Design and Synthesis of Guanidinoglycosides Directed against the TAR RNA of HIV-1

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Replication of human immunodeficiency virus type 1 (HIV-1) requires specific interactions of the Tat protein with the transactivation responsive region (TAR) RNA. Tat-TAR RNA Interaction is mediated by a short arginine-rich domain of the protein. Disruption of this interaction could, in theory, create a state of complete viral latency. Here, four novel 6-amino-6-deoxytrehalose guanidinoglycoside derivatives (**10** and **13**–**15**) as target molecules have been designed to bind to TAR RNA for blocking the interaction of Tat-TAR RNA. They were obtained by coupling 6-amino-6-deoxy-α,α-trehalose (**6**) with the protected amino acids, deprotection by catalytic hydrogenation, followed by guanidinylated with *S*-methylisothiourea sulfate. Their abilities to inhibit Tat-TAR RNA interaction were determined by a Tat-dependent HIV-1 long terminal repeats (LTR)-driven chloramphenicol acetyltransferase (CAT) assays.

Introduction. – Human immunodeficiency virus type 1 (HIV-1) gene expression is regulated by a number of virus-encoded proteins, including the transactivator of transcription (Tat) protein that plays a critical role in virus replication [1]. Tat is responsible for high levels of expression from the viral long terminal repeat (LTR) promoter, involving the interaction of Tat with the viral RNA at a specific region called transactivation responsive element (TAR). Discovery of therapeutic agents that disrupt the Tat-TAR interaction, therefore, would provide a strategy for inhibiting HIV-1 replication.

TAR RNA comprises the first 59 nucleotides at the nascent end of the retrovirus RNA. It forms a hairpin stem-loop structure containing a six-nucleotide loop (residues 30–35), a three-nucleotide pyrimidine bulge (residues 23–25), and two single-nucleotide bulges (residues 5 and 17). The Tat-binding region of TAR centers on the three-nucleotide bulge [2]. It is also known that the binding domain of the Tat protein is an arginine-rich sequence (residues 49–57: ArgLysLysArgArgGlnArgArgArg). Arginine 52 in the protein is the only sequence contact mediating the complex formation between Tat and TAR [3], and its guanidino group interacts with residue U23 of the three-nucleotide bulge.

With structural information from the HIV-1 Tat-TAR interaction, attempts to produce antiviral peptide or peptidomimetic substances that inhibit Tat binding to TAR element have been 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* [4][5]. Tat-mimetic compounds ALX40-4C [6] and CGP64222 [7] that target TAR RNA, demonstrate pronounced antiviral activity.

Among natural RNA-binding molecules, aminoglycoside antibiotics have interesting properties that make them similar to peptide RNA-binders. They may inhibit Tat binding to TAR RNA in the mM range of concentration [8]. Recent studies have shown that the conjugates of aminoglycoside–arginine display high affinity for TAR RNA *in vitro* in the range of 20–400 nM [9]. These molecules were biologically evaluated, and R3G and R4K exhibited antiviral activity *in vitro* in μM concentrations, with undetected cytotoxicity at the active concentrations ($CC_{50} > 3940$ μM for R3G and 1130 μM for R4K) [10][11].

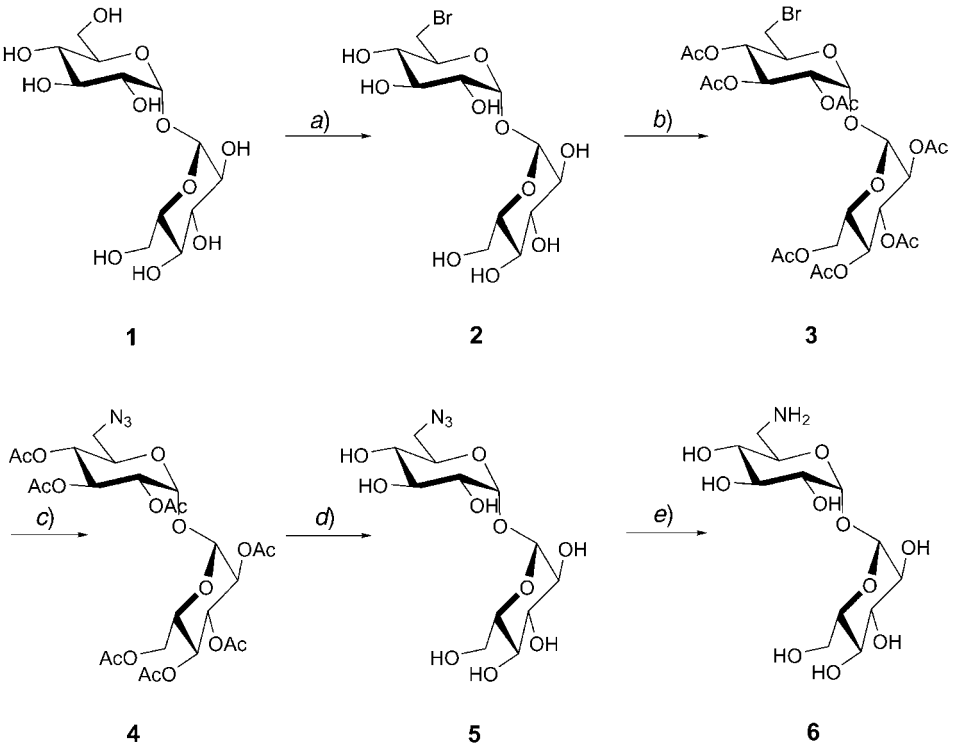
The above prompted us to design novel compounds capable of binding to TAR RNA, by combining a carbohydrate skeleton similar to aminoglycoside antibiotics with side chains of variable length bearing a guanidine or an arginine moiety, resembling peptide side chains. Here, we report the synthesis of 6-amino-6-deoxy-α,α-trehalose guanidinoglycoside derivatives **10** and **13**–**15**, and the inhibition of Tat-TAR interaction by Tat-dependent HIV-1 LTR-driven chloramphenicol acetyltransferase (CAT) assays [12–14].

Results and Discussion. – The syntheses of the 6-amino-6-deoxy-α,α-trehalose guanidinoglycoside derivatives were carried out in three principal steps. First, 6-amino-6-deoxy-α,α-trehalose (**6**) as a precursor was synthesized. Second, coupling of the protected amino acids and the precursor **6**, followed by deprotection, formed amide molecules **10**–**12** with variable-length side chains containing an amino group or an arginine residue. Third, conversion of the amino groups to guanidino groups yielded the final compounds **13**–**15**.

6-amino-6-deoxy-α,α-trehalose (**6**) was synthesized according to the procedures described in [15–17] with some improvements (*Scheme 1*). α,α-Trehalose dihydrate (**1**) was dehydrated directly in DMF instead of pyridine, and then brominated with Ph_3P and *N*-bromosuccinimide (NBS) to give predominantly the 6-bromo-6-deoxy-α,α-trehalose (**2**), which, on conventional acetylation, afforded the 2,3,4,2′,3′,4′,6′-hepta-*O*-acetyl-6-bromo-6-deoxy-α,α-trehalose (**3**) in 58% yield. In the ^{13}C-NMR spectrum of **3**, the signal due to C(6) was shifted from 61.0 to 30.4 ppm, indicating the presence of a Br-atom at C(6). The ^{1}H-NMR spectrum showed seven single peaks for *O*-Ac groups at 1.99–2.12 ppm, with the corresponding resonance at 169.5–170.6 ppm (CO groups of Ac) and 20.6–20.9 ppm (Me groups of Ac) in the ^{13}C-NMR spectrum. The replacement reaction of compound **3** with NaN_3 in DMF provided the 2,3,4,2′,3′,4′,6′-hepta-*O*-acetyl-6-azido-6-deoxy-α,α-trehalose (**4**) in 74% yield. The chemical shift of C(6) was shifted to 50.9 ppm, strongly supporting the fact that the Br-atom was replaced by azide. Deacetylation of compound **4** with MeONa in MeOH afforded 6-azido-6-deoxy-α,α-trehalose (**5**) [18] in 78% yield. Analysis by ^{1}H- and ^{13}C-NMR spectra showed no signals for *O*-Ac groups, confirming the removal of Ac groups. To avoid high pressure and to decrease reaction time, compound **5** was reduced with NH_2NH_2 in the presence of 20% $Pd(OH)_2$/C catalyst to give the 6-amino-6-deoxy-α,α-trehalose (**6**) [19] in 68% yield. The ^{13}C-NMR spectrum of **6** revealed an upfield shift of C(6) (from 50.9 to 41.1 ppm), indicating that reduction occurred under this reaction.

Compounds **10**–**12** were obtained in two steps (*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**, in 39–61% yields. (Benzyloxy)car-

Scheme 1

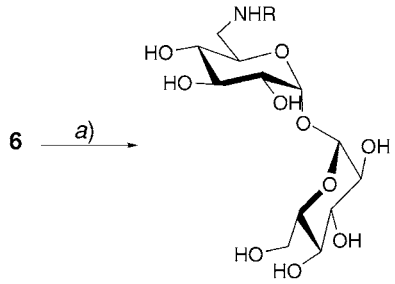


a) Ph_3P, NBS, DMF, 48 h. *b*) Ac_2O, pyridine, 24 h. *c*) NaN_3, DMF, 95°, 14 h. *d*) NaOMe, MeOH, 16 h. *e*) NH_2NH_2, 20% $Pd(OH)_2/C$, MeOH, reflux, 6 h.

bonyl (Cbz) and NO_2 groups were used for protection of the amino and guanidino functions, respectively. The ^{1}H-NMR spectra of **7**, **8**, and **9** revealed the presence of the ^{1}H signals of (benzyloxy)carbonyl group at 7.25–7.36 ppm (arom. H) and 5.02–5.15 ppm (CH_2 groups of Cbz), respectively. All of the characteristic α,α-trehalose ^{1}H signals, in particular the anomeric H-atoms (as *doublet*s at 5.07 ppm), were observed. In addition, the appearance of the signals of the amide C-atoms at 172.6–174.9 ppm (NHCO) and 158.5–159.2 ppm (CO of Cbz), those of the (benzyloxy)-carbonyl group C-atoms at 128.8–138.4 ppm (arom. C) and 67.4–67.9 ppm (CH_2 of Cbz), and those of the α,α-trehalose anomeric C-atoms at 95.2–95.5 ppm confirmed the structures of compounds **7**, **8**, and **9**. Deprotection was realized by catalytic hydrogenation in the presence of 10% Pd/C to give compounds **10**–**12** in 77–84% yields. The disappearance of the ^{1}H and ^{13}C signals of Cbz groups in the ^{1}H- and ^{13}C-NMR spectra of compounds **10**–**12**, and appearance of the signal of the guanidino C-atom at 159.4 ppm in the ^{13}C-NMR spectrum of compound **10** verified the success of this reaction.

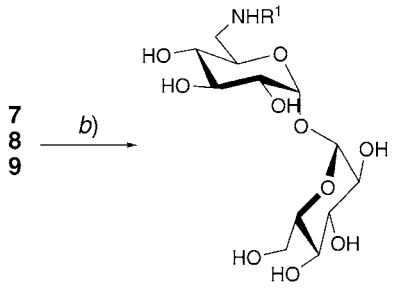
Attempts to prepare guanidinoglycoside derivatives with *N*,*N*′-Bis(benzyloxycarbonyl)-*S*-methylisothiourea in DMF were unsuccessful [20]. Compounds **6**, **11**, and **12**

Scheme 2



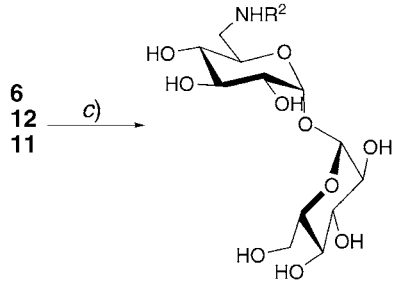
6 —*a*)→

	R
7	$COCH(NH)(Cbz)CH_2CH_2CH_2NHCNH(NH)(NO_2)$
8	$COCH_2CH_2CH_2NH(Cbz)$
9	$COCH_2NH(Cbz)$



7, **8**, **9** —*b*)→

	R^1
10	$COCH(NH_2)CH_2CH_2CH_2NHC(NH)NH_2$
11	$COCH_2CH_2CH_2NH_2$
12	$COCH_2NH_2$



6, **12**, **11** —*c*)→

	R^2
13	$C(NH)NH_2$
14	$COCH_2NHC(NH)NH_2$
15	$COCH_2CH_2CH_2NHC(NH)NH_2$

a) DCC, DMF, MeOH, 0° → r.t., 16 h. *b*) H_2, 10% Pd/C, MeOH. *c*) *S*-Methylisothiourea sulfate, $NH_3 \cdot H_2O$, 85°, 48 h.

were successfully guanidinylated with *S*-methylisothiourea sulfate at 85° for 48 h. The crude products were subjected to reversed-phase *C-18* chromatography and to a subsequent *Sephadex LH-20* column to provide guanidinoglycoside derivatives **13**–**15**, in 42–55% yields (*Scheme 2*). The guanidino ^{13}C signals at 160.4, 158.1, and 157.4 ppm were detected in the ^{13}C-NMR spectra.

The inhibitory activities of Tat-TAR RNA interaction by target compounds **10**, **13**, **14**, and **15** were examined by using Tat-dependent HIV-1 LTR-driven CAT gene-expression colorimetric enzyme assays. Human embryonic kidney cells (293T) 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, and CAT protein

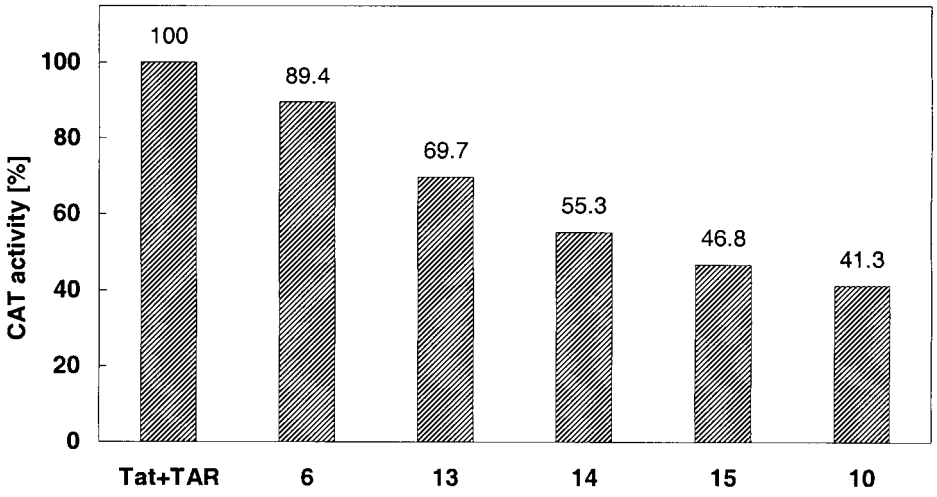


Figure. *Effect of compounds* **6**, **10**, **13**, **14**, *and* **15** *on Tat-dependent HIV-1 LTR-driven CAT expression*

expression is dependent on Tat. As shown in the *Figure*, the decreased CAT activities in the presence of target compounds **10**, **13**, **14**, and **15** show that they compete with Tat for TAR RNA binding and lead to inhibition of Tat function *in vivo*. Compared with the amino precursor **6**, compounds **10**, **13**, **14**, and **15** are more potent inhibitors of Tat-TAR RNA interaction. These results indicate that the compounds comprised of a carbohydrate core with side chains of variable length bearing a guanidino group may serve as specific inhibitors of Tat binding to TAR RNA. The CAT activity of compounds **13**, **14**, **15**, and **10** decreased from 69.7 to 41.3 with the side-chain length increased at 3 μM concentration; compounds **10** and **15** reduced CAT activity more than 50%. Based on these findings, we can conclude that not only a carbohydrate core containing a guanidino group facilitates the binding to TAR RNA, but a longer chain, which connects the core and the charge-bearing moiety, is also beneficial.

Experimental Part

General. All solvents were dried and distilled prior to use. Evaporations were carried out under reduced pressure at a bath temp. of $< 45°$. TLC: precoated silica-gel *60* F_{254} plates; detection by irradiation with UV light (254 nm), by absorption of I_2 vapor, or by spraying with 10% H_2SO_4 in EtOH and ninhydrin. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Chemical Co.*). M.p.: *Electrothermal XT-4* digital melting-point apparatus; uncorrected. Optical rotations: *Perkin-Elmer 243B* polarimeter. NMR Spectra: *Varian VXR-300* instrument; δ in ppm with Me_4Si as an internal standard (= 0 ppm), J in Hz. FAB-MS: *ZAB-HS* instrument; in m/z.

2,3,4,2′,3′,4′,6′-Hepta-O-*acetyl-6-bromo-6-deoxy-α,α-trehalose* (**3**). α,α-Trehalose dihydrate (10.0 g, 26 mmol) was dehydrated by dissolving in DMF (250 ml) and concentrating the resulting soln. to 150 ml. The concentrated soln. was cooled with an ice bath to 0° and *N*-bromosuccinimide (NBS; 9.4 g, 52 mmol) was added with stirring. After 0.5 h, Ph_3P (13.6 g, 52 mmol) was added gradually, and the mixture was stirred for another 48 h at r.t. MeOH (150 ml) was added to decompose excess reagent, and the solvents were removed under reduced pressure and lyophilized to give a yellow solid. H_2O was added, and the soln. was extracted with $CHCl_3$. The combined org. phases were concentrated and lyophilized to give a white solid product of which the chief component was compound **2**. The product was acetylated with Ac_2O (100 ml) in pyridine (350 ml) without

purification. After 24 h at r.t., the soln. was poured slowly into ice-water (2 l), and the resulting precipitate was filtered. The solid was washed with 5% $NaHCO_3$, then H_2O, and lyophilized. The product was purified by CC (CH_2Cl_2/AcOEt 4:1), and **3** (10.72 g, 58%) was isolated. White powder. M.p. 117.0–118.0° ([17]: 119–120°). $[\alpha]_D = +176$ ($c = 0.84$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 5.50–5.41 (*m*, 2 H–C(6′)); 5.29 (*dd*, $J = 3.6$, 2 H–C(1)); 5.13–5.09 (*m*, 2 H–C(5)); 5.04–4.90 (*m*, H–C(6)); 4.21–4.10 (*m*, 2 H–C(3)); 4.09–3.96 (*m*, 2 H–C(2)); 3.38–3.25 (*m*, 2 H–C(4)); 2.12 (*s*, MeCO); 2.09 (*s*, MeCO); 2.05 (*s*, MeCO); 2.04 (*s*, MeCO); 2.01 (*s*, MeCO); 2.00 (*s*, MeCO); 1.99 (*s*, MeCO). ^{13}C-NMR ($CDCl_3$): 170.6, 169.9, 169.8, 169.6, 169.5 (CO); 92.2, 92.1, 71.1, 70.1, 69.8, 69.7, 69.6, 69.3, 68.5, 68.2, 61.7, 30.4 (sugar ring C); 20.9, 20.6 (*Me*CO). FAB-MS: 700.6 ($C_{26}H_{36}BrO_{17}^+$, $[M+H]^+$; calc. 700.4).

2,3,4,2′,3′,4′,6′-Hepta-O*-acetyl-6-azido-6-deoxy-α,α-trehalose* (**4**). A mixture of **3** (9.10 g, 13 mmol) and NaN_3 (2.54 g, 39 mmol) in DMF (100 ml) was heated for 20 h at 95°. After cooling, the insoluble material was filtered off. The filtrate was concentrated under reduced pressure to afford a syrup. $CHCl_3$ and H_2O were added, and the org. layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was further purified by CC (CH_2Cl_2/AcOEt 4:1), and **4** was obtained (6.40 g, 74%). White crystals. M.p. 119.0–120.0° ([17]: 119–120.5°). $[\alpha]_D = +117$ ($c = 0.96$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 5.49–5.41 (*m*, 2 H–C(6′)); 5.29 (*dd*, $J = 3.6$, 2 H–C(1)); 5.08–4.92 (*m*, 2 H–C(5), 2 H–C(6)); 4.25–4.05 (*m*, 2 H–C(3)); 4.04–3.95 (*m*, 2 H–C(2)); 3.36–3.11 (*m*, 2 H–C(4)); 2.09 (*s*, MeCO); 2.06 (*s*, MeCO); 2.05 (*s*, MeCO); 2.03 (*s*, MeCO); 2.02 (*s*, MeCO); 2.01 (*s*, MeCO); 2.00 (*s*, MeCO). ^{13}C-NMR ($CDCl_3$): 170.6, 170.0, 169.9, 169.7, 169.6, 169.5 (CO); 92.8, 92.4, 69.9, 69.8, 69.7, 69.6, 68.4, 68.2, 61.7, 50.9 (sugar ring C): 20.6 (*Me*CO). FAB-MS: 662.0 ($C_{26}H_{36}N_3O_{17}^+$, $[M+H]^+$; calc. 662.6).

6-Azido-6-deoxy-α,α-trehalose (**5**). To a suspension of **4** (5.95 g, 9 mmol) in MeOH (120 ml), a catalytic amount of MeONa was added, and the mixture was stirred for 14 h at r.t. The soln. was neutralized by addition of *Amberlite IR-120* (H^+) resin. The resin was washed with MeOH, and the combined soln. was concentrated under reduced pressure. Purification of the residue by CC ($CHCl_3$/MeOH 1.5:1) afforded **5** (2.57 g, 78%). White powder. M.p. 192.0–194.2°. $[\alpha]_D = +149$ ($c = 0.81$, MeOH). ^{1}H-NMR (CD_3OD): 5.08 (*dd*, $J = 3.6$, 2 H–C(1)); 3.80–3.71 (*m*, 2 H–C(6′), 2 H–C(5)); 3.66–3.60 (*m*, 2 H–C(6)); 3.48–3.23 (*m*, 2 H–C(3), 2 H–C(2), 2 H–C(4)). ^{13}C-NMR (CD_3OD): 95.3; 95.1; 74.5; 74.2; 73.9; 73.1; 72.9; 72.6; 71.8; 62.6; 52.6 (sugar ring C). FAB-MS: 368.2 ($C_{12}H_{22}N_3O_{10}^+$, $[M+H]^+$; calc. 368.3).

6-Amino-6-deoxy-α,α-trehalose (**6**). A soln. of **5** (2.39 g, 6.5 mmol) in MeOH (50 ml) was degassed by evacuating the flask and backfilling with N_2. NH_2NH_2 (0.55 ml, 16.6 mmol) was added, followed immediately by 20% $Pd(OH)_2$/C (130 mg), and the mixture was heated at reflux for 8 h. The soln. was cooled to r.t., filtered, and concentrated under reduced pressure. The residue was purified with *Sephadex LH-20* resin with 5% MeOH and lyophilized to afford **6** (1.51 g, 68%). White powder. M.p. 203° (dec.) $[\alpha]_D = +127$ ($c = 0.95$, H_2O). ^{1}H-NMR (D_2O): 5.04 (*dd*, $J = 3.6$, 2 H–C(1)); 3.86–3.70 (*m*, 2 H–C(6′), 3.68–3.56 (*m*, 2 H–C(5), 2 H–C(6)); 3.53–3.46 (*m*, 2 H–C(3)); 3.31–3.17 (*m*, 2 H–C(2), 2 H–C(4)). ^{13}C-NMR (D_2O): 94.1; 94.0; 73.1; 72.8; 72.6; 72.0; 71.6; 71.3; 70.2; 68.8; 61.0; 41.1 (sugar ring C). FAB-MS: 342.1 ($C_{12}H_{24}NO_{10}^+$, $[M+H]^+$; calc. 342.3).

*6-({*N$^{\alpha}$*-[(Benzyloxy)carbonyl]-*N$^{\omega}$*-nitro-*L*-arginyl}amino)-6-deoxy-α,α-trehalose* (**7**). DCC (270 mg, 1.32 mmol) was added to 10 ml of MeOH soln. containing N^{α}-benzyloxycarbonyl-N^{ω}-nitroarginine (470 mg, 1.32 mmol). After stirring for 30 min at 0°, a soln. of **6** (150 mg, 0.44 mmol) in 5 ml of DMF was added dropwise. The mixture was allowed to slowly warm to ambient temp. and stirred for an additional 16 h. Precipitated *N,N′*-dicyclohexylurea (DCU) was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography (silica gel; $CHCl_3$/MeOH 1.5:1) to afford **7** (110 mg, 39%). Colorless powder. M.p. 144.0–145.2°. $[\alpha]_D = +155$ ($c = 0.92$, MeOH). ^{1}H-NMR (CD_3OD): 7.35–7.25 (*m*, 5 arom. H); 5.15–5.02 (*m*, 4 H, CH_2(Cbz) and 2 H–C(1) overlap); 4.12 (*t*, $J = 5.2$, α-CH); 3.88–3.82 (*m*, 2 H–C(6′)); 3.79–3.73 (*m*, 2 H–(5)); 3.68–3.57 (*m*, 2 H–C(6)); 3.53–3.45 (*m*, 2 H–C(3)); 3.44–3.42 (*m*, 2 H–C(2)); 3.40–3.29 (*m*, 2 H–C(4)); 3.13 (*t*, $J = 9.0$, δ-CH_2); 1.66 (br., β-CH_2, γ-CH_2). ^{13}C-NMR (CD_3OD): 174.9 (NHCO); 160.9 (NO_2C); 158.5 (CO(Cbz)); 138.0, 129.5, 129.1, 129.0 (arom. C); 95.3, 95.2, 74.5, 74.2, 74.1, 73.9, 73.5, 73.2, 72.0, 71.9, 62.6, 41.8 (sugar ring C); 67.9 (CH_2(Cbz)); 56.2 (α-CH); 41.5 (δ-CH_2); 30.5 (β-CH_2); 26.0 (γ-CH_2). FAB-MS: 677.0 ($C_{26}H_{41}N_6O_{15}^+$, $[M+H]^+$; calc. 677.6).

*6-[*N*-({[(Benzyloxy)carbonyl]amino}butanoyl)amino]-6-deoxy-α,α-trehalose* (**8**). From 4-{[(benzyloxy)-carbonyl]}butanoic acid (313 mg, 1.32 mmol) by a method analogous to that for **7**: **8** (150 mg, 61%). Colorless powder. M.p. 113.0–114.0°. $[\alpha]_D = +136$ ($c = 0.84$, MeOH). ^{1}H-NMR (CD_3OD): 7.33–7.26 (*m*, 5 arom. H); 5.07 (*d*, $J = 3.6$, 2 H–C(1)); 5.05 (*s*, CH_2(Cbz)); 3.88–3.81 (*m*, 2 H–C(6′)); 3.77–3.73 (*m*, 2 H–C(5)); 3.68–3.62 (*m*, 2H–C(6)); 3.51–3.47 (*m*, 2 H–C(3)); 3.44–3.42 (*m*, 2 H–C(2)); 3.39–3.27 (*m*, 2 H–C(4)); 3.12 (*q*, $J = 7.2$, α-CH_2), 2.23 (*t*, $J = 7.5$, γ-CH_2); 1.76 (*m*, $J = 7.2$, β-CH_2). ^{13}C-NMR (CD_3OD): 176.2 (NHCO); 159.0 (CO(Cbz)); 138.4, 129.5, 129.0, 128.8 (arom. C); 95.3, 95.2, 80.0, 74.6, 74.1, 73.9, 73.2, 72.0, 71.9, 62.6, 41.4 (sugar

ring C); 67.4 (CH_2(Cbz)); 41.2 (γ-CH_2); 34.1 (α-CH_2); 27.4 (β-CH_2). FAB-MS: 561.0 ($C_{24}H_{37}N_2O_{13}^+$, $[M+H]^+$; calc. 561.6).

*6-([*N*-[(Benzyloxy)carbonyl]glycyl}amino)-6-deoxy-α,α-trehalose* (**9**). From *N*-[(benzyloxy)carbonyl]glycine (276 mg, 1.32 mmol) by a method analogous to that for **7**: **9** (139 mg, 59%). Colorless powder. M.p. 124.0–125.4°. $[\alpha]_D = +158$ ($c = 0.83$, MeOH). ^{1}H-NMR (CD_3OD): 7.36–7.29 (*m*, 5 arom. H); 5.10 (*s*, CH_2(Cbz)); 5.07 (*d*, $J = 3.6$, 2 H–C(1)); 3.92–3.81 (*m*, 2 H–C(6′)); 3.77–3.74 (*m*, 2 H–C(5)); 3.71–3.59 (*m*, 2 H–C(6)); 3.52–3.45 (*m*, 2 H–C(3)); 3.34–3.27 (*m*, 2 H–C(2), 2 H–C(4)); 3.14 (*t*, $J = 9.9$, CH_2). ^{13}C-NMR (CD_3OD): 172.6 (NHCO); 159.2 (CO(Cbz)); 138.0, 129.5, 129.1, 129.0, 128.9 (arom. C); 95.5, 95.3, 74.5, 74.3, 73.9, 73.4, 73.3, 73.2, 71.9, 62.6, 44.9 (sugar ring C); 67.4 (CH_2(Cbz)); 41.5 (α-CH_2). FAB-MS: 533.0 ($C_{22}H_{33}N_2O_{13}^+$, $[M+H]^+$; calc. 533.5).

*6-[(*L*-Arginyl)amino]-6-deoxy-α,α-trehalose* (**10**). Compound **7** (100 mg, 0.15 mmol) was dissolved in 15 ml of MeOH and hydrogenated in the presence of 10% Pd/C (16 mg) at 0.4 Mpa for 48 h. The mixture was then filtered through *Celite*, and the solid was washed thoroughly with MeOH and H_2O. The filtrate was then concentrated. Purification of the residue on a *Sephadex LH-20* column (50 × 1 cm, eluted with 5% MeOH) afforded, after lypophilization, **10** (57 mg, 77%). White powder. M.p. 154.0–156.0°. $[\alpha]_D = +147$ ($c = 0.92$, H_2O). ^{1}H-NMR (D_2O): 5.00 (*dd*, $J = 3.6$, 2 H–C(1)); 3.84 (*t*, $J = 6.4$, α-CH); 3.77–3.69 (*m*, 2 H–C(6′), 2 H–C(5), 2 H–C(6)); 3.65–3.31 (*m*, 2 H–C(3)); 3.28 (*t*, $J = 9.3$, δ-CH_2), 3.20–3.08 (*m*, 2 H–C(2), 2 H–C(4)); 1.78–1.73 (*m*, β-CH_2); 1.53 (*m*, γ-CH_2). ^{13}C-NMR (D_2O): 172.9 (NHCO); 159.4 (guanidino C); 96.2, 96.1, 75.2, 75.0, 74.2, 74.1, 73.8, 73.7, 73.1, 72.4, 63.2, 43.2 (sugar ring C); 55.8 (α-CH); 42.7 (δ-CH_2); 31.0 (β-CH_2); 26.5 (γ-CH_2). FAB-MS: 498.0 ($C_{18}H_{36}N_5O_{11}^+$, $[M+H]^+$; calc. 498.5).

6-[(4-Aminobutanoyl)amino]-6-deoxy-α,α-trehalose (**11**). Compound **8** (100 mg, 0.18 mmol) was dissolved in 15 ml of MeOH and hydrogenated in the presence of 10% Pd/C (16 mg) at atmospheric pressure for 5 h. The mixture was treated and purified as described above for **10** to provide **11** (64 mg, 84%). White powder. M.p. 122.4–124.3°. $[\alpha]_D = +163$ ($c = 0.85$, H_2O). ^{1}H-NMR (D_2O): 4.98 (*dd*, $J = 3.6$, 2 H–C(1)); 3.70–3.62 (*m*, 2 H–C(6′), 2 H–C(5), 2 H–C(6)); 3.49–3.43 (*m*, 2 H–C(3)); 3.29–3.25 (*m*, 2 H–C(2)); 3.17–3.10 (*m*, 2 H–C(4)); 2.84 (*t*, $J = 7.5$, α-CH_2); 2.23 (*t*, $J = 7.5$, γ-CH_2); 1.78 (*m*, $J = 7.5$, β-CH_2). ^{13}C-NMR (D_2O): 177.9 (NHCO), 96.1, 95.9, 75.3, 75.0, 74.9, 73.9, 73.8, 73.4, 72.4, 63.2, 42.5 (sugar ring C); 41.6 (γ-CH_2); 35.2 (α-CH_2); 26.0 (β-CH_2). FAB-MS: 427.0 ($C_{16}H_{31}N_2O_{11}^+$, $[M+H]^+$; calc. 427.4).

6-Deoxy-6-(glycylamino)-α,α-trehalose (**12**). According to a method analogous to that for **11**, **9** (100 mg, 0.19 mmol) was hydrogenated to afford **12** (61 mg, 82%). White powder. M.p. 115.0–117.0°. $[\alpha]_D = +140$ ($c = 0.80$, H_2O). ^{1}H-NMR (D_2O): 4.99 (*d*, $J = 3.6$, 2 H–C(1)); 3.71–3.60 (*m*, 2 H–C(6′), 2 H–C(5), 2 H–C(6)); 3.55–3.44 (*m*, 2 H–C(3), 2 H–C(2)); 3.35–3.25 (*m*, 2 H–C(4)); 3.16 (*t*, $J = 9.3$, CH_2). ^{13}C-NMR (D_2O): 171.3 (NHCO), 96.2, 96.0, 75.2, 75.0, 74.9, 73.7, 73.3, 72.3, 63.1, 43.4 (sugar ring C); 42.5 (α-CH_2). FAB-MS: 399.0 ($C_{14}H_{27}N_2O_{11}^+$, $[M+H]^+$; calc. 399.3).

6-Deoxy-6-guanidino-α,α-trehalose (**13**). To a soln. of the S-methylisothiourea sulfate (245 mg, 1.76 mmol) in H_2O (5 ml) was added the conc. aq. NH_3 (0.20 ml) with stirring at 0°. After 1 h, **6** (150 mg, 0.44 mmol) was added. The mixture was stirred at 85° for 48 h. The solvent was concentrated under reduced pressure, and the residue was applied to reversed-phase *C-18* chromatography (30 × 1 cm, eluted with H_2O). The product-containing fraction was further purified on a *Sephadex LH-20* column (50 × 1 cm, eluted with 5% MeOH). After concentration of the eluate, the residue was dissolved in a small amount of H_2O. The frozen aq. soln. was lyophilized to give **13** (93 mg, 55%). Colorless powder. M.p. 122.0–124.0°. $[\alpha]_D = +139$ ($c = 0.84$, H_2O). ^{1}H-NMR (D_2O): 5.03 (*dd*, $J = 3.6$, 2 H–C(1)); 3.80–3.60 (*m*, 2 H–C(6′), 2 H–C(5), 2 H–C(6)); 3.53–3.46 (*m*, 2 H–C(3)); 3.42–3.32 (*m*, 2 H–C(2)); 3.25 (*t*, $J = 9.6$, 2 H–C(4)). ^{13}C-NMR (D_2O): 160.4 (guanidino C); 96.3, 96.1, 75.2, 75.0, 74.9, 73.7, 73.6, 73.5, 73.3, 72.3, 63.2, 44.7 (sugar ring C). FAB-MS: 384.0 ($C_{13}H_{26}N_3O_{10}^+$, $[M+H]^+$; calc. 384.4).

6-Deoxy-6-{[(guanidino)acetyl]amino}-α,α-trehalose (**14**). From **12** (50 mg, 0.13 mmol) by a method analogous to that for **13**: **14** (25 mg, 45%). Colorless powder. M.p. 214.0° (dec.). $[\alpha]_D = 162$ ($c = 0.92$, H_2O). ^{1}H-NMR (D_2O): 4.98 (*dd*, $J = 3.6$, 2 H–C(1)); 3.71–3.60 (*m*, 2 H–C(6′), 2 H–C(5), 2 H–C(5)); 3.55–3.44 (*m*, 2 H–C(3), 2 H–C(2)); 3.35–3.25 (*m*, 2 H–C(4)); 3.16 (*t*, $J = 9.3$, CH_2). ^{13}C-NMR (D_2O): 170.9 (NHCO); 158.1 (guanidino C); 94.0, 93.8, 73.2, 72.9, 72.8, 71.8, 71.6, 71.5, 71.2, 70.2, 61.0, 44.3 (sugar ring C); 40.4 (α-CH_2). FAB-MS: 441.0 ($C_{15}H_{29}N_4O_{11}^+$, $[M+H]^+$; calc. 441.4).

6-Deoxy-6-[(4-guanidinobutanoyl)amino]-α,α-trehalose (**15**). From **11** (50 mg, 0.12 mmol) by a method analogous to that for **13**: **15** (23 mg, 42%). Colorless powder. M.p. 176.0–178.0°. $[\alpha]_D = +142$ ($c = 0.86$, H_2O). ^{1}H-NMR (D_2O): 4.98 (*dd*, $J = 3.6$, 2 H–C(1)); 3.70–3.57 (*m*, 2 H–C(6′), 2 H–C(5), 2 H–C(6)); 3.49–3.44 (*m*, 2 H–C(3)); 3.29–3.22 (*m*, 2 H–C(2)); 3.18–3.11 (*m*, 2 H–C(4)); 3.04 (*t*, $J = 7.2$, α-CH_2); 2.19 (*t*, $J = 7.5$, γ-CH_2); 1.71 (*m*, $J = 7.2$, β-CH_2). ^{13}C-NMR (D_2O): 176.5 (NHCO); 157.4 (guanidino C); 93.9, 93.7, 73.2, 72.9, 72.8,

71.8, 71.7, 71.6, 71.2, 70.2, 61.1, 40.9 (sugar ring C); 40.4 (γ-CH_2); 33.1 (α-CH_2); 25.0 (β-CH_2). FAB-MS: 469.4 ($C_{17}H_{33}N_4O_{11}^+$, $[M+H]^+$; calc. 469.5).

Tat-Dependent HIV-1 LTR-Driven CAT Assays. 293T Cells were maintained in *Dulbecco*'s modified *Eagle* medium (DMEM; *GIBCO BRL*) supplemented with 10% fetal calf serum and antibiotics at 37° in 5% CO_2 in an incubator. Cells were seeded into 6-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 of 1:1 by the calcium phosphate method. After transfection for 24 h at 37°, the medium was discarded and replaced with fresh medium containing the tested compounds (final concentration 30 μM). All tested compounds were cultured for an additional 24 h. CAT Expression was determined with a commercial CAT ELISA kit (*Boehringer Mannheim*).

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