



## Supporting Information

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### **Tetrahydropyranyl: A Non-aromatic, Mild-Acid-Labile Group for Hydroxyl Protection in Solid-Phase Peptide Synthesis**

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## Supporting Information

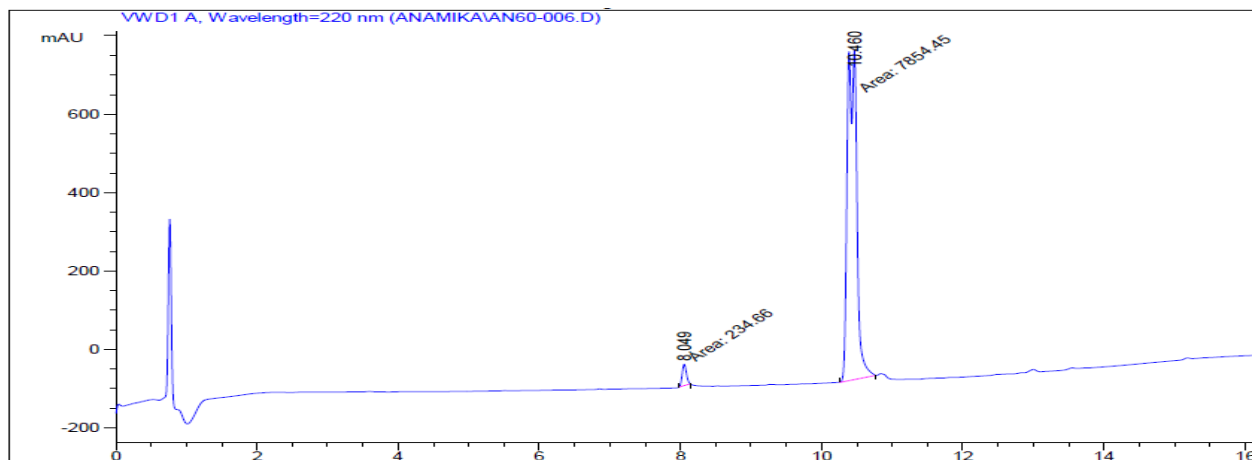
### Materials

All reagents and solvents were obtained from commercial suppliers and were used without further purification unless otherwise stated. 2-CTC resin and Fmoc-amino acids were purchased from GL Biochem Pvt. Ltd., China. Ellman resin was purchased from Merck. NMR spectra ( $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR) were recorded on a Bruker AVANCE III 400 MHz spectrometer. Chemical shift values are expressed in ppm. At short reaction times (2-5 min), the reactions were manually stirred with a Teflon stick, meanwhile for long reactions times (>30 min) the reactions were stirred on a Unimax 1010 shaker from Heidolph instruments. Solvents were removed from the reactor by vacuum suction. All the reactions were carried out at room temperature (~25 °C). Every reaction step was followed of washings of the peptide-resin with DMF (4×1 min) and DCM (4×1 min). Analytical HPLC was performed on an Agilent 1100 system using a Phenomenex  $\text{C}_{18}$  (3  $\mu\text{m}$ , 4.6 x 50 mm) column. Buffer A: 0.1 % TFA in  $\text{H}_2\text{O}$ ; buffer B: 0.1 % TFA in  $\text{CH}_3\text{CN}$ . LC-MS was performed on a Shimadzu 2020 UFLC-MS using an YMC-Triart  $\text{C}_{18}$  (5  $\mu\text{m}$ , 4.6 × 150 mm) column and data processing was carried out by LabSolution software. Buffer A: 0.1 % formic acid in  $\text{H}_2\text{O}$ ; buffer B: 0.1 % formic acid in  $\text{CH}_3\text{CN}$ . High resolution mass spectrometry (HRMS) was performed using a Bruker ESI-QTOF mass spectrometer in positive mode.

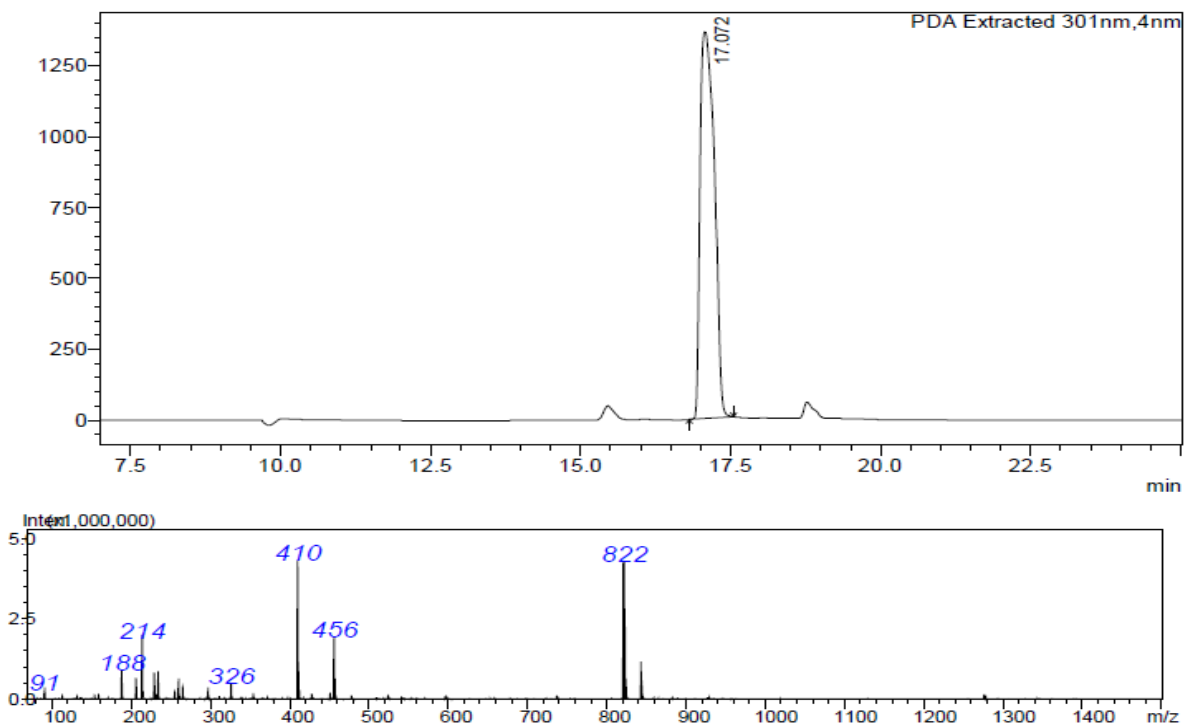
## Spectroscopic Characterization

**Fmoc-Ser(Thp)-OH:** It was obtained as pure white solid (478 mg, 76 %).

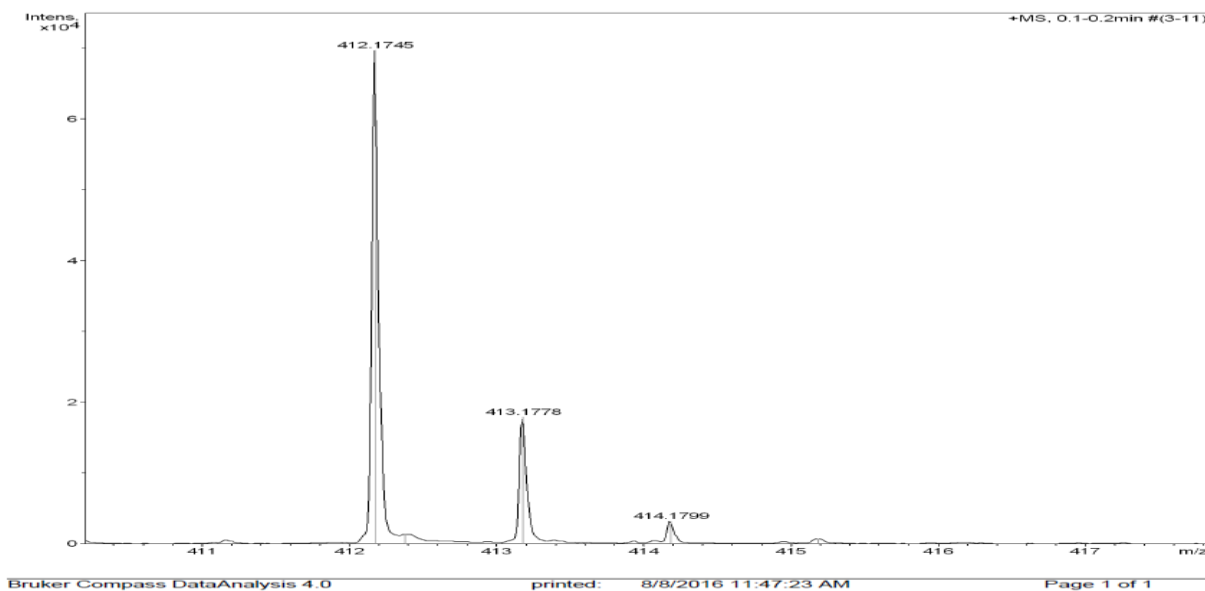
**HPLC:** (H<sub>2</sub>O/MeCN 5:95) *t<sub>R</sub>*: 10.4 min.



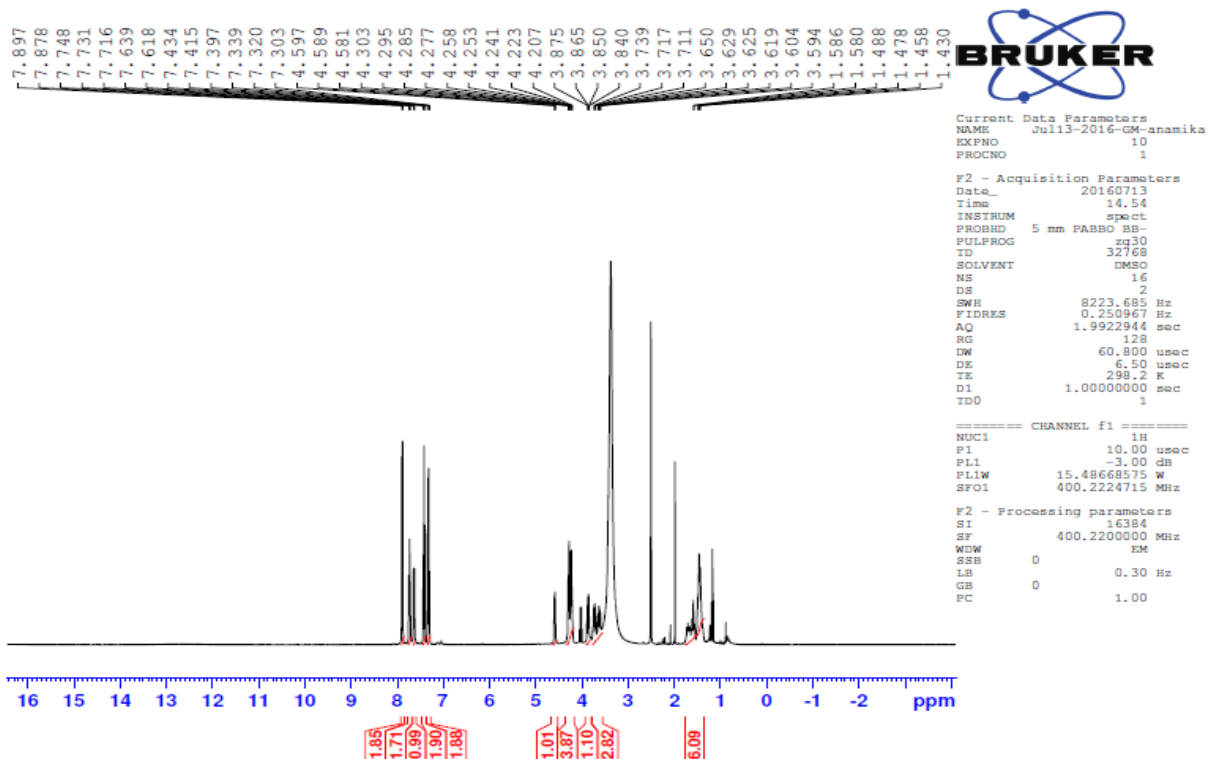
**LCMS:** *m/z* calculated for C<sub>23</sub>H<sub>25</sub>NO<sub>6</sub> = 411.45; found = 410 [M-H]<sup>+</sup>, being M the molecular weight of Fmoc-Ser(Thp)-OH.



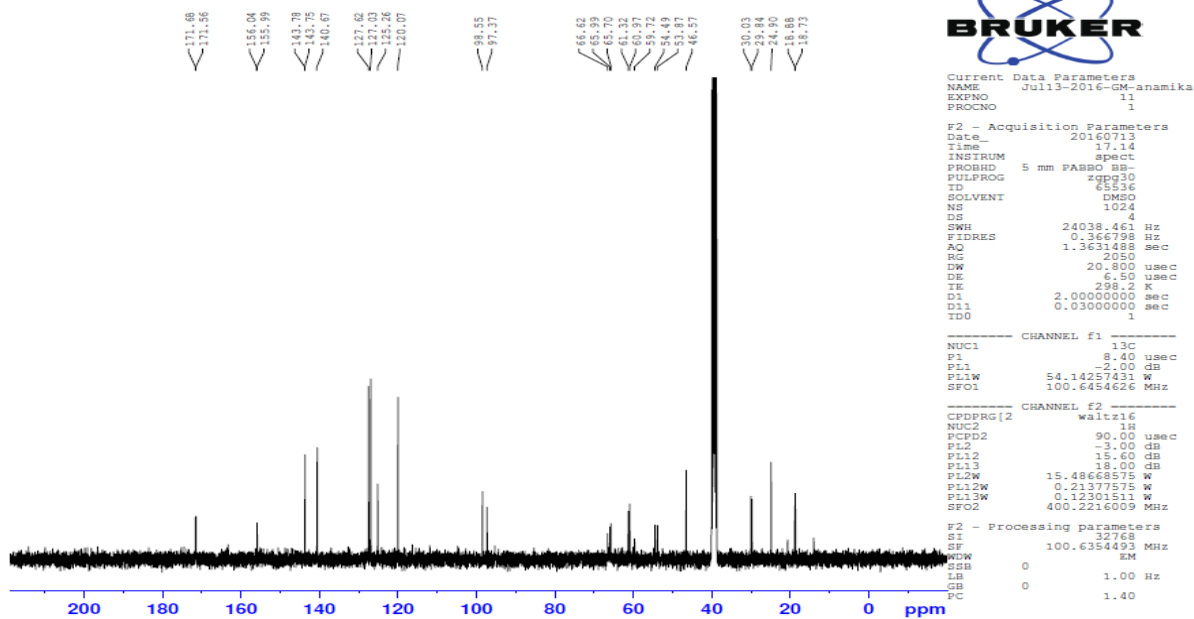
**HRMS:** Calculated for  $C_{23}H_{26}NO_6$   $[M+H] = 412.1760$ ; found = 412.1745  $[M+H]$ ; where M is the molecular weight of Fmoc-Ser(Thp)-OH.



**$^1H$  NMR (400 MHz, DMSO- $d_6$ ):**



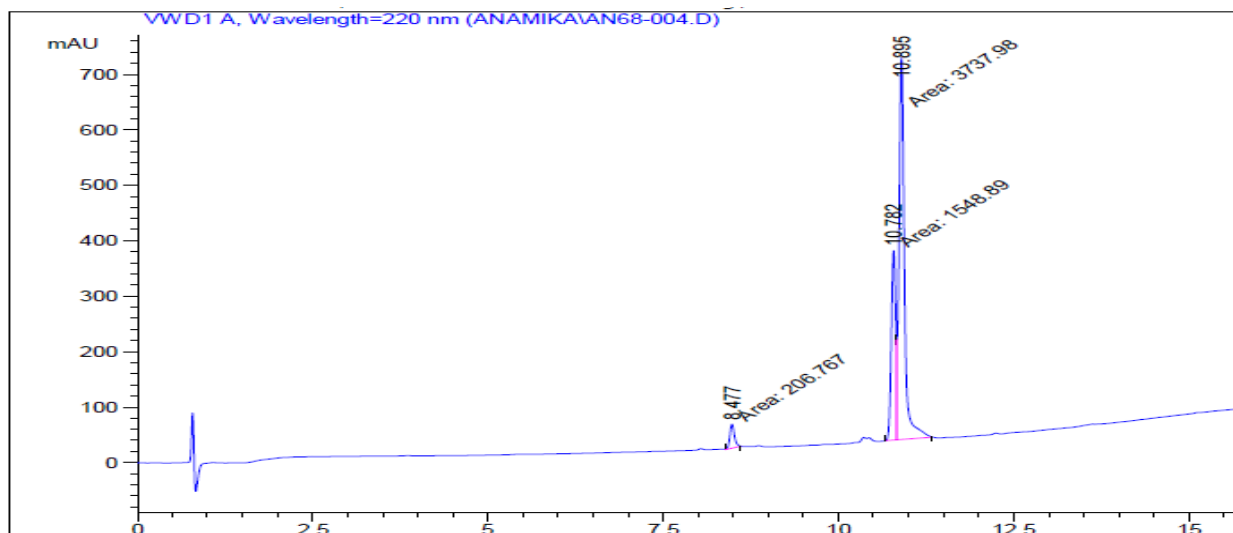
# <sup>13</sup>C NMR(100 MHz, DMSO-d<sub>6</sub>):



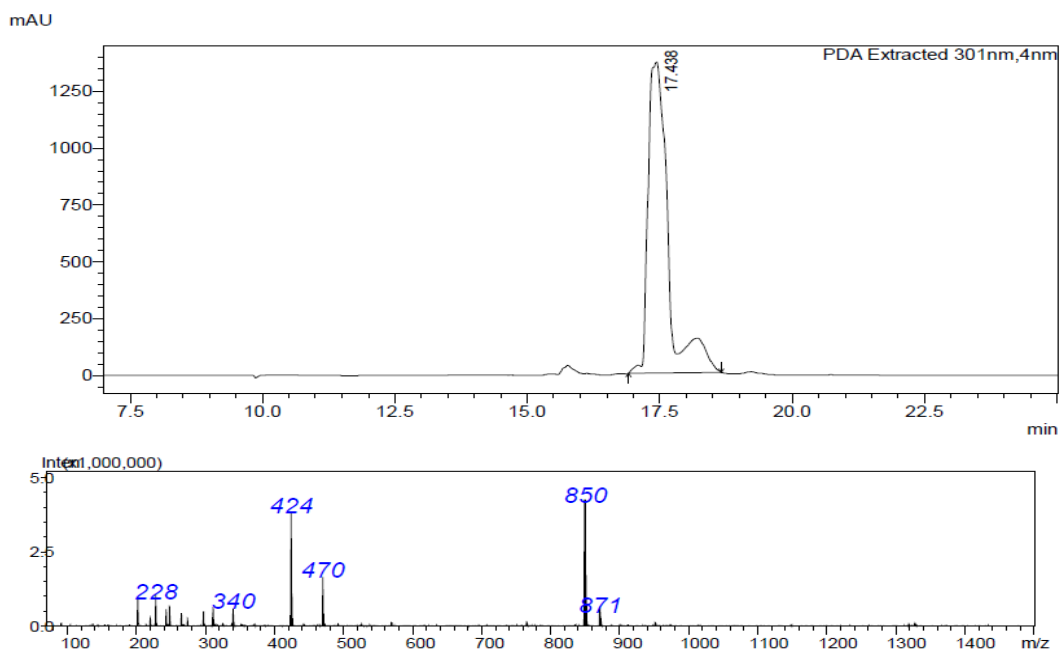
## Spectroscopic Characterization

**Fmoc-Thr(Thp)-OH:** It was obtained as pure white solid (453 mg, 73 %).

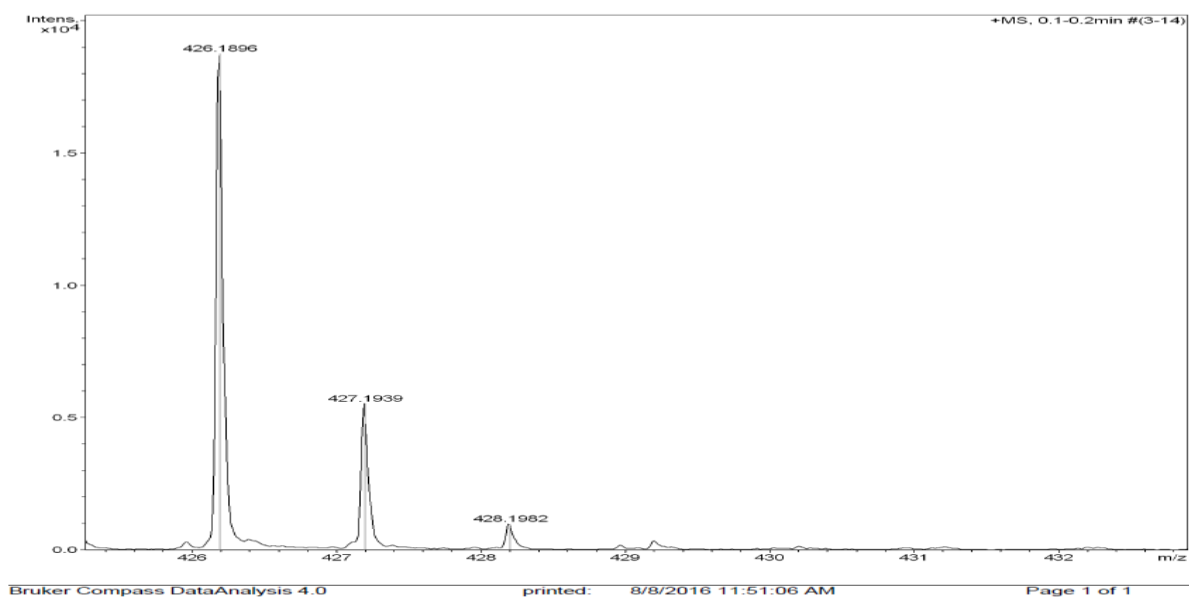
**HPLC:** (H<sub>2</sub>O/MeCN 5:95) *t<sub>R</sub>*: 10.8 min.



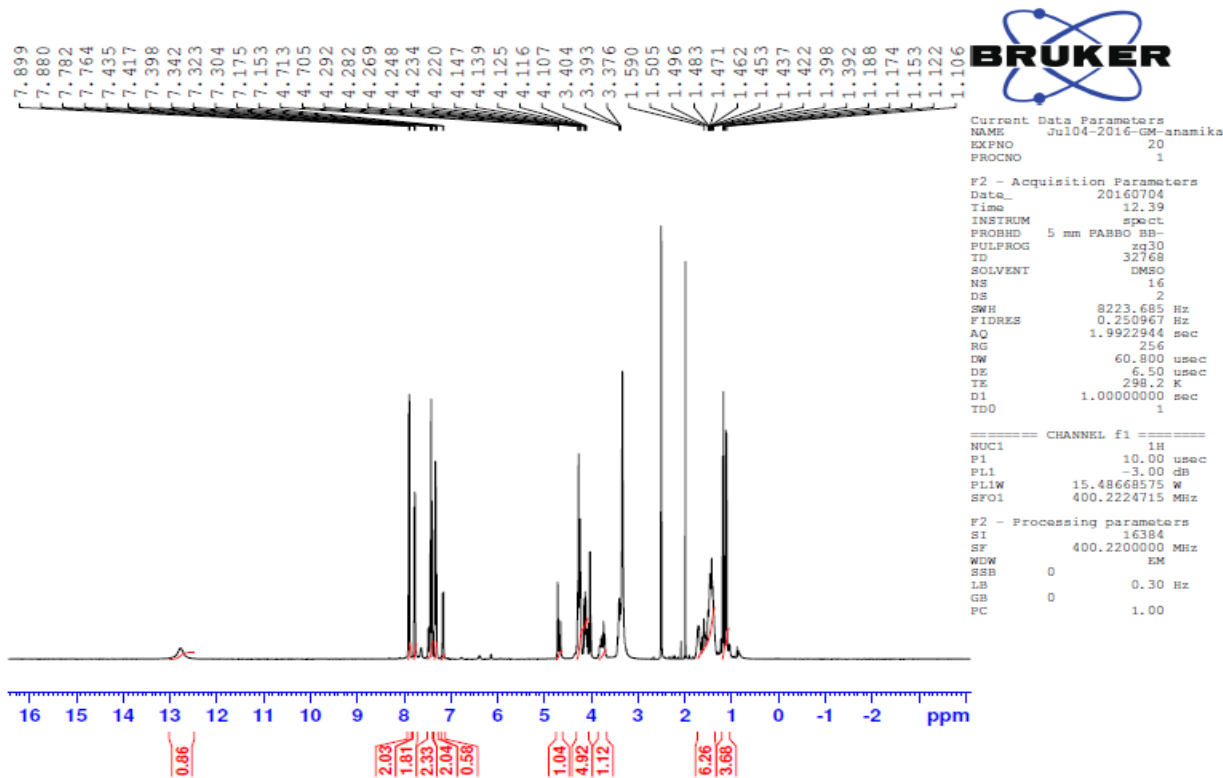
**LCMS:**  $m/z$  calculated for  $C_{23}H_{25}NO_6 = 425.18$ ; found = 424  $[M-H]^+$ , being M the molecular weight of Fmoc-Thr(Thp)-OH.



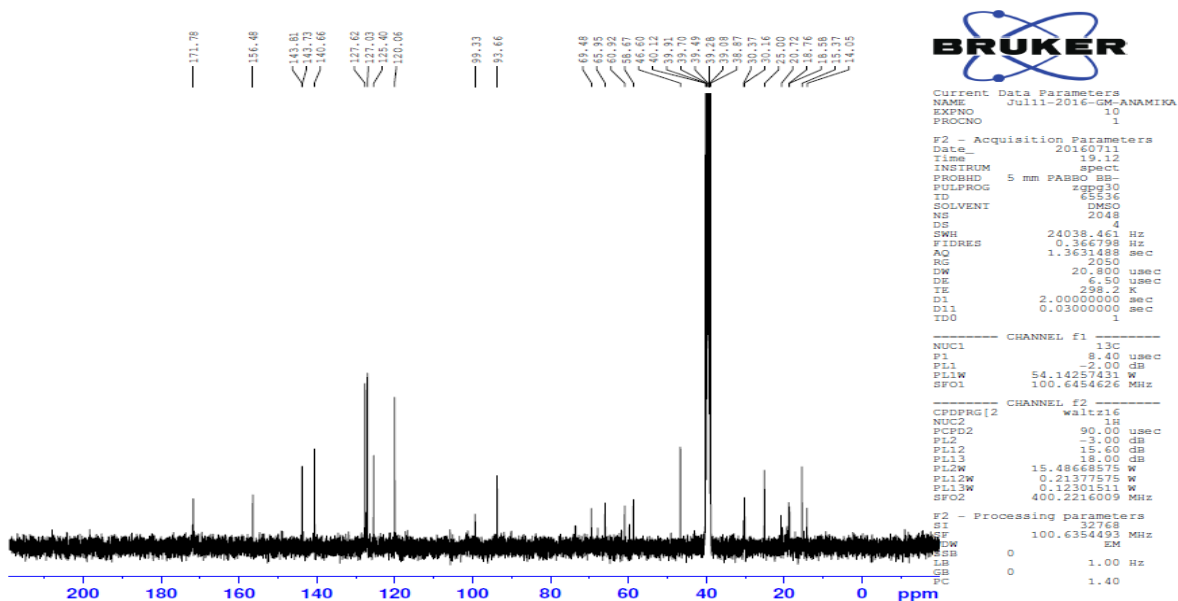
**HRMS:** Calculated for  $C_{24}H_{28}NO_6 [M+H] = 426.1917$ ; found = 426.1896  $[M+H]$ , where M is the molecular weight of Fmoc-Thr(Thp)-OH.



<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):



<sup>13</sup>C NMR(100 MHz, DMSO-d<sub>6</sub>):



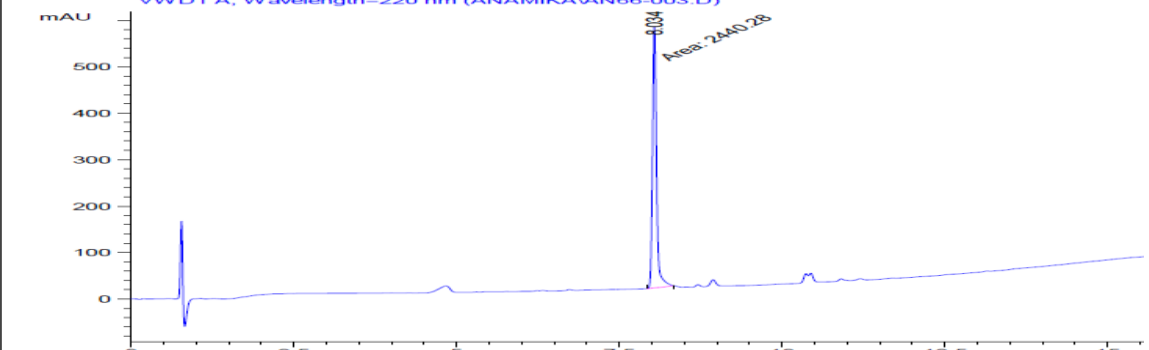
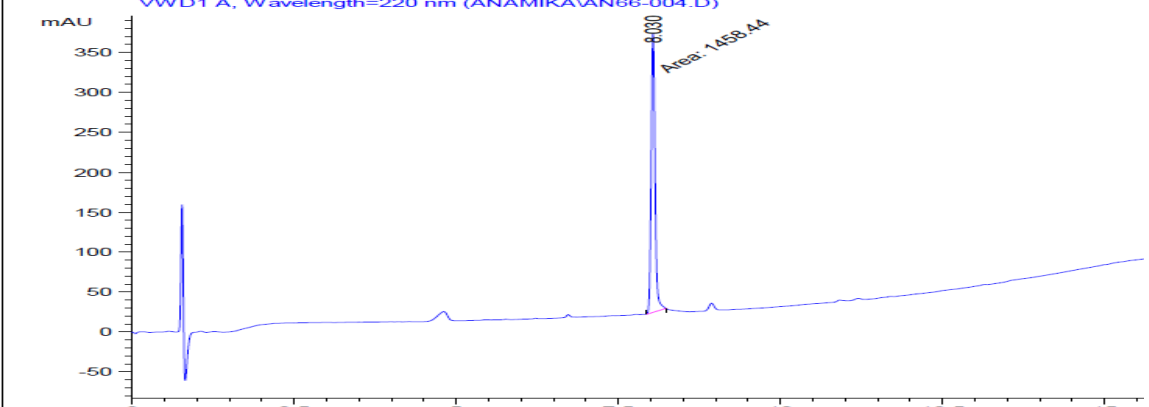
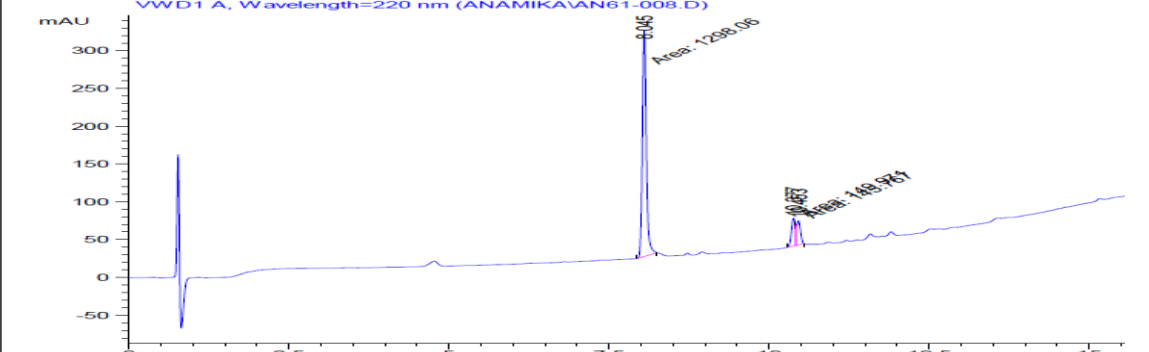
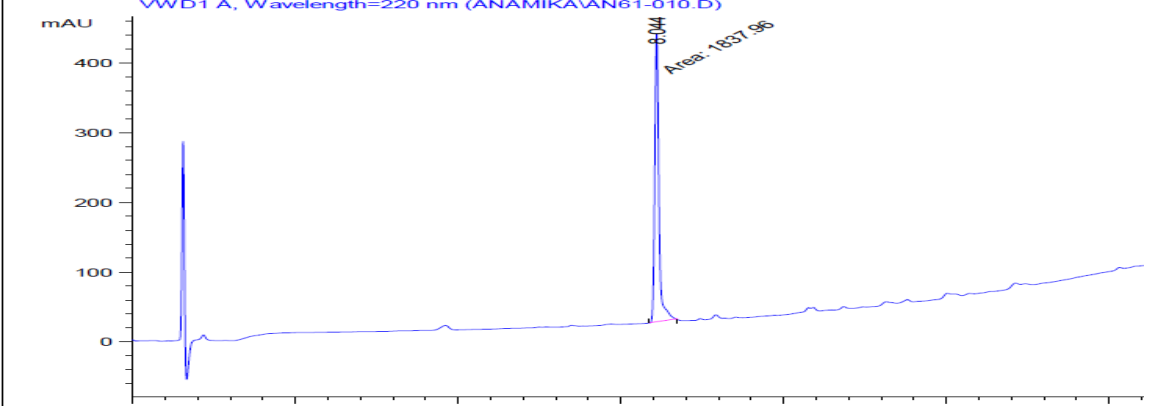
## ACID LABILITY STUDIES OF Fmoc-Ser(Thp)-OH

1. 1 mg of compound was weighed in vial.
2. Added 200  $\mu$ L of cocktail.
3. After 15 min, 30 min and 60 min 20  $\mu$ L was taken, diluted with acetonitrile (400  $\mu$ L) and injected in HPLC (5:95 gradient).

Cocktail	Min	CHROMATOGRAM
1:99 (TFA:DCM)	15	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN61-001.D)</p>
	30	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN61-002.D)</p>
	60	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN61-003.D)</p>
2:98	15	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\SERINE\AN60-011.D)</p>



	30	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\SERINEVAN60-013.D)</p> <p>8.030 Area: 2184.73</p> <p>10.360 Area: 720.797</p>
	60	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\SERINEVAN60-014.D)</p> <p>8.029 Area: 3519.34</p> <p>10.413 Area: 141.586</p>
2:0.5:97.5 (TFA:H <sub>2</sub> O: DCM)	15	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN66-001.D)</p> <p>8.038 Area: 2402.9</p> <p>10.445 Area: 154.381</p>
	30	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN66-002.D)</p> <p>8.025 Area: 2336.01</p> <p>10.430 Area: 108.846</p>

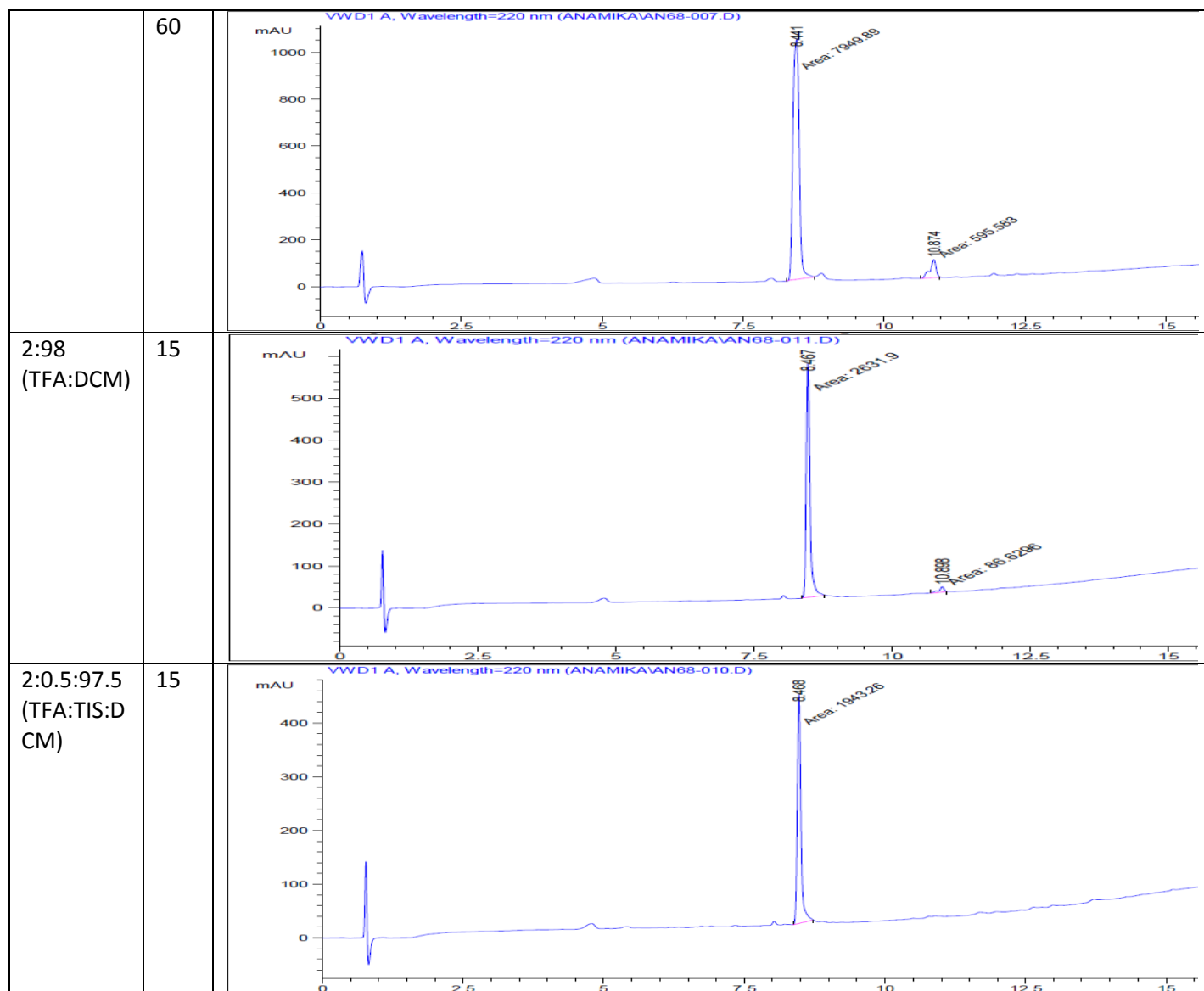
	60	 <p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN66-003.D)</p> <p>mAU</p> <p>500 400 300 200 100 0</p> <p>0 2.5 5 7.5 10 12.5 15</p> <p>8.004 Area: 2440.28</p>
2:0.5:97.5 (TFA:TIS:D CM)	15	 <p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN66-004.D)</p> <p>mAU</p> <p>350 300 250 200 150 100 50 0 -50</p> <p>0 2.5 5 7.5 10 12.5 15</p> <p>8.000 Area: 1458.44</p>
5:95 (TFA:DCM)	15	 <p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN61-008.D)</p> <p>mAU</p> <p>300 250 200 150 100 50 0 -50</p> <p>0 2.5 5 7.5 10 12.5 15</p> <p>8.046 Area: 1288.08</p> <p>10.983 Area: 149.974</p>
	30	 <p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN61-010.D)</p> <p>mAU</p> <p>400 300 200 100 0</p> <p>0 2.5 5 7.5 10 12.5 15</p> <p>8.044 Area: 1837.96</p>

<p>10:90 (TFA:DCM)</p>	<p>15</p>	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN61-006.D)</p>
	<p>30</p>	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN61-005.D)</p>
<p>10:2:88 (TFA:H<sub>2</sub>O: DCM)</p>	<p>15</p>	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN61-007.D)</p>
<p>10:2:88 (TFA:TIS:D CM)</p>	<p>15</p>	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN61-009.D)</p>

## ACID LABILITY STUDIES OF Fmoc-Thr(Thp)-OH

1. 1 mg of compound was weighed in vial.
2. Added 200  $\mu$ L of cocktail.
3. After 15 min, 30 min and 60 min 20  $\mu$ L was taken, diluted with acetonitrile (400  $\mu$ L) and injected in HPLC (5:95 gradient).

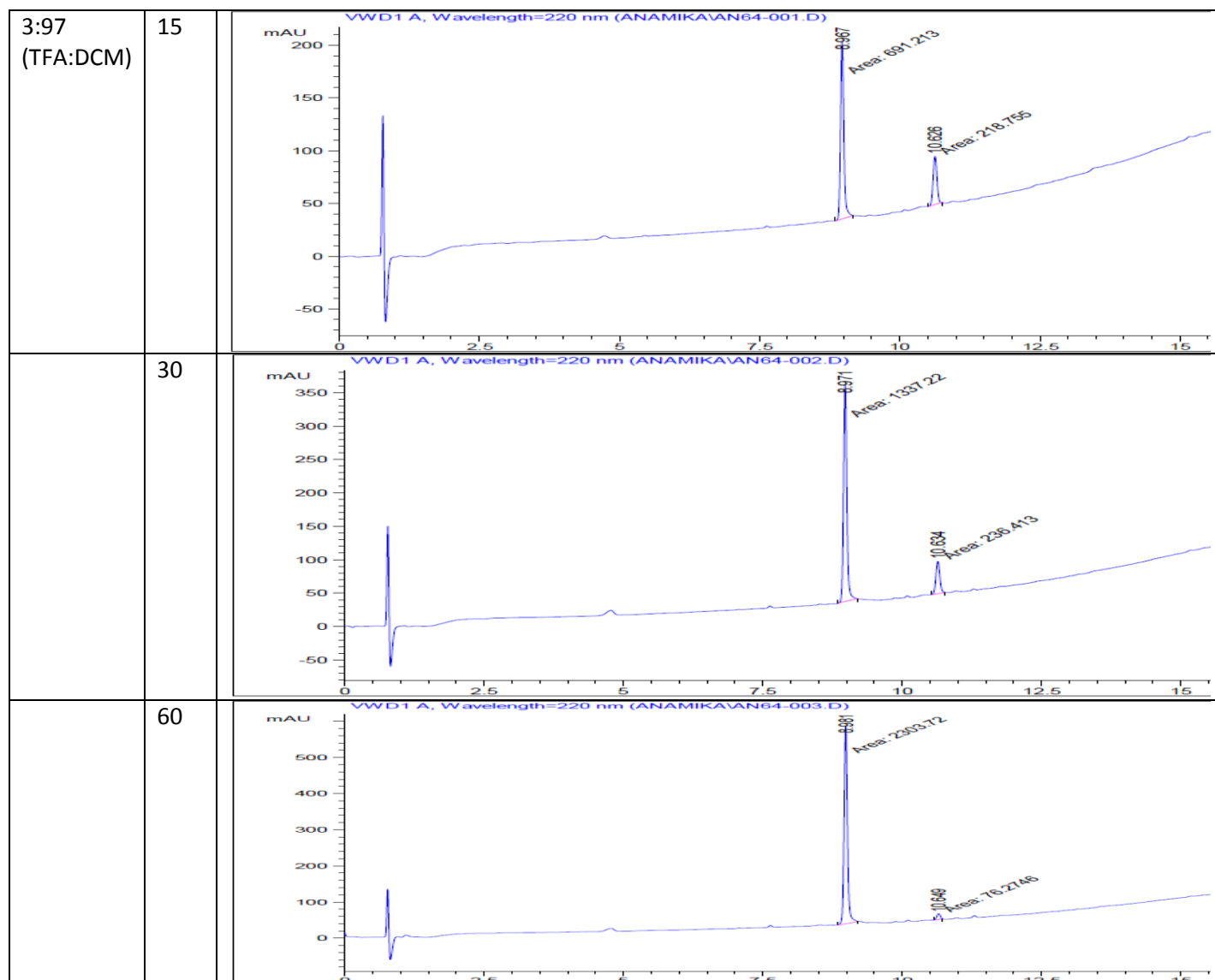
Cocktail	Mi n	CHROMATOGRAM
Fmoc-Thr-OH		<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN68-002.D)</p> <p>8.473 Area: 9853.88</p>
1:99 (TFA:DCM)	15	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN68-005.D)</p> <p>8.423 Area: 8208.73</p> <p>10.858 Area: 1803</p>
	30	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN68-006.D)</p> <p>8.413 Area: 5265.4</p> <p>10.852 Area: 1221.05</p>



### Synthesis and acid lability studies of Fmoc-Ala-Ser(Thp)-Leu-OH

1. CTC resin was used for synthesis of Fmoc-Ala-Ser(Thp)-Leu-OH using DIPCDI/Oxyma Pure as coupling reagent.
2. Peptide was used to study acid lability studies.
3. 5 mg of resin was weighed in vial.
4. Added 500  $\mu$ L of cocktail.
5. After 15 min, 30 min and 60 min 20  $\mu$ L was taken, diluted with acetonitrile (400  $\mu$ L) and injected in HPLC (5:95 gradient).

<p>2:0.5:97.5 (TFA:H<sub>2</sub>O: DCM)</p>	<p>15</p>	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN66-007.D)</p> <p>0.981 Area: 17538.16</p> <p>10.635 Area: 104.891</p>
	<p>30</p>	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN66-008.D)</p> <p>0.994 Area: 2892.25</p> <p>10.647 Area: 114.949</p>
	<p>60</p>	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN66-009.D)</p> <p>0.978 Area: 2891.08</p> <p>10.636 Area: 106.532</p>
<p>2:0.5:97.5 (TFA:TIS:D CM)</p>	<p>15</p>	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN66-013.D)</p> <p>0.973 Area: 1015.5</p>



### Synthesis and acid lability studies of Fmoc-Ala-Thr(Thp)-Leu-OH

1. CTC resin was used for synthesis of Fmoc-Ala-Thr(Thp)-Leu-OH using DIPCDI/Oxyma Pure as coupling reagent.
2. Peptide was used to study acid lability studies.
3. 5 mg of resin was weighed in vial.
4. Added 500  $\mu$ L of cocktail.
5. After 15 min, 30 min and 60 min 20  $\mu$ L was taken, diluted with acetonitrile (400  $\mu$ L) and injected in HPLC (5:95 gradient).

Cocktail	Min	CHROMATOGRAM
1:99 (TFA:DCM)	15	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN69-001.D)</p> <p>9.277 Area: 1557.21</p> <p>11.182 Area: 585.452</p>
	30	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN69-003.D)</p> <p>9.287 Area: 2310.96</p> <p>11.204 Area: 231.981</p>
	60	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN69-004.D)</p> <p>9.293 Area: 2697.13</p> <p>11.188 Area: 183.143</p>
2:98 (TFA:DCM)	15	<p>VWD1 A, Wavelength=220 nm (ANAMIKA\AN69-002.D)</p> <p>9.288 Area: 2322.96</p>