Supporting Information

Structural Determinants for Affinity Enhancement of a Dual Antagonist Peptide Entry Inhibitor of Human Immunodeficiency Virus Type-1

Hosahudya Gopi[1], M.Umashankara[1], Vanessa Pirrone[2], Judith LaLonde[3], Navid Madani[4], Ferit Tuzer[1], Sabine Baxter[1], Isaac Zentner[1], Simon Cocklin[1], Navneet Jawanda[1], Shendra R. Miller[2], Arne Schön[5], Jeffrey C. Klein[5], Ernesto Freire[5], Fred C. Krebs[2], Amos B. Smith III[6], Joseph Sodroski[4] and Irwin Chaiken[1]*

[1]Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA 19102; [2]Department of Microbiology and Immunology, and Center for Molecular Therapeutics, Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA 19102; [3]Department of Chemistry, Bryn Mawr College, Bryn Mawr, PA; [4]Dana-Farber Cancer Institute, Division of AIDS, Harvard Medical School, Boston, MA 02115; [5]Department of Biology, The Johns Hopkins University, Baltimore, MD 21218; [6]Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104

Table of Contents:

I. Reverse phase HPLC profiles for key compounds 1, 2, 4, 8 and 15.

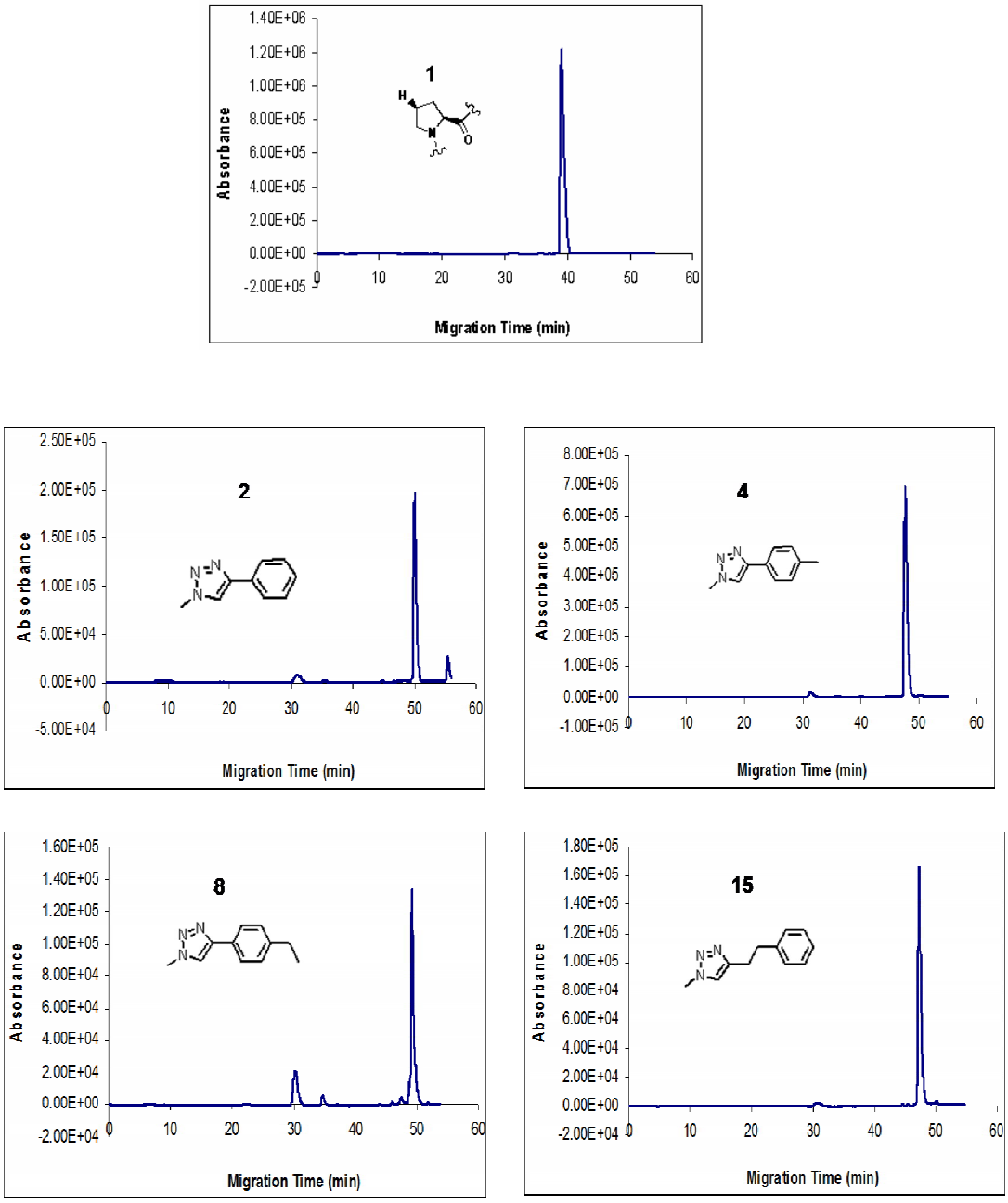
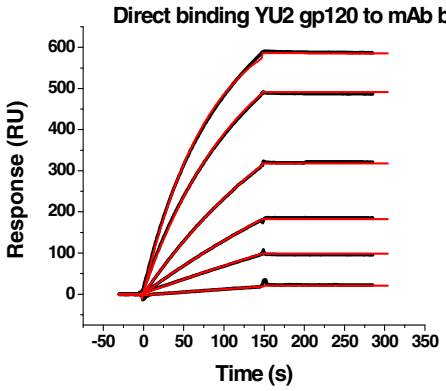
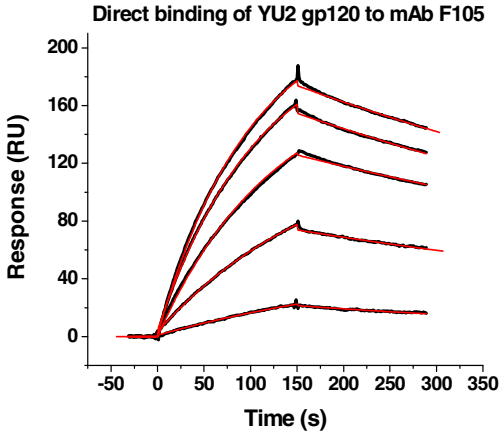


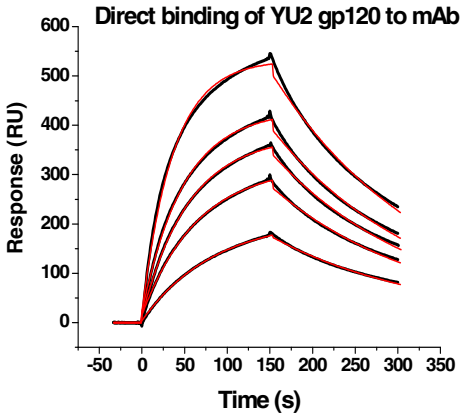
Figure S1. RP-HPLC profiles of triazole conjugate peptides **1**, **2**, **4**, **8** and **15**. The purity of these peptides (side chains shown) was ascertained by analytical RP-HPLC on a Merck Lichospher RP-18c (5µm particle size) column by using a gradient of 5%-75% B in 60 min at a flow rate of 0.75mL/min. The absorbance of the eluting fractions was monitored at 280 nm. (Gradient A= deionized water with 0.1% TFA, and gradient B= Acetonitrile with 0.1% TFA).

II. Direct binding of YU2gp120 to immobilized CD4 site binding antibodies (CD4bs) and the inhibition of binding of YU2 gp120 to CD4bs by compound 4.

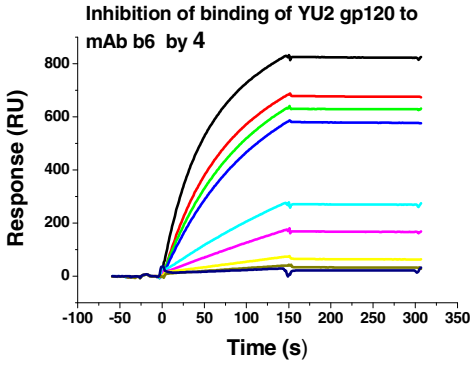
A

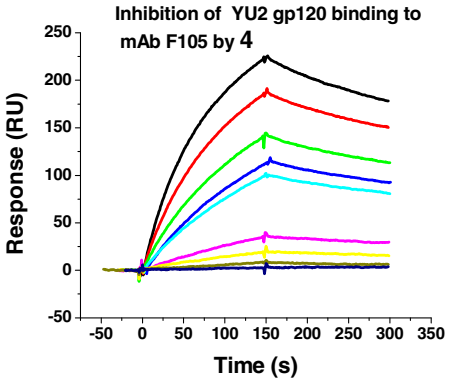






B





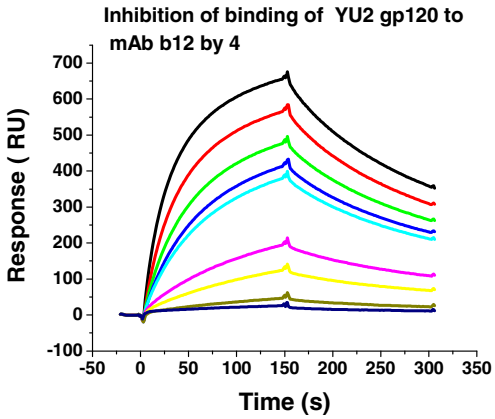
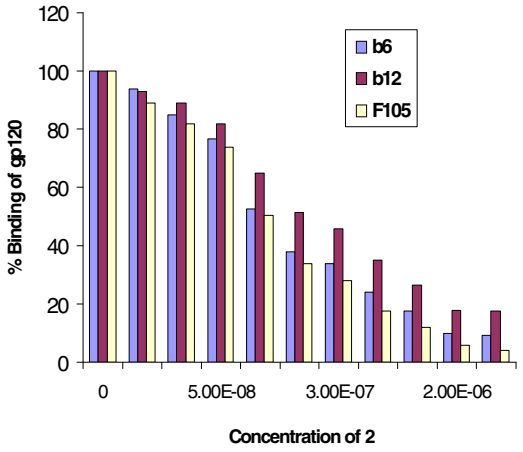
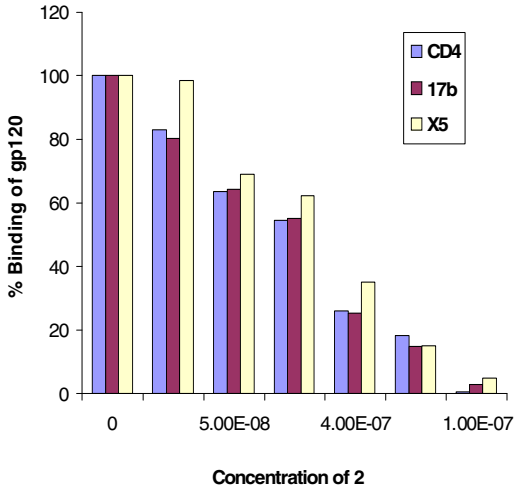


Figure S2. Effect of increasing concentration of **4** on the binding of YU2 gp120 to monoclonal antibodies b6, b12, and F105. **A**. Direct binding of YU2 gp120 to immobilized CD4 and antibodies. Approximately 2000 RUs of either sCD4, b12 , F105 or b6 were immobilized on separate flow cells of biosensor chip and exposed to increasing concentrations (1, 5, 10, 50 and 100) of YU-2 gp120. Black lines indicate experimental data, whereas red lines indicate fitting to a 1:1 Langmuir binding model. **B**. Inhibition of the binding of YU2 gp120 (100 nM) to CD4 and antibodies with increasing concentration of peptide **4** (top to bottom, 0 nM to 4 μM).

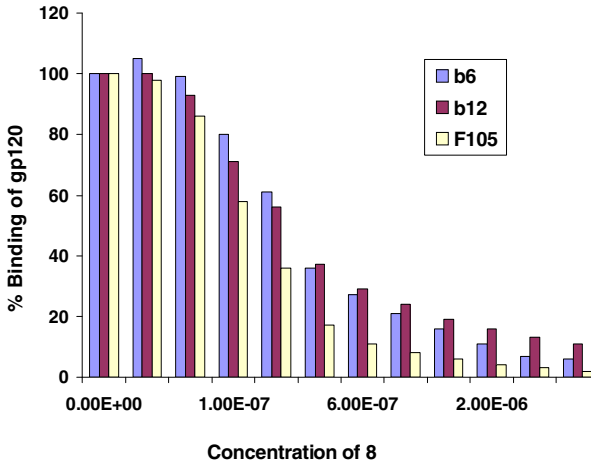
III. Profiles of Inhibition of gp120 Binding by Residue 6 Triazole Conjugates 2, 8 and 15.

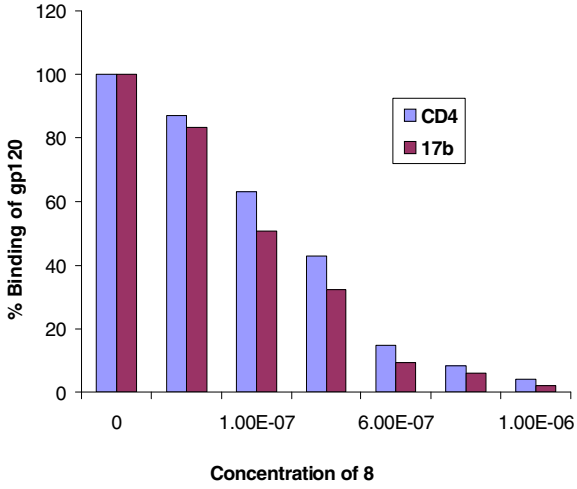
A. Inhibition of binding of gp120 to CD4, and CD4bs and CD4i antibodies by 2.





B. Inhibition of binding of gp120 to CD4, 17b and CD4bs antibodies by 8.





C. Inhibition of binding of gp120 to CD4, 17b and CD4bs antibodies by 15.

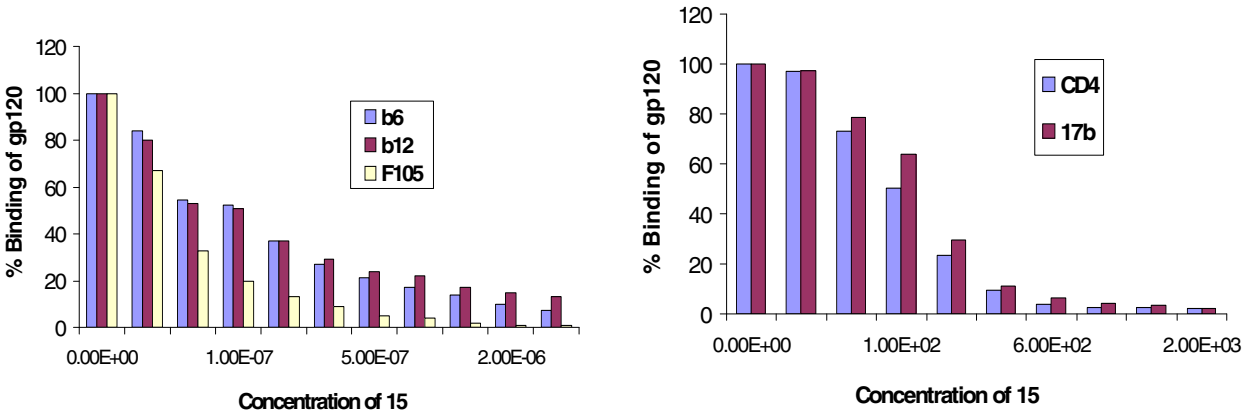


Figure S3. Inhibition of binding of YU2 gp120 to monoclonal antibodies b6, b12, F105 and 17b, Fab X5 (in the case of **2** only) and sCD4 by (A) **2**, (B) **8** and (C) **15**. All antibodies and sCD4 were immobilized on biosensor CM5 chip. Indicated increasing concentrations of inhibitors were passed over immobilized antibodies and CD4 with constant concentration (100 nM) of YU2 gp120. The percent (%) binding of gp120 to immobilized antibodies was plotted against the concentration of the peptide.

IV. Inhibition of binding of gp120 to CD4bs by compound 1.

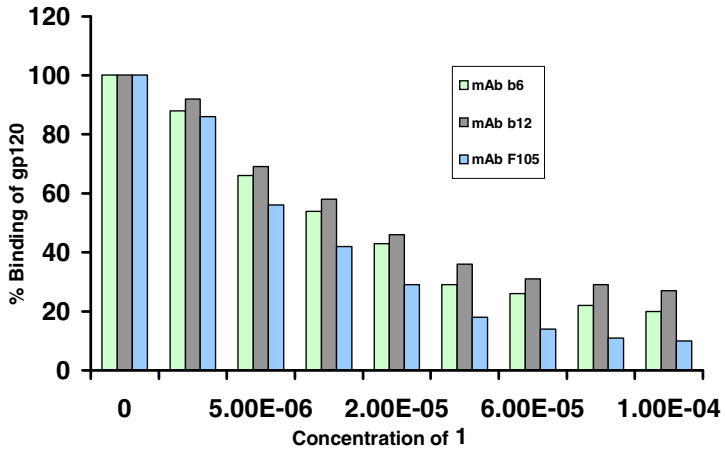


Figure S4. Inhibition of binding of YU2 gp120 to monoclonal antibodies b6, b12 and F105 by **1**. All antibodies were immobilized on biosensor CM5 chip. Indicated increasing concentrations of inhibitors were passed over immobilized antibodies and CD4 with constant concentration (100 nM) of YU2 gp120. The percent (%) binding of gp120 to immobilized antibodies was plotted against the concentration of the peptide.

V. Cytotoxicity of Triazole Conjugates

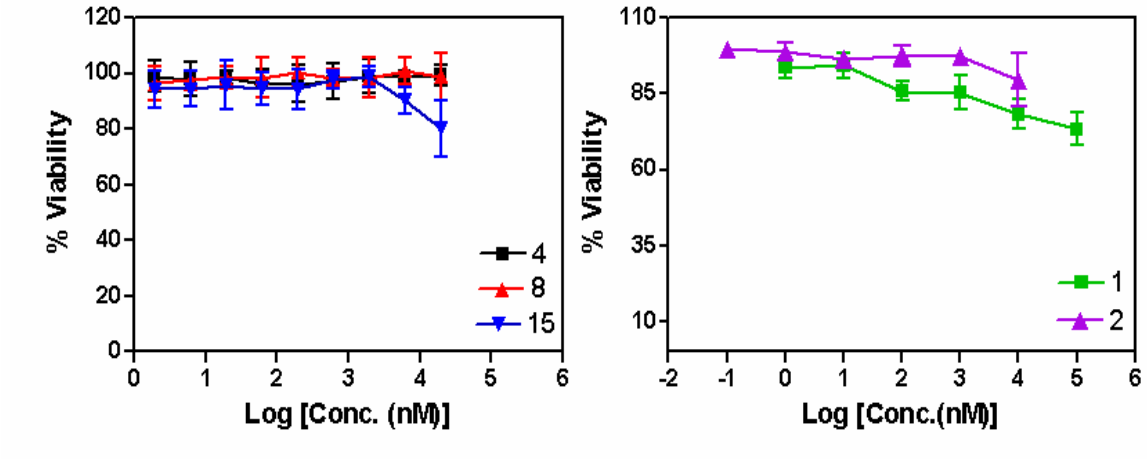


Figure S5. In vitro cytotoxicity of **1** and the high-affinity triazole conjugates **2**, **4**, **8** and **15**. P4-CCR5 cells were seeded at a density of $4x10^4$ cells/well in a 96 well plate approximately 18 h prior to experiment. Cells were then exposed to the indicated concentrations of **1**, **2**, **4**, **8** and **15** for 2 h. The cells were subsequently washed and assessed for viability using a 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay of viability (previously described in Krebs, F. C.; Miller, S. R.; Malamud, D.; Howett, M. K.; Wigdahl, B. Inactivation of human immunodeficiency virus type 1 by nonoxynol-9, C31G, or an alkyl sulfate, sodium dodecyl sulfate. Antiviral Res 1999, 43 (3), 157-73. Concentrations were tested in triplicate in two independent assays.

VI. Low-Affinity Peptide Derivatives Included in SAR Analysis

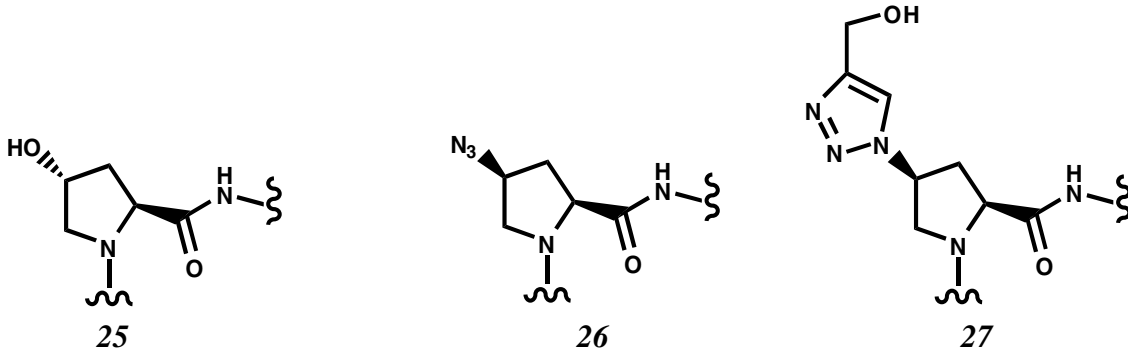


Figure S6. Structures of residue 6 side chains in low-affinity residue 6 derivatives included for SAR analysis but not included in Table 1. Compound numbers and masses are: **25** (**HNG-101**), 1476.5 Da; **26** (**HNG-103**), 1501.9 Da; **27** (**HNG-109**), 1571.8 Da.