SUPPLEMENTARY MATERIAL:

Peptides from the second extracellular loop of the C-C chemokine receptor type 5 (CCR5) inhibit diverse strains of HIV-1

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EXPERIMENTAL PROCEDURES

HIV-1 infectivity assays

Cell lines and molecular clones. HIV-1 expression plasmid SG3Δenv (catalog no. 11051), HIV-1 Env molecular clone pCAGGS SF162 gp160 (catalog no. 10463), indicator cells TZM-b1 (or JC53BL-13, catalog no. 8129), recombinant vaccinia virus vSC60 (encoding HIV-1 IIIB Env protein, catalog no. 3377), vCB41 (encoding HIV-1 LAV Env protein, catalog no. 3375), vBD3 (encoding HIV-1 89.6 Env protein, catalogue no. 4082), vCB21R-LacZ (catalog no. 3365), and vCBYF1-fusin (encoding CXCR4 coreceptor, catalog no. 3364) were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program. 293T cells were obtained from the American Type Culture Collection. B-SC-1 cells and recombinant vaccinia virus vP11T7 gene1 (encoding phage T7 polymerase), vCB32 (encoding HIV-1 SF162 Env protein), and vCCR5 (encoding CCR5 co-receptor) were provided by P. Kennedy and Dr. E. Berger, Laboratory of Viral Diseases, NIAID. gp160 expression plasmids pSVIII HBXc2, 89.6, and YU2 were provided by Dr. J. Sodroski, Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute (1,2). NL4-3 gp160 expression plasmid pHenv was provided by Dr. E. Freed, HIV Drug Resistance Program, NCI (3). Gp160 expression plasmid pSVIII BaL26 was provided by Dr. J. Mascola, BSL-3 Core Virology Laboratory, Vaccine Research Center, NIAID.

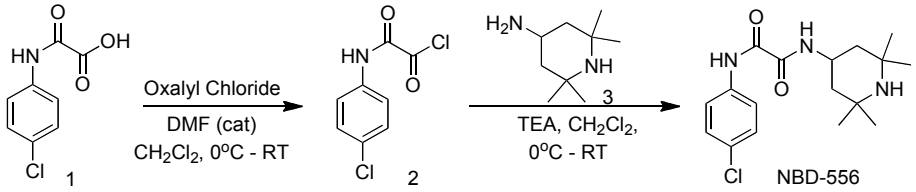
Env-pseudotyped virus preparation- Pseudovirus stocks were prepared essentially as described. **REF** Exponentially dividing 293T cells were transfected using X-treme GENE HP DNA transfection reagent (Roche, Nutley, NJ) with Env-deficient HIV-1 expression plasmid SG3Δenv and Env-expressing plasmid in the ratios corresponding approximately to the ratio of the vector sizes (approximately 16 μg total DNA per T-150 culture flask). Culture supernatants were collected 2 days post-transfection, filtered through a 0.45 μm filter, and stored at -80 °C.

Generation of HIV-1 ΔV1V2 gp120 envelope glycoproteins

Mammalian codon-optimized genes encoding HIV-1 ΔV1V2 gp120 envelope glycoproteins from strains YU2, HxB2 and 89.6 were synthesized and cloned into mammalian expression vector pcDNA2.1 (Invitrogen, Carlsbad, CA). For preparation of each envelope glycoprotein, 500 μg of the

plasmid DNA in 25 ml of OptiMEM (Invitrogen, Carlsbad, CA) was mixed with 1 ml of 293fectin (Invitrogen, Carlsbad, CA) in 25 ml of OptiMEM for 20 minutes before 50ml of the DNA-293fectin complex was added into 850 ml of FreeStyle 293F cells (1.2 x 10^6 cells/ml) in a 2-L shaking flask. After transfection, the cells were returned to suspension incubation for 24 hours at 37 °C, 8% CO_2, at 125 rpm. The culture was fed with 50 ml of the enriched medium CellBoost-5 (HyClone, Logan, UT) and sodium butyrate (Sigma, St. Louis, MO) at a final concentration of 2 mM. After 5 days of suspension culture post transfection, the supernatant was harvested by centrifugation, filtered through a 0.22 μm filter, and purified through an affinity column of 17b (4). The affinity column of 17b was made through cross-linking 17b antibody with Protein A Plus Agarose (Pierce, Thermo, Rockford, IL). Purified proteins were concentrated and dialyzed against PBS, and characterized with SDS-PAGE.

Synthesis of NBD-556._All reactions were performed under a positive flow of nitrogen in flasks that were flame dried under vacuum. SureSeal anhydrous solvents (CH_2Cl_2 and DMF) and all other reagents were purchased from Sigma Aldrich and used without further purification.



2-(4-chlorophenylamino)-2-oxoacetic acid **1** prepared according to a previously described procedure (5) was suspended in dichloromethane and cooled to 0 °C. Oxalyl chloride was added dropwise followed by the addition of 2 drops of DMF. The mixture was allowed to warm to room temperature and stirred for 5 hours during which time the mixture became a clear solution. The solvent was removed under reduced pressure and dried under vacuum. Crude acid chloride **2** was resuspended in dry dichloromethane, and cooled to 0 °C followed by addition of 4-amino tetra methyl piperidine and triethylamine. The mixture was allowed to warm to room temperature overnight, washed with water, and extracted with ethyl acetate. The organics were dried ($MgSO_4$) and the solvent evaporated under reduced pressure to give an off white solid that was recrystallized from dichloromethane/hexanes to yield a white solid whose analytical data matched NBD-556.

Reagents used in NMR experiments. Soluble CD4 (two-domain) was obtained from the AIDS Research and Reference Reagent Program (Cat. No. 4615: Progenics Pharmaceuticals). YU2 and HxB2 gp120, with a Gly-Ala-Gly tripeptide replacing the V1/V2 region was produced by a Drosophila Schneider 2 cell line under control of an inducible metallothionein promoter and purified by affinity chromatography as described previously (6,7). All proteins and protein complexes were passed through a Superdex 200 16/60 gel filtration column in 20 mM Na_2PO_4/50 mM NaCl, pH 6.85, and quantified by A280 measurements prior to use.

Fig. S1. Representative inhibition curves for **2C** against five HIV-1 strains including two R5, two X4 and one dual tropic strain. At least two sets of assays for each strain and inhibitor were performed in duplicate, and standard errors approximated 10-15%. Inhibitors are presented in the legend in the plot.

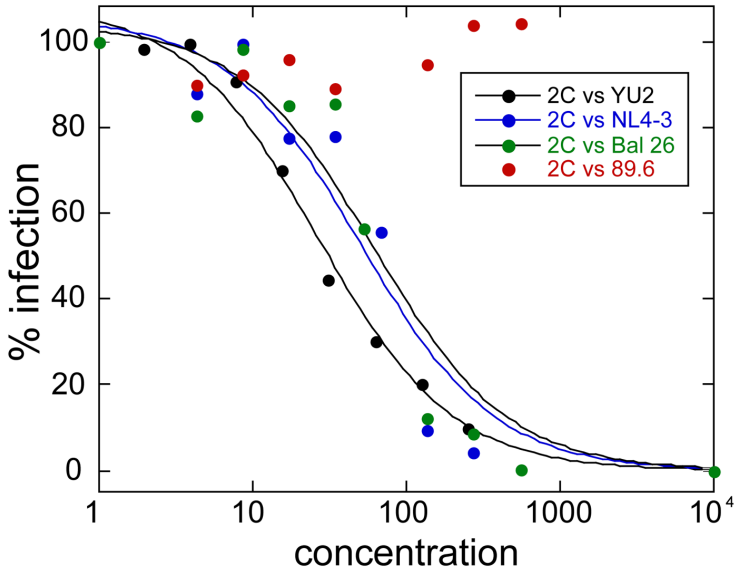


Fig. S2. Inhibition curves for **2C** and cysteine analogs Cys178Ala and Cys-ACM. Analogs were tested against HIV-1 YU2. Experiments were performed as described above and data represent two separate experiments. Inhibitors are presented in the legend in the plot.

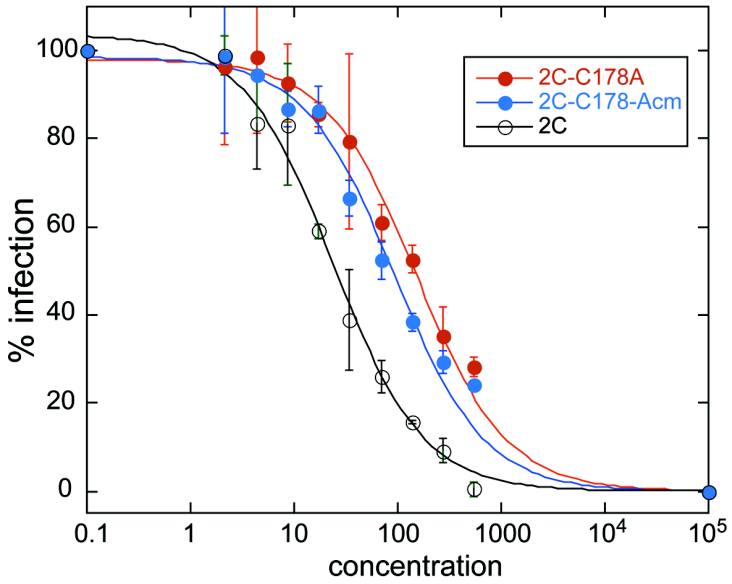


Fig. S3. STD NMR H^1 spectra of peptide **2C** in the presence of CD4-YU2 gp120 complex. Expansions of reference (black) and difference (red) spectra showing the aromatic region. The spectrum shows enhancements nearly identical to those observed when **2C** is in the presence of YU2 gp120 alone.

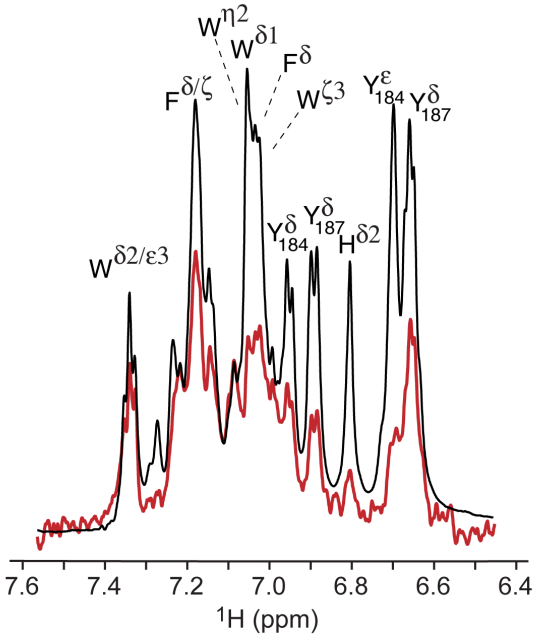


Fig. S4. ^{1}H STD NMR spectra of peptide **2C** in the presence of 89.6 gp120. Expansions are colored as in Fig. S1; the absence of peaks in the difference spectrum indicate a lack of binding of **2C** to 89.6 gp120.

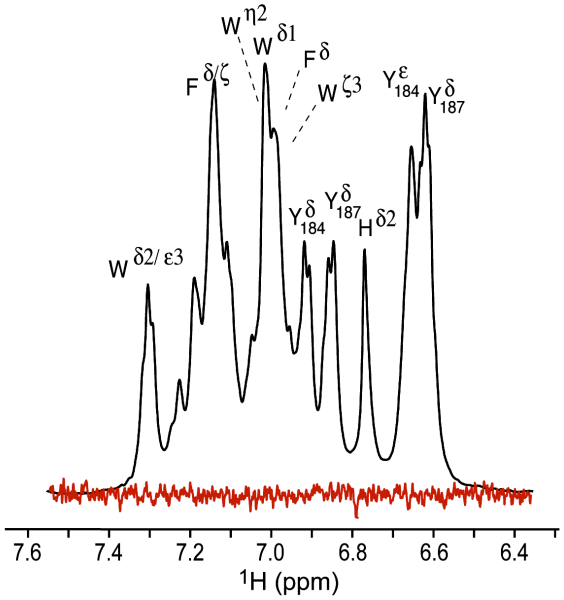
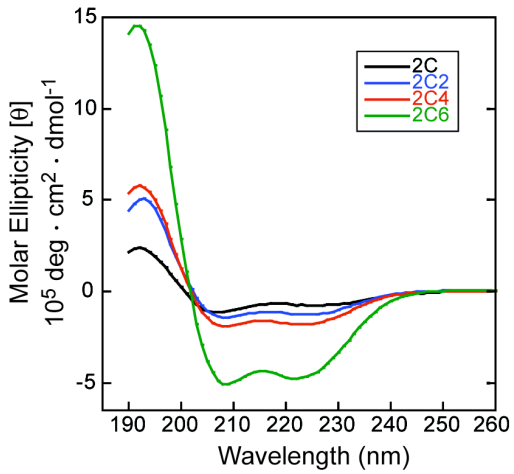


Fig. S5. Circular dichroism data for 2C-TM5 peptides. (*A*) Peptide **2C** shows little secondary structure in 50% TFE used as a stabilizing agent. Only slight increases in molar ellipticity are observed for **2C2** and **2C4** in 50% TFE, which contain two and four residues that are predicted to be located in the transmembrane region. A larger increase in molar ellipticity is observed with addition of six transmembrane residues in peptide **2C6**. (*B*) Numerical values for % helicity per residue. Experimental details are provided in the Experimental Procedures section of the accompanying paper.

A



B

Peptide	% Helicity
1	39
2N	26
2C	8
2C2	22
2C4	28
2C6	63

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