

## *Supporting information*

### **Novel Temporin L antimicrobial peptides: promoting self-assembling by lipidic tags to tackle superbugs**

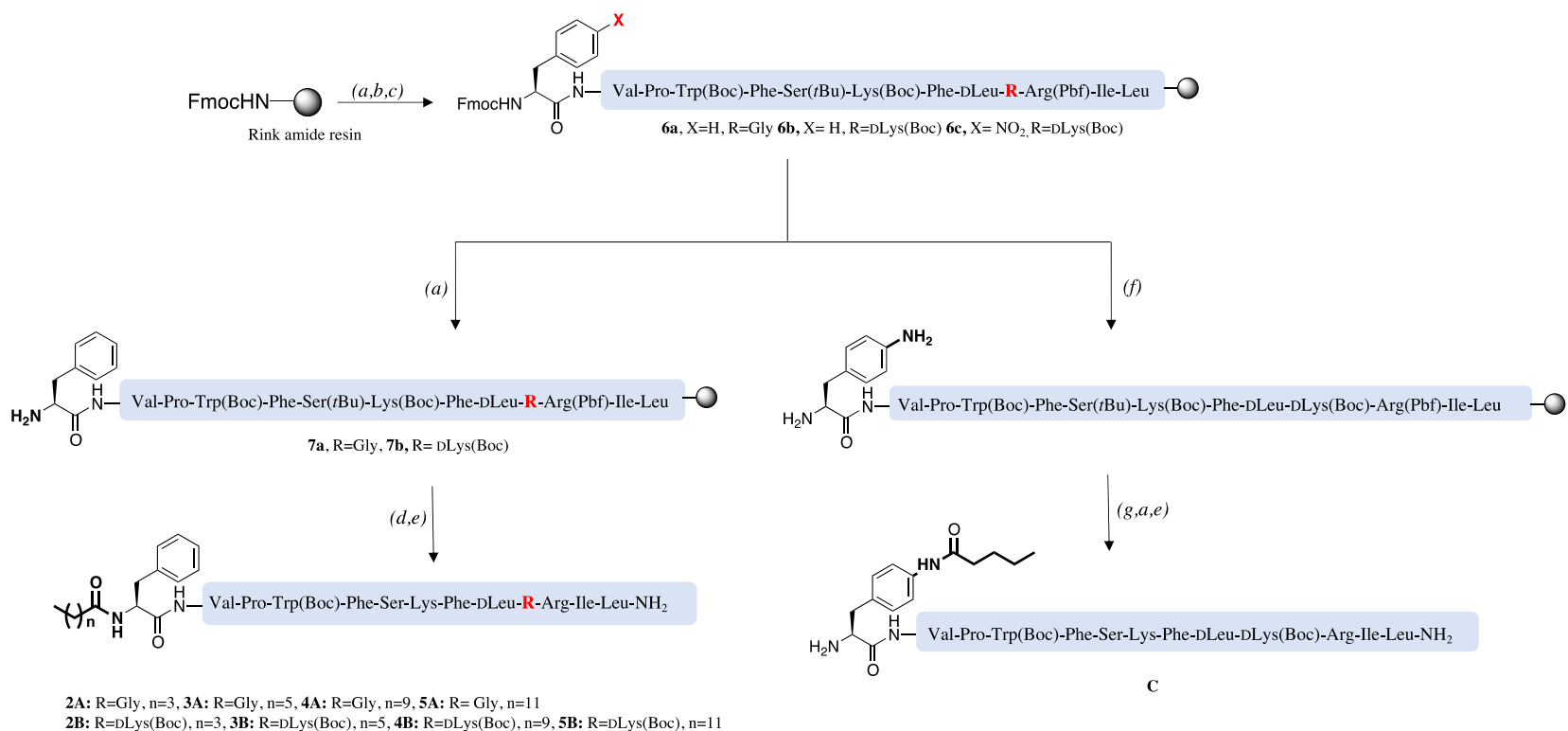
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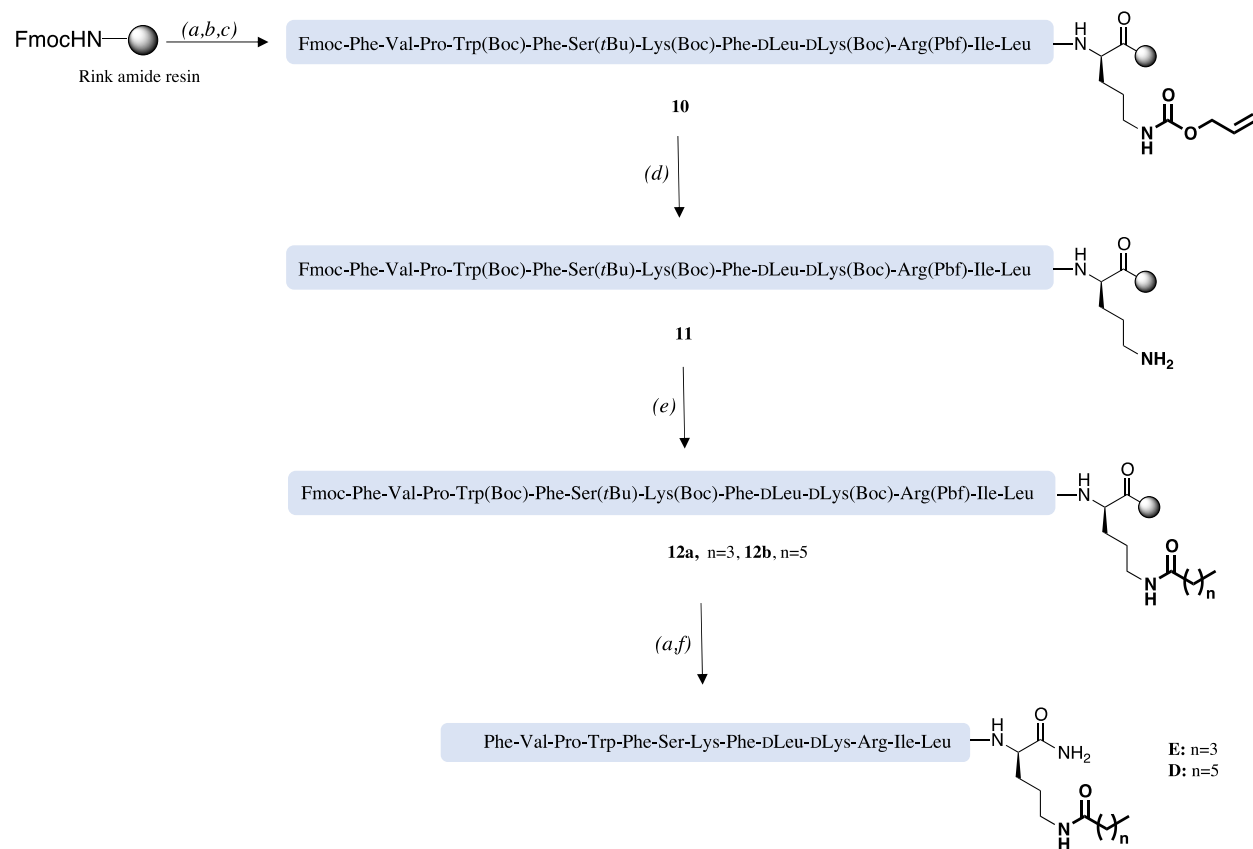
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**Scheme S1.** (a) 20% piperidine in DMF, 0.5 + 1 min, ultrasonic irradiation; (b) Fmoc-Leu-OH (3 equiv), HBTU (3 equiv), HOBT (3equiv), DIEA (6 equiv), 1:1 DMF/DCM, 5 min, ultrasonic irradiation; (c) US-SPPS; (d) Fatty acid (3 equiv), HBTU (3 equiv), HOBT (3equiv), DIEA (6equiv), 1:1 DMF/DCM, 15 min, ultrasonic irradiation; (e) TFA/TIS/H<sub>2</sub>O (95:2.5:2.5 v/v/v), 3h, rt; (f) SnCl<sub>2</sub> 1M in DMF, 12h, rt; (g) Valeric acid (3 equiv), HBTU (3 equiv), HOBT (3 equiv), DIEA (6 equiv), 2h, rt.

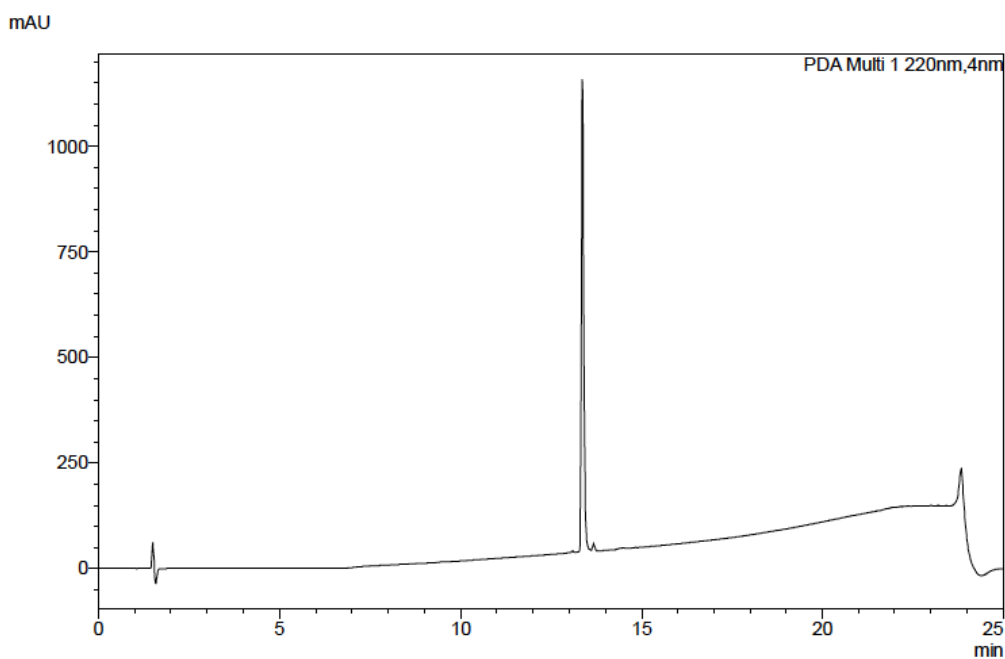


**Scheme 2.** (a) 20% piperidine in DMF, 0.5 + 1 min, ultrasonic irradiation; (b) Fmoc-Orn(Alloc)-OH (3 equiv), HBTU (3equiv), HOBt (3equiv), DIEA (6equiv), 1:1 DMF/DCM, 5 min, ultrasonic irradiation; (c) US-SPPS; (d) Pd(PPh<sub>3</sub>)<sub>4</sub> (0.15 equiv), dimethylbarbituric acid (3equiv), DCM/DMF (3:2 v/v), 1h (x2), rt; (e) Fatty acid (3 equiv), HBTU (3 equiv), HOBt (3 equiv), DIEA (6 equiv), 1:1 DMF/DCM, 15 min, ultrasonic irradiation; (f) TFA/TIS/H<sub>2</sub>O (95:2.5:2.5 v/v/v), 3h, rt.

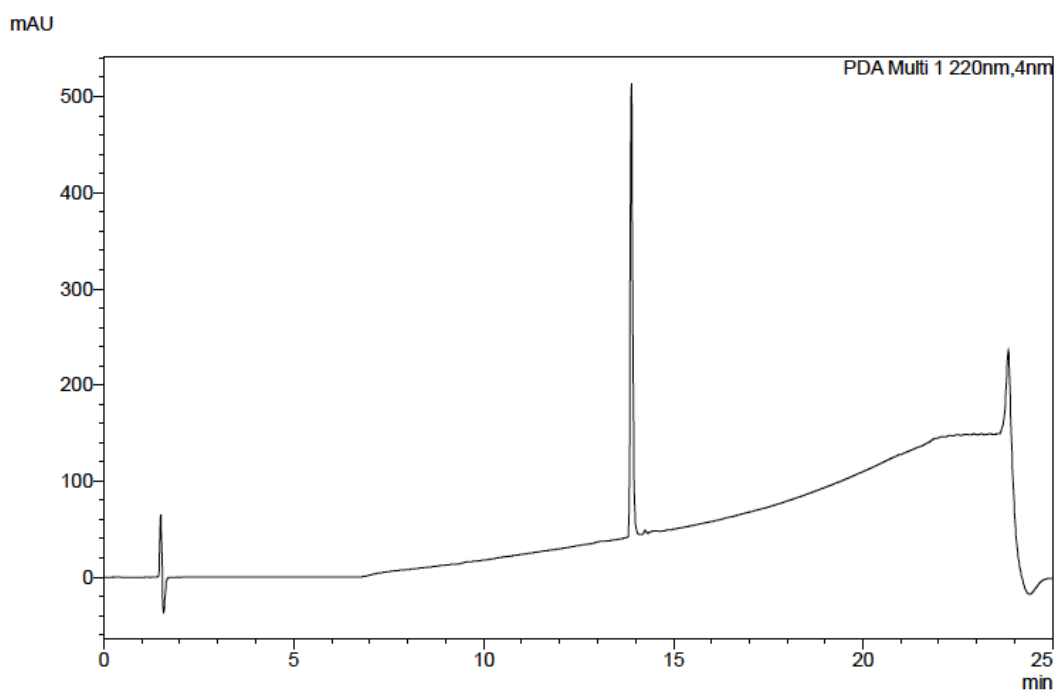
HRMS m/z		
Compound	calcd.	obsd.
2A	1694.0027	[M+H/2] <sup>+</sup> = 847.5036
3A	1722.0340	[M+H/2] <sup>+</sup> =861.5185
4A	1778.0966	[M+H/2] <sup>+</sup> = 889.551
5A	1806.1279	[M+H/2] <sup>+</sup> = 903.5670
2B	1765.0762	[M+2H/2] <sup>+</sup> =883.0450
3B	1793.1075	[M+2H/2] <sup>+</sup> = 897.0603
4B	1849.1701	[M+H/2] <sup>+</sup> =925.0869
5B	1877.2014	[M+2H/2] <sup>+</sup> = 939.1046
C	1780.0871	[M+H/2] <sup>+</sup> = 890.5464
D	1879.1555	[M+2H/2] <sup>+</sup> =940.0730
E	1907.1868	[M+2H/2] <sup>+</sup> = 954.0976

**Table S1.** HRMS calculated and observed for peptides **2A-5A**, **2B-5B**, **C**, **D**, **E**.

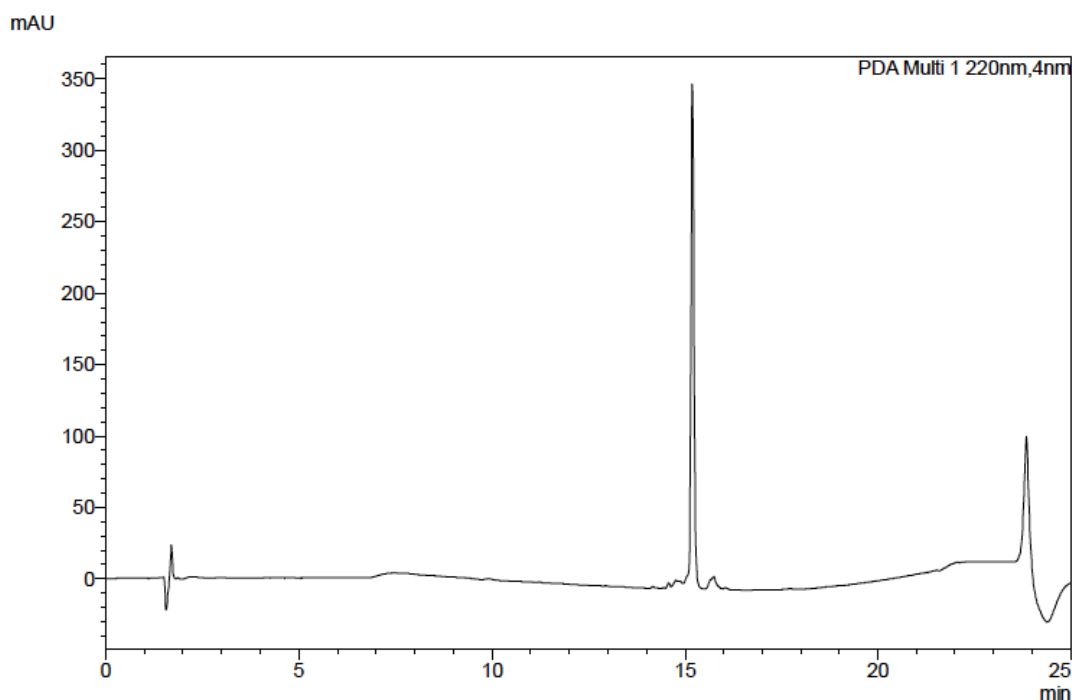
### HPLC chromatograms of peptides **2A-5A**, **2B-5B**, **C**, **D**, **E**



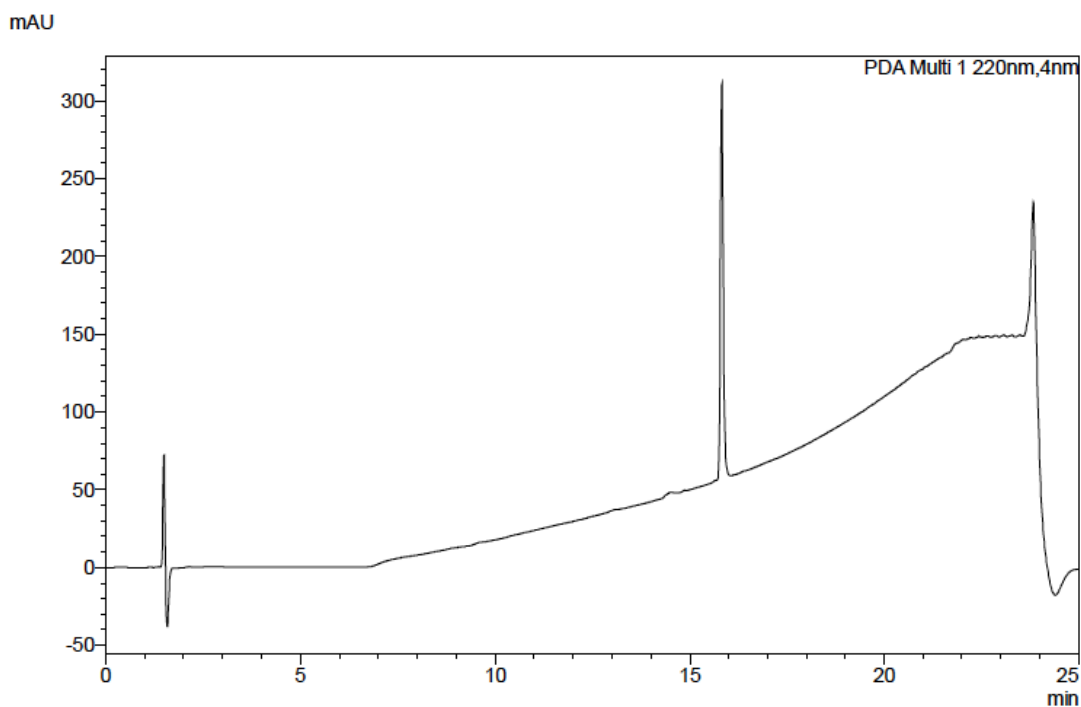
**Figure S3.** Chromatogram of **2A** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm.  $t_R$ : 13.4 min [linear gradient 10-90% MeCN (0.1% TFA) in H<sub>2</sub>O (0.1% TFA) over 20 min, flow rate of 1 mL/min].



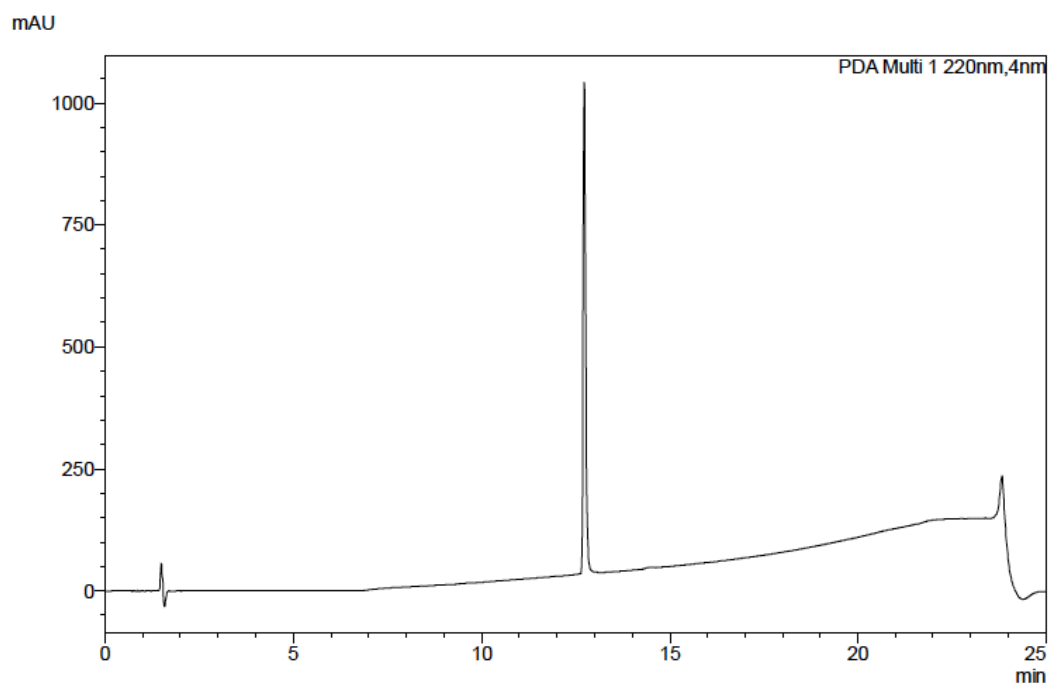
**Figure S4.** Chromatogram of **3A** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm  $\times$  4.6 mm, 5  $\mu$ m, 100  $\text{\AA}$ ), and monitored by UV detection at 220 nm.  $t_R$ : 13.9 min [linear gradient 10-90% MeCN (0.1% TFA) in H<sub>2</sub>O (0.1% TFA) over 20 min, flow rate of 1 mL/min].



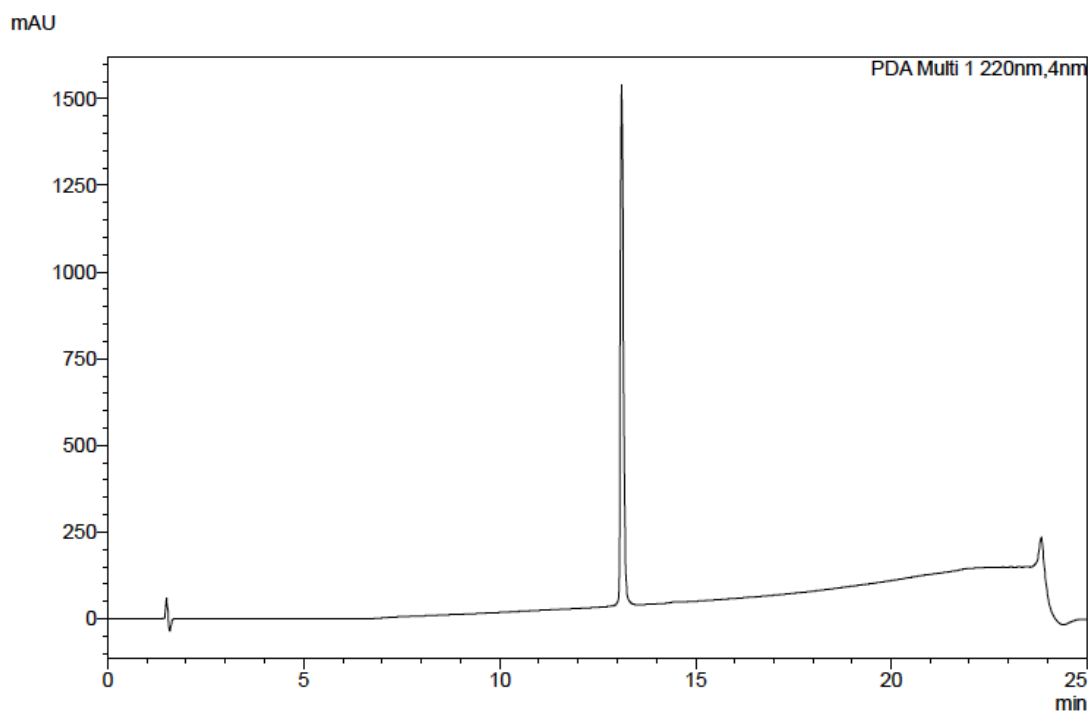
**Figure S5.** Chromatogram of **4A** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm  $\times$  4.6 mm, 5  $\mu$ m, 100  $\text{\AA}$ ), and monitored by UV detection at 220 nm.  $t_R$ : 15.8 min [linear gradient 10-90% MeCN (0.1% TFA) in H<sub>2</sub>O (0.1% TFA) over 20 min, flow rate of 1 mL/min].



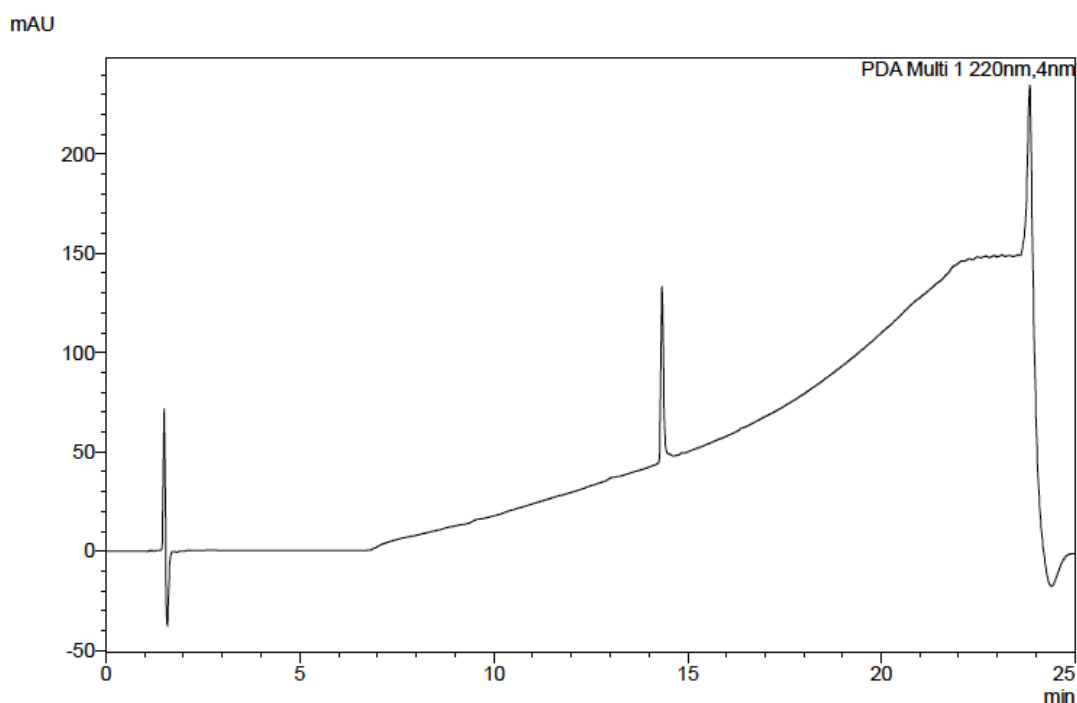
**Figure S6.** Chromatogram of **5A** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm.  $t_R$ : 15.2 min [linear gradient 10-90% MeCN (0.1% TFA) in H<sub>2</sub>O (0.1% TFA) over 20 min, flow rate of 1 mL/min].



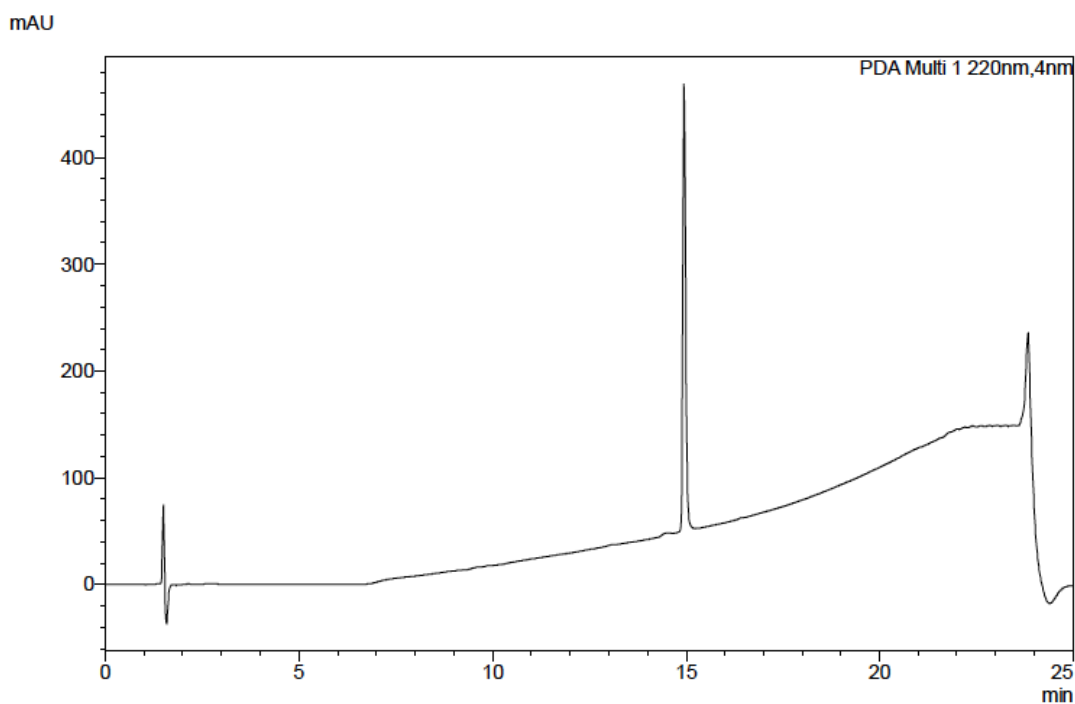
**Figure S7.** Chromatogram of **2B** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm.  $t_R$ : 12.7 min [linear gradient 10-90% MeCN (0.1% TFA) in H<sub>2</sub>O (0.1% TFA) over 20 min, flow rate of 1 mL/min].



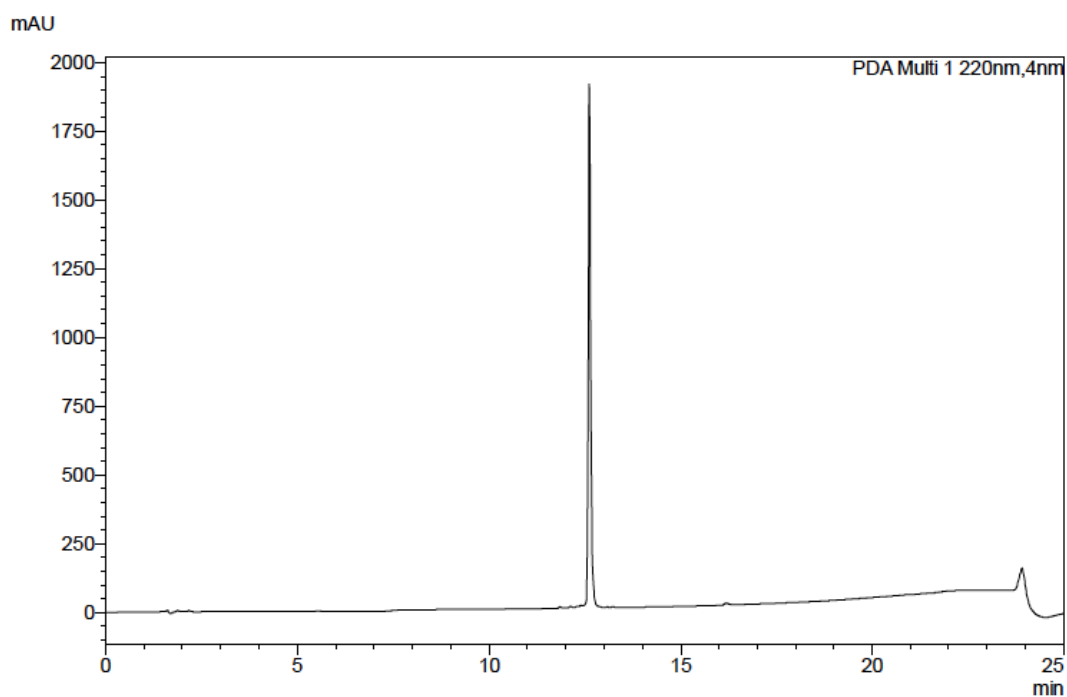
**Figure S8.** Chromatogram of **3B** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm.  $t_R$ : 13.1 min [linear gradient 10-90% MeCN (0.1% TFA) in H<sub>2</sub>O (0.1% TFA) over 20 min, flow rate of 1 mL/min].



**Figure S9.** Chromatogram of **4B** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm × 4.6 mm, 5 μm, 100 Å), and monitored by UV detection at 220 nm.  $t_R$ : 14.9 min [linear gradient 10-90% MeCN (0.1% TFA) in H<sub>2</sub>O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

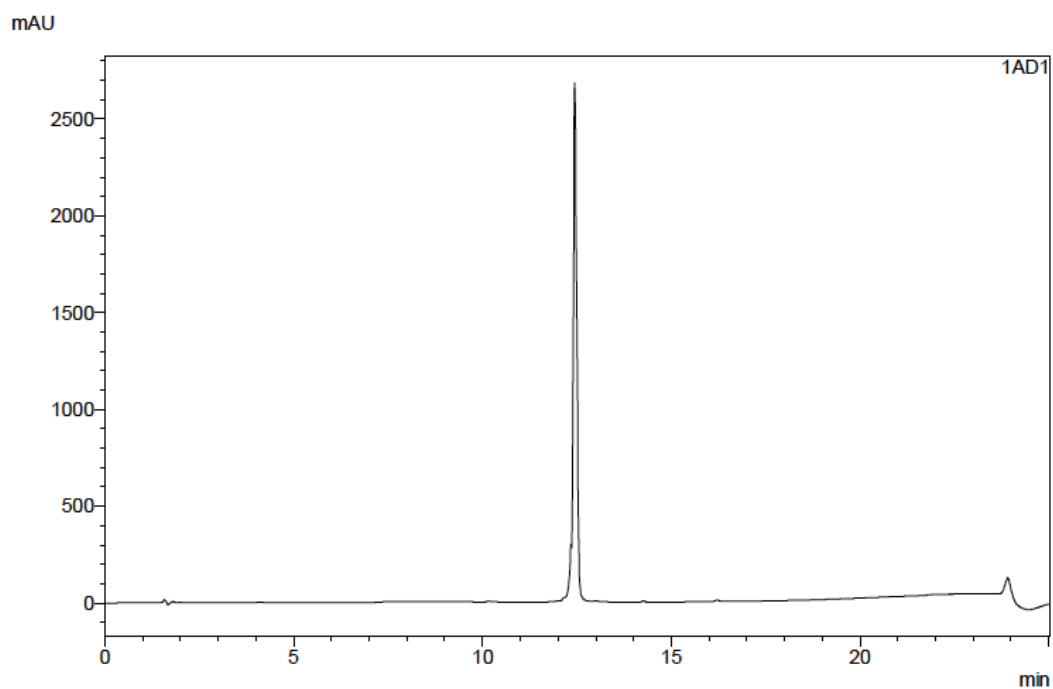


**Figure S10.** Chromatogram of **5B** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm  $\times$  4.6 mm, 5  $\mu$ m, 100  $\text{\AA}$ ), and monitored by UV detection at 220 nm.  $t_R$ : 14.3 min [linear gradient 10-90% MeCN (0.1% TFA) in H<sub>2</sub>O (0.1% TFA) over 20 min, flow rate of 1 mL/min].

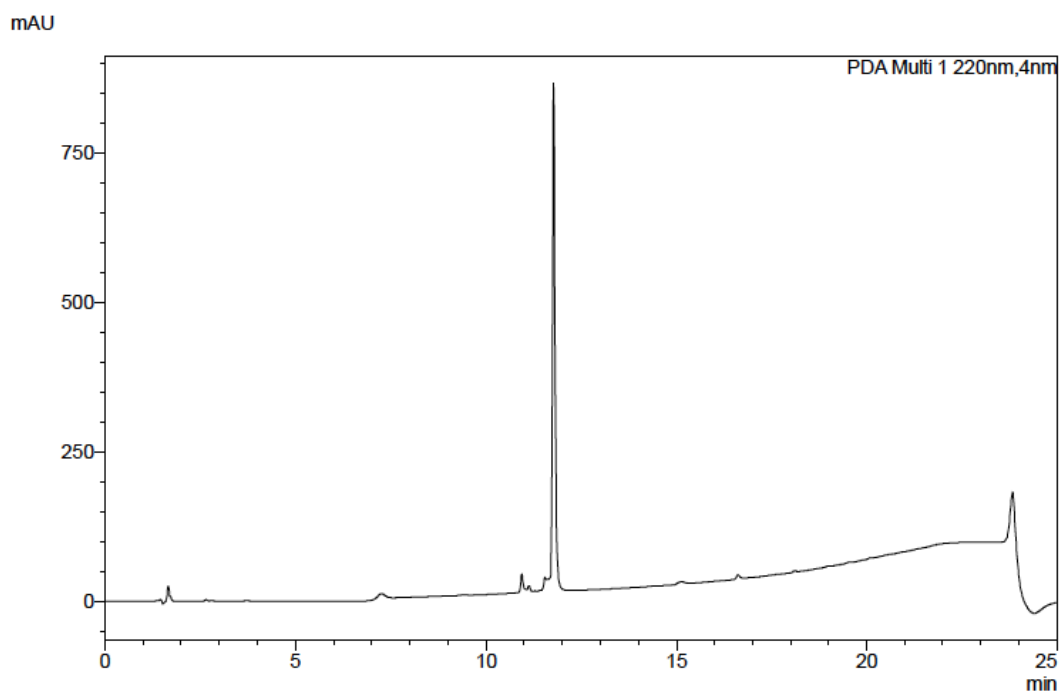


**Figure S11.** Chromatogram of **C** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm  $\times$  4.6 mm, 5  $\mu$ m, 100  $\text{\AA}$ ), and monitored by UV detection at 220 nm.  $t_R$ : 12.6 min [linear gradient 10-90% MeCN (0.1% TFA) in H<sub>2</sub>O (0.1% TFA) over 20 min, flow rate of 1 mL/min].





**Figure S12.** Chromatogram of **D** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm  $\times$  4.6 mm, 5  $\mu$ m, 100  $\text{\AA}$ ), and monitored by UV detection at 220 nm.  $t_R$ : 12.4 min [linear gradient 10-90% MeCN (0.1% TFA) in H<sub>2</sub>O (0.1% TFA) over 20 min, flow rate of 1 mL/min].



**Figure S13.** Chromatogram of **E** obtained by an analytical HPLC (Shimadzu UFLC SPD-M20A/DGU-20A3R/LC-20AD) equipped with a Phenomenex Kinetex C18 column (150 mm  $\times$  4.6 mm, 5  $\mu$ m, 100  $\text{\AA}$ ), and monitored by UV detection at 220 nm.  $t_R$ : 11.8 min [linear gradient 10-90% MeCN (0.1% TFA) in H<sub>2</sub>O (0.1% TFA) over 20 min, flow rate of 1 mL/min].