Essential Factors for Stabilization of the Predominant C3′-*endo* Conformation in Dinucleoside Phosphotriester Derivatives with Cyclonucleotide Bridge Structures at the Downstream 3′-Position

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This paper describes the synthesis of conformationally constrained dinucleoside cyclic phosphotriester derivatives and related compounds, such as Tpc3Um (**2**), Tpc3dU (**3**), Tpc2dU (**4**), pc3dU (**16**), and pc2dU (**25**), where c2 and c3 refer to ethylene and propylene bridges between the 5′-phosphate of the 3′-downstream nucleoside and the 5-position of the uracil residue. These studies found that the c3 linker is essential for fixing the 3′-downstream nucleoside residue in the C3′-*endo* puckering. The presence of the 2′-hydroxyl group at the 3′-downstream nucleoside is also crucial for stabilization of the C3′-*endo* conformation. Two sets of diastereomeric oligonucleotides, T_4(Tpc3Um)T_4 (**30**) and T_4 (Tpc3dU)T_4 (**33**), were synthesized, and each stereoisomer was isolated. The hybridization capability of these oligonucleotides was analyzed by melting point experiments.

Introduction

In the naturally occurring RNA structures analyzed to date, a large variety of conformations have been observed in the ribose moiety and the phosphodiester linkage. In nature, such complex variability of conformation allows for the creation of numerous functional biomolecules.[1−5] As the first step towards the creation of artificial RNA molecules of planned and designed three dimensional structure, for purposes of development of new functions, it should be advantageous to produce various kinds of dinucleotide building blocks capable of inducing particular changes in RNA backbone structure. Therefore, we have recently been investigating the synthesis of dinucleotide blocks possessing an unambiguously confirmed conformational rigidity, through the introduction of a cyclic structure that controls not only the sugar puckering, but also the direction of the backbone phosphodiester linkage. In the preceding paper,[6] we reported the synthesis of Umpc3Um (**1**) as a conformationally constrained dinucleotide monophosphate derivative that should be useful for fixing the normal backbone structure of RNA, as well as certain RNA bending structures.[7−10] The cyclic structure was introduced as a stable mimic of the intramolecularly hydrogen bound structure of 5-[(methylamino)methyl]-5′-uridylic acid[11] and a water-bridged 5′-pseudouridylic acid,[12] both of which have been identified as tRNA structural motifs predominantly possessing the C3′-*endo* conformation. Our study showed that the two diastereomers (diastereoisomerism due to phosphorus chirality) of **1** predominantly display C2′-*endo* (*S*-type) and C3′-*endo* (*N*-type) conformations at the ribose moieties of the 5′-upstream and 3′-downstream 2′-*O*-methyluridines, respectively.[6]

This paper deals with the synthesis of Tpc3Um (**2**), Tpc3dU (**3**), and Tpc2dU (**4**), in which the 5′-upstream or 3′-downstream Um of **1** is replaced with thymidine (T) or 2′-deoxyuridine (dU) (Figure 1). This was intended to clarify essential factors that regulate the sugar conformation in such cyclic phosphotriester-type dinucleotide derivatives (symbols c2 and c3 refer to the ethylene and propylene bridge linkers, respectively, bridging between the 5′-phosphate and the 5-position of the uracil residue). This study found that the rigidity of the C3′-*endo* form in the cyclic bridged structure is essentially a function both of the cyclic propylene linker and of the presence of the 2′-methoxy group.

Results and Discussion

Synthesis and ^{1}H NMR Analysis of Tpc3Um 2a and 2b, with S/N Junctions

Generally, nucleosides have two major sugar puckering conformers (C3′-*endo* and C2′-*endo,* Figure 2). More specifically, the C2′-*endo* conformation tends to be more predominant in deoxyribonucleosides than in ribonucleosides, because of the electronic effect of the 2′-hydroxyl group.[1a] As shown in Table 1, the percentage population of the C3′-*endo* form in thymidine was calculated by ^{1}H NMR analysis to be 29%,[13] while uridine (U) and 2′-*O*-methyluridine (Um) exhibited moderate C3′-*endo* predominance of 56% and 60%, respectively.

On the basis of these facts, the 5′-upstream Um of Umpc3Um (**1**) was replaced with thymidine to see if the conformation of the 5′-upstream nucleoside can be more

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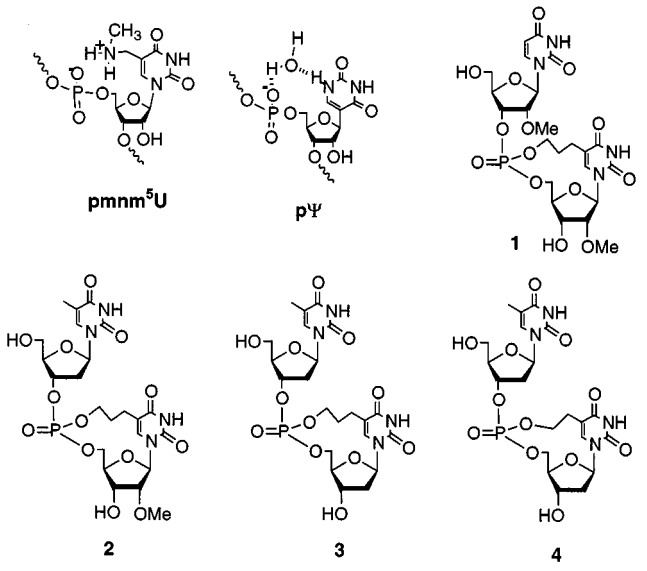


Figure 1. Intramolecular hydrogen bonds found in tRNAs and chemically synthesized dinucleotide blocks **1**−**4**.

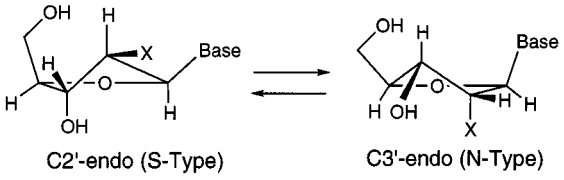
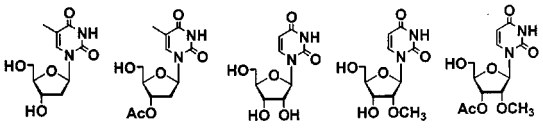


Figure 2. Equilibrium of two major conformers (C2′-*endo* and C3′-*endo*) of nucleosides.

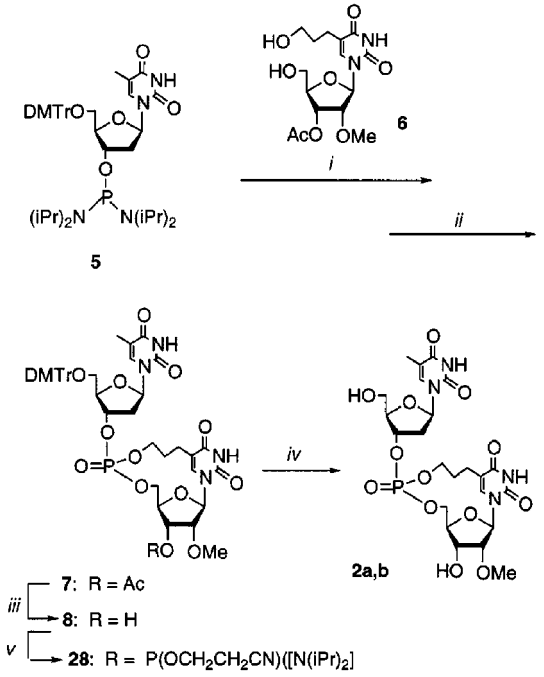
Table 1. Percentage C3′-*endo* conformation populations in 3′-*O*-acetylated thymidine, 2′-*O*-methyluridine and related compounds



	T[a]	Tac	U[a]	Um[a]	Um,ac
P(C3'-endo)[b]	29%	19%	56%	60%	50%
P(C2'-endo)[b]	71%	81%	44%	40%	50%

[a] Ref.[13]. − [b] The percentage population was calculated according to the following references: ref.[14] for thymidine derivatives and ref.[15] for uridine derivatives.

oriented to the C2′-*endo* form than in the 5′-upstream Um of **1**. To synthesize Tpc3Um (**2**), the thymidine 3′-phosphorodiamidite derivative **5** was prepared according to the method reported by van Boom et al.[16] Compound **5** was condensed with 3′-*O*-acetyl-5-(hydroxypropyl)-2′-*O*-methyluridine (**6**)[10] in the presence of 1*H*-tetrazole[17] to give the triester block **7** as a diastereomeric mixture in 85% yield, as shown in Scheme 1.

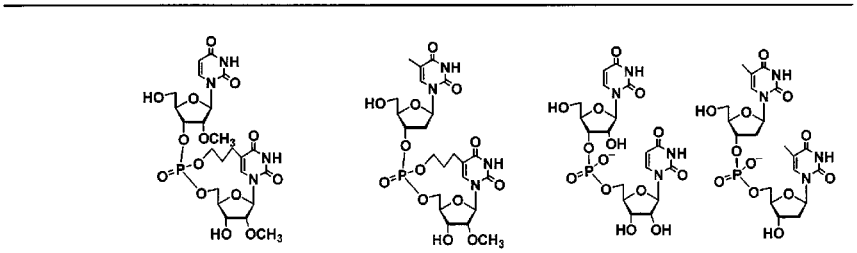


Scheme 1. (i) 1*H*-tetrazole/CH_3CN/dioxane, room temp., 2 h; (ii) *i*BuOOH /CH_3CN/dioxane, room temp., 1 h; (iii) aqueous NH_3-pyridine (1:1, v/v), room temp., 3 h; (iv) 80% AcOH, room temp., 30 min; (v) $ClP(OCH_2CH_2CN)(N{i}Pr_2)$,$Et_3N$, CH_2Cl_2, room temp., 2 h.

Deacetylation of **7** with ammonia gave the 3′-free compound **8**, which was in turn converted in good yield into the desired dimer **2** by treatment with 80% acetic acid. Attempted separation of the diastereomers of **2** or **8** by silica gel column chromatography was unsuccessful. Finally, however, separation of the diastereomers **2a** and **2b** by HPLC resulted in successful isolation of each diastereomer (Symbols "**a**" and "**b**", respectively, refer to rapidly eluted compounds and slowly eluted compounds from a reversed phase HPLC column).

Table 2 shows the results of the ^{1}H NMR analysis of Tpc3Um(fast) **2a**, and Tpc3Um(slow) **2b**. The C3′-*endo* conformer percentage populations of the 5′-upstream nucleoside in the two diastereoisomers **2a** and **2b** were estimated to be 22% and 27%, while those in **1a** and **1b** were reported to be 30% and 27%, respectively. These results show that, as expected, thymidine is a slightly better nucleoside from the point of view of enrichment of the C2′-*endo* conformation at the 5′-upstream nucleoside in order to constrict the conformation of a dinucleotide block as the S/N junction, which is equivalent to the C2′-*endo*/C3′-*endo* junction. The percentage populations of the C3′-*endo* conformation of the 5′-upstream T in Tpc3Um **2a** and **2b** are lower by 14−19% than that (41%) of *T*pT (Table 2). The same is true for the difference between Umpc3Um **1a** and **1b** and UpU, as shown in Table 2: the C2′-*endo* population of the 5′-upstream U of UpU was reported to be 53%[18] or 56%.[13] On the other hand, the 5′-upstream Um of **1** becomes enriched in the C2′-*endo* conformer. These results reflect the fact that the 3′-phosphoryl residue indeed affects

Table 2. Percentage C3′-*endo* conformation populations in the 5′-upstream and 3′-downstream nucleosides of dimers containing a cyclouridylic acid derivative and related compounds



	Umpc3Um(fast) 1a		Umpc3Um(slow) 1b		Tpc3Um(fast) 2a		Tpc3Um(slow) 2b		UpU		TpT	
	Ump[a]	pc3Um[a]	Ump[a]	pc3Um[a]	Tp	pc3Um	Tp	pc3Um	Up[b]	-pU[b]	Tp[b]	pT[b]
P(C3′-endo)[d]	30%	82%	27%	~90%	22%	82%	27%	89%	56% 53%[c]	56% 63%[c]	41%	33%

[a] Ref.[6]. – [b] Ref.[13]. – [c] Ref.[18]. – [d] The percentage populations of ribose and deoxyribose residues were calculated according to the following equations: ref.[14] for the thymidine and ref.[15] for the uridine residues.

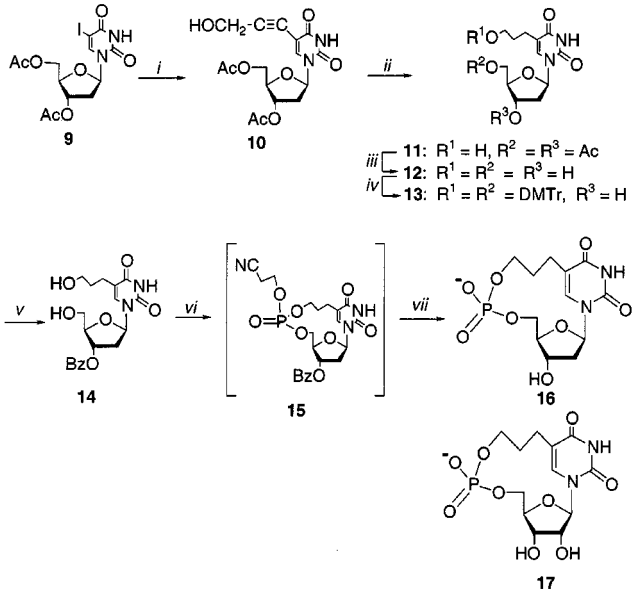
the sugar moiety like an acetyl group, which induces the C2′-*endo* conformation more effectively when attached to the 3′-hydroxy, as shown in Table 1. The effect of the acetyl group in contributing to the C3′-*endo* conformation was estimated to be as much as 10% in both 3′-*O*-acetylthymidine and 3′-*O*-acetyl-2′-*O*-methyluridine.

On the other hand, the percentage populations of the C3′-*endo* conformations of the 3′-downstream Um in **2a** and **2b** were estimated to be 82% and 89%, respectively. In addition, the sums of $J_{1',2'}$ and $J_{3',4'}$ of the 3′-downstream pc3Um of **2a** and **2b** are 9.6 and 9.3 Hz, respectively. These values are fairly normal; in comparison, the average sum value of $J_{1',2'}$ and $J_{3',4'}$ in typical ribonucleosides is 9.4 ± 0.2 Hz.[19] It was therefore to be expected that Tpc3Um **2a** or **2b** should be usable as a good building block as regards the ideal sugar conformation to mimic a bending motif with the rigid S/N junction, although this combination produces a chimeric mixture comprising a deoxynucleoside (T) and a ribonucleoside (Um).

Requirement of the 2′-Hydroxy Group for Stabilization of the C3′-*endo* Conformation in 5′-Cyclouridylic Acid Derivatives

Should it be possible to replace the 3′-downstream pc3Um of Um*pc3Um* **1** or T*pc3Um* **2**, possessing an S/N junction site, with its deoxy counterpart pc3dU **(16)** without loss of conformational rigidity, the synthesis of bent RNA would be facilitated, since the 3′-downstream component **14** can be easily prepared. In addition, it would also engender a possible new route to flexible single-strand DNA oligomers possessing a unique ridged S/N junction at a certain point.

Treatment of 3′,5′-di-*O*-acetyl-5-iododeoxyuridine (**9**)[20] with 2-propyn-1-ol in the presence of a palladium catalyst gave the alkynylated product **10** in 71% yield. Hydrogenation of **10** gave the reduction product **11** in 95% yield. Alkaline hydrolysis of **11**, followed by the bis-dimethoxytritylation of the resulting triol **12**, gave the dimethoxytritylated product **13**. In situ acylation of **13** with benzoic anhydride in the presence of DMAP, followed by treatment with 80% acetic acid, gave the 3′-*O*-benzoylated product **14** in 64% overall yield from **11**. Cyclization of **14** with bis(diisopropylamino)(2-cyanoethoxy)phosphane,[16] followed by oxidation with *t*BuOOH,[21] gave the desired product **15**, which was converted into **16** in situ, in 64% overall yield from **14** (Scheme 2).

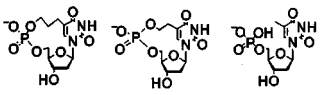


Scheme 2. (*i*) 2-propyn-1-ol, DMF, Et_3N, CuI, $Pd(PPh_3)_4$, room temp., 6 h; (*ii*) H_2/Pd, dioxane/MeOH (1:1, v/v), room temp., 43 h; (*iii*) 25% aqueous NH_3/pyridine (1:1, v/v), room temp., 10 h; (*iv*) DMTrCl, pyridine, room temp., 3 h; (v) Bz_2O, DMAP, pyridine, room temp., 7 h; then 80% AcOH, room temp., 30 min; (*vi*) $NCCH_2CH_2OP(NiPr_2)_2$, 1*H*-tetrazole, CH_3CN/dioxane, room temp., 1 h; *t*BuOOH, CH_3CN/dioxane, room temp., 30 min; (*vii*) 25% aqueous NH_3/pyridine (3:1, v/v), room temp., 24 h.

Conformational Analysis of Deoxy Counterpart 16

To determine the ratio of the C3′-*endo* and C2′-*endo* conformations over a series of deoxyribonucleosides, the well-known Altona equation[14] P(C3′-*endo*) =

Table 3. ^{1}H NMR spectroscopic data and conformational analysis of cyclodeoxyuridylic acid derivatives (**16** and **25**) and 5′-thymidylic acid



	pc3dU 16	pc2dU 25	pT[a]
$J_{4',5'}+J_{4',5''}$	—	9.0	7.4
$J_{1',2'}+J_{3',4'}$	10.6	6.4	10.6
P(C3′-endo)[b]	73%	unusual conformation	33%
P(g$^+$)[c]	—	44%	61%

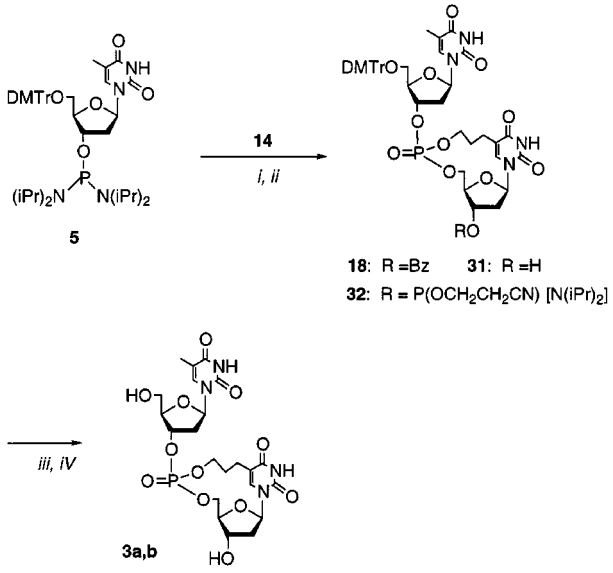
[a] Ref.[13]. — [b] The percentage population was calculated according to the following equation reported by Rinkel and Altona.[14] — [c] The torsion angle P(g+) was calculated according to the following equation reported by Altona.[22]

[1 − ($J_{1',2'}$ + $J_{1',2''}$ − 9.8)/5.9] was used. Generally, this equation applies only for deoxyribonucleoside derivatives with the sum of $J_{1',2'}$ and $J_{3',4'}$ of 10.7 ± 0.2 Hz.[19] We analyzed in detail the conformation of the deoxy counterpart **16**, with a propylene bridge. Consequently, it turned out that the sum of $J_{1',2'}$ and $J_{3',4'}$ of **16** is 10.6 Hz, as shown in Table 3.

This value is within the standard deviation and is effectively the same as that of 5′-thymidylic acid. This result suggests that compound **16** has a fairly natural structure without strain. The percentage population of the C3′-*endo* conformer was calculated by the Altona equation to be 73%.[14] On the other hand, the C3′-*endo* conformer percentage population in 5′-thymidylic acid was calculated to be 33%, while that of the ribose derivative **17** had been reported as 88%.[8] These results indicate that the cyclic structure contributes significantly to the predominance of the C3′-*endo* conformation, but is insufficient for complete fixing of this conformation. Therefore, it can clearly be concluded that both the cyclic structure and the 2′-hydroxy group are essential factors for adequate stabilization of the C3′-*endo* conformation of 5′-thymidylic acid.

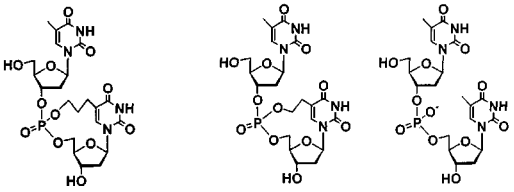
Synthesis and Conformational Analysis of Dimer Tpc3dU (3)

Although the above results indicate that the use of **16** as the 3′-downstream component causes loss of conformational rigidity, which is unfavorable for design of a bent motif, we synthesized Tpc3dU **3a** and **3b** as reference compounds for comprehensive studies, as shown in Scheme 3. Condensation of **5** with **14** gave the product **18** in 71% yield. Compound **18** was deprotected in the usual manner to give **3**, and the two isomers **3a** and **3b** were separated by HPLC. The results of the conformational analysis are summarized in Table 4.



Scheme 3. (*i*) 1*H*-tetrazole, CH_3CN/dioxane (1:1, v/v), room temp., 2 h; (*ii*) *t*BuOOH, CH_3CN/dioxane (1:1, v/v), room temp., 30 min; (*iii*) 25% aqueous NH_3/pyridine (1:3, v/v), room temp., 18 h; (*iv*) 80% AcOH, room temp., 1 h.

Table 4. ^{1}H NMR spectroscopic data and conformational analysis of Tpc3dU **3a** and **3b**, Tpc2dU **4a** and **4b**, and TpT



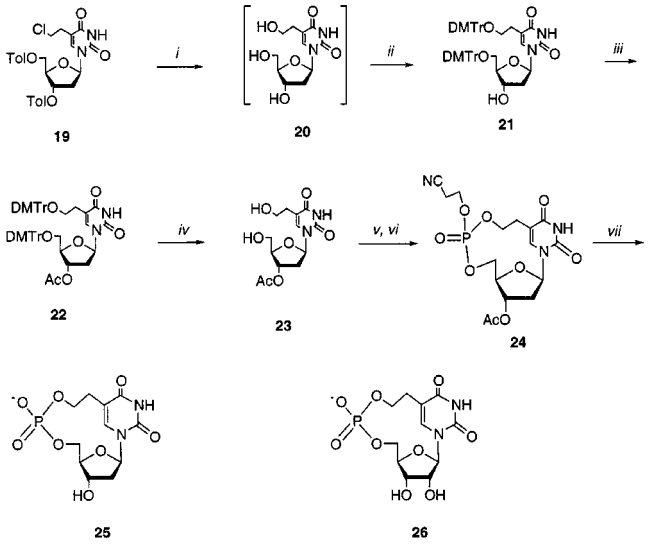
	Tpc3dU(fast) 3a		Tpc3dU(slow) 3b		Tpc2dU(fast) 4a		Tpc2dU(slow) 4b		TpT	
	Tp	pc3dU	Tp	pc3dU	Tp	pc2dU	Tp	pc2dU	Tp[a]	pT[a]
P(C3′-endo)[b]	25%	82%	25%	96%	23%	—	17%	—	41%	33%

[a] Ref.[13]. — [b] The percentage population was calculated according to the following equation reported by Rinkel and Altona.[14]

In Tpc3dU(fast) **3a** and Tpc3dU(slow) **3b**, the C3′-*endo* content of the 5′-upstream thymidine was in each case calculated to be 25%. The sum of $J_{1',2'}$ and $J_{3',4'}$ in the 3′-downstream thymidine in **3a** is 9.0 Hz, which deviates somewhat from that (10.9 Hz) of TpT or the average value, 10.7 ± 0.2 Hz, in typical deoxyribonucleoside derivatives.[19] These differences are considerable, so some disordered form must be contemplated. Therefore, it seems that the calculated value − 82% − for the C3′-*endo* conformation in **3a** might be an overestimate as compared with that (73%) of the 5′-deoxycyclouridylic acid derivative pc3dU **16**. Similar reasoning should be true for **3b**, although the sum of $J_{1',2'}$ and $J_{3',4'}$ in the 3′-downstream thymidine in **3b** was not measurable.

Synthesis of 5′-Cyclodeoxyuridylic Acid Derivative pc2dU (25), Possessing an Ethylene Bridge

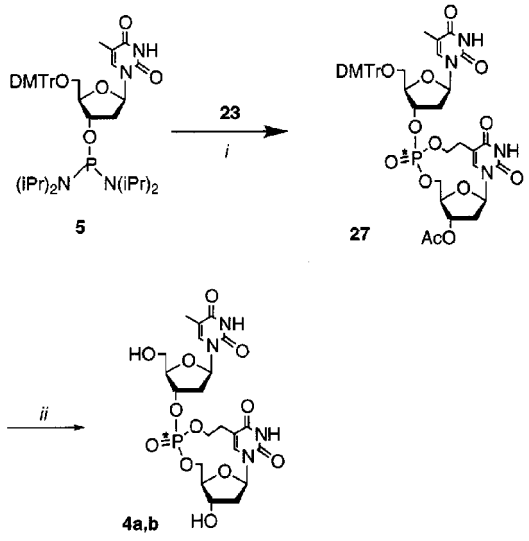
Finally, we examined the effect of the number of methylene groups in the covalent bridge structure on conformational properties at the dimer level. To this end, a shorter ethylene chain was used in place of the propylene moiety. In order to obtain pc2dU (**25**), 5-(2-chloroethyl)-3′,5′-di-*O*-toluoyldeoxyuridine (**19**) was prepared according to the method reported by Griengl et al.[23] Alkaline hydrolysis of **19** gave the triol **20**, which was protected in situ with DMTrCl to give the bis-dimethoxytritylated product **21**, in 90% yield from **19**. Acetylation of **21** gave the 3′-acetate **22** in 90% yield. Treatment of **22** with 80% acetic acid gave the diol **23** in 84% yield (Scheme 4).



Scheme 4. (*i*) 0.5 M NaOH/pyridine (1:1, v/v), room temp., 12 h; (*ii*) DMTrCl, pyridine, room temp., 2 h; (*iii*) Ac_2O, pyridine, room temp., 8 h; (*iv*) 80% AcOH, room temp., 30 min; (*v*) $NCCH_2CH_2OP(NiPr_2)_2$, 1*H*-tetrazole, CH_3CN, room temp., 1 h; (*vi*) *t*BuOOH, CH_3CN, room temp., 1 h; (*vii*) 25% aqueous NH_3/pyridine (1:1, v/v), room temp., 2.5 h.

Phosphitylation of **23** with bis(diisopropylamino)(2-cyanoethoxy)phosphane,[24] followed by oxidation with *t*BuOOH, gave the protected cyclodeoxyuridylic acid derivative **24** in 68% yield, as a diastereomeric mixture due to the chirality of the phosphotriester-type phosphorus atom. The ratio of the two isomers was 66:34. Treatment of **24** with ammonia gave the cyclodeoxyuridylic acid derivative **25** in 50% yield.

The ^{1}H NMR spectroscopic data of pc2dU **25** are shown in Table 3. The $J_{1',2'}$ value observed is 3.9 Hz and the $J_{3',4'}$ value is 2.5 Hz. The sum of $J_{1',2'} + J_{3',4'}$ in **25** is 6.4 Hz, deviating far from the standard. Actually, calculation using the equation described in the literature[14] produced an abnormal figure of 103% as the C3′-*endo* conformation percentage population in **25**. This result appears to be brought about by the presence of the cyclic ethylene bridge structure. The abnormal $J_{1',2'} + J_{3',4'}$ value in **25** suggests that **25** has a disordered structure in the deoxyribose ring. In the case of the previously reported ribose counterpart pc2U **26**, the sum of $J_{1',2'} + J_{3',4'}$ was 8.6 Hz.[7] Since the average value



Scheme 5. (*i*) 1*H*-tetrazole, CH_3CN, room temp., 40 min, *t*BuOOH, CH_3CN, room. temp., 1 h; (*ii*) 25% aqueous NH_3/pyridine (1:1, v/v), room temp., 9 h.

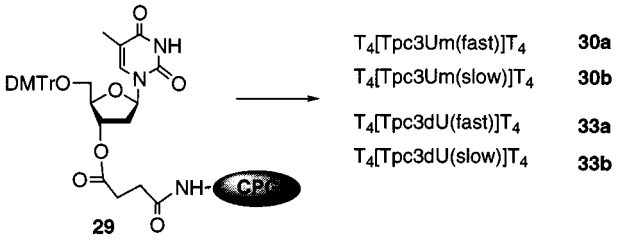
of the sum of $J_{1',2'}$ and $J_{3',4'}$ in ribonucleosides is reported to be 9.4 ± 0.2 Hz,[19] there is a considerable gap, of 0.8 Hz, between these values. It is therefore concluded that the ethylene bridge must induce some conformational disorder in both cyclodeoxy and cycloribo nucleotides pc2dU **25** and pc2U **26**.

Compounds Tp2dU **4a** and **4b** were synthesized by condensation of **5** with **23**, affording product **27** as shown in Scheme 5, and were isolated by HPLC and analyzed by ^{1}H NMR.

In both compound **4a** and **4b**, the C3′-*endo* content of the 5′-upstream thymidine is considerably less than that in TpT, as shown in Table 4. Since the sugar pucker of the 3′-downstream cyclothymidine in **4a** or **4b** is unusual because of the ethylene bridge, the exact ratio of the C3′-*endo*/C2′-*endo* conformers cannot be estimated by the standard equation as shown in Table 1. Actually, the sum (8.4 Hz) of $J_{1',2'}$ and $J_{3',4'}$ is extremely small, as shown in the case of **4a**. Therefore, we did not incorporate this dimer block into oligonucleotides.

Synthesis of T_4[Tpc3Um(fast)]T_4 (30a), T_4[Tpc3Um(slow)]T_4 (30b), T_4[Tpc3dU(fast)]T_4 (33a) and T_4[Tpc3dU(slow)]T_4 (33b)

For incorporation of Tpc3Um into DNA, compound **7** was converted into the 3′-free hydroxyl derivative **8**. This



Scheme 6

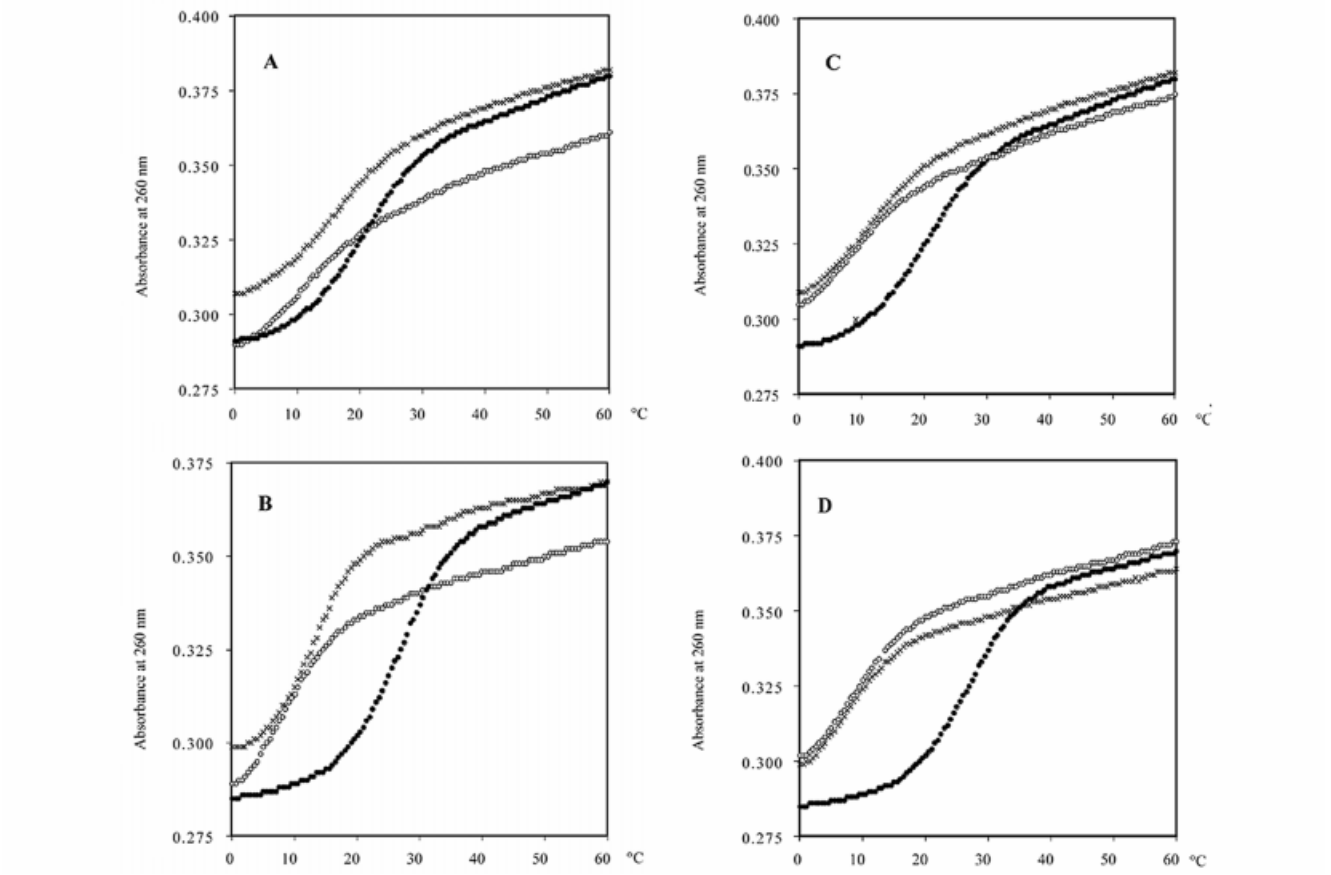


Figure 3. Melting curves of the duplexes formed between $A(pA)_9$ or $d[A(pA)_9]$ and oligonucleotides **30** or **33**. Panel **A**: ○ $A(pA)_9-T_4$[Tpc3Um(fast)]T_4(**30a**), × $A(pA)_9-T_4$[Tpc3Um(slow)]T_4(**30b**), • $A(pA)_9-T(pT)_9$; Panel **B**: ○ $d[A(pA)_9]-T_4$-[Tpc3Um(fast)]T_4(**30a**), × $d[A(pA)_9]-T_4$[Tpc3Um(slow)]T_4(**30b**), • $d[A(pA)_9]-T(pT)_9$; Panel **C**: ○ $A(pA)_9-T_4$[Tpc3dU(fast)]T_4(**33a**), × $A(pA)_9-T_4$[Tpc3dU(slow)]T_4(**33b**), • $A(pA)_9-T(pT)_9$; Panel **D**: ○ $d[A(pA)_9]-T_4$[Tpc3dU(fast)]T_4(**33a**), × $d[A(pA)_9]-T_4$[Tpc3dU(slow)]T_4(**33b**), • $d[A(pA)_9]-T(pT)_9$.

compound was phosphitylated to give the amidite unit **28**. The decadeoxynucleotides T_4[Tpc3Um(fast)]T_4 (**30a**) and T_4[Tpc3Um(slow)]T_4 (**30b**) were synthesized as a diastereomeric mixture according to standard DNA synthesis methodology, by use of **28** and aminopropyl-CPG resin **29**. Using HPLC, it was possible to separate and isolate stereoisomers **30a** and **30b** completely. Both isomers **30a** and **30b** were analyzed by enzymatic degradation using snake venom phosphodiesterase and calf intestinal alkaline phosphatase to give a mixture of T and Tpc3Um(fast) and a mixture of T and Tpc3Um(slow) in the correct ratios, as described in the previous paper.[10]

In a similar manner, the decadeoxynucleotides T_4[Tpc3dU(fast)]T_4 (**33a**) and T_4[Tpc3dU(slow)]T_4 (**33b**) were synthesized from the amidite unit **32**, which was derived from **31** (Scheme 3), and characterized by the enzymatic analysis.

Hybridization with $A(pA)_9$ and $d[A(pA)_9]$

The results of melting point experiments on the duplexes formed between $[A(pA)_9]-T_4$[Tpc3Um(fast)]T_4 **30a** and $[A(pA)_9]-T_4$[Tpc3Um(slow)]T_4 **30b** are summarized in Table 5 and Figure 3 (A). In these experiments, the oligonucleotide **30a** derived from the rapidly eluted Tpc3Um has a melting point lower than that of T_4[Tpc3Um(slow)]T_4 **30b**. The same is true for hybridization with the deoxy counterpart $d[A(pA)_9]$, as shown in Figure 3 (B). These results are similar to those found for the duplexes of U_4[Umpc3Um(-fast)]U_4 with $d[A(pA)_9]$ and $A(pA)_9$, but the difference in ΔΔmelting points has become slightly smaller than that relating to U_4[Umpc3Um (fast)]U_4.[6] These results imply that T_4[Tpc3Um(fast)]T_4 **30a** has an S/N bending structure with the *S* configuration on the phosphorus atom.

Table 5. Melting points values of the duplexes between modified oligonucleotides and decaadenylate or decadeoxyadenylate

Oligonucleotides		Melting temperature of duplex (°C)			
		$A(pA)_9$	ΔTm	$d[A(pA)_9]$	ΔTm
$T(pT)_9$		20.3		27.0	
T_4[Tpc3Um(fast)]T_4	**30a**	11.0	−9.3	8.0	−19.0
T_4[Tpc3Um(slow)]T_4	**30b**	15.3	−5.0	13.4	−13.6
T_4[Tpc3dU(fast)]T_4	**33a**	8.0	−12.3	8.6	−18.4
T_4[Tpc3dU(slow)]T_4	**33b**	11.1	9.2	7.1	−19.9

In contrast, there was less difference in melting points between $A(pA)_9-T_4$[Tpc3dU(fast)]T_4 **33a** and $A(pA)_9-T_4$[Tpc3dU(slow)]T_4 **33b**, as well as between $d[A(pA)_9]-T_4$[Tpc3dU(fast)]T_4 **33a** and $d[A(pA)_9]-T_4$-

[Tpc3dU(slow)]T_4 **33b**, as shown in Table 5 and Figure 3 (C,D). These results imply that a more flexible pc3dU residue incorporated into DNA is no longer sufficient to maintain rigidity.

Conclusion

This study found that the rigidity of the C3′-*endo* form in the cyclic bridged structure designed by us is essentially a function both of the cyclic propylene linker and of the presence of the 2′-methoxy group. It was also shown that the dimer building block of Tpc3Um(fast) **30a** can be used as an S/N bending motif, while Tpc3Um(slow) **30b** could be used as an S/N linear structural motif. Further study is under way.

Experimental Section

General Remarks: ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded at 270, 68 and 109 MHz, respectively. The chemical shifts were measured from tetramethylsilane or sodium dodecyl sulfate (DSS) for ^{1}H NMR spectra, $CDCl_3$ (77 ppm) or DSS (0 ppm) for ^{13}C NMR spectra, and 85% phosphoric acid (0 ppm) for ^{31}P NMR spectra. The ^{1}H-^{1}H coupling constants were measured at 400 MHz using a Varian Unity 400 spectrometer at 20 °C, except for those of **4a** and **4b**, which were measured at 30 °C to avoid overlapping with the HOD signal. − UV spectra were recorded on a U-2000 spectrometer. − CD spectra were recorded on a J-500 C spectrometer, using a 0.5 cm cell. The synthesis of oligonucleotides was performed on a 381A DNA synthesizer or 392 DNA/RNA synthesizer. − TLC was performed with 60-F_{254} Kieselgel (0.25 mm). − Column chromatography was performed with C-200 silica gel purchased from Wako Co. Ltd., and an aquarium minipump was a convenient means to attain sufficient pressure for rapid chromatographic separation. − Reversed phase column chromatography was performed using μBondapak C-18 silica gel (prep S-500, Waters). Reversed phase HPLC was performed using the following systems: System 1: An LC module 1 was used, with an M-741 data module and a μBondasphere 5 μ C18 100 m column (3.9 × 150 mm) at 50 °C with a linear gradient (0−30%) of CH_3CN in 0.1 M NH_4OAc, pH 7.0 at a flow rate of 1.0 mL/min for 30 min; System 2: A 2690 separation module was used, with a 996 photodiode array detector and a Millennium 2010 chromatography manager. The other conditions were the same as those in System 1; System 3: An SCL-6A system was used, with a combination of LC-6A, SPD-6A, and C-R3A. The other conditions were the same as those in System 1, except for a flow rate of 3.0 mL/min. Anion-exchange HPLC was performed on an LC module 1 with a Waters M-741 data module and a Waters column heater, with a 10−67% linear gradient of 25 mM phosphate, 1 M sodium chloride buffer (pH 6.9) in 25 mM phosphate buffer (pH 6.0) at a flow rate of 1.0 mL/min for 40 min. Pyridine was distilled twice from *p*-toluenesulfonyl chloride and from calcium hydride and then stored over 4Å molecular sieves. Elemental analyses were performed by the Microanalytical Laboratory, Tokyo Institute of Technology at Nagatsuta. The spectral data of all the dinucleotide derivatives can be seen in the supplementary material

5′-*O*-(4,4′-Dimethoxytrityl)thymidine 3′-(*N,N,N′,N′*-Tetraisopropyl)phosphorodiamidite (5): 5′-*O*-(4,4′-Dimethoxytrityl)thymidine (545 mg, 1.0 mmol) was rendered anhydrous by repeated coevaporation with dry toluene, and finally dissolved in dry dioxane (5.0 mL). To the mixture were added triethylamine (0.210 mL, 1.5 mmol) and bis(diisopropylamino)chlorophosphane (315 mg, 1.2 mmol). The solution was stirred at room temperature for 30 min and the reaction was quenched by addition of ethanol (1 mL). After standing for 1 min, the mixture was partitioned between $CHCl_3$ (20 mL) and 5% $NaHCO_3$ (20 mL). The organic layer was collected, washed twice with 5% $NaHCO_3$ (10 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The resulting foam was used for the next reaction without further purification.

Synthesis of the Fully Protected Thymidylyl(3′→5′)2′-*O*-methyluridine Derivative 7, Possessing a Cyclic Structure with a Propylene Bridge: A mixture of **6**[6] (179 mg, 0.5 mmol) and 1*H*-tetrazole (168 mg, 2.4 mmol) was rendered anhydrous by coevaporation with dry toluene, and the residue was dissolved in dry acetonitrile (25 mL) and dioxane (25 mL). A solution of **5** (0.60 mmol) in acetonitrile (3.0 mL) was added dropwise to the mixture over a period of 10 min. The resulting mixture was stirred at room temperature for 40 min and a solution of **5** (0.20 mmol) in acetonitrile (1.0 mL) was added. Stirring was then continued for an additional 1 h. The resulting mixture was stirred at room temperature for 40 min, and then *t*BuOOH (0.562 mL, 5.0 mmol) was added to the mixture. After stirring for 1 h, the solution was evaporated under reduced pressure and the residue was extracted with $CHCl_3$ (100 mL) and 5% $NaHCO_3$ (100 mL). The organic layer was collected, washed twice with 5% $NaHCO_3$ (10 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (25 g) with $CHCl_3$/MeOH (100:2, v/v) containing 1% pyridine to give **7** as a foam (403 mg, 85%, the ratio of the diastereomers 1.1:1).

Synthesis of the Partially Protected Thymidylyl(3′→5′)2′-*O*-methyluridine Derivative 8, Possessing a Cyclic Structure with a Propylene Bridge: The fully protected dimer **7** (334 mg, 0.335 mmol) was dissolved in pyridine (5.0 mL) and 25% aqueous ammonia (5.0 mL). After having been stirred at room temperature for 3 h, the mixture was evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (15 g) with $CHCl_3$/MeOH (100:3, v/v) containing 1% pyridine to give **8** as a foam (287 mg, 90%, ratio of the diastereoisomers 1.1:1).

Unprotected Thymidylyl(3′→5′)2′-*O*-methyluridine Derivatives 2a and 2b, Possessing Cyclic Structures with Propylene Bridges: Compound **8** (81.4 mg, 90.0 μmol) was dissolved in 80% acetic acid (2 mL). After having been kept at room temperature for 30 min, the mixture was evaporated under reduced pressure. The residue was extracted with ether (10 mL) and water (10 mL). The aqueous layer was collected, washed twice with ether, and lyophilized to give a mixture of **2a** and **2b** (51 mg, 93%): − $C_{23}H_{31}N_4O_{13}P{\cdot}1.5H_2O$: calcd. C 43.89, H 5.44, N 8.90; found C 43.67, H 5.46, N 8.65. This mixture was chromatographed on a reversed phase HPLC column using System 3 to give **2a** (334 A_{260}, 19%) and **2b** (406 A_{260}, 23%).

Synthesis of 5′-*O*-(4,4′-Dimethoxytrityl)thymidylyl(3′→5′)2′-*O*-methyluridine Phosphoramidite Derivative 28: DMT-Tpc3Um **8** (258 mg, 0.0.285 mmol) was rendered anhydrous by repeated coevaporation with dry toluene and finally dissolved in CH_2Cl_2 (5 mL). To the mixture were added triethylamine (120 μL, 0.86 mmol) and (2-cyanoethoxy)(diisopropylamino)chlorophosphane (94 μL, 0.423 mmol). The mixture was stirred at room temperature for 2 h and then quenched by addition of ethanol

(0.5 mL). The mixture was diluted with $CHCl_3$ (15 mL). The $CHCl_3$ solution was washed three times with 5% $NaHCO_3$ (15 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (1 mL) and this solution was added portionwise to hexane (80 mL)-ether (20 mL)-pyridine (1 mL) with vigorous stirring. The resulting white precipitate was collected by removal of the solvent by a pipette and dried in vacuo to give DMTrTpc3Ump(a,ce) **28** (273 mg, 87%).

3′,5′-Di-*O*-acetyl-5-(3-hydroxypropynyl)-2′-deoxyuridine (10): A solution of **9** (1.0 g, 2.28 mmol) and 2-propyn-1-ol (0.400 mL, 6.85 mmol) in DMF (25 mL) was degassed under ultrasound irradiation with supersonic wave under reduced pressure, and the reaction vessel was then filled with argon. This manipulation was repeated five times to remove the last traces of air. Triethylamine (640 µl, 4.56 mmol), CuI (87 mg, 0.456 mmol), and tetrakis(triphenylphosphane)palladium (263 mg, 0.228 mmol) were added to the solution. Stirring was continued for 6 h, and the mixture was then evaporated under reduced pressure. The residue was coevaporated with xylene under reduced pressure and chromatographed on a column of silica gel (40 g) with $CHCl_3$/MeOH (100:2, v/v). The fractions containing **10** were combined and evaporated under reduced pressure. The residue was dissolved in water-MeOH (1:1, v/v, 20 mL), and the solution was passed through a Dowex 1-X8 column (CO_3^{2-} form, 15 mL). The eluate and washings were combined and evaporated under reduced pressure to give **10** as a foam (597 mg, 71%). − ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.12 (s, 3 H), 2.18 (s, 3 H), 2.26−2.60 (m, 2 H), 3.80 (1 H, br), 4.29−4.41 (m, 5 H), 5.25 (m, 1 H), 6.28 (t, 1 H, $J_{1',2'} = J_{1',2''} = 6.8$ Hz), 7.86 (s, 1 H), 10.06 (1 H, br). − ^{13}C NMR (68 MHz, $CDCl_3$): δ = 20.8, 20.8, 378.0, 51.0, 63.7, 73.9, 82.5, 85.7, 92.9, 99.8, 142.2, 149.3, 162.1, 170.5, 170.5. − $C_{16}H_{18}N_2O_8 \cdot 0.5H_2O$: calcd. C 51.20, H 5.10, N 7.47; found C 51.62, H 4.85, N 7.39.

3′,5′-Di-*O*-acetyl-5-(3-hydroxypropyl)-2′-deoxyuridine (11): A solution of **10** (597 mg, 1.63 mmol) in dioxane/MeOH (1:1, v/v, 20 mL) was treated with Pd/C (240 mg) under hydrogen with shaking at room temperature for 43 h. The solution was filtered over Celite, and the filtrate was evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (12 g) with $CHCl_3$/AcOEt (2:8, v/v) to give **11** as a foam (576 mg, 95%). − ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.73−1.79 (m, 2 H), 2.12−2.66 (10 H), 3.62 (m, 2 H), 4.26−4.45 (m, 4 H), 5.21−5.23 (m, 1 H), 6.32 (dd, 1 H, $J_{1',2'} = 8.6$ Hz, $J_{1',2'} = 5.6$ Hz), 7.35 (s, 1 H), 9.43, (1 H, br). − ^{13}C NMR (68 MHz, $CDCl_3$): δ = 20.8, 23.0, 31.5, 37.5, 60.7, 63.8, 74.1, 82.2, 84.9, 114.9, 135.5, 150.2, 163.8, 170.4, 170.5. − $C_{16}H_{22}N_2O_8 \cdot 0.75H_2O$: C 50.01, H 6.17, N 7.30; found C 49.92, H 5.82, N 7.32.

3′-*O*-Benzoyl-5-(3-hydroxypropyl)-2′-deoxyuridine (14): Compound **11** (452 mg, 1.25 mmol) was dissolved in pyridine (6 mL) and 25% aqueous ammonia (6 mL). The mixture was stirred at room temperature for 10 h. The solvent was then removed under reduced pressure. The residue was rendered anhydrous by coevaporation three times with dry pyridine and finally dissolved in dry pyridine (9 mL). 4,4′-Dimethoxytrityl chloride (554 mg, 1.63 mmol) was added, and the mixture was stirred at room temperature for 3 h. Extraction was performed with $CHCl_3$ (50 mL) and 5% $NaHCO_3$ (50 mL). The organic layer was collected, washed twice with 5% $NaHCO_3$ (25 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was rendered anhydrous by repeated coevaporation with dry pyridine and finally dissolved in dry pyridine (9 mL). To the solution were added benzoic anhydride (504 mg, 2.23 mmol) and 4-dimethylaminopyridine (5 mg, 41 µmol). After stirring at room temperature for 7 h, the mixture was quenched by addition of water. Extraction was performed with $CHCl_3$ (50 mL) and 5% $NaHCO_3$ (50 mL). The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was coevaporated three times with toluene under reduced pressure and dissolved in 80% acetic acid (10 mL). The mixture was stirred at room temperature for 30 min and then evaporated under vacuo. The residue was chromatographed on a column of silica gel (12 g) with $CHCl_3$/MeOH (100:3, v/v) to give **14** as a foam (186 mg, 64%). − ^{1}H NMR (270 MHz, [D_5]pyridine): δ = 2.02−2.06 (m, 2 H), 2.66−2.82 (m, 4 H), 3.82−3.86 (m, 2 H), 4.20 (m, 2 H), 4.51 (m, 1 H), 5.93−5.95 (m, 2 H), 7.05 (t, 1 H, $J_{1',2'} = J_{1',2''} = 7.3$ Hz), 7.38−8.10 (m, 5 H), 8.32 (s, 1 H), 13.28, (1 H, br). − ^{13}C NMR (68 MHz, [D_5]pyridine): δ = 24.1, 32.1, 38.1, 61.2, 62.5, 77.0, 85.4, 86.2, 115.1, 128.9, 129.9, 130.2, 133.6, 151.9, 164.8, 166.1. − $C_{19}H_{22}N_2O_7 \cdot 0.1H_2O$: C 58.18, H 5.70, N 7.14; found C 57.90, H 5.42, N 7.12.

Cyclodeoxyuridylic Acid Derivative 16, Possessing a Propylene Bridge: A mixture of **14** (48 mg, 0.123 mmol) and 1*H*-tetrazole (41 mg, 0.488 mmol) was rendered anhydrous by repeated coevaporation with dry toluene and finally dissolved in dry acetonitrile (6 mL) and dry dioxane (6 mL). Bis(diisopropylamino)(2-cyanoethoxy)phosphane (46 µL, 0.146 mmol) was added dropwise with stirring over a period of 5 min. After the mixture had been stirred for 1 h, *t*BuOOH (0.122 mL, 1.22 mmol) was added. The resulting mixture was stirred for an additional 30 min and evaporated under reduced pressure. The residue was extracted with $CHCl_3$ (10 mL) and water (10 mL). The organic layer was collected and dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was dissolved in pyridine (1.5 mL) and 25% aqueous ammonia (4.5 mL). The mixture was kept at room temperature for 24 h. After removal of the solvent under reduced pressure, the residue was chromatographed on Whatman 3MM papers with *i*PrOH/ammonia-water (7:2:1, v/v/v). Elution of the band containing the product and lyophilization gave **16** (661 A260, 64%). − UV (H_2O): $\lambda_{max.}$ 267 nm, λmin. 235 nm. − PC: R_f = 0.38 (*i*PrOH/25% NH_3 aq/H_2O = 7:1:2). − Reversed phase HPLC: 5.9 min. − ^{1}H NMR (400 MHz, D_2O): δ = 1.50−1.55 (m, 1 H), 1.59−1.64 (m, 1 H), 2.24−2.38 (m, 4 H), 3.56−3.62 (m, 1 H), 3.64−3.68 (m, 1 H), 3.90 (1 H, dt, $J_{4',5''} = J_{5',P} = 3.3$ Hz, $J_{5',5''} = 12.1$ Hz), 3.97−4.00 (m, 2 H), 4.49−4.53 (m, 1 H, $J_{2',3'} = J_{2'',3'} = 6.2$ Hz, $J_{3',4'} = 4.9$ Hz), 6.19 (t, 1 H, $J_{1',2'} = J_{1',2''} = 5.7$ Hz), 7.61 (s, 1 H). − ^{13}C NMR (101 MHz, D_2O, [D_8]dioxane): δ = 21.6, 27.4, 39.5, 63.7, 64.1, 70.2, 86.0, 86.4, 86.5, 112.3, 140.9, 152.5, 166.8. − ^{31}P NMR (109 MHz, D_2O): δ = 1.44. − MS (FAB+) $C_{12}H_{18}N_2O_8P$ (M + H) calcd. 349.0801; found 349.0818.

Synthesis of Fully Protected Thymidylyl(3′→5′)deoxyuridine Derivative 18, Possessing a Cyclic Structure with a Propylene Bridge: A mixture of **14** (46 mg, 0.118 mmol) and 1*H*-tetrazole (50 mg, 0.708 mmol) was rendered anhydrous by coevaporation with dry toluene, and the residue was dissolved in dry acetonitrile-dioxane (1:1, v/v, 12 mL). A solution of **5** (138 mg, 0.178 mmol) in acetonitrile (1.5 mL) was added dropwise to the mixture over a period of 5 min. The resulting mixture was stirred at room temperature for 2 h, and *t*BuOOH (0.12 mL, 1.2 mmol) was then added to the mixture. After stirring for 30 min, the solution was evaporated under reduced pressure and the residue was extracted with $CHCl_3$ (20 mL) and 5% $NaHCO_3$ (20 mL). The organic layer was collected, washed twice with 5% $NaHCO_3$ (10 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (12 g) with $CHCl_3$/MeOH (100:2, v/v) containing 1% pyridine to give **18** as a foam (82 mg, 71%, ratio of the diastereomers 1.5:1).

Unprotected Thymidylyl(3′→5′)thymidine Derivatives 3a and 3b, Possessing Cyclic Structures with Propylene Bridges: Compound **18** (72 mg, 73.5 μmol) was dissolved in pyridine (1.5 mL), and 2% aqueous ammonia (4.5 mL) was added. The mixture was stirred at room temperature for 18 h. The mixture containing the debenzoylated product **31** was then evaporated under reduced pressure, and the residue was coevaporated three times with toluene to remove the last traces of pyridine and dissolved in 80% acetic acid (6 mL). After having been kept at room temperature for 1 h, the mixture was evaporated under reduced pressure. The residue was extracted with ether (2.5 mL) and water (2.5 mL). The aqueous layer was collected, washed twice with ether, lyophilized, and loaded onto a reversed phase HPLC column, using System 3, to give **3a** (358 A_{260}, 28%) and **3b** (332 A_{260}, 26%).

Synthesis of Partially Protected Thymidylyl(3′→5′)deoxyuridine Derivative 31, Possessing a Cyclic Structure with a Propylene Bridge: Compound **18** (337 mg, 0.356 mmol) was dissolved in pyridine (5 mL), and concentrated ammonia (25%. 15 mL) was added. After having been kept at room temperature for 18 h, the mixture was evaporated under reduced pressure. The residue was coevaporated with toluene and chromatographed on a column of silica gel with $CHCl_3$/MeOH (100:2.5, v/v) containing 1% pyridine to give **31** as a foam (284 mg, 91%, ratio of the diastereomers 1.3:1).

Synthesis of 5′-*O*-(4,4′-Dimethoxytrityl)thymidylyl(3′→5′)-deoxyuridine Phosphoramidite Derivative 32: DMT-Tpc3dU **31** (279 mg, 0.319 mmol) was rendered anhydrous by repeated coevaporation with dry toluene and finally dissolved in CH_2Cl_2 (5 mL). To the mixture were added triethylamine (134 μL, 0.96 mmol) and 2-cyanoethoxydiisopropylaminochlorophosphane (106 μL, 0.478 mmol). The mixture was stirred at room temperature for 2 h and then quenched by addition of ethanol (0.5 mL) and diluted with $CHCl_3$ (15 mL). The $CHCl_3$ solution was washed three times with 5% $NaHCO_3$ (15 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (1 mL) and this solution was added portionwise, with vigorous stirring, to hexane (80 mL)-ether (20 mL)-pyridine (1 mL). The resulting white precipitate was collected by removal of the solvent by pipette and dried in vacuo to give **32** (322 mg, 94%).

5′-*O*-(4,4′-Dimethoxytrityl)-5-[2-(4,4′-dimethoxytrityloxy)ethyl]-2′-deoxyuridine (21): Compound **19** (2.0 g, 3.80 mmol) was dissolved in pyridine (150 mL), and 0.5 M NaOH aqueous solution (150 mL) was added. After having been stirred at room temperature for 12 h, the mixture was neutralized by addition of Dowex 50 W X8 (free form, 200 mL). The resin was removed by filtration and washed with water-pyridine (1:1, v/v, 250 mL). The filtrate and washings were combined and concentrated under reduced pressure. The residue was partitioned between water (100 mL) and ether (100 mL). The aqueous layer was collected, washed twice with ether (50 mL), and evaporated under reduced pressure. The residue was rendered anhydrous by repeated coevaporation with pyridine (10 mL), coevaporated twice with dry pyridine (10 mL) to remove the last traces of water, and finally dissolved in dry pyridine (40 mL). 4,4′-Dimethoxytrityl chloride (2.84 g, 8.36 mmol) was added, and the resulting solution was stirred at room temperature for 2 h. Extraction was performed with $CHCl_3$ (100 mL) and 5% $NaHCO_3$ (100 mL). The organic phase was collected, washed twice with 5% $NaHCO_3$ (50 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (150 g), with $CHCl_3$/MeOH (97:3, v/v) containing 1% pyridine, to give **21** as a foam (3.02 g, 90%). − ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.15−2.23 (m, 1 H), 2.31−2.35 (m, 3 H), 3.15 (t, 2 H, J = 5.8 Hz), 3.41 (m, 2 H), 3.70 (s, 6 H), 3.74 (s, 6 H), 3.97 (m, 1 H), 4.38 (m, 1 H), 6.32 (t, 1 H, $J_{1′,2′} = J_{1′,2"}$ = 6.4 Hz), 6.73−6.79 (m, 8 H), 7.14−7.45 (19 H, 6-H), 8.54 (1 H, br). − ^{13}C NMR (68 MHz, $CDCl_3$): δ = 27.8, 40.4, 55.2, 61.3, 63.5, 72.1, 84.5, 85.3, 85.9, 86.8, 112.4, 113.0, 113.3, 126.7, 127.1, 127.7, 128.0, 128.1, 130.0, 135.5, 136.2, 136.9, 145.1, 145.5, 150.1, 158.3, 158.6, 162.8. − $C_{53}H_{52}N_2O_{10}$: C 72.58, H 5.98, N 3.20; found C 72.19, H 6.02, N 3.33.

3′-*O*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-5-[2-(4,4′-dimethoxytrityloxy)ethyl]-2′-deoxyuridine (22): Compound **21** (2.6 g, 2.96 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine (15 mL × 3) and finally dissolved in dry pyridine (20 mL). Acetic anhydride (0.84 mL, 8.88 mmol) was added and the solution was stirred at room temperature for 8 h. Water was then added to quench the reaction. The solution was diluted with $CHCl_3$ (200 mL) and washed with 5% $NaHCO_3$ (200 mL). The organic phase was collected, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (60 g) with hexane/$CHCl_3$ (3:7, v/v) containing 1% pyridine to give **22** as a foam (2.44 g, 90%). − ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.08 (s, 3 H), 2.20−2.41 (m, 4 H), 3.13 (m, 2 H), 3.44 (m, 2 H), 3.69 (s, 6 H), 3.75 (s, 6 H), 4.13 (m, 1 H), 5.33 (m, 1 H), 6.34 (t, 1 H, $J_{1′,2′} = J_{1′,2"}$ = 7.2 Hz), 6.73−6.76 (m, 8 H), 7.15−7.41 (18 H), 7.54 (s, 1 H), 8.04 (1 H, br). − ^{13}C NMR (68 MHz, $CDCl_3$): δ = 21.0, 27.9, 37.8, 55.2, 61.3, 63.7, 74.9, 83.8, 84.5, 85.8, 87.0, 112.8, 113.0, 113.3, 126.7, 127.1, 127.7, 128.0, 128.2, 130.0, 130.1, 135.4, 136.3, 136.6, 144.5, 145.1, 150.2, 158.4, 158.7, 162.6, 170.3. − $C_{55}H_{54}N_2O_{11}$·1.2H_2O: C 70.22, H 6.04, N 2.98; found C 69.89, H 5.94, N 2.99.

3′-*O*-Acetyl-5-(2-hydroxyethyl)-2′-deoxyuridine (23): Compound **22** (2.32 g, 2.52 mmol) was dissolved in 80% acetic acid (40 mL) and the resulting solution was kept at room temperature for 30 min. The solvent was then removed under reduced pressure. The residue was chromatographed on a column of silica gel (40 g) with $CHCl_3$/MeOH (100:4, v/v) to give **23** as a foam (666 mg, 84%). − ^{1}H NMR (270 MHz, CD_3OD): δ = 2.08 (s, 3 H, CH_3COO−), 2.33−2.37 (m, 2 H, 2′-H, 2"-H), 2.51 (t, 2 H, J = 6.4 Hz), 3.13 (t, 2 H, J = 6.3 Hz), 3.79 (m, 2 H), 4.07 (1 H, dd), 5.30 (m, 1 H), 6.27 (t, 1 H, $J_{1′,2′} = J_{1′,2"}$ = 7.1 Hz), 7.85 (s, 1 H). − ^{13}C NMR (68 MHz, CD_3OD): δ = 21.4, 31.8, 38.9, 61.6, 63.5, 76.9, 86.8, 87.3, 113.2, 139.4, 152.8, 166.4, 172.7. − $C_{13}H_{18}N_2O_7$: C 49.68, H 5.77, N 8.92; found C 49.42, H 5.74, N 8.91.

Protected Cyclodeoxyuridylic Acid Derivative 24, Possessing an Ethylene Bridge: A mixture of **23** (50 mg, 0.16 mmol) and 1*H*-tetrazole (45 mg, 0.64 mmol) was rendered anhydrous by repeated coevaporation with dry toluene and finally dissolved in dry acetonitrile (16 mL). Bis(diisopropylamino)(2-cyanoethoxy)phosphane (60.3 mg, 0.19 mmol) was added dropwise with stirring over a period of 10 min. After the mixture had been stirred for 1 h, *t*BuOOH (0.16 mL, 1.6 mmol) was added. The resulting mixture was stirred for an additional 1 h and evaporated under reduced pressure. The residue was extracted with $CHCl_3$/pyridine (3:2, v/v, 10 mL) and water (10 mL). The organic layer was collected, and the aqueous layer was back-extracted with $CHCl_3$/pyridine (3:2, v/v, 10 mL). The combined extract was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on preparative TLC plates with $CHCl_3$/MeOH (9:1, v/v) to give **24** as a foam (46 mg, 68%, ratio of the diastereoisomers = 1.9:1). **Diastereomer 24a:** TLC, R_f = 0.37 ($CHCl_3$/CH_3OH = 20:1, v/v, developed 3 times). − ^{1}H NMR (270 MHz, [D_5]pyridine): δ = 1.97 (s, 3 H), 2.59−3.04 (m, 6 H), 4.37−4.81 (m, 7 H), 5.70 (t, 1 H, $J_{2′,3′} = J_{3′,4′}$ = 8.1 Hz), 6.78 (t, 1 H, $J_{1′,2′} = J_{1′,2"}$ = 6.1 Hz), 7.94 (s, 1 H), 13.33 (1 H, br). − ^{13}C NMR (68 MHz, [D_5]pyridine):

δ = 19.7, 19.8, 20.6, 26.3, 26.5, 37.3, 62.6, 62.7, 67.7, 67.8, 68.1, 68.2, 73.7, 84.3, 84.4, 85.9, 110.2, 117.9, 136.9, 151.7, 164.5, 170.1. − ^{31}P NMR (109 MHz, [D$_5$]pyridine): δ = −0.58. − $C_{16}H_{20}N_3O_9{\cdot}0.5H_2O$: C 43.84, H 4.83, N 9.52; found C 44.10, H 5.02, N 9.23.

Diastereoisomer 24b: TLC, R_f = 0.32 ($CHCl_3/CH_3OH$ = 20:1, v/v, developed 3 times). − ^{1}H NMR (270 MHz, [D$_5$]pyridine): δ = 1.96 (s, 3 H), 2.59−2.62, (m, 1 H), 2.75 (1 H, dt), 2.98−3.03 (m, 4 H), 4.37−4.67 (m, 7 H), 5.49 (m, 1 H), 6.45 (t, 1 H, $J_{1',2'} = J_{1',2''}$ = 5.9 Hz), 7.91 (s, 1 H), 13.32 (1 H, br). − ^{13}C NMR (68 MHz, [D$_5$]pyridine): δ = 19.7, 19.8, 20.6, 26.4, 40.1, 62.6, 62.6, 67.8, 68.0, 68.5, 68.6, 75.2, 85.4, 85.5, 88.5, 108.3, 117.8, 137.0, 151.3, 164.6, 170.3. − ^{31}P NMR (109 MHz, [D$_5$]pyridine): δ = −1.58. − $C_{16}H_{20}N_3O_9{\cdot}0.5H_2O$: C 43.84, H 4.83, N 9.52; found C 44.10, H 5.02, N 9.23.

Unprotected Cyclodeoxyuridylic Acid Derivative 25, Possessing an Ethylene Bridge: The fully protected cyclodeoxyuridylic acid derivative **24** (17 mg, 38.7 μmol) was dissolved in pyridine (1.5 mL) and 25% ammonia (1.5 mL) was added. The mixture was stirred at room temperature for 2.5 h and then evaporated under reduced pressure. Chromatography of the residue on Whatman 3MM papers with *i*PrOH/ammonia/water (7:2:1, v/v/v) gave **25** (189 A_{260}, 53%). − UV (H_2O): $\lambda_{max.}$ = 266 nm, $\lambda_{min.}$ = 244 nm. − PC: R_f = 0.43 (*i*PrOH/25% NH_3 aq/H_2O = 7:1:2)]. − Reversed phase HPLC: 10.2 min. − ^{1}H NMR (400 MHz, D_2O): δ = 2.40 (1 H, dt, $J_{1',2'} = J_{2',3'}$ = 5.61 Hz, $J_{2',2''}$ = 14.0 Hz), 2.54 (m, 1 H), 2.61−2.68 (m, 2 H), 3.81 (1 H, ddd, $J_{4',5''}$ = 3.2 Hz, $^2J_{5',5''}$ = 11.6 Hz, $J_{5',P}$ = 2.9 Hz), 3.81 (1 H, ddd, $J_{4',5'}$ = 2.7 Hz, $J_{5',5''}$ = 11.7 Hz, $J_{5',P}$ = 2.7 Hz), 4.21 (m, 2 H), 4.37 (m, 1 H), 4.53 (m, 1 H, $J_{2',3'}$ = 4.5 Hz, $J_{3',4'}$ = 2.5 Hz), 6.19 (dd, 1 H, $J_{1',2'}$ = 3.9 Hz, $J_{1',2''}$ = 5.7 Hz), 8.00 (s, 1 H). − ^{13}C NMR (68 MHz, D_2O): δ = 26.2, 26.3, 41.6, 64.5, 64.6, 65.7, 65.8, 71.6, 88.1, 88.2, 88.4, 110.9, 138.1, 152.0, 167.2. − ^{31}P NMR (109 MHz, D_2O): δ = 0.11. − MS (FAB+): $C_{11}H_{15}N_2NaO_8P$ (M + H): calcd. 357.0464; found 357.0492.

Synthesis of the Fully Protected Thymidylyl(3′→5′)thymidine Derivative 27, Possessing a Cyclic Structure with an Ethylene Bridge: A mixture of **23** (50 mg, 0.16 mmol) and 1*H*-tetrazole (45 mg, 0.64 mmol) was rendered anhydrous by coevaporation with dry toluene and the residue was dissolved in dry acetonitrile (8.5 mL). A solution of **5** (0.234 mmol) in acetonitrile (7.5 mL) was added dropwise to the mixture over a period of 10 min. The resulting mixture was stirred at room temperature for 40 min and a solution of **5** (0.079 mmol) in acetonitrile (2.5 mL) was added. After stirring had been continued for an additional 20 min, *t*BuOOH (0.16 mL, 1.6 mmol) was added to the mixture. After stirring for 1 h, the solution was evaporated under reduced pressure and the residue was extracted with $CHCl_3$ (50 mL) and 5% $NaHCO_3$ (50 mL). The organic layer was collected, washed twice with 5% $NaHCO_3$ (25 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (12 g), with $CHCl_3$/MeOH (100:2, v/v) containing 1% pyridine, to give **27** as a foam (101 mg, 70%, ratio of the diastereomers 1.2:1).

Unprotected Thymidylyl(3′→5′)thymidine Derivatives 4a and 4b, Possessing Cyclic Structures with Ethylene Bridges: Compound **27** (87.1 mg, 96.4 μmol) was dissolved in pyridine (1.5 mL), and 2% aqueous ammonia (1.5 mL) was added. The mixture was stirred at room temperature for 9 h. The mixture was then evaporated under reduced pressure, and the residue was coevaporated three times with toluene to remove the last traces of pyridine, and dissolved in 80% acetic acid (4 mL). After having been kept at room temperature for 30 min, the mixture was evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (12 g), with $CHCl_3$/MeOH (100:6, v/v) containing 1% pyridine, to give **4a** and **4b** as a foam (46 mg, 86%).

The diastereomers **4a** and **4b** were separated by reversed phase HPLC with System 3 as described in the general procedure, and isolated to give **4a** and **4b**. Compound **4a**: Reversed phase HPLC 18.0 min (System 1).

Typical Procedure for the Synthesis of Oligonucleotides Incorporating Umc3Um in the Solid Phase Approach: The standard protocol described in the ABI manual for the ABI 381 synthesizer was used. For the incorporation of Umpc3Um into the middle position of dodecanucleotides, the Umpc3Um amidite was used in a glass vessel with a filter. A T-loaded CPG resin (1 μmol, 44 μmol/g, Gren Research) or U-loaded CPG resin (19.7 μmol/g) was used. When the modified dimer was inserted, the following manual manipulation procedure was used: (1) detritylation with 3% TCA/CH_2Cl_2, (2) washing with pyridine, (3) dry up, CH_3CN for 10 min, (4) condensation with the amidite unit (0.1 M, 20 equiv.) in the presence of 1*H*-tetrazole (0.5 M,100 equiv.) /CH_3CN for 10 min, (5) washing with CH_3CN, (6) capping with Ac_2O-Py (1:9, v/v) in the presence of 0.1 M DMAP for 1 min, (7) washing with pyridine (8), oxidation with 0.05 M I_2/THF/Py-H_2O for 1 min (7:2:1, v/v/v), (9) washing with pyridine, (10) washing with CH_2Cl_2. After the final condensation was complete, the CPG gel was removed from the synthesizer and treated with 25% NH_3 aq. Py (9:1, v/v, 2 mL) for 20 h. The excess ammonia was removed under reduced pressure and the resin was suspended in water (1 mL). The mixture was evaporated under reduced pressure and lyophilized. (When the synthesis of RNA oligomers was carried out, the resin was treated with 1 M TBAF·H_2O in THF (1 mL) 16 h, after which desalting was performed by gel filtration using Sephadex G-15. The eluate was lyophilized.) The lyophilized material was separated by anion-exchange HPLC, using a FAX column, followed by reversed phase HPLC:

Compounds T_4[Tpc3Um(fast)]T_4 **30a** and T_4[Tpc3Um(slow)]T_4 **30b** were synthesized by using the phosphoroamidite unit **28** and finally isolated in 3% (2.3 A_{260}) and 3% (2.0 A_{260}) yields, respectively, by HPLC. Similarly, T_4[Tpc3dUm(fast)]T_4 **33a** and T_4[Tpc3dUm(slow)]T_4 **33b** were synthesized in 12% (8.4 A_{260}) and 7% (5.0 A_{260}) yields, respectively, by using the phosphoroamidite **32**.

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