# Cytotoxic withanolides from *Physalis angulata var. villosa* and the apoptosis-inducing effect via ROS generation and the activation of MAPK in human osteosarcoma cells

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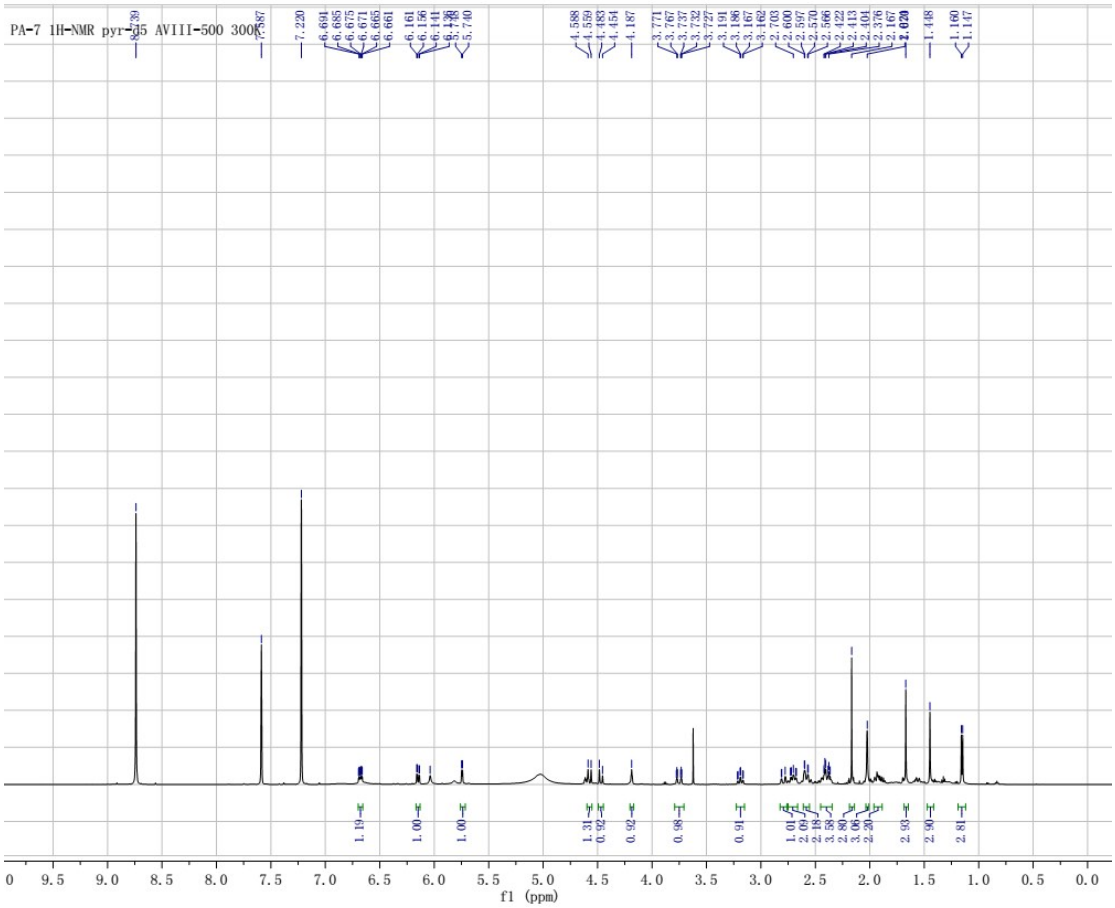
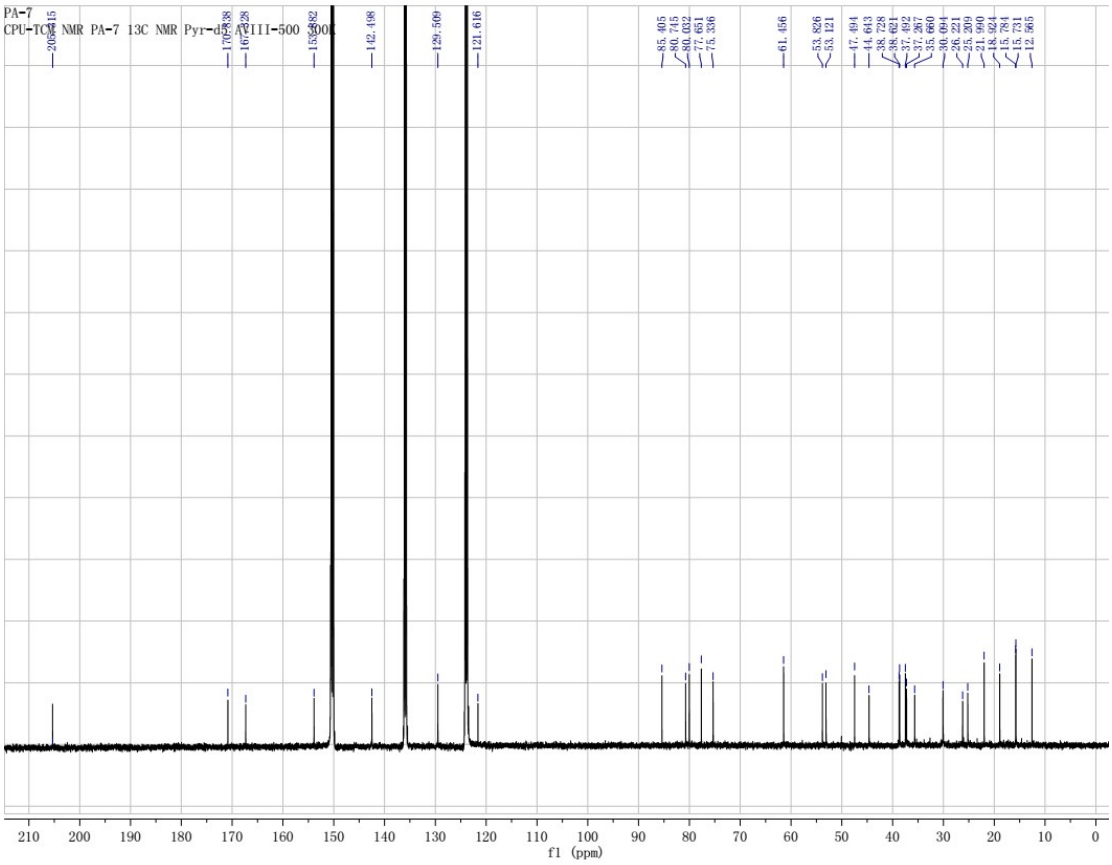
**Author Contributions**

‡T. Ma and W. N, Zhang contributed equally.

## Supporting Information：




S1. $^{1}$H NMR spectrum of physagulide A (**1**) in pyridine-$d_5$ (500MHz)

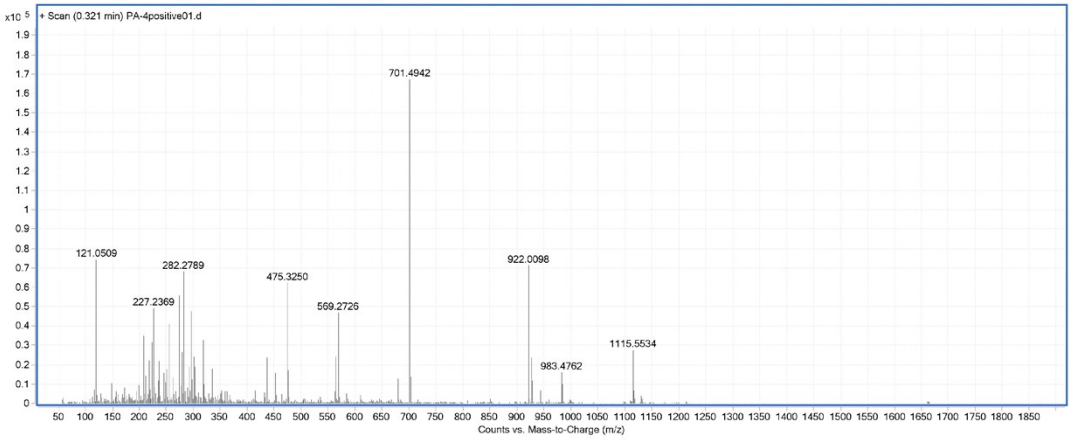


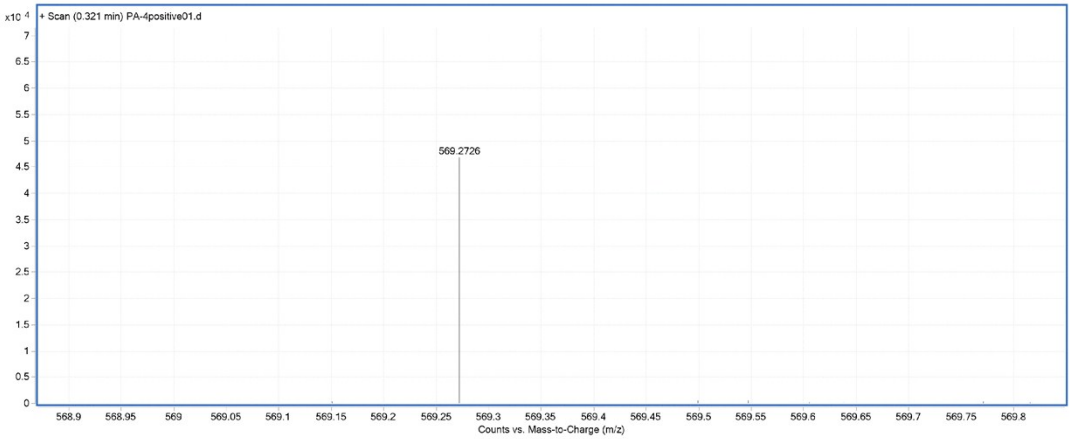

S2. $^{13}C$ NMR spectrum of physagulide A (1) in pyridine-$d_5$ (125MHz)

# TCM-CPU HR-ESI-MS Display Report

**Sample Name**: PA-4 **Instrument:** Agilent 6520B Q-TOF

**Acq. Date:** 04/27 /2013 **Operator:** Administrator




**Elemental Composition Calculator**

| **Target m/z:** | 569.2726 | **Result type:** | Positive ions | **Species:** | $[M+Na]^+$ |
|---|---|---|---|---|---|
| **Elements:** | | C (0-100); H (0-150); O (0-50); N(0-10); Na (0-5); S (0-5) | | | |
| **Ion Formula** | | **Calculated m/z** | | **PPM Error** | |
| C30H42NaO9 | | 569.2721 | | -0.80 | |

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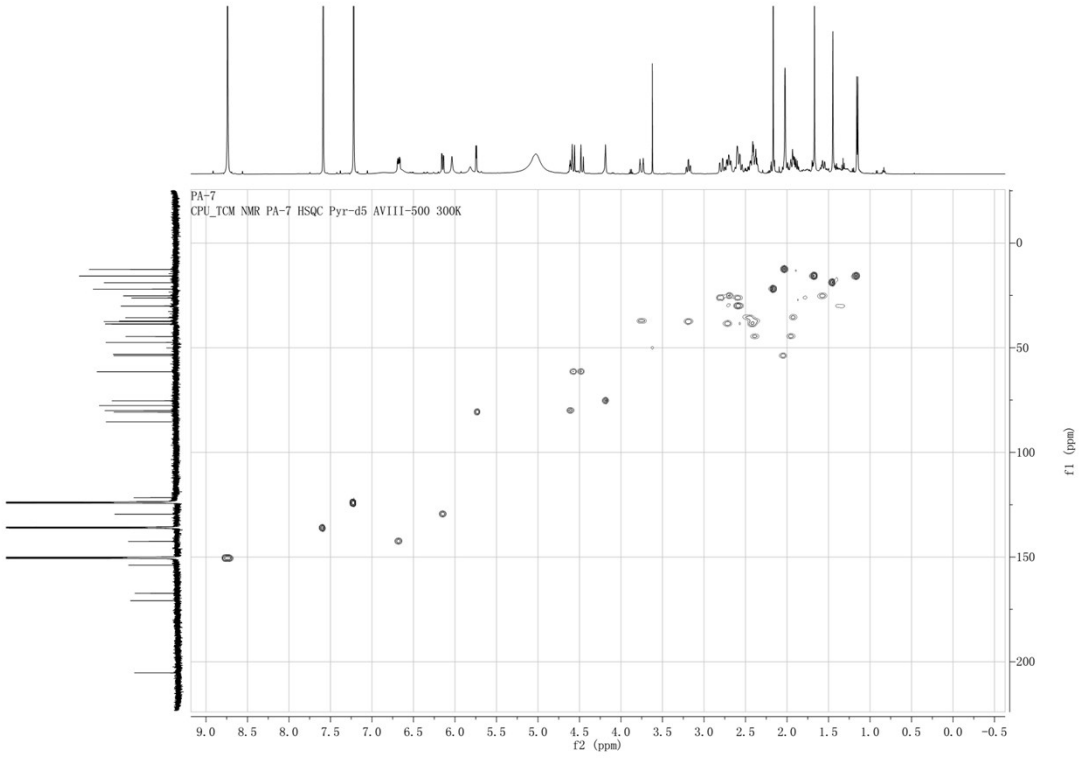
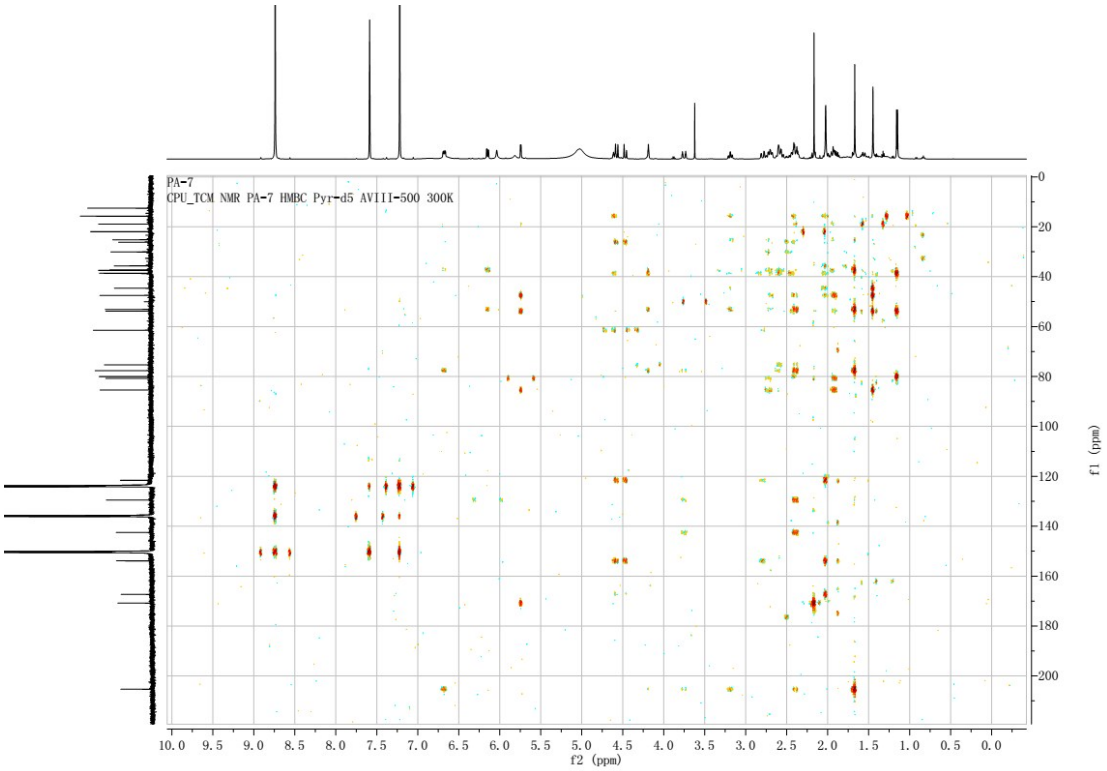
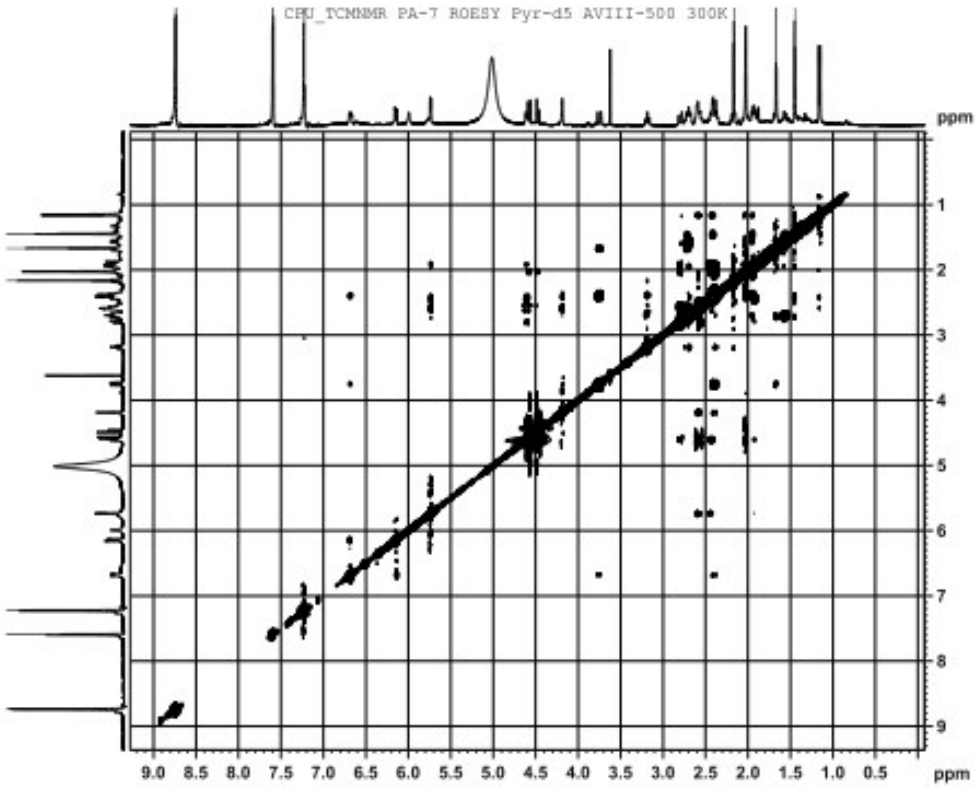
S3. HRESIMS spectrum of physagulide A (1)



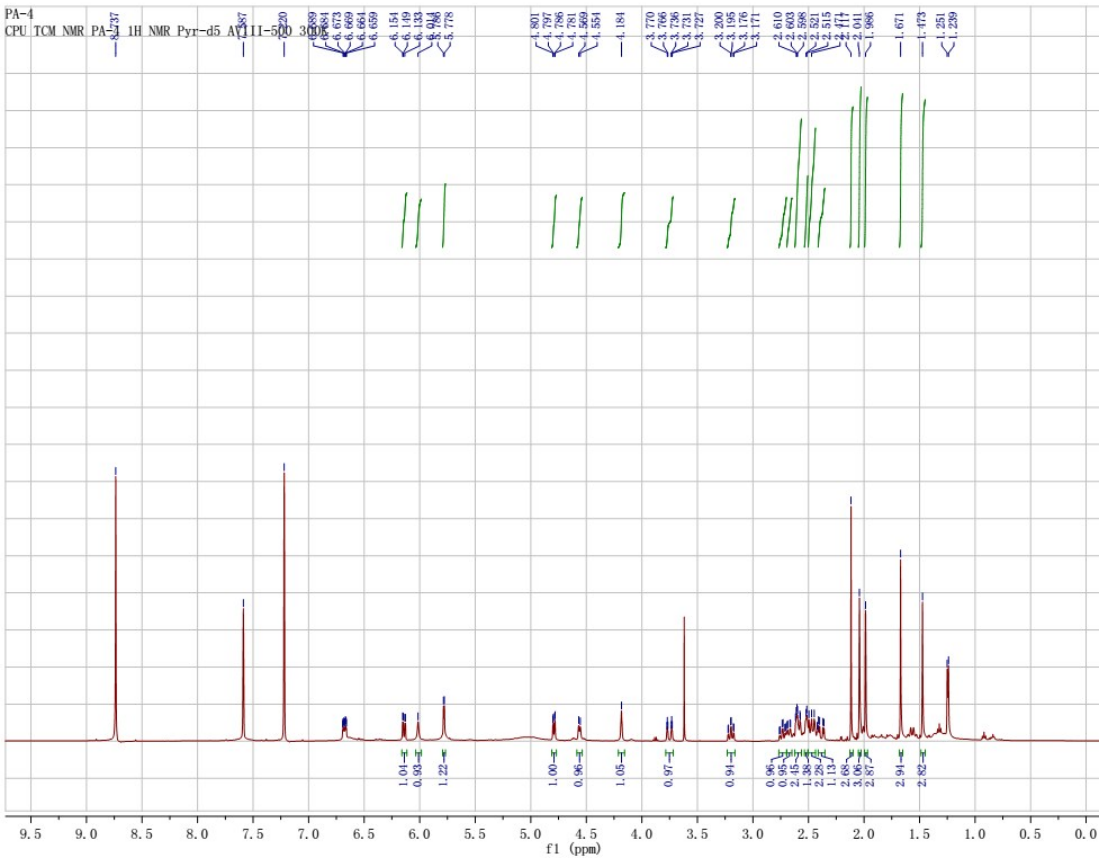

S4. HSQC spectrum of physagulide A (1) in pyridine-$d_5$



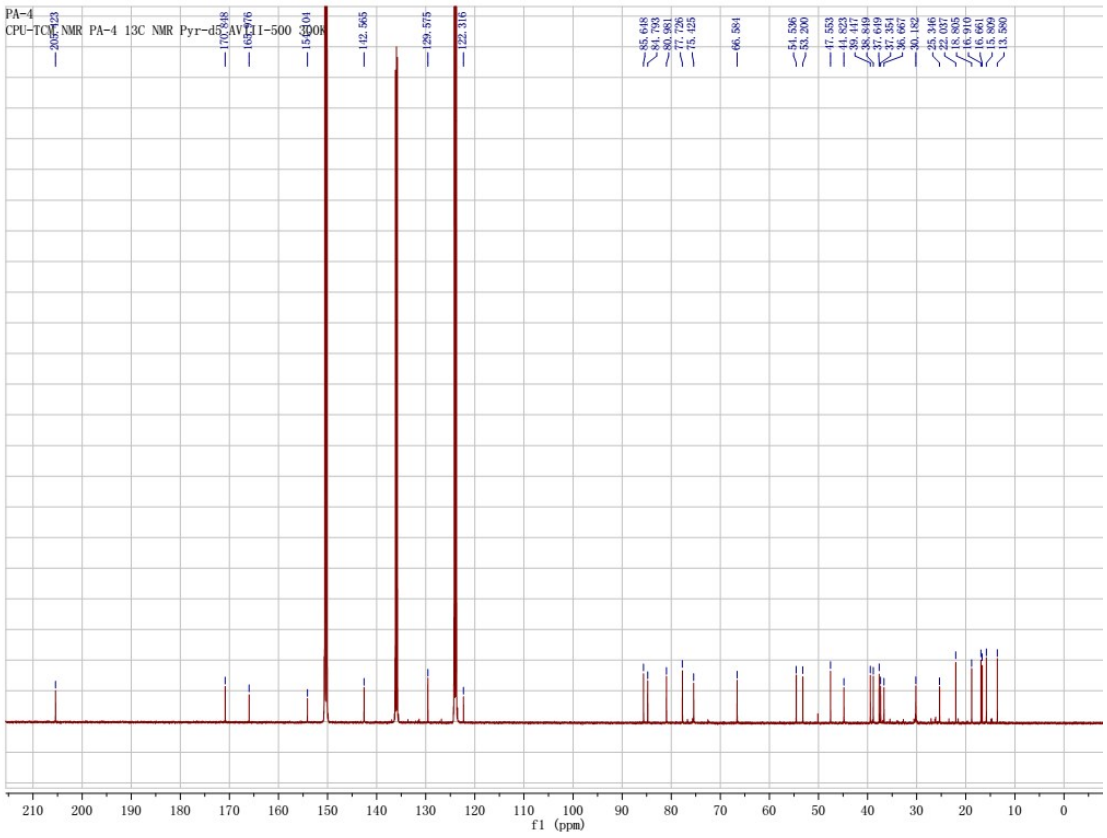

S5. HMBC spectrum of physagulide A (1) in pyridine-$d_5$




S6. ROESY spectrum of of physagulide A (1) in pyridine-$d_5$




S7. $^{1}$H NMR spectrum of physagulide B (**2**) in pyridine-$d_5$ (500MHz)

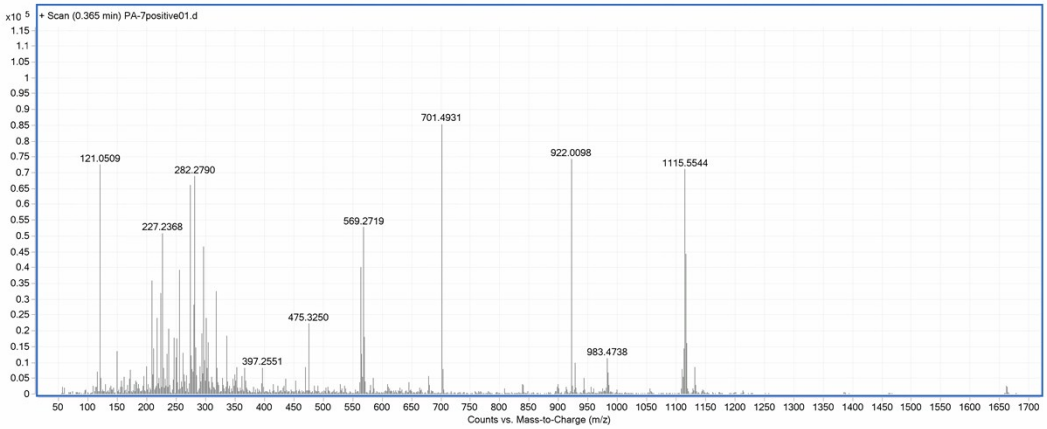


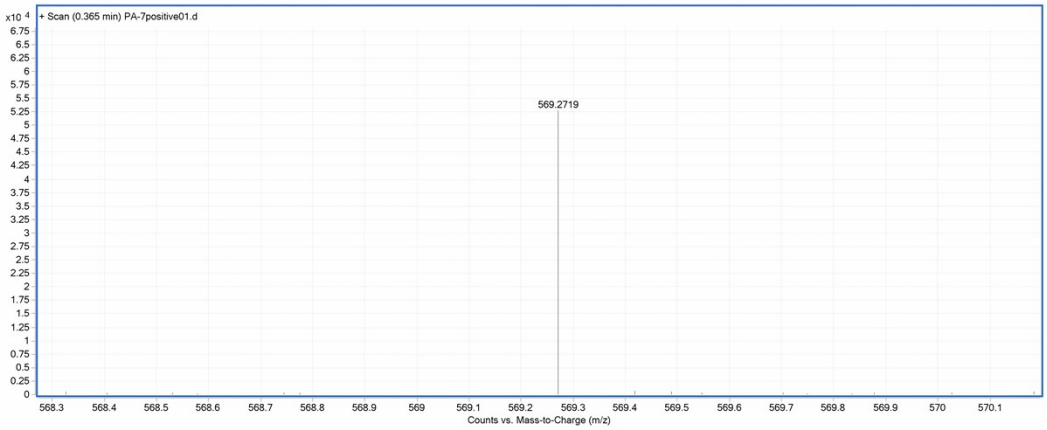

S8. $^{13}$C NMR spectrum of physagulide B (**2**) in pyridine-$d_5$ (125MHz)

# TCM-CPU HR-ESI-MS Display Report

**Sample Name**: PA-7 **Instrument:** Agilent 6520B Q-TOF

**Acq. Date:** 04/27 /2013 **Operator:** Administrator




**Elemental Composition Calculator**

| **Target m/z:** | 569.2719 | **Result type:** | Positive ions | **Species:** | $[M+Na]^+$ |
|---|---|---|---|---|---|
| **Elements:** | | C (0-100); H (0-150); O (0-50); N(0-10); Na (0-5); S (0-5) | | | |
| **Ion Formula** | | **Calculated m/z** | | **PPM Error** | |
| C30H42NaO9 | | 569.2721 | | 0.43 | |

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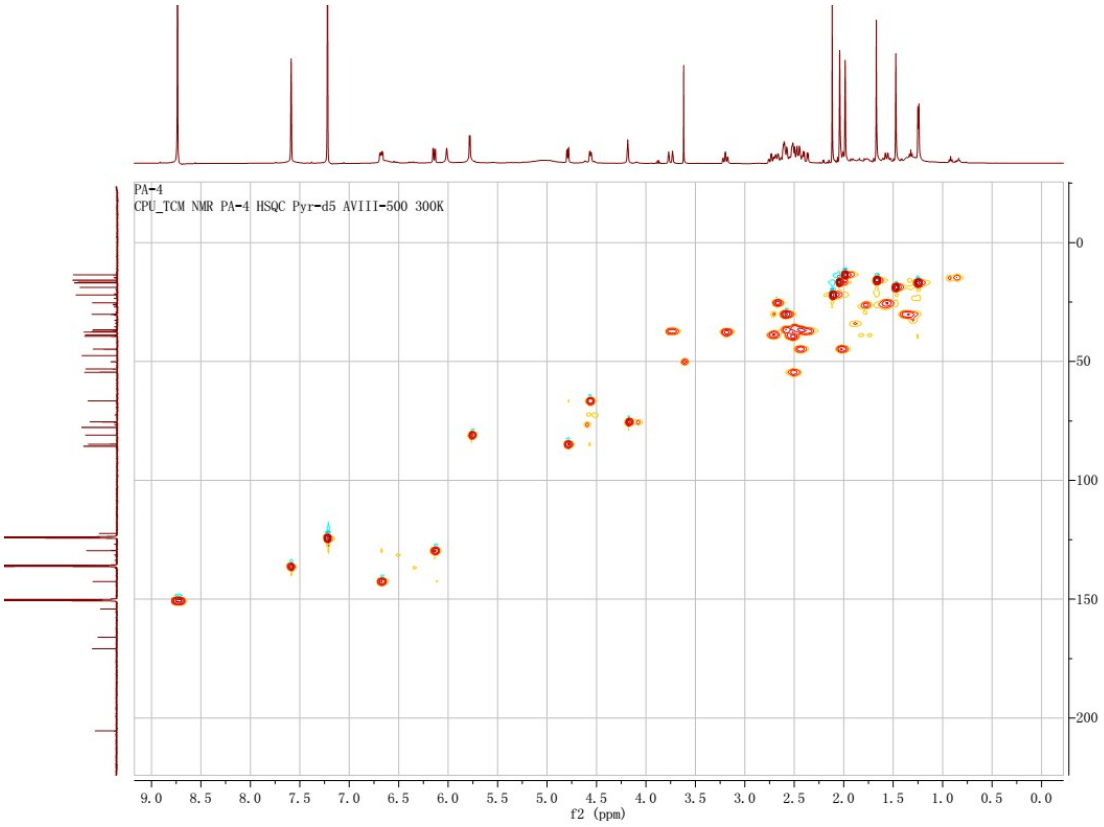
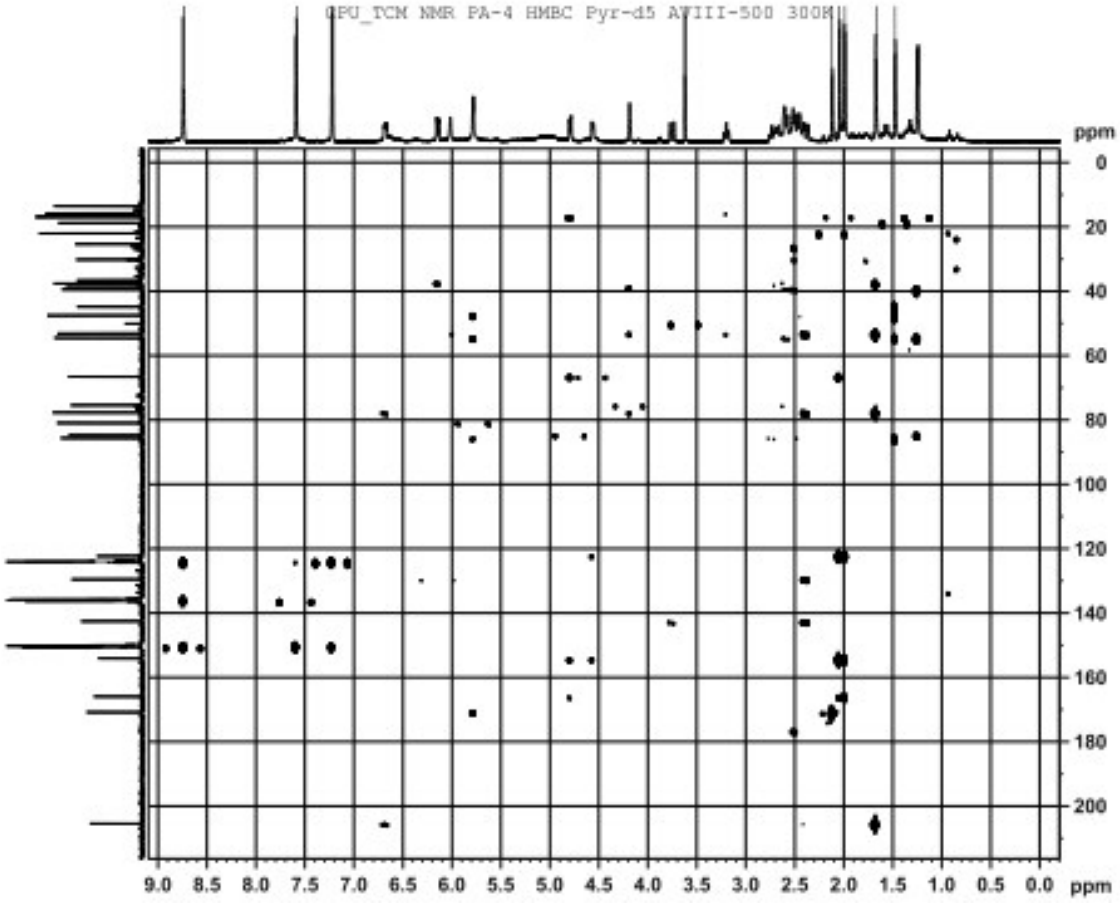
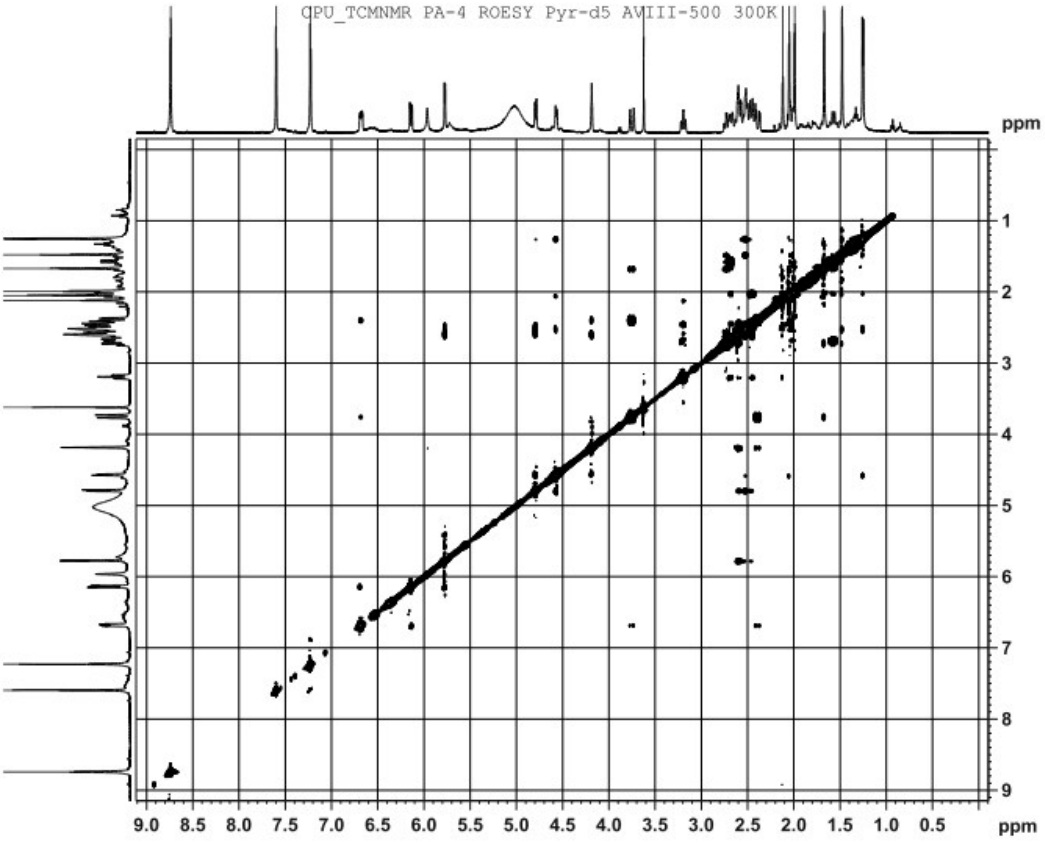
S9. HRESIMS spectrum of physagulide B (**2**)



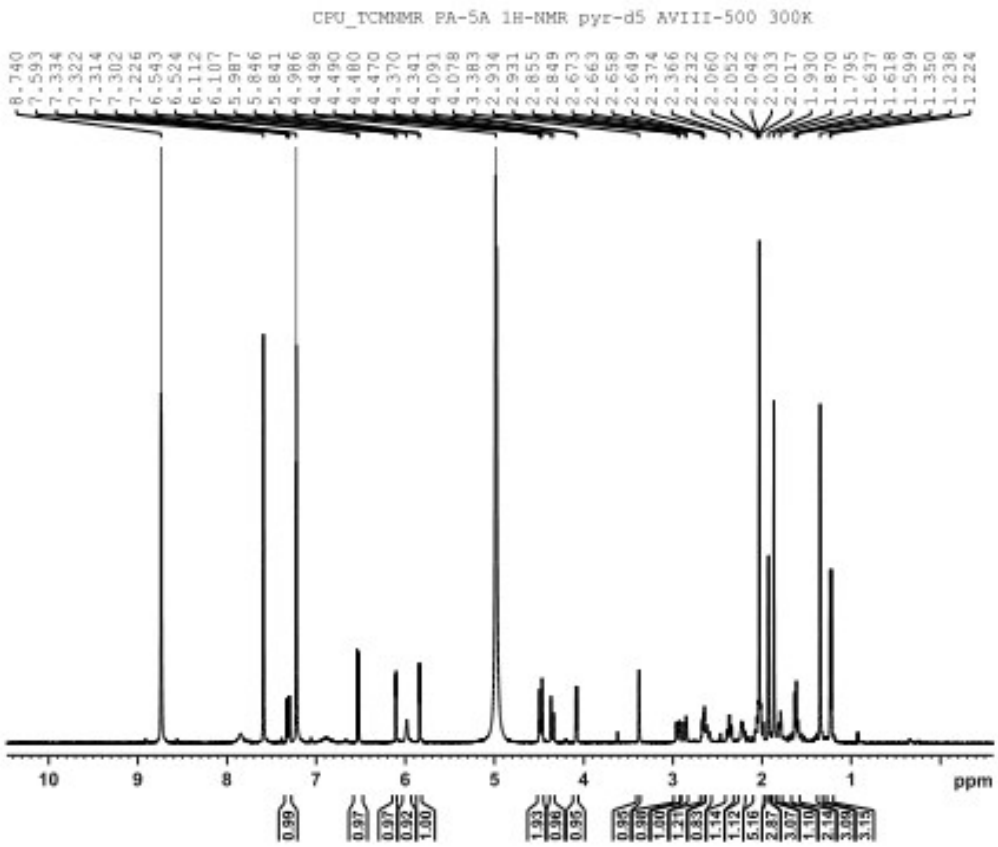

S10. HSQC spectrum of physagulide B (**2**) in pyridine-$d_5$



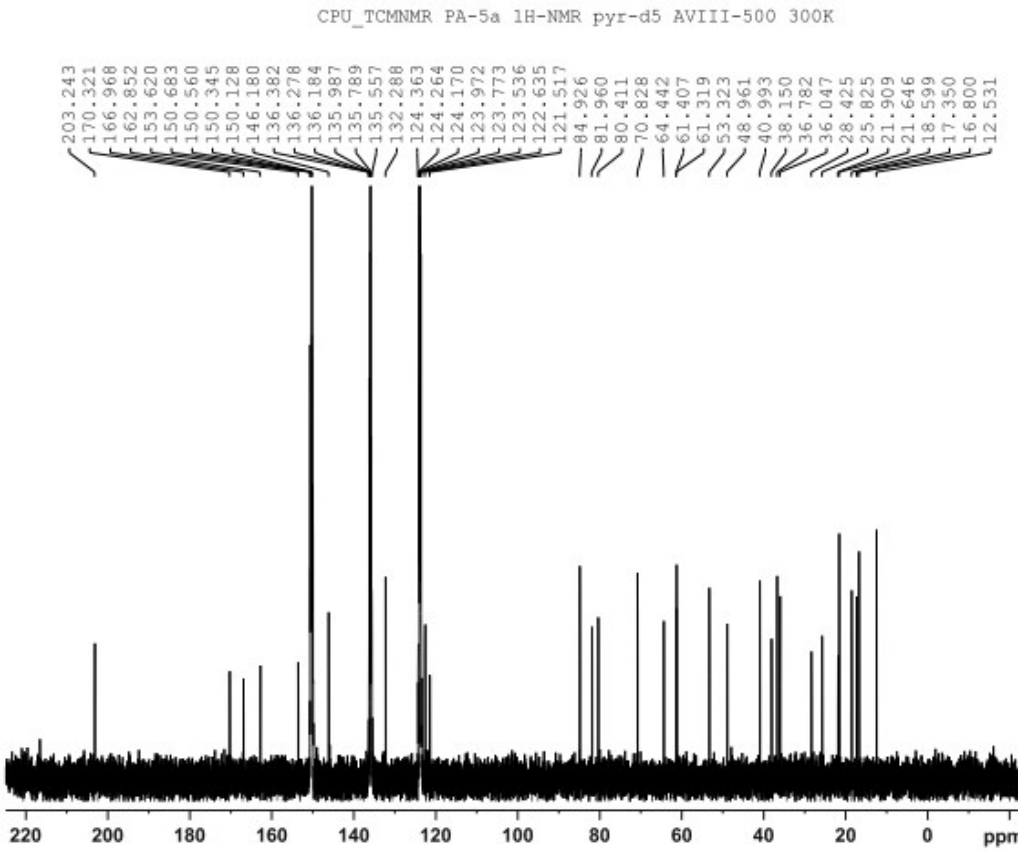

S11. HMBC spectrum of physagulide B (**2**) in pyridine-$d_5$




S12. ROESY spectrum of physagulide B (**2**) in pyridine-$d_5$




S13. $^{1}$H NMR spectrum of physagulide C (**3**) in pyridine-$d_5$ (500MHz)

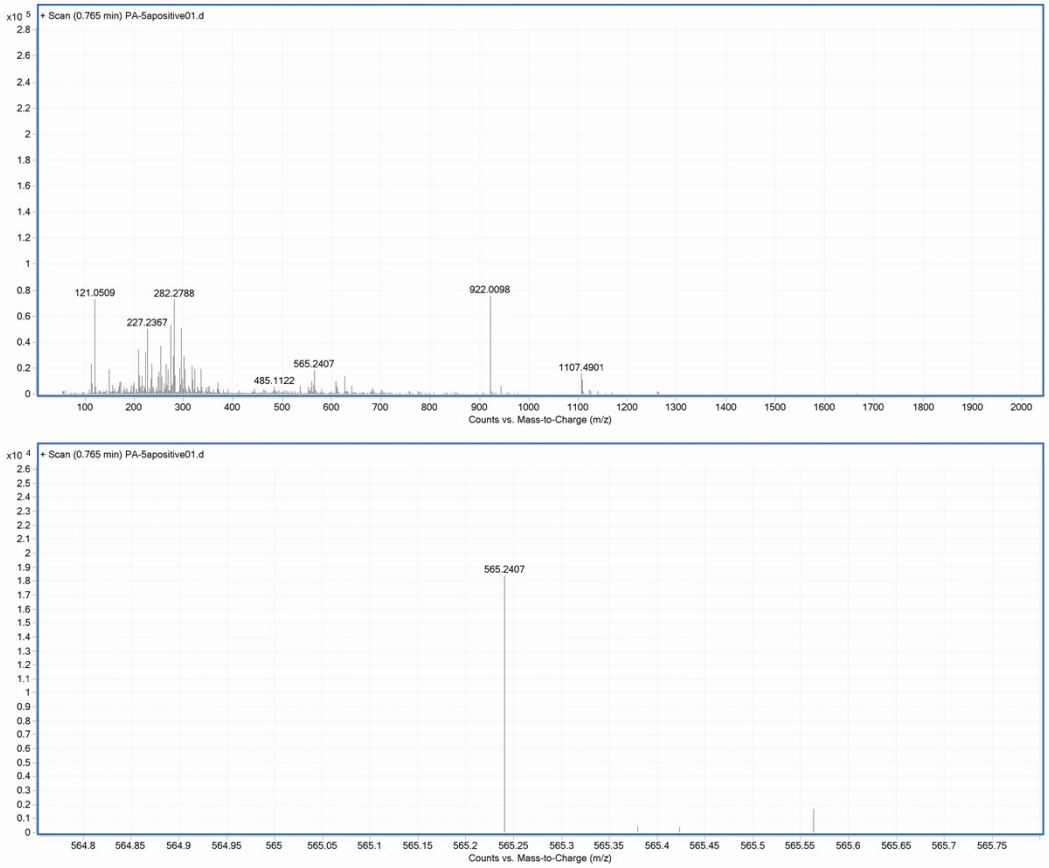



S14. $^{13}$C NMR spectrum of physagulide C (**3**) in pyridine-$d_5$ (125MHz)

# TCM-CPU HR-ESI-MS Display Report

**Sample Name**: PA-5a **Instrument:** Agilent 6520B Q-TOF

**Acq. Date:** 04/27 /2013 **Operator:** Administrator




**Elemental Composition Calculator**

| **Target m/z:** | 565.2407 | **Result type:** | Positive ions | **Species:** | $[M+Na]^+$ |
|---|---|---|---|---|---|
| **Elements:** | | C (0-100); H (0-150); O (0-50); N(0-10); Na (0-5); S (0-5) | | | |
| **Ion Formula** | | **Calculated m/z** | | **PPM Error** | |
| C30H38NaO9 | | 565.2408 | | 0.17 | |

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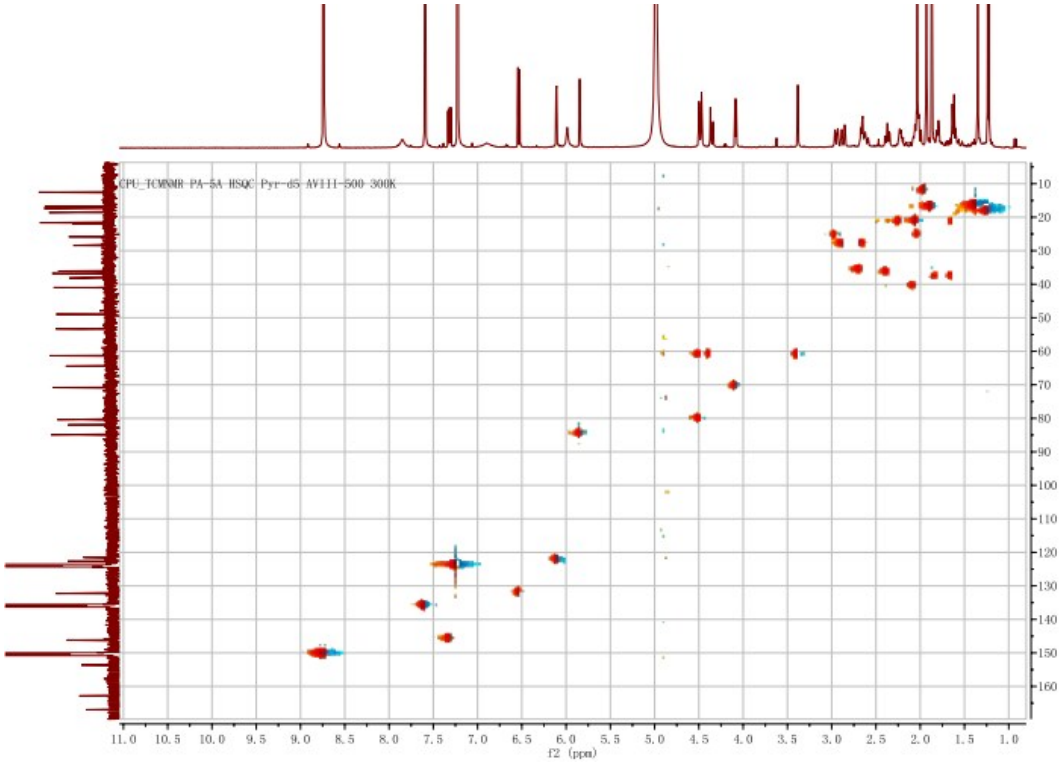
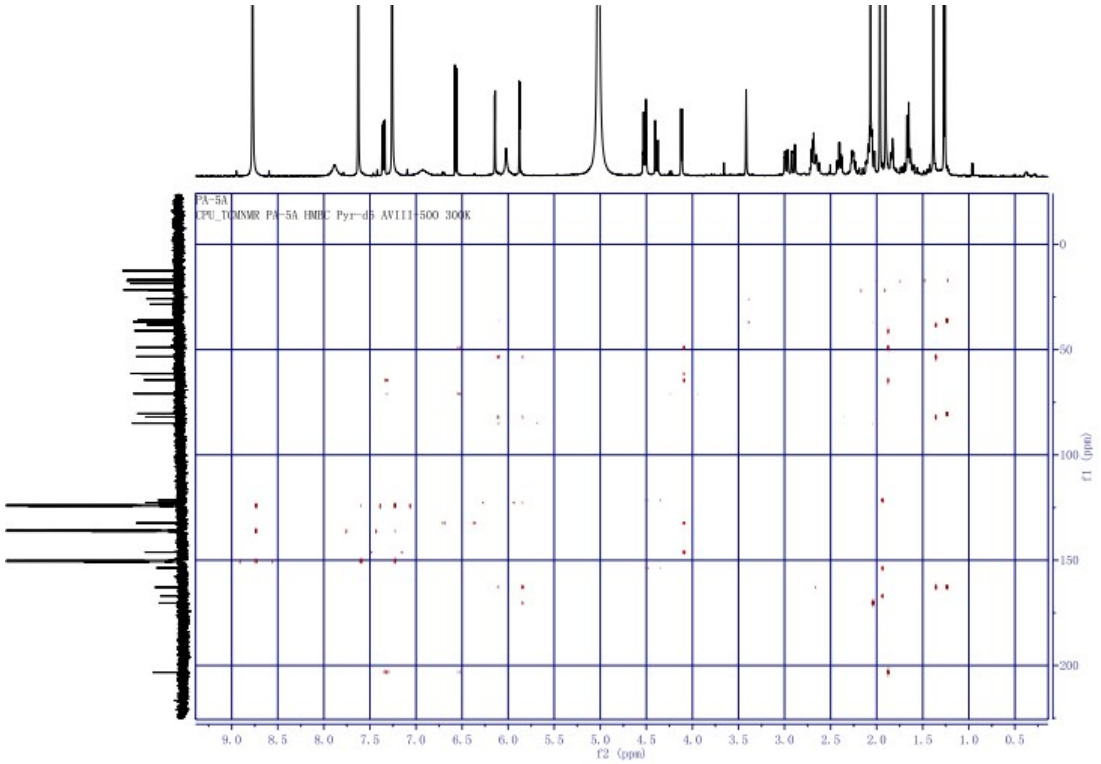
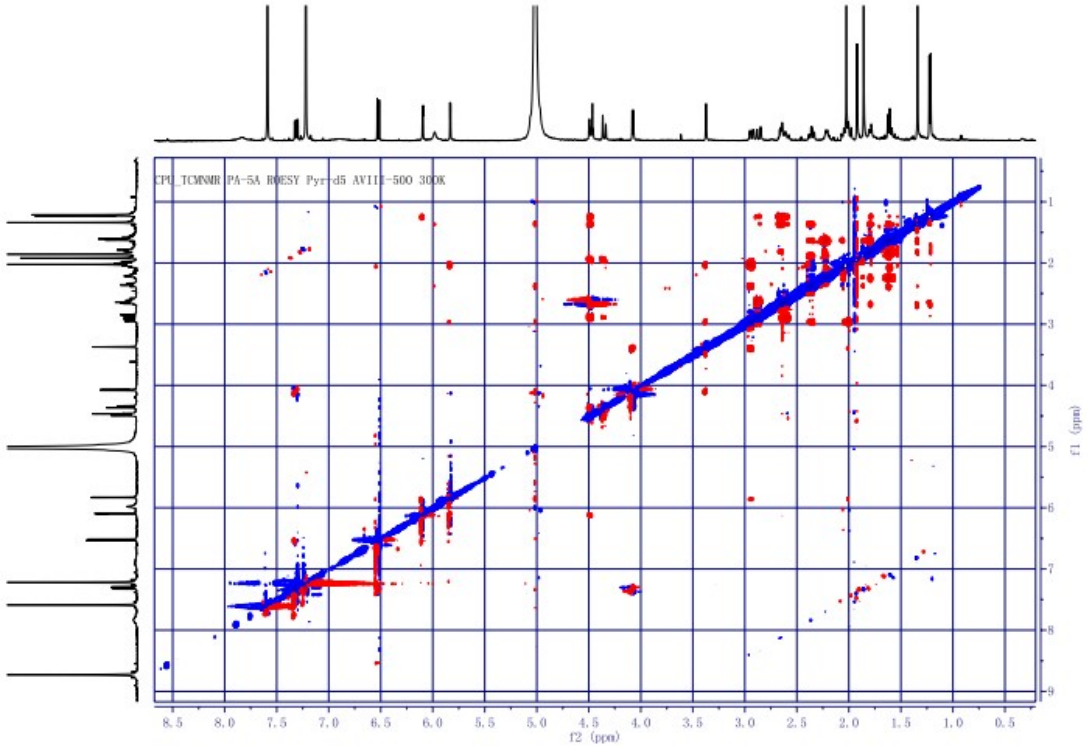
S15. HRESIMS spectrum of physagulide C (**3**)



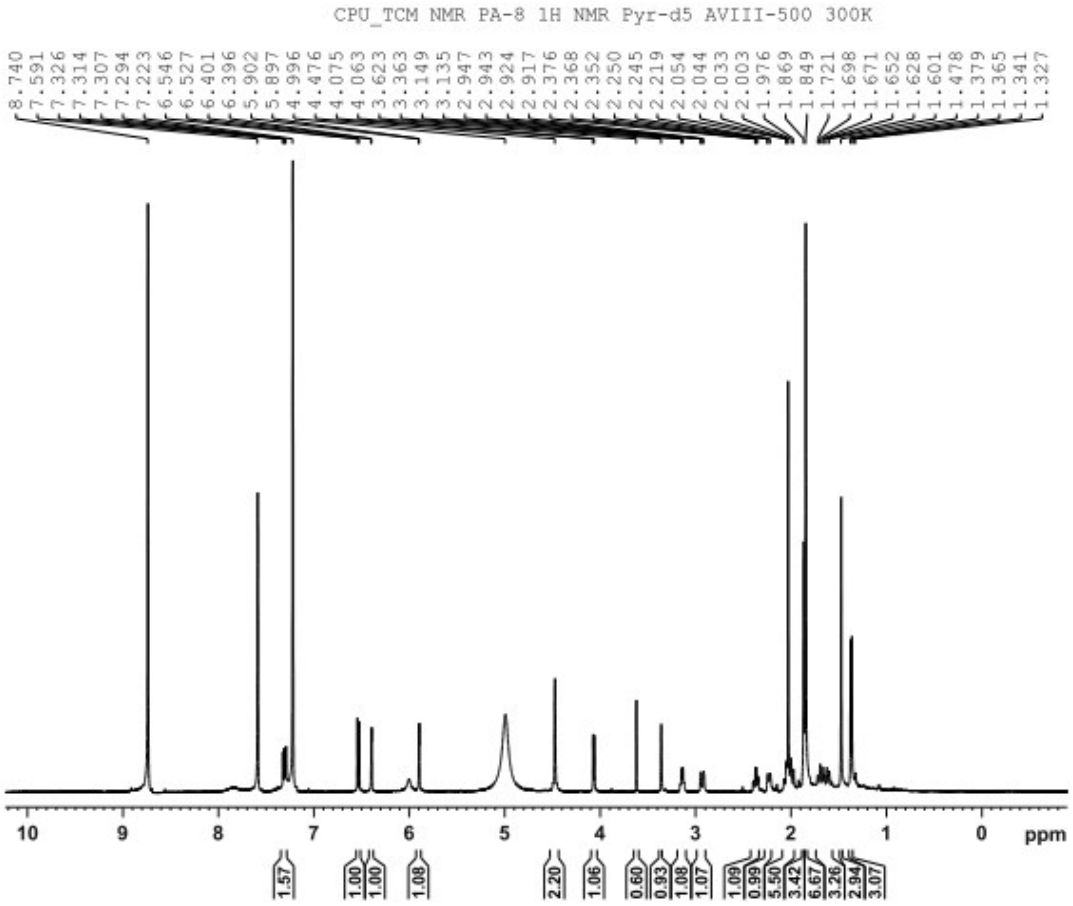

S16. HSQC spectrum of physagulide C (**3**) in pyridine-$d_5$



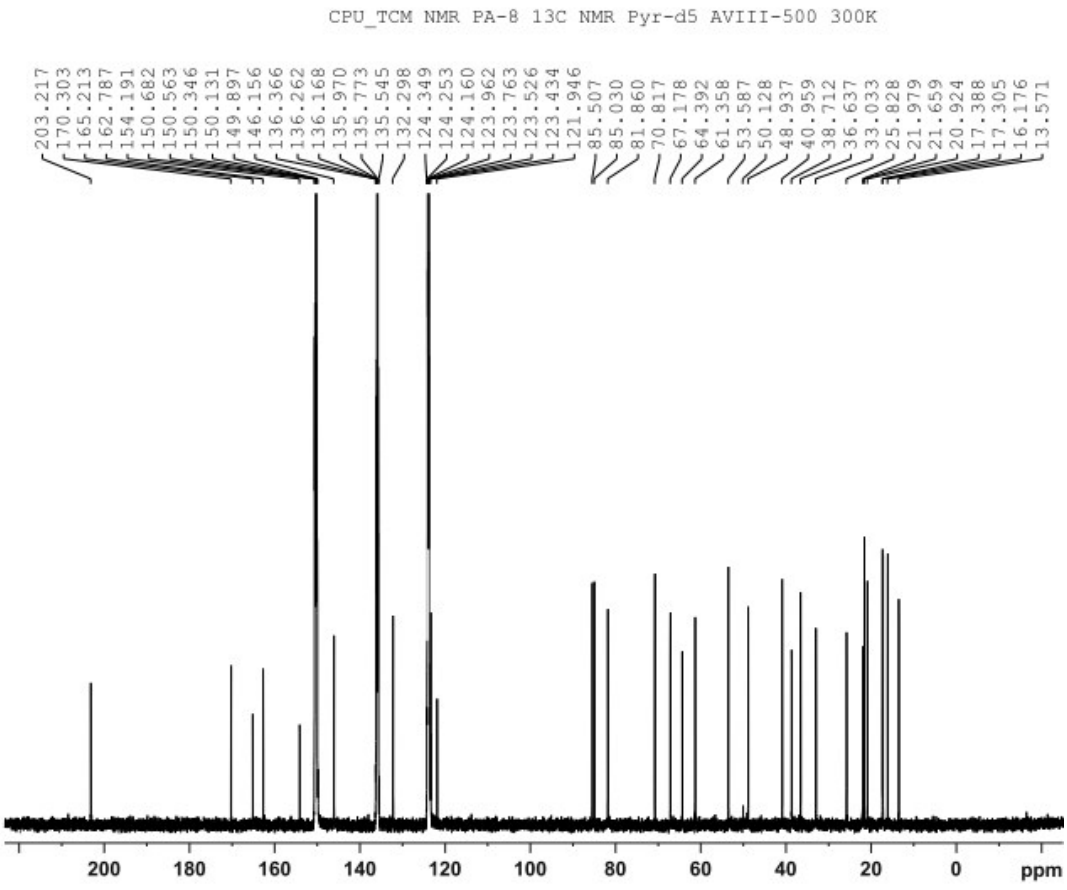

S17. HMBC spectrum of physagulide C (**3**) in pyridine-$d_5$




S18. ROESY spectrum of physagulide C (**3**) in pyridine-$d_5$




S19. $^{1}$H NMR spectrum of physagulide D (**4**) in pyridine-$d_5$ (500MHz)

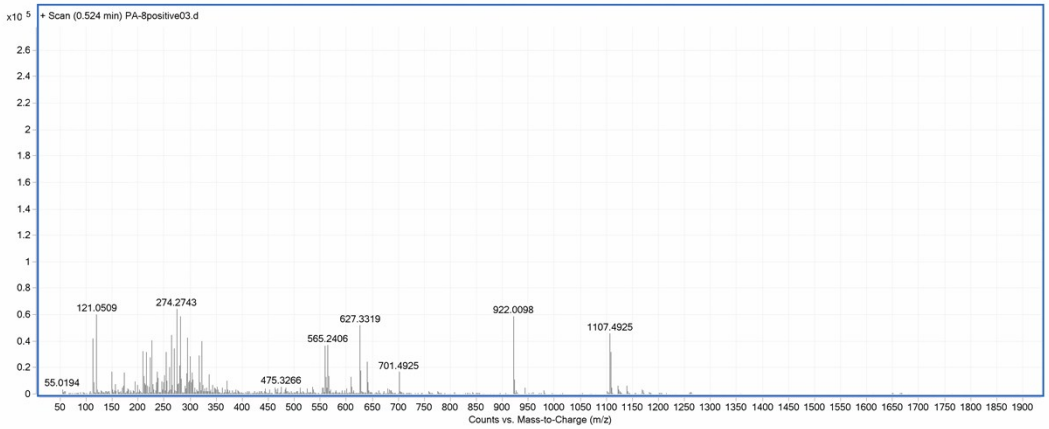


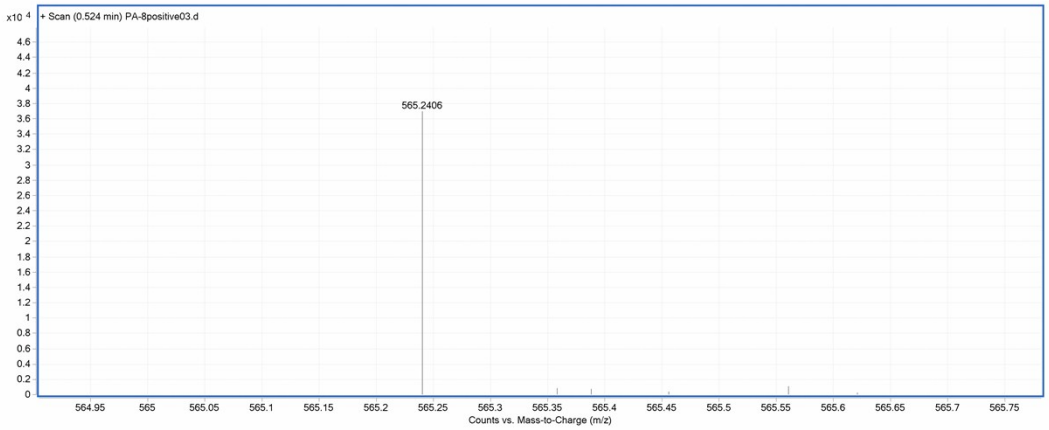

S20. $^{13}$C NMR spectrum of physagulide D (**4**) in pyridine-$d_5$ (125MHz)

# TCM-CPU HR-ESI-MS Display Report

**Sample Name**: PA-8 **Instrument:** Agilent 6520B Q-TOF

**Acq. Date:** 04/27 /2013 **Operator:** Administrator




**Elemental Composition Calculator**

| **Target m/z:** | 565.2406 | **Result type:** | Positive ions | **Species:** | $[M+Na]^+$ |
|---|---|---|---|---|---|
| **Elements:** | | C (0-100); H (0-150); O (0-50); N(0-10); Na (0-5); S (0-5) | | | |
| **Ion Formula** | | **Calculated m/z** | | **PPM Error** | |
| C30H38NaO9 | | 565.2408 | | 0.41 | |

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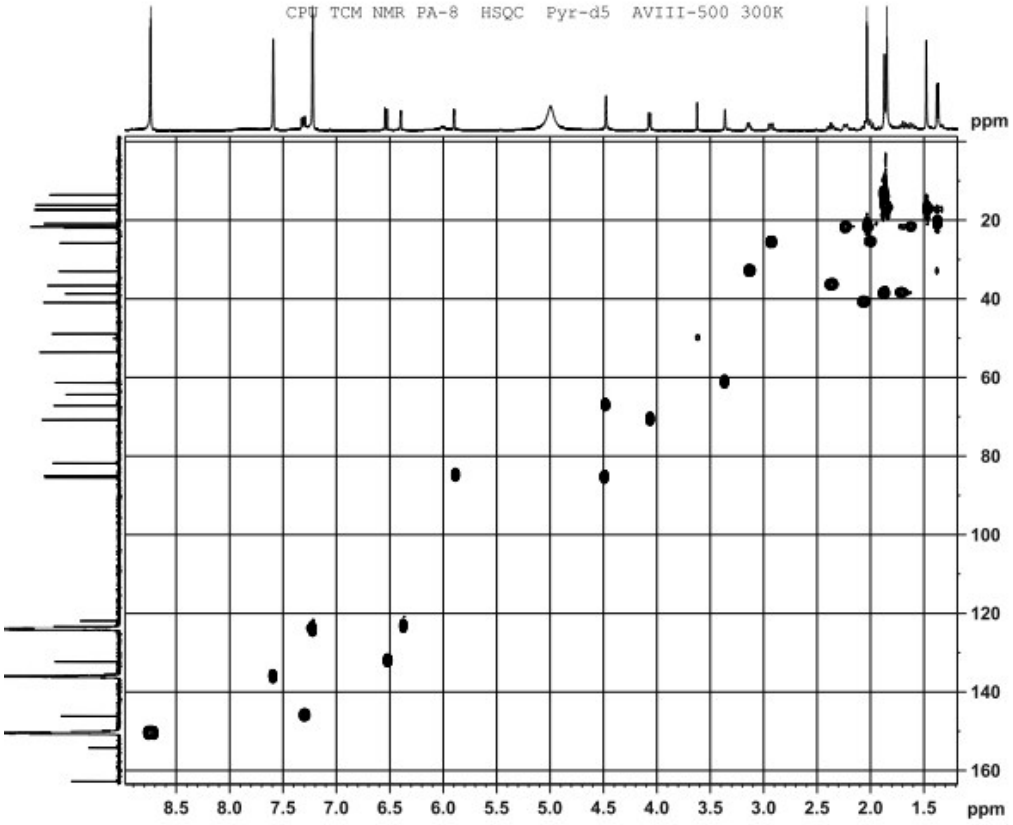
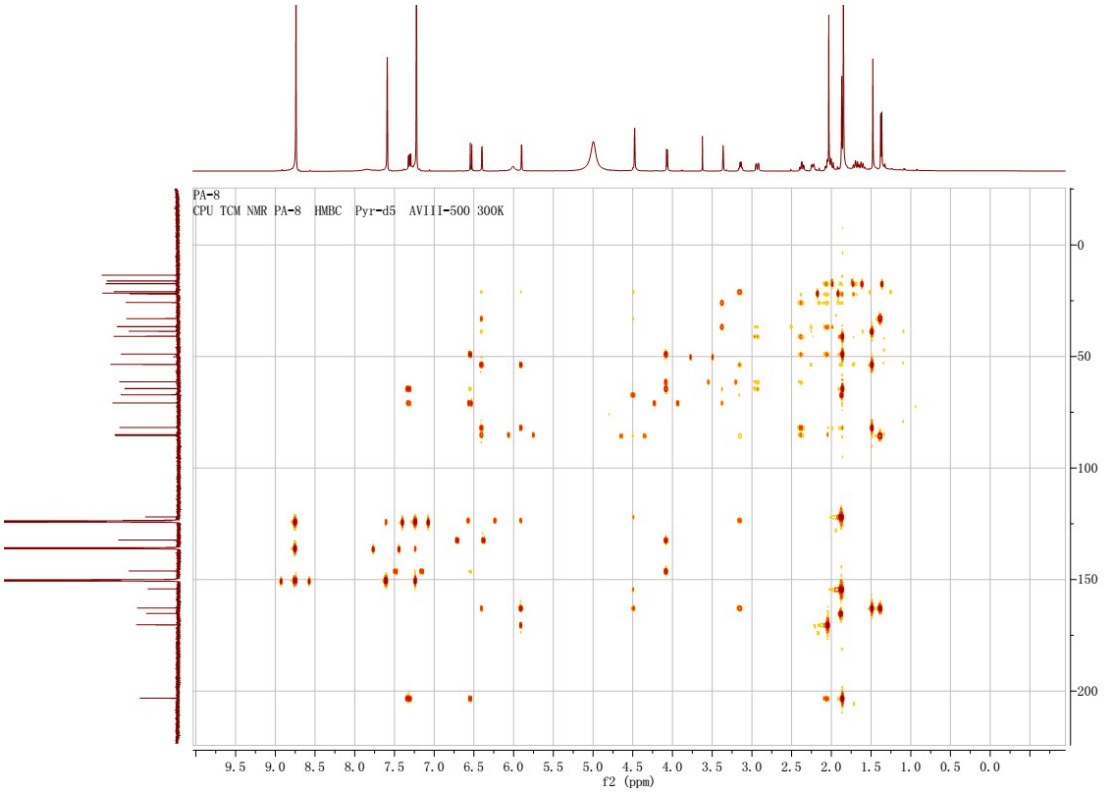
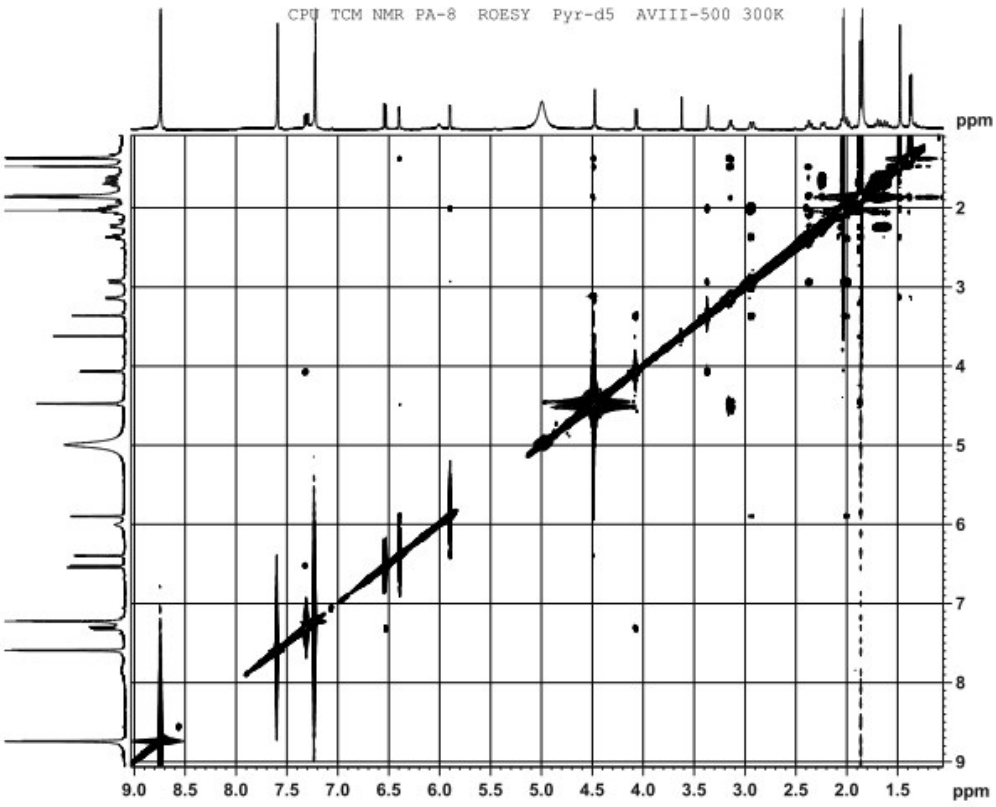
S21. HRESIMS spectrum of physagulide D **(4)**



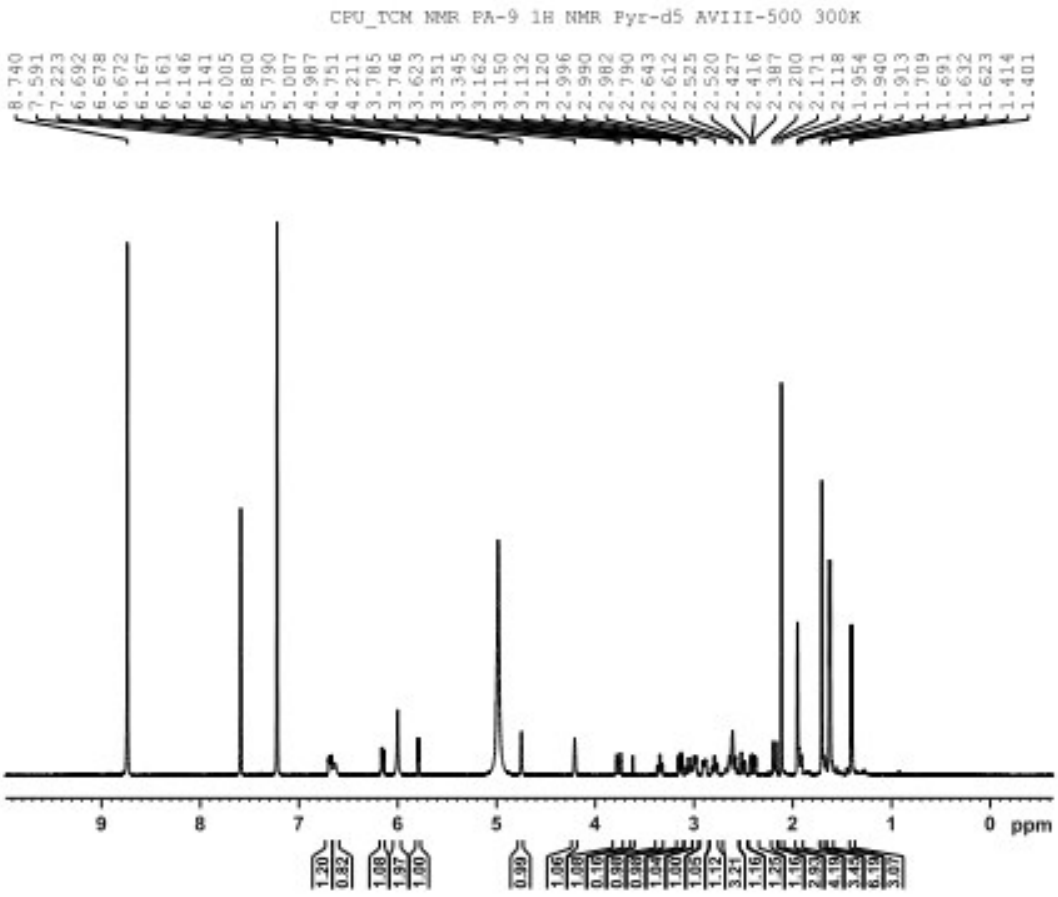

S22. HSQC spectrum of physagulide D **(4)** in pyridine-$d_5$



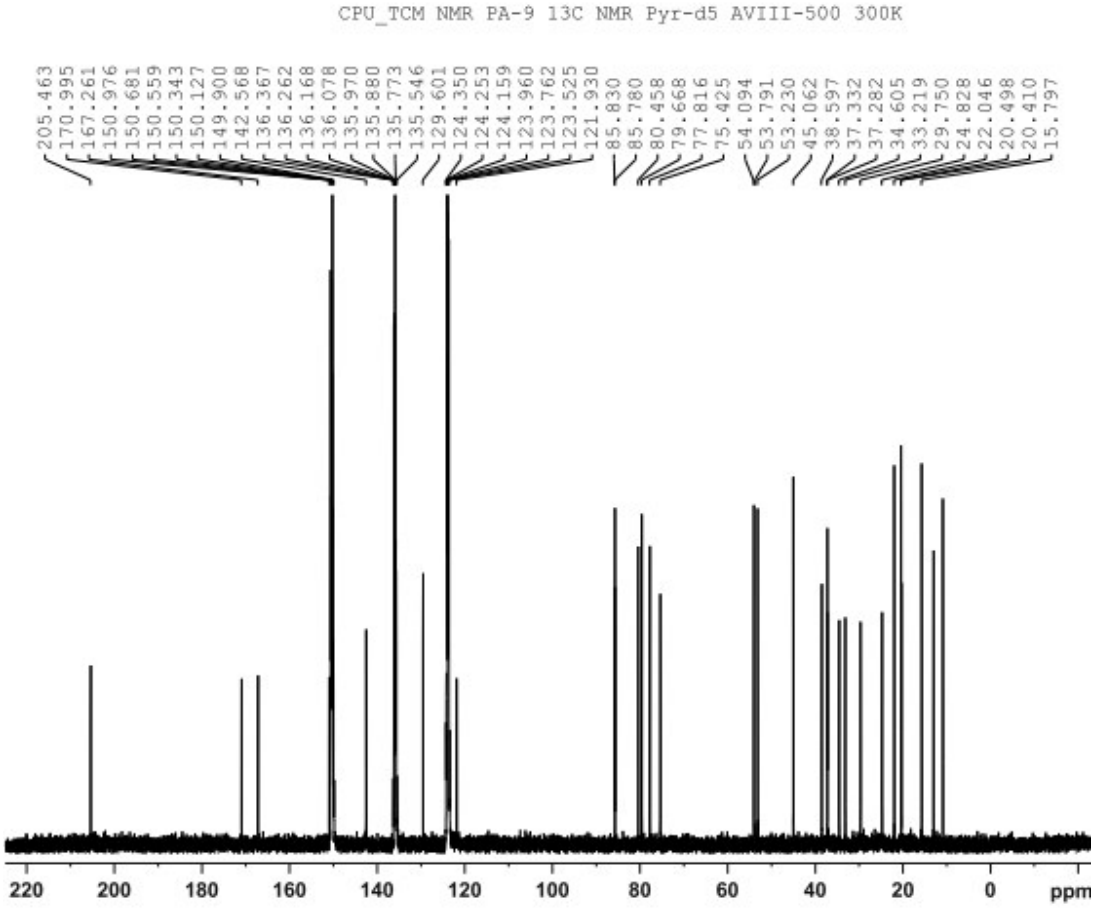

S23. HMBC spectrum of physagulide D (**4**) in pyridine-$d_5$




S24. ROESY spectrum of physagulide D (**4**) in pyridine-$d_5$




S25. $^{1}$H NMR spectrum of physagulide E (**5**) in pyridine-$d_5$ (500MHz)

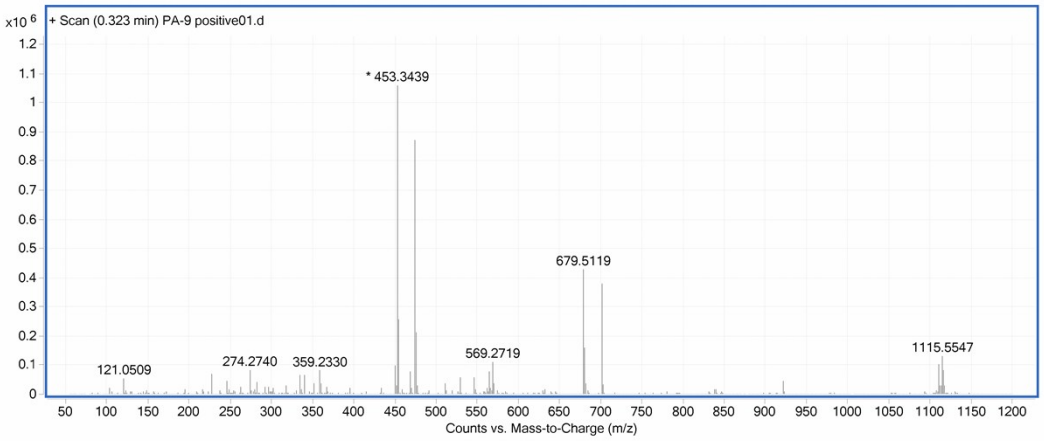


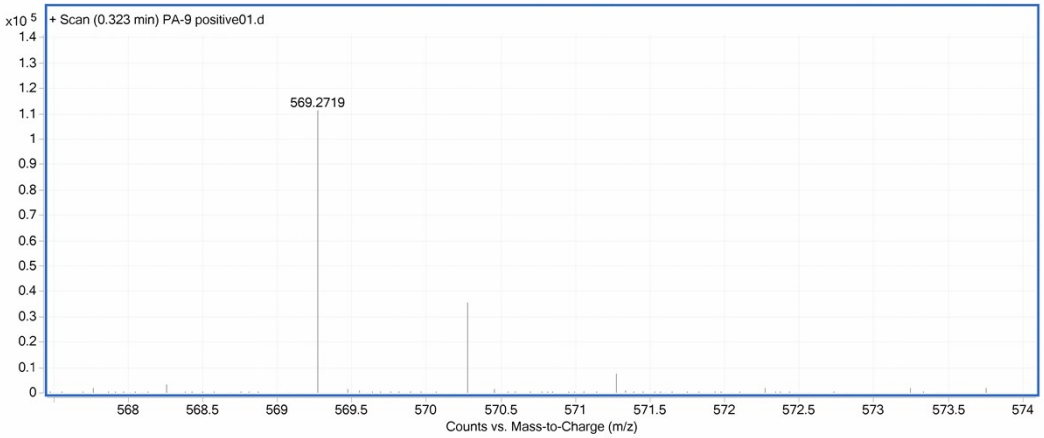

S26. $^{13}$C NMR spectrum of physagulide E (**5**) in pyridine-$d_5$ (125MHz)

# TCM-CPU HR-ESI-MS Display Report

**Sample Name**: PA-9 **Instrument:** Agilent 6520B Q-TOF

**Acq. Date:** 01/18 /2013 **Operator:** Administrator




**Elemental Composition Calculator**

| **Target m/z:** | 569.2719 | **Result type:** | Positive ions | **Species:** | $[M+Na]^+$ |
|---|---|---|---|---|---|
| **Elements:** | | C (0-80); H (0-120); O (0-30); N(0-10); Na (0-5); S (0-5) | | | |
| **Ion Formula** | | **Calculated m/z** | | **PPM Error** | |
| C30H42NaO9 | | 569.2721 | | 0.32 | |

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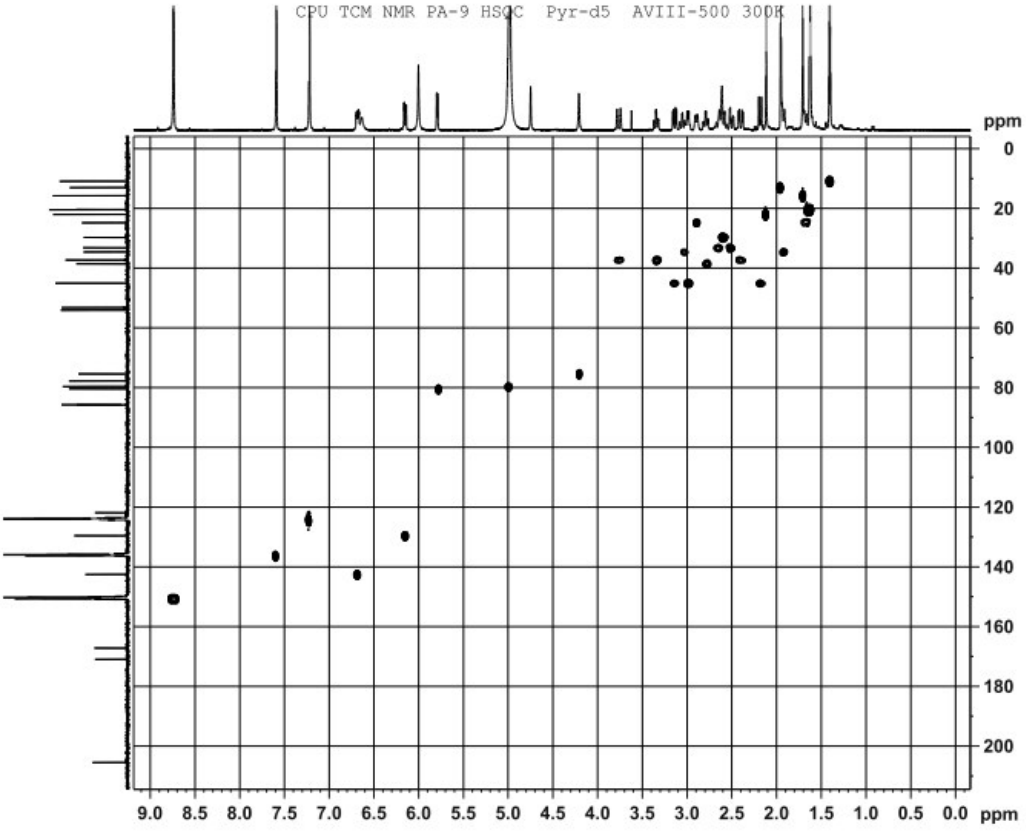
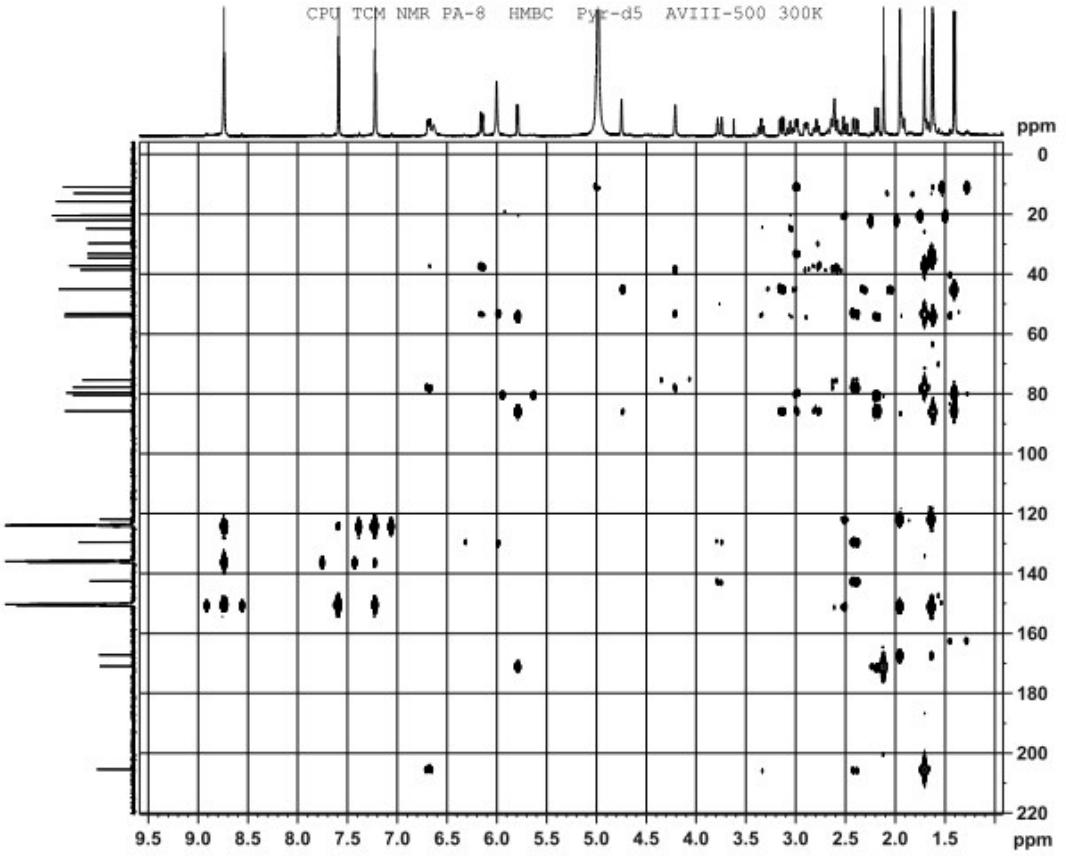
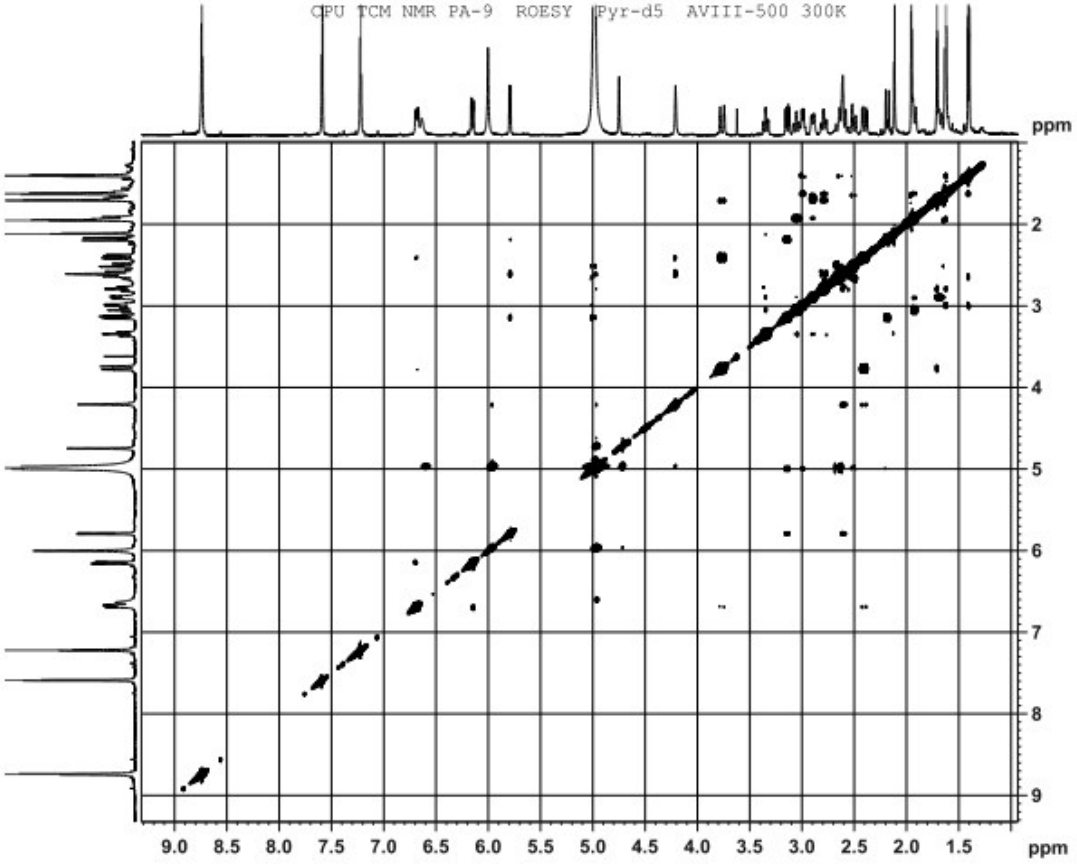
S27. HRESIMS spectrum of physagulide E (**5**)



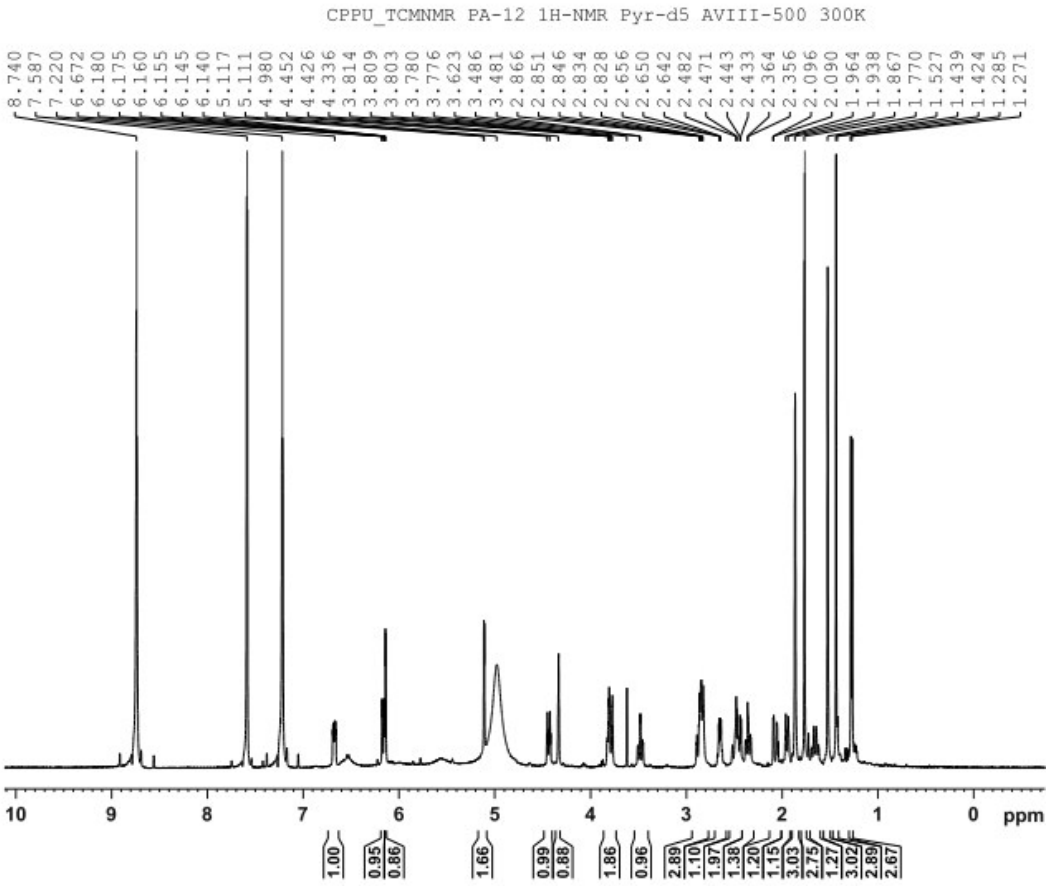

S28. HSQC spectrum of physagulide E **(5)** in pyridine-$d_5$



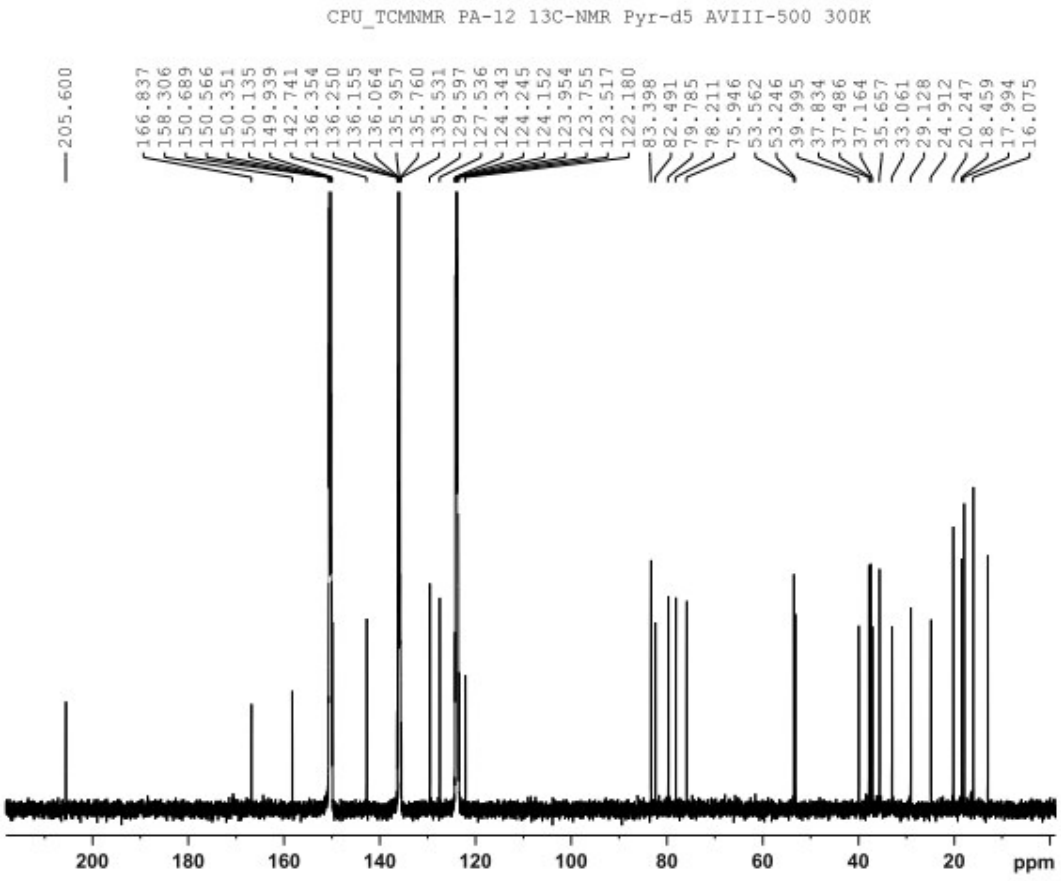

S29. HMBC spectrum of physagulide E (**5**) in pyridine-$d_5$




S30. ROESY spectrum of physagulide E **(5)** in pyridine-$d_5$




S31. $^{1}$H NMR spectrum of physagulide F (**6**) in pyridine-$d_5$ (500MHz)

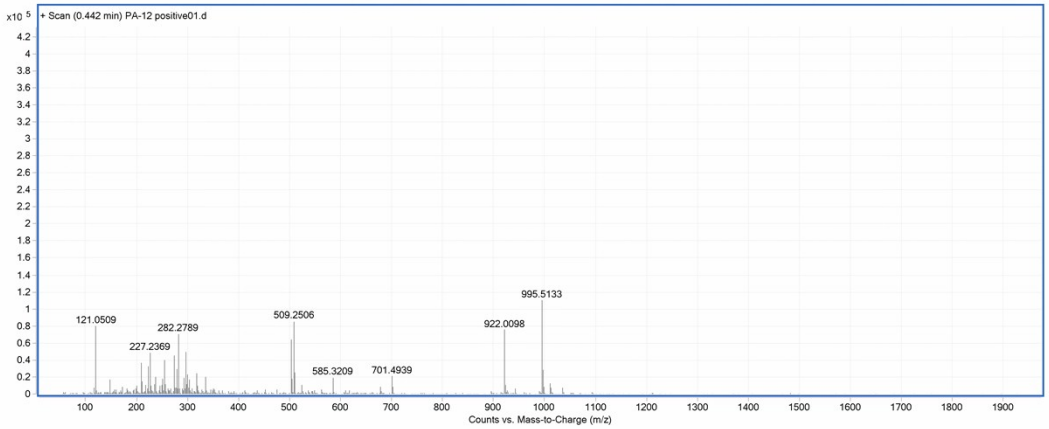


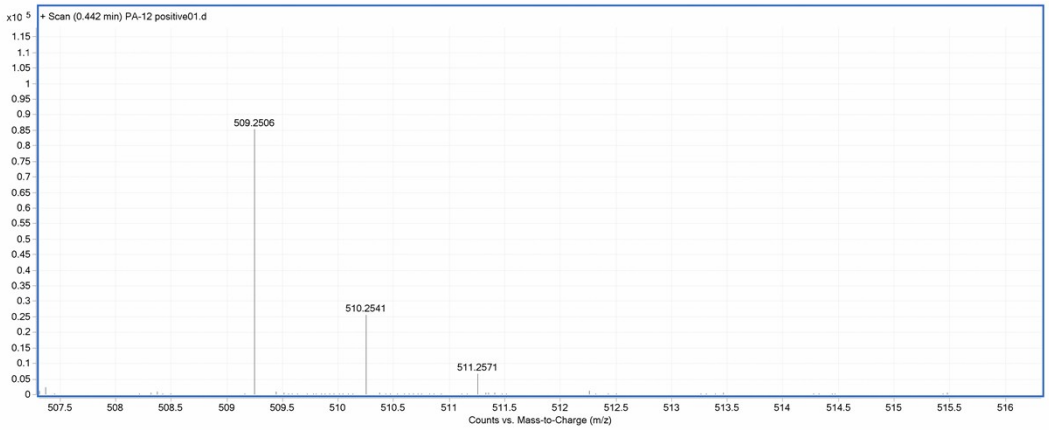

S32. $^{13}$C NMR spectrum of physagulide F **(6)** in pyridine-$d_5$ (125MHz)

# TCM-CPU HR-ESI-MS Display Report

**Sample Name**: PA-12 **Instrument:** Agilent 6520B Q-TOF

**Acq. Date:** 04/27 /2013 **Operator:** Administrator




**Elemental Composition Calculator**

| **Target m/z:** | 509.2506 | **Result type:** | Positive ions | **Species:** | $[M+Na]^+$ |
|---|---|---|---|---|---|
| **Elements:** | | C (0-100); H (0-150); O (0-50); N(0-10); Na (0-5); S (0-5); | | | |
| **Ion Formula** | | **Calculated m/z** | | **PPM Error** | |
| C28H38NaO7 | | 509.2510 | | 0.66 | |

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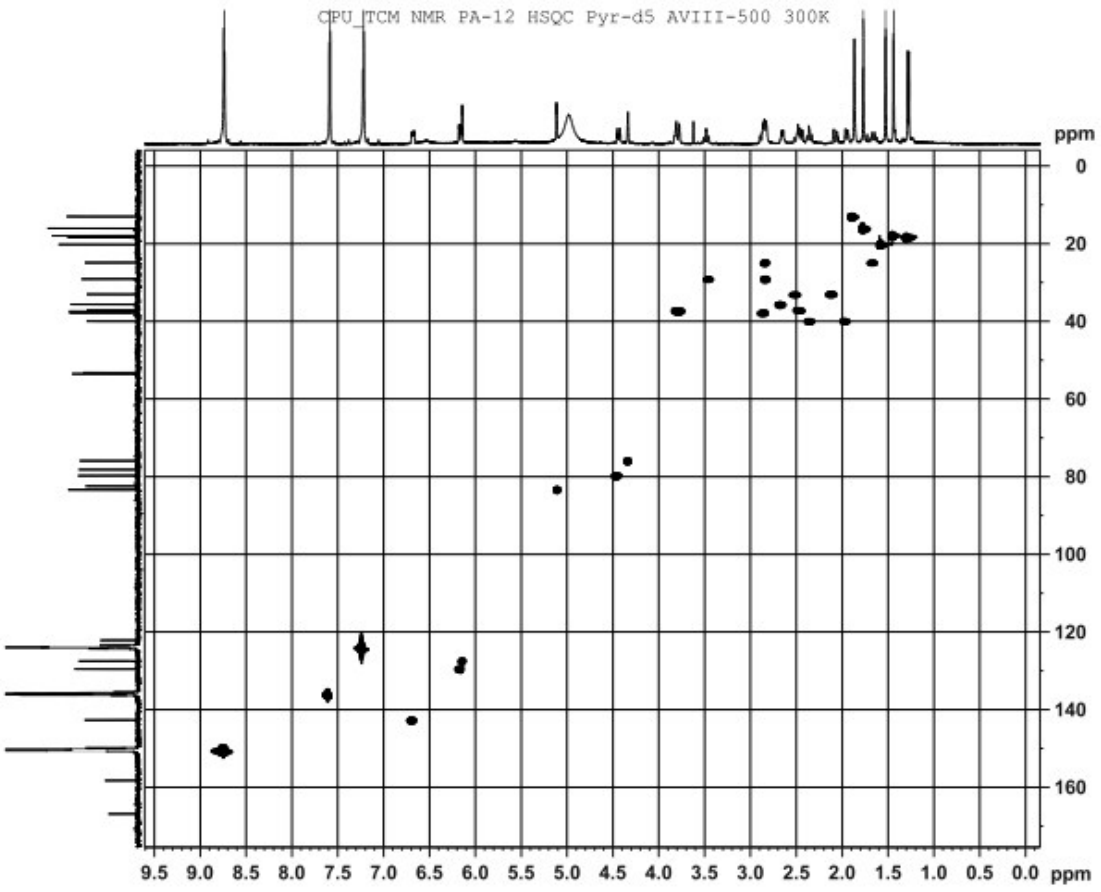
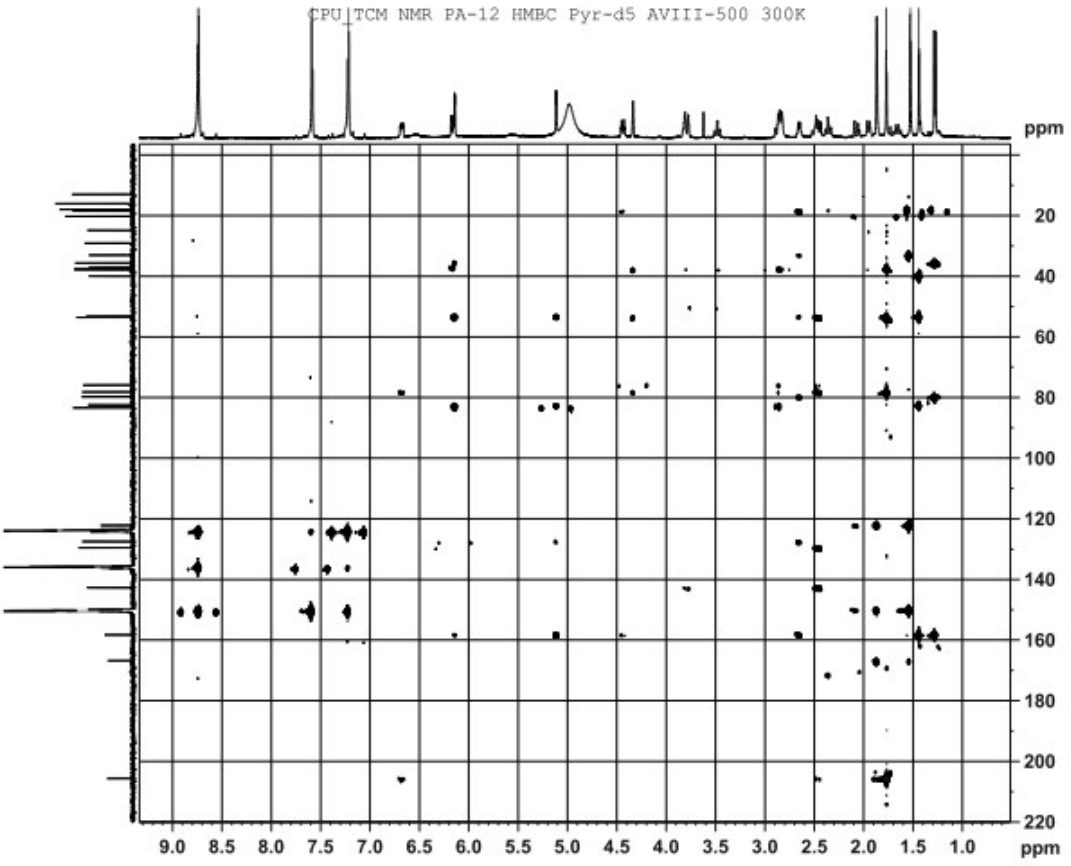
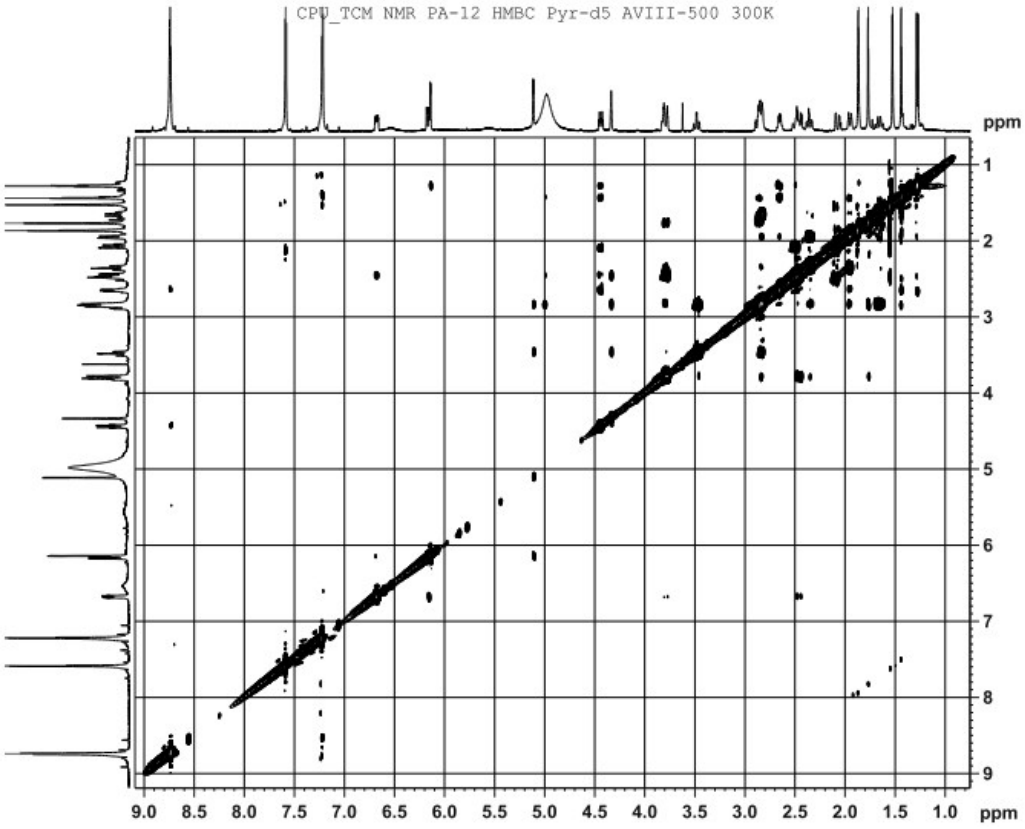
S33. HRESIMS spectrum of physagulide F (**6**)



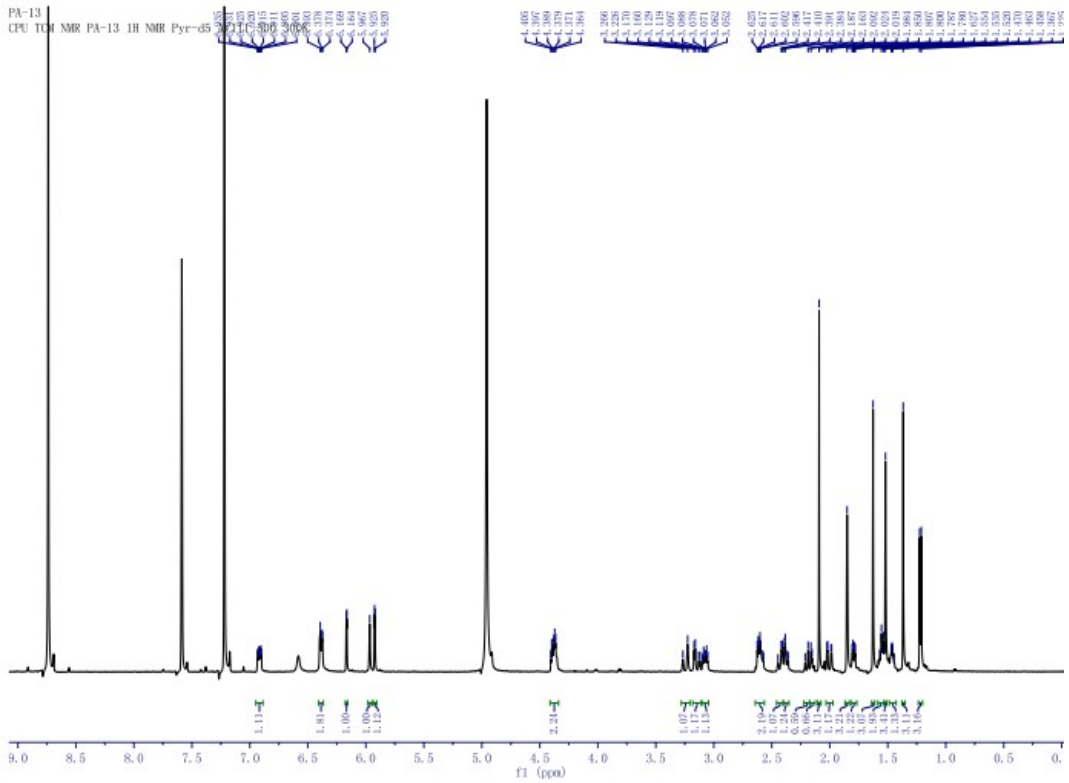

S34. HSQC spectrum of physagulide F (**6**) in pyridine-$d_5$



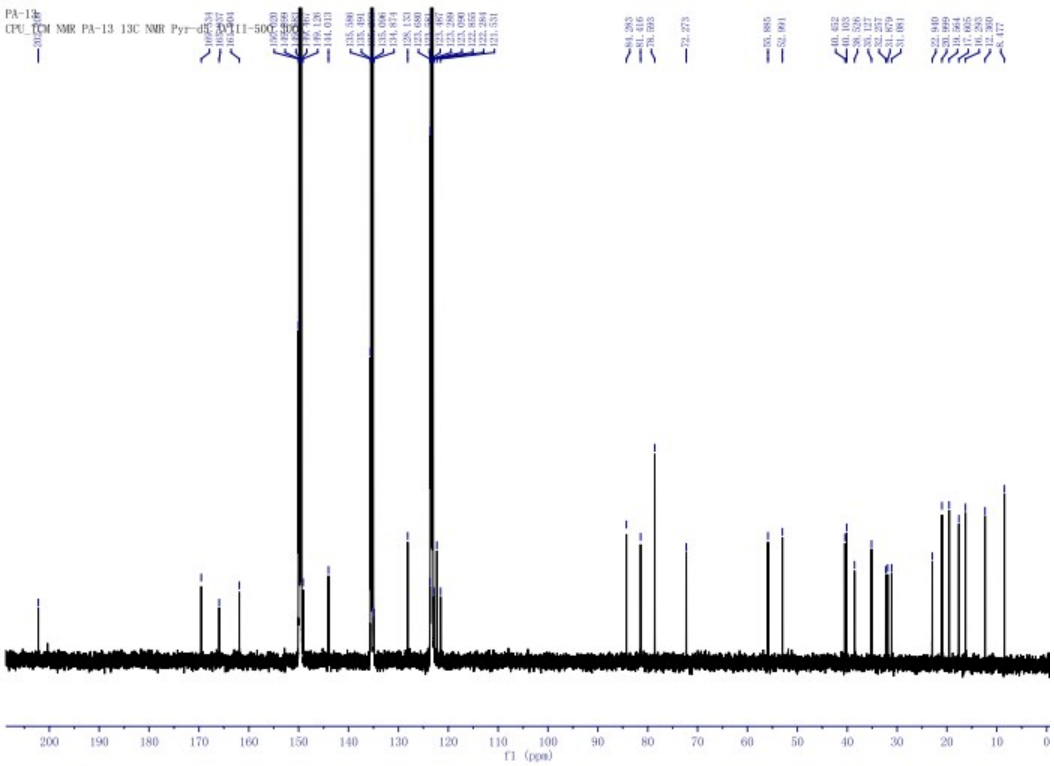

S35. HMBC spectrum of physagulide F (**6**) in pyridine-$d_5$




S36. ROESY spectrum of physagulide F (**6**) in pyridine-$d_5$



S37. $^{1}$H NMR spectrum of physagulide G (**7**) in pyridine-$d_5$ (500MHz)

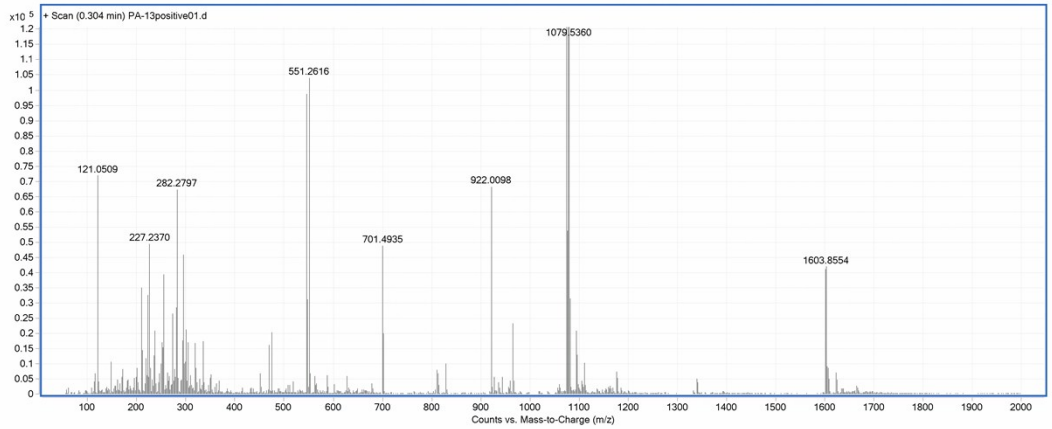


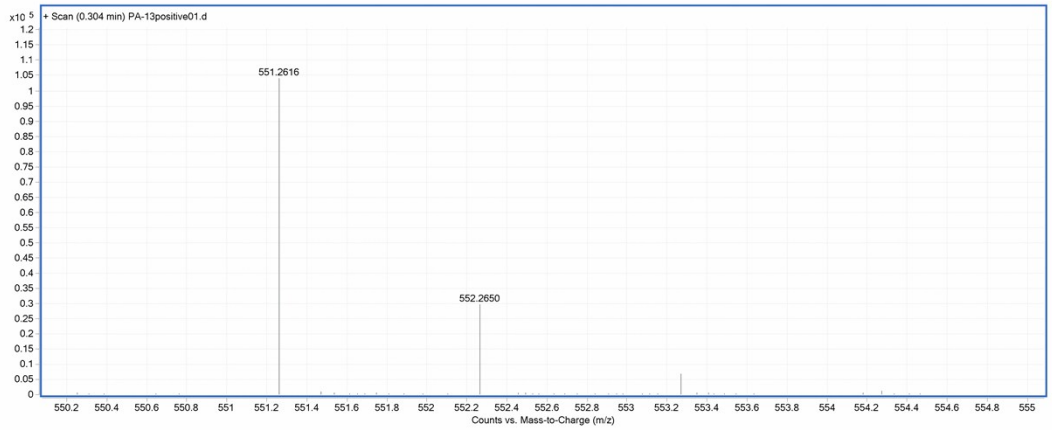

S38. $^{13}$C NMR spectrum of physagulide G (**7**) in pyridine-$d_5$ (125MHz)

# TCM-CPU HR-ESI-MS Display Report

**Sample Name**: PA-13 **Instrument:** Agilent 6520B Q-TOF

**Acq. Date:** 04/27 /2013 **Operator:** Administrator




**Elemental Composition Calculator**

| **Target m/z:** | 551.2616 | **Result type:** | Positive ions | **Species:** | $[M+Na]^+$ |
|---|---|---|---|---|---|
| **Elements:** | | C (0-100); H (0-150); O (0-50); N(0-10); Na (0-5); S (0-5) | | | |
| **Ion Formula** | | **Calculated m/z** | | **PPM Error** | |
| C30H40NaO8 | | 551.2615 | | -0.17 | |

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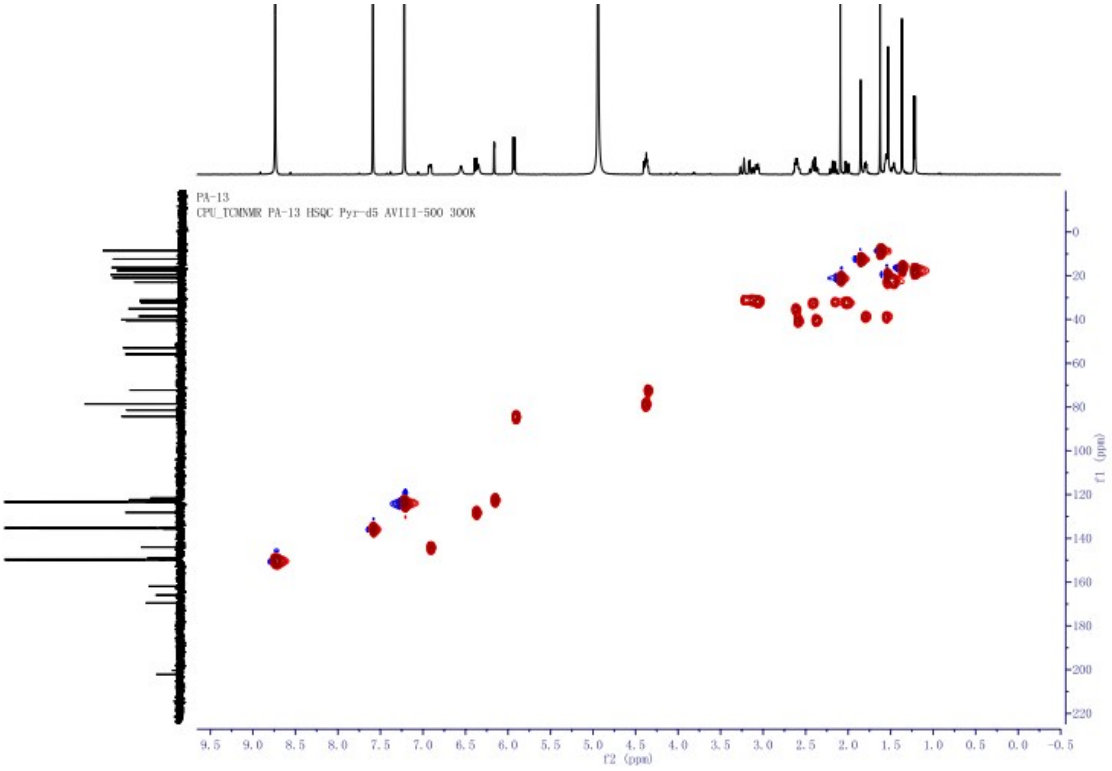
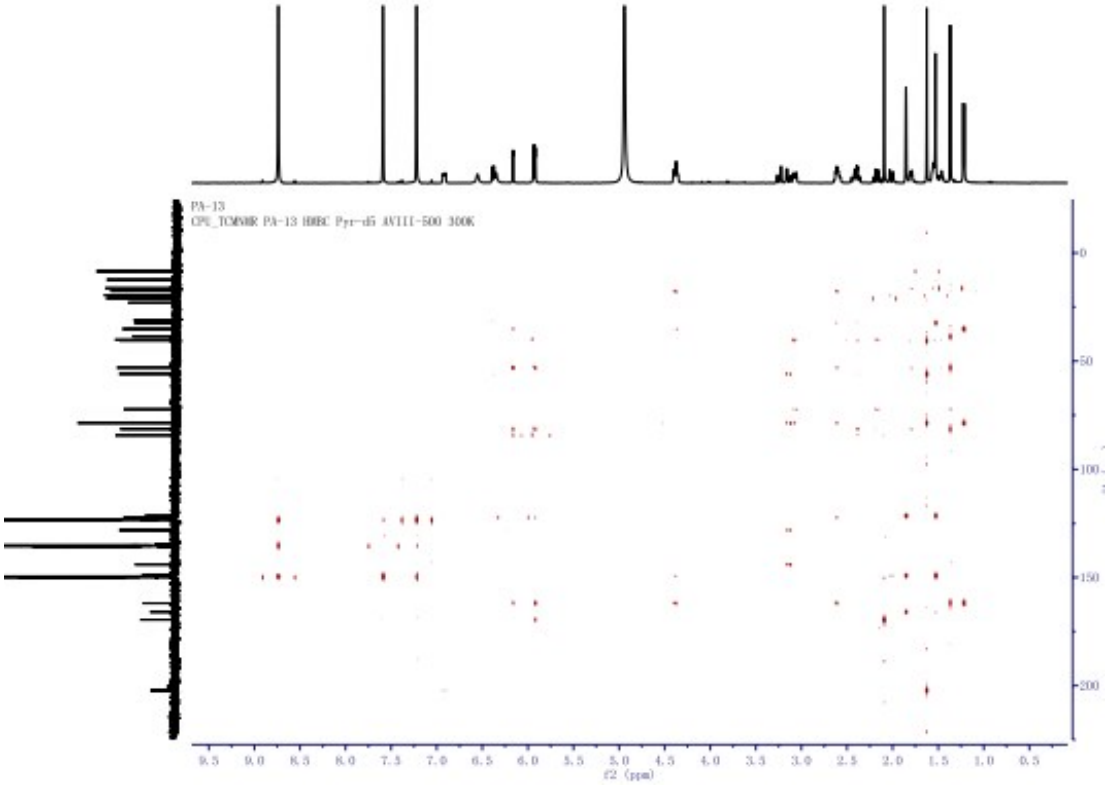
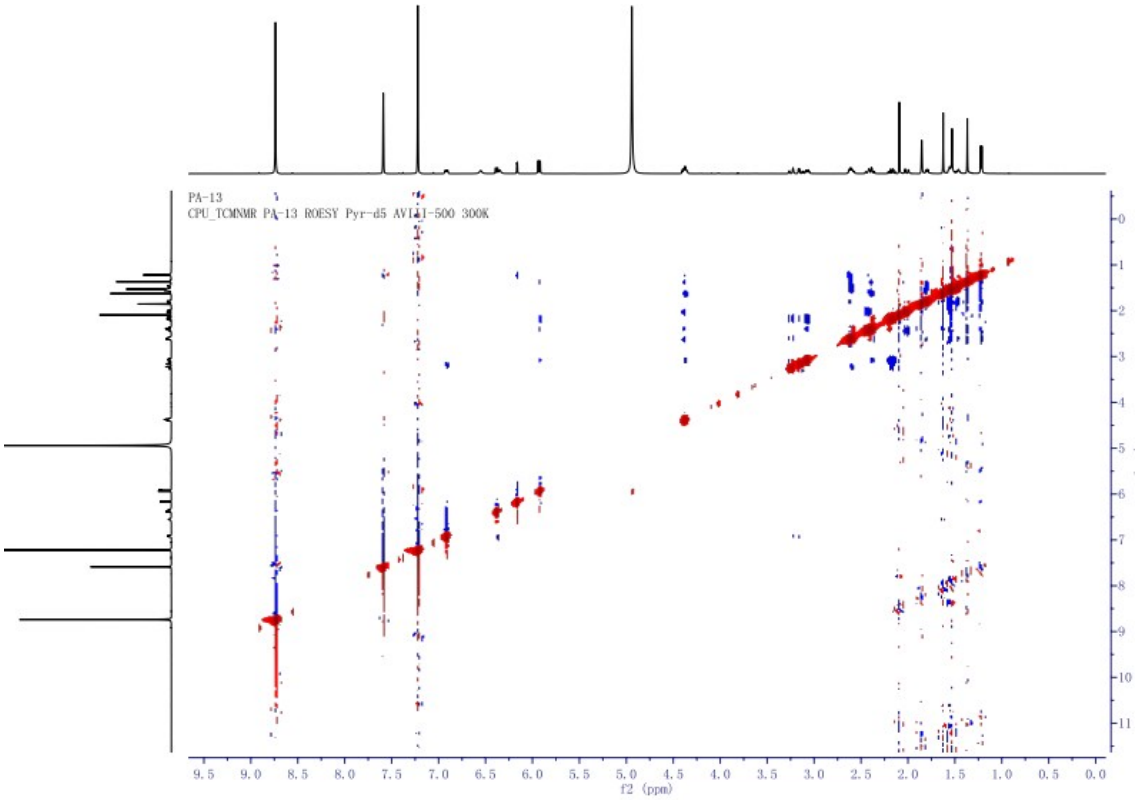
S39. HRESIMS spectrum of physagulide G (**7**)



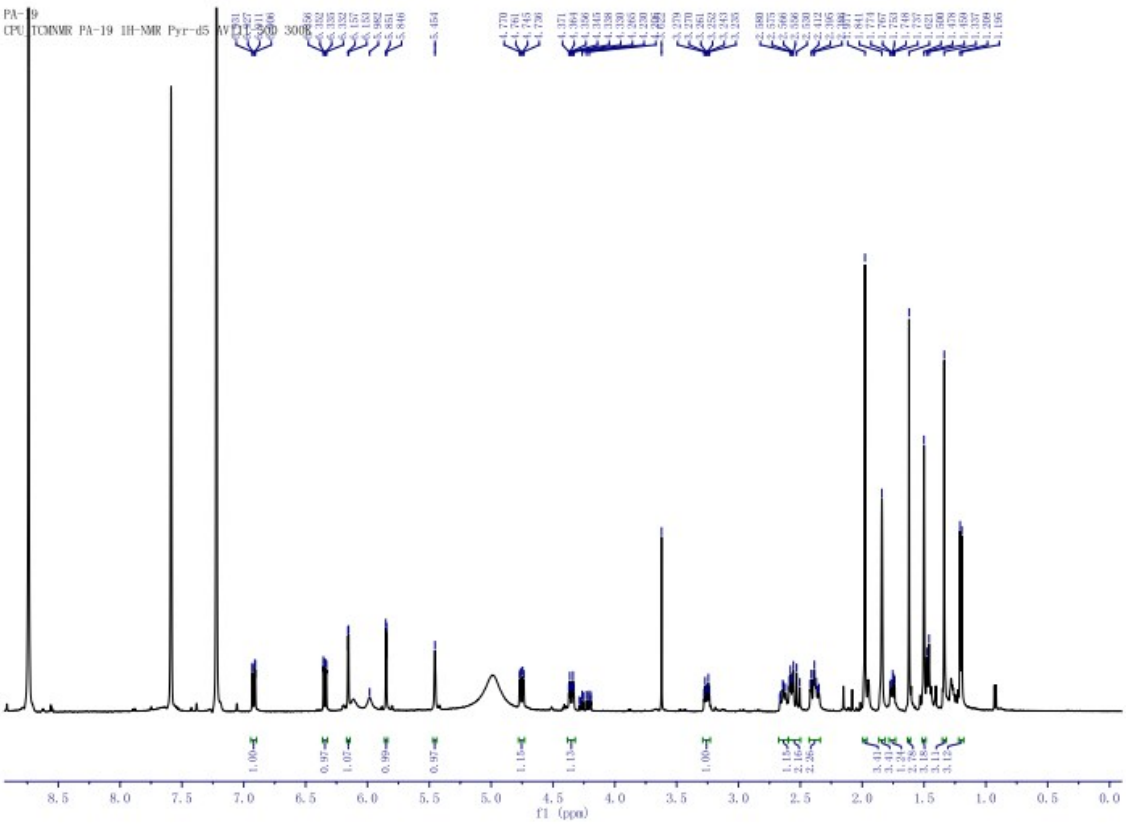

S40. HSQC spectrum of physagulide G **(7)** in pyridine-$d_5$



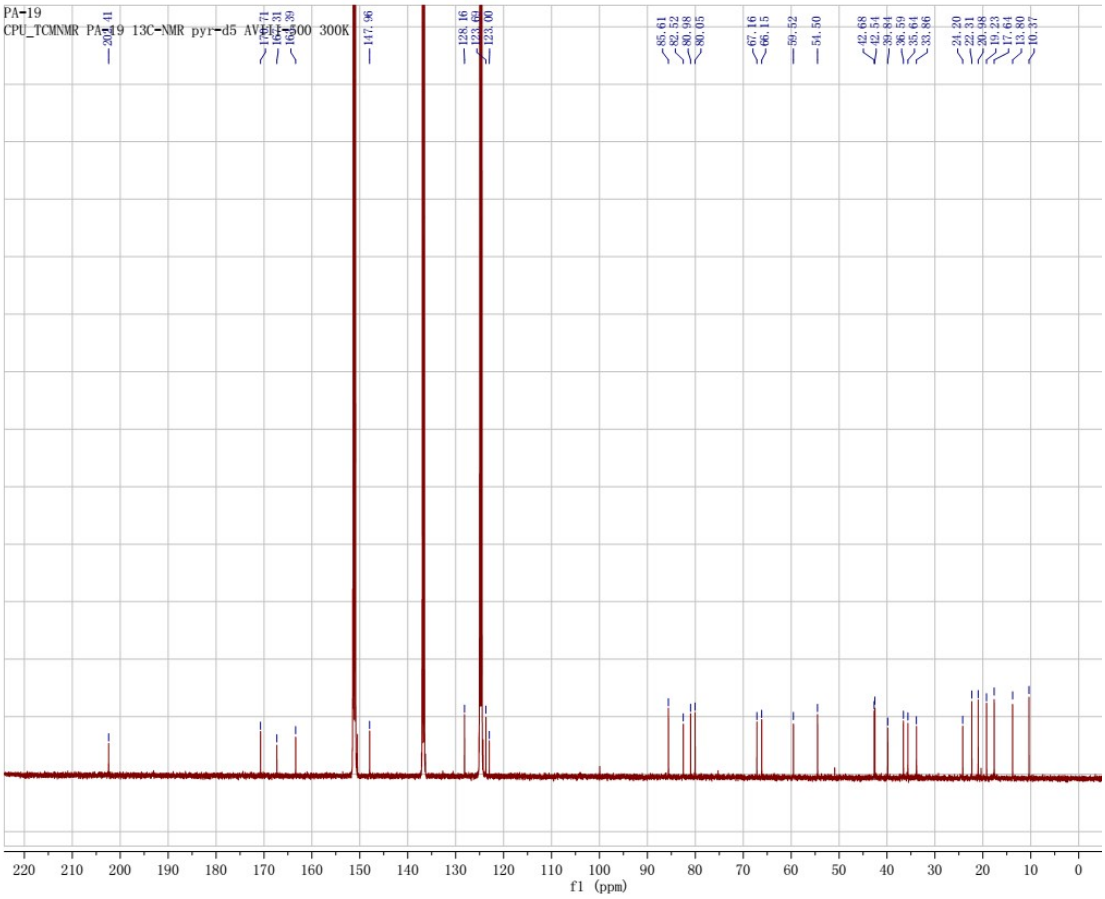

S41. HMBC spectrum of physagulide G (**7**) in pyridine-$d_5$




S42. ROESY spectrum of physagulide G (**7**) in pyridine-$d_5$




S43. $^{1}$H NMR spectrum of physagulide H (**8**) in pyridine-$d_5$ (500MHz)

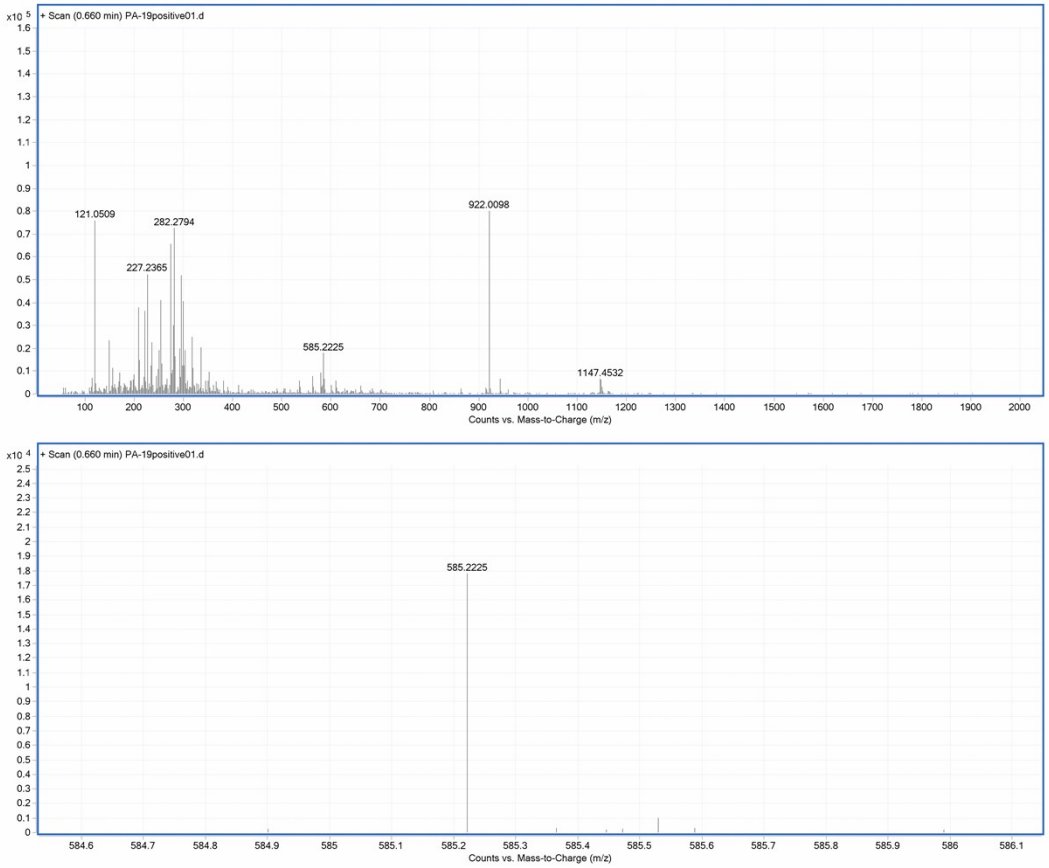



S44. $^{13}$C NMR spectrum physagulide H (**8**) in pyridine-$d_5$ (125MHz)

# TCM-CPU HR-ESI-MS Display Report

**Sample Name**: PA-19 **Instrument:** Agilent 6520B Q-TOF

**Acq. Date:** 04/27 /2013 **Operator:** Administrator




**Elemental Composition Calculator**

| **Target m/z:** | 585.2225 | **Result type:** | Positive ions | **Species:** | $[M+Na]^+$ |
|---|---|---|---|---|---|
| **Elements:** | | C (0-100); H (0-150); O (0-50); N(0-10); Na (0-5); S (0-5);Cl(0-5) | | | |
| **Ion Formula** | | **Calculated m/z** | | **PPM Error** | |
| C30H39ClNaO8 | | 585.2226 | | 0.15 | |

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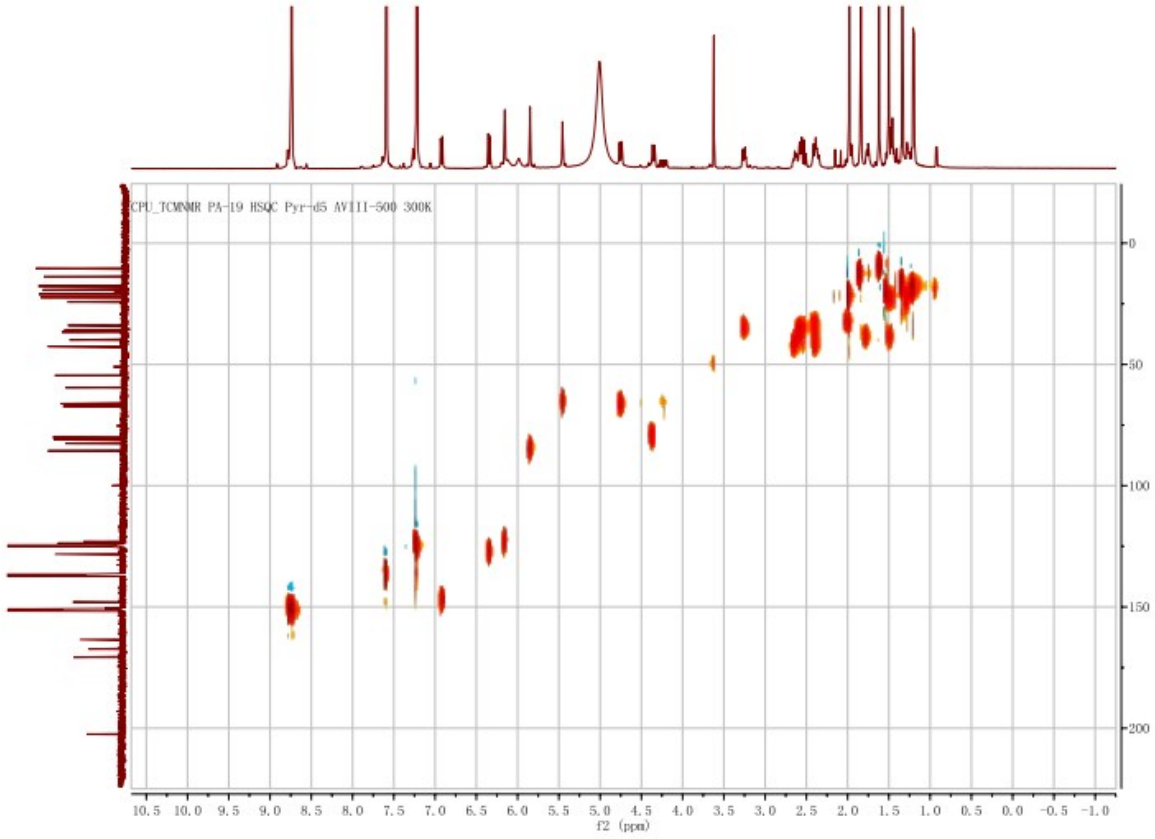
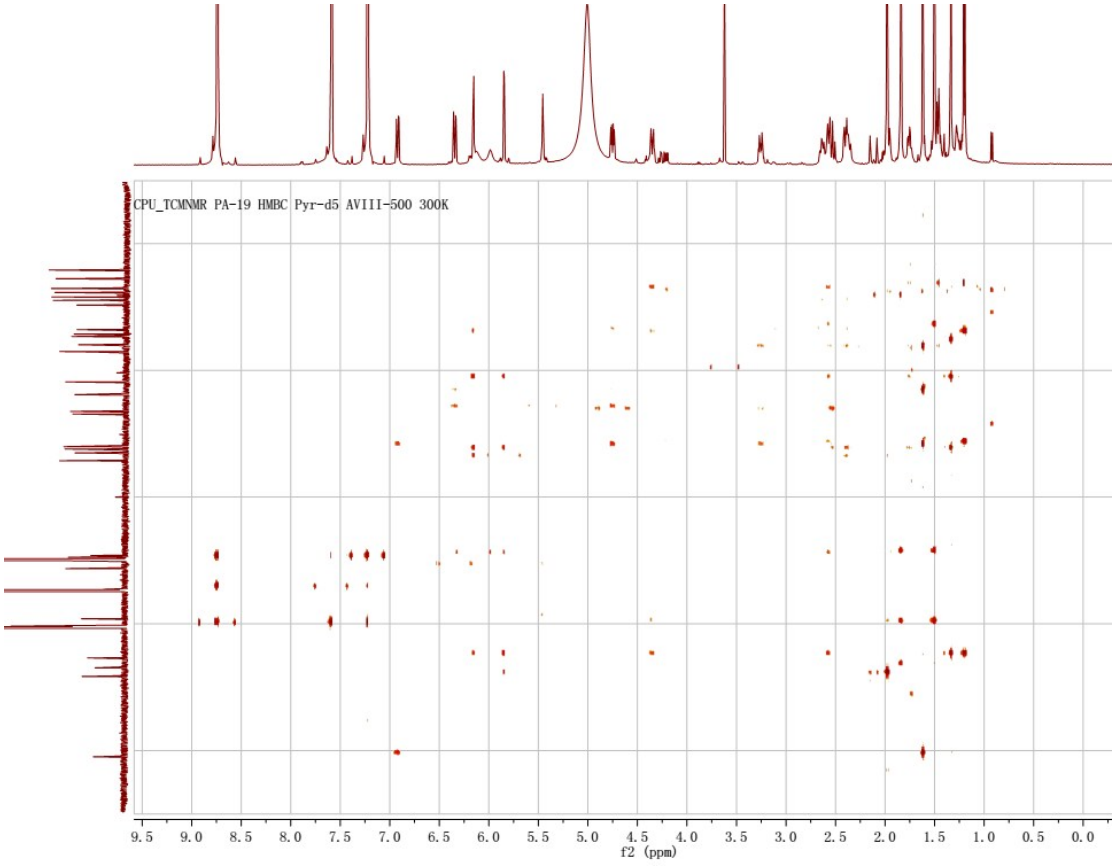
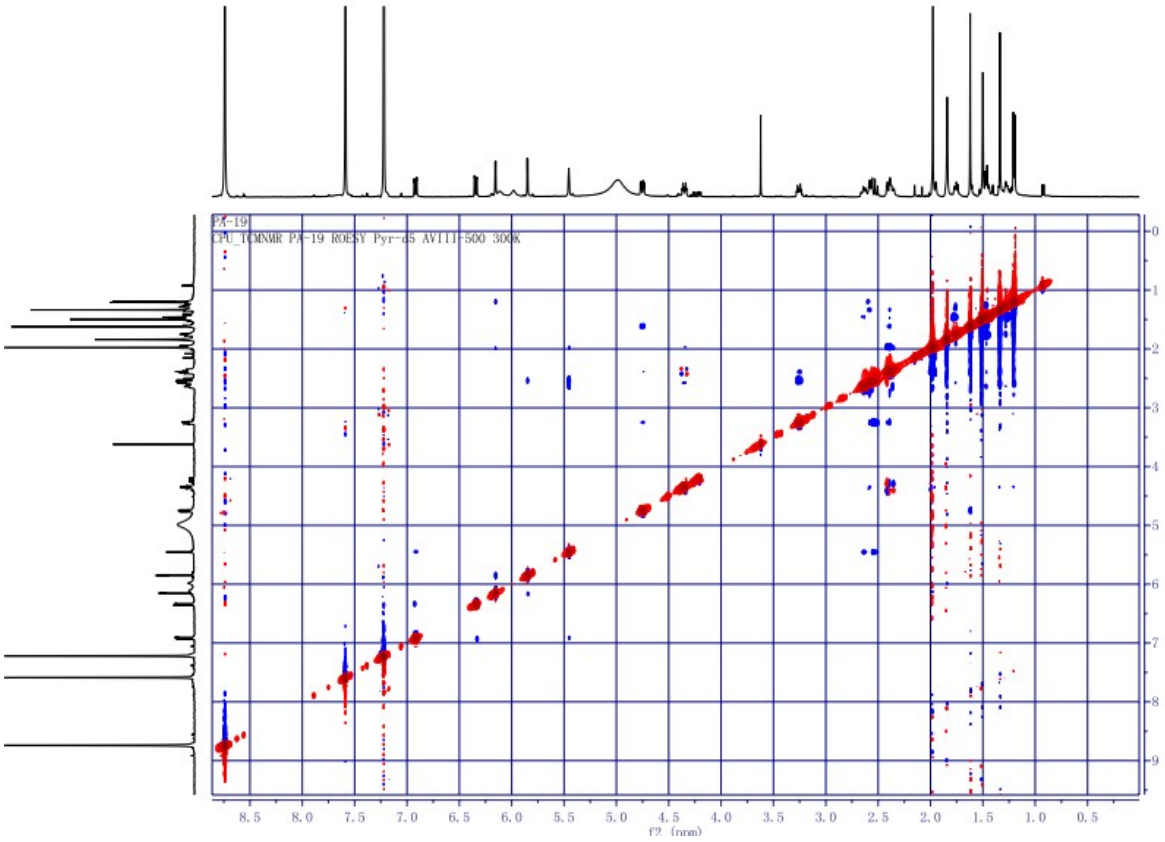
S45. HRESIMS spectrum of physagulide H (**8**)




S46. HSQC spectrum of physagulide H (**8**) in pyridine-$d_5$




S47. HMBC spectrum of physagulide H (**8**) in pyridine-$d_5$




S48. ROESY spectrum of physagulide H (**8**) in pyridine-$d_5$