

Supporting Information

for

Oligovalent Amyloid-binding Agents Reduce SEVI-mediated Enhancement of HIV-1 Infection

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Complete Ref 1:

(1) Munch, J.; Rucker, E.; Standker, L.; Adermann, K.; Goffinet, C.; Schindler, M.; Wildum, S.; Chinnadurai, R.; Rajan, D.; Specht, A.; Gimenez-Gallego, G.; Sanchez, P. C.; Fowler, D. M.; Koulov, A.; Kelly, J. W.; Mothes, W.; Grivel, J. C.; Margolis, L.; Keppler, O. T.; Forssmann, W. G.; Kirchhoff, F. *Cell* **2007**, *131*, 1059.

Materials

Reagents were purchased from Sigma-Aldrich unless otherwise stated. 2-(*p*-aminophenyl)-6-methyl benzothiazole (BTA) was purchased from City Chemical LLC. Amino-dPEG[®]-4-acid was purchased from Quanta BioDesign, Ltd. 2,2',2''-triaminotriethylamine (TREN) was purchased from STREM Chemicals. 4-(dimethylamino)pyridine (DMAP) was purchased from Alfa Aesar. Ethylamine-HCl was from Fluka. HEPES (free acid) was purchased from EMD Biosciences, Inc. Sodium Phosphate Monobasic and NaCl were purchased from Fisher Scientific. KCl was purchased from JT Baker Chemicals. All reagents were used without further purification.

A β (1-42) peptide was purchased from GL Biochem (Shanghai) Ltd. PAP248–286 peptide was synthesized by New England Peptide.

All solvents used for reactions were obtained from Fisher Scientific. Solvents used for regular silica chromatography were ACS technical grade and used without further purification. Solvents used for amine-functionalized silica chromatography (Teledyne Isco, Inc.) were HPLC grade and used without further purification. Water (18.2 $\mu\Omega$ /cm) was filtered through a NANOPure Diamond[™] (Barnstead) water purification system before use.

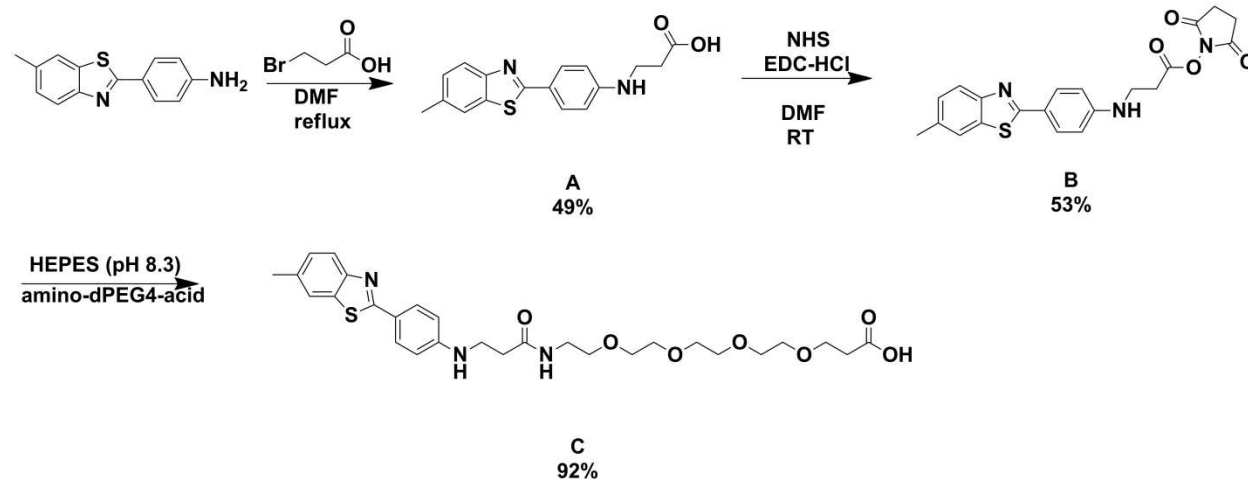
NMR spectra were obtained on a Varian 400 MHz spectrometer. Chemical shifts are reported in ppm relative to residual solvent. Low resolution MS analysis was performed on a Micromass Quattro Ultima triple quadrupole mass spectrometer with an electrospray ionization

(ESI) source. High resolution MS analysis was performed on an Agilent 6230 Accurate-Mass TOFMS with an ESI source.

Dulbecco's Modified Eagle Medium (DMEM) was purchased from Invitrogen (Catalog # 11965). Fetal bovine serum was purchased from Atlas Biologicals (Catalog # F-0500-A), Pen-strep Glutamine was purchased from Invitrogen (Catalog #10378). Britelite Plus was purchased from Perkin Elmer (Catalog # 6016761). DPBS was purchased from Invitrogen (Catalog #14190).

TZM-bl cells were obtained from the NIH AIDS Research & Reference Reagent Program. HIV-1_{IIIB} was obtained from Zeptometrix.

Experimental Methods

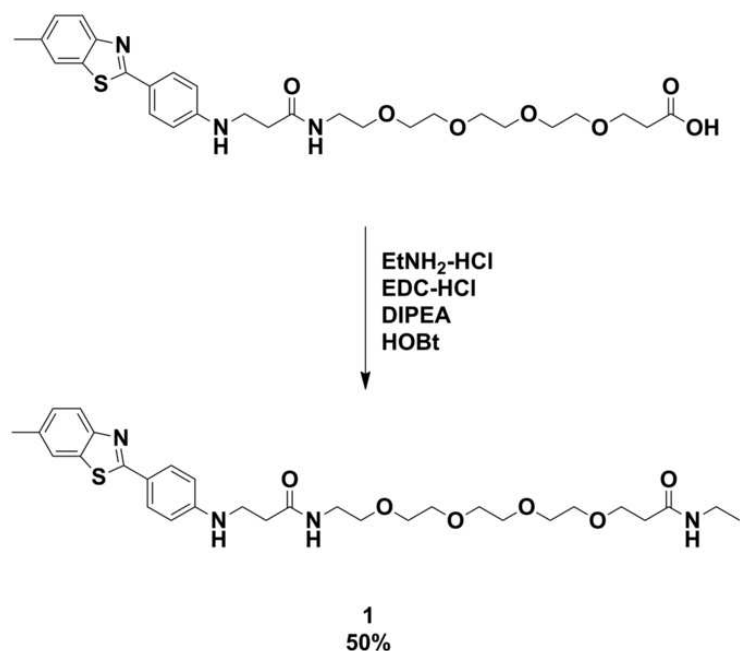


Synthesis of BTA Acid (A): 2-(*p*-aminophenyl)-6-methyl benzothiazole (BTA) (2.00 g, 8.33 mmol) and 3-bromopropionic acid (0.69 g, 4.51 mmol) were added to 25 mL dry dimethyl formamide (DMF) and refluxed for 12 h. The reaction was concentrated *in vacuo* and an excess volume of H₂O was added to the mixture to precipitate the product. The product was filtered and purified by re-crystallization in hot dichloromethane (DCM). The solid was filtered and washed with cold DCM to afford a yellow product (0.69 g, 49% yield). ¹H-NMR (400 MHz, CD₃OD): δ = 2.47 (s, 3H), 2.63 (t, *J*=6.8 Hz, 2H), 3.48 (t, *J*=6.8 Hz, 2H), 6.71 (d, *J*=8.8 Hz, 2H), 7.28 (d, *J*=8.4 Hz, 1H), 7.72 (s, 1H), 7.75 (d, *J*=8.4 Hz, 1H), 7.81 (d, *J*=8.8 Hz, 2H). ESI-MS (*m/z*) calculated for C₁₇H₁₇N₂O₂S [M+H]⁺ 313.10; found 313.35.

Synthesis of BTA-NHS-Ester (B): Acid A (0.62 g, 1.99 mmol), N-hydroxy succinamide (NHS) (0.69 g, 6.00 mmol), and 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide-Hydrochloride (EDC-HCl) (1.14 g, 5.94 mmol) were added to dry DMF and stirred for 12 h at room temperature. The reaction was concentrated *in vacuo* and an excess volume of H₂O was added to precipitate the product. The precipitate was filtered and washed with H₂O to afford a

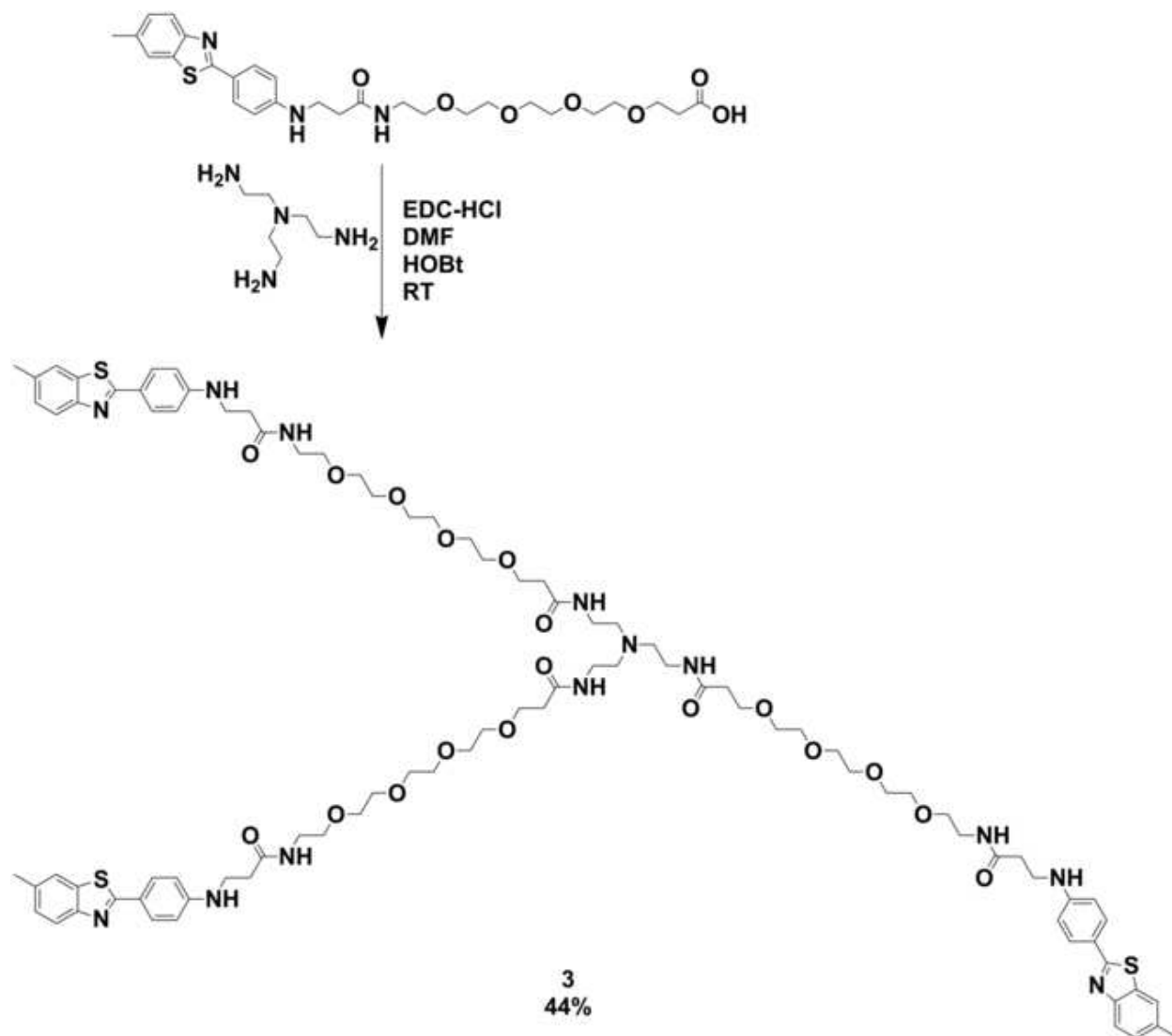
tan product (0.43 g, 53% isolated yield). ¹H-NMR (400 MHz, CDCl₃): δ = 2.47 (s, 3H), 2.85 (s, 4H), 2.93 (t, *J*= 6.4 Hz, 2H), 3.68 (t, *J*=6.4 Hz, 2H), 6.68 (d, *J*=8.8 Hz, 2H), 7.24 (d, *J*= 9.6 Hz, 1H), 7.63 (s, 1H), 7.86 (d, *J*= 8.4 Hz, 1H), 7.89 (d, *J*=8.4 Hz, 2H). ESI-MS (*m/z*) calculated for C₂₁H₂₀N₃O₄S [M+H]⁺ 410.11; found 410.21.

Synthesis of BTA-dPEG4-Acid (C): Ester **B** (0.17 g, 0.42 mmol) was dissolved in 6 mL 1,4-dioxane and was added in 3 portions to a round bottom flask containing Amino-dPEG[®]₄-acid (0.09 g, 0.35 mmol) in 0.1 M HEPES buffer (pH 8.3, 4 mL). The pH was monitored and maintained between 8.2-8.4 to optimize product yield. After the pH stabilized in the 8.2-8.4 range, the solvent was evaporated. The remaining brown, oily residue was taken up in Methanol (MeOH) and purified by silica chromatography (6:1:1:1 mixture of ethyl acetate (EtOAc):acetonitrile (ACN):H₂O:MeOH as eluent) giving the acid **C** as a brown, sticky residue (0.18 g, 92% yield). ¹H-NMR (400 MHz, acetone-d₆): δ = 2.44 (s, 3H), 2.54 (t, *J*= 6.4 Hz, 2H), 2.56 (t, *J*= 6.8 Hz, 2H), 3.36 (q, *J*= 5.6 Hz, 2H), 3.48-3.57 (m, 16H), 3.71 (t, *J*= 6.4 Hz, 2H), 6.75 (d, *J*= 8.4 Hz, 2H), 7.26 (d, *J*= 7.6 Hz, 1H), 7.56 (br. s, 1H), 7.72 (s, 1H), 7.77 (d, *J*= 8 Hz, 1H), 7.84 (d, *J*= 8.8 Hz, 2H). ¹³C-NMR (400 MHz, acetone-d₆): δ = 20.71, 35.02, 35.27, 39.08, 39.65, 66.89, 69.79, 70.18, 70.32, 70.42, 70.46, 70.48, 112.37, 121.48, 121.86, 127.66, 128.86, 134.44, 134.73, 151.54, 152.91, 167.40, 171.07, 172.68. HR-MS (*m/z*) calculated for C₂₈H₃₈N₃O₇S [M+H]⁺ 560.2425; found 560.2426.



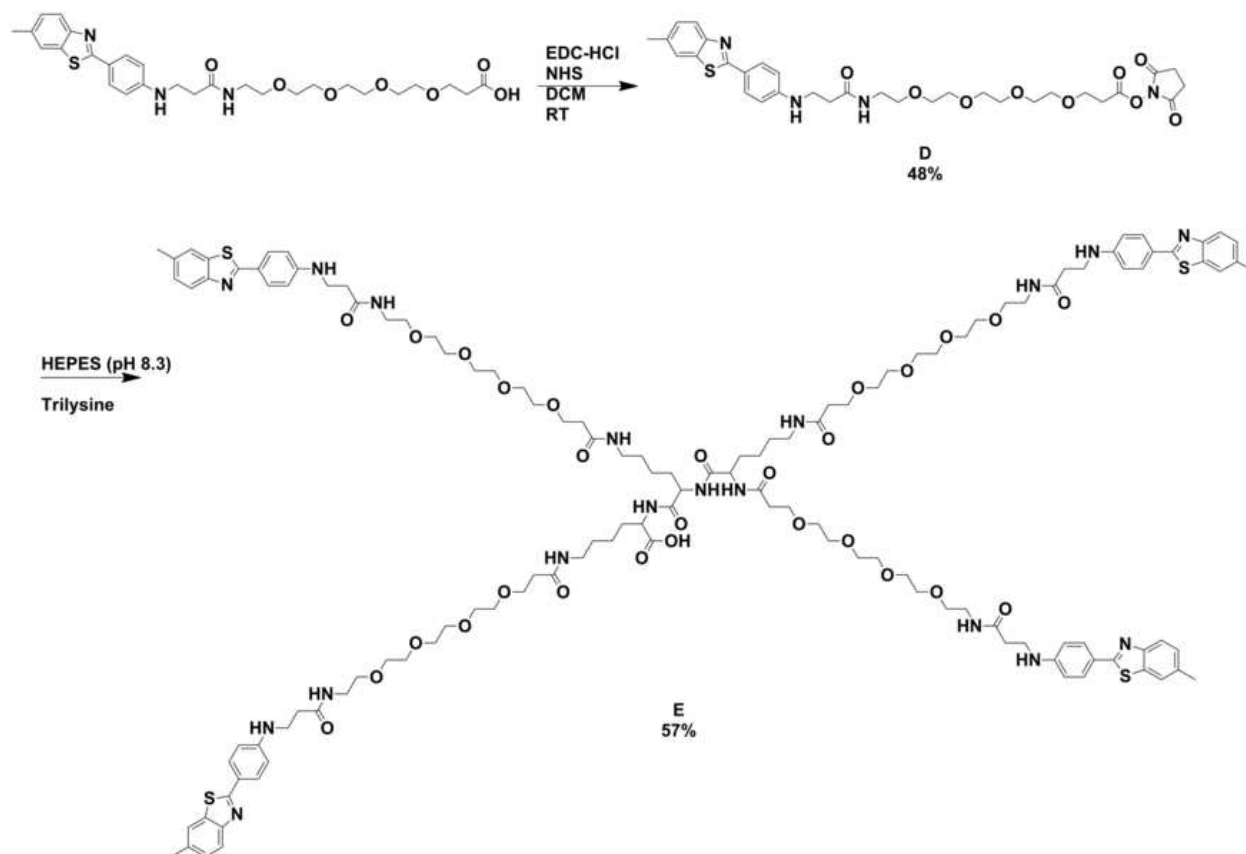
Synthesis of BTA Monomer (1): Compound C (6.3 mg, 11 μmol), N-hydroxybenzotriazole (HOBt) (1 mg, 7.3 μmol), ethylamine-HCl (5.6 mg), EDC-HCl, and diisopropylethylamine (DIPEA) were added to dry DMF and stirred for 12 h at room temperature. The solvent was evaporated *in vacuo* and the reaction mixture was taken up in DCM and then washed with brine, saturated NaHCO_3 , brine once more, and then dried over Na_2SO_4 . The residue was further purified by silica chromatography using a gradient of 8:1:0.25:0.25 to 6:1:1:1 mixture of EtOAc:ACN:H₂O:MeOH as eluent. Monomer **1** was isolated as a yellow, sticky residue (3.3 mg, 50% yield). ¹H-NMR (400 MHz, acetone-d₆): δ = 1.06 (t, J = 7.2 Hz, 2H), 2.38 (t, J = 7.2 Hz, 2H), 2.45 (s, 3H), 2.54 (t, J = 6.4 Hz, 2H), 3.19 (m, J = 5.6 Hz, 2H), 3.48-3.57 (m, 16H), 3.68 (t, J = 6 Hz, 2H), 6.76 (d, J = 8.4 Hz, 2H), 7.27 (d, J = 8.4 Hz, 1H), 7.63 (br. s, 1H), 7.72 (s, 1H), 7.78 (d, J = 8 Hz, 1H), 7.85 (d, J = 8.8 Hz, 2H). ¹³C-NMR (400 MHz, acetone-d₆): δ = 14.53, 20.71, 33.87, 35.33, 36.72, 39.20, 39.72, 67.33, 69.90, 70.17, 70.20, 70.31, 70.41, 70.43, 70.45, 112.38, 121.49, 121.86, 127.68, 128.86, 134.47, 134.70,

171.28. LR-ESI-MS (m/z) calculated for $C_{58}H_{78}N_8O_{12}S_2$ $[M]^+$ 1142.52; found $[M+H]^+$ 1143.47 and $[M+Na]^+$ 1165.55. HR-MS (m/z) calculated for $C_{58}H_{78}N_8O_{12}S_2Na$ $[M+Na]^+$ 1165.5073; found 1165.5065.



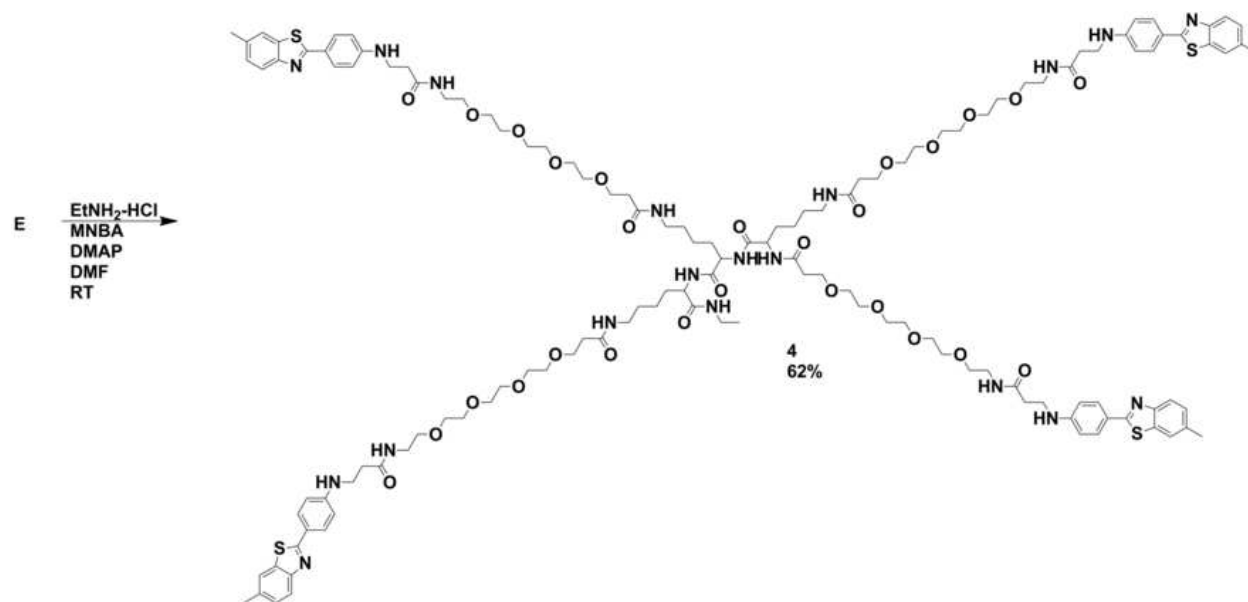
Synthesis of BTA Trimer (3): A mixture of HOBt hydrate (24 mg, 178 μmol) and 2,2',2''-triaminotriethylamine (TREN) (2.1 μl , 14 μmol) in 1 ml dry DMF was added to a vial containing **C** (26 mg, 47 μmol) in 2 mL of dry DMF. EDC-HCl (19 mg, 99 μmol) was added in

3 portions and the reaction was stirred for 12 h at room temperature. The reaction solvent was evaporated *in vacuo* and the residue was taken up in DCM. The DCM layer was washed with brine, 1M HCl, saturated NaHCO₃, brine once more, then dried over anhydrous Na₂SO₄. The DCM layer was concentrated *in vacuo*, then purified by an amine-functionalized silica column (Redisep R_f Gold[®], Teledyne Isco, Inc.) using a gradient of 0% to 25% MeOH in EtOAc over 50 minutes. The product was isolated as a sticky, yellow residue (11 mg, 44% yield). ¹H-NMR (400 MHz, acetone-d₆): δ = 2.44 (s, 9H), 2.46-2.56 (m, 18H), 3.21 (q, *J* = 5.6 Hz, 6H), 3.36 (q, *J* = 5.6 Hz, 6H), 3.49-3.56 (m, 51 H), 3.71 (t, *J* = 6.4 Hz, 6H), 6.75 (d, *J* = 8.4 Hz, 6H), 7.26 (d, *J* = 7.2 Hz, 3H), 7.42 (br.s, 3H), 7.47 (br. s, 3H), 7.73 (s, 3H), 7.76 (d, *J* = 8 Hz, 3H), 7.84 (d, *J* = 8.4 Hz, 6H). ¹³C-NMR (400 MHz, acetone-d₆): δ = 20.74, 35.37, 36.78, 37.86, 39.30, 39.75, 54.50, 67.44, 69.78, 70.29, 70.40, 70.54, 112.40, 121.50, 121.87, 127.67, 128.90, 134.43, 134.75, 151.57, 152.92, 167.41, 171.10, 171.14. ESI-MS (*m/z*) calculated for C₉₀H₁₂₃N₁₃O₁₈S₃ [M]⁺ 1769.83; found [M+H]⁺ 1770.71, [M+Na]⁺ 1792.83. HR-MS (*m/z*) calculated for C₉₀H₁₂₃N₁₃O₁₈S₃Na [M+Na]⁺ 1792.8163; found 1792.8157.

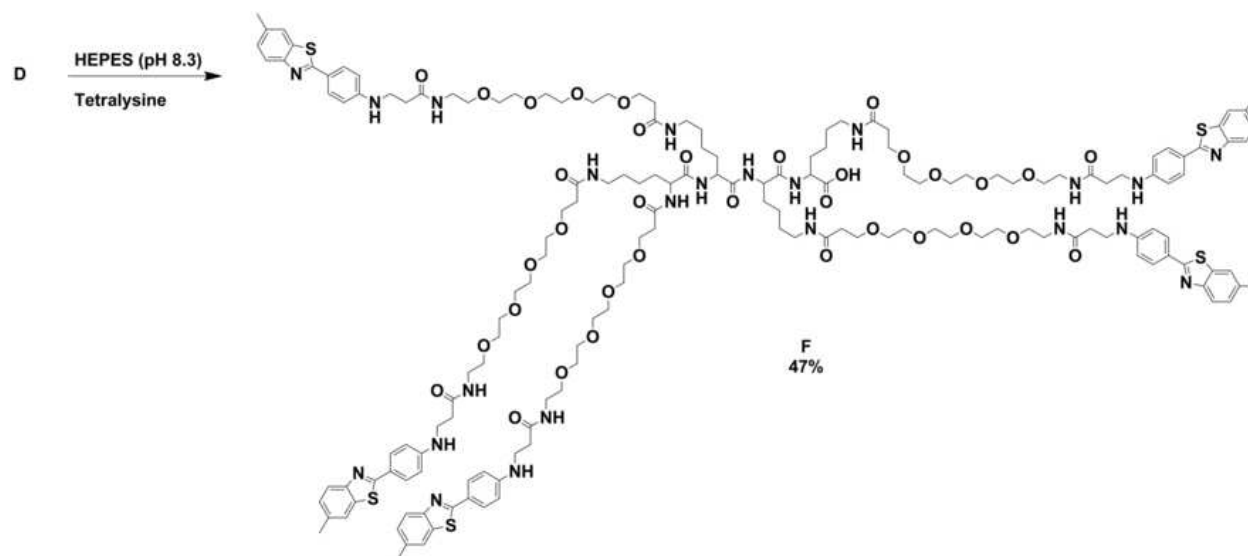


Synthesis of BTA-dPEG4-NHS Ester (D): Compound C (0.26 g, 0.47 mmol), NHS (0.16 g, 1.39 mmol) and EDC-HCl (0.90 g, 4.69 mmol) were added to dry DCM and stirred for 12 h at room temperature. The reaction mixture was washed 3 times with brine and the DCM layer was dried over anhydrous Na_2SO_4 . The DCM layer was concentrated *in vacuo* and purified by silica chromatography (8:1:0.25:0.25 mixture of ethyl acetate (EtOAc):acetonitrile (ACN): H_2O :MeOH as eluent) to afford the product as a sticky, brown residue (0.14 g, 48% yield). $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ = 2.47 (s, 3H), 2.52 (t, J = 6.4 Hz, 2H), 2.78 (s, 4H), 2.87 (t, J = 6.4 Hz, 2H), 3.45 (q, J = 5.2 Hz, 2H), 3.50-3.63 (m, 16H), 3.81 (t, J = 6.4 Hz, 2H), 6.66 (d, J = 8.8 Hz, 2H), 6.74 (br. s, 1H), 7.23 (d, J = 7.6 Hz, 1H), 7.63 (s, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 8.4 Hz, 2H). ESI-MS (m/z) calculated for $\text{C}_{32}\text{H}_{40}\text{N}_4\text{O}_9\text{S}$ $[\text{M}]^+$ 656.3; found $[\text{M}+\text{H}]^+$ 657.2 and $[\text{M}+\text{Na}]^+$ 679.2.

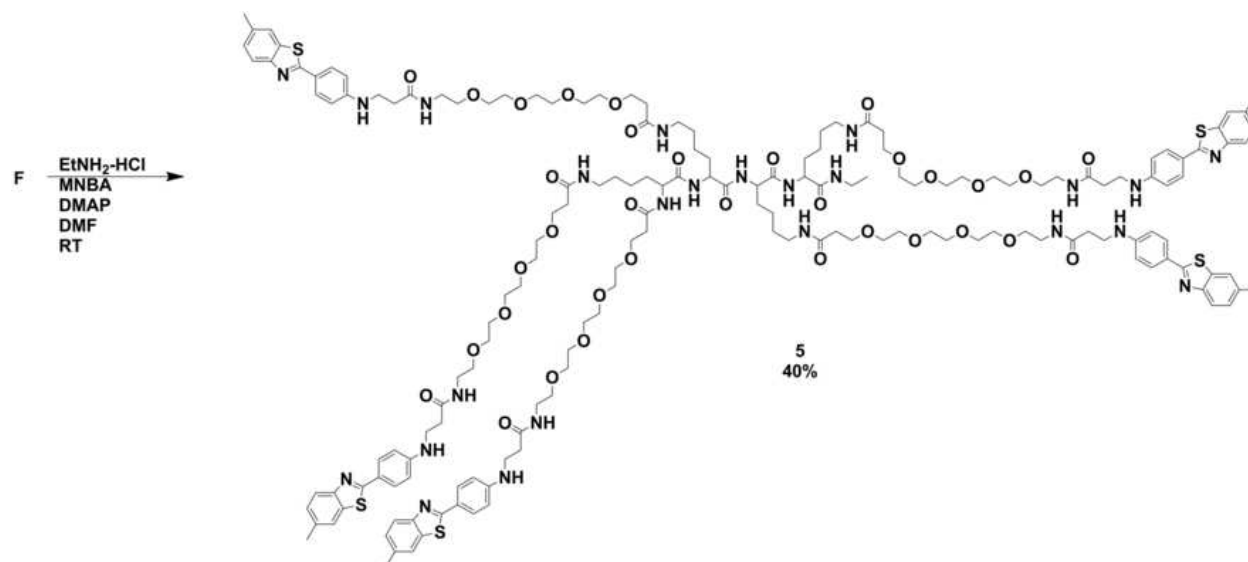
Synthesis of BTA-Tetramer-Acid (E): Compound **D** (81 mg, 123 μmol) in 2 mL 1,4-dioxane was added to 0.1 M HEPES buffer (pH 8.3, 2 mL) containing trily sine (9 mg, 22 μmol). The pH was maintained between 8.2-8.4 to favor complete acylation of all amines. The solvent was evaporated via compressed air and the remaining brown, oily residue was taken up in DCM and purified by silica chromatography (6:1:1:1 mixture of EtOAc:ACN:H₂O:MeOH as eluent) giving **E** as a brown, sticky residue (32 mg, 57% yield). ¹³C-NMR (400 MHz, CDCl₃): δ = 21.67, 22.76, 22.94, 28.99-29.20 (br), 29.90, 31.54 (br), 35.63, 36.83(br), 39.10, 39.48, 39.98, 67.47, 70.33 (br), 112.65, 121.45, 121.86, 122.47, 127.77, 129.12, 134.54, 134.74, 150.80, 152.49, 168.06, 171.94 (br), 172.19 (br). HR-MS (m/z) calculated for C₁₃₀H₁₈₀N₁₈O₂₈S₄ [M+2H]²⁺ 1284.6043; found 1284.6035.



Synthesis of BTA Tetramer (4): Compound **E** (48 mg, 19 μ mol), 2-Methyl-6-nitrobenzoic anhydride (MNBA) (15 mg, 44 μ mol), DMAP (15 mg, 123 μ mol), Ethylamine hydrochloride (EtNH₂-HCl) (8 mg, 99 μ mol) were stirred at room temperature for 12 h in 2 mL dry DMF. The DMF was evaporated *in vacuo* and the residue was taken up in DCM, washed with saturated NaHCO₃, washed three times with brine, and dried over anhydrous Na₂SO₄. The crude product was purified by silica chromatography (6:1:1:1 mixture of EtOAc:ACN:H₂O:MeOH as eluent) to afford **4** as a sticky, yellow residue (30 mg, 62% yield). ¹³C-NMR (400 MHz, CDCl₃): δ = 14.76 (br), 23.31 (br), 29.04 (br), 35.62, 36.90, 39.46, 40.02, 67.49, 70.25 (br), 112.65, 121.48, 121.88, 122.48, 127.78, 129.12, 134.57, 134.76, 150.83, 152.51, 168.08, 172.05-172.20 (br). HPLC analysis was performed on a Spheri-5 phenyl column (5 μ m, 250x4.6 mm) using a gradient of 0 to 100% MeOH in ACN and a flow rate of 1 mL/min over 50 minutes. Retention time of **5** was at 10.3 minutes. HR-MS (*m/z*) calculated for C₁₃₂H₁₈₅N₁₉O₂₇S₄ [M+2H]²⁺ 1298.1280; found 1298.1234.



Synthesis of BTA-Pentamer-Acid (F): Compound **D** (78 mg, 119 μmol) in 2 mL 1,4-dioxane was added to 0.1 M HEPES buffer (pH 8.3, 2 mL) containing tetralysine (10 mg, 19 μmol). The pH was maintained between 8.2-8.4 to favor complete acylation of all amines. The solvent was evaporated with compressed air and the remaining brown, oily residue was taken up in DCM and purified by silica chromatography (6:1:1:1 mixture of EtOAc:ACN:H₂O:MeOH as eluent) giving the BTA pentamer acid **F** as a brown, sticky residue (29 mg, 47% yield). ¹³C-NMR (400 MHz, CDCl₃): δ = 21.67, 22.90, 23.10, 29.05-29.20 (br), 29.90, 31.53 (br), 35.65, 36.80(br), 39.00, 39.48, 40.01, 67.49, 70.32 (br), 112.66, 121.45, 121.88, 122.48, 127.76, 129.12, 134.54, 134.77, 150.82, 152.52, 168.04, 172.05 (br), 172.25 (br). HR-MS (*m/z*) calculated for C₁₆₄H₂₂₅N₂₃O₃₅S₅Na₂ [M+2Na]²⁺ 1641.2461; found 1641.2443.



Synthesis of BTA Pentamer (5): Compound **F** (46 mg, 14 μ mol), MNBA (12 mg, 35 μ mol), DMAP (23 mg, 188 μ mol), and EtNH₂·HCl (20 mg, 245 μ mol) were stirred at room temperature for 12 h in 2 mL dry DMF. DMF was evaporated *in vacuo* and the residue was taken up in DCM, washed with saturated NaHCO₃, washed three times with brine, and dried over anhydrous Na₂SO₄. The crude product was purified by silica chromatography (6:1:1:1 mixture of EtOAc:ACN:H₂O:MeOH as eluent) to afford **5** as a sticky, yellow residue (18 mg, 40% yield). ¹³C-NMR (400 MHz, CDCl₃): δ = 14.42(br), 21.69, 23.20, 29.17 (br), 35.64, 37.00 (br), 39.50 (br), 40.00, 67.48, 70.30 (br), 112.70, 121.46, 121.94, 122.61, 127.77, 129.14, 134.53, 134.82, 150.73, 152.57, 167.95, 172.02-172.20 (br). HPLC analysis was performed on a Spheri-5 phenyl column (5 μ m, 250 x 4.6 mm) using a gradient of 0 to 100% MeOH in ACN and a flow rate of 1 mL/min over 50 minutes. Retention time of **5** was at 9.6 minutes. HR-MS (*m/z*) calculated for C₁₆₆H₂₃₂N₂₄O₃₄S₅ [M+2H]²⁺ 1632.7878; found 1632.7850.

Growth of A β Fibrils: A β fibrils were grown from synthetic A β (1-42) peptides by incubating the peptides (111 μ M final concentration) in PBS at 37 °C for 24 h, with stirring. The presence of fibrils was confirmed by a previously described Congo Red spectroscopic assay.¹

Growth of SEVI Fibrils: PAP248–286 was dissolved in PBS at a concentration of 10 mg/mL. Fibrils were formed by agitation in an Eppendorf Thermomixer at 1400 rpm and 37 °C for 72 h. The presence of fibrils was confirmed by a previously described Congo Red spectroscopic assay.¹

Congo Red spectroscopic assay: Fibril formation was characterized by a Congo Red assay.¹ A fresh solution of 7 mg/ml Congo Red (CR) was prepared in PBS and filtered through a 0.2 μ m syringe filter. 5 μ l of this solution was pipetted in 1 mL PBS to make a dilute solution of Congo Red. 160 μ l of the dilute CR solution was pipetted into wells of a 96-well plate. To each well was added 40 μ l of fibrils or 40 μ l PBS. The microplate was covered in parafilm and incubated for 30 minutes at room temperature. The contents of the wells were pipet-mixed then the spectrum of each well (400-700 nm) was recorded on a UV-Vis microplate reader (SpectraMax 190, Molecular Devices, LLC). A maximal absorbance shift (Congo Red has a maximal absorbance at 490 nm) to approximately 540 nm indicates the presence of fibrils.

Measurement of the Binding Affinity of BTA monomer and oligomers to amyloid fibrils. The binding of BTA monomer oligomer to amyloid fibrils was measured according to the centrifugation assay described by Levine² for BTA-1 to A β fibrils. Briefly, 200 μ L of various concentrations of BTA derivatives **1-5** in 5 % DMSO/PBS were incubated in the presence or

absence of 10 μg of fibrils to give a final volume of 220 μL of solution. These incubations were performed in duplicate runs and allowed to equilibrate for 12 h at room temperature. After equilibration, each solution was centrifuged at 16,000 $\times g$ for 20 min at 4°C. The supernatants were separated from the pelleted fibrils, and 220 μL of fresh 5% DMSO/PBS was added to re-suspend the pellets. 100 μl aliquots of each re-suspended pellet was pipetted into a cuvette (ultramicrocuvette, 10-mm light path, Hellma[®], Müllheim, Germany), and the fluorescence of the bound molecule was determined at 355 nm excitation and 420 nm emission using a spectrofluorometer (Photon Technology International, Inc., Birmingham, NJ). Each experiment was repeated at least 3 times. Error bars represent standard deviations from the mean. Graphs shown in Figures S1 and S2 of fluorescence intensity versus concentration of compounds **1-5** were plotted and fitted using the following one-site specific binding algorithm to determine K_d : $Y = B_{max} \times X / (K_d + X)$, where X is the concentration of BTA oligomer, Y is the specific binding fluorescence intensity, and B_{max} corresponds to the apparent maximal observable fluorescence upon binding of BTA oligomer to A β or SEVI fibrils. The data were processed using Origin 7.0 (MicroCal Software, Inc., Northampton, MA).

Evaluation of SEVI-mediated enhancement of HIV-1 infectivity of TZM-bl cells in the presence of BTA monomer and oligomers: TZM-bl cells (in DMEM supplemented with 10% FBS, 50 units/mL penicillin, and 50 $\mu\text{g}/\text{mL}$ streptomycin) were seeded on 96-well flat-bottomed tissue culture plates at a density of 4×10^3 cells/well. Plates were incubated for 12 h (in a humidified atmosphere of 95% air, 5% CO₂ at 37°C) to promote attachment of cells to the wells. HIV-1_{IIIIB} was pretreated for 10 min at room temperature with 15 $\mu\text{g}/\text{mL}$ SEVI fibrils in the presence or absence of **1-5**. Treated virions were then added to the plated TZM-bl cells and

incubated for 2 h at 37°C. After incubation, the cells were washed with DPBS and the media was replaced. Infection was assayed after 72 hours by quantifying luciferase expression with PerkinElmer Britelite Plus and measuring luminescence with a microplate reader (DTX880, Beckman Coulter). All data are represented as the mean \pm S.D. of triplicate measurements. ANOVA with Tukey's post test was employed in all analyses of data. A *p*-value < 0.05 was considered statistically significant compared to control cells.

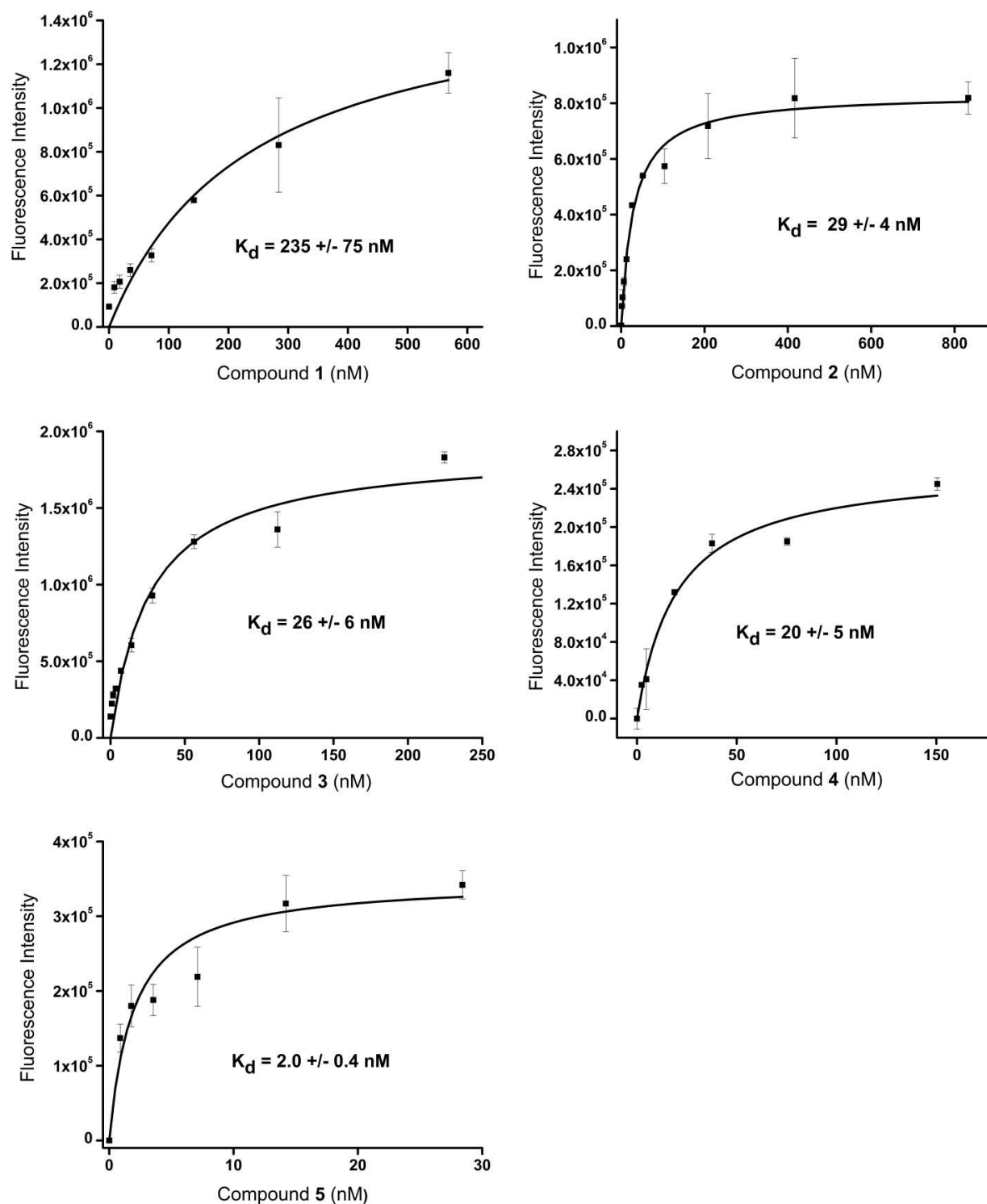
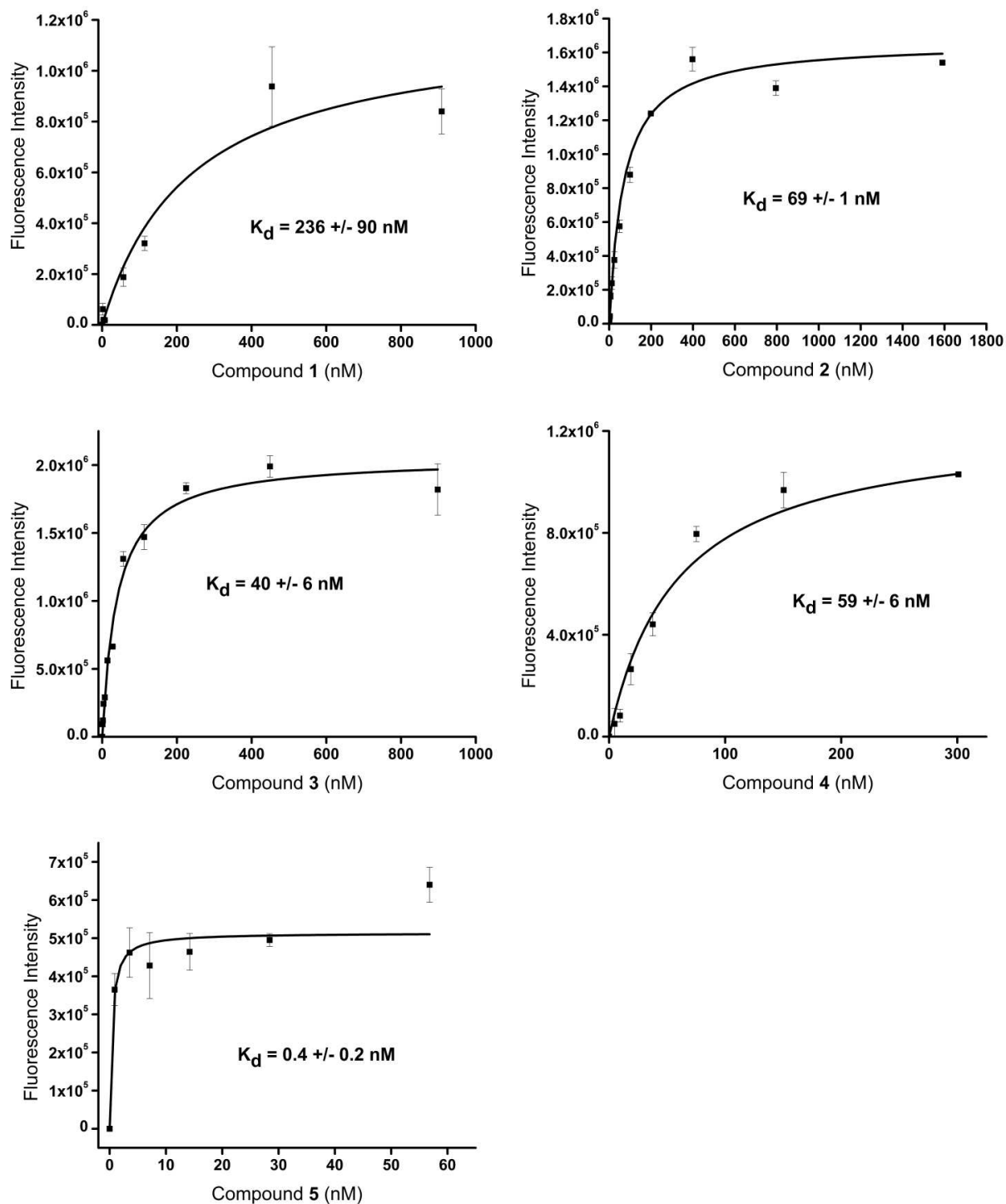


Figure S1. Fluorescence saturation binding curves of compounds 1-5 to Aβ fibrils. λ_{ex} : 355 nm; λ_{em} : 420 nm.



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Figure S2. Fluorescence saturation binding curves of BTA monomer and oligomers to SEVI fibrils. λ_{ex} : 355 nm; λ_{em} : 420 nm.

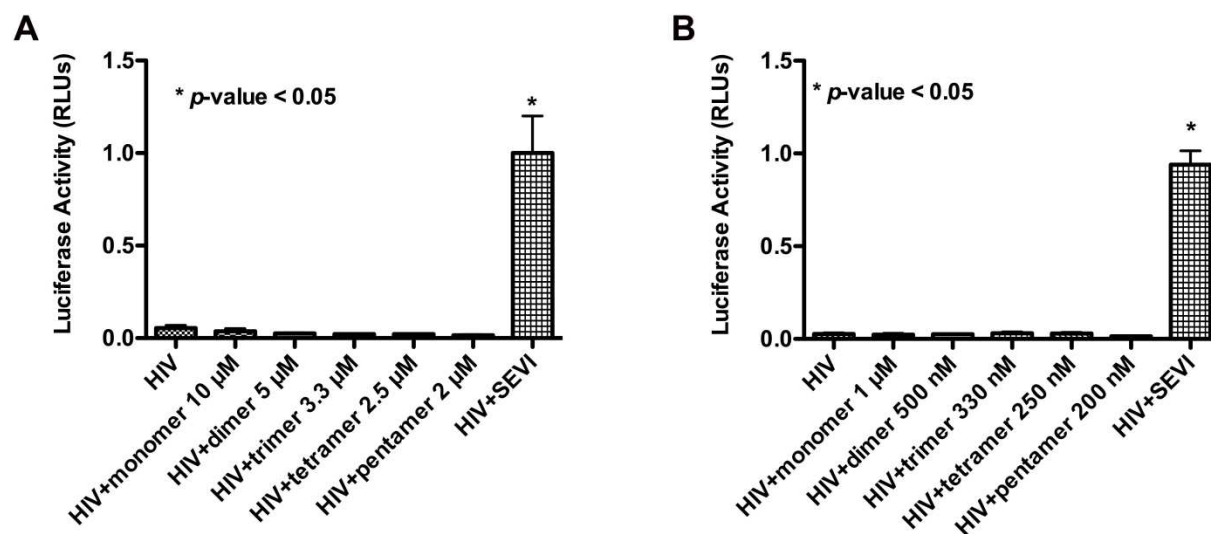


Figure S3. Control studies demonstrating that compounds **1-5** do not affect HIV-1 infection in TZM-bl cells in the absence of SEVI fibrils. RLUs, relative luciferase units. Analyses of the data by 1-way ANOVA with Tukey's post-test revealed that luciferase activity in cells treated with HIV only and cells treated with HIV+compound were not statistically significantly different from one another. * indicates $p < 0.05$ compared to cells treated with only HIV.

Supporting References

- (1) Nilsson, M. R. *Methods* **2004**, *34*, 151-160.
- (2) LeVine, H., III. *Amyloid* **2005**, *12*, 5-14.