Solid-phase synthesis and evaluation of TAR RNA targeted β-carboline–nucleoside conjugates

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Four types of β-carboline–nucleoside conjugates were synthesized. The binding affinities of these β-carboline–nucleoside conjugates **4–11**, **13** and **15** to TAR RNA were evaluated by affinity capillary electrophoresis. The data of binding affinities to TAR RNA show that conjugates **9** and **13** are stronger binders than the parent compound **MC3**. Computer modeling indicates that the β-carboline–nucleoside conjugate **13** can fit to the UCU three-nucleotide bulge region of TAR RNA.

Introduction

The interaction between the trans-activating region (TAR) RNA and the cognate peptide Tat could activate the transcription of the HIV-1 genome.[1] Therefore, blocking Tat–TAR complex formation seems to be a promising target for inhibiting the multiplication of the HIV-1 virus.[2] Intensive research over the past decade has enriched the structural and biological knowledge of the trans-activation mechanism involving a Tat–TAR interaction.[3] Many small molecules targeted to TAR RNA, such as aminoglycosides and their derivatives,[4] 2,4-diaminoquinozaline or quinoxaline-2,3-diones,[5] aminoalkyl-linked acridine-based compounds,[6] β-carboline[7] and isoquinoline[8] derivatives, have been developed through high-throughput screening or rational drug design. The interaction of small molecule to RNA target is usually governed by the mutual electrostatic properties and the π–π stacking between aromatic rings. Hydrogen bonding between the nucleobases is a naturally existing specific interaction in the recognition of DNA or RNA. Introduction of a nucleobase into a small molecule may offer an additional specific interaction with the residue targeted to an RNA sequence in the binding pocket. Several research groups have reported on the synthesis of aminoglycoside–nucleobase conjugates and revealed that the introduction of a nucleobase could increase the affinity and specificity of the interaction between aminoglycoside and RNA target.[9] Yang's group described the synthesis of β-carboline derivatives and found that some β-carboline derivatives can interact with TAR RNA and the planar β-carboline ring system could insert into the active pocket created by the UCU bulge.[10] In this report, to increase the specific binding affinity, four types of nucleoside, such as deoxynucleoside, isonucleoside, acyclic nucleoside and peptide nucleoside, were introduced into the β-carboline molecule by solid-phase or solution synthesis and their binding activities to TAR RNA were evaluated by capillary electrophoresis and the results were discussed by molecular modeling.

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Results and discussion

Synthesis of β-carboline–nucleoside conjugates

Four types of nucleoside derivatives: deoxynucleoside, isonucleoside, acyclic nucleoside and peptide nucleoside (PNA) (Fig. 1), were designed as building blocks to construct β-carboline–nucleoside conjugates. Their different chemical structures may give various contributions to the binding affinity to RNA. Compounds **3a**, **3b** and **3c** were synthesized by the procedures developed by our and other laboratories.[11] Compounds **1a**, **1b** and **1c** were reacted with tosyl chloride in pyridine followed by NaN_3 to yield compounds **2a**, **2b** and **2c** respectively. The azido group of nucleoside **2a**, **2b** or **2c** was reduced by hydrogenation and protected using FmocCl to obtain compound **3a**, **3b** or **3c** in good yield (Scheme 1).

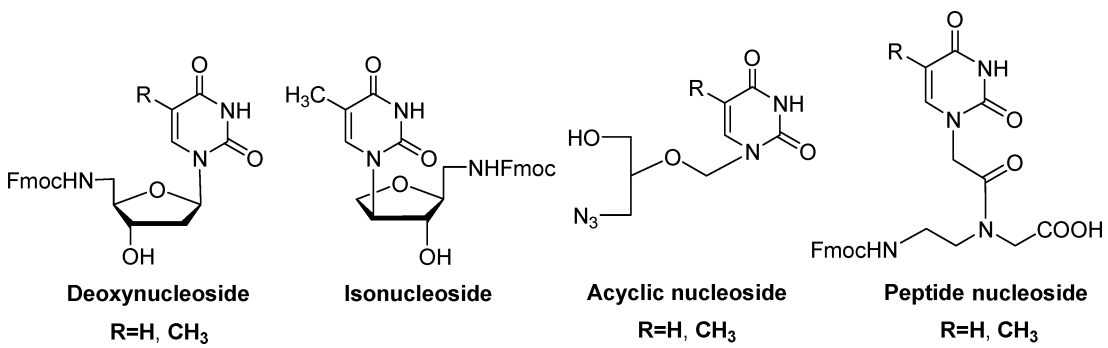
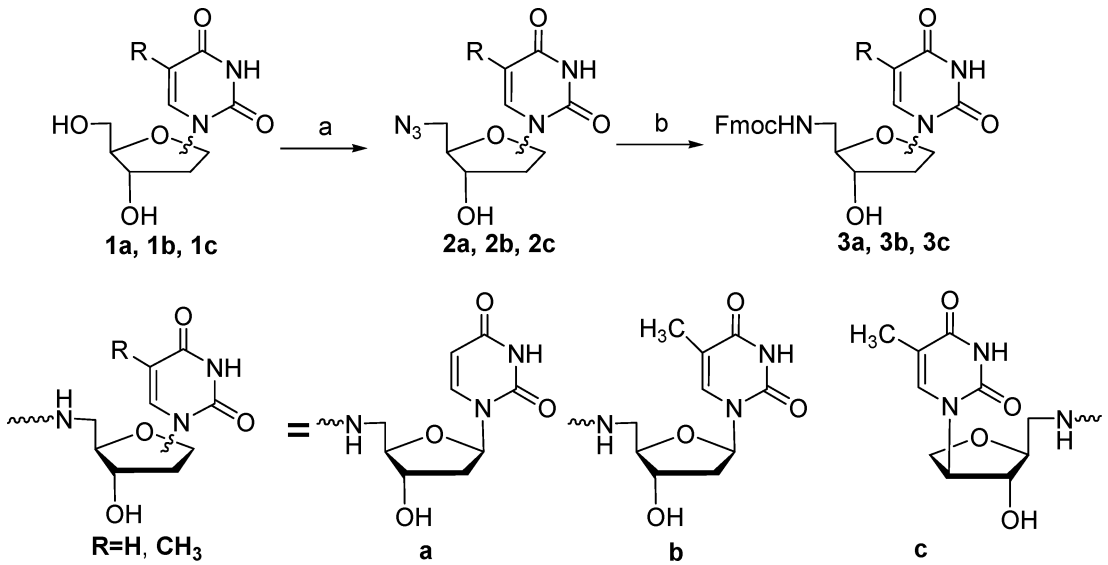


Fig. 1 Structures of nucleoside derivatives.



Scheme 1 Synthesis of deoxynucleoside and isonucleoside building blocks. *Reagents and conditions:* (a) (1) TsCl, Py (2) NaN_3, DMF, 80 °C; (b) (1) 10% Pd/C, H_2, EtOH, 0.4 MPa (2) FmocCl, THF–10% Na_2CO_3.

Solid-phase synthesis has proved to be a powerful tool in drug discovery. But compared to the solid-phase synthesis of other organic small molecules, the development of solid-phase synthesis of nucleoside analogues is very tardy.[12] Nucleoside and its derivatives are multi-functional group containing and acid-sensitive substances. Thus, a universal and highly selective solid-phase synthetic method for nucleoside derivatives should be developed. As part of our research work, we found that parallel solid phase synthesis can be used for the preparation of β-carboline–nucleoside conjugates.

Polystyrene butyl(diethyl)silane (PS-DES) resin has been applied to immobilize nucleosides on a solid support[12d,h] Recently, Tan's group developed an acid-stable *tert*-butyldiarylsilyl (TBDAS) linker for solid phase synthesis[13] and the silyl-ether linker seemed to have the ability to tolerate the common conditions for the modification of nucleosides. In this report, TBDAS resin was used for the synthesis of β-carboline–nucleoside conjugates. One of the major difficulties met in nucleoside synthesis on a solid support is their poor solubility in commonly used solvents. After screening, DCM–2,6-lutidine (1 : 1, v/v) was used in this solid-phase synthesis. Some improvements were also made to increase the loading of nucleoside on the TBDAS resin. The TBDAS resin was treated with TMSiCl in DCM for 30 min before use and addition of DMAP for the loading of nucleosides on the resin proved to be effective. The loading of nucleoside on TBDAS resin can reach 0.200 mmol g^{-1}.

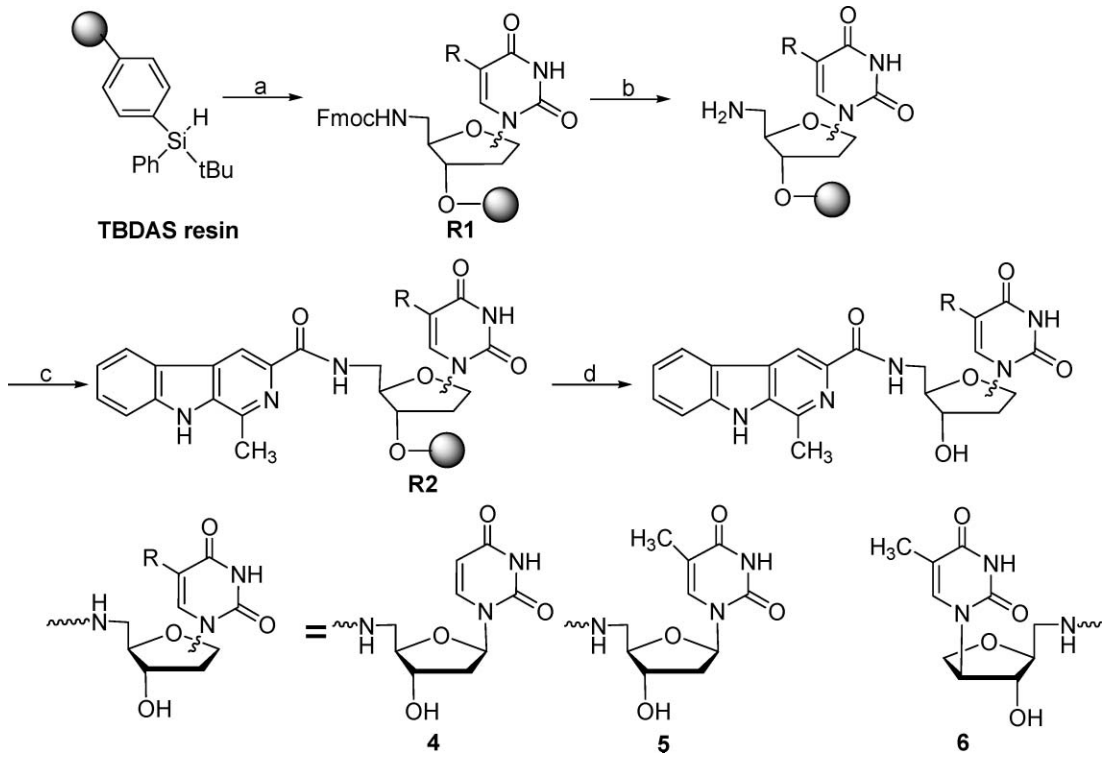
Deoxynucleosides **3a** and **3b** and isonucleoside **3c** were loaded on TBDAS resin to afford **R1**, after deprotection of Fmoc, the resin was condensed with 1-methyl-β-carboline-3-carboxylic acid to provide resin **R2**. The corresponding β-carboline conjugates **4–6** were obtained from **R2** by effective cleavage using 1 M TBAF–THF and the products were purified by column chromatography in about 90% yield (Scheme 2).

For the synthesis of β-carboline–peptide nucleoside conjugates **7–11**, PAL resin can conveniently be used. Peptide nucleoside can be obtained on PAL resin by standard stepwise solid-phase peptide synthesis. After deprotection of **R3**, a solution of 1-methyl-β-carboline-3-carboxylic acid, DCC, and HOBT in DMF was added to the PAL resin. After cleavage step by 95% TFA–Et_3SiH, β-carboline–peptide nucleoside conjugates **7–11** were obtained in yields of 63–96%.

β-Carboline–acyclic nucleoside conjugates **13**, **15** were obtained by solution-phase synthesis. The azido group on acyclic nucleosides **12** or **14** was reduced by hydrogenation, and condensed with 1-methyl-β-carboline-3-carboxylic acid to yield 79% of compound **13** or 72% of compound **15** respectively (Scheme 3).

Evaluation of binding affinity

Many highly affinitive and specific analytical methods have been developed to study RNA–small molecule interactions. These methods include structure-based X-ray diffraction[14] and NMR,[15] electrospray ionization mass spectrometry,[16] traditional biochemical techniques (such as gel mobility, filter binding interference assay, mutagenesis analysis *etc.*),[17] scintillation proximity assay (SPA),[18] surface plasmon resonance (SPR),[19] intramolecular fluorescence resonance energy transfer (FRET)[20] and capillary electrophoresis.[21] The interaction of aminoglycoside analogues and RNA was investigated by the SPR method in our laboratory and some interesting results were obtained[9e,f] Due to the poor solubility of compounds (**4–11**, **13** and **15**) in water, SPR method could not give good results in this case. Theoretically, 10% DMSO may be used for SPR through solvent correction. But in our case, solvent correction cannot provide satisfactory results even using 1% DMSO during SPR screening. Yang, Zhang and coworkers reported the interaction of TAR RNA and water-soluble β-carboline and isoquinoline derivatives by affinity capillary electrophoresis.[21] In this report, we found that the synthetic β-carboline–nucleoside conjugates can be dissolved into 10% (v/v) DMSO and this solution was satisfactory for the evaluation of the binding affinity to TAR RNA by a modified capillary electrophoresis method. Therefore, a concentration of 10% (v/v) dimethyl sulfoxide (DMSO) in PBS buffer was used as the stock sample solution. DMSO can increase the solubility of



Scheme 2 Solid-phase synthesis of β-carboline–deoxynucleoside and β-carboline–isonucleoside conjugates on TBDAS resin. *Reagents and conditions*: (a) (1) TMSiCl, DCM, 0.5 h; (2) 1,3-dichloro-5,5-dimethylhydantoin, DCM, Ar, 4 h; (3) loading of nucleoside derivatives on TBDAS resin: for compound **3a**, **3b**, imidazole, DMAP, DCM, 2,6-lutidine, Ar; for compound **3c**, imidazole, DMAP, DCM, Ar; (b) 20% piperidine–DMF; (c) 1-methyl-β-carboline-3-carboxlic acid, DCC, HOBT, DMF; (d) 1.0 M TBAF–THF, AcOH.

β-carboline–nucleoside conjugates in PBS buffer and also be used as electroosmotic flow (EOF) marker. To avoid the perturbation of DMSO in this determination, UV 254 nm was chosen as detector wavelength. The RNA sample was dissolved in pH = 8.0 PBS buffer and different concentrations of RNA in pH = 8.0 PBS buffer were used as running buffer. 0.01 μmol of sample and 10 OD TAR RNA were used for this determination. K_b is the binding constant calculated from the migration time of the samples on affinity capillary electrophoresis and used for the evaluation of the binding affinity to TAR RNA. It was reported that β-carboline compound **MC3** (Fig. 2) showed inhibitory activity to the interaction between Tat and TAR RNA in the transient cotransfection assay.[10] In this report, **MC3** was used as a positive control.

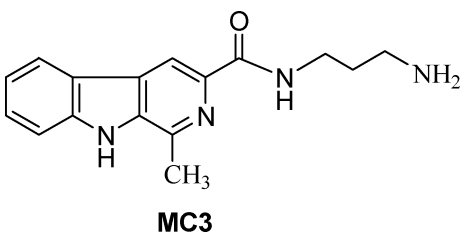


Fig. 2 Structure of **MC3**.

Screening results obtained from affinity capillary electrophoresis showed that β-carboline–nucleoside conjugates **4–9**, **11**, **13** and **15** interacted with TAR RNA more tightly than the positive control **MC3** (Table 1, Fig. 3). Compounds **4** and **5** are β-carboline–nucleoside conjugates, which differ only in nucleobase, and exhibit very similar binding affinities. However, β-carboline-isonucleoside conjugate **6** is a structural analogue of compound **5** in which the thymine base is shifted from C-1 to C-3 and the 5-amino group is moved to C-1 of the deoxyribose ring. The conformation of conjugate **6** shows a big change compared to its partner **5** and the lower binding affinity to TAR RNA of conjugate **6** is expected. Interestingly, the structural difference between conjugate **13** and **15** is also on nucleobase only and they exhibit significantly different binding affinities. In the series

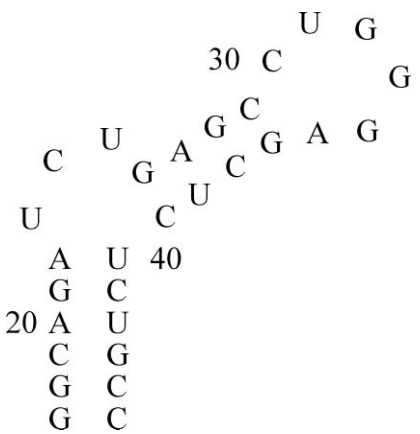
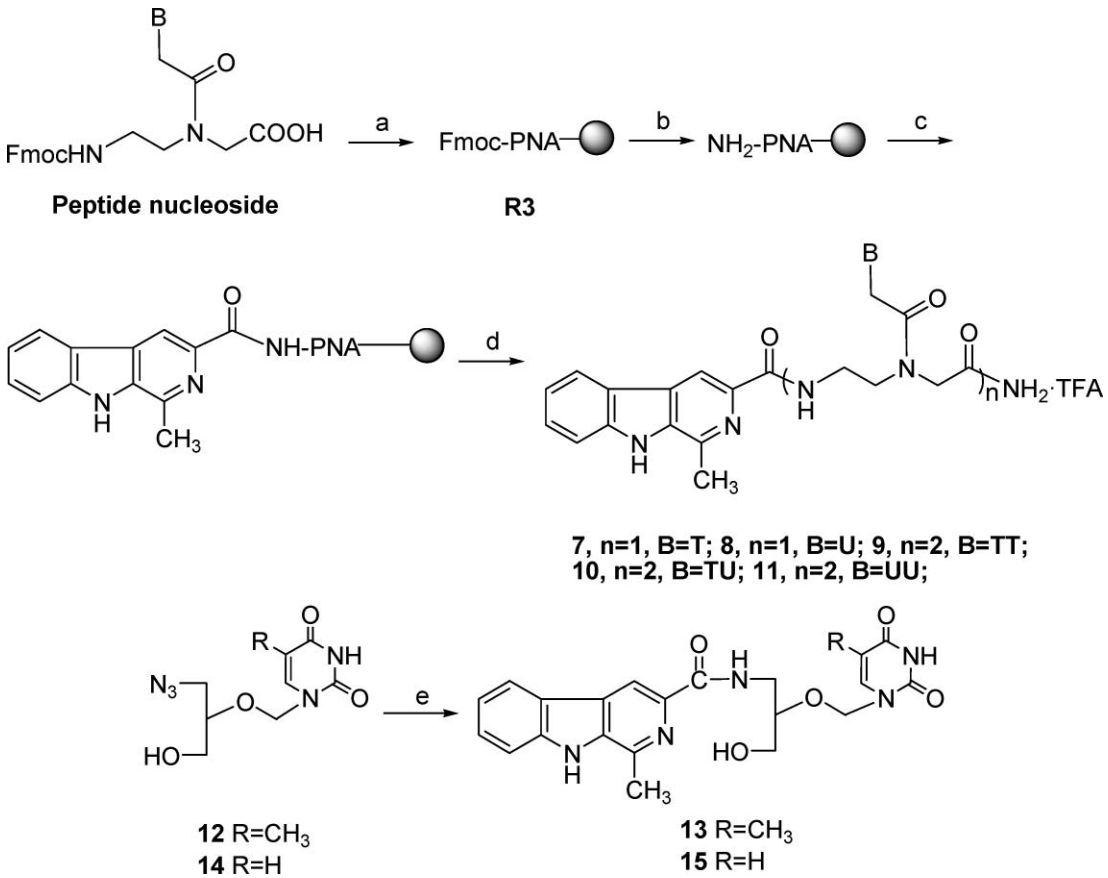


Fig. 3 Sequence and secondary structure of the HIV-1 TAR RNA.



Scheme 3 Solid-phase synthesis of β-carboline–peptide nucleoside conjugates on PAL resin and solution-phase synthesis of β-carboline–acyclic nucleoside conjugates. *Reagents and conditions*: (a) stepwise solid-phase peptide nucleoside synthesis on PAL resin: (1) PAL resin, 20% piperidine–DMF; (2) Fmoc-peptide nucleoside, DCC, HOBT, DMF; (b) 20% piperidine–DMF; (c) 1-methyl-β-carboline-3-carboxlic acid, DCC, HOBT, DMF; (d) 95% TFA–Et_3SiH; (e) (1) 10% Pd/C, H_2, EtOH, 0.4 atm; (2) 1-methyl-β-carboline-3-carboxylic acid, DCC, HOBT, DMF. Note: For compound **9–11**, the second peptide nucleoside was attached to the resin by repeating the deprotection step and condensation step: (1) 20% piperidine–DMF; (2) Fmoc peptide nucleoside, DCC, HOBT, DMF.

of β-carboline-peptide nucleoside conjugates, the data are more confused. Conjugates **7** and **8** show moderate binding affinities to TAR RNA, but there is no relationship between the structures of **9–11** and their binding affinities (Table 1).

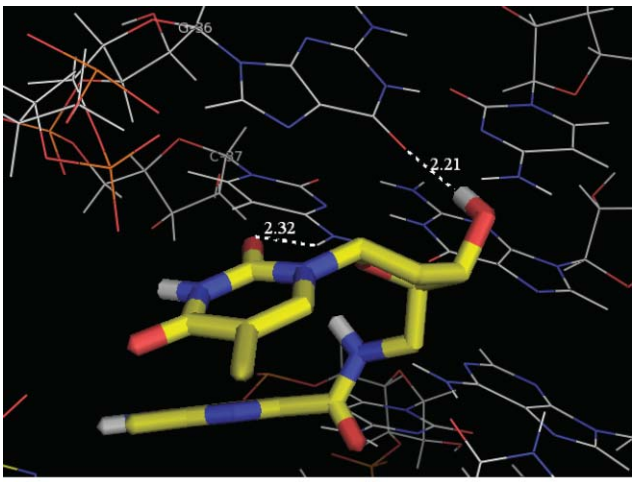
For elucidating the binding mode of the studied conjugates to TAR RNA, a molecular docking study was performed using the program AutoDock 3.0. The binding ability of the ligand to TAR RNA was evaluated by the docking energy and the conformation with the lowest docking energy was used for the analysis of the final docked conformation.[22]

Fig. 4 shows that the synthetic compounds **5** and **6** can fit into the major groove of TAR RNA. For compound **5**, two hydrogen bonds are formed, one is formed by O^2 of thymine and 4-NH_2 of cytosine of the residue C37 in TAR RNA (2.21 Å) and the second is formed by OH of deoxyribose and O^6 of guanine of residue G36 in TAR RNA (2.32 Å), and compound **4** indicates the identical binding model with TAR RNA. These hydrogen bonds restrict the molecular orientation and movement. However, β-carboline–isonucleoside conjugate **6** shows a contrary binding mode to compounds **4** and **5**, in which only one hydrogen bond between N–H (β-carboline rings) and N^7 of guanine of residue G34 of TAR RNA (2.13 Å) was observed.

Compound **13** shows very high affinity to TAR RNA in comparison with the nucleobase-substituted analogue **15**. According to the molecular modeling, compound **13** can fit very well with the UCU three-nucleotide bulge of TAR RNA, β-carboline can intercalate into the space of UCU bulge and three strong hydrogen bonds are observed by computer simulation. The imino group of

Table 1 The binding constants K_b of compounds with TAR RNA. Note: K_b is the binding constant and N. A., no data available. **MC3** was used as positive control

No.	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**13**	**15**	**MC3**
$K_b \times 10^3/M^{-1}$	29.3	23.8	19.6	18.7	21.7	53.9	N. A.	28.1	50.6	11.6	8.9



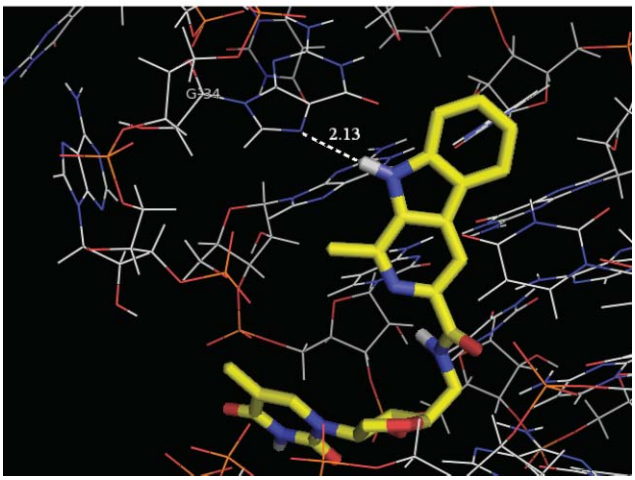
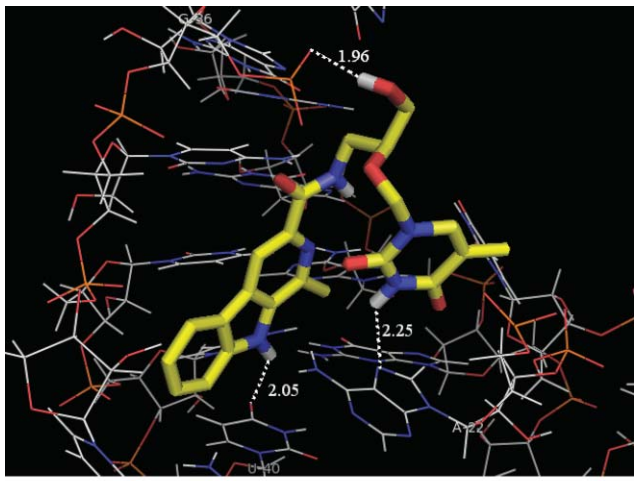


Fig. 4 Interaction of compounds **5** (top) and **6** (bottom) with TAR RNA.



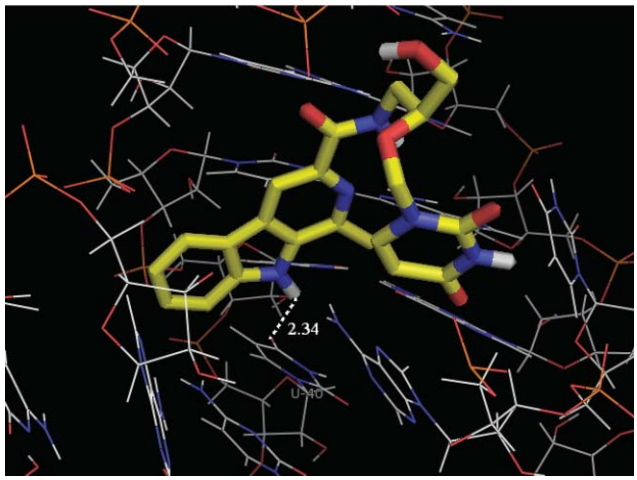


Fig. 5 Interaction of compounds **13** (top) and **15** (bottom) with TAR RNA.

the β-carboline ring and the N^3 thymine of compound **13** form two hydrogen bonds with the O^4 of uracil of the residue U40 (2.05 Å) and the N^7 of adenine of the residue A22 (2.25 Å) in TAR RNA, respectively, whereas the hydroxyl group in compound **13** forms a hydrogen bond (1.96 Å) with the phosphate group of the residue G36 in TAR RNA. Interestingly, compound **15** cannot form the same conformation as compound **13** to fit this model. Only a weak hydrogen bond (2.34 Å) was observed in the case of compound **15**, which indicates its weak binding affinity to TAR RNA (Fig. 5).

For β-carboline–peptide nucleoside conjugates **7** and **8**, their binding modes are similar to compound **6**. Compounds **9–11** are dipeptide nucleoside conjugates, compound **9** shows a strong binding affinity to TAR RNA, but compound **10** exhibits no binding activity. Structurally, in the cases of molecules **9–11**, the peptide linkage is more flexible and it makes it more difficult to restrict the conformation and movement of the molecule in the RNA pocket. Unfortunately, the binding model established here could not be used to elucidate the interaction of such flexible molecules to TAR RNA.

It was reported that the residues U23, C24 and U25 were stacked on A22 in TAR RNA in the absence of Tat and while Tat bound to TAR RNA in the region of the UCU bulge in which U23 was moved in direct proximity to G26 and C24 and U25 became unstacked.[23] The modeling result of conjugate **13** with TAR RNA is in good agreement with the proposed model and the experimental binding affinity, and the designed conjugates **4–11** cannot fit well to this active UCU three-nucleotide bulge region. It is obvious that in comparison with conjugate **13**, the deoxyribose moiety in conjugates **4–6** is a more restrictive linker between β-carboline and nucleobase and the peptide linkage in conjugates **7–11** is more flexible. It seems that the flexibility of the structure of the proper β-carboline–nucleoside conjugate is very important for the recognition of the UCU three-nucleotide bulge region of TAR RNA. β-Carboline–acyclic nucleoside conjugates **13** and **15** would be the interesting models for further investigation of more active inhibitors for the formation of the tat–TAR complex.

In conclusion, four types of β-carboline–nucleoside conjugates were synthesized. The binding affinities of these β-carboline–nucleoside conjugates **4–11**, **13** and **15** to TAR RNA were evaluated by affinity capillary electrophoresis. The binding affinities to TAR RNA show that conjugates **9** and **13** are stronger binders than the parent compound **MC3**. Computer modeling indicates that the β-carboline–acyclic nucleoside conjugate **13** can fit to the UCU three-nucleotide bulge region of TAR RNA. The docking model of compound **13** to TAR RNA is in good agreement with the experimental data.

Experimental

General

Commercial reagents were purchased from Acros and Aldrich Chemical Co. and were used without further purification. PL-PBS resin (1.97 mmol g^{-1}, 150–300 μm) was purchased from Polymer Laboratories USA and PAL resin (1% DVB crossed-linked, 100–200 mesh, 0.4–0.8 mmol g^{-1}) was purchased from Advanced ChemTech Inc., USA. All solvents were dispensed from a solvent purification system prior to use. Yields referred to chromatographic and spectroscopically pure compounds. Silica gel (200–300 mesh) manufactured by Qingdao Haiyang Chemical Company (China) was used for the column chromatography. Solution phase reactions were performed in flame-dried glassware under positive argon pressure with magnetic stirring. Proton magnetic resonance spectra were recorded on a JEOL JNM-AL300 (300 MHz for ^{1}H, 75.5 MHz for ^{13}C) spectrometer or Varian VXR-500 (500 MHz for ^{1}H). All NMR spectra were recorded at ambient temperature. Chemical shifts (δ values) were reported as parts per million (ppm) and referenced to TMS (^{1}H NMR, 0) or CD_3OD (^{13}C NMR, 49.0, center line) or DMSO-d_6 (^{13}C NMR, 39.5, center line). Coupling constants (J values) are expressed in Hz. High-resolution mass spectra were obtained at Bruker DALTONICS APEX IV 70e mass spectrometer by electrospray ionization (ESI). The data are reported in m/z. Melting points were taken on an Electrothermal® Melting Point Apparatus and are uncorrected. Optical rotation was recorded on a Rudolph Research Analytical Auto Pol III automatic polarimeter (sodium D-line, 589 nm). UV spectra were recorded on a UNICO UV-4802 spectrometer (UNICO (Shanghai) Instruments Co., Ltd.). Solid phase reactions were run in a home-made solid-phase synthesizer under positive argon pressure. Resin loading levels are expressed in mmol g^{-1}.

Compound **1c**,[11a] compound **2a**,[24] compound **2b**,[25] compound **3b**,[26] compound **12**,[27] compound **14**,[28] Fmoc-peptide nucleoside,[29] 1-methyl-β-carboline-3-carboxylic acid, 1-methyl-β-carboline-3-carboxylic acid (3-amino-propyl)-amide (**MC3**)[30] and TBDAS resin[13] were prepared according to the literature in agreement with the analytical results. Fmoc quantification was determined by the method of Meienhofer *et al*.[31]

General procedure for the solid-phase synthesis of β-carboline–deoxynucleoside and β-carboline–isonucleoside conjugates on TBDAS resin

Activation of TBDAS resin: To a suspension of TBDAS resin (400 mg, 0.76 mmol) in dry CH_2Cl_2 (10 mL), TMSiCl (1.0 mL) was added and the mixture was agitated for 30 min. The solution was drained from the column under positive argon pressure, and the resin was washed with anhydrous CH_2Cl_2. 1,3-Dichloro-5,5-dimethylhydantoin (10 mL, 1.0 M in CH_2Cl_2) was added by syringe and the resulting slurry was agitated for 60 min. The solution was drained from the column under positive argon pressure and the resin was washed with anhydrous CH_2Cl_2.

Loading of deoxynucleoside and isonucleoside on TBDAS resin: Deoxynucleoside (3 eq.), imidazole (3 eq.) and DMAP (1 eq.) were dissolved in anhydrous CH_2Cl_2 (2 mL) and 2,6-lutidine (2 mL) (for isonucleoside, only 5 mL anhydrous CH_2Cl_2 was used). The solution was added by syringe and the reaction was agitated overnight. The solution was drained from the column under positive argon pressure and dried under high vacuum to give **R1** resin for the next step.

Deprotection of Fmoc on **R1** resin: **R1** resin (200 mg) was treated with 20% piperidine in DMF (4 mL) for 30 min and washed with CH_2Cl_2, dried under vacuum.

Coupling of 1-methyl-β-carboline-3-carboxylic acid: To a suspension of deprotected **R1** resin in DMF (3 mL), 1-methyl-β-carboline-3-carboxylic acid (2 eq.), DCC (2 eq.) and HOBT (2 eq.) were added and agitated overnight. The reaction was stopped when monitoring showed a negative ninhydrin test. The resin was washed with MeOH, CH_2Cl_2 and dried under vacuum to afford **R2** resin.

Cleavage and purification of products from **R2** resin: **R2** resin (starting from 200 mg **R1** resin) was suspended in 1 M TBAF–THF (4 mL) which was pre-neutralized with AcOH to pH = 7.0 and agitated overnight. The mixture was filtered and the resin was washed thoroughly with MeOH. The filtrate was concentrated under vacuum and purified by column chromatography eluting with petroleum ether : ethyl acetate 3 : 1 or CH_2Cl_2 : MeOH 30 : 1 to afford the target compounds.

General procedure for the solid-phase synthesis of β-carboline–peptide nucleoside conjugates on PAL resin

Solid-phase synthesis of β-carboline–peptide nucleoside conjugates was followed the standard stepwise solid-phase peptide synthesis on PAL resin: (1) Fmoc of PAL resin was removed by 20% piperidine–DMF for 30 min. (2) Coupling of peptide nucleoside on PAL resin was carried out in the presence of peptide nucleoside, DCC and HOBT in DMF. (3) Deprotection of Fmoc group of the peptide nucleoside containing resin by 20% piperidine–DMF for 30 min (for the preparation of compounds **9–11**, repeat steps 2 and 3 again). (4) Coupling of the peptide nucleoside containing resin with 1-methyl-β-carboline-3-carboxylic acid was carried out in the presence of DCC, HOBT in DMF. (5) Cleavage of the products from PAL resin was completed by 95% TFA–Et_3SiH for 30 min. The crude product was dissolved in MeOH and the product was purified by reverse-phase HPLC using a C_{18} semi-preparative column (Venusil XBP-C18, 10 μm, 100 Å, 21.5 × 250 mm, Agela Technologies Inc. USA) as the stationary phase and a gradient of 0.1% TFA–H_2O (A) and 0.1% TFA–CH_3CN (B) as the mobile phase, monitoring at 254 nm. The gradient conditions were 0 min, 100% A followed by linear gradient 0–80% B over 30 min.

(3*S*,4*R*,5*S*)-5-Azidomethyl-3-thymine-1-yl-4-hydroxyl-tetrahydrofuran 2c

To an ice-cold stirred solution of isonucleoside **1c**[11a] (360 mg, 1.49 mmol) in anhydrous pyridine (15 mL) was added *p*-toluenesulfonyl chloride (312 mg, 15.8 mmol), and the reaction mixture was left overnight. Solvent was removed under reduced pressure and the resultant syrup was extracted with CH_2Cl_2 and saturated sodium bicarbonate. The organic solution was dried with sodium sulfate. Removing the solvents under reduced vacuum afforded a yellow solid, 5′-*O*-tosyl-isonucleoside. The crude product was dissolved in DMF (10 mL) and sodium azide (480 mg, 7.5 mmol) was added and the mixture was stirred at 80 °C for 8 h. After cooling, the solution was filtered and the filtrate was

evaporated under vacuum, the resultant residue was purified by silica gel chromatography eluting with CH_2Cl_2 : MeOH 50 : 1 to afford colorless liquid **2c** (242 mg) in 62% yield for two steps. $[\alpha]_D^{25}$ –88.9 (*c* 0.027 in MeOH); UV λ_{max} (MeOH)/nm 213 (ε/dm^3 mol^{-1} cm^{-1} 11 400) and 270 (11 100);^{1}H NMR (300 MHz; CD_3OD) δ 7.48 (s, 1H, H-6′), 4.20 (dd, J = 4.5, 6.3, 1H, H-2a), 4.13–4.16 (m, 1H, H-3), 4.06 (dd, J = 4.5, 10.5, 1H, H-2b), 3.78–3.83 (m, 1H, H-5), 3.63 (dd, J = 3.0, 12.0, 1H, $CH_{2a}N_3$), 3.46 (dd, J = 5.1, 13.5, 1H, $CH_{2b}N_3$), 1.89 (s, 3H, 5′-CH_3); ^{13}C NMR (75.5 MHz; CD_3OD) δ 166.3 (C-4′), 153.0 (C-2′), 139.6 (C-6′), 111.9 (C-5′), 85.2 (C-5), 78.0 (C-4), 70.2 (C-2), 65.5 (C-3), 52.6 (CH_2N_3), 12.5 (5′-CH_3). HR-MS (ESI-TOF, *m*/*z*): Calcd. for $C_{10}H_{14}N_5O_4$ (M + H)$^+$ 268.1040. Found 268.1039.

5′-*N*-(9*H*-Fluorenylmethoxycarbonyl)-2′,5′-dideoxyuridine 3a

A mixture of **2a**[24] (1.22 g, 4.82 mmol), 10% Pd/C (0.30 g) and EtOH (10 mL) was stirred under a H_2 atmosphere (0.4 atm) for 4 h, the solvent was removed under vacuum and a mixture of FmocCl (1.37 g, 5.30 mmol) in THF (10 mL) and 10% Na_2CO_3 (5 mL) was added. The mixture was allowed to stir overnight. After removal of the solvent under vacuum, the residue was purified by chromatography eluting with CH_2Cl_2 : MeOH 30 : 1 to obtain white solid of 1.47 g in 68% yield for two steps. Mp 168–170 °C; $[\alpha]_D^{25}$ 9.8 (*c* 0.123 in MeOH); UV λ_{max} (MeOH)/nm 210 (ε/dm^3 mol^{-1} cm^{-1} 48 200) and 264 (31 100);^{1}H NMR (500 MHz; DMSO-d_6) δ 11.31 (br, 1H, N-3 H), 7.30–7.89 (m, 9H, Ar-H, H-6), 6.11 (t, J = 6.5, 1H, H-1′), 5.57 (dd, J = 8.0, 2.0, 1H, CONH), 5.30 (d, J = 4.5, 1H, H-5), 4.14–4.35 (m, 4H, $CHCH_2OCO$, H-3′), 3.76–3.80 (m, 1H, H-4′), 3.15–3.31 (m, 2H, H-5′), 1.99–2.09 (m, 2H, H-2′); ^{13}C NMR (75.5 MHz; DMSO-d_6) δ 163.7 (C-4), 156.4 (OCONH), 150.4 (C-2), 143.9, 140.7 (C-6), 127.6, 127.1, 125.2, 120.1, 101.8 (C-5), 85.2 (C-4′), 84.3 (C-1′), 71.1 (C-3′), 65.4 (C-5′), 46.7 ($CHCH_2OCO$), 42.8 (C-1′). HR-MS (ESI-TOF, *m*/*z*): Calcd. for $C_{24}H_{24}N_3O_6$ (M + H)$^+$ 450.1660. Found 450.1674.

(3*S*,4*R*,5*R*)-*N*-5-(9*H*-Fluorenylmethoxycarbonyl)methyl-3-thymine-1-yl-4-hydroxyl-tetrahydrofuran 3c

This compound was synthesized through the same procedure as described for compound **3a** in 52% yield from compound **2c** (141 mg), colorless liquid. $[\alpha]_D^{25}$ 3.7 (*c* 0.162 in MeOH); UV λ_{max} (MeOH)/nm 210 (ε/dm^3 mol^{-1} cm^{-1} 81 700) and 265 (54 400); ^{1}H NMR (300 MHz; DMSO-d_6) δ 11.33 (s, 1H, N-3′ H), 7.32–7.91 (m, 9H, Ar-H, H-6′), 5.76 (d, J = 1.5, 2H, $CHCH_2OCO$), 5.66 (d, J = 2.7, 1H, N*H*COO), 4.76 (br, 1H, H-2a), 4.20–4.27 (m, 3H, C*H*CH_2OCO, H-3, OH), 3.83–4.04 (m, 2H, H-2b, H-4), 3.63–3.64 (m, 1H, 5-CH_{2a}NH), 1.78 (s, 3H, 5′-CH_3); ^{13}C NMR (75.5 MHz; DMSO-d_6) δ 163.8 (C-4′), 156.4 (NHCOO), 151.1 (C-2′), 143.9, 143.9, 140.7 (C-6′), 137.8, 127.7, 127.1, 125.3, 120.2, 109.4 (C-5′), 83.0 (C-5), 76.3 (C-4), 68.3 (C-2), 65.6 (C-3), 62.6 (CH_2OCONH), 55.0 (CH_2N_3), 46.7 ($CHCH_2OCO$), 12.2 (5′-CH_3). HR-MS (ESI-TOF, *m*/*z*): Calcd. for $C_{25}H_{25}N_3O_6Na$ 486.1636 (M + Na)$^+$. Found 486.1656.

5′-*N*-(1-Methyl-β-carboline-3-carbonyl)-2′,5′-dideoxyuridine 4

This compound was synthesized according to the general procedure for the solid-phase synthesis of β-carboline–deoxynucleoside conjugates on TBDAS resin in 89% yield from 201 mg resin **R1** (deoxynucleoside **3a** on TBDAS resin with the loading 0.157 mmol g^{-1}). Mp 180–182 °C; $[\alpha]_D^{25}$ 10.5 (*c* 0.057 in MeOH); UV λ_{max} (MeOH)/nm 213 (ε/dm^3 mol^{-1} cm^{-1} 14 400), 234 (9800) and 270 (12 100);^{1}H NMR (300 MHz; DMSO-d_6) δ 11.97 (s, 1H, N-3 H), 11.35 (s, 1H, Ar-NH), 8.73 (t, J = 6.0, 1H, Ar-H), 8.70 (s, 1H, Ar-H), 8.36 (d, J = 7.8, 1H, H-6), 7.77 (d, J = 8.4, 1H, Ar-H), 7.56–7.66 (m, 2H, Ar-H), 7.29 (t, J = 7.5, 1H, CON*H*CH_2), 6.16 (t, J = 6.6, 1H, H-1′), 5.61 (d, J = 7.8, 1H, H-5), 5.38 (d, J = 4.2, 1H, OH), 4.25–4.30 (m, 1H, H-3′), 3.97–3.98 (m, 1H, H-4′), 3.56–3.64 (m, 2H, 5′-CH_2), 2.84 (s, 3H, Ar-CH_3), 2.11–2.49 (m, 2H, 2′-CH_2); ^{13}C NMR (75.5 MHz; DMSO-d_6) δ 164.9 (C-4), 163.1 (CONH), 150.4 (C-2), 140.9, 140.9, 140.8 (C-6), 135.9, 128.4, 127.5, 122.2, 121.4, 120.0, 112.3, 102.0 (C-5), 85.2 (C-4′), 84.3 (C-1′), 71.2 (C-3′), 41.2 (C-5′), 38.2 (C-2′), 20.3 (CH_3). HR-MS (ESI-TOF, *m*/*z*): Calcd. for $C_{22}H_{22}N_5O_5$ 436.1616 (M + H)$^+$. Found 436.1614.

5′-*N*-(1-Methyl-β-carboline-3-carbonyl)-5′-deoxythymidine 5

This compound was synthesized according to the general procedure for the solid-phase synthesis of β-carboline–deoxynucleoside conjugates on TBDAS resin in 93% yield from 218 mg resin **R1** (deoxynucleoside **3b** on TBDAS resin with the loading 0.140 mmol g^{-1}). Mp 186–190 °C; $[\alpha]_D^{25}$ –3.5 (*c* 0.114 in MeOH); UV λ_{max} (MeOH)/nm 211 (ε/dm^3 mol^{-1} cm^{-1} 9700), 232 (7600) and 270 (12 900);^{1}H NMR (300 MHz; DMSO-d_6) δ 11.97 (s, 1H, N-3 H), 11.32 (s, 1H, Ar-NH), 8.69–8.78 (m, 2H, Ar-H), 8.35 (d, J = 7.8, 1H, H-6), 7.55–7.66 (m, 3H, Ar-H), 7.29 (t, J = 8.4, 1H, CON*H*CH_2), 6.18 (t, J = 8.4, 1H, H-1′), 5.37 (d, J = 4.5, 1H, OH), 4.30 (br, 1H, H-3′), 3.97 (br, 1H, H-4′), 3.35–3.68 (m, 2H, 5′-CH_2), 2.82 (s, 3H, Ar-CH_3), 2.10–2.17 (m, 2H, 2′-CH_2), 1.74 (s, 3H, 5-CH_3); ^{13}C NMR (75.5 MHz; DMSO-d_6) δ 165.3 (C-4), 164.0, 150.7 (C-2), 141.2, 141.1 (C-6), 139.1, 136.2, 128.6, 127.7, 122.4, 121.7, 120.2, 112.5, 110.0 (C-5), 85.3 (C-4′), 84.2 (C-1′), 71.5 (C-3′), 41.4 (C-5′), 38.7 (C-2′), 20.7 (Ar-CH_3), 12.3 (5-CH_3). HR-MS (ESI-TOF, *m*/*z*): Calcd. for $C_{23}H_{24}N_5O_5$ 450.1772 (M + H)$^+$. Found 450.1765.

(3*S*,4*R*,5*R*)-*N*-6-(1-Methyl-β-carboline-3-carbonyl)-3-thymine-1-yl-4-hydroxyl-tetrahydrofuran 6

This compound was synthesized according to the general procedure for the solid-phase synthesis of β-carboline–isonucleoside conjugates on TBDAS resin in 90% yield from 200 mg resin **R1** (isonucleoside **3c** on TBDAS resin with the loading 0.198 mmol g^{-1}). Mp 160–162 °C; $[\alpha]_D^{25}$ –32.3 (*c* 0.031 in MeOH); UV λ_{max} (MeOH)/nm 215 (ε/dm^3 mol^{-1} cm^{-1} 103 200), 233 (135 800) and 270 (168 400);^{1}H NMR (300 MHz; DMSO-d_6) δ 11.96 (s, 1H, N-3′ H), 11.30 (s, 1H, Ar-NH), 8.62–8.69 (m, 2H, Ar-H), 8.36 (d, J = 7.8, 1H, H-6′), 7.56–7.66 (m, 2H, Ar-H), 7.47 (s, 1H, Ar-H), 7.29 (t, J = 7.8, 1H, CON*H*CH_2), 5.75 (d, J = 5.4, 1H, OH), 4.81 (dd, J = 4.8, 12.0, 1H, H-2a), 4.03–4.19 (m, 2H, H-3, H-4), 3.93 (dd, J = 5.1, 10.2, 1H, H-2a), 3.59–3.81 (m, 3H, H-5, CH_2NH), 2.84 (s, 3H, Ar-CH_3), 1.74 (s, 3H, 5′-CH_3); ^{13}C NMR (75.5 MHz; DMSO-d_6) δ 164.9 (C-4′), 163.8, 151.1 (C-2′), 141.0, 140.8 (C-6′), 138.7, 137.7, 136.0, 128.4, 127.5, 122.2, 121.4, 120.0, 112.2, 109.4 (C-5′), 83.1 (C-5), 76.9 (C-4), 68.5 (C-2), 62.8 (C-3), 40.5 (CH_2NH), 20.5 (Ar-CH_3), 12.0 (5′-CH_3). HR-MS (ESI-TOF,

m/z): Calcd. for $C_{23}H_{25}N_5O_5$ 450.1772 $[M + H]^+$. Found 450.1763.

N-(1-Methyl-β-carboline-3-carbonyl)-*N*-(thymine-N^1-methylenecarbonoyl)-glycinamide 7

This compound was synthesized according to the general procedure for the solid-phase synthesis of β-carboline–peptide nucleoside conjugates on PAL resin in 63.4% yield from 170 mg resin **R3** (peptide nucleoside on PAL resin with the loading 0.344 mmol g^{-1}). Mp 178–180 °C; $[\alpha]_D^{25}$ 0.0 (*c* 0.075 in MeOH); UV λ_{max} (MeOH)/nm 215 (ε/dm^3 mol^{-1} cm^{-1} 28 100) and 265 (14 400);^{1}H NMR (300 MHz; DMSO-d_6, D_2O exchange) δ 8.71 + 8.80 (s, 1H, H-6), 8.30–8.37 (m, 1H, Ar-H), 7.77 (d, J = 3.6, 2H, Ar-H), 7.47 (br, 1H, Ar-H), 7.00 + 7.14 (s, 1H, Ar-H), 4.73 (s, 1H, N1-CH_{2a}CO), 4.24 (s, 1H, N1-CH_{2b}CO), 4.01 (s, 1H, NHCH_{2a}CONH$_2$), 3.63–3.70 (m, 4H, NHCH_2CH_2N), 2.98 (s, 3H, Ar-CH_3), 1.20 + 1.61 (s, 3H, 5-CH_3); HR-MS (ESI-TOF, *m/z*): Calcd. For $C_{25}H_{26}N_7O_5$ 492.1990 $(M + H)^+$. Found 492.2002.

N-(1-Methyl-β-carboline-3-carbonyl)-*N*-(uracil-N^1-methylenecarbonoyl)-glycinamide 8

This compound was synthesized according to the general procedure for the solid-phase synthesis of β-carboline–peptide nucleoside conjugates on PAL resin in 90% yield from 200 mg resin **R3** (peptide nucleoside on PAL resin with the loading 0.391 mmol g^{-1}). Mp 140–142 °C; $[\alpha]_D^{25}$ −11.4 (*c* 0.176 in MeOH); UV λ_{max} (MeOH)/nm 207(ε/dm^3 mol^{-1} cm^{-1} 6000), 238 (10 500) and 270 (14 400);^{1}H NMR (300 MHz, DMSO-d_6, D_2O exchange) δ 8.79 + 8.84 (s, 1H, H-6), 8.35–8.40 (m, 1H, Ar-H), 7.76 (d, J = 6.0, 2H, Ar-H), 7.35–7.48 (m, 2H, Ar-H), 5.48–5.57 (d, J = 6.0, 1H, H-5), 4.55 + 4.77 (s, 2H, N^1-CH_2CO), 4.00 (s, 1H, NHCH_2CONH$_2$), 3.57–3.66 (m, 4H, NHCH_2CH_2N), 2.98 (s, 3H, Ar-CH_3); HR-MS (ESI-TOF, *m/z*): Calcd. for $C_{23}H_{24}N_7O_5$ 478.1834 $(M + H)^+$. Found 478.1834.

Compound 9

This compound was synthesized according to the general procedure for the solid-phase synthesis of β-carboline–peptide nucleoside conjugates on PAL resin in 95% yield from 177 mg resin **R3** (peptide nucleoside on PAL resin with the loading 0.357 mmol g^{-1}). Mp 148–150 °C; $[\alpha]_D^{25}$ −15.1 (*c* 0.106 in MeOH); UV λ_{max} (MeOH)/nm 210 (ε/dm^3 mol^{-1} cm^{-1} 26 100), 237 (31 800) and 270 (49 500);^{1}H NMR (300 MHz; DMSO-d_6, D_2O exchange) δ 8.60 + 8.69 (s, 1H, H-6), 8.16–8.28 (m, 1H, H-6′), 7.65–7.83 (m, 3H, Ar-H), 7.46–7.54 (m, 1H, Ar-H), 7.35–7.36 (m, 1H, Ar-H), 7.16–7.22 (m, 1H), 6.81–7.01 (m, 1H), 4.65 (br, 2H, both are N1-CH_2CO + N1′-CH_2CO), 3.22–4.02 (m, 12H, CONHCH_2CH_2NCH_2CONHCH_2CH_2NCH_2CONH$_2$), 2.86 (s, 3H, Ar-CH_3), 1.43 + 1.58 (s, 6H, 5-CH_3 + 5′-CH_3); HR-MS (ESI-TOF, *m/z*): Calcd. for $C_{35}H_{40}N_{11}O_9$ 758.3005 $(M + H)^+$. Found 758.2988.

Compound 10

This compound was synthesized according to the general procedure for the solid-phase synthesis of β-carboline–peptide nucleoside conjugates on PAL resin in 95% yield from 157 mg resin **R3** (peptide nucleoside on PAL resin with the loading 0.32 mmol g^{-1}). Mp 178–180 °C; $[\alpha]_D^{25}$ −4.8 (*c* 0.125 in MeOH); UV λ_{max} (MeOH)/nm 213 (ε/dm^3 mol^{-1} cm^{-1} 28 100), 234 (16 400) and 269 (24 300);^{1}H NMR (300 MHz; DMSO-d_6, D_2O exchange) δ 8.59 + 8.71 (s, 1H, H-6), 8.35–8.40 (m, 1H, H-6′), 7.76 (d, J = 6.0, 2H, Ar-H), 7.37–7.48 (m, 2H, Ar-H), 7.35–7.48 (m, 1H, Ar-H), 5.48–5.57 (d, J = 6.0, 1H, H-5), 4.55 + 4.77 (s, 2H, N1-CH_2CONH), 4.00 (s, 1H, N1′-CH_{2a}CONH), 3.36–4.23 (m, 12H, CONHCH_2CH_2NCH_2CONHCH_2CH_2NCH_2CONH$_2$), 2.88 (s, 3H, Ar-CH_3), 1.37 + 1.50 (s, 3H, 5-CH_3); HR-MS (ESI-TOF, *m/z*): Calcd. for $C_{34}H_{39}N_{11}O_9$ 744.2849 $(M + H)^+$. Found 744.2824.

Compound 11

This compound was synthesized according to the general procedure for the solid-phase synthesis of β-carboline–peptide nucleoside conjugates on PAL resin in 96% yield from 230 mg resin **R3** (peptide nucleoside on PAL resin with the loading 0.331 mmol g^{-1}). Mp 178–180 °C; $[\alpha]_D^{25}$ −25.4 (*c* 0.032 in MeOH); UV λ_{max} (MeOH)/nm 204 (ε/dm^3 mol^{-1} cm^{-1} 15 600), 276 (18 500);^{1}H NMR (300 MHz; DMSO-d_6, D_2O exchange) δ 8.60 + 8.68 (s, 1H, H-6), 8.23–8.26 (m, 1H, H-6′), 7.61–7.68 (m, 2H, Ar-H), 7.25–7.38 (m, 2H, Ar-H), 7.15–7.18 (m, 1H, Ar-H), 5.41–5.54 (m, 2H, H-5 + H-5′), 4.69 (br, 1H, N1-CH_{2a}CONH), 4.47–4.58 (m, 2H, N1′-CH_2CONH), 3.14–4.00 (m, 8H, some hydrogen on peptide nucleoside main chain CONHCH_2CH_2NCH_2CONHCH_2CH_2NCH_2CONH$_2$), 2.85 (s, 3H, Ar-CH_3); HR-MS (ESI-TOF, *m/z*): Calcd. for $C_{33}H_{36}N_{11}O_9$ 730.2692 $(M + H)^+$. Found 730.2690.

(*R*,*S*)-1-{[*N*-1-(1-Methyl-β-carboline-3-carbonyl)-3-hydroxy-2-propoxy]methyl}-thymine 13

A mixture of compound **12**[27] (46 mg, 0.18 mmol), 10% Pd/C (10 mg) and ethanol (3 mL) was stirred under an H_2 atmosphere (0.4 atm) for 4 h. The mixture was filtered through Celite and the solvent was evaporated off to give a crude solid. To this residue, a mixture of 1-methyl-β-carboline-3-carboxylic acid (49 mg, 0.22 mmol), DCC (45 mg, 0.22 mmol), HOBT (29 mg, 0.22 mmol) and DMF (3 mL) was added in an ice bath. The reaction was allowed to stir overnight. The solvents were evaporated under vacuum and the residue was purified by silica gel chromatography eluting by CH_2Cl_2 : MeOH 30 : 1 to give a white solid 62 mg in 79% yield for two steps. Mp 154–158 °C; $[\alpha]_D^{25}$ 2.7 (*c* 0.075 in MeOH); UV λ_{max} (MeOH)/nm 216 (ε/dm^3 mol^{-1} cm^{-1} 28 100), 236 (93 500) and 270 (14 400);^{1}H NMR (300 MHz; DMSO-d_6) δ 11.93 (s, 1H, N-3 H), 11.22 (s, 1H, Ar-NH), 8.64 (s, 1H, H-6), 8.53 (t, 1H, J = 6.0, Ar-H), 8.34 (d, J = 8.1, 1H, Ar-H), 7.56–7.66 (m, 3H, Ar-H), 7.29 (t, J = 6.9, 1H, CON*H*CH$_2$), 5.18 (d, d, J = 10.5, 2H, N-1-CH_2-O), 4.89 (t, J = 5.4, 1H, OH), 3.78–3.81 (m, 1H, C*H*CH$_2$OH), 3.44–3.64 (m, 3H, CONHCH_2CHCH_{2a}), 2.81 (s, 3H, Ar-CH_3), 1.47 (s, 3H, 5-CH_3); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 164.9 (C-4), 164.1, 151.1 (C-2), 141.0, 140.8, 140.4, 138.7, 135.9, 128.3, 127.4, 122.2, 121.5, 119.9, 112.2, 109.1 (C-5), 78.1 (CONH*C*H$_2$CH), 75.3 (N-1-CH_2O), 61.7 (CH_2*C*HCH$_2$OH), 54.9 (CONH*C*H$_2$CH), 20.5 (Ar-CH_3), 11.7 (5-CH_3). HR-MS (ESI-TOF, *m/z*): Calcd. for $C_{22}H_{24}N_5O_5$ 438.1772 $(M + H)^+$. Found 438.1773.

(*R*,*S*)-1-{[*N*-1-(1-Methyl-β-carboline-3-carbonyl)-3-hydroxy-2-propoxy]methyl}-uracil 15

This compound was synthesized through the same procedure as described for compound **13** in 72% yield from compound **14**[28] (21mg). Mp 150–152 °C; $[\alpha]_D^{25}$ 0.0 (*c* 0.082 in MeOH); UV λ_{max} (MeOH)/nm 211 (ε/dm^3 mol^{-1} cm^{-1} 57 100), 241 (49 300) and 263 (64 400);^{1}H NMR (300 MHz; DMSO-d_6) δ 11.94 (s, 1H, N-3 H), 11.22 (s, 1H, Ar-NH), 8.66 (s, 1H, Ar-H), 8.55 (t, J = 6.0, 1H, H-6), 8.35 (d, J = 7.8, 1H, Ar-H), 7.55–7.71 (m, 3H, Ar-H), 7.29 (t, J = 7.2, 1H, CON*H*CH$_2$), 5.43 (d, J = 7.8, 1H, H-5), 5.22 (d, J = 10.5, 2H, N1-CH$_2$-O), 4.90 (t, J = 5.4, 1H, OH), 4.12 (m, 1H, C*H*CH$_2$OH), 3.79–3.82 (m, 1H), 3.52–3.67 (m, 1H), 3.43–3.50 (m, 1H), 3.17 (d, J = 4.8, 1H, these 4H all are from C*H*$_2$CHC*H*$_2$OH), 2.83 (s, 3H, Ar-CH$_3$); ^{13}C NMR (75.5 MHz; DMSO-d_6) δ 164.9 (C-4), 163.5, 151.1 (C-2), 144.8, 141.0, 140.8 (C-6), 138.7, 135.9, 128.3, 127.4, 122.2, 121.5, 119.9, 112.2, 112.1, 101.5 (C-5), 78.4 (NHCH$_2$*C*HCH$_2$OH), 75.6 (N1-CH$_2$-OH), 61.7 (NHCH$_2$CH*C*H$_2$OH), 48.6 (NH*C*H$_2$CHCH$_2$OH), 20.5 (Ar-CH$_3$). HR-MS (ESI-TOF, m/z): Calcd. for $C_{21}H_{22}N_5O_5$ 424.1616 (M + H)$^+$. Found 424.1613.

Capillary electrophoresis

Apparatus. Capillary electrophoresis was performed on a Beckman Proteomelab™ PA800 system (Beckman Coulter, Inc. Fullerton, CA, USA). The Beckman uncoated fused-silica capillary tubing 30 cm × 50 μm i.d., with a length of 20 cm to the detector, was used. The temperature of capillary chamber was controlled at 20.0 ± 0.1 °C by forced liquid cooling. A run voltage of +15 kV in the normal polarity mode was applied. UV detection was performed at 254 nm. 32 Karat was used as analytical software.

Solution and sample preparation. 31nt TAR RNA: 5′-GGC CAG AUC UGA GCC UGG GAG CUC UCU GGCC-3′ (10 OD), was purchased from Shanghai GenePharma Co., Ltd (Shanghai, China). De-ionized water was pretreated with DEPC (diethyl pyrocarbonate) and used in this test. The stock solution of compounds **4–11**, **13** and **15** were prepared according as follows: accurately weighed compound (**4–11**, **13** and **15**) was dissolved in 100 μL DMSO and 900 μL phosphate buffer saline (PBS, pH = 8.0) was added carefully to get the 10% DMSO in PBS buffer solution. The concentration of TAR RNA stock solution was 100 μM and was stored at 4 °C. The investigated compounds and TAR RNA solutions in pH = 8.0 PBS buffer were prepared by diluting the stock solutions to the constant volume with buffer solution. The constant concentrations of each sample were 100 μM. The varying concentrations of TAR RNA in the running buffer were prepared from 0 to 25 μM (0, 2, 5, 7.5, 10, 15, 25 μM). All of the solution was filtered through 0.22 μm cellulose acetate membrane filters and the air was removed from solution by supersonic wave prior to use.

Procedures for CE experiments. A new capillary was conditioned by following steps: with MeOH for 15 min, with H_2O for 5 min, with 0.1M HCl for 15 min, with H_2O for 5 min, with 0.1 M NaOH for 15 min, with H_2O for 5 min and with phosphate buffer saline (PBS, pH = 8.0) for 30 min respectively. Between each measurement, the capillary was flushed with 0.1 M NaOH for 2 min, with H_2O for 1 min, and with running buffer for 1 min at 20 psi. The sample containing compounds and DMSO was introduced into the capillary by low-pressure injection (0.5 psi for 5 s). Separation time was 4 min and all separations were repeated twice. The migration times of each analyte (t) and DMSO (t_0) were measured in running buffer solutions. The binding constant K_b was calculated according to the method reported by Zhang and co-workers[21a] (Table 2).

Docking study

AutoDock 3.0 program was used for the molecular docking studies of a flexible ligand (*i.e.*, compounds **4–11**, **13**, **15**) to a rigid target (TAR RNA). The target structure of TAR RNA was extracted from the protein databank (PDB code 1ANR). Starting models of ligands were built using the Insight II package, and then minimized with AMBER force field using the steepest decent

Table 2 The binding constants K_b of samples with TAR RNA Note: K_b is the binding constant, r^2 the correlation coefficient, n the number of data points and N. A., no data available

	No.					
	4	**5**	**6**	**7**	**8**	**9**
K_b/M^{-1}	29288	23754	19642	18686	21736	53933
k_d/μM	34.1	42.1	50.9	53.5	46	18.5
n	7	6	6	5	5	7
a	685.35	553.49	359.46	310.19	465.16	895.28
b	0.0234	0.0233	0.0183	0.0166	0.0214	0.0166
r^2	0.9267	0.9688	0.9724	0.9959	0.9755	0.9085
	No.					
	10	**11**	**13**	**15**	**MC3**	
K_b/M^{-1}	N. A.	28115	50647	11577	8918	
k_d/μM	N. A.	35.6	19.7	86.4	112	
n	N. A.	6	6	7	7	
a	N. A.	711.31	779.97	706.09	–2047.5	
b	N. A.	0.0253	0.0154	0.061	–0.2296	
r^2	N. A.	0.9832	0.9338	0.9943	0.9449	

and conjugated gradient methods consecutively. The obtained optimized structures were used for the following docking.

The molecular docking calculations were carried out using an empirical free energy function and Lamarckian Genetic Algorithm. Each dihedral angle of the ligands was chosen to be flexible. The number of generations, energy evaluation, and docking runs were set to 370 000, 1 500 000, and 50 respectively, and the kinds of atomic charges were taken as Kollman-all-atom for Tar RNA and Gasteiger–Hucel for the ligands.

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