Supporting Information

An Artificial Peptide-based Bifunctional HIV-1 Entry Inhibitor That Interferes with Viral Glycoprotein-41 Six-Helix Bundle Formation and Antagonizes CCR5 on the Host Cell Membrane

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Table of Contents

Supplemental Tables and Figures

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

Time (min)	Solvent A (%)	Solvent B (%)
5	70	30
13	53	47
30	17	83
35	17	83
55	70	30
60	70	30

[a] The crude peptide products were purified by preparative reverse phase HPLC with a Waters preparative HPLC system (PrepLC 4000) on a Waters X-bridge C8 column (19.5mm × 250mm, 10μm) at constant flow rate of 16 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
	23	0	100
	25	90	10

[a] The peptide compounds were analyzed by analytical RP-HPLC was performed on a RP-C8 column (Zorbax Eclipse XDB-C8, 4.6 × 150 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.

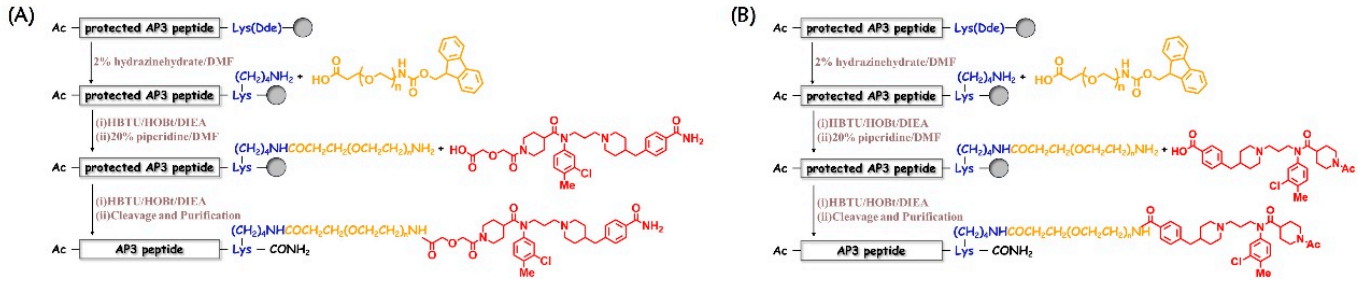


Figure S1. Schematic representation of the strategy used for preparation of bifunctional molecules

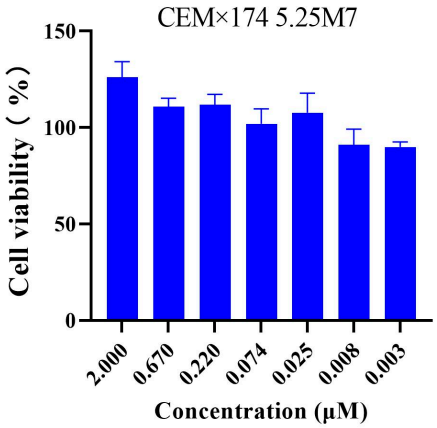
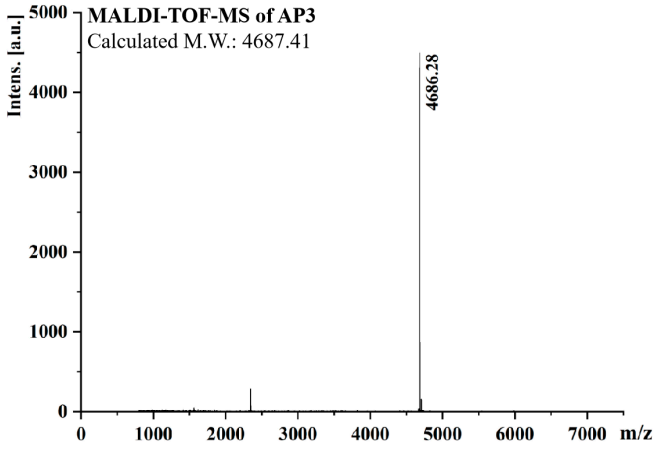
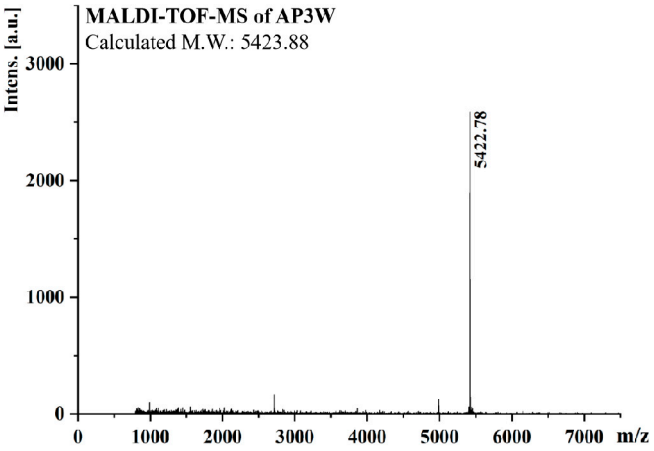
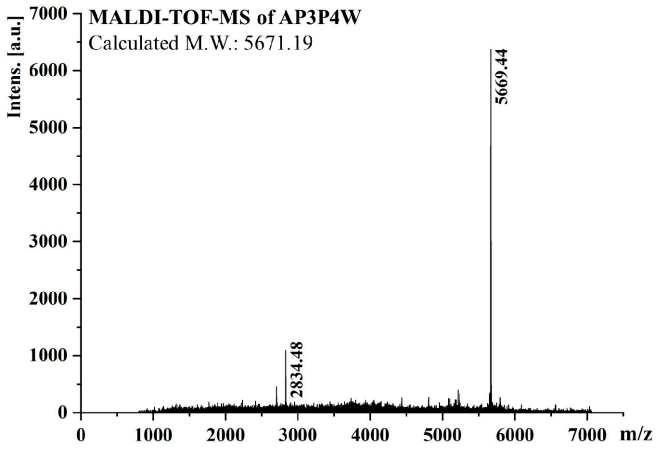


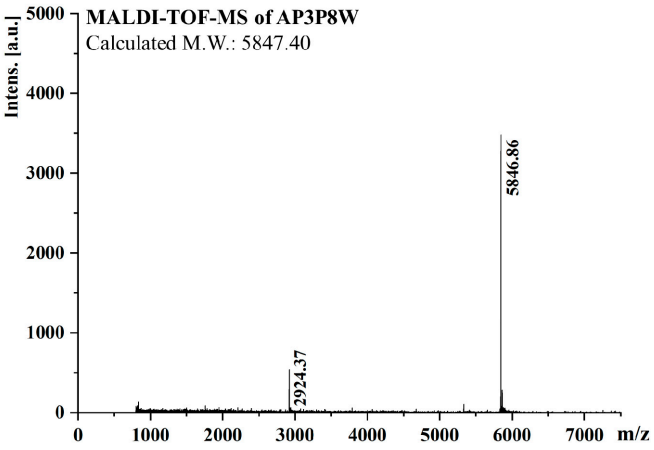
Figure S2. The potential cytotoxicity of AP3P4E on the CEMx174 5.25M7 cells.

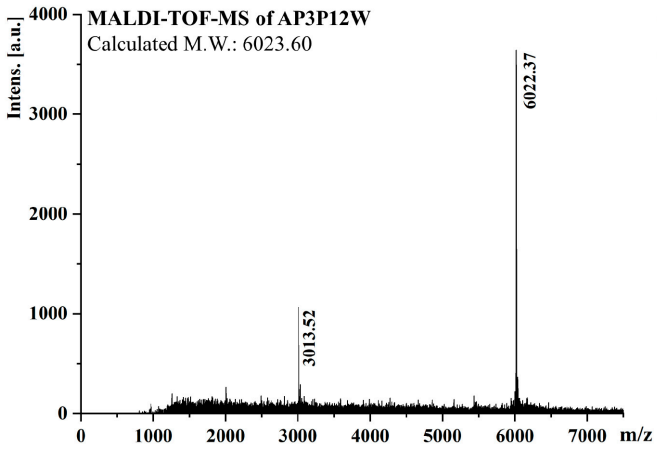
MALDI-TOF-MS and analytical HPLC of designed peptides

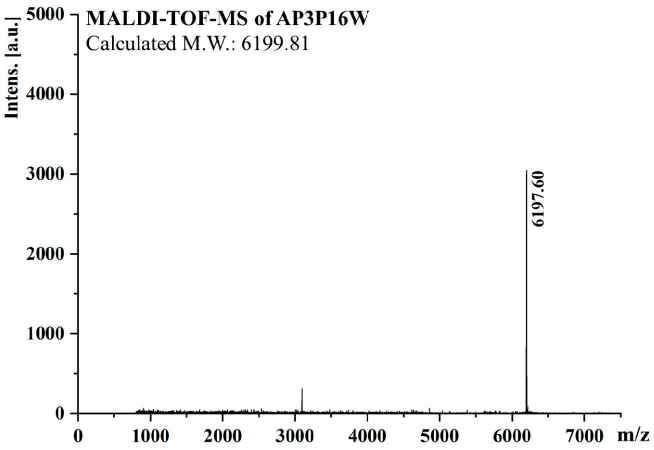


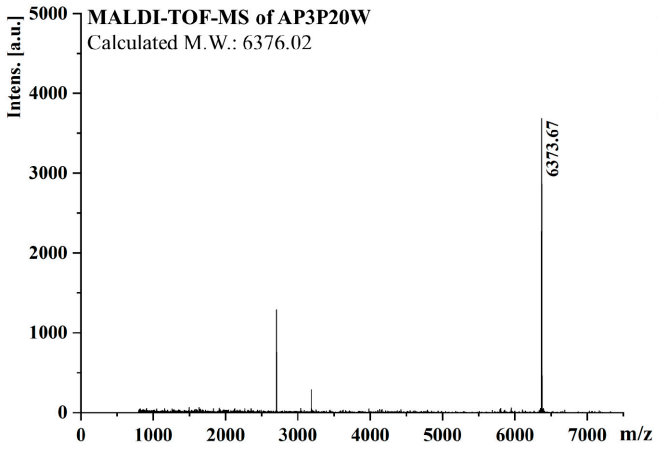


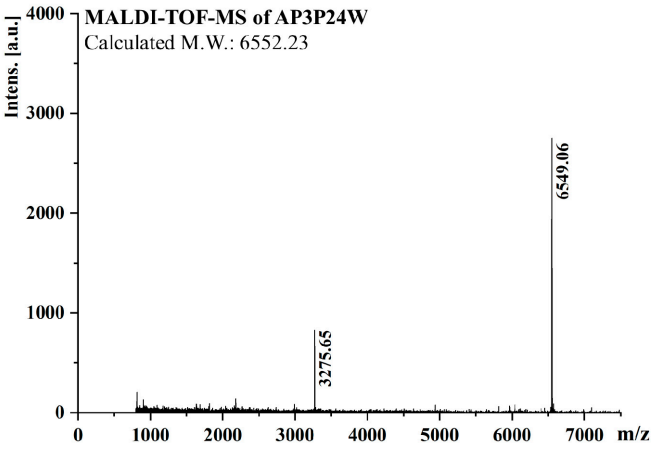









MALDI-TOF-MS of AP3P20W
Calculated M.W.: 6376.02
Intens. [a.u.]
6373.67
m/z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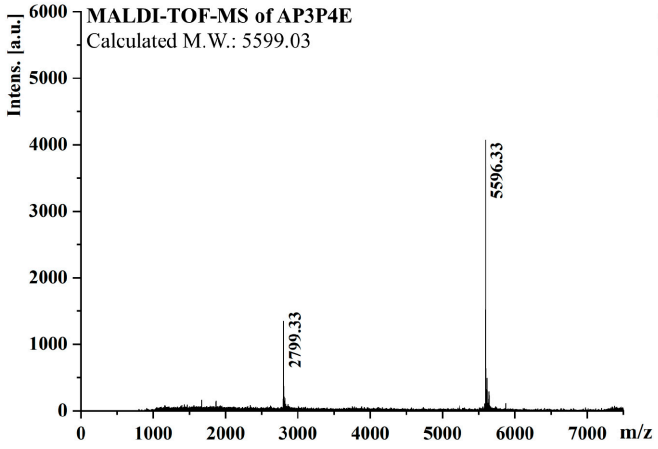
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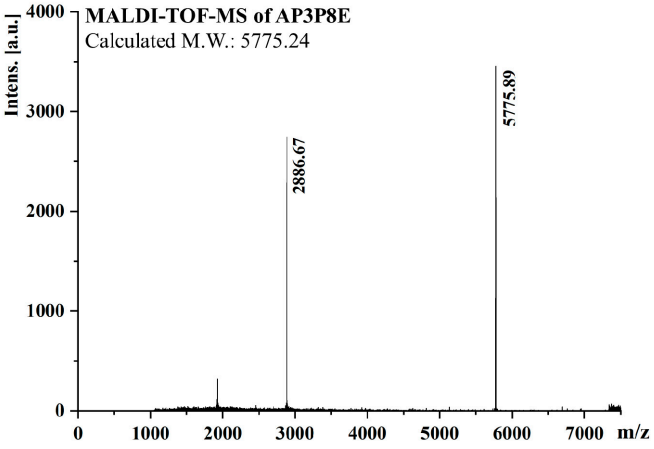
m/z


MALDI-TOF-MS of AP3P8E
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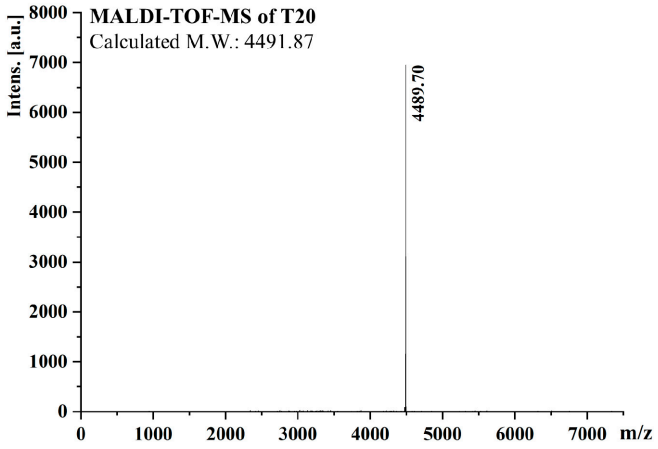
2886.67
m/z


MALDI-TOF-MS of T20
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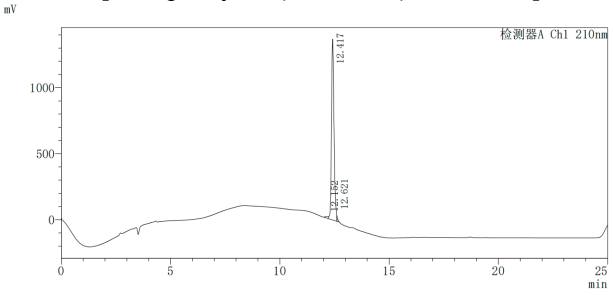
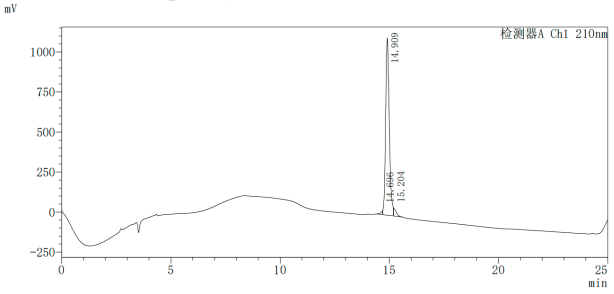
m/z

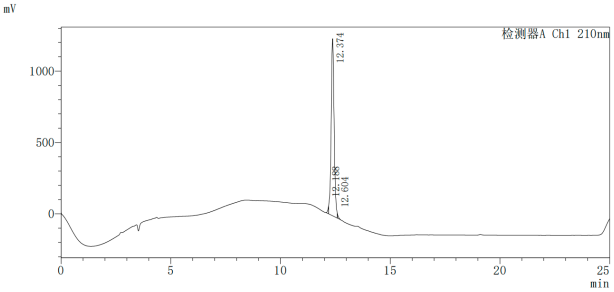
Analytical HPLC of AP3
[97% purity, t_R (Method A)= 12.4 min]



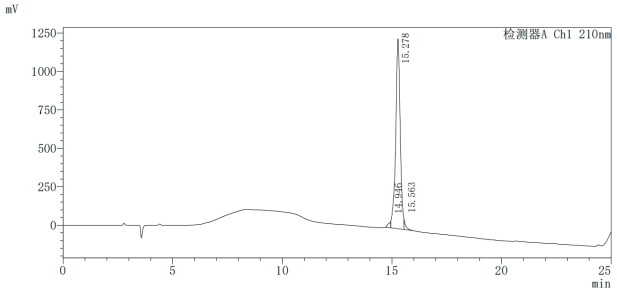
Analytical HPLC of AP3
[96% purity, t_R (Method B)= 14.9 min]



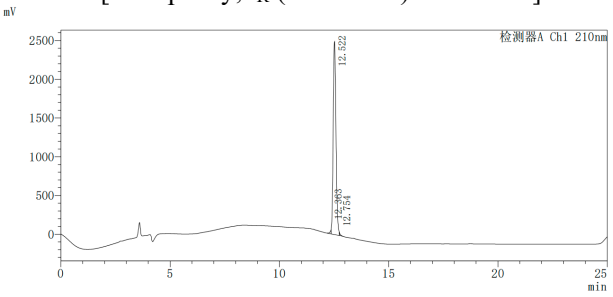
Analytical HPLC of AP3W
[99% purity, t_R (Method A)= 12.4 min]



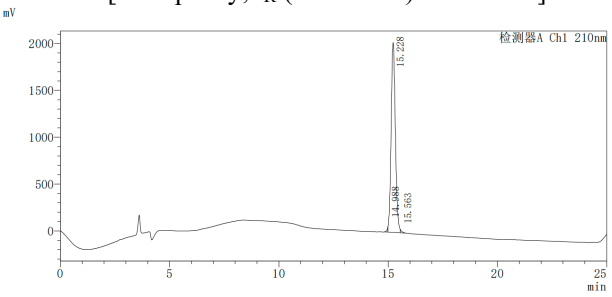
Analytical HPLC of AP3W
[96% purity, t_R (Method B)= 15.3 min]



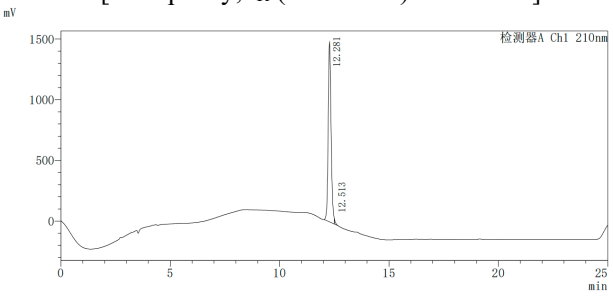
Analytical HPLC of AP3P4W
[99% purity, t_R (Method A)= 12.5 min]



Analytical HPLC of AP3P4W
[99% purity, t_R (Method B)= 15.2 min]



Analytical HPLC of AP3P8W
[99% purity, t_R (Method A)= 12.3 min]



Analytical HPLC of AP3P8W
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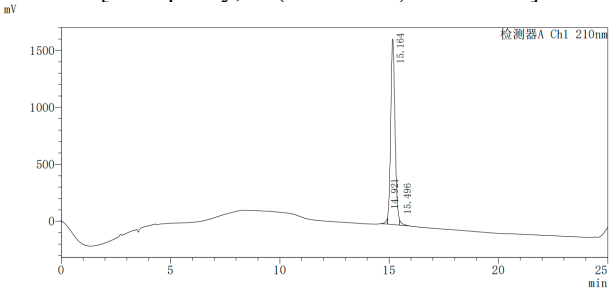
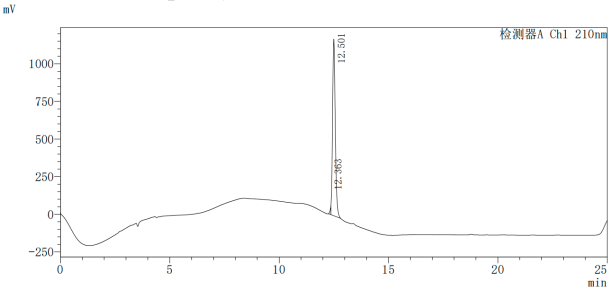


Analytical HPLC of AP3P12W
[99% purity, t_R (Method A)= 12.5 min]



Analytical HPLC of AP3P12W
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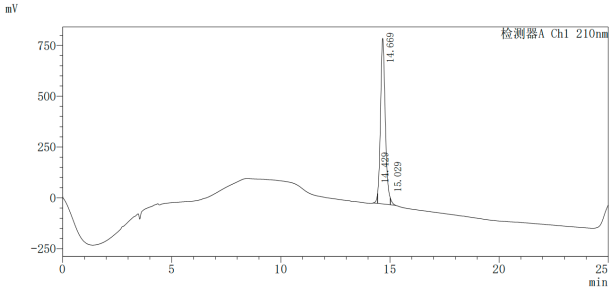

Analytical HPLC of AP3P16W
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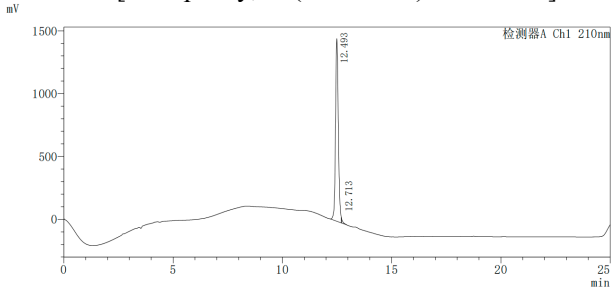
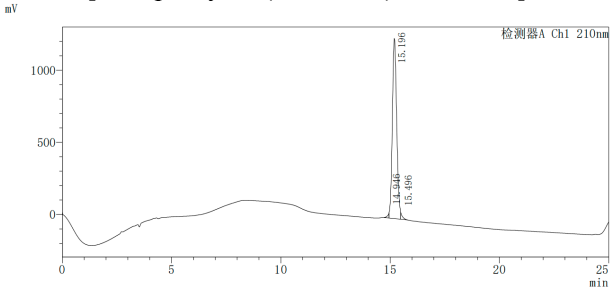
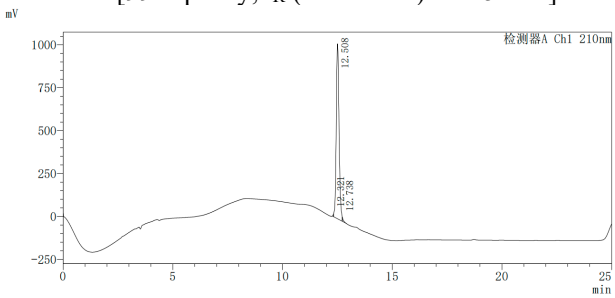


Analytical HPLC of AP3P16W
[98% purity, t_R (Method B)= 15.2 min]



Analytical HPLC of AP3P20W
[99% purity, t_R (Method A)= 12.5 min]



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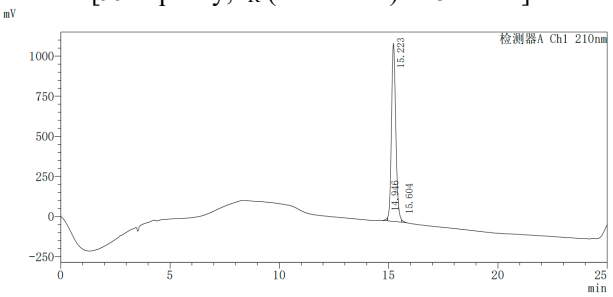

Analytical HPLC of AP3P24W
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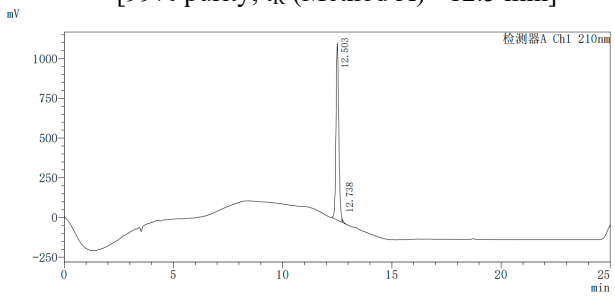

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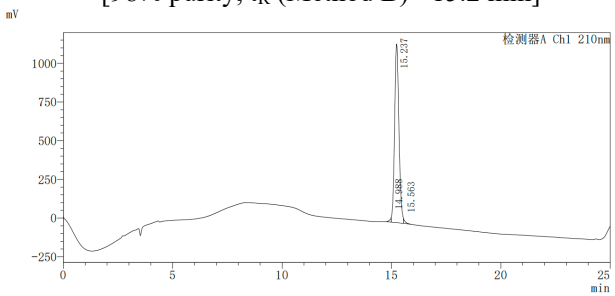

Analytical HPLC of AP3P4E
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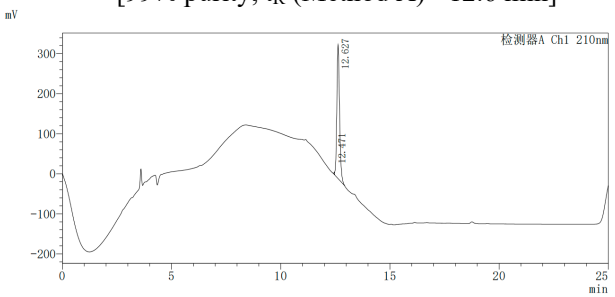

Analytical HPLC of AP3P4E
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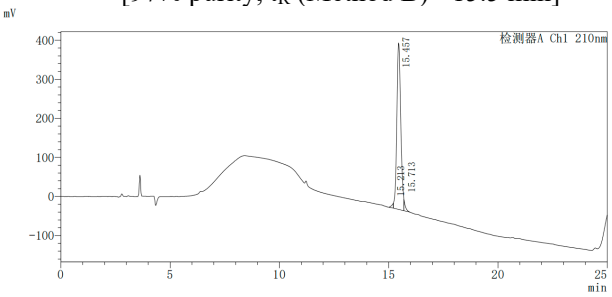

Analytical HPLC of AP3P8E
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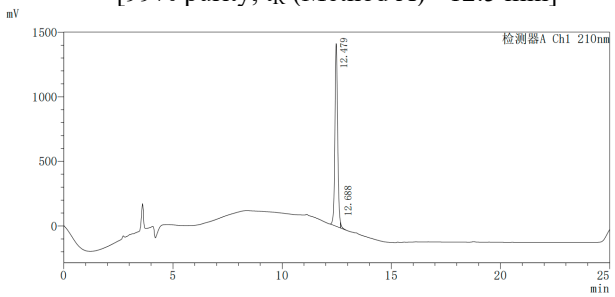
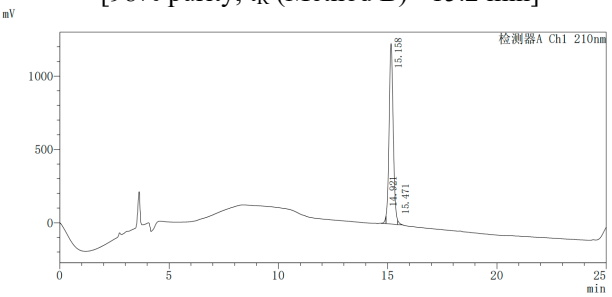


Analytical HPLC of AP3P8E
[98% purity, t_R (Method B)= 15.2 min]



Analytical HPLC of T20

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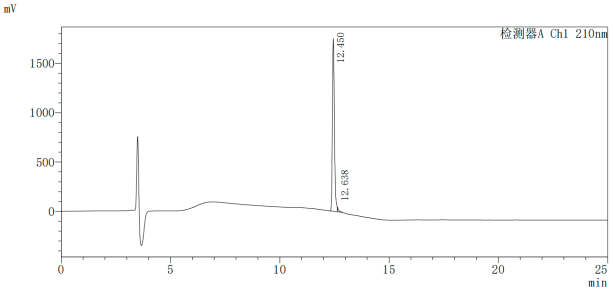

Analytical HPLC of T20

[96% purity, t_R (Method B)= 16.8 min]