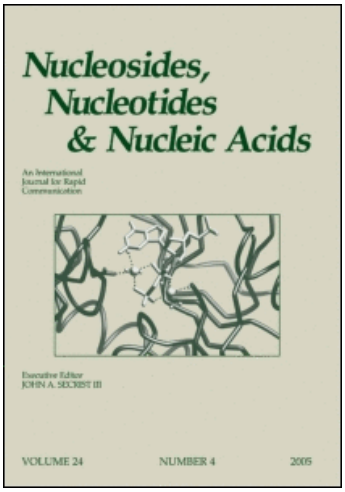

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Isopoly-L-ornithine Derivatives of Thymine and Thymidine

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ISOPOLY-L-ORNITHINE DERIVATIVES OF THYMINE AND THYMIDINE †

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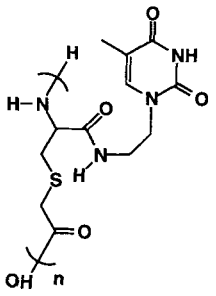
ABSTRACT: L-Ornithine derivatives of thymine, and thymidine gave oligomers by solid phase elongation reactions. These oligomers **2** and **3**, however, hardly interact with the complementary polynucleotide. Conformational studies of the oligomers with CD and NMR revealed that stable intramolecular hydrogen bonding was formed between thymine base and ornithine unit.

In recent years, considerable attention has been directed towards synthetic nucleic acid analogues in the hope of discovering new and more effective antisense compounds.[1] Various synthetic polymers containing nucleic acid bases were prepared, and the interactions of these polymers were studied with polynucleotide DNA and RNA.[2] These synthetic polymers with neither ribose nor phosphodiester were found to interact with DNA and polynucleotides. Recently, cysteine derivative of thymine **1** was prepared, and condensation reaction on the resin was found to give oligomers. The cysteine oligomer was also found to form a complex with poly A.[3] This paper deals with the preparation and properties of isopoly-L-ornithine derivatives of thymine **2** and thymidine **3**.



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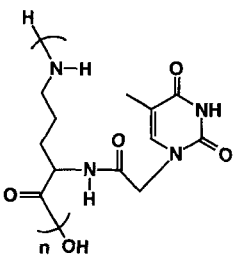

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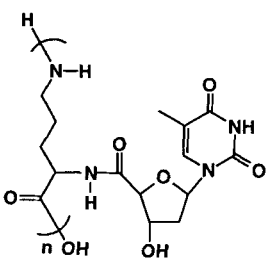

3

† This paper is dedicated to the late Professor Tsujiaki Hata, an extraordinary chemist.

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Preparation of Oligomers

Thymine derivative of L-ornithine was prepared according to SCHEME 1. 2-(Thymin-1-yl)acetic acid **6** was prepared by the reaction of thymine **4** with ethyl bromoacetate in *N, N*-dimethylformamide (DMF) with K_2CO_3 followed by hydrolysis.[4] The coupling reaction of **6** with the protected L-ornithine (N^{δ}-carbobenzyloxy-L-ornithine benzyl ester: L-Orn(Z)Bzl) was carried out with *N, N'*-carbonyldiimidazole (CDI) in DMF solution to give **7** in 67% yield. After deprotection of the protecting groups, δ-amino group was again protected with *tert*-butoxycarbonyl (Boc) group by di-*tert*-butyl dicarbonate [$(Boc)_2O$] to give the monomer **9** in 63% yield. The model compound of monomer **10** was prepared from N^{δ}-acetyl-L-ornithyl methylamine and **6** by condensation with CDI.

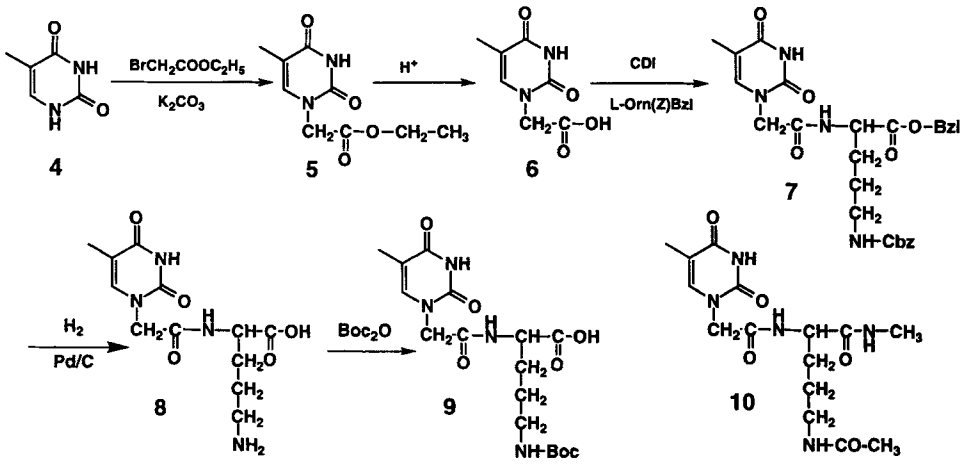
The oligomers **2** was prepared by solid phase method using *p*-nitrobenzophenone oxime resin prepared from BioBeads SX-1(200-400 mesh).[5] Glycine was used as a spacer between the resin and the first monomer, because the direct reaction of monomer with the resin was slow. As the coupling reagents, 1, 3-dicyclohexylcarbodiimide (DCC) was used for glycine, and benzotriazole-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) was used for the thymine monomer elongation reactions. The oligomer was removed from the resin using *N*-hydroxypiperidine solution. The obtained oligomer was analyzed by HPLC using adenosine immobilized silica gel.[6] Degree of polymerization of the oligomer was determined from the peak ratio in NMR spectra (CH_2 of glycine and CH of ornithine).

Thymidine derivative of L-ornithine was prepared according to SCHEME 2. Thymidine-5'-carboxylic acid **12** was obtained from thymidine **11** by oxidation with platinum/carbon catalyst according to the literature.[7] The coupling reaction of **12** with the protected L-ornithine was carried out with the activated ester method. The reaction of **12** with pentachlorophenyl trichloroacetate (PCP-TCA) in DMF gave the activated ester **13** as precipitate. After the coupling reaction, deprotection of all blocking groups and reprotection of the δ-amino group with $(Boc)_2O$ gave the monomer **16** in 52% yield from **12**. The oligomer of the thymidine derivative of L-ornithine **3** was prepared using the same method as the thymine derivatives **2**.

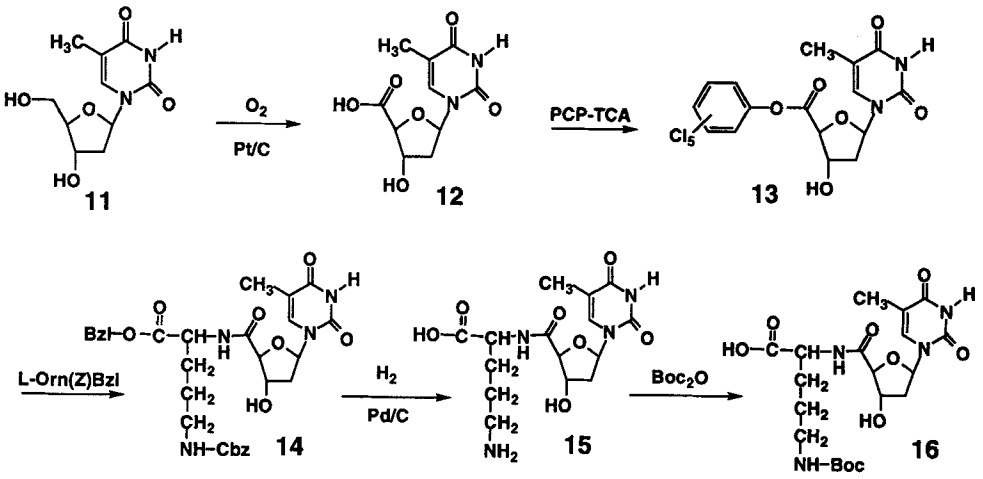
Interaction of the L-Ornithine Derivatives with Oligonucleotides

The complex formation of these oligomers **2** and **3** with polynucleotide (poly A) was studied in buffer solution using mixing curve method of UV spectra. Unfortunately, the hypochromicity was not observed between the octamer **2** with poly A. Heating of the solution at 50°C for 3 min caused increase of absorbance, but the same mixing curve was obtained after cooling. These facts suggested strong intramolecular interaction of the thymine derivative.

The hypochromicity with poly A was not observed for the thymidine derivative **3** either. The measurements were carried out at 15, 10, and 5°C for oligonucleotide (dA_8) and



SCHEME 1



SCHEME 2

poly A. At 5°C, only slight hypochromicity was observed. In this case, intramolecular interaction of thymidine and/or *syn*-conformation of thymidine might prevent the intermolecular interaction of thymidine with adenosine.

Structure of the Thymine Derivatives in Aqueous Solution

Conformation of the octamer **2** was studied by CD spectra as shown in FIG. 1. A negative Cotton effect was observed at 271 nm in Kolthoff buffer solution (pH 7.1). How-

ever, in water without buffer, positive Cotton effect was observed at pH 6.7, and decreased with increases of pH value. The monomer model compound **10** gave also the similar CD spectra to the octamer as shown in FIG. 2. As the solubility of **10** in water was low, the CD spectra were measured in alkaline aqueous solution. Decrease of positive peak at high pH value may be caused by deprotonation of thymine base. In buffer solution, however, **10** gave different CD spectra from the spectra in water without buffer as shown in FIG. 2. When these two solutions (aqueous solution at pH 9 and buffer solution) were mixed, the positive peak changed slowly to negative peak.

These induced Cotton effects may be caused by the interaction between the transition moment of thymine base and the chiral L-ornithine unit, where free rotation of thymine base is strictly inhibited by intramolecular interaction. The intramolecular interaction may be hydrogen bonding between thymine base and amide bonds of L-ornithine unit. The

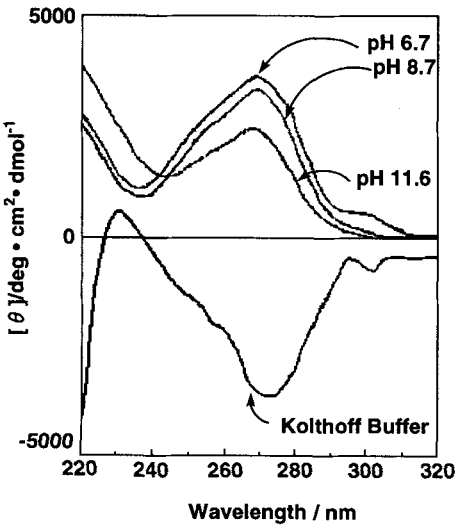


FIG. 1. CD spectra of octamer **2** in water and in Kolthoff buffer (pH 7.1) solutions.

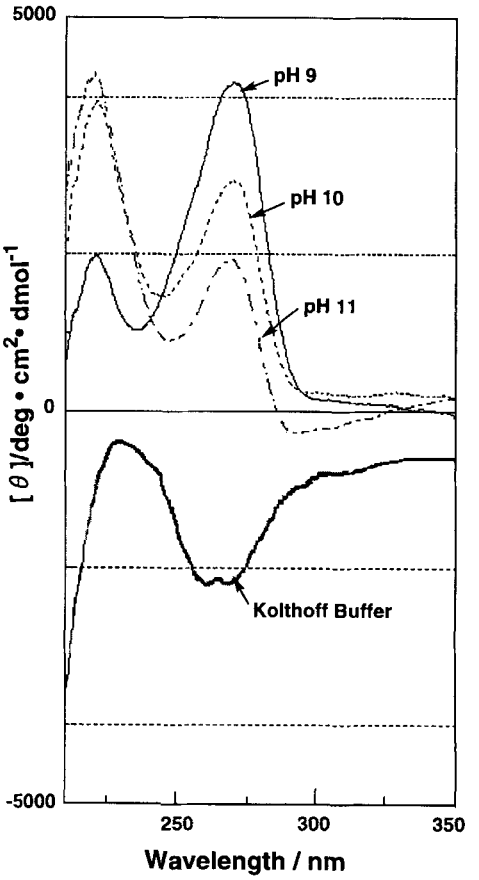
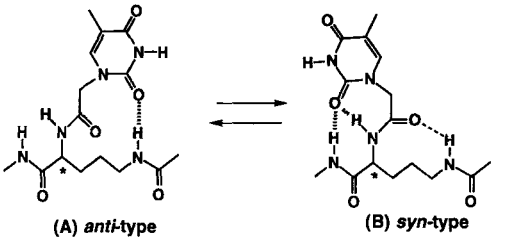
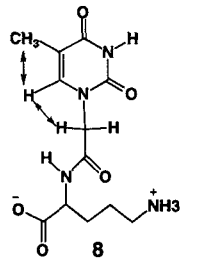


FIG. 2. CD spectra of monomer model **10** in water and in Kolthoff buffer (pH 7.1) solutions.

positive and negative Cotton effects suggest that two kinds of intramolecular interactions exist between the thymine base and the ornithine unit. One may be the hydrogen bonding interaction between thymine and δ-amide bond of ornithine (**A**: *anti*-type), the other may be the hydrogen bonding between thymine and the α-amide group and/or the side chain in L-ornithine unit (**B**: *syn*-type). However, it is difficult to determine which type exists in buffer solution.



Inhibition of free rotation of thymine base was also found in NMR spectra (600 MHz) in D_2O at various temperatures. The methylene protons at N1 of thymine in **8** were found to split into doublet by 17.6 Hz. This doublet was stable from 20° through 80°C. For one of these peaks at higher field, NOESY spectrum revealed the relation with 6-H of thymine. NMR spectrum of **8** in dimethyl sulfoxide-d_6, however, showed singlet peak for the methylene protons at N1 of thymine, suggesting free rotation of thymine base. These findings indicate that rotation of thymine base in **8** is tightly restricted in aqueous solution.



For the compound **7**, NMR spectra were studied in chloroform solution at 25, 35, 45, and 55°C. The peak of methylene protons at N1 of thymine (**d** in FIG. 3) was doublet (15.8 Hz) and became broad doublet at 55°C. While upfield shifts were observed for three amide protons (**a**, **b**, and **c**) from 25 to 55°C, shift for **c** was smaller than **a** and **b**. From these findings with modeling study, fixation of thymine base was concluded to be caused by hydrogen bonding between O-2 of thymine and δ-NH of ornithine (**c**) (**A**: *anti*-type). For the peak around 8-9 ppm assigned to thymine N^3-H (**a**), the highest upfield shift and broadening were observed from 25 to 55°C. This result is explained on the basis of intermolecular hydrogen bonding between thymine units.

Structure of the Thymidine Derivatives in Aqueous Solution

UV and CD spectra of thymidine **11**, thymidine-5'-carboxylic acid **12**, and the L-ornithine derivative **15** were measured in buffer solution as shown in FIG. 4 and TABLE 1. Similar spectra of these compounds suggest the same conformation of thymidine unit. Since the positive Cotton effect of thymidine **11** is known to be due to *anti*-conformation, conformation of the ornithine derivative **15** should be *anti*-conformation.

For these thymidine derivatives, NOE (nuclear Overhauser effect) was measured in D_2O, Kolthoff buffer-D_2O, and phosphate buffer-D_2O to give TABLE 2. Pyrimidine nucleoside in *anti*-conformation is known to show NOE between 6-H of thymine and 2' and 3' protons of ribose. For the thymidine derivatives in this work, NOE was observed for 2' and

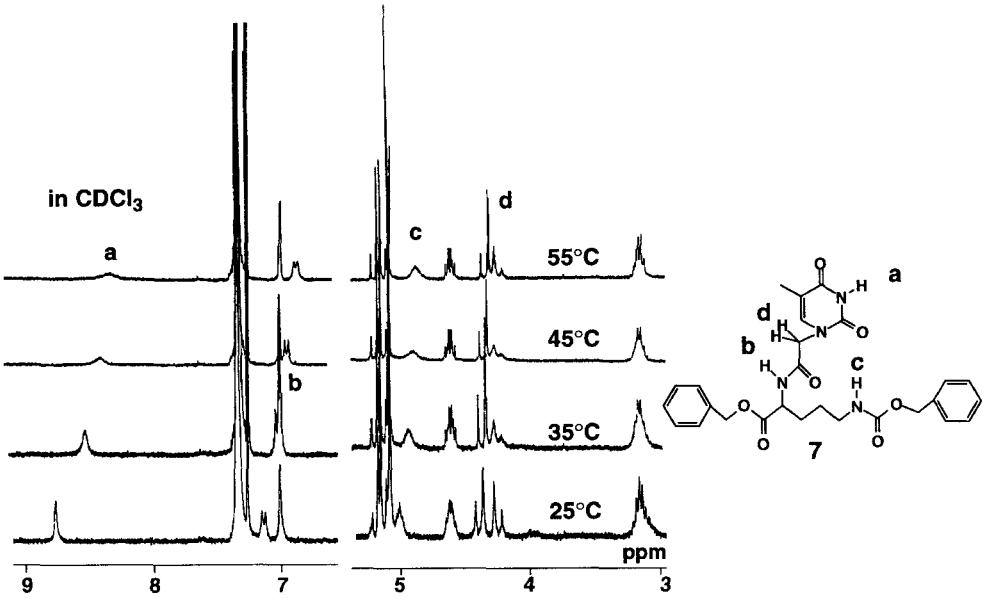


FIG. 3. ^{1}H-NMR spectra of **7** in $CDCl_3$ (270 MHz).

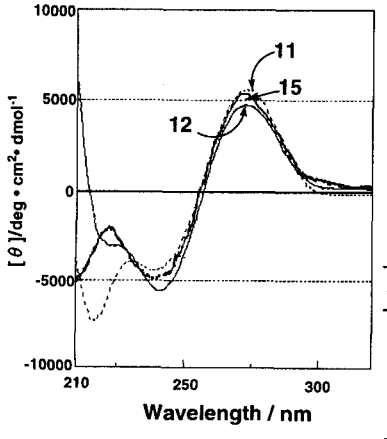


FIG. 4. CD spectra in Kolthoff buffer solution at pH 7.1.

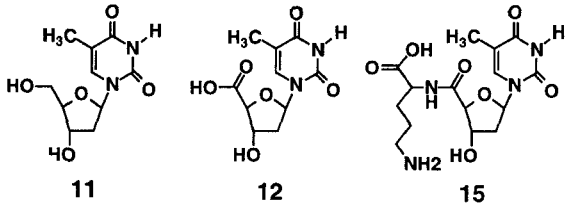


TABLE 1. CD and UV data.

Compound	B_{2u} [1]	B_{1u} [1]	UV: ε
11	5600[2] (273)[3]	-4500[2] (239)[3]	9700 (267)[3]
12	4700 (273)	-5700 (241)	11000 (267)
15	5400 (272)	-4900 (239)	9300 (265)

1) B_{2u}, B_{1u} ; π - π^* transitions in the nucleic acid bases.
2) deg • cm^2• $dmol^{-1}$; [θ] of each compound.
3) nm ; max wavelength.

TABLE 2. NOE data for **12** and **15** by irradiation at thymine 6-H (600 MHz).

	Solvent	1'	2'	3'	4'	5Me
12	D_2O	5.41	**6.54**	—	—	8.85
	Kolthoff buffer [a]	2.47	**6.80**	**0.89**	0.92	7.23
	Phosphate Buffer [b]	2.48	**6.86**	**1.06**	0.96	7.35
15	D_2O	5.48	**6.92**	**6.50**	—	8.10
	Kolthoff buffer [a]	9.99	**5.8**	**2.56**	—	7.21
	Phosphate Buffer [b]	10.27	**6.11**	**2.94**	—	7.47

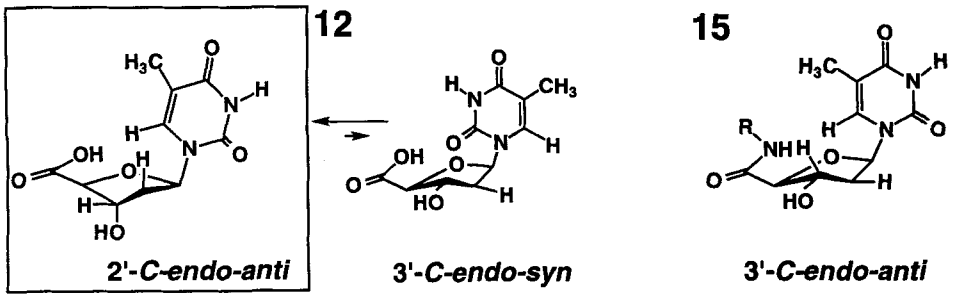
a) 1/10M KH2PO4-1/20M Na2B4O7 pH 7.1; b) 1/15M KH2PO4-1/15M Na2HPO4 pH 7.0

TABLE 3. Coupling constants of **12** and **15** at pH 7.1 (600 MHz).

Compound	1', 2'	1', 2"	2', 3'	2", 3'	3', 4'
12	**8.85**	—	—	**0.61**	**1.53**
15	**6.71**	**6.60**	**3.36**	**6.41**	**3.05**

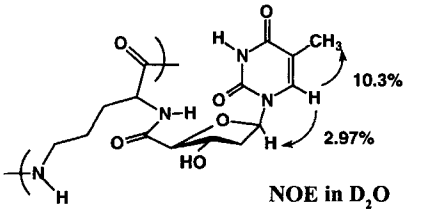
3' protons of ribose with 6-H of thymine suggesting *anti*-conformation. Coupling constants of the thymine derivatives in Kolthoff buffer-D_2O were tabulated in TABLE 3. From these data, percentage of 2'-*C-endo* was calculated to be 85.3% for **12**, and 68.8% for **15**.[8] Higher value of NOE between 6-H and 3'-H for **15** (TABLE 2) may be due to increase of 3'-*C-endo-anti* conformation.

These data of CD, NOE and coupling constants indicated that the thymidine derivatives **12** and **15** existed in the 2'-*C-endo-anti*-conformation. For the ornithine derivative **15**, however, proportion of 3'-*C-endo-anti*-conformation was high compared with **12**.



The octamer of the thymidine derivative **3** gave negative Cotton effect, while the monomer **15** gave positive Cotton effect (FIG. 5). Structural difference of the octamer **3** from the monomer **15** is the amide bonds. Therefore, *anti-* conformation of the thymidine monomer **15** changed to *syn*-conformation in the octamer **3** by intramolecular hydrogen bonding between thymine and the amide units. The hydrogen bonding may be formed between thymine O-2 and NH of α-amide bond in ornithine ($^{\delta}$NH of the next ornithine unit), because the interaction of thymine was not observed with the δ-amino group in **15**.

For the octamer of the thymidine derivative **3** in D_2O, NOE (600 MHz) of thymine 6-H was observed to be 2.94 % for 1'-H, and 10.3 % for 5-CH_3. However, NOE of 6-H was observed neither for 2'-H nor for 3'-H, suggesting *syn*-conformation for the oligomer **3**. Negative Cotton effect of the octamer **3** (FIG. 5) also supported the *syn*-conformation in aqueous solution. This conformational change observed for the octamer **3** may be due to the hydrogen bonding between O-2 of thymine and α-amide NH of ornithine (FIG. 5). The *syn*-conformation in the oligomer may inhibit the intermolecular interaction between the oligomer and poly A as mentioned above.



Thymine derivative of L-ornithine was concluded to form a stable intramolecular hydrogen bonding from CD and NMR spectra. The stable intramolecular interaction inhibits the intermolecular interaction between the oligomer and poly A. In the case of monomeric thymidine derivative, 2'-*C-endo-anti*-conformation was suggested from CD and NMR data. However, the *anti*-conformation of the monomer changed to the *syn*-conformation in the oligomer. The *syn*-conformation of the oligomer, therefore, inhibited the intermolecular interaction of the oligomer with poly A.

EXPERIMENTAL

^{1}H-NMR Spectra were recorded with a Varian unity INOVA600 and JEOL GSX270. UV Spectra were recorded with a JASCO UVIDEC 660. Circular dichroism spectra were obtained using a JASCO J-720S in concentration around 10^{-4} mol/L. HPLC was performed with a Tosoh CCP 8000 with a thermostated water bath (5-25 °C) and UV detector operating at 254 nm. The column used was the deoxyadenosine immobilized silica gel (0.3 mmol/g), and the mobile phase was 10 % methanol/phosphate buffer (pH 7).[6]

***N*$^{\alpha}$-{(Thymin-1-yl)acetyl}amino-*N*$^{\delta}$-carbobenzyloxy-L-ornithine benzyl ester (7).** 2-(Thymin-1-yl)acetic acid[4] **6** (3.0 g, 16 mmol) was dissolved into *N, N*-dimethylformamide (DMF, 5 mL) and was dried under reduced pressure at room temperature, and *N, N'*-carbonyldiimidazole (CDI, 2.8 g, 17 mmol)) was added to the solution. After CO_2 evolu-

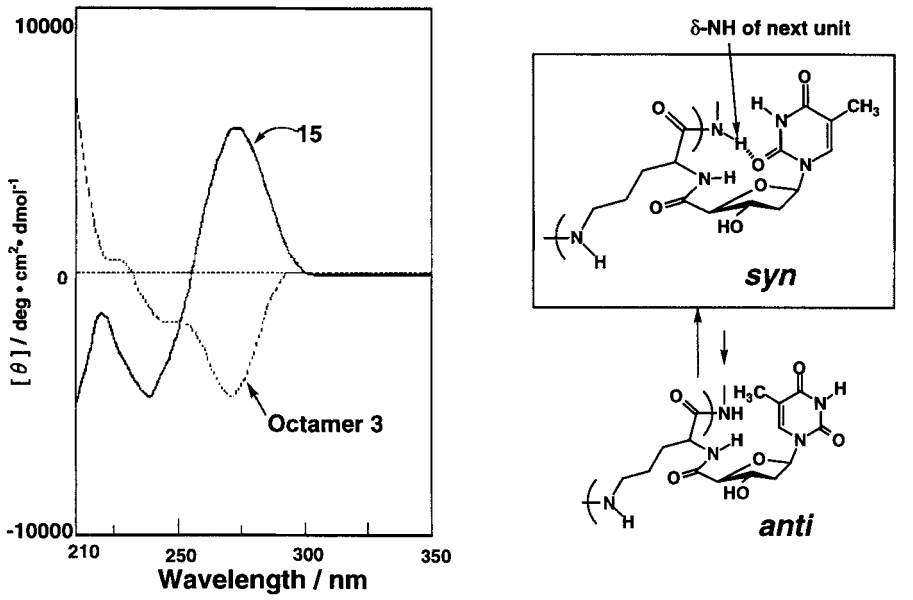


FIG. 5. CD spectra of monomer **15** and octamer **3** in Kolthoff buffer solution at pH 7.1.

tion was ceased, N^{δ}-carbobenzyloxy-L-ornithine benzyl ester in DMF (5 mL) prepared from the hydrochloride (5.6 g, 15 mmol)[9, 10] was added to the solution. The mixture was stirring for 2 days at room temperature, and solvent was removed under reduced pressure. The residue was reprecipitated with chloroform (300 mL), and recrystallized with ethanol to give **7** (5.0 g, yield 67%). R_f 0.82 in 1-butanol/acetic acid/water (5:2:3); mp 172-173 °C. MS (+FAB) 523 $[M+1]^+$. ^{1}H-NMR (270 MHz, DMSO-d_6) δ 11.28 (1H, s, ^{3}NH), 8.65 (1H, d, J = 7.56 Hz, $^{\alpha}$NH), 7.37 (5H, m, $^{\alpha}$Ph), 7.29 (5H, m, $^{\delta}$Ph), 7.01 (1H, s, ^{6}H), 5.11 (2H, s, $^{\alpha}$Bzl), 5.00 (2H, s, $^{\delta}$Bzl), 4.39 (2H, s, ^{1}NCH$_2$), 4.25 (1H, q, J = 4.32 Hz, $^{\alpha}$H), 3.00 (2H, q, J = 6.21 Hz, $^{\delta}$H), 1.73 (3H, s, ^{5}CH$_3$), 1.64 (2H, m, $^{\beta}$H), 1.44 (2H, m, $^{\gamma}$H).

N^{α}{(Thymin-1-yl)acetyl}amino-N^{δ}-*tert*-butoxycarbonyl-L-ornithine (9). The compound **7** (5.0 g, 9.8 mmol) dissolved in ethanol (500 mL) was added palladium on activated carbon (1.1 g), and the mixture was stirring for 2 h with hydrogen blowing. After filtration of the catalyst, filtrate was evaporated to give 2.8 g of N^{α}-{(thymin-1-yl)acetyl}amino-L-ornithine **(8)**. To the solution of **8** (2.8 g, 9.5 mmol) in 1N NaOH (9.5 mL) and water/dioxane (9.5/19 mL), di-*tert*-butyl dicarbonate ($(Boc)_2O$, 2 g, 10 mmol) was added. After stirring for 2 days, the solution was adjusted to pH 2 with 5 % $KHSO_4$. The product was extracted with ethyl acetate (three times), washed with water (several times), and dried with $MgSO_4$. Solvent was removed to yield 2.4 g of N^{δ}-protected compound **9** (63%). R_f

0.41 in 1-butanol/acetic acid/water (5:2:3); mp 159-162 °C. MS (+FAB) 399 $[M+1]^+$. ^{1}H-NMR (270 MHz, DMSO-d_6) δ 12.68 (1H, br, $^{\alpha}$COOH), 11.26 (1H, s, ^{3}NH), 8.47 (1H, d, J = 7.83 Hz, $^{\alpha}$NH), 7.41 (1H, s, ^{6}H), 6.82 (1H, t, J = 4.86 Hz, $^{\delta}$NH), 4.33 (2H, s, ^{1}NCH$_2$), 4.19 (1H, q, J = 5.13 Hz, $^{\alpha}$H), 2.91 (2H, q, J = 6.21 Hz, $^{\delta}$H), 1.74 (3H, s, ^{5}CH$_3$), 1.68 (2H, m, $^{\beta}$H), 1.52 (2H, m, $^{\gamma}$H), 1,37 (9H, s, *t*- Butyl).

***N*$^{\alpha}${(Thymin-1-yl)acetyl}amino-*N*$^{\delta}$-acetyl-L-ornithine methylamide (10).** To the solution of (thymin-1-yl)acetic acid **6** (0.55 g, 3 mmol) in DMF (10 mL), CDI (0.29 g, 3.3 mmol) was added. After the reaction was complete, the solution was added to the DMF (10 mL) solution of *N*$^{\delta}$-acetyl-L-ornithine methylamide (0.56 g, 3.0 mmol), and stirred for 24 h. The product **10** precipitated was collected and washed with DMF to yield 0.72 g (67 %). R_f 0.06 in benzene/ethanol (3:1). ^{1}H-NMR (270 MHz, DMSO-d_6) δ 11.27 (1H, s, ^{3}NH), 8.38 (1H, d, J = 8.10 Hz, $^{\alpha}$NH), 7.87 (1H, q, J = 4.86 Hz, $^{\alpha}$CONH), 7.82 (1H, t, J = 4.32 Hz, $^{\delta}$NH), 7.42 (1H, s, ^{6}H), 4.32 (2H, s, ^{1}NCH$_2$), 4.17 (1H, q, J = 5.13 Hz, $^{\alpha}$H), 2.99 (2H, q, J = 5.94 Hz, $^{\delta}$H), 2.58 (3H, d, J = 4.59 Hz, $^{\alpha}$NCH$_3$), 1.77 (3H, s, ^{5}CH$_3$), 1.74 (3H, s, $^{\delta}$NCOCH$_3$), 1.62 (2H, m, $^{\beta}$H), 1.43 (2H, m, $^{\gamma}$H).

***N*$^{\alpha}${(Thymidin-5'-yl)acetyl}amino-*N*$^{\delta}$-carbobenzyloxy-L-ornithine benzyl ester (14).** Thymidine-5'-carboxylic acid **12** was prepared according to the literature[7]. The compound **12** (0.51 g, 2.0 mmol) dissolved in DMF (50 mL) was sufficiently dried under reduced pressure. Pentachlorophenyl trichloroacetate (TCA-PCP, 0.82 g, 2.0 mmol) was added to the solution, and the mixture was stirred overnight. The activated ester precipitated was collected and washed with chloroform to give **13** (0.95 g). The compound **13** (0.95 g, 1.9 mmol) and *N*$^{\delta}$-carbobenzyloxyl-L-ornithine benzyl ester [11] (0.71 g, 2.0 mmol) were dissolved into DMF and stirred for 2 days at room temperature. The solvent was removed under reduced pressure, and the residue was reprecipitated with chloroform (300 mL), and recrystallized with ethanol to give **14** (0.88 g, yield 74 %). R_f 0.73 in chloroform/methanol (7:1); mp 140-142 °C. MS (+FAB) 595 $[M+1]^+$. ^{1}H-NMR (270 MHz, DMSO-d_6) δ 11.33 (1H, s, ^{3}NH), 8.71 (1H, d, J = 7.29 Hz, $^{\alpha}$NH), 8.10 (1H, s, ^{6}H), 7.35 (5H, m, $^{\alpha}$Ph), 7.27 (5H, m, $^{\delta}$Ph), 6.33 (1H, t, J = 7.29 Hz, $^{1'}$H), 5.66 (1H, d, J = 4.32 Hz, $^{\delta}$NH), 5.13 (2H, s, $^{\alpha}$Bzl), 4.99 (2H, s, $^{\delta}$Bzl), 4.33 (1H, br, $^{3'}$H, $^{4'}$H), 4.23 (1H, m, $^{\alpha}$H), 3.00 (2H, q, J = 6.48 Hz, $^{\delta}$H), 2.04 (2H, m, $^{2'}$H), 1.74 (3H, s, ^{5}CH$_3$), 1.64 (2H, m, $^{\beta}$H), 1.46 (2H, m, $^{\gamma}$H).

N$^{\alpha}${(Thymidin-5'-yl)acetyl}amino-N$^{\delta}$-*tert*-butoxycarbonyl-L-ornithine (16). The preparation of **16** from **14** was carried out using the same method as shown in **9**. The protecting groups of **14** were removed with palladium on activated carbon to give **15** in 88 % yield. The amino group of **15** was again protected with $(Boc)_2O$ to give **16** (85 % yield). R_f 0.15 in chloroform/methanol/acetic acid (95:5:3); mp 143-145 °C. MS (+FAB) 471 $[M+1]^+$. ^{1}H-NMR (270 MHz, DMSO-d_6) δ 12.59 (1H, br, $^{\alpha}$COOH), 11.36 (1H, s, ^{3}NH), 8.51 (1H, d, J = 7.29 Hz, $^{\alpha}$NH), 8.12 (1H, s, ^{6}H), 6.83 (1H, t, J = 5.40 Hz, $^{\delta}$NH), 6.31 (1H, t, J = 6.21 Hz,

$^{1'}$H), 5.64 (1H, d, $^{3'}$OH), 4.33 (2H, br, $^{3'}$H, $^{4'}$H), 4.20 (1H, m, $^{\alpha}$H), 2.91 (2H, q, J = 6.48 Hz, $^{\delta}$H), 2.10 (2H, m, $^{2'}$H), 1.75 (3H, s, ^{5}CH$_3$), 1.62 (2H, m, $^{\beta}$H), 1.46 (2H, m, $^{\gamma}$H), 1,31 (9H, s, *t*- Butyl).

Elongation of N^{α}–{(thymin-1-yl)acetyl}amino-N^{δ}-*tert*-butoxy-carbonyl-L-ornithine (2). *p*-Nitrobenzophenone oxime resin was prepared from BioBeads SX-1(200-400 mesh) according to Kaiser[5]. Glycine was introduced to the resin as the first amino acid with 1, 3-dicyclohexylcarbodiimide (DCC). Content of *N*-(*tert*-butoxycarbonyl)glycine (Boc-gly) in the resin was determined by the picric acid method[12] to be 0.314 mmol/g. The resin containing Boc-gly (0.5g) was washed in glass filter with dichloromethane (DCM, 8 mL, 2 times) and 25% trifluoroacetic acid (TFA)/dichloromethane (DCM). Deprotection of Boc group on the resin was performed with stirring in 25 % TFA/DCM for 25 min. After the reaction, the resin was washed with DCM (2 times), *iso*-propanol, DCM (3 times), and DMF. To the solution of **9** (0.19 g, 0.47 mmol) in DMF, benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent, 0.21 g, 0.47 mmol), 1-hydroxybenzotriazole hydrate (HOBt•H_2O, 64 mg, 0.47 mmol) in DMF, diisopropylethylamine (DIEA, 0.14 mL, 0.79 mmol) were added. The deprotected Boc-gly-resin was added to the DMF solution, and stirred for 30 min. The reaction was followed by Kaiser test[13] for a very small amount of the resin. Elongation of **9** was performed with repeating a series of operation for 8 times. The octamer was removed from the resin using *N*-hydroxypiperidine (HOPip) solution. The oxime resin containing oligomer and HOPip (47 mg, 0.23 mmol) were added into DMF (3 mL), and was stirred for 24 h at room temperature. The exhausted resin was filtered, and washed with DMF (3 mL, 3 times). The filtrate and the washing solution were combined and evaporated under reduced pressure. The residue was dissolved into a small amount of acetic acid, and $Na_2S_2O_4$ (136 mg, 2.35 mmol) in water (2 mL) was poured into the acetic acid solution. The solution was stirred for 60 min, and the solvent was evaporated. The solid product was obtained by addition of water to the residue. Reprecipitation with ether from methanol gave 0.42 mg of octamer **2**. The obtained oligomer was analyzed by HPLC using adenosine immobilized silica gel[6] to be a single peak. Degree of polymerization of the oligomer was determined from the NMR spectra ($^{\alpha}$CH$_2$ of glycine and $^{\alpha}$CH of ornithine). ^{1}H-NMR (270 MHz, D_2O) δ 7.52 (8H, br, ^{6}H), 4.34 (16H, br, ^{1}NCH$_2$), 4.09 (8H, br, $^{\alpha}$H), 3.71 (2H, s, ^{Gly}CH$_2$), 3.04 (16H, br, $^{\delta}$H), 1.68 (24H, s, ^{5}CH$_3$), 1.58 (16H, br, $^{\beta}$H), 1.47 (16H, br, $^{\gamma}$H), 1,23 (9H, s, *t*- Butyl).

Elongation of N^{α}–{(thymidin-5'-yl)acetyl}amino-N^{δ}-*tert*-butoxycarbonyl-L-ornithine (3). Preparation of octamer **3** of thymidine derivative was carried out according to the method used for preparation of **2**. ^{1}H-NMR (270 MHz, DMSO-d_6) δ 11.29 (8H, s, ^{3}NH), 8.29 (8H, m, $^{\alpha}$NH), 8.09 (1H, s, ^{6}H), 7.98 (8H, br, $^{\delta}$NH), 6.67 (1H, t, $^{\delta}$NHCOO), 6.31 (8H,

t, $^{1'}$H), 5.54 (8H, br, $^{3'}$OH), 4.29 (24H, br, $^{3'}$H, $^{4'}$H, $^{\alpha}$H), 3.75 (2H, d, $^{Gly}CH_2$), 3.02 (16H, br, $^{\delta}$H), 2.08 (16H, br, $^{2'}$H), 1.76 (24H, s, $^{5}CH_3$), 1.59 (32H, br, $^{\beta}$H, $^{\gamma}$H), 1,34 (9H, s, *t*-Butyl).

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