Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry


## Supporting information

# Cellular uptake of an α-AApeptide

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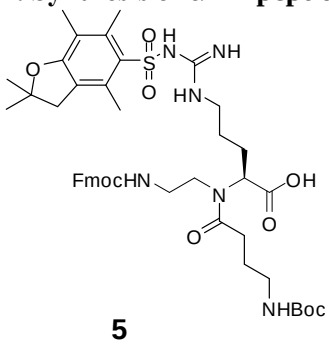
**1. General experimental methods.** Fmoc protected α-amino acids and Knorr resin were obtained from Chem-Impex International, Inc. All other reagents and solvents were provided by either Sigma-Aldrich or Fisher Scientific. The NMR spectrum of the α-AApeptide building block **5** was obtained on a Varian Inova 400. The α-AApeptide sequence was prepared on a Knorr resin in peptide synthesis vessels on a Burrell Wrist-Action shaker. The α-AApeptide was analyzed and purified on a Waters HPLC with both analytical and preparative modules, respectively, and the desired fraction was lyophilized using a Labconco lyophilizer. Molecular weight of the α-AApeptide was identified on a Bruker AutoFlex MALDI-TOF mass spectrometer. Fluorescent flow cytometry was performed using a Zeiss fluorescent imaging system.

**2. Synthesis of α-AApeptide building block 5.**




**5**

Compound **5**. The compound was synthesized following our previous reported protocol.[1, 2] Yield 62% (two steps). $^{1}$H NMR (DMSO-$d_6$, 400MHz) δ = 7.88 (d, *J* = 7.2 Hz, 2H), 7.68-7.65-7.65 (m, 2H), 7.41-7.30 (m,4H), 6.85-6.77 (m, 1H) 6.49 (br.s, 1H), 4.31-4.19 (m, 3H), 3.23-3.15 (m, 1H), 3.05-2.89 (m, 7H), 2.91 (s, 3H), 2.50 (s, 3H), 2.47 (s, 3H), 2.41 (s, 3H), 2.30-2.21 (m, 1H), 2.02-1.97 (m, 3H), 2.02-2.00 (m, 3H ), 1.41-1.1.37 (m, 6.0H), 1.38-1.33 (m, 12 H), 0.89-0.82 (m, 0.5 H) $^{13}$C NMR (DMSO-$d_6$, 100MHz) δ 173.51, 157.81, 155.99, 144.31, 143.0, 144.3, 141.1, 137.6, 134.7, 131.8, 129.3, 128.0, 127.5, 125.7, 124.71, 124.69, 121.8, 120.5, 116.6, 86.6, 79.6, 77.7, 72.7, 65.9, 60.7, 47.2, 42.9, HR-ESI: $[M+H]^+$ cacl: 877.4164 , found: 877.4148.

### 3. Solid phase synthesis, purification and characterization of α -AApeptides.

The fluorescein conjugated Tat 48-57 peptide **1** was synthesized and analyzed by the USF peptide facility, and was used without further purification. The γ-AApeptide building block **5** was synthesized as reported previously. [1, 2] The α-AApeptide **2** and the regular peptide **3** were prepared on a Knorr resin in peptide synthesis vessels on a Burrell Wrist-Action shaker following the standard Fmoc chemistry of solid phase peptide synthesis protocol. [1, 2] Each coupling cycle included an Fmoc deprotection using 20% Piperidine in DMF, and 4 h coupling of 1.5 equiv of building blocks onto resin in the presence of 2 equiv of diisopropylcarbodiimide(DIC) / Oxohydroxybenzotriazole in DMF. After the desired sequences were assembled, they were transferred into a 4 mL vial and cleaved from solid support in 48:50:2 TFA/$CH_2Cl_2$/triisopropylsilane overnight. Then solvent was evaporated and the residues were analyzed and purified on an analytical (1 mL/min) and a preparative Waters (20 mL/min) HPLC systems, respectively. The same methods were used by running 5% to 100% linear gradient of solvent B (0.1% TFA in acetonitrile) in solvent A (0.1% TFA in water) over 40 min, followed by 100% solvent B over 10 min. The desired fractions were collected and lyophilized. The molecular weights of oligomers were obtained on a Bruker AutoFlex MALDI-TOF mass spectrometer using α–cyano-4-hydroxy-cinnamic acid as the matrix.

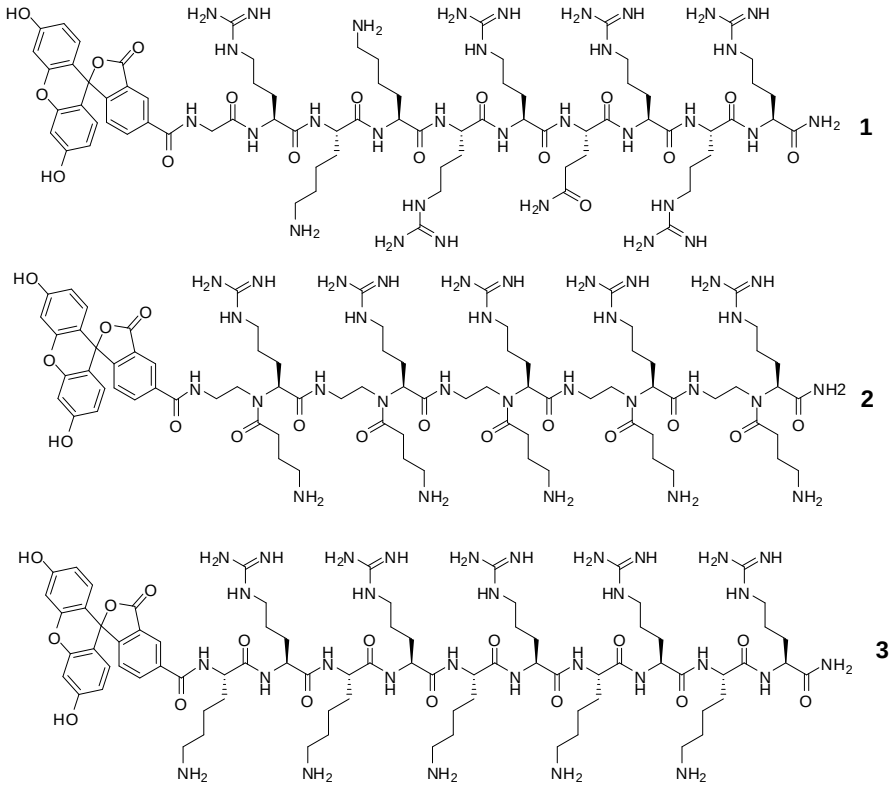
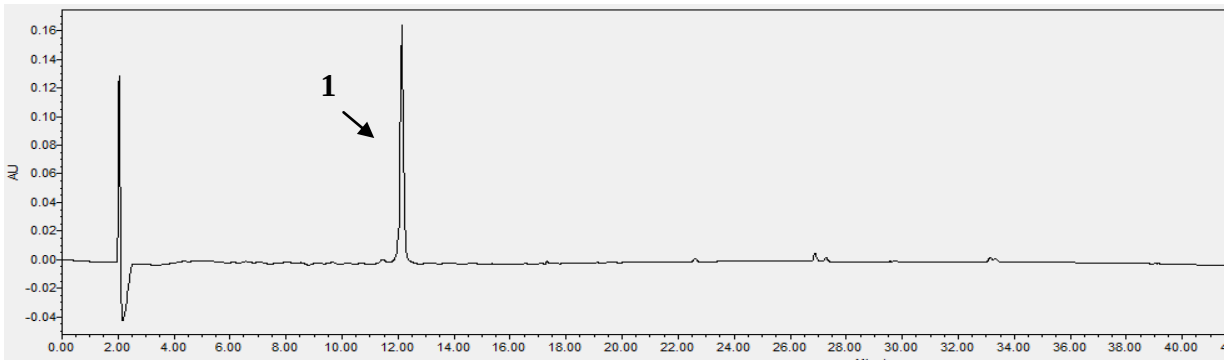


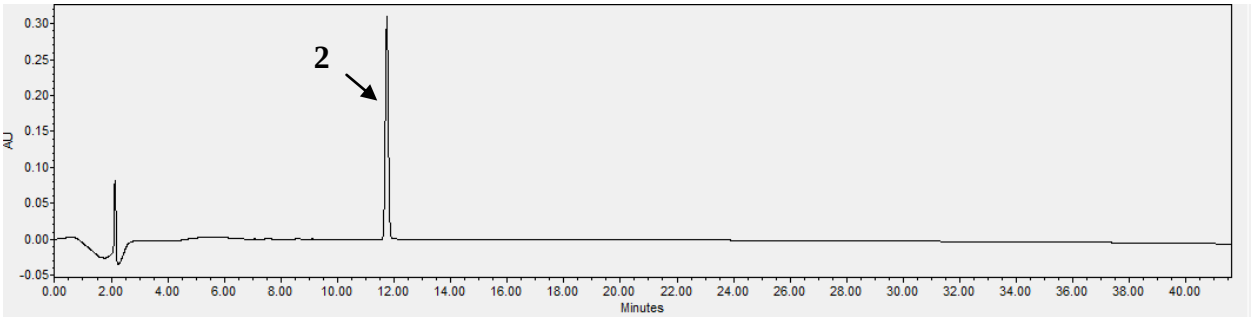

**Figure S1**. The structures of oligomers used in the experiments.

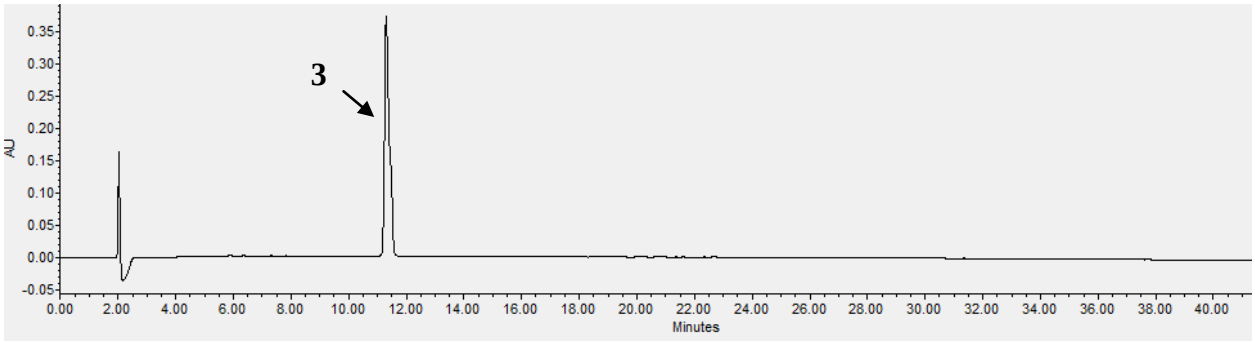
**Table S1.** MALDI-TOF MS analysis of AApeptides.

| Sequences | Formula | Mass calcd. | Mass found |
|---|---|---|---|
| **1** | $C_{76}H_{120}N_{32}O_{17}$ | 1753.0 | 1754.3 $(M + H)^+$ |
| **2** | $C_{81}H_{133}N_{31}O_{16}$ | 1797.1 | 1799.4 $(M + H)^+$ |
| **3** | $C_{81}H_{133}N_{31}O_{16}$ | 1797.1 | 1798.3 $(M + H)^+$ |

**HPLC traces of oligomeric sequences.**




## 4. Cellular uptake assay by fluorescent flow cytometry. [3]

The oligomers **1, 2, 3** were each dissolved in PBS buffer (pH 7.4), and their concentration was determined by UV-vis spectrometer at 490 nm (ε = 67,000, absorption of fluorescein). Jurkat cells (human T cell line) were used for cellular uptake experiments. Cells were seeded to 96 well plate with 6 X $10^5$ cells per well. Different volume of oligomer stock solution (2% FBS in PBS solution) and PBS buffer were added to the 96-well plate (combined total of 200 µl) and incubated for 10 min at 37 ℃. The cells were then washed with PBS buffer for three times, and then analyzed by using fluorescent flow cytometry and the data presented are the mean fluorescent signal for the 5,000 cells collected.

## 5. Confocal Microscopy. [4, 5]

***Co-localization studies.*** A cell plate (with 4 slots) was polylysine-coated by applying 0.01 % Poly-L Lysine in PBS. 160,000 Hela cells were plated in 1mL of DMEM with 10% FBS and 1% PSG onto one

slot of plate. Oligomers were diluted to 5 μM in 1mL of appropriate DMEM in 1.5 mL microcentrifuge tubes. Media was aspirated from plates and replaced with 500uL complete DMEM containing peptide. Cells were incubated at 37 $^{o}$C for 25 minutes and then washed gently with 2 mL PBS. 1mL of 4% PFA was added to all wells. Plate was covered and cells were incubated at room temperature for 15 minutes, after which 10 μL of DAPI/mounting medium was added directly to cells in wells. The cells were extensively washed with PBS buffer and then imaged at 63 X magnification in oil emersion.

***Inhibition of Cellular Uptake with Sodium Azide.*** The assays were performed as described above with the exception that the cells used were pre-treated for 30 min with 0.5% sodium azide in 2% FBS/PBS buffer before the addition of oligomers, and then viewed by confocal microscopy.

***Inhibition of Cellular Uptake with filipin.*** The assays were performed as described above with the exception that the cells used were pre-treated for 10 min with 3 μg/ml filipin in serum free DMEM medium before the addition of oligomers, and then viewed by confocal microscopy.

***Inhibition of Cellular Uptake with sucrose.*** The assays were performed as described above with the exception that the cells used were pre-treated for 10 min with 200 mM sucrose in serum free DMEM medium before the addition of oligomers, and then viewed by confocal microscopy

**6. MTT proliferation assay**

**Jurkat cells** (**CRL-1990, ATCC**) were cultured in RPMI 1640 media (containing 10% FBS and Penicillin and Streptomycin). After 12 hours before treatment, cells were separated by pipetting and plated in 96-well plate with ≈5,0000 cells in 100 μL media per well. Control and Blank wells were prepared accordingly. Serial dilutions of oligomers at concentrations of 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 μM (50 μL per well at 2x concentration) were prepared by diluting stock solution with media. 50 μL media were removed from each well without disturbing Jurkat cells, which were loosely attached to the plate. Then 50uL diluted oligomer solutions (2x) were transferred to cells, resulting in a final 1x concentration for each well. After 36 hours, all media in cells was carefully removed. 10 μL MTT reagent (Roche) and 100 μL pre-warmed media were then added. The plate was incubated at 37 ℃ for 4 h, to which was added 100 μL pre-warmed solubilization solutions each well. After overnight incubation, the absorbance of each well at 550nm was read and recorded. The data was processed based on the equation: Percentage of viability =[ $(A - A_{\text{bland}})/(A_{\text{control}} - A_{\text{blank}})$] × 100%, where $A$ is the average absorbance of the blank wells with only media and MTT solutions, and $A_{\text{control}}$ is the average absorbance of wells of cells without treatment of oligomers. The measurements were repeated at least three times.

**Hela cells** were cultured in DMEM media (contain 10% FBS and Penicillin and Streptomycin). After 12 hours before treatment, cells were separated by pipetting and plated in 96-well plate with ≈1,0000 cells in 100 μL media per well. Control and Blank wells were prepared accordingly. Serial dilutions of oligomers at concentrations of 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 μM (100μL per well at 2x concentration) were prepared by diluting stock solution with media. All media in each well was removed. Then 50 μL media and 50 μL diluted oligomer solutions (2x) were transferred to cells, resulting in a final 1x concentration in each well. After 36 hours, all media in cells was carefully removed. 10 μL MTT reagent (Roche) and 100 μL pre-warmed media were then added. The plate was incubated at 37 ℃ for 4 h, to which was added 100 μL pre-warmed solubilization solutions each well. After overnight incubation, the absorbance of each well at 550nm was read and recorded. The data was processed based on the equation: Percentage of viability =[ $(A - A_{\text{bland}})/(A_{\text{control}} - A_{\text{blank}})$] × 100%, where $A$ is the average absorbance of the blank wells with only media and MTT solutions, and $A_{\text{control}}$ is the average absorbance of wells of cells without treatment of oligomers. The measurements were repeated at least three times.

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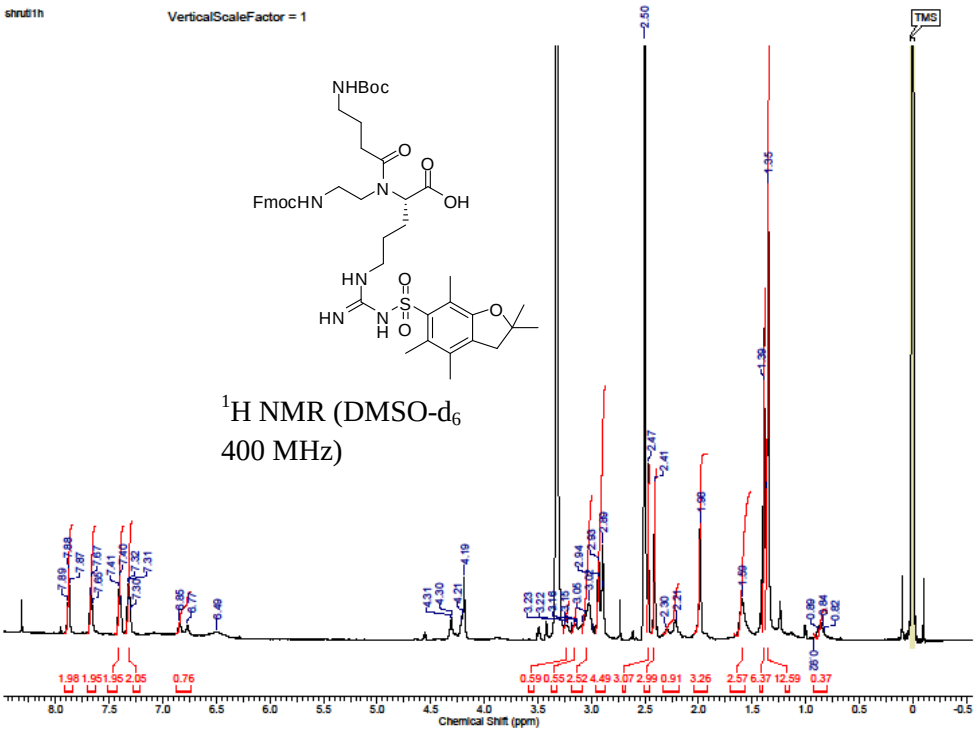
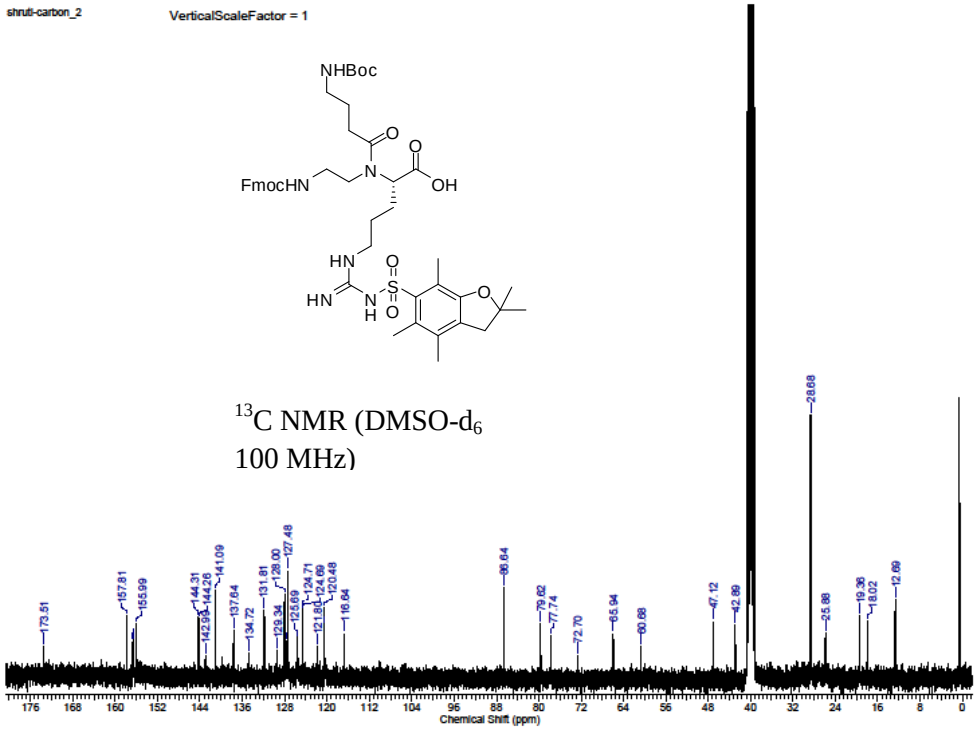



$^{1}$H NMR (DMSO-d$_6$ 400 MHz)




$^{13}$C NMR (DMSO-d$_6$ 100 MHz)