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α,α-Trehalose derivatives bearing guanidino groups as inhibitors to HIV-1 Tat–TAR RNA interaction in human cells

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Abstract—Replication of HIV-1 requires specific interactions of Tat protein with TAR RNA. Disruption of Tat–TAR RNA interaction could inhibit HIV-1 replication. Here four target compounds were designed and synthesized to bind to TAR RNA for blocking the interaction of Tat–TAR RNA. The core molecule 6,6′-diamino-6,6′-dideoxy-α,α-trehalose was obtained from selective bromination of, α,α-trehalose at C-6,6′, followed by acetylation, azide displacement, deacetylation, and reduction. Coupling of the core molecule with the protected amino acid, then deprotection and guanidinylation generated the novel α,α-trehalose derivatives. Their abilities to inhibit Tat–TAR RNA interaction in human cells were determined by a Tat-dependent HIV-1 LTR-driven CAT assays.

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) gene expression depends on the interaction of the viral regulatory protein, *trans*-activator of transcription (Tat) with the *trans*-activation responsive region (TAR) RNA, a 59-base stem-loop structure located at the 5′-end of all nascent HIV-1 transcripts.[1–3] Therefore, a molecule that interrupts Tat–TAR interaction must work as an antagonist to the replication of HIV-1. It could be developed into therapeutic agents.

HIV-1 TAR contains a six-nucleotide loop, a three-nucleotide pyrimidine bulge and two single-nucleotide bulges. The trinucleotide bulge containing residues U23, C24, and U25 is essential for high affinity and specific binding of the Tat protein.[4,5] The TAR RNA binding domain of the Tat protein is known as an arginine-rich sequence (RKKRRQRRR, residues 49–57).[6–8] Arginine residue at position 52 is the only sequence-specific contact mediating the complex formation between Tat and TAR, and its guanidino group interacts with the residue U23 of the trinucleotide bulge.[9,10]

Based on the structural information from HIV-1 Tat–TAR interaction, Tat-antagonistic compounds that inhibit Tat binding to TAR element were undertaken by a number of researchers. Tat-derived basic peptides as well as the oligocarbamates and oligoureas bind to TAR RNA specifically with high affinities in vitro.[11,12] Tat-mimetic compounds ALX40-4C and CGP64222 that target TAR RNA, display efficient suppression of HIV-1 replication.[13,14]

Among natural RNA-binding molecules, aminoglycoside antibiotics have interesting properties that make them similar to peptide RNA binders. They may competitively block the binding of the Tat protein to TAR RNA.[15] However, these molecules lack site specificity to the TAR RNA; therefore, new compounds with selective binding to the TAR must be designed and synthesized. Recent studies have shown that the conjugates of aminoglycoside–arginine and guanidinylated derivatives of aminoglycoside enhance the affinity and selectivity to the HIV-1 TAR as compared to the parent aminoglycoside.[16–18]

The above study prompts us to design and prepare novel compounds by combining a carbohydrate skeleton similar to aminoglycoside antibiotics with side chains of variable length bearing guanidino group or an arginine moiety to inhibit HIV Tat–TAR interaction selectively. Here we report the synthesis of 6,6′-diamino-6,6′-dideoxy-α,α-trehalose derivatives bearing guanidino groups

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(**10** and **13**–**15**) and the inhibition to Tat–TAR RNA interaction by Tat-dependent HIV-1 long terminal repeats (LTR)-driven chloramphenicol acetyltransferase (CAT) assays.

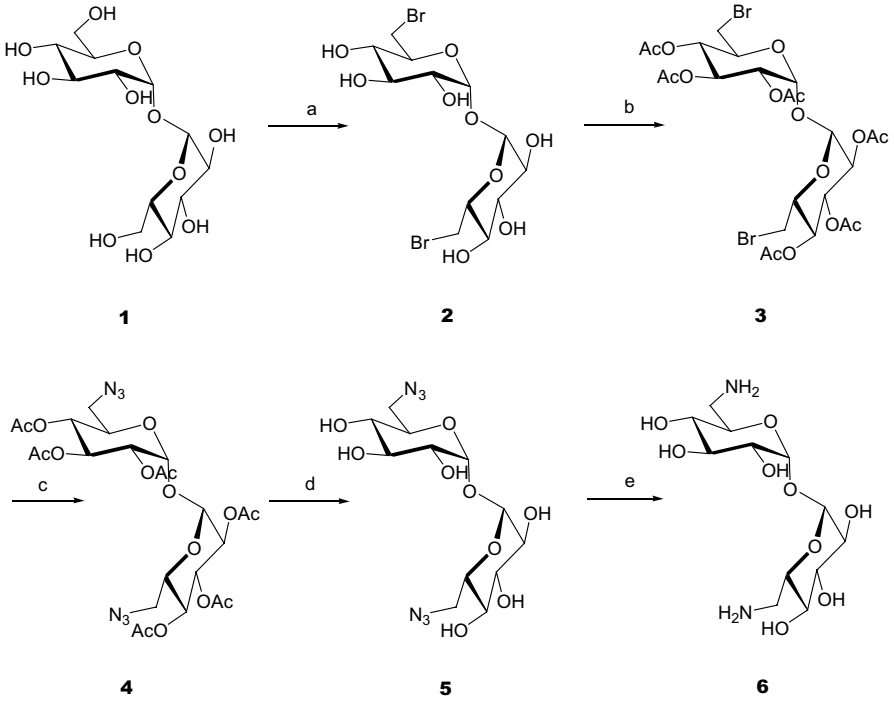
2. Results and discussion

Scheme 1 outlines the preparation of 6,6′-diamino-6,6′-dideoxy-α,α-trehalose (**6**). Compound **6** was synthesized using previously described procedures with some improvements.[19,20] α,α-trehalose dihydrate (**1**) was dehydrated directly in DMF instead of pyridine, and then brominated with 4.3 equiv of Ph_3P and NBS, to give predominantly the 6,6′-dibromo-6,6′-dideoxy-α,α-trehalose (**2**), which on conventional acetylation afforded the 2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dibromo-6,6′-dideoxy-α,α-trehalose (**3**). The displacement reaction of compound **3** with NaN_3 in DMF provided the 2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-diazido-6,6′-dideoxy-α,α-trehalose (**4**). Deacetylation of compound **4** with MeONa in MeOH afforded 6,6′-diazido-6,6′-dideoxy-α,α-trehalose (**5**).[21] In order to avoid high pressure and decrease reaction time, the azide was reduced with H_2NNH_2 in the presence of 20% $Pd(OH)_2$/C catalyst to give the 6,6′-diamino-6,6′-dideoxy-α,α-trehalose (**6**).[22]

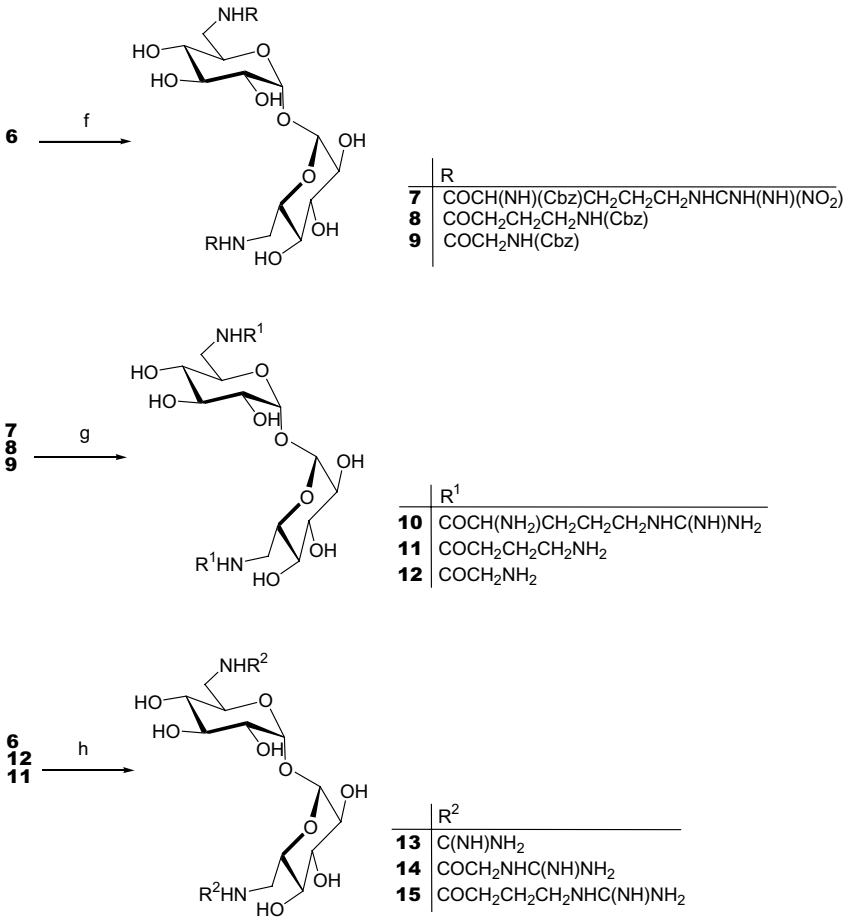
A general synthesis for compounds **10**–**12** was performed according to the following procedure (Scheme 2). The protected amino acids were activated with *N*,*N*′-dicyclohexylcarbodiimide (DCC) and coupled with compound **6** in DMF to lead to compounds **7**–**9**, with 46.1–52.8% yields. Benzyloxycarbonyl (Cbz) and nitro groups were used for protection of the amino and guanidino functions, respectively. Deprotection was realized using catalytic hydrogenation in the presence of 10% Pd/C to give compounds **10**–**12**, with 73.2–77.4% yields. The disappearance of the proton and carbon signals of benzyloxycarbonyl groups in the ^1H and ^{13}C NMR spectra of compounds **10**–**12** and appearance of guanidino carbon at 157.3 ppm in the ^{13}C NMR spectrum of target compound **10** verified the success of this reaction.[23]

In the guanidinylation reaction, a number of reagents were explored. The most commonly used reagent *N*,*N*′-bis(benzyloxycarbonyl)-*S*-methylisothiourea, failed.[24,25] Compounds **6**, **11**, and **12** were successfully guanidinylated with *S*-methylisothiourea sulfate at 85 °C for 48 h. The crude products were subjected to reversed-phase chromatography and subsequent Sephadex LH-20 column to provide the target compounds **13**–**15**, with 44.4–47.8% yields (Scheme 2). The guanidino carbon signals at 158.2, 157.4, and 158.1 ppm were detected in the ^{13}C NMR spectra, respectively.[23]

The activities of target compounds **10** and **13**–**15** to inhibit Tat–TAR RNA interaction in human cells were examined by using Tat-dependent HIV-1 LTR-driven CAT gene expression colorimetric enzyme assays.[26–28] Human embryonic kidney cells (293T) were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum and antibiotics at 37 °C in 5% CO_2 in a incubator. Cells were seeded into six-well plates the day prior to transfection. 293T cells were co-transfected with plasmid pCepIII-CAT containing the HIV-1 LTR linked to a CAT reporter gene and plasmid pSV2-Tat expressing Tat protein in a ratio



Scheme 1. Reagents and conditions: (a) Ph_3P, NBS, DMF, 48 h; (b) Ac_2O, pyridine, 24 h; (c) NaN_3, DMF, 95 °C, 27 h; (d) NaOMe, MeOH, 17 h; (e) H_2NNH_2, 20% $Pd(OH)_2$/C, MeOH, reflux, 8 h.



Scheme 2. Reagents and conditions: (f) DCC, DMF, MeOH, 0 °C to rt, 16 h; (g) H_2, 10% Pd/C, MeOH; (h) *S*-methylisothiourea sulfate, $NH_3 \cdot H_2O$, 85 °C, 48 h.

of 1:1 by the calcium phosphate method. After transfection for 24 h at 37 °C, the medium was discarded and replaced with fresh medium containing the tested compounds (at final 30 μM concentrations). All tested compounds were cultured for an additional 24 h. CAT expression was determined using a commercial CAT ELISA kit.

As shown in Figure 1, the decreased CAT activities in the presence of target compounds **10** and **13**–**15** show that they competed with Tat for TAR RNA binding and led to inhibition of Tat function in vivo. Compared with the amino precursor **6**, compounds **10** and **13**–**15** were more potent to inhibit Tat–TAR RNA interaction. The result presented here suggest that the compounds comprised of a carbohydrate core with side chains of variable length bearing a guanidino group, may serve as a specific inhibitor of Tat binding to TAR RNA. This may be ascribed to the strong basicity of the guanidino groups. They are fully protonated and positively charged under physiological conditions, providing a favorable electrostatic environment for interaction with nucleic acids. Both the electrostatic and potential to form hydrogen bonds are essential for the compound interaction with TAR. The CAT activity of compounds **13**–**15** and **10** decreased from 69.5 to 44.2 as the side chain length increased at 3 μM concentrations, and

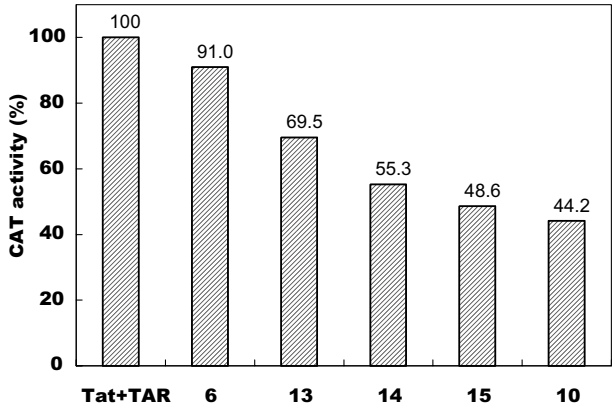


Figure 1. Inhibition to Tat–TAR RNA interaction by compounds **6**, **10**, and **13**–**15** in 293T cells.

compounds **10** and **15** reduced CAT activity more than 50%. Based on this, we can find that not only a carbohydrate core containing a guanidino group facilitates the binding to TAR RNA, but a longer chain, connecting the core and the charge-bearing moiety, is also beneficial. Hence the most potent compound inhibiting Tat–TAR interaction in 293T cells was considered to be the 6,6′-diamino-6,6′-dideoxy-α,α-trehalose-arginine conjugate **10**.

3. Conclusion

We have designed and synthesized four 6,6′-diamino-6,6′-dideoxy-α,α-trehalose derivatives bearing guanidino groups. All the target compounds exhibited inhibiting activities to Tat–TAR RNA interaction in 293T cells, and the derivative bearing arginine moiety was the most potent agent in this series.

Acknowledgements

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23. Compound **10**: mp 156.0–158.0 °C. $[\alpha]_D$ +179 (*c* 0.87, H_2O). ^1H NMR (D_2O) δ 4.90 (d, 2H, *J* 3.6 Hz, H-1), 3.81 (m, 2H, α-CH), 3.66–3.10 (m, 12H, H-6, H-5, H-6′, H-3, H-2, and H-4), 3.04 (t, 4H, *J* 8.4 Hz, δ-CH_2), 1.51 (m, 4H, γ-CH_2), 1.46 (m, 4H, β-CH_2). ^{13}C NMR (D_2O) δ 176.8 (NH–CO), 157.3 (guanidino C), 94.5, 94.1, 72.9, 71.9, 71.6, 71.3 (sugar C), 67.4 (Cbz-CH_2), 54.7 (α-CH), 41.3 (C-6), 40.4 (δ-CH_2), 31.4 (β-CH_2), 24.8 (γ-CH_2). FABMS: *m/z* 653.0 $[M+H]^+$. Anal. Calcd for $C_{24}H_{48}N_{10}O_{11}\cdot 2HCl\cdot 2H_2O$: C, 37.84; H, 7.15; N, 18.39. Found: C, 37.92; H, 7.23; N, 18.47. Compound **13**: mp 226.0 °C (decomp.). $[\alpha]_D$ +147 (*c* 0.85, H_2O). ^1H NMR (D_2O) δ 5.00 (d, 2H, *J* 3.6 Hz, H-1), 3.78–3.73 (m, 2H, H-6), 3.69–3.63 (m, 2H, H-5), 3.49–3.39 (m, 4H, H-6′ and H-3), 3.31–3.26 (m, 2H, H-2), 3.24–3.17 (t, 2H, *J* 9.0 Hz, H-4). ^{13}C NMR (D_2O): δ 158.2 (guanidino C), 94.3, 72.9, 72.6, 71.4, 71.2 (sugar C), 42.5 (C-6). FABMS: *m/z* 425.0 $[M+H]^+$. Anal. Calcd for $C_{14}H_{28}N_6O_9\cdot H_2SO_4\cdot 2H_2O$: C, 30.11; H, 6.14; N, 15.05. Found: C, 30.05; H, 6.10; N, 15.18. Compound **14**: mp 214.0 °C (decomp.). $[\alpha]_D$ +157 (*c* 0.83, H_2O). ^1H NMR (D_2O) δ 4.93 (d, 2H, *J* 3.6 Hz, H-1), 3.85 (m, 2H, H-6) 3.67–3.60 (m, 4H, H-5, and H-6′), 3.45–3.40 (m, 4H, H-2, and H-3), 3.34–3.29 (m, 2H, H-4), 3.15 (t, 4H, *J* 9.3 Hz, CH_2). ^{13}C NMR (D_2O) δ 171.0 (NH–CO), 158.1 (guanidino C), 94.4, 94.2, 73.0, 71.8, 71.6, 71.2 (sugar C), 44.3 (α-CH_2), 40.5 (C-6). FABMS: *m/z* 539.5 $[M+H]^+$. Anal. Calcd for $C_{18}H_{34}N_8O_{11}\cdot H_2SO_4\cdot 2H_2O$: C, 32.14; H, 5.99; N, 16.66. Found: 32.07; H, 5.73; N, 16.72. Compound **15**: mp 185 °C (decomp.). $[\alpha]_D$ +172 (*c* 0.96, H_2O). ^1H NMR (D_2O) δ 4.92 (d, 2H, *J* 3.6 Hz, H-1), 3.67–3.60 (m, 4H, H-6, and H-5), 3.46–3.39 (m, 4H, H-6′, and H-3), 3.29–3.22 (m, 2H, H-2), 3.17–3.17 (m, 2H, H-4), 3.03 (t, 4H, *J* 7.2 Hz, α-CH_2), 2.18 (t, 4H, *J* 7.5 Hz, γ-CH_2), 1.75 (m, 4H, *J* 7.5 Hz, β-CH_2). ^{13}C NMR (D_2O) δ 176.5, (NH–CO), 157.4 (guanidino C), 93.9, 73.0, 71.8, 71.6, 71.3 (sugar C), 41.0 (C-6), 40.4 (γ-CH_2), 33.1 (α-CH_2), 25.0 (β-CH_2). FABMS: *m/z* 595.4 $[M+H]^+$. Anal. Calcd for $C_{22}H_{42}N_8O_{11}\cdot H_2SO_4\cdot 3H_2O$: C, 35.39; H, 6.75; N, 15.01. Found: C, 35.21; H, 6.89; N, 14.90.
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