Peptide Ribonucleic Acids (PRNA). 2. A Novel Strategy for Active Control of DNA Recognition through Borate Ester Formation

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Received October 4, 1999

Abstract: The effect of adding borax and boric acids on the nucleobase orientation and recognition behavior of novel mono- and oligomeric peptide ribonucleic acids (PRNAs) has been investigated. The base orientation of 5′-amino-5′-deoxyuridine and 5′-amino-5′-deoxycytidine was shown by CD and NOE difference spectral studies to switch from anti to syn in borate buffer or upon addition of borax. The origin of this phenomenon is elucidated to be the cooperative effect of the cyclic borate esterification of the sugar's *cis*-2′,3′-diol and the hydrogen-bonding interaction between the sugar's 5′-amino proton and the base's 2-carbonyl oxygen. Because this new strategy for switching the base orientation through the addition of borate is potentially applicable to the recognition control of nucleic acids if the sugar's 5′-proton and *cis*-2′,3′-diol remain unmodified, we synthesized a series of PRNAs, in which the 5′-amino-5′-deoxypyrimidine ribonucleoside moiety was appended to a mono- or oligo(γ-L-glutamic acid) backbone through the 5′-amino group. The orientation switching through the addition of borate was also confirmed with the monomeric model, and the switching efficiency was enhanced for oligomeric γ-PRNA. Finally, it was unambiguously demonstrated that the γ-PRNA 8-mer with an isopoly(L-glutamic acid) backbone can form a tight complex with DNA, and further, the recognition of DNA with γ-PRNA 8-mer is controlled by the borate added as an external factor.

Introduction

To elucidate and mimic the versatile functions of DNA and RNA, a wide variety of nucleic acid model compounds which show sequence-specific recognition/binding to mRNA and dsDNA have been designed.[1] Several models of both normal and antisense nucleic acids have been proposed, not only to improve the stability of the oligomers and hybrids in the presence of nuclease but also to enhance the hybridization affinity,[2] including phosphodiesters,[3] phosphorothioate,[4] alkylphosphonate,[5] and phosphoramidate.[6] Even though it is difficult in general to control the stereogenic center of the phosphorus in the preparation of these model compounds,[7] its stereochemistry is known to significantly influence the hybridization efficiency.[8] An inherent, crucial drawback of these model systems is the lack of a direct means to actively control the function of these nucleic acids.

It is well-documented that the recognition/binding abilities of nucleic acids and their analogues are critically affected by their conformational variations,[9] especially through the syn−anti orientational change of the nucleic acid base moiety. Certainly, the anti orientation of the nucleobase is an essential factor for efficient recognition, since syn-orientated nucleobases are unfavorable for forming intermolecular hydrogen bonds with the complementary base. Thus, if nucleobase orientation could be switched by an additive, the external control of recognition behavior can be readily applied. Unfortunately, no external agent or factor, which affects the syn−anti orientation and therefore the recognition behavior of nucleic acids and analogues has been found or proposed to date, although the unusual syn orientation is known to be induced by several internal factors, such as increased steric hindrance and altered sugar puckering.[10,11]

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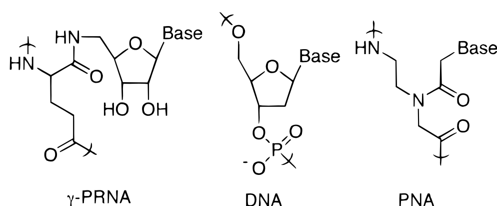
10.1021/ja9935456 CCC: $19.00 © 2000 American Chemical Society
Published on Web 07/06/2000

Chart 1



Indeed, bulky substituents introduced at the 6-position of the pyrimidine base induce the syn orientation in the pyrimidine nucleosides,[10] while ketalization of the *cis*-2′,3′-diol of uridine (**1**) enhances the syn/anti ratio of the resulting 2′,3′-*c*UMP or 2′,3′-*O*-isopropylidene uridine through the imposed 2′,3′-planar-$O_{4'}$-*exo*-furanose structure.[12] However, the energetically disfavored syn orientation has not been induced by external agents.

It is also well-known that boric acids form cyclic esters with a variety of *cis*-1,2-diols, including sugars and ribonucleosides, and this esterification process is reversible in aqueous solutions at moderate pH.[13] Recently, we have reported that the nucleobase orientation of 5′-amino-5′-deoxyuridine (**2**) is switched from anti to syn simply by using borate buffer in place of conventional phosphate buffer, while the parent uridine **1** does not undergo such an orientational change in borate buffer, as determined by circular dichroism (CD) spectroscopy.[14] This unprecedented orientation-switching phenomenon is expected to provide us with a unique methodology for externally controlling nucleic acid recognition, if a nucleic acid analogue, in which these functionalities are carrying a ribose unit with free 2′,3′-diol and 5′-amino/amide proton, can be prepared, since both are indispensable to the orientation-switching process.[14] These structural and functional requirements are not satisfied through conventional modifications of RNA/DNA or peptide nucleic acids (PNA). For this purpose, we wish to propose a new category of nucleic acid analogues, that is, peptide ribonucleic acids (PRNA), in which the ribonucleoside units are not directly incorporated into the main chain, as is the case with RNA, but are instead attached to the peptide backbone as pendant groups. In a previous paper,[15] we have demonstrated that this strategy works well with an α-PRNA which carries 5′-amino-5′-deoxyuridine units appended to poly(L-glutamic acid). However, the efficiency of external control was not very high as a result of the mismatched repetition distance of the nucleobases. In the present study, 5′-amino-5′-deoxyuridine (**2**) and 5′-amino-5′-deoxycytidine (**3**) units are tethered to the isopoly(L-glutamic acid) backbone at the remaining α-carboxyl group of glutamic acid through the 5′-amino group of **2** and **3** (γ-PRNA, Chart 1), thus reserving the ribose *cis*-2′,3′-diol for cyclic borate formation as well as the 5′-amide proton for hydrogen bonding with the pyrimidine nucleobase 2-carbonyl. In the γ-PRNA molecule, the nucleobases are located at the correct position for RNA/DNA recognition, and the intervening ribose unit is expected to greatly improve the poor solubility of the PNA and its RNA/DNA complex in aqueous solution.

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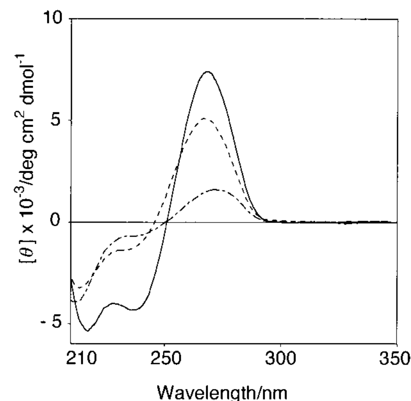

Figure 1. CD spectra of **1** in borate buffer (0.1 M KH_2PO_4 − 0.05 M $Na_2B_4O_7$, pH 7.2; solid line) and **2** in phosphate buffer (0.033 M KH_2PO_4 − 0.033 M Na_2HPO_4, pH 7.2; dashed line) and borate buffer (chain line).

Results and Discussion

5′-Amino-5′-deoxyuridine (2) and 5′-Amino-5′-deoxycytidine (3) as Essential Units. In the recognition process involving DNA/RNA, the base orientation of nucleoside attached to the glycosyl group plays a major role, where an anti orientation is essential for base recognition. Hence, if the syn−anti orientation of nucleoside analogues can be switched by an external factor, this will be a convenient and powerful tool for control of nucleic acid recognition.

Pyrimidine nucleosides are known to favor an anti nucleobase orientation in solution phase.[16] One plausible strategy to induce the unfavorable syn orientation is to utilize the cooperative effects of a hydrogen-bonding interaction between 2-carbonyl and 5′-hydroxyl groups and a bridging substitution of the *cis*-2′,3′-diol. The use of the 5′-hydroxyl proton, which is inevitably lost in conventional nucleotides and nucleic acid model compounds, is unrealistic as a candidate for the possible hydrogen-bonding interaction with 2-carbonyl. We therefore substituted the 5′-hydroxyl group of uridine with an amino group in this study, and elucidated the orientational change and its effect in the resultant 5′-amino-5′-deoxyuridine in the presence/absence of borate, which was added as an external switching agent.

A new route to the selective transformation of uridine to 5′-amino-5′-deoxyuridine (**2**) was also developed, which is an extension of the conventional synthetic procedure employed in the synthesis of 2′-amino-2′-deoxyuridine starting from 2,2′-cyclouridine.[17]

5′-Amino-5′-deoxycytidine (**3**) was synthesized from cytidine (**4**) through the reduction and deprotection of 5′-azido-5′-deoxy-N^4-benzoylcytidine.[18]

CD Spectral Study on Monomers. CD spectroscopy has been used to evaluate the syn-anti orientation of nucleobases.[14] Hence, the CD spectra of uridine (**1**) and 5′-amino-5′-deoxyuridine (**2**) were measured in phosphate and borate buffer solutions at pH 7.2 in order to examine the effects of borate buffer and the amino substitution. As can be seen from the CD spectra shown in Figure 1, the $[\theta]_{max}$ value of **1** decreased from 9700 deg cm^2 $dmol^{-1}$ in phosphate buffer to 7400 deg cm^2 $dmol^{-1}$ (76% of the original value) in a borate buffer. This value, which is similar to that observed for 2′,3′-*O*-isopropylidene uridine in phosphate buffer (6000 deg cm^2 $dmol^{-1}$),[14] suggests that the

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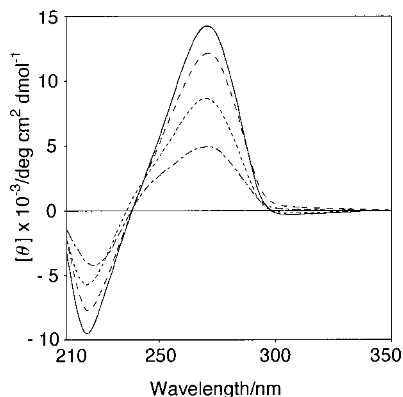

Figure 2. CD spectra of **2** in phosphate buffer (0.033 M KH_2PO_4 − 0.033 M Na_2HPO_4, pH 7; solid line) and borate buffer (0.1 M KH_2PO_4 − 0.05 M $Na_2B_4O_7$, pH 7.2; dashed line) and **3** in phosphate buffer (dotted line) and borate buffer (chain line).

borate added to the solution appreciably induces the syn orientation through ester formation, although this effect is moderate. Conversely, 5′-amino-5′-deoxyuridine (**2**) gave a smaller $[\theta]_{max}$ value (5100 deg cm^2 dmol^{-1}, 53% of that for **1** in phosphate buffer) than that observed for **1** even in a phosphate buffer (9700 deg cm^2 dmol^{-1}), probably as a result of an intramolecular hydrogen-bonding interaction. Interestingly, the use of a borate buffer led to a further decrease of the $[\theta]_{max}$ value to 1600 deg cm^2 dmol^{-1} (16% of that for **1** in phosphate buffer) which is almost comparable to the value observed for 5′-amino-5′-deoxy-2′,3′-*O*-isopropylidene uridine (1200 deg cm^2 dmol^{-1}) in a phosphate buffer.[14]

A similar tendency was observed for cytidine **4** and 5′-amino-5′-deoxycytidine (**3**) (Figure 2). When the solvent was changed from phosphate to borate buffer, the $[\theta]_{max}$ value of **4** was reduced from 14200 to 12200 deg cm^2 dmol^{-1} (86% of the original value). This suggests that the syn/anti ratio was increased by borate ester formation, although the effect is moderate. In the case of 5′-amino-5′-deoxycytidine (**3**), the $[\theta]_{max}$ value was 8400 deg cm^2 dmol^{-1} in phosphate buffer, which was smaller than that for **4** in phosphate buffer, probably as a result of the intramolecular hydrogen bonding interaction. In borate buffer, the $[\theta]_{max}$ value of **3** was further decreased to 4900 deg cm^2 dmol^{-1} (39% of that for **4** in phosphate buffer) as a consequence of the combined effect of the borate ester formation and the intramolecular hydrogen bonding interaction. This demonstrates that the simultaneous use of the 5′-amino substitution and the borate buffer is quite effective in inducing a syn orientation in both uridine and cytidine derivatives.

Nuclear Overhauser Effect. The unique borate-driven orientation switching of nucleobase from anti to syn was further confirmed by NMR NOE measurements made on **2**. Figure 3 shows the ^{1}H NMR and NOE difference spectra of **2** in (a) phosphate and (b) borate buffers. It is noted that the ^{1}H NMR spectrum in borate buffer is similar to that of 2′,3′-*O*-isopropylideneuridine,[12] and the spin coupling constants for **2** of $J_{2',3'}$ (6.8 Hz) and $J_{1',2'}$ and $J_{3',4'}$ (8.2 Hz) in borate buffer are consistent with those of 2′,3′-*c*UMP.[19] This indicates that in borate buffer the sugar puckering of **2** is quite similar to that of 2′,3′-*c*UMP. In the NOE spectra in phosphate buffer, where uracil's H6 at δ 7.6 is presaturated, uracil's H5 and/or furanose's H1′ at δ 5.8 (26.7%), H2′ at δ 4.4 (7.9%), H3′ at δ 4.1 (2.7%), and H5′ at δ 3.0 (2.0%) gave notable NOE peaks, as shown in Figure 3a. This NOE profile is entirely consistent with the anti orientation of **2**, shown in Figure 3 (top right).

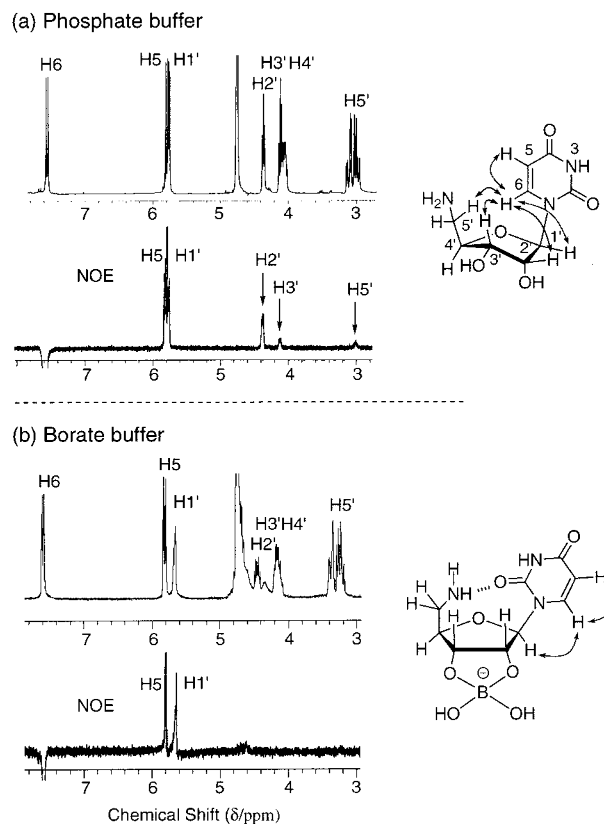

Figure 3. ^{1}H NMR and NOE differential spectra (600 MHz) of **2** obtained with presaturation at H6 (δ_H 7.6) in (a) phosphate buffer (0.033 M KH_2PO_4 − 0.033 M Na_2HPO_4, pH 7.2) and (b) borate buffer (0.1 M KH_2PO_4 − 0.05 M $Na_2B_4O_7$, pH 7.2).

In borate buffer, **2** afforded a completely different NOE spectrum. As shown in Figure 3b, the presaturation of uracil's H6 gave NOE signals only with uracil's H5 (14.5%) and furanose's H1′ (12.5%). This unique NOE profile, in addition to the decreased CD intensity discussed above, provides evidence in support of the predominant contribution of the syn orientation for **2** in borate buffer, which is driven by the borate ester formation, as illustrated in Figure 3 (bottom right).

In the case of 5′-amino-5′-deoxycytidine (**3**), the syn orientation induced by borate ester formation was unambiguously confirmed by the NOE difference spectra obtained in similar NMR measurements in phosphate and borate buffers at pH 7.2. In addition to the chemical shift changes and the varied coupling constants which are characteristic of the 2′,3′-planar puckering of the sugar, the NOE difference spectra provide good evidence in support of the syn orientation of the pyrimidine base of **3** in borate buffer. Measurements made in phosphate and borate buffers afforded distinctly different NOE difference spectra upon presaturation of the cytosine's H6. In phosphate buffer, several significant NOE peaks were observed for the cytosine's H5 at δ 6.0 (15.2%) and/or furanose's H1′ at δ 5.7 (12.1%), H2′ at δ 4.3 (7.2%), H3′ at δ 4.1 (2.8%), and H5′ at δ 3.0 (1.9%). These NOE peaks are compatible with the anti-orientated pyrimidine base in **3**. In contrast, in borate buffer, only the cytosine's H5 (7.9%) and furanose's H1′ (12.8%) gave moderate NOE peaks upon irradiation of cytosine's H6. This characteristic NOE profile, as well as the decreased $[\theta]_{max}$ value of CD spectrum observed in borate buffer, demonstrate that the syn orientation of **3** is efficiently induced by the cooperation of the 2′,3′-cyclic

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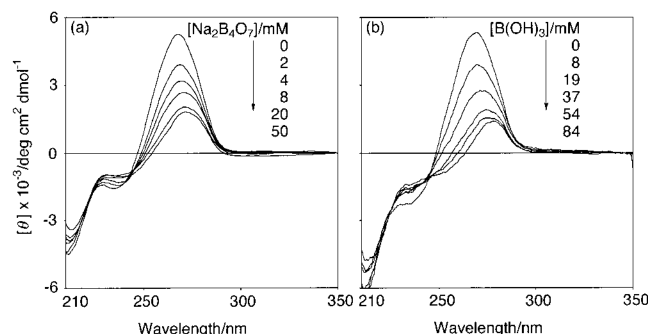

Figure 4. CD spectra changes of **2** with increasing concentrations of (a) $Na_2B_4O_7$ and (b) $B(OH)_3$ in phosphate buffer (0.033 M KH_2PO_4 − 0.033 M Na_2HPO_4, pH 7.2); (a) [**1**] = 1.0×10^{-4} M, (b) [**1**] = 7.1×10^{-5} M.

esterification and the hydrogen bonding interaction between the 5′-amino and 2-carbonyl groups.

Effects of Borate Ester Formation upon Orientation of 5′-Amino-5′-deoxyuridine (2). In agreement with the observation that **2** displays distinctly different CD spectra in phosphate and borate buffers, the gradual addition of borax to the phosphate buffer solution of **2** (pH 7.2) induced large CD spectral changes, as shown in Figure 4a. Isosbestic points that are observed at ∼220 and 240 nm indicate that no multistep processes or intermediates are involved but that a single step is responsible for these changes. The molar ellipticity at the peak around 270 nm ($[\theta]_{max}$) was substantially reduced from 5200 to 1600 deg cm^2 $dmol^{-1}$ as the borax concentration was increased from 0 to 50 mM. The final $[\theta]_{max}$ value of 1600 deg cm^2 $dmol^{-1}$ is close to that (1400 deg cm^2 $dmol^{-1}$) observed for 5′-amino-5′-deoxy-2′,3′-*O*-isopropylidene uridine under the same conditions.[14] This confirms that the addition of borax enhances the syn/anti ratio of nucleobase in **2** through the reversible formation of a borate ester with the *cis*-2′,3′-diol of ribose. A quantitative analysis of the CD spectral changes, using the nonlinear least-squares fit to the curve for 1:1 stoichiometric complexation, gave the equilibrium constant of 75 M^{-1} for the reversible esterification of **2** with borax. This value is well within the typical range ($10-10^4$ M^{-1}) of the equilibrium constant for borate ester formation with *cis*-1,2-diols in aqueous solutions around pH 7.[13b,20]

Analogous CD spectral changes were observed upon addition of boric acid of up to 84 mM concentration to a buffer solution at pH 7.2 containing **2**, again with accompanying isosbestic points at the same wavelengths (Figure 4b). An identical final $[\theta]_{max}$ value of 1400 deg cm^2 $dmol^{-1}$ was obtained for 84 mM boric acid, indicating a high syn content, induced by borate ester formation. An equilibrium constant of 49 M^{-1} was calculated for boric acid, and methylboric acid gave a similar equilibrium constant of 52 M^{-1} in a buffer solution at pH 8.3. The addition of phenylboric acid of up to 6 mM induced comparable CD spectral changes at 270 nm, indicating a more efficient ester formation, although the absorption of this reagent in the same wavelength region could not permit us to make a quantitative analysis of these results.

Peptide Ribonucleic Acid (PRNA) Monomer (5). The above methodology, employing the reversible borate ester formation to switch the orientation of the nucleobase, is interesting and potentially useful. However, it is obvious that this strategy cannot immediately be applied to the external control of the recognition and complex formation of natural nucleic acids, since they do not possess 5′-amino or free 3′-hydroxyl groups on the furanose unit, both of which are the prerequisites for this borate-mediated switch. Nevertheless, the switching phenomenon observed for **2** encouraged us to explore the scope and limitations of this new methodology in the control of the recognition behavior of nucleic acids and their analogues.

To satisfy the structural requirements described above, we decided to synthesize a series of peptide ribonucleic acids (PRNAs). In the present study, the 5′-amino-5′-deoxyuridine (**2**) unit is tethered to an isopoly(L-glutamic acid) backbone at the remaining α-carboxylic group of glutamic acid through the 5′-amino group of **2**, thus reserving the *cis*-2′,3′-diol of ribose for cyclic borate formation, as well as leaving the 5′-amide proton free to hydrogen bond with the pyrimidine 2-carbonyl. In addition to the recognition switching ability, PRNAs may possess several advantages over a conventional peptide nucleic acid (PNA),[1a,b] such as a flexible side chain, which has built-in chirality, a structural similarity to native RNA, and an improved water solubility.

Before considering a polymeric system, we examined whether the same switching strategy would work well with a monomer model of PRNA. For this purpose, we synthesized the protected monomer **5** by reacting **2** with *N*- and γ-*C*-protected L-glutamic acid in 88% yield, using benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent), as shown in Scheme 1.[21] The benzyl ester of **5** was then deprotected to give the PRNA monomer (**6**) (Scheme 3), in which the 5′-amino proton was replaced by a more acidic amide proton located at a position suitable for hydrogen bonding to the uracil's 2-carbonyl. To confirm the possible orientation switching induced by borate, CD and NMR spectral studies were performed with **6**.

The CD spectra of **6** were recorded in borate and phosphate buffers under comparable conditions, but gave distinctly different molar ellipticities at the CD maxima at ∼270 nm, as shown in Figure 5. Thus, the $[\theta]_{max}$ value obtained in borate buffer was 5000 deg cm^2 $dmol^{-1}$, which was significantly smaller than the value of 6500 deg cm^2 $dmol^{-1}$ obtained in phosphate buffer. This CD spectral behavior is similar to, but less intense than, that observed for **2** (Figure 1). These observations suggest that the borate-induced anti-to-syn orientational change occurs in the PRNA monomer **6**, but to a lesser extent.

The orientation switching of **6** by borate was further verified by NOE difference spectra recorded in (a) phosphate and (b) borate buffers (Figure 6). In phosphate buffer, presaturation of uracil's H6 at δ 7.62 led to significant NOE peaks for uracil's H5 at δ 5.86 (19.7%) and furanose's H1′ at δ 5.78 (5.7%), H2′ at δ 4.30 (8%) and H3′ at δ 4.08 (1%), verifying the anti orientation of the base. In contrast, NOE enhancement was observed only for H5 at δ 5.86 (10.5%) and H1′ at δ 5.76 (4.5%) in borate buffer upon presaturation of H6 at δ 7.61, which is consistent with a syn orientation. It is interesting to note that small NOE peaks (<1% enhancement) at δ 4.1 and 4.3, attributable to H3′ and H2′, are also observed for **6** in borate buffer, although no such NOE peaks are observed for **2** under the same conditions. These small NOE peaks, in conjunction with the above-mentioned smaller CD spectral change observed in borate buffer for **6** than formation for **2**, indicate a less-effective cooperation of the borate ester formation and the hydrogen bonding interaction in the uridine-based **6**. This lower

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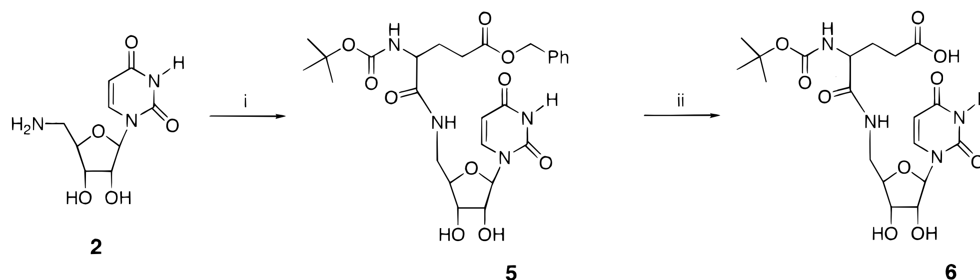
Scheme 1[a]



[a] *Reagents and conditions*: i, Boc-Glu(OBzl), BOP reagent, HOBt, $(i\text{-Pr})_2NEt$, DMF, rt, 1 h; ii, H_2, Pd/C(10%), MeOH, rt, 2 h

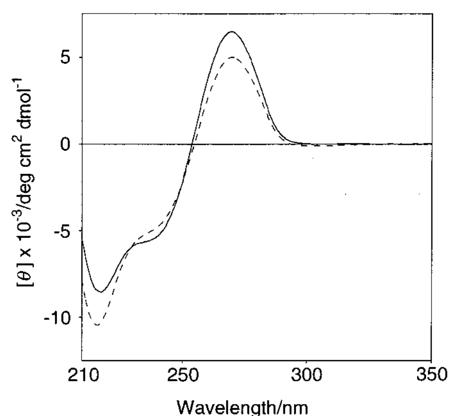


Figure 5. CD spectra of **6** in phosphate buffer (0.033 M KH_2PO_4 − 0.033 M Na_2HPO_4, pH 7.2; solid line) and borate buffer (0.1 M KH_2PO_4 − 0.05 M $Na_2B_4O_7$, pH 7.2; dashed line).

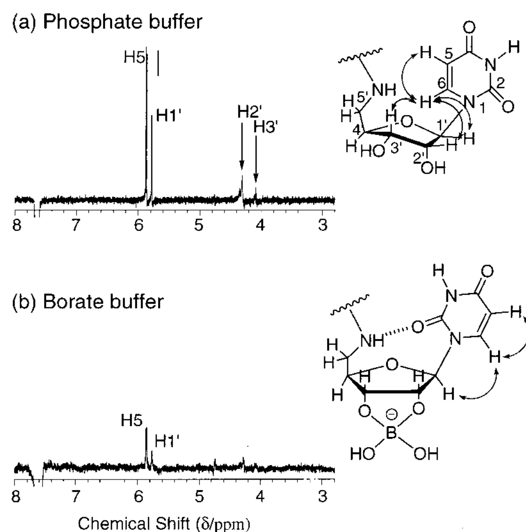


Figure 6. NOE differential spectra (600 MHz) of **6** obtained with presaturation at H6 (δ_H 7.6) in (a) phosphate buffer (0.033 M KH_2PO_4 − 0.033 M Na_2HPO_4, pH 7.2) and (b) borate buffer (0.1 M KH_2PO_4 − 0.05 M $Na_2B_4O_7$, pH 7.2).

efficiency may be the result of the steric effect of the *tert*-butoxycarbonyl protecting group and the increased hydrophilicity and extensive hydration around the peptide backbone.

Synthesis of Peptide Ribonucleic Acids with Oligo(γ-L-glutamic acid) Backbone (γ-PRNA). A series of oligomeric PRNAs were prepared by progressive elongation reactions composed of a repeated selective deprotection-condensation cycle, as shown in Scheme 2. In the first step, the benzyl group of the protected PRNA monomer (**5**) was removed by catalytic hydrogenation to give **6** with a free carboxyl terminus, while the Boc group of **5** was removed by treatment with TFA, affording **7** with a free amino terminus. The subsequent condensation of **6** and **7** using the BOP reagent yielded the dimeric PRNA (**8**). This synthetic cycle was repeated three times to give the protected 8-mer (**14**). Each coupling process proceeded in 70−98% yield. The benzyl group of **14** was subsequently removed to give the *N*-protected 8-mer (**15**), to the *C*-terminus of which was introduced N^{ϵ}-(2-chlorobenzyloxycarbonyl)-*O*-benzyl-L-lysine (Lys(ClZ)-OBzl), as shown in Scheme 3. **16** was completely deprotected using trimethylsilyl triflate (TMSOTf),[22] affording the γ-PRNA oligomer (**17**).

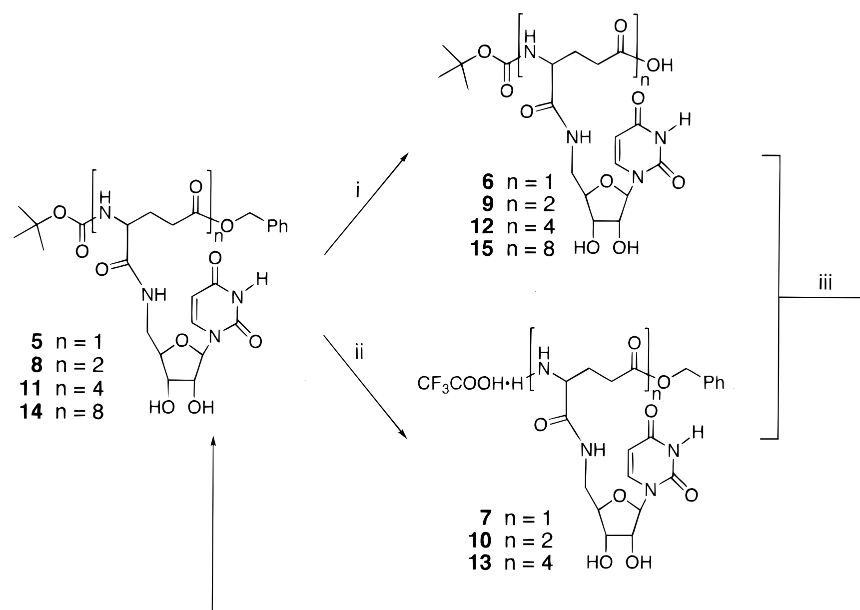
Using the above synthetic strategy, it is possible to introduce different bases into the sequence of γ-PRNA, because the oligomeric γ-PRNA was obtained by elongation of monomeric γ-PRNA. In the present study, the γ-PRNA monomer (**18**), which carries a cytidine unit, was also prepared in a manner similar to that employed in the preparation of **5**, and the Boc group of **18** was removed by treatment with TFA to afford **19,** which has a free amino group. The BOP mediated condensation of **19** with **6** yielded a dimeric γ-PRNA (**20**), which has a uridine unit and a cytidine unit at the amino and carboxyl terminals of glutamic acid, respectively. Repeated elongation, using **20**, followed by the subsequent introduction of lysine derivative and final deprotection, afforded the 8-mer (**21**), which is composed of alternate uridine and cytidine units with a lysine at the *C*-terminus (Scheme 4). After gel filtration using Sephadex G-25, the oligomeric γ-PRNAs **17** and **21** were purified by reverse phase HPLC.

CD Spectral Study on γ-PRNA Oligomers. The CD spectra of γ-PRNA 8-mer (**17**) were measured in both borate and phosphate buffers, under comparable conditions (Figure 7). In phosphate buffer, the $[\theta]_{max}$ value obtained (5200 deg cm^2 dmol^{-1}) was nearly the same as that observed for 5′-amino-5′-dexoyuridine (**2**), and this is compatible with the preferred anti orientation in phosphate buffer. In contrast, the $[\theta]_{max}$ value of this oligomer was greatly reduced to 650 deg cm^2 dmol^{-1} in borate buffer, which is significantly smaller than the value observed for **2** in borate buffer (1600 deg cm^2 dmol^{-1}). The reduced $[\theta]_{max}$ value observed for PRNA 8-mer **17** in borate buffer is important, since the use of the borate buffer, in place of phosphate buffer, caused only a small decrease from 6500 to 5000 deg cm^2 dmol^{-1} with the PRNA monomer (**6**). These results strongly indicate the predominant syn orientation of nucleobase in borate buffer, probably through cooperative effects of adjacent nucleobases in this oligomer.

A similar tendency was also observed with observed oligomer **21**. As shown in Figure 8, the $[\theta]_{max}$ value (2100 deg cm^2 dmol^{-1}) in borate buffer was much smaller than that observed in phosphate buffer (7800 deg cm^2 dmol^{-1}). This indicates that the change in base orientation from anti to syn in **21** is induced by borate ester formation at each nucleoside unit.

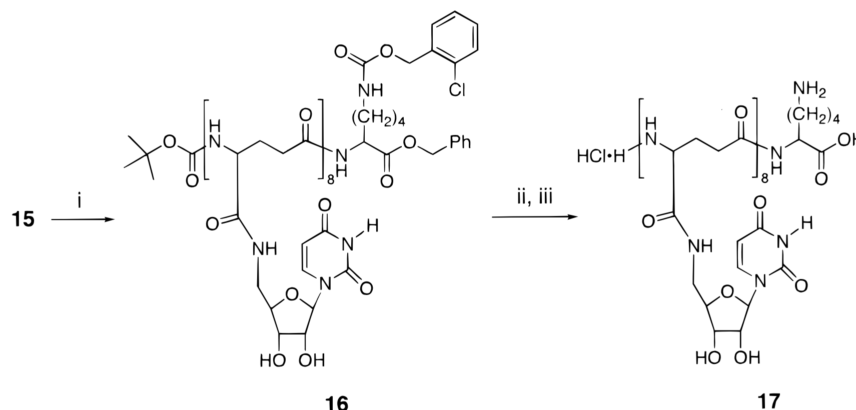
(22) Fujii, N.; Otaka, A.; Ikemura, O.; Akaji, K.; Funakoshi, S.; Hayashi, Y.; Kuroda, Y.; Yajima, H. *J. Chem. Soc., Chem. Commun.* **1987**, 274.

Scheme 2[a]



[a] *Reagents and conditions*: i, H_2, Pd/C(10%), MeOH−DMF (10:0−1: 3 v/v), rt, 2−6 h; ii, TFA, 0 °C, 30 min; iii, BOP reagent, HOBt, (*i*-Pr)$_2$NEt, DMF, rt, 2−4 h

Scheme 3[a]

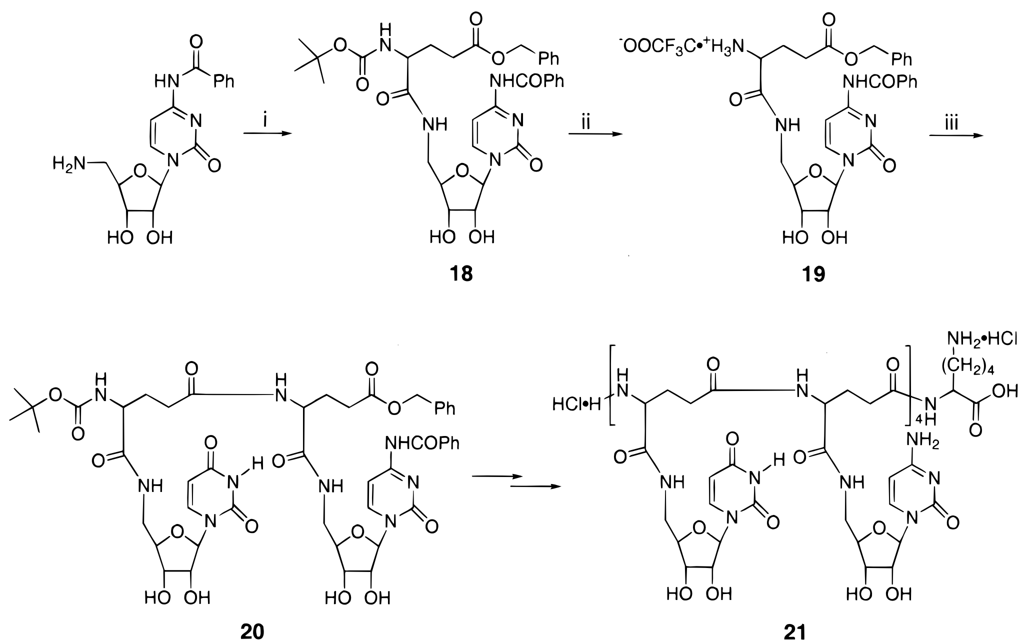


[a] *Reagents and conditions*: i, Lys(ClZ)-OBzl, BOP reagent, HOBt, (*i*-Pr)$_2$NEt, DMF, rt, 4 h; ii, *m*-cresol, PhSMe, TMSOTf, TFA, 0 °C, 1 h; iii, 5% NH_3 aq 0 °C, 30 min, then 4 *N* HCl/dioxane, 0 °C, 30 min

The CD spectra was also measured at various concentrations of borax added to a phosphate buffer solution of **17**. As shown in Figure 9, the CD intensity continuously decreased as the concentration of borax increased, leveling off between 3.7 and 10 mM. The CD spectra observed at borax concentrations of borax >3.7 mM is almost superimposable upon that obtained in borate buffer which contains 20 mM of borax. The gradual CD spectral changes, accompanied by the isosbestic points observed at ∼225 and 250 nm, indicate clearly that the reversible anti-to-syn orientation switching process is caused by a cooperative cyclic borate ester formation and hydrogen bonding interaction, as observed for the 5′-amino-5′-deoxyuridine and the *γ*-PRNA monomer **6**. A quantitative treatment of these CD spectral changes, using the nonlinear least-squares fitting to the curve for 1:1 stoichiometric complexation, gave the equilibrium constant of 750 M^{-1} for the formation of borate ester for each ribonucleoside, which is exactly 10 times greater than that obtained for **2** under the comparable conditions. The higher binding constant and much reduced CD intensity at high concentrations of borax observed for **17** indicate that the borate ester formation and the hydrogen-bonding interaction synergetically enhance the anti-to-syn orientation switching of the uracil moiety in oligomeric *γ*-PRNA. This may be attributed to the increased hydrophobicity, and thus a reduced hydration around the peptide backbone, which is thought to form a right-handed, loose helical conformation based on the weak, positive exciton coupling of the major CD band at 270 nm, and also from the hypochromic change of 5% observed in the UV spectrum.

Control of Hybridization of *γ*-PRNA with d(A)$_8$ by Borate Ester Formation. The hybridization ability of *γ*-PRNA **17** with complementary d(A)$_8$ was evaluated from the melting temperature, T_m, which was determined from hypochromic changes observed in the UV spectrum upon mixing **17** with the target oligonucleotide 8-mer. The T_m value, taken as a quantitative measure of the binding affinity, is known to be affected by the conformation of a nucleic acid or its analogue,[9] as well as the ionic strength of the aqueous solution used. To elucidate the effects of borate on the formation and stability of the PRNA-DNA hybrid, the melting profiles for the *γ*-PRNA **17** pairs and the reference compound (T)$_8$ with the complementary d(A)$_8$ were measured independently in phosphate buffer with and

Scheme 4[a]



[a] *Reagents and conditions*: i, Boc-Glu(OBzl), BOP reagent, HOBt, (i-Pr)$_2$NEt, DMF, rt, 1 h; ii, TFA, 0 °C, 30 min; iii, **6**, BOP reagent, HOBt, (i-Pr)$_2$NEt, DMF, rt, 2 h

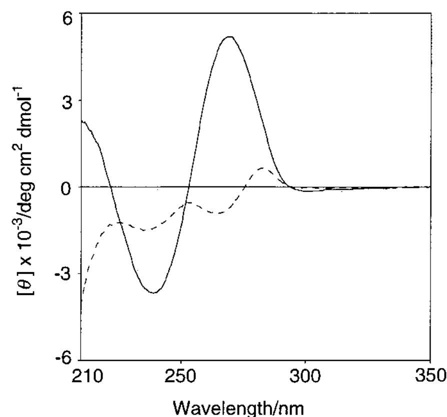


Figure 7. CD Spectra of **17** in phosphate buffer (0.033 M KH_2PO_4 − 0.033 M Na_2HPO_4, pH 7.2; solid line) and borate buffer (0.1 M KH_2PO_4 − 0.05 M $Na_2B_4O_7$, pH 7.2; dashed line).

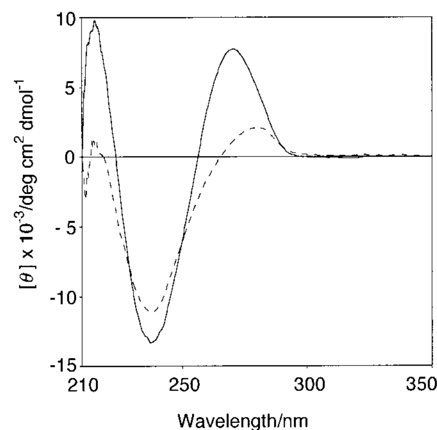


Figure 8. CD Spectra of **21** in phosphate buffer (0.033 M KH_2PO_4 − 0.033 M Na_2HPO_4, pH 7.2; solid line) and borate buffer (0.1 M KH_2PO_4 − 0.05 M $Na_2B_4O_7$, pH 7.2; dashed line).

without borax (20 mM). Table 1 summarizes the T_m values and maximum hypochromicity observed in these two buffer solutions. The stoichiometry of the complex with d(A)$_8$ was determined to be 1:1 in each case (uracil or thymine:adenine unit ratio) using the Job plot of the hypochromic change upon mixing (see Experimental), and triplex formation is therefore not to occur under the conditions employed. Control runs using noncomplementary (T)$_8$ in place of d(A)$_8$ were also carried out under comparable conditions and found to show no hypochromicity or appreciable melting behavior. This confirms the base-specific interaction of γ-PRNA **17** with the complementary d(A)$_8$. In borax-free buffer, the hybrid complex between γ-PRNA **17** and d(A)$_8$ gave a considerably higher T_m of 52.8 °C than the complementary (T)$_8$-d(A)$_8$ duplex (T_m = 45.0 °C), indicating a stronger interaction in the hybrid than in the natural pair. To the best of our knowledge, this is the first example of tight binding of the oligomers of nucleobases attached to a γ-glutamyl peptide backbone.

In contrast, in the borax-containing buffer solution, the hybrid complex did not exhibit any melting behavior above 0 °C, or hypochromic changes, while the complementary (T)$_8$−d(A)$_8$ duplex gave an even higher T_m of 56.9 °C and hypochromicity of 40%, presumably due to the slight increase in the ionic strength. This contrasting behavior between the natural and hybrid pairs in the presence/absence of borax is most likely attributable to the anti-to-syn orientation switching of the uracil base in γ-PRNA **17**, for which a cooperative borate ester formation at the *cis*-2′,3′-diol and a hydrogen bonding interaction between the 5′-amide proton and the 2-carbonyl oxygen are responsible. However, the electrostatic repulsion between the adjacent anionic borate esters makes some contribution, as illustrated in Scheme 5.

The complexation behavior of γ-PRNA **21**, which possesses alternating uridine and cytidine units, was studied by using two types of complementary oligonucleotides with a "parallel" or 5′-d(AG)$_4$-3′ sequence and an "antiparallel" 5′-d(GA)$_4$-3′ sequence. The antiparallel oligonucleotide is expected to form a complex with γ-PRNA **21** from the *N*-terminus to the 3′-end, while the parallel oligonucleotide should bind **21** from the *N*-terminus to 5′-end. If γ-PRNA **21** is able to recognize the

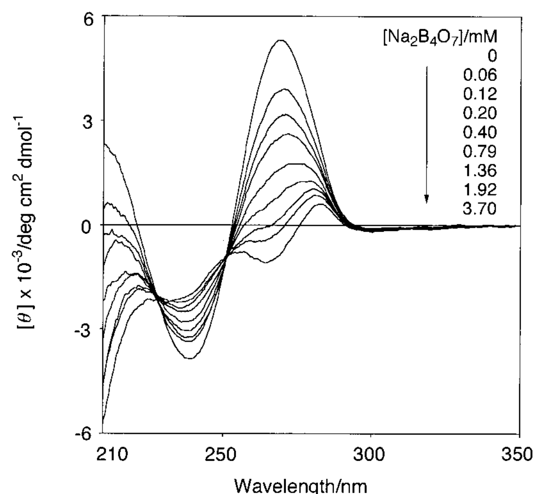


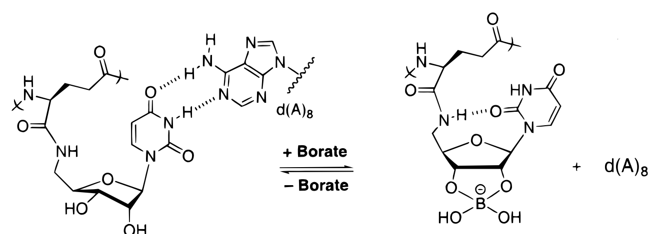
Figure 9. CD spectra changes of **17** with increasing concentrations of $Na_2B_4O_7$ in phosphate buffer (0.033 M KH_2PO_4 − 0.033 M Na_2HPO_4, pH 7.2); [**17**] = 1.0×10^{-4} M.

Table 1. Melting Temperatures (T_m) of γ-PRNA−Oligonucleotide Complexes[a]

γ-PRNA or oligodeoxyribonucleotide	complement	T_m/°C additive none	20 mM borax
17	d(A)$_8$	52.8	<0
17	(T)$_8$	<0	<0
(T)$_8$	d(A)$_8$	45	56.9
21	d(ApGpApGpApGpApG)	41.6	<0
21	d(GpApGpApGpApGpA)	49.2	<0

[a] Solvent: phosphate buffer (0.033 M KH_2PO_4 − 0.033 M Na_2HPO_4, pH 7.2).

Scheme 5. Proposed Recognition Control Model of **17** with Complementary d(A)



antiparallel orientation correctly, the parallel complex should not be observed.[23] As shown in Table 1, the antiparallel target gives a T_m of 49.2 °C, which is appreciably higher than that obtained for the parallel complex (T_m = 41.6 °C). This result may indicate that γ-PRNA **21** tightly binds to antiparallel target sequence rather than parallel one, although the possibility of one-base shifted binding to the parallel target in the antiparallel fashion cannot rigorously be ruled out.

Interestingly, the addition of borax to these systems resulted in a complete loss of T_m, indicating the dissociation of the complex through the anti-to-syn orientation switching of the base. It should be emphasized that oligomeric γ-PRNA and the complementary antiparallel DNA form a complex that is more stable than the equivalent natural DNA-DNA duplex. Furthermore, the on−off switching of nucleic acid recognition has been realized for the first time using the borate-induced orientation switching of γ-PRNA.

(23) Egholm, M.; Nielsen, P. E.; Buchardt, O.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 9677.

Conclusions

Current studies on antisense molecules are directed mostly toward the simple inhibition of genetic information transfer. Little effort has been devoted to the active control of the DNA/RNA recognition processes, possibly as a result of a lack of suitable fundamental strategy and the necessary practical tools. In this and preceding studies,[14,15] we have proposed a new methodology and effective tools for controlling DNA/RNA recognition through the use of an external agent. This strategy employs novel nucleic acid analogues, that is, α- and γ-peptide ribonucleic acids (α- and γ-PRNAs), as the recognition moiety which has a built-in switch triggered by an external factor. The formation of a borate ester of the ribose's *cis*-2′,3′-diol and the simultaneous hydrogen-bonding interaction between the 5′-amide proton and the 2-carbonyl oxygen act as the external and internal switching devices. The results obtained in both studies are encouraging, demonstrating that α- and γ-PRNA form stable hybrid complexes with the complementary oligonucleotides, which are readily dissociated upon addition of borax or boric acid. Certainly, a definition of the scope and limitations of this strategy remain to be addressed, including the study of sequence selectivity and the search for other switching devices/agents. Moreover, the present concept of the external control of DNA/RNA recognition has the potential to be widely employed in the next generation of antisense molecules.

Experimental Section

Standard abbreviations for amino acids and protecting groups are as recommended by the IUPAC-IUB Commission on Biochemical Nomenclature. Other abbreviations include: HOBt, 1-hydroxybenzotriazole; BOP, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; TFA, trifluoroacetic acid; isoGln(5′U), N^5-(5′-deoxy-5′-uridyl)-L-isoglutamine residue; ClZ, 2-chlorobenzyloxycarbonyl group. All starting materials, reagents, and solvents were commercially available and used without further purification. Uridine was purchased from Seikagaku Co. (Tokyo, Japan). Boc-L-amino acids and 1-hydroxybenzotriazole (HOBt) were purchased from Peptide Institute, Inc. (Osaka, Japan). Oligonucleotides were purchased from Takara Co. (Kyoto, Japan). Other chemicals of guaranteed grade were purchased Tokyo Kasei Kogyo Co. (Tokyo, Japan) or Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Column chromatography was performed over 70−230 mesh silica gel from Merck. Gel filtration was performed over Sephadex G-25 from Pharmacia. Nucleic acid model was purified on a pre-packed Hiber column RT by elution with 10% acetonitrile in water at a flow rate of 3 mL/min, using a recycling preparative HPLC instrument LC-908 from Japan Analytical Industries. IR spectra were recorded on a JASCO FT/IR-230 spectrometer. ^{1}H NMR spectra were obtained on a JEOL GSX-270 at 270 MHz or a Varian INOVA-600 at 600 MHz. Chemical shifts are reported as δ value in parts per million (ppm) relative to tetramethylsilane (δ_H = 0.0 ppm) as internal standard. Mass spectral measurements were performed on a JEOL AX-500 instrument by fast-atom bombardment (FAB) ionization with nitrobenzyl alcohol (NBA) as a matrix and a Voyager RP from PerSeptive Biosystems with α-cyano-4-hydroxycinnamic acid (α-CHCA) or picolinic acid as a matrix (MALDI-TOF). CD spectra were recorded on a JASCO J-720W spectrophotometer. Thermal dissociation experiments were performed on a JASCO V-550 UV/vis spectrophotometer equipped with a temperature controller. Absorbance data at 260 nm was collected at 10 s intervals upon heating a solution of nucleic acid model (6×10^{-5} M) and oligonucleotide (6×10^{-5} M) at a rate of 0.5 °C/min to give the melting curve.

Equilibrium constants (K_S) were determined for the borate ester formation of γ-PRNAs from the quantitative analysis of the CD spectral changes induced by adding boric acid or borax (3.7−84 mM) to a borate-free buffer solution of 5′-aminonucleoside or γ-PRNA (0.7−1 $\times 10^{-4}$ M). Nonlinear least-squares analysis of the differential molar ellipticity data ($\Delta[\theta]_{max}$) led to an excellent fit to the 1:1 stoichiometric

curve, affording the equilibrium constants for the esterification of **2**, **3**, and **17** with boric acid or borax.

Complex stoichiometry was determined from the Job plot, where the interaction of PRNA and oligonucleotide was monitored by the hypochromic changes at 260 nm in UV spectra, as reported previously.[24] In these experiments, the molar fractions of γ-PRNA and oligonucleotide were continuously varied, while the combined concentration was fixed at 10^{-4} M. In all cases examined, the induced hypochromicity was maximized at a molar fraction of 0.5, indicating the formation of 1:1 complex between PRNA and oligonucleotide.

***N*$^{\alpha}$-*tert*-Butoxycarbonyl-*N*^{5}-(5′-deoxy-5′-uridyl)-L-isoglutamine Benzyl Ester (Boc-isoGln(5′U)-OBzl) (5).** To a solution of *N*-*tert*-butoxycarbonyl-L-glutamic acid γ-benzyl ester (2.52 g, 7.47 mmol), HOBt (1.01 g, 7.47 mmol), and BOP reagent (3.30 g, 7.47 mmol) in DMF (100 mL) was added diisopropylethylamine (1.30 mL, 7.47 mmol). After 30 s of stirring at 0 °C, 5′-amino-5′-deoxyuridine (**2**)[18] (2.00 g, 8.22 mmol) was added, and the mixture was stirred for 1 h at room temperature. The solvent was removed under reduced pressure and the residue was suspended in ethyl acetate (200 mL). The suspension was successively washed with equal-volume portions of 4% sodium hydrogen carbonate, 5% potassium hydrogen sulfate, and brine. The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with chloroform−methanol (30:1 v/v) to give compound **5** as a powder (3.70 g, 88%), ν_{max}(KBr)/cm^{-1} 3420, 1680, 1520, 1460, 1390, 1250, and 1170; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.37 (9 H, s, *t*-Bu-H), 1.77−1.88 (2 H, m, β-CH$_2$), 2.36 (2 H, t, $J_{\gamma,\beta}$ = 7.8, γ-CH$_2$), 3.29 (2 H, m, 5′-H), 3.83 (2 H, m, 3′-H and 4′-H), 3.94 (1 H, q, $J_{CH,NH}$ = $J_{CH,\beta}$ = 7.3, Boc-NHC<u>H</u>), 4.01 (1 H, q, $J_{2',1'}$ = $J_{2',3'}$ = 5.4, 2′-H), 5.07 (2 H, s, PhCH$_2$), 5.18 (1 H, d, $J_{OH,3'}$ = 4.9, 3′-OH), 5.40 (1 H, d, $J_{OH,2'}$ = 5.4, 2′-OH), 5.63 (1 H, d, $J_{5,6}$ 8.3, 5-H), 5.74 (1 H, d, $J_{1',2'}$ = 5.9, 1′-H), 6.94 (1 H, d, $J_{NH,CH}$ = 7.8, Boc-NH), 7.35 (5 H, m, Ar-H), 7.64 (1 H, d, $J_{6,5}$ = 7.8, 6-H), 8.00 (1 H, t, $J_{NH,5'}$ = 5.6, 5′-NH), 11.35 (1 H, s, 3-NH); Found: C, 55.06; H, 6.02; N, 9.76. Calcd for $C_{26}H_{34}N_4O_{10}\cdot 1/4H_2O$: C, 55.07; H, 6.13; N, 9.88%; MALDI-TOF HRMS (α-CHCA), *m/z* found 585.22 (M + Na), calculated 585.217.

***N*$^{\alpha}$-*tert*-Butoxycarbonyl-*N*^{5}-(5′-deoxy-5′-uridyl)-L-isoglutamine (Boc-isoGln(5′U)-OH) (6).** Palladium on activated carbon (10%; ∼0.2 g) was added to a solution of **5** (1.80 g, 3.20 mmol) in methanol (50 mL). After 2 h of continued stirring under hydrogen atmosphere (1atm), the reaction mixture was filtered, and the filtrate was evaporated under reduced pressure to give compound **6** as a powder (1.44 g, 95%), ν_{max}(KBr)/cm^{-1} 3320, 1680, 1530, 1460, 1390, 1250, and 1170; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.37 (9 H, s, *t*-Bu-H), 1.69−1.82 (2 H, m, β-CH$_2$), 2.19 (2 H, t, $J_{\gamma,\beta}$ =7.3, γ-CH$_2$), 3.29−3.39 (2 H, m, 5′-H), 3.83 (2 H, m, 3′-H and 4′-H), 3.92 (1 H, q, $J_{CH,NH}$ = $J_{CH,\beta}$ = 5.9, Boc-NHC<u>H</u>), 4.02 (1 H, t, $J_{2',1'}$ = $J_{2',3'}$ = 4.9, 2′-H), 5.63 (1 H, d, $J_{5,6}$ = 8.3, 5-H), 5.73 (1 H, d, $J_{1',2'}$ = 5.9, 1′-H), 6.92 (1 H, d, $J_{NH,CH}$ = 8.3, Boc-NH), 7.65 (1 H, d, $J_{6,5}$ = 7.8, 6-H), 7.98 (1 H, t, $J_{NH,5'}$ = 5.4, 5′-NH), 11.35 (1 H, s, 3-NH); MALDI-TOF HRMS (α-CHCA), *m/z* found 495.17 (M + Na), calculated 495.170.

***N*^{5}-(5′-Deoxy-5′-uridyl)-L-isoglutamine Benzyl Ester Trifluoroacetic Acid Salt (TFA·isoGln(5′U)-OBzl) (7).** **5** (1.80 g, 3.20 mmol) was dissolved in TFA (10 mL), and the solution was kept at 0 °C for 30 min. TFA was removed under reduced pressure, and ether (200 mL) was added to give compound **12** as a powder (1.81 g, 98%), ν_{max}(KBr)/cm^{-1} 3420, 1680, 1560, 1460, 1270, 1200, and 1130; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.97 (2 H, m, β-CH$_2$), 2.44 (2 H, t, $J_{\gamma,\beta}$ = 8.1, γ-CH$_2$), 3.82 (3 H, m, NH$_3^+$CH, 3′-H and 4′-H), 4.11 (1 H, q, $J_{2',1'}$ = $J_{2',3'}$ = 5.2, 2′-H), 5.08 (2 H, s, PhCH$_2$), 5.24 (1 H, d, $J_{OH,3'}$ = 4.9, 3′-OH), 5.47 (1 H, d, $J_{OH,2'}$ = 5.4, 2′-OH), 5.63 (1 H, d, $J_{5,6}$ = 8.3, 5-H), 5.75 (1 H, d, $J_{1',2'}$ = 5.4, 1′-H), 7.36 (5 H, m, Ar-H), 7.66 (1 H, d, $J_{6,5}$ = 8.3, 6-H), 8.10 (3 H, br, NH$_3^+$), 8.62 (1 H, t, $J_{NH,5'}$ 5.4, 5′-NH), 11.37 (1 H, s, 3-NH); MALDI-TOF HRMS (α-CHCA), *m/z* found 463.17 (M − CF$_3$COO), calculated 463.183.

Boc-(isoGln(5′U))$_2$-OBzl (8). To a solution of **6** (1.25 g, 2.65 mmol), HOBt (0.358 g, 2.65 mmol), and BOP reagent (1.17 g, 2.65 mmol) in DMF (50 mL) was added diisopropylethylamine (0.97 mL, 5.57 mmol). After 30 s of stirring at 0 °C, **7** (1.68 g, 2.92 mmol) was added, and the resultant mixture was stirred for 2 h at room temperature. The solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel with chloroform−methanol (10:1 v/v) to give compound **8** as a powder (1.70 g, 70%), ν_{max}(KBr)/cm^{-1} 3420, 1680, 1540, 1380, 1270, and 1200; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.36 (9 H, s, *t*-Bu-H), 1.80 (4 H, m, β-CH$_2$), 2.15 (2 H, t, $J_{\gamma,\beta}$ = 7.1, γ-CH$_2$), 2.35 (2 H, t, $J_{\gamma,\beta}$ = 7.8, γ-CH$_2$), 3.83 (5 H, m, Boc-NHC<u>H</u>, 3′-H and 4′-H), 4.03 (2 H, q, $J_{2',1'}$ = $J_{2',3'}$ = 4.4, 2′-H), 4.26 (1 H, q, $J_{CH,NH}$ = $J_{CH,\beta}$ = 7.3, α-CH), 5.06 (2 H, s, PhCH$_2$), 5.16 (2 H, s, 3′-OH), 5.39 (2 H, d, $J_{OH,2'}$ = 4.4, 2′-OH), 5.62 (1 H, d, $J_{5,6}$ = 8.3, 5-H), 5.64 (1 H, d, $J_{5,6}$ = 7.8, 5-H), 5.73 (2 H, d, $J_{1',2'}$ = 5.9, 1′-H), 6.85 (1 H, d, $J_{NH,CH}$ = 8.3, Boc-NH), 7.35 (5 H, m, Ar-H), 7.63 (1 H, d, $J_{6,5}$ = 7.8, 6-H), 7.64 (1 H, d, $J_{6,5}$ = 7.8, 6-H), 7.95 (2 H, m, 5′-NH), 8.14 (1 H, t, $J_{NH,CH}$ = 10.7, α-NH), 11.33 (2 H, s, 3-NH); MALDI-TOF HRMS (α-CHCA), *m/z* found 939.34 (M + Na), calculated 939.335.

Boc-(isoGln(5′U))$_2$-OH (9). Palladium on activated carbon (10%; ∼0.1 g) was added to a solution of **8** (0.850 g, 0.927 mmol) in methanol (50 mL). After 4 h of continued stirring under hydrogen atmosphere (1 bar) at room temperature, the reaction mixture was filtered, and the filtrate was evaporated under reduced pressure to give compound **9** as a powder (0.736 g, 96%), ν_{max}(KBr)/cm^{-1} 3390, 1680, 1540, 1470, 1390, and 1280; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.37 (9 H, s, *t*-Bu-H), 1.67−1.81 (4 H, m, β-CH$_2$), 2.14 (4 H, m, γ-CH$_2$), 3.58 (4 H, m, 5′-H), 3.92 (4 H, m, 3′-H and 4′-H), 4.03−4.24 (3 H, m,2′-H and Boc-NHC<u>H</u>), 4.42 (1 H, q, $J_{CH,NH}$ = $J_{CH,\beta}$ = 7.1, α-CH), 5.17−5.39 (4 H, m, 2′-OH and 3′-OH), 5.67 (2 H, d, $J_{5,6}$ = 8.3, 5-H), 5.86 (2 H, d, $J_{1',2'}$ = 7.8, 1′-H), 6.99 (1 H, d, $J_{NH,CH}$ = 8.8, Boc-NH), 7.67 (2 H, d, $J_{6,5}$ = 8.8, 6-H), 7.88 (2 H, m, 5′-NH), 8.16 (1 H, m, α-NH), 11.35 (2 H, s, 3-NH); MALDI-TOF HRMS (α-CHCA), *m/z* found 849.29 (M + Na), calculated 849.288.

TFA·(isoGln(5′U))$_2$-OBzl (10). **8** (0.850 g, 0.927 mmol) was dissolved in TFA (5 mL), and the solution was kept at 0 °C for 30 min. TFA was removed under reduced pressure, and ether (100 mL) was added to give compound **10** as a powder (0.846 g, 98%), ν_{max}(KBr)/cm^{-1} 3420, 1680, 1540, 1470, 1280, 1200, and 1140; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.89 (4 H, m, β-CH$_2$), 2.25−2.34 (4 H, m, γ-CH$_2$), 3.59 (4 H, m, 5′-H), 3.86 (6 H, m, NH$_3^+$CH, α-CH, 3′-H and 4′-H), 4.11 (2 H, m, 2′-H), 5.08 (2 H, s, PhCH$_2$), 5.20 (4 H, m, 3′-, 2′-OH), 5.70 (2 H, d, $J_{5,6}$ = 7.8, 5-H), 5.77 (2 H, d, $J_{1',2'}$ = 5.9, 1′-H), 7.36 (5 H, m, Ar-H), 7.89 (1 H, m, 5′-NH), 8.10 (4 H, m, α-NH and NH$_3^+$), 8.56 (1 H, m, 5′-NH), 11.32 (2 H, s, 3-NH); MALDI-TOF HRMS (α-CHCA), *m/z* found 817.30 (M − CF$_3$COO), calculated 817.301.

Boc-(isoGln(5′U))$_4$-OBzl (11). To a solution of **9** (0.662 g, 0.801 mmol), HOBt (0.108 g, 0.801 mmol), and BOP reagent (0.354 g, 0.801 mmol) in DMF (30 mL) was added diisopropylethylamine (0.29 mL, 1.68 mmol). After 30 s of stirring at 0 °C, **10** (0.820 g, 0.881 mmol) was added, and the mixture was stirred for 2 h at room temperature. The solvent was removed under reduced pressure, and ethanol was added to give compound **11** as a powder (1.22 g, 94%), ν_{max}(KBr)/cm^{-1} 3300, 1680, 1540, 1460, 1390, 1270, and 1080; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.37 (9 H, s, *t*-Bu-H), 1.71−1.82 (8 H, m, β-CH$_2$), 2.14 (6 H, m, γ-CH$_2$), 2.35 (2 H, t, $J_{\gamma,\beta}$ = 7.6, γ-CH$_2$), 3.26−3.44 (8 H, m, 5′-H), 3.83 (9 H, m, Boc-NHC<u>H</u>, 3′-H and 4′-H), 4.04 (4 H, m, 2′-H), 4.22 (3 H, m, α-CH), 5.06 (2 H, s, PhCH$_2$), 5.16 (4 H, d, $J_{OH,3'}$ = 4.4, 3′-OH), 5.39 (4 H, d, $J_{OH,2'}$ = 5.4, 2′-OH), 5.65 (4 H, d, $J_{5,6}$ = 7.3, 5-H), 5.73 (4 H, d, $J_{1',2'}$ = 3.9, 1′-H), 6.87 (1 H, d, $J_{NH,CH}$ = 7.8, Boc-NH), 7.35 (5 H, m, Ar-H), 7.65 (4 H, d, $J_{6,5}$ = 8.3, 6-H), 7.96 (4 H, m, 5′-NH), 8.12 (3 H, m, α-NH), 11.34 (4 H, s, 3-NH); MALDI-TOF HRMS (α-CHCA), *m/z* found 1647.57 (M + Na), calculated 164.570.

Boc-(isoGln(5′U))$_4$-OH (12). Palladium on activated carbon (10%; ∼0.1 g) was added to a solution of **11** (0.600 g, 0.369 mmol) in methanol−DMF (1:1 v/v) (30 mL). After 4 h of continued stirring under hydrogen atmosphere (1 atm) at room temperature, the reaction mixture was filtered, and the filtrate was evaporated under reduced pressure to give compound **12** as a powder (0.550 g, 97%), ν_{max}(KBr)/cm^{-1} 3420, 1680, 1540, 1490, 1380, 1210, and 1130; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.37 (9 H, s, *t*-Bu-H), 1.69−1.82 (8 H, m, β-CH$_2$), 2.15 (8 H, m,

(24) Thomas, G. J.; Kyogoku, Y. *J. Am. Chem. Soc.* **1967**, *89*, 4170.

γ-CH$_2$), 3.85 (9 H, m, Boc-NHCH, 3′-H and 4′-H), 4.04 (4 H, m, 2′-H), 4.20 (3 H, m, α-CH), 5.21 (4 H, br, 3′-OH), 5.37 (4 H, br, 2′-OH), 5.64 (4 H, d, $J_{5,6}$ = 7.8, 5-H), 5.73 (4 H, d, $J_{1',2'}$ = 5.9, 1′-H), 6.87 (1 H, d, $J_{NH,CH}$ = 8.3, Boc-NH), 7.65 (4 H, d, $J_{6,5}$ = 8.3, 6-H), 7.95 (4 H, m, 5′-NH), 8.13 (3 H, m, α-NH), 11.33 (4 H, s, 3-NH); MALDI-TOF HRMS (α-CHCA), *m/z* found 1557.52 (M + Na), calculated 1557.523.

TFA·(isoGln(5′U))$_4$-OBzl (13). **11** (0.600 g, 0.369 mmol) was dissolved in TFA (5 mL), and the solution was kept at 0 °C for 30 min. TFA was removed under reduced pressure, and ether (100 mL) was added to give compound **13** as a powder (0.593 g, 98%), ν_{max}(KBr)/cm^{-1} 3420, 1680, 1540, 1460, 1390, 1270, and 1130; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.76−1.89 (8 H, m, β-CH$_2$), 2.13−2.25 (6 H, m, γ-CH$_2$), 2.34 (2 H, t, $J_{\gamma,\beta}$ = 8.3, γ-CH$_2$), 3.83 (9 H, m, NH$_3^+$CH, 3′-H and 4′-H), 4.05−4.22 (7 H, m, α-CH and 2′-H), 5.06 (2 H, s, PhCH$_2$), 5.16 (3 H, m, 3′-OH), 5.24 (1 H, d, $J_{OH,3'}$ = 4.9, 3′-OH), 5.42 (3 H, m, 2′-OH), 5.48 (1 H, d, $J_{OH,2'}$ = 5.4, 2′-OH), 5.64 (4 H, m, 5-H), 5.73 (4 H, m, 1′-H), 7.35 (5 H, m, Ar-H), 7.66 (4 H, m, 6-H), 7.95 (3 H, m, 5′-NH), 8.13 (6 H, m, α-NH and NH$_3^+$), 8.61 (1 H, m, 5′-NH), 11.35 (4 H, m, 3-NH); MALDI-TOF HRMS (α-CHCA), *m/z* found 1548.52 (M − CF$_3$COO + Na), calculated 1548.526.

Boc-(isoGln(5′U))$_8$-OBzl (14). To a solution of **12** (0.468 g, 0.305 mmol), HOBt (0.041 g, 0.305 mmol), and BOP reagent (0.135 g, 0.305 mmol) in DMF (30 mL) was added diisopropylethylamine (0.11 mL, 0.641 mmol). After 30 s of stirring at 0 °C, **13** (0.550 g, 0.335 mmol) was added, and the mixture was stirred for 4 h at room temperature. The solvent was removed under reduced pressure, and methanol was added to give compound **14** as a powder (0.854 g, 92%), ν_{max}(KBr)/cm^{-1} 3420, 1680, 1530, 1460, 1400, 1270, and 1130; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.37 (9 H, s, *t*-Bu-H), 1.71−1.82 (16 H, m, β-CH$_2$), 2.14 (14 H, m, γ-CH$_2$), 2.34 (2 H, t, $J_{\gamma,\beta}$ = 7.8, γ-CH$_2$), 3.25−3.42 (16 H, m, 5′-H), 3.83 (17 H, m, Boc-NHCH, 3′-H and 4′-H), 4.05 (8 H, m, 2′-H), 4.20 (7 H, m, α-CH), 5.06 (2 H, s, PhCH$_2$), 5.15 (8 H, d, $J_{OH,3'}$ = 3.4, 3′-OH), 5.39 (8 H, d, $J_{OH,2'}$ = 5.4, 2′-OH), 5.64 (8 H, d, $J_{5,6}$ = 7.8, 5-H), 5.73 (8 H, d, $J_{1',2'}$ = 5.4, 1′-H), 6.87 (1 H, d, $J_{NH,CH}$ = 7.3, Boc-NH), 7.35 (5 H, m, Ar-H), 7.64 (8 H, d, $J_{6,5}$ = 7.8, 6-H), 7.95 (8 H, m, 5′-NH), 8.13 (7 H, m, α-NH), 11.33 (8 H, s, 3-NH); MALDI-TOF HRMS (α-CHCA), *m/z* found 3064.04 (M + Na), calculated 3064.041.

Boc-(isoGln(5′U))$_8$-OH (15). Palladium on activated carbon (10%; ∼0.1 g) was added to a solution of **14** (0.800 g, 0.263 mmol) in methanol−DMF (1:3 v/v) (50 mL). After 6 h of continued stirring under hydrogen atmosphere (1 atm) at room temperature, the reaction mixture was filtered, and the filtrate was evaporated under reduced pressure to give compound **15** as a powder (0.745 g, 96%), ν_{max}(KBr)/cm^{-1} 3410, 1680, 1540, 1470, 1390, 1260, and 1110; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.37 (9 H, s, *t*-Bu-H), 1.70−1.82 (16 H, m, β-CH$_2$), 2.15 (16 H, m, γ-CH$_2$), 3.83 (17 H, m, Boc-NHCH, 3′-H and 4′-H), 4.05 (8 H, m, 2′-H), 4.19 (7 H, m, α-CH), 5.18 (8 H, m, 3′-OH), 5.29 (8 H, m, 2′-OH), 5.65 (8 H, d, $J_{5,6}$ = 7.8, 5-H), 5.73 (8 H, d, $J_{1',2'}$ = 5.9, 1′-H), 6.88 (1 H, d, $J_{NH,CH}$ = 8.8, Boc-NH), 7.65 (8 H, d, $J_{6,5}$ = 8.3, 6-H), 7.95 (8 H, m, 5′-NH), 8.14 (8 H, m, α-NH), 11.33 (8 H, s, 3-NH); MALDI-TOF HRMS (α-CHCA), *m/z* found 2973.99 (M + Na), calculated 2973.994.

Boc-(isoGln(5′U))$_8$-Lys(ClZ)-OBzl (16). To a solution of **15** (0.700 g, 0.237 mmol), HOBt (0.032 g, 0.237 mmol), and BOP reagent (0.105 g, 0.237 mmol) in DMF (30 mL) was added diisopropylethylamine (0.04 mL, 0.237 mmol). After 30 s of stirring at 0 °C, N^{ϵ}-2-chlorobenzyloxycarbonyl-*O*-benzyl-L-lysine (0.106 g, 0.261 mmol) was added, and the mixture was stirred for 4 h at room temperature. The solvent was removed under reduced pressure, and methanol was added to give compound **16** as a powder (0.712 g, 90%), ν_{max}(KBr)/cm^{-1} 3330, 1680, 1540, 1470, 1390, 1270, and 1130; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.36 (13 H, m, *t*-Bu-H, γ-CH$_2$(Lys) and δ-CH$_2$(Lys)), 1.57−1.84 (18 H, m, β-CH$_2$(Glu) and β-CH$_2$(Lys)), 2.14 (16 H, m, β-CH$_2$(Glu)), 2.98 (2 H, m, ϵ-CH$_2$(Lys)), 3.83 (17 H, m, Boc-NHCH, 3′-H and 4′-H), 4.05 (8 H, m, 2′-H), 4.20 (8 H, m, α-CH), 5.07 (2 H, s, PhCH$_2$), 5.08 (2 H, s, Cl−PhCH$_2$), 5.16 (8 H, d, $J_{OH,3'}$ = 3.9, 3′-OH), 5.39 (8 H, d, $J_{OH,2'}$ = 5.4, 2′-OH), 5.65 (8 H, d, $J_{5,6}$ = 7.8, 5-H), 5.73 (8 H, d, $J_{1',2'}$ = 5.9, 1′-H), 6.87 (1 H, d, $J_{NH,CH}$ = 7.3, Boc-NH), 6.99 (1 H, m, ϵ-NH(Lys)), 7.35 (5 H, m, Ar-H(Bzl)), 7.45 (4 H, m, Ar-H(Cl-Z)), 7.65 (8 H, d, $J_{6,5}$ = 7.8, 6-H), 7.96 (8 H, m, 5′-NH), 8.14 (8 H, m, α-NH), 11.33 (8 H, s, 3-NH); MALDI-TOF HRMS (α-CHCA), *m/z* found 3360.13 (M + Na), calculated 3360.134.

HCl·(isoGln(5′U))$_8$-Lys-OH (17). **16** (0.300 g, 0.090 mmol) was dissolved in TFA (10 mL), and the solution was kept at −10 °C under nitrogen atmosphere. Thioanisole (1.84 mL, 15.7 mmol), *m*-cresol (0.97 mL, 9.27 mmol), and trimethylsilyl triflate (3.00 mL, 16.6 mmol) were successively added, and the mixture was stirred for 1 h at 0 °C. Ether (200 mL) was added, and the precipitate was filtered. The precipitate was dissolved in 5% aqueous ammonia. After 30 min, the solvent was removed under reduced pressure, and the residue was treated with 4 M HCl in dioxane for 30 min. The solvent was removed under reduced pressure, and the residue was purified by gel filtration and reverse phase HPLC to give compound **17** as a powder (0.223 g, 82%), ν_{max}(KBr)/cm^{-1} 3420, 1680, 1540, 1460, 1390, 1270, and 1110; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.35 (4 H, m, γ-CH$_2$(Lys) and δ-CH$_2$(Lys)), 1.70−1.83 (16 H, m, β-CH$_2$(Glu)), 2.14 (16 H, m, γ-CH$_2$(Glu)), 2.98 (2 H, m, ϵ-CH$_2$(Lys)), 3.84 (17 H, m, NH$_3^+$CH, 3′-H and 4′-H), 4.06 (8 H, m, 2′-H), 4.20 (8 H, m, α-CH), 5.17 (8 H, m, 3′-OH), 5.41 (8 H, m, 2′-OH), 5.65 (8 H, d, $J_{5,6}$ = 7.8, 5-H), 5.73 (8 H, d, $J_{1',2'}$ = 5.9, 1′-H), 7.24 (2 H, br, ϵ-NH$_2$), 7.65 (8 H, d, $J_{6,5}$ = 7.8, 6-H), 7.95 (8 H, m, 5′-NH), 8.14 (11 H, m, α-NH and NH$_3^+$), 8.59 (1 H, m, 5′-NH), 11.34 (8 H, m, 3-NH); MALDI-TOF HRMS (α-CHCA), *m/z* found 2980.06 (M − HCl), calculated 2980.055.

***N*$^{\alpha}$-*t*-Butoxycarbonyl-*N*^{5}-(*N*^{4}-benzoyl-5′-deoxy-5′-cytidyl)-L-isoglutamine benzyl ester (Boc-isoGln(5′BzC)-OBzl) (18).** To a solution of *N*-*t*-butoxycarbonyl-L-glutamic acid γ-benzyl ester (2.52 g, 7.47 mmol), HOBt (1.01 g, 7.47 mmol), and BOP reagent (3.30 g, 7.47 mmol) in DMF (100 mL) was added diisopropylethylamine (1.30 mL, 7.47 mmol). After 30 s of stirring at 0 °C, 5′-amino-5′-deoxycytidine (**3**)[18] (2.85 g, 8.22 mmol) was added, and the mixture was stirred for 1 h at room temperature. The solvent was removed under reduced pressure, and methanol was added to precipitate a white solid. After filtration, the product was recrystallized from methanol to give compound **18** (4.48 g, 90%), ν_{max}(KBr)/cm^{-1} 3420, 1690, 1560, 1480, 1320, 1260, and 1140; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.36 (9 H, s, *t*-Bu-H), 1.77−1.90 (2 H, m, β-CH$_2$), 2.37 (2 H, t, $J_{\gamma,\beta}$ = 7.8, γ-CH$_2$), 3.43 (2 H, m, 5′-H), 3.83 (1 H, q, $J_{3',2'} = J_{3',4'}$ = 5.5, 3′-H), 3.92 (1 H, q, $J_{4',3'} = J_{4',5'}$ = 5.5, 4′-H), 3.98 (1 H, q, $J_{CH,NH} = J_{CH,\beta}$ = 4.9, Boc-NHCH), 4.07 (1 H, q, $J_{2',1'} = J_{2',3'}$ = 4.7, 2′-H), 5.06 (2 H, s, PhCH$_2$), 5.17 (1 H, d, $J_{OH,3'}$ = 5.9, 3′-OH), 5.49 (1 H, d, $J_{OH,2'}$ = 5.4, 2′-OH), 5.80 (1 H, d, $J_{1',2'}$ = 3.9, 1′-H), 6.95 (1 H, d, $J_{NH,CH}$ = 7.8, Boc-NH), 7.34 (5 H, m, Ar-H), 7.40 (1 H, d, $J_{5,6}$ = 7.8, 5-H), 7.52 (2 H, t, $J_{m,o} = J_{m,p}$ = 7.6, Ar-*m*-H), 7.63 (1 H, t, $J_{p,m}$ = 7.6, Ar-*p*-H), 8.01 (2 H, d, $J_{o,m}$ = 7.3, Ar-*o*-H), 8.07 (1 H, t, $J_{NH,5'}$ = 5.6, 5′-NH), 8.19 (1 H, d, $J_{6,5}$ = 7.3, 6-H); MALDI-TOF HRMS (α-CHCA), *m/z* found 688.26 (M + Na), calculated 688.260.

***N*^{5}-(*N*^{4}-benzoyl-5′-deoxy-5′-cytidyl)-L-isoglutamine benzyl ester trifluoroacetic acid salt (TFA·isoGln(5′BzC)-OBzl) (19).** **18** (4.20 g, 6.31 mmol) was dissolved in TFA (50 mL), and the solution was kept at 0 °C for 30 min. TFA was removed under reduced pressure, and ether (200 mL) was added to give compound **19** as a powder (4.20 g, 98%), ν_{max}(KBr)/cm^{-1} 3400, 1680, 1560, 1480, 1320, 1250, 1200, and 1140; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.79−1.92 (2 H, m, β-CH$_2$), 2.42 (2 H, t, $J_{\gamma,\beta}$ = 7.9, γ-CH$_2$), 3.82 (3 H, m, NH$_3^+$CH, 3′-H and 4′-H), 4.10 (1 H, q, $J_{2',1'} = J_{2',3'}$ = 4.9, 2′-H), 5.07 (2 H, s, PhCH$_2$), 5.20 (1 H, d, $J_{OH,3'}$ = 5.6, 3′-OH), 5.52 (1 H, d, $J_{OH,2'}$ = 5.4, 2′-OH), 5.78 (1 H, d, $J_{1',2'}$ 4.2, 1′-H), 7.33 (5 H, m, Ar-H), 7.41 (1 H, d, $J_{5,6}$ = 7.7, 5-H), 7.51 (2 H, t, $J_{m,o} = J_{m,p}$ = 7.6, Ar-*m*-H), 7.62 (1 H, t, $J_{p,m}$ = 7.6, Ar-*p*-H), 8.03 (2 H, d, $J_{o,m}$ = 7.4, Ar-*o*-H), 8.05 (1 H, t, $J_{NH,5'}$ = 5.8, 5′-NH), 8.20 (4 H, m, NH$_3^+$ and 6-H), 11.45 (1 H, s, 4-NH); MALDI-TOF HRMS (α-CHCA), *m/z* found 566.22 (M − CF$_3$COO), calculated 566.225.

Boc-isoGln(5′U)-isoGln(5′BzC)-OBzl (20). To a solution of **9** (1.25 g, 2.65 mmol), HOBt (0.358 g, 2.65 mmol), and BOP reagent (1.17 g, 2.65 mmol) in DMF (50 mL) was added diisopropylethylamine (0.97 mL, 5.57 mmol). After 30 s of stirring at 0 °C, **19** (1.98 g, 2.92 mmol) was added, and the mixture was stirred for 2 h at room temperature. The solvent was removed under reduced pressure, and methanol was added to give compound **20** as a powder (2.65 g, 98%), ν_{max}(KBr)/cm^{-1} 3420, 1650, 1560, 1490, 1390, 1260, 1170, and 1080; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.35 (9 H, s, *t*-Bu-H), 1.78−1.92 (4 H, m,

β-CH$_2$), 2.16 (2 H, m, γ-CH$_2$), 2.36 (2 H, t, $J_{\gamma,\beta} = 7.8$, γ-CH$_2$), 3.42 (4 H, m, 5′-H(Urd) and 5′-H(Cyd)), 3.83 (3 H, m, 3′-H(Urd), 3′-H(Cyd) and 4′-H(Urd)), 3.91 (2 H, m, Boc-NHC$\underline{H}$ and 4′-H(Cyd)), 4.03 (1 H, q, $J_{2',1'} = J_{2',3'} = 5.4$, 2′-H(Urd)), 4.09 (1 H, q, $J_{2',1'} = J_{2',3'} = 4.7$, 2′-H(Cyd)), 4.29 (1 H, q, $J_{CH,NH} = J_{CH,\beta} = 7.3$, α-CH), 5.05 (2 H, s, PhCH$_2$), 5.17 (2 H, m, 3′-OH(Urd), and 3′-OH(Cyd)), 5.39 (1 H, d, $J_{OH,2'} = 5.9$, 2′-OH(Urd)), 5.51 (1 H, d, $J_{OH,2'} = 5.4$, 2′-OH(Cyd)), 5.64 (1 H, d, $J_{5,6} = 7.8$, 5-H(Urd)), 5.73 (1 H, d, $J_{1',2'} = 5.9$, 1′-H(Urd)), 5.78 (1 H, d, $J_{1',2'} = 3.9$, 1′-H(Cyd)), 6.87 (1 H, d, $J_{NH,CH} = 8.3$, Boc-NH), 7.34 (6 H, m, Ar-H and 5-H(Cyd)), 7.52 (2 H, t, $J_{m,o} = J_{m,p} = 7.8$, Ar-*m*-H), 7.63 (2 H, m, Ar-*p*-H and 6-H(Urd)), 7.95 (1 H, t, $J_{NH,5'} = 5.9$, 5′-NH(Urd)), 8.02 (3 H, m, Ar-*o*-H and α-NH), 8.20 (2 H, m, 6-H(Cyd) and 5′-NH(Cyd)), 11.31 (2 H, m, 3-NH(Urd) and 3-NH-(Cyd)); MALDI-TOF HRMS (α-CHCA), *m/z* found 1042.38 (M + Na), calculated 1042.377.

HCl·(isoGln(5′U)-isoGln(5′C))$_4$-Lys-OH (21). This compound was synthesized in a similar manner as the synthesis of **17** using hetero dimer **20** instead of homo dimer **8**. Each deprotection and coupling process was done with >95% and 80−95% yields, respectively; ν_{max}-(KBr)/cm^{-1} 3400, 1660, 1550, 1480, 1400, 1230, 1150, and 1050; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.34 (4 H, m, γ-CH$_2$(Lys) and δ-CH$_2$-(Lys)), 1.76−1.90 (16 H, m, β-CH$_2$(Glu)), 2.14 (16 H, m, γ-CH$_2$(Glu)), 2.99 (2 H, m, ϵ-CH$_2$(Lys)), 3.43 (16 H, m, 5′-H(Urd), and 5′-H(Cyd)), 3.84 (17 H, m, NH$_3^+$CH, 3′-H(Urd), 3′-H(Cyd), 4′-H(Urd) and 4′-H(Cyd)), 4.04 (4 H, m, 2′-H(Urd)), 4.10 (4 H, m, 2′-H(Cyd)), 4.27 (8 H, m, α-CH), 5.17 (8 H, m, 3′-OH(Urd) and 3′-OH(Cyd)), 5.40 (4 H, m, 2′-OH(Urd)), 5.52 (4 H, m, 2′-OH(Cyd)), 5.64 (4 H, m, 5-H(Urd)), 5.72 (4 H, m, 1′-H(Urd)), 5.79 (4 H, m, 1′-H(Cyd)), 7.23 (2 H, br, ϵ-NH$_2$(Lys)), 7.32 (4 H, m, 5-H(Cyd)), 7.64 (4 H, m, 6-H(Urd)), 7.94 (4 H, m, 5′-NH(Urd)), 8.03 (11 H, m, α-NH and NH$_3^+$), 8.22 (8 H, m, 6-H(Cyd) and 5′-NH(Cyd)), 11.33 (8 H, m, 3-NH(Urd) and 4-NH(Cyd)); MALDI-TOF HRMS (α-CHCA), *m/z* found 2976.12 (M − HCl), calculated 2976.119.

Acknowledgment. This paper is dedicated to the memory of Dr. Narutoshi Minamimoto, who has made a great deal of contribution to this work and unexpectedly deceased of cancer on November 14, 1999. We are grateful to the generous support of this work by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture, and also by a grant from the Asahi Glass Foundation. We would also like to thank Dr. Simon R. L. Everitt of the Inoue Photochirogenesis Project and Dr. George Lem of Columbia University for assistance in the preparation of this manuscript.

Supporting Information Available: Schemes of the synthetic routes and the experimental procedures for **2** and **3**; Tables of the $[\theta]_{max}$ values for **1**, **2**, **3**, and **4** in phosphate and borate buffers, the results of NOE experiments with **2** and **3** in phosphate and borate buffers, and the $[\theta]_{max}$ values for **2**, **6**, **17**, and **21** in phosphate and borate buffers (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA9935456