

Supplementary Materials

Figure S1
Checkerboard assays

Compound ID	Inhibition (%) Medians	Antimicrobial compound activity removed	AMK IC50/80	AMK Fold Potentiation	EC 2/3-Fold IC50/80																																																																																																																																																															
2700.001	<table><tr><th colspan="7">Amk (µg/ml)</th></tr><tr><th>µM</th><th>0</th><th>4</th><th>8</th><th>16</th><th>32</th><th>64</th></tr><tr><td>24.0</td><td>6.5%</td><td>84.6%</td><td>87.0%</td><td>90.1%</td><td>76.6%</td><td></td></tr><tr><td>16.0</td><td>13.1%</td><td>64.3%</td><td>85.2%</td><td>86.9%</td><td>84.2%</td><td></td></tr><tr><td>8.0</td><td>13.8%</td><td>31.7%</td><td>44.7%</td><td>80.0%</td><td>80.9%</td><td></td></tr><tr><td>4.0</td><td>14.2%</td><td>20.8%</td><td>29.8%</td><td>47.1%</td><td>75.9%</td><td></td></tr><tr><td>2.0</td><td>8.8%</td><td>14.4%</td><td>18.7%</td><td>31.5%</td><td>83.3%</td><td></td></tr><tr><td>0.0</td><td>0.0%</td><td>2.5%</td><td>5.5%</td><td>10.9%</td><td>71.1%</td><td></td></tr></table>	Amk (µg/ml)							µM	0	4	8	16	32	64	24.0	6.5%	84.6%	87.0%	90.1%	76.6%		16.0	13.1%	64.3%	85.2%	86.9%	84.2%		8.0	13.8%	31.7%	44.7%	80.0%	80.9%		4.0	14.2%	20.8%	29.8%	47.1%	75.9%		2.0	8.8%	14.4%	18.7%	31.5%	83.3%		0.0	0.0%	2.5%	5.5%	10.9%	71.1%		<table><tr><th colspan="7">Amk (µg/ml)</th></tr><tr><th>µM</th><th>0</th><th>4</th><th>8</th><th>16</th><th>32</th><th>64</th></tr><tr><td>24.0</td><td>0.0%</td><td>83.6%</td><td>86.1%</td><td>89.4%</td><td>74.9%</td><td></td></tr><tr><td>16.0</td><td>0.0%</td><td>58.9%</td><td>83.0%</td><td>84.9%</td><td>81.8%</td><td></td></tr><tr><td>8.0</td><td>0.0%</td><td>20.8%</td><td>35.9%</td><td>76.7%</td><td>77.8%</td><td></td></tr><tr><td>4.0</td><td>0.0%</td><td>7.6%</td><td>18.2%</td><td>38.3%</td><td>71.9%</td><td></td></tr><tr><td>2.0</td><td>0.0%</td><td>6.2%</td><td>10.9%</td><td>24.9%</td><td>81.7%</td><td></td></tr><tr><td>0.0</td><td>0.0%</td><td>2.5%</td><td>5.5%</td><td>10.9%</td><td>71.1%</td><td></td></tr></table>	Amk (µg/ml)							µM	0	4	8	16	32	64	24.0	0.0%	83.6%	86.1%	89.4%	74.9%		16.0	0.0%	58.9%	83.0%	84.9%	81.8%		8.0	0.0%	20.8%	35.9%	76.7%	77.8%		4.0	0.0%	7.6%	18.2%	38.3%	71.9%		2.0	0.0%	6.2%	10.9%	24.9%	81.7%		0.0	0.0%	2.5%	5.5%	10.9%	71.1%		<table><tr><th colspan="4">Effective Concentration</th><th colspan="4">Fold Potentiation</th></tr><tr><th>50%</th><th>80%</th><th>50%</th><th>80%</th></tr><tr><td>2.4</td><td>3.8</td><td>11.0</td><td>8.4</td></tr><tr><td>3.4</td><td>7.5</td><td>7.8</td><td>4.3</td></tr><tr><td>10.8</td><td>> 32</td><td>2.5</td><td>1.0</td></tr><tr><td>21.6</td><td>> 32</td><td>1.2</td><td>1.0</td></tr><tr><td>23.1</td><td>31.5</td><td>1.1</td><td>1.0</td></tr><tr><td>26.4</td><td>> 32</td><td>1.0</td><td>1.0</td></tr></table>	Effective Concentration				Fold Potentiation				50%	80%	50%	80%	2.4	3.8	11.0	8.4	3.4	7.5	7.8	4.3	10.8	> 32	2.5	1.0	21.6	> 32	1.2	1.0	23.1	31.5	1.1	1.0	26.4	> 32	1.0	1.0	<table><tr><th colspan="2">2-Fold</th><th colspan="2">3-Fold</th></tr><tr><th>50%</th><th>80%</th><th>50%</th><th>80%</th></tr><tr><td>6.5</td><td>10.4</td><td>8.8</td><td>12.9</td></tr></table>	2-Fold		3-Fold		50%	80%	50%	80%	6.5	10.4	8.8	12.9
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Figure S1. *Checkerboard assays*. The leftmost column shows the measured values (medians %, $n \geq 6$). The adjacent column shows the adjusted values after removing any antimicrobial activity exerted by the testing compound alone. Adjustment was carried out as described in Materials and Methods. The third column shows the amikacin inhibitory concentration 50 and 80% and the fold potentiation at the corresponding concentrations of compound. The fold potentiation was calculated dividing the IC50 and IC80 values in the absence of compound by the values obtained in the presence of each compound concentration. In those cases where the IC50 or IC80 values were >32, the value used for the calculation was 32. The rightmost column indicates the compound concentration needed for 2- or 3-fold potentiation of both the 50% and 80% inhibitory dose points.

Figure S2
Molecular docking

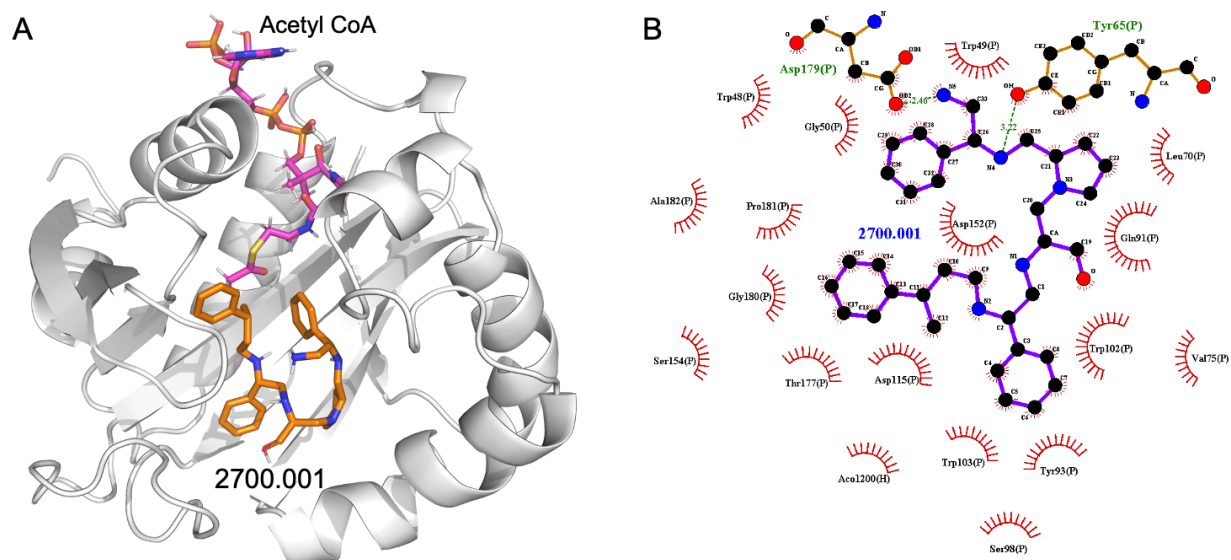


Figure S2a. Complex between compound 2700.001 and AAC(6')-Ib. (A) The complex of 2700.001 and AAC(6')-Ib obtained from molecular docking. The bound acetyl CoA is also shown. (B) Interaction map of the ligand in its binding site of the AAC(6')-Ib receptor. The map shows that the phenol group of Tyr65 forms a hydrogen bond with 2700.001. The primary amine of 2700.001 forms a hydrogen bond with Asp179. The pictures show the top-ranked, lowest energy conformation.

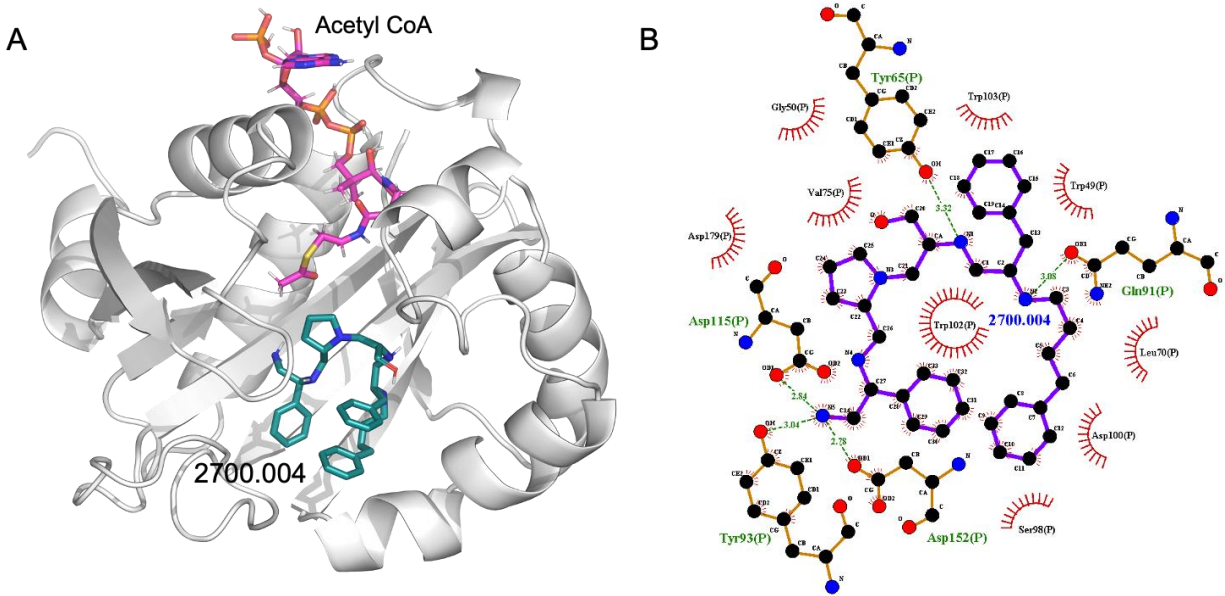


Figure S2b. *Complex between compound 2700.004 and AAC(6')-Ib.* (A) The complex of 2700.004 and AAC(6')-Ib obtained from molecular docking. The bound acetyl CoA is also shown. The figure shows a potential for intramolecular pi stacking between two phenol groups. (B) Interaction map of the ligand in its binding site of the AAC(6')-Ib receptor. The map shows that 2700.004 forms several bonds with the receptor, particularly the primary amine, which forms hydrogen bonds with Asp115, Asp152, and the phenol group of Tyr93. Gln91 and the phenol group of Tyr65 also form hydrogen bonds with the other side of the molecule.

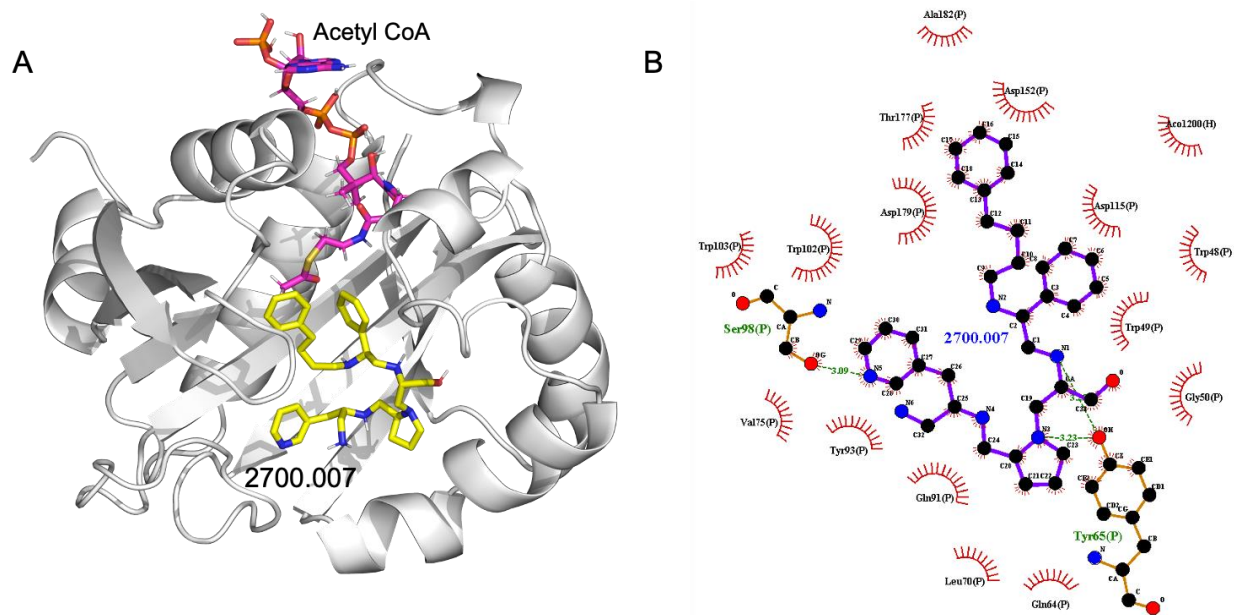


Figure S2c. Complex between compound 2700.007 and AAC(6')-Ib. (A) The complex of 2700.007 and AAC(6')-Ib obtained from molecular docking. The bound acetyl CoA is also shown. (B) Interaction map of the ligand in its binding site of the AAC(6')-Ib receptor. The map shows that although there is conformational change, 2700.007 maintains a hydrogen bond with the phenol group of Tyr65 and hydrogen bonding with Ser98.

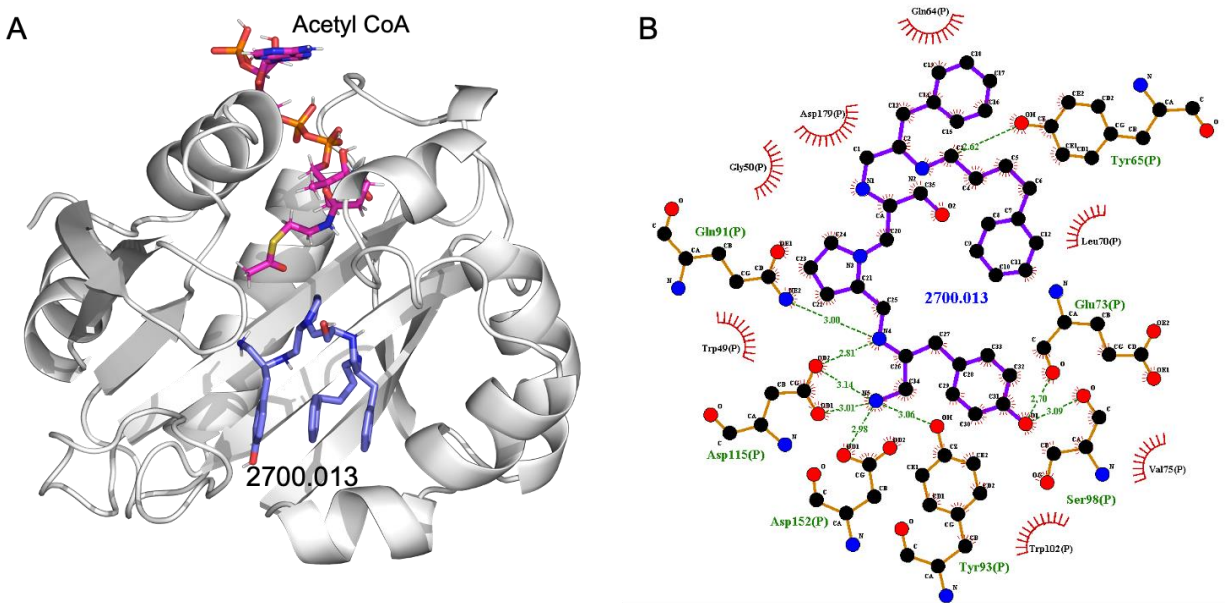


Figure S2d. *Complex between compound 2700.013 and AAC(6')-Ib.* (A) The complex of 2700.013 and AAC(6')-Ib obtained from molecular docking. The bound acetyl CoA is also shown. (B) Interaction map of the ligand in its binding site of the AAC(6')-Ib receptor. The map shows 2700.013 forms hydrogen bonds through its primary amine with Tyr 93, Asp115, and Asp152. Ser98 and Glu73 shows hydroxyl group functionality while the Ser98 and the phenol group of Tyr65 show hydrogen bonding with 2700.013.

Figure S3
Synthetic scheme

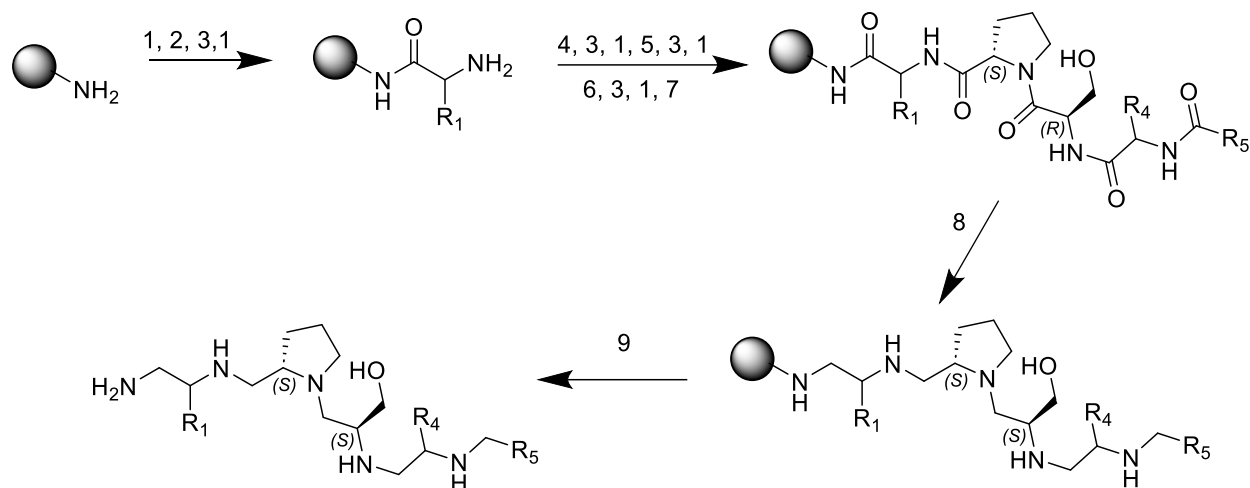


Figure S3. *Synthetic method.* Starting from mBHA resin. 1) 5% DIEA/DCM. 2) 6-fold excess Boc-Amino acid (R₁), DIC, and HOBt in 0.1M DMF 2hr. 3) 55% TFA/DCM. 4) 6-fold excess Boc-L-Pro-OH, DIC, and HOBt in 0.1M DMF 2hr. 5) 6-fold excess Boc-D-Ser-OH, DIC, and HOBt in 0.1M DMF 2hr. 6) 6-fold excess Boc-Amino acid (R₄), DIC, and HOBt in 0.1M DMF 2hr. 7) 10-fold excess Carboxylic acid (R₅), DIC, and HOBt in 0.1M DMF 2hr. 8) 40-fold excess of 0.1M BH₃/THF 60°C 72hr followed by Piperidine 60°C 24hr. 9) HF 0°C 7hr.

Figure S4

Compound synthesis, purification, and characterization.

All reagents were commercially available and used without further purification. The final compounds were purified using preparative HPLC with a dual pump Shimadzu LC-20AB system equipped with a Luna C18 preparative column (21.5 x 150 mm, 5 micron) at $\lambda = 214$ nm, with a mobile phase of (A) H₂O (+0.1% trifluoroacetic acid)/(B) acetonitrile (ACN) (+0.1% trifluoroacetic acid) at a flow rate of 15 mL/min; gradients varied by compound based on hydrophobicity. The purities of synthesized compounds were confirmed to be greater than 90% by liquid chromatography and mass spectrometry on a Shimadzu LCMS-2020 instrument with ESI Mass Spec and SPD-20A Liquid Chromatograph with a mobile phase of (A) H₂O (+0.1% formic acid)/(B) ACN (+0.1% formic acid) (5-95% over 6 min with a 4 min rinse).

Compound **2700.001** **(2S)-3-((2S)-2-(((2-amino-1-phenylethyl)amino)methyl)pyrrolidin-1-yl)-2-((2-phenyl-2-((3-phenylbutyl)amino)ethyl)amino)propan-1-ol** Using General Scheme (scheme 1) for the synthesis of pentamines, compound 2700.001 was synthesized using the following reagents: (100mg) MBHA resin starting material, Boc-L-Phenylglycine-OH (R₁), Boc-L-Phenylglycine-OH (R₄), and 3-Phenylbutyric Acid (R₅). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/2, 2/2, 5/5, 40/20, 43/98, 45/98. **Isolated Mass** 4.8mg, **m/z** calculated C₃₄H₄₉N₅O [M+H]⁺ 543.8, found 544.45 (MS ESI) **Purity** LC-MS: 91.20% (214 nm, peak area).

Compound **2700.002** **(2S)-3-((S)-2-(((S)-1-amino-3-phenylpropan-2-yl)amino)methyl)pyrrolidin-1-yl)-2-((2-phenyl-2-((3-phenylbutyl)amino)ethyl)amino)propan-1-ol**. Using General Scheme (scheme 1) for the synthesis of pentamines, compound 2700.002 was synthesized using the following reagents: (100mg) MBHA resin starting material, Boc-L-Phenylalanine-OH (R₁), Boc-L-Phenylglycine-OH (R₄), and 3-Phenylbutyric Acid (R₅). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/2, 2/2, 5/5, 40/20, 43/98, 45/98. **Isolated Mass** 8.6mg, **m/z** calculated C₃₅H₅₁N₅O [M+H]⁺ 557.83, found 558.45 (MS ESI) **Purity** LC-MS: 90.10% (214 nm, peak area).

Compound **2700.003** **(2S)-3-((2S)-2-(((2-amino-1-phenylethyl)amino)methyl)pyrrolidin-1-yl)-2-((2-phenyl-2-((4-phenylbutyl)amino)ethyl)amino)propan-1-ol**. Using General Scheme (scheme 1) for the synthesis of pentamines, compound 2700.003 was synthesized using the following reagents: (100mg) MBHA resin starting material, Boc-L-Phenylglycine-OH (R₁), Boc-L-Phenylglycine-OH (R₄), and 4-Phenylbutyric Acid (R₅). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/2, 2/2, 5/5, 40/20, 43/98, 45/98. **Isolated Mass** 5.1mg, **m/z** calculated C₃₄H₄₉N₅ [M+H]⁺ 543.8, found 544.45 (MS ESI) **Purity** LC-MS: 90.01% (214 nm, peak area).

Figure S4 continued

Compound **2700.004** **(2S)-3-((2S)-2-(((2-amino-1-phenylethyl)amino)methyl)pyrrolidin-1-yl)-2-(((S)-3-phenyl-2-((4-phenylbutyl)amino)propyl)amino)propan-1-ol**. Using General Scheme (scheme 1) for the synthesis of pentamines, compound 2700.004 was synthesized using the following reagents: (100mg) MBHA resin starting material, Boc-L-Phenylglycine-OH (R_1), Boc-L-Phenylalanine-OH (R_4), and 4-Phenylbutyric Acid (R_5). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/2, 2/2, 5/5, 40/20, 43/98, 45/98. **Isolated Mass** 5.5mg, **m/z** calculated $C_{35}H_{51}N_5O$ $[M+H]^+$ 557.83, found 558.4 (MS ESI) **Purity** LC-MS: 92.19% (214 nm, peak area).

Compound **2700.005** **(2S)-3-((2S)-2-(((2-amino-1-phenylethyl)amino)methyl)pyrrolidin-1-yl)-2-(((2S)-3-phenyl-2-((3-phenylbutyl)amino)propyl)amino)propan-1-ol**. Using General Scheme (scheme 1) for the synthesis of pentamines, compound 2700.005 was synthesized using the following reagents: (100mg) MBHA resin starting material, Boc-L-Phenylglycine-OH (R_1), Boc-L-Phenylalanine-OH (R_4), and 3-Phenylbutyric Acid (R_5). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/2, 2/2, 5/5, 40/20, 43/98, 45/98. **Isolated Mass** 5.6mg, **m/z** calculated $C_{35}H_{51}N_5O$ $[M+H]^+$ 557.83, found 558.4 (MS ESI) **Purity** LC-MS: 90.59% (214 nm, peak area).

Compound **2700.006** **(2S)-3-((S)-2-(((S)-1-amino-3-(pyridin-3-yl)propan-2-yl)amino)methyl)pyrrolidin-1-yl)-2-((2-phenyl-2-((3-phenylbutyl)amino)ethyl)amino)propan-1-ol**. Using General Scheme (scheme 1) for the synthesis of pentamines, compound 2700.006 was synthesized using the following reagents: (100mg) MBHA resin starting material, Boc-L-Alanine(3-pyridyl)-OH (R_1), Boc-L-Phenylglycine-OH (R_4), and 3-Phenylbutyric Acid (R_5). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/2, 2/2, 5/5, 40/20, 43/98, 45/98. **Isolated Mass** 5.6mg, **m/z** calculated $C_{34}H_{50}N_6O$ $[M+H]^+$ 558.82, found 559.45 (MS ESI) **Purity** LC-MS: 92.18% (214 nm, peak area).

Compound **2700.007** **(2S)-3-((S)-2-(((S)-1-amino-3-(pyridin-3-yl)propan-2-yl)amino)methyl)pyrrolidin-1-yl)-2-((2-phenyl-2-((4-phenylbutyl)amino)ethyl)amino)propan-1-ol**. Using General Scheme (scheme 1) for the synthesis of pentamines, compound 2700.007 was synthesized using the following reagents: (100mg) MBHA resin starting material, Boc-L-Alanine(3-pyridyl)-OH (R_1), Boc-L-Phenylglycine-OH (R_4), and 4-Phenylbutyric Acid (R_5). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/2, 2/2, 5/5, 40/20, 43/98, 45/98. **Isolated Mass** 6.4mg, **m/z** calculated $C_{34}H_{50}N_6O$ $[M+H]^+$ 558.82, found 559.4 (MS ESI) **Purity** LC-MS: 84.46% (214 nm, peak area).

Figure S4 continued

Compound 2700.008 (S)-3-((S)-2-(((S)-1-amino-3-(pyridin-3-yl)propan-2-yl)amino)methyl)pyrrolidine-1-yl)-2-(((S)-3-phenyl-2-((4-phenylbutyl)amino)propyl)amino)propan-1-ol. Using General Scheme (scheme 1) for the synthesis of pentamines, compound 2700.008 was synthesized using the following reagents: (100mg) MBHA resin starting material, Boc-L-Alanine(3-pyridyl)-OH (R₁), Boc-L-Phenylalanine-OH (R₄), and 4-Phenylbutyric Acid (R₅). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/2, 2/2, 5/5, 40/20, 43/98, 45/98. **Isolated Mass** 6.4mg, **m/z** calculated C₃₅H₅₂N₆O [M+H]⁺ 572.84, found 573.45 (MS ESI) **Purity** LC-MS: 96.22% (214 nm, peak area).

Compound 2700.009 (2S)-3-((S)-2-(((S)-1-amino-3-phenylpropan-2-yl)amino)methyl)pyrrolidin-1-yl)-2-((2-phenyl-2-((4-phenylbutyl)amino)ethyl)amino)propan-1-ol. Using General Scheme (scheme 1) for the synthesis of pentamines, compound 2700.009 was synthesized using the following reagents: (100mg) MBHA resin starting material, Boc-L-Phenylalanine-OH (R₁), Boc-L-Phenylglycine-OH (R₄), and 4-Phenylbutyric Acid (R₅). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/2, 2/2, 5/5, 40/20, 43/98, 45/98. **Isolated Mass** 6.1mg, **m/z** calculated C₃₅H₅₁N₅O [M+H]⁺ 557.83, found 558.4 (MS ESI) **Purity** LC-MS: 92.26% (214 nm, peak area).

Compound 2700.010 (S)-3-((S)-2-(((S)-1-amino-3-phenylpropan-2-yl)amino)methyl)pyrrolidin-1-yl)-2-(((S)-3-phenyl-2-((4-phenylbutyl)amino)propyl)amino)propan-1-ol. Using General Scheme (scheme 1) for the synthesis of pentamines, compound 2700.010 was synthesized using the following reagents: (100mg) MBHA resin starting material, Boc-L-Phenylalanine-OH (R₁), Boc-L-Phenylalanine-OH (R₄), and 4-Phenylbutyric Acid (R₅). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/2, 2/2, 5/5, 40/20, 43/98, 45/98. **Isolated Mass** 6.1mg, **m/z** calculated C₃₆H₅₃N₅O [M+H]⁺ 571.85, found 572.5 (MS ESI) **Purity** LC-MS: 93.97% (214 nm, peak area).

Compound 2700.011 4-((2S)-3-amino-2-(((2S)-1-((2S)-3-hydroxy-2-((2-phenyl-2-((3-phenylbutyl)amino)ethyl)amino)propyl)pyrrolidin-2-yl)methyl)amino)propyl)phenol. Using General Scheme (scheme 1) for the synthesis of pentamines, compound 2700.011 was synthesized using the following reagents: (100mg) MBHA resin starting material, Boc-L-Tyrosine-OH (R₁), Boc-L-Phenylglycine-OH (R₄), and 3-Phenylbutyric Acid (R₅). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/2, 2/2, 5/5, 40/20, 43/98, 45/98. **Isolated Mass** 5.6mg, **m/z** calculated C₃₅H₅₁N₅O₂ [M+H]⁺ 573.83, found 574.45 (MS ESI) **Purity** LC-MS: 92.79% (214 nm, peak area).

Figure S4 continued

Compound 2700.012 4-((2S)-3-amino-2-((((2S)-1-((2S)-3-hydroxy-2-((2-phenyl-2-((4-phenylbutyl)amino)ethyl)amino)propyl)pyrrolidin-2-yl)methyl)amino)propyl)phenol. Using General Scheme (scheme 1) for the synthesis of pentamines, compound 2700.012 was synthesized using the following reagents: (100mg) MBHA resin starting material, Boc-L-Tyrosine-OH (R₁), Boc-L-Phenylglycine-OH (R₄), and 4-Phenylbutyric Acid (R₅). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/2, 2/2, 5/5, 40/20, 43/98, 45/98. **Isolated Mass** 4.7mg, **m/z** calculated C₃₅H₅₁N₅O₂ [M+H]⁺ 573.83, found 574.45 (MS ESI) **Purity** LC-MS: 92.94% (214 nm, peak area).

Compound 2700.013 4-((S)-3-amino-2-((((S)-1-((S)-3-hydroxy-2-(((S)-3-phenyl-2-((4-phenylbutyl)amino)propyl)amino)propyl)pyrrolidin-2-yl)methyl)amino)propyl)phenol. Using General Scheme (scheme 1) for the synthesis of pentamines, compound 2700.013 was synthesized using the following reagents: (100mg) MBHA resin starting material, Boc-L-Tyrosine-OH (R₁), Boc-L-Phenylalanine-OH (R₄), and 4-Phenylbutyric Acid (R₅). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/2, 2/2, 5/5, 40/20, 43/98, 45/98. **Isolated Mass** 6.0mg, **m/z** calculated C₃₆H₅₃N₅O₂ [M+H]⁺ 587.85, found 588.25 (MS ESI) **Purity** LC-MS: 92.59% (214 nm, peak area).

Compound 2700.014 (2S)-3-((S)-2-((((R)-1-aminohexan-2-yl)amino)methyl)pyrrolidin-1-yl)-2-((2-phenyl-2-((4-phenylbutyl)amino)ethyl)amino)propan-1-ol. Using General Scheme (scheme 1) for the synthesis of pentamines, compound 2700.014 was synthesized using the following reagents: (100mg) MBHA resin starting material, Boc-D-Norleucine-OH (R₁), Boc-L-Phenylglycine-OH (R₄), and 4-Phenylbutyric Acid (R₅). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/2, 2/2, 5/5, 40/20, 43/98, 45/98. **Isolated Mass** 5.1mg, **m/z** calculated C₃₂H₅₃N₅O [M+H]⁺ 523.81, found 524.25 (MS ESI) **Purity** LC-MS: 76.03% (214 nm, peak area).

Compound 2700.015 (S)-3-((S)-2-((((R)-1-aminohexan-2-yl)amino)methyl)pyrrolidin-1-yl)-2-(((S)-3-phenyl-2-((4-phenylbutyl)amino)propyl)amino)propan-1-ol. Using General Scheme (scheme 1) for the synthesis of pentamines, compound 2700.015 was synthesized using the following reagents: (100mg) MBHA resin starting material, Boc-D-Norleucine-OH (R₁), and Boc-L-Phenylalanine-OH (R₄), and 4-Phenylbutyric Acid (R₅). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/2, 2/2, 5/5, 40/20, 43/98, 45/98. **Isolated Mass** 5.4mg, **m/z** calculated C₃₃H₅₅N₅O [M+H]⁺ 537.84, found 538.25 (MS ESI) **Purity** LC-MS: 82.97% (214 nm, peak area).

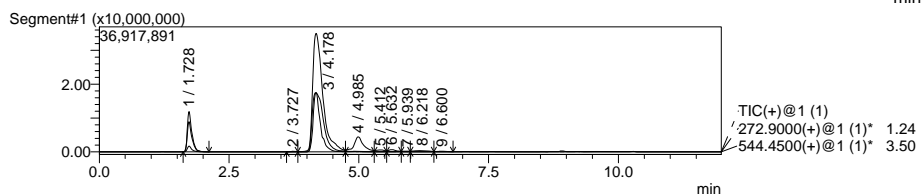
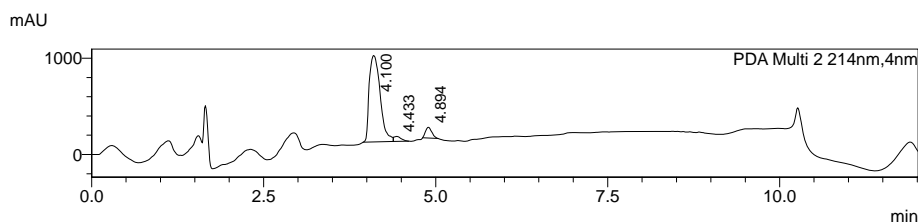
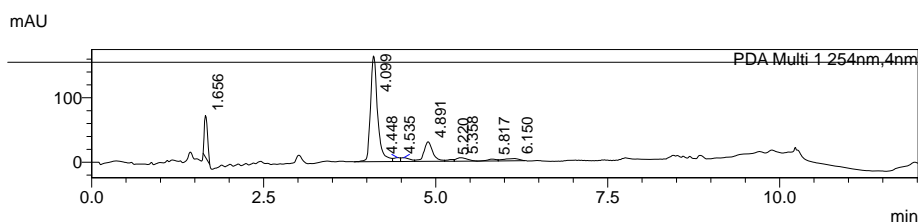
Figure S5
Liquid chromatography-mass spectrometry analysis
2700.001

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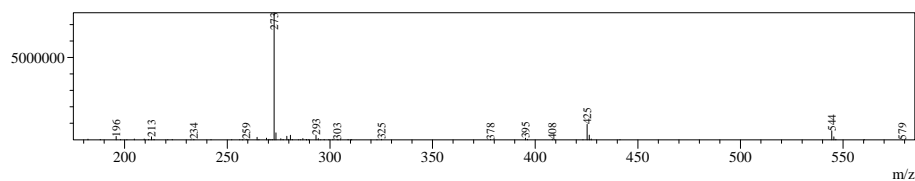
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Method Filename	: 6min 5-95, 1min clean(254)_(214) Col_1_LCMS3.lcm		
Batch Filename	: 080421.lcb		
Vial #	: 1-1		
Injection Volume	: 10 uL		
Date Acquired	: 8/4/2021 4:12:21 PM		
Date Processed	: 9/1/2021 3:08:34 PM		

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Spectrum Mode:Averaged 1.723-1.744(160-162)
BG Mode:Calc Segment 1 - Event 1



D:\2700 LCMS\2700-A1 peak 1 080421.lcd

Figure S5 continued
2700.002

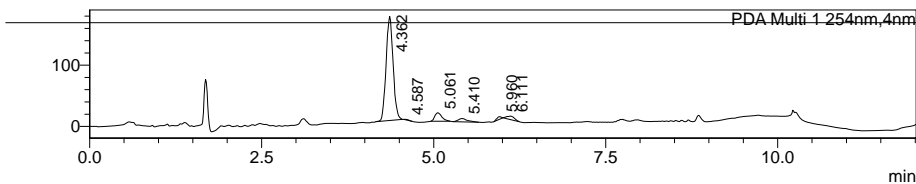
4/16/2024 2:38:15 PM Page 1 / 3

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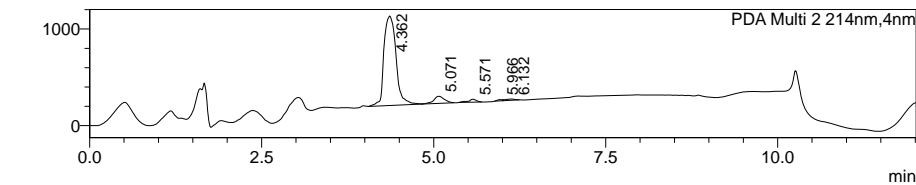
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Method Filename	: 6min 5-95, 1min clean(254)_(214) Col_1_LCMS3.lcm		
Batch Filename	: 080421.lcb		
Vial #	: 1-5		
Injection Volume	: 10 uL		
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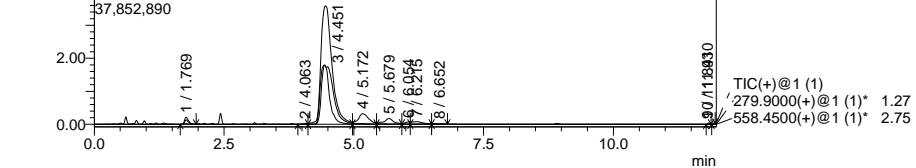
mAU



mAU

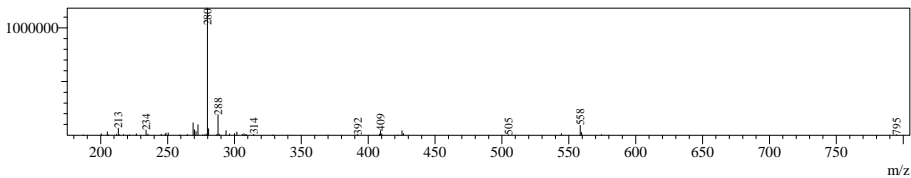


Segment#1 (x10,000,000)



MS Spectrum

Peak#1 R.Time:1.769(Scan#:164)
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Spectrum Mode:Averaged 1.755-1.777(163-165)
BG Mode:Calc Segment 1 - Event 1



D:\2700 LCMS\2700-A2 peak 1 080421.lcd

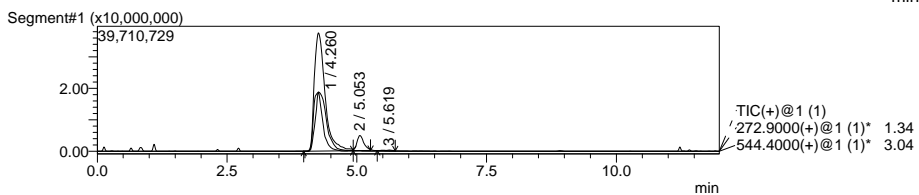
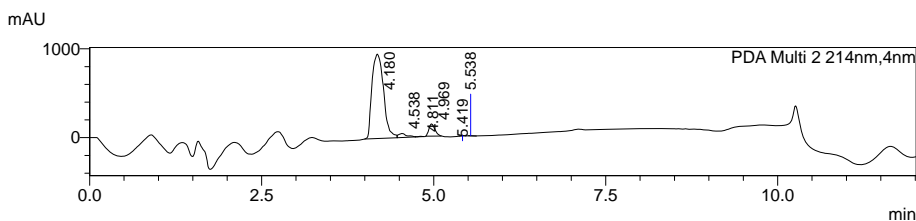
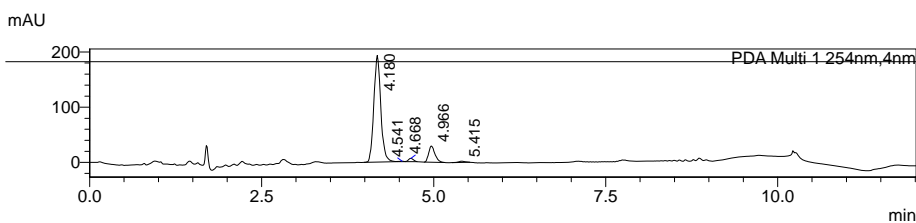
Figure S5 continued
2700.003

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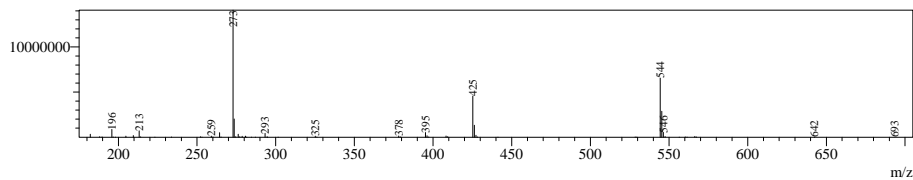
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Batch Filename	: 080421.lcb		
Vial #	: 1-7		
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Date Processed	: 4/16/2024 2:46:33 PM	Processed by	: System Administrator

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Peak#:1 R.Time:4.260(Scan#:394)
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BG Mode:Calc Segment 1 - Event 1

MS Spectrum



D:\2700 LCMS\2700-A3 peak 1 080421.lcd

Figure S5 continued
2700.004

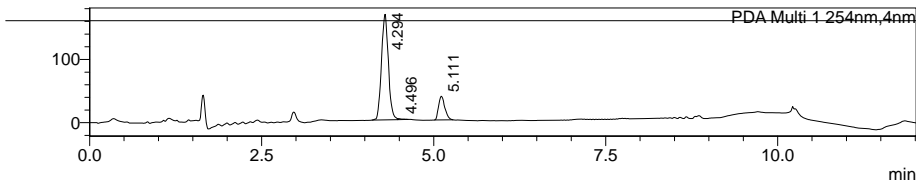
4/16/2024 2:50:36 PM Page 1 / 3

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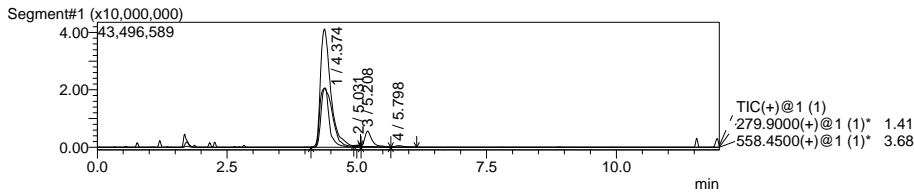
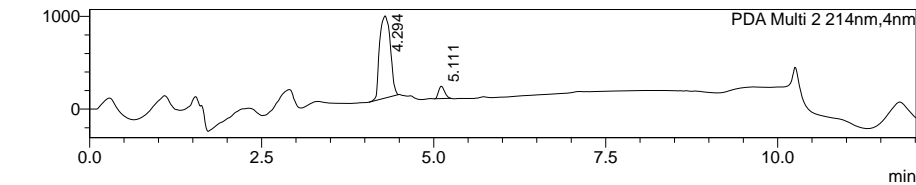
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Vial #	: 1-10		
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mAU

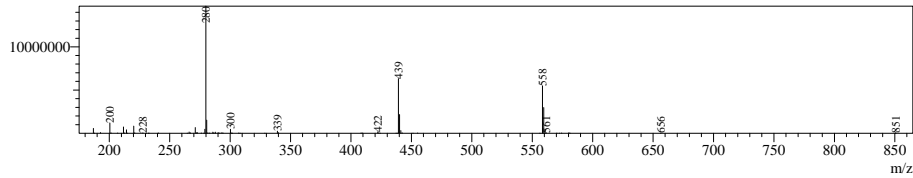


mAU



Peak#:1 R.Time:4.374(Scan#:405)
MassPeaks:77
Spectrum Mode:Averaged 4.366-4.388(404-406)
BG Mode:Calc Segment 1 - Event 1

MS Spectrum



D:\2700 LCMS\2700-A4 peak 1 080421.lcd

Figure S5 continued
2700.005

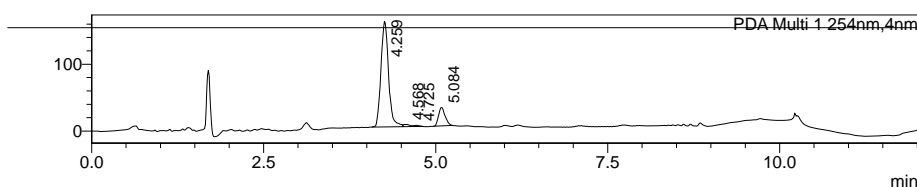
4/16/2024 2:58:55 PM Page 1 / 3

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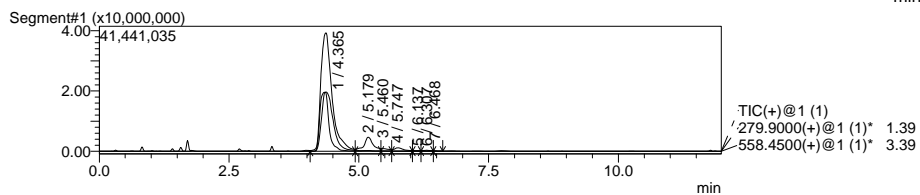
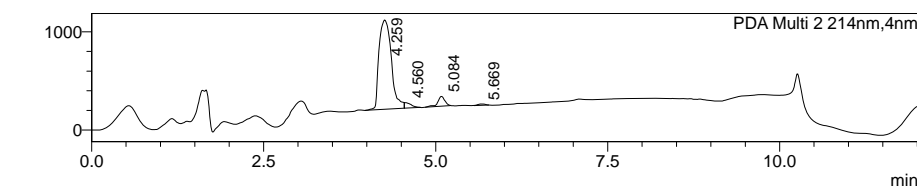
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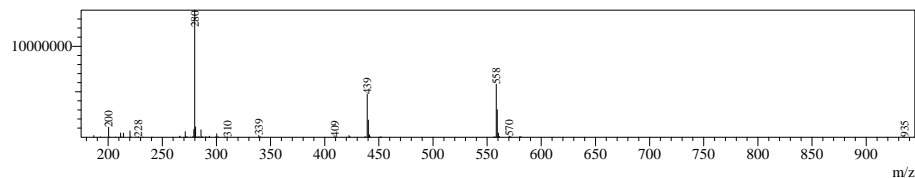


mAU



Peak#:1 R.Time:4.365(Scan#:404)
MassPeaks:74
Spectrum Mode:Averaged 4.355-4.377(403-405)
BG Mode:Calc Segment 1 - Event 1

MS Spectrum



D:\2700 LCMS\2700-A5 peak 1 080421.lcd

Figure S5 continued
2700.006

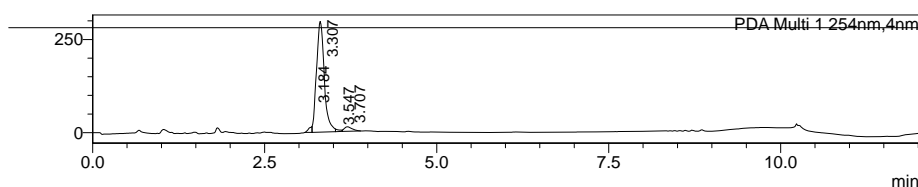
4/16/2024 3:03:09 PM Page 1 / 3

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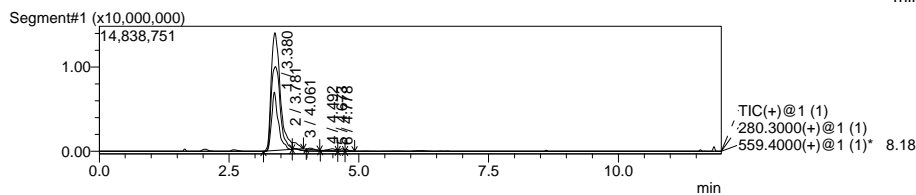
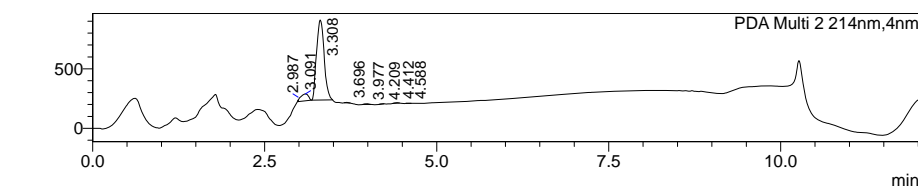
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Data Filename : 2700-A6 peak 1 080521.lcd
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Batch Filename : 080521.lcb
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mAU

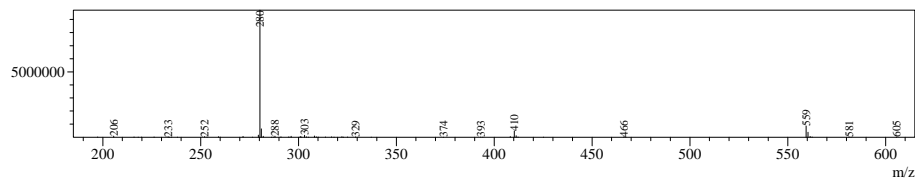


mAU



MS Spectrum

Peak#:1 R.Time:3.380(Scan#:313)
MassPeaks:69
Spectrum Mode:Averaged 3.369-3.391(312-314)
BG Mode:Calc Segment 1 - Event 1



D:\2700 LCMS\2700-A6 peak 1 080521.lcd

Figure S5 continued
2700.007

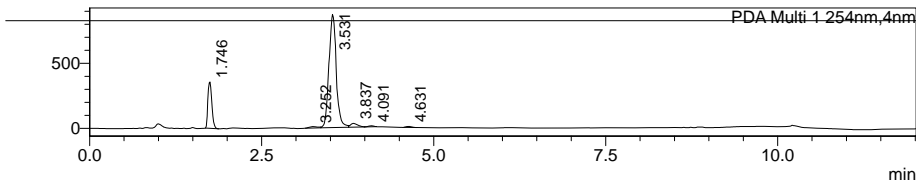
4/16/2024 3:07:22 PM Page 1 / 3

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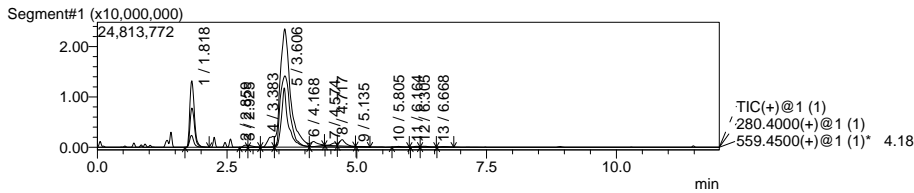
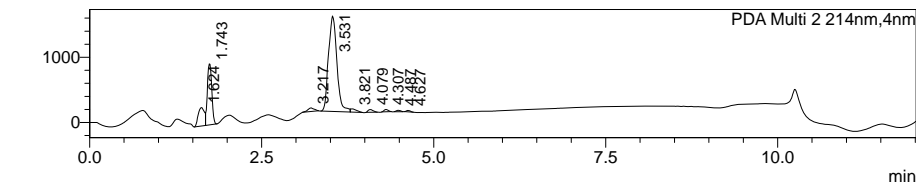
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Sample ID : 2700-A7 peak 1
Data Filename : 2700-A7 peak 1 080421b.lcd
Method Filename : 6min 5-95, 1min clean(254)_(214) Col_1_LCMS3.lcm
Batch Filename : 080421.lcb
Vial # : 1-19
Injection Volume : 10 uL
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mAU

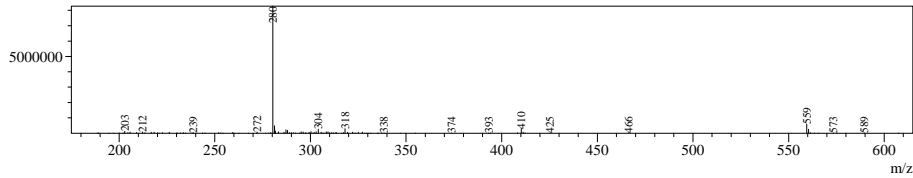


mAU



Peak#1 R.Time:1.818(Scan#:169)
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BG Mode:Calc Segment 1 - Event 1

MS Spectrum



D:\2700 LCMS\2700-A7 peak 1 080421b.lcd

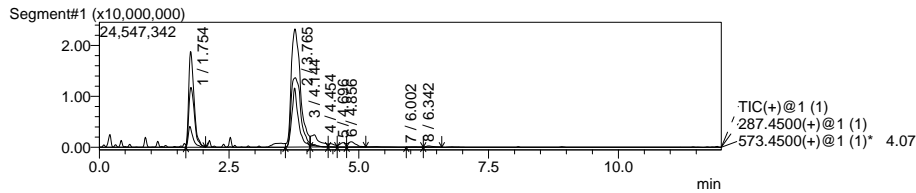
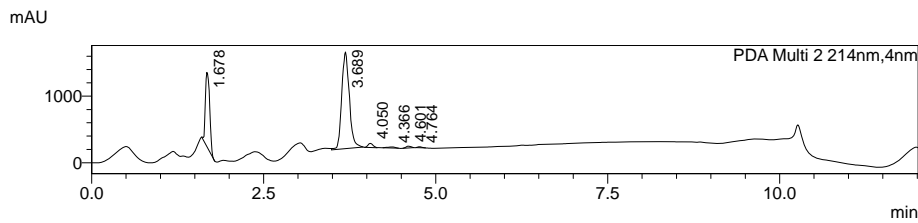
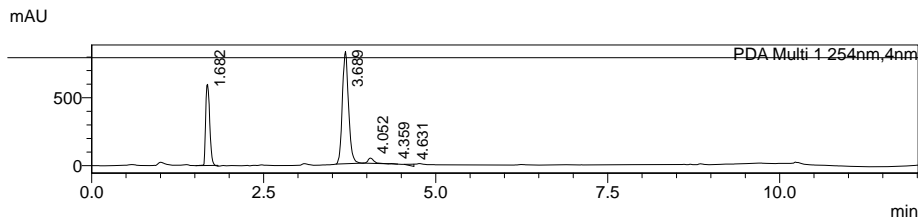
Figure S5 continued
2700.008

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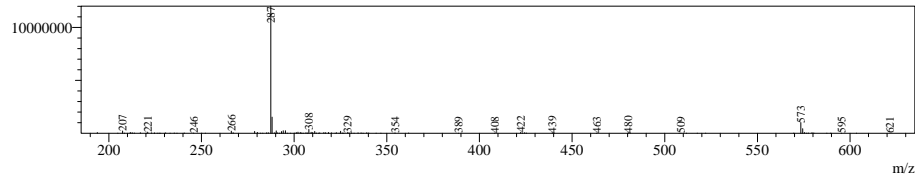
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Batch Filename	: 080421.lcb		
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BG Mode:Calc Segment 1 - Event 1

MS Spectrum



D:\2700 LCMS\2700-A8 peak 1 080421.lcd

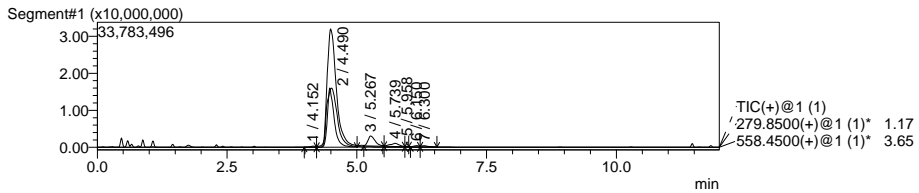
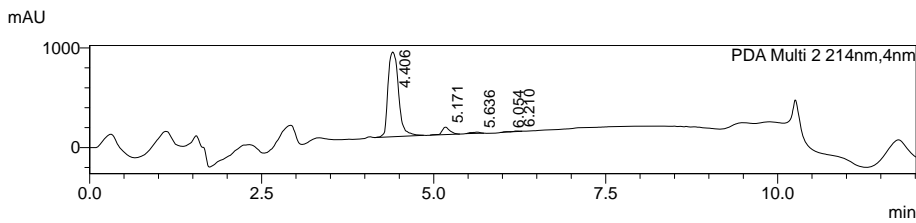
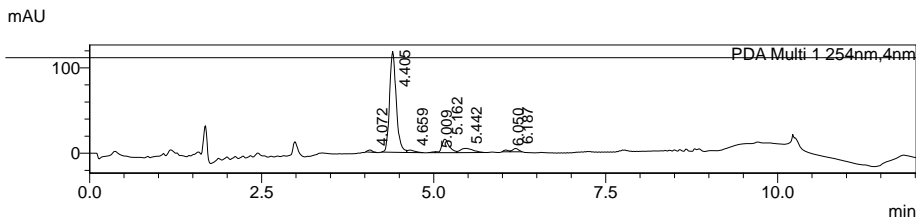
Figure S5 continued
2700.009

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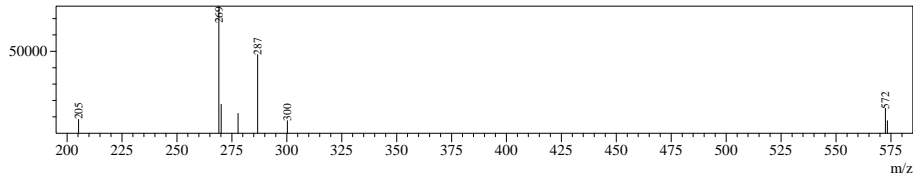
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BG Mode:Calc Segment 1 - Event 1

MS Spectrum



D:\2700 LCMS\2700-A9 peak 1 080421.lcd

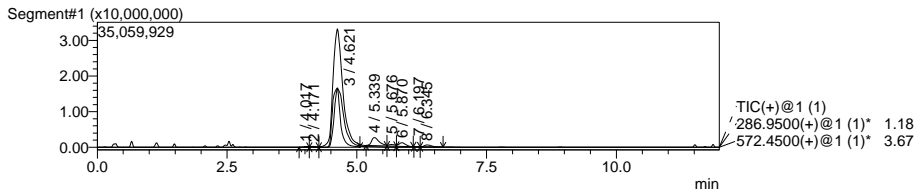
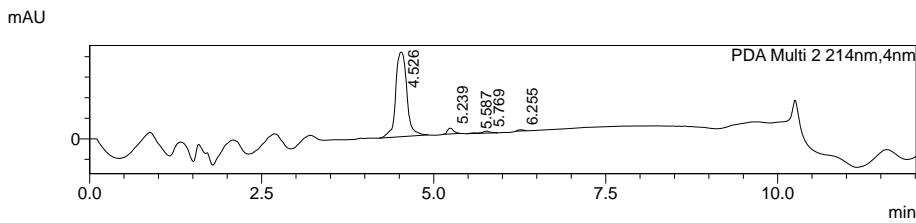
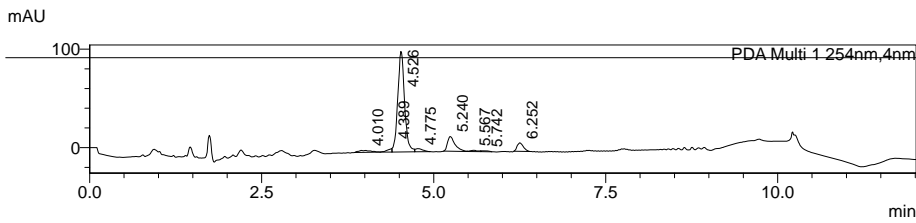
Figure S5 continued
2700.010

4/17/2024 12:41:29 PM Page 1 / 3

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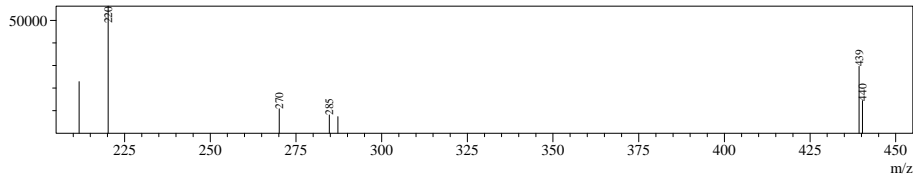
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Spectrum Mode:Averaged 4.008-4.030(371-373)
BG Mode:Calc Segment 1 - Event 1

MS Spectrum



D:\2700 LCMS\2700-A10 peak 1 080421.lcd

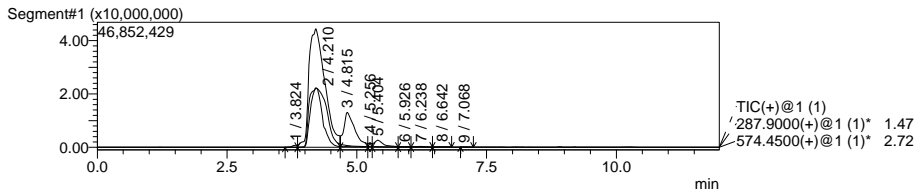
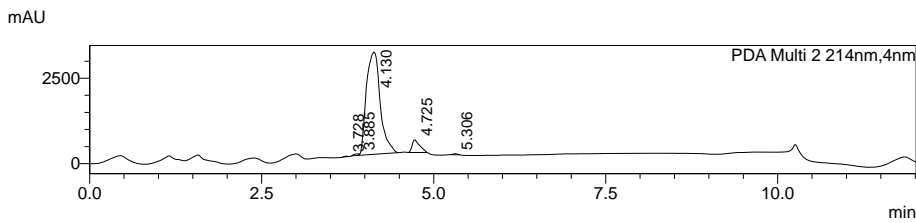
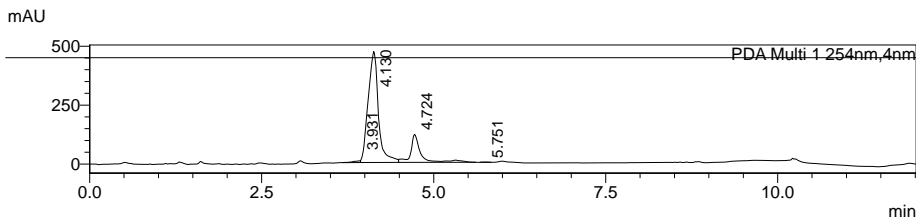
Figure S5 continued
2700.011

4/17/2024 2:26:12 PM Page 1 / 3

<Sample Information>

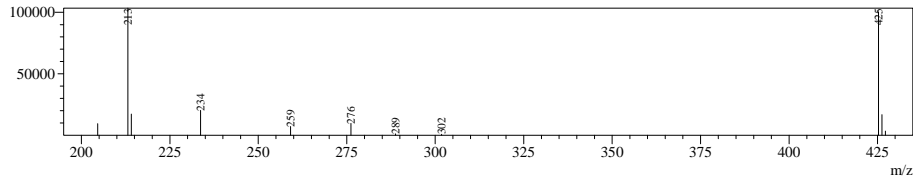
Sample Name	: 2700	Sample Type	: Unknown
Sample ID	: 2700-A11 peak 1		
Data Filename	: 2700-A11 peak 1 080521.lcd		
Method Filename	: 6min 5-95, 1min clean(254)_(214) Col_1_LCMS3.lcm		
Batch Filename	: 080421.lcb		
Vial #	: 1-26		
Injection Volume	: 5 uL		
Date Acquired	: 8/5/2021 1:11:03 PM	Acquired by	: System Administrator
Date Processed	: 9/7/2021 12:12:59 PM	Processed by	: System Administrator

<Chromatogram>



Peak#:1 R.Time:3.824(Scan#:354)
MassPeaks:11
Spectrum Mode:Averaged 3.813-3.835(353-355)
BG Mode:Calc Segment 1 - Event 1

MS Spectrum



D:\2700 LCMS\2700-A11 peak 1 080521.lcd

Figure S5 continued
2700.012

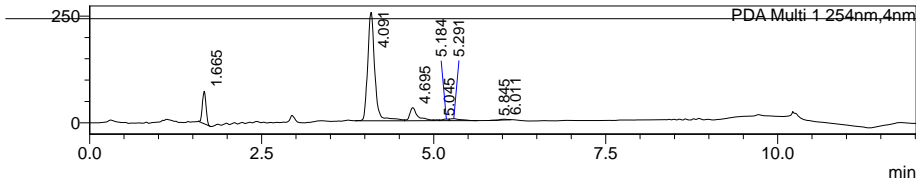
4/17/2024 2:58:14 PM Page 1 / 3

<Sample Information>

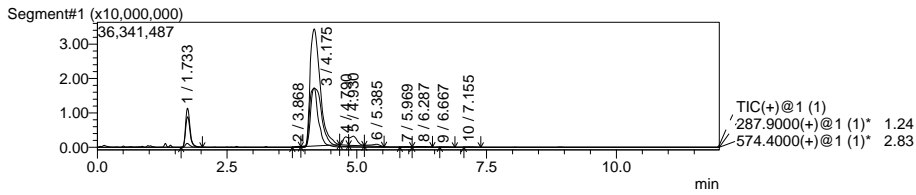
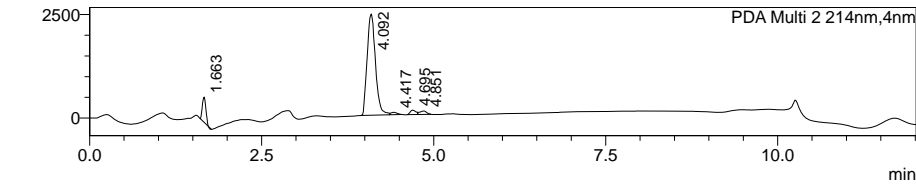
Sample Name	: 2700	Sample Type	: Unknown
Sample ID	: 2700-A12 peak 1		
Data Filename	: 2700-A12 peak 1 080421.lcd		
Method Filename	: 6min 5-95, 1min clean(254)_(214) Col_1_LCMS3.lcm		
Batch Filename	: 080421.lcb		
Vial #	: 1-29		
Injection Volume	: 10 uL		
Date Acquired	: 8/4/2021 10:26:37 PM	Acquired by	: System Administrator
Date Processed	: 8/5/2021 3:29:29 PM	Processed by	: System Administrator

<Chromatogram>

mAU

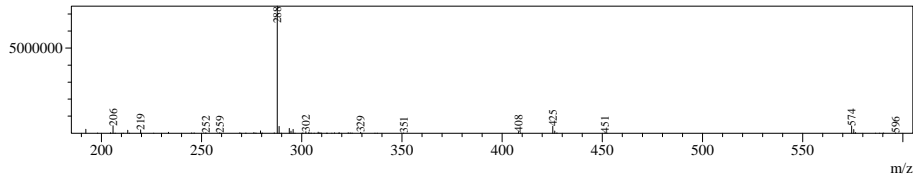


mAU



MS Spectrum

Peak#1 R.Time:1.733(Scan#:161)
MassPeaks:67
Spectrum Mode:Averaged 1.723-1.744(160-162)
BG Mode:Calc Segment 1 - Event 1



D:\2700 LCMS\2700-A12 peak 1 080421.lcd

Figure S5 continued
2700.013

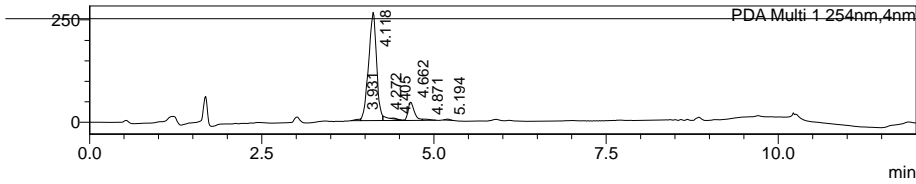
4/17/2024 2:34:39 PM Page 1 / 3

<Sample Information>

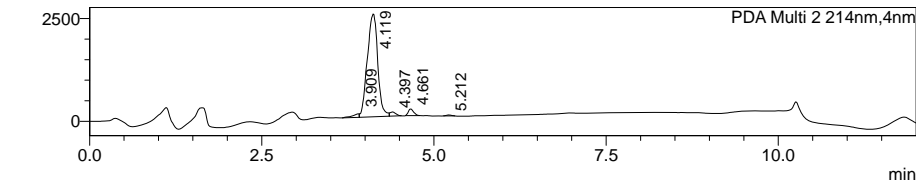
Sample Name	: 2700	Sample Type	: Unknown
Sample ID	: 2700-A13 peak 1		
Data Filename	: 2700-A13 peak 1 080421.lcd		
Method Filename	: 6min 5-95, 1min clean(254)_(214) Col_1_LCMS3.lcm		
Batch Filename	: 080421.lcb		
Vial #	: 1-30		
Injection Volume	: 10 uL		
Date Acquired	: 8/5/2021 10:04:14 AM	Acquired by	: System Administrator
Date Processed	: 4/17/2024 2:34:20 PM	Processed by	: System Administrator

<Chromatogram>

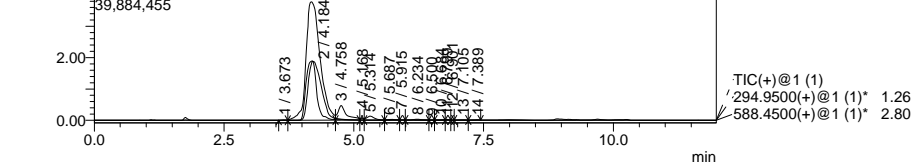
mAU



mAU



Segment#1 (x10,000,000)



2700.014

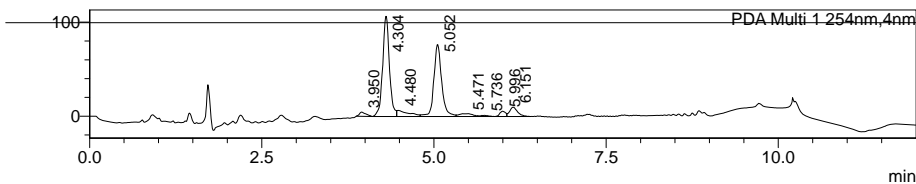
4/17/2024 2:37:33 PM Page 1 / 3

<Sample Information>

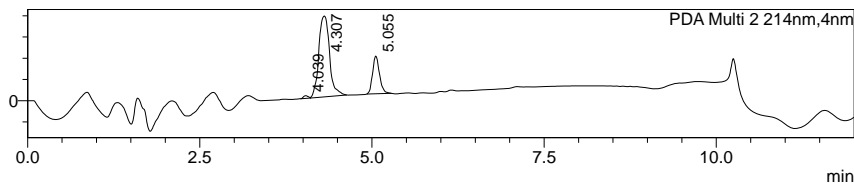
Sample Name	: 2700		
Sample ID	: 2700-A14 peak 1 <90		
Data Filename	: 2700-A14 peak 1 less than 90 080421.lcd		
Method Filename	: 6min 5-95, 1min clean(254)_(214) Col_1_LCMS3.lcm		
Batch Filename	: 080421.lcb		
Vial #	: 1-32	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 8/5/2021 10:41:25 AM	Acquired by	: System Administrator
Date Processed	: 9/20/2021 9:36:13 PM	Processed by	: System Administrator

<Chromatogram>

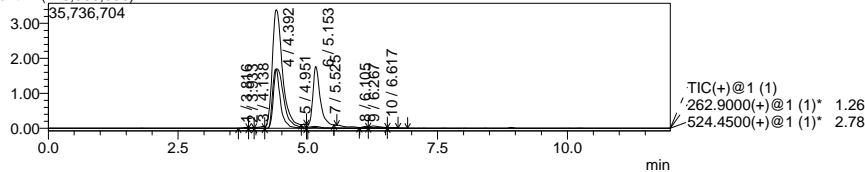
mAU



mAU

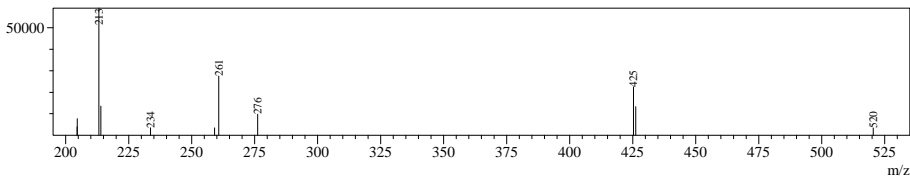


Segment#1 (x10,000,000)



Peak#:1 R.Time:3.816(Scan#:353)
MassPeaks:11
Spectrum Mode:Averaged 3.803-3.824(352-354)
BG Mode:Calc Segment 1 - Event 1

MS Spectrum



D:\2700 LCMS\2700-A14 peak 1 less than 90 080421.lcd

Figure S5 continued
2700.015

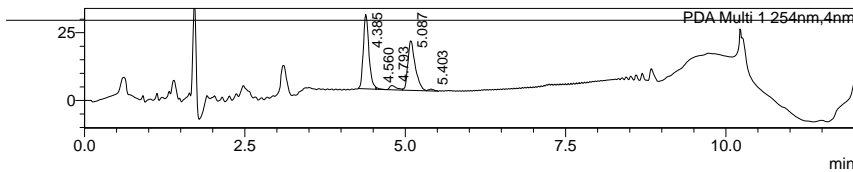
4/17/2024 2:40:11 PM Page 1 / 3

<Sample Information>

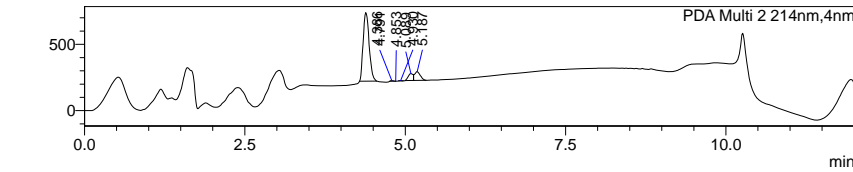
Sample Name	: 2700	Sample Type	: Unknown
Sample ID	: 2700-A15 peak 1		
Data Filename	: 2700-A15 peak 1 080521.lcd		
Method Filename	: 6min 5-95, 1min clean(254)_(214) Col_1_LCMS3.lcm		
Batch Filename	: 080521.lcb		
Vial #	: 1-5		
Injection Volume	: 5 uL		
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Date Processed	: 9/20/2021 9:34:19 PM	Processed by	: System Administrator

<Chromatogram>

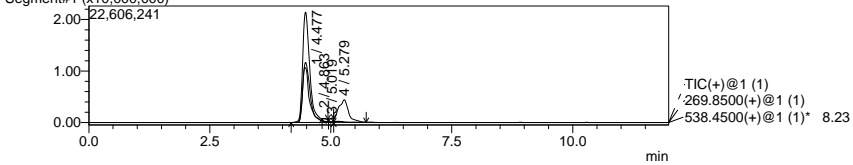
mAU



mAU

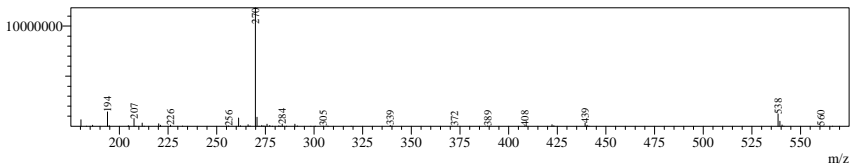


Segment#1 (x10,000,000)



MS Spectrum

Peak#1 R.Time:4.477(Scan#:414)
MassPeaks:64
Spectrum Mode:Averaged 4.463-4.485(413-415)
BG Mode:Calc Segment 1 - Event 1



D:\2700 LCMS\2700-A15 peak 1 080521.lcd

Figure S5. *Liquid chromatography-mass spectrometry (LCMS) analysis.* The figure shows UV traces at 254 nm, % purity integration data, Total ion current (TIC) data, mass spectrum (MS), and MS peak table.

Figure S6

Compound ID	SMILES
2700.001 2637.001	<chem>NC[C@H](C1=CC=CC=C1)NC[C@H](CCC2)N2C[C@@H](CO)NC[C@H](C3=CC=CC=C3)NCCCC(C)C4=CC=CC=C4</chem>
2700.002 2637.003	<chem>NC[C@H](CC1=CC=CC=C1)NC[C@H](CCC2)N2C[C@@H](CO)NC[C@H](C3=CC=CC=C3)NCCCC(C)C4=CC=CC=C4</chem>
2700.003 2637.011	<chem>NC[C@H](C1=CC=CC=C1)NC[C@H](CCC2)N2C[C@@H](CO)NC[C@H](C3=CC=CC=C3)NCCCCC4=CC=CC=C4</chem>
2700.004	<chem>NC[C@H](C1=CC=CC=C1)NC[C@H](CCC2)N2C[C@@H](CO)NC[C@H](CC3=CC=CC=C3)NCCCCC4=CC=CC=C4</chem>
2700.005	<chem>NC[C@H](C1=CC=CC=C1)NC[C@H](CCC2)N2C[C@@H](CO)NC[C@H](CC3=CC=CC=C3)NCCCC(C)C4=CC=CC=C4</chem>
2700.006	<chem>NC[C@H](CC1=CC=CC=C1)NC[C@H](CCC2)N2C[C@@H](CO)NC[C@H](C3=CC=CC=C3)NCCCC(C)C4=CC=CC=C4</chem>
2700.007	<chem>NC[C@H](CC1=CC=CC=C1)NC[C@H](CCC2)N2C[C@@H](CO)NC[C@H](C3=CC=CC=C3)NCCCCC4=CC=CC=C4</chem>
2700.008	<chem>NC[C@H](CC1=CC=CC=C1)NC[C@H](CCC2)N2C[C@@H](CO)NC[C@H](CC3=CC=CC=C3)NCCCCC4=CC=CC=C4</chem>
2700.009	<chem>NC[C@H](CC1=CC=CC=C1)NC[C@H](CCC2)N2C[C@@H](CO)NC[C@H](C3=CC=CC=C3)NCCCCC4=CC=CC=C4</chem>
2700.010	<chem>NC[C@H](CC1=CC=CC=C1)NC[C@H](CCC2)N2C[C@@H](CO)NC[C@H](CC3=CC=CC=C3)NCCCCC4=CC=CC=C4</chem>
2700.011	<chem>NC[C@H](CC1=CC=C(O)C=C1)NC[C@H](CCC2)N2C[C@@H](CO)NC[C@H](C3=CC=CC=C3)NCCCC(C)C4=CC=CC=C4</chem>
2700.012	<chem>NC[C@H](CC1=CC=C(O)C=C1)NC[C@H](CCC2)N2C[C@@H](CO)NC[C@H](C3=CC=CC=C3)NCCCCC4=CC=CC=C4</chem>
2700.013	<chem>NC[C@H](CC1=CC=C(O)C=C1)NC[C@H](CCC2)N2C[C@@H](CO)NC[C@H](CC3=CC=CC=C3)NCCCCC4=CC=CC=C4</chem>
2700.014	<chem>NC[C@@H](CCCC)NC[C@H](CCC1)N1C[C@@H](CO)NC[C@H](C2=CC=CC=C2)NCCCCC3=CC=CC=C3</chem>
2700.015	<chem>NC[C@@H](CCCC)NC[C@H](CCC1)N1C[C@@H](CO)NC[C@H](CC2=CC=CC=C2)NCCCCC3=CC=CC=C3</chem>

Figure S6. SMILES representation of the compounds used in this work.

Table S1

AAC(6')-Ib residues that form hydrogen bonds with 2700.001 and 2700.004

Compound	Residue	Occupancy (%)
2700.001	TYR65	32.50
	GLN91	16.00

Compound	Residue	Occupancy (%)
2700.004	TYR65	23.50
	GLN91	21.50