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Synthesis and Properties of Oligonucleotides with Iodo-Substituted Aromatic Aglycons: Investigation of Possible Halogen Bonding Base Pairs

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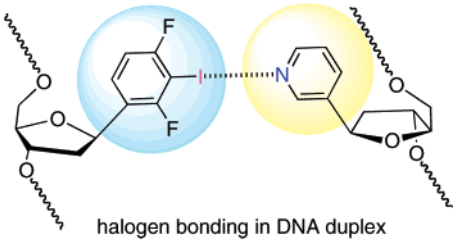
Received August 6, 2007



Ab initio calculations of halogen bond energies of artificial base pairs constructed between iodinated aromatic nucleobase mimics and nitrogen-containing acceptor molecules such as pyridine and imidazole suggest that modified base pairs are converted to optimized planar base pairs with weak ΔE values of −0.19 to −3.93 kcal/mol. To evaluate the contribution of halogen bonding toward duplex stabilization of such modified nucleobase mimics introduced into artificial base pairs, we synthesized three C-nucleoside analogues **1**−**3** with several iodinated aromatic rings and an imidazole nucleoside derivative **4** and incorporated them into oligodeoxynucleotides. Hybridization studies of modified oligodeoxynucleotides incorporating iodoaromatic bases showed their unique universal base-like ability; however, no indication of halogen bond formation was observed. A more sophisticated design is required for the development of new base pairs stabilized by halogen bonding.

Introduction

Recently, many nucleoside derivatives having a functional aromatic group as their aglycon have been reported.[1−3] The biological properties and base pairing abilities of these modified bases upon incorporation into oligodeoxynucleotides have been studied.[4,5] For example, Shionoya et al. reported nucleotide analogues as inhibitors of DNA polymerases using phenol- and catechol-type modified bases.[6] Wielckens et al. reported ribosylated derivatives of benzamide as nicotinamide adenine dinucleotide (NAD) analogues.[7] Moreover, Romesberg et al. reported new base pairs stabilized by hydrophobic interactions between aromatic bases.[8] In these studies of nucleosides with aromatic bases, there are some examples of aromatic rings modified by halogen atoms because of their inherent unique properties. Seela and Kröshel reported that the bromo and iodo groups enhanced base stacking on substitution into position 7 of 7-deazaguanine derivatives.[9] 5-Bromouracil has been used instead of thymine because the bromo group affects the stacking effect with the 5′-upstream and 3′-downstream bases.[10] 5-Fluorouracil is known to exhibit strong antitumor activity.[11] Other examples of halogenated bases have also been reported for diverse functions.[12,13]

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10.1021/jo701634t CCC: $40.75
Published on Web 12/15/2007

Among halogens, the iodo group has a potentially interesting property to form halogen bonds[14] with other atoms having lone electron pairs. The order of the strength of halogen bonding is I > Br > Cl > F for the respective halogens. If such halogen bonding can be achieved in DNA duplexes, it can be used instead of hydrogen bonding for connecting different nucleobase analogues in the development of new base pairing motifs. To the best of our knowledge, this possibility has not yet been investigated.

The iodo group when present in aromatic rings is reactive toward various reagents such as Pd catalysts[15] and butyl lithium.[16] Therefore, the synthesis of such modified nucleosides having iodoaromatic rings and oligodeoxynucleotides incorporating them can be challenging. In this paper, we report that it is theoretically possible to construct such base pairs using halogen bonding and that oligodeoxynucleotides incorporating iodoaromatic deoxynucleoside analogues can be successfully synthesized. However, based on our results of hybridization experiments with the halogenated oligodeoxynucleotides, it seems that additional design modifications are required prior to application of this new chemical bond for the synthesis of base pairs with increased stability.

Results and Discussion

Theoretical Evaluation of Stability of Base Pairs of Iodoaromatic Base Analogues and Heteroaromatic Base Analogues Using Halogen Bonding. Halogen bonding has been found in several crystallized organic compounds in naturally occurring substances.[17] A typical example is a weak but very unique interaction between a halogen atom and a heteroaromatic nitrogen atom. We focused on the possibility that this new interaction might be used for stabilizing a base pair formed between a set of artificially designed nucleobase analogues. As an initial trial to evaluate this possibility, we calculated halogen bonding energies of various possible combinations of iodoaromatic base donors and nitrogen-containing heteroaromatic acceptors.

Because iodine is the 53rd element in the periodic table, we could not use a high-level basis set, such as the 6-31G* level. We had previously compared the results of the hydrogen bond energies of several modified base pairs obtained using MO calculations at the 3-21G* to 6-31G* levels. It was observed that the relative stability remained unchanged. Therefore, in this study, the theoretical calculation was performed at the HF/3-21G* level in the gas phase considering basis set superposition errors (BSSE). We also performed calculations considering the effects of solvation using the conductor-like polarizable continuum (CPCM) model.[18] These results are listed in Table 1 and Figure 2.

For calculation, the structures were initially arranged to keep the two aromatic rings in a planar configuration. As a result, after calculations, the halogen bonding structures of all combinations were found to be conversed, and the planarity of the aromatic rings was maintained. The typical geometry of the halogen bond, the bond length, and the bond angle have been reported in various literature.[14] These studies revealed that the optimal bond length was 3 Å and that the optimal bond angle was 160−180°. In the case of the optimized structures shown in Table 1 and Figure 2, the bond length and angle were close to these optimal values.

Gas phase calculations revealed that the halogen bonding energies without BSSE correction (ΔE_{gas} in Table 1) varied from −5.46 to −11.30 kcal/mol and that those with BSSE correction ($\Delta E^{BSSE}{}_{gas}$ in Table 1) varied from −1.98 to −6.63 kcal/mol. These results suggested that the binding energies were overestimated by 3.46 kcal/mol (Table 1, entry 9) to 4.76 kcal/mol

TABLE 1. Theoretical Calculation of Energy (kcal/mol) of Halogen Bonding between Iodoaromatic Compounds and N-Containing Donor Compounds[a]

entry	acceptor	donor	ΔE_{gas}	ΔE^{BSSE}_{gas}	ΔE_{aq}	ΔE^{BSSE}_{aq}
1			-5.46	-1.98	-3.67	-0.19
2			-6.53	-2.59	-4.25	-0.31
3			-7.66	-3.79	-5.78	-1.91
4			-9.29	-4.76	-6.95	-2.42
5			-8.66	-4.66	-6.57	-2.57
6			-10.60	-5.84	-7.99	-3.23
7			-9.41	-5.26	-7.02	-2.87
8			-11.30	-6.63	-8.60	-3.93
9			-5.58	-2.12	-4.26	-0.80
10			-6.57	-2.81	-4.97	-1.21

[a] $\Delta E^{BSSE}{}_{aq} = \Delta E_{aq} + (\Delta E^{BSSE}{}_{gas} - \Delta E_{gas})$.

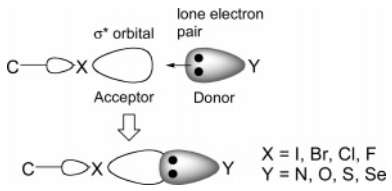

FIGURE 1. Halogen bonding between halogenated compounds and acceptor molecules with heteroatoms (N, O, S, and Se).

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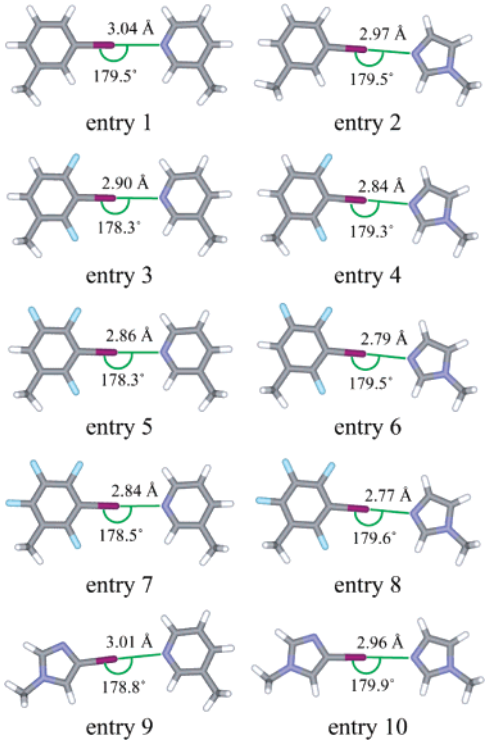

FIGURE 2. Optimized structures of gas phase calculations based on data in Table 1.

(Table 1, entry 6), assuming that the BSSE corrections were accurate. In the case of aqueous phase calculations, the energies (ΔE_{aq}) varied from −3.67 to −8.60 kcal/mol. Using the BSSEs corresponding to the gas phase, ΔE_{aq} was further corrected to result in the interaction energies in the aqueous phase ΔE^{BSSE}_{aq}. The simplest example, as depicted in Table 1, entry 1, showed the smallest ΔE^{BSSE}_{aq} value of −0.19 kcal/mol, and the halogen bond formed between a tetra-fluoroiodophenyl derivative and imidazole, as shown in Table 1, entry 8, resulted in the largest ΔE^{BSSE}_{aq} value of −3.93 kcal/mol. Because all of the interaction energy values are consistently smaller than the canonical base pairs, there is a possibility of destabilization of the double-stranded oligonucleotides when halogen bonding base pairs are incorporated. However, optimized structures, as shown in Figure 2, indicate that these types of specific interactions are possible.

In addition, by molecular modeling, we also confirmed that the glycosyl bonds in these optimized base pair structures could be superimposed over those in the natural base pairs, as shown in the case of the superposition with an A−T base pair (see Supporting Information). Further, we performed calculations of partially fixed molecular mechanics for double-stranded DNA containing the modified nucleosides iPh and Py (Figure 3) at the central position of each single strand. The results showed that iPh and Py maintained the halogen bonded base pair shown in entry 1 of Figure 2 without a large distortion of the B-type duplex structure (see Supporting Information). These data suggest that there is a possibility of formation of such base pairs in double-stranded oligonucleotides.

Synthesis of C- and N-Nucleosides Having Iodoaromatic Aglycon and Their Phosphoramidite Building Blocks. We synthesized several new deoxynucleoside derivatives **1**−**4** with iodoaromatic nucleobase analogues. They include 3-iodophenyl (iPh), 2,4-difluoro-3-iodophenyl (2F), 2,4,5-trifluoro-3-iodophenyl (3F), and 4-iodoimidazol-1-yl (iIm) moieties as the aglycon

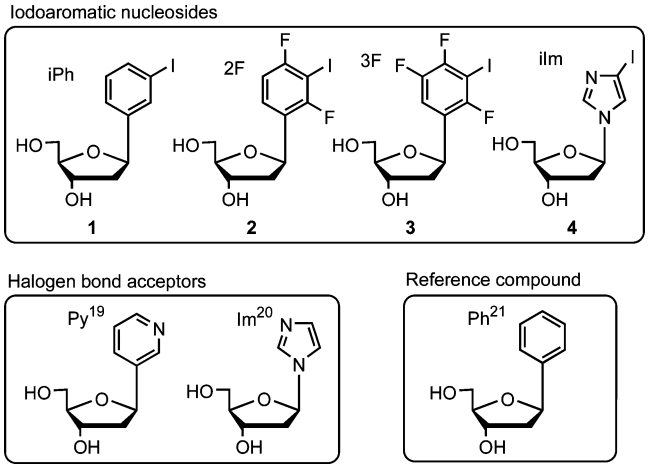

FIGURE 3. Nucleoside derivatives with iodoaromatic rings (iPh, 2F, and 3F), 4-iodoimidazole (iIm), pyridine-3-yl (Py),[19] imidazol-1-yl ring (Im),[20] and phenyl (Ph)[21] moieties.

components, as shown in Figure 3. We adopted Woski and Wichai's method for preparing the three C-glycosides **1**−**3**.[22]

For the synthesis of **1**, 1,3-diiodobenzene **5** was lithiated with *n*-BuLi, and then silyl-protected 2-deoxyribonolactone **6** was added to a solution of the 1-iodo-3-lithiobenzene intermediate. The resulting hemiacetal was allowed to react in situ with excess Et_3SiH and $BF_3{\cdot}OEt_2$ to produce an inseparable anomeric mixture (α/β = 1:5.6) of **8** with a total yield of 32%. The free C-nucleoside **9** (an anomeric mixture of **1**) was obtained by treatment of **8** with $Et_3N{\cdot}3HF$ and Et_3N. Compound **10**, obtained by dimethoxytritylation of **9**, could be easily isolated with an overall yield of 57% by column chromatography. For preparing oligodeoxynucleotides using standard β-cyanoethyl phosphoramidite chemistry, the building block **11** was synthesized by phosphitylation of **10** with 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetra-isopropylphosphordiamidite[23] (Scheme 1).

Next, we attempted to synthesize the C-nucleoside **2** as an anomeric mixture of **19** and its diastereomerically pure phosphoramidite derivative **21**, as shown in Scheme 3. Schlosser and Rausis reported that the treatment of 1,3-difluorobenzene **12** with *s*-BuLi led to selective 2-lithiation and that the lithiated species **13** could react with iodine to give the iodinated compound 1,3-difluoro-2-iodobenzene **14** quantitatively (Scheme 2).[24]

Therefore, we first attempted to synthesize the 1-(2-deoxy-β-D-ribofuranosyl)-2,4-difluorobenzene derivative (**18**)[25] as a key intermediate to obtain **19**. For the synthesis of **18**, we attempted to generate the organolithium compound by halogen−lithium exchange of 1-bromo-2,4-difluorobenzene (**15**) with *n*-BuLi. However, lithiation of the 3-H proton of **15** occurred simultaneously to give a mixture of the desired and undesired lithiated species.

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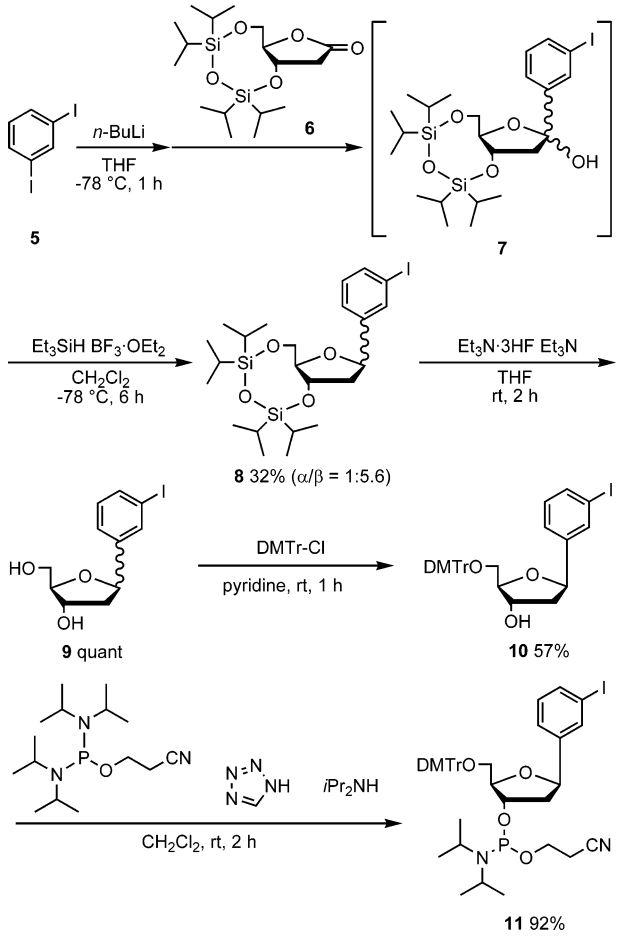
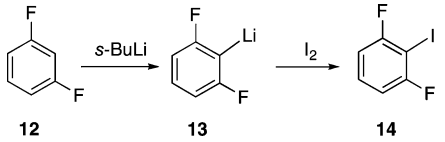
SCHEME 1. Synthesis of C-Nucleoside 9 (Anomeric Mixture of 1) and Its Diastereomerically Pure Phosphoramidite Building Block 11

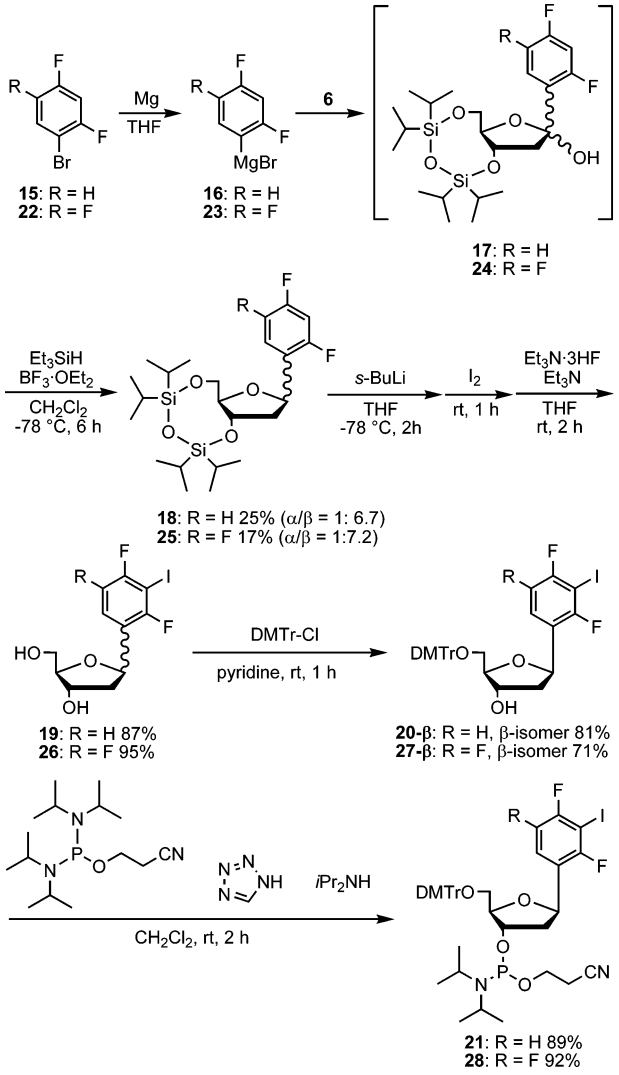


SCHEME 2. Lithiation of 1,3-Difluorobenzene



Therefore, we chose an alternative way to synthesize **19**. Compound **15** was converted to the Grignard reagent **16**, which in turn was allowed to react with **6**. Treatment of the resulting hemiacetal **17** with Et_3SiH and $BF_3{\cdot}OEt_2$ gave an anomeric mixture of **18** in 25% yield (α/β = 1:6.7). Although the yield of the C-glycosylation product seemed rather low, it was comparable to those obtained by similar C-glycosylation reactions.[24] The selective lithiation of **18** with *s*-BuLi followed by reaction with I_2 gave the iodinated product. Successive in situ treatments of this product with $Et_3N{\cdot}3HF$ and Et_3N resulted in the desired nucleoside analogue **19** in 87% yield. The 5′-hydroxyl group of **19** was protected with a 5′-dimethoxytrityl (DMTr) group. At this stage, the desired β-isomer **20**-β could be easily separated from the α-isomer **20**-α by silica gel column chromatography. The α- and β-isomers were isolated in 9 and 81% yields, respectively. To incorporate the C-nucleoside analogues **2** into oligodeoxynucleotides, the 3′-phosphoramidite building block **21** was prepared in the usual manner in 92% yield The phosphoramidite building block **28** of the 2,4,5-trifluoro-3-iodophenyl derivatives **3** could be obtained by a series of reactions starting from **22** via **23**−**26** and **27**-β.

SCHEME 3. Synthesis of C-Nucleoside Derivatives 19 (Anomeric Mixture of 2), 26 (Anomeric Mixture of 3), and Their Diastereomerically Pure Phosphoramidite Building Blocks 21 and 28



Synthesis of Nucleoside Analogue 4 with Iodoimidazole Aglycon and Its Phosphoramidite Unit. The synthesis of **4** was carried out using a stereospecific glycosylation procedure.[26] The reaction of the sodium salt of 4,5-diiodoimidazole (**29**)[27] with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose **30** in acetonitrile afforded the corresponding protected nucleoside analogue **31** in 89% yield. Removal of the toluoyl groups was accomplished by treatment with NaOMe in MeOH to give the free nucleoside **32** in 90% yield, which was further converted to the corresponding 5-*O*-dimethoxytrityl derivative **33** in 86% yield.

Previously, Lovely reported on the selective removal of iodine atoms from 1-substituted 4,5-diiodoimidazole derivatives using EtMgBr.[28] We applied this reagent to the synthesis of the monoiodinated derivative **34**. It was found that treatment of **33** with 2.0 equiv of EtMgBr in THF followed by the addition of

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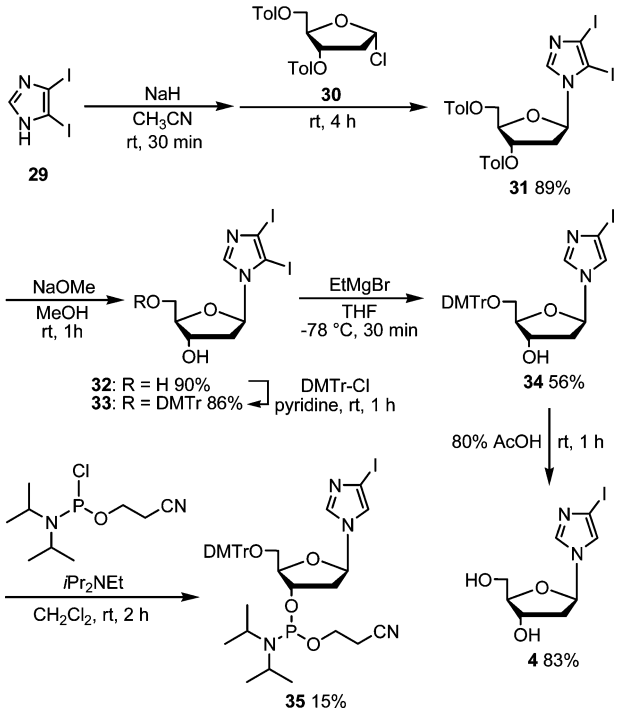
SCHEME 4. Synthesis of Nucleoside Derivative 4 and Its Phosphoramidite Building Block 35



water produced the desired product **34**. To confirm the structure of this product by NMR, it was detritylated with 80% acetic acid to give the 3′,5′-O-free nucleoside **4** in 83% yield. All resonance signal peaks of the ^{1}H and ^{13}C NMR spectra of **4** were unambiguously assigned by DEPT, COSY, and HMQC. In the HMBC measurement, a cross-peak was observed between the anomeric protons with the carbon on the dehalogenated carbon. On the other hand, no cross-peak was observed between the anomeric protons with the carbon on the iodinated carbon. The usual phosphitylation of **34** gave the building block **35** in 15% yield.

Synthesis of Modified Oligodeoxynucleotides. Oligodeoxynucleotide 13-mers **39**−**42** 5′-d(GGACTAXACTGCG)-3′ and **45**−**48** 5′-d(CGCAGTYTAGTCC)-3′ incorporating the iodoaromatic nucleosides **1**−**4** at the central positions were synthesized on a DNA/RNA synthesizer using standard β-cyanoethyl phosphoramidite chemistry (Table 2). Each coupling yield was more than 96%, as estimated by a DMTr cation assay. All oligonucleotides were synthesized according to the DMTr-ON procedure and purified by the use of C_{18} cartridge columns. All oligonucleotides were characterized by MALDI-TOF mass spectrometry, and their purities were checked by anion-exchange HPLC (Table 2).

Interestingly, we found that minor peaks appeared as byproducts in the anion-exchange HPLC charts of **40**, **41**, **46**, and **47**. MALDI-TOF mass spectrometry suggested that these peaks had molecular weights corresponding to the products lacking the iodo group. These results strongly implied the unexpected removal of the iodine atom from the difluoro derivatives **40** and **46** and the trifluoro derivatives **41** and **47** during oligonucleotide synthesis. In contrast to the results of the oligonucleotide synthesis, nucleoside analogues **19** and **26** were stable under the conditions used for oligonucleotide synthesis such as 3% TFA in CH_2Cl_2, I_2 in THF/pyridine/H_2O, and concentrated aqueous NH_3. Moreover, these nucleosides did not react with 1 equiv of each of the T-phosphoramidite building block, triphenylphosphine, and triethyl phosphite in acetonitrile at room temperature for 1 h. The reason for this dehalogenation is unclear, and further studies are necessary.

TABLE 2. Synthesis of Oligodeoxynucleotides with Iodoaromatic Rings as Aglycons[a]

d(GGACTAXACTGCG)				
			MALDI-TOF-mass	
	base X	isolated yield (%)	calculated	found
39	X = iPh	46	4049.6	4049.8
40	2F	16	4085.6	4088.7
41	3F	15	4103.6	4108.7
42	iIm	51	4039.6	4038.8
d(CGCAGTYTAGTCC)				
			MALDI-TOF-mass	
	base Y	isolated yield (%)	calculated	found
45	Y = iPh	42	4000.6	3997.8
46	2F	30	4036.6	4036.4
47	3F	22	4054.6	4055.9
48	iIm	36	3990.5	3990.1

[a] Oligodeoxynucleotides **45**−**48** and **39**−**42** were hybridized with d(GGACTAXACTGCG) (**36**: X = Py, **37**: X = Im, and **38**: X = Ph) and d(CGCAGTYTAGTCC) (**43**: Y = Py and **44**: Y = Im) containing acceptor deoxynucleoside analogues, respectively, to measure T_m values.

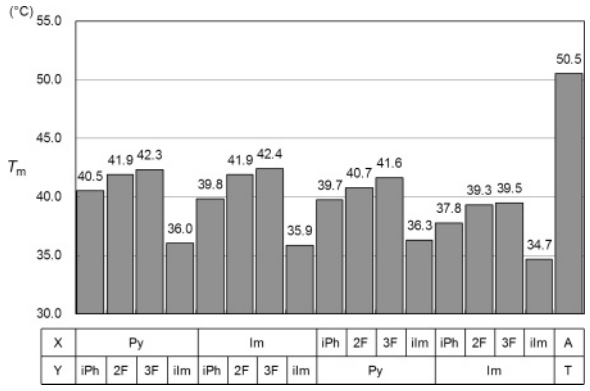


FIGURE 4. Thermal stability of duplexes formed between modified oligodeoxynucleotides with X and Y.

Base Recognition Abilities of Iodoaromatic Bases in DNA Duplexes. To analyze the thermal stability of the base pairs that include the iodoaromatic bases in double-stranded oligodeoxynucleotides, UV-melting experiments were performed. All T_m values were obtained using 2 μM each oligonucleotide in 10 mM sodium phosphate buffer (pH 7.0), 100 mM NaCl, and 0.1 mM EDTA.

We measured the T_m values of oligodeoxynucleotide duplexes containing a pyridine[19] (Py) or imidazole[20] (Im) residue as the aglycon with oligodeoxynucleotides containing a simple iodo donor deoxynucleoside analogue (iPh or iIm). As shown in Figure 4, the duplexes containing iPh exhibited higher T_m values than those containing iIm (see X/Y = Py/iPh vs Py/iIm and Im/iPh vs Im/iIm). When X/Y is reversed to Y/X, a similar tendency was observed (see X/Y = iPh/Py vs iIm/Py and iPh/Im vs iIm/Im). Iodo donor bases, iPh, theoretically form the weakest halogen bond with pyridine (Py) and the second weakest halogen bond with imidazole (Im) (Table 1). Therefore, the hybridization affinity of the oligodeoxynucleotides **40**, **41**, **46**, and **47** for incorporating 2F or 3F was examined to determine

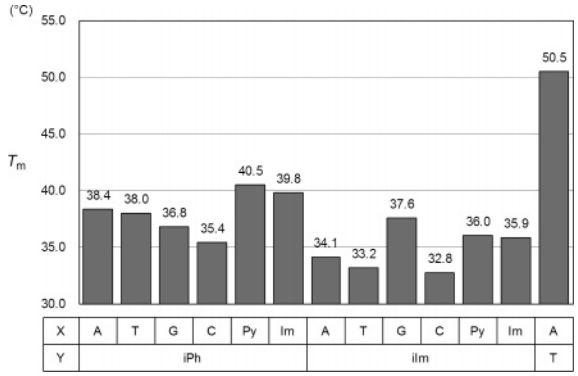


FIGURE 5. Thermal stability of duplexes formed between modified and unmodified oligodeoxynucleotides.

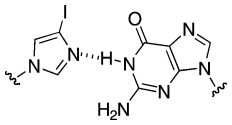


FIGURE 6. Possible hydrogen bond formation between iIm and G.

if they could act as iodo donors capable of forming more stable halogen bonds with Py and Im. These results are also shown in Figure 4.

For the halogen bond acceptors Py and Im, the highest T_m value was observed when they were paired with 3F, and stability decreased in the order of their pairing with 2F > iPh > iIm. To further check if the halogen bond actually contributed to the most stable base pair, we synthesized an oligodeoxynucleotide d(GGACTA[Ph]ACTGCG), **38**, incorporating an unsubstituted phenyl (Ph) base instead of Py using the phosphoramidite unit, as previously reported.[21] The T_m value (42.3 °C) of the duplex (X/Y = Ph/3F) formed between **38** and d(CGCAGT[3F]TAGTCC) **47** was essentially the same as that (42.1 °C) of the duplex (X/Y = Py/3F) formed between **36** and **47**. This result implies that the contribution to stabilization of the halogen bond was negligible and that more sophisticated designs should be considered.

We also studied the hybridization affinity of **45** (Y = iPh) and **48** (Y = iIm) for the complementary oligodeoxynucleotides having naturally occurring nucleobases at the site opposite to Y. For comparison, the data of non-natural base pairs—iPh/Py, iPh/Im, iIm/Py, and iIm/Im—are also shown in Figure 5. The iodinated bases iPh and iIm considerably destabilized the DNA duplexes as compared to their natural, fully matched duplex, for which T_m = 50.5 °C. Interestingly, the iIm moiety specifically recognized guanine and not the other three natural bases, as suggested by T_m = 37.6 °C for X/Y = G/iIm and T_m = 34.1, 33.2, and 33.8 °C for the other pairs—A/iIm, T/iIm, and C/iIm, respectively. This specificity might be explained in terms of a possible hydrogen bond between the 1-N nitrogen of the guanine base and the 3-N nitrogen of iIm (Figure 6).

Moreover, iPh formed duplexes with higher stabilities when paired with synthetic bases Py and Im than when paired with the natural bases. In addition, it should be noted that iPh performs as a universal base because modified DNA duplexes showed similar T_m values with the differences being within 3 °C.

Conclusion

We synthesized a series of new C-deoxynucleoside derivatives with iodinated aromatic rings—iPh, 2F, 3F, and iIm. Modified oligonucleotides incorporating these iodoaromatic nucleosides were also synthesized. Interestingly, it was found that the iodine atoms of oligodeoxynucleotides **40**, **41**, **46**, and **47** were partly removed when the oligonucleotides were synthesized by the standard phosphoroamidite method; however, the reason for the loss of the iodo group is unclear.

We compared the thermal stability of the modified oligodeoxynucleotides with that of the unmodified oligodeoxynucleotides with the canonical bases. The results show that the T_m value of X/Y = G/iIm was higher than the other pairs with the natural bases, thus suggesting the possibility of a base pair shown in Figure 6.

In this model, iIm should be in a syn conformation (i.e., the iodine atom should not face the side of the Watson−Crick base pair but that of the 5′-hydroxyl group). Ab initio studies showed that the energy of the syn conformation equals that of the anti conformation (see Supporting Information). To the best of our knowledge, this is the first published work on halogen bonded base pairs in double-stranded DNA. On the basis of theoretical and experimental data, our results imply that the halogen bond interactions do not contribute as much to the increase in the stability of the double-stranded DNA as the A−T pair. There are several possibilities for this destabilization. In general, halogen bonds have energies of 2−50 kcal/mol, which indicates that a halogen bond could have an interaction energy comparable to a canonical hydrogen bond, depending on the environment.[14] The calculation of interaction energy of halogen bonded base pairs suggested a much lower stability of halogen bonds in the aqueous phase. This result gives a good account of destabilization of double-stranded DNA incorporating the iodoaromatic and halogen bond acceptor bases. In addition, there is a possibility that the glycosyl bond of the iodoaromatic and halogen bond acceptor nucleosides rotates so that the iodo group and the nitrogen atoms move away from each other. Nevertheless, in the case of 2F and 3F, the iodo groups are expected to face opposite strands because the fluorine atom at position 2 is responsible for fixing the glycosyl bond in an anti conformation similar to the carbonyl oxygens at position 2 of thymidine and deoxycytidine. Therefore, a better design of the halogen bond acceptor nucleosides, and possibly the iodoaromatic nucleotides, can lead to the development of new artificial base pairs stabilized by a halogen bond. Studies are currently underway to explore this further.

Experimental Section

3,5-*O*-[(1,1,3,3-Tetra-isopropyl)disiloxanediyl]-1,2-dideoxy-1-(3-iodophenyl)-D-ribofuranose (8). To a solution of 1,3-diiodobenzene (**5**) (4.95 g, 15 mmol) in dry THF (30 mL) under argon at −78 °C was added slowly *n*-BuLi (1.54 M in hexane, 9.74 mL, 15.0 mmol). The mixture was stirred at −78 °C for 30 min. The mixure was added via a syringe to a solution of 3,5-*O*-[(1,1,3,3-tetra-isopropyl)disiloxanediyl]-2-deoxy-D-ribono-1,4-lactone (**6**) (3.74 g, 10.0 mmol) in dry THF (30 mL) at −78 °C. After 1 h, the reaction mixture was quenched with saturated aq NH_4Cl and extracted with ethyl acetate. The combined organic layer was washed with saturated aq NH_4Cl, water, and brine. The organic solution was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting oil containing the initial product **7** was used without further purification in the next reaction.

A solution of the crude oil in CH_2Cl_2 (35 mL) under argon at −78 °C was treated with Et_3SiH (4.79 mL, 30.0 mol) and $BF_3{\cdot}OEt_2$ (3.80 mL, 30.0 mmol). The resulting solution was stirred at −78 °C for 6 h, and the reaction was quenched by the addition of saturated aq $NaHCO_3$ at −78 °C. The resulting mixture was

extracted with ethyl acetate. The combined organic layer was washed with saturated aq $NaHCO_3$ water and brine. The solution was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (C-200, 100 g, hexane/ethyl acetate, 0−1%) to give anomeric mixture **8** (1.80 g, 32%, α/β = 1:5.6). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.73 (0.15H, s), 7.68 (0.85H, s), 7.60−7.58 (1H, m), 7.30−7.29 (1H, m), 7.07−7.04 (1H, m), 5.02 (0.85H, t, J = 7.33 Hz), 4.96 (0.15H, dd, J = 6.10, 9.77 Hz), 4.59−4.54 (0.15H, m), 4.52−4.48 (0.85H, m), 4.14−4.10 (0.85H, m), 4.07−4.03 (0.15H, m), 3.93−3.86 (2H, m), 2.67−2.62 (0.15H, m), 2.39−2.34 (0.85H, m), 2.12−2.00 (1H, m), 1.12−0.95 (28H, m); ^{13}C NMR ($CDCl_3$, 126 MHz, β-isomer) δ 144.8, 136.7, 134.9, 130.3, 125.3, 94.7, 86.7, 78.3, 73.2, 63.7, 43.3, 17.8, 17.7, 17.7, 17.5, 17.3, 17.3, 17.2, 13.7, 13.6, 13.2, 12.8. ESI MS calcd $C_{23}H_{43}INO_4Si_2^+$ $[M + NH_4]^+$ 580.1770, found 580.2539.

1,2-Dideoxy-1-(3-iodophenyl)-D-ribofuranose (9). To a solution of an α,β-anomeric mixture of **8** (1.68 g, 3.18 mmol) in THF (16 mL) were added Et_3N (799 μL, 5.73 mmol) and $Et_3N{\cdot}3HF$ (1.81 mL, 11.2 mmol) at room temperature. After stirring for 1 h, the mixture was extracted with $CHCl_3-H_2O$, and the organic layer was washed with H_2O. The organic solution was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (C-200, 30 g, $CHCl_3$/MeOH, 0−3%) to give an anomeric mixture of **9** as a colorless oil (951 mg, quant., α/β = 1:5.6). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.74 (0.15H, s), 7.70 (0.85H, s), 7.63−7.60 (1H, m), 7.33−7.29 (1H, m), 7.10−7.05 (1H, m), 5.10 (0.85H, dd, J = 5.62, 10.26 Hz), 5.38 (0.15H, t, J = 7.81 Hz), 4.47−4.42 (1H, m), 4.07 (0.15H, dd, J = 4.88, 9.28 Hz), 4.02−4.00 (0.85H, m), 3.85−3.81 (1H, m), 3.78−3.71 (1H, m), 2.70−2.65 (0.15H, m), 2.28−2.65 (0.85H, m), 2.04−1.94 (3H, m); ^{13}C NMR ($CDCl_3$, 126 MHz, β-isomer) δ 143.7, 136.9, 135.0, 130.4, 125.4, 94.6, 87.5, 79.3, 73.6, 63.4, 43.9. ESI MS calcd. $C_{11}H_{13}INaO_3^+$ $[M + Na]^+$ 342.9802, found 342.9808.

3,5-*O*-[(1,1,3,3-Tetra-isopropyl)disiloxanediyl]-1,2-dideoxy-1-(2,4-difluorophenyl)-D-ribofuranose (18). Magnesium (384 mg, 15.8 mmol) and iodine (200 mg, 0.79 mmol) were added to dry THF (15 mL) under argon at room temperature. 2,4-Difluorobromobenzene (**15**) (1.89 mL, 15.8 mmol) was added dropwise via a syringe to the mixture. The mixture was stirred for 1 h at room temperature. After formation of the Grignard reagent **16** was complete, 3,5-*O*-[(1,1,3,3-tetra-isopropyl)disiloxanediyl]-2-deoxy-D-ribono-1,4-lactone (**6**) (2.00 g, 5.27 mmol) in dry THF (5 mL) was added via a syringe to the mixture at 0 °C. After stirring for 2 h, the reaction mixture was quenched with saturated aq NH_4Cl and extracted with ethyl acetate. The combined organic layer was washed with saturated aq NH_4Cl, water, and brine. The organic solution was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting oil containing the hemiacetal **17** was used without further purification in the next reaction. A solution of the crude oil in CH_2Cl_2 (15 mL) under argon at −78 °C was treated with Et_3SiH (2.53 mL, 15.8 mol) and $BF_3{\cdot}OEt_2$ (2.00 mL, 15.8 mmol). The resulting solution was stirred at −78 °C for 6 h, and the reaction was quenched by the addition of saturated aq $NaHCO_3$ at −78 °C. The resulting mixture was extracted with ethyl acetate. The combined organic layer was washed with saturated aq $NaHCO_3$, water, and brine. The solution was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (C-200, 80 g, hexane/ethyl acetate, 0−1%) to give an anomeric mixture of **18** (627 mg, 25%, α/β = 1:6.7). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.51−7.47 (1H, m), 6.86−6.82 (1H, m), 6.79−6.75 (1H, m), 5.28 (0.86H, t, J = 7.32 Hz), 5.21 (0.14H, dd, J = 6.09, 9.64 Hz), 4.59−4.54 (0.14H, m), 4.09 (0.86H, dd, J = 5.86, 13.43), 4.13−4.04 (1H, m), 3.95−3.83 (2H, m, $\alpha\beta$-5H), 2.74−2.69 (0.14H, m), 2.46−2.41 (0.86H, m), 2.07−2.02 (1H, m), 1.13−0.92 (28H, m); ^{13}C NMR ($CDCl_3$, 126 MHz, β-isomer) δ 162.5 (dd, J = 12.48, 247.60 Hz), 159.8 (dd, J = 12.48, 249.52 Hz), 128.3 (dd, J = 6.72, 9.60 Hz), 126.0 (dd, J = 3.84, 13.44 Hz), 111.3 (dd, J = 3.84, 21.11 Hz), 103.8 (t, J = 24.95 Hz), 85.9, 72.9 (d, J = 2.88 Hz), 72.3, 63.1, 41.9, 17.8, 17.7, 17.6, 17.5, 17.3, 17.3, 17.2, 13.7, 13.6, 13.2, 12.8. ESI MS calcd $C_{23}H_{38}F_2NaO_4Si_2^+$ $[M + Na]^+$ 495.2169, found 495.2170.

1,2-Dideoxy-1-(2,4-difluoro-3-iodophenyl)-D-ribofuranose (19). To a solution of an anomeric mixture of **18** (460 mg, 0.97 mmol) in dry THF (10 mL) was added dropwise via a syringe *s*-BuLi (0.95 M in hexane, 1.13 mL, 1.07 mmol) under argon at −78 °C. The mixture was stirred at −78 °C for 2 h and then added via a syringe to a solution of iodine (272 mg, 1.07 mmol) in dry THF (550 μL). The mixture was warmed to room temperature and stirred for 1 h. The reaction was quenched by the addition of saturated aq Na_2SO_3. The resulting mixture was extracted with ethyl acetate−H_2O. The organic extracts were combined, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The resulting oil was used without further purification. A solution of the residue in THF (5 mL) was treated with Et_3N (251 μL, 1.80 mmol) and $Et_3N{\cdot}3HF$ (570 μL, 3.50 mmol) at room temperature. After stirring for 6 h, the mixture was extracted with ethyl acetate−H_2O, and the organic layer was washed with saturated aq $NaHCO_3$ and brine. The organic extract was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (C-200, 30 g, $CHCl_3$/MeOH, 0−2%) to give an anomeric mixture of **19** as a colorless oil (301 mg, 87%). ^{1}H NMR (DMSO-d_6, 500 MHz, β-isomer) δ 7.61 (1H, dd, J = 8.54, 15.13 Hz), 7.13 (1H, t, J = 7.56 Hz), 5.20 (1H, dd, J = 5.61, 10.25 Hz), 5.12 (1H, d, J = 3.90 Hz), 4.78 (1H, t, J = 5.86 Hz), 4.20−4.18 (1H, m), 3.81−3.74 (1H, m), 3.50−3.40 (2H, m), 2.14−2.11 (1H, m), 1.80−1.74 (1H, m); ^{13}C NMR (DMSO-d_6, 126 MHz, β-isomer) δ 161.2 (dd, J = 6.72, 242.81 Hz), 158.7 (dd, J = 6.72, 244.24 Hz), 128.6 (dd, J = 3.84, 16.32 Hz), 111.5 (dd, J = 2.88, 12.00 Hz), 87.7, 72.9, 72.4 (t, J = 30.71 Hz), 72.2, 62.2, 42.0. ESI MS calcd $C_{11}H_{11}F_2INaO_3^+$ $[M + Na]^+$ 378.9613, found 378.9607.

1-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-D-ribofuranose-1-yl]-4,5-diiodoimidazole (31). To a solution of 4,5-diiodoimidazole (**29**) (2.22 g, 6.95 mmol) in dry acetonitrile (70 mL) was added sodium hydride (167 mg, 6.95 mmol). After the mixture had been stirred at room temperature for 30 min, 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose (**30**) (500 mg × 4, 6.32 mmol) was added in four portions at 15 min intervals. After a total 2 h of stirring, the solvent was evaporated under reduced pressure. The residue was diluted with ethyl acetate (200 mL). The organic solution was washed with H_2O (200 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel colum chromatography using a gradient (0−12%) of $CHCl_3$/hexane to give **31** (3.80 g, 89%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.95−7.85 (5H, m), 7.29−7.23 (4H, m), 6.78 (1H, dd, J = 5.62, 7.81 Hz), 5.64 (1H, m), 4.67, (2H, t, J = 2.69, 3.42 Hz), 4.61 (1H, m), 2.81−2.80 (1H, m), 2.58−2.53 (1H, m), 2.44 (3H, s), 2.41 (3H, s); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 166.3, 166.0, 144.8, 144.5, 139.1, 130.0, 129.7, 129.6, 129.5, 126.6, 126.4, 97.3, 89.6, 83.3, 79.8, 74.7, 63.9, 39.9, 21.9, 21.9. ESI MS calcd $C_{24}H_{23}I_2N_2O_5^+$ $[M + H]^+$ 672.9696, found 672.9682.

1-(2-Deoxy-β-D-ribofuranose-1-yl)-4,5-diiodoimidazole (32). To a solution of **31** (3.79 g, 5.46 mmol) in MeOH (56 mL) was added NaOMe (305 mg, 5.46 mmol). After this, the mixture was stirred at room temperature for 1 h. The mixture was purified by dry silica gel column chromatography (C-200, 80 g, methanol/chloroform, 0−3%) to give **32** (2.21 g, 90%) as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.14 (1H, s), 5.88 (1H, t, J = 6.59 Hz), 5.34 (1H, d, J = 4.15 Hz), 4.95 (1H, t, J = 5.13 Hz), 4.30−4.28 (1H, m), 3.83−4.82 (1H, m), 3.54−3.45 (2H, m), 2.41−2.27 (2H, m); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 140.1, 97.2, 88.6, 88.0, 84.4, 70.3, 61.2, 40.3. ESI MS calcd $C_8H_{11}I_2N_2O_3^+$ $[M + H]^+$ 436.8859, found 436.8845.

1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranose-1-yl]-4,5-diiodoimidazole (33). The free nucleotide **32** (2.18 g, 5.00 mmol) was dried by coevaporation with dry pyridine (2 mL × 3)

and dissolved dry pyridine (50 mL). 4,4′-Dimethoxytrityl chloride (1.86 g, 5.50 mmol) was added, and the mixture was stirred under argon atmosphere at room temperature for 6 h. The reaction was quenched by the addition of MeOH (5 mL), and the solvent was evaporated under reduced pressure. The residue was diluted with ethyl acetate (100 mL), and the organic solution was extracted with saturated aq $NaHCO_3$ (100 mL × 2). The organic layer was dried by use of anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (C-200, 50 g, methanol/chloroform, 0−2% with 1% triethylamine) to give **33** as a foam (3.17 g, 86%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.77 (1H, s), 7.42−7.20 (9H, m), 6.84−6.82 (4H, m), 5.97 (1H, t, J = 6.35 Hz), 4.50 (1H, m), 3.79 (6H, s), 3.41−3.30 (2H, m), 2.53−2.48 (1H, m), 2.39−2.34 (1H, m), 2.09 (1H, br); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 158.8, 144.4, 139.3, 135.6, 135.6, 130.1, 130.1, 128.2, 128.2, 127.2, 113.4, 96.9, 89.2, 86.9, 86.1, 79.9, 72.2, 63.6, 55.4, 41.7. ESI MS calcd $C_{29}H_{29}I_2N_2O_5^+$ $[M + H]^+$ 739.0166, found 739.0226.

1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranose-1-yl]-4-iodoimidazole (34). The 1.0 M EtMgBr in THF (1.0 mL, 1.0 mmol) was instilled into a solution of **33** (738 mg, 1.0 mmol) in dry THF (10 mL) at −78 °C under argon atmosphere. After stirring for 5 min, the mixture was warmed up to 0 °C and stirred for 5 min. A 1.0 M solution of EtMgBr in THF (1.0 mL, 1.0 mmol) was added dropwise to the solution at 0 °C and stirred for 15 min. The reaction mixture was quenched by the addition of H_2O (3 mL), and the residue was extracted with ethyl acetate and saturated aq $NaHCO_3$. The organic layer was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (C-200, 15 g, methanol/chloroform, 0−1% with 0.5% triethylamine) to give **34** as a foam (334 mg, 56%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.50 (1H, s), 7.40−7.22 (9H, m), 7.11 (1H, s), 6.85−6.83 (4H, m), 5.97 (1H, t, J = 6.59 Hz), 4.50 (1H, m), 4.05 (1H, dd, J = 4.40, 8.06 Hz), 3.80 (6H, s), 3.39−3.26 (2H, m), 2.41 (2H, dd), 2.18 (1H, br); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 158.8, 144.5, 137.5, 135.7, 135.6, 130.7, 130.4, 128.2, 128.2, 127.2, 122.4, 133.4, 86.9, 86.2, 86.1, 82.8, 72.5, 63.9, 55.4, 41.7. ESI MS calcd $C_{29}H_{30}IN_2O_5^+$ $[M + H]^+$ 613.1200, found 613.1118.

***T*$_m$ Measurement.** Each oligonucleotide was dissolved in 10 mM sodium phosphate (pH 7.0) containing 100 mM NaCl and 0.1 mM EDTA so that the final concentration of each oligonucleotide became 2 μM. The solutions were separated into quartz cells (10 mm) and incubated at 85 °C. After 10 min, the solutions were cooled to 5 °C at 0.5 °C/min and then heated until the temperature reached 85 °C at the same rate. During this annealing and melting, the absorptions at 260 nm were recorded and used to draw UV melting curves. The T_m values were calculated as the temperature that gave maximum first derivatives of the UV melting curves. The same experiment was repeated 4 times, and the average of the T_m values is given in Figures 4 and 5. The oligonucleotide concentration incorporating a modified nucleobase was determined on the assumption that ϵ_{260} is identical to that of thymidine.

Acknowledgment. We thank Drs. Akihiro Ohkubo and Haruhiko Taguchi for helpful discussions. This work was supported by a grant from CREST of the Japan Science and Technology Agency and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan. This work was supported in part by a grant of the Genome Network Project from the Ministry of Education, Culture, Sports, Science and Technology, Japan and by the COE21 project.

Supporting Information Available: ^{1}H, ^{13}C, and ^{31}P NMR data of all new products and experimental procedures and characterization of **4**, **10**, **11**, **20**-β, **21**, **25**, **26**, **27**-β, **28**, and **35**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO701634T