Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry 14 (2006) 2799–2809
Bioorganic & Medicinal Chemistry

Neamine derivatives having a nucleobase with a lysine or an arginine as a linker, their synthesis and evaluation as potential inhibitors for HIV TAR–Tat

Saki Yajima, Hirohito Shionoya, Takashi Akagi and Keita Hamasaki*

Department of Applied Chemistry, Shibaura Institute of Technology, 3-4-9 Shibaura, Minato-ku, Tokyo 108-8548, Japan

Received 5 October 2005; revised 30 November 2005; accepted 30 November 2005
Available online 18 January 2006

Abstract—Neamine derivatives bearing a nucleobase, adenine, cytosine, guanine or thymine with a lysine or an arginine as a linker have been synthesized and its potential as the inhibitor for HIV TAR–Tat interaction examined. Among them, modified neamine having an arginine-nucleobase showed a higher inhibition than that of the one having a lysine-nucleobase. The difference of the nucleobase anchor did not characterize inhibition specificity. Also, stereochemistry of the amino acid in the compounds causes no difference in the inhibition for TAR–Tat.

1. Introduction

The discovery of the protease inhibitor was the main strategy in the treatment for acquired immunodeficiency syndrome (AIDS). Recently, some kind of human immunodeficiency virus (HIV) having resistance for those protease inhibitors was found. In the replication scheme of HIV, protease acts in the bottom of HIV replication process. This means HIV has many chances to impart drug resistance against protease inhibitor in the HIV gene regulation process. An inhibition strategy in the earlier stage of the replication process should be more effective. It is known that transcription of the HIV genome is regulated by virally encoded regulatory proteins, trans-activator protein (Tat) and retroviral protein (Rev), those two proteins which bind to the specific region of the viral RNA, trans-activator responsive region (TAR) and Rev responsive element (RRE), respectively.[1] On the other hand, some naturally occurring molecules have been known as drugs that disturb cell growth. They are known as antibiotics. Among them, some aminoglycoside antibiotics are known to bind specific RNA fragment. Neomycin, kanamycin, and their derivatives with similar chemical structure bind the A-site of 16S RNA in the 30S subunit of the bacterial ribosome and leads to misreading of the gene that causes bacterial death.[2–7] Also, those aminoglycoside antibiotics inhibit the binding of regulatory proteins of HIV, Tat, and Rev for TAR and RRE RNA, respectively.[8–11] We have known neamine as a consensus unit of the aminoglycoside which inhibits HIV Rev–RRE RNA interaction with micromolar of dissociation constant.[12] Also, an appropriate modification to aminoglycoside enhances its binding to RRE–Rev, TAR–Tat or 16S rRNA.[13–17] On this basis we have been studying the modification of neamine. On the other and, DNA and RNA binding proteins include a number of argnines or lysines. An arginine-modified neamine enhanced its inhibition for TAR–Tat over 30 times higher than native neamine.[18] Due to this advantage, basic amino acid is one of the good building blocks to design new drugs with neamine as a scaffold. For the sake of enhancement of inhibition specificity, further modification on neamine was designed. Obviously, nucleobase is also one of the appropriate building blocks to construct a drug which has specific binding with the RNA. Here, we have synthesized 12 neamine derivatives having a nucleobase with a lysine or an arginine as a linker and their inhibitory effect for TAR–Tat were examined.

Keywords: Aminoglycoside; Neamine; HIV TAR; HIV Tat; Drug design; Inhibition.
* Corresponding author. Tel./fax: +81 3 5476 2432; e-mail: hamie@sic.shibaura-it.ac.jp

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.11.056

2. Results and discussion

2.1. Design and synthesis of the neamine-based drugs

We have been attempting the modification at the 6′-amino group of neamine. It is known that amino groups of neamine are necessary for electrostatic interaction with the phosphate anion of the RNA. When, the 6′-amino group is used for the conjugation with building blocks, this amino group should be compensated with an alternate cationic group for net positive charge. For this purpose, a lysine or an arginine is employed as a linker of which conjugate neamine and a nucleobase, the nucleobase introduced to the α-amino group of arginine or lysine. Then the nucleobase-amino acid unit is introduced to the 6′-amino group of neamine. Amino acid works not only as the linker of which conjugate nucleobase and neamine, but also provides a positive charge with a ε-amino group or a guanidino group of arginine and lysine, respectively. With this strategy, eight neamine derivatives have been designed as shown in Figure 1.

Nucleobase-acetic acid derivatives (N^6-Cbz-adenine-9-yl)-, (N^4-Cbz-cytosine-1-yl)-, and thymine-1-yl-acetic acid (**1**, **2**, and **3**, respectively) were prepared by a previously reported method.[19] As shown in Figure 2, these purine or pyrimidine derivatives were introduced to the α-amino group of arginine or lysine. After deprotection of the α-carbonyl group, nucleobase-amino acid unit was introduced to the 6′-amino group of neamine by the condensation reaction. Finally, all protection group was removed and then the desired product was obtained (Fig. 2).

On this condensation reaction, cytosine–arginine unit, guanine–arginine unit, thymine–arginine, and cytosine–lysine unit are partially converted to their D-isomers. We have estimated L-isomer as the major product, because the synthesis started from the L amino acid. D-Isomers and L-isomers were obtained separately by purification with the reversed-phase HPLC, except for thymine–arginine and thymine–lysine-conjugated neamines. Conversion ratios were estimated by the integral of ^{1}H NMR spectrum derived from anomeric proton of the neamine unit. Irrespective of either arginine or lysine, D/L ratios were 2:1 and 1:1 for cytosine, guanine, and the thymine-modified one, respectively. However, either adenine–arginine unit or adeninine–lysine unit did not give a D-form. Adenine unit has a benzyloxycarbonyl (Cbz) at the ^{6}N-amino group as a protection group. Consequently, α-amino group of the amino acid becomes considerably bulky. This would not be favorable for stereochemical conversion. On the other hand, cytosine unit, even if it has Cbz as a protection group at the 4′-amino group, both cytosinyl lysine and cytosinyl arginine gave D-form on the condensation reaction. These results suggest that steric hindrance of this unit was not enough to protect stereochemical conversion. Obviously, the thymine unit which does not have any bulk protection group is small enough

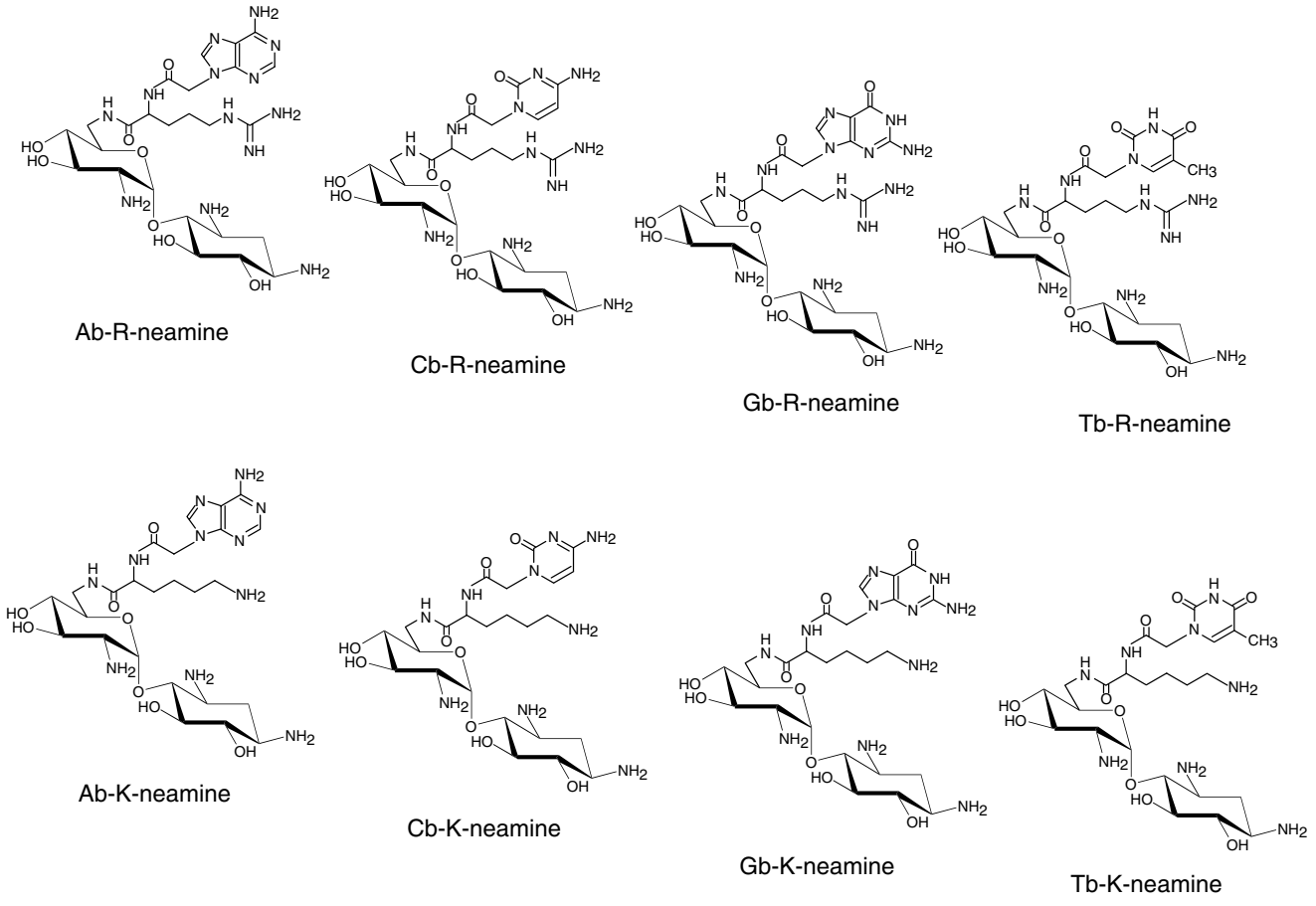

Figure 1. Designed neamine derivatives.

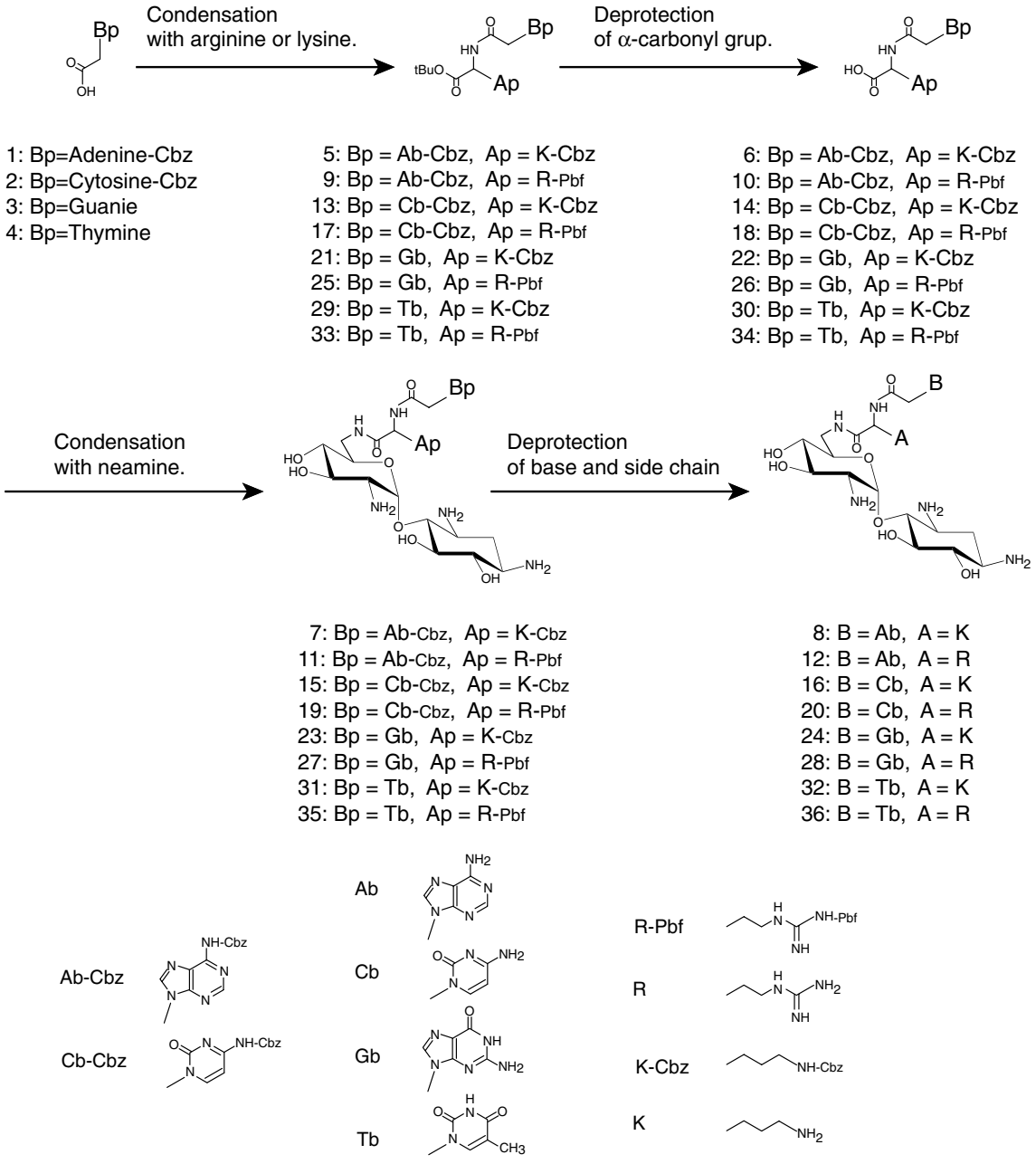

Figure 2. Synthetic route of neamine derivatives.

to effect D-form conversion. Due to poor difference in affinity to the ODS column on HPLC, both Tb-(D)K-neamine and Tb-(L)K-neamine had identical retention times on the HPLC chromatogram. The compound was obtained as a mixture with the 1:1 ratio. Same as above, Tb-(D)R-neamine and Tb-(L)R-neamine could not be separated by HPLC and it was obtained as a mixture with the 1:1 ratio.

2.2. Evaluation of the neamine derivatives as a potential inhibitor for TAR–Tat

Fluorescently labeled tat peptide, FtatRhd[20] (Fig. 3), was employed as the tracer to evaluate modified neamine which was synthesized in this study.

The solution containing 100 nM of FtatRhd and 800 nM TAR RNA was titrated with Ab-(L)K-neamine. As shown in Figure 4, fluorescence intensity of FtatRhd was diminished upon the addition of drug. This result implies that Ab-(L)K-neamine successfully competes with FtatRhd. Since, we could monitor the drug inhibition to TAR–Tat as the fluorescence decrease of FtatRhd as a probe. Inhibition constants (IC) for all neamine derivatives were determined as apparent dissociation constants by the competitive binding assay.[20]

Results are summarized in Table 1. No matter what kind of nucleobase, modified neamines with lysine as a linker have 1.7–1.9 μM as inhibition constants. These ICs were 10 times better than that of lysine-modified neamine (K-neamine, IC = 19 μM) and 16 times better than that

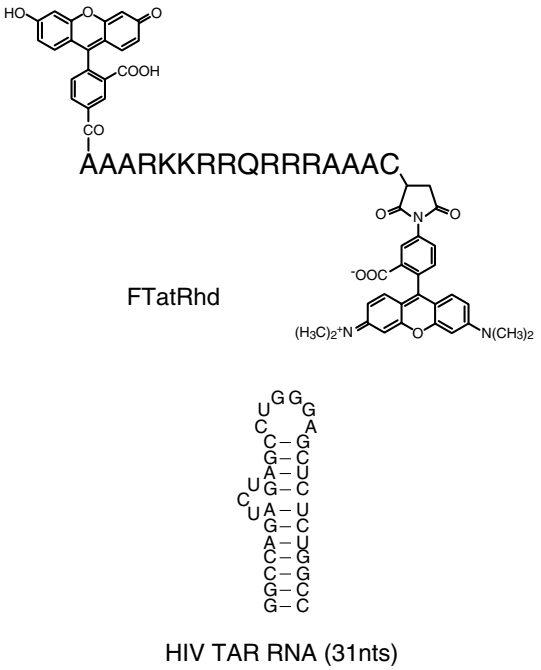

Figure 3. The tracer, FtatRhd, its amino acid sequence, and the position of the dye labeled and secondary structure of HIV TAR RNA.

of native neamine. These results suggest that the nucleobase moiety in the neamine derivatives could not interact with specific base in the RNA. But the nucleobase may have some interaction, such as an aromatic stacking or an electrostatic interaction, with the bases in the RNA. The IC of arginine-modified neamine (R-neamine) was 0.60 μM. On the other hand, nucleobase-modified neamines with arginine as a linker have 0.25–0.29 μM as IC, except for the thymine-modified one. These results were only 2 times better than that of the arginine-modified neamine and 100 times better than that of neamine itself. Thymine–arginine-modified neamine has 0.54 as IC. This IC is very close to that of R-neamine. The above result suggests that thymine residue does not contribute much for the inhibition for TAR–Tat and the contribution of arginine linker for IC is much greater than that of the thymine

Table 1. Inhibition constants (IC) of the neamine derivatives

Inhibitors	IC (μM)
Neamine	28
K-Neamine	19
Ab-(L)K-Neamine	1.8
Cb-(D)K-Neamine	1.8
Cb-(L)K-Neamine	1.9
Gb-(D)K-Neamine	1.8
Gb-(L)K-Neamine	1.8
Tb-(D,L)K-Neamine	1.7
R-Neamine	0.60
Ab-(L)R-Neamine	0.29
Cb-(D)R-Neamine	0.28
Cb-(L)R-Neamine	0.29
Gb-(D)R-Neamine	0.26
Gb-(L)R-Neamine	0.25
Tb-(D,L)R-Neamine	0.54

unit. Only thymine residue does not have a primary amino group. That makes it difficult to form the electrostatic interaction. Those results imply that the enhancement for the inhibition of those compounds mostly depends on an amino acid linker, but not on a nucleobase part. Even though, the nucleobase part supports modified neamine by an enhancement of the inhibition for TAR–Tat complex. Especially, the effect of the nucleobase is obvious in the modified neamine having lysine as a linker. Previous study showed that the synthetic peptide containing a 9-arginine stretch binds TAR RNA but the 9-lysine stretch significantly abolishes its binding affinity and an arginine residue in Tat peptide is required for specific binding and transactivation of TAR–Tat.[21] Also, the NMR structural study showed that an arginine reside interacts with guanosine-26, just below a base triple of $U^{23}{\cdot}A^{27}$–U^{38}.[22,23] Therefore, it is very reasonable that the arginine linker showed a better effect than the lysine linker one. No matter arginine linker or lysine linker, D-isomer showed almost identical IC as L-isomer. These results suggest that stereochemistry of the amino acid linker does not cause inhibition specificity in this case. The rotation between the carbons in the methylene may allow the interaction of the side chain of the amino acid to the bases in the RNA.

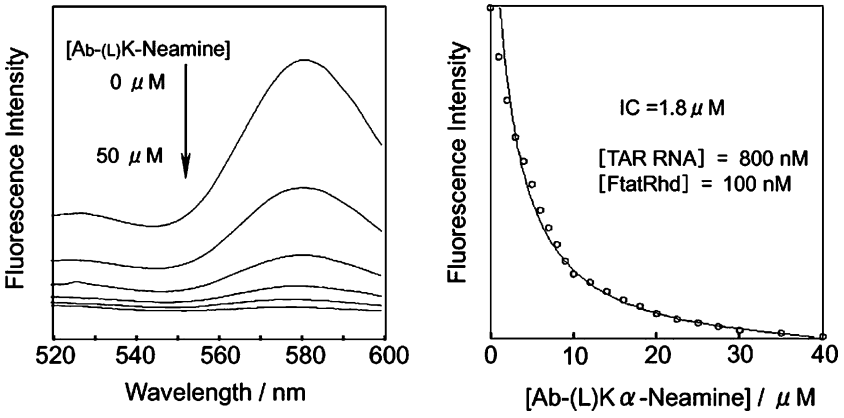

Figure 4. Fluorescence spectra of FtatRhd in the absence and in the presence of various concentrations of Ab-(L)K-neamine (left) and fluorescence titration curve as a function of the concentration of Ab-(L)K-neamine (right). The concentrations of TAR RNA and FtatRHd were fixed at 800 and 100 nM, respectively.

3. Conclusion

Twelve novel neamine derivatives were synthesized and their inhibition for HIV TAR–Tat was studied. The enhancement of inhibition depends on the linker part rather than nucleobase. Among them, neamine derivatives having an arginine as a linker showed better inhibition than the one having a lysine as a linker. The stereochemistry of the amino acid linker does not cause any difference for the inhibition magnitude.

4. Experimental

Amino acids were purchased either from Novabiochem or Kokusan Chemical Co., Ltd. All other chemicals were from Wako Pure Chemicals. TLC was performed on Silica gel 60 F_{254} from Merck. TLC solvents, solvent 1: $CHCl_3$/MeOH = 5/1; solvent 2: $CHCl_3$/MeOH = 3/2; solvent 3: $CHCl_3$/MeOH/NH_3 (aq) = 5/3/1; solvent 43: $CHCl_3$/MeOH/NH_3 (aq) = 2/2/1. HPLC was carried out with HITACH L7100 pump and L7400 UV detector on YMC-Pack Pro C18 RS column. ^{1}H NMR spectra were recorded with a JEOL JNM-LA300 spectrometer operating at 300 MHz. PCR was carried out with Perkin Elmer DNA thermal cycler 480. Steady-state fluorescence spectroscopy was performed at 25 °C on a HITACH F3000 fluorescence spectrophotometer.

4.1. Evaluation as binding inhibitor for TAR–Tat

Fluorescent Tat peptide, FtatRhd, was employed as a tracer to monitor the inhibition for TAR–Tat.[10] On the addition of the drugs, fluorescence intensity of FtatRhd around 577 nm was decreased. Inhibition constants of the drugs were determined by the curve-fitting analysis with the Eq. 1 of 1:1 stoichiometry.

$$[\mathrm{Drug}]_0 = (K_D(i_\infty - i)/K_d(i - i_0) + 1) \times ([\mathrm{RNA}]_0 - K_d(i - i_0)/(i_\infty - i) - [\mathrm{FtatRhd}]_0 \times (i - i_0)/(i_\infty - i_0)), \quad (1)$$

where i and i_0 are fluorescence intensities of FtatRhd in the presence and absence of the RNA, respectively, and i_∞ is the fluorescence intensity in the presence of infinite concentration of the drug. $[\mathrm{Drug}]_0$ is the initial concentration of the neamine derivatives, K_D and K_d are the dissociation constants of TAR–Drug and TAR–FtatRhd complex, respectively. $[\mathrm{FtatRhd}]_0$ and $[\mathrm{TAR}]_0$ were fixed at 100 and 800 nM, respectively. The drug concentrations were varied from 0 to 40 μM. Fluorescence intensity of FtatRhd was measured at 580 nm for each concentration of drugs.

4.2. Synthesis

Neamine was obtained by the hydrolysis of neomycin[17] and desalted by ion exchange chromatography with Sephadex QAE A-25.

4.3. Nucleobase-conjugated amino acids

For the sake of conjugation of the nucleobase unit with arginine or lysine, an acetic acid unit was introduced to the nucleobase unit. This unit was then conjugated to the amino acid by the condensation reaction. Nucleobase-acetic acid derivatives (N^6-Cbz-adenine-9-yl)-, (N^4-Cbz-cytosine-1-yl)-, guanine-1-yl-acetic acid, and thymine-1-yl-acetic acid (**1**, **2**, **3**, and **4**, respectively), were prepared as previously reported.[21]

4.4. *tert*-Butyl$^{\alpha}$*N*[[6-*N*-(benzyloxycarbonyl)adenine-9-yl[acetyl]-$^{\varepsilon}$*N*-(benzyloxycarbonyl)lysinate (Cbz-A_b-K(Cbz)-OtBu) (5)

To a suspension of **1** (0.131 g, 0.400 mmol) in DMF (3.5 ml) was added H-Lys(Cbz)-OtBu HCl (0.150 g, 0.402 mmol). After cooling to 0 °C, to this suspension was added a solution of PyBrop (0.224 g, 0.449 mmol) in DMF (1.0 ml) and DIEA (0.36 ml, 2.0 mmol) in small portion. After stirring for 2 h in an ice bath, the reaction mixture was stirred for another 21 h in room temperature. This reaction mixture was poured into cold water (70 ml) and stored in a refrigerator overnight. The precipitate was collected on the filter, washed with water, and dried in vacuum. The desired product, 5, was obtained as a white powder (0.189 g, 0.292 mmol, yield 73.0%). R_f = 0.887 (solvent 2), ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.7 (br s 1H), δ 8.75 (d, 1H), δ 8.59 (s, 1H), δ 8.38 (s, 1H), δ 7.45–7.33 (m, 11H), δ 5.22 (s, 2H), δ 5.02 (s, 2H), δ 5.01 (s, 2H), δ 4.13 (m, 2H), δ 3.00 (m, 2H), δ 1.62 (m, 2H), δ 1.40 (m, 13H).

4.5. $^{\alpha}$*N*[[6-*N*-(Benzyloxycarbonyl)adenine-9-yl[acetyl]-$^{\varepsilon}$*N*-(benzyloxycarbonyl)lysine (Cbz-A_b-K(Cbz)-OH) (6)

A solution of **5** (0.0924 g, 0.143 mmol) in CH_2Cl_2 was cooled to 0 °C, TFA (1.5 ml) was added to this solution and stirred for 4 h. The reaction mixture was concentrated to about 20% of initial volume and poured into Et_2O (6.0 ml). The precipitate was collected by centrifuging (6000 rpm, 20 min.) and dried in vacuum. The crude product was obtained as a white powder (0.088 g, 0.149 mmol). R_f = 0.438 (solvent 3).

4.6. $^{\varepsilon}$6-*N*-[$^{\alpha}$*N*[[6-*N*-(Benzyloxycarbonyl)adenine-9-yl[acetyl]-$^{\varepsilon}$*N*-(benzyloxycarbonyl)lysinyl]neamine (Cbz-Ab-K(Cbz)-neamine) (7)

A solution of **6** (0.072 g, 0.122 mmol) in DMF (1.0 ml) was cooled to 0 °C. WSC HCl (0.0236 g, 0.123 mmol) was added to this solution and stirred for 2 h. This solution was added to a solution of neamine in DMF (0.5 ml) and water (1.0 ml), and stirred for 2 h at 0 °C and another 24 h at room temperature. The reaction mixture was evaporated. The crude product was dissolved in 10% acetic acid aqueous solution, purified by reversed-phase HPLC (YMC-Pack Pro C18 RS, p 10 mm × 250 mm). The desired product was obtained as a white powder (0.025 g, 0.0279 mmol, yield 22.8%). R_f = 0.375 (solvent 2), ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.45 (s, 1H), δ 8.17 (s, 1H), δ 7.45–7.33 (m, 5H), δ 7.13 (m, 5H), δ 5.42

(d, 1H), δ 5.18 (s, 2H), δ 4.99 (s, 2H), δ 4.91 (s, 2H), δ 4.12 (m, 1H), δ 3.73–3.08 (m, majority), δ 3.00 (m, 2H), δ 2.27 (m, 1H), δ 1.65 (m, 2H), δ 1.35 (m, 2H), δ 1.27 (m, 2H).

4.7. 6′-*N*[[$^{\alpha}$*N*-(Adenine-9-yl)acetyl]lysinyl]neamine (Ab-K-neamine) (8)

A solution of **7** (0.029 g, 0.027 mmol) in FTA (1.5 ml) were added *m*-cresol (0.20 ml, 1.91 mmol), thioanisol (0.34 ml, 2.91 mmol), and trimethylsilyl trifluoromethanesulfonate (TMSOTF, 0.56 ml, 2.90 mmol) and stirred for 1 h at 0 °C, 3 h at room temperature. The reaction mixture was concentrated to around 1 ml, and cold Et_2O (10 ml) was added to this solution. The resultant precipitate was collected by centrifugation and dried in vacuum. The crude product was obtained as a white powder and purified by reversed-phase HPLC. The desired product was obtained as a white powder (0.011 g, 0.169 mmol, 60.8%). R_f = 0.025 (solvent 3), ^{1}H NMR (D_2O, 300 MHz) δ 8.21 (s, 1H), δ 8.10 (s, 1H), δ 5.47 (d, 1H), δ 5.02 (s, 2H), δ 4.12 (m, 1H), δ 3.66–3.18 (m, majority), δ 2.83 (m, 2H), δ 2.26 (m, 1H), δ 1.67 (m, 2H), δ 1.53 (m, 2H), δ 1.29 (m, 2H), MALDI-TOF MS, found M+H$^+$, M+Na$^+$ (calcd M+H$^+$, M+Na$^+$), 626.29, 648.29 (625.69, 647.69).

4.8. Methyl-$^{\alpha}$*N*-[[6-*N*-(benzyloxycarbonyl)adenine-9-yl]acetyl]-N^{G}-(2,2,4,6,7-pentamethyl-dehydrobenzofuran-5-sulfonyl)arginate (Cbz-Ab-R(Pbf)Ome, 9)

A suspension of **1** (0.104 g, 0.319 mmol) and H-Arg(Pbf)-OMe (0.100 g, 0.227 mmol) in DMF (1.0 ml) was added a solution of PyBrop (0.180 g, 0.383 mmol, in DMF 1 ml) and DIEA (0.16 ml, 0.908 mmol) and stirred overnight. The reaction mixture was poured into cold water then stored in a refrigerator. The white precipitate was collected and dried in vacuum. The desired product was obtained as a white powder (0.106 g, 0.142 mmol, 62.29%). R_f = 0.80 (solvent 1), ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.3 (br s 1H), δ 8.7 (d, 1H), δ 8.6 (s, 1H), δ 8.4 (s, 1H), δ 7.5–7.3 (m, 5H), δ 5.2 (s, 2H), δ 5.0 (s, 2H), δ 3.6 (s, 3H), δ 3.0 (q, 2H), δ 2.5 (s, 3H), δ 2.4 (s, 3H), δ 2.0 (s, 3H), 1.4 (s, 6H).

4.9. $^{\alpha}$*N*-[[6-*N*-(Benzyloxycarbonyl)adenine-9-yl]acetyl]-N^{G}-(2,2,4,6,7-pentamethyl-dehydrobenzofuran-5-sulfonyl)arginate (Cbz-Ab-R(Pbf)Ome, 10)

A solution of **9** in methanol (3 ml) was added 2 ml of 1 M NaOH aq and stirred for 1 h. The pH of the reaction mixture was adjusted to 3 with 0.25 M HCl then stored in a refrigerator overnight. White pellet was washed with Et_2O and dried in vacuum. The desired product was obtained as a white powder (0.073 g, 0.10 mmol, 70.04%). R_f = 0.50 (solvent 3), ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.6 (br s 1H), δ 8.7 (d, 1H), δ 8.6 (s, 1H), δ 8.4 (s, 1H), δ 7.5–7.3 (m, 5H), δ 5.2 (s, 2H), δ 5.0 (s, 2H), δ 2.9 (s, 2H), δ 2.5 (s, 3H), δ 2.4 (s, 3H), δ 2.0 (s, 3H), 1.4 (s, 6H).

4.10. [6′-*N*-$^{\alpha}$*N*-[[6-*N*-(Benzyloxycarbonyl)adenine-9-yl]acetyl]-N^{G}-(2,2,4,6,7-pentamethyl-dehydrobenzofuran-5-sulfonyl)arginyl]neamine (Cbz-Ab-R(Pbf)-neamine, 11)

A solution of **10** (0.041 g, 0.0054 mmol) and WSC HCl (0.01 g, 0.054 mmol) in DMF (3 ml) was added to the aqueous solution of neamine (0.052 g, 0.162 mmol, 1.5 ml in water). This reaction mixture was stirred overnight. Then, all the solvent was removed and dried. Resultant yellow powder was dissolved in 10% acetic acid solution and then purified by reversed-phase HPLC. The desired product was obtained as a white powder (0.018 g, 0.017 mmol, 32.32%). R_f = 0.40 (solvent 3), ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.5 (s, 1H), δ 8.4 (s, 1H), δ 7.3–7.2 (m, 5H), δ 5.4 (d, 1H), δ 5.2 (s, 2H), δ 5.1 (s, 2H), δ 37–3.0 (m, majority), δ 2.97 (s, 2H), δ 2.3 (s, 3H), δ 2.2 (s, 3H), δ 1.8 (s, 3H), 1.2 (s, 6H).

4.11. 6′-*N*-[$^{\alpha}$*N*-[(Adenine-9-yl)acetyl]arginyl]neamine (Ab-(L)R-neamine, 12)

A solution of **11** (15.6 mg, 0.015 mmol) was dissolved in TFA (0.323 ml), added TMSOTF (0.087 ml, 0.45 mmol), *m*-cresol (0.01 ml, 0.095 mmol), and thioanisol (0.0537 ml, 0.45 mmol), and stirred for 2 h at 0 °C. This reaction mixture was poured into Et_2O. Resultant white precipitate was dissolved in 10% acetic acid solution and then purified by reversed-phase HPLC. The desired product was obtained as a white powder (0.018 g, 0.017 mmol, 32.32%). ^{1}H NMR (D_2O, 300 MHz) δ 8.3 (s, 1H) δ 5.5 (d, 1H), δ 5.1 (s, 2H), δ 4.2 (m, 1H), δ 3.8–3.1 (m, majority), δ 2.3 (m, 1H), δ 1.2 (m, 2H).

4.12. *tert*-Butyl$^{\alpha}$*N*[[4-*N*-(Benzyloxycarbonyl)cytosine-1-yl]acetyl]-$^{\varepsilon}$*N*-(benzyloxycarbonyl)lysinate (Z-Cb-Lys(Z)-OtBu, 13)

Compound **2** (0.140 g, 0.463 mmol) was suspended in DMF (0.5 ml), H-Lys(Z)-OtBu HCl (0.173 g, 0.464 mmol) was added to the solution and cooled to 0 °C. A DMF solution (1.5 ml) of PyBrop (0.260 g, 0.554 mmol) was combined to the above solution and DIEA (0.33 ml, 1.86 mmol) was added. This solution was stirred for 2 h in an ice bath and for 21 h at room temperature. The reaction mixture was poured into cold water and stored in a refrigerator overnight. Resultant precipitate was collected by centrifugation and dried in vacuum. Yield: 0.209 g, 0.336 mmol, 72.4%, ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.76 (s 1H), δ 8.54 (d, 1H), δ 7.958 (d, 1H), δ 7.41–7.35 (m, 11H), δ 7.01 (d, 1H), δ 5.19 (s, 2H), δ 5.00 (s, 1H), δ 4.51 (s, 2H), δ 4.11 (m, 1H), δ 3.00 (m, 2H), δ 1.63 (m, 2H), δ 1.40 (s, 9H), δ 1.25 (m, 4H).

4.13. $^{\alpha}$*N*[[4-*N*-(Benzyloxycarbonyl)cytosine-1-yl]acetyl]-$^{\varepsilon}$*N*-(benzyloxycarbonyl)lysine (Z-Cb-Lys(Z)-OH, 14)

Compound **13** (0.092 g, 0.15 mmol) was dissolved in CH_2Cl_2 (2 ml) and cooled to 0 °C. TFA (2.0 ml) was added to this solution and stirred for 3.5 h. This reaction mixture was concentrated to 20% of original volume and poured into cold Et_2O. Resultant precipitate was collected by centrifugation and dried in vacuum. The crude

product was obtained as a white powder, 0.088 g, 0.16 mmol. $R_f = 0.49$ (solvent 4).

4.14. 6′-*N*-[$^{\alpha}$*N*[[4-*N*-(Benzyloxycarbonyl)cytosine-1-yl]acetyl]-$^{\varepsilon}$*N*-(benzyloxycarbonyl)lysinyl]neamine (Z-Cb-Lys(Z)-neamine, 15D and 15L)

Compound **14** (0.077 g, 0.135 mmol) was dissolved in DMF (1 ml) and cooled to 0 °C. WSC HCl (0.032 g, 0.158 mmol) was added to this solution and stirred for 30 min. This solution was added to the neamine solution (0.085 g, 0.263 mmol, in water 1 ml, DMF 0.5 ml) and stirred for 2 h at 0 °C and for 24 h at room temperature. After the solvent was removed by evaporation, the crude product was dissolved in 10% acetic acid solution and purified by reversed-phase HPLC. **15D** and **15L** were obtained as white powder separately. **15D**: 0.019 g, 0.022 mmol, 16.2%. $R_f = 0.55$ (solvent 4), ^{1}H NMR (D_2O, 300 MHz) δ 7.78 (d, 1H), δ 7.28–7.21 (m, 10H), δ 6.98 (d, 1H), δ 5.27 (d, 1H), δ 5.09 (s, 2H), δ 4.91 (s, 2H), δ 4.47 (s, 2H), δ 3.93 (m, 1H), δ 3.67–2.94 (m, majority), δ 2.69 (m, 2H), δ 2.24 (m, 1H), δ 1.63–1.59 (m, 2H), δ 1.30 (m, 2H), δ 1.22 (m, 2H). **15L**: 0.032 g, 0.037 mmol, 27.4%. $R_f = 0.55$ (solvent 4), ^{1}H NMR (D_2O, 300 MHz) δ 7.72 (d, 1H), δ 7.28–7.20 (m, 10H), δ 6.90 (d, 1H), δ 5.52 (d, 1H), δ 5.09 (s, 2H), δ 4.91 (s, 2H), δ 4.07 (s, 2H), δ 3.72–3.16 (m, majority), δ 2.94 (m, 2H), δ 2.25 (m, 1H), δ 1.68–1.64 (m, 2H), δ 1.32 (m, 2H), δ 1.21 (m, 2H).

4.15. 6′-*N*-[$^{\alpha}$*N*-(Cytosine-1-yl)acetyl]-$^{\varepsilon}$*N*-lysinyl]neamine (Cb-Lys-neamine, 16D)

Compound **15D** (0.019 g, 0.022 mmol) was dissolved in TFA (1.0 ml) and *m*-cresol (0.137, 1.31 mmol), thioanisol (0.153 ml, 1.31 mmol), and TMSOTF (0.252 ml, 1.31 mmol) were added to the solution and stirred for 30 min at 0 °C and for 2 h in room temperature. The reaction mixture was added cold Et_2O (10 ml). Resultant precipitate was collected by centrifugation and dried. This crude product was dissolved in 10% acetic acid solution and purified by HPLC. Compound **16D** was obtained as a white powder, 0.010 g, 0.016 mmol, 74.7%. $R_f = 0.0375$ (solvent 4), ^{1}H NMR (D_2O, 300 MHz) δ 7.56 (d, 1H), δ 5.96 (d, 1H), δ 5.44 (d, 1H), δ 4.43 (s, 2H), δ 4.03 (m, 1H), δ 3.67–3.13 (m, majority), δ 2.78 (m, 2H), δ 2.28 (m, 1H), δ 1.61 (m, 2H), δ 1.48 (m, 2H), δ 1.26 (m, 2H), MALDI-TOF MS, found $M+H^+$, $M+Na^+$ (calcd $M+H^+$, $M+Na^+$), 602.32, 624.26 (602.68, 624.66).

4.16. 6′-*N*-[$^{\alpha}$I-(Cytosine-1-yl)acetyl]-$^{\varepsilon}$I-lysinyl]neamine (Cb-Lys-neamine, 16L)

Compound **16L** was obtained in the same manner as **16D** as a white powder, 0.016 g, 0.027 mmol, 73.8%. $R_f = 0.0375$ (solvent 4), ^{1}H NMR (D_2O, 300 MHz) δ 7.58 (d, 1H), δ 6.01 (d, 1H), δ 5.56 (d, 1H), δ 4.47 (s, 2H), δ 4.15 (m, 1H), δ 3.76–3.18 (m, majority), δ 2.81 (m, 2H), δ 2.31 (m, 1H), δ 1.71 (m, 2H), δ 1.67 (m, 2H), δ 1.52 (m, 2H), δ 1.29 (m, 2H), MALDI-TOF MS, found $M+H^+$, $M+Na^+$ (calcd $M+H^+$, $M+Na^+$), 602.19, 624.18 (602.68, 624.66).

4.17. Methyl-$^{\alpha}$*N*-[[4-*N*-(benzyloxycarbonyl)cytosine-1-yl]-acetyl]-N^G-(2,2,4,6,7-pentamethylene-dihydrobenzofuran-5-sulfonyl)arginate (Z-Cb-R(Pbf)-OMe, 17)

Compound **2** (0.250 g, 0.824 mmol) and H-Arg(Pbf)-OMe HCl were dissolved in DNF (5 ml). A DMF solution (5 ml) of PyBrop (0.466 g, 1.00 mmol) and DIEA (0.42 ml, 2.36 mmol) was added to the above solution dropwise and stirred for 24 h. The reaction mixture was poured into cold water. The precipitate was collected by centrifugation and dried in vacuum. The desired product was obtained as a white powder (0.442 g, 0.609 mmol, 73.95%). $R_f = 0.83$ (solvent 3), ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.3 (br s 1H), δ 8.5 (d, 1H), δ 7.8 (d, 1H), δ 7.3–7.2 (m, 5H), δ 7.0 (d, 1H), δ 6.8 (br s, 1H), δ 6.5 (br s, 1H), δ 5.2 (s, 2H), δ 4.5 (s, 2H), δ 4.3 (m, 1H), δ 3.6 (s, 3H), δ 3.0 (s, 2H), δ 2.5 (s, 3H), δ 2.4 (s, 3H), δ 2.0 (s, 3H), δ 1.4 (s, 6H).

4.18. $^{\alpha}$*N*-[[4-*N*-(Benzyloxycarbonyl)cytosine-1-yl]acetyl]-N^G-(2,2,4,6,7-pentamethylene-dihydrobenzofuran-5-sulfonyl)arginate (Z-Cb-R(Pbf)-OH, 18)

Compound **17** (0.089 g, 0.122 mmol) was dissolved in methanol (3 ml). 1 M NaOH solution (1 ml) was added to this solution and then stirred for 30 min. Methanol was removed by an evaporator. The pH was adjusted to 3 with 0.25 M HCl and stored in a refrigerator overnight. Resultant white oil was washed with ether. White precipitate was collected by centrifugation and dried in vacuum. The desired product was obtained as a white powder (0.043 g, 0.059 mmol, 49.3%). $R_f = 0.075$ (solvent 3), ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.8 (br s 1H), δ 8.5 (d, 1H), δ 7.8 (d, 1H), δ 7.3–7.2 (m, 5H), δ 7.0 (d, 1H), δ 6.8 (br s, 1H), δ 6.5 (br s, 1H), δ 5.2 (s, 2H), δ 4.5 (s, 2H), δ 4.3 (m, 1H), δ 3.0 (s, 2H), δ 2.5 (s, 3H), δ 2.4 (s, 3H), δ 2.0 (s, 3H), δ 1.4 (s, 6H).

4.19. 6′-*N*-[$^{\alpha}$*N*-[[4-*N*-(Benzyloxycarbonyl)cytosine-1-yl]acetyl]-N^G-(2,2,4,6,7-pentamethylene-dihydrobenzofuran-5-sulfonyl)arginyl]neamine (Z-Cb-R(Pbf)-neamine, 19)

Compound **18** (0.043, 0.059 mmol) was dissolved in DMF (2.5 ml). WSC HCl (0.012 g, 0.060 mmol) was dissolved in this solution and then stirred for 2 h in an ice bath. Neamine (0.058 g, 0.18 mmol) was dissolved in ether (3.0 ml) added above DMA solution dropwise, and stirred for 21 h. The reaction mixture was evaporated and dried in vacuum. The crude product was obtained as a white powder (0.028 g, 0.028 mmol, 46.3%).

4.20. Z-Cb-(D)R(Pbf)-Neamine, 19D, and Z-Cb-(L)R(Pbf)-neamine, 19L

14 mg of **19** was dissolved in 10% acetic acid solution. Z-Cb-(L)R(Pbf)-neamine and Z-Cb-(D)R(Pbf)-neamine were isolated by reversed-phase HPLC and then dried in vacuum. Z-Cb-(D)R(Pbf)-neamine, **19D** (6 mg, 5.9 μmol), and Cb-(L)R(Pbf)-neamine, **19L** (3 mg, 3.0 μmol) were obtained as white powder.

4.21. 6′-*N*-[[$^{\alpha}$*N*-(Cytosine-1-yl)acetyl]-D-arginyl]neamine (Cb-(D)R-neamine, 20D)

6.0 mg of **19D** was dissolved in TFA (1.0 ml), and *m*-cresol (0.051 ml, 0.482 mmol), thioanisol (0.046 ml, 0.482 mmol), and TMSOTF (0.076 ml, 0.482 mmol) were added to the solution and then stirred for 3 h. The reaction mixture was poured into diethyl ether and the crude product was obtained as a white powder. This crude product was dissolved in 10% acetic acid solution and purified by reversed-phase HPLC. After removal of the solvent, a desired product was obtained as white powder (4.0 mg, 6.4 μmol). $R_f = 0.013$ (solvent 3), ^{1}H NMR (D_2O, 300 MHz) δ 7.59 (d, 1H), δ 5.59 (d, 1H), δ 5.47 (d, 1H), δ 4.46 (s, 2H), δ 4.09 (t, 1H), δ 3.70 (m, 3 H), δ 3.37–3.06 (m, majority), δ 2.31 (m, 1H), δ 1.64 (m, 2H), δ 1.49 (m, 2H), MALDI-TOF MS, found M+H$^+$ (calcd M+H$^+$), 630.20, (629.68).

4.22. 6′-*N*-[[$^{\alpha}$*N*-(Cytosine-1-yl)acetyl]-L-arginyl]neamine (Cb-(L)R-neamine, 20L)

Cb-(L)R-neamine, **19L** was deprotected and purified in the same manner as Cb-(D)R-neamine, **20D**. The desired product was obtained as a white powder (1.2 mg, 1.9 μmol). $R_f = 0.013$ (solvent 3), ^{1}H NMR (D_2O, 300 MHz) δ 7.60 (d, 1H), δ 6.03 (d, 1H), δ 5.55 (d, 1H), δ 4.48 (s, 2H), δ 4.18 (t, 1H), δ 3.69 (m, 3 H), δ 3.51–3.04 (m, majority), δ 2.30 (m, 1H), δ 1.64 (m, 2H), δ 1.49 (m, 2H), MALDI-TOF MS, found M+H$^+$ (calcd M+H$^+$), 630.22 (629.68).

4.23. *tert*-Butyl$^{\alpha}$*N*-[(guanine-9-yl)acetyl]-$^{\varepsilon}$*N*-(benzyloxycarbonyl)lysinate (Gb-K(Z)-OtBu, 21)

Compound **3** (0.0657 g, 0.314 mmol) was suspended in DMF (2.0 mL). H-Lys(Z)-OtBu was added to this solution and then PyBrop (0.146 g, 0.313 mmol) was added as DMF (1.5 ml) solution. The reaction mixture was stirred in an ice bath for 2 h and in room temperature for 21 h. This solution was poured into cold water (50 ml) and stored in a refrigerator overnight. The precipitate was collected and dried. The product was obtained as a pale yellow powder (0.101 g, 0.192 mmol, 61.1%). $R_f = 0.75$ (solvent 2), ^{1}H NMR H NMR (DMSO-d_6, 300 MHz) δ 10.5 (br s, 1H), δ 8.54 (d, 1H), δ 7.60 (s, 1H), δ 7.36–7.33 (m, 6H), δ 6.42 (s, 2H), δ 5.00 (s, 2H), δ 4.68 (s, 2H), δ 4.12 (m, 1H), δ 3.00 (m, 2H), δ 1.62 (m, 2H), δ 1.39 (s, 9H), δ 1.29 (m, 4H).

4.24. $^{\alpha}$*N*-[(Guanine-9-yl)acetyl]-$^{\varepsilon}$*N*-(benzyloxycarbonyl)lysine (Gb-K(Z)-OH, 22)

Compound **21** was dissolved in acetic acid (1.5 mL) and then cooled in an ice bath. TFA was added to the above solution and stirred for 3.5 h. The reaction mixture was concentrated to about 20% volume of the original and then poured into cold ether. Resultant precipitate was collected and dried. The crude product was obtained as a white powder (0.0799 g, 0.179 mmol). $R_f = 0.41$ (solvent 4).

4.25. 6-*N*-[$^{\alpha}$*N*-[(Guanine-9-yl)acetyl]-$^{\varepsilon}$*N*-(benzyloxycarbonyl)-D-lysinyl]neamine, and 6$^{\partial}$-*N*-[$^{\alpha}$*N*-[(guanine-9-yl)acetyl]-$^{\varepsilon}$*N*-(benzyloxycarbonyl)-L-lysinyl]neamine (Gb-(D)K(Z)-neamine, 23D and Gb-(L)K(Z)-neamine, 23L)

Compound **22** (0.0656 g, 0.147 mmol) was dissolved in DMF (1.0 ml), WSC HCl (0.029 g, 0.151 mml) was added and stirred for 2.5 h. This solution was added to the DMF aqueous solution of neamine (0.0930 g, 0.289 mmol) and stirred for 2 h in an ice bath and another 24 h in room temperature. The solvent of the reaction was evaporated. The crude product was dissolved in 10% acetic acid and then purified by reversed-phase HPLC and dried in vacuum. The desired product was obtained as a white powder. $R_f = 0.375$ (solvent 4), ^{1}H NMR (D_2O, 300 MHz) δ 7.89 (s, 1H), δ 7.19 (m, 5H), δ 5.43 (d, 1H), δ 4.95 (s, 2H), δ 4.75 (s, 2H), δ 4.05 (m, 1H), δ 3.73–3.09 (m, majority), δ 3.00 (m, 2H), δ 2.21 (m, 1H), δ 1.64–1.60 (m, 2H), δ 1.36–1.33 (m, 2H), δ 1.25 (m, 2H).

4.26. Gb-(D)K(Z)-neamine, 23D and Gb-(L)K(Pbf)-neamine, 23L

Compound **23** was dissolved in 10% acetic acid solution. Gb-(D)K(Z)-neamine, **23D** and Gb-(L)K(Pbf)-neamine, **23L** were isolated by reversed-phase HPLC and then dried in vacuum. Gb-(L)K(Pbf)-neamine, **23D** (14 mg, 5.9 μmol) and Gb-(L)K(Pbf)-neamine, **23L** (7 mg, 3.0 μmol) were obtained as white powder.

4.27. 6′*N*-[$^{\alpha}$*N*-[(Guanine-9-yl)acetyl]-L-lysinyl]neamine (Gb-(L)K-neamine, 24L)

Compound **23L** was dissolved in TFA (0.5 ml), and *m*-cresol (0.07 ml, 0.699 mmol), thioanisol (0.095 ml, 0.812 mmol), and trimethylsilyl trifluoromethanesulfonate (TMSOTF, 0.155 ml, 0.803 mmol) were added to this solution and stirred for 30 min in an ice bath and for 2 h in room temperature. The reaction mixture was concentrated to half the original volume and then the solution was poured into cold ether. Precipitate was collected by centrifugation and then dried in vacuum. The crude product (white powder) was dissolved in 10% acetic acid and then purified by reversed-phase HPLC. The desired product was obtained as a white powder (0.017 g, 0.0265 mmol, 100%). $R_f = 0.375$ (solvent 4), ^{1}H NMR (D_2O, 300 MHz) δ 7.72 (s, 1H), δ 5.38 (d, 1H), δ 4.76 (s, 2H), δ 4.03 (m, 1H), δ 3.69–3.01 (m, majority), δ 2.80–2.79 (m, 2H), δ 2.16 (m, 1H), δ 1.60 (m, 2H), δ 1.51 (m, 2H), δ 1.29 (m, 2H), MALDI-TOF MS, found M+H$^+$, M+Na$^+$, M+K$^+$ (calcd M+H$^+$, M+Na$^+$, M+K$^+$) 643.18, 665.23, 683.19 (641.69, 663.67, 679.78).

4.28. 6′*N*-[$^{\alpha}$*N*-[(Guanine-9-yl)acetyl]-D-lysinyl]neamine (Gb-(D)K-neamine, 24D)

Compound **23D** was deprotected and purified by reversed-phase HPLC in the same manner as compound **31L**. The desired product was obtained as a white powder (0.017 g, 0.0265 mml, 100%). $R_f = 0.375$ (solvent 4), ^{1}H NMR (D_2O, 300 MHz) δ 7.95 (s, 1H), δ 4.95 (d, 1H),

δ 4.88 (s, 2H), δ 4.69 (m, 1H), δ 3.72–2.89 (m, majority), δ 2.55 (m, 1H), δ 1.88 (m, 2H), δ 1.76 (m, 2H), δ 1.69 (m, 2H), MALDI-TOF MS, found $M+H^+$, $M+Na^+$, $M+K^+$ (calcd $M+H^+$, $M+Na^+$, $M+K^+$) 642.10, 664.16, 680.17 (641.69, 663.67, 679.78).

4.29. Methyl$^{\alpha}$*N*[(Guanine-9-yl)acetyl]-*N*^{G}-(2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl)arginate (Gb-R(Pbf)-OMe, 25)

Compound **4** (0.093 g, 0.454 mmol) and H-Arg(Pbf)-OMe HCl (0.100 g, 0.227 mmol) were suspended in DMF (1.0 ml), a solution of PyBrop (0.275 g, 0.590 mmol, in DMF 1.0 ml) added at 0 °C. Subsequently, a DMF solution (1.0 ml) of DIEA (0.16 ml, 0.908 mmol) was added slowly and stirred for overnight at room temperature. The reaction mixture was added 20 ml of $CHCl_3$ and 80 ml of water, extracted three times, concentrated, and dried. The product was obtained as a yellow oil. Yield: 0.226 g. $R_f = 0.675$ (solvent 3), ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.54 (br s, 1H), δ 8.68 (d, 1H), δ 7.57 (s, 1H), δ 6.39 (s, 2H), δ 4.67 (s, 2H), δ 3.60 (s, 3H), δ 3.32 (q, 1H), δ 2.93 (s, 2H) 2.50 (s, 3H), δ 2.46 (s, 3H), δ 1.99 (s, 3H), δ 1.39 (s, 6H).

4.30. $^{\alpha}$*N*[(Guanine-9-yl)acetyl]-*N*^{G}-(2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl)arginate (Gb-R(Pbf)-OH, 26)

Compound **25** (0.226 g, 0.357 mmol) was dissolved in methanol (5 ml), 1 M NaOH soln was added to the solution of 12 and stirred for 1 h at room temperature. The pH of the solution was adjusted to 3 by HCl soln and stored in a refrigerator overnight. Resultant precipitate was collected and washed with water, and dried in vacuum in the presence of P_2O_5. Yield: 0.064, 0.103 mmol, 28.9%. $R_f = 0.6$ (solvent 3), ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.58 (br s, 1H), δ 8.49 (d, 1H), δ 7.60 (s, 1H), δ 6.44 (s, 2H), δ 4.68 (s, 2H), δ 3.01 (q, 1H), δ 2.95 (s, 2H) 2.48 (s, 3H), δ 2.42 (s, 3H), δ 2.00 (s, 3H), δ 1.40 (s, 6H).

4.31. 6′-*N*-[$^{\alpha}$*N*](Guanine-9-yl)acetyl]-*N*^{G}-(2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl) arginyl]neamine (Gb-R(Pbf)-neamine, 27)

Compound **26** (0.050 g, 0.081 mmol) was dissolved in DMF (1.5 ml) and cooled to 0 °C, WSC HCl (0.018 g, 0.097 mmol). Neamine (0.078 g, 0.243 mmol) was dissolved in water (1.5 ml), the reaction mixture of compound **13** added slowly and stirred overnight. The reaction mixture was concentrated and dried. The compound **14** (mixture of D and L forms) was obtained as a white powder. This crude product was dissolved in 10% acetic acid solution and purified by HPLC. Gb-(D)R(Pbf)-Neamine. Yield: 10.6 mg, 13.1 μmol, 16%. $R_f = 0.275$ (solvent 4), ^{1}H NMR (D_2O, 300 MHz) δ 7.92 (s, 1H), δ 4.90 (d, 1H), δ 4.77 (s, 2H), δ 3.85 (t, 1H), δ 3.65–2.96 (m, majority), δ 2.86 (sm, 2H), δ 2.35 (s, 3H), δ 2.28 (s, 3H), δ 1.89 (s, 3H), δ 1.28 (s, 6H). Gb-(L)R(Pbf)-Neamine. Yield: 12.1 mg, 15.0 μmol, 19%. $R_f = 0.275$ (solvent 4), ^{1}H NMR (D_2O, 300 MHz) δ 8.10 (s, 1H), δ 5.41 (d, 1H), δ 4.79 (s, 2H), δ 4.05 (t, 1H), δ 3.67–3.02 (m, majority), δ 2.83 (m, 2H), δ 2.33 (s, 3H), δ 2.27 (s, 3H), δ 1.87 (s, 3H), δ 1.26 (s, 6H).

4.32. 6′-*N*-[$^{\alpha}$*N*](Guanine-9-yl)acetyl]-D- or L-arginyl]neamine (Gb-(D)R-neamine, 28D and Gb-(L)R-neamine, 28L)

Compound **27** (10.6 mg, 13.1 μmol) was dissolved in TFA (0.8 ml), cooled to 0 °C, and stirred for 2 h. The reaction mixture was poured into cold diethyl ether. The precipitated was collected by centrifuge and washed with diethyl ether 7 times. The crude compound was dissolved in 10% acetic acid solution and purified by HPLC. Gb-(D)R-Neamine, **28D**. Yield: 6.9 mg, 10.3 mmol, 79.2%, ^{1}H NMR (D_2O, 300 MHz) δ 7.81 (s, 1H), δ 4.88 (d, 1H), δ 4.82 (s, 2H), δ 3.94 (t, 1H), δ 3.74–2.88 (m, majority), δ 2.22 (m, 1H), δ 1.73–1.59 (m, 2H), MALDI-TOF MS, found $M+H^+$, $M+Na^+$, $M+K^+$ (calcd $M+H^+$, $M+Na^+$,$M+K^+$), 670.1, 692.2, 708.2 (669.9, 692.9, 708.9).

Gb-(L)R-Neamine, **30L** was obtained in the same manner as Gb-(D)R-neamine. Yield: 6.0 mg, 9.0 μmol, 69.2% from 13, ^{1}H NMR (D_2O, 300 MHz) δ 7.87 (s, 1H), δ 5.49 (d, 1H), δ 4.85 (s, 2H), δ 4.16 (t, 1H), δ 3.79–3.08 (m, majority), δ 2.27 (m, 1H), δ 1.70–1.57 (m, 2H), MALDI-TOF MS, found $M+H^+$, $M+Na^+$ (calcd $M+H^+$, $M+Na^+$), 670.1, 692.0 (669.9, 692.9).

4.33. *tert*-Butyl $^{\alpha}$*N*[(thymine-1-yl)acetyl]-$^{\varepsilon}$*N*-(benzyloxycarbonyl) lysinate (Tb-K(Cbz)-OH, 29)

Thymine acetic acid, **4** (0.182 g, 0.986 mmol) and H-Lys(Cbz)-OtBU HCl (0.242 g, 0.655 mmol) were dissolved in DMF (6.0 ml). This solution was cooled to 0 °C, then a DMF solution of PyBrop (0.362 g, 0.777 mmol) and a DIEA (0.57 ml, 3.26 mmol) was added and stirred for 2 h at 0 °C and another 21 h at room temperature. The reaction mixture was poured into water (200 ml) and stored in a refrigerator overnight. Resultant precipitate was collected by centrifugation and dried in vacuum. The product was obtained as a white powder, 0.196 g, 0.390 mmol, 59.7%. $R_f = 0.83$ (solvent 2), ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.25 (s 1H), δ 8.48 (d, 1H), δ 7.42 (s, 1H), δ 7.35 (m, 6H), δ 5.00 (s 2H), δ 4.33 (s, 2H), δ 4.09 (m, 1H), δ 2.98 (m, 2H), δ 1.74 (s 3H), δ 1.62 (m, 2H), δ 1.39 (s, 9H), δ 1.73 (s, 3H), δ 1.31 (m, 4H).

4.34. $^{\alpha}$*N*[(Thymine-1-yl)acetyl]-$^{\varepsilon}$*N*-(benzyloxycarbonyl)lysinate (Tb-K(Cbz)-OH, 30)

Compound **29** (0.090 g, 0.179 mmol) was dissolved in dichloromethane (1.5 ml) and TFA (1.5 ml) was added and stirred for 30 min. at 0 °C and 3 h at room temperature. The reaction mixture was concentrated. The crude product was crystallized from cold ethanol. White precipitate was collected and dried. Yield 0.079 g, 0.178 mmol, 99.2%. $R_f = 0.54$ (solvent 4).

4.35. [$^{\alpha}$*N*][(Thymine-1-yl)acetyl]-$^{\varepsilon}$*N*-(benzyloxycarbonyl)lysinyl]eamine (Tb-(D,L)K(Cbz)-neamine, 31)

Compound **30** (0.072 g, 0.160 mmol) was dissolved in DMF (1.0 ml) and WSC HCl (0.039 g, 0.204 mmol) was added at 0 °C and stirred for 2 h. Neamine (0.0853 g, 0.264 mmol) was dissolved in water (1.0 ml) and DMF (0.5 ml). The reaction mixture of **33** was added to the neamine solution and stirred for 24 h. All the solvent was removed and then residue was dissolved in 10% acetic acid and purified by HPLC. The desired product was obtained as a white powder. Yield: 0.044 g, 0.058 mmol, 36.4%. $R_f = 0.55$ (solvent 4), ^{1}H NMR (D_2O, 300 MHz) δ 7.23 (m, 18H), δ 75.51 (d, 2H), δ 5.29 (d, 1H), δ 4.92 (s 6H), δ 4.29 (m, 4H), δ 4.07 (m, 2H), δ 3.92 (m, 2H), δ 3.72–3.13 (m, majority), δ 2.94 (m 6H), δ 2.56 (m, 3H), δ 1.70 (s, 3H), δ 1.68 (s, 6H), δ 1.62 (m, 6H), δ 1.26 (m, 12H).

4.36. 6′-*N*-[[$^{\alpha}$*N*][(Thymine-1-yl)acetyl]-D-, and L-lysinyl]neamine (Tb-(D and L)K-neamine, 32)

Compound **31** (0.0343 g, 0.0573 mmol) was dissolved in TFA (1.0 ml), *m*-cresol (0.18 ml, 1.72 mmol), thioanisol (0.201 ml, 1.72 mmol), and TMSOTf (0.332 ml, 1.72 mmol) were added to the solution and stirred for 30 min at 0 °C and another 2 h at room temperature. The reaction mixture was concentrated and poured into diethyl ether (10 ml), the white precipitate was collected and dried. The white powder was dissolved in 10% acetic acid solution and purified by reversed-phase HPLC. The desired product was obtained as a white powder. Yield: 0.09 g, 0.014 mmol, 24.6%. $R_f = 0.05$ (solvent 4), ^{1}H NMR (D_2O, 300 MHz) δ 7.31 (s, 1H), δ 7.24 (s, 2H), δ 5.53 (d, 2H), δ 5.31 (d 1H), δ 4.36 (m, 6H), δ 4.12 (m, 2H), δ 4.00 (m, 1H), δ 3.740–3.15 (m, majority), δ 2.81 (m 6H), δ 2.27 (m, 3H), δ 1.70 (s, 9H), δ 1.58 (m, 12H), δ 1.28 (m, 6H), MALDI-TOF MS, found $M+H^+$, $M+Na^+$, $M+K^+$ (calcd $M+H^+$, $M+Na^+$, $M+K^+$) 617.26, 639.27, 655.22 (616.68, 638.66, 654.77).

4.37. Methyl$^{\alpha}$*N*][(thymine-1-yl)acetyl]-$^{\varepsilon}$*N*-(2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl)arginate (Tb-R(Pbf)-OMe, 33)

Thymine acetic acid, **4** (0.233 g, 1.21 mmol) and H-Arg(Pbf)-OMe HCl (0.214 g, 0.485 mmol) were dissolved in DMF (4 ml). This solution was cooled to 0 °C, then a DMF solution of PyBrop (0.735 g, 1.58 mmol) and a DIEA (0.52 ml, 2.91 mmol) was added and stirred for 25 h. Water (180 ml) was added to the reaction mixture. Resultant precipitate was collected by centrifugation, yielding a crude product 0.427 g, 0.704 mmol, 58.1%.

4.38. $^{\alpha}$*N*][(Thymine-1-yl)acetyl]-$^{\varepsilon}$*N*-(2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl)arginate (Tb-R(Pbf)-OH, 34)

A MeOH (1.5 ml) solution of **33** (0.427 g, 0.704 mmol) was added 1 M NaOH soln (1.5 ml) and stirred for 30 min. After MeOH was removed by evaporation, pH of the reaction mixture was adjusted to 3 by HCl solution and stored in a refrigerator overnight. White precipitate was collected and dried, washed with Et_2O, and dried again. Yield 0.249 g, 0.421 mmol, 59.8%. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.25 (s 1H), δ 8.49 (d, 1H), δ 7.40 (s, 1H), δ 6.83 (br s, 1H), δ 6.42 (br s 1H), δ 4.31 (s, 2H), δ 4.15 (br s, 1H), δ 3.00 (s, 3H), δ 2.51 (s 3H), δ 2.42 (s, 3H), δ 2.00 (s, 3H), δ 1 .73 (s, 3H), δ 1.41 (s, 6H).

4.39. 6′-*N*-[[$^{\alpha}$*N*][(Thymine-1-yl)acetyl]-$^{\varepsilon}$*N*-(2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl)arginyl]neamine (Tb-R(Pbf)-neamine, 35)

Compound **34** (0.088 g, 0.148 mmol) was dissolved in DMF (2.5 ml) and WSC HCl(0.29 g, 0.150 mmol) was added at 0 °C and stirred for 3 h. Neamine (0.145 g, 0.450 mmol) was dissolved in water (3.0 ml) and DMF (3.0 ml) was added. This neamine solution was added to the solution of Tb-R(Pbf)-OH and stirred for 20 h. All the solvent was removed by evaporation, residue was dissolved in 10% acetic acid and then purified by HPLC. The desired product was obtained as a white powder. Yield: 0.078 g, 0.086 mmol, 58.3%. ^{1}H NMR (D_2O, 300 MHz) δ 7.49 (s, 1H), δ 7.41 (s, 1H), δ 5.70 (d, 1H), δ 5.51 (br s 1H), δ 4.53 (m, 4H), δ 4.28 (t, 1H), δ 4.16 (t, 1H), δ 3.86–3.20 (m, majority), δ 3.08 (s 4H), δ 2.56 (s, 6H), δ 2.49 (s, 6H), δ 2.10 (s, 6H), δ 1.89 (s, 6H), raceme product (D/L = 1:1).

4.40. 6′-*N*-[[$^{\alpha}$*N*][(Thymine-1-yl)acetyl]-D- and L-arginyl]neamine (Tb-(D and L)R-neamine, 36)

Compound **35** (0.033 g, 0.0368 mmol) was dissolved in TFA (1 ml) and stirred for 3 h. TFA was removed by evaporation, dissolved in water (2 ml), and then purified by HPLC. The desired product was obtained as a white powder. Yield: 0.019 g, 0.029 mmol, 79.0%. ^{1}H NMR (D_2O, 300 MHz) δ 7.50 (s, 1H), δ 7.43 (s, 1H), δ 5.69 (d, 1H), δ 5.50 (br s 1H), δ 4.55 (m, 4H), δ 4.33 (t, 1H), δ 4.21 (t, 1H), δ 3.90–3.23 (m, majority), δ 2.44 (m 1H), δ 1.90–δ 1.68 (m, 12H), MALDI-TOF MS, found $M+H^+$, $M+Na^+$ (calcd $M+H^+$, $M+Na^+$) 645.23, 666.20 (644.69, 666.6).

Acknowledgments

This study was supported by Saneyoshi Scholarship Foundation. The authors thank Dr. Koutarou Goto (The Noguchi Institute) for measuring MALDI-TOF MS.

References and notes

1. Vashinov, Y. N.; Wong-Staal, F. *Annu. Rev. Biochem.* **1991**, *60*, 577–630.
2. Noller, H. F. *Ann. Rev. Biochem.* **1991**, *60*, 191–227.
3. Zapp, M. L.; Stern, S.; Green, M. R. *Cell* **1993**, *74*, 969–978.
4. Purofit, P.; Stern, S. *Nature* **1994**, *370*, 659–662.
5. Ma, C.; Baker, N. A.; Joseph, S.; McCammon, J. A. *J. Am. Chem. Soc.* **2002**, *124*, 1438–1442.

6. Yokogawa, S.; Fourmy, D.; Paglisi, J. D. *EMBO J.* **1998**, *17*, 6437–6448.
7. Mei, H.-Y.; Cui, M.; Heldsinger, A.; Lemrow, S. M.; Loo, J. A.; Sannes-Lowrey, K. A.; Sharmeen, L.; Czarnik, A. W. *Biochemistry* **1998**, *37*, 14204–14212.
8. Faber, C.; Sticht, H.; Schweimer, S.; Rosch, P. *J. Biol. Chem.* **2000**, *275*, 20660–20666.
9. Wang, S.; Huber, P. W.; Cui, M.; Czarnik, A. W.; Mei, H.-Y. *Biochemistry* **1998**, *37*, 5549–5557.
10. Fourmy, D.; Recht, M. I.; Blanchard, S. C.; Puglisi, J. D. *Science* **1996**, *274*, 1367–1371.
11. Blount, K. F.; Tor, Y. *Nucleic Acid Res.* **2003**, *31*, 4590–5500.
12. Hamasaki, K.; Woo, M.-C.; Ueno, A. *Tetrahedron Lett.* **2000**, *41*, 8327–8332.
13. Riguet, E.; Desire, J.; Bailly, C.; Decout, J.-L. *Tetrahedron* **2004**, *60*, 8053–8064.
14. Ding, Y.; Hofstader, S. A.; Swayze, E.; Risen, L.; Griffey, R. H. *Angew. Chem. Int. Ed.* **2003**, *42*, 3409–3412.
15. Lee, J.; Kwon, M.; Lee, K. H.; Jeong, S.; Hyn, S.; Shin, K. J.; Yu, J. *J. Am. Chem. Soc.* **2004**, *126*, 1956–1957.
16. Agnelli, F.; Sucheck, S. J.; Marby, K. A.; Rabuka, D.; Yao, S.-L.; Sers, P. S.; Ling, F.-S.; Wong, C.-H. *Angew. Chem. Int. Ed.* **2004**, *43*, 1562–1566.
17. Park, W. C. K.; Auer, M.; Jaksche, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 10150–10155.
18. Hamasaki, K.; Ueno, A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 591–594.
19. Dueholm, K. L.; Egholm, M.; Behrens, C.; Christensen, L.; Hansen, H. F.; Vulpius, T.; Petersen, K. H.; Berg, R. H.; Nielsen, P. E.; Buchardt, O. *J. Org. Chem.* **1994**, *59*, 5767–5773.
20. Matsumoto, C.; Hamasaki, K.; Mihara, H.; Ueno, A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1857–1861.
21. Calnan, B. L.; Tider, B.; Biancalana, S.; Hudson, D.; Frankel, A. D. *Science* **1991**, *252*, 1167–1171.
22. Puglisi, D. J.; Tan, R.; Calnan, B. J.; Frankel, A. D.; Williamson, J. R. *Science* **1992**, *257*, 76–80.
23. Puglisi, D. J.; Lily Chen, L.; Frankel, A. D.; Williamson, J. R. *Proc. Natl. Acad Sci. U.S.A.* **1993**, *90*, 3680–3684.