Supplementary Data

A Bifunctional SARS-CoV-2 Entry Inhibitor Targeting the Host Protease TMPRSS2 and Viral Spike Protein HR1 Region

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Table S1. HPLC method used for the purification of peptide compounds[a]

Time (min)	Solvent A (%)	Solvent B (%)
5	60	40
25	50	50
50	35	65
60	35	65
65	60	40

[a] Crude peptide products were purified by preparative reverse-phase HPLC with a Shimadzu preparative HPLC system (LC-20A) on a Thermo Scientific HYPERSIL PREP HS C8 column (30mm × 250mm, 5μm) at constant flow rate of 15 mL/min. Solvent A: 0.1% trifluoroacetic acid in H_2O; Solvent B: 0.1% trifluoroacetic acid in 70% CH_3CN/H_2O.

Table S2. HPLC method used for the analysis of peptide compounds[a]

Methods	Time (min)	Solvent A (%)	Solvent B (%)
Method A	5	50	50
	10	0	100
	15	0	100
	20	0	100
	23	0	100
	25	90	10
Method B	5	50	50
	10	30	70
	15	10	90
	20	0	100
	34	0	100
	35	90	10

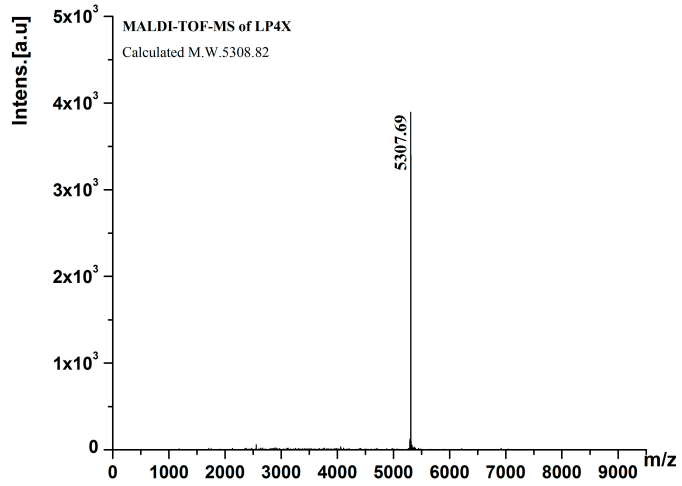
[a] Peptide compounds were analyzed by analytical RP-HPLC performed on a RP-C8 column (X-Bridge C8, 4.6 × 250 mm, 5 μm) using two different solvent systems (Methods A and B) and a flow rate of 1 mL/min with detection at 210 nm. Solvent A: 0.1% trifluoroacetic acid in H_2O; Solvent B: 0.1% trifluoroacetic acid in 70% CH_3CN/H_2O.

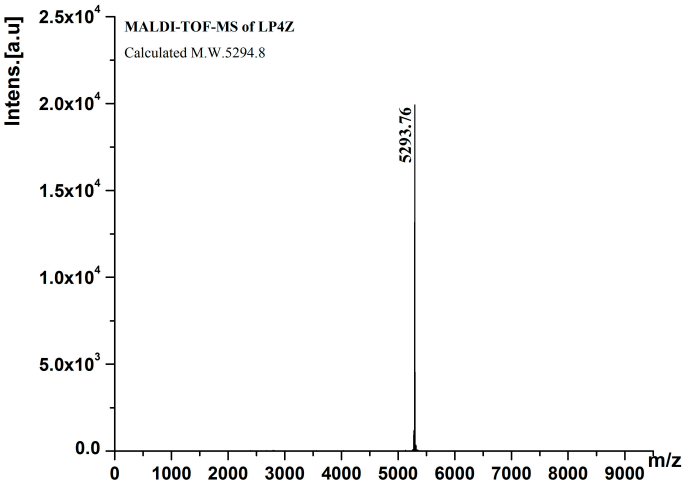
Table S3. Antiviral activity of entry inhibitors against pseudotyped SARS-CoV-2 Omicron strain [a]

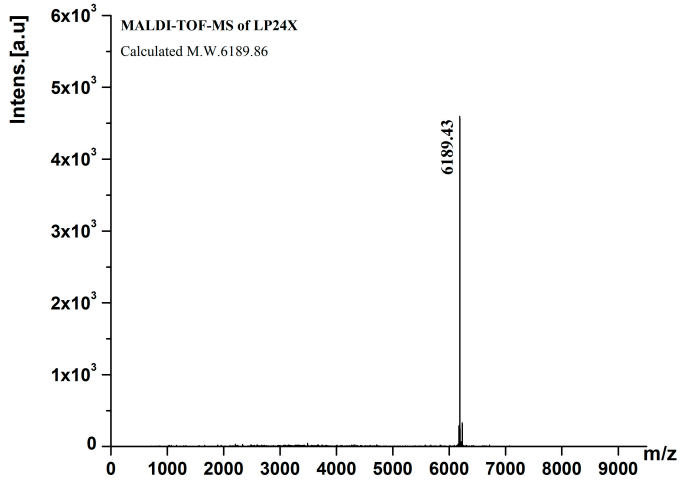
Name	IC_{50} (μM)
IP4X	0.83±0.01
IP4Z	0.76±0.08

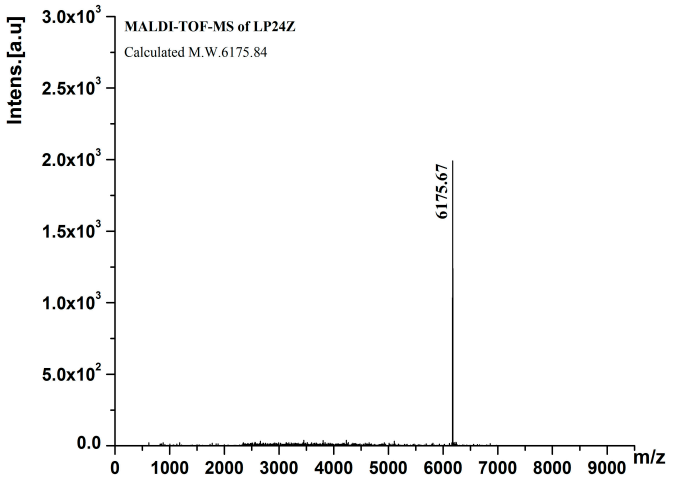
[a]IC_{50} values were obtained with pseudotyped SARS-CoV-2 Omicron strain in Caco2 cells that express TMPRSS2 on the surface. The assay was performed in triplicate, and the data are expressed as the mean ± standard deviation.

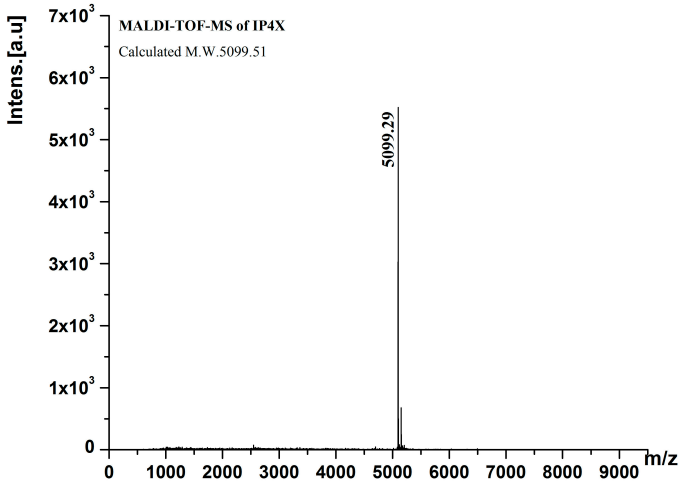
MALDI-TOF-MS and analytical HPLC of designed peptides

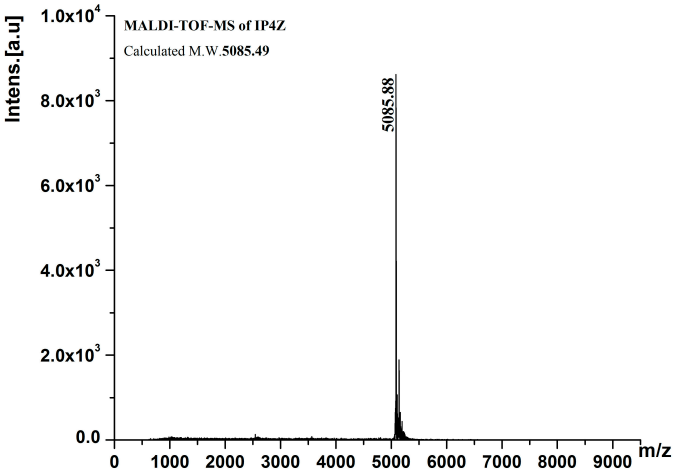


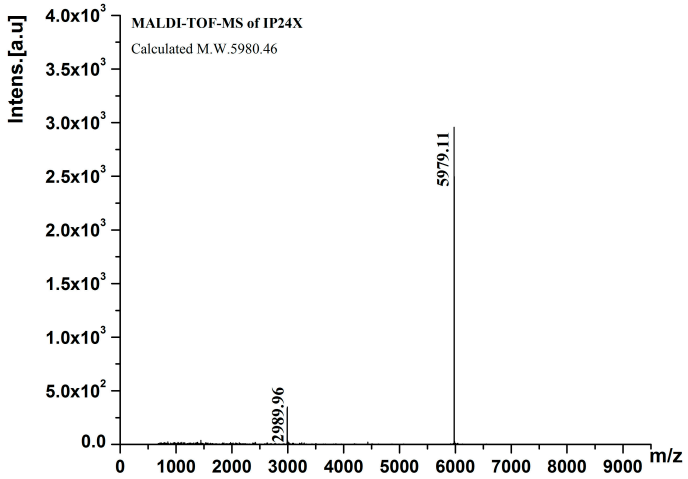


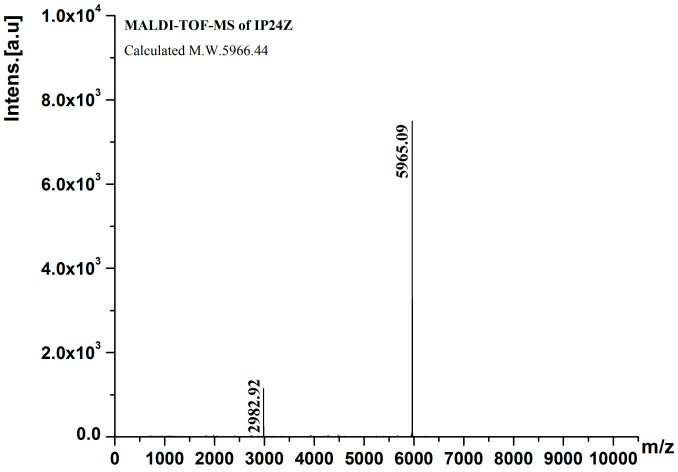









MALDI-TOF-MS of IP24X
Calculated M.W.5980.46
Intens.[a.u]
m/z
2989.96
5979.11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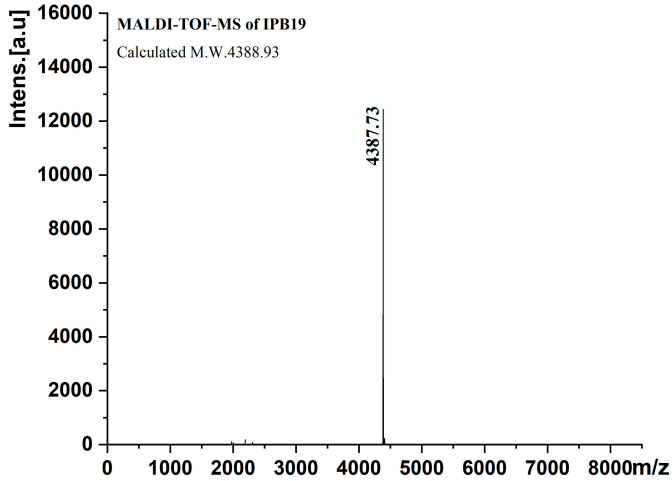
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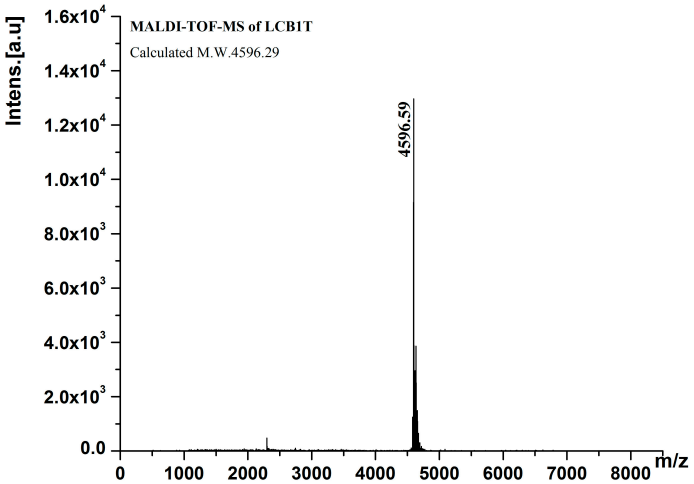
MALDI-TOF-MS of LCB1T
Calculated M.W.4596.29
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m/z
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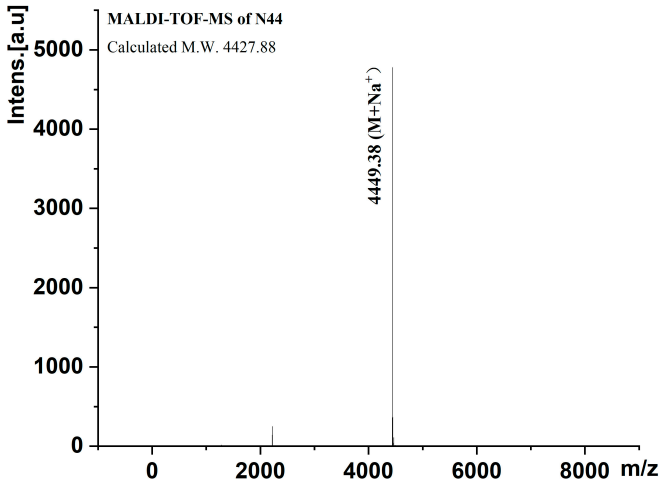
MALDI-TOF-MS of N44
Calculated M.W. 4427.88
Intens.[a.u]
m/z
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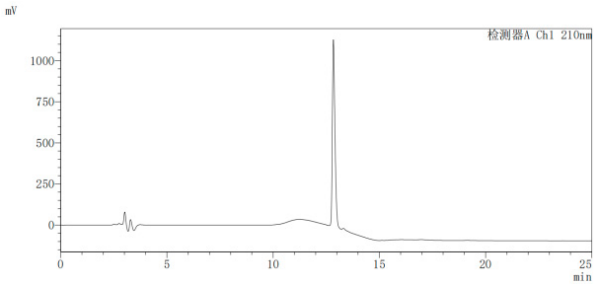
Analytical HPLC of LP4X
[98 % purity, t_R (Method A) = 12.83 min]



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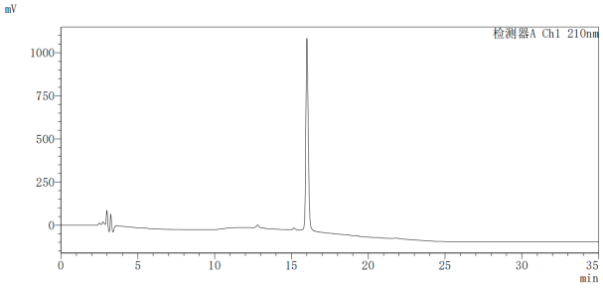

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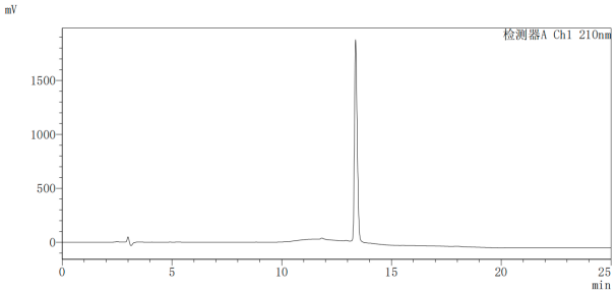

Analytical HPLC of LP4Z
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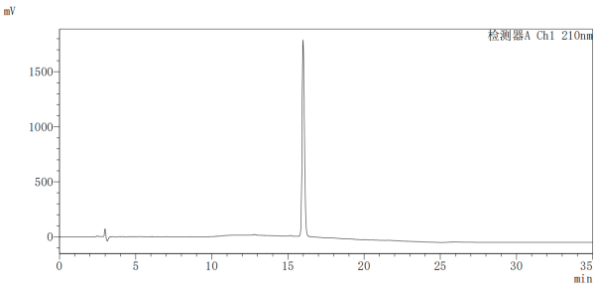

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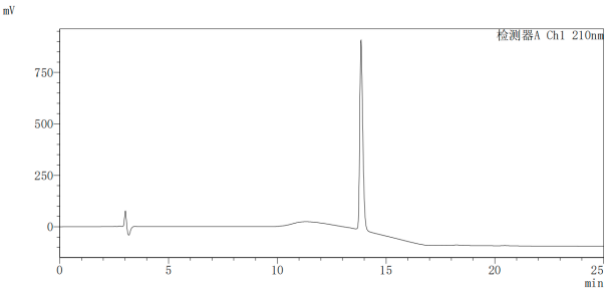

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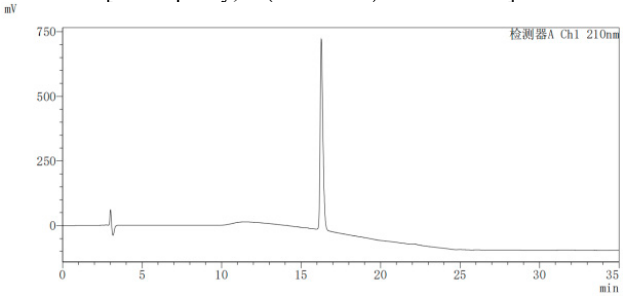
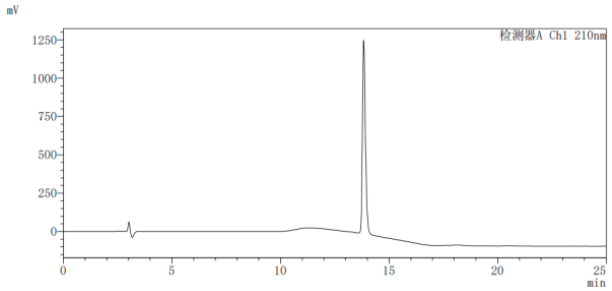


Analytical HPLC of LP24Z
[99 % purity, t_R (Method A) = 13.83 min]



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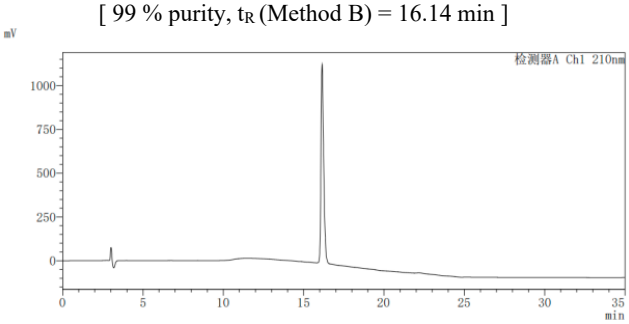

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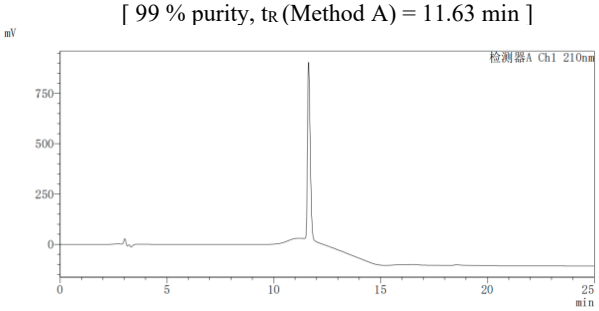

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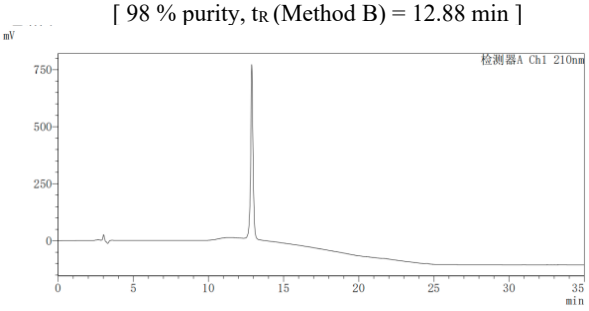
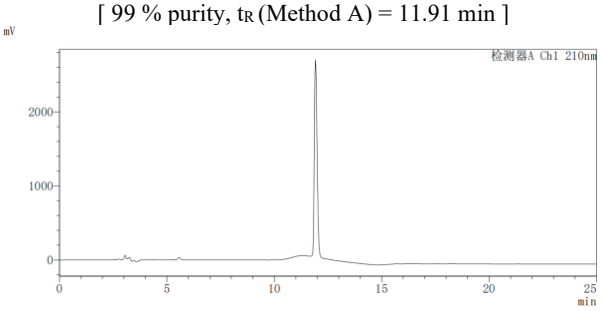
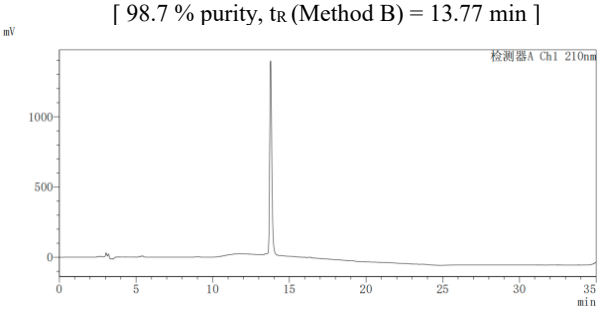


Analytical HPLC of IP4Z
[99 % purity, t_R (Method A) = 11.91 min]



Analytical HPLC of IP4Z
[98.7 % purity, t_R (Method B) = 13.77 min]



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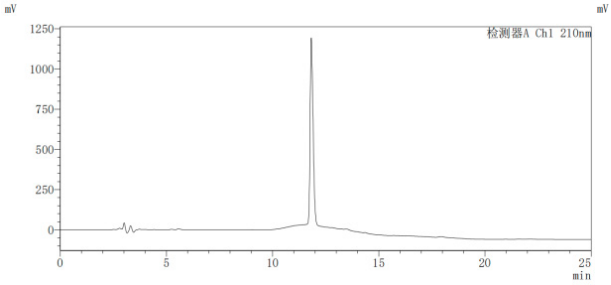
[99 % purity, t_R (Method A) =11.82 min]



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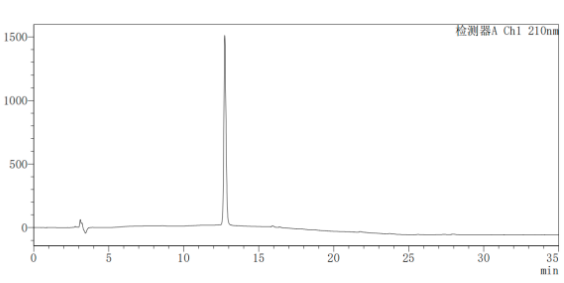

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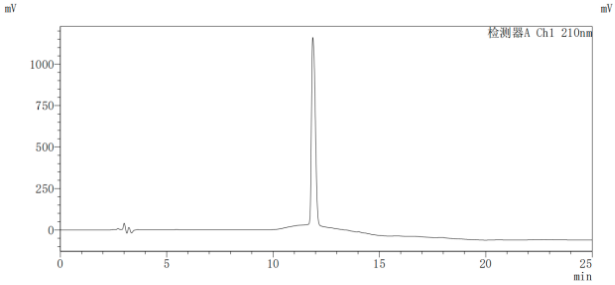

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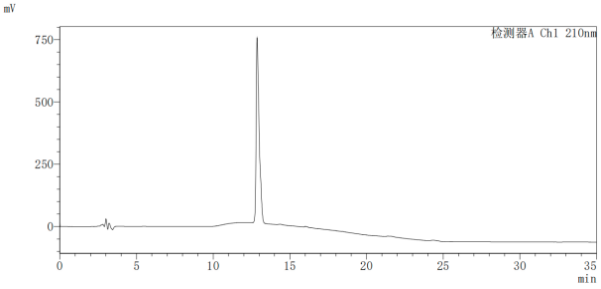

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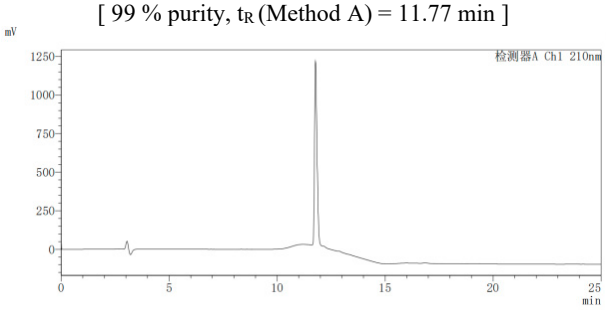

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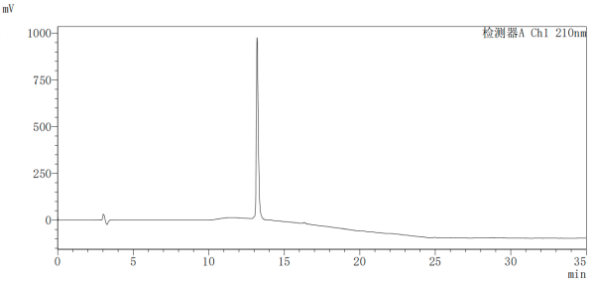
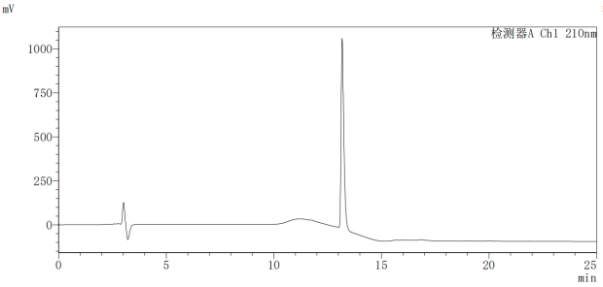


Analytical HPLC of LCB1T
[99 % purity, t_R (Method A) = 13.17 min]



Analytical HPLC of LCB1T
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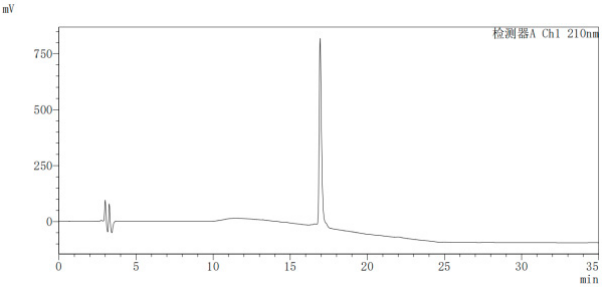

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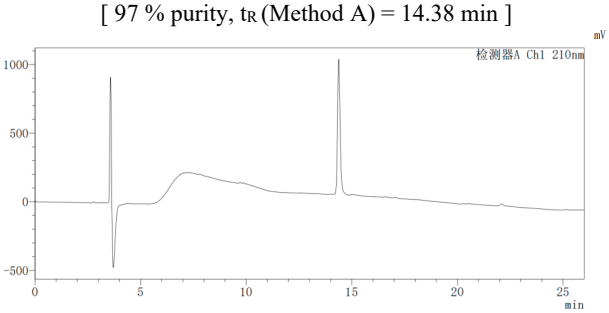

Analytical HPLC of N44
[98 % purity, t_R (Method B) = 16.21 min]

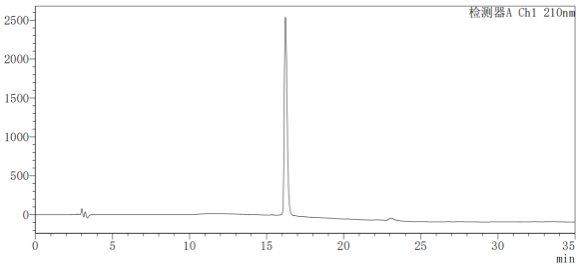

Figure S1. MALDI-TOF-MS and Analytical HPLC of designed peptides