Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 6604

www.rsc.org/obc

PAPER

γ-AApeptides bind to RNA by mimicking RNA-binding proteins†

Youhong Niu,‡[a] Alisha "Jonesy" Jones,‡[b] Haifan Wu,[a] Gabriele Varani*[b,c] and Jianfeng Cai*[a]

Received 11th May 2011, Accepted 1st July 2011
DOI: 10.1039/c1ob05738c

The interactions between proteins and RNAs are of vital importance for many cellular processes, including transcription and processing of RNA, translation, and viral infections. Here we report an γ-AApeptide that can mimic HIV-1 Tat protein and bind to TAR RNAs of HIV and BIV with nanomolar affinity, comparable to that of the RNA-binding fragment of Tat (amino acids 49–58). The interaction is resistant to the presence of a large excess of tRNA. With resistance to proteolytic hydrolysis and limitless potential for diversification, γ-AApeptides may emerge as a new class of peptidomimetics to modulate RNA-protein interactions.

Introduction

The discovery of novel molecules that can bind strongly to RNA and regulate RNA-protein interactions could have very broad application in chemical and molecular biology, since RNA-protein interactions are important in many aspects of cellular functions.[1–3] These RNA-binding molecules could be very promising tools or novel therapeutics in the biological sciences and medicine. A well-known approach to discovering such molecules is to develop oligonucleotides to directly target RNA or to regulate gene expression by antisense or RNAi mechanisms.[4] However, there are considerable limitations for clinical development, including cost, delivery and metabolic stability.[4] Another attractive approach is to develop small molecules which can mimic RNA binding proteins, since many RNAs are highly structured and create unique binding sites to specific proteins. In contrast to oligonucleotides, small molecules are more easily adaptable to clinical therapeutic development. Nonetheless, the development of small molecules that can bind to RNAs with high affinity is very challenging. This is because RNA-protein interactions are similar to protein-protein interactions, involving a large surface area for recognition and tight binding. Thus, competition of small molecular weight drugs with large macromolecular complexes has so far been a very difficult task to achieve. An alternative approach that relies instead of using larger molecular weight peptides has been advocated. This approach could be more effective because peptides are much larger than traditional small molecular weight drugs, and therefore more likely to compete with a protein for binding to RNA. Additionally, they are also much more amenable to clinical development than oligonucleotides. In the past, a variety of peptide mimetics of RNA-binding proteins have been introduced,[4–11] but none has reached even the pre-clinical stage of development as drug candidates. Here we report the discovery of γ-AApeptides, mimics of a well-known RNA-binding protein.

The HIV TAR RNA-Tat complex is one of the best studied protein-RNA interactions because of its involvement in transcriptional activation and essential role for viral replication of human immunodeficiency virus type 1 (HIV-1).[6] The Tat (transactivator) viral protein specifically binds to the transactivator response element (TAR) RNA and stimulates the transcription of the viral genome.[12] TAR has been found to be extremely conserved among viral isolates, and the Tat-TAR interaction is unique and essential to the virus,[5] without which HIV would fail to replicate. Therefore, the TAR-Tat complex is a promising target for the development of new antiviral agents through the disruption of the TAR-Tat interaction, which would inhibit viral replication at both latent and active stages of infected cells.[5] Therefore, the TAR-Tat interaction is an excellent testing ground as well as a promising therapeutic target for the development of novel peptidomimetics to disrupt RNA-protein interactions.

In order to develop inhibitors of Tat-TAR interaction, significant effort has been dedicated to synthesize and evaluate short peptides that can mimic Tat protein and disrupt Tat binding to TAR.[4–11] Among them, oligopeptidomimetics such as oligocarbamates,[13] oligoureas,[14] β-peptides,[15] peptoids[16] and templated cyclic peptides[5] were considered, since these structures are resistant to proteolytic degradation. However, more than a decade's exploration has not led to any clinical drugs, in part because a structure of the HIV-1 TAR/Tat complex remains to be determined, due to its highly conformational dynamics.[17] Recently, our group has developed a new class of peptide mimics – γ-AApeptides,[18] based on the γ-PNA backbone.[19] These γ-AApeptides can project the same number of functional groups as peptides of equivalent length, suggesting that they could

[a]*Department of Chemistry, University of South Florida, 4202 E. Fowler Ave., Tampa, FL, 33620, USA. E-mail: jianfengcai@usf.edu*
[b]*Department of Chemistry, University of Washington, Box 351700, Seattle, WA, 98195, USA*
[c]*Department of Biochemistry, University of Washington, Box 357350, Seattle, WA, 98195, USA. E-mail: varani@chem.washington.edu*

† Electronic supplementary information (ESI) available: NMR and MS spectra of building blocks, HPLC traces and MALDI of oligomeric sequences. See DOI: 10.1039/c1ob05738c

‡ These authors contributed equally to this work.

structurally mimic an RNA-binding protein. They can be modified with virtually limitless potential by introducing a wide variety of functional groups and are resistant to proteolytic degradation.[18] Their potential biomedical application has been demonstrated by their capability to disrupt the p53-MDM2 protein-protein interaction.[18] To further explore the applications of γ-AApeptides, we demonstrate here that a γ-AApeptide analogue of Tat 48–57 can bind to HIV TAR RNA with nanomolar affinity. The results indicate that γ-AApeptides are valid peptide mimics of RNA binding proteins, and they can potentially be further developed to modulate RNA-protein interactions in the future.

The arginine-rich segment of HIV-1 Tat (residues 48–57) makes direct contacts with the TAR trinucleotide bulge region, and is the key determinant of the Tat-TAR interaction.[17] Many oligopeptidomimetic inhibitors were designed based on this fragment of Tat.[5,8,12–16] Since HIV Tat 48–57 adopts an extended conformation,[20] we hypothesized that γ-AApeptide **γ-AA1** (Fig. 1) would be able to mimic HIV Tat 48–57. **γ-AA1** and Tat 48–57 have identical molecular weight and project exactly the same functional groups; the relative positions of these functional groups are similar to each other when the peptide conformation is extended. To test this hypothesis, we synthesized γ-AApeptide **γ-AA1** and γ-AApeptide **γ-AA2** (a truncated sequence mimicking Tat 48–53). The control HIV Tat 48–57 peptide **P1** was also prepared for comparison.

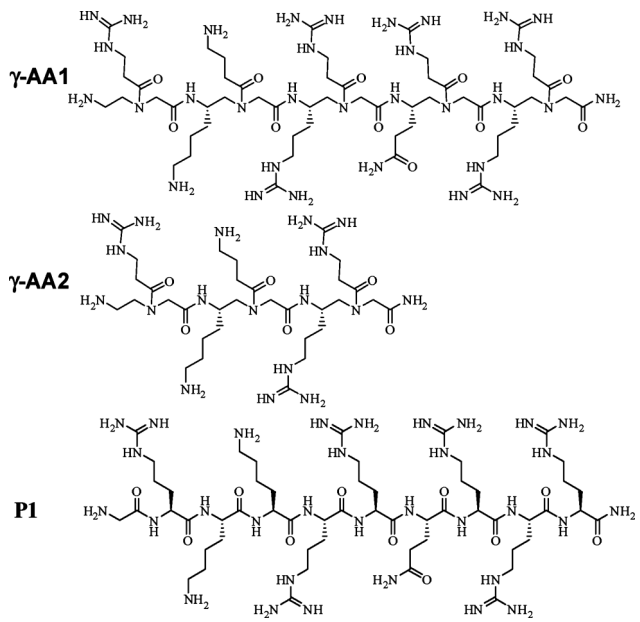


Fig. 1 γ-AApeptides γ-AA1 and γ-AA2 and control Tat 48-57 peptide P1.

Results and discussion

The synthesis of γ-AApeptides was carried out by manual solid-phase synthesis from Fmoc-protected γ-AApeptide building blocks, a method developed by our group recently to synthesize AApeptide sequences.[18,21] Typically (Fig. 2), a Fmoc protected amino aldehyde **1** reacted with benzyl glycinate to form secondary amine **2**, which was acylated by either γ-Boc-amino butyric acid or di-Boc-guanidinopropionic acid to give **3**. Subsequent

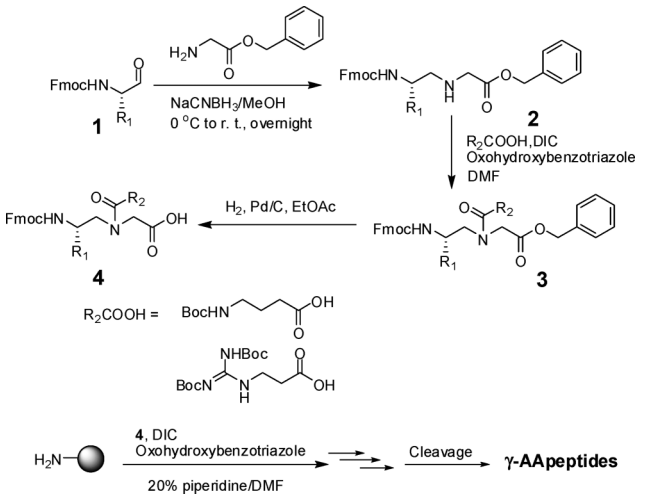


Fig. 2 Synthesis of γ-AApeptide building blocks and of γ-AApeptides.

hydrogenation provided Fmoc protected γ-AApeptide building blocks **4**. These γ-AApeptide building blocks were assembled on solid phase, and the desired sequences were cleaved from the solid support, purified by HPLC and characterized by MALDI (see ESI for details†).

To investigate whether γ-AApeptides could mimic the Tat 48–57 peptide **P1**, we tested their binding to HIV-1 TAR RNA by measuring K_d's using EMSA (electrophoretic mobility shift assay); the closely related BIV TAR RNA (bovine immunodeficiency virus) was used as a control for specific binding. Both HIV-1 and BIV are lentiviruses, and the functions of Tat and TAR are conserved; the sequences and secondary structures of HIV-1 and BIV TAR are also highly similar (Fig. 3). Because of these similarities, we anticipated that these Tat γ-AApeptide mimetics should bind tightly to both HIV-1 and BIV TAR by recognizing their TAR-Tat binding regions.

The EMSA experiments demonstrated that γ-AApeptide **γ-AA1** (Fig. 4) can bind to both HIV-1 TAR and BIV TAR RNAs with nanomolar affinity. Furthermore, binding in the low μM regime is retained even in the presence of 25,000-fold excess of tRNA (Fig. 4), suggesting that the interaction is partially specific for the TAR RNA structures, in that even a huge excess of tRNA fails to completely abolish complex formation. Binding affinities are listed in Table 1 and compared with those of γ-AApeptide **γ-AA2** and Tat 48–57 peptide **P1**, which were also obtained by EMSA.

K_d' values shown in Table 1 were calculated from data as shown in Fig. 4, B and D, where a 250-fold excess of tRNA was present. In the absence of tRNA, band smearing was observed. This smearing may occur due to partial dissociation of complexes during gel electrophoresis,[15] as well as to the likely presence of non-specific, lower affinity complexes between the peptides and the TAR RNAs,

Table 1 Summary of the affinity of different peptide and peptide mimetic sequences for their interaction with HIV-1 and BIV TAR, as determined by EMSA

Sequences	K_d' (HIV), nM	K_d' (BIV), nM
AA1	166	300
AA2	>33000	>33000
P1	166	333

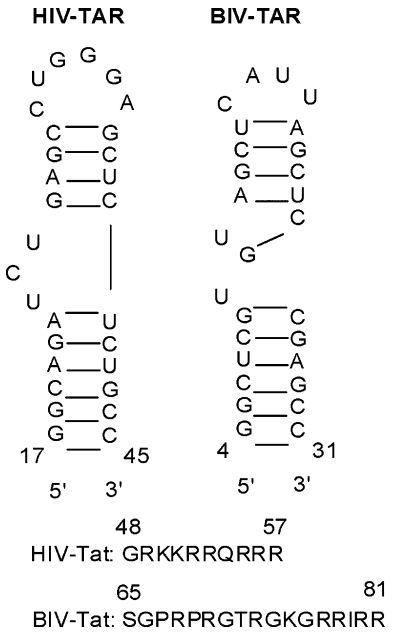


Fig. 3 Secondary structures of HIV-1 and BIV TAR RNA, and partial sequences of HIV-1 Tat protein (48–47) and BIV Tat protein (65–81); these fragments are largely responsible for the interaction with their respective TAR RNAs.

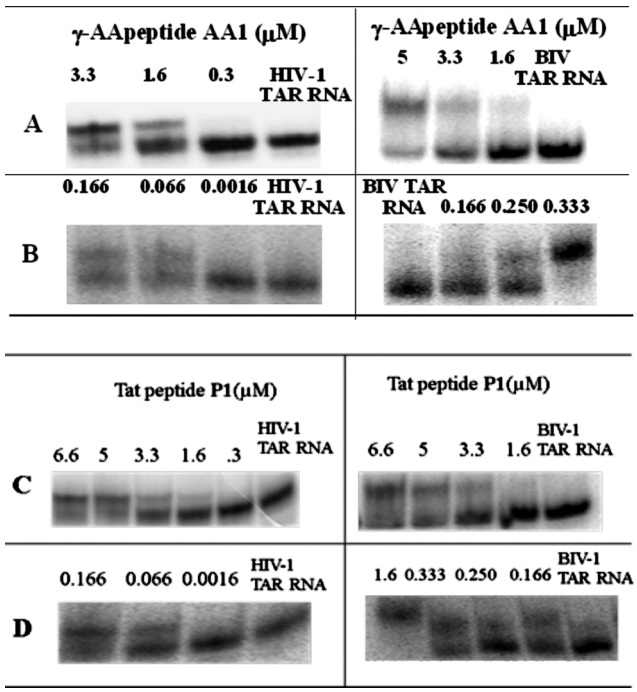


Fig. 4 Binding of γ-AApeptide **γ-AA1** to HIV-1 and BIV TAR RNAs assayed by EMSA. **A**, Binding of γ-AApeptide 1 to HIV-1 (0.4 nM) and BIV TAR (0.4 nM); the buffer contains a 25,000-fold excess of tRNA to reduce non-specific binding. **B**, Binding of γ-AApeptide **γ-AA1** to HIV-1 (0.4 nM) and BIV TAR (0.4 nM) RNAs; the buffer contains a smaller excess of tRNA (250-fold) to measure K_d's more accurately. **C**, Binding of Tat peptide **P1** to HIV-1 (0.4 nM) and BIV TAR (0.4 nM); the buffer contains a 25,000-fold excess of tRNA to reduce non-specific binding. **D**, Binding of Tat peptide **P1** to HIV-1 (0.4 nM) and BIV TAR (0.4 nM) RNAs; the buffer contains a smaller excess of tRNA (250-fold) to measure K_d's more accurately.

which were reduced or eliminated in the presence of the tRNA, resulting only in the presence of a specific peptide-TAR complex. The EMSA results show that γ-AApeptide **γ-AA1** binds to HIV TAR with K_d of 166 nM, 2-fold more tighly than binding to BIV TAR (K_d' = 300 nM). As expected, Tat peptide **P1** can bind to HIV and BIV TAR tightly with the same K_d' of 166 nM and 333 nM, respectively. Interestingly, γ-AApeptide **γ-AA2**, although carrying a few postively charged side functional groups, failed to provide any binding capability to both HIV and BIV TAR under our experimental conditions. The EMSA results show that γ-AApeptide **γ-AA1** binds to HIV-1 and BIV TAR RNAs as tightly as the Tat-derived **P1** peptide 48–57, even if the γ-AApeptide backbone is more flexible than the conventional peptide backbone.

Although structural information is not yet available for the complete HIV TAR/Tat complex, we have previously used NMR to investigate the conformational change of HIV TAR when binding to Tat and other small molecules and peptides.[12,22,23] In the presence of Tat and other ligands, the bulge region of TAR undergoes a local conformational rearrangement and forms a more stable structure (Fig. 5). This folding process can be induced by any ligand containing a guanidinium group and even by the single amino acid analogue argininamide. However, the interaction of this guanidinium group with TAR is not the only source of binding affinity and specificity for Tat recognition. NMR studies demonstrated that there are multiple points of contacts between base functional groups and phosphate groups of HIV TAR and amino acid residues of Tat. These interactions contribute not only to the affinity of the interaction but also to its specificity. Therefore, based on the experimental results, we postulate that **γ-AA1** binds to HIV TAR by mimicking the Tat peptide **P1**, because they posses the same side functional groups. Following the mechanism of Tat-TAR interaction, **γ-AA1** could recognize the bulge of HIV TAR using one of the guanidinium groups, which would re-fold

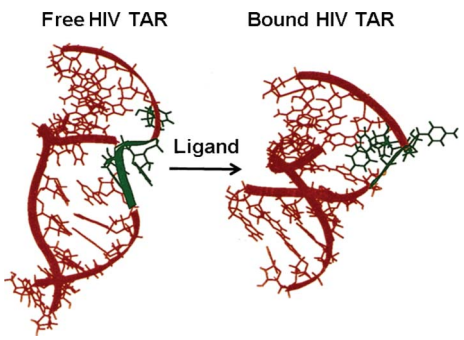


Fig. 5 Schematic representation of the mechanism of HIV TAR/Tat recognition (adapted from reference22). Binding of Tat re-folds the bulge of TAR into a locally different conformation.

the HIV TAR bulge and define the precise positioning of critical functional groups in the major groove. Compared to α-peptides, half of the C=O groups have been relocated to side chains, which leads to increased comformational freedom for the γ-AApeptide backbone. As a result, **γ-AA1** may be able to more easily adjust its conformation in the TAR- γ-AApeptide complex by mimicking that of the Tat peptide so as to achieve optimal binding.[17]

Satisfactorily, the truncated γ-AApeptide **γ-AA2** has completely lost its binding capability to both HIV-1 and BIV TAR RNAs, strongly supporting the vitally important presence of three

neighboring guanidino functional groups for binding. This result also further suggests that the TAR- γ-AApeptide interaction is not purely driven by electrostatic interactions, since the truncated γ-AApeptide **γ-AA2** retains as many guanidino functional groups as small molecules that were shown to bind to TAR with nM affinity.[9] The sequence is highly positively charged, yet it shows no interaction with TAR RNA in the presence or absence of tRNA. This result indirectly shows the importance of multiple points of interactions between HIV TAR and **γ-AA1**, similar to the HIV TAR-Tat interaction. It is also noteworthy that there is a small binding preference of **γ-AA1** for HIV-1 compared to BIV TAR (2-fold), which may be due to **γ-AA1** being the mimic of HIV-1 Tat protein and not of BIV Tat. Altogether, these observations provide a starting point for the rational design of more potent and selective RNA-binding γ-AApeptides in the future.

Conclusions

We have developed a new peptide mimetic structure, the γ-AApeptides, which mimics the Tat peptide and binds to TAR RNAs with nanomolar affinity. Our findings suggest that γ-AApeptide structures are capable of binding to RNAs by mimicking RNA-binding proteins. These structures can be developed further to probe or disrupt RNA-protein interactions in the future. This is a further demonstration of the promising biological activity of γ-AApeptides, that have already been shown to disrupt protein-protein interactions.[18,21] Due to their resistance to proteolysis, convenient synthesis and limitless diversification, there is great potential for γ-AApeptide **AA1** to be further optimized to identify new anti-viral leads. A challenge will be increasing the specificity of these flexible structures, which could be achieved by restraining the conformation of γ-AApeptide by forming cyclic ring structures. NMR studies of TAR- γ-AApeptide complex are currently under investigation.

Experimental

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. NMR spectra of intermediates and γ-AApeptide building blocks were obtained on a Varian Inova 400. γ-AApeptide sequences were prepared on a Knorr resin in peptide synthesis vessels on a Burrell Wrist-Action shaker. The γ-AApeptides were analyzed and purified on a Waters HPLC with both analytical and preparative modules, respectively, and the desired fractions were lyophilized using a Labconco lyophilizer. Molecular weights of γ-AApeptides were identified on a Bruker AutoFlex MALDI-TOF mass spectrometer.

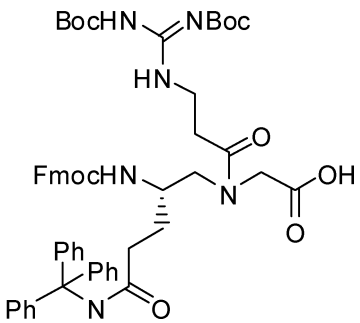
2. Synthesis of γ-AApeptide building blocks

General procedure. Typical synthesis of 2. To glycine benzyl ester hydrochloride in 20 ml methanol in a 100 ml round bottom flask was added 1.2 equiv. of triethylamine and stirred at 0 °C for 15 min. Stoichiometric amount of a Fmoc protected amino acid aldehyde[24,25] was added and the solution mixture was stirred for another 30 min. Catalytic amount of acetic acid was then added, followed by 2 equivalents of $NaBH_3CN$. The solution was allowed to stir at 0 °C for 1 h and continue at room temperature overnight. The solvent was evaporated and 100 ml ethyl acetate and 100 ml saturated sodium bicarbonate solution were added to the residue. The organic layer was separated and washed with 100 ml brine, dried over anhydrous sodium sulfate, and removed *in vacuo*. Flash chromatography using ethyl acetate/hexane 1 : 1 gave **2** as a colorless oil.

Typical synthesis of 3. Compound **2**, 1.2 equiv. of DIC, Oxohydroxybenzotriazole, and R_2COOH were stirred in 20 ml DMF overnight. The solution was then partitioned in 100 ml ethyl acetate and 100 ml water. The organic layer was separated and washed with water (3 ×100 ml) and Brine (2 ×100 ml), dried over anhydrous sodium sulfate, and then concentrated *in vacuo*. Flash chromatography using ethyl acetate/hexane 1 : 3 gave **3** as a colorless oil.

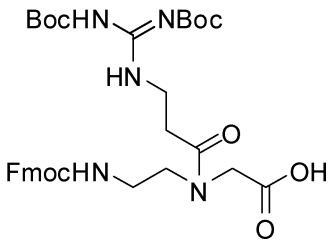
Typical synthesis of 4. **3** in 20 ml ethyl acetate was added to 10% Pd/C and hydrogenated at atmospheric pressure and room temperature overnight. The solution was evaporated and the residue was purified by flash chromatography 5–7% $MeOH/CH_2Cl_2$ to give **4** as a white foam solid.

4a



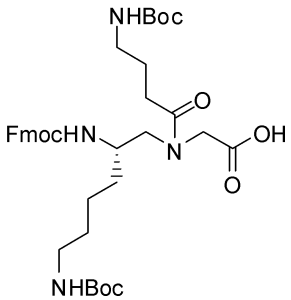
Yield was 41% in three steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ (two rotamers) 11.39 (m, 1H), 10.71 (d, J = 4.0 Hz, 1H), 8.66–8.60 (m, 1H), 8.53–8.50 (m, 1H), 8.29–8.23 (m, 1H), 7.85 (d, J = 8.0 Hz, 2H), 7.65–7.62 (m, 2H), 7.41–7.35 (m, 2H), 7.29–7.09 (m, 15H), 4.30–4.14 (m, 3H), 4.05–4.03 (m, 1H), 3.98–3.75 (m, 2H) 3.46–3.40 (m, 2H), 3.30–3.17 (m, 2H), 3.02–2.56 (m, 2H), 2.35–2.22 (m, 2H), 1.65–1.19 (m, 20H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (two rotamers) 171.8, 170.8, 156.5, 155.3, 153.5, 152.1, 151.9, 145.3, 144.3, 144.2, 141.2, 128.9, 128.0, 127.8, 127.4, 126.7, 126.7, 125.5, 125.4, 120.6, 120.5, 84.0, 83.3, 69.6, 65.8, 56.4, 52.3, 49.9, 47.3, 47.2, 37.1, 33.0, 31.7, 28.4, 28.0, 27.97, 27.90, 18.97. HRMS for $[M+Na]^+$ Calc: 989.4420, found: 989.4428.

4b



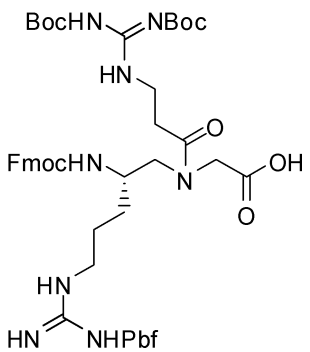
Yield was 38% in three steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ (two rotamers) 11.43 (s, 1H), 8.58–8.50 (m, 1H), 7.86–7.80 (m, 2H), 7.64–7.61 (m, 2H), 7.40–7.25 (m, 4H), 4.25 (m, 3H), 2.75–3.67 (m, 2H), 3.55–3.42 (m, 5H), 3.15–3.10 (m, 2H), 2.55–2.54 (m, 1H), 2.45–2.42 (m, 1H), 1.42&1.40&1.34&1.33 (4 s, 18H) ^{13}C NMR (100 MHz, DMSO-d_6) δ (two rotamers) 172.3, 163.6, 156.52, 156.5, 152.3, 144.3, 143.0, 141.1, 139.9, 137.9, 129.4, 127.7, 127.3, 125.7, 129.4, 127.7, 127.5, 125.7, 120.5, 83.2, 79.63, 78.5, 65.9, 56.5, 53.4, 47.2, 38.6, 36.8, 32.5, 28.4, 28.04. HRMS for $[M+H]^+$ Calc: 654.3137, found: 654.3138.

4c



Yield was 69% in three steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ (two rotamers) 7.84 (d, J = 8.0 Hz, 2H), 7.64–7.61 (dd, J = 4.0, 8.0 Hz, 2H), 7.37 (t, J = 8.0 Hz, 2H), 7.28 (t, J = 8.0 Hz, 2H), 7.14 & 6.98 (2d, J = 8.0 Hz, 1H), 6.74–6.69 (m, 1H), 4.29–4.14 (m, 3H), 3.40–3.81 (m, 2H), 3.62–3.52 (m, 2H), 3.28–3.16 (m, 1H), 2.95–2.69 (m, 4H), 2.36–2.22 (m, 1H), 2.12–2.01 (m, 1H), 1.70–1.13 (m, 26H). ^{13}C NMR (100 MHz, CD_3OH) δ (two rotamers) 174.7, 174.4, 171.5, 171.2, 157.32, 157.3, 157.1, 143.9, 143.8, 141.23, 141.20, 127.3, 126.7, 124.7, 124.6, 119.5 78.5, 78.4, 66.1, 65.9, 53.1, 51.2, 50.1, 49.9, 49.8, 39.8, 39.5, 39.3, 31.6, 31.2, 29.7, 29.5, 29.2, 27.4, 25.2, 22.9, 22.8. HRMS for $[M+H]^+$ Calc: 697.3807, found: 697.3796.

4d



Yield was 43% in three steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ (two rotamers) 11.37 (s, 1H), 10.80 (d, J = 8.0 Hz, 1H), 8.83–8.77 (m, 1H), 8.36–8.32 (m, 1H), 7.83 (d, J = 8.0 Hz, 2H), 7.64–7.59 (m, 2H), 7.38–7.34 (m, 2H), 7.30–7.25 (m, 2H), 7.20–7.16 (m, 1H), 7.04 (d, J = 8.0 Hz, 1H), 6.72 (br.s, 1H), 6.40 (br.s, 1H), 4.28–4.11 (m, 4H), 4.10–3.81 (m, 2H), 3.64–3.54 (m, 1H), 3.53–3.15 (m, 3H), 3.04–2.94 (m, 2H), 2.90 (s, 2H), 2.76–2.62 (m, 2H), 2.50–2.42 (m, 4H), 2.39 (s, 3H), 1.96 (s, 3H), 1.49–1.19 (m, 28H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (two rotamers) 172.2, 171.8, 163.5, 157.9, 156.6, 152.3, 144.4, 144.25, 144.23, 144.2, 141.2, 137.7, 134.7, 131.8, 127.98, 127.43, 125.6, 125.5, 124.7, 120.5, 116.7, 86.7, 83.2, 78.5, 65.7, 65.5, 55.3, 52.41, 52.39, 49.9, 47.9, 47.3, 47.2, 42.9, 36.96, 36.8, 32.5, 32.0, 29.8, 29.5, 28.7, 28.4, 28.0, 27.98, 26.2, 19.4, 18.0, 18.0, 12.7. HRMS for $[M+H]^+$ Calc: 1004.4677, found: 1004.4677.

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

The Tat 48–57 peptide **P1** was synthesized and analyzed by the USF peptide facility, and was used without further purification. The two γ-AApeptides 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. Each coupling cycle included an Fmoc deprotection using 20% Piperidine in DMF, and 4 h coupling of 1.5 equiv of γ-AApeptide building blocks onto resin in the presence 2 equiv of DIC (diisopropylcarbodiimide)/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^{-1}) and a preparative Waters (20 ml min^{-1}) HPLC systems, respectively. The same methods were used by running 5% to 100% linear gradient of solvent B (0.1% TFA in acetonitrile) in A (0.1% TFA in water) over 40 min, followed by 100% solvent B over 10 min. The desired fractions were >70% in crude (as determined by HPLC) and eluted as single peaks at > 95% purity. They were collected and lyophilized. The molecular weights of γ-AApeptides and Tat peptide were obtained on Bruker AutoFlex MALDI-TOF mass spectrometer using α–cyano-4-hydroxy-cinnamic acid as the matrix.

4. HIV-1 and BIV TAR RNA preparation[26]

Body-labeled RNA was prepared by *in vitro* transcription using T7 RNA polymerase, a synthetic oligonucleotide template, and a nucleotide mixture containing [R-^{32}P]-CTP (3000 Ci/mmol). The RNA oligonucleotides were purified by denaturing PAGE, and concentrations were determined by UV at 260 nm. RNA was annealed by heating at 90 °C and slow cooling to room temperature in sterile H_2O at a concentration of 20–100 nM. Binding assays were performed at 4 °C.

5. EMSA (Electrophoretic mobility shift assay)[4]

γ-AAPeptides or Tat 48–57 peptide and RNA were incubated in a buffer (10 μL) containing Tris-HCl (50 mM, pH 8.0), KCl (50 mM), DTT (200 mM), tRNA (*Escherichia coli*) and Triton X-100 (0.05%). The samples were fractionated by loading onto 12% native polyacrylamide gels in 0.5% TB buffer and electrophoresed at 15 W and 4 °C. Dried gels were exposed to a phosphor imaging plate and scanned with a Molecular Dynamics phosphor imager. Bands corresponding to free and bound RNA were quantified using ImageQuant.

Acknowledgements

This work is supported by USF start-up fund to JC and by a grant from NIH-NIAID to GV.

References

1 N. Leulliot and G. Varani, *Biochemistry*, 2001, **40**, 7947–7956.
2 H. M. Al-Hashimi, *ChemBioChem*, 2005, **6**, 1506–1519.
3 D. E. Draper, *J. Mol. Biol.*, 1999, **293**, 255–270.
4 Z. Athanassiou, K. Patora, R. L. Dias, K. Moehle, J. A. Robinson and G. Varani, *Biochemistry*, 2007, **46**, 741–751.
5 A. Davidson, T. C. Leeper, Z. Athanassiou, K. Patora-Komisarska, J. Karn, J. A. Robinson and G. Varani, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 11931–11936.
6 M. F. Bardaro, Jr., Z. Shajani, K. Patora-Komisarska, J. A. Robinson and G. Varani, *Nucleic Acids Res.*, 2009, **37**, 1529–1540.
7 M. S. Lalonde, M. A. Lobritz, A. Ratcliff, M. Chamanian, Z. Athanassiou, M. Tyagi, J. Wong J, J. A. Robinson, J. Karn, G. Varani and E. J. Arts, *PLoS Pathog.*, 2011, **7**, e1002038.
8 K. Moehle, Z. Athanassiou, K. Patora, A. Davidson, G. Varani and J. A. Robinson, *Angew. Chem., Int. Ed.*, 2007, **46**, 9101–9104.
9 B. Davis, M. Afshar, G. Varani, A. I. H. Murchie, J. Karn, G. Lentzen, M. Drysdale, J. Bower, A. J. Potter, I. D. Starkey, T. Swarbrick and F. Aboul-ela, *J. Mol. Biol.*, 2004, **336**, 343–356.
10 R. Nathans, H. Cao, N. Sharova, A. Ali, M. Sharkey, R. Stranska, M. Stevenson and T. M. Rana, *Nat. Biotechnol.*, 2008, **26**, 1187–1192.
11 I. Huq, X. Wang and T. M. Rana, *Nat. Struct. Biol.*, 1997, **4**, 881–882.
12 A. Davidson, K. Patora-Komisarska, J. A. Robinson and G. Varani, *Nucleic Acids Res.*, 2010, **39**, 248–256.
13 X. L. Wang, I. Huq and T. M. Rana, *J. Am. Chem. Soc.*, 1997, **119**, 6444–6445.
14 N. Tamilarasu, I. Huq and T. M. Rana, *J. Am. Chem. Soc.*, 1999, **121**, 1597–1598.
15 M. A. Gelman, S. Richter, H. Cao, N. Umezawa, S. H. Gellman and T. M. Rana, *Org. Lett.*, 2003, **5**, 3563–3565.
16 F. Hamy, E. R. Felder, G. Heizmann, J. Lazdins, F. Aboul-ela, G. Varani, J. Karn and T. Klimkait, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 3548–3553.
17 W. Huang, G. Varani and G. P. Drobny, *J. Am. Chem. Soc.*, 2010, **132**, 17643–17645.
18 Y. Niu, Y. Hu, X. Li, J. Chen and J. Cai, *New J. Chem.*, 2011, **35**, 542–545.
19 S. Rapireddy, G. He, S. Roy, B. A. Armitage and D. H. Ly, *J. Am. Chem. Soc.*, 2007, **129**, 15596–15600.
20 C. Gregoire, J. M. Peloponese, D. Esquieu, S. Opi, G. Campbell, M. Solomiac, E. Lebrun, J. Lebreton and E. P. Loret, *Biopolymers*, 2001, **62**, 324–335.
21 Y. Hu, X. Li, S. M. Sebti, J. Chen and J. Cai, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 1469–1471.
22 F. Aboulela, J. Karn and G. Varani, *J. Mol. Biol.*, 1995, **253**, 313–332.
23 F. Aboul-ela, J. Karn J and G. Varani, *Nucleic Acids Res.*, 1996, **24**, 3974–3981.
24 F. Debaene, J. A. Da Silva, Z. Pianowski, F. J. Duran and N. Winssinger, *Tetrahedron*, 2007, **63**, 6577–6586.
25 F. Debaene, L. Mejias, J. L. Harris and N. Winssinger, *Tetrahedron*, 2004, **60**, 8677–8690.
26 Z. Athanassiou, R. L. A. Dias, K. Moehle, N. Dobson, G. Varani and J. A. Robinson, *J. Am. Chem. Soc.*, 2004, **126**, 6906–6913.