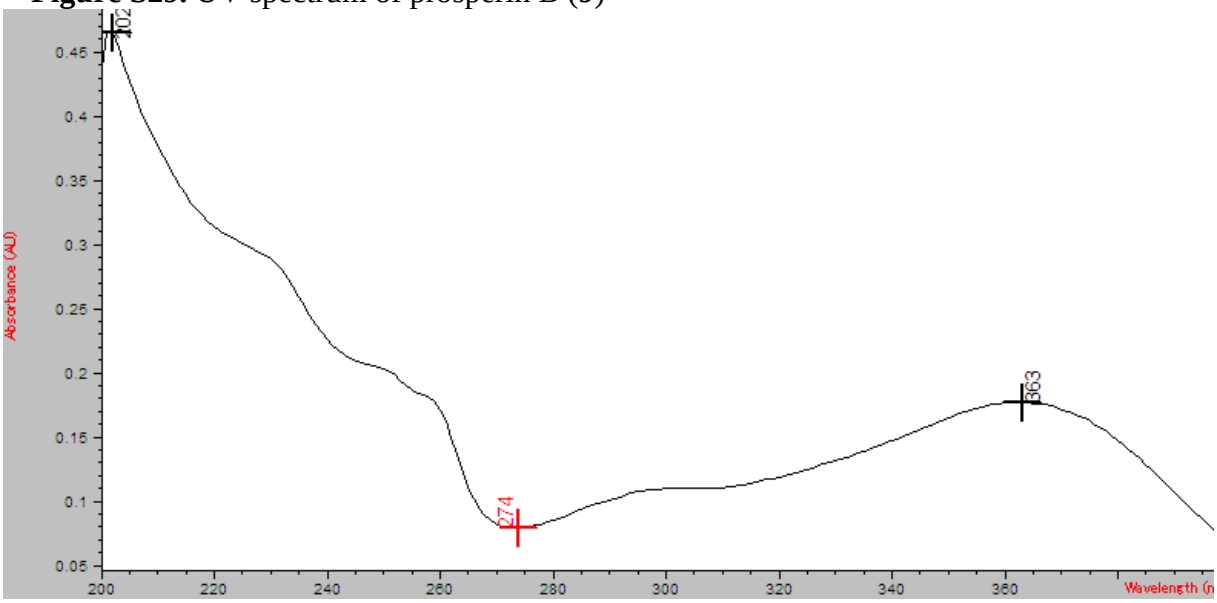
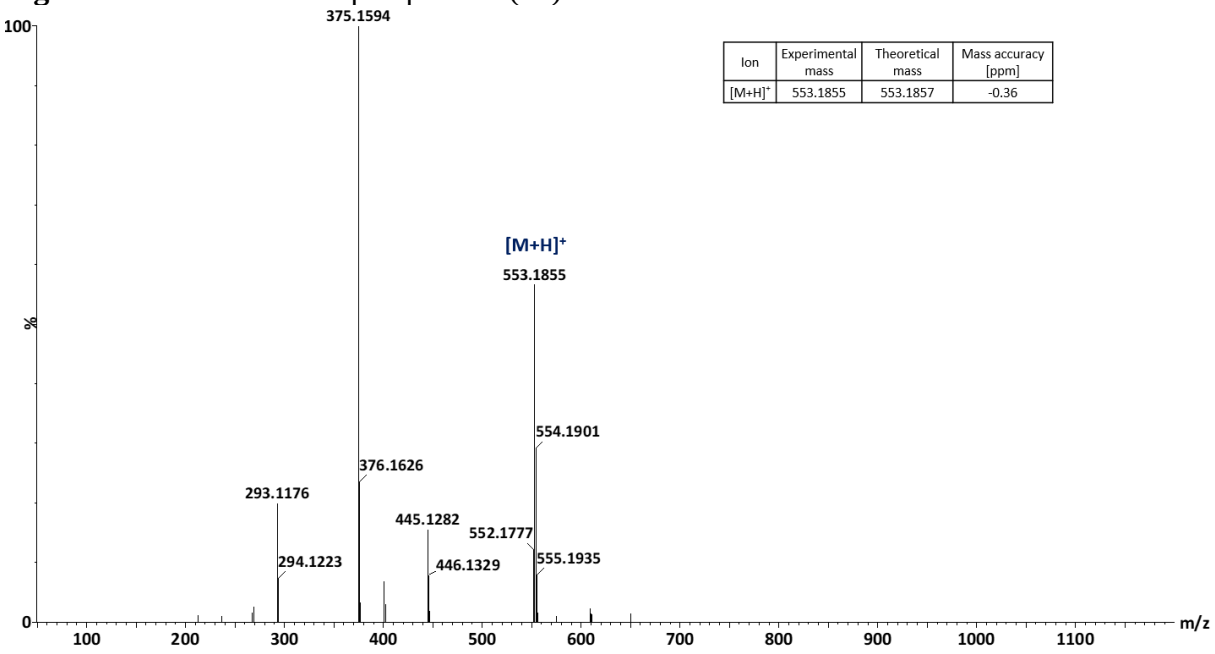


**Figure S30.** HRESIMS of prosperin C (**10**)




| Ion | Experimental mass | Theoretical mass | Mass accuracy [ppm] |
|---|---|---|---|
| $[M+H]^+$ | 553.1855 | 553.1857 | -0.36 |

**Figure S31.** $^1$H NMR (500 MHz) spectrum of prosperin C (**10**) in $CD_3COCD_3$

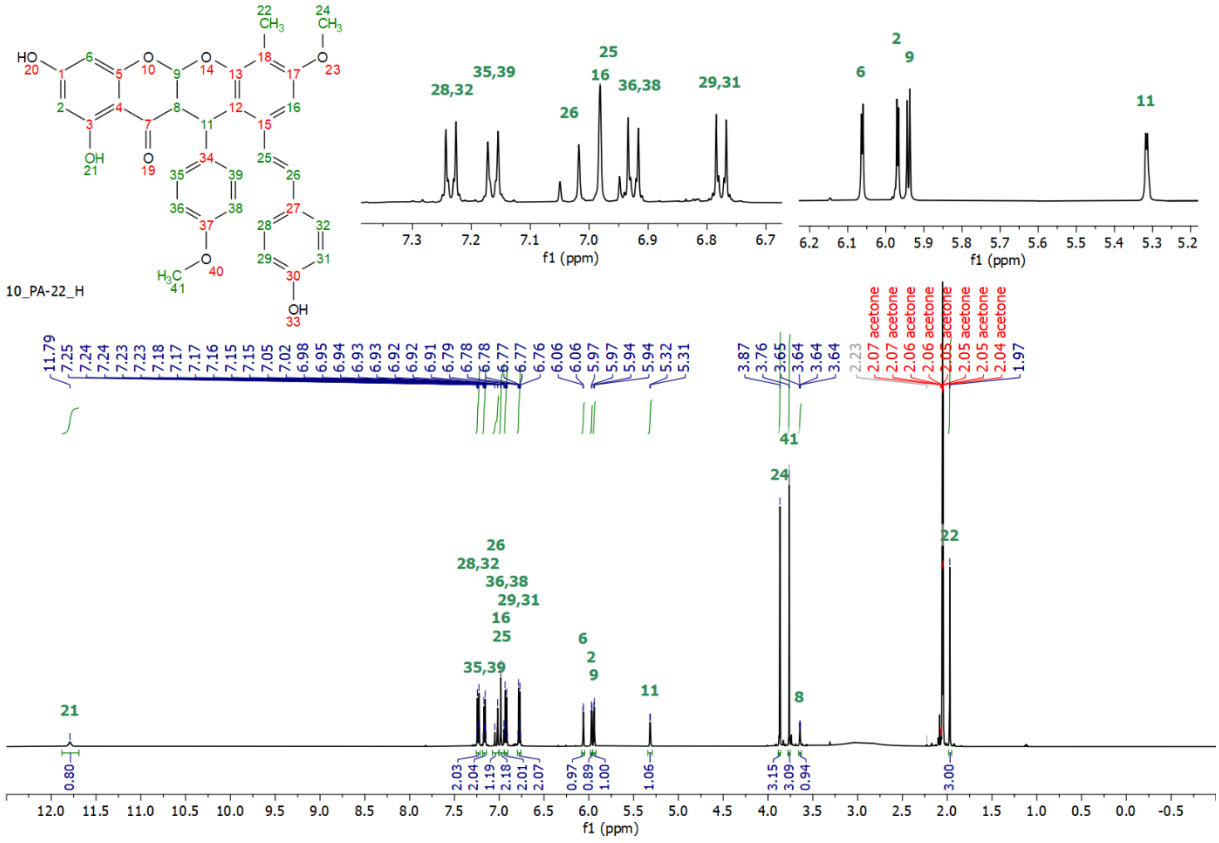


**Figure S32.** $^{13}$C NMR (125.7 MHz) spectrum of prosperin C (**10**) in $CD_3COCD_3$

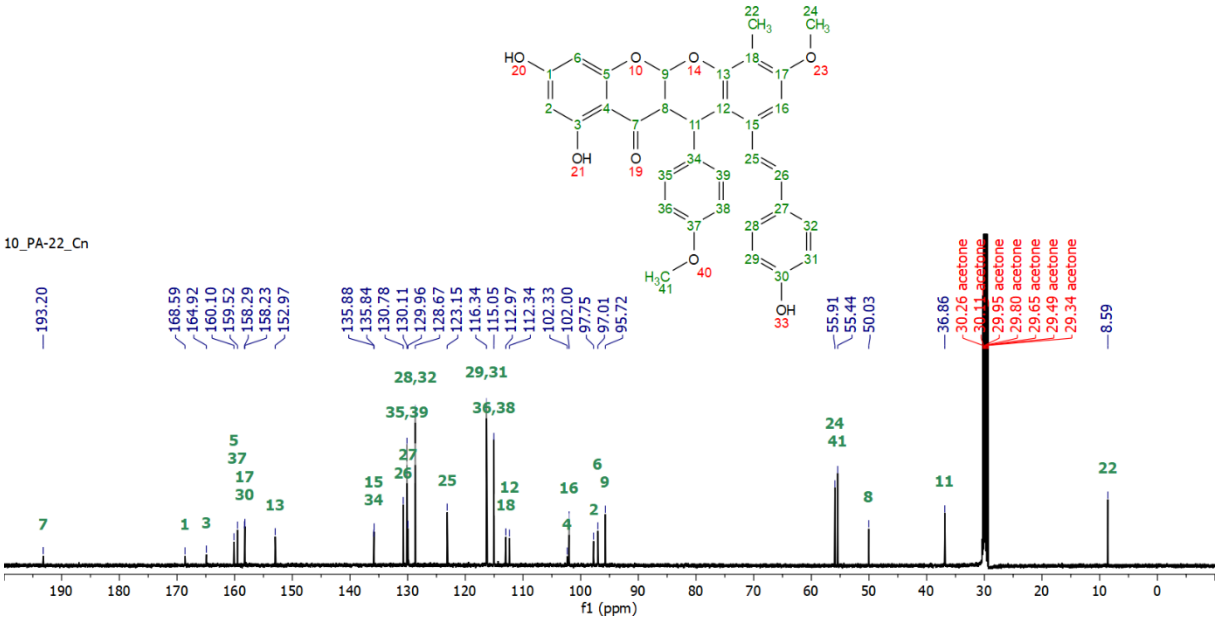



**Figure S33.** HSQC spectrum of prosperin C (**10**)

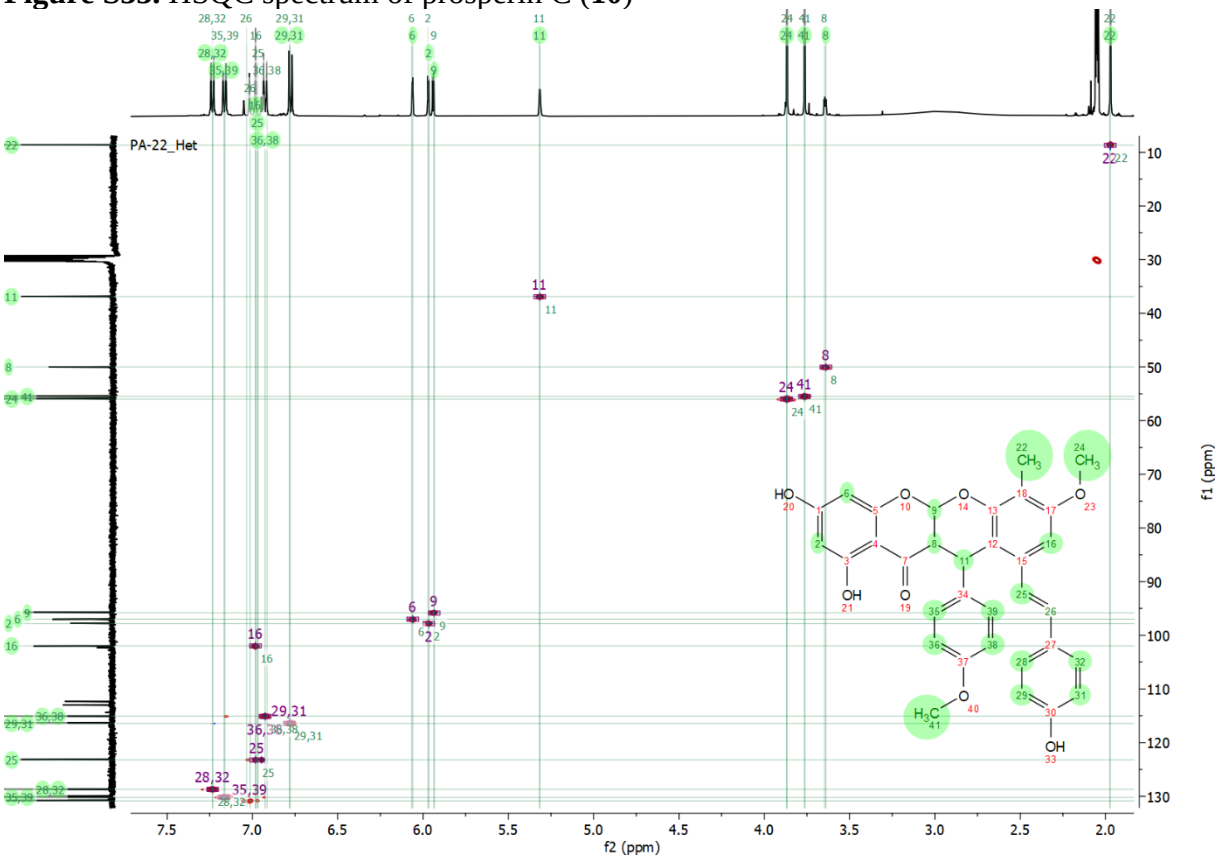


**Figure S34.** COSY spectrum of prosperin C (**10**)

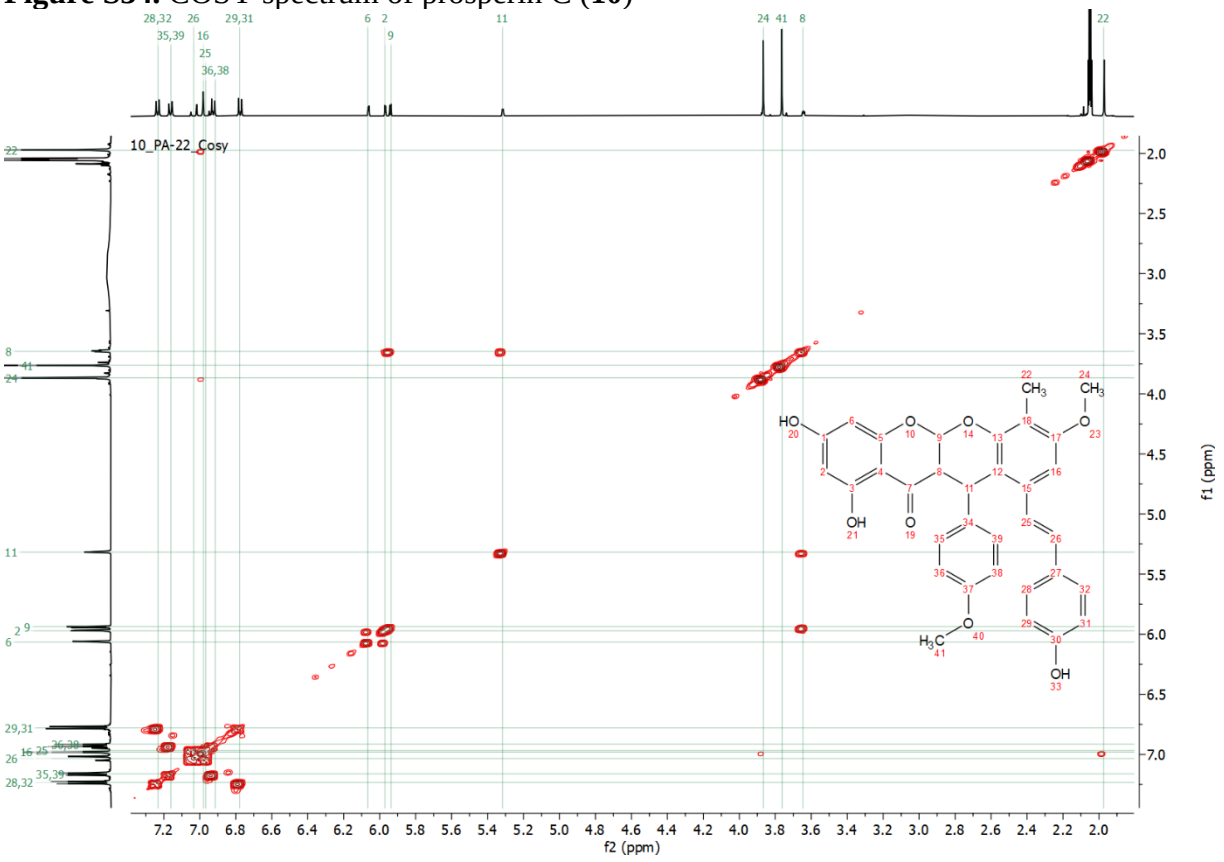



**Figure S35.** H2BC spectrum of prosperin C (**10**)

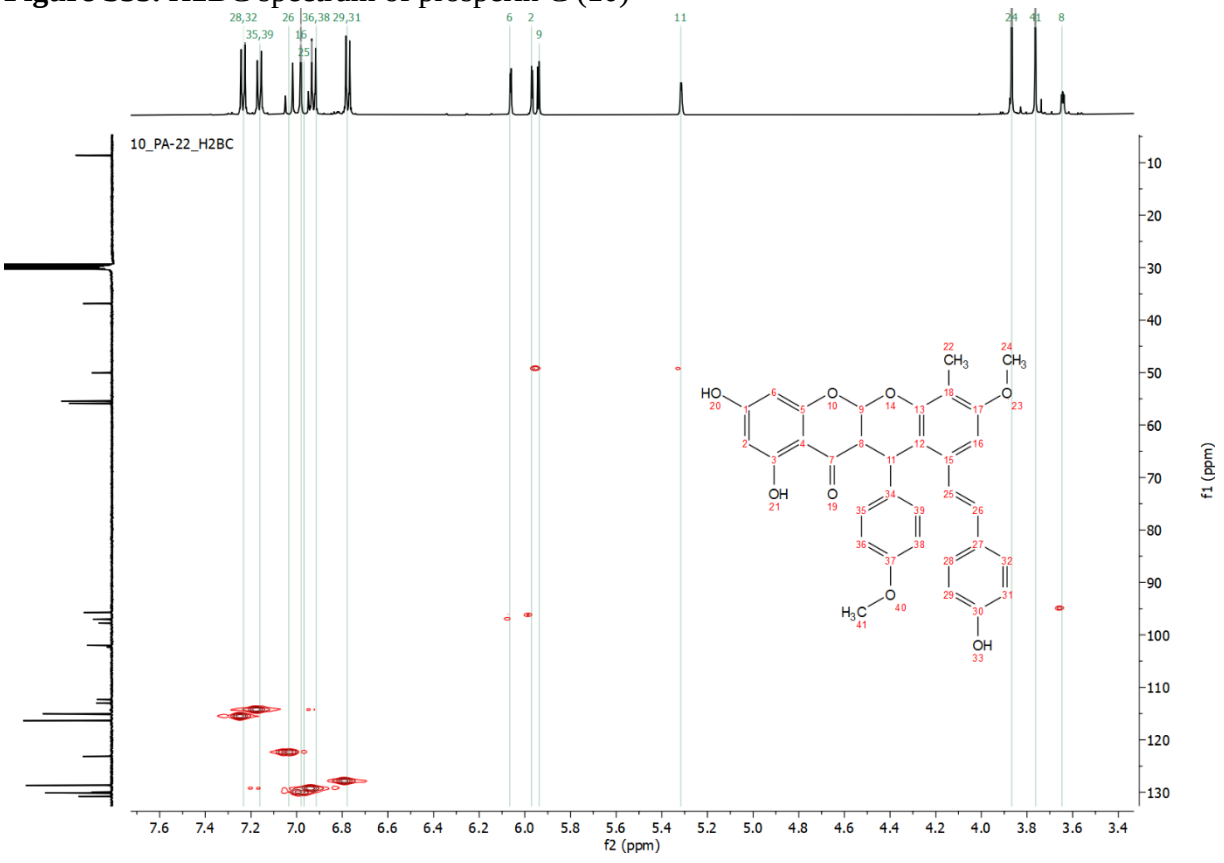


**Figure S36.** HMBC spectrum of prosperin C (**10**)

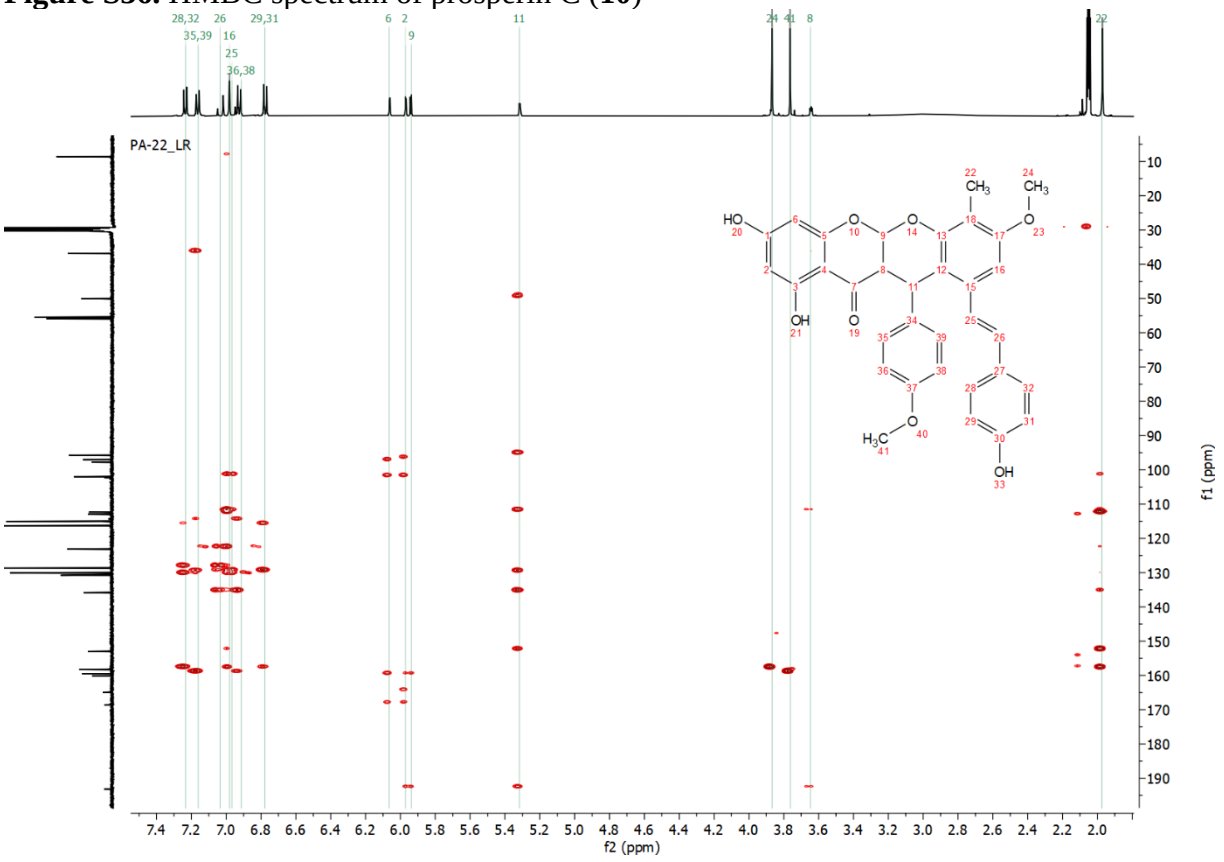



**Figure S37.** NOESY spectrum of prosperin C (**10**)

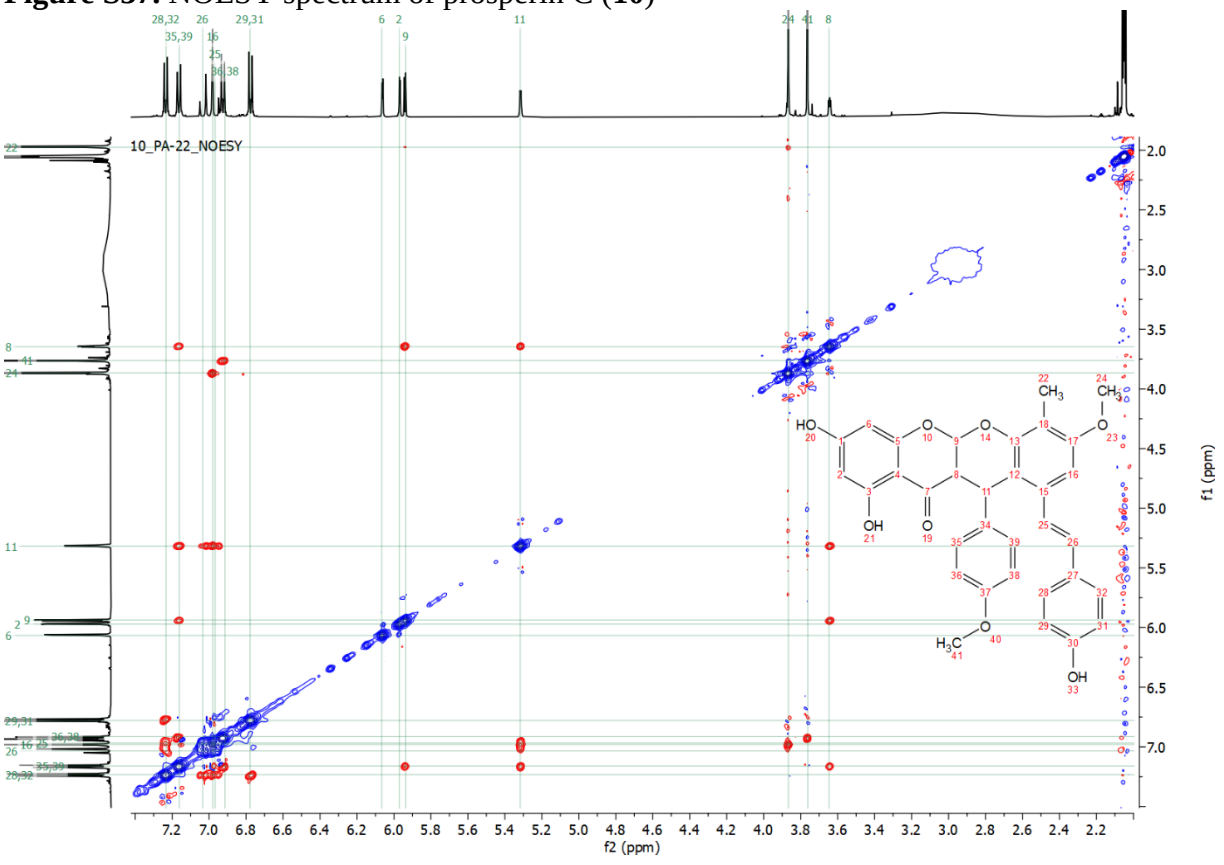
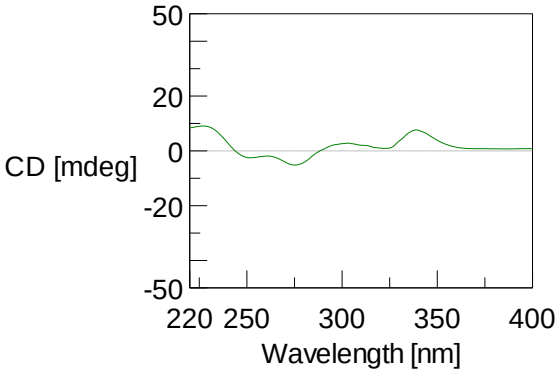


**Figure S38.** ECD spectrum of prosperin C (**10**) in MeOH




ECD (c 0.2 mg/ml, MeOH)

$\Delta\varepsilon_{227} = +7.56$ $[\theta]_{227} = +24958$

$\Delta\varepsilon_{252} = -2.13$ $[\theta]_{252} = -7012$

$\Delta\varepsilon_{275} = -4.34$ $[\theta]_{275} = -14301$

$\Delta\varepsilon_{339} = +6.38$ $[\theta]_{339} = +21038$

**Figure S39.** IR spectrum of prosperin C (**10**)

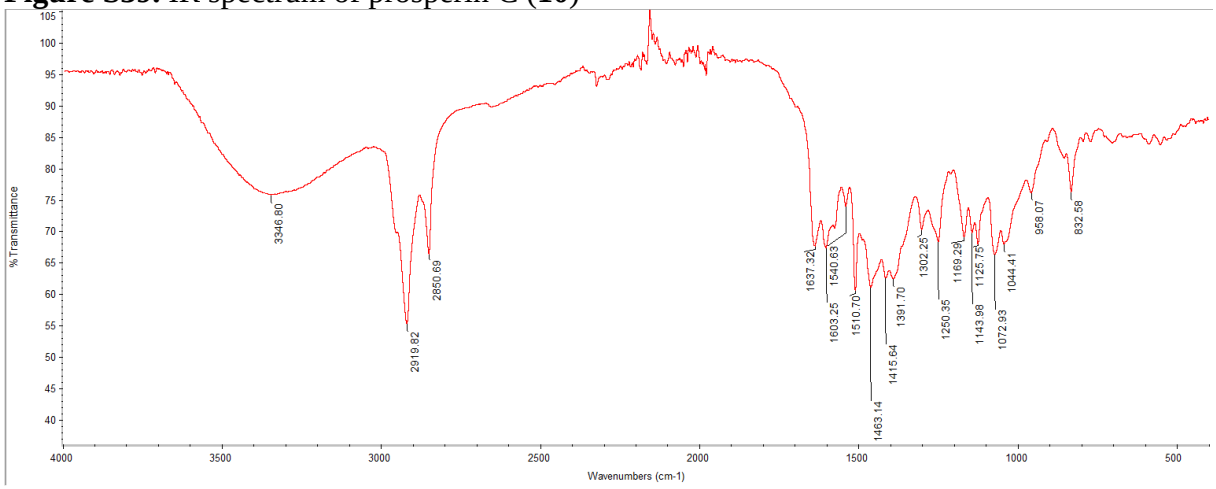
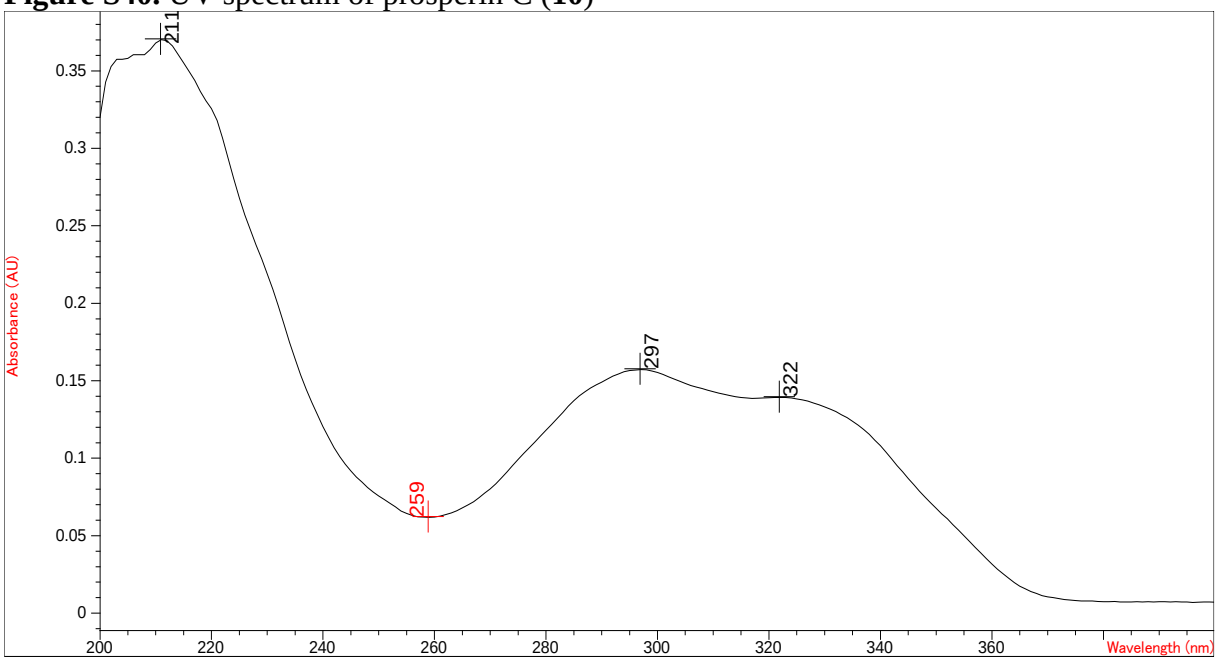



**Figure S40.** UV spectrum of prosperin C (**10**)



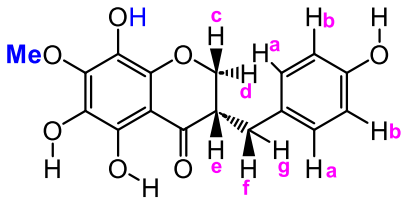
**Details about computational NMR calculations of prosperin A (8) in chloroform**

In addition to modeling prosperin A in actone (see main text), we calculated the NMR shifts in chloroform as well. The conformer library was generated manually resulting in 24 confomers for both form A and B. Geometry optimizations done using the same level of theory used in the main text, but NMR calulcations were done at the mPW1PW91/6-311+G(2d,p) scrf=(smd,solvent=chloroform) level of theory. Conformers for structure A ranged from 0 to 4.12 kcal/mol while structure B had conformers ranging from 0 to 3.80 kcal/mol. Comparison between the hydrogen and carbon NMR are shown for the highlighted hydrogens and carbons are below.

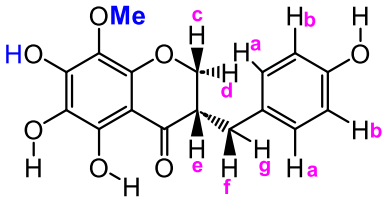
**Table S2.** Experimental and computed $^{1}$H NMR of prosperin A (**8**) in $CDCl_3$

| | Me | a | b | c | d | f | e | g |
|---|---|---|---|---|---|---|---|---|
| Measured | 4.14 | 7.11 | 6.79 | 4.34 | 4.19 | 3.15 | 2.81-2.87 | 2.75 |
| **A (computed)** | 4.13 | 7.20 | 6.77 | **4.12** | 4.02 | **3.33** | 2.77 | **2.53** |
| **B (computed)** | **3.75** | 7.20 | 6.74 | 4.19 | **3.91** | 3.03 | 2.86 | **3.00** |

Difference from the measured values: **red** > 0.30 ppm, **blue** 0.20 ~ 0.30 ppm



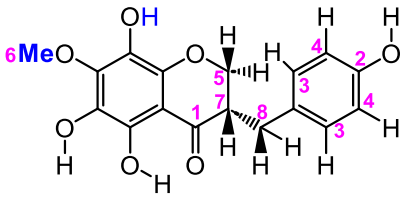

A



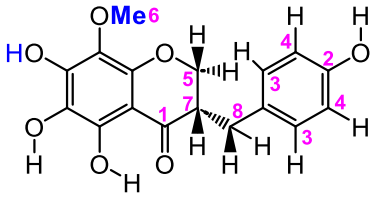

B

**Table S3.** Experimental and computed $^{13}C$ NMR of prosperin A (**8**) in $CDCl_3$

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Measured | 199.1 | 154.4 | 130.4 | 115.6 | 69.6 | 61.1 | 47.7 | 32.0 |
| **A (computed)** | 199.1 | 155.3 | 130.7 | 113.9 | 68.9 | 58.8 | 50.0 | 32.7 |
| **B (computed)** | 197.6 | 155.3 | 130.5 | 113.8 | 69.3 | 58.7 | 49.3 | 32.4 |



A



B

**Table S4.** Comparison of $^{1}H$ and $^{13}C$ NMR of prosperin B (**9**) in $CD_3COCD_3$

| **no.** | $\delta_C$, type (measured) | $\delta_C$ (calculated) | $\delta_H$, mult. (*J* in Hz) (measured) | $\delta_H$ (calculated) |
|---|---|---|---|---|
| 2 | 157.3, CH | 158.2 | 7.78, d (1.1) | 8.12 |
| 3 | 125.1, C | 123.8 | | |
| 4 | 182.2, C | 179.5 | | |
| 4a | 105.6, C | 102.2 | | |
| 5 | 163.5, C | 162.0 | | |
| 6 | 99.8, CH | 98.3 | 6.25, d (2.1) | 6.16 |
| 7 | 165.1, C | 162.0 | | |
| 8 | 94.4, CH | 93.0 | 6.38, d (2.1) | 6.42 |
| 8a | 159.12, C | 157.1 | | |
| 9 | 35.9, CH | 38.7 | 5.97, bs | 5.64 |
| 10 | 133.2, C | 129.8 | | |
| 11/15 | 130.2, CH | 127.5 | 7.24–7.21, m | 7.09 |
| 12/14 | 114.3, CH | 112.1 | 6.86–6.83, m | 6.79 |
| 13 | 159.08, C | 157.6 | | |

| | | | | |
|---|---|---|---|---|
| 5-OH | - | | 12.76, s | - |
| 7-OH | - | | 10.00–9.00, bs | - |
| 13-$OCH_3$ | 55.4, $CH_3$ | 53.0 | 3.76, s | 3.64 |
| 2′ | 68.1, $CH_2$ | 67.6 | 5.38, d (1.8) | 5.20 |
| 3′ | 128.1, C | 127.1 | | |
| 4′ | 185.9, C | 181.6 | | |
| 4a′ | 103.2, C | 102.2 | | |
| 5′ | 164.2, C | 163.9 | | |
| 6′ | 109.6, C | 107.9 | | |
| 7′ | 165.9, C | 164.6 | | |
| 8′ | 96.1, CH | 96.3 | 5.99, s | 5.89 |
| 8a′ | 161.8, C | 160.5 | | |
| 9′ | 137.5, C | 138.3 | 7.75–7.73, m | 7.89 |
| 5′-OH | - | | 13.62, s | - |
| 7′-OH | - | | 10.00–9.00, bs | - |
| 1″ | 126.7, C | 126.2 | | |
| 2″/6″ | 133.5, CH | 133.4 | 7.36–7.33, m | 7.29 |

$^{13}C$ CMAD: 1.6; $^{1}H$ CMAD: 0.2

**Figure S41.** ECD calculations for prosperin B (**9**) with experimental values (green) and computed *S* and *R* enantiomers (blue and red respectively)

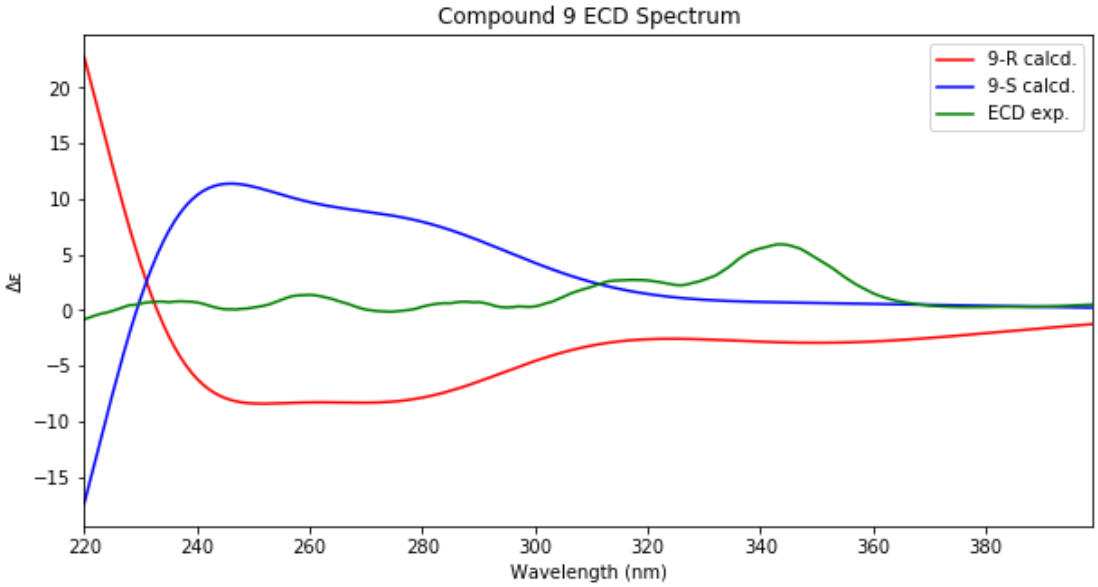
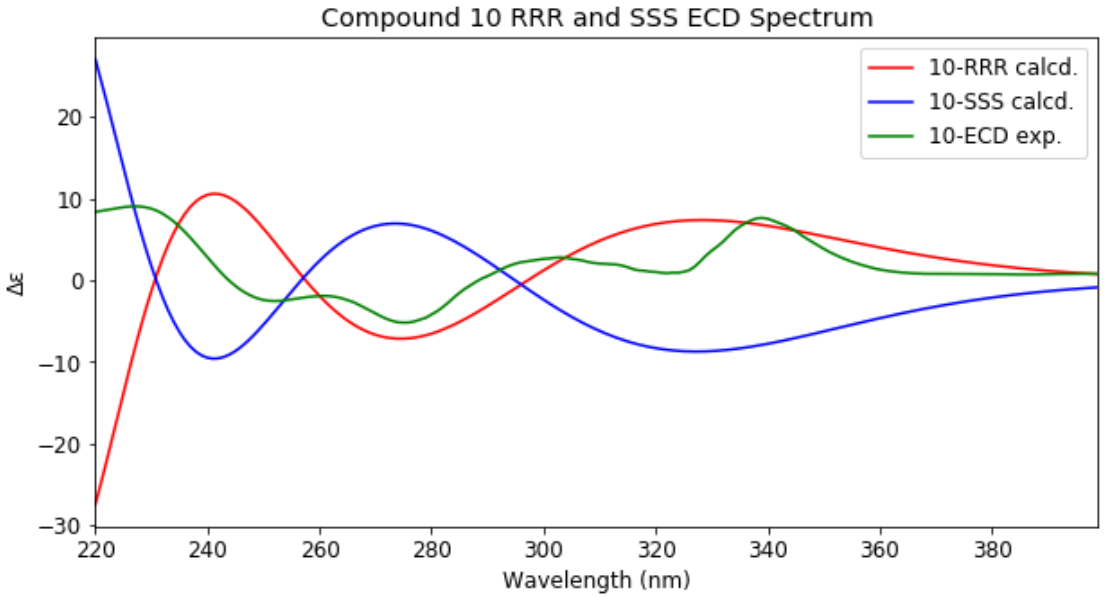



**Figure S42.** ECD calculations for alternative prosperin C (**10**) stereoisomers



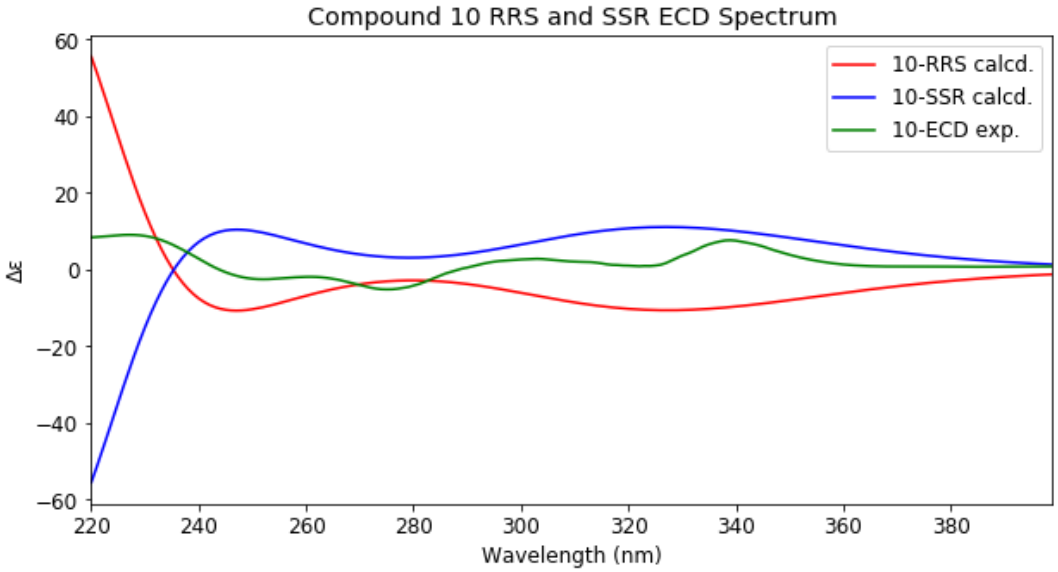

Compound 10 RRS and SSR ECD Spectrum
10-RRS calcd.
10-SSR calcd.
10-ECD exp.
Δε
Wavelength (nm)
60
40
20
0
−20
−40
−60
220
240
260
280
300
320
340
360
380

**Figure S43.** NMR calculations for alternative prosperin C (**10**) stereoisomers

| **no.** | **10** | | | | | |
|---|---|---|---|---|---|---|
| | $\delta_C$, type, exp. | $\delta_C$, Computed as (2S,3R,9S) | $\delta_C$, Computed as (2S,3R,9R) | $\delta_H$, mult. (*J* in Hz), exp. | $\delta_H$ Computed as (2S,3R,9S) | $\delta_H$ Computed as (2*S*,3*R*,9*R*) |
| 2 | 95.7, CH | 94.3 | 96.3 | 5.94, d (3.6) | 5.9 | 6.0 |
| 3 | 50.0, CH | 51.5 | 49.0 | 3.64, dd (3.6, 2.3) | 3.4 | 3.3 |
| 4 | 193.2, C | 192.2 | 196.2 | | | |
| 4a | 102.3, C | 102.2 | 103.7 | | | |
| 5 | 164.9, C | 163.9 | 163.6 | | | |
| 6 | 97.8, CH | 95.1 | 94.5 | 5.97, d (2.1) | 6.0 | 5.8 |
| 7 | 168.6, C | 164.6 | 164.1 | | | |
| 8 | 97.0, CH | 94.4 | 93.5 | 6.06, d (2.1) | 6.1 | 5.7 |
| 8a | 160.1, C | 159.4 | 160.1 | | | |
| 9 | 36.9, CH | 38.8 | 40.1 | **5.32, d (2.3)** | **5.4** | **4.8** |
| 10 | 135.8, C | 137.0 | 132.4 | | | |
| 11/15 | 130.1, CH | 129.8 | 131.2 | 7.20–7.16, m | 7.2 | 7.1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 12/14 | 115.1, CH | 112.5 | 111.7 | 6.96–6.92, m | 6.9 | 6.7 |
| 13 | 159.5, C | 158.1 | 158.1 | | | |
| 5-OH | - | | | 11.79, s | 11.6 | 11.7 |
| 13-$OCH_3$ | 55.4, $CH_3$ | 52.9 | 52.7 | 3.76, s | 3.7 | 3.5 |
| 1′ | 135.9, C | 136.0 | 136.0 | | | |
| 2′ | 112.3, C | 111.3 | 113.6 | | | |
| 3′ | 153.0, C | 152.1 | 149.9 | | | |
| 4′ | 113.0, C | 113.1 | 113.4 | | | |
| 5′ | 158.3, C | 156.7 | 155.9 | | | |
| 6′ | 102.0, CH | 99.8 | 100.8 | 6.98, s, overlap | 6.8 | 6.9 |
| 7′ | 123.2, CH | 124.3 | 123.3 | 6.96, d (16.1), overlap | 7.2 | 7.1 |
| 8′ | 130.8, CH | 130.5 | 130.7 | 7.03 d (16.1) | 7.0 | 6.9 |
| 4′-$CH_3$ | 8.6, $CH_3$ | 8.4 | 8.8 | 1.97, s | 2.0 | 2.3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 5′-$OCH_3$ | 55.9, $CH_3$ | 53.0 | 53.0 | 3.87, s | 3.8 | 3.8 |
| 1″ | 130.0, C | 129.0 | 128.9 | | | |
| 2″/6″ | 128.7, CH | 128.4 | 128.5 | 7.26–7.22, m | 7.4 | 7.4 |
| 3″/5″ | 116.3, CH | 113.9 | 113.9 | 6.80–6.76, m | 6.9 | 6.9 |
| 4″ | 158.2, C | 156.2 | 156.2 | | | |
| CMAD | | 1.4 | 1.7 | | 0.10 | 0.18 |
| largest outlier | | Δδ 4.0 | Δδ 4.5 | | Δδ 0.24 | Δδ 0.54 |