Supporting Information

Sulfono-γ-AA modified peptides that inhibit HIV-1 fusion

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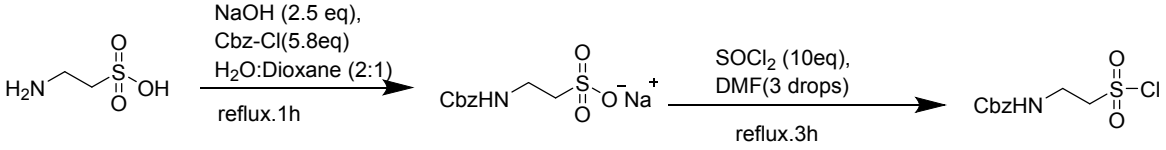
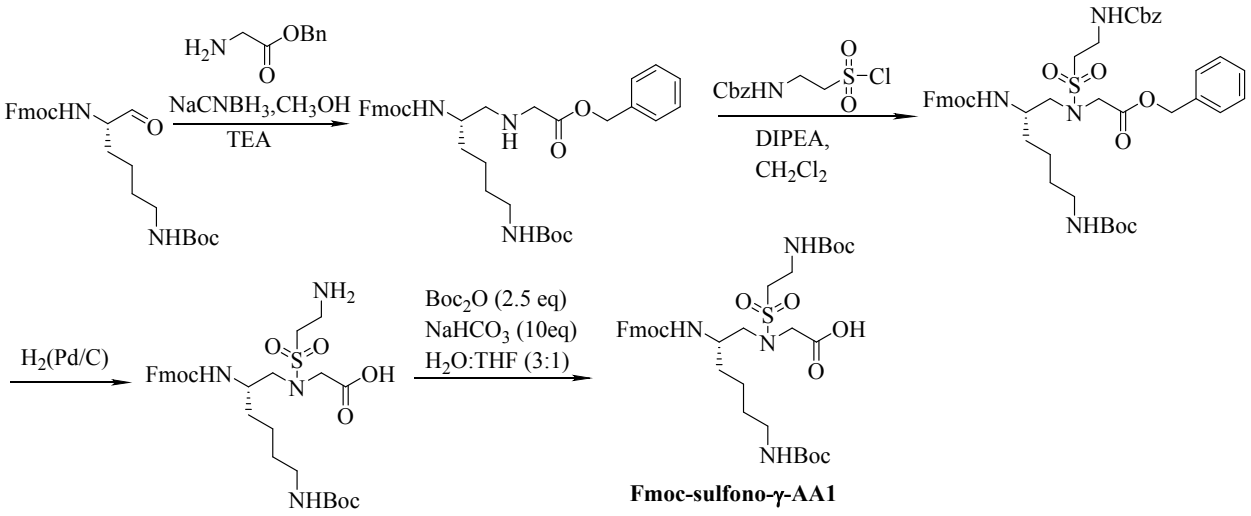
1. General information

All peptides used in this study were synthesized by the standard Fmoc-based solid phase peptide synthesis protocol using the Burrell Wrist-Action shaker. All Fmoc protected α-amino acids and Rink amide resin (0.7mmol/g, 200-400 mesh) were purchased from Chem-Impex International, Inc. All other Solvents and reagents were purchased from either Sigma Aldrich or Fisher Scientific and used without further purification. The peptides were analyzed and purified on a Waters Breeze 2 HPLC System, and then lyophilized on a Labconco Lyophilizer

2. Synthesis of sulfono- γ-AApeptides



Scheme S1. Synthetic schemes for Cbz-aminoethanesulfonyl chloride



Scheme S2. Synthetic schemes for Fmoc-sulfono-γ-AA1.

2.1 Aminoethanesulfonylchloride. Taurine (5g) and NaOH (2.5 eq) were dissolved in water and was kept stirring at 0°C.Benzylchloroformate (5.8 eq) was mixed with Dioxane and added dropwise to the solution. The ice bath was then removed and the reaction was stirred for 1h. The solution was washed with EtOAc three times. The aqueous layer was co-evaporated with

Toluene (2 times), Ethanol (once) and DCM (3 times).The resulting white solid, Cbz-protected aminoethane sodium sulfonate was suspended in EtOAc and filtered. To the white solid, Thionyl Chloride (10eq) and DMF (3 drops) were added and the reaction was refluxed for three hours and then concentrated on rotavap to obtain the Cbz-protected aminoethanesulfonyl chloride (Scheme S1).

2.2 Fmoc-sulfono-γ-AA1. Fmoc-Lysine amino aldehyde and Glycine benzyl ester were dissolved in methanol. Triethylamine was added and the reaction was stirred for one hour. Sodium cyanoborohydride was then added to the reaction and left to stir for another hour. The solution was concentrated, extracted with EtOAc and further concentrated on the rotavap. The crude secondary amine was purified on silica gel with Hexanes/EtOAc (1:1) as eluent. After that, the secondary amine was dissolved in DCM, to which Cbz-protected aminoethanesulfonylchloride and DIPEA were added. The reaction was stirred overnight and the resulting product was purified on silica gel (eluent was hexanes/EtOAc 2:1). Next, hydrogenation was done in methanol and under hydrogen with 20% Pd-C for 1 h to remove the benzyl and carbamate group to yield the mono-Boc protected Fmoc-sulfono- γ-AA1. Boc protection of the amino group on the sulfonyl side chain was achieved by adding Boc anhydride in THF to a stirred solution of Fmoc-sulfono- γ-AA1 (Boc) and NaHCO3 in water in a dropwise manner. The reaction was allowed to continue overnight at room temperature. 1N citric acid was added, followed by extraction with EtOAc and drying over Na_2SO4.The solution was then concentrated and purified on silica gel. The product, Fmoc-sulfono- γ-AA1 was eluted with Hexanes/EtOAc 1:1(Scheme S2) to give a white solid.

2.3 Solid Phase synthesis. The synthesis was conducted on 200 mg Rink amide resin (0.7 mmol/g) at room temperature and atmospheric pressure. The Fmoc protecting group was

removed by treatment with 3mL 20% Piperidine in DMF solution for 15 mins(x2).The resin was washed with DCM and DMF (three times each).A solution of Fmoc-aa-OH/ Fmoc-sulfono-γ-AA1/ Fmoc-(S)-2-(4-pentenyl)Ala-OH (2 eq),HOBt (4 eq) and DIC (4 eq) was first premixed in 2mL DMF and then transferred into the peptide vessel containing the deprotected resin. The mixture was allowed to shake for 4h. The resin is then washed with DCM and DMF (3x each) and capped with acetic anhydride (1mL) and Pyridine (2mL) for another 15 min. After capping, deprotection was done and the cycle was repeated until the desired product was obtained.

2.4 Olefin Metathesis and Purification. Ring-closing metathesis (RCM) of resin-bound N-Fmoc, side chain protected peptides was performed using 20 mol% of Grubbs I catalyst in degassed DCE for 2 hours at room temperature. The resin-bound peptide was washed in the peptide vessel and filtered. The RCM reaction was repeated once again to complete the cross-linking reaction. The reaction was monitored by LC/MS after cleavage of peptide from an aliquot of resin. The resulting stapled peptide was deprotected and cleaved from the resin with a cleavage cocktail mixture of TFA/Phenol/H2O/thioanisole/1, 2-ethanedithiol (82.5%/5% /5% / 5%/2.5% v/v) for 3h and then precipitated with diethyl ether. The crude peptides(stapled and unstapled) were analyzed and purified on an analytical(1mL/min) and a preparative (16mL/min) RP-HPLC respectively.5% to 100% linear gradient of solvent B(0.1% TFA in acetonitrile) in A(0.1 % TFA in water) over 40 min was used. The HPLC traces were detected at 215nm.The fraction containing the desired peptides/stapled peptide was collected, confirmed on an Applied Biosystems 4700 Proteomics Analyzer and lyophilized.

3. Circular Dichroism Analysis

Circular dichroism (CD) spectra were measured on an Aviv 215 circular dichroism spectrometer using a 1 nm bandwidth and 1nm step resolution from 195 to 260nm at 25°C. Peptide solutions

were prepared in water to a final concentration of 300μM.The samples were loaded in a 1mm path length quartz cell, and the CD spectra were acquired with an averaging time of 1s; Settling time of 0.333s and multi-scan wait time of 1s. 3 scans were averaged for each sample and the final spectra were normalized by subtracting the average blank spectra. The data unit was converted from machine units (millidegree,θ) into mean residue ellipticity [θ].Mean Residue Ellipticity [θ] (deg.cm^2.dmol^{-1}) was calculated using the equation:

$$[\theta] = \theta_{obs} / (p \times c \times 10 \times n) \quad \text{(i)}$$

Where θ_{obs} is the measured ellipticity in millidegrees, while p is the path length in centimeter, n is the number of side chains and c is the concentration of the peptide in Molar.

The temperature-dependent unfolding was carried out by monitoring the change in ellipticity [θ] at 222nm at increasing temperature (5-70°C) after 1.6 mins equilibration at the desired temperature and an integration time of 1s.The temperature was increased at the rate of 1°C per min; data acquisition was done with the following parameters: 1-nm bandwidth; λ = 222nm

4. HIV-1 Neutralization Assay

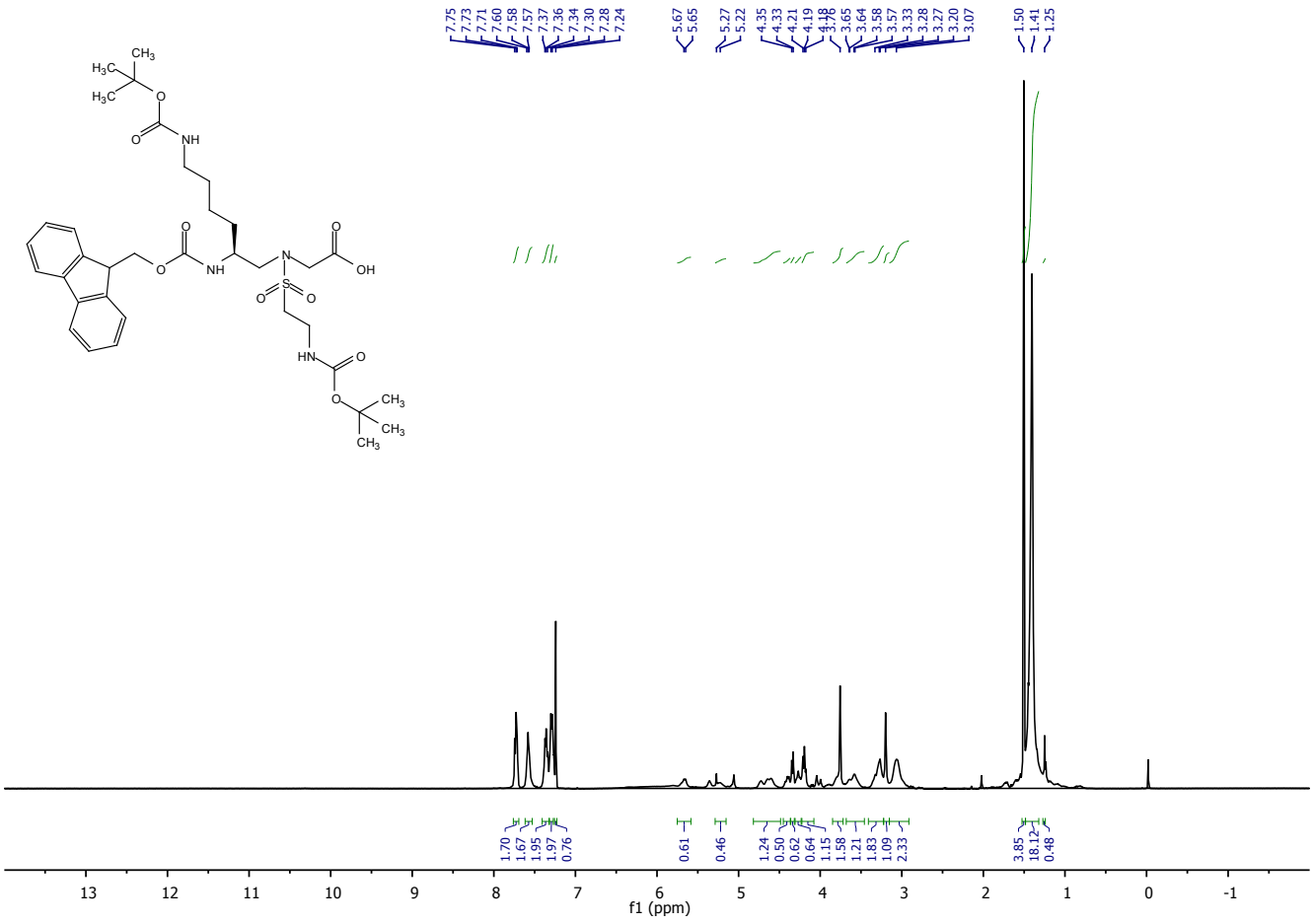
The preparation of Env-pseudotyped virus stocks in 293T cells by calcium phosphate transfection and the use of the engineered HIV-1 coreceptor-bearing cell line U87-CD4$^+$-CCR5$^+$ (3,000 cells per well) for Env-pseudotyped virion infection were described previously.[1, 2] Briefly, the envelope genes were amplified by PCR, cloned into an expression vector and cotransfected with an envelope-defective proviral plasmid to generate pseudotyped luciferase-encoding viruses. Recombinant viruses were used to infect U87-CD4$^+$-CCR5$^+$ cells (3,000 cells per well) in the presence of varying concentrations of peptide. The amount of input pseudovirus

was normalized by infectivity (virus titer) rather than by p24 antigen content. Luciferase activity in the target cells was measured in relative light units (RLU) 72 h after infection. Data from duplicate measurements were fit to the variable-slope-sigmoid equation to obtain IC_{50} values.[3]

5. Protease Stability Assay

To evaluate the stability of the peptides against enzyme degradation, the peptides (0.1 mg/ml) were incubated with Chymotrypsin (0.1mg/ml) in 100mM pH 7.8 ammonium bicarbonate buffer for 30 mins. The residual substrate and digested products were quantified by LC-based peak detection at 280nm. Each experiment was done in duplicate.

6. The NMR Spectrum of Fmoc-sulfono-γ-AA1



^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71-7.75 (t, *J* = 8.00 Hz, 2H), 7.57 – 7.60 (t, *J* = 8.00 Hz, 2H), 7.34 - 7.37 (t, *J* = 4.00 Hz, 2H), 7.28 – 7.3 (d, *J* = 8.00 Hz, 2H), 7.24 (s, 1H), 5.65 – 5.67 (d, *J* = 8.00 Hz, 1H), 5.22 – 5.27 (m, *J* = 8.00 Hz, 1H),4.60 – 4.73 (dd, *J* = 4.00, 16.00 Hz, 1H), 4.38 – 4.43 (t, *J* = 12.00 Hz,1H) ,4.33 – 4.35 (d, *J* = 8.00 Hz, 1H), 4.25 – 4.29 (t, *J* = 8.00 Hz,1H), 4.18 – 4.21 (t, *J* = 8.00 Hz,1H), 3.76(s,2H), 3.57 – 3.65 (dd, *J* = 4.00 Hz, 1H), 3.27 – 3.33 (t, *J* = 16.00 Hz, 2H),3.20 (s,1H), 3.07 (s,2H), 1.50 (s, 4H), 1.41 (s,18H), 1.25 (s,1H).

^{13}C NMR (400MHz, DMSO-d_6): δ 156.4, 155.9, 155.7, 144.4, 141.4, 127.9, 127.45, 125.6, 120.5, 78.4, 77.7, 70.2, 65.7, 65.3, 51.5, 47.2, 40.5, 40.3, 40.1, 39.9, 39.7, 39.5, 39.3, 35.3, 31.8, 29.8, 28.7, 23.2. HRMS (ESI): ($[M+H]^+$) calculated for $C_{35}H_{50}N_4O_{10}S$, 719.3281; found, 719.3336.

Fig S1. ^{1}H NMR (400 MHz, $CDCl_3$) and MS of Fmoc-sulfono-γ-AA1.

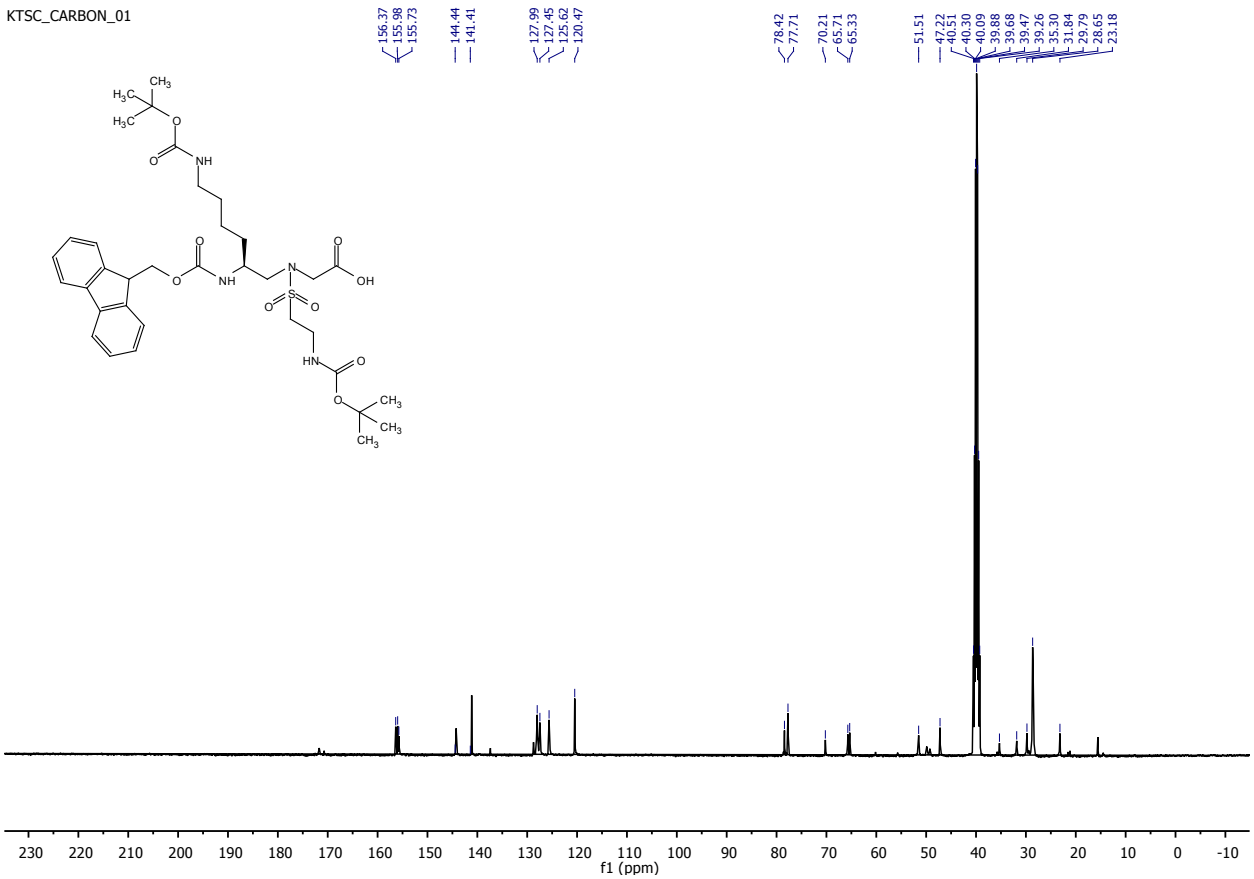
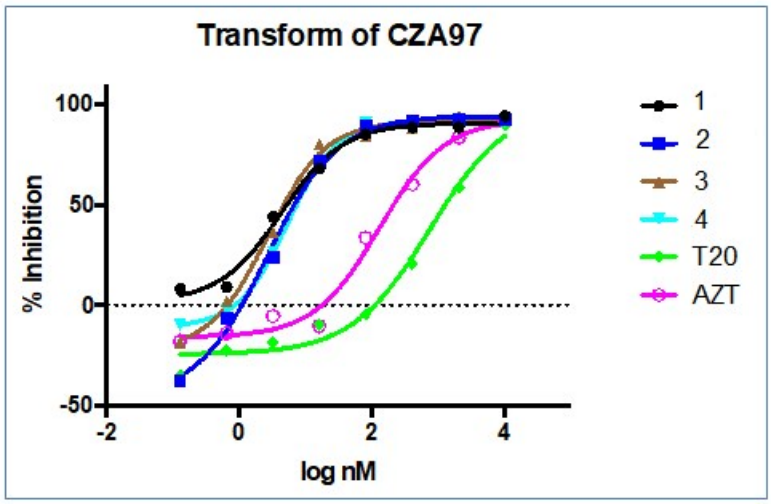


Fig S2. ^{13}C NMR (400 MHz, DMSO-d_6) of Fmoc-sulfono-γ-AA1.

Table S1. HIV-Neutralisation Assay – Raw Data

IC50 (nM)	1	2	3	4	T20	AZT
CZA97	8.43	8.60	4.50	5.70	612.67	158.24
	5.22	6.70	4.65	7.31	1269.34	210.99
B41	270.15	269.66	122.73	166.65	198.46	123.13
	91.96	151.88	114.80	175.86	230.82	89.59
BG505	15.08	13.50	8.75	7.05	8.34	85.81
	15.40	14.48	9.18	12.28	28.55	82.25
MLV	>10000	>10000	>10000	>10000	>10000	94.22
	>10000	>10000	>10000	>10000	>10000	94.99
SF162	13.52	16.49	8.91	10.64	21.83	133.4
	9.07	14.68	5.40	12.26	25.95	86.06
MN	72.50	57.98	75.50	41.03	5.85	192.50
	132.27	76.27	80.34	61.36	7.14	195.78
DU422	15.04	17.13	8.67	11.14	54.38	181.45
	14.54	14.91	3.31	11.42	57.55	185.22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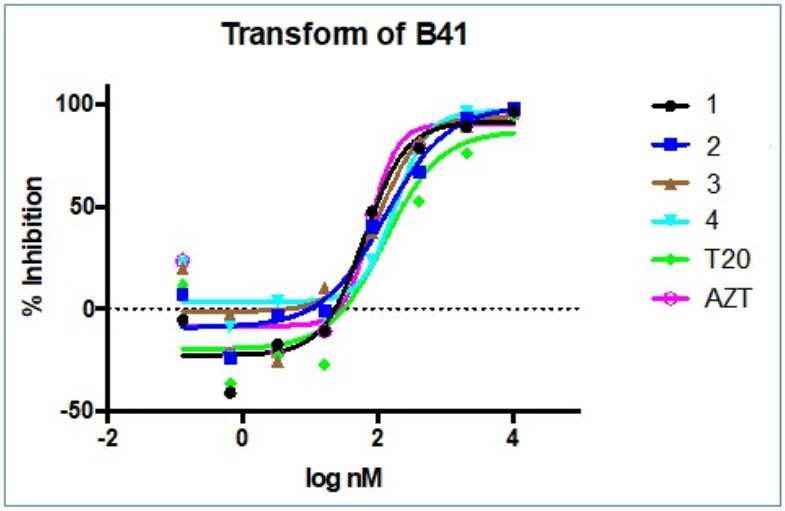
T20
AZT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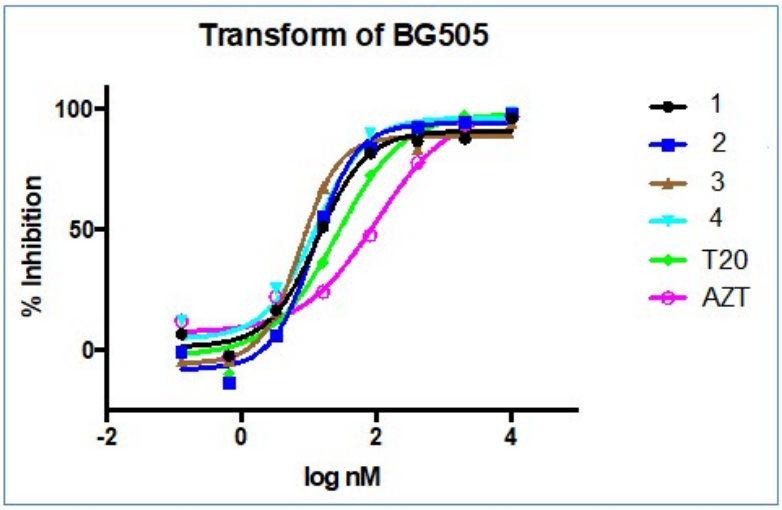
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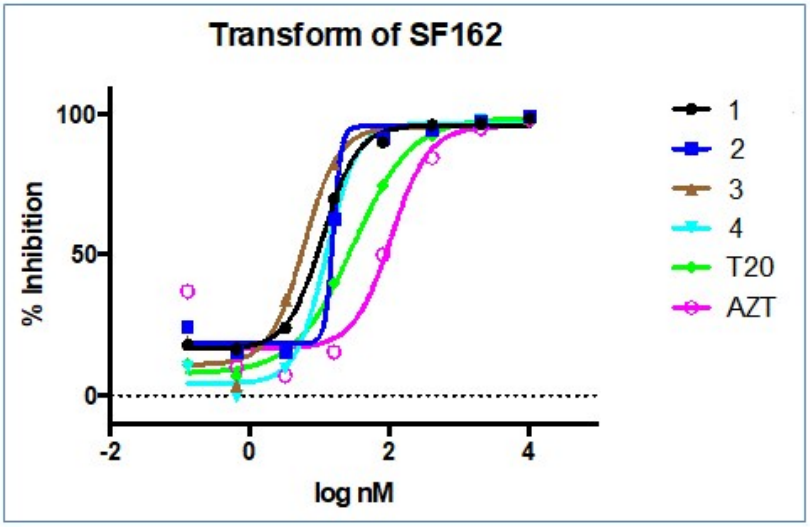
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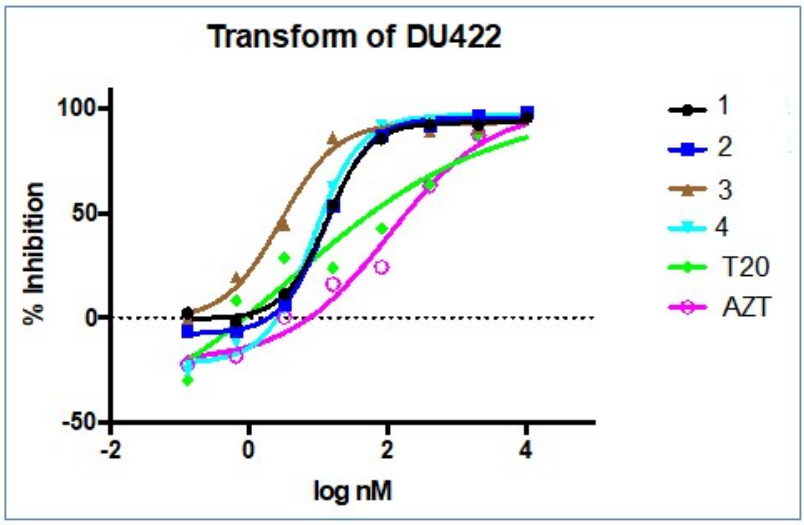
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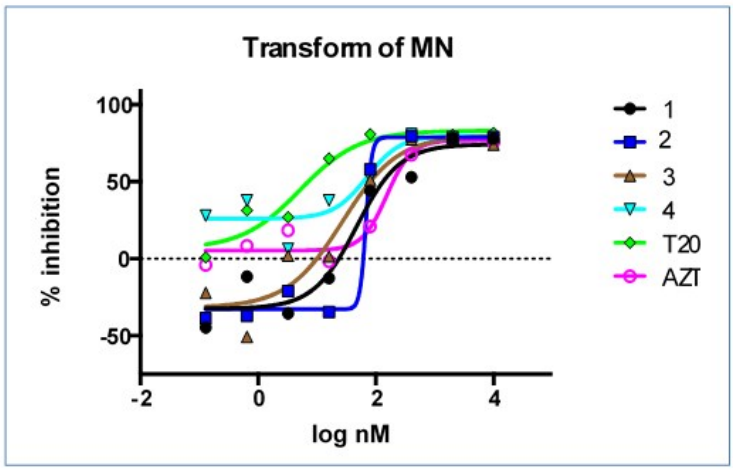
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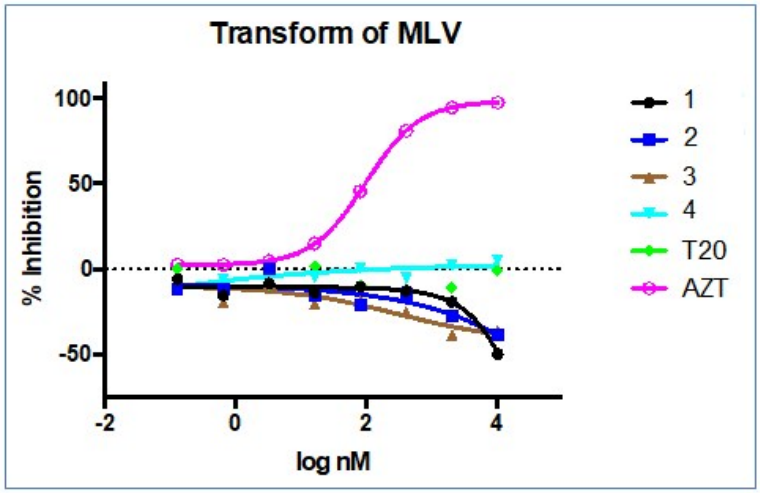


Fig S3. Anti-HIV activity of peptides.

Table S2 The sequences and antiviral activities of previously synthesized peptides

Peptide	Sequence	IC50 (nM)
P1	MTW**γE**WD**γK**I**γE**YT**γK**I**γE**LI**γK**S	>10000 for all strains tested
P2	MTW**EE**WD**KK**I**EE**YT**γK**I**EE**LI**KK**S	>10000 for all strains tested
P3	MTW**γE**WD**KK**I**EE**YT**KK**I**EE**LI**γK**S	>10000 for all strains tested
P4	MTW**EE**WD**KK**I**EE**YT**KK**I**γE**LI**γK**S	>10000 for all strains tested

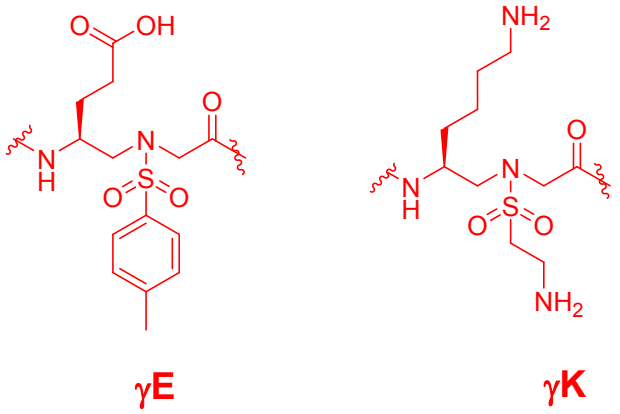


Fig S4 Structures of sulfono-γAA monomers **- γE and γK**

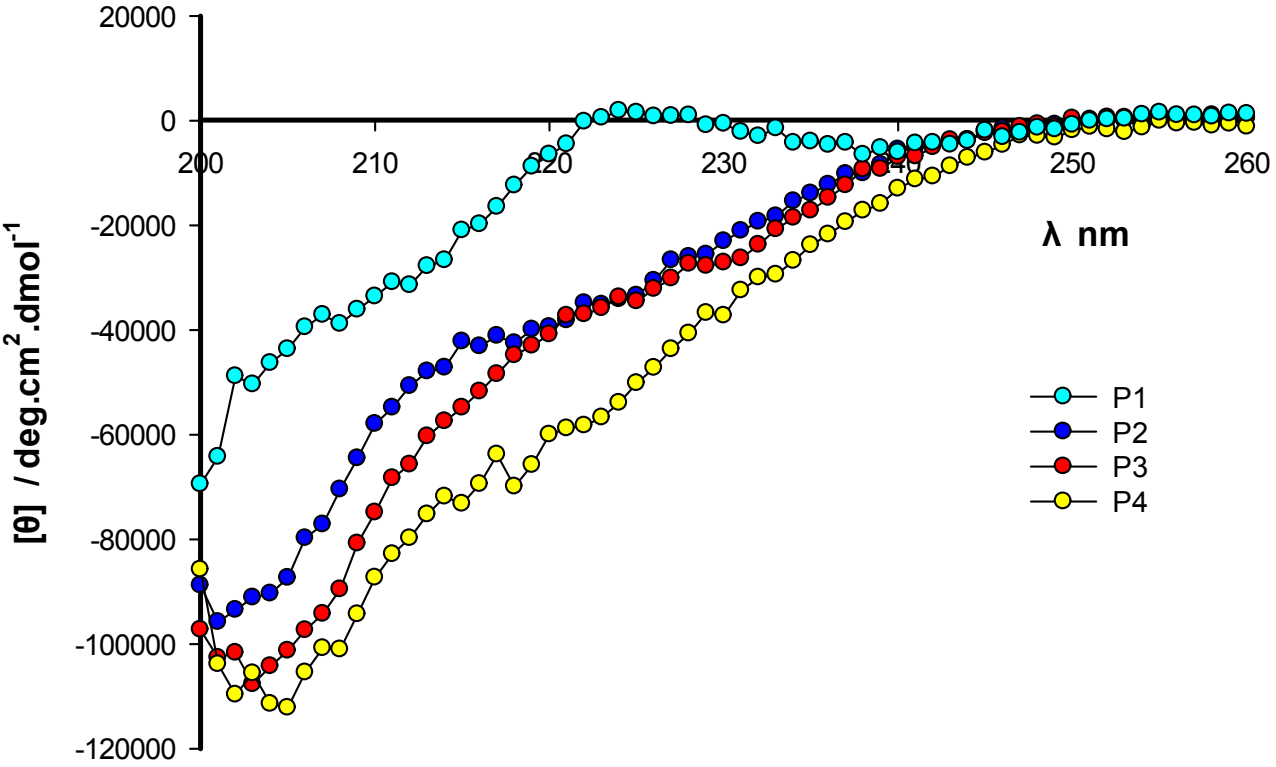


Fig S5 CD spectra of peptides P1-P4

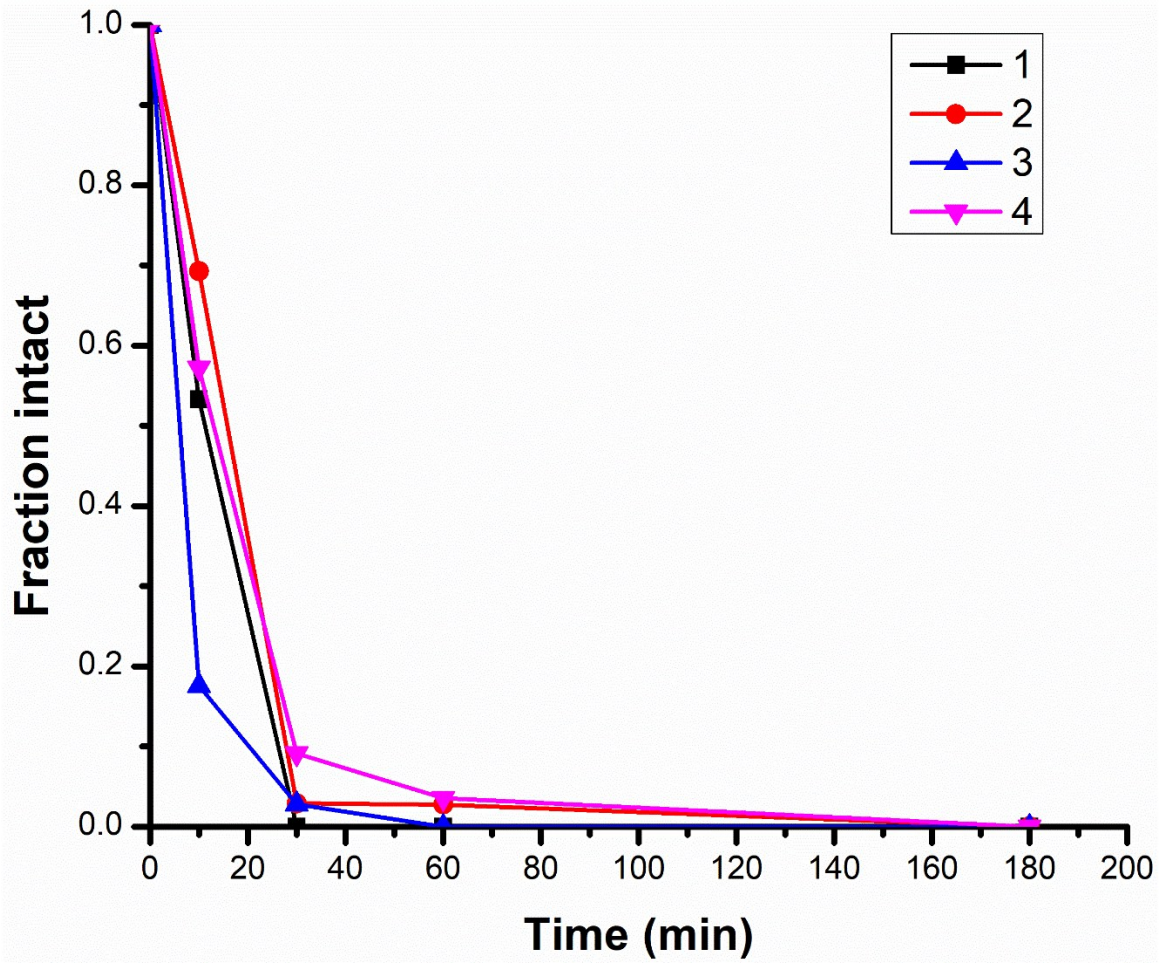


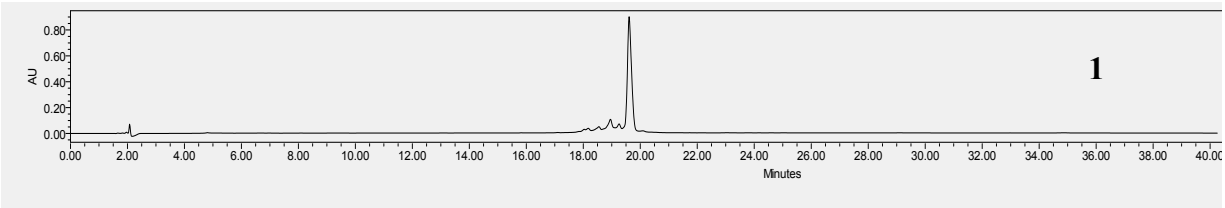
Fig S6 The degradation curve of **1-4**

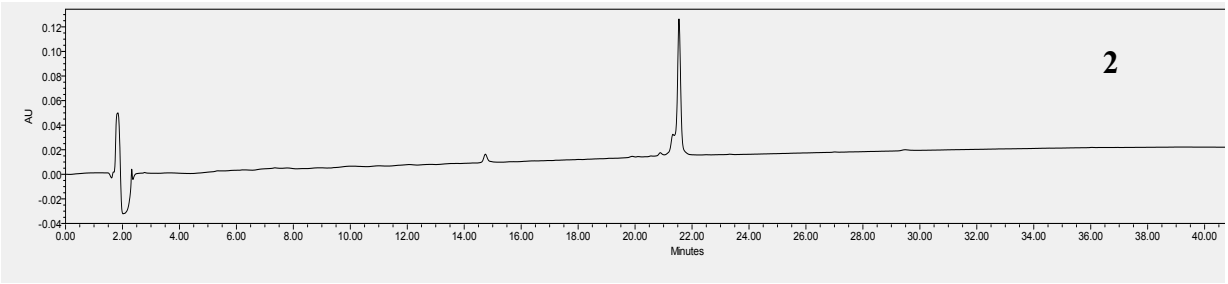
HPLC analysis of peptides derived from the C-HR of gp41

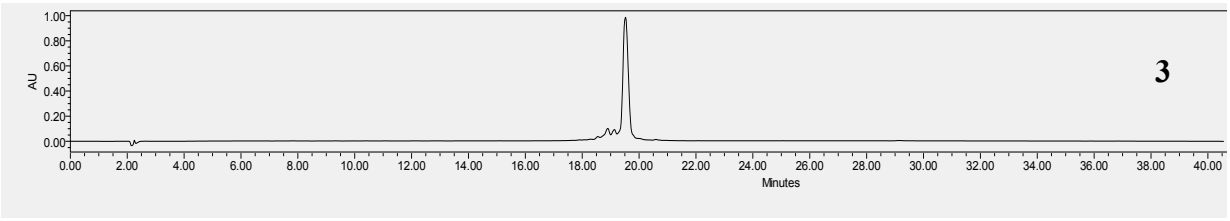
Table S3. HPLC purities and retention time of peptides derived from the C-HR of gp41

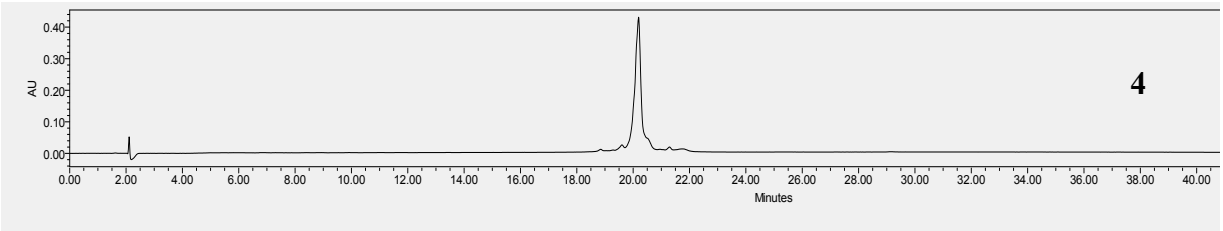
Peptide	Molecular Mass [M+H]+ (expected/observed)	Purity (based on HPLC) %	Retention Time (min)	Yield (%)
1	3125.6431/3125.8845	93.55	19.99	67
2	3118.6494/3118.3872	95.45	21.53	54
3	3147.5668/3147.8384	93.62	19.82	63
4	3140.6007/3140.3728	98.89	20.02	48
P1	3395.4948/3395.7952	99.01	19.16	70
P2	3147.5668/3147.9421	98.31	17.32	55
P3	3215.5753/3215.8425	96.36	18.96	82
P4	3215.5753/3215.9702	98.97	18.63	79

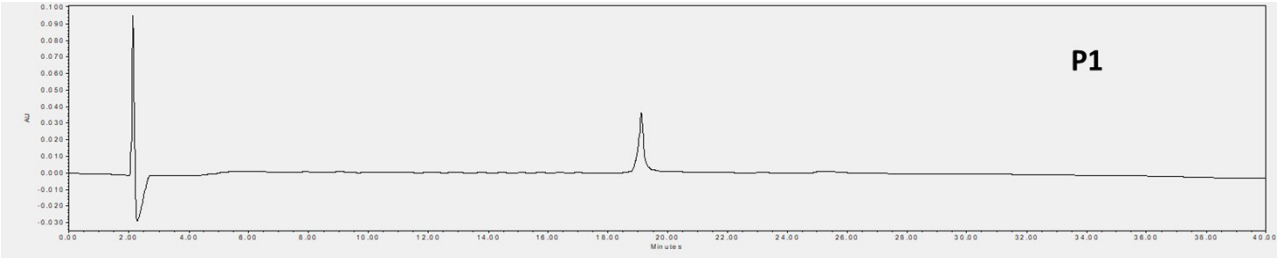
HPLC spectra of peptides **1-4**

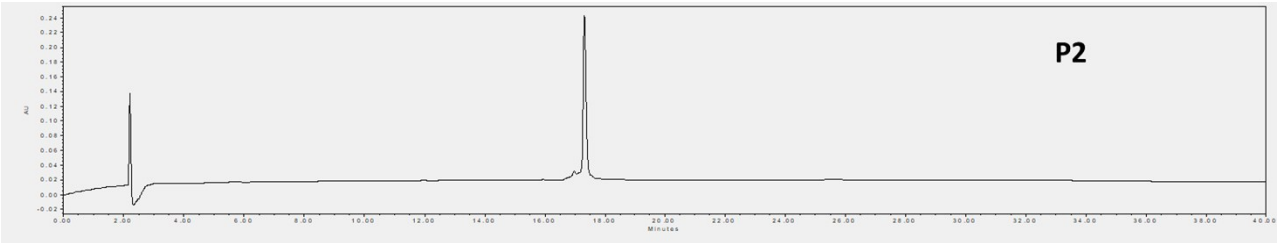


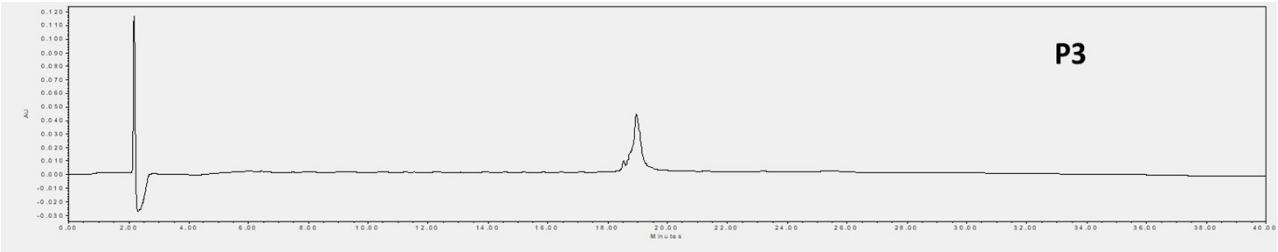


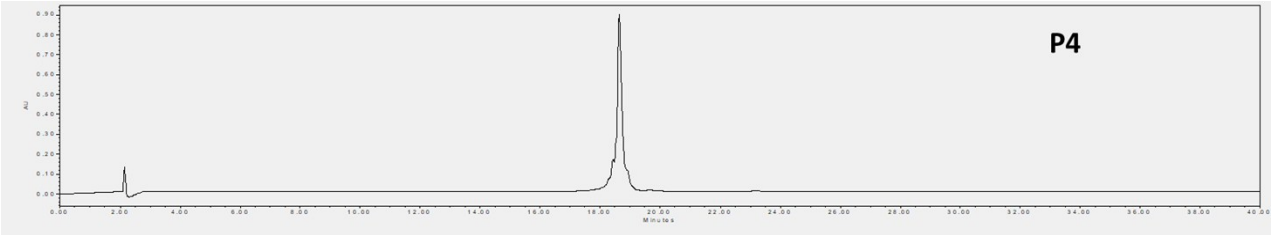












References

1. C. J. Gordon, M. A. Muesing, A. E. I. Proudfoot, C. A. Power, J. P. Moore and A. Trkola, *J. Virol.*, 1999, **73**, 684.
2. C. Herrera, C. Spenlehauer, M. S. Fung, D. R. Burton, S. Beddows and J. P. Moore, *J. Virol.*, 2003, **77**, 1084.
3. S. Beddows, N. Schülke, M. Kirschner, K. Barnes, M. Franti, E. Michael, T. Ketas, R. W. Sanders, P. J. Maddon, W. C. Olson and J. P. Moore, *J. Virol.*, 2005, **79**, 8812.