

Supplementary Information

Identification and Structure-Activity Relationship Analysis of Minimal Fusion
Inhibitors Targeting Measles Virus F Protein

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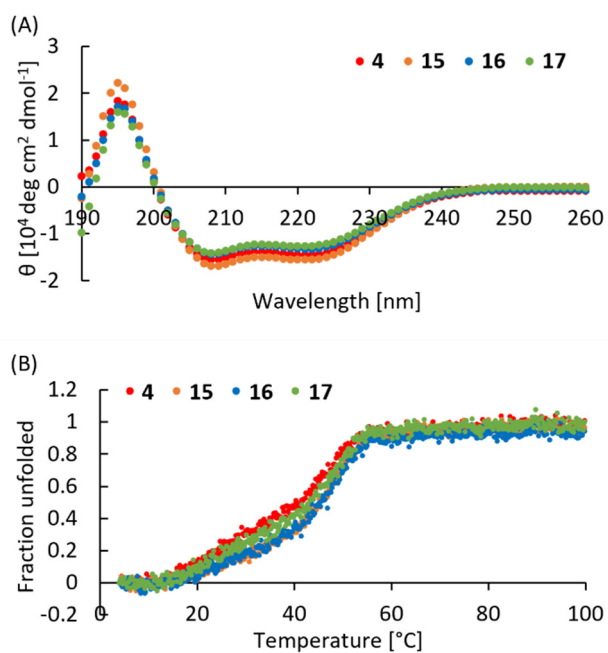
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Table S1. Antiviral activity of peptides **3** and **4** against wild-type and resistant measles viral strains.

Peptide	EC ₅₀ (μM) ^a [pEC ₅₀ ± SD]			
	MeV _{WT}	MeV _{M1^R_{p29}} ^b	MeV _{M1^R_{p62}} ^b	MeV _{M2^R_{p44}} ^b
MEK35GT (3)	0.0021 [8.7 ± 0.3]	0.0022 [8.7 ± 0.3]	0.0015 [8.8 ± 0.4]	0.0027 [8.6 ± 0.4]
MEK28GT (4)	0.0092 [8.0 ± 0.3]	1.5 [5.8 ± 0.6]	0.97 [6.0 ± 0.3]	1.3 [5.9 ± 0.2]

^aThe concentration required to inhibit viral replication by 50%. ^bMeV_{M1^R_{p29}}, MeV_{M1^R_{p62}}, and MeV_{M2^R_{p44}} carry the A167E mutation, N158S/A167E/Y349H mutations, and A167E/K244R mutations, respectively, in the fusion peptide (FP) region.^{S1}

**Fig. S1.** CD analysis of peptides **4** and **15–17** in the presence of the HR1-40. (A) CD spectra. (B) Thermal stability of the six-helical bundles.

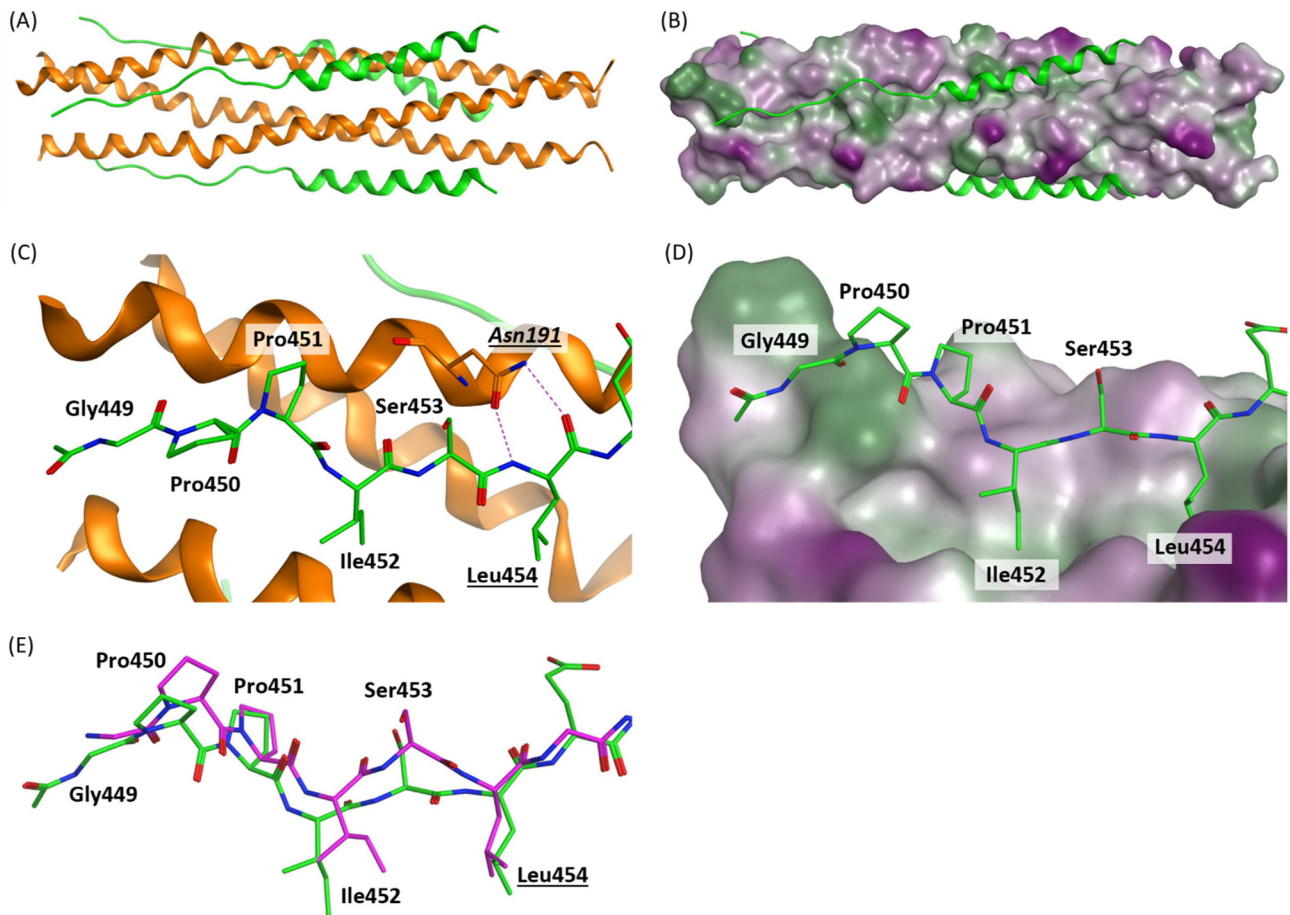


Fig. S2. Molecular models of the six-helical bundle formed by peptide **17** (green) and HR1Y+8 (orange). HR1Y+8 residues are shown in italics. (A) Overall view of the six-helical bundle. (B) Overall view of the six-helical bundle with the surface of the HR1Y+8 trimer rendered according to lipophilicity (purple, hydrophilic; gray, neutral; green, lipophilic). (C) Close-up view of the N-terminal region of **17**. Residues involved in hydrogen bonding are underlined. (D) Close-up view of the N-terminal region of **17** with the surface of the HR1Y+8 trimer rendered according to lipophilicity. (E, F) Superposition of the N-terminal region of **17** with the corresponding region in the postfusion MV F protein structure (PDB ID: 8UTF, purple).

Table S2. Characterization data of synthetic peptides by electrospray ionization mass spectrometry (ESI-MS).

Peptide	Formula	MW	<i>m/z</i>		
				Calcd.	Observed
HR1-40	C ₁₈₈ H ₃₁₁ N ₅₇ O ₆₅ S	4441.95	[M+4H] ⁴⁺	1111.50	1112.13
			[M+3H] ³⁺	1481.66	1482.26
MEK35GT (3)	C ₁₈₂ H ₃₂₃ N ₄₉ O ₅₆	4093.87	[M+5H] ⁵⁺	819.78	819.93
			[M+4H] ⁴⁺	1024.48	1024.70
			[M+3H] ³⁺	1365.63	1365.75
MEK28GT (4)	C ₁₄₅ H ₂₅₈ N ₄₀ O ₄₄	3265.90	[M+4H] ⁴⁺	817.48	817.58
			[M+3H] ³⁺	1089.64	1089.82
			[M+2H] ²⁺	1633.96	1633.74
MEK21GT (5)	C ₁₀₈ H ₁₉₃ N ₃₁ O ₃₃	2453.92	[M+3H] ³⁺	818.98	818.56
			[M+2H] ²⁺	1227.97	1227.51
MEK14GT (6)	C ₇₁ H ₁₂₇ N ₂₁ O ₂₂	1626.92	[M+2H] ²⁺	814.47	814.01
MEK28GT-1 (7)	C ₁₃₉ H ₂₄₇ N ₃₉ O ₄₃	3152.74	[M+4H] ⁴⁺	789.19	789.08
			[M+3H] ³⁺	1051.92	1051.86
MEK28GT-2 (8)	C ₁₃₆ H ₂₄₂ N ₃₈ O ₄₁	3065.66	[M+4H] ⁴⁺	767.42	767.28
			[M+3H] ³⁺	1022.89	1022.84
MEK28GT-3 (9)	C ₁₃₀ H ₂₃₁ N ₃₇ O ₄₀	2952.50	[M+4H] ⁴⁺	739.13	738.96
			[M+3H] ³⁺	985.17	985.01
			[M+2H] ²⁺	1477.26	1477.15
MEK28GT-4 (10)	C ₁₂₅ H ₂₂₄ N ₃₆ O ₃₇	2823.38	[M+4H] ⁴⁺	706.85	706.63
			[M+3H] ³⁺	942.13	941.91
			[M+2H] ²⁺	1412.70	1412.60
MEK28GT-5 (11)	C ₁₁₉ H ₂₁₂ N ₃₂ O ₃₆	2667.19	[M+3H] ³⁺	890.07	889.95
			[M+2H] ²⁺	1334.60	1334.59
MEK28GT-6 (12)	C ₁₁₃ H ₂₀₁ N ₃₁ O ₃₅	2554.03	[M+3H] ³⁺	852.35	852.24
			[M+2H] ²⁺	1278.02	1278.06
MEK28GT-7 (13)	C ₁₀₉ H ₁₉₆ N ₃₀ O ₃₂	2438.95	[M+3H] ³⁺	813.99	813.83
			[M+2H] ²⁺	1220.48	1220.48
MEK28GT-8 (14)	C ₁₀₄ H ₁₈₇ N ₂₉ O ₃₁	2339.81	[M+3H] ³⁺	780.94	780.79
			[M+2H] ²⁺	1170.91	1170.93
MEK28GT+1 (15)	C ₁₅₀ H ₂₆₅ N ₄₁ O ₄₅	3363.01	[M+4H] ⁴⁺	841.76	841.25
			[M+3H] ³⁺	1122.01	1121.59
MEK28GT+2 (16)	C ₁₅₅ H ₂₇₂ N ₄₂ O ₄₆	3460.13	[M+4H] ⁴⁺	866.04	865.57
			[M+3H] ³⁺	1154.38	1153.99
MEK28GT+3 (17)	C ₁₅₇ H ₂₇₅ N ₄₃ O ₄₇	3517.18	[M+4H] ⁴⁺	880.30	879.85
			[M+3H] ³⁺	1173.40	1173.02

Table S2. (continued) Characterization data of synthetic peptides by ESI-MS.

Peptide	Formula	MW	<i>m/z</i>		
				Calcd.	Observed
MEK28GT-A466V (18)	C ₁₄₇ H ₂₆₂ N ₄₀ O ₄₄	3293.95	[M+4H] ⁴⁺	824.50	824.07
			[M+3H] ³⁺	1098.99	1098.88
MEK28GT-A473V (19)	C ₁₄₇ H ₂₆₂ N ₄₀ O ₄₄	3293.95	[M+4H] ⁴⁺	824.50	824.09
			[M+3H] ³⁺	1098.99	1099.14
MEK28GT-L463F (20)	C ₁₄₈ H ₂₅₆ N ₄₀ O ₄₄	3299.91	[M+4H] ⁴⁺	825.99	825.54
			[M+3H] ³⁺	1100.98	1102.75
MEK28GT-L470F (21)	C ₁₄₈ H ₂₅₆ N ₄₀ O ₄₄	3299.91	[M+4H] ⁴⁺	825.99	825.47
MEK28GT-L477F (22)	C ₁₄₈ H ₂₅₆ N ₄₀ O ₄₄	3299.91	[M+4H] ⁴⁺	825.99	825.48
MEK28GT+S480 (23)	C ₁₄₈ H ₂₆₃ N ₄₁ O ₄₆	3352.97	[M+4H] ⁴⁺	839.25	838.80
HR1X	C ₂₂₂ H ₃₆₇ N ₆₅ O ₇₆ S ₂	5226.88	[M+6H] ⁶⁺	872.15	872.41
			[M+5H] ⁵⁺	1046.38	1045.78
			[M+4H] ⁴⁺	1307.73	1307.85
HR1Y	C ₂₃₆ H ₃₉₁ N ₆₉ O ₈₁ S ₂	5555.25	[M+6H] ⁶⁺	926.88	926.72
			[M+5H] ⁵⁺	1112.06	1112.02
			[M+4H] ⁴⁺	1389.82	1389.76

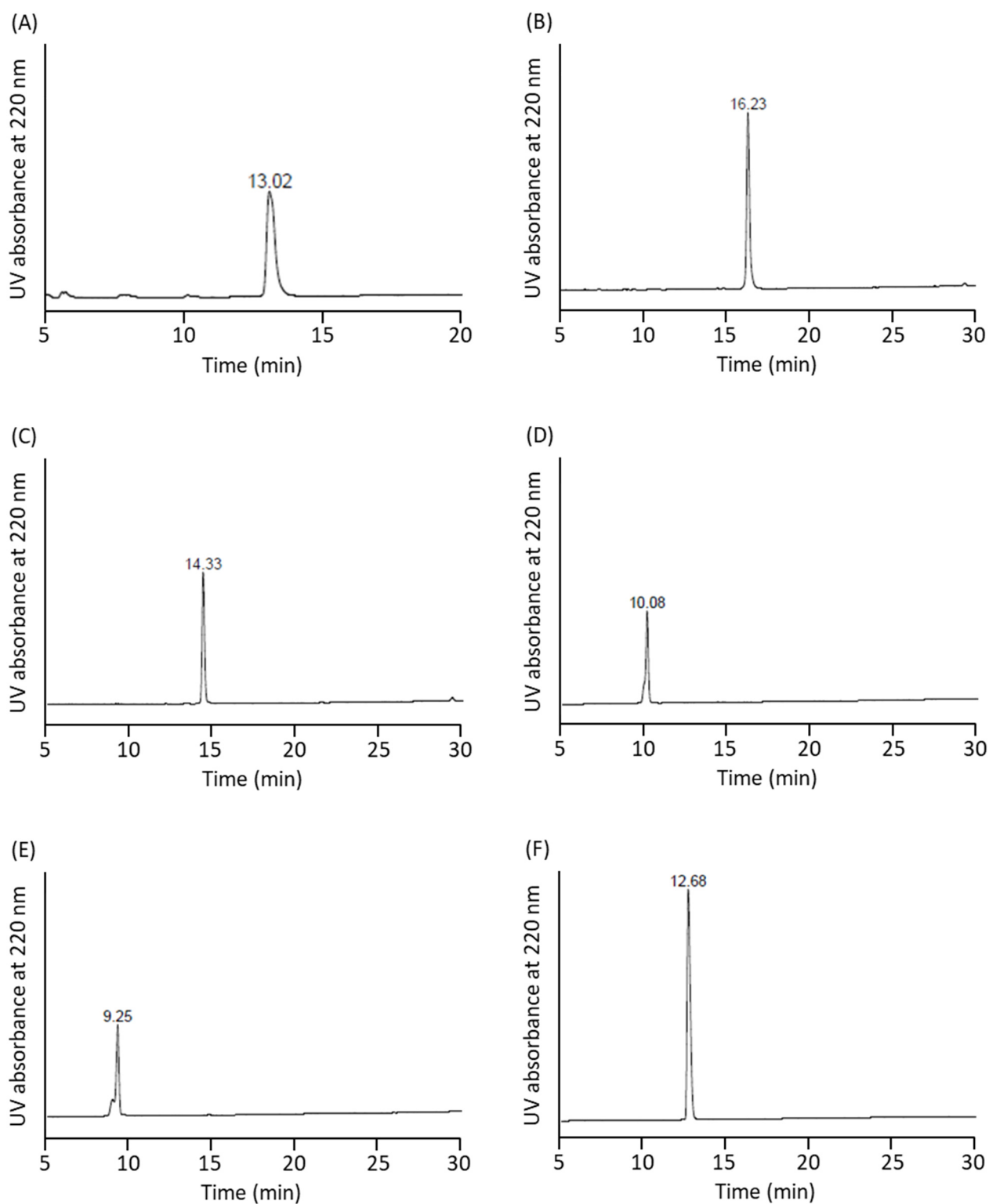


Fig. S3. HPLC chromatograms of purified synthetic peptides. (A) HR1-40; (B–F) peptides 3–7. *HPLC conditions:* (A) Linear gradient of 40–60% CH₃CN in H₂O over 20 min; (B–F) Linear gradient of 20–50% CH₃CN in H₂O over 30 min. †A shoulder peak with identical mass was observed in peptides 5 (D) and 6 (E); the species responsible for this peak was not identified.

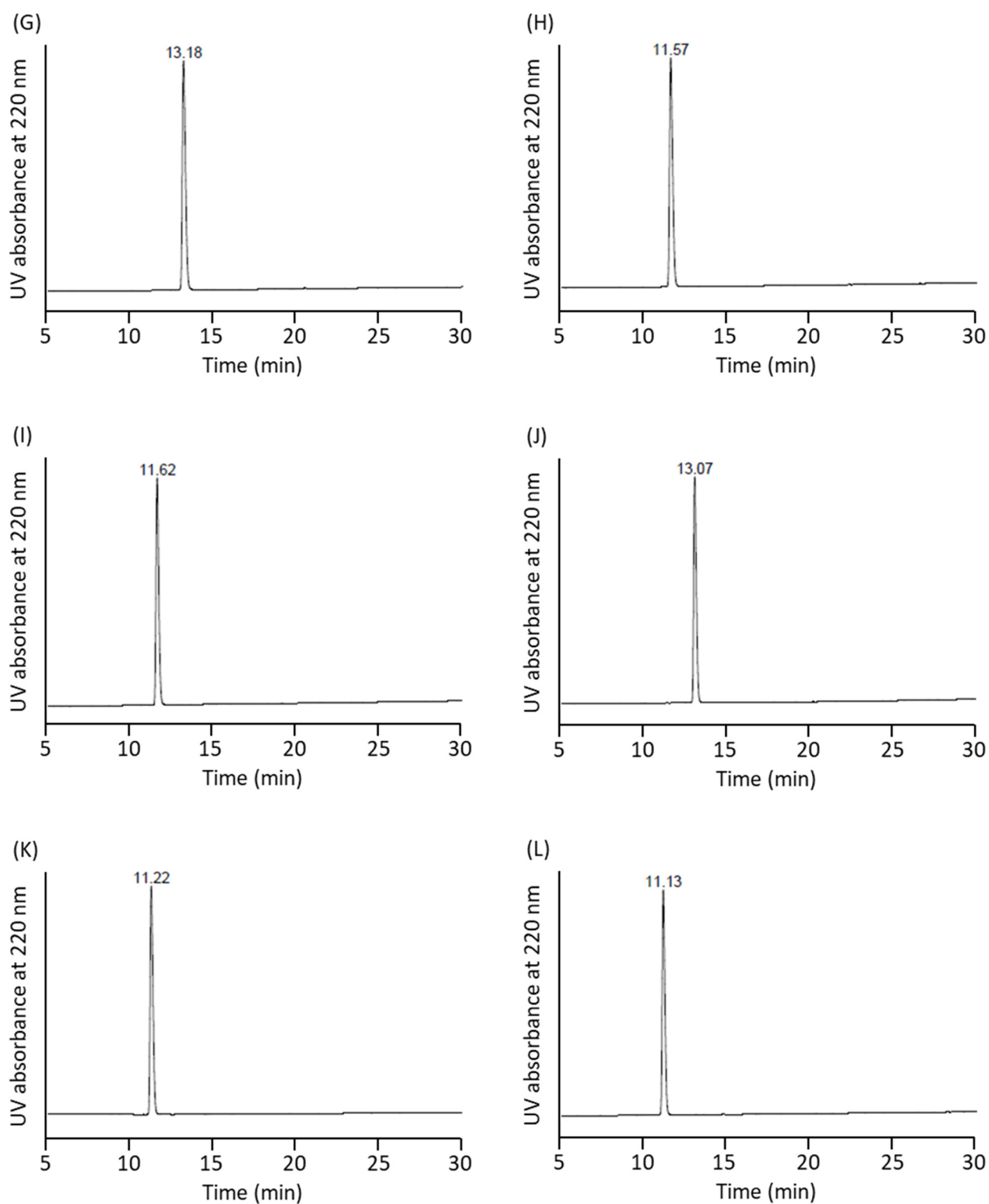


Fig. S3. (continued) HPLC chromatograms of purified synthetic peptides. (G–L) peptides **8–13**. HPLC conditions: Linear gradient of 20–50% CH₃CN in H₂O over 30 min.

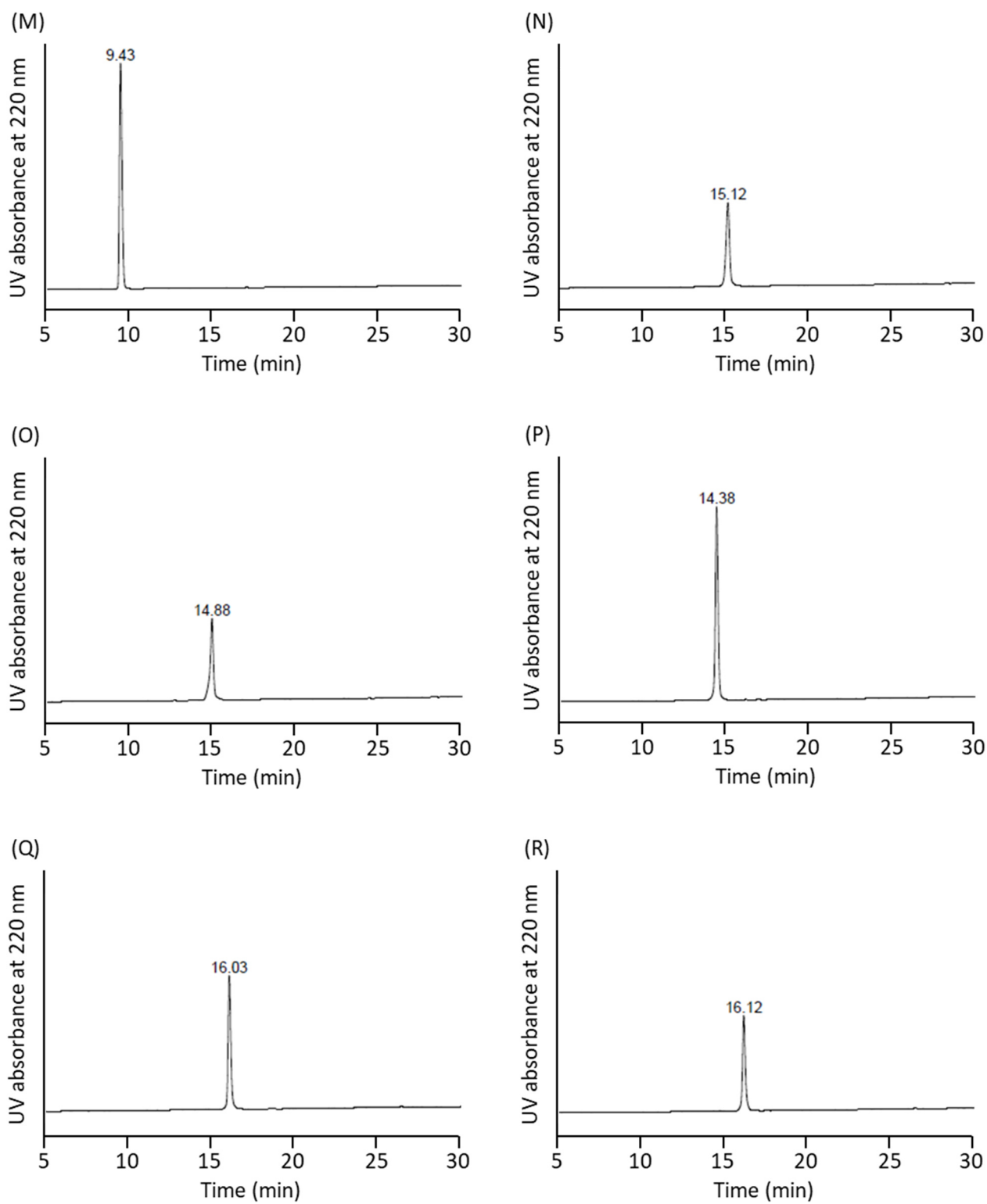


Fig. S3. (continued) HPLC chromatograms of purified synthetic peptides. (M–R) peptides 14–19. *HPLC conditions:* Linear gradient of 20–50% CH₃CN in H₂O over 30 min.

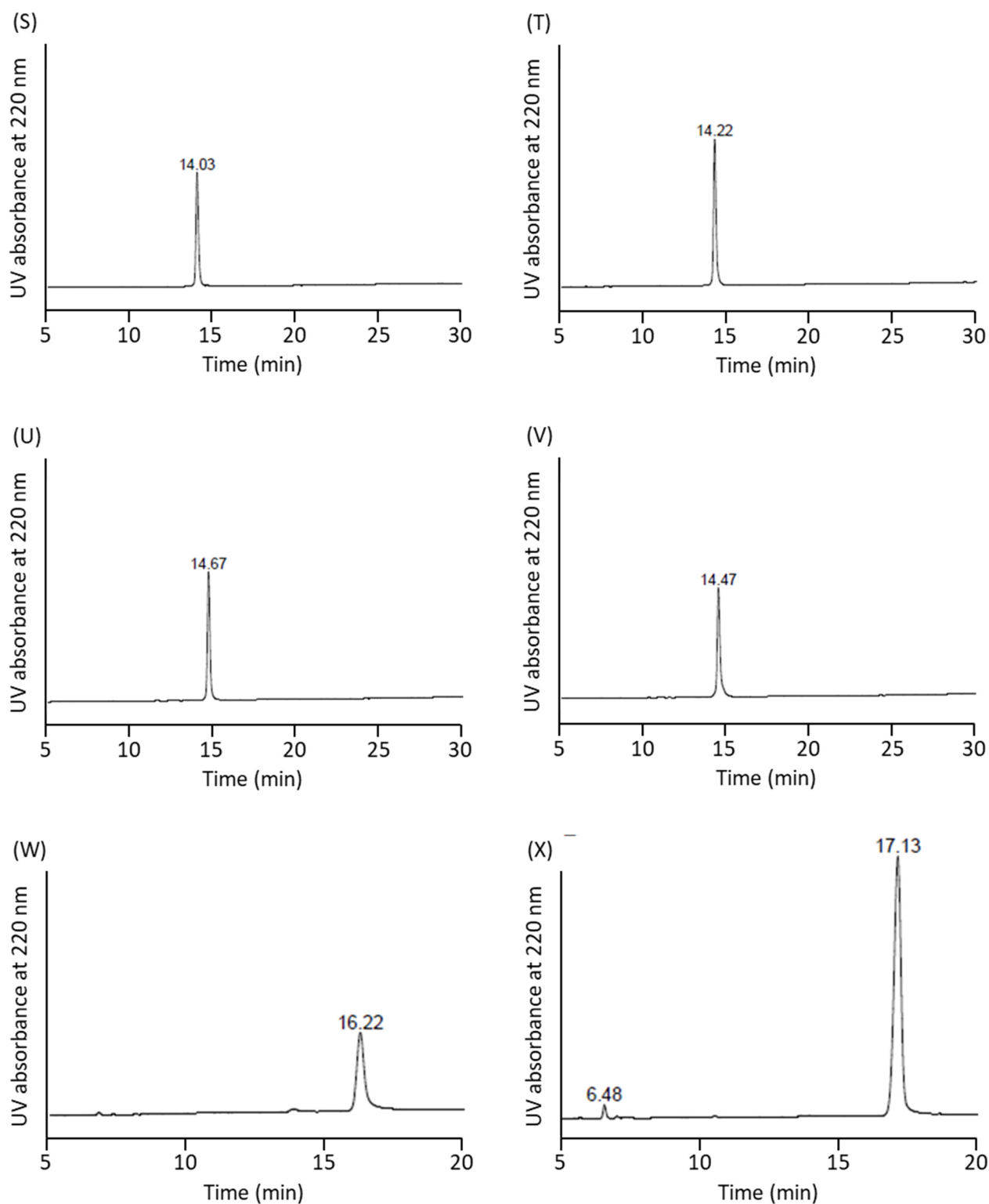


Fig. S3. (continued) HPLC chromatograms of purified synthetic peptides. (S–V) peptides **20–23**; (W) HR1X; (X) HR1Y. *HPLC conditions:* (S–V) Linear gradient of 20-50% CH₃CN in H₂O over 30 min; (W, X) Linear gradient of 40-60% CH₃CN in H₂O over 20 min.

Reference

- S1. K. Hirata, A. Takahara, S. Suzuki, S. Murakami, K. Kawaji, A. Nishiyama, M. Sasano, M. Shoji-Ueno, E. Usui, K. Murayama, H. Hayashi, S. Oishi, and E. N. Kodama, *iScience*, 2024, **27**, 108961.